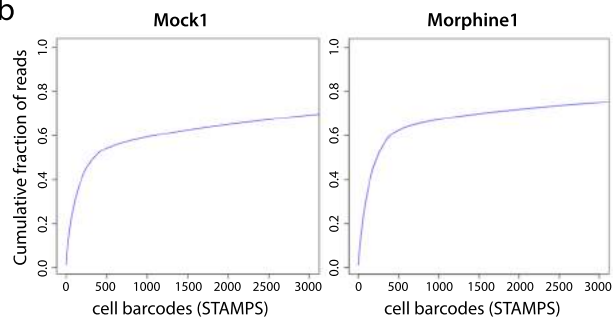


**Figure S1**

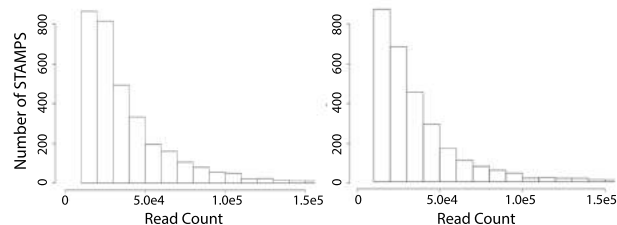
**a**

| Replicate | Treatment (# mice) | Age (Days $\pm$ SD) |
|-----------|--------------------|---------------------|
| 1         | Mock (2)           | 43.5 $\pm$ 2.1      |
|           | Morphine (2)       | 43.5 $\pm$ 2.1      |
| 2         | Mock (2)           | 39.5 $\pm$ 3.5      |
|           | Morphine (2)       | 39 $\pm$ 2.1        |
| 3         | Mock (2)           | 80 $\pm$ 2.8        |
|           | Morphine (2)       | 80 $\pm$ 2.8        |
| 4         | Mock (2)           | 78 $\pm$ 0          |
|           | Morphine (2)       | 78 $\pm$ 0          |

