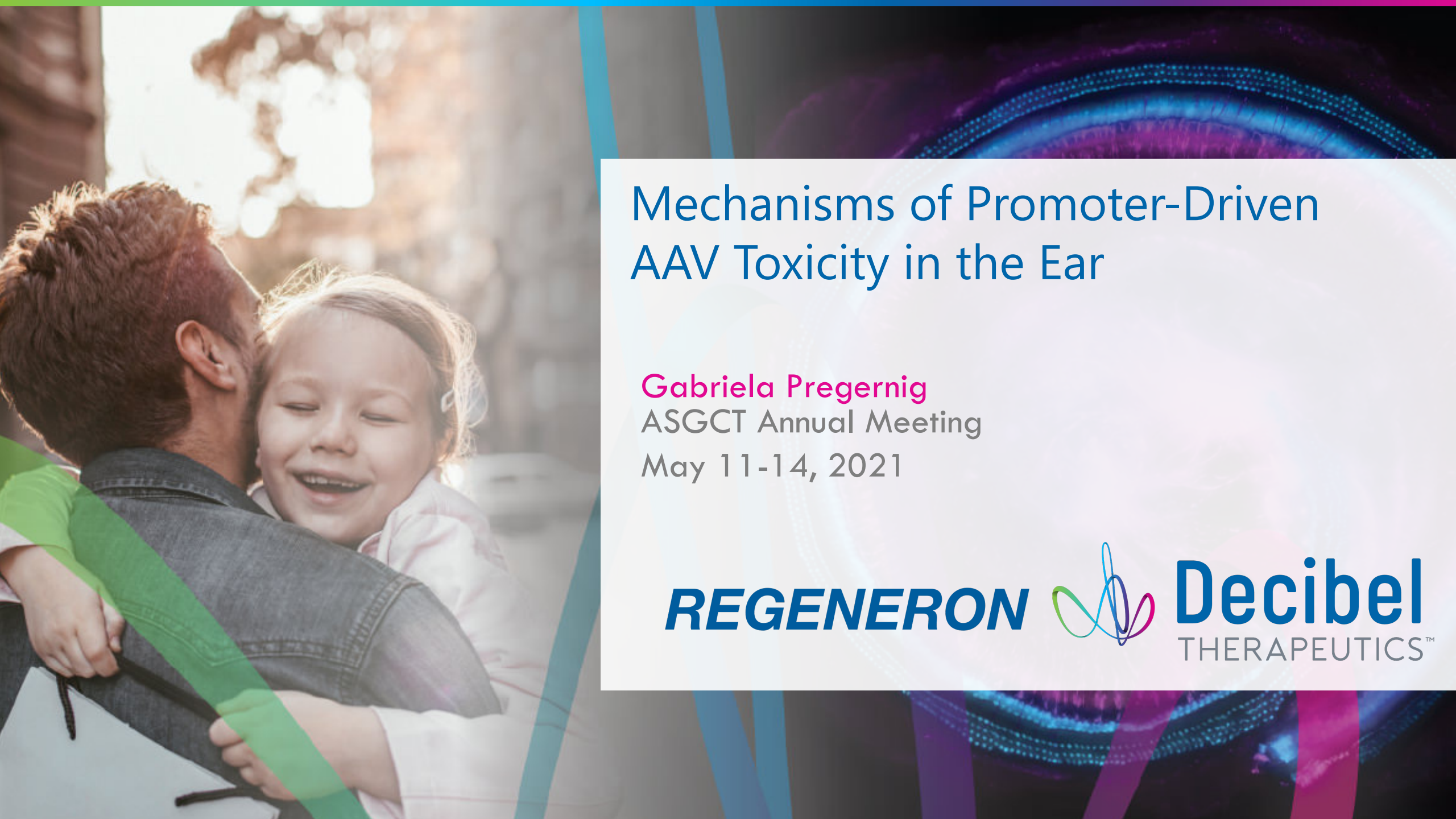

Mechanisms of Promoter-Driven AAV Toxicity in the Ear

Gabriela Pregernig

ASGCT Annual Meeting

May 11-14, 2021

REGENERON

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Abstract

Background

Adeno-associated virus (AAV) is a promising approach to the treatment of a variety of inner ear disorders. These gene delivery vectors, with which there is deep clinical experience in other tissues, are being developed both for the correction of inner ear genetic disease and for regenerative therapy of common conditions where hair cells are lost. Several products are on their way to the clinic across these areas.

An AAV gene therapeutic usually consists of a capsid, a coding sequence, and one or more regulatory elements that may impact when and where the transgene is expressed. We have previously shown that the choice of synthetic promoter can have a substantial impact on the therapeutic efficacy or durability of a gene therapy for genetic disease. First, in the context of Tmc1-mutant mice treated with AAV vectors expressing functional Tmc1, we demonstrated an improvement in ABR threshold recovery when a non-cell-specific CMV promoter was replaced with a hair-cell specific promoter derived from the Myo15 gene. Second, in the context of Otof-mutant mice, we showed that threshold recovery was not durable when a functional copy of Otof was delivered driven by a non-cell-specific smCBA promoter, but was durable when driven by the Myo15 promoter. In both of these contexts, loss of inner hair cells was observed with ubiquitous promoters that was not observed with the Myo15 promoter.

Methods

AAV-Myo15-hOTOF and AAV-smCBA-hOTOF were administered to the inner ear of mice and gene expression profiling of the cochlea was performed one month later.

Results

Consistent with previous results, animals treated with the smCBA-containing vector had impaired long-term hearing relative to those treated with the Myo15-containing vector. Several hypotheses may explain the apparent benefit of using the Myo15 promoter here. First, Myo15 may express at a lower level than the ubiquitous promoters even in the target hair cells, thus reducing the risk of overexpression-driven toxicity. Second, the hair cell-specificity of the promoter may be important through some mechanism. We performed RNA sequencing on cochleae from animals in this study in order to disentangle these two potential mechanisms of toxicity and loss of durability. High quality expression data was obtained, with marked differences between the two treatment groups.

Conclusions

We report the results of these analyses here, including examination of cell pathways and immune system involvement that may drive them.

