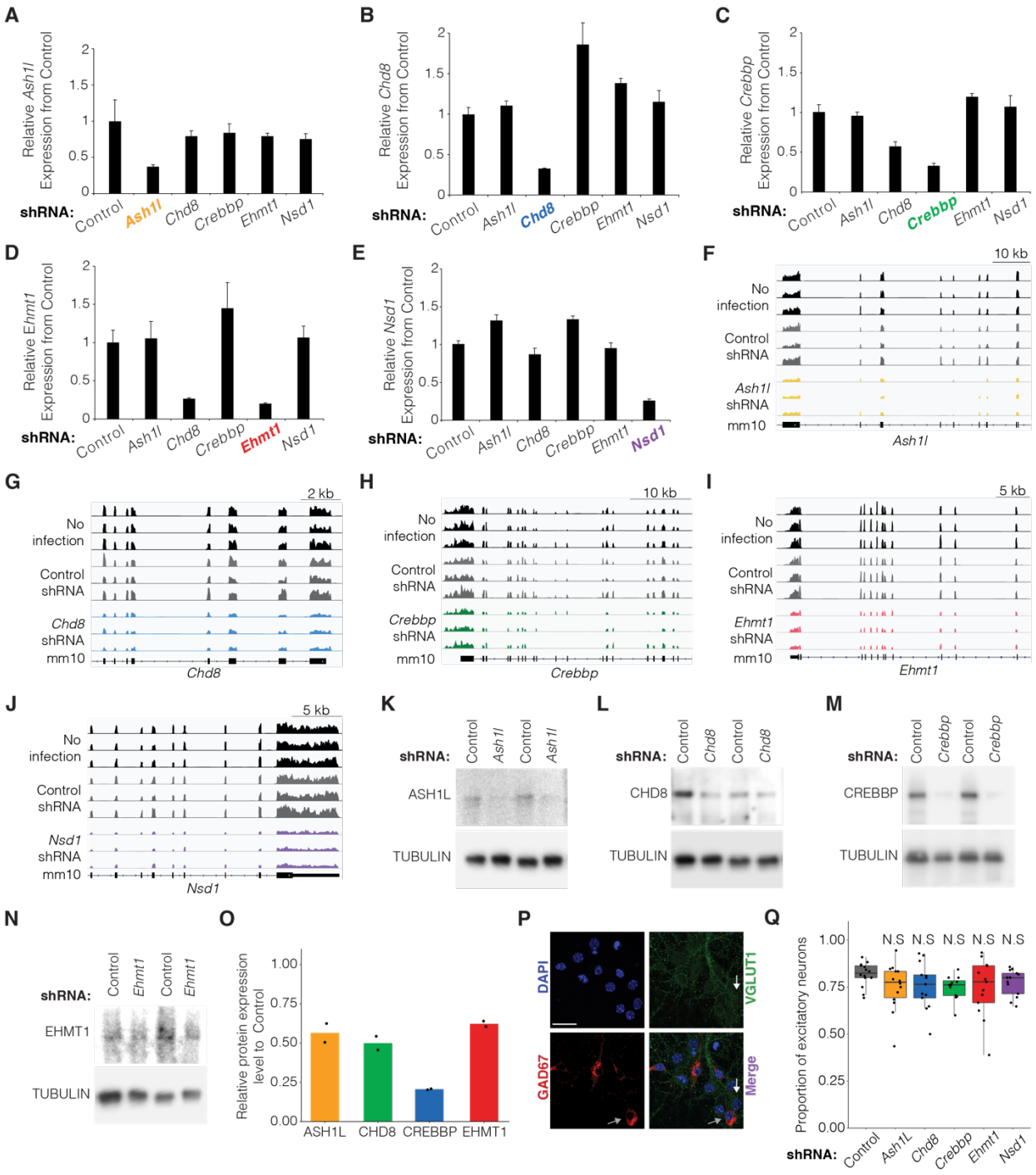
Supplemental Data

Supplemental Figures 1-14

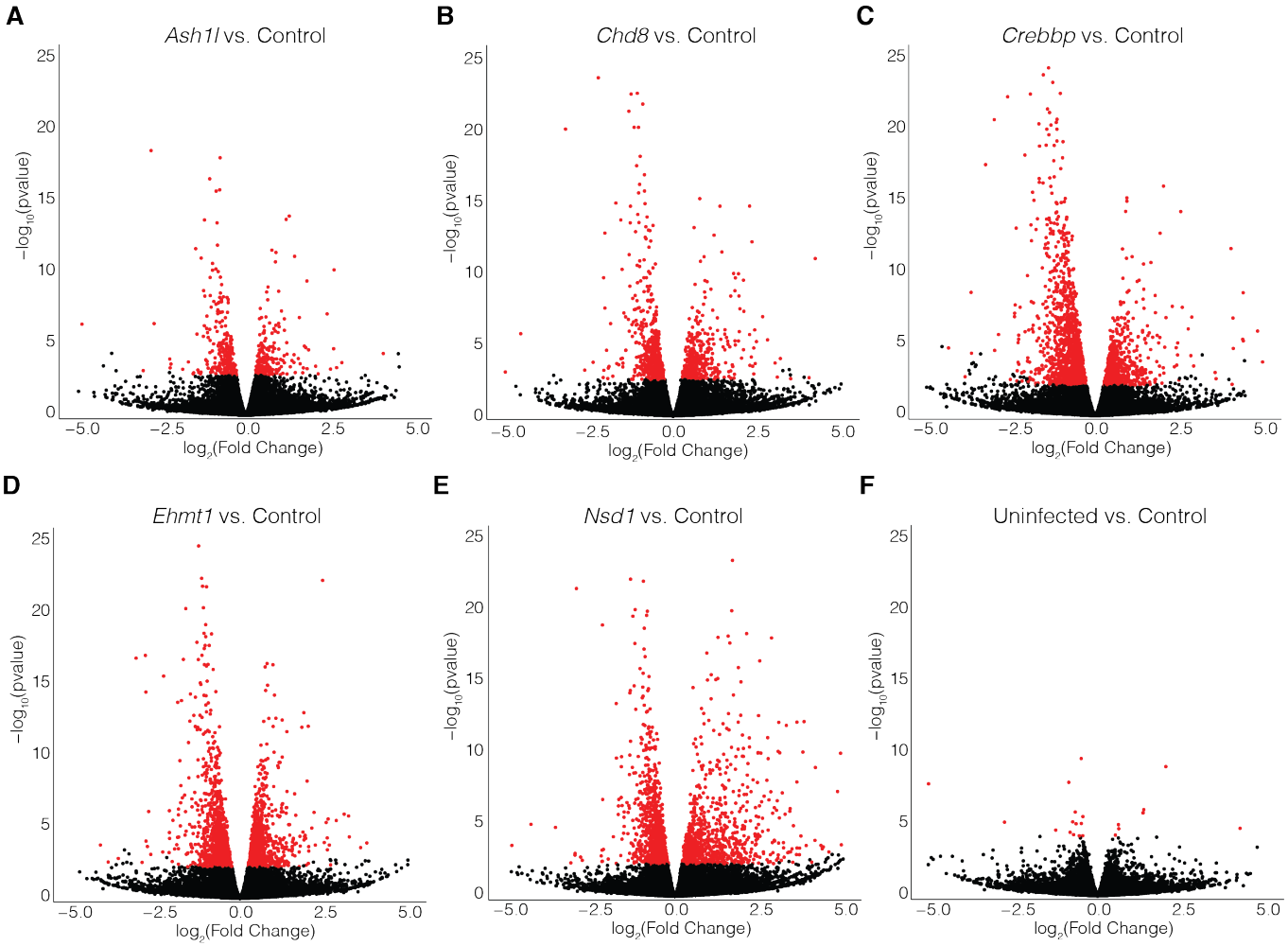
Supplemental Data Tables 1-14

Supplemental Figure 1



Supplemental Figure 1. Confirmation of knockdown of target chromatin modifiers. (A-E) RT-qPCR analysis of knockdown after infection with shRNA lentiviral vectors. N = 3. (F-J) RNA-sequencing gene tracks for each targeted chromatin modifier following knockdown. (K-N) Western blot analysis of protein levels of ASD-linked chromatin modifiers targeted by shRNA lentiviruses. NT indicates non-targeting control lentivirus infection. (O) Quantification of western blots with 2 replicates analyzed per target. (P) Example images of neurons from primary neuronal culture system stained for excitatory (VGLUT1) or inhibitory (GAD67) neuronal markers. (Q) Quantification of percentages of excitatory and inhibitory neurons indicate that knockdown of chromatin modifiers does not change the composition of primary neuronal cultures. N=4-5 images per condition per replicate for each of 3 biological replicates. N.S indicates not significant by one-way ANOVA or by two-sided *t*-test with Bonferroni multiple testing correction.

