Supplemental Figure legends

Figure S1, related to Figure 1. mRNA levels

(A) Relative mRNA expression of three FoxO isoforms in normal islets.

(B) mRNA levels of three FoxO isoforms in islets of mice fed normal chow (NC) or high-fat diet (HFD) for 30 weeks.

(C) Quantification of mRNA encoding the main pancreatic hormones in wildtype and β-FoxO KO mice.

(D) mRNA analysis of markers of β-cell function, pluripotency, and progenitor cells in 10-month-old β-FoxO KO and wildtype mice. Each experiment (n=3 per group) was performed with pooled islets from 3-4 mice per genotype. Each data point consists of 4 replicates. Data represent means ± SEM. *$p<0.05$, **$p<0.01$, ***$p<0.001$ by Student's *t* test.

Figure S2, related to Figure 2. Insulin tolerance tests and glucose levels during hyperglycemic clamp

(A) Glucose levels, plotted as % of basal values, following intraperitoneal injection of insulin (0.15 U/kg) in wildtype and β-FoxO KO mice (n=8 mice per genotype).

(B) Clamp glucose level. The data represent means ± SEM.

Figure S3, related to Figure 3. Marker analysis of β-FoxO KO islets

(A) Immunohistochemistry showing a representative image with antibodies to Pcsk1, Pcsk2, Glut-2 and Pdx-1 in 24-week-old (n=4 mice per group, 3 sections per mouse).

(B) Quantification of mRNA encoding β-cell transcription factors. Data represent means ± SEM of qPCR analyses from mRNA extracted from 16-wk-old mice (n=3 per group).

Each experiment was performed with pooled islets from 3 mice per genotype. Each data point consists of 4 replicates.

Figure S4, related to Figure 4. Lipid and glucose metabolism in KO islets

(A) Phospholipid generation in isolated islets using ^{14}C-labeled palmitate incorporation in the presence of low (2.8 mM) and high (16.7 mM) glucose concentrations.

(B) Free fatty acid (FFA) generation from labeled palmitate in isolated islets.

(C) Glucose or pyruvate stimulated insulin secretion in the absence or presence of etomoxir (1uM) in primary islets. Each experiment (n=3 per group) was performed with pooled islets from 5 mice per genotype. Each data point consists of 6-9 replicates. All data are presented as means ± SEM. *$p<0.05$ by Student's *t* test.

Figure S5, related to Figure 6. Validation of RNA profiling data

mRNA levels of selected target genes, including isoforms of L-type voltage-gated Ca^{2+} channels, protein kinase C, inositol-phosphate receptors, and phospholipase C. Each experiment (n=3 per group) was performed with pooled islets from 3 mice per genotype. Each data point consists of 3 replicates. All data are presented as means ± SEM with p-< 0.05 by Student's *t* test. Asterisks are omitted for simplicity.

Table S1, related to Table 1. Predicted activation state of cellular and molecular functions

Category	*Functions Annotation*	*p-value*	*Predicted Activation State*	*z-score*
Cell Signaling / Vitamin and Mineral Metabolism	Translocation of Ca2+	5.09×10^{-3}	Decreased	-3.095
Gene Expression	Expression of RNA	3.20×10^{-2}	Decreased	-2.631
Cell Death	Survival of tumor cell line	9.20×10^{-3}	Decreased	-2.319
Cellular Assembly and Organization	Transport of vesicles	1.76×10^{-4}	Decreased	-2.178
Organismal Survival	Organism death	1.79×10^{-2}	Increased	2.050
Tissue Development	Extracellular matrix adhesion	1.40×10^{-3}	Increased	2.081
Molecular Transport	Transport of fatty acid	3.45×10^{-2}	Increased	2.167
Energy production/ Small Molecule Biochemistry/ Lipid Metabolism	Oxidation of lipid	4.43×10^{-3}	Increased	2.170
Energy production/ Small Molecule Biochemistry/ Lipid Metabolism	Oxidation of fatty acid	2.28×10^{-2}	Increased	2.211
Small Molecule Biochemistry/ Lipid Metabolism	Cleavage of glycosphingolipid	7.53×10^{-3}	Increased	2.401
Small Molecule Biochemistry/ Lipid Metabolism	Cleavage of sphingolipid	1.91×10^{-3}	Increased	2.599

The activation state of cellular and molecular functions in the described biological categories, selected from the analysis of the β-FoxO KO transcriptome.

