

Supplemental Information

Activation of HERV-K(HML-2) disrupts cortical patterning and neuronal differentiation by increasing NTRK3

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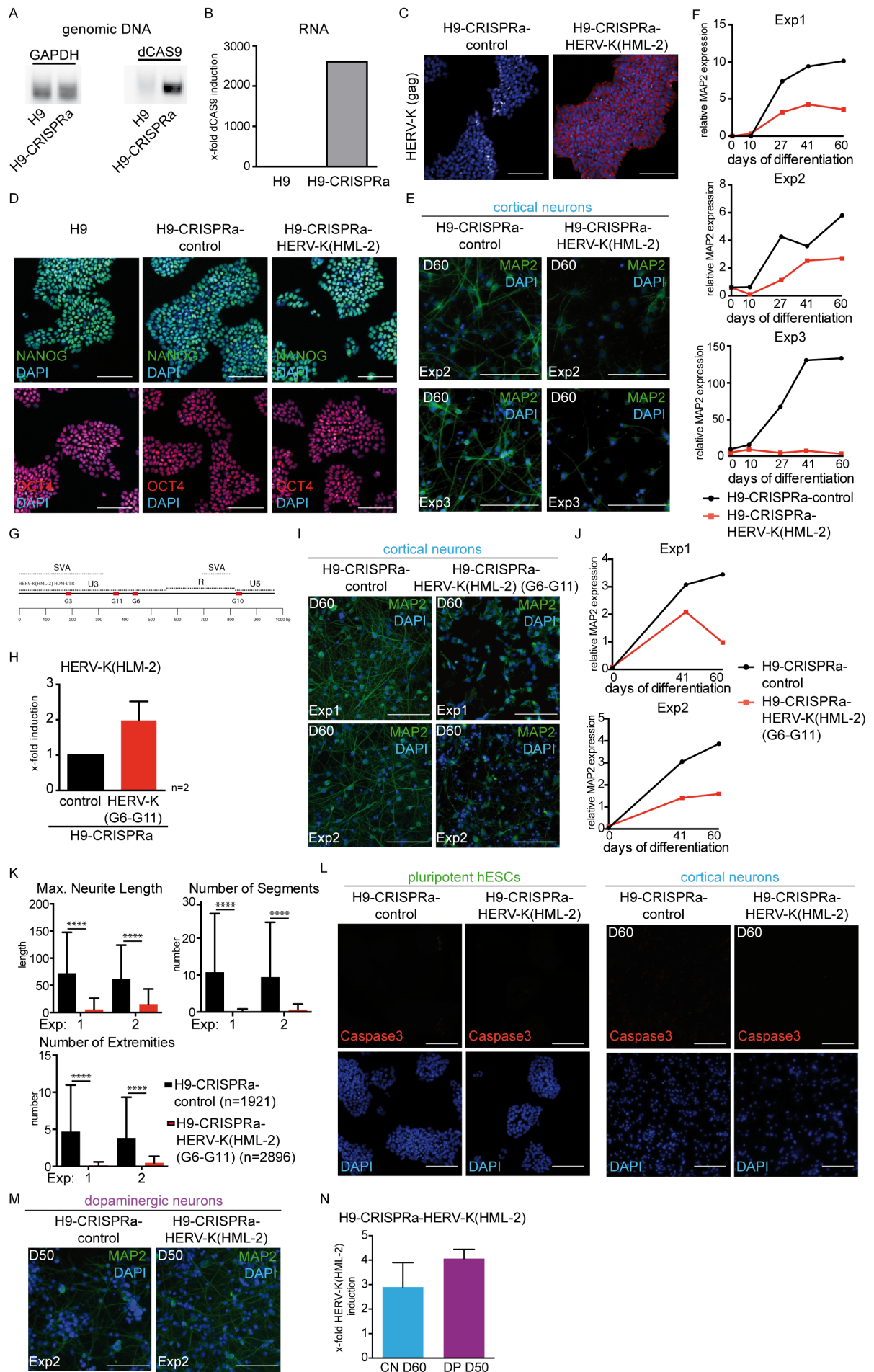


Figure S1: HERV-K(HML-2) activation does not affect pluripotency but reduces MAP2 expression in cortical neurons. Related to Figure 1.

(A) H9 (WA09) cells were transduced with dCas9-VP64. PCR analysis of genomic DNA confirmed integration of dCas9-VP64. GAPDH served as a loading control. n=3 biological replicates.

(B) Expression of dCas9-VP64 was quantified on the RNA level by qRT-PCR. Data were normalized to housekeeping gene GAPDH. n=3 biological replicates.

(C) HERV-K(HML-2) expression was detected by immunofluorescence using a HERV-K(HML-2) Gag antibody. n=3 biological replicates. Scale bars, 100 μ m.

(D) Pluripotency markers NANOG and OCT4 were analyzed in HERV-K(HML-2) activated and control cells by immunofluorescence. n=3 biological replicates. Scale bars, 100 μ m.

(E) MAP2 expression was tested in HERV-K(HML-2) activated and control cells by immunofluorescence. Two biological replicates are shown. Scale bars, 100 μ m.

(F) MAP2 repression was confirmed by qRT-PCR during the course of cortical differentiation in HERV-K(HML-2) activated and control cells. Three biological replicates are shown.

