Expanded View Figures

Figure EV1. AAV9.NDP vector transduction in the cochlea and retina.

A–E Organ of Corti and lateral wall wholemounts stained with anti-GFP antibody. (A-A″) Untreated WT cochlea; (B-B″) P2-L dose; (C-C″) P21-L dose; (D-D″) P21-H dose; (E-E″) P30-H dose. Scale bars = 500 μm (A-E), 500 μm (A′-E′), enlarged view of boxed region shown in A″-E″. SGN, spiral ganglia region; arrowheads, fibrocyte shaped cells in the lateral wall.

F P30-H dose; cochlea stained with anti-MyoVIIa antibody showing that hair cells are not transduced. Scale bar 100 μm.

G, H EGFP immunostaining at 2 months in retinal cryosections after AAV9.NDP treatment at P2 (G) and P21 (H); optic nerve: o.n. Arrows indicate transduced region of the retina; arrowheads indicate transduced cells in the RPE. Scale bar 500 μm.

Source data are available online for this figure.

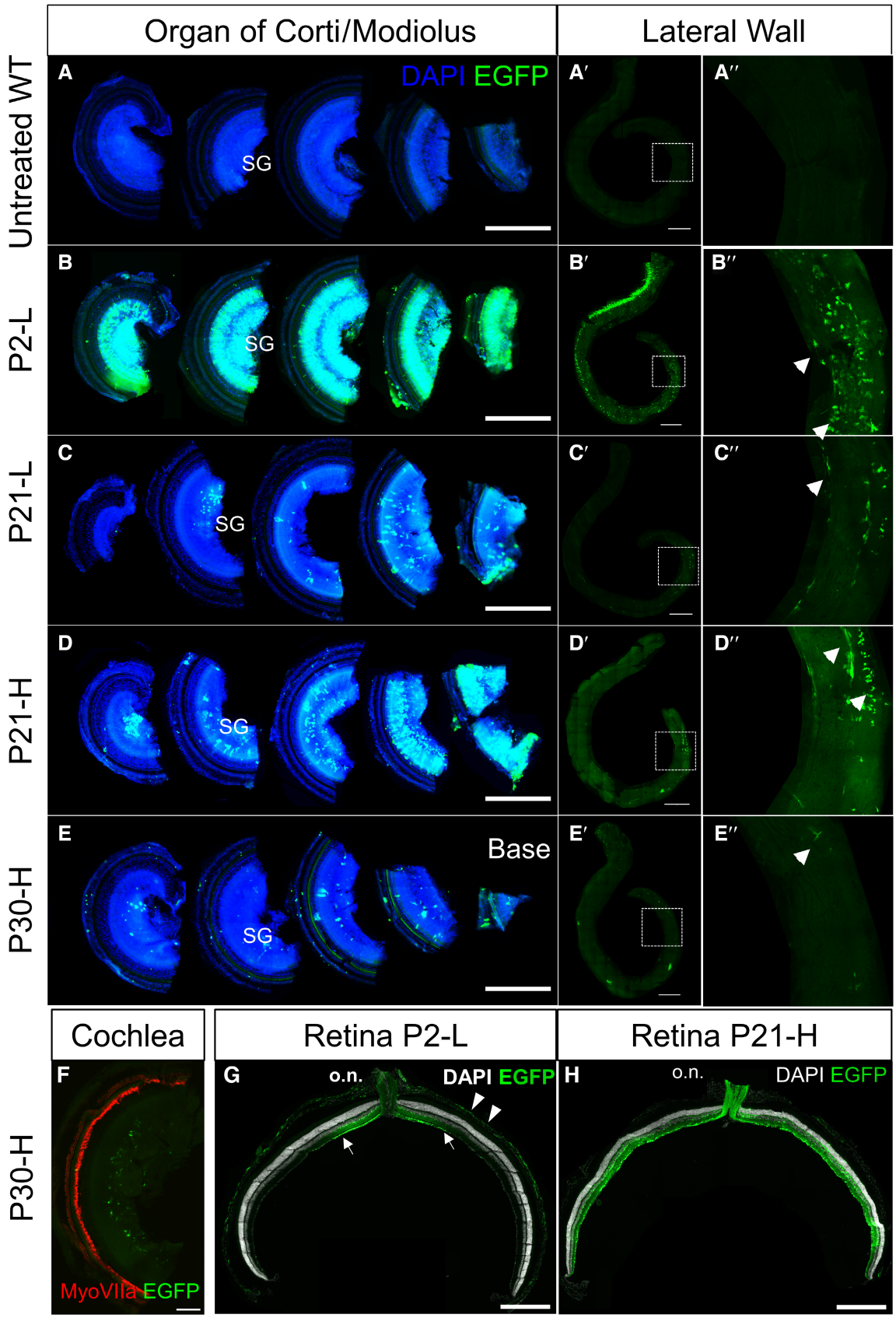

Figure EV1.

