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Article

Aberrant splicing of *INS* impairs beta-cell differentiation and proliferation by ER stress in the isogenic iPSC model of neonatal diabetes

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Table S1. List of sgRNAs and oligonucleotides for sgRNA cloning.

Name	Sequence, 5′-3′	PAM	Oligonucleotide sequence for cloning
sgRNA-1	GTTCCAGAACCTGCTCTGCG	CGG	Fwd: GTTCCAGAACCTGCTCTGCGGTTTT Rev: CGCAGAGCAGGTTCTGGAACCGGTG
sgRNA-2	CAGGTTCTGGAACAGCGGCG	AGG	Fwd: CAGGTTCTGGAACAGCGGCGGTTTT Rev: CGCCGCTGTTCCAGAACCTGCGGTG
sgRNA-3	CAGAGCAGGTTCTGGAACAG	CGG	Fwd: CAGAGCAGGTTCTGGAACAGGTTTT Rev: CTGTTCCAGAACCTGCTCTGCGGTG

Single-stranded oligodeoxynucleotide (ssODN):

5′-TCCTGTGTCCCTCTGCCTCGCCGCTGTTCCGGAACCTGCTCTGCGCGGCACGTCCTGGCAG-TCGGGCAGGTGGAGCTGGGCGGGGGCCCTGGT-3′

Table S2. Comparison of culture conditions for iPSC single cell cloning after FACS analysis.

Matrix	Medium	Viability, %
Feeder cells on Matrigel	mTESR1	2.1%
Feeder cells on Matrigel	½ mTESR1 + ½ conditioned mTESR1	5.2%
Feeder cells on Matrigel	½ mTESR1 + ½ conditioned mTESR1 + 10% KSR	8.3%
Matrigel	½ mTESR1 + ½ conditioned mTESR1	0%

Table S3. Summary of sequencing results of iPSC clone screening.

Clone number	Intron genotype	Presence of cleavage
patient	Heterozygous mutation c.188-31G>A	N/A
	sgRNA-3	

3	Heterozygous mutation c.188-31G>A	no
13	Heterozygous mutation c.188-31G>A	no
16	Heterozygous mutation c.188-31G>A	no
17	Heterozygous mutation c.188-31G>A	no
18	Del/ins at the position c.188-31G>A	yes
27	Heterozygous mutation c.188-31G>A	no
42	Heterozygous mutation c.188-31G>A	no
2	Heterozygous mutation c.188-31G>A	no
3	Del/ins at the position c.188-31G>A	yes
4	Heterozygous mutation c.188-31G>A	no
6	Heterozygous mutation c.188-31G>A	no
7	Del/ins at the position c.188-31G>A	yes
9	Del/ins at the position c.188-31G>A	yes
10	Heterozygous mutation c.188-31G>A	no
11	Heterozygous mutation c.188-31G>A	no
12	Heterozygous mutation c.188-31G>A	no
13	Heterozygous mutation c.188-31G>A	no
14	Heterozygous mutation c.188-31G>A	no
15	Heterozygous mutation c.188-31G>A	no
16	Heterozygous mutation c.188-31G>A	no
18	Del/ins at the position c.188-31G>A	yes
19	Heterozygous mutation c.188-31G>A	no
20	Heterozygous mutation c.188-31G>A	no
21	Heterozygous mutation c.188-31G>A	no
22	Del/ins at the position c.188-31G>A	yes
	sgRNA-2	
1	Heterozygous mutation c.188-31G>A	no
2	Heterozygous mutation c.188-31G>A	no

3	Del/ins at the position c.188-31G>A	yes
4	Heterozygous mutation c.188-31G>A	no
5	Heterozygous mutation c.188-31G>A	no
6	Heterozygous mutation c.188-31G>A	no
7	Heterozygous mutation c.188-31G>A	no
8	Heterozygous mutation c.188-31G>A	no
9	Heterozygous mutation c.188-31G>A	no
10	Heterozygous mutation c.188-31G>A	no
11	Heterozygous mutation c.188-31G>A	no
12	Del/ins at the position c.188-31G>A	yes
13	Heterozygous mutation c.188-31G>A	no
14	Heterozygous mutation c.188-31G>A	no
15	Heterozygous mutation c.188-31G>A	no
16	Heterozygous mutation c.188-31G>A	no
17	Del/ins at the position c.188-31G>A	yes
18	Heterozygous mutation c.188-31G>A	no
19	Duplication	yes
20	Insertion 7bp	yes
21	Heterozygous mutation c.188-31G>A	no
22	Heterozygous mutation c.188-31G>A	no
23	Heterozygous mutation c.188-31G>A	no
24	Heterozygous mutation c.188-31G>A	no
36	Heterozygous mutation c.188-31G>A	no
37	Del/ins at the position c.188-31G>A	yes
38	Heterozygous mutation c.188-31G>A	no
39	Heterozygous mutation c.188-31G>A	no
40	Heterozygous mutation c.188-31G>A	no
41	Heterozygous mutation c.188-31G>A	no

