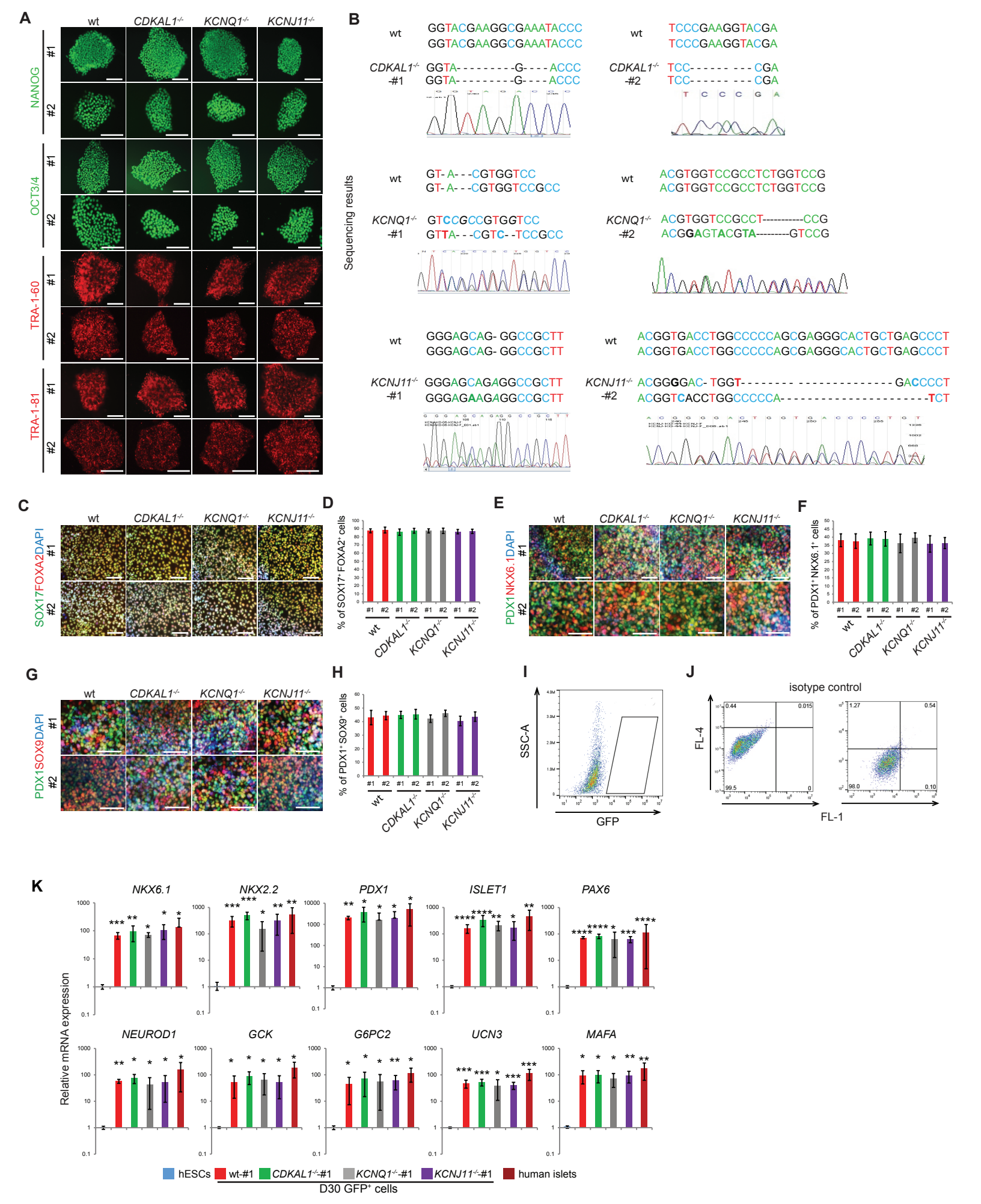
A
wt
CDKAL1-/-
KCNQ1-/-
KCNJ11-/-
NANOG
OCT3/4
TRA-1-60
TRA-1-81
#1
#2
B
Sequencing results
wt
CDKAL1-/- -#1
CDKAL1-/- -#2
KCNQ1-/- -#1
KCNQ1-/- -#2
KCNJ11-/- -#1
KCNJ11-/- -#2
C
SOX17FOXA2DAPI
D
% of SOX17+FOXA2+ cells
E
PDX1NKX6.1DAPI
F
% of PDX1+NKX6.1+ cells
G
PDX1SOX9DAPI
H
% of PDX1+SOX9+ cells
I
SSC-A
GFP
J
isotype control
FL-4
FL-1
0.44
0.015
99.5
0
1.27
0.54
98.0
0.10
K
NKX6.1
NKX2.2
PDX1
ISLET1
PAX6
NEUROD1
GCK
G6PC2
UCN3
MAFA
Relative mRNA expression
hESCs
wt-#1
CDKAL1-/--#1
KCNQ1-/--#1
KCNJ11-/--#1
human islets
D30 GFP+ cells

Figure S1. Related to Figure 1. Generation of isogenic mutant hESCs and stepwise differentiation of hESC derived day 30 beta-like cells. (A) Immunocytochemistry of markers for pluripotency in wildtype (wt) $INS^{GFP/W}$ HES3 and isogenic mutant hESC lines. (B) Schematic DNA sequencing results of six isogenic mutant hESC lines. Dashes indicate deleted bases and italic bases are inserts or insertions. Thus, the mutations are indels that generate early frame-shifts and are presumed null. (C and D) Immunocytochemistry analysis (C) and quantification (D) of wt and isogenic mutant hESC-derived definitive endoderm. (E-H) Immunocytochemistry analysis (E, G) and quantification (F, H) of hESC-derived pancreatic progenitors. (I) FACS analysis control of Figure 1C. (J) FACS analysis control of Figure 1E. (K) qRT-PCR analysis of wildtype and isogenic mutant hESC-derived insulin-GFP^+ cells purified at day 30 (D30) by cell sorting, n=4. Clone #1 and #2 are two independent isogenic hESC clones carrying different frameshift mutations. The expression in primary human beta cells was calculated by dividing the expression level in primary human islets by the percentage of $insulin^+$ cells. The data is presented as mean±S.D. n=3 independent experiments if not specified. hESCs were differentiated using protocol 2. n.s. indicates a non-significant difference. Scale bar = 100 µm. p values calculated by unpaired two-tailed Student's t-test were *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

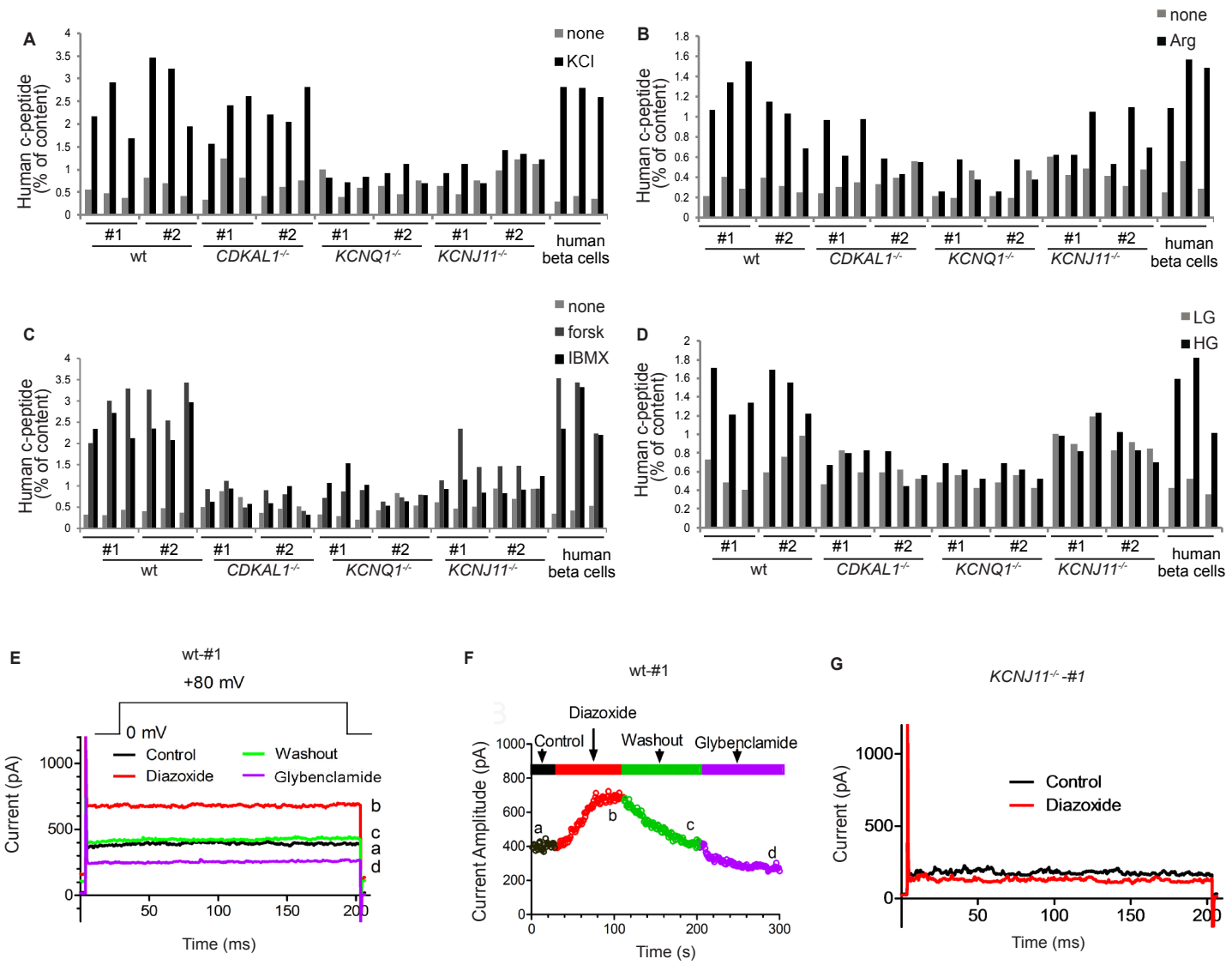
A
none
KCl
Human c-peptide (% of content)
#1 #2 #1 #2 #1 #2 #1 #2
wt
CDKAL1-/-
KCNQ1-/-
KCNJ11-/-
human beta cells
B
none
Arg
Human c-peptide (% of content)
C
none
forsk
IBMX
Human c-peptide (% of content)
D
LG
HG
Human c-peptide (% of content)
E
wt-#1
+80 mV
0 mV
Control
Diazoxide
Washout
Glybenclamide
Current (pA)
Time (ms)
F
wt-#1
Diazoxide
Control
Washout
Glybenclamide
Current Amplitude (pA)
Time (s)
G
KCNJ11-/--#1
Control
Diazoxide
Current (pA)
Time (ms)