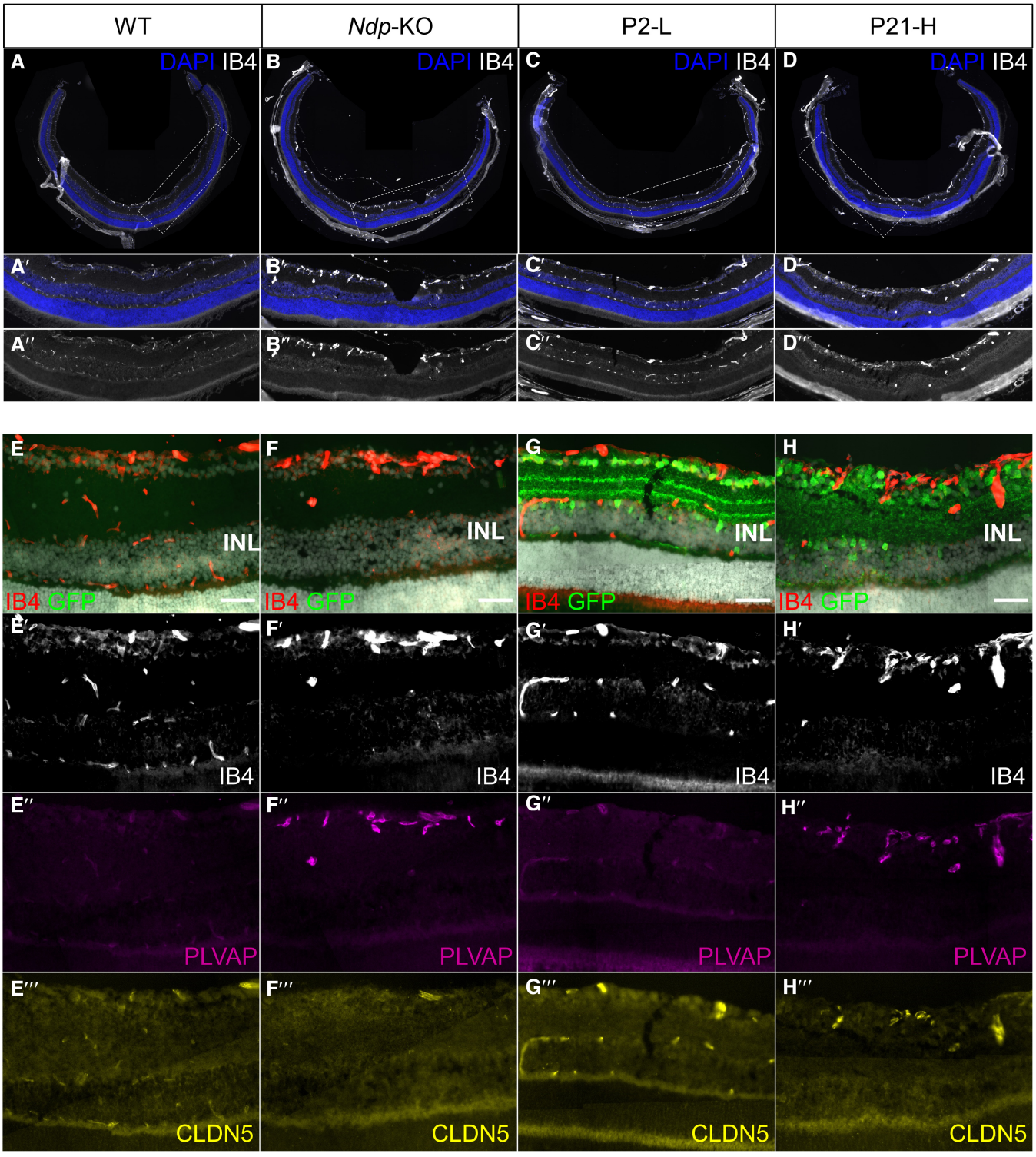

Figure EV2. Effects of AAV9.NDP treatment on the retinal vessel morphology and barrier proteins.

A–H Retinal cryosections at 2 months stained with IB4 (A–D, E–G, E–G′), anti-PLVAP and anti-CLDN5 antibodies (E″–G″) and anti-EGFP antibody (E–H). Note the presence of three layers of vessels on WT (A-A″, E-E′) and P2-L (C-C″, G-G′) groups and only one layer in *Ndp*-KO (B-B″, F-F′) and P21-H (D-D″, H-H′) groups. CLDN5 expression is visible in vessels in WT (E‴), P2-L (G‴) and P21-H (H‴) groups. Scale bar 50 μm (E–H).

Source data are available online for this figure.

Figure EV3. Electroretinograms showing effects of early and late treatments.

A Representative ERG traces in response to flashes of light of increasing intensity (an average of 10 flashes shown for each trace).
B, C Flash ERG, ratio of b-wave to a-wave amplitude for P2-L and P21-H treatment groups.
D, E Flash ERG, a-wave amplitudes for P2-L and P21-H treatment groups.

n = biological replicates. Data information: *n* (WT) = 10, *n* (*Ndp*-KO) = 10, *n* (P2-L) = 7, *n* (P21-H) = 10. Data are shown as mean ± SD. Statistical analysis was performed by one-way ANOVA with Sidak's *post hoc* test, comparing each treatment group with WT (blue asterisks) and *Ndp*-KO (red asterisks), between WT and *Ndp*-KO (black asterisks). *Post hoc* test values: *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$: ns, not significant.
Source data are available online for this figure.

