Cell Reports, Volume *24*

Supplemental Information

Single-Cell RNA-Seq Uncovers a Robust Transcriptional Response to Morphine by Glia

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

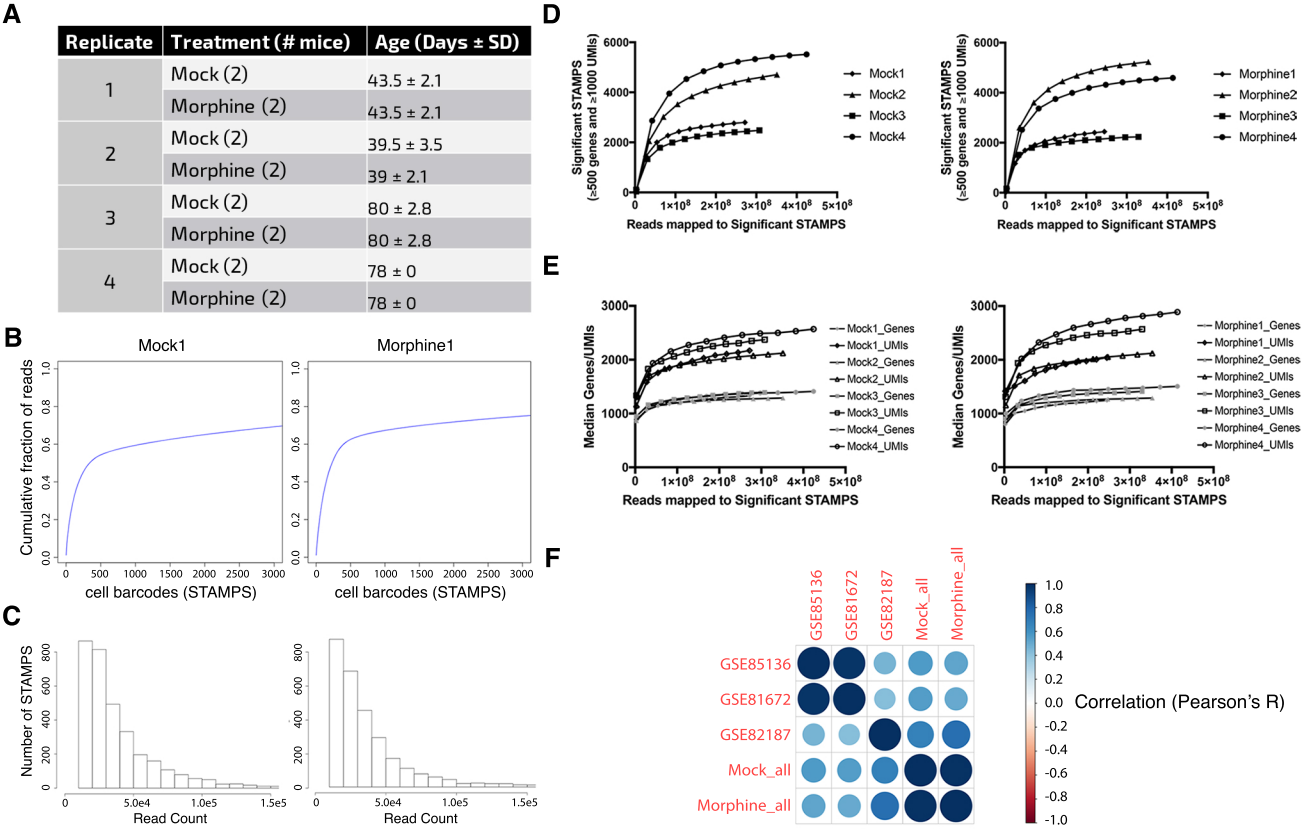

Figure S1. Quality control of Drop-seq libraries and correlation with previous RNA-seq datasets of the mouse nucleus accumbens, Related to Figure 1. (**A**) Age of mice for each biological replicate. (**B**) Cumulative read distribution for a subset of STAMPS (~500) from the first biological replicate. (**C**) Read count distribution for Mock1 and Morphine1. (**D-E**) Number of Significant STAMPS (**D**) or median genes and UMIs (**E**) were plotted for each sample as a function of sequencing depth. (**F**) Our samples (log-transformed gene expression averaged across all single cells) correlate well with three previously published gene expression datasets of the mouse nucleus accumbens.

