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Supplemental Information

A Cas9 Variant for Efficient Generation of Indel-Free Knockin or Gene-Corrected Human Pluripotent Stem Cells

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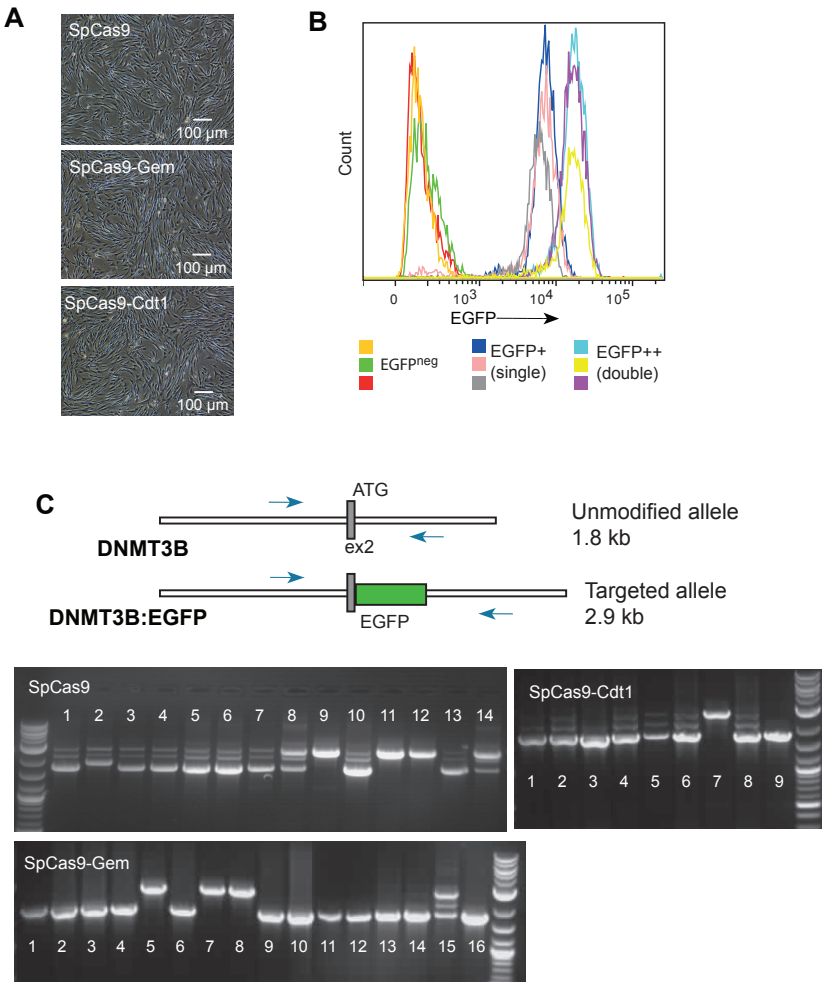

Figure S1, related to Figure 2. (A) *Micrographs of fibroblast cultures 24 h following transfection of mRNA encoding SpCas9, SpCas9-Gem or SpCas9-Cdt1.* ***(B)*** *Flow cytometric analysis of EGFP-expressing clones with either a single DNMT3B allele ($EGFP^{+}$) or both DNMT3B alleles ($EGFP^{++}$) targeted by homologous recombination.* ***(C)*** *Schematic of the DNMT3B locus showing location of PCR primers used to screen EGFP-expressing clones and subsequent gel analysis of the PCR products amplified from EGFP-expressing clones. These primers preferentially amplify the untargeted allele in EGFP+ (mono-allelic) clones, whereas only the larger 2.9 kb band is visible following PCR analysis of EGFP++ (bi-allelic) clones. Clones where two bands are clearly visible (clones 8 and 14 in SpCas9 panel and clone 15 in SpCas9-Gem panel) exhibited low levels (<15%) of contamination with* EGFP *non-expressing cells observed by flow cytometry (not shown).*

