

Single-cell landscape of the mouse medial vestibular nucleus after unilateral vestibular loss and the effect of sequencing depth

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Method Article

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Abstract

Acute unilateral vestibular loss initiates central vestibular compensation, but its single-cell mechanism is unresolved. We reanalyzed the public mouse medial vestibular nucleus (MVN) single-cell dataset GSE143701 under a four-tier sequencing-depth control to map the cell-type-resolved response and to test how depth shapes the direction of differential expression. Among 12,436 cells assigned to 8 types, the fraction of down-regulated genes fell from 81.2% at full depth to 50.4% after depth matching, indicating that widespread down-regulation was largely a depth artifact. The microglial IL1_TLR module showed the largest increment (2.9–3.5-fold vs the next cell type, consistent across three depth settings); 70% derived from *Cd14* (45%) and *Tlr2* (24%), whereas *Il1r1* detection of 0.6% rendered the classical IL-1 receptor axis unmeasurable. Cell-cell communication was globally weakened (25%–38% of axes positive), with a direction-robust gained *Cd14–Itgb1* axis on the lesioned side. Cross-model comparison showed no synchronous *Cd14/Tlr2* up-regulation in a facial-axotomy model. We conclude that the acute MVN response centers on a microglial *Cd14/Tlr2* program rather than IL-1 receptor activation, and that pathway-dominance claims in acute single-cell data require per-gene attribution and depth-sensitivity testing.

1. Introduction

Acute unilateral vestibular loss (vestibular neuritis being its most common form) is a frequent vestibular emergency, and roughly a third of patients go on to develop persistent dizziness^[1–3]. The only evidence-based rehabilitation is vestibular rehabilitation training, yet its efficacy varies widely between individuals and no early stratification marker exists^[4–7]. The deeper reason is that the central mechanism of vestibular compensation remains uncharacterized at single-cell resolution. Previous studies relied on bulk transcriptomics and immunohistochemistry^[8–11]; because bulk signals are a weighted average of multiple cell types, the responding cell type cannot be resolved. Although associations between peripheral and central interleukin (IL)-18 and vestibular compensation have generated a series of hypotheses, direct evidence on the cellular origin and direction of change is lacking^[12–15], and vestibular-nucleus communication remodeling has not been systematically described.

Prior single-cell studies rarely report the effect of sequencing depth, yet depth differences are pervasive in acute-phase samples and can systematically change the direction and proportion of differential expression. We systematically reanalyzed GSE143701 to build a cell-type-resolved response map and localize the inflammatory signal, comparing it with rat vestibular-nucleus microarray data and facial-nerve axotomy microglial data. We pre-specified four hypotheses to be tested (Hypotheses 1–4, see Results 3.5); Hypothesis 2' is a revised hypothesis raised after Hypothesis 2 was not supported.

2. Materials and methods

2.1 Data sources and study design

This study is a secondary analysis of public databases and involves no human subjects and no new animal experiments; therefore no ethical approval or informed consent was required for this re-analysis (ethics approval for the original studies was obtained by the original authors). Reporting follows the STROBE statement (Strengthening the Reporting of Observational Studies in Epidemiology) for secondary analyses, and we declare no dual submission. The core dataset GSE143701 (NCBI GEO; Drop-seq^[16]) comprises the MVN of C57BL/6 mice with three groups: a naïve control (VN), the lesion-ipsilateral side (ULI), and the lesion-contralateral side (ULC)^[17]. GSE32437 is an Agilent microarray (GPL7294) of the rat MVN with NL, D1, and D7 groups^[8]. GSE90975 is a single-cell dataset of microglia sorted from the facial nucleus of CX3CR1-GFP mice after axotomy^[18]; methods are in 2.7 (technical roadmap in Supplementary Fig. S9).

2.2 Data processing and quality control

GSE143701 was supplied as a pre-computed count matrix covering 14,876 cells. We admitted a cell only if it expressed more than 200 genes and carried a mitochondrial read fraction under 20%, and removed any gene detected in fewer than 3 cells; this quality filter yielded 12,436 cells and 23,802 genes. Sequencing depth varied sharply across the three groups (median UMI [unique molecular identifier]: VN 3,272, ULI 2,181, ULC 880 (a 3.7-fold span). One log-normalized matrix supported every expression comparison; a distinct normalization was computed solely to pick variable features and to drive principal component analysis and clustering, and was never used for differential testing. We deliberately omitted three steps: doublet calling, ambient-RNA correction (omitted because *Ttr* appeared strongly in off-target clusters, leaving those conclusions uncertain), and batch integration (omitted because condition-specific clusters dominated, so we describe only compositional shifts). Cell-level metadata and quality metrics are in Supplementary Material S5.

2.3 Cell-type annotation and validation

Eight cell types were assigned by merging 23 transcriptomic clusters according to canonical markers (microglia: *Cx3cr1/C1qa/P2ry12*; astrocytes: *Gfap/Aldh1l1*; oligodendrocytes: *Mbp/Mag*; neurons: *Snap25/Syt1*; endothelial: *Pecam1*; pericytes: *Pdgfrb*; ependymal: *Foxj1*, etc.), plus an unassigned class (846 cells, 6.8%). Markers were strongly positive only within the corresponding type and negative in all others, with no crossover. Neurons were excluded from between-group quantitative comparison (VN only 6, ULI 96, ULC 68; median UMI in lesion groups 6.3-fold higher; sample size and depth are both non-comparable).

2.4 Differential expression and four-tier depth control

For the 6 principal cell types we contrasted ULI and ULC with VN separately using Wilcoxon rank-sum statistics, declaring significance at a Bonferroni-adjusted corrected $P < 0.05$ together with $|\log_2FC| > 0.25$. Because the three groups differed markedly in depth, a four-tier depth control was applied: FD (full depth, baseline); DS (conventional downsampling to 800/cell, applied only to cells with ≥ 800 counts, partial equalization); CDS (depth subset keeping only cells with ≥ 800 counts without downsampling, reducing the depth difference from 3.7 $\times$ to a residual 1.29 $\times$); CDU (depth-consistent: within the ≥ 800 subset,

uniformly downsampled to 800/cell). Depth-consistent differentially expressed genes were those significant and directionally concordant in both FD and CDU (1,043 unique genes, 1,745 records).

2.5 Cell-cell communication analysis

Communication inference relied on liana, taking CellPhoneDB's permutation P value as the significance measure and the ligand–receptor mean as the magnitude measure^[19, 20]; ligand–receptor pairs formed the analytical unit. Because scores are sensitive to both cell composition and depth, a three-tier design was used: D0 (random $\leq 1,600$ cells/condition, neither composition nor depth controlled); D1 (proportional subsampling by type, composition matched); D2' (first depth-matched within the ≥ 800 subset, then proportional subsampling by type, both composition and depth matched). Built-in magnitude rankings were disabled and a dual metric was reported; 100 permutations per group. The differential list was ordered by effect size and is presented only as hypothesis-generating evidence.

To avoid supporting mechanistic claims with technical artifacts or unreliable cell populations, two removal criteria were pre-specified and reported axis by axis: (1) depth confounding: the apparent change at the ligand/receptor end converged substantially after re-computation within the shared depth interval, or gene detection rate was strongly coupled to depth; (2) unreliable population: too few cells in the control group or too large a depth difference. Raw values of removed axes are in Supplementary Material S4, S10, S11.

