Supplementary information for

Connect-seq to superimpose molecular on anatomical neural circuit maps

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This PDF file includes: Figs. S1 to S9

Supplementary figure 1

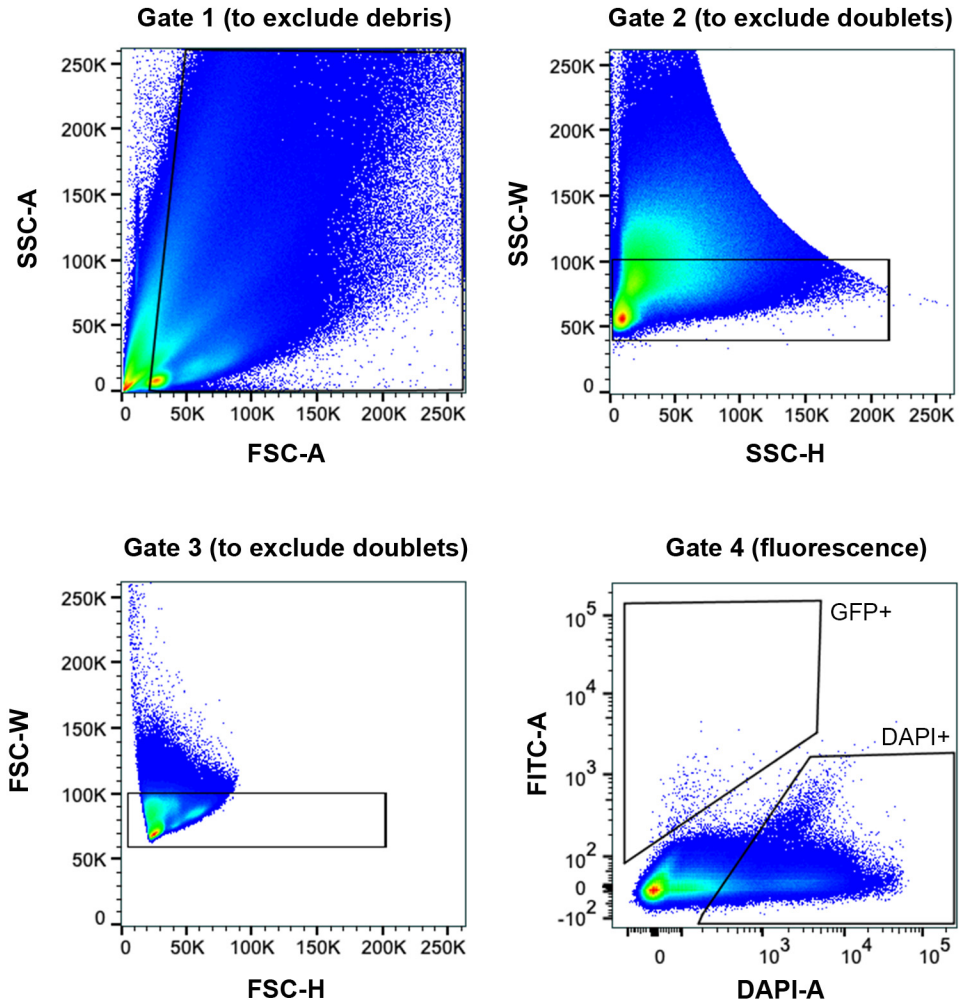

Fig. S1. Workflow of flow cytometry. Dissociated cells from cell suspensions were sorted using FACS in a "single cell sorting mode". A representative of FACS illustrating different gates to place individual GFP+ DAPI- (live PRV+) cells singly into wells of 96 well plates. Gate 1 was applied to exclude debris and sort cells based on size and granularity using forward and side scatter area pulse parameters, Gates 2 and 3 were applied to exclude doublets using pulse height (H) and width (W) parameters, and Gate 4 was applied to sort cells based on their fluorescence. Live cell-impermeable DAPI was used to stain and exclude dead cells. On average, 2.8±0.6% of cells were found within the DAPI+ gate settings. Single cells in the boxed GFP+ area were isolated in 96 well plates.

