Initiative 3: New Delivery Systems

Project Team Leader: PJ Brooks (NCATS)

Goal:
Develop and evaluate innovative approaches to deliver genome editing machinery into somatic cells in vivo

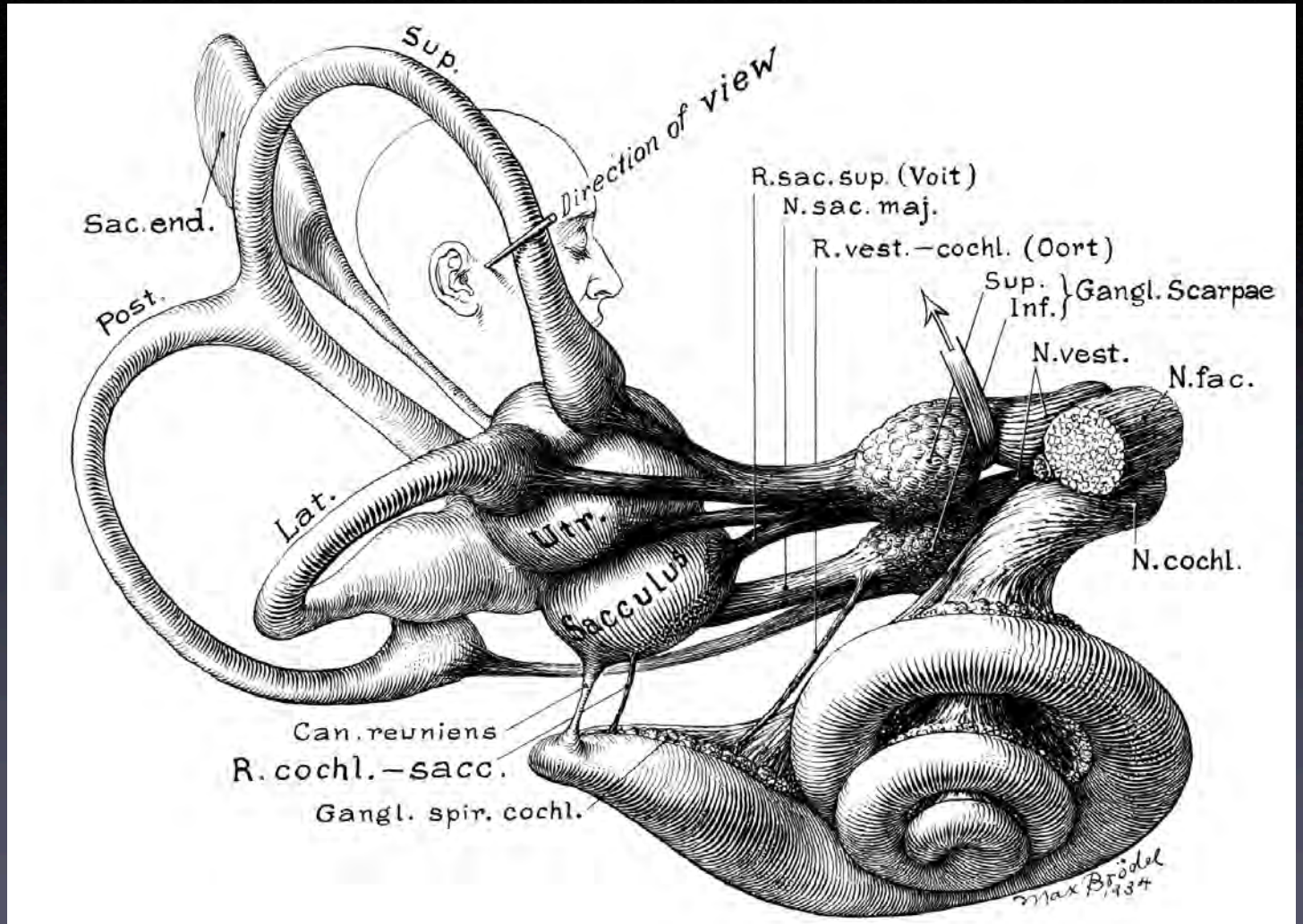
Awardees:
Aravind Asokan; Duke University: Adeno-associated viruses (AAVs)
Zheng-Yi Chen; Massachusetts Eye & Ear Infirmary: Lipid nanoparticles
Benjamin Deverman; Broad Institute: AAVs
Guangping Gao; University of Massachusetts-Worcester: AAVs & nanoparticles
Ionita Ghiran; Beth Israel: Red blood cell-derived extracellular vesicles
Shaoqin Gong; University of Wisconsin-Madison: Nanocapsules
Paul McCray; University of Iowa: Amphiphilic peptides
Mark Saltzman; Yale University: Peptide nucleic acids
Erik Sontheimer; University of Massachusetts-Worcester: Chemically-modified nucleic acids

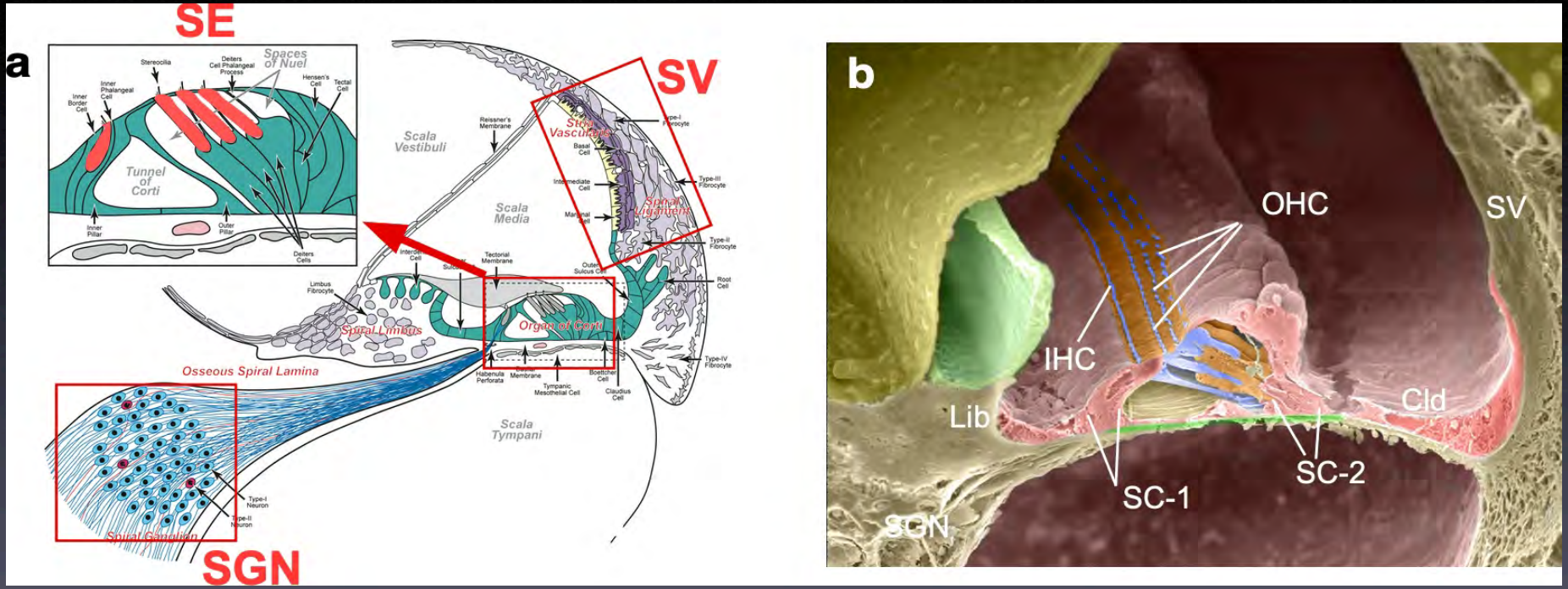
Efficient in Vivo RNP-based Gene Editing in the Sensory Organ Inner Ear Using Bioreducible Lipid Nanoparticles (bLNPs)

Mass Eye & Ear Infirmary/Harvard Medical School
Zheng-Yi Chen ,Mingqian Huang,Wan Du,Yiran Li, Veronica Lamas

