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# Supplementary Materials for

## Cell type–specific epigenetic priming of gene expression in nucleus accumbens by cocaine

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## SUPPLEMENTARY MATERIALS

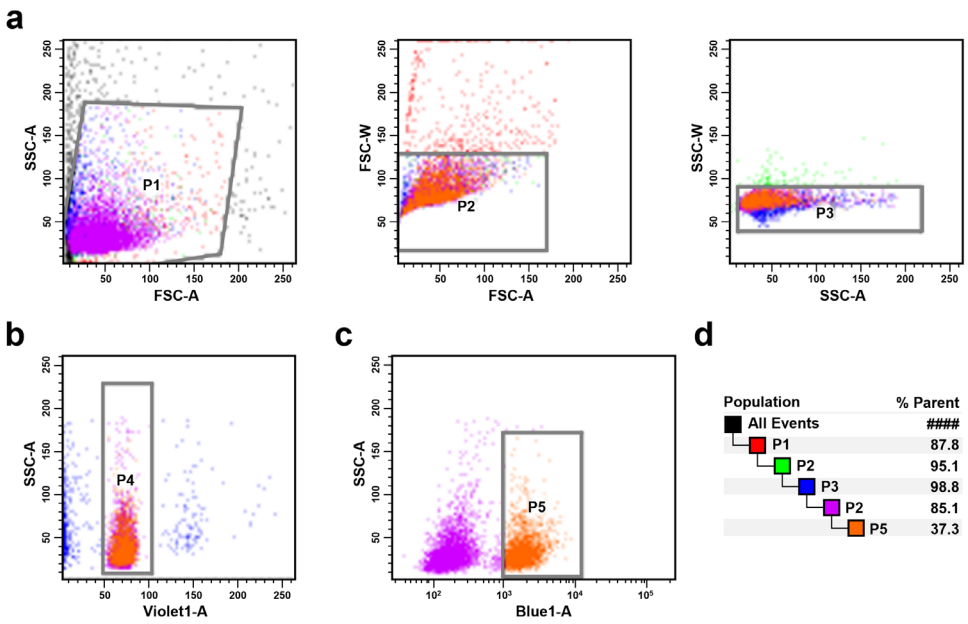


**Figure S1.** Fluorescence-activated nuclei sorting (FANS) to purify D1 and D2 MSN nuclei from NAc. Representative gating strategy for FANS. **(a)** First three gates on FSC-A vs. SSC-A (P1), FSC-A vs. FSC-W (P2), and SSC-A vs SSC-W (P3) retrieve nuclei as opposed to debris. **(b)** Gating strategy on Violet1-A vs. SSC-A (P4) retrieves single nuclei (DAPI+ events) as opposed to duplets. **(c)** The final gate FSC-A vs. Blue1-A (FITC channel) separates transgenically labeled nuclei (P5, GFP+ events) from wild-type nuclei. **(d)** Summary table of representative hierarchical gating strategy.

