PI: **Halfmann, Peter Joseph**	Title: The Building Resistance Against Viral Entities (BRAVE) Innovation Center	
Received: 08/05/2024	Opportunity: PAR-24-251	Council: 08/2024
Competition ID: PAR-24-251	FOA Title: Animal and Veterinary Innovation Centers	
1U18FD008464-01	Dual:	Accession Number: 5033390
IPF: 578503	Organization: UNIVERSITY OF WISCONSIN-MADISON	
Former Number:	Department: Pathobiological Sciences	
IRG/SRG: ZFD1 CVM-T (06)1	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 601,673 Year 2: 808,673 Year 3: 847,173 Year 4: 847,173 Year 5: 789,673	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N HFT: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
Peter Halfmann	The Board of Regents of the University of Wisconsin System	PD/PI
Yoshihiro Kawaoka	The Board of Regents of the University of Wisconsin System	Co-Investigator
Adel Talaat	The Board of Regents of the University of Wisconsin System	Co-Investigator
Andres Mejia	The Board of Regents of the University of Wisconsin System	Co-Investigator
Zhongde Wang	Utah State University	MPI

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ❍ Application ● Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED 2024-08-05

Application Identifier

c. Previous Grants.gov Tracking Number GRANT14225350

5. APPLICANT INFORMATION **UEI*:** LCLSJAGTNZQ7

Legal Name*: The Board of Regents of the University of Wisconsin System
Department:
Division:
Street1*: 21 N Park St, Suite 6301
Street2:
City*: Madison
County: Dane
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 53715-1218

Person to be contacted on matters involving this application
Prefix: First Name*: Darlene Middle Name: A Last Name*: Holte Suffix:
Position/Title: University Grants & Contracts Specialist, Sr
Street1*: 21 N. Park St.
Street2: Suite 6301
City*: Madison
County: Dane
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 53715-1218
Phone Number*: 608-262-3822 Fax Number: 608-262-5111 Email: johnsonholte@rsp.wisc.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 396006492

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).

❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
Food and Drug Administration

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
93.103
TITLE: Food and Drug Administration Research

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
The Building Resistance Against Viral Entities (BRAVE) Innovation Center

12. PROPOSED PROJECT
Start Date* 10/01/2024
Ending Date* 09/30/2029

13. CONGRESSIONAL DISTRICTS OF APPLICANT
WI-002

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Peter Middle Name: Last Name*: Halfmann Suffix:
Position/Title: Assistant Professor
Organization Name*: The Board of Regents of the University of Wisconsin System
Department: Pathobiological Sciences
Division: School of Veterinary Medicine
Street1*: Influenza Research Institute
Street2: 575 Science Drive
City*: Madison
County: Dane
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 53711-1060
Phone Number*: 608-262-2019 Fax Number: 608-709-2189 Email*: peter.halfmann@wisc.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$5,858,979.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$5,858,979.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Brenda Middle Name: Last Name*: Egan Suffix:
Position/Title*: Managing Officer
Organization Name*: The Board of Regents of the University of Wisconsin System
Department: Research & Sponsored Programs
Division:
Street1*: 21 N. Park Street, Suite 6301
Street2:
City*: Madison
County: Dane
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 53715-1218
Phone Number*: 608-262-3822 Fax Number: 608-262-5111 Email*: preaward@rsp.wisc.edu

Signature of Authorized Representative*
Brenda Egan

Date Signed*
08/05/2024

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_Letter_U181049190229.pdf

424 R&R and PHS-398 Specific

Table Of Contents

OMB Number: 4040-0010
Expiration Date: 11/30/2025

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	The Board of Regents of the University of Wisconsin System
UEI:	LCLSJAGTNZQ7
Street1*:	21 N. Park Street
Street2:	Suite 6301
City*:	Madison
County:	Dane
State*:	WI: Wisconsin
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	53715-1218
Project/Performance Site Congressional District*:	WI-002

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Utah State University
UEI:	SPE2YDWHDYU4
Street1*:	1415 Old Main Hill
Street2:	
City*:	Logan
County:	
State*:	UT: Utah
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	84322-1415
Project/Performance Site Congressional District*:	UT-001

Additional Location(s) File Name:

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

OMB Number: 4040-0010
Expiration Date: 11/30/2025

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number D16-00239

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Summary_U181049190225.pdf
8. Project Narrative*	Project_Narrative_U181049190220.pdf
9. Bibliography & References Cited	References_U181049190221.pdf
10.Facilities & Other Resources	Facilities_and_Other_Resources_U181049190217.pdf
11.Equipment	Major_Equipment_U181049190228.pdf

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

SUMMARY

The introduction of highly pathogenic avian influenza (HPAI) virus of the Guangdong H5N1 lineage has resulted in severe disease outbreaks with widespread mortality in wild birds and poultry in the United States. Current control measures, including massive culling of infected flocks, vaccination efforts, and biosecurity procedures have failed to stop the spread of avian viruses including HPAI viruses resulting in significant economic losses and public health risks due to their zoonotic potential. There is a pressing need to explore alternative strategies to combat viral pathogens that inflict massive losses on the poultry industry.

To tackle the susceptibility of chickens to avian viruses, we propose establishing the BRAVE (Building Resistance Against Viral Entities) Innovation Center. This initiative aims to generate chickens with intentional genome alterations that are resistant to viral pathogens of significant biological and economic importance.

To identify the host factors that will be targeted for intentional genome alterations in chickens, we will perform a genome-wide CRISPR/Cas9 study with a sgRNA library in **Aim 1**. These experiments will focus on identifying pro-viral chicken genes that are essential for virus replication. We will begin with HPAI H5N1 virus, and then expand the study to HPAI H7N9 virus and other avian viruses including infectious bronchitis virus (IBV). **Aim 1** will also identify host factor targets for intentional genome alterations to identify unique and common antiviral proteins that directly inhibit the replication of the viruses in this proposal. We will identify and rank the best host factors when knocked out (pro-viral factors) or overexpressed (antiviral factors) for the greatest inhibitory effect on virus replication. We will monitor cell viability and the lack of escape viruses that would mutate away from the resistance profile.

Using the data collected in Aim 1, in **Aim 2**, we will generate an intentional genomically altered (IGA) chicken line resistant to HPAI virus infection and second line that has a multi-virus resistance phenotype. We will employ CRISPR/Cas9 or transposase methods to modify the genomes in chicken primordial germ cells (PGCs) and then inject the modified PGCs into recipient chicken embryos to establish germline-modified chickens and eventually flocks of birds for infection studies in **Aim 3**. Once an IGA chicken line is established, we will compare the susceptibility of the altered birds with that of wild-type birds to HPAI H5N1 and H7N9 virus infections. Virus replication, associated pathology, transmission, and emergence of mutant viruses associated with breakthrough infections will be assessed and compared to wild-type birds. We will also evaluate the susceptibility of the multi-virus-resistant chicken line to H5N1 and H7N9 viruses, along with other avian viruses including IBV. Non-infected IGA chicken lines will be characterized to ensure proper growth, longevity, and fertility along with any pathological changes compared to their wild-type counterparts.

PROJECT NARRATIVE

Highly pathogenic avian influenza (HPAI) viruses are highly transmissible in chickens, causing 100% mortality within days and leading to significant economic losses and potentially severe public health risks if transmitted to humans. This project aims to develop gene modified chickens with reduced susceptibility or resistance to HPAI and other avian viruses by genetically targeting pro-viral host factors, antiviral proteins, or viral genes. Innovatively creating a single gene modified chicken line resistant to multiple viral pathogens could dramatically reduce disease burden and economic losses in the poultry industry.

FACILITIES & OTHER RESOURCES

University of Wisconsin-Madison

Environment – Contribution to Success. Drs. Halfmann, Kawaoka, and Talaat are part of the Pathobiological Sciences Department (PBS) in the School of Veterinary Medicine (SVM) while Dr. Mejia is part of the Comparative Pathology Laboratory at the Research Animal Resources Center (RARC) the University of Wisconsin (UW). As one of the leading research universities in the country, UW ranks 8th national in research expenditures, a statistic that indicate the positive climate for research provided by the university.

The Influenza Research Institute

The laboratories of Drs. Halfmann and Kawaoka are housed at Influenza Research Institute (IRI), a state-of-the-art facility with biological safety level (BSL)-3 Enhanced and BSL-3 Agriculture (BSL-3 Ag) laboratories providing an outstanding environment for research on respiratory viral pathogens including highly pathogenic avian influenza viruses.

An important component of the research program at the IRI is the exceptional oversight for Select Agent projects and biosafety provided by UW. The university has a 'Biosecurity Task Force' that includes personnel with a range of expertise including biosafety, Select Agent compliance, security, facility engineering, law, and communications. The task force maintains regular contact with Drs. Halfmann and Kawaoka along with their staff to ensure compliance and provides answers to questions as they arise.

In addition to administrative support for his program (grants financial manager, administrative assistant, and financial specialist) and operational support from the PBS department and business services office in SVM, salary support for key research staff provided by the UW Graduate School and SVM has enabled the retention of experienced staff and build a strong team with valuable skills to conduct research on highly pathogenic viruses.

Laboratory: The IRI facility houses (b) (4) sq. ft. of BSL-2 laboratory space with an addition (b) (4) sq. ft. of high-throughput BSL-2 laboratory space in the adjacent IRI Annex that houses liquid-handling robots, ll-sorter, and other instruments to support high-throughput projects in this proposal. There is an additional (b) (4) q. ft. of BSL-2 space dedicated to RNA isolation, cDNA synthesis, and library preparation for deep-sequencing on a miSeq platform which will be utilized in this project.

The IRI also houses a (b) (4) sq. f nced BSL-3 laboratory space (including (b) (4) sq. ft. of animal holding, and (b) (4) sq. ft. of laboratory) and (b) (4) sq. ft of BSL-3 agriculture laboratory space (including (b) (4) sq. ft. of animal holding, (b) (4) sq. ft. of lab space, and a (b) (4) sq. ft. procedure room). These designations meet regulatory standards governing the use of pathogenic human viruses. These facilities contain a double-door autoclave and shower facilities. Additionally, laboratory practices, including the use of positive air purifying respirators (PAPRs) and exit showers, meet and exceed guidelines established in Biosafety in Microbiological and Biomedical Laboratories (BMBL, 6th edition) for working with pathogenic viruses. In addition, the IRI has (b) (4) sq. ft. of BSL-3 Ag laboratory space, a designation assigned to laboratories meeting strict, highly regulated biocontainment guidelines. In facilities qualifying for this designation, both supply and exhaust air are filtered, effluent wastes are decontaminated, and the structure is pressure decay tested to ensure facility integrity. All of these BSL-3 facilities have been inspected and approved for use by officials from the Centers for Disease Control (CDC) and the USDA and are registered with these agencies as a select agent facility.

Animals. The IRI also houses a total of (b) (4) sq. ft. of enhanced BSL-3 space that inclu (b) (4) sq. ft. of animal space and (b) (4) sq. ft. of BSL-3 Ag space that includes (b) (4) sq. ft. of animal space and (b) (4) -sq. ft. of procedure space. The animal spaces are approved for coronavirus research and approved by the CDC and USDA for select agent use.

BSL-2 animal space is available at the Charmany Instructional Facility, located within two miles of the IRI. Charmany houses over 20 isolation rooms for various animals. The researchers at the IRI currently occupies four isolation rooms (b) (4) sq. ft. -(b) (4) sq. ft.) at Charmany, and additional rooms can be assigned upon request. Gene edit chicken lines will be raised at Charmany once they are received from Dr. Wang at Utah State University. The staff duties at Charmany include cage changes, health monitoring, breeding, pup weaning, changing and monitoring of food and water, and autoclaving of cages and water.

Veterinary oversight is provided through the UW Graduate School's Research Animal Resource Center. The School of Veterinary Medicine is assigned a Senior Program Veterinarian and Veterinary Technician who report

to the Chief Campus Veterinarian. The Research Animal Resources Center also offers pathology, surveillance, breeding and training services.

Computers. The researchers at the IRH have computers and printers along with an established shared resource area with computers linked to printers and a photocopier scanner for administrative and laboratory personnel use. All administrative staff and scientists in the group have individual laptop computers. All computers are networked into the mainframe computing facilities of UW and are used for data acquisition and analysis, as well as record-keeping. The School of Veterinary Medicine provides information technology (IT) support with a dedicated IT specialist located at the IRI. The IRI server is backed up every night to safeguard data.

Office. Office space for IRI staff is available in areas adjacent to the laboratories.

Meeting space. The IRI has two meeting rooms located in the office area adjacent to the laboratories. The larger meeting room accommodates weekly lab meetings (20–30 people). Wireless access is available for meeting visitors. Both meeting rooms are equipped with projectors, webcams, and speakerphones to facilitate regular webcasts with collaborators.

Hanson Laboratories

Laboratory. Dr. Talaat has research space at Hanson laboratories. His group's space consists of three general BSL-2 laboratory rooms comprising (b) (4) q. ft. for tissue culture, cDNA cloning and sequencing, and handling risk group 2 viruses.

Animals. Similar to Drs. Halfmann and Kawaoka, Dr. Talaat has animal space at Charmany Instructional Facility which houses animal isolation rooms for infection studies of chickens with risk group 2 pathogens under ABSL-2 containment.

Office and meeting space. Dr. Talaat's office is adjacent to the laboratories. Adequate office space for research assistants and associate researcher is available next to the Dr. Talaat's office.

Comparative Pathology Laboratory

Laboratory. Dr. Mejia has laboratory space at the RARC which supports the fixation, processing, slide preparation, staining, and evaluation of tissues from normal and diseases animals.

Office space. Dr. Mejia's office is adjacent to the laboratory space at the RARC.

Utah State University

Laboratory. Dr. Wang has an approximately (b) (4) ft^2 of laboratory space on the 3rd floor of the USTAR (Utah Science Technology And Research initiative) building located on the Innovation Campus**,** 650 E. Grand Avenue, in North Logan at Utah State University (USU). This floor also houses four other research laboratories within the USTAR program with shared core facilities, such as two chemical rooms, two 4^{o}C cold rooms, two cell culture rooms, a microscopy room housing a confocal microscope, and a "freezer farm" room housing multiple ultracool (-80^{o}C) freezers. All the facilities and equipment on the 3rd floor are available 24/7 for use by members of the research teams. Dr. Wang's lab also has an additional cell culture room, a medium preparation room, and a bacteria culture room.

Animals. Dr. Wang has eight animal housing rooms (each is about (b) (4) ft^2), four for Syrian hamsters, one for guinea pigs, one for deer mice, one for rats, and one for other rodents, and two (b) (4) ft^2 animal surgery and micromanipulation rooms located in the animal vivarium on the second floor of the USTAR building. There are additional rooms that can house gene edited chickens generated in this proposal.

The surgery and micromanipulation room located on the 2nd floor of the USTAR building is equipped with two Narishige micromanipulators, two isoflurane anesthesia units, an inverted Nikon microscope, an inverted Olympus microscope, two Nikon dissecting microscopes, a pipette puller, K-System IVF workstation (equipped with Nikon dissecting microscope), two CO_2 embryo culture incubators, one refrigerator and one freezer, and two portable liquid N2 tanks.

Computers. Dr. Wang has multiple desktop computers and laptop computers installed with DNASTAR (Lagergene 9) software and one desktop computer installed with Gel Doc^{TM} EZ imager software for gel imaging.

Office. Dr. Wang has office space ((b) (4) ft^2) on the 3rd floor of the USTAR building.

MAJOR EQUIPMENT

Influenza Research Institute (Drs. Halfmann and Kawaoka, University of Wisconsin-Madison)

The following major equipment is available during the funding period:

- Multiple -80°C Freezers, -20°C Freezers and 4°C Refrigerators
- Multiple Liquid Nitrogen Tanks
- Animal Cage Unit - Nexgen 900 PNC
- Six HEPA-filtered Transmission Isolator Units
- Two Animal Transfer Stations, 6 Sterilgard Class 11 Type A1
- Three BSS310 Biospot Vivas Bioaerosol Sampler
- Twenty Sterilgard E3 Biological Safety Cabinets
- Five Allegra X30R Centrifuges
- Beckman Ultracentrifuge L70
- Four Eppendorf 5417R Refrigerated Centrifuges
- Eppendorf 5810R Centrifuge
- Two Optima L-100XP Ultracentrifuge
- Two 4110 Forma Series 3 Water Jacketed CO_2 Incubators
- Twenty-three CO_2 Water Jacketed, Series II Incubator
- Two Forma 203110 Dual Water Jacketed CO_2 Water Jacketed Incubator
- LabConco CentriVap Micro IR Concentrator
- Thermo Savant Instruments SPD121 SpeedVac Enhanced Evaporator
- Gene Pulser XCell Electroporator
- BD Accuri C6 Plus System Flow Cytometer and BD FACSaria II Cell Sorter
- ABAXIS iStat1 Blood Analyzer
- HESKA Hematrue Veterinary Hematology System
- Evos M5000 Light Cube Imaging System
- BioRad Gel Doc Go Imaging System
- ImmunoSpot S6 Ultra M2 Analyzer
- Three I-24, Eppendorf Benchtop Incubator Shakers and 4x I-Series I26 Shakers
- Bio-plex 200 Pro Microplate Wash Station
- BioTek Automated Microplate Stacker, MicroFlo Dispenser, Microplate Reader ELX800 and Microplate TS Washer
- Infinite M1000 Premium Quad4 Monochromator Microplate Reader
- Two Matrix WELLMATE Microplate Dispenser and Stacker Units
- Microplate Reader 30086376 Spark
- NanoQuant Microplate Reader M200 and F200 Pro
- Promega E6521 GloMAX 96 Microplate Luminometer
- Spectramax ID5 Standard Multi-mode Microplate Reader
- 2100 Bioanalyzer Microscope with Laptop
- AMG Fluorescent Digital Inverted Evos Microscope
- Two AMG Fluorescent Ultra Xtra Wide View Microscope
- BioTek Cytation 7 Cell Imaging Multimode Reader
- Nanodrop 8000 and Lite Spectrophotometer
- Misonix Sonicator Q800R
- 384-Wellblock QuantStudio 6 Flex Real-Time PCR System
- Applied Biosystems PCR system, QuantStudio6 Flex Real-Time 96-well
- Three BioRad C1000 Touch Thermal Cycler

- Three BioRad S1000 Thermocycler with 48/48 well block
- Two Thermal Cycler - PTC-0200 DNA Thermal Cycler
- Three Tissue Lyser - Qiagen TissueLyser II Disrupter #85300
- Vacuum System UVS800DDA115
- Purelab Ultra-Pure Water Purification
- Automated Liquid Handling System 4730201 Workstation
- Automated Microplate Barcode Labeler and Benchtop Integrated Workstation
- Bravo Liquid Handling Platform LT Liquid Handling Pipetting Head
- Hamilton Liquid-Handling Robot Microlab Starlet Single Arm Manual Load Workstation
- Pico 8 Digital Dispenser for Enhanced Dispensing Precision Workstation
- Platemaster P220 Pipetting System for 96 well plates
- Qiagen QIAcube HT Robotic Workstation for mid to high throughput nucleic acid purification in 96-well Format with UV lamp, HEPA Filter and Laptop.

In addition, the lab is well stocked in various glassware, pipettors, flasks, centrifuge rotors, balances, shakers, microwaves, vacuum hookups, vortexes, water baths, etc., required for general lab work and preparation.

Hanson Laboratories (Dr. Talaat, University of Wisconsin-Madison)

- A Sorvall RC5 Centrifuge, two Benchtop Centrifuges and four Microcentrifuges.
- DNA/RNA Electrophoresis Boxes with two Power Supplies
- Protein Electrophoresis Boxes and Power Supplies
- Two Fisher’s Sonicators
- A BioRad UV/Visible Spectrophotometer
- Two Chemical Hoods
- Three Perkin Elmer PCR Machines for 96-well Format Amplification
- An Olympus (BX51) Bright Field Microscope with a Digital Camera and Fluorescent Optics
- Three Freezers (-20°C), Two freezers (–70°C) and Three Refrigerators
- Three Biosafety Cabinets
- Four CO_2 Incubators
- A Nanodrop
- A Nanopore Sequencing Device
- Eight Benchtop Bacterial Incubators

As part of shared equipment, Dr. Talaat has access to the following:

- Two Real-time PCR Instruments (iCycler, BioRad and ABI prism 7300)
- GenePix Axon Screen and a MicroGrid II DNA Spotter for Microarrays
- Sanger and Next-Generation Sequencing Platforms

Comparative Pathology Laboratory (Dr. Mejia, University of Wisconsin-Madison)

- Biosafety level-2 Necropsy Suite
- Two Downdraft Tables
- Electronic Analytical Balances and Scales
- Multiple Refrigerators and Freezers
- Sakura Tissue Tek VIP Cassette Processor
- Sakura Tissue Tek Embedding Station
- Two Leica RM2235 Microtomes
- A Tissue TEK DRS Automated Slide Stainer
- Refrigerated Centrifuges

- Chemical Hoods
- Class-A2 Biosafety Cabinets
- An Olympus BX43 Microscope with a DP23 Camera and CellSense Olympus Software

Utah Science Technology and Research Initiative Center (Dr. Wang, Utha State University)

- One Real-Time PCR LightCycler
- Three Eppendorf Mastercycle ProGradient Thermal Cyclers
- Two BioRad Therma Cyclers
- Two Applied Biosystem Therma Cyclers
- Horizontal Agarose Gel Electrophoresis Apparatuses and Power Supplies
- A western blotting system
- UV/fluorescence Gel Documentation System
- Five CO_2 Cell Culture Incubators
- Two Inverted Fluorescence Microscopes
- A Dissecting Microscope
- Water Baths
- Multiple -80°C Freezers, -20°C Freezers and 4°C Refrigerators
- Multiple Liquid Nitrogen Tanks
- Benchtop Centrifuges
- RS 2000 X-ray Irradiator
- BD Accuri C6 Plus Benchtop Flow Cytometer
- Two Narishig Micromanipulators
- Two Isoflurane Anesthesia Units
- Pipette Puller
- A K-System In Vivo Fertilization Workstation
- Two CO_2 Embryo Culture Incubators

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Peter Middle Name Last Name*: Halfmann Suffix:

Position/Title*: Assistant Professor
Organization Name*: The Board of Regents of the University of Wisconsin System
Department: Pathobiological Sciences
Division: School of Veterinary Medicine
Street1*: Influenza Research Institute
Street2: 575 Science Drive
City*: Madison
County: Dane
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 53711-1060

Phone Number*: 608-262-2019 Fax Number: 608-709-2189

E-Mail*: peter.halfmann@wisc.edu

Credential, e.g., agency login: (b) (4)

Project Role*: PD/PI Other Project Role Category:

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Biosketch___Halfmann___overall1049083817.pdf

Attach Current & Pending Support: File Name:

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

PROFILE - Senior/Key Person

Prefix: First Name*: Zhongde Middle Name Last Name*: Wang Suffix:

Position/Title*: Professor
Organization Name*: Utah State University
Department: Animal, Dairy and Veterinary S
Division: College of Agriculture and App
Street1*: 9825 Old Main Hill
Street2:
City*: Logan
County:
State*: UT: Utah
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 84322-1415

Phone Number*: 435-797-9668 Fax Number:

E-Mail*: zonda.wange@usu.edu

Credential, e.g., agency login (b) (4)

Project Role*: PD/PI Other Project Role Category:

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Wang___Biosketch___general___07_20241049083208.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: Yoshihiro Middle Name Last Name*: Kawaoka Suffix:

Position/Title*: Professor
Organization Name*: The Board of Regents of the University of Wisconsin System
Department: Pathobiological Sciences
Division: School of Veterinary Medicine
Street1*: Influenza Research Institute
Street2: 575 Science Drive
City*: Madison
County: Dane
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 53711-1060

Phone Number*: 608-265-4925 Fax Number: 608-709-2189

E-Mail*: yoshihiro.kawaoka@wisc.edu

Credential, e.g., agency login (b) (4)

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Biosketch___Kawaoka____06_20241049083249.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: Andres Middle Name Last Name*: Mejia Suffix:

Position/Title*: Chief of Comparative Pathology
Organization Name*: The Board of Regents of the University of Wisconsin System
Department: Research Animal Resource Center
Division: Office of Vice-Chancellor for Research
Street1*: Enzyme Institute
Street2: 1710 University Ave
City*: Madison
County: Dane
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 53726-4087

Phone Number*: (608) 209-2517 Fax Number:

E-Mail*: amejia2@wisc.edu

Credential, e.g., agency login (b) (4)

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Mejia___Biosketch___07_20241049083251.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: Adel Middle Name Last Name*: Talaat Suffix:

Position/Title*: Professor
Organization Name*: The Board of Regents of the University of Wisconsin System
Department: Pathobiological Sciences
Division: School of Veterinary Medicine
Street1*: 1656 Linden Drive
Street2: 337 Hanson Building
City*: Madison
County:
State*: WI: Wisconsin
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 53706-1520

Phone Number*: 608-262-2861 Fax Number: 608-262-7420

E-Mail*: atalaat@wisc.edu

Credential, e.g., agency login: (b) (4)

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Talaat___Biosketch__07_20241049083250.pdf

Attach Current & Pending Support: File Name:

BIOGRAPHICAL SKETCH

NAME: Peter Joseph Halfmann

eRA COMMONS USER NAME (credential, e.g., agency login): (b) (4)

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date	FIELD OF STUDY
University of Wisconsin, Madison, WI	B.S.	2000	Bacteriology
University of Wisconsin, Madison, WI	Ph.D.	2008	Molecular Virology

A. Personal Statement

Over the last 18 years, my research has focused on using different animal models to study virus pathogenesis and to evaluate medical countermeasures. I have focused on RNA viruses including influenza viruses, coronaviruses and filoviruses. Given the continued threat of influenza virus for both humans and animals, I have maintained my involvement in influenza virus research. My introduction into virology began with learning the reverse genetics system for influenza virus, which was used to generate and characterize H5N1 isolates associated with the first six known H5N1 deaths in humans to study viral determinants of pathogenicity in mice. My interest in highly pathogenic avian influenza virus continues with my involvement with mouse and ferret pathogenicity and transmission studies related to bovine H5N1 isolates.

My research has also focused on the development of a novel Ebola virus system that can be utilized outside of biosafety level-4 (BSL-4) containment. This biologically contained Ebola virus (EbolaΔVP30 virus) mimics authentic virus in morphology, protein composition, and growth kinetics. Using this system as a whole-virus vaccine, I have evaluated the efficacy of the Ebola vaccine candidate in immunized animals include mice hamsters, and nonhuman primates at the high containment BSL-4 facility at NIAID's Rocky Mountain Laboratories in Hamilton, MT.

When COVID-19 emerged, I led the nearly complete transition of the infectious disease research at the Influenza Research Institute to SARS-CoV-2. This shift allowed our group to quickly characterize the virus and identify animal models for SARS-CoV-2 to study pathogenicity and transmission, and therapeutics. My research on the transmission of SARS-CoV-2 in domestic cats provided valuable information to veterinarians and cat owners on symptoms of disease in felines. As the pandemic continued, we characterized new SARS-CoV-2 variants in Syrian hamsters to determine changes in pathogenicity and susceptibility to therapeutic countermeasures, directly informing decision and policy makers in the Federal government to assist in their response to the pandemic.

In my current position as Assistant Professor, I will train, supervise, and mentor post-doctoral associates and scientists. In addition, I am highly experienced in managing collaborative projects that require tight integration of research groups across different institutions. In summary, I have established a successful and productive record i n the research field which allows me to play an active role in the current project.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2024- Assistant Professor, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI

2021-2024 Research Associate Professor, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI

2013-2021 Associate Scientist, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI

2010-2013 Assistant Scientist, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI

2009- Special Volunteer, NIAID's Rocky Mountain High Containment BSL-4 Laboratories, Hamilton, MT

2008-2010 Research Associate, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI

2002-2008 Graduate Student, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI
2000-2002 Associate Research Specialist, Department of Pathobiological Sciences, University of Wisconsin, Madison, WI
2000-2002 Associate Research Specialist, Department of Pathobiological Sciences, University of Wisconsin, Madison, WI

C. Contribution to Science

1. **Ebola virus research.** Because Ebola virus is a BSL-4 agent, I sought to develop an *in vitro* system to study the Ebola virus life cycle under non-BSL-4 containment. I engineered a biologically contained virus, termed EbolaΔVP30 virus (Ref. a) that lacks an essential viral gene but grows to high titers in a helper cell line. This system provided a breakthrough in the field because EbolaΔVP30 virus is indistinguishable in shape and growth kinetics from authentic Ebola virus, but can be used in BSL-3 containment. My colleagues and I have used the EbolaΔVP30 virus system to study Ebola virus in detail; for example, to examine the neutralization of monoclonal antibodies to the viral glycoprotein (Ref. b), to determine the expression kinetics of viral proteins and RNA during infection, to determine the role of cellular genes during infection (Ref. c), and to identify cellular factors involved in virus entry and compounds that can inhibit this entry step (Ref. d). Most importantly, I have explored the potential of the EbolaΔVP30 virus system as a novel vaccine platform (see Section 3).

   a. Halfmann P, Kim JH, (+4 other authors) and Kawaoka Y. Generation of biologically contained Ebola viruses. **Proc Natl Acad Sci U S A** 105:1129-1133, 2008. PMCID: PMC2234103.
   b. Dias JM, Kuehne AI, (Halfmann P +11 other authors) and Saphire EO. A shared structural solution for neutralizing ebolaviruses. **Nat Struct Mol Biol** 18:1424-1427, 2011. PMCID: PMC3230659.
   c. Takahashi K, Halfmann P, (+3 other authors) and Kawaoka, Y. DNA topoisomerase 1 facilitates the transcription and replication of the Ebola virus genome. **J Virol** 87:8862-8869, 2013. PMCID: PMC3754039.
   d. Kuroda M, Halfmann P, Kawaoka Y. HER2-mediated enhancement of Ebola virus entry. **PLoS Pathog** 16:e1008900, 2020. PMCID: PMC7556532.

