Cell Reports, Volume *36*

Supplemental information

Patient-derived iPSC-cerebral organoid modeling of the 17q11.2 microdeletion syndrome establishes *CRLF3* as a critical regulator of neurogenesis

Michelle L. Wegscheid, Corina Anastasaki, Kelly A. Hartigan, Olivia M. Cobb, Jason B. Papke, Jennifer N. Traber, Stephanie M. Morris, and David H. Gutmann

Supplemental Data Contents

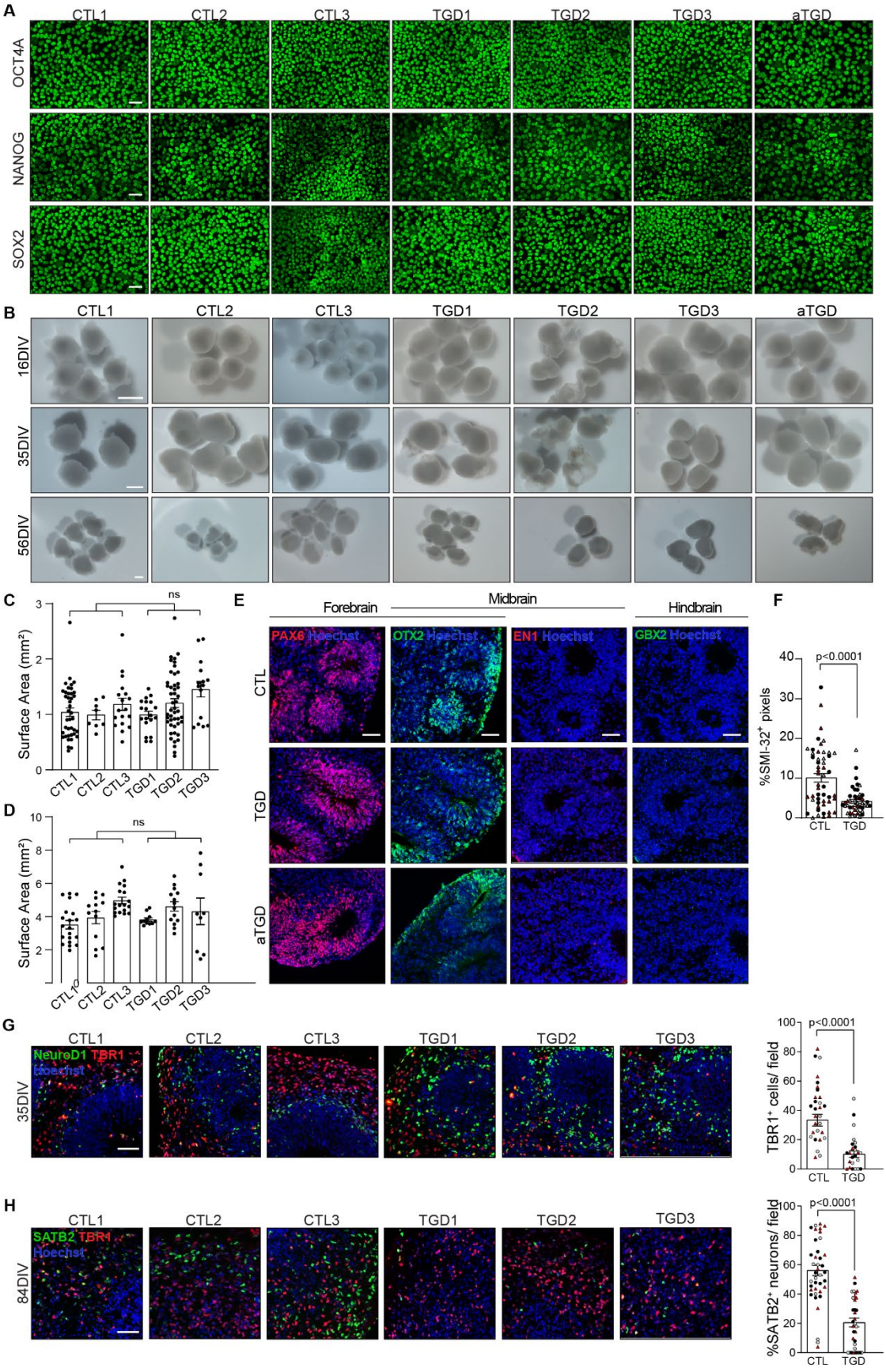

A
CTL1
CTL2
CTL3
TGD1
TGD2
TGD3
aTGD
OCT4A
NANOG
SOX2
B
16DIV
35DIV
56DIV
C
Surface Area (mm²)
ns
D
E
Forebrain
Midbrain
Hindbrain
PAX6 Hoechst
OTX2 Hoechst
EN1 Hoechst
GBX2 Hoechst
CTL
TGD
aTGD
F
%SMI-32⁺ pixels
p<0.0001
G
NeuroD1 TBR1
Hoechst
TBR1⁺ cells/ field
H
SATB2 TBR1
Hoechst
84DIV
%SATB2⁺ neurons/ field

Figure S1 (related to Figure 1). Patient-derived hiPSCs and hCOs.