Figure S2. Related to Figure 2. Data of each individual experiment shown in Figure 2A-2H. Wildtype (wt) and isogenic mutant D30 cells were exposed to secretalogue stimulation (A) KCl (30 mM); (B) arginine (10 mM); (C) forskolin (20 μM) or IBMX (50 μM); (D) D-glucose (2 mM or 20 mM; LG and HG, respectively). Arg: arginine; forsk: forskolin. (E) K_{ATP} Currents elicited in wildtype insulin-GFP^+ cells by depolarization from holding potential (HP) of 0 mV to +80 mV (see the protocol on the top) under various recording conditions, including Control (a), after the cells were exposed to 100 μM diazoxide (b), Washout (c), and after 10 μM Glybenclamide (d). (F) K_{ATP} current amplitude measured at +80 mV in the presence or absence of K_{ATP} channel activator diazoxide and inhibitor glybenclamide. Note that diazoxide increased K_{ATP} current amplitude by almost 100%, and its effect was reversible. Glybenclamide at 10 uM further inhibited K_{ATP} current from 400 pA to 220 pA, indicating that K_{ATP} channels were activated under basal (control) condition, presumably by the pipette solution used in the recording of K_{ATP} currents. The time points a, b, c, and d represent the time when the original traces shown in A were recorded. (G) K_{ATP} current elicited in KCNJ11$^{-/-}$-#1 insulin-GFP^+ cells. (n=6~8). hESCs were differentiated using protocol 2.

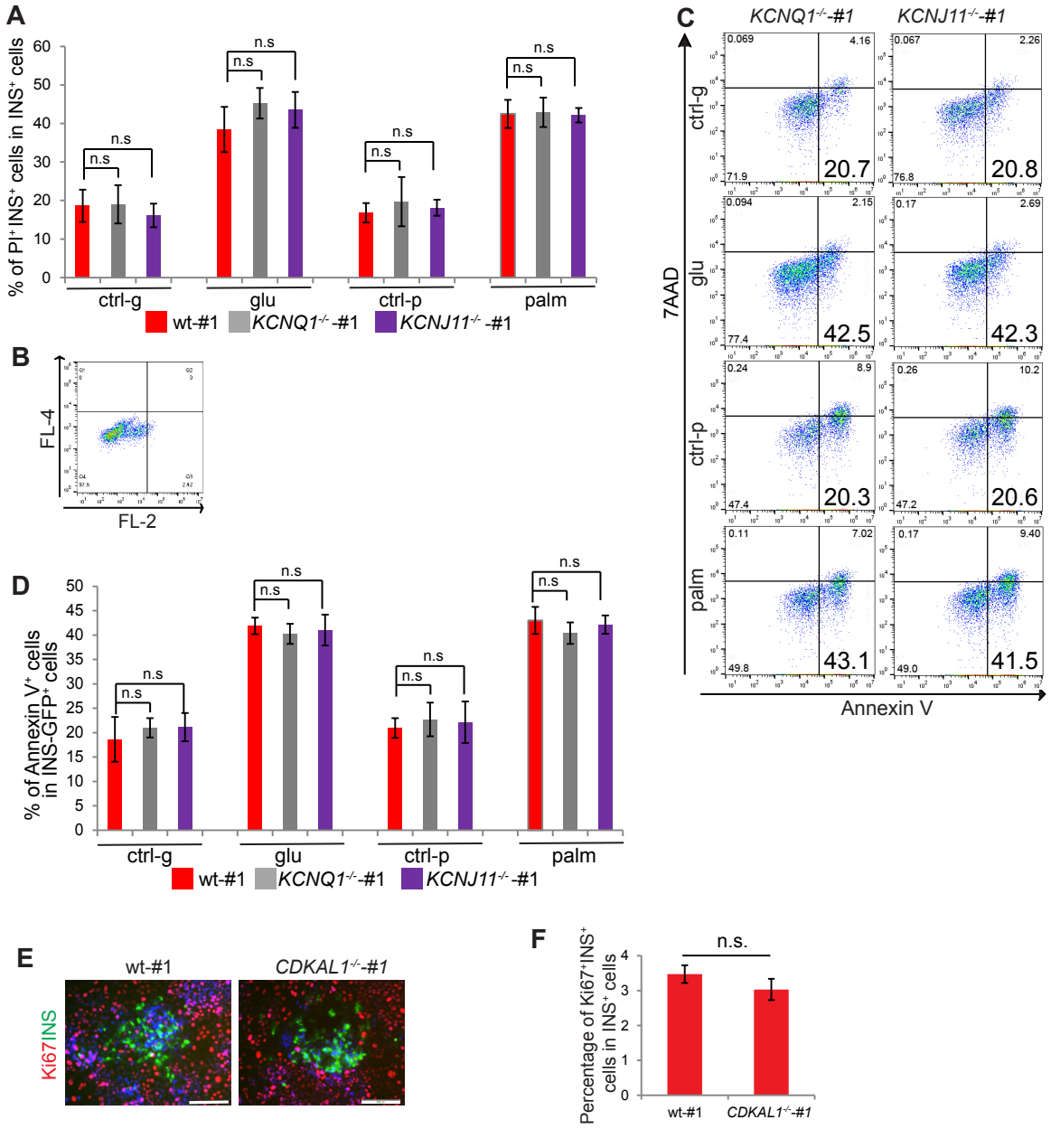
A
% of PI+ INS+ cells in INS+ cells
n.s
ctrl-g
glu
ctrl-p
palm
wt-#1
KCNQ1-/- -#1
KCNJ11-/- -#1
B
FL-4
FL-2
C
KCNQ1-/--#1
KCNJ11-/--#1
7AAD
Annexin V
0.069
4.16
71.9
20.7
0.067
2.26
76.8
20.8
0.094
2.15
77.4
42.5
0.17
2.69
42.3
0.24
8.9
47.4
20.3
0.26
10.2
47.2
20.6
0.11
7.02
49.8
43.1
0.17
9.40
49.0
41.5
D
% of Annexin V+ cells in INS-GFP+ cells
E
wt-#1
CDKAL1-/--#1
Ki67INS
F
Percentage of Ki67+INS+ cells in INS+ cells
n.s.

Figure S3. Related to Figure 3. CDKAL1$^{-/-}$ insulin-GFP^{+} cells are hypersensitive to glucotoxicity and lipotoxicity. (A) The quantification of the percentage of PI^{+}/insulin^{+} cells in wildtype (wt), KCNQ1$^{-/-}$ and KCNJ11$^{-/-}$ insulin^{+} cells cultured in the presence of 2 mM D-Glucose (ctrl-g), 35 mM D-Glucose (glu), no palmitate (ctrl-p) and 1 mM palmitate (palm). (B) FACS analysis control of Figure 3C. (C and D) Flow cytometry analysis (C) and quantification of the percentage (D) of Annexin V^{+} cells in wt, KCNQ1$^{-/-}$, and KCNJ11$^{-/-}$ insulin-GFP^{+} cells cultured as in (A). (E and F) Immunocytochemistry analysis (E) and the quantification (F) of Ki67 and insulin staining in D30 wildtype and CDKAL1$^{-/-}$ cells. n=3 independent biological replicates. n.s. indicates a non-significant difference. Scale bar=100 μm. hESCs were differentiated using protocol 2.