Supplementary figure 2

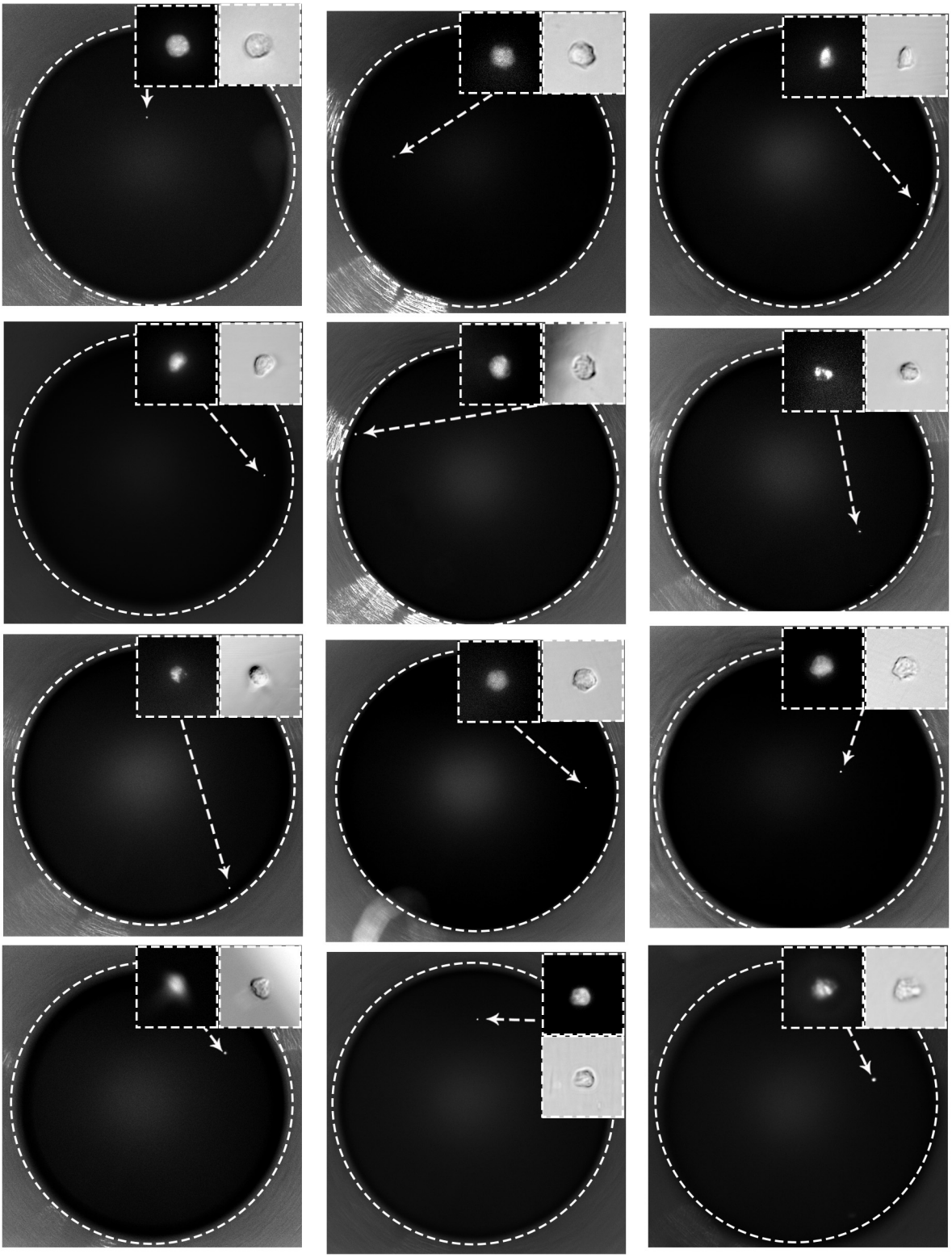

Fig. S2. Quality control to assess the use of flow cytometry to sort one cell per well in multiwell plates. CRHNs were infected with PRVB180 and dissociated cells from the hypothalamus subjected to flow cytometry. Individual GFP+/DAPI- cells were sorted one per well into wells of 60-well Terasaki plates and microscopy used to examine individual wells for fluorescent and non-fluorescent cells. A total of 125 wells contained a single GFP+ cell. None contained more than one cell. Shown are representative low magnification images of individual wells with insets showing higher magnification bright-field (white background) and fluorescent images (black background) of the cell found in each well. Arrows indicate the location of the cell observed in each well.

