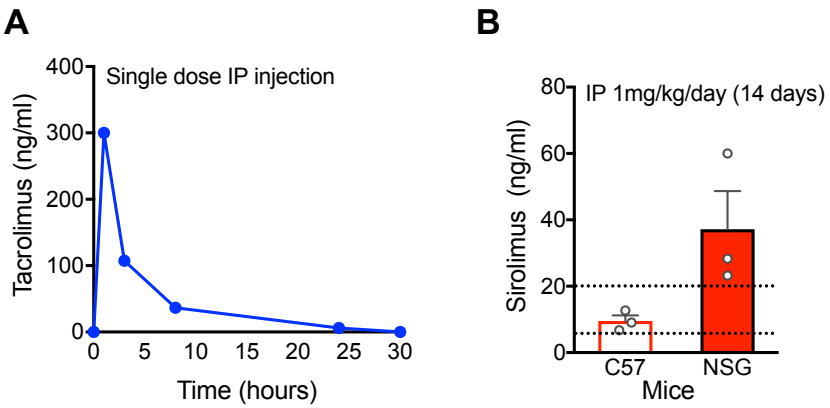


Figure S1. **Pharmacokinetics of TAC and SIR *in vivo*** Time course of TAC blood levels with single dose (0.25mg/kg) injection in NSG mice to establish drug half-life (A). Comparison of the SIR blood levels in C57BL/6 and NSG mice (B, n=3/group). Data represent mean ± SEM.

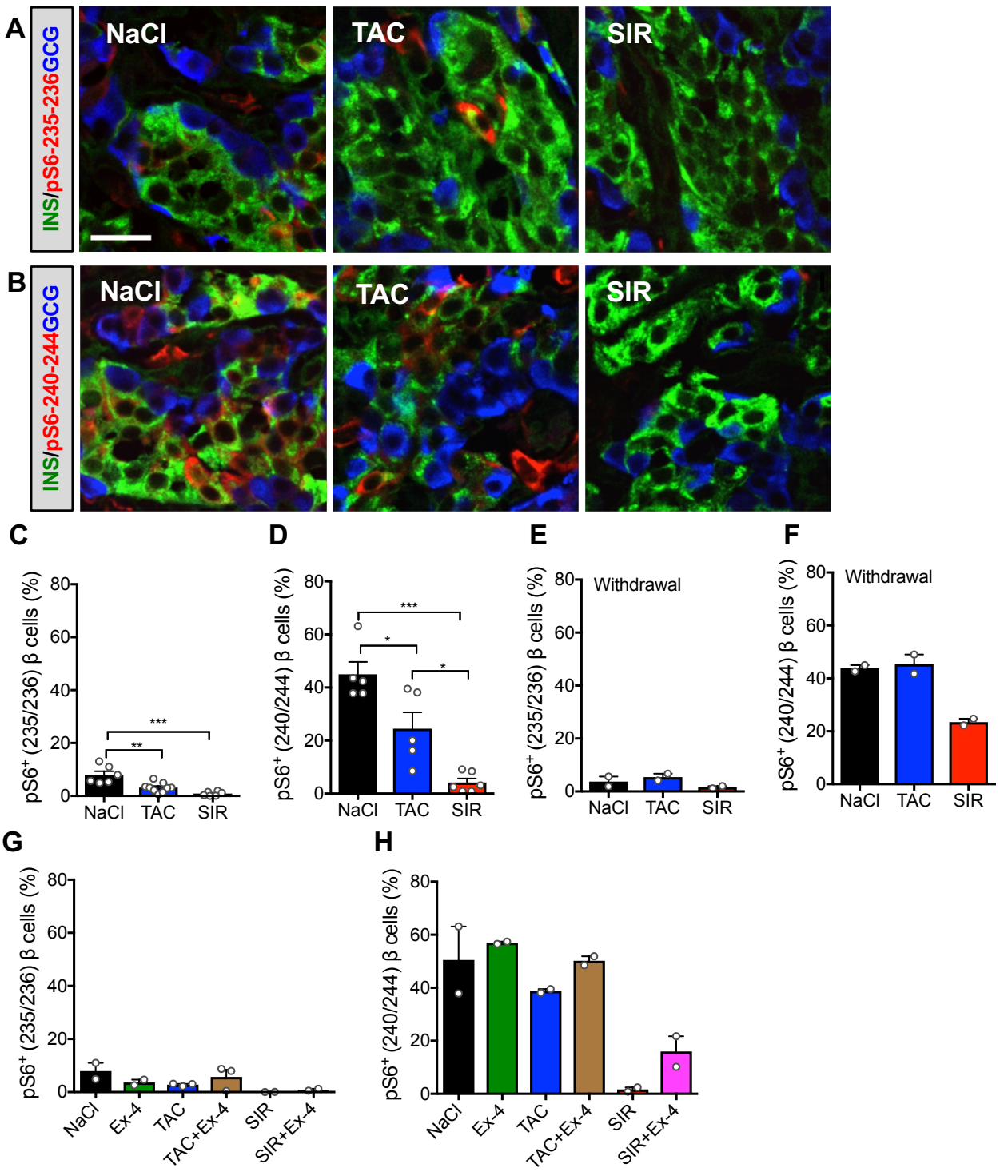


Figure S2. **TAC and SIR treatment reduces S6 phosphorylation in human grafts.** Representative images (A-B) of human grafts labeled with INS (green), pS6-235/236 or 240/244(red), GCG (blue). Scale bar=20 μm and applies to all images in (A) and (B). % of positive pS6 β cells in human grafts (C-D, n=6-9 grafts/treatment from 3 donors, #3, #5, #7). % of positive pS6 β cells in human grafts after 4 weeks withdrawal (E-F, n=2 grafts/treatment from donor #7). % of positive pS6 β cells in human grafts with Ex-4 cotreatment (G,H, n=2-3 grafts/treatment from donor #5). * p<0.05, ** p<0.01, *** p<0.001. Data represent mean ± SEM. One-way ANOVA was used for analysis of statistical significance.

