Supporting Information

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SI Materials and Methods

Mammalian Cell Culture and Cell Line Generation. JumpIN TI 293 (catalog no. M4455; Life Technologies, Inc.) and SH-SY5Y (CRL-2266; American Type Culture Collection) cell lines were confirmed by SNP analysis and verified to be mycoplasma free by routine testing. All cells were kept in a humidified incubator at 37 °C and 5% CO_2. JumpIN TI 293 cells were cultured according to the manufacturer's instructions. SH-SY5Y cells were maintained in DMEM Nutrient Mixture F-12 (no. 10565–018; Life Technologies) supplemented with 10% FBS. Cell culture reagents were obtained from Invitrogen. JumpIN TI 293 cells expressing 3xFlagCas9 were generated by lentiviral delivery of Cas9 in pNGx-LV-c003 (Dataset S4) followed by selection with zeocin and isolation of single clones. endoGFP-PARKIN cells were generated by transfecting JumpIN TI 293 Cas9+ cells with pcDNA3.1(+) in which EGFP-PARKIN was inserted using NheI and HindIII restriction sites. The CMV promoter was replaced by 4.5 kb of the genomic DNA sequence upstream of the PARKIN translation start site using NruI and NheI restriction sites to generate pcDNA3.1(+)-endoGFP-PARKIN, which was sequence verified. Transfected cells were selected with geneticin and single clones were isolated. SH-SY5Y cells expressing 3xFlagCas9 were generated by lentiviral delivery of Cas9 in pNGx-LV-c004 (19) followed by selection with blasticidin and isolation of single clones. Individual clones were expanded, tested for Cas9 and/or GFP-PARKIN expression by Western blot and flow-cytometry, and JumpIN TI 293 Cas9+ endoGFP-PARKIN clone 9/2 (endoGFP-PARKIN cells) was picked for the screen and confirmation experiments. JumpIN TI 293 Cas9+ clone 9 (parental cells) was picked for validation experiments. SH-SY5Y Cas9+ clone 10 was picked for validation experiments.

Pooled CRISPR Screening.

sgRNA library design and construction. The genome-wide sgRNA library targeting 18,360 protein-coding genes was constructed using chip-based oligonucleotide synthesis to generate spacer-tracrRNA–encoding fragments that were PCR-amplified and cloned as a pool into the BpiI site of the pRSI16 lentiviral plasmid (Cellecta). Olfactory receptors were omitted from the library. The sgRNA designs were based on published sequences (52), and five sgRNAs were selected per gene targeting the most proximal 5′ exons. A total of 277 genes did not have published sgRNA sequence information, and new sgRNAs were designed for these targets that contained an NGG PAM motif, filtering for GC content >40% and <80%, eliminating homopolymer stretches >4, and removing any guides with off-target locations having <4 mismatches across the genome. Sequencing of the plasmid pool showed robust normalization, with >90% clones present at a representation of ±fivefold from the median counts in the pool.

Viral packaging. sgRNA libraries were packaged into lentiviral particles using HEK293T cells as described previously (53). Packaging was scaled up by growing cells in cell stacks (Corning). For each cell stack, 210 million cells were transfected 24 h after plating using 510.3 μL TransIT reagent (Mirus) diluted in 18.4 mL OPTI-MEM that was combined with 75.6 μg of the sgRNA library and 94.5 μg of lentiviral packaging mix (psPAX2 and pMD2 plasmids that encode Gag/Pol and VSV-G, respectively; Cellecta). Seventy-two hours posttransfection, lentivirus was harvested, aliquoted, and frozen at −80 °C. Viral titer was measured by FACS in HCT116 cells and was typically in the range of 5×10^6 transforming units/mL.

FACS-based screening procedure. The screen was run in duplicate. sgRNA libraries were transduced at a multiplicity of infection (MOI) of 0.5, aiming for coverage of, on average, 1,000 cells per sgRNA reagent. MOI was determined by using a 12-point dose-response ranging from 0 to 400 μL of viral supernatants in the presence of 5 μg/mL polybrene and measuring infection rate by FACS as a percentage of red fluorescent protein (RFP)-positive cells. Selection was optimized by determining the puromycin dose required to achieve >95% cell killing in 96 h. Cell viability was measured for a six-point dose-response ranging from 0 to 10 μg/mL puromycin using the Incucyte Live Cell Analysis System (Essen BioScience). For the genome-wide screen, cells were seeded at 167.5 million cells per cell stack (Corning) in media containing 5 μg/mL polybrene and lentivirus at an MOI of 0.5. Twenty-four hours after infection, cells were trypsinized and reseeded in media containing puromycin. Ninety-six hours after puromycin addition, cells were trypsinized and plated into new cell stacks at 167 million cells per cell stack. An aliquot of cells was analyzed by FACS to confirm infection and selection efficiency, and the percentage of RFP-positive cells was typically >95%. Cells were maintained in culture and split as needed to ensure confluence did not exceed 90%. The sgRNA libraries were screened 8 d and 15 d postinfection. For FACS, cells were harvested, resuspended at 30 million cells/mL, and fixed using BD Cytofix (diluted 1:1 in PBS; BD Biosciences) according to the manufacturer's instructions. Single, RFP-positive cells were sorted (BD ARIA Fusion) from the lower GFP quartile (GFP-low) or from the upper GFP quartile (GFP-high). For each replicate, 100 million cells were sorted by FACS from the lower GFP quartile or from the upper GFP quartile. One hundred million unsorted cells were also collected as an input sample.