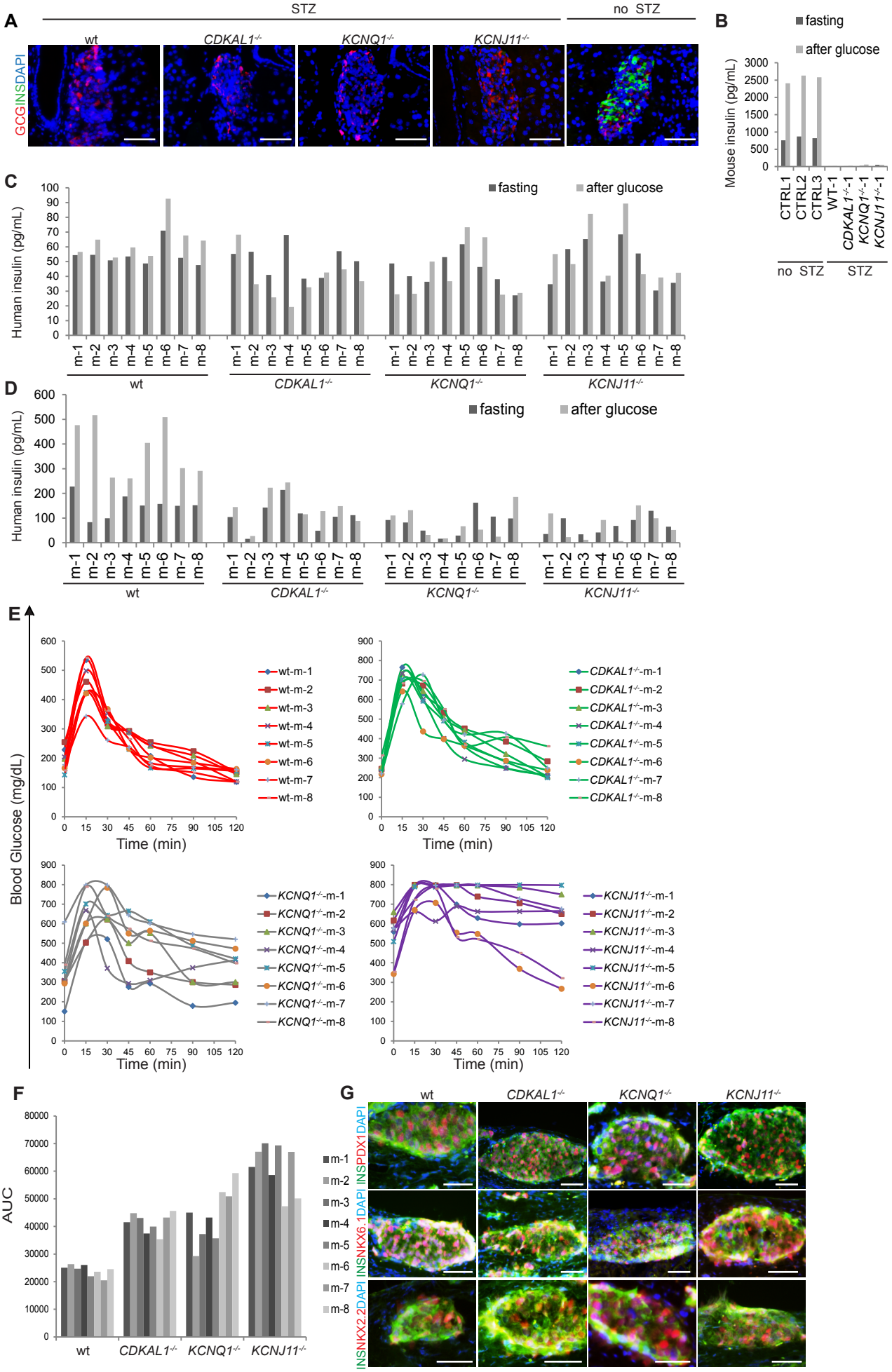
A
STZ
no STZ
wt
CDKAL1-/-
KCNQ1-/-
KCNJ11-/-
GCG INS DAPI
B
fasting
after glucose
Mouse insulin (pg/mL)
CTRL1
CTRL2
CTRL3
WT-1
CDKAL1-/--1
KCNQ1-/--1
KCNJ11-/--1
no STZ
STZ
C
Human insulin (pg/mL)
fasting
after glucose
m-1 m-2 m-3 m-4 m-5 m-6 m-7 m-8
wt
CDKAL1-/-
KCNQ1-/-
KCNJ11-/-
D
Human insulin (pg/mL)
fasting
after glucose
E
Blood Glucose (mg/dL)
Time (min)
wt-m-1
wt-m-2
wt-m-3
wt-m-4
wt-m-5
wt-m-6
wt-m-7
wt-m-8
CDKAL1-/--m-1
CDKAL1-/--m-2
CDKAL1-/--m-3
CDKAL1-/--m-4
CDKAL1-/--m-5
CDKAL1-/--m-6
CDKAL1-/--m-7
CDKAL1-/--m-8
KCNQ1-/--m-1
KCNQ1-/--m-2
KCNQ1-/--m-3
KCNQ1-/--m-4
KCNQ1-/--m-5
KCNQ1-/--m-6
KCNQ1-/--m-7
KCNQ1-/--m-8
KCNJ11-/--m-1
KCNJ11-/--m-2
KCNJ11-/--m-3
KCNJ11-/--m-4
KCNJ11-/--m-5
KCNJ11-/--m-6
KCNJ11-/--m-7
KCNJ11-/--m-8
F
AUC
wt
CDKAL1-/-
KCNQ1-/-
KCNJ11-/-
m-1
m-2
m-3
m-4
m-5
m-6
m-7
m-8
G
wt
CDKAL1-/-
KCNQ1-/-
KCNJ11-/-
INS PDX1 DAPI
INS NKX6.1 DAPI
INS NKX2.2 DAPI

Figure S4. Related to Figure 4. CDKAL1$^{-/-}$, KCNQ1$^{-/-}$, and KCNJ11$^{-/-}$ cells show defective glucose stimulated insulin secretion and impaired ability to maintain glucose homeostasis after transplantation into streptozotocin-treated immunedeficient mice. (A) Immunohistochemistry analysis of the pancreas of STZ treated mice. Untreated mice (no STZ) were used as a positive control for staining. (B) GSIS of SCID-beige mice with and without STZ treatment. (C and D) GSIS data for individual mice at two weeks (C) and six weeks (D) post transplantation. (E and F) IPGTT curves (E) and AUC (F) for individual mice at 6 weeks post transplantation. (G) Immunohistochemistry analysis of transplanted human grafts. GCG: glucagon, INS: insulin. Scale bar=200 μm. hESCs were differentiated using protocol 2.