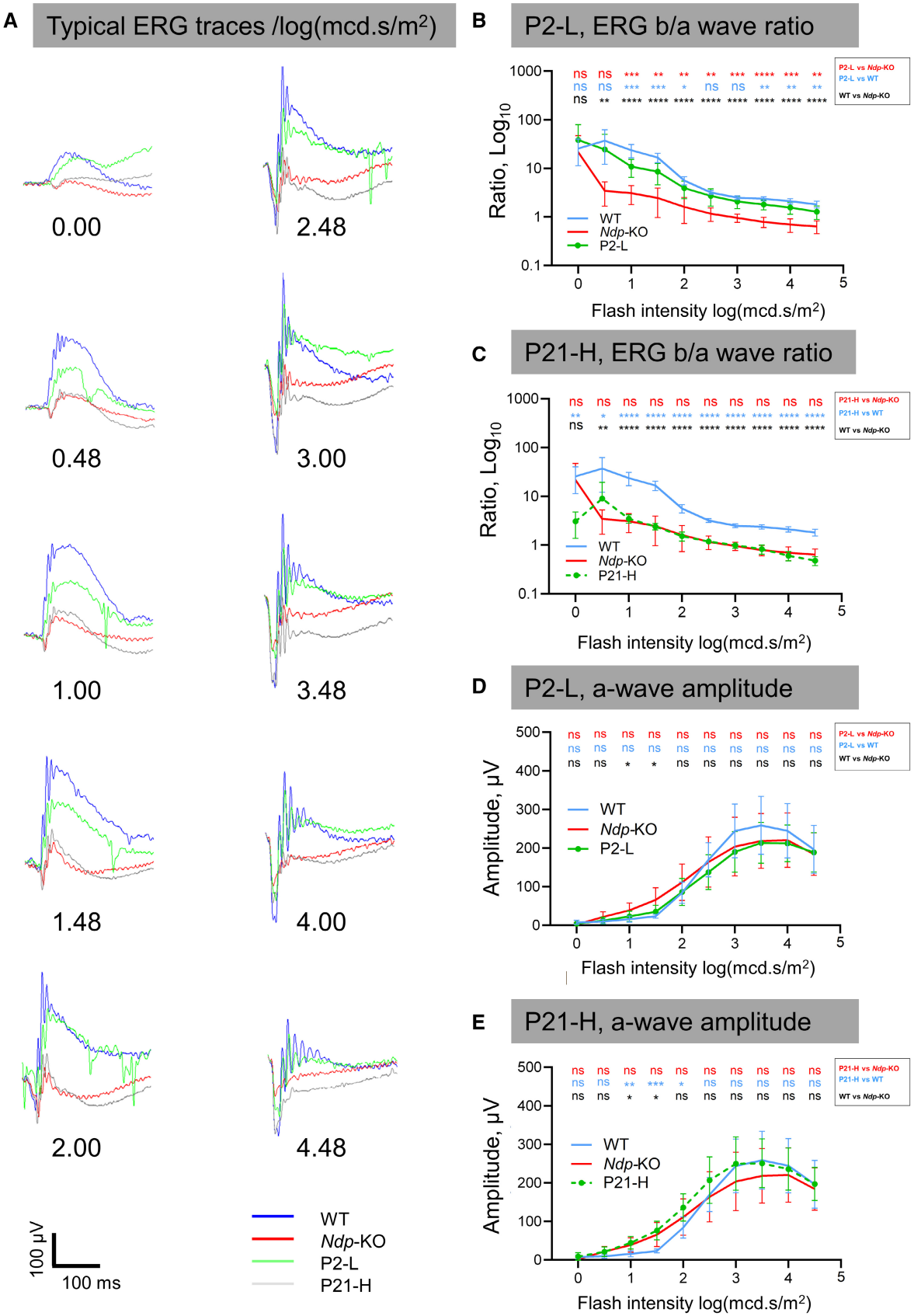
A
Typical ERG traces /log(mcd.s/m²)
0.00
2.48
0.48
3.00
1.00
3.48
1.48
4.00
2.00
4.48
100 µV
100 ms
WT
Ndp-KO
P2-L
P21-H
B
P2-L, ERG b/a wave ratio
Ratio, Log10
Flash intensity log(mcd.s/m²)
C
P21-H, ERG b/a wave ratio
Ratio, Log10
Flash intensity log(mcd.s/m²)
D
P2-L, a-wave amplitude
Amplitude, µV
Flash intensity log(mcd.s/m²)
E
P21-H, a-wave amplitude
Amplitude, µV
Flash intensity log(mcd.s/m²)

Figure EV3.

Figure EV4. Differential gene expression in the cochlea by RNA sequencing and qRT-PCR.

A Venn diagram showing overlap between *Ndp*-KO versus WT DEGs and *Ndp*-KO versus P2-L DEGs.

B Heatmap showing levels of expression of the 18 pathology-related DEGs identified between *Ndp*-KO (red) versus P2-L (blue) in WT, *Ndp*-KO and P2-L treated cochleas (green). Note that these genes showed trends of differential expression between *Ndp*-KO and WT cochleas that did not reach significance. No genes were found to be significantly differentially expressed between WT and P2-L cochleas, indicating rescue by the treatment. In heat map, red indicates upregulated and blue indicates downregulated gene expression in *Ndp*-KO.

C Gene set enrichment analyses of differentially expressed genes between WT and *Ndp*-KO using gene sets were previously defined by transcriptome profiling of FACS sorted CNS vs peripheral endothelial cells (Daneman *et al*, 2010) and previously used to assess transcriptomes of WT and *Ndp*-KO retinas (Zhou *et al*, 2014). Gene sets characterising barrier vasculature, BBB1 and 2, BBB endothelial transporters, CNS endothelial and CNS pericyte were significantly positively correlated (FDR < 0.25) with the WT genotype.

D Dot plot using scRNA seq data of the adult mouse cochlear lateral wall from GEO database: accession numbers GSM5124299, GSM5124300, GSM5124301, and GSM5124302. Gene markers used to distinguish 30 cell type clusters in the UMAP were as previously reported (Gu *et al*, 2020; Bryant *et al*, 2022).

