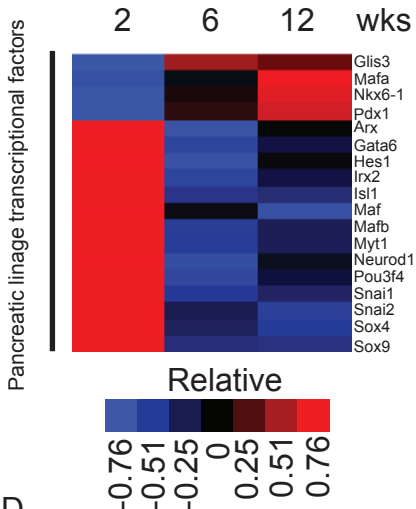
A



B

Enriched Pathway in neonate islets

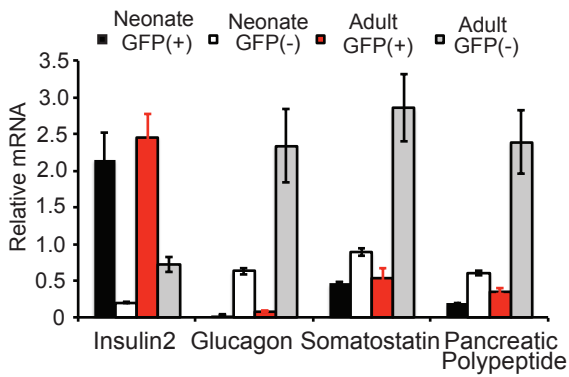
GO Biological Function	Count	p value
cell adhesion	114	1.40E-25
vasculature matrix organization	57	3.80E-15
extracellular matrix organization	31	5.40E-12
regulation of cell proliferation	84	5.70E-12
cell motion	65	8.50E-12
regulation of cell migration	26	2.50E-09
response to hypoxia	18	1.30E-06
regulation of Wnt receptor signaling pathway	9	5.00E-03

C

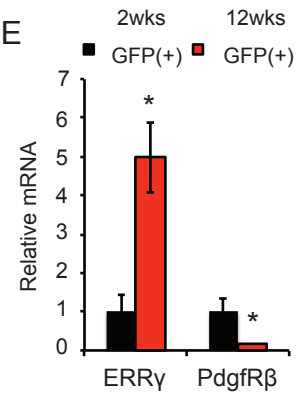
Enriched Pathway in adult islets

GO Biological Function	Count	p value
vesicle-mediated transport	57	1.60E-08
monosaccharide metabolic process	26	3.90E-05
oxidation reduction	60	1.60E-04
secretion	27	1.60E-04
cation transport	49	1.70E-04
generation of precursor metabolites and energy	26	4.10E-03
ATP biosynthetic process	10	3.30E-02
electron transpot chain	8	4.10E-01

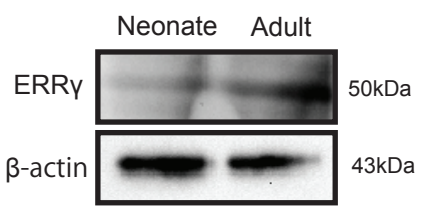
D



E



F



G

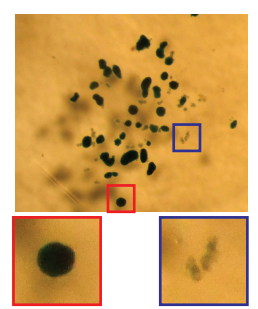


Figure S1

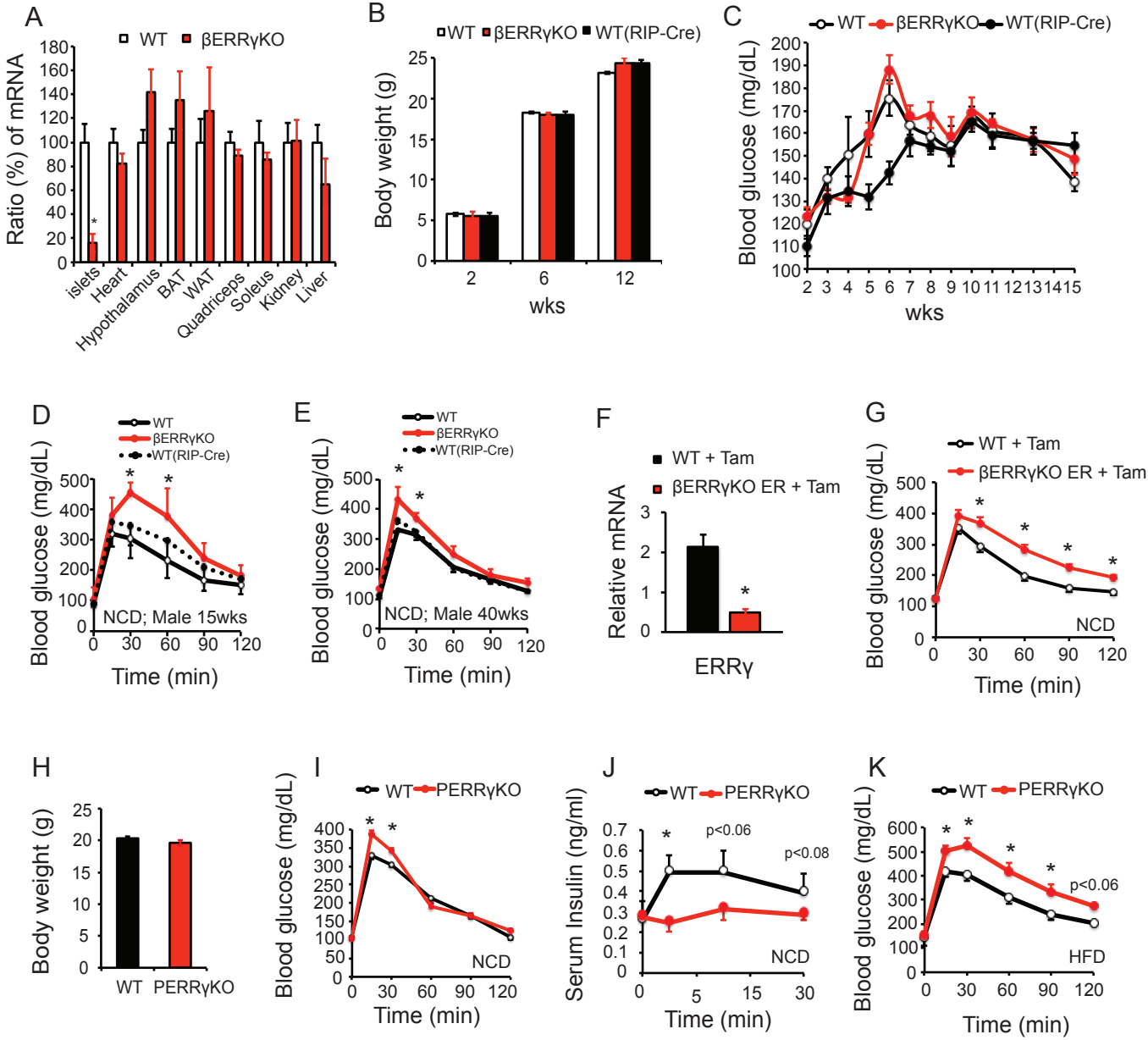

A
WT
βERRγKO
Ratio (%) of mRNA
islets
Heart
Hypothalamus
BAT
WAT
Quadriceps
Soleus
Kidney
Liver
B
WT
βERRγKO
WT(RIP-Cre)
Body weight (g)
wks
C
WT
βERRγKO
WT(RIP-Cre)
Blood glucose (mg/dL)
wks
D
WT
βERRγKO
WT(RIP-Cre)
Blood glucose (mg/dL)
NCD; Male 15wks
Time (min)
E
WT
βERRγKO
WT(RIP-Cre)
Blood glucose (mg/dL)
NCD; Male 40wks
Time (min)
F
WT + Tam
βERRγKO ER + Tam
Relative mRNA
ERRγ
G
WT + Tam
βERRγKO ER + Tam
Blood glucose (mg/dL)
NCD
Time (min)
H
Body weight (g)
WT
PERRγKO
I
WT
PERRγKO
Blood glucose (mg/dL)
NCD
Time (min)
J
WT
PERRγKO
p<0.06
p<0.08
Serum Insulin (ng/ml)
NCD
Time (min)
K
WT
PERRγKO
p<0.06
Blood glucose (mg/dL)
HFD
Time (min)