Tufts University
Qiaobing Xu,Yamin Li, Feihe Ma, Jinjin Chen

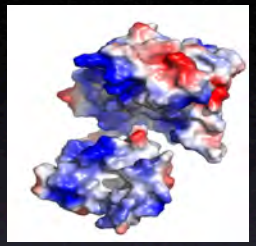
Broad Institute
David Liu


Sup.
Direction of view
Sac. end.
Post.
R. sac. sup. (Voit)
N. sac. maj.
R. vest. – cochl. (Oort)
Sup.
Inf.
Gangl. Scarpae
N. vest.
N. fac.
Lat.
Utr.
Sacculus
N. cochl.
Can. reuniens
R. cochl. – sacc.
Gangl. spir. cochl.
Max Brödel
1934


a
SE
Spaces of Nuel
Stereocilia
Deiters Cell Phalangeal Process
Hensen's Cell
Tectal Cell
Inner Phalangeal Cell
Inner Border Cell
Tunnel of Corti
Inner Pillar
Outer Pillar
Deiters Cells
SV
Scala Vestibuli
Reissner's Membrane
Stria Vascularis
Basal Cell
Intermediate Cell
Marginal Cell
Spiral Ligament
Type-I Fibrocyte
Type-III Fibrocyte
Type-II Fibrocyte
Root Cell
Type-IV Fibrocyte
Scala Media
Tectorial Membrane
Limbus Fibrocyte
Spiral Limbus
Organ of Corti
Osseous Spiral Lamina
Habenula Perforata
Basilar Membrane
Tympanic Mesothelial Cell
Boettcher Cell
Claudius Cell
Scala Tympani
Type-I Neuron
Type-II Neuron
Spiral Ganglion
SGN
b
OHC
SV
IHC
Lib
Cld
SC-1
SC-2
SGN

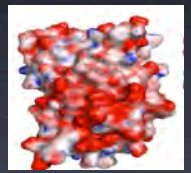
Bio-Engineering of proteins for delivery

Cre recombinase



(net charge: +11)

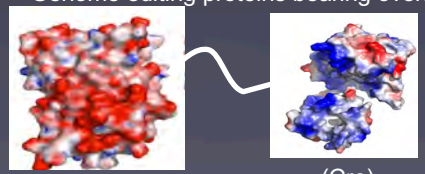
Fusion with (-30)GFP or other polyanionic species such as nucleic acids



(GFP)

(net charge: -30)

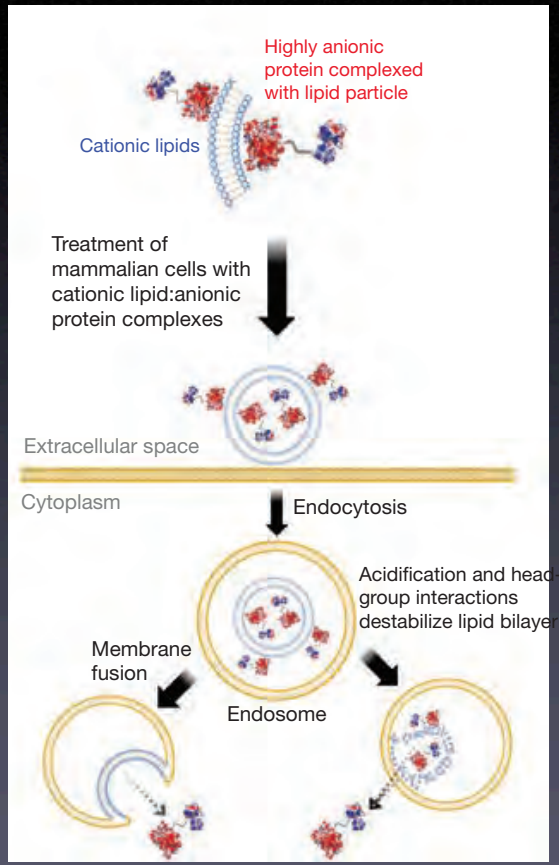
Genome editing proteins bearing overall negative charge



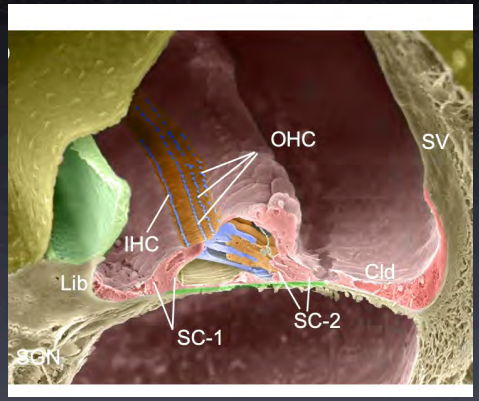
(GFP)

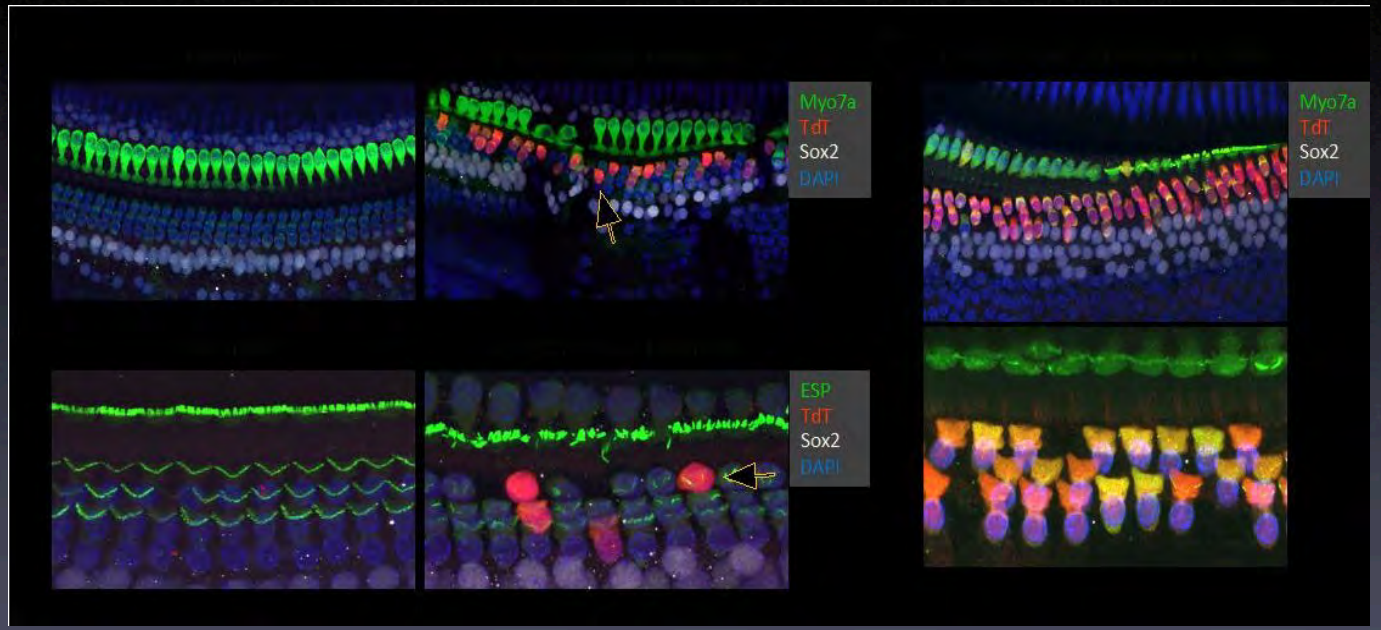
(Cre)

(-19)



