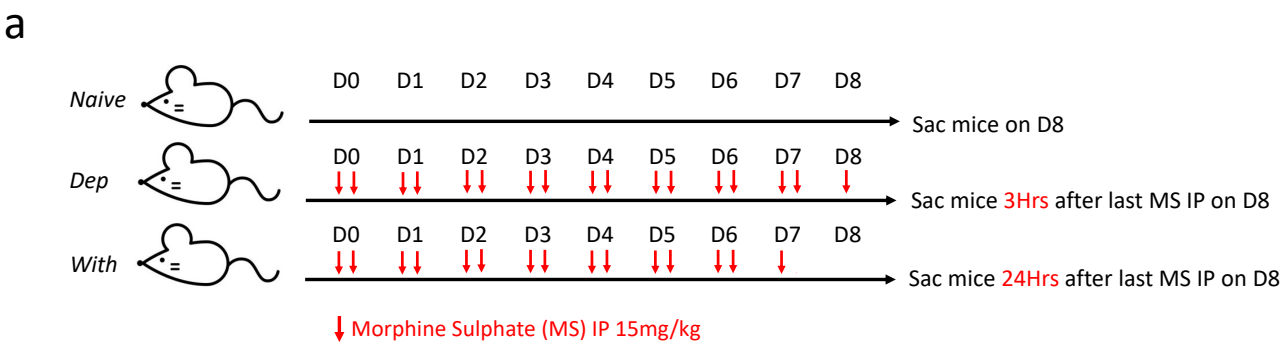
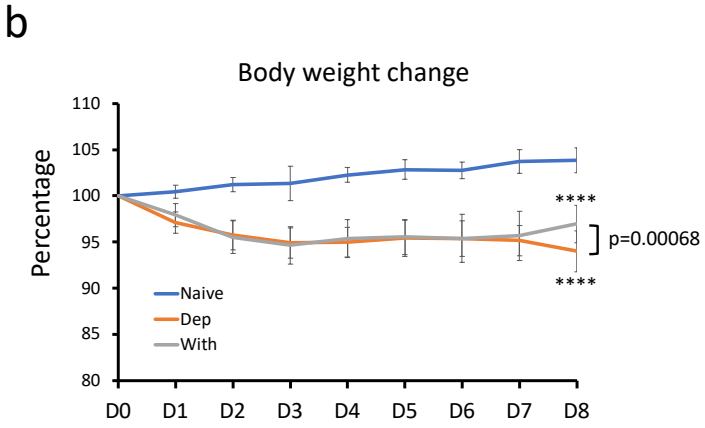
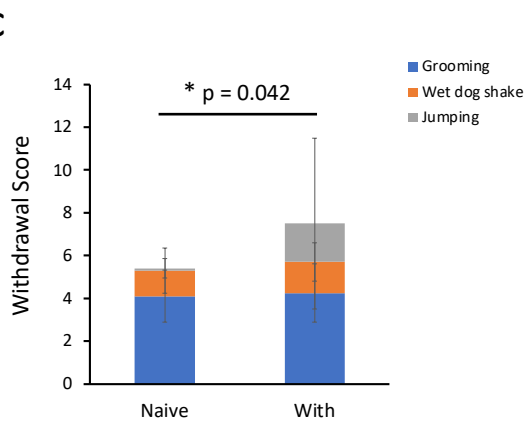
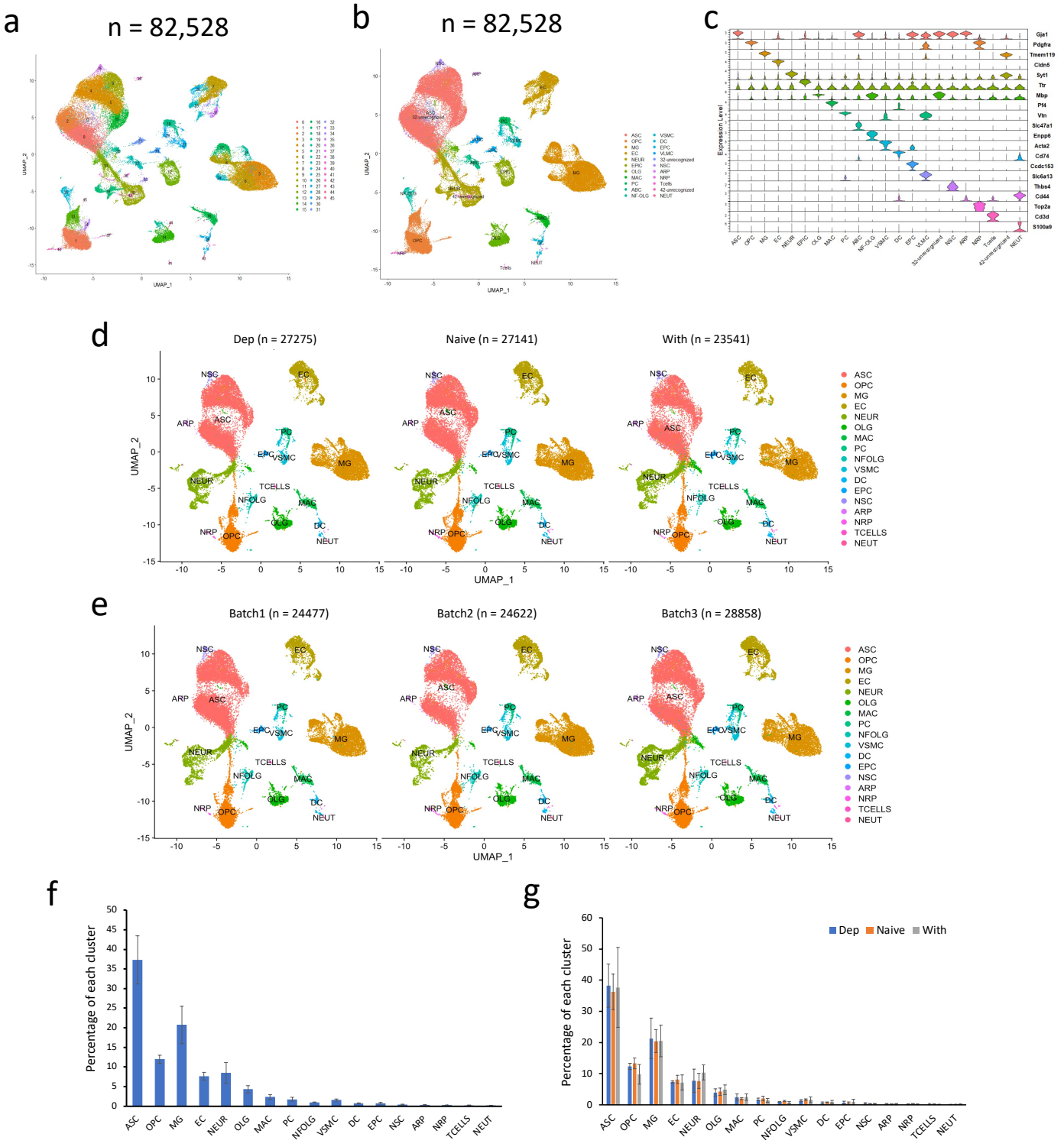


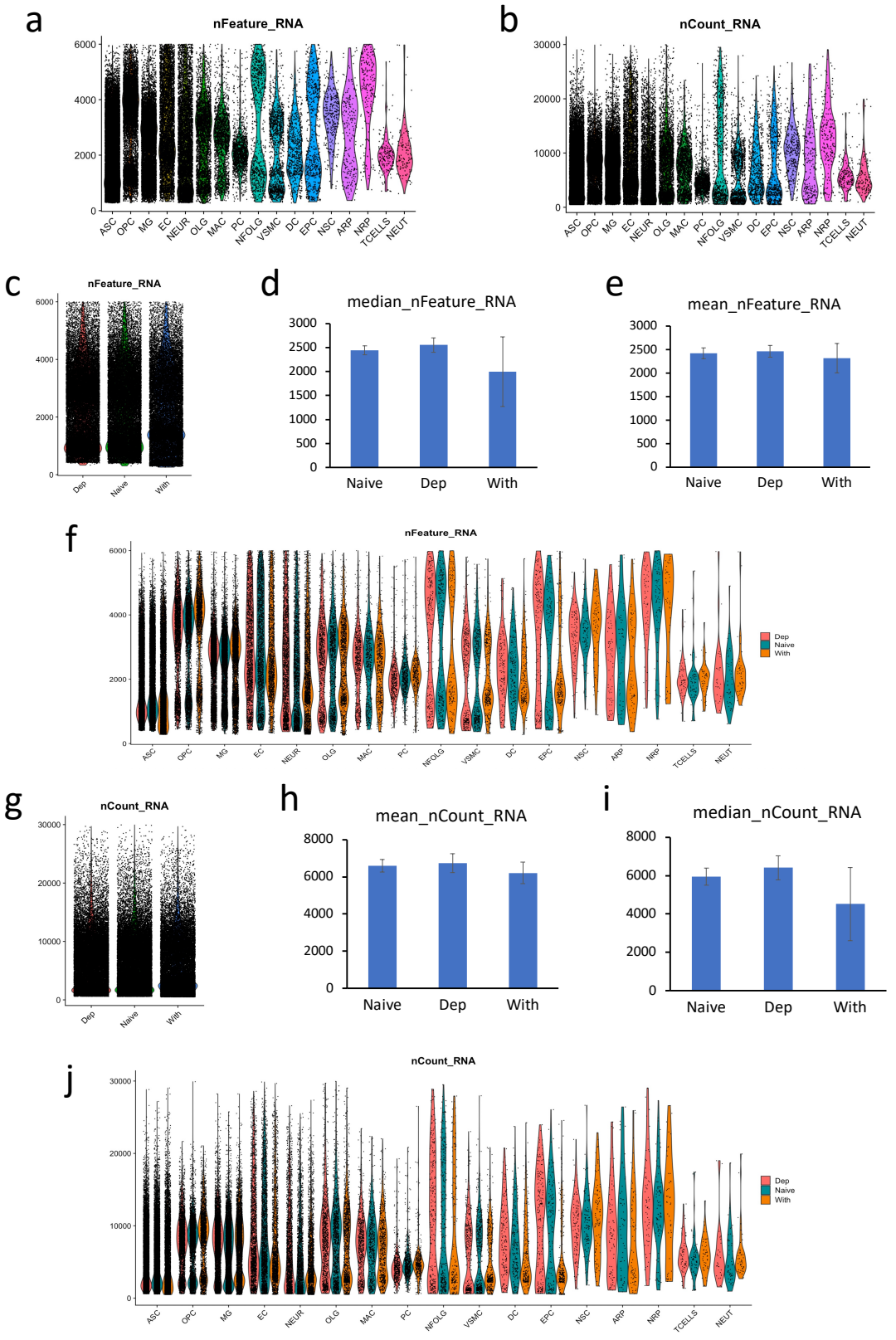




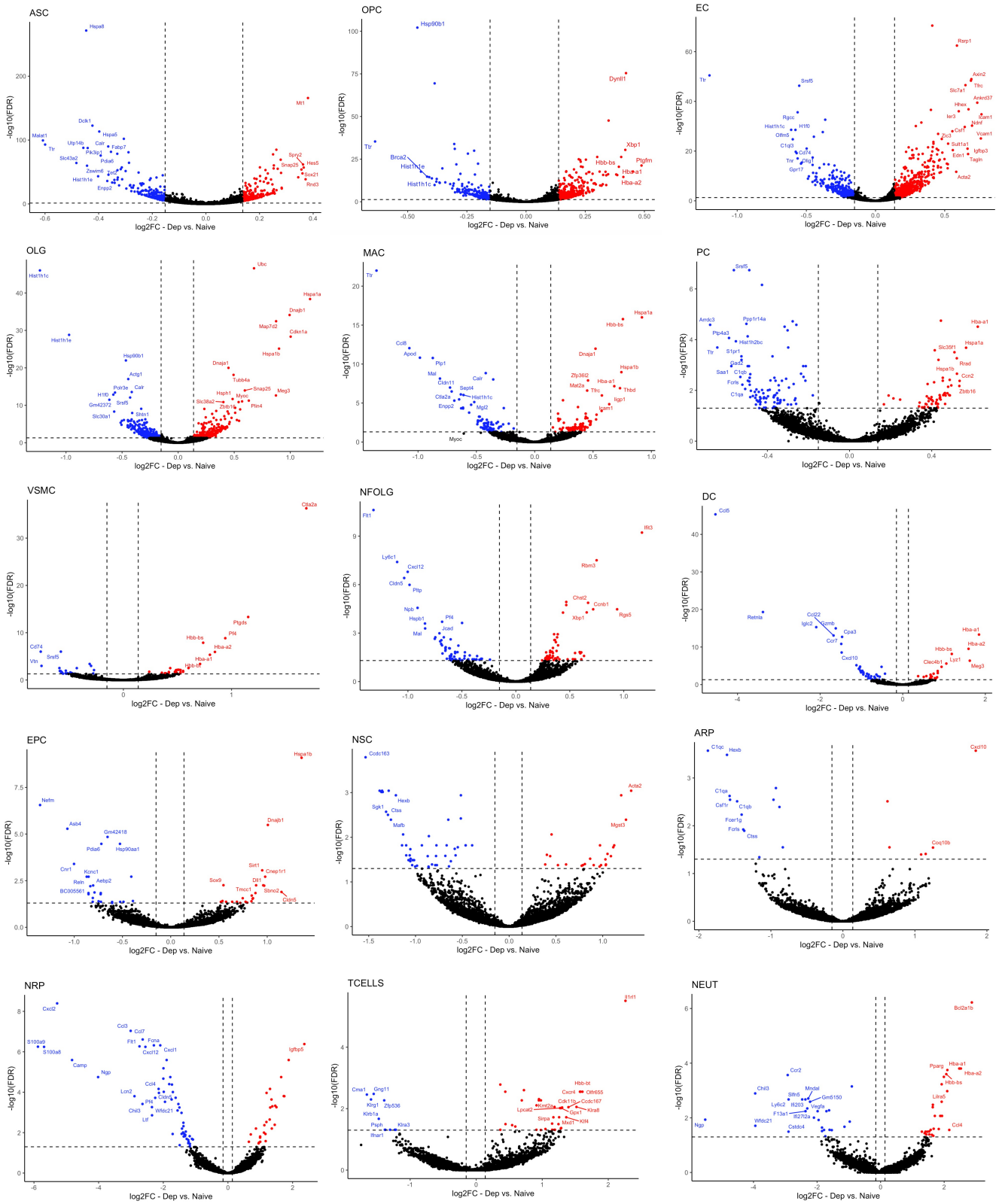
Supplementary Fig. 1 Animal experiments. a, The experiment plan of morphine injection. One arrow indicates one morphine sulphate IP injection. **b,** Changes of body weights of the mice in each experiment group (n = 15 per group). **** p value < 0.0001 by two tailed Student's t-test comparing Dep or With to Naive. p value = 0.00068 comparing With to Dep. Data presents mean ± SEM. **c,** The total withdrawal scores and individual behavior scores of the naïve mice and the mice under the morphine withdrawal condition. * p value < 0.05 by two tailed Student's t-test. Data presents mean ± SEM.