E Dot plot showing expression of the 45 DEGs identified in WT versus *Ndp*-KO analysis in the 30 cell type clusters identified in the adult mouse cochlear lateral wall at the single cell level. Blue arrows indicate endothelial cell genes (*Cldn5*, *Abcb1a* and *Flt1*) downregulated in *Ndp*-KO, and orange arrows indicate upregulated genes in *Ndp*-KO. Box indicates genes significantly different in the P2-L versus *Ndp*-KO comparison. Shaded DEGs are downregulated in the *Ndp*-KO cochlea. Note expression of some DEGs across several different cell type clusters.

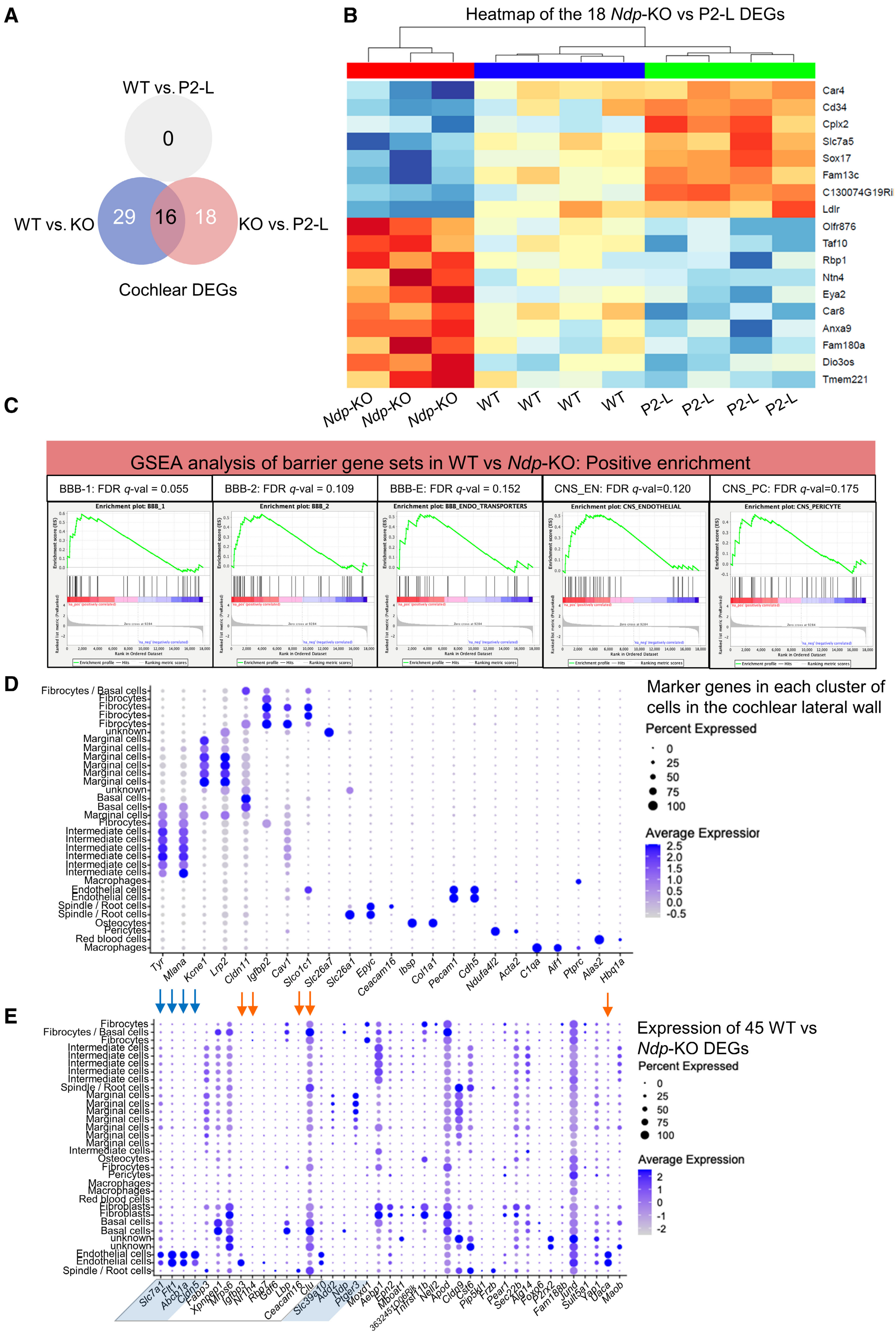
A
WT vs. P2-L
0
WT vs. KO
29
16
18
KO vs. P2-L
Cochlear DEGs
B
Heatmap of the 18 Ndp-KO vs P2-L DEGs
Car4
Cd34
Cplx2
Slc7a5
Sox17
Fam13c
C130074G19Rik
Ldlr
Olfr876
Taf10
Rbp1
Ntn4
Eya2
Car8
Anxa9
Fam180a
Dio3os
Tmem221
Ndp-KO
Ndp-KO
