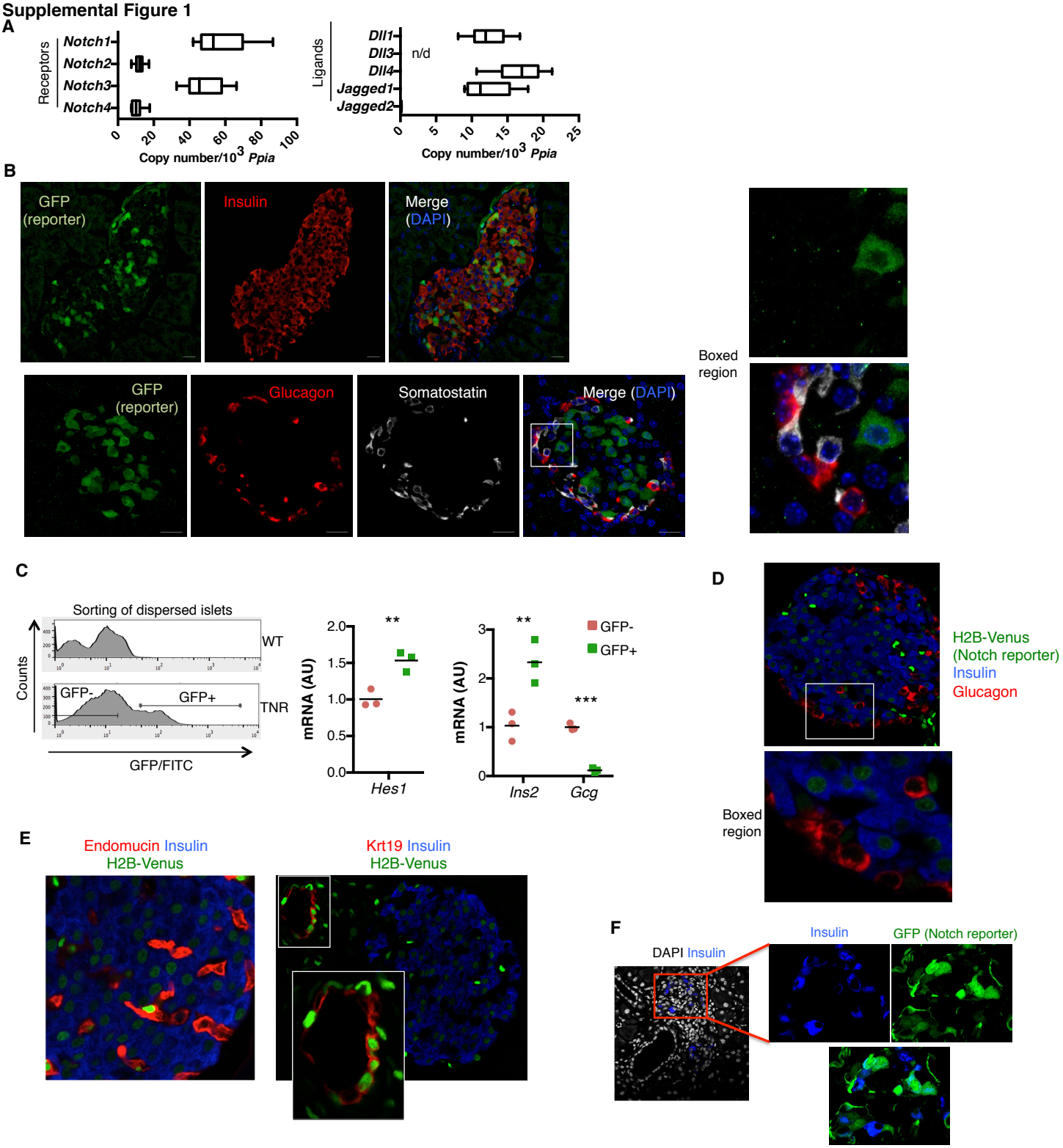
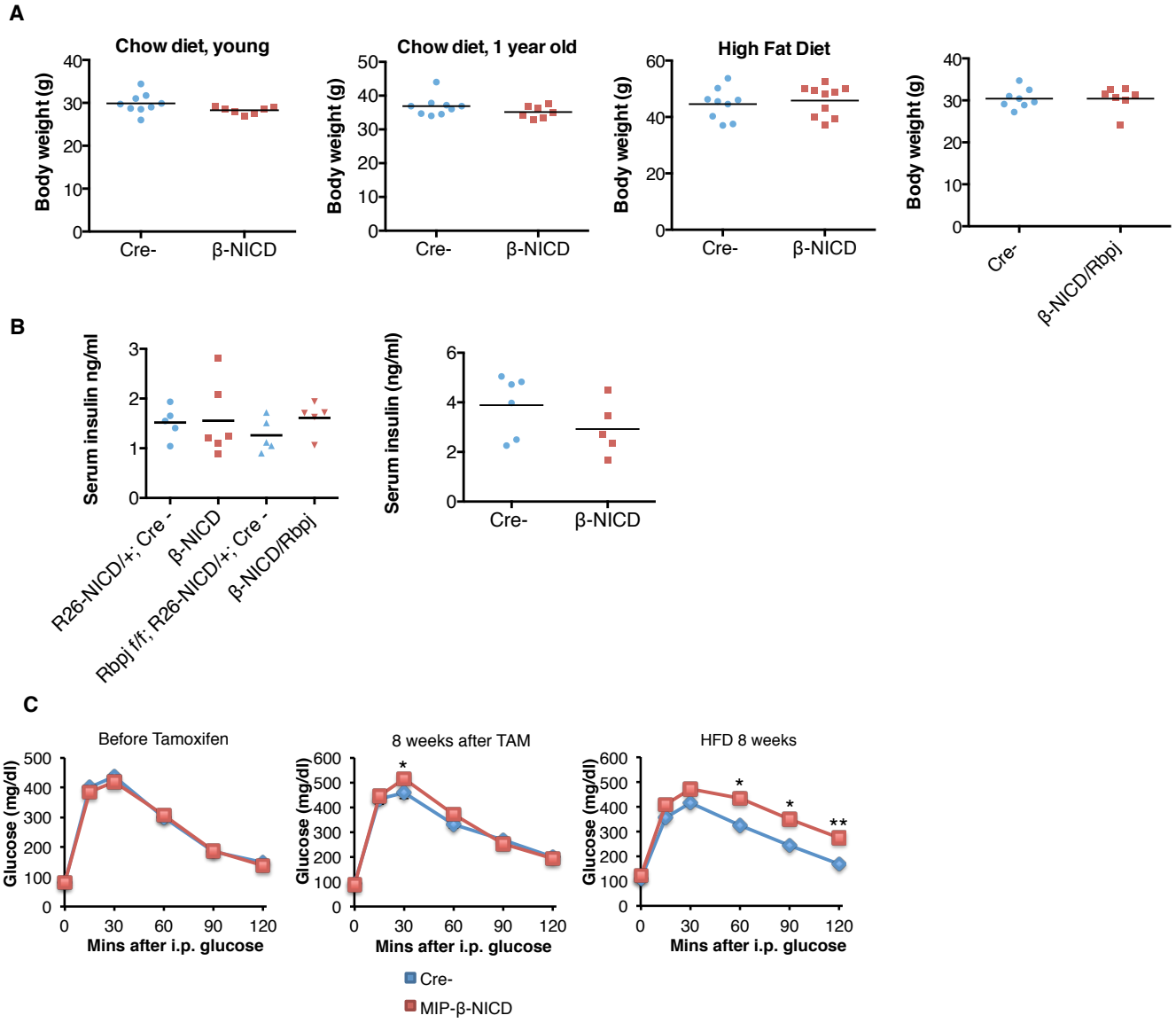
Supplemental Figure 1



