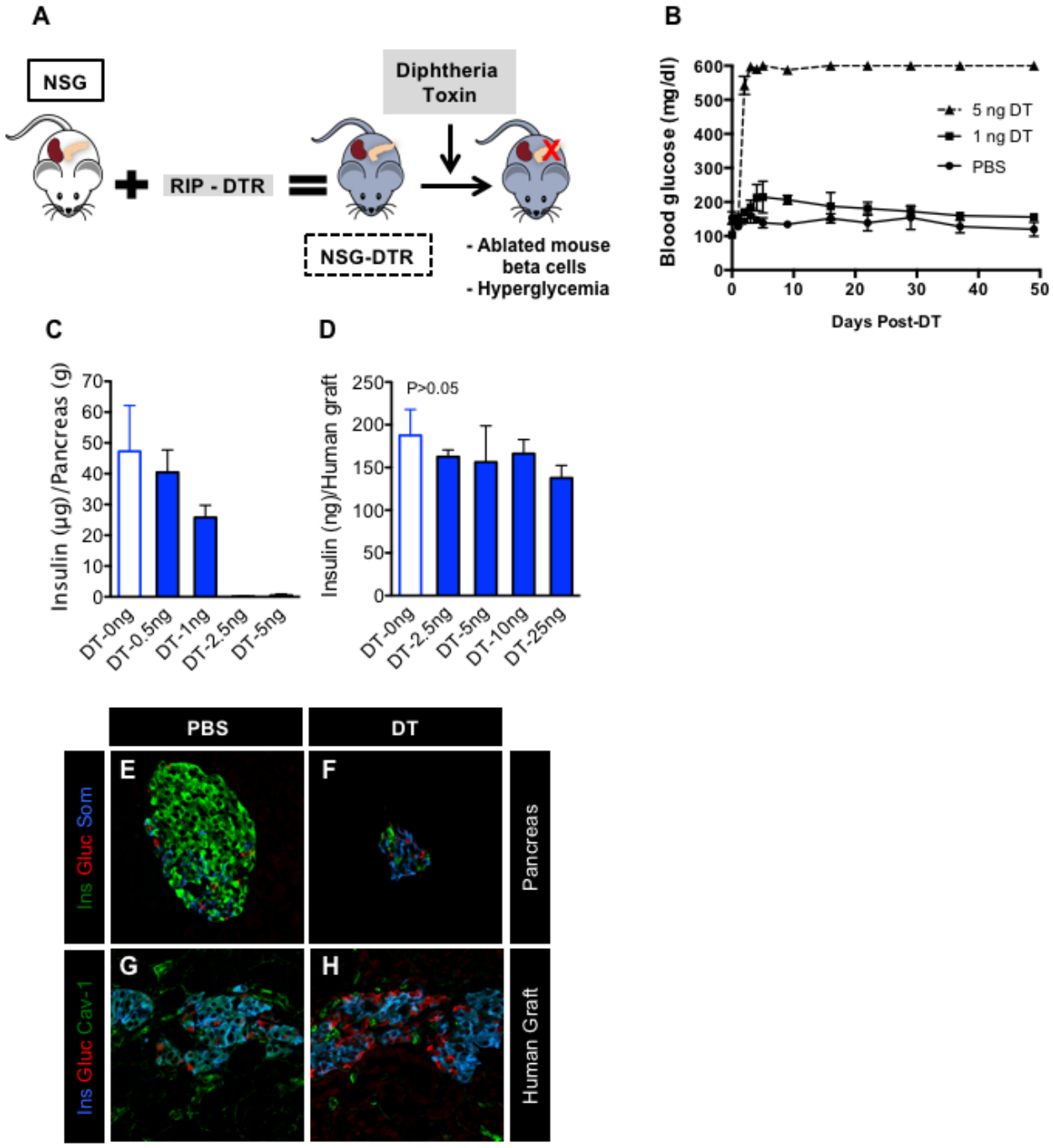


Figure S1. Establishment of chronic hyperglycemia model (NSG-DTR). (A) Breeding of NSG mouse with RIP- DTR transgenic mouse yields NSG- DTR mouse. Injection of diphtheria toxin ablates mouse β cells and results in hyperglycemia. **(B)** Blood glucose values of NSG-DTR mice in response to a single injection of 5ng DT, 1ng DT, or PBS. **(C)** Pancreatic insulin content of NSG-DTR mice (no human islets) injected with different DT doses (n= 3). **(D)** Human graft insulin content after injection with different DT doses (n=3). Representative images of NSG-DTR pancreata or human grafts after treatment with PBS **(E, G)** or DT **(F, H)**. (E-F) Green = insulin, red = glucagon, blue = somatostatin. (G, H) Blue = insulin, red = glucagon, green = caveolin-1.

