
Supplementary information

Single-cell and spatial atlases of spinal cord injury in the *Tabulae Paralytica*

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Supplementary Notes

Supplementary Note 1 | Cell type annotation

The immune compartment comprised 106,619 immune cells spanning both central nervous system-resident and infiltrating immune cells, which are known to coordinate sequential phases of the acute response to SCI (Fig. 2a and Supplementary Fig. 6a-c)¹. *P2ry12*-expressing homeostatic microglia were transcriptionally distinguishable from reactive microglia, which expressed *Tgfb β 1*. Peripheral lymphocytes comprised B cells, NK cells, and CD8 T cells, whereas myeloid cells subdivided into proliferating and fate-committed subpopulations, as well as neutrophils, macrophages, and dendritic cells. Macrophages encompassed border-associated macrophages (expressing *H2-Aa*) that mediate immune responses in perivascular spaces², as well as distinct inflammatory (*Ifi211*), chemotaxis-inducing (*Cd300a*), and thrombospondin-sensing (*Htr2b*) subpopulations³.

The astroependymal compartment comprised 25,211 nuclei that were subclustered into grey matter-resident protoplasmic astrocytes (*Gpc5*, *Gfap*^{low}), white matter-resident fibrous astrocytes (*Vim*, *Gfap*^{high}), reactive subpopulations that we subdivided based on the expression of *Aldoc*, *Tmem47*, and *Gja1*, and ependymal cells (*Dnah12*) (Fig. 2c and Supplementary Fig. 7a-g). Expression of these marker genes suggested continuous gradients in astrocyte transcriptional phenotypes, rather than the existence of discrete subpopulations (Supplementary Fig. 7a,c-g).

The vascular compartment comprised 40,620 cells, which subdivided into endothelial cells, vascular leptomeningeal cells (VLMCs, *Pdgfra*^{high})^{4,5} and pericytes (Fig. 2e and Supplementary Fig. 8a-c). Endothelial cells were further subdivided by their arteriovenous zonation, with distinct arterial (*Emcn*), capillary (*Meox1*), and venous (*Slc38a5*) subpopulations. VLMCs, which are fibroblast-like cells located between astrocyte end-feet and endothelia that express fibril-forming collagens^{4,5}, were further separated into homeostatic and extracellular matrix-forming subpopulations, as well as arachnoid barrier cells (*Slc47a1*) that are involved in cerebrospinal fluid maintenance^{4,6}.

The oligodendrocyte lineage comprised 182,334 cells that were distributed along a continuous developmental trajectory, spanning oligodendrocyte precursor cells (OPCs), differentiation-committed oligodendrocyte precursors (COPs), newly formed oligodendrocytes (NFOLs), myelin-forming oligodendrocytes (MFOLs), and mature oligodendrocytes (MOLs) (Fig. 2g and Supplementary Fig. 10a-c)^{4,7}.

Neuronal subpopulations were parcellated into dorsal versus ventral, excitatory versus inhibitory, and local (*Nfib*) versus long-projecting (*Zfhlx3*) populations⁸. We identified well-studied dorsal excitatory (*Tac1*, *Reln*, *Cck*) and inhibitory (*Rory*, *Npy*, *Gal*) subpopulations, as well as dl5/dIL deep dorsal subpopulations expressing *Lmx1b*^{4,9-11}. A group of ventral neuron subpopulations included developmentally defined V0, V1, V2, V3 (*Sim1*) neurons^{10,12}; Renshaw cells (*Calb1*, *Chrna2*, *Slco5a1*)^{13,14}; cerebrospinal fluid-contacting neurons (CSF-N; *Pdk1l2*)^{4,15,16}; and motor neurons (*Isl1*)¹⁷. V0 neurons subclustered into V0c (*Chat*, *Pitx2*)¹⁸, V0v (*Evx1*)^{19,20}, V0g (*Slc17a6*, *Pitx2*)¹⁸, and V0d (*Chat*, *Evx1*^{OFF}, *Gabra1*)^{19,20} subpopulations. V1 (*En1*) neurons subclustered into four subpopulations expressing *Pou6f2*, *Foxp2*, *Mafa*, and *Sp8*, respectively²¹. Within V2 neurons, we identified one inhibitory subpopulation of V2b neurons expressing *Gata2* and *Gata3*^{22,23}, as well as multiple subpopulations of developmentally defined V2a neurons (*Vsx2*)^{10,24-27} that could be separated into local (*Nfib*) and long-projecting (*Zfhlx3*) subpopulations⁸.

Supplementary Note 2 | Growth-facilitating and inhibiting molecule expression

Our snRNA-seq atlas provides a resource to explore the production of inhibitory and facilitating molecules across the entire repertoire of cell types in the injured spinal cord. For example, we and others previously showed that laminins provide a permissive substrate for axon growth²⁸⁻³⁰, but the cells that express these molecules have not been identified. We found that laminins (*Lama1*, *Lama2*, *Lama4*, *Lama5*) were expressed primarily by VLMCs, identifying these cells as a target to upregulate the expression of axon growth-supporting molecules for spinal cord repair (Supplementary Fig. 12a-d). A second example comes from the family of chondroitin sulfate proteoglycans (CSPGs), which are known to play contrasting roles in inhibiting or supporting axon growth^{28,29,31-34}. Among inhibitory CSPGs, we found that OPCs are responsible for the expression of *Acan*, *Vcan*, and *Ncan*, whereas astrocytes were the dominant producers of *Bcan* (Supplementary

Fig. 12e-h). OPCs were also the dominant producer of axon growth-supportive CSPGs, such as *Cspg4* and *Cspg5* (Supplementary Fig. 12i-j).

Various studies reported that the enzyme ChABC can digest CSPGs³⁵⁻³⁹, which are a main constituent of perineuronal nets^{40,41}, and that this digestion may open opportunities for circuit reorganization^{35,36,42,43}. We employed cell type prioritization to identify the cell types that were most transcriptionally perturbed by ChABC treatment, which pointed to CSPG-producing cell types including OPCs and VLMCs (Supplementary Fig. 12k). We then asked how ChABC influences genes associated with circuit reorganization. After ChABC administration, neurons upregulated genes associated with focal adhesion, as previously described⁴⁴, including *Itgav*, *Sorbs3*, *Itgb5*, and *Ilk* (Supplementary Fig. 12l-m). Despite these changes, however, we did not detect differential expression of the circuit reorganization module (Supplementary Fig. 12n-o). This data raises the possibility that the axonal sprouting that has been observed after ChABC treatment^{35,37,39,45} may involve local transcription outside the nucleus, or transcription-independent mechanisms. Notwithstanding these possibilities, high-resolution kinematics showed no significant benefit for the recovery of walking (Supplementary Fig. 3e).

Collectively, these results illustrate how our snRNA-seq atlas can be used as a resource to identify the cell-types that produce growth-promoting or inhibitory molecules following SCI, and dissect cell-type-specific responses to potential therapies that target these molecules.

Supplementary Note 3 | Sexually dimorphic responses to SCI are subtle

We applied cell type prioritization to evaluate whether specific cell types are differentially perturbed by SCI in male versus female mice. As anticipated, this analysis prioritized immune cell types as having the most prominent sex-dependent transcriptional differences (Supplementary Fig. 14a). Moreover, comparison of immune cell proportions revealed an increase in NK cells within female mice, consistent with established sex differences in the adaptive immune response to neurotrauma^{46,47} (Supplementary Fig. 14b-c).

Despite these dimorphic immune responses, however, high-resolution kinematics failed to demonstrate any sexual dimorphism in neurological recovery (Supplementary Fig. 3g). Moreover, the average magnitude of the transcriptional perturbation between male and female mice was among the most subtle in our snRNA-seq atlas (Supplementary Fig. 14d). Accordingly, we failed to detect sex differences in the overall proportion of surviving neurons (Supplementary Fig. 14e-f).

Taken together, these results suggest that sexual dimorphism does not impact early neuronal death or survival, and consequently, has no detectable influence on neurological recovery⁴⁸.

Supplementary Note 4 | Distinct molecular programs between lesion compartments

To identify molecular differences between lesion compartments, we demarcated spatial barcodes corresponding to the fibrotic scar, the astrocyte barrier, and the adjacent neural tissue (Fig. 6c and Supplementary Fig. 28). We then performed DE analysis to identify genes specific to each lesion compartment (Fig. 6d-e and Extended Data Fig. 6). This analysis showed that the fibrotic scar was differentiated from other lesion compartments by upregulation of extracellular matrix molecules (*Col6a3*, *Col4a2*) and matrix metalloproteinases (*Mmp2*, *Mmp14*), and a sustained expression of cytokines (*Cd74*, *Pf4*) and complement proteins (*C1s1*, *C1ra*). The astrocyte barrier was differentiated by genes associated with cytoskeletal organization (*Tubal1c*, *Arpc1b*) and cell proliferation (*Fxyd5*, *Id3*) at 7 days, and by genes related to radial glial fiber development (*Fabp7*), extracellular matrix molecules (*Ucma*, *Prelp*), synaptic reorganization (*Sctr*), and neuronal survival (*Rnase4*) at 2 months after SCI. Spared reactive neural tissue was differentiated by genes associated with neuronal functions, including solute transport (*Slc12a5*, *Kcnc2*), neuron growth and projection formation (*Nrsn1*, *Bend6*), and synapse maturation (*Scrt1*, *Nptx1*), whereas levels of growth factors and their receptors demonstrated little change.

We next sought to dissect the cellular composition of each lesion compartment by deconvolving the cell types within each spatial barcode⁴⁹ (Fig. 6f-h and Extended Data Fig. 7). We found that the fibrotic lesion core was composed almost exclusively of immune and vascular cells (Fig. 6g). The density of astroependymal cells abruptly increased towards the edge of the lesion, and thereafter gradually decreased with increasing distance from the

lesion core. As the distance from the lesion core increased, the proportions of each cell type approximated those observed in uninjured spinal cords, mirroring immunohistochemical observations^{50,51}. Immunohistochemistry validated the spatial localization of key cell types and subtypes inferred through cell type deconvolution (**Supplementary Fig. 29**).

Supplementary Note 5 | Validation of the three-dimensional spatial atlas

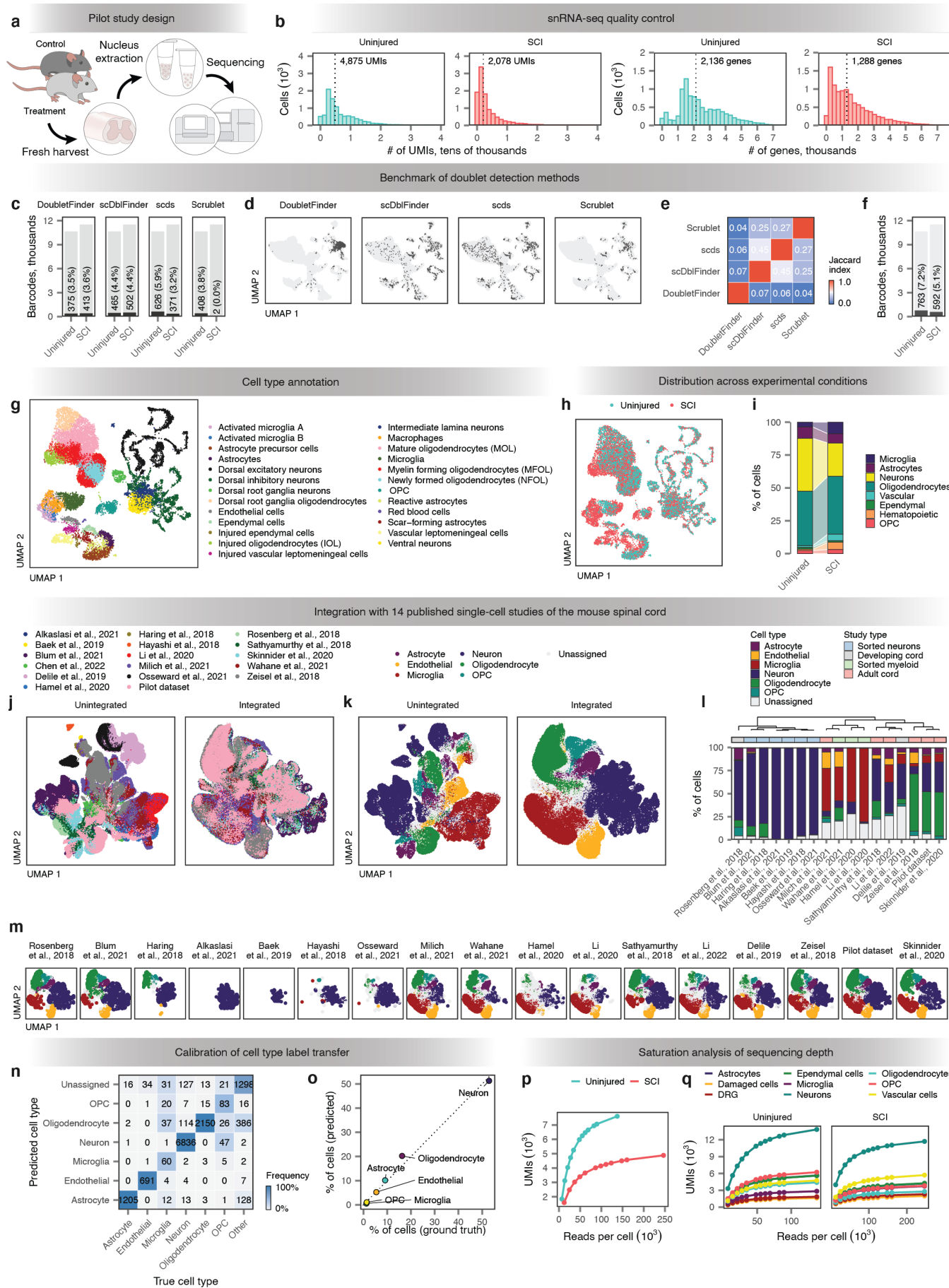
Three-dimensional projections of gene expression distinguished the canonical lesion compartments, including the fibrotic scar (*Col4a2*), the astrocyte barrier (*Gfap*), and spared but reactive neural tissue (*Slc12a5*; **Fig. 7b** and **Supplementary Fig. 36**). Moreover, neurons and immune cells occupied their appropriate locations in the injured spinal cord, whereby the fibrotic scar was invaded by peripheral immune cells and devoid of neurons. We next inspected the expression of well-studied inhibitory and facilitating molecules (**Extended Data Fig. 9**), finding the inhibitory CSPG aggrecan (*Acan*) was expressed within spared neural tissue that surrounded the lesion core, whereas brevican (*Bcan*) was expressed in both the lesion core and neural tissue. The growth-permissive molecule *Cspg4* was expressed predominantly in the lesion core, whereas *Cspg5* was expressed within the astrocyte barrier. Finally, the permissive extracellular matrix molecule *Lama1* was upregulated both within the lesion core and by border-forming astrocytes.

Supplementary Note 6 | Supplementary Discussion

The *Tabulae Paralytica* provide a resource that not only allowed us to resolve outstanding questions within the field of SCI, but also uncovered previously unknown biological principles. For example, we revealed a dichotomy between the early-conserved and late-diverging neuronal responses to SCI, the latter of which reflect differential capacity to upregulate genes associated with circuit reorganization across neuronal subpopulations. We found that this capacity is encoded in the basal transcriptional repertoires of each subpopulation prior to SCI, raising the possibility that specific neuronal subpopulations are primed to become circuit-reorganizing neurons after injury. Moreover, we show that the ability of neuronal subpopulations activate transcriptional programs involved in circuit reorganization is inversely correlated with the activation of cellular stress responses. Further studies will be needed to establish causal links between these transcriptional changes and neurological recovery. We also demonstrate that the profound impairment of neurological recovery after SCI in old mice reflects a failure to coordinate the formation of a tripartite neuroprotective barrier between immune-privileged neural tissue and extra-neural lesion environments. This failure leads to overwhelming immune infiltration into neural tissue and neuronal death, precluding neurological recovery. We built on this discovery to develop a new gene therapy strategy that reactivated border-forming cells, prevented lesion expansion, and improved neurological recovery in old mice. This mechanism-based therapeutic strategy opens new avenues to accelerate wound repair and increase neurological recovery in humans with SCI.

Our work also has limitations. We leveraged snRNA-seq to profile the injured spinal cord across an extensive set of experimental conditions, but these experiments took place in a single strain of inbred mice. Moreover, our multiome and spatial transcriptomics experiments were limited to a subset of these experimental conditions, and this may have limited the resolution with which we could overlay our snRNA-seq atlas onto a three-dimensional model of the injured spinal cord. We profiled spinal cords without specific enrichment or depletion protocols, which enabled broad coverage of the cellular responses to SCI, but limited our ability to dissect these responses in less abundant cell types. These limitations define opportunities to refine the *Tabulae Paralytica* in future work, for example by investigating how genetic differences modulate transcriptional responses to injury; by mapping the regulatory programs activated in response to injury at finer temporal resolution; or by targeting specific cellular subpopulations for more fine-grained characterization.

Supplementary figures



Supplementary Fig. 1 | Pilot study of snRNA-seq in the injured mouse spinal cord.

a, Schematic overview of experimental design.

b, Quality control (number of UMIs per cell and number of genes detected per cell) of single-nucleus libraries from injured and uninjured mouse spinal cords.

c-f, Detection and prevalence of cell doublets in the pilot study.

c, Number of doublets detected in each library by four computational methods for doublet detection.

d, Visualization of doublets detected by each of four computational methods on a UMAP plot of 22,157 cell barcodes from the mouse spinal cord.

e, Overlap between doublets identified by each method, as quantified by the Jaccard index. DoubletFinder calls a largely distinct set of cell barcodes as doublets, as compared to the other three methods.

f, Number and proportion of doublets detected in each library by the final method for doublet identification, comprising the union of doublet calls from scDbtFinder and scds.

g, UMAP visualization of 18,269 cells, revealing 25 transcriptionally distinct clusters of cells in the injured and uninjured mouse spinal cord.

h, UMAP visualization of 18,269 cells, colored by the experimental condition of origin for each cell (uninjured or SCI).

i, Proportions of eight major cell types of the mouse spinal cord detected in either experimental condition.

j, UMAP visualization of 420,786 cells, including the pilot dataset and sixteen published single-cell studies of the mouse spinal cord, colored by the study of origin. Left, before data integration with Seurat; right, after data integration.

k, As in **j**, but showing major cell types inferred by label transfer with Seurat.

l, Proportions of six major cell types, and unassigned cells, recovered by sixteen published single-cell studies of the mouse spinal cord and the pilot dataset. Datasets are hierarchically clustered by cell type composition and annotated according to the study type.

m, As in **k**, but showing cells from each study individually.

n-o, Calibration of the label transfer functionality used to assign coarse cell types in published studies.

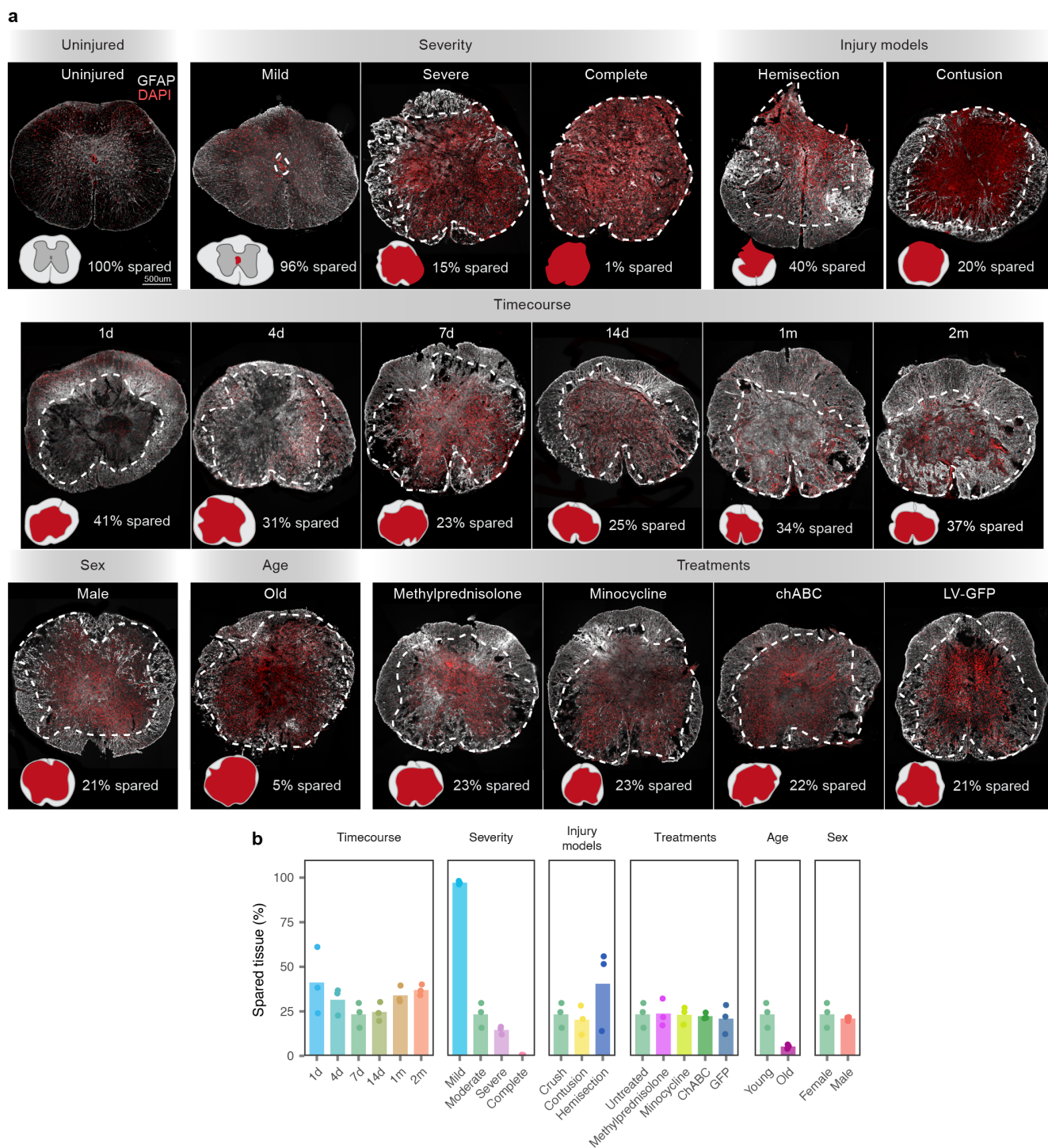
n, Concordance between cell types automatically assigned by label transfer and manually assigned by the authors of the original study in the Sathya-murthy et al. dataset.

o, Correlation between the cell type proportions recovered by label transfer and those manually assigned by the authors of the original study in the Sathya-murthy et al. dataset.

p-q, Analysis of the minimum sequencing depth required to detect cell-type-specific transcriptional changes in the injured mouse spinal cord.

p, Proportion of the complete UMI count matrix recovered after downsampling the number of reads in the injured and uninjured libraries, respectively, as compared to the complete dataset.