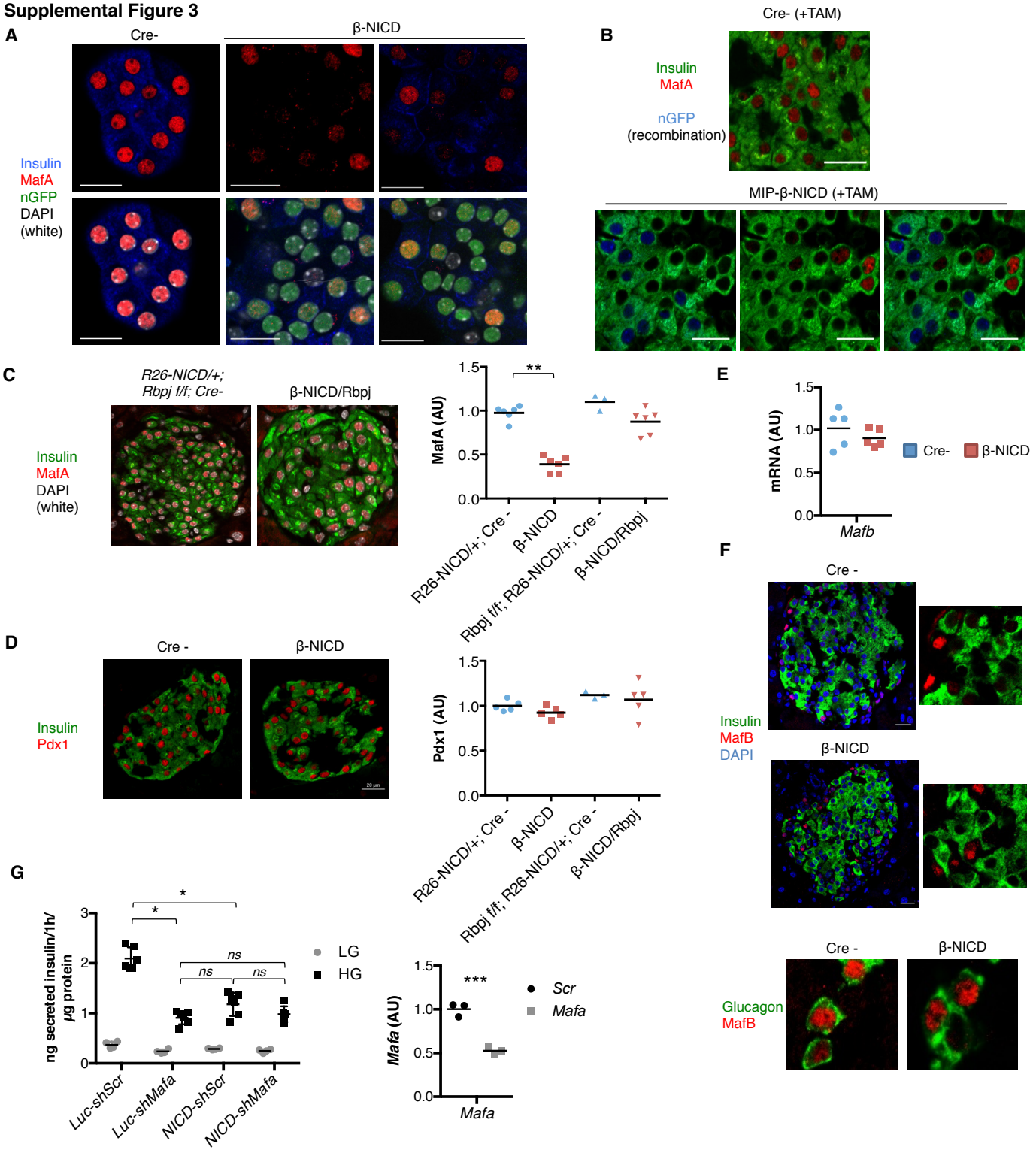
Supplemental Figure 1. Islet Notch activity is mostly restricted to β cells.
(A) Notch receptor and ligand expression in islets isolated from adult WT mice. Results show absolute quantitation of gene copies relative to *Ppia* (N=10 mice/group)
(B) Representative images of pancreatic sections from adult TNR mice (N=9 mice).
(C) FACS histogram from dispersed islet cells from WT and TNR mice, and gene expression from GFP- and GFP+ cells sorted from TNR mice (N=3 mice/group).
(D) Representative image of pancreas sections from H2B-Venus Notch reporter mice showing Venus fluorescence and insulin/glucagon staining (N=5 mice).
(E) Representative images of pancreatic sections from H2B-Venus Notch reporter mice showing Venus fluorescence and insulin co-staining with endomucin (left) or Krt19 (right), (N=5 mice).
(F) Representative images of pancreatic sections from TNR mice treated with low-dose STZ (N=5 mice).
Scale bars: 20 μm. All data are shown with group means; **, $P < 0.01$, ***, $P < 0.001$ by two-tailed *t* test.