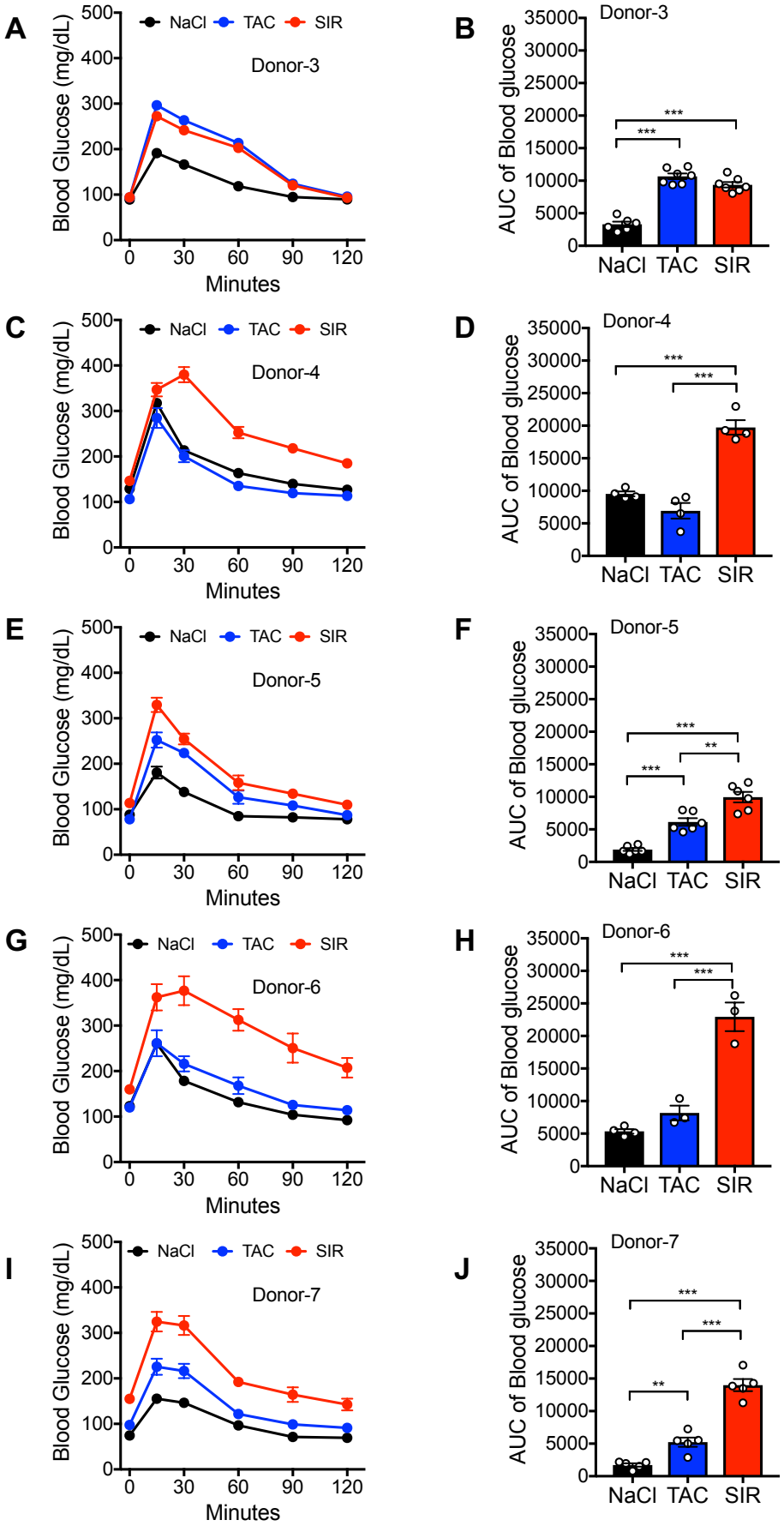


Figure S3. Glucose tolerance test by individual donor in mice transplanted with human islets. GTT (A,C,E,G,I) or AUC (B,D,F,H,J) shown by donor; corresponds to Fig. 1D-E. n=3-7samples/treatment/donor. GTTs were not performed in mice from Donor-1 or Donor-2.

