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Supplemental information

Specific knockdown of *Htra2* by CRISPR-CasRx prevents acquired sensorineural hearing loss in mice

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Supplementary information

Supplemental text

Guide-RNA expressing plasmid: gRNA sequences are colored in blue

GCTAGCGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAA
GGCTGTTAGAGAGATAATTGGAATTAATTTGACTGTAAACACAAAGATATTA
GTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTT
TAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTAT
TTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGCAAGTA
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TCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACA

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CGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGA

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GGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTG

GCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGAAGCCGCGGTA

TCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTA

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ACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAA

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CGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCA

RfxCas13d-mCherry plasmid: RfxCas13d and mCherry are colored in blue and red respectively

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ATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCC
ATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTC
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CATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTA
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CCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTT
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ACATTAACCTATAAAAATCAAATAATGATTTTATTTTGACTGATAGTGACCTG
TTCGTTGCAACAAATTGATGAGCAATGCTTTTTTATAATGCCAACTTTGTAC
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GGGTGCCAGGGCGTGCCCTTGGGCTCCCCGGGCGCGTACTCCACCTCACC
CATCTGGTCCATCATGATGAACGGGTCGAGGTGGCGGTAGTTGATCCCGGC

Htra2-EGFP plasmid: the sequence of Htra2 and EGFP are colored in blue and green respectively

GACGGATCGGGAGATCTCCCGATCCCCTATGGTGCACTCTCAGTACAATCT
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TGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGC
TTCGCGATGTACGGGCCAGATATACGCGTTGACATTGATTATTGACTAGTTAT
TAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCG
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Supplemental figures

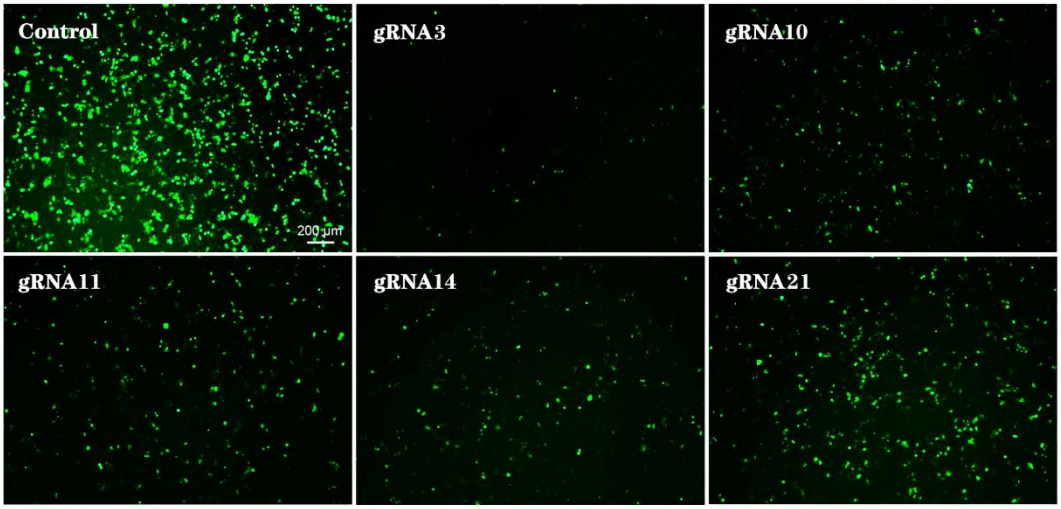

Figure S1. The fluorescent images of HEK293T-cells at 48 hours after transfection with CasRx-mCherry plasmid, Htra2-EGFP plasmid, and the top five gRNAs of high knockdown efficiency. Scale bar: 200 μm.