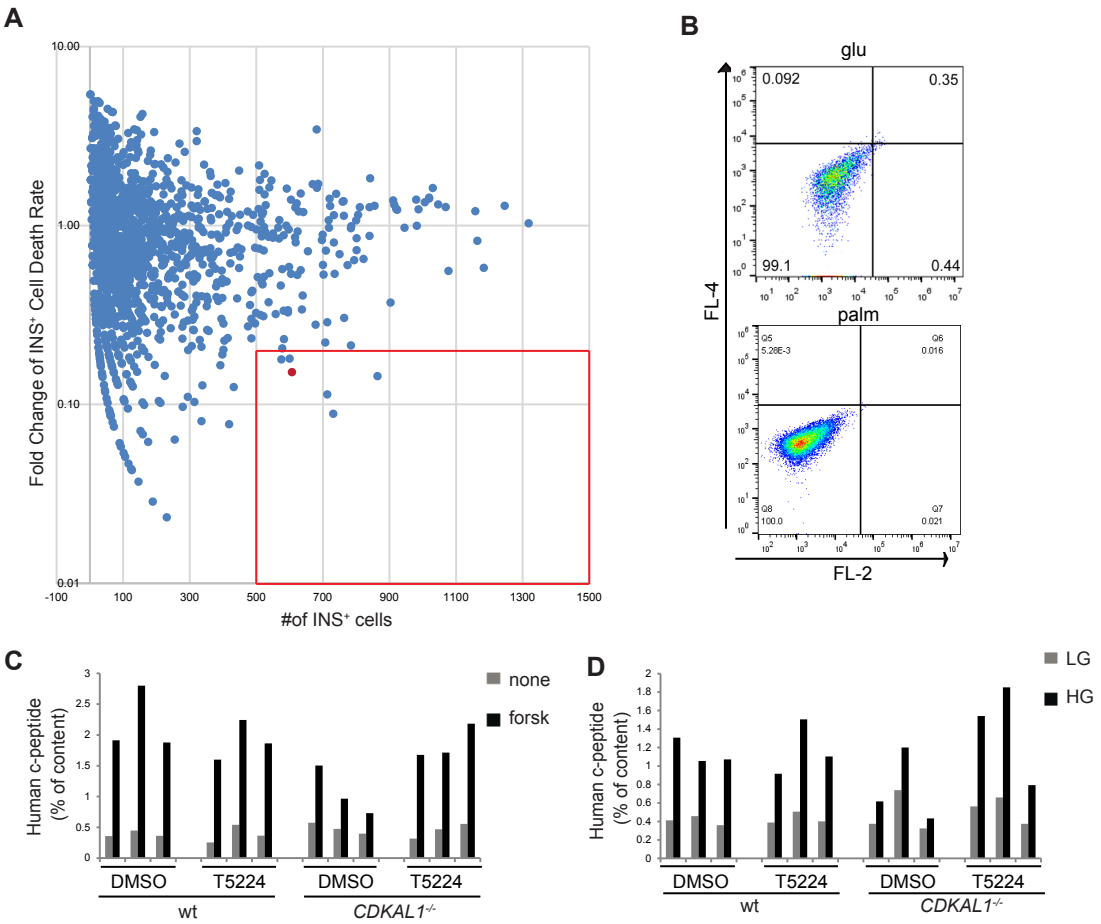
A
Fold Change of INS+ Cell Death Rate
#of INS+ cells
10.00
1.00
0.10
0.01
-100
100
300
500
700
900
1100
1300
1500
B
glu
0.092
0.35
99.1
0.44
palm
FL-4
FL-2
Q5
5.28E-3
Q6
0.016
Q8
100.0
Q7
0.021
C
Human c-peptide (% of content)
none
forsk
DMSO
T5224
DMSO
T5224
wt
CDKAL1-/-
D
Human c-peptide (% of content)
LG
HG
DMSO
T5224
DMSO
T5224
wt
CDKAL1-/-

Figure S5. Related to Figure 5. **A high content chemical screen identifies a drug candidate that rescues lipotoxicity caused specifically by mutations in CDKAL1.** (A) Primary screening results**.** Fold change of insulin$^+$ cell death rate: the percentage of PI$^+$INS$^+$ cells in insulin$^+$ cells of each well was normalized by the average percentage of PI$^+$INS$^+$ cells in insulin$^+$ cells; The red square highlights the lead compounds selected, showing suppressed cell death (20% or less of control) and increased INS$^+$ cells (at least 2-fold). T5224 is highlighted as the dark red dot. (B) FACS analysis unstained control for Figure 5F. (C and D) Data for individual experiments summarized in Figure 5H (C) and 5I (D). Data from experiments shown in Figure S5A was performed using protocol 1. Data from experiments shown in Figure S5B-S5D was performed using protocol 2.

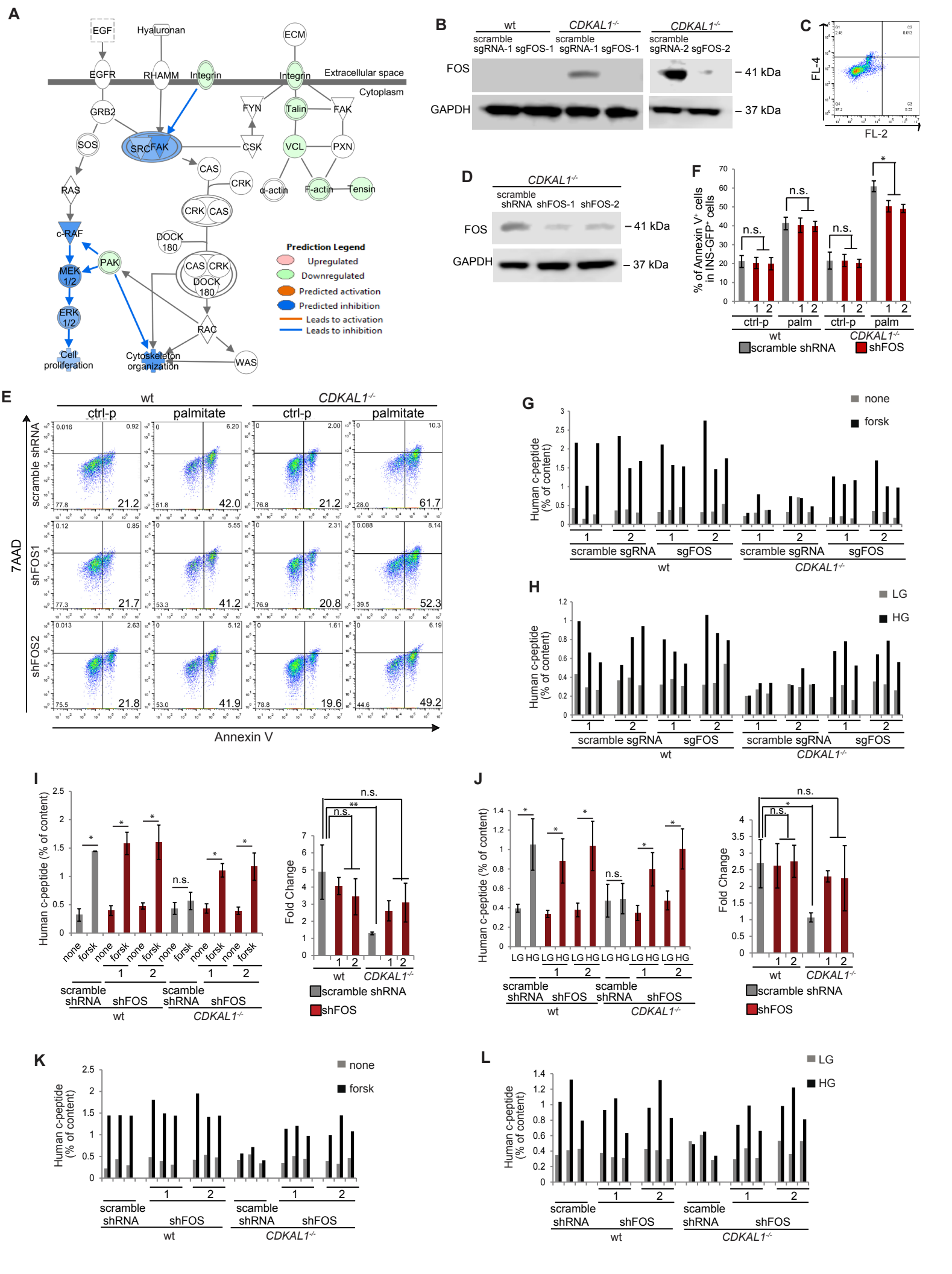
A
EGF
Hyaluronan
ECM
EGFR
RHAMM
Integrin
Integrin
Extracellular space
Cytoplasm
GRB2
FYN
Talin
FAK
SOS
SRC
FAK
CSK
VCL
PXN
CAS
CRK
RAS
α-actin
F-actin
Tensin
CRK
CAS
c-RAF
DOCK 180
PAK
CAS
CRK
DOCK 180
MEK 1/2
ERK 1/2
RAC
Cell proliferation
Cytoskeleton organization
WAS
Prediction Legend
Upregulated
Downregulated
Predicted activation
Predicted inhibition
Leads to activation
Leads to inhibition
B
wt
CDKAL1-/-
CDKAL1-/-
scramble sgRNA-1 sgFOS-1
scramble sgRNA-1 sgFOS-1
scramble sgRNA-2 sgFOS-2
FOS
GAPDH
– 41 kDa
– 37 kDa
C
FL-4
FL-2
D
CDKAL1-/-
scramble shRNA shFOS-1 shFOS-2
FOS
GAPDH
– 41 kDa
– 37 kDa
F
% of Annexin V+ cells in INS-GFP+ cells
n.s.
n.s.
n.s.
*
ctrl-p
palm
ctrl-p
palm
wt
CDKAL1-/-
scramble shRNA
shFOS
E
wt
CDKAL1-/-
ctrl-p
palmitate
ctrl-p
palmitate
scramble shRNA
shFOS1
shFOS2
7AAD
Annexin V
21.2
42.0
21.2
61.7
21.7
41.2
20.8
52.3
21.8
41.9
19.6
49.2
G
none
forsk
Human c-peptide (% of content)
scramble sgRNA
sgFOS
scramble sgRNA
sgFOS
wt
CDKAL1-/-
H
LG
HG
Human c-peptide (% of content)
scramble sgRNA
sgFOS
scramble sgRNA
sgFOS
wt
CDKAL1-/-
I
Human c-peptide (% of content)
none
forsk
scramble shRNA
shFOS
scramble shRNA
shFOS
wt
CDKAL1-/-
Fold Change
n.s.
**
n.s.
wt
CDKAL1-/-
scramble shRNA
shFOS
J
Human c-peptide (% of content)
LG HG
scramble shRNA
shFOS
scramble shRNA
shFOS
wt
CDKAL1-/-
Fold Change
n.s.
*
n.s.
wt
CDKAL1-/-
scramble shRNA
shFOS
K
Human c-peptide (% of content)
none
forsk
scramble shRNA
shFOS
scramble shRNA
shFOS
wt
CDKAL1-/-
L
Human c-peptide (% of content)
LG
HG
scramble shRNA
shFOS
scramble shRNA
shFOS
wt
CDKAL1-/-

