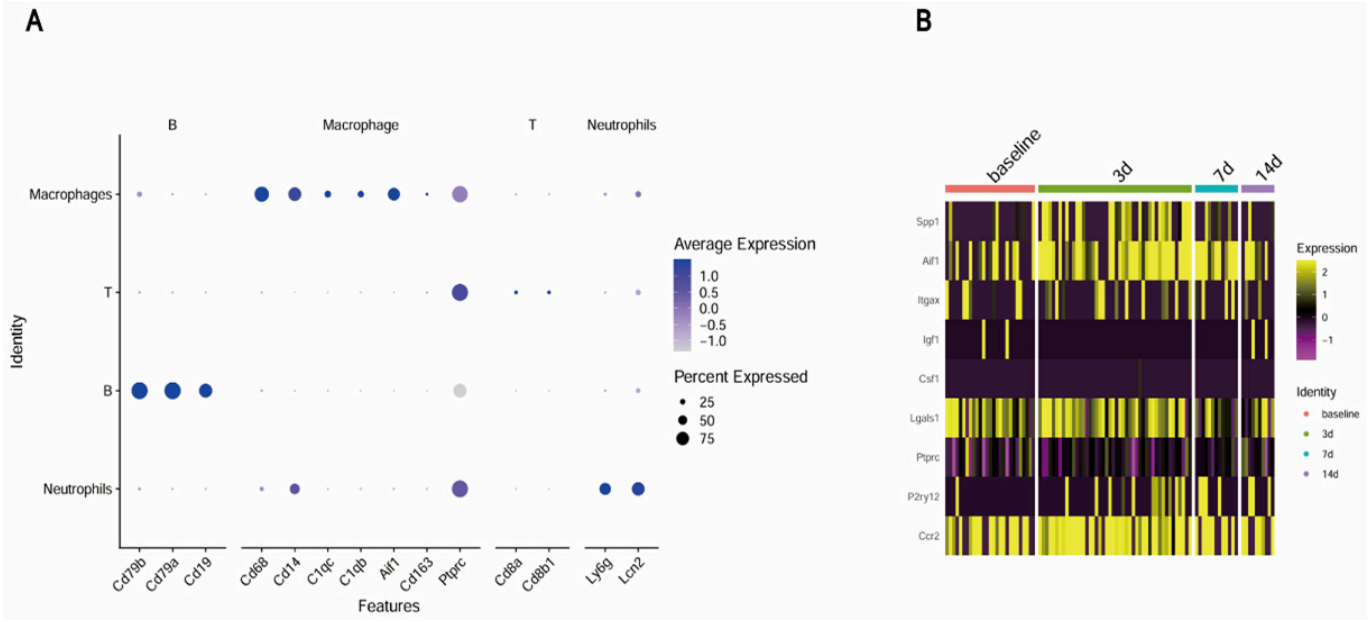


Fig. S1. Bubble plot of cell marker genes and heatmap of macrophage origin-related genes.

(A) Dot plot showing marker gene expression for B cells, macrophages, T cells, and neutrophils. (B) Heatmap of gene expression related to macrophage origin.

