Tailoring Regulatory Elements in Gene Therapies for Hearing Loss

Danielle R. Lenz[1], Hao Chiang[1], Yukako Asai[1], Shimon Francis[1], Enping Qu[1], Priya Bansode[1], Katherine D. Gribble[1], Richard Churchill[1], Francesc Puig-Basagoiti[1], Yuanzhao Darcy[1], Bifeng Pan[1], Junaid Syed[1], Pascal Schamber[1], Eva Andres-Mateos[1], Michelle D. Valero[1], Emmanuel J. Simons[1], Gregory S. Robinson[1], Robert Ng[1]

[1]Akouos, Inc., Boston, MA

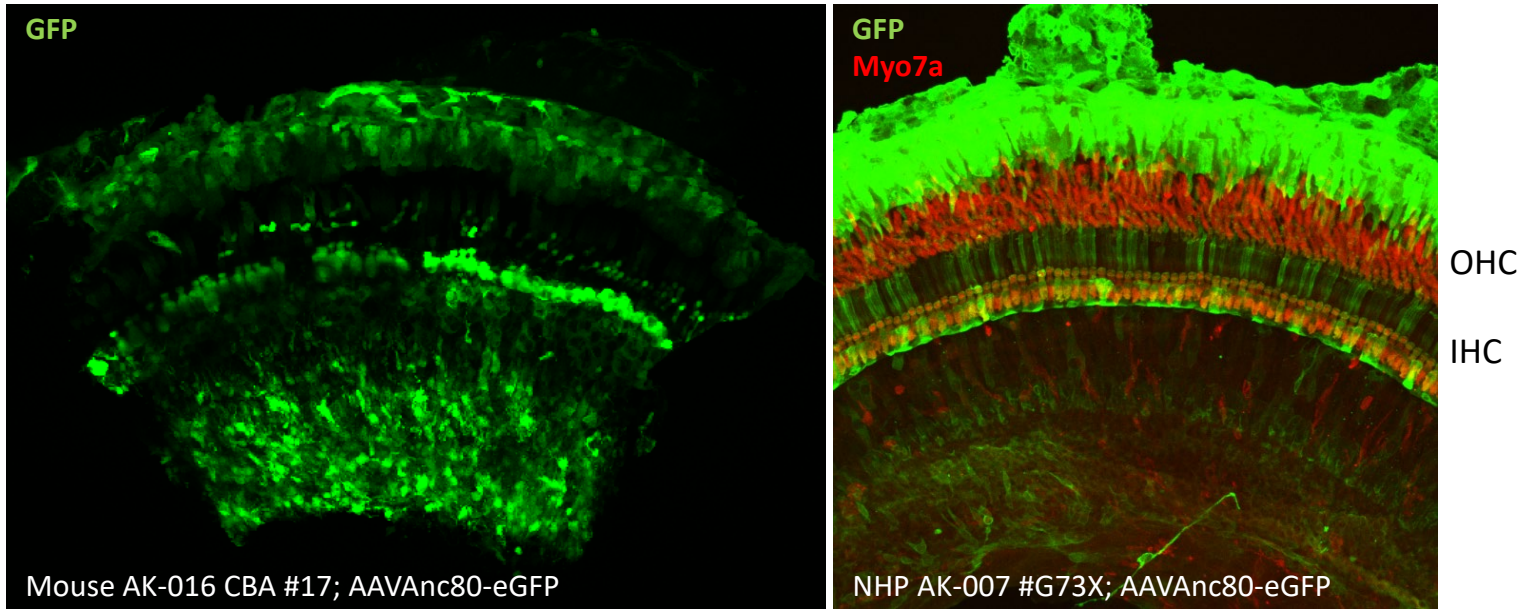
Association for Research in Otolaryngology | February 5 to 9, 2022

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 to use our precision genetic medicine platform to build a product candidate pipeline. 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 November 12, 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.

