PI: **Lomvardas, Stavros**	Title: Principles of zonal olfactory receptor gene expression	
Received: 08/16/2019	FOA: PA19-056 Clinical Trial:Not Allowed	Council: 01/2020
Competition ID: FORMS-E	FOA Title: Research Project Grant (Parent R01 Clinical Trial Not Allowed)	
1 R01 DC018745-01	Dual:	Accession Number: 4339093
IPF: 1833205	Organization: COLUMBIA UNIVERSITY HEALTH SCIENCES	
Former Number:	Department: Biochemistry and Mol. Biology	
IRG/SRG: ZRG1 IFCN-U (02)M	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: [redacted] Year 2: [redacted] Year 3: [redacted] Year 4: [redacted] Year 5: [redacted]	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Stavros Lomvardas	The Trustees of Columbia University in the City of New York	PD/PI

OMB Number 4040-0001
Expiration Date 10/31/2019

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 | **Application Identifier**

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION **Organizational DUNS***: [redacted]

Legal Name*: The Trustees of Columbia University in the City of New York
Department: Zuckerman Institute
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application
Prefix: First Name*: Rudina Middle Name: Last Name*: Odeh-Ramadan Suffix:
Position/Title: Vice President for Research Administration
Street1*: [redacted]
Street2:
City*: New York
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** [redacted]

7. TYPE OF APPLICANT* O: Private 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*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Principles of zonal olfactory receptor gene expression

12. PROPOSED PROJECT
Start Date* Ending Date*
04/01/2020 03/31/2025

13. CONGRESSIONAL DISTRICTS OF APPLICANT
[redacted]

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Stavros Middle Name: Last Name*: Lomvardas Suffix:
Position/Title: Professor
Organization Name*: The Trustees of Columbia University in the City of New York
Department: Biochemistry and Mol. Biology
Division: Zuckerman Institute
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP/ Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested* [redacted]
b. Total Non-Federal Funds* [redacted]
c. Total Federal & Non-Federal Funds* [redacted]
d. Estimated Program Income* [redacted]

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*: Madhavi Middle Name: Last Name*: Nambiar Suffix: Ph.D
Position/Title*: Senior Project Officer
Organization Name*: The Trustees of Columbia University in the City of New York
Department: Sponsored Projects Administrat
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
ZIP/ Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

Signature of Authorized Representative*
Elba Suarez

Date Signed*
08/16/2019

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_Letter_Zones.pdf

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 10/31/2019

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 Trustees of Columbia University in the City of New York

Duns Number: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: [redacted]

Additional Location(s) File Name:

Tracking Number: GRANT12921503
Funding Opportunity Number: PA-19-056. Received Date: 2019-08-16T10:05:36.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

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 A3007-01

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*	Abstractzones.pdf
8. Project Narrative*	Project_Narrativezones.pdf
9. Bibliography & References Cited	ZonesBibliography.pdf
10.Facilities & Other Resources	Facilities_and_Other_Resources.pdf
11.Equipment	Major_Equipment.pdf

Funding Opportunity Number: PA-19-056. Received Date: 2019-08-16T10:05:36.000-04:00

Abstract

The monogenic, monoallelic, and seemingly stochastic transcriptional choice of one out of > 1000 olfactory receptor (OR) genes remained elusive for decades after the discovery of the largest mammalian gene family. However, in the past few years we obtained significant understanding on the molecular underpinnings of this enigmatic gene regulatory process. Specifically, we showed that OR gene clusters become heterochromatic at the early stages of olfactory sensory neuron (OSN) differentiation and then they aggregate in distinct nuclear compartments that assure their stable repression. As a result of this interchromosomal convergence, intergenic OR enhancers (known as Greek Islands) that are found in most OR gene clusters come in close nuclear proximity and form a multi-chromosomal super-enhancer that in each OSN associates with the transcriptionally active OR allele. The formation of the Greek Island hub is dependent upon the recruitment of the adaptor protein Ldb1, which is essential for the stable interchromosomal interactions between Greek Islands and for OR transcription. This intricate network of activating and repressive interchromosomal interactions, together with a feedback signal elicited by the expression of the chosen OR, likely generate the regulatory framework for transcriptional singularity. However, what remains unknown how this seemingly stochastic process operates under deterministic restrictions related to the spatial location of the OSN along the dorso-ventral and apico-basal axes of the MOE. These restrictions, known as zonal pattern of OR expression, restrict the expression of each OR gene in one of five zones of expression. Here we identified putative mechanisms of zonal restriction, by uncovering the molecular mechanisms that enable only zone 5 ORs to be expressed in zone 5. We show that transcription factors of the NFI family enable the transcriptional activation of zone 5 ORs, by mediating the recruitment of these ORs to the interchromosomal OR compartment. Moreover, we show that the repressive histone modification H3K79me3 prevents the expression of out of zone ORs, possibly under the control of NFI factors, as well. We propose experiments that will decipher which NFI factors are required and sufficient for specification of zone 5 transcription programs, and experiment that will determine how NFI proteins accomplish these zonal restrictions. Our experiments will reveal novel mechanisms of regulation of nuclear architecture, and will uncover generally applicable principles for the regulation of developmental patterning.

Project Narrative

Developmental patterning is a crucial biological mechanism perturbations of which are related to a plethora of developmental disorders. Developmental patterning in the olfactory epithelium, as manifested by the zonal expression of olfactory receptor genes remains mysterious for decades. Here, we describe genetic and epigenetic factors regulating zonal olfactory receptor expression, and we seek to uncover regulatory principles that are applicable to other systems undergoing developmental patterning.

FACILITIES AND OTHER RESOURCES

Lomvardas Laboratory

Laboratory: The laboratory is housed in ~2500 sq. feet in the 4th floor of the Jerome L. Green Science building that houses the Zuckerman mind brain and behavior institute (ZMBBI) at the new Manhattanville campus of Columbia University. Included in this area are the PI's office, administrator's office, 12 benches 12 computer stations, one room for microscopy and histology, one room for cell culture and one room for electrophysiology and surgery. Shared facilities include dark room, cold room, bacterial incubator and shakers. There are 3 rooms for seminars and social gathering in the floor. My lab neighbors the laboratory of Dr. Tom Maniatis's lab (open space configuration).

Animal Facilities: We have access to the state of the art barrier mouse facility in the basement of the Jerome L. Green Science building with space for up to 1000 cages. Moreover, we have a satellite mouse facility next to our laboratory with space for up to 250 cages. This facility is ideal for time-sensitive experiments that require continuous access to the mice. Next to the satellite mouse facility we also have a behavior room that is shared between the Maniatis and Lomvardas lab. All mice will be under the constant supervision of veterinary staff as well as the IACUC at Columbia. Moreover, we have access to the transgenic core facility of the Cancer Center at CUMC, which we used for the genetically modified mice described in this proposal.

Computational resources: For analysis of deep sequencing data, every student and postdoc in the lab has their own personal computer and access to a Dual Xeon E5-2600 V3 Workstation, which has 24 cores, 4 x 32 GB RAM, and 24TB of local storage. An additional 60 TB of storage is available on backup workstations and digitally in an Amazon Cloud account. Additional large-scale computation resources are available through the Zuckerman Institute Research Computing Core. Finally, to keep up with our ever expanding needs for "scratch memory" in the analysis of deep sequencing databases by Next-seq we are renting 40TB of memory from Habanero, the high performance computing cluster at Columbia University.

Office: In addition to the PI's office, there is office space for one administrator and one lab manager and desks for every student and postdoc in the lab (1desk per bench). We also have two color printers and one scanner that are used by members of the Lomvardas laboratory.

Intellectual Environment: As a member of the Zuckerman Mind, Brain, and Behavior Institute I have the privilege to interact with some of the most creative and influential neuroscientists of our generation. First, Dr. Richard Axel, my postdoctoral mentor is located at the 8th floor of our building and we have weekly meetings to discuss common interests and ideas regarding olfaction (including the idea of using the olfactory system as a model for neurodegeneration). Furthermore, Tom Maniatis, with whom I share lab space, seeks to investigate the molecular mechanisms of monoallelic Protocadherin gene choice. As in OR gene choice, long range genomic interactions between intergenic enhancers and stochastically chosen promoters determine which Protocadherin allele will be expressed from each genomic cluster. The two laboratories have joined lab meetings and collaborate in various projects, including the development of powerful single cell HiC approaches. Moreover, we have monthly joint meetings with Dr. Richard Mann, Dr. Minoree Kohwi and their labs, which work on different aspects on genetic, epigenetic and nuclear architecture-based control of gene regulation. Finally, I meet regularly with Dr. Laura Landwerber and Dr. Sam Sternberg my colleague at the biochemistry department who are experts in regulated chromosomal DSBs (Landwerber) and CRISPR/Cas9 technologies (Sternberg). As of recently, I am communicating with Dr. Gautier, who is an expert of DSB-induced chromatin mobility, discussing possible applications of his findings in our system.

ZMBBI Jerome L. Greene Science Center

The Cellular Imaging Core (Darcy Peterka, PhD, Director of Cellular Imaging) will provide access to the state-of-the-art imaging methods, broadly categorized into two critical imaging areas in neuroscience, high-speed functional imaging, and high resolution structural imaging, both across multiple scales, from sub-cellular structures, to circuits and entire brains. Second, the core will provide expert staff trained in sample preparation, microcopy, and image processing to assist investigators in complex experiments. Lastly, the core will be the central repository of imaging and image analysis expertise, within ZMBBI, and the greater Columbia Community, for both the hardware and the algorithms and software necessary for success. The Core will also provide strategic consulting services on current imaging modalities, feasibility and experimental design, and data processing and archiving. The Cellular Imaging Core has ~900 sq. ft. of dedicated imaging space on the lower level of the Greene Science Center, which currently houses a laser scanning confocal microscope, and a robotic slide scanner. Two-photon microscopes capable of in vivo imaging in awake animals, and a laser

scanning confocal microscope will be added to the facility before the commencement of this grant. A full animal monitoring suite will be maintained in this room to support *in vivo* imaging. The Core equipment on the 3rd floor includes the Zeiss Sigma HV electron microscope, and is the planned location of the lightsheet microscope budgeted for a Fall 2017 acquisition.

The Cellular Imaging Core maintains local computer resources and workstations for image processing and analysis. There is also a suite of diagnostic instruments and electronics for support of advanced imaging including spectrophotometers and optical detectors. A large pool of optomechanics and optics are available to modify and enhance existing imaging platforms.

The Cellular Imaging Core maintains a listing of all existing lasers, microscopes, and imaging related equipment for ZMBBI, with close relationships to the investigators. This allows Cellular Imaging to leverage a large pool of resources to bear on imaging intensive projects.

The Zuckerman Institute Virology Core (ZIVIROLOGY) (Julia Sable, Director) is a full-service viral vector production organization that provides ZMMBI investigators access to state-of-the-art vector technology for basic animal model research applications. The core provides internal pricing structures for Z-MBBI faculty and Columbia University investigators which provides reduced costs compared to those currently incurred from purchasing via external viral vector cores. The core provides on-site same day pick up for in-stock catalog items. The Director, Julia Sable, has 18 years of experience in viral production and advanced imaging design. The Director and staff provide expert consultation services for advanced study design, safe use of viral vector technologies and viral construction services for multiple viral vector types.

The ZIVIROLOGY Core is located in JLG in rooms L3.017. L3.010 and L3.014 and provides lenti and retro virus, rAAV and N2C G-deleted rabies customized for individual investigators needs; production and distribution of catalog/stock rAAV and N2C G-deleted rabies vectors expressing reporter and functional transgenes (e.g.) XFPs, GECIs, GEVIs and optogenetic tools. It provides consultation services on experimental design, development and use of custom viral vectors as well as novel client specific viral transfer plasmids. An example is the development of a fish Rhabdovirus genetic system to study cerebellar circuits in Mormyrid electric fish. The Center will soon provide local distribution of cloning vectors and cell lines with relevant MTA information to investigators prior to vector design and production (e.g. complete AAV plasmid collections housed on site from Deisseroth, Boyden, Roth, Uchida and Wickersham Labs).

Zuckerman Institute Research Computing Core (Rajendra Bose, Director) is a five-person team that provides research computing and IT services, support and infrastructure to faculty, their research labs and other scientific service groups in the Greene Science Center. The Institute has launched a dedicated, shared, multi-petabyte storage system to house images and other research data and allow collaboration by Zuckerman researchers. This storage platform has an initial capacity of roughly two usable petabytes (PB, equal to 2000 terabytes (TB)) and is designed to accommodate growth up to tens of PB over the next years. All three initial tiers of storage include remote backup for disaster recovery purposes. The Research Computing group also provides access to the university's newest state-of-the-art high performance computing (HPC) cluster, launched in November 2016. This cluster has over 200 computer systems providing 5500 central processing unit (CPU) cores and 140,000 graphics processing unit (GPU) hardware accelerators for highly parallelized applications. In addition, a scalable virtual machine infrastructure (VMI) is in place to provide computational resources for data analysis approaches for which a shared HPC system is unsuited. The storage, VMI, HPC system and other local equipment is is housed in the dedicated, climate-controlled 1400 square foot Zuckerman Institute Data Center in the Greene Science Center with the ability to power and cool up to 30 cabinets of equipment. The computational resources in the server room are accessed through a data network that has up to 10 Gigabit per second (Gbps) data transfer speeds within the building, and that connects to the 40 Gbps campus backbone network and beyond to regional, national and international research networks. In addition, Columbia's multiple commodity Internet connections provide an aggregate bandwidth of over 9 Gbps to the public Internet.

The *Advanced Instrumentation Core* (Tanya Tabachnik, MEng, director) is available to help design, build and test instruments, tools, and software that are not available commercially. The group has complementary skills spanning mechanical, electrical, and optical engineering including manufacturing. The Advanced Instrumentation core supports an in-house machine and fabrication shop operated by and for the researchers themselves after completing a short training course. The Facility will continuously improve its design engineering and manufacturing capability to allow rapid and cost effective delivery of functional solutions.

In all cases, the AIC's philosophy is to collaborate closely with the research community to understand the neuroengineering problem to be solved. We believe that the synergy between life science and engineering will bring next-generation design solutions. We will support shared use of equipment and software by all interested Z-MBBI researchers. The AI group will have complementary skills spanning mechanical, electrical, and optical engineering including manufacturing. The Facility's goal is to provide in-house solutions and training to assist researchers through a complete life cycle of system development.

The Advanced Instrumentation core is located with the Jerome L Greene Center encompassing 2,200 Sq. feet in the basement level and 2 rows of the wet benches at the laboratory 3 level. Facilities include a fully equipped modern machine shop as well as electronic shop and engineering area. The shop includes a storage room, and two manufacturing and assembly spaces. The Manufacturing Shop is equipped with CNC and a manual milling machine, manual lathe, table and vertical saws, drill press, laser cutter and 3D printers. The Shop will also offer accessible hardware storage. Electrical equipment includes a Digital Storage Oscilloscope 1Gs/s, with 4 channels capable of 100MHz and FFT Mathematical analysis, Logic Analyzer of 16 Channels at 100MHz, decoding of I2C, SPI, RS232 interfaces, 25MHz Math Function and Arbitrary Signal Generator, Triple Channel intelligent programmable DC Power Supply 30V 1.5A, 5V 6A, with max load of 120W, 300 ft. Electrical and communications TDR Hand held meter, hot Air rework soldering station. The Facility will also have a metrology and optics lab, shared with the Cellular Imaging Core, suitable for optical design, integration, and assembly.

Major Equipment:

General Equipment: Our laboratory is equipped with the standard biochemistry, molecular biology and imaging equipment: 1 ultrancentrifuge (Sorval), 3 minicentrifuges, eppendorf thermomixer, hybridization ovens, cryostat (Leica), 4 PCR machines, two real time PCR machines, one epifluorescence microscope, one confocal microscope (Zeiss 710) with 4 lasers and 10x, 20x and 100x (oil immersion) lenses, tissue culture room (with 4 incubators, 2 hoods etch), an automatic sonicator (Covaris M220 fosused ultrasonicator), bioanalyzer, DNA library preparation hood (Air clean PCR workstation), gel documentation system, gel dryer, blue pipen DNA extraction instrument. We also have free access to a 96 well head Biomek FX and 384 head Biomek FXp, purposed for single cell library preparation, which are owned by the Maniatis lab but are freely shared with us.

FACS: We have access to he MoFlo XDP from Beckman Coulter, which is a high speed, high accuracy cell sorter with customizable lasers. It has a 32-bit high-resolution 5-decade multi-channel digital system to enable accurate sorting of rare events. It also has robust and stable microfluidics that enable both long sorts as well as high precision, for example, when sorting into 96, 384, and 1536-well plates. We also have direct access to a Sony SH800 flow cytometer, enabling direct sorting 24 hours a day, 7 days a week. Both instruments are ideal for our sorting needs achieving high viability, yield and purity combined with very high sensitivity.