Figure S2

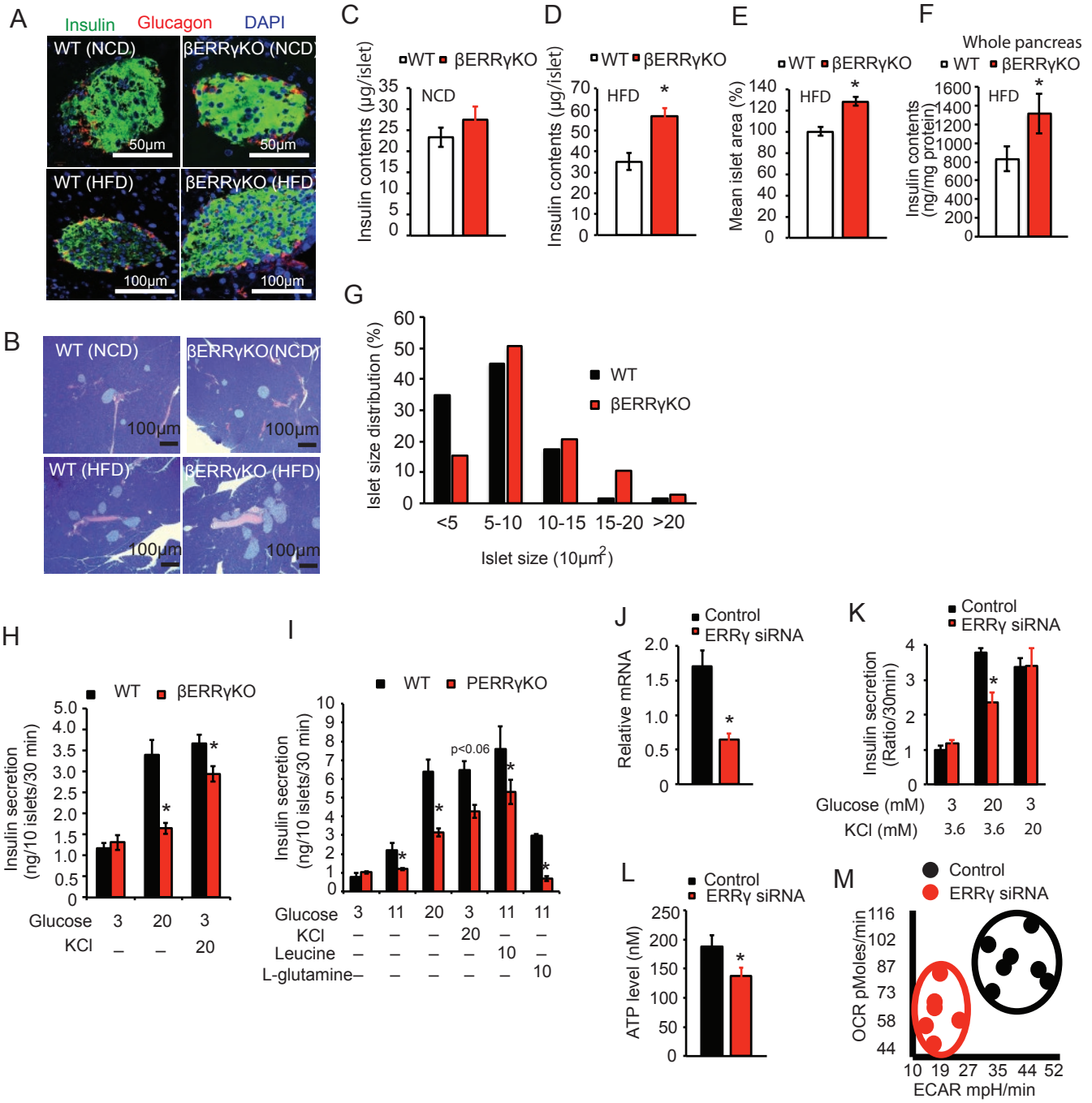
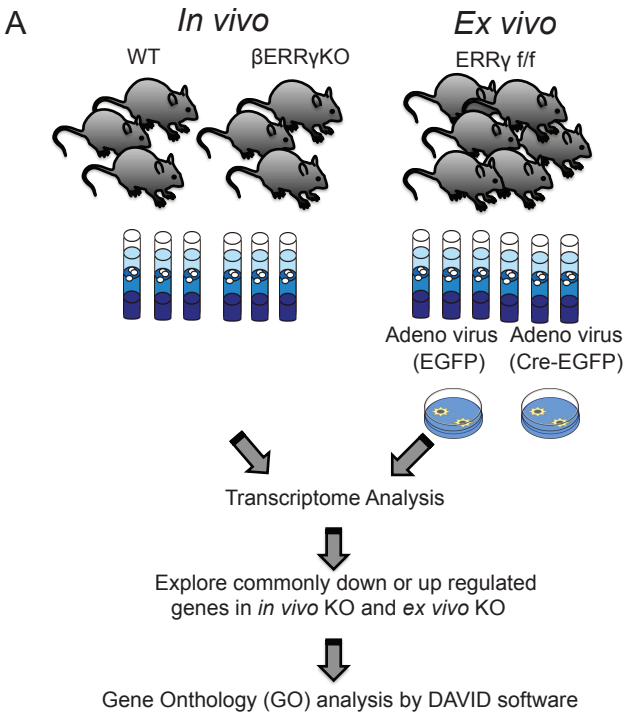
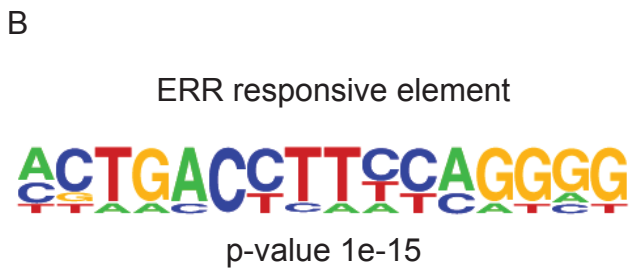
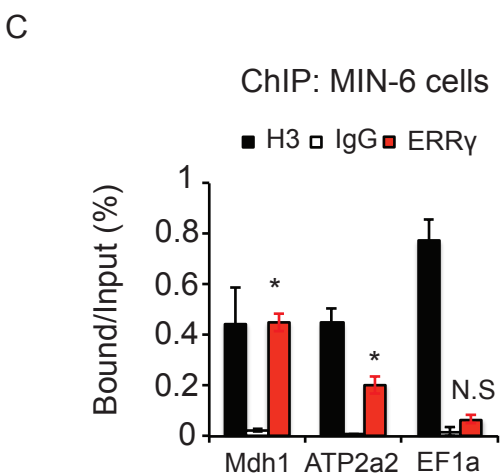

A
Insulin
Glucagon
DAPI
WT (NCD)
βERRγKO (NCD)
WT (HFD)
βERRγKO (HFD)
50μm
100μm
B
WT (NCD)
βERRγKO(NCD)
WT (HFD)
βERRγKO (HFD)
100μm
C
WT βERRγKO
NCD
Insulin contents (μg/islet)
D
WT βERRγKO
HFD
Insulin contents (μg/islet)
E
WT βERRγKO
HFD
Mean islet area (%)
F
Whole pancreas
WT βERRγKO
HFD
Insulin contents (ng/mg protein)
G
WT
βERRγKO
Islet size distribution (%)
<5
5-10
10-15
15-20
>20
Islet size (10μm²)
H
WT βERRγKO
Insulin secretion (ng/10 islets/30 min)
Glucose 3 20 3
KCl – – 20
I
WT PERRγKO
p<0.06
Insulin secretion (ng/10 islets/30 min)
Glucose 3 11 20 3 11 11
KCl – – – 20 – –
Leucine – – – – 10 –
L-glutamine – – – – – 10
J
Control
ERRγ siRNA
Relative mRNA
K
Control
ERRγ siRNA
Insulin secretion (Ratio/30min)
Glucose (mM) 3 20 3
KCl (mM) 3.6 3.6 20
L
Control
ERRγ siRNA
ATP level (nM)
M
Control
ERRγ siRNA
OCR pMoles/min
ECAR mpH/min

Figure S3


A
In vivo
Ex vivo
WT
βERRγKO
ERRγ f/f
Adeno virus (EGFP)
Adeno virus (Cre-EGFP)
Transcriptome Analysis
Explore commonly down or up regulated genes in in vivo KO and ex vivo KO
Gene Onthology (GO) analysis by DAVID software


B
ERR responsive element
p-value 1e-15


C
ChIP: MIN-6 cells
H3
IgG
ERRγ
Bound/Input (%)
1
0.8
0.6
0.4
0.2
0
*
*
N.S
Mdh1
ATP2a2
EF1a