Illumina library construction and sequencing. Fixed cells were treated in de-crosslinking buffer (TNES: 10 mM Tris-Cl pH 8.0, 100 mM NaCl, 1 mM EDTA, 1% SDS; overnight 65 °C) and genomic DNA was extracted using phenol:chloroform:isoamylalcohol (PCIA 25:24:1; Sigma) and quantified using PicoGreen (Invitrogen) following the manufacturer's instructions. Illumina sequencing libraries were generated using PCR amplification with primers specific to the genome integrated lentiviral vector backbone sequence. A total of 24 × 4 μg PCR reactions were performed per transduced sample. PCR reactions were performed in a volume of 100 μL containing a final concentration of 0.5 μM of each PCR primer (Integrated DNA Technologies), 0.5 mM dNTPs (Clontech), and 1× titanium Taq and buffer (Clontech). PCR primer sequences are reported in DeJesus et al. (19). PCR cycling conditions used were as follows: 1× 98 °C for 5 min; 28× 95 °C for 15 s, 65 °C for 15 s, 72 °C for 30 s; 1× 72 °C for 5 min. The resulting Illumina libraries were purified using 1.8× SPRI AMPure XL beads (Beckman Coulter) following the manufacturer's recommendations and qPCR quantified using primers specific to the Illumina sequences using standard methods. Illumina sequencing libraries were pooled and sequenced with an HiSEq. 2500 instrument (Illumina). sgRNA libraries were sequenced with 1 × 30 b (sgRNA) and 1 × 11 b (sample index) reads. Sequencing was performed following the manufacturer's recommendations using custom sequencing primers (19). The number of reads was adjusted to cover each sgRNA with ∼1,000 reads.

CRISPR screening data analysis. Raw sequencing reads were aligned to the appropriate library using Bowtie (54), allowing for no mismatches, and counts were generated. Differential fold-change estimates between samples were generated for each sgRNA using DESeq2 (55). Effects on proliferation were assessed by

comparison of the unsorted cell population to the input library. For gene-based hit calling, consistency of all sgRNAs per gene was considered [RSA (25)] and plotted against the second strongest sgRNA as representative.

Gene-set enrichment analyses. The gene-set database was compiled from multiple sources including Reactome, NCBI Biosystems, and Gene Ontology. Enrichments for CRISPR screening hits were calculated using a hypergeometric overrepresentation test. The top 500 best genes were considered as input. Benjamini-Hochberg–corrected *P* values were calculated for each gene set and combination of input gene list size. For RNAseq data, gene-set enrichment analyses were performed using a python script that conduct a series of (non)parametric tests for each contrast. We performed all of the analyses using two gene rankings, where the first one is by fold-change only and the second is by the fold-change multiplied by the negative logarithm of the *P* value; the latter version puts more weight on genes that move less, but significantly. Within a contrast, results were adjusted using the Benjamini-Hochberg method.

Confirmation of Individual sgRNAs. Gene scores were assigned by multiplying fold-change values of the second strongest sgRNA of a particular gene with its RSA value. Genes were ranked according to their scores and selected for confirmation. For confirmation of individual candidate hit genes, sgRNA sequences were cloned into the BbsI site of the pNGx-LV-g003 lentiviral plasmid (19) and plasmid DNA was obtained from transformed bacterial clones. sgRNAs directed against *THAP11* (and a non-targeting SCR sgRNA) were also cloned into pNGx-LV-gc006 (Dataset S4) for use in iPSCs. Plasmids were sequence verified. Lentiviral particles were generated in HEK293T cells using the ViraPower Lentiviral Packaging Mix (Invitrogen) following the manufacturer's instructions. endoGFP-PARKIN cells and parental JumpIN cells were seeded at 25,000 cells/well in 96 wells in media containing 5 μg/mL polybrene and infected with 10 μL of virus supernatant, respectively. Infected cells were selected with 1 μg/mL puromycin for 96 h and analyzed 8 or 15 d postinfection. SH-SY5Y Cas9+ cells were seeded at 500,000 cells/well in a six-well plate in media containing 5 μg/mL polybrene and infected with 25 μL of virus supernatant. Twenty-four hours postinfection, media were replaced with fresh media and incubated for 24 h. Subsequently, infected cells were selected with 1 μg/mL puromycin for 72 h and analyzed 15 or 16 d postinfection.

Where indicated, cells were treated with 10 μM CCCP (Sigma-Aldrich), 10 μM/5 μM A/O (Sigma-Aldrich), 1 μM valinomycin (Sigma-Aldrich), or 0.1% DMSO (Sigma-Aldrich).

Analysis of Gene Editing Efficiency Using the Software TIDE. The TIDE editing efficiency assay was described by Brinkman et al. (27). Briefly, genomic DNA was isolated from cultured cells using the AllPrep DNA/RNA Kit (catalog no. 80204; Qiagen) following the manufacturer's instructions and 10 ng genomic DNA was processed by PCR using the 2X GoTaq Polymerase Mastermix (Cat M7122; Promega). Primers, number of cycles, and annealing temperature are listed in Dataset S4. PCR amplification conditions were as follows: initial denaturation step of 2 min at 95 °C, amplification cycles at 94 °C for 30 s, primer-specific annealing temperature for 30 s and 72 °C for 1 min, and extension step at 72 °C for 7 min. The final PCR products were electrophoresed on 1% agarose gel and size-specific bands purified with the Wizard SV gel & PCR kit (catalog no. A9281; Promega). Purified PCR products were sequenced and gene editing was compared with controls (SCR) by the TIDE webtool software available at https://tide.nki.nl/ (27).

***PARK2, SIRT4,* and *MTRF1* qPCR.** Total RNA was extracted using the AllPrep DNA/RNA Kit (catalog no. N80204; Qiagen) and cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (catalog no. 4368814; Thermo Fischer Scientific) following the manufacturer's instructions. Each cDNA was amplified using qPCR Maxima Probe (catalog no. K0231; Thermo Fischer Scientific) on the ViiA7 real-time qPCR system. Primers and probes were purchased from Applied Biosystems (GAPDH HS99999905_M1, PARK2 HS01038322_M1, PACRG HS01125334_M,1 HPRT1 HS02800695_M1, SIRT4 HS00202033_M1, MTRF1 HS00939225_M1). Briefly, 10 ng cDNA was amplified at 50 °C for 2 min, 95 °C for 10 min, and 40 cycles at 94 °C for 15 s and 60 °C for 45 s. Relative quantification of mRNA was performed using *HPRT1* or *GAPDH* mRNA as normalization control using the $\Delta\Delta$-C_T method. Student's *t* test was applied to calculate *P* values.