AAVAnc80 With a Ubiquitous Promoter Transduces Hair Cells and Supporting Cells in Mice and Non-human Primates

- Gene therapy using adeno-associated viral (AAV) vectors is a promising therapeutic modality for inner ear conditions, enabling delivery of potentially therapeutic genes directly to the cochlea
- Hearing loss can be a result of mutation(s) in different genes that are expressed in various cells, requiring transduction of multiple cells types in the cochlea for a broad range of conditions
- The broad cochlear tropism of AAVAnc80 transduction allows for multiple programs with different relevant cell types



Whole mount immunofluorescent staining from the middle turn of the cochlea.
Myo7a stains inner and outer hair cells (red), eGFP-reporter in green.

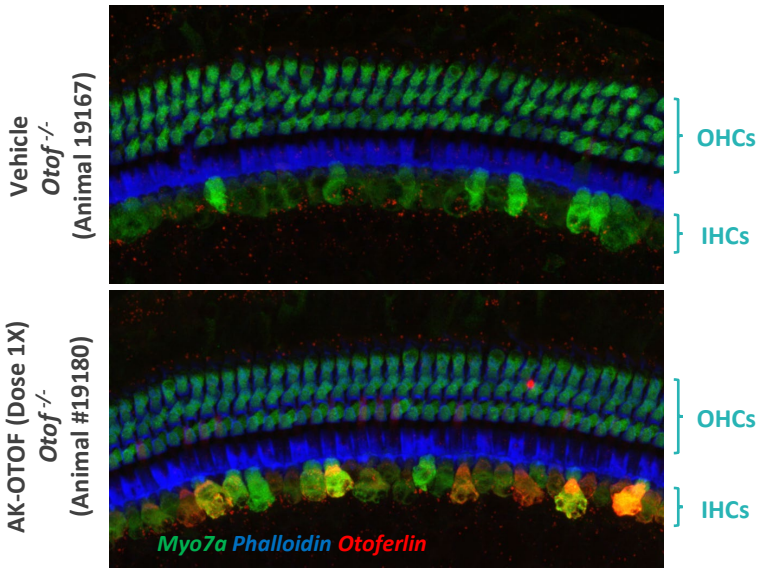
Abbreviations: GFP = green fluorescent protein; IHC = inner hair cells; NHP = non-human primate; OHC = outer hair cells.

Ubiquitous Promoter Drives Robust, Selective, and Well-tolerated Expression of hOTOF in Mice and NHPs

- Otoferlin protein is endogenously expressed in inner hair cells (IHCs)
- AAVAnc80-hOTOF under the control of a strong ubiquitous promoter drives otoferlin expression in IHCs
- Otoferlin expression detected only in IHCs in both mice and non-human primates (NHPs)
- Otoferlin expression using a ubiquitous promoter does not result in hair cell loss 6 months post-administration