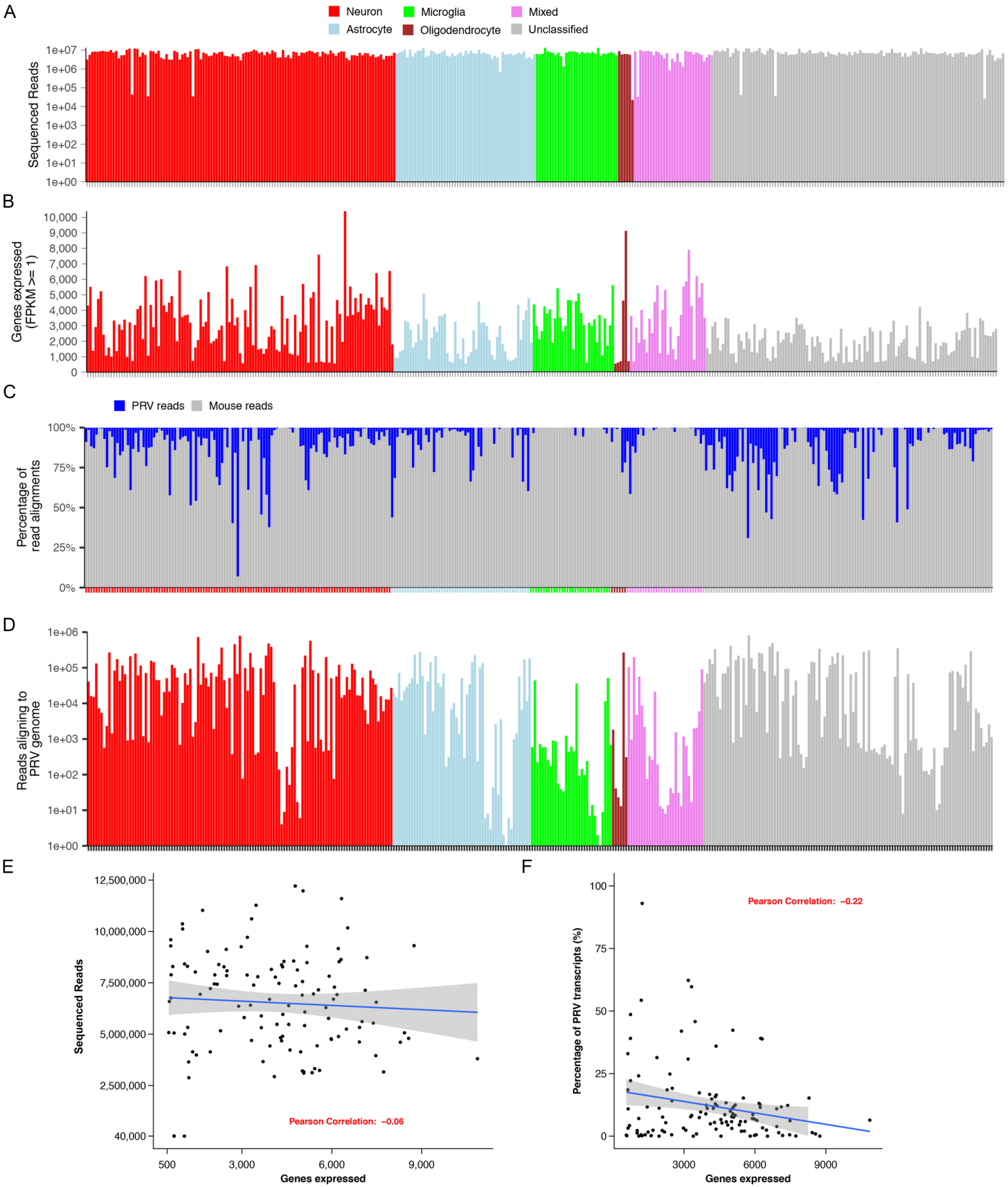

Fig. S3. Quality metrics for single cell RNA-seq libraries. A-D. Graphs show the number of sequenced reads per cell (A), number of genes detected at or above one FPKM (B), percentage of reads mapping to exons of mouse genome (GENCODE M15, mouse genome build mm10) (grey) or PRV genome (NC_006151.1) (blue) (C), and number of reads mapping to the PRV genome (D). Individual cells are indicated by bars and colored according to cell type, as indicated. E-F. Scatterplots show comparison of the number of genes detected in neurons and sequencing depth (sequenced reads) (E) or level of PRV infection (percentage of PRV transcripts) (F). Each dot indicates a single neuron. Blue lines indicate linear regression of correlation.