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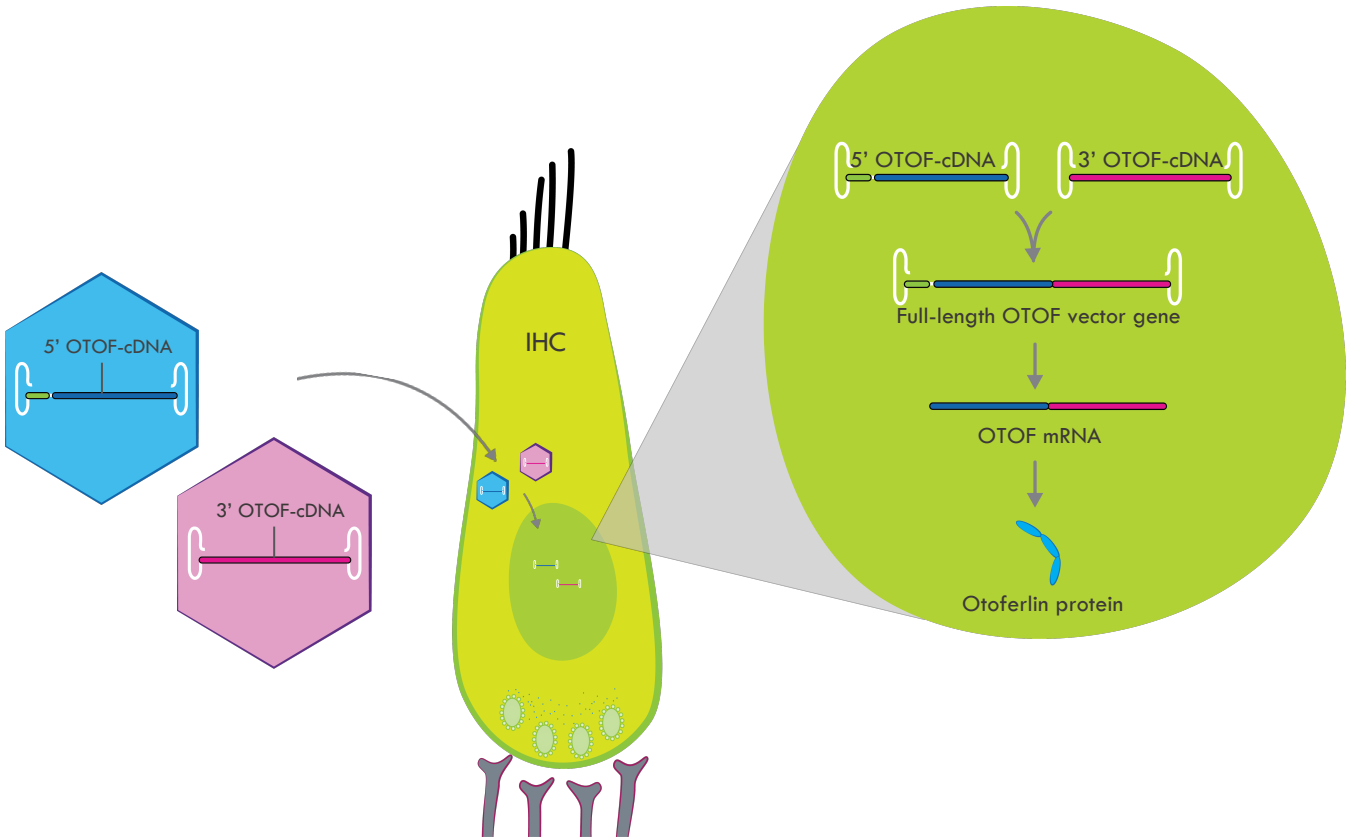
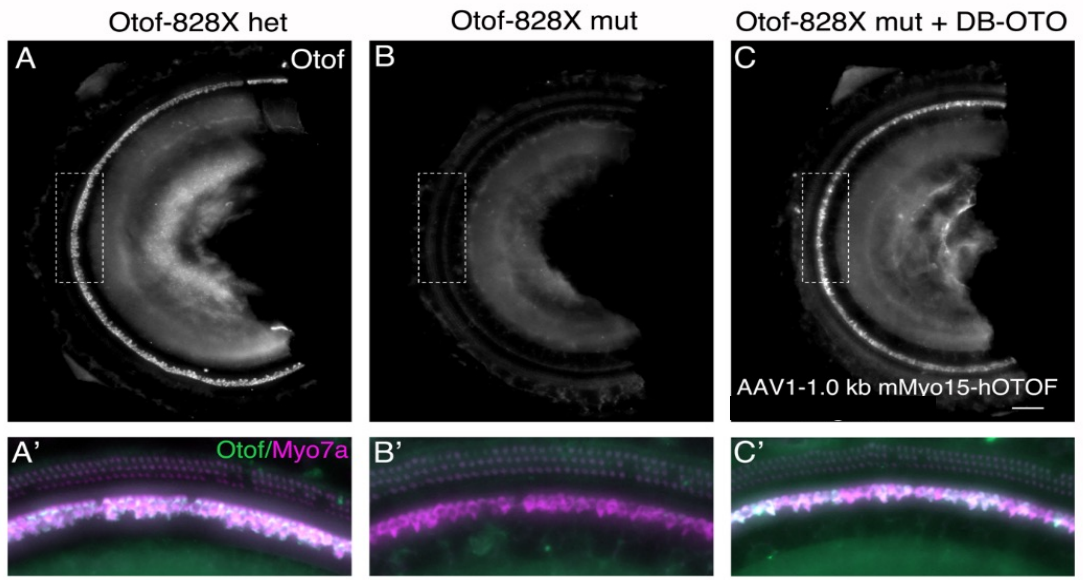
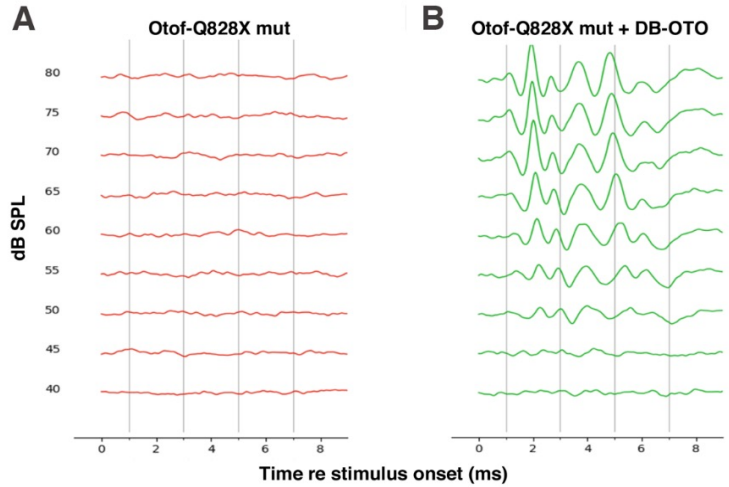
AAV Delivery of *Otoferlin* Rescues Hearing In Deaf Mice

Illustration of dual vector strategy for AAV Delivery of *Otoferlin*





Expression and functional rescue with Decibel vector.



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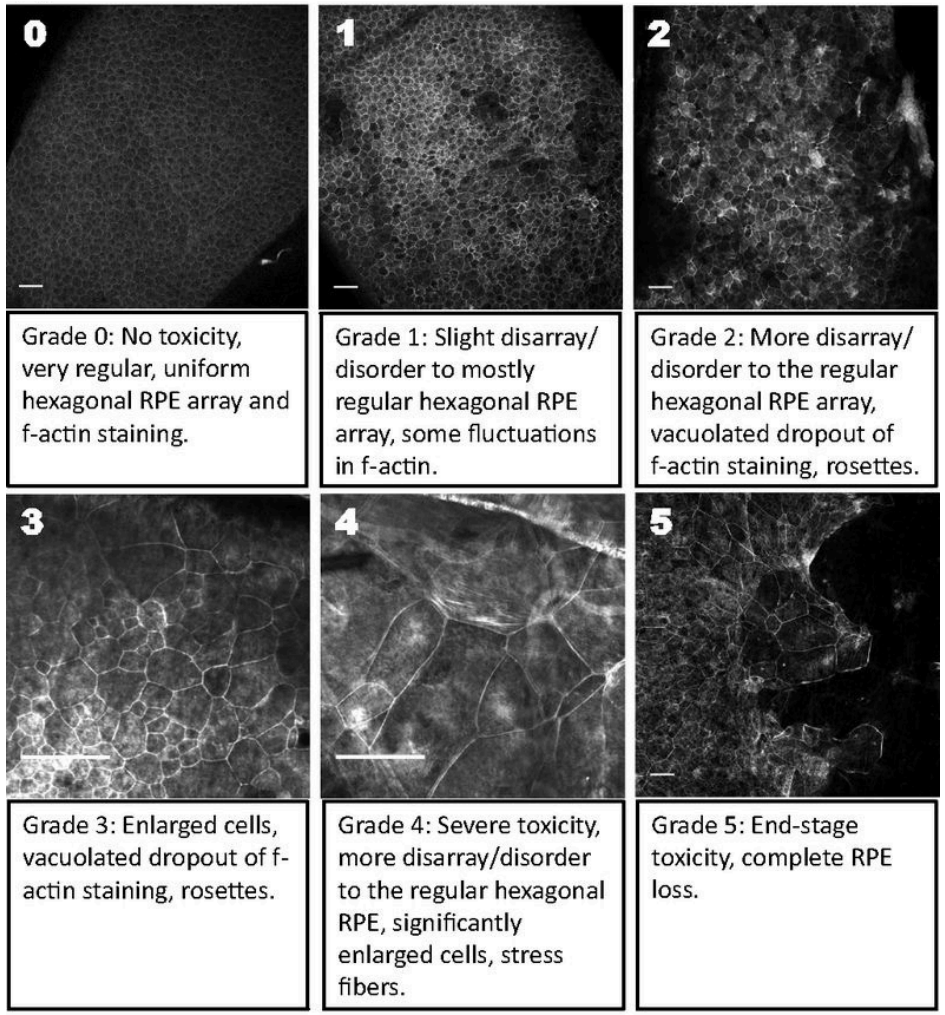
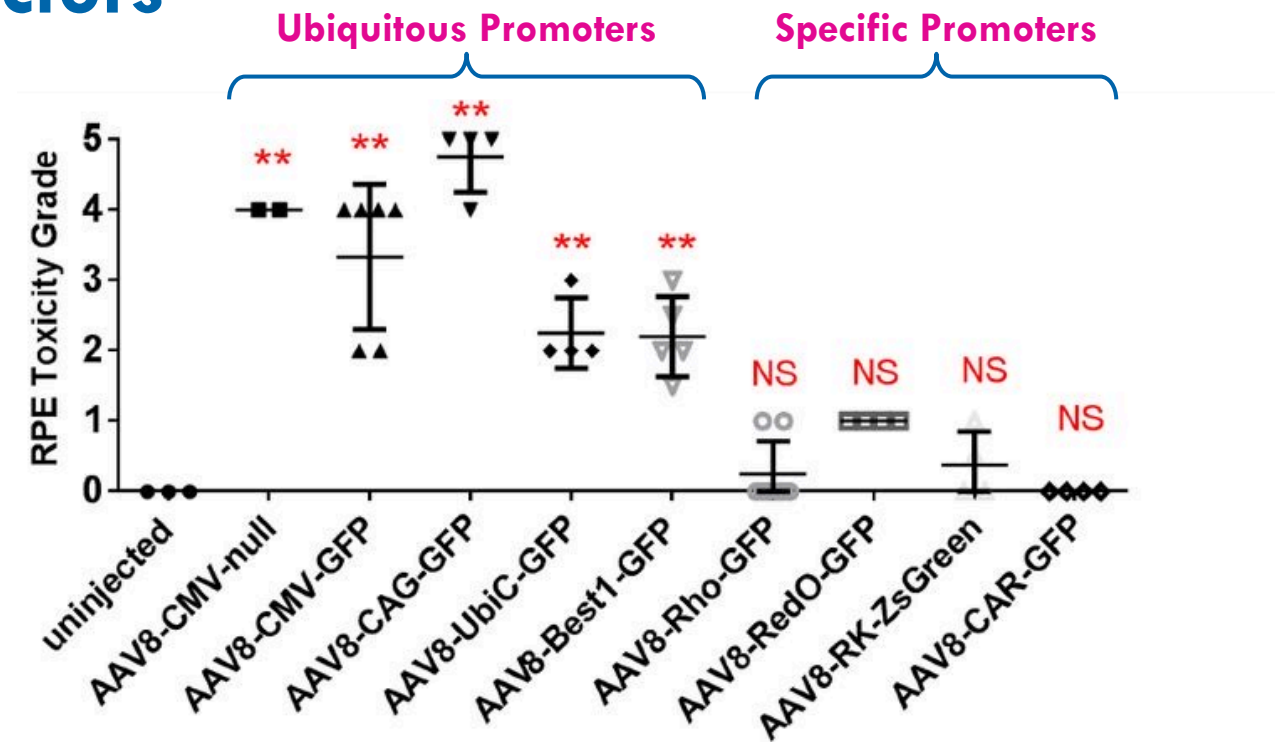
AAV Off-Target Expression is a Risk that Should be Mitigated When Using Broadly-Tropic Vectors





Xiong et al (2019) PNAS

Data from Cepko lab are shown, demonstrating deleterious effects in the retina when cell-specific promoters are not used.

