In Vitro and In Vivo Analyses of Dual Vector Otoferlin Expression to Support the Clinical Development of AK-OTOF (AAVAnc80-hOTOF Vector)

Eva Andres-Mateos[1], Francesc Puig-Basagoiti[1], Danielle R. Lenz[1], Yukako Asai[1], Ivy K. Hughes[1], Hao Chiang[1], Richard M. Churchill Jr. [1], Shimon P. Francis[1], Yuanzhao Darcy[1], Michelle D. Valero[1], Robert Ng[1], Michael J. McKenna[1], Emmanuel J. Simons[1], Gregory S. Robinson[1], Jennifer A. Wellman[1]

[1]Akouos, Inc.

Bellucci Symposia | June 3-4, 2021

Disclosures

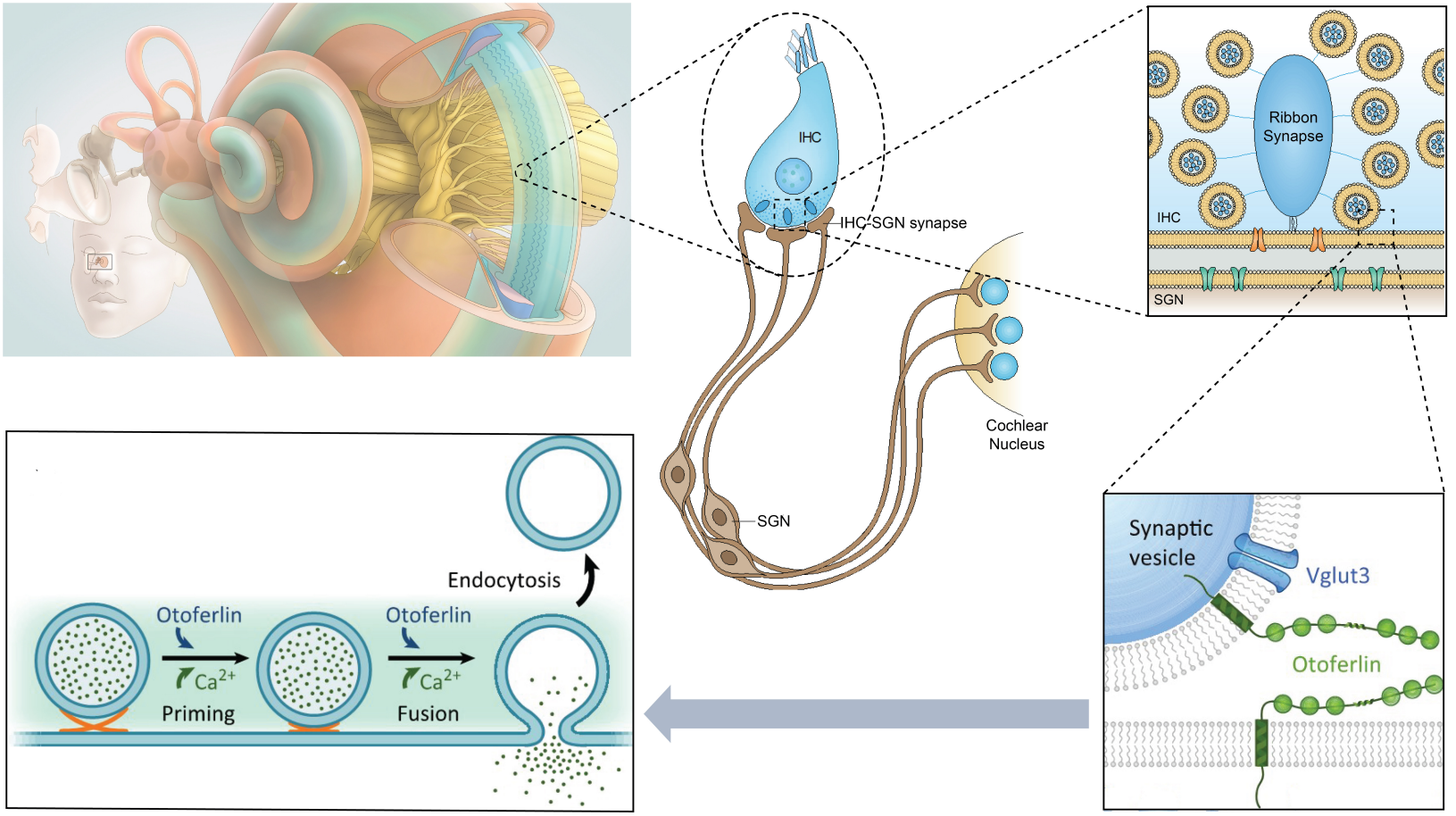
Eva Andres-Mateos is an employee of Akouos, Inc., and has received, and is receiving, compensation and equity from Akouos, Inc.