Supplemental Figure 2



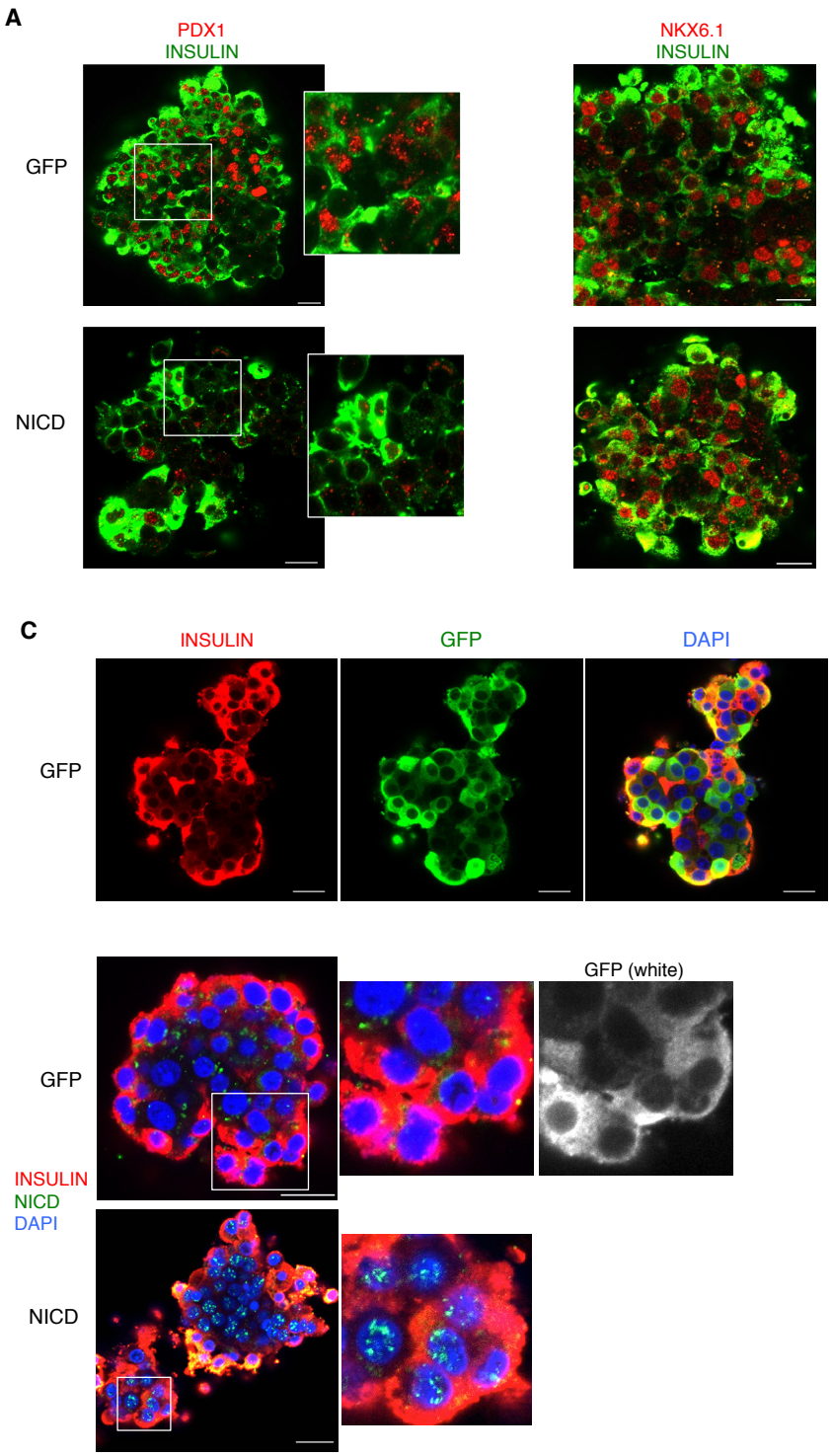
Supplemental Figure 2. β-NICD and MIP-β-NICD mice show normal body weight and fasting insulin, but glucose intolerance.
(A) Body weight of Cre-, β-NICD and β-NICD/Rbpj mouse cohorts from Figure 2.
(B) Plasma insulin in adult Cre-, β-NICD and β-NICD/Rbpj mice (N=5-7 mice/group), or in 1-year-old Cre- and β-NICD mice (N=5-6 mice/group), all taken after 5h fasting.
(C) GTT in MIP-β-NICD and Cre- control mice before and 8 weeks after tamoxifen-induced recombination (same cohort), or in an independent cohort of HFD-fed mice 8 weeks after tamoxifen treatment (N=5-10 mice/group).
All data are shown with group means ± s.e.m; *, $P < 0.05$, **, $P < 0.01$ by two-tailed *t* test.