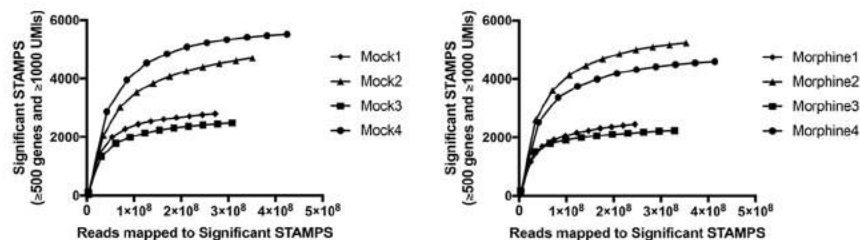
**b**



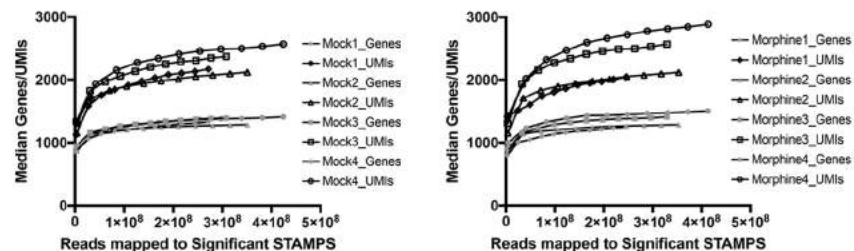
**c**



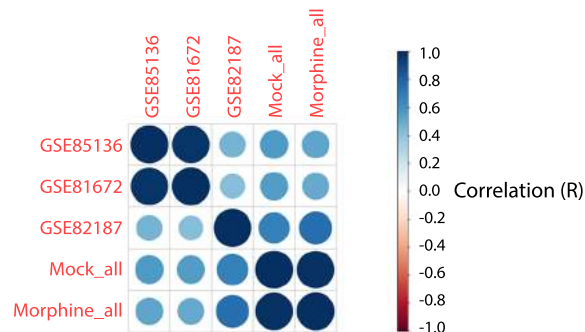
**d**



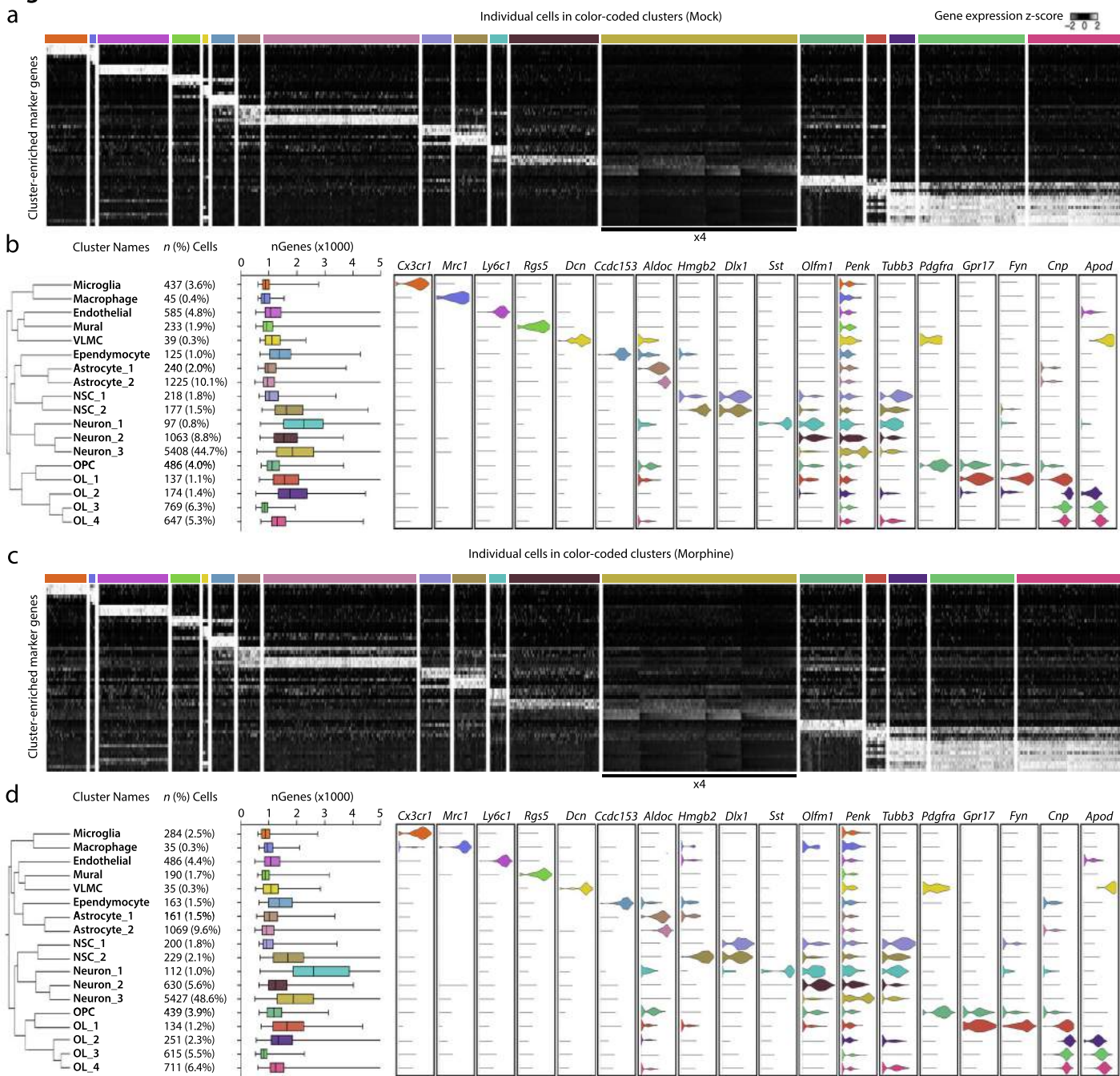
**e**



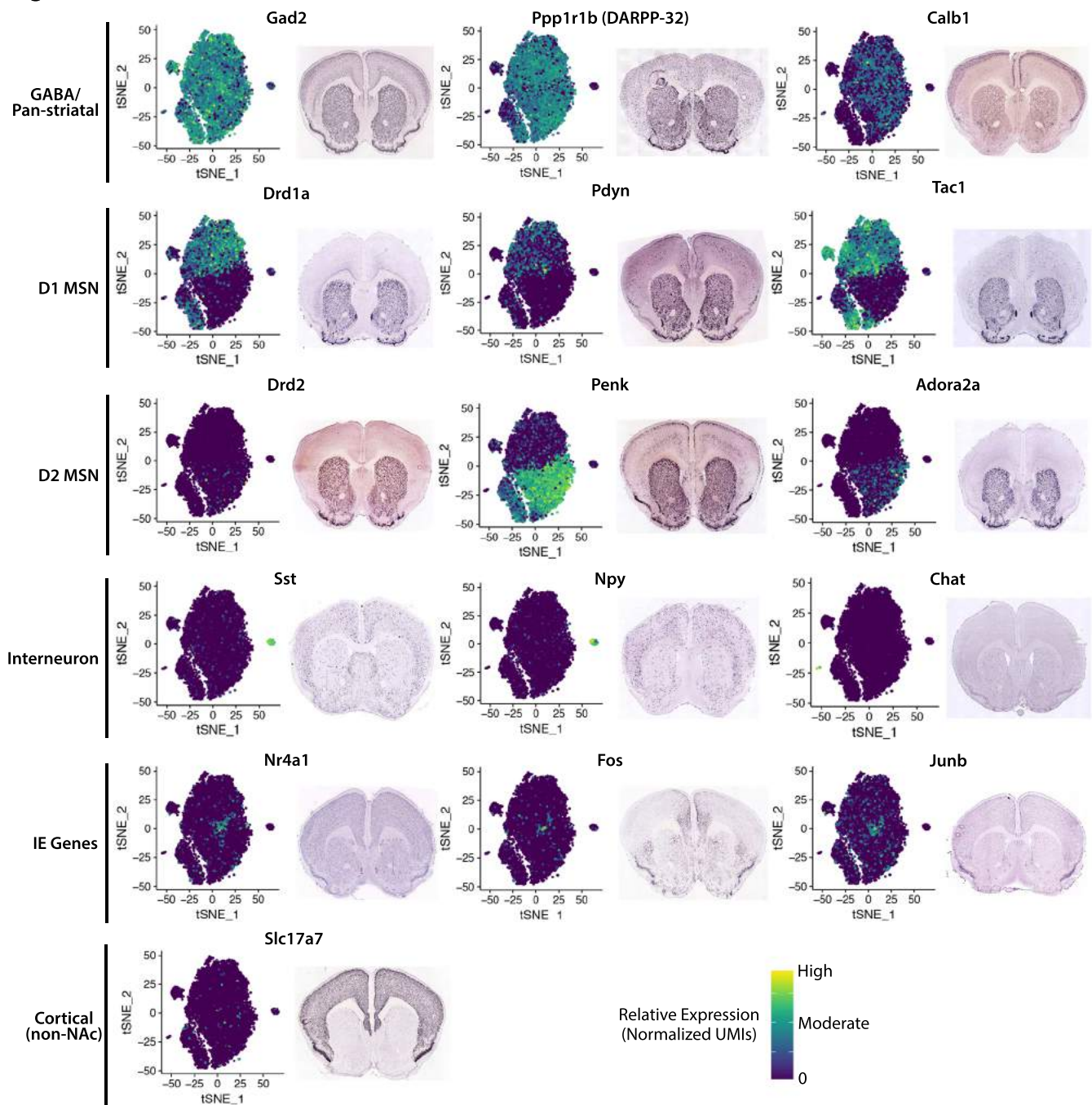
**f**



**Supplementary Figure 1** Ages of mice, quality control of Drop-seq libraries, and correlation with previous RNA-seq datasets of the mouse nucleus accumbens.

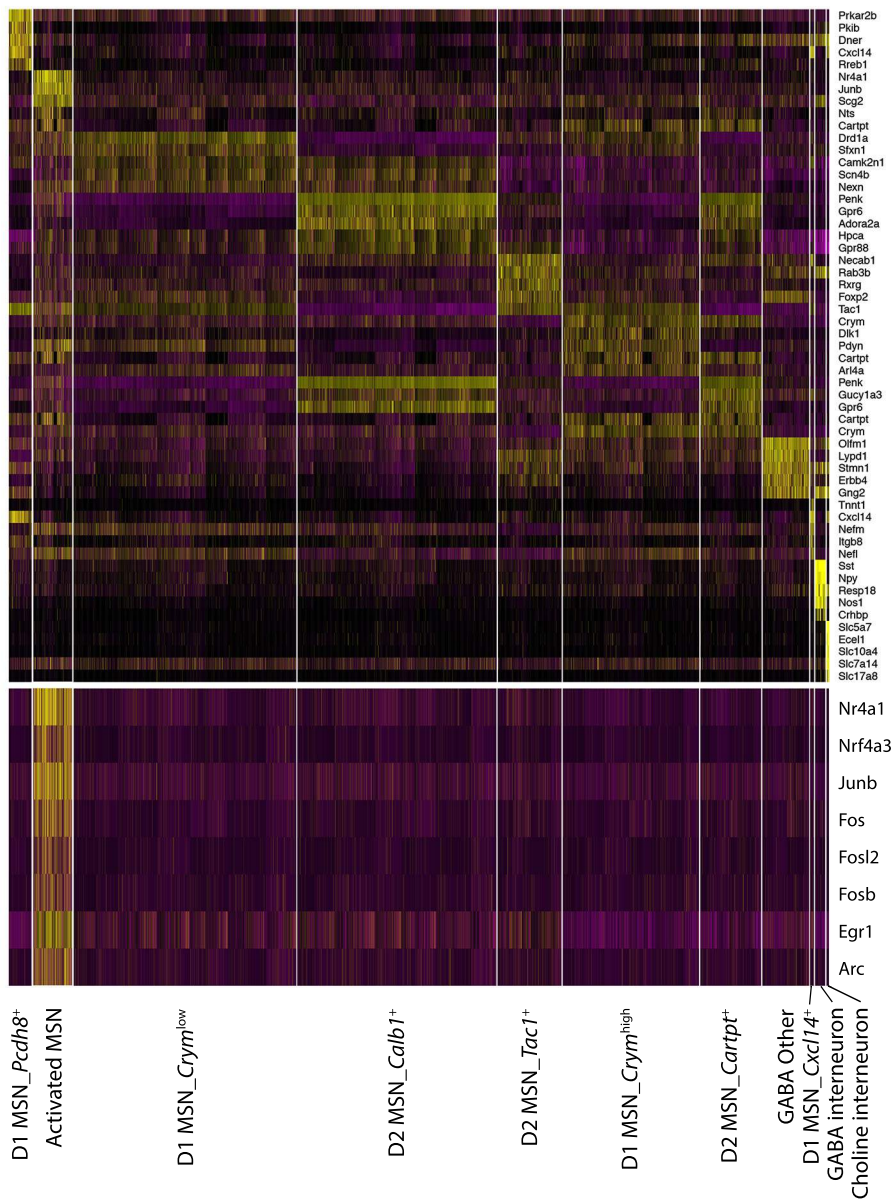
**Figure S2****Supplementary Figure 2** Proportions and marker genes of major CNS cell types are unchanged by acute morphine treatment.

**Figure S3**



**Supplementary Figure 3** Markers of neuronal subclusters are consistent with ISH data from the Allen Brain Atlas.

Figure S4

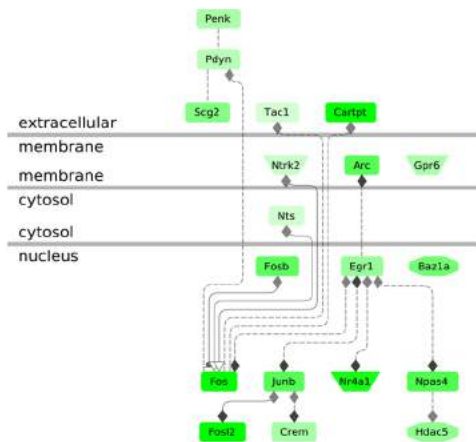


Supplementary Figure 4 Heatmaps showing single-cell relative expression of neuronal cluster-enriched genes.