Supplementary figure 4

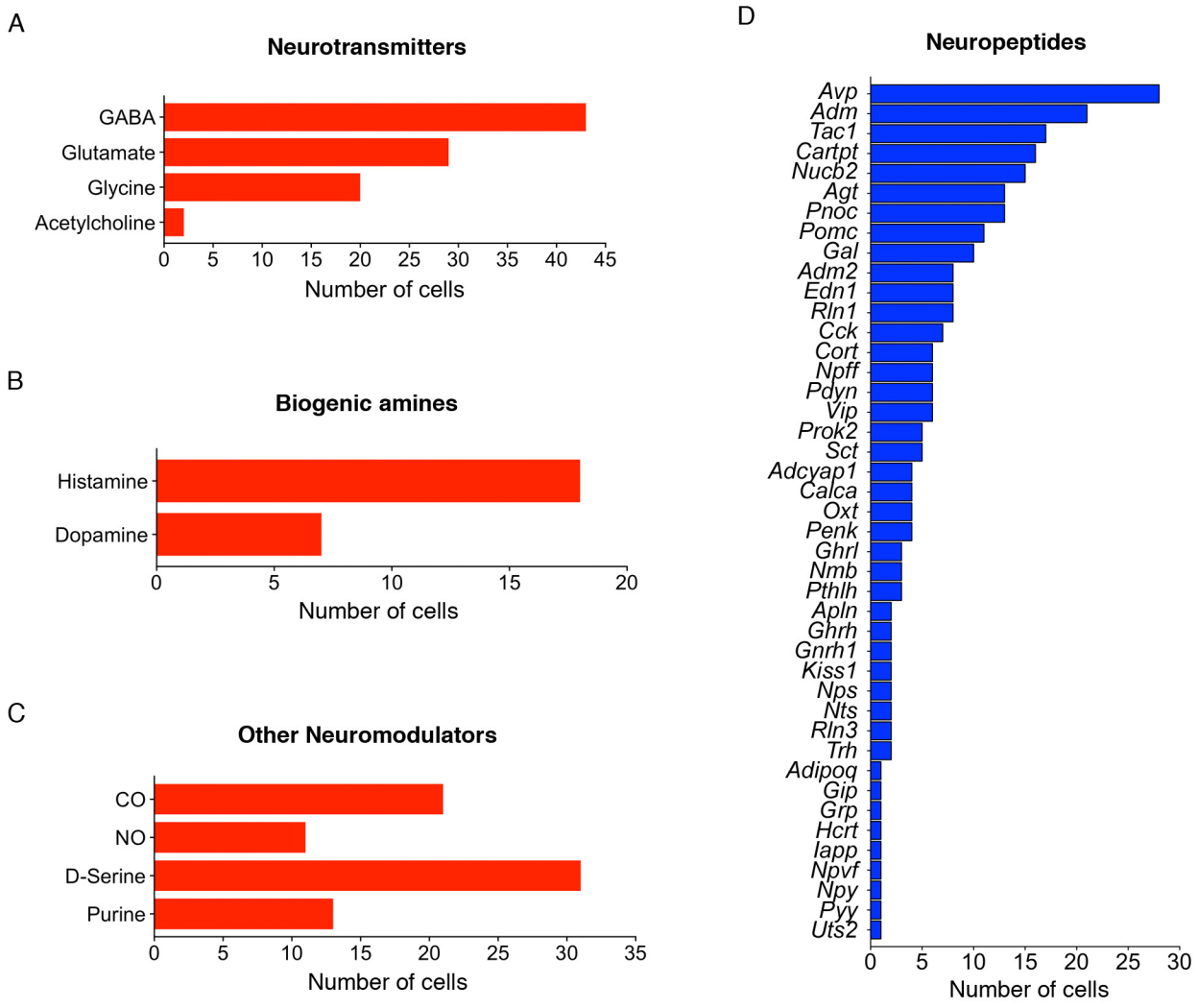

Fig. S4. Variations in proportions of upstream neurons expressing different signaling molecules. Graphs show the number of upstream neurons expressing different neurotransmitters (A), biogenic amines (B), other neuromodulators (C), and neuropeptides (D).