(G) Schematic depiction of an HERV-K(HML-2) LTR5_{Hs} including locations of the different HERV-K(HML-2) gRNAs, U3, R and U5 regions, and LTR regions also present in SVA elements (see also the main paper).

(H) HERV-K(HML-2) transcript levels quantified by qRT-PCR after HERV-K(HML-2) activation using gRNA6 and gRNA11. n=2 biological replicates. The control was set to 1 and values represent mean \pm SD.

(I) MAP2 expression was examined by immunofluorescence in HERV-K(HML-2) activated cortical neurons using gRNA6 and gRNA11. Two biological replicates are shown. Scale bars, 100 μ m.

(J) MAP2 repression was confirmed by qRT-PCR during the course of cortical differentiation in HERV-K(HML-2) gRNA 6 and gRNA11 activated and control cells. Two biological replicates are shown.

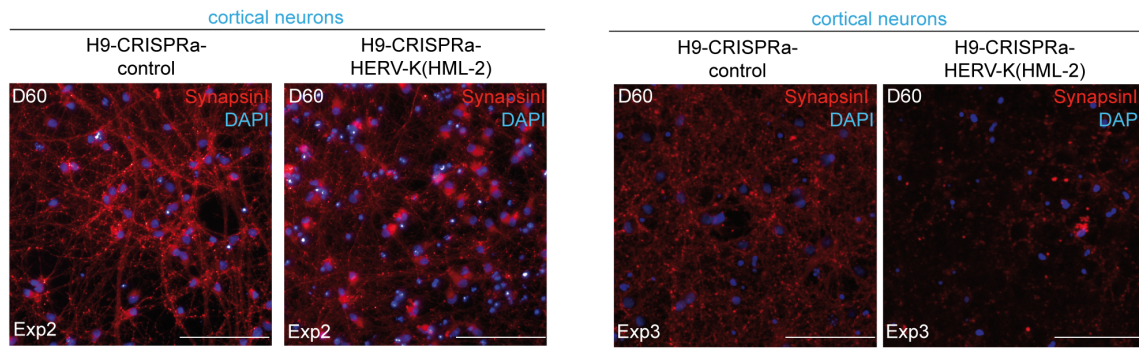
(K) Morphological analysis of cortical neurons upon HERV-K(HML-2) activation using gRNA 6 and gRNA11. Two biological replicates were quantified. Values represent mean \pm SD. Multiple t test using the Holm-Sidak method (****p<0.0001).

(L) Caspase 3 expression was analyzed in HERV-K(HML-2) activated as well as control pluripotent human embryonic stem cells. Moreover, Caspase3 expression was analyzed in day 60 cortical neurons generated from HERV-K(HML-2) activated cells and control cells using immunofluorescence. n=3 biological replicates. Scale bars, 100 μ m.

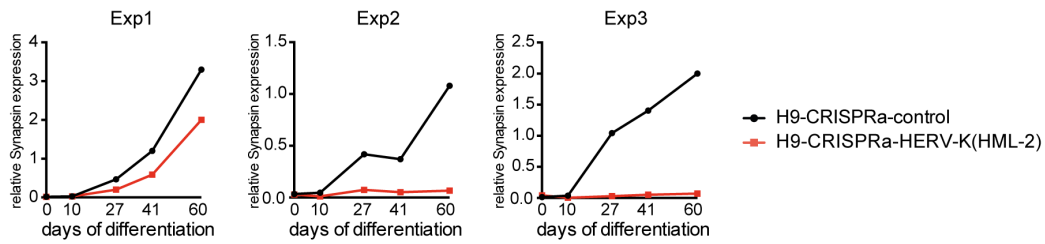
(M) Day 50 dopaminergic neurons generated from HERV-K(HML-2) activated cells as well as control cells were stained for MAP2 using immunofluorescence. Scale bars, 100 μ m.

(N) Equal activation of HERV-K(HML-2) was confirmed in day 60 cortical neurons as well as day 50 dopaminergic neurons generated from HERV-K(HML-2) activated cells using qRT-PCR.