**Figure S5**

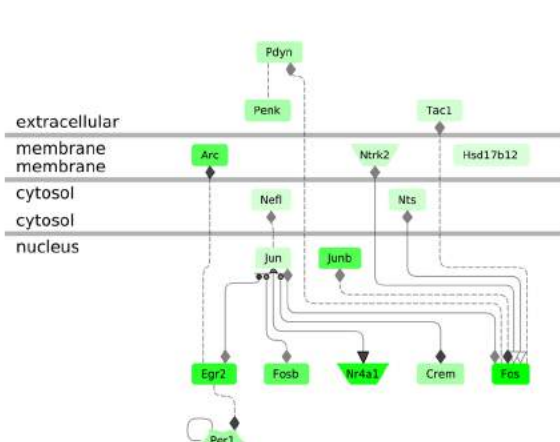
**Nucleus Accumbens**

( $p = 1.12e-9$ )



**Morphine Dependence**

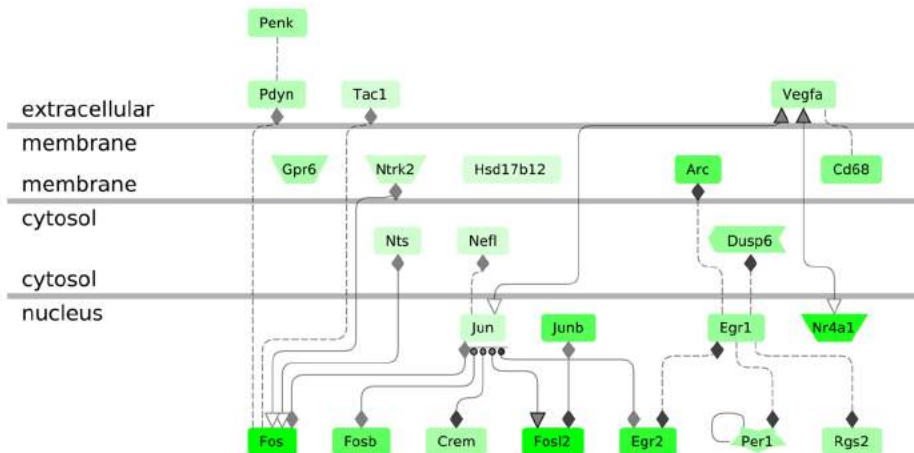
( $p = 2.75e-10$ )



**Opioid-Related Disorders**

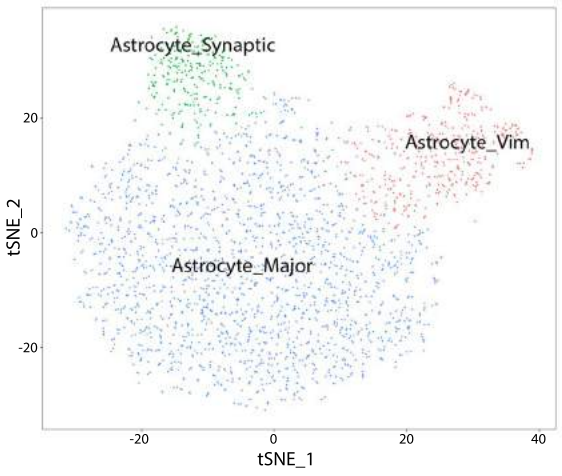
( $p = 5.10e-13$ )

4 SCDE  
2  $\log_2$ FC  
0

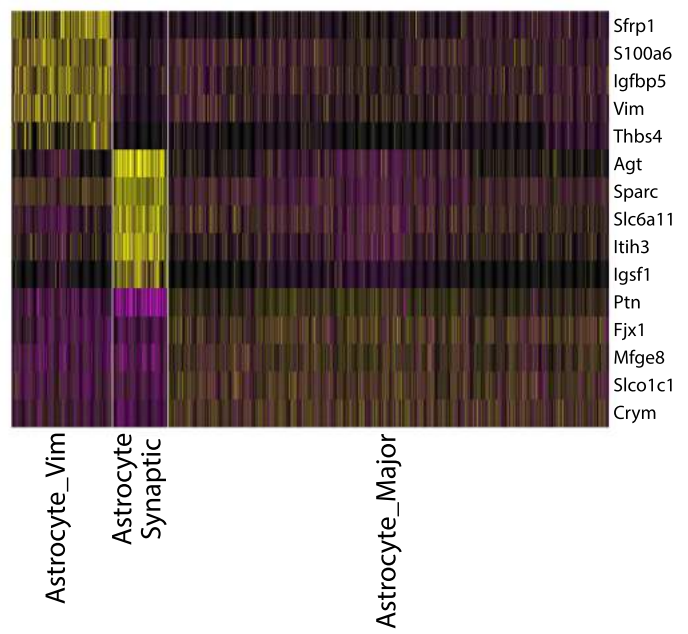


**Supplementary Figure 5** Pathway analysis of genes enriched in morphine-treated Drd1-expressing cells of the Activated MSN cluster.