Table S2, related to Table 1. Predicted activation state of transcription factors

Transcription Regulator	*Predicted Activation State*	*z-score*	*p-value*
Hic1	Activated	2.796	1.8×10^{-3}
Pparα	Activated	2.417	1.3×10^{-1}
Pparγ	Activated	2.385	2.4×10^{-1}
Mycn	Activated	2.300	3.9×10^{-1}
Rb1	Activated	2.137	1.1×10^{-1}
Foxm1	Inhibited	-2.000	3.4×10^{-2}
Irf7	Inhibited	-2.004	1.7×10^{-1}

Computational prediction of the activation state of transcriptional networks in β-FoxO KO mice, based on transcriptome analysis. A Z-score >2 is predictive of activation, while a z-score < –2 is predictive of inhibition.

Table S3, related to Table 1. Top 25 networks

Name	*Score*
Gene Expression, Post-Translational Modification, Cellular Compromise	44
Cellular Function and Maintenance, Molecular Transport, Cell-To-Cell Signaling and Interaction	41
Cell Signaling, Nucleic Acid Metabolism, Small Molecule Biochemistry	41
RNA Post-Transcriptional Modification, Cellular Movement, Post-Translational Modification	41
Cell-To-Cell Signaling and Interaction, Cellular Assembly and Organization, Nervous System Development and Function	40
Carbohydrate Metabolism, Lipid Metabolism, Post-Translational Modification	39
Drug Metabolism, Endocrine System Development and Function, Lipid Metabolism	39
RNA Post-Transcriptional Modification, Hereditary Disorder, Neurological Disease	38
Hereditary Disorder, Metabolic Disease, Cardiovascular Disease	38
Embryonic Development, Nervous System Development and Function, Organ Development	38
DNA Replication, Recombination, and Repair, Cell Cycle, Cell Death	37
Nucleic Acid Metabolism, Small Molecule Biochemistry, Cardiovascular Disease	37
Cell Cycle, DNA Replication, Recombination, and Repair, Cell-To-Cell Signaling and Interaction	35
Cancer, Cell-To-Cell Signaling and Interaction, Inflammatory Response	35
Dermatological Diseases and Conditions, Developmental Disorder, Organismal Injury and Abnormalities	34
Drug Metabolism, Protein Synthesis, Cancer	34
Protein Synthesis, Gene Expression, Cardiovascular System Development and Function	34
Cell Death, Inflammatory Response, Infectious Disease	33
Developmental Disorder, Hereditary Disorder, Neurological Disease	32
Cell-To-Cell Signaling and Interaction, Cellular Function and Maintenance, Molecular Transport	32
Cellular Assembly and Organization, Cellular Movement, Nervous System Development and Function	32
Cell-To-Cell Signaling and Interaction, Nervous System Development and Function, Cell Morphology	31
Tissue Development, Cell-To-Cell Signaling and Interaction, Embryonic Development	31
Cellular Function and Maintenance, Cellular Assembly and Organization, Gastrointestinal Disease	30
Hereditary Disorder, Infectious Disease, Carbohydrate Metabolism	30

Non-directional networks from the analysis of β-FoxO KO transcriptome were generated based on the connectivity of Focus Genes in the ingenuity knowledge base. A higher score indicates greater statistical significance with < 1% chance of the Focus Genes being together in a network due to random chance.