Supplementary figure 5

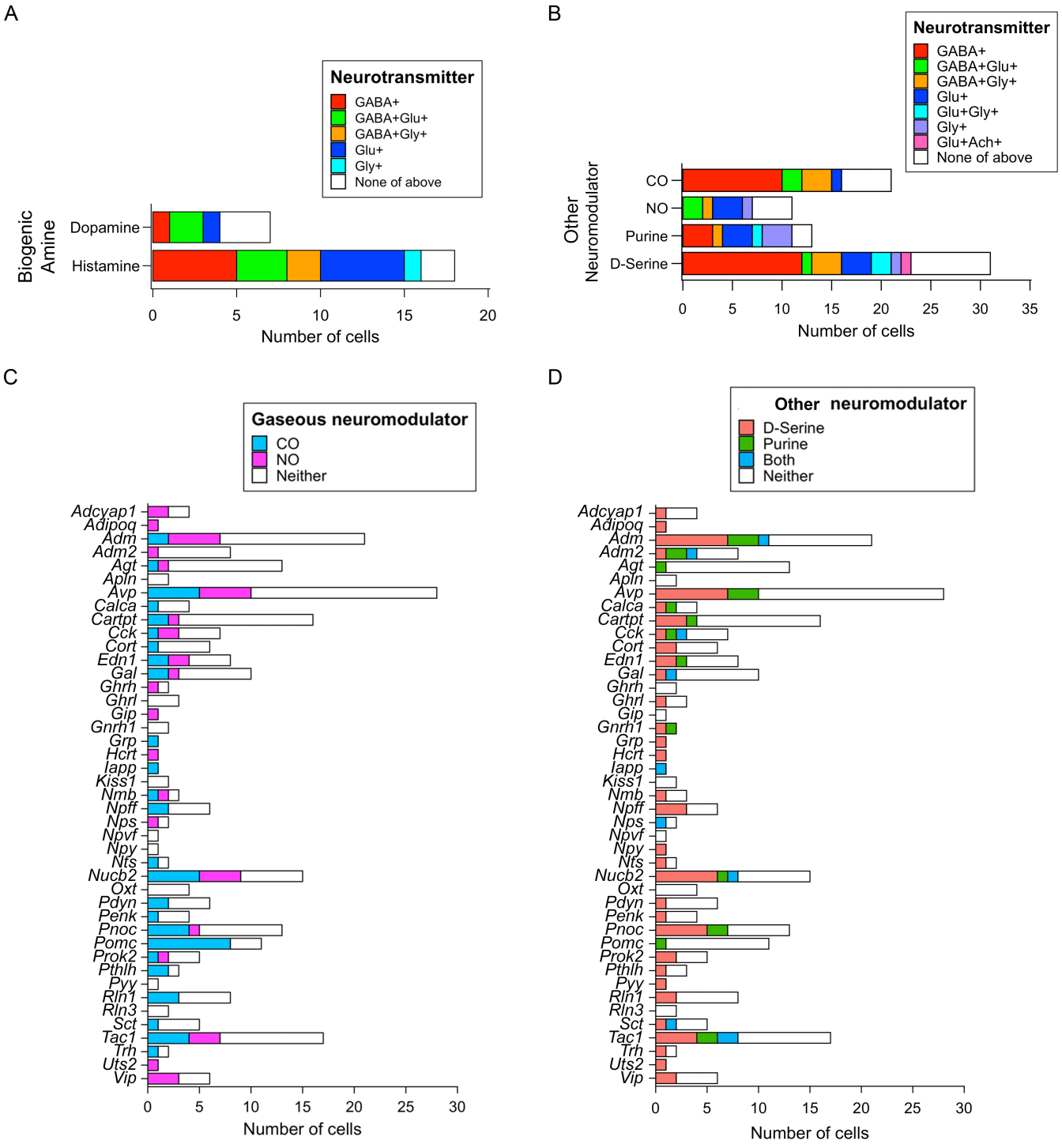

Fig. S5. Coexpression of different signaling molecules in upstream neurons. Graphs show the number of upstream neurons that coexpressed biogenic amines (A) or other neuromodulators (B) with different neurotransmitters. Graphs show the number of upstream neurons that coexpressed individual neuropeptides with gaseous neuromodulators (C) or other neuromodulators (D).

