SUPPLEMENTAL FIGURE LEGENDS

Figure S1

A) UpSet plot of *Consensus-ASD* genes. **B)** Genic constraint scores for each *Consensus-ASD* category (see also **Table S1**). * adj. p < 0.05; ** adj. p < 0.01; *** adj. p < 0.001; *** adj. p < 0.0001. **C)** Cross-enrichment of *Consensus-ASD* genes across variant classes. Throughout the figure, CNVs are mapped to “known ASD genes”.

Figure S2

A) Expression of *Consensus-ASD* genes within BrainSpan. **B)** Expression of *Consensus-ASD* genes within different cell types during mid-gestation neocortical development (data from Polioudakis et al., 2019). **C)** Enrichment of genetic variant classes for fifty Molecular Signature Database Hallmark pathways.

Figure S3

A) Dot plot of relative expression of neighboring PCG and lncRNA pairs in successful KDs of PCGs. Significance of KD of the neighboring gene is given by the color of the dot. **B)** Dot plot of relative expression of neighboring PCG and lncRNA pairs in successful KDs of lncRNAs. Significance of KD of the neighboring gene is given by the color of the dot.

Figure S4

Log_2 fold change upon KD of a PCG versus KD of its neighboring lncRNA, considering all expressed genes. The identity line is shown as a dashed black line. KD target genes that have been directly implicated in ASD are labeled in bold text.

Figure S5

A) Bar plot depicting enrichment of *Consensus-ASD* genes or SysID genes within the DEGs of

each KD condition. **B)** Heatmap showing the relative expression of HC-SFARI genes that are DEGs in at least 1 KD condition. Statistically significant DEGs are indicated with *. **C)** MAGIC analysis identifying transcriptional regulators whose targets are enriched within the DEGs from each KD. Throughout the figure, KD target genes that have been directly implicated in ASD are labeled in bold text.

Figure S6

Volcano plots depicting differential expression upon KD of the gene indicated above the plot. Statistically significant DEGs with decreased expression are shown in blue, while DEGs with increased expression are shown in red. Genes within the 1st and 2nd degree regulons, derived from an ARACNe GRN constructed with these KD samples held out, are highlighted in pink. Genes within the 1st degree regulon are depicted with dark borders. Statistically significant enrichment of 1st and 2nd degree regulon genes within the downregulated DEGs is indicated with the adjusted p-value above the plot.

Figure S7

Circular network visualization depicting the 78 regulators that meet the following three criteria: 1) identified as a significantly differentially active regulator between control (NTC) samples and samples in which genes known to be associated with ASD had been perturbed; 2) has a regulon that is enriched for genes with high PageRank scores; and 3) has a regulon that is enriched for genes in *Consensus-ASD*. Genes from *Consensus-ASD* are highlighted in yellow, and HC-SFARI genes are depicted with a dark border. In the upper right corner, a bar graph depicts mean normalized degree, a measure of centrality, across regulators from different gene sets.

Figure S8

Depiction of protein-protein interactions between the top candidate regulators (data from the

STRING database).

Figure S9

Depiction of protein-protein interactions between the top candidate regulators of each module (data from the STRING database).

Figure S10

A) Bar plot depicting the enrichment of candidate ASD risk genes within the regulons of GRN-identified, X-linked regulators. **B)** Enrichment of gene sets relevant to ASD within *ZFX* targets identified from ChIP-seq data in Rhie et al., 2018.

Figure S11

Relative CytoTrack intensity ($-\log_2$) over time for each KD condition that did not exhibit statistically significant proliferation phenotypes or exhibited phenotypes that were inconsistent over time. KD target genes that have been directly implicated in ASD are labeled in bold text. Error bars indicate standard error of the mean. Statistical significance was determined using 2-way ANOVA. See **Table S8** for full statistical results.

Figure S12

PCA plot of RNA-seq from each KD, colored by the effect of that KD on proliferation. KD target genes that have been directly implicated in ASD are labeled in bold text.

Figure S13

A) KD efficiency of FLICK constructs. Bar plot depicts the relative expression of a gene of interest (ENSG00000261840) in iPSCs harboring FLICK constructs with non-targeting controls (NTC) or gRNAs that target the gene for degradation (KD). **B)** Immunohistochemistry of FLICK

organoid at day 15. Nuclei are labeled with DAPI (blue), cells with active FLICK constructs are labeled with the reporter EGFP (green), and NPCs express PAX6 (red). **C)** UMAP of organoid scRNA-seq data colored by timepoint. **D)** UMAP of organoid scRNA-seq data colored by targeted gene. KD target genes that have been directly implicated in ASD are labeled in bold text.

Figure S14

A) Heatmap of cross-enrichment of differentially expressed genes (DEGs) from intermediate progenitor cells. **B)** Bar plot of cross-enrichment of DEGs from radial glia (RG) from one-month organoids versus DEGs from previous NPC cultures upon KD of the same target gene. Only perturbations with at least 20 DEGs were tested for cross-enrichment. **C)** Down-sampling the number of cells per perturbation decreases the significant cross-enrichment (transcriptomic convergence) observed between sets of DEGs. An equal number of NTCs was used for DEG analysis as the number of down-sampled cells per perturbation. **D)** Gene set enrichment analysis results for KD samples. C-ASD = *Consensus-ASD*. Blue module obtained from unsupervised clustering of top candidate regulators from GRN analysis.

Figure S15

MAGIC analysis to identify drivers of transcriptomic convergence across different cell types and stages of organoid development.

Figure S16

A) UMAP of scRNA-seq data from acute KD organoids, colored by targeted gene. **B)** Expression of canonical brain marker genes used for identifying cell types. **C)** Cell type composition across the different perturbations in acute KD organoids. **D)** Convergence of differentially expressed genes (DEGs) between distinct KDs in radial glia following acute KD. **E)**

Convergence of differentially expressed genes (DEGs) between distinct KDs in intermediate progenitor cells following acute KD. **F)** Gene set enrichment analysis results for KD samples. C-ASD = *Consensus-ASD*. Green and purple modules obtained from unsupervised clustering of top candidate regulators from GRN analysis.

SUPPLEMENTAL TABLE LEGENDS

Table S1. Raw data associated with **Fig. S1B**.

Table S2. Full set of KD target genes.

Table S3. Guide RNA (gRNA) sequences.

Table S4. Raw data associated with **Fig. 1D**.

Table S5. Candidate ASD risk genes.

Table S6. Constrained MAGIC regulators whose targets were significantly enriched for candidate ASD risk genes.

Table S7. Raw data associated with **Fig. 4B**.

Table S8. MAGIC regulators of candidate autism risk genes.

Table S9. Oligo sequences used for libraries for bulk RNA-sequencing.

Table S10. Primers for ddPCR to confirm knockdown efficiency.

Table S11. The exact source and curation criteria for each dataset included in *Consensus-ASD*.