(**A**) Representative images of hiPSCs immunolabeled for pluripotency markers OCT4A, NANOG, and SOX2. Scale bars: 50 µm. (**B**) Representative bright-field images of hCOs at 16, 35 and 56DIV. Scale bars: 1 mm. (**C-D**) Quantification of surface areas of hCOs at (**C**) 16DIV and (**D**) 84DIV. (**E**) Representative immunofluorescence images of 16DIV CTL (CTL1), TGD (TGD1) and aTGD hCOs immunolabeled for dorsal forebrain (PAX6, OTX2), midbrain (OTX2, EN1) and hindbrain (GBX2) markers. Scale bars: 50 µm. (**F**) (related to **Figure 1I**) Quantitation of SMI-32$^+$ immunopositive dendrites in 35DIV TGD relative to CTL hCOs. (**G**) Representative images of 35DIV CTL and TGD hCOs immunolabeled for early-stage immature neurons (NeuroD1) and deep-layer cortical neurons (TBR1) and quantification of the number of TBR1$^+$ deep-layer neurons per image field in hCOs at 35DIV. (**H**) Representative images of 84DIV CTL and TGD hCOs immunolabeled for deep-layer (TBR1) and upper-layer (SATB2) neurons and quantification of %SATB2$^+$ upper-layer neurons in hCOs at 84DIV. Scale bars, 100 µm. Independent hiPSC lines representing three different CTL or TGD lines (black, CTL1 / TGD1; white, CTL2 / TGD2; red, CTL3 / TGD3) are shown. Data are expressed as the mean ± SEM. Each data point represents one hCO, 2-6 hCOs per experimental replicate, 3-5 experimental replicates per genotype. Statistical analysis by unpaired, two-tailed *t*-test or one-way ANOVA with Bonferroni multiple comparisons test.

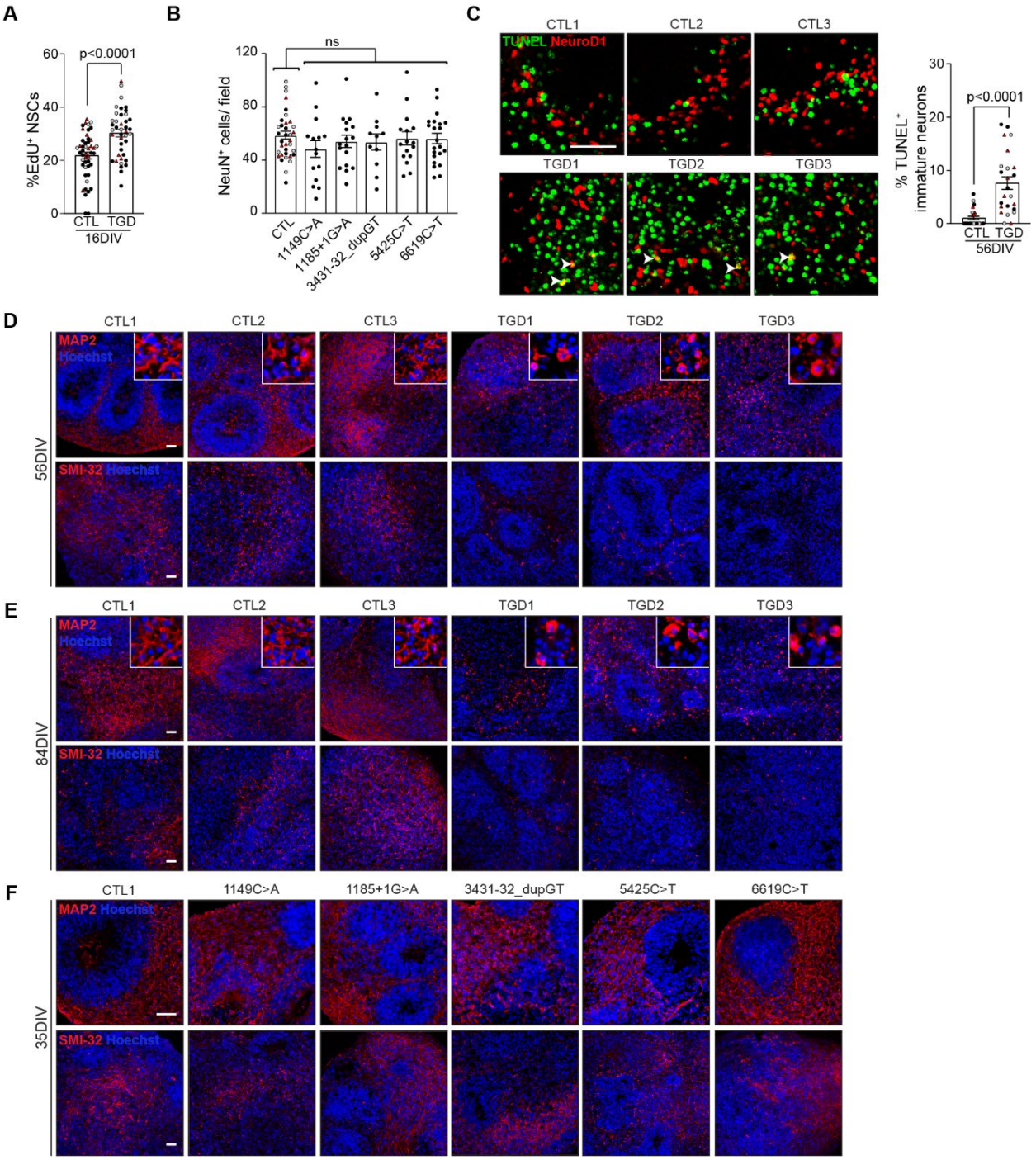
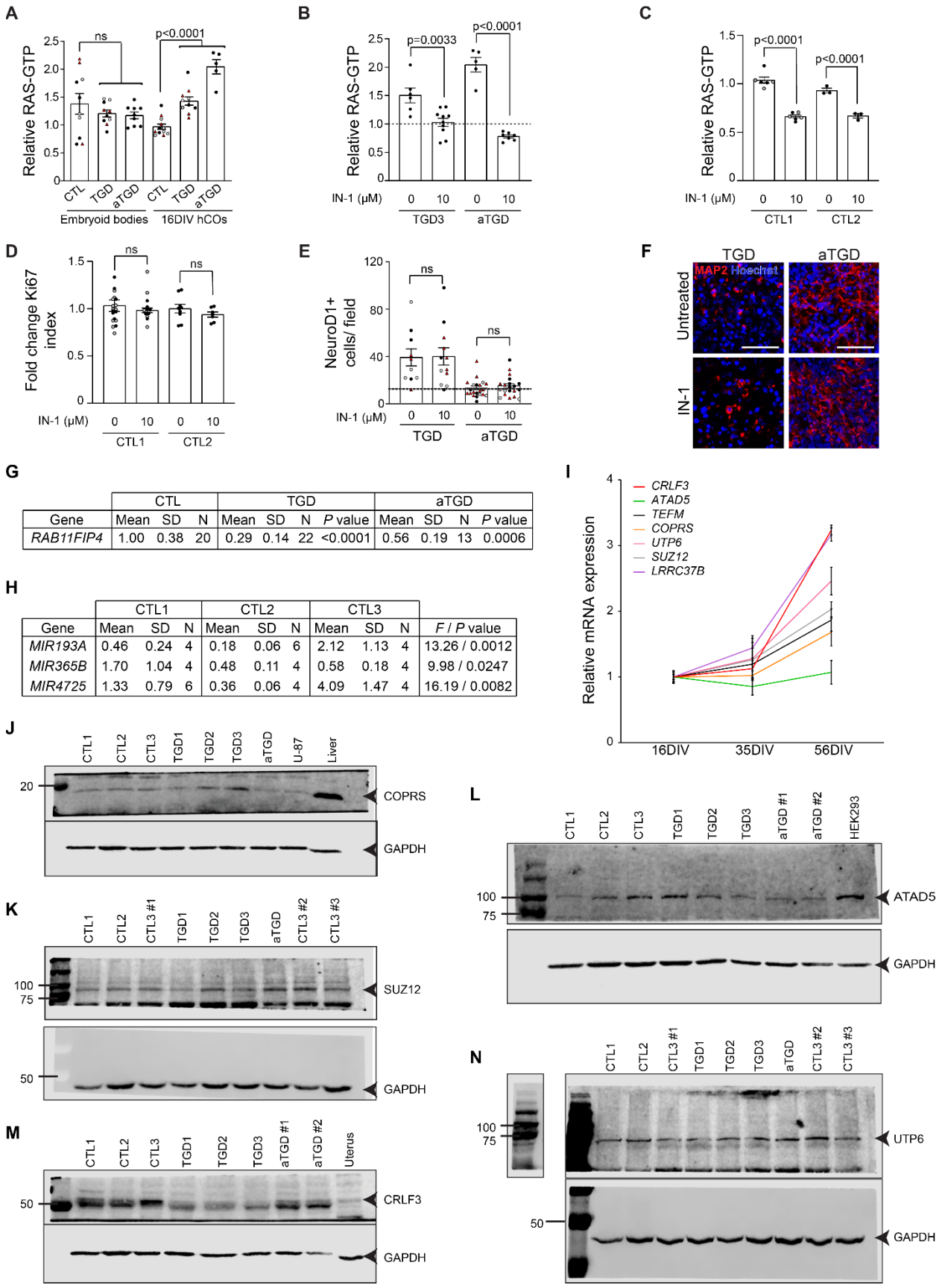