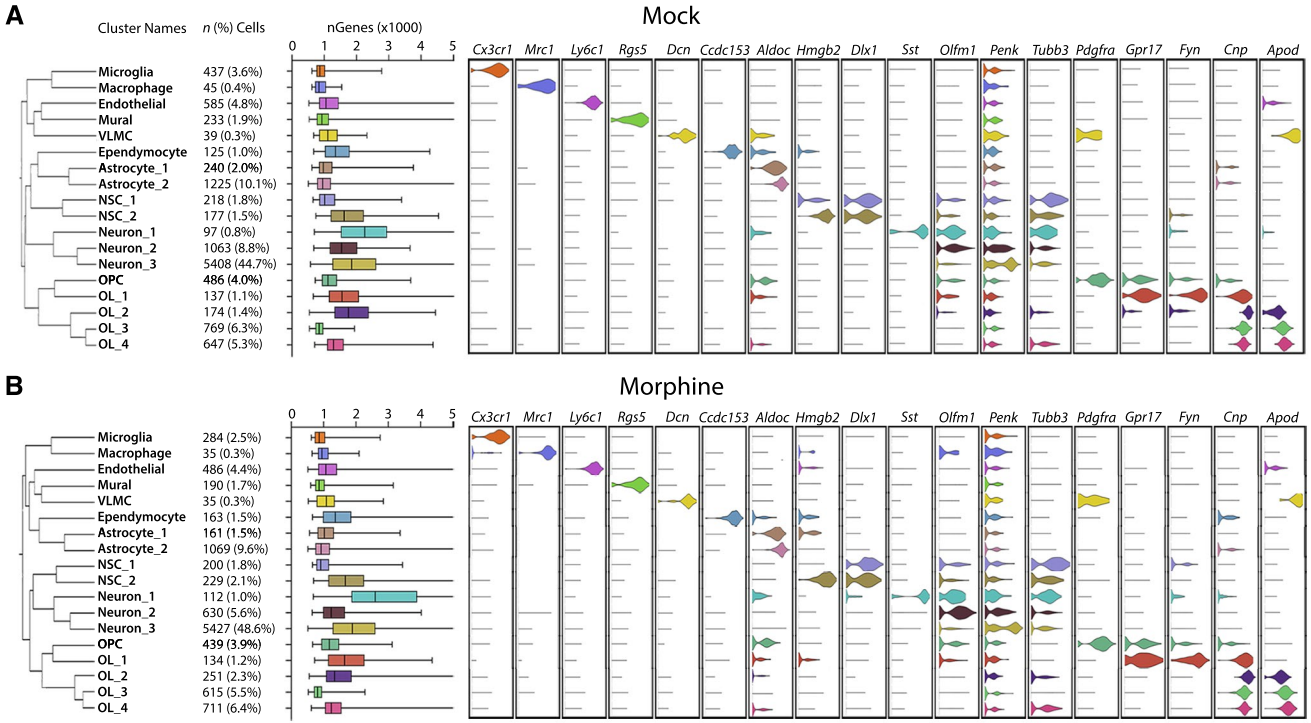

Figure S2. Proportions and marker genes of major CNS cell types are unchanged by acute morphine treatment, Related to Figure 1. On left, unbiased hierarchical clustering yields highly similar trees. The number (and percentage) of cells identified per cluster, number of genes (nGenes), and expression of cell type marker genes are also comparable between mock and morphine samples (clusters are color-coordinated as in Figure 1). In the nGenes box-and-whisker plots, the vertical line represents the median across all single cells in a given cluster, the box extends from the 25th to 75th percentile, and the whiskers extend from the minimum to maximum. Those with an open-ended whisker have a max number of genes greater than 5000.