Direct protein (-30)GFP-Cre delivery into mouse inner ear

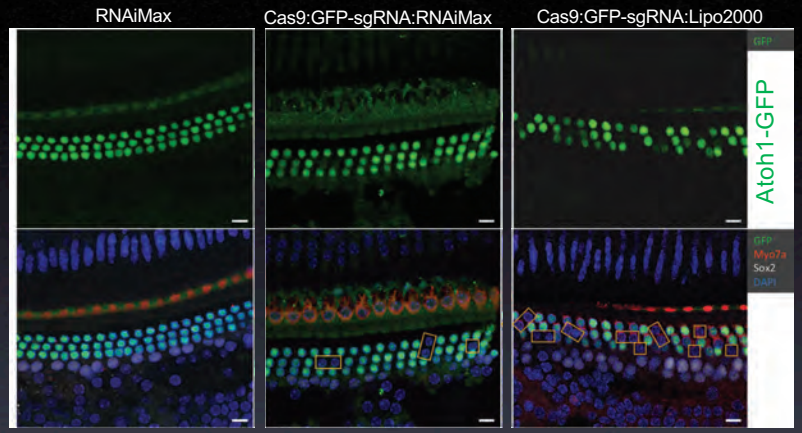


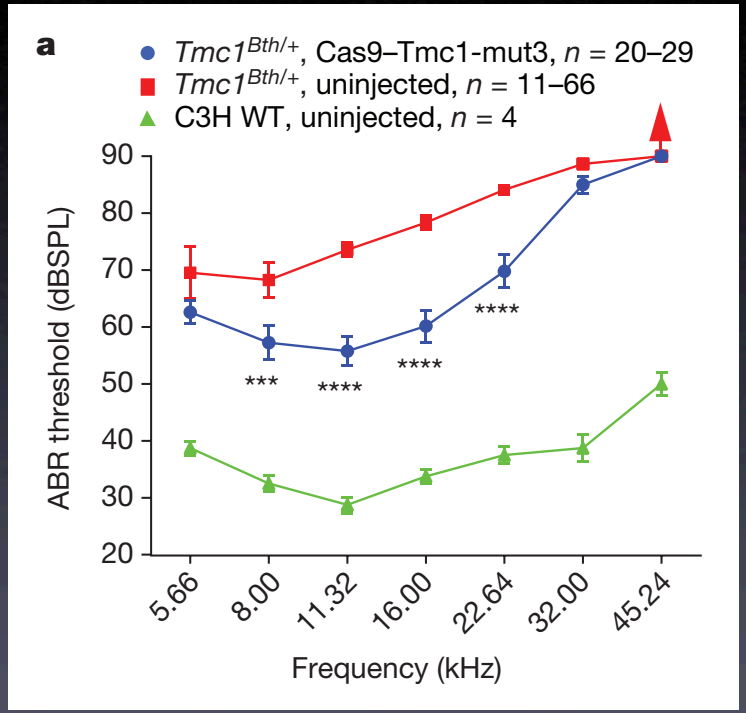


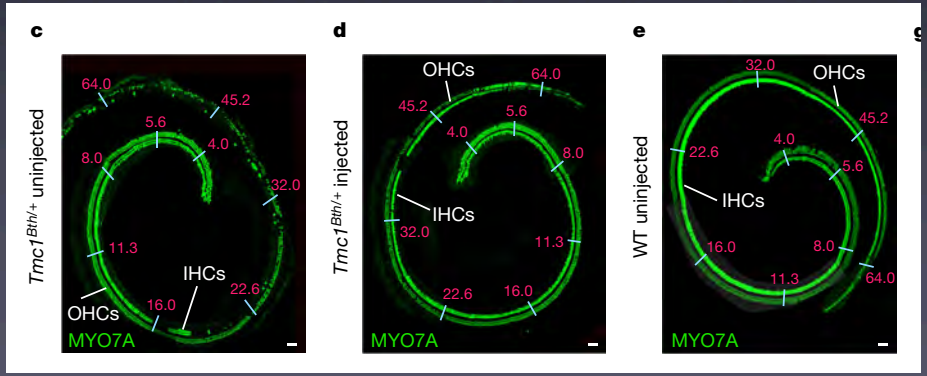
Lipids only RNAimax+protein Lipofectamine2000+protein

Zuris, et al., Nat Biotech 2015

RNP (Cas9 protein+gRNA) delivery into mouse inner ear for genome editing and rescue of hearing from genetic deafness



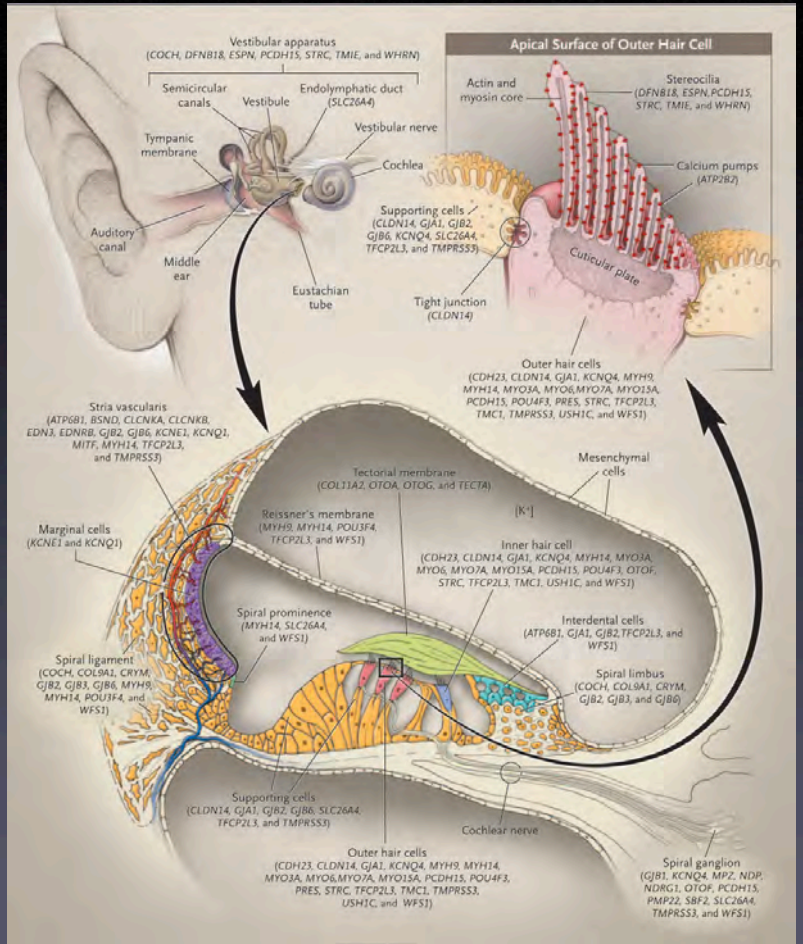




Rescue of hair cells

Zuris, et al., Nat Biotech 2015
Gao et al, Nature 2017

Large number of genes implicated in hearing loss



Non-syndromic deafness genes

Identified: 112
DFNA (dominant): 45
DFNB (Recessive): 71
DFN(x-linked): 5

Estimated deafness genes

400-800

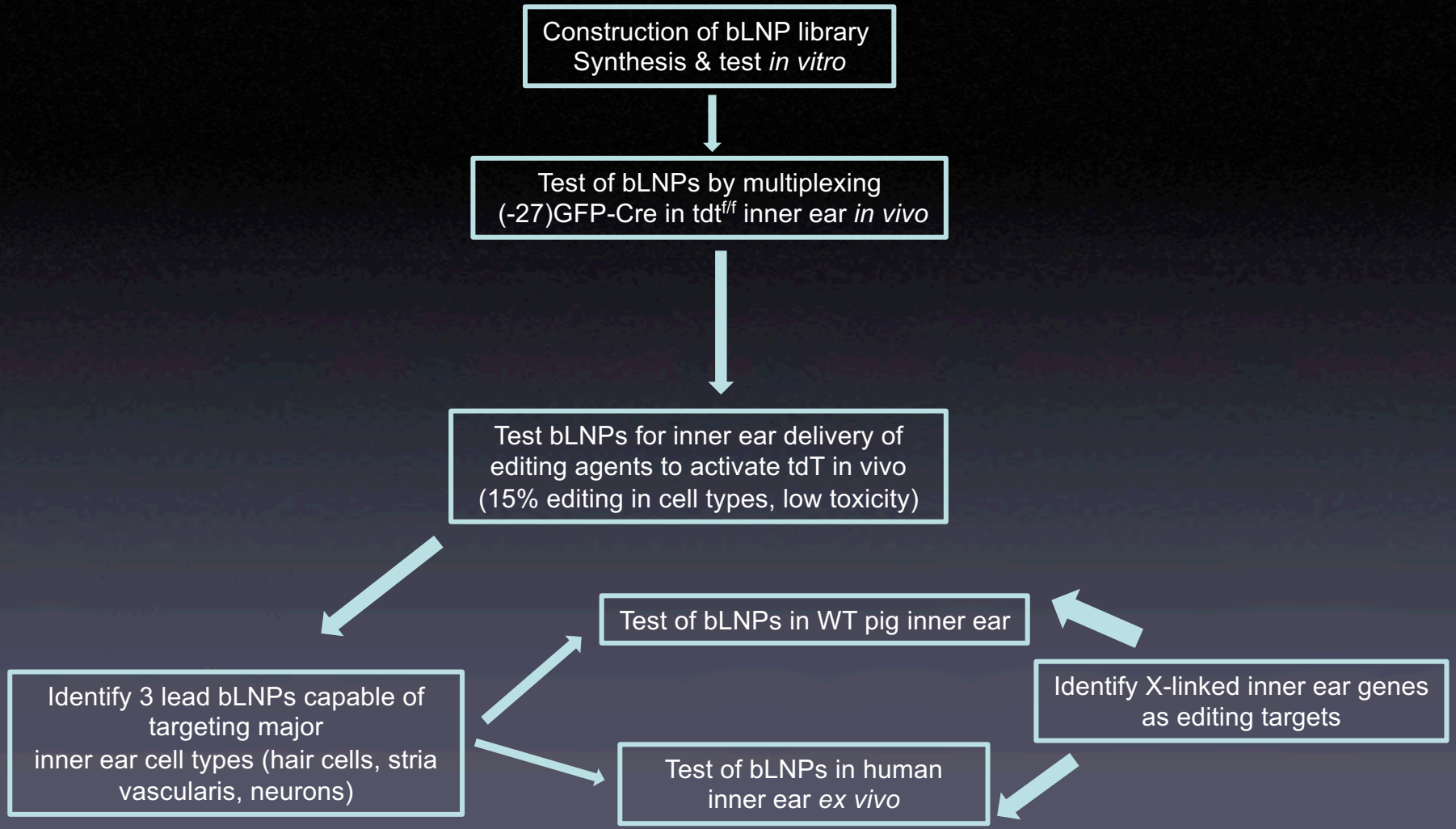
It is necessary to target diverse cell types in mature inner ear with low toxicity

