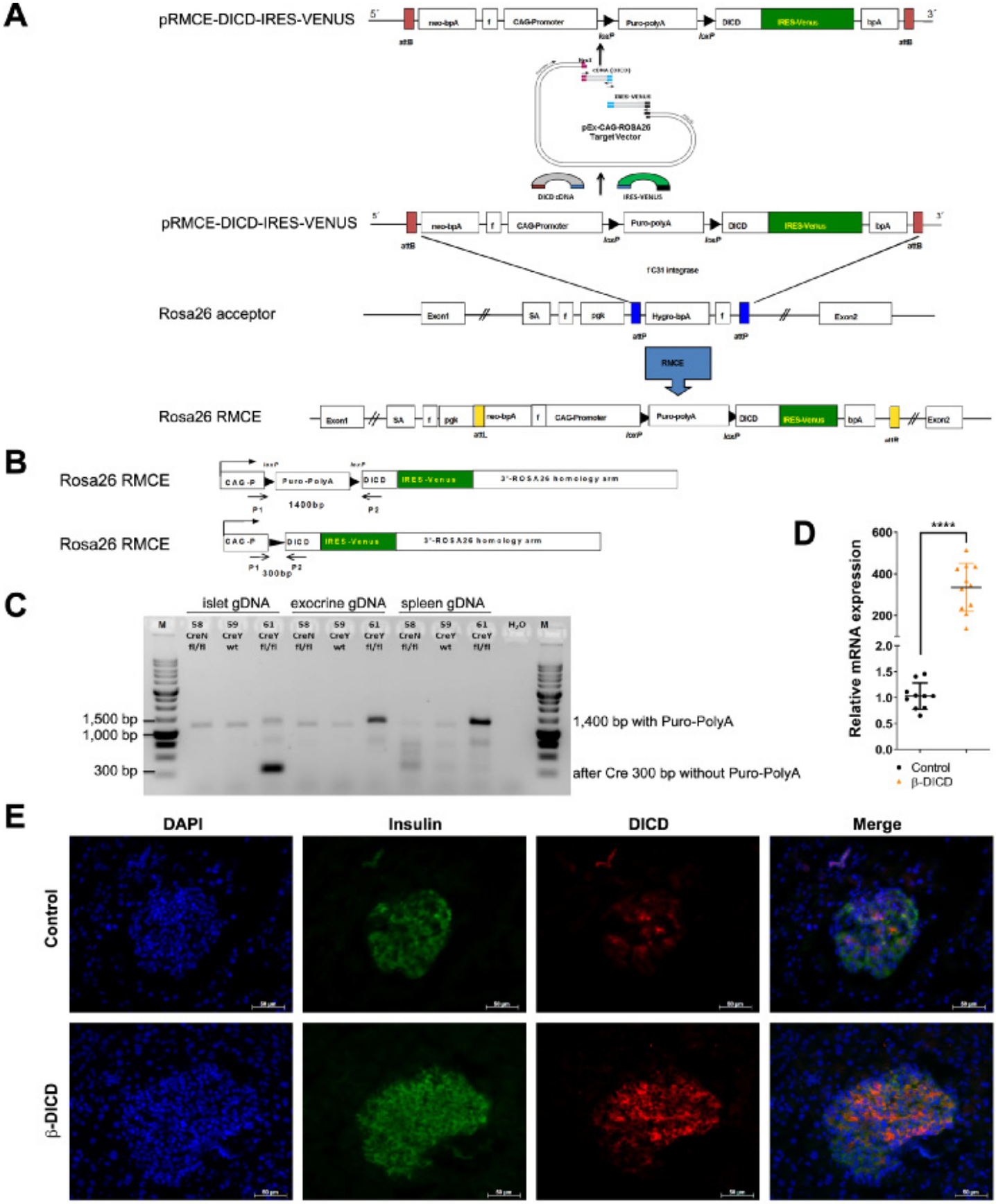