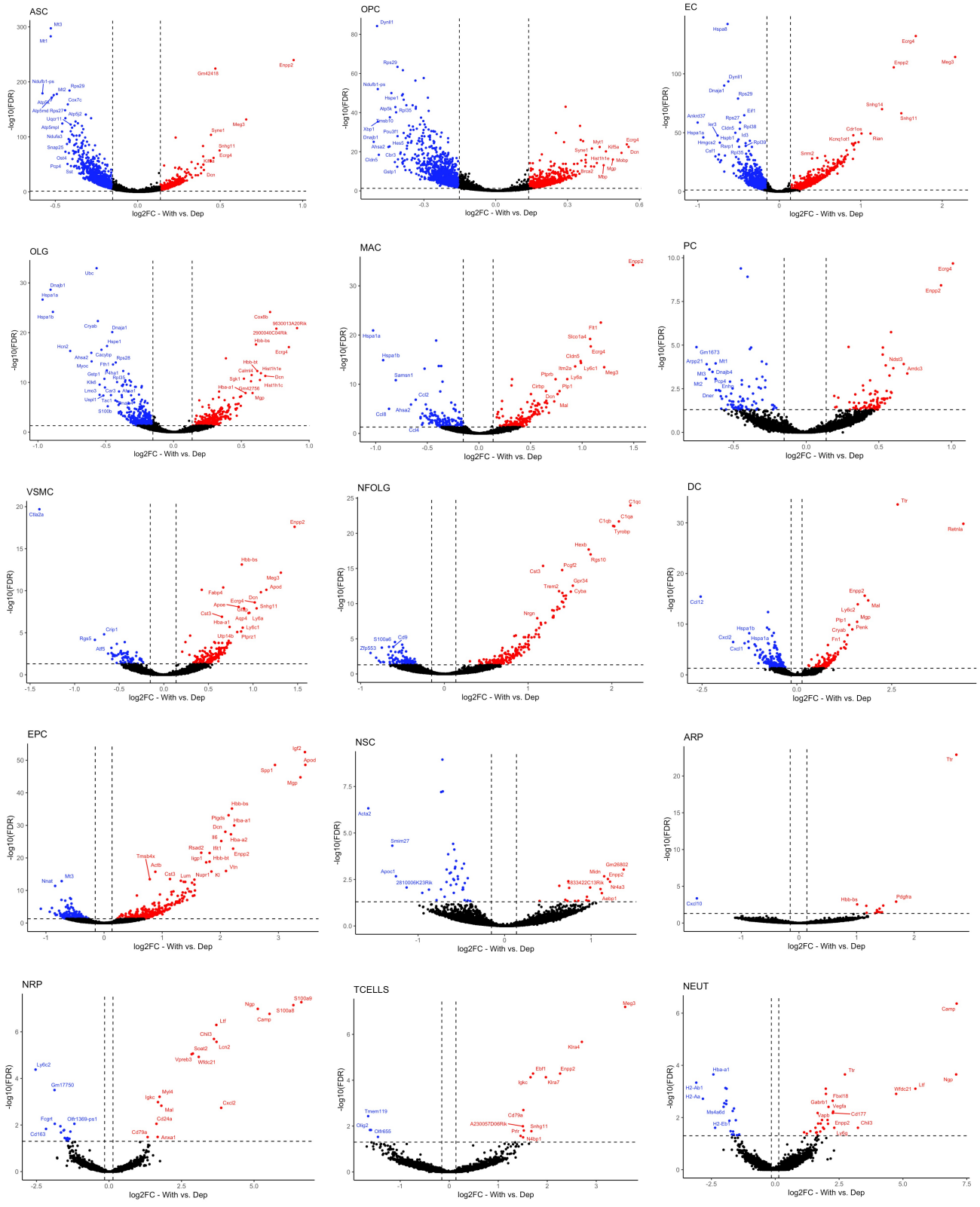
Supplementary Fig. 2 Cell clustering. a, UMAP plot showing the clustering of 82,528 cells based on transcriptome. Cell debris and potential doublets have been removed. 46 initial clusters were identified. **b, c,** 22 clusters were identified (**b**) after assigning each of the 46 initial clusters (**a**) to corresponding cell type based on the cell-type specific/enriched marker genes (**c**). **d,** UMAP plot showing the clusters in each experiment group. **e,** UMAP plot showing the clusters in each batch of experiment. Note that all clusters are represented by cells from all groups and batches. **f,** Percentage of each cluster in the whole cell population. Data presents mean ± SEM of 3 batches of experiments. **g,** Percentage of each cluster in the whole cell population in each experiment group. Data presents mean ± SEM of 3 batches of experiments.