Supplemental Figure 2

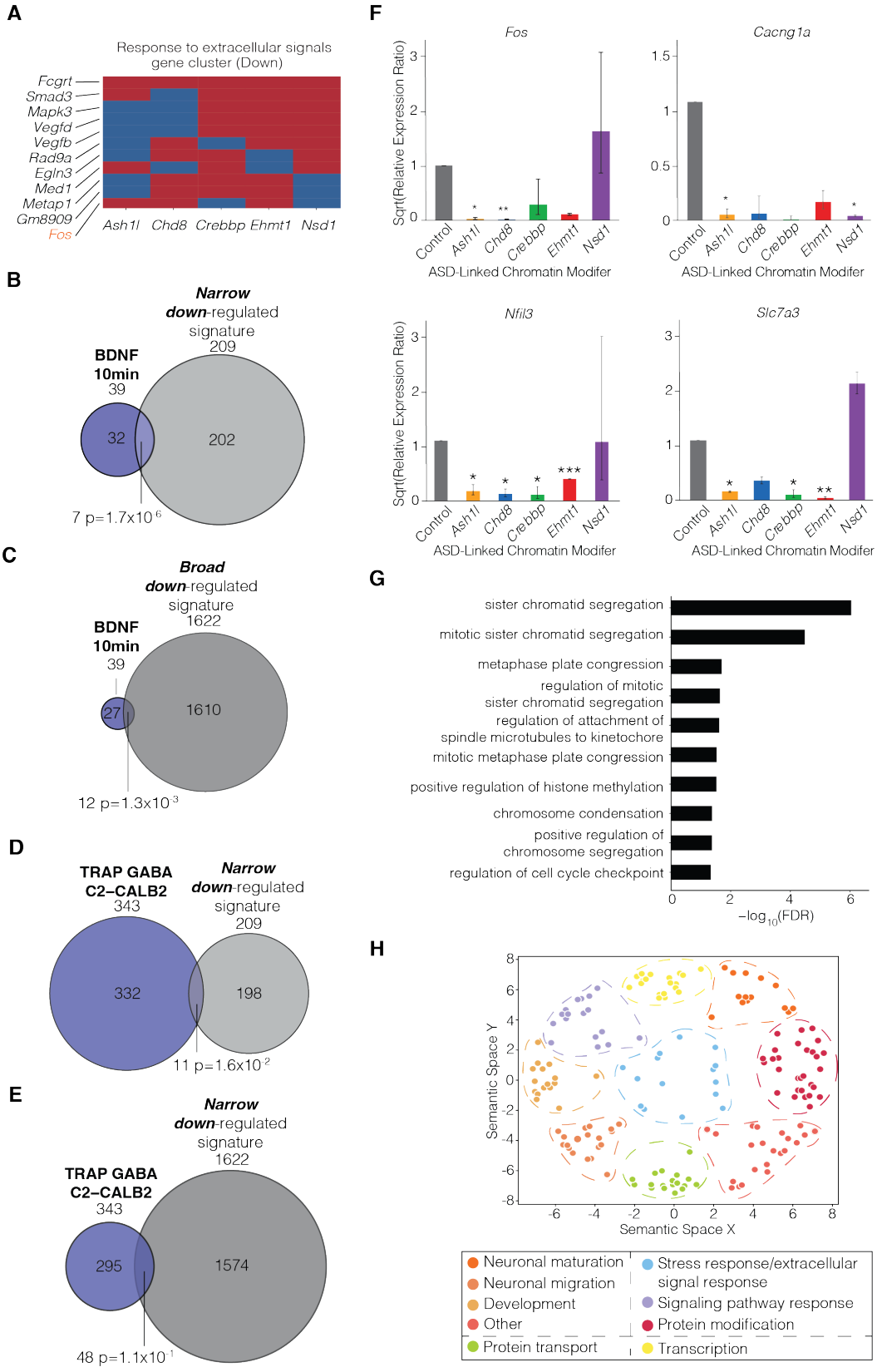


Supplemental Figure 2. Differential gene expression following knockdown of chromatin modifiers. (A-E) Volcano plot of gene expression changes comparing knockdown shRNA to non-targeting shRNA lentiviral infection. (F) Volcano plot of gene expression changes comparing non-targeting shRNA lentiviral infection to uninfected control neurons. Genes that were differentially expressed in this comparison and removed from transcriptional signatures are included in Supplemental Table 2. DESeq2 analysis. Red indicates adjusted $p<0.05$. N = 3.

Supplemental Figure 3

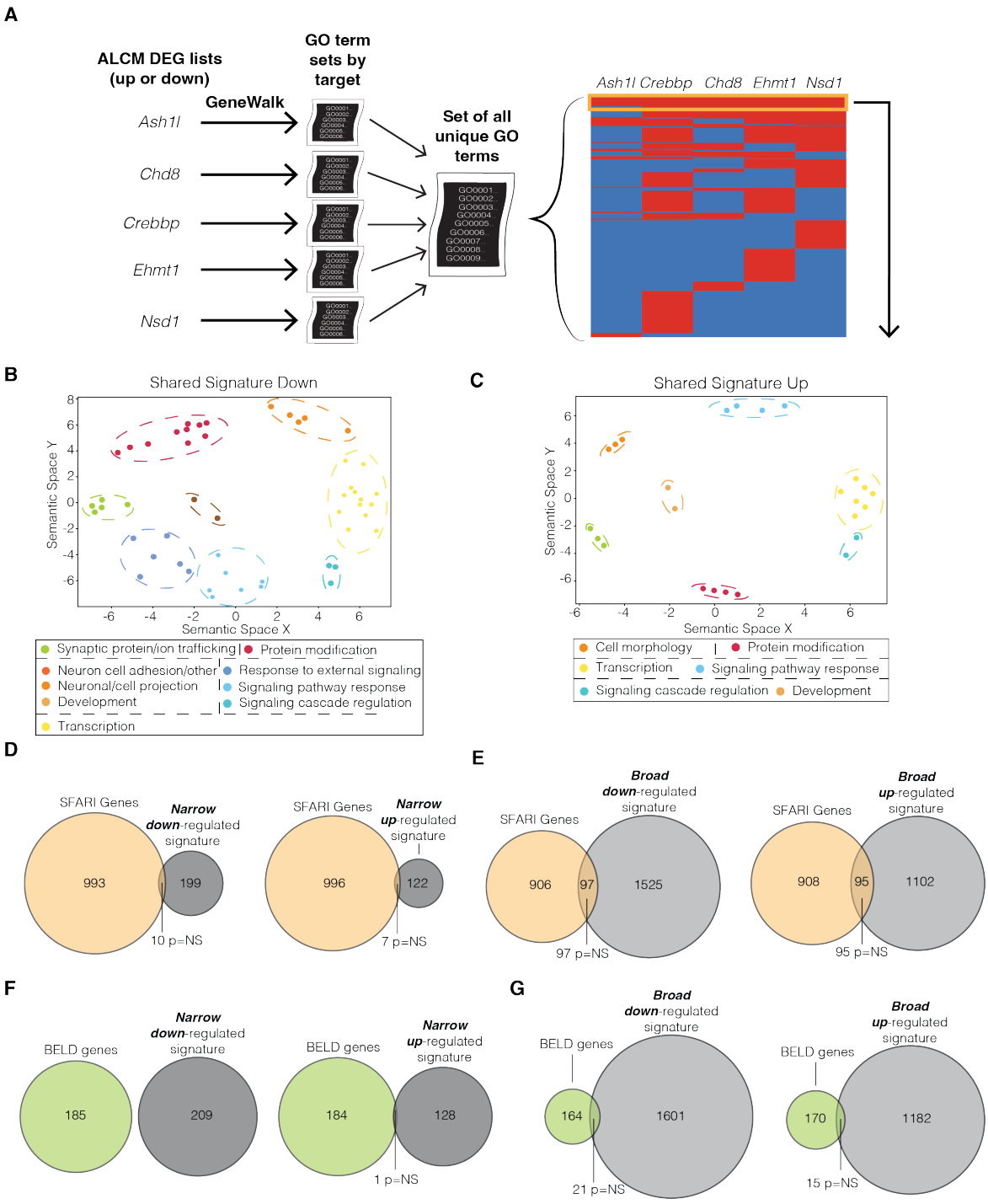
Supplemental Figure 3. Comparison of control conditions used for DEG analysis. (A) Comparison of down-regulated differentially expressed genes when each knockdown condition is compared to either non-targeting shRNA lentivirus infection or uninfected cells. (B) Comparison of up-regulated differentially expressed genes when each knockdown condition is compared to either non-targeting shRNA lentivirus infection or uninfected cells. Overlap significance; hypergeometric tests.