Figure S2 (related to Figure 1). Neuronal differentiation defects in TGD and intragenic *NF1*-mutant hCOs.

(**A**) %EdU$^+$ neural stem cells (NSCs) in 16DIV CTL and TGD hCOs. (**B**) Quantification of late-stage immature (NeuN$^+$) neurons per image field in the SVZ of intragenic *NF1*-mutant hCOs

relative to CTL hCOs at 35DIV. (**C**) Representative images and quantification of CTL and TGD hCOs immunolabeled for TUNEL (green) and NeuroD1 (red) (co-localization indicated by white arrows) at 56DIV. (**A-C**) Independent hiPSC lines (black, CTL1 / TGD1; white, CTL2 / TGD2; red, CTL3 / TGD3) are shown. (**D-E**) Representative images of CTL and TGD hCOs immunolabeled for $MAP2^+$ and $SMI\text{-}32^+$ dendrites at (**D**) 56DIV and (**E**) 84DIV. (**F**) Representative control (CTL1) and intragenic *NF1*-mutant hCOs immunolabeled for dendrite-specific markers ($MAP2^+$, $SMI\text{-}32^+$) at 35DIV. Data are shown as the mean ± SEM. Each data point represents one biological replicate (hCO), 2-6 biological replicates per experimental replicate, 3-5 experimental replicates per genotype. Statistical analysis by unpaired, two-tailed *t*-test or one-way ANOVA with Dunnett's multiple comparisons test. Scale bars: 50 μm.



G

	CTL			TGD				aTGD			
Gene	Mean	SD	N	Mean	SD	N	*P* value	Mean	SD	N	*P* value
RAB11FIP4	1.00	0.38	20	0.29	0.14	22	<0.0001	0.56	0.19	13	0.0006

H

	CTL1			CTL2			CTL3			
Gene	Mean	SD	N	Mean	SD	N	Mean	SD	N	*F* / *P* value
MIR193A	0.46	0.24	4	0.18	0.06	6	2.12	1.13	4	13.26 / 0.0012
MIR365B	1.70	1.04	4	0.48	0.11	4	0.58	0.18	4	9.98 / 0.0247
MIR4725	1.33	0.79	6	0.36	0.06	4	4.09	1.47	4	16.19 / 0.0082

Figure S3 (related to Figure 2 and 3). RAS activity and differential gene expression analysis of TGD and CTL hCOs.