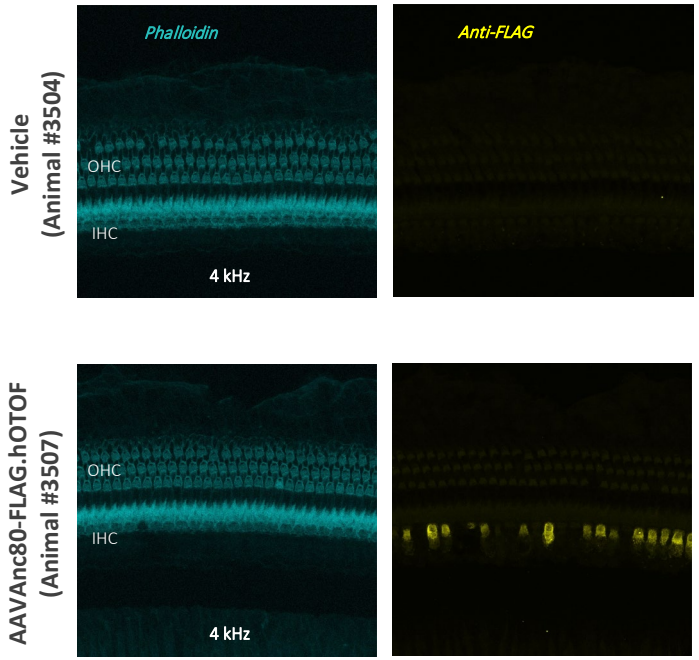
Mice

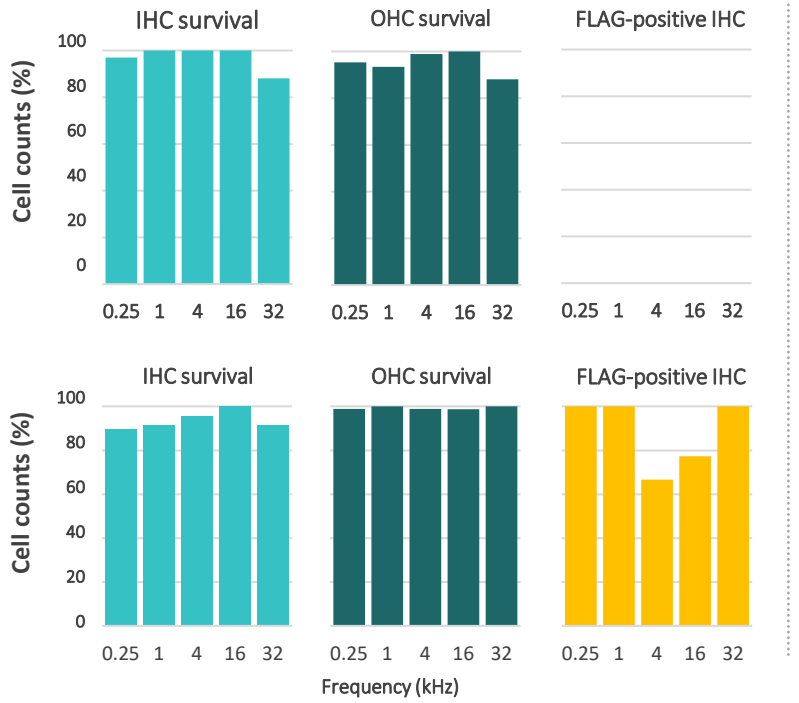
16-kHz Cochlear Micrographs
6 Months Post-administration



Cochlear micrographs represent maximum projections through confocal image stacks

NHPs





Abbreviations: hOTOF = human otoferlin; kHz = kilohertz; OHC = outer hair cell.

Ubiquitous Promoters Can Drive Safe Expression of Multiple Transgenes, and are Used in Commercially Approved and Development-Stage Gene Therapies

Approved AAV gene therapies use ubiquitous promoters

Commercially approved gene therapy for treatment of patients with confirmed biallelic *RPE65* mutation-associated retinal dystrophy

LUXTURNA® voretigene neparvovec-rzyl for subretinal injection

Illuminating possibilities.

Spark Therapeutics™, a member of the Roche Group

Commercially approved gene therapy for treatment of patients with spinal muscular atrophy with biallelic mutations in the *SMN1* gene

zolgensma® (onasemnogene abeparvovec-xioi)

AveXis, a Novartis company

Multiple inner-ear targeted AAV gene therapies in discovery and preclinical development use ubiquitous promoters and do not exhibit tolerability concerns

Article

SOURCE DATA | TRANSPARENT PROCESS | OPEN ACCESS

EMBO Molecular Medicine

Neonatal AAV gene therapy rescues hearing in a mouse model of *SYNE4* deafness

Shahar Taiber[1], Roie Cohen[2], Ofer Yizhar-Barnea[1], David Sprinzak[2], Jeffrey R Holt[3,*] & Karen B Avraham[1,**]

SCIENTIFIC REPORTS

OPEN

Modeling and Preventing Progressive Hearing Loss in Usher Syndrome III

Received: 21 April 2017
Accepted: 29 September 2017
Published online: 18 October 2017

Ruishuang Geng[1], Akil Omar[2], Suhasini R. Gopal[1], Daniel H.-C. Chen[1], Ruben Stepanyan[1], Martin L. Basch[1], Astra Dinculescu[3], David N. Furness[4], David Saperstein[5], William Hauswirth[3], Lawrence R.