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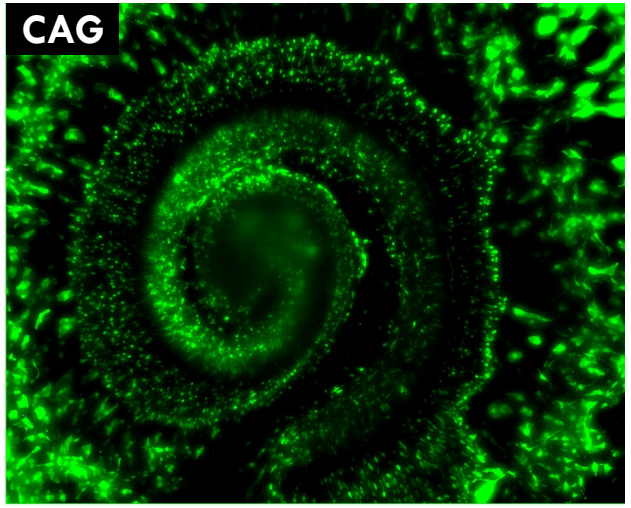
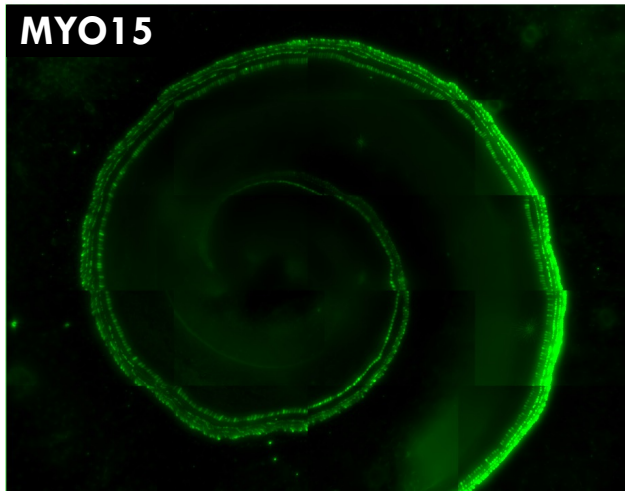
DB-OTO Leverages a MYO15-based Hair Cell-Specific Promoter

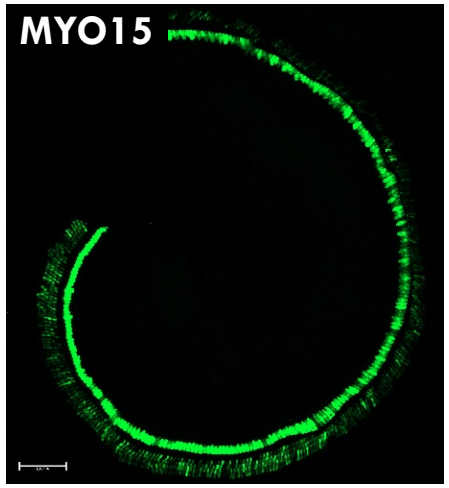
GFP Expression Driven by MYO15 Promoter Shown Across Species

Mouse Explant

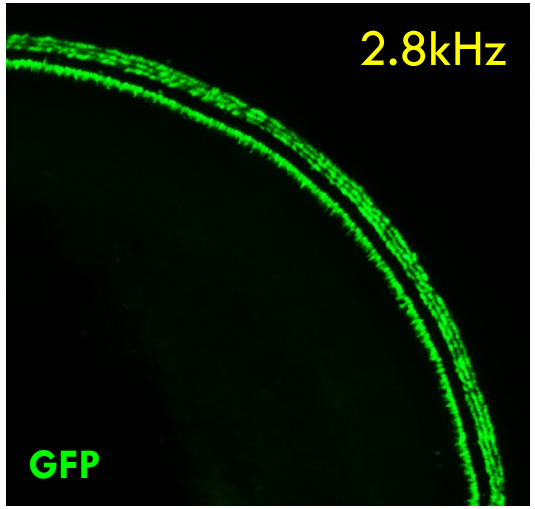


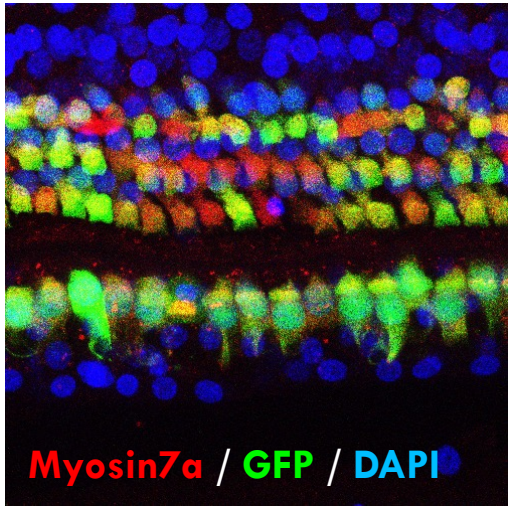


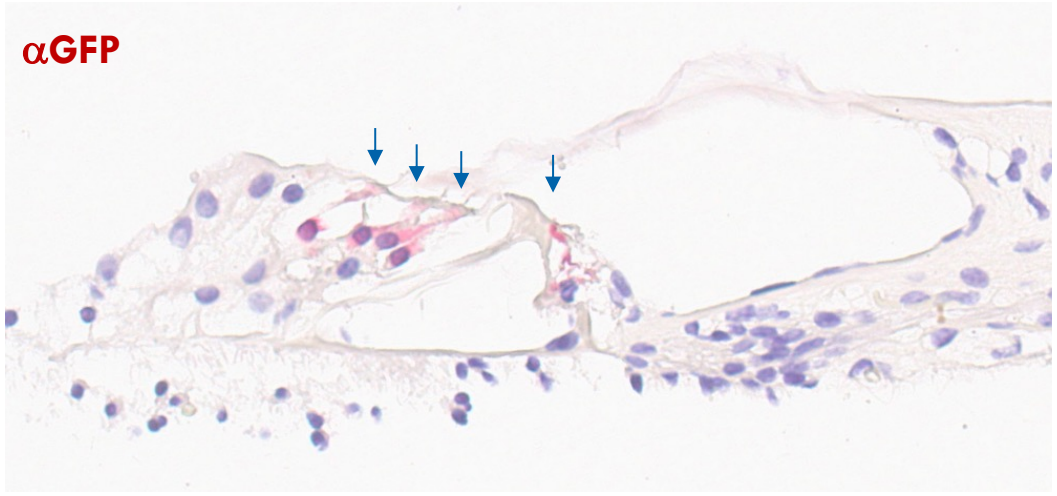
Mouse *In Vivo*



Nonhuman Primate *In Vivo*







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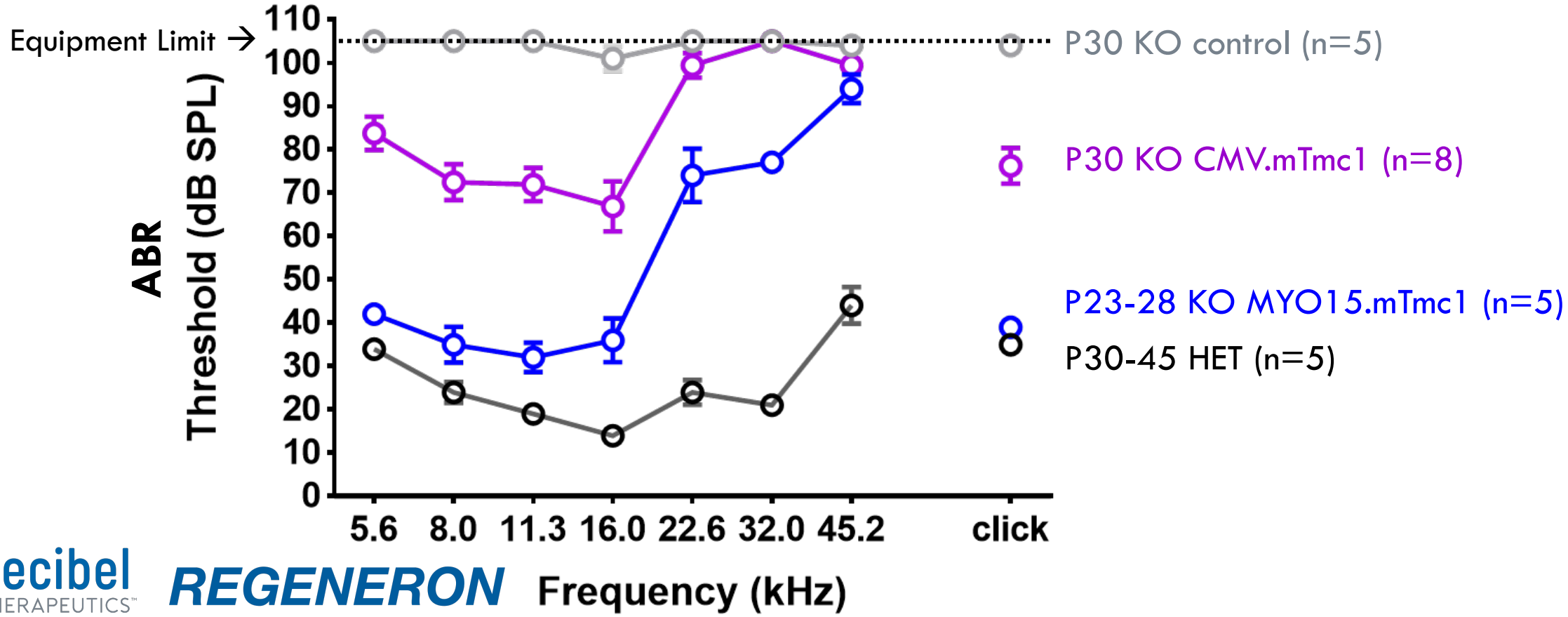
The MYO15 Promoter Improves Tmc1 Gene Replacement Therapy Relative to a CMV Ubiquitous Promoter