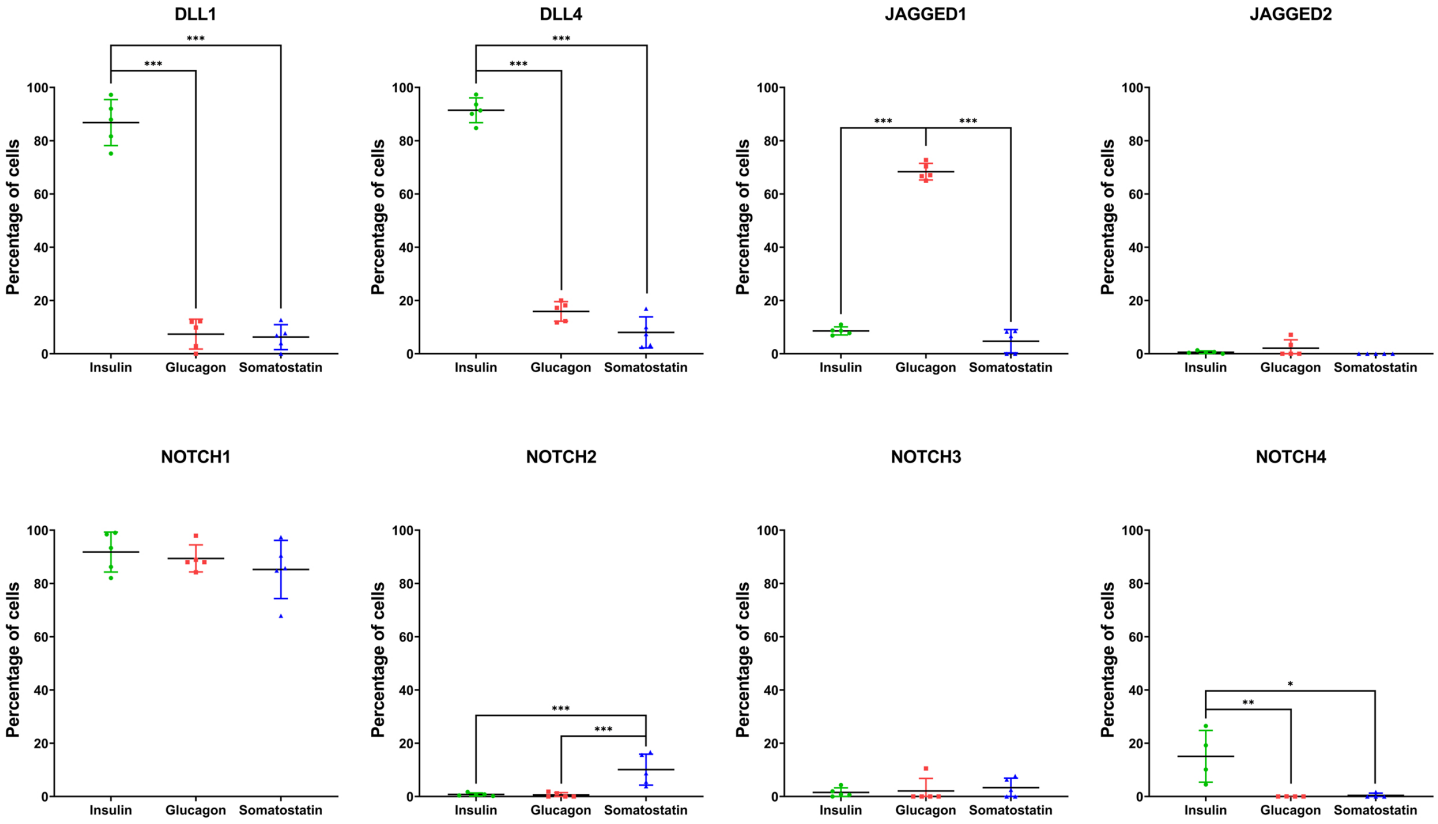
SUPPLEMENTARY DATA