2. **Development of a novel vaccine and therapeutics against Ebola virus.** There are no licensed vaccines to combat Ebola virus infections. To address this need, I tested the protective efficacy of the EbolaΔVP30 virus as a whole-virus vaccine platform in various animal models. I demonstrated protection of mice and guinea pigs immunized three or two times, respectively, with this whole-virus vaccine from a lethal challenge of Ebola virus (Ref. a). More importantly, I recently demonstrated the protective efficacy of this whole-virus vaccine against a lethal challenge of Ebola virus in nonhuman primates (the “gold standard” in Ebola virus vaccine research) after a single immunization (Ref. b). In addition to a vaccine against Ebola virus, I have played a role in the identification and evaluation of therapeutic antibodies against Ebola viruses within a large consortium of investigators (Refs. c and d).

   a. Halfmann P, Ebihara H, (+6 other authors) and Kawaoka, Y. Replication-deficient ebolavirus as a vaccine candidate. **J Virol** 83:3810-3815, 2009. PMCID: PMC2663241.
   b. Marzi A, Halfmann P, (+5 other authors) and Kawaoka Y. An Ebola whole-virus vaccine is protective in nonhuman primates. **Science** 348:439-442, 2015. PMCID: PMC4565490.
   c. Gunn BM, Yu WH, (Halfmann P +22 other authors) and Alter, G. A Role of Fc Function in Therapeutic Monoclonal Antibody-Mediated Protection against Ebola Virus. **Cell Host Microbe**. 24:221-233, 2018. PMID: 30092199.
   d. Saphire EO, Schendel SL, (Halfmann P +44 other authors) and Dye, JM. Systematic Analysis of Monoclonal Antibodies against Ebola Virus GP Defines Features that Contribute to Protection. **Cell**. 174:938-952, 2018. PMCID: PMC6102396.

3. **Determinants of Ebola virus pathogenicity in humans.** Ebola virus is a highly pathogenic virus in humans, but the molecular basis of its pathogenicity is not well understood. During the 2013–2016 West Africa Ebola outbreak, I was part of a study that used integrative “OMICS” approaches to reveal how novel mechanisms such as the induction of pancreatic enzymes and the activity of aberrant neutrophils may contribute to the virulent phenotype of Ebola virus disease in humans (Ref. a). In a follow-up study, we found that levels of plasma lipids in Ebola fatalities are profoundly altered compared with those of Ebola survivors, a finding that could be useful in diagnostic and therapeutic development (Ref. b). After the Ebola outbreak, we monitored

virus-specific antibody titers in Ebola survivors and their close contacts (family members and members of the healthcare community that cared for infected individuals) to determine the rate of potential subclinical cases of Ebola infection in Sierra Leone (Ref. c). The role host factors play to counteract Ebola virus infection can dictate viral pathogenicity. Therefore, we screened a library of over 400 interferon-stimulated genes (ISGs) to identify genes that can attenuate Ebola virus replication (Ref. d). From this screen, we identified the top 10 ISGs that attenuated virus titers by up to 1000-fold and explored the mechanism of one ISG that inhibited both virus transcription and budding (Ref. d).

a. Eisfeld AJ, Halfmann PJ, (+27 other authors) and Kawaoka Y. Multi-platform 'Omics Analysis of Human Ebola Virus Disease Pathogenesis. **Cell Host Microbe** 22(6):817-829, 2017. PMCID: PMC5730472.
b. Kyle JE, Burnum-Johnson KE, (Halfmann PJ + 7 other authors) and Metz TO. Plasma lipidome reveals critical illness and recovery from human Ebola virus disease. **Proc Natl Acad Sci U S A** 116:3919-3928, 2019. PMCID: PMC6397561.
c. Halfmann, PJ, Eisfeld AJ, (+8 other authors) and Kawaoka Y, Sahr F. Serological analysis of Ebola virus survivors and close contacts in Sierra Leone: A cross-sectional study. **PLoS Negl Trop Dis** 13:e0007654, 2019. PMCID: PMC6692041.
d. Kuroda M, Halfmann P (+7 other authors). Identification of interferon-stimulated genes that attenuate Ebola virus infection. **Nat Commun** 11:2953, 2002. PMCID: PMC7289892.

4. **SARS-CoV-2 research.** My nearly 20-year scientific career provided me with an invaluable background in molecular virology and disease modeling in animals that was useful in my transition to coronavirus research. A study in cats demonstrated that cats easily transmit SARS-CoV-2 between infected and naïve cats without showing any outward signs of infection (Ref. a). To establish a small animal model for SARS-CoV-2 infection that resembles human infection, golden Syrian hamsters were infected with virus, and we found efficient virus replication in the lung leading to severe lung pathology (Ref. b). These findings demonstrated that Syrian hamsters are a useful infection model to understand SARS-CoV-2 pathogenesis and to evaluate vaccines and antiviral drugs. (Ref. b). Using the hamster model for SARS-CoV-2 infection, I helped develop a transmission cage system for hamsters to study the transmission kinetics of a new variant of the virus (D614G) and found this new variant transmits faster than the original virus (Ref. c). In a collaborative project, I took the lead to characterize the pathogenicity of the Omicron variant (B.1.1.529: BA.1) in mice and hamsters (Ref. d).

a. Halfmann PJ, Hatta M, Kawaoka Y. Transmission of SARS-CoV-2 in Domestic Cats. **N Engl J Med** 83:592-594, 2020. PMID: 32402157.
b. Imai M, Iwatsuki-Horimoto K, Hatta M, Loeber S, Halfmann PJ, (+28 other authors) and Kawaoka Y. Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. **Proc Natl Acad Sci U S A** 117:16587-16595, 2020. PMCID: PMC7368255.
c. Hou YJ, Chiba S, Halfmann PJ, (+ 21 other authors). SARS-CoV-2 D614G variant exhibitis efficient replication ex vivo and transmission in vivo. **Science** 370:1464-1468, 2020. PMCID: PMC7775736
d. Halfmann PJ, (+ 51 other authors). SARS-CoV-2 Omicron virus causes attenuated disease in mice and hamsters. **Nature** doi: 10.1038/s41586-022-04441-6, 2022. PMID: 35062015.

5. **Influenza virus research.** My scientific career in virology began with the study of highly pathogenic influenza viruses, focusing on the replicative abilities and pathogenicity of these viruses in various animal models. I contributed to studies that identified a single amino acid change as a critical marker of influenza virulence (Ref. a). In another project, I assessed the human influenza A viral genes that restricted the replication of an avian influenza virus in ducks (Ref. b). I was also part of a project that recreated the 1918 pandemic influenza virus and demonstrates the importance of the influenza viral hemagglutinin gene for the virulence of this virus (Ref. c). Recently, we identified several amino changes in the influenza viral PB2 polymerase protein that act synergistically to enhance virulence (Ref. d). These studies formed my technical foundation in virology and provided me with a solid background in reverse genetics and disease modeling in animals.

a. Hatta M, Gao P, Halfmann PJ, and Kawaoka Y. Molecular basis for high virulence of Hong Kong H5N1 influenza viruses. **Science** 293:1840-1842, 2001.
b. Hatta M, Halfmann PJ, Wells K, and Kawaoka Y. Human influenza A viral genes responsible for the restriction of its replication in duck intestine. **Virology** 295:250-255, 2002.
c. Kobasa D, Takada A, (Halfmann PJ +13 other authors) and Kawaoka Y. Enhanced virulence of influenza A viruses with the haemagglutinin of the 1918 pandemic virus. **Nature** 431:703-707, 2004.

d. Fan S, Hatta M, (Halfmann PJ +7 other authors) and Kawaoka Y. Novel residues in avian influenza virus PB2 protein affect virulence in mammalian hosts. **Nat Commun** 5:5021, 2014. PMID: 25289523.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/peter.halfmann.1/bibliography/52175209/public/?sort=date&direction=ascending.

BIOGRAPHICAL SKETCH

NAME: Zhongde Wang

eRA COMMONS USER NAME (credential, e.g., agency login): (b) (4)

POSITION TITLE: Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date	FIELD OF STUDY
Jilin University	B.S.	1986	Chemistry and Molecular Biology
Dalian Polytechnic University	M.S.	1991	Biochemistry
University of Massachusetts at Amherst	Ph.D.	2000	Molecular and Cellular Biology
University of Massachusetts at Amherst	Post. Doc.	2001	Genome engineering in cattle, cloning of transgenic cattle
Whitehead Institute/MIT, Cambridge	Post. Doc.	2004	Transgenic mouse model, epigenetics of mouse stem cells, and mouse cloning

A. Personal Statement

I am a professor in the Department of Animal, Dairy and Veterinary Sciences at Utah State University (USU). My laboratory is specialized in developing genetically engineered animal models in nonconventional laboratory animal species. To address the unmet needs to model human diseases, my laboratory has established genetic engineering techniques in the golden Syrian hamster, guinea pig, P. maniculatus, and P. leucopus, and has created over 50 genetically engineered animal models across all four species, each representing a world-first achievement. These animal models have been used in several countries (e.g., Belgium, Spain, Canada, and USA) by government agencies (e.g., the NIH, the PHAC) and many universities for studying infectious diseases. I also have extensive genetic engineering expertise in livestock, including cattle, sheep and goats, and have developed multiple genetically engineered herds/flocks in these species; we are also in the process of developing genetic engineering techniques in camelids (alpacas), naked mole rats, cotton rats and P. californicus. Directly related to this proposed project, my laboratory also has extensive experience in working with chicken primordial germ cells (PGCs), which provides the assurance for the success of this proposed project. I will be serving as a PI at the USU study site to produce gene-edited chickens resistant to some of the costliest chicken viral infections.

Ongoing and recently completed projects that I would like to highlight include:

1R01AI157513 McGregor (PI) 09/2020-08/2025
Development of a universal DISC vaccine strategy against congenital cytomegalovirus.

1R01AI155561-01 Long (PI) 04/2021-08/2026
Genetic architecture of host response to tickborne disease in Peromyscus leucopus.

1R21AI176683-01 Lowen (PI) 04/2023-03/2025
Role of type I and III interferons in shaping influenza A virus dynamics within and between hosts.

1R01AI173043-01A1 Langlois (PI) 07/2023-06/2028
Natural model for evaluating within- and cross-species virus transmission.

1R01AI132633-01 Chandran (PI) 07/2017-06/2022
Dissecting the receptor-mediated infection mechanisms of hantaviruses.

1R03CA234201-01 Cormier (PI) 01/2019–12/2020
A novel cancer syndrome in KCNQ1-deficient Syrian Golden hamsters.

a. Uraki R, Kiso M, (+ 49 other authors) and Kawaoka Y. Characterization and antiviral susceptibility of SARS-CoV-2 Omicron BA.2. **Nature.** 2022, 607(7917):119-127. PMID: 35576972.
b. Jangra RK, Herbert AS, (+ 26 other authors) and Chandran K. Protocadherin-1 is essential for cell entry by New World hantaviruses. **Nature**. 2018, 563(7732):559-563. PMCID: PMC6556216.
c. Boudewijns R, Thibaut HJ, (+ 39 other authors) and Dallmeier K. STAT2 signaling restricts viral dissemination but drives severe pneumonia in SARS-CoV-2 infected hamsters. **Nat Commun**. 2020, 11(1):5838. PMCID: PMC7672082.
d. Sanchez-Felipe L, Vercruysse T, (+ 45 other authors) and Dallmeier K. A single-dose live-attenuated YF17D-vectored SARS-CoV-2 vaccine candidate. **Nature**. 2021, 590(7845):320-325. PMID: 33260195.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2018 -	Professor, Utah State University, Logan, UT
2012 - 2018	Associate Professor, Utah State University, Logan, UT
2010 - 2011	Executive Director, Hematech, Inc., Sioux Falls, SD
2006 - 2010	Director and Sr. Director, Hematech, Inc., Sioux Falls, SD
2004 - 2006	Senior Scientist, Hematech, Inc., Sioux Falls, SD
2001 - 2004	Postdoctoral fellow, Whitehead Institute/MIT, Cambridge, MA
2000 - 2001	Postdoctoral fellow, University of Massachusetts at Amherst, Amherst, MA
1993 - 2000	Research Assistant, University of Massachusetts at Amherst, Amherst, MA

Review:

2024	Ad hoc reviewer, NIH/NIAID
2021	Ad hoc reviewer, NIH/NIAID
2019	Ad hoc reviewer, USDA, AFRI
2017	Ad hoc reviewer, NIH/ORIP
2017	Ad hoc reviewer, BBSRC

Awards:

2018	Faculty Researcher of the Year Award, College of Agriculture and Applied Sciences, USU
2017	Faculty Researcher of the Year Award, Department of Animal, Dairy and Veterinary Sciences, USU
2004	Postdoctoral Fellowships, Lalor Foundation
2003	Postdoctoral Fellowships, Lalor Foundation

C. Contributions to Science

1. **Development of genetically engineered animal models for human disease.** Genetically engineered murine models have contributed greatly to the understanding of many human diseases, as well as to the development of therapeutics or vaccines. However, they are inadequate for some of the most devastating human diseases, as evidenced by the high percentage of clinical trial failures of therapeutics or vaccines based the preclinical studies in these models. My laboratory has created and characterized several novel genetic animal models in non-murine species that more faithfully recapitulate the pathology of human diseases and provide better predictions for the safety and efficacy of drugs and vaccines in humans.
   a. Halfmann PJ, Iida S, (+ 64 other authors) and Kawaoka Y. SARS-CoV-2 Omicron virus causes attenuated disease in mice and hamsters. **Nature.** 2022, 603(7902):687-692. PMCID: PMC8942849.
   b. Golden JW, Li R, (+ 17 other authors) and Kawaoka Y. Hamsters Expressing Human Angiotensin-Converting Enzyme 2 Develop Severe Disease following Exposure to SARS-CoV-2. **mBio.** 2022 PMCID: PMC8787465.
   c. Brocato RL, Principe LM, (+ 12 other authors) and Hooper JW. Disruption of Adaptive Immunity Enhances Disease in SARS-CoV-2-Infected Syrian Hamsters. **J Virol**. 2020, 94(22):e01683-20. PMC7592214.
   d. Li R, Ying B, (+ 8 other authors) and Toth K. Generation and characterization of an *IL2rg* knockout Syrian hamster model for XSCID and HAdV-C6 infection in immunocompromised patients. **Dis Model Mech.** 2020, 13(8): dmm044602. PMCID: PMC7592214.

2. **Genome engineering for human medicine and agriculture.** Genome engineering tools are currently only available in several animal species, severely limiting the application of these technologies in human

medicine. To address this challenge, my laboratory has embarked on establishing genome engineering technologies, such as CRISPR/Cas9 and TALEN, in several unconventional animal model species. We are the first to succeed in developing gene-targeting technologies in the Syrian hamster, the guinea pig, and Peromyscus. We are also the first to produce a genetic goat model for lung cancer and a genetic sheep model for cystic fibrosis. In addition, my group has produced one of the most extensively genetically engineered cattle for human pharmaceutical production.

a. Li R, Miao J, (+ 4 other authors) and **Wang Z**. Production of Genetically Engineered Golden Syrian Hamsters by Pronuclear Injection of the CRISPR/Cas9 Complex. **J Vis Exp**. 2018, (131):56263. PMCID: PMC5908450.
b. Wu H, Fan Z, (+ 11 other authors) and Sullivan E. Generation of H7N9-specific human polyclonal antibodies from a transchromosomic goat (caprine) system. **Sci Rep.** 2019, 9(1):366. PMCID: PMC6344498.
c. Viotti Perisse I, Fan Z, (+ 11 other authors) and Polejaeva IA. Sheep models of F508del and G542X cystic fibrosis mutations show cellular responses to human therapeutics. **FASEB Bioadv.** 2021, 3(10):841-854. PMCID: PMC8493969.
d. Wang Z. Genome engineering in cattle: recent technological advancements. **Chrom Res**. 2015, 23(1):17-29. PMID: 25596824.

3. **Develop novel non-murine models for viral infections.** My lab has made the unique contributions to the development of novel non-murine animal models for viral infection diseases, including those caused by SARS-CoV-2, Zika virus, Hantavirus, Rift Valley Fever virus, Marburg virus, human adenovirus, etc.
   a. Uraki R, Halfmann PJ, (+ 23 other authors) and Kawaoka Y. Characterization of SARS-CoV-2 Omicron BA.4 and BA.5 isolates in rodents. **Nature.** 2022, 612(7940):540-545. PMID: 36323336.
   b. Uraki R, Iida S, (+ 27 other authors) and Kawaoka Y. Characterization of SARS-CoV-2 Omicron BA.**2**.75 clinical isolates. **Nat Commun.** 2023 Mar 23;14(1):1620. PMID: 36959194.
   c. Ranadheera C, Valcourt EJ, (+ 12 other authors) and Safronetz D. Characterization of a novel STAT 2 knock-out hamster model of Crimean-Congo hemorrhagic fever virus pathogenesis. Sci Rep. 2020, 10(1):12378. PMCID: PMC7378551.
   d. Atkins C, Miao J, (+ 9 other authors) and Freiberg AN. Natural History and Pathogenesis of Wild-Type Marburg Virus Infection in STAT2 Knockout Hamsters. **J Infect Dis.** 2018, (suppl_5):S438-S447. PMCID: PMC6249581.

4. **Develop novel non-murine models for cancer and immunodeficiency.** My laboratory established the world first genetically engineered hamster models for cancer and immunodeficiency. We recently also succeeded in establishing genome engineering techniques in the guinea pig and Peromyscus for these and other human diseases.
   a. Miao J, Wang J, (+ 8 other authors) and Wang Y. Promising xenograft animal model recapitulating the features of human pancreatic cancer. **World J Gastroenterol.** 2020, 26(32):4802-4816. PMCID: PMC7459204
   b. Li R, Miao J, (+ 5 other authors) and Cormier RT. A novel cancer syndrome caused by *KCNQ1*-deficiency in the golden Syrian hamster. **J Carcinog.** 2018, 17:6. PMCID: PMC6187935.
   c. Miao J, Ying B, (+ 8 other authors) and **Wang Z**. Characterization of an N-Terminal Non-Core Domain of RAG1 Gene Disrupted Syrian Hamster Model Generated by CRISPR Cas9. **Viruses.** 2018, 10(5):243. PMCID: PMC5977236.

Completed List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1X5HGj7IL3BAF/bibliography/public/

BIOGRAPHICAL SKETCH

NAME: Yoshihiro Kawaoka

eRA COMMONS USER NAME (credential, e.g., agency login) (b) (4)

POSITION TITLE: Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date	FIELD OF STUDY
The Ministry of Agriculture and Fishery, Japan	D.V.M.	1978	
Hokkaido University, Japan	B.S.	1978	Veterinary Medicine
Hokkaido University, Japan	M.S.	1980	Microbiology
Hokkaido University, Japan	Ph.D.	1983	Microbiology

A. Personal Statement

I have worked on negative-strand RNA viruses, including influenza virus and Ebola virus, for more than 40 years and have published more than 800 peer-reviewed publications. My research has been supported by more than 30 federal grants, contracts, and sponsored research agreements. My major research interests are the development of novel influenza vaccines, improving our understanding of the virulence and transmissibility of influenza viruses, and furthering our understanding of influenza virus antigenic evolution. In addition, my research has focused on systems biology studies. I am currently Director of the Influenza Research Institute at the University of Wisconsin-Madison, Madison, WI, and oversee a staff of over 25 researchers including six individuals in the trainee category. Hence, I have demonstrated my research productivity, my ability to train scientists and staff, and my ability to obtain and administer research funds. In February 2020, I initiated research on SARS-CoV-2 that included development of viral assays and the establishment of animal models of infection. Hence, I have demonstrated my research productivity, my ability to train scientists and staff, and my ability to obtain and administer research funds.

Ongoing and recently completed projects that I would like to highlight include:

OPP1212929 Kawaoka (PI) 09/01/19-04/30/23
A new strategy to elicit broadly protective antibodies to influenza viruses
Development of universal influenza vaccines.

75D30121C12027 Kawaoka (PI) 08/01/21-02/01/24
Generation of Antigenically Advanced Influenza Viruses
We will generate influenza virus libraries possessing (random) mutations at random or predetermined positions of the viral hemagglutinin protein.

P01AI165077 Kawaoka (PI) 09/16/21-08/31/24
PanCorVac (Center for Pan-Coronavirus Vaccine Development)

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2021- Director, Center for Global Viral Infections, National Center for Global Health and Medicine, Japan
2021- Project Professor, Institute of Medical Science, University of Tokyo, Tokyo, Japan
1999-2021 Professor, Institute of Medical Science, University of Tokyo, Tokyo, Japan
1997- Professor, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, Wisconsin
1996-1997 Member, Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee
1991-1997 Associate Professor, Department of Pathology, University of Tennessee, Memphis, Tennessee
1989-1995 Associate Member, Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee
1985-1989 Assistant Member, Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee

1983-1985 Postdoctoral Fellow, Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee
1980-1983 Research Associate, Department of Veterinary Microbiology, Faculty of Agriculture, Tottori University, Tottori, Japan

Review Panel:

10/01	Virology Study Section Ad-hoc Member, NIH
07/94-06/98	Virology Study Section Member, NIH
1992	Special Review Committee, NIH

Editorial Boards:

2006 to date	PLoS Pathogens
2004 to 2006	Journal of Clinical Investigation
2002 to 2006	Journal of General Virology
2001 to date	Virology
1999-2001	American Journal of Veterinary Research
1996 to date	Journal of Virology

Other professional responsibilities:
International Union of Microbiological Societies, Virology Division, Chair (2011-2014)
International Union of Microbiological Societies, Virology Division, Vice Chair (2008-2011)
International Union of Microbiological Societies, Virology Division, Advisory Council Member (2002-2008)
International Committee on Taxonomy of Viruses, Chair, Orthomyxoviridae Study Group (1999-2006)

Awards:

2015	UNESCO Carlos J. Finlay Prize for Microbiology
2014	Excellence in Research Award, Association of American Veterinary Medical Colleges
2013	International Member of the United States National Academy of Sciences
2006	Robert Koch Award

C. Contributions to Science

1. **Molecular Virology.** Until the late 1990s, no 'reverse genetics' system existed for influenza viruses that would allow the generation of wild-type, reassortant, or mutant influenza viruses from scratch. The lack of such a system considerably hindered influenza virus research and the development of better influenza vaccines. In 1999, we developed the first plasmid-based reverse genetics system for influenza viruses (Ref. a). This system has revolutionized influenza virus research, is used around the world in countless laboratories, has led to thousands of research publications (many of which reported seminal discoveries in influenza virus research), and is used for the production of seasonal and pandemic influenza vaccines. We have used this system extensively to study the basic virology of influenza virus. For example, we found that the eight different viral ribonucleoprotein complexes are packaged in a particular configuration (Ref. b). This knowledge will lead to a better understanding of reassortment (i.e., the exchange of viral RNA segments among influenza viruses) and could be exploited to develop a new class of antiviral compounds. Reverse genetics has also allowed us to study the antigenic evolution of influenza viruses at the molecular level (Refs. c, d), which may facilitate the development of improved influenza vaccines.

a. Neumann G, Watanabe T, (+9 other authors) and Kawaoka Y. Generation of influenza A viruses entirely from cloned cDNAs. **Proc Natl Acad Sci U S A** 96:9345-9350, 1999. PMCID: PMC17785.
b. Noda T, Murakami S, (+ 5 other authors) and Kawaoka Y. Importance of the 1+7 configuration of ribonucleoprotein complexes for influenza A virus genome packaging. **Nature Commun** 9:54, 2018. PMCID:PMC5754346.
c. Li C, Hatta M, (+ 32 other authors) and Kawaoka Y. Selection of antigenically advanced variants of seasonal influenza viruses. **Nature Microbiol** 1:16058, 2016. PMCID: PMC5087998.
d. Yasuhara A, Yamayoshi S, (+ 7 other authors) and Kawaoka Y. Antigenic drift originating from changes to the lateral surface of the neuraminidase head of influenza A virus. **Nature Microbiol** 4:1024-1034, 2019. PMID: 30886361.

2. **Virus Pathogenesis.** Over the last 30 years, I have comprehensively studied influenza virus pathogenesis, leading me to identify the viral hemagglutinin (HA) and polymerase subunit PB2 proteins as major determinants of influenza virus pathogenicity and host range. Specifically, I discovered that the sequence at the HA cleavage site determines the level of pathogenicity [this information is now used by the USDA and Organisation Mondiale de la Santé Animale (World Organisation for Animal Health) as a criterion for rapidly identifying high and low pathogenic avian influenza viruses]. Moreover, my group identified mutations in HA that increase the ability of highly pathogenic H5N1 influenza viruses to bind to human cells, which may enable these viruses to infect humans. Further support for the critical role of HA in influenza virus pathogenesis came from our finding that the HA gene of the 1918 pandemic virus (which was recreated by using reverse genetics; see above) increased the pathogenicity of human influenza viruses. To improve our ability to monitor the progression of the pathophysiological changes that occur in the lungs of infected animals, we established an in vivo imaging system that combines two-photon excitation microscopy with the use of fluorescent influenza viruses. This approach allowed us to monitor and correlate several parameters and physiological changes including the spread of infection, pulmonary permeability, and neutrophil motility in the lungs of live influenza virus-infected mice (Ref. a). Recently, I expanded my research to SARS-CoV-2 and demonstrated that Syrian hamsters are a robust small animal model for SARS-CoV-2 (Ref. b). We also found that SARS-CoV-2 can be transmitted among cats, which may remain asymptomatic upon infection (Ref. c). Importantly, we also demonstrated that the recently emerged and now dominant SARS-CoV-2 Omicron variant causes attenuated disease in mice and hamsters (Ref. d).

   a. Ueki H, Wang I, (+2 other authors) and Kawaoka Y. Multicolor two-photon imaging of in vivo cellular pathophysiology upon influenza virus infection using the two-photon IMPRESS. **Nature Protocol** 15:1041-1065, 2020. PMCID: PMC7086515.
   b. Imai M, Iwatsuki-Horimoto (+31 other authors) and Kawaoka Y. Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. **Proc Natl Acad Sci U S A** 117:16587-16595, 2020. PMCID: PMC7368255.
   c. Halfmann P, Hatta M, (+9 other authors) and Kawaoka Y. Transmission of SARS-CoV-2 in Domestic Cats. **N Engl J Med** 383:592-594, 2020. PMID: 32402157.
   d. Halfmann PJ, Iida S, (+65 other authors) and Kawaoka Y. Omicron virus causes attenuated disease in mice and hamsters. **Nature** 2022 Jan 21. PMID:35062015.

3. **Emerging Viruses.** I have made essential contributions to the characterization of newly emerging viruses. Our characterization of the 2009 pandemic H1N1 virus showed that this virus is more virulent in several animal models than 'conventional' human influenza viruses (Ref. a). To better understand the pandemic potential of H5N1 viruses, we generated large numbers of viruses with random mutations in HA and selected one variant that bound to human receptors. A virus with this mutant HA and its remaining viral genes derived from a human influenza virus did not transmit among ferrets via respiratory droplets, but acquired this property after two passages in ferrets during which additional mutations in HA occurred (Ref. b). This study proved that H5 viruses can acquire the ability to transmit among mammals. Importantly, we were the first to discover that HA stability is an important factor in virus transmission among mammals. In addition to demonstrating that H5N1 viruses can acquire respiratory droplet transmissibility in ferrets, we found that the recently emerged H7N9 viruses of low (Ref. c) or high pathogenicity transmit via respiratory droplets among ferrets without the need for additional mammalian-adapting mutations. H7N9 viruses may therefore have a higher pandemic potential than highly pathogenic H5N1 influenza viruses. These studies have greatly advanced our understanding of the potential threat posed by novel influenza viruses. With the emergence of SARS-CoV-2, I also initiated studies on the pandemic virus. Among other studies, I characterized the Omicron (BA.2) variant which rapidly spread around the world and infected millions of people, including SARS-CoV-2 vaccinated people and people infected with an earlier SARS-CoV-2 variant (Ref. d).

   a. Itoh Y, Shinya K, (+49 other authors) and Kawaoka Y. In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. **Nature** 460:1021-1025, 2009. PMCID: PMC2748827.
   b. Imai M, Watanabe H, (+15 other authors) and Kawaoka Y. Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. **Nature** 486:420-428, 2012. PMCID: PMC3388103.
   c. Watanabe T, Kiso M, (+47 other authors) and Kawaoka Y. Characterization of H7N9 influenza A viruses isolated from humans. **Nature** 501:551-555, 2013. PMCID: PMC3891892.

d. Uraki R, Kiso M, (+51 other authors) and Kawaoka Y. Characterization and antiviral susceptibility of SARS-CoV-2 Omicron/BA.2. **Nature**. 2022 May 16. doi: 10.1038/s41586-022-04856-1. Online ahead of print. PMID: 35576972.