- Tmc1 KO mice were infused at P2-P3 with AAV.CMV.mTmc1 or AAV.MYO15.mTmc1
- ABR thresholds are shown for the various groups ~4 weeks post-injection. Both AAV treatments led to improved thresholds compared to age-matched controls, however significantly better recovery was observed with the MYO15 promoter as compared to the ubiquitous CMV promoter.



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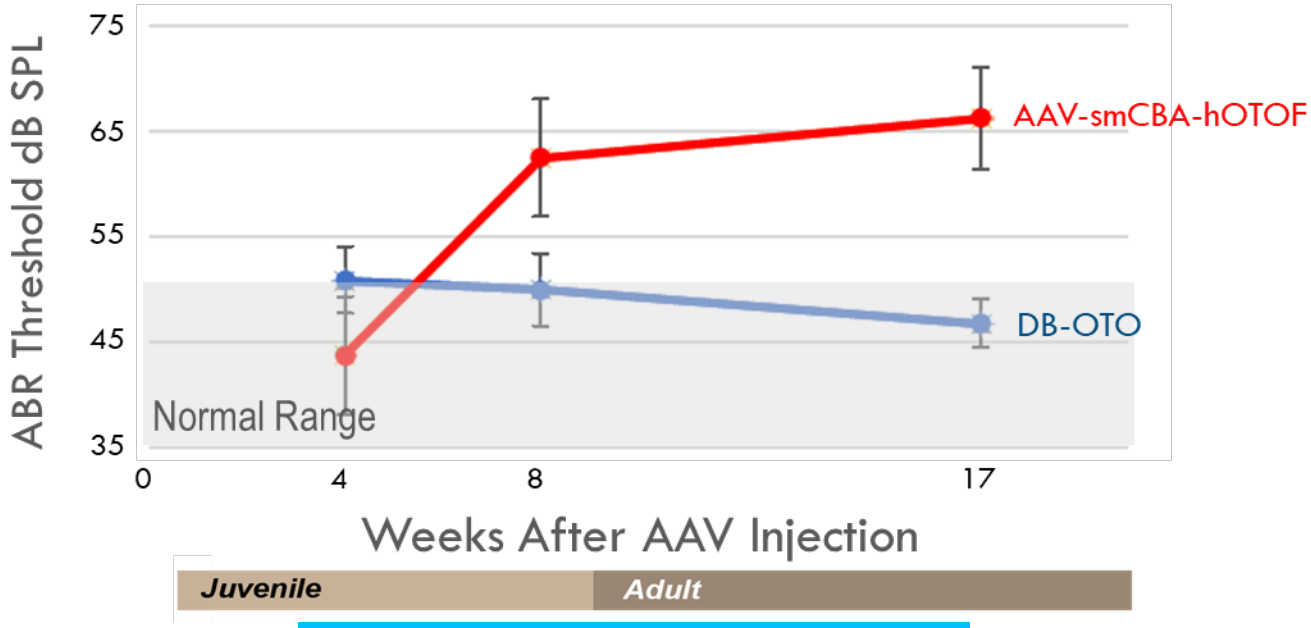
DB-OTO: MYO15 Promoter Provided Cell-Selective Expression and Enabled Improved Durability in Preclinical Studies

- Q828X adult HOM animals were injected with DB-OTO or AAV-smCBA-hOTOF (same serotype). ABR Thresholds were measured at 4, 8 and 17 weeks post-injection.
- ABR recovery with DB-OTO remained stable up to 17 weeks after treatment.
- Reduced durability was seen with AAV-smCBA-hOTOF treatment, as evidenced by increased ABR thresholds 8 and 17 weeks after treatment.

Durability of ABR Sensitivity with DB-OTO vs. AAV Vector with Ubiquitous Promoter in Q828X Mice



Auditory Brainstem Response
22 kHz Thresholds in dB SPL

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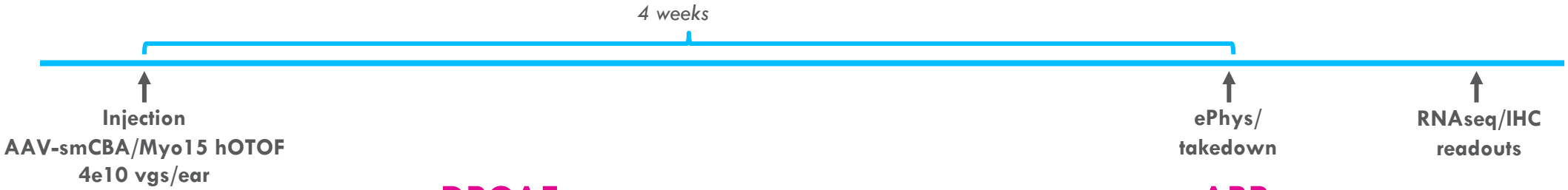
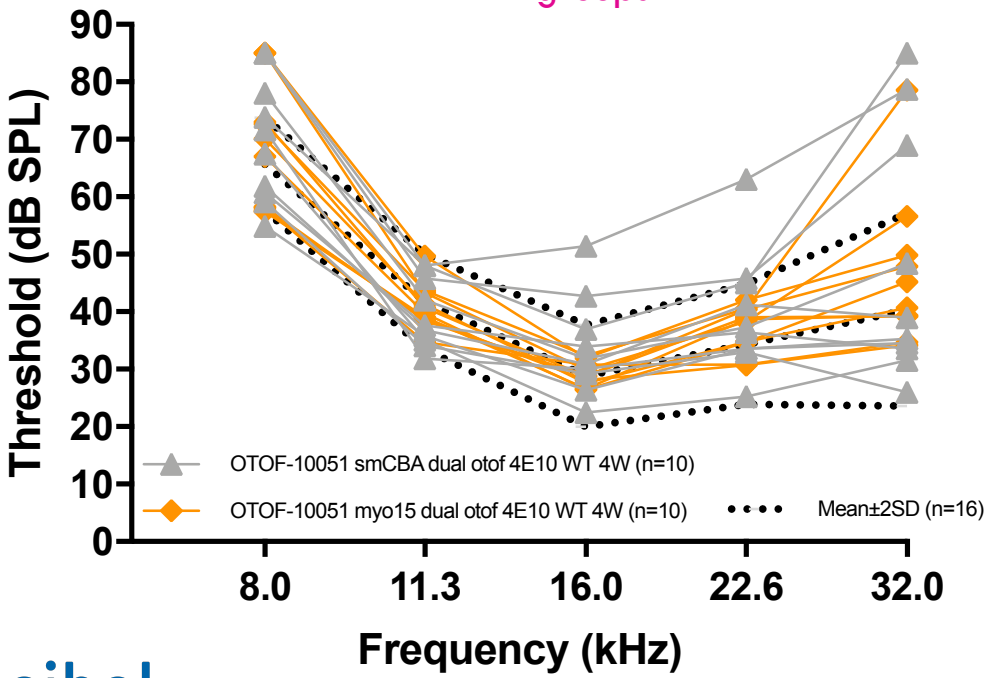
hOTOF Overexpression with a smCBA Ubiquitous Promoter in Wild-Type Animals Leads to Elevated Hearing Thresholds