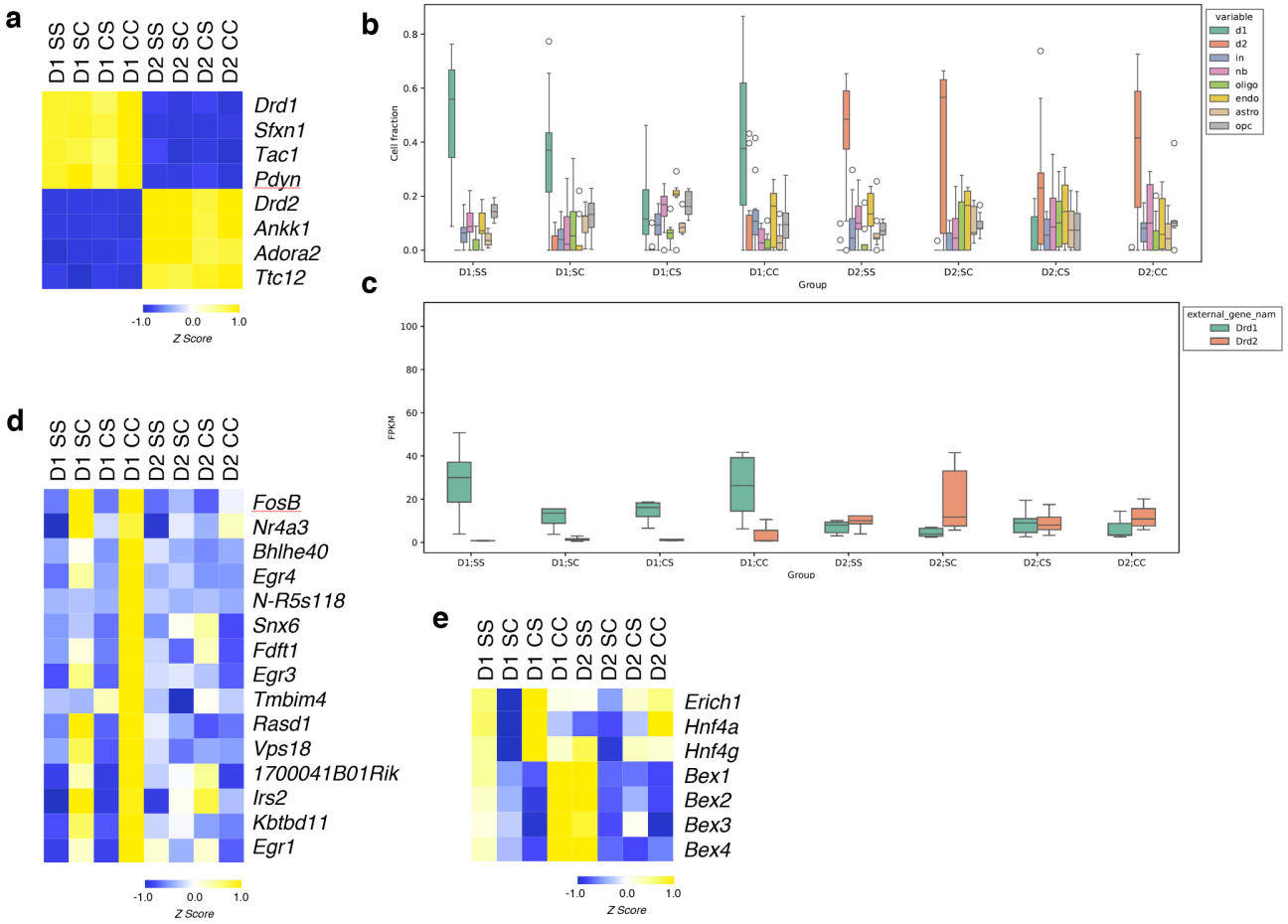


**Figure S2. (a)** Heatmap of mRNA expression (cpm) of well-characterized genes specific to D1 MSNs (*Drd1, Sfcn1, Tac1, Pdyn*) or to D2 MSNs (*Drd2, Ankk1, Adora2, Ttc12*), confirming successful sorting and purification of D1 and D2 MSN nuclei. **(b)** To evaluate the purity of the FACS sorted cells from the perspective of the RNA-seq libraries, the cell type components of the RNA-seq samples were estimated using a deconvolution approach using Unicell. The sorted D1 RNA-seq samples exhibit a high predicted D1 cell type proportion, whereas the sorted D2 RNA-seq samples exhibit a high predicted D2 cell type proportion. **(c)** When examining the Drd1 and Drd2 expression of the sorted libraries, the sorted D1 cells show a vastly higher expression of Drd1 compared to Drd2. Conversely, the sorted D2 cells show higher overall expression of Drd2 compared to Drd1. **(d)** mRNA expression heatmap of top genes upregulated in D1 MSNs with cocaine challenge in withdrawal animals (D1 CC), indicating priming of cocaine-responsive genes when compared to acute cocaine (D1 SC). **(e)** mRNA expression heatmap of genes previously shown to be upregulated upon withdrawal from cocaine (*Erich1, Hnf4a, Hnf4g*) or depressed with cocaine or alcohol addiction in humans (Bex gene family, *Bex1-4*) (*62, 63*).

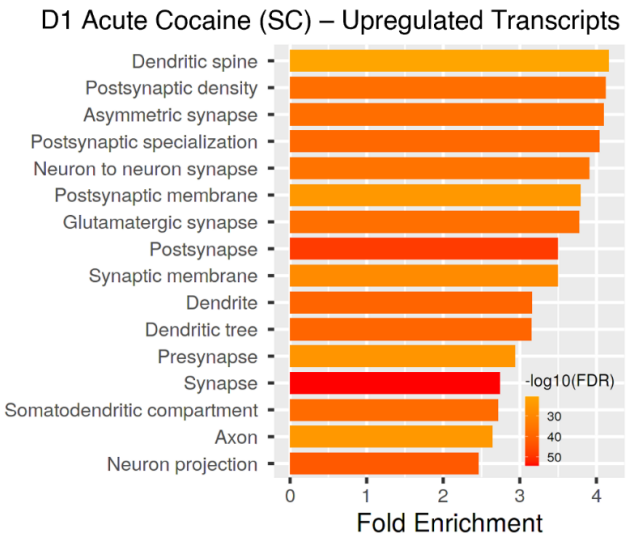


**Figure S3.** Gene ontology (GO) enrichment of genes that become upregulated with acute cocaine in D1 MSNs using ShinyGO 0.75 (2871 upregulated genes shown in top panel Fig. 1C).