Forward Looking Statements

This presentation includes “forward-looking statements” within the meaning of the Private Securities Litigation Reform Act of 1995, including, but not limited to: the ability of our product candidate AK-OTOF to potentially restore, improve, and preserve high-acuity physiologic hearing; statements relating to the initiation, plans, and timing of our future clinical trials and our research and development programs; and the timing of our planned IND submission for AK-OTOF. These forward-looking statements may be accompanied by such words as “aim,” “anticipate,” “believe,” “continue,” “could,” “estimate,” “expect,” “forecast,” “goal,” “intend,” “may,” “might,” “plan,” “potential,” “possible,” “target,” “will,” “would,” and other words and terms of similar meaning. Akouos may not actually achieve the plans, intentions, or expectations disclosed in these forward-looking statements, and you should not place undue reliance on these forward-looking statements. Actual results or events could differ materially from the plans, intentions, and expectations disclosed in these forward-looking statements as a result of various factors, including: our limited operating history; uncertainties inherent in the development of product candidates, including the initiation and completion of nonclinical studies and clinical trials; the timing of and our ability to submit and obtain regulatory approval; whether results from nonclinical studies will be predictive of results or success of clinical trials; our ability to obtain sufficient cash resources to fund our foreseeable and unforeseeable operating expenses and capital expenditure requirements; our ability to obtain, maintain, and enforce our intellectual property; the impact of the COVID-19 pandemic on our business, results of operations, and financial condition; the potential that our internal manufacturing capabilities and/or external manufacturing supply may experience delays; risks related to competitive programs; and the other risks and uncertainties that are described in the Risk Factors section of the Company's Quarterly Report on Form 10-Q for the quarter ended March 31, 2021, which is on file with the Securities and Exchange Commission, and in other filings that Akouos may make with the U.S. Securities and Exchange Commission. These statements are based on our current beliefs and expectations as of the date of this presentation. We do not undertake any obligation to publicly update any forward-looking statements except as required by law. By attending or receiving this presentation, you acknowledge that: you are cautioned not to place undue reliance on these forward-looking statements; you will be solely responsible for your own assessment of the market and our market position; and you will conduct your own analysis and be solely responsible for forming your own view of the potential future performance of Akouos, Inc.

Otoferlin Plays an Essential Role in Hearing

- Otoferlin plays a critical role in exocytosis of synaptic vesicles at the inner hair cell synapse
- Mutations in the *OTOF* gene are associated with autosomal recessive sensorineural hearing loss

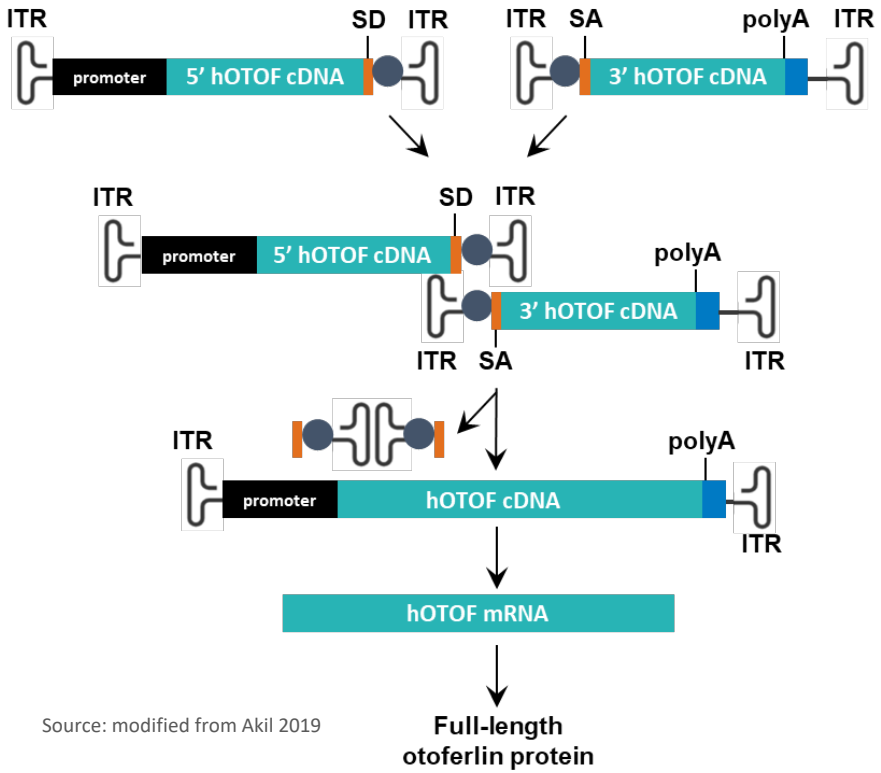


Source: modified from Pangrsic 2012 and Moser 2016

IHC: inner hair cell; SGN: spiral ganglion neuron (also referred to as cochlear nerve); VGlut3: vesicular glutamate transporter 3

AK-OTOF Restores Auditory Function in Knockout Mice

- AK-OTOF (AAVAnc80-hOTOF) utilizes a dual vector approach and encodes both the 5’ and the 3’ components of the *OTOF* gene
- Intracochlear delivery of AK-OTOF, under a ubiquitous promoter, demonstrated long-term recovery of auditory function in otoferlin knockout mice (right panel below and ASGCT 2021 Abstract #569)