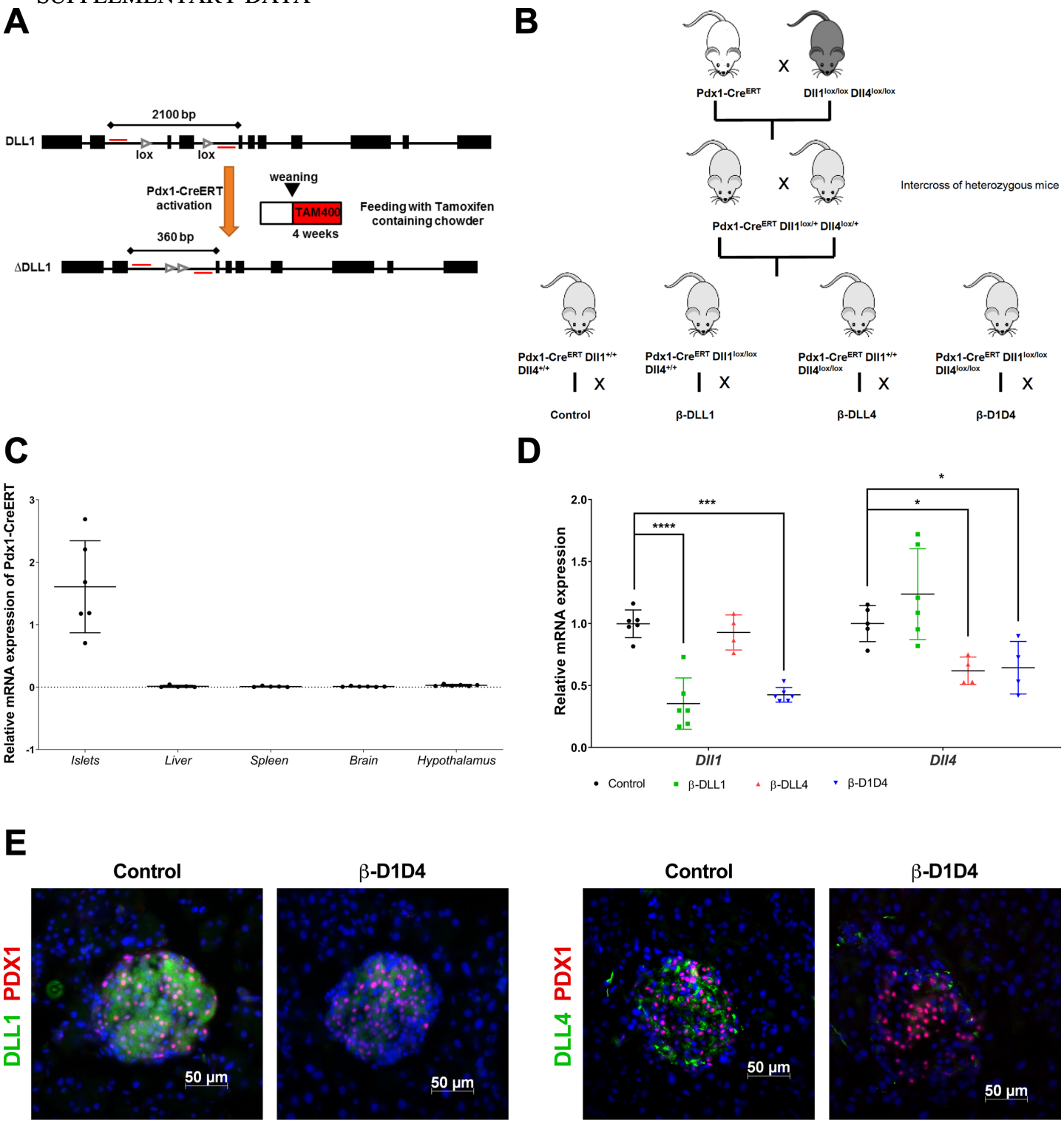


ESM Fig. 1: (**A**) DICD vector construct within the *Rosa26* locus; (**B**) *Cre*-mediated removal of the puro-stop cassette. (**C**) Gene recombination in genomic DNA (gDNA) of islets, exocrine tissue and spleen as control tissue. (**D**) Relative gene expression of *DICD* in islets of β-DICD and control mice; control n=10 and β-DICD n=11. Data are shown as mean±SD. (**E**) Co-immunostaining of insulin (green) and DICD (red) by using an antibody that specifically recognizes the DLL1 intracellular domain in pancreatic sections; n=4. Nuclei were counterstained with DAPI (blue). 8 weeks old male mice were used in the study. The scale bar represents 50 µm. Data are shown as mean ±SEM. Differences were considered statistically significant at $p<0.05$ using a two-tailed student´s t-test (**** $p<0.0001$).

