

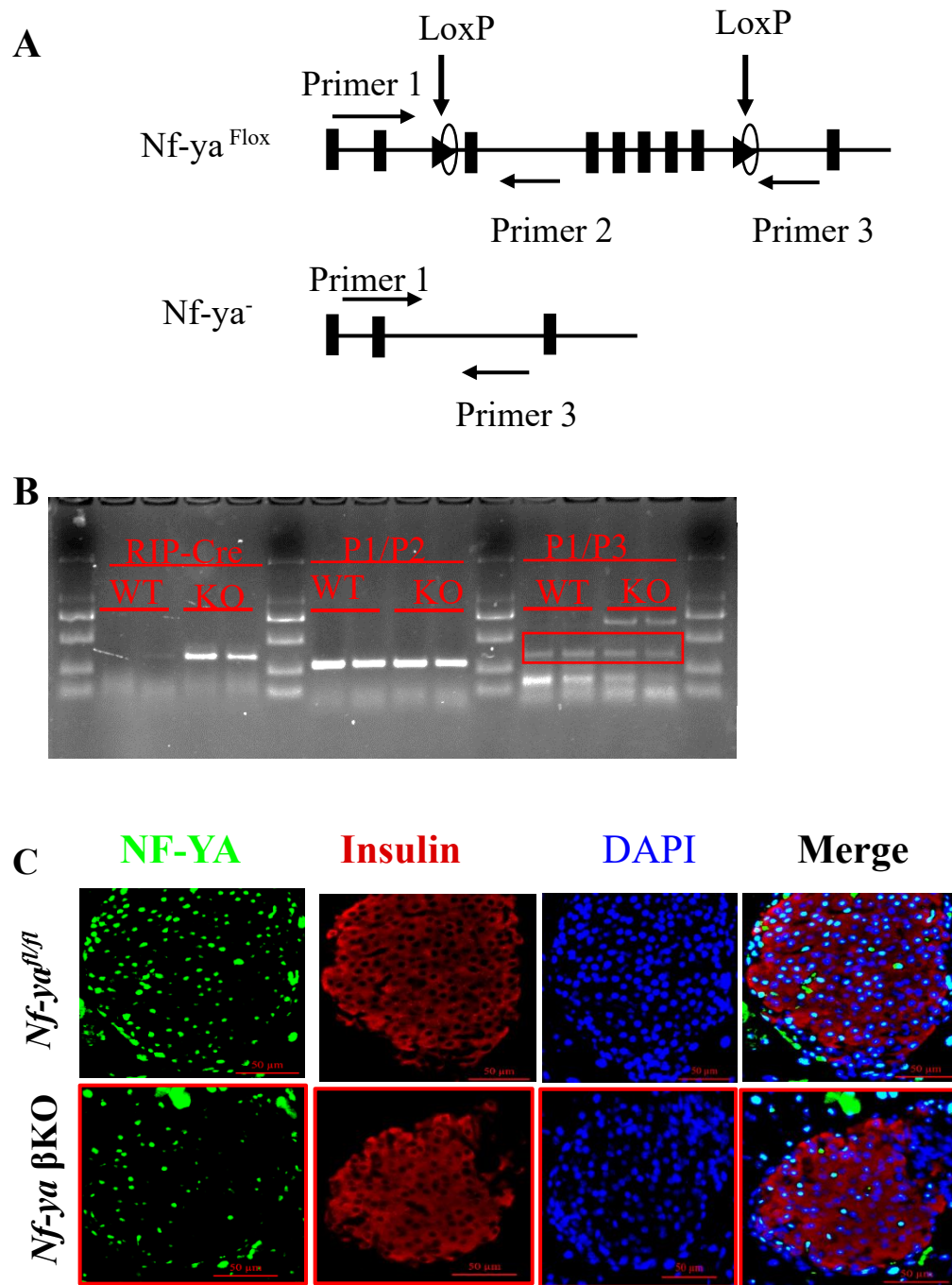
**Nuclear factor Y in mouse pancreatic  $\beta$ -cells plays a crucial role in glucose homeostasis by  
regulating  $\beta$ -cell mass and insulin secretion**