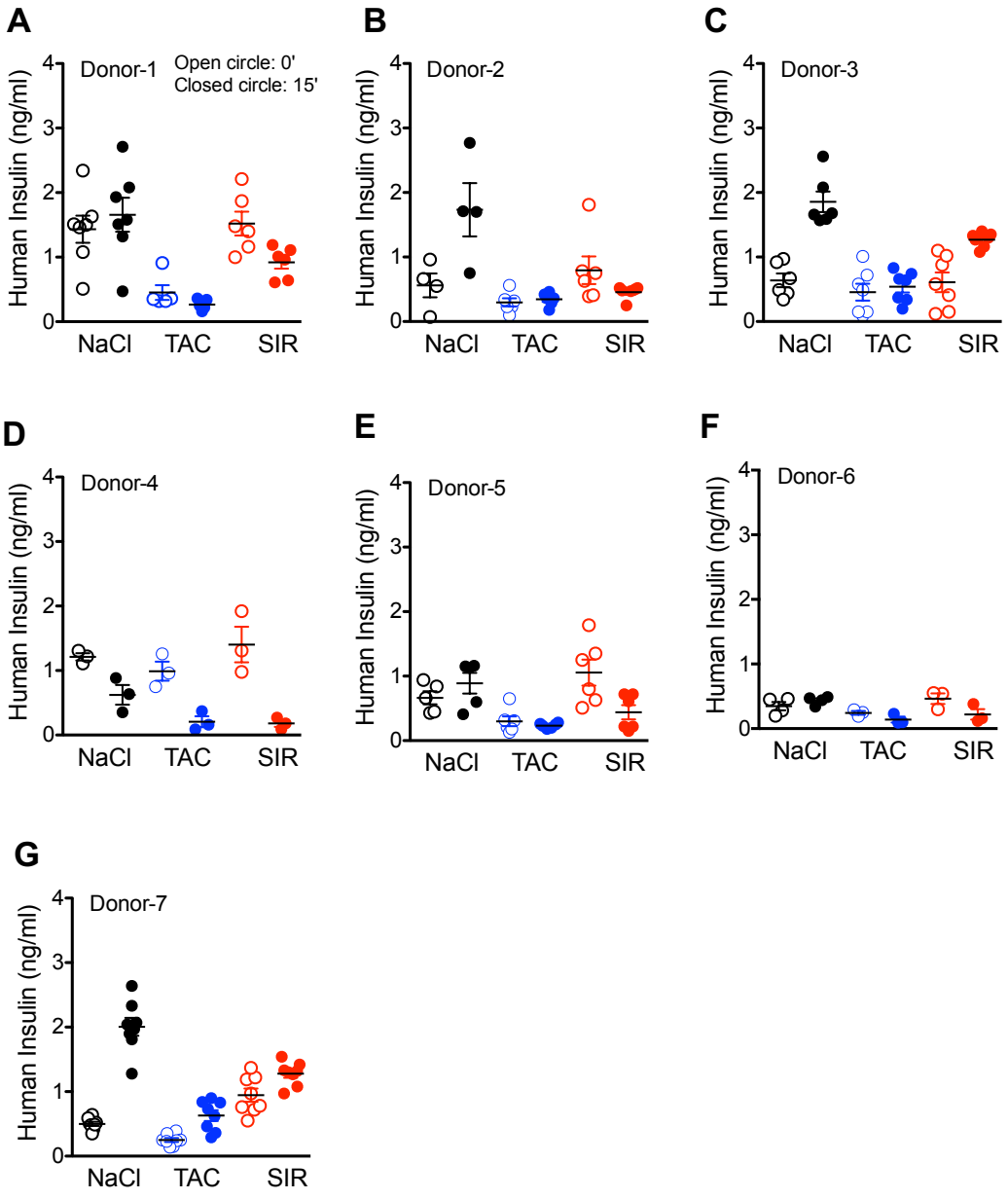


Figure S4. Effects of TAC and SIR treatment for 4 weeks. Data corresponds to Fig. 1G,J. Human insulin levels before and after stimulation from individual donors with TAC or SIR treatment for 4 weeks. n=3-8 serum samples/treatment/donor, donors #1-7.

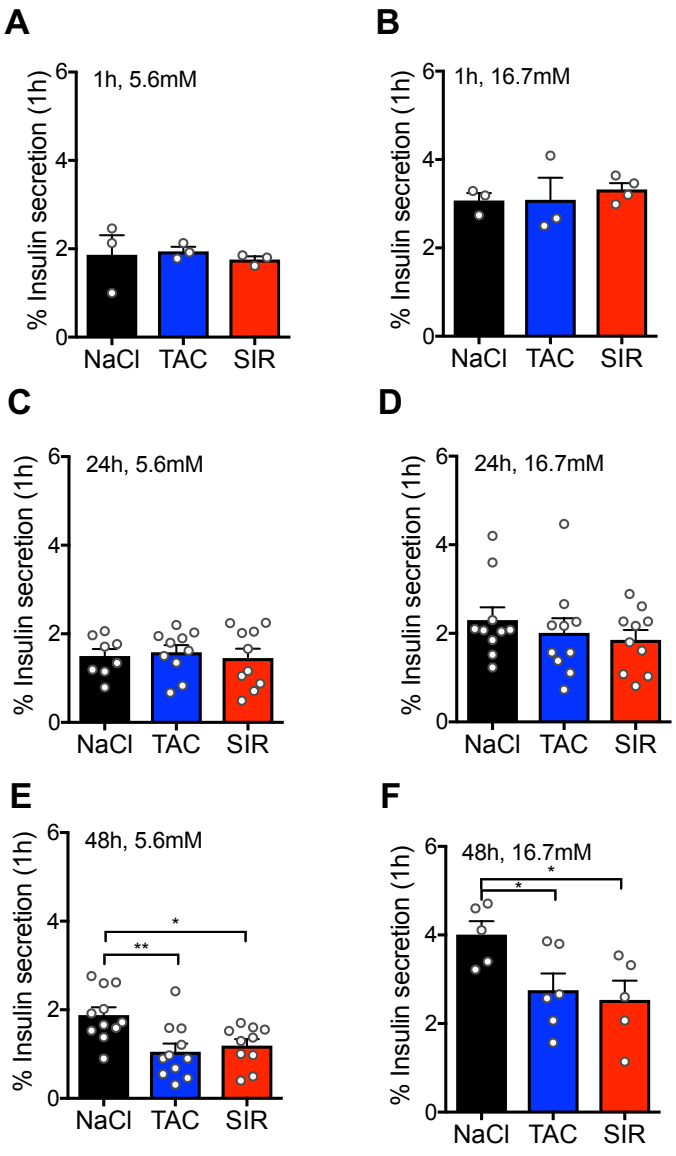
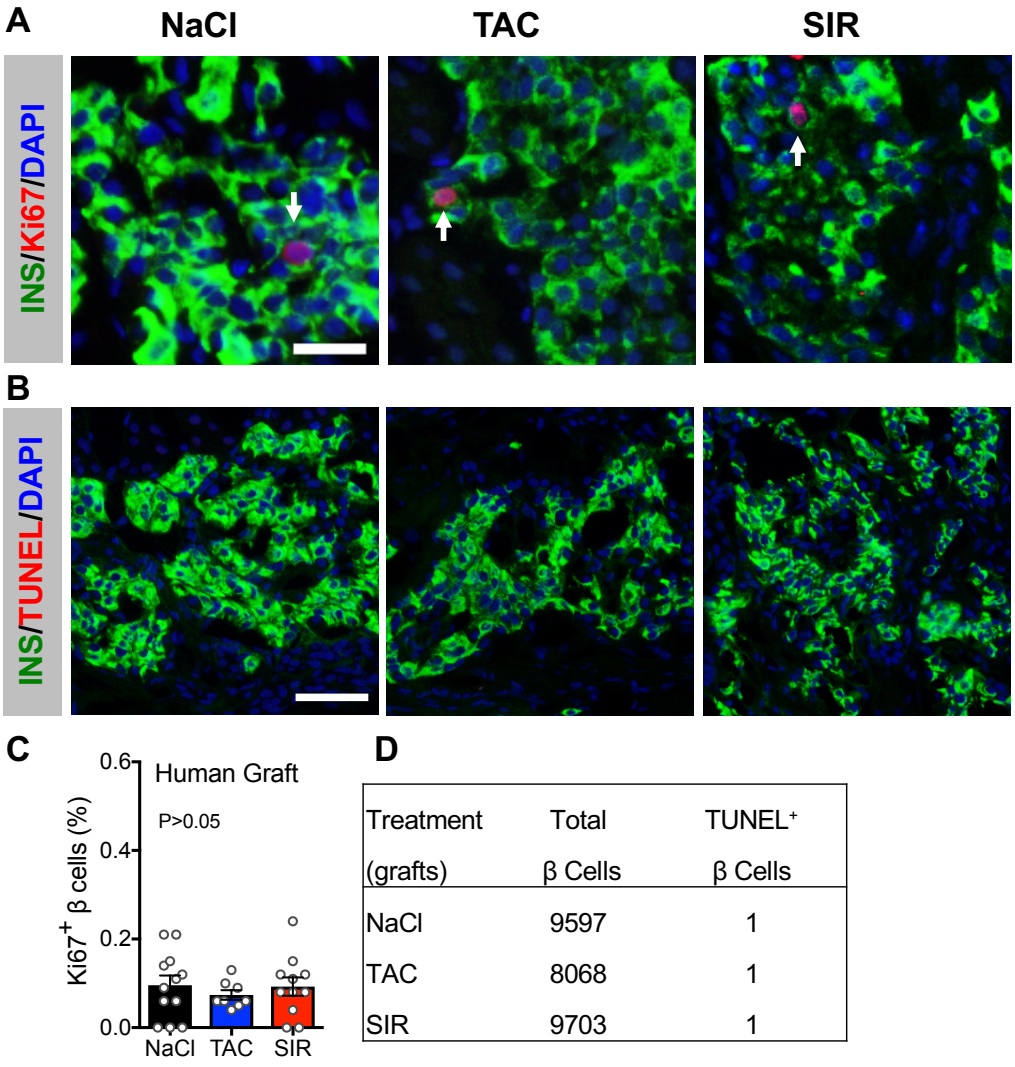