Morton CC & Nance WE, NEJM, 2006

Development of synthetic biodegradable lipid-based nanoparticles for editing agents to target major inner ear cell types

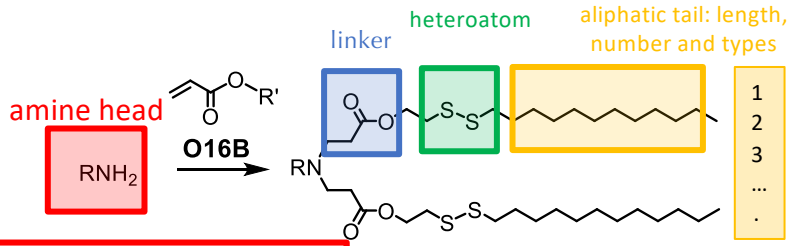
Detection of editing at cellular resolution in non-transgenic animal models

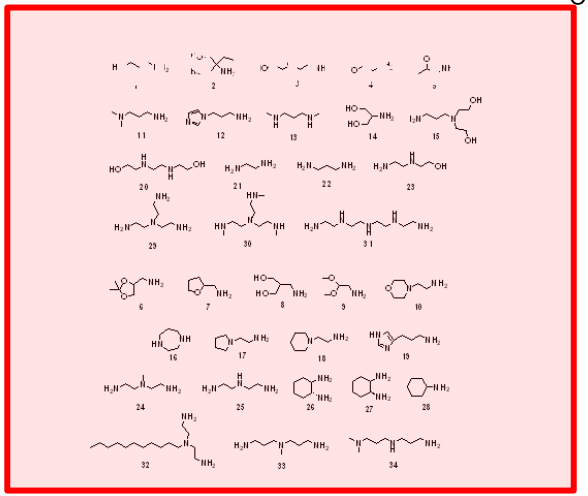
Demonstration of application of delivery vehicles in other species and in human inner ear


Construction of bLNP library
Synthesis & test *in vitro*
Test of bLNPs by multiplexing
(-27)GFP-Cre in tdt$^{f/f}$ inner ear *in vivo*
Test bLNPs for inner ear delivery of
editing agents to activate tdT in vivo
(15% editing in cell types, low toxicity)
Test of bLNPs in WT pig inner ear
Identify 3 lead bLNPs capable of
targeting major
inner ear cell types (hair cells, stria
vascularis, neurons)
Identify X-linked inner ear genes
as editing targets
Test of bLNPs in human
inner ear *ex vivo*

Design of Combinatorial Library of Synthetic Biodegradable Lipid-based Nanoparticles

Design of Combinatorial Library of Synthetic Biodegradable Lipid-based Nanoparticles





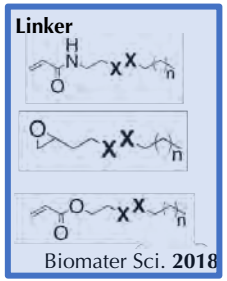
Tail with heteroatoms

O

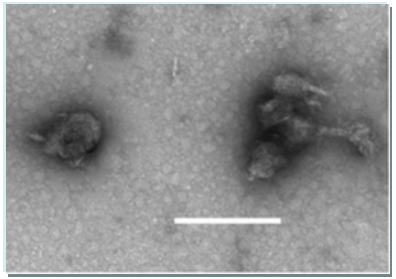
S

Se

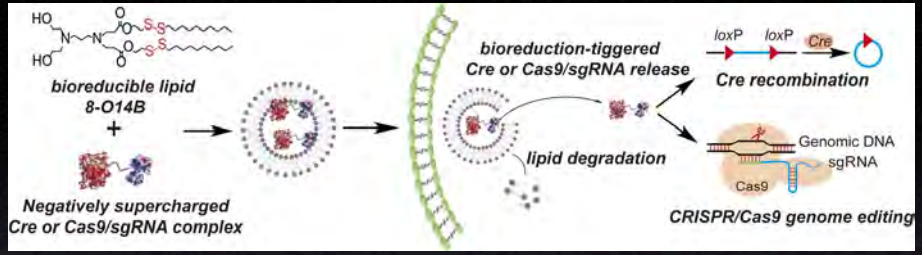
Biomaterials **2018**



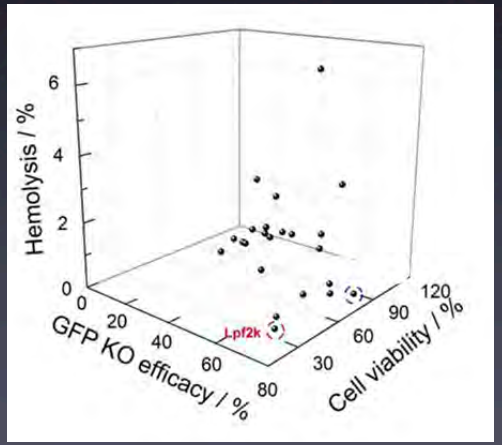
- Positive charge after protonization
- Degradable at reducible environment
- S-S bond to replace C-C bond, equals to a double bond to provide the fluidity of the lipid assembly



Strategy for Library Screening



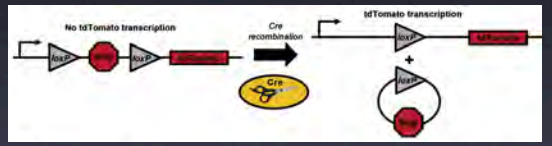
In vitro screening



- Efficiency
- Toxicity
- Hemolytic capability

Lead compounds

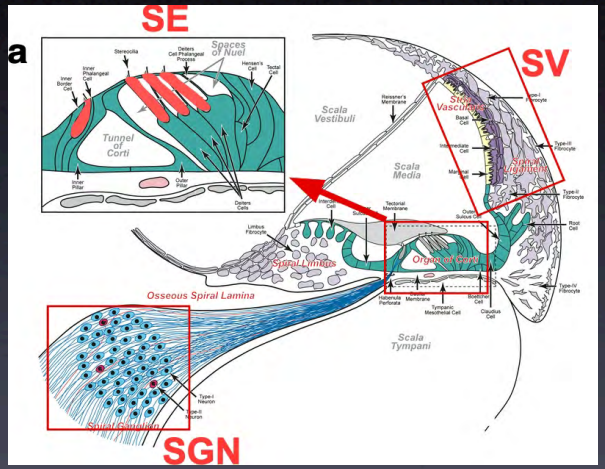
Ai9 mouse model:

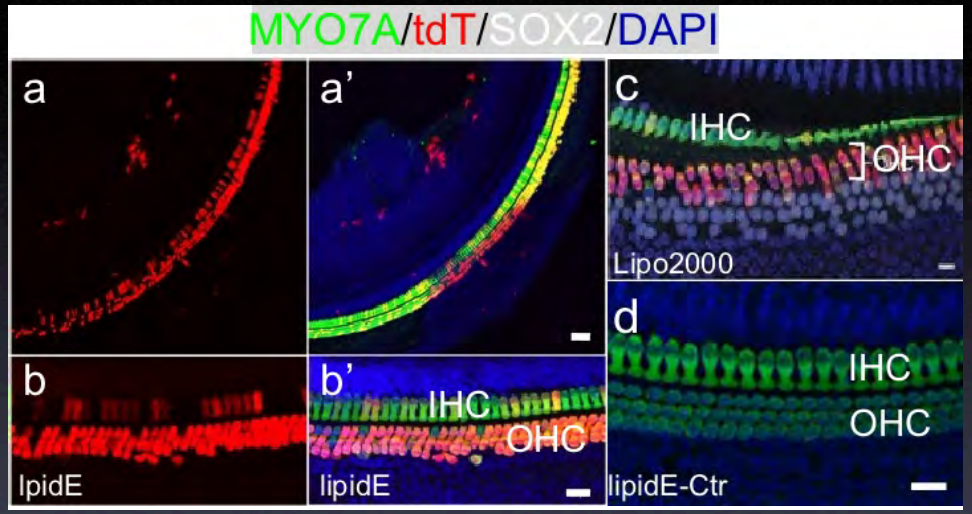


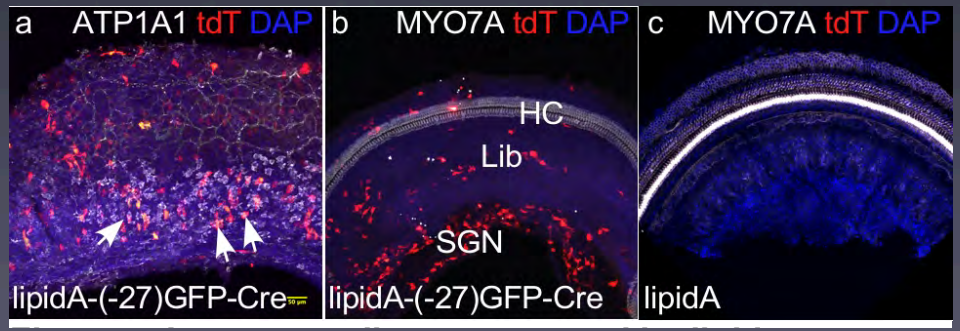
Rapid screening in vivo by multiplexing and inner ear injection