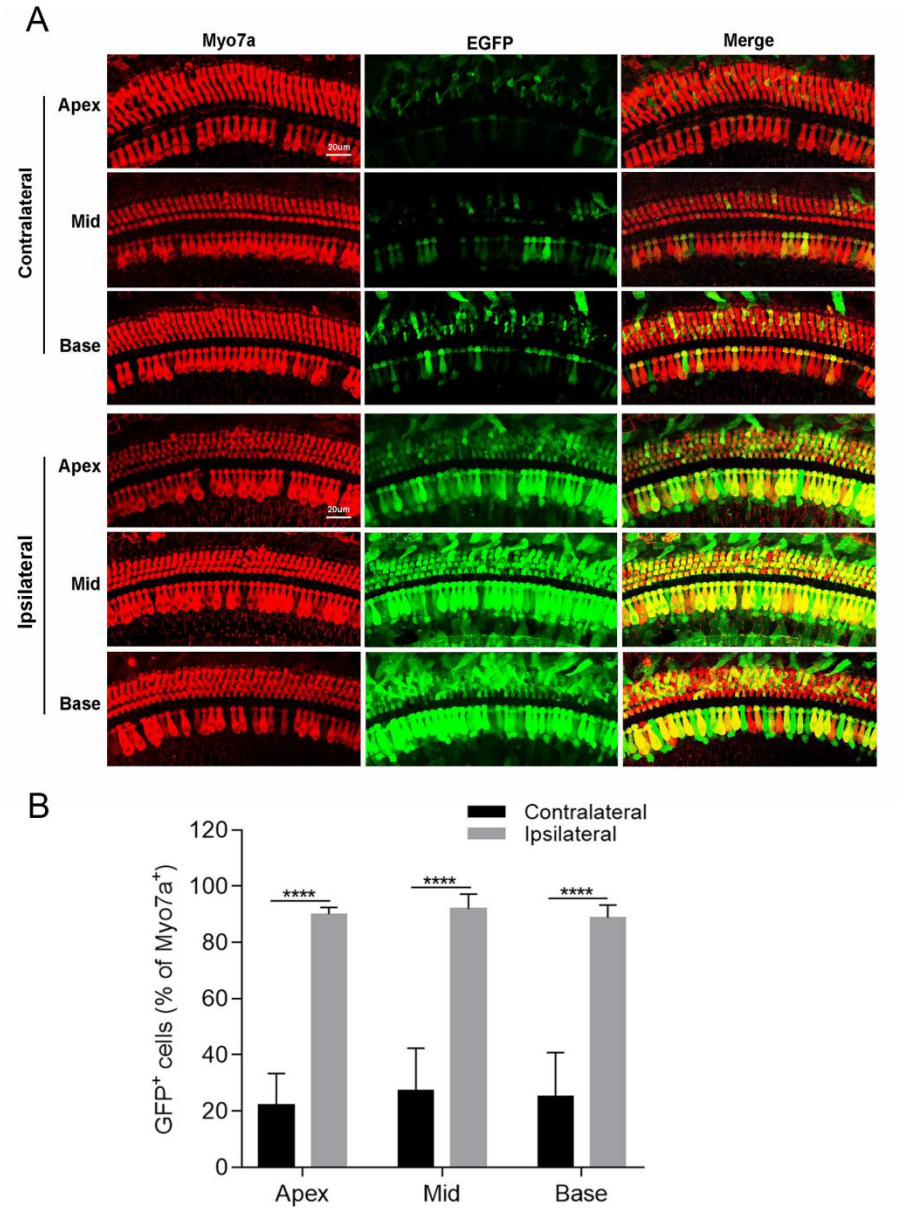

Figure S2. The transduction efficiency of AAV-PHP.eB-EGFP in cochlear hair cells. (A) The cochleae from both ears were dissected for immunostaining with Myo7a (red) and EGFP (green) antibodies. The respective confocal images were taken in the apical, middle, and basal turns of AAV-injected and non-injected ears. Scale bar = 20 μm. Ipsilateral: the cochleae with AAV-PHP.eB-EGFP injection; contralateral: the cochleae without AAV-PHP.eB-EGFP injection. (B) The percentage of EGFP positive cells per 100 μm sensory hair cells in apex, mid and base turns in AAV-PHP.eB-EGFP-injected cochleae. Data were presented as the mean ± SD. Unpaired two-tailed Student's *t*-test was used for statistical analysis, ****$p < 0.0001$ *vs* contralateral.