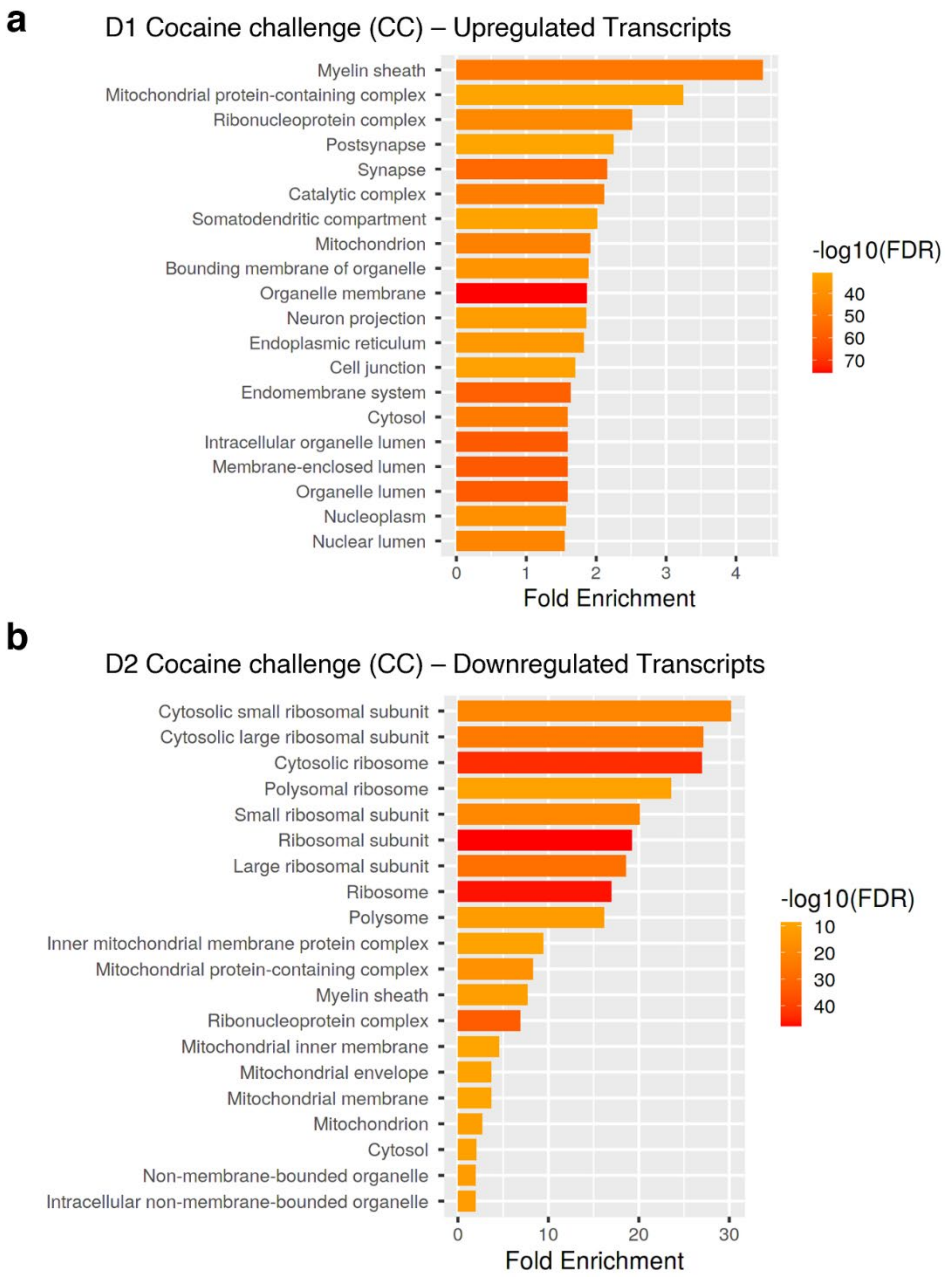


**Figure S4.** GO term enrichment analysis shows opposite gene regulation in D1 vs. D2 MSNs of NAc with an acute cocaine challenge after prolonged withdrawal from chronic cocaine. **(a)** GO term enrichment of genes that are upregulated in D1 MSNs with cocaine challenge after withdrawal from chronic cocaine using ShinyGO 0.75 (4704 genes shown in lower right panel Fig. 1C). **(b)** GO term enrichment of genes that are downregulated in D2 MSNs with cocaine challenge after withdrawal from chronic cocaine using ShinyGO 0.75 (583 genes shown in lower right panel Fig. 1C).