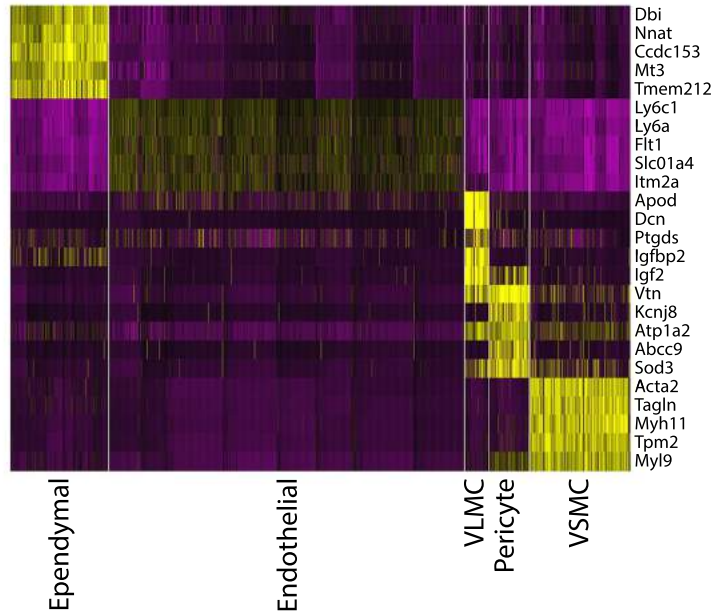
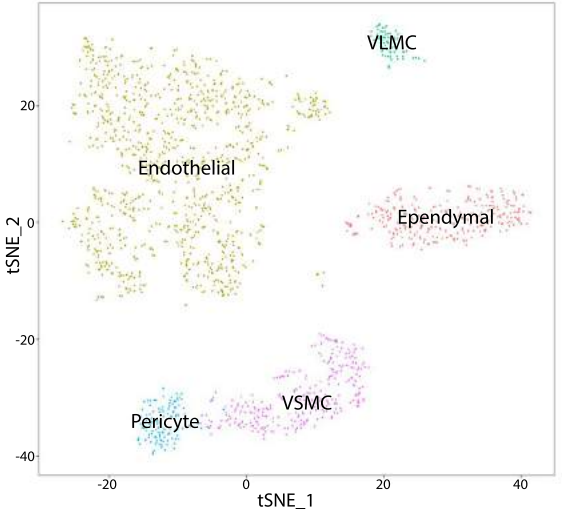
**Figure S6**



**Astrocytes**



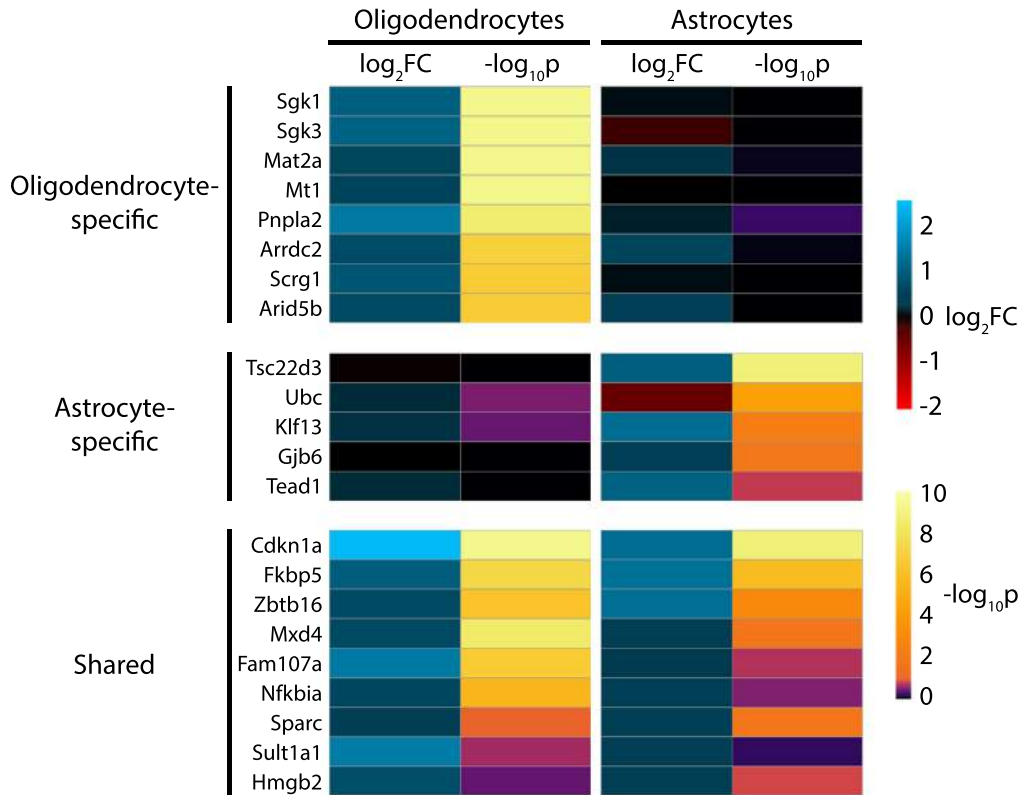
**Endothelial, Ependymal, Mural and VLM Cells**



