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Supplementary Materials for

GTF2I dosage regulates neuronal differentiation and social behavior in 7q11.23 neurodevelopmental disorders

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This PDF file includes:

Supplementary Material

Figure S1

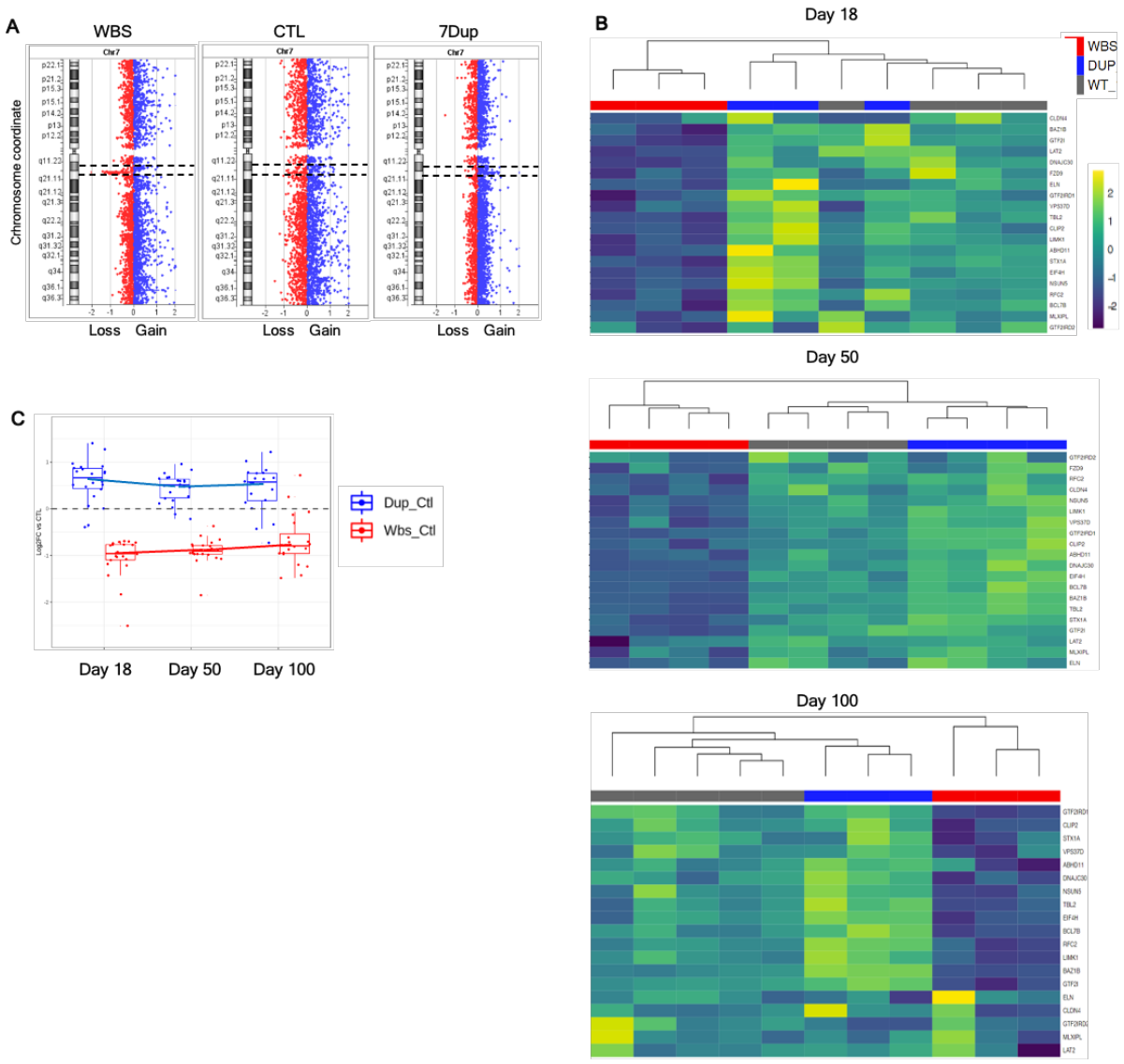


Figure S1. Expression levels of 7q11.23 genes in cortical brain organoids

(A) Array-CGH analysis for chromosome 7, representative of 1 iPSC line per genotype (WBS / CTL / 7DUP) dotted line highlights 7q11.23 locus. For details of the lines used in this study, refer to supplementary table 2. **(B)** Heatmaps reporting the expression levels of the genes located in the 7q11.23 CNV (z-score calculated on the Log2Cpm) from RNASeq transcriptome profiling in WBS, CTL and 7Dup cortical organoids at Day 18, Day 50 and Day 100 of differentiation. **(C)** Log2 fold-change of the genes located in the 7q11.23 CNV from stage-wise RNASeq differential expression analysis, comparing either WBS (in red) or 7Dup (in blue) to CTL. Each dot represents the log2FC value of a single gene of the region at a specific stage, while the boxplots summarize at each stage and in each genotype the overall behavior of the region. Trend lines connect the median for different stages of the same genotype.