2.6 Inflammatory pathway module scoring

Based on Gene Ontology and Reactome annotations combined with the literature^[12–15, 22], 7 inflammatory pathway gene sets were built (IL1_TLR, IL-18_axis, TNF_NFkB, chemokines, type I interferon, antigen presentation, complement; 5–9 genes each, full lists in Supplementary Table S2). After per-cell scoring, values were summarized by type $\times$ condition as the mean change relative to VN. Control genes corrected depth differences only partially, so changes were compared relatively only within the same cell type. A module's conclusion was retained only when its direction was consistent across three depth settings (FD, the ≥ 800 -count subset, and CDU) (Table 1). Pathway-level "dominance" additionally required per-gene attribution: the module signal in microglia was decomposed gene by gene, and a leave-one-out test examined its concentration; if attribution failed, the conclusion was narrowed to specific genes and the module name was not retained. The full matrix is in Supplementary Table S1.

Table 1
Consistency of microglial inflammatory module changes across 3 depth settings.

Module	Full depth (FD)	≥ 800 UMI subset	Depth-consistent (CDU)	Verdict
IL1_TLR	+ 0.301	+ 0.330	+ 0.314	Consistent direction
IL-18 axis	+ 0.119	+ 0.123	+ 0.096	Consistent direction
Chemokines	+ 0.214	+ 0.199	+ 0.249	Consistent direction
TNF_NFkB	+ 0.029	+ 0.024	+ 0.121	Consistent direction, magnitude near zero
Type I interferon	+ 0.014	+ 0.012	+ 0.014	Consistent direction, magnitude near zero
Complement (ULI side)	-0.036	-0.010	-0.094	Consistent direction (negative in all 3 settings)
Complement (ULC side)	+ 0.100	+ 0.176	+ 0.015	Opposite to ipsilateral; excluded
Antigen presentation	-0.123	-0.125	+ 0.025	Inconsistent direction; excluded

2.7 Cross-dataset and cross-model comparison

The Agilent raw files of GSE32437 were parsed and mapped to a gene-level matrix; after $\log_2$ transformation and quantile normalization, $\log_2$ FC of D1 and D7 relative to NL was computed. Analysis had four layers: direction concordance of single-cell differentially expressed genes, Fisher test of overlap between depth-consistent and bulk-level changed genes, per-gene $\log_2$ FC of 18 pre-defined inflammatory core genes, and mean $\log_2$ FC of four pathway features.

GSE90975 is a unilateral facial-nerve axotomy model in CX3CR1-GFP mice; microglia from the facial nucleus (GFP⁺CD45^{lo}CD11b⁺) were sorted and sequenced, with Day 0, Day 7, and Day 30 time points, bilaterally sampled from the same mice; 1,526 cells remained after quality control. The analysis pipeline matched the primary dataset, with changes referenced to Day 0 and depth sensitivity re-computed by uniform downsampling of UMI ≥ 800 cells to 800. In this dataset "lesioned vs control side" is a paired comparison within the same mouse, whereas in the primary dataset "contralateral vs VN" measures the contralateral lesion relative to the normal control; the baselines differ, so ratios cannot be directly subtracted or divided, and only directional hints are given. Its median UMI was only 126–353, far lower than the primary dataset, so "no up-regulation observed" is treated as a negative finding under the current detection sensitivity. The two datasets differ in five respects (species, platform, time point, sampling scope, count version), so cross-model comparison is limited to the qualitative level.

2.8 Statistical methods and reproducibility

This is an exploratory secondary analysis. The core dataset uses a single-library pooled design per condition with no independent biological replicate; therefore cell-level rank-sum and permutation tests serve only for signal filtering and ranking, not for between-group inference. The main evidence is effect size ($\log_2FC$, module change, expression-magnitude rank) and cross-analysis consistency (4 depth settings, 3-tier communication design, single-cell vs bulk direction comparison); corrected P and permutation P are used for threshold filtering. Differential testing used Wilcoxon rank-sum with Bonferroni adjustment. Communication permutation tests were reported without multiplicity correction and ranked by effect size as hypothesis-generating evidence. Cross-dataset overlap was assessed by Fisher's exact test, and associations by Spearman rank correlation. All analyses were performed in Python 3.13 with scanpy 1.12^[21] and liana, random seed 0, Leiden resolution 0.5, and conclusions are based on 8 cell types.

3. Results

3.1 Single-cell landscape and cell composition after lesion (Fig. 1)

The three groups comprised 14,876 cells; after quality control 12,436 cells remained, yielding 23 clusters annotated into 8 types. In relative composition, ULI microglia fell from 46.7% to 25.7% and pericytes rose from 3.4% to 9.5%. Because depth differences were large and no batch integration was performed, proportional changes cannot be separated from dissociation and capture efficiency and are presented descriptively only (cluster assignment and markers in Supplementary Table S2).

3.2 Depth dependence of response direction (Fig. 2)

At full depth, every cell type showed an asymmetric predominance of down-regulation (e.g., oligodendrocytes ULC: 469 up / 5,206 down; microglia ULI: 121 / 1,211). The four-tier depth control showed this asymmetry was mainly related to depth differences: across 12 "type × comparison" blocks, the down-regulated proportion fell from 81.2% at FD through 55.0% at DS and 66.3% at CDS to 50.4% at CDU; an extreme case reversed (oligodendrocyte ULC changed from 91.7% down at FD to 53.0% up at CDU), and the direction concordance of co-significant genes was 0.999–1.000, indicating depth control changed the set and proportion but not the direction. We therefore do not describe "widespread down-regulation" as an overall feature.

Depth sensitivity also served as an internal control: oligodendrocytes had the largest inter-group depth difference (median UMI 6,251 vs 721, 8.7×) and their differential set contracted most (Jaccard index of FD vs CDU only 0.066–0.121); microglia showed the strongest integrated response (1,332 significant at FD, 245 retained in the depth-consistent set) and were the type that did not markedly contract after depth control. Full differential-expression lists per type are in Supplementary Material S3.

3.3 Inflammatory modules: microglial IL1_TLR highest, signal concentrated in *Cd14/Tlr2* (Figs. 3, 8)

Among the 7 inflammatory pathway modules (Fig. 1), the microglial IL1_TLR was highest overall (+ 0.30/+0.31); the IL-18 axis and chemokines were in the same direction but smaller (+ 0.12/+0.09, + 0.21/+0.07), and TNF_NFkB and type I interferon were negligible; antigen presentation and complement showed no consistent signal (sign flip or opposite direction) and were excluded from conclusions (Table 1).

IL1_TLR elevation was concentrated in microglia: on the ipsilateral side it was 2.9–3.5 times the next-highest cell type and 2.9 times on the contralateral side, with others near zero; across three depth settings + 0.301/+0.330/+0.314, the highest magnitude and consistent direction; Hypothesis 1 supported. The IL-18 axis was about one third to one half of its magnitude, with no marked up-regulation; type I interferon was flat.

Per-gene attribution. The IL1_TLR gene set contains 7 genes (*Il1a*, *Il1b*, *Il1r1*, *Tlr2*, *Tlr4*, *Myd88*, *Cd14*). Decomposition in microglia (from VN to ULC): *Cd14* + 0.995, *Tlr2* + 0.522, *Il1b* + 0.390, *Il1a* + 0.241, whereas *Tlr4* (– 0.062) and *Myd88* (– 0.010) were negative and *Il1r1* (+ 0.010) near zero. By absolute increment, *Cd14* accounted for 45% and *Tlr2* for 24%, together 70%. Leave-one-out showed: removing *Cd14* reduced the module change from + 0.298 to + 0.182 (– 39%), removing *Tlr2* to + 0.261 (– 13%); removal of the other 5 genes changed – 5% to + 20%, so the module signal was concentrated in 2 genes rather than spread across 7.