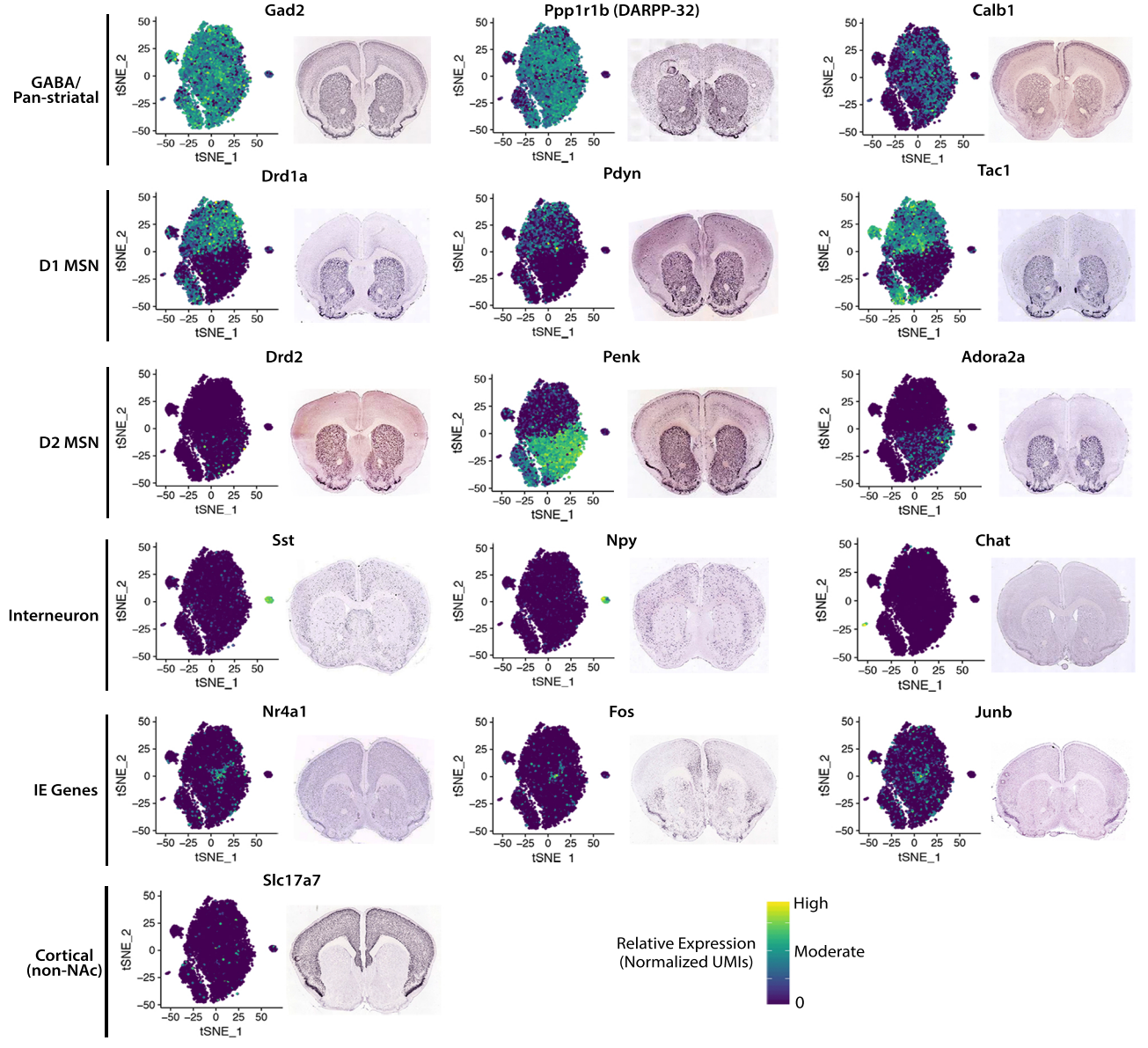

Figure S3. Neuronal subcluster-enriched genes are expressed in the nucleus accumbens, Related to Figure 2. t-SNE plots of neuron subclusters were overlaid with single-cell expression of cluster-enriched marker genes, which correlates well with published ISH data (at Bregma +1; Allen Brain Atlas).