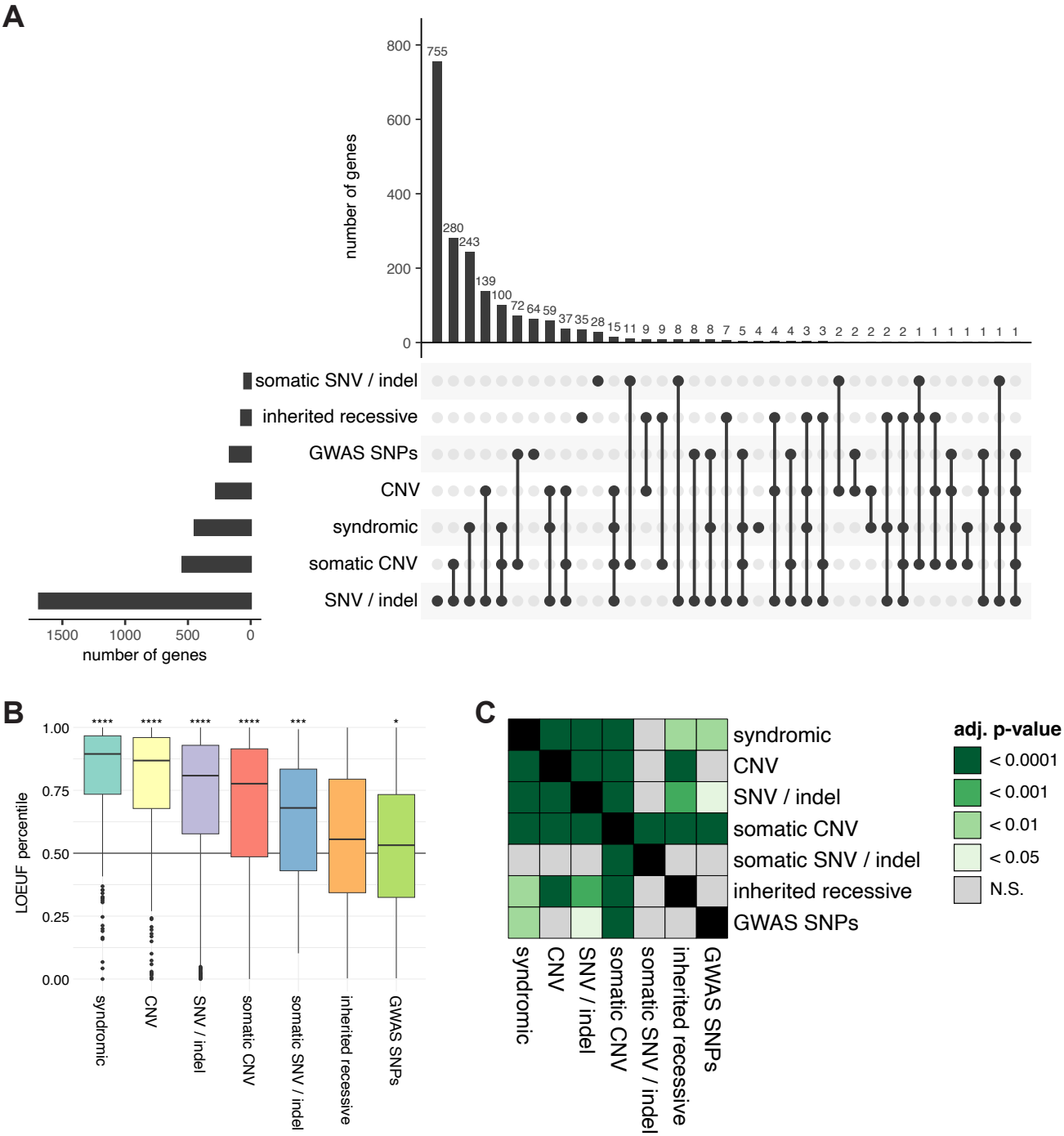
A
number of genes
755
280
243
139
100
72
64
59
37
35
28
15
11
9
9
8
8
8
7
5
4
4
4
3
3
2
2
2
2
2
1
1
1
1
1
1
1
800
600
400
200
0
somatic SNV / indel
inherited recessive
GWAS SNPs
CNV
syndromic
somatic CNV
SNV / indel
1500
1000
500
0
number of genes
B
LOEUF percentile
1.00
0.75
0.50
0.25
0.00
****
****
****
****
***
*
syndromic
CNV
SNV / indel
somatic CNV
somatic SNV / indel
inherited recessive
GWAS SNPs
C
syndromic
CNV
SNV / indel
somatic CNV
somatic SNV / indel
inherited recessive
GWAS SNPs
adj. p-value
< 0.0001
< 0.001
< 0.01
< 0.05
N.S.

Figure S1

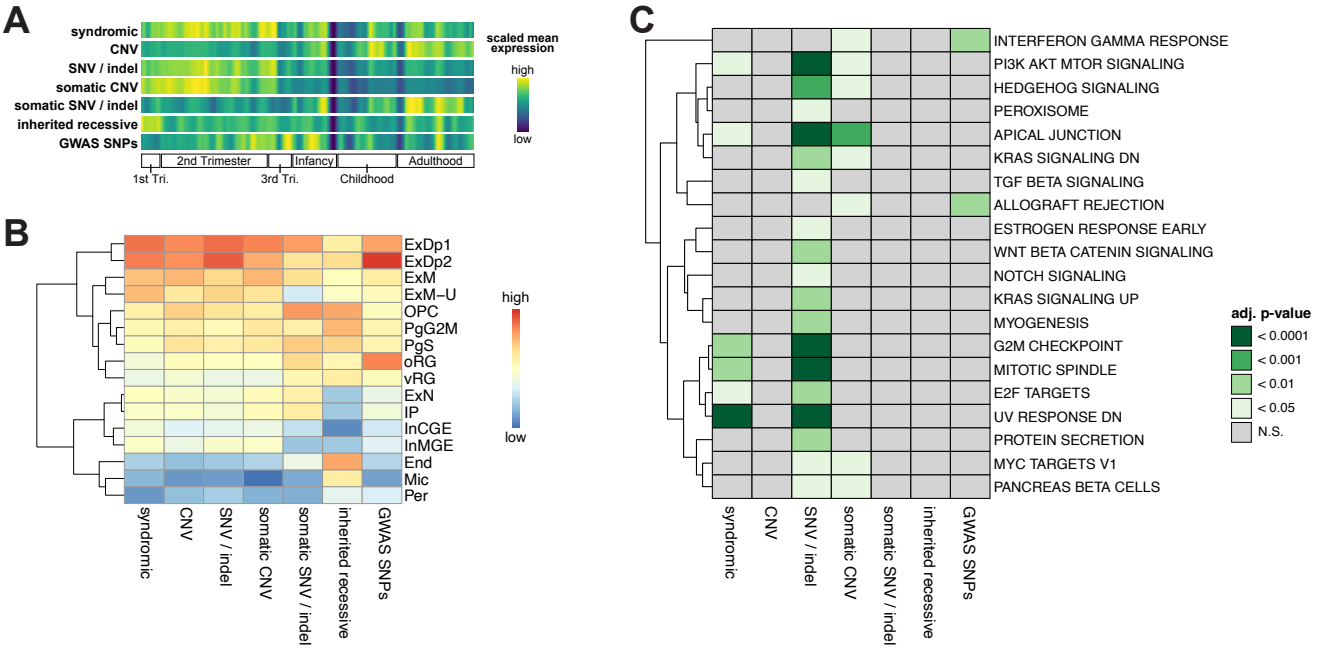
A
syndromic
CNV
SNV / indel
somatic CNV
somatic SNV / indel
inherited recessive
GWAS SNPs
2nd Trimester
Infancy
Adulthood
1st Tri.
3rd Tri.
Childhood
scaled mean expression
high
low
B
ExDp1
ExDp2
ExM
ExM–U
OPC
PgG2M
PgS
oRG
vRG
ExN
IP
InCGE
InMGE
End
Mic
Per
high
low
syndromic
CNV
SNV / indel
somatic CNV
somatic SNV / indel
inherited recessive
GWAS SNPs
C
INTERFERON GAMMA RESPONSE
PI3K AKT MTOR SIGNALING
HEDGEHOG SIGNALING
PEROXISOME
APICAL JUNCTION
KRAS SIGNALING DN
TGF BETA SIGNALING
ALLOGRAFT REJECTION
ESTROGEN RESPONSE EARLY
WNT BETA CATENIN SIGNALING
NOTCH SIGNALING
KRAS SIGNALING UP
MYOGENESIS
G2M CHECKPOINT
MITOTIC SPINDLE
E2F TARGETS
UV RESPONSE DN
PROTEIN SECRETION
MYC TARGETS V1
PANCREAS BETA CELLS
adj. p-value
< 0.0001
< 0.001
< 0.01
< 0.05
N.S.
syndromic
CNV
SNV / indel
somatic CNV
somatic SNV / indel
inherited recessive
GWAS SNPs