RNA Sequencing. RNA sequencing libraries were prepared using the Illumina TruSeq RNA Sample Prep kit v2 and sequenced using the Illumina HiSeq2500 platform. Samples were sequenced to a length of 2 × 76 bp. A total of 615 million 76-bp paired-end reads were mapped to the *Homo sapiens* genome (HG19) (56) (release 59; May 3, 2013) and a custom junction database by using an in-house gene quantification pipeline (57). On average, 97.8% of the total reads were mapped to the genome or the transcripts, and 92.8% of the aligned reads mapped to expressed sequences. The genome and the transcript alignments were used to derive gene counts based on human Ensembl gene IDs (v76; August 2014). Gene counts were divided by the total number of mapped reads for each sample and multiplied by one million to obtain cpm to account for varying library sizes. Differential expression analysis was performed on the cpm using a limma/voom workflow with R (58). The following test was performed: THAP11 knockout vs. control. Results are reported in terms of log2 fold-changes and negative log10-adjusted *P* values (Benjamini Hochberg false discovery rate) in Dataset S3.

DNA Motif Enrichment Analysis. To search for functional DNA motifs, Ensembl Gene IDs were used to obtain promoter sequences located within 2,000 bases upstream and 2,000 bases downstream of each transcription start site using the Regulatory Sequence Analysis Tools (59) available at rsat.eu. Sequences were masked for the presence of repeats, and redundant sequences due to alternative transcripts were also avoided. The obtained sequences were scanned for the presence of the known THAP11 DNA binding motif [previously identified by ChIP-seq in Hnisz et al. (60)] using Centrimo (61) of the MEME suite (62).

Cell Reprogramming, iPSC Maintenance, and Differentiation (hDF 83/22 NGN2 #9). Neonatal human dermal fibroblasts from Invitrogen were used for reprogramming using Sendai virus with the help of the CytoTune-iPS reprogramming kit according to the standard protocol. Colonies with hallmarks of pluripotent morphology were readily visible between days 17 and 20 after transduction. These were picked and subcloned multiple times on plates coated with Matrigel (BD Biosciences) in mTeSR medium (STEMCELL Technologies) until Sendai virus RNA could no longer be detected and the morphology looked stable. Pluripotency was controlled by FACS analyses. Potential to differentiation into the three germ layers was approved by using the Applied Biosystems TaqMan hPSC Scorecard Panel according to the supplier's guideline. Karyotype analysis was performed by full-genome SNP analyses done by life&brain and showed no larger chromosomal aberrations.

Generation of iNGN2 iPSCs. Human *NGN2* cDNA was synthesized using sequence information from the Ensembl database (Ensembl Gene ID ENSG00000178403 or accession number NM_024019.3) and cloned under the control of TRE tight (Tetracycline Response Element) promoter in a PiggyBac/Tet-ON all-in-one

vector (63). This vector contains a CAG rtTA16 cassette allowing constitutive expression of Tet-ON system and an Hsv-tkNeo cassette for generation of stable iPS clones. After trypsinization into single cells with TrypLE express reagent (catalog no. 12604013; ThermoFisher), ~1×10^6 iPS cells were nucleofected by the Amaxa nucleofector device using the Human Stem Cell Nucleofector Kit 1 (VPH-5012; Lonza) and Prg#B-016 with 4 μg of NGN2 plasmid and 1 μg of the dual helper plasmid. Subsequently, cells were replated on Matrigel plates with mTeSR medium containing 10 μM of Rock inhibitor. Antibiotic selection (G418, 0.1 mg/mL) was applied 48 h later. Stable clones appeared within 1 wk.

Generation of iNGN2 SCR iPSCs and Thap11$^{+/-}$ iPSCs. Viral particles expressing sgRNAs and Cas9 were produced as described above and used to infect iNGN2 iPSCs. 1×10^6 iNgn2 iPSCs were seeded on Matrigel-coated six-well plates in mTeSR medium containing 10 μM of Rock inhibitor and infected at confluency (~2 d after seeding). A total of 100 μL of viral supernatant per well was used for infection. Twenty-four hours postinfection, cells were selected in mTeSR medium containing 1 μg/mL puromycin. Puromycin-containing mTeSR medium was refreshed every day for 4 d. Resistant clones were allowed to expand in mTeSR medium until visible colonies formed. Individual colonies were picked and transferred to Matrigel-coated 35-mm dishes in mTeSR medium containing 10 μM of Rock inhibitor. Single clones were allowed to expand in mTeSR medium before assessment of editing efficiency.

Differentiation of iNGN2 Neurons. After trypsinization, 1×10^6 iPS cells were plated on a 6-cm Matrigel plate in proliferation medium (DMEM/F12 with Glutamax supplemented with 2% B27; catalog no. 17504-044; ThermoFisher) and 1% N2 (catalog no. 17502-048; ThermoFisher), 10 ng/mL hEGF (catalog no. PHG0315; ThermoFisher), 10 ng/mL hFGF (catalog no. CTP0263; ThermoFisher), and 1% Pen/Strep (catalog no. 15070-063; ThermoFisher) containing Rock inhibitor (10 μM) for 1 d and doxycycline (1 μg/mL) for 3 d. Three days later, induced neurons were given differentiation medium (Neurobasal supplemented with 2% B27, 1% N2, Pen/Strep, 0.05 mg/mL uridine, 1 mM sodium pyruvate; catalog no. 11360-039; ThermoFisher) and the following growth factors at 10 ng/mL: BDNF (catalog no. 450-02), GDNF (catalog no. 450-10), and hNT3 (catalog no. 450-03) (all from PeproTech).

