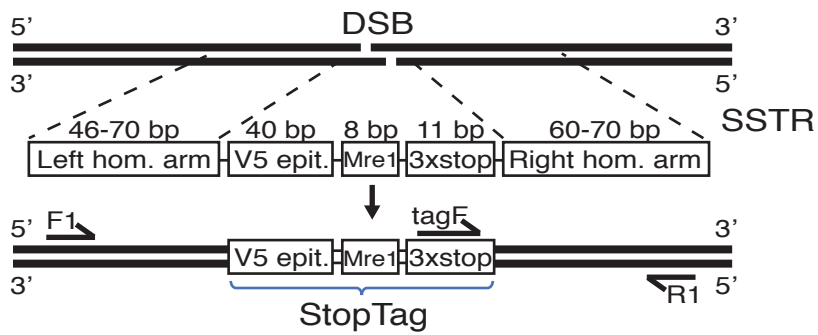


A



B

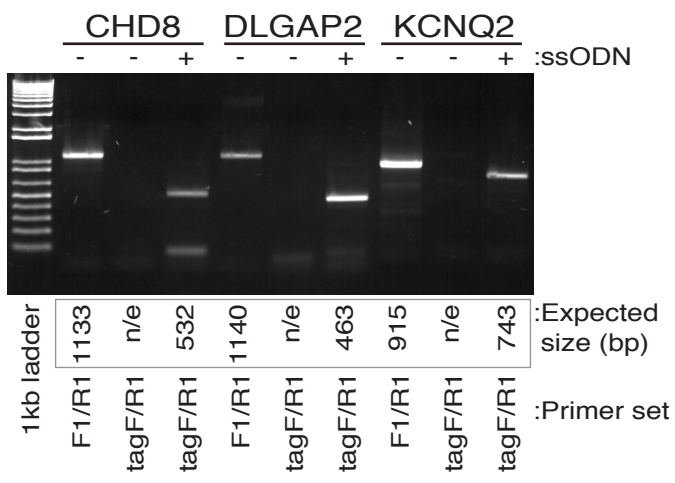


Figure S1 Validation of StopTag insertion in HEK293T cells. (A) Scheme of the single-stranded oligonucleotide DNA (ssODN) template used to splice the StopTag sequence into target genomic DNA; DBS = double-strand break; SSTR = single-stranded template repair; hom. = homology; epit. = epitope; F1, tagF, R1 = PCR primers. (B) Validation of StopTag insertion by PCR amplification of three selected target loci, i.e., *CHD8*, *DLGAP2* and *KCNQ2*, in HEK293T cells using corresponding primer sets [F1, tagF, R1 referring to (A)]. bp = base pair

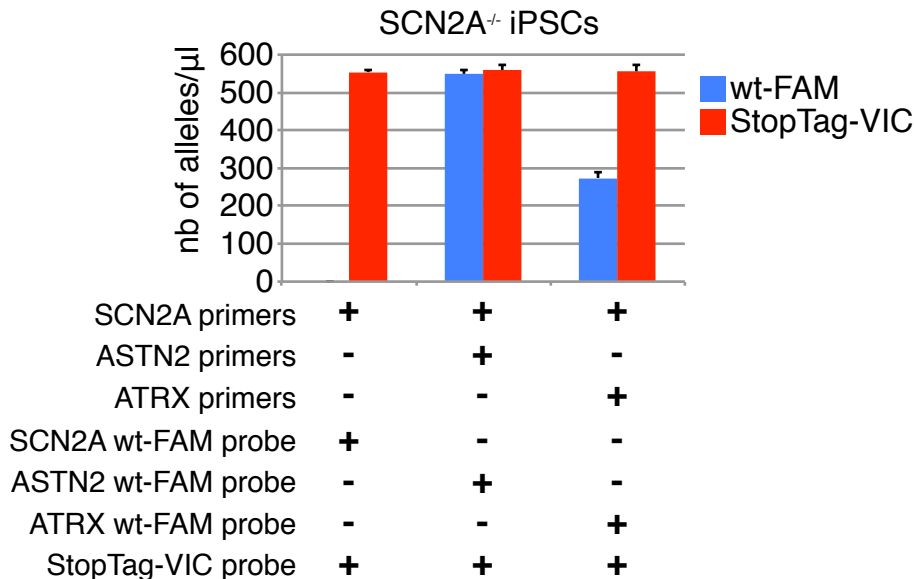


Figure S2 Confirmation of StopTag insertion and zygosity in different target loci in the human iPSC line 19-2. Representative verification of homozygosity of StopTag insertion in SCN2A^{StopTag/StopTag} (SCN2A^{-/-}) iPSCs using SCN2A primers/StopTag-VIC probe along with ASTN2 primers/ASTN2wt-FAM probe. No significant difference was observed between the number of SCN2A-associated StopTag-VIC alleles and the number of ASTN2-associated wt-FAM alleles, i.e., both were homozygous. The same comparison was performed with the hemizygous allele ATRX, where only half of the wt-FAM alleles were detected. Values are presented as mean \pm SD of three amplification reactions. wt = wild-type