4. **Role of Host Factors in Virus Infection.** Influenza viruses, like other viruses, require host machineries for their life cycle. To define host factors involved in influenza virus replication, we performed RNAi screens (Ref. a), and identified cellular interaction partners of influenza viral proteins. The latter study was followed by extensive validation (siRNA knock-down) and mechanistic studies that resulted in a comprehensive picture of the virus-host interactome, which we then exploited for antiviral drug development. We also performed a multi-omics analysis of peripheral blood mononuclear cells and plasma from people who survived or succumbed to Ebola virus infection (Ref. b) and identified biomarkers that differentiated survivor and fatalities early after infection (Ref. b). Moreover, we screened a library of interferon-stimulated genes (ISGs) against a biologically contained Ebola virus and identified ISGs that attenuated Ebola virus infection (Ref. c). We also developed an MDCK cell line, hCK, which expresses increased levels of α2,6-sialoglycans (human virus receptors) and very low levels of α2,3-sialoglycans (avian virus receptors) (Ref. d). hCK cells are markedly better than MDCK cells in supporting the efficient replication of human H3N2 influenza viruses (Ref. d).

a. Hao L, Sakurai A, (+5 other authors) and Kawaoka Y. *Drosophila* RNAi screen identifies host genes important for influenza virus replication. **Nature** 454:890-893, 2008. PMCID: PMC2574945.
b. Eisfeld AJ, Halfmann PJ, (+28 other authors) and Kawaoka Y. Multi-platform 'Omics Analysis of Human Ebola Virus Disease Pathogenesis. **Cell Host Microbe** 22:817-829, 2017. PMCID: PMC5730472.
c. Kuroda M, Halfmann PJ, (+6 other authors) and Kawaoka Y. Identification of interferon-stimulated genes that attenuate Ebola virus infections. **Nature Communications** 11:2953, 2020. PMCID:PMC7289892.
d. Takada K, Kawakami C, (+11 other authors) and Kawaoka Y. A humanized MDCK cell line for the efficient isolation and propagation of human influenza viruses. **Nature Microbiol** 4:268-1273, 2019. PMID: 31036910.

5. **Virus Infection Control.** The treatment of influenza virus infections relies primarily on the use of neuraminidase inhibitors; however, the emergence of viruses resistant to these compounds will considerably limit treatment options in cases of (severe) influenza virus infections. For example, we showed that oseltamivir-resistant highly pathogenic H5N1 viruses emerge in patients treated with this drug. Given that the case fatality rate of human H5N1 virus infections exceeds 50%, the isolation of H5N1 viruses resistant to the most widely used neuraminidase inhibitor is of great concern. Since relatively little is known about the frequency of emergence of influenza B viruses with reduced sensitivity to neuraminidase inhibitors, we tested the neuraminidase inhibitor sensitivity of influenza B isolates before and after oseltamivir therapy. We found that neuraminidase inhibitor-resistant viruses circulate in children and adults and are transmitted among people, suggesting that neuraminidase-resistant viruses could cause an epidemic. This scenario became a reality with the emergence and spread of oseltamivir-resistant H1N1 viruses in 2007–2008. Recently, we tested influenza A variants that show reduced susceptibility to the novel viral polymerase inhibitor baloxavir and found that they are fit and transmit among ferrets via respiratory droplets (Ref. a). In another study, we identified mammalian-adapting mutations in the polymerase complex of an avian H5N1 influenza virus. Influenza vaccine production can be hindered by low yield of the vaccine virus. To overcome this limitation, we developed a high-yield influenza A virus vaccine 'backbone' that can be combined with the HA and NA genes of the selected vaccine viruses and confers high yield in cultured cells and embryonated chicken eggs.

   In addition to influenza virus, we study Ebola virus. Currently, no vaccines or antiviral compounds exist to prevent or treat Ebola virus infections. This lack of preventative and therapeutic measures contributed to the large number of (fatal) human cases during the Ebola virus outbreak in West Africa in 2014–2015. To this end, we developed a replication-incompetent, whole virus Ebola vaccine that protected mice, guinea pigs, and nonhuman primates (the gold standard animal model for Ebola virus protection studies) against challenge with a lethal dose of Ebola virus (Ref. b). Our candidate vaccine represents a safe and efficacious Ebola virus vaccine that differs from other candidate Ebola virus vaccine platforms in that it presents all of the viral proteins and viral RNA to the host immune system, which might provide greater and/or broader immune responses than vaccine candidates that present single viral proteins.

   The recently emerged SARS-CoV-2 Omicron variant differs substantially from earlier variants. We, therefore, tested the efficacy of approved and investigational therapeutic monoclonal antibodies and antiviral compounds against Omicron and other SARS-CoV-2 variants (Ref. c). Some of the monoclonal antibodies

may not be effective against the Omicron variant, but the antiviral compounds remained effective against the Omicron variant (Ref. c).

An H5N1 highly pathogenic avian influenza A virus transmitted to cattle in the US and spread to multiple states. We isolated these H5N1 viruses from milk samples from virus-infected cows and tested their heat stability in milk and infectivity to mice (Ref. d).

a. Imai M, Yamashita M, (+ 24 other authors) and Kawaoka Y. Influenza A variants with reduced susceptibility to baloxavir isolated from Japanese patients are fit and transmit through respiratory droplets. **Nature Microbiol** 5:27-33, 2020. PMID: 31768027
b. Marzi A, Halfmann P, (+5 other authors) and Kawaoka Y. An Ebola whole-virus vaccine is protective in nonhuman primates. **Science** 348:439-442, 2015. PMCID: PMC4565490.
c. Imai M, Ito M, (+9 other authors) and Kawaoka Y. Efficacy of Antiviral Agents against Omicron BQ.1.1 and XBB Subvariants. **N Engl J Med** 23(1):30-32, 2023.
d. Guan L, Eisfeld A, (+ 15 other authors) and Kawaoka Y. Cow's milk containing avian influenza A (H5N1) virus – heat inactivation and infectivity in mice. **N Engl J Med** doi: 10.1056, 2024.

Completed List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/yoshihiro.kawaoka.1/bibliography/public/

BIOGRAPHICAL SKETCH

NAME: Andres F. Mejia

eRA COMMONS USER NAME (credential, e.g., agency login): (b) (4)

POSITION TITLE: Veterinary Pathologist

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date	FIELD OF STUDY
Universidad de Antioquia	D.V.M.	1997	Veterinary Medicine
The Pennsylvania State University	M.S.	2006	Lab Animal Medicine
The Pennsylvania State University	Postdoc	2006	Comparative Medicine
Harvard University	Postdoc Fellow	2010	Veterinary Pathology
American College of Lab. Animal Medicine	DACLAM	2009	Lab Animal Medicine
American College of Veterinary Pathology	DACVP	2018	Veterinary Pathology

A. Personal Statement

I have participated in numerous research projects as a pathologist, veterinarian and collaborator from colony pathology surveillance and diagnoses, aging, toxicological studies, basic research projects in the areas of vaccines, HIV, SIV, opportunistic infections research, ZIKAV, artificial organs, organ transplantation, toxicology, pharmacology, immune modulation and neuropathology among others. I was also the Comparative Medicine and Pathology Unit Director at the Caribbean Primate Research Center. I have conducted and executed my own research in Human Papillomavirus where we develop a unique animal model to test HPV vaccines and standardized the rabbit animal model method of challenge. My responsibilities at the Wisconsin National Primate Research Center is to assess the health status of the colony, develop the Veterinary Pathology division within the WNPRC and develop collaborative research projects from different scientific disciplines including infectious disease (such as ZIKA, HIV/SIV, Listeria, Sars-Cov-2 and Pegivirus) and neuropathology (traumatic spinal cord injury, and Parkinson's). I'm also involved in teaching Veterinary Pathology and Laboratory Animal Medicine and have developed my own research in infectious and non-infectious diseases models of human conditions. With the Veterinary Pathology and Laboratory Animal Medicine, Diplomate of the American College of laboratory animal Medicine (DACLAM) and American College of Veterinary Pathology (DACVP), training and knowledge, Dr. Mejia brings a unique strength the UW, Veterinary Medicine and Pathology. Dr. Mejia is an avid science ambassador with frequently teaches from elementary, intermediate school to residents, including University of Wisconsin Vet School, University of Missouri and Mayo.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2011- Veterinary Pathologist-Pathology Unit, Wisconsin National Primate Research Center, University of Wisconsin-Madison
2010-2011 Director, Comparative Medicine and Pathology Unit-Caribbean Primate Research Center,
2010-2011 Veterinary Pathologist/Adjunct Professor, Caribbean Primate Research Center, University of Puerto Rico
2006-2010 Postdoctoral/Pathology Residency/Research Fellow, New England Primate Research Center, Harvard Medical School
2004-2006 Postdoctoral Scholar/Resident, Pennsylvania State University, Hershey, Pennsylvania
2003-2004 Animal Laboratory Technologist, Charles River Laboratories, Wilmington, Massachusetts
2000-2004 Adjunct Professor, Quincy College, Quincy, Massachusetts

Other professional responsibilities: American Association for Laboratory Animal Sciences (AALAS), Member (2004-present)
New England Brach of AALAS (NEBAALAS), Member (2006-present)
American Association for the Advancement of Science (AAAS),

Member (2006-present)
Latin Comparative Pathology Group (LCPG),
Executive Member (2008-present)
Cl Davis Foundation, Puerto Rico,
Contact Member (2006-present)
American Society of Laboratory Animal Practitioners (ASLAP),
Member (2004-present)
Puerto Rican Pathology Academy,
Member (2010-present)
American College of Laboratory Animal Medicine (ACLAM),
Diplomate and Member (2009-present)
Association of Primate Veterinarians
Member (2009-present)
American College of Veterinary Pathology (ACVP),
Diplomate and Member (2018-present)

Awards:

2005	UNESCO Carlos J. Finlay Prize for Microbiology
2006-2010	Institutional Training Awards, Postdoctoral Program for Veterinarians (T32) RR007000

C. Contributions to Science

Dr. Mejia has contributed to numerous studies including infectious and non-infectious diseases, pathogenesis, and stem cells. Additionally, Dr. Mejia has a great passion for education, including hosting and mentoring numerous veterinary students, and Laboratory animal and Pathology Veterinary residents. Dr. Mejia is the founder and director since its inception of the WNPRC and CL Davis Foundation Lab Animal Medicine and Pathology Seminar, now in its 8th edition where more than 2,000 attendees have benefitted from this event including Veterinary Technicians, Veterinary Students, Residents and Researchers. In the 2020 the seminar changed to be online with 488 people from 31 countries and in the last edition on May 1st, 2021, the seminar audience grew to 690 attendees from 35 countries. Furthermore Dr. Mejia has coordinated the International Mock examination since 2009 to aid in the preparation of numerous veterinarians across the globe. Dr. Mejia is an avid science ambassador with frequent teaching activities from elementary, intermediate school to teaching residents at University of Wisconsin-Madison Veterinary School, University of Missouri and Mayo.

Completed List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/10emdptnynuAw/bibliography/public/

BIOGRAPHICAL SKETCH

NAME: Adel Talaat

eRA COMMONS USER NAME (credential, e.g., agency login) (b) (4)

POSITION TITLE: Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date	FIELD OF STUDY
Cairo University, Cairo	B.V.Sc.	1986	Veterinary Medicine
Cairo University, Cairo	M.V.Sc.	1990	Microbiology
University of Maryland School of Medicine, Baltimore, MD	Ph.D.	1998	Molecular Pathogenesis
University of Texas Southwestern Medical Center, Dallas, TX	Postdoc Fellow	2002	Genomics and vaccine development

A. Personal Statement

The current project is designed to take advantage of our teams experience in microbiology, animal models (including poultry and ruminants), molecular biology and pathogenesis to develop disease resistant breeds of poultry. For the last 30 years, I focused my attention on immunopathogenesis of mycobacterial infections (tuberculosis and paratuberculosis) to reduce their impact on human and animal health. During the last 10 years, I started to focus my attention on novel approaches to solve real-life problems that face poultry health by developing novel vaccines against Avian Influenza Virus (AIV) and infectious Bronchitis Virus (IBV). The aims of this project are the continuation for my long-term quest to improve poultry health strategies during outbreaks with transboundary infection such as high and low pathogenic strains of AIV. My experience in microbiology, molecular biology, animal models, pathogenesis and vaccine development for the last 30 years will enable me to serve as a co-PI with Dr. Peter Halfmann, a world-class expert in virology and immunology. Currently, both Dr. Halfmann and I co-lead 2 research projects for the USDA and NIH programs. Previously, I enjoyed working with the project collaborators and I am sure the current collaborative project will advance the animal and human health fields.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2013 -	Professor of Microbiology, University of Wisconsin-Madison, School of Veterinary Medicine, Madison, WI
2011 -	Founder, Pan Genome Systems, INC, Madison, WI
2008 - 2013	Associate Professor of Microbiology, University of Wisconsin-Madison, School of Veterinary Medicine, Madison, WI
2002 - 2008	Assistant Professor, University of Wisconsin-Madison, School of Veterinary Medicine, Madison, WI
1998 - 2002	Post-Doctoral Fellow, University of Texas, Southwestern Medical Center, Department of Internal Medicine, Dallas, TX
1993 - 1998	Graduate Research Assistant, University of Maryland School of Medicine, Departments of Pathology and Medicine, Baltimore, MD
1990 - 1992	Lecturer, Cairo University, Department of Food Hygiene & Technology, Cairo
1986 - 1990	Demonstrator, Cairo University, Department of Food Hygiene & Technology, Cairo

Other professional responsibilities: American Society of Microbiology, Member (1995)
American Association for the Advancement of Science, Member (2001)

Awards:

1998	J. Howard Brown Award, American Society of Microbiology, Maryland Branch
1998	Student Travel Award, American Society of Microbiology

1998 Arthur Schwartz Award, University of Maryland Graduate School
1998 Graduate Merit Award, University of Maryland
1997 President's Certificate of Commendation, University of Maryland
1995 President's Certificate of Commendation, University of Maryland
1987 Outstanding Veterinarian, Brooks Animal Hospital
1986 Award for Outstanding Veterinary Student, Egyptian Veterinary Medical Association
1986 Valedictorian, Cairo University, Faculty of Veterinary Medicine

C. Contributions to Science

1. **Novel vaccines against old diseases.** Early on during my post-doctoral training, I realized the importance of vaccines to improve the health of humans and animals. I started my effort to develop better vaccine by using DNA immunization against important pathogens such human influenza, herpes simplex and respiratory Cynthia virus. When I started my own group at the University of Wisconsin, my search has led me to select live-attenuated vaccine technology to develop vaccine against mycobacterial infections, generally, because of the difficulty to reduce the pathogenicity of such organisms with a few antigens. The list below represents a few my publication in the field of vaccine development.

   a. Kingstad-Bakke BA, Chandrasekar SS, (+7 other authors) and Talaat AM. Effective mosaic-based nanovaccines against avian influenza in poultry. **Vaccine**. 2019 Aug 14;37(35):5051-5058. PMID: 31300285.
   b. Shippy DC, Lemke JJ, (+3 other authors) and Talaat AM. Superior Protection from Live- Attenuated Vaccines Directed against Johne's Disease. **Clin Vaccine Immunol**. 2017 Jan;24(1) PMCID: PMC5216426.
   c. Ghosh P, Steinberg H, Talaat AM. Virulence and immunity orchestrated by the global gene regulator sigL in Mycobacterium avium subsp. paratuberculosis. **Infect Immun**. 2014 Jul;82(7):3066-75. PMCID: PMC4097628.
   d. Settles EW, Kink JA, Talaat A. Attenuated strains of Mycobacterium avium subspecies paratuberculosis as vaccine candidates against Johne's disease. **Vaccine**. 2014 Apr 11;32(18):2062-9. PubMed PMID: 24565753.

2. **Mycobacterial Pathogenesis**. At UW-Madison, I focused my efforts on examining the pathogenesis of tuberculosis and paratuberculosis in more relevant models of infection to identify novel mechanisms of virulence. The list below represents a few of my publication in the field of mycobacterial pathogenesis.

   a. Marcus SA, Sidiropoulos SW, Steinberg H, Talaat AM. CsoR Is Essential for Maintaining Copper Homeostasis in Mycobacterium tuberculosis**. PLoS One**. 2016;11(3):e0151816. PMC4801387.
   b. Franklin RK, Marcus SA, (+4 other authors) and Heath TD. Correction to: "A Novel Loading Method for Doxycycline Liposomes for Intracellular Drug Delivery: Characterization of In Vitro and In Vivo Release Kinetics and Efficacy in a J774A.1 Cell Line Model of Mycobacterium smegmatis Infection". **Drug Metab Dispos**. 2015 Nov;43(11):1805. PMCID: PMC4613953.
   c. Abomoelak B, Marcus SA, (+3 other authors) and Talaat AM. Characterization of a novel heat shock protein (Hsp22.5) involved in the pathogenesis of Mycobacterium tuberculosis. **J Bacteriol**. 2011 Jul;193(14):3497-505. PMCID: PMC3133320.
   d. Talaat AM, Ward SK, (+5 other authors) and Johnston SA. Mycobacterial bacilli are metabolically active during chronic tuberculosis in murine lungs: insights from genome-wide transcriptional profiling. **J Bacteriol**. 2007 Jun;189(11):4265-74. PMCID: PMC1913421.

3. **Gene expression analysis during infection (*in vivo*).** I have worked in the mycobacterial field for almost 20 years starting from developing novel animal models for tuberculosis pathogenesis using *M. marinum* into working with the genome and transcriptome of *M. tuberculosis* during infection. The following list of publication is selected to reflect my research on developing novel approaches to examine *in vivo* gene expression during bacterial infections.

   a. Berry A, Wu CW, Venturino AJ, Talaat AM. Biomarkers for Early Stages of Johne's Disease Infection and Immunization in Goats. **Front Microbiol**. 2018;9:2284. PMCID: PMC6172484.
   b. Marcus SA, Sidiropoulos SW, Steinberg H, Talaat AM. CsoR Is Essential for Maintaining Copper Homeostasis in Mycobacterium tuberculosis. **PLoS One**. 2016;11(3):e0151816. PMCID: PMC4801387.

c. Wu CW, Schmoller SK, Shin SJ, Talaat AM. Defining the stressome of Mycobacterium avium subsp. paratuberculosis in vitro and in naturally infected cows. **J Bacteriol**. 2007 Nov;189(21):7877-86. PMCID: PMC2168719.
d. Talaat AM, Lyons R, Howard ST, Johnston SA. The temporal expression profile of Mycobacterium tuberculosis infection in mice. **Proc Natl Acad Sci U S A**. 2004 Mar 30;101(13):4602-7. PMCID: PMC384793.

4. **Genomic analysis of mycobacterial infections**. Strain to strain variations and drug resistance are very important for the outcome of the infection and hence, the efficacy of used vaccine. To better design vaccination strategy directed towards mycobacterial infections, I started to characterize transcriptome, gene and genome-wide variations among mycobacterial isolates circulating in both humans and animals. The list below represents a few of my publication in the field of mycobacterial genomics.

a. Alyamani EJ, Marcus SA, (+9 other authors) and Talaat AM. Genomic analysis of the emergence of drug-resistant strains of Mycobacterium tuberculosis in the Middle East. **Sci Rep**. 2019 Mar 14;9(1):4474. PMCID: PMC6418154.
b. Amin AS, Hsu CY, (+4 other authors) and Talaat AM. Ecology and genomic features of infection with Mycobacterium avium subspecies paratuberculosis in Egypt. **Microbiology**. 2015 Apr;161(Pt 4):807-18. PMID: 25667007.
c. Ghosh P, Hsu C, (+9 other authors) and Talaat AM. Genome-wide analysis of the emerging infection with Mycobacterium avium subspecies paratuberculosis in the Arabian camels (Camelus dromedarius). **PLoS One**. 2012;7(2):e31947. PMCID: PMC3290536.
d. Wu CW, Glasner J, Collins M, Naser S, Talaat AM. Whole-genome plasticity among Mycobacterium avium subspecies: insights from comparative genomic hybridizations. **J Bacteriol**. 2006 Jan;188(2):711-23. PMCID: PMC1347307.

Completed List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/myncbi/adel.talaat.1/bibliography/41139603/public/?sort=date&direction=ascending

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

UEI*: LCLSJAGTNZQ7

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 10-01-2024 **End Date*:** 09-30-2025 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Peter		Halfmann		PD/PI							(b) (4)
2.		Yoshihiro		Kawaoka		co-I							
3.		Adel		Talaat		co-I							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person (b) (4)

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
3	Post Doctoral Associates						(b) (4)
1	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Scientist						
1	Lab Animal Care Supervisor						
1	Instrumentation Engineer						
1	Accountant						
1	Technician						
9	**Total Number Other Personnel**						

Total Other Personnel (b) (4)

Total Salary, Wages and Fringe Benefits (A+B)

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

UEI*: LCLSJAGTNZQ7

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 10-01-2024 **End Date*:** 09-30-2025 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
1 . -80 Freezer	16,000.00
Total funds requested for all equipment listed in the attached file	
Total Equipment	**16,000.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	7,000.00
2. Foreign Travel Costs	3,000.00
Total Travel Cost	**10,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

UEI*: LCLSJAGTNZQ7

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 10-01-2024 **End Date*:** 09-30-2025 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	100,820.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	292,000.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Tuition Remission	12,000.00
9. Service contracts	10,650.00
10. Animal per diems	3,000.00
Total Other Direct Costs	**418,470.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**693,673.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	55.5	398,673.00	221,264.00
		Total Indirect Costs	**221,264.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif Karim, Dallas, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**914,937.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	914,937.00

L. Budget Justification* File Name: Budget_Justification_8_4_241049190253.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

UEI*: LCLSJAGTNZQ7

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 10-01-2025 **End Date*:** 09-30-2026 **Budget Period:** 2

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Peter		Halfmann		PD/PI							
2.		Yoshihiro		Kawaoka		co-I							
3.		Andres		Mejia		co-I							
4.		Adel		Talaat		co-I							

(b) (4)

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person (b) (4)

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
3	Post Doctoral Associates						
1	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Scientist						
1	Lab Animal Care Supervisor						
1	Instrumentation Engineer						
1	Accountant						
1	Technician						
9	**Total Number Other Personnel**						

(b) (4)

Total Other Personnel

Total Salary, Wages and Fringe Benefits (A+B) (b) (4)

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

UEI*: LCLSJAGTNZQ7

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 10-01-2025 **End Date*:** 09-30-2026 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,800.00
2. Foreign Travel Costs	3,000.00
Total Travel Cost	**7,800.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

UEI*: LCLSJAGTNZQ7

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 10-01-2025 **End Date*:** 09-30-2026 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	100,820.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	432,250.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Tuition Remission	12,000.00
9. Service contracts	23,650.00
10. Animal per diems	20,000.00
Total Other Direct Costs	**590,720.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**940,923.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	55.5	496,673.00	275,654.00
		Total Indirect Costs	**275,654.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif Karim, Dallas, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**1,216,577.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	1,216,577.00

L. Budget Justification* File Name: Budget_Justification_8_4_241049190253.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

UEI*: LCLSJAGTNZQ7

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 10-01-2026 **End Date*:** 09-30-2027 **Budget Period:** 3

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Peter		Halfmann		PD/PI							
2.		Yoshihiro		Kawaoka		co-I							
3.		Andres		Mejia		co-I							
4.		Adel		Talaat		co-I							

(b) (4)

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person (b) (4)

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
3	Post Doctoral Associates						
1	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Scientist						
1	Lab Animal Care Supervisor						
1	Instrumentation Engineer						
1	Accountant						
1	Technician						
9	**Total Number Other Personnel**						

(b) (4)

Total Other Personnel

Total Salary, Wages and Fringe Benefits (A+B) (b) (4)

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

UEI*: LCLSJAGTNZQ7

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 10-01-2026 **End Date*:** 09-30-2027 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
1 . Biosafety cabinet	15,000.00
Total funds requested for all equipment listed in the attached file	
Total Equipment	**15,000.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,800.00
2. Foreign Travel Costs	3,000.00
Total Travel Cost	**7,800.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

UEI*: LCLSJAGTNZQ7

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 10-01-2026 **End Date*:** 09-30-2027 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	125,820.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	432,250.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Tuition Remission	12,000.00
9. Service contracts	10,650.00
10. Animal per diems	30,500.00
Total Other Direct Costs	**614,220.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**979,423.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	55.5	520,173.00	288,696.00
		Total Indirect Costs	**288,696.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif Karim, Dallas, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**1,268,119.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	1,268,119.00

L. Budget Justification*	File Name:
	Budget_Justification_8_4_241049190253.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

UEI*: LCLSJAGTNZQ7

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 10-01-2027 **End Date*:** 09-30-2028 **Budget Period:** 4

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base S	Calendar	Academic	Summer	Requested	Fringe	Funds Requested ($)*
1.		Peter		Halfmann		PD/PI							
2.		Yoshihiro		Kawaoka		co-I							
3.		Andres		Mejia		co-I							
4.		Adel		Talat		co-I							

(b) (4)

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person (b) (4)

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
3	Post Doctoral Associates						
1	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Scientist						
1	Lab Animal Care Supervisor						
1	Instrumentation Engineer						
1	Accountant						
1	Technician						

(b) (4)

9	**Total Number Other Personnel**	**Total Other Personnel** (b) (4)
		Total Salary, Wages and Fringe Benefits (A+B)

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

UEI*: LCLSJAGTNZQ7

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 10-01-2027 **End Date*:** 09-30-2028 **Budget Period: 4**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
1 . -80 freezer	16,000.00
Total funds requested for all equipment listed in the attached file	
Total Equipment	**16,000.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,800.00
2. Foreign Travel Costs	3,000.00
Total Travel Cost	**7,800.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

UEI*: LCLSJAGTNZQ7

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 10-01-2027 **End Date*:** 09-30-2028 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	125,820.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	432,250.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Tuition Remission	12,000.00
9. Service contracts	10,650.00
10. Animal per diems	30,500.00
Total Other Direct Costs	**613,220.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**979,423.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	55.5	519,173.00	288,141.00
		Total Indirect Costs	**288,141.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif Karim, Dallas, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**1,267,564.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	1,267,564.00

L. Budget Justification* File Name: Budget_Justification_8_4_241049190253.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

UEI*: LCLSJAGTNZQ7

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 10-01-2028 **End Date*:** 09-30-2029 **Budget Period:** 5

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Peter		Halfmann		PD/PI							
2.		Yoshihiro		Kawaoka		co-I							
3.		Andres		Mejia		co-I							
4.		Adel		Talaat		co-I							

(b) (4)

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person (b) (4)

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
3	Post Doctoral Associates						
1	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Scientist						
1	Lab Animal Care Supervisor						
1	Instrumentation Engineer						
1	Accountant						
1	Technician						

(b) (4)

9	**Total Number Other Personnel**	**Total Other Personnel** (b) (4)
		Total Salary, Wages and Fringe Benefits (A+B)

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

UEI*: LCLSJAGTNZQ7

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 10-01-2028 **End Date*:** 09-30-2029 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,800.00
2. Foreign Travel Costs	3,000.00
Total Travel Cost	**7,800.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

UEI*: LCLSJAGTNZQ7

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Board of Regents of the University of Wisconsin System

Start Date*: 10-01-2028 **End Date*:** 09-30-2029 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	125,820.00
2. Publication Costs	3,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	359,250.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Tuition Remission	12,000.00
9. Service contracts	26,650.00
10. Animal per diems	22,000.00
Total Other Direct Costs	**548,720.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**898,923.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	55.5	527,673.00	292,859.00
		Total Indirect Costs	**292,859.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Arif Karim, Dallas, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**1,191,782.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	1,191,782.00

L. Budget Justification*	File Name:
	Budget_Justification_8_4_241049190253.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

BUDGET JUSTIFICATION – UNIVERSITY OF WISCONSIN-MADISON (Halfmann)

SENIOR/KEY PERSONNEL: (b) (4) Years 2-5)

Peter Halfmann, PhD, Assistant Professor, principal investigator (contact) (b) (4) effort, (b) (4) calendar months, Years 1-5) is a molecular virologist with an extensive experience with different animal models to study the pathogenicity of emerging viruses and with the development of medical countermeasures. His research focuses on RNA viruses including filoviruses, coronaviruses, and influenza viruses. He will work together with Dr. Wang (co-PI) to identify the best candidate host factors for gene modification in chickens. Dr. Halfmann will oversee the studies to characterize the resistance to viral infection of the gene edited chicken lines in collaboration with Drs. Kawaoka and Talaat. Dr. Halfmann has extensive experience with grants management along with training and mentoring research personnel to ensure the successful completion of the aims in this proposal.

