Neuron

Supplemental Information

LINE-1 activation in the cerebellum drives ataxia

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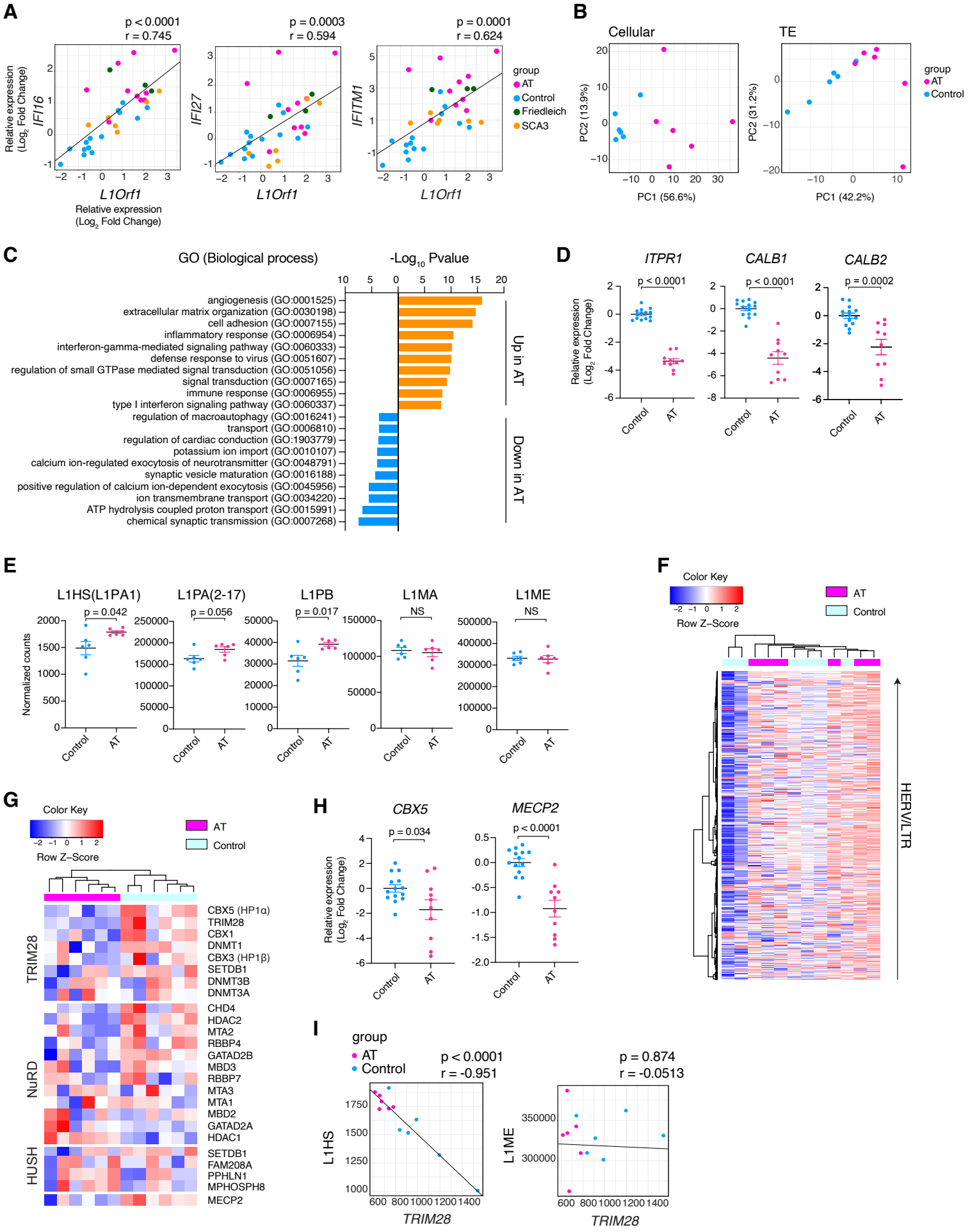
A
p < 0.0001
r = 0.745
p = 0.0003
r = 0.594
p = 0.0001
r = 0.624
IFI16
IFI27
IFITM1
L1Orf1
Relative expression (Log2 Fold Change)
group
AT
Control
Friedleich
SCA3
B
Cellular
TE
PC1 (56.6%)
PC2 (13.9%)
PC1 (42.2%)
PC2 (31.2%)
C
GO (Biological process)
-Log10 Pvalue
angiogenesis (GO:0001525)
extracellular matrix organization (GO:0030198)
cell adhesion (GO:0007155)
inflammatory response (GO:0006954)
interferon-gamma-mediated signaling pathway (GO:0060333)
defense response to virus (GO:0051607)
regulation of small GTPase mediated signal transduction (GO:0051056)
signal transduction (GO:0007165)
immune response (GO:0006955)
type I interferon signaling pathway (GO:0060337)
regulation of macroautophagy (GO:0016241)
transport (GO:0006810)
regulation of cardiac conduction (GO:1903779)
potassium ion import (GO:0010107)
calcium ion-regulated exocytosis of neurotransmitter (GO:0048791)
synaptic vesicle maturation (GO:0016188)
positive regulation of calcium ion-dependent exocytosis (GO:0045956)
ion transmembrane transport (GO:0034220)
ATP hydrolysis coupled proton transport (GO:0015991)
chemical synaptic transmission (GO:0007268)
Up in AT
Down in AT
D
ITPR1
CALB1
CALB2
p < 0.0001
p < 0.0001
p = 0.0002
Relative expression (Log2 Fold Change)
Control
AT
E
L1HS(L1PA1)
L1PA(2-17)
L1PB
L1MA
L1ME
p = 0.042
p = 0.056
p = 0.017
NS
NS
Normalized counts
F
Color Key
Row Z-Score
AT
Control
HERVL TR
G
Color Key
Row Z-Score
AT
Control
TRIM28
CBX5 (HP1α)
TRIM28
CBX1
DNMT1
CBX3 (HP1β)
SETDB1
DNMT3B
DNMT3A
NuRD
CHD4
HDAC2
MTA2
RBBP4
GATAD2B
MBD3
RBBP7
MTA3
MTA1
MBD2
GATAD2A
HDAC1
HUSH
SETDB1
FAM208A
PPHLN1
MPHOSPH8
MECP2
H
CBX5
MECP2
p = 0.034
p < 0.0001
Relative expression (Log2 Fold Change)
Control
AT
I
group
AT
Control
p < 0.0001
r = -0.951
p = 0.874
r = -0.0513
L1HS
L1ME
TRIM28
TRIM28