Western Blotting. Cells were lysed in RIPA buffer (Thermo Scientific) supplemented with protease inhibitor mixture (Roche) and 1% Triton X-100 (Sigma) by rigorous vortexing. Protein concentration was determined with the Pierce BCA protein assay kit (Thermo Scientific). Samples were mixed with NuPAGE LDS sample buffer (Invitrogen) containing 5 mM DTT (Sigma), denatured for 20 min at 40 °C, and loaded onto NuPAGE Novex 12% Bis-Tris protein gels (Invitrogen). Proteins were transferred to nitrocellulose membranes using the NuPage blotting system (Bio-Rad). After blocking for 1 h in Odyssey blocking buffer (LICOR), membranes were incubated overnight at 4 °C with primary antibodies in Odyssey blocking buffer supplemented with 0.1% Tween-20 (Bio-Rad). Secondary antibodies (anti-rabbit IRDye 800CW from LICOR; anti-mouse Alexa Fluor 680 from Invitrogen) were diluted in Odyssey blocking buffer supplemented with 0.1% Tween-20 and incubated with membranes for 1 h at room temperature. Proteins were visualized using the Odyssey infrared imaging system. Alternatively, membranes were probed with secondary (anti-rabbit HRP or anti-mouse HRP from GE Healthcare) antibodies diluted 1:2,000 for 1 h at room temperature. Proteins were visualized using the SuperSignal West Pico chemiluminescent substrate (Thermo Scientific) by autoradiography. The primary antibodies used in this study are reported in Dataset S4.

Confocal Immunofluorescence Microscopy. A total of 2,000 endoGFP-PARKIN cells were seeded on day 7 postinfection into 384 clear bottom plates coated with poly-D-lysine in fresh growth media. Upon 24 h incubation, cells were treated with 10 μM/5 μM A/O (Sigma-Aldrich) or DMSO for 6 h before fixation in 4% paraformaldehyde, blocking (Odyssey buffer; LICOR), and permeabilization in 0.1% TX-100 (Sigma). Fixed cells were incubated overnight at 4 °C with primary anti-pUb antibodies (A110 1:400; R&D) and for 3 h with secondary anti-rabbit AlexaFLuor647 (1:800; Life Technologies) supplemented with Hoechst 33342 (Sigma-Aldrich). After three washes with PBS, cells were subjected to confocal laser scanning microscopy (Zeiss LSM700). Images were acquired using 40× magnification, DAPI, and far-red laser settings.

Statistical Analyses. Statistical analyses were performed with a two-tailed unpaired *t* test or as indicated in the figure legends and Methods. *P* values are indicated by asterisks in figures: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Differences lower than $P = 0.05$ were considered significant.

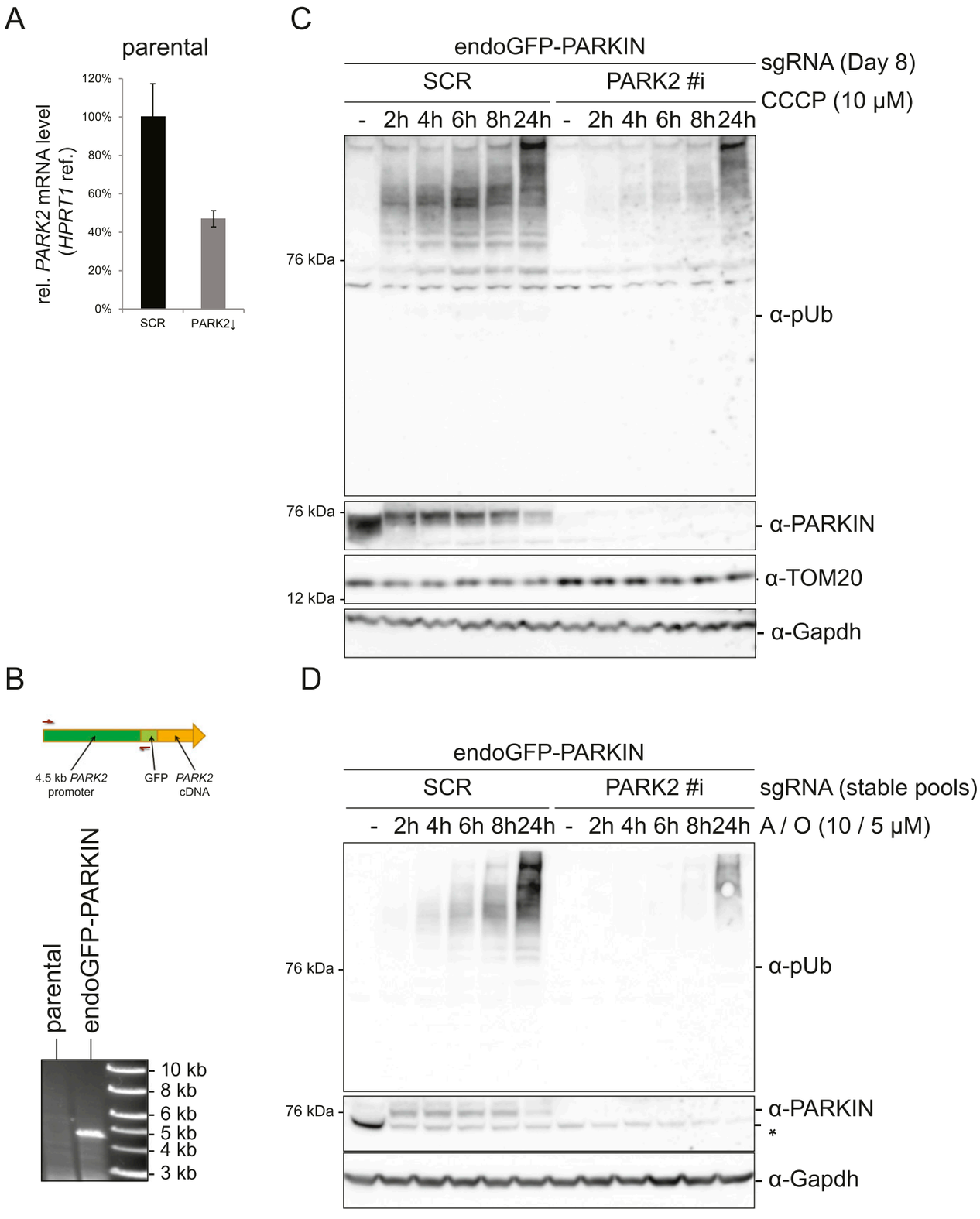
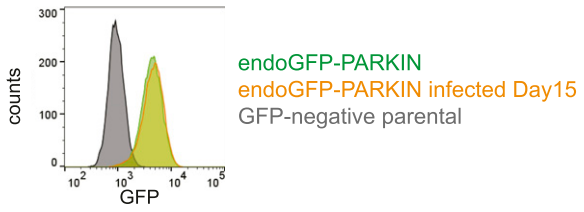
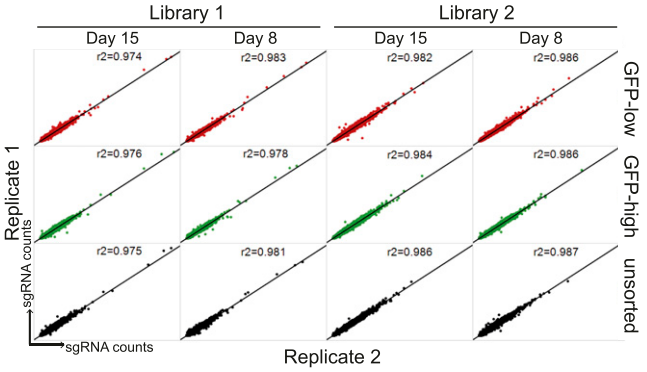
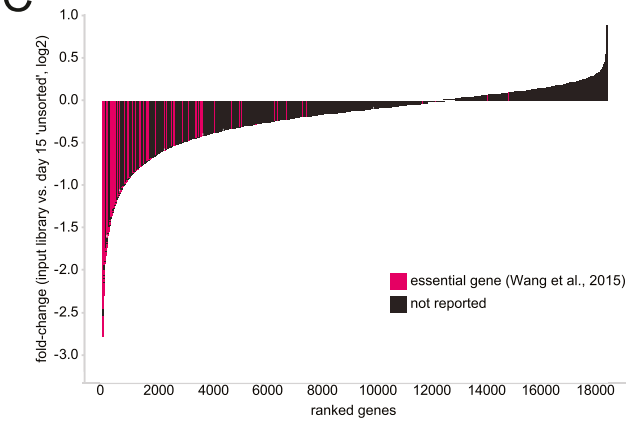