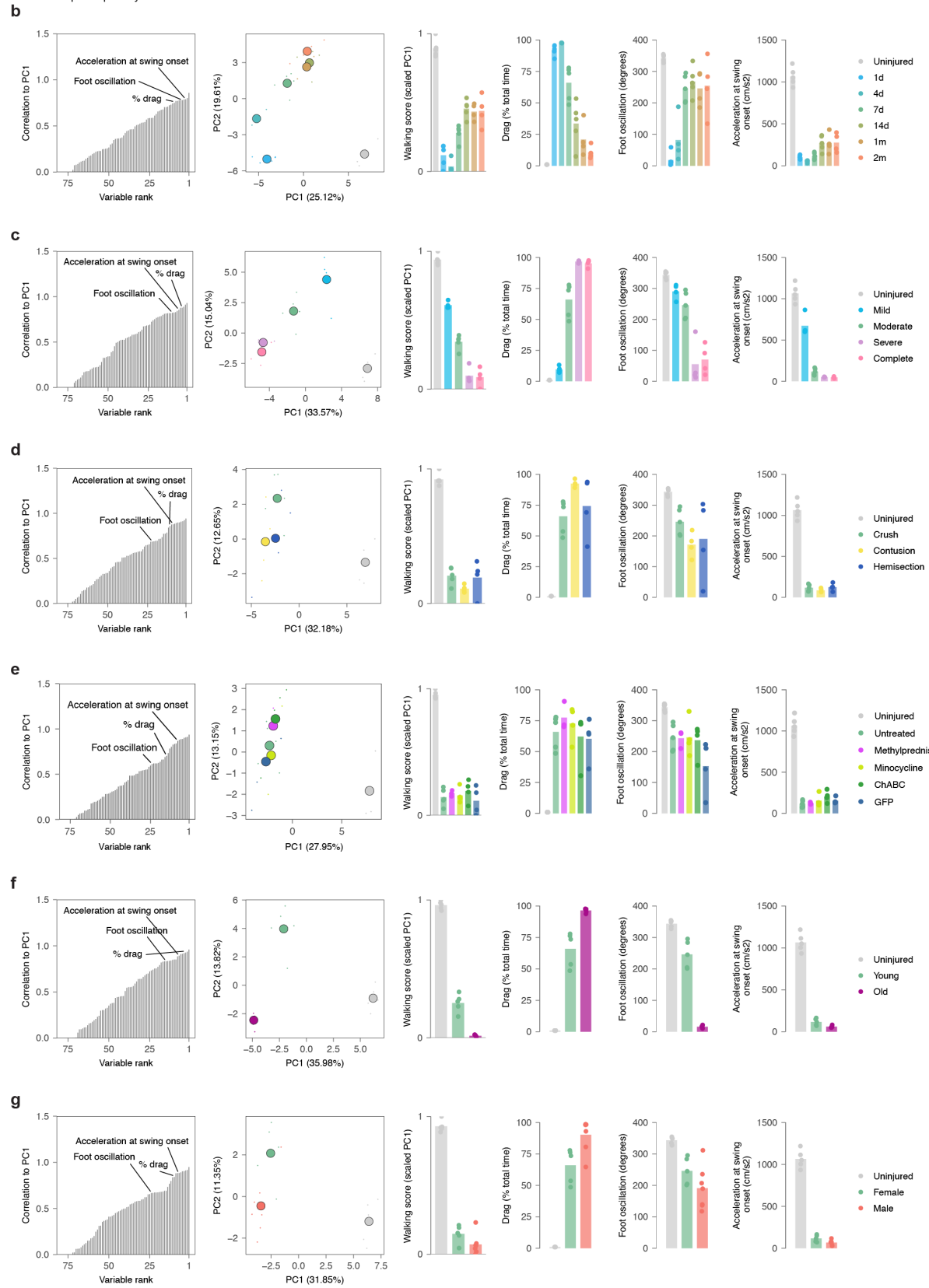
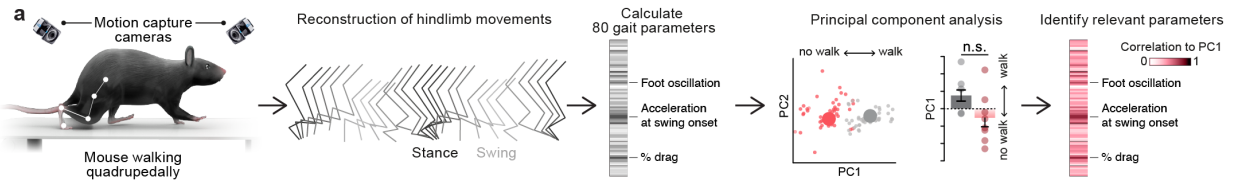
q, Proportion of the complete UMI count matrix recovered in each major cell type after downsampling the number of reads in the injured and uninjured libraries, respectively, as compared to the complete dataset.



Supplementary Fig. 2 | Representative histological images across experimental conditions.

a, Histological photomicrographs show two-dimensional views of injured spinal cords stained with glial fibrillary acidic protein (GFAP).

b, Quantification of the SCI lesion epicentre. The extent of spared spinal cord tissue was quantified from coronal sections immunolabeled against GFAP.



Supplementary Fig. 3 | High-resolution kinematic analysis across experimental conditions.

a, Summary of kinematic gait analysis workflow. Walking performance was evaluated during quadrupedal walking on a treadmill. Bilateral leg kinematics were captured with twelve infrared cameras and gait parameters were calculated from the recordings using markerless tracking software. All gait parameters are reported in **Supplementary Table 2**. Steps 3-6: To evaluate differences between experimental conditions, as well as to identify the most relevant parameters to account for these differences, we implemented a multistep multifactorial analysis based on principal component analysis. The first principal component (PC1) was used to summarize differences in walking performance, accounting for all parameters and the covariance between them. Finally, factor loadings on PC1 were used to identify the kinematic parameters with the most pronounced contribution to differences in locomotor performance between experimental groups.

b, Locomotor performance of mice across timepoints after SCI. Locomotor performance was quantified using principal component analysis applied to 55 gait parameters calculated from kinematic recordings. Small points show individual gait cycles ($n > 10$ per mouse, $n = 4-7$ mice per group). Large points show the mean of each experimental group. The first principal component (PC1) distinguished gaits from mice across different experimental groups. Analysis of factor loadings on PC1 revealed that the percentage of paw dragging, the extent of foot oscillation, and acceleration of the limb at swing onset were among the parameters that showed the highest correlation with PC1. Bars report the mean values of these gait parameters.

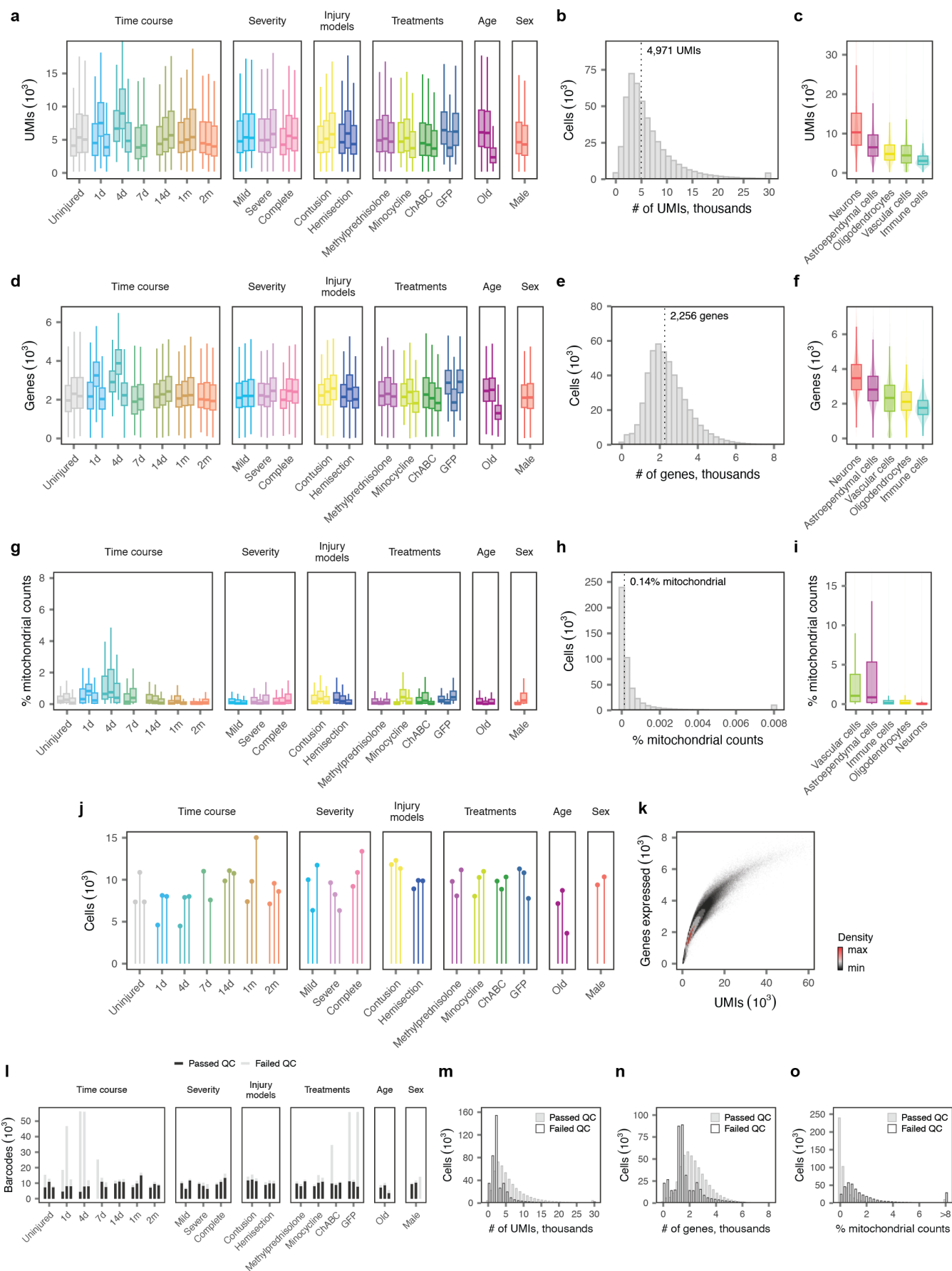
c, As in **b**, for mice subjected to different severities of crush SCI.

d, As in **b**, for mice subjected to different injury mechanisms.

e, As in **b**, for mice treated with various clinical and experimental interventions.

f, As in **b**, for old versus young mice.

g, As in **b**, for male versus female mice.



Supplementary Fig. 4 | Quality control of the snRNA-seq atlas.

a-i, Quality control statistics for 435,099 single-nucleus transcriptomes from the uninjured and injured mouse spinal cord.

a, Number of unique molecular identifiers (UMIs) per nucleus in individual libraries from each experimental condition.

b, Number of UMIs per nucleus, aggregated across all libraries. Inset text shows the median number of UMIs.

c, Number of UMIs per nucleus in each major cell type.

d, Number of genes detected per nucleus in individual libraries from each experimental condition.

e, Number of genes detected per nucleus, aggregated across all libraries. Inset text shows the median number of genes detected.

f, Number of genes detected per nucleus in each major cell type.

g, Proportion of mitochondrial counts per nucleus in individual libraries from each experimental condition.

h, Proportion of mitochondrial counts per nucleus, aggregated across all libraries. Inset text shows the median proportion of mitochondrial counts.

i, Proportion of mitochondrial counts per nucleus in each major cell type.

j, Number of nuclei passing quality control in each of the 52 libraries comprising the snRNA-seq atlas.

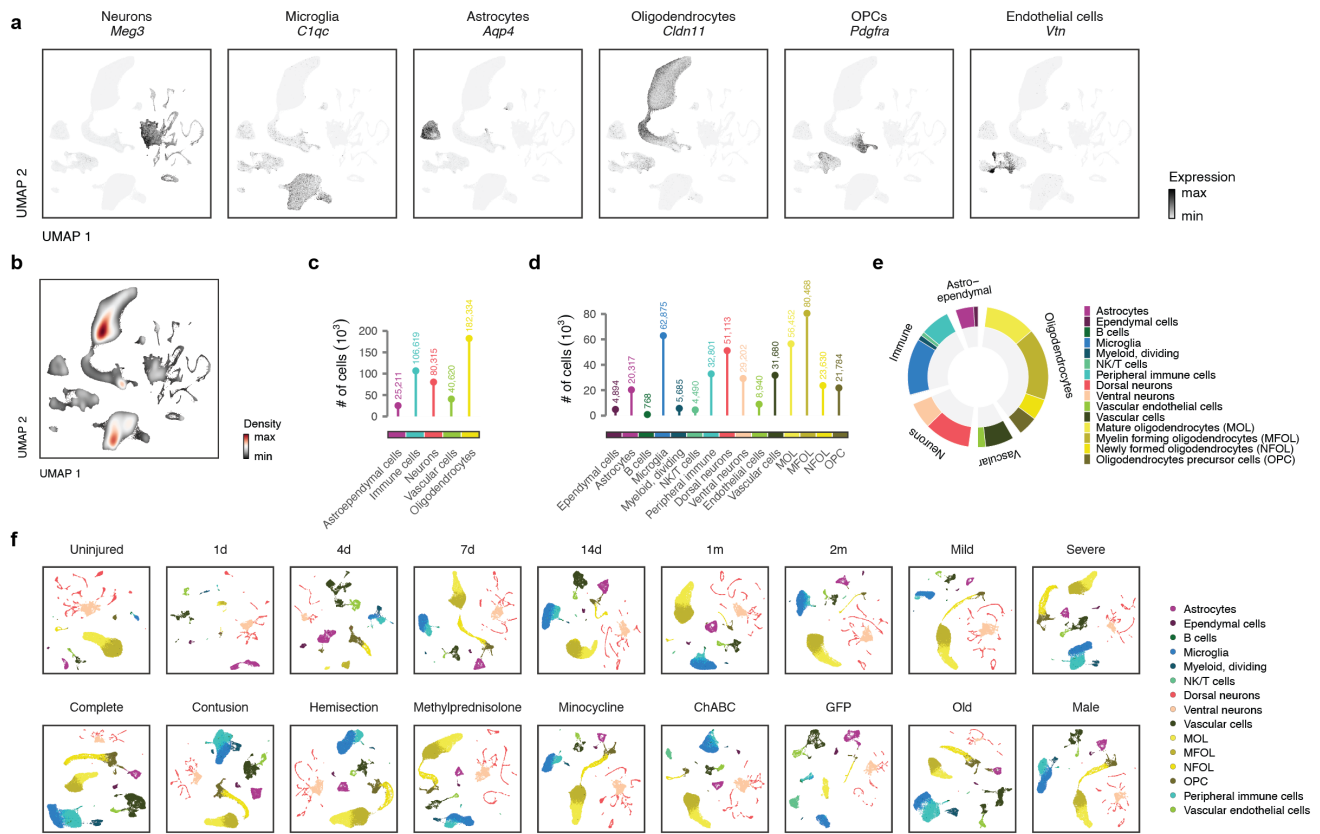
k, Relationship between the number of UMIs and the number of genes detected per nucleus across all 435,099 nuclei.

l, Number of nuclei that passed or failed quality control in libraries sequenced from all 54 mice.

m, Number of UMIs per nucleus, aggregated across all libraries and shown separately for nuclei that passed or failed quality control.

n, Number of genes detected per nucleus, aggregated across all libraries and shown separately for nuclei that passed or failed quality control.

o, Proportion of mitochondrial counts, aggregated across all libraries and shown separately for nuclei that passed or failed quality control.



Supplementary Fig. 5 | Annotation of major cell types in the snRNA-seq atlas.

a, UMAP visualization showing expression of key marker genes for the major cell types of the mouse spinal cord.

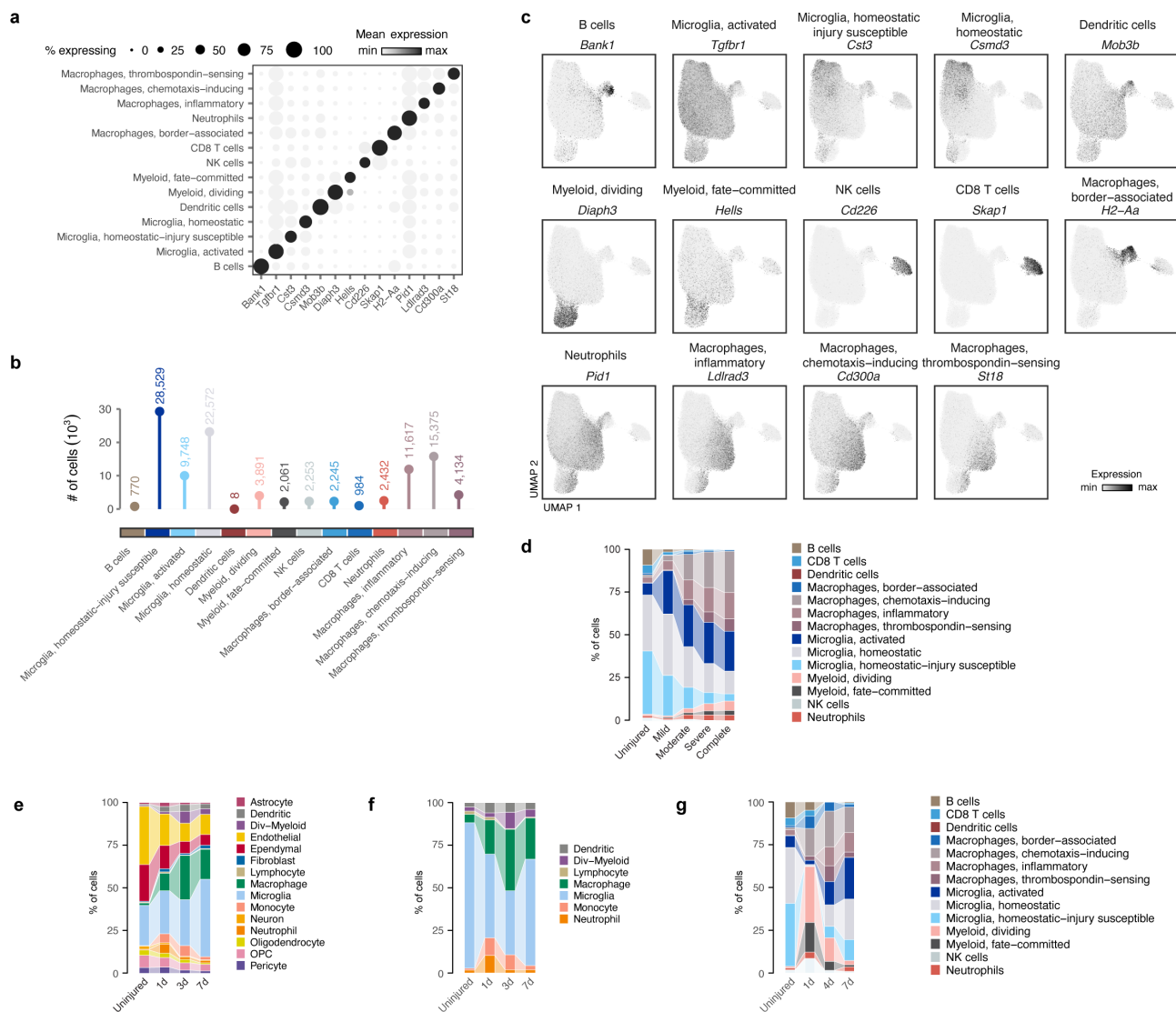
b, UMAP visualization showing the density of individual nuclei within the UMAP embedding.

c, Number of nuclei from each major (level 1) cell type identified across all experimental conditions.

d, Number of nuclei from each level 2 cell type identified across all experimental conditions.

e, Proportions of nuclei from each level 1 (inner circle) and level 2 (outer circle) across all experimental conditions.

f, UMAP visualizations of each individual experimental condition in the snRNA-seq atlas, colored by level 2 cell type.



Supplementary Fig. 6 | Annotation of immune cell subtypes in the snRNA-seq atlas.

a, Dot plot showing expression of key marker genes for immune cell subtypes.

b, Number of nuclei from each immune cell subtype identified across all experimental conditions.