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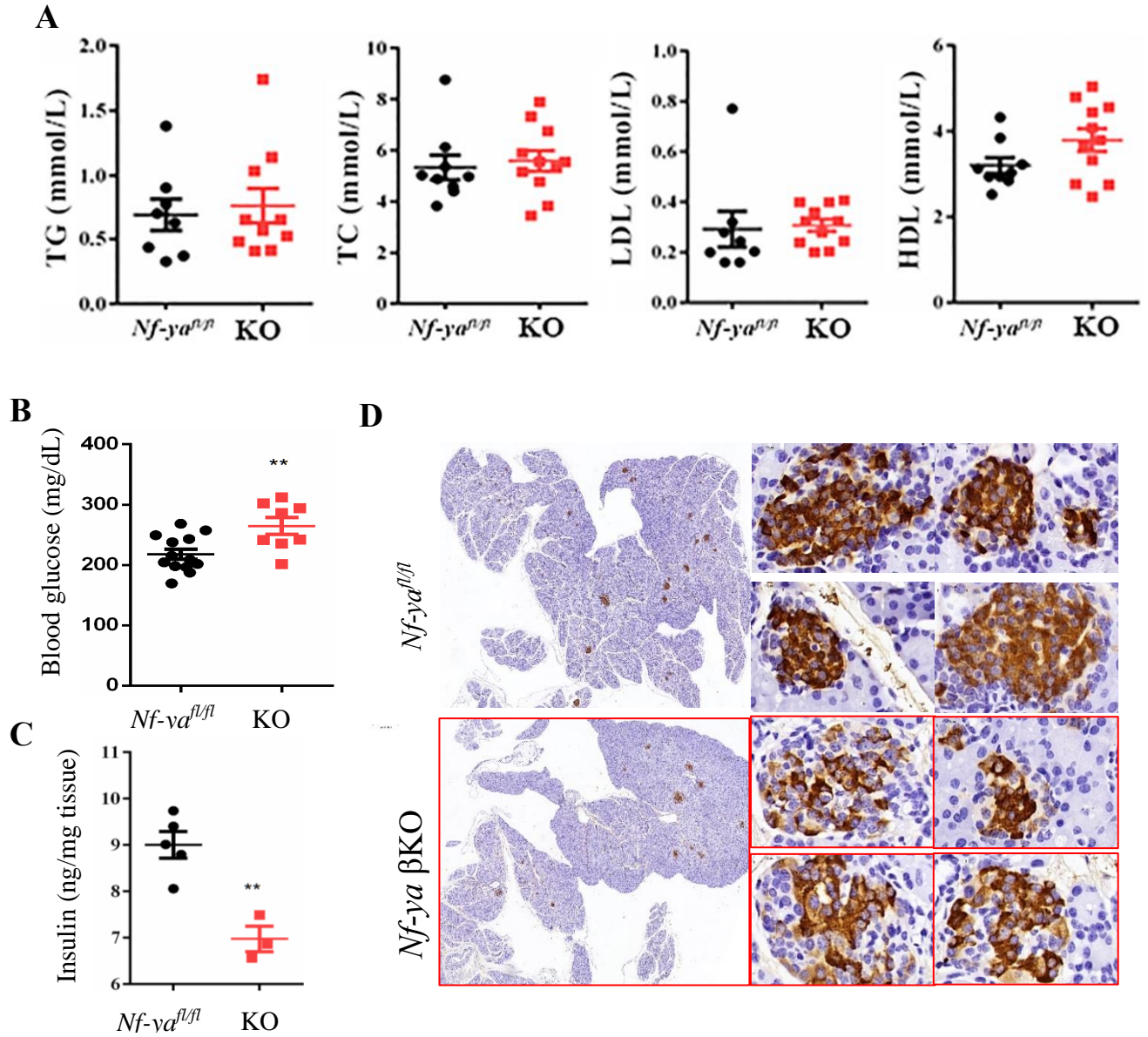
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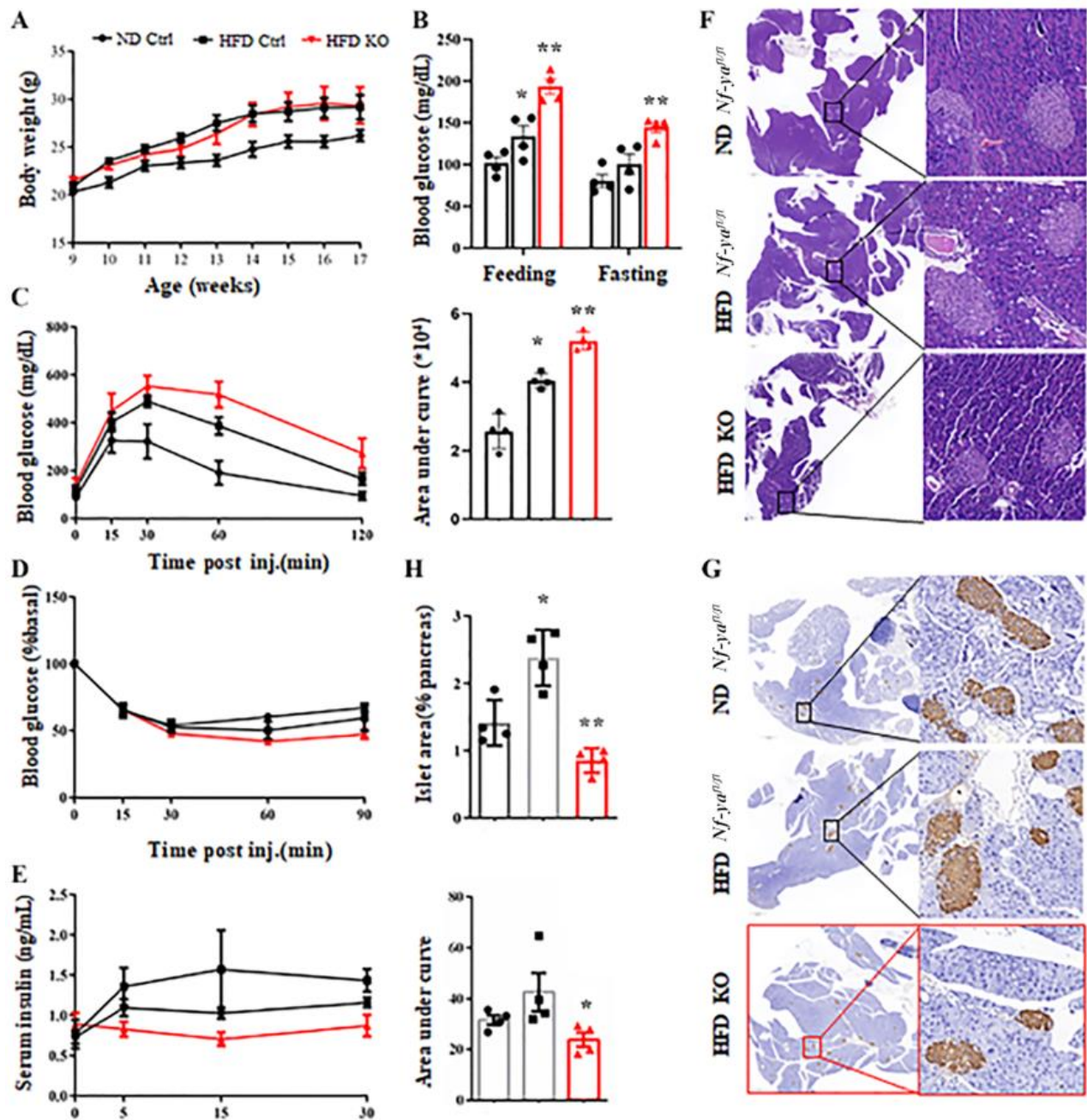
**Supplementary data consist of Supplementary Figures S1-7 and Tables S1-6.**



