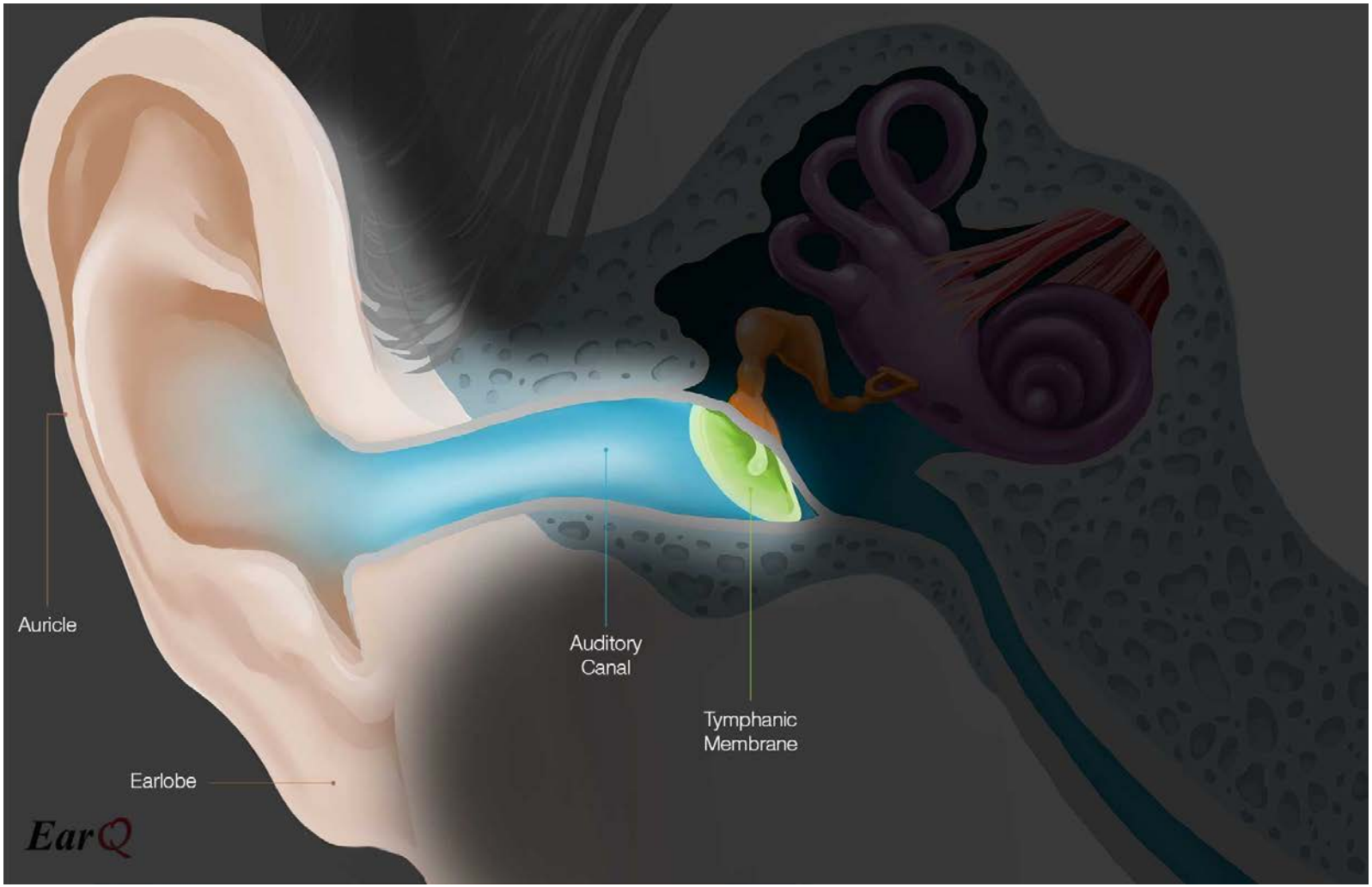
Progress in Gene Therapy - Focus on Hearing Loss