Supplemental Figure 3



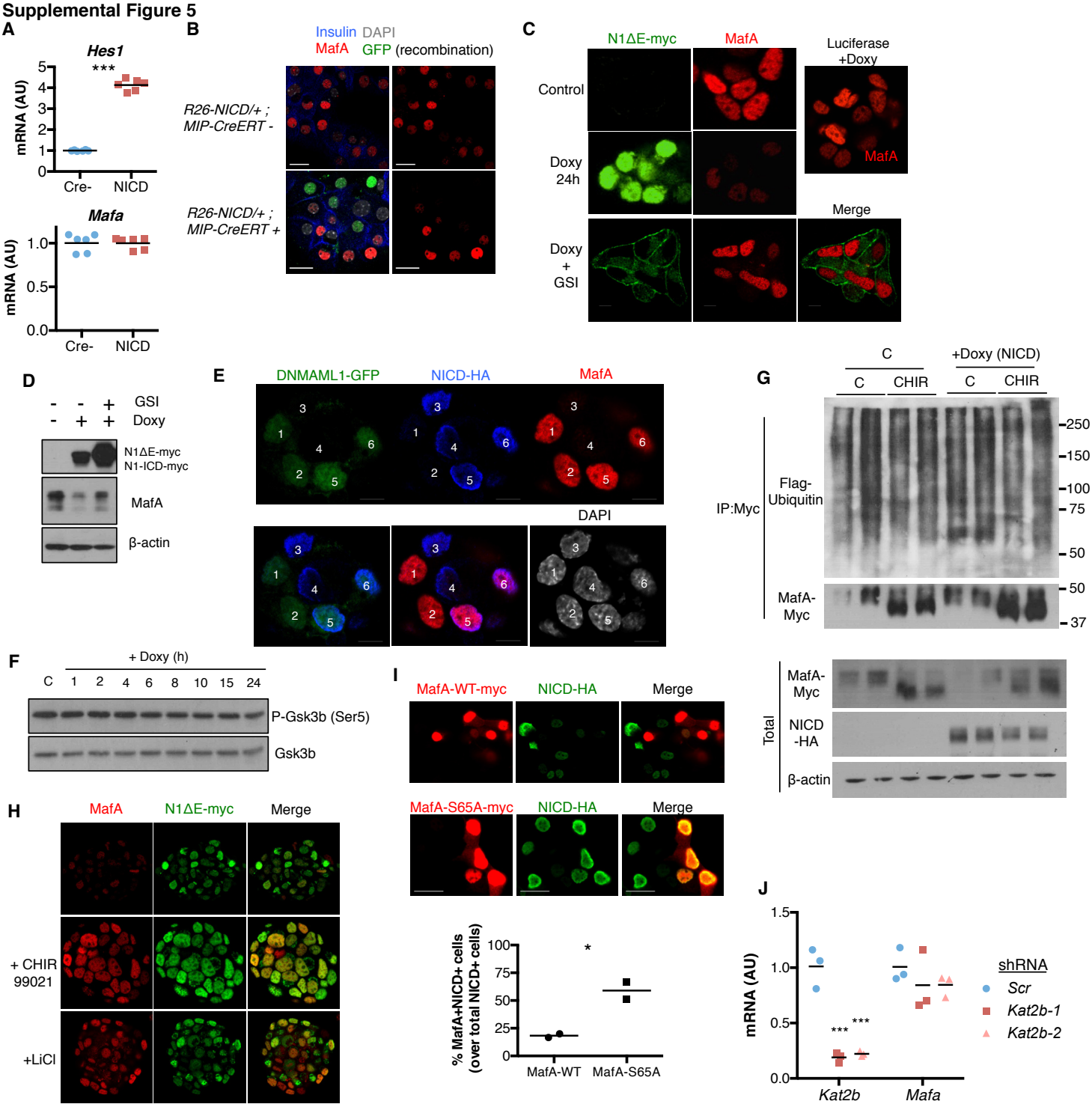
Supplemental Figure 3. Canonical Notch signaling induces a selective loss of MafA in β cells.
(A) Representative images of MafA staining in dispersed islet cells from β-NICD and Cre- control mice, cultured in vitro for 96h. Nuclear GFP (nGFP, co-expressed with NICD) is indicative of recombination in the Rosa26 locus.
(B) Representative images of MafA staining in pancreatic sections from Cre- and MIP-β-NICD mice, 8 weeks after tamoxifen treatment (N=5 mice/group).
(C) Representative images of MafA staining in pancreatic sections from β-NICD/Rbpj and Cre- control mice, with quantitation of MafA fluorescence intensity, including sections from Figure 3A (N=3-6 mice/group).
(D) Representative images of Pdx1 staining in pancreatic sections from β-NICD and Cre- control mice, with quantitation of Pdx1 fluorescence intensity (N=3-5 mice/group).
(E) *Mafb* gene expression in islets isolated from β-NICD and Cre- control mice (N=5 mice/group).
(F) MafB staining in pancreatic sections from β-NICD and Cre- control mice, co-stained with insulin (top), and glucagon (bottom), (N= 3 mice/group).
(G) GSIS from MIN6 cells transduced with lentivirus encoding NICD or Luciferase (Luc), with shRNA against MafA (shMafa) or scrambled control (shScr), in medium containing low (1 mM, LG) or high glucose (25 mM, HG), and *Mafa* gene expression (N=3 independent experiments).
Scale bars: 20 μm. All data are shown with group means ± s.e.m.; *, $P < 0.05$, **, $P < 0.01$ by two-tailed *t* test.

Supplemental Figure 4



Supplemental Figure 4. NICD expression in human islets
(A) Confocal images of whole islets transduced with lentivirus expressing GFP or NICD, stained with antibodies against insulin and PDX1 (left) and NKX6.1 (right).
(B) MAFB levels in dispersed human β cells transduced with lentivirus expressing GFP or NICD.
(C) Confocal images of whole islets transduced with lentivirus expressing GFP (top) or NICD (bottom).
Representative images from a total of 6 donors. Scale bars: 20 μm.

Supplemental Figure 5



Supplemental Figure 5. NICD-mediated loss of MafA

(A) Gene expression in islets isolated from tamoxifen-naïve Cre- or MIP-β-NICD mice, cultured with 1 μM 4-OHT for 4 days (N=6 mice/group).

(B) Representative immunofluorescence images from dispersed islet cells isolated from tamoxifen-naïve Cre- or MIP-β-NICD mice, cultured with 1 μM 4-OHT for 4 days (N=6 mice/group).

(C) Representative immunofluorescence images of MIN6-rTTA3 N1ΔE-myc Tet-On cells, stimulated with doxycycline (Doxy) with or without a γ-secretase inhibitor (GSI: dibenzazepine 100 nM). (N=4 independent experiments).

(D) Western blots of MIN6-rTTA3 N1ΔE-myc Tet-On cells stimulated with Doxy and/or GSI.

(E) Representative immunofluorescence images of MIN6-rTTA3 cells transduced with DNMAML-GFP, then transfected with NICD-HA. Cells labeled #1 and 2 are DNAMAML+, #3 and 4 are NICD+ (and MafA-) while #5 and 6 are DNMAML+ NICD+ and show preserved MafA levels.

(F) Western blots of MIN6-rTTA3 N1ΔE-myc Tet-On cells stimulated with Doxy for the indicated times.

(G) Western blots of MafA-Myc immunoprecipitate (top) and total lysate (bottom) in 293T cells with stable integration of the rTTA3 transcriptional trans-activator, transfected with MafA-Myc, Ubiquitin-Flag and Tet-On NICD-HA, with or without stimulation with Doxy for 6h, and treated with MG132 for the last 3h of the experiments.