Figure S6. Related to Figure 6. T5224 rescues beta cell defects caused by CDKAL1 mutation through inhibiting the FOS/JUN pathway. (A) Ingenuity Pathway Analysis of genes that are >2 fold upregulated in CDKAL1$^{-/-}$ insulin^{+} cells. (B) Western blotting data validating the effective knockout of FOS. (C) FACS analysis unstained control for Figure 6H. (D) Western blotting data validating the effective knockdown of FOS. (E and F) Flow cytometry analysis (E) and quantification of apoptotic rate (F) of CDKAL1$^{-/-}$ insulin-GFP^{+} cells expressing either scrambled shRNA or shFOS. (G and H) Data for individual experiments summarized in Figure 6J (G) and 6K (H). (I and J) Knockout FOS rescues the impaired forskolin-induced (I) and glucose-induced (J) insulin secretion that is caused by mutation of CDKAL1. (K and L) Data for individual experiments summarized in Figure S6I (K) and S6J (L). hESCs were differentiated using protocol 2. The data is presented as mean±S.D. n.s. indicates a non-significant difference. p values calculated by unpaired two-tailed Student's t-test were *$p<0.05$, **$p<0.01$.

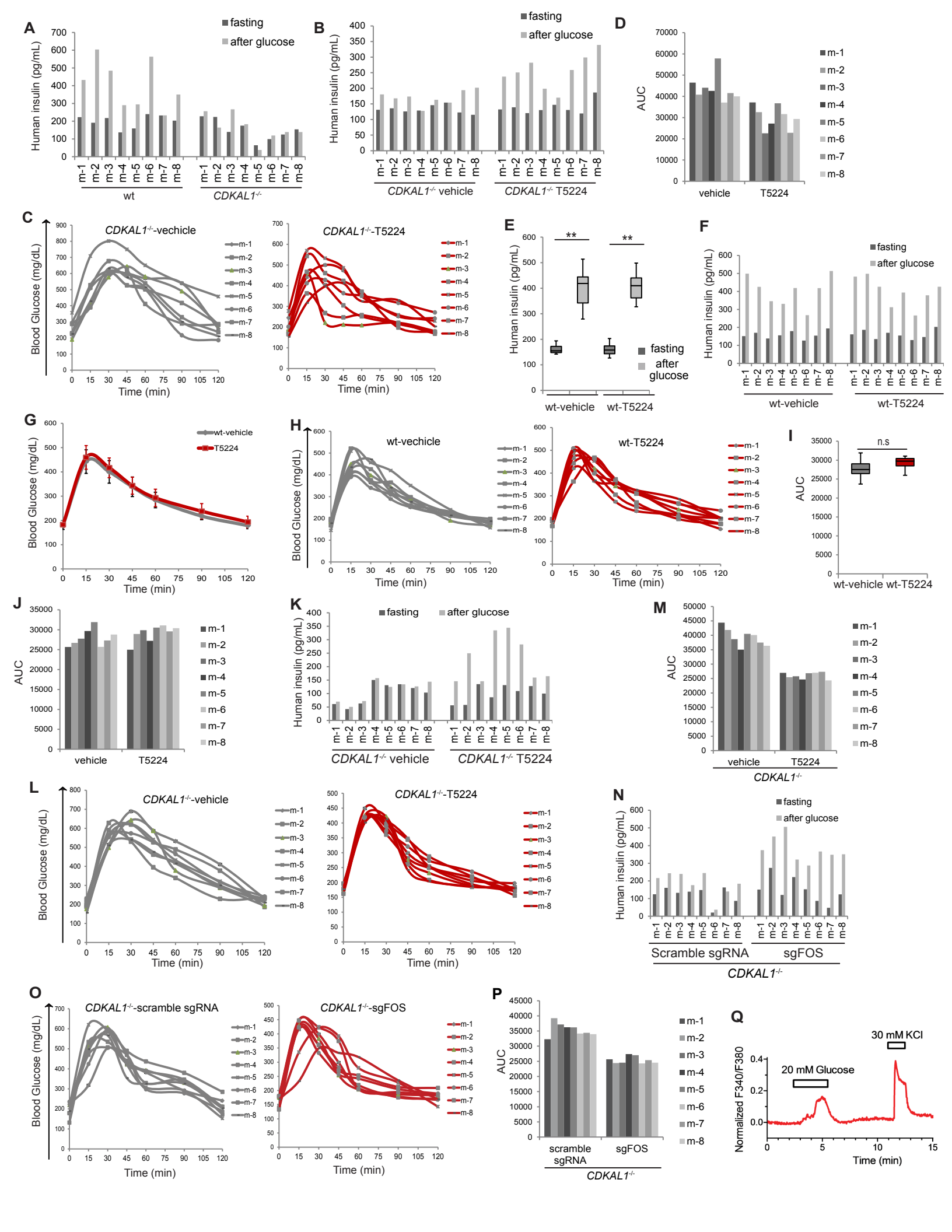
A
fasting
after glucose
Human insulin (pg/mL)
wt
CDKAL1-/-
B
fasting
after glucose
Human insulin (pg/mL)
CDKAL1-/- vehicle
CDKAL1-/- T5224
D
AUC
vehicle
T5224
C
CDKAL1-/--vechicle
CDKAL1-/--T5224
Blood Glucose (mg/dL)
Time (min)
E
Human insulin (pg/mL)
fasting
after glucose
wt-vehicle
wt-T5224
F
Human insulin (pg/mL)
fasting
after glucose
wt-vehicle
wt-T5224
G
wt-vehicle
T5224
Blood Glucose (mg/dL)
Time (min)
H
wt-vechicle
wt-T5224
Blood Glucose (mg/dL)
Time (min)
I
AUC
n.s
wt-vehicle wt-T5224
J
AUC
vehicle
T5224
K
fasting
after glucose
Human insulin (pg/mL)
CDKAL1-/- vehicle
CDKAL1-/- T5224
M
AUC
vehicle
T5224
CDKAL1-/-
L
CDKAL1-/--vehicle
CDKAL1-/--T5224
Blood Glucose (mg/dL)
Time (min)
N
fasting
after glucose
Human insulin (pg/mL)
Scramble sgRNA
sgFOS
CDKAL1-/-
O
CDKAL1-/--scramble sgRNA
CDKAL1-/--sgFOS
Blood Glucose (mg/dL)
Time (min)
P
AUC
scramble sgRNA
sgFOS
CDKAL1-/-
Q
30 mM KCl
20 mM Glucose
Normalized F340/F380
Time (min)

Figure S7. Related to Figure 7. T5224 rescues the function of CDKAL1$^{-/-}$ cells in vivo. (A) GSIS data of individual mice at 10 weeks post transplantation. (B) GSIS data of individual mice carrying CDKAL1$^{-/-}$ cells treated with 300 mg/kg T5224 or vehicle. (C, D) IPGTT curves (C) and AUC (D) of individual mice transplanted with CDKAL1$^{-/-}$ cells 48 hours after treated with 300 mg/kg T5224 or control vehicle. (E, F) GSIS secretion of SCID-beige mice (E) and individual mice (F) carrying wildtype human cells after 48 hours treatment with 300 mg/kg T5224 or vehicle; (G-J) IPGTT (G) and AUC (I) of mice transplanted with wildtype cells after 48 hours treated with 300 mg/kg T5224 or vehicle. IPGTT (H) and AUC (J) of individual mice transplanted with wildtype cells after 48 hr treated with 300 mg/kg T5224 or vehicle. (K) GSIS data of individual mice carrying CDKAL1$^{-/-}$ cells treated with 300 mg/kg T5224 or vehicle twice a week for four weeks. (L and M) IPGTT curves (L) and AUC (M) of individual mice transplanted with CDKAL1$^{-/-}$ cells treated with 300 mg/kg T5224 or control vehicle twice a week for four weeks. (N) GSIS data of individual mice transplanted with CDKAL1$^{-/-}$ cells carrying scramble sgRNA or sgFOS. (O and P) IPGTT curves (O) and AUC (P) of individual mice transplanted with CDKAL1$^{-/-}$ cells carrying scramble sgRNA or sgFOS. (Q) Ca^{2+} flux assay using wildtype D30 insulin-GFP^{+} cells upon 20 mM D glucose or 30 mM KCl stimulation. Calcium influx was elicited by 20 mM glucose and 30 mM KCl in HES3 cells. Cells were perfused with normal Tyrode solution at basal level, and Ca^{2+} influx was first elicited by 20 mM glucose. After washout with 30 mM glucose with normal Tyrode solution, and upon return of Ca^{2+} to the basal level, cells were perfused with 30 mM K^{+} solution to induce Ca^{2+} influx. The changes of Ca^{2+} influx elicited by 20 mM glucose and 30 mM KCl, as represented by the changes of F340/F380, were normalized to changes of F340/F380 induced by Ionomycin, The peak normalized F340/F380 was 0.259±0.33 for glucose and 0.45±0.02 for KCl (mean±SEM, n=17 from three replicated experiments). There was about 30-40% insulin-GFP^{+} cells responsive to 20 mM glucose and 30 mM KCl. n=8 mice for each condition. hESCs were differentiated using protocol 2. In GSIS assay, p values were calculated by one-way repeated measures ANOVA. In IPGTT assay, p values were calculated by two-way repeated measures ANOVA with a Bonferroni test for multiple comparisons between DMSO and T5224 treated conditions. p values were *$p<0.05$, ** $p<0.01$.