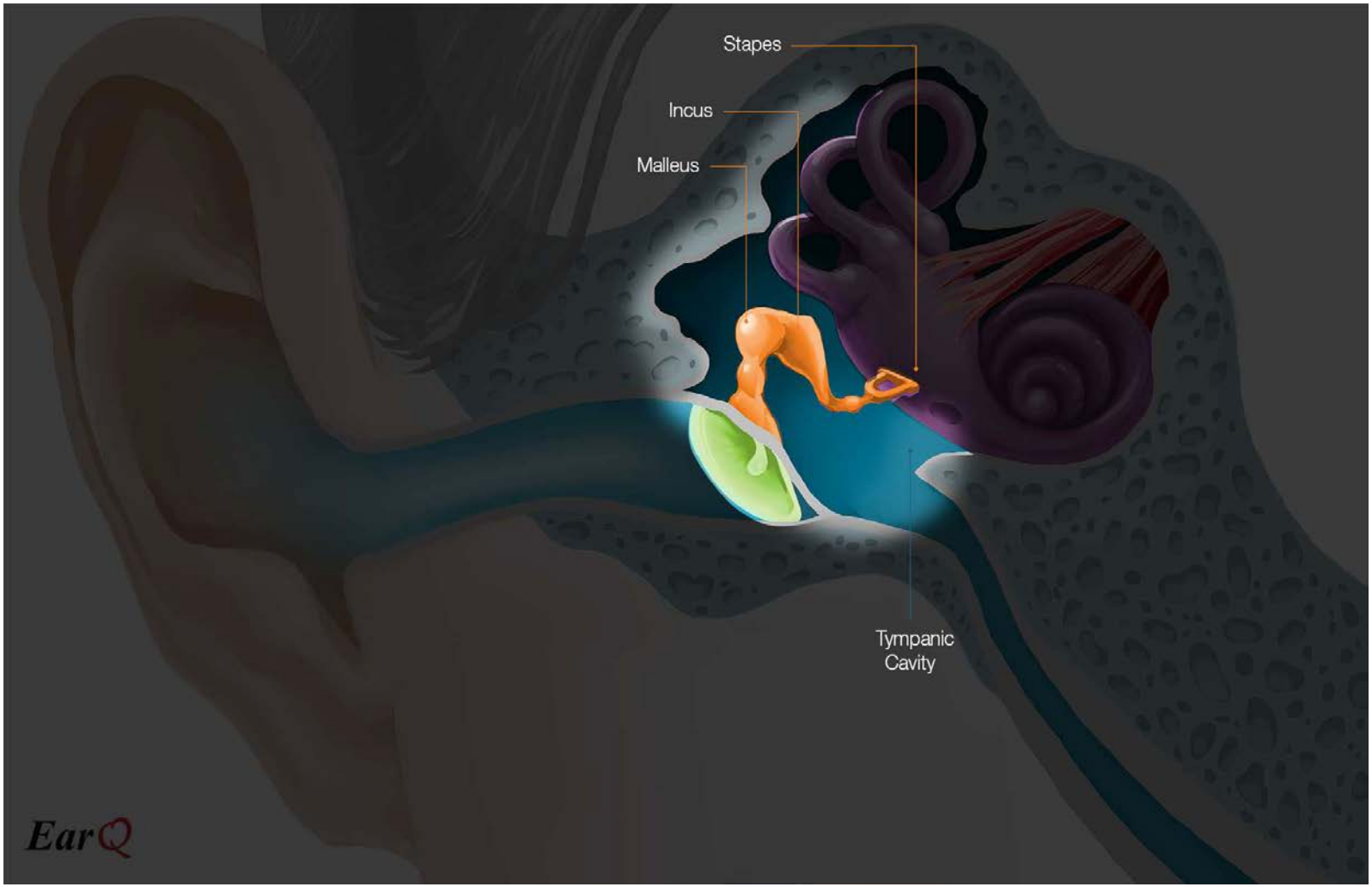
08/08/17

Claudia Scheckel

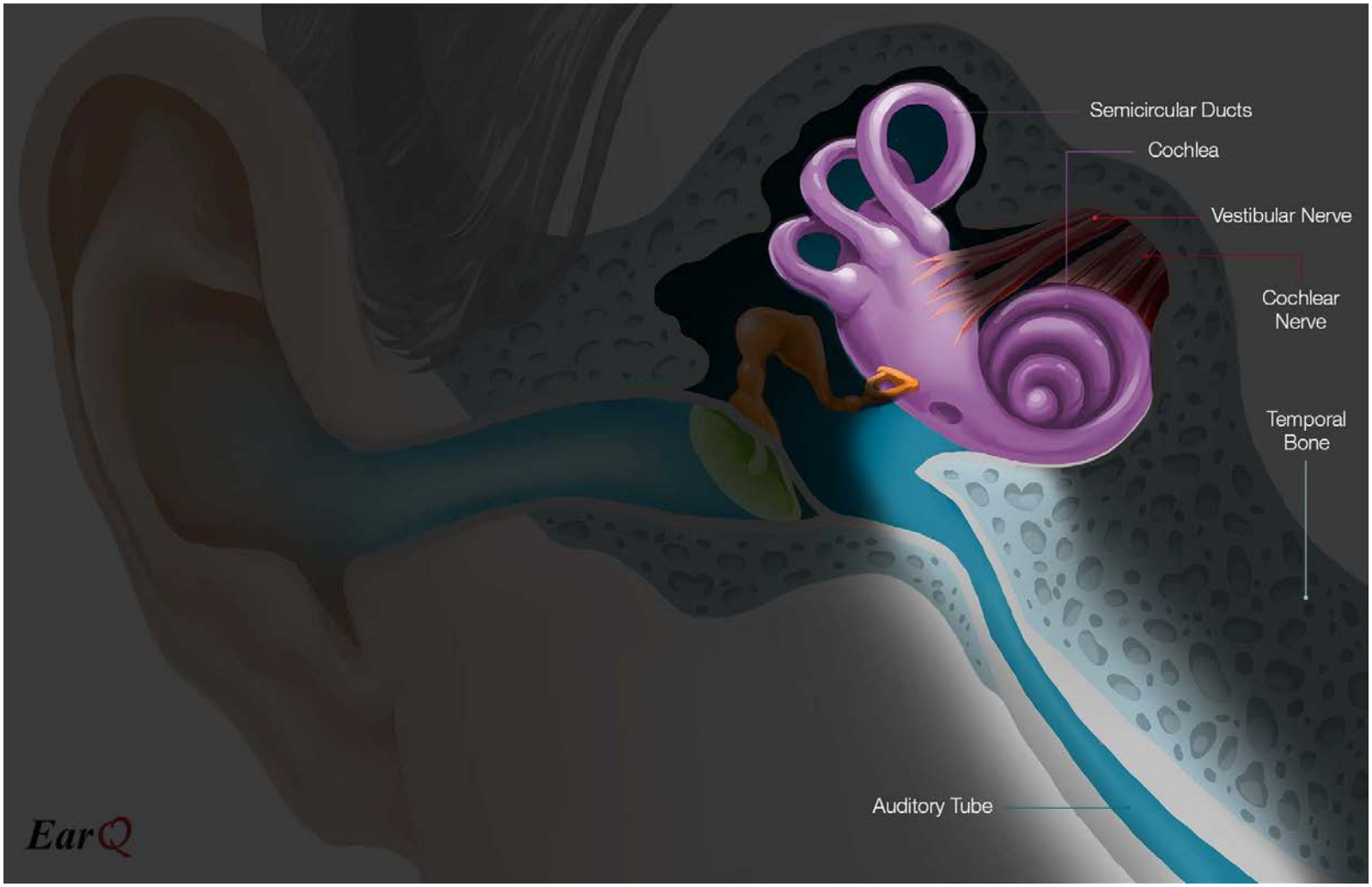
The Auditory System – The outer ear



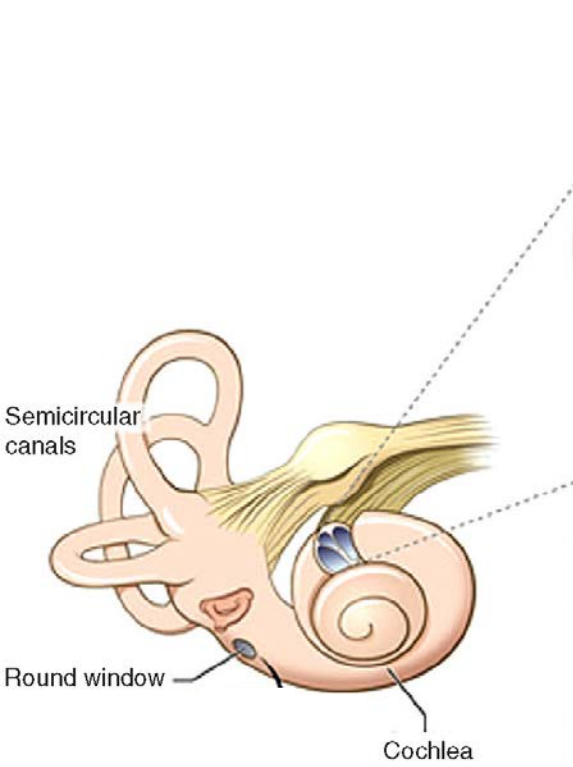
The Auditory System – The middle ear

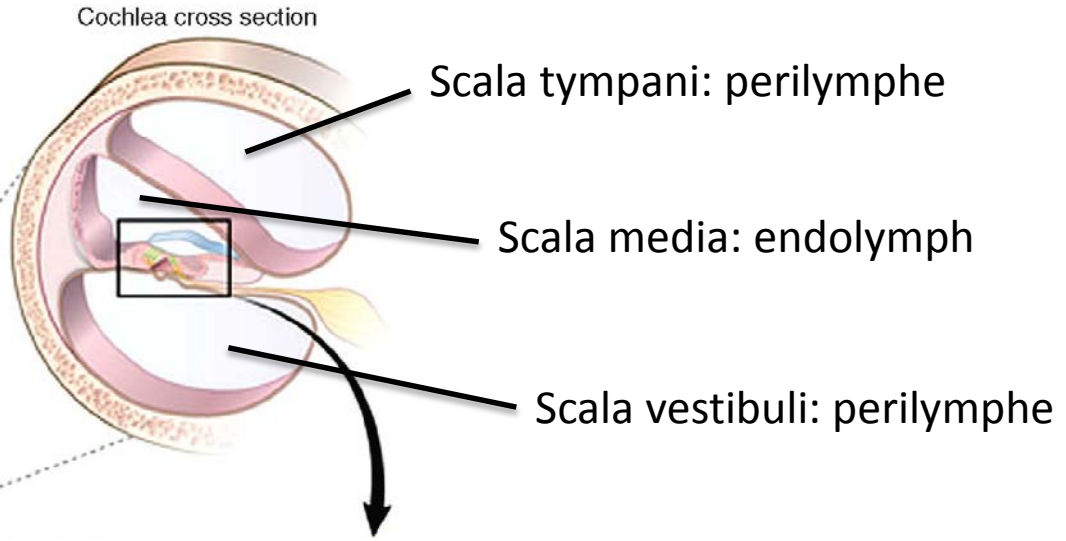


The Auditory System – The inner ear



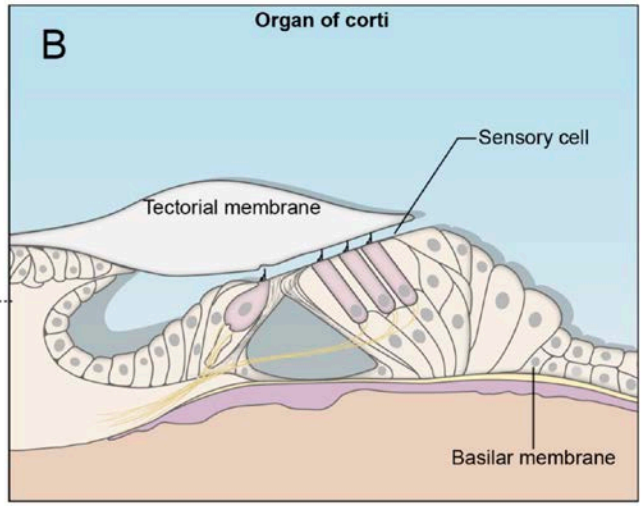
The Inner Ear – The cochlea

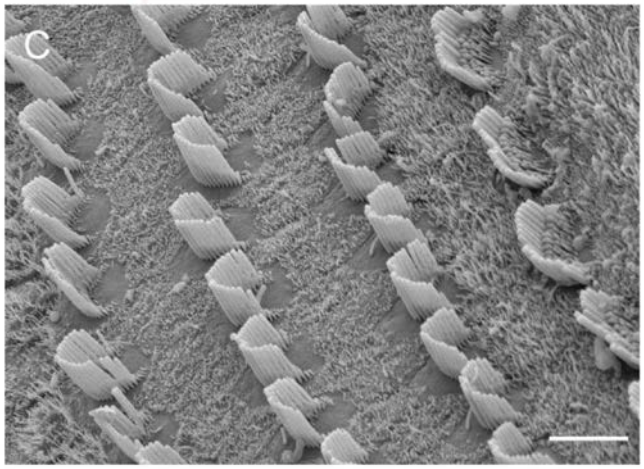




- Conversion of oscillations in air to fluid pressure traveling down the cochlear duct
- Induction of basilar membrane vibrations
- 100x amplification of signal
- Perception of frequencies from 20Hz – 20kHz

Inner ear – Organ of Conti





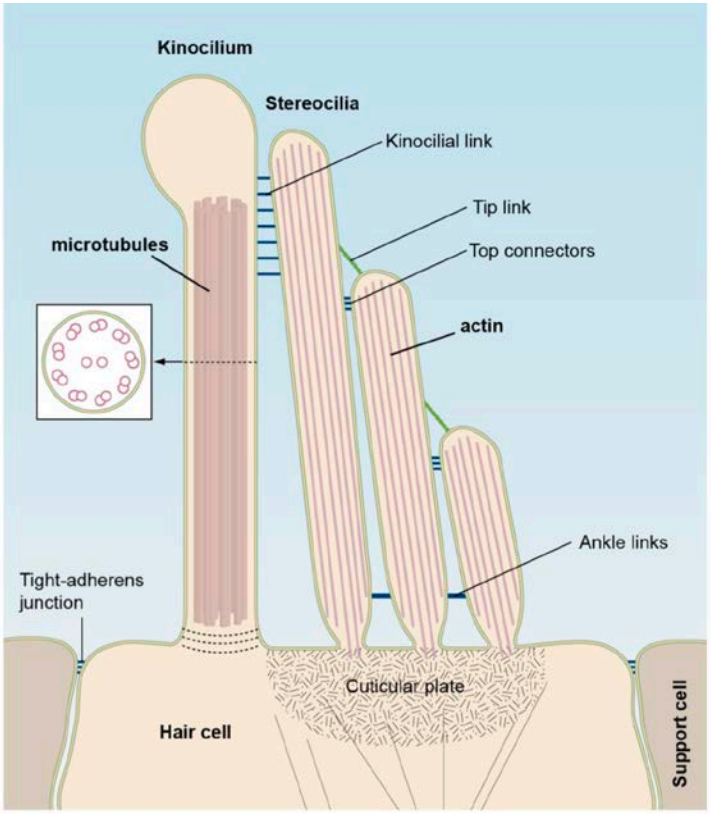
- 16 000 hair cells
  - 3 outer hair cell row: enhance basilar membrane vibrations (sensitivity and frequency selectivity)
  - 1 inner hair cell row: activate afferent neurons
- Supporting pillar cells form tight junctions with hair cells and attach to the basilar membrane
- Perception of different frequencies
  - differential height of stereocilia
  - differential width/thickness of basilar membrane

➔ each frequency induces a maximal motion at a particular position (low-frequency travels farthest)

➔ hair cells at the position of the wave's peaks transduce basilar membrane oscillations into receptor potentials

Inner Ear – Hair Cells

stimulation



- Stereocilia are attached to tectorial membrane: mechanically sensitive hair cell bundle
- Staircase structure: established during development by kinocilium (sometimes lost after maturation)
- Stereocilia are linked at the tips: required for mechanical sensitivity

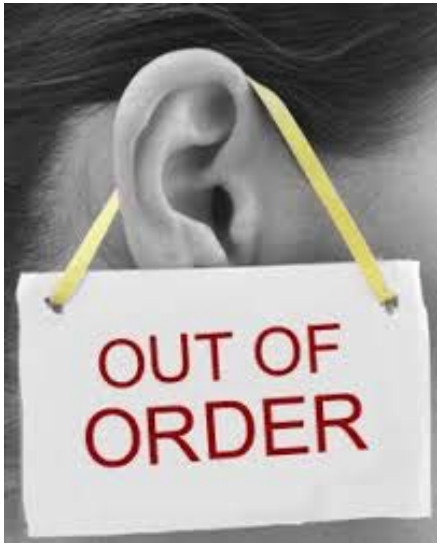
➔ Deflection of hair bundles

➔ Opening of mechanically gated ion channels

➔ Hair cell depolarization

➔ Excitation of nerve fibers that innervate the particular regions

Hearing Loss



Environmental
- High-intensity sound/acoustic trauma
- Infection
- Drugs

Hereditary
- 1 in 1000 newborns suffers from hearing loss
- 30% of cases are accompanied with other clinical features (syndromic hearing loss)
- 70% of cases are nonsyndromic
  - 130 linked genetic loci
  - 60 genes with causative mutations

Current treatments
- Hearing Amplification
- Cochlear Implant
- Gene Therapy

Mouse Models of Hearing Loss

Dancing/Waltzing Mice

- First described 80 BC in China
- Domesticated in 18th century in Japan
- Phenotype linked to hearing loss

Whirler Mouse

- Complete loss of Whirlin function
- Model for human type 2 Usher syndrome
- Hearing loss, blindness, vestibular defects
- Circling, head-tossing

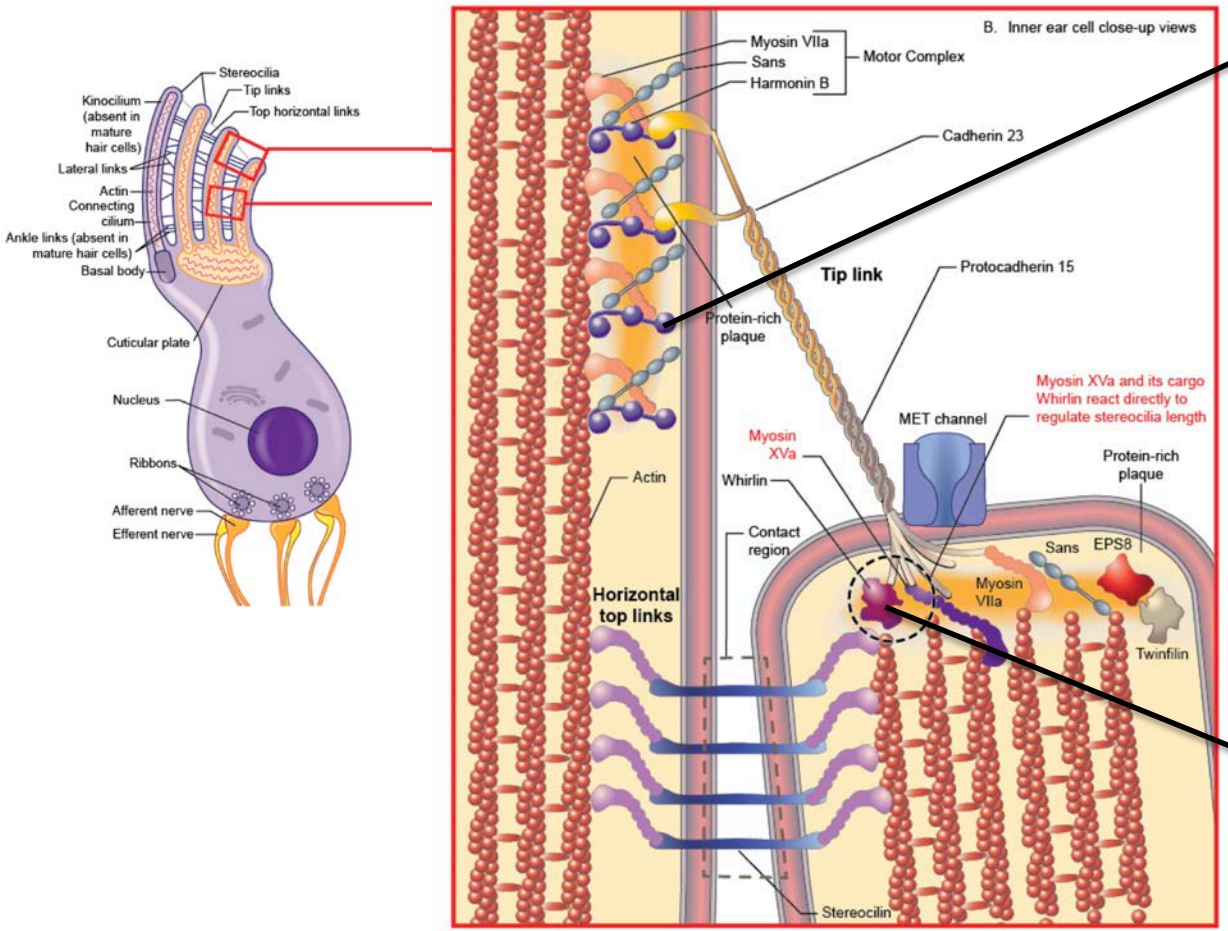
Ush1c Mouse

- Complete loss of Harmonin function
- Model for human type 1c Usher syndrome
- Severe hearing loss, retinal and vestibular dysfunction
- Circling, head-tossing

Ush proteins

- localize to the apex of hair cells
- morphogenesis of sensory hair bundles

Harmonin B: type 1c Usher syndrome
Structural role and sensory transduction



WHRN: DFNB3 or type II Usher syndrome
Scaffold required for stereocilia development

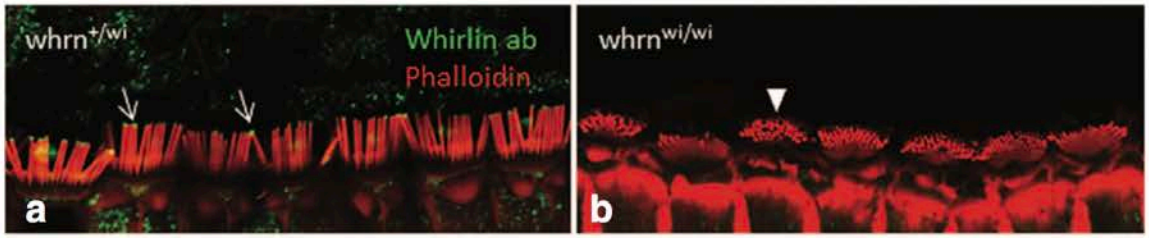
original article

Gene Therapy Restores Hair Cell Stereocilia Morphology in Inner Ears of Deaf Whirler Mice