Supplemental Figure 4



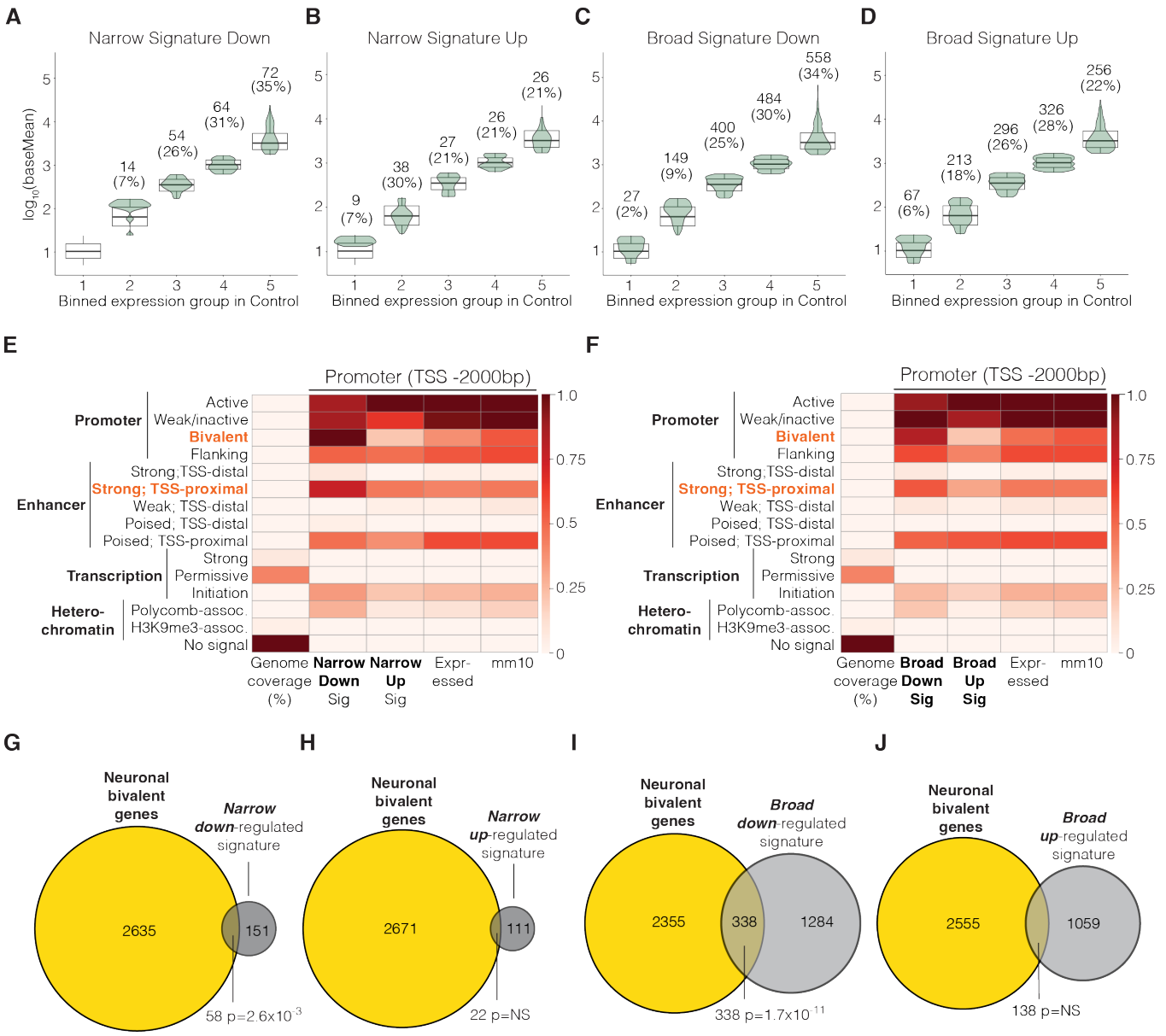
Supplemental Figure 4. Function of down- and up-regulated transcriptional signature genes. (A) Genes contributing to 'Response to extracellular signals' cluster that are differentially expressed after knockdown of 3 or more ASD-linked chromatin modifiers. Activity dependent genes shown in orange. (B-C) Overlap of narrow down-regulated transcriptional signature genes with (B) genes induced in response to a 10-minute BDNF stimulation in primary cultured neurons or (C) genes induced in a fear conditioning memory paradigm in neurons activated in a TRAP2 mouse model. (D-E) Overlap of broad down-regulated transcriptional signature genes with (D) genes induced in response to a 10-minute BDNF stimulation in primary cultured neurons or (E) genes induced in a fear conditioning memory paradigm in neurons activated in a TRAP2 mouse model. (F) RT-qPCR validation of genes that are disrupted by at least 3 of the 5 ASD-linked chromatin modifiers and that contribute to gene ontology clusters. N=3. (G) Gene Ontology analysis of up-regulated broad transcriptional signature gene function. (H) GeneWalk analysis followed by Revigo clustering of up-regulated narrow transcriptional signature genes. Overlap significance determined by hypergeometric tests. RT-qPCR statistics determined by two-sided *t*-test of means of CT values normalized to *Gapdh* relative to control infection, * indicates <0.05, ** indicates <0.01, *** indicates <0.001. BDNF indicates Brain Derived Neurotrophic Factor.

Supplemental Figure 5



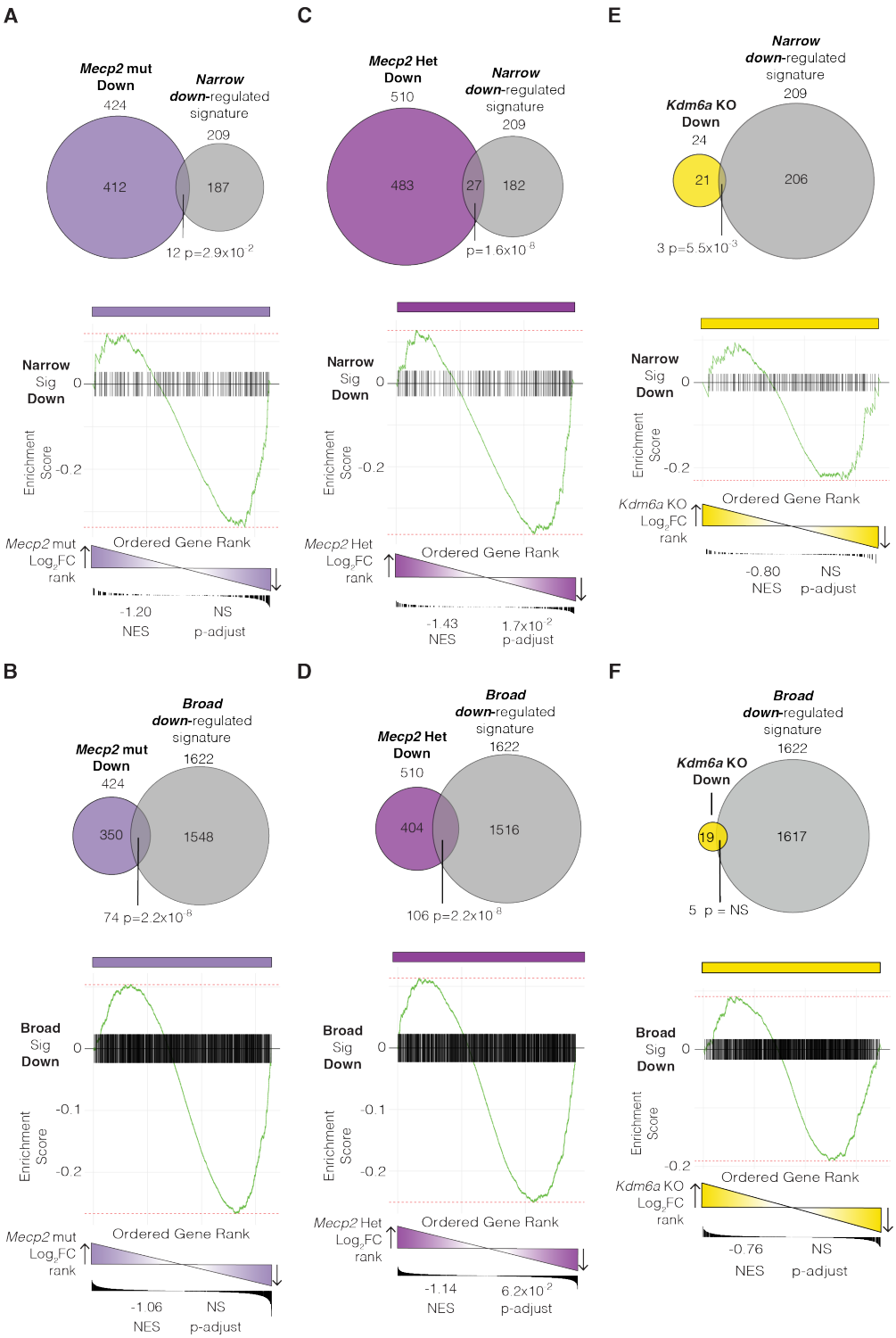
Supplemental Figure 5. Function of down- and up-regulated genes for each ASD-linked chromatin modifier. (A) Analysis schematic of GeneWalk performed on each separate set of differentially expressed genes following knockdown of 5 ASD-linked chromatin modifiers (ALCM). GO terms were then overlapped to find common functions and clustered by REVIGO. (B) Analysis of separate GeneWalk analysis and overlapping outputs of genes down-regulated following knockdown of ASD-linked chromatin modifiers. (C) Analysis of separate GeneWalk analysis and overlapping outputs of genes up-regulated following knockdown of ASD-linked chromatin modifiers. DEG indicates differentially expressed genes following knockdown of an ALCM target compared to non-targeting control lentiviral infection. (D-E) Overlaps of SFARI genes with narrow (D) or broad (E) gene signatures. (F-G) Overlaps of BELD genes with narrow (F) or broad (G) gene signatures.