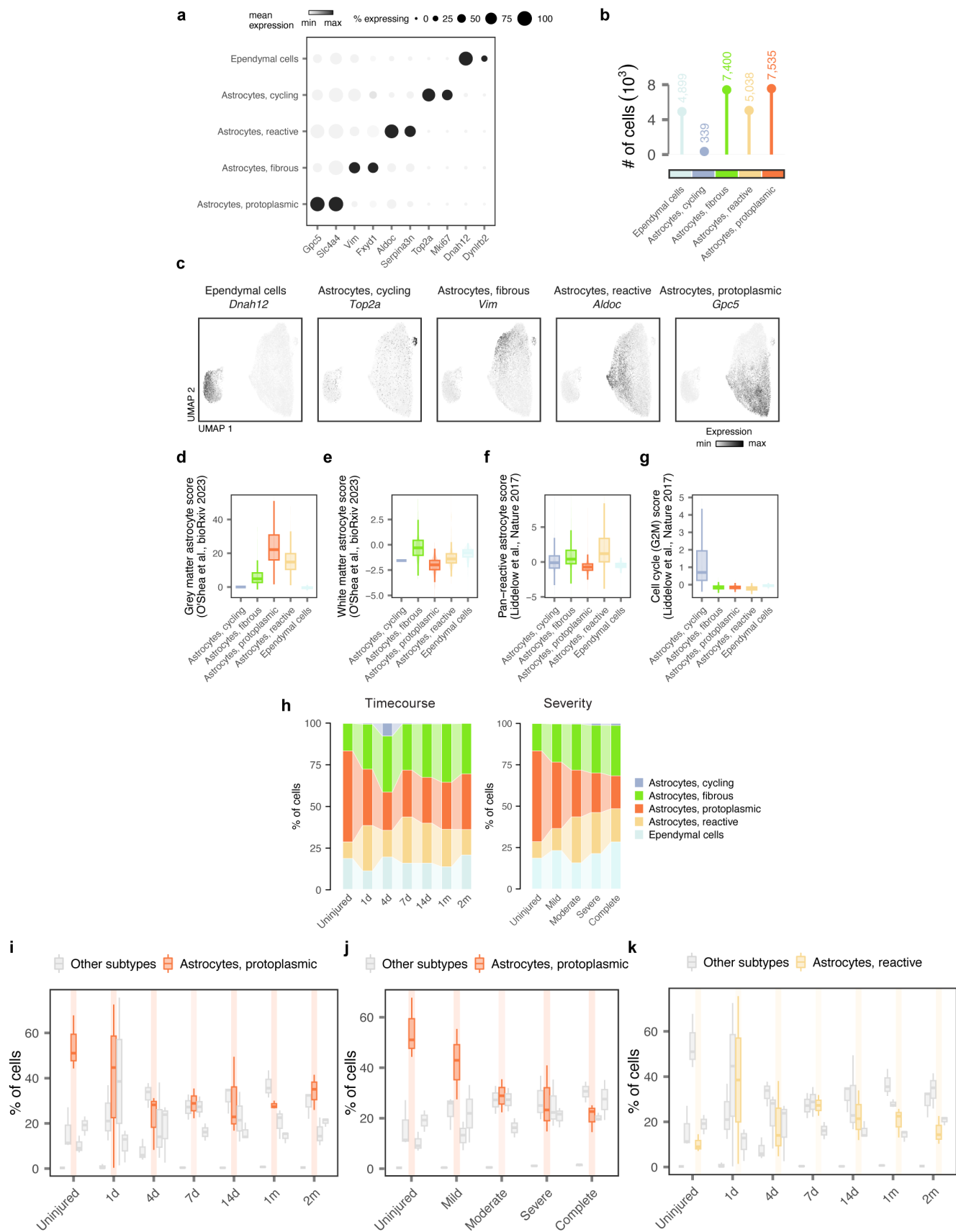
c, UMAP visualization showing expression of key marker genes for immune cell subtypes.

d, Sankey diagram showing the proportions of each immune cell subtype across injury severities.

e, Sankey diagram showing the proportions of each cell subtype across timepoints in the dataset of Milich *et al.*

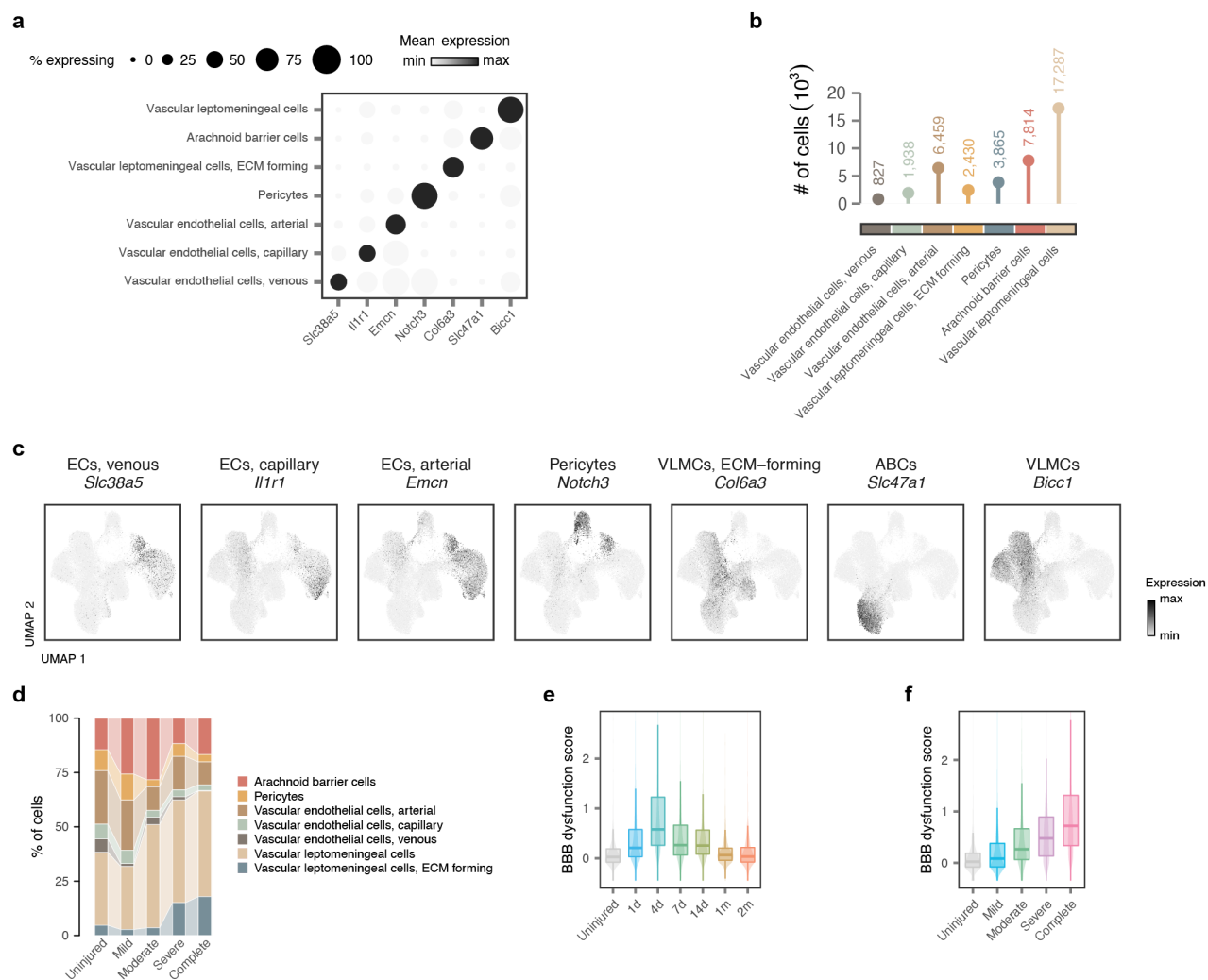
f, As in **e**, but showing only the relative proportions of immune cell subtypes.

g, Sankey diagram showing the proportions of each immune cell subtype across timepoints in the snRNA-seq atlas, as in Fig. 2b but here showing only uninjured, 1d, 4d, and 7d.



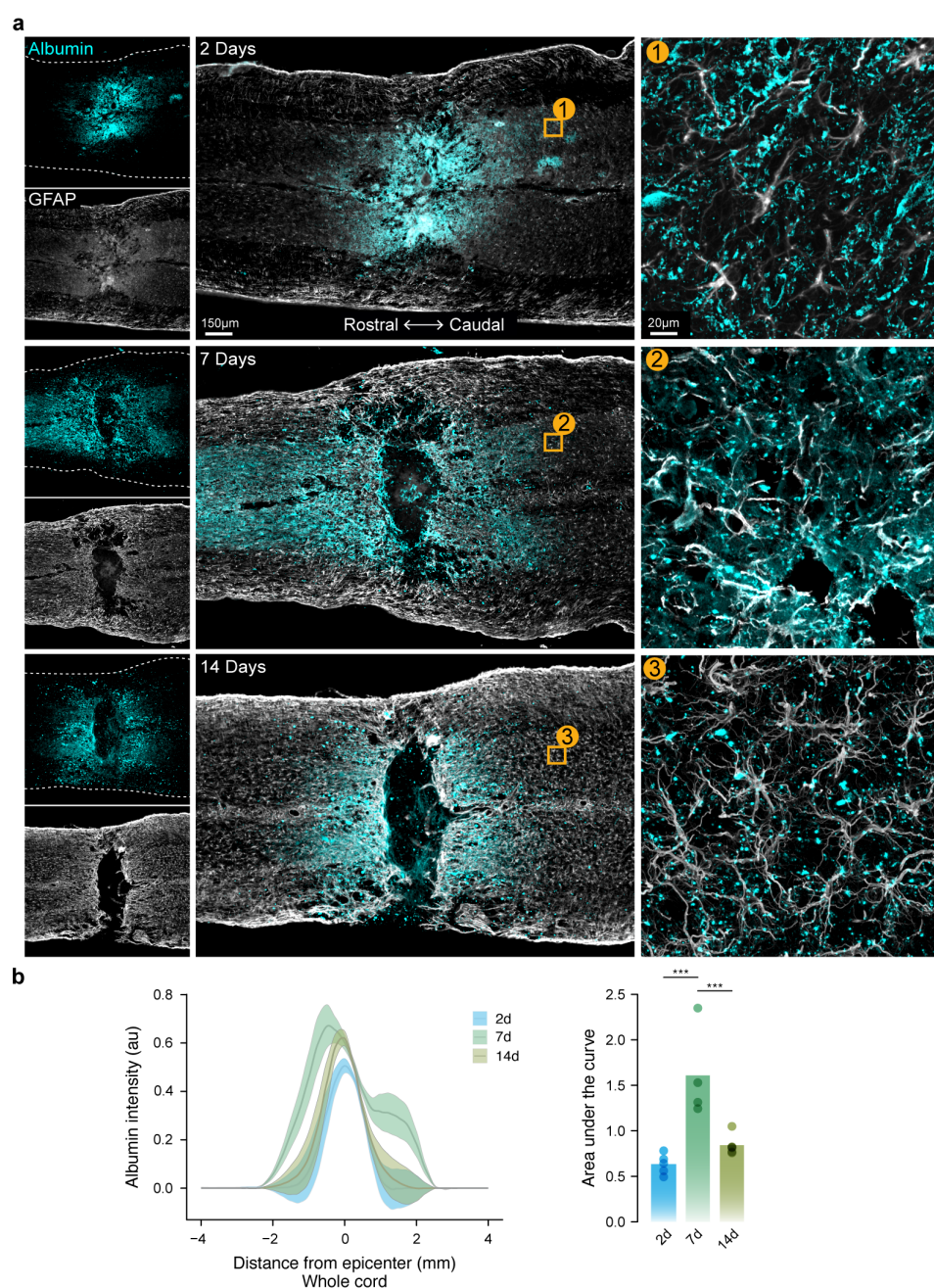
Supplementary Fig. 7 | Annotation of astroependymal cell subtypes in the snRNA-seq atlas.

- a**, Dot plot showing expression of key marker genes for astroependymal cell subtypes.
- b**, Number of nuclei from each astroependymal cell subtype identified across all experimental conditions.
- c**, UMAP visualization showing expression of key marker genes for astroependymal cell subtypes.
- d**, Expression of the grey matter astrocyte gene signature from O'Shea *et al.* in astrocytes from the uninjured spinal cord.
- e**, Expression of the white matter astrocyte gene signature from O'Shea *et al.* in astrocytes from the uninjured spinal cord.
- f**, Expression of the pan-reactive astrocyte gene signature from Liddelow *et al.* in astrocytes from the injured spinal cord.
- g**, Expression of the cell cycle (G2M) gene module from Tirosh *et al.* in astrocytes from the injured spinal cord.
- h**, Sankey diagrams showing the proportions of each astroependymal cell subtype across timepoints, left, and injury severities, right.
- i**, Boxplot highlighting the proportions of protoplasmic astrocytes within individual libraries from the timecourse experiment, as compared to all other astroependymal cell subtypes.
- j**, Boxplot highlighting the proportion of protoplasmic astrocytes within individual libraries from the severity experiment, as compared to all other astroependymal cell subtypes.
- k**, Boxplot highlighting the proportion of reactive astrocytes within individual libraries from the timecourse experiment, as compared to all other astroependymal cell subtypes.



Supplementary Fig. 8 | Annotation of vascular cell subtypes in the snRNA-seq atlas.

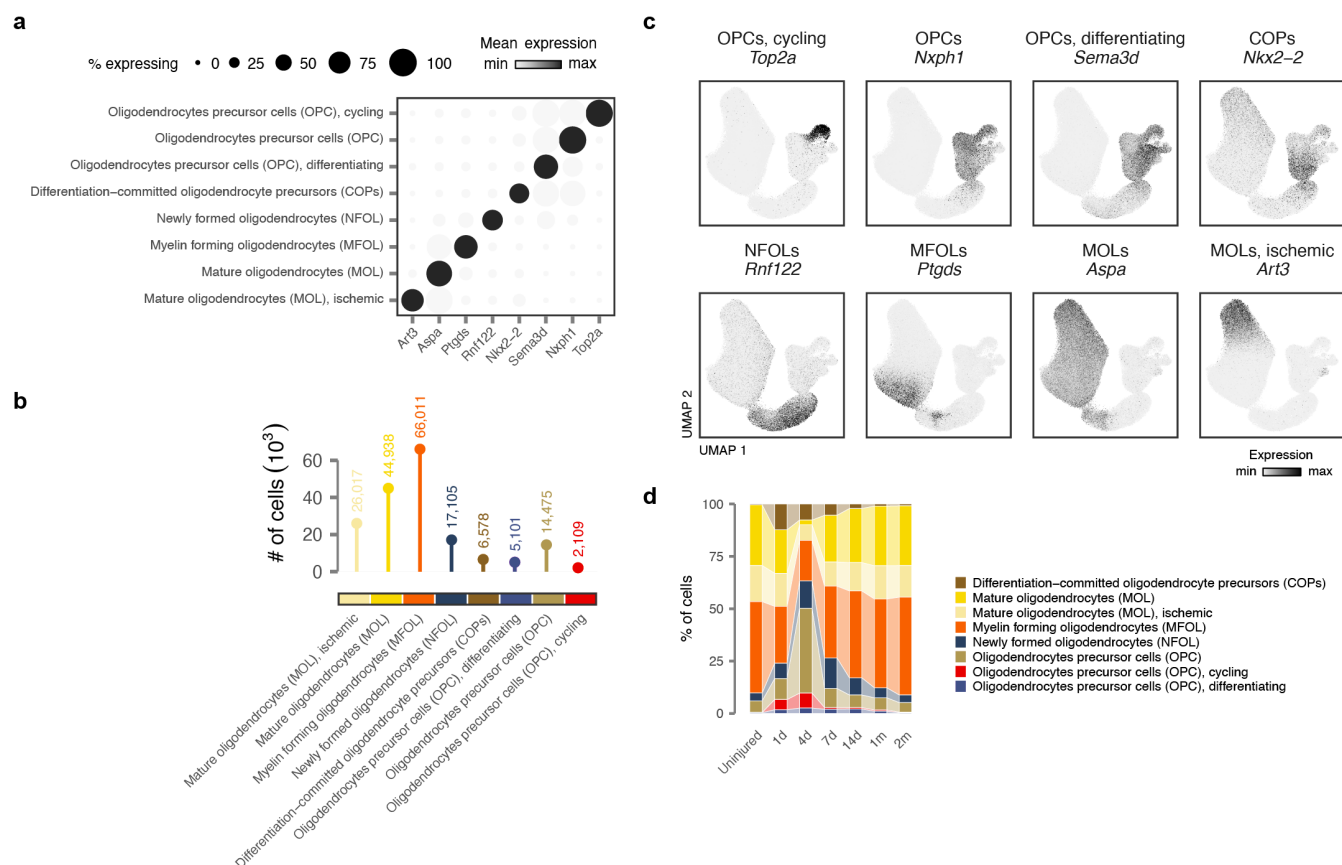
- a**, Dot plot showing expression of key marker genes for vascular cell subtypes.
- b**, Number of nuclei from each vascular cell subtype identified across all experimental conditions.
- c**, UMAP visualization showing expression of key marker genes for vascular cell subtypes.
- d**, Sankey diagram showing the proportions of each vascular cell subtype across injury severities.
- e**, Average expression of the BBB dysfunction module in vascular cells across timepoints.
- f**, Average expression of the BBB dysfunction module in vascular cells across injury severities.



Supplementary Fig. 9 | Immunohistochemical analysis of blood-spinal cord barrier dysfunction after SCI.

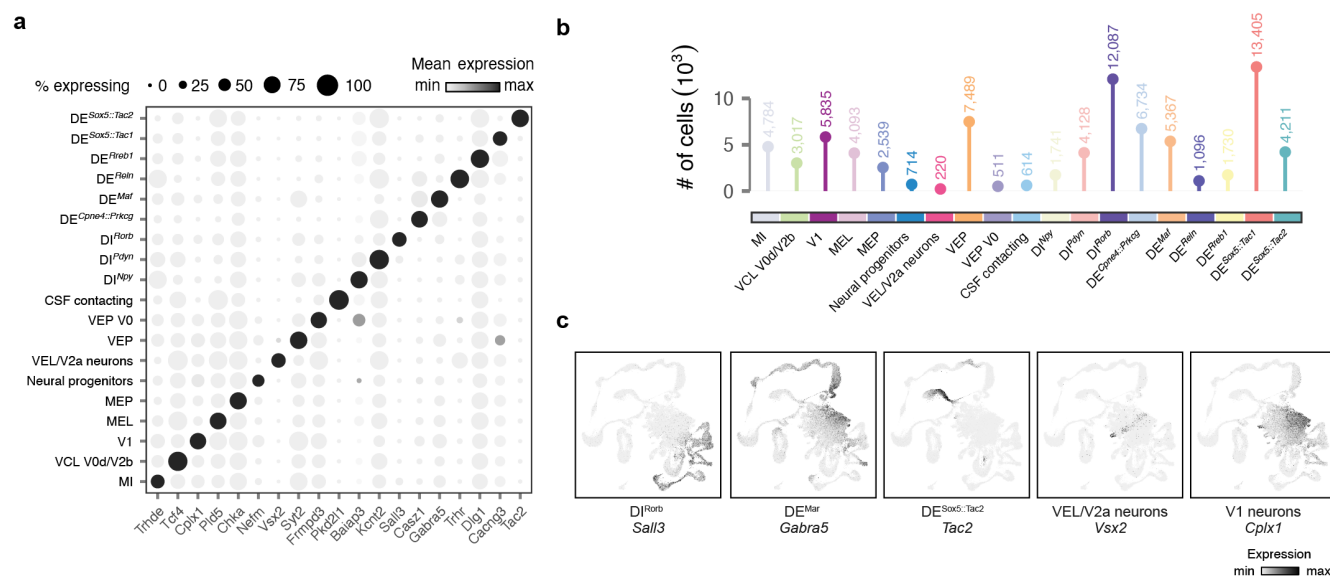
a, Composite tiled scans and confocal insets of albumin and GFAP in horizontal sections from representative mice at 2 days, 7 days, and 14 days after SCI.

b, Line graph denotes albumin intensity at specific distances rostral and caudal to lesion centers. Bottom right, bar graph indicates the area under the curve (Tukey's honestly significant difference test, $p = 0.0008$ (2d vs. 7d), 0.49 (2d vs. 14d), and 0.005 (7d vs. 14d)).



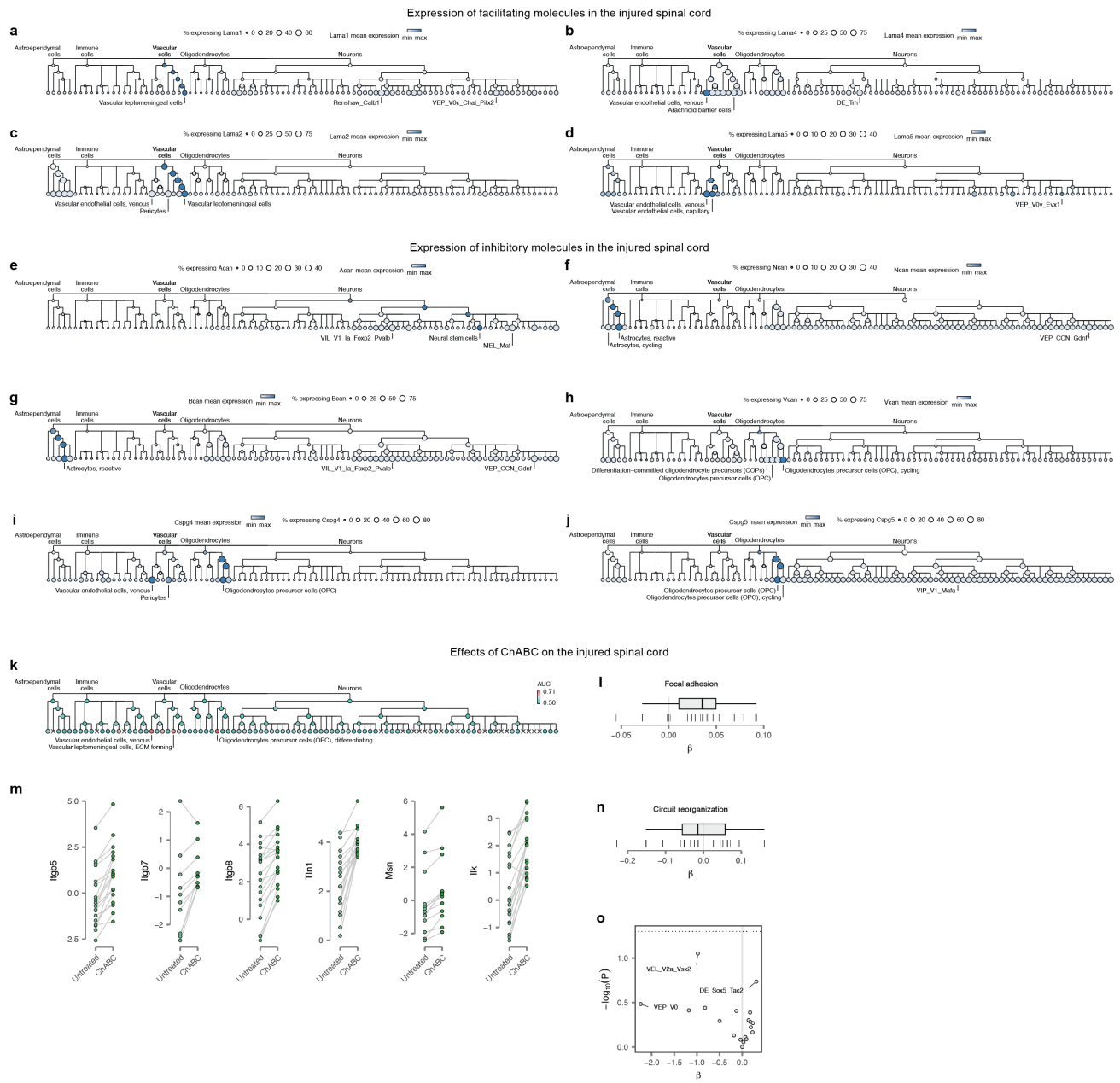
Supplementary Fig. 10 | Annotation of oligodendrocyte subtypes in the snRNA-seq atlas.

- a**, Dot plot showing expression of key marker genes for oligodendrocyte subtypes.
b, Number of nuclei from each oligodendrocyte subtype identified across all experimental conditions.
c, UMAP visualization showing expression of key marker genes for oligodendrocyte subtypes.
d, Sankey diagram showing the proportions of each oligodendrocyte subtype across timepoints.



a, Dot plot showing expression of one key marker gene for each level 4 neuron subtype in the mouse spinal cord.

c, UMAP visualization showing expression of classical excitatory-inhibitory and dorsal-ventral marker genes within neurons in the mouse spinal cord.



Supplementary Fig. 12 | Facilitating and inhibiting molecule expression in the injured spinal cord.

a-j, Dendrograms showing expression of selected facilitating and inhibiting molecules across the cellular taxonomy of the spinal cord. Point color shows mean expression in each cell type, while point size reflects the proportion of cells with detectable expression.