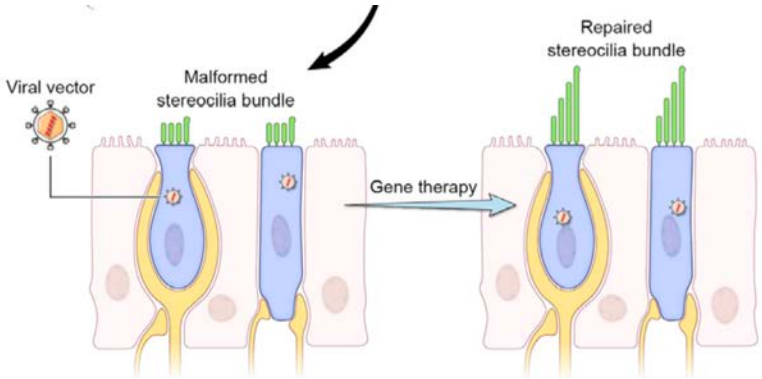
Wade W Chien[1,2], Kevin Isgrig[3], Soumen Roy[3], Inna A Belyantseva[4], Meghan C Drummond[4], Lindsey A May[3], Tracy S Fitzgerald[5], Thomas B Friedman[4] and Lisa L Cunningham[3]

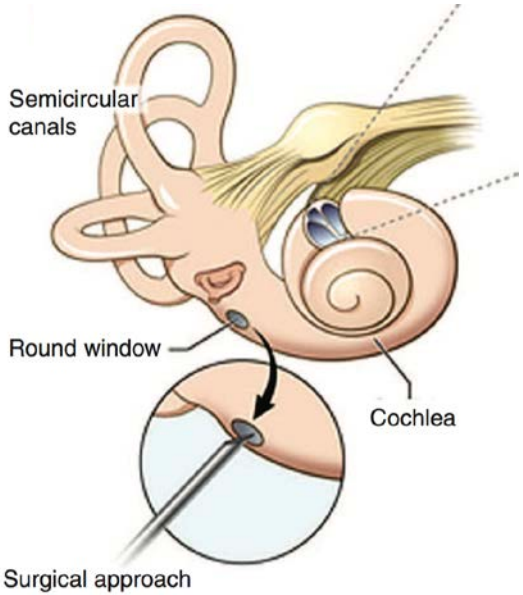
[1]Neurotology Program, National Institute on Deafness and Other Communication Disorders (NIDCD), National Institutes of Health, Bethesda, Maryland, USA; [2]Department of Otolaryngology-Head & Neck Surgery, Johns Hopkins School of Medicine, Baltimore, Maryland, USA; [3]Section on Sensory Cell Biology, NIDCD, National Institutes of Health, Bethesda, Maryland, USA; [4]Laboratory of Molecular Genetics, NIDCD, National Institutes of Health, Bethesda, Maryland, USA; [5]Mouse Auditory Testing Core Facility, NIDCD, Bethesda, Maryland, USA

Abnormal stereocilia morphology in *whirlin* mice

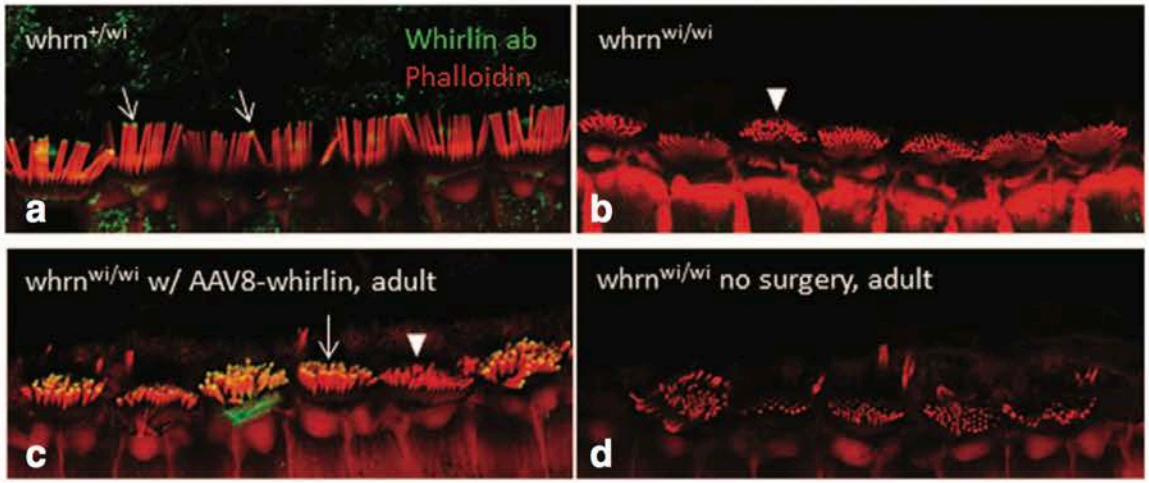


- Hair cell degeneration starts only at P30
- Administration of wild-type *whirlin* cDNA via an adeno-associated virus serotype 2/8 (AAV8) at P30

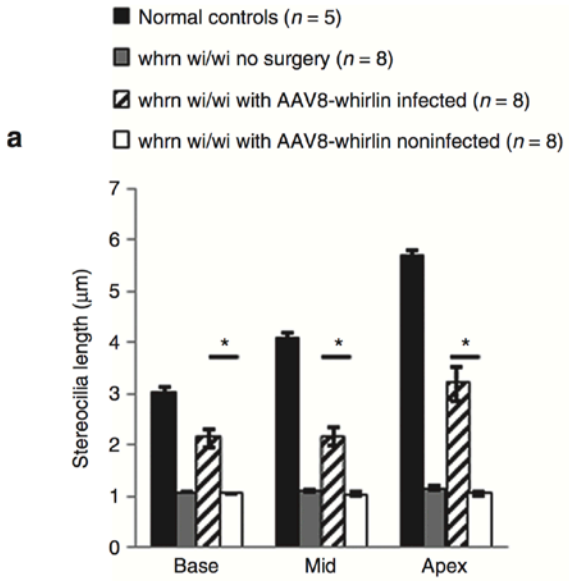




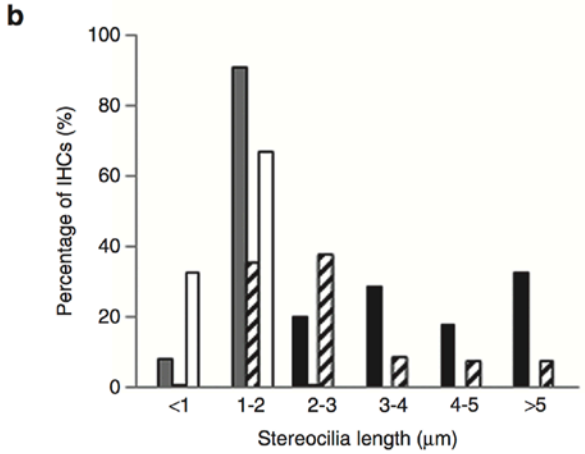
Whirlin Gene Therapy restores *whirlin* expression

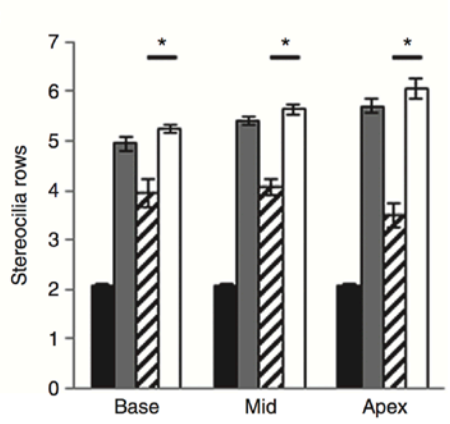


Whirlin Gene Therapy partially restores stereocilia length/row number

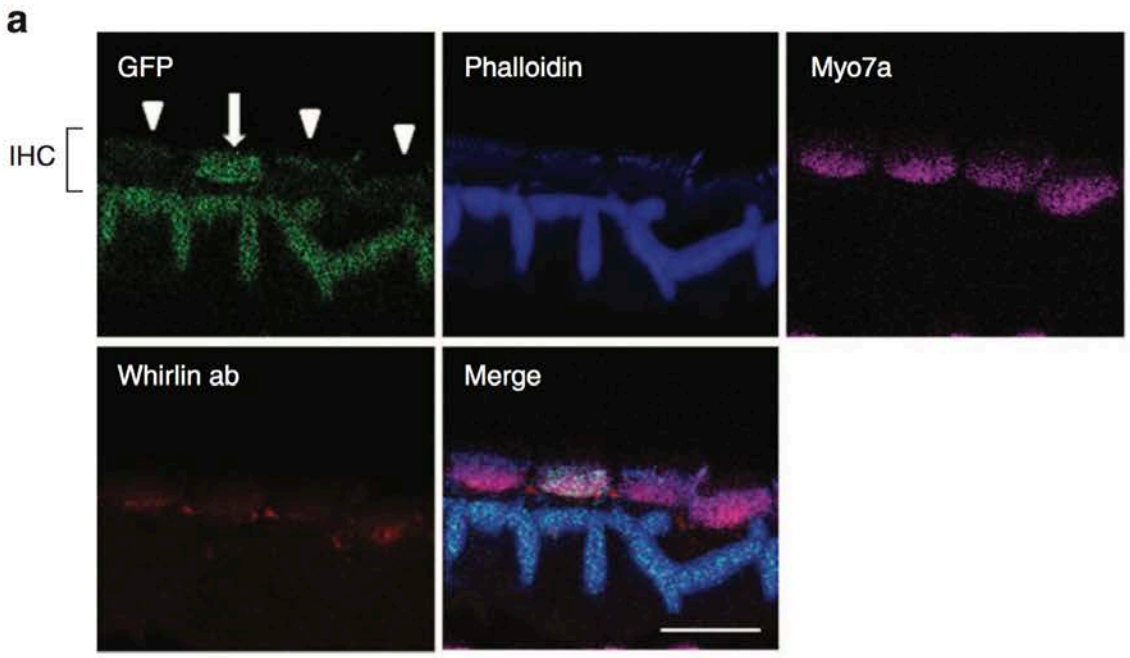


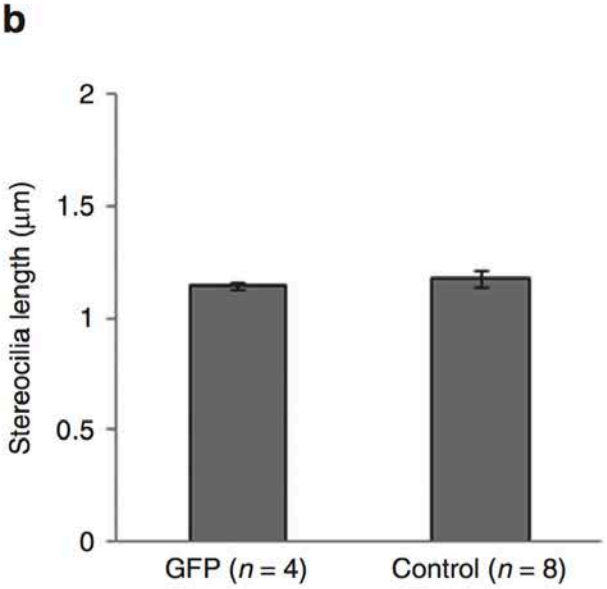
n IHCs/n animals	Base	Mid	Apex
Normal controls	15/5	15/5	15/5
whrn wi/wi controls	19/5	21/6	28/8
whrn wi/wi with AAV8-whirlin infected	17/5	16/5	20/6
whrn wi/wi with AAV8-whirlin non-infected	21/5	15/5	20/6





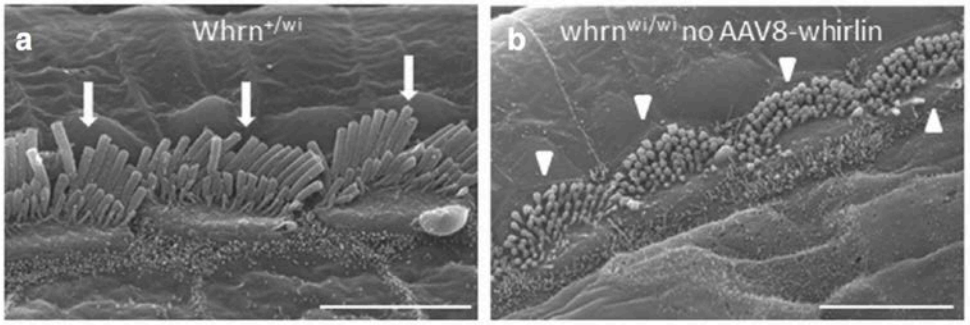
GFP Gene Therapy does not alter stereocilia morphology





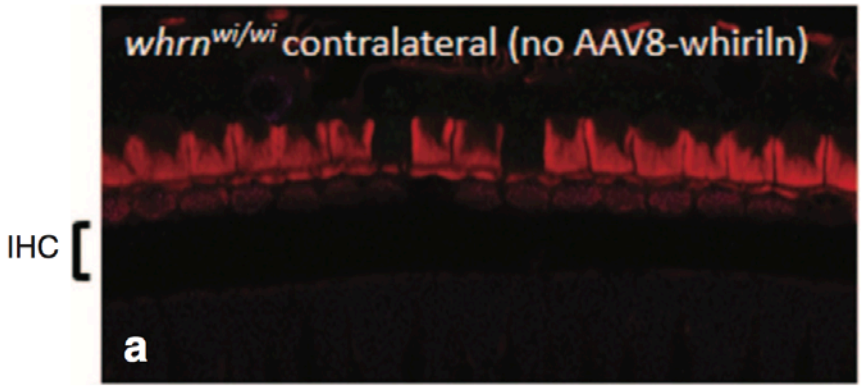
Whirlin Gene Therapy partially restores stereocilia morphology