Wang et al., PNAS 2016

Rapid in vivo screening by tdT reporter

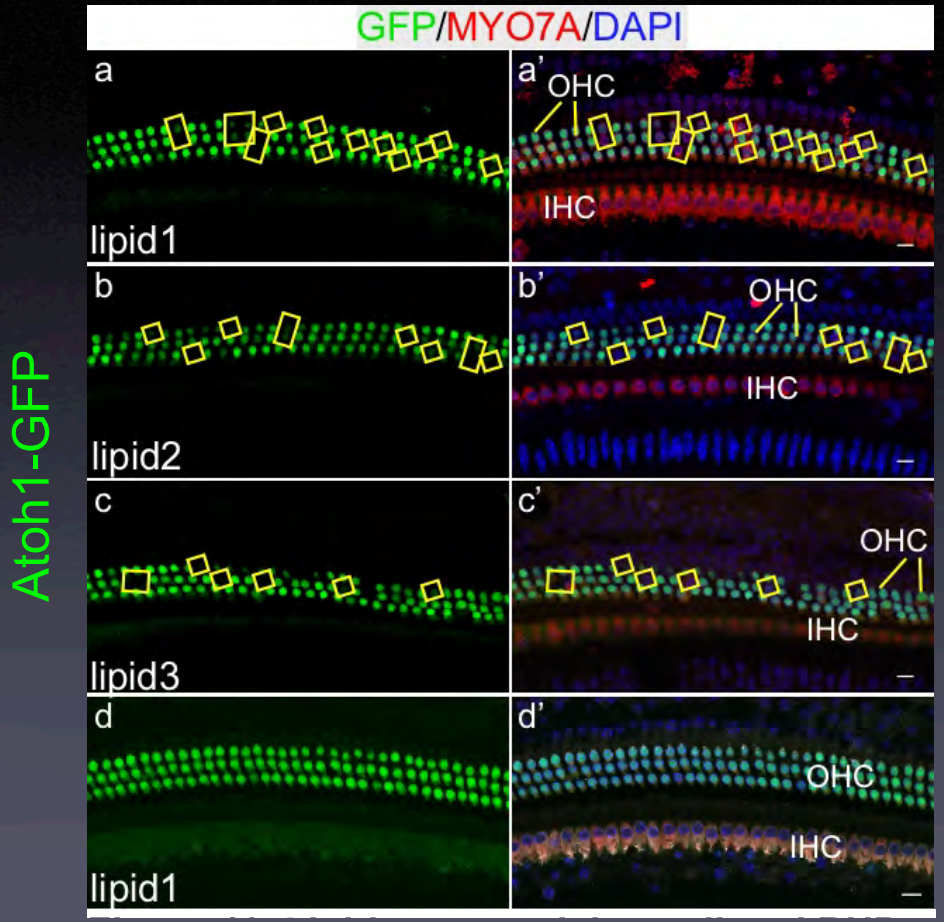




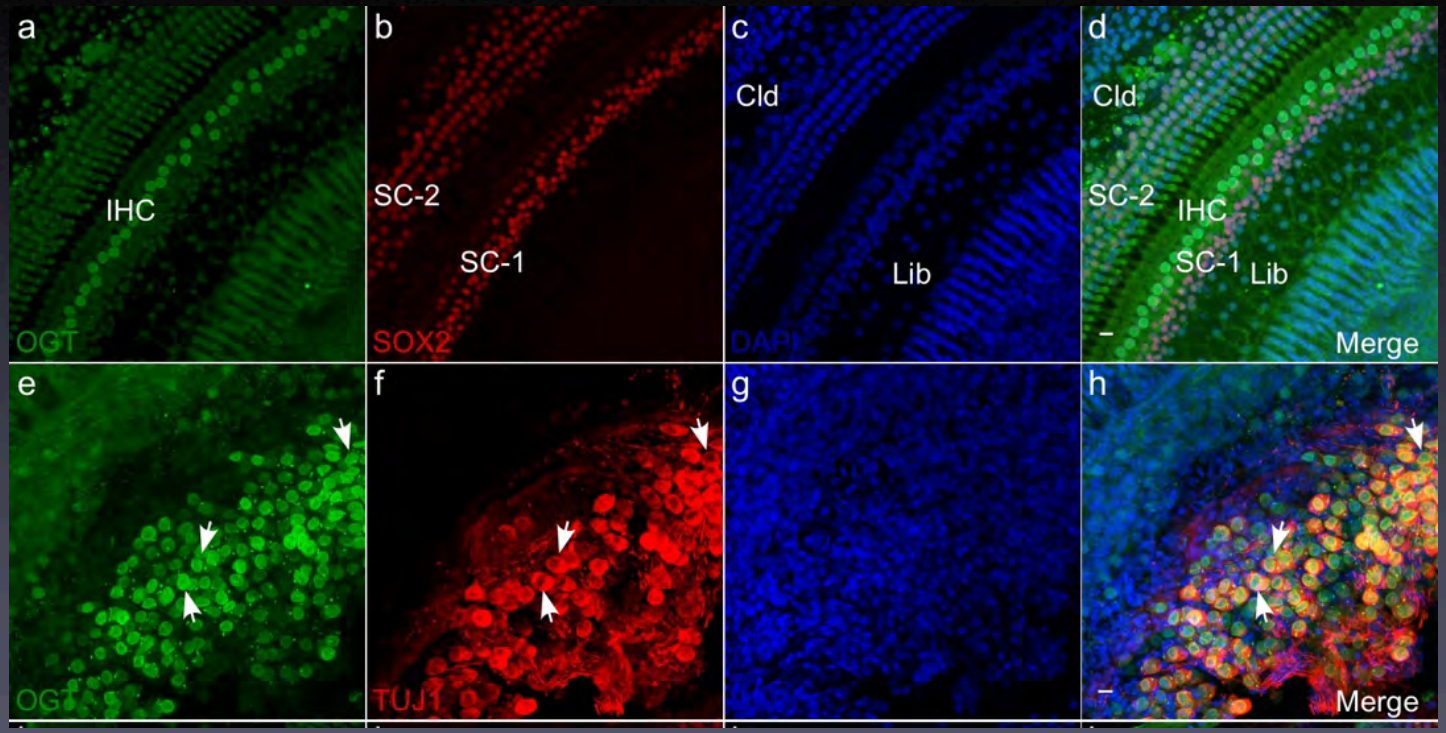


bLNP-mediated genome editing in vivo

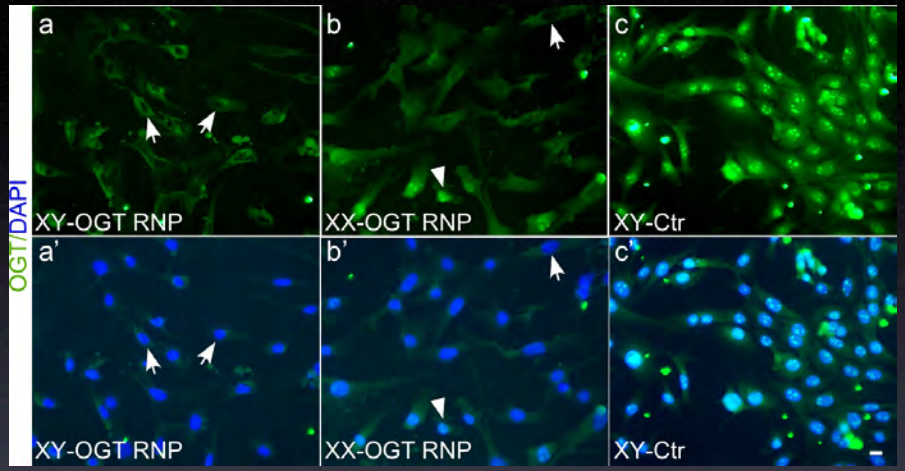
bLNP-mediated genome editing in vivo

