SUPPLEMENTAL DATA

SUPPLEMENTAL METHODS

Auditory-evoked brainstem response (ABR)

Hearing assessment was performed as previously described (1). Mice were anesthetized with tribromoethanol, and then placed on a heating pad in a sound-attenuating chamber. Needle electrodes were placed just under the skin, with the active electrode placed between the ears just above the vertex of the skull, the ground electrode between the eyes, and the reference electrode underneath the left ear. High frequency transducers were placed just inside the ear canal and computer-generated sound stimuli were presented at defined intervals. Thresholds were determined for a broad-band click stimulus and for 8, 16 and 32 kHz pure-tone stimuli by increasing the sound pressure level (SPL) in 10 dB increments followed by 5 dB increases and decreases to determine the lowest level at which a distinct ABR wave pattern could be recognized. Stimulus presentation and data acquisition were performed using the Smart EP evoked potential system (Intelligent Hearing Systems, Miami, FL).

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. The *nmf333* mutation of *Cyba* does not influence hearing. (**A**) Representative ABR waveforms are shown for A.B6-Tyr^{+}/J (WT), heterozygous (*nmf333*/+), and *nmf333/333* mice exposed to broad-band click stimuli at 50, 55, 60, 70, 80, 90, and 100 dB sound pressure levels (SPL). Thresholds for all three genotypes are similar. (**B**) Statistical analysis of auditory-evoked brain stem response thresholds. Mean ABR thresholds (dB SPL) are shown for wild-type (○, n=5), *nmf333*/+ (□, n=7), and *nmf333/nmf333* (■, n=9) mice for broad-band click, 8 kHz, 16 kHz, and 32 kHz frequencies. Error bars are ± 1 S.D. Mice with ABR thresholds above 55 dB SPL (click and 8 kHz stimuli), 35 dB SPL (16 kHz stimulus), and

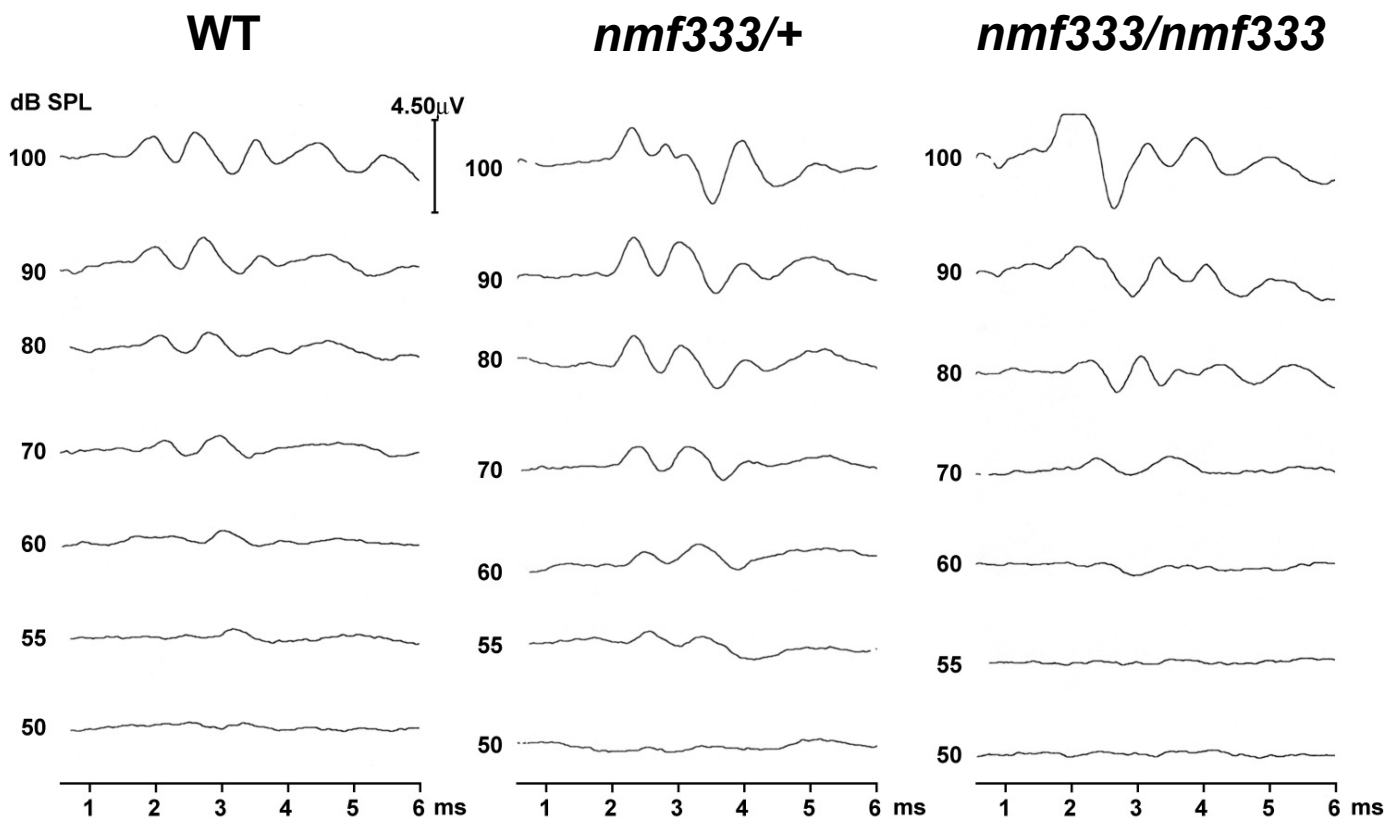
60 dB SPL (32 kHz stimulus) are considered hearing-impaired. Mice with ABR thresholds above 100 dB SPL are considered deaf. Audiograms for each of the three genotypes are very similar. All three genotypes are hearing impaired as expected in the A.B6-Tyr^{+} genetic background.

SUPPLEMENTAL REFERENCES

1. Zheng, Q.Y., Johnson, K.R., and Erway, L.C. 1999. Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses. *Hear. Res.* **130**:94-107.

Supplemental Figure 1

A



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