**Figure S1. Generation of  $\beta$  cell-specific *Nf-ya* KO (*Nf-ya*  $\beta$ KO) mice.** *Nf-ya*  $\beta$ KO mice were generated by crossing *Nf-ya* flox/flox mice with albumin-Cre transgenic mice, Cre-negative *Nf-ya* flox/flox animals were used as control. (A) Schematic diagram of the *Nf-ya* gene showing exons 3-8 flanked by two loxP sites indicated as triangles and the subsequent excision of exons 3-8 by Cre-mediated gene recombination. Vertical thick bars show relative locations of exons. (B) The genomic DNA extracted from the tails was used for PCR with primers detecting Cre, *Nf-ya* flox, *Nf-ya* WT or the deleted *Nf-ya*. (C)  $\beta$ -cells specific deletion of *Nf-ya* was confirmed using the immunofluorescent staining for NF-YA. Scale bars, 50 $\mu$ m.



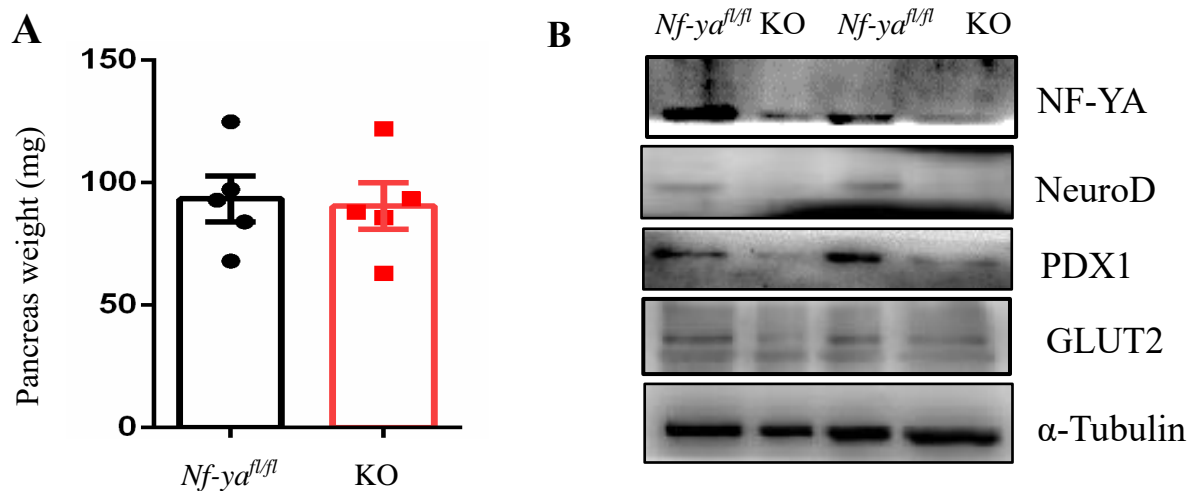
**Figure S2. Effects of  $\beta$ -cell-specific *Nf-ya* deletion on islets insulin content and plasma levels of glucose and lipids.** (A) Plasma lipids levels in 12-week-old male mice fed a normal chow diet (n=7-11 mice/group). (B-D) Plasma levels of glucose (B), pancreatic insulin contents (C), and immunohistochemical staining for insulin in pancreatic islets (D) in 2-week old mice (n = 12-15 mice per group). The data are means  $\pm$  SD.