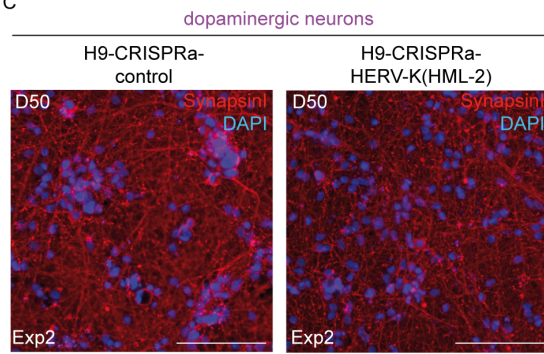
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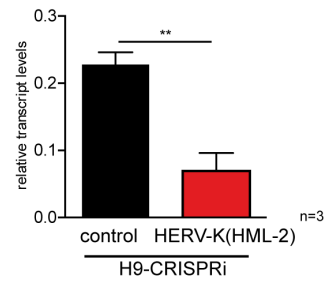
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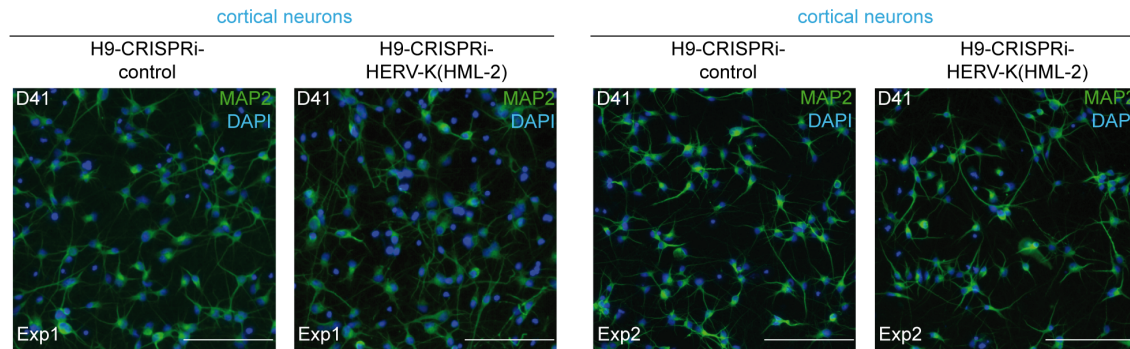
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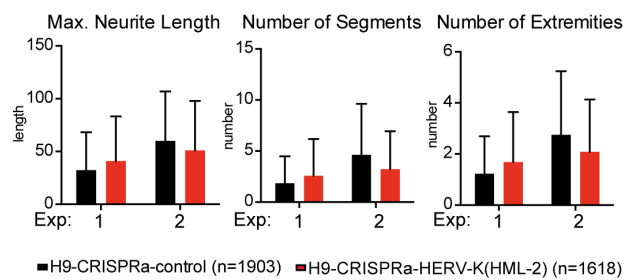


Figure S2: HERV-K(HML-2) activation reduces Synapsin I expression and functionality in cortical neurons. Related to Figure 2.

(A) Synapsin I was analyzed in HERV-K(HML-2) activated and control derived neurons by immunofluorescence. Two biological replicates are displayed. Scale bars, 100 μ m.

(B) *SYN1* gene expression was validated by qRT-PCR in HERV-K(HML-2) activated and control cells during the course of cortical differentiation. Three biological replicates are shown.

(C) Synapsin I expression in dopaminergic neurons derived from HERV-K(HML-2) activated cells was analyzed by immunofluorescence at day 50. One out of three biological replicates is shown. Scale bars, 100 μ m.

(D) HERV-K(HML-2) transcript levels were quantified by qRT-PCR, targeting a HERV-K(HML-2) *gag* region, in H9 cells stably expressing dCas9-KRAB together with HERV-K(HML-2) specific gRNAs or control gRNAs. n=3 biological replicates. Values represent mean \pm SD. Unpaired two-tailed Student's t test (**p<0.01).

(E) MAP2 expression was analyzed by immunofluorescence in HERV-K(HML2) repressed cells at day 41. n=2 biological replicates.

(F) Morphological analysis in HERV-K(HML-2) repressed and control cortical neurons using high-content image analysis. Two biological replicates were quantified. Values represent mean \pm SD. Multiple t test using the Holm-Sidak method.