Demonstration of Secreted Protein Expression Levels Following Intracochlear Delivery of AK-antiVEGF (AAVAnc80-antiVEGF Vector) Across Multiple Doses in Non-human Primates

AKOUOS

Michelle D. Valero[1], John Connelly[1], Francesc Puig-Basagoiti[1], Brian Lin[1], Ann E. Hickox[1], Timothy Boyd[1], Ignacio Navas Enamorado[1], Shimon Francis[1], Ivy K. Hughes[1], Christopher A. Shera[2], Eric D. Horowitz[1], Kathleen Lennon[1], Jean Phillips[1], Jenna Soper[1], Jennifer A. Wellman[1], Gregory S. Robinson[1], Emmanuel J. Simons[1], William F. Sewell[3], Michael J. McKenna[1], Eva Andres-Mateos[1]

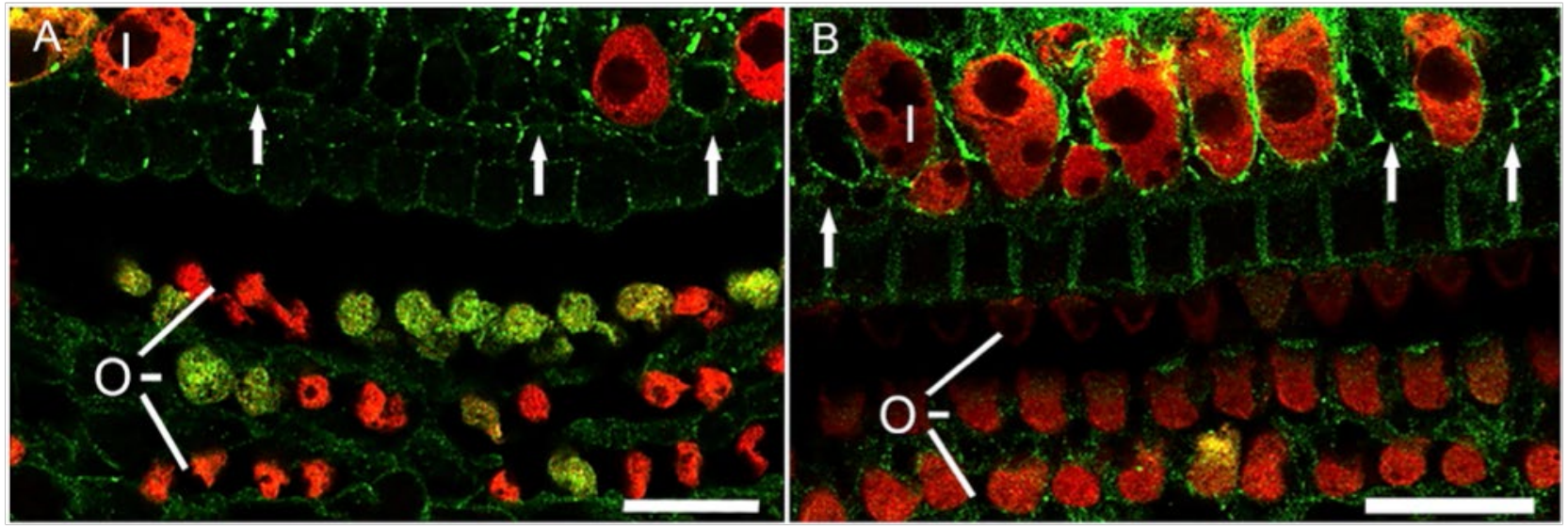
[1]Akouos Inc., Boston, MA
[2]Consultant to Akouos, Inc.
[3]Co-founder and consultant to Akouos, Inc.

Association for Research in Otolaryngology | February 5 to 9, 2022

Abbreviations: AAV = adeno-associated viral; FDA = U.S. Food and Drug Administration.

However, Promoter Choice Can Be Transgene Dependent

- The *GJB2* gene encodes the connexin 26 (Cx26) protein
- Cx26 is endogenously expressed in supporting cells (SCs) of the inner ear
- Ubiquitous promoter-driven expression of Cx26 in inner hair cells results in cell loss
- A tailored expression pattern may be warranted for this transgene



Cx26 Myo7a

Guo J, et al. *Mol Ther Methods Clin Dev.* 2021;23:319-333.