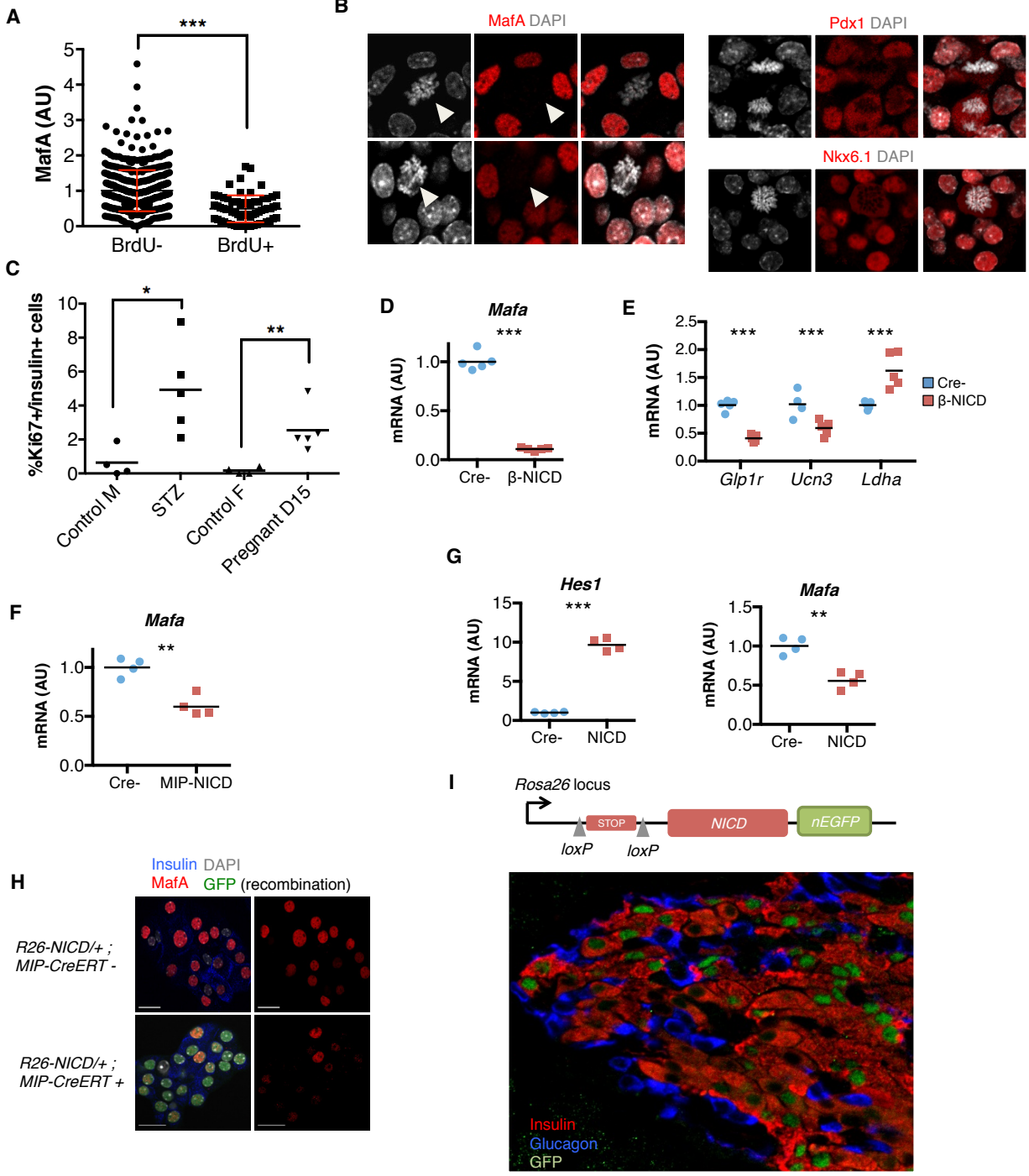
(H) Immunofluorescence images from MIN6-rTTA3 cells after 24h of N1ΔE expression, with or without 5 μM CHIR99021 or 10 mM LiCl. (N=4 independent experiments).

(I) Representative immunofluorescence images of MIN6-rTTA3 cells co-transfected with NICD-HA and MafA-WT-myc or MafA-S65A-myc, from 2 independent experiments, ~500 cells counted per experiment.

(J) Gene expression in MIN6 cells transduced with shRNA targeting *Kat2b* (2 different sequences: #1 and #2), or scrambled shRNA (Scr) control.

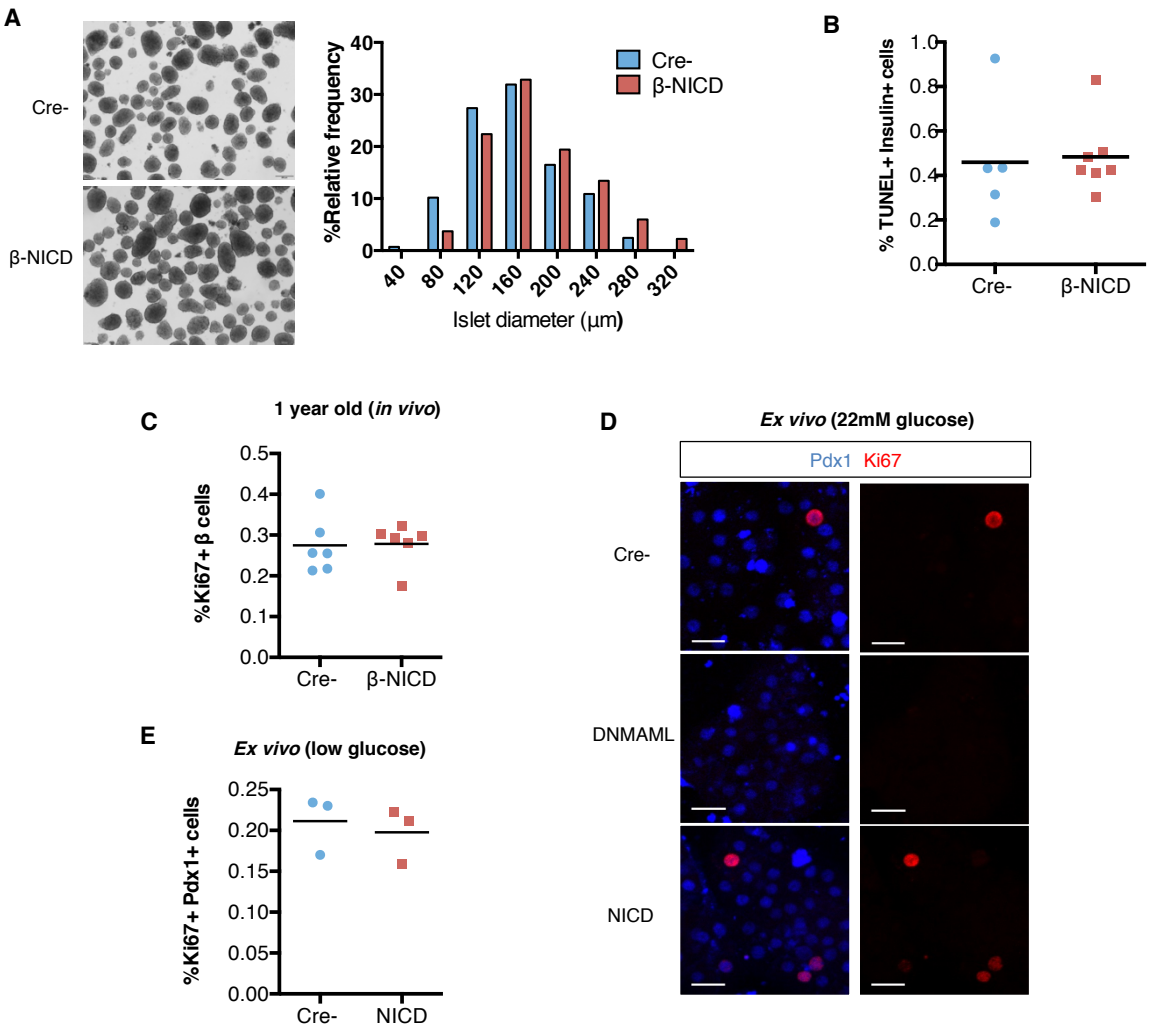
Scale bars: 20 μm. All data are shown with group means; ***, $P < 0.001$ by two-tailed *t* test.

