

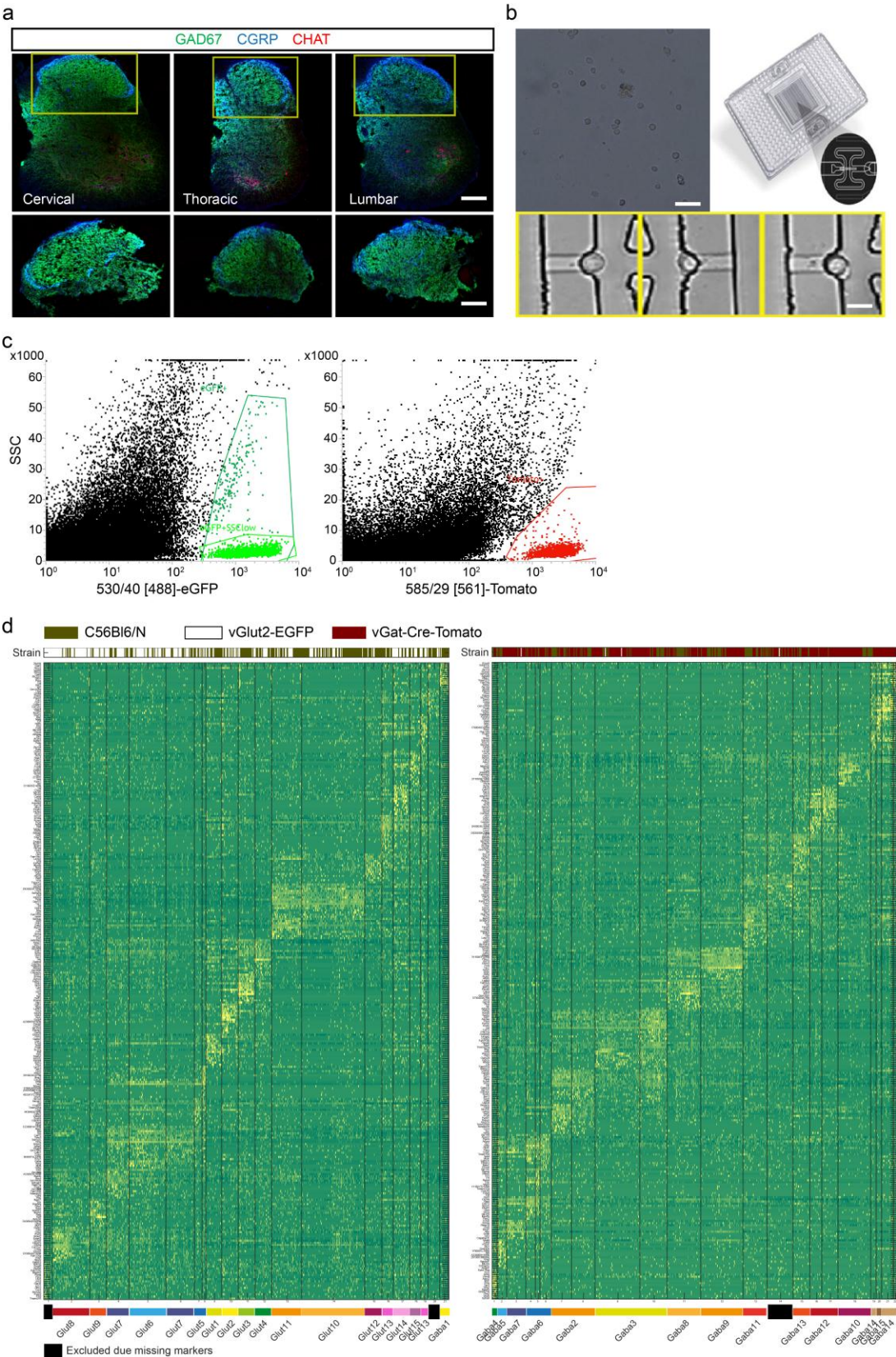
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Neuronal atlas of the dorsal horn defines its architecture and links sensory input to transcriptional cell types

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Häring et al., Supplementary Fig. 1