Table S4, related to Table 1. List of genes regulated by ≥ ± 2-fold

Gene Symbol	*RefSeq*	*p-value*	*Fold-Change*	*KO*
Serpina7	NM_177920	0.0111642	6.10514	Up
Rsl1	NM_001013769	0.0414689	4.99012	Up
Tcea1	NM_011541	0.0428449	4.72036	Up
Ly96	NM_016923	0.0145388	3.54036	Up
Asb11	NM_026853	0.00140206	2.9386	Up
Tc2n	NM_001082976	0.0290396	2.91161	Up
Aldh1a3	NM_053080	4.90E-06	2.87677	Up
Fabp3	NM_010174	0.0107567	2.7083	Up
Bet1	NM_009748	0.0446089	2.69339	Up
Naa38	NM_133939	0.0279857	2.68719	Up
Aass	NM_013930	0.00663965	2.58233	Up
Gnpda2	NM_001038015	0.0200575	2.54645	Up
Tmed6	NM_025458	0.0154591	2.52494	Up
Arsk	NM_029847	0.00890659	2.5013	Up
4930432O21Rik	NM_001025373	0.0163108	2.47144	Up
S100a1	NM_011309	0.00886515	2.44876	Up
Gtf3c6	NM_026113	0.00778928	2.42173	Up
Gm3604	NM_001162910	0.00843881	2.41623	Up
Cetn2	NM_019405	0.00555014	2.40101	Up
0610009B22Rik	BC024353	0.0124628	2.39262	Up
Vamp7	NM_011515	0.0383625	2.39248	Up
Fam96a	NM_026635	0.0331078	2.33822	Up
Ppp2r3c	NM_021529	0.0271285	2.33742	Up
Arl6	NM_019665	0.020463	2.29758	Up
Tceal1	NM_146236	0.0173341	2.28281	Up
Fam107a	NM_183187	0.00143458	2.28199	Up
Nxt2	NM_172782	0.0230735	2.24335	Up
Dhrs1	NM_026819	0.0400549	2.24112	Up
Mc5r	NM_013596	0.0277206	2.24063	Up
Zfp758	NM_145484	0.00877272	2.23204	Up
Gabra4	NM_010251	0.0029828	2.21679	Up
Gstk1	NM_029555	0.0250684	2.21367	Up
Gc	NM_008096	0.0302735	2.2136	Up
Ube2g1	NM_025985	0.0196621	2.1793	Up
Sumo1	NM_009460	0.0492272	2.17814	Up
Tnfrsf11b	NM_008764	0.00451015	2.17536	Up
Ndufs8	NM_144870	0.0204631	2.15693	Up
Pou3f4	NM_008901	0.0356638	2.14325	Up
Gatc	NM_029645	0.0308833	2.13377	Up
Il1r2	NM_010555	0.00557243	2.12099	Up
Nsun3	NM_178925	0.0233136	2.12087	Up
Ubl5	NM_025401	0.0238611	2.10567	Up
Zfp758	NM_145484	0.00554606	2.09893	Up
Spc25	NM_025565	0.0219171	2.08955	Up
Pfn2	NM_019410	0.0435835	2.08917	Up
BC048546	NM_001001179	0.0291405	2.07485	Up
Eapp	NM_025456	0.0344546	2.06206	Up
Srek1ip1	NM_026075	0.0176001	2.05832	Up
Fundc1	NM_028058	0.0131075	2.04164	Up
Ndufs4	NM_010887	0.0318198	2.03858	Up
Trappc2	NM_025432	0.0156154	2.0358	Up
Cript	NM_019936	0.0399088	2.02949	Up
Crbn	NM_021449	0.0194331	2.02776	Up
Ndufb9	NM_023172	0.0354024	2.02202	Up
Nupr1	NM_019738	0.0143316	2.01786	Up
Nudt11	NM_021431	0.0470274	2.00691	Up
Eml5	NM_001081191	0.0174169	-2.04931	Down
Muc4	NM_080457	0.0121668	-2.04958	Down

Meg3	NR_027651	0.010206	-2.22119	Down
Myl7	NM_022879	0.010106	-2.33234	Down
6330403K07Rik	NM_134022	8.77E-05	-2.68071	Down
Eid3	NM_025499	0.0280126	-3.14097	Down
Gm3579	AY140896	0.0334865	-3.53078	Down