46	Del/ins at the position c.188-31G>A	yes
47	Heterozygous mutation c.188-31G>A	no
48	Heterozygous mutation c.188-31G>A	no
49	Heterozygous mutation c.188-31G>A	no
50	Del/ins at the position c.188-31G>A	yes
51	Heterozygous mutation c.188-31G>A	no
52	Del/ins at the position c.188-31G>A	yes
53	Heterozygous mutation c.188-31G>A	no
54	Heterozygous mutation c.188-31G>A	no
55	Del/ins at the position c.188-31G>A	yes
56	Heterozygous mutation c.188-31G>A	no
57	Heterozygous mutation c.188-31G>A	no
58	Heterozygous mutation c.188-31G>A	no
59	Heterozygous mutation c.188-31G>A	no
60	Heterozygous mutation c.188-31G>A	no
61	Homozygous c.188-31G	yes
62	Heterozygous mutation c.188-31G>A	no
63	Heterozygous mutation c.188-31G>A	no
64	Heterozygous mutation c.188-31G>A	no
65	Heterozygous mutation c.188-31G>A	no

Table S4. Key points of pancreatic differentiation protocol.

Stage 1	Day 1	Basa medium + supplement A + supplement B
	Day 2	Basal medium + supplement B
	Day 3	
	Day 4	

Stage 2 (posterior foregut induction)	Day 5 Day 6	RPMI + B27 (1×) +FGF7 50ng/ml
Stage 3 (pancreatic endoderm induction)	Day 7 Day 8 Day 9	DMEM, Glutamax (1x), sodium pyruvate (1x), B27 (1×) +KAAD 0.25μM +RA 2μM +LDN 250nM
Stage 4 (pancreatic progenitor induction)	Day 10 Day 11 Day 12	DMEM, Glutamax (1x), sodium pyruvate (1x), B27 (1×) +EGF 50ng/ml
Stage 5	Day 13 Day 14	DMEM, Glutamax (1x), sodium pyruvate (1x), B27 (1×) + KAAD 0.25μM + T3 1μM + Alk5i 10μM + Zinc-Sulfate 10μM + Heparin 10μg/ml + Y-27632 5μM

Table S5. List of reagents for beta-cell culturing.

Reagent	Company	Final concentration	Stock concentration
Sodium bicarbonate	Merck	1.5 g/l	150g/L (x100)
BSA	Gibco	2%	100 %
SANT-1	Millipore	0.25 μM	0,25mM (x1000)

Retinoic acid	Stemcell Technologies	0.05 μM	1mM (x20.000) Or 50 mkM (x1000)
LDN193189	Stemcell Technologies	100 nM	1mM (x10.000)
ITS-X	Gibco	1:200	100% (x200)
T3	Millipore	1 μM	1mM (x1000)
ALK5 inhibitor II	Abcam	10 μM	10 mM (x1000)
Heparin	Sigma-Aldrich	10 μg/ml	10 mg/ml (x1000)
EGF	R&D Systems	50 ng/ml	500 μg/ml (x10.000)
Cyclopamine	Stemcell Technologies	0,25 μM	5mM (x20.000)
KGF (FGF7)	Abcam	50 ng/ml	100 μg/ml (x2.000)
B27	Gibco	1:100	100% (x100)

Table S6. List of primers for the *INS* splice variant isoform cloning.

Name	**Sequence, 5′-3′**
Ins-CDS-RI-F	ATCGAATTCACTGTCCTTCTGCCATGGCCCTG
R1InsEx3-R	TTGAATTCCATCTCTCTCGGTGCAGG
GRP78-LucF	TCCGGTACCCGAGATAGACAGCTGCTGAACCA
Grp78-LucR	TTCCCTCGAGCTTCATCTTGCCAGCCAGT
UPRE-1-For	CACAGGTGCTGACGTGGCATTCACAGGTGCTGACGTGGCATTCACAGGT
UPRE-1-Rev	GTGAATGCCACGTCAGCACCTGTGAATGCCACGTCAGCACCTGTGGTAC
UPRE-2-For	GCTGACGTGGCATTCACAGGTGCTGACGTGGCATTCACAGGTGCTGACGTGGCATTC
UPRE-2-Rev	TCGAGAATGCCACGTCAGCACCTGTGAATGCCACGTCAGCACCTGTGAATGCCAC-GTCAGCACCT
ERSE-For	CTTCACCAATCGGCGGCCTCCACGACGGC
ERSE-Rev	TCGAGCCGTCGTGGAGGCCGCCGATTGGTGAAGGTAC

Table S7. Primers for iPSC clone screening after CRISPR/Cas9 genome editing (371-bp PCR product).