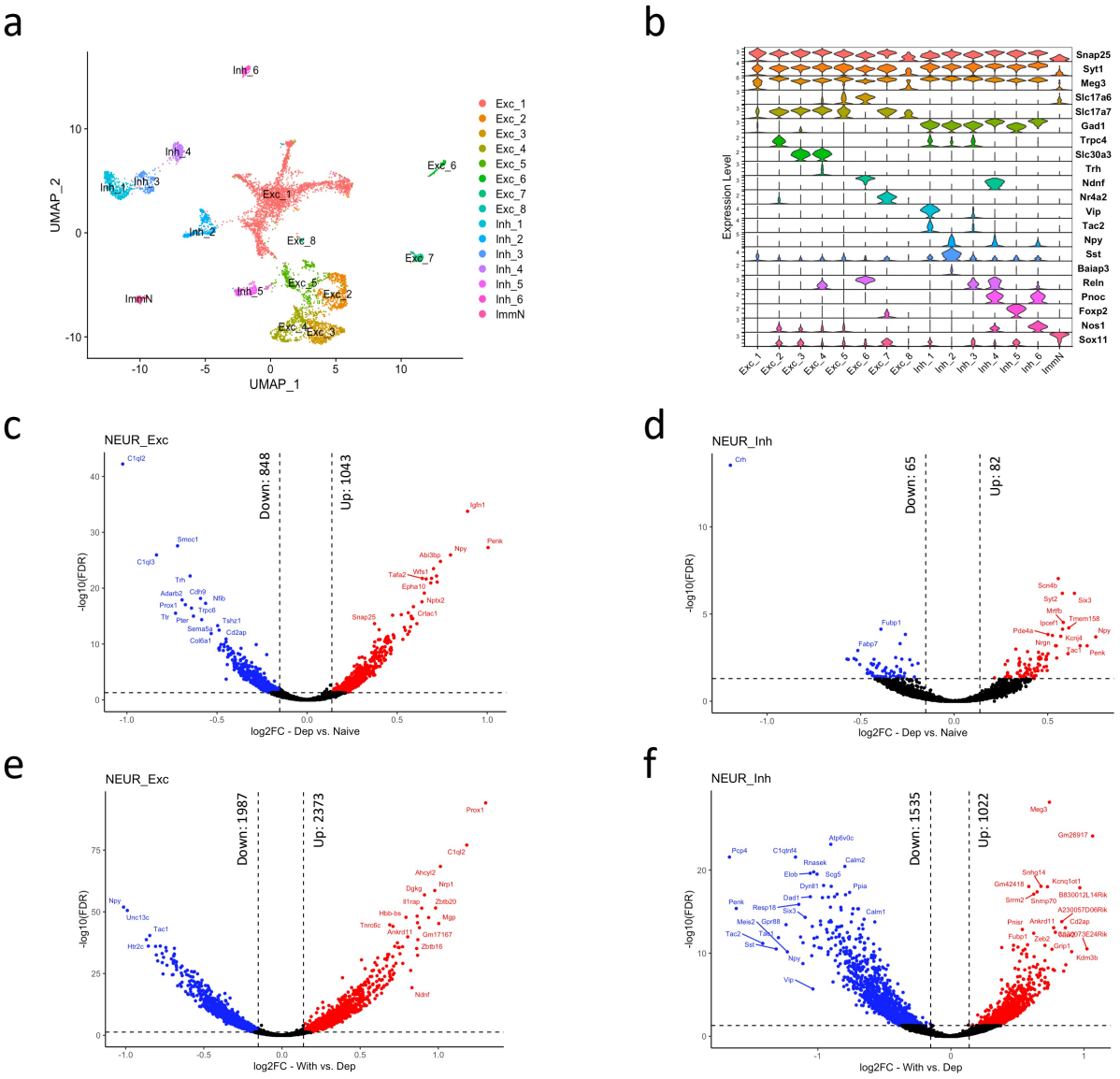
Supplementary Fig. 3 Primary data analysis. Violin plots overlaid with dot plots showing QC metrics: plots in (**a, b**) showing aggregated data of cells from all three experiment groups and plots in (**c, f, g, j**) showing data separated by groups. **a, c, f,** showing number of genes detected per cell; **b, g, j,** showing number of UMI counts per cell. Bar plots **(d, e, h, i)** showing median (**d**) or mean (**e**) number of genes detected per cell by group, or median (**h**) or mean (**i**) number of UMI counts detected per cell by group. Data presents mean ± SEM of 3 batches of experiments.