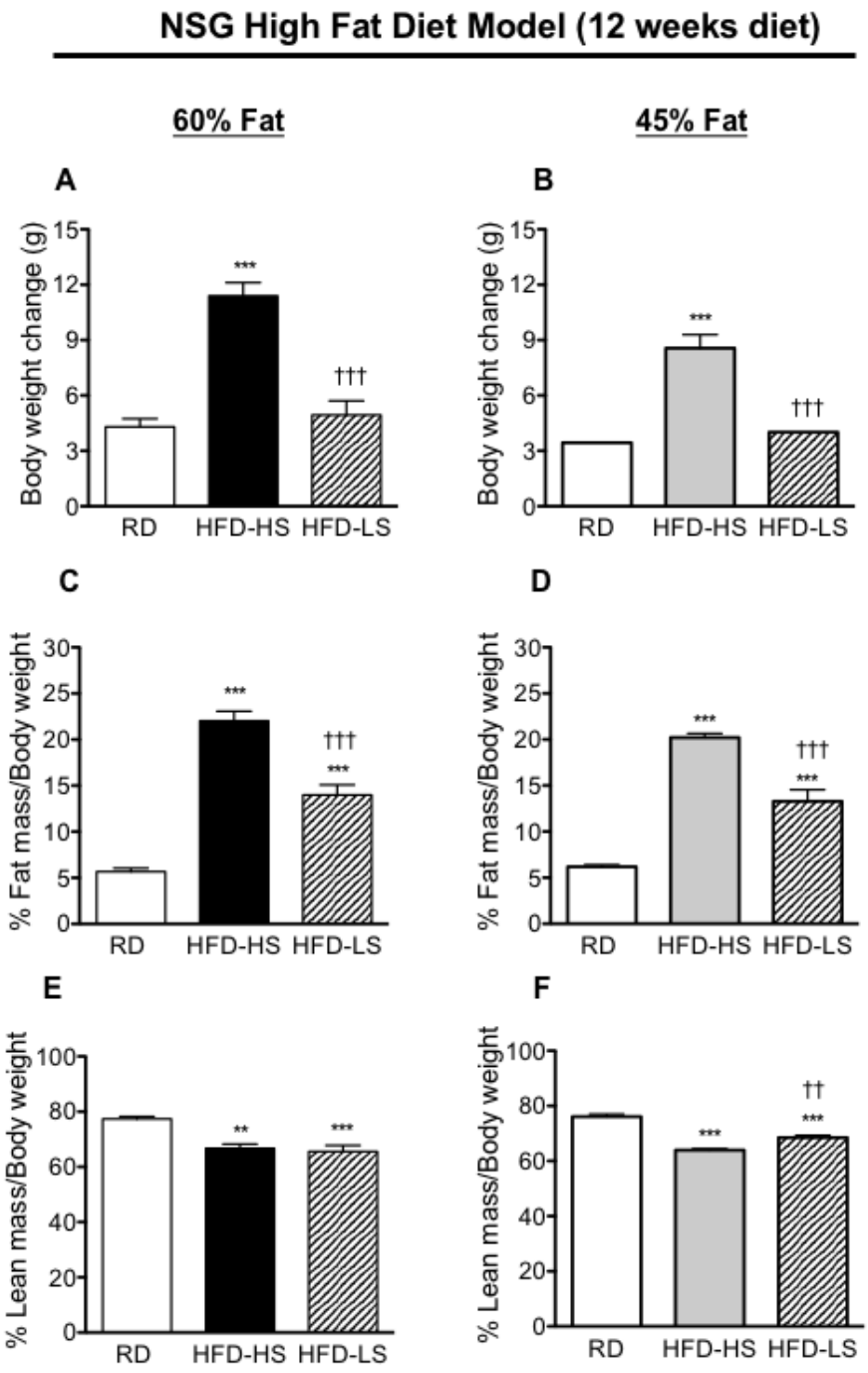


Figure S2. **Feeding with either 60% or 45% of high fat diet (HFD) for 12 weeks induces obesity in NSG mice, with some mice more sensitive (high sensitivity-HS) than others (low sensitivity – LS).** (**A, B**) Mouse body weight change is higher on HFD (**A**. RD, n=8; 60% HFD-HS, n=14, 60% HFD-LS, n=6; **B**. RD, n=10, 45% HFD-HS, n=10; 45% HFD- LS, n=5). (**C-F**) Fat mass and lean mass (**C, E**. RD, n=10; 60% HFD-HS, n=13, 60% HFD- LS, n=5; **D, F**. RD, n=10, 45% HFD-HS, n=10; 45% HFD-LS, n=5). ** p<0.01, *** p<0.001, HFD vs RD; †† P<0.01, ††† P<0.001, HFD-HS vs HFD-LS. 1-way ANOVA followed by Newman-Keuls Multiple Comparison test was used for statistical significant analysis.

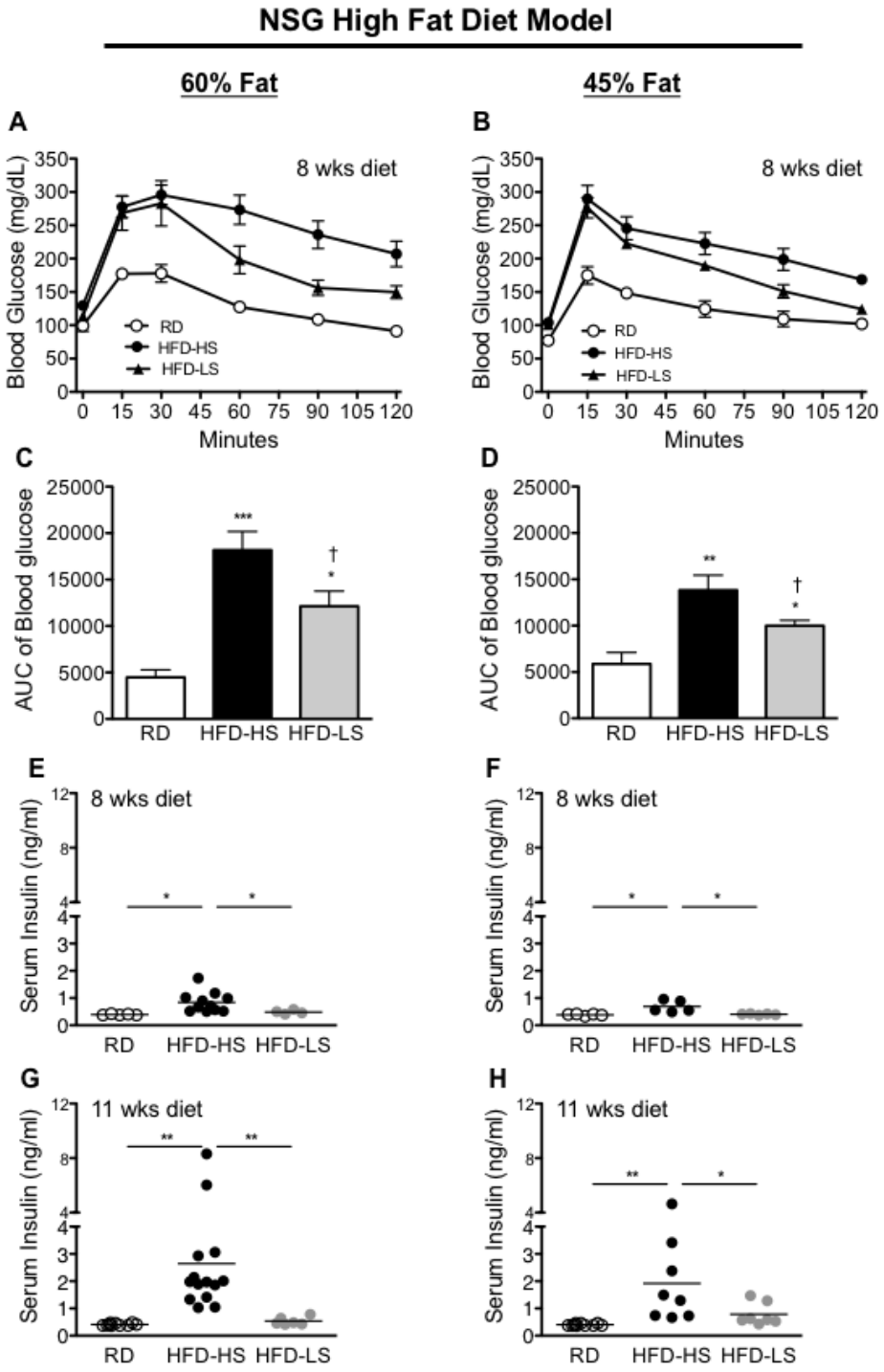


Figure S3. HFD induces insulin resistance in NSG mice. (**A, B**) Eight weeks of 60% or 45% HFD impaired GTT in the NSG mice. However, the mice with 60% HFD had more severely impaired glucose clearance. (RD, n=10; 60% HFD-HS, n=15; 60% HFD-LS, n=5; 45% HFD- HS, n=10; 45% HFD=LS, n=10) (**C, D**) Blood glucose area under curve of GTT ** p<0.01, *** p<0.001, HFD vs RD; † P<0.05, HFD-HS vs HFD-LS. **(E-H)** Mouse serum insulin increases in response to 8 weeks **(E, F)** and 11 weeks **(G, H)** 60% (E, G) or 45% (F, H) fat diet. * p<0.05, ** p<0.01. No significant difference showed between RD-LS and HFD-LS. 1-way ANOVA followed by Newman-Keuls Multiple Comparison test was used for statistical significant analysis.