Figure S2

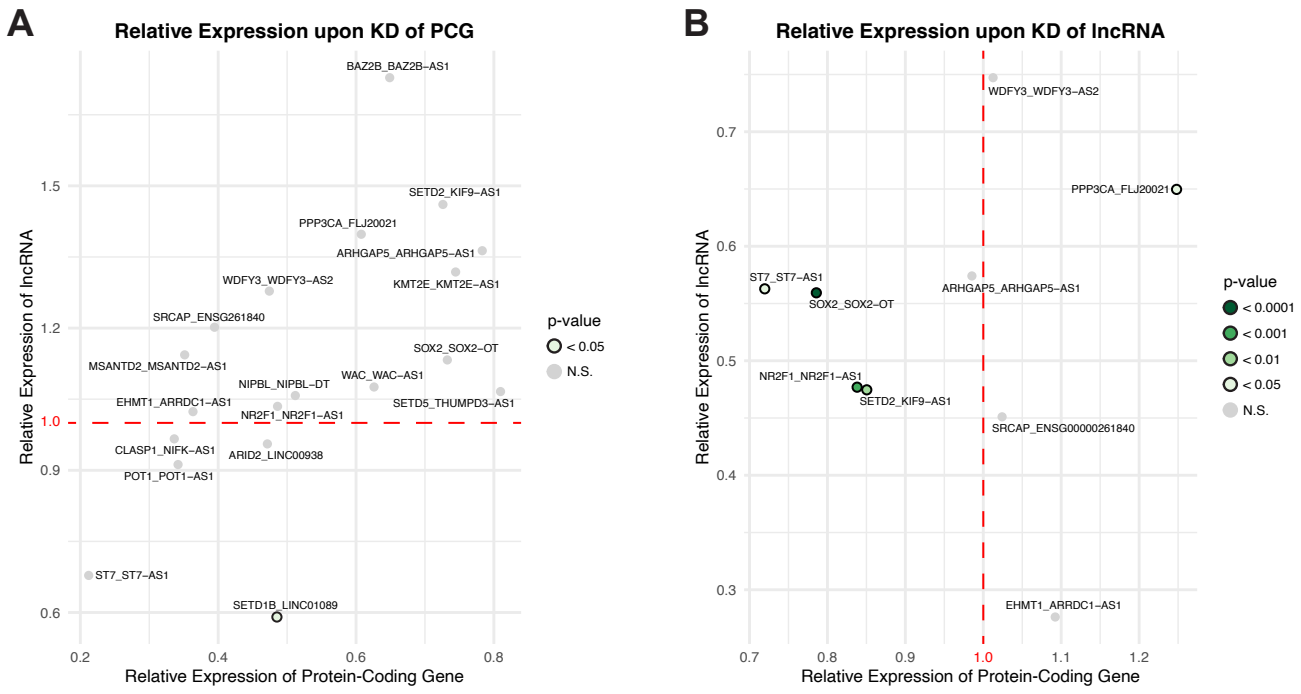
A
Relative Expression upon KD of PCG
BAZ2B_BAZ2B−AS1
SETD2_KIF9−AS1
PPP3CA_FLJ20021
ARHGAP5_ARHGAP5−AS1
KMT2E_KMT2E−AS1
WDFY3_WDFY3−AS2
SRCAP_ENSG261840
SOX2_SOX2−OT
MSANTD2_MSANTD2−AS1
WAC_WAC−AS1
NIPBL_NIPBL−DT
SETD5_THUMPD3−AS1
EHMT1_ARRDC1−AS1
NR2F1_NR2F1−AS1
CLASP1_NIFK−AS1
ARID2_LINC00938
POT1_POT1−AS1
ST7_ST7−AS1
SETD1B_LINC01089
Relative Expression of lncRNA
Relative Expression of Protein-Coding Gene
p-value
< 0.05
N.S.
B
Relative Expression upon KD of lncRNA
WDFY3_WDFY3−AS2
PPP3CA_FLJ20021
ST7_ST7−AS1
ARHGAP5_ARHGAP5−AS1
SOX2_SOX2−OT
NR2F1_NR2F1−AS1
SETD2_KIF9−AS1
SRCAP_ENSG00000261840
EHMT1_ARRDC1−AS1
Relative Expression of lncRNA
Relative Expression of Protein-Coding Gene
p-value
< 0.0001
< 0.001
< 0.01
< 0.05
N.S.

Figure S3

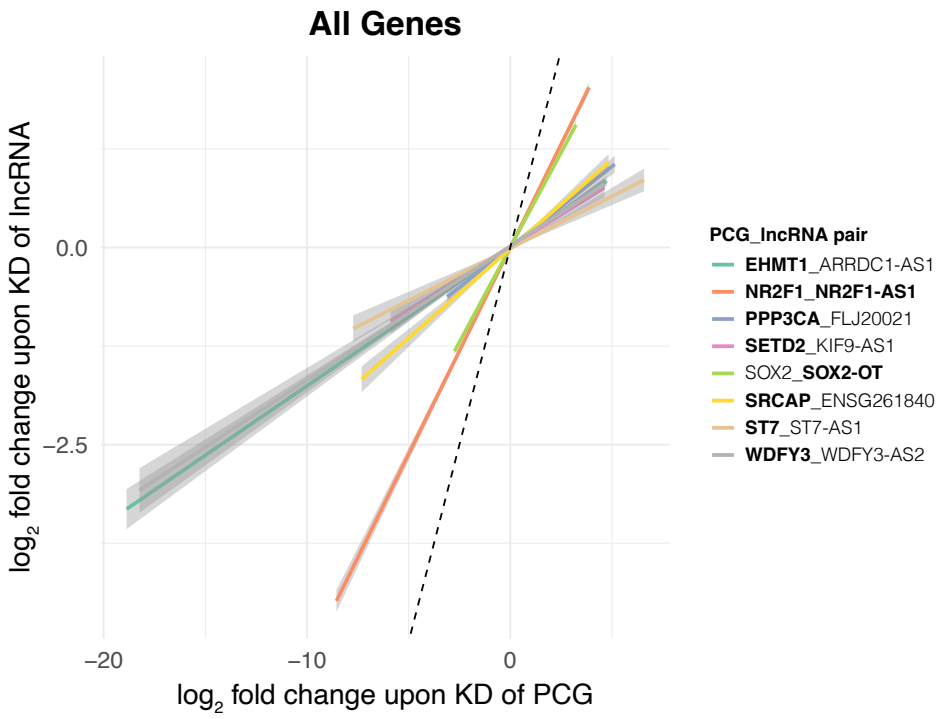
All Genes
log2 fold change upon KD of lncRNA
0.0
−2.5
−20
−10
0
log2 fold change upon KD of PCG
PCG_lncRNA pair
EHMT1_ARRDC1-AS1
NR2F1_NR2F1-AS1
PPP3CA_FLJ20021
SETD2_KIF9-AS1
SOX2_SOX2-OT
SRCAP_ENSG261840
ST7_ST7-AS1
WDFY3_WDFY3-AS2

Figure S4

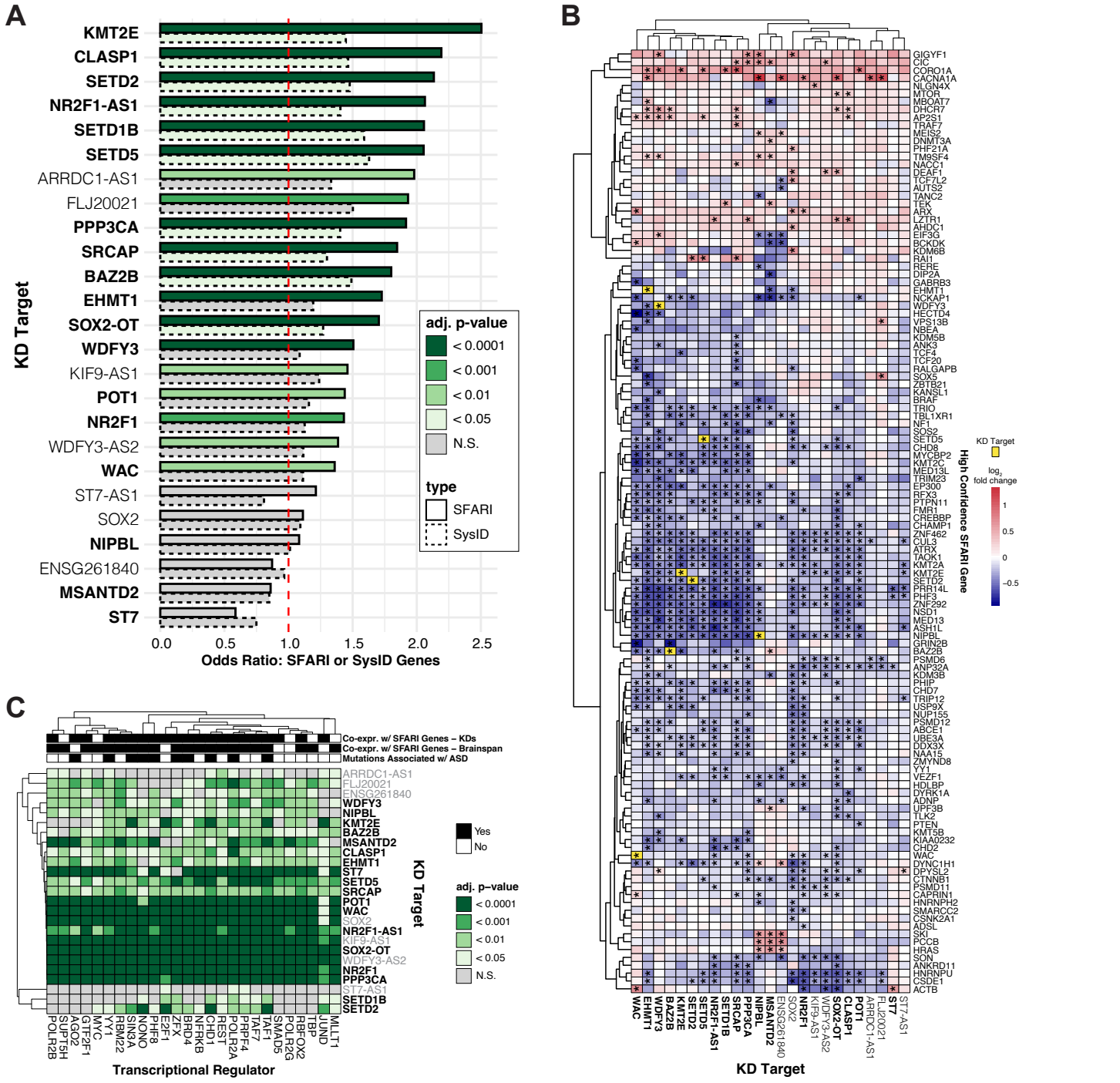
A
KMT2E
CLASP1
SETD2
NR2F1-AS1
SETD1B
SETD5
ARRDC1-AS1
FLJ20021
PPP3CA
SRCAP
BAZ2B
EHMT1
SOX2-OT
WDFY3
KIF9-AS1
POT1
NR2F1
WDFY3-AS2
WAC
ST7-AS1
SOX2
NIPBL
ENSG261840
MSANTD2
ST7
KD Target
0.0
0.5
1.0
1.5
2.0
2.5
Odds Ratio: SFARI or SysID Genes
adj. p-value
< 0.0001
< 0.001
< 0.01
< 0.05
N.S.
type
SFARI
SysID
B
KD Target
log2 fold change
1
0.5
0
−0.5
High Confidence SFARI Gene
KD Target
C
Co-expr. w/ SFARI Genes – KDs
Co-expr. w/ SFARI Genes – Brainspan
Mutations Associated w/ ASD
Yes
No
adj. p−value
< 0.0001
< 0.001
< 0.01
< 0.05
N.S.
KD Target
Transcriptional Regulator