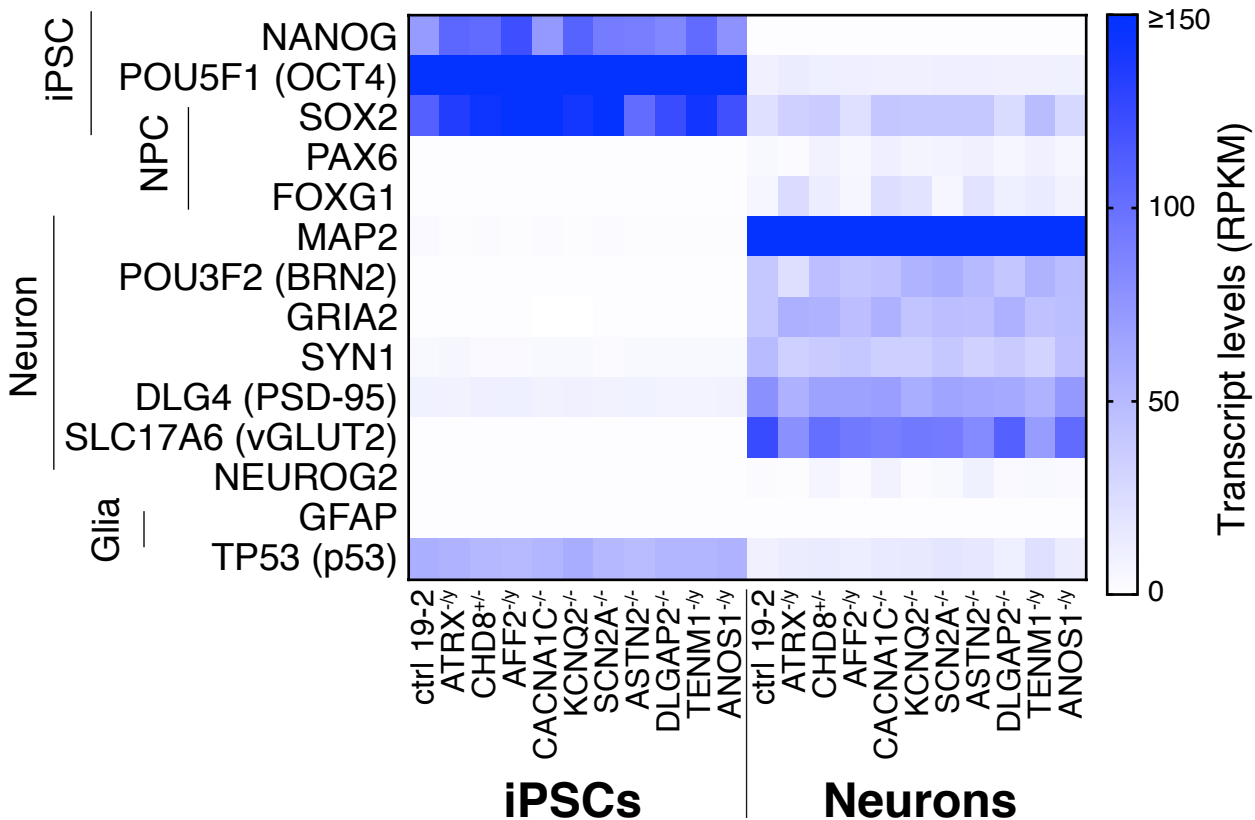
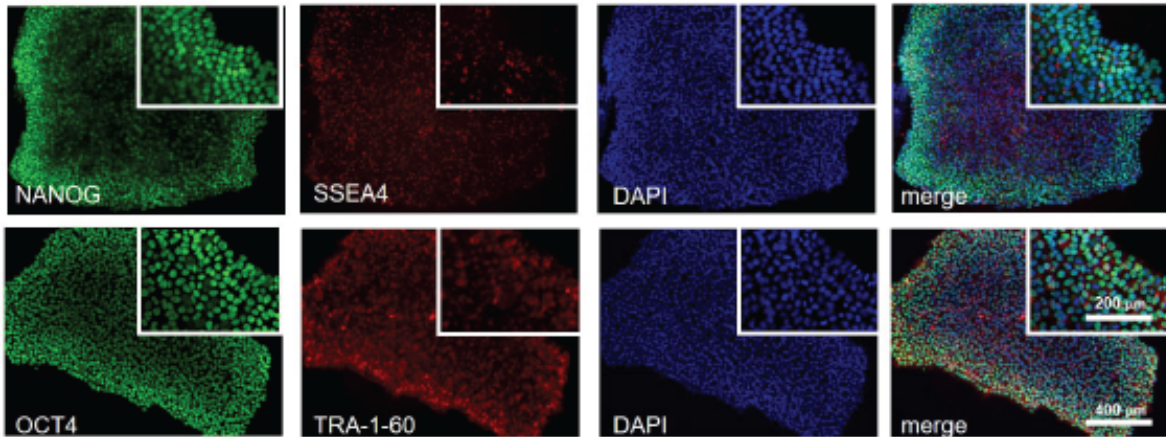