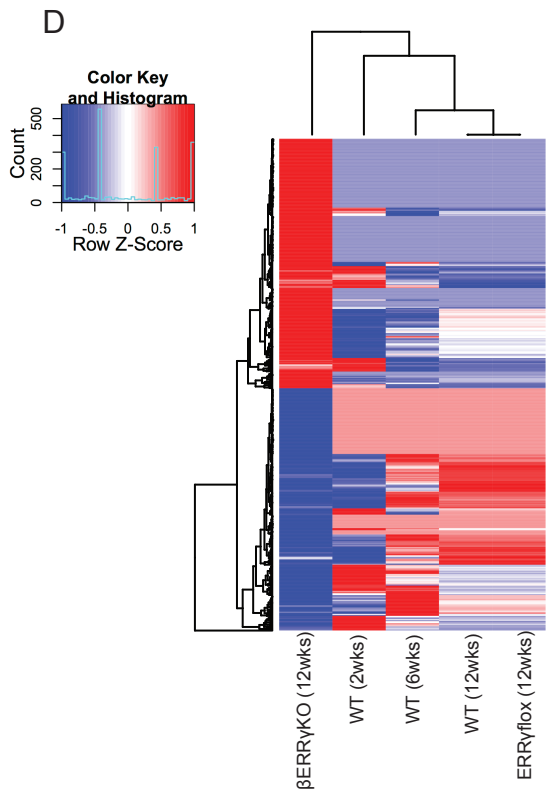

D
Color Key and Histogram
Count
500
200
0
-1
-0.5
0
0.5
1
Row Z-Score
βERRγKO (12wks)
WT (2wks)
WT (6wks)
WT (12wks)
ERRγflox (12wks)

Figure S4

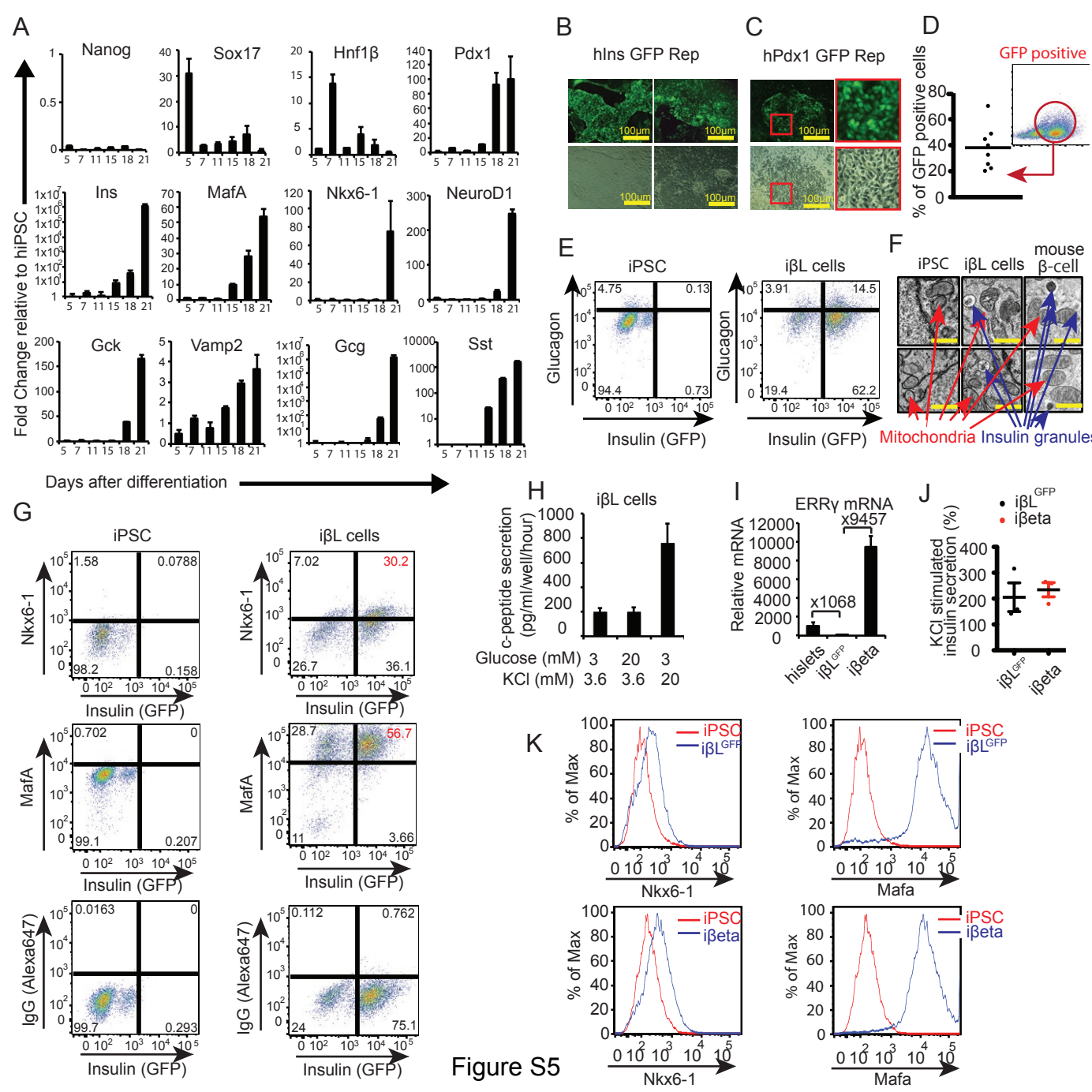
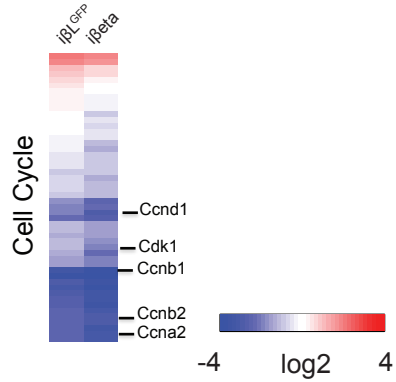

A
Nanog
Sox17
Hnf1β
Pdx1
Ins
MafA
Nkx6-1
NeuroD1
Gck
Vamp2
Gcg
Sst
Fold Change relative to hiPSC
Days after differentiation
B
hIns GFP Rep
C
hPdx1 GFP Rep
100μm
D
GFP positive
% of GFP positive cells
E
iPSC
iβL cells
Glucagon
Insulin (GFP)
4.75
0.13
94.4
0.73
3.91
14.5
19.4
62.2
F
iPSC
iβL cells
mouse β-cell
Mitochondria
Insulin granules
G
iPSC
iβL cells
Nkx6-1
MafA
IgG (Alexa647)
Insulin (GFP)
1.58
0.0788
98.2
0.158
7.02
30.2
26.7
36.1
0.702
0
99.1
0.207
28.7
56.7
11
3.66
0.0163
0
99.7
0.293
0.112
0.762
24
75.1
H
iβL cells
c-peptide secretion (pg/ml/well/hour)
Glucose (mM) 3 20 3
KCl (mM) 3.6 3.6 20
I
ERRγ mRNA
Relative mRNA
x9457
x1068
hislets
iβL GFP
iβeta
J
iβL GFP
iβeta
KCl stimulated insulin secretion (%)
K
iPSC
iβL GFP
iβeta
% of Max
Nkx6-1
Mafa

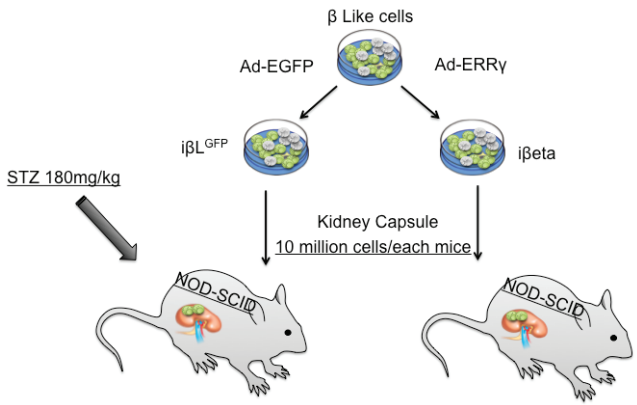
Figure S5

A

GO Biological Function	Count	p-value
response to organic substance	204	2.00E-17
M phase of mitotic cell cycle	75	2.60E-10
nuclear division	74	2.80E-10
mitosis	74	2.80E-10
intracellular signaling cascade	283	5.50E-10
response to hormone stimulus	106	7.80E-10
cell cycle	190	7.90E-10
organelle fission	75	8.20E-10
regulation of apoptosis	195	1.10E-09
cell division	89	2.00E-09



