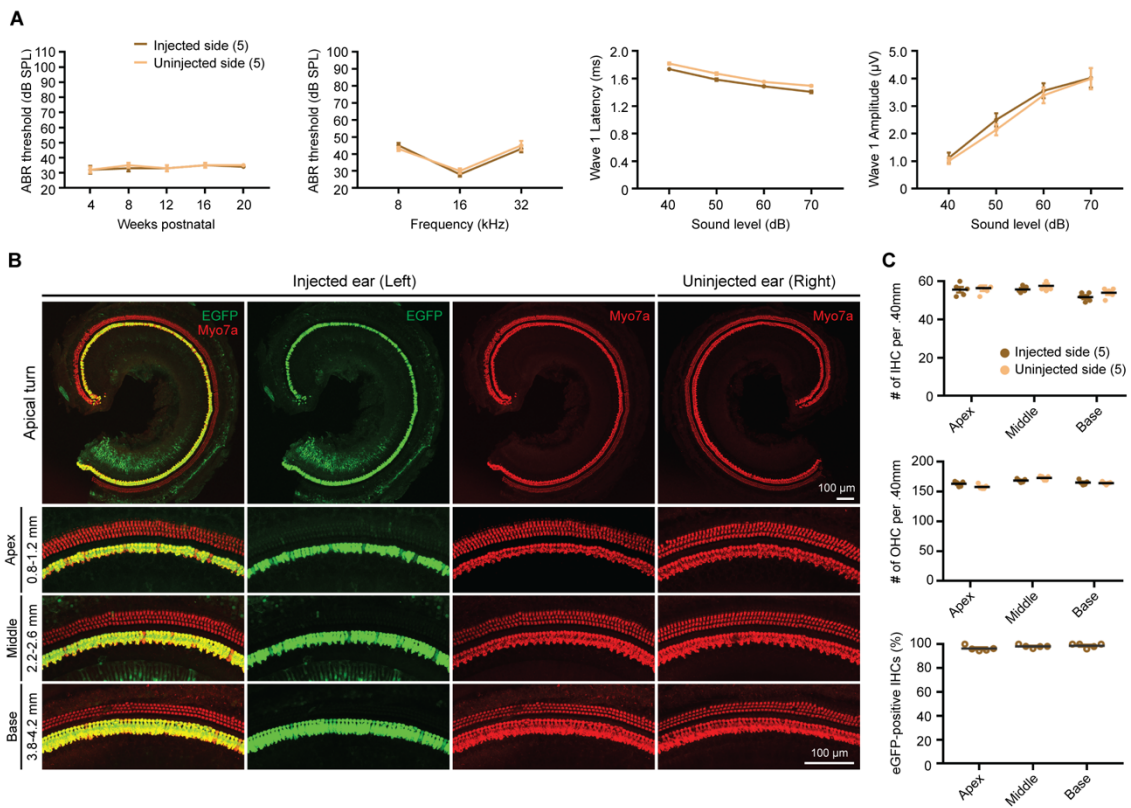


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## **Supplemental Information**

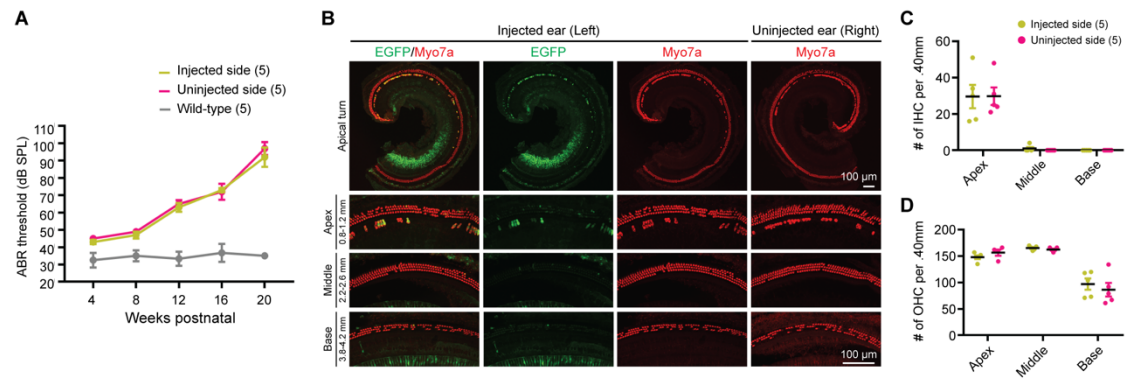
### **Targeted Allele Suppression Prevents Progressive Hearing Loss in the Mature Murine Model of Human *TMC1* Deafness**