**Supplementary Figure 6** Subclustering further resolves non-neuronal cell types of the NAc.

**Figure S7**

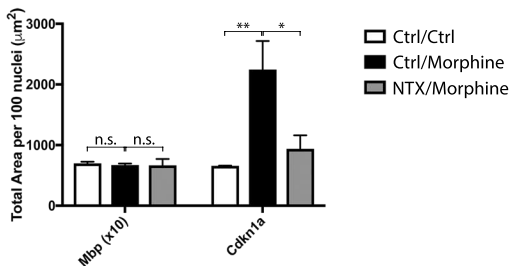
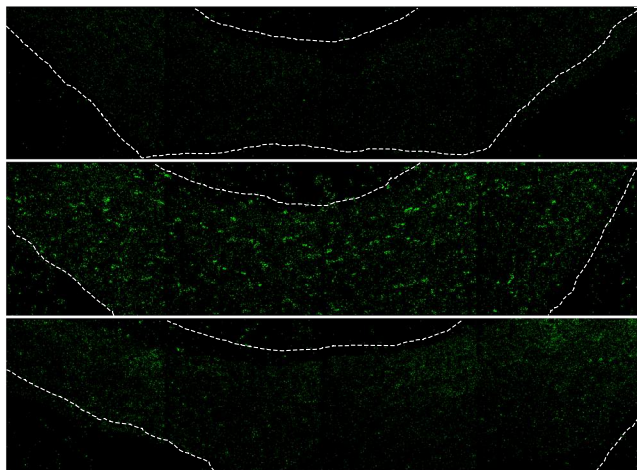
**SCDE Result (Morphine vs. Mock)**



**Supplementary Figure 7** Distinct subsets of glucocorticoid receptor (GR) target genes are induced by morphine in oligodendrocytes (MO\_all) and astrocytes (Astrocyte\_all).

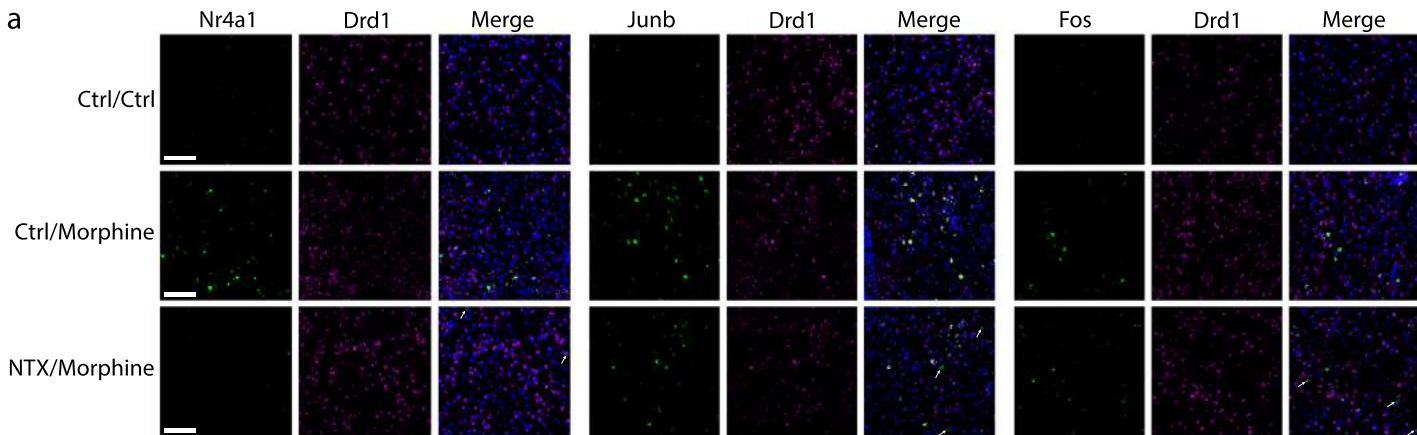
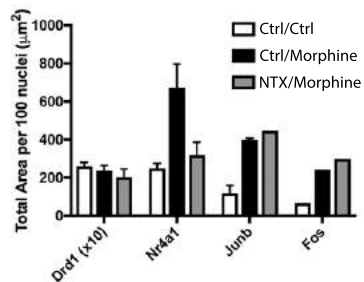
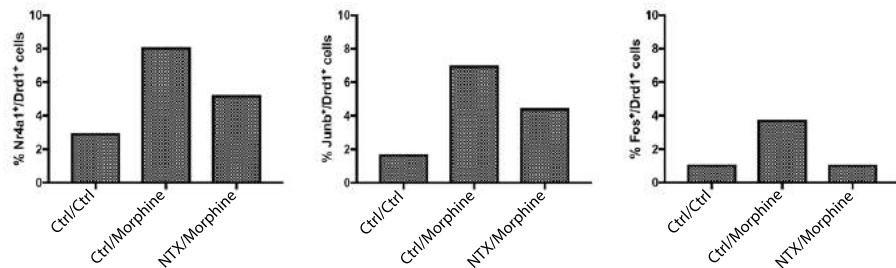
Figure S8

Cdkn1a - Corpus Callosum (CC)