B



C

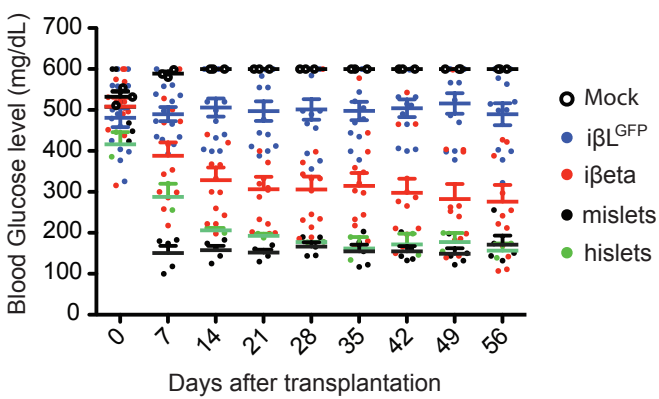


Figure S6

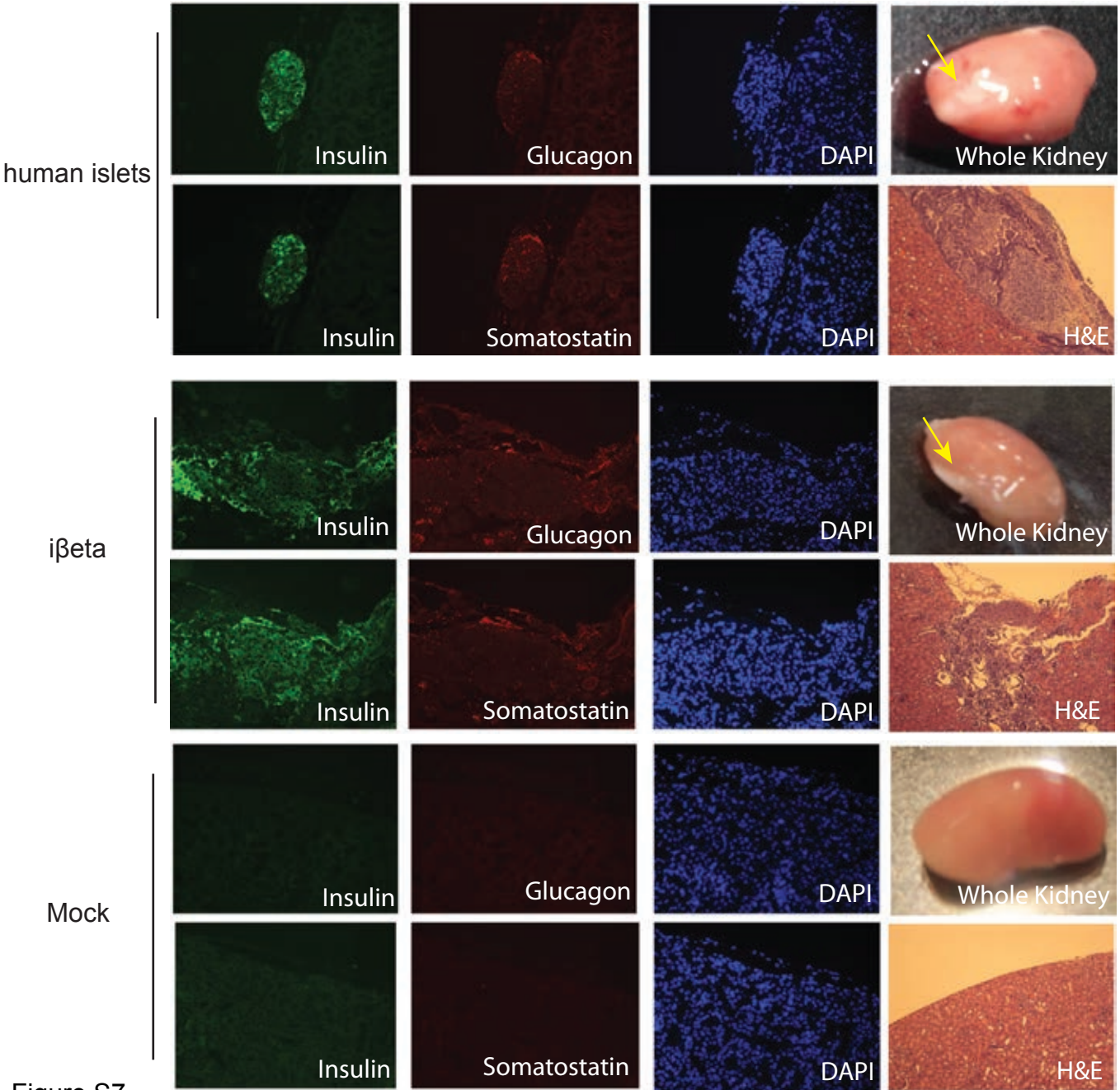

human islets
Insulin
Glucagon
DAPI
Whole Kidney
Insulin
Somatostatin
DAPI
H&E
iβeta
Insulin
Glucagon
DAPI
Whole Kidney
Insulin
Somatostatin
DAPI
H&E
Mock
Insulin
Glucagon
DAPI
Whole Kidney
Insulin
Somatostatin
DAPI
H&E

Figure S7

Table S2

	Log2 Fold change	
ETC/OxPhos/TCA	vs iPSC	vs iPSC
GeneSymbol	EGFP	ERRg
ACO2	1.59873	2.80003
ALDH5A1	1.49639	3.37664
ATP5A1	Not Sig	0.277102
ATP5B	Not Sig	0.846419
ATP5E	0.731179	1.25099
ATP5G1	0.254222	1.05967
ATP6V0E2	Not Sig	0.900001
ATP6V1E1	0.626112	1.30561
ATP6V1F	0.45153	1.02396
CYC1	0.603286	0.896287
DLAT	0.334244	0.961947
DLD	0.441694	0.921321
DLST	Not Sig	0.780315
ENOX1	2.7392	4.74469
ERO1LB	0.672097	1.29379
FDX1L	Not Sig	0.434021
FDXR	Not Sig	3.67598
FH	0.241744	0.74715
FXN	-0.948575	Not Sig
GLRX	0.501042	1.11671
IDH3B	Not Sig	1.14196
IDH3G	0.950187	1.75898
MDH1	-0.255856	1.10709
NDUFA10	0.499061	0.948454
NDUFA11	Not Sig	0.564872
NDUFA12	0.25208	0.704452
NDUFA2	Not Sig	0.914633
NDUFA3	-0.220469	0.553712
NDUFA5	Not Sig	1.07434
NDUFA6	0.379207	0.874344
NDUFA7	Not Sig	0.75212
NDUFA8	Not Sig	0.413066
NDUFA9	0.755382	1.65441
NDUFAB1	-0.381263	Not Sig
NDUFB2	0.597611	1.033
NDUFB5	0.273809	0.774613
NDUFB6	-0.311921	0.189206
NDUFB8	Not Sig	0.544258
NDUFB9	0.263353	0.994521
NDUFS1	0.379948	1.35876
NDUFS2	Not Sig	0.50899
NDUFS3	Not Sig	0.671524
NDUFS5	-0.917236	-0.466569
NDUFV1	0.551033	1.44145
PDHB	-0.842791	0.669281
SDHA	0.790141	1.60913
SDHB	0.407439	1.09377
SDHC	-0.273585	0.383452
SLC1A3	-0.511466	2.37408
SLC25A12	Not Sig	0.839587
SLC25A13	Not Sig	0.566767
SNCA	Not Sig	1.43413
TMX4	2.1645	3.28654
TXNRD3	Not Sig	1.52149
UQCR10	0.592409	1.2018
UQCRB	Not Sig	0.632791
UQCRC2	0.34232	0.837592
UQCRFS1	0.309875	0.760593

Cell Cycle	vs iPSC	vs iPSC
GeneSymbol	EGFP	ERRg
CDC14A	-0.380578	-1.03937
CDC14B	-0.69238	-1.25311
CREBBP	0	-0.833229
DBF4	-1.1856	-1.47334
EP300	-0.688392	-1.09124
E2F1	-1.12537	-1.97869
E2F3	-1.09357	-1.52456
E2F4	-0.265279	-0.470933
MAD2L1	-2.44662	-3.14233
RAD21	-1.36874	-2.20653
SMAD3	2.08416	1.84569
SMAD4	-0.553343	-0.94641
TTK	-2.55557	-3.36766
ANAPC2	1.05548	0.704531
BUB1	-3.38933	-3.70847
BUB1B	-3.69111	-4.09542
ABL1	-0.813672	-1.13291
CDK1	-2.26848	-2.6219
CDC20	-2.48852	-2.90934
CDC25B	0.751421	0.343281
CDC26	-0.348854	-1.21828
CDC27	0.376144	0
CCNA2	-2.40664	-3.15337
CCNB1	-2.51698	-2.88801
CCNB2	-2.98725	-3.35154
CCND2	-0.847802	-0.899491
CDKN1B	0.42953	-0.0322305
CDKN2D	1.98428	1.68098
HDAC1	0	-0.62393
HDAC2	-1.24824	-1.43757
MCM2	-2.56831	-3.01465
MCM3	-1.91484	-2.34638
MCM4	-1.68462	-2.42024

MCM5	-3.09236	-3.60412
MCM7	-1.99523	-2.8826
PTTG1	-2.75808	-3.34462
PLK1	-2.78904	-3.41241
PCNA	-1.12241	-1.77944
CHEK2	-2.99836	-3.54351
RB1	0.839276	0.601377
RBL1	-0.402046	-0.853579
STAG1	0.269041	-0.287144
STAG2	-1.47451	-1.94558
SMC1A	-0.759328	-1.12585
SMC3	-1.5107	-1.83194
TFDP1	0.237269	-0.327076
YWHAB	-0.166558	-0.424803
YWHAG	-0.0469328	-0.429083
YWHAQ	-0.599707	-0.802293
YWHAZ	0.370218	-0.281107