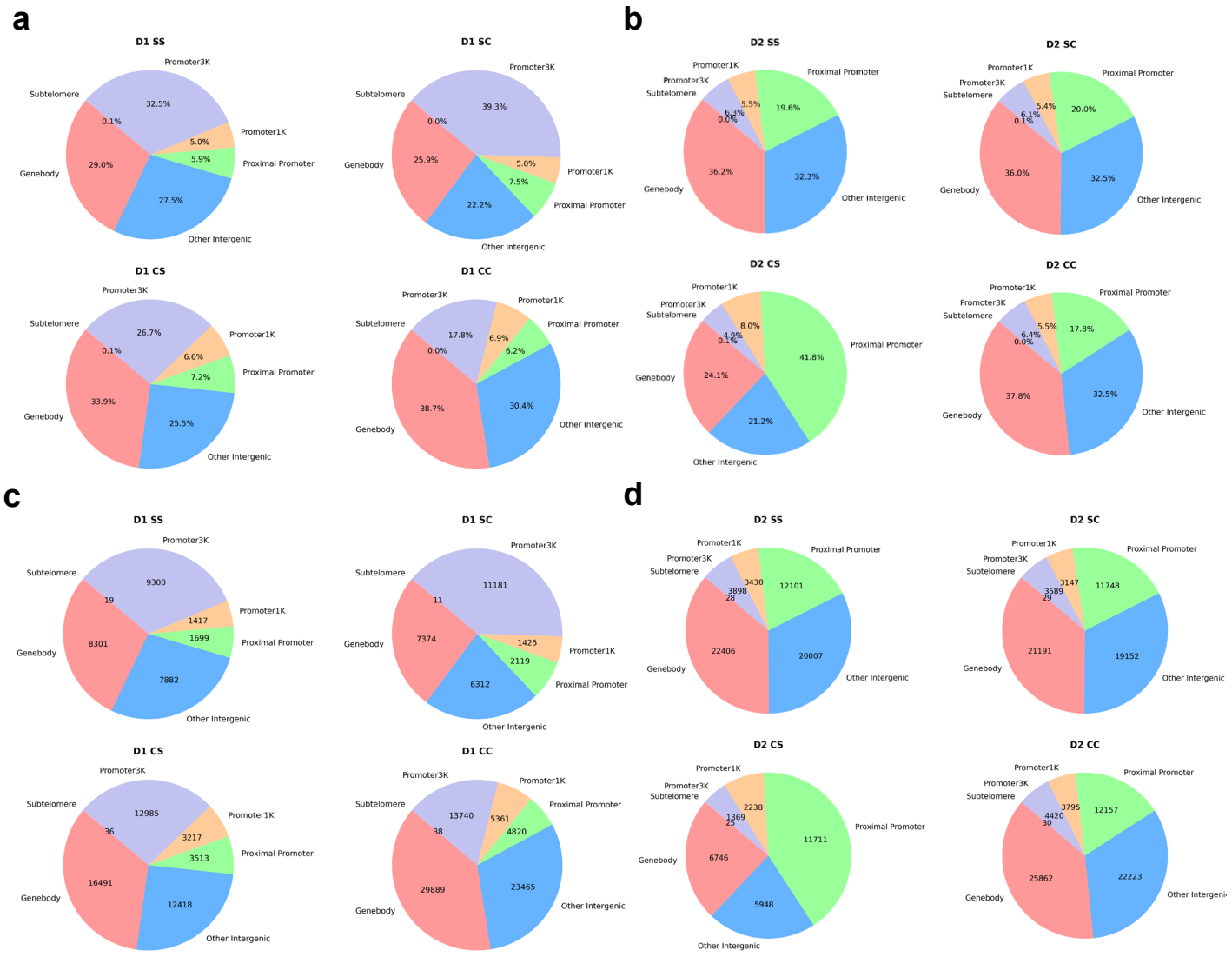


**Figure S5.** Detailed annotation of ATAC-seq peaks across different conditions as illustrated in pie charts that depict: **(a)** relative D1 MSN and **(b)** relative D2 MSN peak distribution across several genomic features, including proximal promoters, longer promoter regions, gene body regions, subtelomeres, and intergenic regions. The respective peak counts across different conditions are shown in **(c)** for D1 MSNs and **(d)** for D2 MSNs.

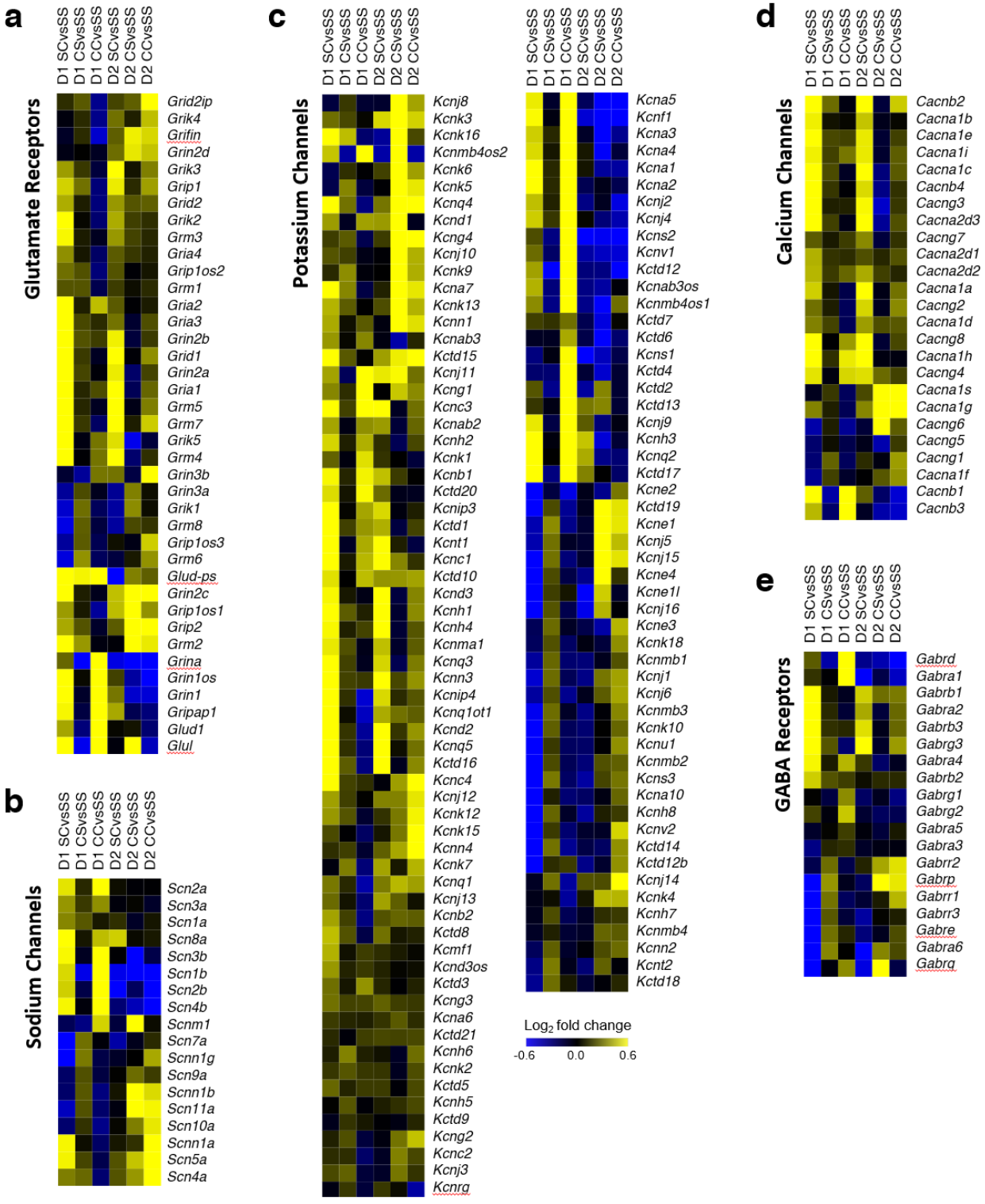


**Figure S6.** Excitability- and plasticity-related genes exhibit cell-type-specific regulation by cocaine. Heatmaps show mRNA gene expression of key ionotropic receptor and ion channel subunits upon acute cocaine (SC), prolonged cocaine withdrawal (CS), and cocaine challenge after withdrawal (CC), in both D1 and D2 MSNs of NAc. These plasticity-related transcripts include subunits for **(a)** glutamate receptors, **(b)** sodium channels, **(c)** potassium channels, **(d)** calcium channels, and **(e)** GABA receptors.