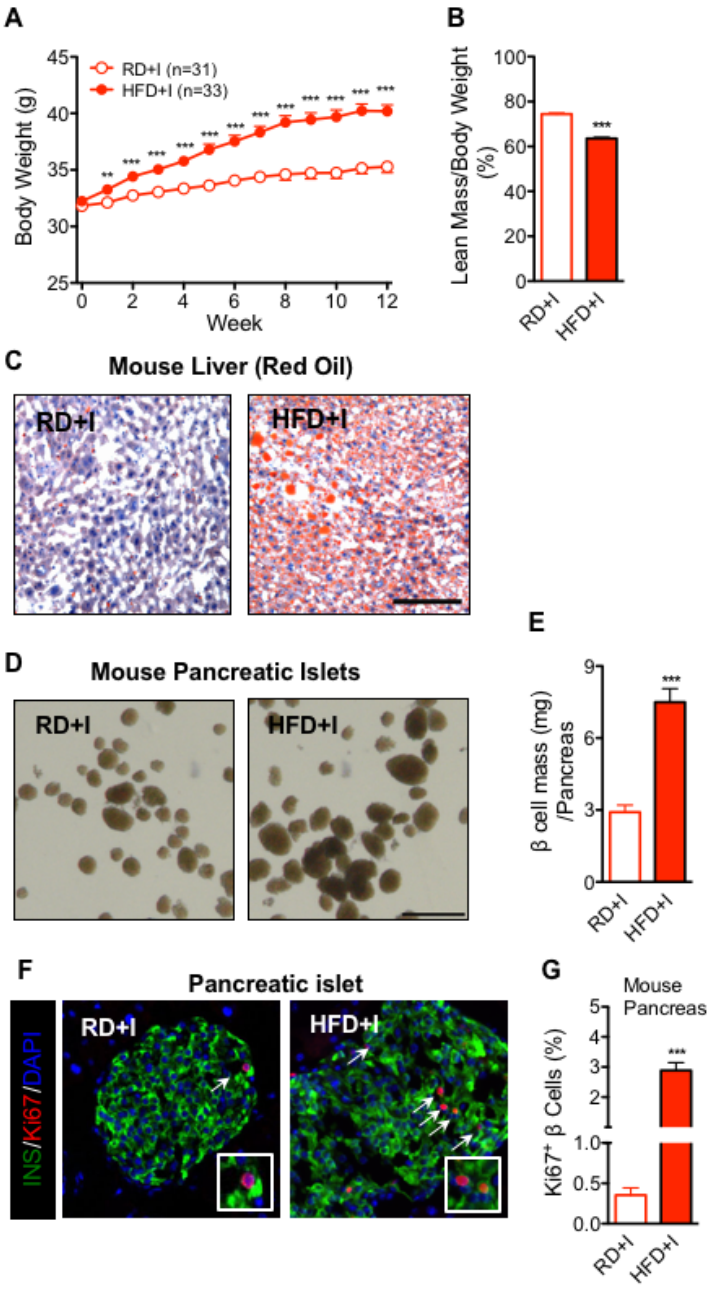


Figure S4. Greater β cell proliferation and larger mouse islet size in response to HFD. (A) Body weight in NSG mice with transplanted human islets from day 1 to 12 weeks on RD and HFD. *** p<0.001 **(B)** Lean mass in RD+I and HFD+I. *** p<0.001 (**C**) Dramatically increased lipid deposit in mouse liver after fed with high fat diet for 12 weeks (Oil Red O stain, lipid is red). Scale bar = 250 mm and also applies to RD+I. **(D)** Size of mouse islets in response to 12 weeks of HFD. Islets are from one mouse/each diet. Scale bar = 500 mm and also applies to RD+I. **(E)** β cell mass of mouse pancreas (n=4/diet group). *** p<0.001. (**F**) Representative mouse pancreatic islet images. White arrows point to proliferating Ki67-positive cells. (**G)** Quantification of Ki67-positive β cells in mouse pancreas (n=9/each diet). The number of β cells counted in each group was 7,000 to 16,000. *** p<0.001. Unpaired 2-tailed Student's *t* test was used for statistical significant analysis.

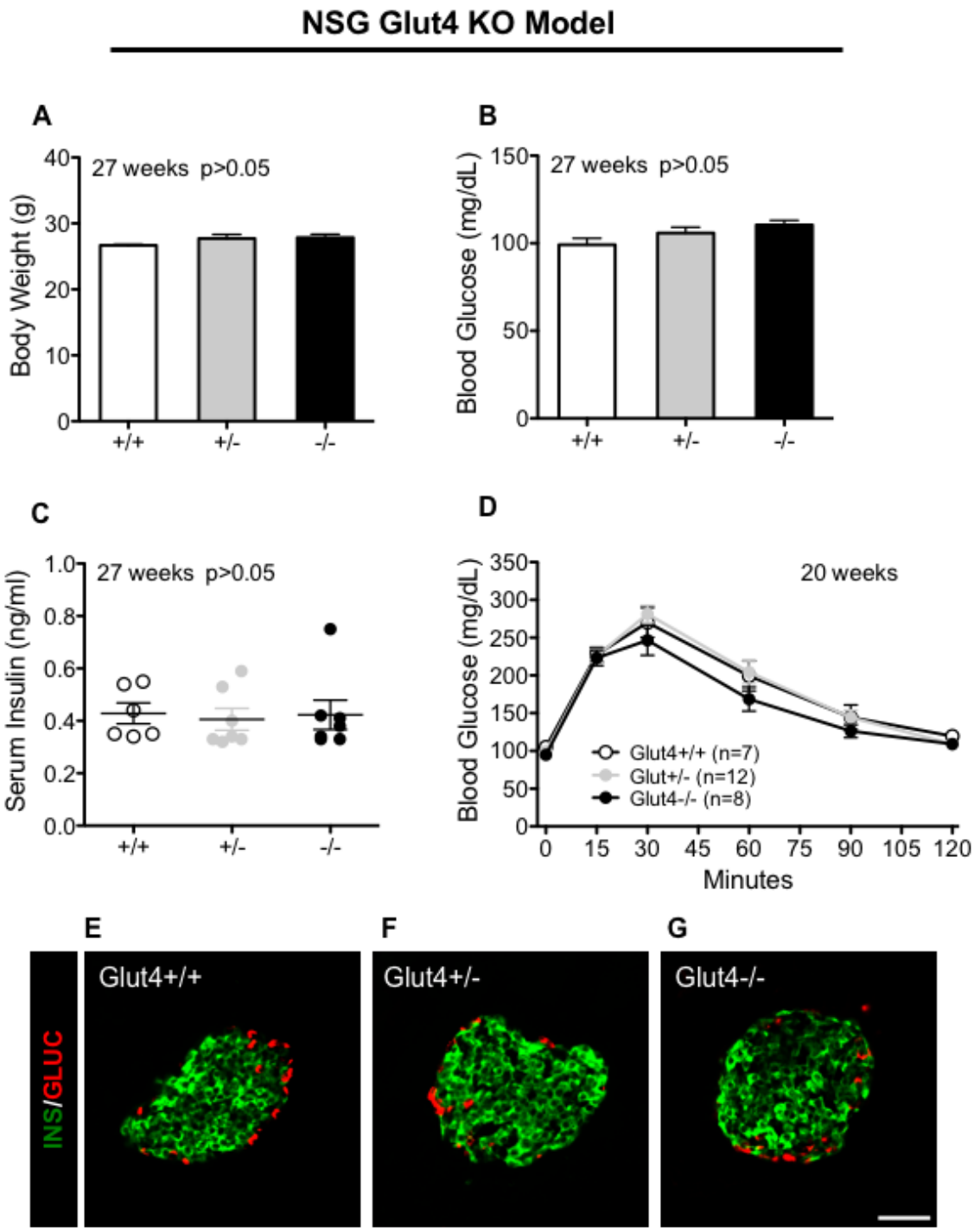


Figure S5. **No phenotype in NSG mice with GLUT4 deficiency**. (**A**) Body weight, (**B**) blood glucose (6 hour fast), (**C**) serum insulin (6 hour fast) in wild type (+/+), heterozygotes (+/-), and homozygotes (-/-) at 27 weeks old. n=6-7/genotype/age. p>0.05 (**D**) GTT at 20 weeks. P>0.05 at all time points. (**E-G**) Islet images of three genotypes labeled with insulin (INS, green), glucagon (GLUC, red). Scale bar = 100 µm and applies to E and F. 1-way ANOVA followed by Newman-Keuls Multiple Comparison test was used for statistical significant analysis.