Yoshihiro Kawaoka, DVM, PhD, Professor, co-Investigator (b) (4) effort, (b) (4) calendar months, Years 1-5) is the director of the Influenza Research Institute (IRI), a state-of-the art biosafety level (BSL)-3, Select Agent registered facility that focuses on research with respiratory viral pathogens including highly pathogenic avian influenza viruses. Dr. Kawaoka will provide intellectual and conceptual direction, as well as guidance in the design and interpretation of the proposed experiments.

Andres Mejia, MS, DVM, DACLAM, DACVP, Chief of Comparative Pathology, co-Investigator (b) (4) effort, (b) (4) alendar months, Years 2-5) is an expert animal pathologist with infectious disease experience with tissues from SARS-CoV-2, Zika virus and SIV/HIV infected animals. Dr. Mejia determine if there are any adverse pathological changes associated with the gene edited chickens compared to wild-type birds.

Adel M. Talaat, MS, PhD, Professor, co-Investigator (b) (4) effort, (b) (4) calendar months, Years 1-5) has extensive experience in developing disease resistant poultry breeds. Dr. Talaat will lead the team working on aspects of avian viral pathogens including infectious bronchitis virus (IBV) infection and pathogenesis. He will organize the collaborative efforts among members of the research team and coordinate with other involved scientists. With the help of other investigators, Dr. Talaat will help with the reporting of the research findings to the scientific community, through publications and conference presentations.

OTHER PERSONNEL: ((b) (4) Year 1; (b) (4) Years 2-5)

Tadashi, Maemura, PhD, Assistant Scientist ((b) (4) % effort, (b) (4) calendar months, Years 1-5) has extensive experience with studies on influenza viruses at BSL-3 containment. Dr. Maemura has experience in generating gene knockout cell lines using CRISPR/Cas9 to allow for improved influenza virus replication for vaccine production studies. This experience will be beneficial in studies to be carried out in Aim 1 of this proposal. In addition, he has received BSL-3 training by Dr. Halfmann, thus he is well-suited to carry out infections, sample collections, and transmission studies in chickens.

Tong Wang, PhD, Research Associate (b) (4) % effort, (b) (4) calendar month, Year 1; (b) (4) effort, (b) (4) calendar months, Years 2-5) is a virology with recent bovine H5N1 virus research experience at the IRI. She will perform studies to identify and validate influenza virus specific pro-viral and antiviral proteins that inhibit virus replication. She will be trained by Dr. Maemura on CRISPR/Cas9 techniques to generate knockout cell lines for virus validations studies. She will also receive BSL-3 training to assist with chicken infection studies.

Sangam Kandel, PhD, Research Associates (b) (4) % effort, (b) (4) calendar month, Year 1; (b) (4) % effort, (b) (4) calendar months, Years 2-5). Dr. Kandel recently joined Dr. Halfmann's group after completing his thesis project which focused on next-generation sequencing of SARS-CoV-2 nasopharyngeal samples. In this proposal, he will focus on influenza virus sequencing to determine if mutant viruses arise during cell culture passaging or during infection of gene edited chickens. Dr. Kandel will be responsible for the generation, analysis, and visualization of sequence data from this project.

Fakry Ramadan, MS, PhD, Postdoctoral Research Associate (b) (4) % effort, (b) (4) calendar months Years 1-5) is a Research Associate with a PhD in molecular virology will be responsible for experimental design, construction, and characterization of infection outcomes.

To-Be-Named, Graduate Student, Research Assistant (b) (4) % effort, (b) (4) calendar months, Years 1-5): A graduate student (pursing PhD degree) in the Comparative Biomedical Sciences Program will dedicate (b) (4) % of

their time for this project. The graduate student will help in the design and execution of experiments including immunological analyses planned for this project.

Kate Skogen, AS, Lab Animal Care Supervisor (b) (4)% effort, (b) (4)calendar month, Year 1; (b) (4)% effort, (b) (4) calendar months, Years 2-5). Ms. Skogen will oversee the daily care of animals in the BSL-3 facility including health monitoring along with cage cleaning. She will also coordinate critical procedures (infections, swab collection, and necropsies) with researchers. She will be responsible for record keeping and compliances with animal requirements for the use of chickens in the studies outlined in this proposal.

Mark Olson, MS, Instrumentation Engineer (b) (4)% effort, (b) (4)calendar month, Year 1; (b) (4)% effort, (b) (4)calendar months, Years 2-5). Mr. Olson will be responsible for the mechanical aspects of scientific equipment and/or instrumentation aspects of studies in the BSL-3 laboratories. This includes the isolator units that house the infected chickens.

Stephen Schmitt, BS, Accountant, (**(b) (4)% effort, (b) (4)calendar months Years 1-5).** Mr. Schmitt will be responsible for all financial aspects of this grant including subawards, supervising purchasing, payroll, and process-required reporting.

Michael Phillips, BA, Microbiology Laboratory Technician ((b) (4)% effort, (b) (4)calendar months, Years 1-5), will perform supportive and facility maintenance roles, including media preparation, glassware upkeep, disposal of biohazardous waste, and supply ordering.

FRINGE COSTS:

Fringe benefits are budgeted in accordance with university policy as follows: Faculty/Academic, 36.5%; University staff 37.8%, and Research Associates, 19.8%.

EQUIPMENT: ($16,000 Year 1; $15,000 Year 3; $16,000 Year 4)

We request to purchase a -80℃ freezer in Years 1 and 4 of the project to help in the storage of samples and viral isolates. Additional funding is requested for a biosafety cabinet level II in Year 3 to replace an existing cabinet.

TRAVEL: ($10,000 Year 1; $7,800 Years 2-5)

We request funds to send up to four persons to attend and/or present work to the greater scientific community at domestic or international conferences. Examples of some meetings that would be relevant to this proposal are Annual World Poultry Science Association and Biosecurity Program/NIFA meetings. Funds will also be used for any meetings with the funding agency.

OTHER DIRECT COSTS:
($418,470 Year 1; $590,720 Year 2; $614,220 Year 3; $613,220 Year 4; $548,720 Year 5)

Tissue culture supplies: We request funds for cell culture media and reagents including fetal bovine serum, screw cap tubes for sample collection, assay plates, tissue culture plates, and flasks.

Molecular biology and supplies: We request funds for virus isolation and detection including RNA isolation kits, RT-PCR kits, Sanger sequencing, cloning kits, and DNA polymerases. For the identification and validation of host factors associated with virus infection, we request funds for transfection reagents, antibodies, primers, enzymes, guide RNAs, a lentivirus-based chicken CRISPR whole-genome gRNA library, and plasmid DNA isolation kits. In addition, we request funds for consumables associated with the transmission isolator units and reagents for immunological assays.

BSL-2/3 supplies: We request funds for personal protective equipment used in BSL-2/3 containment, including scrubs, dedicated shoes, shoe covers, Tyvek suits, powered air purifying respirators, and gloves.

Services: We request funds to partially cover service contracts for equipment that will be used for the proposed studies, third-party data analysis, and technical writing.

Animals: We request funds to purchase specific-pathogen free (SPF) eggs and wild-type chicken for virus infection studies with gene edited birds. In addition, we request funds for the associated *per diem* charges associated with the birds for the duration of the studies including the establishment of gene edited chicken flocks once they are received from Dr. Wang at Utah State University (USU).

Publications: Funding is requested to publish scientific findings in a peer-reviewed journal.

Tuition Remission: As required by University of Wisconsin-Madison policy, we request (b) (4) per year for the to-be-named graduate student research assistant in tuition remission costs.

Subaward: We request funding to issue a subaward to Utah State University (USU) to meet the objectives of our proposal. Dr. Wang at USU is a highly accomplished researcher in the field of gene editing of animals and is essential for the success of this project.

DIRECT COSTS: ($693,673 Year 1; $940,923 Year 2; $979,423 Year 3; $979,423 Year 4; $898,923 Year 5)

INDIRECT COSTS:
($221,264 Year 1; $275,654 Year 2; $288,696 Year 3; $288,141 Year 4; $292,859 Year 5)

The facilities and administrative rates are the Modified Total Direct Costs as approved by the agreement with the Department of Health & Human Services, dated November 21, 2023, of 55.5% after July 1, 2021. Modified Total Direct Costs exclude equipment, capital expenditure, charges for patient care, rental costs, tuition remission, scholarships and fellowships, the portion of each subaward in excess of $25,000 and participant support costs.

TOTAL COSTS:
($914,937 Year 1; $1,216,577 Year 2; $1,268,119 Year 3; $1,267,564 Year 4; $1,191,782 Year 5)

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		(b) (4)
Section B, Other Personnel		
Total Number Other Personnel	45	
Total Salary, Wages and Fringe Benefits (A+B)		
Section C, Equipment		47,000.00
Section D, Travel		41,200.00
1. Domestic	26,200.00	
2. Foreign	15,000.00	
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		2,785,350.00
1. Materials and Supplies	579,100.00	
2. Publication Costs	10,000.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs	1,948,000.00	
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	60,000.00	
9. Other 2	82,250.00	
10. Other 3	106,000.00	
11. Other 4		
12. Other 5		
13. Other 6		
14. Other 7		
15. Other 8		
16. Other 9		
17. Other 10		
Section G, Direct Costs (A thru F)		4,492,365.00
Section H, Indirect Costs		1,366,614.00

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

Section I, Total Direct and Indirect Costs (G + H)	5,858,979.00
Section J, Fee	
Section K, Total Costs and Fee (I + J)	5,858,979.00

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

UEI*: SPE2YDWHDYU4

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Utah State University

Start Date*: 10-01-2024 **End Date*:** 09-30-2025 **Budget Period:** 1

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Zhongde		Wang		PD/PI					(b) (4)		

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person (b) (4)

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates				(b) (4)		
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4)
						Total Salary, Wages and Fringe Benefits (A+B)	

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

UEI*: SPE2YDWHDYU4

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Utah State University

Start Date*: 10-01-2024 **End Date*:** 09-30-2025 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

UEI*: SPE2YDWHDYU4

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Utah State University

Start Date*: 10-01-2024 **End Date*:** 09-30-2025 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	50,905.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**50,905.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**200,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	46	200,000.00	92,000.00
		Total Indirect Costs	**92,000.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Arif Karim, Cost Allocation Services Director Department of Health and Human Services Phone: (214) 767-3600 Email: Arif.Karim@psc.hhs.gov

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**292,000.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	292,000.00

L. Budget Justification*	File Name:
	Wang___Budget_Justification1049083428.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

UEI*: SPE2YDWHDYU4

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Utah State University

Start Date*: 10-01-2025 **End Date*:** 09-30-2026 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Zhongde		Wang		PD/PI					(b) (4)		

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** (b) (4)

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates				(b) (4)		
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician						
2	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4)
						Total Salary, Wages and Fringe Benefits (A+B)	

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

UEI*: SPE2YDWHDYU4

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Utah State University

Start Date*: 10-01-2025 **End Date*:** 09-30-2026 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
1 . chicken caging system	12,500.00
Total funds requested for all equipment listed in the attached file	
Total Equipment	**12,500.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,200.00
2. Foreign Travel Costs	
Total Travel Cost	**2,200.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

UEI*: SPE2YDWHDYU4

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Utah State University

Start Date*: 10-01-2025 **End Date*:** 09-30-2026 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	49,328.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal per diem	8,432.00
Total Other Direct Costs	**57,760.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**300,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	46	287,500.00	132,250.00
		Total Indirect Costs	**132,250.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Arif Karim, Cost Allocation Services Director Department of Health and Human Services Phone: (214) 767-3600 Email: Arif.Karim@psc.hhs.gov

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**432,250.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	432,250.00

L. Budget Justification*	File Name:
	Wang___Budget_Justification1049083428.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

UEI*: SPE2YDWHDYU4

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Utah State University

Start Date*: 10-01-2026 **End Date*:** 09-30-2027 **Budget Period: 3**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Zhongde		Wang		PD/PI					(b) (4)		

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person (b) (4)

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates				(b) (4)		
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician						
2	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4)
						Total Salary, Wages and Fringe Benefits (A+B)	

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

UEI*: SPE2YDWHDYU4

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Utah State University

Start Date*: 10-01-2026 **End Date*:** 09-30-2027 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
1 . chicken caging system	12,500.00
Total funds requested for all equipment listed in the attached file	
Total Equipment	**12,500.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,200.00
2. Foreign Travel Costs	
Total Travel Cost	**2,200.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

UEI*: SPE2YDWHDYU4

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Utah State University

Start Date*: 10-01-2026 **End Date*:** 09-30-2027 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	29,800.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. animal per diem	8,432.00
9. animal shipping	13,500.00
Total Other Direct Costs	**51,732.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**300,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	46	287,500.00	132,250.00
		Total Indirect Costs	**132,250.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): Arif Karim, Cost Allocation Services Director Department of Health and Human Services Phone: (214) 767-3600 Email: Arif.Karim@psc.hhs.gov

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**432,250.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	432,250.00

L. Budget Justification*	File Name:
	Wang___Budget_Justification1049083428.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

UEI*: SPE2YDWHDYU4

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Utah State University

Start Date*: 10-01-2027 **End Date*:** 09-30-2028 **Budget Period:** 4

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Zhongde		Wang		PD/PI					(b) (4)		

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person (b) (4)

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates				(b) (4)		
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician						
2	**Total Number Other Personnel**						

Total Other Personnel (b) (4)

Total Salary, Wages and Fringe Benefits (A+B)

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

UEI*: SPE2YDWHDYU4

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Utah State University

Start Date*: 10-01-2027 **End Date*:** 09-30-2028 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
1 . chicken caging system	12,500.00
Total funds requested for all equipment listed in the attached file	
Total Equipment	**12,500.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,200.00
2. Foreign Travel Costs	
Total Travel Cost	**2,200.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

UEI*: SPE2YDWHDYU4

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Utah State University

Start Date*: 10-01-2027 **End Date*:** 09-30-2028 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	23,579.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. animal per diem	8,432.00
9. animal shipping	13,500.00
Total Other Direct Costs	**45,511.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**300,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	46	287,500.00	132,250.00
		Total Indirect Costs	**132,250.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Arif Karim, Cost Allocation Services Director Department of Health and Human Services Phone: (214) 767-3600 Email: Arif.Karim@psc.hhs.gov

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**432,250.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	432,250.00

L. Budget Justification*	File Name:
	Wang___Budget_Justification1049083428.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

UEI*: SPE2YDWHDYU4

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Utah State University

Start Date*: 10-01-2028 **End Date*:** 09-30-2029 **Budget Period:** 5

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Zhongde		Wang		PD/PI					(b) (4)		

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person (b) (4)

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates				(b) (4)		
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4)
						Total Salary, Wages and Fringe Benefits (A+B)	

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

UEI*: SPE2YDWHDYU4

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Utah State University

Start Date*: 10-01-2028 **End Date*:** 09-30-2029 **Budget Period: 5**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
1 . chicken caging system	12,500.00
Total funds requested for all equipment listed in the attached file	
Total Equipment	**12,500.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,200.00
2. Foreign Travel Costs	
Total Travel Cost	**2,200.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Opportunity Number: PAR-24-251 . Received Date: 2024-08-05T12:08:26.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

UEI*: SPE2YDWHDYU4

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Utah State University

Start Date*: 10-01-2028 **End Date*:** 09-30-2029 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	49,912.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. animal per diem	8,432.00
9. animal shipping	13,500.00
Total Other Direct Costs	**71,844.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**250,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	46	237,500.00	109,250.00
		Total Indirect Costs	**109,250.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Arif Karim, Cost Allocation Services Director Department of Health and Human Services Phone: (214) 767-3600 Email: Arif.Karim@psc.hhs.gov

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**359,250.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	359,250.00

L. Budget Justification*	File Name:
	Wang___Budget_Justification1049083428.pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION – UTAH STATE UNIVERSITY (Wang)

SENIOR/KEY PERSONNEL:

(b) (4)

Zhongde Wang, Ph.D., Professor, (b) (4) % effort, (b) (4) calendar months Years 1-5), will oversee the execution and management of the project at Utah State University (USU).

OTHER PERSONNEL:

(b) (4)

MyeongDon Joo, PhD, Postdoctoral Associate (b) (4) % effort, (b) (4) calendar months Years 1-5), will be responsible for chicken PGC isolation, purification, in vitro culture, and gene editing to produce gene edited chickens.

Nathan Merrill, Technician (b) (4) % effort, (b) (4) calendar months Years 2-4), will assist Dr. Joo to produce gene-edited chickens, breeding and maintaining the chicken flock, and shipping animals to the University of Wisconsin Madison study site.

FRINGE COSTS:

Fringe benefits for faculty and staff are calculated at (b) (4) % FY25, with (b) (4) % increase annually.

EQUIPMENT: ($12,500 Years 2-5)

Chicken caging system: Allentown Inc or Alternative design. Four Poultry 9-cage housing units, cat# SG9-24x24x18-SS @ $11,000 + $1,500 shipping each.

TRAVEL: ($2,200 Years 2-5)

Annual Scientific Meetings: We request annual funds to send 1-2 persons to meetings with FDA program staff, with the research team at University of Wisconsin Madison and scientific conferences starting year 2.

OTHER COSTS: ($50,905 Year 1; $49,328 Year 2; $ 29,800 Year 3; $ 23,579 Year 4; $ 49,912 Year 5)

Molecular biology kits and reagents: Reagents include CRISPR/Cas, electroporation kits, anesthetics, analgesics, hormones, restriction enzymes, gel electrophoresis, imaging reagents, genomic DNA isolation and RNA extraction reagents, protein purification kits, PCR (genomic PCR, RT-PCR, quantitative PCR) reagents, primers, DNA subcloning, DNA sequencing, reagents for real-time PCR, Western blots, immunohistochemistry, antibodies for flowcytometry**,** pathology analysis, SDS-PAGE and Western blot reagents for detecting genetically edited chicken genes and the genes in the same pathways, etc.

Chemicals: Chemicals and buffers for, electrophoresis, gel transfer, etc.

Small equipment: This budget will be used to replace aged or defective benchtop small equipment during the project period, for example, electrophoresis apparatus, power supply, benchtop centrifuge, electronic transfer pipet, heat blocks, pH meter, vortex, shaker, rotisseries, micromanipulator, microscopes, etc.

Disposables: A variety of disposable plastic pipet tips (0.5-1,000µl), centrifuge and ultracentrifuge tubes with different volumes (1.5-50ml), PCR and qPCR tubes and plates, gloves, etc.

Animal costs ($8,432 Year 2; $21,932 Years 3-5)

Cost of chicken per diem:

Note: Per Diem rate at USU is $0.77 per chicken per day (may be subjected to 6% raise annually).

On average, we will house 30 chickens on any given day throughout year 2 to year 5. The cost of per diem in each year is $0.77 X 30 (chickens) X 365 (days) = $8,432.

Cost of shipping animals:

The cost of shipping experimental animals will be evenly distributed among Y3-5. Based on the quote provided by World Courier Inc. (www.WorldCourier.com; New Hyde Park, NY), it costs $4,500 per shipment for up to 15 crates. It is estimated that 3 shipments of 15 crates per year will be made to other study site. Shipping cost for each year is $4,500 X 3 = $13,500

DIRECT COSTS: ($200,000 Year 1; $300,000 Year 2-4; $250,00 Year 5)

INDIRECT COSTS: ($92,000 Year 1; Year $132,250 Year 2-4; $109,250 Year 5)

Indirect Costs have been included according to USU's federally negotiated rate of 46% Modified Direct Total Costs.

TOTAL COSTS: ($292,000 Year 1; $432,250 Year 2-4; $359,250 Year 5)

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	(b) (4)
Section A, Senior/Key Person		
Section B, Other Personnel		
Total Number Other Personnel	8	
Total Salary, Wages and Fringe Benefits (A+B)		
Section C, Equipment		50,000.00
Section D, Travel		8,800.00
1. Domestic	8,800.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		277,752.00
1. Materials and Supplies	203,524.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	33,728.00	
9. Other 2	40,500.00	
10. Other 3		
11. Other 4		
12. Other 5		
13. Other 6		
14. Other 7		
15. Other 8		
16. Other 9		
17. Other 10		
Section G, Direct Costs (A thru F)		1,350,000.00
Section H, Indirect Costs		598,000.00

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Section I, Total Direct and Indirect Costs (G + H)	1,948,000.00
Section J, Fee	
Section K, Total Costs and Fee (I + J)	1,948,000.00

OMB Number: 0925-0001

Expiration Date: 01/31/2026

PHS 398 Cover Page Supplement

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Human Fetal Tissue Section

*Does the proposed project involve human fetal tissue obtained from elective abortions? ○ Yes ● No

If "yes" then provide the HFT Compliance Assurance

If "yes" then provide the HFT Sample IRB Consent Form

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

6. Change of Investigator/Change of Institution Section

☐ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

☐ Change of Grantee Institution

*Name of former institution:

OMB Number: 0925-0001
Expiration Date: 01/31/2026

PHS 398 Research Plan

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	Specific_Aims_U181049190226.pdf
3. Research Strategy*	Research_Strategy_U181049190222.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	Vertebrate_Animals_U181049190224.pdf
6. Select Agent Research	Select_Agent_Research_U181049190223.pdf
7. Multiple PD/PI Leadership Plan	Multi_PI_leadership_plan_U181049190219.pdf
8. Consortium/Contractual Arrangements	consortintent_letter_print1049083336.pdf
9. Letters of Support	
10. Resource Sharing Plan(s)	Resource_sharing_plan_U181049190227.pdf
11. Other Plan(s)	Data_U181049190216.pdf
12. Authentication of Key Biological and/or Chemical Resources	Authentication_U181049190213.pdf
Appendix	
13. Appendix	

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SPECIFIC AIMS

Avian viruses cause substantial economic losses due to high mortality and morbidity rates, but also representing a severe public health risk through zoonotic transmission. Highly pathogenic avian influenza (HPAI) viruses are among the most formidable threats. Outbreaks HPAI viruses specifically of the Guangdong H5N1 lineage, can lead to the death of chickens and the rapid spread of virus through flocks, resulting in massive culling efforts and deaths of thousands to millions of birds.

Given the limitations and failures of current control approaches to control avian viruses including HPAI viruses, there is a pressing need to explore alternative strategies. To tackle the susceptibility of chickens to avian viruses, we propose establishing the BRAVE (Building Resistance Against Viral Entities) Innovation Center. This initiative aims to generate chickens that are resistant to viral pathogens of significant biological and economic importance though intentional genomic alterations. This proposal is in response to the Animal and Veterinary Innovation Centers funding opportunity with one area of specific interest that seeks to identify genome targets associated with virus disease resistance allowing for gene editing to decrease the susceptibility of animals to HPAI viruses.

Our objective is to first identify host factors, both pro-viral and antiviral genes, that when genetically modified in cell culture result in inhibition of viral replication. Using genomic modification approaches, we will generate chicken lines with intentional genome alterations to produce birds with resistance to viral infections. While our primary focus is on developing a modified chicken line with reduced susceptibility to HPAI viruses, the center will also identify common antiviral restriction factors that inhibit HPAI virus along with other critical virus pathogens including the avian gammacoronavirus, infectious bronchitis virus (IBV). Creating a single gene modified chicken line resistant to multiple viral pathogens would be innovative to the poultry field, significantly reducing disease burden and economic losses. The following aims are designed to successfully accomplish the objectives of the BRAVE Innovation Center.

Specific Aim 1: Identify pro-viral and antiviral host factors that reduce infection of avian viruses in cell culture. Viruses rely on host factors for replication and spread, while hosts use antiviral factors to combat infections. A CRISPR/Cas9 genome-wide study using a library of chicken-specific sgRNAs will be performed in a chicken cell line to identify unique and common pro-viral host factors essential for replication of avian viruses. A cDNA library of human interferon-stimulated genes will be utilized to identify antiviral factors that inhibit the avian viruses in this proposal to develop a multi-virus resistant chicken line. Once host factors of interest are identified in the initial studies, we will perform confirmatory studies in stable cell lines for up to 10 of the best candidates in each group tested against multiple avian viruses. These studies will allow us to rank and identify host target candidates for gene modifications in chickens.

Specific Aim 2. Generate virus-resistant chicken lines by intentional genomic alterations. We will generate intentional genomic altered (IGA) chicken lines that target individual or a combination of pro-viral and antiviral host factors along with virus genome targets to ensure comprehensive and long-lasting resistance without breakthrough infections. We will employ CRISPR/Cas9 or transposase methods to modify the genomes in chicken primordial germ cells (PGCs) followed by the injection of modified PGCs into recipient chicken embryos to establish germline-modified chickens. Our primary goal is to generate an influenza-specific IGA chicken line resistant to HPAI virus infection along with a second IGA chicken line resistant to more than one avian virus.

Specific Aim 3. Characterize infection of IGA chicken lines with avian viruses. Once an IGA chicken line is established, we will compare its susceptibility of the IGA birds with that of wild-type birds with HPAI H5N1 and H7N9 virus infections. We will assess virus replication in respiratory and other tissues along with swabs and evaluate associated pathology. Additionally, we will examine virus transmission from infected wild-type chickens to IGA chickens and monitor for the emergence of escape viruses. We will also evaluate the susceptibility of a multi-virus resistant IGA line HPAI viruses along with other avian viruses including IBV. Non-infected IGA chicken lines will be characterized to ensure proper growth, longevity, and fertility along with any pathological changes compared to their wild-type counterparts.

A comprehensive evaluation of pro-viral and antiviral host factors in cell culture will provide a strong scientific rational to identify targets for genome modifications in chickens. These genome-modified birds with a resistance phenotype to viral diseases will reduce significant economic losses and health risk through zoonotic transmission.

A. BACKGROUND

Given the recurrence of avian influenza virus outbreaks in the United States, particularly those caused by H5N1 viruses in commercial and backyard chicken flocks, the goal of the BRAVE (Building Resistance Against Viral Entities) Innovation Center is to develop an influenza-specific chicken line with a phenotype of resistance to infection with highly pathogenic avian influenza (HPAI) viruses through intentional genomic alterations. This will be achieved by gene edits or knockouts in the chicken genome that target host factors or the genomes of avian viruses of interest. Host factors such as antiviral proteins may have broad inhibitory effects on multiple viral pathogens. Therefore, a second goal is to develop a multi-virus intentional genomically altered (IGA) chicken line focusing on the genetic modification of antiviral genes to establish a phenotype of resistance to infection with three avian viruses. A potential third IGA chicken line will be developed in case of unforeseen issues such as breakthrough infections with our primary influenza-specific IGA line.

The concept of gene-edited organisms with a resistance phenotype is not new. Modified soybeans with a tolerance to herbicide, corn resistant to the European corn borer, and plums resistant to the plum pox virus are examples of gene-edited plants. In livestock, scientists have developed IGA calves resistant to bovine viral diarrhea virus by slightly altering the cellular receptor CD46, so the virus does not bind efficiently while CD46 still retains normal bovine functions (1). Although this IGA cow line is not in the human food chain, the FDA has approved IGA salmon and pigs for human consumption. Therefore, there is a path forward for eventual FDA approval of an IGA chicken line after the completion of the proof-of-concept studies in this proposal.

Avian viruses cause devastating economic losses to the poultry industry and compromise food security. HPAI viruses, infectious bronchitis virus (IBV), and infectious bursal disease virus (IBDV) are among the most concerning poultry viral pathogens. HPAI H5N1 viruses have high mortality rates and outbreaks have led to the culling of millions of birds. H5N1 outbreaks not only disrupt poultry production, they trigger stringent biosecurity measures and trade restrictions, further exacerbating financial strain. The ongoing challenges to control these viral diseases underscores the urgent need for new strategies to safeguard the poultry industry.

HPAI viruses: Influenza A viruses are classified based on their surface proteins hemagglutinin (HA) and neuraminidase (NA). Among the various HA subtypes, H5 and H7 subtypes have a significantly impact on the poultry industry due to their potential to cause high mortality and are particularly lethal.

Since 2021, HPAI viruses of the Guangdong H5N1 lineage have caused severe outbreaks with widespread mortality in wild birds and poultry in the United States. First identified in 1997 in Hong Kong, H5N1 virus has led to the culling of millions of chickens (2). But despite culling measures, H5N1 viruses have spread from Hong Kong to other parts of Asia, Europe, Africa, and North America, facilitated by migratory birds and global poultry trade. By 2023, H5N1 had caused outbreaks in more than 80 countries, threating the poultry industries and wild bird populations worldwide. The high pathogenicity of H5N1 viruses in chickens is attributed to its ability to infect multiple organs, leading to systemic infection, with mortality rates reaching 100% within a few days. More recently, other H5 subtypes, such as H5N2, H5N6, and H5N8, have emerged, causing significant outbreaks in Asia, Europe, and North America (3). These viruses can reassort with other influenza viruses, leading to new variants that also threaten poultry health.