Deep Sequencing: We perform our sequencing reactions at ZMBBI using two NextSeq 550 instruments that are shared by a total of 4 labs in the institute. We also have access to the Columbia Cancer center core, which offers two more NextSeq 550 and two 10xGenomics single cell RNA-seq platforms. Thus, we have enough instrumentation for the completion of the proposed experiments and for the first time in my career we will not need to wait for sequencing. In the case that we need significantly deeper sequencing depths we will use the facilities of the NYC genome center, which is affiliated with Columbia University and directed by Dr. Maniatis. This facility has 80 Illumina Hi-seq stations,7 Nova-seq stations on two PAC-bio stations in a 30,000 square feet sequencing facility in NYC.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix:	First Name*: Stavros	Middle Name	Last Name*: Lomvardas	Suffix:

Position/Title*:	Professor
Organization Name*:	The Trustees of Columbia University in the City of New York
Department:	Biochemistry and Mol. Biology
Division:	Zuckerman Institute
Street1*:	[redacted]
Street2:	
City*:	
County:	
State*:	[redacted]
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	[redacted]
Phone Number*:	[redacted]
Fax Number:	
E-Mail*:	[redacted]
Credential, e.g., agency login:	[redacted]
Project Role*:	PD/PI
Other Project Role Category:	
Degree Type:	PHD
Degree Year:	2002
Attach Biographical Sketch*: File Name:	Biosketch_Lomvardas_08.12.19.pdf
Attach Current & Pending Support: File Name:	

Funding Opportunity Number: PA-19-0560 Received Date: 2019-08-16T10:05:36.000-04:00

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contr butors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Lomvardas, Stavros

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Professor of Biochemistry and Molecular Biophysics and Neuroscience

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Crete, Heraklion, Greece	B.S	06/1998	Molecular Biology
Columbia University, New York, NY	M.A.	09/1999	Genetics and Development
Columbia University, New York, NY	Ph.D.	09/2002	Biochemistry and Molecular Biophysics
Columbia University, New York, NY	Postdoctoral	12/2006	Biochemistry and Molecular Biophysics

A. Personal Statement

My research program aims to understand the molecular mechanisms that generate the astounding diveristy of cell types that characterise the mammalian nervous system. As a model system for our studies we use the olfactory system and the study of the epigenetic mechanisms regulating olfactory receptor (OR) gene choice. OR genes compose the largest mammalian gene family consisted of >1000 members. These genes are expressed in a a mutually exclusive fashion, in such a way, that only one OR allele is expressed in each olfactory sensory neuron. In our effort to understand this extreme mode of stochastic gene expression we uncovered novel genetic and epigenetic principles that are likely deployed by other neuronal populations. For example, we previously showed that OR translation activates the unfolded protein response pathway (UPR) toward the generation of a feedback signal that prevents the expression of other OR alleles by silencing Lsd1. Moreover, we showed that the singularity of OR gene choice is orchestrated by an intricate network of interchromosomal interactions that culminates in the formation of a singular, multienhancer complex over the transcriptionally active OR. Our published work clearly indicates our expertise in genetics, epigenetics, nuclear architecture, gene regulation and developmental biology to lead a project seeking to decipher the zonal gene expression of olfactory receptor genes and the molecular mechanisms that recruit a single OR allele to the mult-enhancer hub. My move from UCSF to Columbia in the end of 2014 posed significant delays to our research progress due to issue related to the mouse house of our interim space at the main campus at Columbia University. Due to technical problems at the vivarium we had limited mouse space that drastically delayed our genetic experiments. Importantly, due to delays in the completion of the Jerome L. Green Science building we spent 3 years instead of 6 months at this interim space. Since our move to the new state-of-the-art science building we have expanded our colony and accelarated our productivity (one manuscript accepted in Nature, one in Cell and one in eLife in 2019 and three other manuscripts in preparatoin)

1. Clowney E.J., LeGross M.A., Mosley C.M., Clowney F.G., Markenskoff-Papadimitriou E.C., Myllys M., Barnea G., Larabell C.A. and **Lomvardas, S.** (2012). Nuclear aggregation of olfactory receptor genes regulates their monogenic expression. *Cell*, 151(4), 724-737. PMCID: PMC3659163.
2. Lyons, B.D., Allen, W.E., Goh, T., Tsai, L., Barnea, G. & **Lomvardas, S.** (2013). An epigenetic trap stabilizes singular olfactory receptor expression. *Cell*, 154(2), 325-336. PMCID: PMC3929589.

3. Markenscoff-Papadimitriou, E.C., Allen, W.E., Colquitt, B.M., Goh, T., Monahan, K., Murphy, K.K., Mosley, C.P., Ahituv, N., & **Lomvardas S.** (2014). Enhancer interaction networks as a means of singular olfactory receptor expression. *Cell*, 159(3), 543-557. PMCID: PMC4243057.
4. Monahan, K., Horta A., and **Lomvardas S** (2019)**.** Lhx2/Ldb1-mediated trans interactions regulate olfactory receptor choice. *Nature*, 565(7740): 448-453. PMCID: PMC6436840.

B. Positions and Honors

Positions and Employment

2007-2013 Assistant Professor, Department of Anatomy, University of California, San Francisco, CA
2013-2014 Associate Professor, Department of Anatomy, University of California, San Francisco, CA
2014- Professor, Biochemistry and Molecular Biophysics and Neuroscience, Columbia University, NY
2014- Principal Investigator, Zuckerman Mind Brain Behavior Institute, Columbia University, NY

Other Experience and Professional Memberships

2010-2014 Scientific Board of the International Rett Syndrome Foundation
2011-2014 European Research Council: Referee in Peer Review Evaluations
2013 NIDA CEBRA review roster member (NIH)
2012-2015 Somatosensory and Chemosensory Study Section, Ad Hoc Roster member (NIH)
2015-2018 NIDA AVENIR phase II review roster member (NIH)
2015 Simons Foundation Autism Research Initiative (SFARI) review roster member
2015 4D Nucleome Program, review roster member for the Nucleomics Tools RFA (NIH)
2015-2021 Chemosensory Study Section (NIH), regular member (chairperson from 2019)

Honors

2002 Harold M. Weintraub Award for outstand achievement during graduate studies in Biological Sciences, Fred Hutch Cancer Center, Seattle, WA
2002 Ph.D. with highest distinction from Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY
2002 Samuel W. Rover and Lewis Rover Awards for outstanding achievement in Biochemistry and Molecular Biophysics, Columbia University, New York, NY
2003 Helen Hay Whitney Foundation Fellowship, Helen Hay Whitney Foundation, New City, NY
2007 Innovations in Basic Sciences Award, Program for Breakthrough Biomedical Research, San Francisco, CA
2008 Rett Syndrome Trust New Investigator Award, Rett Syndrome Research Trust, Trumbull, CT
2009 NIH Director's New Innovator Award, National Institutes of Health, Bethesda, MD
2010 EUREKA Award, National Institutes of Health, Bethesda, MD
2010 McKnight Scholar Award, McKnight Endowment for Neuroscience, Minneapolis, MN
2014 Vilcek Prize for Creative Promise in Biomedical Promise, Vilcek Foundation, New York, NY
2014 Young Investigator Award for Research in Olfaction, Association for Chemoreception Sciences, Glenview, IL
2016 HHMI Faculty Scholar, Howard Hughes Medical Institute
2017 Blavatnik Foundation National Award Finalist, Blavatnik Foundation

C. Contribution to Science

My scientific career is focused on deciphering regulatory mechanisms of gene expression, with major contributions in the understanding of mechanisms of inducible and stochastic gene activation.

1. As a graduate student I examined the role of chromatin structure in the transcriptional activation of the IFNβ gene. My experiments revealed the order of events that lead to the activation of IFNβ transcription *in vivo*, established the role of histone acetylation in recruitment of nucleosome remodeling complexes, and demonstrated that TBP binding induces nucleosome sliding away from the core promoter, providing a novel mechanism by which general transcription factors facilitate transcription. Moreover, I showed that chromatin imposes specificity in gene activation; the position of a nucleosome determines the range of stimuli that lead to activation of IFNβ expression and defines the order of molecular events required for

initiation of transcription. These experiments provided the first insight into the role of chromatin in regulation of inducible gene transcription and constitute the textbook view of transcriptional activation in the context of chromatin. I had major conceptual and technical contribution to most of these experiments.

a. Agalioti, T*., **Lomvardas, S*.**, Parekh, B., Yie, J., Maniatis, T., & Thanos, D. (2000). Ordered recruitment of chromatin modifying and general transcription factors to the IFN-beta promoter. *Cell*, 103(4), 667-678. *Equally contributing authors
b. **Lomvardas, S**. & Thanos, D. (2001). Nucleosome sliding via TBP DNA binding in vivo. *Cell,* 106(6), 685-696.
c. **Lomvardas, S**. & Thanos, D. (2002). Modifying gene expression programs by altering core promoter chromatin architecture. *Cell*, 110(2), 261-271.

2. As an independent investigator I study the regulatory mechanisms of the monogenic and monoallelic olfactory receptor (OR) choice. Our experiments revealed an elegant molecular pathway that starts with the heterochromatic silencing of OR genes, continues with enzymatic de-silencing of one OR allele by lysine demethylase Lsd1, and culminates with the OR-dependent downregulation of Lsd1 expression, which stabilizes the singular OR choice. We also discovered that OR elicit this feedback signal via components of the unfolded protein response pathway, which detect OR protein expression in the endoplasmic reticulum. This regulatory loop, combined with our analysis of the unusual organization of OR loci in nuclei of olfactory sensory neurons provide molecular insight to a process that remained elusive since the cloning of olfactory receptor genes. These experiments, which I supervised and designed, provided significant insight to the elusive process of olfactory receptor choice, which remained mysterious since the discovery of olfactory receptor genes by Buck and Axel.
   a. Magklara, A., Yen, A., Colquitt, B.M., Clowney, E.J., Allen, W., Markenscoff-Papadimitriou, E., Evans, Z.A., Kheradpour, P., Mountoufaris, G., Carey, C., Barnea, G., Kellis, M., & **Lomvardas, S**. (2011). An epigenetic signature for monoallelic olfactory receptor expression. *Cell*, 145(4), 555-570. PMCID: PMC3094500.
   b. Lyons, B.D., Allen, W.E., Goh, T., Tsai, L., Barnea, G. & **Lomvardas, S.** (2013). An epigenetic trap stabilizes singular olfactory receptor expression. *Cell*, 154(2), 325-336. PMCID: PMC3929589.
   c. Dalton, P.R., Lyons, B.D & **Lomvardas S**. (2013). Co-opting the unfolded protein response to elicit olfactory receptor feedback. *Cell,* 155(2), 321-32 PMCID: PMC3843319.
   d. Lyons, B.D., Magklara, A., Goh, T., Sampath, S., Schaefer, A., Schotta, G. & **Lomvardas, S**. (2014). Heterochromatic silencing facilitates the diversification of olfactory neurons. *Cell Rep*, 9(3), 884-892. PMCID: PMC4251488.

3. In addition to our epigenetic studies focused on OR gene regulation we have performed comprehensive analyses on the epigenetic transitions occurring during the differentiation of olfactory neurons in vivo. By performing ChIP-seq and DIP-seq on FACsorted cells we provided the first detailed description of the changes in DNA methylation, DNA hydroxymethylation, chromatin accessibility occurring during differentiation. With genetic manipulation (loss of function and overexpression experiments) we demonstrated the role of hydroxylmethylcytosine in neuronal transcription as a facilitator of transcriptional elongation than a mere intermediate of cytosine de-methylation. Finally, more recently by performing ChIP-seq for transcription factors we showed that transcription factors Lhx2 and Ebf bind cooperatively to OR enhancers and enable their function.
   a. Colquitt, B.M., Allen, W.E., Barnea, G., **Lomvardas, S.** (2013). Alteration of genic 5-hydroxymethylcytosine patterning in olfactory neurons correlates with changes in gene expression and cell identity. *Proc Natl Acad Sci U S A*, 110(36), 14682-14687 PMCID: PMC3767503.
   b. Colquitt, B.M., Markenscoff-Papadimitriou, E., Duffié, R., **Lomvardas, S.** (2014). Dnmt3a regulates global gene expression in olfactory sensory neurons and enables odorant-induced transcription. *Neuron*, 83(4), 823-838. PMCID: PMC4153871.
   c. Monahan K.,Schieren I., Cheung J., Mumbey-Wafula A., Monuki E.S., and **Lomvardas S**. (2017). Cooperative interactions enable singular olfactory receptor expression in mouse olfactory neurons. *Elife* PMCID: PMC5608512.
   d. Canzio D., Nwakeze C.,L., Horta A., Rajkumar S., M., Coffey E., L., Duffy E., E., Duffie R., Monahan K., O'Keeffe S., Simon M., D., **Lomvardas S**., and Maniatis T., (2019) Antisense lncRNA transcription mediates DNA demethylation to drive stochastic Protocadherin a promoter choice. *Cell*. 2019 Apr 18;177(3):639-653.e15. PubMed PMID: 30955885.

4. Importantly, my research program has revealed significant insight to the understanding of the role of nuclear architecture in epigenetic processes and gene regulation during neuronal differentiation. Using sequence capture technologies to generate a complex DNA FISH probe that detects the thousands of olfactory receptor alleles simultaneously we showed that during neuronal differentiation olfactory receptor loci converge to unique heterochromatic nuclear foci that contribute to the complete transcriptional silencing of olfactory receptor genes. Our experiments revealed that essential for this unusual nuclear reorganization is the downregulation of lamin b receptor from the differentiating neurons, which normally tethers olfactory receptor genes to the nuclear envelope. In each olfactory neuron, the transcriptionally active olfactory receptor allele resides outside of these repressive foci and is surrounded by a large number of intergenic enhancers that converge over the active allele from many different chromosomes, a discovery that constitutes the continuation of work that I started as post-doc in Richard Axel's lab. These findings demonstrate the decisive role of nuclear architecture in gene regulation in vivo. I designed and supervised the experiments that led to these discoveries from my own lab and designed and executed the experiments that I published as post-doc.
    a. Clowney E.J., LeGross M.A., Mosley C.M., Clowney F.G., Markenskoff-Papadimitriou E.C., Myllys M., Barnea G., Larabell C.A. and **Lomvardas, S.** (2012). Nuclear aggregation of olfactory receptor genes regulates their monogenic expression. *Cell,* 151(4), 724-737. PMCID: PMC3659163.
    b. Markenscoff-Papadimitriou, E.C., Allen, W.E., Colquitt, B.M., Goh, T., Monahan, K., Murphy, K.K., Mosley, C.P., Ahituv, N., & **Lomvardas S.** (2014). Enhancer interaction networks as a means of singular olfactory receptor expression. *Cell*, 159(3), 543-557. PMCID: PMC4243057.
    c. Le Gros, M.A., Clowney, E.J., Magklara, A., Yen, A., Markenscoff-Papadimitriou, E., Colquitt, B., Myllys, M., Kellis, M., **Lomvardas, S.,** & Larabell, C.A. (2016). Soft X-Ray Tomography reveals gradual chromatin compaction and reorganization during neurogenesis in vivo. *Cell Rep*, 17(8), 2125-2136. PMCID: PMC5135017.
    d. Monahan, K., Horta A., and **Lomvardas S** (2019)**.** Lhx2/Ldb1-mediated trans interactions regulate olfactory receptor choice. *Nature* 565(7740): 448-453. PMCID: PMC6436840.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/stavros.lomvardas.1/bibliography/40755668/public/?sort=date&direction=descending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R01 DC015451 Lomvardas (PI) 07/01/2016–06/30/2021
Deciphering the Molecular Principles of Olfactory Receptor Gene Choice
This grant seeks to decipher the molecular mechanisms by which transcription factors Lhx2 and Ebf activate intergenic transcriptional enhancers towards the singular olfactory receptor transcription.
Role: PI

U01 DA040582 Lomvardas, Brown, Larabell (MPI) 09/01/2015–08/30/2020
Deciphering nuclear bodies and compartments that govern singular olfactory receptor expression
This grant seeks to develop new technologies for the imaging, genomic, and biochemical analysis of the heterochromatic nuclear bodies formed by silent olfactory receptor genes.
Role: MPI

NSF 1921500 Narlikar (PI), Larabell, Lomvardas 08/01/2019–07/31/2024
Collaborative Research: URoL: Epigenetics 2: Phase separated genome compartments as drivers of epigenetic phenotypes
This grant seeks to decipher how droplet mediated DNA organization is regulated by cellular signals and how it impacts epigenetic phenotypes.
Role: Co-PI

R56 AG062454 Lomvardas (PI) 08/15/2019–07/31/2020
Anosmia as a predictor of preclinical Alzheimer's disease
This grant seeks to we explore the possibility that olfactory neurons could be used as early molecular sensors for neurodegenerative disorders.
Role: PI

HHMI Faculty Scholar Lomvardas (PI) 11/01/2016–10/31/2021
Molecular Mechanisms of Stochastic Gene Expression
Role: PI

Completed Research Support

R01 DC014144 Lomvardas (PI) 08/01/2014–07/31/2019
The Unfolded Protein Response as an Organizer of Chemosensory Response
This grant seeks to characterize a newly discovered molecular pathway that uses components of the unfolded protein response for the stabilization of olfactory receptor gene choice.
Role: PI

R01 DC013560 Lomvardas (PI) 08/01/2014–07/31/2019
Understanding the Nuclear Architecture in Olfactory Receptor Choice.
This grant seeks to characterize a network of interchromosomal interactions formed between the transcriptionally active OR allele and distant regulatory enhancers in olfactory neurons.
Role: PI

R01 DA036894 Lomvardas, Barnea (MPI) 09/15/2013–9/14/2018
Controling epigenetic states and nuclear architecture in the brain
This grant seeks to develop technologies that will allow the cell type and sequence specific manipulation of gene loci, either at the level of epigenetic regulation and at the level of relative nuclear positioning.
Role: MPI

OMB Number: 4040-0001
Expiration Date: 10/31/2019

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Trustees of Columbia University in the City of New York

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **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 .		Stavros		Lomvardas		PD/PI	[redacted]	2.0			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	6.0			[redacted]	[redacted]	[redacted]
1	Graduate Students	12.0			[redacted]	[redacted]	[redacted]
	Undergraduate Students						
	Secretarial/Clerical						
1	Staff Associate	6.0			[redacted]	[redacted]	[redacted]
3	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-19-056 . Received Date: 2019-08-16T10:05:36.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Organization: The Trustees of Columbia University in the City of New York

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **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	[redacted]

Additional Equipment: File Name:

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

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**	[redacted]

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

Tracking Number: GRANT12921503 Funding Opportunity Number: PA-19-056 . Received Date: 2019-08-16T10:05:36.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Organization: The Trustees of Columbia University in the City of New York

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	[redacted]
3. Consultant Services	
4. ADP/Computer Services	[redacted]
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal costs	[redacted]
9 . Illumina sequencing, FACS Core Costs, Equipment Maintenance contracts	[redacted]
10 . GRA tuition plus GRA fees	[redacted]
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, [redacted]

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification*	File Name: Lomvardas.Zonal.R01.Budget.Justification.pdf (Only attach one file.)