Supplemental Figure 6



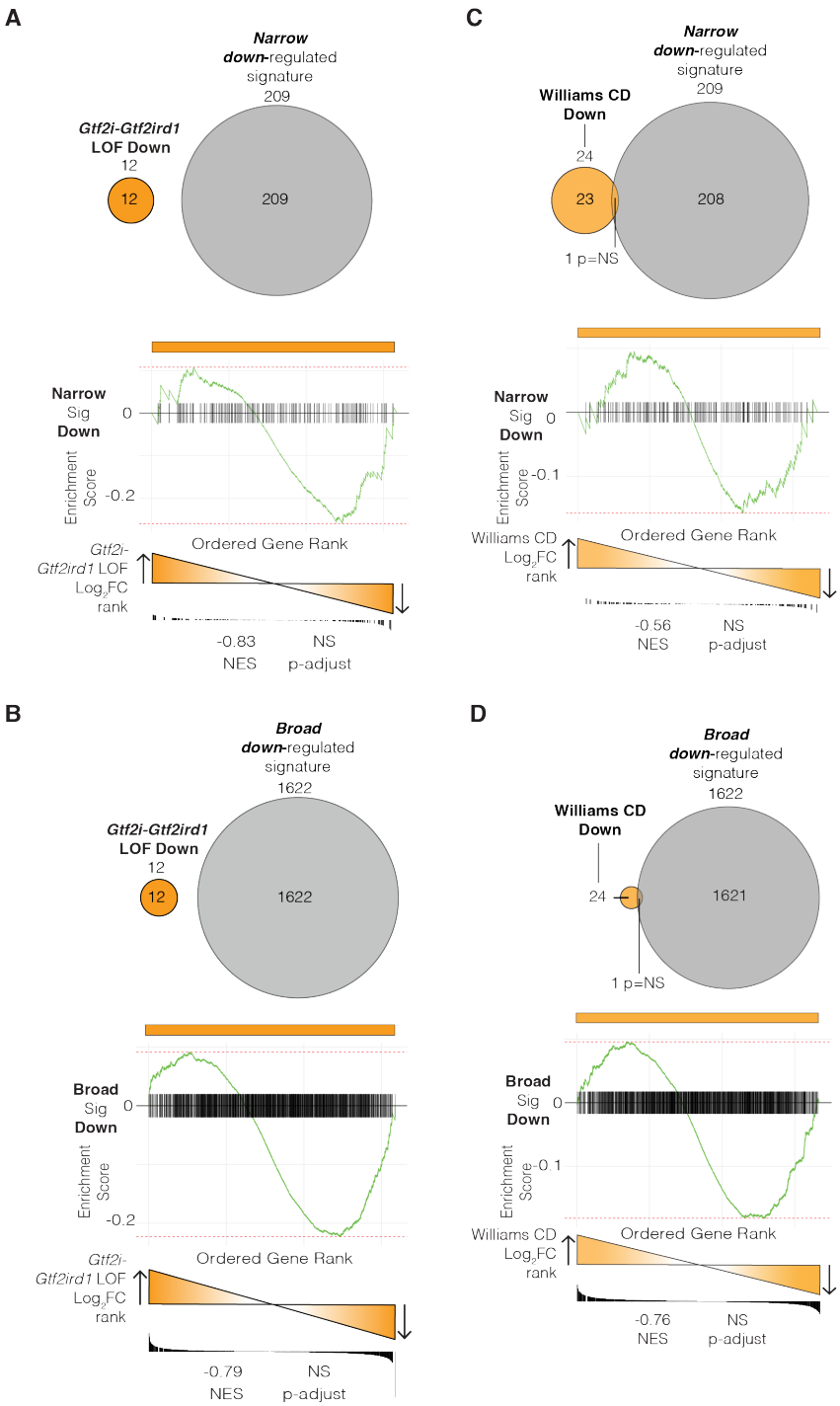
Supplemental Figure 6. Chromatin states in transcriptional signature genes. (A-D) Expression of all genes from control neurons binned into 5 equal groups with distribution of narrow (A-B) and broad (C-D) ASD transcription signature genes shown in green. (E) ChromHMM analysis of promoter region of narrow transcriptional signature genes using an expanded upstream region up to 2000 basepairs upstream of the TSS. (F) ChromHMM analysis of promoter region of broad transcriptional signature genes using an expanded upstream region up to 2000 basepairs upstream of the TSS. Expressed indicates genes expressed in neuronal culture system. Displayed heatmaps represent overlap enrichment output values range-normalized by column. (G-H) Down- and up-regulated broad transcriptional signature genes overlap with bivalent genes expressed in neurons. Overlap significance based on a hypergeometric test. (I-J) Down- and up-regulated narrow transcriptional signature genes overlap with bivalent genes expressed in neurons. Overlap significance based on a hypergeometric test.