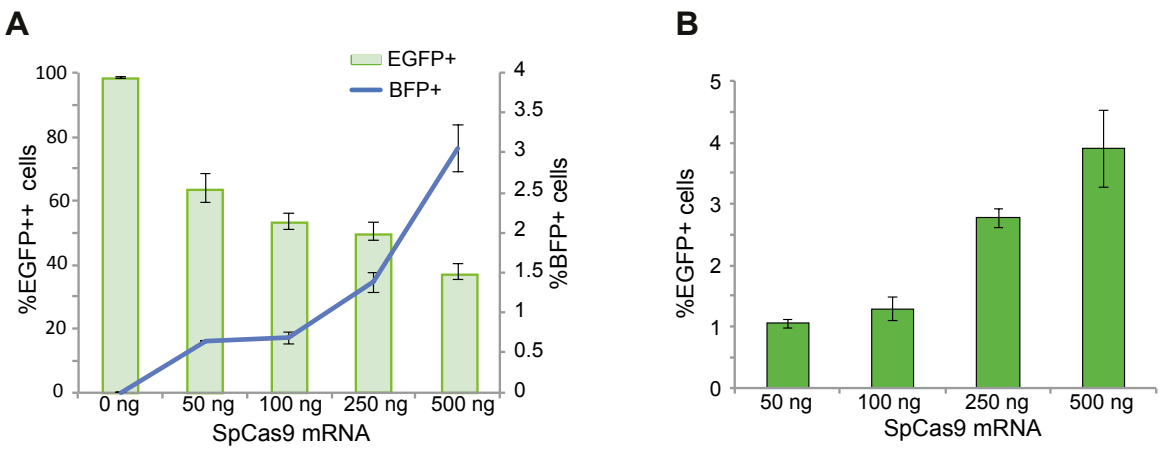

Figure S2, related to Figure 4. (A) *Graphical plot depicting the proportion of BFP+ (targeting efficiency) and EGFP^{neg} cells (NHEJ) assessed 3 days after the co-transfection of 0, 50 ng, 100 ng, 250 ng or 500 ng mRNA encoding SpCas9 with a BFP homologous template in iPSCs constitutively expressing EGFP. Data represent an average ± SEM of three independent experiment* ***(B)*** *Graphical plot depicting the proportion of EGFP+ (targeting efficiency) assessed 3 days after the co-transfection of 0, 50 ng, 100 ng, 250 ng or 500 ng mRNA encoding SpCas9 with the DNMT3B-EGFP homologous template H9 cells. Data represent an average ± SEM of three independent experiments.*