Detection-power limitation: microglial *Il1r1* detection was 0.6% and *Il1b* 4.6%, so the classical IL-1 receptor signal was unmeasurable; "IL1_TLR module dominance" means *Cd14/Tlr2* dominance (detection rising from 31.8% to 59.2% and from 38.9% to 52.3%), not classical IL-1 receptor activation, which also explains the absence of an *Il1b–Il1r1* axis in communication.

3.4 Cell-cell communication remodeling: global weakening with a direction-robust gained axis on the lesioned side (Fig. 4)

The three-tier comparison showed the binary "gain/loss" sets were design-sensitive (gained ULI changed from 108 pairs at D0 to 12 at D2', Jaccard as low as 0.008), whereas magnitude Spearman correlation reached 0.940–0.984 and Top20 source–target axis overlap was 67%–82%.

The global background was weakening: under D2', of 4,070 comparable communication axes only 30.0% (ULC) and 25.1% (ULI) were positive, and across 4 depth levels 25%–38% were positive with consistent direction. We therefore do not use gain/loss counts for quantitative conclusions, but report each axis's magnitude, direction, and depth sensitivity; *P*-filtered gave 14 gained and 37 diminished axes (Supplementary Table S3).

The direction-robust gained axis on the lesioned side was *Cd14–Itgb1* (microglia-to-glia, 3 axes), with the smallest magnitude decay across 4 downsamplings (ratio 1.04) and ligand-side within-band verification: *Cd14* + 0.995, re-computed + 1.102 within the ULI–VN shared depth interval (UMI 1,000–3,000), unaffected by depth confounding; the receptor *Itgb1* decreased in pericytes (– 0.335). *Ptn–Ncl*, *Ptn–*

Ptprz1, and *Psap–Gpr37l1* diminished axes were negative at all depth levels, forming a "nutritive/survival axis weakening."

By pre-specified criteria, 8 axes were removed, the most important 5 involving neurons: neurons had only 6 in VN, 96 in ULI, 68 in ULC, with median UMI 6.3× higher in lesion groups, so any axis involving this population was removed (3 as source, 2 as target). Source axes re-computed within the shared depth interval (UMI 900–5,400): *Hsp90b1* neuron + 0.138 (only 3 cells on VN side), ULC – 0.309 opposite direction, with within-band microglia + 0.121, suggesting the apparent gain was receptor-end driven; this gene had the highest depth coupling (Spearman + 0.540) and the ligand end was insufficient to support a specific interpretation. Two more were removed for depth confounding and one for definitional contradiction; after removal the 8 gained and the top 12 diminished axes involved no neurons.

Hypothesis 3

was partially supported: against a globally weakened background, a direction-robust gained axis *Cd14–Itgb1* existed on the lesioned side, and the *Ptn/Psap* nutritive/survival axes weakened consistently on the lesioned side, consistent with damage-associated molecular patterns amplifying neuroinflammation via Toll-like receptors^[25, 26]; the contralateral side was dominated by reparative ligand enhancement (list in S4).

3.5 Cross-dataset concordance and hypothesis testing (Fig. 5, Table 2)

Table 2
Summary of hypothesis testing.

Hypothesis	Statement	Result	Evidence
1	Microglia are the main cell type responsible for innate immune response	Supported (molecularly restricted to <i>Cd14/Tlr2</i>)	The increase in IL1_TLR is 2.9–3.5-fold that of the second-highest cell type and shows a consistent direction across the 3 depth settings; per-gene attribution shows that 70% of the increase derives from <i>Cd14</i> (45%) and <i>Tlr2</i> (24%); the IL-1 receptor axis is not measurable because the detection rate of <i>Il1r1</i> is only 0.6%
2	IL-18 family signalling is involved and up-regulated	Not supported	Microglia only + 0.12/+0.09; down-regulated at both time points in the rat bulk-level dataset
2'	Central IL-18 is not characterised by transcriptional up-regulation; the value of peripheral markers requires validation at the protein and cleavage level	Proposed for future testing	The transcriptome alone cannot determine the peripheral origin; measurements of mature IL-18, caspase-1 products and paired serum/CSF samples are required
3	Communication remodelling can be distinguished between the ipsilateral and contralateral sides	Partially supported (descriptive)	The global background is a decrease (only 25%–38% of 4,070 axes are positive); a directionally robust gained axis <i>Cd14-Itgb1</i> is present on the ipsilateral side (depth decay 1/1.04, ligand-side within-band verification + 1.102); binary counts are unstable and no multiple-comparison correction was applied
4	Up-regulation of <i>Cd14/Tlr2</i> is associated with the vestibular-loss context rather than a generic program of deafferentation	Partially supported (directional)	Markedly up-regulated in the MVN (2.74/1.71-fold, each baselined on its own control); no synchronous up-regulation in the facial nucleus (0.84/0.73-fold); negative correlation in module composition ($\rho = -0.81$). However, the two datasets use different baseline definitions, so the ratios cannot be directly subtracted; reported as a directional hint only

Compared with independent rat bulk-level data: the direction concordance of single-cell up-regulated genes was only 33%–52% (no better than random); the depth-consistent differentially expressed genes (1,043) overlapped 406 bulk-level changed genes but were significantly depleted (odds ratio 0.46, $P = 1.1 \times 10^{-33}$); the whole-transcriptome scatter ($n = 11,807$, Spearman = -0.02) suggests cross-species, cross-platform extrapolation is inappropriate. Among 18 inflammatory core genes, at D7 *Il1b* (+ 1.09), *Casp1* (+ 1.48), *Ccl3* (+ 1.46), and *C1qa* (+ 1.02) were up-regulated, IL-18 was down at both time points ($-0.53/-1.20$), interferons were negative at both time points, and antigen presentation persistently

negative. Synthesis: the *Il1b/Casp1*/chemokine axis direction converged as the priority candidate; the IL-18 axis showed no consistent up-regulation (Hypothesis 2 not supported), and overall low concordance defines the boundary of reproducibility.

Marker validation and annotation confidence: the 8 cell types' canonical markers were the only strong positive signal within the corresponding type with little cross-type crossover (Fig. 6); homeostatic markers were highest in VN and decreased after lesion, whereas activation-related markers were highest in ULC, suggesting a shift of microglia toward an activated phenotype (magnitude is depth-sensitive and described qualitatively only); neuronal clusters (20/22) highly expressed neuronal markers and were negative for microglial markers, consistent with annotation.

3.6 Cross-model comparison: *Cd14/Tlr2* not synchronously up-regulated in the facial nucleus model (Figs. 7, 8)

The same scoring scheme was repeated in the facial axotomy model: its acute microglial response was dominated by "antigen presentation plus complement" (Day 7 ipsilateral vs Day 0: antigen presentation + 0.26, complement + 0.18, interferon + 0.07, all passing the unified-downsampling sign-consistency test, same-mouse paired $n = 3$); the IL1_TLR axis showed no up-regulation (Day 7 full depth - 0.02, downsampled + 0.06, Day 30 + 0.02, all near zero (the MVN's + 0.30/+0.31 is 5–15 times larger); the two models' module composition was negatively correlated (Spearman = - 0.81, $n = 7$).