Supplemental Figure 7



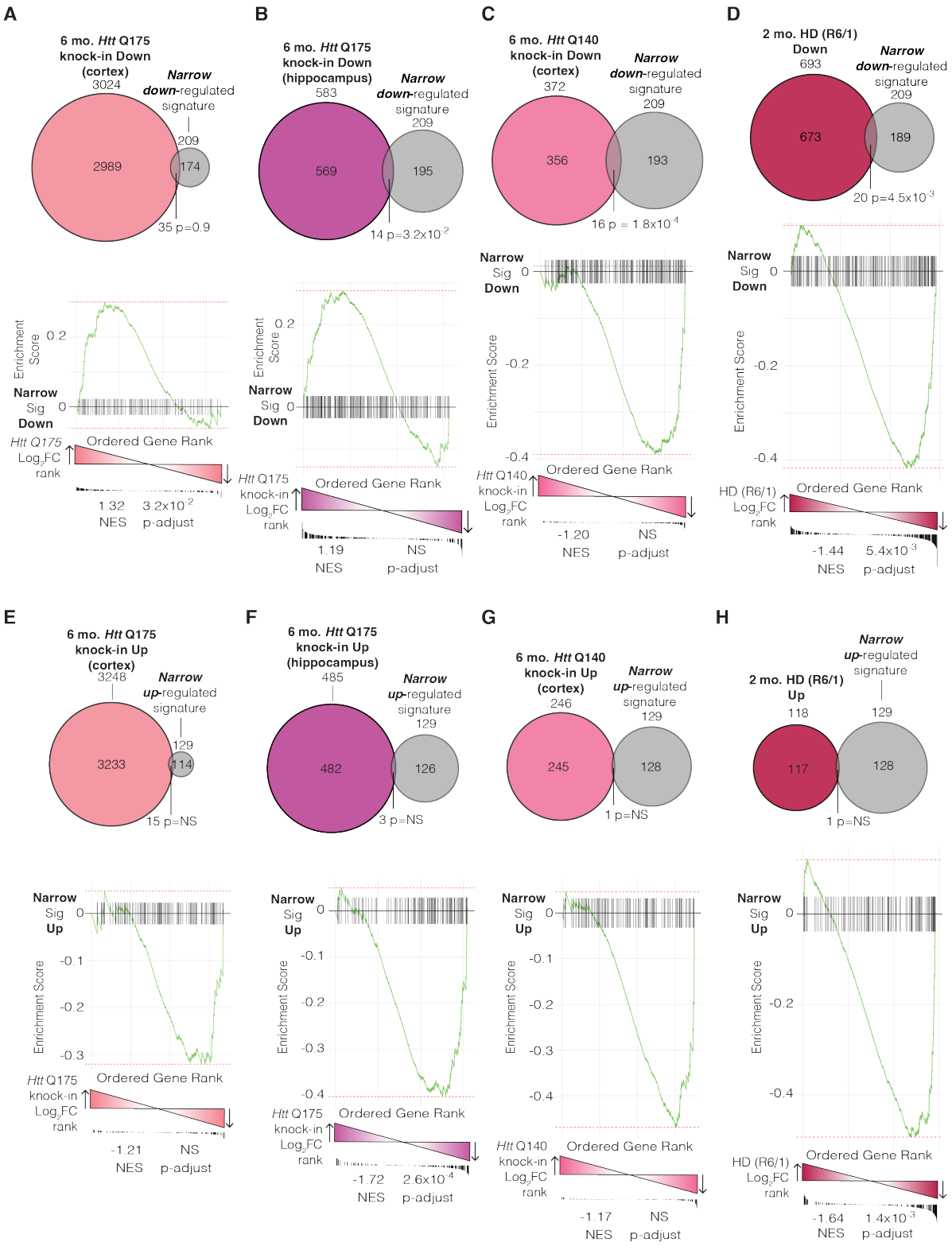
Supplemental Figure 7. Examination of down-regulated transcriptional signature in additional mouse models of NDDs. (A-B) Overlap (top) and GSEA (bottom) analysis of the narrow (A) and broad (B) down-regulated transcriptional signature compared to differentially expressed genes in a mutant *Mecp2* mouse model of Rett Syndrome. (C-D) Overlap and GSEA analysis of the narrow (C) and broad (D) down-regulated transcriptional signature compared to differentially expressed genes in an additional *Mecp2* KO mouse model of Rett Syndrome. (E-F) Overlap and GSEA analysis of the narrow (E) and broad (F) down-regulated transcriptional signature compared to differentially expressed genes in a *Kdm6a* KO mouse model of Kabuki Syndrome. Overlap significance based on hypergeometric tests. NES indicates normalized enrichment score. LOF indicates loss of function. KO indicates knockout.

Supplemental Figure 8



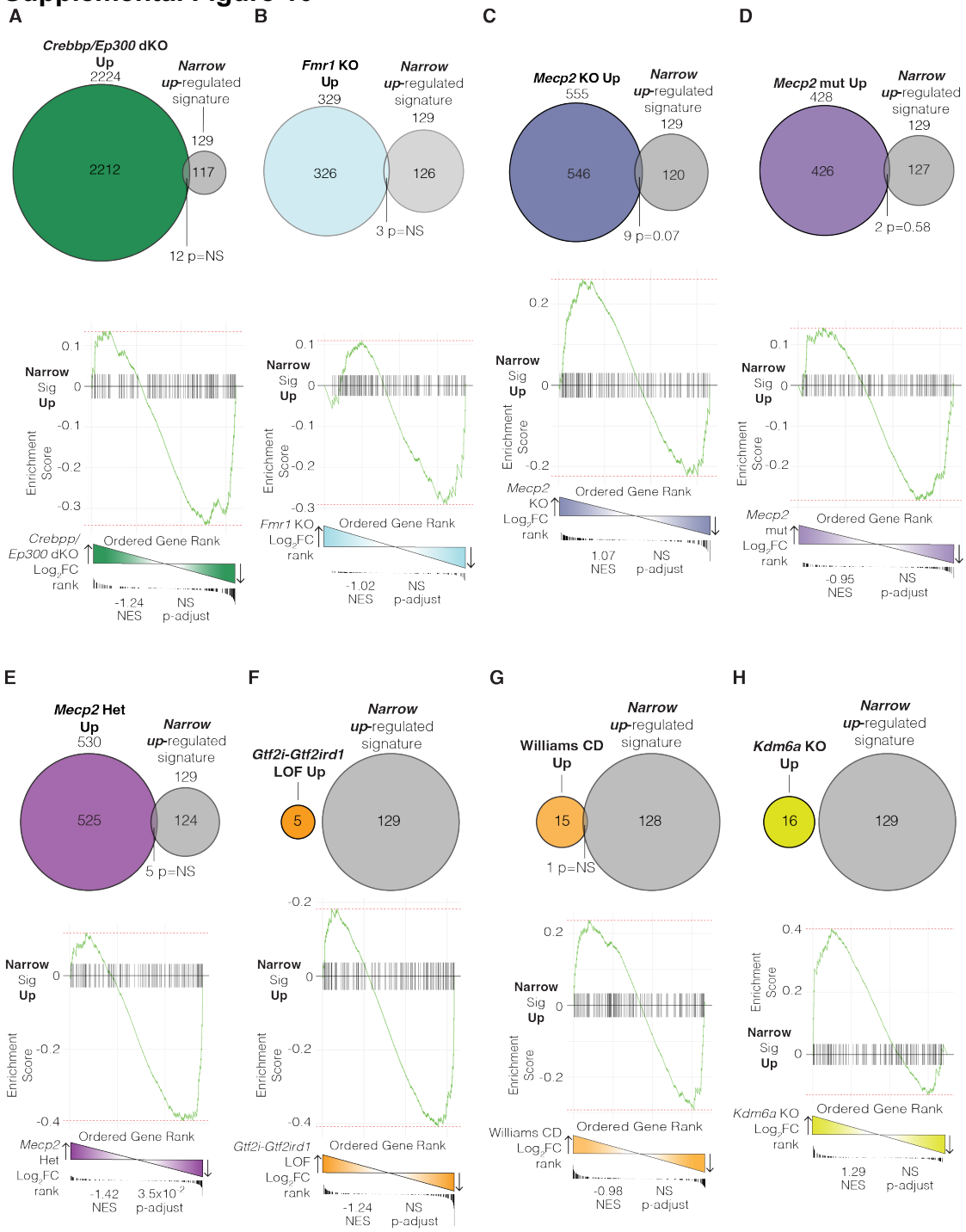
Supplemental Figure 8. Examination of down-regulated transcriptional signature in additional mouse models ID. (A-B) Overlap (top) and GSEA (bottom) analysis of the narrow (A) and broad (B) down-regulated transcriptional signature compared to differentially expressed genes in a *Gtf2i* and *Gtf2ird1* double LOF mouse model of Williams Syndrome. (C-D) Overlap and GSEA analysis of the narrow (C) and broad (D) down-regulated transcriptional signature compared to differentially expressed genes in an additional mouse model of Williams Syndrome containing the full deletion. Overlap significance based on hypergeometric tests. NES indicates normalized enrichment score. LOF indicates loss of function. KO indicates knockout.