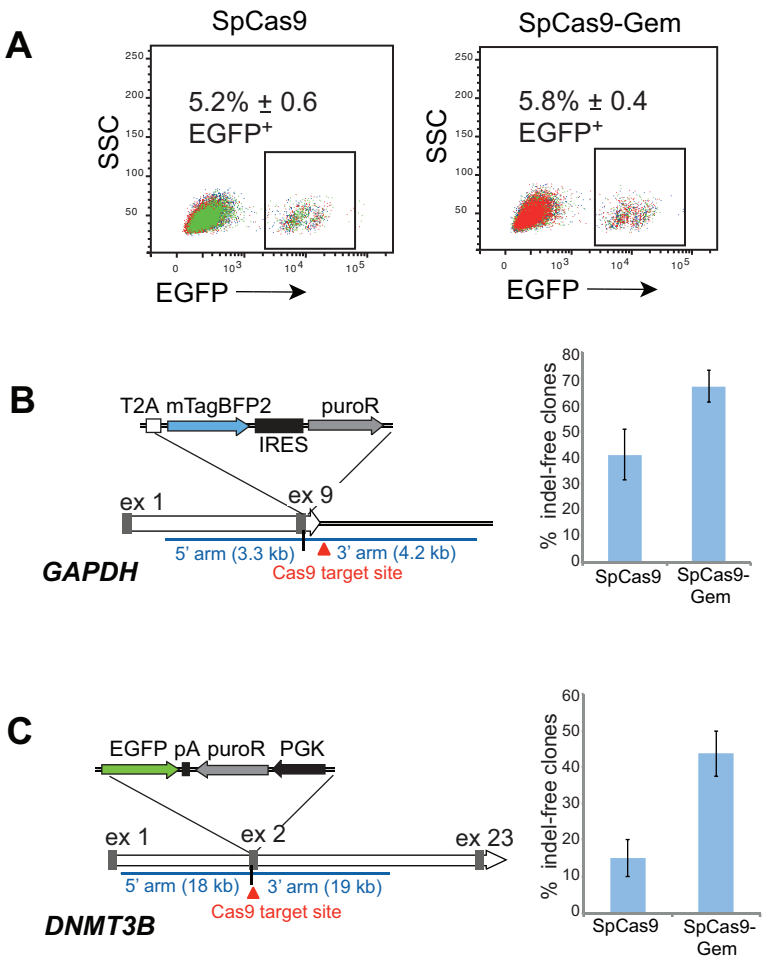

Figure S3, related to Figure 4. (A) *Targeting efficiency associated with SpCas9 and SpCas9-Gem assessed by flow cytometric analysis following targeting of EGFP to the DNMT3B locus in H9 cells. An overlay of three independent experiments is shown.* ***(B)*** *Schematic diagram of the GAPDH targeting strategy and proportion of puromycin resistant/mTagBFP2+ clones identified without disruption of the other untargeted GAPDH allele associated with SpCas9 or SpCas9-Gem. A total of 78 colonies were isolated and screened (40 for SpCas9, 38 for SpCas9-Gem) from three independent experiments.* ***(C)*** *Schematic diagram of the DNMT3B targeting strategy and proportion of puromycin resistant/EGFP+ clones identified without disruption of the other untargeted DNMT3B allele associated with SpCas9 or SpCas9-Gem. A total of 40 colonies were isolated and screened (20 for SpCas9, 20 for SpCas9-Gem) from two independent experiments. Data represent mean ± SEM.*

Table S1, related to Figure 2. *Analysis of EGFP^{+} clones isolated and expanded following simultaneous reprogramming and targeting of EGFP to DNMT3B locus. Three independent experiments were performed, as indicated. Clones that had EGFP targeted to both DNMT3B alleles are highlighted green. Clones that had EGFP targeted to one DNMT3B allele with no indels on the second DNMT3B allele are highlighted blue.*

	SpCas9		***SpCas9-Gem***		***SpCas9-Cdt1***	
Experiment 3	**CLN**	**INDEL (Y/N)**	**CLN**	**INDEL (Y/N)**	**CLN**	**INDEL (Y/N)**
	1	Y - 1bp INS	1	Y - 1bp INS	1	Y - 1bp INS
	2	Y - 180bp INS	2	Y - 1bp INS	2	N
	3	Y - 23bp DEL	3	Y- 9bp DEL	3	Y
	4	Y - 1bp INS	4	N	4	Y - 1bp INS
	5	Y - 2bp INS	5	N/A	5	Y - 1bp INS
	6	Y - 1bp INS	6	Y - 8bp DEL	6	Y - 2bp DEL
	7	Y - 1bp DEL	7	N/A	7	N/A
	8	N/A	8	N/A	8	Y - 1bp INS
	9	N/A	9	Y - 22bp DEL		
	10	Y - 1bp INS	10	N		
	11	N/A	11	N		
	12	N/A	12	Y - 60bp DEL		
	13	Y - 1bp INS	13	Y- 3bp DEL		
	14	N/A	14	Y - 1bp INS		
	15	N/A	15	N/A		
	16	N/A	16	N		
	17	N/A				
Experiment 2	18	Y - 6pb DEL	17	Y - 3bp DEL	9	Y- 400bp INS
	19	Y - 8bp DEL	18	Y- 7bp DEL	10	N
	20	N/A	19	N	11	Y - 3bp INS
	21	Y - 1bp INS	20	N	12	Y - 20bp DEL
	22	Y - 22bp DEL	21	N/A	13	Y
	23	Y - >1kb INS				
	24	indel within EGFP				
Experiment 1	25	Y- 8bp DEL	22	Y- 1bp INS	14	Y- 2bp DEL
	26	N/A	23	N	15	Y- 1bp INS
	27	Y- 1bp INS	24	Y- 1bp INS	16	Y- 1bp INS
	28	Y- 1bp INS	25	N	17	Y- 3bp DEL
			26	Y - 6p DEL		
			27	N/A		
			28	Y- 1bp INS		