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

Tracking Number: GRANT12921503 Funding Opportunity Number: PA-19-056 . Received Date: 2019-08-16T10:05:36.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Trustees of Columbia University in the City of New York

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **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 .		Stavros		Lomvardas		PD/PI	[redacted]	2.0			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	6.0			[redacted]	[redacted]	[redacted]
1	Graduate Students	12.0			[redacted]	[redacted]	[redacted]
	Undergraduate Students						
	Secretarial/Clerical						
1	Staff Associate	6.0			[redacted]	[redacted]	[redacted]
3	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-19-056 . Received Date: 2019-08-16T10:05:36.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Organization: The Trustees of Columbia University in the City of New York

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **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	[redacted]

Additional Equipment: File Name:

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

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**	[redacted]

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

Tracking Number: GRANT12921503 Funding Opportunity Number: PA-19-056 . Received Date: 2019-08-16T10:05:36.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Organization: The Trustees of Columbia University in the City of New York

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	[redacted]
3. Consultant Services	
4. ADP/Computer Services	[redacted]
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal costs	[redacted]
9 . Illumina sequencing, FACS Core Costs, Equipment Maintenance contracts	[redacted]
10 . GRA tuition plus GRA fees	[redacted]
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, [redacted]

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification*	File Name: Lomvardas.Zonal.R01.Budget.Justification.pdf (Only attach one file.)

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

Tracking Number: GRANT12921503 Funding Opportunity Number: PA-19-056 . Received Date: 2019-08-16T10:05:36.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Trustees of Columbia University in the City of New York

Start Date*: 04-01-2022 **End Date*:** 03-31-2023 **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 .		Stavros		Lomvardas		PD/PI	[redacted]	2.0			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	6.0			[redacted]	[redacted]	[redacted]
1	Graduate Students	12.0			[redacted]	[redacted]	[redacted]
	Undergraduate Students						
	Secretarial/Clerical						
1	Staff Associate	6.0			[redacted]	[redacted]	[redacted]
3	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-19-056 . Received Date: 2019-08-16T10:05:36.000-04:00

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

ORGANIZATIONAL DUNS* [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Organization: The Trustees of Columbia University in the City of New York

Start Date*: 04-01-2022 **End Date*:** 03-31-2023 **Budget Period: 3**

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	[redacted]

Additional Equipment: File Name:

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

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**	[redacted]

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

Tracking Number: GRANT12921503 Funding Opportunity Number: PA-19-056 . Received Date: 2019-08-16T10:05:36.000-04:00

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

ORGANIZATIONAL DUNS* [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Organization: The Trustees of Columbia University in the City of New York

Start Date*: 04-01-2022 **End Date*:** 03-31-2023 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	[redacted]
3. Consultant Services	
4. ADP/Computer Services	[redacted]
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal costs	[redacted]
9 . Illumina sequencing, FACS Core Costs, Equipment Maintenance contracts	[redacted]
10 . GRA tuition plus GRA fees	[redacted]
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, [redacted]

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification*	File Name: Lomvardas.Zonal.R01.Budget.Justification.pdf (Only attach one file.)

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

Tracking Number: GRANT12921503 Funding Opportunity Number: PA-19-056 . Received Date: 2019-08-16T10:05:36.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Trustees of Columbia University in the City of New York

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **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 .		Stavros		Lomvardas		PD/PI	[redacted]	2.0			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	6.0			[redacted]	[redacted]	[redacted]
1	Graduate Students	12.0			[redacted]	[redacted]	[redacted]
	Undergraduate Students						
	Secretarial/Clerical						
1	Staff Associate	6.0			[redacted]	[redacted]	[redacted]
3	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-19-056 . Received Date: 2019-08-16T10:05:36.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Organization: The Trustees of Columbia University in the City of New York

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **Budget Period: 4**

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	[redacted]

Additional Equipment: File Name:

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

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**	[redacted]

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

Tracking Number: GRANT12921503 Funding Opportunity Number: PA-19-056 . Received Date: 2019-08-16T10:05:36.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Organization: The Trustees of Columbia University in the City of New York

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	[redacted]
3. Consultant Services	
4. ADP/Computer Services	[redacted]
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal costs	[redacted]
9 . Illumina sequencing, FACS Core Costs, Equipment Maintenance contracts	[redacted]
10 . GRA tuition plus GRA fees	[redacted]
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, [redacted]

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification*	File Name: Lomvardas.Zonal.R01.Budget.Justification.pdf (Only attach one file.)

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

Tracking Number: GRANT12921503 Funding Opportunity Number: PA-19-056 . Received Date: 2019-08-16T10:05:36.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Trustees of Columbia University in the City of New York

Start Date*: 04-01-2024 **End Date*:** 03-31-2025 **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 .		Stavros		Lomvardas		PD/PI	[redacted]	2.0			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	6.0			[redacted]	[redacted]	[redacted]
1	Graduate Students	12.0			[redacted]	[redacted]	[redacted]
	Undergraduate Students						
	Secretarial/Clerical						
1	Staff Associate	6.0			[redacted]	[redacted]	[redacted]
3	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-19-056 . Received Date: 2019-08-16T10:05:36.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Organization: The Trustees of Columbia University in the City of New York

Start Date*: 04-01-2024 **End Date*:** 03-31-2025 **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	[redacted]

Additional Equipment: File Name:

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

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**	[redacted]

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

Tracking Number: GRANT12921503 Funding Opportunity Number: PA-19-056 . Received Date: 2019-08-16T10:05:36.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Organization: The Trustees of Columbia University in the City of New York

Start Date*: 04-01-2024 **End Date*:** 03-31-2025 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	[redacted]
3. Consultant Services	
4. ADP/Computer Services	[redacted]
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal costs	[redacted]
9 . Illumina sequencing, FACS Core Costs, Equipment Maintenance contracts	[redacted]
10 . GRA tuition plus GRA fees	[redacted]
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	62.0	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS, [redacted]

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification*	File Name: Lomvardas.Zonal.R01.Budget.Justification.pdf (Only attach one file.)

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

Tracking Number: GRANT12921503 Funding Opportunity Number: PA-19-056 . Received Date: 2019-08-16T10:05:36.000-04:00

Budget Justification

Key Personnel

Stavros Lomvardas, P.I. (2.0 calendar months). Dr. Lomvardas has extensive experience in genetic and epigenetic analysis of the olfactory system and has an established track record in the study of interchromosomal genomic interactions in gene regulation. Dr. Lomvardas will supervise the proposed experiments, will provide technical assistance and troubleshooting, and will help with the computational analysis and interpretation of the data.

Other Personnel

Lisa Bashkirova, Graduate Research Assistant (12 calendar months). Ms. Bashkirova is a senior graduate student that performed all the zonal experiments presented in this grant and she has the skills to perform the proposed experiments and to analyze them computationally.

Marianna Zazhytska, Postdoctoral Research Scientist (6 calendar months). Dr. Zazhytska is a new postdoc in my lab that has experience with HiC experiments and computational analysis from her graduate work that included an extended collaboration with Erez Lieberman-Aiden. She will assist Ms. Bashklrova with her HiC experiments.

Alice Mumbey-Wafula, M.S., Research Staff Associate. (6 calendar months). Ms. Mumbey-Wafula has a Master's degree in Molecular Biology and Biochemistry and has been working as a research staff associate in my lab for several years- she will be responsible for managing the vast mouse colony related to this project, by assisting with genotyping, setting crosses, performing methimazol ablation experiments and preparing MOE sections for IF and RNA ISH experiments.

Other Direct Costs

General Lab Supplies. The proposed experiments require mouse genotyping via PCR (Taq polymerase+primer costs), use of primary and secondary antibodies, papain dissociation kits for FACS, generation of RNA probes for ISH experiments, primary and secondary antibodies, cDNA synthesis and cloning (for making riboprobes), mRNA purification kits and other standard lab supplies such as slides, coverslips, pipet tips, tubes, glassware and plasticware, tissue culture plates, serum and media. We calculated the sum of all these expenses as **$20,000/year.**

Illumina Sequencing: The proposed experiments include multiple in situ HiC, ChIP-seq, Cut and Run and RNA-seq experiments for various mutants. Every experiment will be performed in biological triplicates. Moreover for our proposed HiC experiments, we seek the genomic resolution afforded by 1 billion HiC reads, that we will be using at least 4 Next-seq flow cells per condition. Importantly, for each mutant strain we will be micro-dissecting and comparing zones 1 and 5, and in certain experiments we will be characterizing the various developmental stages of each zone.

Total sequencing costs are estimated at: [redacted]

FACS Core Costs.
The proposed experiments require extensive FAC-sorted populations of GFP or iRFP labeled olfactory sensory neurons. We calculate needing 100 hours of FAC-sorting per year with hourly cost of $100/hour
Total FACS core costs are estimated at [redacted]

Animal Costs. The current per diem cost for mouse cages at Columbia is [redacted]
We have proposed analysis of the following genotypes:
1. NFIAfl/fl, Krt5CreER, TdTomato
2. NFIB fl/fl; Krt5CreER, TdTomato
3. NFIX fl/fl; Krt5CreER, TdTomato
4. NFIA fl/fl; NFIX fl/fl; Krt5CreER, TdTomato
5. NFIB fl/fl; NFIX fl/fl; Krt5CreER, TdTomato
6. Gg8tTA; tetO-NFIAT2ANFIBiresmCherry

7. Krt5CreER; Cre-inducible tTA; tetO-NFIAT2ANFIBiresmCherry
8. Krt5CreER; NFIA,B fl/fl; Cre-inducible tTA; tetO-NFIAT2ANFIBiresmCherry
9. Gg8-Cre; Dot1lfl/fl; TdTomato
10. Mash1-CreER; Dot1lfl/fl; TdTomato
11. Olfr1507iresCre; Dot1lfl/fl; TdTomato

In total we have 11 different genotypes that contain 3 or 4 different alleles. To obtain sufficient number of animals from each genotype we anticipate having 8 breeding cages for each genotype (88 cages total). Moreover, we will have 3 cages for maintenance of each one of the different alleles (11 different alleles described here, 33 cages). Finally, in addition to the breeding cages, male and female mice will be housed separately until the reach the desired age, thus we anticipate another 8 cages per genotype (88 cages). Total number of cages anticipated for this project in the coming year is (88+33+88=209).

Total mouse costs: [redacted] in year 1 with anticipated increases of [redacted] per year.

Publications. We anticipate publishing one manuscript per year describing these findings with an estimated publication cost of [redacted] per year.

ADP/Computer costs. An amount of [redacted] per year is requested to access Columbia's Habanero high performance computing server for the computational processing power required for data analysis.

Equipment Maintenance Contracts.
Confocal Service Contract (Zeiss). Our yearly service contract is [redacted] and we have calculated that 1/3 of our microscope use will be dedicated to this project, thus we calculate a yearly cost of [redacted]

Cryostar Service Contract (Leica). Our yearly service contract for the cryostat is [redacted] and we anticipate that 1/3 of its use will be dedicated to this project thus the yearly cost will be [redacted]

Conference travel. We are requesting [redacted] per year to attend and present results at Neuroscience and Nuclear Architecture conferences. We envision a total of up to 2 conferences per year spread between the PI, Graduate Research Assistant and Postdoctoral Research Scientist. ([redacted]/conference on average).

Graduate student tuition and fees. Funds are requested to cover the Graduate Research Assistant's tuition and fees according to the schedule required by the Columbia University Graduate School of Biomedical Sciences. ([redacted] tuition and [redacted] fees, in year 1 with [redacted] annual increases.)

Indirect Costs:
Sponsored Projects Administration certifies that the Primary Place of Performance for the proposed research will occur at Jerome L. Green Science Center, 3227 Broadway New York NY, 10027, 4th floor L4.005, an on-campus location, and that the appropriate F&A rate is [redacted]

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)
Section A, Senior/Key Person	[redacted]
Section B, Other Personnel	[redacted]
Total Number Other Personnel	15
Total Salary, Wages and Fringe Benefits (A+B)	[redacted]
Section C, Equipment	[redacted]
Section D, Travel	[redacted]
1. Domestic	[redacted]
2. Foreign	[redacted]
Section E, Participant/Trainee Support Costs	[redacted]
1. Tuition/Fees/Health Insurance	[redacted]
2. Stipends	[redacted]
3. Travel	[redacted]
4. Subsistence	[redacted]
5. Other	[redacted]
6. Number of Participants/Trainees	[redacted]
Section F, Other Direct Costs	[redacted]
1. Materials and Supplies	[redacted]
[redacted] Costs	[redacted]
3. Consultant Services	[redacted]
4. ADP/Computer Services	[redacted]
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	[redacted]
7. Alterations and Renovations	[redacted]
8. Other 1	[redacted]
9. Other 2	[redacted]
10. Other 3	[redacted]
Section G, Direct Costs (A thru F)	[redacted]
Section H, Indirect Costs	[redacted]
Section I, Total Direct and Indirect Costs (G + H)	[redacted]
Section J, Fee	[redacted]
Section K, Total Costs and Fee (I + J)	[redacted]

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

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)

Tracking Number: GRANT12921503 Funding Opportunity Number: PA-19-056 . Received Date: 2019-08-16T10:05:36.000-04:00

PHS 398 Cover Page Supplement

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. 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

5. 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:

Tracking Number: GRANT12921503 Funding Opportunity Number: PA-19-056 . Received Date: 2019-08-16T10:05:36.000-04:00

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 03/31/2020

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

Tracking Number: GRANT12921503
Funding Opportunity Number: PA-19-056. Received Date: 2019-08-16T10:05:36.000-04:00

Specific Aims

Olfactory receptor (OR) genes are expressed in a monogenic, monoallelic, and seemingly stochastic fashion in olfactory sensory neurons (OSNs) of the main olfactory epithelium (MOE)[1]. This singular expression pattern is orchestrated by the formation of a multi-chromosomal enhancer hub over the transcriptionally chosen OR[2], followed by a feedback signal that prevents the transcriptional activation of additional OR alleles[3]. Although great progress was achieved towards the understanding of these two regulatory mechanisms, the process that restricts the transcription of specific OR gene repertoires to continuous "zones" along the dorso-ventral and apico-basal axes of the MOE[4] remains elusive. The zonal pattern of OR gene expression was realized more than two decades ago[5,6], yet very little is known about *cis* regulatory elements and *trans* factors imposing these spatial restrictions.