Supplemental Figure 9



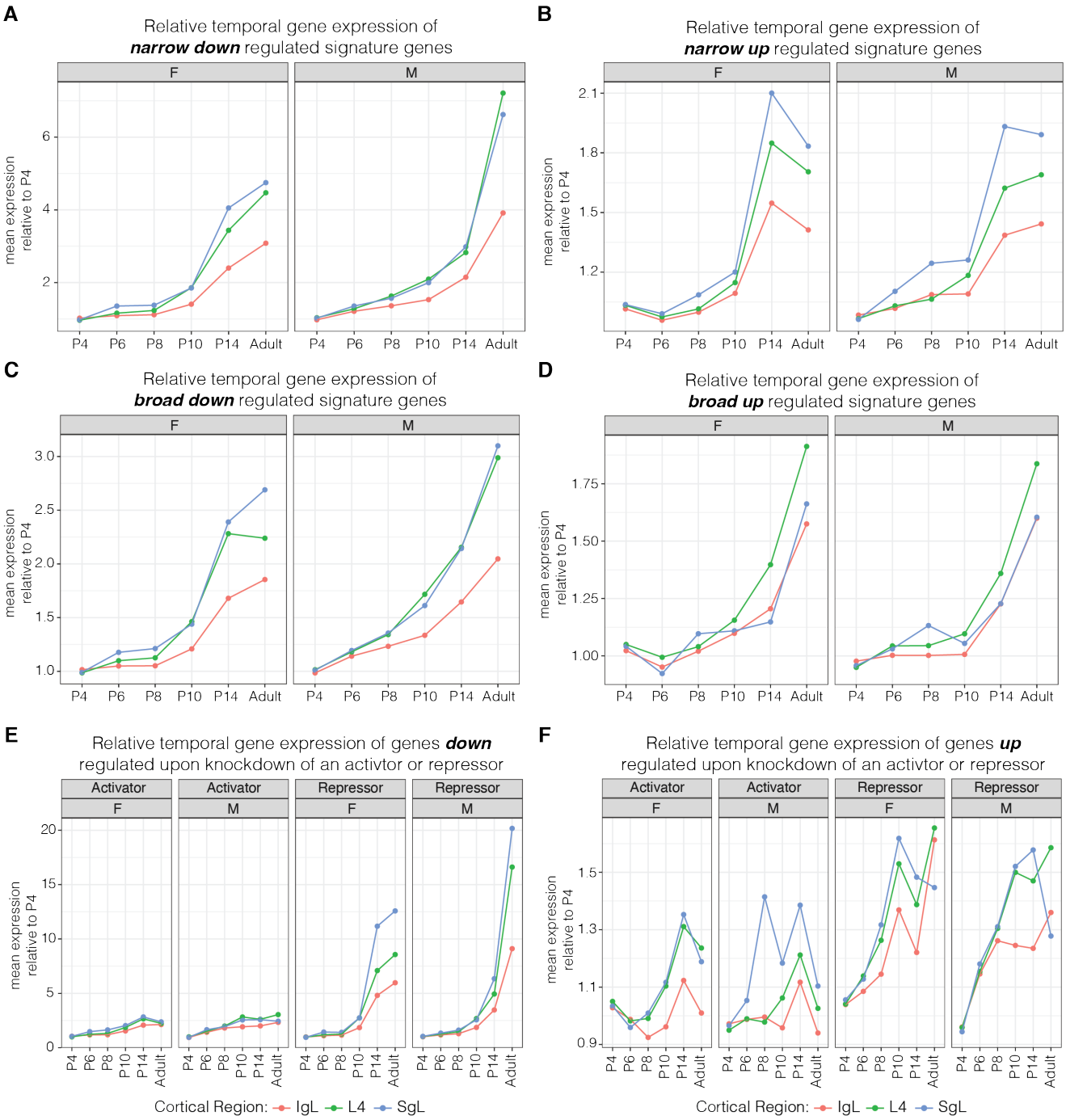
Supplemental Figure 9. Examination of transcriptional signature in mouse models of Huntington's Disease. (A-B) Overlap and GSEA analysis of down-regulated narrow signature genes compared to differentially expressed genes from the cortex (A) or hippocampus (B) of a mouse model of Huntington's Disease containing 175 glutamine repeats. Mice were aged 6 months along with littermate WT controls. (C) Overlap and GSEA analysis of down-regulated transcriptional signature compared to differentially expressed genes from the cortex of a mouse model of Huntington's Disease containing 140 glutamine repeats. Mice were aged 6 months along with littermate WT controls. (D) Overlap and GSEA analysis of down-regulated transcriptional signature compared to differentially expressed genes from the striatum of R6/1 mouse model of Huntington's Disease containing 115 glutamine repeats. Mice were aged 2 months along with age-matched controls. (E-F) Overlap and GSEA analysis of up-regulated transcriptional signature compared to differentially expressed genes from the cortex (E) or hippocampus (F) of a mouse model of Huntington's Disease containing 175 repeats. (G) Overlap and GSEA analysis of up-regulated transcriptional signature compared to differentially expressed genes from the cortex of a mouse model of Huntington's Disease containing 140 repeats. (H) Overlap and GSEA analysis of up-regulated transcriptional signature compared to differentially expressed genes from the striatum of R6/1 mouse model of Huntington's Disease containing 115 repeats. Overlap significance based on hypergeometric tests. NES indicates normalized enrichment score. Broad down- and up-regulated signature analyses are summarized in Supplemental Table 9.

Supplemental Figure 10



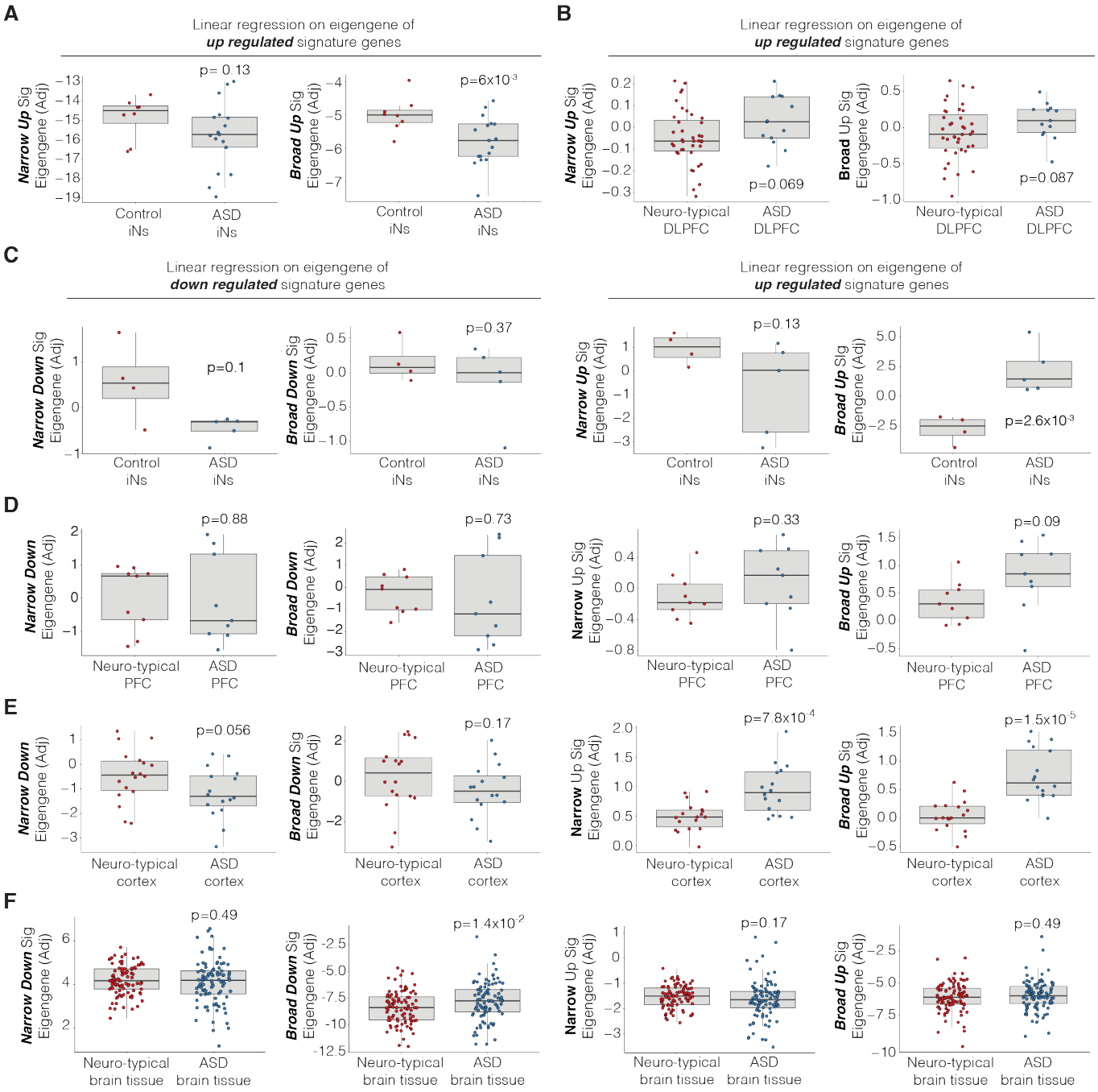
Supplemental Figure 10. Examination of upregulated transcriptional signature in mouse models of NDDs. (A-H) Overlap (top) and GSEA (bottom) analysis of the narrow up-regulated transcriptional signature compared to differentially expressed genes in (A) a *Crebbp/Ep300* (*Kat3a/b)* double KO mouse model; (B) a *Fmr1* KO mouse model of FXS; (C) a *Mecp2* KO mouse model of Rett Syndrome; (D) a mouse model of Rett Syndrome (RTT) containing a mutated *Mecp2* gene (T158M); (E) a mouse model of Rett Syndrome containing a heterozygous knockout of *Mecp2;* (F) a *Gtf2i* and *Gtf2ird1* double LOF mouse model of Williams Syndrome; (G) a mouse model of Williams Syndrome containing the full deletion comparable to that seen in human patients; (H) a *Kdm6a KO* mouse model of Kabuki Syndrome. Overlap significance based on hypergeometric tests. CD indicates complete deletion on 5G2 analogous to the human Williams Syndrome Critical Region on 7q11.23. 'mut' indicates *Mecp2* T158M mutation commonly found in cases of RTT. NES indicates normalized enrichment score. Broad up-regulated signature analyses are summarized in Supplemental Table 9.