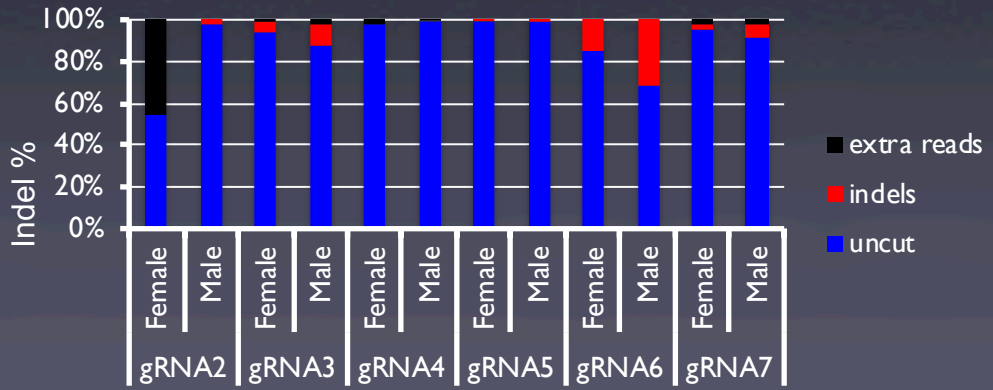


Study editing at cellular level using X-linked genes

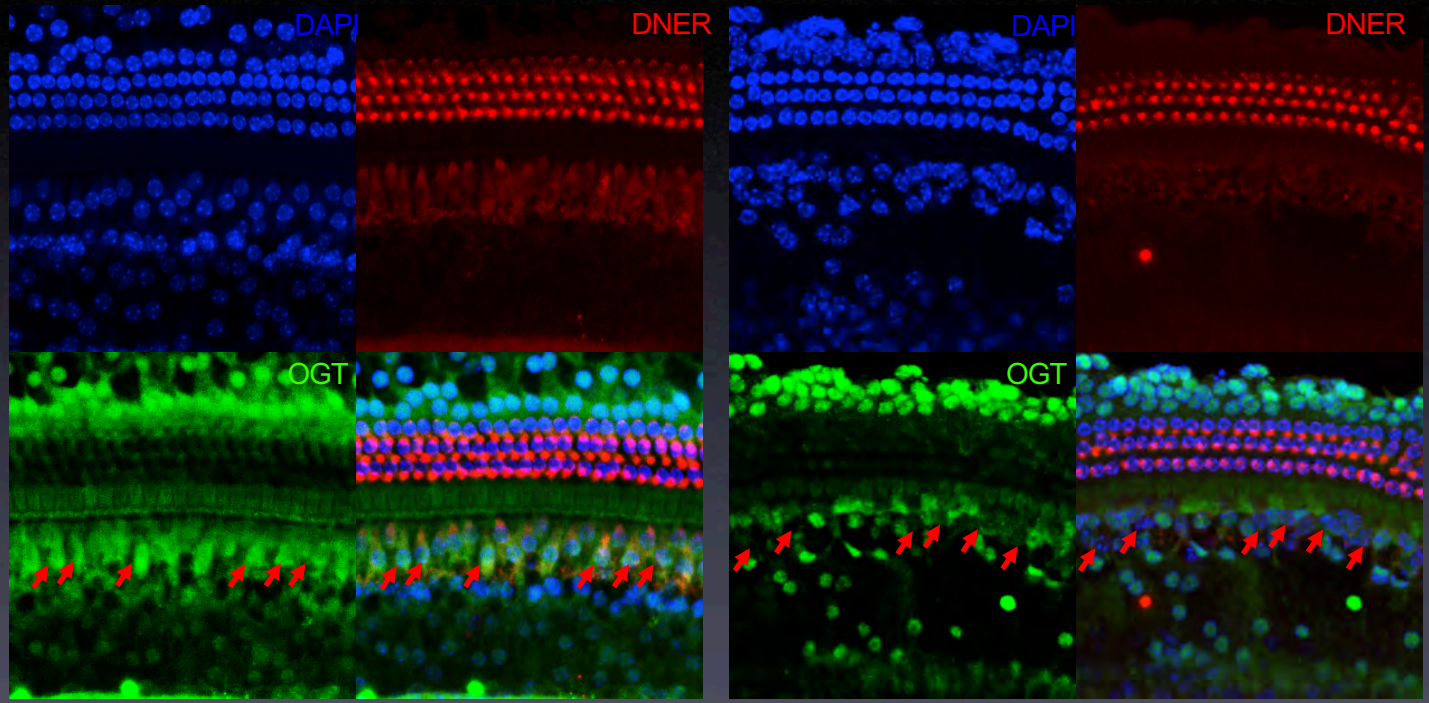


Study editing at cellular level using X-linked genes





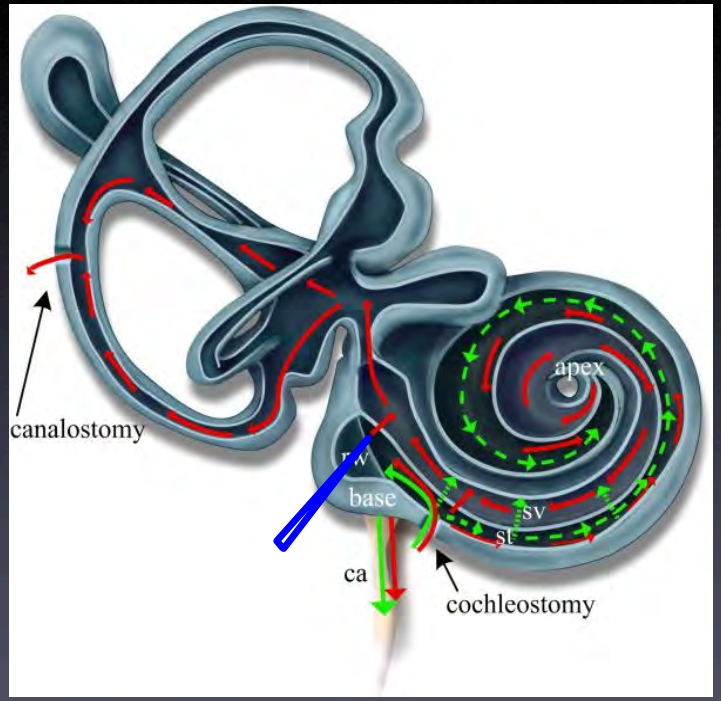
RNP delivery and editing of X-linked genes in vivo