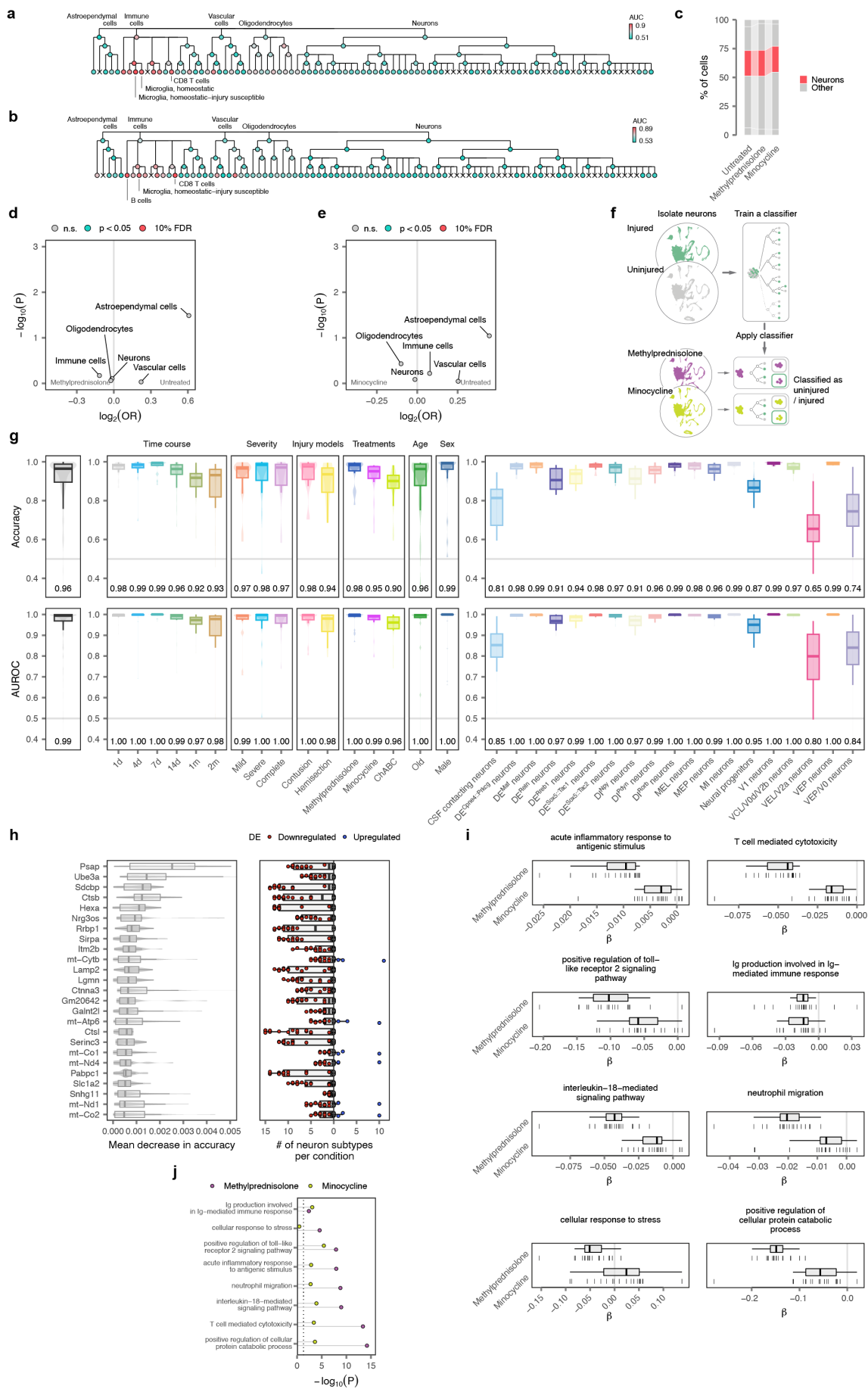
k, Dendrogram showing cell type prioritizations assigned by Augur across the cellular taxonomy of the spinal cord after treatment with ChABC. The three level 5 cell types with the highest AUCs are annotated.

l, Upregulation of genes associated with focal adhesion across level 4 neuron subtypes after ChABC treatment. Vertical lines show coefficients estimated by a linear mixed model within each neuron subtype.

m, Differential expression of individual genes associated with focal adhesion across level 4 neuron subtypes after ChABC treatment. Points show pseudobulk gene expression values in counts per million. Lines connect each neuron subtype across experimental conditions.

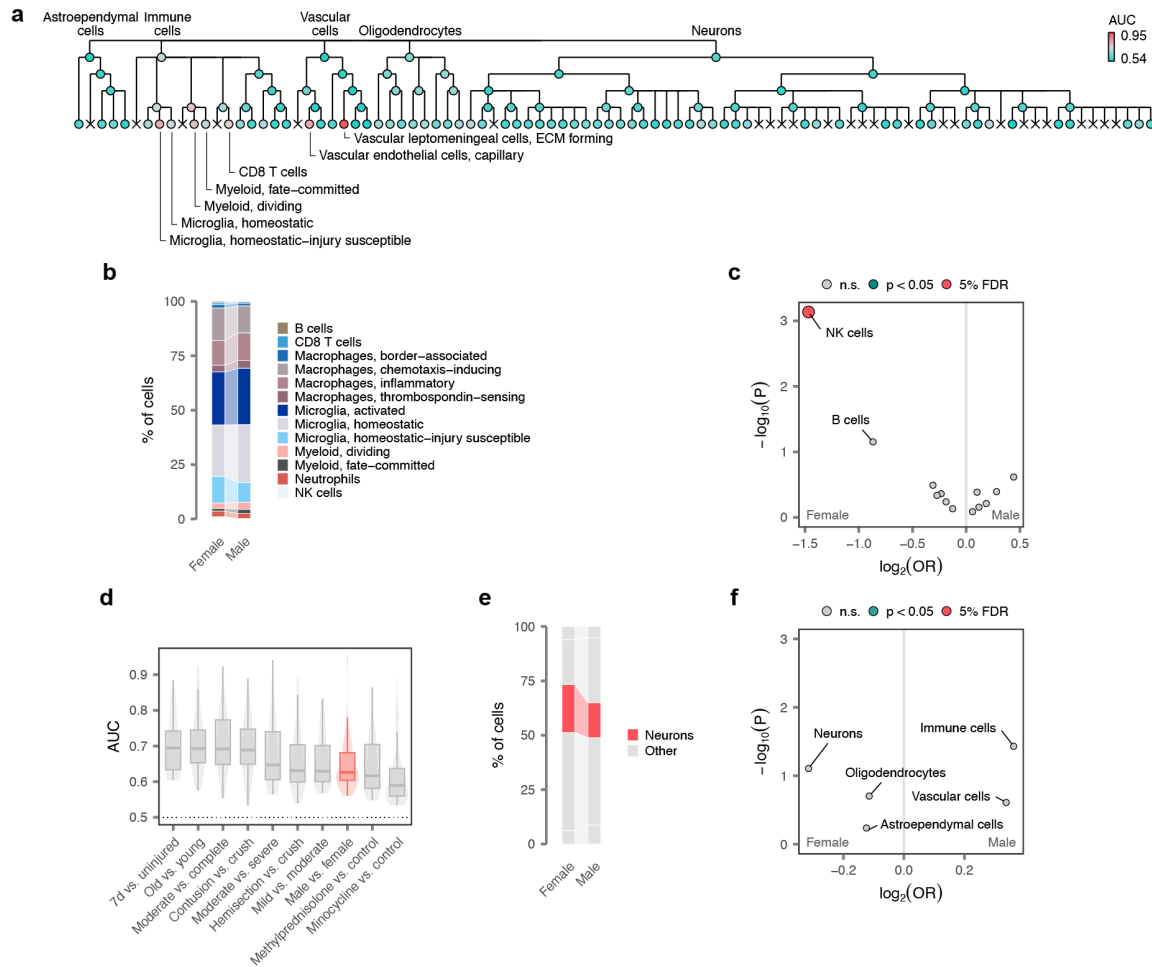
n, Upregulation of genes associated with circuit reorganization across level 4 neuron subtypes after ChABC treatment. Vertical lines show coefficients estimated by a linear mixed model within each neuron subtype.

o, As in **n**, but showing the coefficients estimated by a linear mixed model within each neuron subtype (x-axis) against the p-value derived from the same model (y-axis). Dotted line shows nominal $p = 0.05$.



Supplementary Fig. 13 | Immunomodulation does not confer neuroprotection after spinal cord injury.

- a**, Dendrogram showing cell type prioritizations assigned by Augur across the cellular taxonomy of the spinal cord after treatment with methylprednisolone. The three level 5 cell types with the highest AUCs are annotated.
- b**, Dendrogram showing cell type prioritizations assigned by Augur across the cellular taxonomy of the spinal cord after treatment with minocycline. The three level 5 cell types with the highest AUCs are annotated.
- c**, Sankey diagram showing the proportions of neurons recovered from the injured spinal cord after treatment with methylprednisolone or minocycline, as compared to untreated controls.
- d**, Volcano plot showing the statistical significance of changes in major cell type proportions after treatment with methylprednisolone, as compared to untreated controls. No statistically significant differences in cell type proportions are detected after treatment.
- e**, Volcano plot showing the statistical significance of changes in major cell type proportions after treatment with minocycline, as compared to untreated controls. No statistically significant differences in cell type proportions are detected after treatment.
- f**, Schematic depicting the procedure by which neurons are assigned to uninjured versus injured transcriptional phenotypes by a machine-learning classifier.
- g**, Performance of the uninjured neuron classifier in ten-fold cross-validation (top, accuracy; bottom, area under the receiver operating characteristic curve). Left, aggregate performance over all neuron subtypes and injury conditions. Middle, performance by injury condition. Right, performance by neuron subtype.
- h**, Left, feature importance of the top 25 genes used in the uninjured neuron classifier, ranked by mean decrease in accuracy across neuron subtypes and injury conditions. Right, number of neuron subtypes in which each gene was up- or downregulated in comparisons of uninjured mice and each injury condition.
- i**, Downregulation of gene modules associated with cellular stress across level 4 neuron subtypes after methylprednisolone or minocycline treatment. Vertical lines show coefficients estimated by a linear mixed model within each neuron subtype.
- j**, Lollipop plot showing the statistical significance of downregulation for each of the gene modules shown in **i** after methylprednisolone or minocycline treatment.



Supplementary Fig. 14 | Sexual dimorphism in the response to SCI.

a, Dendrogram showing cell type prioritizations assigned by Augur across the cellular taxonomy of the spinal cord in comparisons of male and female mice. The seven level 5 cell types with the highest AUCs are annotated.

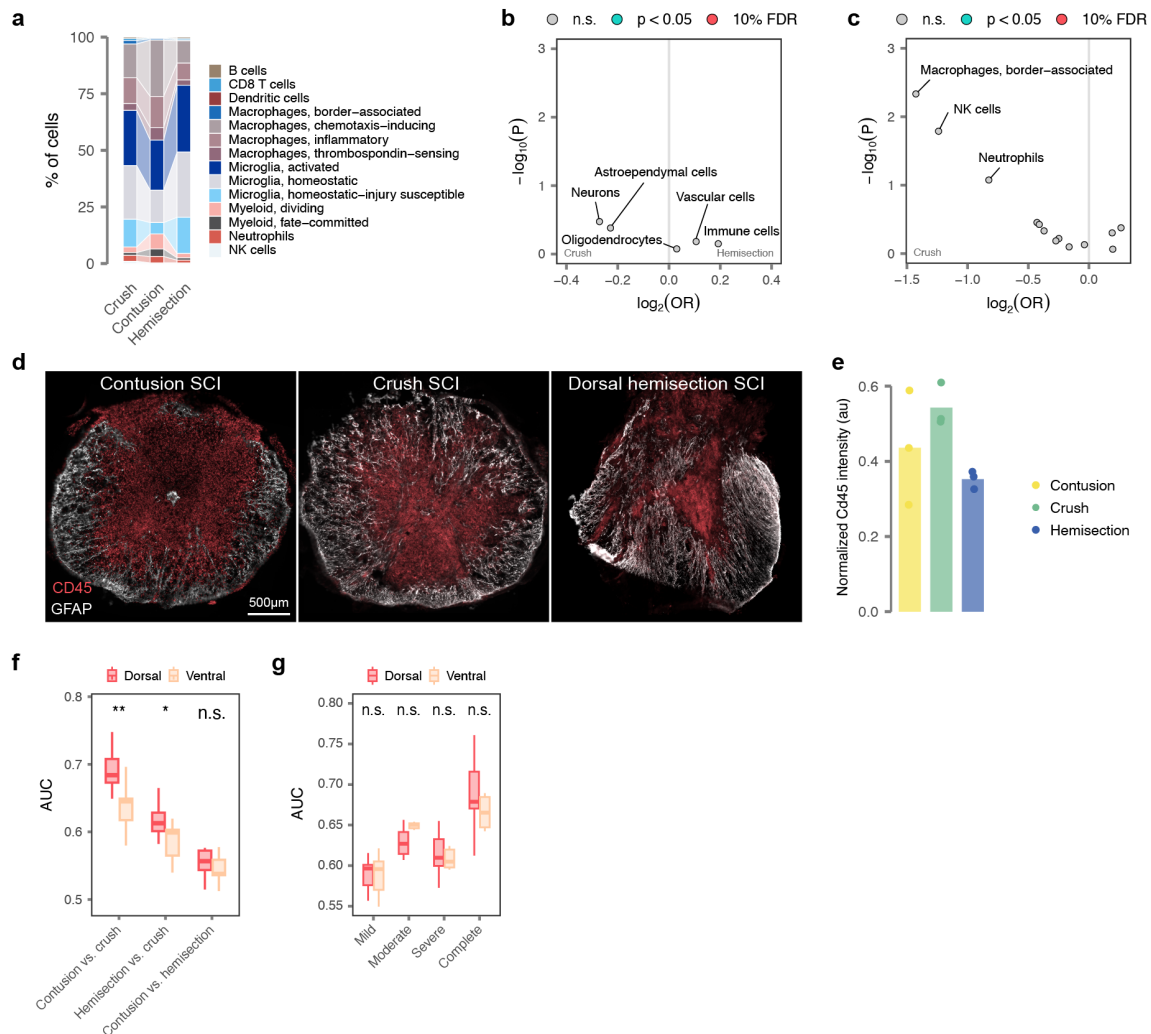
b, Sankey diagram showing the proportions of immune cell subtypes recovered from the spinal cords of male versus female mice.

c, Volcano plot showing the statistical significance of changes in immune cell subtype proportions between male and female mice.

d, Boxplot showing the intensity of transcriptional perturbations within level 4 cell types, as quantified by Augur, in all comparisons involving the injured spinal cord at 7 days post-injury. The transcriptional perturbation between male and female mice is among the most subtle in the snRNA-seq atlas.

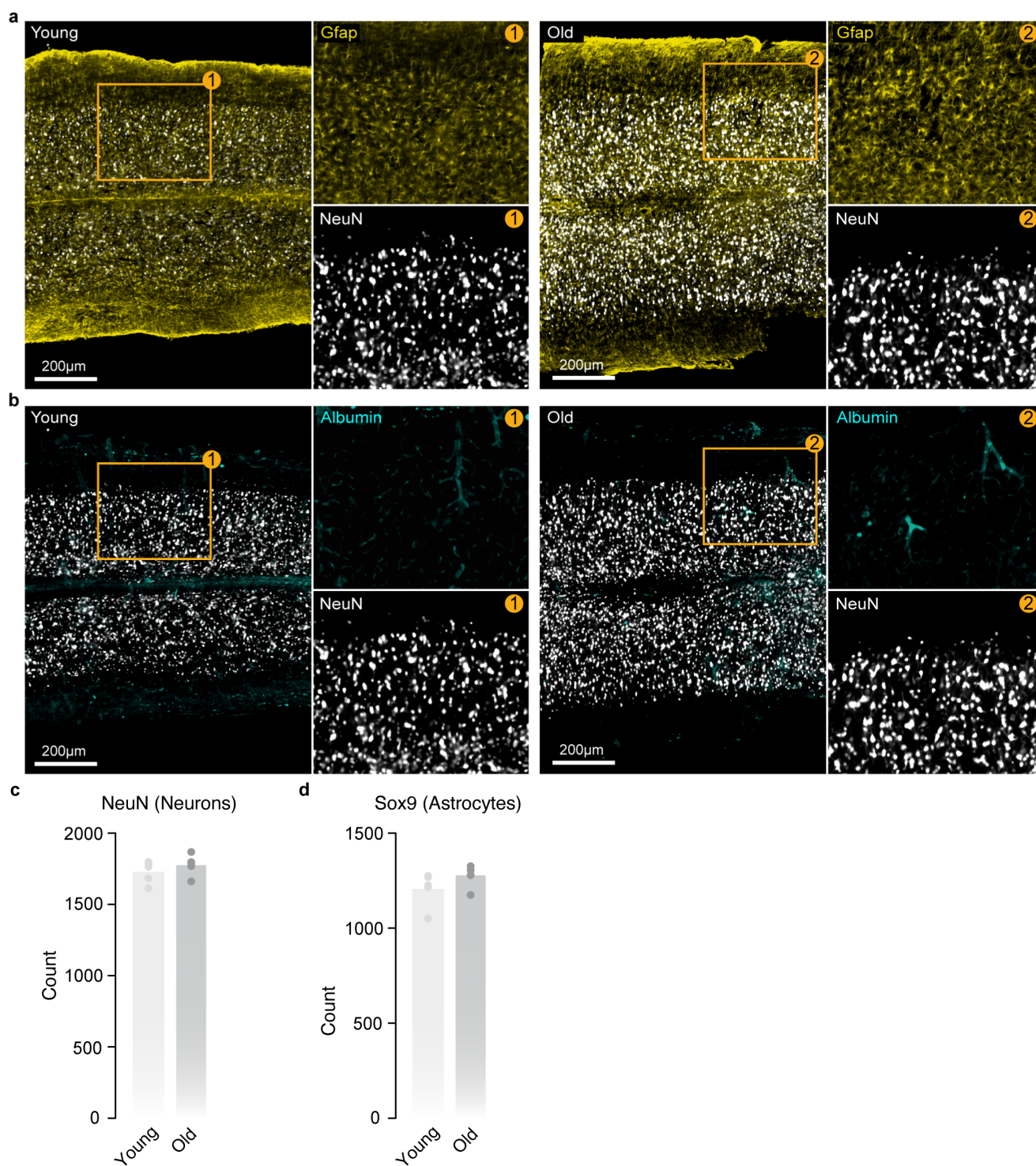
e, Sankey diagram showing the proportions of major cell types recovered from the spinal cords of male versus female mice.

f, Volcano plot showing the statistical significance of changes in major cell type proportions between male and female mice. No statistically significant differences in cell type proportions are detected.



Supplementary Fig. 15 | Cellular divergence between animal models of SCI.

- a**, Sankey diagram showing the proportions of immune cell subtypes across crush, contusion, and hemisection injury models.
- b**, Volcano plot showing the statistical significance of changes in major cell type proportions between crush and hemisection injuries. No statistically significant differences in cell type proportions are detected.
- c**, Volcano plot showing the statistical significance of changes in immune cell subtype proportions between crush and hemisection injuries. No statistically significant differences in cell type proportions are detected.
- d**, Histological photomicrographs show injured spinal cords across injury models after staining for GFAP and CD45.
- e**, Quantification of immune infiltration at the lesion epicentre across injury models. The extent of immune infiltration was quantified from coronal sections immunolabeled for CD45. No comparisons were significant (all $p > 0.05$, Tukey's honestly significant difference test).
- f**, Boxplots showing the average intensity of transcriptional perturbations in dorsal versus ventral neurons, as quantified by Augur, in comparisons of mice subjected to crush, contusion, and hemisection injuries. *, $p < 0.05$; **, $p < 0.01$; n.s., not significant.
- g**, Boxplots showing the average intensity of transcriptional perturbations in dorsal versus ventral neurons, as quantified by Augur, in comparisons of mice subjected to crush injuries of increasing severity. n.s., not significant.

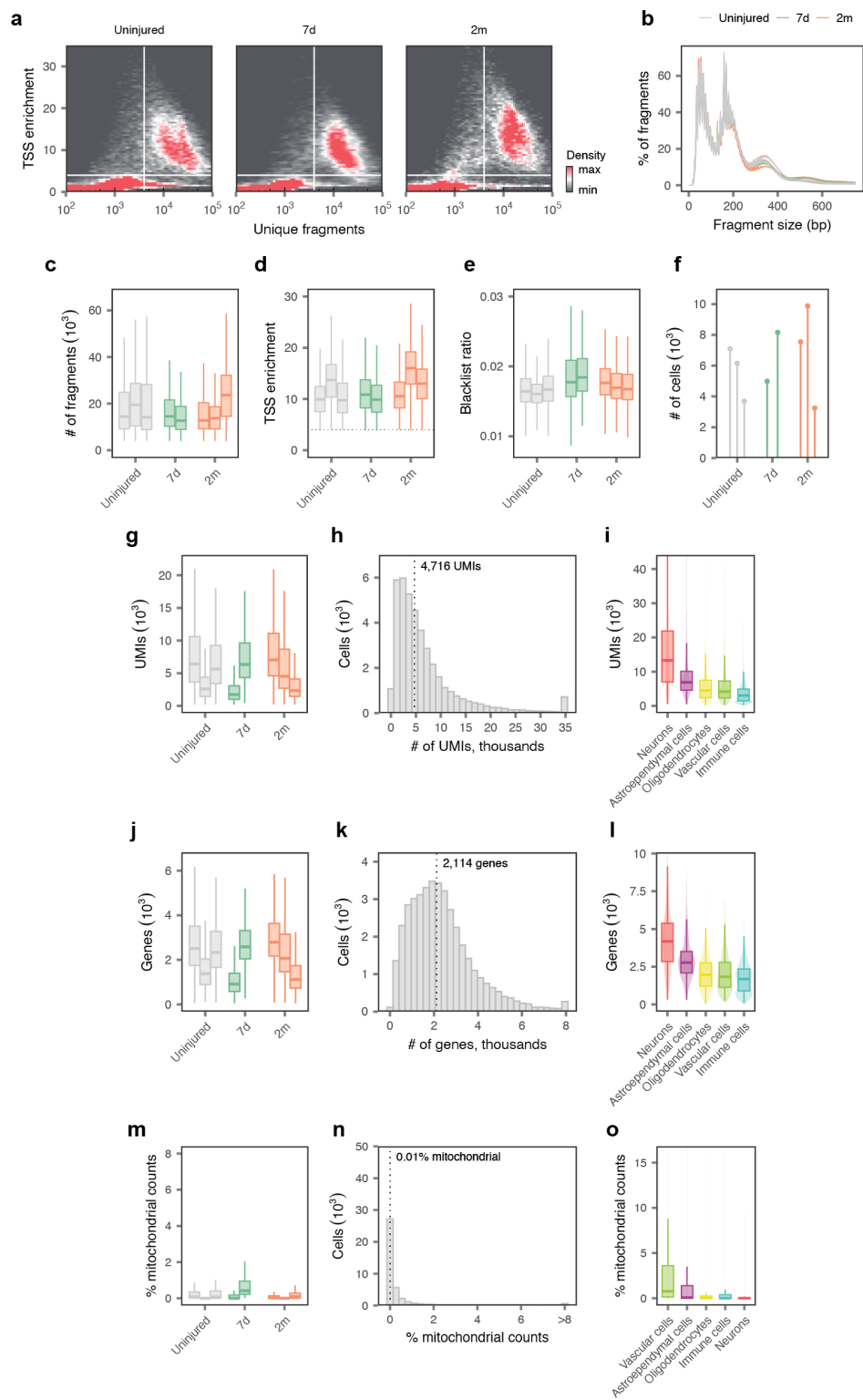


Supplementary Fig. 16 | Cellular composition and blood-spinal cord barrier integrity in uninjured young and old mice.

a, Composite tiled scans and confocal insets of NeuN and GFAP in horizontal sections from representative uninjured young and old mice after SCI.

b, Composite tiled scans and confocal insets of albumin and NeuN in horizontal sections from representative uninjured young and old mice after SCI.

c-d, Number of NeuN^{ON}, **c**, and Sox9^{ON}, **d**, cells in the spinal cords of uninjured young and old mice (independent samples two-tailed t-test; $n = 5$ per group; Sox9 [$t = 1.45$, $p = 0.191$]; NeuN [$t = 0.98$, $p = 0.358$]).



