Supplementary Materials

Supplementary results

Cisplatin induces a dose dependent ABR threshold shift which was prevented by HNK

With a dosage of 15 mg/kg, apparent ABR threshold shift occurred on Day 7 at 32 kHz (Figure S1A, the blue triangles). By Day 14, ABR threshold elevation was even more significant at 32 kHz (green diamonds, $p<0.001$, one-way ANOVA, same in the following). Note that the number of animals was less over time because some were removed from the studies during the treatment due to health issue. When the animals were pre-treated with honokiol (HNK) with a total dose of 20 mg/kg, the ABR threshold elevation induced by cisplatin was significantly reduced (Figure S1B). No significant ABR elevations were measured up to Day14 ($p=0.35$). ABR thresholds on Day 0 for controls (C0H0, Day 0 and Day 14) and all other experimental groups are plotted in Figure S1C. The ABR thresholds on Day 14 (D14) of different treatment groups are also plotted in Figure S1D (D7 for the animals treated with cisplatin 20 mg/kg, C20H0) for direct comparison.

Cisplatin induces a significant DPOAE loss which was prevented by HNK pre-treatment

A typical example of DPOAE measurements is shown in Figure S2A, where the amplitude of the cubic distortion product ($2f_1$-f_2) was plotted as a function of the frequency of f_2. Cisplatin treatment (15 mg/kg) induced a reduction in DPOAE magnitude at high frequencies between 17.8 kHz to 35.6 kHz on Day 7 (the blue curve). No further changes were observed on Day 14 (the green curve). The changes of DPOAE magnitude of the 2 animals treated with cisplatin alone (15 mg/kg) and the averaged changes are normalized to the baseline and plotted in Figure S2B. A 30-48 dB amplitude decrease was observed from 17.8 to 35.6 kHz. Treatment with HNK alone (20 mg/kg) did not affect DPOAE magnitude (Figure S2C). When the animals were pre-treated with HNK, the decrease of DPOAE magnitude was largely prevented (Figure S2D and S2E). A slight drop was observed in the animals pre-treated with HNK 10 mg/kg HNK, with a decrease of 10-15 dB at frequencies higher than 27.0 kHz (Figure S2D). In the animals pre-treated with HNK 20 mg/kg, no apparent decrease of DPOAE magnitude was observed at all frequencies (Figure S2E).

Supplementary materials and methods

Distortion product otoacoustic emission (DPOAE) measurement

DPOAEs were evoked by paired tones presented at 70 dB SPL and with a frequency ratio of $f_1/f_2=1.2$. The amplitude of $2f_1$-f_2 was measured pre- and post-treatment. Because the details of these procedures are published elsewhere [1, 2], we present here an abbreviated version. Animals were again sedated with ketamine/xylazine and their temperature maintained. The emission probe was placed in the ear canal forming a tight seal. After calibration was verified, iso-input functions were collected for f_2 frequencies between 3.8 and 35.6 kHz for cisplatin and HNK alone, as well as for the various combinations of cisplatin and HNK. Each acoustic stimulus was repeated 3000 times.

Supplementary references

[1] Cheatham MA, Goodyear RJ, Homma K, Legan PK, Korchagina J, Naskar S, Siegel JH, Dallos P, Zheng J and Richardson GP. Loss of the tectorial membrane protein CEACAM16 enhances spontaneous, stimulus-frequency, and transiently evoked otoacoustic emissions. J Neurosci 2014; 34: 10325-10338.

[2] Cheatham MA, Ahmad A, Zhou Y, Goodyear RJ, Dallos P and Richardson GP. Increased spontaneous otoacoustic emissions in mice with a detached tectorial membrane. J Assoc Res Otolaryngol 2016; 17: 81-88.