Source: modified from Akil 2019

Transduction

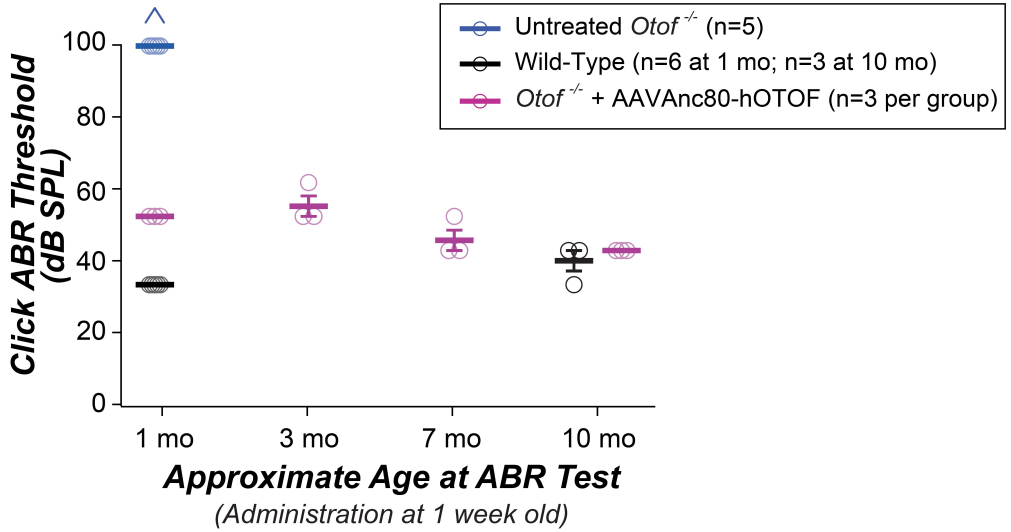
DNA sequence alignment

Recombination

Transcription

Splicing

Translation



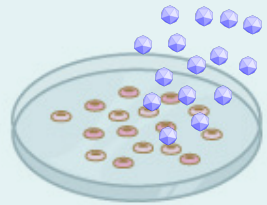
ABR: auditory brainstem response; cDNA: complementary DNA; h: human; dB: decibels; ITR: inverted terminal repeats; mRNA: messenger RNA; n: number; mo: month; polyA: polyadenylation tail; SA: splice acceptor; SD: splice donor; SPL: sound pressure level

Objectives

- Evaluate otoferlin mRNA transcripts by next generation sequencing (NGS) following transduction of HEK293 cells with AK-OTOF
- Assess full-length human otoferlin protein expression in HEK293 cells following *in vitro* transduction with AK-OTOF
- Determine optimal ratio of dual vector components (5’ and 3’) through RNA and protein analyses following *in vitro* transduction of HEK293 cells
- Verify full-length otoferlin protein expression in inner hair cells of non-human primate (NHP) cochleae

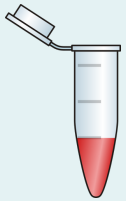
HEK293: human embryonic kidney 293; mRNA: messenger RNA; NGS: next generation sequencing; NHP: non-human primate

RNA Transcript Evaluation following *In Vitro* Transduction



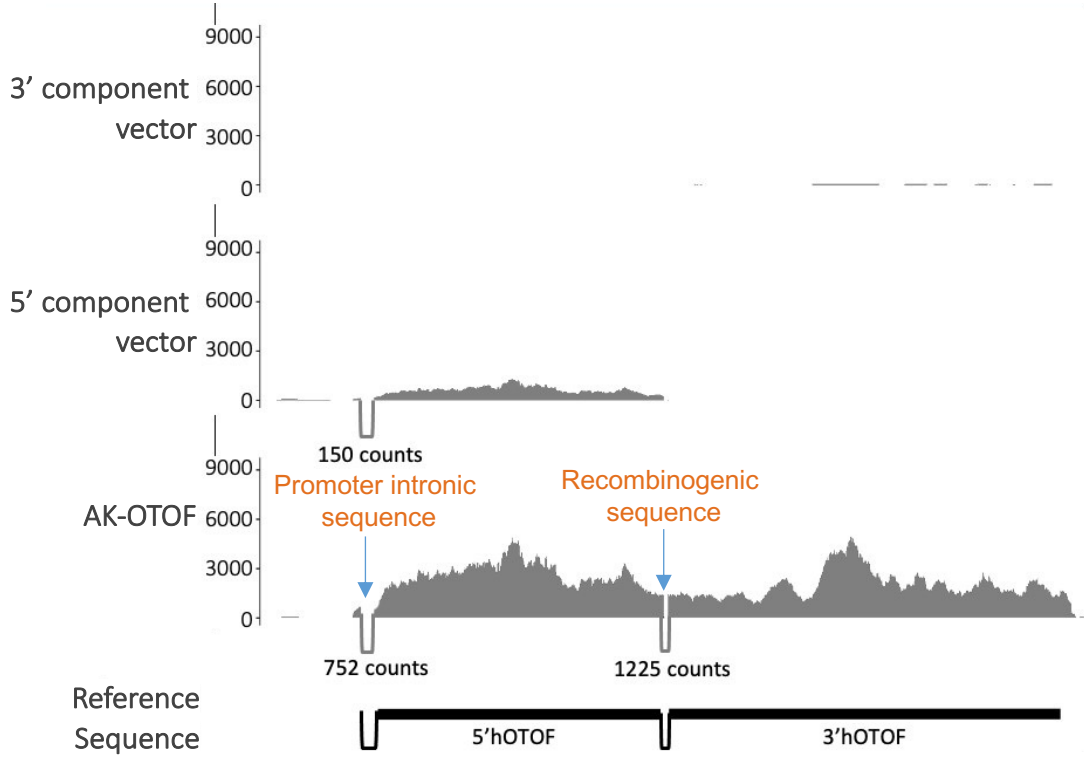
HEK293 cells transduced with:
- AK-OTOF or
- 5’ component vector or
- 3’ component vector