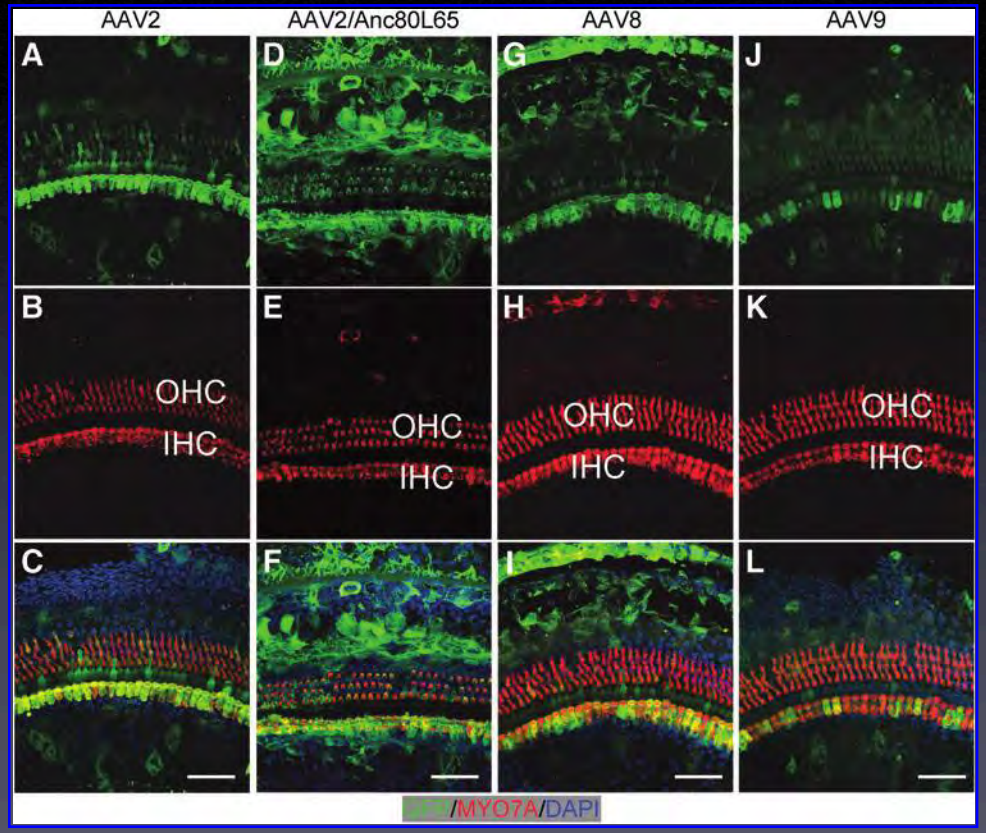


Lipo:Cas9

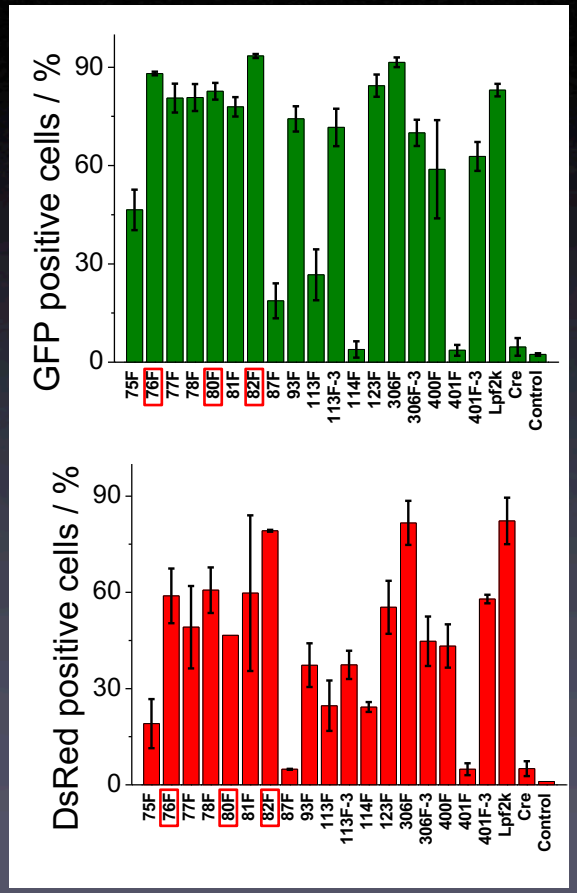
Lipo:Cas9:gRNA-OGT

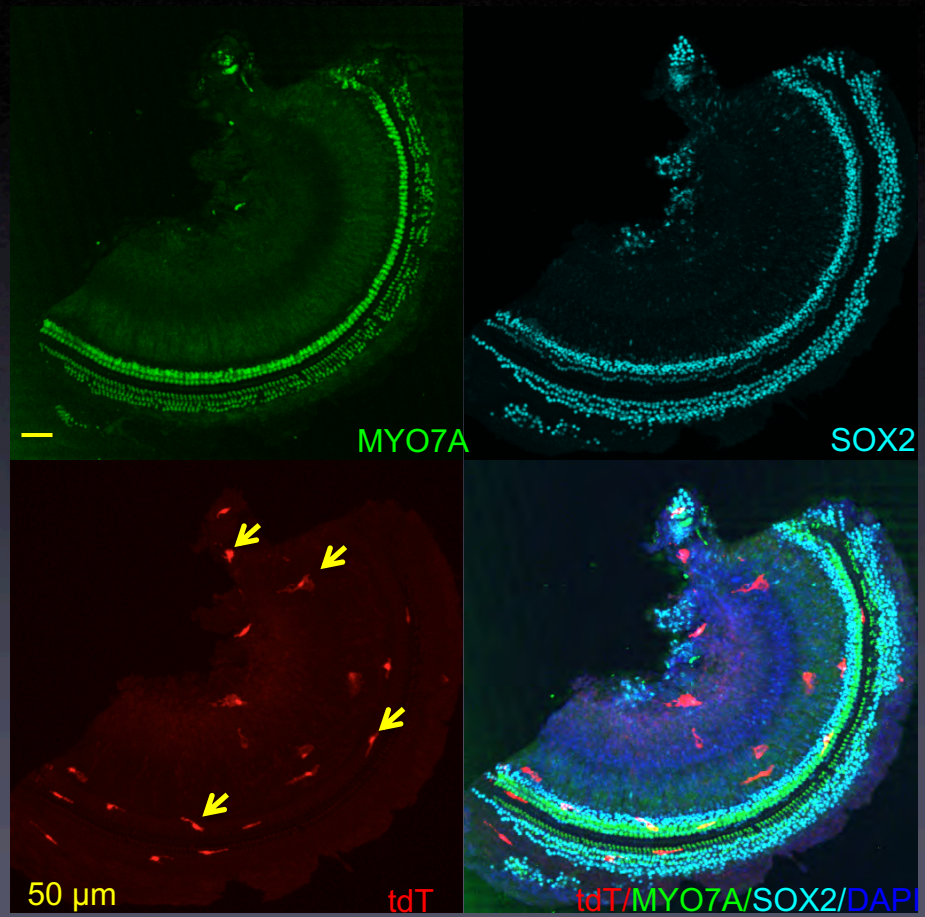
Nanoparticle-mediated adult inner ear delivery





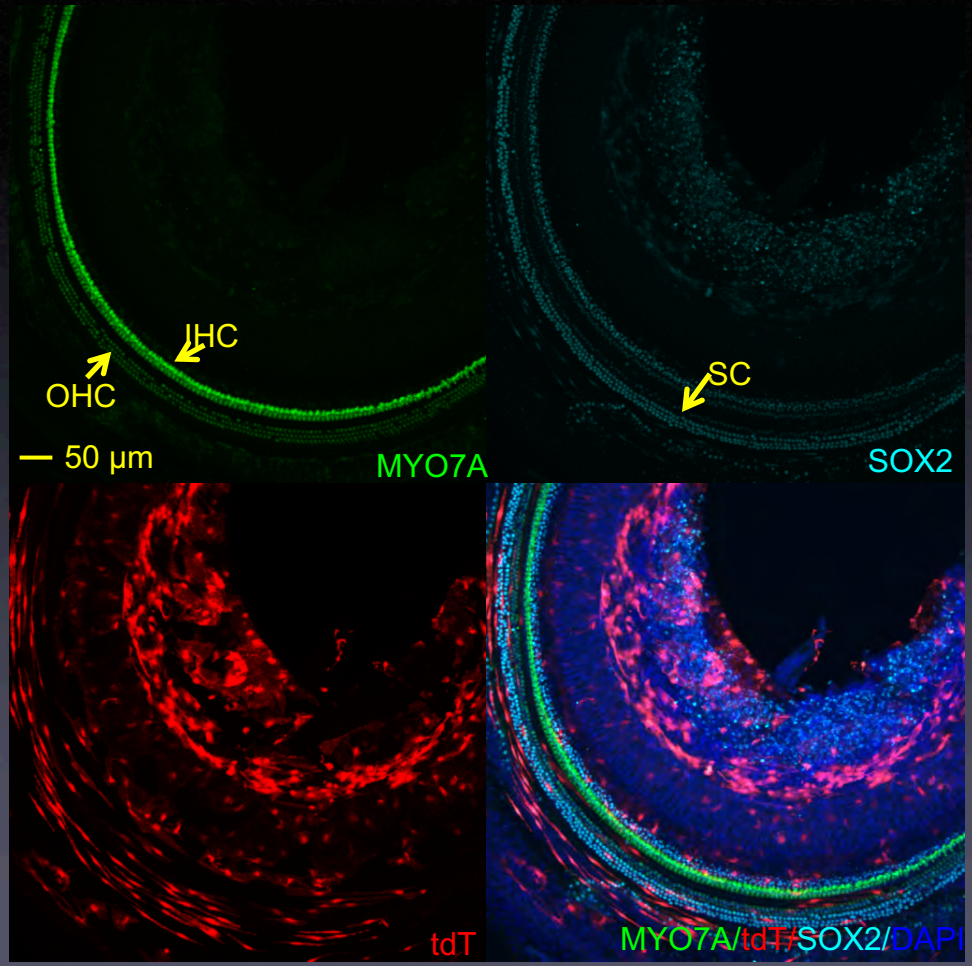
Nanoparticle-mediated inner ear delivery



