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Supplementary Materials for

A dopamine-induced gene expression signature regulates neuronal function and cocaine response

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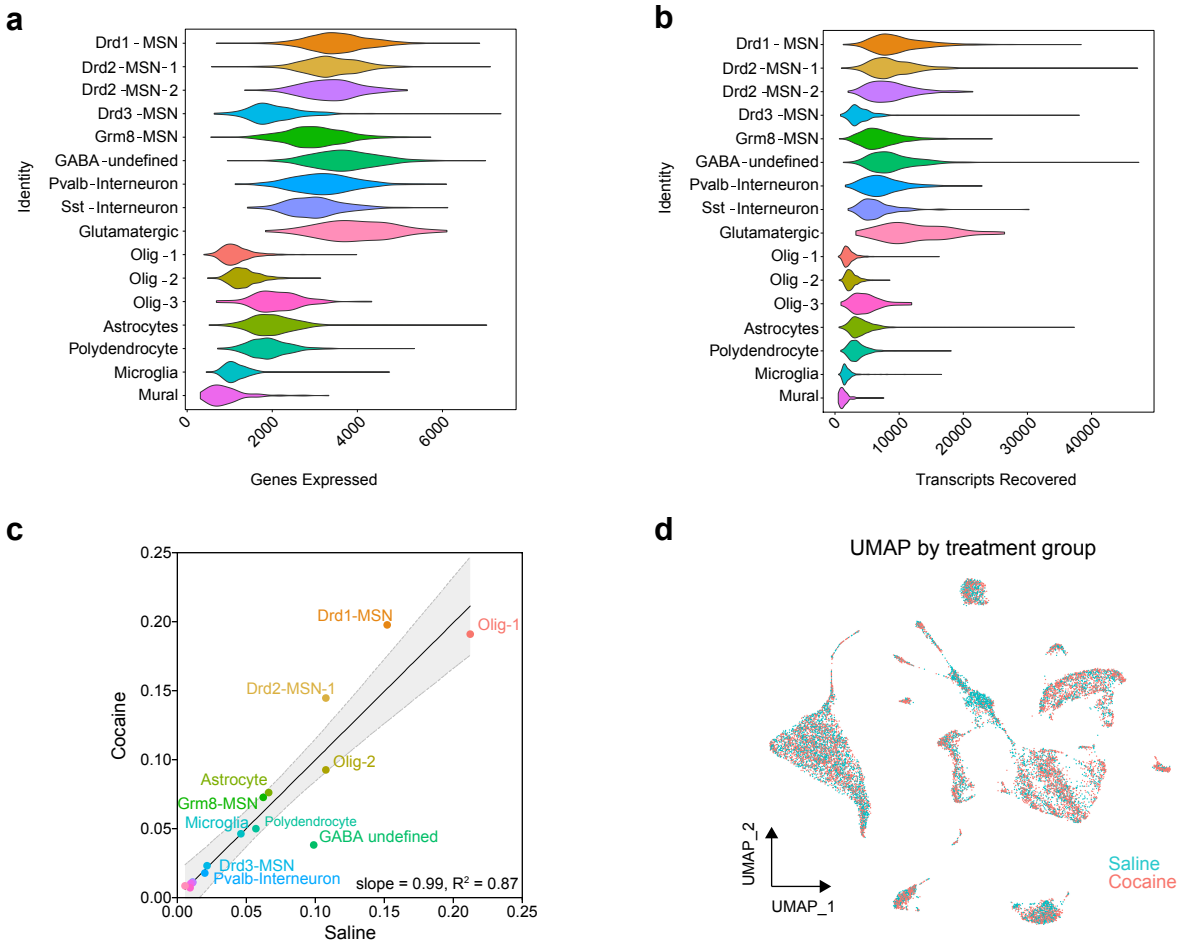
The PDF file includes:

Figs. S1 to S7

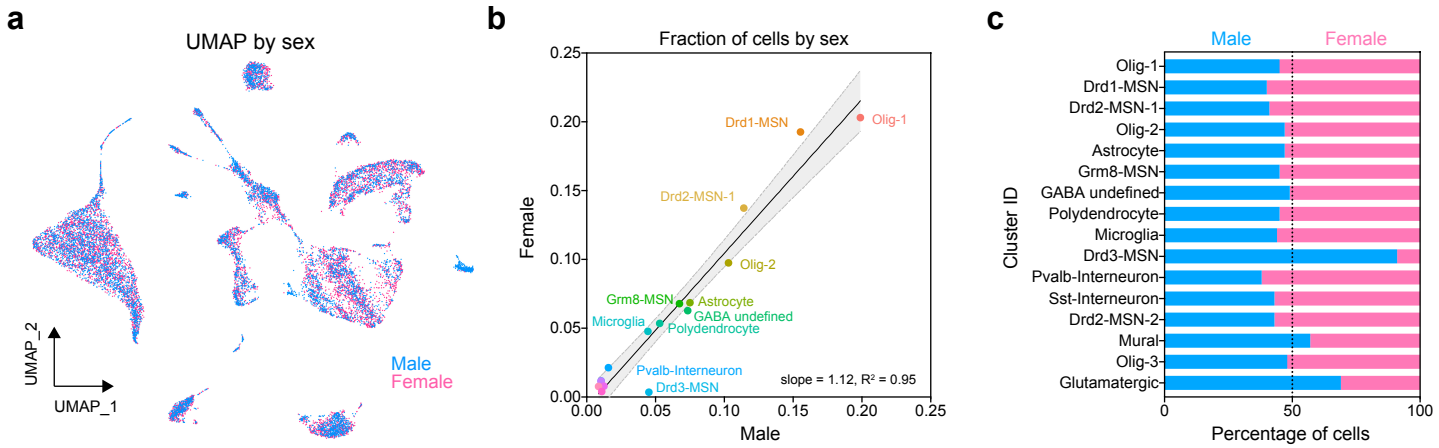
Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/26/eaba4221/DC1)