Supplementary Fig. 17 | Quality control of the multiome atlas.

a-f, Quality control of the ATAC modality.

a, TSS enrichment score versus the number of unique fragments per cell in barcodes from each experimental condition. Solid lines show the thresholds used for initial quality control of the ATAC modality in the multiome atlas.

b, Aggregate fragment size distributions for barcodes passing initial quality control thresholds in the ATAC modality of the multiome atlas.

c, Number of fragments per nucleus in individual libraries from each experimental condition.

d, TSS enrichment scores per nucleus in individual libraries from each experimental condition.

e, Proportion of reads mapping to blacklist regions per nucleus in individual libraries from each experimental condition.

f, Number of nuclei passing quality control for each individual library in the multiome atlas.

g-o, Quality control of the RNA modality.

g, Number of unique molecular identifiers (UMIs) per nucleus in individual libraries from each experimental condition.

h, Number of UMIs per nucleus, aggregated across all libraries. Inset text shows the median number of UMIs.

i, Number of UMIs per nucleus in each major cell type.

j, Number of genes detected per nucleus in individual libraries from each experimental condition.

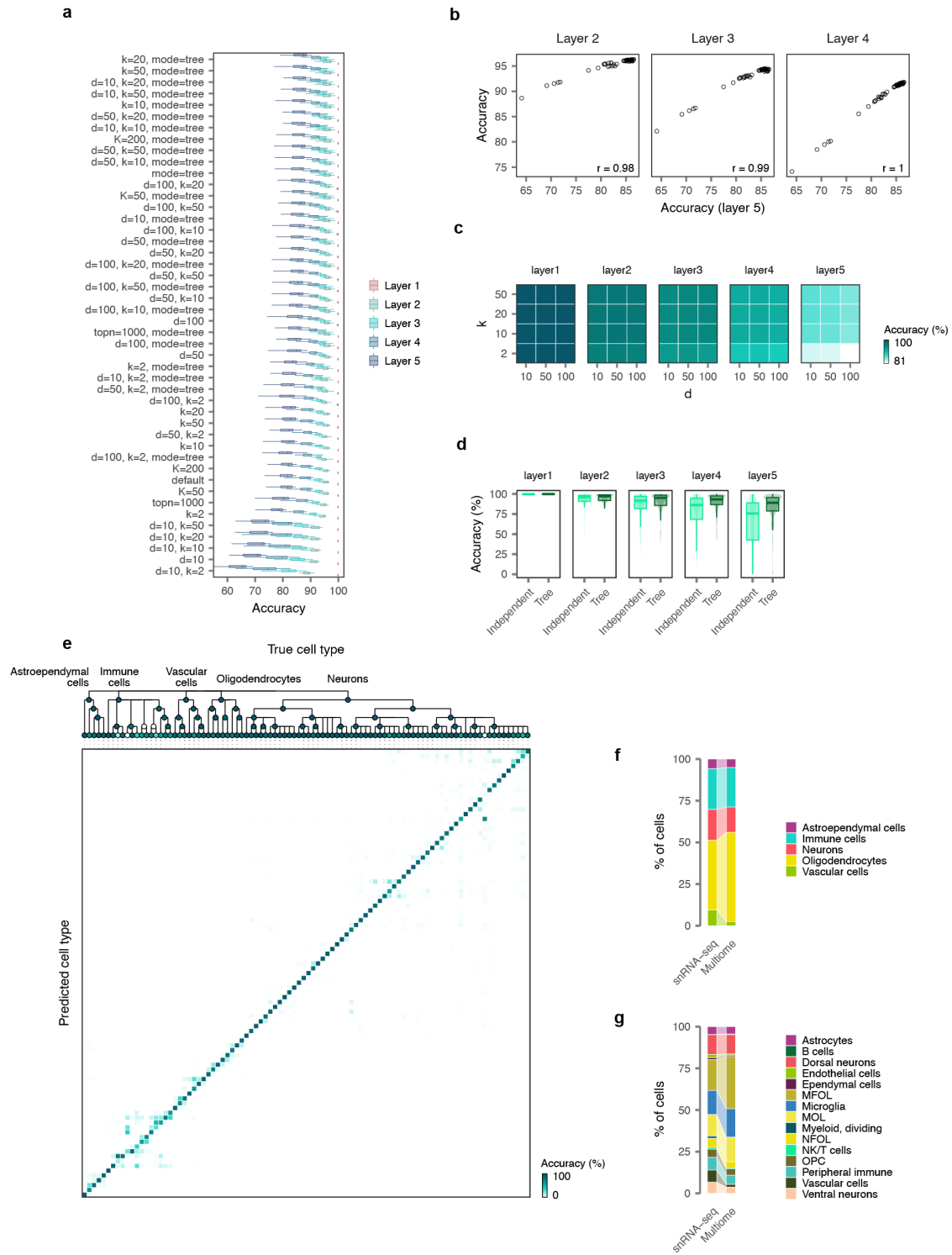
k, Number of genes detected per nucleus, aggregated across all libraries. Inset text shows the median number of genes detected.

l, Number of genes detected per nucleus in each major cell type.

m, Proportion of mitochondrial counts per nucleus in individual libraries from each experimental condition.

n, Proportion of mitochondrial counts per nucleus, aggregated across all libraries. Inset text shows the median proportion of mitochondrial counts.

o, Proportion of mitochondrial counts per nucleus in each major cell type.



Supplementary Fig. 18 | Hierarchical transfer of cell type annotations from the snRNA-seq atlas to the multiome atlas.

a, Benchmarking the accuracy of automated cell type assignment by leave-library-out cross-validation on the snRNA-seq atlas. Boxplot shows per-cell-type accuracies in each held-out library after systematically varying the number of nearest-neighbors, k ; the number of principal components, d ; and the number of soft cluster centroids, K . In addition, we experimented with constraining the potential matches at finer levels of our cellular taxonomy on the basis of cell types assigned at higher levels ("mode=tree").

b, Correlation between the accuracy of cell type annotation at the finest level of the clustering tree (level 5) and levels 2, 3, and 4, for each hyperparameter combination shown in a. The results for level 1 are not shown because accuracy at this level approximated 100%. **c**, Impact of varying the number of nearest-neighbors, k , and the number of principal components, d , on the accuracy of automated cell type assignment in leave-library-out cross-validation.

d, Impact of the hierarchical adaptation of cell type annotation transfer, whereby all possible cell types are considered as potential matches at a given resolution ("independent"), or potential matches are constrained based on the cell type annotation at the previous resolution of the clustering tree ("tree"). The hierarchical approach consistently improves accuracy, particularly for lower levels of the clustering tree.

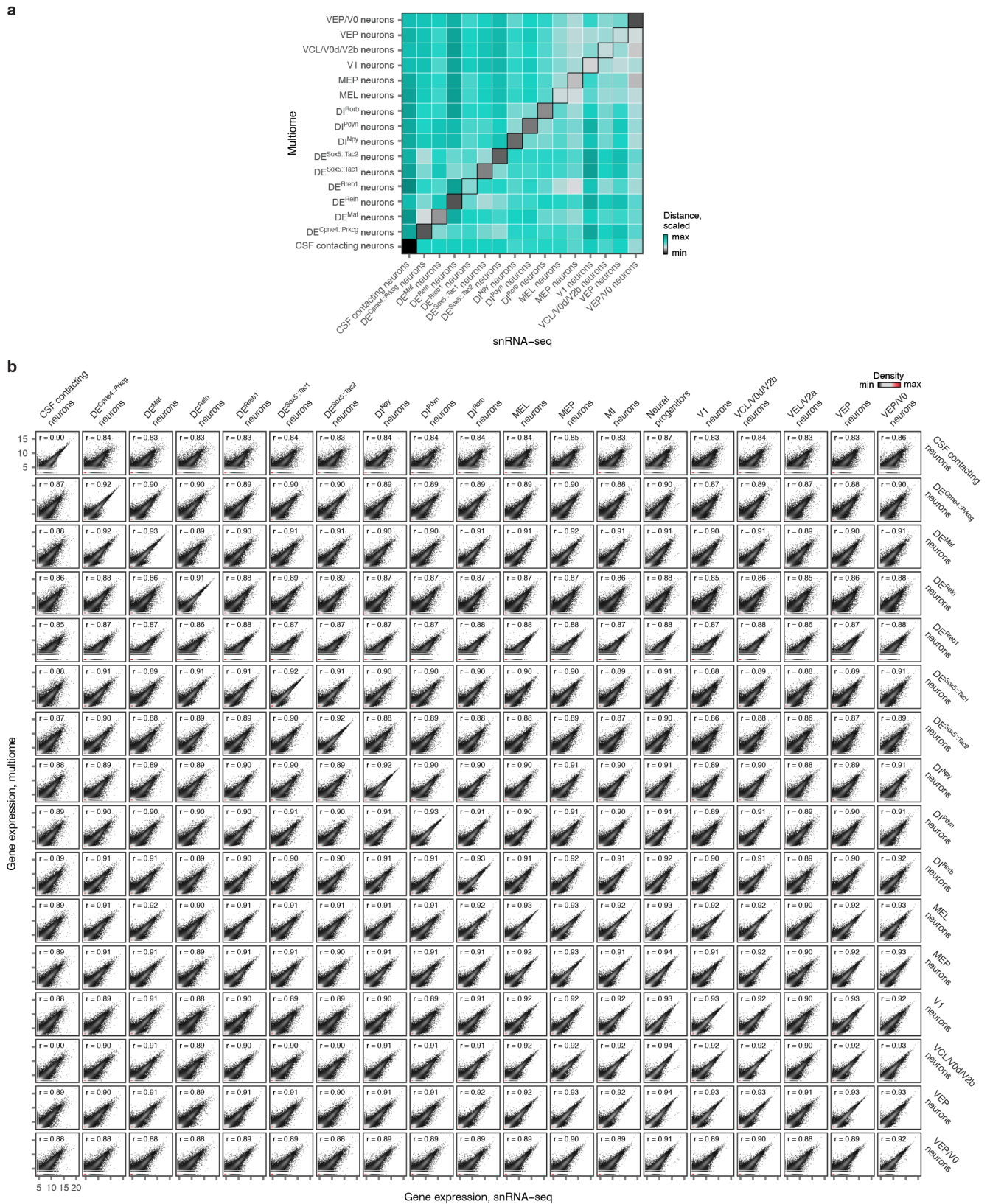
e, Performance of automated cell type annotation under the specific parameters used for cell type annotation in the multiome dataset. Top, dendrogram showing the average accuracy of cell type annotation for the complete cellular taxonomy in leave-library-out cross-validation on the snRNA-seq atlas. Bottom, confusion matrix at level 5 of the cellular taxonomy.

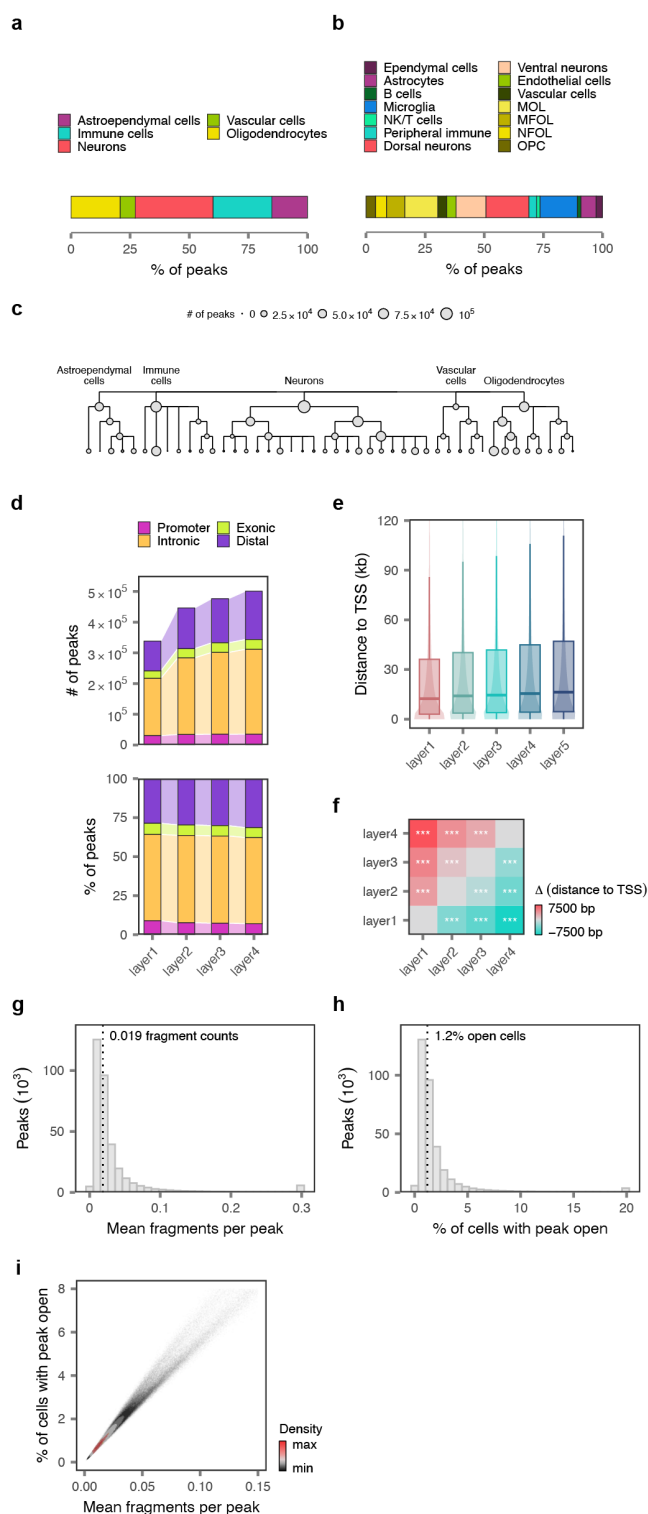
f, Sankey diagram showing the proportions of major (level 1) cell types in the snRNA-seq versus multiome atlases.

g, Sankey diagram showing the proportions of level 2 cell types in the snRNA-seq versus multiome atlases.

Supplementary Fig. 19 | Cell types and subtypes in the multiome atlas.

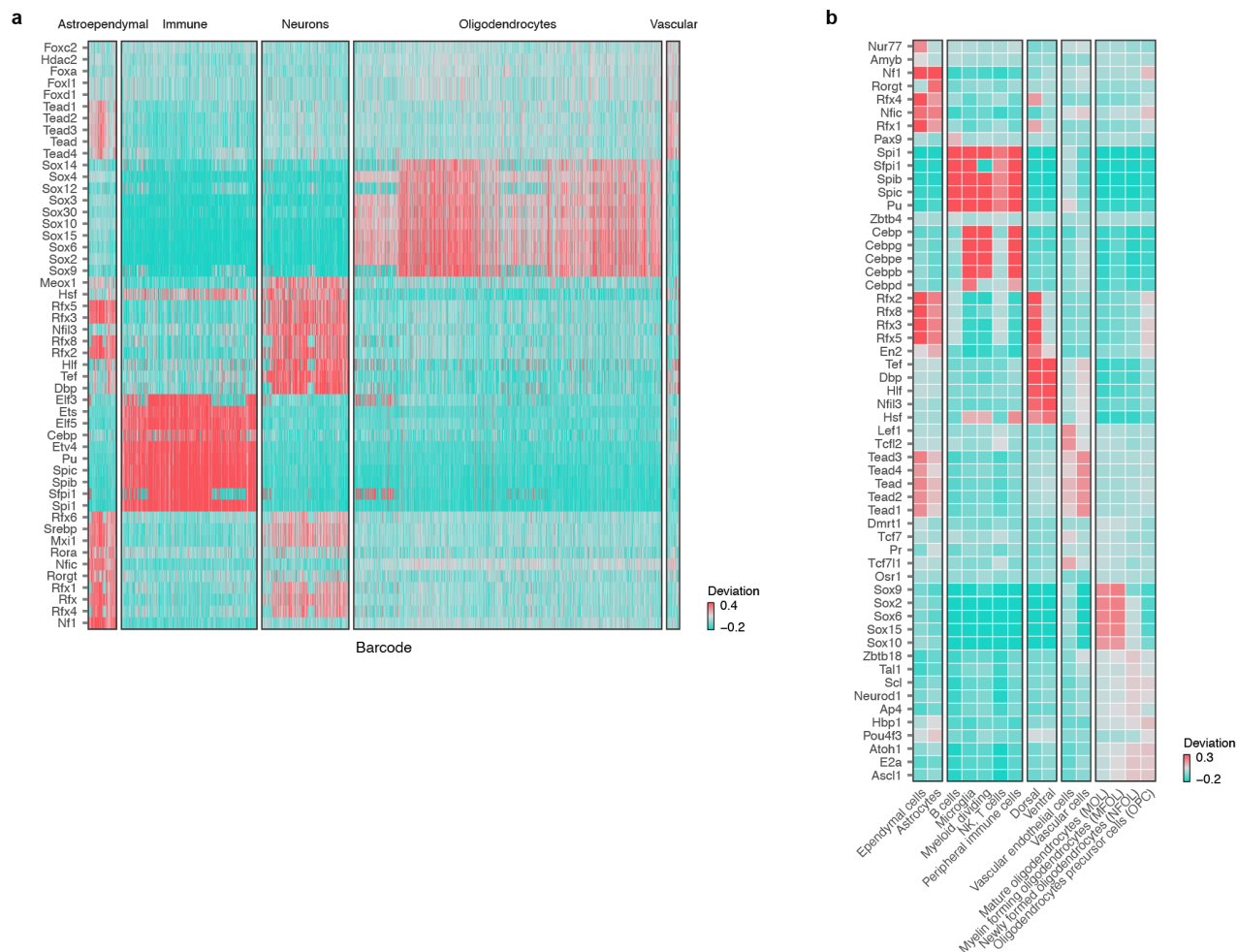
- a**, UMAP visualization showing nuclei separately for each experimental condition in the multiome atlas, based on gene expression in the RNA modality and colored by level 2 cell type.
- b**, UMAP visualization showing expression of key marker genes for major cell types in the RNA modality of the multiome atlas.
- c**, UMAP visualization showing nuclei separately for each experimental condition in the multiome atlas, based on chromatin accessibility in the ATAC modality and colored by level 2 cell type.
- d**, UMAP visualization showing gene activity scores of key marker genes for major cell types in the ATAC modality of the multiome atlas.
- e**, UMAP visualization of immune cells in the multiome atlas based on gene expression in the RNA modality or chromatin accessibility in the ATAC modality, and colored by level 2 cell type or experimental condition.
- f**, As in **e**, but for astroependymal cells.
- g**, As in **e**, but for vascular cells.
- h**, As in **e**, but for oligodendrocytes.
- i**, As in **e**, but for neurons.
- j**, Number of nuclei from each major (level 1) cell type identified in the multiome atlas.
- k**, Number of nuclei from each level 2 cell type identified in the multiome atlas.





Supplementary Fig. 21 | Peak calling in the multiome atlas.

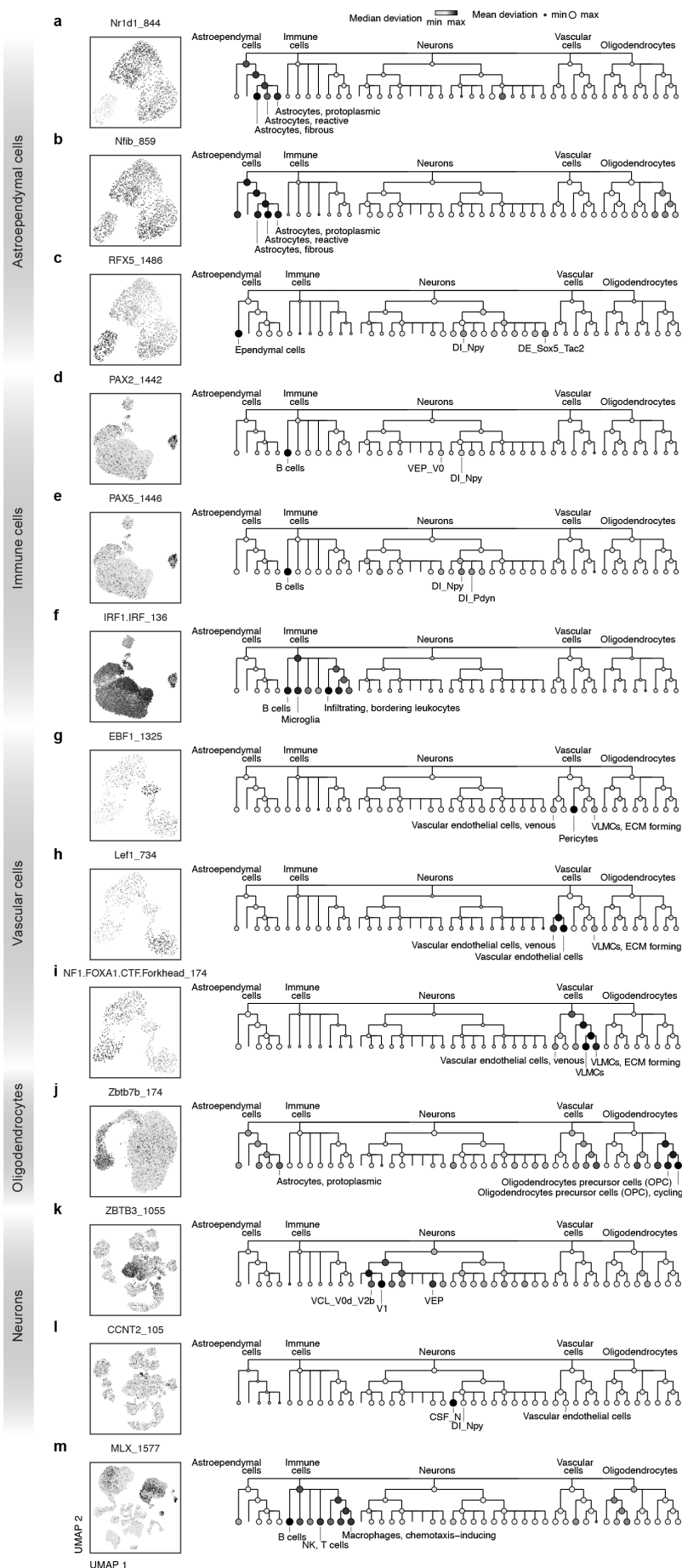
- a**, Number of peaks attributed to each major (level 1) cell type by the iterative overlap-merging procedure in ArchR.
- b**, Number of peaks attributed to each level 2 cell type by the iterative overlap-merging procedure in ArchR.
- c**, Number of peaks attributed to each cell type throughout the cellular taxonomy of the spinal cord by the iterative overlap-merging procedure in ArchR.
- d**, Number, top, and proportion, bottom, of promoter, exonic, intronic, or distal peaks when calling peaks at levels 1 to 4 of the cellular taxonomy.
- e**, Distance to the nearest TSS for peaks called at levels 1 to 4 of the cellular taxonomy.
- f**, Statistical comparison of distance to the nearest TSS for peaks called at levels 1 to 4 of the cellular taxonomy, as shown in **e**. Calling peaks for cell subtypes defined at more granular resolutions leads to improved detection of distal regulatory elements.
- g**, Distribution of the mean fragment counts per cell, for all peaks called at level 4 of the cellular taxonomy.
- h**, Distribution of the mean proportion of cells in which each peak is accessible, for all peaks called at level 4 of the cellular taxonomy.
- i**, Scatterplot showing the relationship between the mean fragment counts per cell and the mean, for all peaks called at level 4 of the cellular taxonomy.