Figure S3 Transcript levels in RPKM of selected iPSC, NPC and neuronal markers in all KO cell lines. Values are presented as mean \pm SD of (n) independent experiments; n = 8 for ctrl 19-2, n = 4 for ATRX^{-ly}, CHD8^{+/-}, KCNQ2^{-/-}, SCN2A^{-/-}, ASTN2^{-/-}, DLGAP2^{-/-}, ANOS1^{-ly}; n = 2 for AFF2^{-ly}, CACNA1C^{-/-} and TENM1^{-ly} iPSCs; n = 5 for AFF2^{-ly} and TENM1^{-ly}; n = 4 for ctrl 19-2, ATRX^{-ly}, CHD8^{+/-}, KCNQ2^{-/-}, SCN2A^{-/-}, DLGAP2^{-/-}, ANOS1^{-ly}; n = 3 for CACNA1C^{-/-} and ASTN2^{-/-} neurons. Note that color gradient ranges from 0 to 150 RPKM; all RPKM values \geq 150 are presented with the same blue intensity as 150.

Control 50B

A



B

Control 50B

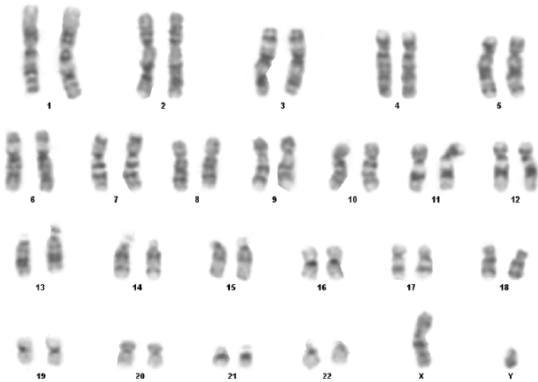


Figure S4 Characterization of the control 50B iPSC line. (A) Immunohistochemistry revealing expression of the pluripotency markers NANOG, SSEA4, OCT4 and TRA-1-60. (B) 50B iPSCs presented a normal male karyotype; 20 cells were examined.

