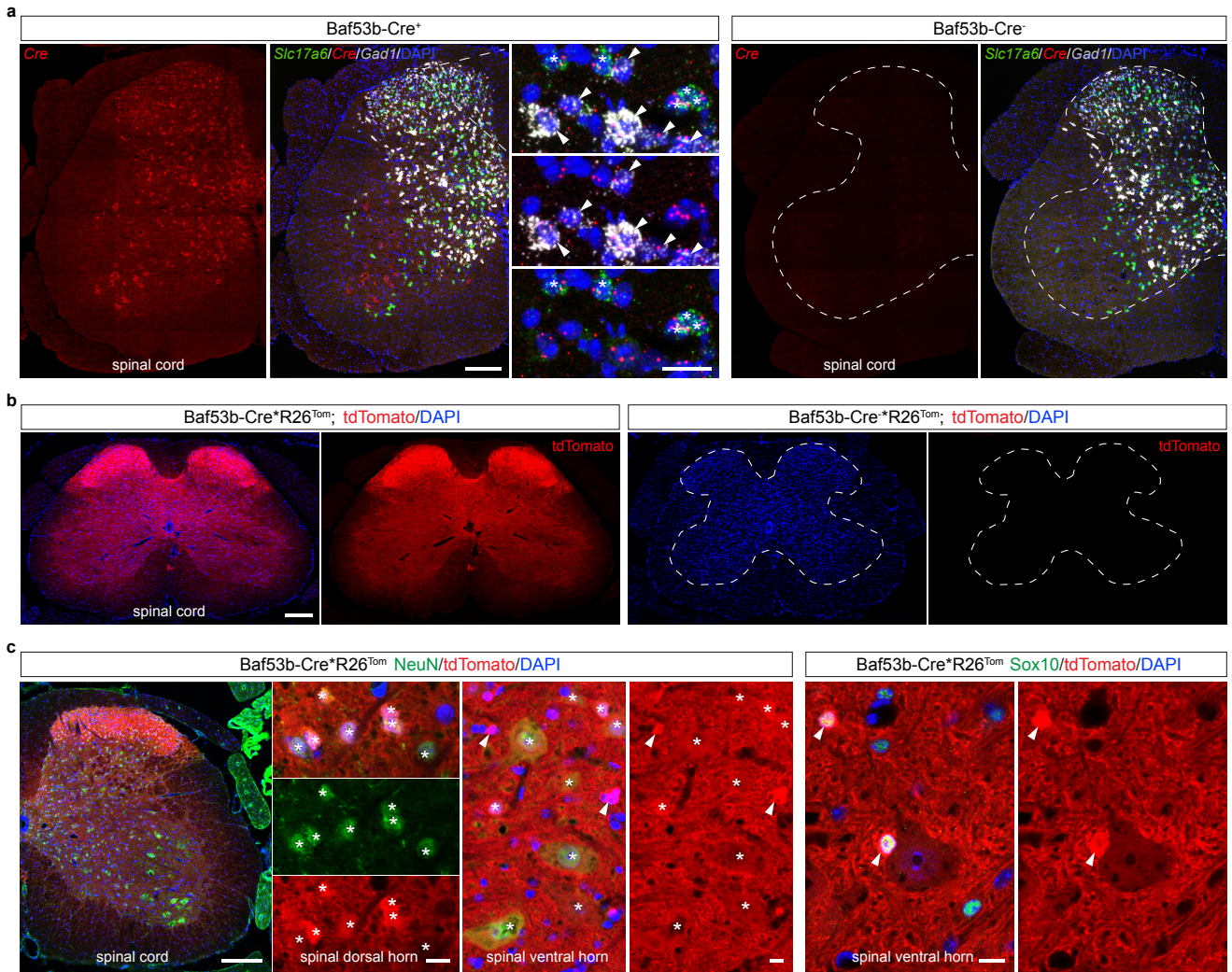
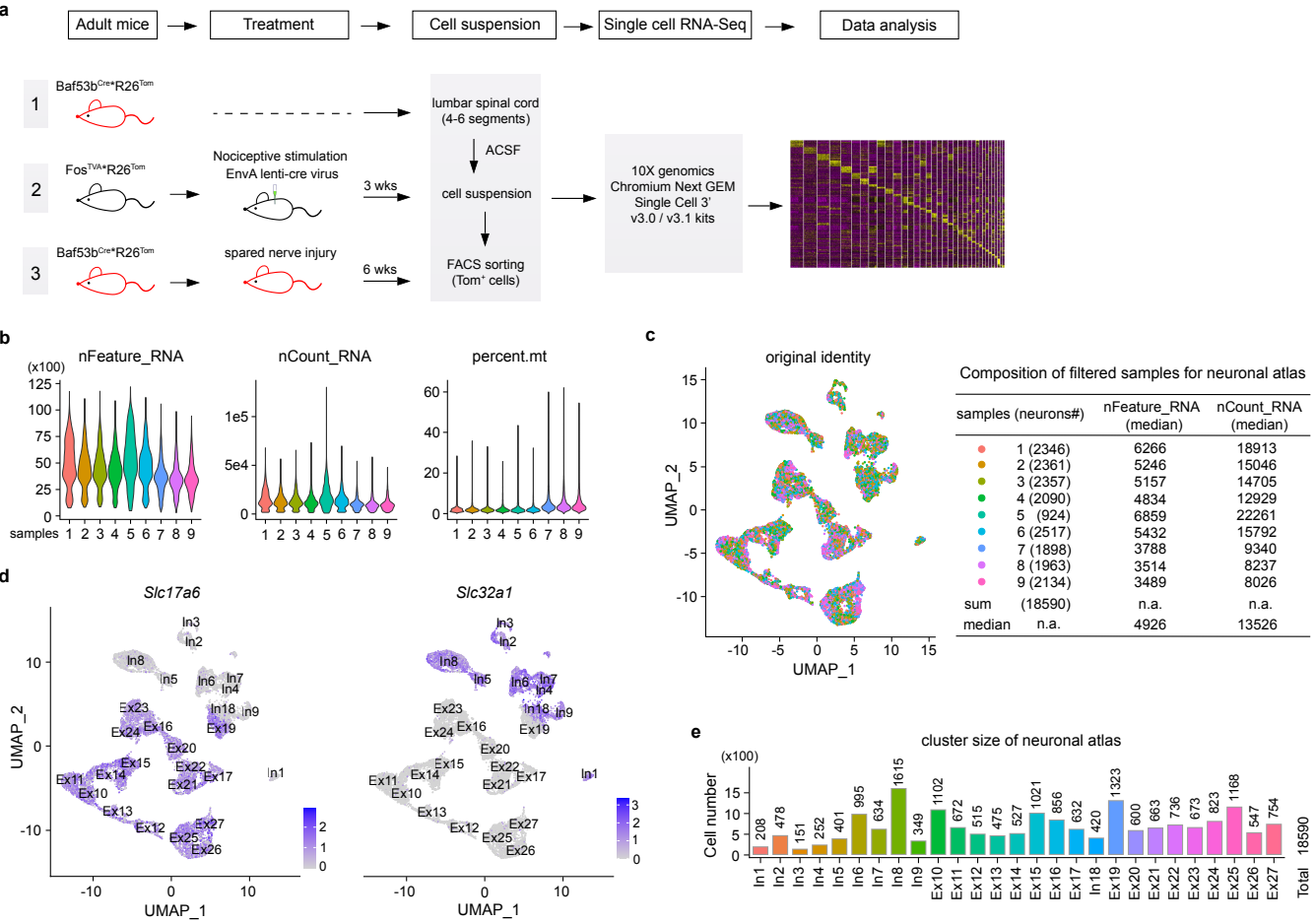


Neural ensembles that encode nocifensive mechanical and heat pain in mouse spinal cord

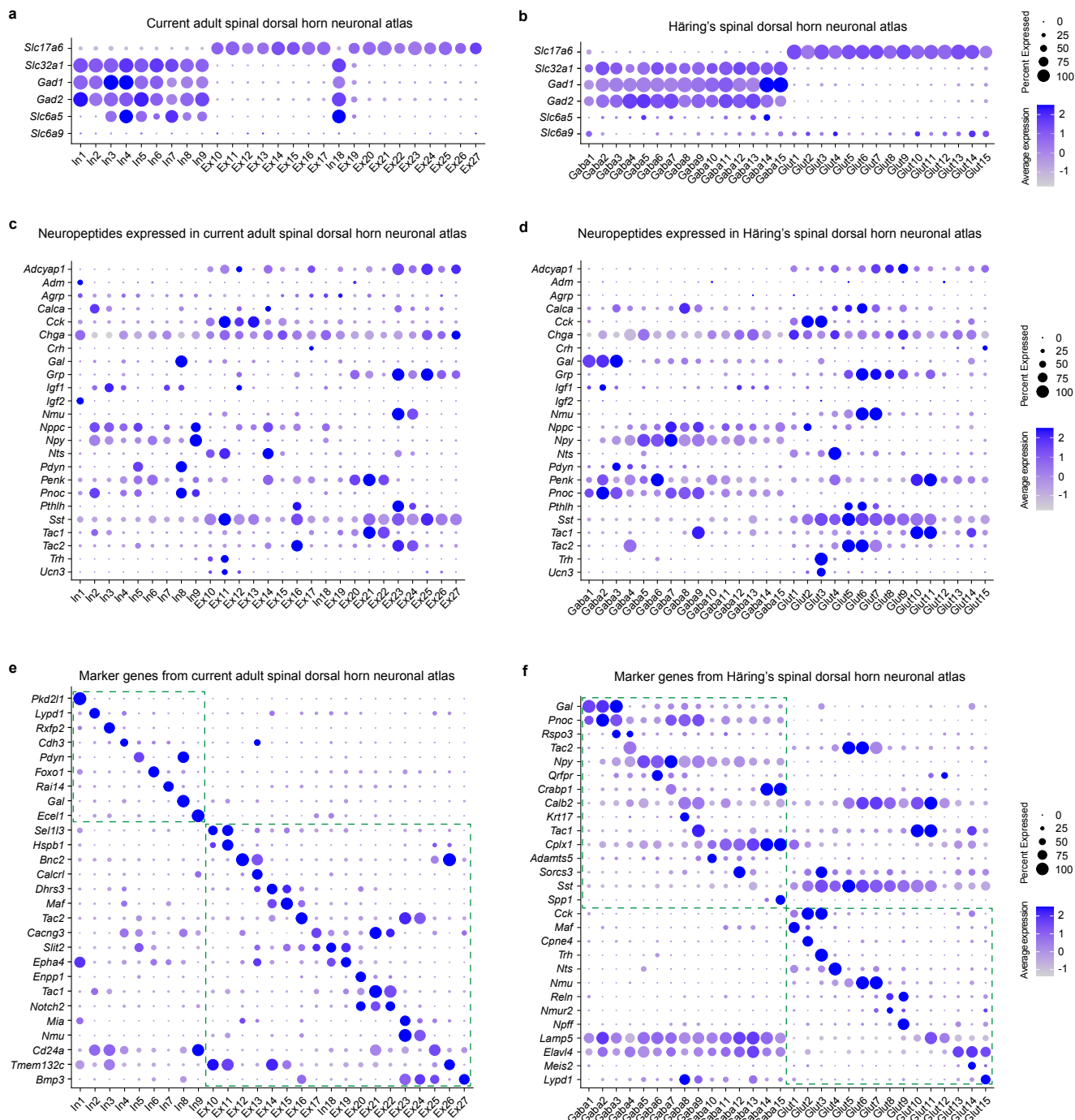
In the format provided by the
authors and unedited



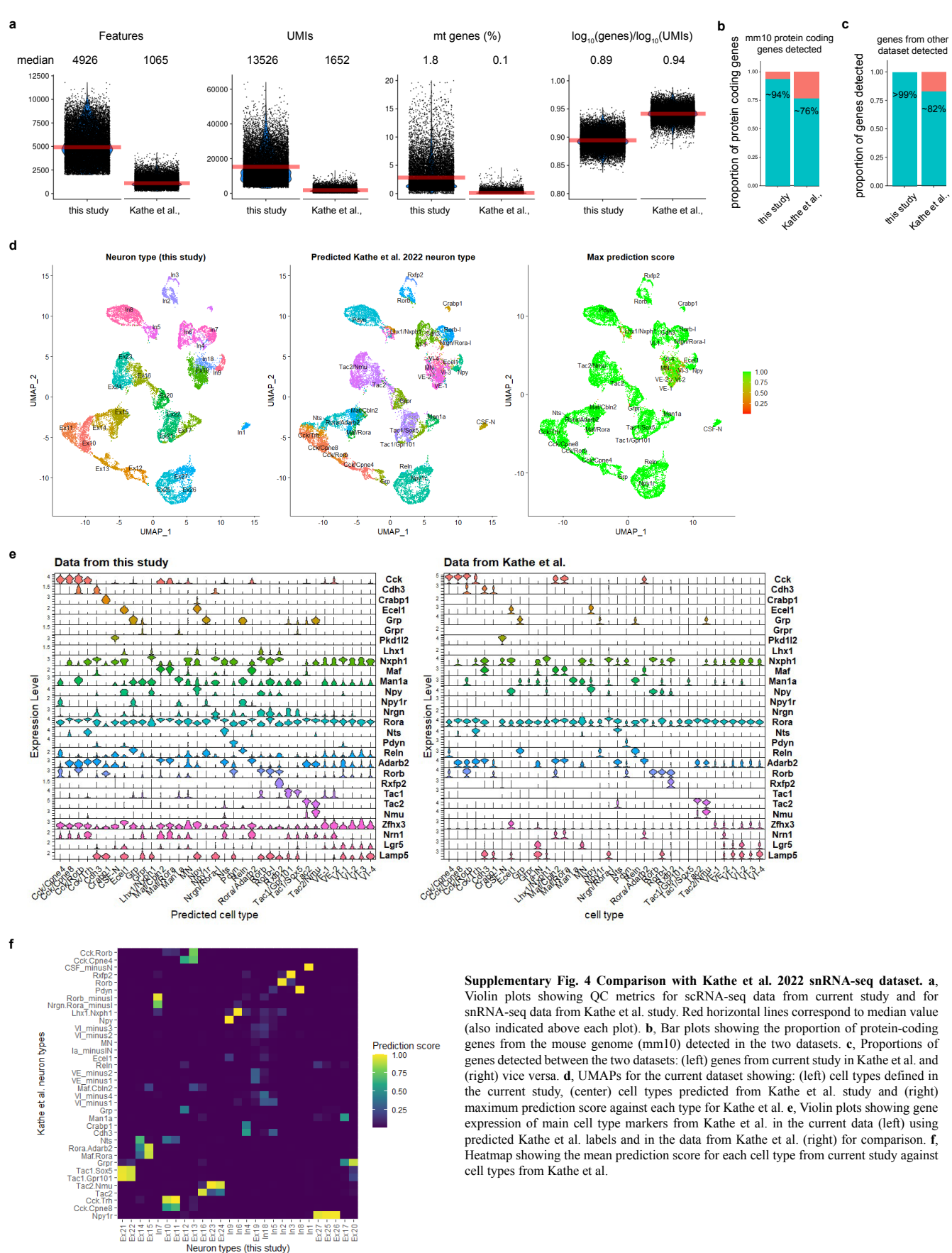
Supplementary Fig. 1 Validation of tdTomato expression in spinal cord from Baf53b-Cre⁺R26^{Tom} mice. **a**, Representative RNAscope