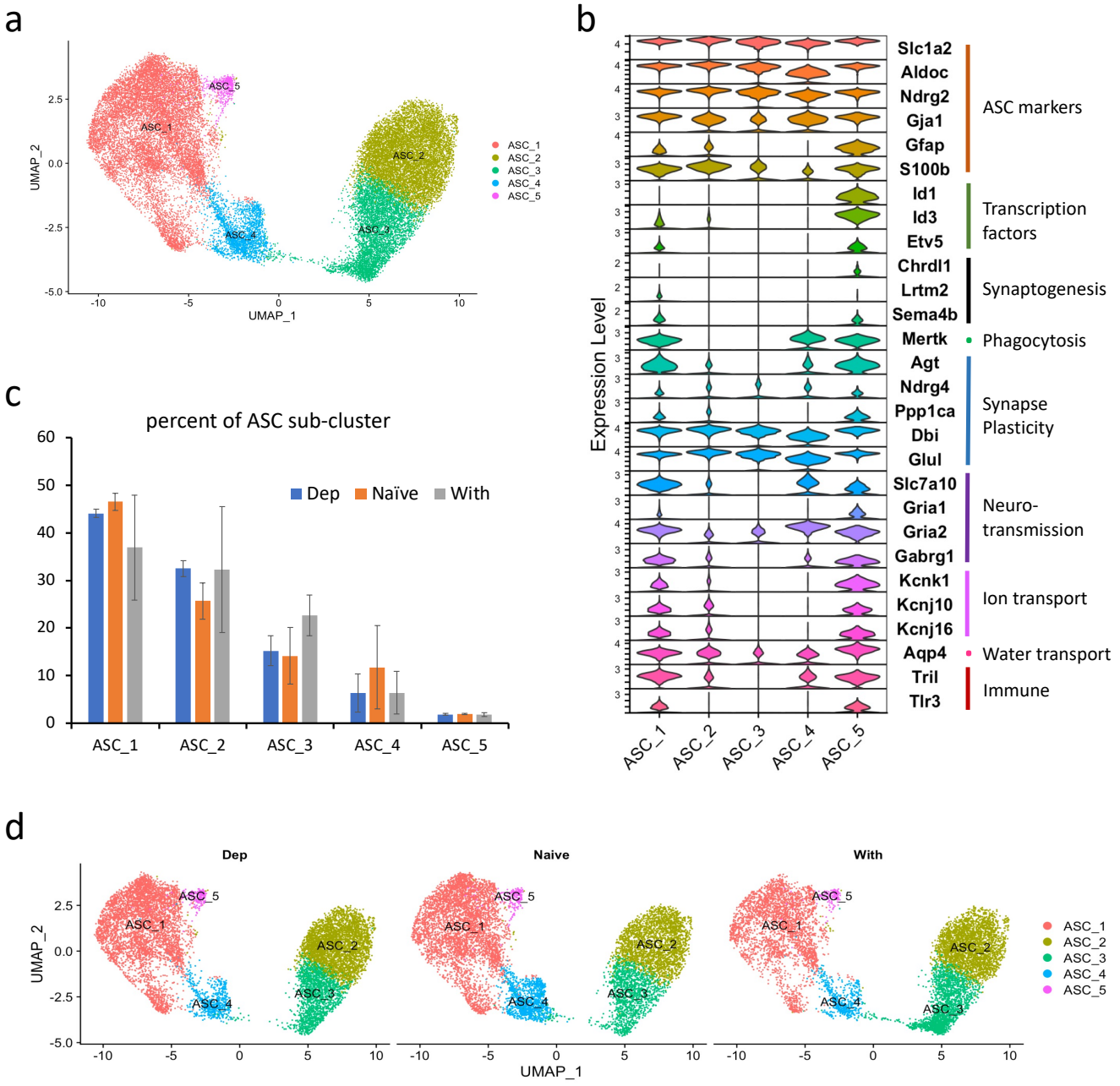
Supplementary Fig. 4 Differentially expressed genes under morphine dependence condition. Volcano plots showing the log2FC and -log10(FDR) of all detected genes in 15 cell types, comparing Dep to Naive. Significantly down-regulated genes are dots in blue, up-regulated genes are in red and genes in black are not significantly changed.