Figure S5. **The effect of TAC and SIR on human β cell function in vitro.** Insulin levels after human islets cultured with TAC or SIR for 1 hour (A-B, n=3 samples/treatment from donor #8), 24 hours (C-D, n=8-10 samples/treatment from donor #9,10,11), or 48 hours (E-F, n=10-11 samples/treatment from donor #9,10,11) with 5.6mM or 16.7mM glucose. * $p<0.05$, ** $p<0.01$. Data represent mean ± SEM. One-way ANOVA was used for analysis of statistical significance.



Treatment (grafts)	Total β Cells	TUNEL⁺ β Cells
NaCl	9597	1
TAC	8068	1
SIR	9703	1

Figure S6. TAC or SIR treatment do not change in vivo human β cell proliferation or apoptosis.(A) Representative images of Ki67 assay in human grafts after 4 weeks treatment with insulin (green), Ki67 (red), and DAPI (blue). Arrows showed Ki67 positive β cells. Scale bar: 25 μm applies to all images in (A). (B) Representative images of TUNEL assay in human grafts after 4 weeks treatment with insulin (green), TUNEL (red), and DAPI (blue). Scale bar: 50 μm applies to all images in (B). (C) Percentage of Ki67 positive β cells in transplanted human grafts (n=8-12 grafts/treatment from donors #1, #3, #5). (D) Quantification of TUNEL positive β cells (donor #3, #4 and #5). Data represent mean ± SEM. One way ANOVA was used for analysis of statistical significance.