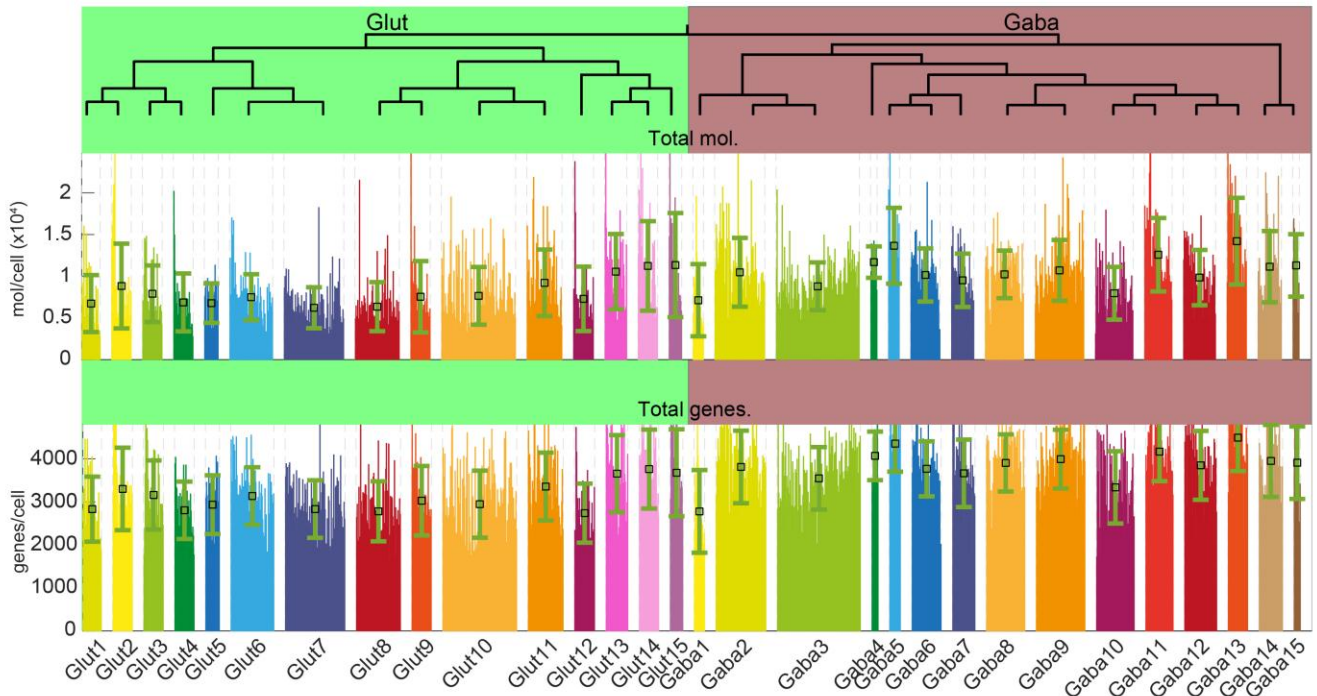
Supplementary Figure 1

Dissection and dissociation of the spinal cord dorsal horn (related to Fig. 1).

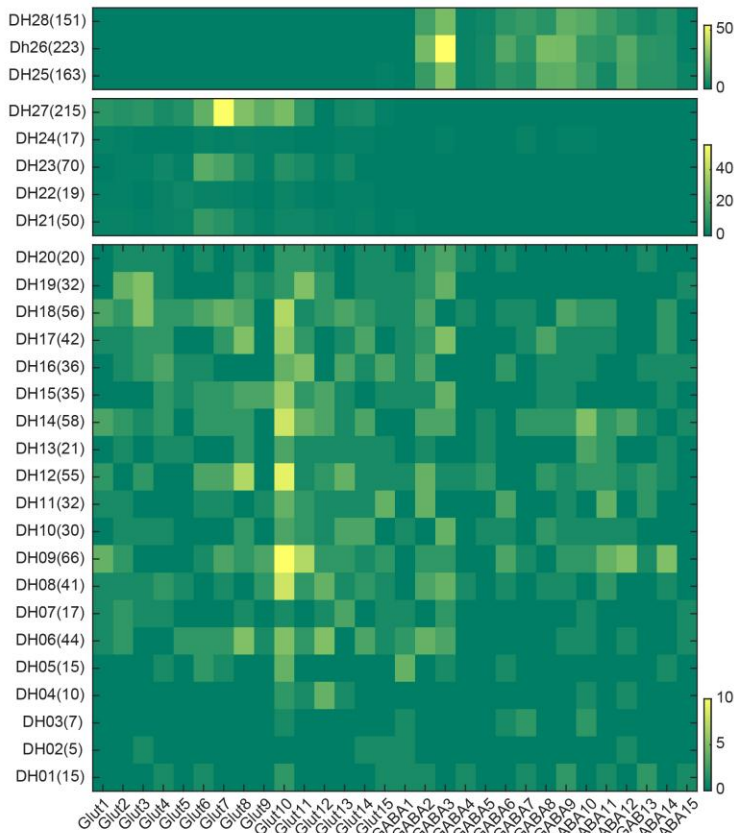
a) Upper row shows 3 representative sections from an intact hemisphere of spinal cord (from left to right: Cervical, thoracical, lumbar) The lower row shows 3 corresponding sections of only the dorsal horn after dissection. The dissection included the dorsal horn. Neurons ventral to the dorsal horn such as Clarke's column was not part of the analyzed tissue. Scale bar = 200µm for upper and 300µm lower panels. **b)** Illustration of single cell suspension and the capturing of single cells in the C1 Fluidigm Chip. Scale bar = 50µm for upper panel and 12µm lower panels. **c)** Illustration of FACS from $Vglut^{GFP}$ (left) and $Vgat^{TdTomato}$ (right) dorsal horn suspensions. **d)** Heat map displaying the single cell expression profile of 1639 dorsal horn spinal cord neurons divided into glutamatergic (left) and GABAergic (right) neurons. Heat map is based on relative expression of each gene, dark green representing minimal and yellow maximal expression ("summer" color scheme). Neurons passed quality control, namely, containing at least 3000 molecules. Black vertical lines represent the 43 clusters defined by backspin. The color code below illustrates the final clustering after manual control. Clusters without distinguishable differences were fused (i.e. Cluster 4 and 6 of the glutamatergic neurons) and clusters without defining genes (thick black bars) were excluded resulting in a total number of 30 clusters.