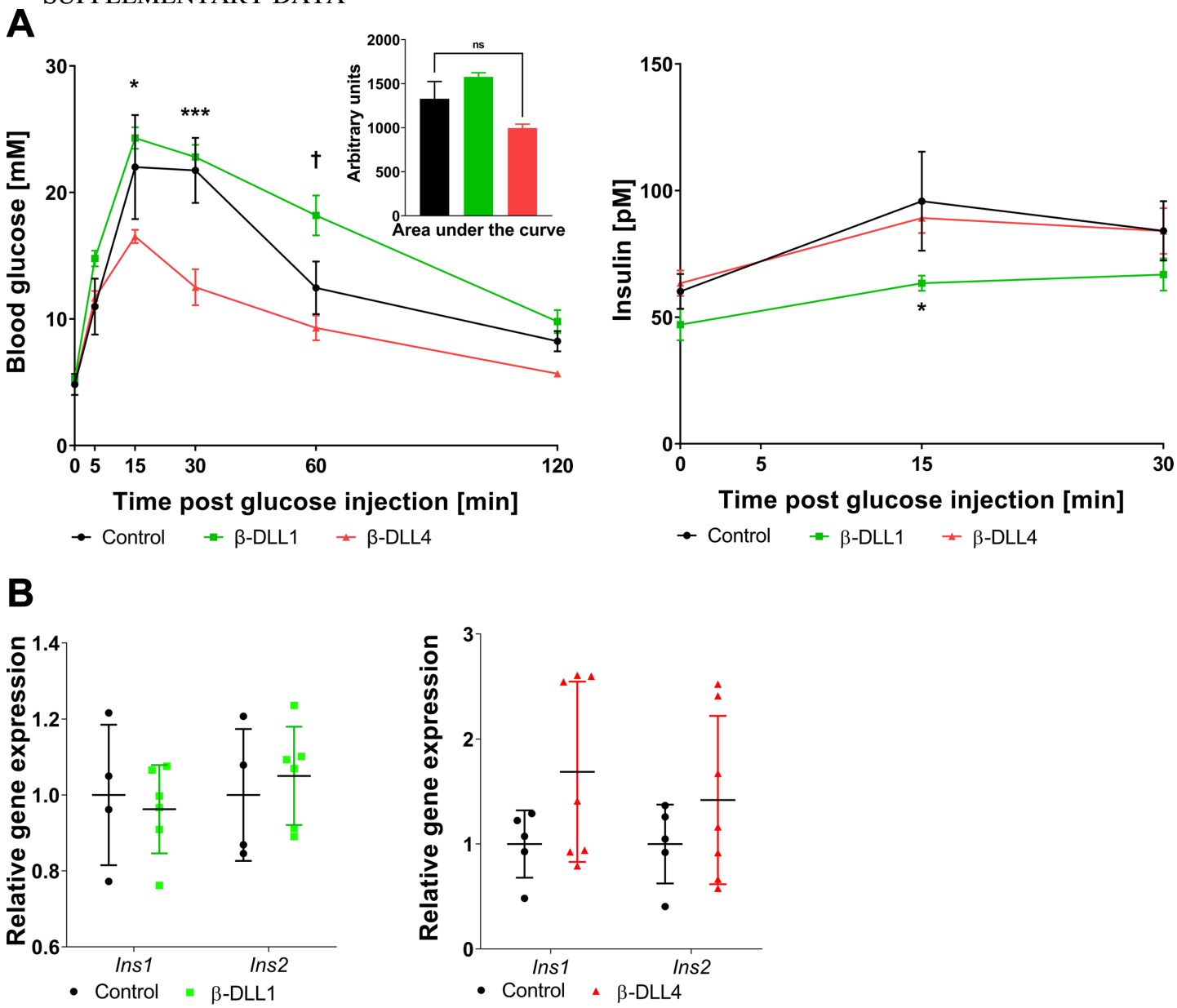
SUPPLEMENTARY DATA



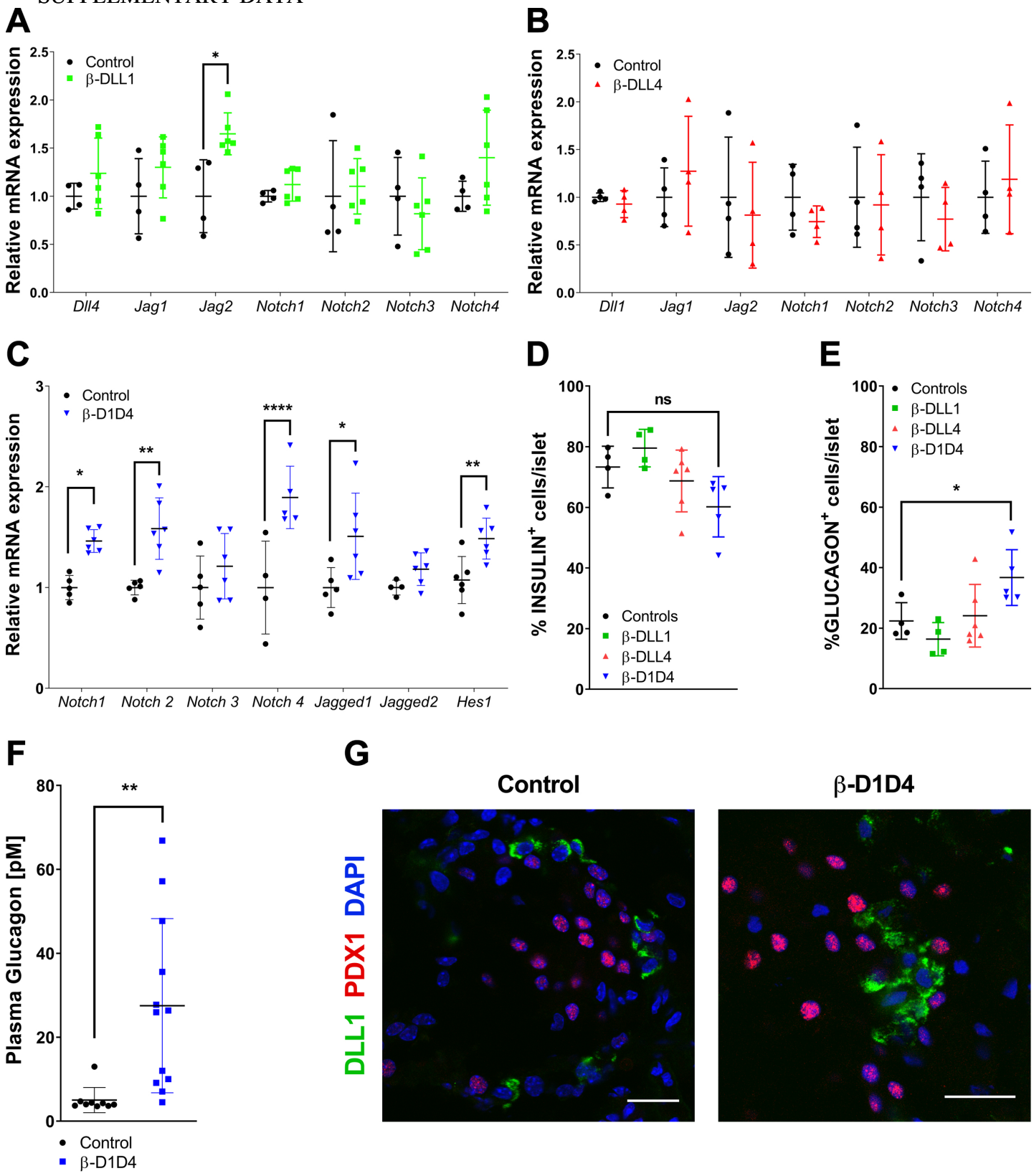
ESM Fig. 2: Quantifications displaying percentage of insulin positive β-cells, glucagon positive α-cells and somatostatin positive δ-cells expressing Notch receptors and ligands, as acquired by immunostaining of dispersed islet cells. 13-week-old male C3HeB/FeJ mice were used for this study; n=5 (NOTCH4 n=4). Data are shown as mean±SD. Differences were considered statistically significant at $p<0.05$ using a one-way ANOVA with Bonferroni *post hoc* test (**** $p<0.001$, **** $p<0.0001$).