Like H5, the H7 subtype includes strains that can shift from low pathogenic avian influenza (LPAI) to highly pathogenic avian influenza (HPAI) forms. H7N9 is a prime example; first detected in China in 2013, it has caused severe disease in poultry and humans (4). H7N3 and H7N7 are other notable subtypes that have led to significant outbreaks. The transformation of LPAI to HPAI in H7 viruses often results in high mortality rates in chickens, with rapid spread and severe clinical symptoms.

The first introduction of an HPAI H5 virus (of the H5N8 subtype) into North America occurred in 2014 through the Pacific flyway. Reassortment with North American LPAI viruses resulted in an HPAI H5N2 virus that caused substantial mortality in poultry with huge economic losses for the poultry industry. The outbreak was eliminated in 2015 through culling and quarantine. In late 2021, HPAI H5 viruses were reintroduced into North America, this

time from Europe through the Atlantic flyway (5). Multiple reassortment events occurred with LPAI viruses, resulting by now in more than 100 different genotypes. Rapid virus spread occurred throughout North America, and into Central and South America, followed by the first HPAI H5N1 virus isolations from Antarctica in early 2024 (6). By July 18, 2024, there were 9,552 confirmed HPAI detections in wild birds in the US alone. In addition, 1,145 commercial and backyard flocks in 48 US States have reported HPAI H5N1 viruses with >96 million birds affected.

Other important avian viruses: In addition to HPAI viruses, IBV and IBDV are significant pathogens in the poultry industry. IBV infection targets the respiratory and urogenital systems of chickens, impairing growth and egg production. IBDV infection primarily targets the bursa of Fabricius of chickens, an essential organ for the development of the immune system in birds. Infection with IBDV leads to immunosuppression, making chickens more susceptible to other diseases, and can cause high morbidity and mortality rates, particularly in young birds. Effective control of these pathogens through IGA chickens would be an important development for maintaining poultry health, productivity, and overall industry sustainability.

Zoonotic potential and pandemic concerns: HPAI viruses pose a significant public health risk due to their zoonotic potential. The H5N1 and H7N9 subtypes can infect humans and cause severe illness and death. The continuous circulation of H5N1 virus in the United States has allowed the virus to evolve, resulting in transmission to cows in over 164 herds in 13 states. The biggest concern with transmission and replication of H5N1 in mammals is the eventually adaption of the virus to mammalian hosts resulting in an H5N1 pandemic in humans. In Colorado, nine poultry workers may have been exposed to the virus while culling infected chickens, and dairy workers have contracted the virus from cows on farms.

Impact of outbreaks: Effective prevention and control strategies are essential to protect poultry health and ensure food security. The poultry industry is valued at $312 billion and is a critical component of global agriculture, providing major sources of protein in the form of meat and eggs. It supports the livelihoods of millions of people, from small-scale farmers to large commercial enterprises. The industry's success relies on maintaining healthy flocks; as such, the control of diseases like influenza is crucial for economic stability.

One of the most immediate economic impacts of avian viruses is the mortality of infected birds. HPAI viruses cause rapid and widespread death within poultry flocks. Infected birds that do not die from the virus are often culled to prevent further spread, leading to substantial losses. The cost of culling includes not only the loss of poultry but also the expenses related to disposal and disinfection. In addition, avian influenza outbreaks can lead to decreased productivity among surviving birds. Infected birds may experience reduced egg production, poor growth rates, and other health issues that diminish their economic value. Avian influenza outbreaks in 2022 in the United States resulted in the loss of 40 million animals and an economic cost of $3 billion.

Failure of control measures: Effective management of avian viruses requires continuous surveillance and prevention efforts. These include monitoring wild bird populations, testing poultry flocks, maintaining biosecurity, and culling entire flocks in attempts to prevent spread. The costs associated with these activities are substantial but necessary to prevent and control outbreaks. Biosecurity measures include controlling access to poultry farms, disinfecting equipment and vehicles, and ensuring proper waste management. The costs of biosecurity can be significant, but they are essential for protecting poultry health and minimizing economic losses.

Vaccination can effective for controlling avian viruses. However, this control measure faces significant challenges that often undermine its effectiveness. Viruses like HPAI mutate rapidly under immune pressures resulting in virus isolates that are less susceptible or resistant to the vaccine. Additionally, vaccinated birds can still become infected and shed the virus asymptomatically, complicating disease control by allowing the virus to circulate unnoticed within poultry populations.

Logistic and economic challenges further limit the effectiveness of vaccination. Asymptomatic spread in vaccinated birds complicates surveillance, making it harder to detect and monitor outbreaks. This impedes swift and effective responses to contain the virus. Vaccination programs can also lead to trade restrictions, as some

countries prefer poultry products from regions declared free of avian influenza without vaccination. These trade barriers impact the poultry export market and discourage the adoption of vaccination as a control measure.

Current control measures have proven inadequate in curbing the spread of avian viruses, particularly HPAI viruses. This has led to significant economic and health impacts within the poultry industry. Therefore, it is crucial to explore and implement innovative strategies and advanced technologies to enhance the resistance of poultry, especially chickens, to HPAI viruses and other avian pathogens. This grant proposal seeks to address this need by advancing cutting-edge solutions such as the generation of IGA chicken lines resistant to virus infection to mitigate the devastating effects of these viral diseases.

B. INNOVATION

IGA chicken lines resistant to pathogens such as avian influenza viruses offer a promising and potentially transformative approach to securing the poultry industry. Unlike the traditional vaccination strategy, which faces challenges such as viral mutation, incomplete protection, and logistic hurdles, genetic modification could provide a more robust and permanent solution. By generating a phenotype resistant to infection directly in the chicken genome, we can create birds that are inherently resistant to avian influenza viruses and other avian pathogens, reducing the risk of outbreaks regardless of strain changes. Such genetic resistance would eliminate the need for repeated vaccinations and the associated costs, making it more economical for large-scale poultry operations.

IGA chicken lines would significantly enhance biosecurity and disease surveillance. Resistant birds would not become asymptomatic carriers, thereby reducing the risk of undetected virus within flocks. This could lead to more stable and predictable poultry production, minimizing the economic losses associated with disease outbreaks. With the increasing precision and safety of genetic engineering technologies, developing and deploying gene-edited chickens offers a sustainable, long-term strategy to protect the poultry industry from the devastating impact of avian influenza virus. This platform can be leveraged to include other viral pathogens of high consequence to chickens including IBV and IBDV as well as bacterial pathogens.

Given the limitations and failures of current approaches to control avian viruses including HPAI viruses, there is a pressing need to explore alternative strategies. One promising avenue is the development of IGA chickens with different pathogen resistant phenotypes. By leveraging gene-editing technologies like CRISPR, we can create chicken lines that exhibit different pathogen-resistance phenotypes, potentially taking a large step forward in disease control within the poultry industry and mitigating the significant economic and health impacts of avian viruses.

C. APPROACH

The BRAVE Innovation Center: To advance our novel control strategy against HPAI viruses and other avian pathogens through the generation of virus resistant IGA chicken lines, we have assembled a team of multidisciplinary expert researchers in their respective fields of study.

Dr. Peter Halfmann, assistant professor at the School of Veterinary Medicine at UW-Madison, will serve as lead (contact) principal investigator of the center of this multiple PI (MPI) proposal. His research career has focused on utilizing different animal models to study virus pathogenicity and to evaluate vaccine efficacy. Dr. Halfmann was involved in the characterization of the early H5N1 influenza isolates responsible for the Hong Kong outbreak in humans in 1997 (7) and the more recent clade 2.3.4.4b isolates (8, 9) in particular the bovine H5N1 isolates affecting the dairy industry in the United States (10). His research efforts also include evaluating a novel Ebola virus vaccine in different animal models (11, 12), and characterizing SARS-CoV-2 variants and coronavirus vaccines and therapeutics (13-26).

Dr. Zhongde Wang (PI), professor at Utah State University, is a leader in the generation of IGA animal models and his group was the first to produce genetically engineered hamsters. His extensive expertise extends to both laboratory and livestock animals, including Syrian hamsters, guinea pigs, *Peromyscus maniculatus, P. leucopus*, cows, goats, and sheep. In response to the COVID-19 pandemic, his group generated human ACE2 transgenic

hamsters, which were widely utilized to study SARS-CoV-2 pathogenicity and countermeasures (27, 28). Drs. Wang, Halfmann, and Kawaoka have collaborated over the last four years on the transgenic hamster model for SARS-CoV-2 infections (16, 17, 29-31).

Dr. Yoshihiro Kawaoka (co-investigator), professor and director of the Influenza Research Institute, has over 40 years of research experience on influenza viruses with over 800 peer-reviewed publications. His pioneering reverse genetic system for influenza viruses has been used in the development of influenza vaccines. Dr. Kawaoka will collaborate with Dr. Halfmann on the study design for HPAI virus challenge studies in chickens.

Dr. Adel Talaat (co-investigator), professor at the School of Veterinary Medicine, studies human and animal pathogens including the use of nanotechnology in the development of vaccines and novel adjuvant systems against important poultry diseases including IBV (32, 33). Dr. Talaat will oversee the cell culture studies to identify host proteins and restriction factors associated with reduced susceptibility or resistance of avian viruses including IBV and will confirm the phenotype in the multi-virus resistant IGA chicken line.

Dr. Andres Mejia Montoya, (co-investigator) is chief veterinary pathologist of the research animal resources and compliance's comparative pathology laboratory at UW-Madison. He has participated in studies of Zika virus, HIV/SIV, and SARS-CoV-2 in array of animal models from mice to nonhuman primates. Besides examining the pathology of infected chickens, Dr. Montoya will also conduct a preliminary histopathological analysis to assess tissue morphology and identify any pathological changes associated with the gene edits in non-infected IGA chicken lines.

Our goal is to identify key host factors that significantly reduce or prevent virus infection in cell culture. Leveraging this knowledge, we aim to develop IGA chicken lines with resistance phenotypes to viral pathogens through the aims illustrated in **Figure 1**.

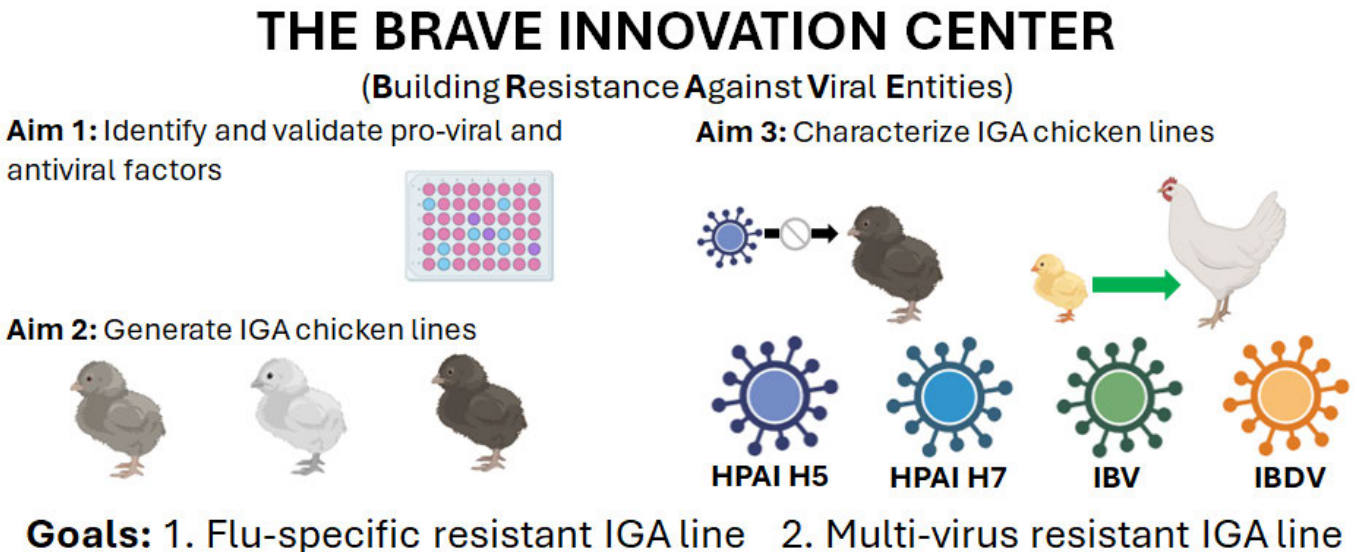

Figure 1. An illustration of the aims and goals of the BRAVE Innovation Center to generate intentional genomic alterations in chicken lines resistant to virus infection.

Biosafety Considerations: Given the biosafety risks associated with HPAI virus research, all studies with virus-infected cells and chickens will be carried out at the Influenza Research Institute (IRI), which houses shower-out, biosafety level-3 containment labs including a BSL-3 agriculture (AG) suite for animal research. The IRI is a Select Agent registered facility and is inspected annual by the CDC and USDA to ensure compliance with federal regulations. Although each animal room in the BSL-3 AG suite is designated primary containment, infected chickens will be housed in HEPA-filtered isolator units and all procedures will be performed in a biosafety cabinet

or on a ducted necropsy table. Research on IBV and IBDV will be carried out under BSL-2 or animal (A)BSL-2 containment. More details are available in the Biohazards and Select Agent document.

C.1 Specific Aim 1: Identify pro-viral and antiviral host factors that reduce avian virus infection in cell culture.

Rationale: RNA viruses, including influenza viruses, IBV, and IBDV, rely on host proteins for efficient replication. These host factors can be targeted by gene editing to generated chickens resistant to infection. For example, the host-encoded family of ANP32 proteins, specifically ANP32A and ANP32B, support influenza virus polymerase activity in human cells (34). In chicken cells, ANP32A is the primary facilitator of virus replication due to the nonfunctional nature of chicken APN32B. The pro-viral activity of ANP32A is dependent on its interaction with the viral polymerase proteins, a function mapped to two critical amino acids, 129N and 130D (35). N129I and D130N substitutions disrupt this interaction, significantly reducing virus replication (36).

Building on this knowledge, Idoko-Akon et al. generated an IGA chicken line with the APN32A mutations N129I-D130N to inhibit virus replication (37). When challenged with an LPAI virus, 90% of the IGA chickens were resistant to infection. However, replicating virus was detected in one bird and breakthrough infections were detected at higher doses of virus, suggesting potential limitations with this specific IGA chicken line. In a real-world setting, a mutant virus that adapts to overcome the edited host factor could spread throughout the flock, ultimately compromising the effectiveness of the IGA line.

This proof-of-concept study by Idoko-Akon et al. demonstrates the feasibility of creating IGA chickens resistant to an LPAI virus. However, it also highlights the need to identify and validate additional pro-viral and antiviral host factor targets for gene modification that are effective against viruses, including HPAI viruses, and to ensure comprehensive and long-lasting resistance without breakthrough infections.

Experimental Design: There are published genome-wide studies that identified host factors involved in the influenza virus life cycle by using human cell lines or Drosophila cells with human influenza isolates and lab-adapted viruses (38, 39). In this aim, we will focus our genomic-wide studies on a chicken cell line infected with an HPAI H5N1 virus. A single-guide (sg)RNA CRISPR chicken-specific genome library will be used to identify pro-viral host factors and a human interferon-stimulated gene (ISG) cDNA library to identify antiviral host factors.

C.1.1. A CRISPR/Cas9 genome-wide study to identify essential pro-viral host factors for virus replication. To identify pro-viral host factors that are essential for virus replication, we will use a sgRNA CRISPR library from Cellecta that targets approximately 17,000 chicken protein-coding genes with 3-4 sgRNA sequences that target each gene. The library is packaged in a lentivirus and contains a red fluorescent protein (RFP) reporter gene and a puromycin resistance gene.

A chicken embryonic fibroblast cell line (DF1 cells) that stable expresses Cas9 will be generated for the CRISPR study. After generating a kill curve for the antibiotic blasticidin, a DF1-Cas9 cell line will be generated by transfection of a plasmid expressing the Cas9 and blasticidin resistance genes. After plasmid transfection and drug selection, single cell clones will be isolated by limiting dilution. Cas9 expression will be confirmed by immunostaining and western blot analysis using an antibody against Cas9, followed by a functional Cas9 assay using a reporter gene along with a sgRNA targeting the reporter gene.

A DF1-Cas9 clone with confirmed Cas9 expression and function will be used for the CRISPR library study. First, the transduction efficiency of the cell line will be determined by using an RFP-lentivirus and infection at multiple concentrations of virus. At 72 h after transduction, cell suspensions will be used to determine the percentage infection using flow cytometry at an excitation wavelength of 561 nm. Based on the percent infection, a multiplicity of infection (MOI) of 0.3 to achieve a transduction efficiency of 25%–30% of the cells will be used for transduction of DF1-Cas9 cells with the CRISPR library to ensure cells receive more than one sgRNA at the same time to avoid multiple integrations. After the establishment of a kill curve for puromycin in the DF1-Cas9 cells, the cells will be transduced with the CRISPR library and selected for puromycin resistance 72 h after transduction. Puromycin-resistant cells will be expanded and then infected with an H5N1 virus at an MOI of 3 to ensure

complete infection. Since DF1 cells are highly susceptible to H5N1 virus infection, cell death will be the readout of the CRISPR library study. The media will be changed daily to remove dead cell debris and provide fresh media to cells that survive infection.

Cells that survive infection will have a sgRNA integrated in their genome responsible for the knockout of a pro-viral factor associated with resistance to infection. To reveal these sgRNAs, genomic DNA from the surviving cells will be harvested and next-generation sequencing will be used to identify the sequences of the indels induced by the sgRNAs, which will be aligned to known genes for hit identification. Mock-infected cells will be harvested 48 h after seeding and will serve as a reference for downstream sgRNA enrichment analysis to improve the quality and interpretation of results.

To confirm the pro-viral activity of factors identified in the screen, we will knockout the top ranked genes with individual sgRNAs (2-4 sgRNAs/gene) in DF1-Cas9 cells. Cells will then be infected with an H5N1 virus at a low MOI of 0.001 to examine the growth kinetics of the virus. Treatment with a non-targeting sgRNA and one that targets the viral NP gene will serve as controls. Separate groups of sgRNA-treated DF1-Cas9 cells will be used to monitor cell viability and virus growth. Up to 10 of the best pro-viral factors will be identified based on reduction in virus titers (100-fold or greater) without significant impact on cell viability (> 90% viability). The doubling times of the stable knockout DF1 cells and the control cell line will be compared to ensure that there are no significant changes in cell growth that might indicate issues in the generation of healthy IGA chicken lines in **Aim 2**.

To examine resistance to infection over time, stable knockout DF1 cell lines will be generated using CRISPR/Cas9 for the top host factors with the greatest reduction in influenza virus replication. To confirm the gene modification or knockout of the host factor, we will perform sequencing to confirm the editing event (e.g., inactivation of a gene by the introduction of premature stop codons or non-function mutations), qRT-PCR to confirm a lack of a mRNA transcription, western blot analysis to confirm the lack of protein, or a combination of these techniques. Once the gene modification is confirmed, cells will be infected with virus at a low MOI of 0.001. In parallel, a stable cell line with non-targeting sgRNA will be infected to serve as a control to confirm the reduction of virus replication in the host-specific knockout cell lines. Virus will be passaged five times in each knockout cell line, and virus titers will be examined after each passage. If the pro-viral factor that is knocked out remains essential for the virus, we will observe reduced virus titers compared to the control cell line or even a loss of detectable virus after passaging. However, if the virus mutates to modify its interaction with the pro-viral factor or finds an alternative factor, then we will observe virus titers similar to the ones in the control cell line. We will perform deep sequencing on the viral genome to identify potential mutations responsible for this change and the host target will no longer be considered for editing in **Aim 2**.

In addition to identifying and validating HPAI H5N1-specific pro-viral factors, we will incorporate other avian viruses into this study. Using the CRISPR/Cas9 pro-viral knockout cell lines, we will examine the replication of an HPAI H7N9 virus, IBV, and IBDV. Dr. Talaat's group will perform a sgRNA CRISPR library study with a IBV-GFP reporter virus (40). After infection, GFP-negative cells resistant to infection will be collected under biosafety level-2 containment using a sorter at the Influenza Research Institute. The genomic DNA from the GFP-negative cells will be used to identify the sequences of the sgRNA inserts, which will be aligned to known genes for hit identification. Studies to validate the hits from the CRISPR library study will be performed in a similar manner as described above for HPAI H5N1 virus.

Collectively, these studies will identify unique and common pro-viral factors; at least one of the top candidates will be genetically edited in chickens in **Aim 2** to abolish its pro-viral activity.

C.1.2. Other potential host factors for gene modification. While the chicken CRISPR library study will yield new important information on pro-viral host factors associated with avian virus infection of chicken cells, as well as potential new targets for editing, it is important to start validating host targets for gene editing immediately in the first year of this proposal. Therefore, host factors (pro-viral and antiviral) that have been reported by others in peer-reviewed publications will be identified. Before generating an IGA chicken line with one of these factors, we will confirm the function of these previously identified factors against HPAI H5N1 virus in chicken DF1 cells as

well as potential escape from resistance in stable cells as described in **C.1.1**. If the previously identified factor is an antiviral protein, we will examine its activity against IBV and IBDV in studies described in Section **C.1.3**.

We have already identified two previously published factors that we will investigate immediately in our study. The first is metabotropic glutamate receptor subtype 2 (mGluR2). This host factor was identified as a endocytic receptor for influenza virus entering cells (41). Both mice and rats lacking mGluR2 have been successfully generated and are healthy without any apparent defects (42, 43); knockout mice were partially protected (60% survival) from a lethal H5N1 infection compared to wild-type mice (0% survival) (41).

The second potential pro-viral factor that could be targeted for gene modification in chickens is transmembrane protein immunoglobulin superfamily DCC subclass member 4 (IGDCC4). This host factor was identified by a genome-wide CRISPR/Cas9 study in human lung epithelial cells after infection with an H5N1 virus (44). Knockout of IGDCC4 significantly reduced virus replication in cell culture. Infection with H5N1 virus was completely lethal in WT mice, but only 50% lethal in IGDCC4 knockout mice with reduced virus replication in the respiratory tissues. Like mGluR2, IGDCC4 plays a role in virus internalization. In year 1 of this proposal, we will determine whether knocking out the chicken homologs of these two human pro-viral host factors has an inhibitory effect on virus replication in DF-1 cells.

C.1.3. A human ISG cDNA library to identify antiviral restriction factors that inhibit virus replication. Antiviral proteins including ISGs play an important role in inhibiting virus replication. Several studies using a human ISG cDNA library have been performed to identify virus-specific antiviral proteins (45, 46). Using this library of nearly 400 ISG cDNA plasmids, we identified 22 ISGs that significantly reduced infection of an Ebola reporter virus, including 3 ISGs that decreased Ebola virus titers by nearly 1000-fold in cell culture on day 6 after infection (47) (**Figure 2**). Although this cDNA library study was performed with Ebola virus, it demonstrates our ability to identify antiviral proteins using this cDNA library. Antiviral proteins against influenza virus have been identified but using human cell lines and/or human isolates or lab-adapted viruses (48).

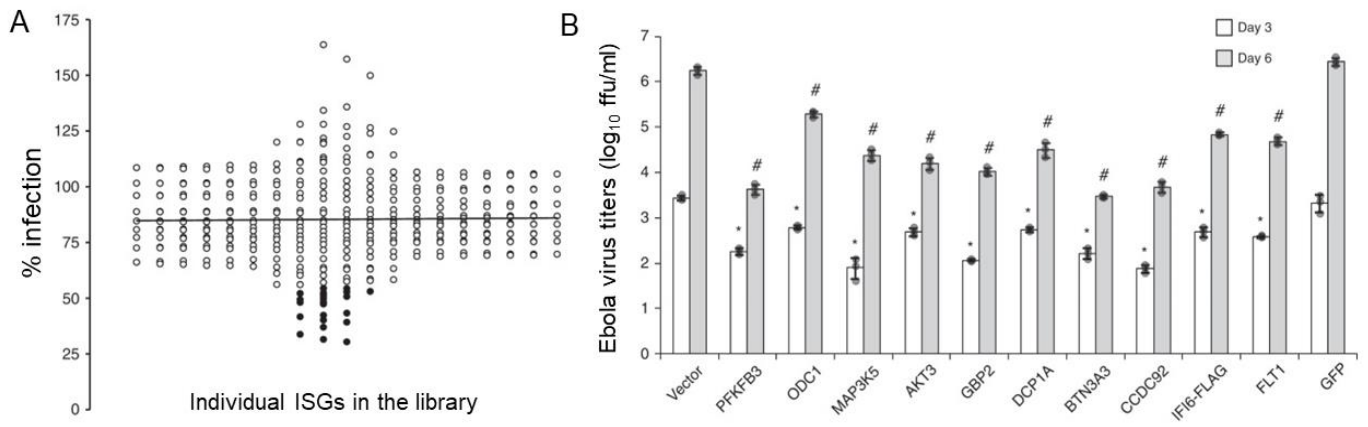

Figure 2. A. Dot plot of an Ebola-driven luciferase activity in the presense of overexpressed ISGs. HEK-293T cells were transfected with expression vectors for each ISG for 24 h prior to infection with a luciferase reporter Ebola virus at an MOI of 1.0. Virus-driven luciferase was measured 48 h after infeciton and compared to the luciferase activity of infected, control cells that were transfected wth a control vector. The black line indicates the ISG poplation mean, and the black filled-in dots represent the top 22 ISGs with signficant anti-Ebola virus activity. **B.** Titers of Ebola virus from HEK-293T cells after overexpression of selected ISGs. Virus titers were measured on days 3 and 6 after infection. * indicates a statistically significant difference (p-value $\leq$ 0.05) from the day 3 control; # indicates a statistically signifcant difference (p-valve $\leq$ 0.05) from the day 6 control.

To identify human ISGs with antiviral activity against HPAI H5N1 virus in chicken cells, 96-well plates will be seeded with DF1 cells and transfected with individual ISG constructs in each well, while a construct expressing only RFP will be used as a control. Twenty-four hours later, transfected cells will be infected an H5N1 virus at an

MOI of 0.001 and cell culture supernatants will be harvested 48 h after infection. Virus titers will be determined by standard plaque assays to identify ISGs that inhibit virus replication. Up to 10 of the best antiviral proteins will be identified based on the reduction in virus titers (100-fold or greater) without significant impact on cell viability.

Similar to the studies with the pro-viral host factors, stable knock-in antiviral gene cell lines in chicken DF1 cells will be generated for up to 10 of the best antiviral ISGs. Expression in the stable knock-in lines will be confirmed by sequencing and/or western blot analysis. Using these stable knock-in cell lines, we will confirm inhibition of virus replication, potential resistance to the antiviral factor during passaging, and broad antiviral activity against other avian influenza viruses. Up to three of the top candidates (i.e., those with the highest antiviral activities) will be chosen to produce IGA chicken lines expressing the ISGs in **Aim 2**.

Expected Outcomes, Potential Problems and Alternatives: Performing genome-wide studies using CRISPR and cDNA libraries is expected to provide important new insights into host-virus interactions. These screens will identify pro-viral host proteins essential for virus replication and antiviral host proteins that inhibit viral replication. While similar studies have been conducted by others in human or other cell lines but not chicken cells, our research focuses specifically on HPAI H5N1 virus infection of a chicken DF1 cell line, relevant for downstream experiments to generate IGA chicken lines resistant to HPAI virus infection.

Given that only 60% of chicken genes have human homologs, these studies must be done in a chicken cell line rather than relying on data collected with human cell lines. This approach ensures that we identify relevant host factors specific to chickens. Our lab and others have successfully carried out similar genome-wide screening studies, so we anticipate no significant technical issues in performing these studies.

Because HPAI viruses, IBV, and IBDV are all RNA viruses, we may identify common pro-viral and antiviral host factors. However, essential pro-viral factors may be difficult to detect if cell death results from knocking out the gene. It is more likely that we will identify antiviral host factors that restrict the replication of these three RNA viruses, enabling the development of an IGA chicken line with a multi-virus resistance phenotype.

Ranking and identifying the appropriate host factors to target for gene modification in chickens in **Aim 2** will be important for the success of this proposal. We will narrow down host factors based on their ability to significantly reduce virus titers without having a negative effect on cell viability.

Some pro-viral genes not associated with a negative effect on cell viability may be essential for embryogenesis. Attempting to edit these genes *in ovo* could result in a lethal phenotype for the embryo, thus preventing us from generating the desired IGA chicken line. To address this issue, we will utilize knockout mouse data to determine if a gene-edited mouse line that targets the same gene of interest in this project was created and if any undesirable phenotypes (e.g., lethality of the embryo, premature death, infertility, and abnormal growth) were observed. There are data for approximately 9,7000 knockout strains produced by the International Mouse Phenotyping Project (KOMP2) and the International Mouse Phenotyping Consortium (IMPC). We will use these databases to help determine any potential issues with the IGA chicken lines that we want to produce if the same host factor was targeted in a mouse model.