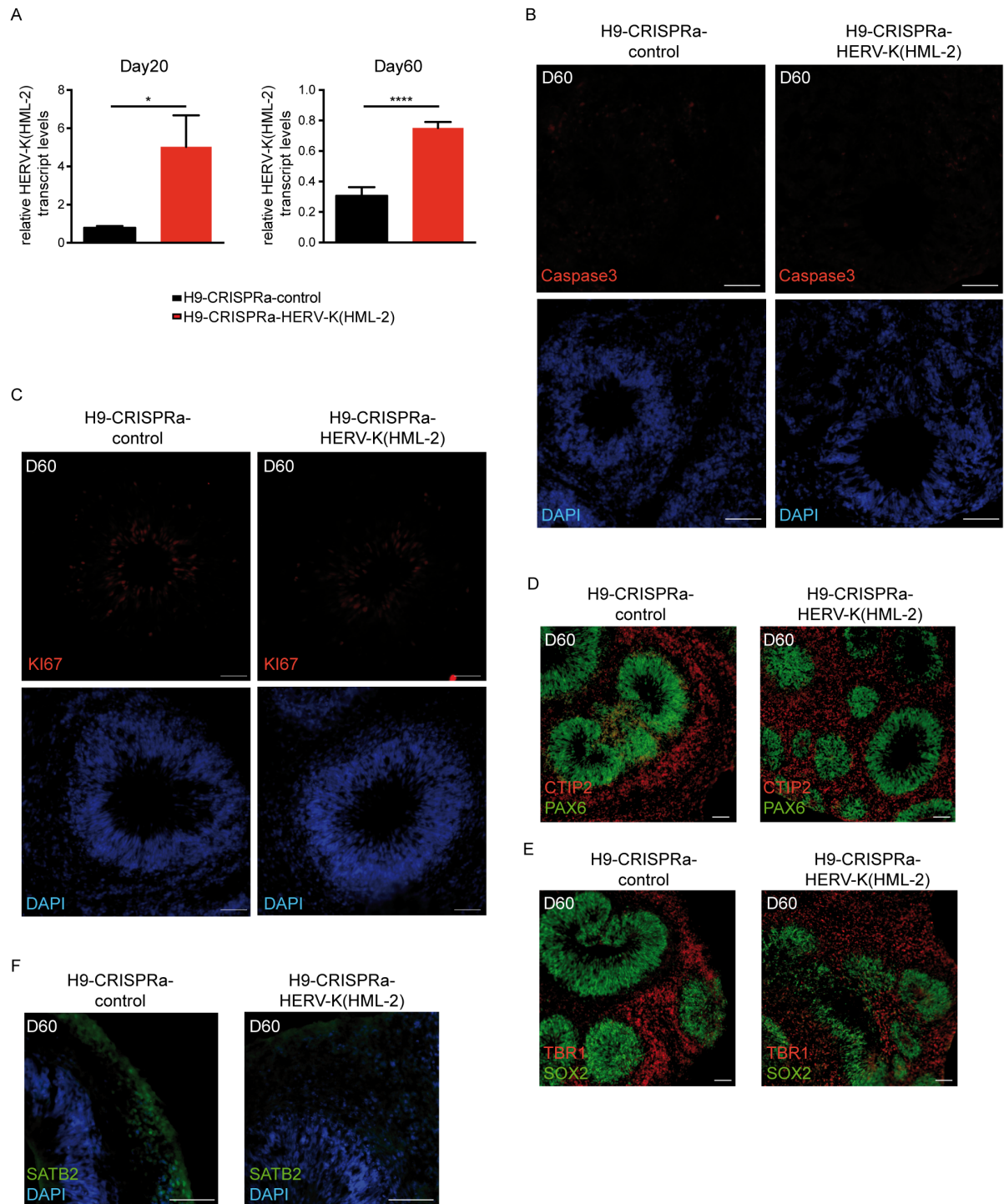


Figure S3: HERV-K(HML-2) activation has no effect on toxicity and leads to less organized CTIP2⁺ and TBR1⁺ layers in forebrain organoids. Related to Figure 3.

(A) HERV-K(HML-2) transcript levels were quantified by qRT-PCR in forebrain organoids. $n=3$ biological replicates. Values represent mean \pm SD. Unpaired two-tailed Student's t test (* $p<0.05$ and **** $p<0.0001$).

(B and C) Organoids from HERV-K(HML-2) activated and control cells were stained for Caspase 3 and KI67 by immunofluorescence ($n=3$). Scale bars, 50 μ m.

(D, E and F) Sections of HERV-K(HML-2) activated and control forebrain organoids at day 60 stained for CTIP2/PAX6 (E), TBR1/SOX2 (F) and SATB2/DAPI cells by immunofluorescence. $n=3$ biological replicates. Scale bars, 50 μ m.