ESM Fig. 3: (**A**) Schematic representation of the *Dll1* locus and its recombination in beta-cells of the β-DLL1 mouse. Upon activation of the Pdx1-CreERT recombinase with tamoxifen, exon 3 and 4 are excised and a novel termination codon is generated. The scheme is similar for the *Dll4* locus in β-DLL4 mice. (**B**) Mating strategy to acquire four homozygous mouse lines expressing different variants of the *Dll1* and *Dll4* locus as well as Pdx1-Cre^{ERT}. (**C**) Relative gene expression of *Cre* recombinase in islets, liver, spleen, brain and hypothalamus tissue of tamoxifen-induced mice, normalized to the housekeeping gene *Hprt*; n=3. (**D**) Relative gene expression of *Dll1* and *Dll4* in islets of β-DLL1, β-DLL4, β-D1D4 and control mice, achieving 60% recombination for Dll1 and 45% for Dll4; *Dll1* control, β-DLL1 and β-D1D4 n=6 and β-DLL4 n=5; *Dll4* control n=5, β-DLL1=6, β-DLL4 and β-D1D4 n=4. (**E**) Co-immunostaining for PDX1, DLL1 and DLL4, respectively, on pancreatic sections from 8 weeks old male β-D1D4 mice; n=4. Nuclei were counterstained with DAPI (blue). The scale bar represents 50 µm. Data are shown as mean ±SD. Differences were considered statistically significant at $p<0.05$ using a two-tailed student´s t-test (* $p<0.05$, *** $p<0.001$, **** $p<0.0001$).



ESM Fig 4: (**A**) Measurement of blood glucose levels and insulin during an intraperitoneal glucose tolerance test in 8-10-week-old mice; control n=4, β-DLL1 and β-DLL4 n=5. (**B**) Relative gene expression levels of insulin gene isoforms in isolated islets from 8 weeks-old male; control n=4 and β-DLL1 n=6; control n=5 and β-DLL4 n=7. Data are shown as mean ±SEM for (**A**) and ±SD for (**B**). Differences were considered statistically significant at $p<0.05$ using a two-way ANOVA with Bonferroni *post hoc* test and a two-tailed student´s t-test (*, † $p<0.05$, *** $p<0.001$, †= control vs β-DLL1, *=control vs β-DLL4 and n.s – non-significant).