Gene-level directional comparison: baselined on each model's own control, *Cd14* in the MVN contralateral/normal was 2.74 and in the facial nucleus lesioned/contralateral 0.84; *Tlr2* was 1.71 and 0.73, respectively (the two genes markedly up-regulated in the MVN showed no synchronous up-regulation in the facial nucleus).

Boundary of this comparison: the MVN's VN is the normal control, whereas the facial nucleus "contralateral side" is the uninjured side of the same mouse, so 0.84 measures the ipsilateral lesion relative to the ipsilateral uninjured side; baselined on Day 0, *Cd14* 1.13 and *Tlr2* 1.07, i.e., the facial nucleus showed mild up-regulation. Its median UMI was only 126–353, and the two genes' detection (38%–62% and 11%–23%) was sufficient to support "no marked up-regulation" but not "true down-regulation", so only a directional conclusion is claimed; *Il1b* was in the same direction in both models (5.48 and 3.75) but detection was very low (0.4%–1.3%) and not used as independent evidence. All cross-model comparison values are in Supplementary Material S8.

4. Discussion

4.1 Main findings and literature comparison

We found the microglial IL1_TLR module was highest among the 7 inflammatory pathways and directionally consistent across three depth settings; per-gene attribution showed 70% of the increment came from *Cd14* (45%) and *Tlr2* (24%), and the classical IL-1 receptor axis was unmeasurable because

Il1r1 detection was 0.6%. Our "IL1_TLR module" is thus a *Cd14/Tlr2* two-gene program rather than IL-1 receptor pathway activation (a restriction that provides actionable targets) for protein and intervention studies.

Previous bulk-level studies reported up-regulated inflammatory genes in the vestibular nucleus after lesion^[8, 9, 22], but tissue homogenization left "inflammation" without a cellular subject. At single-cell resolution we confined the main cell type to microglia and the main signal to *Cd14/Tlr2* rather than pan-inflammation: type I interferon was flat, IL-18 and chemokines were less than half its magnitude, and complement and antigen presentation failed the depth-consistency criterion.

Through triple evidence (module scoring, rat bulk-level, and single-cell threshold analysis), we did not support Hypothesis 2 and propose the revised Hypothesis 2': if the compensatory value of peripheral IL-18 holds, the mechanism should point to post-transcriptional steps (caspase-1 cleavage to mature IL-18, IL-18-binding protein neutralization) or a peripheral immune-cell origin, not an expected central transcriptional up-regulation; this defines the object, sampling site, and expected direction of testing.

From the otology-clinical view, this study narrows the molecular object of vestibular compensation from "whole tissue" to a two-gene program in microglia, offering candidate directions for monitoring compensation after canalith repositioning and in the acute phase of vestibular neuritis: the timing of anti-inflammatory intervention in the compensation period should be based on the microglial activation window, and peripheral marker screening should prioritize the co-changing *Cd14/Tlr2* combination rather than IL-18 alone. We note this is a public-data secondary analysis and the above inference lacks case validation. Contralateral MVN communication was dominated by reparative ligands, and activation markers were highest in ULC (qualitative), consistent with "rebalancing" evidence^[23, 24]; the global communication background was weakened (only 25%–38% positive), with the ipsilateral *Cd14-Itgb1* enhancement as the exception.

4.2 Methodological implications and limitations

The four-tier design demonstrated the bias chain in data with a 3.7× inter-group depth difference (8.7× stratified): "predominantly down" (81.2%) was mainly produced by depth differences, returning close to balance (50.4%) only after uniform downsampling, so directional conclusions across conditions should report the up/down proportion before and after depth matching. This study produced three reusable resources (a catalog of 1,043 depth-consistent differentially expressed genes; module scores with depth-consistency records; and a script chain with parameters and random seed); peripheral IL-18 marker research gained the restriction "central corresponding event", and the coexistence of a "reparative window and an inflammation-amplification circuit" on the lesioned side suggests the key to acute-phase intervention is "when to shift to pre-repair".

This study has the following limitations. (1) It is a single-time-point acute-phase secondary analysis and cannot track compensation outcomes; the pooled-design prevents estimating inter-individual variation. (2) No independent biological replicate (one library per condition); cell-level *P* values are small, so effect

size is prioritized and conclusions still need independent-cohort testing. (3) Depth harmonization sacrifices statistical power (CDU significant genes only 11%–19% of FD), weak effects may be missed, and module-level conclusions use "sign consistency across 3 depth settings" as the threshold. (4) No doublet or ambient-RNA correction (27.6% of retained cells fell in the high-risk range; *Ttr* etc. were highly expressed in non-corresponding clusters, so conclusions must be cautious). (5) The neuronal population is unreliable (VN only 6 cells, median UMI 6.3× higher in lesion groups); all related communication axes were removed and no claim is made on neuronal molecular response or communication remodeling. (6) Communication and cell composition are described qualitatively only (binary-set Jaccard as low as 0.008 and not multiple-corrected; gain/loss judged by *P* value while expression product only ranked, the two may disagree (see Supplementary Table S3 note). (7) Cross-model comparison baselines are not comparable and only directional hints are given; this dataset's median UMI 126–353 means "IL1_TLR not up-regulated" is a negative reading under detection power. (8) The two datasets differ in five respects (species, platform, time point, sampling scope, count version). (9) Transcript ≠ protein; no spatial information or wet-lab validation. (10) IL1_TLR signal concentrated in 2 genes (70% of increment) and *Il1r1* detection 0.6% made the classical IL-1 receptor signal unmeasurable, so conclusions are limited to the *Cd14/Tlr2* program.

5. Conclusions

After unilateral vestibular loss, the early molecular response of the mouse medial vestibular nucleus is dominated by a microglial *Cd14/Tlr2* program rather than the classical IL-1 receptor pathway (*Il1r1* detection 0.6% makes it unmeasurable); cell communication has a globally weakened background, with a direction-robust gained axis represented by *Cd14-Itgb1* on the lesioned side. Cross-model directional comparison suggests this up-regulation is associated with the vestibular-loss context, but is limited by baseline definition and detection power and does not constitute a cross-model quantitative conclusion; the IL-18 family showed no marked transcriptional up-regulation and Hypothesis 2 was not supported.

This study quantitatively shows that the direction of differential expression depends strongly on sequencing depth control: 70% of the IL1_TLR increment concentrated in 2 genes, and 5 communication axes involving unreliable populations were removed overall, indicating that acute-phase single-cell pathway "dominance" conclusions must rest on per-gene attribution and depth-sensitivity testing. We thereby provide a methodological note and a reusable pipeline for acute-phase single-cell research, a cell-resolution framework for the mechanism of vestibular compensation, and cell-origin and evidence-grade restrictions for *Cd14/Tlr2* and IL-18 peripheral-marker research.

Declarations

Ethics approval

This study is a secondary analysis of publicly available datasets (GSE143701, GSE32437, and GSE90975) obtained from the NCBI Gene Expression Omnibus. The original studies were approved by

the respective institutional animal-care committees; no new animals were used and no additional ethical approval was required for this re-analysis.

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Author Contribution

Liling Cui: conceptualization, methodology, formal analysis, investigation, writing—original draft, writing—review and editing. Wenjun Qi: supervision, project administration, funding acquisition, writing—review and editing. Both authors read and approved the final manuscript.