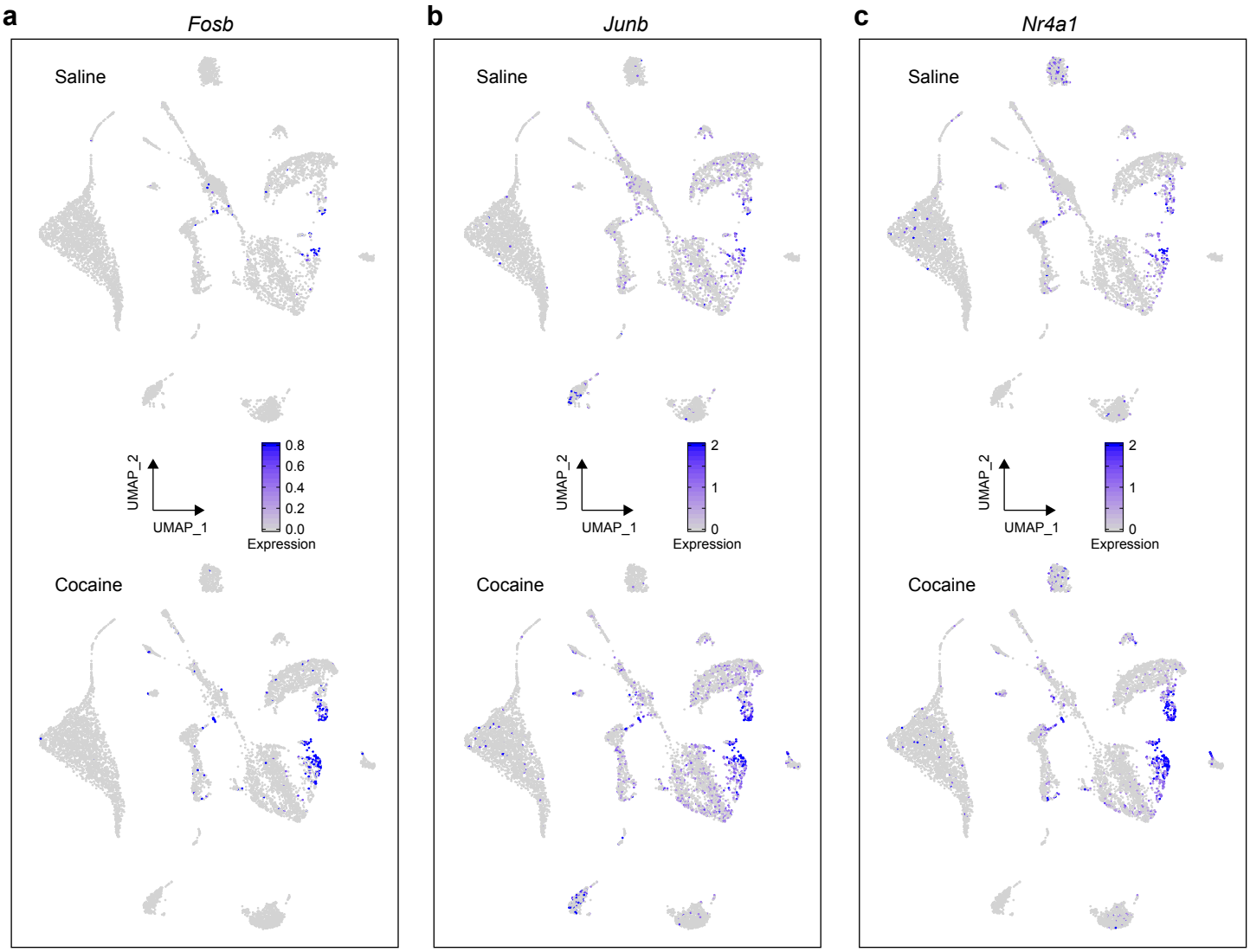
Tables S1 to S12



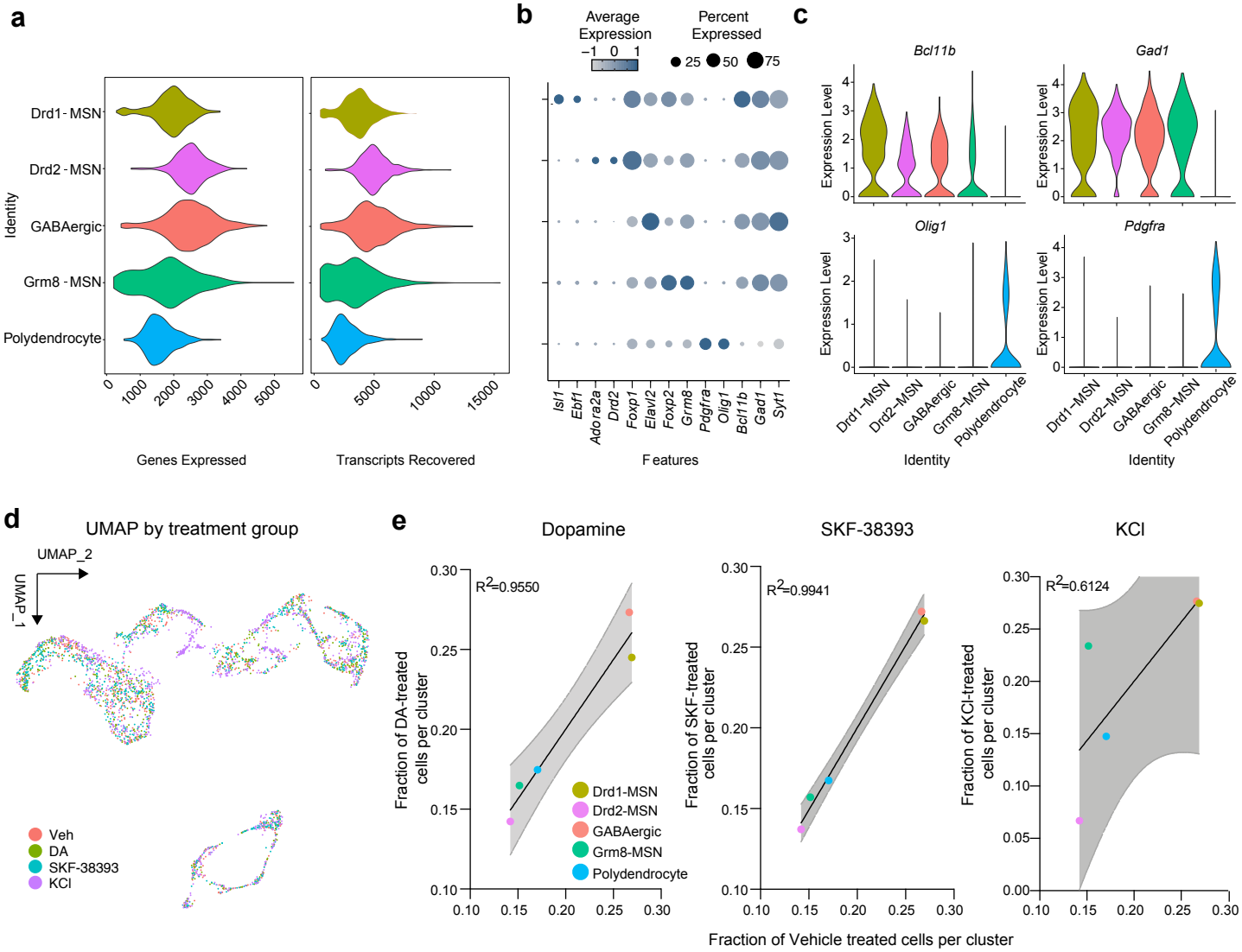
Supplementary Figure 1. Identification of cell types within the rat nucleus accumbens. **a**, Violin plots indicating the distribution of number of total genes expressed by cell class. **b**, Violin plot indicating the distribution of number of transcripts recovered by cell class. **c**, Fraction of cocaine and saline -treated neurons within each cluster. **d**, UMAP showing distribution of cells from saline- and cocaine-treated rats.