Supplementary Fig. 22 | Cell-type-specific transcription factors in the multiome atlas.

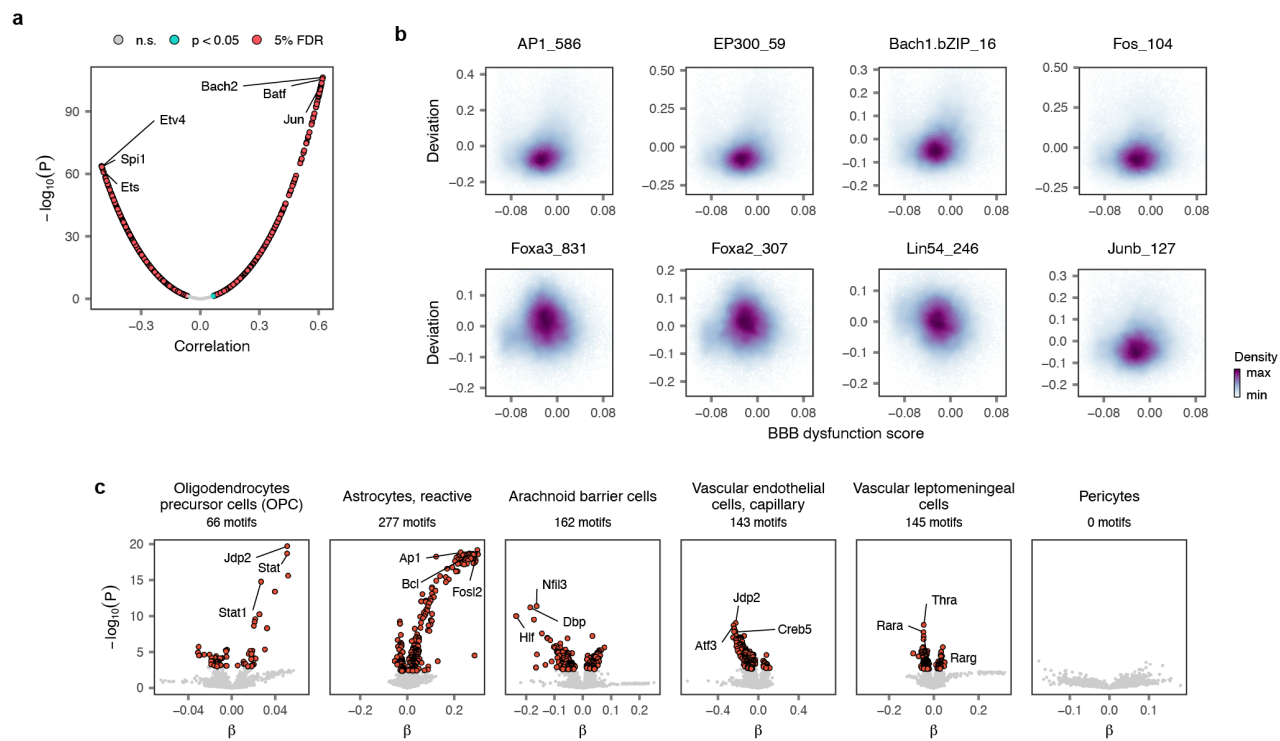
a, Deviations of the top ten cell-type-specific transcription factors for major (level 1) cell types identified by chromVAR, shown for each individual barcode in the multiome atlas.

b, Median deviations of the top five cell-type-specific transcription factors for level 2 cell types identified by chromVAR.



Supplementary Fig. 23 | Cell type- and subtype-specific transcription factors in the multiome atlas.

Cell-type-specific transcription factors discussed in the main text. Left, UMAP visualizations of chromVAR motif deviations for cells subset to the relevant major (level 1) cell type, **a-l**, or for all cells in the dataset, **m**. Right, dendrograms showing mean and median chromVAR motif deviations per cell type for levels 1-4 of the clustering tree.

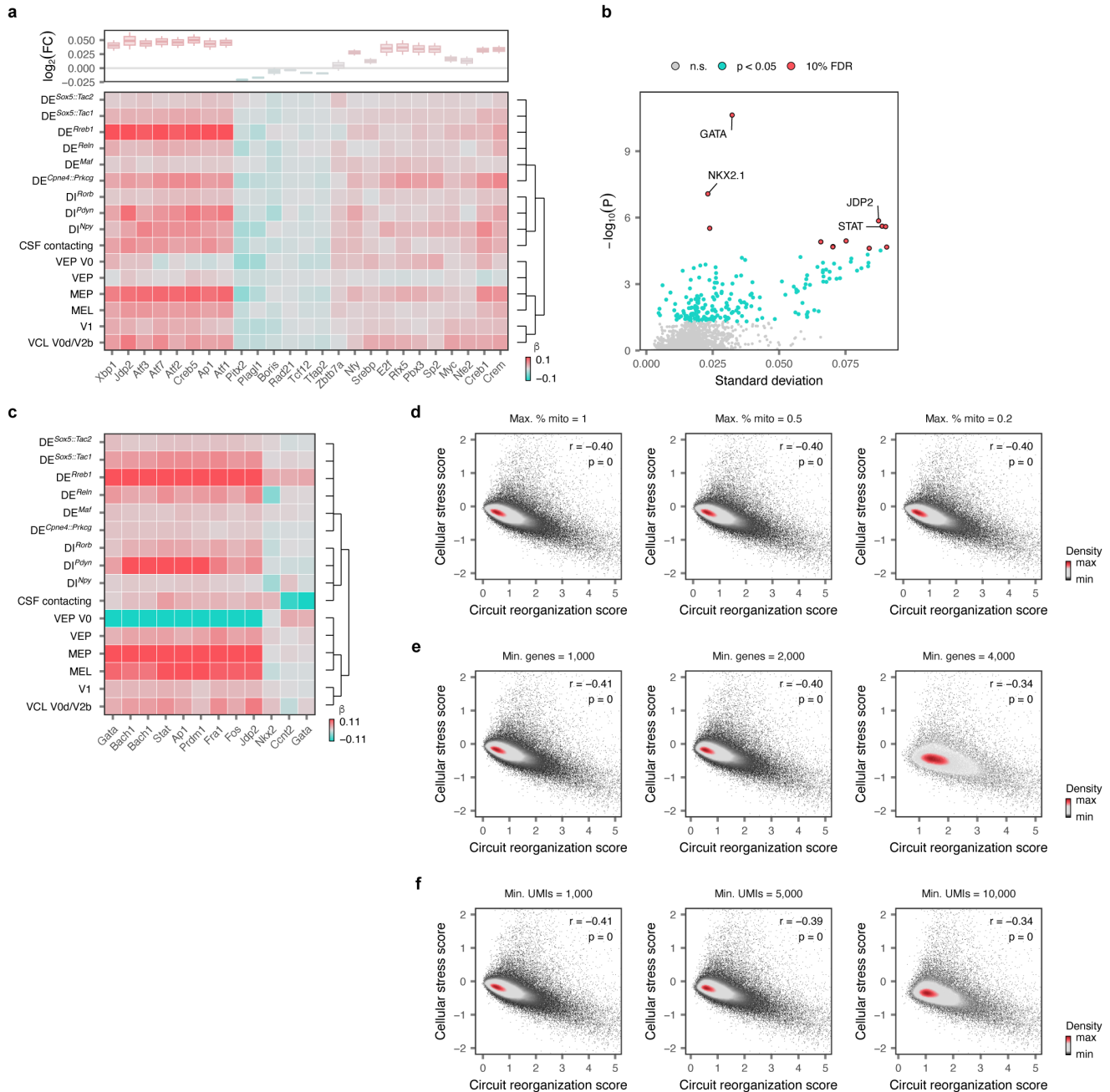


Supplementary Fig. 24 | Regulatory programs underlying tripartite barrier formation.

a, Volcano plot showing correlations between transcription factor binding motif accessibility, in the ATAC modality, and expression of the blood-brain barrier dysfunction score, in the RNA modality, across all vascular cells. The top three correlated and anti-correlated motifs are annotated.

b, Scatterplots highlighting relationships between expression of the blood-brain barrier dysfunction score and transcription factor binding motif accessibility for selected motifs discussed in the main text. Points are colored by the density of individual nuclei.

c, Volcano plots showing differentially accessible transcription factor binding motifs in the other cell types that establish the tripartite barrier.



Supplementary Fig. 25 | Conserved and divergent regulatory programs in injured neurons.

a, Top, boxplot showing the estimated effect of SCI on transcription factor binding motif accessibility across all level 4 neuron subtypes at 7 days post-injury. Bottom, heatmap showing the estimated effect of SCI on transcription factor binding motif accessibility within each level 4 neuron subtype individually.

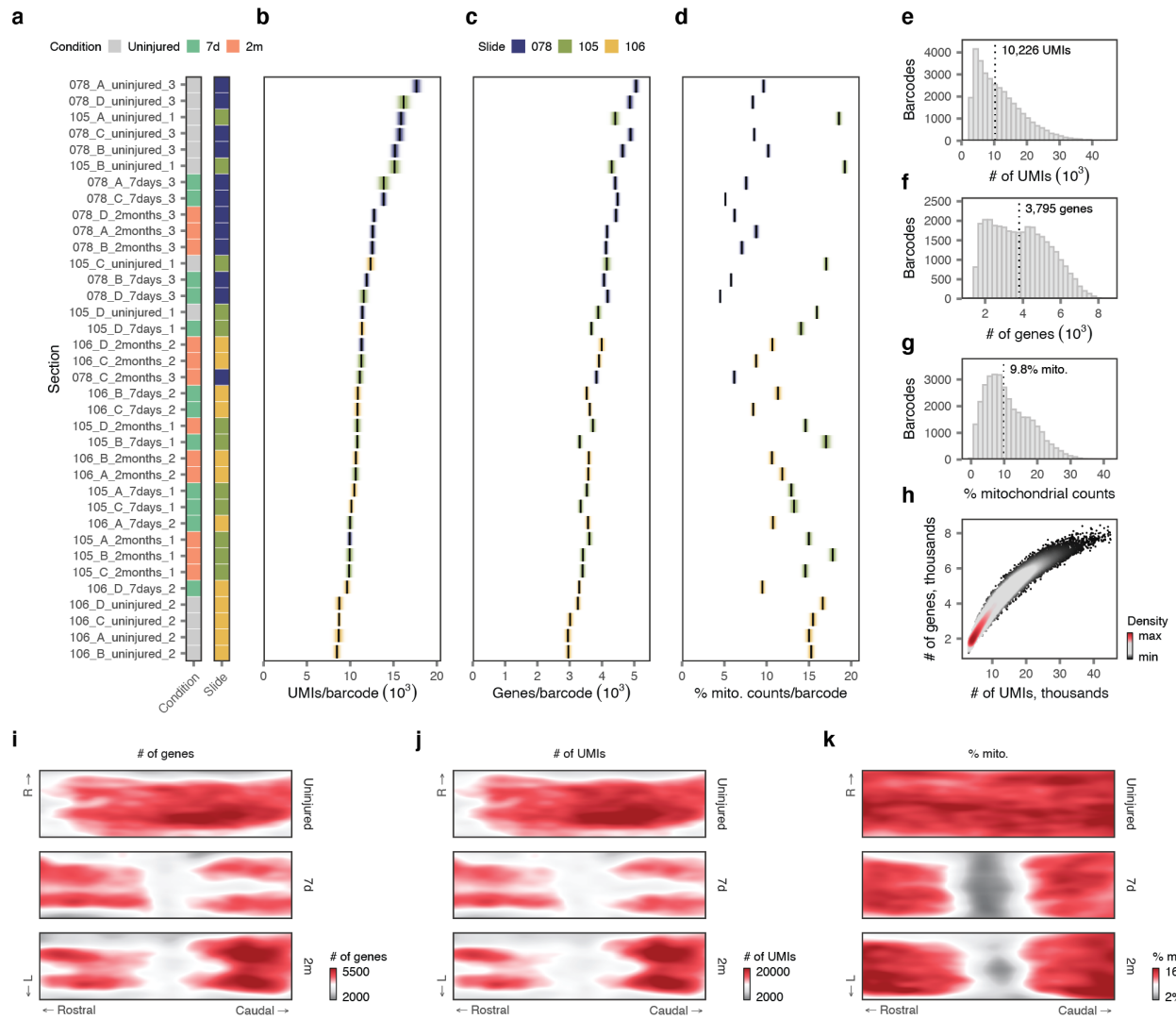
b, Identification of transcription factor binding motifs with significant variation across neuron subtypes in their patterns of accessibility at 2 months post-injury. The x-axis shows the standard deviation of coefficients estimated by linear mixed models for each neuron subtype independently, while the y-axis shows the statistical significance of the standard deviation compared to linear mixed models fit with randomized neuron subtype assignments.

c, Heatmap showing the estimated effect of SCI on transcription factor binding motif accessibility across level 4 neuron subtypes for the significantly variable motif shown in **b**.

d, Anticorrelated expression of the circuit reorganization module, x-axis, versus that of the cellular stress module, y-axis, in 80,315 neurons from the snRNA-seq atlas, as in **Fig. 5g** but after imposing increasingly stringent filters on the minimum number of UMIs per neuron.

e, As in **d**, but after imposing increasingly stringent filters on the minimum number of genes expressed per neuron.

f, As in **d**, but after imposing increasingly stringent filters on the maximum proportion of mitochondrial counts per neuron.



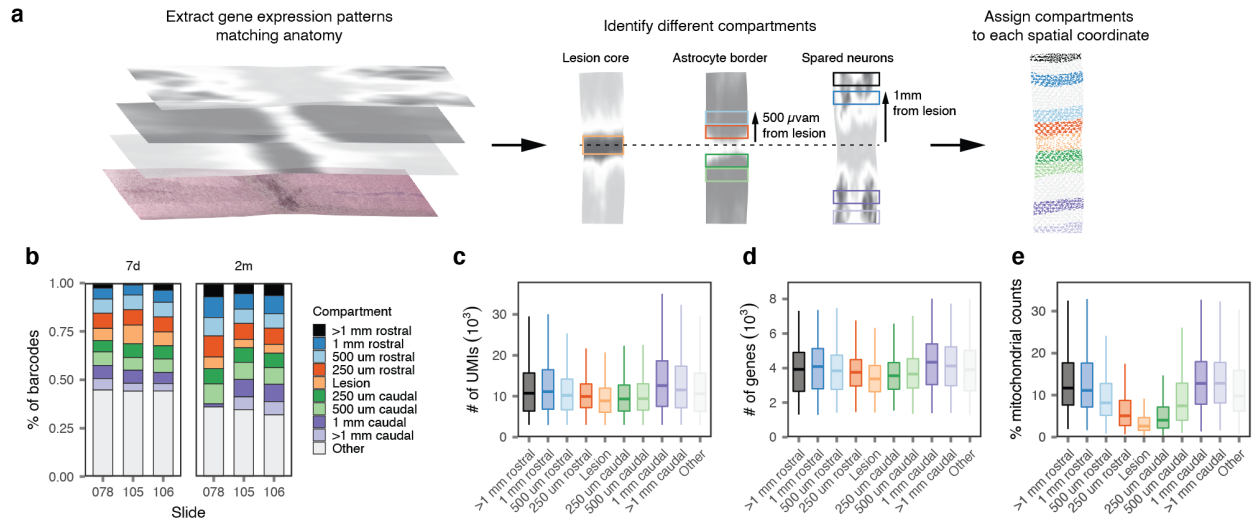
Supplementary Fig. 27 | Quality control of the 2D spatial atlas.

a-d, Quality control statistics for 36 sections profiled by spatial transcriptomics (**a**, legend showing the experimental condition and slide number for each section; **b**, mean number of UMIs per barcode; **c**, mean number of genes detected per barcode; **d**, mean proportion of mitochondrial counts per barcode). Shaded areas show the standard deviation. Sections are colored by the slide on which each section was sequenced.

e-g, Aggregate distributions of quality control statistics over all sections (**e**, number of UMIs per barcode; **f**, number of genes detected per barcode; **g**, proportion of mitochondrial counts per barcode).

h, Relationship between the number of UMIs and number of genes detected per spatial barcode.

i-k, Quality control statistics visualized on the common coordinate system of the spinal cord, separately for each experimental condition (**i**, mean number of UMIs per barcode; **j**, mean number of genes detected per barcode; **k**, mean proportion of mitochondrial counts per barcode).

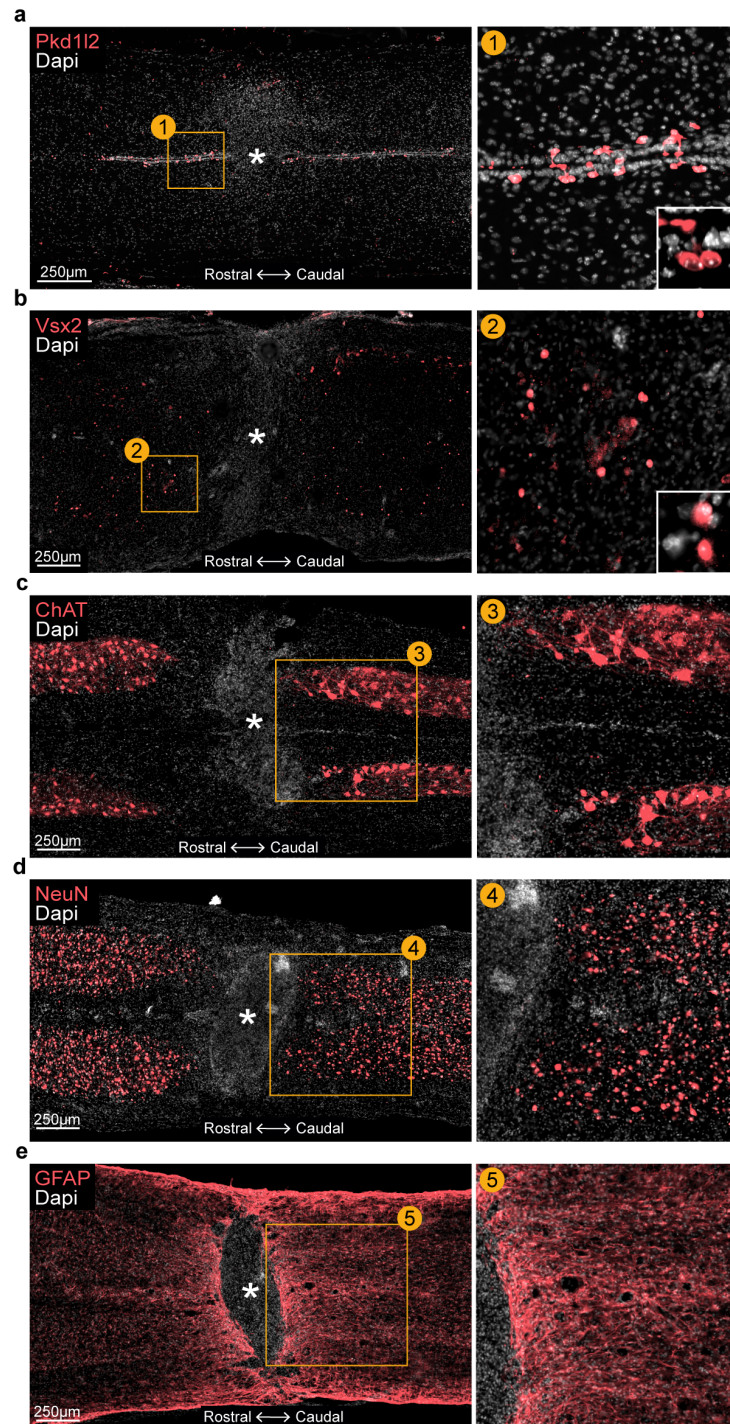


Supplementary Fig. 28 | Definition and quality control of lesion compartments.

a, Schematic overview of the procedure for definition of lesion compartments on the common coordinate system of the spinal cord.

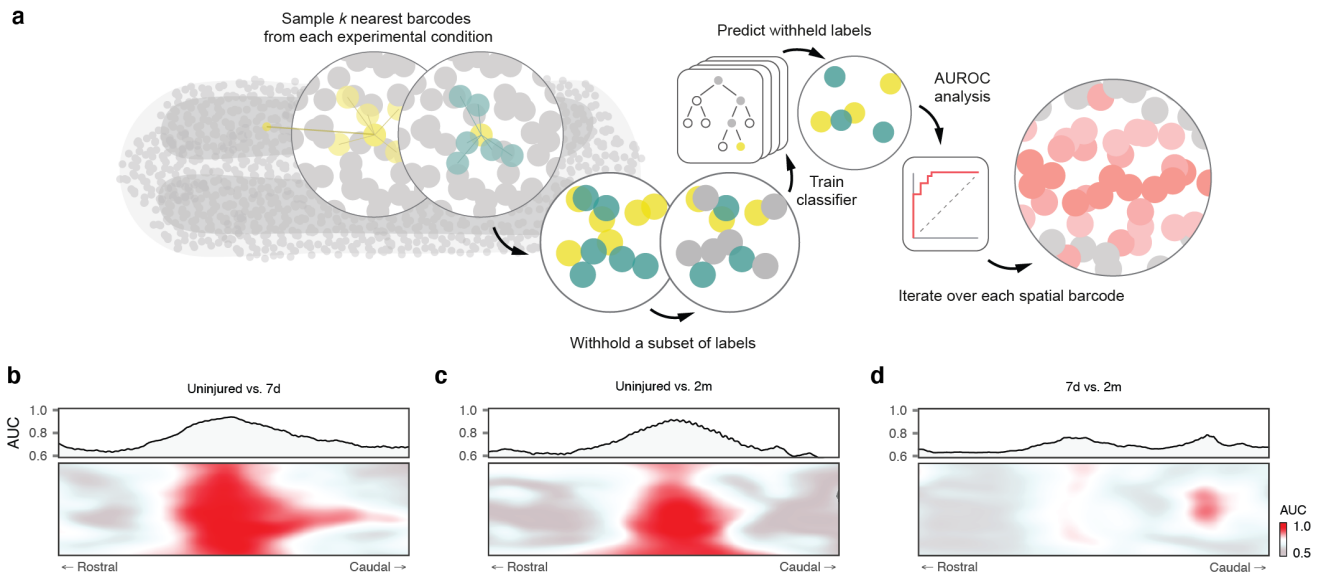
b, Proportion of spatial barcodes assigned to each lesion compartment within each slide.

c-e, Distributions of quality control statistics (**c**, number of UMIs per barcode; **d**, number of genes detected per barcode; **e**, proportion of mitochondrial counts per barcode) for spatial barcodes within each lesion compartment.



Supplementary Fig. 29 | Immunohistochemical validation of cell type deconvolution.