72 hours



Cell collection and mRNA extraction

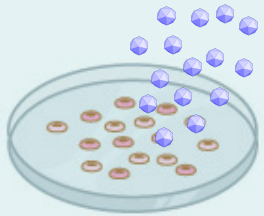
NGS evaluation of mRNA transcripts



- Sashimi plots demonstrate robust full-length OTOF transcript detection following transduction with AK-OTOF
- Minimal transcript representation was detected following transduction with the 3’ component vector
- As expected, due to the promoter region, low transcript representation was detected following transduction with the 5’ component vector
- NGS read-count alignment did not identify consistently expressed undesired mRNA transcripts in cells transduced with individual component vectors or with AK-OTOF, thus decreasing the likelihood of expression of truncated proteins

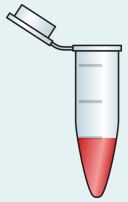
h: human; HEK293: human embryonic kidney 293; mRNA: messenger RNA; NGS: next generation sequencing

Full-Length Otoferlin Protein Detection after *In Vitro* Transduction



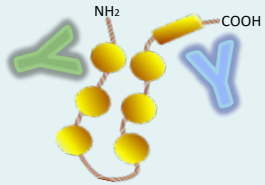
HEK293 cells transduced with:
- AK-OTOF or
- 5’ component vector or
- 3’ component vector

72 hours

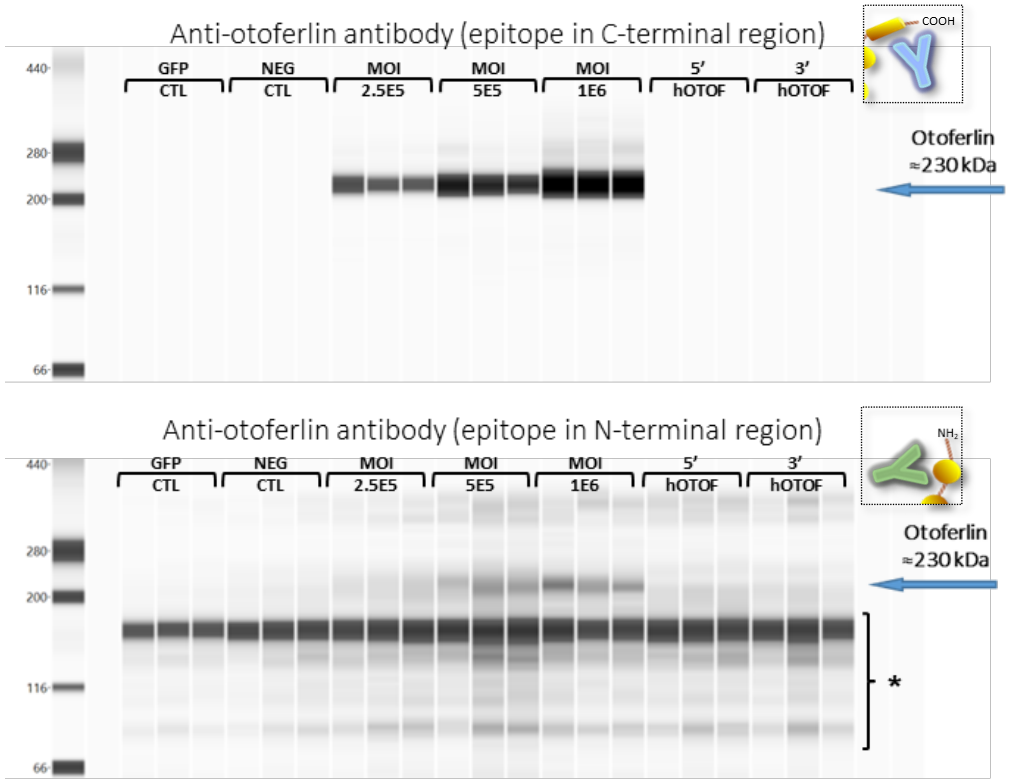


Cell collection and protein extraction

Anti-otoferlin antibodies
(against C-terminal and N-terminal domains)