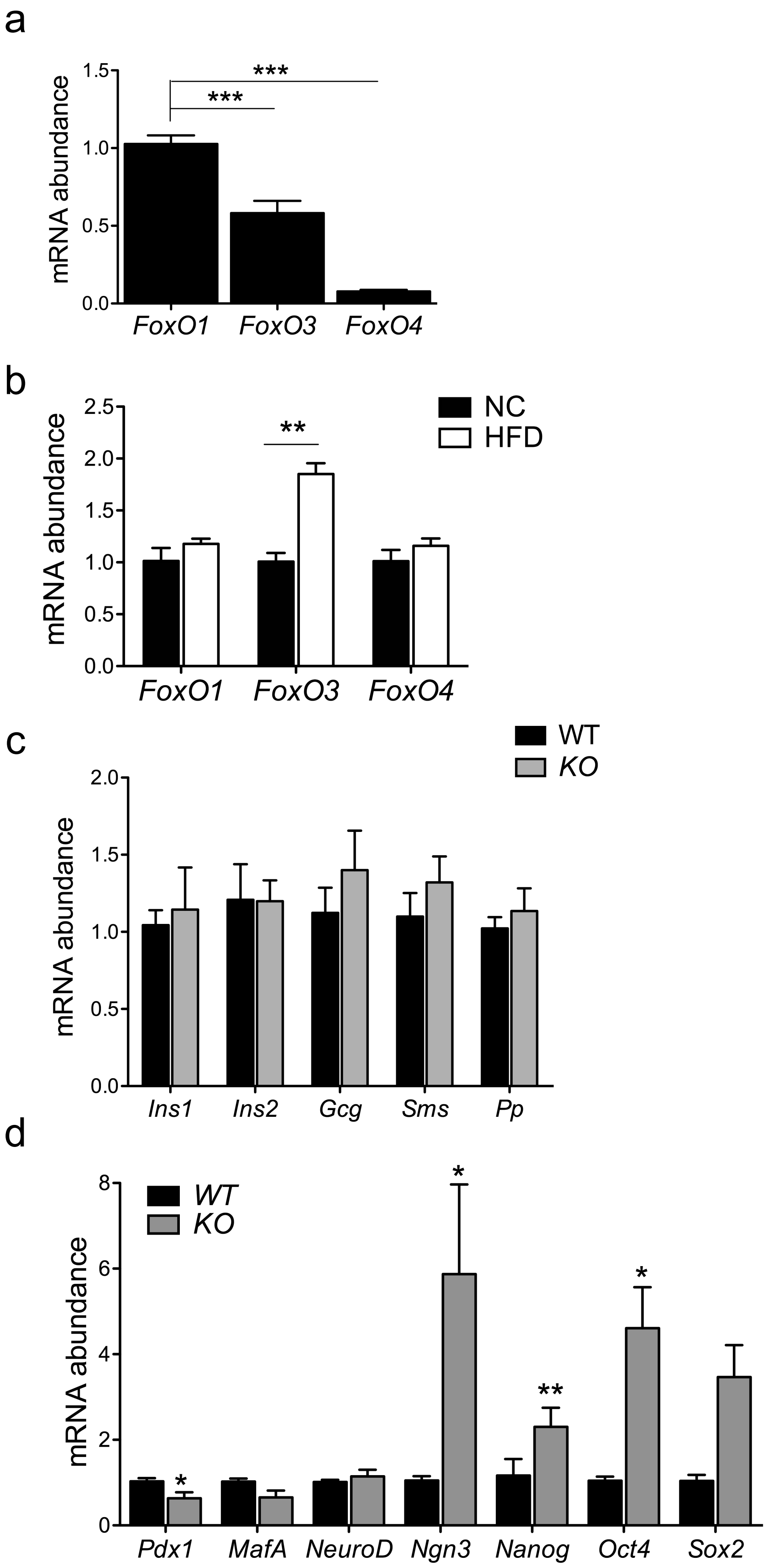
a
mRNA abundance
1.5
1.0
0.5
0.0
***
***
FoxO1
FoxO3
FoxO4
b
mRNA abundance
2.5
2.0
1.5
1.0
0.5
0.0
NC
HFD
**
FoxO1
FoxO3
FoxO4
c
WT
KO
mRNA abundance
2.0
1.5
1.0
0.5
0.0
Ins1
Ins2
Gcg
Sms
Pp
d
WT
KO
mRNA abundance
8
6
4
2
0
*
*
**
*
Pdx1
MafA
NeuroD
Ngn3
Nanog
Oct4
Sox2