**Hidekane Yoshimura, Seiji B. Shibata, Paul T. Ranum, Hideaki Moteki, and Richard J.H. Smith**



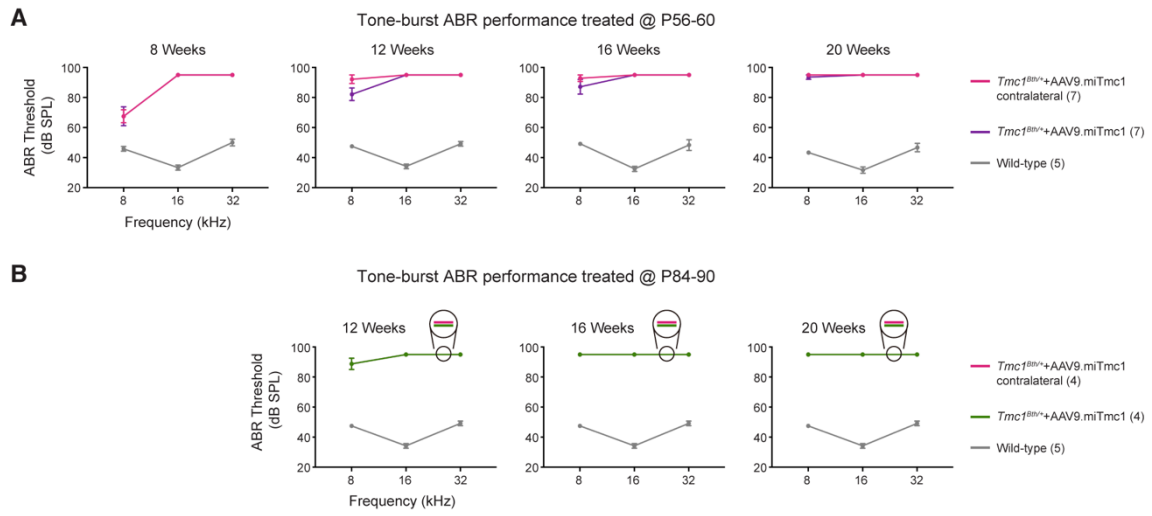
**Figure S1. AAV9.miTmc delivery in wild-type cochlea showing robust long-term transduction without IHC and OHC losses and auditory dysfunction.**

- (A) Click-evoked ABR thresholds, Peak 1 latencies and amplitudes in AAV9.miTmc1-injected and uninjected ears in wild-type mice at 20 weeks of age. Data are means  $\pm$  SEM.
- (B) Cochlear whole-mount images of AAV9.miTmc1-injected and uninjected ears in wild-type mice sacrificed at 20 weeks. Representative low magnification images of whole-mount apical turns and high magnification images of each turn of the cochlea. Distance from the apical tip is indicated.
- (C) Quantitative comparison of IHC and OHC survival and IHC transduction efficiency in AAV9.miTmc1-injected and uninjected ears in wild-type mice assessed in 400  $\mu$ m segments across different regions of the cochlea (apex, middle and base). Data are means  $\pm$  S.E.M.



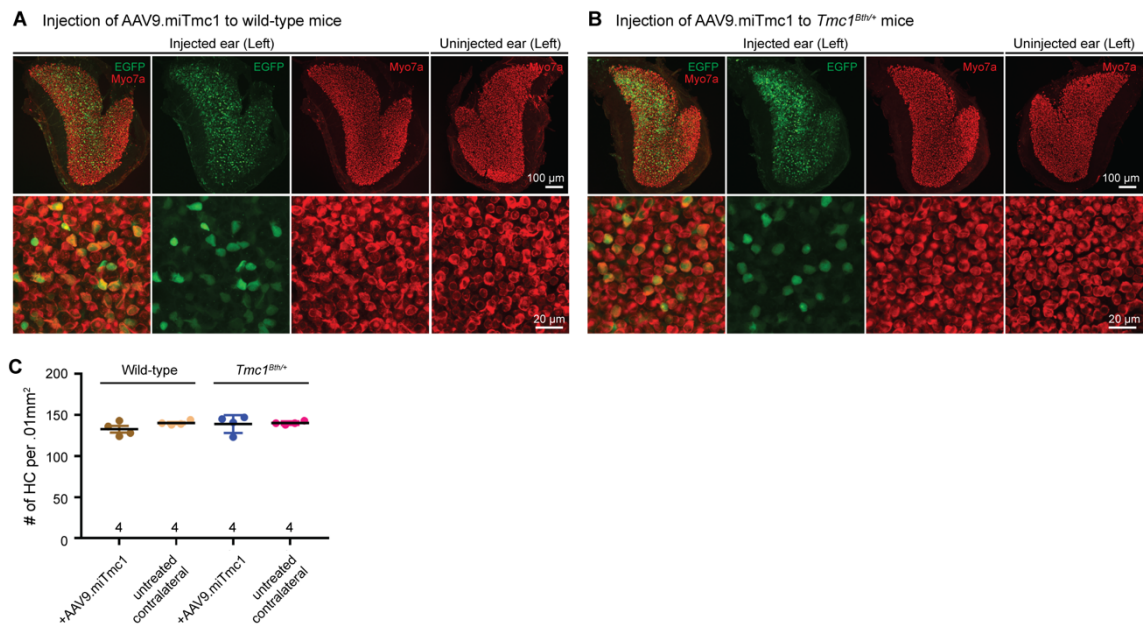
**Figure S2. Auditory function and morphology of *Tmc1<sup>Bth/+</sup>*+AAV9.miSafe injected ears at P15-16 are indistinguishable from that in *Tmc1<sup>Bth/+</sup>* untreated contralateral ears.**