Figure S1. Transcriptomic feature and downregulation of TE regulators in AT patients. Related to Figure 1. (A) Correlation of L1Orf1 and ISG mRNA expressions. Pearson correlation (r) and p-values for the correlations are indicated above each panel. Values are shown as the log-2 fold changes from the mean values of control group as in Figure 1A. (B) PCA plot for cellular gene expression (left**)** and TE expression (right**)** with RNA-seq data from control individuals and AT patients (n = 6). (C) GO term analysis (Biological Process) of DEGs between AT and controls. DEGs (padj < 0.01, |Log2-fold change|>1) are used for the analysis. Top-10 GO terms for upregulated and downregulated genes are shown. (D) qRT-PCR validation of RNAseq results on the calcium homeostasis-related genes, *ITPR1*, *CALB1*, and *CALB2*. n = 14 for control and 10 for AT. (E) Comparisons of normalized counts for each LINE-1 family. The counts for subfamilies were added up to calculate the counts per each family (L1HS=L1PA1, L1PA: L1PA2-17, L1PB: L1PB/PB1-4, L1MA: L1MA1-10, L1ME: L1ME1-5/L1MEa-j. p-values shown are with unpaired two-sided t-test. (F) Heatmap of ERVs in AT and controls. Each row represents one ERV or LTR family. (G) Heatmap for representative TE regulators. (H) qRT-PCR of *CBX5* and *MECP2* in controls and AT. n = 14 for controls and 10 for AT. (I) Representative correlation plots from Figure 1E, showing correlations between TRIM28 and L1HS or L1ME expressions. Pearson correlation coefficients and p-values are shown. Data are mean ± SEM. p-values shown are with unpaired two-sided t-test in (D), (E), and (H).