Fig. S1. (*A*) Parental JumpIN Cas9+ cells express *PARK2*. qPCR on total RNA extracted from parental JumpIN Cas9+ cells treated with either nontargeting SCR or a *PARK2*-specific siRNA (PARK2↓). *PARK2*-specific Taqman probes were used with *HPRT1* probes as a reference. Technical triplicates; error bars indicate SDs. (*B*) GFP-PARKIN is expressed from 4.5 kb of endogenous *PARK2* promoter. Genomic DNA from endoGFP-PARKIN and parental cells was extracted and subjected to PCR using a transgene-specific primer set comprising the entire 4.5-kb *PARK2* promoter (*Upper*). PCR products were analyzed by agarose gel electrophoresis (*Lower*). (*C*) Time-course analysis of PARKIN-dependent pUb accumulation in endoGFP-PARKIN cells treated with CCCP. WB analysis of endoGFP-PARKIN expressing *PARK2*-specific (PARK2 #i) or control (SCR) sgRNAs treated with 10 μM CCCP for times indicated on day 8 postinfection. The membrane was probed with antibodies indicated. (*D*) Time-course analysis of PARKIN-dependent pUb accumulation in endoGFP-PARKIN cells treated with A/O. WB analysis of stable pools of endoGFP-PARKIN cells expressing *PARK2*-specific (PARK2 #i) or control (SCR) sgRNAs by lentiviral delivery treated with A/O for times and concentrations given. The membrane was probed with antibodies indicated. *An unspecific cross-reaction. rel., relative; ref., reference transcript; WB, Western blot.







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Day 8

GFP-low

Gene set	-log (*p*-value)	set size	common #
poly(A) RNA binding	44.94	1120	125
nucleoplasm	38.03	2740	185
protein binding	23.56	8651	328
translational initiation	17.21	132	30
rRNA processing	16.46	203	35
nucleolus	16.2	820	69
mediator complex	12.49	34	15
mRNA catabolism, nonsense-med. decay	12.49	117	24
cytosolic small ribosomal subunit	12.17	43	16
mitochondrion	10.96	1226	76

Day 15

GFP-low

Gene set	-log (*p*-value)	set size	common #
mitochondrion	47.75	1226	134
mitochondrial inner membrane	43.06	410	78
mitochondrial translational elongation	28.96	85	34
mitochondrial translational termination	28.87	86	34
poly(A) RNA binding	27.74	1120	101
mitochondrial respiratory chain C I assembly	19.61	55	23
nucleoplasm	17.93	2740	146
mitochondrial matrix	17.2	324	44
mitochondrial large ribosomal subunit	16.31	32	17
protein binding	12.81	8651	298

GFP-high

Gene set	-log (*p*-value)	set size	common #
nucleoplasm	24.77	2740	162
nucleus	12.33	5116	206
protein binding	9.72	8651	290
interstrand cross-link repair	8.4	47	14
poly(A) RNA binding	6.03	1120	62
INTS6/SAGE1/DDX26B/CT45, C-terminal	5.68	12	7
Fanconi anaemia nuclear complex	5.16	14	7
DNA repair	5.16	196	21
transcription, DNA-templated	4.71	1782	80
telomere organization	4.23	27	8

GFP-high

Gene set	-log (*p*-value)	set size	common #
nucleoplasm	22.77	2740	158
nucleus	13.77	5116	210
transcription, DNA-templated	8.93	1782	93
DSB repair via homologous recombination	7.65	74	16
protein binding	7.21	8651	280
DNA repair	5.86	196	22
DNA binding	5.48	1438	71
RNA recognition motif domain	5.02	220	22
regulation of transcription, DNA-templated	4.52	1288	63
Nucleotide-binding alpha-beta plait domain	4.52	257	23

