Figure S1; Related to Figure 1

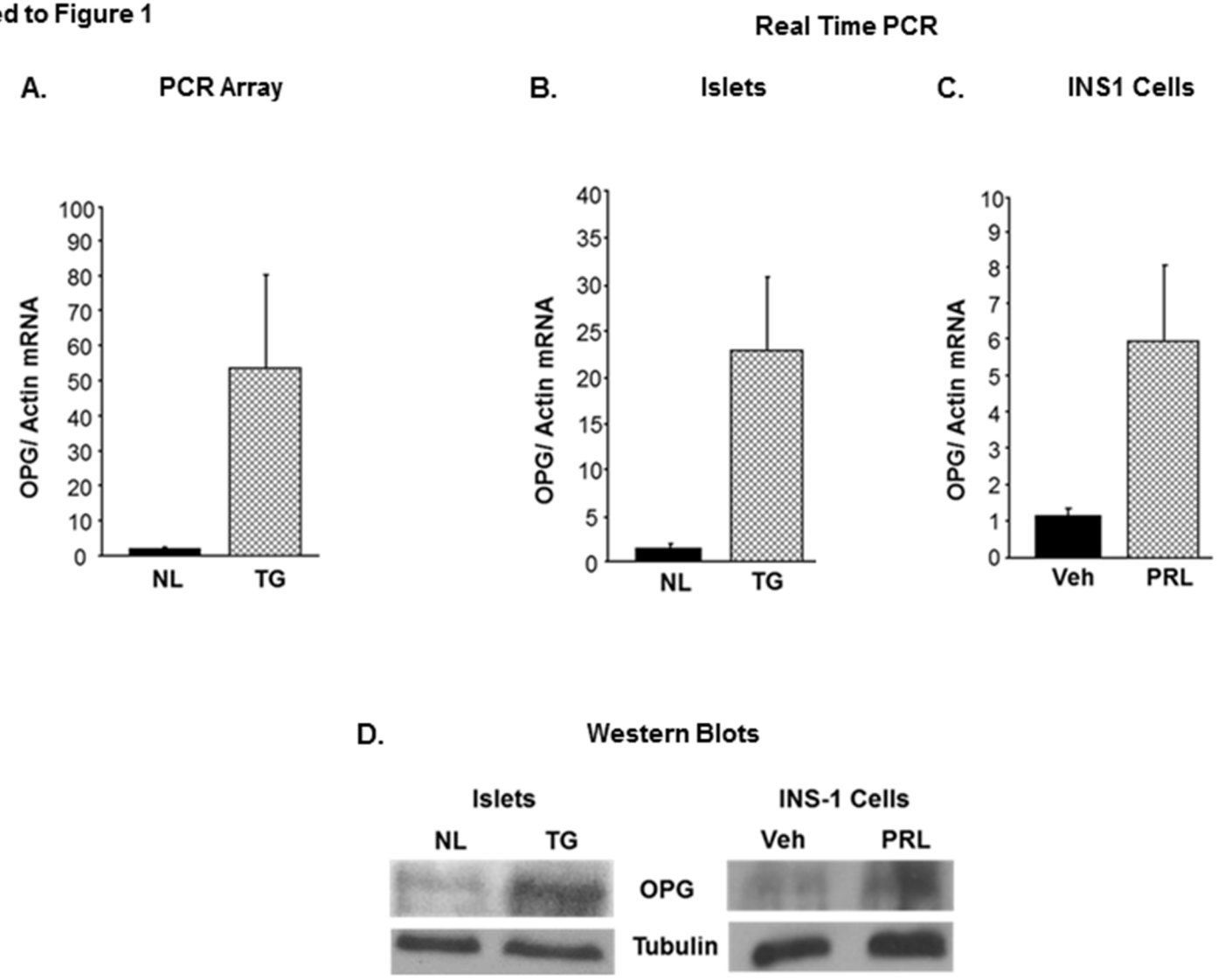


Figure S2; Related