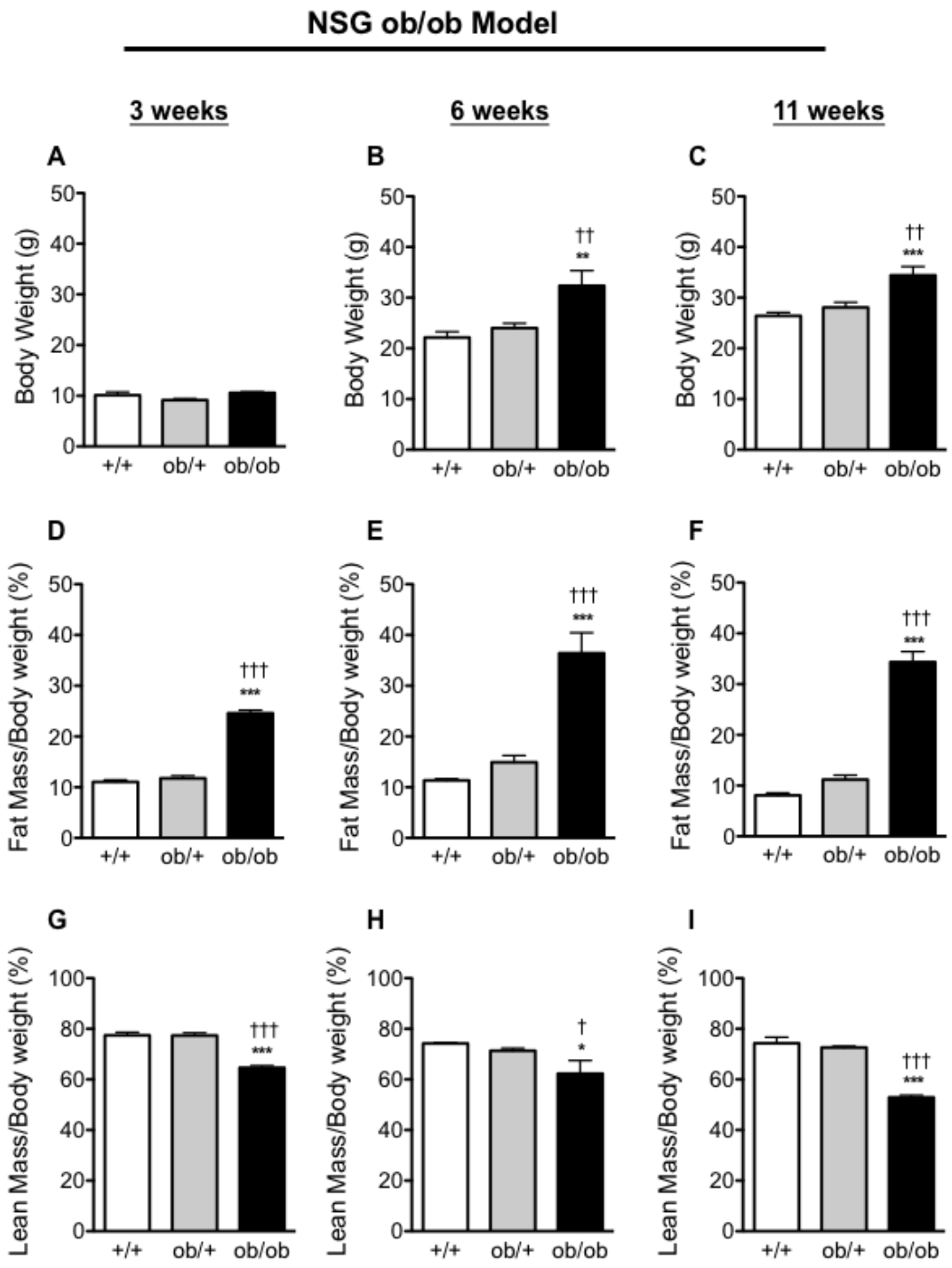


Figure S6. **Leptin deficiency causes obesity in NSG mice.** NSG mice crossed with C57BL/J6 *ob*/+ mice and backcrossed for more than 10 generations before study. (**A-C**) Body weight of wild type (+/+), heterozygotes (*ob*/+), and homozygotes (*ob/ob*) at 3, 6, and 11 weeks old. (**D-F**) Fat mass and (**G-I**) lean mass in three genotyping groups at age of 3, 6, and 11 weeks. (3 weeks: n=6, +/+; n=8 (+/ob); n=4, *ob/ob*; 6 weeks: n=5, +/+; n=12, +/*ob*; n=5, *ob/ob*; 11 weeks: n=15, +/+; n=13, +/*ob*; n=17, ob/ob). * p<0.05, ** p<0.01, *** p<0.001, *ob*/+ and *ob/ob* vs +/+; † p<0.05, †† P<0.01, ††† p<0.001, ob/+ vs *ob/ob*. 1-way ANOVA followed by Newman-Keuls Multiple Comparison test was used for statistical significant analysis.