Table S2, related to Figure 2. *Analysis of EGFP^{neg} clones isolated and expanded following simultaneous reprogramming and targeting of EGFP to DNMT3B locus. Three independent experiments were performed, as indicated.*

	SpCas9		***SpCas9-Gem***		***SpCas9-Cdt1***	
Exp3	**CLN**	**INDEL (Y/N)**	**CLN**	**INDEL (Y/N)**	**CLN**	**INDEL (Y/N)**
	A	Y- both alleles	A	N	A	Y- both alleles
	B	Y- both alleles	B	N	B	Y- one allele
	C	Y- both alleles	C	Y- both alleles	C	Y- both alleles
	D	Y - both alleles	D	N	D	Y- one allele
	E	Y- both alleles	E	Y- both alleles	E	Y- one allele
	F	Y- both alleles	F	Y- one allele	F	Y- one allele
	G	Y- both alleles	G	N	G	Y- both alleles
	H	Y- both alleles	H	N	H	Y- both alleles
	I	Y- both alleles	I	N	I	Y- one allele
	J	Y- both alleles	J	N	J	Y- both alleles
Exp2	K	Y- both alleles	K	Y - both alleles	K	Y- both alleles
	L	Y - both alleles	L	Y- both alleles	L	Y- both alleles
	M	Y - both alleles	M	N	M	N
	N	Y - both alleles	N	N	N	Y- one allele
	O	Y- both alleles	O	N	O	Y- both alleles
	P	Y- both alleles	P	Y- one allele	P	Y- both alleles
Exp1	Q	Y - both alleles	Q	N	Q	Y- both alleles
	R	Y - one allele	R	N	R	Y- one allele
	S	Y - both alleles	S	Y - one allele	S	Y- one allele
	T	Y - both alleles	T	N	T	Y- one allele
	U	Y - one allele	U	N	U	Y- both alleles
	V	Y - both alleles	V	N	V	Y- one allele
	W	Y - both alleles	W	Y - both alleles		
	X	Y - both alleles				
	Y	Y - both alleles				

Table S3, related to Figure 3. *Analysis of iPSC clones isolated and expanded following simultaneous reprogramming and gene correction of fibroblasts from patient with an autosomal dominant mutation in SCN2A*

SpCas9			***SpCas9-Gem***			***SpCas9-Cdt1***		
CLN	**INDEL**	**REPAIR**	**CLN**	**INDEL**	**REPAIR**	**CLN**	**INDEL**	**REPAIR**
1	Y- both alleles	N	1	N	N	1	Y- wt allele	N
2	N	N	2	N	N	2	Y- mut allele	N
3	N	N	3	Y- mut allele	N	3	Y- wt allele	N
4	Y- wt allele	**Y**	4	N	N	4	Y- mut allele	N
5	Y- both alleles	N	5	**N**	**Y- both alleles**	5	N	N
6	Y- both alleles	N	6	N	no	6	Y- both alleles	N
7	Y- both alleles	N	7	N	no	7	Y- wt allele	N
8	Y- both alleles	N	8	N	no	8	Y- mut allele	N
9	Y- both alleles	N	9	Y- mut allele	N	9	N	N
10	Y- mut allele	N	10	**N**	**Y- both alleles**	10	Y- mut allele	N
11	Y- both alleles	N	11	N	N	11	Y- mut allele	N
12	Y- both alleles	N	12	N	N	12	Y- mut allele	N
13	Y- both alleles	N	13	N	N	13	N	N
14	Y- both alleles	N	14	N	N	14	Y- mut allele	N
15	Y- both alleles	N	15	N	N	15	Y- both alleles	N
16	Y- both alleles	N	16	N	N	16	N	N
17	**N**	**Y**	17	N	N	17	Y- wt allele	N
18	Y- both alleles	N	18	N	N	18	N	N
19	Y- both alleles	N	19	N	N	19	N	N
20	Y- both alleles	N	20	N	N	20	Y- mut allele	N
21	Y- mut allele	N	21	Y – wt allele	**Y**	21	Y- mut allele	N
22	**Y- wt allele**	**Y**	22	N	N	22	Y- mut allele	N
23	Y- both alleles	N	23	N	N	23	Y- both alleles	N
24	Y- mut allele	N	24	N	N	24	Y- both alleles	N
25	Y- both alleles	N	25	**N**	**Y- both alleles**	25	N	N
26	Y- both alleles	N	26	N	N	26	Y- mut allele	N
27	Y- both alleles	N	27	**N**	**Y- both alleles**	27	N	N
28	Y- both alleles	N	28	Y-mut allele	N	28	Y- both alleles	N
29	N	N	29	Y- both alleles	N	29	Y- wt allele	N
30	Y- both alleles	N	30	Y- wt allele	N	30	N	**Y- both alleles**
31	Y- both alleles	N				31	Y- mut allele	N