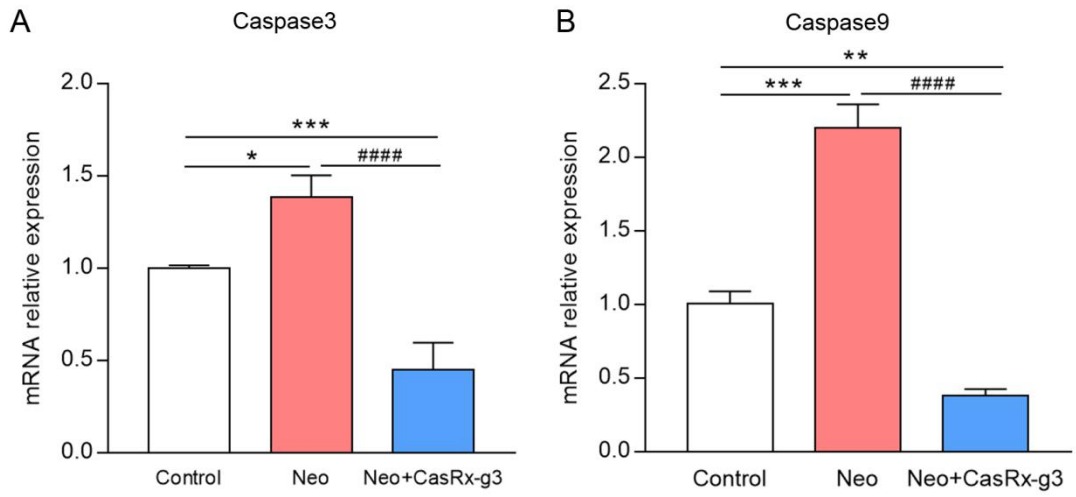

Figure S3. The expression of apoptosis-related genes in HEI-OC1 cells. The relative mRNA expression of *Caspase3* (A) and *Caspase9* (B) in HEI-OC1 cells. Three groups were shown in the graph including HEI-OC1 cells without any treatments (Control), the neomycin-treated group (Neo), and the neomycin+CasRx-g3-treated group (Neo+CasRx-g3). Each group had more than three biologically independent samples. Statistical analysis was performed by One-way ANOVA with Bonferroni's multiple comparisons test. All data were presented as the mean ± SD. *, **,***$p < 0.05, 0.01, 0.001$ *vs* control; $^{\#\#\#\#}p < 0.0001$ *vs* Neo.

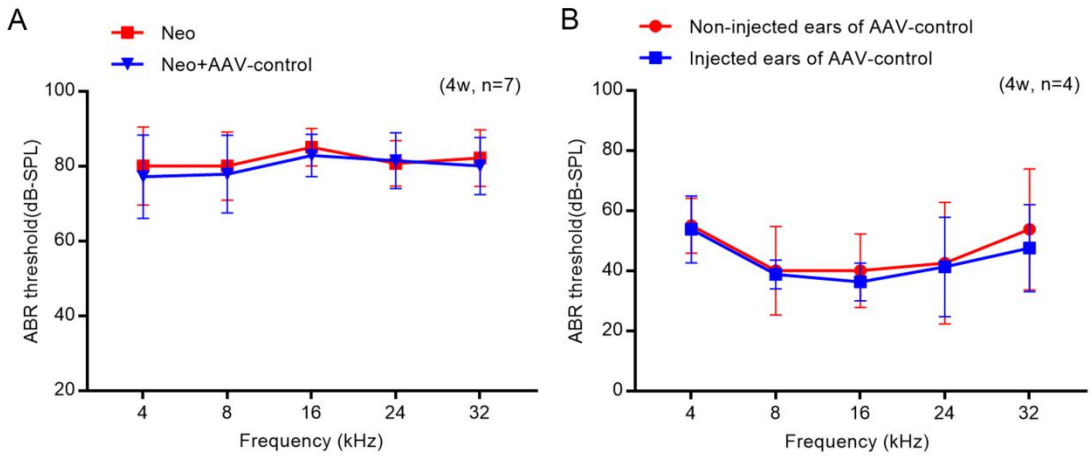

Figure S4. The ABR thresholds of both ears after AAV-control injection with or without neomycin exposure. (A) The ABR thresholds of the contralateral and ipsilateral ears were recorded after the treatment of AAV-control with neomycin exposure and were from 70 dB to 90 dB. (B) The ABR thresholds of the contralateral and ipsilateral ears were recorded after the treatment of AAV-control without neomycin exposure. The ABR thresholds were from 30 dB to 60 dB, and no significant differences were seen between the two sides.

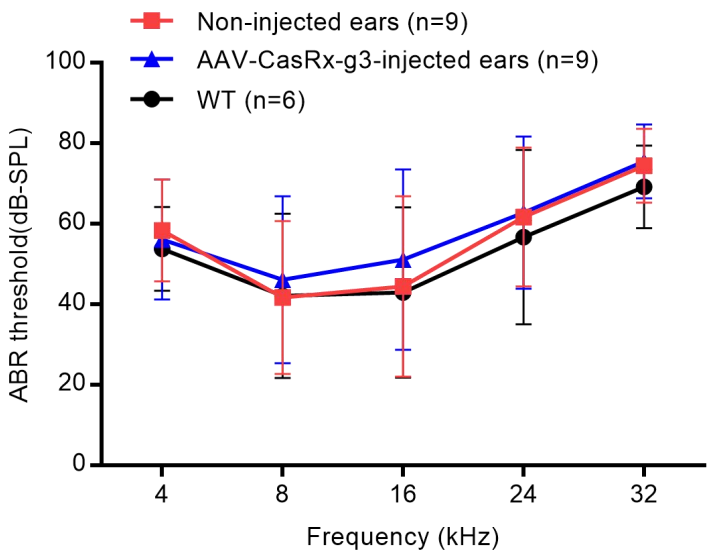

Figure S5. The ABR thresholds of 10-week mice. The ABR thresholds of the bilateral ears were recorded after the treatment of AAV-CasRx-g3 without neomycin exposure, which showed no significant difference with that of WT mice at all frequencies. All data were presented as the mean ± SD.