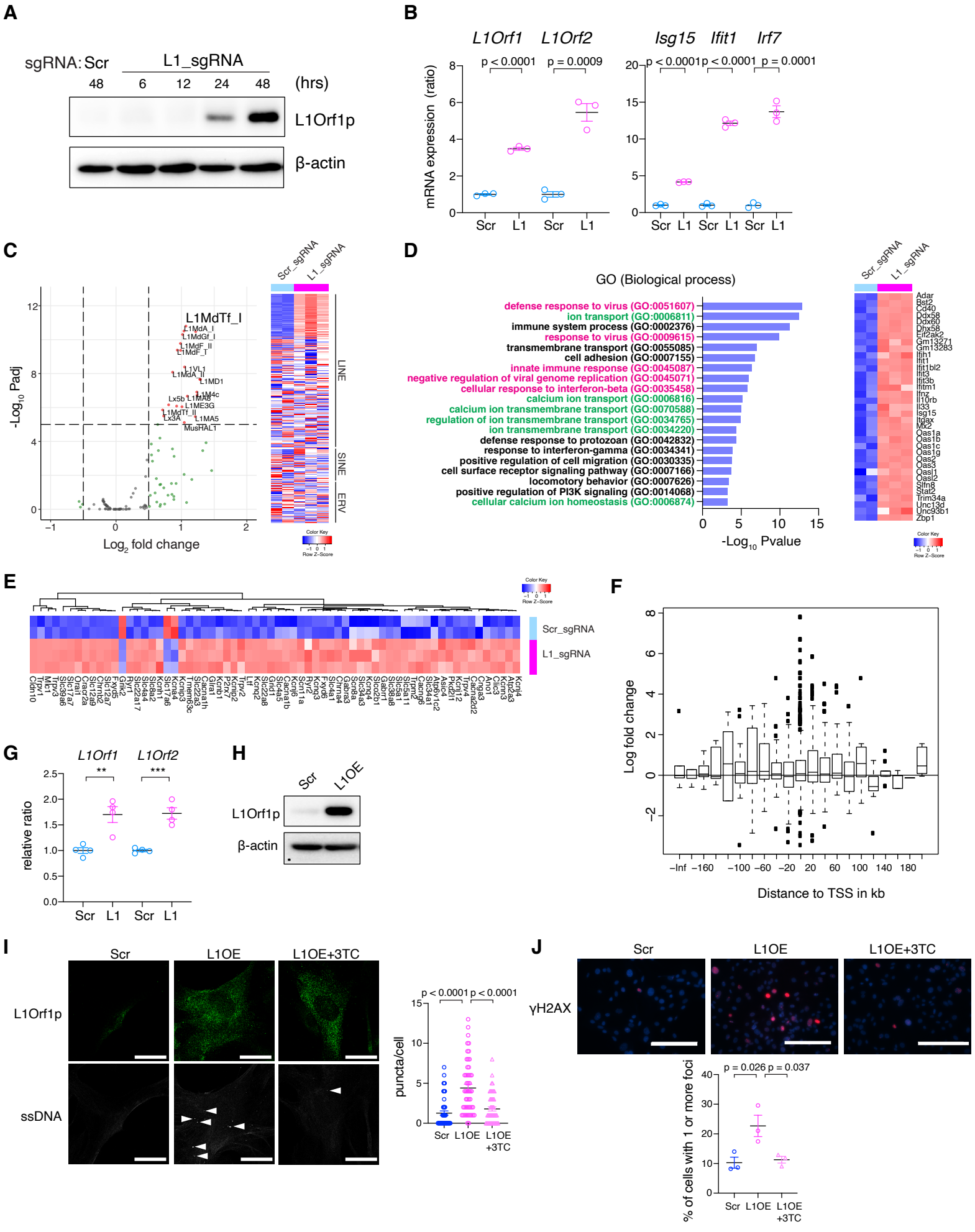
A
sgRNA: Scr
L1_sgRNA
48
6
12
24
48
(hrs)
L1Orf1p
β-actin
B
L1Orf1
L1Orf2
Isg15
Ifit1
Irf7
p < 0.0001
p = 0.0009
p < 0.0001
p < 0.0001
p = 0.0001
mRNA expression (ratio)
Scr
L1
C
Scr_sgRNA
L1_sgRNA
L1MdTf_I
L1MdA_I
L1MdGf_I
L1MdF_II
L1MdF_I
L1VL1
L1MdA_II
L1MD1
L1M4c
Lx5b
L1MA8
L1ME3G
L1MdTf_II
Lx3A
L1MA5
MusHAL1
-Log10 Padj
Log2 fold change
LINE
SINE
ERV
Color Key
Row Z-Score
D
GO (Biological process)
defense response to virus (GO:0051607)
ion transport (GO:0006811)
immune system process (GO:0002376)
response to virus (GO:0009615)
transmembrane transport (GO:0055085)
cell adhesion (GO:0007155)
innate immune response (GO:0045087)
negative regulation of viral genome replication (GO:0045071)
cellular response to interferon-beta (GO:0035458)
calcium ion transport (GO:0006816)
calcium ion transmembrane transport (GO:0070588)
regulation of ion transmembrane transport (GO:0034765)
ion transmembrane transport (GO:0034220)
defense response to protozoan (GO:0042832)
response to interferon-gamma (GO:0034341)
positive regulation of cell migration (GO:0030335)
cell surface receptor signaling pathway (GO:0007166)
locomotory behavior (GO:0007626)
positive regulation of PI3K signaling (GO:0014068)
cellular calcium ion homeostasis (GO:0006874)
-Log10 Pvalue
Adar
Bst2
Cd40
Ddx58
Ddx60
Dhx58
Eif2ak2
Gm13271
Gm13283
Ifih1
Ifit1
Ifit1bl2
Ifit3
Ifit3b
Ifitm1
Ifnz
Il10rb
Il33
Isg15
Itgax
Mx2
Oas1a
Oas1b
Oas1c
Oas1g
Oas2
Oas3
Oasl1
Oasl2
Stfn8
Stat2
Trim34a
Unc13d
Unc93b1
Zbp1
E
Color Key
Row Z-Score
Scr_sgRNA
L1_sgRNA
F
Log fold change
Distance to TSS in kb
−Inf −160 −100 −60 −20 20 60 100 140 180
G
L1Orf1
L1Orf2
**
***
relative ratio
Scr
L1
H
Scr
L1OE
L1Orf1p
β-actin
I
Scr
L1OE
L1OE+3TC
L1Orf1p
ssDNA
p < 0.0001
p < 0.0001
puncta/cell
Scr
L1OE
L1OE +3TC
J
Scr
L1OE
L1OE+3TC
γH2AX
p = 0.026
p = 0.037
% of cells with 1 or more foci
Scr
L1OE
L1OE +3TC