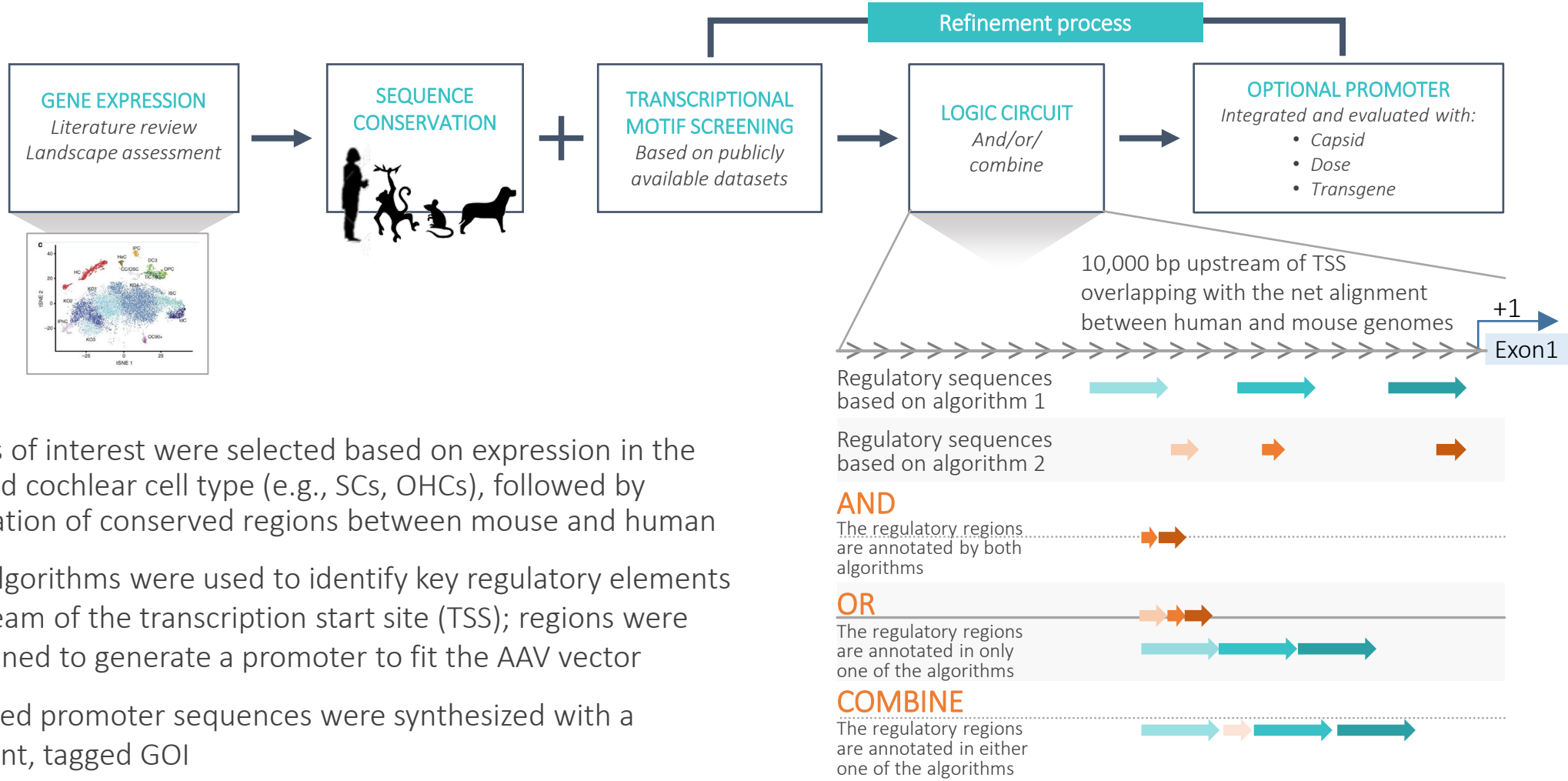
Gene Therapy Regulation

- Customization of regulatory elements can induce strong transgene expression in cells where this is desired and minimize expression in a subset of cells where such expression may not be well tolerated

- Regulatory elements can be incorporated to support selective transgene expression:
  - Gene of Interest (GOI) – may provide selectivity based on endogenous cell regulation, as observed for *OTOF*
  - Promoter – may be specific to the transgene or selective for a cell population
  - 5’ and 3’ untranslated region (UTR) – may support endogenous cell regulation machinery to enhance expression in cells where this is desired and reduce expression in cells where expression may not be well tolerated
  - Enhancer sequences – could be added to the promoter to enhance expression
  - Additional regulatory elements, such as microRNA target sites (miR-TS), splicing elements, and degrons can be added for improved spatiotemporal regulation

Abbreviations: *OTOF* = otoferlin gene.