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Data and code availability

This study generated no new raw data; the single-cell RNA-seq datasets GSE143701, GSE32437, and GSE90975 were obtained publicly from the NCBI Gene Expression Omnibus (<https://www.ncbi.nlm.nih.gov/geo/>). The reproducible script chain is provided in Supplementary Material S6.

Supplementary Material

- S1 Full matrix of inflammatory module changes
- S2 Full gene lists of the 7 module gene sets
- S3 Complete differential-expression lists
- S4 Complete cell-communication list (including the 8 removed axes with raw values, depth tests, and removal rationale)
- S5 Cell-level metadata and quality control
- S6 Reproducible scripts (runtime environment and random-seed description)
- S7 Quantitative values of marker dot plots

- S8 All values and scripts for the cross-model comparison (GSE90975), including ratios under both Day-0 and same-day-control baselines
- S9 Technical roadmap
- S10 Depth-sensitivity details of communication axes under 4 downsamplings (raw/1,200/900/700 counts) and ligand-side within-depth re-computation
- S11 Reliability tests of neuron-related communication axes (3 as source, 2 as target): population size and depth verification, ligand/receptor re-computation within the shared depth interval (UMI 900–5,400), removal list and rationale

Note: Fig. S9 shows the technical roadmap; the main color figures (Figs. 1–8) are provided as separate high-resolution PNG/PDF files for submission.

Conflict of interest

The authors declare no conflict of interest.

AI assistance statement

During the preparation of this manuscript, the authors used a generative-AI tool to assist with language drafting and formatting. The scientific content, data analyses, and all conclusions were authored by the authors, who take full responsibility for the final content.

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Figures

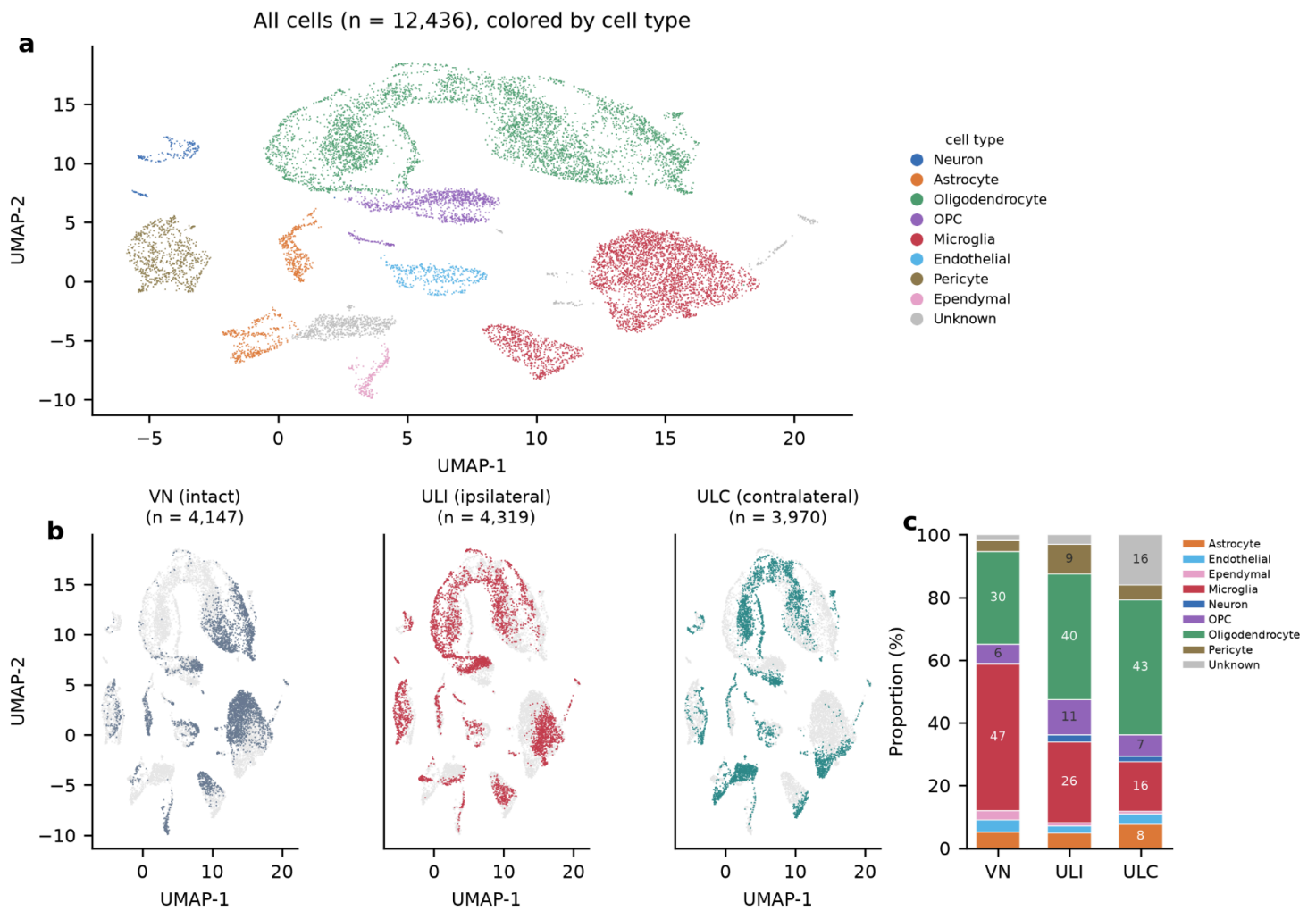


Figure 1

Single-cell landscape and cell-type composition of the mouse MVN after unilateral vestibular loss. A. UMAP projection of 12,436 cells from the VN, ULI, and ULC groups, coloured by cell type. B. Cell distribution stratified by condition. C. Relative composition of the 8 cell types (an unknown cluster is listed separately) across the three groups. Because sequencing depth differs markedly among the three groups and no batch integration was performed, differences in cell proportions are presented descriptively only.