ESM Fig. 5: Relative mRNA expression of Delta-Notch components: (**A**) Control n=4 and β-DLL1 n=6; (**B**) Control and β-DLL4 n=4 and (**C**) Control n=4-5 and β-D1D4 n=6. Percentage of (**D**) insulin positive and (**E**) glucagon positive cells per islet. Control, β-DLL1 n=4, β-DLL4 n=6 and β-D1D4 n=5. (**F**) Concentration of glucagon in blood plasma: Control n=9 and β-D1D4 n=12. (**G**) Immunostaining for glucagon and PDX1; n=5. The scale bar represents 50 µm. 8-weeks-old male mice were used in the study. Data are shown as mean ±SD. Differences were considered statistically significant at $p<0.05$ using a two-tailed student´s t-test (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, n.s – non-significant).

SUPPLEMENTARY DATA

Electronic Supplementary Tables

Supplementary Table 1. Primer pairs used in this study

Primer for Genotyping		
Gene name	**forward primer 5′-3′**	**reverse primer 5′-3′**
DICD lox	GCACTTGCTCTCCCAAAGTC	GATACCGTCGATCCCCACT
Dll1lox	CACACCTCCTACTTACCTGA	GAGAGTACTGGATGGAGCAAG
Dll4lox	GTGCTGGGACTGTAGCCACT	TGTTAGGGATGTCGCTCTCC
Pdx1 CreERT	AACCTGGATAGTGAAACAGGGGC	TTCCATGGAGCGAACGACGAGACC
Primer for qPCR		
Gene name	**forward primer 5′-3′**	**reverse primer 5′-3′**
Cdkn1a	GCAGACCAGCCTGACAGATT	CACACAGAGTGAGGGCTAAGG
Cdkn1c	CCAATGCGAACGACTTCTT	GCCGTTAGCCTCTAAACTAACTCA
Ctgf	AGTGTGCACTGCCAAAGATG	TTCCAGTCGGTAGGCAGCTA
DICD	CACTTAGGGGTGGGGAGATT	CGCTTCCATCTTACACCTCAG
Dll1	TGGCCAGGTACCTTCTCTCT	TCTTTCTGGGTTTTCTGTTGC
Dll4	CACAGTGAGAAGCCAGAGTGTC	TCCTGCCTTATACCTCTGTGG
Glucagon	AGGCTCACAAGGCAGAAAAA	CAATGTTGTTCCGGTTCCTC
Hes1	GAGCACAGAAAGTCATCAAAG	ATGCCGGGAGCTATCTTTCT
Hes6	CCAATCTTGAGACTGAGCATTAGG	TCATAGCCAAAGTAGCAAATCTGAAC
Hey1	GAAAAGACGGAGAGGCATCA	AGCAGATCCCTGCTTCTCAA
Hey2	ATTACCCTGGGCACGCTAC	TTTTCTATGATCCCTCTCCTTTTC
Hprt	CCTAAGATGAGCGCAAGTTGAA	CCACAGGACTAGAACACCTGCTAA
Ins1	GCAAGCAGGTCATTGTTTCA	CACTTGTGGGTCCTCCACTT
Ins2	CAGCAAGCAGGAAGCCTATC	GCTCCAGTTGTGCCACTTGT
Jagged1	GCCAGACTGCAGGATAAACA	CCCTGAAACTTCATGGCACT
Jagged2	GCCAGGAAGTGGTCATATTCA	ATCCGCACCATACCTTGCTA
Mafb	TAGCGATGGCCGCGGAG	CTTCACGTCGAACTTGAGAAGG
Msln	CATCCCCAAGGATGTCAAAG	GCAGGCTTTCTGTTCTGCAT
Mtor	CAAGCAGGCAACATCTCACG	CAGAAGGGACACCAGCCAAT
Neurog3	GTCGGGAGAACTAGGATGGC	GGAGCAGTCCCTAGGTATG
Notch1	TCAGGGTGTCTTCCAGATCC	CGACTTGCCTAGGTCATCCA
Notch2	GCAGTGGATGACCATGGAA	GGTGTCTCTTCCTTATTGTCCTG
Notch3	TGCACTGGGAATGAAGAACA	CCGGCTCCTCTACCTTCAGT
Notch4	GGATAAAAGGGGAAAAACTGC	CGTCTGTTCCCTACTGTCCTG
Pdx1 Cre	TGCAACGAGTGATGAGGTTC	GCAAACGGACAGAAGCATTT
Sdha	GCAATTTCTACTCAATACCCAGTG	CTCCCTGTGCTGCAACAGTA
Smad2	GGGAGCAGAATATCGGAGGC	TGCAGAGGGCCATTCAGATG
Smad7	CTGCAACCCCCATCACCTTA	CAGCCTGCAGTTGGTTTGAG
Ubc	AGCCCAGTGTTACCACCAAG	ACCCAAGAACAAGCACAAGG