To obtain insight into this enigmatic process we performed a comprehensive comparison of zone 1 and zone 5 OSNs and progenitor cells, which revealed striking differences in epigenetic marks and nuclear architecture of zone 1 and 5 OR genes at their respective zones. To identify sequence-specific factors that orchestrate these differences we searched for zonally enriched motifs on OR promoters that correspond to zonally expressed transcription factors. We identified NFI motifs as being enriched on zone 5 OR promoters and paralogous transcription factors NFIA, B, and X as being enriched in zone 5 OSN progenitors (NFIA,B) and OSNs (NFIX). Conditional triple deletion of NFIA, B, and X in the developing MOE resulted in strong downregulation of zone 4-5 OR transcription and ectopic expression of zone 2-3 OR genes in zone 5, providing a striking example of homeotic transformation. *In situ* HiC of NFI KO OSNs revealed a similar transformation in nuclear architecture, supporting a role of NFI proteins as master regulators of zone 5 OR identity. We propose experiments that will determine the role of each NFI gene in establishing zonal OR identities and will define the developmental timing of their action, as well as experiments that will explore the molecular mechanism by which NFI proteins regulate zonal OR expression.

Aim1: Determine <u>which</u> NFI genes are required and sufficient for establishment of zone 5 identity.
Triple deletion of NFIA, B, and X in the stem cells of the MOE perturbs the expression of zone 4 and 5 OR genes and allows ectopic expression of zone 2-3 ORs. Deletion of only NFIA, B also results in downregulation of zone 4-5 ORs, but not in ectopic expression of zone 2 ORs, suggesting a role of NFIX in repressing these ORs in zone 5. Finally, triple deletion of NFI in mature OSNs does not alter zonal OR transcription, suggesting a transient role of these factors in specifying zone 5 OR identity. We seek to identify which of the 3 NFI genes are required and sufficient for zone 5 OR expression and for repression of ectopic OR expression in zone 5.

A. We will generate individual NFIA, B and X KO mice and ask which NFI genes are required for activation of zone 4-5 OR genes and for repression of ectopic ORs.
B. We will ectopically express NFIA and NFIB proteins in progenitors and OSNs of zones 1-4 to determine the sufficiency of these factors in specifying zone 5 identity.

Aim2: Determine <u>how</u> NFI proteins regulate zonal OR expression.
Given that continuous NFI activity is not required for stable zone 5 OR expression in mature OSNs, NFI proteins do not act as traditional transcriptional activators but as factors that establish an early epigenetic "imprint" that enables transcription of zone 5 ORs in mature OSNs. Our data suggest that NFI-dependent recruitment to nuclear OR compartments contributes to this epigenetic memory. Furthermore, preliminary analysis suggests that zonal deposition of repressing histone modification H3K79me3 prevents "out of zone" ORs from being expressed in this zone. Thus, we seek to identify the mechanisms by which NFI proteins enable zone 4-5 ORs and prevent zone 2-3 ORs from expression in zone 5. We have three major goals:

A. First, using Cut&Run, we seek to identify the direct genomic targets of NFI proteins on FAC-sorted progenitors and OSNs from zone 5 as well as in OSNs expressing the zone 5 OR Olfr1507.
B. We wish to determine how NFI proteins affect the nuclear architecture of zone 4-5 and zone 2-3 ORs by performing zonal *in situ* HiC in NFI KO and transgenic mice expressing NFI in every zone.
C. We will explore whether the repressive mark H3K79me3 keeps "out of zone" ORs silent in zone 5, and whether NFI proteins are mediating H3K79me3 deposition. We will also seek additional repressive histone and DNA modifications deposited on ORs in a zonal fashion.

Our experiments will provide mechanistic insight to a fundamental process of developmental patterning that remained intractable since 1993 and will reveal general principles for the regulation of genomic interactions and epigenetic changes that govern transcriptional choices and cellular identities during differentiation.

Significance

Pattern formation is a fascinating phenomenon that governs fundamental developmental processes in plants and animals, vertebrates and invertebrates[7-9]. From the classic theoretical predictions on the chemical basis of morphogenesis[10] to the seminal demonstration of the genetic control of segmentation in drosophila[11,12]; and from the elucidation of motor neuron pool identity along the spinal cord axes[13] to the explanation of photoreceptor patterning in invertebrate omatidia[14], developmental biologists have made strides in understanding various forms of patterning. However, patterning in the main olfactory epithelium (MOE), as manifested by the zonal expression of olfactory receptor (OR) genes[5,6], remains intractable. This constitutes a glaring gap of knowledge for sensory biology for two main reasons: First, we cannot fully comprehend how an OR is chosen if we do not decipher why this choice is restricted to a zonal OR repertoire. The realization that OR gene choice is influenced by the position of an OSN begs the fundamental question: what mechanisms impose deterministic restrictions to an otherwise stochastic process? This question becomes even more pressing upon our recent discovery that OR gene choice is mediated by a multi-chromosomal enhancer hub that interacts in *trans* with the active OR allele, regardless of the zonal identity of the chosen OR[15,16]. Therefore, determining how this zonally invariant enhancer hub exhibits differential affinity for distinct OR combinations along the 5 zones, may provide valuable insight into the mechanism of OR choice itself.

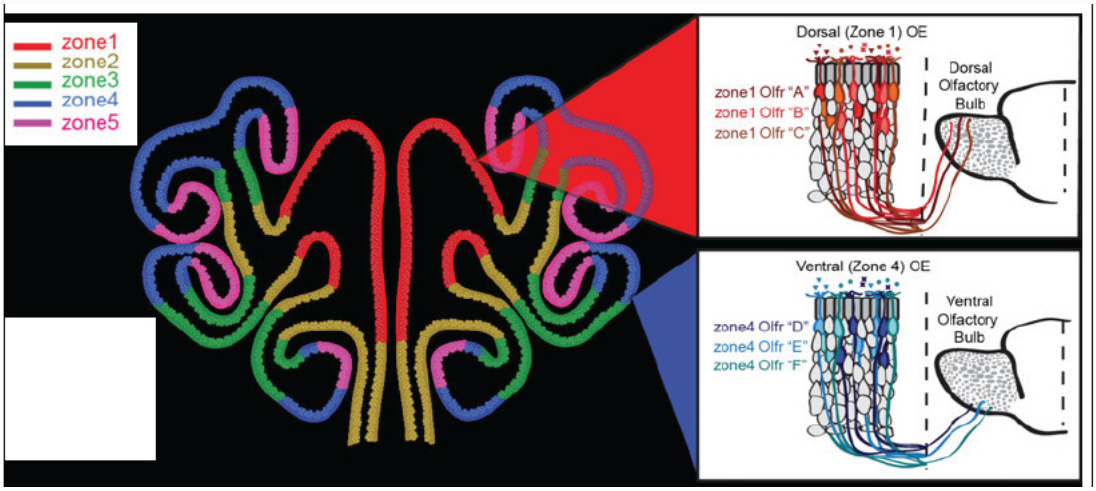

Figure1: The 5 major zones of OR gene expression. Coronal MOE section depicting the 5 zones (left) and the projections of zonal OSNs to the olfactory bulb.

Second, without knowing the regulatory mechanism that orchestrate zonal OR expression, we cannot address the biological significance of this expression pattern. The conservation of zonal OR expression in most vertebrates suggests that spatial restrictions of OR gene choice is selected through evolution. The fact that zonal coordinates are preserved in the olfactory bulb raises possible implications in olfactory perception[17]. For example, there is evidence that dorsal glomeruli receiving input from zone I respond to odors associated with innate fear responses[18]. Thus, although most odorants lack strong innate valance[19,20] and activate dispersed glomeruli[21], zonal boundaries may influence odor perception. Even if zonal OR expression is not contributing to odor perception, it may have different functional roles. For example, zonal restrictions may facilitate axon fasciculation and convergence of like axons to a common glomerulus; if all the OSNs expressing the same OR were evenly distributed across the MOE axes, then they may fail to converge to distinct glomeruli, because like axons will be rarely proximal to each other. Finally, we cannot exclude the possibility that zonal restrictions facilitate the singularity of OR gene choice; if ~1200 OR genes could be activated in each OSN, then the processes assuring singularity of choice may not be adequate. The OR-elicited feedback signal may not be fast enough if 2400 OR alleles are transcriptionally competent in each OSN, and more than one OR alleles may be able to associate simultaneously with the activating enhancer hub, violating the "one receptor per neuron rule". Therefore, our work is creating the foundation for a future experimental interrogation of the role of zones in olfactory coding, OSN targeting, and singular OR choice.

In addition to its importance for olfaction, this project has general significance for developmental biology and for the emerging field of nuclear architecture. The role of long-range and *trans* genomic interactions in developmental patterning is starting to be appreciated in numerous fields. In developing cardiomyocytes, a multi-chromosomal gene hub coordinates the alternative splicing of cardiomyocyte-specific genes[22], promoting cardiogenesis. Further, photoreceptor gene choice in flies[23], Th1/Th2 lymphocyte differentiation in mice[24], and X-chromosome inactivation in humans[25,26] are orchestrated by interchromosomal contacts, highlighting the diversity of cell specification processes and organisms utilizing this mechanism. Super-enhancers (SEs) tend to form interchromosomal compartments, likely promoting transcriptional

robbustness[27,28]. SPRITE, an alternative to HiC, revealed multi-chromosomal interactions organized by nuclear RNA speckles[29], and similarly, the non-coding RNA Firre promotes interchromosomal interactions[30,31]. In the case of human antiviral responses, multi-chromosomal transcription factor "repositories" appear essential for the activation of INFβ[32,33]. Multi-chromosomal interactions are also used by trypanosome[34] and plasmodium[35,36] during the process of antigenic variation[34,37]. Therefore, deciphering mechanisms that regulate the formation of interchromosomal interactions across the axes of the MOE will have general impact in understanding 3D genome organization, especially since NFI proteins regulate a plethora of developmental processes[38-44].

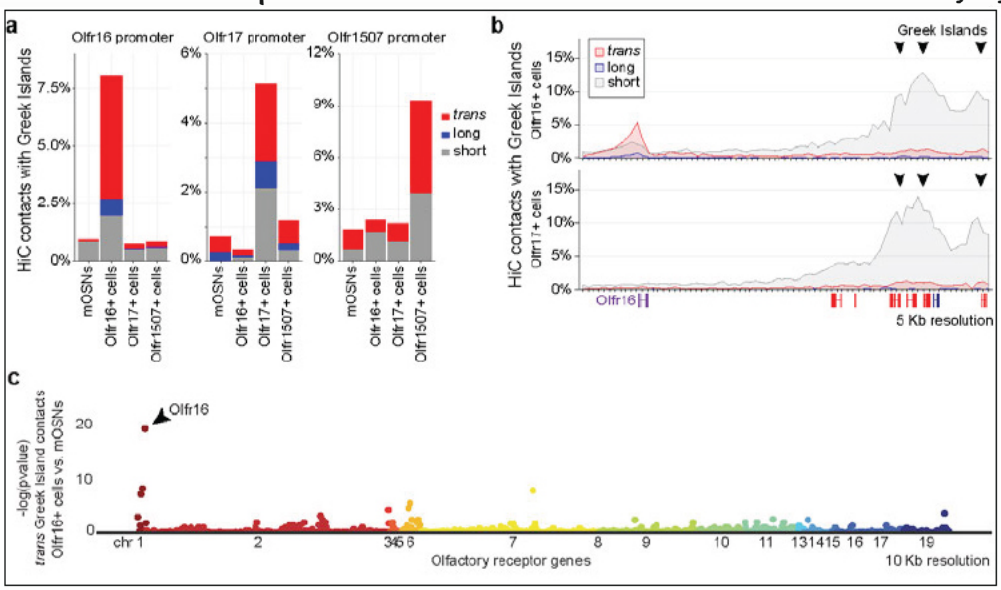

Figure2: Transcriptionally Active ORs interact with multiple *trans* and *cis* Greek Islands regardless of zonal identity. a, Increased contacts between the active OR promoter and Greek Islands located in short range *cis* (grey), long range *cis* (>5Mb, blue) and *trans* (red). Greek Island interactions are expressed as the fraction of the total HiC contacts mapped to each promoter. Olfr16 is a zone 1 OR, Olfr17 is zone 2, and Olfr1507 is zone 5 **b,** Profile of the OR cluster containing Olfr16 reveals increased contacts, expressed as fraction of the total HiC contacts mapped to each position (5 Kb resolution), in Olfr16^{+} or Olfr17^{+} cells. **c,** Manhattan plot of Greek Island contacts with OR genes reveals that in Olfr16^{+} cells the Olfr16 locus is the most significantly enriched for Greek Island contacts.

Finally, there is potential translational significance of our proposal. Recent experiments revealed the feasibility of cell transplantation experiments in restoring olfactory sensitivity in a mouse model for congenital anosmia[45]. With anosmia and hyposmia affecting the emotional wellness[46] and longevity[47] of affected individuals, such cell-based approaches may soon reach the clinic. However, without being able to restore the full spectrum of odorant perception, such approaches will fall short. Deciphering the molecular regulators of zonal OR expression will eventually allow us to generate "designer" OSN progenitors that will produce zonal OSNs of our choice, allowing us to "correct" specific odor detection deficiencies or to restore damaged MOEs with the proper zonal consistency.

Innovation

Our proposal is conceptually and technically innovative. On the conceptual front we tackle a problem that has not been solved in any model organism, thus, by default, we break new ground. The correlation between formation of zone-dependent interchromosomal interactions in OSN progenitors and zonal transcriptional restrictions in mature OSNs (mOSNs) suggests that we uncovered a completely novel mechanism for establishing epigenetic memory during developmental patterning. In this note, this is the first demonstration that interchromosomal interactions can be subject to deterministic spatial restrictions that change along the dorso-ventral axis of an organ in a reproducible and stereotypic fashion. Until now such super long range genomic interactions were considered highly variable and appropriate only for stochastic transcriptional outcomes[48]. Moreover, this is the first ever demonstration of a role of NFI proteins in the regulation of nuclear architecture and in establishing epigenetic memory, opening new routes for the study of these protein at the context of cell type specification. Finally, gradients of H3K79me3 along developmental axes represent a completely novel finding, adding a new potential function of this histone modification in patterning.

From the technical standpoint we utilize cutting edge genomic approaches, such as *in situ* HiC and Cut&Run[49], which are applied in zonally dissected, FAC-sorted populations from mice carrying numerous genetically modified alleles. To our knowledge, we are the first to combine these state-of-the-art genomic approaches with sophisticated and complex genetics in any model organism. Thus, we approach an elusive and fundamental biological problem with a multi-disciplinary approach that deploys state of the art techniques and is poised to produce novel regulatory principles with general impact in developmental biology.

Approach

Soon after the identification of the mammalian OR superfamily[50], it became apparent that the expression of a given OR gene is spatially restricted to a specific segment, or zone, along the dorso-ventral and apico-basal axes of the MOE[5,6]. In mice there are 5 broad zones of OR expression[51] (Figure 1) plus the septal organ[52,53] and the Gruenemberg ganglion[54,55]. Each zone represents an area that expresses only 50-200 ORs, in a highly reproducible and stereotypic fashion. Zones do not have sharp borders, but continuous boundaries in which ORs from neighboring zones are expressed in an intermingled fashion[56]. Zonal OR expression is conserved across evolution and reported in flies[57], zebrafish[58,59], frogs[60], salamanders[61], and various mammalian species, from rodents to goats[62]. This expression pattern is not an evolutionary remnant inherited from aquatic vertebrates; although about 100 of the mammalian ORs are class I[63], or fish-like ORs, the majority of the mammalian chemoreceptors are class II ORs that emerged in amphibians[60,64]. Thus, as the OR repertoire expanded dramatically during terrestrial adaptation[65], the "logic" of zonal expression was preserved. Yet, nothing is known about the mechanisms that generate zonal boundaries. In fact, we only understand the spatial separations among the major chemoreceptor subfamilies, olfactory and vomeronasal receptors (VRs)[66].