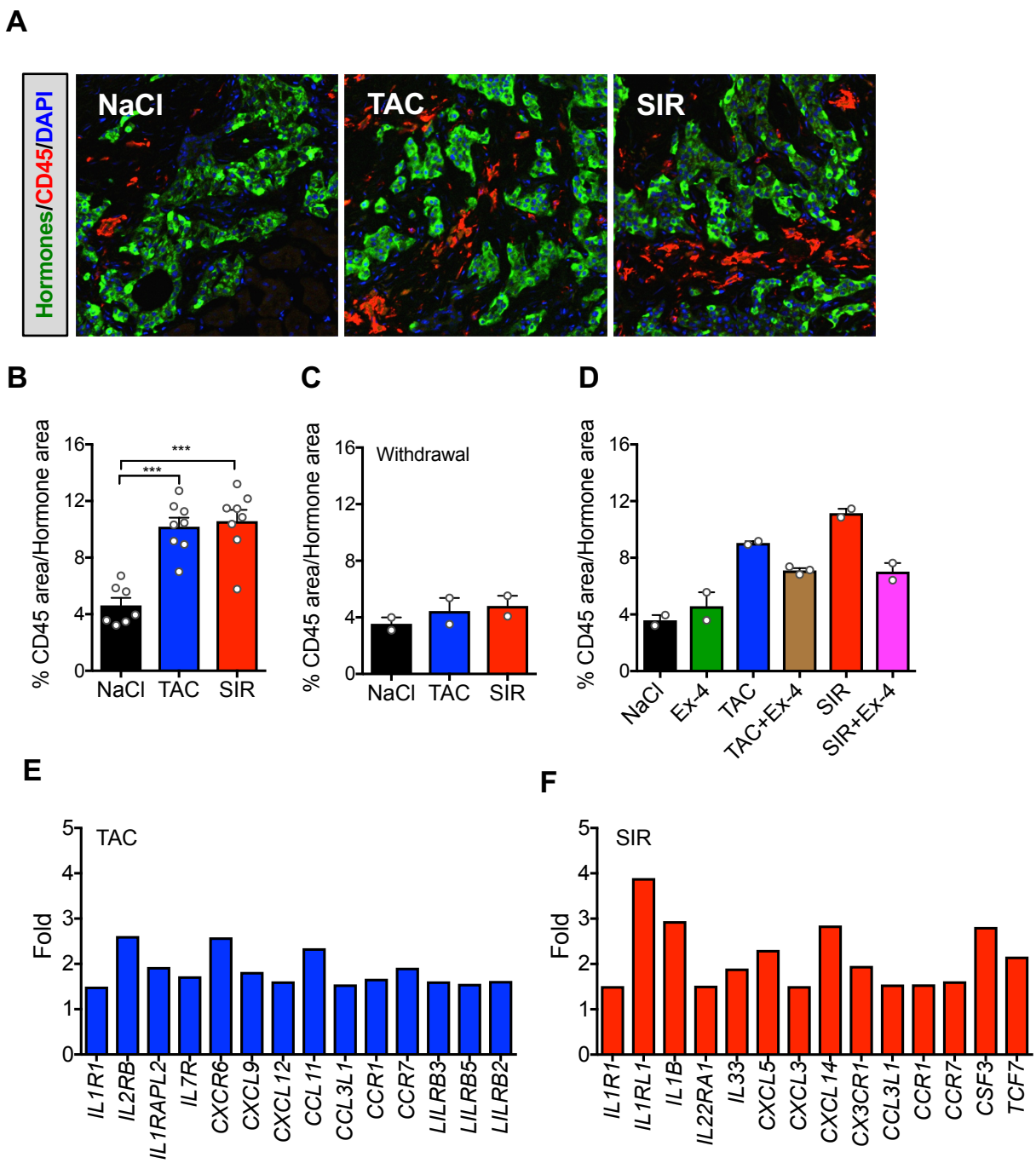


Figure S7. **TAC and SIR increase CD45⁺ cells in human grafts.** Representative images (A) of CD45⁺ cells in human grafts with green (INS, GCG, SOM), red (CD45) and blue (DAPI). Quantification % of CD45 area after TAC and SIR treatment for 4 weeks (B, n=7-8 grafts/treatment from 3 donors, #3,#5,#7), 4 weeks withdrawal (C, n=2 grafts/treatment from donor #7) or with Ex-4 treatment (D, n=2-3 grafts/treatment from donor #5). (E, F) RNAseq data from human graft samples. Fold change transcripts related to inflammation stimulated by TAC (E) and SIR (F) compared to control group (n=5 samples from 2 donor transplantations). * p<0.05, ** p<0.01, *** p<0.001. Data represent mean ± SEM. One-way ANOVA was used for analysis of statistical significance.

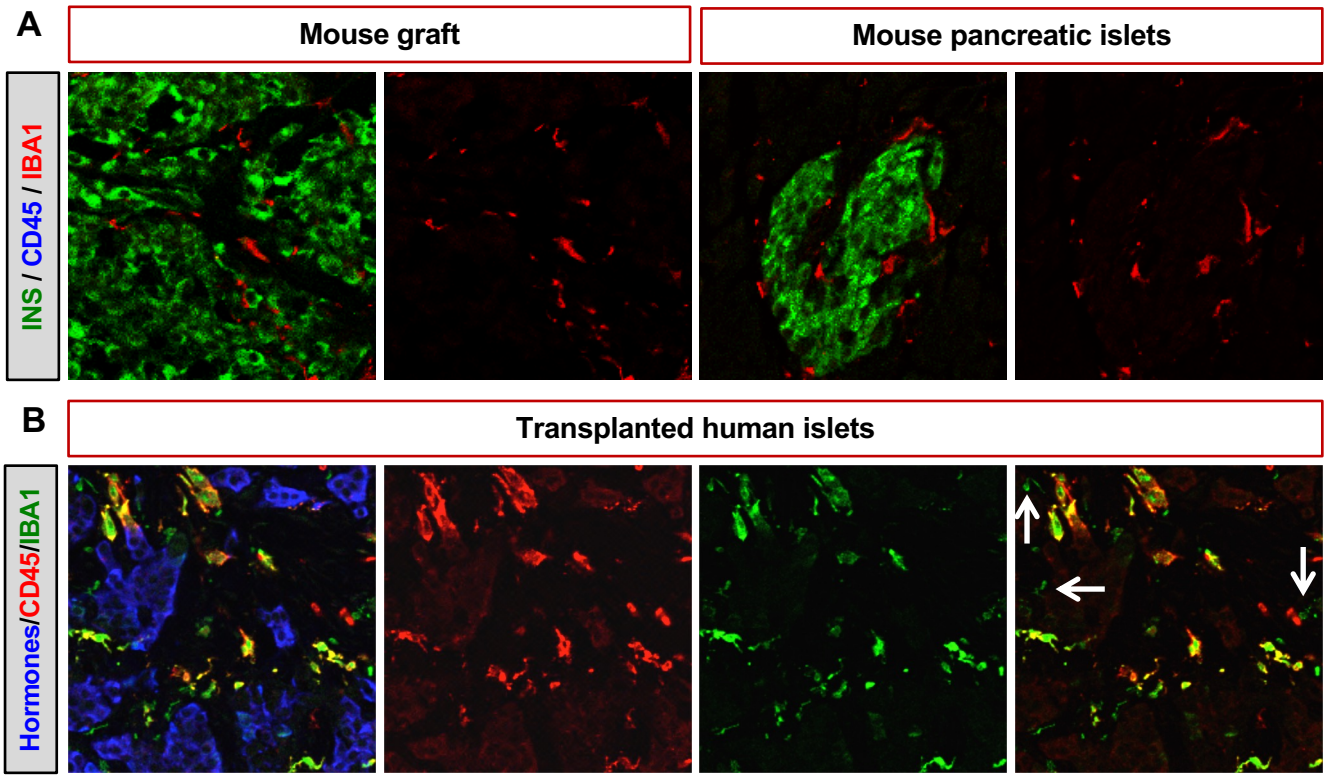


Figure S8. **CD45⁺ cells in human grafts were derived from human.** (A) Images showing macrophages in mouse grafts or mouse pancreatic islets, green (INS), red (IBA1, antibody reacts with human and mouse macrophages), blue (CD45, reacts only to human tissue, negative in both mouse graft and mouse pancreatic islet). (B) Macrophages in human graft derived from human (co-labeled CD45 and IBA1, yellow) and mouse (IBA1 only, green, pointed with arrows): blue (INS, GCG, and SOM), red (CD45), green (IBA1).