to Figure 1

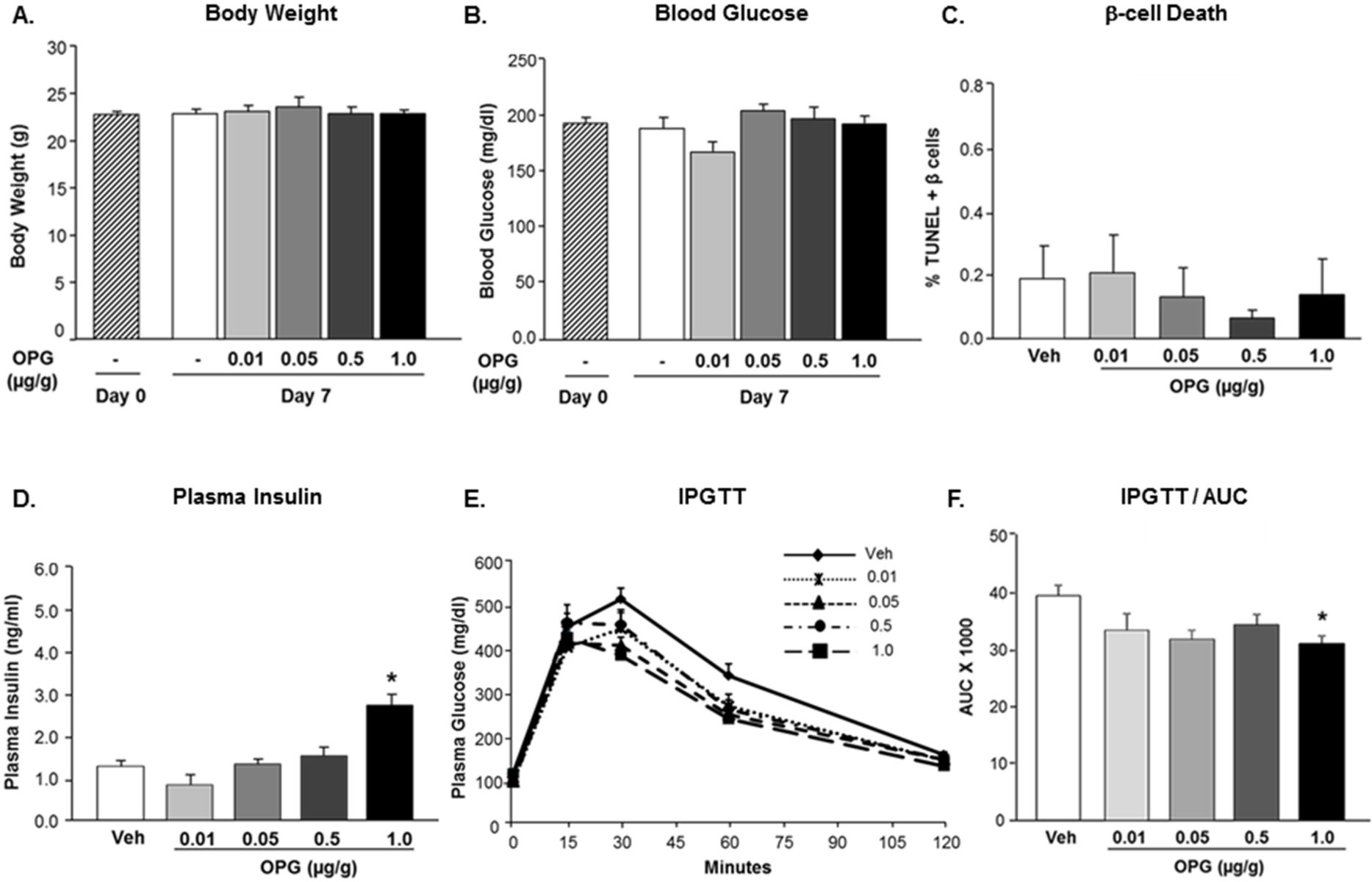


Figure S3; Related to Figure 1