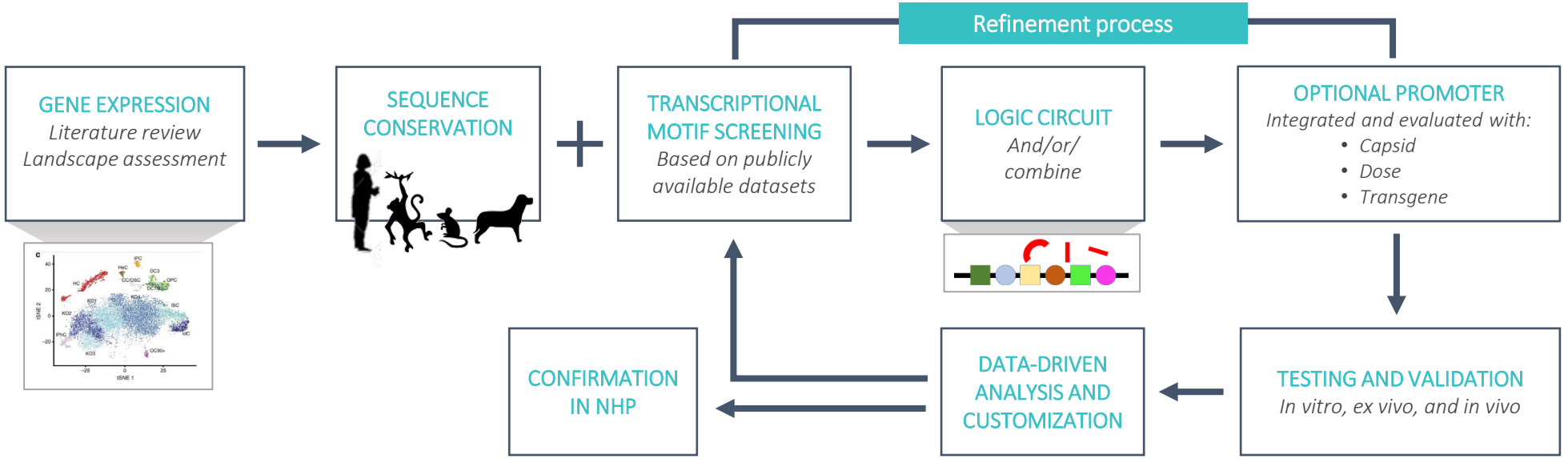
Identification of Regulatory Sequences



- Genes of interest were selected based on expression in the desired cochlear cell type (e.g., SCs, OHCs), followed by evaluation of conserved regions between mouse and human
- Two algorithms were used to identify key regulatory elements upstream of the transcription start site (TSS); regions were combined to generate a promoter to fit the AAV vector
- Selected promoter sequences were synthesized with a relevant, tagged GOI

Abbreviations: AAV = adeno-associated viral; GOI = gene of interest; OHC = outer hair cells; SCs = supporting cells.