Figure S1

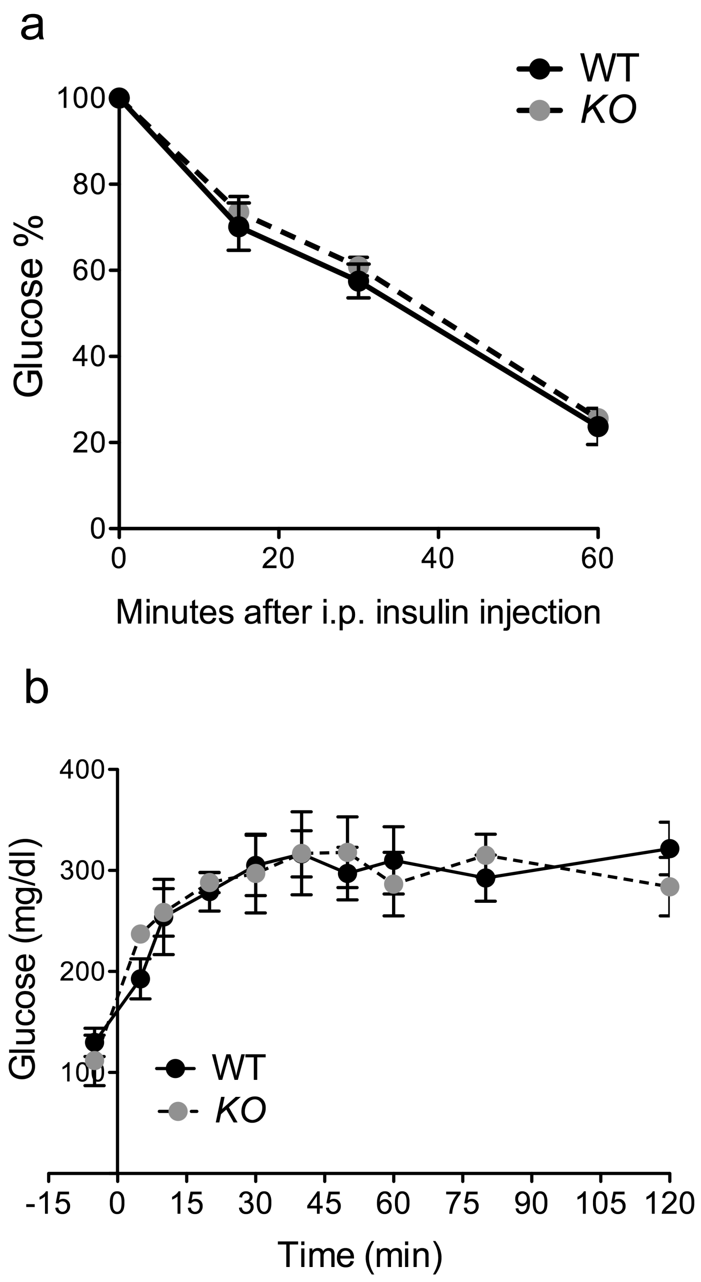
a
WT
KO
Glucose %
100
80
60
40
20
0
0
20
40
60
Minutes after i.p. insulin injection
b
Glucose (mg/dl)
400
300
200
100
WT
KO
-15
0
15
30
45
60
75
90
105
120
Time (min)