Supplementary figure 6

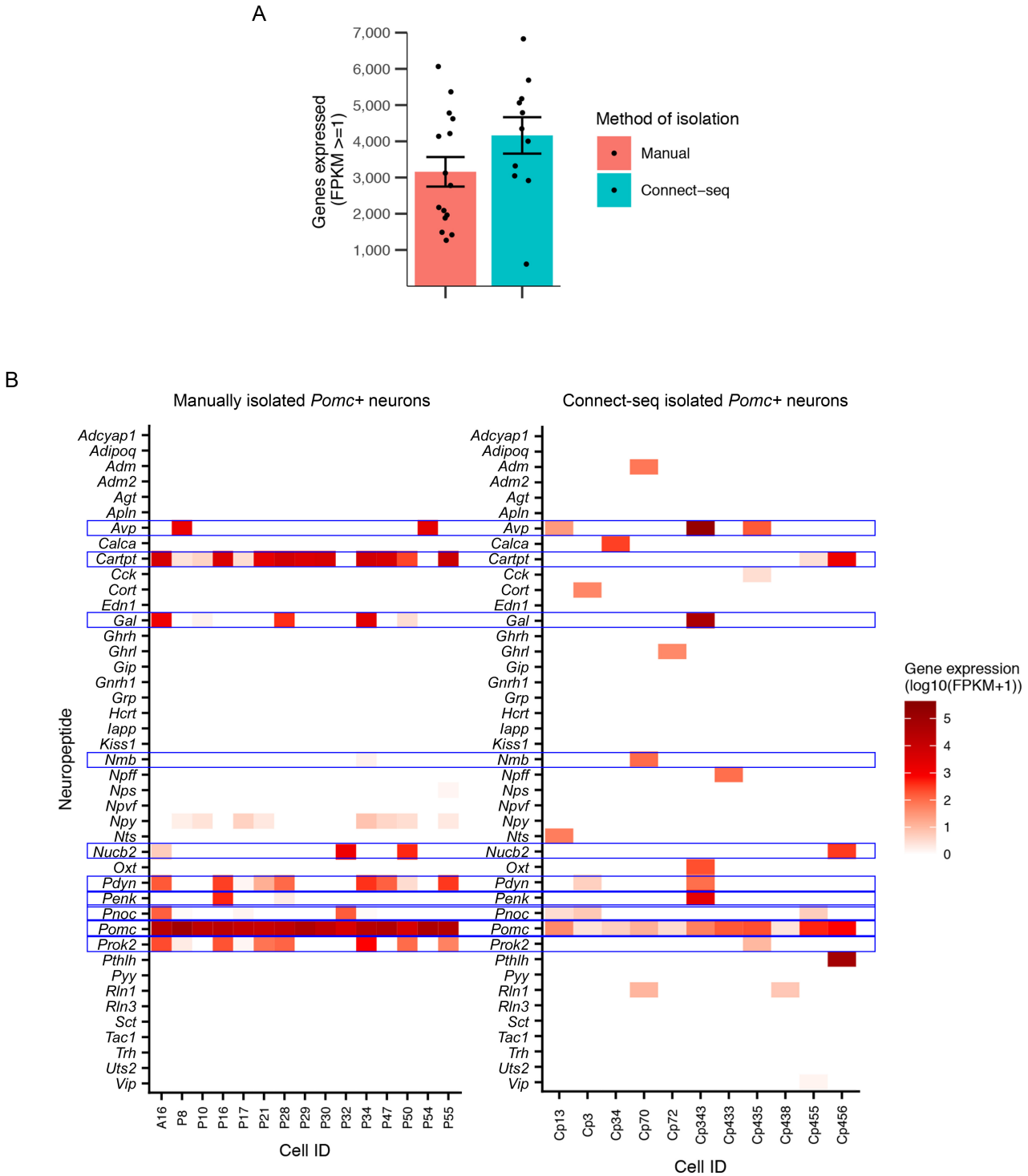

Fig. S6. Comparison of neuropeptides expressed in *Pomc*+ neurons isolated by Connect-seq or manual isolation. (A) Graph shows the average (colored bars) number of genes detected in individual *Pomc*+ cells (black dots) isolated manually from *Pomc*-eGFP mice or by using Connect-seq. Error bars indicate s.e.m. (B) Heatmaps illustrate diverse coexpression of neuropeptides in *Pomc*+ cells. Neuropeptides are indicated on the y-axis and individual cell identificaton numbers on the x-axis. Blue boxes indicate neuropeptides coexpressed in *Pomc*+ neurons obtained using both methods.