images for triple labeling of *Cre* (BAF53), *Slc17a6* (excitatory) and *Gad1* (inhibitory) in spinal cord sections from Baf53b-Cre⁺ (n = 2) and Baf53b-Cre⁻ (n = 1) mice. *Cre* was detected in both excitatory and inhibitory neurons and most probably cholinergic neurons (negative for both *Slc17a6* and *Gad1*) from spinal ventral horn. No *Cre* signal was detected in spinal cord from Baf53b-Cre⁻ mice. Arrowheads indicate co-localization between *Cre* and *Gad1*. Asterisks indicate co-localization between *Cre* and *Slc17a6*. Dashed line shapes the spinal grey matter. **b**, Representative images of tdTomato expression in spinal cord from Baf53b-Cre⁺R26^{Tom} mice (n = 2). No tdTomato signal was observed in Baf53b-Cre negative mice (n = 2). **c**, Representative images of co-localization between tdTomato and NeuN (left panel, 4 μ m projection image for high magnification) or Sox10 (right panel). Asterisks indicate co-localization between tdTomato and NeuN, whereas arrowheads indicate tdTomato⁺ cells negative for NeuN but positive for Sox10 from spinal ventral horn (n = 2 mice). DAPI was used for the nucleus counter staining. Scale bars: 200 μ m for spinal cord overview in (a-c), 20 μ m for the high magnification from rectangle with dashed line in (a) and 10 μ m for high magnification in (c).