Figure S2

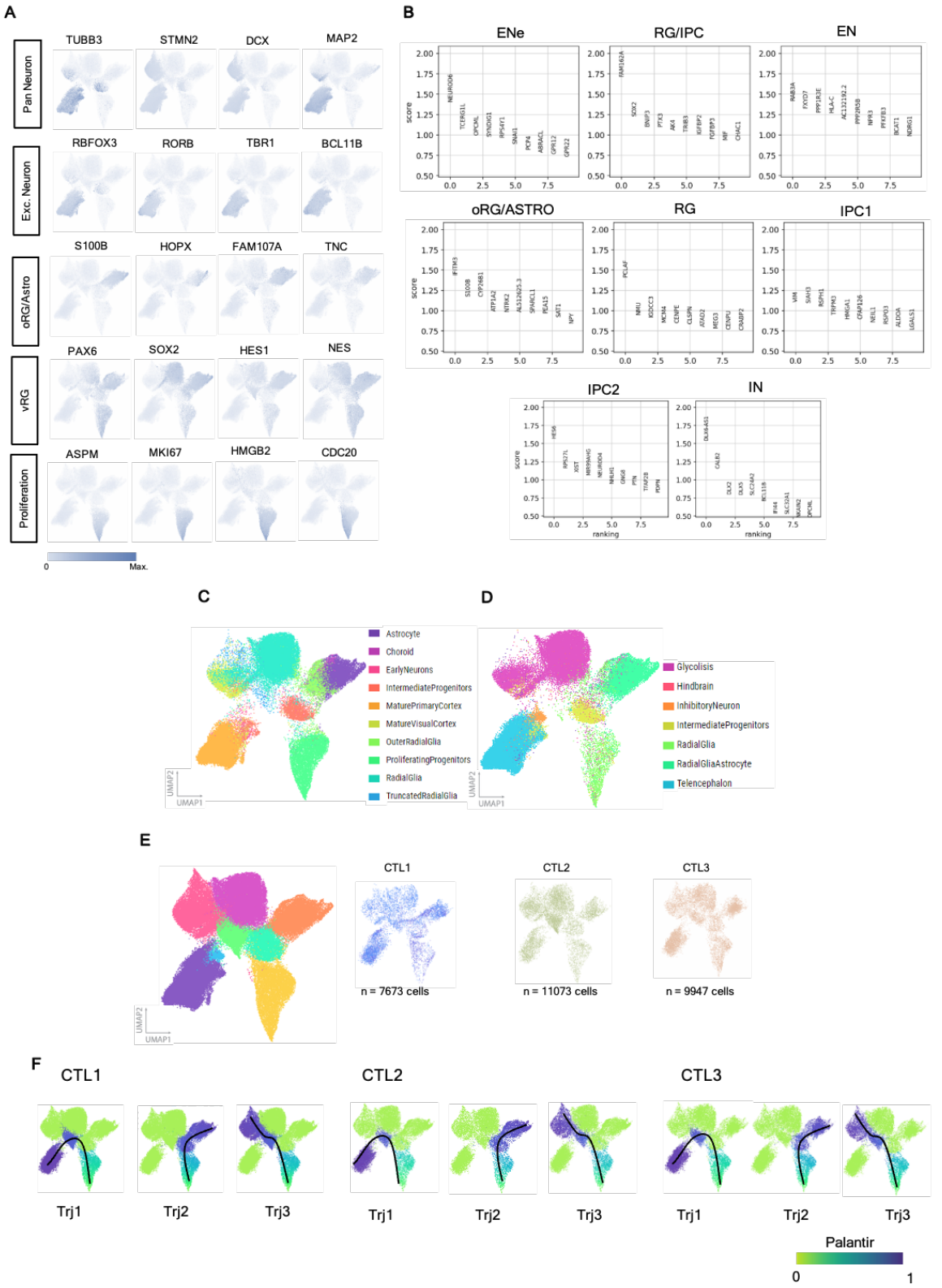

A
Pan Neuron
TUBB3
STMN2
DCX
MAP2
Exc. Neuron
RBFOX3
RORB
TBR1
BCL11B
oRG/Astro
S100B
HOPX
FAM107A
TNC
vRG
PAX6
SOX2
HES1
NES
Proliferation
ASPM
MKI67
HMGB2
CDC20
0
Max.
B
ENe
RG/IPC
EN
oRG/ASTRO
RG
IPC1
IPC2
IN
score
ranking
C
Astrocyte
Choroid
EarlyNeurons
IntermediateProgenitors
MaturePrimaryCortex
MatureVisualCortex
OuterRadialGlia
ProliferatingProgenitors
RadialGlia
TruncatedRadialGlia
UMAP1
UMAP2
D
Glycolisis
Hindbrain
InhibitoryNeuron
IntermediateProgenitors
RadialGlia
RadialGliaAstrocyte
Telencephalon
E
CTL1
CTL2
CTL3
n = 7673 cells
n = 11073 cells
n = 9947 cells
F
CTL1
CTL2
CTL3
Trj1
Trj2
Trj3
Palantir
0
1

Figure S2. Cortical brain organoids show prototypical cell populations of cortex development

(A) Uniform manifold approximation and projection (UMAP) with color coded the most representative marker per population. **(B)** Top 10 marker genes for each identified cluster used for the manual annotations. **(C-D)** UMAP with color code representing automatically annotated cells using gene signature extracted from *(30, 87)* using SCINA *(86)*. **(E)** UMAP with color code representing cell distribution in the UMAP among the 3 CTL lines. **(F)** UMAP with color code representing the 3 main differentiation trajectories, in all control genotypes are the same (Trj = Palantir trajectory).