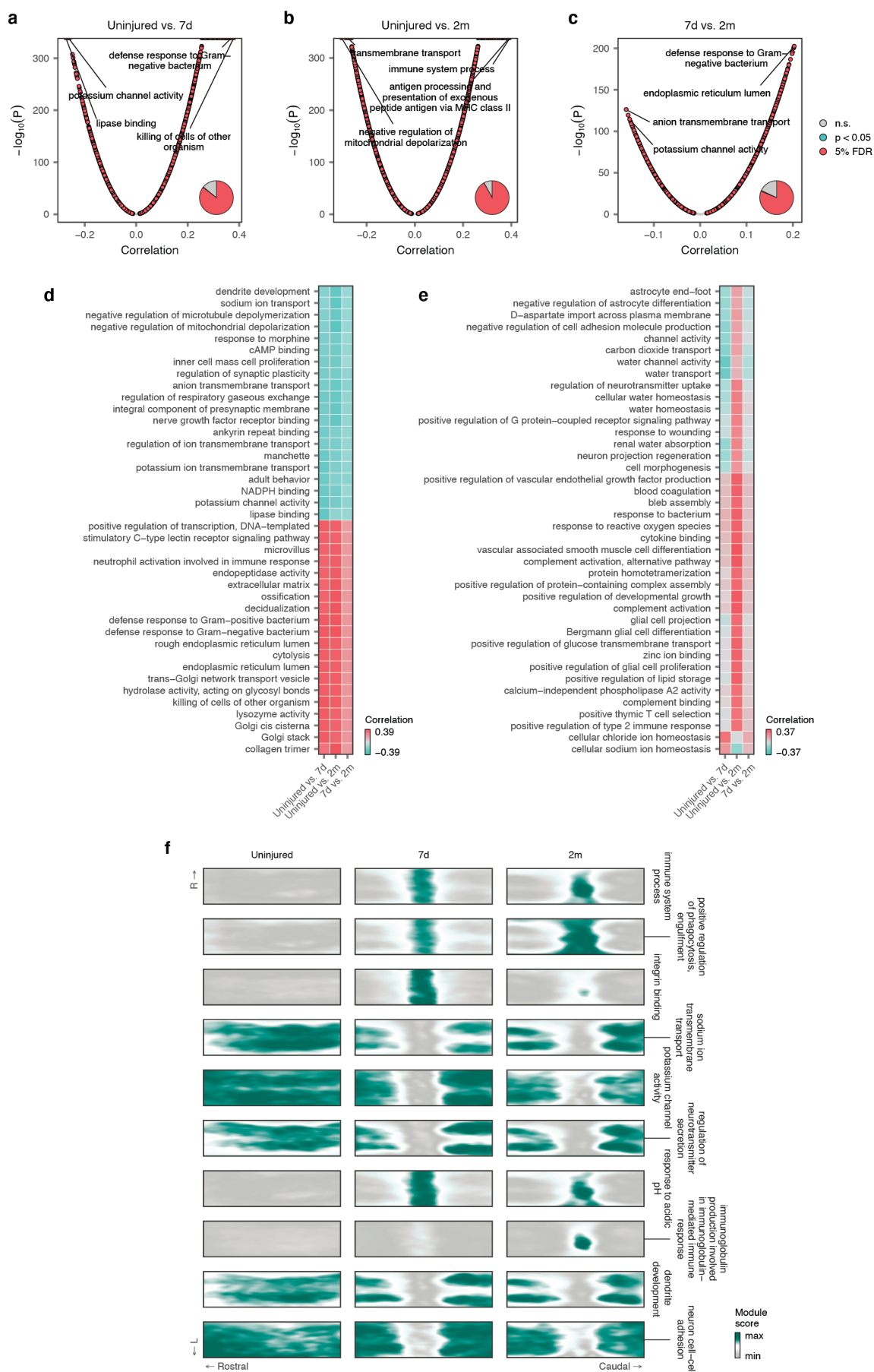
a-e, Immunohistochemical staining validating the spatial distributions of cell types in the injured spinal cord at 7 days after SCI. **a**, Pkd1l2, CSF-contacting neurons; **b**, Vsx2, VEP/VEL V2a neurons; **c**, ChAT, motor neurons; **d**, NeuN, neurons; **e**, GFAP, astrocytes.



Supplementary Fig. 30 | Spatial prioritization of the 2D spatial atlas.

a, Schematic overview illustrating the concept of spatial prioritization, as captured in Magellan.

b-d, Spatial prioritizations assigned by Magellan to each pairwise comparison of experimental conditions in the 2D spatial atlas (**b**, 7 days versus uninjured; **c**, 2 months versus uninjured, **d**, 7 days versus 2 months). Bottom, AUCs of spatial prioritization visualized on the two-dimensional coordinate system of the injured spinal cord. Top, average AUCs of spatial prioritization along the rostrocaudal axis, highlighting prioritization of the lesion site.



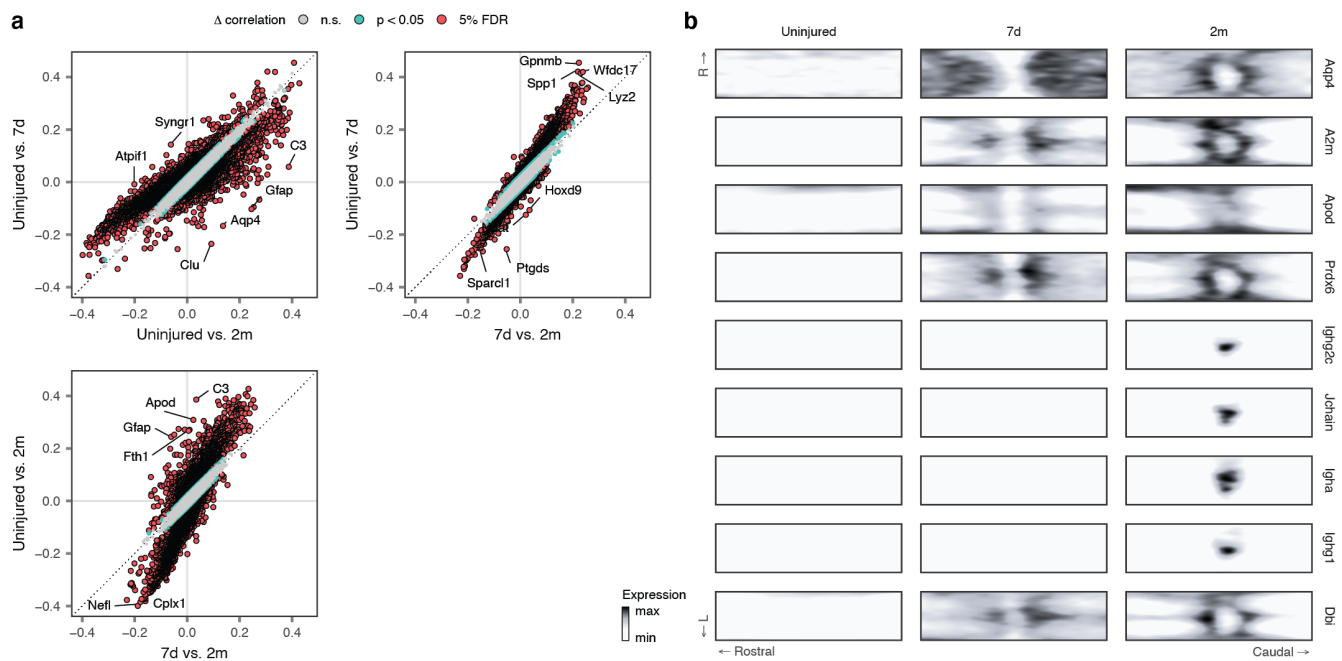
Supplementary Fig. 31 | Molecular basis of spatial prioritization at the gene module level.

a-c, Volcano plots showing correlations between the AUCs assigned by Magellan at each spatial barcode and the average expression of genes associated with a given GO term ("GO modules") across the same spatial barcodes (**a**, 7 days versus uninjured; **b**, 2 months versus uninjured; **c**, 7 days versus 2 months). Inset pie charts show the proportions of all tested GO modules that are significantly correlated with the spatial prioritizations.

d, Heatmap showing Pearson correlations between spatial prioritizations and GO module scores for each pairwise comparison of experimental conditions, for the top 40 most recurrently correlated GO modules across all comparisons.

e, Heatmap showing Pearson correlations between spatial prioritizations and GO module scores for each pairwise comparison of experimental conditions, for the top 40 most variably correlated GO modules across all comparisons.

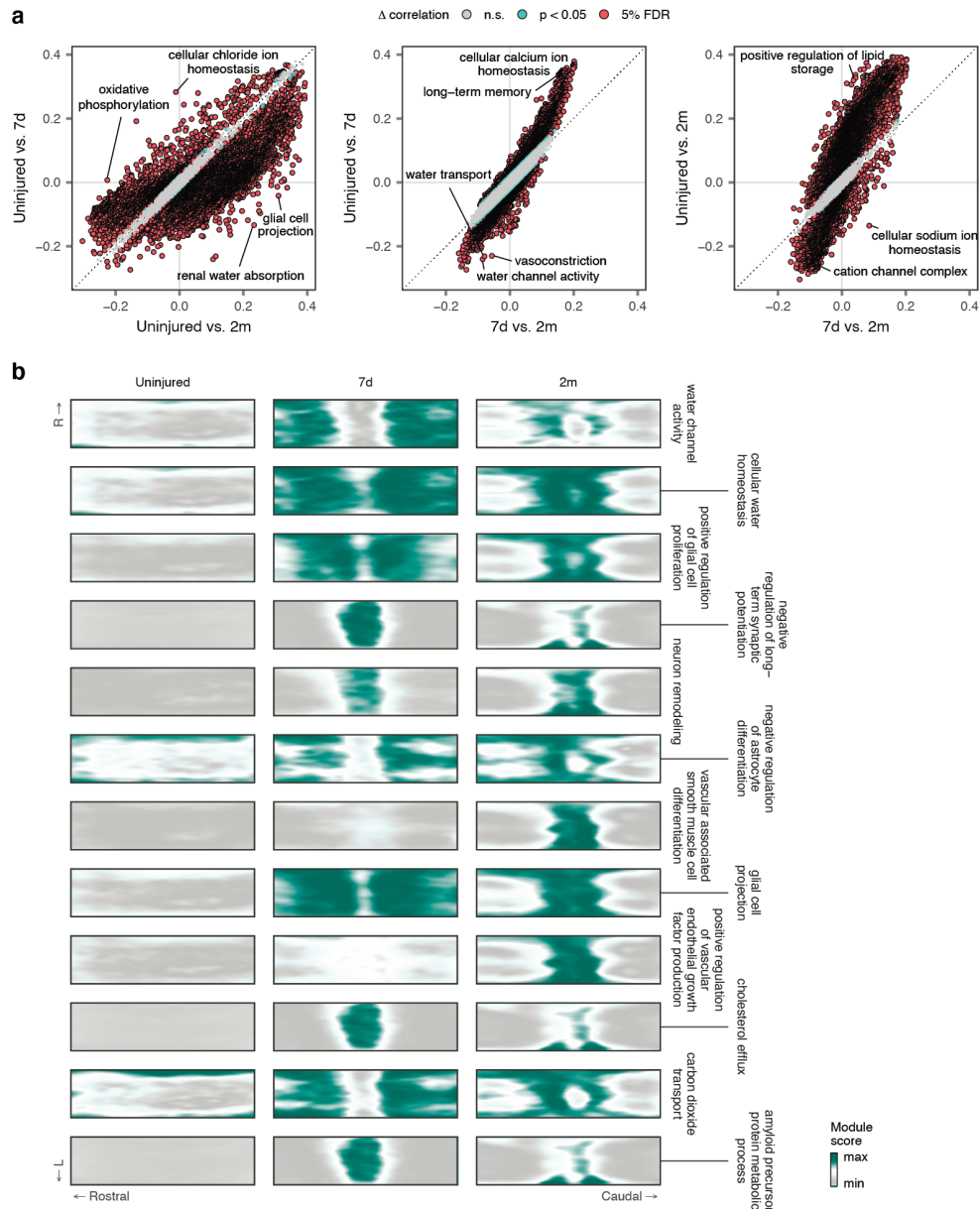
f, Visualization of selected GO modules prioritized by their correlation to spatial prioritizations on the two-dimensional coordinate system of the injured spinal cord.



Supplementary Fig. 32 | Molecular basis of differential spatial prioritization at the gene level.

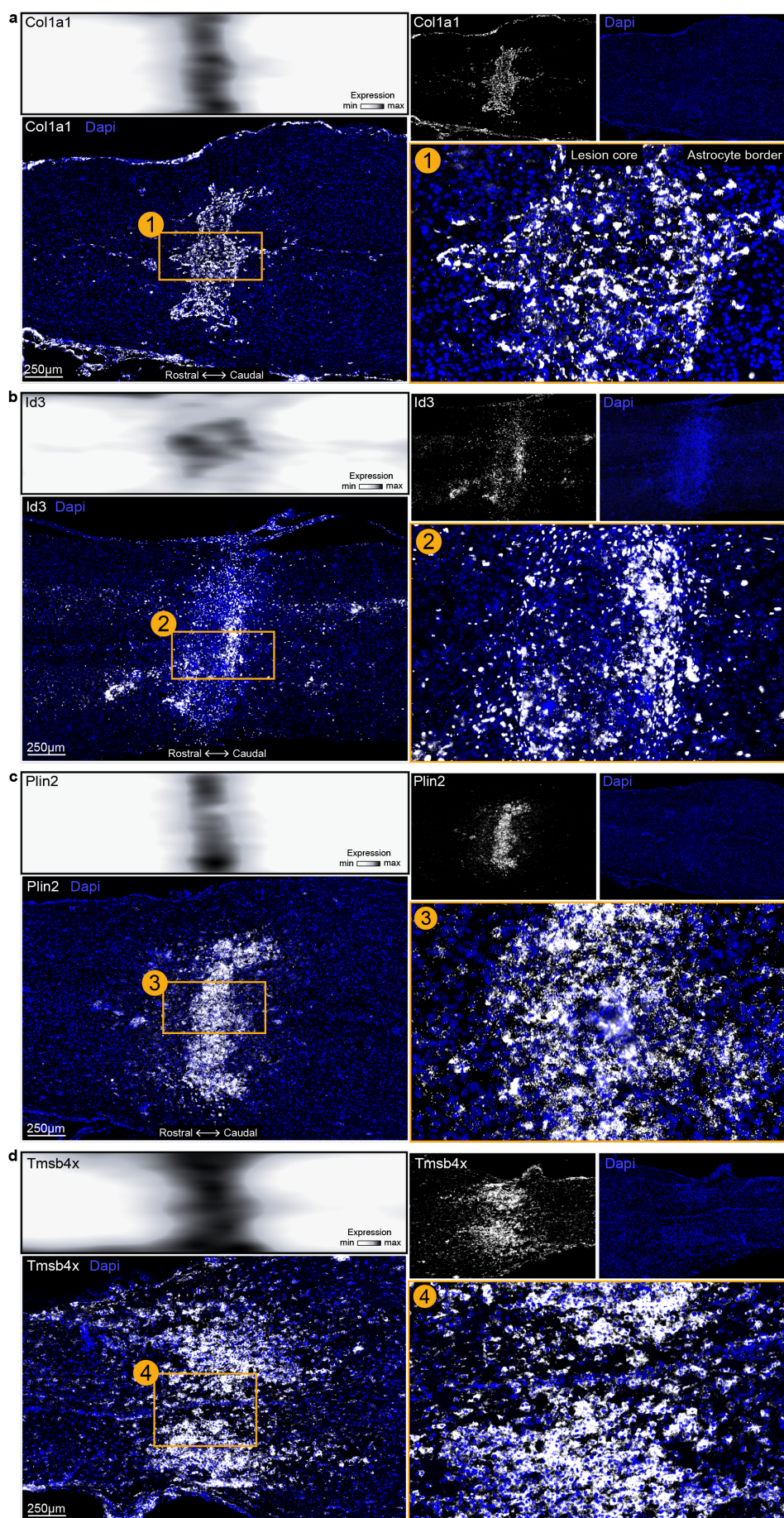
a, Scatterplots highlighting genes with differential correlations to spatial prioritizations between pairwise comparisons of experimental conditions.

b, Visualization of selected genes prioritized by their differential correlation to spatial prioritizations between comparisons on the two-dimensional coordinate system of the injured spinal cord.



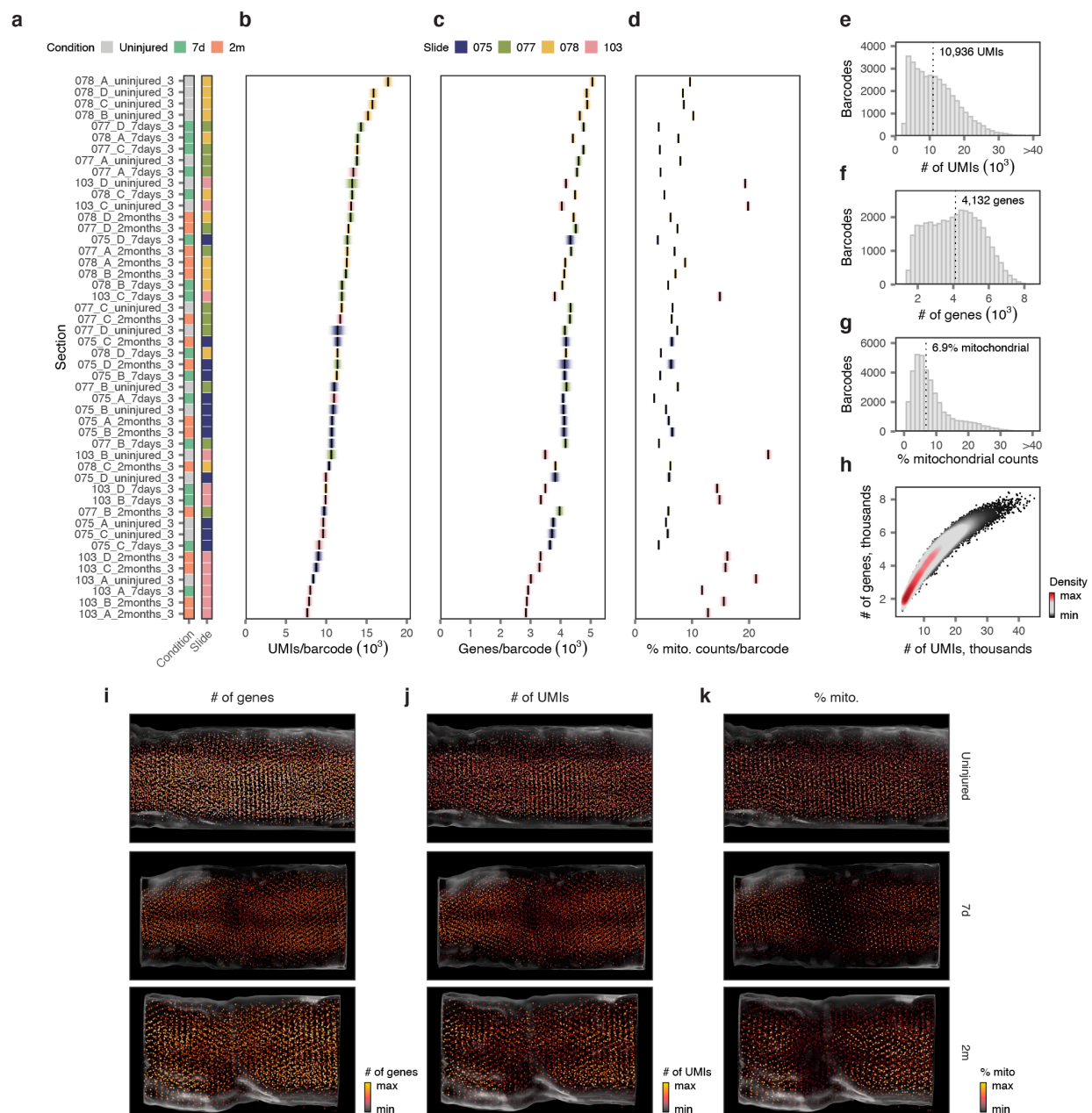
Supplementary Fig. 33 | Molecular basis of differential spatial prioritization at the gene module level.

a, Scatterplots highlighting GO modules with differential correlations to spatial prioritizations between pairwise comparisons of experimental conditions.
b, Visualization of selected GO modules prioritized by their differential correlation to spatial prioritizations between comparisons on the two-dimensional coordinate system of the injured spinal cord.



Supplementary Fig. 34 | Spatial localization of genes identified by compartment-specific DE and spatial prioritization analyses.

- a**, Expression of *Col1a1* in the injured spinal cord at 7 days as measured with spatial transcriptomics, top left, and RNAscope in situ hybridization, as a representative gene upregulated within the lesion core.
- b**, As in **a**, but showing *Id3* as a representative gene upregulated within the astrocyte barrier.
- c**, As in **a**, but showing *Plin2* as a representative gene prioritized by Magellan.
- d**, As in **a**, but showing *Tmsb4x* as a representative gene prioritized by Magellan.



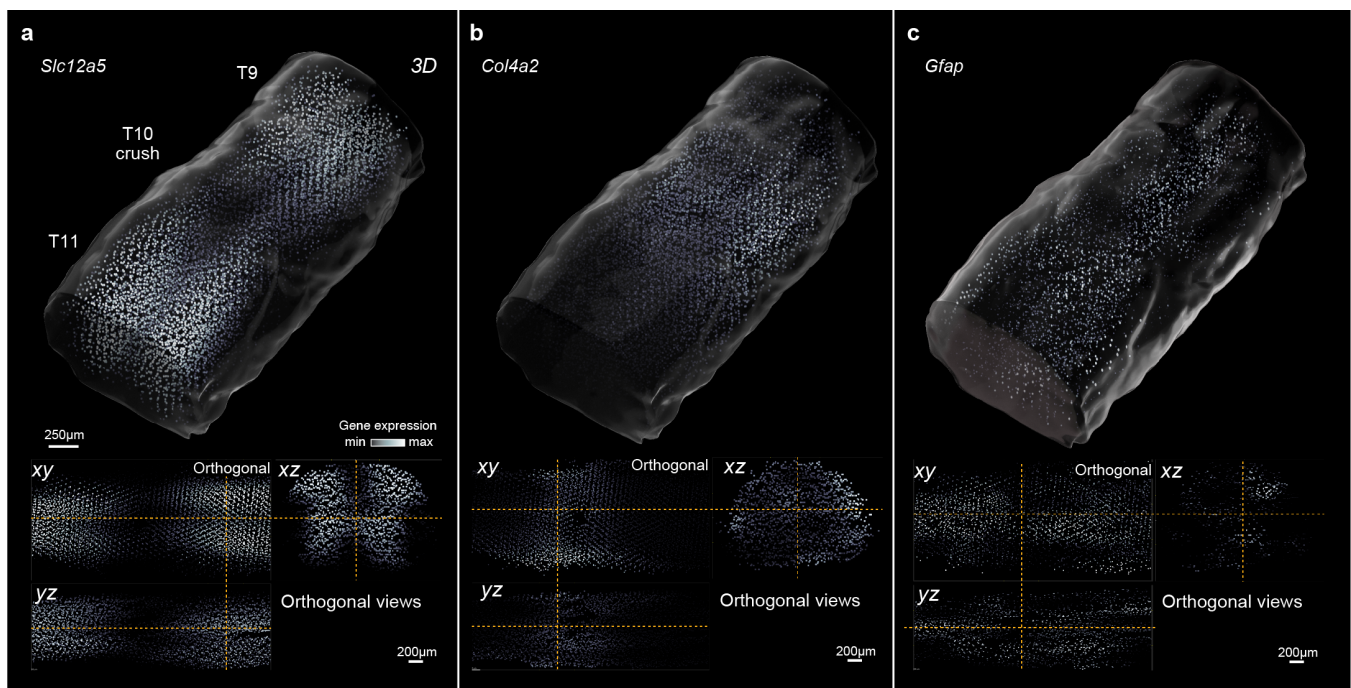
Supplementary Fig. 35 | Quality control of the 3D spatial atlas.

a-d, Quality control statistics for 16 sections profiled by three-dimensional spatial transcriptomics (**a**, legend showing the experimental condition and slide number for each section; **b**, mean number of UMIs per barcode; **c**, mean number of genes detected per barcode; **d**, mean proportion of mitochondrial counts per barcode). Shaded areas show the standard deviation. Sections are colored by the slide on which each section was sequenced.

e-g, Aggregate distributions of quality control statistics over all sections in the 3D spatial atlas (**e**, number of UMIs per barcode; **f**, number of genes detected per barcode; **g**, proportion of mitochondrial counts per barcode).