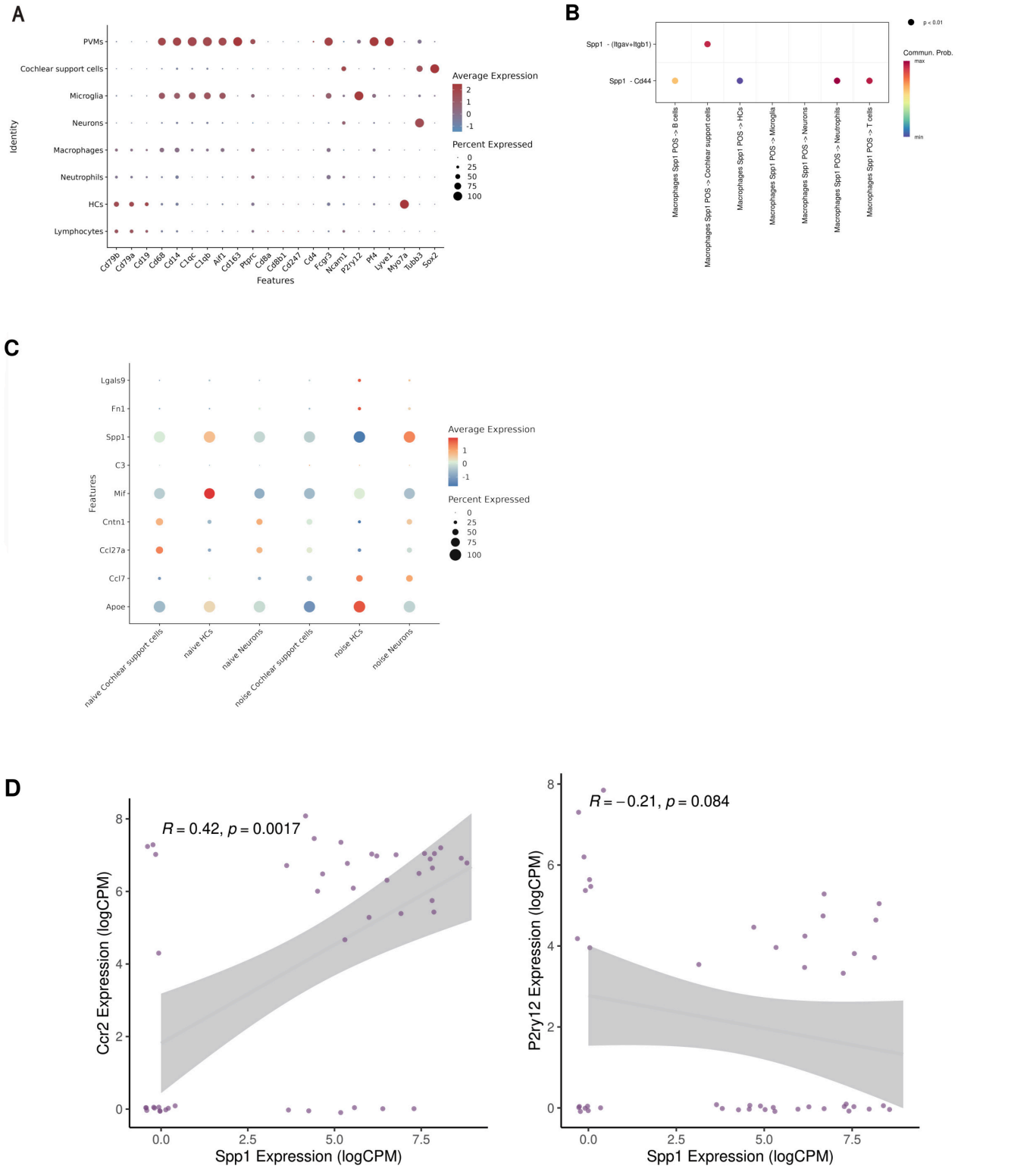


Fig. S2. Cell–cell communication analysis between SPP1⁺ macrophages and other cochlear cell types (CellChat).

(A) Dot plot showing the marker genes used to annotate cochlear cell types (the input to the CellChat inference). (B) Dot plot showing ligand–receptor interactions between SPP1⁺ macrophages and other cochlear cell types. SPP1⁺ macrophages are predicted to communicate with cochlear supporting cells through SPP1–ITGAV+ITGB1, and with neutrophils, T cells, B cells and macrophages themselves through SPP1–CD44. Communication networks were inferred with CellChat [22] using its curated mouse ligand–receptor database; the SPP1 pathway as annotated in that database comprises precisely these two receptor complexes, and this panel therefore reports the full pathway rather than a selected subset. All interactions shown are computationally predicted from single-cell transcript abundance and were not validated at the protein or functional level; they should be read as hypothesis-generating (see Discussion). (C) Changes in ligand expression in cochlear supporting cells, hair cells, and neurons before and after noise exposure.