Figure S3

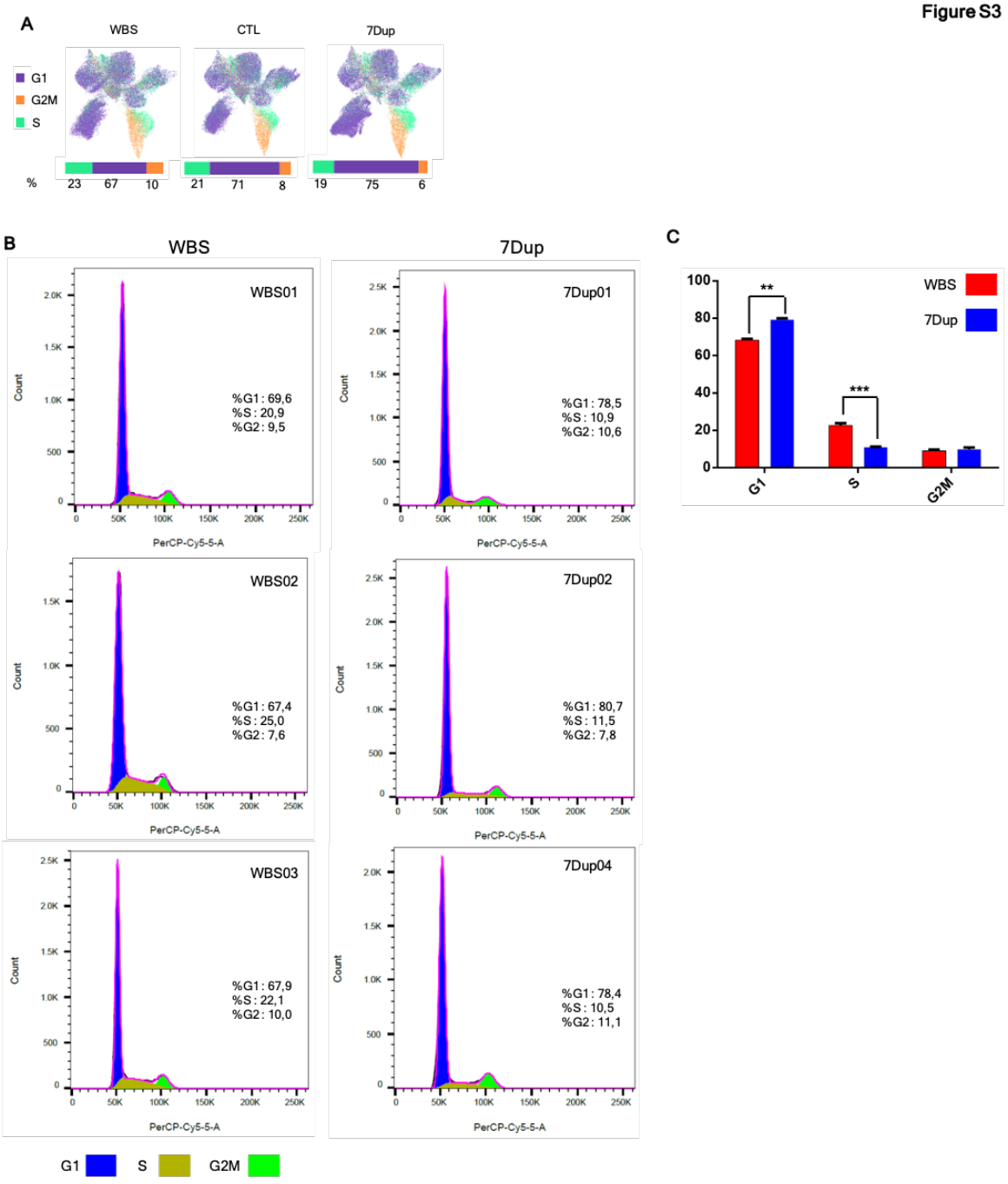
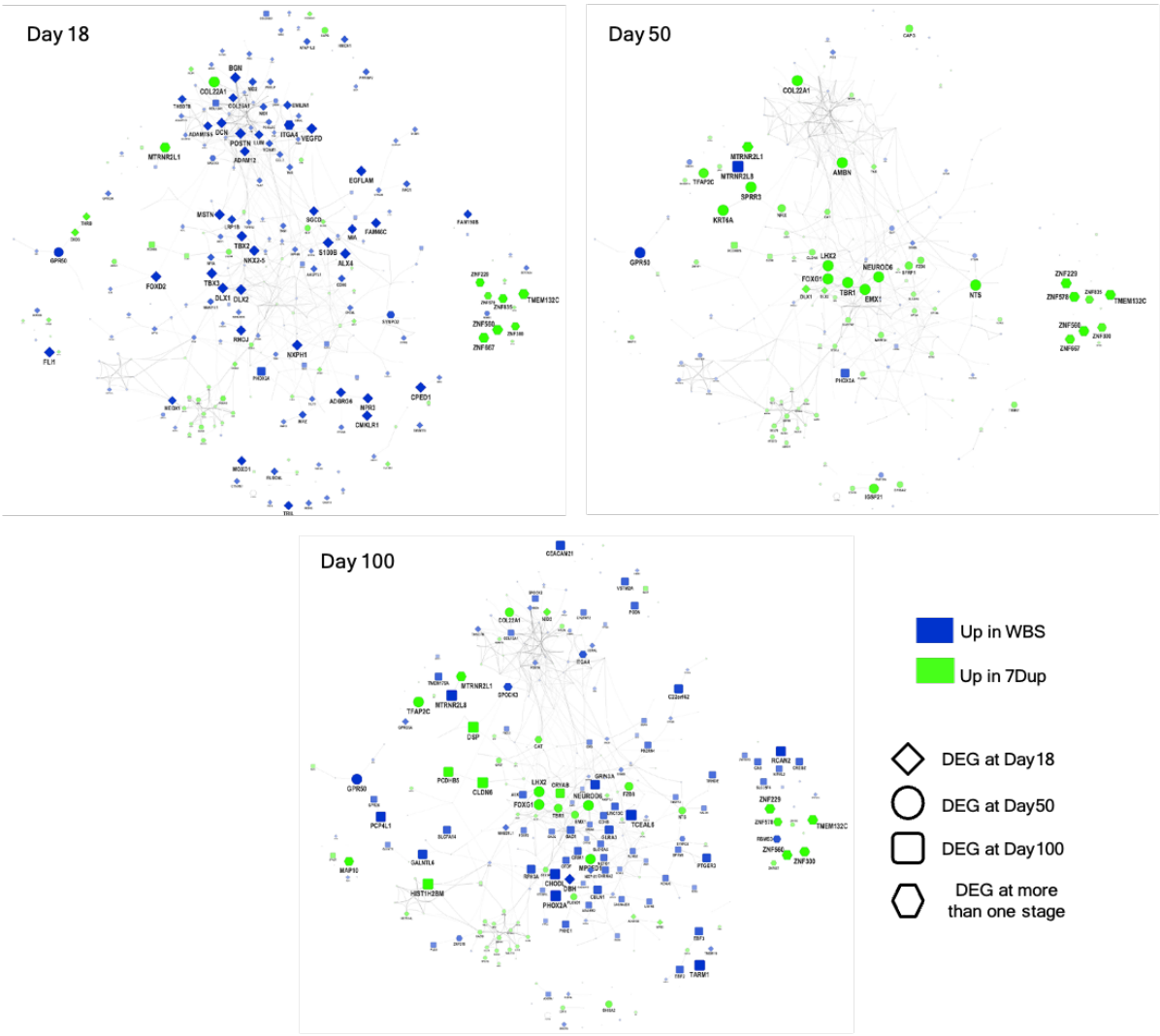

A
WBS
CTL
7Dup
G1
G2M
S
%
23 67 10
21 71 8
19 75 6
B
WBS
7Dup
WBS01
%G1 : 69,6
%S : 20,9
%G2 : 9,5
7Dup01
%G1 : 78,5
%S : 10,9
%G2 : 10,6
WBS02
%G1 : 67,4
%S : 25,0
%G2 : 7,6
7Dup02
%G1 : 80,7
%S : 11,5
%G2 : 7,8
WBS03
%G1 : 67,9
%S : 22,1
%G2 : 10,0
7Dup04
%G1 : 78,4
%S : 10,5
%G2 : 11,1
Count
PerCP-Cy5-5-A
G1
S
G2M
C
WBS
7Dup
**
***
G1
S
G2M

Figure S3. Distribution of cell cycle phases in WBS and 7Dup COs

(**A**) Percentage of total number of cells in each cell cycle phase and distribution in UMAP at day 50 stratified by condition. (**B**) Histogram plot of propidium iodine-stained single cells from Day 18 orgs quantified by flow cytometry comparing WBS and 7DUP samples from different individuals (WBS01, WBS02, WBS03 and 7DUP01, 7DUP02, 7DUP04). Each plot is representative of a pool of 3 organoids per individual. (N = 4 - WBS, 4- CTL and 4- 7Dup). Student's t-test comparing WBS and 7DUP using the aggregated percentages of 3 individuals per condition ** $p > 0.01$ *** $p > 0.005$.