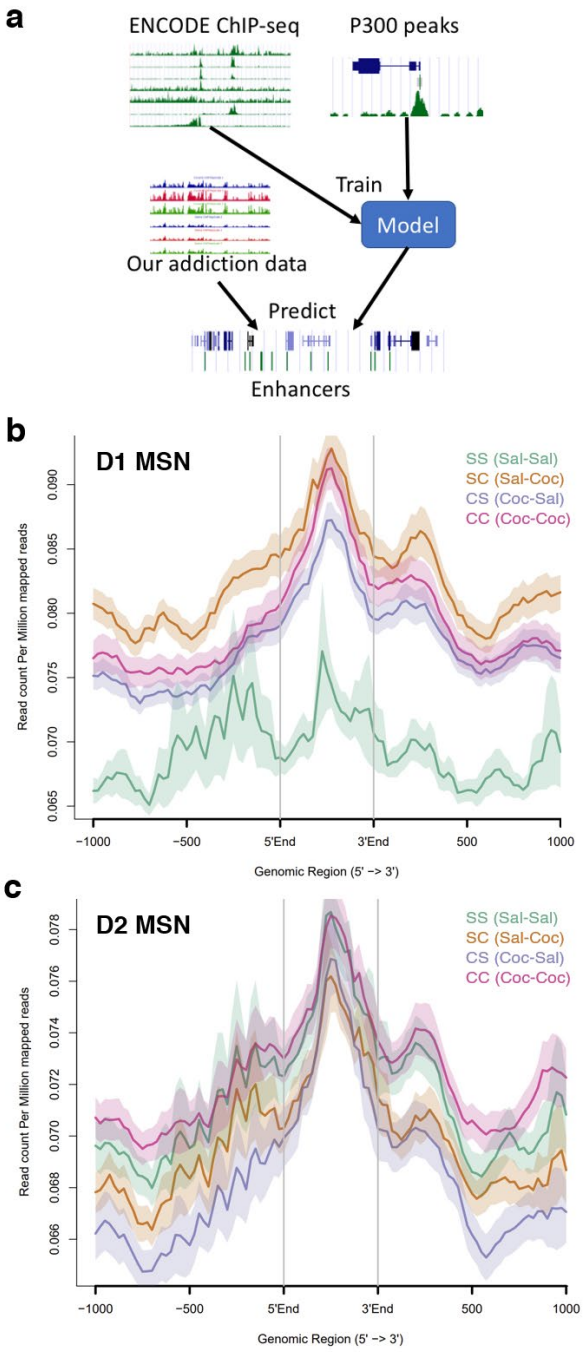


**Figure S7.** Identification of cocaine-regulated enhancers shows D1-specific accessibility changes. **(a)** Machine learning model, DeepRegFinder (*44*), identifies putative cocaine-regulated enhancer regions. DeepRegFinder utilizes 1-D convolutional and recurrent neural networks to extract features that represent characteristic chromatin modification patterns of representative enhancers (defined by P300) from ENCODE ChIP-seq data. The trained model was then used to identify enhancers on the whole genome. We used ChIP-seq data from a previous cocaine study (*18*) to build a model to identify 7,796 cocaine-related enhancers in NAc for this analysis. Accessibility of these 7,796 putative enhancers shown for **(b)** D1 MSNs and **(c)** D2 MSNs in saline control animals (SS), after acute cocaine in drug-naïve mice (SC), after prolonged withdrawal from chronic cocaine (CS), and upon cocaine challenge in withdrawal animals (CC).