- (A) Click ABR thresholds recorded longitudinally from 4 to 20 weeks in *Tmc1<sup>Bth/+</sup>* untreated contralateral, *Tmc1<sup>Bth/+</sup>*+AAV9.miSafe animals.
- (B) Cochlear whole-mount images of *Tmc1<sup>Bth/+</sup>*+AAV9.miSafe treated at P15-16 and *Tmc1<sup>Bth/+</sup>* untreated contralateral animals sacrificed at 20 weeks. Representative low magnification images of apical turns and high magnification images of each turn of the cochlea. Distance from the apical tip is indicated.
- (C, D) Quantitative comparison of IHC (C) and OHC (D) survival in *Tmc1<sup>Bth/+</sup>*+AAV9.miSafe and *Tmc1<sup>Bth/+</sup>* untreated contralateral animals assessed in 400  $\mu$ m segments across different regions of the cochlea (apex, middle and base). Data are means  $\pm$  S.E.M.



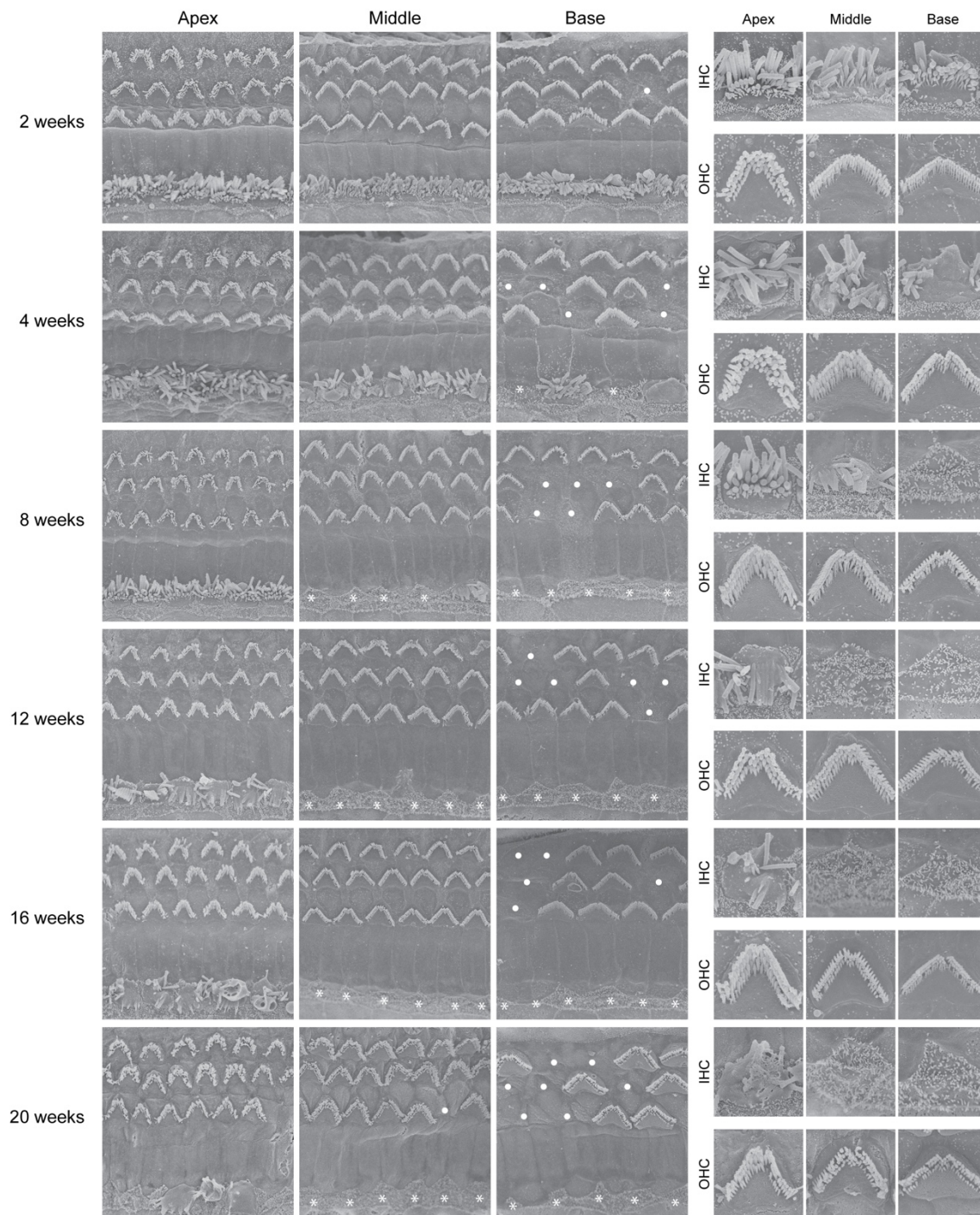
**Figure S3. Frequency-specific ABR thresholds in the P56-60 and P84-90 treatment groups.**