Supplemental Tables

Supplemental Table 1. Related to Figure 1. sgRNAs for targeted mutations using the CRISPR-Cas9 system.

Target Gene	sgRNA sequence
CDKAL1	CATCGAAGATATCGTGTCTC
KCNQ1	GTTCTTCGGGACGGAGTACG
KCNJ11	CGCCTTCGCCCACGGTGACC

Supplemental Table 2. Related to Figure 1. Table of mutation efficiency.

Target Genes	mutation efficiency
CDKAL1	6/42, 14.29%
KCNJ11	8/70, 11.43%
KCNQ1	12/82, 14.63%

Supplemental Table 3. Related to Figure 1. Scheme of directed differentiation protocols. Differences are highlighted.

	Stage 1 Definitive Endoderm	Stage 2 Primitive Endoderm	Stage 3 Posterior Foregut	Stage 4 Pancreatic Endoderm	Stage 5 Pancreatic Endocrine Precursors	Stage 6 Immature beta cells	Stage 7 Immature beta cells
	10 mM glucose; planar culture				20 mM glucose' air-liquid interface		
Rezaniar et al	3 d	2 d	2 d	3 d	3 d	7-15 d	7-15 d
	GDF8, GSK3β inh	FGF7 VitC	FGF7 VitC 1 μM RA SANT TPB LDN	FGF7 VitC 100 nM RA SANT TPB LDN	SANT 50 nM RA ALK5 inh II T3 LDN	ALK5 inh II T3 LDN GS inh XX	ALK5 inh II T3 N-Cys AXL inh
Zeng et al protocol 1	3 d	2 d	2 d	3 d	3 d	15 d	2 d
	GDF8, GSK3β inh	FGF7 VitC	FGF7 VitC 1 μM RA SANT TPB LDN	FGF7 VitC 100 nM RA SANT TPB LDN	SANT 50 nM RA ALK5 inh II T3 LDN	ALK5 inh II T3 LDN GS inh XX	ALK5 inh II T3 N-Cys AXL inh
Zeng et al protocol 2	3 d	2 d	4 d	4 d	3 d	12 d	2 d
	100 ng/ml Activin A 2 μM GSK3β inh	FGF7 VitC	FGF7 VitC 1 μM RA SANT TPB LDN	FGF7 VitC SANT TPB LDN	SANT 50 nM RA ALK5 inh II T3 LDN	ALK5 inh II T3 LDN GS inh XX	ALK5 inh II T3 N-Cys AXL inh

Supplemental Table 4. Related to Figure 1. The total c-peptide of primary human beta cells.

$$\text{Total insulin per cell}=\frac{\text{Total c-peptide}}{\text{Total number of islet cells X the percentage of insulin+cells X cellular viability}}$$

	Total c-peptide (ng/ml)	Total number of islet cells (#/ml)	The percentage of insulin$^+$ cells	Cellular viability	Total insulin per cell (ng/1k cells)
Islet preparation 1	23.65	54873	34.5%	32.4%	3.856
Islet preparation 2	19.17	69043	29.5%	29.1%	3.234
Islet preparation 3	34.8	109324	31.2%	45.5%	2.245

Supplemental Table 5. Related to Figure 1. P**rimers used for expression level analysis by qRT-PCR.**

Gene name	**Primer sequences**
CDKAL1	F:GTCGGTAGAGCATCAGACTT R:CCTGGACCCTCAGATTAAAA
KCNJ11	F: CTGTCCCGAAAGGGCATTAT R: CGTTGCAGTTGCCTTTCTTG
KCNQ1	F: CATCACCCACATCTCACAGC R: GTCCCGCACATCGTAAGG
PDX1	F: CCTTTCCCATGGATGAAGTC R: CGTCCGCTTGTTCTCCTC
NKX6.1	F: GGGGATGACAGAGAGTCAGG R: CGAGTCCTGCTTCTTCTTGG
NKX2.2	F: TCTACGACAGCAGCGACAAC R: GGAGCTTGAGTCCTGAGGG
PAX6	F: GTTGGTATCCGGGGACTT R: TCCGTTGGAACTGATGGAGT
GLUT2	F: GCCTGGTTCCTATGTATATCGGT R: GCCACAGATCATAATTGCCCAAG
GCK	F: GCAGAAGGGAACAATGTCGTG R: CGTAGTAGCAGGAGATCATCGT
MAFA	F: GAGTTGGCACTTCTCGCTCT R: TTCAGCAAGGAGGAGGTCAT
NEUROD1	F: ATGACCAAATCGTACAGCGAG R: GTTCATGGCTTCGAGGTCGT
ISL1	F: GCGGAGTGTAATCAGTATTTGGA R: GCATTTGATCCCGTACAACCT
UCN3	F: GATGGGCTTGGCTTTGTAGA R: GGAGGGAAGTCCATCCTCG
G6PC2	F: ACACTCCAAAGAAATGACCAGG R: CGCATCCTGTGTCTGGTATG
FOSB	F: ACCAGCTACTCAACCCCAG R: GGGTAAGTGTCTCTTCTCGGG
FOS	F: AGCTCTGCTTCACAGCGC R: GGCCTCCTGTCATGGTCTT
JUNB	F: CACCAAGTGCCGGAAGCGGA R: AGGGGCAGGGGACGTTCAGA
GAPDH	F: AATCCCATCACCATCTTCCA R: TGGACTCCACGACGTACTCA

Supplemental Table 6. Related to Figure 6. sgRNAs and shRNAs targeting for c-FOS.

Name	Sequence
sgFOS-1	CGTTTCTCTTCACTGCTGCA
sgFOS-2	GGGCTCGCCTGTCAACGCGC
Scramble sgRNA-1	CACCGACGGAGGCTAAGCGTCGCAA
Scramble sgRNA-2	CACCGAAGAGCGAATCGATTTCGTG
shFOS-1	GCGGAGACAGACCAACTAGAA
shFOS-2	TCTGCTTTGCAGACCGAGATT
Scramble shRNA-1	CCTAAGGTTAAGTCGCCCTCG