Ndp-KO
WT
WT
WT
WT
P2-L
P2-L
P2-L
P2-L
C
GSEA analysis of barrier gene sets in WT vs Ndp-KO: Positive enrichment
BBB-1: FDR q-val = 0.055
BBB-2: FDR q-val = 0.109
BBB-E: FDR q-val = 0.152
CNS_EN: FDR q-val=0.120
CNS_PC: FDR q-val=0.175
D
Marker genes in each cluster of cells in the cochlear lateral wall
Percent Expressed
0
25
50
75
100
Average Expression
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
Tyr
Mlana
Kcne1
Lrp2
Cldn11
Igfbp2
Cav1
Slco1c1
Slc26a7
Slc26a1
Epyc
Ceacam16
Ibsp
Col1a1
Pecam1
Cdh5
Ndufa4l2
Acta2
C1qa
Aif1
Ptprc
Alas2
Hbq1a
Fibrocytes / Basal cells
Fibrocytes
Marginal cells
unknown
Basal cells
Intermediate cells
Macrophages
Endothelial cells
Spindle / Root cells
Osteocytes
Pericytes
Red blood cells
E
Expression of 45 WT vs Ndp-KO DEGs
Percent Expressed
0
25
50
75
100
Average Expression
2
1
0
-1
-2
Fibroblasts