If a particular knockout mouse line associated with a gene of interest in this project is available (live animals, not cryopreserved materials), we could obtain these knockout mice first for *in vivo* validation studies to ensure a similar resistance phenotype in the mice that was observed in the genetically modified DF1 chicken cell lines. If the knockout mouse is partially or completely resistant to HPAI H5N1 virus infection, we would have additional data for ranking candidate host factors for **Aim 2**.

If we want to move forward with a particular pro-viral gene without the aid of mouse background information, we can perform protein-protein interaction studies with the pro-viral factor and viral proteins. Such studies will identify the viral protein that interacts with the essential host protein and then map important amino acids in the pro-viral protein essential for the interaction with the viral proteins. If identified, these amino acids in the pro-viral protein can be changed during *in ovo* editing for a loss-of-function of the pro-viral protein instead trying to completely

prevent the expression of the protein. This approach may alleviate potential embryo lethality issues if the mutated amino acids are only essential for the virus and not for embryonic development.

C.2. Specific Aim 2. Generate virus-resistant chicken lines by intentional genomic alterations.

Rationale: Given the results of breakthrough infection of low pathogenic avian influenza virus in APN32A gene-edited chickens, different strategies are needed to successfully generate birds with a stable resistance phenotype to HPAI viruses and other economically important avian viruses.

Here, our objective is to generate intentional genomically altered (IGA) chicken lines that target individual or a combination of pro-viral and antiviral host factors along with virus genome targets to ensure comprehensive and sustained resistance without breakthrough infections.

We will achieve this objective by leveraging the extensive expertise of Dr. Wang and his group at Utah State University, who has modified the genomes of animals in multiple livestock species and model organisms. The Wang lab has been working with several livestock species including sheep, goats, and cattle for both agricultural and biomedical applications and has generated a list of genetically engineered herds and flocks (49-60). His lab also pioneered genetic engineering techniques in the golden Syrian hamster, guinea pig, *Peromyscus maniculatus* and *P. leucopus* and has developed over 50 genetically engineered animal models across these species, each representing world-first achievements; some of these models have been widely used for viral infection studies (61-63).

Dr. Wang's group has extensive experience with chicken primordial germ cells (PGCs), particularly with the techniques of isolating, growing, and purifying chicken PGCs from donor embryos and injecting them to recipient embryos, all of which are required to produce IGA chicken lines.

General Experimental Design to Generate IGA Chicken Lines: As depicted in **Figure 3**, IGA lines will be generated through in vitro genetic manipulation of PGC in culture, followed by injecting the PGCs carrying the desired genetic alteration into surrogate chicken embryos to establish germline-modified chickens. Genotyping and sequencing methods will be used to identity positive genome-altered founder chickens, which will be further confirmed at the protein level with Western blotting analysis. Chicken PGCs will be isolated from fertilized chicken embryonic blood at stage HH 14-17 (Hamburger-Hamilton stages (64)), amplified in vitro and genetically modified by different techniques. Single cell colonies from positive genome-altered PGCs will be established by limiting dilution, screened via genomic DNA PCR genotyping assays, and the genome modification will be confirmed by Sanger sequencing and protein expression levels will be confirmed using western blot analysis. Positive genome-altered founder chickens will be produced by injecting positive PGCs into recipient chicken embryos. The produced chickens will be genotyped to identify the genome-altered positive founder chickens, which will be bred to establish genome-altered positive flocks. The first generation (F1) of these breeding pairs will be genotyped and positive birds will then be bred (sister-brother) to establish breeding flocks. This general method will be used to generate IGA chicken lines using the gene modifications described below.

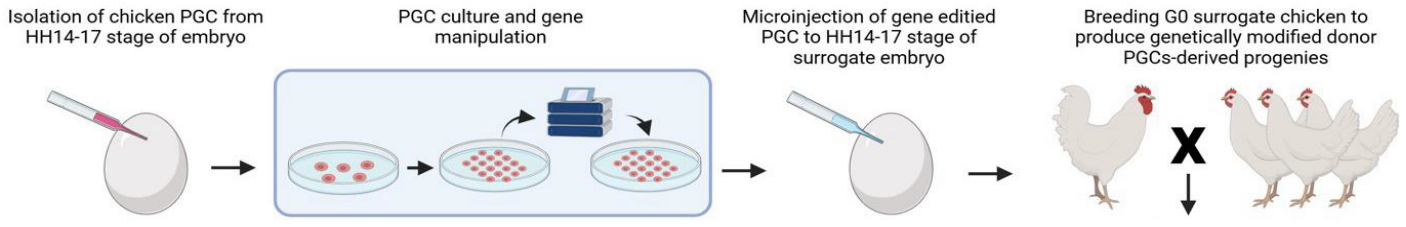

Figure 3. Workflow for generating intentional genome-altered chickens. Primordial germ cells (PGC) will be isolated from chicken embryos at stage HH 14-17, expanded in cell culture, and genetically modified. Once the desired genotype is identified from a clone, it will be injected into surrogate chicken embryos to generate an IGA chicken line. Founder chickens carrying the desired intentional genome alteration will be bred to establish a flock of IGA birds for experimental studies.

C.2.1. Generate an IGA chicken line resistant to virus infection by targeting pro-viral host factors. One approach to generate an IGA chicken line resistant to virus infection is by disabling at least one pro-viral host factor (**C.1.1 –C.1.2**) using CRISPR/Cas9-mediated genome editing. This technique targets the gene encoding the host factor by designing a single guide RNA (sgRNA) with a 20-nucleotide sequence that complements the target gene's DNA sequence, guiding the Cas9 enzyme to the specific site in the chicken genome (65). The sgRNAs used in **Aim 1** to generate knockout DF1 chicken cell lines will be employed here since they have been validated to target the genes of interest in the chicken genome. When Cas9 introduces a double-strand break in the DNA, the cell attempts to repair the damage through non-homologous end joining, an error-prone process that often results in insertions or deletions (indels). These indels disrupt the reading frame of the gene, leading to the production of a nonfunctional protein and effectively knocking out the gene product (66).

If the specific amino acids of the host protein associated with inhibition of virus replication are known, CRISPR/Cas9 base editing can introduce point mutations into the gene. This more targeted approach will introduce specific amino acid substitutions in the protein resulting in a loss of function of the host factor due to disruption of the interaction between the host factor and viral proteins, similar to studies with ANP32A and viral polymerase proteins (35, 36).

C.2.2. Generate an IGA chicken line resistant to virus infection with antiviral ISG restriction factors. A second approach to generate an IGA chicken line resistant to virus infection involves the transgenic expression of ISGs identified in **C.1.3**. We will select up to three top-ranked ISGs with the most potent antiviral activity against HPAI H5N1 virus. Priority will be given to ISGs that also demonstrate significant antiviral activity against IBV and IBDV, as determined in DF1 chicken cells in **Aim 1**.

To generate an IGA chicken line expressing these ISGs, we will use a piggyBac transposon-mediated editing approach. Dr. Wang's group has successfully employed this method to create multiple transgenic animal models including the human ACE2 transgenic Syrian hamster (27). The gene expression cassette will consist of the coding sequences of the selected ISGs, along with GSG linkers and self-cleaving 2A peptide sequences to enable simultaneous expression of the desired ISG proteins.

First, the piggyBac construct will be used to generate a DF1 cell line expressing these ISGs. We will confirm the expression of the ISG proteins and verify their antiviral function against the target viruses. Once the antiviral efficacy of the piggyBac-ISG construct is confirmed in vitro, it will be used to generate an IGA chicken line. The piggyBAC transposon method ensures the effective and stable integration of ISG genes into the chicken genome, providing a robust and expandable approach through the insertion of multiple ISGs to enhance viral resistance in poultry.

H5N1 PA:
gRNA1: 5'-AGTTGAACCAAGATGCATTAAGCAAAACCCAGGGATCATT-3'
gRNA2: 5'-TTTCGACATTTGAGAAAGCTTGCCCTCAATGCAGCCGTTC-3'
gRNA3: 5'-GGTGTTTCAATAATGCATTGGGATCGCCAGATTCTATAAT-3'
gRNA4: 5'-TACATGAAACAGACTTCTAAGTGCGTGCATATTGCGGCAA-3'
gRNA5: 5'-TTTCGACATTTGAGAAAGCTTGCCCTCAATGCAGCCGTTC-3'
gRNA6: 5'-AAGGGCATCCATCAGCAAAAACTTCGACCGCTGAGAGCAG-3'

IBV NSP9:
gRNA1: 5'-ACTGTTTGCATAAGCAGTAGTAGCATCACCACTGCTAGTG-3'
gRNA2: 5'-ACTGTTTGCATAAGCAGTAGTAGCATCACCACTGCTAGTG-3'
gRNA3: 5'-ATCACGCGTTATAACACTCAAAAGACGCGCAACATTAGCA-3'
gRNA4: 5'-TGCTCTATCACACTTAGGATAATCCCAACCCATAAGAATT-3'
gRNA5: 5'-TCCGAACTCAATAGCATTCAATAAAGCACGTCGTACAATA-3'
gRNA6: 5'-AGAATAATACGTATCAAAAACAGGAACACCAGCACCAGGT-3'

IBDV VP1:
gRNA1: 5'-GGGATACGCAGCAGAGCAAAATAAGCGTTCCTTGTCAAGC-3'
gRNA2: 5'-TGTTGGAGTGAACAATGTATATGTTGTCAGCATATACTAA-3'
gRNA3: 5'-AAGGTTCATAATCAGACATGATGAGTCCACAACTAGGGCT-3'
gRNA4: 5'-TTATCCACTCCACGATCCTGTTTAGCCCACCTCTAAACGG-3'
gRNA5: 5'-AAGGTTCATAATCAGACATGATGAGTCCACAACTAGGGCT-3'
gRNA6: 5'-TTATCCACTCCACGATCCTGTTTAGCCCACCTCTAAACGG-3'

Figure 4. The sequences of six sgRNAs (green) targeting the gene sequences of the polymerase protein PA of HPAI H5 virus, NSP9 of IBV, and VP1 of IBDV, which will be evaluated for inhibition of virus replication first in cell culture and then in an IGA chicken line.

C.2.3. Generate an IGA chicken line resistant to virus infection by targeting the viral genome. The first two approaches to generate IGA chicken lines resistant to virus infection focus on host factors. An alternative method is to use sgRNAs and Cas13d to target viral RNA sequences that encode the viral polymerase proteins for each virus. This approach is independent of the studies in **Aim 1** and can be initiated in the first year of this proposal.

We have designed sgRNAs targeting the polymerase proteins of HPAI H5N1 virus (PA protein), IBV (NSP9), and IBDV (VP1) using the online tool from Integrated DNA Technologies. Six sgRNA sequences for each viral polymerase protein were selected based on high on-target

scores and low off-target scores (**Figure 4**). These sgRNAs were confirmed not to target chicken-specific sequences.

First, these sgRNAs will be tested against each respective virus in DF1 cells that stably express a chicken codon-optimized, high-fidelity variant of Cas13d. This enzyme will ensure high specificity for on-target RNA degradation, which is important for this sgRNA targeting approach to limit off-target effects (67). DF1-Cas13d cells will be transfected with individual sgRNAs. Twenty-four hours later, transfected cells will be infected with the appropriate virus at a low MOI, and cell culture supernatants will be harvested at 24, 48, and 72 h after infection. Virus titers will be determined to identify the sgRNAs with the best inhibition of virus replication.

We will repeat these growth kinetic studies with the top two sgRNAs for each virus with the greatest reduction in titers using different virus isolates to ensure breath of coverage. For example, we will evaluate the influenza-specific sgRNAs against HPAI H7N9, and H7N7 viruses along with different genotype clades of H5N1 viruses. The potential development of resistance to the sgRNA will also be evaluated by virus passaging up to five times in DF1-Cas13d cells expressing the virus-specific sgRNAs.

Once one sgRNA for each of the three viruses is identified from the cell culture studies, a piggyBac transposon construct will be engineered for *in ovo* manipulation of the chicken genome. The transposon construct will comprise the following DNA elements: 1) a gene expression cassette containing the CMV promoter, the coding sequences of chicken codon-optimized hfCas13d, and the late polyadenylation signal from simian virus 40 to allow for the transcription termination and polyadenylation of the hfCas13d mRNA; and 2) a sgRNA expression cassette containing the three sgRNAs each targeting a different viral polymerase sequence under the control of the characterized chicken U6 promoter (68). After introduction of the modified piggyBac transposon into chicken PGC culture, the integrity of the construct will be validated by Sanger sequencing.

Expected Outcomes, Potential Problems and Alternatives: Based on Dr. Wang's experience with techniques and procedures to generate gene-edited and transgenic animals in multiple species spanning from rodents to livestock, we do not anticipate technical difficulties in accomplishing the proposed experiments. Targeted sequencing, transcriptomics, and protein analysis will be performed to characterize the IGA cells to ensure no unwanted mutations at off-target sites in the DNA sequence are present, no alterations in the protein level of the targeted host factor, and no significant changes in the transcriptome.

We are taking three different approaches to improve our likelihood of generating one IGA chicken line that is resistant to HPAI H5N1 viruses and potentially a second line that is resistant to multiple avian viruses. However, there are potential issues that we may encounter with these approaches.

We will perform assays to identify pro-viral host factors whose knockout could potentially have negative effects on cell viability. However, the knockout of certain factors *in ovo* may still result in lethality during the development of the embryo, which would result in a failed attempt to generate an IGA chicken line. To help avoid any potential issues, we will use the huge amount of informative data that is available in the field for knockout mice and rats. The methods used to generate these knockout rodents may guide us on the exact type of gene editing method (premature stop codons, deletions of exons, or point mutations) we should use to produce viable IGA chicken lines.

If such data are not available on our specific host factor of interest, mechanistic studies are proposed in **Aim 1**. These mechanistic studies are aimed to identify the specific animo acids in the host protein that are essential for the interaction with the viral protein and responsible for the pro-viral function of the protein. Mutations at these specific amino acid positions should prevent the host-viral protein interaction thus inhibit virus replication but still producing a functional protein for the development of a healthy IGA chicken. It is possible that the mutations associated with inhibition of virus replication are also associated with lethality during embryo development, in which case that specific host factor will be abandon and the next highest ranked pro-viral host factors will be evaluated.

Antiviral ISG restriction factors are known to inhibit virus infections but can potentially cause an inflammatory response. The ISG retinoic acid-inducible gene I (RIG-1) is missing from chickens but conserved in mallard ducks. When RIG-1 was “re-installed” in an IGA chicken line, an avian influenza H7N1 virus infection in these transgenic chickens was more severe. Modified birds had higher virus replication compared to wild-type birds, potentially due to inflammation caused by virus infection. Continuous expression of antiviral ISG proteins in an IGA chicken line might result in a similar, more severe disease phenotype.

An alternative approach to an IGA chicken line with constitutively expressed ISGs is to generate a virus-inducible system. Lutz et al. generated an influenza-inducible reporter gene using the RNA polymerase I promoter and terminator cassette to express an RNA transcript of the luciferase reporter gene flanked by the untranslated regions of the influenza NP segment (69). Expression of luciferase was induced by influenza virus as early as six hours after infection in a dose-dependent manner. Following a similar approach, the RNA polymerase I regulator sequences along with the sequence of the untranslated regions of the influenza segment could be incorporated into the piggyBac-ISG transposon construct described in **C.2.2** resulting in an influenza virus inducible ISG-specific IGA chicken line if the constitutive expression of ISGs in chickens is detrimental. However, this alternative approach would not allow for a multi-virus resistant IGA chicken line.

Escape mutant viruses pose a significant challenge for interventions such as vaccines and antiviral drugs, and this concern extends to IGA chicken lines. To address this, we propose a strategy analogous to combination drug therapies, which are known to suppress viral escape effectively. Specifically, we plan to target multiple pro-viral and antiviral factors when generating IGA chicken lines. By simultaneously targeting two pro-viral proteins and factors involved in early steps of the virus life cycle, such as entry, we aim to prevent virus replication and the chance to generate mutant viruses. In addition, an IGA chicken line could be generated with three different sgRNAs targeting three different viral proteins in **C.2.3**. For example, an influenza-specific IGA chicken line could express individual sgRNAs against the three viral polymerase proteins, PA, PB1, and PB2. These approaches should reduce the likelihood of breakthrough infections and the emergence of escape mutant viruses, thereby enhancing the overall efficacy and resilience of the IGA chicken lines against viral infections.

Specific Aim 3. Characterize infection of IGA chicken lines with avian viruses.

Rationale: Once an IGA chicken line is generated and the gene modification is confirmed, we will need to confirm the degree of resistance to virus infection, first focusing on infection with a HPAI H5N1 virus. These studies will involve challenge experiments to assess viral replication, clinical outcomes, and pathogenicity in the IGA chickens compared to wild-type (WT) counterparts. Additionally, we will examine direct contact transmission from infected IGA chickens to naïve WT animals to evaluate virus shedding and spread. We will monitor for the occurrence of breakthrough infections and the emergence of viral mutants, providing crucial insights into the robustness of each IGA chicken line. This evaluation will not only validate the protective phenotype of the genetic modifications but also contribute to our understanding of evolutionary pressures exerted by gene-editing strategies.

Experimental Design for the Infection of Chickens with Influenza Virus: To compare HPAI H5N1 virus infection between groups of WT and IGA chickens, mixed-sex birds (5–10-day-old, antibody-negative for influenza virus) will be used with 12 birds in each group. Birds will be inoculated intranasally equally disturbed into each nare. A H5N1 clade 2.3.4.4b virus associated with the current outbreaks in poultry and dairy cows in the US will be used for the challenge studies (9). An initial study will be performed with an inoculation dose of 10^3 plaque-forming units (PFU) and then increased to a higher dose of 10^6 PFU if the chicken line is resistant to the lower dose.

Twenty-four hours after infection, six birds in each group will be paired with an equal number of mixed-sex, naïve WT birds to examine direct contact transmission. Signs of illness including lethargy and neurological signs will be monitored and mortality and morbidity will be recorded. Every day after infection, birds will be monitored for clinical illness, and oropharyngeal and cloacal cavity swabs will be collected from all birds for virus detection and sequencing. On day 14 after infection or exposure, any remaining birds will be humanely euthanized, and blood and tissue samples (trachea, lung, brain, kidney, and heart) will be collected. Samples will be divided and either

frozen for virus titrations or fixed in 4% paraformaldehyde for histopathology. Serum collected from the blood will be used to determine antibody seroconversion if infectious virus is not detected in the tissue samples. The other set of 6 infected birds in each group that are not paired will be humanely euthanized on day 2 after infection. The same tissue samples will be collected to examine virus replication and spread to the different organs in the body.

If an IGA chicken line is resistant to HPAI H5N1 virus infection, then we will repeat these studies to include two more HPAI or LPAI virus isolates (H7N9, H7N7, H5N2, H5N6, or H9N2). These additional studies will confirm the resistance profile of the IGA chicken line to different influenza viruses of importance in the poultry industry.

Experimental Design for the Infection of Chickens with Other Avian Viruses: In collaboration with Dr. Talaat, IGA chicken lines designed to be resistant to multiple avian viruses (developed in **C.2.2** and **C.2.3**) will be evaluated. Groups of WT and IGA chicken (mixed-sex birds, 5–10-day-old; 12 in each group) will be inoculated intranasally with IBV (Ark serotype) at 10^6 egg infectious dose 50 (EID50). Oropharyngeal swabs will be collected every day to examine virus shedding. Clinial signs of infection will also be recorded daily to monitor for watery feces, nasal exudates, and head shaking. On days three and six after infection, groups of WT and IGA chickens (6 birds in each group per timepoint) will be humanly euthanized. Tissue samples from the brain, thymus, trachea, heart, spleen, lungs, kidneys, liver, and bursa of Fabricius will be collected for histopathological analysis. Virus titers will also be examined in these tissues to determine if viral loads and spread are reduced in the IGA chicken line compared to WT birds. If an IGA chicken line is resistant to infection with HPAI viruses or IBV, a similar study design will be carried to infect chickens with a virulent, serotype 1 IBDV isolate (IM-IBDV).

Pathology in Non-infected Chickens: To ensure that intentional genomic alterations in animals do not result in unintended phenotypical changes, it is important to gather the necessary data outlined in the FDA guidelines for IGA animals early in the process. In addition to examining the pathology in WT and IGA chickens after virus infection, we will also perform comprehensive analyses on non-infected birds to begin the phenotypic characterization of the IGA chicken lines. These studies are crucial for any potential regulatory review process of successful IGA chicken lines resistant to virus infection in accordance with FDA guidance on the regulation of IGAs in animals.

Dr. Andres Mejia, Chief of Comparative Pathology, and his team will harvest tissues from both WT and IGA chickens for extensive molecular and histopathological analyses. They will employ various fixation methods and staining procedures to ensure thorough examination. The analysis of gross and histopathological features will contribute significantly to the phenotypic characterization of the IGA birds compared to their WT counterparts. This will include assessing tissue morphology (e.g., size of the bursa and thymus), gross pathology (organ weights), and detailed histopathological changes, such as immune cell infiltrates and any other morphological differences.

These detailed characterizations will help identify any unintended phenotypical changes resulting from the genomic alterations, thereby ensuring the IGA chickens meet the stringent safety and efficacy standards required for regulatory approval. If an IGA chicken line has abnormal phenotypical changes compared WT counterparts, that line could be terminated.

Statistically Analysis: In **Aims 1-2**, the outcome measurements including viral titers and pathogenicity will be analyzed using parametric (viral titers) or non-parametric (semi-quantitative severity scores) approaches, as appropriate based on model diagnostic evaluations. Parametric approaches will include t-tests, analysis of variance (ANOVA), and linear mixed effects analysis. Non-parametric approaches will include Wilcoxon rank sum tests, Kruskal Wallis tests, and linear/nonlinear regression analyses. Tukey's Honestly significant difference method will be used to control the type I error (to be < 0.05) when conducting multiple comparisons. The proposed sample sizes of animals for the various experiments are sufficient to detect anticipated biologically important differences with adequate power (>80%) at the two-sided 0.05 significant level. To compare transmission rates, we will use Fisher's exact test.

Expected Outcomes, Potential Problems and Alternatives: Infection with HPAI virus results in sudden and high mortality in chickens. WT birds exhibit severe lethargy, pronounced swelling around the eyes, and diarrhea, typically leading to death within two days of infection. WT birds also show high levels of virus shedding in swab samples and elevated viral titers in multiple organs, as HPAI virus efficiently replicates in tissues beyond the respiratory tract. Histopathological analysis will reveal severe pneumonia in the lungs of WT birds, characterized by a significant influx of inflammatory cells.

If we successfully modify the appropriate influenza-specific pro-viral host factor(s) in an IGA chicken line, we anticipate that the birds will exhibit complete protection against H5N1 virus infection, even at a high virus dose. We expect 100% survival with no detectable infectious virus in tissues or swabs from both infected and contact birds. Should any virus be detected in a swab sample, the viral titer is expected to be minimal, just above the limit of detection, and only detectable on a single sampling day rather than multiple days. Sequencing from these swab samples should reveal only the wild-type virus, with subpopulations of virus consistent with the sequence and population of the stock virus used for infection.

If a virus resistant to the gene modification emerges, a breakthrough infection may occur in the IGA chicken line. In such a scenario, we anticipate detecting replicating virus in swab samples across multiple days, with increasing viral titers and detectable infectious virus in tissues by day 2 after infection. If virus replication is robust, there may be a delay in the time of death of infected IGA chickens compared to their WT counterparts, as well as direct contact transmission to naïve WT cage mates.

We will sequence viruses from breakthrough infections to identify genetic changes associated with resistance to the IGA chicken line. This information will be useful to understand the mechanisms of resistance and inform future gene modification strategies and selection of host factors for additional targeting. Additionally, we will infect knockout cell lines developed in **Aim 1**, and primary cells from the IGA chicken line, using both WT and mutant viruses isolated from the breakthrough infection. These studies will allow us to examine growth kinetics in greater detail and assess the robustness of the breakthrough infection in cell culture.

Similar to the results from HPAI virus infection, we anticipate a lack of clinical signs of infection (watery feces or heading shaking) and a lack of detectable infectious virus in target tissues and swabs taken from multi-virus resistant IGA chickens after infection with IBV and IBDV. While WT birds infected with IBV and IBDV will exhibit high virus titers and virus-induced damage in the bursal, IGA birds should show no such damage on pathological examination if the IGA chicken line is resistant to infection.

If the initial IGA chicken line fails, alternative strategies will include targeting different host genes involved in viral replication, employing a combination of gene modifications, or targeting both pro-viral and antiviral factors; strategies will create a more resilient genetic barrier to breakthrough infection. Any negative data will be leveraged to refine our understanding of viral adaptation and host interactions, guiding iterative improvements in our gene-editing approach. This iterative process will strengthen our overall strategy and enhance the likelihood of developing an IGA chicken like with durable resistance to viral pathogens.

Summary: Collectively, the studies in this proposal aim to provide proof-of-concept data demonstrating the feasibility of generating at least one IGA chicken line with resistance to HPAI virus infection, with a potential second line having a multi-pathogen resistance phenotype. In addition, these IGA birds will be tested to ensure a lack of negative and adverse phenotypic traits that can arise in IGA birds due to the genetic modifications. Successful outcomes will pave the way for future efforts to expand the characterization of an IGA chicken line for additional pathogen resistance and characterization studies in response to FDA's guidance on the regulation of intentional genome alterations in animals.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001

Expiration Date: 01/31/2026

Use of Human Specimens and/or Data

Does any of the proposed research in the application involve human specimens and/or data *	❍ Yes ● No
Provide an explanation for any use of human specimens and/or data not considered to be human subjects research.	
Are Human Subjects Involved	❍ Yes ● No
Is the Project Exempt from Federal regulations?	❍ Yes ❍ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Other Requested Information	

Delayed Onset Studies

Delayed Onset Study#	Study Title	Anticipated Clinical Trial?	Justification
The form does not have any delayed onset studies			

VERTEBRATE ANIMALS

At the Utah State University (USU) performance site, genetically engineered chickens will be generated and initially raised for the infection experiments to be carried out at the University of Wisconsin-Madison (UW). All animals will be housed at AAALAC accredited facilities at both performance sites. Experiments will be conducted in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* (NIH publication no. 80-23).

Description of Procedures

Genome modifications (Aim 2): Dr. Wang's group at USU will develop genetically engineered chickens with CRISPR-mediated gene-editing and piggyBac-mediated transgenesis techniques using embryotic chicken eggs. After the establishment of the genetically engineered chicken lines, we will breed the flocks to produce experimental animals to be used for infection studies.

Infection studies (Aim 3): Birds will be infected with virus by intranasal administration while under anesthesia (isoflurane). Swabs do not require any anesthesia. A terminal blood by cardiac puncture will be performed while under deep anesthesia. If any animal develops a sign of illness (see criteria in **Section 4**), they will be euthanized (see below, **Method of Euthanasia**).

Specific Aim 1: Identify pro-viral and antiviral host factors that reduce infection of avian viruses in cell culture.

No animals

Specific Aim 2. Generate virus-resistant chicken lines by intentional genomic alterations.

Our goal is to generate two different virus-resistant chicken lines: an influenza-specific resistant line and a multi-virus resistant line. For each transgenic line, we anticipate using 60 chickens.

2 transgenic lines x 60 birds = <u>120 animals for Aim 2</u>

Specific Aim 3. Characterize infection of IGA chicken lines with avian viruses.

For the <u>influenza-specific resistant chicken line</u>, we plan to test three different influenza viruses at a low and high dose of virus.

6 IGA chickens and 6 wild-type chickens will be infected, and tissues collected at an earlier time point to examine virus replication = 12 birds are needed.

6 IGA chickens and 6 wild-type chickens will be infected, and paired with an equal number of naïve, wild-type birds to examine virus replication and survival = 24 birds are needed

A total of 36 birds are needed for one virus infection at one dose.

36 birds x 3 viruses x 2 doses = 216 birds for an influenza-specific resistant chicken line

Another 216 birds would be needed to evaluate a second influenza-specific resistant chicken line.

For a <u>multi-virus resistant chicken line</u>, we plan to test two influenza viruses (H5N1 and H7N9) for virus replication at an early timepoint after infection and transmission to naïve birds at only one dose of virus.

36 birds x 2 viruses x 1 dose = 72 birds for influenza infection of a multi-virus resistant chicken line

For infection of a multi-virus resistant chicken line, we will also test two other avian viruses, IBV and IBDV, at one dose of virus and two timepoints after infection (days 3 and 6), but no transmission component.

6 birds per group x 2 groups (IGA and WT birds) x 2 viruses x 2 timepoints = **48 birds for IBV and IBDV infection of a multi-virus resistant chicken line**

Summary of number of birds needed:

For infection studies of two IGA chicken lines: 216 birds (influenza-specific resistant line) + 120 birds (multi-virus resistant line) = <u>336 total birds for Aim 3.</u>

Justification

While work with human cells (both continuous cell lines and primary cells) is both useful and valuable, a complete immune system and necessary components are required to fully evaluate viral pathogenicity; a complex immune system cannot be reproduced in cell culture. Therefore, animal models are needed. Chickens are an excellent animal model for avian viruses including highly pathogenic avian influenza viruses. The numbers of animals proposed in this study will provide robust statistical confidence of our results.