Table S4, related to experimental procedures. *Oligonucleotide sequences*

ODN	Sequence	Purpose
1	Cas9ecoF	Amplification of SpCas9 sequence
2	ctcgagcggccgccagtgtgatggatatcccttgtcagcc ctgctgtctccac	Amplification of SpCas9 sequence
3	agggatatccatcacactgg	Amplification of Geminin/Cdt1
4	cagtaccggtagattacagcgcctttctcc	Amplification of Geminin
5	cagtaccggtagattagatggtgtcctgg	Amplification of Cdt1
6	cagtagatctagcaaaggcaagtgacttgg	Amplification of 5' DNMT3B arm and part of EFPP for targeting vector
7	cagtcatatgaacttcagggt	Amplification of 5' DNMT3B arm and part of EFPP for targeting vector
8	caccgctcccacaggaaagcatga	Generation of DNMT3B sgRNA
9	aaactcatgctttcctgtgggagc	Generation of DNMT3B sgRNA
10	ctcgttctctatctaatcctgg	Amplification of DNMT3B (binds upstream of start codon)
11	ggaccactaactacatttcc	Amplification of DNMT3B (binds downstream of start codon)
12	ggcaagagcatcaccctaag	Sequencing of DNMT3B PCR product
13	caccgatttaccagctggaatgat	Generation of SCN2A sgRNA
14	aaacatcattccagctggtaaatc	Generation of SCN2A sgRNA
15	gccagtagtagaaatgttgg	Amplification of SCN2A gene (exon 14)
16	ctaagaagagaagtgtagac	Amplification of SCN2A gene (exon 14)
17	gacttaatccgtgtactc	Sequencing of SCN2A PCR product
18	caccgcactgcacgccgtaggtca	Generation of EGFP sgRNA
19	aaactgacctacggcgtgcagtgc	Generation of EGFP sgRNA

Table S5, related to experimental procedures. *DNA/mRNA concentrations used for transfection experiments*

	DNMT3B knockin (fib)	***SCN2A gene correction (fib)***	***EGFP to BFP conversion (iPSC)***	***GAPDH and DNMT3B (H9)***
pEP4EO2SEN2L	2.5 µg	2.5 µg	-	
pEP4EO2SET2K	2.5 µg	2.5 µg	-	
pEP4EO2SEM2K	2.5 µg	2.5 µg	-	
pSimple-miR302/367	2.5 µg	2.5 µg	-	
EBNA1 mRNA	5 µg	5 µg	-	
sgRNA plasmid	2 µg	2 µg	0.5 µg	2 µg
Cas9 mRNA	5 µg	5 µg	0.5 µg	5 µg
Gene targeting plasmid	5 µg	5 µg	2 µg	5 µg

DNMT3B gblock

PHOS//AAGTTagatctcatatgCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACC
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TCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGA
CGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCAT
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CAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTG
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GATCCTCGTCAACGGGGCCTGCAGCGACCAGTCCTCCGACTCGCCCCCAATCCTGGAGGC
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GCCACACTCTACATGGGAGCACTTGAGAAGCGGCT//PHOS

U6Sptracr gblock

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SCN2A 5’ gblock

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SCN2A 3' gblock

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BFP gene block

GCAGTgacgtcATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTC
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