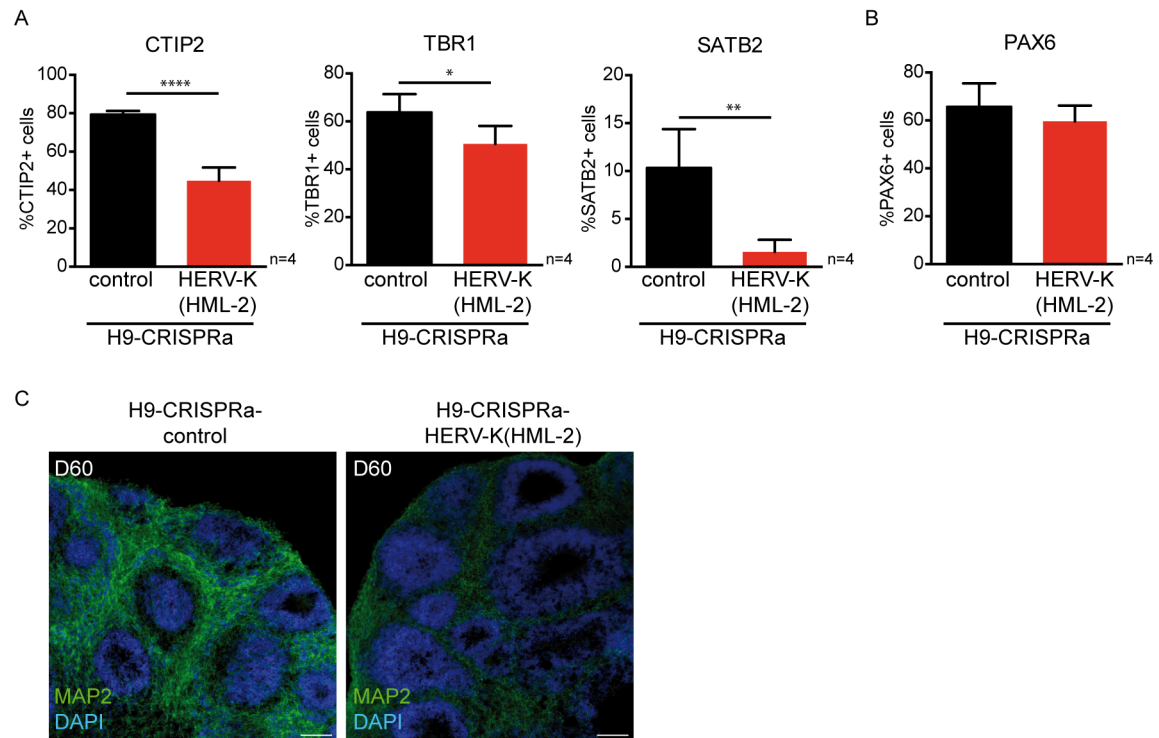


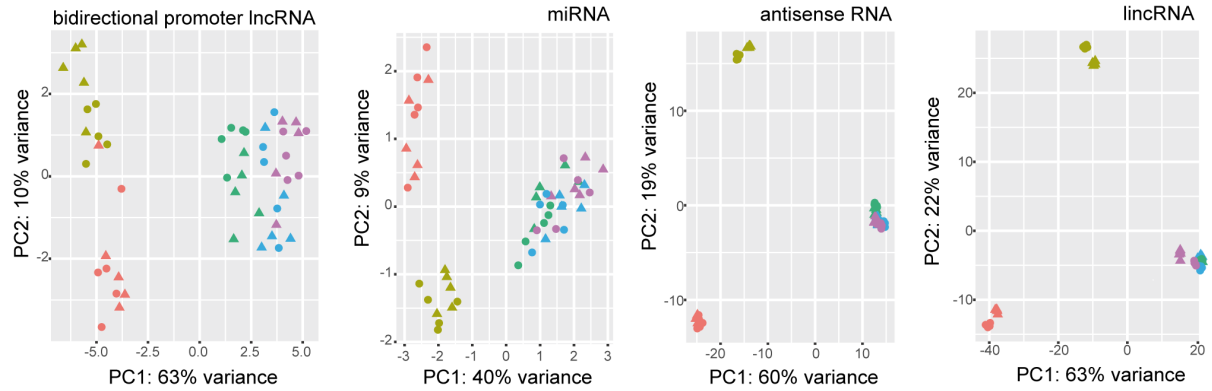
Figure S4: HERV-K(HML-2) activation leads to reduced CTIP2, TBR1 and SATB2 positive cells as well as reduced MAP2 expression. Related to Figure 4.

(A and B) Flow cytometry analysis of day 60 forebrain organoids upon HERV-K(HML-2) activation. n=4 biological replicates. Values represent mean \pm SD. Unpaired two-tailed Student's t test (* $p < 0.05$, ** $p < 0.01$ and **** $p < 0.0001$).

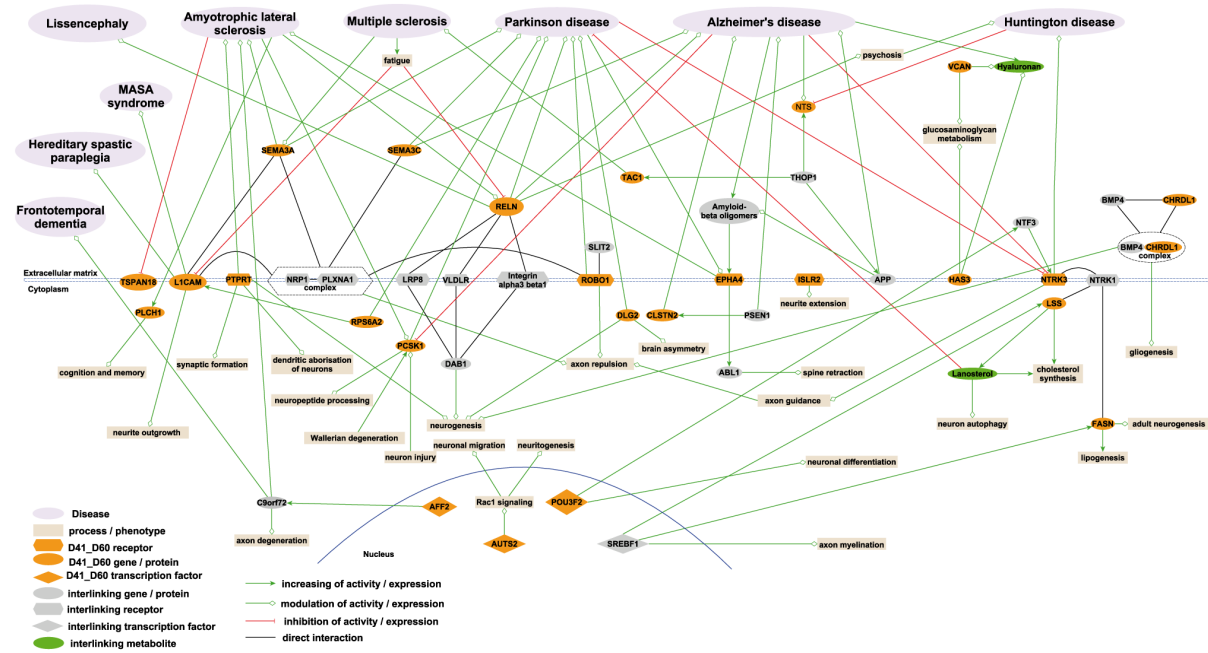
(C) Organoids from HERV-K(HML-2) activated and control cells were analyzed for MAP2 expression by immunofluorescence. n=3 biological replicates. Scale bars, 50 μ m.

Induction of HERV-K(HML-2) activation at day 5, day 10 and day 21 by doxycycline was examined by qRT-PCR at day 0, day 41, and day 55 during cortical differentiation. n=2 biological replicates. Values represent mean \pm SD. 2way ANOVA multiple comparison (*p<0.05, **p<0.01 and ***p<0.001).