Häring et al., Supplementary Fig. 2

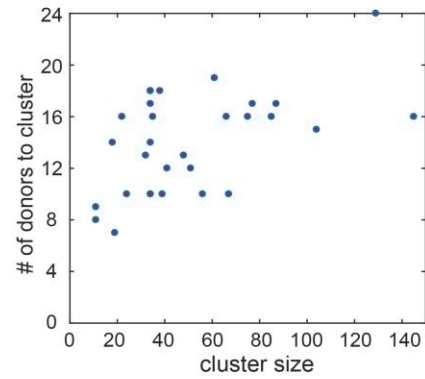
a



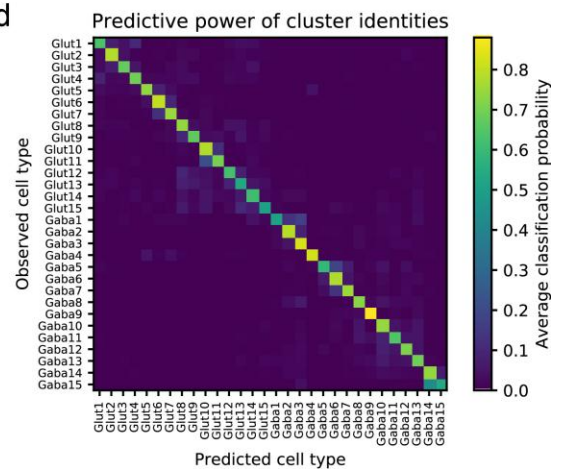
b



c



d

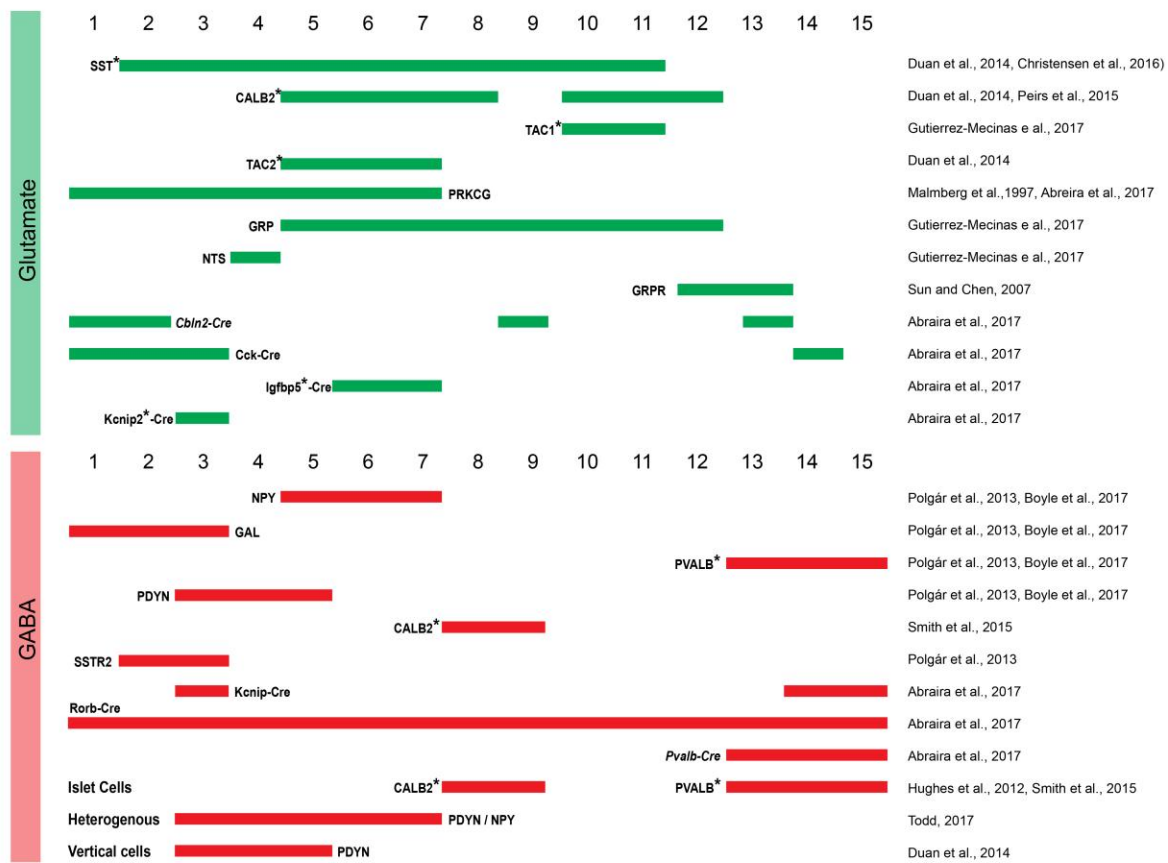


Supplementary Figure 2

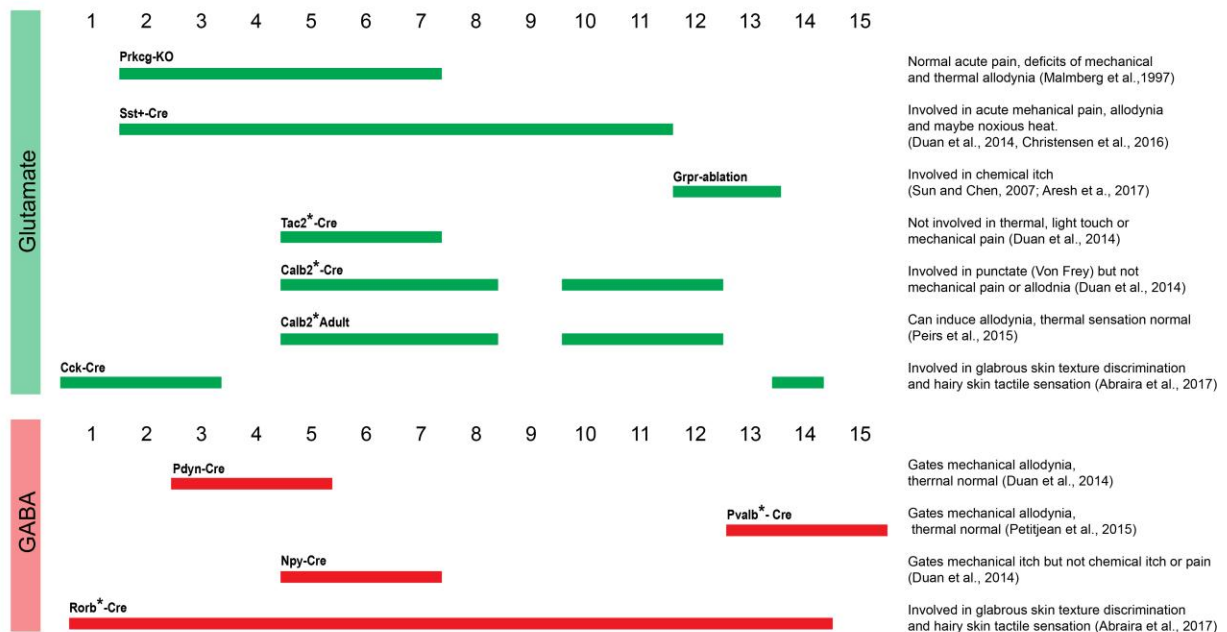
Determination of cell types by marker genes (related to Figs. 1, 2 and 3)