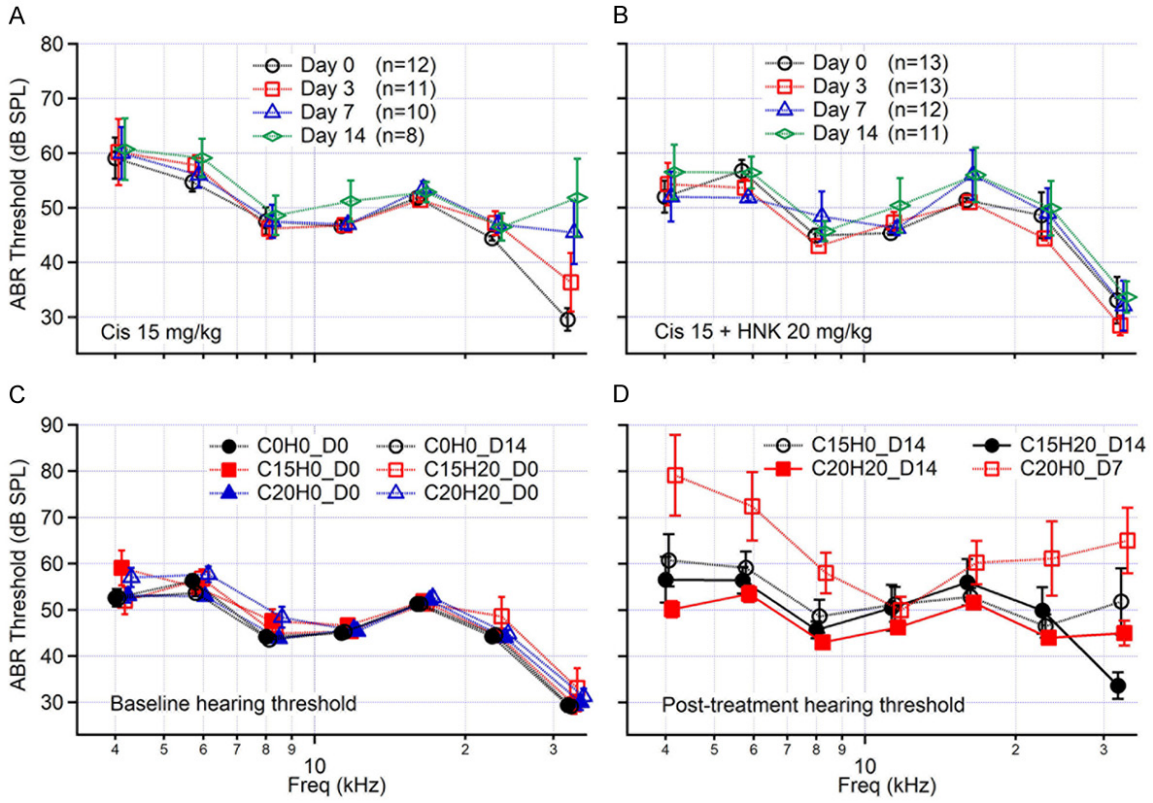

Figure S1. ABR threshold shift in cisplatin and HNK treatment. A. ABR threshold changes in Cisplatin 15 mg/kg treatment group. An ABR threshold elevation was induced at 32 kHz. B. HNK 20 mg/kg pre-treatment prevented the threshold shift induced by cisplatin 15 mg/kg. C. ABR threshold baseline (on Day 0) of all the testing groups. No significant difference was observed across different experimental groups. D. A direct comparison of ABR threshold shift on Day 14 among different cisplatin (15 and 20 mg/kg) and HNK (0 and 20 mg/kg) groups. Note that cisplatin 20 mg/kg group (C20H0) only has the data on Day 7 because most animals were removed from the study after day 8. Data represent mean ± SEM.