Figure EV4.

Figure EV5. Statistical analysis of auditory function analysis of DPOAE and ABR thresholds for all groups at 3 months.

A, B DPOAE thresholds of P2-L, P30-H and control groups. *P* values indicate statistically significant difference from WT (blue) and *Ndp*-KO (red). *n* = biological replicates. *n* (WT) = 11, *n* (*Ndp*-KO) = 6, *n* (P2-L) = 9, *n* (P30-H) = 8.

C–E ABR thresholds of P2-L, P-30H and control groups. *P* values (blue) indicate statistically significant difference from WT. *n* = biological replicates. *n* (WT) = 12, *n* (*Ndp*-KO) = 7, *n* (P2-L) = 10, *n* (P21-H) = 8, *n* (P30-H) = 8.

E ABR wave latency for each treatment group compared to *Ndp*-KO and WT. *n* = biological replicates. *n* (WT) = 12, *n* (*Ndp*-KO) = 7, *n* (P2-L) = 10, *n* (P21-H) = 8, *n* (P30-H) = 8.

F ABR wave 1 amplitude for each treatment group compared to *Ndp*-KO and WT. *n* = biological replicates. *n* (WT) = 12, *n* (*Ndp*-KO) = 7, *n* (P2-L) = 10, *n* (P21-H) = 8, *n* (P30-H) = 8.

Data information: Data are shown as mean ± SD. Statistical analysis was performed by two-way repeated measures ANOVA with Tukey's *post hoc* test, comparing each treatment group with WT (blue asterisks) and *Ndp*-KO (red asterisks), and comparing between WT and *Ndp*-KO (black asterisks). *Post hoc* test values: *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$.
Source data are available online for this figure.