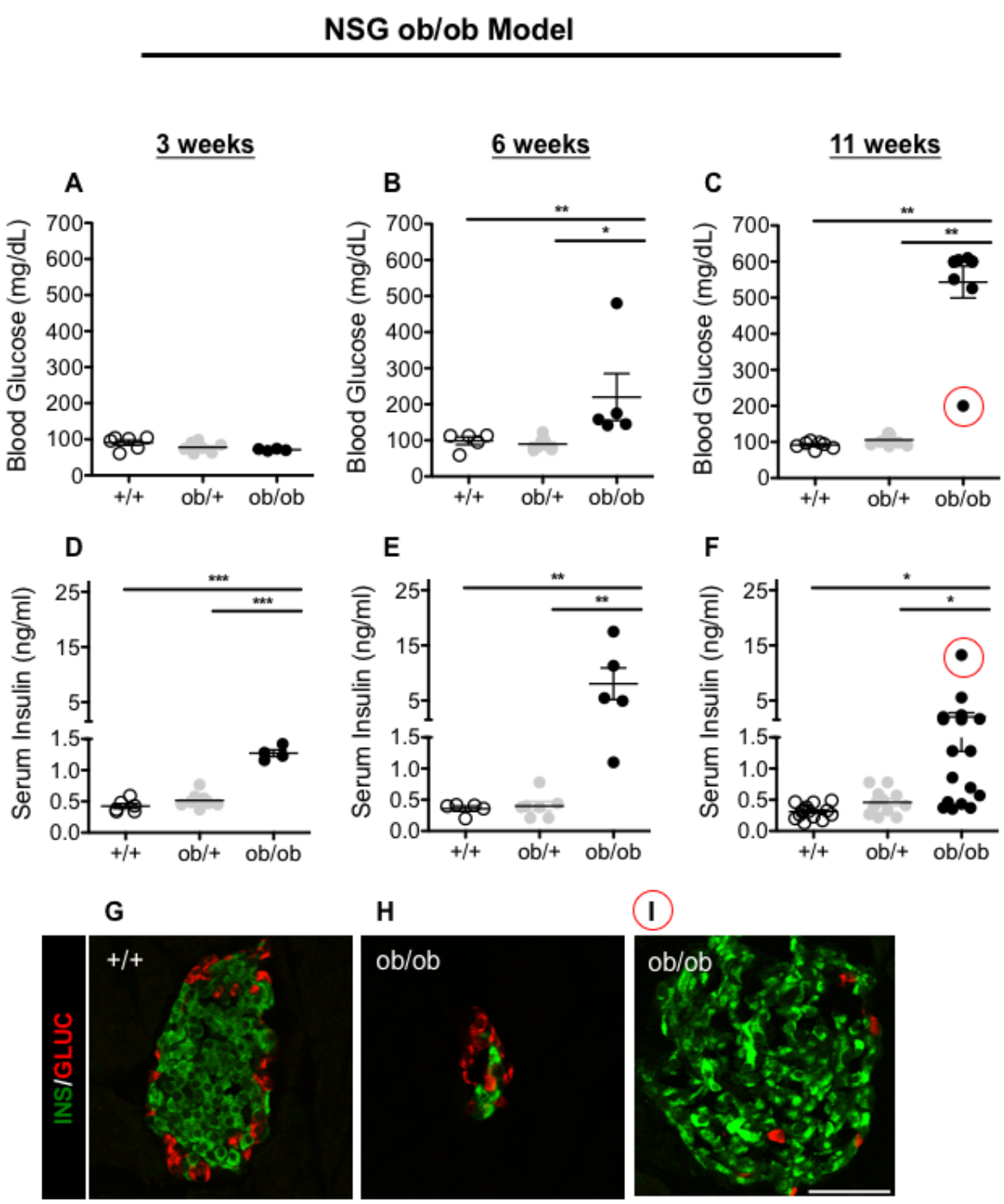


Figure S7. **Diabetes occurs earlier in NSG-ob/ob mice. (A-C)** Blood glucose after fasted for 6 hours. All mice developed to diabetes (glucose > 500mg/dL, except one mouse – 200 mg/ dL). **(D-F)** Serum insulin (fasted) significantly increased at 3 weeks old and was dramatically elevated at 6 weeks. However, at 11 weeks the insulin level declined. **(G-I)** Images of +/+ and *ob/ob* islets labeled with insulin (INS, green), glucagon (GLUC, red). In most mice at 11 weeks, islet size and the number of β cells decreases in ob/ob mice while alpha cell number increases **(H)**. Red circles represent the data and islet image **(I)** from same *ob/ob* mouse that has higher insulin, lower glucose, and larger islets. 1-way ANOVA followed by Newman-Keuls Multiple Comparison test was used for statistical significant analysis.

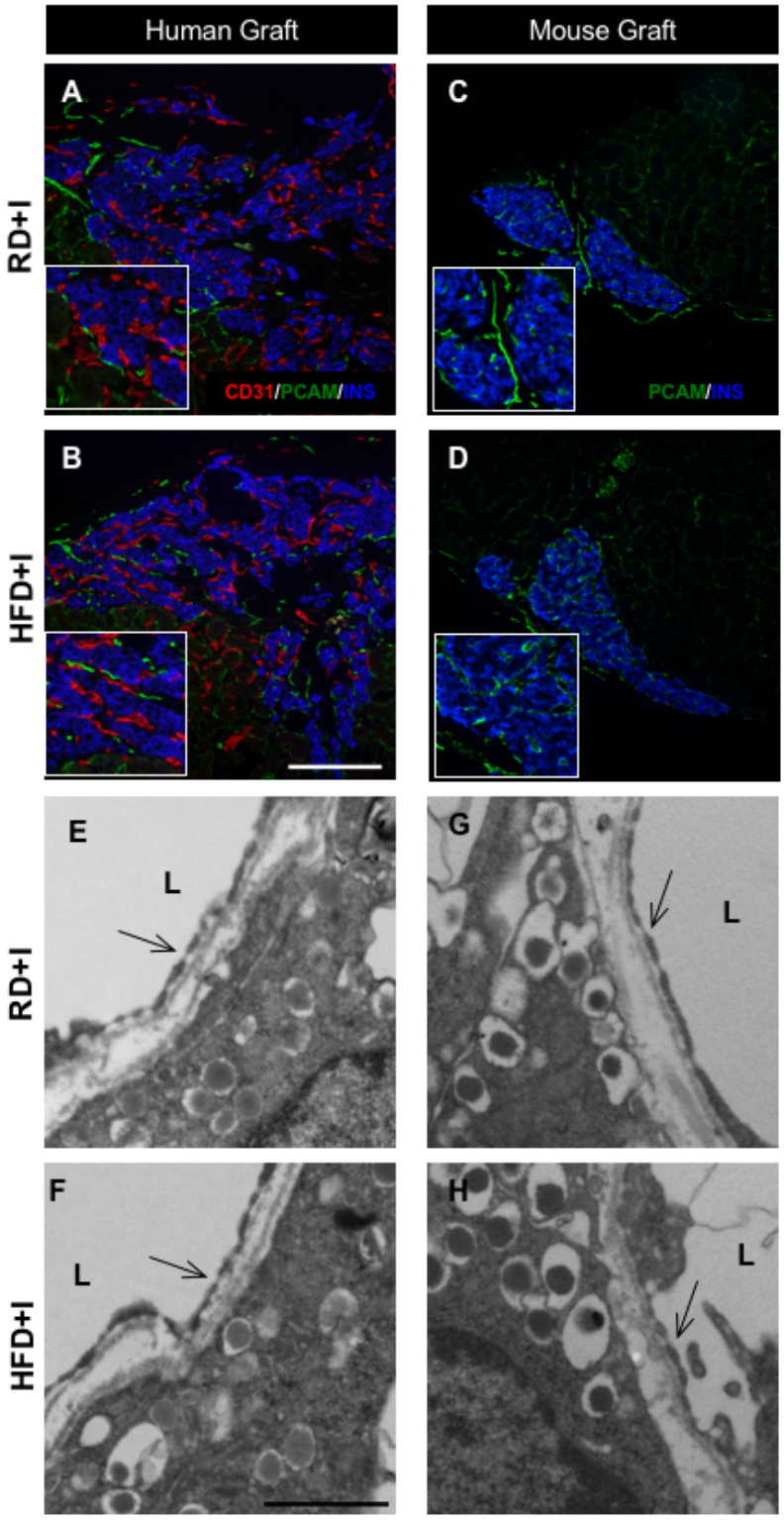


Figure S8. **Graft vasculature does not change in mice on high fat diet.** Representative images of human (**A, B**) and mouse grafts (**C, D**) labeled for insulin (blue), mouse vessels (green), and human vessels (red). Scale bar = 200 μm and applies to A,C,D. Representative EM images of fenestration in human **(E, F)** and mouse grafts **(G, H).** Arrows point to fenestration. L = lumen. Scale bar = 1 μm and applies to E-G.