Supplemental Experimental Procedures

Directed differentiation protocol 1. Briefly, hESCs (~5x10^4 cells/cm^2) were plated on Matrigel-coated plates and cultured for 4 days to reach ~70% confluence (defined as day 0). Cells were first rinsed with 1× DPBS without Mg^{2+} and Ca^{2+} and then exposed to basal medium (MCDB 131 medium, Life Science), 1.5 g/l sodium bicarbonate (Sigma), 1× Glutamax (Life Science), supplemented with 10 mM glucose (Sigma), 0.5% BSA (fatty acid free BSA, Proliant), plus 100 ng/ml GDF8 (PeproTech), and 3 μM of CHIR99021 (GSK3β inhibitor3, SelleckChem) for 1 day. The concentration of CHIR was reduced to 0.3 μM at day 2 and withdrawn at day 3. The resulting definitive endoderm cells were treated with 0.25 mM ascorbic acid (Sigma) and 50 ng/ml FGF7 (R & D Systems) for 2 days, followed by 0.25 mM ascorbic acid, 50 ng/ml of FGF7, 0.25 μM SANT-1 (Sigma), 1 μM retinoic acid (RA; Sigma), 100 nM LDN193189 (LDN; BMP receptor inhibitor, Stemgent), 1:200 ITS-X (Life Science), and 200 nM TPB (PKC activator, Sigma) for an additional 2 days. At day 8, cells were exposed to basal medium plus 10 mM glucose, 2% BSA, 0.25 mM ascorbic acid, 2 ng/ml of FGF7, 0.25 μM SANT-1, 0.1 μM retinoic acid, 200 nM LDN193189, 1:200 ITS-X, and 100 nM TPB for 3 days. After 3 days in culture, the resulting pancreatic endoderm cells were treated for 4 h with 10 μM Y-27632. Cells were then rinsed with 1× DPBS without Mg^{2+} and Ca^{2+} and then exposed to TrypLE (1×) for 3–5 min at room temperature. The resulting cell pellet was resuspended as single cells at a density of ~0.5 × 10^5 cells/μl on filter inserts (Corning) using 10 μl per spot for a total of 0.25–0.5 × 10^6 cells/spot at an air-liquid interface. For 6-well filter inserts, 1.5 ml/well was added to the bottom of each insert whereas 8 ml was added for 10-cm filter inserts (Corning). Typically 10–15 spots were used per well of a 6-well insert. The medium supplements were changed to basal medium supplemented with 20 mM glucose, 2% BSA, 0.25 μM SANT-1, 0.05 μM retinoic acid, 100 nM LDN193189, 1:200 ITS-X, 1 μM T3 (3,3′,5-Triiodo-L-thyronine sodium salt, Sigma,), 10 μM ALK5 inhibitor II (Enzo Life Sciences), 10 μM zinc sulfate (Sigma) and 10 μg/ml of heparin (Sigma). Three days later, cells were exposed to basal medium supplemented with 20 mM final glucose concentration, 2% BSA, 100 nM LDN193189, 1:200 ITS-X, 1 μM T3, 10 μM ALK5 inhibitor II, 10 μM zinc sulfate, 100 nM gamma secretase inhibitor XX for the first 7 days only (EMD MilliPore) and 10 μg/ml of heparin for additional 8 days. Cells were cultured in basal medium supplemented with 20 mM glucose, 2% BSA, 1:200 ITS-X, 1 μM T3, 10 μM ALK5 inhibitor II, 10 μM zinc sulfate, 1 mM N-acetyl cysteine (N-Cys, Sigma), 10 μM Trolox (Vitamin E analogue, EMD), 2 μM R428 (AXL inhibitor, SelleckChem) and 10 μg/ml of heparin for a further 2 days.

Directed differentiation protocol 2. Briefly, hESCs (~5x10^4 cells/cm^2) were plated on Matrigel-coated plates and cultured for 4 days to reach ~70% confluence (defined as day 0). Cells were first rinsed with 1× DPBS without Mg^{2+} and Ca^{2+} and then exposed to basal medium (MCDB 131 medium, Life Science), 1.5 g/l sodium bicarbonate (Sigma), 1× Glutamax (Life Science), supplemented with 10 mM glucose (Sigma), 0.5% BSA (fatty acid free BSA, Proliant), plus 100 ng/ml Activin A (RnD), and 2 μM of CHIR99021 (GSK3β inhibitor3, SelleckChem) for 1 day. CHIR99021 was withdrawn at day 2. The resulting definitive endoderm cells were treated with 0.25 mM ascorbic acid (Sigma) and 50 ng/ml FGF7 (R & D Systems) for 2 days, followed by 0.25 mM ascorbic acid, 50 ng/ml of FGF7, 0.25 μM SANT-1 (Sigma), 1 μM retinoic acid (RA; Sigma), 100 nM LDN193189 (LDN; BMP receptor inhibitor, Stemgent), 1:200 ITS-X (Life Science), and 200 nM TPB (PKC activator, Sigma) for an additional 4 days. At day 10, cells were exposed to basal medium plus 10 mM glucose, 2% BSA, 0.25 mM ascorbic acid, 10 ng/ml of FGF7, 0.25 μM SANT-1, 200 nM LDN193189, 1:200 ITS-X, and 100 nM TPB for 4 days. After 4 days in culture, the resulting pancreatic endoderm cells were treated for 4 hours with 10 μM Y-27632. Cells were then rinsed with 1× DPBS without Mg^{2+} and Ca^{2+} and then exposed to TrypLE (1×) for 3–5 min at room temperature. The resulting cell pellet was resuspended as single cells at a density of ~0.5 × 10^5 cells/μl on filter inserts (Corning) using 10 μl per spot for a total of 0.25–0.5 × 10^6 cells/spot at an air-liquid interface. For 6-well filter inserts, 1.5 ml/well was added to the bottom of each insert whereas 8 ml was added for 10-cm filter inserts (Corning). Typically 10–15 spots were used per well of a 6-well insert. The medium supplements were changed to basal medium supplemented with 20 mM glucose, 2% BSA, 0.25 μM SANT-1, 0.05 μM retinoic acid, 100 nM LDN193189, 1:200 ITS-X, 1 μM T3 (3,3′,5-Triiodo-L-thyronine sodium salt, Sigma,), 10 μM RepSox (ALK5 inhibitor II, Tocris or Enzo Life Sciences), 10 μM zinc sulfate (Sigma) and 10 μg/ml of heparin (Sigma). Three days later, cells were exposed to basal medium supplemented with 20 mM final glucose concentration, 2% BSA, 100 nM LDN193189, 1:200 ITS-X, 1 μM T3, 10 μM RepSox (ALK5 inhibitor II, Tocris or Enzo Life Sciences), 10 μM zinc sulfate, 100 nM gamma secretase inhibitor XX for the first 7 days only (EMD MilliPore) and 10 μg/ml of heparin to 12 days. Cells were cultured in basal medium supplemented with 20 mM glucose, 2% BSA, 1:200 ITS-X, 1 μM T3, 10 μM ALK5 inhibitor II, 10 μM zinc sulfate, 1 mM N-acetyl cysteine (N-Cys, Sigma), 10 μM Trolox (Vitamin E analogue, EMD), 2 μM R428 (AXL inhibitor, SelleckChem) and 10 μg/ml of heparin for a further 2 days. For all stages, the cultures were fed daily.

Targeted mutation of c-FOS in hESCs derived pancreatic progenitors using lentiviral-based sgRNA. The resulting sgRNA cassettes of FOS were cloned into the plentiCRISPR v2 vector that contains a puromycin cassette for selection (Addgene plasmid#52961). Two independent sgRNAs targeted different locations of exon1 of c-FOS. Alternatively, two mock plentiCRISPR v2-sgRNAs were constructed as negative controls. Two independent scramble ''target'' controls were designed to have low homology to the human genome and are used as a non-targeting control as well. Each of the seven plasmids was used to prepare lentivirus in the 293T cell line (Invitrogen) with Lipofectamine 2000 (Thermo Fisher Scientific). The lentiviral supernatants were harvested, pooled, and supplemented with 6 μg/ml polybrene (Sigma) for infection of hESCs-derived pancreatic endoderm on day 8-10 of induction. Forty-eight hours after infection, 500 ng/ml puromycin was added daily for selection of cell clones expressing the transgenes. Cells were trypsinized and transferred to air-liquid interface as previously described 4-6 days after puromycin selection.

Insulin secretion assay. Cells were starved in 1 ml glucose-free DMEM (with GluMax) for 1 hour in a 5% CO2 /37° C incubator. After washing with warmed KRBH, cells were incubated in KRBH (with 0.1% BSA) for 1 hour in air incubator at 37°C. Cells were then incubated in 1 ml KRBH with combinations of 2 mM glucose, 20 mM glucose, 30 mM KCl, 10 mM arginine (Sigma A5006), 20 μM forskolin (Sigma, F6886) or 50 μM IBMX (Sigma, 28822) in an air incubator at 37°C for 1 hour. 500-1000 μl medium were collected from each well and filtered by a 0.45 μm filter for ELISA analysis (Human C-peptide ELISA kit, Millipore, EZHCP-20K). Cells were lysed to measure the protein levels in each sample for normalization to total c-peptide content (Human C-peptide ELISA kit, Millipore, EZHCP-20K).

Annexin V cellular apoptosis analysis. Cells were dissociated by Accutase (Stem Cell Technologies) and washed with cold DPBS, subjected to the PE/Annexin V apoptosis detection Kit 1 (BD Bioscience, 559763), and then analyzed by flow cytometry (BD Bioscience, FASCvantage) within 1 hour.

RNA-seq. The quality of RNA samples was analyzed, and cDNA libraries were synthesized and sequenced by the Weill Cornell Genomics Core. In brief, the quality of RNA samples was examined by Agilent bioanalyzer (Agilent). cDNA libraries were generated using TruSeq RNA Sample Preparation (Illumina). Each library was sequenced using single-reads in HiSeq2000/1000 (Illumina). Gene expression levels were analyzed using Cufflinks(Trapnell et al., 2012; Trapnell et al., 2010).

Bioinformatics Analysis. For heat map normalization, RPKM values were normalized per gene over all samples. Specifically, mean and standard deviation (stdev) of RPKM over all samples were calculated for each gene, and used to linearly transform RPKM using the formula (rpkm-mean)/stdev. The heat map was then generated by heatmap.2 in the R(Team., 2015) gplots(Gregory R. Warnes, 2015) package. Pathway analysis on ER stress was performed using QIAGEN's Ingenuity® Pathway Analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity). The upstream and/or downstream effects of activation or inhibition of molecules in the pathway were predicted using the Molecule Activity Predictor tool in the same tool suite. Pathway enrichment analysis on up/down-regulated genes was performed using the DAVID function annotation tool(Huang da et al., 2009a, b).