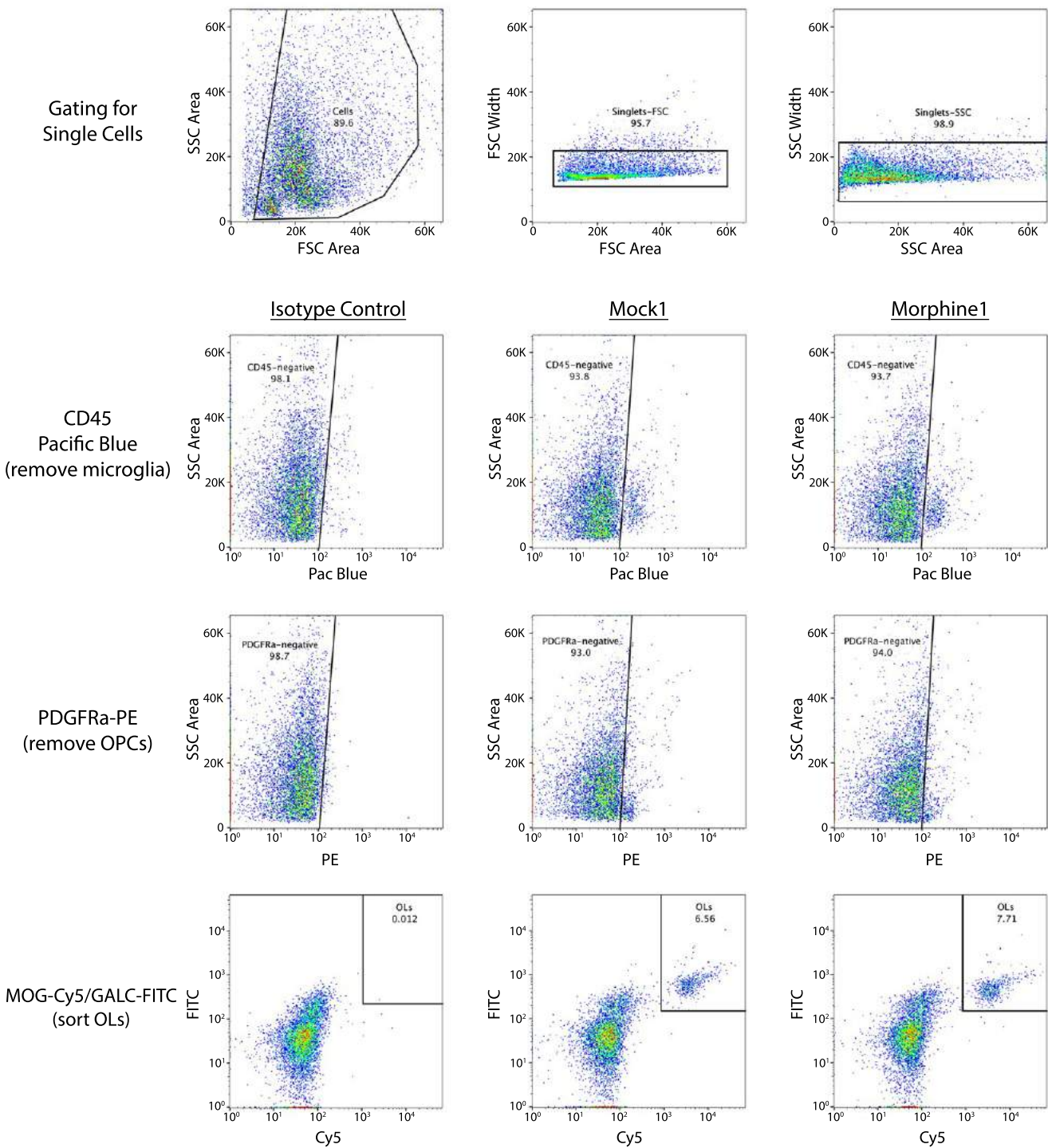
Supplementary Figure 8 FISH of Cdkn1a in the corpus callosum (outlined by dotted line).



**Figure S9****a****b****c**

**Supplementary Figure 9** FISH of neuronal genes in the NAC. Arrows in NTX/Morphine Merge panels indicate cells positive for the indicated IEG (Nr4a1, Junb, or Fos), but negative for Drd1.