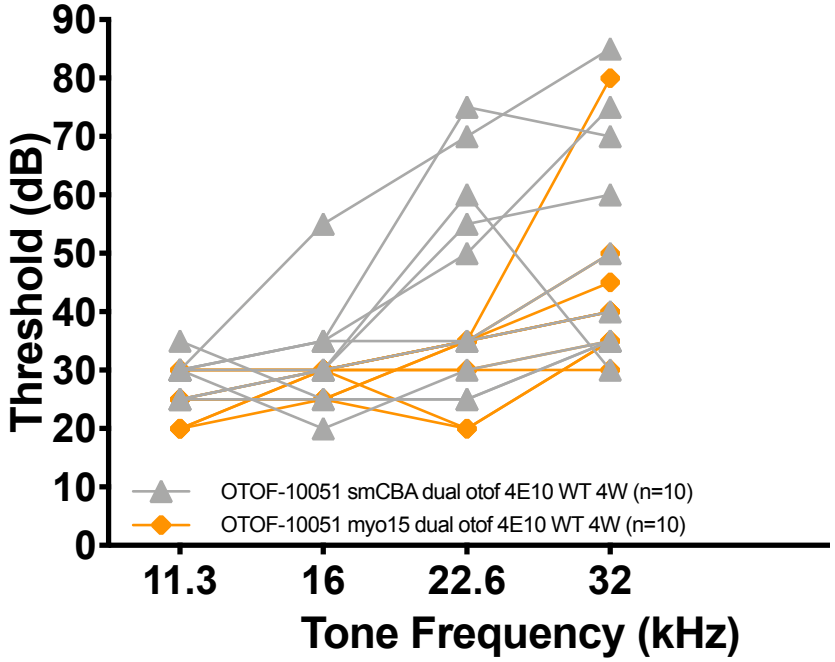
DPOAE

Thresholds comparable in both groups



ABR

Elevated thresholds observed in some AAV-smCBA animals



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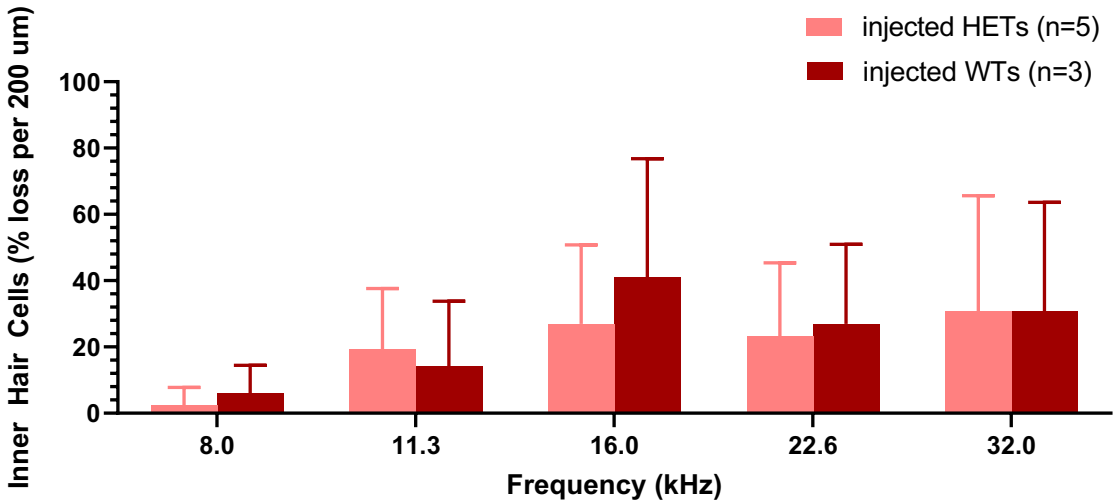
Elevated ABR Thresholds after AAV-smCBA Gene Therapy Can Be Explained by Inner Hair Cell Loss

Ubiquitous Promoter

~30% IHCs lost across frequencies

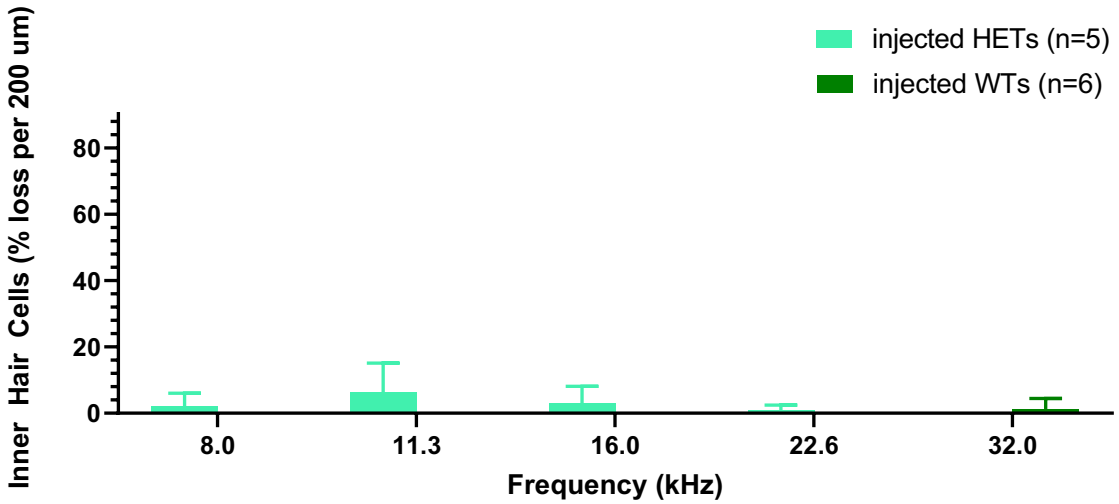
IHC loss in animals injected with smCBA-OTOF



MYO15 Specific Promoter

Little to no IHC loss observed across frequencies

IHC loss in animals injected with mMyo15-OTOF



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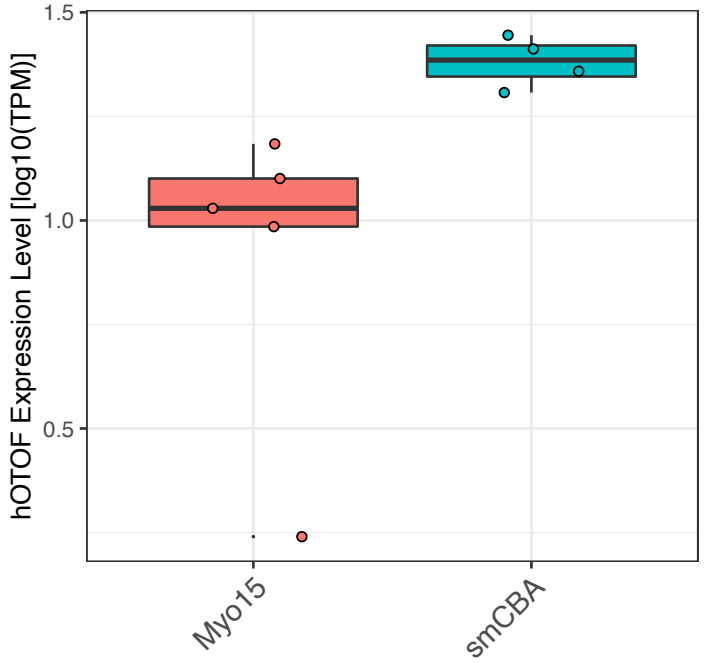
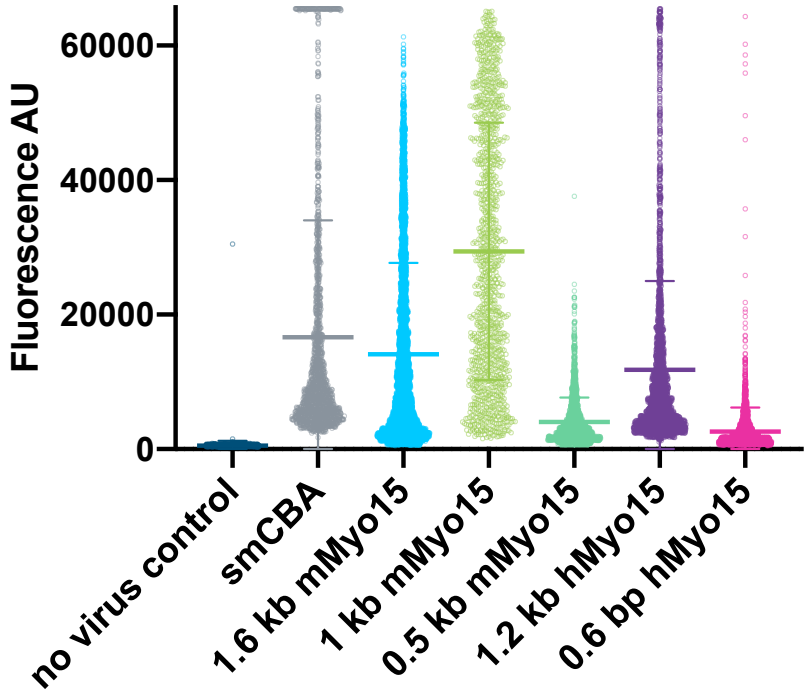
Differences in Transgene Expression Between Promoters Could Contribute to Observed IHC Loss

Bulk RNA-seq quantification of hOTOF expression



- hOTOF expression levels were quantified from whole cochlea bulk RNA-seq of animals injected with either DB-OTO or AAV-smCBA-hOTOF.
- hOTOF expression was lower after DB-OTO treatment. However, we cannot exclude that this difference is due to smCBA-driven expression in non-IHC cell types.

Quantitative image analysis was used to determine GFP expression levels in myosin 7a positive hair cells



- GFP expression levels driven in cochlear explants by AAV-Myo15 promoters or smCBA were quantified in myosin 7a-positive hair cells.
- While some variability was observed across the different Myo15 promoters, overall smCBA-driven GFP expression in hair cells was not definitively higher.