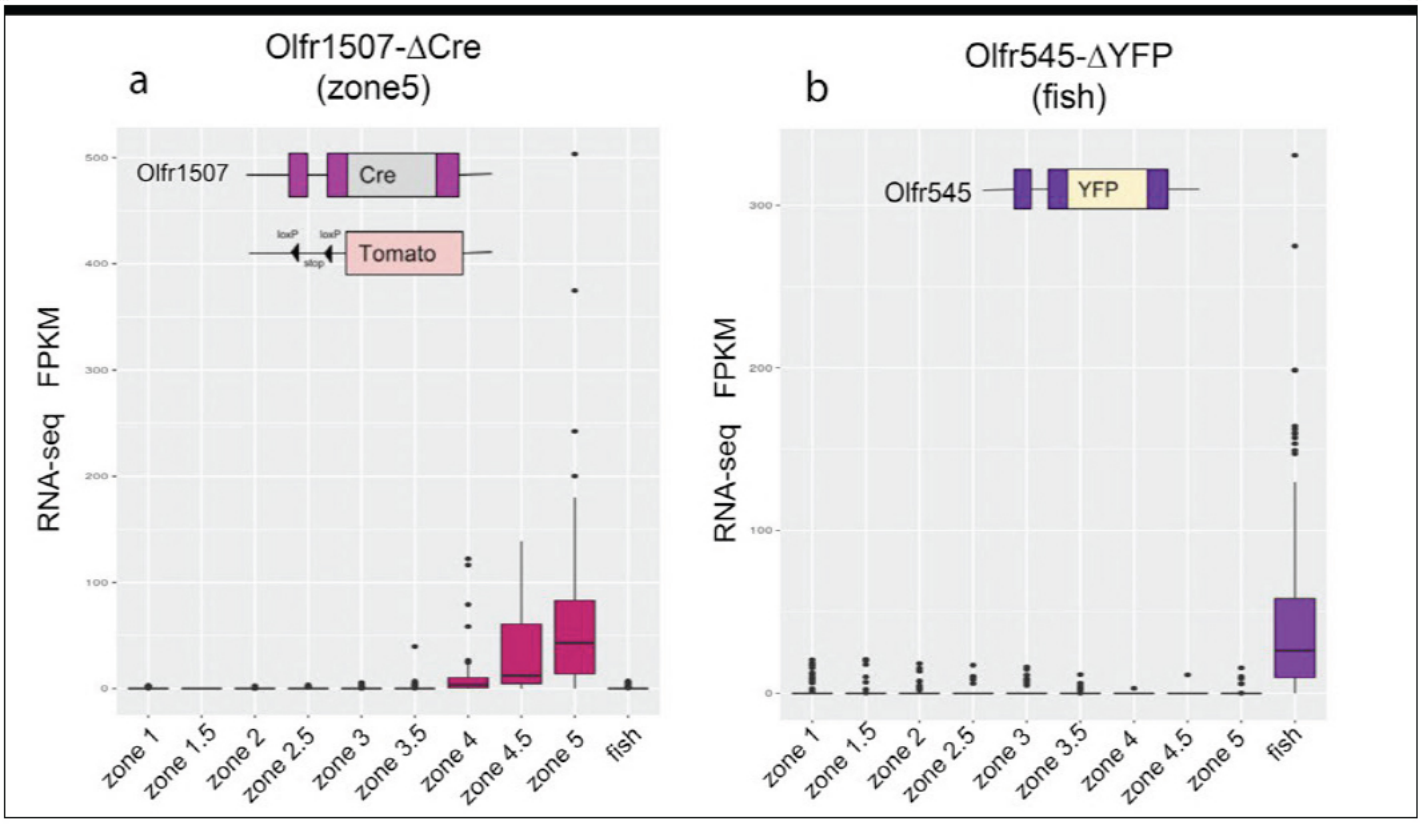

Figure3: An OSN has the potential to express any OR from its zone. a, lineage tracing experiment from a pseudogenized zone 5 OR (Olfr1507) and RNA-seq analysis of the OSNs that initially choose Olfr1507, reveals preferential switching to zone 5 and 4.5 (border between zones 4-5), but also infrequent but significant switch to zone 4 ORs. **b**, In contrast a class I (fish-like OR) switches only to class I ORs.

Briefly, ORs and VRs become restricted to the MOE and vomeronasal organ (VNO) respectively, under the control of transcription factors Fezf1 and Fezf2[67,68], during embryonic development. Within these chemosensory organs there are two additional major divisions. In the VNO, the two main classes of VRs (V1Rs and V2Rs) become spatially segregated in basally and apical sensory neurons (VSNs). This spatial distinction is initiated by transcription factors Bcl11b[69] and maintained by transcription factor AP2-ε[70]. Bcl11b appears to also act in the MOE, where it regulates the distinction between class I and II ORs[71]. There are additional fundamental differences in the regulation of these ORs. Class II OR gene activation is mediated by multiple *trans*-interacting enhancers, the Greek Islands, which form a multi-chromosomal enhancer hub over the active OR in an Lhx2- and Ldb1-dependent fashion. In contrast a single enhancer, the J element that does not interact with Greek Islands in *cis* or *trans*, appears sufficient for the regulation of class I ORs[72,73], which all reside in a single genomic cluster. Consistent with a distinct regulatory logic between the two major OR classes, straight Lhx2 KO mice express class I but not class II ORs[74], and the same is true for Ldb1 KO

OSNs[16]. Finally, unclear are the mechanisms that determine which sensory neurons express other major types of chemoreceptors, such as MS4A[75] and TAAR[76] genes in the MOE, and FPRs[77] in the VNO. However these gene families are also organized in a single cluster each, and have different nuclear organization than class II ORs[16,78], suggesting that class I-like mechanisms may by involved in their regulation. In contrast, the >1000 class II ORs are organized in 60 genomic clusters distributed across 18 chromosomes making it difficult to regulate them as a group by targeting a single *cis* regulatory element.

A challenge in deciphering zonal OR expression stems from the observation that the multi-chromosomal enhancer hub, which mediates class II OR choice, has similar constitution across zones[79] and interacts at similar frequencies with the active OR irrespective of zonal identity[15,16] (Figure 2). Moreover, the intergenic class II enhancers (known as Greek Islands) are homogeneously bound by Lhx2, Ebf and Ldb1, and have comparable accessibility in these 3 zones[79]. Instead, what may be subject to zonal restrictions is not the activity of the Greek Islands, but their affinity for different OR genes. Thus, class II OR choice may be accomplished by a combination of deterministic and stochastic mechanisms: a deterministic mechanism that uses spatial cues to "mark" the ORs that can be chosen in each zone, and a stochastic mechanism that deploys the same multi-enhancer hub, across zones for a random selection among enabled ORs. Alternatively, the Greek Island hub could interact with any OR in any OSN, but transcription factor combinations would determine the consequences of hub-OR interactions via specific binding to OR promoters.

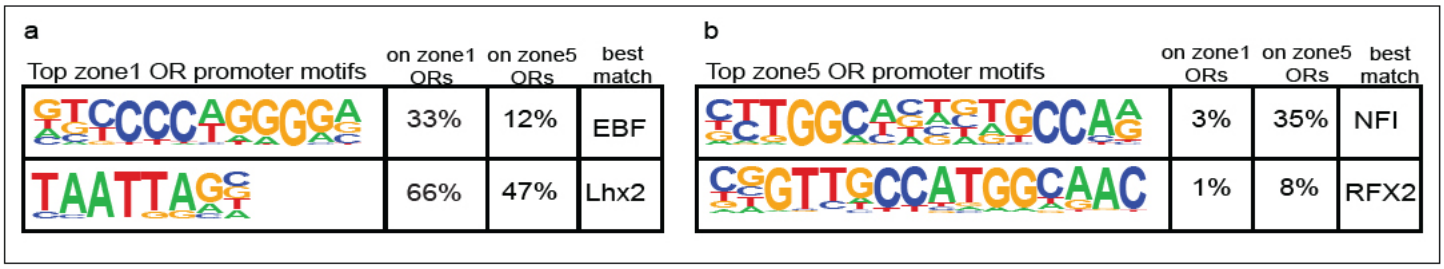

Figure4: NFI motifs enriched on promoters of zone 5 ORs. Using HOMER for *de novo* motif analysis we identified NFI motifs as being enriched on zone 5 promoters over zone 1 promoters, which are enriched for Ebf and Lhx2. We used zone 1 and 5 OR definitions from Tan and Xie 2018.

Our data suggest that a combination of both models is in place: In zone I, only zone I ORs have the proper genomic positioning that would allow association with the Greek Island hub, whereas in zone 5 every OR is privy to these interactions and, therefore, eligible for activation. However, only zone 5 OR genes are chosen because zone 1-4 OR genes are repressed via H3K79me3. Our data suggest that different NFI family members are responsible for the recruitment of zone 5 ORs to the OR compartments and, possibly, for the zone 5-specific repression of "out of zone" ORs. Thus, an elegant mechanism for zonal OR expression deploys two competing processes: gradual "enabling" of an increasing number of OR genes via recruitment to the OR compartment and selective repression of the enabled "out of zone" ORs).

Aim1: Determine <u>which</u> NFI genes are required and sufficient for establishment of zone 5 identity.

<u>Preliminary data:</u> Before attempting to identify common features between ORs from the same zone we clarified whether an OSN has the potential to transcribe every OR from its respective zone. To answer this, we performed RNA seq on FAC-sorted OSNs that had originally chosen a pseudogenized Olfr1507 allele (Olfr1507deliresCre from zone 5), using a lineage tracing strategy[80]. RNA seq of these FAC-sorted OSNs revealed that any zone 5 OR[51] can replace the mutant Olfr1507 allele (Figure 3a). Interestingly, zone 4 ORs are also detected in these cells, albeit at lower levels, consistent with the continuous nature of zones. The same analysis with a pseudogenized class I OR[81], revealed expression of other class I ORs only, without "leakage" to class II ORs, consistent with a sharp cell fate distinction between class I and II OSNs (Figure 3b).

We searched for unique features that distinguish zone 5 ORs from ORs that cannot be expressed in Olfr1507^{+} OSNs. We compared the promoters of ORs expressed in the most distant zones, zones 1 and 5, by performing *de novo* motif analysis on 1Kb fragments centered at the transcriptional start site (TSS) of these genes, since these sequences were previously shown to contain zonal information[82,83]. This revealed enrichment of Ebf and Lhx2 motifs on zone 1 promoters, consistent with previous reports on zone 1 OR Olfr151[83,84] (Figure 4a). Zone 5 promoters, however, have low quality Ebf motifs and strong enrichment for nuclear factor I (NFI) motifs (Figure 4b). NFI motifs were previously reported on genes expressed in mOSNs[85] but the functional significance of these elements in the MOE is unclear.

The NFI family of transcription factors has 4 members NFIA, B, C and X, which bind on DNA as homo- or hetero-dimers and act as activators or repressors of transcription[86,87]. Zonal RNA-seq comparing the levels of NFI genes in mature OSNs and in various OSN progenitors from zones 1 and 5 revealed increased expression of NFIA, B, and X in zone 5, with distinct transcriptional dynamics: NFIA and B are more highly expressed in zone 5 progenitor cells, whereas NFIX is upregulated in zone 5 OSNs (Figure 5a, b). Zone-specific OMNI-ATAC-seq analysis[88] in the same FAC-sorted populations revealed NFI as the motif that is most highly enriched in zone 5 specific peaks, supporting a functional role for these zonally expressed factors (Figure 5c). To achieve these zonal dissections we used Olfr16iresGFP (zone 1) and Olfr1507iresGFP (zone 5) mice to identify these zones. The identity of OR genes in each micro-dissection, as determined by RNA-seq, was used in every experiment to confirm the accuracy of our dissection. These micro-dissections had low level contamination from the neighboring zones, however, there was no zone 1-3 ORs in micro-dissected zone 5 and not zone 3-5 ORs in micro-dissected zone 1.

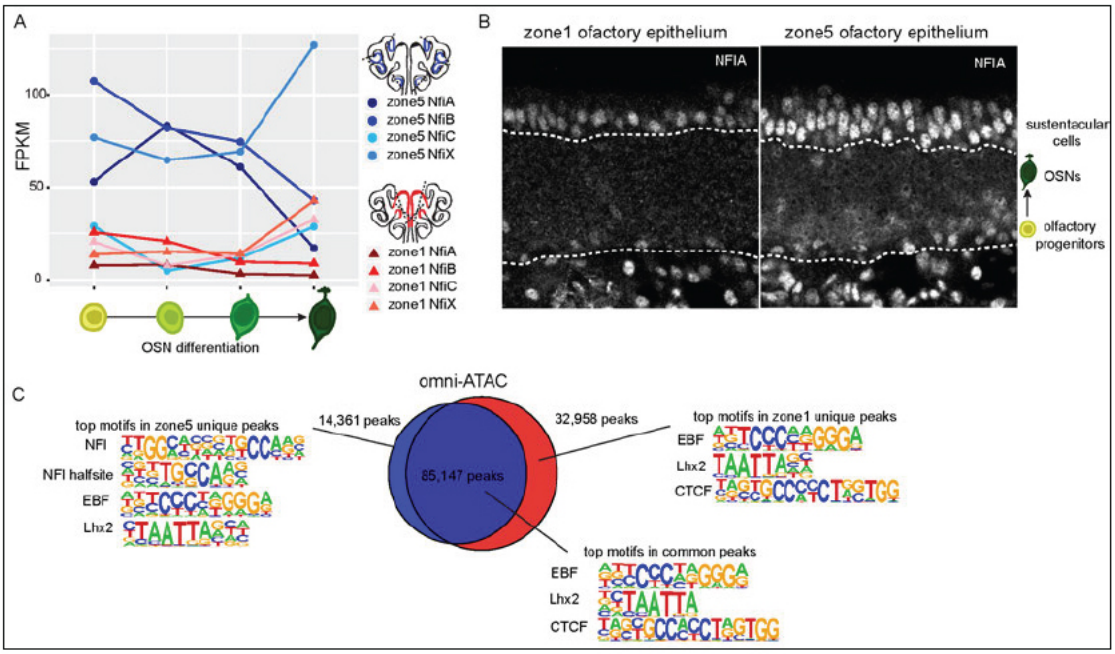

Figure5: Zone 5 expression of NFI proteins. A, expression of NFI genes in zone 1 (red) and 5 (blue) during differentiation. From left to right cells are Mash1^{+}, Mash1^{+}/Ngn1^{+}, Ngn1^{+}, Omp^{+} **B**, NFIA immunofluorescence shows strong NFIA expression in apically located Sustentacular cells of both zone1 and zone 5, and weaker expression in the basal (progenitor) cell layers of zone 5. The area between the doted lines contains OSNs and OSN progenitors. C, omni-ATAC-seq from zone 1 and zone 5 shows that in zone 5 specific peaks, the most common motif is NFI, and the second most common is a half site of NFI.

We embarked in a genetic analysis of the functional role of the three zonal NFI genes, using conditional KO mice for the three zonal NFI genes, shared with us from Dr. Gronostasjki. We first performed a homozygote triple NFI deletion in the stem cells (HBCs) of the MOE, using the Krt5CreER mice. Deletion was induced by tamoxifen injection of adult mice[16]. A Cre-inducible reporter (TdTomato) was used to mark the recombined stem cells and of all their progeny[16]. We induced differentiation of the NFI KO stem cells via methimazol ablation of the MOE[89-92]. We let the MOE to fully recover by waiting for 40 days, and then we FAC-sorted tomato+ cells from control and triple NFI KO mice. As previously described, we used tomato signal intensity to isolate OSNs, which were subjected to expression analysis[16].

RNA-seq comparing control and triple KO OSNs sorted from the whole MOE revealed a strong reduction in the levels of zone 4 and 5 ORs (Figure 6a), consistent with the hypothesis that NFI proteins regulate zone 4-5 OR expression. We also noticed a significant increase in zone 2 OR mRNA, suggesting ectopic expression in zones 4-5. Indeed, RNA-seq on OSNs sorted from micro-dissected zones 4-5 revealed strong expression of zone 2-3 ORs in the triple NFI KO OSNs, together with strong downregulation of zone 4-5 ORs (Figure 6b). This ectopic OR expression explains why KO OSNs differentiate properly and express mOSN markers, since OR expression is required for OSN differentiation[93,94]. Thus, triple NFI deletion in HBCs results in a homeotic transformation of zone 4 and 5 OSNs to zone 2 and 3 OSNs, in regards of OR expression. Intriguingly, in NFIA, B double KO mice, this homeotic transformation shifted towards higher zone indexes, with zone 3 and 4 ORs replacing zone 5 ORs (Figure 6c). Thus, NFIX may be repressing zone 2 ORs in zone 5, while NFIA and B are responsible for zone 5 OR expression. Finally, it should be noted that among 200 other genes that are significantly enriched in zone 5 OSN and progenitors, only 30 are significantly downregulated in triple NFI KO (Figure 6e), with no transcriptional regulators being included in this list. Thus, most likely we are

not describing a drastic change in cellular identity but a specific alteration in zone 5 OR gene regulation that is likely mediated by the transiently expressed NFIA and B.