(A, B) Tone-burst ABRs at 8, 12, 16 and 20 weeks in wild-type mice, *Tmc1<sup>Bth/+</sup>* untreated contralateral ears, and *Tmc1<sup>Bth/+</sup>*+AAV9.miTmc1 ears treated at P56-60 (A) and P84-90 (B). Data are means  $\pm$  S.E.M.



**Figure S4. Hair cell survival in vestibular organ of wild-type and *Tmc1<sup>Bth/+</sup>* mice with and without cochlear transgene delivery**

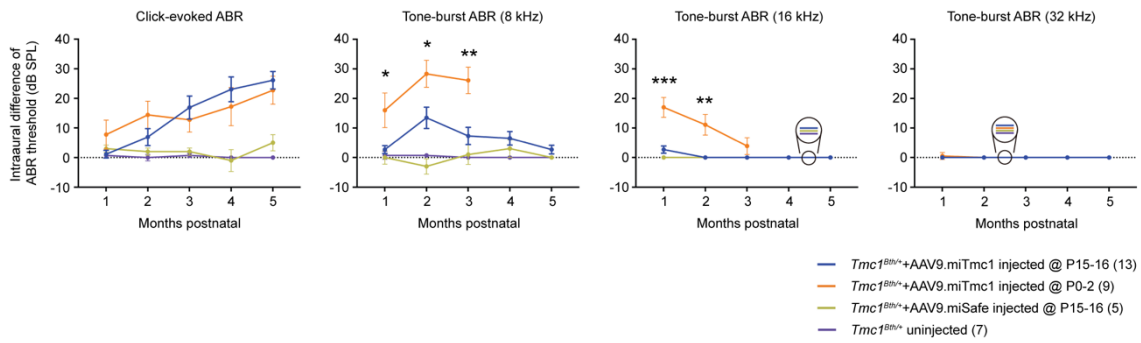
- (A, B) Representative whole-mount images of the saccule of wild-type (A) and *Tmc1<sup>Bth/+</sup>* (B) mice sacrificed at 20 weeks of age. High-magnification images show transduced HCs (eGFP-positive; AAV transduction).
- (C) Quantitative comparison of HC survival in wild-type and *Tmc1<sup>Bth/+</sup>* animals with and without the injection of AAV9.miTmc1.



**Figure S5. Scanning electron micrographs of *Tmc1<sup>Bth/+</sup>* mice of the organ of Corti.**

Representative SEM images of the organ of Corti at the apex, middle and base obtained from untreated *Tmc1<sup>Bth/+</sup>* mice at 2, 4, 8, 12, 16 and 20 weeks of age. Asterisk and circles show IHC and OHC losses, respectively.





**Figure S6. Click and tone-burst ABR thresholds.**

Click and tone-burst ABR thresholds are shown as the intra-aural differences between ears in *Tmc1<sup>Bth/+</sup>*+AAV9.miTmc1 animals injected at P0-2<sup>7</sup> and P15-16, *Tmc1<sup>Bth/+</sup>*+AAV9.miSafe animals injected at P15-16, and in *Tmc1<sup>Bth/+</sup>* uninjected controls (\*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \* $P < 0.05$ ; Student's *t*-test). In the *Tmc1<sup>Bth/+</sup>*+AAV9.miSafe animals and in *Tmc1<sup>Bth/+</sup>* uninjected controls, there is no difference in ABR thresholds between ears and hence the intra-aural difference is zero. The maximum intra-aural difference observed is seen in *Tmc1<sup>Bth/+</sup>*+AAV9.miTmc1 animals injected at P0-2. These results show that phenotypic rescue as measured by tone-burst ABR is directly related to the age of the animal at the time of injection.