Immunocytochemistry analysis. Cells were fixed with 10% (v/v) formalin for 20 min at room temperature (RT) and blocked in a solution of Ca^{++}/Mg^{++}-free PBS containing 5% horse serum and 0.3% TritonX for 1 hour at room temperature and followed by incubation with primary antibody at 4C overnight. Primary antibodies were anti-NANOG (1:100, R&D), anti-SOX2 (1:200, Santa Cruz), anti-TRA-1-60 (1:100, Millipore), anti-TRA-1-81 (1:100, Millipore), anti-SOX17 (1:500, R&D), anti-FOXA2 (1:500, 07-633 Millipore), anti-PDX1 (1:500, R&D), anti-SOX9 (1:500, Millipore), anti-NKX6.1 (1:500, University of Iowa Hybridoma bank), anti-NKX2.2 (1:500, U of lowa Hybridoma bank), and anti-insulin (1:500, DAKO). After washing three times, cells were incubated with secondary antibodies for 1 hour at room temperature. Alexa fluor secondary antibodies were obtained from Invitrogen (1:500). The images were quantified using MetaMorph Image Analysis Software (Molecular Device).

Flow Cytometry and intracellular FACS analysis. hESC-derived D30 cells were dissociated by Accutase (STEM CELL Technologies) and the resulting single cell suspensions was directly analyzed with an Accuri C6 flow cytometry instrument to detect the percentage of GFP^+ cells. In addition, the D30 cells were fixed and stained with primary antibody at 4C overnight. Primary antibodies were anti-NKX6.1 (1:500), anti-NKX2.2 (1:500), and anti-insulin (1:500). After washing three times, cells were incubated with secondary antibodies for 1 hour at room temperature. Alexa 488 conjugated donkey anti-mouse or Alexa 647 conjugated donkey anti-guinea pig secondary antibody (1:500) using cytofix/cytoperm (BD Bioscience) and wash buffer (BD Bioscience) according to

instructions by the manufacturer. Flow cytometry data were obtained using an Accuri C6 flow cytometry instrument and analyzed with FlowJo.

Western blotting analysis. Cells were collected in complete RIPA buffer (Millipore). Lysates were separated electrophoretically on 4-12% Bis-Tris gels (Life technologies and proteins transferred to nitrocellulose membrane (Life technologies). Membranes were blocked (5% milk in TBS-T) and incubated with primary antibody overnight. Primary antibodies used were rabbit anti-cFOS (1:500, Abcam) and GAPDH (Abcam). Membranes were washed and incubated with secondary antibody for 1 hour at room temperature in TBS-T, 5% milk and developed using Chemiluminescent substrate (LI-COR).

Quantitative RT-PCR analysis. Total RNA was isolated using Qiagen RNeasy mini kit according to instructions by the manufacturer. 0.5 μg of total RNA was used to generate first strand cDNA using the Superscript III First Strand Synthesis System (Thermofisher). First strand cDNA products were diluted 4-fold and used as qPCR templates. SYBR Green-based qPCR was carried out using the Roche 480 Lightcycler. Triplicate reactions were carried out for each sample. GAPDH was used as a control to normalize target gene expression. Sequences of primers used are listed in Table S3.

Patch-Clamp experiments. Whole-cell currents were recorded using an Axopatch 200B amplifier. Data were digitized at 5 or 10 kHz, and digitally filtered off-line at 1 kHz. Patch electrodes were pulled from borosilicate glass and fire-polished to a resistance of ~3 MΩ when filled with internal solutions. Series resistance (Rs) was compensated up to 90% to reduce series resistance errors to <5 mV. Cells with Rs bigger than 10 MΩ were discarded (Du et al., 2010). The whole cell currents were elicited by voltage stimuli lasting 200 ms delivered at 1 s intervals. In order to avoid activation of voltage-gated ion channels, a depolarization step protocol from holding potential of 0 mV to +80 mV was used to record K_{ATP} currents. A fast perfusion system was used to exchange extracellular solutions containing K_{ATP} channel activator diazoxide or inhibitor glybenclamide.

The internal pipette solution for K_{ATP} whole cell current recordings (Pasyk et al., 2004) contained (in mM): 115 K^+-Aspartate 115, 10 K^+-Chloride, 1 $MgCl_2$, 10 HEPES, 1 EGTA-K^+, 0.5 GTP, and 0.5 ATP, with pH adjusted to 7.2 with KOH. The standard extracellular Tyrode's solution for whole cell current recording contained (mM): 145 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES and 10 glucose; pH was adjusted to 7.4 with NaOH.

All the chemicals, diazoxide and glybenclamide were purchased from Sigma.

Ca^{2+} flux. Cells plated on 25 mm glass coverslips were loaded with 5 μM Fura-2 for 45 minutes. Non-incorporated dye was washed away using a HEPES-buffered Saline Solution (HBSS) containing (in mM) 20 HEPES, 10 glucose, 1.2 $MgCl_2$, 1.2 KH_2PO_4, 4.7 KCl, 140 NaCl, 1.3 Ca^{2+} (pH 7.4). At basal level, cells were perfused with normal Tyrode solution containing (in mM) 145 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES and 3 glucose; pH was adjusted to 7.4 with NaOH. The NaCl concentration was reduced in the modified Tyrode solutions containing 30 mM KCl or 20 mM glucose in order to keep the similar osmolarity (290~300 Osmole).

Cells were first perfused with normal Tyrode solution for 10-15 min to reach a steady-state basal level. Before treatment with 20 mM glucose or 30 mM KCl, 2~3 min recording at basal level was performed to ensure the staeady-state basal level. The effects of 20 mM glucose or 30 mM KCl were washout to make sure the recovery of Ca^{2+} sinal to the basal level. Ionomycin at 1 μM was applied at the end of each experiment as an internal control. Fluorescence intensities at 510 nm with 340 nm and 380 nm excitation were collected at a rate of 1 Hz using CoolSNAP HQ2 (Photometrics) and data were analyzed using NIS-Elements (Nikon). The fluorescence intensity ratio at 340:380 nm (F340/F380) in the presence of 20 mM glucose or 30 mM KCl was normalized to that of the Ca^{2+} signal elicited by 1μM Ionomycin as we previously reported (Du et al., 2010).

Targeted c-FOS in hESCs derived pancreatic progenitors shRNA Knockdown Assay. The shRNA constructs were constructed with Puro 2.0 plasmids (Addgene plasmid#24970) to facilitate the production of lentiviral particles following co-transfection with appropriate packaging vectors in 293T cells. Alternatively, one mock Puro 2.0-scrambleRNA was constructed as a negative control. Lentiviral particles were tittered by qPCR and used to transduce 0.5 million hESCs-derieved human pancreatic progenitors in biological duplicate at a MOI of 5~10. Two days after transduction, puromycin (0.5 μg/ml) was added to the media, and the cells were subjected to selection for 4 to 6 days after which cells were transferred to air-liquid interface membrane for further experiments.

Supplemental References.

Du, J., Xie, J., Zhang, Z., Tsujikawa, H., Fusco, D., Silverman, D., Liang, B., and Yue, L. (2010). TRPM7-mediated Ca2+ signals confer fibrogenesis in human atrial fibrillation. Circulation research 106, 992-1003.

Gregory R. Warnes, B.B., Lodewijk Bonebakker, Robert Gentleman, Wolfgang Huber Andy Liaw, Thomas Lumley, Martin Maechler, Arni Magnusson, Steffen Moeller, Marc Schwartz, and Bill Venables. (2015). gplots: Various R Programming Tools for Plotting Data

Huang da, W., Sherman, B.T., and Lempicki, R.A. (2009a). Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic acids research 37, 1-13.

Huang da, W., Sherman, B.T., and Lempicki, R.A. (2009b). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature protocols 4, 44-57.

Pasyk, E.A., Kang, Y., Huang, X., Cui, N., Sheu, L., and Gaisano, H.Y. (2004). Syntaxin-1A binds the nucleotide-binding folds of sulphonylurea receptor 1 to regulate the KATP channel. The Journal of biological chemistry 279, 4234-4240.

Team., R.C. (2015). R: A Language and Environment for Statistical Computing. In R Foundation for Statistical Computing (Vienna, Austria,).

Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D.R., Pimentel, H., Salzberg, S.L., Rinn, J.L., and Pachter, L. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nature protocols 7, 562-578.

Trapnell, C., Williams, B.A., Pertea, G., Mortazavi, A., Kwan, G., van Baren, M.J., Salzberg, S.L., Wold, B.J., and Pachter, L. (2010). Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nature biotechnology 28, 511-515.