Supplemental Figure 6

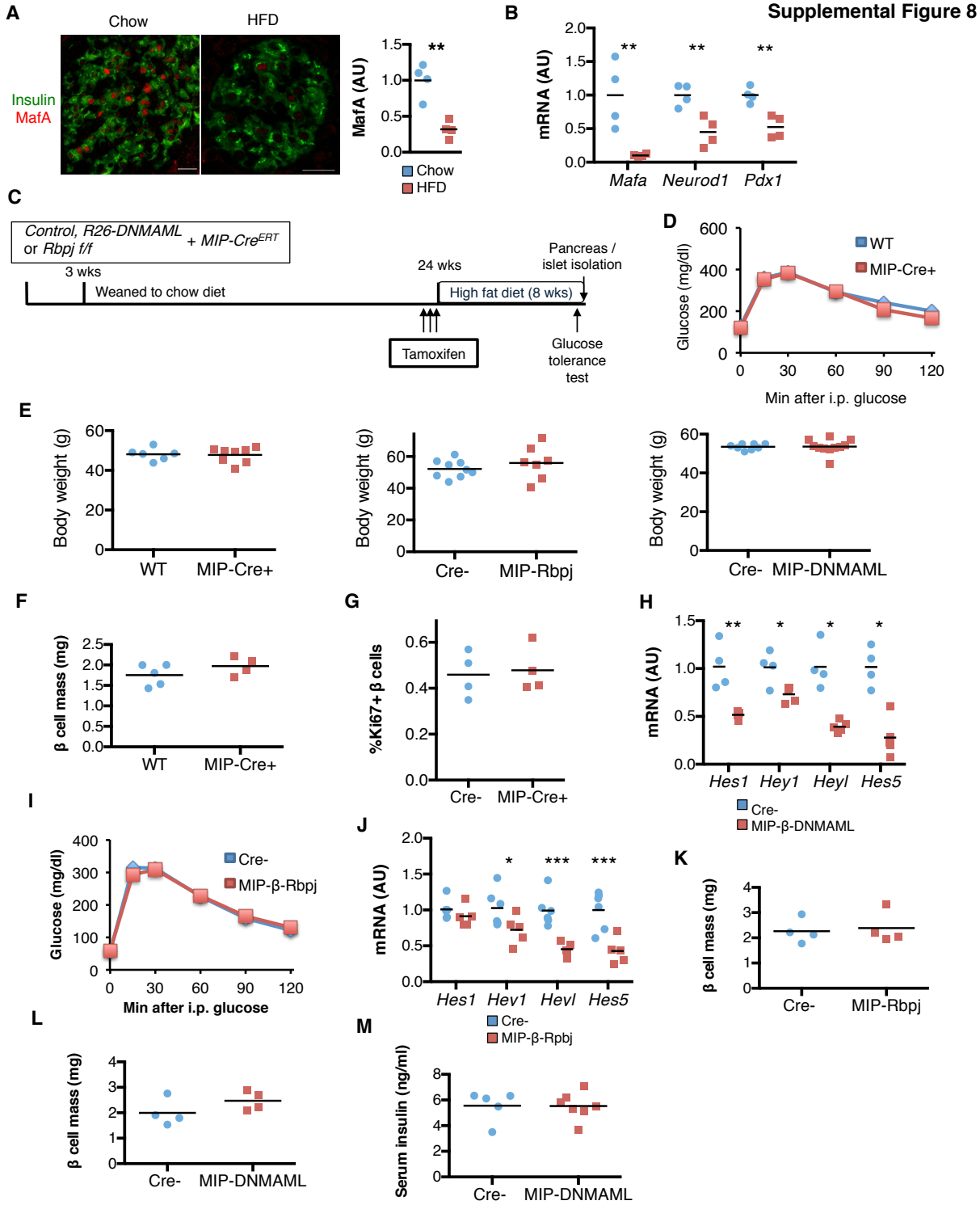


Supplemental Figure 6. Chronic Notch activation leads to loss of *Mafa*, but not β cell dedifferentiation or transdifferentiation.
(A) Quantitation of fluorescence intensity of MafA staining per cell in pancreatic sections from WT D15 pregnant mice, normalized to average fluorescence in BrdU- cells. Overall count of 472 BrdU- cells, 75 BrdU+ cells ($P < 0.0001$ by Mann-Whitney test for non-parametric data).
(B) Representative images from MIN6 cells, stained with antibodies directed against MafA, Pdx1 or Nkx6.1, showing chromatin condensation (white arrows).
(C) Quantitation of Ki67+ β cells in pancreatic sections from vehicle- or STZ-treated TNR male (M) mice, and virgin or D15 pregnant female (F) mice.
(D) *Mafa* expression in islets isolated from β-NICD and Cre- control mice (N=5 mice/group).
(E) Gene expression in islets isolated from β-NICD and Cre- control mice (N=5 mice/group).
(F) *Mafa* expression in islets isolated from MIP-β-NICD and Cre- mice, 8 weeks after tamoxifen treatment (N=4 mice/group).
(G) Gene expression in islets isolated from tamoxifen-naïve control and MIP-β-NICD mice, cultured with 1 μM 4-OHT for 21 days (N=6 mice/group).
(H) Representative images from islets isolated from tamoxifen-untreated control or MIP-β-NICD mice, cultured with 1 μM 4-OHT for 21 days (N=6 mice/group).
(I) In *R26-NICD* transgenic mice, transcription is blocked by an STOP cassette which is flanked by *loxP* sequences. Cre-mediated deletion allows expression of NICD and nuclear EGFP. As shown in representative image of 1 year old β-NICD mice, no GFP+ nuclei are observed in insulin negative cells. Scale bars: 20 μm. All data are shown with group means; *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$ by two-tailed *t* test.