Figure S2. Establishment of LINE-1 CRISPRa system in vitro. Related to Figure 2. (A) Whole cell lysates of dCasVP-3T3 cells transfected with Scr_sgRNA or L1_sgRNA expression plasmids were collected at the indicated time points after the transfection and assayed for LINE-1Orf1p detection. (B) mRNA of dCasVP-3T3 cells transfected with Scr_sgRNA or L1_sgRNA expression plasmid (48-hour post-transfection) were analyzed with qRT-PCR (n =3). Expression levels were normalized to *Gapdh* expression and shown as the ratios to scramble condition. (C) RNA-seq was performed with mRNAs from dCas-CRISPRa NIH3T3 cells transfected with Scr_sgRNA or L1_sgRNA plasmids (48-hour post-transfection, n = 2 and 3, respectively). Fold changes and padj for each LINE-1 subfamily plotted as a volcano plot (Left). Heatmap of the TEs across the samples. Each row represents TE subfamily. ERVs are uniquely mappable ERVs in mice (Tokuyama et al., 2018; Treger et al., 2019). (D) Gene Ontology analysis (Biological Process) of the DEGs. Top 20 GO terms are shown. Anti-viral/type-I interferon e-related terms and ion homeostasis-related terms are highlighted. Heatmap of DEGs in the "defense response to virus" shown in right (the top GO term in (D)). (E) Heatmap of DEGs between L1_sgRNA and scr_sgRNA conditions in the "ion transport" term in (D). (F) The fold changes of cellular gene expression in L1 CRISPRa cells were plotted in relation to the distance between TSS of each gene and its closest L1_sgRNA target sequences (1 mismatch allowed). For all box plots, genes are binned into 20 kb bins centered around the indicated integer by distance from the TSS to the nearest L1_sgRNA target sites. Plots show median, with interquartile range (IQR), and whiskers show points within 1.5x IQR. (G) Primary MEFs from C57BL/6J mice were established, and dCas9-VP64, MPH, and either scr_sgRNA or L1_sgRNA were transduced. The transduced cells were selected with antibiotics for 4 days, passaged, and 48 hours later, mRNA expression levels were assayed by qRT-PCR and normalized to *Gapdh* expression. n = 3 independent biological samples. (H) The whole cell lysates from the same conditions were assayed for L1Orf1p expression. (I) Cells treated with 3TC or DMSO for 48 hours were stained for L1Orf1p and ssDNA. Representative images are shown. Scale bar = 20 μm. ssDNA puncta number inside the cells were counted and summarized (right panel). (J) The L1 CRISPRa cells or control cells treated with either 3TC or DMSO were stained for γH2AX. Representative images are shown (left). The foci number were counted. More than 100 cells were analyzed per independent replicate, and % of cells with more than one focus were calculated. Result from 3 independent replicates is summarized (right). Scale bar = 200 μm. Data are mean ± SEM. p-values shown are with unpaired two-sided t-test in (B), and (F), and one-way ANOVA with Tukey's post-hoc test in (H) and (I).