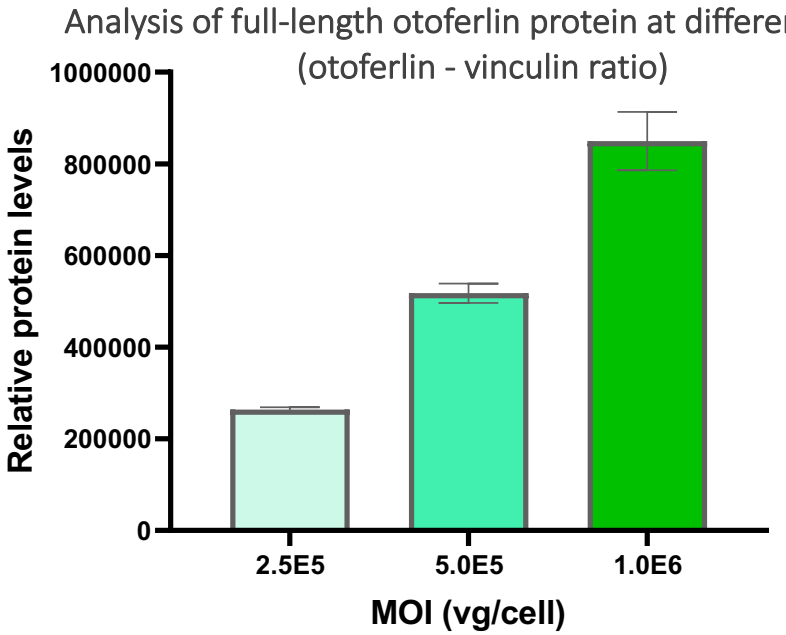


Immunodetection of full-length otoferlin protein



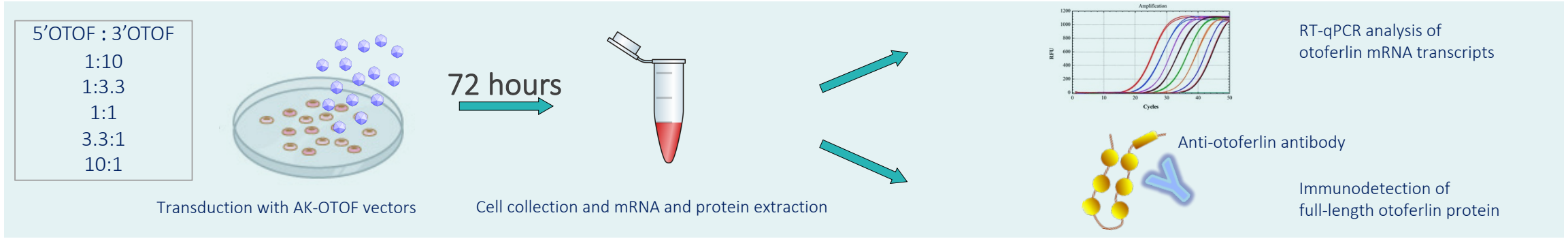
* Non-specific antibody detection. Bands also detected in samples that were not transduced with AAVAnc80-hOTOF vectors (NEG and GFP controls)

- Full-length otoferlin protein was detected after *in vitro* transduction with dual vector and its 5’ and 3’ component vectors at different MOIs
- No detectable truncated proteins were observed when cells were transduced with dual vector or individual component vectors



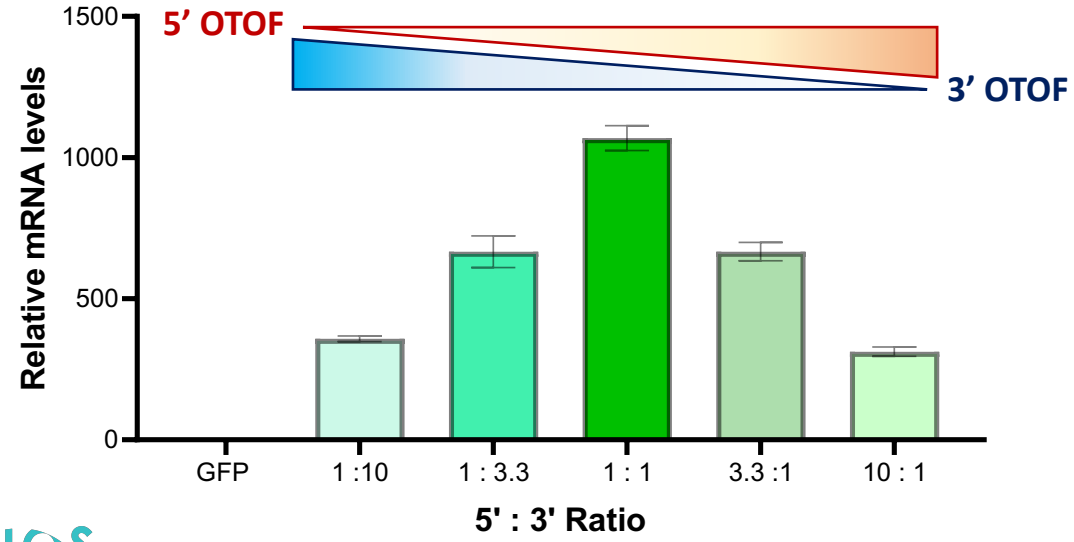
GFP: green fluorescent protein; h: human; HEK293: human embryonic kidney 293; kDa: kilodalton; MOI: multiplicity of infection; NEG: negative control; vg: vector genome