Supplementary figure 7

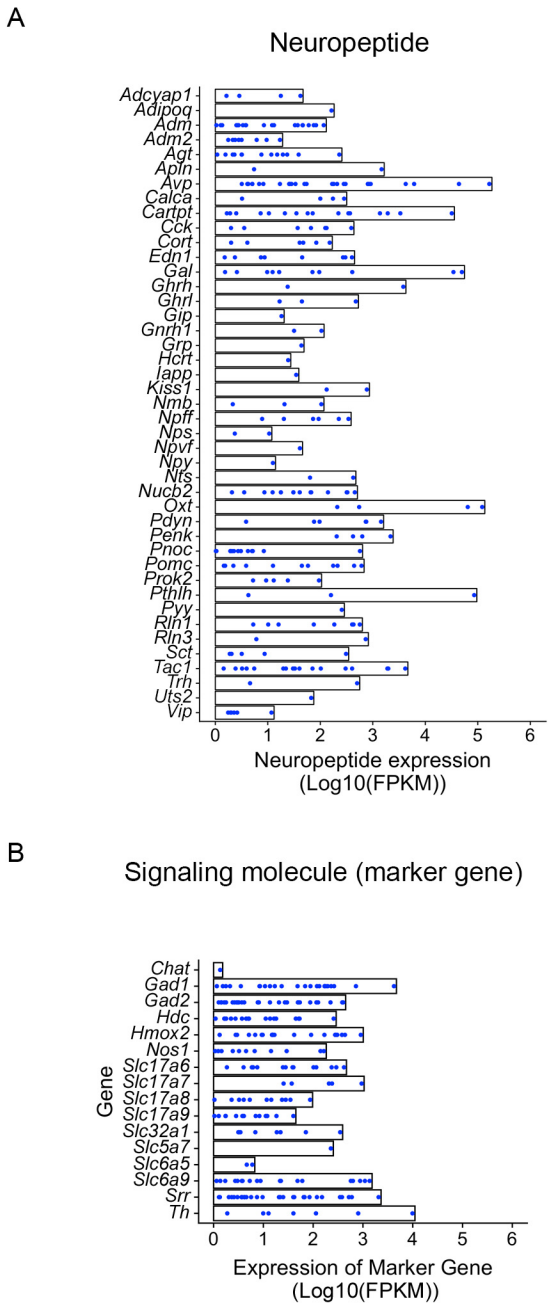

Fig. S7. Expression levels of signaling molecules vary in upstream neurons

Graphs show the expression levels of different neuropeptides (A) and marker genes for other signaling molecules (B) in individual neurons (blue dots) upstream of CRHNs. Data are shown as log-transformed FPKM.