SUPPLEMENTARY DATA

Supplemetary Table 2. Primary antibodies used in this study

Primary antibody	**Host**	**Clonality**	**Cataloge number**	**Company**	**Dilution**
CRE	rabbit	polyclonal	69050-3	Millipore	1:200
Dll1 (155-173) extracellular	rabbit	polyclonal	ab10554	Abcam	1:200
Dll1 intracellular	rat	monoclonal		Gift from Dr. E. Kremmer	1:5
Dll4	rabbit	polyclonal	ab7280	Abcam	1:200
Glucagon	mouse	monoclonal	G2654	Sigma Aldrich	1:1000-1:5000
Insulin	guinea pig	polyclonal	A0564	Dako	1:200
Jagged1	rabbit	polyclonal	ab7771	Abcam	1:200
Jagged2	rabbit	polyclonal	sc-5604	Santa Cruz Biotechnology	1:50
Ki67	rabbit	monoclonal	RM-9106-S	Thermo Fisher	1:200
Mafb	rabbit	polyclonal	IHC-00351	biomol	1:200
Notch1 (immunohistochemistry)	rabbit	polyclonal	ab27526	Abcam	1:200
Notch1 (immunocytochemistry)	rabbit	polyclonal	ab8925	Abcam	1:100
Notch2	goat	polyclonal	sc-7423	Santa Cruz Biotechnology	1:200
Notch3	rabbit	polyclonal	ab23426	Abcam	1:200
Notch4	rabbit	polyclonal	N5163-100UG	Sigma Aldrich	1:200
PDX1	rabbit	monoclonal	5679	Cell Signaling	1:300
Somatostatin	rabbit	polyclonal	A0566	Dako	1:200
Somatostatin	goat	polyclonal	sc-7819	Santa Cruz Biotechnology	1:200
Somatostatin	mouse	monoclonal	14-9751-82	Affymetrix eBioscience	1:200

Supplementary Table 3. Secondary antibodies used in this study

Secondary antibody	Cataloge number	Company	Dilution
Alexa 488 - donkey-anti-goat	A11055	Invitrogen	1:500
Alexa 488 - donkey-anti-mouse	A21202	Invitrogen	1:500
Alexa 488 - donkey-anti-rabbit	A21206	Invitrogen	1:500
Alexa 488 - donkey-anti-rat	A21208	Invitrogen	1:500
Alexa 488 - goat-anti-guinea pig	A11073	Invitrogen	1:500
Alexa 594 - donkey-anti-goat	A11058	Invitrogen	1:500
Alexa 594 - donkey-anti-rabbit	A21207	Invitrogen	1:500
Alexa 594 - donkey-anti-mouse	A21203	Invitrogen	1:500
Alexa 594 - goat-anti-rat	ab96965	Abcam	1:500
DAPI	D9542	Sigma Aldrich	1:1000