Determination of Optimal AK-OTOF Component Vector Ratio

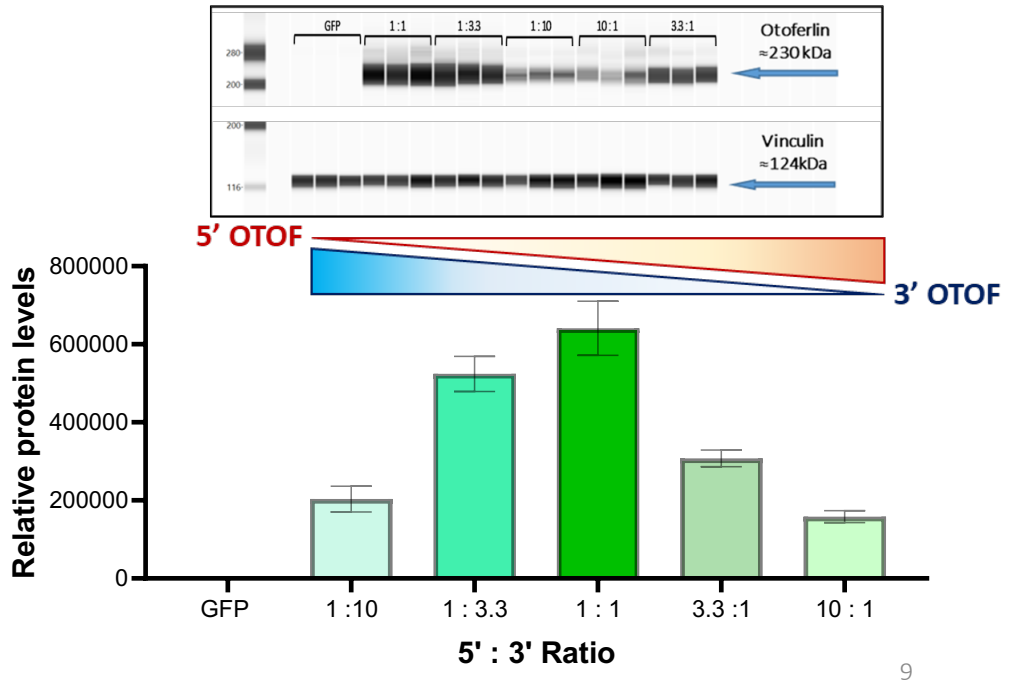


- A 1:1 ratio showed the highest levels of full-length otoferlin RNA compared to the other ratios tested
- Consistent with RNA levels, a 1:1 ratio also showed the highest levels of full-length otoferlin protein expression

Analysis of otoferlin mRNA transcripts (otoferlin - GAPDH ratio)

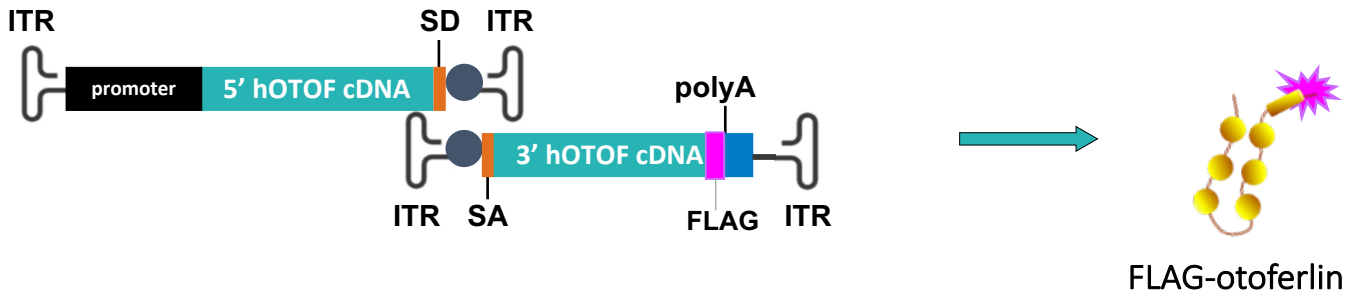


Analysis of full-length otoferlin protein (otoferlin - vinculin ratio)



GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; GFP: green fluorescent protein; kDa: kilodalton; MOI: multiplicity of infection; mRNA: messenger RNA; NEG: negative control

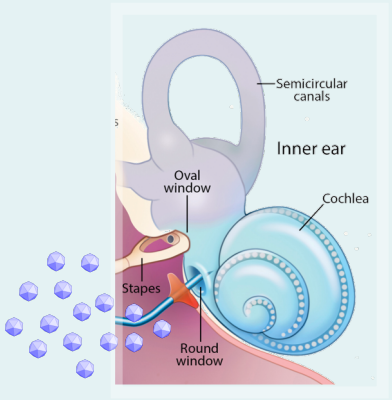
Otoferlin Expression Following Intracochlear Administration in NHP



Generation of AAVAnc80-FLAG.hOTOF vectors with FLAG-tag sequence in the C-terminal region

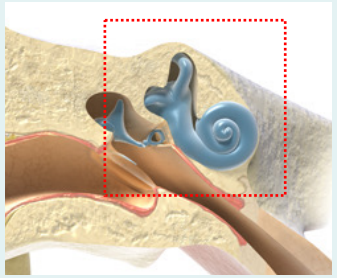
Intracochlear administration in Cynomolgus macaques (male and female, 1.8 to 2.4 years old)

AAVAnc80-FLAG.hOTOF delivered through the round window membrane

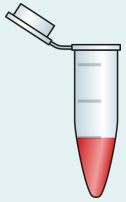


Three experimental groups:
Vehicle control (2 ears)
Dose 1 (4 ears)
Dose 2 (4 ears)