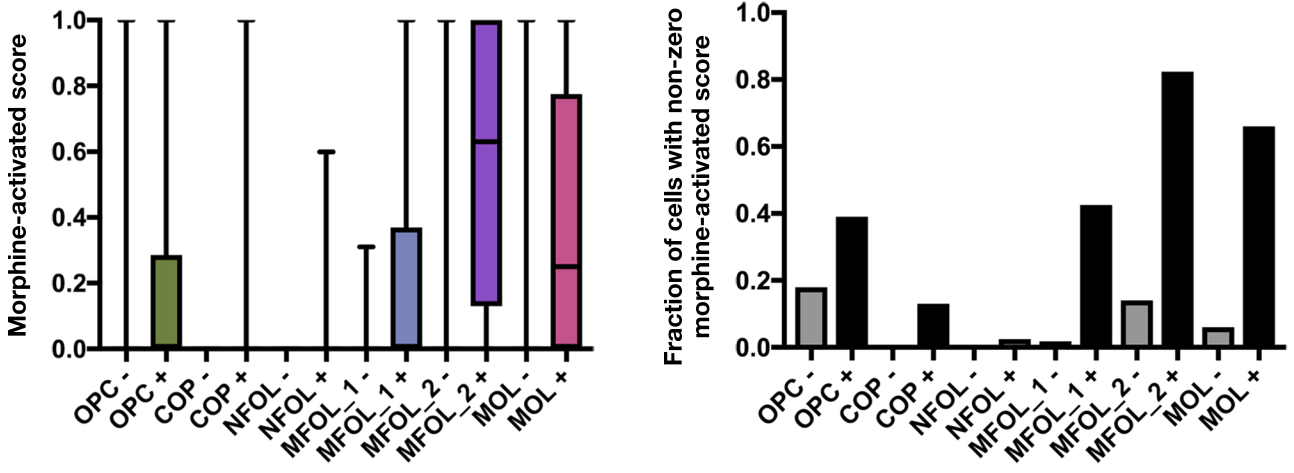

Figure S4. Morphine-activated score for oligodendrocyte subclusters, Related to Figure 4. A morphine-activated score was generated for each cell from the OL lineage (see Methods). (left panel) The distribution of scores is plotted for cells from each oligodendrocyte subcluster from mock (-) or morphine (+) treated mice. Horizantal lines, box boundaries, and error bars denote median, 25th/75th percentile, and min/max, respectively. (right panel) The fraction of cells from each cluster with a non-zero morphine-activated score is plotted. OPC: OL progenitor cell; COP: differentiation-committed OL progenitor; NFOL: newly formed OL; MFOL: myelin-forming OL; MOL: mature OL.

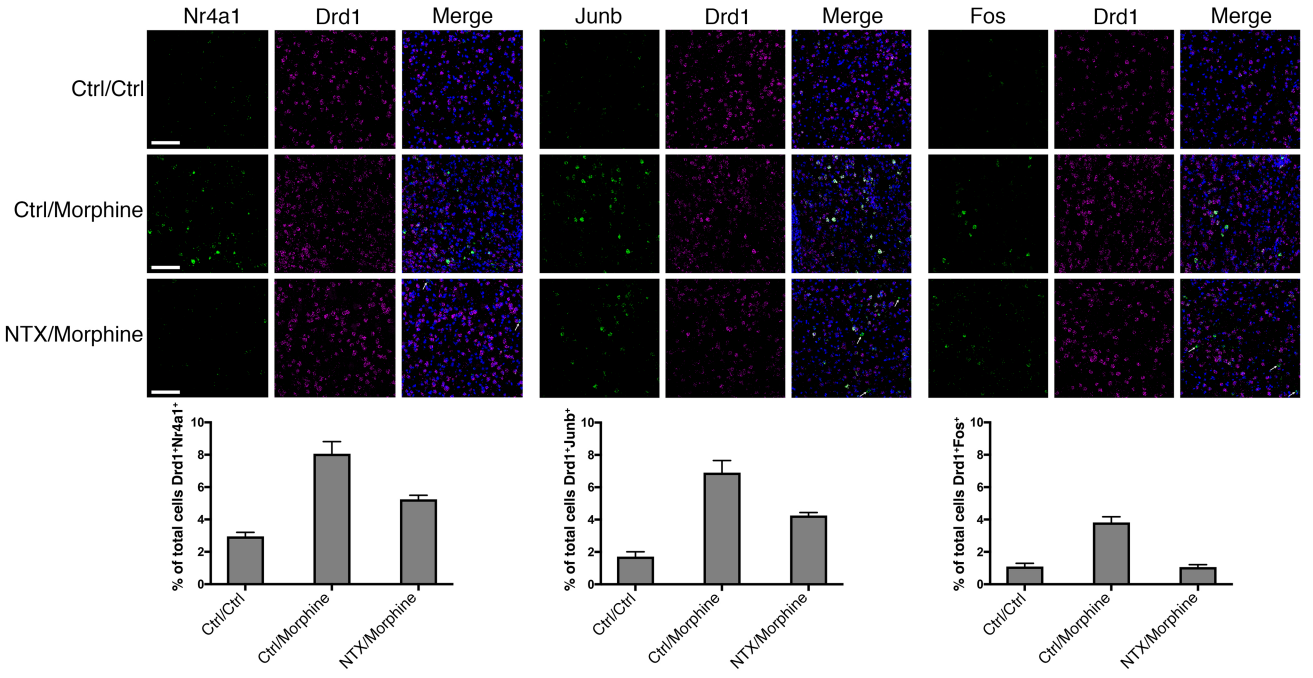

Figure S5. Morphine induces the expression of immediate-early genes (IEGs) in the nucleus accumbens in an opioid receptor-dependent manner, Related to Figure 5. Arrows in NTX/Morphine Merge panels indicate cells positive for the indicated IEG (*Nr4a1*, *Junb*, or *Fos*), but negative for *Drd1*. Quantification is shown as percentage of total cells in the nucleus accumbens that are double-positive for *Drd1* and the designated IEG. Error bars are the standard deviation among three biological replicates (n=1000 cells per probe pair per replicate). NTX: Naltrexone.