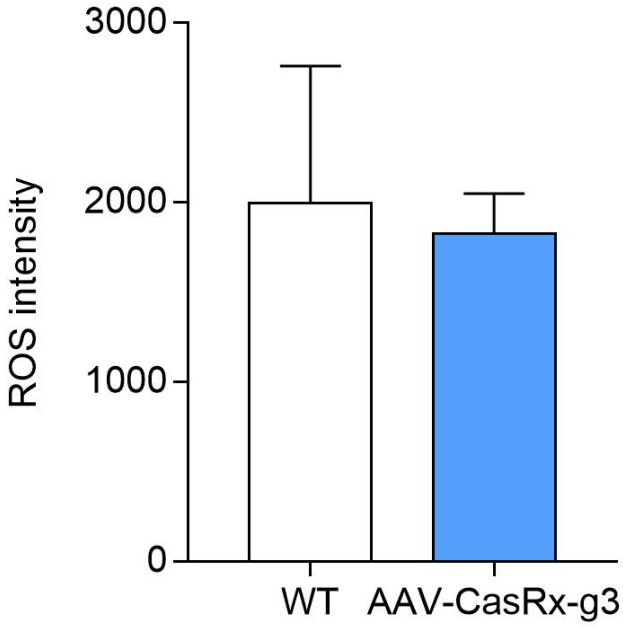

Figure S6. The ROS intensity in cochleae. The cochleae from 10-week mice were collected and prepared for ROS activity according to the ROS detection kit (Biolab Technology Co., Ltd. Beijing). The O12 probe was co-incubated with samples for 30 min, and the fluorescence in 530nm wavelength was examined. The ROS intensity was determined by the fluorescence per microgram protein. No significant difference in ROS intensity between WT and AAV-CasRx-injected mice. Each group had five biologically independent samples(n=5), data were presented as the mean ± SD.

Supplemental tables

Table S1. Guide sequences for *Htra2* mRNA targeting

Targets	Sequences
gRNA1	5' TCCCCACATTCAATCGTGCCCAGAGATCCG 3’
gRNA2	5' TGAAGTCCCCACATTCAATCGTGCCCAGAG 3'
gRNA3	5' CCCACATTCAATCGTGCCCAGAGATCCGGG 3'
gRNA4	5' CTTCCAGGGCTCCTGCGGGCCTCCTGGTCA 3’
gRNA5	5' GATCTCGATATAGACCACAGCAGGGGCTGT 3’
gRNA6	5' CCACTACGAATCCTGATCCGTTTGAGATGG 3’
gRNA7	5' TGGGCGTTGGTAACGATGAGCCCATCTGAA 3’
gRNA8	5' AGCTGTGACCATGGCCTCATAAGTATCCCC 3’
gRNA9	5' AATCCTCAGTGTGGCAATGTCTGCTACGGG 3’
gRNA10	5' GCAACAACAAACTCCCCTTGCCGGACATCA 3’
gRNA11	5' GGCTTCCCATGGCAACAACAAACTCCCCTT 3’
gRNA12	5' CATGGCAACAACAAACTCCCCTTGCCGGAC 3’
gRNA13	5' GGTTAACCAGGGGACCACCAGAATTTCCAA 3’
gRNA14	5' CCAGAATTTCCAAAATCAATAGCTGCATCG 3’
gRNA15	5' TTCCAAAATCAATAGCTGCATCGGTCTGAA 3’
gRNA16	5' AAGGCGATCAGAAGGGATGGCAAAGGAGAT 3’
gRNA17	5' AGTCAGGGTCAGCATCATCACTCCAATGTA 3’
gRNA18	5' ACCATGCTGAACATCAGGGAAGCTTGGCTC 3’
gRNA19	5' TGCGGGGGAGCCCAGGATAACTTTATGAAT 3’
gRNA20	5' TAACTTTATGAATGAGGACACCATGCTGAA 3’
gRNA21	5' GCCAAGATCACATCACCAGGCCGCAGACCA 3’
gRNA22	5' ATAAACATCTTCAGCATTTTGTGCCAATTT 3’
gRNA23	5' TAAGGTCAGTGTCTCTGATCCGCGCCGGAT 3’

Table S2. DEGs between Neo and AAV-CasRx-g3 groups