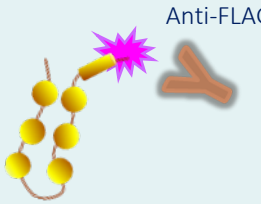
In life: one month



Temporal bones collected bilaterally;
Inner ears isolated from temporal bone

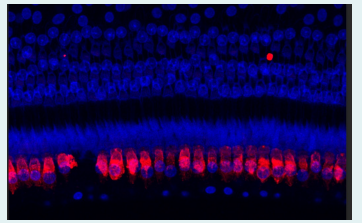


Fresh tissue collection;
Organ of Corti dissection
and protein extraction



Immunodetection of full-length FLAG-otoferlin protein

Cochleae perfused and fixed;
Cochlear decalcification
and inner-ear dissection

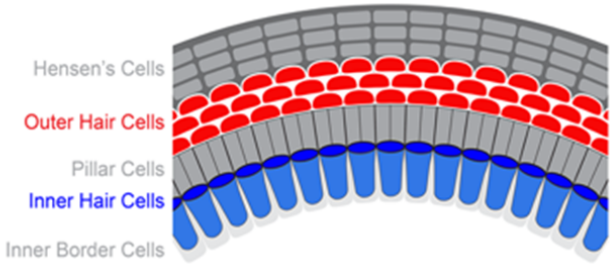


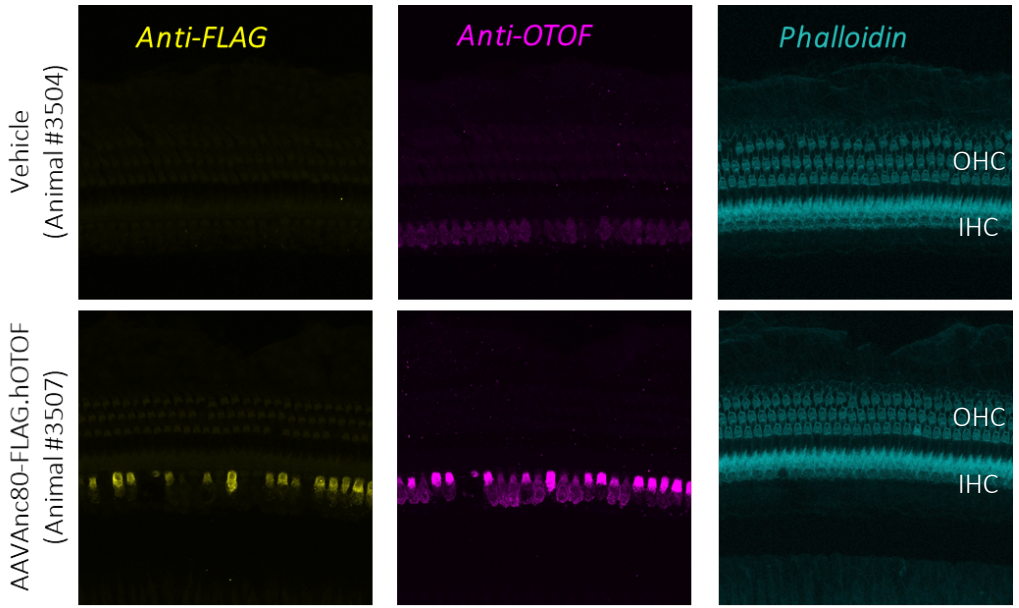
Immunostaining of FLAG-otoferlin using anti-FLAG antibody

cDNA: complementary DNA; h: human; ITR: inverted terminal repeats; NHP: Non-human primate; polyA: polyadenylation tail; SA: splice acceptor; SD: splice donor

Full-Length Otoferlin Expression was Limited to Inner Hair Cells

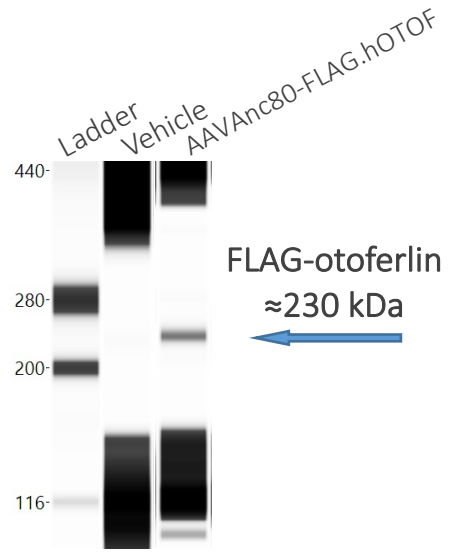
Cochlear micrographs from mid-cochlear region (4 kHz) from animals administered vehicle or AAVAnc80-FLAG.hOTOF (Dose 1)





- FLAG-otoferlin was detected one month following intracochlear administration of AAVAnc80-FLAG.hOTOF in NHP at two different doses
- FLAG-otoferlin expression in NHP cochleae was only detected in inner hair cells (IHC)
- Full-length FLAG-otoferlin (230 kilodalton band) expression was detected in cochlear sensory epithelium lysates

Full-length FLAG-otoferlin detection using anti-FLAG antibody from NHPs administered vehicle or AAVAnc80-FLAG.hOTOF (Dose 2)