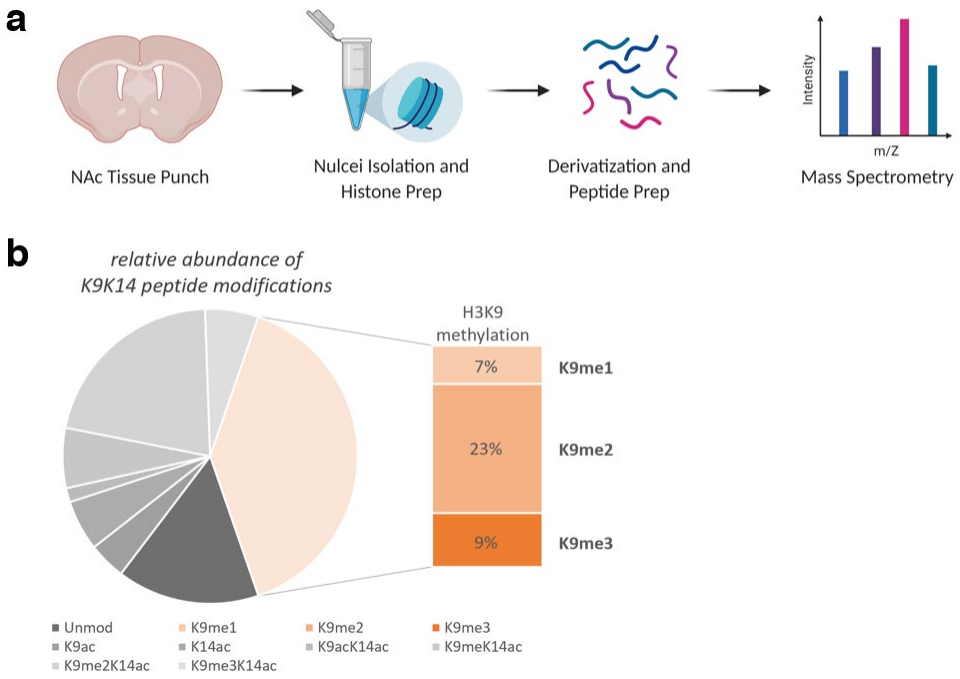


**Figure S8.** Mass spectrometry reveals changes in chromatin in NAc. **(a)** Experimental outline for histone mass spectrometry starting from NAc tissue punches, which is the starting material for nuclei isolation and histone protein extraction. The prepared histones are first derivatized using propionic anhydride to neutralize charge and block unmodified and monomethylated lysine residues, and are subsequently digested using trypsin, which, under these conditions, cleaves only arginine residues. The generated peptides are then analyzed using online LC-electrospray ionization-tandem mass spectrometry to identify modification sites. **(b)** Relative abundance of H3 lysine 9 (H3K9) and lysine 14 (H3K14) acetylation and methylation (me1/2/3 – mono/di/tri-methylation).

**a**

D1 CC-specific upregulated genes

| GO Term | P-value |
|---|---|
| Regulation of synaptic plasticity | 1.43E-12 |
| Anterograde trans-synaptic signaling | 2.26E-10 |
| Chemical synaptic transmission | 2.26E-10 |
| Trans-synaptic signaling | 3.56E-10 |
| Neuron projection morphogenesis | 1.51E-08 |
| Neuron projection development | 3.11E-08 |

**b**

D1 CC-specific upregulated genes

| Upstream TF Motif | P-value |
|---|---|
| CREB1 | 3.62E-05 |
| CTCF | 1.53E-03 |
| CEBPB | 3.14E-03 |
| EGR1 | 4.27E-03 |
| NFIC | 6.78E-03 |

**Figure S9. (a)** GO term enrichment analysis of gene sets only induced with cocaine challenge after withdrawal from chronic cocaine, and not with acute cocaine in drug-naïve mice. **(b)** Predicted upstream transcription factors of gene sets only induced with cocaine challenge in withdrawal, and not with acute cocaine in drug-naïve mice.

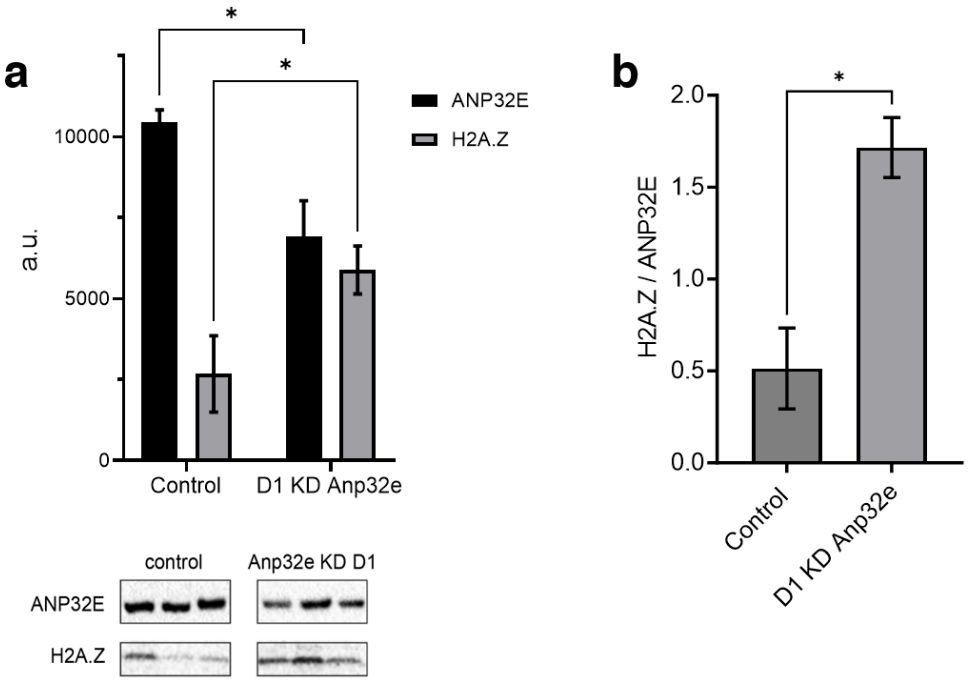


**Figure S10. (a)** Western blot revealed that ANP32E knockdown (KD) in D1 MSNs prevents H2A.Z depletion in the NAc after chronic cocaine exposure (6 days of cocaine following D1 MSN ANP32E KD using Cre-dependent AAV1-siAnp32e delivered to the NAc). Note that all six animals shown in this figure received cocaine treatment (2way ANOVA Interaction $p$ =0.0058; control - Anp32e D1 KD: ANP32E $p$ = 0.049, H2A.Z $p$ = 0.049, Holm-Šídák's multiple comparisons test). **(b)** Plotted is the relative abundance H2A.Z in control and D1 ANP32E KD animals normalized to ANP32E ($p$ = 0.012 unpaired T test). Data are mean ± s.e.m.