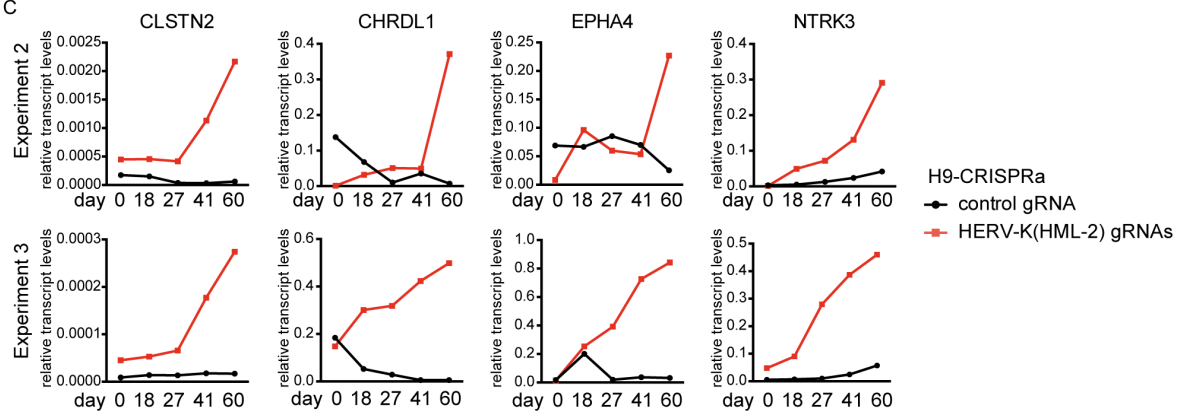
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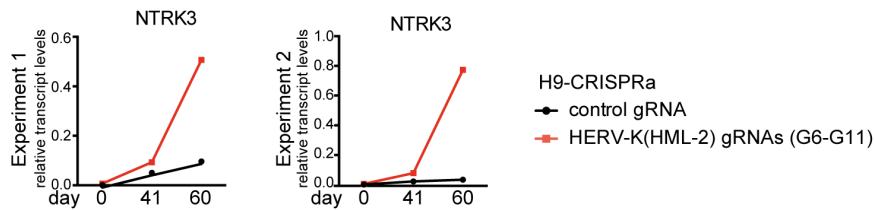


Figure S6: Network interactions between the identified genes and neurodegenerative diseases. Related to Figure 6.

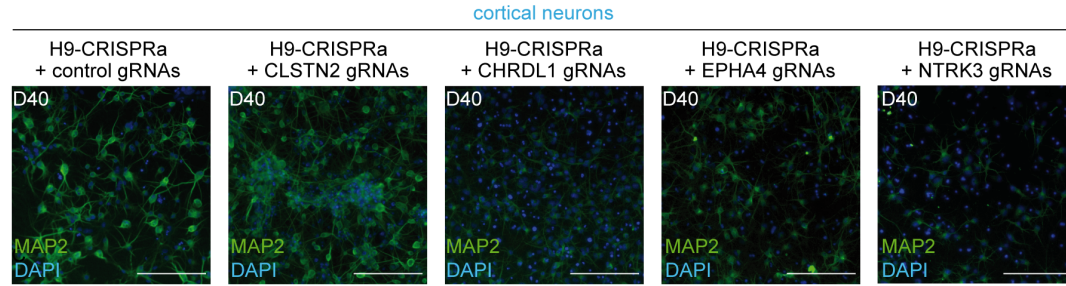
(A) Results from analyses of RNAseq data for bidirectional promoter lncRNA, miRNA, antisense RNA and lincRNA were visualized by PCA.

(B) Depiction of a network of interactions between the identified gene set and neurodegenerative diseases as per the multifactorial database CIDEr.

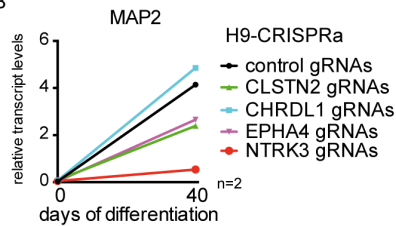
(C) Expression levels of *Calsyntenin 2* (*CLSTN2*), *Chordin Like 1* (*CHRD1*) and *Neurotrophic Tyrosine Receptor Kinase 3* (*NTRK3*) in HERV-K(HML-2) activated and control cells, measured by qRT-PCR, during the course of cortical neuron differentiation. Two further biological replicates are shown (see also Figure 6E).

(D) Expression level of *Neurotrophic Tyrosine Receptor Kinase 3* (*NTRK3*) in HERV-K(HML-2) activated cells, using gRNA6 and gRNA11 analyzed by qRT-PCR during the course of cortical neuron differentiation. Two biological replicates are shown.