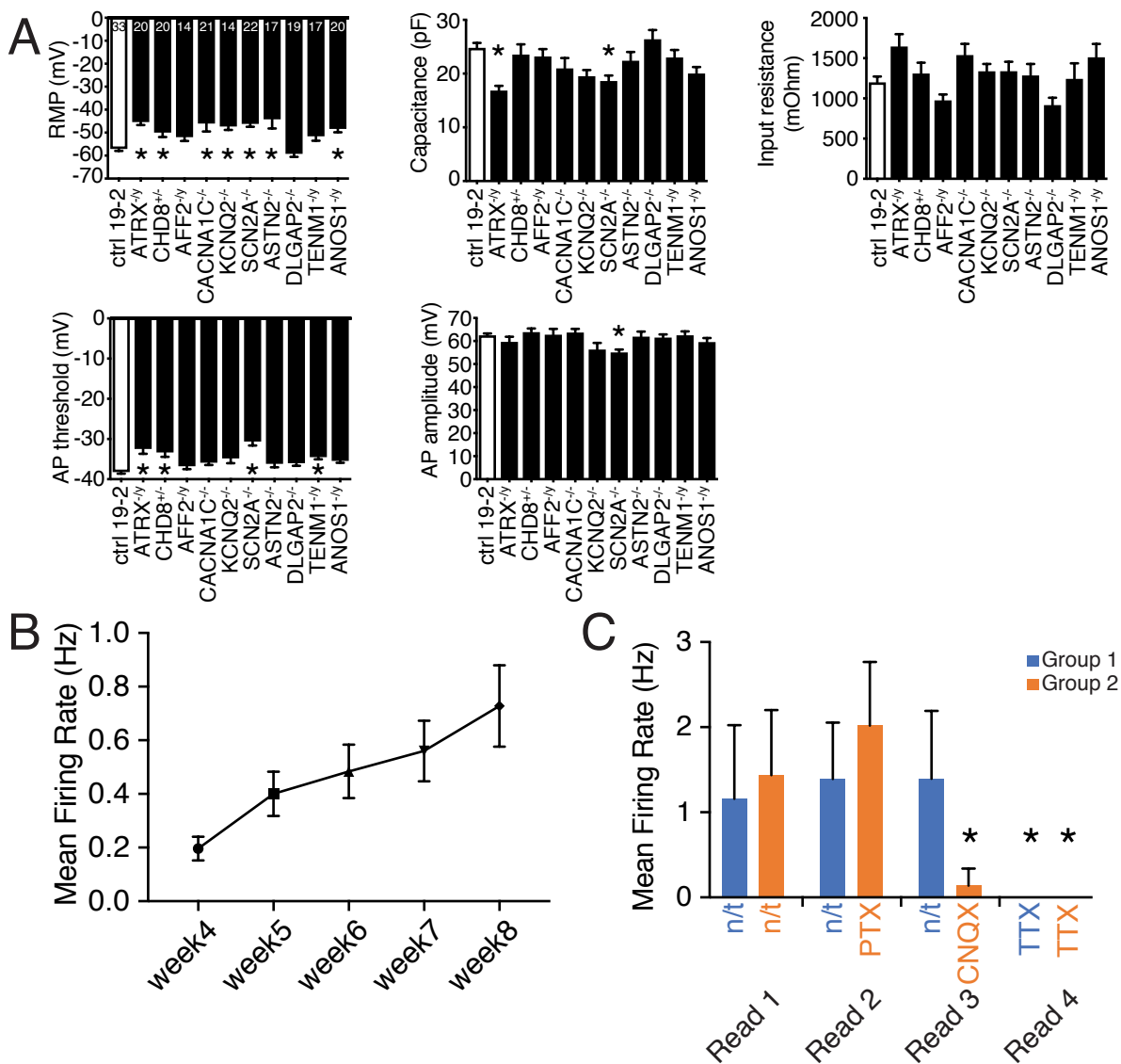


Figure S5 Electrophysiological phenotyping of KO iPSC-derived neurons. (A) Different intrinsic properties detected by patch-clamp recordings; the number of recorded neurons is indicated in the bars of the top left graph; values are presented as mean \pm SEM of three independent experiments, recorded at day 21-28 post-NEUROG2-induction (PNI); * $p < 0.05$. (B) Multi-electrode array recording of the mean firing rate at one-week interval from week 4 to week 8 PNI. 43 different wells were recorded from 6 independent experiments. Values are presented as mean \pm SEM. (C) Multi-electrode array recording of the mean firing rate of control 19-2 neurons upon treatments with different receptor inhibitors. Values were acquired in four consecutive readings, i.e., before (read 1) and after addition of GABA receptor inhibitor PTX (read 2), after addition of AMPA receptor inhibitor CNQX (read 3), and after addition of sodium channel blocker TTX (read 4). Four selected active wells were recorded for each group at week 8 PNI. Values are presented as mean \pm SD from a single experiment; * $p < 0.05$ compared to n/t; n/t = not treated; mV = millivolt; pF = picofarad; mOhm = milliohm; Hz = hertz

Tables S1 Description of selected target transcript and exon for each candidate gene to knockout, as well as the sequence of each guide RNA (gRNA) and template single-stranded oligonucleotide (ssODN) used for gene editing, and sequence of primers and probes used for enrichment of edited cell by droplet digital (ddPCR). Chr = chromosome; ID = identifier; bp = base pair; n/a = not available.