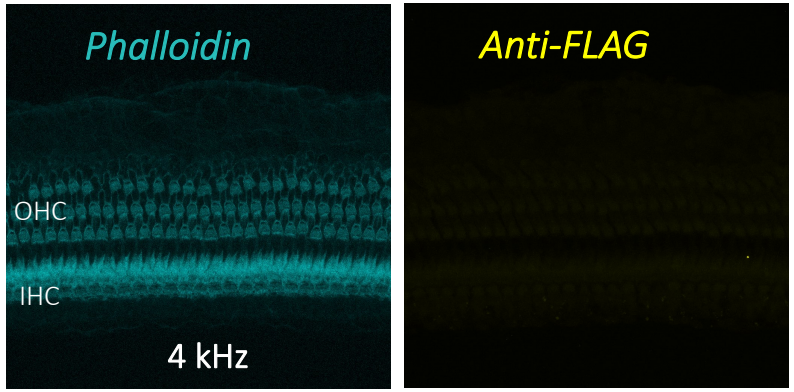


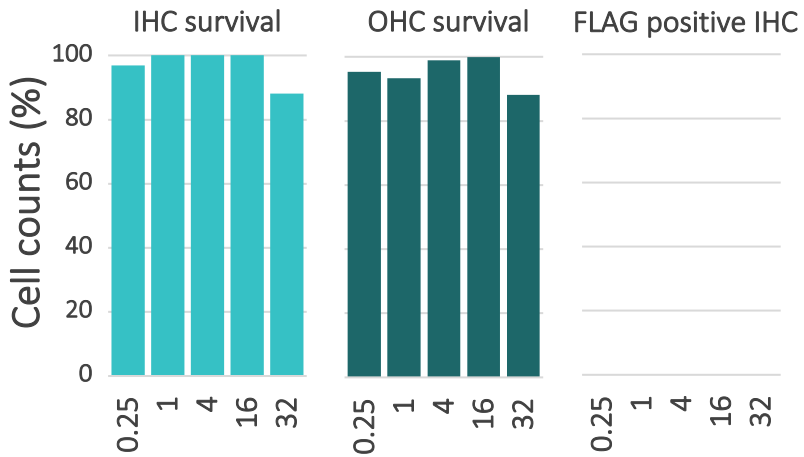
h: human; IHC: inner hair cells; kDa: kilodalton; kHz: kilohertz; NHP: non-human primate; OHC: outer hair cells

Full-Length Otoferlin Expression was Well Tolerated in NHP Cochlea

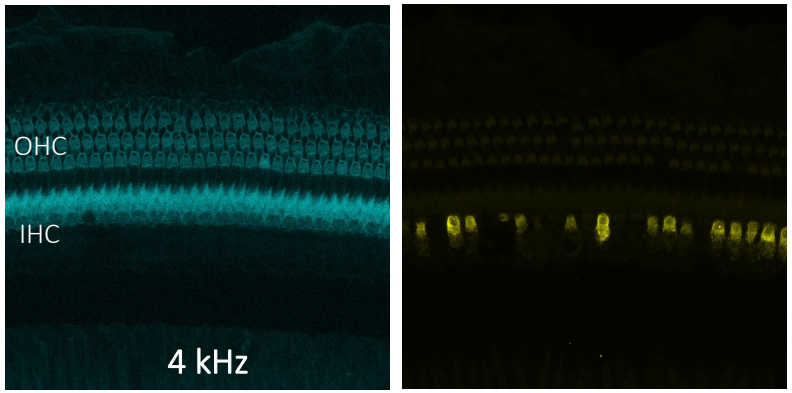
Cochlear micrographs from mid-cochlear region (4 kHz; left panels) and hair cell survival and FLAG positive cell quantification (right panels) from animals administered vehicle (top panels) or AAVAnc80-FLAG.hOTOF (bottom panels); inner hair cell (IHC) and outer hair cell (OHC) survival was robust in animals expressing FLAG-otoferlin

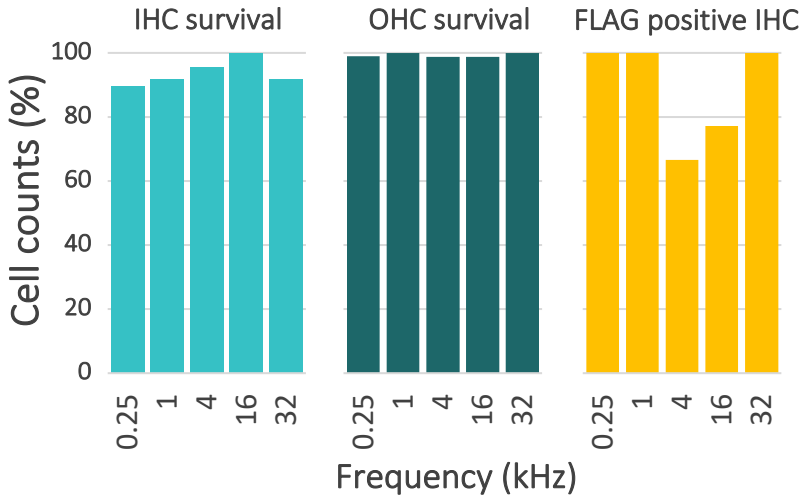
Vehicle
(Animal #3504)





AAVAnc80-FLAG.hOTOF
(Animal #3507)





h: human; IHC: inner hair cell; kHz: kilohertz; NHP: non-human primate; OHC: outer hair cell

Conclusions

- Full-length otoferlin mRNA and protein were detected in transduced HEK293 cells at different MOIs
- No consistently expressed undesired mRNA transcripts were observed, and no truncated otoferlin proteins were detected, following *in vitro* transduction with either AK-OTOF or its component vectors
- A 1:1 ratio of the AK-OTOF component vectors appears to be optimal for efficient reconstitution of full-length human otoferlin
- Full-length otoferlin protein expression was detected by immunohistochemistry and immunodetection one month following intracochlear administration of AAVAnc80-FLAG.hOTOF in inner hair cells of NHP cochleae

HEK293: human embryonic kidney 293; MOI: multiplicity of infection; mRNA: messenger RNA; NGS: next generation sequencing; NHP: non-human primate


AKOUOS