(**A**) RAS activation in CTL and TGD 8DIV embryoid bodies and 16DIV hCOs. (**B-C**) Reduced RAS activity in (**B**) TGD3 and aTGD 16DIV hCOs and (**C**) CTL1 and CTL2 hCOs following 10 μM pan-RAS-IN-1 (IN-1) treatment. The mean CTL hCO RAS activity was assigned a value of 1 (dotted line). (**A-C**) Each data point represents an independent experimental replicate consisting of 20 pooled embryoid bodies or 4 pooled hCOs. Statistical analysis by unpaired, two-tailed *t*-test or one-way ANOVA with Dunnett's multiple comparisons. (**D**) Quantification of NSC proliferation (fold change in %Ki67$^+$ NSCs) in control hCOs at 16DIV with or without IN-1 treatment. Each data point represents one hCO, 2-6 hCOs per experimental replicate, 3-5 experimental replicates per genotype. Statistical analysis by unpaired, two-tailed *t*-test. (**E**) Number of early-stage immature (NeuroD1$^+$) neurons per image field in the SVZ of 16DIV TGD3 and aTGD hCOs with and without IN-1 treatment. Each data point represents one hCO, 3-12 hCOs per clone. Statistical analysis by unpaired, two-tailed *t*-test comparing TGD3 and aTGD hCOs with control values (indicated by dotted line). (**A-E**) All data are shown as the mean ± SEM. Independent (**A, E**) hiPSC lines (black, CTL1 / TGD1 / aTGD1; white, CTL2 / TGD2 / aTGD2; red, CTL3 / TGD3, aTGD-3), or (**C-D**) independent hiPSC clones (black, clone 1; white, clone 2; red, clone 3) are shown. (**F**) Representative images of 16DIV TGD3 and aTGD hCOs with and without RAS-IN-1 treatment immunolabeled for MAP2$^+$ dendrites. Scale bars: 50 μm. (**G**) mRNA expression of *RAB11FIP4* in 56DIV hCOs showing gene deletion status in TGD1-3 and aTGD. Statistical analysis by unpaired, two-tailed *t*-test. (**H**) RT-qPCR analysis of microRNA gene expression in CTL hCOs at the time point of highest expression (16DIV). Statistical analysis by one-way ANOVA; *F*-ratio / *P* values reported. *MIR4733* was not expressed in CTL hCOs. Each mRNA expression data point represents one biological replicate (hCO), 2-3 hCOs per experimental replicate. (**I**) Time course analysis of mRNA expression in 16, 35 and 56DIV CTL hCOs for 7 protein-coding genes included in differential gene expression

analysis, illustrating highest transcript expression levels for 6 of the 7 genes at 56DIV. *ATAD5* had no change in expression over time. Each time point represents 2 independent experimental replicates of CTL1 hCOs with each experimental replicate containing 2 biological replicates (hCOs). Data are shown as the mean ± SEM. (**J-N**) Representative unprocessed western blots of CTL and TGD protein expression including (**J**) COPRS, (**K**) SUZ12, (**L**) ATAD5, (**M**) CRLF3 and (**N**) UTP6.