Gene Symbol	chr	Transcript ID	Exon	Exon ID	gRNA- (5'-3')	gRNA+ (5'-3')	Offset (bp)	FAM probe (5'-3')	VIC probe (5'-3')	Forward primer ddPCR (5'-3')	Reverse primer ddPCR (5'-3')
ANKRD11	16	ENST00000378330.6	7	ENSE00003504243	TCAAAGACTCTCAGCAGAGGG	CGTCCCGCGGATGGCGCTCGG	9	ATCCCGGGGGACGCC	CAACCGCGGTAACATA	AGAACGAGCTGGAGAGAC	GCTCTGGCCCTTGCC
AUTS2	7	ENST00000406775.6	1	ENSE00001671293	GCGGCTTTTGGCGAGTCCATGG	GTCGCGGTCCGACGAGACCGGG	1	CGGAAAAAGCGGGG	CAACCGCGGTAACATA	GAACCATGGATGGCCCGAC	CGACGAGGAGCGAGTG
ATRX	X	ENST00000373344.9	5	ENSE00001713901	ATCAGATGATGAAAACCTTTGG	ACATCTTTGTTAACAATTGAAGG	3	TGTAATAAGTATGTAGA	CAACCGCGGTAACATA	TCCAGTTGCAAGAACATTCATAATTC	TGGCAAGCTGTGATGTAAT
CHD8	14	ENST00000430710.7	4	ENSE00001422532	GTTGCCAGCGTTCGCGCAGAGG	TGCTCTCAATTAAGACTGAGG	0	TCTTAAATGAGGACGAGTTG	CAACCGCGGTAACATA	CGAGGAGCAGAACAATCTCG	GAGGACCAAGTGTCTACTCT
AF2	X	ENST00000370460.6	3	ENSE00003780313	TGACTACTCACGCTCCACTGAGG	TAGCACTGATCGGCAAGCGGG	6	TCCAGTATGATCATAAC	CAACCGCGGTAACATA	ACACGACACAGAAAATAAAACC	AGCTCTCCAGTTGGCTTGAGA
CAPRN1	11	ENST00000341394.8	5	ENSE00003682460	AACCTCTCTTCGGCAATATTGG	TAAAGTAGTAGACCTGAACGG	21	CGAAGAGGAGTTGTAT	CAACCGCGGTAACATA	GAAATGAAGTCCCAATATTGTC	TTGTCAACTAAATCTCAGAA
CACNA1C	12	ENST00000399655.2	2	ENSE00000936250	CGATGGCCGCTCCACGACAGG	GCCCGGAGGCTAAGCTGATGG	5	AGCGCCCACTCGACG	CAACCGCGGTAACATA	CTGAGCACATCCCACCC	GGTTTCCATATCTGCTCG
KCNQ2	20	ENST00000626839.2	1	ENSE00003772915	GAATTTCTCTCAACGCTGTGG	CGGTGAAGGCTTGTGCTTGGG	9	ACGCTTCTACGCAA	CAACCGCGGTAACATA	CGGGCCGGGGAAGC	CGATGGGGGCTGCG
SCN2A	2	ENST00000375437.6	2	ENSE00003607689	TGCGTTGTTCAATGACGCAAGG	GAGACCAAACGAGGAACGCAAGG	19	AAGCATTGACAGAGA	CAACCGCGGTAACATA	CTTCCGCTCTTACACGGGA	GGAAGAGATTTCTGCTTCCA
ASTN2	9	ENST00000356787.8	16	ENSE00003567878	GCTGCTGGAAATCAACGCTGTGG	CCTTGCTGGCGGGGCGCAAGG	22	CAAGGAGACGTGAGC	CAACCGCGGTAACATA	TCCATCTACTTCCACCTTTCG	GGTAGGAGGGGCTGCTCAAGT
DLGAP2	8	ENST00000637795.1	5	ENSE00003797478	CGGGGCTGGTCCGACTTCCGG	CACGCTGGTCTGGGCGCCCCGG	14	AGCCCGCTGTGTT	CAACCGCGGTAACATA	CCCAGGACATGAAGGGC	GCTCAGCAGTAGGGCCG
CHTNAP2	7	ENST00000351727.7	4	ENSE00001369431	TCGGATCTCTAATTGTGCGGG	CTATGTGGCATAGTGTGCTTGG	10	TACAGCATCGATTA	CAACCGCGGTAACATA	CGGAAACATTAACTTGACGG	ACCTCCCAAAAHTTAAACATACG
TENM1	X	ENST00000371130.7	1	ENSE00000857625	GACCTGCACAGATATACCAGG	CTGGAGTGTATGACTGCTTGG	3	CAAGACATCATAAC	CAACCGCGGTAACATA	ACCAAAGCAAGATGAAATGGA	AGCTCTGTATTACTCTGTC
ANOS1	X	ENST0000026248.7	6	ENSE00001090930	GTAATGTGATGGAACCTCGAGG	TGGAACCTGTACATCGCTGG	9	CCAGTTCGATGGCT	CAACCGCGGTAACATA	CTCAGATAGAACACAGCCGA	ATTCGTGTGCGGAGAAAGGA
AF2 (validation in 50B line)	X	ENST00000370460.6	3	ENSE00003780313	n/a	GGCTTGGCAGTACAGTCTGAGG	n/a	TCACTGTATGATCATAAC	CAACCGCGGTAACATA	ACACGACAAAGAAAATAAAACC	AGTCTTCCAGTTGGCTTGAGA