Beta Cell	vs iPSC	vs iPSC
GeneSymbol	EGFP	ERRg
INS	infinity	infinity
PDX1	infinity	infinity
IAPP	-1.0251	1.54488
FOXA2	7.08098	6.07311
GATA4	7.10581	8.19732
GATA6	9.68187	9.71362
MAF	4.1695	5.41346
MAFA	No Test	No Test
MAFB	2.32924	1.69467
NEUROD1	5.23953	3.96233
PAX4	1.00481	0.0836628
PAX6	0.891676	0.309519
NKX2-2	3.73834	3.46179
NKX6-1	2.55188	2.40718
NKX6-2	7.04707	5.21865
NANOG	-9.38333	-10.2497

Table S3

IIDP Donor ID	Internal ID	Sex	Age (years)	Race	BMI	Cause of Death	Experiment	Collesponding Figures
785	#1	Male	50	White	28.99	Cerebrobascular/stroke	cDNA library for qPCR	Data not shown
790	#2	Female	24	Hispanic/Latino	34.9	Head trauma	cDNA library for qPCR	Data not shown
794	#3	Female	41	White	35.5	Head trauma	cDNA library for qPCR	Data not shown
807	#4	Female	26	White	46.6	Head trauma	cDNA library for qPCR	Data not shown
821	#5	Male	27	Asian	22.1	Anoxia	cDNA library for qPCR	Data not shown
824	#6	Female	47	White	20.6	Cerebrobascular/stroke	cDNA library for qPCR	Data not shown
882	#7	Male	50	Hispanic/Latino	25.7	Cerebrobascular/stroke	cDNA library for qPCR	Data not shown
915	#8	Male	31	White	31.7	Head trauma	cDNA library for qPCR	Data not shown
937	#9	Male	52	White	34.3	Anoxia	glucose stimulated c-peptide secretion	Data not shown
975	#10	Male	54	Black or African American	23.3	Cerebrobascular/stroke	glucose stimulated c-peptide secretion	Figure 4E
979	#11	Male	53	White	27.2	Cerebrobascular/stroke	cDNA library for qPCR	Figure S5J
985	#12	Male	40	White	38.91	No info	cDNA library for qPCR	Data not shown
1042	#13	Male	37	White	26.3	Anoxia	islets transplantation in NOD-SCID	Figure 6B, 6C, Figure S6B
1050	#14	Male	36	White	26	Anoxia	islets transplantation in NOD-SCID	Figure 6B, 6C, Figure S6B
1059	#15	Male	32	White	23.1	Anoxia	cDNA library for qPCR	Figure 5A,5B,5C
1060	#16	Female	47	White	25	Cerebrobascular/stroke	cDNA library for qPCR	Figure 5A,5B,5C
1061	#17	Male	33	Hispanic/Latino	31.5	Head trauma	cDNA library for qPCR	Figure 5A,5B,5C
1082	#18	Female	49	White	30.3	Cerebrobascular/stroke	glucose stimulated c-peptide secretion	Figure 4E
1089	#19	Female	51	Hispanic/Latino	25.4	Cerebrovascular/stroke	EM study	Data not shown

Table S4

qPCR primers

NCBI or Primer bank ID	Genes	Species	Primers (Fw)	Primers (Rv)
NM_001243792.1	ERRγ	mouse	gcaaggcattcttcaagagg	ggctgggcagctgtactcta
NM_001136069.2	Ldha	mouse	ccgttacctgatgggagaga	gtaggcactgtccaccacct
NM_008618.3	Mdh1	mouse	gaagccctgaaagacgacag	tcgacacgaactctccctct
NM_009943.2	Cox6a2	mouse	ctctcgactgggtgaaggag	gaagagccagcacaaaggtc
L09192.1	Pcx	mouse	cctctcagagcgagcagact	atagggaagccgaaggtgtt
NM_010886.2	Ndufa4	mouse	ctgcgtgtaggtaggcctgt	catggctctgggttgttctt
NM_153064.4	Ndufs2	mouse	gatccgagtgctctttggag	atgtcatccagaagcccaag
NM_009722.3	Atp2a2	mouse	ctgtggagacccttggttgt	cagagcacagatggtggcta
NM_011596.5	Atp6v0a2	mouse	cacgaagaccttcctcaagc	ccagctctgcataggtcaca
NM_001185083.1	Ins2	mouse	tttgtcaagcagcacctttg	tctacaatgccacgcttctg
NM_001039710.1	Coq10b	mouse	ccccgttctggagagataca	tagcgagctgagagtgcaga
NM_023179.3	Atp6v1g2	mouse	ctggaagcctcagtgtcctc	tcacagtggctgaacaaagc
NM_001103157.1	Steap2	mouse	gcagagcaggagaaatggac	ctttctttcgggatgccata
NM_001177752.1	Pfkfb3	mouse	ccgacagaactggagaggag	agcggagacacgagacagat
NM_000207.2	Insulin	human	agcctttgtgaaccaacacc	gctggtagagggagcagatg
NM_206594.2	ERRγ	human	gctaacactgtcgcagtttga	cgaacagctggaatcaatgtg
NM_024865.2	Nanog	human	ttccttcctccatggatctg	tctgctggaggctgaggtat
NM_001165923.2	HNF1β	human	tcacagataccagcagcatcagt	gggcatcaccaggcttgta
NM_022454.3	Sox17	human	cctgggtttttgttgttgct	gaggaagctgttttgggaca
NM_000209.3	Pdx1	human	ggatgaagtctaccaaagctcacgc	ccagatcttgatgtgtctctcggtc
NM_201589	Mafa	human	cttcagcaaggaggaggtcatc	ctcgtatttctccttgtacaggtcc
NM_006168.2	Nkx6.1	human	attcgttggggatgacagag	tcaacagctgcgtgattttc
NM_002500.4	NeuroD1	human	gttctcaggacgaggagcac	cttgggcttttgatcgtcat
NM_000162.3	Gck	human	gctggaatcaatttcccaga	ctccccacacaggatgagtt
NM_014232.2	Vamp2	human	tcccccagctggtatgtaag	ccacacacacactggtagcc
NM_002054.4	Gcg	human	aggcagacccactcagtga	aacaatggcgacctcttctg
NM_001048.3	Sst	human	gtacttcttggcagagctgctg	cagaagaaattcttgcagccag
260099724c1	Ldha	human	atggcaactctaaaggatcagc	ccaaccccaacaactgtaatct
NM_001199111	Mdh1	human	cccagtaatgggaagggatt	ccgtaacgtcctttggaaaa
34147589c1	Ndufa4	human	atgatcggcttaatctgcctg	tccgggttgttctttctgtcc
NM_000920.3	Pcx	human	ccagaggcaggtcttctttg	gggtgaggtcaccacagtct
NM_145230.3	Atp6v0e2	human	agtatcctgttccgcctcct	ctctcatcccagctctggtc
25121968c1	Kcnj8	human	aacctggcgcataagaacatc	ccacatgatagcgaagagcag
20336240c1	Pcsk1	human	accccgagctgttgaggta	gggtctctaggcgtttcaca
206725425c1	Kcnk1	human	cctggggaaggctacaatcaa	ccagaactaccaacatggcaa
197245365c1	Kcnk3	human	ctacgagcactggaccttctt	cgtaaggatgtagacgaagctga
NM_001002.3	U36B4 (RPLP0)	human/mouse/rat	gtgctgatgggcaagaac	aggtcctccttggtgaac
NM_203336	ERRγ	Rat	cagctgttcgtccttcatca	tctggggatcctctacgatg

ChIP primers

Reference	Genes	Species	Primers (Fw)	Primers (Rv)
Dofour et al, Cell Metab, 2007	Mdh1	mouse	cgccagaggtcgccggaagaactacac	ccaggagcccacactcaccattattgc
Dofour et al, Cell Metab, 2007	Atp2a2	mouse	gtatgttttagacaaggtccaacgtgg	caaggtaattttgccaatataaagagg
Active Motif	EF1a	mouse	ChIP-IT Control Kit-Mouse No53011	

Genotyping Primers

Reference	Genes	Animal model	Primers (Fw)	Primers (Rv)
-	ERRγ	ERRγ flox/flox	gttttaaaggcccttggtgatctcgc	ctgcaacccttggactgccagaac
-	Cre	Cre positive	gcattaccggtcgatgcaacgagtgatgag	gagtgaacgaacctggtcgaaatcagtgcg
-	FABP3	internal control for Cre	tggacaggactggacctctgc	tagagctttgccacatcacag
-	Insulin GFP promoter	MIP-GFP	aagttcatctgcaccaccg	tccttgaagaagatggtgc
-	Insulin GFP promoter	internal control for MIPGFP	ctaggccacagaattgaaagatct	gtaggtggaaattctagcatcatcc