To examine whether continuous NFI activity in mOSNs is required for stable zone 5 OR transcription we deleted NFIA, B, and X with OmpiresCre, which is only expressed in mOSNs. Although NFIA and B become downregulated with differentiation, they are still expressed in mOSNs, thus, they may still be required for OR expression. This is the case for Lhx2, which is downregulated during OSN differentiation yet, its continuous expression is necessary for OR transcription[16,79]. However, triple NFI deletion with OMPiresCre, which is expressed only in mOSNs, revealed small downregulation of zone 4-5 ORs (Figure 6d) suggesting that NFI activity is predominantly required in OSN progenitors. Thus, our data confirm a role of NFI proteins in establishing zone 5 OR expression during differentiation and raise the following questions:

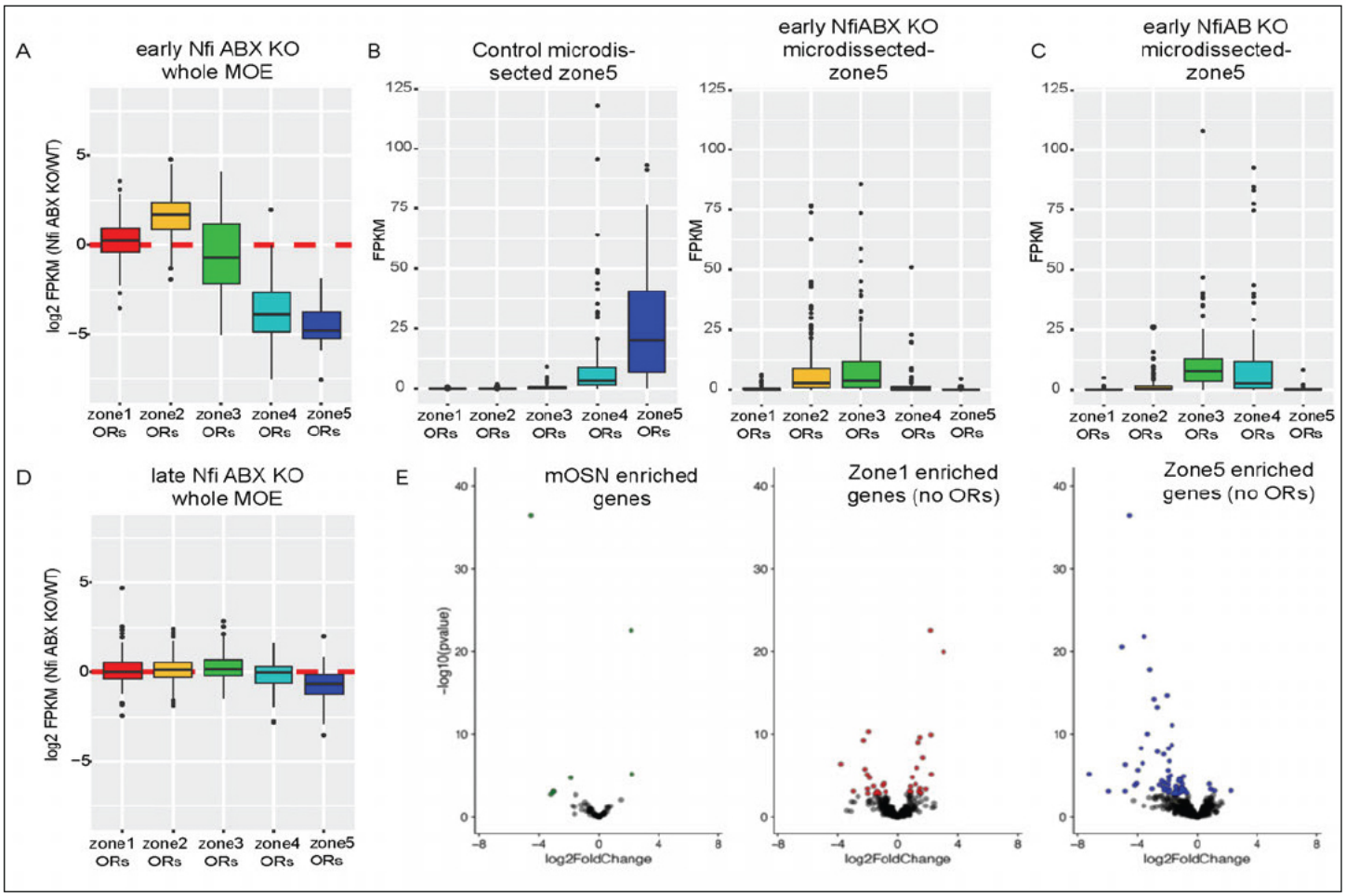

Figure6: Triple NFI deletion abolishes zone 4 and 5 OR expression and allows ectopic expression of zone 2 and 3 ORs. A, RNA-seq in mOSNs sorted from whole MOE shows reduction of zone 4 and 5 OR mRNAs and increase in zone 2 OR expression, in early (Krt5CreER driver) NIF triple deletion. **B**, RNA-seq in mOSNs sorted from micro-dissected zone 5 shows significant downregulation of zone 4 and 5 ORs and concomitant upregulation of zone 2 and 3 ORs. **C**, RNA-seq in mOSNs sorted from micro-dissected zone 5 shows strong downregulation of zone 5 ORs, sustained expression of zone 4 ORs, ectopic expression of zone 3 but not zone 2 ORs in early NFIA, B double KO mice. **D**, RNA-seq in mOSNs sorted from whole MOE shows small effects on zone 4 and 5 ORs in late (OmpiresCre) triple NFI KO mice. **E**, volcano plots showing the mOSN markers and zone 1-enriched genes are not affected by early triple NFI deletion. Only a small fraction of zone 5-enriched genes is downregulated in early triple NFI KO mice, and non of those downregulated genes is a transcriptional regulator. Data represent averages from biological triplicates

1A. NFIA, NFIB, or both NFIA and NFIB are required for zone 5 OR transcription? Generation of individual NFIA and NFIB KO mice with Krt5CreER, will reveal which NFI gene regulates zone 4 and 5 OR transcription. Control and KO littermates will be characterized by RNA-seq performed on FAC-sorted OSNs from the whole MOE or from micro-dissected zone 5. RNA-seq will be verified with IF and RNA ISH for zone 5 ORs. If the two factors were fully redundant, then individual NFI deletion would have a small effect in zone 4-5 OR transcription. If only one of the two factors is essential for zone 5 transcription, then its deletion will recapitulate the double deletion. The NFI gene that does not enable zone 5 ORs may be responsible for repression of zone 1 or 3 OR genes, similarly with the role of NFIX in repressing zone 2 ORs. A third possibility is that NFIA and B

have differential affinity for zone 4 and 5 ORs, with each deletion affecting a fraction of the ORs that are downregulated in the double and triple KO.

1B. Are NFI proteins sufficient for specifying zone 5 OR expression? To explore if NFI proteins are sufficient for enabling the choice of zone 4-5 ORs we will induce ectopic NFI expression in OSN progenitors from other zones. We generated a tetO-NFIA2aNFIBiresmCherry transgenic line, which will allow conditional expression of the two key NFI genes in a non-zonal fashion. We have one transgenic line established (data not shown) and we are repeating injections for additional ones. We will cross the transgenic NFI mice with Gg8tTA mice, which will allow ectopic expression of the transgene in OSN progenitors throughout the MOE. If NFIA and B are sufficient for zone 5 OR transcription, then a transient pulse of these proteins should generate mOSNs that transcribe ectopically zone 4-5 ORs. If we fail to induce ectopic zone 5 OR expression, then either additional zone 5 specific factors are required, or repressors for "out of zone" ORs prevent ectopic induction of zone 5 ORs. Either result will be insightful and will not impact other aims.

Caveats, pitfalls, alternative approaches: Given the effects of the triple and double NFI deletions, we do not anticipate technical issues or unexpected outcomes. An extreme scenario predicts that at least 2 NFI genes are needed for zone 5 OR expression, explaining why NFIX alone cannot replace the other two genes. This is highly unlikely, since our data support a different role for NFIX. However, if individual KO mice do not have strong phenotypes, we will generate NFIA, X and NFIB, X double KO to test this scenario. Finally, we will generate individual NFIX KO mice that will allow us to test if zone2 ORs can be ectopically expressed in zone 5 with zone 5 ORs, revealing whether ectopic OR expression can occur when native zonal ORs are transcribed.

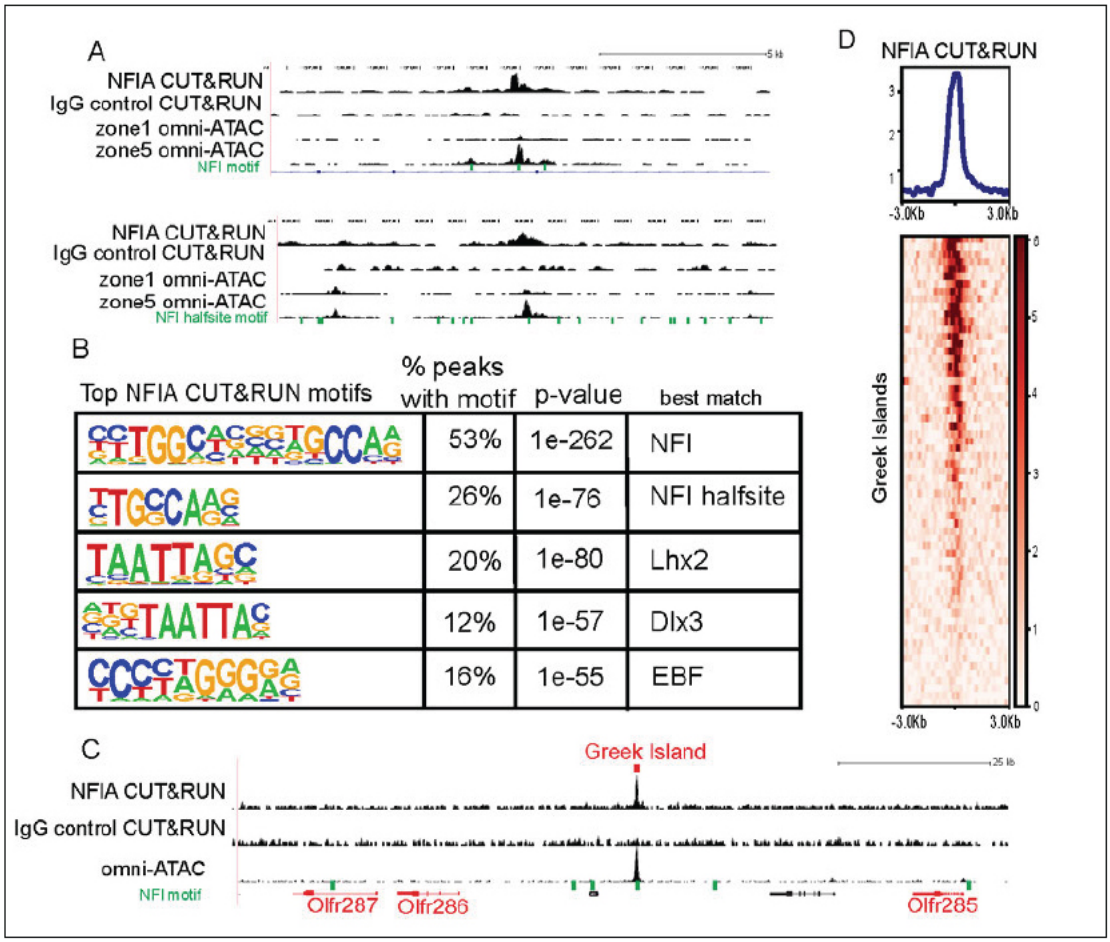

Figure7: Cut&Run on micro-dissected zone 5 reveals NFI binding on Greek Islands. A, NFIA Cut&Run peaks that coincide with zone 5 specific omni-ATAC-seq peaks **B**, NFI motifs are the most highly enriched motifs on NFIA Cut&Run peaks. **C**, NFIA peaks are detected on Greek Islands. **D**, Heat map of NFIA Cut&Run peaks on the 63 Greek Islands.

Induction of transgenic NFI proteins by Gg8tTA[95,96] may not be induced early enough, failing to enable ectopic zone 5 OR transcription. In this case we will induce NFI earlier, using Krt5CreER crossed to a Cre-inducible tTA. Repeated tamoxifen injections will induce permanent tTA expression in HBCs and their progeny, resulting in stable expression of the transgenic NFI proteins. Methimazol ablation of the MOE will be used to induce HBC differentiation into OSNs. If needed, doxycycline will turn off the expression of transgenic NFI, generating mOSNs that experienced a pulse of NFI at progenitor stages. To assure that transgenic NFI is expressed at physiological levels we will screen transgenic founders for ectopic NFI expression at the levels of the endogenous NFI proteins in zone 5, using IF. To exclude transgenic artifacts we will rescue NFIA, B double KO mice with transgenic NFIA, B expression. If the transgene restores expression of zone 4 and 5 ORs then the

timing and levels of transgenic NFI expression are appropriate. Alternatively, a CRISPR/dCAS9-AD strategy[97] will be deployed for the activation of the endogenous NFI proteins at physiological levels. We will use dCas9-AD mice (Jackson Laboratories) and transgenic expression of sgRNAs under control of the tetO promoter induced by Gg8-tTA. The level of induction will be controlled by the number of sgRNAs used to recruit the activating dCAS9 to the NFI loci[97].

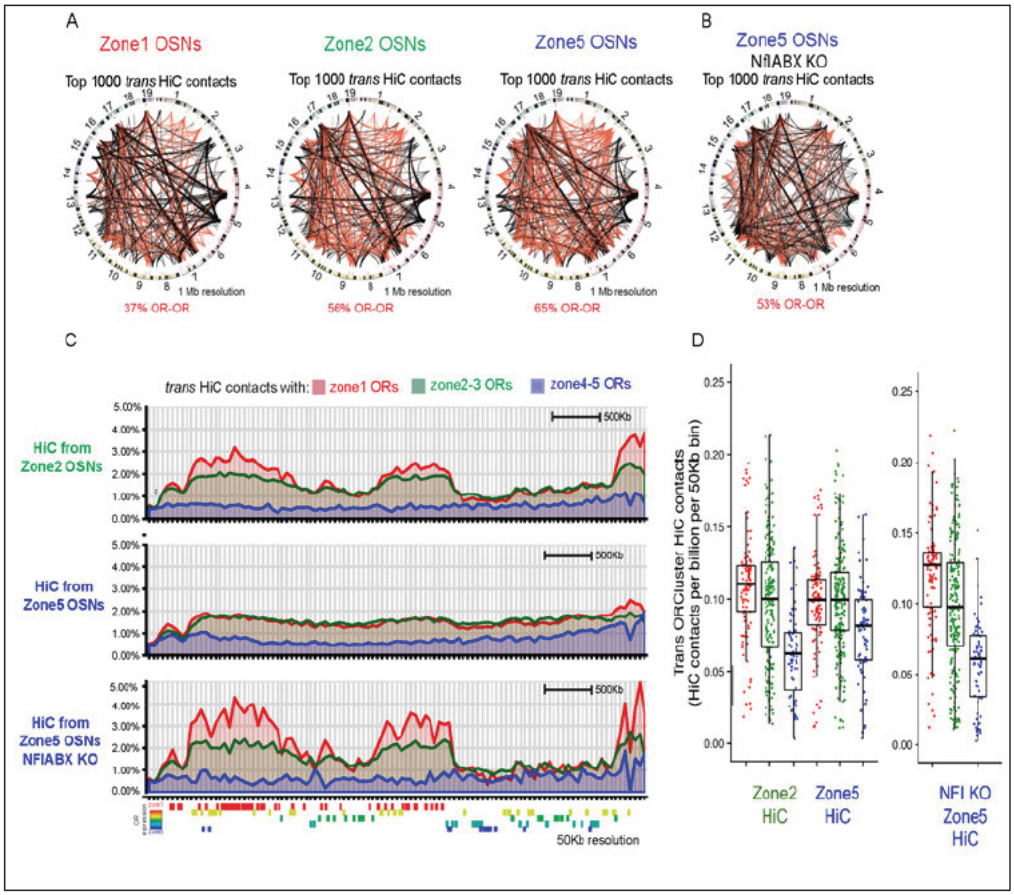

Figure8: Zonal HiC reveals that NFI regulates nuclear architecture of Zone 5 OSNs A, Circos plots depicting the top 1000 trans interactions in zone 1, 2 and 5 OSNs, with OR-OR interactions being depicted in red. **B,** Same circos plot from triple NFI KO OSNs from zone 5, shows reduction of trans OR-OR contacts to zone 2 levels **C,** Plotting cumulative trans contacts of all the ORs, separated by their zonal indexes, across a large OR cluster in chromosome 2 that contains OR genes from every zone (depicted with our standard color code at the bottom of the plot. In wild type Zone 5 OSNs the distribution among zones is homogeneous, but in early NFIABX KO zone 5 OSNs zone 1 and 2 ORs have enriched contacts with each other. **D,** A zone 2-like transformation is obvious in a genomewide analysis of OR-OR contacts according to zone index. Data are averages of biological duplicates

Aim2: Determine how NFI proteins regulate zonal OR expression.

Preliminary data: A question emerging from our studies is whether NFI proteins enable the expression of zone 5 ORs by direct binding, or by activating secondary transcription factors. Due to the enrichment of NFI motifs on OR promoters of zone 5 ORs, we favor a model of direct NFI binding. In support of this, the 4 other transcription factors that are specifically expressed in zone 5 OSNs, Dlx4, Msx1, Meis1 and Meis2, do not change significantly in triple NFI KO. Obtaining evidence for direct binding of NFI proteins on zone 5 ORs is challenging since these factors are zonally expressed and dynamically regulated. Crosslinked ChIPs require hundreds of thousands of cells, which cannot be obtained for zonally isolated FAC-sorted cells. We therefore propose a novel approach for the detection of protein-DNA interactions named "Cut&Run", which was recently applied on populations of a few hundred cells[49,98-100].