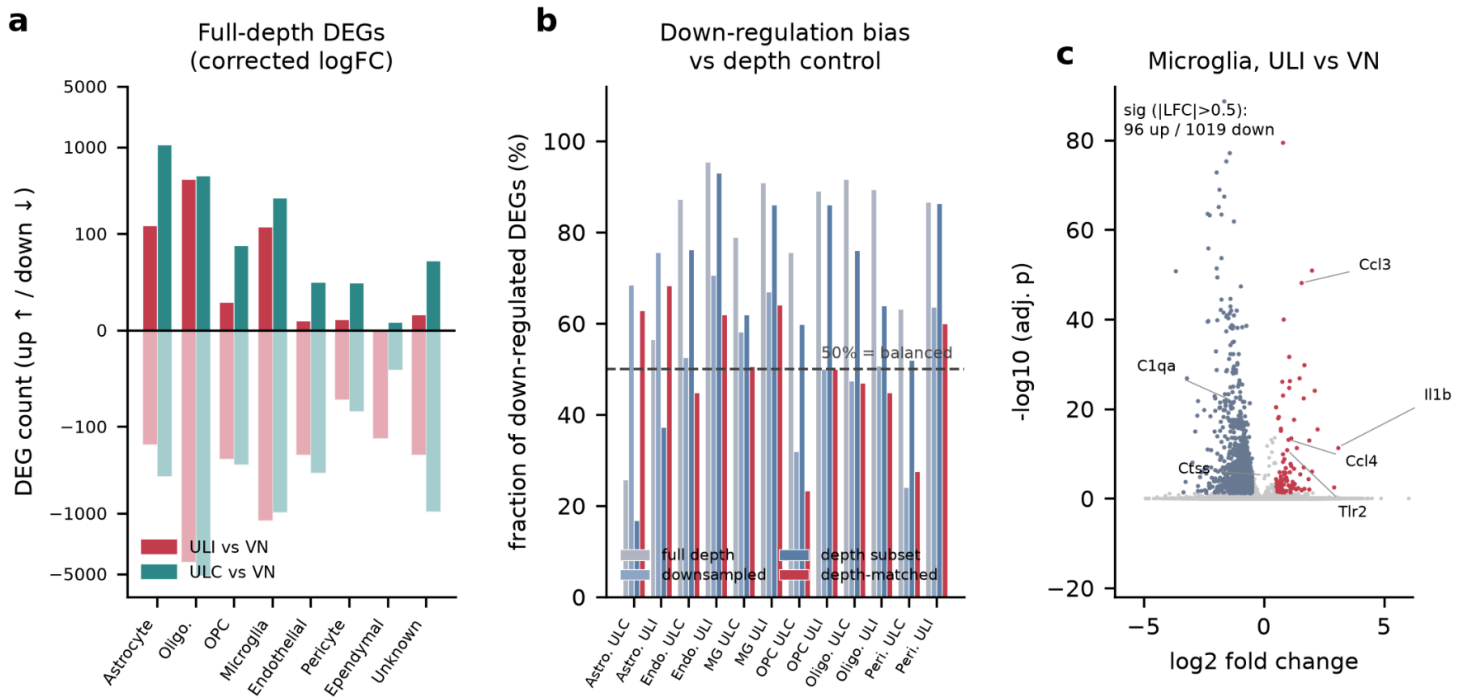


Figure 2

Cell-type-resolved differential expression and depth control. A. Number of differentially expressed genes per cell type for ULI vs VN and ULC vs VN (full depth; $P < 0.05$ after correction and $|\log_2\text{FC}| > 0.25$; the y axis uses a symmetric log scale). B. Proportion of down-regulated genes under the 4-level depth control (FD/DS/CDS/CDU); the dashed line marks the 50% balance point (81.2% at full depth, reduced to 50.4% under CDU). C. Contraction of the differential gene sets after depth control for each cell type (Jaccard index); oligodendrocytes show the most marked contraction.

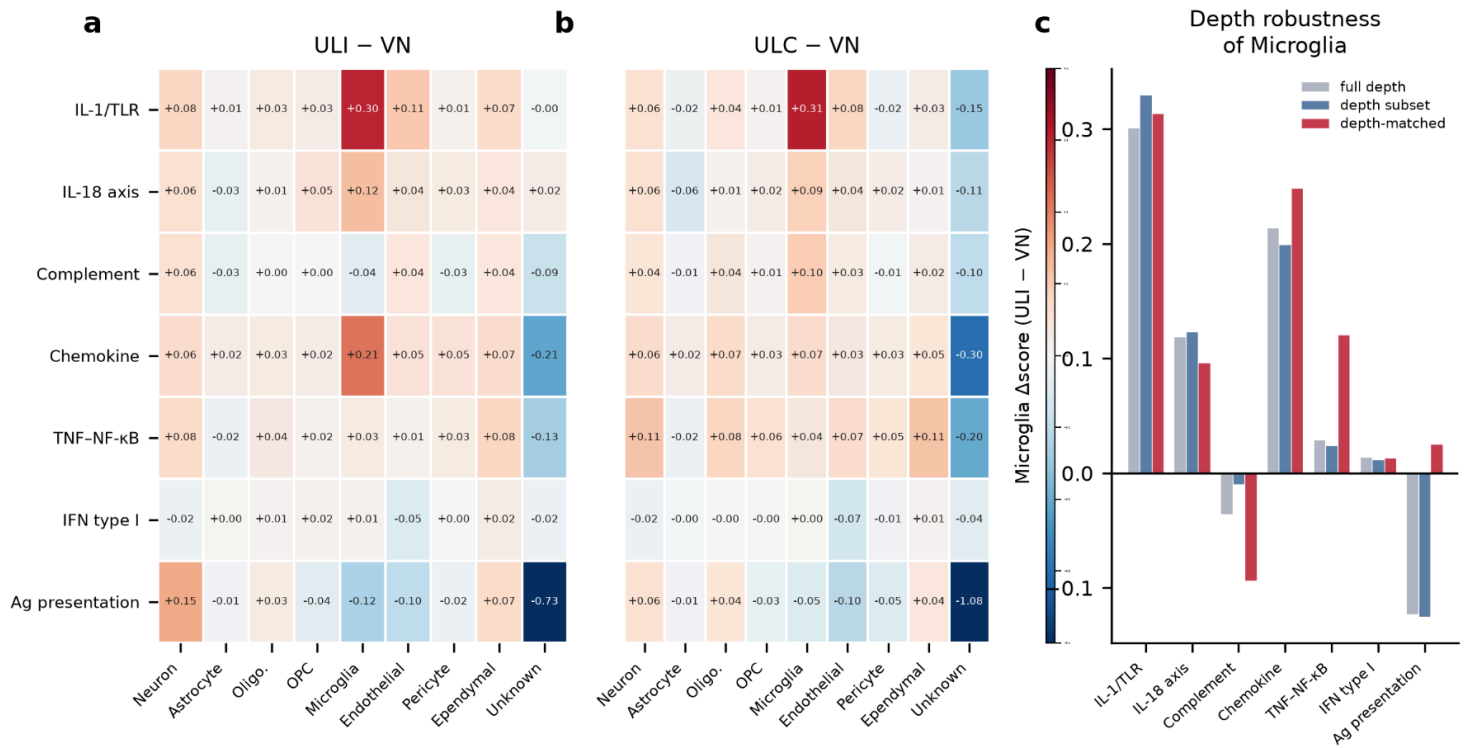


Figure 3

Cell type × inflammatory pathway module scores and depth consistency. A, B. Mean changes of the 7 inflammatory pathways in ULI and ULC relative to VN; the microglial IL1_TLR module is highest (+0.30/+0.31), with per-gene attribution shown in Fig. 8. C. Comparison of microglial module changes across the 3 depth settings. Complete values are given in Supplementary Table S1.