Table S5

Material	Dilution	Company	Catalog Number	STOCK solution	Final Concentration	Expected effect
rh/m/r Activin A	0.1% BSA PBS	R&D systems	338-AC	100ug/ml	100ng/ml	TGFβ super family
rhWnt3a	0.1% BSA PBS	R&D systems	5036-WN	25ug/ml	25ng/ml	Wnt singnaling
CHIR99021	DMSO	Axon	Axon 1386	3mM	3uM	GSK3β inhibitor
Wortmannin	DMSO	Invivo Gen	10C22-MM	100uM	100nM	PI3K Inhibitor
Retinoic acid	DMSO	SIGMA	R2625-1G	2uM	2mM	Retinoic acid signal
SB431542	DMSO	SIGMA	S4317-5MG	10uM	10mM	TGFβRI inhibitor
Dorsomorphin	DMSO	Bio vision	1686-5	1mM	1uM	BMP type I R inhibitor
rhFGF10	0.1% BSA PBS	R&D systems	345-FG	50ug/ml	50ng/ml	FGF10 signaling
rhFGF7/KGF	0.1% BSA PBS	R&D systems	251-KG/CF	25ug/ml	25-50ng/ml	FGF7 signaling
KAAD-cyclopamine	DMSO	STEMGENT	04-0028	0.25mM	0.25uM	Hedgehog signaling inhibitor
rhNoggin	0.1% BSA PBS	R&D systems	6057-NG	50ug/ml	50ng/ml	BMP4 inhibitor
rhFGF2	0.1% BSA PBS	Peprotech	100-18B	10ug/ml	10ng/ml	FGF2 signaling
rhBMP4	0.1% BSA PBS	R&D systems	314-BP	10ug/ml	10ng/ml	BMP4 signaling
Forskolin	DMSO	SIGMA	F6886-25MG	10mM	10uM	Adenylate cyclase activator
Dexametazon	DMSO	SIGMA	D4902-100MG	10mM	10uM	Glucocorticoid receptor signaling
TGF-β RI kinase inhibitor II (Alk5i II)	DMSO	Calbiochem or Enzo	616452 or ALX-270-445	10mM	10uM	TGFβRI inhibitor
Nicotinamide	H2O or PBS	SIGMA	72340-100G	1M	10mM	Vitamin B3
Exendin4	H2O or PBS	SIGMA	E7144	1mg/ml	1ug/ml	GLP1 analog
human GLP1	0.1% BSA PBS	Peprotech	130-08	1mg/ml	1ug/ml	GLP1 signaling/cAMP activation
human IGF2	0.1% BSA PBS	Prospec	cyt-265-b	10ug/ml	10ng/ml	IGF2 signaling
B27 Supplement	-	GIBCO	17504-044	-	1%	Growth supplement
N2 Supplement	-	GIBCO	17502-048	-	1%	Growth supplement
Insulin-Transferrin-Selenium	-	GIBCO	41400-045	-	1%	Growth supplement
Reserpine	DMSO	TOCRIS	2742	0.63mM	0.63uM	VMAT inhibitor
Tetrabenazine (TBZ)	DMSO	TOCRIS	2175	1.25mM	1.25uM	VMAT inhibitor
dBu-cAMP	DMSO	ENZO	BML-CN125-0100	0.6mM	0.6uM	cAMP activation
R428	DMSO	Selleckchem	S2841	2mM	2uM	Axl inhibitor
3,3′,5-Triiodo-L-thyronine sodium salt (T3)	DMSO	SIGMA	T6397-100MG	1mM	1uM	Thyloid hormone
Stemolecule™ LDN-193189	DMSO	Stemgent	**04-0074-10**	100uM	100nM	TGFβ/Smad inhibitor
SANT-1	DMSO	SIGMA	S4572-5MG	0.25mM	0.25uM	Hedgehog/Smoothened antagonist
N-acythyl cysteine	H2O	SIGMA	A9165	100mM	1mM	Anti oxidants
Gamma Secretase inhibitor XX (GSiXX)	DMSO	Millipore	565789	100uM	100nM	Notch inhibitor
Betacellulin (BTC)	0.1% BSA PBS	Millipore EMD	200496-10UG	20ug/ml	20ng/ml	EGFR ligand
Heparin	H2O	SIGMA	H3149-10KU	10mg/ml	10ug/ml	Enhance Growth hormone binding

Days for differentiation	0 day	1 ~2 day	3~4 day	5~11 day	12 ~21 day	21 day~
Virus or Reagents	Infection Human Insulin Reporter Lenti Virus (pGreenZeo System) 800g 1 hour spin fection Chenge media to Fresh TeSR Media Red; Essential small molecules Blue; Additional small molecules	Atvivin A 100ng/ml Wnt3a 25ng/ml CHIR99021 3uM (Replacable to Wnt3a)	Atvivin 100ng/ml	Retinoic Acid 2uM SB431542 10uM Dorsomorphin 1uM B27 supplement 1%	Forskolin 10uM Dexametasone 10uM Alk5i II 10uM Nicotinamide 10mM B27 supplement 1% T3 1uM	Forskolin 10uM Dexametasone 10uM Alk5i II 10uM Nicotinamide 10mM B27 supplement 1% dBu-cAMP 0.6uM T3 1uM
	Change media every day Matrigel coated well	Change media every day Matrigel coated well	Change media every day Matrigel coated well	Change media every other day Matrigel coated well	Change media every 2~3 days Matrigel coated well	Change media every 2~3 days Matrigel coated well
Base Midea	TeSR media	Custam TeSR media (w/o growth Factors, containing Vit C and GABA)	Custam TeSR media (w/o growth Factors, containing Vit C and GABA)	Custam TeSR media (w/o growth Factors, containing Vit C and GABA)	Custam TeSR media (w/o growth Factors, containing Vit C and GABA)	Custam TeSR media (w/o growth Factors, containing Vit C and GABA)

Table S6

	Treatment	C-peptide (ng/ml) 3mM glucose	20mM glucose	C-peptide (%) 20mM/3mM Glucose	C-peptide (ng/ml) 20mM KCl
Batch1	iβL 1	0.231	0.231	100	0.587
	iβL 2	0.203	0.207	102	0.811
	iβL 3	0.169	0.158	93	0.891
Batch2	iβL GFP1	0.215	0.215	100	
	iβL GFP2	0.205	0.227	111	
	iβL GFP3	0.197	0.233	118	
	iβL GFP4	0.205	0.223	109	
	iβeta1	0.21	0.33	157	
	iβeta2	0.205	0.517	252	
	iβeta3	0.246	0.366	149	
	iβeta4	0.207	0.484	234	
Batch3	iβL GFP1	0.173	0.173	100	
	iβL GFP2	0.161	0.176	109	
	iβL GFP3	0.164	0.173	105	
	iβeta1	0.172	0.413	240	
	iβeta2	0.175	0.307	175	
	iβeta3	0.168	0.312	186	
	human islets 1	1.455	5.27	362	
	human islets 2	1.414	6.833	483	
	human islets 3	1.298	5.921	456	
Batch4	iβL GFP1	0.173	0.173	100	
	iβL GFP2	0.161	0.176	109	
	iβL GFP3	0.164	0.173	105	
	iβeta1	0.172	0.213	124	
	iβeta2	0.175	0.207	118	
	iβeta3	0.168	0.212	126	
Batch5	iβL GFP1	1.659	1.833	110	2.627
	iβL GFP2	1.61	1.645	102	2.281
	iβL GFP3	1.574	1.635	104	1.973
	iβeta1	1.539	2.258	147	2.762
	iβeta2	1.597	2.406	151	4.131
	iβeta3	1.572	2.858	182	4.163
Batch6	iβL GFP1	1.184	0.677	57	
	iβL GFP2	0.777	0.729	94	
	iβL GFP3	0.867	0.52	60	
	iβeta1	0.583	4.206	721	
	iβeta2	0.573	3.336	582	
	iβeta3	1.044	2.405	230	
Batch7	human islets 1	0.318	1.362	428	
	human islets 2	0.836	2.168	259	
	human islets 3	0.324	1.01	312	
	human islets 4	1.182	1.44	122	
	human islets 5	0.887	1.101	124	
	human islets 6	1.302	1.064	82	

Figure S1, Related to Figure 1 and Table S1. Transcriptional differences between neonatal and adult islets (A) Heatmap of differentially expressed transcriptional factors involved in pancreatic lineage determination in islets from 2, 6, and 12 wk old C57BL/6J mice. 53 pancreatic lineage genes were selected based on published literature (Hrvatin *et al*., 2014); the full gene list and expression data are provided in Table S1A. **(B-C)** Biological pathways enriched in neonatal (2 wk old) and adult (12 wk old) islets, determined by DAVID gene ontology (GO). **(D-E)** Relative expression of cell type-specific markers and *ERRγ* and the proliferative marker *PdgfR*β (n= 3) in FACS-sorted cell populations from mouse insulin promoter GFP (MIP-GFP) islets at 2 and 12 wks. ERRγ is induced during postnatal β cell development. **(F)** Immunoblot analysis for ERRγ and β-actin in neonate (2wk, n=6) and adult (9wk, n=3) islets. **(G)** X-gal staining showing enriched ERRγ expression in isolated islets (endocrine) but not pancreatic exocrine acini cells from 12 wk old ERRγ lacZ knock-in mice. Data represent the mean ±s.e.m. *p<0.05 Student's unpaired t-test.