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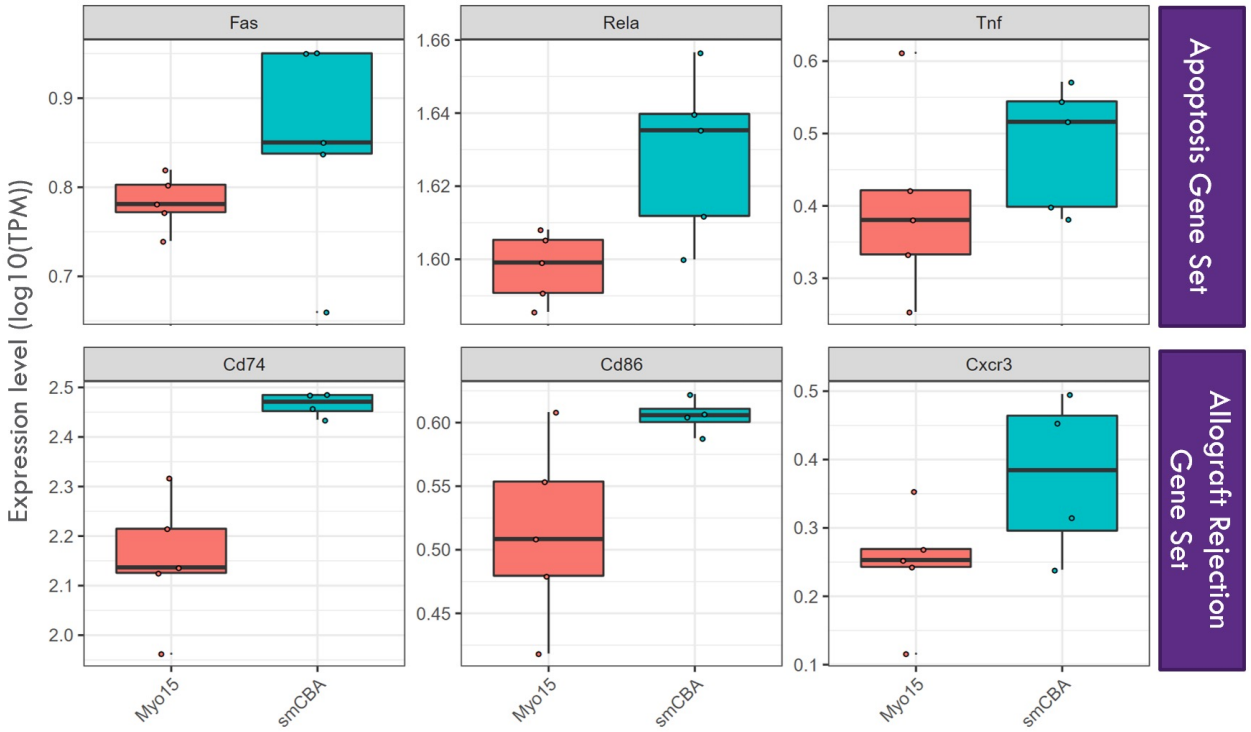
Bulk RNA-seq Analysis of smCBA vs Myo15 hOTOF Treated Animals Provides Clues about Mechanistic Differences

- Differential expression followed by gene set enrichment analysis were performed between whole cochlea bulk RNA-seq samples of animals injected with DB-OTO or AAV-smCBA-hOTOF. Plots on the right show differences in expression levels between groups for selected genes.
- An **apoptosis signature** was found to be elevated in smCBA-hOTOF-treated mice.
- We also observed smCBA-specific elevation of gene sets associated with **antigen-directed immunity**, including MHC and T cell genes.
- This could suggest that expression of exogenous hOTOF in professional antigen-presenting cells may be **directing an immune response against cochlear sensory cells.**



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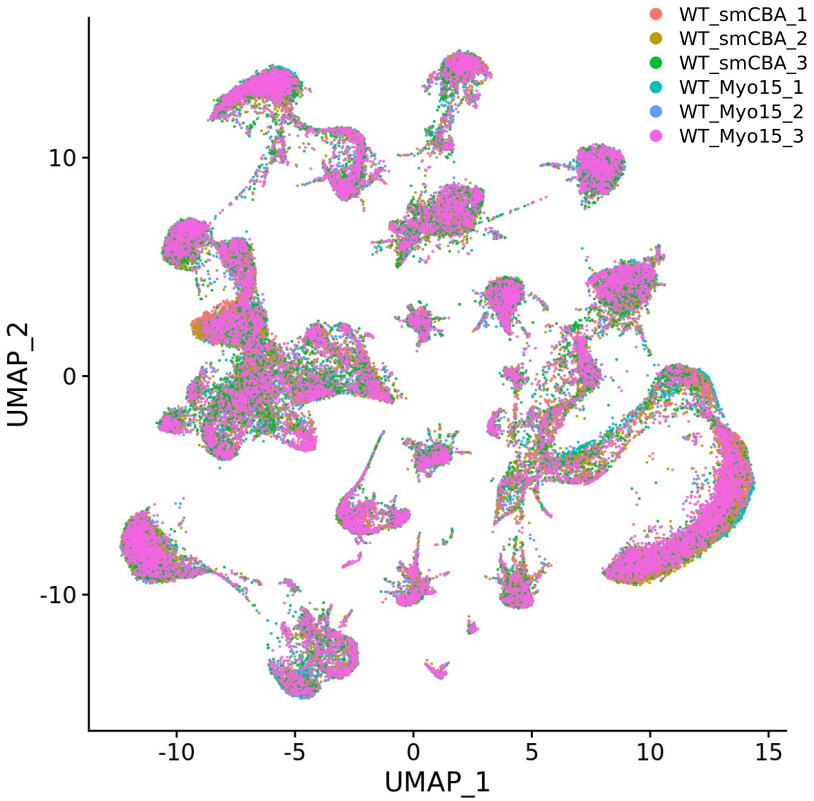
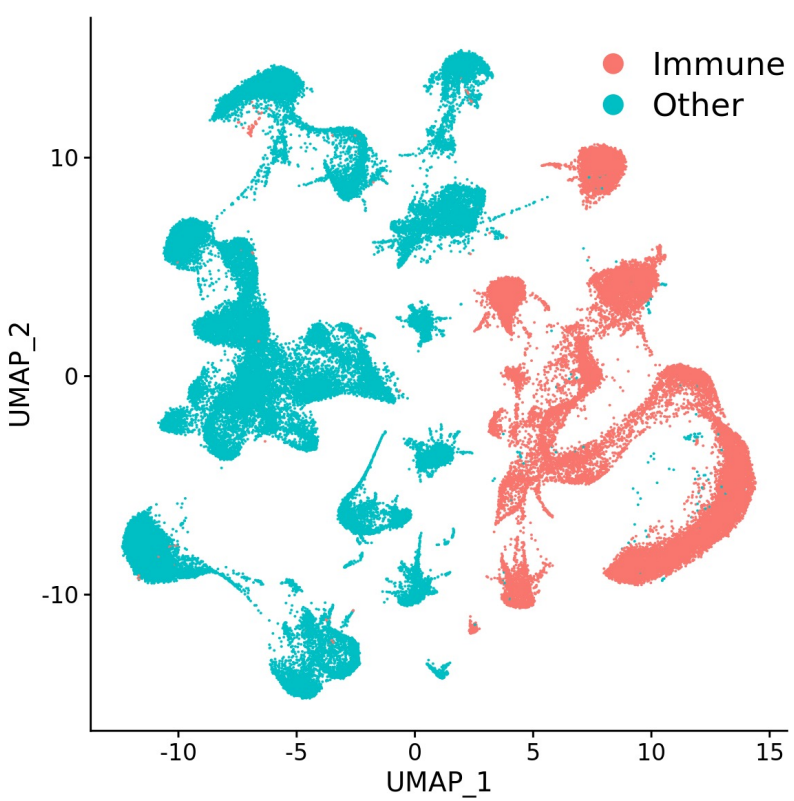
scRNA-seq Profiling Captures >25,000 Immune Cells

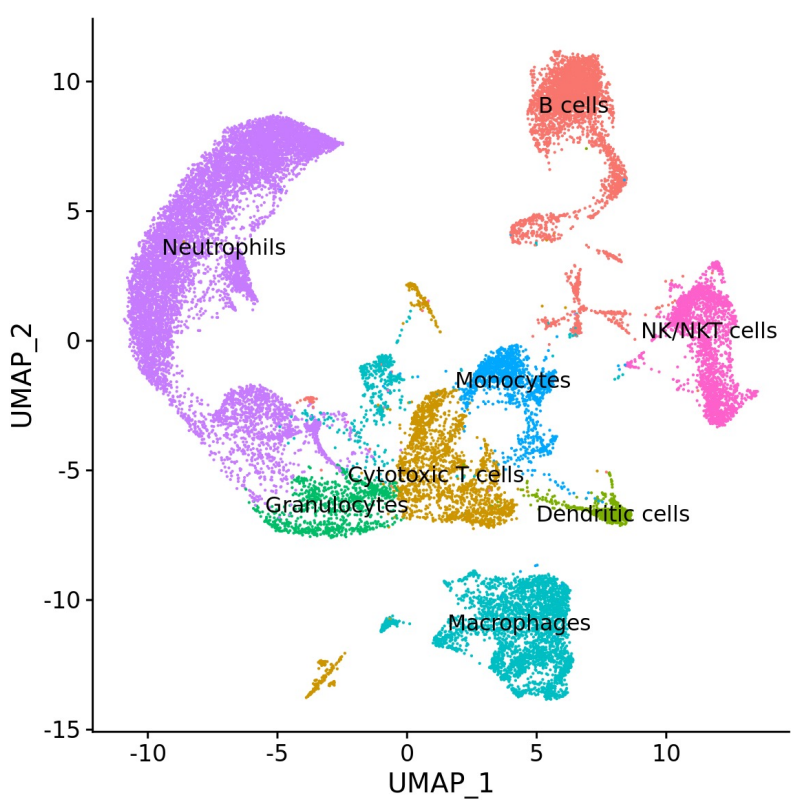
Colored by sample



Colored by immune vs other



Immune cells only



- Whole cochlea single cell RNA-seq was performed on animals injected with either DB-OTO or AAV-smCBA-hOTOF (10X Genomics). Gene by cell matrices were generated using Cellranger software, and downstream processing (QC, filtering, clustering) was done using Seurat.
- No signs of batch effects were observed as evidenced by the sample-colored UMAP (left). A large proportion of clusters were identified as immune cells, corresponding to >25,000 total cells (center). Immune cells were extracted, re-clustered, and labeled based on known markers (right).