Supplemental Figure 7



Supplemental Figure 7. Notch activation increases β cell proliferative capacity.
(A) Isolated islets from adult β-NICD and Cre- visualized by light microscopy, with quantified distribution of islet diameter (pooled islets from 4 mice/group).
(B) Quantitation of TUNEL+ β cells in pancreatic sections from adult β-NICD and Cre- mice (N=5-7 mice/group).
(C) Quantitation of Ki67+ β cells in pancreatic sections from 1-year-old β-NICD and Cre- mice (N=5-6 mice/group).
(D) Representative images of dispersed islet cells from control (Cre-), DNMAML (*MIP-Cre^{ERT}+; R26-DNMAML/+*), or NICD (*MIP-Cre^{ERT}+; R26-NICD/+*) mice, grown in full medium containing 22 mM glucose and 1 μM 4-OHT for 4 days (N=5-7 mice/group).
(E) Quantitation of Pdx1+ Ki67+ of dispersed islet cells grown in 5.5mM glucose (N=3 mice/group).
Scale bars: 20 μm. All data are shown with group means; *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$ by two-tailed *t* test.



Supplemental Figure 8. MIP-Cre^{ERT} transgenic mice show unchanged glucose tolerance or β cell mass if HFD-feeding is delayed until 24-weeks.
(A) Representative images of pancreatic sections from WT mice fed chow or HFD for 6 months, with quantitation of MafA fluorescence intensity (N=4 mice/group).
(B) Gene expression of islets isolated from WT mice fed chow or HFD for 6 months (N=4 mice/group).
(C) Diagram that illustrates the experimental protocol used with MIP-Cre^{ERT} lines.
(D) GTT in MIP-Cre^{ERT} or WT mice treated with tamoxifen at 24 weeks, then challenged with 8 weeks of HFD feeding (N=5-7 mice/group).
(E) Body weight at sacrifice of WT, MIP-Cre, MIP-*DNMAML*, MIP-*Rbpj* and Cre- controls in the experiments described in Supplemental Figure 8C.
(F) β cell mass in WT or MIP-Cre^{ERT} mice treated with tamoxifen at 24 weeks, then challenged with 8 weeks of HFD feeding (N=4 mice/group).
(G) Ki67+ β cells in pancreatic sections from WT or MIP-Cre^{ERT} mice fed chow or HFD for 6 months (N=4 mice/group).
(H) Gene expression in islets isolated from HFD-fed MIP-β-DNMAML and Cre- control mice (N=4-6 mice/group)
(I) GTT in chow-fed MIP-β-Rbpj and Cre- mice at 16 weeks of age, 8 weeks after tamoxifen (N=10 mice/group).
(J) Gene expression in islets isolated from HFD-fed MIP-β-Rbpj and Cre- control mice (N=5-6 mice/group).
(K) β cell mass in HFD-fed MIP-β-Rbpj and Cre- mice treated with tamoxifen at 24 weeks, then challenged with 8 weeks of HFD feeding (N=4 mice/group)
(L) β cell mass in HFD-fed MIP-β-DNMAML and Cre- mice treated with tamoxifen at 24 weeks, then challenged with 8 weeks of HFD feeding (N=4 mice/group)
(M) Serum insulin in 5h fasted, HFD-fed MIP-β-DNMAML and Cre- mice treated with tamoxifen at 24 weeks, then challenged with 8 weeks of HFD feeding (N=5-8 mice/group)
All data are shown with group means ± s.e.m.; *, $P < 0.05$, **, $P < 0.01$ by two-tailed *t* test.

Supplemental Table 1 - Antibodies

Antibody	Company	Catalogue number
Rabbit anti-Somatostatin	Dako	A0566
Guinea pig anti-Insulin	Dako	A056401-2
Rabbit anti Phospho-GSK-3 (Ser9)	Cell Signaling Technology	9336
Rabbit anti GSK-3beta	Cell Signaling Technology	9315
Rabbit anti-PCAF (Kat2b)	Cell Signaling Technology	3378S
Rabbit anti-RBPJ	Cell Signaling Technology	5442S
Rabbit anti-HA tag	Cell Signaling Technology	2367
Rabbit anti-Pdx1	Cell Signaling Technology	5679
Rabbit anti-Notch1 (to overexpressed NICD)	Cell Signaling Technology	4380
Rabbit anti-Slc2a2 (Glut2)	Millipore-Sigma	07-1402
Mouse anti-FLAG	Millipore-Sigma	F3165
Rabbit anti-MAFB (anti Hs)	Millipore-Sigma	HPA005653
Rabbit anti-NeuroD1	Millipore-Sigma	ABE991
Rabbit anti-MafB (anti Ms)	Bethyl	IHC-00351
Mouse anti-Nkx6.1	DSHB	F55A12
Rabbit anti-Ki67	Abcam	ab16667
Rat anti-BrdU	Abcam	ab6326
Rabbit anti-MafA (Ms/Hs)	Abcam	ab26405
Goat anti-GFP	Abcam	ab6673
Rabbit Anti-Cytokeratin 19	Abcam	ab15463
Mouse anti-MYC (9E10)	Santa Cruz Biotechnology	sc-40
Rabbit anti-HES1	Santa Cruz Biotechnology	sc-25392
Rat anti-Endomucin	Santa Cruz Biotechnology	sc-65495
Mouse anti-HA	Roche	11666606001