Figure S4

A


Day 18
Day 50
Day 100
Up in WBS
Up in 7Dup
DEG at Day18
DEG at Day50
DEG at Day100
DEG at more than one stage

B

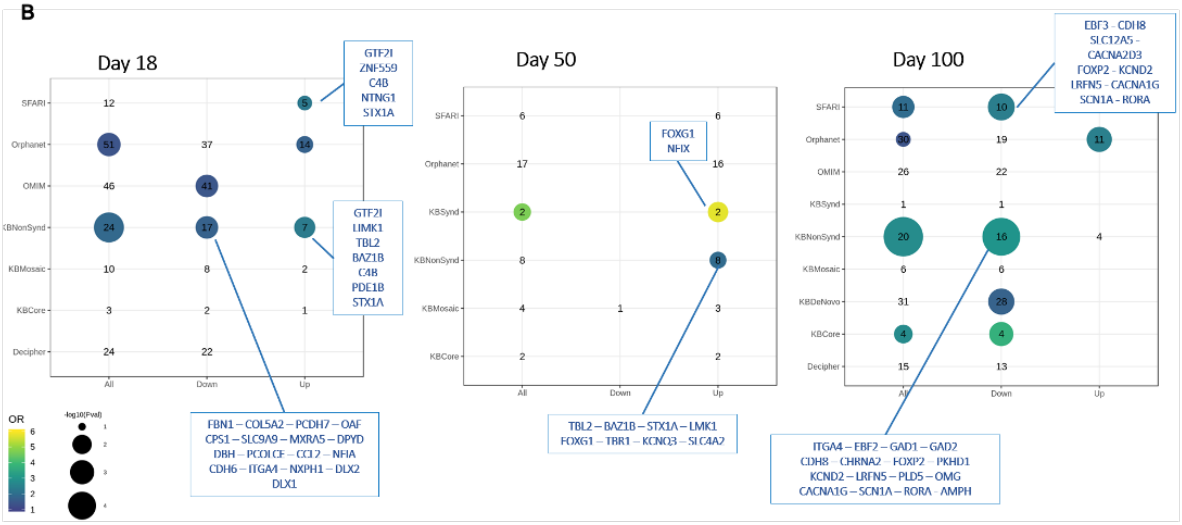

Day 18
Day 50
Day 100
SFARI
Orphanet
OMIM
KBNonSynd
KBMosaic
KBCore
KBDeNovo
Decipher
All
Down
Up
OR
GTF2I
ZNF559
C4B
NTNG1
STX1A
GTF2I
LIMK1
TBL2
BAZ1B
C4B
PDE1B
STX1A
FBN1 – COL5A2 – PCDH7 – OAF
CPS1 – SLC9A9 – MXRA5 – DPYD
DBH – PCOLCE – CCL2 – NFIA
CDH6 – ITGA4 – NXPH1 – DLX2
DLX1
FOXG1
NFIX
TBL2 – BAZ1B – STX1A – LMK1
FOXG1 – TBR1 – KCNQ3 – SLC4A2
EBF3 – CDH8
SLC12A5 – CACNA2D3
FOXP2 – KCND2
LRFN5 – CACNA1G
SCN1A – RORA
ITGA4 – EBF2 – GAD1 – GAD2
CDH8 – CHRNA2 – FOXP2 – PKHD1
KCND2 – LRFN5 – PLD5 – OMG
CACNA1G – SCN1A – RORA – AMPH

Figure S4. Genes modulated by 7q11.23 gene dosage imbalance significantly overlap with disease-related knowledge bases

(A) STRING-based (https://string-db.org/) network reconstructed for the genes found modulated (FDR < 0.1, log2FC > 1) in at least one time-point by stage-wise differential expression analysis. At each examined stage, node (gene) size and color represent respectively the magnitude and direction of Log2FC, with genes more expressed in 7Dup and WBS in green and blue respectively. Across the three visualizations, node shape highlights the stage in which the genes are significantly modulated: diamond for Day 18, circle for Day 50, square for Day100 and hexagon for genes significant in more than one stage. **(B)** Dotplot showing the results of the overlap analysis between genes differentially expressed in 7Dup compared to WBS and disease-related knowledge bases. DEGs (FDR < 0.1, log2FC > 1 as absolute value, stage-wise DEA in cortical organoids) are analyzed either grouped or split in up- and down-regulated. The plot reports the number of overlapping genes for Odds Ratio (OR) > 1 and the dot for p-values < 0.1. Dot size and color represent the p-value and the OR respectively.