Figure S5

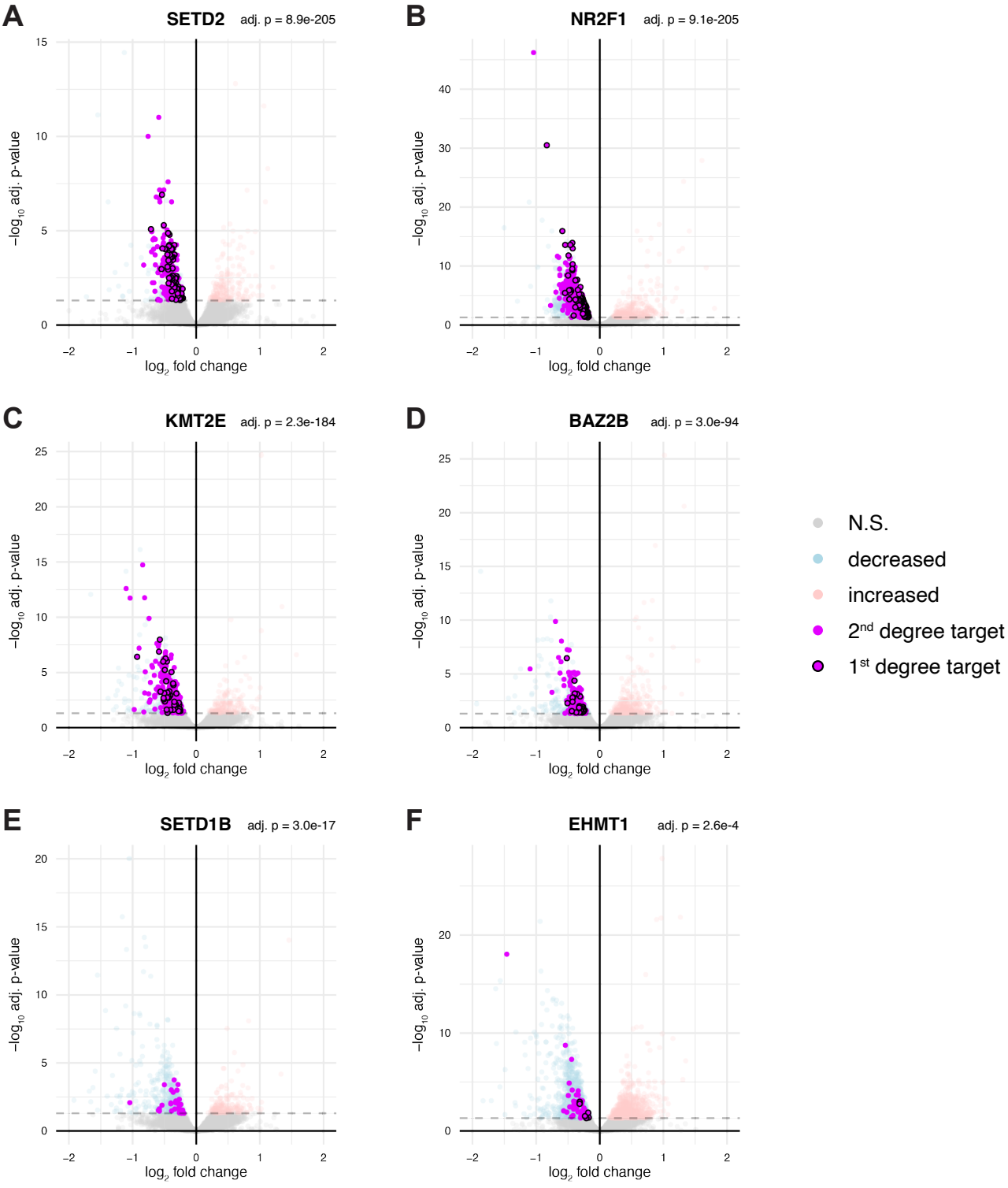
A
SETD2
adj. p = 8.9e-205
B
NR2F1
adj. p = 9.1e-205
C
KMT2E
adj. p = 2.3e-184
D
BAZ2B
adj. p = 3.0e-94
E
SETD1B
adj. p = 3.0e-17
F
EHMT1
adj. p = 2.6e-4
−log10 adj. p-value
log2 fold change
N.S.
decreased
increased
2nd degree target
1st degree target

Figure S6

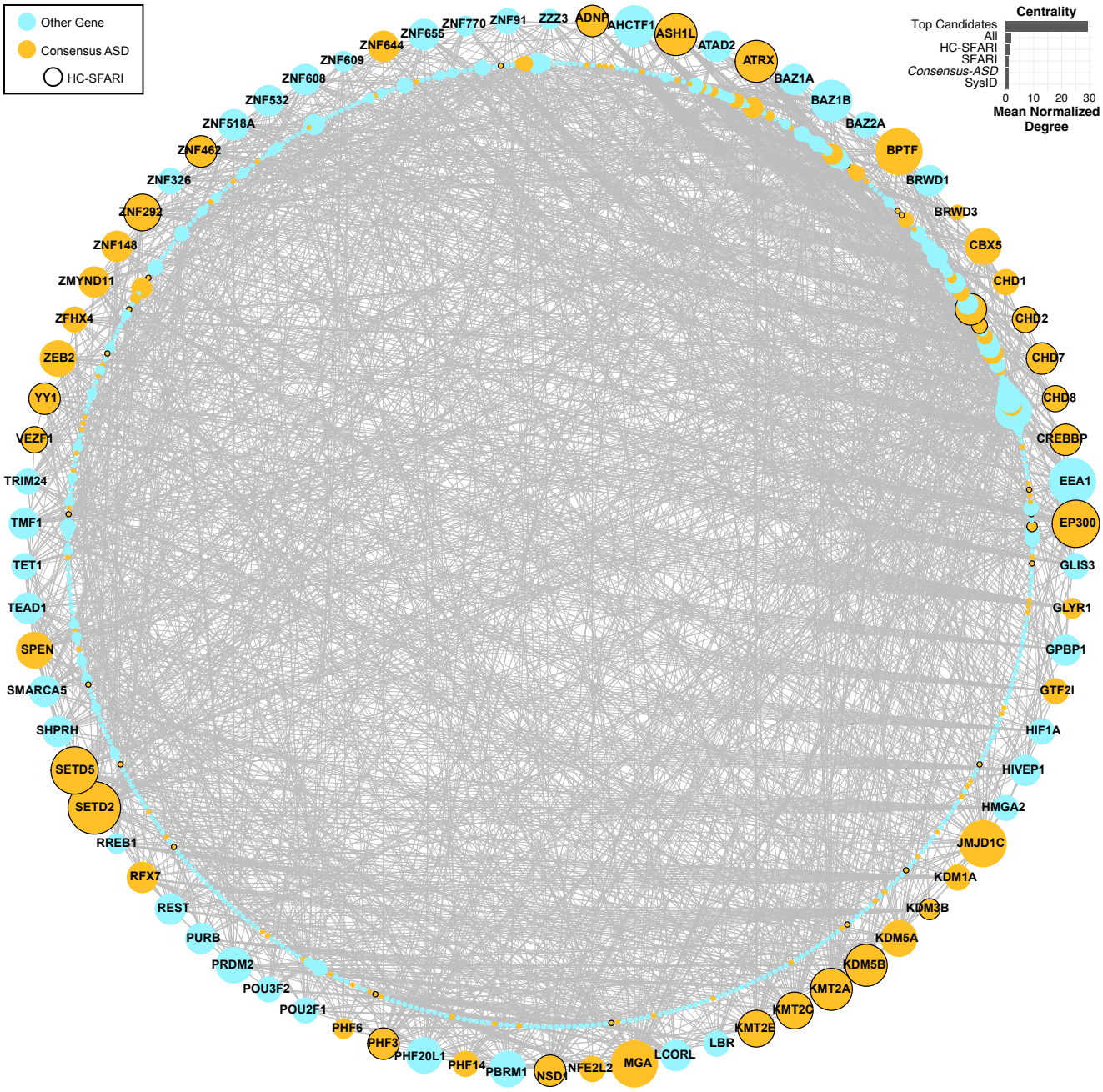
Other Gene
Consensus ASD
HC-SFARI
Centrality
Top Candidates
All
HC-SFARI
SFARI
Consensus-ASD
SysID
0
10
20
30
Mean Normalized Degree
ZNF770
ZNF91
ZZZ3
ADNP
AHCTF1
ASH1L
ATAD2
ATRX
BAZ1A
BAZ1B
BAZ2A
BPTF
BRWD1
BRWD3
CBX5
CHD1
CHD2
CHD7
CHD8
CREBBP
EEA1
EP300
GLIS3
GLYR1
GPBP1
GTF2I
HIF1A
HIVEP1
HMGA2
JMJD1C
KDM1A
KDM3B
KDM5A
KDM5B
KMT2A
KMT2C
KMT2E
LBR
LCORL
MGA
NFE2L2
NSD1
PBRM1
PHF14
PHF20L1
PHF3
PHF6
POU2F1
POU3F2
PRDM2
PURB
REST
RFX7
RREB1
SETD2
SETD5
SHPRH
SMARCA5
SPEN
TEAD1
TET1
TMF1
TRIM24
VEZF1
YY1
ZEB2
ZFHX4
ZMYND11
ZNF148
ZNF292
ZNF326
ZNF462
ZNF518A
ZNF532
ZNF608
ZNF609
ZNF644
ZNF655