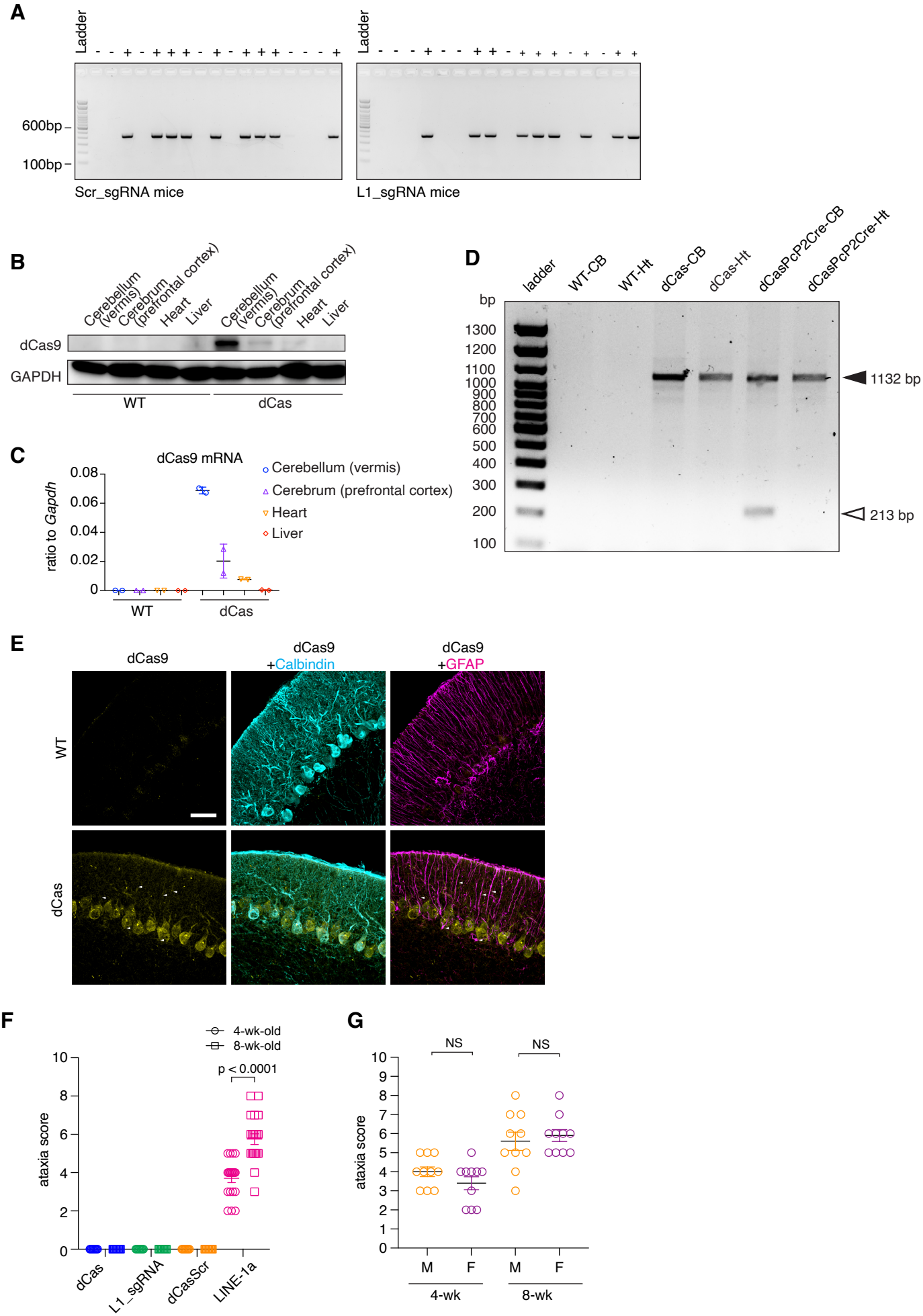
A
Ladder
600bp
100bp
Scr_sgRNA mice
L1_sgRNA mice
B
Cerebellum (vermis)
Cerebrum (prefrontal cortex)
Heart
Liver
dCas9
GAPDH
WT
dCas
C
dCas9 mRNA
ratio to Gapdh
Cerebellum (vermis)
Cerebrum (prefrontal cortex)
Heart
Liver
D
ladder
WT-CB
WT-Ht
dCas-CB
dCas-Ht
dCasPcP2Cre-CB
dCasPcP2Cre-Ht
bp
1132 bp
213 bp
E
dCas9
dCas9 +Calbindin
dCas9 +GFAP
WT
dCas
F
4-wk-old
8-wk-old
p < 0.0001
ataxia score
dCas
L1_sgRNA
dCasScr
LINE-1a
G
NS
NS
ataxia score
M
F
M
F
4-wk
8-wk

Figure S3. Generation of Scr_sgRNA mice and L1_sgRNA mice and basal expression of dCas9 in the cerebellum of dCas mice. Related to Figure 2. (A) Genotyping of Scr_sgRNA and L1_sgRNA mice. "+" indicates positive mice. Expected band size for both strain is 424 bp. (B) Tissues from 8-wk-old WT ($dCas^{-/-}$) mice and dCas ($dCas^{+/-}$) mice were harvested, homogenized, lysed, and the lysates were subject to Western blotting. Representative results are shown here. (n=2 mice for each group). Similar results were obtained in two independent experiments. (C) Tissues from 8-wk-old WT mice and dCas mice were harvested, homogenized, and mRNA was extracted. (d)Cas9 mRNA expression was assessed with qRT-PCR using specific primers for Cas9 in indicated organs. (n=2 for each group). (D) Genomic DNAs extracted from the cerebellum (CB) and heart (Ht) from WT, dCas, and dCas-PcP2Cre (Purkinje cell-specific Cre) double transgenic mice were subject to PCR with primers flanking LSL sequence in the dCas mice transgene. Expected band sizes for the amplification product of non-recombined and recombined sequence are 1132 bp and 213 bp, respectively (black and white arrowhead, respectively). Only the cerebellum of dCasPcP2Cre mice has the 213 bp product. (E) Frozen sections of cerebella from 8-week-old dCas mice and WT mice were stained for (d)Cas9 using specific antibodies and co-stained for Calbindin and GFAP. Representative images from n=3 for each strain are shown. Scale bar = 30 μm. (F) Composite scores for ataxia were scored at 4- and 8-wk-old time points. n = 20 (n=10 for both male/female) for LINE-1a mice. For other genotypes, n = 10 for each group. (G) Ataxia scores for LINE-1a males (M, n=10) and females (F, n=10) were compared at 4- and 8-wk of age. Data are mean ± SEM. p-values shown are with unpaired two-sided t-test.