Fig. S2. (*A*) GFP-fluorescence overlay of endoGFP-Parkin cells 15 d post infection with the pooled lentiviral sgRNA library, noninfected endoGFP-Parkin cells, and GFP-negative parental cells. (*B*) Plot of replicate scores of normalized sgRNA counts for each pool analyzed during the screen with corresponding r^2 values. (*C*) An essential gene set is enriched among genes with strong negative fold-changes. Fold-change values were calculated from replicate-binned sgRNA counts in the day 15 "unsorted" population in respect to the input library. Genes were ranked (*x* axis) according to their fold-changes (*y* axis; second strongest sgRNA, log2). The essential gene set from KBM7 cells (24) is highlighted in pink. (*D*) Hypergeometric analysis using the 500 strongest scoring genes (either GFP-low or -high population) for gene set enrichment. The 10 most significantly enriched gene sets for GFP-low and -high populations are shown for the day 8 (*Left*) and day 15 (*Right*) time points. Gene-set sizes are indicated. "Common #" indicates the number of genes of that gene set in the 500.

symbol	Day 8	Day 15	symbol	Day 8	Day 15
PACRG	-1.86	-2.15	THAP11	1.43	1.77
KAT7	-1.16	-0.97	FBXW7	1.05	1.05
BRD1	-1.14	-0.89	HCFC1	0.96	0.34
NCBP2	-1.12	-0.71	MRGBP	0.85	0.75
DHX29	-0.99	-0.41	OGT	0.85	0.50
SMCHD1	-0.83	-1.54	WAPL	0.75	0.66
CCDC71L	-0.72	-0.54	FANCD2	0.72	0.58
MED24	-0.71	-0.58	NF2	0.71	0.26
DHX36	-0.67	-0.34	INTS12	0.71	0.55
USP48	-0.65	-0.59	PDS5A	0.59	0.39
TRIP12	-0.64	-0.23	ELAVL1	0.57	0.39
UBAP2L	-0.64	-0.46	UBE4B	0.57	0.48
MEAF6	-0.63	-0.36	CCZ1B	0.57	0.37
WDR82	-0.63	-0.06	LOC648044	0.55	0.08
CTBP1	-0.62	-0.78	FBXL12	0.55	0.38
THAP12	-0.62	-0.91	INTS6	0.52	0.53
ATXN7L3	-0.62	-0.51	PCGF1	0.49	0.34
RNMT	-0.62	-0.38	CAB39	0.44	0.26
UBE3D	-0.62	-0.64	FAM60A	0.43	0.44
CBLL1	-0.60	-0.77	STAG2	0.42	0.42
CSNK2A1	-0.56	-0.51	DIDO1	0.40	0.45
TAF5L	-0.54	-0.96	TFDP1	0.39	0.20
CEP350	-0.50	0.14	MPHOSPH8	0.39	0.25
SUPT20H	-0.49	-1.02	PDS5B	0.39	0.04
MED13	-0.48	-0.64	INTS6L	0.38	0.08
TTC14	-0.45	-0.95	RBM12	0.35	0.45
SRRT	-0.45	-0.52	ICE1	0.35	0.01
NCBP1	-0.43	-0.64	PPHLN1	0.34	0.12
ZFAT	-0.42	-0.43	G3BP1	0.33	0.03
HNRNPK	-0.40	-0.02	SRRM2	0.31	0.49
EIF4G2	-0.38	-0.79	RERE	0.31	0.26
CTBP2	-0.36	-0.45	NPRL3	0.30	0.23
FGFR1OP	-0.36	-0.73	KLF16	0.30	0.28
MBTD1	-0.33	-0.40	NCLN	0.29	0.36
PPP2R3C	-0.33	-0.64	RNASEH2B	0.29	0.31
OTUD5	-0.29	-0.40	KDM4B	0.28	0.61
TADA2B	-0.28	-0.53	EPC2	0.27	0.03
FZR1	-0.25	-0.39	MAU2	0.27	0.32
YWHAE	-0.24	-0.58	CNOT1	0.26	0.04
SMG7	-0.22	-0.20	PCDH9	0.21	0.25
UBR5	-0.22	-0.34	UCHL3	0.21	0.14
ZC3H18	-0.22	-0.10	CNOT10	0.20	0.25
AMBRA1	-0.18	-0.26	SUB1	0.20	0.16
TP53	-0.16	-0.28	BRD8	0.20	0.17
HMGN2	-0.14	-0.14	KDM3B	0.19	0.37
RPS25	-0.13	-0.23	COMMD6	0.18	0.03
DPH1	-0.12	-0.38	SIAH1	0.18	0.41
DRG2	-0.10	-0.31	ASB3	0.17	0.54
OXSM	-0.10	-0.09	PAXIP1	0.17	0.24
DPH6	-0.08	-0.32	RANBP3	0.16	0.19
NOB1	-0.08	-0.24	E2F3	0.15	-0.06
CLN5	-0.06	0.15	RING1	0.14	0.25
RNF219	-0.01	-0.07	ZFP36L2	0.13	0.54
SCR	0.00	0.00	HSPA4	0.11	0.14
CSNK2B	nd	-0.72	HDGFRP2	0.06	0.26
MED1	nd	-0.53	**SCR**	0.00	0.00
			ELOF1	nd	0.32
			GATA6	nd	0.12
			IWS1	nd	0.49
			PTBP1	nd	0.28

Fig. S3. Assessment of GFP-PARKIN fluorescence in single sgRNA confirmation experiments comprising 114 primary screen hits. endoGFP-PARKIN cells individually expressing the strongest scoring sgRNA for 114 hit genes were assessed for GFP-dependent fluorescence on days 8 and 15. GFP fluorescence is expressed as background-corrected (PARK2) median fold-changes (log2) over control (SCR). Gray highlighting: hits identified in the GFP-low population. nd, not determined.