a) Molecule and gene count of the remaining 1545 cells. Black squares and error bars within each cluster represent the average values and standard deviation. Clusters were hierarchically reordered to represent the relation between the 30 clusters (Number of cells for each cluster from left to right: 32, 34, 34, 34, 24, 75, 105, 77, 34, 129, 61, 35, 38, 34, 22, 18, 87, 145, 11, 19, 51, 39, 67, 85, 66, 48, 56, 34, 41, 11). **b)** Heatmaps showing the contribution of cells to each cluster by each of the donors (donors = different animals). Donors are named DH followed by a number that identifies the donor and the number of cells that were captured in each experiment is indicated in parenthesis. There are three heatmaps. The first is for $Vgat^{tdTomato}$ FACS sorted cells (n=3 animals), the second for $Vglut2^{GFP}$ FACS sorted cells (n=5 animals) and the bottom is unbiased dissociation of the dorsal horn and collection of cells (without FACS sorting) (n=20 animals). This shows that cells are contributed to most clusters in each experiment and that this can be repeated over and over with similar results. The distribution of cells in each experiment is found by reading the heatmap horizontally. Some cell types are expected to be more abundant than others and will because of this appear stronger in the heatmap. Examining the heatmap vertically shows that there is no strong bias in contribution of cells between experiments. **c)** Contribution of donors (animals) to each cluster (neuron type) vs the cluster size. Large clusters are expected to have more donors than small clusters simply as the probability of capturing a cell belonging to that cluster is greater. If batch effects were strong, cell types should appear in certain batches (donors) but not in the other batches. Please note that even the smallest cluster with around 15 cells is composed of >7 donors (experiments). This can be taken as an indicator of the biological reproducibility of the clusters and the ability to identify all cell types across different experiments. **d)** Assessment of the predicted strength of clusters. Random forest classifier (max depth 30) was trained on 80% of the cells and testing its performance on the remaining 20% of the cells. The heatmap shows the predicted strength of the cluster identity. The rows are our "observed" cell types (i.e. the ones we assigned). The columns are the predictions the classifier makes. The color shows the probability of assigning each label, averaged across all the test cases. The mean probability along the diagonal is 69.8%.

a Neurochemistry



b Predicted function of neuron types



Supplementary Figure 3

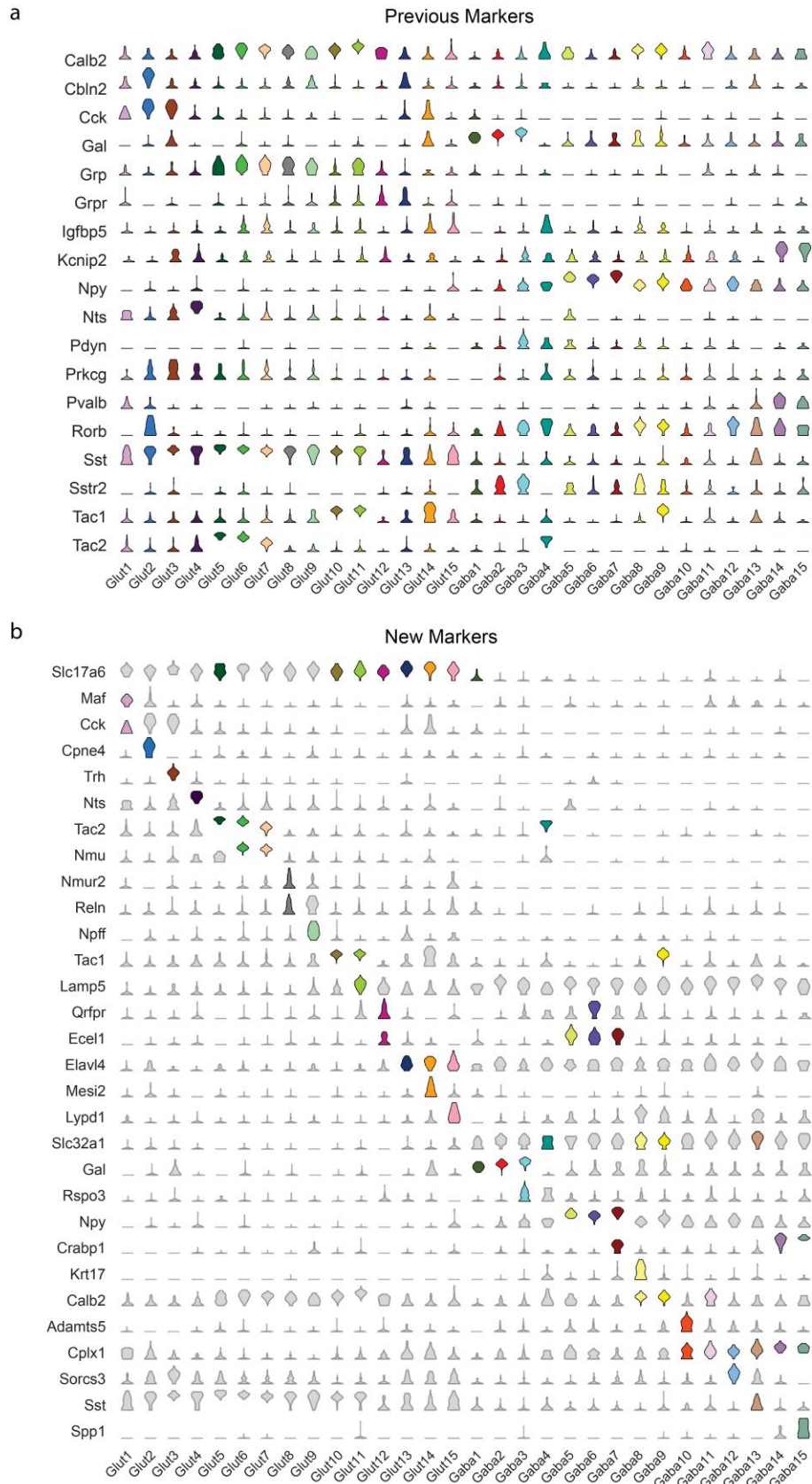
Relation of the identified neuron types to known literature (related to Figs. 1, 2 and 3)