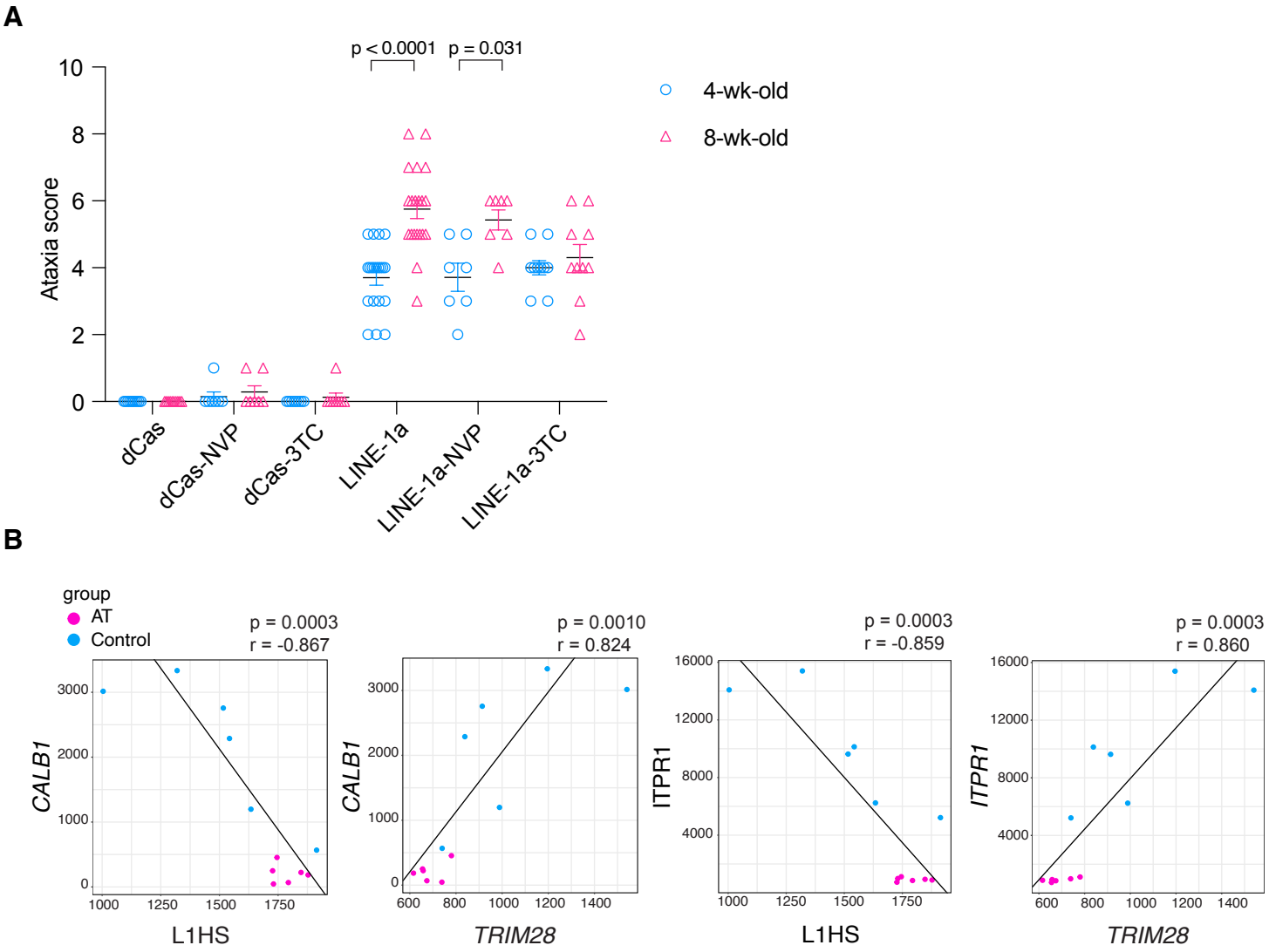

Figure S4. Attenuation of ataxia in LINE-1a mice with NRTI treatment and correlations between L1HS, *TRIM28*, *CALB1*, and *ITPR1* in human RNA-seq data. Related to Figure 4. (A) Ataxia scores of at 4- and 8-wk-old naïve, 3TC-treated- (4 to 8-wk), and NEV-treated (4-8-wk) dCas and LINE-1a mice. n for dCas-naïve, dCas-NEV, dCas-3TC, LINE-1a-naïve, LINE-1a-NEV, LINE-1a-3TC group are 10, 7, 8, 20, 7, and 10, respectively Data are mean ± SEM. Scores were compared with multiple t-test with Holm-Sidak correction. (B) Correlations between expression (normalized counts) of L1HS, TRIM28, CALB1, and ITPR1 in RNA-seq data of AT patients and control individuals (same datasets as in Figure 1). Pearson correlation coefficients and p-values are shown.