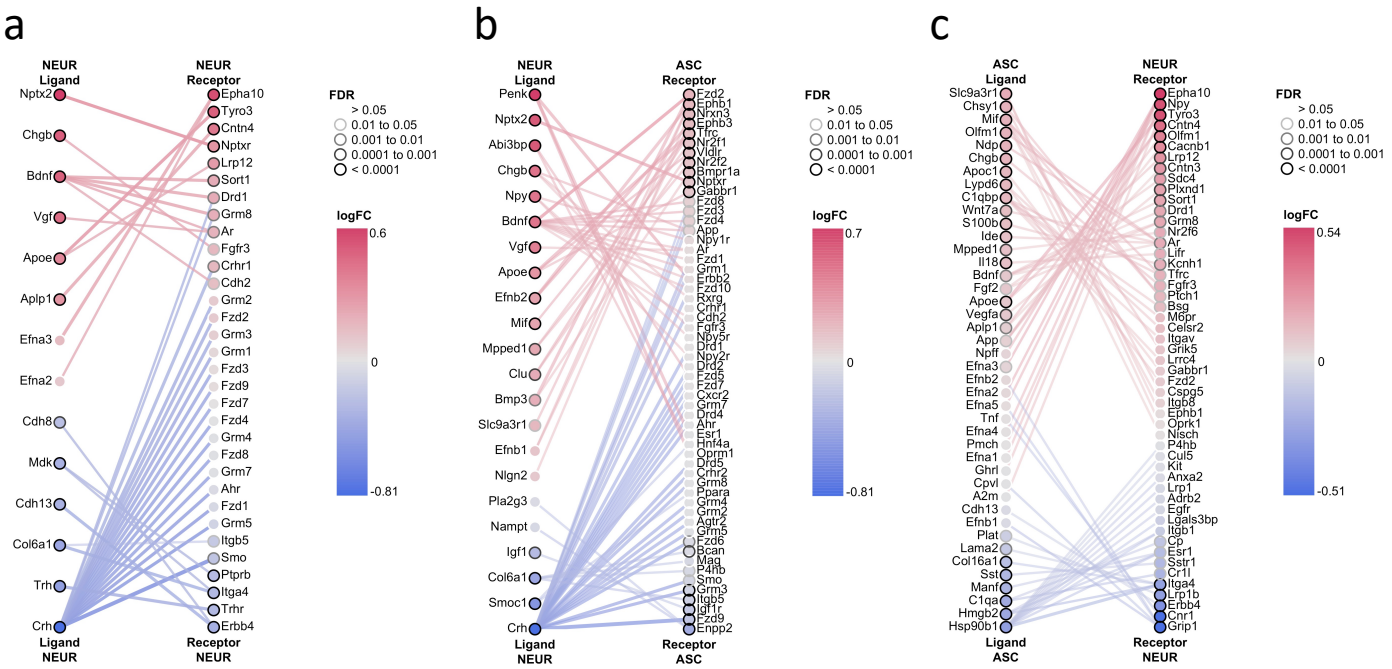
Supplementary Fig. 5 Differentially expressed genes under morphine withdrawal condition. Volcano plots showing the log2FC and -log10(FDR) of all detected genes in 15 cell types, comparing With to Dep. Significantly down-regulated genes are dots in blue, up-regulated genes are in red and genes in black are not significantly changed.



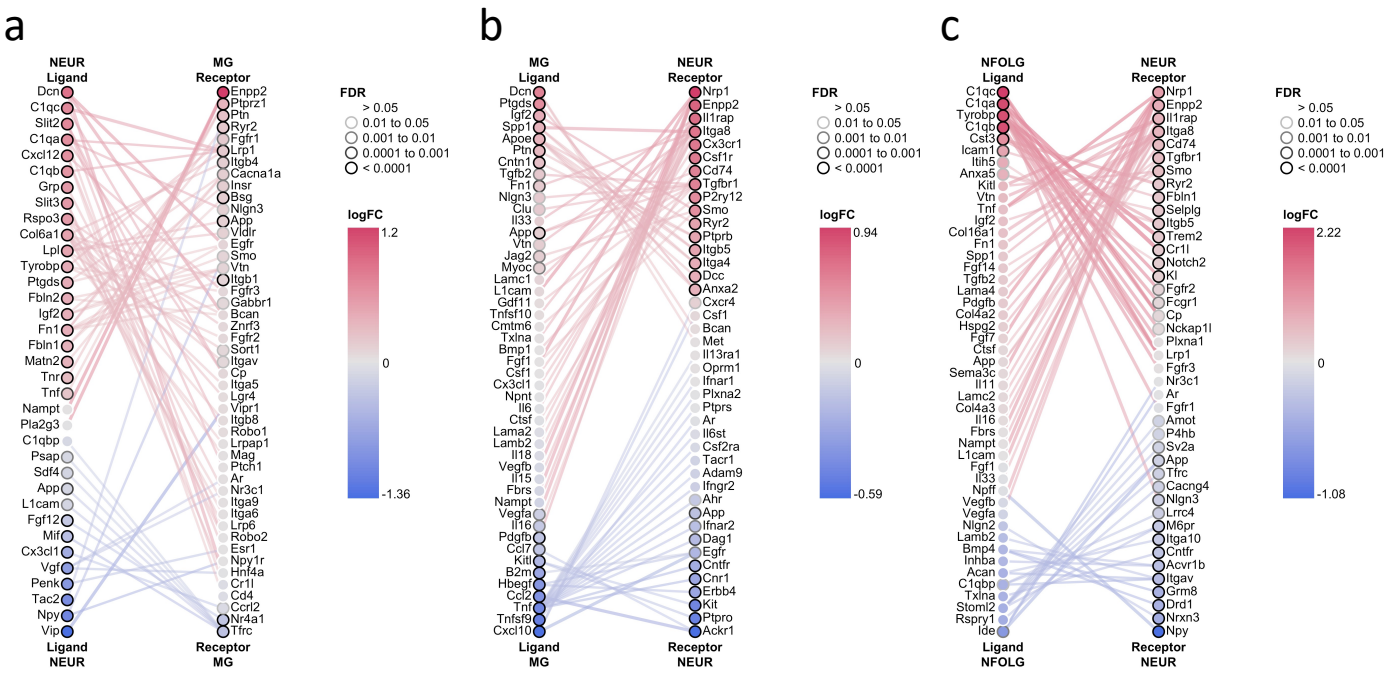
Supplementary Fig. 6 Sub-clustering of the neuron population. a, UMAP plot showing the clustering of NEUR cells (n=6,751) based on transcriptome. Our analysis revealed the existence of 8 excitatory neuron subtypes (Exc_1 - Exc_8), 6 inhibitory neuron subtypes (Inh_1 - Inh_6) and the immature neurons (ImmN) with distinct cellular signatures (see Supplementary Table 6). **b,** Violin plot showing the expression of different neuropeptides, neurotransmitters, neurohormones, etc., across all the neuron subtypes. **c, e,** Volcano plots showing the log2FC and -log10(FDR) of all detected genes in excitatory neurons, comparing Dep to Naive (**c**), or With to Dep (**e**). **d, f,** Volcano plots showing the log2FC and -log10(FDR) of all detected genes in inhibitory neurons, comparing Dep to Naive (**d**), or With to Dep (**f**). Significantly down-regulated genes are dots in blue, up-regulated genes are in red and genes in black are not significantly changed. To be noted, our data showed excitatory and inhibitory neurons responded quite differently to morphine treatment. These data suggests that the transcriptional changes that we observed in the whole neuron population under morphine dependence (Fig. 2e) occurred mainly in the excitatory neurons. Under withdrawal condition, both excitatory and inhibitory neurons showed over two thousand DEG, although there were still more DEG in excitatory neurons than inhibitory neurons. The detailed DEG information was summarized in Supplementary Table 7.