a) Neurochemistry and morphology. Green indicates glutamatergic neurons, red GABAergic neurons. The lines depict predicted detection of the markers using immunohistochemistry. Immunohistochemistry is not as sensitive or quantitative as *in situ* hybridization. This figure therefore only depicts the high-expressing cell-types that we predict have been detected in the cited studies. The actual expression data of these markers is available as Supplementary Fig 4a. For morphology, CALB2⁺ and PVALB⁺ cells are often islet cells, PDYN⁺ and NPY⁺ are heterogenous but never Islet cells, PDYN⁺ cells are sometimes vertical cells. **b)** Predicted function of neuron types. Illustration of neuron types subjectively predicted to have been functionally analyzed. Note that mouse models used Cre driver lines and thus cells expressing the gene at any point in development will recombine and be included in the study and therefore, this summary has to be interpreted with this in mind. Due to transient developmental expression or low levels of expression, studies on Slc17a8, Ret, Bhlhb5, Slc6a5 were excluded. Stars indicate the gene also to be expressed in neurons belonging to the other neurotransmitter type which is not shown in the figure. For the actual expression profile of these genes in both neurotransmitter types, see Supplementary Fig. 4a.

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Håring et al., Supplementary Fig. 4



Supplementary Figure 4

Expression of previously used markers and new markers for identification of dorsal horn neuron types (related to Figs. 1, 2 and 3)

a) Violin plots of genes previously used as markers to characterize cell types involved in sensation. Note broad expression in many neuronal types, often also crossing neurotransmitter boundaries. **b)** Violin plots of minimal set of new markers to uniquely identify each neuronal type (colored by neuronal type). Vertical gives the proposed markers, horizontally the expression in other neuron types.