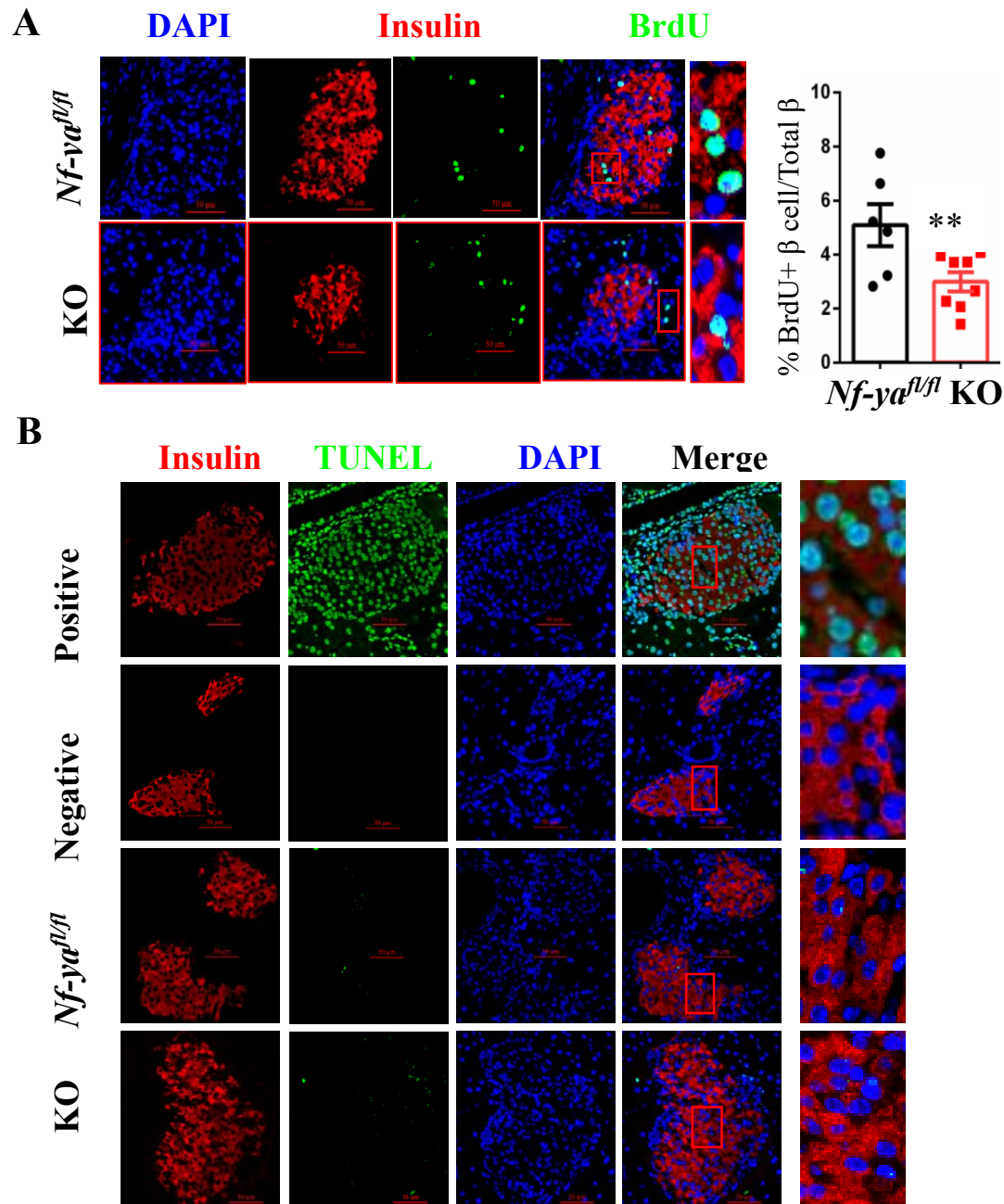
**Figure S3. *Nf-ya*  $\beta$ KO mice exhibited accelerated impaired glucose tolerance and diminished islets compensation to HFD.** Male mice of 8 weeks old were fed a normal chow diet (ND) or high fat diet (HFD) for 9 weeks, as marked. (A) Mice body weight was monitored over time (n = 4-5 mice per group). (B) Blood levels of glucose were examined in mice under either feeding states or 6-h fasting conditions (n = 4 for each group). (C) Plasma glucose concentrations (left) were measured at the designated time points during GTT in 12-h fasted mice given intraperitoneally glucose (2 g/kg body weight), and the areas under the curve (AUC) of GTT were calculated (right) (n = 4 for each group). (D) Plasma glucose concentrations were monitored at the designated time