Figure S2

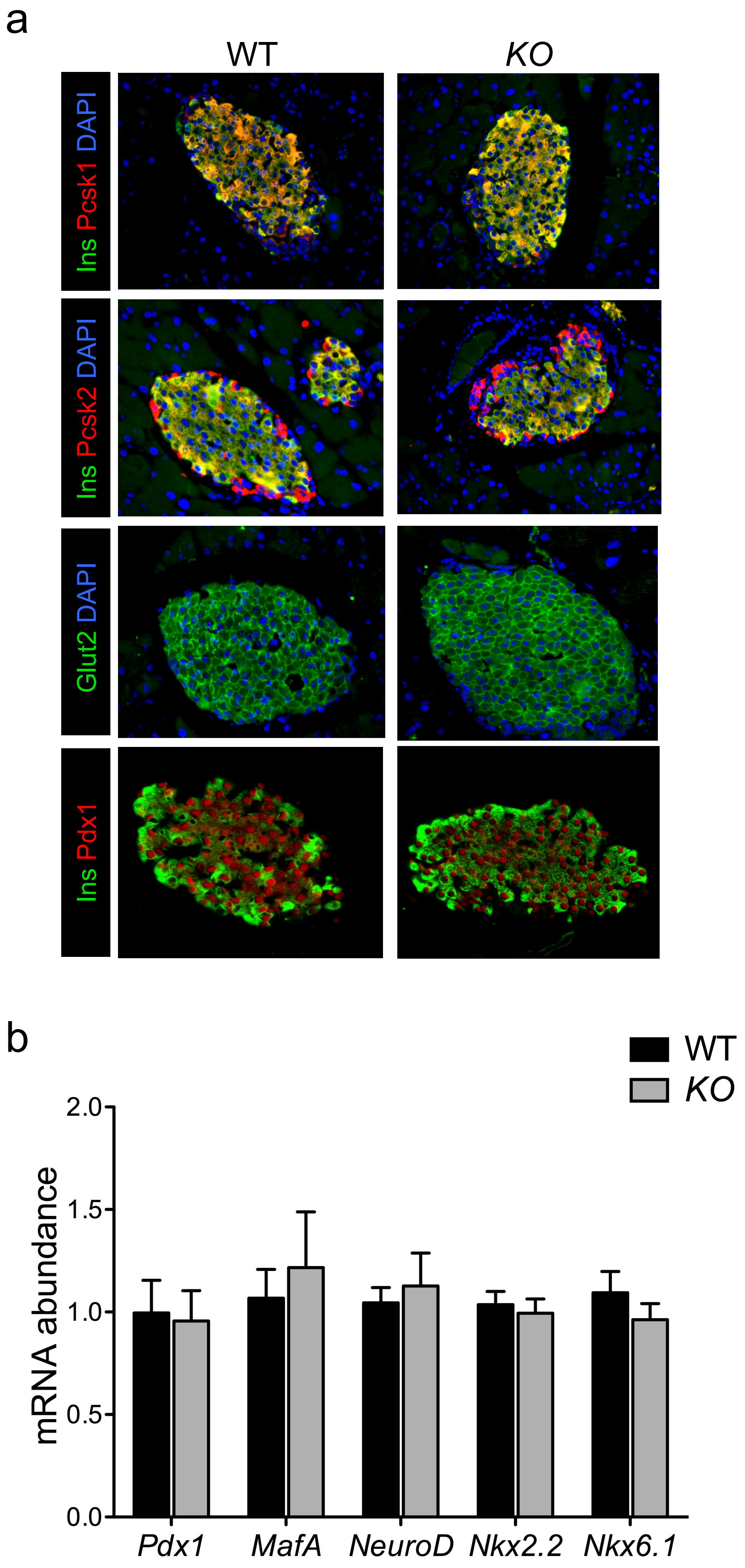
a
WT
KO
Ins Pcsk1 DAPI
Ins Pcsk2 DAPI
Glut2 DAPI
Ins Pdx1
b
WT
KO
mRNA abundance
2.0
1.5
1.0
0.5
0.0
Pdx1
MafA
NeuroD
Nkx2.2
Nkx6.1