Figure S7

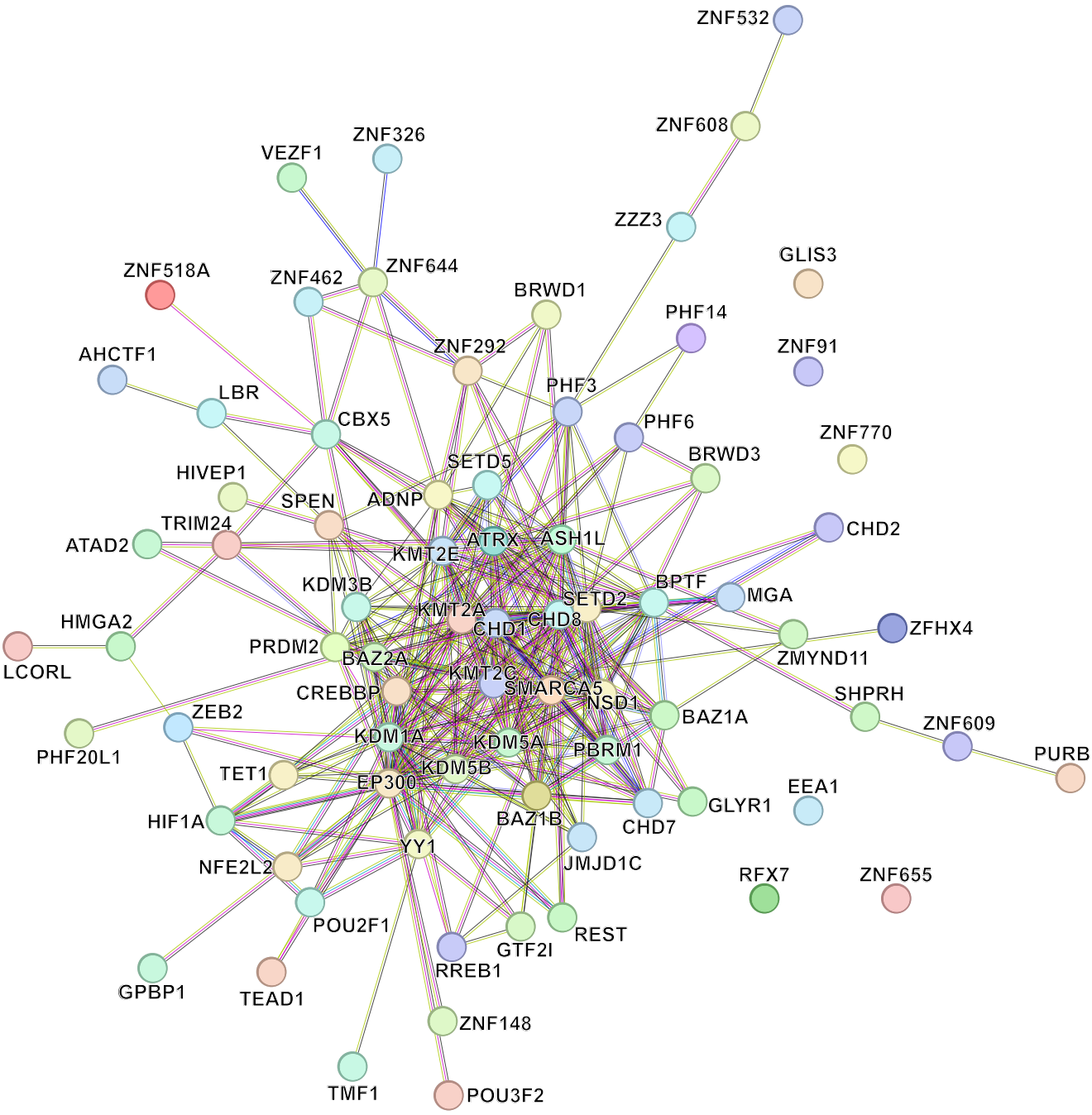
ZNF532
ZNF608
ZNF326
VEZF1
ZZZ3
GLIS3
ZNF518A
ZNF462
ZNF644
BRWD1
PHF14
ZNF292
ZNF91
AHCTF1
PHF3
LBR
CBX5
PHF6
ZNF770
BRWD3
HIVEP1
SETD5
SPEN
ADNP
TRIM24
CHD2
ATAD2
ATRX
ASH1L
KMT2E
KDM3B
BPTF
MGA
SETD2
KMT2A
CHD8
HMGA2
CHD1
ZFHX4
PRDM2
BAZ2A
ZMYND11
LCORL
KMT2C
SMARCA5
CREBBP
NSD1
SHPRH
ZEB2
BAZ1A
ZNF609
KDM1A
KDM5A
PBRM1
PHF20L1
PURB
TET1
KDM5B
EP300
EEA1
GLYR1
HIF1A
BAZ1B
CHD7
YY1
JMJD1C
NFE2L2
RFX7
ZNF655
POU2F1
REST
GTF2I
RREB1
GPBP1
TEAD1
ZNF148
TMF1
POU3F2

Figure S8

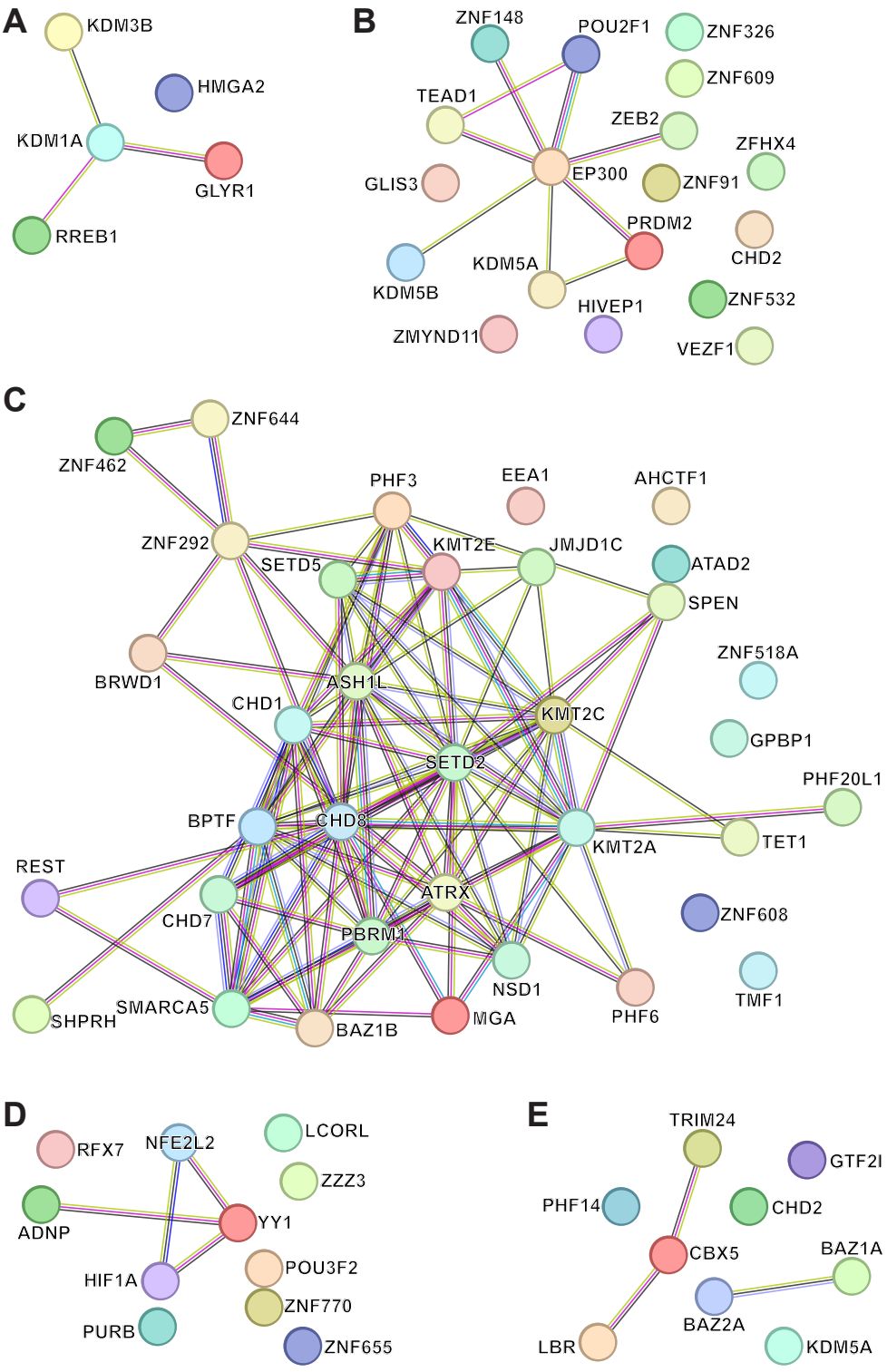
A
KDM3B
HMGA2
KDM1A
GLYR1
RREB1
B
ZNF148
POU2F1
ZNF326
ZNF609
TEAD1
ZEB2
ZFHX4
GLIS3
EP300
ZNF91
PRDM2
CHD2
KDM5B
KDM5A
ZNF532
HIVEP1
ZMYND11
VEZF1
C
ZNF644
ZNF462
PHF3
EEA1
AHCTF1
ZNF292
KMT2E
JMJD1C
SETD5
ATAD2
SPEN
ZNF518A
BRWD1
ASH1L
CHD1
KMT2C
GPBP1
SETD2
PHF20L1
BPTF
CHD8
KMT2A
TET1
REST
ATRX
CHD7
ZNF608
PBRM1
NSD1
TMF1
SHPRH
SMARCA5
BAZ1B
MGA
PHF6
D
RFX7
NFE2L2
LCORL
ZZZ3
ADNP
YY1
HIF1A
POU3F2
ZNF770
PURB
ZNF655
E
TRIM24
GTF2I
PHF14
CHD2
CBX5
BAZ1A
BAZ2A
LBR
KDM5A