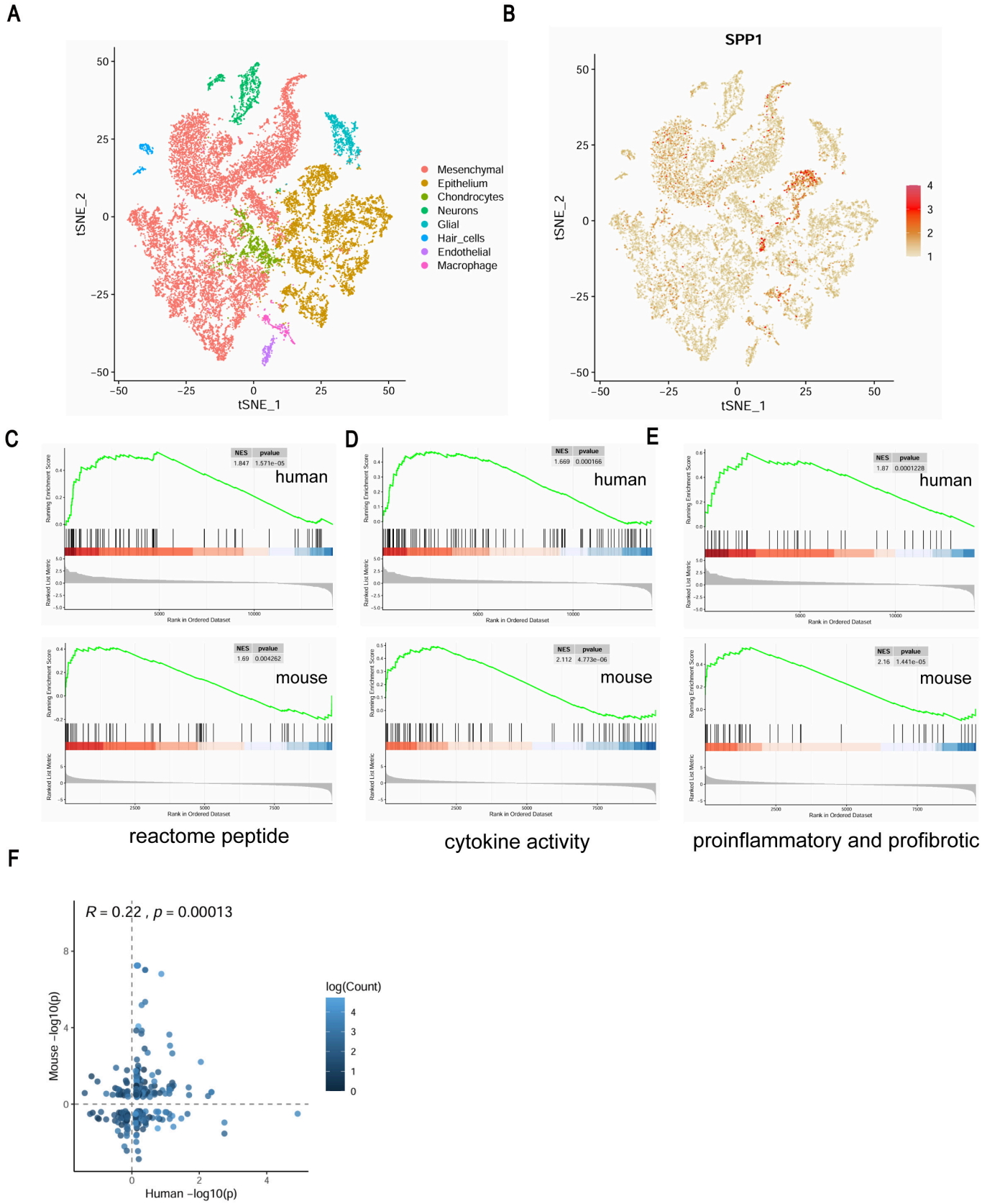


Fig. S3. Single-cell RNA sequencing analysis of the human inner ear.

Data source: GSE213796 (single-nucleus RNA-sequencing of two human fetal inner ears at 7.5 and 9.2 weeks of fetal age and one human adult inner ear) [21]. (A) t-SNE plot showing the distribution of major cell types, including mesenchymal cells, epithelial cells, chondrocytes, neurons, glial cells, hair cells, endothelial cells, and macrophages. (B) t-SNE plot highlighting SPP1 expression across different cell types. (C) Comparison of Reactome peptide-associated pathways between human and mouse. (D) Comparison of cytokine activity-related pathways between human and mouse. (E) Comparison of pro-inflammatory and pro-fibrotic signature-associated pathways between human and mouse. (F) Scatter plot showing the correlation of gene expression between SPP1^{+} macrophages from the human inner ear and Spp1^{+} macrophages from the mouse cochlea after noise exposure (R = 0.22, P = 0.00013). Note that this correlation is weak in magnitude despite reaching statistical significance.