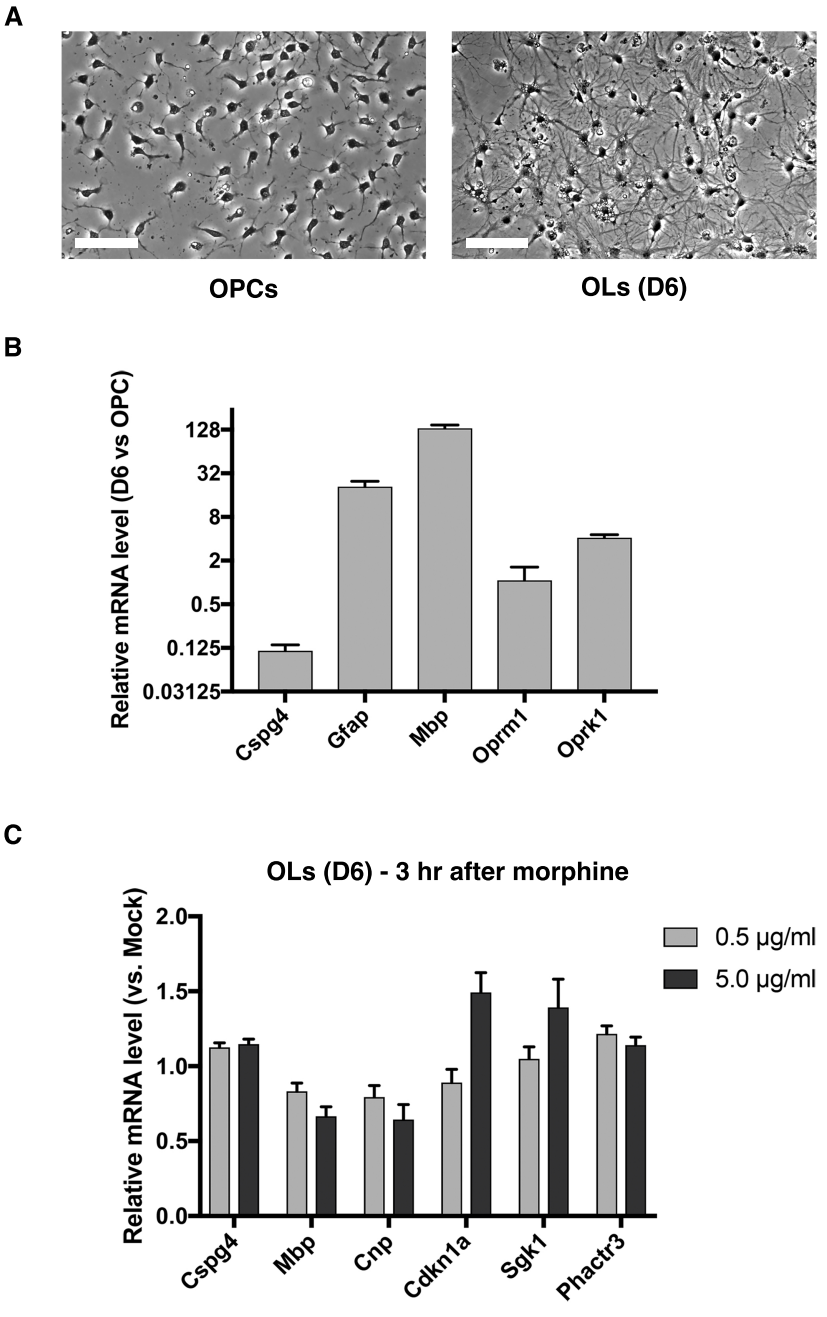

Figure S6. ***In vitro*** **OL culture failed to recapitulate morphine-dependent changes observed** ***in vivo*****, Related to Figures 4 and 5.** (**A**) Bright-field micrographs of purified OPCs (2 days after isolation and plating) and OLs (D4: 4 days of differentiation). Scale bar = 100 µm. (**B**) qRT-PCR of the indicated genes comparing relative expression between OLs (6 days of differentiation) and OPCs. (**C**) qRT-PCR of the indicated genes following a 3-hour treatment of D6 OLs with morphine. In (**B**) and (**C**), values were normalized to *Gapdh*, and error bars represent the standard deviation among three biological replicates.