Figure S5

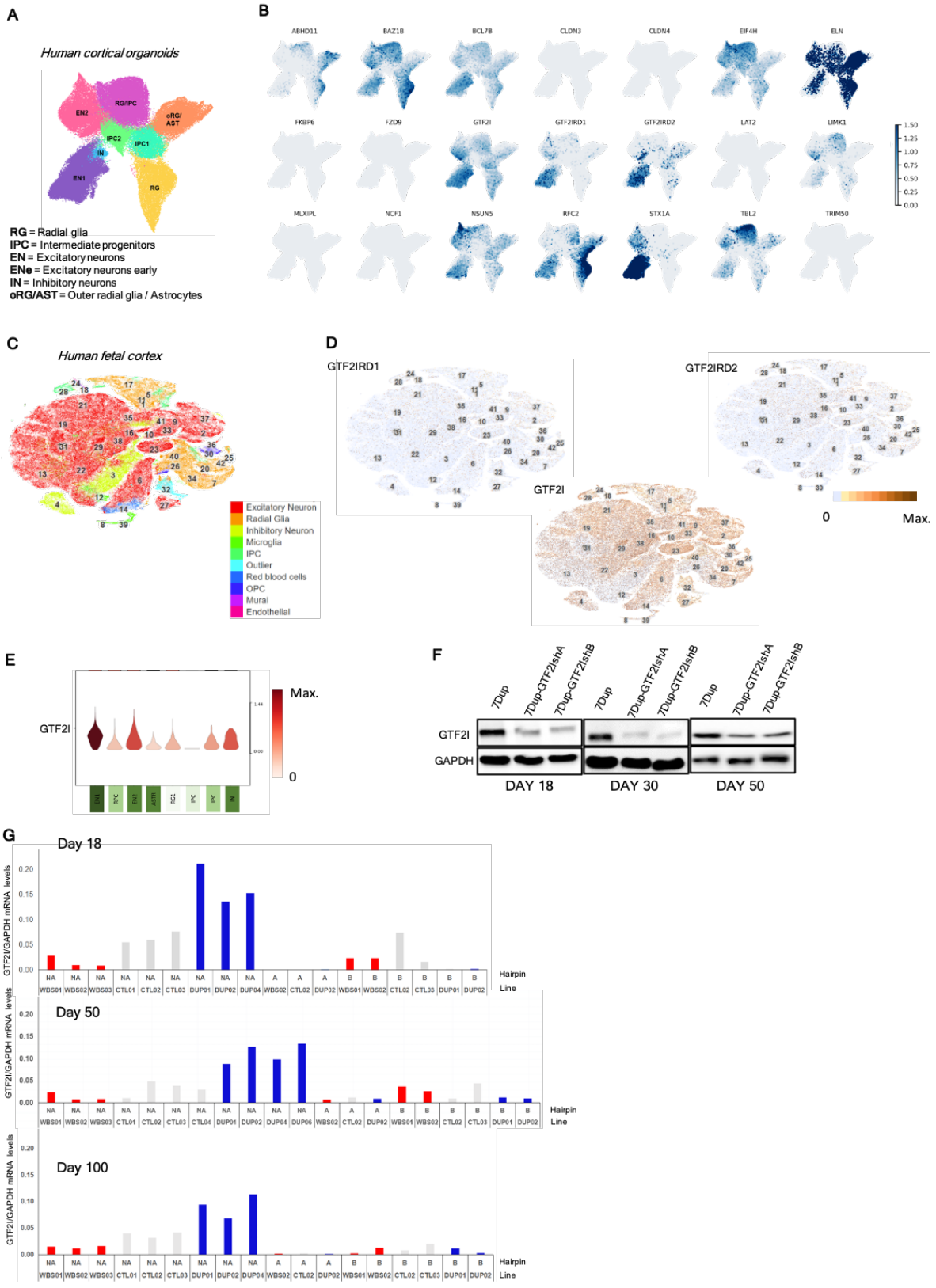

A
Human cortical organoids
EN2
RG/IPC
oRG/ AST
IPC2
IPC1
IN
EN1
RG
RG = Radial glia
IPC = Intermediate progenitors
EN = Excitatory neurons
ENe = Excitatory neurons early
IN = Inhibitory neurons
oRG/AST = Outer radial glia / Astrocytes
B
ABHD11
BAZ1B
BCL7B
CLDN3
CLDN4
EIF4H
ELN
FKBP6
FZD9
GTF2I
GTF2IRD1
GTF2IRD2
LAT2
LIMK1
MLXIPL
NCF1
NSUN5
RFC2
STX1A
TBL2
TRIM50
1.50
1.25
1.00
0.75
0.50
0.25
0.00
C
Human fetal cortex
Excitatory Neuron
Radial Glia
Inhibitory Neuron
Microglia
IPC
Outlier
Red blood cells
OPC
Mural
Endothelial
D
GTF2IRD1
GTF2IRD2
GTF2I
0
Max.
E
GTF2I
Max.
0
F
7Dup
7Dup-GTF2IshA
7Dup-GTF2IshB
GTF2I
GAPDH
DAY 18
DAY 30
DAY 50
G
Day 18
Day 50
Day 100
GTF2I/GAPDH mRNA levels
Hairpin
Line

Figure S5. *GTF2I* expression levels in fetal brain and cortical organoid

(A) UMAP with color coded the identified populations (as shown in Fig. 1B). **(B)** UMAP showing expression levels in control COs for genes in the 7q11.23 locus (missing genes are absent in the integration matrix). **(C)** Seurat tSNE showing the population annotation from nearly 200,000 primary cells sampled from GW6-22 from seven cortical regions, including PFC, V1, motor, somatosensory, temporal, parietal and hippocampus. Annotation taken from the UCSC cell data browser. Cell Browser dataset ID: organoidreportcard/primary10X. Public dataset published here: (*91*) **(D)** Expression levels in human fetal primary brain cells for *GTF2I, GTF2IRD1* and *GTF2IRD2.* **(E)** Breakdown of expression levels in control in COs for GTF*2I* between different populations from a single cell dataset. **(F)** Western blot showing the downregulation of *GTF2I* using two different short hairpins respectively, at Day 18, 30 and 50, using *GAPDH* as loading control. Each lane is representative from a pool of n = 3 organoids/cell line, 2 cell lines/genotype. **(G)** Expression levels of *GTF2I* in COs at Days 18, 30 and 50 by quantitative RT-PCR. Values are expressed as normalized expression over the average for *GAPDH* levels. Each bar represents a pool of n = 3 organoids from a single organoid line.