	Sequence, 5′-3′	Tm	GC %
Forward	GGGGAGAAGTACTGGGATCACC	61.55	59.09
Reverse	ATTGTTCCACAATGCCACGCTT	61.92	45.45

Table S8. List of antibodies for immunocytochemistry.

Antibody	Company, catalog number	Dilution
Ms anti-PDX1 PE-conjugated	BD Biosciences, 562161	1:100
Ms anti-NKX6.1 Alexa Fluor 647-conjugated	BD Biosciences, 563338	1:100
Rb mAb to Insulin, Alexa Fluor 647-conjugated	Cell Signaling Technology, 9008	1:500
Rb pAb to C-Peptide	Cell Signaling Technology, 4593	1:100
Ms pAb to Glucagon	Sigma, 62654	1:1000
Ms mAb to Somatostatin	Santa Cruz SC-55565	1:50
Goat anti-Mouse IgG H&L, Alexa Fluor 488	Abcam, ab150117	1:500
Goat anti-Mouse IgG H&L, Alexa Fluor 594	Abcam, ab150120	1:500
Goat anti-Rabbit IgG H&L, Alexa Fluor 488	Abcam, ab150077	1:500
Goat anti-Rabbit IgG H&L, Alexa Fluor 594	Abcam, ab150084	1:500

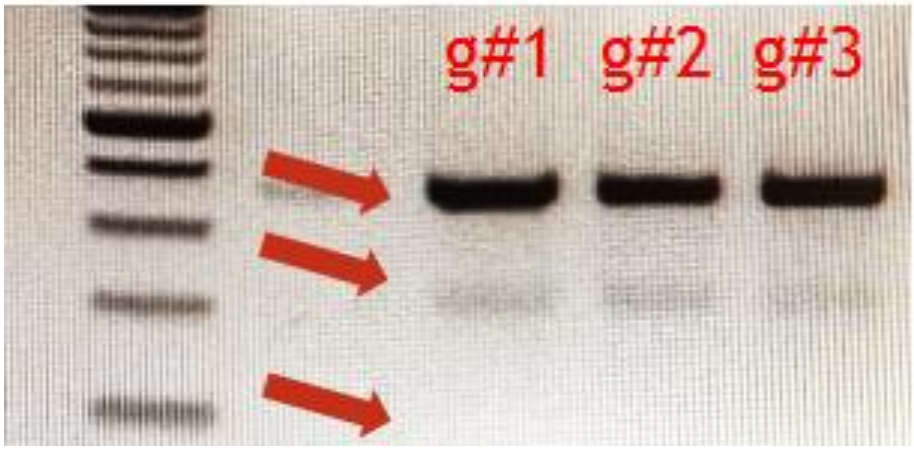

Figure S1. Analysis of cutting efficiency of the *INS* allele-specific sgRNAs by T7E1 assay. sgRNA-1, sgRNA-2 and sgRNA-3 used in our study are marked as g#1, g#2 and g#3 on the electrophoregram. Red arrows show bands which are generated when Cas9 nuclease makes modifications to genomic DNA. The cutting efficiency was calculated based on band intensity and amounted to 10%, 11.1%, and 8.9% for sgRNA-1, sgRNA-2 and sgRNA-3, respectively.