Gene Symbol	ssODN template sequence (5'-3')
ANKRD11	AGATAAGTGAACAGAGAACGAGCGTGAGAGACCCGCTGCACGAGCCGCGCTGGGAAACCCATCCCACTCCCTCTTGGGCTGATTCAACCGCGGTAAGTACTGACGATCAAGAGCTATCAGCGAGGGGGCAGACGTAACGCTAAGGACTTCGAGGTGGACACCCTGTC
AUTS2	ACGGGGAGAACCCCGGGCCGAGCAACATGGATGCGCCGACGCGGGCCTGAGCTGGGAAACCCATCCCACTCCCTCTTGGGCTGATTCAACCGCGGTAAGTACTGACGATCGGGTCCAGCAGACCGGGAAGGCGCTCCCGGGCGGGCCGCGGGCCGGCGGGCGG
ATRX	GTCTCAGAAATTTCAATAATTTATGAATTAATTTCTTATAGGAAAATCCTTCAATGGGAAACCCATCCCACTCCCTCTTGGGCTGATTCAACCGCGGTAAGTACTGACGATAGATGAAAACTTTGGATGATGAACTGAAATGAAGATGCTCTATGAAATTCAGA
CHD8	AGCAGAGCCAAATGCGAGAGCCGTTGCCGGTGGAGCAACAATCACTCGAGTGGGAAACCCATCCCACTCCCTCTTGGGCTGATTCAACCGCGGTAAGTACTGACCCAGCTTGCGCCAGAGGAGGGAAGGAAACCGAAGAAAGGAGTGTGGGAGAGGCTGAAAG
AF2	TGTGATGACTGAATCACTCAATACACGCAACAGAAATCAAACTCGAGTGGGAAACCCATCCCACTCCCTCTTGGGCTGATTCAACCGCGGTAAGTACTGACCTAGACTGATCGGCAAGCGAGGCTGGTCAAGCAACAGATGCAAGTTGACAGGAGGACGACT
CAPRN1	AGTGCGGACTGACCTGAACAAAGGTTTGAATGGAGTGCCAATTTGGGAAACCCATCCCACTCCCTCTTGGGCTGATTCAACCGCGGTAAGTACTGAGAAATTCTAAGCTAGTACCCCTGAACCGGACATGAGCTGGAGTATGGATTGATAATGAAAG
CACNA1C	GGCAGCGGGGCTCGCCCTGAGCACATCCCACCCCGGGGCTGCCCTGTGCTGGGAAACCCATCCCACTCCCTCTTGGGCTGATTCAACCGCGGTAAGTACTGACCCGCGAGGCTAAGCTGATGGGACGCGCTGGCAATGGACCATCTCCACAGTACGCTCCACGACGGGA
KCNQ2	CCTCAGCAAACTCGCGCGGGCCGGCGGGGCGCGGGAACCCCTCAGCGCGGAAACCCATCCCACTCCCTCTTGGGCTGATTCAACCGCGGTAAGTACTGACGAGAAATTCTCTACACGCTGCTGGAGCGCCGGCGGCTGGGCTGTATCTACACGCTCACTGTGTGAG
SCN2A	CACAGTCAAGTGTCTTACCGCCGAGCAGCTGACAGTTCGCTCTTACAGCGAAATCCCTGCTGCGGAAACCCATCCCACTCCCTCTTGGGCTGATTCAACCGCGGTAAGTACTGACGTAAGGCTAAGGACCAACAGAAAGGAAAGGAGGATGATGAATGGCCAAAGCCAAAGCAAGTGAC
ASTN2	TCGCTGTGAGAGAAAGGGCCCTGTCTACTCACTGTACCTTGTGCGGAAACCCATCCCACTCCCTCTTGGGCTGATTCAACCGCGGTAAGTACTGACCTGGAATCAACCGCTGTCAGCTTTACTCTTAACGATGAGAAACAGAA
DLGAP2	GGAGCCGCTACTCCCGCGCGGAGATGAAAGGGCTCTCCGGAATGCGGAAACCCATCCCACTCCCTCTTGGGCTGATTCAACCGCGGTAAGTACTGAGGACACAGCTGTGGCTGGCGGCCCGGAGGACTGGAGACCTCACACCGGGCCGACGGCGGC
CHTNAP2	TTAAATAACTTCTTCTTCCAGCCATTCCCGGAACATTAATCTCAGCGTGTGCTCGGACGAAAGGAAACCCATCCCACTCCCTCTTGGGCTGATTCAACCGCGGTAAGTACTGACCTATGCTGACATGTCCTGTGATGAAAGAGAAAGCTGACAGGACTGGAATGAGTTAT
TENM1	TAGCTTACACAGTCTCTCTGATGAAGTGAAGAGTGAAGGAAACAGACTATACAACTCGAGTGGGAAACCCATCCCACTCCCTCTTGGGCTGATTCAACCGCGGTAAGTACTGACCCCTGACAGATATAACCGAGCTGAGGATGAAATACATAGCCAGAGTGAAGAAAGAAAGAT
ANOS1	TCAGACACAGCGAGGCTGATCACTGACATAGAACACAGCCAGCCGATGGGAAACCCATCCCACTCCCTCTTGGGCTGATTCAACCGCGGTAAGTACTGAGTGAATGATGGAATCGAGGCTTCTGCCCACAAACATCCCTGCTTCTCCCAAGGTGAGTCTCT
AF2 (validation in 50B line)	TGTGATGACTGAATCACTCAATACAGCAACAAAGAAATCAAACTGAGTGGGAAACCCATCCCACTCCCTCTTGGGCTGATTCAACCGCGGTAAGTACTGACCAAGCCAGCGAGTGTACGCAAAAGATGCAAGTGTGACAGGACCGGAGT