Supplemental Table 2 - Oligonucleotides

Gene	Forward (5'-3')	Reverse (5'-3')
Notch1	CACAACGTGGCACGTTCCAGCA	TGAAGCCTGTGTCTGCAGCGGA
Notch2	TCAACGGAGGCACCTGTCGTCA	TGTGGTTGGGGCAGTCGTCGAT
Notch3	CGTTCCCCCATTGAGACACCGA	TGCCCCTTCCCCGTGTCGTAAT
Notch4	GGACCCCGGGCTTCACATTCAT	TCCCAGAGGGACACTCCACCTTTCA
Jag1	TGCACCCGCGACGAGTGTGATA	ACCTCGGCCAGGCGAAACTGAA
Jag2	TTGCAAAAACCTGATTGGCGGC	TCGCAATGGCGACCTCCAAA
Dll1	TCTCCCGATGACCTCGCAACAGA	TCATCCCGAGGTCGGCAGAACA
Dll3	TGTGCCCTTCCGCGATGCTT	CGGGCCCTCCAGCATGCTCT
Dll4	TGGAGAGAGCTGTTCTCGCCTATGCA	CCAACCTGGACGGCAGATGCAC
Ppia	CAGACGCCACTGTCGCTTT	TGTCTTTGGAACTTTGTCTGCAA
Hes1	AGAGGCTGCCAAGGTTTTTG	TCCCACTGTTGCTGGTGTAGA
Hes5	TGGTGGAGAAGATGCGTCGG	GCGAAGGCTTTGCTGTGTTT
Hey1	ACGAGAATGGAAACTTGAGTTC	AACTCCGATAGTCCATAGCAAG
Heyl	TCTGAATTGCGACGATTGGTCCCC	CCAGGGCTCGGGCATCAAAGAA
Pcsk1	TTCTTCCACATTGGCTACCTCC	GCAGGTCAGCGCTTGTTATTC
Pcsk2	TCTGACTGTGCTCACCTCCAA	AGGACTCCGTAGCCAAAGAGG
Slc30a8	TCATGGAAGGTGTTCCAAAGGG	CACGGAGATCACGCCATCAA
Unc3	TGGGCATCAGCATCGCT	GCTGTGCCCCTCGACCT
Glp1r	CGCTGCTGTTCGTTATCCCA	AGTTGACGCCGATAGCAAAGAG
Ldha	GACTGTACTTGACAATGTTGGGA	TGTCTCCAGCAAAGACTACTGT
Mafa	GAGGAGGTCATCCGACTGAAA	GCACTTCTCGCTCTCCAGAAT
Pdx1	GAAATCCACCAAAGCTCACG	CGGGTTCCGCTGTGTAAG
Neurod1	GCCCAGCTTAATGCCATCTTT	CAAAAGGGCTGCCTTCTGTAA
Ins2	TTTGTCAAGCAGCACCTTTG	GGTCTGAAGGTCACCTGCTC
Gcg	CTCACAGGGCACATTCACCA	GGCAATGTTGTTCCGGTTCCT
Kat2b	AGATGGCCGTGTCATTGGTG	GTGGGTTCCATAGCCCTTGAC

Genotyping primers

Targeting	**Forward (5'-3')**	**Reverse (5'-3')**
Cre	GCGGTCTGGCAGTAAAAACTATC	GTGAAACAGCATTGCTGTCACTT
R26-NICD wt	CCAAAGTCGCTCTGAGTTGTTATC	GAGCGGGAGAAATGGATATG
R26-NICD mut	GAAAGACCGCGAAGAGTTTG	AAAGTCGCTCTGAGTTGTTAT
R26-DNMAML wt	AAAGTCGCTCTGAGTTGTTAT	GCGAAGAGTTTGTCCTCAACC
R26-DNMAML mut	AAAGTCGCTCTGAGTTGTTAT	AGCGGGAGAAATGGATATGAA
Rbpj wt	GCATCTGTTTTGTCGTCTTCC	CATCAGTTAGATTGGTCCTGC
Rbpj flox	ATCCGGGGGTACCGCGTCGAG	CATCAGTTAGATTGGTCCTGC

Sequences cloned in pLKO.1-TRC cloning vector (*AgeI + EcoRI*)

Targeting:

Mafa	5' CCGG GAGGAGGTCATCCGACTGAAA CTGCAG TTTCAGTCGGATGACCTCCTC TTTTTG 3', 5' AATTCAAAAA GAGGAGGTCATCCGACTGAAA CTGCAG TTTCAGTCGGATGACCTCCTC 3'
Kat2b (#1)	5' CCGG AGTGGTATCTAGACTATTAAT CTGCAG ATTAATAGTCTAGATACCACT TTTTTG 3', 5' AATTCAAAAA AGTGGTATCTAGACTATTAAT CTGCAG ATTAATAGTCTAGATACCACT 3'
Kat2b (#2)	5' CCGG ATGGAACATGAGAGTTTATTT CTGCAG AAATAAACTCTCATGTTCCAT TTTTTG 3', 5' AATTCAAAAA ATGGAACATGAGAGTTTATTT CTGCAG AAATAAACTCTCATGTTCCAT 3'
Scrambled	5'-CCGGCCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGGTTTTTG-3', 5'-AATTCAAAAACCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG- 3'

Supplemental Table 3 – List of vectors used in this study

Plasmid	Insert	Used for:	Origin
pTRE-Tight-HA-NICD	HA-NICD	Transient transfection of MIN6-rTTA3	Kei Sakamoto, Tokyo Medical and Dental University (1)
pLenti CMV rtTA3 Blast	rTTA3	Lentivirus production; generation of stable rTTA3 line	Addgene #26429 (Eric Campeau)
pMD2.G	lentiviral packaging	Lentivirus production	Addgene #12259 (Didier Trono)
psPAX2	lentiviral envelope	Lentivirus production	Addgene #12260 (Didier Trono)
pLKO.1-TRC cloning vector	-----	Cloning, lentiviral production	Addgene #10879 (David Root) (2)
pLKO.1-puro-scrambled	Scrambled shRNA	Lentivirus production	Original
pLKO.1-puro-Mafa	Mafa shRNA	Lentivirus production	Original
pLKO.1-puro-Kat2b#1	Kat2b shRNA	Lentivirus production	Original
pLKO.1-puro-Kat2b#2	Kat2b shRNA	Lentivirus production	Original
pCS2 Notch1 ΔEMV-6MT	Notch1 ΔE	Cloning Notch1 ΔE into pLVX-Tight-Puro	Addgene #41737 (Raphael Kopan, Jeffrey Nye) (3)
pLVX-Tight-Puro	-----	Cloning, lentivirus production	Clonetech
pLVX-Tight-Puro-Luc	Luciferase	Lentivirus production, generation of MIN6-rTTA3 Luciferase Tet-On cells	Clonetech
pLVX-Tight-Puro-N1ΔE	Notch1 ΔE	Lentivirus production, generation of MIN6-rTTA3 N1ΔE-myc Tet-On cells	Original
pCCL-MNDU3-DNMAML-GFP	DnMAML1-GFP	Lentivirus production, generation of MIN6-DNMAML1-GFP cells	Randy Gascoigne, British Columbia Cancer Research Centre (4)
pCCL-MNDU3-GFP	GFP	Lentivirus production, generation of MIN6-GFP cells	Randy Gascoigne, British Columbia Cancer Research Centre (4)
EF.hICN1.Ubc.GFP	NICD-IRES-GFP	Transient transfection	Addgene #17626 (Linzhao Cheng) (5)
pCI flag PCAF	Kat2b-Flag	Transient transfection	Addgene #8941 (Yoshihiro Nakatani) (6)
pCMV-Mafa-myc-WT	Mafa-myc-WT	Transient transfection	Roland Stein, Vanderbilt University School of Medicine (7)

pCMV-Mafa-myc-S65A	Mafa-myc-S65A	Transient transfection	Roland Stein, Vanderbilt University School of Medicine (7)

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