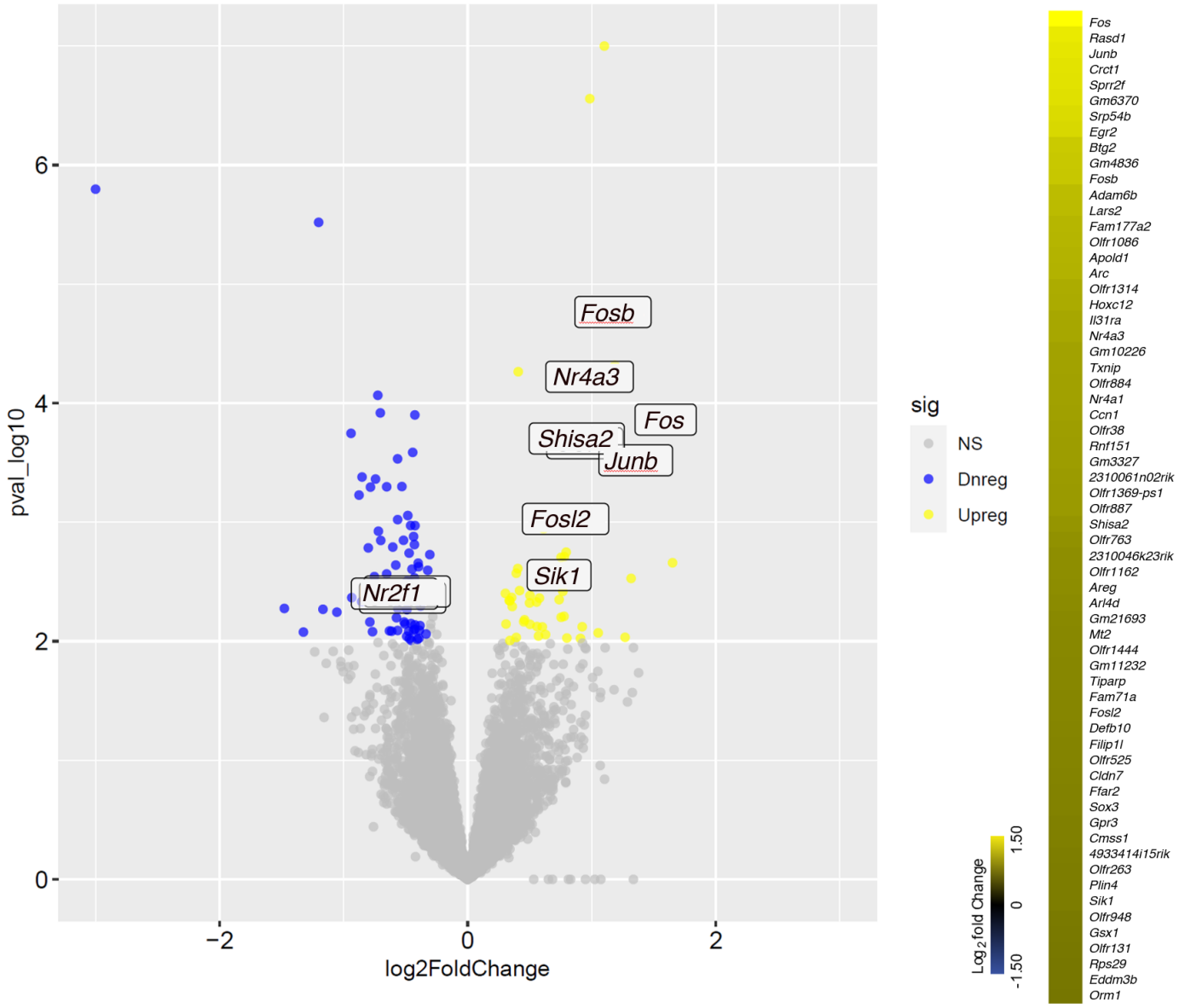


**Figure S11.** Volcano plot shows protein-coding transcripts that become upregulated (Upreg, yellow) or downregulated (Dnreg, blue) in the NAc upon cocaine challenge injection following prolonged withdrawal from chronic cocaine (CC; whole NAc tissue analyzed for RNAseq). The top genes induced by cocaine challenge encompass well-known immediate early genes (IEGs) such as *Fos, Junb, FosB, Arc*, and *Nr4a*, that have been previously implicated in neural plasticity linked to cocaine action in this brain region.

**a**

D1 cocaine challenge (CC) upregulated transcripts | **Network master regulators**

| Network Master Regulator | Type | Predicted Activation | Activation z-score | p-value of overlap |
|---|---|---|---|---|
| **BDNF** | growth factor | Activated | 5.009 | 2.12E-20 |
| **EGF** | growth factor | Activated | 8.598 | 1.85E-19 |
| **CAMK1** | kinase | Activated | 6.591 | 4.02E-19 |
| **RASA2** | other | - | -1.584 | 6.14E-19 |
| **EGF** | growth factor | Activated | 8.391 | 6.91E-19 |
| **CREB1** | trxn regulator | - | 1.279 | 1.15E-18 |
| **ROR2** | kinase | Activated | 6.891 | 3.89E-13 |