Table S1. Sample information of 33 individuals with cerebellar ataxia or unaffected control. Related to Figure 1.

Clinical Brain Diagnosis	Age (yrs)	Ethnicity	Manner of Death	PMI (hrs)	Race	Sex	RNA-seq
Unaffected Control	30	Unknown	Accidental	20	White	M	Yes
Unaffected Control	44	Unknown	Natural	16	White	F	
Unaffected Control	35	Unknown	Natural	17	White	M	Yes
Unaffected Control	56	Unknown	Natural	5	White	M	
Unaffected Control	27	Unknown	Natural	13	White	M	
Unaffected Control	25	Unknown	Natural	4	White	F	Yes
Unaffected Control	18	Unknown	Natural	22	White	F	Yes
Unaffected Control	29	Unknown	Natural	8	White	M	Yes
Unaffected Control	29	Unknown	Accidental	23	White	F	
Unaffected Control	35	Unknown	Natural	27	Black or African-American	M	
Unaffected Control	42	Unknown	Natural	12	White	F	
Unaffected Control	45	Unknown	Natural	19	White	M	
Unaffected Control	27	Unknown	Natural	12	White	M	Yes
Unaffected Control	32	Unknown	Natural	8	White	F	
SCA3	56	Not Hispanic or Latino	Natural	3	White	F	
SCA3	59	Not Hispanic or Latino	Undetermined	18.5	White	M	
SCA3	41	Not Hispanic or Latino	Natural	19	White	M	
SCA3	36	Not Hispanic or Latino	Undetermined	36	White	M	
SCA3	63	Not Hispanic or Latino	Natural	18.9	White	M	
SCA3	37	Not Hispanic or Latino	Natural	11.6	White	F	
Friedreich's ataxia	27	Unknown	Natural	7	White	F	
Friedreich's ataxia	36	Not Hispanic or Latino	Natural	42	White	F	
Friedreich's ataxia	24	Hispanic or Latino	Natural	0	Unknown	M	
Ataxia Telangiectasia	28	Unknown	Natural	4	White	F	
Ataxia Telangiectasia	24	Unknown	Natural	2	White	F	Yes
Ataxia Telangiectasia	29	Unknown	Natural	12	White	M	
Ataxia Telangiectasia	25	Unknown	Natural	15	White	M	Yes
Ataxia Telangiectasia	44	Unknown	ATLL	29	White	M	
Ataxia Telangiectasia	27	Not reported	Natural	14	White	M	Yes
Ataxia Telangiectasia	36	Unknown	Natural	20	White	M	Yes
Ataxia Telangiectasia	27	Not reported	Natural	18	White	M	Yes
Ataxia Telangiectasia	31	Not Hispanic or Latino	Natural	4	White	F	
Ataxia Telangiectasia	16	Unknown	Natural	23	Unknown	F	Yes

Table S2. List of genes that were significantly upregulated in LINE-1 CRISPRa NIH3T3 cells compared to control cells, whose TSS are located within 1kb from the L1_sgRNA target loci allowing 1-mismatch. Related to Figure 2.

Gene	log2 fold change	padj	Gene	log2 fold change	padj
Gramd1b	1.6414848	2.99E-79	Brinp2	6.69996974	0.00017219
Sorcs2	1.81018283	1.82E-61	Dpp6	6.17435426	0.00019767
Iqub	5.84879227	1.00E-31	Nkain2	3.21729015	0.00031878
Sntb1	3.90307704	3.33E-25	Zdhhc15	6.15481183	0.00096709
Gab2	1.05349831	1.79E-23	Atp8a2	1.28016446	0.00161642
Thsd4	4.15102309	2.78E-22	Spag16	3.66524069	0.00202261
Bbs9	1.16842107	4.58E-19	Gabra3	2.17834033	0.0026132
Slc4a4	1.42495761	5.74E-19	Samd12	2.5126567	0.00299411
Ntrk2	7.56533298	1.13E-17	Scn1a	5.62442832	0.00471093
Unc13b	1.02626784	1.25E-17	Sema6d	1.42118843	0.00577004
Ccdc148	1.8781046	1.81E-14	Kcnq3	2.45882509	0.00699431
Grid1	5.57693808	3.48E-14	Tex11	2.41447362	0.01968851
Ptprn2	2.45601787	1.43E-12	Morc1	4.41391969	0.02302515
Me3	2.15863521	1.93E-10	4921539E11Rik	2.07375217	0.02305095
Clca3b	9.39818529	4.96E-10	Syt16	4.4138949	0.02645436
Abcb1a	3.24160788	3.49E-08	Reln	1.5561982	0.02793395
Prkn	2.25157336	7.22E-08	Cntn5	1.82523922	0.03031926
Kcnc2	7.80073917	1.88E-06	Catspere1	1.87839926	0.03712511
Dnah12	2.13215325	1.84E-05	Tenm1	2.21005115	0.04440885
Adgre1	4.21931731	9.86E-05	Ak9	1.24895746	0.04466259