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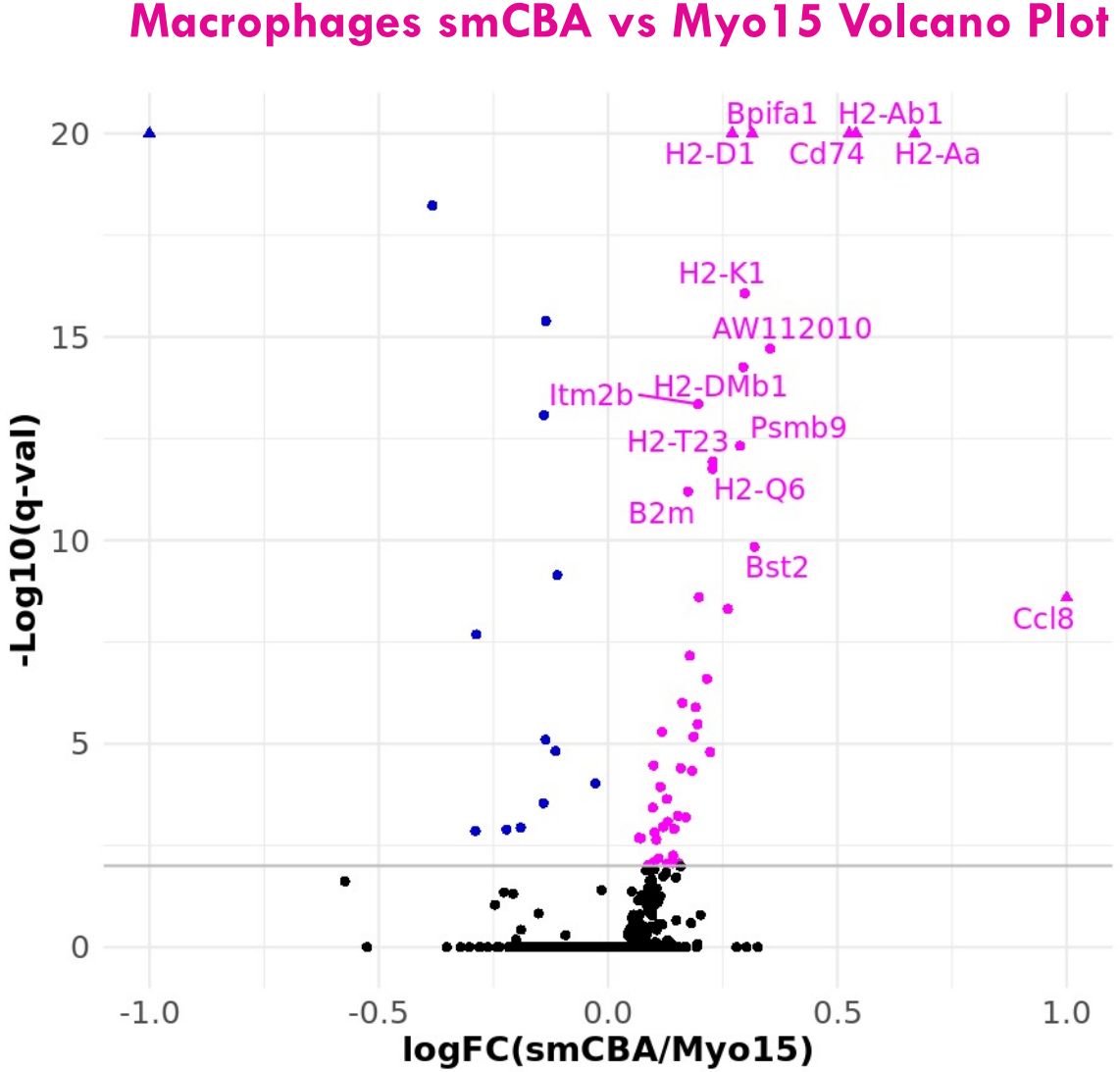
Differential Gene Expression Analysis Points to Elevated Macrophage Activity in smCBA Treated Animals

- We performed **differential expression** analysis between smCBA and Myo15 samples across cell types. Results for macrophages are shown in the volcano plot on the right.
- Several genes related to **antigen presentation** were highly upregulated in macrophages
- Gene set enrichment analysis highlighted **allograft rejection and interferon response** pathways



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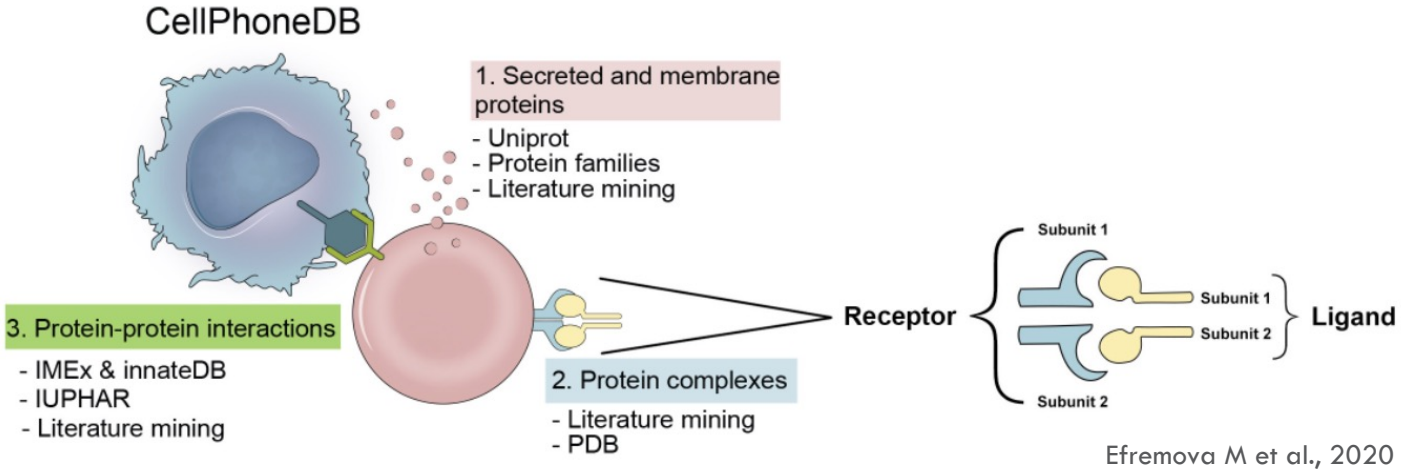
CellphoneDB Identifies Significant Ligand-Receptor Interactions Across Cell Types and Samples

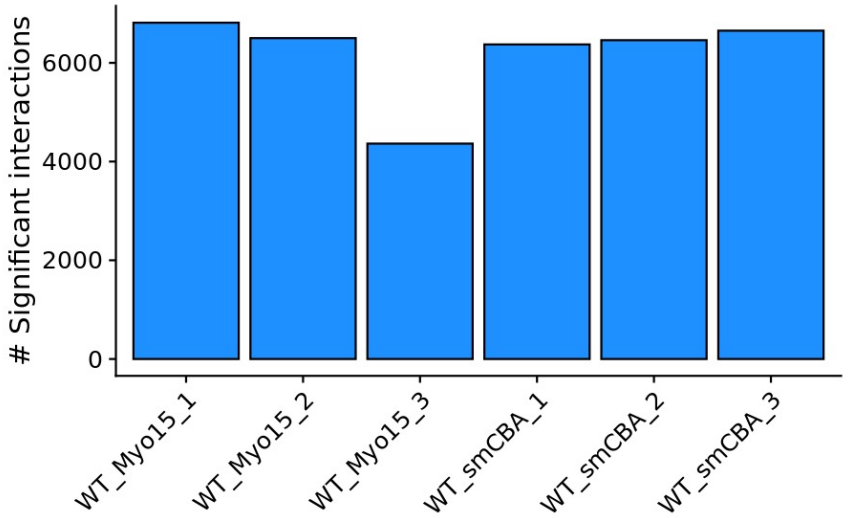
- CellphoneDB was leveraged to identify **ligand-receptor interactions** which could mediate the signs of an immune response seen in smCBA samples
- Over **4,000 significant interactions** were found in each sample
- Of those, >1,300 common to all smCBA samples were not significant across Myo15 samples, compared to only ~500 Myo15-biased interactions