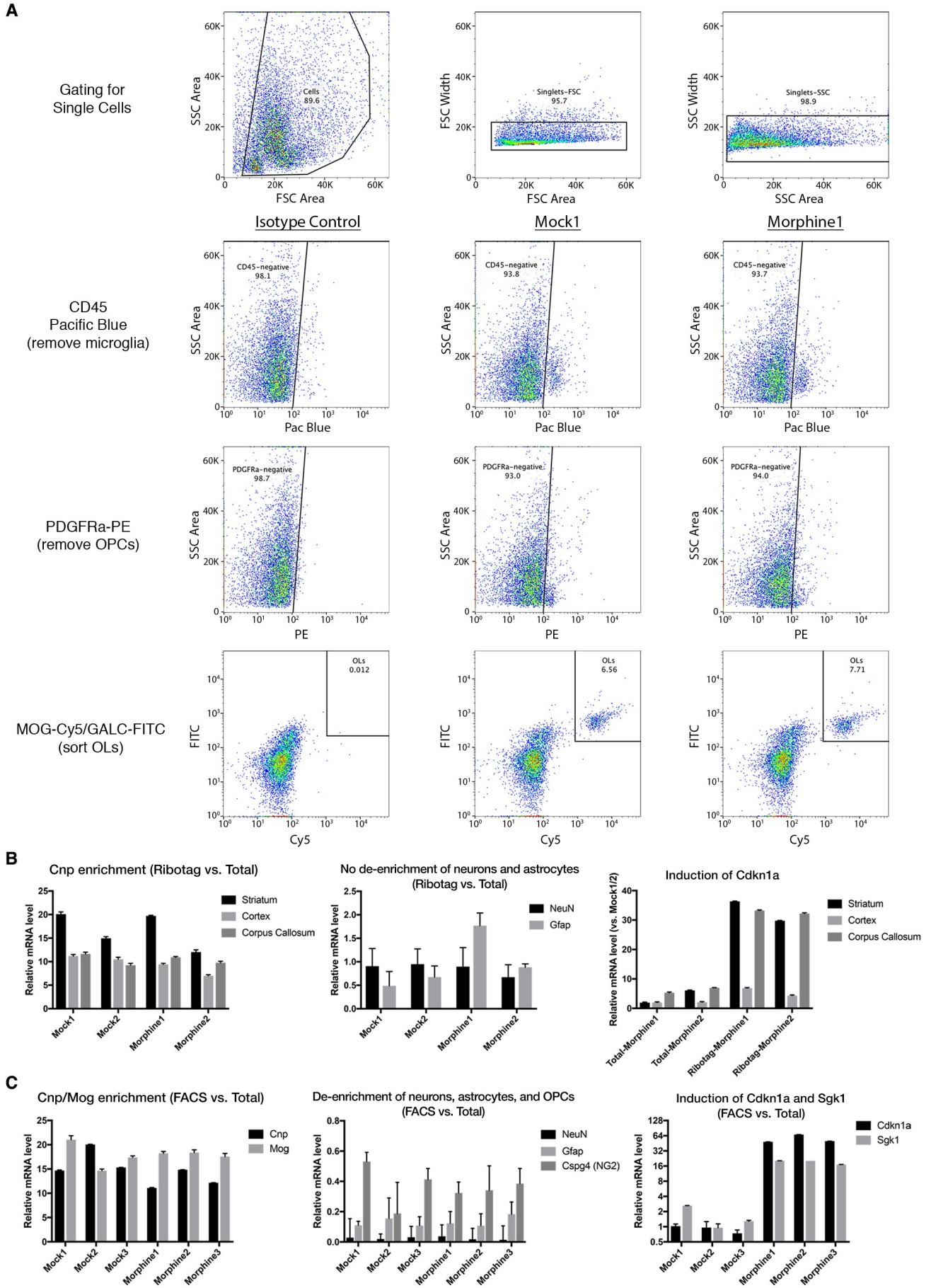

Figure S7. Representative FACS plots illustrating strategy to purify OLs, and qRT-PCR validating enrichment, Related to Figure 6. (**A**) Immunostaining and FACS were performed to obtain GALC^{+}/MOG^{+} oligodendrocytes from whole brains of adult mice (mock- or morphine-treated). (**B and C**) qRT-PCR results showing relative enrichment of OL-specific genes (left panel), de-enrichment of other cell type markers (middle panel), and expression of morphine-induced genes (right panel) using oligodendrocyte RNA isolated from (**B**) Cnp-Cre Ribotag mice or (**C**) the FACS strategy outlined in (**A**). We chose to proceed with FACS rather than Ribotag because of the dramatically improved de-enrichment of neuronal (*NeuN*) and astrocytic (*Gfap*) mRNAs. Error bars represent the SEM among nine technical replicates.