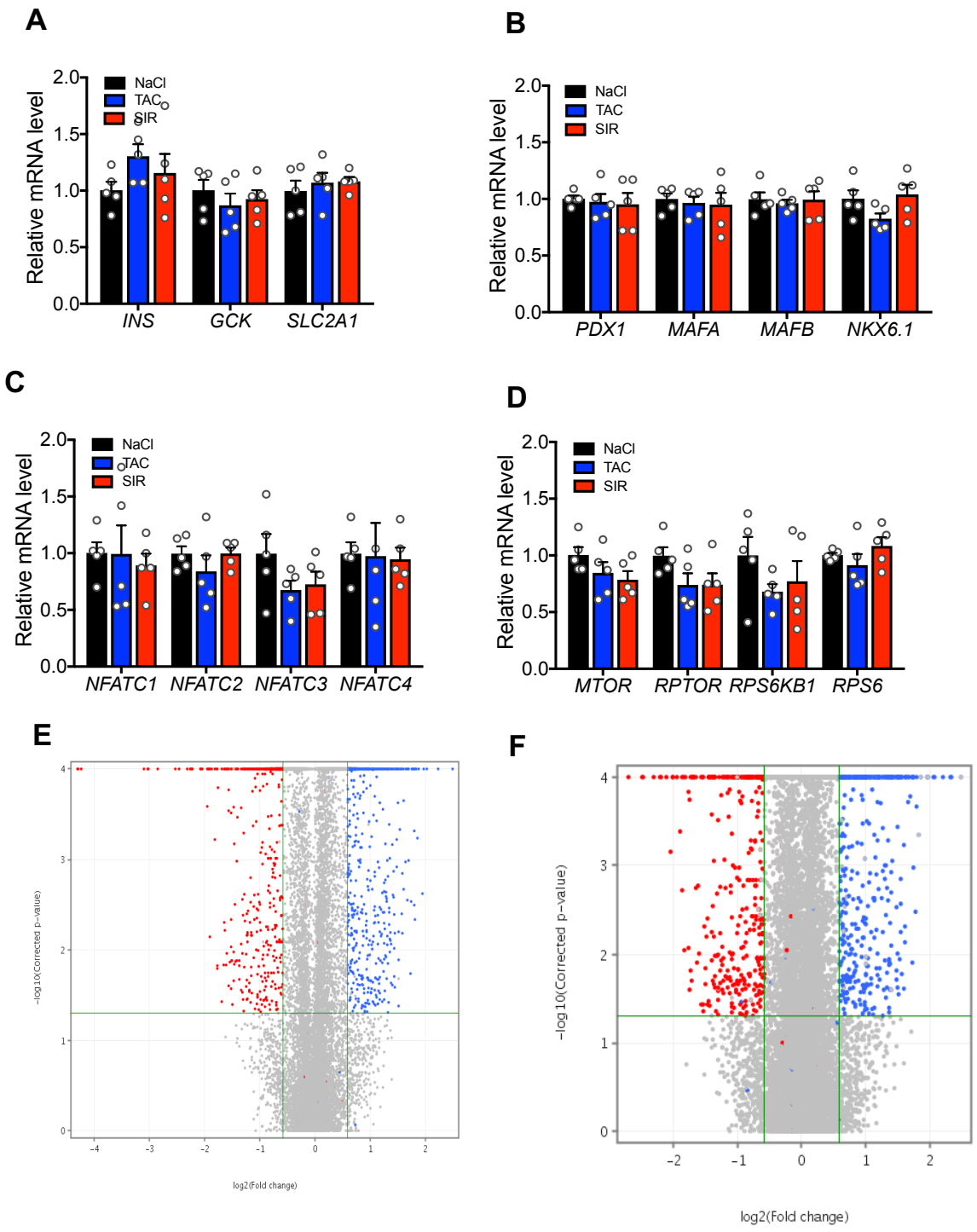


Figure S9. Gene expression in human grafts measured by quantitative real-time RT-PCR (qPCR). Genes are grouped by (A) β cell metabolism, (B) β cell enriched transcription factors, (C) the NFATC family, and (D) mTOR signaling. n=5 grafts/treatment from donor #3 and #5. Volcano plots of RNAseq data, TAC vs PBS (E) and SIR vs PBS (F).

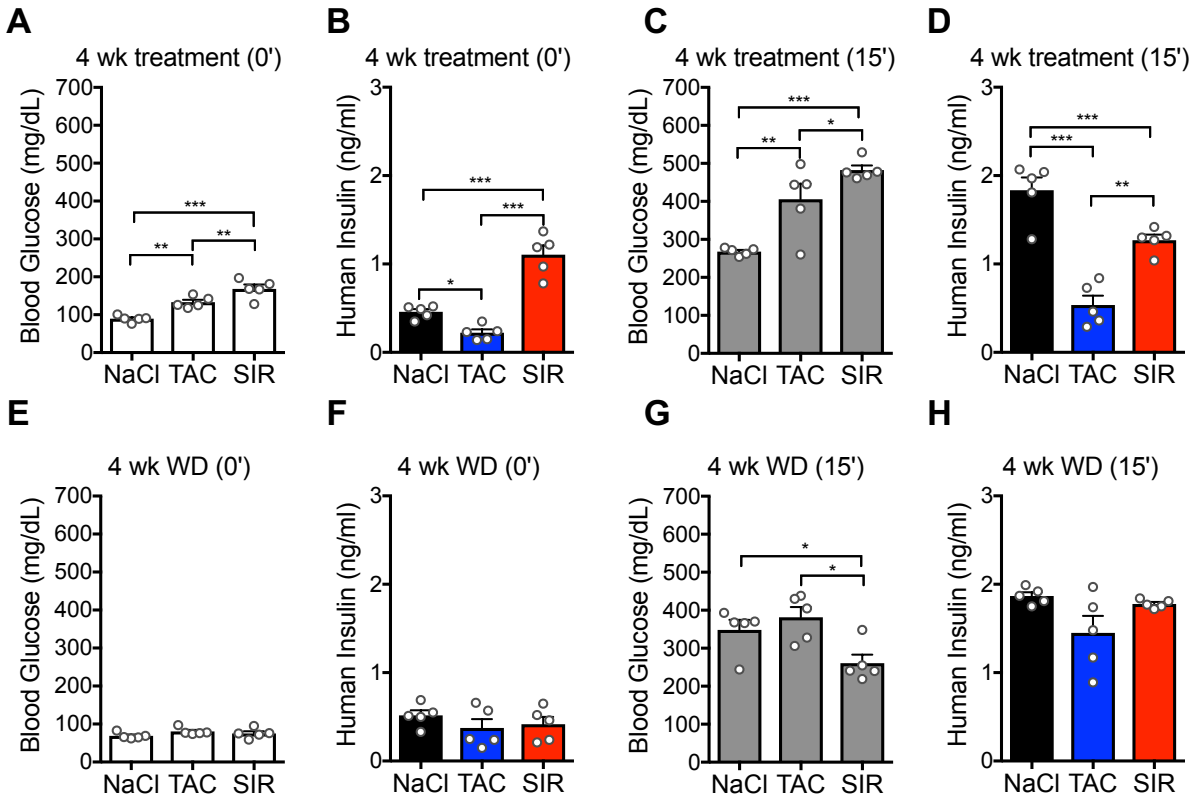


Figure S10. The changes of blood glucose and human insulin levels induced by TAC or SIR are reversed after 4 weeks of withdrawal (donor #7). Fasted and stimulated blood glucose and human insulin analyses after 4 weeks of drug treatment (A-D; corresponds to Fig 3D-E). Fasted and stimulated blood glucose and human insulin analyses after 4 weeks drug withdrawal (E-H; corresponds to Fig 3H-I). * p<0.05, ** p<0.01, *** p<0.001. Data represent mean ± SEM. One-way ANOVA was used for analysis of statistical significance.