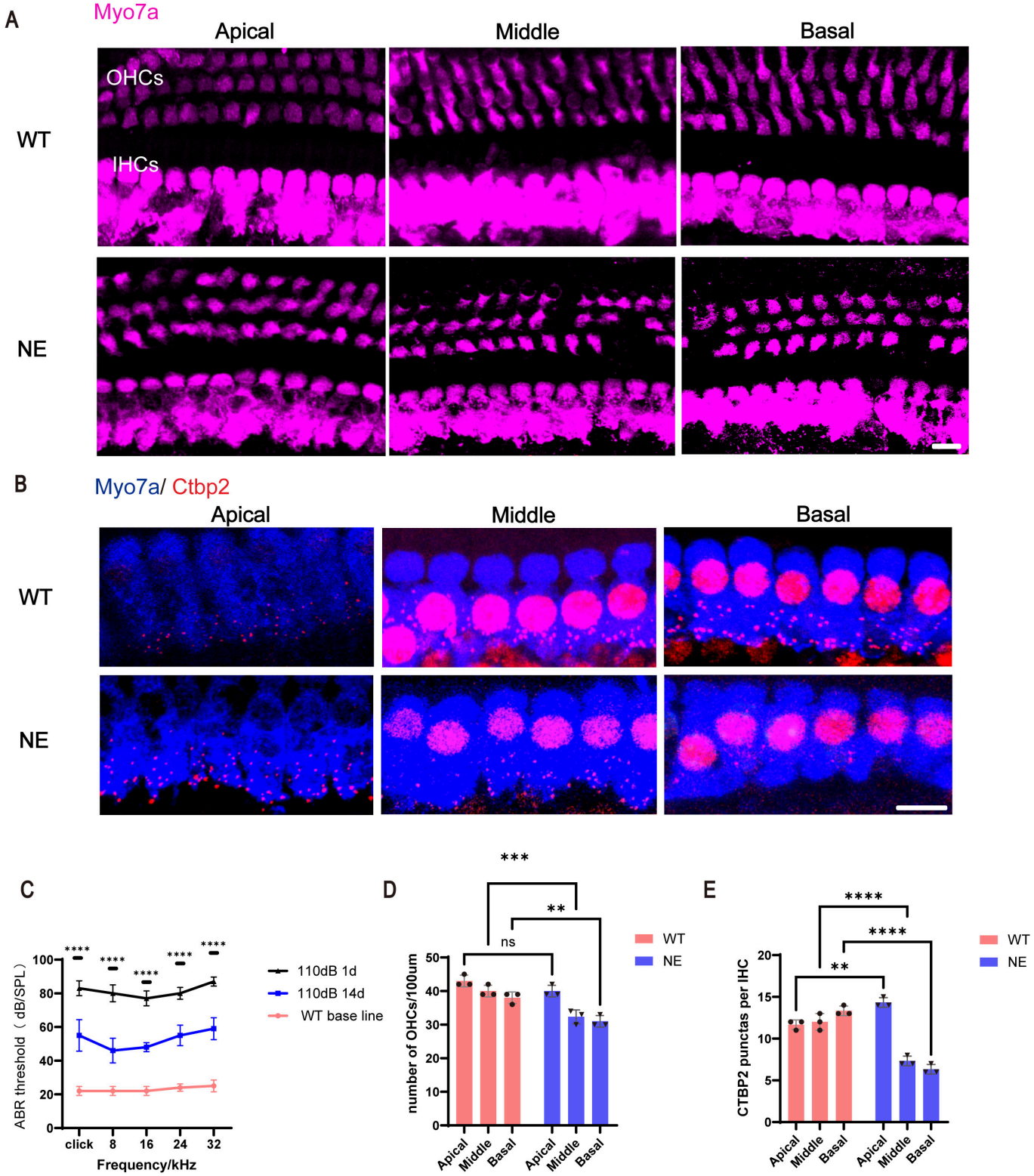


Fig. S4. Hair cell and synapse staining, and auditory threshold shifts in wild-type mice before and after noise exposure.

(A) Immunofluorescence staining of cochlear hair cells (Myo7a) in the apical, middle, and basal turns of wild-type mice before and after noise exposure. (B) Immunofluorescence staining of inner hair cells (Myo7a) and synapses (CtBP2) in the apical, middle, and basal turns of wild-type mice before and after noise exposure. Scale bars = 10 μm (A, B). (C) Auditory brainstem response (ABR) thresholds of WT mice at baseline, 1 day and 14 days post-noise exposure at frequencies of click, 8, 16, 24 and 32 kHz (n = 6). (D) Quantification of hair cell counts from the images shown in (A). Noise exposure induced cochlear hair cell death, leading to a reduction in hair cell number. (n=3) (E) Quantification of synapse counts from the images shown in (B). A significant decrease in synapse number was observed in mice following noise exposure. (n=3) ns, not significant; ** p<0.01; *** p<0.001; **** p<0.0001.