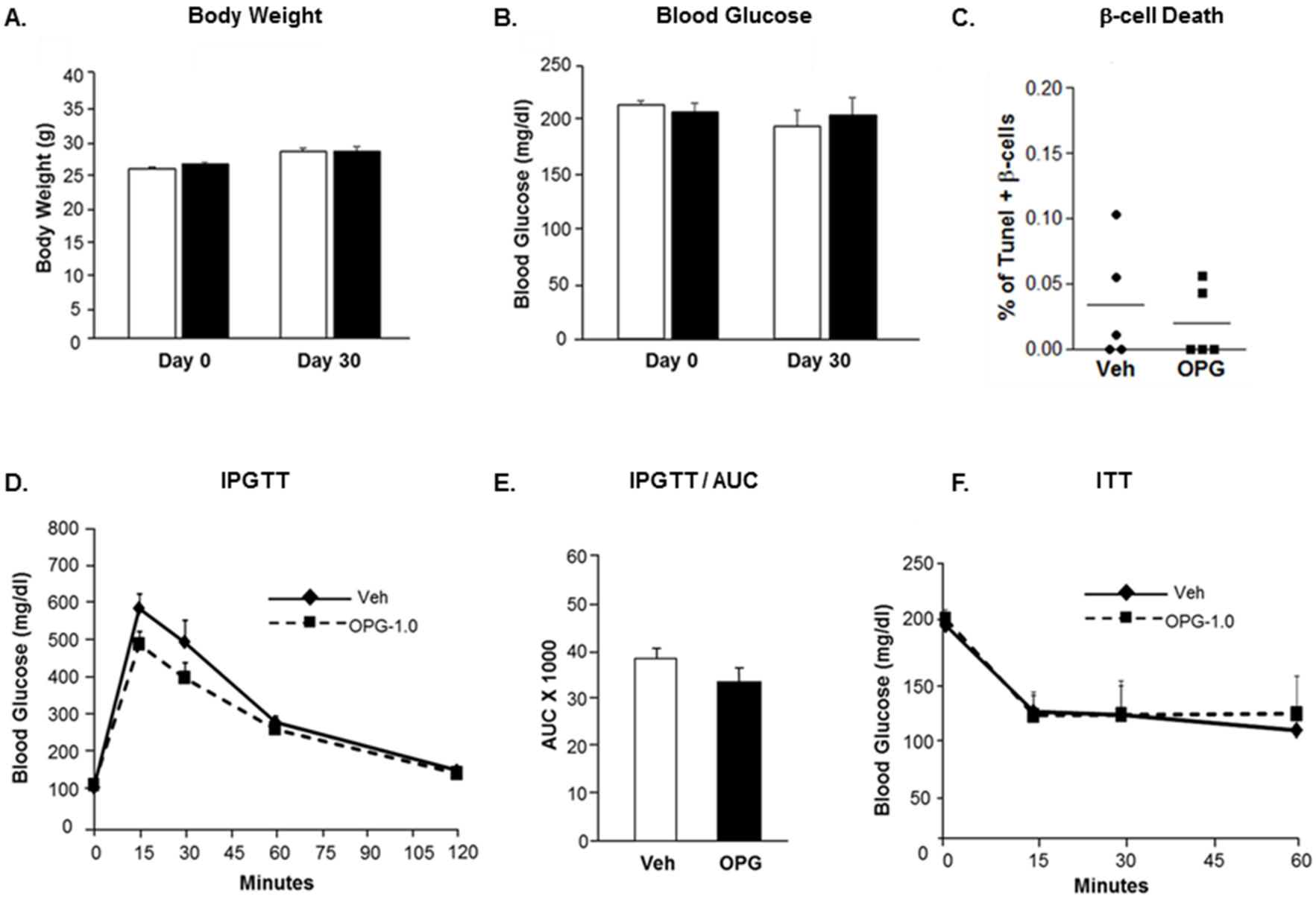
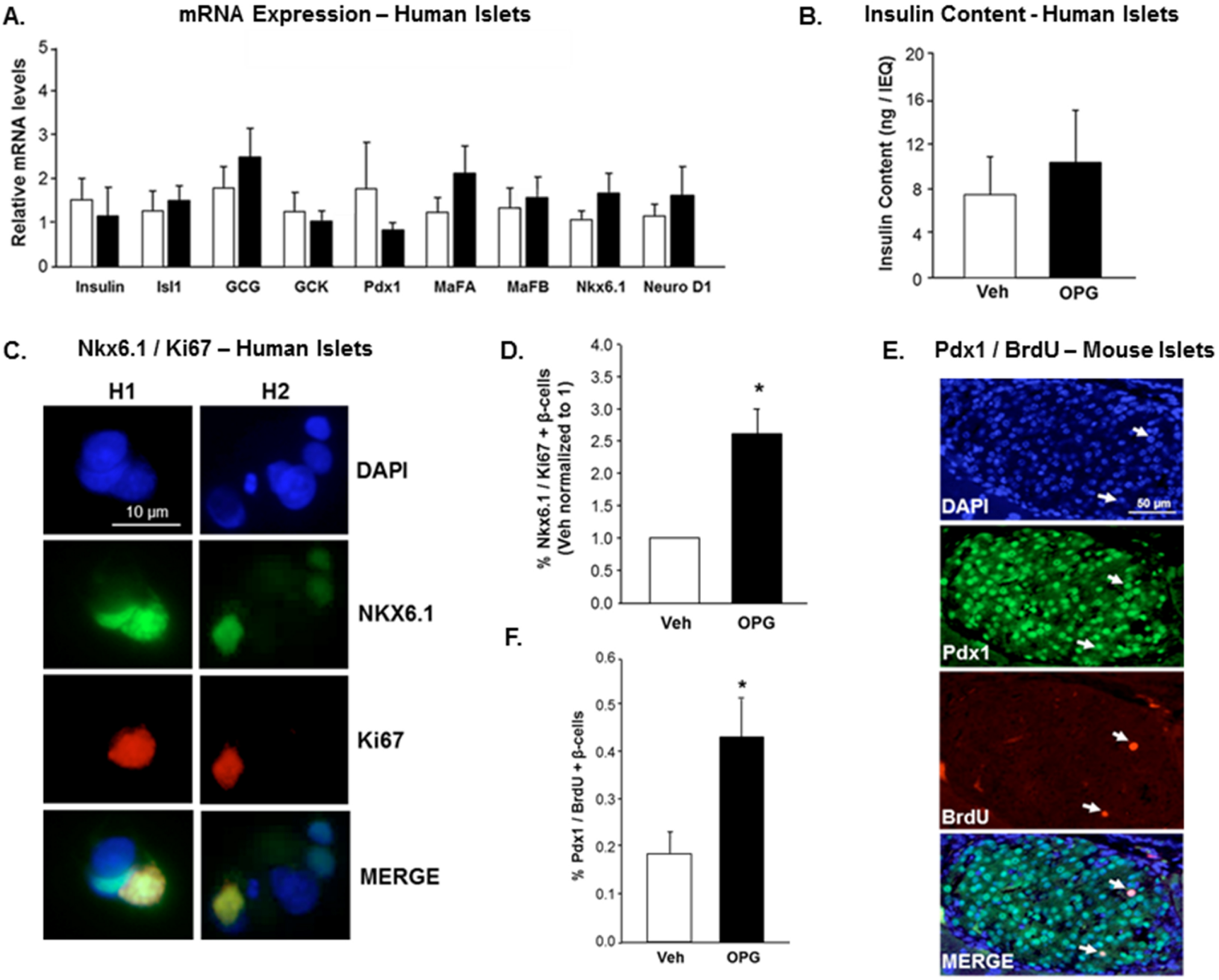