Figure S9

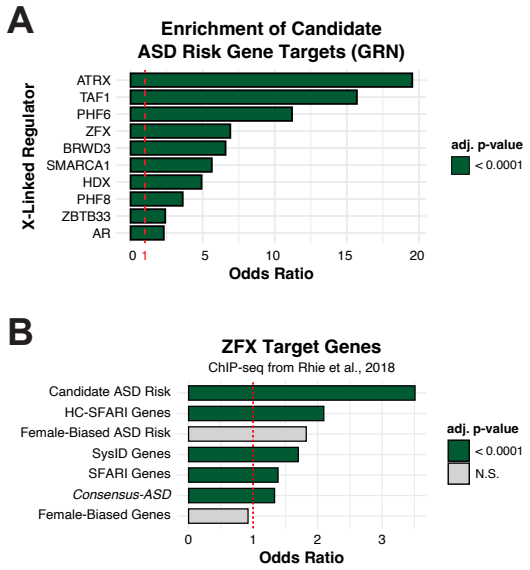
A
Enrichment of Candidate
ASD Risk Gene Targets (GRN)
X-Linked Regulator
ATRX
TAF1
PHF6
ZFX
BRWD3
SMARCA1
HDX
PHF8
ZBTB33
AR
0 1 5 10 15 20
Odds Ratio
adj. p-value
< 0.0001
B
ZFX Target Genes
ChIP-seq from Rhie et al., 2018
Candidate ASD Risk
HC-SFARI Genes
Female-Biased ASD Risk
SysID Genes
SFARI Genes
Consensus-ASD
Female-Biased Genes
0 1 2 3
Odds Ratio
adj. p-value
< 0.0001
N.S.

Figure S10

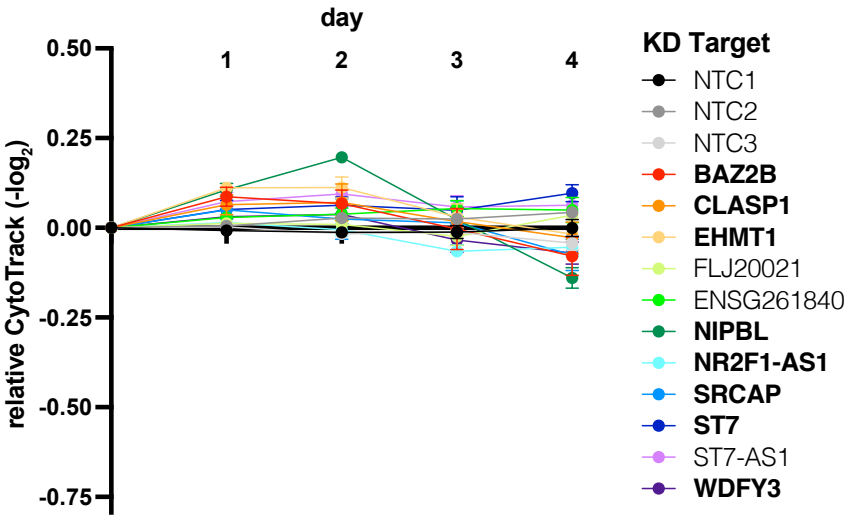
day
1
2
3
4
0.50
0.25
0.00
-0.25
-0.50
-0.75
relative CytoTrack (-log2)
KD Target
NTC1
NTC2
NTC3
BAZ2B
CLASP1
EHMT1
FLJ20021
ENSG261840
NIPBL
NR2F1-AS1
SRCAP
ST7
ST7-AS1
WDFY3

Figure S11

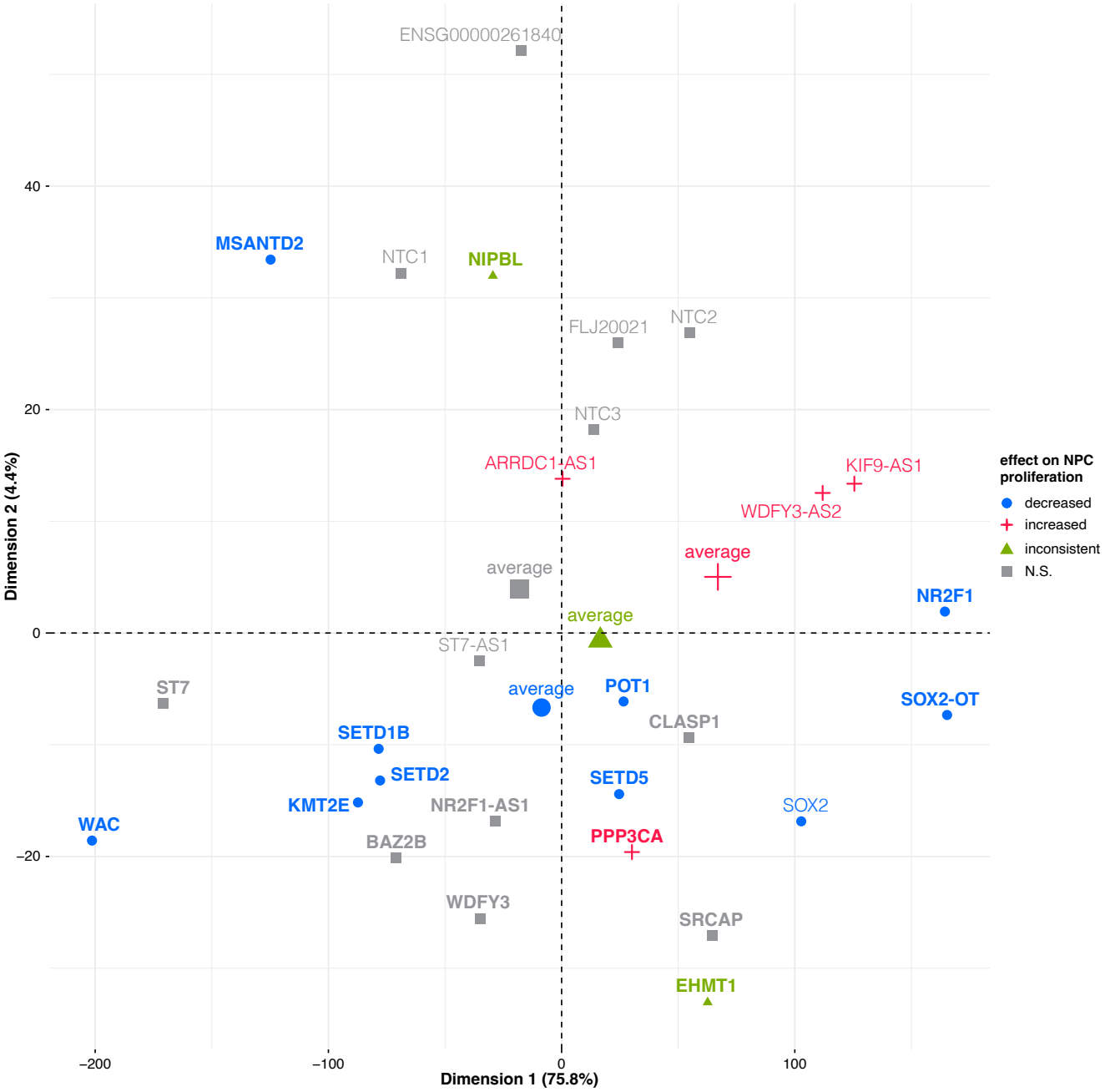
ENSG00000261840
MSANTD2
NTC1
NIPBL
FLJ20021
NTC2
NTC3
ARRDC1-AS1
KIF9-AS1
WDFY3-AS2
average
average
average
NR2F1
ST7-AS1
ST7
average
POT1
SOX2-OT
CLASP1
SETD1B
SETD2
SETD5
KMT2E
NR2F1-AS1
SOX2
WAC
BAZ2B
PPP3CA
WDFY3
SRCAP
EHMT1
40
20
0
−20
−200
−100
0
100
Dimension 2 (4.4%)
Dimension 1 (75.8%)
effect on NPC proliferation
decreased
increased
inconsistent
N.S.