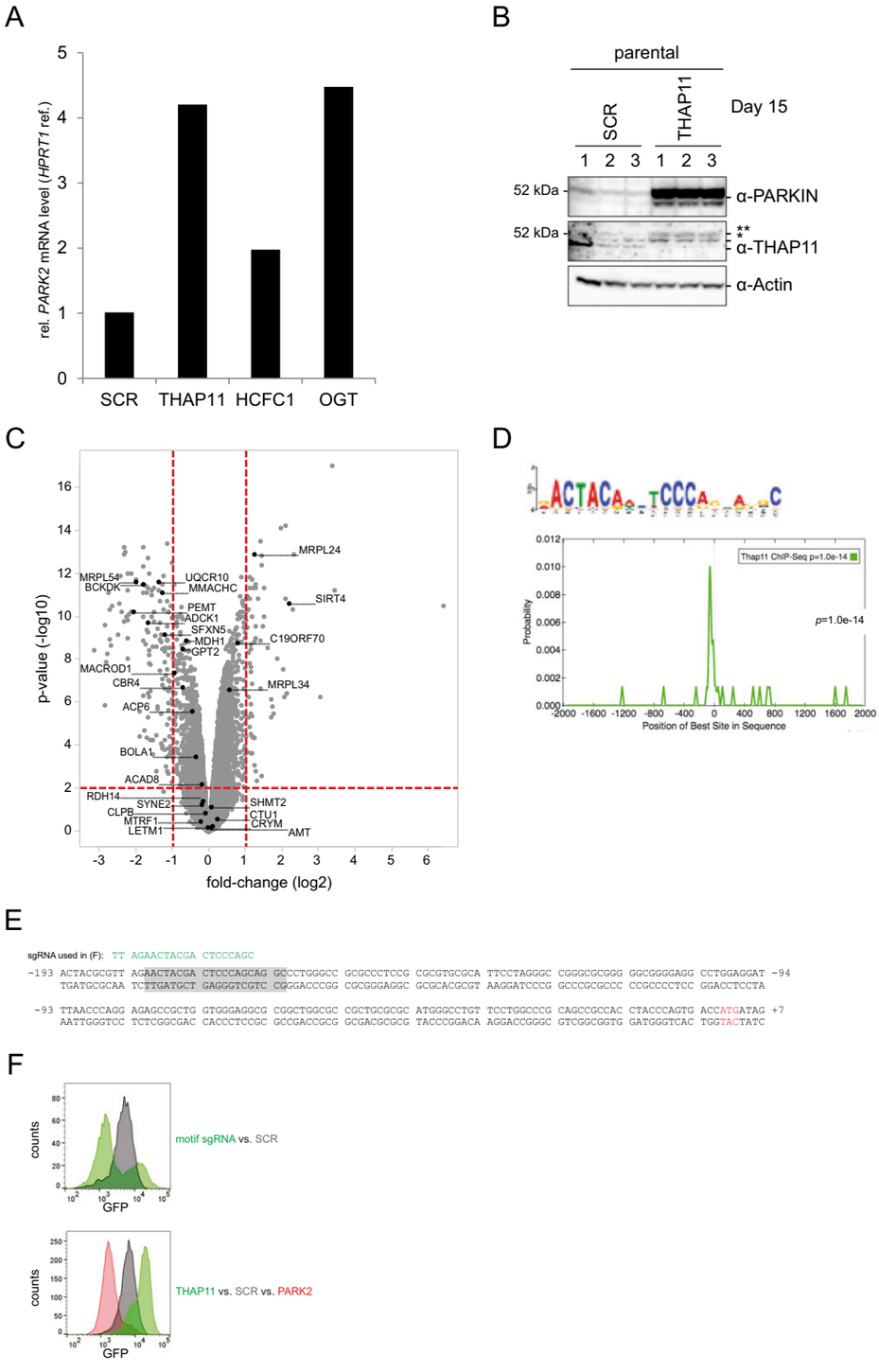

Fig. S4. (*A*) THAP11, HCFC1, and OGT negatively regulate *PARK2* mRNA levels. qPCR on total RNA extracted from parental JumpIN cells expressing *THAP11*-, *HCFC1*-, or *OGT*-specific sgRNAs (strongest sgRNA in primary screen) on day 8 postinfection using *PARK2*-specific Taqman probes and *HPRT1* as a reference. $n = 1$. (*B*) Targeting of *THAP11* negatively affects endogenous PARKIN protein level. Parental JumpIN cells were infected with *THAP11*-specific (second strongest sgRNA in the primary screen) or control (SCR) sgRNAs in triplicates. Total RNA was extracted on day 15 postinfection and subjected to RNAseq. In parallel, protein samples were prepared and analyzed by WB to assess THAP11 depletion and PARKIN protein elevation using PARKIN-, THAP11-, and Actin-specific antibodies. **Residual signal from α-PARKIN antibody that was used to probe the membrane before; *an unspecific cross-reaction of the THAP11 antibody. (*C*) Transcript fold-changes of *THAP11*-targeted vs. control samples (*x* axis, log2) were plotted against *P* values (*y* axis, −log10). Mitochondria-related transcripts identified in a recent study are labeled (29). (*D*) Unbiased analysis of the DNA sequence from −2,000 to +2,000 relative to the transcription start site for the 31 commonly regulated genes in Fig. 4*F* identified the motif shown. The position and relative position of this motif with respect to the transcription start site are shown. (*E*) Identification of motif from *D* within the *PARK2* promoter (gray highlighting; see also ref. 29). The translation start site is labeled in red. (*F*) endoGFP-PARKIN cells expressing either an SCR or an sgRNA specific for the motif within the *PARK2* promoter (*E*) were assessed for GFP fluorescence by flow cytometry 15 d postinfection (*Upper*). For comparison, day 15 GFP-fluorescence profiles of *PARK2*- (#ii) and *THAP11*-targeted (second strongest sgRNA in primary screen) endoGFP-PARKIN cells are shown (*Lower*). rel., relative; ref., reference transcript; WB, Western blot.