Figure S2, Related to Figure 2. Characterization of β cell-specific ERRγ knockout mice (β*ERRγ*KO). (A) Relative expression of *ERRγ* in isolated islets and tissues from ERRγ$^{f/f}$ (WT) and β*ERRγ*KO mice, measured by qPCR at 12 wks old (n=3). **(B)** Body weights of WT (n=9), βERRγKO (n=13) and WT(RIP-Cre) (n=12) mice at indicated developmental ages. **(C)** *Ad lib* fed blood glucose levels in male mice at indicated ages. **(D-E)** Intra-peritoneal glucose tolerance test (2g/kg; IP-GTT) of WT (n=6), βERRγKO (n=5), and WT (RIP-Cre) (n=5) mice fed a normal chow diet (NCD) in 15 and 40 wk old male mice. 12 wk old male WT (n=9) and Tamoxifen-induced β*ERRγ*KO (βERRγKO ER) (n=11) mice were given daily tamoxifen (Tam) injections (2mg/kg in corn oil, i.p.) for 7 days prior to **(F)** analysis of *ERRγ* expression in isolated islets or **(G)** IP-GTT at 16 wks. **(H-J)** Body weights, IP-GTT blood glucose, IP-GTT serum insulin level (ng/ml) of 16 wk old ERRγ$^{f/f}$ (WT, n=9) and pancreatic-specific ERRγKO (PERRγKO; ERRγ$^{f/f}$ x PDX1-Cre, n=7) mice under NCD. **(K)** IP-GTT of 8 wk old in WT (n=7) and PERRγKO (n=6) after 4 wks high fat diet (HFD) after weaning. Data represent the mean ±s.e.m. *p<0.05 Student's unpaired t-test.

Figure S3, Related to Figure 2. HFD-fed βERRγKO islets are hypertrophic while ERRγ deletion disrupts insulin secretion in response to nutrients. (A) Immunostaining (insulin, green and glucagon, red), and **(B)** hematoxylin and eosin (H&E) staining of islets from 10 wk old ERRγ$^{f/f}$ (WT) and βERRγKO mice fed a normal chow diet (NCD) or after 6 wks on a high fat diet (HFD). **(C)** Insulin content of islets from 10 wk old WT and βERRγKO mice fed NCD or **(D)** 6 wks high fat diet (HFD). **(E)** Average area of islet in pancreas, **(F)** whole pancreas insulin content (ng/mg protein from whole pancreas) and **(G)** frequency distribution of islet sizes were measured from 10 wk old WT and βERRγKO mice fed 4-6 wks HFD after weaning. (WT, n=5-7; βERRγKO, n=5-7). Mice were started on HFD at the age of 4 wks. *Ex vivo* insulin secretion from **(H)** WT and βERRγKO or **(I)** WT and pancreatic-specific *ERRγ*KO (P*ERRγ*KO) islets in response to nutrients (glucose, leucine and glutamine) and KCl. Islets were isolated from 10 wk old male mice. **(J)** Relative ERRγ expression, **(K)** glucose-stimulated (20mM) or KCl-stimulated (20mM) insulin secretion assay **(L)** cellular ATP levels, and **(M)** cellular bioenergetics in rat INS-1 cells at 72 hours after transfection with scrambled (Control) or ERRγ-targeted siRNA. Data represent the mean ±s.e.m.*$p<0.05$ Student's unpaired t-test.

Figure S4, Related to Figure 3 and Table S1. ERRγ directly regulates postnatal islet maturation. (A) Schematic of genomic analyses of ERRγ-deleted β cells. Transcriptional changes between islets from ERRγ$^{f/f}$ (WT, n=3) and βERRγKO (n=3) mice were compared to the transcriptional changes between ERRγ$^{f/f}$ islets after adenoviral EGFP or Cre infection (n=10). Islets were isolated from 12 wk old male mice. **(B)** ERR/NR responsive elements identified in 140 down- and 149 up-regulated genes in βERRγKO islets. **(C)** ChIP assay for indicated genes in mouse insulinoma MIN-6 cell line. **(D)** Altered gene expression in ERRγKO islets. Heatmap of 471 hierarchally clustered genes whose expression changed in βERRγKO islets compared to Control islets (ERRγ$^{f/f}$) are compared to postnatal developmental transcriptomic changes in WT islets at indicated ages. Heatmap values were shown as relative expression (Row Z-score). Full gene list with expression data are provided in Table S1C. Data represent the mean ±s.e.m. *$p<0.05$ Student's unpaired t-test.

Figure S5, Related to Figure 4. Functional characterization of iPSC-derived β-like cells.

(A) Expression profiling of differentiating human iPSCs. Relative expression of the pluripotent marker (*NANOG*), endoderm marker (*Sox17*), pre-pancreatic foregut endoderm marker (*Hnf1β*), pancreatic progenitor marker (*Pdx1*), and endocrine/β-cell markers (Insulin, *Mafa*, *Nkx6-1*, *NeuroD1*, *Gck*, *Vamp2*, *Gcg*, *Sst*) in iPSCs during differentiation into β-like cells (iβL). **(B)** Human insulin reporter-driven (hIns) GFP expression (top panels) and phase contrast image (bottom panels) of day 30 iβL cells. **(C)** Human Pdx1 reporter-driven GFP expression (top panels) and phase contrast image (bottom panels) of day 23 iβL cells. **(D)** Percentage of iβL cells expressing hIns-driven GFP, as measured by FACS (n=8). **(E)** Representative FACS analyses of insulin (GFP) and glucagon expression in iPSCs and iβL cells. **(F)** EM analyses of iβL cells (day 22) and mouse primary β cells (arrows indicate representative mitochondria and insulin granules). **(G)** Co-expression of Insulin (GFP reporter) and Nkx6-1 or MafA in iPSCs and iβL cells, as measured by FACS. IgG is shown as a negative control. **(H)** Glucose and potassium-stimulated c-peptide secretion from iβL cells. **(I)** Relative *ERRγ* expression in human islets, β-like cells with adenoviral EGFP expression ($i\beta L^{GFP}$ cells) and β-like cells with adenoviral *ERRγ* expression (i*βeta* cells). **(J)** 20mM KCl-stimulated human c-peptide secretion from $i\beta L^{GFP}$ and i*βeta* cells. Data were shown by % increased c-peptide secretion compared to basal (glucose 3mM, KCl 3.6mM) condition. **(K)** Nkx6-1 and MafA expression in undifferentiated iPSCs, iβL and i*βeta* (ERRγ-expressing) cells. ERRγ overexpression does not enhance β cell marker expression. Data represent the mean ±s.e.m.

Figure S6, Related to Figure 6. Pathways down-regulated in i*βeta* cells and schematic of cell transplantation experiments. (A) Functional annotation of down-regulated gene categories in i*βeta* cells, identified by Gene Ontology (GO). Heatmap of expression changes in selected genes involved in cell cycle. **(B)** Schematic of cell transplantation experiments. Type1 diabetic NOD-SCID mice were prepared by high-

dose (180mg/kg) STZ i.p. injection 7-10 days before transplantation. Day 22-30 iPSC-derived β-like cells were infected with adenoviral *ERRγ* (Ad-ERRγ) or control (Ad-GFP) and 10 million cells/mice were transplanted into kidney capsules of STZ-treated NOD-SCID mice within 5 days after virus infection. **(C)** *Ad lib*-fed blood glucose levels of individual mice described in Figure 6B.

Figure S7, Related to Figure 6. Kidney immunohistochemistry in transplanted mice. Representative immunohistochemistry (IHC) staining and histology of human islets, iβ*eta* cells and Mock grafts from kidney capsules of transplanted mice that were harvested 2 months post-transplant. Immunofluorescence of Insulin (Green), Glucagon (Red), Somatostain (Red), nuclear DAPI (blue) staining and Hematoxylin & Eosin (H&E) staining as well as whole kidney images are provided.

Hrvatin, S., O'Donnell, C.W., Deng, F., Millman, J.R., Pagliuca, F.W., Diiorio, P., Rezania, A., Gifford, D.K., and Melton, D.A. (2014). Differentiated human stem cells resemble fetal, not adult, beta cells. Proceedings of the National Academy of Sciences of the United States of America.