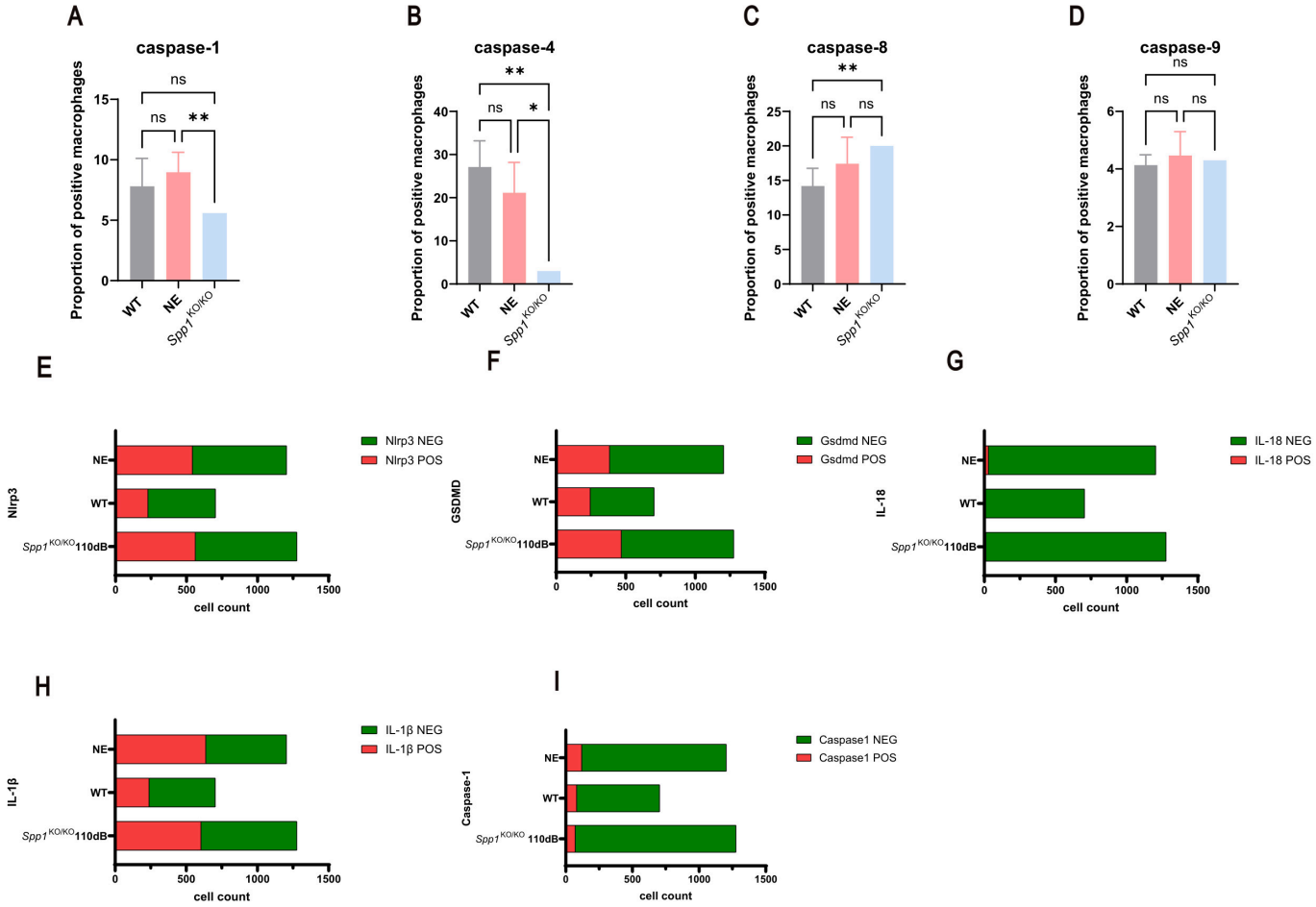


Fig. S5. Proportions of macrophages expressing cell-death-related molecules in WT and Spp1 KO/KO mice (single-cell data).

Sample labels used throughout this figure: WT = wild-type mice without noise exposure; NE = wild-type mice 3 days after noise exposure; Spp1 KO/KO = Spp1 knockout mice 3 days after noise exposure. (A–D) Proportions of macrophages expressing the pyroptosis-related molecules caspase-1 and caspase-11, and the apoptosis-related molecules caspase-8 and caspase-9, in the single-cell sequencing data. The proportions of caspase-1^{+} and caspase-11^{+} macrophages were reduced after Spp1 knockout, whereas caspase-8 and caspase-9 did not decrease (unchanged or slightly higher). WT and NE groups: three independently prepared single-cell samples (N = 3; mean ± SEM). Spp1 KO/KO group: a single pooled sample prepared from the cochleae of eight mice (one biological replicate; no error bars). (E–I) Contingency table showing the proportion of macrophages expressing pyroptosis-related molecules (Nlrp3, GSDMD, caspase-1, IL-18, IL-1β). ns, not significant; **p < 0.01; ****p < 0.0001.