Figure S6

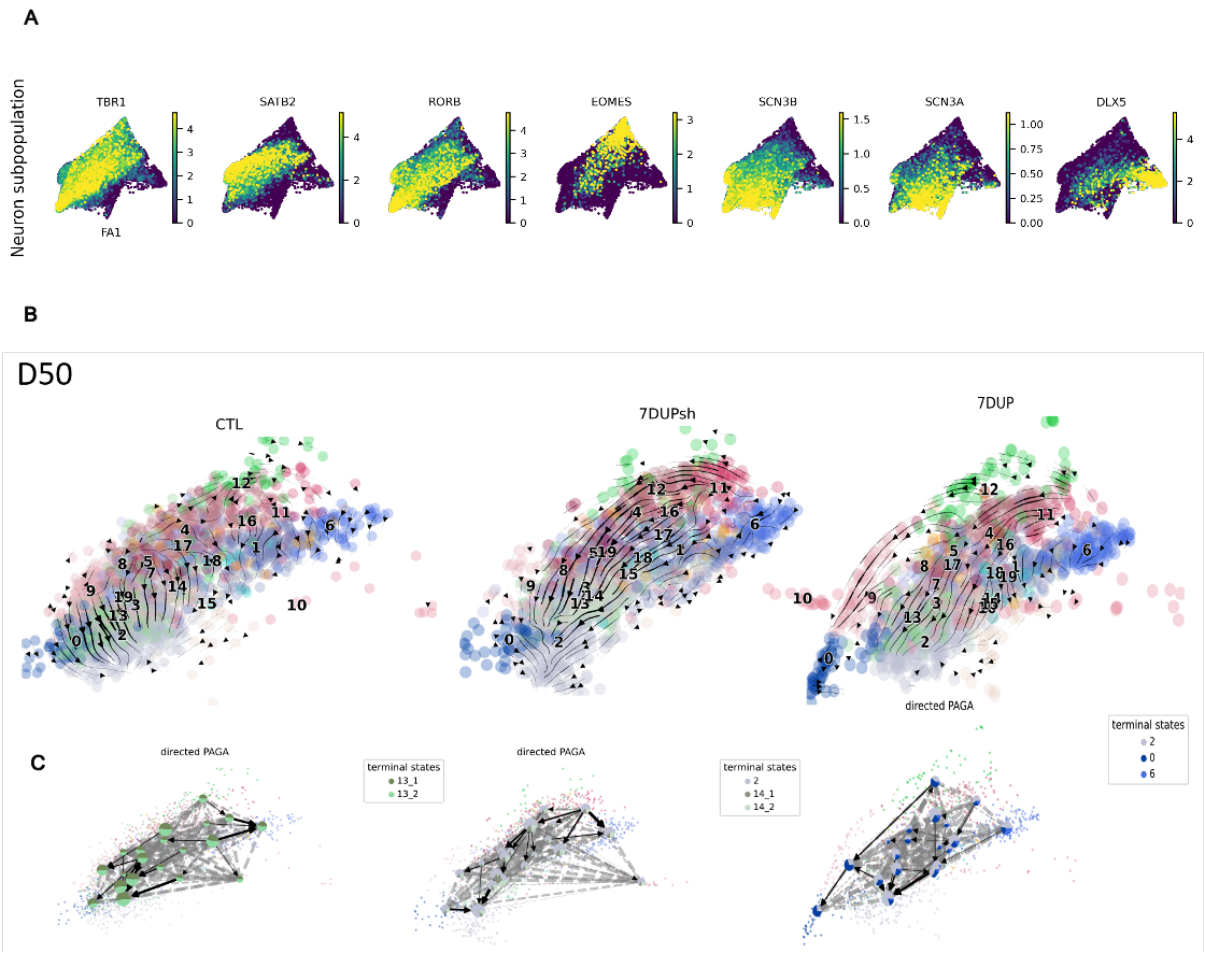

A
Neuron subpopulation
TBR1
SATB2
RORB
EOMES
SCN3B
SCN3A
DLX5
FA1
B
D50
CTL
7DUPsh
7DUP
directed PAGA
C
directed PAGA
terminal states
13_1
13_2
directed PAGA
terminal states
2
14_1
14_2
terminal states
2
0
6

Figure S6. Gene dosage imbalances at 7q11.23 dysregulated normal differentiation trajectory at D50 and partial rescue by the knockdown of *GTF2I*

(A) Force-directed graph stratified for genotype (CTL, 7DupshGTF2I and 7Dup) from left to right at D50, with color coded the identified subpopulations. The arrow defines the inferred differentiation trajectory identified by Velocyto. **(B)** PAGA with position identified by force-directed graph subpopulations location. Color code identifies the number of cells per cluster that will be most probably going in the identified terminal state.

Figure S7

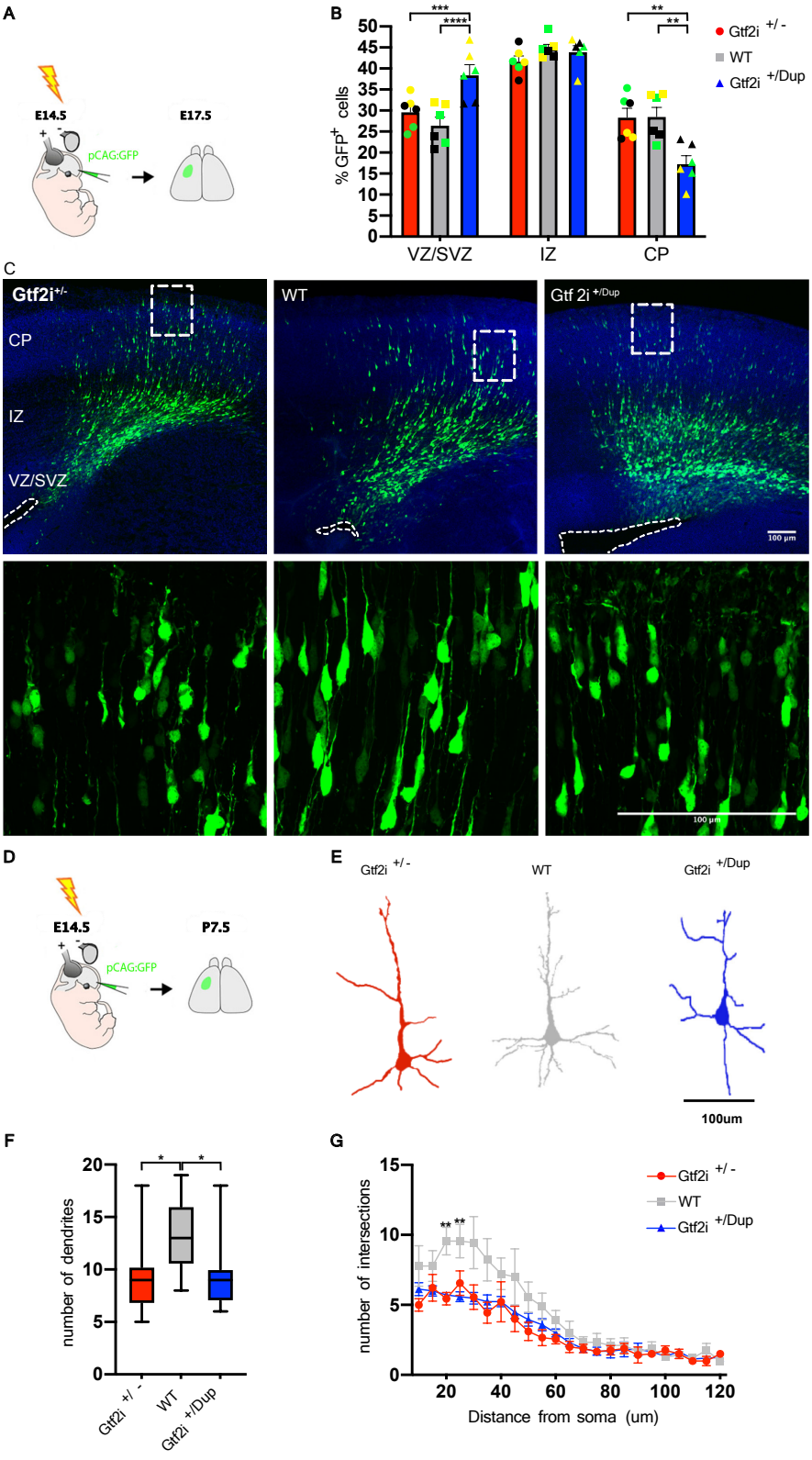

A
E14.5
E17.5
pCAG:GFP
B
% GFP+ cells
VZ/SVZ
IZ
CP
Gtf2i +/ -
WT
Gtf2i +/Dup
C
Gtf2i+/-
WT
Gtf 2i +/Dup
CP
IZ
VZ/SVZ
100 µm
D
E14.5
P7.5
pCAG:GFP
E
Gtf2i +/ -
WT
Gtf2i +/Dup
100um
F
number of dendrites
Gtf2i +/ -
WT
Gtf2i +/Dup
G
number of intersections
Distance from soma (um)
Gtf2i +/ -
WT
Gtf2i +/Dup