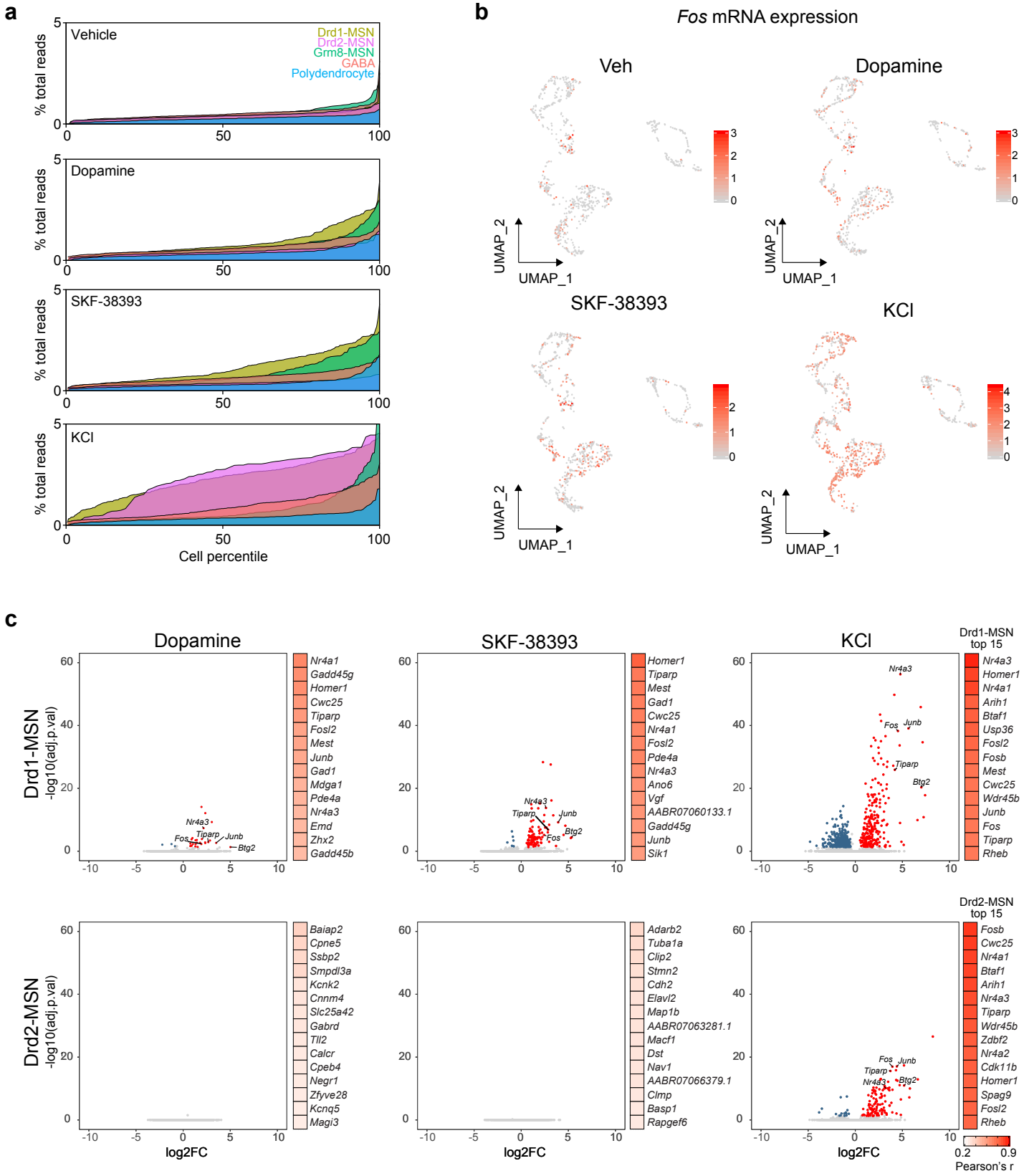
Supplementary Figure 2. **Sex differences in NAc cell distribution. a**, Global UMAP showing male and female cells following Seurat integration. **b**, Linear regression of male and female cell fractions reveals highly similar cell distribution in nearly all cell clusters, with the exception of Drd3-MSNs. **c**, Percentage of each cluster accounted for by male and female cells. Over 90% of Drd3-MSNs were obtained from male animals.