Supplementary Fig. 7 Sub-clustering of the astrocyte population. a, UMAP plot showing the clustering of ASC cells (n= 29,067) based on transcriptome. Our analysis revealed the existence of 5 astrocyte subtypes (ASC_1 - ASC_5) with distinct cellular signatures (see Supplementary Table 8). **b,** Violin plot showing the expression of genes involved in different biological processes across the astrocyte subtypes. **c,** Percentage of each ASC subcluster in the whole astrocyte population in each experiment group. Data presents mean ± SEM of 3 batches of experiments. **d,** UMAP plot showing the astrocyte subclusters in each experiment group. Two of the five subpopulations showed almost the opposite transcriptional profiles: ASC_1 (also the largest ASC subtype) showed high levels of phagocytosis, neurotransmission, ion and water transport, and immune activity, while ASC_3 showed very low levels. Interestingly, there was a trend that compared to Naive and Dep samples, the With samples exhibited a higher percentage of ASC_3 in the whole ASC population (**c, d**), although this was not significant.



Supplementary Fig. 8 Changes of cell-cell interactions with chronic morphine treatment. The comparisons were between Dep and Naive samples. **a,** ligands expressed in NEUR with receptors expressed in NEUR. As the largest glia cell population, ASC exhibited distinct changes of the interactions with NEUR under morphine dependence condition: **b,** ligands expressed in NEUR with receptors expressed in ASC. **c,** ligands expressed in ASC with receptors expressed in NEUR.



Supplementary Fig. 9 Changes of cell-cell interactions under morphine withdrawal condition. The comparisons were between With and Dep samples. **a,** ligands expressed in NEUR with receptors expressed in MG. **b,** ligands expressed in MG with receptors expressed in NEUR. **c,** ligands expressed in NFOLG with receptors expressed in NEUR.