Minimization of pain and distress

Minimization of pain and distress is a critical requirement of regulatory agencies governing the care and use of animals. The researchers working with animals are committed to animal management practices that reflect compliance with this standard. Therefore, to minimize pain and distress, animals will be restrained or anesthetized prior to procedures. Additionally, post-procedure, animals will be monitored with increased frequency until they return to their baseline state. Pre-procedure strategies for certain procedures include inhalant anesthetic or the potential use of injectables such as ketamine. Lidocaine will be used during collection of tracheal samples to relax the trachea and minimize discomfort.

Animals will be monitored at least once a day, at times twice a day during the experiment. With each health check, room conditions (temperature and humidity) will be recorded, and specific illness symptoms will be noted (e.g., rapid body weight loss, lethargy, hunched posture). Monitoring will be performed by staff and/or veterinarians or researchers. Researchers will communicate with veterinarians and staff if any question or need arises.

The illness experienced by the animals exposed to virus cannot be treated because any treatment would interfere with the outcome of the study, thus making the data uninterpretable (i.e., treatment will interfere with the course of disease and the identification of potential correlates of immunity). Nonsteroidal anti-inflammatory drugs cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis, as well as stabilization of lysosomal membranes that may reduce the release of cytokines. Opiates are not indicated since the pain resulting from virus infection is a non-specific malaise which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems.

Method of Euthanasia

If any animal becomes sick during the experiment (see Criteria and Score Sheets), the animal will be painlessly euthanized using methods that are consistent with those defined by the American Veterinary Medicine Association Panel on Euthanasia; death is not an endpoint in this study. The following methods are approved to euthanize animals: deep sedation with ketamine/dexdomitor followed by intravenous or intracardial injection of a euthanasia solution or exsanguination.

All animals will be observed 1-2 times a day for signs of illness, rapid breathing, rapid weight loss, inappetence, and impaired ambulation. If any of the animals are judged to be moribund by the following criteria, they will be anesthetized and then euthanized.

Scoring and Criteria for Euthanasia

Clinical signs to be observed and recorded for hamsters:

1. Ruffled feathers and/or hunched posture
2. Decreased activity
3. Weight loss greater than 10%, but $\leq$ 25%
4. Development of rapid breathing
5. Weight loss >25%, but $\leq$35%
6. Inability to remain upright
7. Weight loss >35%

Responses to birds exhibiting these clinical signs:

- Animals exhibiting clinical signs #1, #2, and #3 will be monitored carefully for further deterioration of health.
- Animals exhibiting clinical sign #4 and #5 will be kept in the study as long as they are active, eating, and hydrated.

  Monitoring will increase to 2 times per day; a morning check and a late afternoon check. If needed, body weights will be determined during the late afternoon check. If an animal shows continued diminishment of health at the afternoon check.
- Animals exhibiting clinical signs #6 and #7 will be considered to have reached the predetermined endpoint and will be euthanized immediately.

SELECT AGENT RESEARCH / BIOHAZARDS

Studies described in this proposal involve the use of highly pathogenic avian H5 and H7 influenza viruses which are Animal and Plant Health Inspection Service (APHIS)-identified Select Agents, as established under 9 CFR Part 121 of federal regulations. As such, all work with highly pathogenic viruses will be performed in (animal) enhanced biosafety level 3 (BSL-3+) or BSL-3 Agriculture (BSL-3 AG) containment facilities at the Influenza Research Institute, following approval by the local Institutional Biosafety Committee (IBC).

Though highly pathogenic avian influenza H5Nx viruses have been temporarily removed from the Select Agent registration, we will continue to treat these viruses as Select Agents since this temporary status may be revoked by the Federal agencies in 3-years or less. The Influenza Research Institute has extensive protocols in place for the handling of Select Agent viruses that includes animal research, inactivation procedures, shipping and receiving of viruses, and inventory of virus stocks. All procedures have been approved by the institutional and Federal Select Agent programs and are updated at least once a year.

Highly pathogenic avian influenza H7Nx viruses are still considered Select Agent viruses and all Select Agent regulations still apply for this group of viruses. Low pathogenic avian influenza viruses and non-influenza avian viruses in this proposal are classified as BSL-2 pathogens and not Select Agent viruses.

Facility Registration: The laboratories at the Influenza Research Institute are registered with the Select Agent Program (registration number #C20191112-101357). The laboratories have been inspected and approved for use by the Federal Select Agent Program.

Risk Assessment: Prior to initiating work with highly pathogenic avian influenza viruses, the University's Select Agent Program, in consultation with the principal investigator, performed a risk assessment and the results were reviewed by the university's Biosecurity Task Force.

Consistent with guidelines set forth by the National Institutes of Health (NIH) governing the use of recombinant DNA and those established in the Biosafety in Microbiological and Biomedical Laboratories (6th edition, 'BMBL6'; http://www.cdc.gov/biosafety/publications/bmbl6/BMBL.pdf), work with highly pathogenic avian influenza viruses will be conducted in (A)BSL-3+ or BSL-3 AG containment.

The IBC reviews research activities involving biologically hazardous materials and/or recombinant DNA molecules/organisms. The Office of Biological Safety and the IBC monitor and review research in the Dr. Halfmann's biological safety protocol which are approved by the University of Wisconsin-Madison's IBC after risk assessments are conducted by the Office of Biological Safety. In addition, the University of Wisconsin-Madison Select Agent Program and Biosecurity Task Force regularly reviews the research program and ongoing activities of the laboratory. The task force has a diverse expertise and provides support in the areas of biosafety, facilities, compliance, security, communication, and health. Members of the Biosecurity Task Force are in frequent contact with the principal investigator and laboratory personnel to provide oversight and assure biosecurity.

Biosafety and Biosecurity: The containment facilities at the Influenza Research Institute were designed to exceed standards outlined in the BMBL, and facility design and biocontainment practices are consistent with currently established guidelines.

Facility: The Influenza Research Institute houses a (b) (4) sq. ft. of (A)BSL3+ suite (including (b) (4) sq. ft. of animal holding, and (b) (4) sq. ft. of laboratory) and a (b) (4) sq. ft. of BSL3 AG suite (including (b) (4) sq. ft. of animal holding, (b) (4) sq. ft. of lab space, and a (b) (4) sq. ft. procedure room). Features of the (A)BSL-3+ suites include entry/exit through a shower change room, effluent decontamination, negative air-pressure laboratories, double-door autoclaves, double HEPA-filtered exhaust air, and gas decontamination ports. The BSL-3Ag suite designation is assigned to laboratories meeting strict, highly regulated biocontainment guidelines. The BSL3 AG suite features include all of those listed for BSL-3+, as well as HEPA-filtered supply and double-HEPA-filtered exhaust air, double-gasketed watertight and airtight seals, airtight dampers on all ductwork, and a structure that was pressure-decay tested during commissioning. The facility has a dedicated alarm system that monitors all building controls and sends alarms (>500 possible alerts). Redundancies and emergency resources are built-in to the facility; these include two air handlers, two compressors, two filters in each place filters are required, two effluent sterilization tanks, two power feeds to the building, an emergency generator in case of a power failure, and other physical containment measures that operate without power. Biosecurity measures include the use of multiple different measures to monitor and control facility entry and exit. Biosecurity monitoring of the facility is ongoing.

Access: Possession, use, and transfer of Select Agents is monitored according to guidelines set forth in 42 CFR 73, 9 CFR 121, and 7 CFR 331. All personnel working with these viruses have undergone Select Agent security risk assessment by the United States Criminal Justice Information Services Division. In addition, they complete rigorous biosafety, (A)BSL3-specific and Select Agent training and demonstrate competence in these areas before working independently on (A)BSL-3-level experiments. Refresher training is conducted on a regular basis. Dr. Halfmann participates in training sessions and emphasizes compliance to maintain safe operations and a responsible research environment.

Biocontainment: In addition to standard microbiological practices for work in (A)BSL-3 facilities, staff working with Select Agents wear disposable jumpsuits and powered air purifying respirators. Exit showers are required. The laboratory has also developed an occupational health plan, which follows the University of Wisconsin-Madison's Occupational Health Program. An incident response plan is in place to notify appropriate personnel in the event of a breach of containment or some other emergency. In addition, inventory reconciliation is performed on a monthly basis, and procedures are in place for reporting unaccounted for changes in the inventory.

Oversight: The research program, procedures, occupational health plan, documentation, security, and facilities are reviewed annually by the University of Wisconsin-Madison Responsible Official and at regular intervals by the Federal Select Agent Program as part of the University of Wisconsin-Madison Select Agent Program.

In summary, the biosafety, biocontainment, and biosecurity plans established in Dr. Halfmann's laboratory meet current regulations governing the possession, use, and transfer of Select Agents.

Dual-Use-Research-of-Concern (DURC) and Pathogens with Enhanced Pandemic Potential Considerations: Prior to the start of the work, the University of Wisconsin-Madison DURC Subcommittee will evaluate all studies for Category 1 (Dual Use Research of Concern) and Category 2 (Pathogens with Enhanced Pandemic Potential) in accordance with the United States Government Policy for Oversight of Dual Use Research of Concern and Pathogens with Enhanced Pandemic Potential. Most studies with wild-type H5 and H7 viruses are unlikely to be Category 1 or Category 2 under the new policy if these viruses are not known to be a pathogen with pandemic potential (as defined by the policy referenced above). Studies with mutant H5 viruses may be Category 1 or 2. If any of the proposed studies are determined to be Category 1 or 2, a risk-benefit analysis will be performed by the DURC Subcommittee and shared with the funding agency. In addition, Dr. Halfmann will work with the DURC Subcommittee to develop a risk mitigation plan that adequately addresses the risks of the proposed research. This too will be shared with the funding agency. In the event the proposed studies are determined to be subject to the *Department of Health and Human Services Framework for Guiding Funding Decision about Proposed Research Involving Potential Pandemic Pathogens* an additional review of the research strategy, risk-benefit analysis, risk mitigation plan, and other materials will be requested by the HHS Committee.

MULTI-PI LEADERSHIP PLAN

Drs. Halfmann and Wang will act as principal investigators (PI) on this multiple PI proposal. Drs. Kawaoka, Talaat, and Montoya will act as co-investigators. Based on virus inhibition data from cell culture studies, Drs. Halfmann and Wang will be responsible deciding what targets will be modified in intentional genomic altered chickens. Drs. Halfmann, Kawaoka and Talaat will oversee the initial virus data in cell culture for the identification and validation of targets and virus infection studies in the modified chickens. Dr. Wang's group will be responsible for the generation of the gene modified chickens. Dr. Montoya will work all members of the group to provide pathology data for both wild-type chickens and chickens with intentional genome alterations.

Dr. Halfmann will be contact PI, and as such will provide overall scientific leadership and direction for the project. Dr. Halfmann will closely coordinate with Drs. Kawaoka and Talaat on the design and overall management of the project and will assist with project oversight and design of experimental studies. All groups also will undertake appropriate quality assurance procedures to ensure that the project operates at a level required for success. The leadership team of Drs. Halfmann and Wang will be jointly responsible for overseeing research agendas, establishing research timelines and deliverables, monitoring progress, and preparing results for publication, and sharing results with stakeholders including the NIH, the scientific community, and the public. All these activities will be undertaken in consultation with the co-investigators, to ensure that all key personnel have input and contribute their expertise to the success of the project. As contact PI, Dr. Halfmann will be the primary point of contact with NIH on behalf of the Research Project. The leadership team will work with collaborators and NIH to assess progress, identify areas for improvement, and refine experimental strategies as new data become available.

Distribution of PI labor

Dr. Halfmann will coordinate the overall project, planning and facilitating meetings, and ensuring that each group's efforts are coordinated and efficient. His group will be responsible for the studies to identify and validate pro-viral and antiviral factors associated with high pathogenic avian influenza viruses in chicken cells. Dr. Talaat will be the primary investigator to carry out similar studies for the other avian viruses in collaboration with Drs. Halfmann and Kawaoka.

Dr. Wang will work closely with Drs. Halfmann, Kawaoka, and Talaat to make decisions on what targets will be modified in chickens based on the cell culture data along with other published data for other gene modified animals. All three virologists will coordinate infection studies in chickens once the gene edited birds are generated by Dr. Wang. Dr. Montoya efforts to identify any negative side effects of gene editing in the birds will be important to decide if certain gen modified chicken lines should be terminated.

All the project leads will collaborate to interpret data, devise alternate approaches when necessary, and write manuscripts. They will also work together to assemble and submit annual progress reports.

Communication plan

If this proposal is awarded, we will plan for 2 sets of regular meetings. The PIs will meet at least once monthly by Zoom to discuss progress of the overall project and plans for future activities. We will also plan regular monthly group meetings that include all investigators and their laboratory staff, which will give students and fellows the opportunity to share data and exchange ideas. Impromptu discussions via telephone or telepresence systems will likely occur on a more frequent basis. We anticipate that most members of this consortium will meet at least once annually. This meeting will include others from the greater veterinary communities at the School of Veterinary Medicine at the University of Wisconsin-Madison or at Utah State University.

Conflict resolution

If a conflict arises, the PIs will meet face-to-face and attempt to resolve the dispute. In the unlikely event of a

dispute that cannot be resolved to the satisfaction of both PIs, a meeting with all investigators (Halfmann, Wang, Kawaoka, Talaat, and Montoya) will be held to make a final decision based on the available data.

Change in PI location / PI incapacitation

If a PI moves, attempts will be made to transfer the relevant portion of the research to the new institution. If a PI is unable to carry out his duties for any reason, the remaining PI will have decision-making authority from that time forward. This includes the authority to enlist a new PI or other senior personnel as a replacement.

Intellectual property

Ownership of inventions conceived and reduced to practice in the performance of this project shall be determined by U.S. patent laws and subject to 35 U.S.C. secs. 200-212 (the “Bayh-Dole Act,” P.L. 96-517 and 98-620) and NIH policies on patents and inventions. PIs will disclose such inventions to the Wisconsin Alumni Research Foundation (WARF), which WARF will hold in confidence so as to not affect the patentability of such inventions.

Publication policy

Authorships on publications will be based on the relative scientific contributions of the PIs and key personnel. If the PIs cannot unanimously resolve an authorship issue, the policies outlined above for “Conflict Resolution” will be employed.

Budgets

If any spending or rebudgeting issues arise, they will first be addressed informally by the PIs. If this does not resolve the issue to the PIs’ satisfaction, the conflict resolution plan will be activated.

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STATEMENT OF INTENT TO ESTABLISH A SUB-AGREEMENT

UW Investigator: Peter Halfmann

Collaborating Institution Investigator:

Dates: 10/1/2024-09/30/2029

Project Title: The Building Resistance Against Virus Entities (BRAVE) Innovation Center

Total project amount:

The appropriate programmatic and administrative personnel of each institution involved in this grant application are aware of the sponsor's grant policy and are prepared to establish the necessary inter-institutional agreement(s) consistent with that policy.

Utah State University
Institution

Zhongde Wang 07/22/2024
Signature of PI

Dr. Zhongde Wang
Typed Name of PI

Mikayla Cook 07/22/2024
Signature of Authorized Official Date

Mikayla Cook, Proposal Analyst II
Typed Name & Title

Form Approved Through 02/28/2023 OMB No. 0925-0001

Department of Health and Human Services
Public Health Services

Grant Application

Do not exceed character length restrictions indicated.

LEAVE BLANK—FOR PHS USE ONLY.

Type	Activity	Number
Review Group		Formerly
Council/Board (Month, Year)		Date Received

1. TITLE OF PROJECT *(Do not exceed 81 characters, including spaces and punctuation.)*
The Building Resistance Against Virus Entities (BRAVE) Innovation Center

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☐ NO ■ YES
(If "Yes," state number and title)
Number: PAR-24-251 Title: Animal and Veterinary Innovation Centers (U18)

3. PROGRAM DIRECTOR/PRINCIPAL INVESTIGATOR

3a. NAME (Last, first, middle)	3b. DEGREE(S)	3h. eRA Commons User Name
Wang, Zhongde	PhD	WANGZHONGDE

3c. POSITION TITLE
Professor

3d. MAILING ADDRESS *(Street, city, state, zip code)*
9825 Old Main Hill
Logan, UT 84322-9825

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
College of Agriculture and Applied Sciences

3f. MAJOR SUBDIVISION
Animal, Dairy, and Veterinary Sciences

3g. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: 435-797-9668 FAX:

E-MAIL ADDRESS:
zonda.wang@usu.edu

4. HUMAN SUBJECTS RESEARCH	4a. Research Exempt	If "Yes," Exemption No.
■ No ☐ Yes	☐ No ☐ Yes	

4b. Federal-Wide Assurance No.	4c. Clinical Trial	4d. NIH-defined Phase III Clinical Trial
	☐ No ☐ Yes	☐ No ☐ Yes

5. VERTEBRATE ANIMALS ☐ No ■ Yes 5a. Animal Welfare Assurance No. A3801-01

6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)*		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT	
From	Through	7a. Direct Costs ($)	7b. Total Costs ($)	8a. Direct Costs ($)	8b. Total Costs ($)
10/01/2024	09/30/2029	$200,000	$292,000	$1,350,000	$1,948,000

9. APPLICANT ORGANIZATION
Name Utah State University
Address 1415 Old Main Hill
Logan, UT 84322-1415

10. TYPE OF ORGANIZATION
Public: → ☐ Federal ■ State ☐ Local
Private: → ☐ Private Nonprofit
For-profit: → ☐ General ☐ Small Business
☐ Woman-owned ☐ Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER
SPE2YDWHDYU4
DUNS NO. 072983455 Cong. District UT-001

12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name Mikayla Cook
Title Grant and Contract Officer
Address 1415 Old Main Hill
Logan, UT 84322-1415
Tel: 435-797-0943 FAX: 435-797-3543
E-Mail: mikayla.cook@usu.edu

13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name Mikayla Cook
Title Grant and Contract Officer
Address 1415 Old Main Hill
Logan, UT 84322-1415
Tel: 435-797-0943 FAX: 435-797-3543
E-Mail: mikayla.cook@usu.edu

14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 13. *(In ink. "Per" signature not acceptable.)* Mikayla Cook

DATE 07/22/2024

RESOURCE SHARING PLAN

Research tools. As for our plan to share materials and our management of intellectual property, we will adhere to the NIH Grant Policy on Sharing of Unique Research Resources including the Sharing of Biomedical Research Resources Principles and Guidelines for Recipients of NIH Grants and Contracts. All research tools including viruses, anti-sera, monoclonal antibodies, tissues, plasmids, cell lines generated by this project will be distributed freely or deposited into a repository/stock center making them available to the broader research community, either before or immediately after publication. Our lab has demonstrated its commitment to sharing by providing several viral stocks, cell lines, antibodies, and plasmids. If we assume responsibility for distributing the newly generated resources, we fill requests in a timely fashion. In addition, we will provide relevant protocols and published genetic and phenotypic data upon request. Material transfers will be made with no more restrictive terms than in the Simple Letter Agreement (SLA) or the Uniform Biological Materials Transfer Agreement (UBMTA) and without reach through requirements. Should any intellectual property arise which requires a patent, we will ensure that the technology (materials and data) remains widely available to the research community in accordance with the NIH Principles and Guidelines document.

Sharing Model Organism. This proposed project will develop multiple genetically engineered chicken lines for viral infection studies. After initial publications, all the reagents generated from this project and genetically engineered chicken lines will be made available to all researchers and the poultry industry upon requests under MTAs through Utah State University. We also have a substantial track record of distributing genetically engineered animal models—several of the genetic hamster and guinea pig models developed in the Wang lab have been provided to more than ten research institutions (including the NIH).

NIH Generated message:

The Other Plan(s) attachment included with the application is not evaluated during the peer review process but will be evaluated prior to a funding decision. Although part of the official submission, the attachment is maintained as a separate document in eRA Commons viewable by authorized users and is not part of this assembled application.

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

All researchers involved in this project will ensure that the highest quality of science, public accountability, and social responsibility in the conduct of science are maintained throughout the work**.** The overall goal is to ensure that the underlying scientific foundation of the project from conception to completion is scientifically sound.

Antibodies. Antibodies will be purchased from commercial vendors who confirm their authenticity. All antisera and antibodies generated in-house will be tested for their authenticity and specificity.

Cell lines. The cell lines used in our studies have been or will be purchased from commercial vendors (e.g., ATCC) who confirm the authenticity of the cells. All cell lines will be tested for the absence of mycoplasma each month while in use. Lines will be discarded after 15-10 passages.

Primers, CRISPR guide RNAs, and plasmids. Primers, CRISPR guide RNAs, and plasmids will be synthesized or provided by established vendors, such as GeneScript, Addgene and IDT DNA Technologies. All plasmids either commercial or in-house will be sequenced after their isolation, after each PCR amplification, and after other modifications (such as site-directed mutagenesis) to verify the appropriate sequence.

Specific pathogen-free (SPF) eggs. SPF chicken eggs will be purchased from the leading chicken egg providers, such as AVS Bio (former Charles River Avian Vaccine service) or Lohmann breeders, depending on the chicken breeds needed. The commercial vendors confirm the authenticity of the eggs they supply.

Genetically engineered chickens and eggs. PCR-RFLP (PCR-fragment length polymorphism) assays and Sanger sequencing will be used to confirm the genotypes of the breeders. Experimental animals will be produced by pairing the breeders. PCR-RFLP assays and Sanger sequencing will also be used to confirm the genotypes of the produced chickens.

Viruses. Stocks of viruses will be tested to ensure that they are negative for mycoplasma contamination. Sequencing of the viral genome will authenticate the identity of the virus and ensure that no unwanted mutations occurred during production.

Other reagents. Specific chemicals and biologicals (including tissue culture media, fetal bovine sera, buffers, PCR primers, enzymes and probes, and molecular biology reagents) will be purchased from commercial sources and stored and used according to the information provided by each manufacturer. Chemicals purchased commercially come with an authentication sheet stating the purity of the chemical. While we do not expect to find inconsistencies caused by commercially acquired reagents, a detailed record of the lot/batch numbers will help us to identify problematic reagents if inconsistent results are obtained.

Notice of Award
FAIN# U18FD008464
Federal Award Date
09/16/2024

Recipient Information

1. Recipient Name
UNIVERSITY OF WISCONSIN SYSTEM
21 N PARK ST STE 6301
MADISON, WI 53715

2. Congressional District of Recipient
02

3. Payment System Identifier (ID)
1396006492A1

4. Employer Identification Number (EIN)
396006492

5. Data Universal Numbering System (DUNS)
161202122

6. Recipient's Unique Entity Identifier
LCLSJAGTNZQ7

7. Project Director or Principal Investigator
Peter Joseph Halfmann, PHD (Contact)

peter.halfmann@wisc.edu
608-262-2019

8. Authorized Official
Darlene A Holte

Federal Agency Information

9. Awarding Agency Contact Information
Kimberly Pendleton

FOOD AND DRUG ADMINISTRATION
kimberly.pendleton@fda.hhs.gov
240-402-7610

10. Program Official Contact Information
Megan Miller

FOOD AND DRUG ADMINISTRATION
megan.miller@fda.hhs.gov

Federal Award Information

11. Award Number
1U18FD008464-01

12. Unique Federal Award Identification Number (FAIN)
U18FD008464

13. Statutory Authority
PHS Act,Sec 1706,42 USC 300u-5,as amended;Sec2(d),PL 98-551

14. Federal Award Project Title
The Building Resistance Against Viral Entities (BRAVE) Innovation Center

15. Assistance Listing Number
93.103

16. Assistance Listing Program Title
Food and Drug Administration Research

17. Award Action Type
New Competing

18. Is the Award R&D?
Yes

Summary Federal Award Financial Information	
19. Budget Period Start Date 09/15/2024 **– End Date** 08/31/2025	
20. Total Amount of Federal Funds Obligated by this Action	$693,673
20 a. Direct Cost Amount	$693,673
20 b. Indirect Cost Amount	$0
21. Authorized Carryover	
22. Offset	
23. Total Amount of Federal Funds Obligated this budget period	$693,673
24. Total Approved Cost Sharing or Matching, where applicable	$0
25. Total Federal and Non-Federal Approved this Budget Period	$693,673
26. Project Period Start Date 09/15/2024 **– End Date** 08/31/2029	
27. Total Amount of the Federal Award including Approved Cost Sharing or Matching this Project Period	$693,673

28. Authorized Treatment of Program Income
Additional Costs

29. Grants Management Officer - Signature
Kimberly Pendleton

30. Remarks

PLEASE REVIEW ALL TERMS AND CONDITIONS IN SECTIONS III AND IV. "Terms and Conditions," is acknowledged by the recipient when funds are drawn down or otherwise requested from the grant payment system.

SECTION I – AWARD DATA – 1U18FD008464-01

Award Calculation (U.S. Dollars)	
Salaries and Wages	(b) (4)
Fringe Benefits	
Personnel Costs (Subtotal)	
Equipment	$16,000
Travel	$10,000
Other	$418,470
Federal Direct Costs	$693,673
Approved Budget	$693,673
Federal Share	$693,673
TOTAL FEDERAL AWARD AMOUNT	$693,673
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$693,673

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$693,673	$693,673
2	$914,937	$914,937
3	$914,937	$914,937
4	$914,937	$914,937
5	$914,937	$914,937

* Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project.

Fiscal Information:

Document Number:	UFD008464A
PMS AccountType:	P(Subaccount)
Fiscal Year:	2024

IC	CAN	2024	2025	2026	2027	2028
FD	6C8R3JS	$693,673	$914,937	$914,937	$914,937	$914,937

* Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project.

FDA Administrative Data:
PCC: / **OC**: 4141 / **Processed**: 09/16/2024

SECTION II – PAYMENT/HOTLINE INFORMATION – 1U18FD008464-01

Acceptance of this award including the "Terms and Conditions" is acknowledged by the recipient when funds are drawn down or otherwise obtained from the grant payment system.

Grant payments will be made available through the DHHS Payment Management System (PMS). Please go to https://pms.psc.gov/ to find more information on user access, payment, reporting and FAQs.

Inquiries should be directed to:

ONE-DHHS—the PMS Help Desk, providing assistance to all system users. Support is available Monday - Friday from 7 a.m. to 9 p.m. ET (except Federal Holidays): 1-877-614-5533 or email PMSSupport@psc.gov.

The HHS Inspector General (IG) maintains a toll-free telephone number, 1-800-447-8477, for receiving information concerning fraud, waste, or abuse under grants and cooperative agreements. Such reports are

kept confidential, and callers may decline to give their names if they choose to remain anonymous.

SECTION III – TERMS AND CONDITIONS – 1U18FD008464-01

Acceptance of this award including the "Terms and Conditions" is acknowledged by the recipient when funds are drawn down or otherwise obtained from the grant payment system.

Failure to adhere and comply with the terms and conditions of award, may result in disallowances, enforcement actions such suspension, termination, withholding of support and/or conversion to a reimbursement payment method.

This award is based on the application submitted to, and as approved by, FDA on the above-title project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Grant Award.
b. The restrictions on the expenditure of federal funds in appropriations acts to the extent those restrictions are pertinent to the award.
c. 2 CFR Part 200 and 45 CFR Part 75, currently in effect or implemented during the period of the award.
d. The HHS Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. The Funding Opportunity Announcement in which this award is issued under.
g. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

This award has been assigned the Federal Award Identification Number (FAIN) U18FD008464. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Expanded Authorities:
This award is covered under Expanded Authorities. An unobligated balance (carryover) may be carried over from one budget period to any subsequent budget period for allowable costs within the original scope of the project without Grants Management Officer prior approval. The recipient is required to indicate as part of the grant's annual progress report (RPPR), whether any estimated unobligated balance (including prior-year carryover) is expected (regardless of whether the percentage of unobligated funds is over or under 25% of the current year's total approved budget) and indicate the carryover amount in the Remarks section of the annual FFR. Carryover from one competitive segment to a new competitive segment will not be allowed under expanded authorities. A recipient may perform a one-time no cost extension (NCE) of the expiration date of the award (Project Period) of up to 12 months in eRA Commons without prior approval. The NCE request must be made prior to the end of the current project period end date but preferably no later than 30 days before the expiration date. The one-time extension may not be exercised to extend Budget Periods, or merely for the purpose of using unobligated balances, nor may recipients extend project periods previously extended by the FDA awarding office. If a second NCE is required beyond the initial Expanded Authority extension, a prior approval request must be submitted to FDA's Grants Management Office.

Reporting Requirements:
All FDA grants require both Financial and Performance reporting.

Financial Reporting:

A. Financial Expenditure Reports

A required Federal Financial Report (FFR) must be submitted annually. All annual FFRs must be submitted electronically using the Payment Management System (PMS). This includes all initial FFRs being prepared for submission and any revised FFRs being submitted or re-submitted to FDA. Paper expenditure/FFR reports will not be accepted.