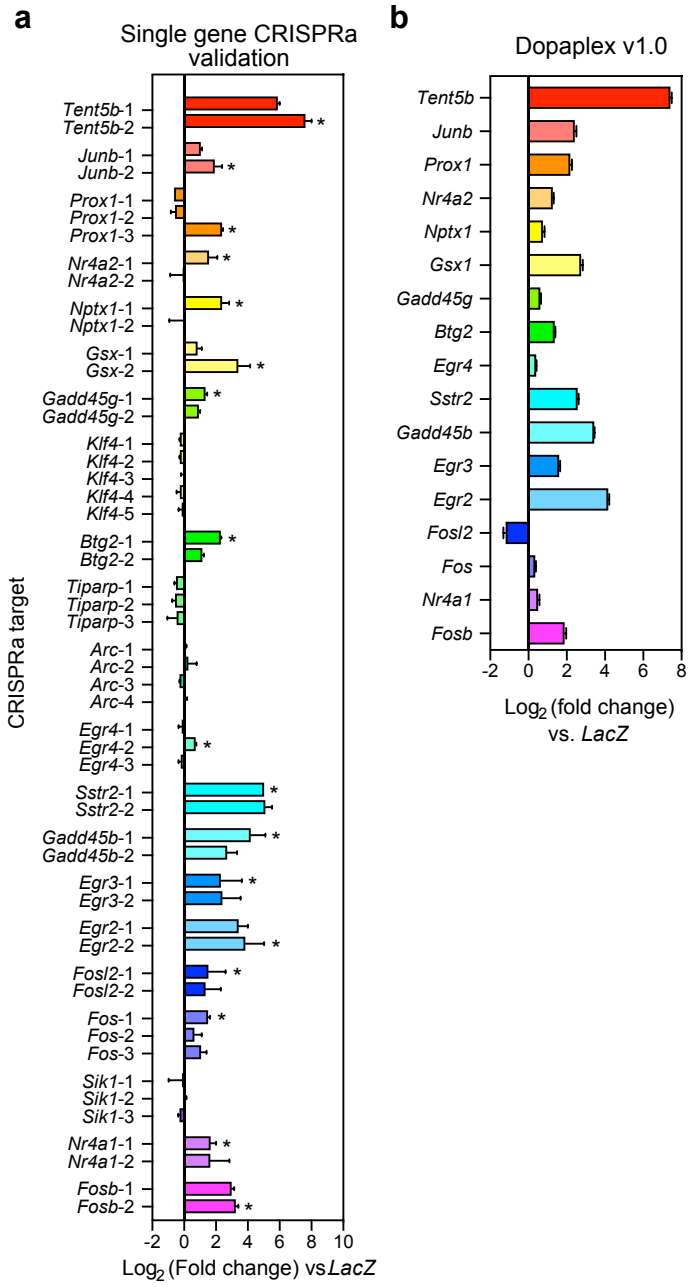
Supplementary Figure 3. **Cocaine induces immediate early gene response in Drd1-MSNs. a-c**, Global UMAPs plotting expression values of *Fosb*, *Junb*, and *Nr4a1*. For each gene, cocaine increases mRNA abundance in the Drd1-MSN population.