Identification of Regulatory Sequences

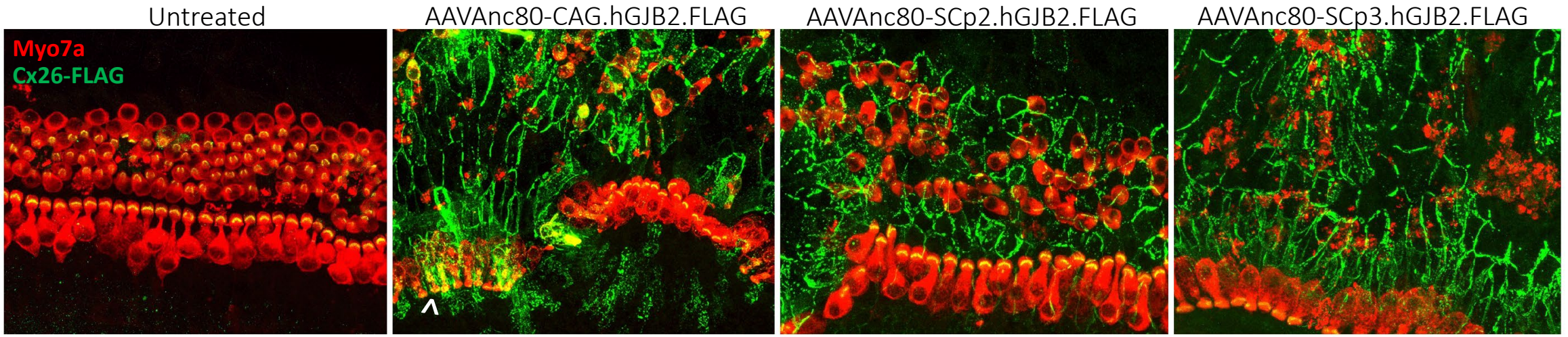


- Plasmids and subsequent AAVAnc80 vectors containing the different promoters were evaluated for expression in vitro and preliminary selective expression in cochlear explants
- Subsequent evaluation in vivo in mature cochleae of wild-type (WT) mice was used to identify promising promoters for future programs

Abbreviations: NHP: non-human primate(s).

Selective Promoters Can Drive *GJB2* Transgene Expression in Cochlear Explants

Explant screening for selective promoters enabled prioritization prior to in vivo studies



Strong ubiquitous promoter

- Robust FLAG expression in SCs
- Expression is also detected in IHCs and OHCs (arrowhead)

SC selective promoters

- Robust FLAG expression in SCs
- No apparent IHC or OHC expression

- Explants were generated from P2 neonate mice and incubated for 5 days with vector at 1E10 vector genomes (vg)/explant

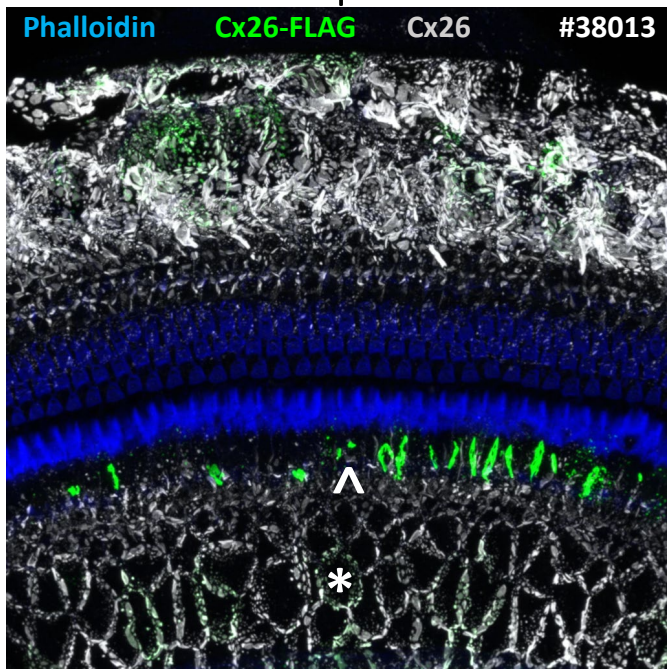
Abbreviations: *GJB2* = gap junction beta 2 gene; IHC = inner hair cells; OHC = outer hair cells; SCs = supporting cells.

Data Cutoff: 30Nov2021

Selective Promoters Can Drive *GJB2* Transgene Expression in Mature Cochleae of WT Mice