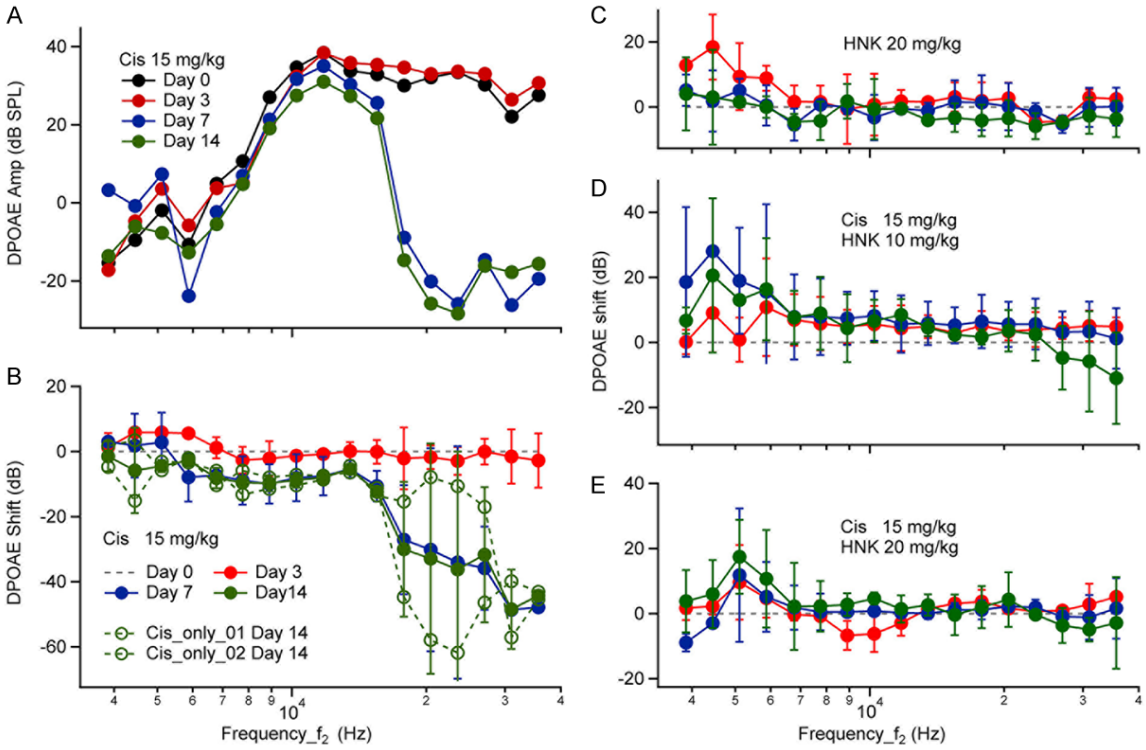

Figure S2. Effects of cisplatin and HNK treatment on DPOAEs. (A) An example of DPOAE amplitude changes after cisplatin 15 mg/kg treatment. An DPOAE amplitude decrease on Day 7 at frequencies over 16 kHz. (B-E) The DPOAE amplitude shift of all the treatment groups, normalized to Day 0. The dashed lines and open circles in (B) are the results of the two animals on Day 14, to show the individual variation in terms of frequency range and amplitude. Data represent mean ± SEM.