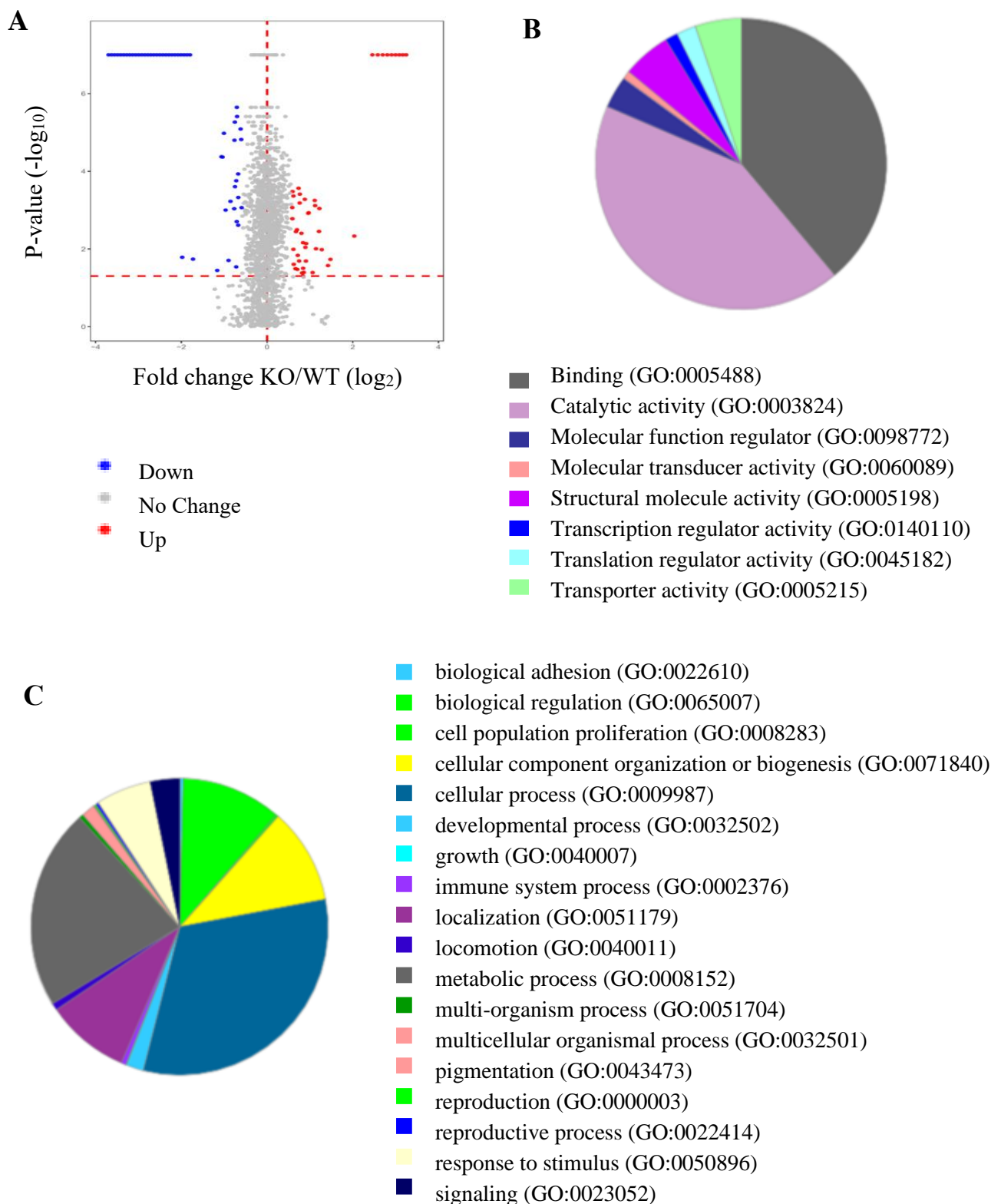
points during ITT in 6-h fasted mice with the intraperitoneal injection of insulin (1 U/kg body weight) (n = 4 mice per group). (E) Plasma insulin concentrations (left) were measured at indicated time points during glucose-stimulated insulin secretion (GSIS) in response to administration of 3 g glucose/kg body weight in 12-h fasted mice, and AUC calculated from GSIS data were compared (right) (n = 4 mice per group). (F) Example H&E-stained pancreatic islets from ND-*Nf-ya*<sup>fl/fl</sup>, HFD-*Nf-ya*<sup>fl/fl</sup> and *Nf-ya* βKO HFD mice. The right-side panels (scale bars = 50 μm) show high-magnification images of the boxed areas in the left-side panels (scale bars = 500μm). (G) Example immunohistochemical staining for insulin (brown color) in pancreatic islets (H) Quantitative assessment of the islet area relative to the total pancreas. Each dot represents an individual mouse (n=4 mice per group). \**P* < 0.05, \*\**P* < 0.01 vs. ND- *Nf-ya*<sup>fl/fl</sup>.