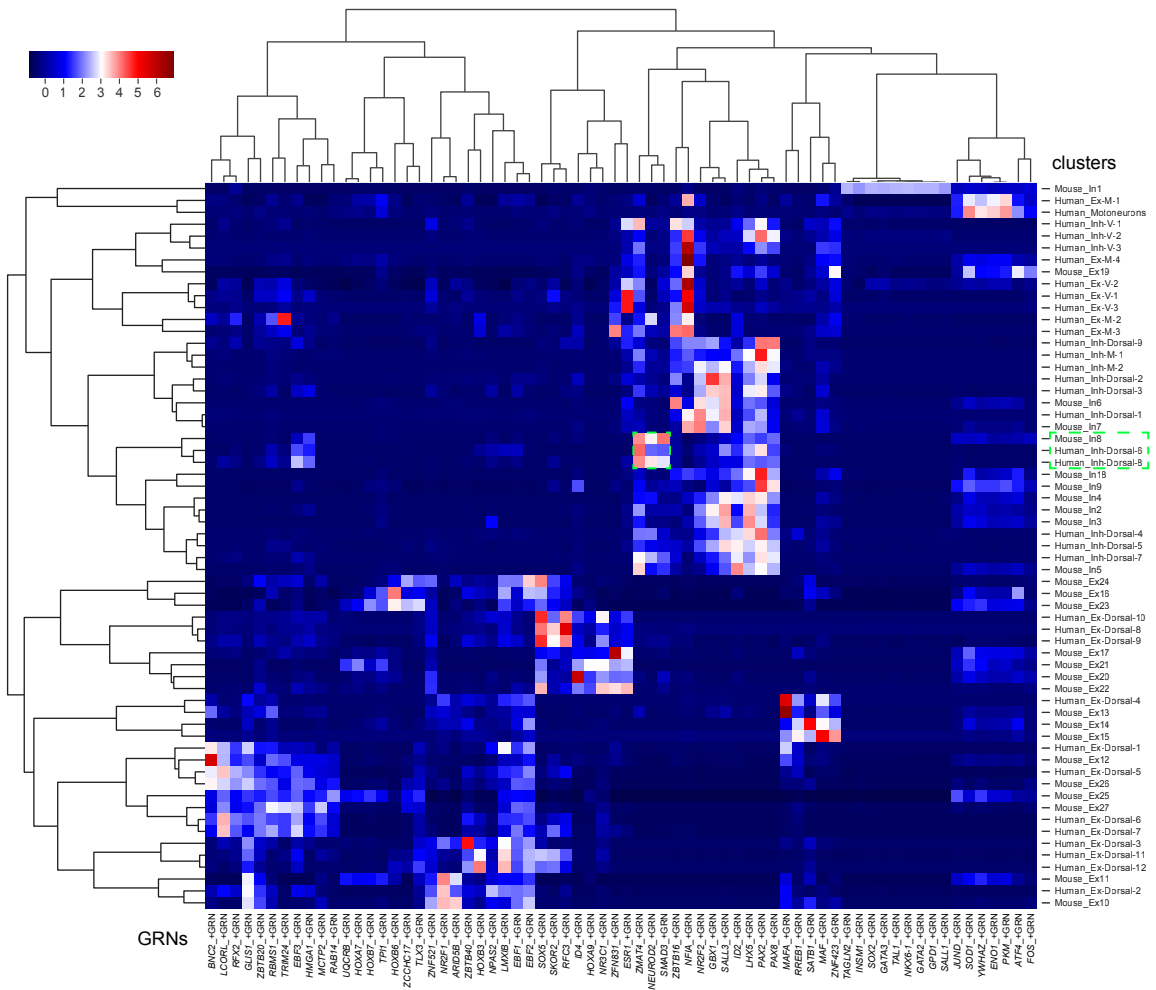


Supplementary Fig. 2 Workflow for single cell RNA-sequencing of neurons from adult mouse spinal cord and quality control of spinal neuronal atlas. **a**, Experimental workflow of single cell suspension preparations from different scenarios and RNA-Seq included in this study. **b**, Overview of sequencing quality including number of detected genes (nFeature_RNA), total reads of detected genes (nCount_RNA) and percentage of mitochondrial genes (percent.mt) for 9 samples (without filtering) from adult mice spinal cord. **c**, Left: UMAP of spinal neuronal atlas composed of cells from different samples. Right: table for numbers of neurons contributed from each sample to spinal dorsal horn neuronal atlas and their corresponding median for number of detected genes and total reads of detected genes. **d**, UMAP plots of excitatory *Slc17a6* (vGLUT2) and inhibitory *Slc32a1* (VGAT) marker gene expression. **e**, Size distribution (number of cells) of individual cluster across the atlas, which was composed of 18,590 neurons.



Supplementary Fig. 3 Comparison of expressions for excitatory/inhibitory genes, neuropeptides and lists of selected genes in current atlas and Häring's dataset. **a** and **b**, Dot plots show expression of *Slc17a6*, *Slc32a1*, *Gad1* (GAD67), *Gad2* (GAD65), *Slc6a5* (GlyT2, neurons) and *Slc6a9* (GlyT1, predominantly astrocytes). **c** and **d**, Dot plots show expression of a list of neuropeptides. **e**, Dot plot shows selected marker genes for neuronal clusters of current atlas. **f**, Dot plot shows published marker genes in Häring's dataset. Dashed line rectangles highlight the enriched gene expression for inhibitory and excitatory clusters in (**e** and **f**).





Supplementary Fig. 5 Conservation of In8 Gal⁺ population between mouse and human spinal cord. Heatmap with hierarchical clustering shows similar gene regulatory networks (GRNs) between mouse In8 and human Inh-Dorsal-6/8 (indicated by the green rectangle) according to Yadav's 2023 annotation.