Table S1, Related to Figure 1, 3 and Figure S1, S3. List showing comparison of gene expression in 2wk and 12wk islets. (A) Gene list for pancreatic lineage-specific gene expression in neonatal and adult islets related to Figure 1B and 1C. **(B)** Gene list for glucose metabolism, mitochondria metabolic genes include ATP Biosynthesis/ETC/OxPhos, Exocytosis and cell proliferation-specific gene expression related to Figure 1B and 1C. **(C)** Gene list for ERRγ regulated gene expression related to Figure 3D, 3F and 3G. Log2 Fold change in expression between samples is compared. "Not Sig" indicates no significant change in expression between the samples and "NO TEST" indicates no expression in at least one of the samples being compared.

Table S2, Related to Figure 4. Gene List of representative up-regulated and down-regulated pathways in i*βeta* cells. Log2 Fold change in expression between samples is compared. "Not Sig" indicates no significant change in expression between the samples and "NO TEST" indicates no expression in at least one of the samples being compared.

Table S3, Related to Figure, 4, 5, 6 and Figure S5, S6, S7. Additional information regarding human islets

Table S4, Related to Figure 1, 2, 3, 4, 5 and Figure S1, S2, S3, S4, S5. Primer information Primer sequence information for qPCR, ChIP and Genotyping

Table S5, Related to Figure 4 Stepwise differentiation protocol and small molecule information for insulin-producing cells. Small molecule information used for optimizing the β cell differentiation protocol. Simple strategy for the differentiation is also provided. Wnt3a can be replaced by CHIR99021. Addition of T3 and dBu-cAMP from 12 days after initiation of differentiation, increases the efficiency of β cell lineage specification.

Table S6, Related to Figure 4. Raw data for in vitro c-peptide secretion assay. Static glucose-stimulated human c-peptide secretion (ng/ml) by iβL, iβLGFP, iβ*eta* and human islets. Details of experimental information are provided in Experimental Procedures.

ON-LINE SUPPLEMENTAL METHODS

INS-1 Cell culture, transfection and insulin secretion assay

INS-1 cells were cultured at 37°C in 5% CO_2 in air in RPMI-1640 (Sigma Aldrich) supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) Antibiotic-Antimycotic (Gibco) 10 mM HEPES, 2 mM glutamax, 1 mM sodium pyruvate, and 50 μM β-mercaptoethanol (RPMI for INS-1 medium). INS-1 cells were transfected with Lipofectamine2000 containing Plus Reagent (Invitrogen). INS-1 cells were transfected with ERRγ siRNA (Qiagen) or negative control scramble siRNA (Qiagen) for 72 hr. Insulin secretion was measured in pre-incubated cells (37°C for 30 min in KRBH with 3 mM glucose, as described in insulin secretion assays for primary islets) after a 30 min glucose challenge (KRBH buffer with 3 mM or 20 mM glucose) using a Rat/mouse Insulin ELISA kit (Millipore).

Histology (H&E staining, Immunohistochemistry and LacZ staining)

H&E staining was performed by Pacific Pathology (San Diego). Immunostaining was visualized by ZEISS confocal microscopy analysis or fluorescence microscopy analysis using the following antibodies on frozen or paraffin sections of pancreas and human islets or i*βeta* cells in the kidney capsule and 4% PFA-fixed cells: Insulin (1/100, Abcam ab7842), glucagon (1/100, Abcam ab10988), somatostatin (1/100, Abcam ab103790). DAPI-containing mounting media (VECTASHIELD mounting medium for fluorescence) was used for nuclear staining. Whole pancreases from ERRγ knock-in mice (Alaynick et al., 2007) were fixed with paraformaldehyde and glutaraldehyde, and frozen sections

stained by X-gal.

Electron microscopy

Pancreatic isolated islets or hiPSC, hiPSC-derived iβL, iβL^{GFP} and i*βeta* cells on Acryl plate were fixed in 0.15M cacodylate buffer (pH 7.4) containing 2% paraformaldehyde and 2.5% glutaraldehyde with 2mM calcium chloride at 37°C for 5 min. Subsequently, EM analysis were performed in Biophotonics Core Facility at Salk institute.

Microarray Analyses

Total RNA was extracted from Ad-GFP or Ad-Cre infected islets using Trizol reagent (Invitrogen) and its quality determined by Agilent 2100 Bioanalyzer. 500 ng of RNA was reverse transcribed into cRNA and biotin-UTP labeled using the Illumina TotalPrep RNA Amplification Kit (Ambion). cRNA was quantified using an Agilent Bioanalyzer 2100 and hybridized to the Illumina mouseRefseq-8v2 Expression BeadChip using standard protocols (Illumina). Image data was converted into unnormalized Sample Probe Profiles using Illumina GenomeStudio. Data were analyzed by GeneSpring GX software. Briefly, per-chip normalizations were set to the 75th percentile, and per-gene normalizations to the median and specific samples. Genes assigned as absent were eliminated from the dataset and genes with an expression difference of 2-fold more than WT were selected. Combination analyses by GO, pathway analyses and cluster analyses were performed using mainly DAVID software (Huang *et al*., 2009a; Huang *et al*., 2009b). The microarray data is deposited in the NCBI Gene Expression Omnibus and accessible through GEO Series accession number GSE56080.

Quantitative RT-PCR analysis

Total RNA was extracted using TRIzol reagent (Invitrogen) and RNeasy KIT (Qiagen). Reverse transcription was performed with a SuperScript III First-Strand Synthesis System kit (Invitrogen) or PrimeScript RT reagent kit (TAKARA). Real time quantitative RT-PCR (qPCR) was performed using SYBR Green (Bio-Rad). PCR analyses were carried out using the oligonucleotide primers listed in Table S4.

RNA-Seq library generation

Total RNA was isolated from cell pellets treated with RNAlater using the RNA mini kit (Qiagen) and treated with DNaseI (Qiagen) for 30 min at room temperature. Sequencing libraries were prepared from 100-500ng total RNA using the TruSeq RNA Sample Preparation Kit v2 (Illumina) according to the manufacturer's protocol. Briefly, mRNA was purified, fragmented, and used for first- and second-strand cDNA synthesis followed by adenylation of 3' ends. Samples were ligated to unique adapters and PCR amplified. Libraries were then validated using the 2100 BioAnalyzer (Agilent), normalized and pooled for sequencing.

High-throughput sequencing and analysis

RNA-Seq libraries prepared from 2-3 biological replicates for each experimental condition were sequenced on the Illumina HiSeq 2500 using bar-coded multiplexing and a 100bp read length. Image analysis and base calling were performed with Illumina CASAVA-1.8.2. This yielded a median of 29.9M usable reads per sample. Short

read sequences were mapped to a UCSC mm9 reference sequence using the RNA-Seq aligner STAR (Dobin *et al*., 2013). Known splice junctions from mm9 were supplied to the aligner and *de novo* junction discovery was also permitted. Differential gene expression analysis, statistical testing and annotation were performed using Cuffdiff 2 (Trapnell *et al*., 2013). Transcript expression was calculated as gene-level relative abundance in **f**ragments **p**er **k**ilobase of exon model per **m**illion (fpkm) mapped fragments and employed correction for transcript abundance bias (Roberts *et al*., 2011). RNA-Seq results for genes of interest were also explored visually using the UCSC Genome Browser. Heatmaps were generated by R-Script with heatmap.2 (gplot) software or Cluster with Javatree view software. Scale of heatmaps was determined by Z-score (Figure 1B, 1C, 3F, S1A and S4D) or fold change with log scale (Figure 3G, 4G and S6A). Motif analyses were performed using HOMER (Heinz et al., 2010). Detailed methods for HOMER are freely available at http:// http://homer.salk.edu/homer/. Briefly, the program searches against the target and background sequences for enrichment of known motifs, returning motifs enriched with a p-value less than 0.05. In this study, the promoter regions, defined as 1kB upstream from the transcription start site, of genes dysregulated in ERRg KO islets were interrogated for enriched motifs of 8-16 bp using HOMER motif analysis. This analysis revealed the ERR response element (ERREs) shown in Figure S4B as the 5th most enriched motif in the proximal 1kb promoter region of 140 down- and 149 up-regulated genes (p=1e-15). Other variations of the ERRE came up ranked 7th, 9th, and 10th.

RNA-Seq data can be accessed on the NCBI Sequence Read Archive under the

accessions SRP048600 and SRP048605.

Tamoxifen-inducible mouse experiments

Glucose tolerance tests were performed before (at 12 wks of age) and 3 wks after treatment (at 16 wks of age) in tamoxifen-inducible β cell-specific ERRγ-knockout mice. Male mice were given daily injections of tamoxifen (2mg/kg in corn oil, i.p.) for 7 days.

References for Online Methods

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