Table S3. Primer and oligo sequences used in this study. Related to STAR Methods.

Primers for qPCR	
Human	
GAPDH Fw ; 5' CAACGGATTTGGTCGTATT	GAPDH Rv ; 5'GATGGCAACAATATCCACTT
LINE1Orf1 (L1HS) Fw ; 5' ACCTGAAAGTGACGGGGAGA	LINE1Orf1 (L1HS) Rv ; 5' CCTGCCTTGCTAGATTGGGG
LINE1Orf2 (L1HS) Fw ; 5'CAAACACCGCATATTCTCACTCA	LINE1Orf2 (L1HS) Rv ; 5'CTTCCTGTGTCCATGTGATCTCA
IFITM1 Fw ; 5'ACTCCGTGAAGTCTAGGGACA	IFITM1 Rv ; 5'TGTCACAGAGCCGAATACCAG
IFI16 Fw ; 5'ATATCCTTCAGAGGCCAGCA	IFI16 Rv ; 5'ATCTGAGGAGTGTGGGGATG
IFI27 Fw ; 5'TGCCCATGGTGCTCAGTG	IFI27 Rv ; 5'GAGAGTCCAGTTGCTCCCAG
Primers for human TRIM28 (Taqman); Hs00232212_m1 (Thermofisher)	
Primers for human DNMT1 (Taqman); Hs00945899_m1 (Thermofisher)	
Primers for human CALB1 (Taqman); Hs01077197_m1 (Thermofisher)	
Primers for human CALB2 (Taqman); Hs00242372_m1 (Thermofisher)	
Primers for human CBX5 (Taqman); Hs01127577_m1 (Thermofisher)	
Primers for human MECP2 (Taqman); Hs05049079_g1 (Thermofisher)	
Primers for human ITPR1 (Taqman); Hs00181881_m1 (Thermofisher)	
Mouse	
Gapdh Fw ; 5'GAAGGGCTCATGACCACAGT	Gapdh Rv ; 5'GGATGCAGGGATGATGTTCT
LINE1Orf1 Fw ; 5'ATGAAAGCCAGAAGAGCCTG	LINE1Orf1 Rv ; 5'TTTGAAGGGCTGGATTCGTG
LINE1Orf2 Fw ; 5'AGAAGACAGCCACAAGAACAGA	LINE1Orf2 Rv ; 5'TATTGTGTGAGGCGCAATGT
Isg15 Fw ; 5'AGCGGAACAAGTCACGAAGAC	Isg15 Rv ; 5'TGGGGCTTTAGGCCATACTC
Irf7 Fw ; 5'TGCTGTTTGGAGACTGGCTA	Irf7 Rv ; 5'TCCAAGCTCCCGGCTAAGT
Ifit1 Fw ; 5'CAAGGCAGGTTTCTGAGGAG	Ifit1 Rv ; 5'GACCTGGTCACCATCAGCAT
Oasl2 Fw ; 5'GGGAGGTCGTCATCAGCTTC	Oasl2 Rv ; 5'CCCTTTTGCCCTCTCTGTGG
Primers for mouse Calb1 (Taqman); Mm00486645_m1 (Thermofisher)	
Primers for mouse Itpr1 (Taqman); Mm00439907_m1 (Thermofisher)	
Other primers	
Primer set for (d)Cas9 (Fw and Rv); System Biosciences (CAS9-PR-1)	
Primers for LSL assessment (Figure S2D) Fw: GCAACGTGCTGGTTATTGTG, Rv: TCACGACACCTGAAATGGAA	
Primers for L1_/Scr_sgRNA mice genotyping Fw: GAGGGCCTATTTCCCATGAT, Rv: CACGCGCTAAAAACGGACTA	
Oligonucleotides used for sgRNA cloning (for lentiSAMv2. Overhangs adjusted to each backbone vector)	
L1_sgRNA_sense : CACCGCGCTGAGGCAGCACCCTGTG	L1_sgRNA_antisense : AAACCACAGGGTGCTGCCTCAGCGC
Scr_sgRNA_sense : CACCGGCACTCACATCGCTACATCA	Scr_sgRNA_antisense : AAACTGATGTAGCGATGTGAGTGCC