Figure S3

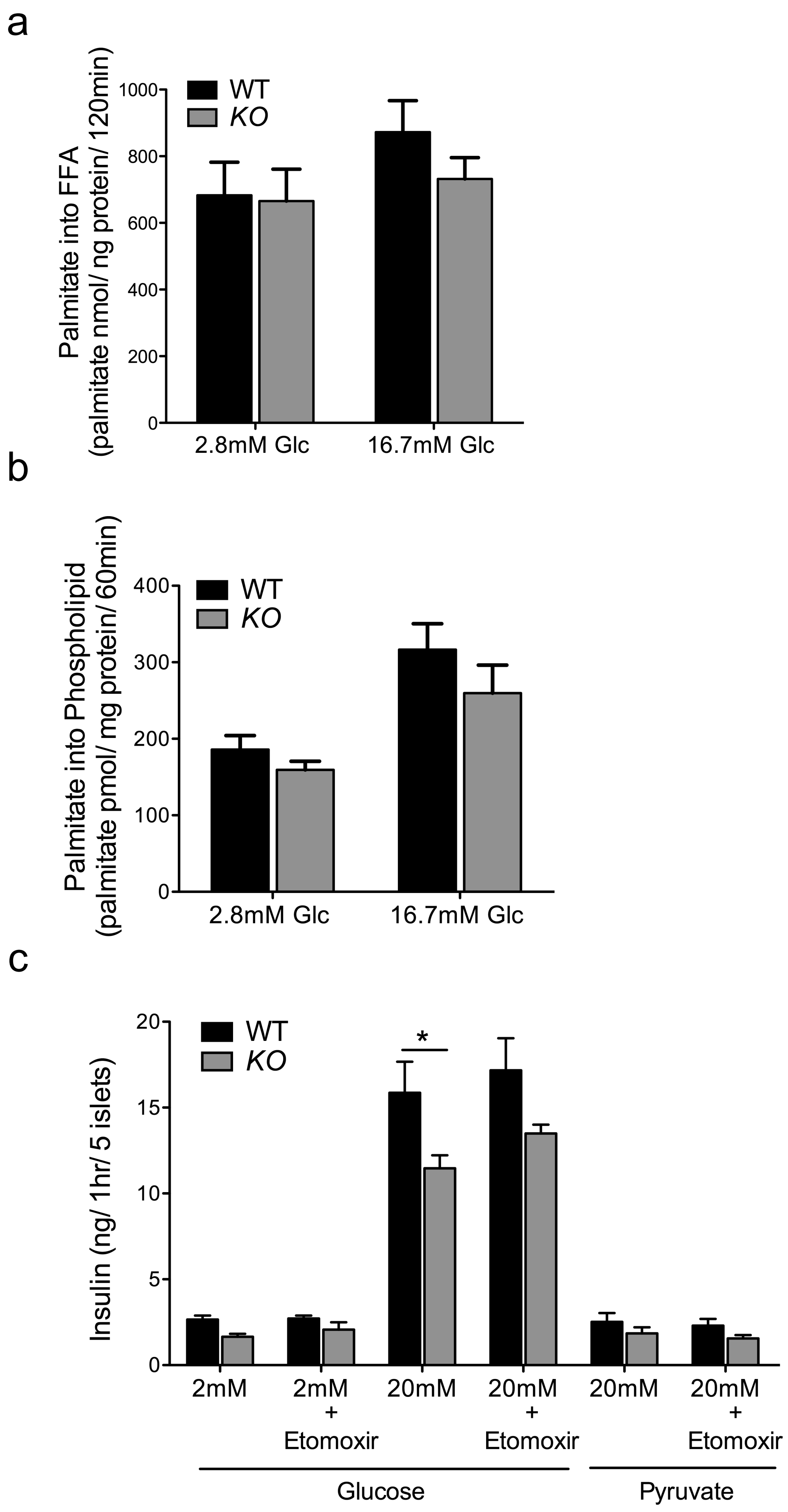
a
Palmitate into FFA
(palmitate nmol/ ng protein/ 120min)
WT
KO
1000
800
600
400
200
0
2.8mM Glc
16.7mM Glc
b
Palmitate into Phospholipid
(palmitate pmol/ mg protein/ 60min)
WT
KO
400
300
200
100
0
2.8mM Glc
16.7mM Glc
c
Insulin (ng/ 1hr/ 5 islets)
WT
KO
*
20
15
10
5
0
2mM
2mM + Etomoxir
20mM
20mM + Etomoxir
20mM
20mM + Etomoxir
Glucose
Pyruvate