Supplemental Figure 11



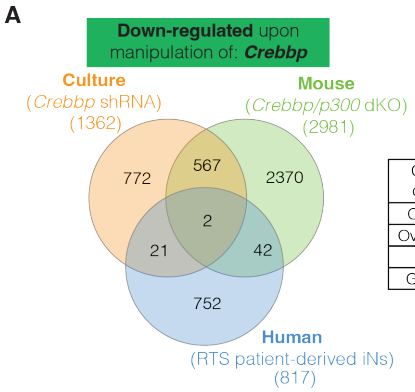
Supplemental Figure 11. Developmental expression of transcriptional signature. (A-D) Relative change in expression during development for narrow down (A) and up (B) and broad down (C) and up (D) signature genes. (E-F) Relative change in expression during development for genes down-regulated (E) or up-regulated (F) specifically by activators or repressors. IgL: L5/6; infragranular layers, L4: layer 4, SgL: L2/3; supragranular layers. Here, 'activators' refers to ASH1L, CREBBP, and NSD1; 'repressors' refers to CHD8 and EHMT1. 'F' indicates female. 'M' indicates male.

Supplemental Figure 12

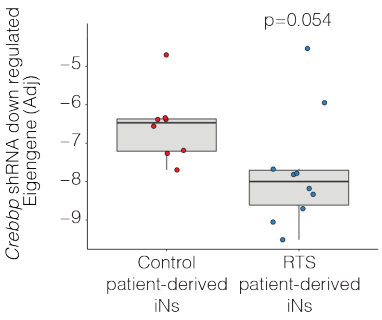


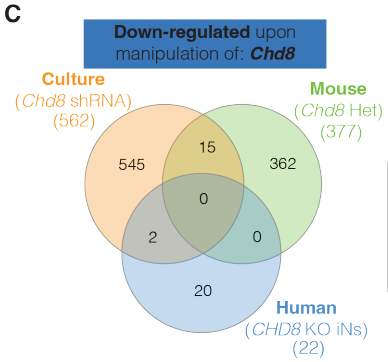
Supplemental Figure 12. Transcriptional signature in human iPSC-derived neurons with idiopathic ASD. (A) The eigengene of up-regulated transcriptional signatures on idiopathic ASD patient iPSC-derived neurons from Marchetto et al., 2017. (B) The eigengene of up-regulated transcriptional signatures on idiopathic ASD patient postmortem DLPFC from Wright et al., 2017. (C) The eigengenes of down- and up-regulated transcriptional signatures across control and idiopathic ASD patient iPSC-derived neurons from DeRosa et al., 2018. (D) The eigengenes of down- and up-regulated transcriptional signatures across control and idiopathic ASD patient postmortem PFC from Velmeshev et al., 2020. (E) The eigengenes of down- and up-regulated transcriptional signatures across control and idiopathic ASD patient postmortem cortex from Velmeshev et al., 2019. (F) The eigengenes of down- and up-regulated transcriptional signatures across control and idiopathic ASD patient postmortem brain tissue from Parikshak et al., 2016. DLPFC indicates dorsolateral prefrontal cortex. PFC indicates prefrontal cortex. 'Adj' indicates adjusted. Significance values computed by linear regression for ASD.

Supplemental Figure 13

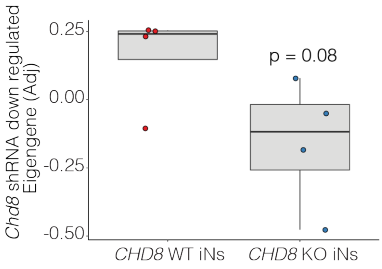


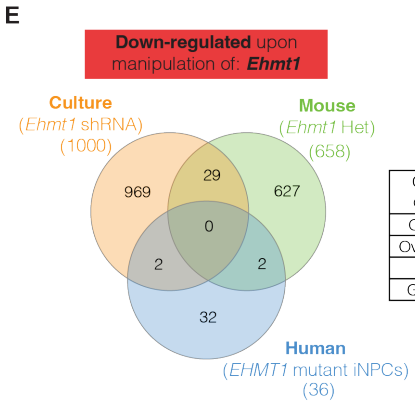
Cross-model comparison	culture vs. mouse	culture vs. human	mouse vs. human
Overlap - Size	569	23	42
Overlap - Pvalue	$2x10^{-74}$	NS	NS
GSEA - NES	-2.48	-1.51	-1.30
GSEA - Pvalue	$3.7x10^{-83}$	$3.7x10^{-10}$	$8.5x10^{-10}$



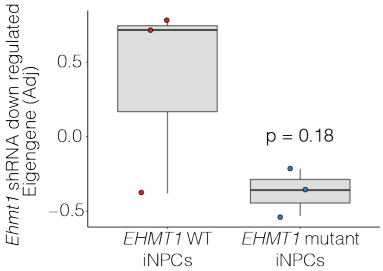


Cross-model comparison	culture vs. mouse	culture vs. human	mouse vs. human
Overlap - Size	15	2	0
Overlap - Pvalue	NS	NS	NS
GSEA - NES	-1.43	-1.31	-0.90
GSEA - Pvalue	$2.2x10^{-3}$	$3.1x10^{-2}$	NS