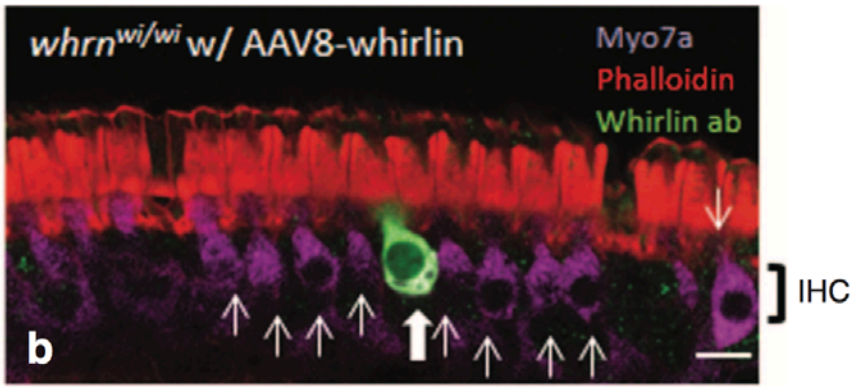
middle

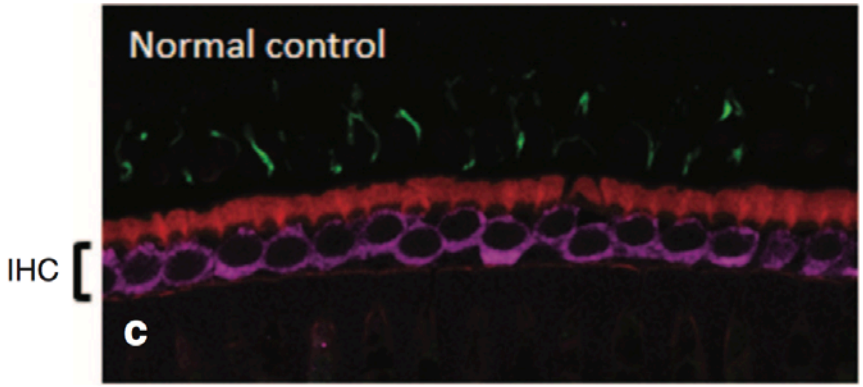


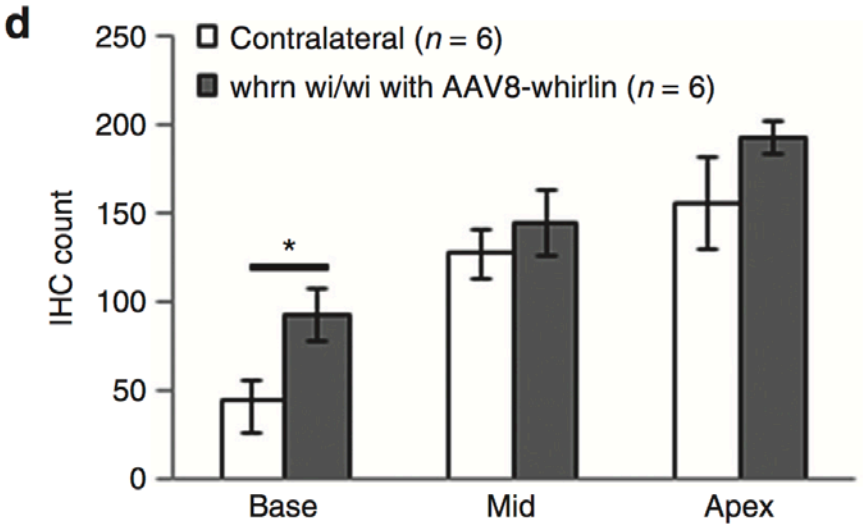
middle

Whirlin Gene Therapy temporarily increases IHC survival



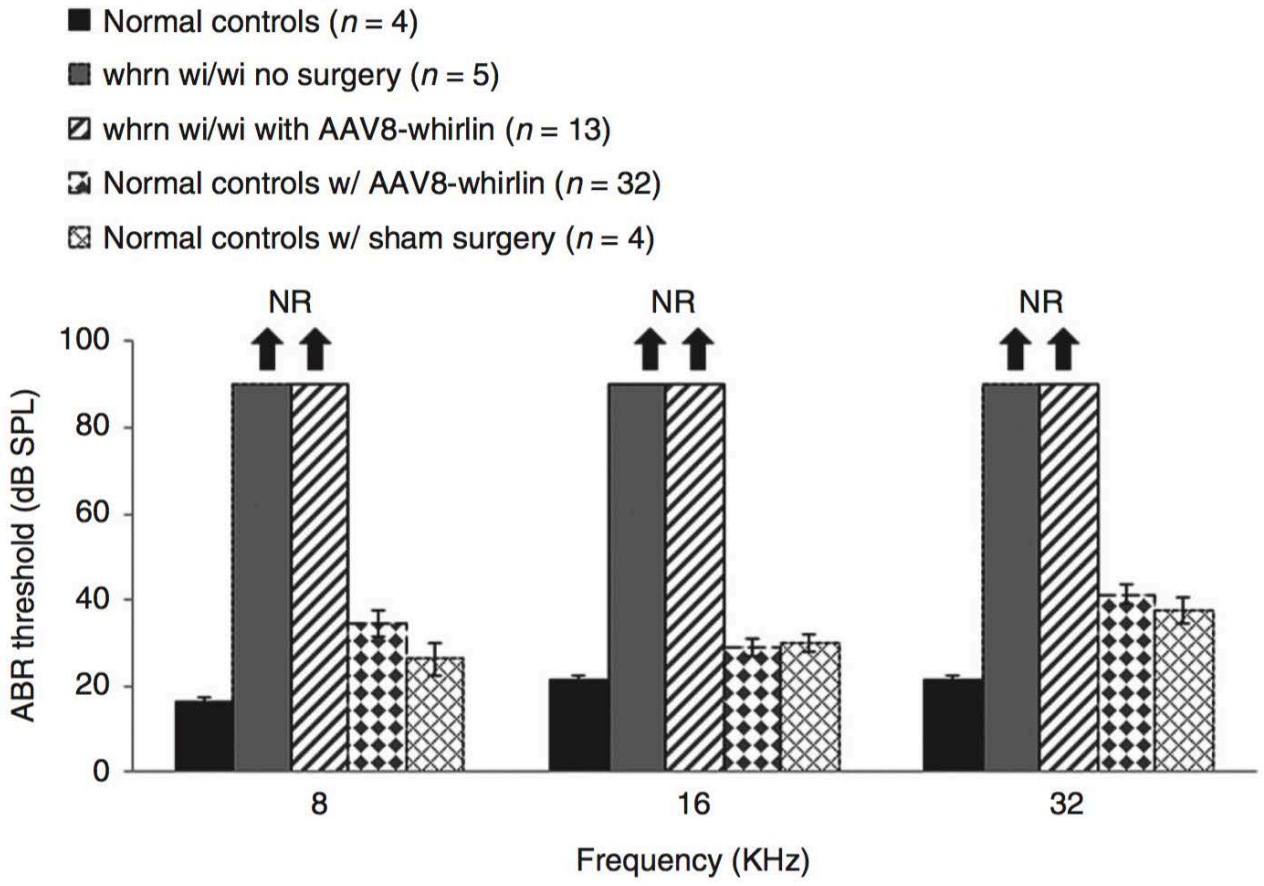






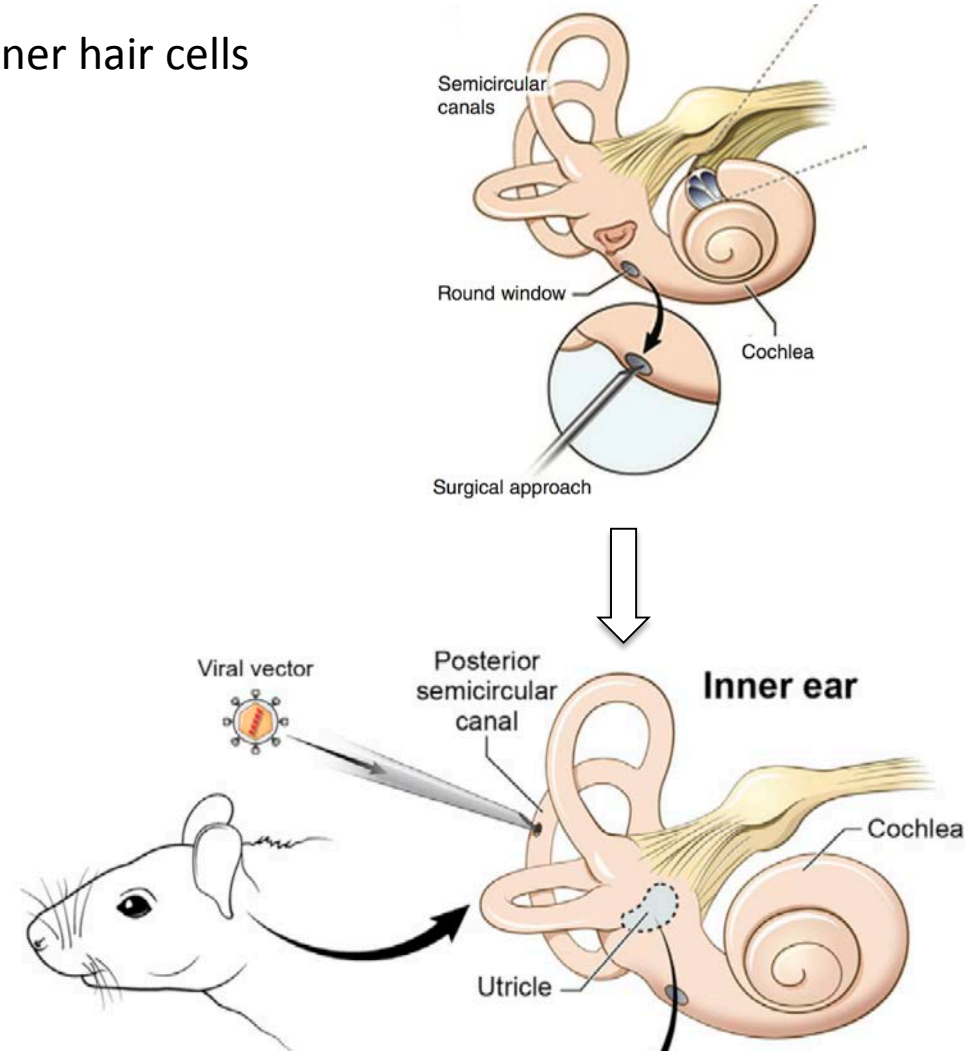
→ No increase in hair cell number at P90

Whirlin Gene Therapy does not restore auditory function



Limitations of current approach

- Whirlin delivery to stereocilia tips of inner hair cells but with a low infection rate (~15%)
- Hair cell survival was only temporary
- No restoration of auditory defects



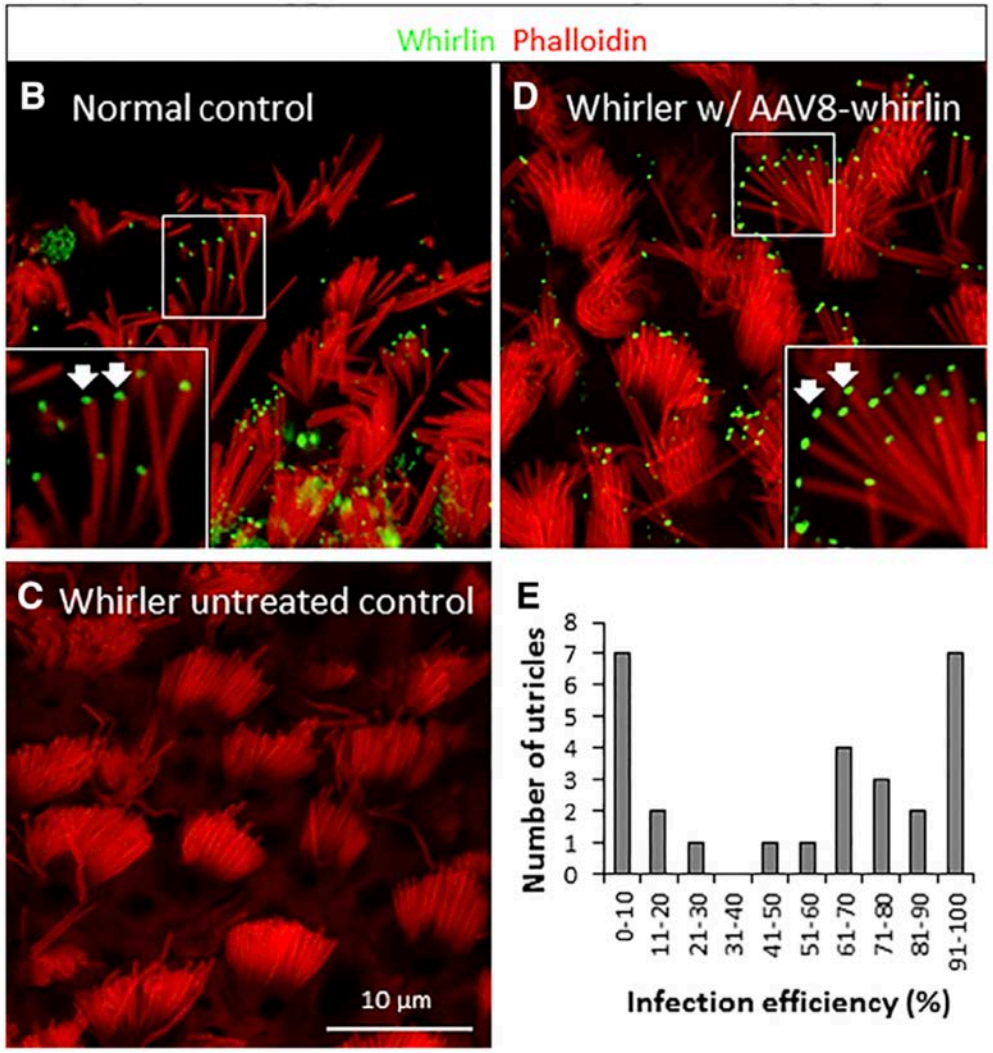
Molecular Therapy
Original Article

AMERICAN SOCIETY of GENE & CELL THERAPY

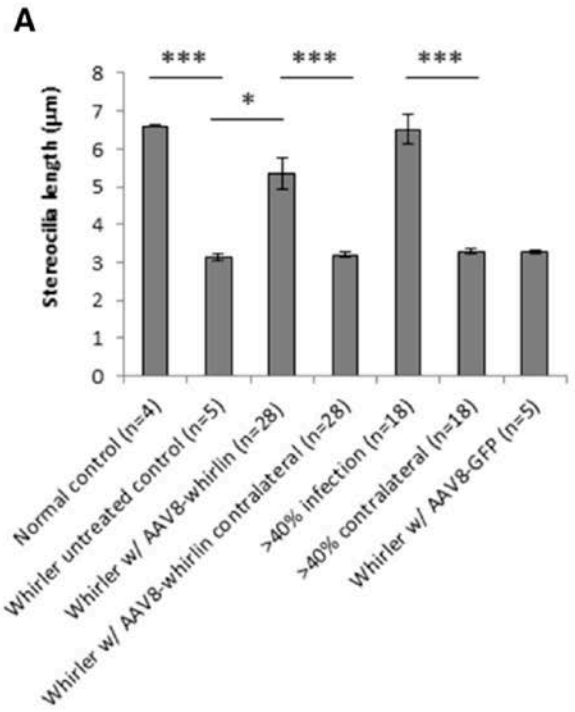
Gene Therapy Restores Balance and Auditory Functions in a Mouse Model of Usher Syndrome