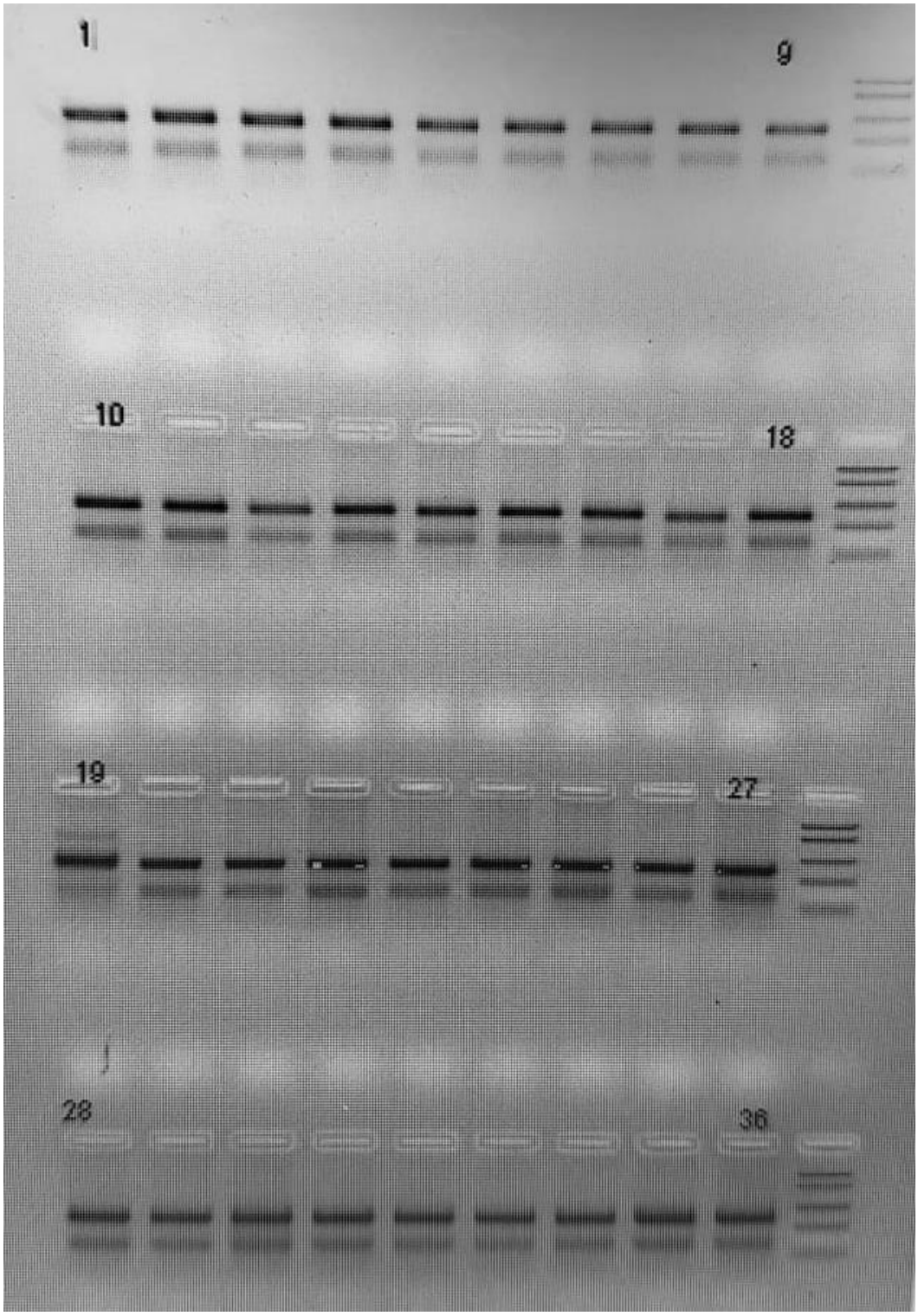

Figure S2. Restriction digest screening of iPSC clones after CRISPR/Cas9 editing using *BtsI* enzyme. The electrophoregram shows screening results for the first 36 clones, wherein an additional 400-bp band expected in the case of HDR is observed in clone #19.

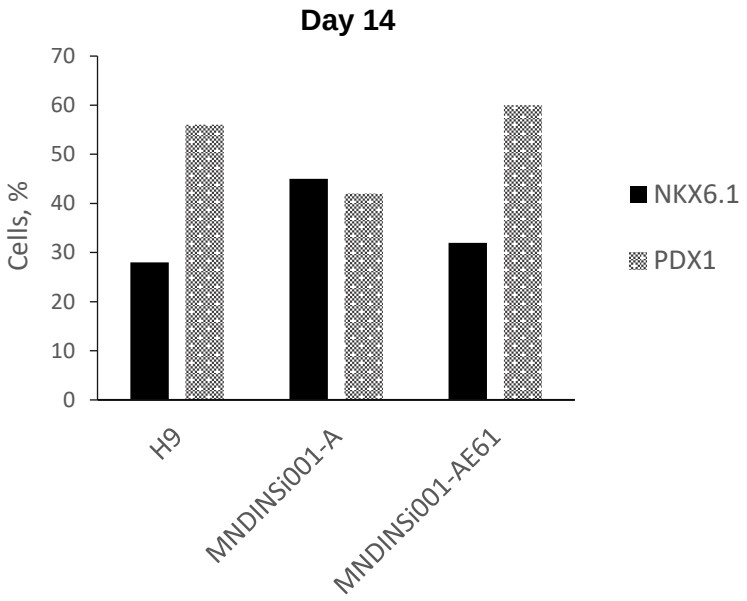

Figure S3. The capacity of established isogenic iPSC lines to differentiate into pancreatic progenitor cells. The data show the amount of NKX6.1- and PDX1-positive cells at Day 14 of differentiation for MNDINSi001-A iPSC line carrying c.188-31G>A *INS* mutation and CRISPR/Cas9-corrected MNDINSi001-AE61 iPSC line. H9 ESC line was used as a gold-standard.