**Figure S4. Effects of *Nf-y* deletion in β-cells on pancreas weight (A) and the amount of islets proteins (B).** Pancreas tissues and islets were isolated from 12-week old mice fed a normal chow diet (n = 4-5 mice per group). The data are means ± SD.

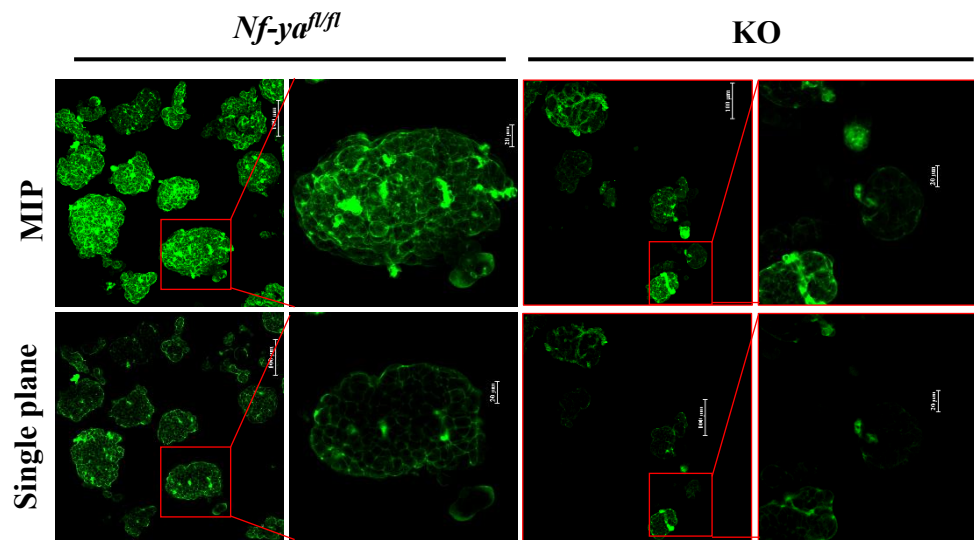


**Figure S5. Impacts of *Nf-y* deletion on the proliferation and apoptosis of pancreatic  $\beta$ -cells.** (A) BrdU labeling of pancreatic  $\beta$ -cells from 12-week old mice. Pancreatic sections stained for anti-insulin are shown in red, for anti-BrdU are shown in green, and for DAPI are shown in blue (left chart). Proliferation of  $\beta$ -cells was quantified as the number of nuclei from both BrdU and insulin positive cells divided by the number of nuclei from only insulin positive cells, multiplied by 100 (right chart). (B)  $\beta$ -cell apoptosis was measured by double staining of TUNEL and insulin and represented by percentage of double positives for TUNEL/insulin. TUNEL staining of pancreatic  $\beta$ -cells from 12-week old mice. Original magnification, X200.



**Figure S6. Proteomics measures.** Islets proteomics was conducted using high-resolution mass spectrometry coupled with nano-flow liquid chromatography. Samples were quantified via Q Exactive Plus LCMS. (A) Among 1530 quantified proteins identified, 54 were down-regulated (blue)

and 46 were up-regulated red) in *Nf-ya*  $\beta$ KO mice as compared with the control mice. (B-C) The functional roles (B) and biologic processes (C) of the differentially abundant proteins were grouped by Gene Ontology (GO) terms.



**Figure S7.** Imaging of F-actin detected by staining with Alexa Fluor 488-conjugated phalloidin in *Nf-ya*  $\beta$ KO and control pancreatic islets. Representative maximum intensity projection (MIP) of consecutive optical sections and single optical section (Single plane) of pancreatic islets (scale bars = 50  $\mu$ m).



**Table S1. Primers for mice genotyping**

Gene	Forward (5' to 3')	Reverse (5' to 3')
Cre	ATTTGCCTGCATTACCGGTC	ATCAACGTTTTCTTTTCGG
Nf-ya	GTAAGTCAGGCTCCAGGG	GGGTTGTCAGGATGTTTCGCAG AGGCAAGGCAGATTTAGGAAGGTC

**Table S2. Antibodies for immunostaining and protein blotting**

Antibody	company	Cat.NO
Anti-NF-YA	Santa Cruz	sc-17753
Anti-NF-YB	Zen-Bioscience	507295
Anti-NF-YC	Zen-Bioscience	509832
Anti-Insulin	HuaBio	EM80714
Anti-Glucagon	HuaBio	ET1702-20
Anti-Ki67	Abcame	ab15580
Anti-Brdu	Abcame	(ab6326)
Anti-PCNA	HuaBio	ET1605-38
Anti-CyclinD1	HuaBio	ET1601-31
Anti-CyclinB1	HuaBio	ET1608-27
Anti-NeuroD	CST	#2833
Anti-PDX1	CST	#5679
Anti-cl-caspase3	CST	#9661
Anti-syntaxin3	HuaBio	ET7109-19
Anti-Glut2	Santa cruz	sc-518022
Anti-Ucp2	ServiceBio	GB11377
Anti-pAKTs473	CST	#4060
Anti- $\alpha$ -tubulin	HuaBio	M1501-1