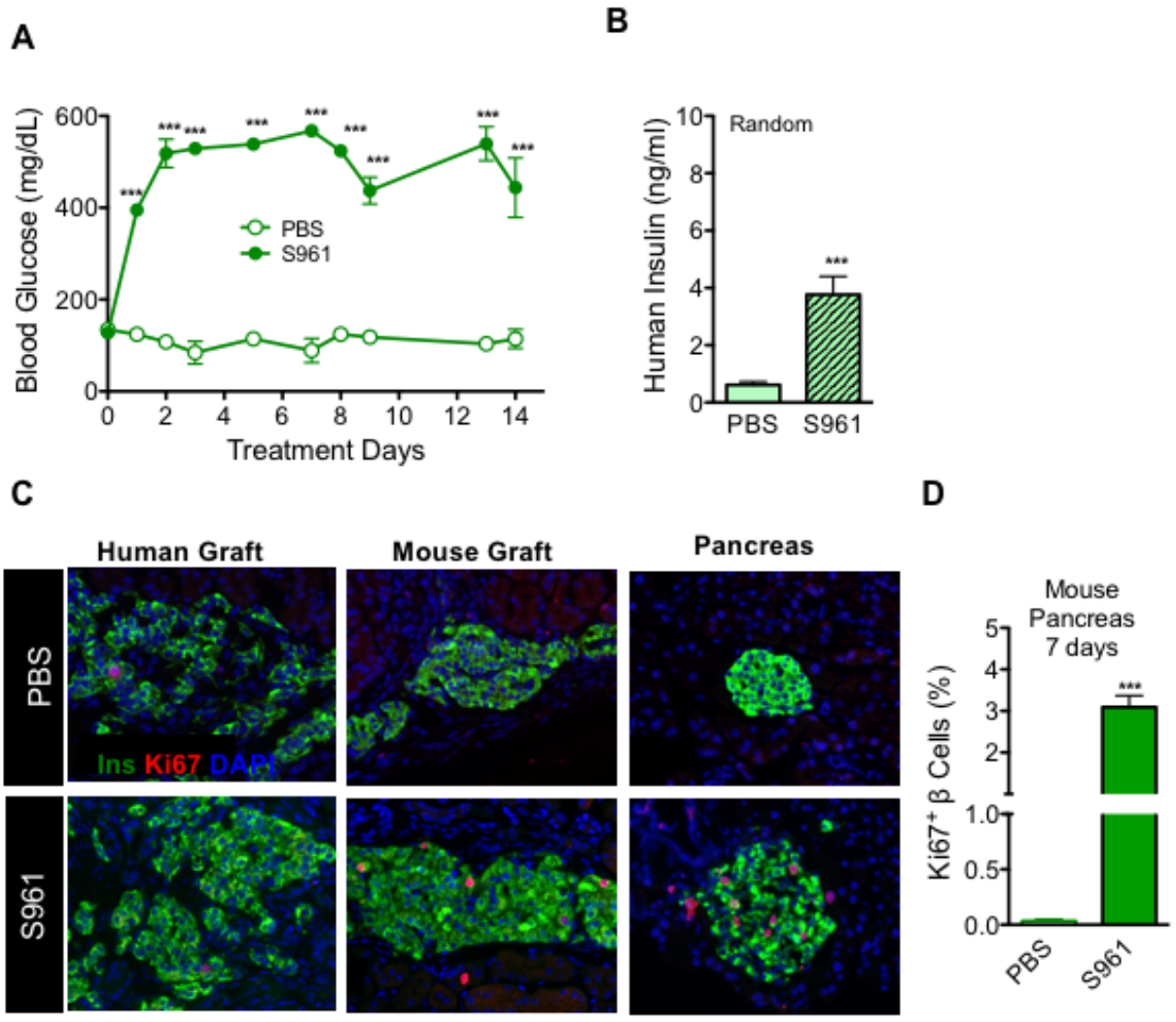
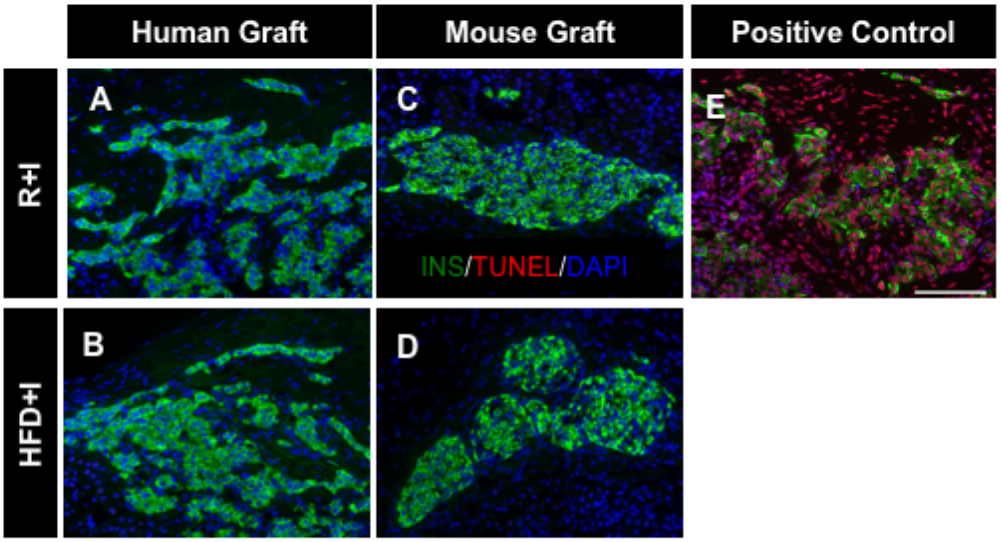


Figure S9. S961 model of acute hyperglycemia and insulin resistance. (A) Random blood glucose measurements of S961 and PBS-treated mice. *** p<0.001, n=4/treatment. **(B)** Random (non-fasting) human insulin values. *** p<0.001, PBS, n=8; S961, n=12). **(C)** Representative images showing relative levels of β cell proliferation in human graft, mouse graft, and pancreas of S961- or PBS-treated mice. Green = insulin, red = Ki67, blue = DAPI. **(D)** Quantification of β cell proliferation in mouse pancreata 7 days after S961 injection. *** p<0.001, n=5/treatment. Unpaired 2-tailed Student's *t* test was used for statistical significant analysis.



F

NSG-HFD		Total β Cells	TUNEL⁺ β Cells
Human Grafts	RD	10860	0
	HFD	11135	1
Mouse Grafts	RD	1455	0
	HFD	1444	0

G

NSG-DTR		Total β Cells	TUNEL⁺ β Cells
Human Grafts	PBS	4943	1
	DT-NG	2931	1
	DT-HG	3767	1

Figure S10. Chronic hyperglycemia and insulin resistance do not increase β cell apoptosis. Representative images showing lack of TUNEL-positive β cells in NSG+HFD **(A, B)** human grafts and **(C, D)** mouse grafts, compared to a positive control. Scale bar = 100 μm and applies to A-D. **(E)** Tabulated quantification of TUNEL+ β cells in NSG+HFD **(F)** and NSG-DTR **(G)** models.