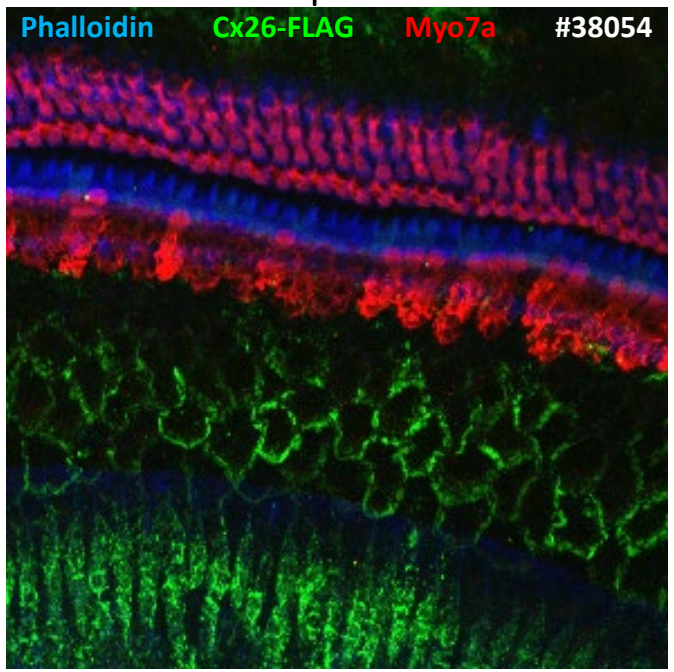
AAVAnc80 vectors with selective promoters can drive *GJB2* expression in SCs, but not in HCs, in mature cochleae of WT mice

AAVAnc80-SCp1.hGJB2.FLAG

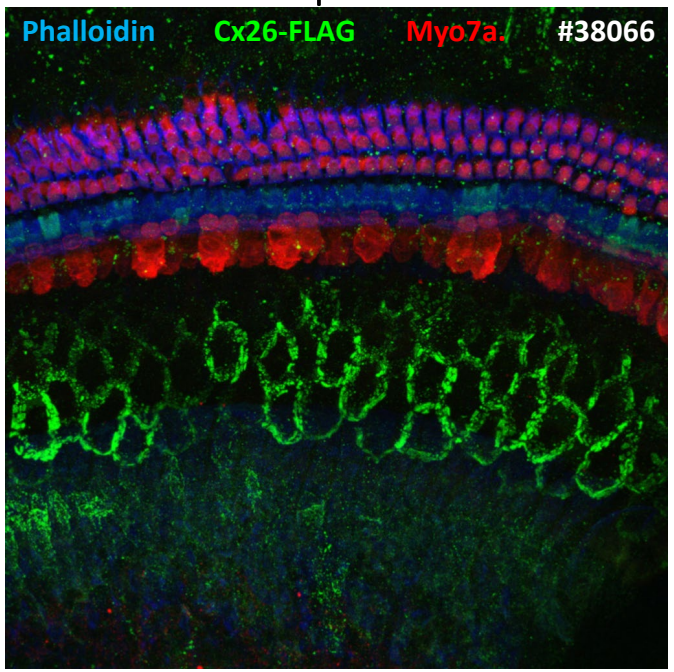


- FLAG expression colocalized with endogenous Cx26 expression (*)
- Expression detected in IHCs (arrowhead)

AAVAnc80-SCp2.hGJB2.FLAG



AAVAnc80-SCp3.hGJB2.FLAG



- Robust FLAG expression in SCs (green)
- No apparent expression in IHCs, or hair cell loss
- Differential expression patterns observed between supporting cell subtypes

AKOUOS

Abbreviations: Cx26 = connexin26; *GJB2* = gap junction beta 2 gene; HCs = hair cells; IHCs = inner hair cells; SCs = supporting cells; WT = wild-type.

Data Cutoff: 09Nov2021

Summary and Conclusions

1. Ubiquitous promoter drives strong selective otoferlin expression in IHCs, and expression is well tolerated across the inner ear
2. Addition of selective cis-regulatory elements, such as promoters, is transgene dependent
3. Inclusion of a selective promoter was evaluated in the case of *GJB2,* where expression in hair cells is not well-tolerated
4. Akouos developed a method to guide customization of regulatory elements based on bioinformatic efforts
5. Akouos identified multiple regulatory elements to drive various differential expression patterns and evaluated them ex vivo and in vivo
6. Supporting cell-selective promoters were evaluated in mice in vivo, demonstrating robust and well-tolerated expression
7. A combination of AAVAnc80 and supporting cell selective promoter can drive widespread *GJB2* expression in supporting cells, while limiting expression in, and loss of, hair cells
8. Future work will include customization of regulatory elements where this may be beneficial

AKOUOS

Abbreviations: *GJB2* = gap junction beta 2 gene; IHCs = inner hair cells.

Thank you!