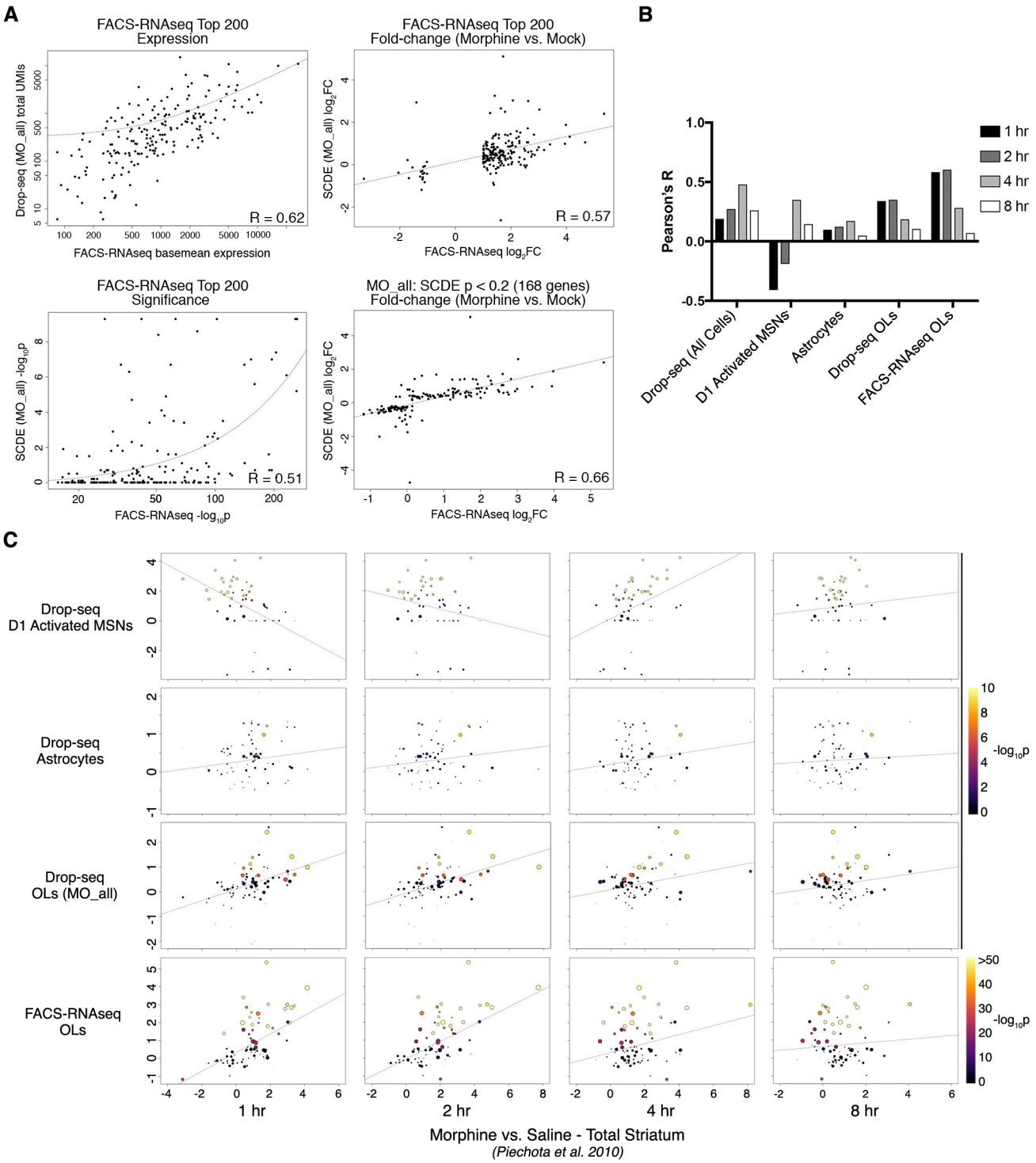

Figure S8. Correlation analyses between single-cell, FACS-RNAseq, and published microarray data, Related to Figure 6. (**A**) Log_2FC correlation plot of OL-specific morphine-regulated genes as assessed by Drop-seq/SCDE (y-axis) or FACS-RNASeq/DESeq ("Top 200"; x-axis). (**B**) Correlation values (Pearson's R) for each pair-wise comparison in (C). (**C**) Log_2FC correlation plots comparing the expression of previously identified morphine-regulated genes at various timepoints (1, 2, 4, or 8 hours; Piechota *et al.*, 2010; x-axis) with their expression in neurons (D1 Activated MSNs: Activated MSNs expressing *Drd1a*), astrocytes, or oligodendrocytes. Points are colored by $-log_{10}(p)$ and sized by their relative expression in our data (larger points denote higher expression). The different patterns observed for distinct cell types suggests temporal differences in their responsiveness to morphine exposure.