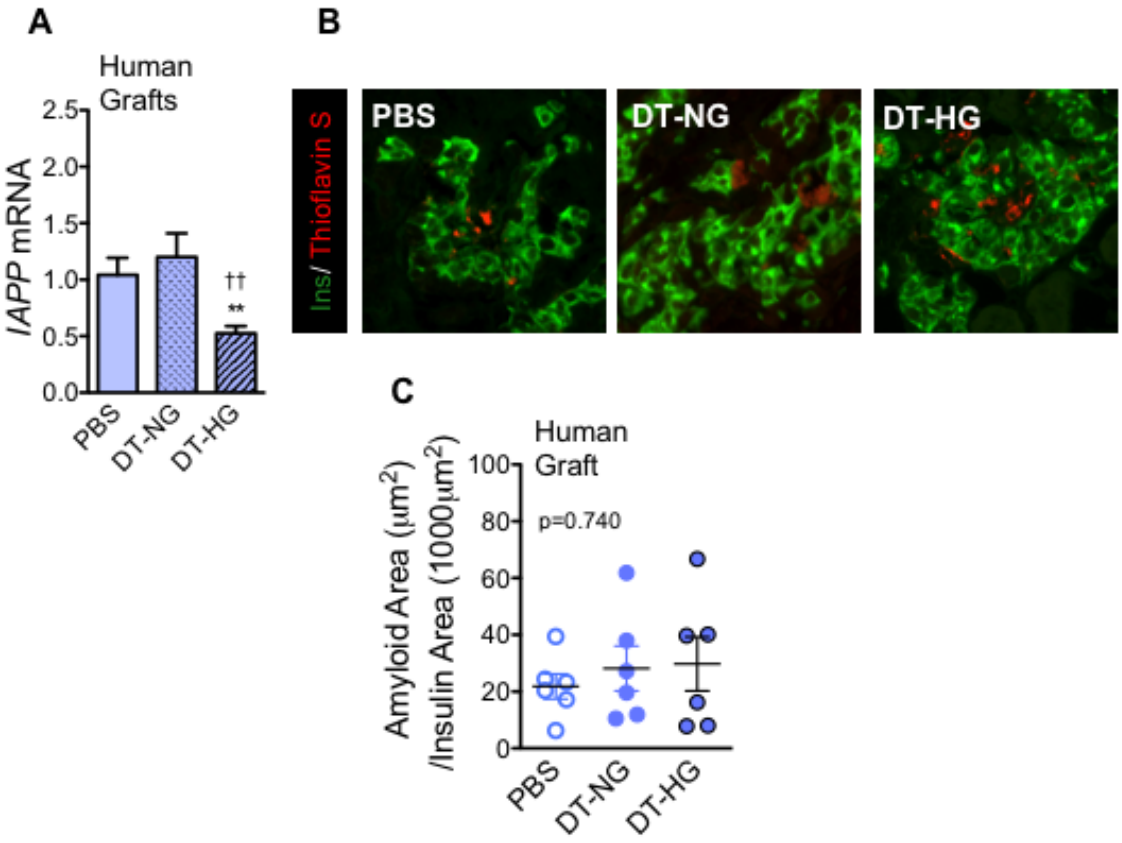


Figure S11. Islet amyloid is not increased by chronic hyperglycemia. (A) mRNA levels of islet amyloid polypeptide (IAPP) in human grafts from DTR mice (n=3-6/group). ** p<0.01 vs PBS group, †† p<0.01 vs DT-NG group. **(B)** Representative images of thioflavin S (red) staining in human islet grafts from each group (green = insulin). **(C)** Quantification of amyloid area per graft section (n=6 grafts per group). 1-way ANOVA followed by Newman-Keuls Multiple Comparison test was used for statistical significant analysis.

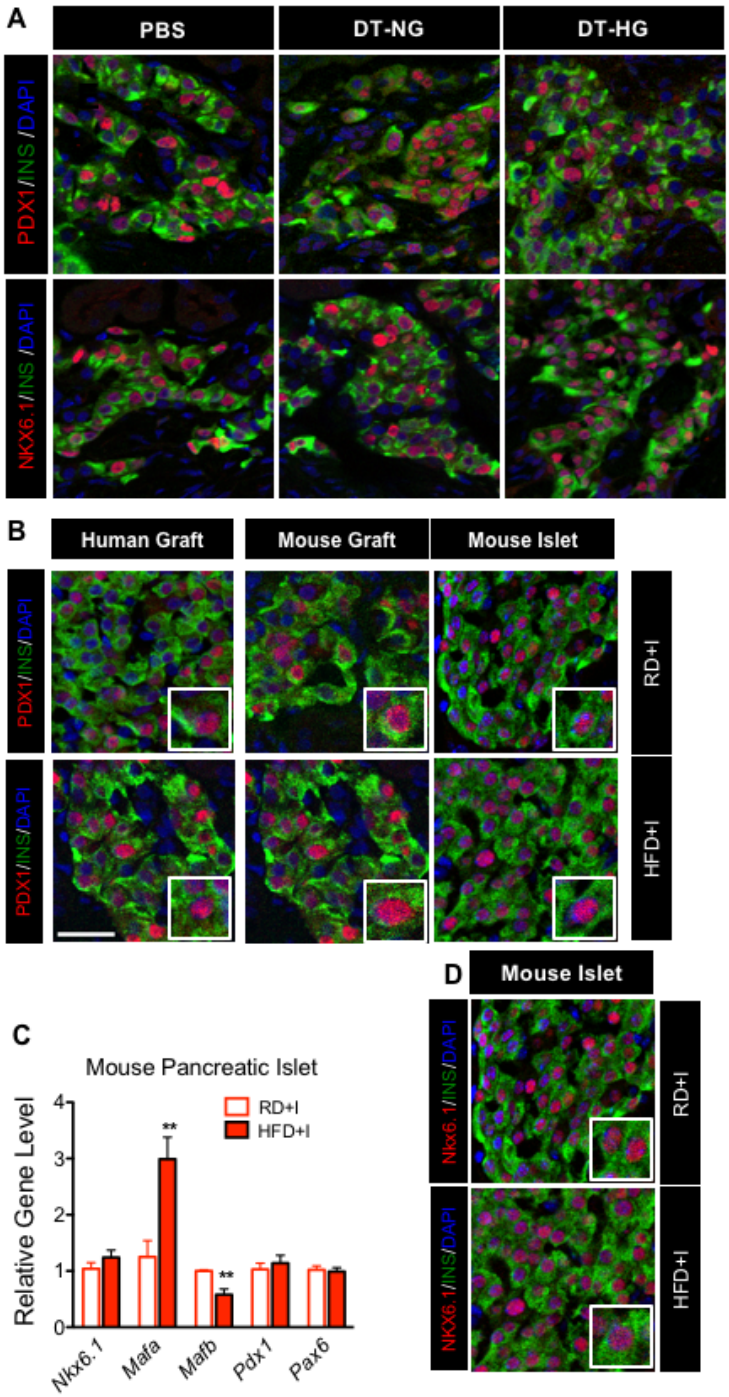


Figure S12. PDX1 protein level does not change in transplanted human β cells in high fat diet. (**A**) Images of PDX1 and NKX6.1 in human grafts of PBS-treated (left panels), DT-NG (center panels), and DT-HG (right panels) mice. (**B**) Images of PDX1 human grafts (left panels) mouse grafts (center panels), and mouse islets (right panels) in response to RD (top panels) or HFD (bottom panels). Scale bar = 50 μm and applies to E. Inserts show an enlargement of β cells. (**C**) mRNA of transcription factors in mouse islets from NSG-HFD model (n=5/diet, ** p<0.01). (**D**) Images of Nkx6.1 in mouse islets on HFD or RD. Green = insulin, red = PDX1, blue = DAPI. Unpaired 2-tailed Student's *t* test was used for statistical significant analysis.