Figure S4; Related to Figure 2



SUPPLEMENTAL FIGURE LEGENDS:

Figure S1, related to Figure 1. OPG is a novel target of lactogens in β-cells.

(A) PCR-array and **(B)** Real time PCR analysis of the ratio of OPG/actin mRNA in islets from normal (NL) and transgenic (TG) RIP-mPL1 mice (n=4-5 mice/group). **(C)** Ratio of OPG/actin mRNA by real time PCR in INS-1 cells treated with vehicle (Veh) or prolactin (PRL) for 24h (n=6). **(D)** NL and TG islets, and INS-1 cells treated with Veh or PRL for 24h, analyzed for OPG and tubulin by western blot (n=2).

Figure S2, related to Figure 1. Glucose homeostasis and β-cell survival in mice treated with OPG for 7 days. (A) Body weight, **(B)** Blood glucose, **(C)** Percent β-cell death, **(D)** Plasma insulin, **(E)** IPGTT at day 5, (solid line with diamond represents Veh-treated, dashed lines are OPG-treated, with cross representing 0.01, triangle representing 0.05, circle representing 0.5, and square representing 1.0μg/g of mOPG-Fc), and **(F)** Area under the curve (AUC) for IPGTT, in mice treated with vehicle (Veh) or different concentrations of mOPG-Fc for 7 days. The body weight and blood glucose at day 0 are the average of all the mice before initiation of treatment. (n=5-15 mice/group); *$p<0.05$ vs Veh.

Figure S3, related to Figure 1. Glucose homeostasis in mice treated with OPG for 30 days. (A) Body weight **(B)** Blood glucose, **(C)** Percent β-cell death (each point represents data from one mouse; 3243±457 β-cells counted/mouse), **(D)** IPGTT at day 25, **(E)** AUC for IPGTT, and **(F)** ITT at day 21, in mice treated with Vehicle or 1.0μg/g of mOPG-Fc every alternate day for 30 days. White bars represent vehicle-treated and black bars represent OPG-treated mice; diamond with solid line represents vehicle-treated, and square with dashed line represents OPG-treated mice. (n=5 mice/group).