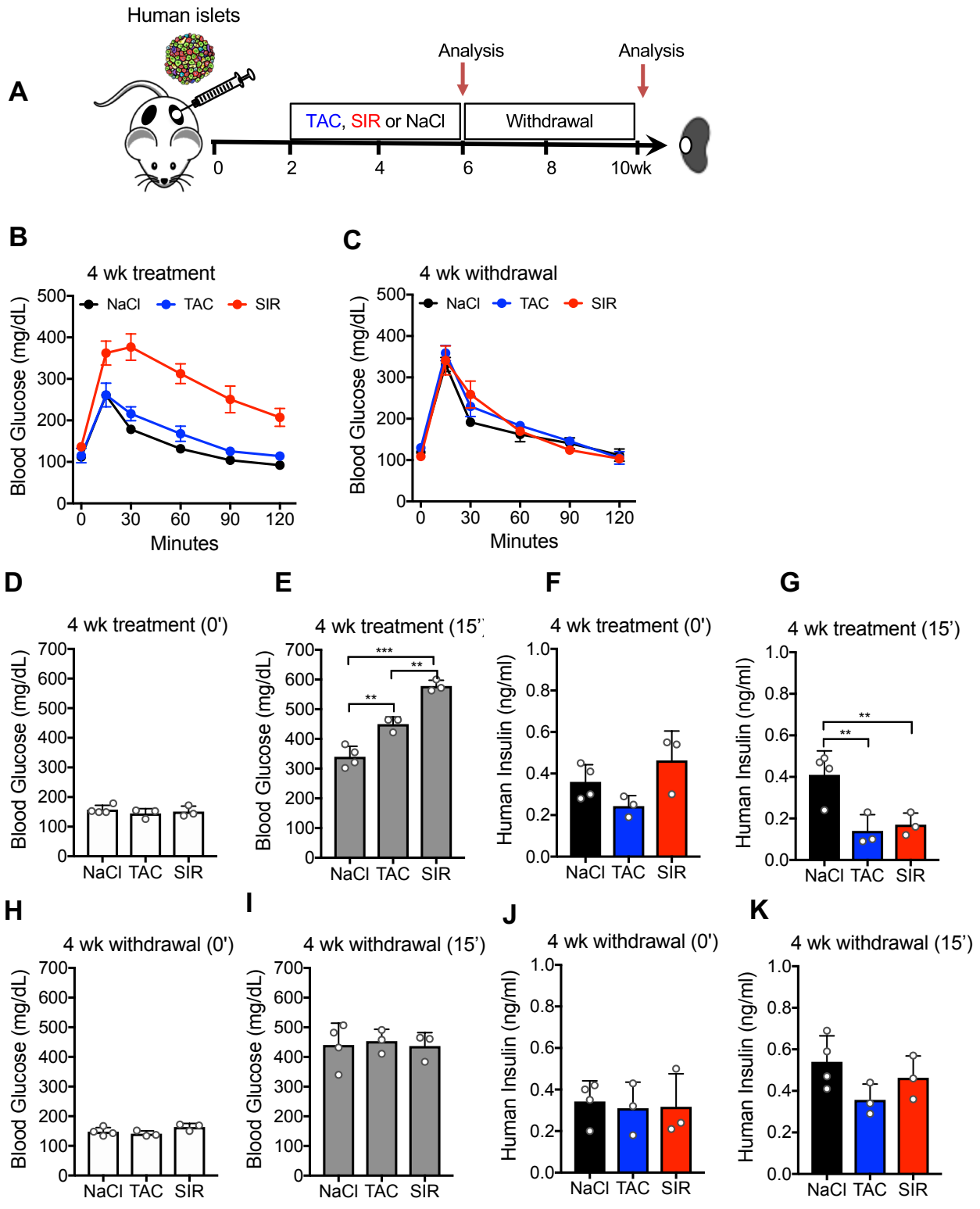


Figure S11. Impaired β cell function by either TAC or SIR normalizes after 4 weeks of withdrawal (Donor #6). Schematic of experimental design (A). Mice were given NaCl, TAC or SIR for 4 weeks followed by withdrawal for 4 weeks. GTT after treatment with TAC or SIR for 4 weeks (B) or 4 weeks after withdrawal (C). Blood glucose and human at 0' and 15' of glucose-arginine stimulation after 4 weeks treatment (D-G) and then 4 weeks after withdrawal (H-K). n=3-4 samples/treatment. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Data represent mean ± SEM. One-way ANOVA was used for analysis of statistical significance.

Checklist for reporting human islet preparations used in research
Adapted from Hart NJ, Powers AC (2019) Progress, challenges, and suggestions for using human islets to understand islet biology and human diabetes. Diabetologia https://doi.org/10.1007/s00125-018-4772-2 and Poitout V, Satin LS, Kahn SE, et al (2019) A call for improved reporting of human islet characteristics in research articles. Diabetologia and Diabetes. https://doi.org/10.2337/dbi18-0055

Supplemental Table 1. Human Islet Donor Information

Islet preparation	1	2	3	4	5	6	7	8
Unique identifier	08785748	08784318	08774468	DON61	08930707	DON75	08768702	08769130
Donor age (years)	46	43	44	55	55	16	59	37
Donor sex (M/F)	M	M	F	M	M	M	F	M
Donor BMI (kg/m^2)	24.3	29.6	23.8	35.6	27.8	23.0	22.0	27.6
Donor HbA1C	N/A	N/A	N/A	N/A	4.9	5.0	5.2	N/A
Origin/source of islets	IIDP	IIDP	IIDP	AHN	IIDP	AHN	IIDP	IIDP
Islet isolation centre	U Penn	U Penn	U Penn	AHN	U Illinois	AHN	U Wisconsin	U Wisconsin
Donor history of diabetes?	No	No	No	No	No	No	No	No
Donor cause of death	Head Trauma	Head Trauma	CVA	CVA	CVA	Overdose	CVA	CVA
Warm ischaemia time (h)	N/A	N/A	N/A	DBD	N/A	DBD	N/A	N/A
Cold ischaemia time (h)	12.2	6.9	6.2	7.5	10.0	20.9	6.5	11.0
Estimated purity (%)	80	90	90	70	90	N/A	98	90
Estimated viability (%)	95	91	95	N/A	99	N/A	95	95
Total culture time (h)	27	16	43	36	24	N/A	36	18