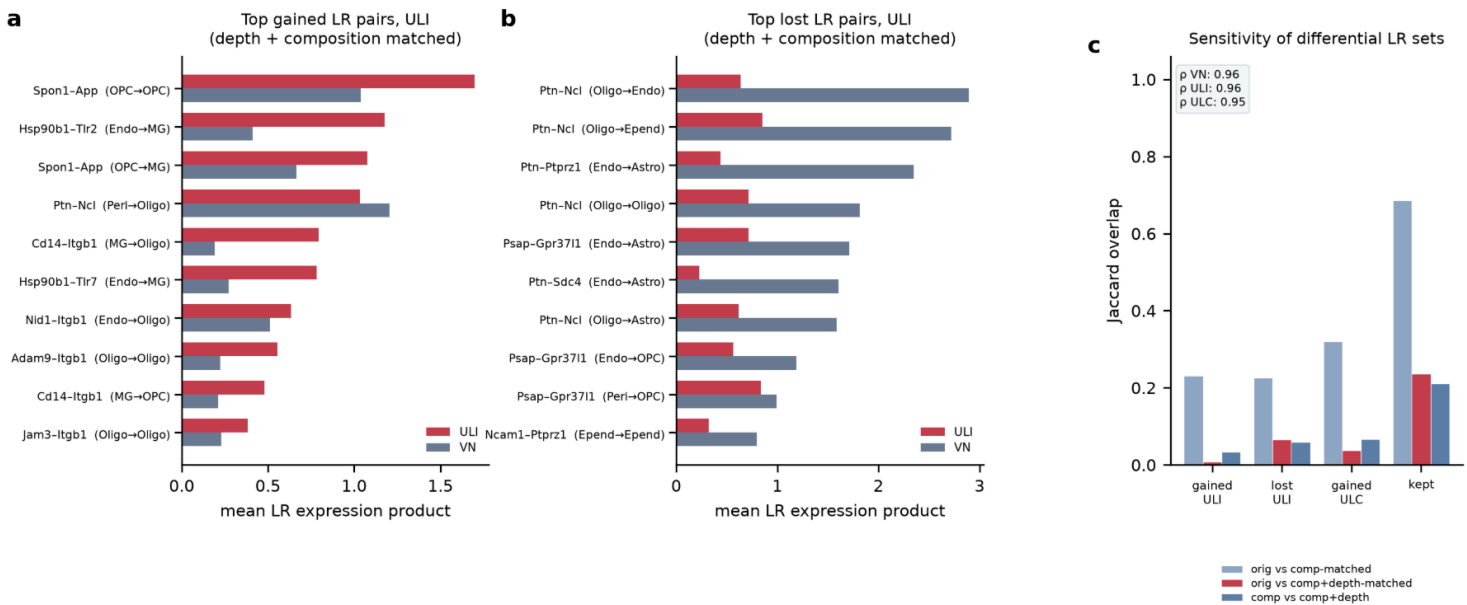


Figure 4

Differential cell-cell communication between groups (composition- and depth-matched). A. Directionally robust gained axes in ULI relative to VN (Supplementary Table 3A, 8 axes). B. Top 12 diminished axes (Supplementary Table 3B, all changes negative); bar length denotes expression-product magnitude. C. Set-level Jaccard consistency across the 3-level design (D0/D1/D2'); binary counts are unstable (Jaccard index as low as 0.008), whereas magnitude correlation coefficients reach 0.940–0.984.

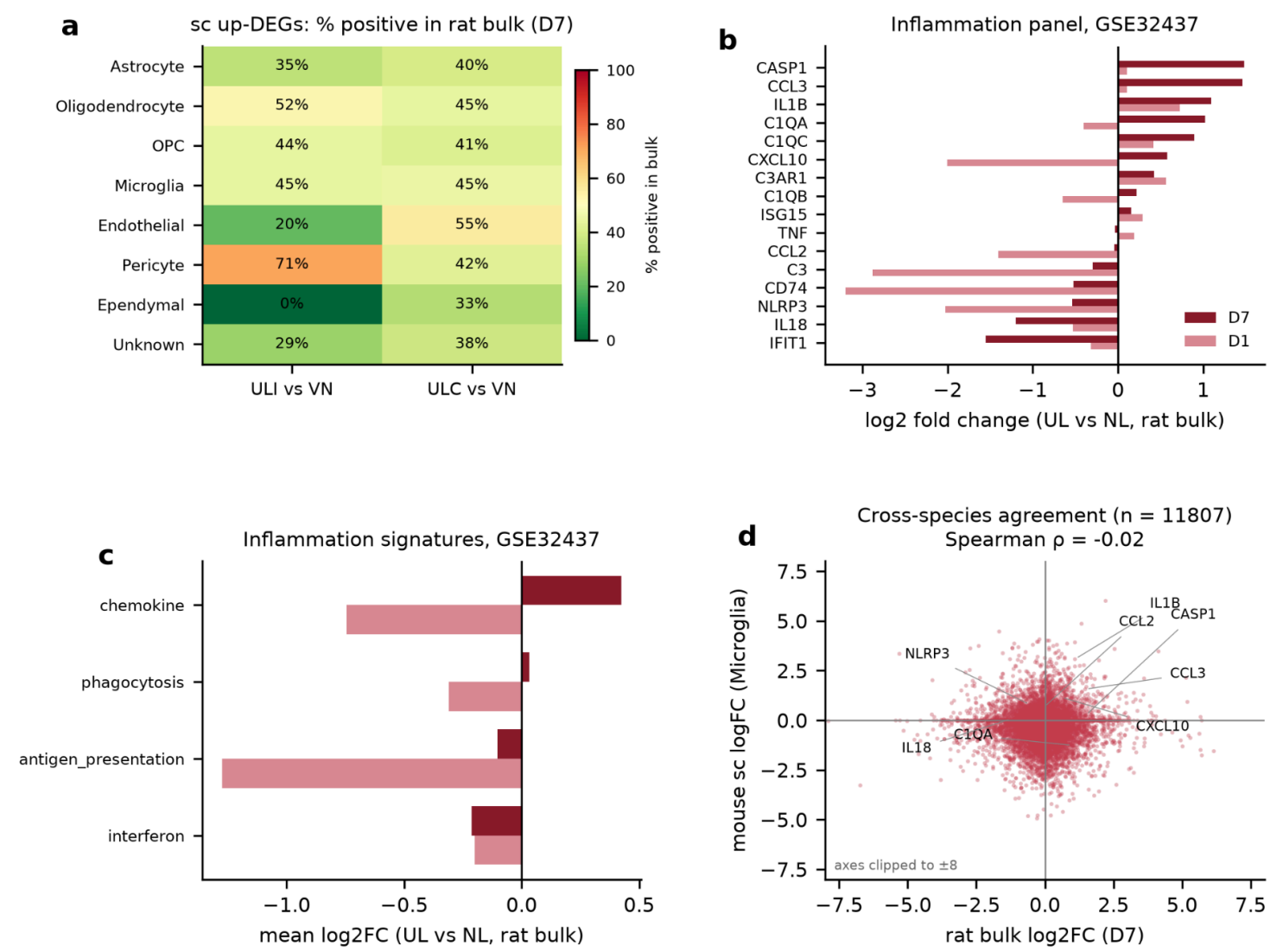


Figure 5

Cross-dataset concordance analysis (GSE32437 rat bulk data, D1/D7). A. Heat map of the direction-concordance rate of single-cell up-regulated genes in the rat data at D7. B. log₂FC of 18 inflammatory core genes at D1 and D7. C. Mean log₂FC of 4 pathway features. D. log₂FC scatter across the whole transcriptome (n = 11,807; Spearman correlation coefficient = -0.02).



Figure 6

Marker validation of cell types and the microglial phenotype. A. Matrix of canonical markers of the 8 cell types against their annotated identities (colour indicates the within-row z-score of mean expression; point size indicates the fraction of expressing cells). B. Changes in microglial homeostatic and activation-related markers across VN/ULI/ULC. C. Cluster-level diagnostic panel: neuronal clusters

(20/22) highly express neuronal markers, whereas microglial markers are negative. Values are given in Supplementary Table S7.