CellphoneDB overview



Efremova M et al., 2020

Total # significant interactions across samples



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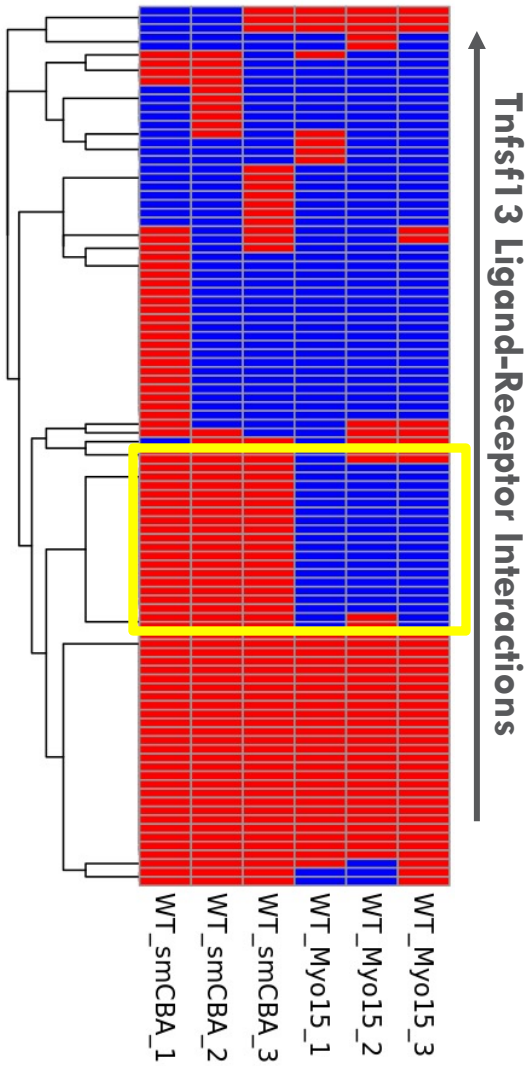
Ligand-Receptor Interactions Analysis Points to Elevated Tnf-Related Apoptosis Pathway Activity with AAV-smCBA

- Condition-specific ligand-receptor interactions were identified by selecting those significant in all 3 samples in one group, and not in any of the other groups' samples

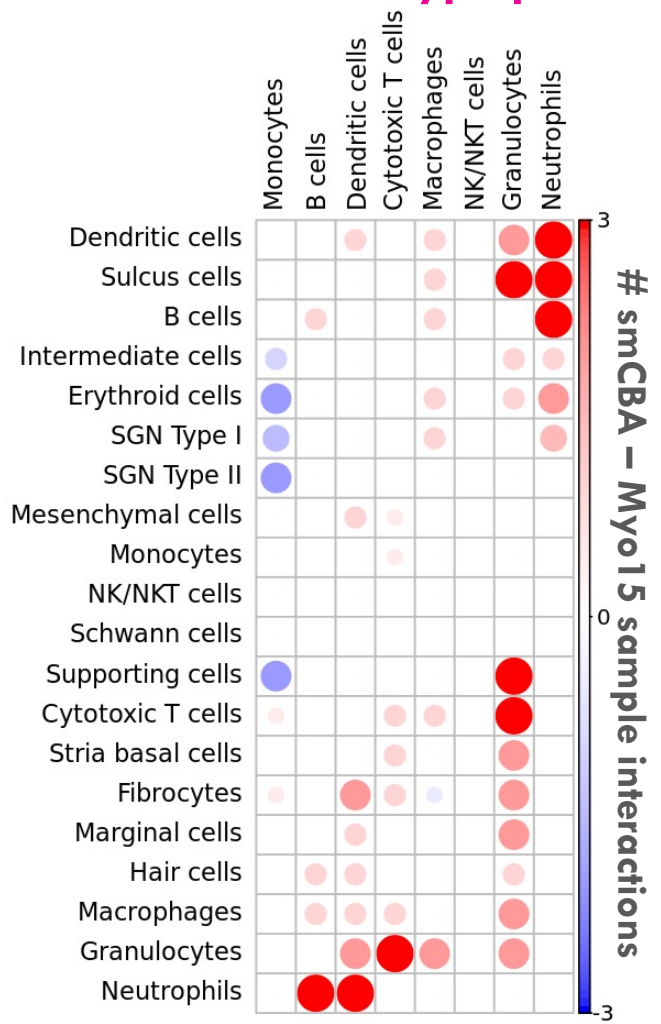
- Many of the smCBA-specific interactions involved Tnfrsf genes, which could indicate elevated apoptosis pathway activity. The heatmap on the left illustrates this by showing the significance of Tnfrsf13-mediated interactions (rows) across samples (columns, red: q-val<0.05). The yellow rectangle highlights smCBA-specific interactions.

- Analysis of cell types involved appears to primarily implicate neutrophils and granulocytes. The figure on the right shows, for each cell type pair, the difference in smCBA vs Myo15-injected samples with a significant Tnfrsf-mediated ligand-receptor interaction.

Significant ligand-receptor interactions across samples



Distribution of interactions across cell type pairs



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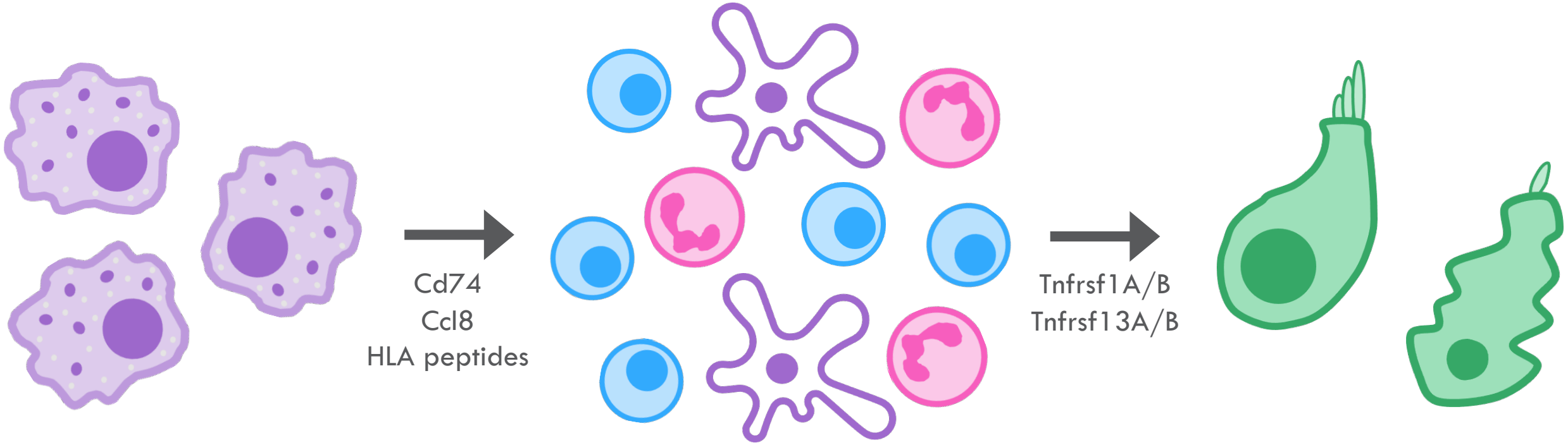
Graphical Summary



Acute response led by macrophage activation

Immune cell recruitment to the cochlea

Immune cell-mediated apoptosis pathway activation leading to tissue damage

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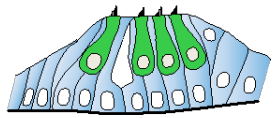
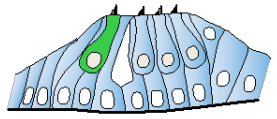
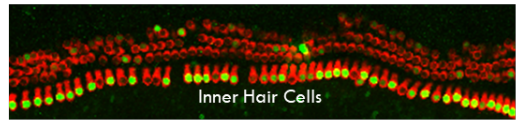
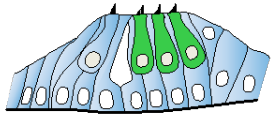
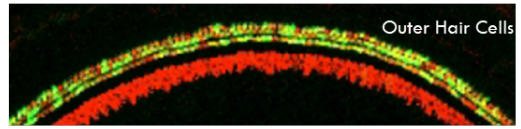
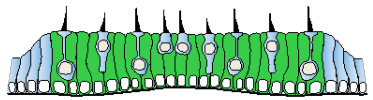
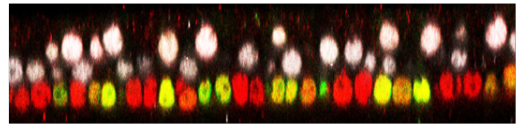
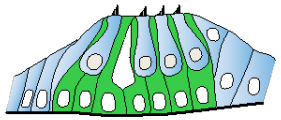
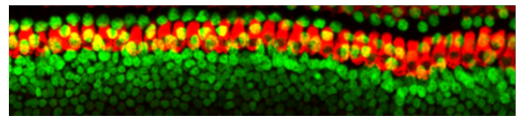
Summary & Future Directions: Working Towards Cell-Selective Expression for Inner Ear Gene Therapies

- The use of **smCBA ubiquitous promoters** for OTOF gene therapy leads to a **lack of durable recovery** due to **IHC loss**

- RNA-seq data suggest this could be caused by the mounting of a **macrophage-mediated immune response**, leading to further **immune cell recruitment** and the activation of **TNF apoptosis mechanisms**. We cannot, however, exclude that differences in transgene expression levels, vs promoter specificity, lead or contribute to the observed IHC loss.

- **Restricting expression** of AAVs to target cell types has the potential to **improve safety margins** and **improve outcome measures** and should be considered essential for gene therapy as a treatment modality in the ear

Cell Type Targeted by Our Cell-Selective Promoter		Cell-Selective Expression
	All Hair Cells	
	Inner Hair Cells	
	Outer Hair Cells	
	Vestibular Supporting Cells	
	Cochlear Supporting Cells	

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