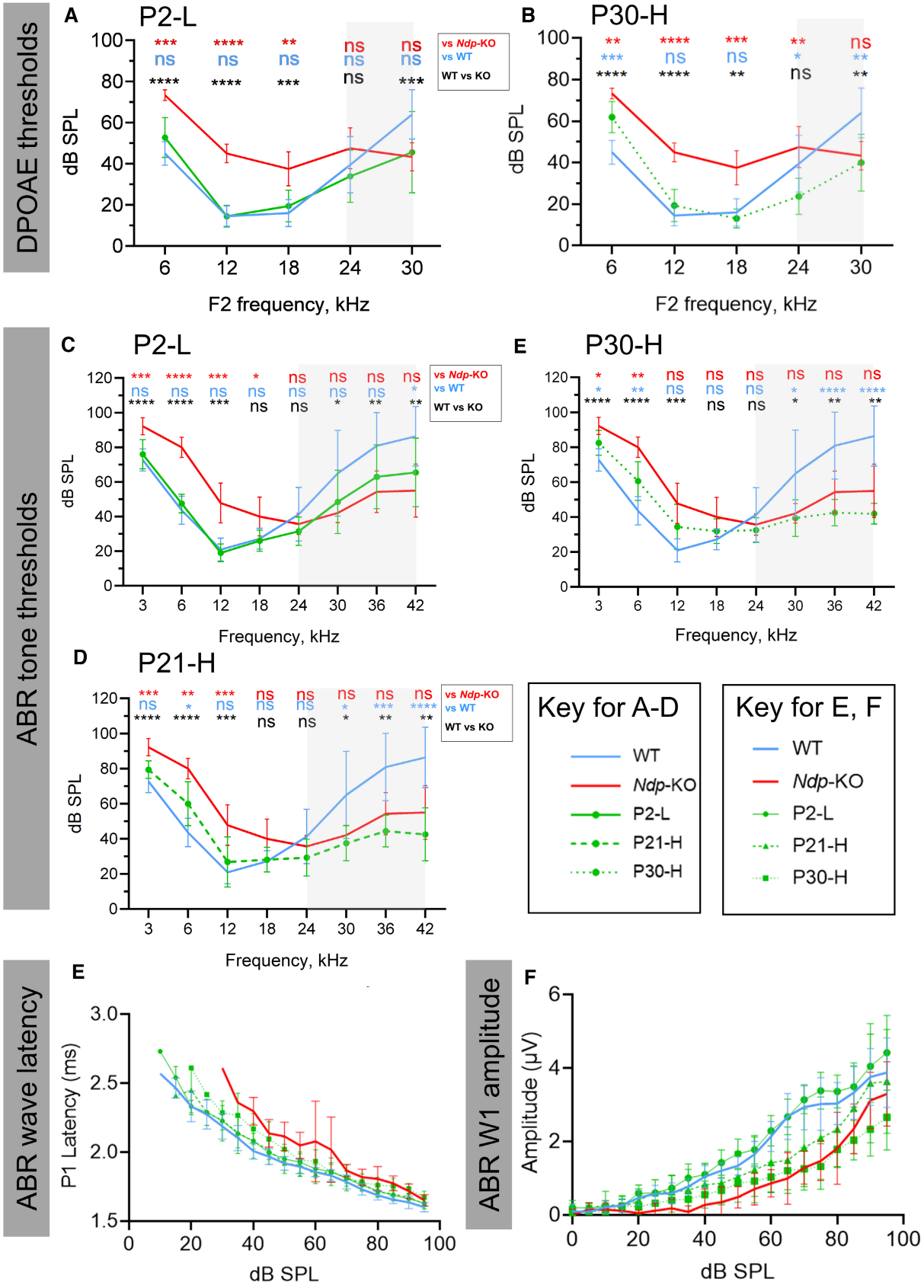
DPOAE thresholds
A
P2-L
B
P30-H
dB SPL
F2 frequency, kHz
vs Ndp-KO
vs WT
WT vs KO
ABR tone thresholds
C
P2-L
E
P30-H
D
P21-H
Frequency, kHz
Key for A-D
WT
Ndp-KO
P2-L
P21-H
P30-H
Key for E, F
ABR wave latency
E
P1 Latency (ms)
dB SPL
ABR W1 amplitude
F
Amplitude (μV)
dB SPL

Figure EV5.