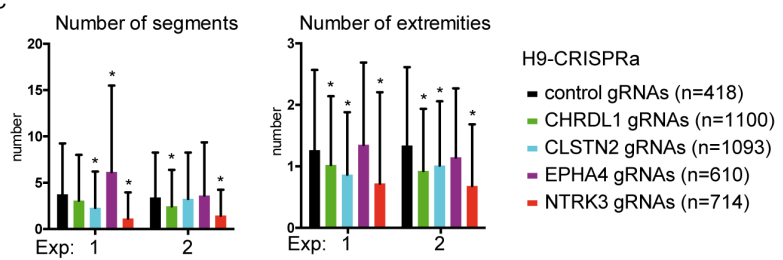
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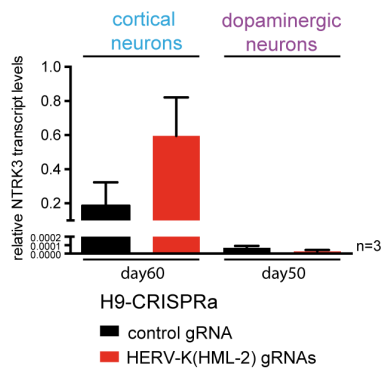
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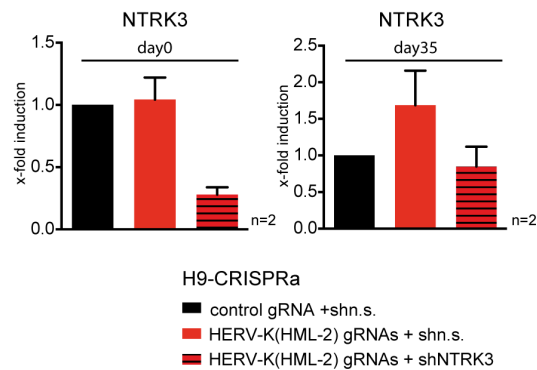
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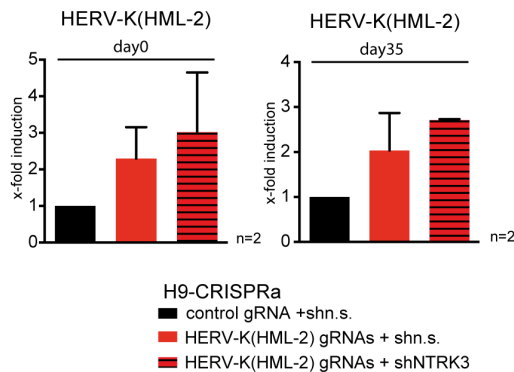
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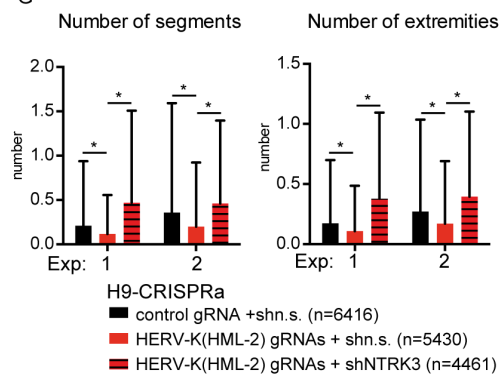


Figure S7: Activation of the Neurotrophic Tyrosine Receptor Kinase 3 (NTRK3) results in changes of MAP2 expression in cortical neurons. Related to Figure 7.

(A) MAP2 expression was analyzed by immunofluorescence in neurons and control cells with transcriptionally activated CLSTN2, CHRDL1 or NTRK3. Two biological replicates were performed. Scale bars, 100 μ m.

(B) MAP2 mRNA levels were quantified by qRT-PCR in neurons and control cells with transcriptionally activated CLSTN2, CHRDL1 or NTRK3. n=2 biological replicates.

(C) Morphological analyses, using high-content image analysis, in neurons with selected candidate genes activated. Two biological replicates were quantified. Values

represent mean \pm SD. Multiple t test using the Holm-Sidak method (*p < 0.05).

(D) *NTRK3* expression on the transcript level was measured by qRT-PCR in cortical neurons as well as dopaminergic neurons upon HERV-K(HML-2) activation. n=3 biological replicates. Values represent mean \pm SD.

(E) *NTRK3* transcript levels were quantified by qRT-PCR after *NTRK3* knockdown in HERV-K(HML-2) activated neurons. n=2 biological replicates. The control was set to 1 and values represent mean \pm SD.

(F) HERV-K(HML-2) transcript levels were quantified by qRT-PCR after *NTRK3* knockdown in HERV-K(HML-2) activated neurons. n=2 biological replicates. The control was set to 1 and values represent mean \pm SD.

(G) Morphological analysis, using high-content image analysis, in HERV-K(HML-2) activated neurons expressing an *NTRK3*-targeting or non-silencing control shRNA. Two biological replicates were quantified. Values represent mean \pm SD. Multiple t test using the Holm-Sidak method (*p<0.05).

Table S1: Top 28 upregulated genes upon HERV-K(HML-2) activation (related to Figure 6 and Figure S6).