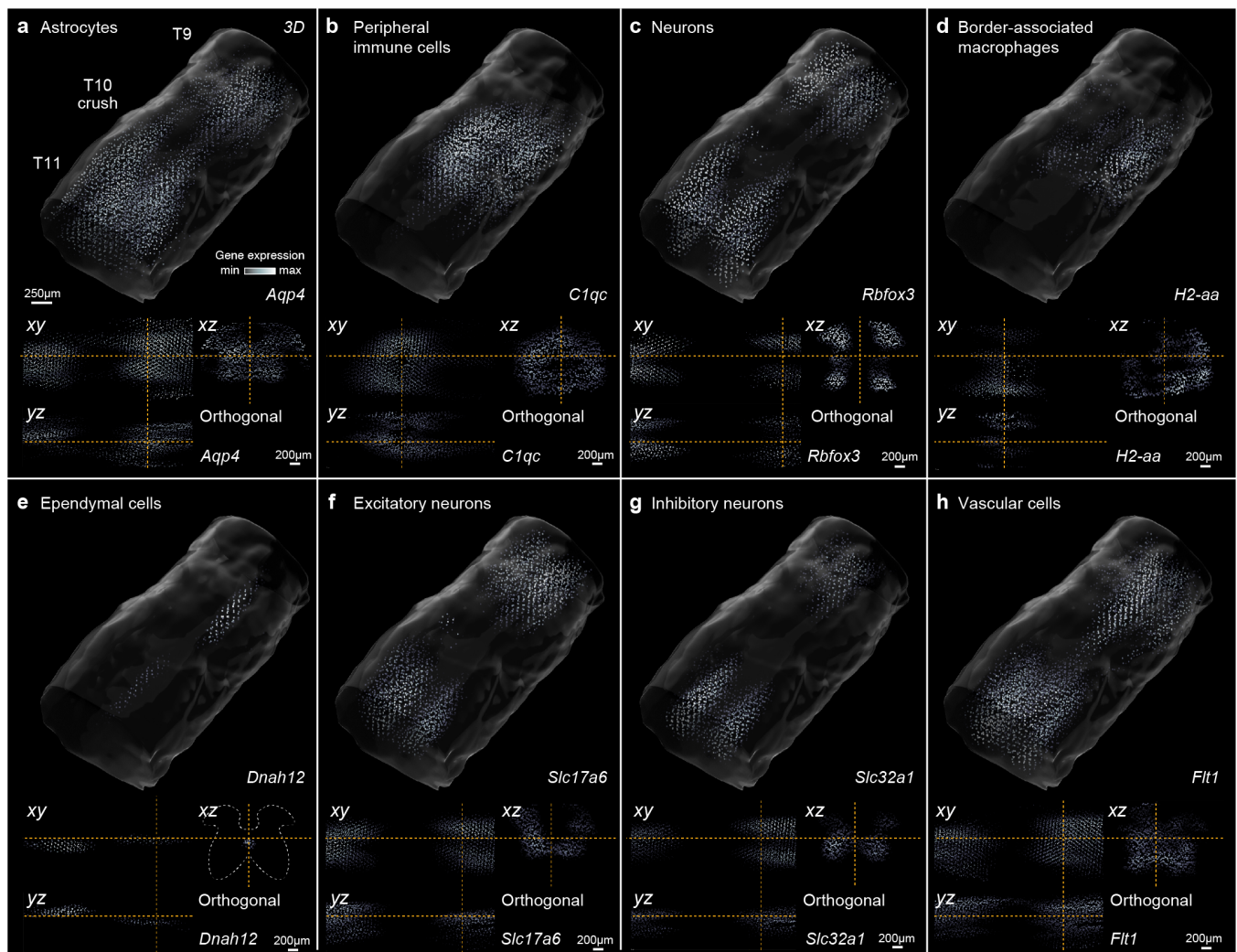
h, Relationship between the number of UMIs and number of genes detected per spatial barcode in the 3D spatial atlas.

i-k, Quality control statistics visualized on the three-dimensional common coordinate system of the spinal cord, separately for each experimental condition (**i**, mean number of UMIs per barcode; **j**, mean number of genes detected per barcode; **k**, mean proportion of mitochondrial counts per barcode).



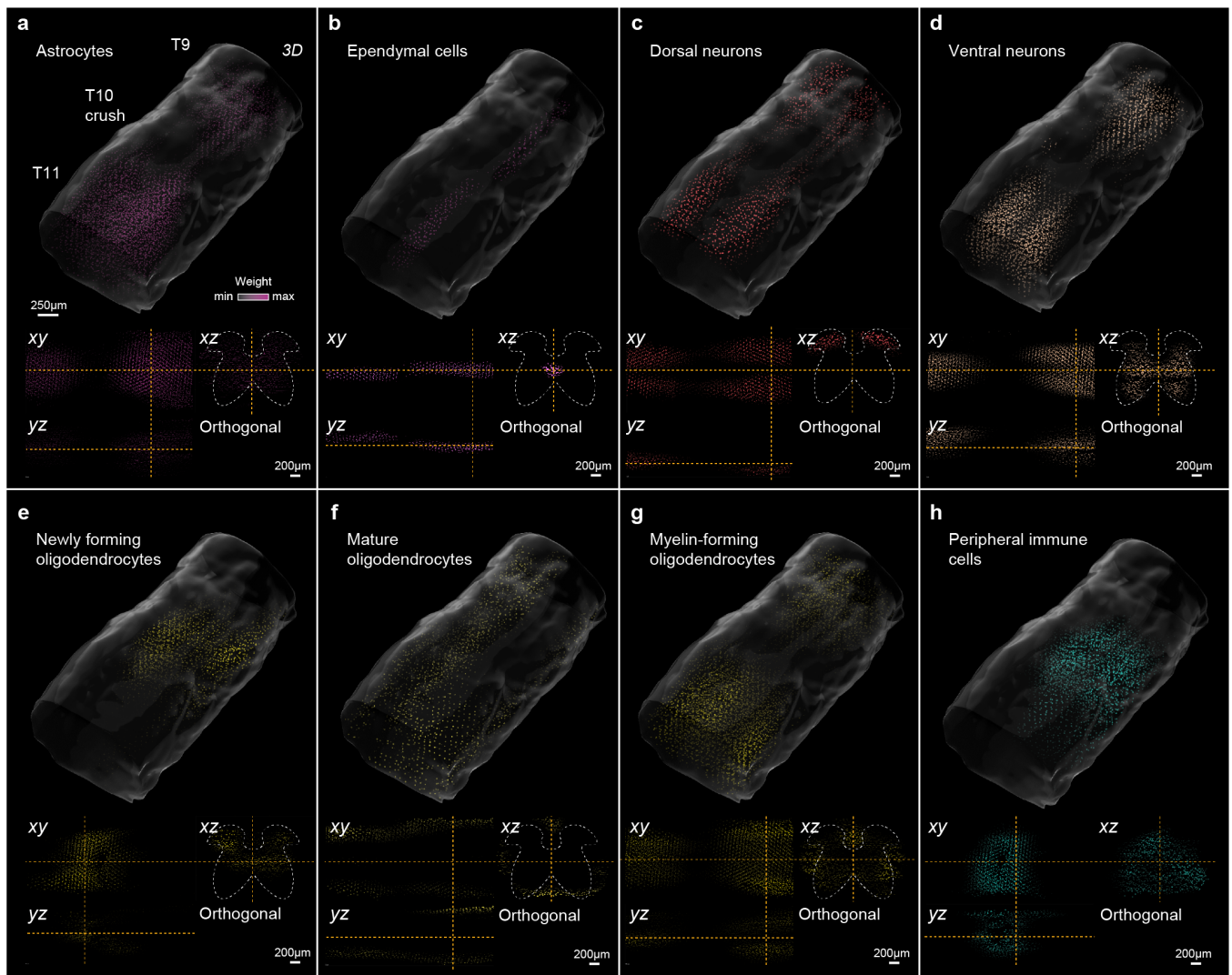
Supplementary Fig. 36 | Lesion compartments in the 3D spatial atlas.

Expression of marker genes associated with distinct lesion compartments across the 3D spatial transcriptomic atlas at 7 days, including spared but reactive neural tissue (*Slc12a5*, **a**) the fibrotic scar (*Col4a2*, **b**), and the astrocyte barrier (*Gfap*, **c**), as shown in **Fig. 8b** but here visualized separately for each gene.



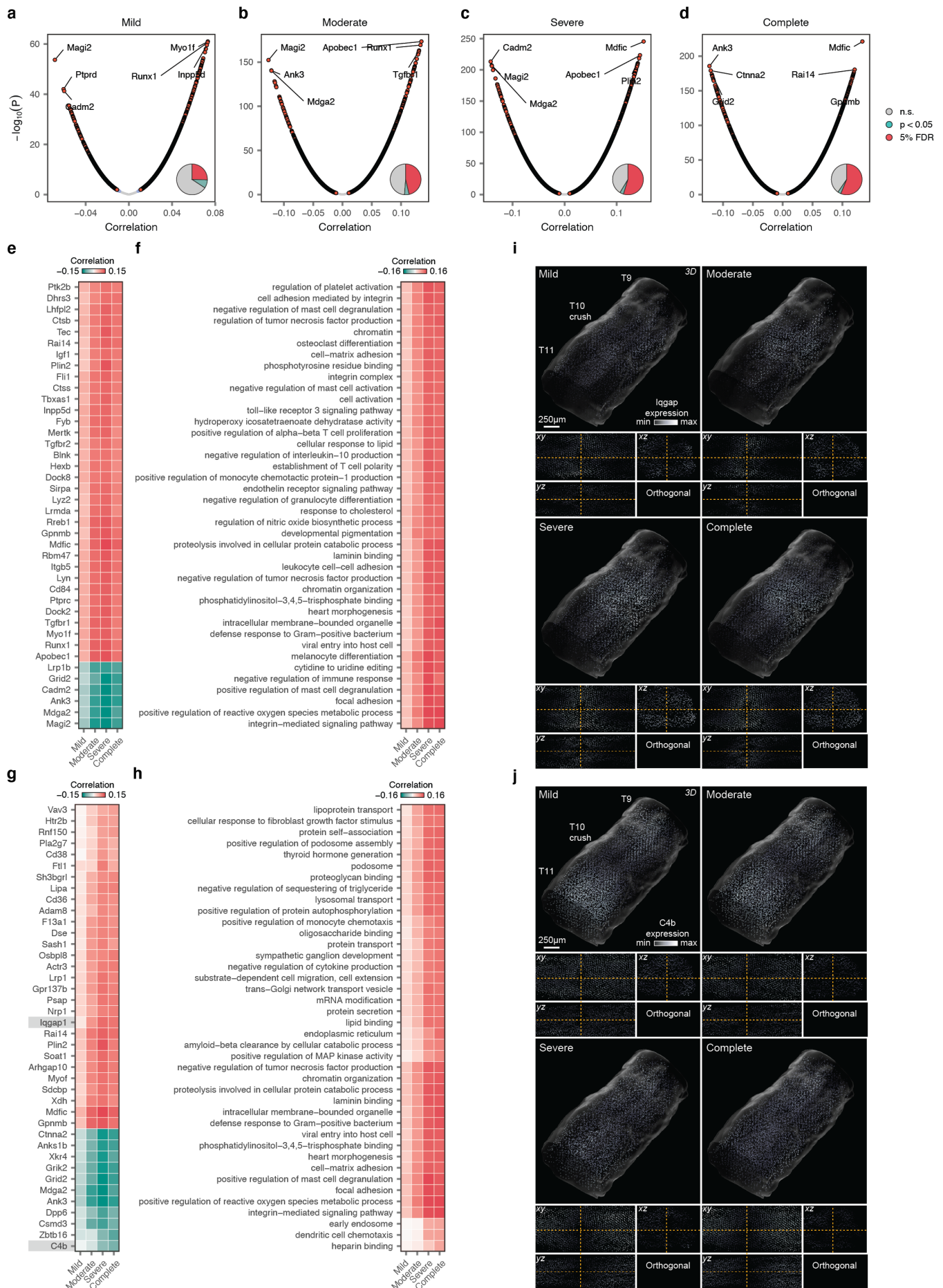
Supplementary Fig. 37 | Cell type marker gene expression in the 3D spatial atlas.

Expression of selected cell type marker genes across the 3D spatial transcriptomic atlas at 7 days. **a**, *Aqp4* (astrocytes); **b**, *C1qc* (peripheral immune cells); **c**, *Rbfox3* (neurons); **d**, *H2-aa* (border-associated macrophages); **e**, *Dnah12* (ependymal cells); **f**, *Slc17a6*, excitatory neurons; **g**, *Slc32a1* (inhibitory neurons); **h**, *Flt1* (vascular cells).



Supplementary Fig. 38 | Cell type deconvolution of the 3D spatial atlas.

Visualization of the deconvolution weights assigned by RCTD for selected level 2 cell types at 7 days, on the two-dimensional coordinate system of the injured spinal cord. **a**, Astrocytes; **b**, ependymal cells; **c**, dorsal neurons; **d**, ventral neurons; **e**, newly forming oligodendrocytes (NFOL); **f**, mature oligodendrocytes (MOL); **g**, myelin-forming oligodendrocytes (MFOL); **h**, peripheral immune cells.



Supplementary Fig. 39 | Molecular basis of three-dimensional spatial prioritization at the gene and gene module levels.

a-d, Volcano plots showing correlations between the AUCs assigned by Magellan to each spatialized barcode from the snRNA-seq and transcriptome-wide gene expression across the same snRNA-seq barcodes (**a**, mild; **b**, moderate; **c**, severe; **d**, complete). Inset pie charts show the proportions of all tested genes that are significantly correlated with the spatial prioritizations.

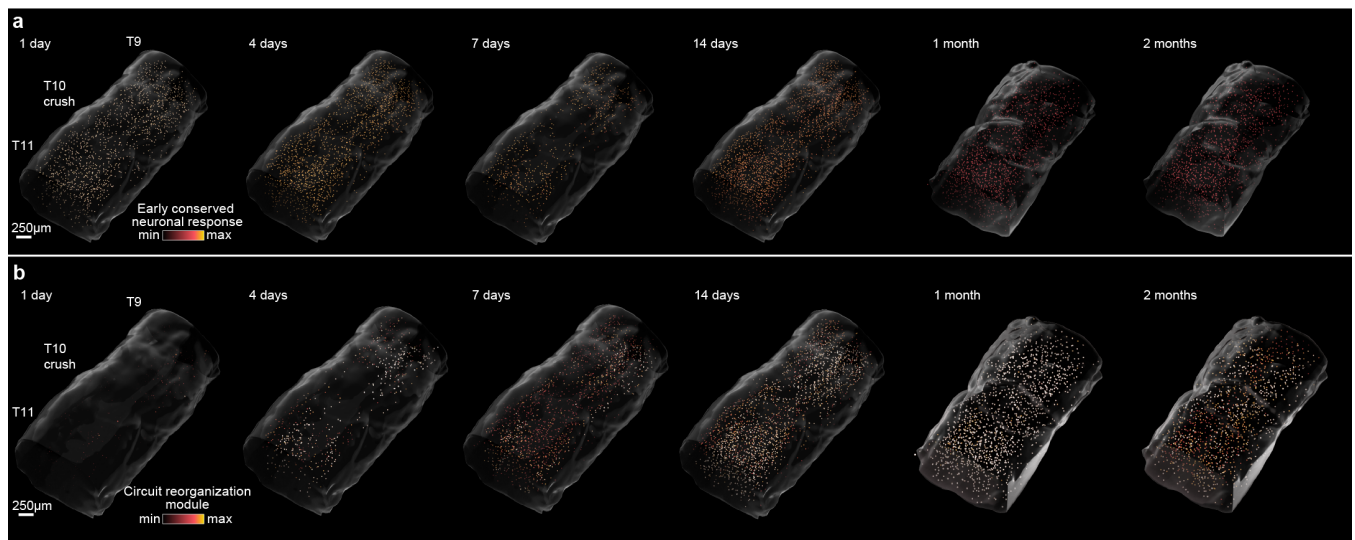
e, Heatmap showing Pearson correlations between spatial prioritizations and gene expression for the top 40 most recurrently correlated genes across injury severities.

f, Heatmap showing Pearson correlations between spatial prioritizations and gene expression for the top 40 most variably correlated genes across injury severities.

g, As in **e**, but for gene modules.

h, As in **f**, but for gene modules.

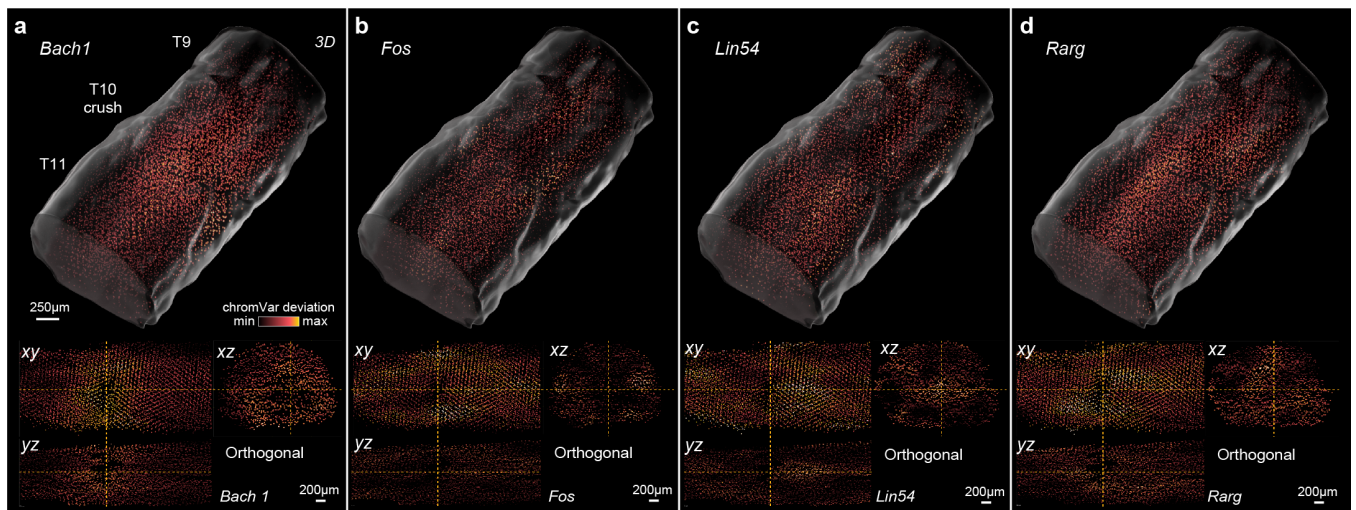
i-j, Expression of selected genes from **f** across injury severities, visualized on the 3D spatial transcriptomic atlas (**i**, *Iqgap*; **j**, *C4b*).



Supplementary Fig. 40 | Early-conserved and late-diverging neuronal responses to SCI in the 3D spatial atlas.

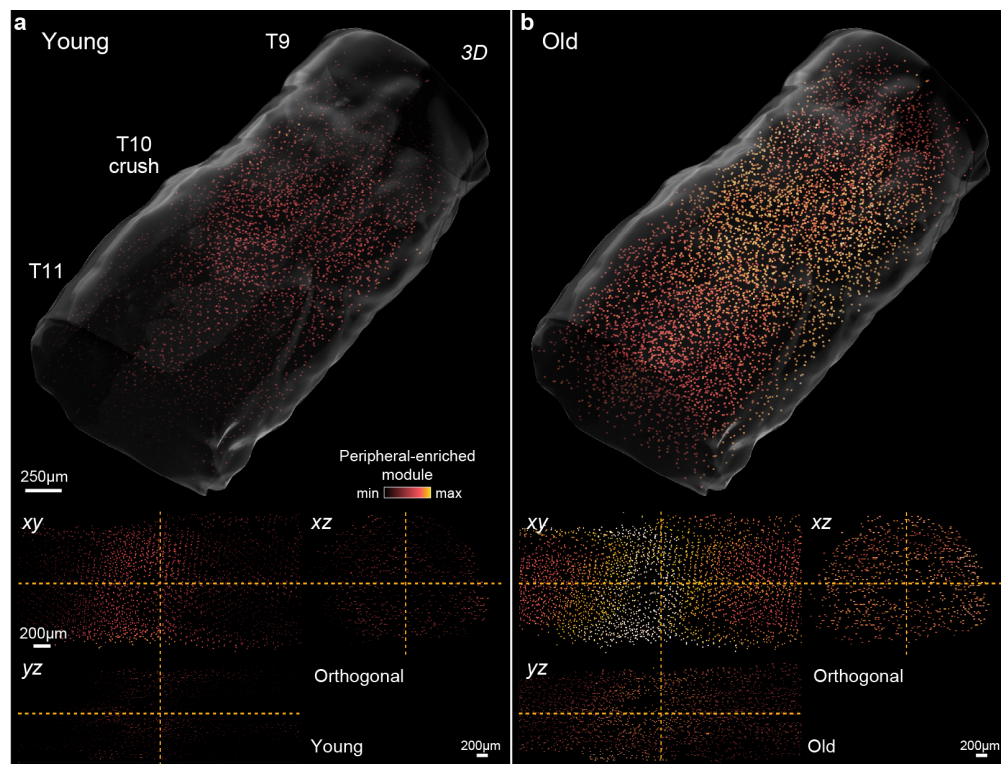
a, Expression of the early conserved neuronal response module in spatialized neurons from the snRNA-seq atlas, visualized on the coordinate system of the 3D spatial transcriptomic atlas.

b, Expression of the circuit reorganization module in spatialized neurons from the snRNA-seq atlas, visualized on the coordinate system of the 3D spatial transcriptomic atlas.



Supplementary Fig. 41 | Three-dimensional mapping of chromatin accessibility.

Accessibility of motifs associated with tripartite barrier formation in spatialized vascular cells from the multiome atlas, visualized on the coordinate system of the 3D spatial transcriptomic atlas. **a**, *Bach1*; **b**, *Fos*; **c**, *Lin54*; **d**, *Rarg*.



Supplementary Fig. 42 | Loss of blood-brain barrier transcriptional identity in old mice.

Expression of genes that distinguish peripheral endothelial cells from cells of the blood-brain barrier in spatialized vascular cells from young versus old mice, visualized on the coordinate system of the 3D spatial transcriptomic atlas.

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