**Table S3. Primers for amplifying Glut2 upstream regions and Nf-y cDNAs**

Gene	Forward (5' to 3')	Reverse (5' to 3')
Glut2-P1	<b>GAGCTC</b> GACACGTAAGCAGAATTC	<b>AAGCTT</b> TGTGTGTGTGTGGAATTGTC CT
Glut2-P2	<b>GAGCTC</b> CATCTGGCTCCGCACTCTC	
Glut2-P3	<b>GAGCTC</b> GGTAAAGGGTGTATTGATT	
Glut2-	AGTCTCCA <b>g</b> TGCACAGCCTCCCCAG	GGCTGTGCA <b>c</b> TGGAGACTCACTGGAT
Glut2-	AAGCCACAAGTCA <b>c</b> TGGGGTAAAGG	TTACCCCA <b>g</b> TGACTTGTGGCTTGAGC
Glut2-	TAAAGGGTGTATTGA <b>c</b> TGGATTACC	TTGATGGTAATCCA <b>g</b> TCAATACACCC
Nf-ya	<b>GAATTC</b> GAGCAGTATACGACAAAC	<b>TCTAGA</b> TTAGGAAACTCGGATGATC
Nf-yb	<b>GAATTC</b> GACAATGGACGGCGACAG	<b>GGATCC</b> TCATGAAAAGTGAATTTGC
Nf-yc	<b>GAATTC</b> GTCCACAGAAGGAGGGTTT	<b>TCTAGA</b> TCAGTCTCCAGTCACCTGGG

**Table S4. Primers used for real-time quantitative PCR analysis**

Gene	Forward (5' to 3')	Reverse (5' to 3')
<i>Cyclin D1</i>	ATGGAAGGACCCTTGAGGC	CTTCACGGCTTGCTCGTTCT
<i>Cyclin B1</i>	AAGGTGCCTGTGTGTGAACC	GTCAGCCCCATCATCTGCG
<i>Cyclin D2</i>	GAGTGGGAACCTGGTAGTGTTG	CGCACAGAGCGATGAAGGT
<i>Slc2a2</i>	TCAGAAGACAAGATCACCGGA	GCTGGTGTGACTGTAAGTGGG
<i>Ucp2</i>	ATGGTTGGTTTCAAGGCCACA	CGGTATCCAGAGGGAAAGTGAT
<i>Ppargc1a</i>	TATGGAGTGACATAGAGTGTGC	CCACTTCAATCCACCCAGAAAG
<i>Nf-ya</i>	GTCCAGACCCTCCAGGTAGT	AGGCACCAACTGTATCTGCT
<i>Ins1</i>	CACTTCCTACCCCTGCTGG	ACCACAAAGATGCTGTTTGACA
<i>Ins2</i>	GCTTCTTCTACACACCCATGTC	AGCACTGATCTACAATGCCAC
<i>Mafa</i>	AGGAGGAGGTCATCCGACTG	CTTCTCGCTCTCCAGAATGTG
<i>Pdx1</i>	CCCCAGTTTACAAGCTCGCT	CTCGGTTCCATTCGGGAAAGG
<i>Neurod1</i>	AGAAGAAGAGGAGGAGGAGG	TGACAGAGCCCAGATGTAG
<i>Nd4</i>	ACCATAGCCTTCTCACTATCAC	TGGAGTTGGAGTTTAGGGAAGT
<i>Nd6</i>	GGTTAGCATTAAGCCTTCACC	CATCAACCAATCTCCCAAACCA
<i>as9</i>	GTCCGCTTTTCGGGTTGTTAGA	CCTCCTTTCCCGTGAGGTA
<i>Cytb</i>	GCCACCTTGACCCGATTCT	TTGCTAGGGCCGCGATAAT
<i>Cr1</i>	AGACCCAGGTCAGCATCTTG	GCCGATTCTTTGTTCCCTTGA
<i>Cytc</i>	CCAAATCTCCACGGTCTGTTC	ATCAGGGTATCCTCTCCCCAG
<i>Cox 1</i>	TCGGAGCCCCAGATATAGCA	TTCCGGCTAGAGGTGGGTA
<i>Cox2</i>	CATGAGCAGTCCCCTCCCTA	TAACCCTGGTCGGTTTGATGT
<i>Cox3</i>	AAGGCCACCACACTCCTATT	ATCATGTGTTGGTACGAGGCT
<i>Cox4</i>	ATTGGCAAGAGAGCCATTTCTA	CACGCCGATCAGCGTAAGT
<i>Cox5b</i>	TTCAAGGTTACTTCGCGGAGT	CGGGACTAGATTAGGGTCTTCC
<i>Atpase6</i>	AGGATTCCCAATCGTTGTAGCC	CCTTTTGGTGTGTGGATTAGCA
<i>Atp5a1</i>	TCTCCATGCCTCTAACACTCG	CCAGGTCAACAGACGTGTCAG
<i>Atp5b</i>	GGTTCATCCTGCCAGAGACTA	AATCCCTCATCGAACTGGACG
<i>Atp5c1</i>	CCAGGAGACTGAAGTCCATCA	AGAACCTGTCCCATACTACTCG
<i>18s rRNA</i>	CGCCGCTAGAGGTGCAATTC	CCAGTCGGCATCGTTTATGG
mtDNA		
<i>16S rRNA</i>	CCGCAAGGGAAAGATGAAAGA	TCGTTTGGTTTCGGGGTTTC
Nuclear DNA		
<i>Nhk2</i>	GCCAGCCTCTCCTGATTTTAGTG	GGGAACACAAAAGACCTCTTCTG