Ensembl gene id	Gene name	Chr.	Gene description
ENSG00000157570	TSPAN18	chr11	tetraspanin 18
ENSG00000075223	SEMA3C	chr7	semaphorin 3C
ENSG00000114805	PLCH1	chr3	phospholipase C eta 1
ENSG00000189056	RELN	chr7	reelin
ENSG00000160285	LSS	chr21	lanosterol synthase
ENSG00000158258	CLSTN2	chr3	calyntenin 2
ENSG00000140538	NTRK3	chr15	neurotrophic receptor tyrosine kinase 3
ENSG00000184486	POU3F2	chr6	POU class 3 homeobox 2
ENSG00000167178	ISLR2	chr15	immunoglobulin superfamily containing leucine rich repeat 2
ENSG00000075213	SEMA3A	chr7	semaphorin 3A
ENSG00000198910	L1CAM	chrX	L1 cell adhesion molecule
ENSG00000071242	RPS6KA2	chr6	ribosomal protein S6 kinase A2
ENSG00000150672	DLG2	chr11	discs large MAGUK scaffold protein 2
ENSG00000093072	ADA2	chr22	adenosine deaminase 2
ENSG00000101938	CHRD1	chrX	chordin like 1
ENSG00000006128	TAC1	chr7	tachykinin precursor 1
ENSG00000103044	HAS3	chr16	hyaluronan synthase 3
ENSG00000175426	PCSK1	chr5	proprotein convertase subtilisin/kexin type 1
ENSG00000133636	NTS	chr12	neurotensin
ENSG00000169855	ROBO1	chr3	roundabout guidance receptor 1
ENSG00000116106	EPHA4	chr2	EPH receptor A4
ENSG00000196090	PTPRT	chr20	protein tyrosine phosphatase%2C receptor type T
ENSG00000169710	FASN	chr17	fatty acid synthase
ENSG00000131016	AKAP12	chr6	A-kinase anchoring protein 12
ENSG00000152818	UTRN	chr6	utrophin
ENSG00000158321	AUTS2	chr7	AUTS2 activator of transcription
ENSG00000155966	AFF2	chrX	AF4/FMR2 family member 2
ENSG00000038427	VCAN	chr5	versican

Table S2: Sequences of gRNAs used in this study (related to the Star Methods).

Name	Source	Sequence
Control gRNA	(Kearns et al., 2014)	GTTCCGCGTTACATAACTTA
HERV-K(HML-2) gRNA 3 U	This study	AAATGGATTAAGGGCGGTGC
HERV-K(HML-2) gRNA 3 L	This study	GCACCGCCCTTAATCCATTTC
HERV-K(HML-2) gRNA 10 U	This study	ATCCTCCATATGCTGAACGC
HERV-K(HML-2) gRNA 10 L	This study	GCGTTCAGCATATGGAGGATC
HERV-K(HML-2) gRNA 6 U	This study	CTGAGGAGGATTAGTAAAAG
HERV-K(HML-2) gRNA 6 L	This study	TTTTACTAATCCTCCTCAG
HERV-K(HML-2) gRNA 11 U	This study	AGCCTGAAATATGGCCTCGT
HERV-K(HML-2) gRNA 11 L	This study	ACGAGGCCATATTTTCAGGTC
CHRD1 gRNA 1 U	This study	GCCGGCTTCTCGGGCCAAGT
CHRD1 gRNA 1 L	This study	ACTTGGCCCGAGAAGCCGGC
CHRD1 gRNA 7 U	This study	GCTCCCTCGTGGTGTGAGGG
CHRD1 gRNA 7 L	This study	CCCTCACACCACGAGGGAGC
CLSTN2 gRNA 3 U	This study	GAGAGTCGGAGTGGAGGCGC
CLSTN2 gRNA 3 L	This study	GCGCCTCCACTCCGACTCTC
CLSTN2 gRNA 23 U	This study	GTGACGTCACGGGCCGTCCC
CLSTN2 gRNA 23 L	This study	GGGACGGCCCGTGACGTCAC
EPHA4 gRNA 2 U	This study	GATCCCCCACGTTACCTCGA
EPHA4 gRNA 2 L	This study	TCGAGGTAACGTGGGGGATC
EPHA4 gRNA 11 U	This study	GACTGGCGGGCTCACGTCAC
EPHA4 gRNA 11 L	This study	GTGACGTGAGCCCGCCAGTC
NTRK3 gRNA 1 U	This study	GATAACCCGTGCGTTTCGTA
NTRK3 gRNA 1 L	This study	TACGAAACGCACGGGTTATC
NTRK3 gRNA 17 U	This study	GCATTTGAGATTGCGAGGGT
NTRK3 gRNA 17 L	This study	ACCCTCGCAATCTCAAATGC

Table S3: Sequences of qRT-PCR primers used in this study (related to the Star Methods).

Primers	SEQUENCE FORWARD (5'-3')	SEQUENCE REVERSE (5'-3')
RNA Polymerase II	GCACCACGTCCAATGACAT	GTCGGCTGCTTCCATAA
dCas9	TCGGATCTGCTACCTGCAGGAGATCTTT AG	CAGCCTTATCAGTACTGTCTACCAGCT TCT
MAP2	TTCCTCCATTCTCCCTCCTC	TCTGCGAATTGGCTCTGAC
SYNAPSIN-I	GGAAGGGATCACATCATTGAGG	TGTTTGTCTTCATCCTGGT
CHRD1	CCTGGAACCTTATGGGTTGGT	AACATTTGGACATCTG ACTCGG
CLSTN2	GCACCGGGAGGCGAG	TGTGCTTATTGACTTTAGCCGC
EPHA4	GGAAGGCGTGGTCACTAAAT	TCTGCCATCATTTTTCTGA
NTRK3	TCCGTCAGGGACACAACCTG	GCACACTCCATAGAACTTGACA