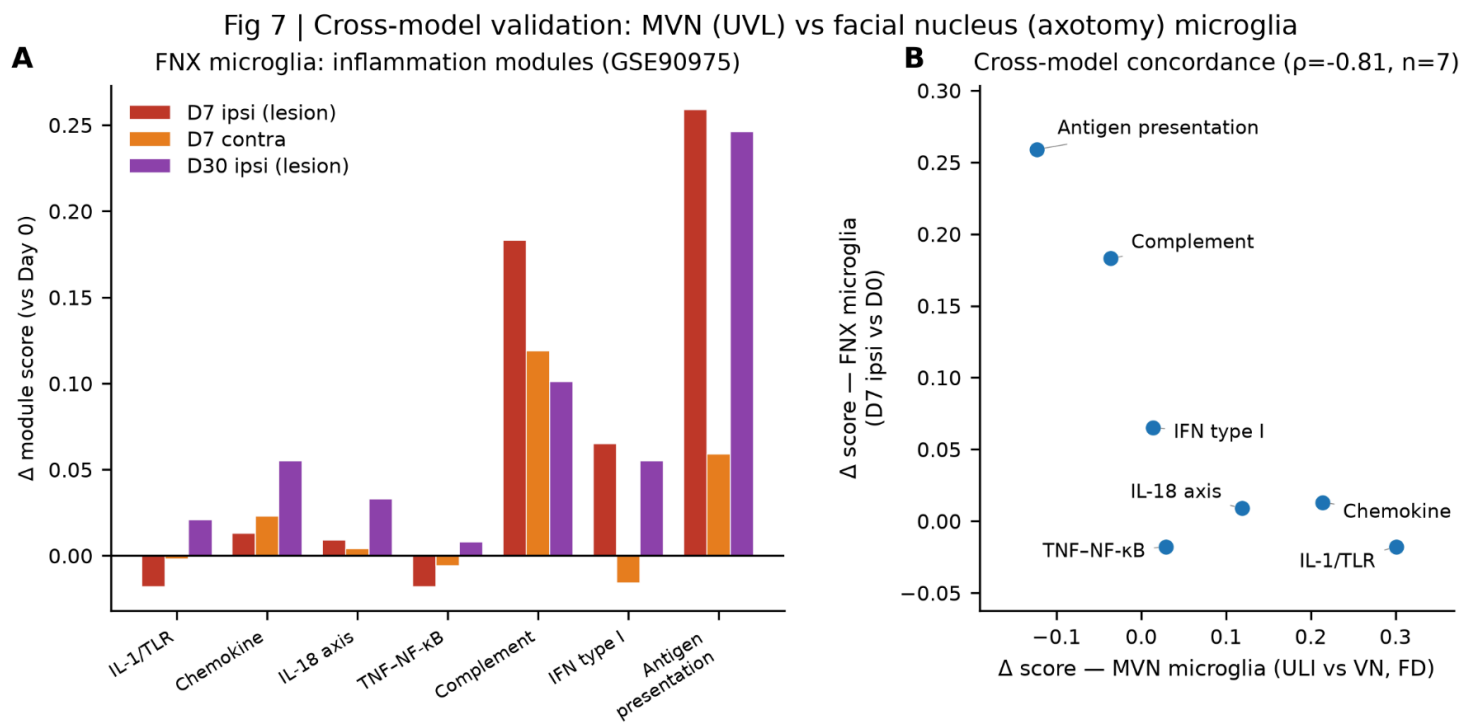


Figure 7

Cross-model comparison of microglial inflammatory modules between the MVN (vestibular loss) and the facial nucleus (axotomy) (GSE90975). A. Changes of the 7 inflammatory pathways in the facial nerve axotomy model (relative to Day 0): Day 7 ipsilateral, Day 7 contralateral and Day 30 ipsilateral. On the ipsilateral side, antigen presentation (+0.26) and complement (+0.18) predominate, whereas IL1_TLR is near zero. B. Comparison of module composition between the two models (Spearman correlation coefficient = $-0.81, n = 7$).

Fig8 Per-gene attribution of *Cd14*/*Tlr2* and cross-model directional comparison

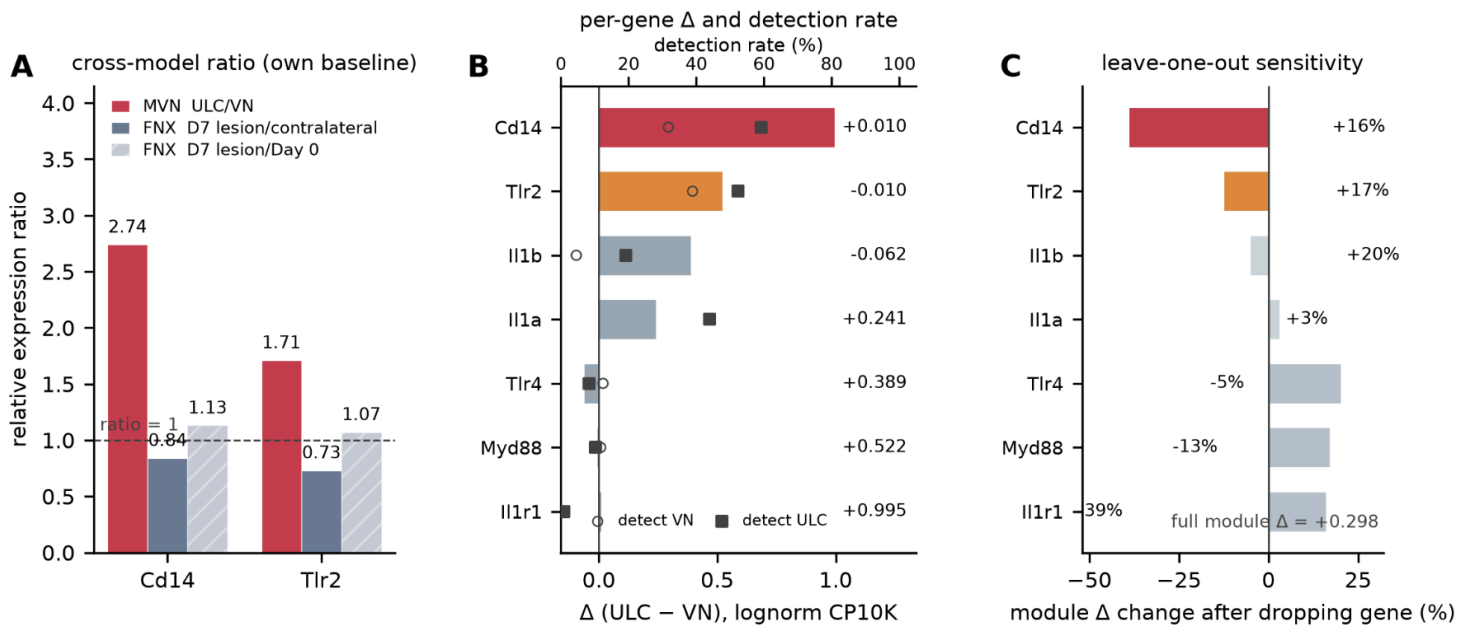


Figure 8

Per-gene attribution of *Cd14*/*Tlr2* and cross-model directional comparison. A. Relative expression ratios of *Cd14* and *Tlr2* in MVN microglia (ULC vs VN) and in the facial nucleus (Day 7 lesioned side vs contralateral side), each baselined on its own within-model control. Ratios baselined on Day 0 in the facial nucleus are additionally shown (hatched) to illustrate the influence of baseline definition; the dashed line indicates 1. B. Per-gene changes of the 7 IL1_TLR module genes in microglia (from VN to ULC) and the shift in the module change value after leave-one-out removal; *Cd14* (45%) and *Tlr2* (24%) together account for 70% of the increase.

Supplementary Files

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