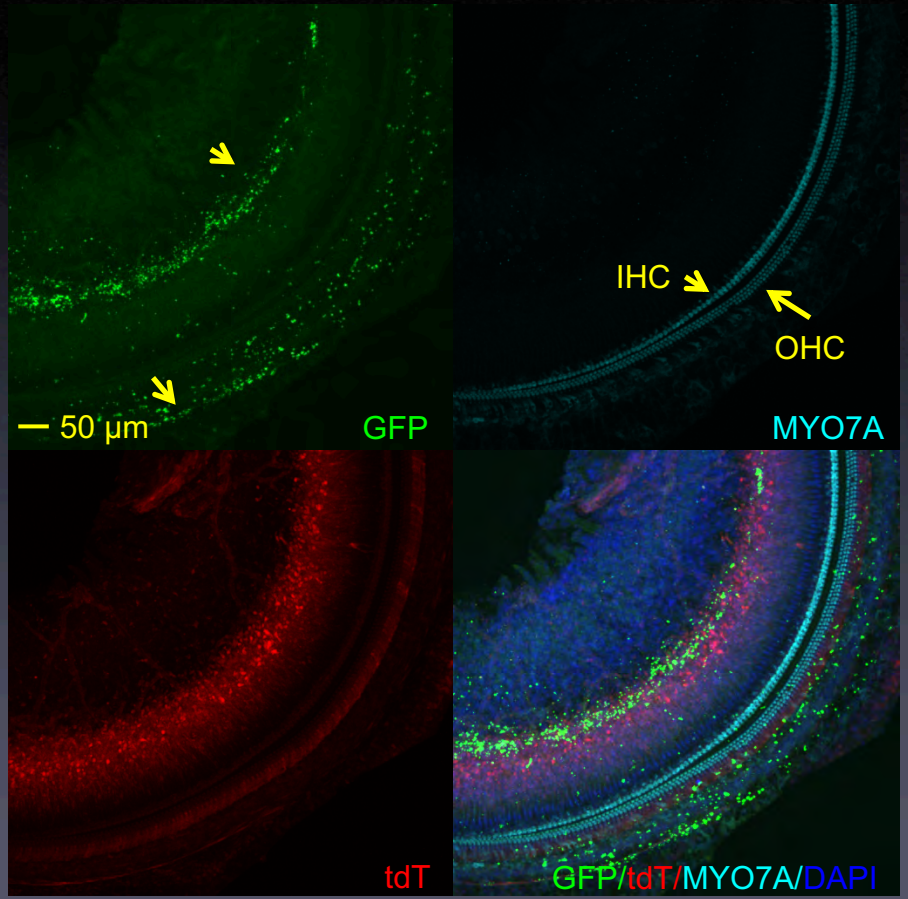


Nanoparticle-mediated inner ear delivery

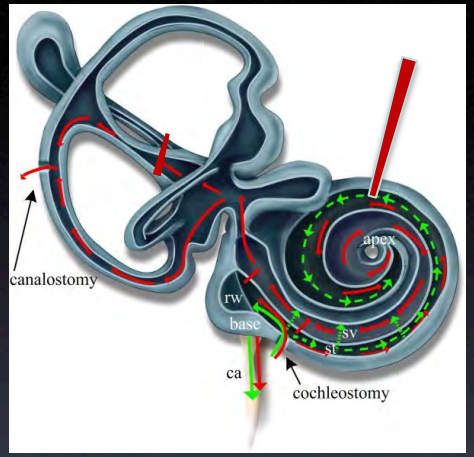
New LNP

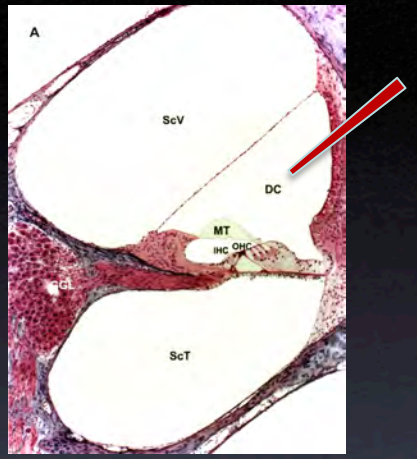


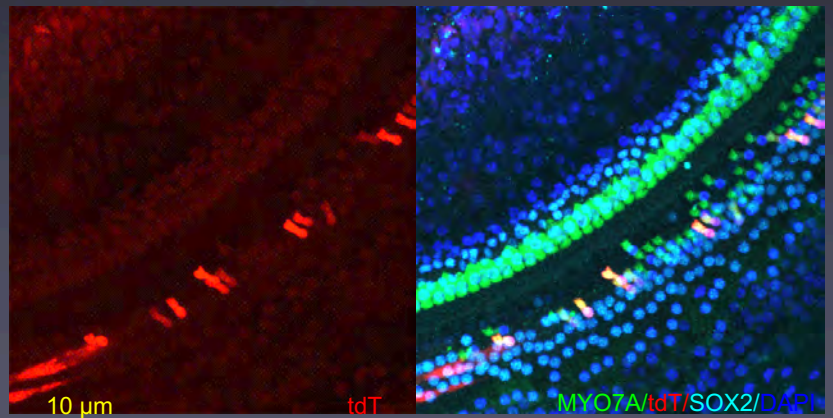
Impact of delivery route

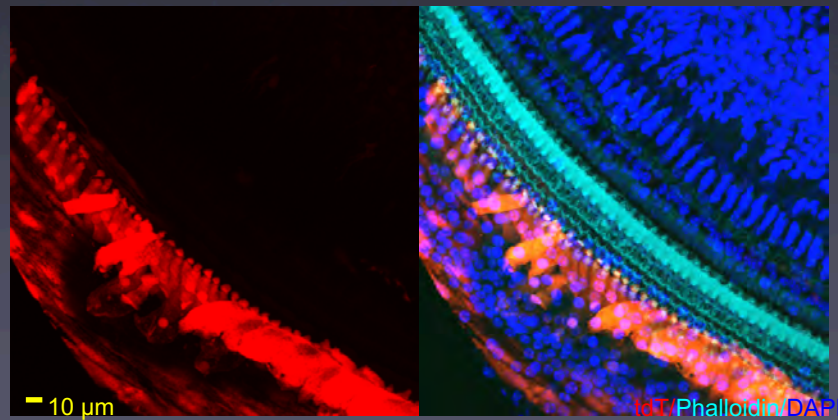


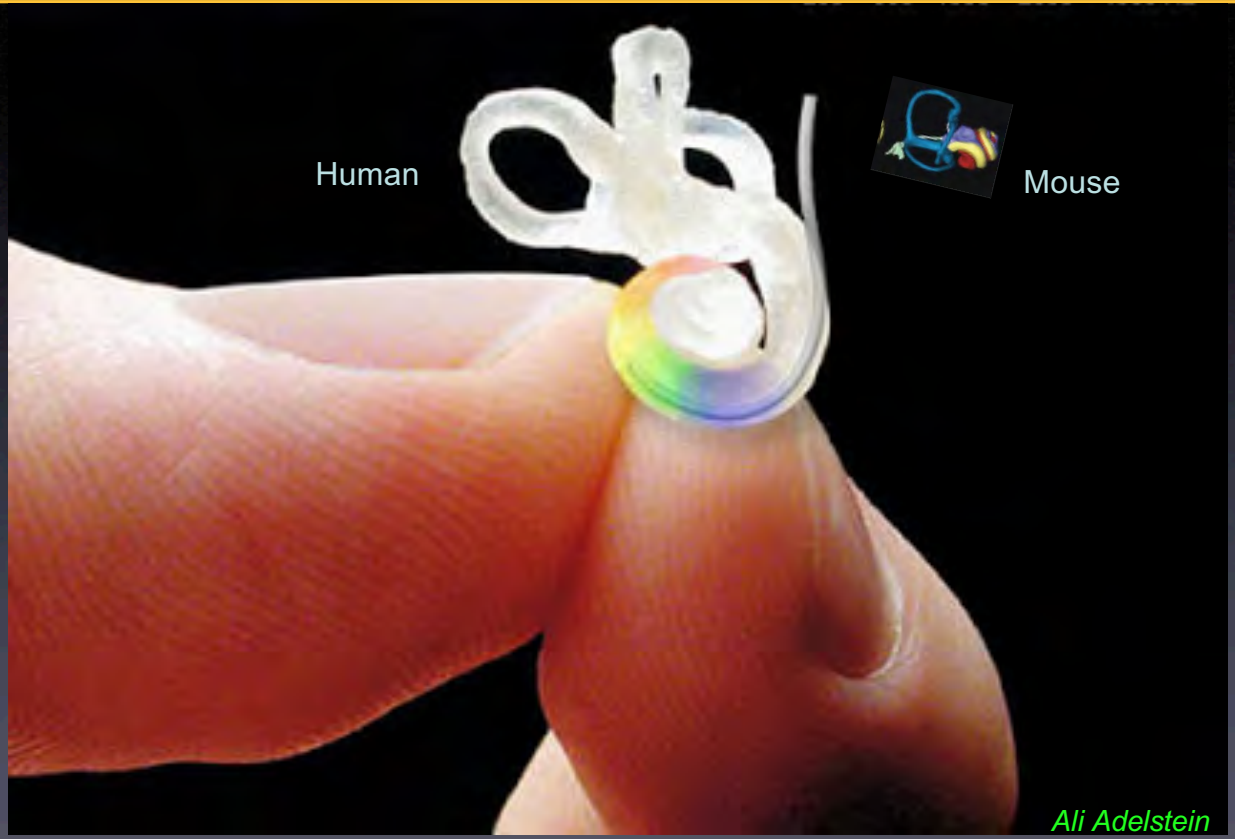
Optimize delivery route and improve permeability







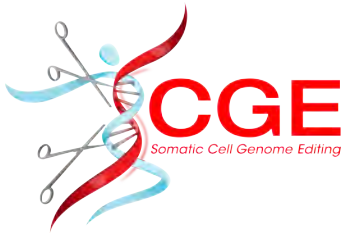



Human
Mouse
Ali Adelstein

Identify three lead bLNPs that mediate editing in major mouse inner ear cell types with high efficiency (>15%) and low toxicity

Test of RNP delivery and editing of X-linked genes in mouse retina *in vivo*

Test of RNP delivery and editing of X-linked genes in pig *in vivo* and human inner ear *ex vivo*



Program Officers
PJ Brooks
Stephanie Morris