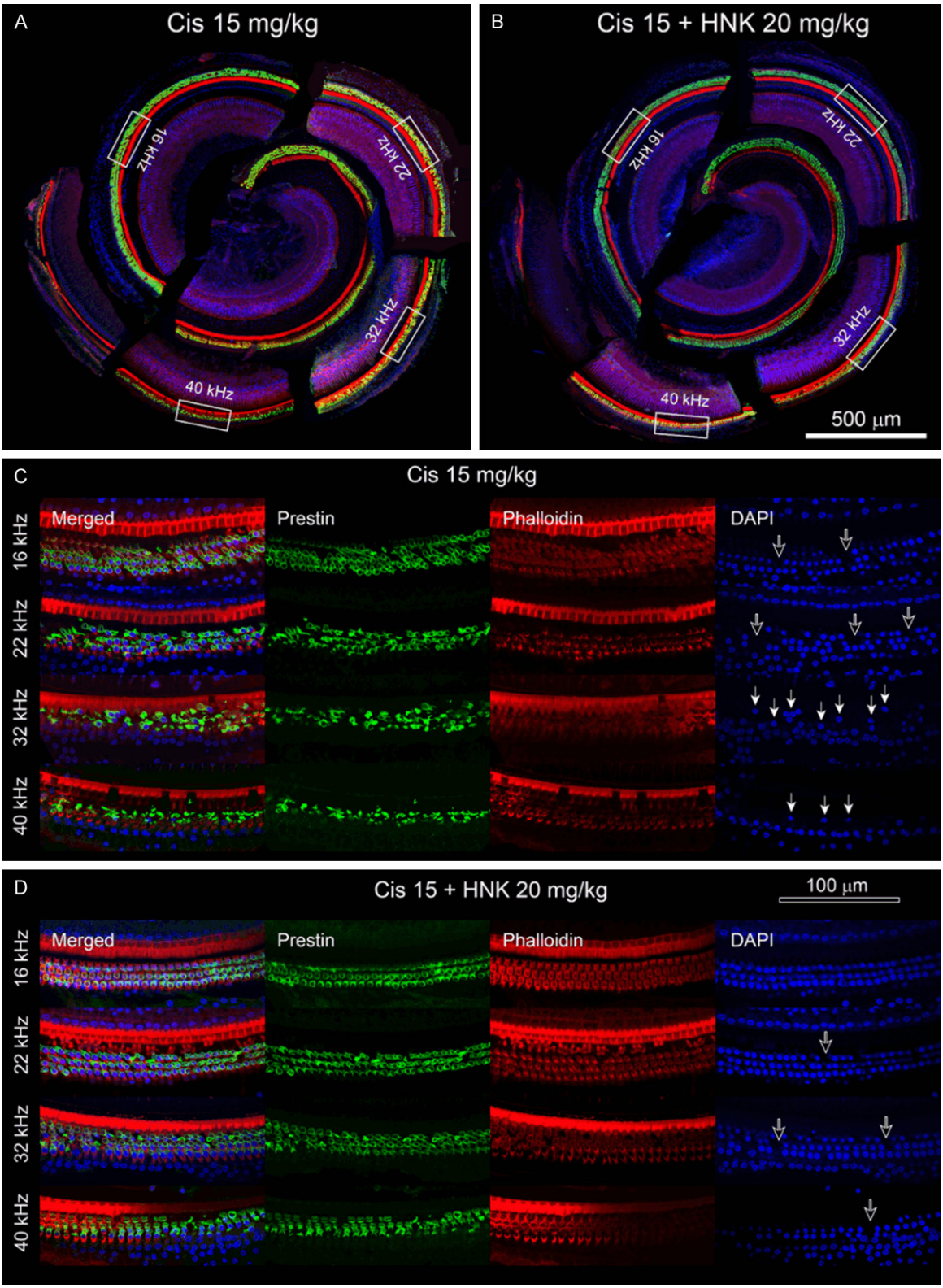

Figure S3. Confocal images of the cochlear whole mount showing the changes with different treatments. The full length of the coil was cut into 5 segments (S1-S5, from apex to base), and S3 was cut again into half right before the mounting. (A) Cochlea treated with cisplatin alone (15 mg/kg), and (B) treated with Cis 15 + HNK 20 mg/kg, under 20 × subjective. High resolution images (60 × subjective) taken at the locations marked by white squares in (A) and (B) are shown in (C) and (D), respectively. Hollow arrows: missing OHCs; white arrows: nuclei of remaining OHCs. The preparations are immunostained with Prestin (N-terminal, for outer hair cells), Phalloidin (for hair bundles) and DAPI (for nuclei).

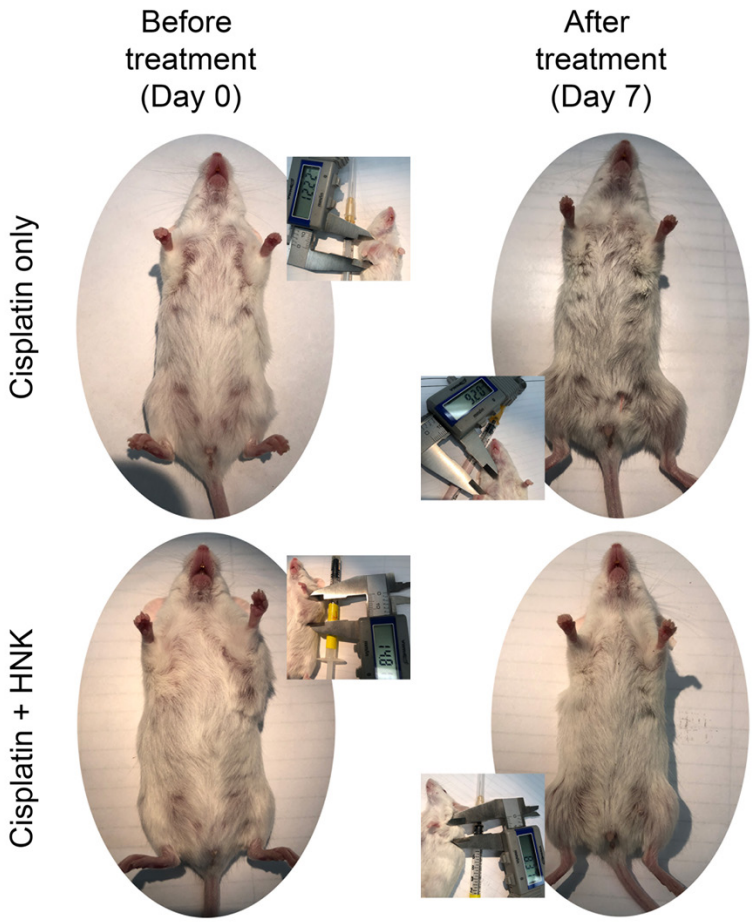

Figure S4. Tumor growth and size measurement in MMTV-PyMT mice before (Day 0) and after (Day 7) cisplatin and cisplatin + HNK treatment.

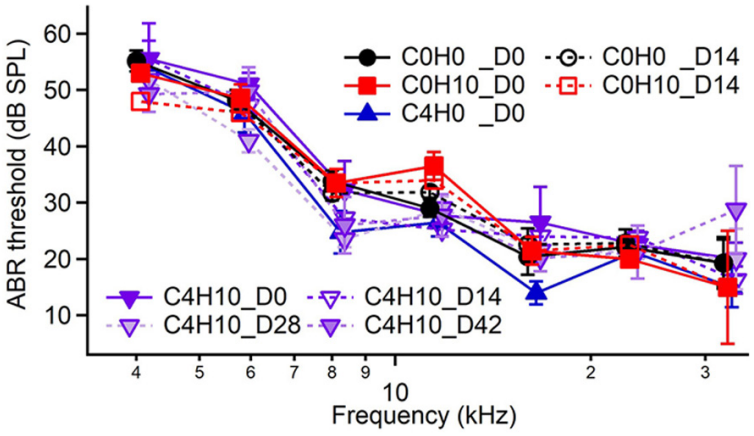

Figure S5. Supplementary ABR threshold of the MMTV-PyMT mice before and after different treatments. C0H0: control group without cisplatin and HNK; C0H10: HNK only (10 mg/kg/day) group; C4H10: cisplatin (4 mg/kg/day) + HNK (10 mg/kg/day) group. D0: Day 0, and so on. Data represent mean ± SEM.