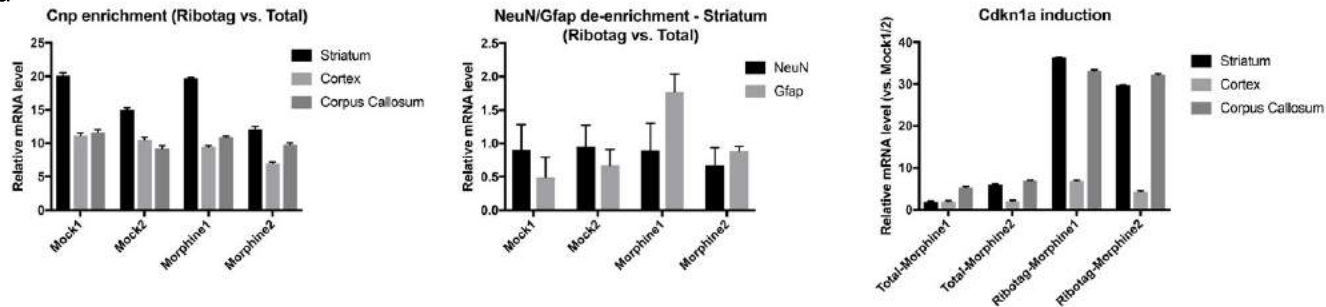
**Figure S10**



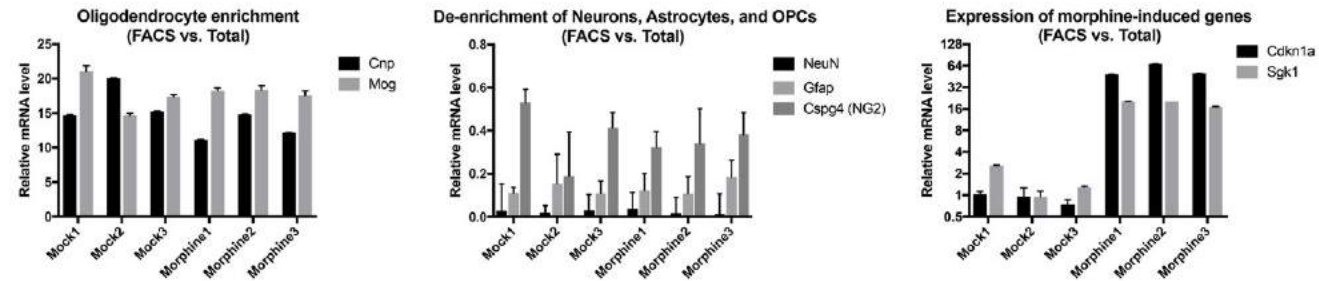
**Supplementary Figure 10** FACS plots illustrating gating for GALC<sup>+</sup>/MOG<sup>+</sup> oligodendrocytes.

# Figure S11

a



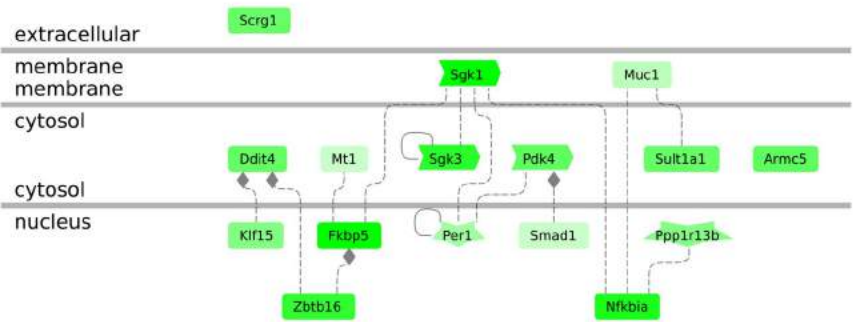
b



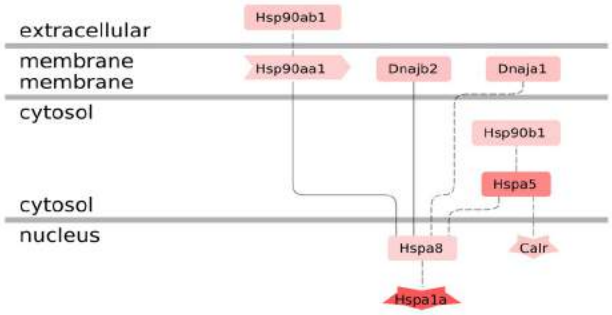
**Supplementary Figure 11** qRT-PCR analysis of RNAs purified from Cnp-Cre x Ribotag mice (a) or from FACS-isolated OLs of WT mice (b).

Figure S12

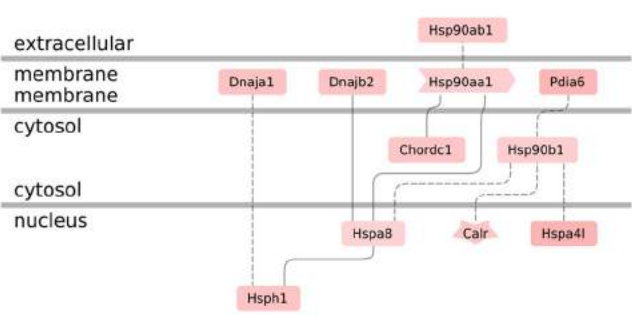
# Glucocorticoid Receptor Signaling



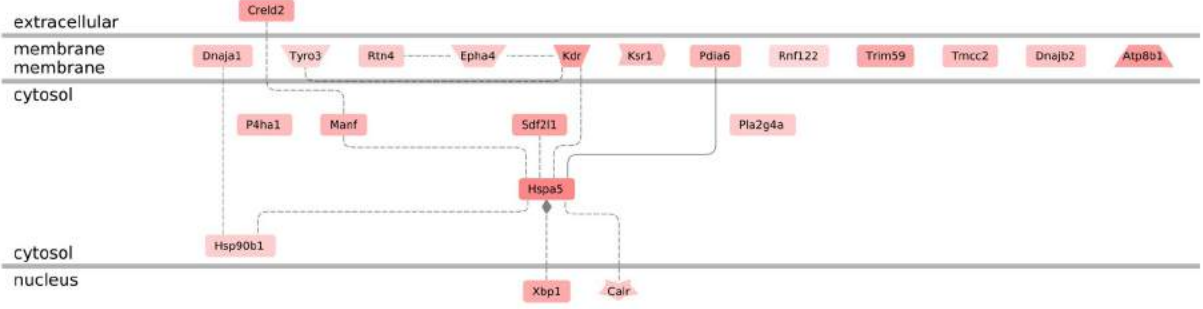
## Unfolded Protein Binding



## Protein Folding



## Endoplasmic Reticulum



Supplementary Figure 12 Pathway analysis of morphine-induced genes in oligodendrocytes as assessed by bulk RNAseq.