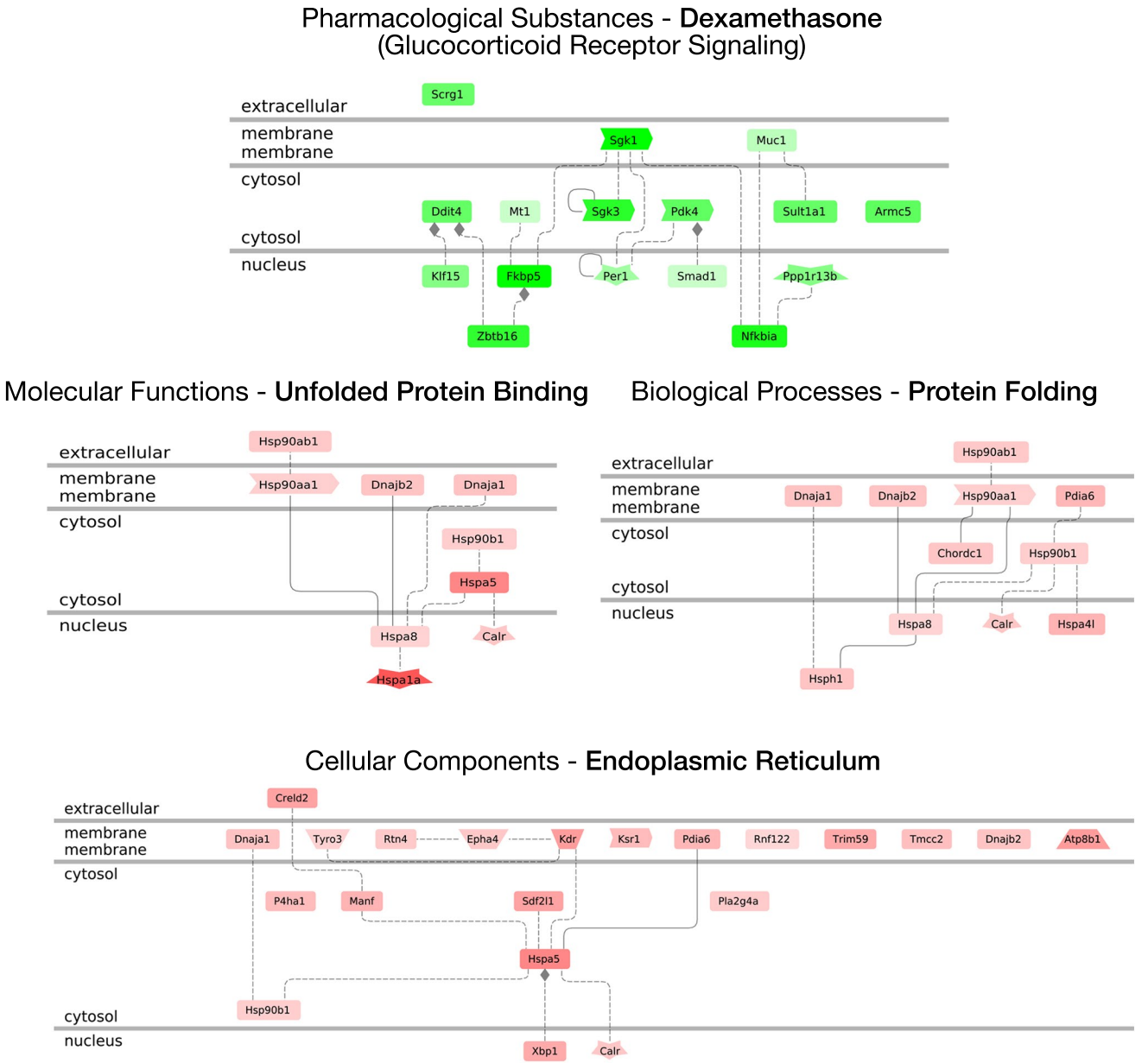

Figure S9. Summary of the most significantly enriched biological pathways and terms among morphine-regulated genes in oligodendrocytes, Related to Figures 6 and 7. These terms are significantly enriched as assessed by GeneRanker software (see also Table S3). Genes are arranged by their reported subcellular localization and interactions and colored by the magnitude of $\log_2$FC (green – upregulated by morphine; red – downregulated by morphine). Dexamethasone p = 5.32e-4; Unfolded Protein Binding p = 1.27e-9; Protein Folding p = 2.43e-9; Endoplasmic Reticulum p = 3.5e-4. Adjustped p-values for these terms are < 0.001 for all.