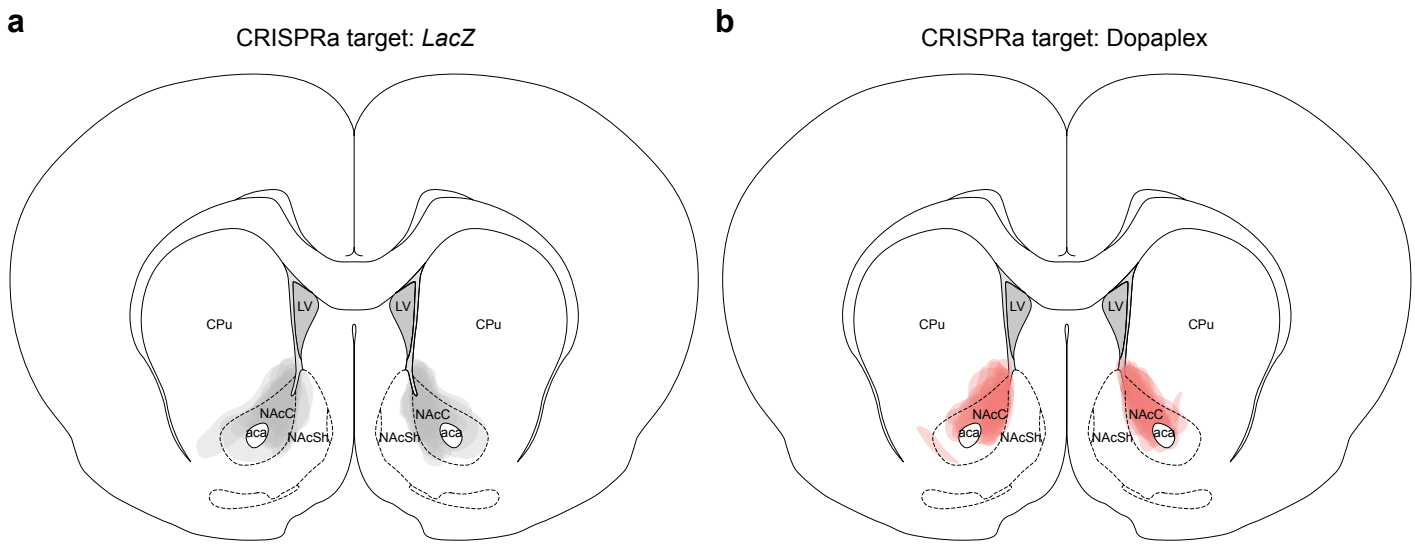
Supplementary Figure 4. Identification of cell types within rat embryonic striatal neurons. **a**, Violin plots indicating the number of genes expressed and transcripts recovered in each cluster **b**, Dot plot indicating the average expression and percent of cells expresing marker genes of each identified cell type. **c**, Violin plots indicating the distribution of neuronal (*Bcl11b*, *Gad1*) and glial (*Olig1*, *Pdgfra*) marker genes. **d**, Distribution of DA-, KCl-, SKF-treated cells. **e**, Fraction of DA-,KCl-, or SKF-treated cells within each cluster.



Supplementary Figure 5. **Treatment-induced changes in gene expression in cultured striatal neurons are cell type-specific. a**, Percent of reads aligning to 100 DA-DEGs for each cell across treatment groups from cell culture snRNA-seq experiment. Cells percentiled due to different numbers in each cluster. **b**, UMAPs of *Fos* gene expression changes across DA, SKF, and KCl treatment groups. **c**, Volcano plots showing all DEGs (adjusted *p* value < 0.05, absolute value of log2(fold change) > 0.5) and top 15 EES correlating genes for Drd1- and Drd2-MSNs. DA and SKF-38393 induce transcriptional responses in Drd1-MSNs only, while KCl induces a large-scale transcriptional response in both cell types.



Supplementary Figure 6. CRISPRa validation at single gene targets. a, Single guide RNAs targeted with dCas9-VPR to DA-induced genes results in validated induction in 16 out of 23 genes, as compared to a *LacZ* control sgRNA (*n* = 2/group). Asterisks indicate sgRNAs selected for multiplexed targeting. **b,** Lentiviral vectors expressing sgRNAs validated individually to upregulate target genes were pooled to target 17 DA-induced genes simultaneously. Simultaneous targeting resulted in induction of all but one gene (*n* = 5/group). All data are expressed as mean ± s.e.m.



Supplementary Figure 7. Viral placement validation for animals infused with CRISPRa constructs. CRISPRa lentivirus targeted to either the bacterial gene LacZ (**a**) or Dopaplex genes (**b**) were infused into the nucleus accumbens core (NAcC). Schematics of target regions illustrate viral spread, as determined from mCherry signal expressed by sgRNA array vector. NAcC: nucleus accumbens core; NAcSh: nucleus accumbens shell; CPu: caudate putamen; LV: lateral ventricle.