Table S2 Number of enrichment steps (or plates) required to isolate a 100% (homozygous or hemizygous) or 50% (heterozygous) StopTag iPSC population for each candidate genes. Shaded area indicates unsuccessful attempts. n/a = not available; RNP = ribonucleoprotein

Gene	vector	plate 1	plate 2	plate 3	plate 4	plate 5	plate 6	RPKM - iPS ctrl 19-2
ANKRD11	plasmids	A4 = 0.4	E8 = 0.3	E10 = 2.6	A4 = 28.2	F9 = 4.8	C1 = 15.1	26.2
AUTS2	plasmids	H3 = 0.5	A12 = 3.1	A10 = 0.4	n/a	n/a	n/a	16.6
ATRX	plasmids	E11 = 1.2	C8 = 2.4	G2 = 13.9	A5 = 100.0	n/a	n/a	17.7
CHD8	plasmids	E1 = 0.8	B9 = 2.5	C11 = 28.5	E1 = 47.7	G8 = 52.4	G1 = 52.7	27.8
AFF2	plasmids	F3 = 7.0	H2 = 100.0	n/a	n/a	n/a	n/a	1.7
CAPRIN1	plasmids	G1 = 0.6	D8 = 0.2	E1 = 0.8	D1 = 0.06	n/a	n/a	110.9
CACNA1C	plasmids	D9 = 1.6	D10 = 6.7	F10 = 15.4	H7F3 = 44.3	A4 = 100.0	n/a	0.2
KCNQ2	plasmids	H1 = 1.6	E9 = 1.4	G5 = 8.4	F11 = 26.5	E1 = 47.2	C1 = 52.4	7.8
SCN2A	plasmids	G6 = 2.0	D2 = 10.9	E8 = 48.5	D2 = 68.9	B6 = 86.2	A8 = 100.0	0.3
ASTN2	plasmids	C4 = 3.3	D6 = 12.1	A2 = 43.0	G1 = 100.0	n/a	n/a	2.9
DLGAP2	plasmids	C1 = 3.9	F10 = 1.7	B8 = 14.8	F9 = 63.0	C4 = 100.0	n/a	0.2
CNTNAP2	plasmids	G7 = 0.2	E2 = 2.0	F9 = 12.7	n/a	n/a	n/a	19.3
TENM1	plasmids	B10 = 11.3	C9 = 7.0	C7 = 19.1	F1 = 100.0	n/a	n/a	0.2
ANOS1	plasmids	A1 = 3.0	C8 = 4.6	E4 = 19.0	C6 = 88.6	H10 = 100.0	n/a	35.8
50B-AFF2	RNP complex	F4 = 7.3	F8 = 89.0	A1 = 100.0	n/a	n/a	n/a	n/a

Table S3 Identification of SNV/indel calls specific to the different KO line genomes with respect to the 19-2 control genome. Only calls flanked with a matching gRNA sequence (up to five mismatches) within 200 bp on each side are presented. REF = reference; ALT = alternative; Nb = number; bp = base pair