Annual FFRs must be submitted for each budget period no later than 90 days after the end of the calendar quarter in which the budget period ended. The reporting period for an annual FFR will be that of the budget period for the particular grant; however, the actual submission date is based on the calendar quarter. If a grant is under expanded authorities, the recipient must indicate the carryover amount in Section 12. Remarks of the annual FFR.

If the budget period end date falls within:	**then annual FFR is due by:**
January, February, March	June 30th
April, May, June	September 30th
July, August, September	December 31st
October, November, December	March 31st

Performance Progress Reporting:

When multiple years (more than one budget period) are involved, recipients will be required to submit the Research Performance Progress Report (RPPR) annually as required in the Notice of Award. Annual RPPRs must be submitted using the RPPR module in eRA Commons. The annual RPPR must include a detailed budget. Annual RPPRs are due no later than 60 days prior to the start of the next budget period.

Failure to submit timely reports may affect future funding. Additional Financial and Performance Progress reports may be required for this award. Any additional reporting requirements will be listed under Section IV – Special Terms and Condition of the Notice of Award.

Salary Caps:
None of the funds in this award shall be used to pay the salary of an individual at a rate in excess of the current Executive Level II of the Federal Executive Pay Scale.

Certificates of Confidentiality – 42 U.S.C. 241(d)
Recipients are responsible for complying with all requirements to protect the confidentiality of identifiable, sensitive information that is collected or used in biomedical, behavioral, clinical, or other research (including research on mental health and research on the use and effect of alcohol and other psychoactive drugs) funded wholly or in part by the Federal Government. See 42 U.S.C. 241(d). All research funded by FDA, in whole or in part, that is within the scope of these requirements is deemed to be issued a "Certificate of Confidentiality" through these Terms and Conditions. Certificates issued in this manner will not be issued as a separate document.

Recipients are expected to ensure that any investigator or institution not funded by FDA who receives a copy of identifiable, sensitive information protected by these requirements, understand they are also subject to the requirements of 42 U.S.C. 241(d). Recipients are also responsible for ensuring that any subrecipient that receives funds to carry out part of the FDA award involving a copy of identifiable, sensitive information protected by these requirements understand they are also subject to subsection 42 U.S.C. 241(d).

Acknowledgment of Federal Support:
When issuing statements, press releases, publications, requests for proposal, bid solicitations and other documents --such as tool-kits, resource guides, websites, and presentations (hereafter "statements")-- describing the projects or programs funded in whole or in part with FDA federal funds, the recipient must clearly state:

1. the percentage and dollar amount of the total costs of the program or project funded with federal money; and,
2. the percentage and dollar amount of the total costs of the project or program funded by non-governmental sources.

When issuing statements resulting from activities supported by FDA financial assistance, the recipient entity must include an acknowledgement of federal assistance using one of the following statements.

If the FDA Grant or Cooperative Agreement is NOT funded with other non-governmental sources:

> This [project/publication/program/website, etc.] [is/was] supported by the Food and Drug Administration (FDA) of the U.S. Department of Health and Human Services (HHS) as part of a

> financial assistance award [FAIN] totaling $XX with 100 percent funded by FDA]/HHS. The contents are those of the author(s) and do not necessarily represent the official views of, nor an endorsement, by FDA/HHS, or the U.S. Government.

If the FDA Grant or Cooperative Agreement IS partially funded with other nongovernmental sources:

> This [project/publication/program/website, etc.] [is/was] supported by the Food and Drug Administration (FDA) of the U.S. Department of Health and Human Services (HHS) as part of a financial assistance award [FAIN] totaling $XX with XX percentage funded by FDA/HHS and $XX amount and XX percentage funded by non-government source(s). The contents are those of the author(s) and do not necessarily represent the official views of, nor an endorsement, by FDA/HHS, or the U.S. Government.

The federal award total must reflect total costs (direct and indirect) for all authorized funds (including supplements and carryover) for the total competitive segment up to the time of the public statement. Any amendments by the recipient to the acknowledgement statement must be coordinated with FDA. If the recipient plans to issue a press release concerning the outcome of activities supported by FDA financial assistance, it should notify FDA in advance to allow for coordination.

Additional prior approval requirements pertaining to Acknowledgement of Federal Support, publications, press statements, etc. may be required, and if applicable, will be listed under Section IV – Special Terms and Condition of the Notice of Award.

Prior Approval:
All prior approval requests must be submitted using the Prior Approval module in eRA Commons. Any requests involving budgetary issues must include a new proposed budget and a narrative justification of the requested changes. If there are any questions regarding the need or requirement for prior approval for any activity or cost, the recipient is to contact the assigned Grants Management Specialist prior to expenditure of funds.

For grant awards not covered under Expanded Authorities, Carryover and No Cost Extension (NCE) requests will require prior approval. All Carryover and NCE requests should be submitted using the Prior Approval module in eRA Commons. ****Please review the section on Expanded Authorities to determine if this award is covered/not covered under Expanded Authorities and whether prior approval is needed for carryover and no cost extension requests.****

The following activities require prior approval from FDA on all awards:

1. Change in Recipient Organization
2. Significant Rebudgeting
3. Change in Scope or Objectives
4. Deviation from Terms and Conditions of Award
5. Change in Key Personnel which includes replacement of the PD/PI or other key personnel as specified on the NoA.
6. Disengagement from the project for more than three months, or a 25 percent reduction in time devoted to the project, by the approved PD/PI. No individual may be committed to more than 100% professional time and effort. In the event that an individual's commitment exceeds 100%, the recipient must make adjustments to reduce effort. For FDA-sponsored projects, significant reductions in effort (i.e., in excess of 25% of the originally proposed level of effort) for the PD/PI and key personnel named on named on this Notice of Award must receive written prior approval from FDA.

Additional prior approval requirements may be required for this award, and if applicable, will be listed under Section IV – Special Terms and Condition of the Notice of Award.

Audits and Monitoring:

Audit Requirements:

1. Recipients of Federal funds are subject to annual audit requirements as specified in 45 CFR 75.501 (https://www.ecfr.gov/cgi-bin/retrieveECFR?gp=1&SID=8040c4036b962cc9d75c3638dedce240&ty=HTML&h=L&r=PART&n=pt45.1.75#se45.1.75_1501). Recipients should refer to this regulation for the current annual Federal fund expenditure threshold level which requires audit.
2. Foreign recipients are subject to the same audit requirements as for-profit organizations (specified in 45 CFR 75.501(h) through 75.501(k).
3. For-profit and foreign entities can email their audit reports to AuditResolution@hhs.gov or mail them to the following address:

   U.S. Department of Health and Human Services
   Audit Resolution Division, Room 549D
   Attention: Robin Aldridge, Director
   200 Independence Avenue, SW
   Washington, DC 20201

Monitoring:

Recipients are responsible for managing the day-to-day operations of grant-supported activities using their established controls and policies, as long as they are consistent with Federal, DHHS and FDA requirements. However, to fulfill their role in regard to the stewardship of Federal funds, FDA monitors our grants to identify potential problems and areas where technical assistance might be necessary. This active monitoring is accomplished through review of reports and correspondence from the recipient, audit reports, site visits, and other information available to FDA.

1. **Desk review:** FDA grants monitoring specialists will periodically reach out to recipients to request information for the completion of desk reviews. Requested information may include:
   - Policies and procedures
   - List of grant expenditures
   - Accounting records
   - Supporting documents (e.g., invoices, receipts, paystubs, timesheets, contracts, etc.)
   - Financial statements
   - Audit reports
   - Other related documentation
2. **Site visits:** FDA will conduct site visits when necessary and will notify the recipient with reasonable advance notice of any such visit(s).
3. **Foreign entities:** All Foreign entities are subject to the same monitoring requirements as domestic entities. Foreign entities covered under immunity Executive Orders will provide supporting documents for monitoring requirements unless such an action is a violation of the Executive Orders. Recipients may discuss with the FDA to come up with an alternate approach to satisfy the award monitoring requirements.

All recipients will make reasonable efforts to resolve issues found, including audit findings. Successful resolutions to issues are important as they are part of the grant performance review. All recipients are responsible for submitting all requested information in an expeditious manner. **Failure to submit timely reports and/or respond to inquiries from FDA may affect future funding or enforcement actions, including withholding, or conversion to a reimbursement payment method.**

Financial Conflict of Interest (FCOI):
This award is subject to the Financial Conflict of Interest (FCOI) regulation at 42 CFR Part 50 Subpart F.

Closeout Requirements (when applicable):
A Final Research Performance Progress Report (FRPPR), Final Invention Statement (FIS) HHS-568 (if applicable), Tangible Personal Property Report SF-428 (if applicable), and Statement of Disposition of

Equipment (if applicable) must be submitted within 120 days after the expiration date of the project period. All closeout documents must be submitted electronically in eRA Commons.

The Final Federal Financial Report (FFFR) SF-425 must be submitted in the Payment Management System (PMS) within 120 days after the expiration date of the project period. Recipients have 90 days after the project period end date to liquidate all obligations in PMS. All obligations must be liquidated prior to the submission of the Final FFR. The Final FFR must indicate the exact balance of unobligated funds and may not reflect unliquidated obligations. There must be no discrepancies between the Final FFR expenditure data and FFR cash transaction data in the Payment Management System (PMS). The expended funds reported on the Final FFR must exactly match the disbursements and the charge advances in PMS. It is the recipient's responsibility to reconcile reports submitted to PMS and to the FDA.

Program Income:
The recipient is required to report any Program Income generated during the Project Period of this grant. Except for royalty income generated from patents and inventions, the amount and disposition of Program Income must be identified on lines 10 (l), (m), (n), and (o) of the recipient's Federal Financial Report (FFR) SF-425.

Examples of Program Income include (but are not limited to): fees for services performed during the grant or sub-grant period, proceeds from sale of tangible personal or real property, usage or rental fees, patent or copyright royalties, and proceeds from the sale of products and technology developed under the grant.

Any Program Income generated during the Project Period of this grant by the recipient or sub-recipient will be treated as identified below.

Treatment of Program Income:
Additional Costs

Prohibition on certain telecommunications and video surveillance services or equipment:
(a) As described in CFR 200.216, recipients and subrecipients are prohibited to obligate or spend grant funds (to include direct and indirect expenditures as well as cost share and program) to:

(1) Procure or obtain,
(2) Extend or renew a contract to procure or obtain; or
(3) Enter into contract (or extend or renew contract) to procure or obtain equipment, services, or systems that use covered telecommunications equipment or services as a substantial or essential component of any system, or as critical technology as part of any system. As described in Pub. L. 115-232, section 889, covered telecommunications equipment is telecommunications equipment produced by Huawei Technologies Company or ZTE Corporation (or any subsidiary or affiliate of such entities).

i. For the purpose of public safety, security of government facilities, physical security surveillance of critical infrastructure, and other national security purposes, video surveillance and telecommunications equipment produced by Hytera Communications Corporation, Hangzhou Hikvision Digital Technology Company, or Dahua Technology Company (or any subsidiary or affiliate of such entities).
ii. Telecommunications or video surveillance services provided by such entities or using such equipment.
iii. Telecommunications or video surveillance equipment or services produced or provided by an entity that the Secretary of Defense, in consultation with the Director of the National Intelligence or the Director of the Federal Bureau of Investigation, reasonably believes to be an entity owned or controlled by, or otherwise, connected to the government of a covered foreign country.

Other:
This award is subject to the requirements of 2 CFR Part 25 for institutions to maintain an active registration in the System of Award Management (SAM). Should a consortium/subaward be issued under this award, a requirement for active registration in SAM must be included.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the

award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75.

You must administer your project in compliance with federal civil rights laws that prohibit discrimination on the basis of race, color, national origin, disability, age and, in some circumstances, religion, conscience, and sex (including gender identity, sexual orientation, and pregnancy). This includes taking reasonable steps to provide meaningful access to persons with limited English proficiency and providing programs that are accessible to and usable by persons with disabilities. The HHS Office for Civil Rights provides guidance on complying with civil rights laws enforced by HHS. Please see https://www.hhs.gov/civil-rights/for-providers/provider-obligations/index.html and https://www.hhs.gov/civil-rights/for-individuals/nondiscrimination/index.html

- You must take reasonable steps to ensure that your project provides meaningful access to persons with limited English proficiency. For guidance on meeting your legal obligation to take reasonable steps to ensure meaningful access to your programs or activities by limited English proficient individuals, see https://www.hhs.gov/civil-rights/for-individuals/special-topics/limited-english-proficiency/fact-sheet-guidance/index.html and https://www.lep.gov.
- For information on your specific legal obligations for serving qualified individuals with disabilities, including providing program access, reasonable modifications, and taking appropriate steps to provide effective communication, see http://www.hhs.gov/ocr/civilrights/understanding/disability/index.html.
- HHS funded health and education programs must be administered in an environment free of sexual harassment, see https://www.hhs.gov/civil-rights/for-individuals/sex-discrimination/index.html.
- For guidance on administering your project in compliance with applicable federal religious nondiscrimination laws and applicable federal conscience protection and associated anti-discrimination laws, see https://www.hhs.gov/conscience/conscience-protections/index.html and https://www.hhs.gov/conscience/religious-freedom/index.html.

SECTION IV – FD Special Terms and Condition – 1U18FD008464-01

Special Terms and Conditions for PAR 24 251 - 1 U18 FD008464-01

Please submit correct F&A breakdown to Kimberly Pendleton@ Kimberly.Pendleton@fda.hhs.gov

The administrative and funding instrument used for this program will be the cooperative agreement, an assistance mechanism (rather than an acquisition mechanism), in which substantial FDA programmatic involvement with the awardees is anticipated during the performance of the activities. Under the cooperative agreement, FDA's purpose is to support and stimulate the recipient's activities by involvement in and otherwise working jointly with the award recipients in a partnership role; it is not to assume direction, prime responsibility, or a dominant role in the activities. Consistent with this concept, the dominant role and prime responsibility resides with the awardees for the project, although specific tasks and activities may be shared among the awardees and FDA as defined below.
Substantive involvement may include, but is not limited to, the following:
(1) General guidance on activities
(2) Site visits from FDA staff
(3) Facilitating cooperation and joint advancement of priorities among multiple grant recipients.
The following conditions of the award will apply to all funded applicants

and must be maintained throughout the cooperative agreement.

Principal Investigator Rights and Responsibilities
The PD(s)/PI(s) will have the primary responsibility for the scientific, technical, and programmatic aspects of the cooperative agreement and for day-to-day management of the project or program. The PD(s)/PI(s) will maintain general oversight for ensuring compliance with the financial and administrative aspects of the award, as well as ensuring that all staff have sufficient clearance and/or background checks to work on this project or program. This individual will work closely with designated officials within the recipient organization to create and maintain necessary documentation, including both technical and administrative reports; prepare justifications; appropriately acknowledge Federal support in publications, announcements, news programs, and other media; and ensure compliance with other Federal and organizational requirements.

Additional conditions:
1. Participate in regularly scheduled status/check in meetings.
2. Cooperate with other Innovation Centers and identify common procedures, as applicable.
3. Participate in site visits or attend meetings as requested by the FDA. A portion of the budget may be reserved for such travel.43. The awardees will provide FDA any samples if requested by FDA.
4. The awardees will provide FDA any data obtained from work under the cooperative agreement as requested. Data will be posted to appropriate or available data repositories as requested by FDA.
5. FDA may also request data be made available through speaking engagements and publications, presentations at scientific symposia and seminars, while making sure that confidentiality and privacy of the data is protected.
6. Any publication or oral presentation of the results of funded work must undergo FDA review and approval process. This process can take 30-90 days.
7. The grantee must provide quarterly reports as requested, to include status and budget updates.

FDA Responsibilities:
An FDA Project Officer (PO) will have substantial programmatic involvement. The PO is the official responsible for monitoring the programmatic, scientific, and/or technical aspects of assigned applications and grants. The PO’s responsibilities include, but are not limited to, post-award monitoring of project/program performance, including review of progress reports and making site visits; and other activities complementary to those of the Grants Management Officer (GMO). The PO and the GMO work as a team in many of these activities.

FDA will provide technical monitoring and/or direction of the work,

including monitoring of data analysis, interpretation of analytical findings and their significance.

FDA will assist and approve (as deemed appropriate) the substance of publications, co-authorship of publications and data release.

Direct inquiries regarding fiscal, grants policy, procedures and/or administrative matters to the grants management specialist listed on page one of the Notice of Award (NoA).

Direct inquiries regarding scientific, technical and programmatic issues to the program official listed on page one of the Notice of Award (NoA).

All formal correspondence/reports regarding the grant should be signed by an authorized institutional official and the Principal Investigator and should be sent to the attention of the grants management specialist, unless otherwise explicitly directed.

SUMMARY STATEMENT

PROGRAM CONTACT:
Megan Miller
megan.miller@fda.hhs.gov

(Privileged Communication)

Release Date: **09/24/2024**
Revised Date:

Application Number: **1U18FD008464-01**

Principal Investigators (Listed Alphabetically):

HALFMANN, PETER JOSEPH (Contact)
WANG, ZHONGDE

Applicant Organization: UNIVERSITY OF WISCONSIN-MADISON

Review Group: **ZFD1 CVM-T (06)**
Food and Drug Administration Special Emphasis Panel
PAR-24-251 - Animal and Veterinary Innovation Centers - Meeting Group 1

Meeting Date: **08/27/2024** ***Opportunity Number:*** **PAR-24-251**
Council: **AUG 2024**

Project Title: **The Building Resistance Against Viral Entities (BRAVE) Innovation Center**

SRG Action: **Score:93**

Human Subjects: **10-No human subjects involved**
Animal Subjects: **20-Live vertebrate animals involved**

1U18FD008464-01 Halfmann, Peter

WARNING: ANIMAL SUBJECT CODE = 20

DESCRIPTION (provided by applicant):
SUMMARY The introduction of highly pathogenic avian influenza (HPAI) virus of the Guangdong H5N1 lineage has resulted in severe disease outbreaks with widespread mortality in wild birds and poultry in the United States. Current control measures, including massive culling of infected flocks, vaccination efforts, and biosecurity procedures have failed to stop the spread of avian viruses including HPAI viruses resulting in significant economic losses and public health risks due to their zoonotic potential. There is a pressing need to explore alternative strategies to combat viral pathogens that inflict massive losses on the poultry industry. To tackle the susceptibility of chickens to avian viruses, we propose establishing the BRAVE (Building Resistance Against Viral Entities) Innovation Center. This initiative aims to generate chickens with intentional genome alterations that are resistant to viral pathogens of significant biological and economic importance. To identify the host factors that will be targeted for intentional genome alterations in chickens, we will perform a genome-wide CRISPR/Cas9 study with a sgRNA library in Aim 1. These experiments will focus on identifying pro-viral chicken genes that are essential for virus replication. We will begin with HPAI H5N1 virus, and then expand the study to HPAI H7N9 virus and other avian viruses including infectious bronchitis virus (IBV). Aim 1 will also identify host factor targets for intentional genome alterations to identify unique and common antiviral proteins that directly inhibit the replication of the viruses in this proposal. We will identify and rank the best host factors when knocked out (pro-viral factors) or overexpressed (antiviral factors) for the greatest inhibitory effect on virus replication. We will monitor cell viability and the lack of escape viruses that would mutate away from the resistance profile. Using the data collected in Aim 1, in Aim 2, we will generate an intentional genomically altered (IGA) chicken line resistant to HPAI virus infection and second line that has a multi-virus resistance phenotype. We will employ CRISPR/Cas9 or transposase methods to modify the genomes in chicken primordial germ cells (PGCs) and then inject the modified PGCs into recipient chicken embryos to establish germline-modified chickens and eventually flocks of birds for infection studies in Aim 3. Once an IGA chicken line is established, we will compare the susceptibility of the altered birds with that of wild-type birds to HPAI H5N1 and H7N9 virus infections. Virus replication, associated pathology, transmission, and emergence of mutant viruses associated with breakthrough infections will be assessed and compared to wild-type birds. We will also evaluate the susceptibility of the multi- virus-resistant chicken line to H5N1 and H7N9 viruses, along with other avian viruses including IBV. Non-infected IGA chicken lines will be characterized to ensure proper growth, longevity, and fertility along with any pathological changes compared to their wild-type counterparts.

PUBLIC HEALTH RELEVANCE
PROJECT NARRATIVE Highly pathogenic avian influenza (HPAI) viruses are highly transmissible in chickens, causing 100% mortality within days and leading to significant economic losses and potentially severe public health risks if transmitted to humans. This project aims to develop gene modified chickens with reduced susceptibility or resistance to HPAI and other avian viruses by genetically targeting pro-viral host factors, antiviral proteins, or viral genes. Innovatively creating a single gene modified chicken line resistant to multiple viral pathogens could dramatically reduce disease burden and economic losses in the poultry industry.

CRITIQUE 1

Significance:	25
Investigators:	13
Innovation:	18

Approach:	25
Environment:	15

Application #: 1U18FD008464-01
Principal Investigator(s): Halfmann, Peter Joseph

1. Significance:

Strengths

- The project addresses an important problem in the field of avian health and public health.
- If the aims of the project are achieved, it would result in the generation of chicken lines that are resistant to HPAI which would have a significant impact on reducing the infection and transmission of HPAI in domestic chicken. Although the project would not make a new therapeutic available for HPAI, the generation of chicken with IGA that makes them resistant to HPAI would be a significant contribution to the field of avian medicine.
- The project demonstrates sufficient long term significance to be established as an Innovation center.

Weaknesses

- None identified

2. Investigators:

Strengths

- The PIs, collaborators and other researchers are well suited for the project.
- The PIs and collaborators have demonstrated accomplishments, with a strong record of publications in high impact journals and made significant contributions to their field.
- The investigators have expertise in complementary research areas as required by the proposed project.
- The proposed approach, governance, and organizational structure are appropriate for the project.

Weaknesses

- The application does not outline the expertise/ experience of the investigators in breeding and maintaining chicken lines, which is a critical component of the proposal.

3. Innovation:

Strengths

- Although not entirely novel, if successful, will result in a significant advancement in generating chickens resistant to HPAI.

Weaknesses

- The proposal is not entirely novel (proposing to use different targets than those previously reported/used for genomic alteration in chickens).

4. Approach:

Strengths

- The overall strategy, methods, and analyses well-reasoned and appropriate to accomplish specific aims.
- Potential weaknesses and pitfalls are clearly identified, and alternative plans are proposed.
- There is IACUC oversight of vertebrate animals used.
- The work proposed is of reasonable scope to be accomplished in the proposed timeline.

Weaknesses

- None identified

5. Environment:

Strengths

- The institutional support, facilities, equipment, and other resources available appear adequate for the project proposed.
- There is ongoing research at the institutions that utilizes the methods proposed in the grant which increases the likelihood of success.
- There is sufficient support to justify the long-term presence as an innovation center.

Weaknesses

- None identified

CRITIQUE 2

Significance:	25
Investigators:	14
Innovation:	18
Approach:	23
Environment:	15

Application #: 1U18FD008464-01
Principal Investigator(s): Halfmann, Peter Joseph

1. Significance:

Strengths

- The grant details the current status of prevention of influenza illness including vaccines and points out the drawbacks of vaccination/vaccination programs
- The idea of a genetically modified chicken line is helpful as another means of limiting infection

Weaknesses

- This is a proof-of-concept endeavor and the adaptability on large scale production is hard to predict because disease resistance is not the only factor considered in chickens - egg production, muscle mass, etc are also factors. Would this be of interest or something that would be considered novel? The other factor is the acceptability of genetically modified species. So while the idea is novel and the approach sound, the overall impact does factor in on the significance.

2. Investigators:

Strengths

- The investigators have an excellent background with viruses and genetic modification

Weaknesses

- none

3. Innovation:

Strengths

- This is an innovative project that would look into development of genetically modified chicken lines that could be resistant to HPAI
- this is a novel approach since there are few GMO animal species (fish, pig)

Weaknesses

- Influenza is prone to re-assortment and the question of whether a GMO could prove resistant with each wave of a new influenza is not addressed

4. Approach:

Strengths

- The grant submitters have proposed a three step process in the development of a chicken line that is resistant to influenza and other viruses (BRAVE - Building Resistance Against Viral Entitities). Steps including identifiy and validated pro-viral and antiviral factors in a cells line, generating IGA chikcnecken lines, and characterization of the IGA lines
- EAch component is described in details including potential pit-falls
- The identification and validatio of pro-viral and anti-viral factors in the cells lines is the crucial step and if the development of a genetically modified chicken is outside the scope of this type of grant, would be an important area to focus on that could provide helpful information

Weaknesses

- there is no histopathologic evaluation of exposed chickens; it appears to be viral tropism only. The understanding of host response primarily in susceptible organs (lung, brain) immune organ response (Bursa of Fabricius, thymus, spleen) of exposed animals would be of interest.
- while there is a look at longevity and fertility, it would be help from the food side to know if any difference in muscle mass/eqg quality
- while exposure studies will be done, the affect of genetic resortment of the virus on resistance is not discussed

5. Environment:

Strengths

- The submitters provided information on the laboratory setup and capabilities
  - lab includes BSL2 and BSL3

Weaknesses

- none

CRITIQUE 3

Significance:	21
Investigators:	15
Innovation:	15
Approach:	23
Environment:	15

Application #: 1U18FD008464-01
Principal Investigator(s): Halfmann, Peter Joseph

1. Significance:

Strengths

- Generation of IGA chicken addressed an important problem in the field.
- The investigators relied on previously published research to support their proposed project.
- They demonstrated their ability to identify the right host's pro-viral and anti-viral factors, and their ability to generate intentional genomically altered (IGA) animal models.
- Scientific knowledge on HPAI and clinical poultry practice will be improved If the aims of the project are achieved.
- Completion of this project will provide poultry farmers the option to use IGA chicken as a preventative intervention against HPAI.
- The outcome of this study will help establish a new path to improving animal health through the use of chicken that are resistant to HPAI.
- It will also serve as the template for generating other IGA chicken that would be resistant to some other important viral diseases.
- Thus, the expected potential future relevance in the development of IGA animals demonstrates a possible long-term importance of designating the center as an innovation center.

Weaknesses

- The impact of this significant work is limited, and it may take decades to achieve.
- HPAI would still remain a problem through other non-IGA birds and animals, thereby, keeping the threats of exposure and susceptibility around for the foreseeable future.

2. Investigators:

Strengths

- The investigators on this study have combination of expertise that can be integrated for the success of the proposed project.
- The investigator and co-investigators' expertise range from virology including expertise on influenza viruses, immunology, especially virus-mediated host immune responses, mycobacterial pathogenesis, animal model development, vaccine development, viral host factors, animal genetic engineering etc..
- The investigator and co-investigators' also have expertise working on multiple model animals including rabbit, nonhuman primate, mice, guinea pigs, embryonated chicken eggs, cattle, sheep, goat and hamsters.
- These investigators have published previous works to demonstrate their abilities to conduct research in these fields.
- There is organizational structure including the Influenza research Institute that is appropriate for this project.

Weaknesses

- None noted

3. Innovation:

Strengths

- The proposed project seeks to shift current research and clinical practice paradigms by generating novel IGA chicken lines to combat HPAI disease and the public health implication.

Weaknesses

- However, the proposed approach is only easily adaptable to poultry viral diseases.
- The novel intervention does not have broad application.
- This innovative approach may have limited impact on public health as HPAI virus may still be transmitted through other birds and animals.

4. Approach:

Strengths

- The overall strategy, methodology, and analyzes proposed for the identification of pro-viral and anti-viral host factors, generation of IGA chickens and characterization of the IGA chickens are well-reasoned and appropriate to accomplish the specific aims of the project.
- The investigators included plans to address weaknesses in the rigor of prior IGA chicken line.
- They presented robust, unbiased strategies to identify and characterize their experimental targets.
- They also anticipated potential problems that may arise with identification of pro-viral and anti-viral host factors, generation of IGA chickens and characterization of the IGA chickens.
- They proposed alternative strategies that may be independent of the success of another Aim in the project proposal.

- The proposed experimental approach are scientifically feasible and possible risks associated with some of the strategies were addressed.
- The investigators had a pending IACUC approval for this project.
- The principal investigator and the co-investigators have existing, multi-year collaborations that will help drive the project forward.
- Considering the number and expertise of investigators involved in this proposal, the proposed research can be accomplished in the planned study period.

Weaknesses

- Although the investigators presented plans to address variabilities due to sex, they did not present adequate plans to address variabilities due to age, species or breed of birds in their proposal.
- There is a clear strategy to address the topic areas described in NOFO, but it is not structured as a multi-year plan.

5. **Environment:**

Strengths

- The proposed research project will be conducted in part, at the Influenza research institute, a scientific environment that can enhance the probability of success of this project.
- The investigator and co-investigators are in highly resourceful laboratories and facilities that are adequate to support the success of the project.
- The University of Wisconsin and the Influenza Research Institute have proper institutional structure and support to justify long-term presence as an innovation center.

Weaknesses

- None noted