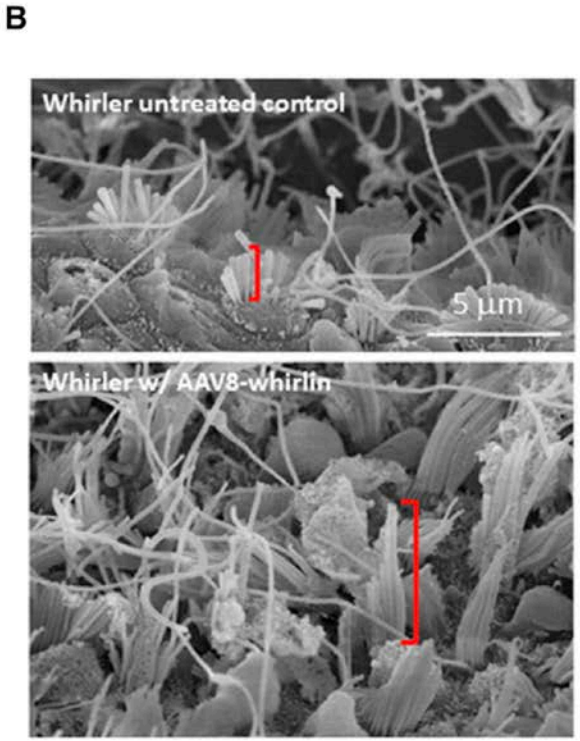
Kevin Isgrig,[1,8] Jack W. Shteamer,[1,8] Inna A. Belyantseva,[2] Meghan C. Drummond,[2,9] Tracy S. Fitzgerald,[3] Sarath Vijayakumar,[4] Sherri M. Jones,[4] Andrew J. Griffith,[5] Thomas B. Friedman,[2] Lisa L. Cunningham,[6] and Wade W. Chien[1,7]

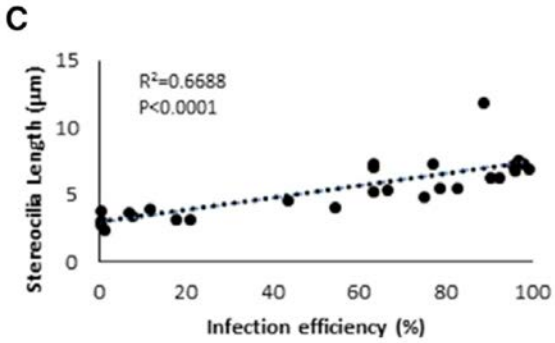
Efficient restoration of *whirlin* expression via gene therapy



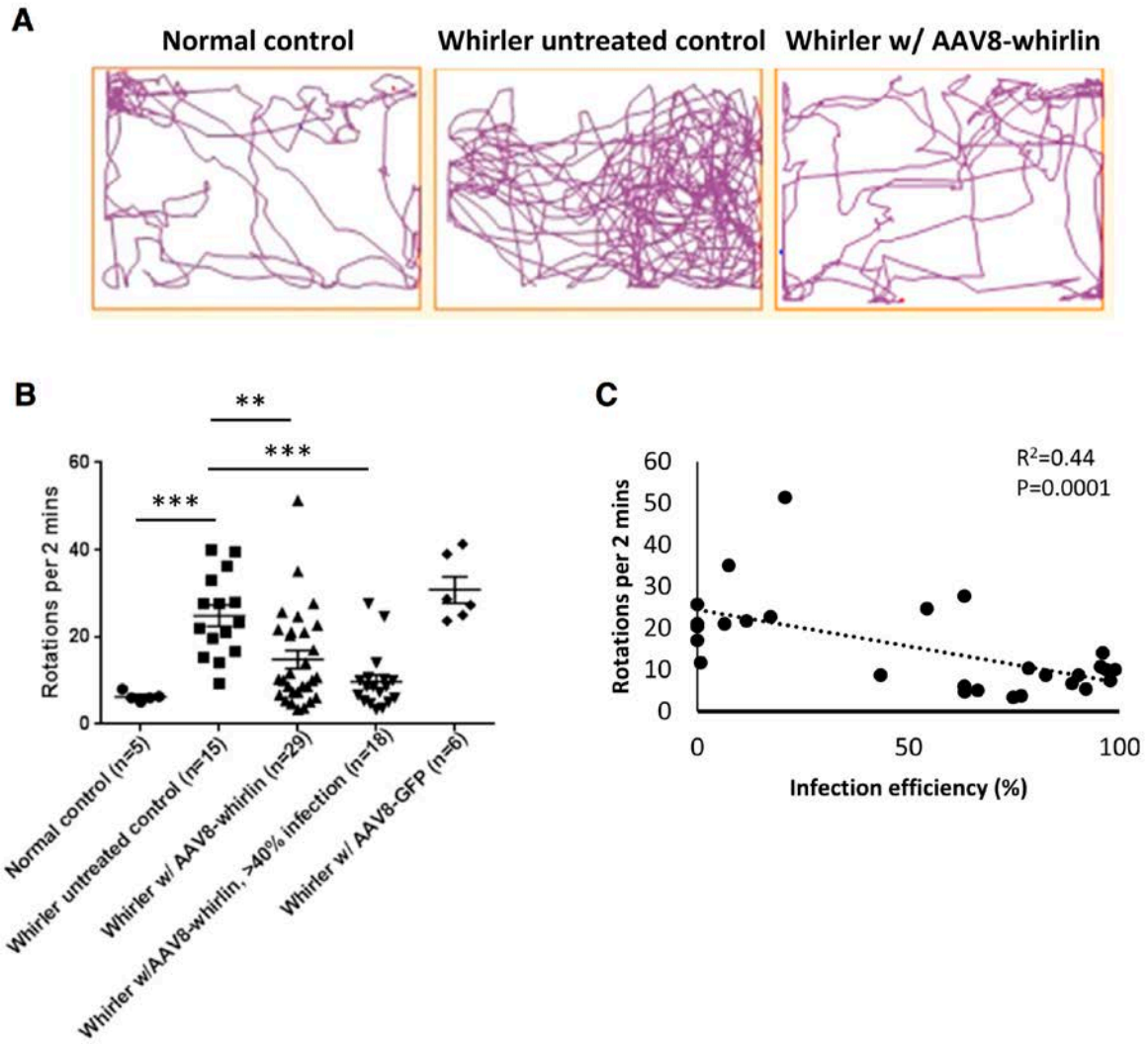
Whirlin expression correlates with stereocilia length



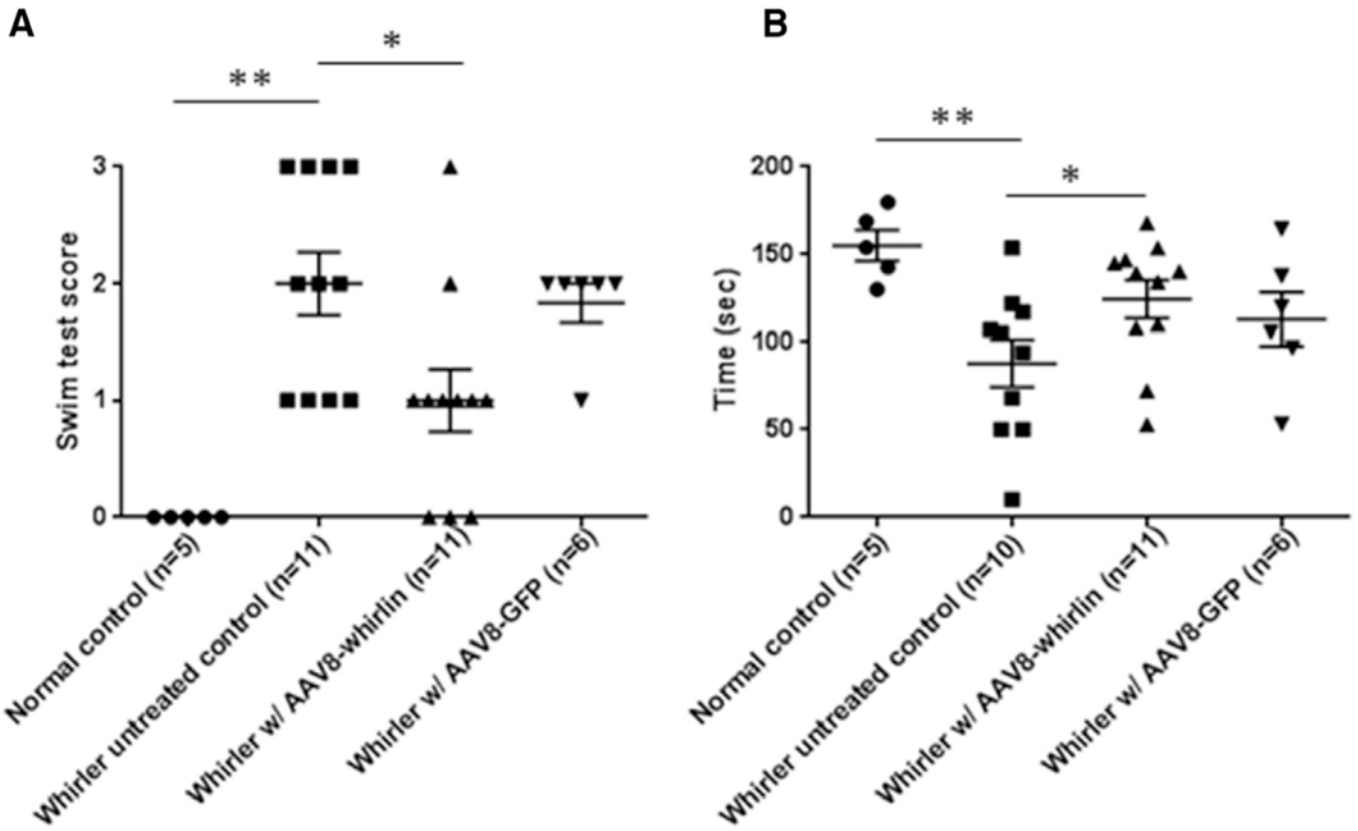




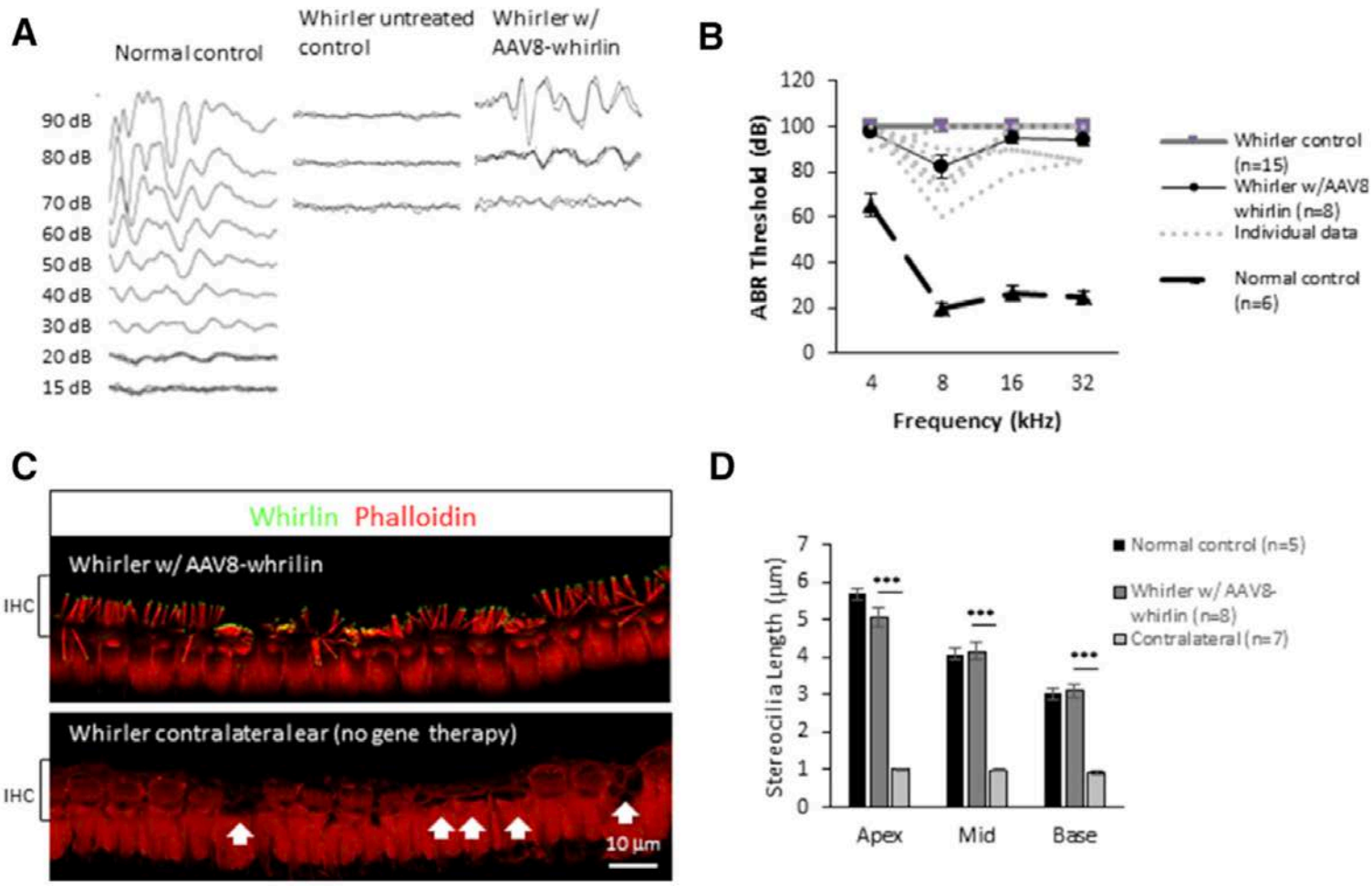
Whirlin gene therapy improves balance dysfunction