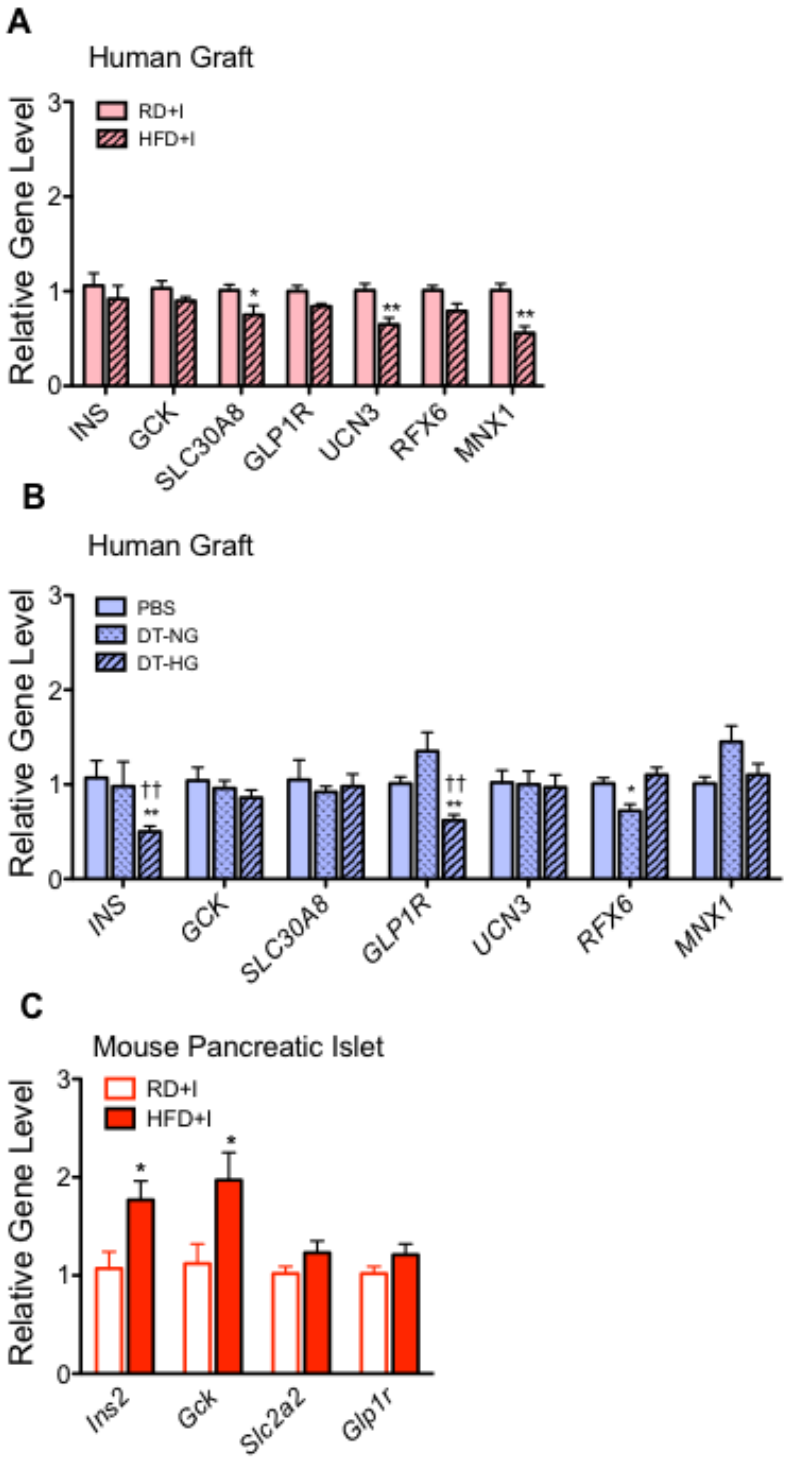


Figure S13. Expression of glucose-sensing genes is decreased in human grafts. Expression of glucose metabolism genes in (**A**) HFD human grafts (n=5/diet), (**B**) NSG-DTR human grafts (n=4-5/group), and (**C**) HFD mouse islets (n=5/diet). * p<0.05, ** p<0.01, *** p<0.001 RD+I vs HFD+I; DT vs PBS, †† p<0.01 vs DT-NG. Unpaired 2-tailed Student's *t* test or 1-way ANOVA followed by Newman-Keuls Multiple Comparison test (B) was used for statistical significant analysis.

Supplemental Table 1. Islet donor information.

Donor	Sex	Age	BMI	Ischemia time (h)	Cause of death
1	F	40	28.4	7.9	N/A
2	M	50	29.1	10.4	N/A
3	F	20	24.6	8.1	Anoxia
4	F	51	21.2	9.3	Stroke
5	M	60	24.5	N/A	N/A
6	F	27	23.9	16.8	Anoxia
7	M	19	26.9	N/A	Head Trauma
8	M	21	28.4	14.5	Anoxia
9	M	26	31.0	7.8	Anoxia
10	M	43	29.6	5.8	Head Trauma
11	N/A	46	N/A	N/A	N/A
12	M	25	35.7	N/A	Anoxia
13	M	43	24.0	N/A	CVA

Supplemental Table 2. PCR primers.

Gene Symbol	Catalog #	Gene Symbol	Catalog #
INS	Hs02741908_m1	*Ins2*	Mm00731595_gh
GCK	Hs01564555_m1	*Gck*	Mm00439129_m1
SLC2A1	Hs00892681_m1		
SLC2A2	Hs01096904_m1	*Slc2a2*	Mm00446229_m1
GLP1R	Hs00157705_m1	*Glp1r*	Mm00445292_m1
BID	Hs00609632_m1	*Bid*	Mm00626981_m1
BAD	Hs00188930_m1	*Bad*	Mm00432042_m1
DDIT3	Hs00358796_g1	*Ddit3*	Mm00492097_m1
SOD1	Hs00533490_m1		
SOD2	Hs00167309_m1		
CAT	Hs00156308_m1		
GPX1	Hs00829989_gH		
UCP2	Hs01075225_m1		
NFE2L2	Hs00975961_g1		
HSPA5	Hs00607129_gH	Hspa5	Mm00517690_g1
HSP90B1	Hs00427665_g1	*Hsp90b1*	Mm00441926_m1
PDIA4	Hs01115905_m1	*Pdia4*	Mm00437958_m1
IAPP	Hs00169095_m1	*Iapp*	Mm00439403_m
NKX6.1	Hs00232355_m1	*Nkx6.1*	Mm00454961_m1
MAFA	Hs01651425_s1	*Mafa*	Mm00845206_s1
MAFB	Hs00534343_s1	*Mafb*	Mm00627481_s1
PDX1	Hs00236830_m1	*Pdx1*	Mm00435565_m1
FOXO1	Hs01054576_m1	*Foxo1*	Mm00490672_m1
ACTB	Hs99999903_m1	*Actb*	Mm00607939_s1
TFRC	Hs99999911_m1	Tfrc	Mm00441941_m1
TBP	Hs99999910_m1	Tbp	Mm00446971_m1
G6PC2	Hs01549773_m1		
G6PD	Hs00166169_m1		
AGT	Hs01586213_m1		
PRKAA1	Hs01562315_m1		
SLC30A8	Hs00545183_m1		
UCN3	Hs00846499_s1		
RFX6	Hs00543100_m1		
MNX1	Hs00907365_m1		