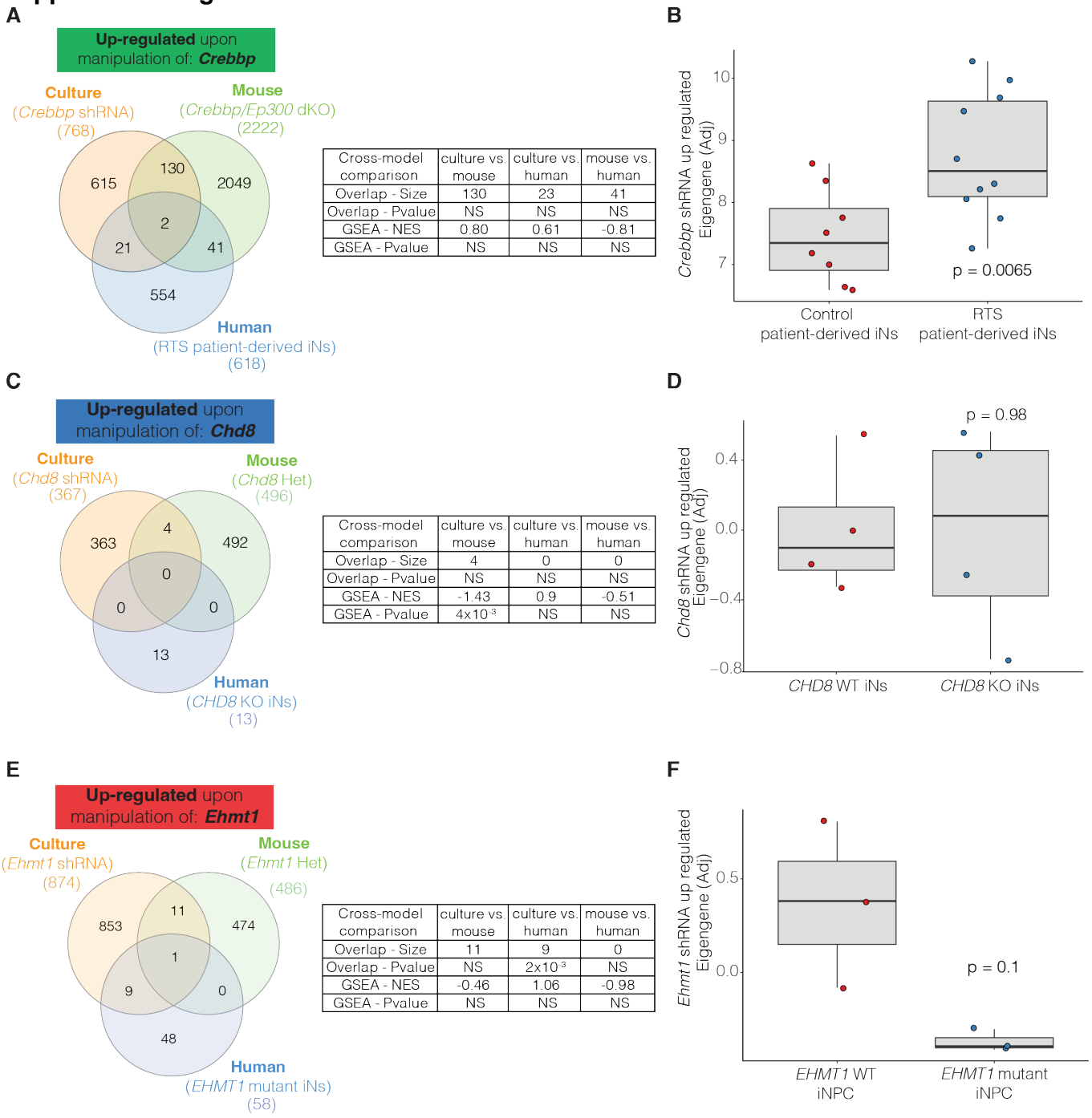


Cross-model comparison	culture vs. mouse	culture vs. human	mouse vs. human
Overlap - Size	29	2	2
Overlap - Pvalue	NS	NS	NS
GSEA - NES	-0.93	-1.00	-1.37
GSEA - Pvalue	NS	NS	$3.5x10^{-2}$



Supplemental Figure 13. Cross-model comparisons of individual down-regulated genes. (A) Overlaps of genes down-regulated by *Crebbp* knockdown in cultured neurons, *Crebbp* knockdown in mouse tissue, or Rubinstein-Taybi Syndrome (RTS) patient iPSCs-derived neurons. (B) Linear regression analysis of RTS iPSCs-derived neurons using genes down-regulated by *Crebbp* knockdown in cultured neurons. (C) Overlaps of genes down-regulated by *Chd8* knockdown in cultured neurons, *Chd8* knockdown in mouse tissue, or CHD8 KO iPSCs-derived neurons. (D) Linear regression analysis of *Chd8* KO iPSCs-derived neurons using genes down-regulated by *Crebbp* knockdown in cultured neurons. (E) Overlaps of genes down-regulated by *Ehmt1* knockdown in cultured neurons, *Ehmt1* knockdown in mouse tissue, or *EHMT1* mutation patient iPSCs-derived neurons. (F) Linear regression analysis of *EHMT1* mutation patient iPSCs-derived neurons using genes down-regulated by *Ehmt1* knockdown in cultured neurons. KO indicates knockout. iNs indicates induced neurons. iNPCs indicates induced neuronal progenitor cells. Significance values computed by linear regression for ASD model.

Supplemental Figure 14



Cross-model comparison	culture vs. mouse	culture vs. human	mouse vs. human
Overlap - Size	130	23	41
Overlap - Pvalue	NS	NS	NS
GSEA - NES	0.80	0.61	-0.81
GSEA - Pvalue	NS	NS	NS

Cross-model comparison	culture vs. mouse	culture vs. human	mouse vs. human
Overlap - Size	4	0	0
Overlap - Pvalue	NS	NS	NS
GSEA - NES	-1.43	0.9	-0.51
GSEA - Pvalue	$4x10^{-3}$	NS	NS

Cross-model comparison	culture vs. mouse	culture vs. human	mouse vs. human
Overlap - Size	11	9	0
Overlap - Pvalue	NS	$2x10^{-3}$	NS
GSEA - NES	-0.46	1.06	-0.98
GSEA - Pvalue	NS	NS	NS

Supplemental Figure 14. Cross-model comparisons of individual up-regulated genes. (A) Overlaps of genes up-regulated by *Crebbp* knockdown in cultured neurons, *Crebbp* knockdown in mouse tissue, or Rubinstein-Taybi Syndrome (RTS) patient iPSCs-derived neurons. (B) Linear regression analysis of RTS iPSCs-derived neurons using genes up-regulated by *Crebbp* knockdown in cultured neurons. (C) Overlaps of genes up-regulated by *Chd8* knockdown in cultured neurons, *Chd8* knockdown in mouse tissue, or CHD8 KO iPSCs-derived neurons. (D) Linear regression analysis of *Chd8* KO iPSCs-derived neurons using genes up-regulated by *Crebbp* knockdown in cultured neurons. (E) Overlaps of genes up-regulated by *Ehmt1* knockdown in cultured neurons, *Ehmt1* knockdown in mouse tissue, or *EHMT1* mutation patient iPSCs-derived neurons. (F) Linear regression analysis of *EHMT1* mutation patient iPSCs-derived neurons using genes up-regulated by *Ehmt1* knockdown in cultured neurons. KO indicates knockout. iNs indicates induced neurons. iNPCs indicates induced neuronal progenitor cells. Significance values computed by linear regression for ASD model.

Supplemental Table 12. shRNA sequences.

Target	Oligo sequence	Clone ID
Ash1l	CCGGAGCTACGTCAGAGACCTAAACCTCGAGGTTTAGGTCTCTGACGTAGCTTTTTTG	TRCN0000304509
Chd8	CCGGATGACCACTTCCTCGTTTCTGCTCGAGCAGAAACGAGGAAGTGGTCATTTTTTG	TRCN0000241050
Crebbp	CCGGCCTCACAATCAACATCTCCTTCTCGAGAAGGAGATGTTGATTGTGAGGTTTTT	TRCN0000012727
Ehmt1	CCGGGCGCTGGCTATATGGAAGTTTCTCGAGAAACTTCCATATAGCCAGCGCTTTTTG	TRCN0000086070
Nsd1	CCGGGAGCTCTCGTACAGATCATTACTCGAGTAATGATCTGTACGAGAGCTCTTTTTTG	TRCN0000413536
Luciferase	CCGGCGCTGAGTACTTCGAAATGTCCTCGAGGACATTTCGAAGTACTCAGCGTTTTT	SHC007

Supplemental Table 13. Antibody information.

Target	Supplier (Cat No.)	Working dilution
Rabbit anti-ASH1L	Bethyl Laboratories (A301-749A)	1:1000
Rabbit anti-CHD8	Cell Signaling Technologies (77694S)	1:1000
Rabbit anti-CREBBP	Cell Signaling Technologies (7389S)	1:1000
Rabbit anti-EHMT1	Thermo Fisher Scientific (PA5114733)	1:1000
Rabbit anti-TUBULIN	Abcam (ab18207)	1:5000
HRP Goat anti-Rabbit	Abcam (ab6721)	1:10000
HRP Sheep anti-Mouse	Millipore Sigma (GENA931-1ML)	1:10000
Rabbit anti-VGLUT1	Abcam (ab180188)	1:125
Mouse anti-GAD67	Millipore Sigma (MAB5406)	1:500
Goat Anti-Rabbit IgG H&L (Alexa Fluor 488)	Abcam (ab150077)	1:500
Goat anti-Mouse IgG H+L (Alexa Fluor 594)	Invitrogen (A11032)	1:500

Supplemental Table 14. Primer sequences.

Target	Sequence
Gapdh Forward	AACTCCCTCAAGATTGTCAGCAA
Gapdh Reverse	GGCATGGACTGTGGTCATGA
Ash1l Forward	CCAACACCTGGTTTCCTGAT
Ash1l Reverse	TCCTCCTTCCAAGTCTTCCA
Chd8 Forward	CACTGAACTTCCCAAAGAATCCA
Chd8 Reverse	GGTGGGCTGAGTGGTATAATCAT
Crebbp Forward	GGCTTCTCCGCGAATGACAA
Crebbp Reverse	GTTTGGACGCAGCATCTGGA
Ehmt1 Forward	GAACAGGAGTCTCCCGACAC
Ehmt1 Reverse	GGGCTGTCAGTCTTCCCTC
Nsd1 Forward	TCCGGTGAATTTAGATGCCTCC
Nsd1 Reverse	CGGTAACTGCATAGTACACCCAT
Cacng1 Forward	GAGACACAGAGTACGGGAGC
Cacng1 Reverse	CACTGTCTGCCTTGGAGCAA
Nfil3 Forward	CAGTGCAGGTGACGAACATT
Nfil3 Reverse	TTCCACCACACCTGTTTTGA
Slc7a3 Forward	ATTTGCTTTCTCCGAGGGCA
Slc7a3 Reverse	AGGATGCTAGCTAGGTTCTCAA
Fos Forward	CCGACTCCTTCTCCAGCAT
Fos Reverse	TCACCGTGGGGATAAAGTTG
HIV-PSI Forward	GGACTCGGCTTGCTGAAG
HIV-PSI Reverse	CCCCCGCTTAATACTGACG