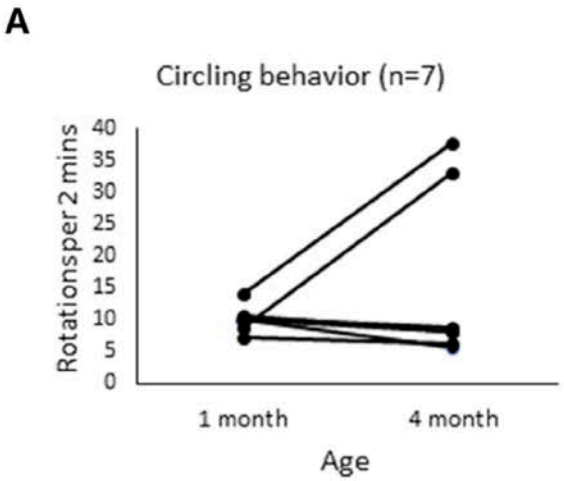
Whirlin gene therapy improves swimming/rotarod performance

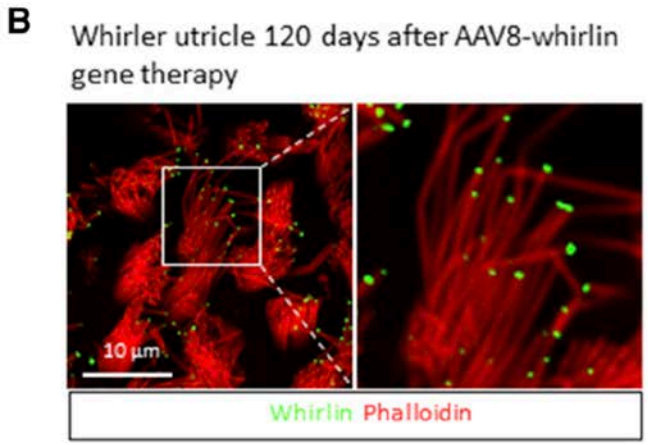


Whirlin gene therapy improves hearing function



Functional Improvements are long-lasting





At 4 months:

reduced circling behaviour
higher Whirlin expression

Improved ABR threshold
Higher IHC numbers
Higher Whirlin expression

ARTICLES

nature biotechnology

Gene therapy restores auditory and vestibular function in a mouse model of Usher syndrome type 1c

Bifeng Pan[1,8], Charles Askew[1,7,8], Alice Galvin[1,8], Selena Heman-Ackah[1,7], Yukako Asai[1], Artur A Indzhykulian[2], Francine M Jodelka[3], Michelle L Hastings[3], Jennifer J Lentz[4], Luk H Vandenberghe[5], Jeffrey R Holt[1,6] & Gwenaëlle S Géléoc[1]

Ush1c mice

Ush1c c.216G➔A

- Cryptic splice cite: complete loss of function of all 10 harmonin isoforms

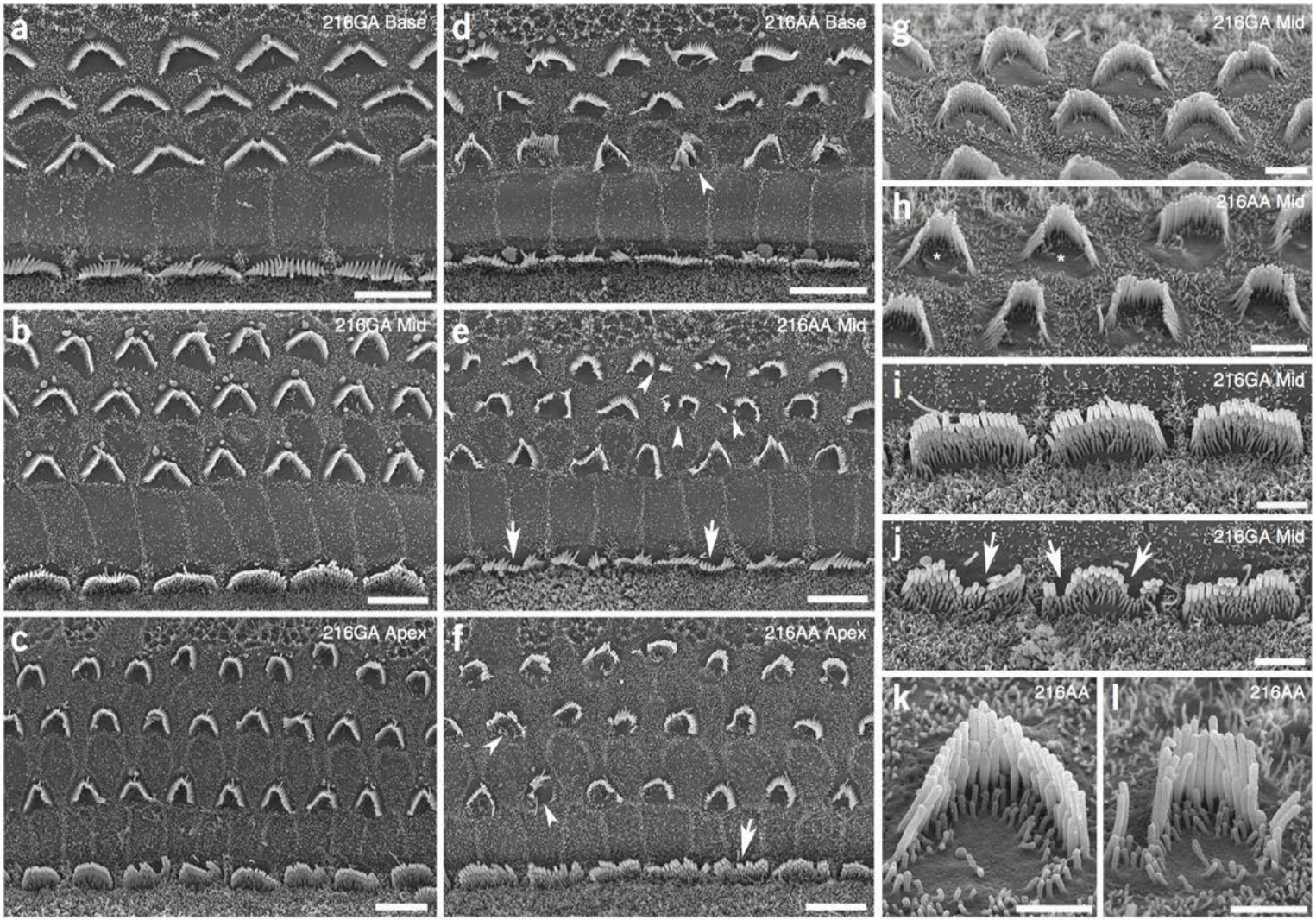
- Harmonin-b: localization at stereocilia tips (sensory transduction in auditory/vestibular hair cells)
- Harmonin-a: localization at synapse (association and regulation of Calcium-channels)

- Severe hearing loss
- Vestibular dysfunction
- Retinal deficits

Ush1c mice show mild hair bundle disorganization at P8

216GA: het (~wt)

216AA: mut

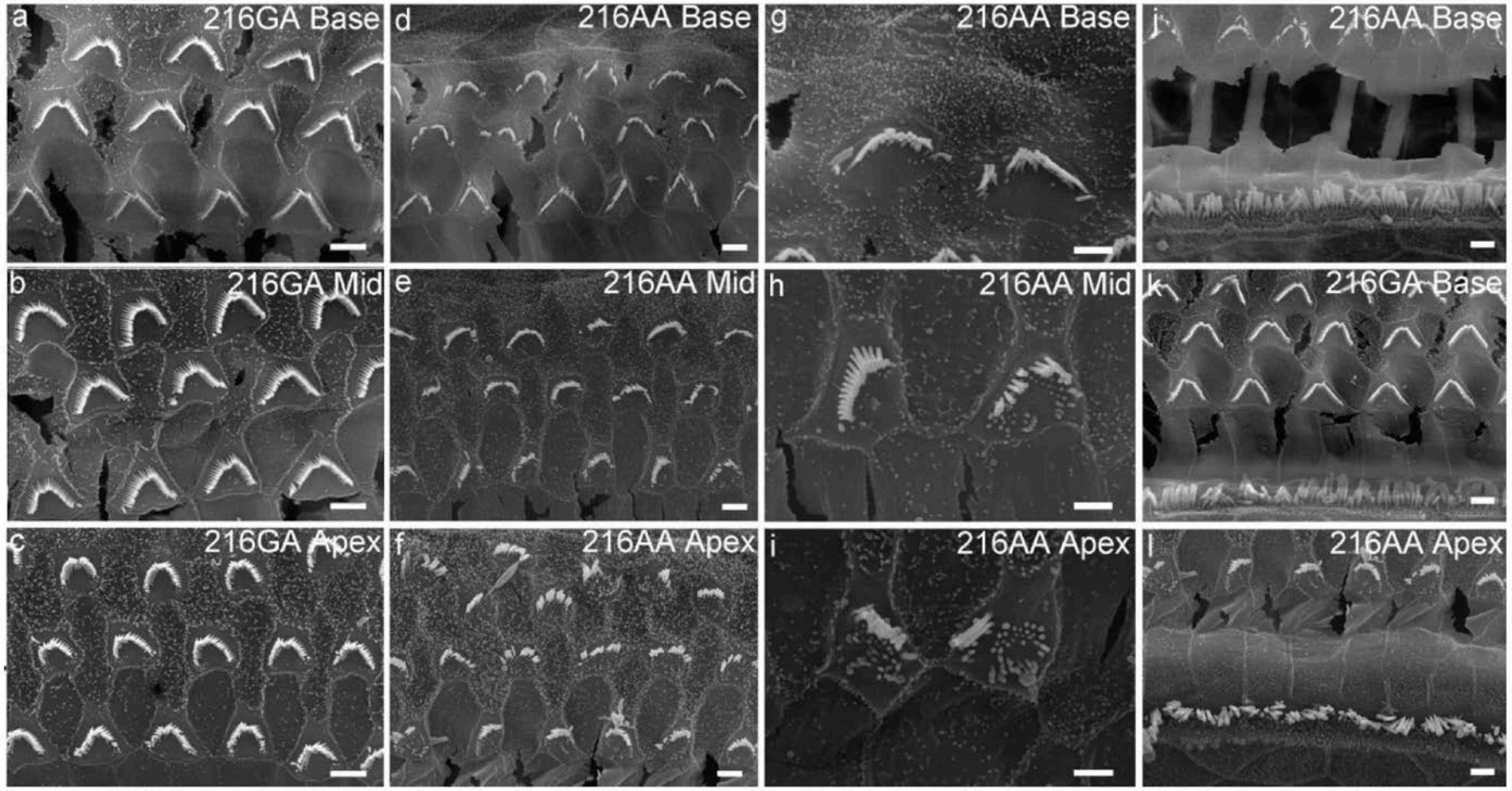


* preserved

↓ disorganized

↓ wavy

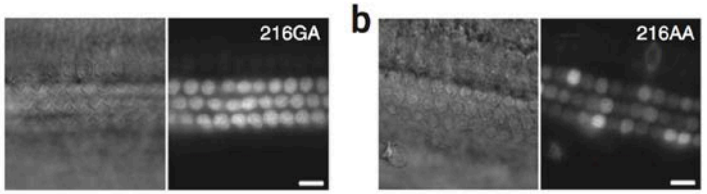
Ush1c mice show severe hair bundle disorganization at P18



Ush1c mice have impaired mechanosensitive channels

Assessment of hair cell functionality via FM1-43 uptake

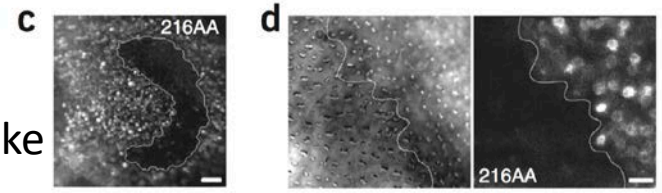
OHCs:



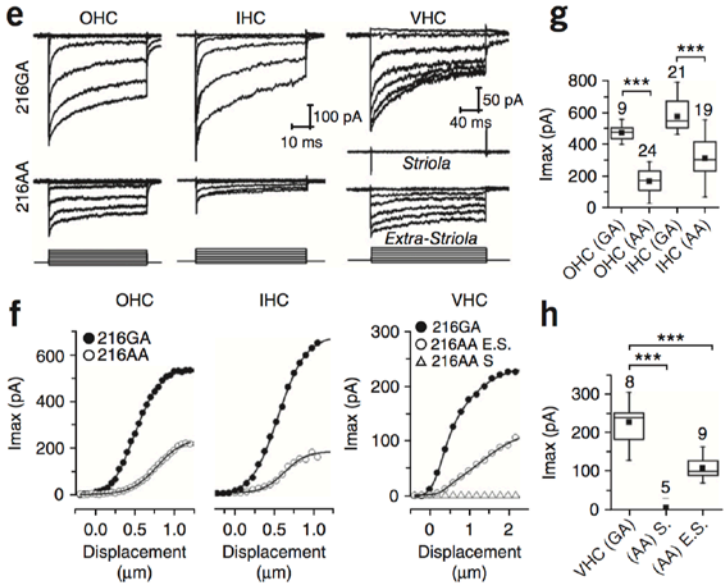
Utricle:

Striola: no uptake

Extra-striola: uptake

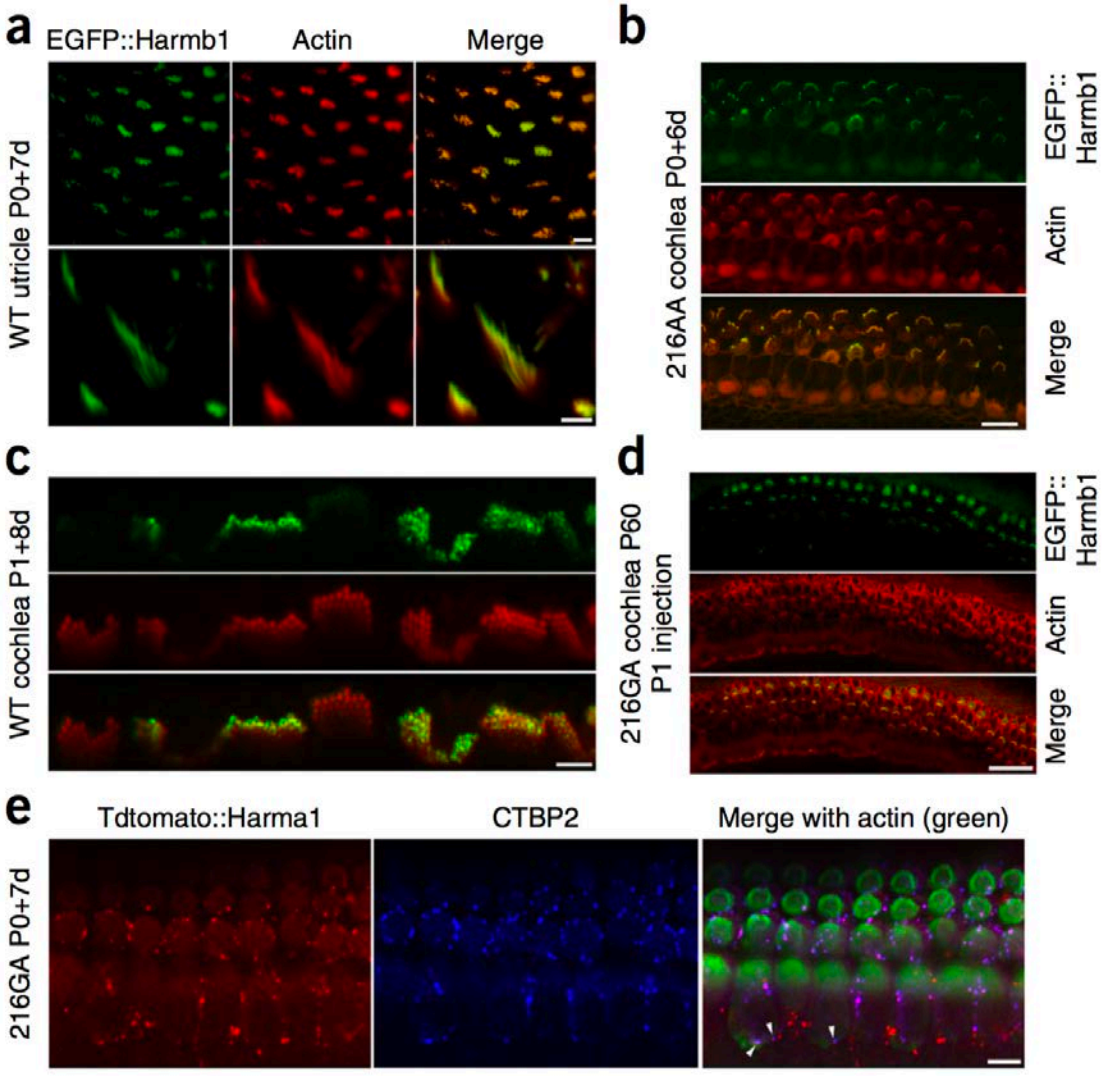


Assessment of mechanotransduction currents



Reduced amplitudes but similar sensitivity

Infection of sensory hair cells *in vitro* and *in vivo*

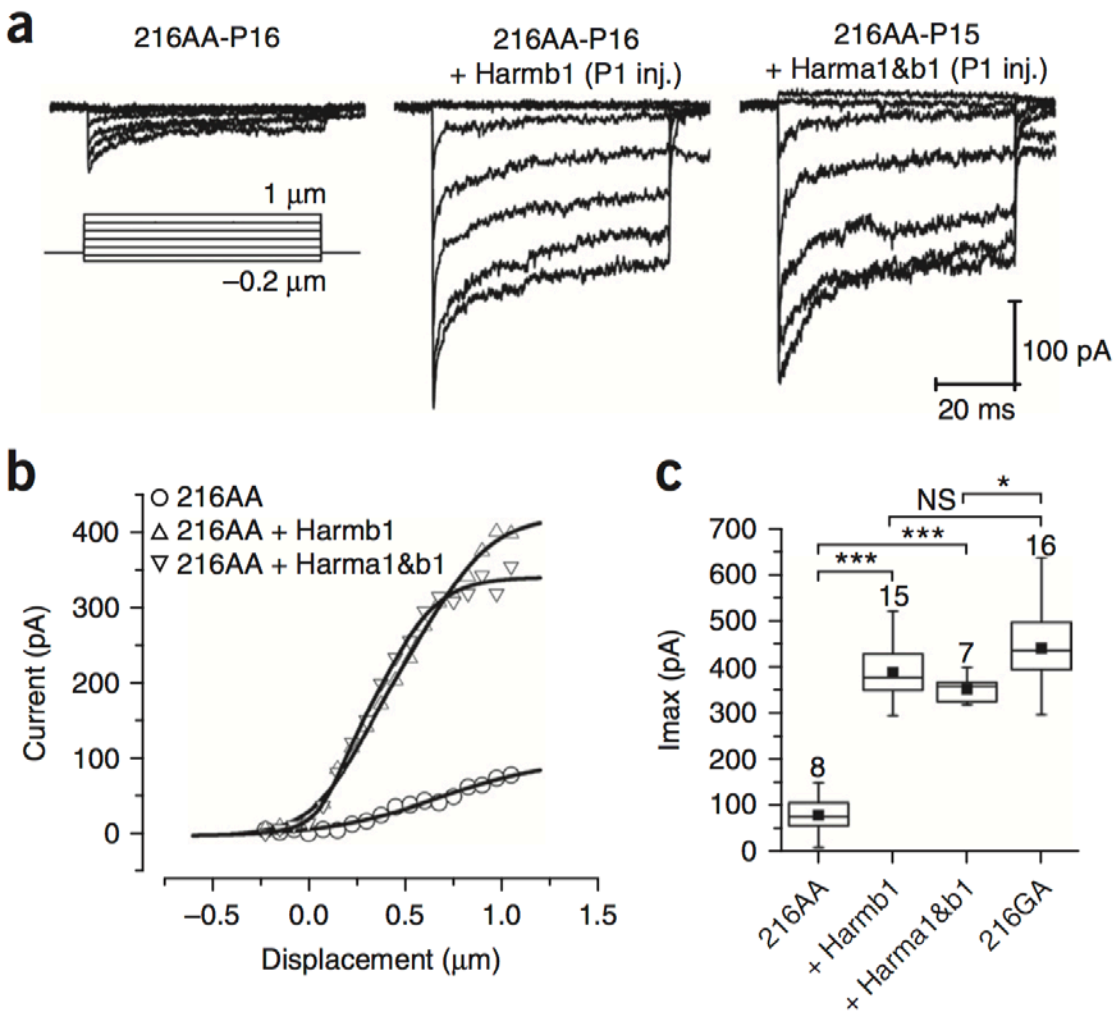


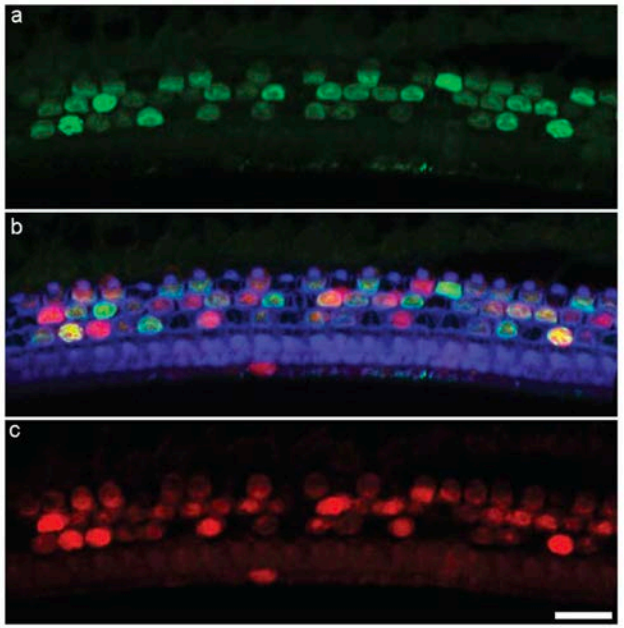
Harmonin-b localizes to the tips of sensory hair sterocilia

Harmonin-a localizes to the base of sensory cells near ribbon synapse (afferent terminal)

Harmonin-infection restores mechanotransduction defects

Recording of mechanotransduction currents





65% of hair cells were co-transduced

No significant improvement by combined infection

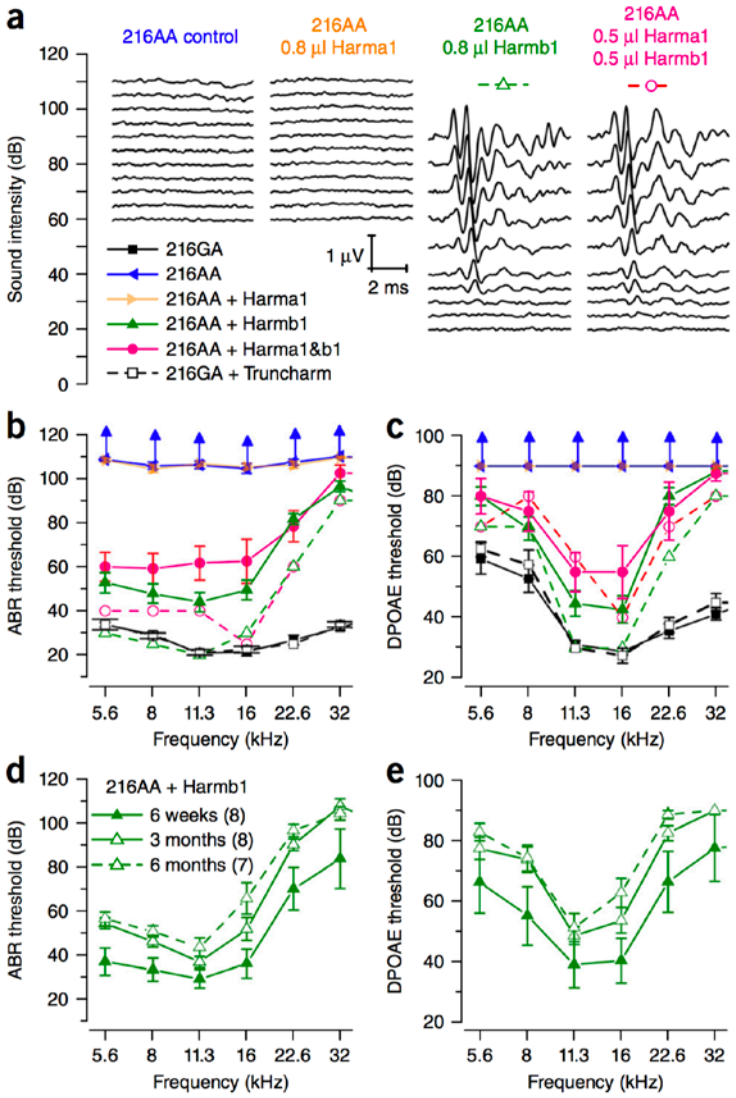
Rescue of auditory dysfunction in *Ush1c* mice

ABR:

auditory brain stem response

DPOAE:

Distortion product of otoacoustic emissions



Harmonin-b but not Harmonin-a injection rescues auditory function

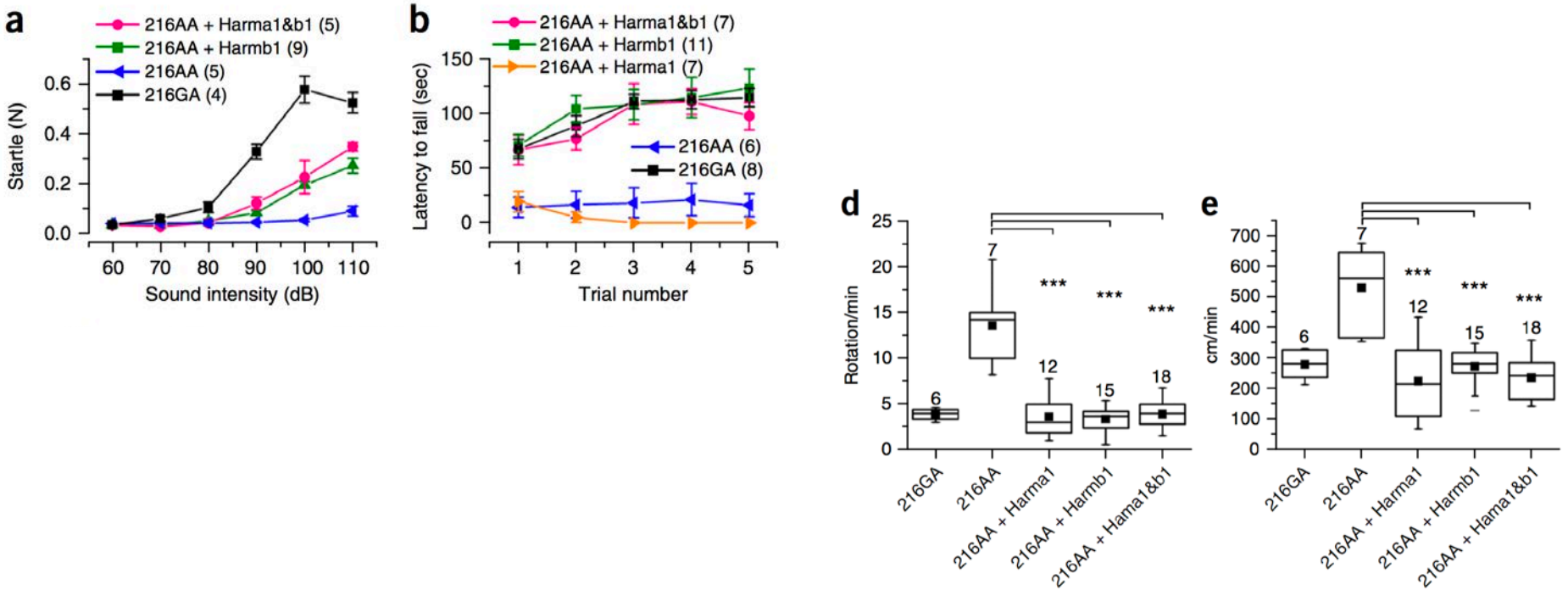
ABR threshold increased slightly at 3 months but then stayed stable

Truncated Harmonin does not affect ABR

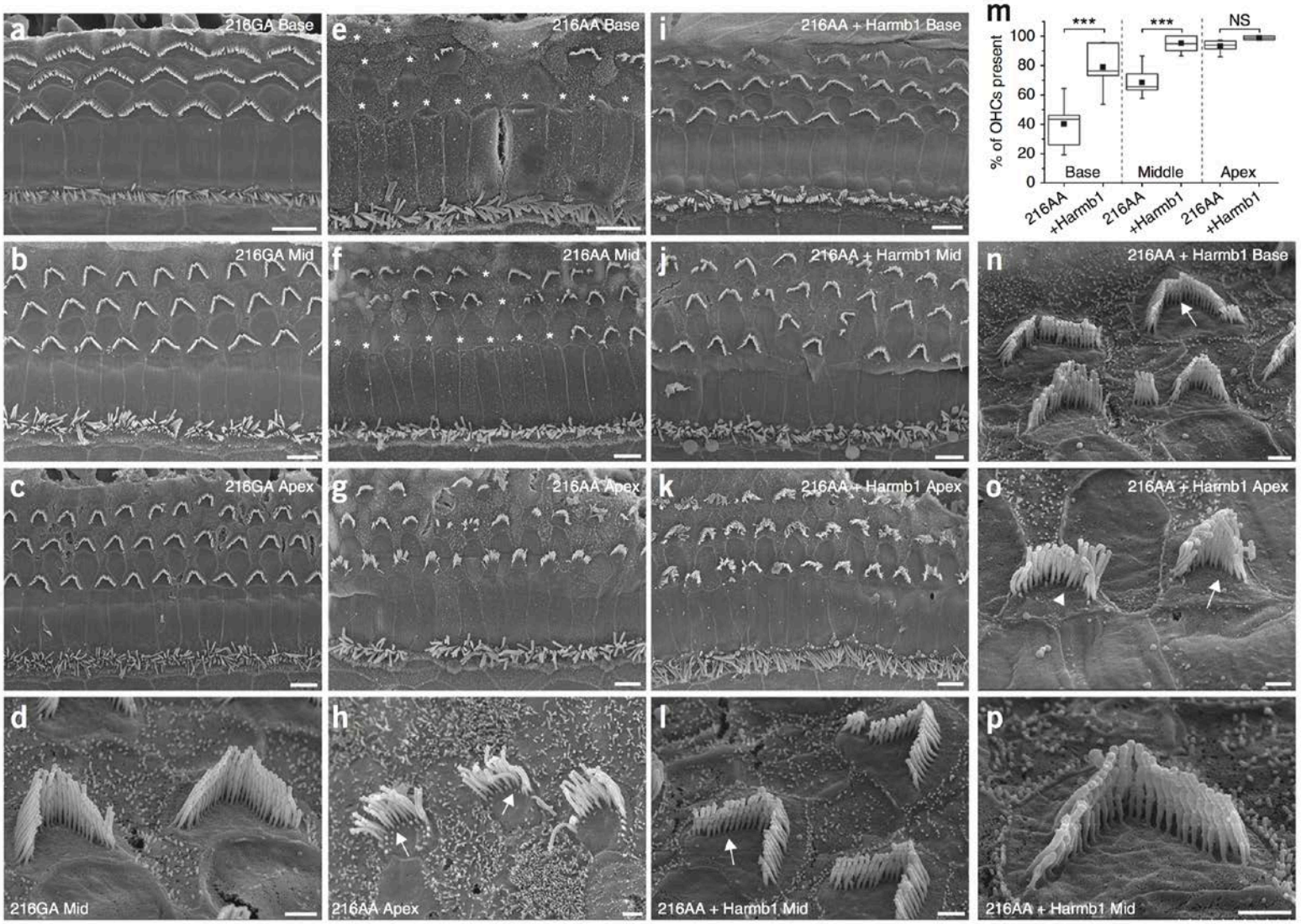
Rescue of auditory dysfunction in contralateral ear

No rescue when injected at 6 months

Rescue of auditory and balance behavior in *Ush1c* mice



Rescue of hair bundle morphology

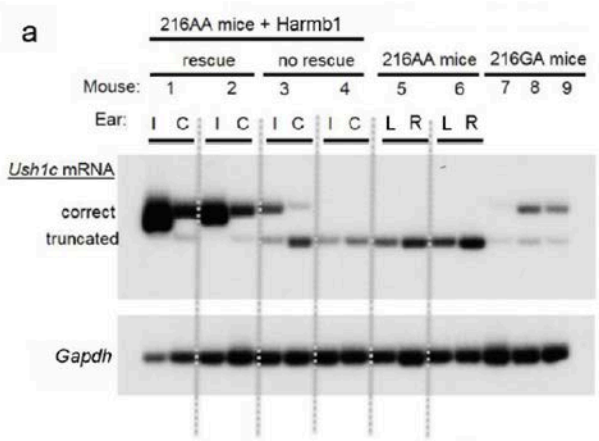


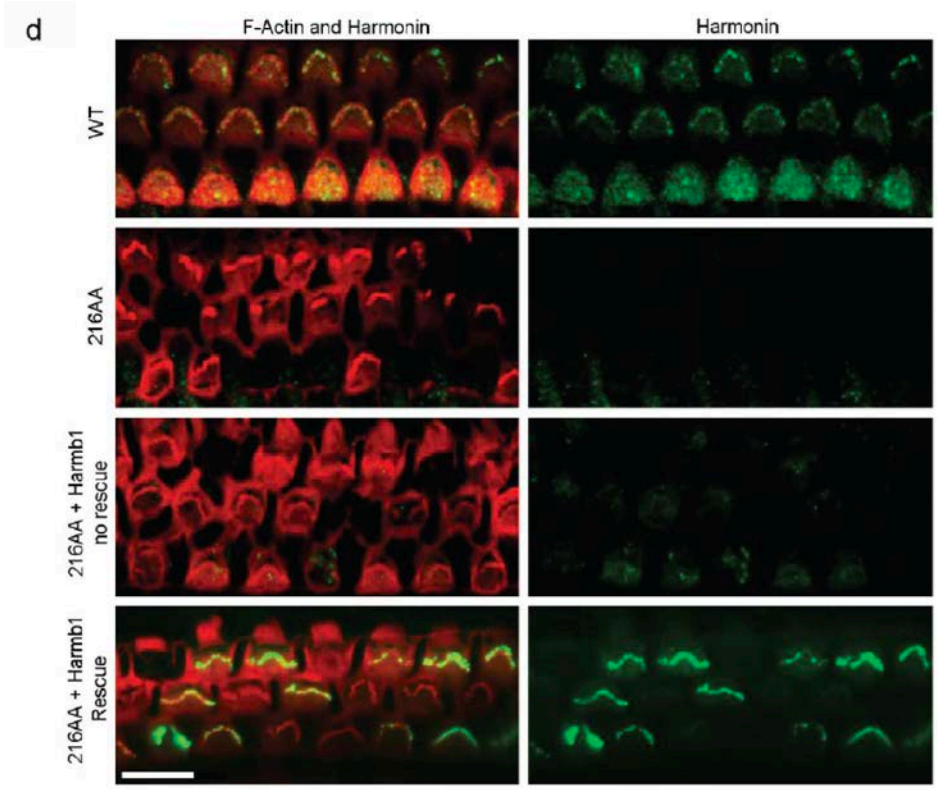
* loss

↓ + staircase

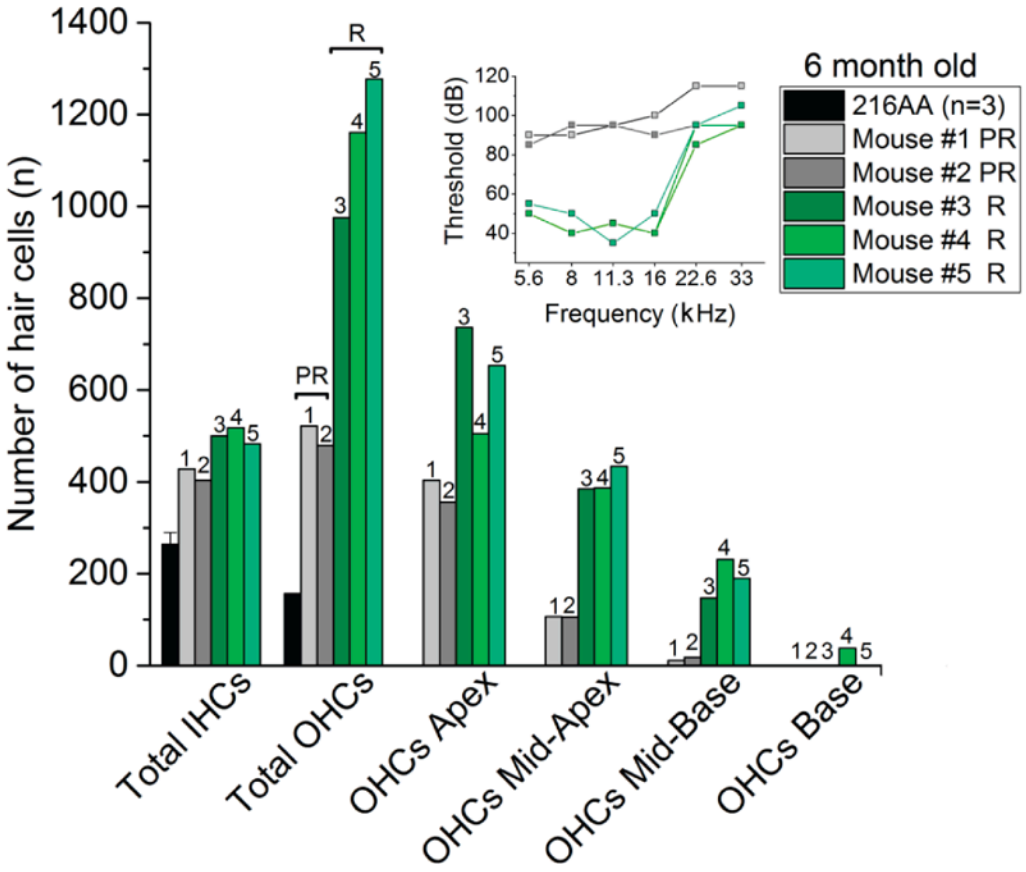
↓ - staircase

Correlation of Harmonin-b1 expression and auditory rescue





Long-term auditory rescue correlates with OHC survival



Conclusions

Therapeutic window in first postnatal week:

- Relatively normal hair bundle morphology
- Auditory system:
  - reduced mechanosensitivity
- Vestibular system:
  - Striola region: defective mechanosensitivity
  - Extra-striola region: reduced mechanosensitivity

AAV2/1 targeting of:

- Harmonin-a1 (regulates Ca^{2+} channels/exocytosis) to ribbon synapses of IHCs but not in the utricle
  ➔ partial vestibular rescue

- Harmonin-b1 (sensory transduction) to tips of auditory/vestibular stereocilia
  ➔ auditory rescue at low frequencies
  ➔ functional vestibular/auditory recovery

Conclusions

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Gene Therapy to treat hearing loss

Viral gene transfer requires optimization of

- delivery vectors
- promotors
- coding sequences
- targeting
- efficacy

- Therapeutic window in mice vs humans:
  - Auditory function is mice is measurable at P11
  - Human inner ear is fully developed at birth – in utero deliver?

- Rescue of stereocilia morphology, hair cell survival
- Restoration of balance/auditory functions
- Lasting effect for at least 4 months

Thank you !