Häring et al., Supplementary Fig. 5

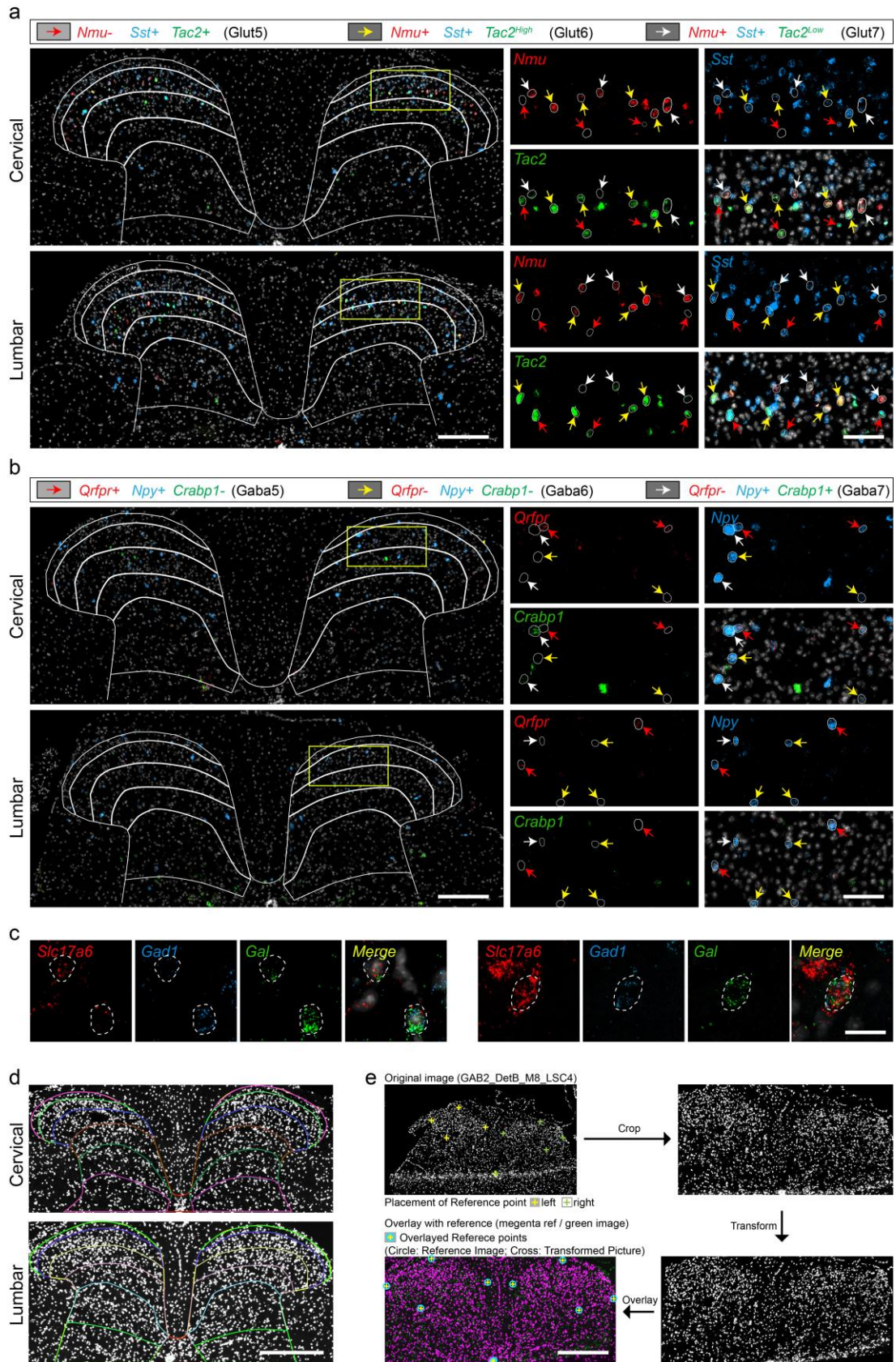


Supplementary Figure 5

Expression of transcription factors, neuropeptides/receptors and ion channels (related to Fig. 4)

Listed are those genes that show significant expression in at least one cluster (see Online Methods, Negative binomial generalized linear regression). Note that this list shows all significantly expressed genes ($p < 0.001$) while in the main Fig. 4, only significantly expressed with a minimum of 3 transcripts are shown. Thus, with the relaxed inclusion criteria, many more genes can be detected in this analysis than in main Fig. 4. (n= 1545 neurons)