KO Line	Chr	Strand	Variant Site	REF	ALT	Explored Locus	gRNA Site	Nb Mismatch	Mismatch Pos.	gRNA Sequence	gRNA Name	Diagnostic
ATRX-/y	X	+	76952179	C	StopTag	76951962-76952397	76952168	0	19	CATACTTTGTTACAATTGA	ATRX gRNA+	StopTag insertion on-target
ATRX-/y	X	-	76952179	G	StopTag	76951962-76952397	76952144	0	19	AAGGTTTTTCATCATCTGA	ATRX gRNA-	StopTag insertion on-target
ATRX-/y	14	+	93462922	TA	T	93462722-93463122	93463005	2	9C4G4	TCAGATGATCAAAAGCCTT	ATRX gRNA-	1-bp deletion off-target, 98 bp away from cut site; intronic ITPK1
CHD8+/-	14	+	21896163	A	StopTag	21895961-21896366	21896168	0	19	CGTCTCATTTAAGACTCG	CHD8 gRNA+	StopTag insertion on-target
CHD8+/-	14	-	21896163	T	StopTag	21895961-21896366	21896147	0	19	CTGGCCGAACGCTGGGCAA	CHD8 gRNA-	StopTag insertion on-target
AFF2-/y	X	-	147743734	A	StopTag	147743525-147743943	147743721	0	19	GAGTGGTCACGTGATAGTC	AFF2 gRNA-	StopTag insertion on-target
AFF2-/y	X	+	147743734	T	StopTag	147743525-147743943	147743748	0	19	AGCACTGTACTGGCAAGCC	AFF2 gRNA+	StopTag insertion on-target
CACNA1C-/-	12	-	2224504	G	StopTag	2224296-2224713	2224491	0	19	GTCGTGGCAGGCGGCCATC	CACNA1C gRNA-	StopTag insertion on-target
CACNA1C-/-	12	+	2224504	C	StopTag	2224296-2224713	2224517	0	19	CCCGCAGGCTAAGCTGAT	CACNA1C gRNA+	StopTag insertion on-target
KCNQ2-/y	20	+	62103590	T	StopTag	62103375-62103806	62103595	0	19	GGTAGAAGCGTTGCGCTT	KCNQ2 gRNA+	StopTag insertion on-target
KCNQ2-/y	20	-	62103590	A	StopTag	62103375-62103806	62103565	0	19	GCACGTTGTAGAGGAAATT	KCNQ2 gRNA-	StopTag insertion on-target
SCN2A-/y	2	-	166152415	G	StopTag	166152205-166152625	166152400	0	19	TGCTGCTATTGAACAACGC	SCN2A gRNA-	StopTag insertion on-target
SCN2A-/y	2	+	166152415	C	StopTag	166152205-166152625	166152440	0	19	AGACCCAAACAGGAACGCA	SCN2A gRNA+	StopTag insertion on-target
ASTN2-/y	9	+	119413904	C	StopTag	119413689-119414120	119413895	0	19	CTGCCTGGCCGGCGGCAA	ASTN2 gRNA+	StopTag insertion on-target
ASTN2-/y	9	-	119413904	G	StopTag	119413689-119414120	119413852	0	19	CACGTTGATTTCCAGCAG	ASTN2 gRNA-	StopTag insertion on-target
DLGAP2-/y	8	-	1496893	G	StopTag	1496683-1497104	1496877	0	19	GAAGTCGGACCCAGCCGCC	DLGAP2 gRNA-	StopTag insertion on-target
DLGAP2-/y	8	+	1496893	C	StopTag	1496683-1497104	1496912	0	19	ACGTGTGGTCTGGCGCCCC	DLGAP2 gRNA+	StopTag insertion on-target
TENM1-/y	X	+	124097473	C	StopTag	124097270-124097676	124097477	0	19	TGGAGTTGTATGACTGTCT	TENM1 gRNA+	StopTag insertion on-target
TENM1-/y	X	-	124097473	G	StopTag	124097270-124097676	124097453	0	19	GGTTATACTGTGCAGGGT	TENM1 gRNA-	StopTag insertion on-target
ANOS1-/y	X	+	8553378	T	StopTag	8553167-8553589	8553380	0	19	CGAAACTGGTACCATCGGC	ANOS1 gRNA+	StopTag insertion on-target
ANOS1-/y	X	-	8553378	A	StopTag	8553167-8553589	8553350	0	19	CGAGTTCATGCACATTCA	ANOS1 gRNA-	StopTag insertion on-target

Table S4 Distance between premature termination codon insertion (StopTag) and the next upstream and downstream exon-exon junctions (e-e) or wildtype (wt) start or stop codons. chr = chromosome; ins. = insertion; dist. = distance; NMD = nonsense-mediated decay

Gene	chr	exon	ins. site	dist. 5'e-e	dist. 3'e-e	decreased transcript	
						levels (Fig. 2b)	possible explanation
ATRX	X	5/35	77,696,639	62	97	yes	NMD
CHD8	14	4/38	21,428,112	243	130	yes	NMD
AFF2	X	3/21	148,661,908	351	546	yes	NMD
CACNA1C	12	2/47	2,115,379	155	197	no	higher mRNA stability
KCNQ2	20	1/16	63,472,426	258	65	no	higher mRNA stability
SCN2A	2	2/27	165,295,894	121	171	no	higher mRNA stability
ASTN2	9	16/22	116,651,743	215	75	yes	NMD
DLGAP2	8	5/15	1,548,767	141	945	no	higher mRNA stability
TENM1	X	1/31	124,963,689	152	85	no	StopTag <200bp downstream of wt AUG
ANOS1	X	6/14	8,585,364	97	61	yes	NMD