Figure S4, related to Figure 2. Gene expression and insulin content in human islets, and co-immunostaining of proliferation marker and β-cell transcription factor in human and rodent islets treated with OPG. (A) Gene expression analysis by real time PCR for genes related to β-cell function and differentiation, relative to actin, the housekeeping gene used as internal control. (n=4-5 human islet preps) **(B)** Measurement of insulin content per islet equivalent (IEQ) in human islets treated with vehicle or 0.1µg/ml of hOPG-Fc for 24h. (n=3 human islet preps). **(C)** Representative images of nuclear DAPI (blue), NKX6.1 (green), and Ki67 (red) staining shown individually and merged for two human islet cell preps (H1, H2) treated with hOPG-Fc for 24h co-stained for Ki67 and NKX6.1. **(D)** Percent Ki67/NKX6.1 double-positive cells relative to NKX6.1-positive cells in Veh- (normalized to 1) versus hOPG-treated human islet cells (n=4 human islet preps); *p<0.05 vs Veh. **(E)** Image of OPG-treated mouse pancreatic section co-stained for DAPI (blue), Pdx1 (green), and BrdU (red) shown individually and merged. The white arrows represent double-positive cells. **(F)** Percent BrdU/Pdx1 double-positive cells relative to Pdx1-positive cells in Veh- versus mOPG-treated pancreatic sections (n=5-6 mice/group); *p<0.05 vs Veh.

Supplemental Table 1 (related to Experimental Procedures): Human islet preps

Human Islet Preps	Age (years)	Gender	BMI	Purity (%)	Viability (%)
AAJC32	54	F	37	90	91
AAJW036A	51	M	23.0	75	98
AAKT155	46	F	36.9	90	90
AAL5258A	61	M	37.4	60	90
ABE1387	58	M	34.8	95	95
ABIK168	53	M	26.4	73	98
ABI3265	38	M	27.40	88	97
ACAM113	18	M	27.90	80	97
ACAX261A	35	F	34.80	90	90
ACBDO86	53	M	20.10	95	95
ACBO251	45	F	32.90	90	98
H-418	59	F	22.6	85	92
H-436	28	M	29.7	91	94
H-2014	61	M	20.9	50	99
H-2027	33	M	23.7	90	90
HP-2048	40	M	23.2	95	92
HP-11310-01	26	M	22.7	90	95
HP-12020-01	56	M	30.9	90	95
HP-12076-01	28	M	32.0	85	98
HP-12166-01	46	M	26.6	95	95
HP-12133-01A	62	M	26.0	90	98
HP-12180-01A	56	F	30.1	95	95
HP-12115-01B	47	M	33.1	50	80
MGH-HI-105	42	M	34.1	60	95
ZCE-161	55	F	22.3	80	94

Supplemental Table 2 (related to Experimental Procedures): Primer sequences for real time PCR

PRIMERS	REVERSE	FORWARD
Actin (Human)	CTCCTTAATGTCACGCACGAT	CATGTACGTTGCTATCCAGGC
Actin (Mouse)	GCATGCAGAAGGAGATCACA	TTGTCGATTGTCGTCCTGAG
Actin (Rat)	TGGGAATGAAGATCCTCCAG	GAGGAAGGAAAGGGCCTATG
Glucagon (Human)	TCACCAGCCAAGCAATGAAT	GATGAACGAGGACAAGCGCC
Glucokinase (Human)	TTGATTCCAGCGAGAAAGGTG	CTTCCCTCAGTTTTTCGGTGG
Insulin (Human)	ACAATGCCACGCTTCTGCAGGGAC	TCACACCTGGTGGAAGCTCTCTA
Isl1 (Human)	CGTTCTTGCTGAAGCCGATG	ATTTCCCTATGTGTTGGTTGCG
Maf A (Human)	CTCGTATTTCTCCTTGTACAGGTCC	CTTCAGCAAGGAGGAGGTCATC
Maf B (Human)	CACGCAGCCGCCGGAGTTTC	TGAACTTTGCGCGTTAAGCC
Neuro D1 (Human)	CGGCGGAGGCTTAACGTGGA	GACTGAACGCGGCGCTAGAC
Nkx6.1 (Human)	TGCTGGACTTGTGCTTCTTCAAC	ACACGAGACCCACTTTTTCCG
Pdx1 (Human)	TGATGTGTCTCTCGGTCAAGTT	ACCAAAGCTCACGCGTGGAAA
Rank (Mouse)	CTTCTTCATTCCAGGTGTCCA	GCACCCAGGAGAGGCATTAT
Rank (Rat)	ATGAGCATCTTGGACGGTGT	CCTTGCCTGCATCACAGAC
TNFRSF11B (OPG; Mouse)	TTGTGAAGCTGTGCAGGAAC	CTGCCTGGGAAGAAGATCAG
TNFRSF11B (OPG; Rat)	CACGGCATTATCACCAACTG	CCGGAGGCATAGAGAGACAG