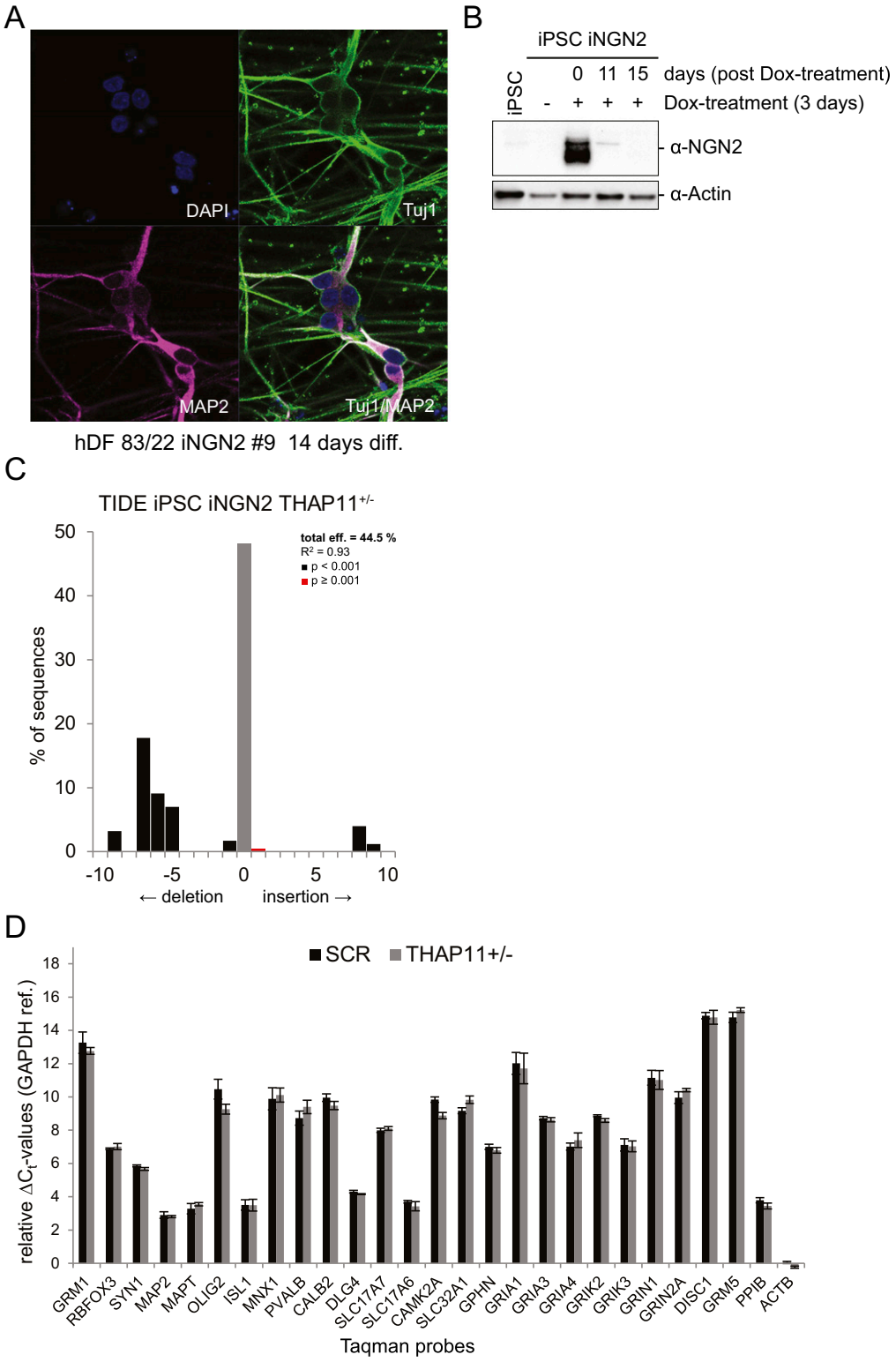

Fig. S5. (*A*) iNGN2-induced neurons display typical neuronal markers. hDF 83/22 iNGN2 #9 iPSCs were differentiated for 14 d and assessed for neuronal markers MAP2, β-tubulin (Tuj1), and DAPI using fluorescence microscopy. (*B*) Immunoblot analysis of NGN2 levels before and after Dox induction. Lysates of iPS cells with and without NGN2 as well as lysates of iPS cells harvested at different time points of neuronal differentiation were analyzed. (*C*) Monoallelic editing in iNGN2 iPSCs infected with lentiviruses allowing *THAP11*-directed sgRNA expression. iNGN2 iPSCs were infected with *THAP11*-specific (strongest sgRNA in screen) or nontargeting (SCR) sgRNAs by lentiviral delivery. Upon selection for viral uptake, clones were picked (iPSC iNGN2 THAP11$^{+/-}$ and iPSC iNGN2 SCR). Upon expansion, genomic DNA was extracted and subjected to sequencing of the *THAP11* sgRNA target region. TIDE analysis (https://tide.nki.nl) revealed 44.5% editing efficiency which is—in the light of the essential role of THAP11 in stem cells where only heterozygous lines could be readily generated (47)—consistent with monoallelic editing. (*D*) Transcriptional profiling of iNGN2 THAP11$^{+/-}$ and iNGN2 SCR neurons revealed a high correlation of expression levels of a set of neuronal markers. iPSC iNGN2 THAP11$^{+/-}$ and iPSC iNGN2 SCR were differentiated and total RNA was extracted on day 10 of differentiation and subjected to qPCR using a custom array of neuronal transcripts (Life Technologies, Inc.) and *GAPDH* as a reference. $n = 3$; error bars indicate SDs. diff., differentiation; eff., efficiency; ref., reference transcript.

Other Supporting Information Files

Dataset S1 (XLSX)
Dataset S2 (XLSX)
Dataset S3 (XLSX)
Dataset S4 (XLSX)