**b**

D1 cocaine challenge (CC) upregulated transcripts | **Upstream chemical regulators**

| Upstream Regulator | Molecule Type | Predicted Activation | Activation z-score | p-value of overlap |
|---|---|---|---|---|
| **levodopa** | chemical - endogenous | Activated | 6.624 | 6.17E-18 |
| **cocaine** | chemical drug | Activated | 4.539 | 1.14E-11 |
| **haloperidol** | chemical drug | Activated | 2.645 | 2.1E-10 |
| **kainic acid** | chemical toxicant | Activated | 3.66 | 3.25E-09 |
| **topotecan** | chemical drug | Inhibited | -3.534 | 9.8E-09 |

**Table S1.** Upstream regulators of cocaine-induced gene programs in D1 MSNs of NAc. Network master regulators **(a)** and upstream chemical regulators **(b)** of gene programs that are induced by acute cocaine in D1 MSNs, as revealed by IPA (2871 upregulated genes shown in top panel Fig. 1C).

**a**

| GO Enrichment \| Genes with increased accessibility in withdrawal (D1 MSNs) | P-value | FDR |
|---|---|---|
| regulation of synaptic transmission, glutamatergic | 4.06E-07 | 2.39E-03 |
| calcium ion regulated exocytosis | 5.83E-07 | 1.71E-03 |
| postsynaptic specialization organization | 7.70E-07 | 1.51E-03 |
| postsynaptic density organization | 7.70E-07 | 1.13E-03 |
| postsynaptic density assembly | 7.70E-07 | 9.05E-04 |
| postsynaptic specialization assembly | 7.70E-07 | 7.54E-04 |
| regulation of AMPA receptor activity | 7.70E-07 | 6.46E-04 |
| regulated exocytosis | 3.85E-06 | 2.51E-03 |
| calcium ion transport | 5.08E-06 | 2.98E-03 |

**b**

| TF name | D1SCvsSS_pvalue | TF name | D1CSvsSS_pvalue | TF name | D1CCvsSS_pvalue |
|---|---|---|---|---|---|
| TCFL5 | 4.63002E-160 | ZNF148 | 5.65836E-172 | CTCF | 1.49896E-169 |
| KLF15 | 8.80881E-190 | ZNF740 | 4.34876E-159 | EGR2 | 3.80405E-147 |
| ZBTB14 | 1.00306E-167 | KLF15 | 3.15907E-162 | KLF15 | 2.23885E-167 |
| NRF1 | 4.43142E-165 | CTCFL | 2.74017E-150 | ZBTB14 | 1.31038E-152 |
| SP9 | 3.86183E-176 | MAZ | 1.57747E-158 | TCFL5 | 1.07547E-136 |
| HINFP | 1.63945E-142 | KLF5 | 3.98330E-172 | EGR3 | 3.70092E-159 |
| TFDP1 | 7.38029E-158 | KLF4 | 1.72389E-173 | NRF1 | 5.69842E-149 |
| SP3 | 1.57084E-178 | SP9 | 5.51040E-162 | CTCFL | 4.06985E-157 |
| HES1 | 6.06382E-142 | KLF2 | 4.64131E-159 | EGR1 | 8.29278E-170 |
| ZNF148 | 2.57292E-181 | KLF16 | 1.63022E-165 | KLF11 | 1.42953E-162 |
| SP4 | 1.33636E-165 | EGR1 | 3.78524E-157 | KLF14 | 1.25149E-153 |
| CTCFL | 5.48915E-161 | SP3 | 1.03533E-158 | SP3 | 6.82874E-169 |
| KLF2 | 3.02082E-170 | Plagl1 | 6.70764E-142 | FOSL2::JUN | 6.36510E-151 |
| KLF14 | 1.06983E-162 | PLAGL2 | 2.91769E-120 | KLF6 | 6.44372E-151 |
| KLF3 | 8.06021E-159 | VEZF1 | 1.24086E-132 | KLF3 | 7.61597E-152 |
| SP1 | 7.30706E-170 | ZIC5 | 1.76407E-140 | FOS::JUND | 1.29246E-156 |
| EGR1 | 4.65410E-164 | ZIC3 | 1.78197E-136 | SP4 | 8.24260E-154 |
| EGR3 | 1.18135E-154 | BHLHE22 | 6.60473E-139 | SP9 | 1.03807E-160 |
| YY2 | 2.30903E-138 | PLAG1 | 7.61472E-143 | FOS::JUNB | 7.95603E-148 |
| KLF5 | 3.48142E-177 | ZBTB14 | 7.01988E-143 | HES1 | 1.30693E-129 |
| KLF6 | 1.60169E-160 | KLF11 | 2.86021E-155 | SP1 | 7.33044E-154 |
| Zfx | 1.03906E-155 | SP8 | 4.97788E-158 | ZIC5 | 6.19579E-140 |
| ZIC5 | 1.83773E-147 | TFAP2A | 2.80698E-131 | FOSL2::JUND | 5.76016E-147 |
| KLF11 | 1.03618E-168 | TFAP2C | 2.07045E-132 | FOSB::JUNB | 5.57231E-147 |
| KLF16 | 7.15986E-172 | EGR3 | 3.89172E-143 | FOS::JUN | 5.99997E-152 |

**Table S2. (a)** GO term enrichment analysis of genes that show increased accessibility in D1 MSNs after cocaine withdrawal (CS)**. (b)** TF footprinting analysis using TOBIAS implicates previously known TF dynamics in the response to cocaine exposure, including the AP-1 TF family members FOS, FOSB, JUN, and JUNB.

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