Figure S7. *Gtf2i* dosage affect neuronal migration and neuronal morphology in mouse cortex

(A) Schematic representation of *in utero* electroporation experiments. Mice were electroporated at E14.5 and analysed at E17.5. **(B)** Bar diagram depicting neuronal migration defects in *Gtf2i*$^{+/Dup}$ mice. Note the difference between the increased number of GFP^{+} neuronal progenitors in VZ/SVZ and reduced number of GFP^{+} neurons in CP. Gtf2i$^{+/-}$, WT, *Gtf2i*$^{+/Dup}$; n=6 sections from 3 mice/group. Same color shapes denote sections from the same mouse. Data shown as mean±SEM. Mixed linear regression model considering sections of the same cortical area (VZ, IZ, CP) as replicates from the same mouse/sample (~individual+genotype), and different brain regions as covariate (~individual+region+genotype). Significance level was set to $p<0.05$. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. **(C)** (Upper panel) Representative images of electroporated brains from *Gtf2i*$^{+/-}$, WT and *Gtf2i*$^{+/Dup}$ mice at E17.5. (Lower panel) Insets of upper panel figures showing GFP^{+} neurons in CP. **(D)** Schematic representation of *in utero* electroporation experiments. Mice were electroporated at E14.5 and analyzed at P7.5. **(E)**. Representative morphology of cortical neurons from *Gtf2i*$^{+/-}$, WT and *Gtf2i*$^{+/Dup}$ mice at P7.5. **(F)** Box plot showing reduced number of dendrites in mutant mice compared to WT. *Gtf2i*$^{+/-}$, n=10 neurons from 3 mice; WT, n=9 from 4 mice; *Gtf2i*$^{+/Dup}$, n=15 from 5 mice. Kruskal-Wallis followed by Dunn's multiple comparisons test. **(G)**. Plot showing number of intersections from Sholl analysis. *Gtf2i*$^{+/-}$, n=19 neurons from 3 mice; WT, n=9 from 4 mice; *Gtf2i*$^{+/Dup}$, n=15 from 5 mice. Data shown as mean±SEM. Kruskal-Wallis followed by Dunn's multiple comparisons test. Significance level was set to $p<0.05$. *p<0.05; **p<0.01.

Figure S8

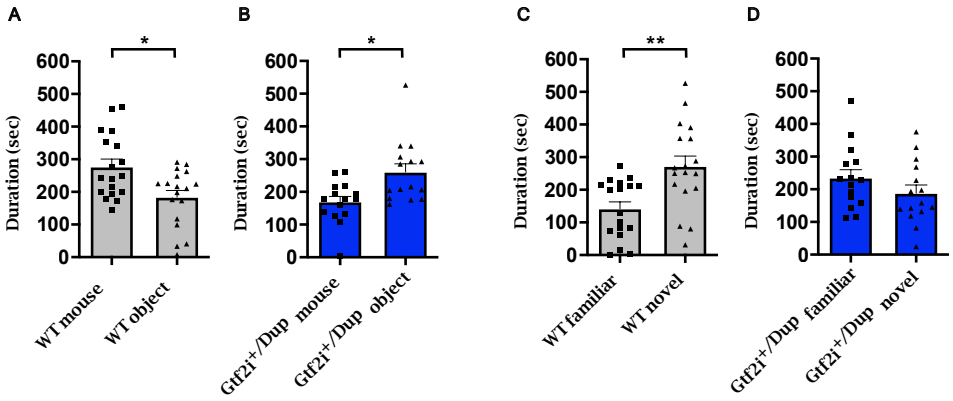

A
B
C
D
Duration (sec)
0
100
200
300
400
500
600
*
*
**
WT mouse
WT object
Gtf2i+/Dup mouse
Gtf2i+/Dup object
WT familiar
WT novel
Gtf2i+/Dup familiar
Gtf2i+/Dup novel

Figure S8. *Gtf2i* duplication causes social preference impairments in female mice

Bar plots with dots depicting the quantification of social preference **(A, B)** and social novelty **(C, D)** in female Wt (n=18) and *Gtf2i*$^{+/Dup}$ mice (n=15). Statistical analyses were performed using paired Student's t-test followed by Holm-Bonferroni correction for multiple testing. Significance level $p<0.05$. $^{*}p<0.05$; $^{**}p<0.01$.

Figure S9

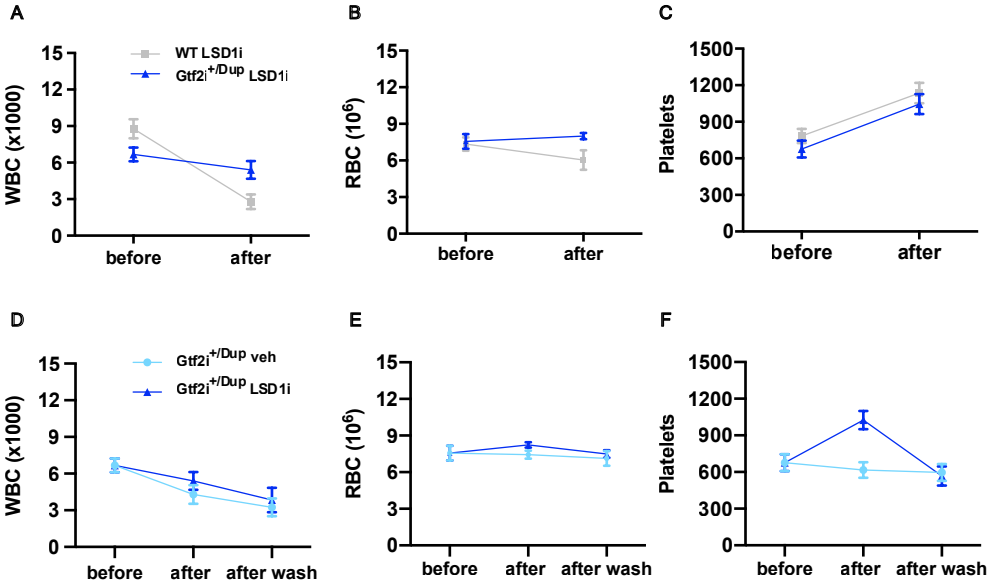

A
WBC (x1000)
WT LSD1i
Gtf2i+/Dup LSD1i
before
after
B
RBC (10^6)
before
after
C
Platelets
before
after
D
WBC (x1000)
Gtf2i+/Dup veh
Gtf2i+/Dup LSD1i
before
after
after wash
E
RBC (10^6)
before
after
after wash
F
Platelets
before
after
after wash

Figure S9. Blood factors remain within normal range following LSD1 inhibitor administration

(A-C) Line charts depicting values respectively, of white blood cell (WBC), red blood cells (RBC) and platelets before (Wt n=18; *Gtf2i*$^{+/Dup}$ n=16), and after (Wt n=8; *Gtf2i*$^{+/Dup}$ n=21) administration of LSD1 inhibitor. **(D-F)** Line charts depicting values respectively, of WBC, RBC and platelets in *Gtf2i*$^{+/Dup}$ mice before (n=16), after administration of LSD1 inhibitor or vehicle (LSD1i n=21, vehicle; n=16), and after drug 'washout' (LSD1i; n=13; vehicle; n=11).