Figure S12

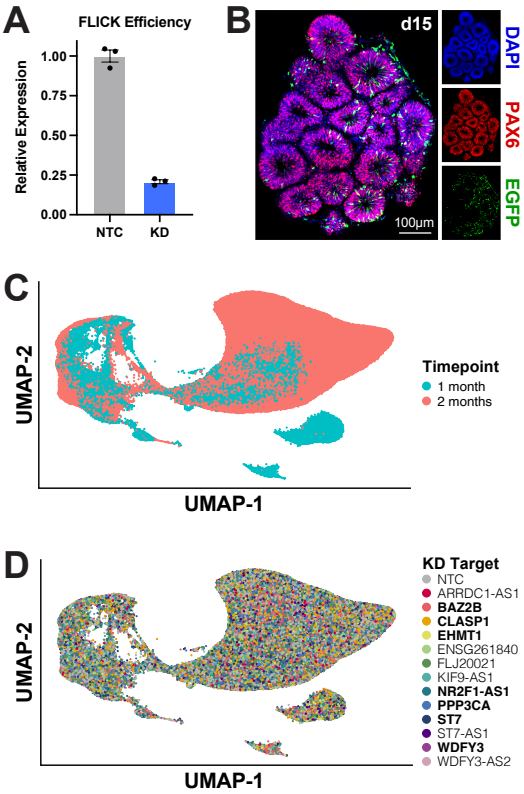
A
FLICK Efficiency
Relative Expression
1.00
0.75
0.50
0.25
0.00
NTC
KD
B
d15
100μm
DAPI
PAX6
EGFP
C
UMAP-2
UMAP-1
Timepoint
1 month
2 months
D
UMAP-2
UMAP-1
KD Target
NTC
ARRDC1-AS1
BAZ2B
CLASP1
EHMT1
ENSG261840
FLJ20021
KIF9-AS1
NR2F1-AS1
PPP3CA
ST7
ST7-AS1
WDFY3
WDFY3-AS2

Figure S13

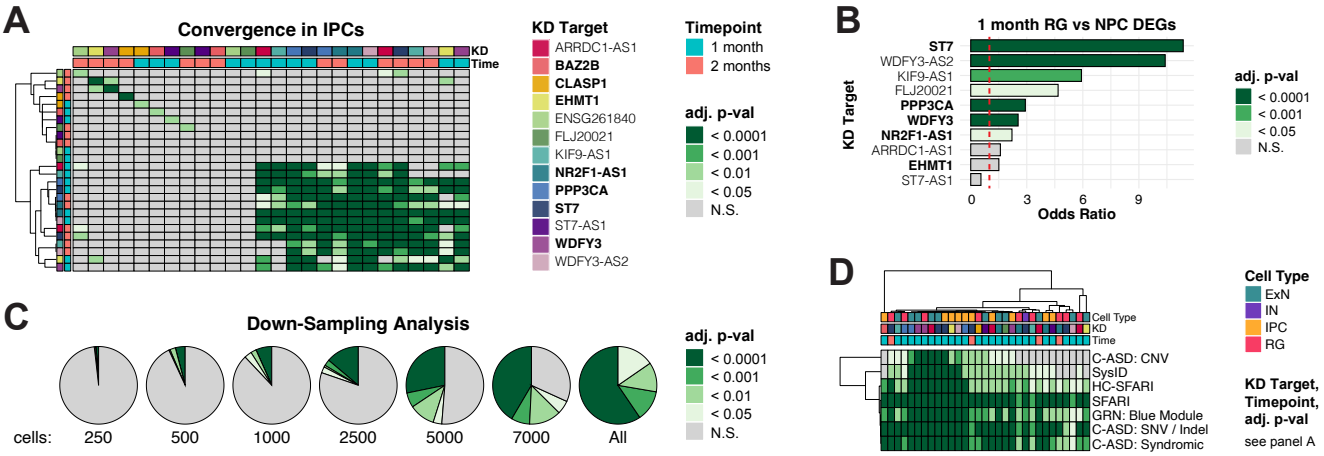
A
Convergence in IPCs
KD
Time
KD Target
ARRDC1-AS1
BAZ2B
CLASP1
EHMT1
ENSG261840
FLJ20021
KIF9-AS1
NR2F1-AS1
PPP3CA
ST7
ST7-AS1
WDFY3
WDFY3-AS2
Timepoint
1 month
2 months
adj. p-val
< 0.0001
< 0.001
< 0.01
< 0.05
N.S.
B
1 month RG vs NPC DEGs
KD Target
ST7
WDFY3-AS2
KIF9-AS1
FLJ20021
PPP3CA
WDFY3
NR2F1-AS1
ARRDC1-AS1
EHMT1
ST7-AS1
0
3
6
9
Odds Ratio
adj. p-val
< 0.0001
< 0.001
< 0.05
N.S.
C
Down-Sampling Analysis
cells:
250
500
1000
2500
5000
7000
All
adj. p-val
< 0.0001
< 0.001
< 0.01
< 0.05
N.S.
D
Cell Type
KD
Time
C-ASD: CNV
SysID
HC-SFARI
SFARI
GRN: Blue Module
C-ASD: SNV / Indel
C-ASD: Syndromic
Cell Type
ExN
IN
IPC
RG
KD Target,
Timepoint,
adj. p-val
see panel A