Häring et al., Supplementary Fig. 6

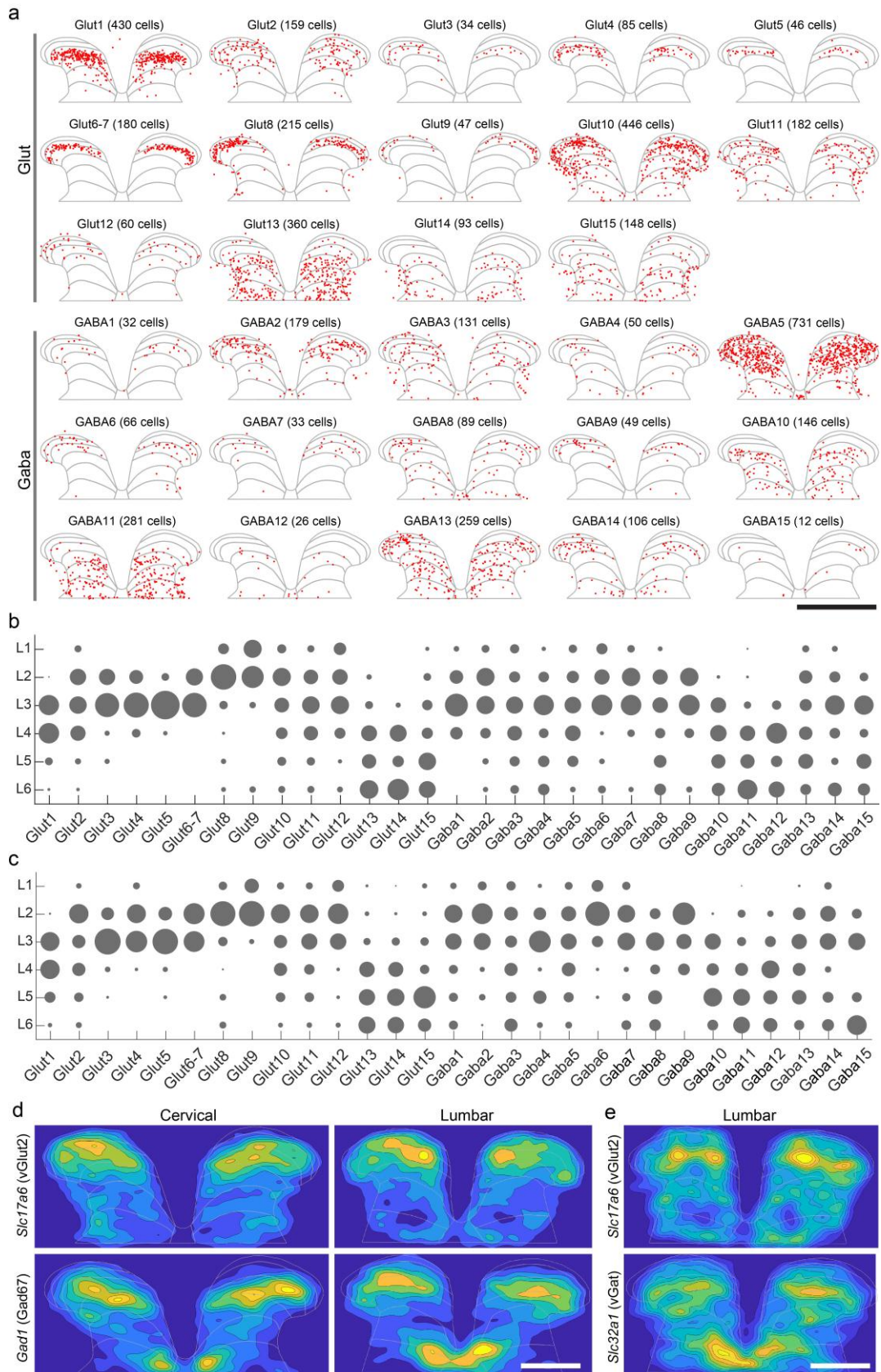


Supplementary Figure 6

***In situ* mRNA staining using RNAScope (related to Fig. 5)**

a) Cervical and lumbar section stained for *Nmu* (red), *Tac2* (green) and *Sst* (blue) in order to detect cells representing the clusters Glut5-7. The celltypes are highlighted in the small panels on the right using arrows (Glut5 [red arrow], Glut6 [yellow arrow], Glut7 [white arrow]). The small panels represent the magnified region depicted by the yellow frame in the corresponding panel on the left. **b)** Cervical and lumbar section stained for *Qrfpr* (red), *Crabp1* (green) and *Npy* (blue) in order to detect cells representing the clusters Gaba5-7. The cell types are highlighted in the small panels on the right using arrows (Gaba5 [red arrow], Gaba6 [yellow arrow], Gaba7 [white arrow]). The small panels represent the magnified region depicted by the yellow frame in the corresponding panel on the left. **c)** Two example cells for cluster Gaba1 expressing beside *Gal* both *Slc17a6* and *Gad1*. **d)** Images of the lumbar and cervical section that were used as reference sections to the Allan Brain Reference Atlas. **e)** In order to align two sections, we defined in total 10 Reference points for each section (including the reference section; 5 for the left and right horn respectively, starting always at the central canal). Each image was then cropped and transformed so that they could be aligned using the reference points. Scale bars = 200µm for overview picture and 50µm for magnifications in panels a) and b), 20µm in panel c) as well as 500µm in panel d) and e).

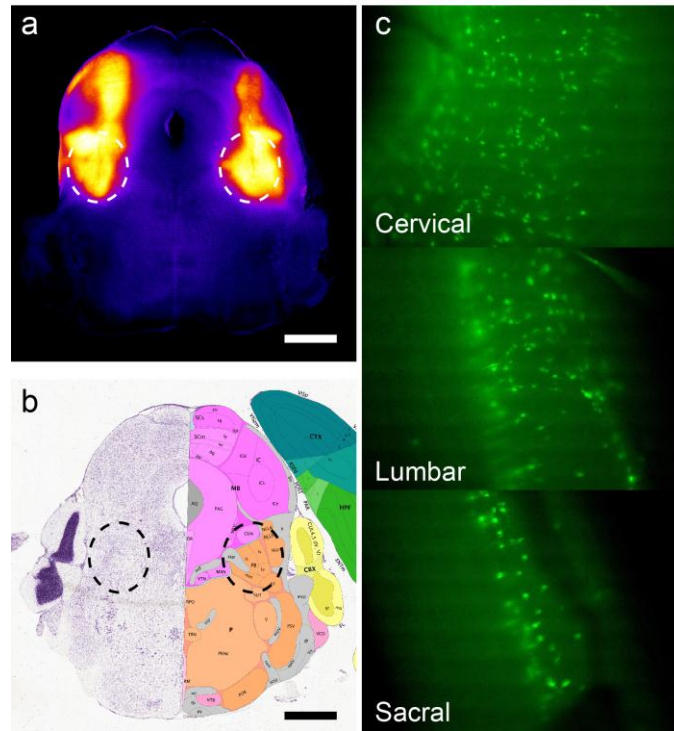
Håring et al., Supplementary Fig. 7



Supplementary Figure 7

Spatial distribution of the neuronal types within the cervical spinal cord (related to Fig. 5)

a) Localization of all the neuronal types within the cervical dorsal spinal cord. Red dots represent single cells expressing the respective marker genes defining the indicated cell type. Lines represent Rexed laminae. Names of each neuronal type and number of identified cells (in parenthesis) are indicated on top of each spinal cord projection. n=2 animals; Spatial information was collected and combined from 6 sections (3 sections per animal per staining) **b)** Quantification of distribution of each neuronal type within the six dorsal Rexed laminae of the cervical dorsal spinal cord. Size of the gray circle represents percentage of cells of a particular type found within a particular layer. **c)** Quantification of distribution of each neuronal type within the six dorsal Rexed laminae of the lumbar dorsal spinal cord. Size of the gray circle represents percentage of cells of a particular type found within a particular layer. **d)** Density heat map of glutamatergic and GABAergic neurons using *Slc17a6* and *Gad1* in lumbar or cervical spinal cord (n=2 animals; Spatial information was collected and combined from total 60 section for *Slc17a6* and 37 sections for *Gad1*) or **e)** *Slc17a6* and *Slc32a1* in the lumbar spinal cord (n=3 animals Spatial information was collected and combined from total 28 section for *Slc17a6* and 19 sections for *Slc32a1*). Scale bars = 1000µm in panels a) and 500µm in panel d) and e). Heat map uses “parula” color scheme, dark blue represent minimal and yellow maximal expression.