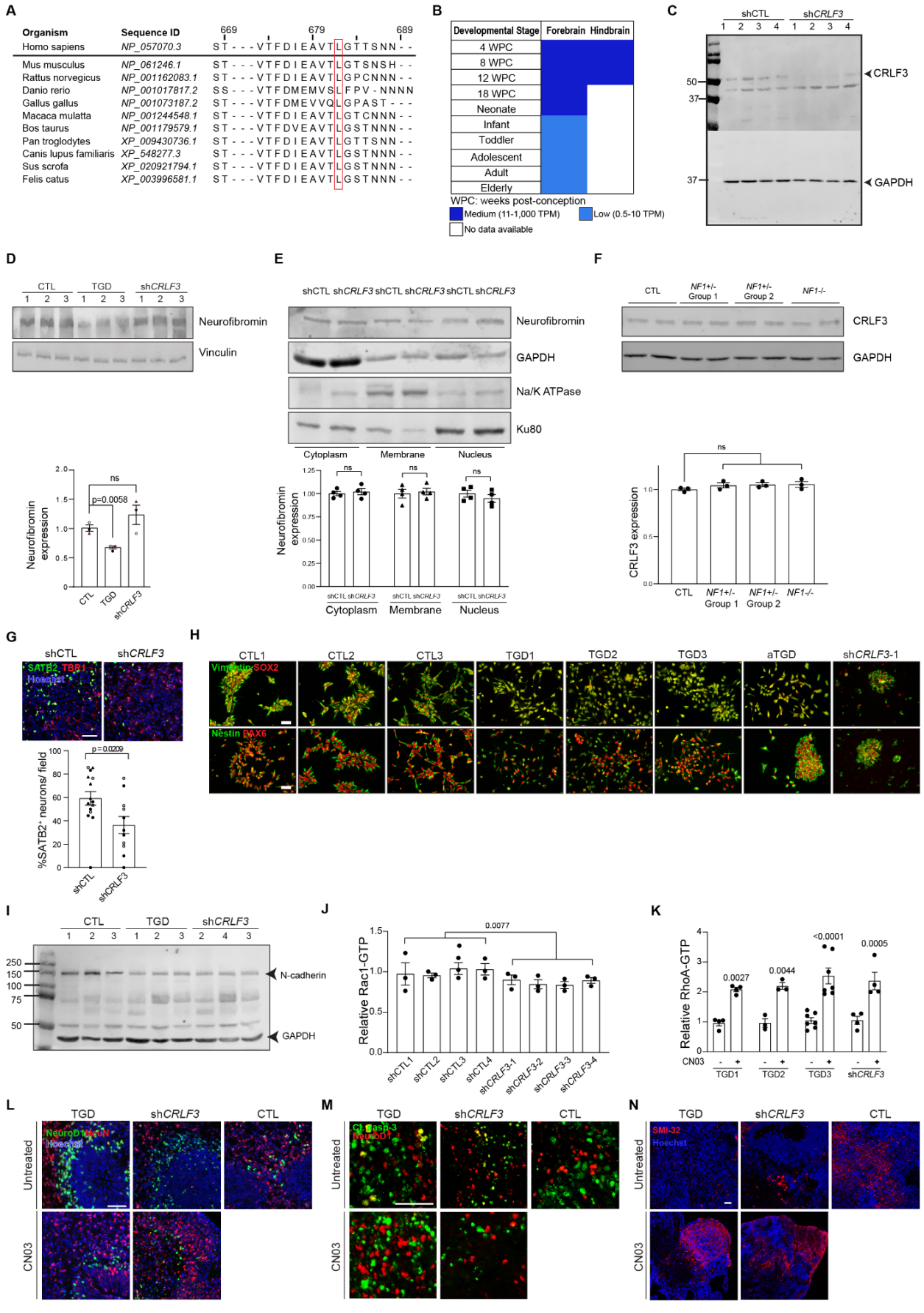

A
Organism
Sequence ID
669
679
689
Homo sapiens NP_057070.3 ST - - - VTFDIEAVTLGTTSNN - -
Mus musculus NP_061246.1 ST - - - VTFDIEAVTLGTSNSH - -
Rattus norvegicus NP_001162083.1 ST - - - VTFDIEAVTLGPCNNN - -
Danio rerio NP_001017817.2 SS - - - VTFDMEMVSLFPV - NNNN
Gallus gallus NP_001073187.2 ST - - - VTFDMEVVQLGPAST - - -
Macaca mulatta NP_001244548.1 ST - - - VTFDIEAVTLGTCNNN - -
Bos taurus NP_001179579.1 ST - - - VTFDVEAVTLGSTNNN - -
Pan troglodytes XP_009430736.1 ST - - - VTFDIEAVTLGTTSNN - -
Canis lupus familiaris XP_548277.3 ST - - - VTFDIEAVTLGSTNNN - -
Sus scrofa XP_020921794.1 ST - - - VTFDIEAVTLGSTNNN - -
Felis catus XP_003996581.1 ST - - - VTFDIEAVTLGSTNNN - -
B
Developmental Stage
Forebrain
Hindbrain
4 WPC
8 WPC
12 WPC
18 WPC
Neonate
Infant
Toddler
Adolescent
Adult
Elderly
WPC: weeks post-conception
Medium (11-1,000 TPM)
Low (0.5-10 TPM)
No data available
C
shCTL
shCRLF3
1 2 3 4 1 2 3 4
50
37
37
CRLF3
GAPDH
D
CTL
TGD
shCRLF3
1 2 3 1 2 3 1 2 3
Neurofibromin
Vinculin
Neurofibromin expression
ns
p=0.0058
CTL
TGD
shCRLF3
E
shCTL shCRLF3 shCTL shCRLF3 shCTL shCRLF3
Neurofibromin
GAPDH
Na/K ATPase
Ku80
Cytoplasm
Membrane
Nucleus
Neurofibromin expression
ns
shCTL shCRLF3
Cytoplasm
Membrane
Nucleus
F
CTL
NF1+/- Group 1
NF1+/- Group 2
NF1-/-
CRLF3
GAPDH
CRLF3 expression
ns
CTL
NF1+/- Group 1
NF1+/- Group 2
NF1-/-
G
shCTL
shCRLF3
SATB2 TBR1 Hoechst
%SATB2+ neurons/ field
p=0.0209
shCTL
shCRLF3
H
CTL1
CTL2
CTL3
TGD1
TGD2
TGD3
aTGD
shCRLF3-1
Vimentin SOX2
Nestin PAX6
I
CTL
TGD
shCRLF3
1 2 3 1 2 3 2 4 3
250
150
100
75
50
N-cadherin
GAPDH
J
Relative Rac1-GTP
0.0077
shCTL1
shCTL2
shCTL3
shCTL4
shCRLF3-1
shCRLF3-2
shCRLF3-3
shCRLF3-4
K
Relative RhoA-GTP
0.0027
0.0044
<0.0001
0.0005
CN03
TGD1
TGD2
TGD3
shCRLF3
L
TGD
shCRLF3
CTL
Untreated
CN03
NeuroD TuJN Hoechst
M
TGD
shCRLF3
CTL
Untreated
CN03
Cl. Casp-3 NeuroD1
N
TGD
shCRLF3
CTL
Untreated
CN03
SMI-32 Hoechst

Figure S4 (related to Figures 3 and 4). *CRLF3* sequence conservation, developmental expression, and downstream signaling.