We established conditions for NFIA Cut&Run on micro-dissected zone 5 cells. Despite using a mixed cell population in our proof of principle experiments we obtained NFIA peaks that, by and large, overlap with computationally predicted NFI motifs. In fact, *de novo* motif analysis of all the NFIA peaks returned NFI motifs as the most enriched in these peaks (Figure 7a, b). Interestingly, we detect NFIA peaks on Greek Islands, further supporting a direct role of this protein on OR gene regulation (Figure 7c, d). In this mixed population we do not detect NFIA peaks on zone 5 OR promoters, which is expected since each promoter is active in at least ~100 fold fewer cells than the Greek Islands. Alternatively, NFIB may be binding on OR promoters and NFIA on Greek Islands, mediating zone 5 OR-Greek Island contacts through NFIA-B interactions. Experiments on pure cell populations will resolve these models.

2A. Perform cut and run for NFI proteins in distinct cell types isolated from zone 5. With Cut&Run protocols in place we will use specific antibodies for NFIA, NFIB, and NFIX to determine their genomic targets in mash1, mash1/Ngn1, Ngn1, and OMP-expressing cells from zone 5. In addition, we will FACS Olfr1507^{+} cells, using an Olfr1507iresGFP knock-in mouse. We expect to detect transient binding of NFIA or NFIB (or both) on the majority of zone 5 ORs at the Mash1^{+}/Ngn1^{+} positive state that will be gradually eliminated from

the non-chosen ORs as cells commit to a single OR. In this case we expect continuous NFI binding only on the transcriptionally active Olfr1507. Based on our genetic observations, we expect NFIX binding on the zone 2 ORs, if NFIX directly represses these ORs in zone 5.

2B. Determine the role of NFI proteins in establishing zonal interchromosomal interactions.

Preliminary data: An important question emerging from our studies is how NFI proteins influence the nuclear architecture of zone 5 ORs, which as we previously showed plays a critical role in OR gene choice[15,16,101]. We performed HiC in mOSNs from zones 1, 2 and 5, which revealed a transient increases in *trans* OR-OR interactions as we transition from zone 1 to zone 5 (Figure 8a). Intriguingly, in triple NFI KO OSNs, *trans* OR-OR interactions are reduced to the levels of zone 2 OSNs (Figure 8b), consistent with the ectopic zone 2 OR transcription in these cells. Virtual 4 C analysis of the largest OR cluster in chr. 2, which contains ORs from every zone, shows that in zone 2 OSNs, zone 1 and 2 ORs from the other chromosomes have increased *trans* contacts with the zone 1 and 2 ORs from this cluster, whereas in zone 5 OSNs the distribution is more uniform. However, in NFIABX KO OSNs from zone 5, this pattern reverts to the pattern observed in zone 2 OSNs (Figure 8c), suggesting that zone 1 and 2 ORs have more robust and selective contacts in the absence of NFI. This transformation in 3D nuclear architecture is confirmed by genomewide quantifications of HiC contacts, showing significant reduction of zone 5 *trans* OR-OR contacts in the NFI KO (Figure 8d). The dramatic differences in zonal genomic interactions is not an epiphenomenon stemming from differences in transcriptional states: Even within their zone each zone 4 and 5 OR is transcribed, on average, in less than 1% of the OSNs, making their interactions undetectable in the mixed population. Furthermore, the zonal differences in OR-OR interactions are first detected prior to OR gene activation by the Greek Island hub. Given the importance of OR-OR interactions in OR transcription, these data suggest that NFI-dependent recruitment of zone 5 ORs to the OR compartment enables subsequent interactions with the Greek Island hub and, thus, transcriptional activation. Unpublished observations suggest the NFIA interacts with Lhx2 and H3K9me3 (data not shown), providing a possible mechanism for the role of this factor in mediating zone 5 OR interactions with the rest of the OR repertoire.

Proposed experiments: To dissect the relationship between zonal OR transcription and NFI-dependent genomic contacts we will perform zonal *in situ* HiC in the various KO mice from Aim1. These experiments will uncover the contribution of every NFI factor in the establishment of interchromosomal OR-OR interactions. We anticipate to identify a strong correlation between transcriptional consequences and *trans* OR contacts in a zone-dependent fashion. Moreover, if ectopic expression of transgenic NFIA, B in progenitor cells induces ectopic expression of zone 4,5 ORs, we expect that we will also detect ectopic recruitment of these genes to the OR compartments, demonstrating sufficiency of NFI proteins in sculpting the OSN nucleome.

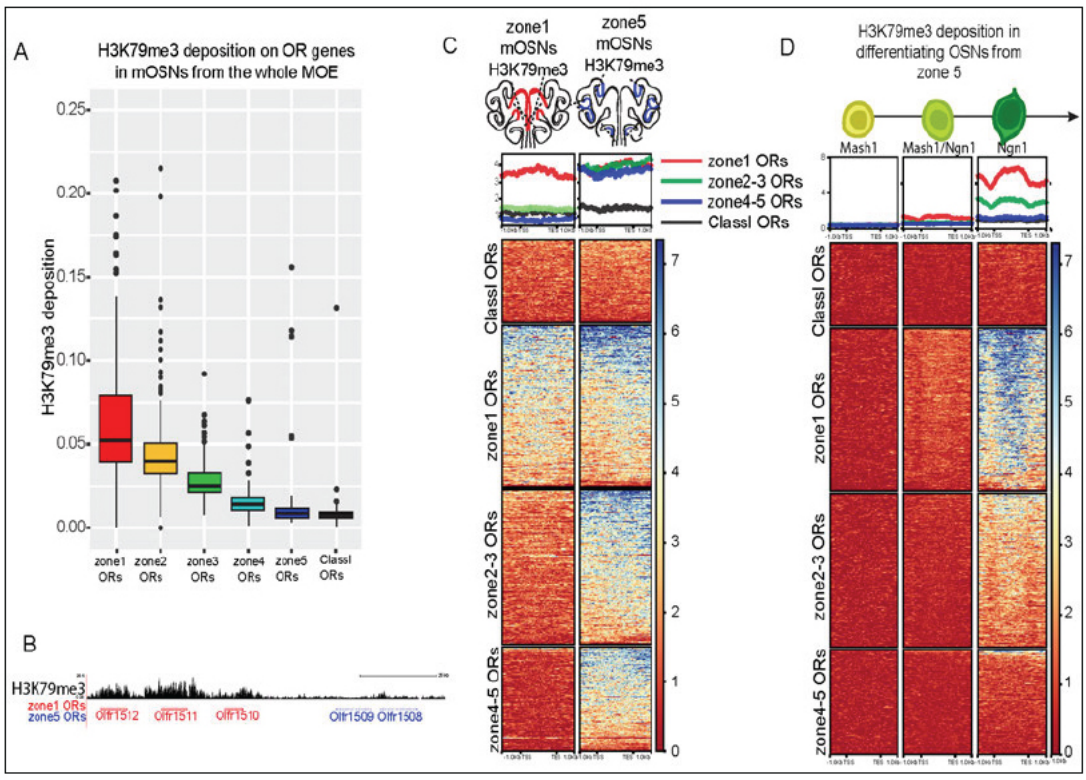

Figure9: Zonal OR marking by H3K79me3 A, H3K79me3 distribution on OR genes from various zones by ChIP-seq on mOSNs from the whole MOE. **B,** same ChIP focused on a cluster with zone 1 and 5 ORs. **C,** Heat map depicting H3K79me3 ChIP-seq signal on OR genes from various zones in mOSNs isolated from micro-disscted zones 1 (red) and 5 (blue). In zone 1, zone 1 ORs have the highest H3K79me3 signal whereas in zone 5 H3K79me3 is evenly distributed across zonal indexes **D,** Zone 5 H3K79me3 ChIP-seq at different OSN-progenitor populations reveals delayed marking of zone 5 ORs. Data are averages of biological duplicates

2C. To explore the role of NFI proteins in H3K79me3-dependent repression of "out of zone" ORs. Our results may explain how zone 5 ORs become enabled for zone 5-specific transcription but they do not account for the lack of zone 1-3 OR expression in zone 5. The observation that NFIX prevents ectopic zone 2 OR expression in double KO mice, suggests that a repressive mechanism may keep "out of zone" ORs silent in zone 5. OR genes are marked by repressive H3K9me3, but in a non-zonal fashion. We, thus, searched for repressive histone modifications with zonal distribution. One such modification is H3K79me3, which is also found on pericentromeric and subtelomeric heterochromatin[102] and was previously implicated in mono-allelic expression[103]. H3K79me3, unlike H3K9me3 and H4K20me3, is irreversible, since no de-methylase for H3K79 has been identified in yeast or higher animals. Thus, we hypothesized that H3K79me3 is used for irreversible repression of enabled ORs that should not be expressed. In this logic, in zone 1 OSNs, the zone 1 ORs that were not chosen for transcription should be the only ORs repressed via both H3K9me3/H3K79me3. In zone 2, all the zone 1 and 2 ORs should be H3K79me3-positive, but not zone 3-5 ORs, and so forth, predicting that zone 1 ORs will have the highest levels of H3K79me3 and zone 5 ORs the lowest. Indeed, native H3K79me3 ChIP-seq in OSNs from the whole MOE revealed this exact distribution (Figure 9a, b). Furthermore, zonal dissections confirmed the zonal dependency on H3K79me3 distribution, with all the ORs having this mark in zone 5 (except class I ORs) and only zone1 ORs in zone 1 (Figure 9c). Importantly, in zone 5 H3K79me3 is deposited first on zone 1 ORs and last on zone 5 ORs during differentiation, consistent with the notion zone 5 OR repression occurs after one is chose for transcription (Figure 9d). Indeed, the transcriptionally active Olfr1507 is devoid of H3K79me3, based on ChIP-seq experiments from sorted Olfr1507+ OSNs (data not shown).

Proposed Experiments: We seek to determine the role of NFI proteins in establishing zonal H3K79me3 deposition and to test genetically the role of H3K79me3. For the first goal, if only ORs recruited to the OR compartment become marked by H3K79me3, then triple NFI deletion should result in loss of H3K79me3 from zone 4-5 ORs and delayed marking of the ectopically expressed zone 2-3 ORs. ChIP-qPCR experiments reveal the reduction of H3K79me3 from zone 5 ORs, without changes in H3K9me3, confirming that only the H3K79me3 is zonally regulated via NFI (Figure 10). Deep sequencing will determine the genomewide effects of NFI deletion in H3K79me3 distribution. We will repeat these ChIP-seq experiments in the double NFIA, B KO mice, determining if NFIX preserves high levels, and early deposition of H3K79me3 on zone 2 ORs. Finally, we will ask if ectopic expression of the NFIA, B transgene alters H3K79me3 deposition in other zones.

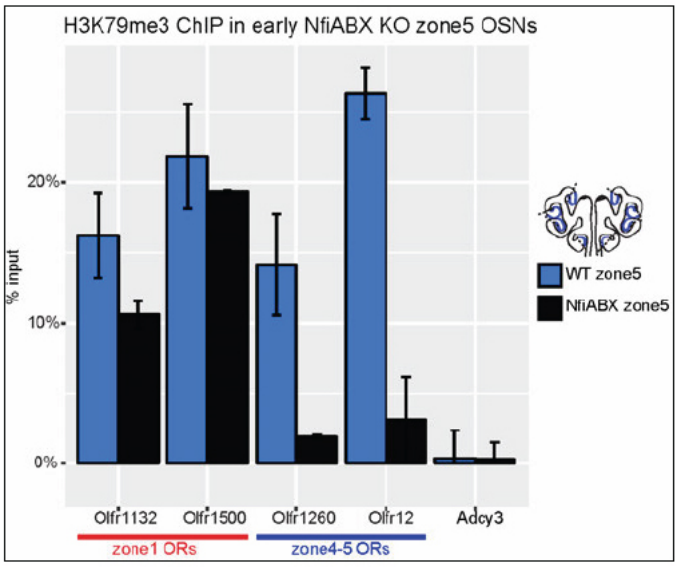

Figure10: NFI deletion depletes H3K79me3 from zone 5 ORs. ChIP-qPCR from control and triple early NFI KO OSNs micro-dissected from zone 5 shows strong reduction of zone 5 ORs. Adcy3, which is not marked by H3K79me3, is used as negative control. Data are averages of two biological replicates. Reduction in zone 5 is significant ($p<10^{-5}$, Student's T-test)

For the functional interrogation of H3K79me3 we will perform conditional deletion of Dot1L[102], the only mammalian enzyme catalyzing H3K79 tri-methylation. Heterozygote Dot1L deletion in HBCs, results in reduction of H3K79me3 on ORs, supporting the role of Dot1L in this process (data not shown). We anticipate that in homozygote KO OSNs we will detect complete loss of H3K79me3 and ectopic OR expression, the extent of which will be dependent on the timing of this deletion: Deletion in HBCs, may allow ORs from every zonal index to be expressed in zone 5; deletion in early GBCs may allow ORs from zones 2-3 to be expressed in zone 5; and deletion at the moment of OR choice may impair only zone 5 ORs, which may be co-expressed in zone 5 OSNs, violating the "one receptor per neuron" rule. Conditional deletions in GBCs will be performed with Mash1-CreER (early GBC) and Gg8-Cre (late GBC), whereas deletion at the stage of OR gene choice will be achieved with Olfr1507iresCre. Ectopic OR expression will be assessed by zonal RNA-seq, IF and RNA ISH, whereas OR co-expression will be determined by single cell RNA seq and two-color fluorescent RNA ISH.

Caveats, pitfalls, alternative approaches: Based on our Cut&Run results we do not anticipate technical issues. There are commercially available antibodies that were successfully used for NFIA, B and X ChIP-seq experiments[104] assuring that this approach will be successful for

each NFI factor. We have also established ChIP-seq experiments on FAC-sorted Olfr1507^{+} OSNs, thus, the more sensitive Cut&Run assay will provide cleaner results. If we discover that NFI binding on zone 5 ORs is so transient that it is not detectable even at pure populations, we will deploy alternative approaches. We could use the transgenic NFI mice to perform Cut&Run under conditions of stable NFI expression. A second approach will be to use CRISPR/Cas9 to generate fusions between NFIA or NFIB and a bacterial adenine methyltransferase[105], allowing a DamID-based[106] approach for the identification of NFIA and NFIB targets. Also, we are currently experimenting with Cut&Tag, a newer version of Cut&Run applicable to single cell analysis, which would overcome issues of heterogeneity in progenitor populations. Finally, we can use CRISPR/Cas9 to delete predicted NFI motifs on zone 5 OR promoters and ask if this affects their expression. In the unlikely event that NFI proteins do not bind on zone 5 OR promoters, the other goals of the grant will not be affected. In fact, with the data presented on 2B and 2C we already have epigenetic mechanisms in place that likely explain these zonal restrictions. Since no other transcription factors are both highly and zonally expressed in an NFI dependent fashion we will explore other possibilities. For example, we will explore whether NFIA binding on Greek Islands alters the affinity of the hub towards zone 4-5 OR promoters. Alternatively, we will search for zonal differences on factors regulating gene expression at the post-transcriptional level.

Regarding the proposed HiC experiments, based on the strong preliminary data, we do not anticipate technical difficulties in the execution and analysis. Similarly, the effects of the triple NFI deletion in 3D nuclear architecture render unexpected results very unlikely.

H3K79me3 ChIP-qPCR from NFI KO mice show our ability to execute the proposed zonal experiments and support our hypothesis, thus, we do not expect challenges. We do not know if Dot1L deletion will affect zonal OR expression, which constitutes the <u>only</u> goal of this proposal not guaranteed to generate positive results. If Dot1L deletion does not affect OR expression, then we will seek additional repressive marks with zonal distribution. We will ask if Polycomb complexes are implicated in zonal OR silencing and whether DNA methylation plays a role in this process. Finally, we will entertain a different scenario: Our data show that zone 1 ORs are recruited first in the OR compartment, followed by zone 2-3 ORs, and culminating in the recruitment of zone 4-5 ORs. Possibly, this gradual process results in a 'layered" assembly of the OR compartment, with zone 1 ORs in the center, surrounded by zone 2-3 ORs in the middle layer and zone 4-5 ORs in the external layer, which is proximal to the Greek Island hub[15,101]. Ongoing super-resolution DNA FISH with zonal pan-OR probes will test this model.