Figure 10

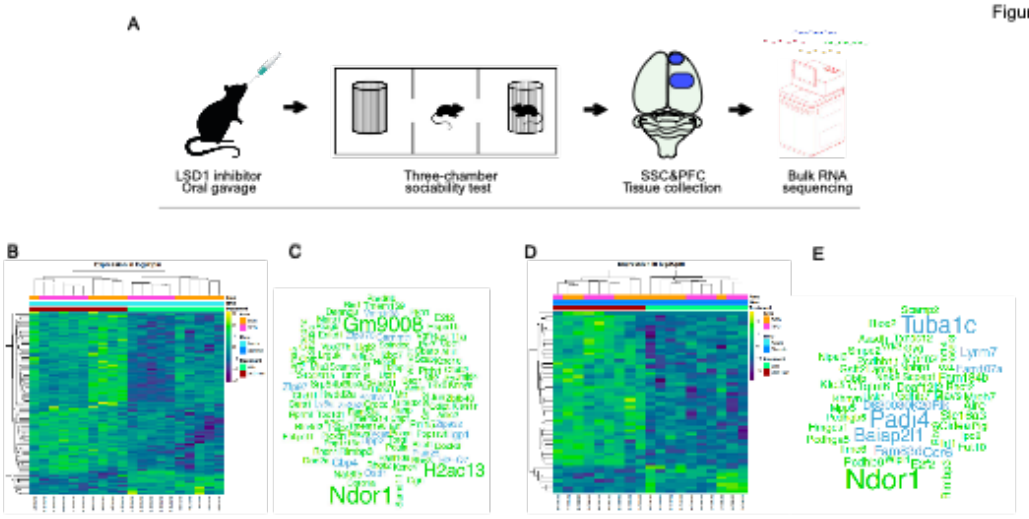

A
LSD1 inhibitor
Oral gavage
Three-chamber
sociability test
SSC&PFC
Tissue collection
Bulk RNA
sequencing
B
C
Gm9008
H2ac13
Ndor1
D
E
Tuba1c
Padi4
Basp2l1
Ndor1

Figure S10. LSD1 inhibition affects neurodevelopment and synaptic organization in the cortex of *Gtf2i*$^{+/Dup}$ mice

(A) Schematic representation of the experimental flow. *Gtf2i*$^{+/Dup}$ mice received either a single (acute) or 2 administrations of LSD1 inhibitor/week over 2 weeks (semi-chronic) followed by three-chambered sociability test. *Prefrontal* (PFC) and *somatosensory* (SSC) cortices were dissected, flash frozen and RNA extracted for bulk transcriptomic profiling. **(B-C)** Heatmap and word cloud respectively depicting the expression levels (as z-scores) and gene symbol (green: up-regulated; blue: down-regulated) of the 114 genes identified as DEGs following a single administration of LSD1 inhibitor compared to vehicle in pre-frontal and somatosensory cortex. Vehicle, n=5 mice and LSD1 inhibitor, n=5 for both cortical areas. **(D-E)** Chronic administration of LSD1 inhibitor results in 54 DEGs as shown in the heatmap and the word cloud. Genes from the protocadherins family were prominently upregulated as a result of chronic LSD1 inhibition. Note in both acute and chronic paradigms, the upregulation of most genes after inhibition of the transcriptional suppressor LSD1. Vehicle, n=5 mice for both areas; LSD1 inhibitor, n=5 mice for SSC and n=4 mice for PFC.

Supplementary Tables

Table S1. Antibodies dilutions

Antibodies	Vendor	Product number	Application	Concentration
Pff3	Abcam	ab5169	IF	1:250

Ki67	Abcam	ab15580	IF	1:500
Tbr2	Abcam	ab23345	IF	1:500
Pax6	Biolegend	901301	IF	1:300
Tbr1	Abcam	ab31940	IF	1:250
Blc11b	Abcam	ab18465	IF	1:500
Cux1	Santa Cruz	sc-13024	IF	1:500
Gfap	Abcam	ab53554	IF	1:500
Gtf2i	BD Biosciences	610942	WB	1:3000
Gapdh	Millipore	ABS16	WB	1:10000

Table S2. iPSC lines used in each experiment

Cell line	Gender	Age	Kinship	Experiment	Reference

WBS01	M		Son of CTL01	Bulk and single cell RNAseq, IF, Proteomics	(*11*)
WBS02	F		-	Bulk and single cell RNAseq, IF, Proteomics	(*11*)
WBS03	F		-	Bulk and single cell RNAseq, IF, Proteomics	(*11*)
WBS04	M		-	Bulk and single cell RNAseq	(*11*)
CTL01	F		Mother of WBS01	Bulk and single cell RNAseq, IF, Proteomics	(*11*)
CTL02	M	Neonate		Bulk and single cell RNAseq, IF, Proteomics	(*11*)
CTL08	M	55-59 years old		Bulk and single cell RNAseq, IF, Proteomics	
CTL04	F			Bulk and single cell RNAseq	
CTL09	M			Bulk RNAseq	
DUP01	M			Bulk and single cell RNAseq, IF, Proteomics	(*11*)
DUP02	F			Bulk and single cell RNAseq,	(*11*)

				IF, Proteomics	
DUP04	M	4 years old	Sibling of DUP06	Bulk and single cell RNAseq, IF, Proteomics	(3)
DUP06	M	9 years old	Sibling of DUP04	Bulk and single cell RNAseq	(3)

Table S3. Collection timepoints for each experiment with cortical organoids

Experiment / Cell line	Day 18	Day 50	Day 100
Bulk RNAseq	X	X	X
scRNAseq		X	X
Immunostaining		X	X
Proteomics		X	
Flow Cytometry	X		