(**A**) Amino acid sequence alignments revealed 92.8% conservation in p.Leu389 between human and 303 vertebrate *CRLF3* orthologs. Ten representative orthologs from NCBI's Eukaryotic Genome Annotation pipeline are shown, with p.Leu389 outlined in red. (**B**) Heat map of *CRLF3* mRNA expression levels in the human forebrain and hindbrain at different developmental stages, as reported by the Expression Atlas: Human RNA-seq time-series of the development of seven major organs. TPM: transcripts per million. (**C**) Uncropped western immunoblot from **Figure 4A**. (**D**) Neurofibromin relative expression in CTL, TGD, and sh*CRLF3* hiPSC-derived NSCs. Independent hiPSC lines (black, CTL1 / TGD1 / sh*CRLF3*-1; white, CTL2 / TGD2 / sh*CRLF3*-2; red, CTL3 / TGD3 / sh*CRLF3*-3) are shown. Statistical analysis by unpaired, two-tailed *t*-test. (**E**) Immunoblots and quantitation of neurofibromin expression in different subcellular fractions (cytoplasm, membrane, nucleus) in shCTL and sh*CRLF3* NPCs. GAPDH (cytoplasm), Na/K ATPase (membrane) and human-specific Ku80 (nucleus) were used as loading controls. (**F**) Immunoblot and quantitation of CRLF3 expression in NPCs harboring *NF1* point mutations, either conferring <30% reduced (Group 1), or >70% reduced (Group 2) neurofibromin levels, NPCs harboring homozygous null NF1 mutations (NF1-/-), or non-mutant controls. GAPDH was used as a loading control. (**E-F**) Data are expressed as the mean ± SEM. Statistical analysis by (**E**) unpaired, two-tailed t-test or (**F**) one-way ANOVA with Bonferroni post-test correction. ns, not significant. (**G**) Representative images of 84DIV shCTL and *shCRLF3* hCOs immunolabeled for deep-layer (TBR1) and upper-layer (SATB2) neurons and quantification of %$SATB2^+$ upper-layer neurons in hCOs at 84DIV. Scale bar: 100 µm. (**H**) hiPSC-derived NSCs immunolabeled for NSC markers SOX2, Vimentin, Nestin and PAX6. Scale bar: 50µm. (**I**) Unprocessed western immunoblot from **Figure 4H**. (**J**) Rac1 activity levels in shCTL and sh*CRLF3* NSCs. Each data point represents individual NSC sample. Statistical analysis by unpaired, two-tailed *t*-test. (**K**) RhoA activity in 2DIV TGD and sh*CRLF3* hCOs with

and without 1 µg/mL CN03 RhoA activator (CN03) treatment for 24 hours. Each data point represents 6 pooled hCOs. Statistical analysis by two-way ANOVA with Sidak's multiple comparison test performed comparing untreated with treated hCOs. All data are shown as the mean ± SEM and the *P* values are shown above each bar. (**L-N**) Representative images of (**L**) NeuroD1^{+} (green)/ NeuN^{+} (red) neurons, (**M**) cleaved caspase-3^{+} apoptotic immature neurons and (**N**) SMI-32^{+} dendrites in 35DIV CTL, TGD and *shCRLF3* hCOs with and without CN03 treatment. Scale bars: 50 µm.

Table S1 (related to Figure 1). Patient-derived CTL1-3, TGD1-3 and aTGD (atypical TGD) hiPSC lines and isogenic hiPSC lines CRISPR/Cas9-engineered to harbor NF1 patient *NF1* gene mutations.

Genotype	Sex	Age (years)	Specimen source	No. clones
CTL1[a]	Male	Fetal	Skin biopsy	2
CTL2[b]	Male	27	Skin biopsy	1
CTL3[c]	Male	41	Skin biopsy	1
TGD1	Male	44	Skin biopsy	2
TGD2	Male	6	Urine	2
TGD3	Male	11	Blood	3
aTGD	Female	16	Blood	3
NF1 patient mutation	**Protein level**		**Mutation Type**	**No. clones**
c.1149C>A	p.Cys383X		Nonsense	2
c.1185+1G>A	p.Asn355_Lys395del		Splice site	2
c.3431-32_dupGT	p.Thr1145Val_FS		Frameshift	2
c.5425C>T	p.Arg1809Cys		Missense	2
c.6619C>T	p.Gln2207X		Nonsense	1

[a]BJFF.6 commercially available
[b]Dr. Matthew B. Harms (WUSM)
[c]Dr. Fumihiko Urano (WUSM)

Table S2 (related to Figure 3). Human genomic DNA whole-exome sequencing.

Patient ID	SRS-2	Age (years)	Sex	*CRLF3*-mutation	*NF1*-mutation
OtB3317	81	10	M	c.1166T>C	c.5305C>T
OtC6610	48	11	F		c.3137_3138delCA
OtB3335	64	11	M		c.1756_1759delACTA
OtB3325	45	11	F		c.3888T>G
OtC6607	70	11	F		c.3449C>T
OtB3313	98	13	M	c.1166T>C	c.7255_7256delCT
OtC6614	48	13	M		c.2965G>T
OtC6612	50	13	M	c.1166T>C	c.910C>T
OtB3333	91	13	M	c.1166T>C	c.204+1G>T
OtB3326	54	15	F	c.1166T>C	c.2125T>C
OtB3321	88	15	M		c.6855C>A
OtB3312	98	15	M	c.1166T>C	c.4514delG
OtC6619	46	16	F		c.4006C>T
OtC6615	76	16	M		c.205-19T>A
OtB3319	74	16	F	c.1166T>C	c.4985G>A
OtB3323	56	17	M		c.1885G>A
OtB3336	46	18	M		c.3520C>T

Table S3 (related to Figure 4). Differentially expressed gene list filtered for non-significant genes in the comparison of TGD vs sh*CRLF3* samples.