Glucose-stimulated insulin secretion or other functional measurement	Perifusion	Perifusion	Perifusion	Perifusion	Perifusion	Perifusion	Perifusion	Perifusion
Handpicked to purity?	No	No	No	No	No	No	No	Yes
Experiment Used in	Transplant	Transplant	Transplant	Transplant	Transplant	Transplant	Transplant	In Vitro

Islet preparation	**9**	**10**	**11**
Unique identifier	HPAP004	DON160	08769035
Donor age (years)	24	15	43
Donor sex (M/F)	F	M	M
Donor BMI (kg/m^2)	32.2	25.1	35.0
Donor HbA1C	N/A	4.7	N/A
Origin/source of islets	HPAP	AHN	IIDP
Islet isolation centre	U Penn	AHN	S California
Donor history of diabetes?	No	No	No
Donor cause of death	Anoxia	Head Trauma	CVA
Warm ischaemia time (h)	N/A	DBD	N/A
Cold ischaemia time (h)	9.0	11.5	N/A
Estimated purity (%)	95	70	85
Estimated viability (%)	90	85	96

Total culture time (h)	87	18	67
Glucose-stimulated insulin secretion or other functional measurement	Perifusion	Perifusion	Perifusion
Handpicked to purity?	Yes	Yes	Yes
Experiment Used in	In Vitro	In Vitro	In Vitro

Abbreviations: N/A – Not available, IIDP – Integrated Islet Distribution Program, AHN – Allegheny Health Network, CVA – cerebrovascular accident (stroke), DBD – donation after brain death (warm ischemia time essentially 0 hours).

Supplemental Table 2: Percent of Amyloid/insulin area per graft – raw data

	----------4 week treatment----------						4 week withdrawal		
	NaCl	TAC	SIR	Ex-4	TAC+Ex-4	SIR+Ex-4	NaCl	TAC	SIR
Donor 3	0.48	1.40	1.23						
	0.43	1.64	1.55						
		1.50	1.51						
Donor 4	0.32	0.69	0.50	0.23	0.38	0.21			
Donor 5	2.85	5.00	7.62	2.52	1.82	6.26			
	2.50	6.16	8.46	2.03	2.53	4.67			
					1.87	3.37			
Donor 6	0.38	0.83	0.84				0.32	0.52	0.31
	0.28	0.71	0.71				0.49	0.42	0.37
Donor 7	1.17	2.34	2.86				1.93	2.68	2.57
	0.95	2.05	3.83				2.05	2.49	3.06
	1.08	1.98	5.38						

Thioflavin S area expressed at a percent of insulin area for 5-6 sections per graft shows varying baseline amyloid formation by donor. Data from columns 1-3 (donors 3-7) is included in Figure 1, columns 1-3 and 7-9 for donors 6 and 7 is included in Figure 2, and columns 1-6 for donors 4 and 5 is included in Figure 3.

Supplemental Table 5. Full statistical analysis of Figure 4C-F and H

	AUC	Blood Glucose	Human Insulin	H Ins/Glucose Ratio	Amyloid /Ins area
NaCl vs Ex-4	ns	ns	ns	ns	ns
NaCl vs TAC	ns	ns	**	*	ns
NaCl vs TAC+Ex-4	ns	ns	ns	ns	ns
NaCl vs SIR	***	**	ns	ns	*
NaCl vs SIR+Ex-4	**	ns	ns	ns	ns
Ex-4 vs TAC	ns	***	***	***	*
Ex-4 vs TAC+Ex-4	ns	ns	ns	ns	ns
Ex-4 vs SIR	***	***	*	***	**
Ex-4 vs SIR+Ex-4	**	***	ns	**	ns
TAC vs TAC+Ex-4	ns	ns	*	*	*
TAC vs SIR	**	ns	ns	ns	ns
TAC vs SIR+Ex-4	ns	ns	ns	ns	ns
TAC+Ex-4 vs SIR	***	**	ns	ns	**
TAC+Ex-4 vs SIR+Ex-4	ns	ns	ns	ns	ns
SIR vs SIR+Ex-4	ns	ns	ns	ns	ns

Groups compared by One-way ANOVA followed by Tukey multiple comparisons test. ns $p>0.05$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$

Supplemental Table 6: Primary antibodies

Antigen	Host	Class	Source	Catalog #
Insulin	Guinea Pig	polyclonal	Dako	A0564
Glucagon	mouse	monoclonal	abcam	ab10988
Glucagon	Rabbit	polyclonal	Cell Signaling	2760
Somatostatin	Goat	polyclonal	Santa Cruz BT	sc-7819
Ki-67	Rabbit	polyclonal	abcam	ab-15580
SER235/236	Rabbit	monoclonal	Cell Signaling	4858s
SER240/244	Rabbit	monoclonal	Cell Signaling	5364s
Iba1	Rabbit	polyclonal	Wako	019-19741
hCD45	mouse	monoclonal	BD Pharmingen	347460

Supplemental Table 7: Primers

Gene Symbol	Catalog #
INS	Hs02741908_m1
GCK	Hs01564555_m1
PDX1	Hs00236830_m1
MAFA	Hs01651425_s1
MAFB	Hs00534343_s1
NKX6.1	Hs00232355_m1
NFATC1	Hs00542678_m1
NFATC2	Hs00905451_m1
NFATC3	Hs00190046_m1
NFATC4	Hs00190037_m1
MTOR	Hs00234508_m1
RPTOR	Hs00375332_m1
RPS6KB1	Hs00356367_m1
RPS6	Hs04195024_g1
CHGA	Hs00900370_m1
TFRC	Hs00951083_m1
SV2A	Hs01059458_m1