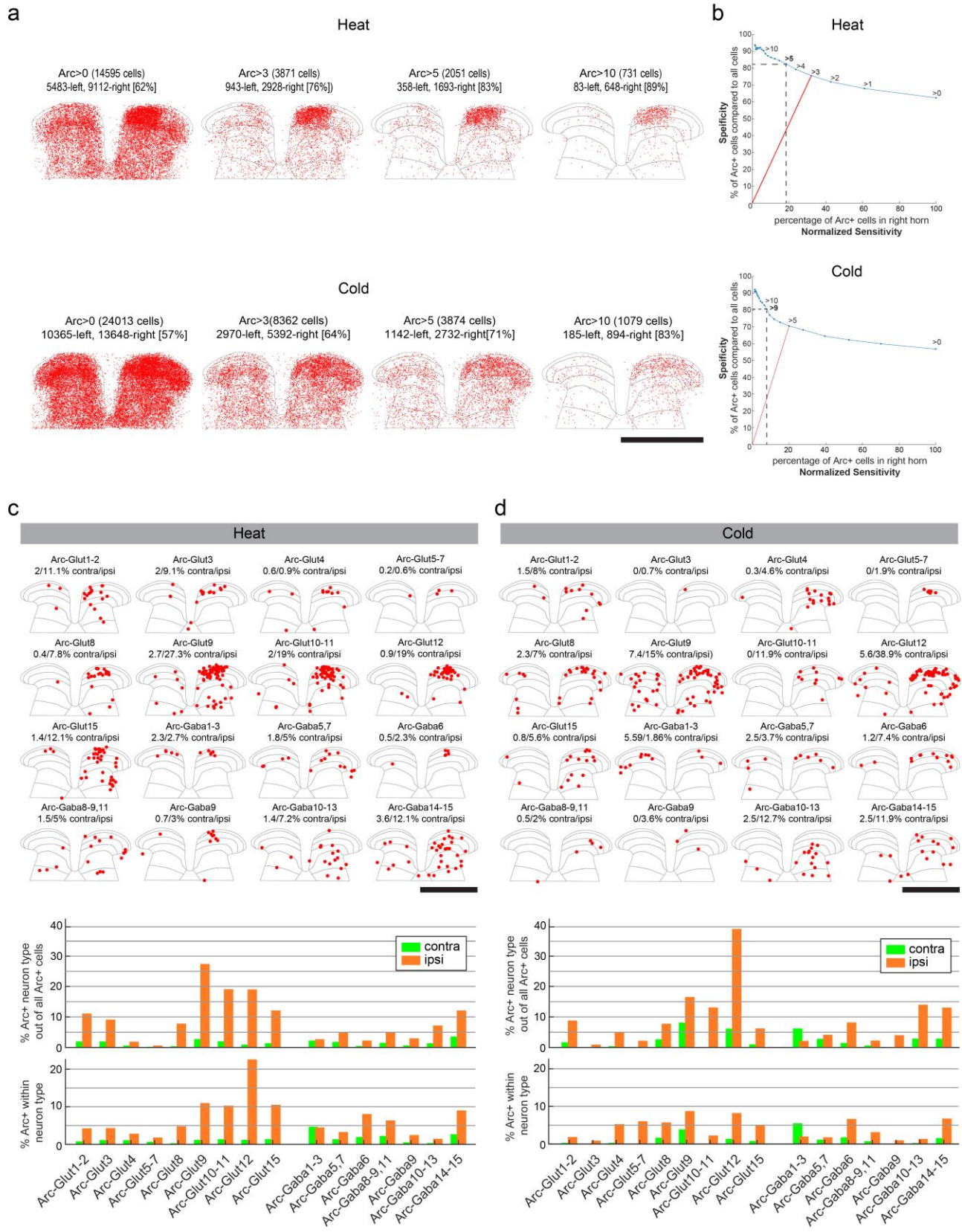


Supplementary Figure 8

Overview of the CTb488 injection area in the pons and traced projection neurons in the spinal cord (related to Fig. 5)

a) Coronal section of the pons displaying the Parabrachial nucleus (white dashed circles) as CTb488 injection site. CTb488 signal (bright yellow/white) can be seen in the Parabrachial nucleus and in the dorsal Inferior colliculus. **b)** Reference section from Allen mouse brain atlas (image 103/132). The Parabrachial nucleus is marked with black dashed circles. **c)** image of dorsal horn surface during dissection at cervical, lumbar and sacral levels. Rostral side is up and caudal side is down. Projection neurons are visible in green. Scale is not available due to variable power objective. Scale bar in a and b is 1000 μ m and was constructed from the Allen mouse brain section.

Häring et al., Supplementary Fig. 9



Supplementary Figure 9

Identification of heat- and cold-activated neurons (related to Fig. 6)

a) Localization the immediate early gene *Arc* within the lumbar dorsal spinal cord following noxious heat or cold as indicated using different thresholds. Red dots represent single cells detected to express *Arc*. With increased stringency (higher threshold) number of detected cells decreases and significance increases. The numbers of neurons detected in left and right dorsal horn and the predicted correctly identified positive cells (presented as %) are indicated at each threshold. **b)** Panel shows the relation between specificity and sensitivity (normalized). Red line depicted closest point to origin as simple optimal threshold criteria. Dash lines illustrate the threshold (>5) chosen for the further analysis for heat and threshold (>8) chosen for cold. **c)** Identification of neuronal types based on marker genes (marker genes>3 transcripts/cell) that become active by heat ($Arc > 5/\text{cell}$). Red dots represent single neurons expressing the respective marker genes together with *Arc*. Below, quantification of the percent of neurons of each neuron types that were active (Arc^+) and percent of Arc^+ neurons belonging to the different neuron types as percent of all Arc^+ neurons in the control (contralateral, left) and experimental (ipsilateral, right) side of the spinal cord following heat. **d)** Identification of neuronal types based on marker genes (marker genes>3 transcripts/cell) that become active by cold ($Arc > 9/\text{cell}$). Red dots represent single neurons expressing the respective marker genes together with *Arc*. Below, quantification of the percent of neurons of each neuron types that were active (Arc^+) and percent of Arc^+ neurons belonging to the different neuron types as percent of all Arc^+ neurons in the control (contralateral, left) and experimental (ipsilateral, right) side of the spinal cord following cold. $n=3$ animals; analysis contains 3 lumbar sections per animal per staining. Scale bars = 1000 μm in panel a), c) and d).