Gene symbol	*P* value (TGD vs. sh*CRLF3*)	FDR step up (TGD vs. sh*CRLF3*)	Fold change (TGD vs. sh*CRLF3*)
KCP	0.1073	0.1689	2.99
SPN	0.2332	0.3201	2.90
THSD7A	0.0109	0.0248	2.84
MMP23B	0.0177	0.0373	2.67
ACOT11	0.0456	0.0826	2.25
ASCL1	0.9106	0.9346	1.82
DACT1	0.0700	0.1184	1.75
LDHAP4	0.7063	0.7715	1.29
ADGRE5	0.7043	0.7696	1.25
RUBCNL	0.8635	0.8992	1.20
NEFM	0.5160	0.6030	1.13
EPB41L4A	0.9420	0.9570	-1.09
TENM2	0.2461	0.3342	-1.27
MDGA2	0.1567	0.2308	-1.27
CAMK4	0.5252	0.6119	-1.31
SORBS2	0.0812	0.1341	-1.64
ATCAY	0.1408	0.2112	-1.69
PTX3	0.0732	0.1229	-1.72
MANEAL	0.0455	0.0825	-1.78
ITGB8	0.0134	0.0296	-1.86
DCLK2	0.0091	0.0214	-1.91
SYT5	0.0236	0.0474	-2.25
SYP	0.0102	0.0234	-2.25
RASGRP1	0.0241	0.0481	-2.58
MSI1	0.0047	0.0121	-2.58
CRABP1	0.0052	0.0134	-2.82
FCHO1	0.0056	0.0142	-2.85
ECEL1	0.0114	0.0257	-2.90
PLEKHA7	0.0097	0.0225	-3.02
ULBP1	0.0051	0.0131	-3.43
MMRN1	0.0103	0.0237	-3.89

Table S4 (related to Figures 1-4). Summary of experimental samples, replicates and statistical tests used.