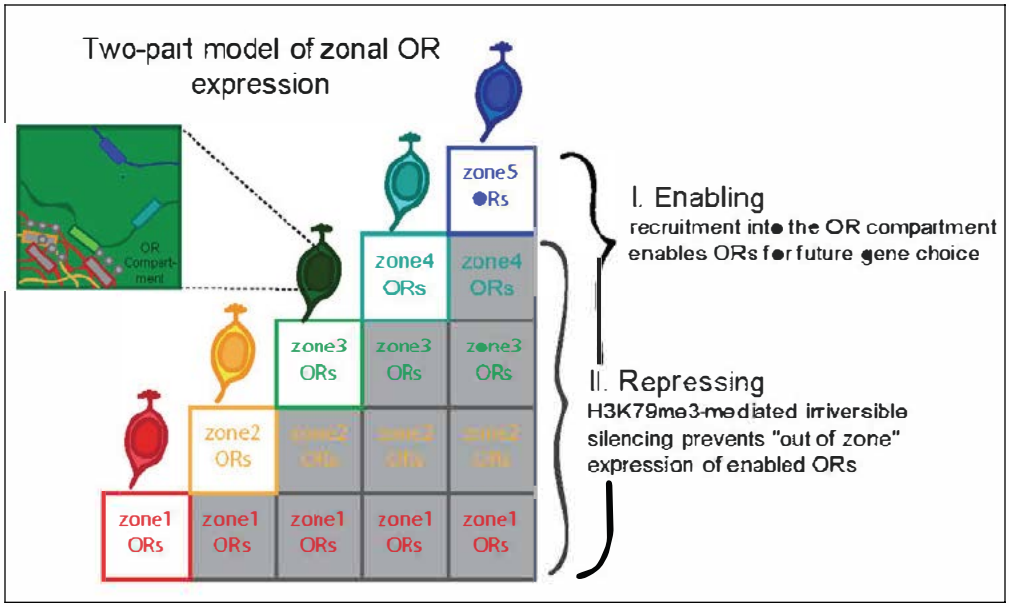

Figure11: A model for zonal OR regulation. In each zone, ORs from that zone and any previous zone are enabled by being recruited to the OR compartment. ORs from the previous zones are repressed by H3K79me3, allowing only ORs from the proper zone to be chosen for transcription.

<u>Measures to assure rigor and reproducibility:</u> RNA-seq will be performed in 4 biological replicates, with each replicate being generated from a separate mouse. PCA will identify outliers among replicates, which will be removed and replaced with new replicates. In addition to including TdTomato in our crosses, efficient deletion of the conditional KO genes will be confirmed by measuring the reduction of reads on the floxed exons, using Sashimi, as previously described. Mice will be at the same exact age when induced by tamoxifen (P30) and after every methimazol ablation we will wait 40 days for the epithelium to be fully restored. Control and experimental mice will always represent littermates. Each HiC experiment will be performed in biological duplicates, with 500 million HiC contacts per replicate. Replicates will be analyzed separately and tested for reproducibility (reproducibility of contact curves, fraction of HiC contacts mapping to OR genes, etch). Additional replicates will be performed needed. Replicates will then be combined to increase

genomic resolution. Our HiC analysis uses a stringent cutoff for removal of all multi-mappers. *In situ* HiC results will be independently confirmed by two- and three-color DNA FISH experiments as we have done in the past[15,107]. Control and experimental sections will be placed on the same slide and imaged under the same settings to minimize variability. Cut&run experiments will be performed in biological triplicates and NFI KO mice will be used as controls for antibody specificity, as well as, IgG isotype controls.

Sex as biological variable: We have not observed sex dependent differences in the zonal expression of ORs. That said, in our 4 replicates we will have 2 males and 2 females, the data will be initially analyzed separately for each sex and only if sex differences are not detected they will be combined. In future publications we will have pre-prints with supplemental figures describing the data in a sex-segregated fashion. Also, we will deposit our data in GEO with each replicate separate, so other researchers will have the means to perform comparisons between sexes. We have not detected sexually dimorphic expression of NFI genes in the MOE, and none of the effects described in the double and triple KO mice are sexually dimorphic. Obviously, we would be very excited to detect sexual dimorphisms in OR expression, and if any of the experiments suggest that such differences exist, we will increase our analyses to properly powered numbers of males and females.
Summary: Our data support a model of gradual increase in the number of ORs that participate in OR compartmentalization according to their zonal index and the zonal location of an OSN. Since OR compartmentalization is a pre-requisite for OR gene choice, we view this recruitment step as “enabling” an OR allele for future activation. Although this model explains how each OR becomes enabled for transcription in its cognate zone, it does not account for the fact that out of zone ORs that are enabled, they are never ectopically expressed. However, the observation that there is a concomitant increase in H3K79me3 along zonal indexes as we transition from zone 1 to zone 5, suggest that there is also a repressive process that prevents out of zone but enabled ORs to be ectopically activated. Our data show zone 5-enriched NFI proteins enable zone 5 (and 4) OR genes and repress zone 2 and 3 ORs. To assist the reviewers with our working model we provide a visual depiction in Figure 11. Our data cannot explain how this process is regulated in zones 1-3, where NFI genes are lowly expressed, thus, future studies will explore if other NFI-like factors mediate a similar function. Our proposed genetic experiments will test this model and will reveal mechanisms by which each NFI protein regulates the epigenetic landscape and nuclear architecture of OR genes in zone 5 OSN.

Timeline: The proposed experiments, although ambitious in regards of the extensive genetic crosses and genomic approaches, are within the expertise of our laboratory and feasible for the timeframe of an R01 proposal. Importantly, all the genetically modified mice are in our colony and the proposed crosses are ongoing, thus no delays or technical challenges are expected for this application.

Aim	Year 1	Year 2	Year 3	Year 4	Year 5
1A	Generate individual NF A NF B and NF X KO mice with Krt5CreER and d omato	Analyze the 3 individual KO strains by RNA-seq on whole MOE and micro-dissected zone 5 Con irm with F and RNA SH	Generate double NF A X and NF B X KO mice with Krt5CreER and d omato	Analyze the 2 double KO strainsby RNA-seq on whole MOE and micro-dissected zone 5 Con irm with F and RNA SH	
1B		Generate multiple tetO-NF A2aNF BiresmCherry lines and screen them or expression levels similar endogenous NF A B in zone 5	Analyse the e ects o ectopic NF expression in zonal OR expression	Cross NF transgenic with NF KO mice	Explore i transgenic NF expression can rescue NF deletion in zone 5 OR expression
2A		Per orm Cut&Run in FAC sorted cells rom micro-dissected zone 5	Per orm Cut&Run in FAC sorted cells rom micro-dissected zone 5 rom triple NF KO mice	Per orm Cut&Run in FAC sorted cells transgenic NF mice	
2B			Per orm in situ HiC in late triple NF KO mice to con irm nuclear architecture is intact	Per orm in situ HiC in individual NF KO mice and in double NF KO mice using zone 5 micro-dissections	Per orm in situ HiC in itransgenic NF mice both in zone 5 and zones 1-3
2C	Per orm H3K79me3 Ch P-seq on triple NF KO mice using zone 5 microdissections	Per orm H3K79me3 Ch P-seq on double and individual NF KO mice using zone 5 microdissections (plus zone 1 and 3 or the NF X KO)	Generate Dot1L KO mice using Krt5CreER Gg8-Cre and Ol r1507iresCre drivers + d omato	Analyze the various Dot1L mutants by RNA-seq and H3K79me3 using micro-dissected zone 5	Analyze the various Dot1L mutants by HiC

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

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
Does the proposed research involve human specimens and/or data	○ Yes ● No
Other Requested information	

Vertebrate Animals

Description of procedures

All 3 aims will require the use of *Mus musculus* (the house mouse). We will study both male and female mice but we will analyze them separately in case there are unexpected sexual dimorphisms. Also in our future publications, we will provide supplemental figures with where data from males and female mice will be segregated. This is not something we have done yet, but we will start following this principle from now on.

Approximately 935 young adult transgenic mice (mixed background) of both sexes will be used per year for the purposes of this proposal. This will amount to approximately 4675 mice over the 5-year period of support requested. This total number of animals includes mice that will be used for breeding, mice used for the retention of the different strains, as well mice that will be used for experimental analysis. Careful planning and periodic meetings between lab members and the PI ensure that animals are effectively shared between lab members, minimizing the numbers of animals that need to be bred.

Justification of animal use

Our proposal aims to study mechanism of olfactory receptor gene choice in mammalian olfactory neurons. Because there are no available olfactory neuron cell lines that recapitulate the *in vivo* gene regulation process our proposed experiments can only be performed in the mouse. We cannot use invertebrates as alternative because the mechanisms of olfactory receptor gene regulation and olfactory neuron differentiation in flies and worms are very different from the mechanisms we uncovered in the mouse. Thus, mice constitute the only model organism that is genetically accessible and utilizes long-range genomic interactions for stochastic OR gene choice. Furthermore, mouse olfaction provides the closet model for human olfaction among genetically tractable model organisms.

Minimization of Pain and Distress

Our animals our housed in the state of the art barrier mouse facility in the basement of the Jerome L. Green Science building. Moreover, we have a satellite mouse facility next to our laboratory with space for up to 250 cages. This facility is ideal for time-sensitive experiments that require continuous access to the mice. There is 24/7 access to the veterinary staff of the campus for any case of emergency. My lab members as well as the animal care staff monitor our mice for any visible infection, injury, visible guarding, sensorimotor abnormalities (as evidenced by abnormal gait or paralysis), seizures or loss of >15% body weight and inform us about it in order to euthanize the animal. Usually, when animal care stuff inform us about a mouse experiencing discomfort, we respond within 15-30minutes by either instructing them to euthanize the animal or by euthanizing the animal in the lab for collection of its olfactory epithelium.

Minimizing Pain and Distress during Genotyping and Identification of animals

We will use metal ear tags to mark our animals when they are 10 days old. All our mice are genotyped by PCR, which requires minimal amount of DNA for the analysis. Therefore we use tail snips, which are performed at the same time with the insertion of ear tags under isofluarane anesthesia to avoid discomfort and stress for the mice. We use sharp sterile scissors and the tail is swabbed with a dilute betadine solution. For all the experiments proposed, except for aims 1C and 2A mice are euthanized prior to experimentation, so there will be no additional source of pain and distress.

Viral infections: We deliver Lenti and adeno-associated viruses through intra-nasal delivery of 20ul high tighter virus using a 1ml syringe. The syringe is placed in the nasal cavity and administered during inhalation on un-anesthetized pups (daily from P4 till P7). Mice are analyzed 23 days later. The procedure is approved by IACUC and abides to AVMA guidelines.

Euthanasia

Mice will be euthanized before the extraction of the olfactory epithelium. All methods are consistent with American Veterinary Medical Association (AVMA) guidelines.

Strains and number of mice

We have proposed analysis of the following genotypes:

1. NFIAfl/fl, Krt5CreER, TdTomato
2. NFIB fl/fl; Krt5CreER, TdTomato
3. NFIX fl/fl; Krt5CreER, TdTomato
4. NFIA fl/fl; NFIX fl/fl; Krt5CreER, TdTomato
5. NFIB fl/fl; NFIX fl/fl; Krt5CreER, TdTomato
6. Gg8tTA; tetO-NFIAT2ANFIBiresmCherry
7. Krt5CreER; Cre-inducible tTA; tetO-NFIAT2ANFIBiresmCherry
8. Krt5CreER; NFIA,B fl/fl; Cre-inducible tTA; tetO-NFIAT2ANFIBiresmCherry
9. Gg8-Cre; Dot1lfl/fl; TdTomato
10. Mash1-CreER; Dot1lfl/fl; TdTomato
11. Olfr1507iresCre; Dot1lfl/fl; TdTomato

In total we have 11 different genotypes that contain 3 or 4 different alleles. To obtain sufficient number of animals from each genotype we anticipate having 8 breeding cages for each genotype (88 cages total). Moreover, we will have 3 cages for maintenance of each one of the different alleles (11 different alleles described here, 33 cages). Finally, in addition to the breeding cages, male and female mice will be housed separately until the reach the desired age, thus we anticipate another 8 cages per genotype (88 cages). Total number of cages anticipated for this project in the coming year is (88+33+88=209).

We house a maximum of 5 mice per cage thus we will use a total of 1045 mice per year. This includes mice that will not be used for experiments and represent genotypes that cannot serve as experimental or control subjects. As described in the proposal, male and female mice will be analyzed separately and if these analyses suggest existence of sexual dimorphisms we will increase the number of mice to statistically significant results.

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Resource Sharing Plan

My laboratory always deposits raw and normalized sequencing data to public databases (GEO for ChIP-seq and RNA-seq, 4D-nucleome portal for HiC). Raw images and the quantifications that we performed on them will be deposited at Mendeley Data, a new practice that my lab has started to follow. Detailed protocols will also be deposited in Mendeley Data plus, as we always do, we will be happy to host scientists that require hands-on training for new protocols.

Mice will be deposited to Jackson laboratories and until the colonies are available for distribution we will be sharing them directly with investigators who request them. We are strong believers in sharing our published mice without requiring authorship or collaboration (for example: Ericksson and colleagues, 2015 and Brooks and colleagues, 2018).

Timeline of Resource Sharing Plan: We are strong advocates of pre-prints, which make discoveries and technologies available to the community prior to the completion of the peer review process. Thus, our manuscripts will be deposited at BioRxiv prior to submission to peer review journals and this will immediately initiate the resource-sharing plan described above. Specifically, as we have done in the past with BioRxiv submissions, we will provide GEO accession numbers for the sequencing data and we will make our mice freely available (without requesting collaboration) to the community.

Authentication of Key Biological and Chemical Resources

1. **Mice**:
A) NFIA, B, and X conditional knockout mice were provided by Dr. Gronostasjki from University of Buffalo. We confirmed that crossing the conditional KO alleles to Cre drivers results in deletion of the floxed exons using RNA-seq and Sashimi software for exon usage analysis. We further confirmed the loss of protein by western blots for the three NFI factors.

B) The Gg8-tTA and Gg8-Cre drivers were donated to us by Dr. Nick Ryba. The expression pattern of Gg8 was described in the following publication:

> Ryba, N. J. & Tirindelli, R. A novel GTP-binding protein gamma-subunit, G gamma 8, is expressed during neurogenesis in the olfactory and vomeronasal neuroepithelia. *The Journal of biological chemistry* **270**, 6757-6767 (1995).

We confirmed that Gg8 is expressed only in GBCs/INPs by performing RNA-seq in HBCs, GBCs, INPs and mOSNs, which detects no Gg8 expression in mOSNs.

The activity of the tTA driver was described in the following publication:

> Nguyen, M. Q., Marks, C. A., Belluscio, L. & Ryba, N. J. Early expression of odorant receptors distorts the olfactory circuitry. *J Neurosci* **30**, 9271-9279, doi:10.1523/JNEUROSCI.1502-10.2010 (2010).

We confirmed that the Gg8-tTA driver is only active in the GBCs/INPs by visualizing the expression pattern of tetO-GFP reporters, as well as the expression pattern of the tetO-OlfrCDSdeliresGFP mice (Fig. 7b) which are both tTA dependent and their expression is completely restricted to basal layers of the MOE corresponding to these cell types. Similar results for the Cre driver.

C) The OMPirestTA driver was gifted to us from the Axel lab, and has been used in numerous publications by us and others to induce expression of tetO-dependent genes specifically in mOSNs.

D) The Olfr1507iresCre and Olfr1507deliresCre lines were gifted by Dr. Axel and behaved exactly as described in Shykind et al 2004.

E) Transgenic expression of NFIA and B will be confirmed by RNA-seq, RNA ISH and IF experiments.

2.**Chemicals:**
A) **Methimazol**: We perform methimazol injections as previously described by performing peritoneal injections of (0.1mg/g) of Methimazol. We have performed careful timecourse experiments that confirm the almost complete ablation of the MOE and the timely reformation after 30 days. RNA-seq analysis of OMP, Ngn1, and Mash1 populations 30 days after methimazol ablation shows that these major MOE populations are extremely similar with the same populations from intact animals.
B) **Tamoxifen**: We perform peritoneal tamoxifen injection of (400mg/Kg) and we confirm the activation of Cre drivers with the use of the cre-dependent fluorescent driver TdTomato
A) **Doxycycline**: We use a high doxycycline feeding diet (200mg/Kg) that turns off tetO-GFP reporters less than a week after initiation of the feeding. We only keep the food pellets for 4 months to assure high Dox potency. Importantly, for every experiment we deploy an OmpirestTA;tetOOlfr17iresGFP control as well as a Gg8-tTA;tetO GFP control. These two lines do not express GFP under dox diet, so they provide assurance for the effectiveness of the dox diet.

3. **Antibodies**:
Antibodies used in ChIP-seq have been evaluated in two ways:
A) H3K9me3, H4K20me3 and H3K79me3 were evaluated with the use of the Modified histone peptide array from active motif, which contains histone tails with various modifications. Every time we purchase a new

antibody lot, we screen it with this array and we reject antibodies that cross react with other modifications than the ones they are supposed to interact with.

B) NFIA, B and X antibodies were evaluated by doing IF in the triple NFI KO MOEs. We continued using only antibodies in which immunoreactivity was diminished from the triple KO mice.