**Table S5. Downregulated proteins in *Nf-ya*  $\beta$ KO islets as compared with *Nf-ya*<sup>fl/fl</sup> islets**

Gene	p.adjust	Fold Change	Gene	p.adjust	Fold Change
Cul4b	1.00E-07	0.07	Prdx4	1.00E-07	0.261
Prdm16	1.00E-07	0.074	Eftud2	1.00E-07	0.274
Ddi2	1.00E-07	0.077	RO60	1.00E-07	0.288
Kiaa1324	1.00E-07	0.081	Uba2	2.25E-06	0.614
Ttn	1.00E-07	0.085	Eif3g	3.87E-06	0.616
Ttc38	1.00E-07	0.089	Clic1	5.40E-06	0.595
Cad	1.00E-07	0.094	Bub3	8.14E-06	0.653
Arxes2	1.00E-07	0.099	Pmm2	1.05E-05	0.499
Safb	1.00E-07	0.103	Snx1	1.52E-05	0.659
Pdia2	1.00E-07	0.109	Vars	1.58E-05	0.591
Gls	1.00E-07	0.114	Psma5	4.22E-05	0.479
Gcn1	1.00E-07	0.119	Psma7	4.29E-05	0.487
Numa1	1.00E-07	0.126	Aifm1	0.000117	0.628
Atp2b1	1.00E-07	0.132	Atp6v0a1	0.000175	0.608
Capn2	1.00E-07	0.139	Ddx39b	0.000249	0.596
Sec22b	1.00E-07	0.146	Ap1m2	0.000472	0.629
Dpysl2	1.00E-07	0.153	Mtmr9	0.000594	0.554
Gtpbp1	1.00E-07	0.160	Arpc1b	0.000862	0.664
Alyref	1.00E-07	0.169	Uso1	0.00092	0.589
Clta	1.00E-07	0.177	Suc1g2	0.000997	0.512
Stxbp1	1.00E-07	0.186	Atp6v1c1	0.001972	0.614
Metap2	1.00E-07	0.195	Eif2s3x	0.002443	0.628
Prdx6	1.00E-07	0.205	Suc1g2	0.01637	0.254
Dld	1.00E-07	0.2150	Slc25a20	0.018286	0.3023
Hsd17b10	1.00E-07	0.2258	Letm1	0.019725	0.5372
Dctn1	1.00E-07	0.2370	Nfs1	0.028925	0.6081
Prkcsh	1.00E-07	0.2489	Hnrnpf	0.035721	0.4474

**Table S6. Upregulated proteins in *Nf-ya*  $\beta$ KO islets as compared with *Nf-ya*<sup>fl/fl</sup> islets**

Gene	p.adjust	Fold Change	Gene	p.adjust	Fold Change
Psmb1	1.00E-07	5.5	Hnrnph1	0.003538	2.318
Man2b1	1.00E-07	6	Wdr1	0.003581	1.598
Rpl21	1.00E-07	6.5	Eif3d	0.003952	1.746
Scarb2	1.00E-07	7	Kpna3	0.004657	4.106
Phb2	1.00E-07	7.5	Dhx9	0.00691	1.793
Psm4	1.00E-07	8	Pgrmc1	0.007256	1.882
Dhx15	1.00E-07	8.5	Copb2	0.009088	1.861
Purb	1.00E-07	9	Api5	0.009886	2.209
Srsf5	1.00E-07	9.5	Ddc	0.010246	1.533
Stx7	0.000273	1.667	C1qbp	0.010414	2.426
Cops4	0.000331	1.512	Gnaz	0.014614	1.646
Eef1b	0.000388	1.702	Capn1	0.018553	2.790
Ppt1	0.000433	1.535	Psma3	0.020448	1.683
Nipsnap2	0.000527	1.844	Impa1	0.020508	1.876
Timm44	0.000563	2.165	Dync1i2	0.024874	1.542
Nmt1	0.000654	1.697	Anp32a	0.026774	2.685
Bcat2	0.000765	2.1787	Atp2a2	0.031422	1.794
Hnrnpa2b1	0.000858	1.505	Papss2	0.032181	1.585
Nudc	0.000908	2.335	Hsd17b12	0.033549	1.633
Ap3d1	0.001169	1.963	Septin7	0.040192	1.8138
Asna1	0.001206	1.949	Zw10	0.040679	2.092
Cops6	0.001663	1.508	Dpm1	0.041563	1.764
Smpd2	0.003193	1.625	Psmb5	0.049924	1.793