Figure S4

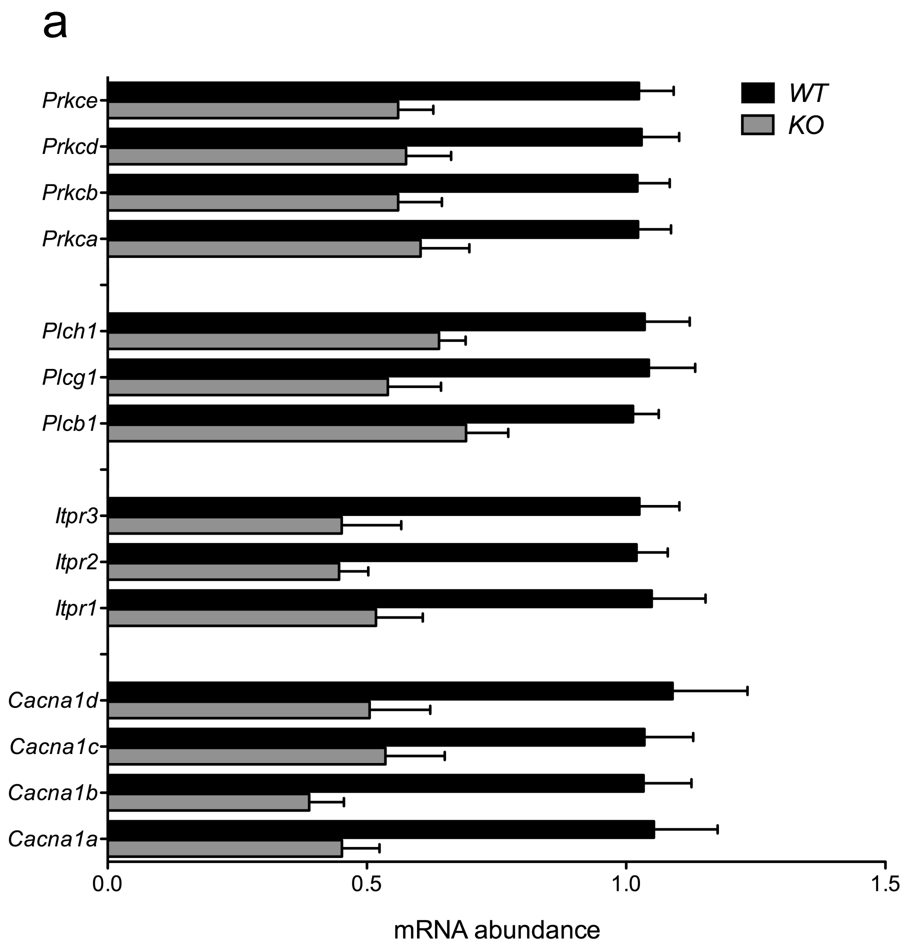
a
WT
KO
Prkce
Prkcd
Prkcb
Prkca
Plch1
Plcg1
Plcb1
Itpr3
Itpr2
Itpr1
Cacna1d
Cacna1c
Cacna1b
Cacna1a
0.0
0.5
1.0
1.5
mRNA abundance

Figure S5