Figure S14

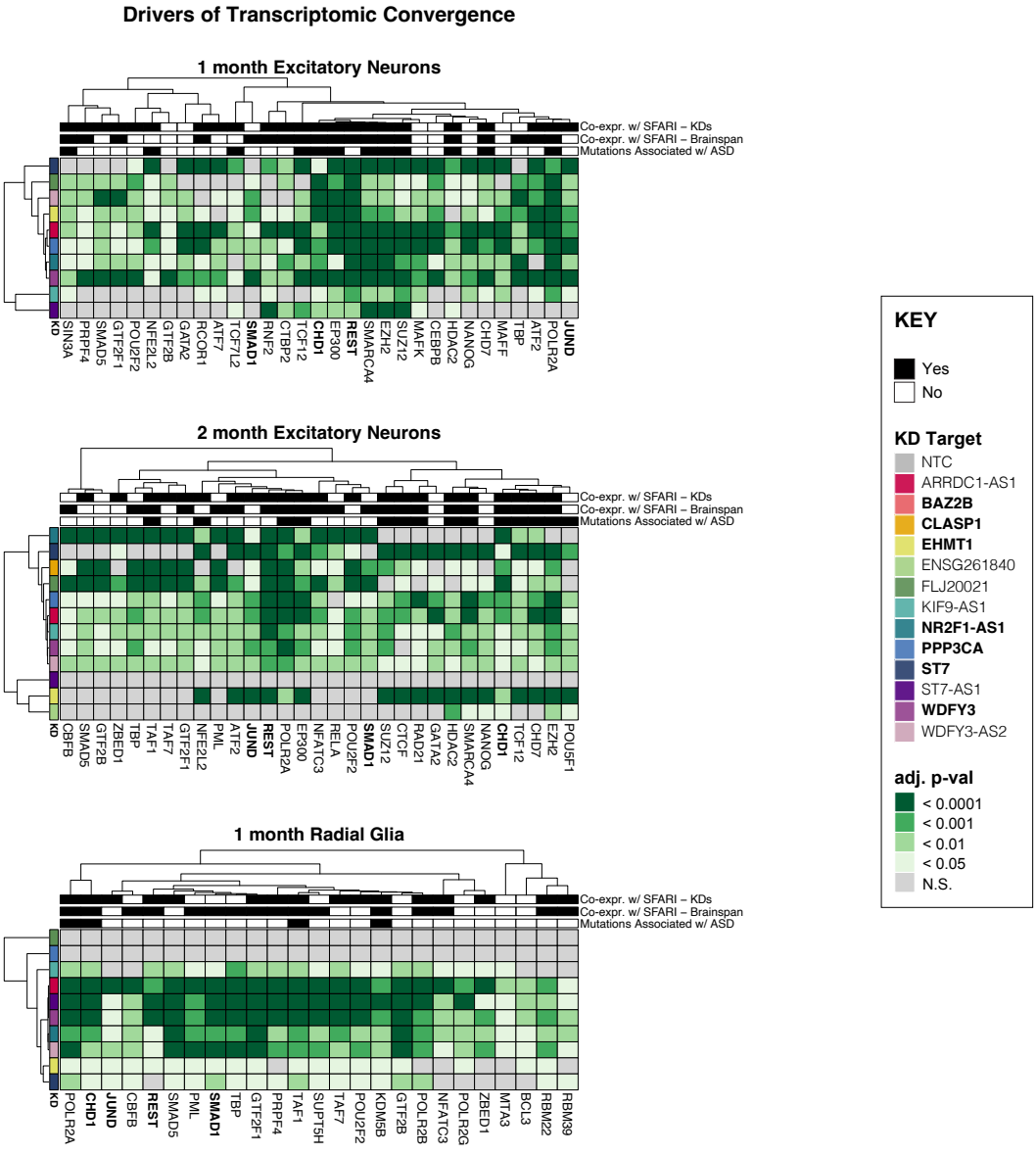
Drivers of Transcriptomic Convergence
1 month Excitatory Neurons
Co-expr. w/ SFARI – KDs
Co-expr. w/ SFARI – Brainspan
Mutations Associated w/ ASD
KD
SIN3A
PRPF4
SMAD5
GTF2F1
POU2F2
NFE2L2
GTF2B
GATA2
RCOR1
ATF7
TCF7L2
SMAD1
RNF2
CTBP2
TCF12
CHD1
EP300
REST
SMARCA4
EZH2
SUZ12
MAFK
CEBPB
HDAC2
NANOG
CHD7
MAFF
TBP
ATF2
POLR2A
JUND
2 month Excitatory Neurons
Co-expr. w/ SFARI – KDs
Co-expr. w/ SFARI – Brainspan
Mutations Associated w/ ASD
KD
CBFB
SMAD5
GTF2B
ZBED1
TBP
TAF1
TAF7
GTF2F1
NFE2L2
PML
ATF2
JUND
REST
POLR2A
EP300
NFATC3
RELA
POU2F2
SMAD1
SUZ12
CTCF
RAD21
GATA2
HDAC2
SMARCA4
NANOG
CHD1
TCF12
CHD7
EZH2
POU5F1
1 month Radial Glia
Co-expr. w/ SFARI – KDs
Co-expr. w/ SFARI – Brainspan
Mutations Associated w/ ASD
KD
POLR2A
CHD1
JUND
CBFB
REST
SMAD5
PML
SMAD1
TBP
GTF2F1
PRPF4
TAF1
SUPT5H
TAF7
POU2F2
KDM5B
GTF2B
POLR2B
NFATC3
POLR2G
ZBED1
MTA3
BCL3
RBM22
RBM39
KEY
Yes
No
KD Target
NTC
ARRDC1-AS1
BAZ2B
CLASP1
EHMT1
ENSG261840
FLJ20021
KIF9-AS1
NR2F1-AS1
PPP3CA
ST7
ST7-AS1
WDFY3
WDFY3-AS2
adj. p-val
< 0.0001
< 0.001
< 0.01
< 0.05
N.S.

Figure S15

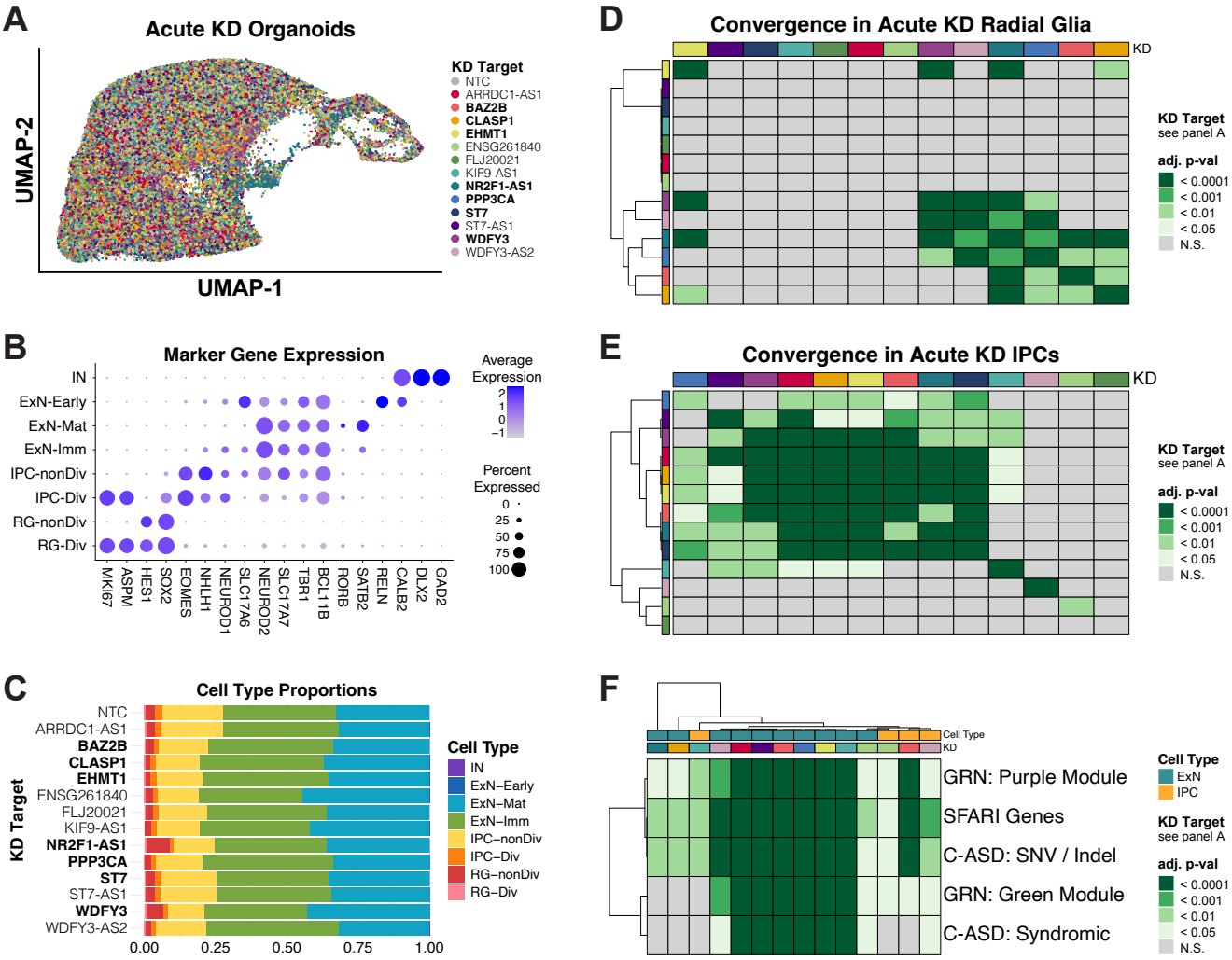
A
Acute KD Organoids
UMAP-2
UMAP-1
KD Target
NTC
ARRDC1-AS1
BAZ2B
CLASP1
EHMT1
ENSG261840
FLJ20021
KIF9-AS1
NR2F1-AS1
PPP3CA
ST7
ST7-AS1
WDFY3
WDFY3-AS2
B
Marker Gene Expression
IN
ExN-Early
ExN-Mat
ExN-Imm
IPC-nonDiv
IPC-Div
RG-nonDiv
RG-Div
MKI67
ASPM
HES1
SOX2
EOMES
NHLH1
NEUROD1
SLC17A6
NEUROD2
SLC17A7
TBR1
BCL11B
RORB
SATB2
RELN
CALB2
DLX2
GAD2
Average Expression
Percent Expressed
C
Cell Type Proportions
KD Target
Cell Type
IN
ExN–Early
ExN–Mat
ExN–Imm
IPC–nonDiv
IPC–Div
RG–nonDiv
RG–Div
0.00
0.25
0.50
0.75
1.00
D
Convergence in Acute KD Radial Glia
KD
KD Target
see panel A
adj. p-val
< 0.0001
< 0.001
< 0.01
< 0.05
N.S.
E
Convergence in Acute KD IPCs
F
Cell Type
KD
GRN: Purple Module
SFARI Genes
C-ASD: SNV / Indel
GRN: Green Module
C-ASD: Syndromic
ExN
IPC

Figure S16