Supplementary figure 8

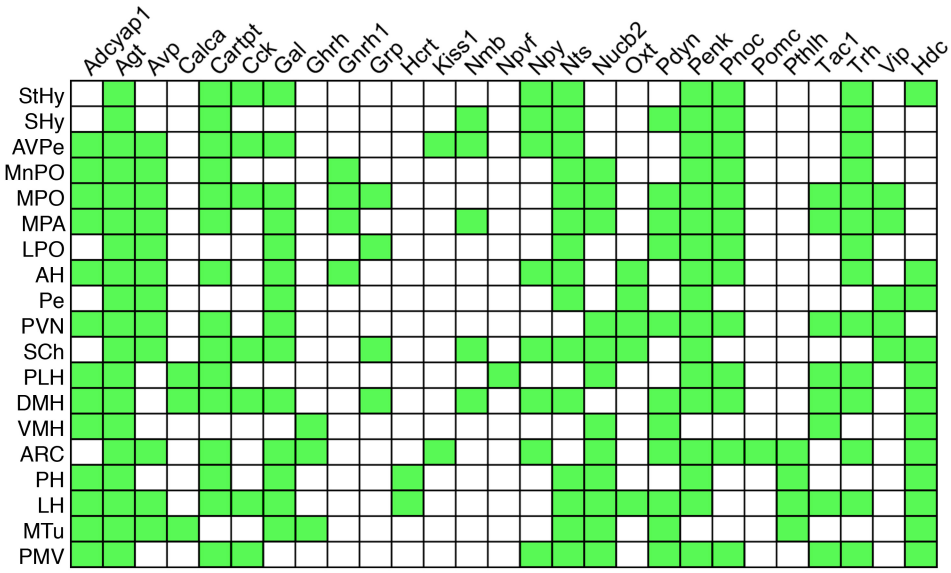

Fig. S8. Expression of genes encoding neuromodulators in different areas of the hypothalamus.

Chart shows data obtained from the Allen Brain Atlas in situ hybridization database indicating expression of specific neuropeptides or histamine in areas of the hypothalamus found to contain neurons upstream of CRHNs (green boxes) in previous viral tracing studies (8).

Supplementary figure 9

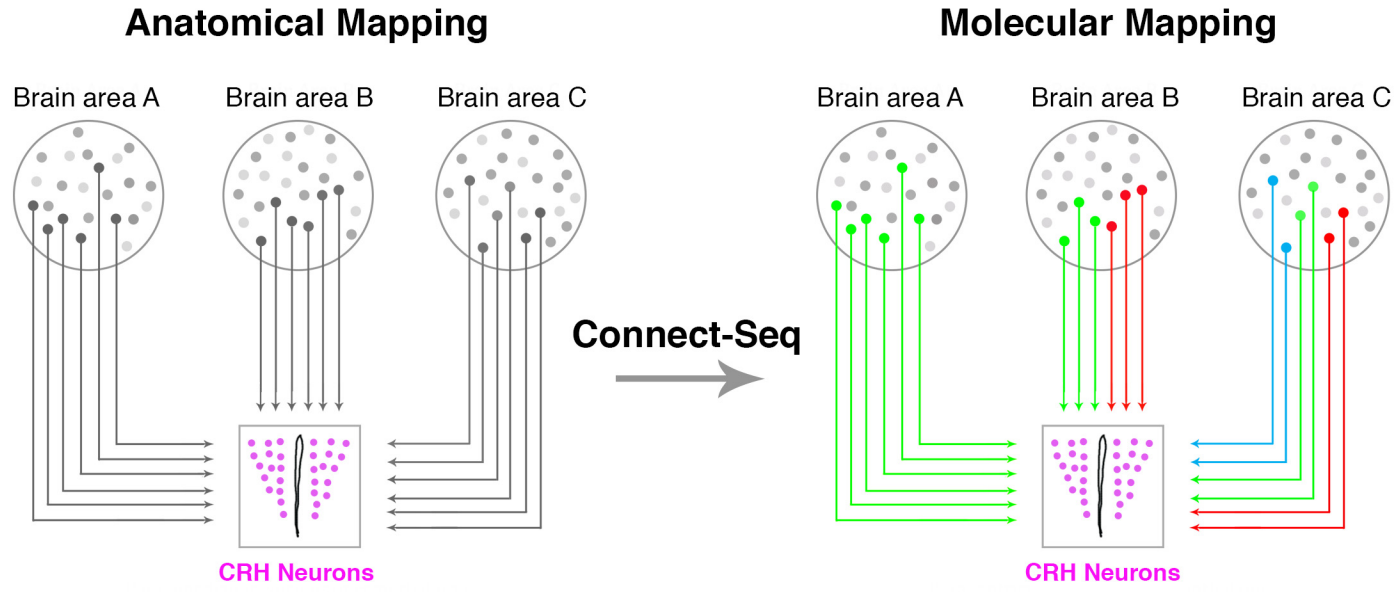

Fig. S9. Connect-seq for superimposing molecular on anatomical circuit maps. Retrograde viral tracing provided an anatomical map of neurons upstream of CRHNs. Connect-seq defined the transcriptomes of single neurons upstream of CRHNs and revealed signaling molecules they express. By mapping the locations of upstream neurons expressing those signaling molecules, it is possible to superimpose a molecular map on the anatomical map of neural circuits upstream of CRHNs.