Relative to main Figure	Readout	Sample type	Assay	Quantitation	Independent CTL lines used	Independent mutant lines used	Min. times experiment performed	Min. biological replicates/ line or clone	Statistical test used
Figure 1	Ki67	hCOs	IF: Figure 1B	Figure 1D	3 CTL cell lines (CTL1, CTL2, CTL3)	3 TGD cell lines (TGD1, TGD2, TGD3)	3	7	unpaired, 2-tailed *t*-test
	NeuroD1	hCOs	IF: Figure 1C	Figure 1E			3	8	unpaired, 2-tailed *t*-test
	NeuN	hCOs	IF: Figure 1C	Figure 1F			3	7	unpaired, 2-tailed *t*-test
	Cleaved Caspase-3	hCOs	IF: Figure 1H	Figure 1G			3	10	unpaired, 2-tailed *t*-test
	MAP2	hCOs	IF: Figure 1I	n/a			3	9	n/a
	SMI-32	hCOs	IF: Figure 1I	Figure S1F			3	11	unpaired, 2-tailed *t*-test
	SMI-312	hCOs	IF: Figure 1I	n/a			3	9	n/a
	SMI-32	2D neurons	IF: Figure 1J	Figure 1J			3	39	1-way ANOVA with Bonferroni correction
	TBR1	hCOs	IF: Figure S1G	Figure S1G			3	6	unpaired, 2-tailed *t*-test
	SATB2	hCOs	IF: Figure S1H	Figure S1H			3	10	unpaired, 2-tailed *t*-test
Figure 2	Ki67	hCOs	IF: Figure 2B	Figure 2B	3 CTL	1 aTGD	3	7	unpaired, 2-tailed *t*-test
	RAS-GTP	hCOs	ELISA: Figure 2C	Figure 2C	3 CTL	4 (TGD1, TGD2, TGD3, aTGD)	3	12	1-way ANOVA with Bonferroni correction
	Ki67	hCOs + IN-1	IF: Figure 2D	Figure 2D	n/a	2 (TGD3, aTGD)	3	3/clone; 9/line	unpaired, 2-tailed *t*-test
	NeuroD1	hCOs	IF: Figure 2E	n/a	3 CTL	1 aTGD	3	9	n/a
	NeuN	hCOs	IF: Figure 2E	Figure 2E	3 CTL	1 aTGD	3	9	unpaired, 2-tailed *t*-test
	Cleaved Caspase-3	hCOs	IF: Figure 2F	Figure 2F	3 CTL	1 aTGD	3	8	unpaired, 2-tailed *t*-test
	MAP2	hCOs	IF: Figure 2G	n/a	3 CTL	1 aTGD	3	9	n/a
	SMI-32	hCOs	IF: Figure 2G	n/a	3 CTL	1 aTGD	3	9	n/a
	SMI-312	hCOs	IF: Figure 2G	n/a	3 CTL	1 aTGD	3	9	n/a
	EdU	hCOs	n/a	Figure S2A	3 CTL	3 TGD	3	8	unpaired, 2-tailed *t*-test
	NeuN	hCOs	n/a	Figure S2B	3 CTL	5 intragenic cell lines	3	15	1-way ANOVA with Bonferroni correction
	TUNEL	hCOs	IF: Figure S2C	Figure S2C	3 CTL	3 TGD	3	7	unpaired, 2-tailed *t*-test
	RAS-GTP	EBs	ELISA: Figure S3A	Figure S3A	3 CTL	4 (TGD1, TGD2, TGD3, aTGD)	3	60	1-way ANOVA with Dunnett's mutiple comparison
	RAS-GTP	hCOs	ELISA: Figure S3A	Figure S3A	3 CTL	4 (TGD1, TGD2, TGD3, aTGD)	3	12	1-way ANOVA with Dunnett's mutiple comparison
	RAS-GTP	hCOs + IN-1	ELISA: Figure S3B	Figure S3B	n/a	2 (TGD3, aTGD)	3	9	unpaired, 2-tailed *t*-test
	RAS-GTP	hCOs + IN-1	ELISA: Figure S3C	Figure S3C	2 CTL (CTL1, CTL2)	n/a	3	12	unpaired, 2-tailed *t*-test
	Ki67	hCOs + IN-1	n/a	Figure S3D	2 CTL (CTL1, CTL2)	n/a	3	6	unpaired, 2-tailed *t*-test
	NeuroD1	hCOs + IN-1	n/a	Figure S3E	n/a	2 (TGD3, aTGD)	3	3/ clone; 10/ line	unpaired, 2-tailed *t*-test
Figure 3	CRLF3	hCOs	WB: Figure 3C	Figure 3C	3 CTL	4 (TGD1, TGD2, TGD3, aTGD)	4	4	unpaired, 2-tailed *t*-test
	SRS-2 Score	patient DNA	SRS-2 analysis	Figure 3E	10 patients without *CRLF3* mutation	7 patients with *CRLF3* mutation	n/a	7	unpaired, 2-tailed *t*-test
	mRNA Expression	hCOs	mRNA expression	Figure S3I	1 CTL (CTL1)	n/a	2	4	n/a
Figure 4	CRLF3	sh*CRLF3* hiPSCs	WB: Figure 4A	Figure 4A	4 shCTL	4 sh*CRLF3*	3	4	unpaired, 2-tailed *t*-test
	Ki67	sh*CRLF3* hCOs	n/a	Figure 4B	4 shCTL	4 sh*CRLF3*	3	9	unpaired, 2-tailed *t*-test
	NeuroD1	sh*CRLF3* hCOs	IF: Figure 4C	Figure 4C	4 shCTL	4 sh*CRLF3*	3	4	unpaired, 2-tailed *t*-test
	NeuN	sh*CRLF3* hCOs	IF: Figure 4C	Figure 4C	4 shCTL	4 sh*CRLF3*	3	4	unpaired, 2-tailed *t*-test
	Cleaved Caspase-3	sh*CRLF3* hCOs	IF: Figure 4D	Figure 4D	4 shCTL	4 sh*CRLF3*	3	3	unpaired, 2-tailed *t*-test
	SMI-32	sh*CRLF3* hCOs	IF: Figure 4E	Figure 4E	4 shCTL	4 sh*CRLF3*	3	3	unpaired, 2-tailed *t*-test
	N-cadherin	NSCs	WB: Figure 4H	Figure 4H	3 CTL	6 (3 TGD; 3 sh*CRLF3*)	3	3	1-way ANOVA with Bonferroni correction
	Rac1-GTP	NSCs	ELISA: Figure 4I	Figure 4I	3 CTL	3 TGD	3	3/clone; 3/line	unpaired, 2-tailed *t*-test
	RhoA-GTP	NSCs	ELISA: Figure 4J	Figure 4J	3 CTL	3 TGD	3	3/clone; 3/line	unpaired, 2-tailed *t*-test
	RhoA-GTP	sh*CRLF3* NSCs	ELISA: Figure 4K	Figure 4K	4 shCTL	4 sh*CRLF3*	3	3	unpaired, 2-tailed *t*-test
	NeuroD1	hCOs + CN03	IF: Figure S4L	Figure 4L	3 CTL	3 TGD; 2 sh*CRLF3*	3	5	1-way ANOVA with Tukey multiple comparison
	NeuN	hCOs + CN03	IF: Figure S4L	Figure 4M	3 CTL	3 TGD; 2 sh*CRLF3*	3	4	1-way ANOVA with Tukey multiple comparison
	Cleaved Caspase-3	hCOs + CN03	IF: Figure S4M	Figure 4N	3 CTL	3 TGD; 2 sh*CRLF3*	3	3	1-way ANOVA with Tukey multiple comparison
	SMI-32	hCOs + CN03	IF: Figure S4N	Figure 4O	3 CTL	3 TGD; 2 sh*CRLF3*	3	3	1-way ANOVA with Tukey multiple comparison
	Neurofibromin	NSCs	WB: Figure S4D	Figure S4D	3 CTL	3 TGD; 3 sh*CRLF3*	3	3	unpaired, 2-tailed *t*-test
	Neurofibromin	NSCs	WB: Figure S4E	Figure S4E	3 shCTL	3 sh*CRLF3*	3	3	unpaired, 2-tailed *t*-test
	CRLF3	NSCs	WB: Figure S4F	Figure S4F	2 CTL	4 *NF1+/-*, 2 *NF1-/-*	3	3	1-way ANOVA with Bonferroni correction
	SATB2	sh*CRLF3* hCOs	IF: Figure S4G	Figure S4F	3 shCTL	2 sh*CRLF3*	3	5	unpaired, 2-tailed *t*-test
	Rac1-GTP	sh*CRLF3* NSCs	ELISA Figure S4J	Figure S4H	4 shCTL	4 sh*CRLF3*	3	3	unpaired, 2-tailed *t*-test
	RhoA-GTP	NSCs + CN03	ELISA: Figure S4K	Figure S4I	n/a	3 TGD; 2 sh*CRLF3*	3	3	2-way ANOVA with Sidak's multiple comparison test