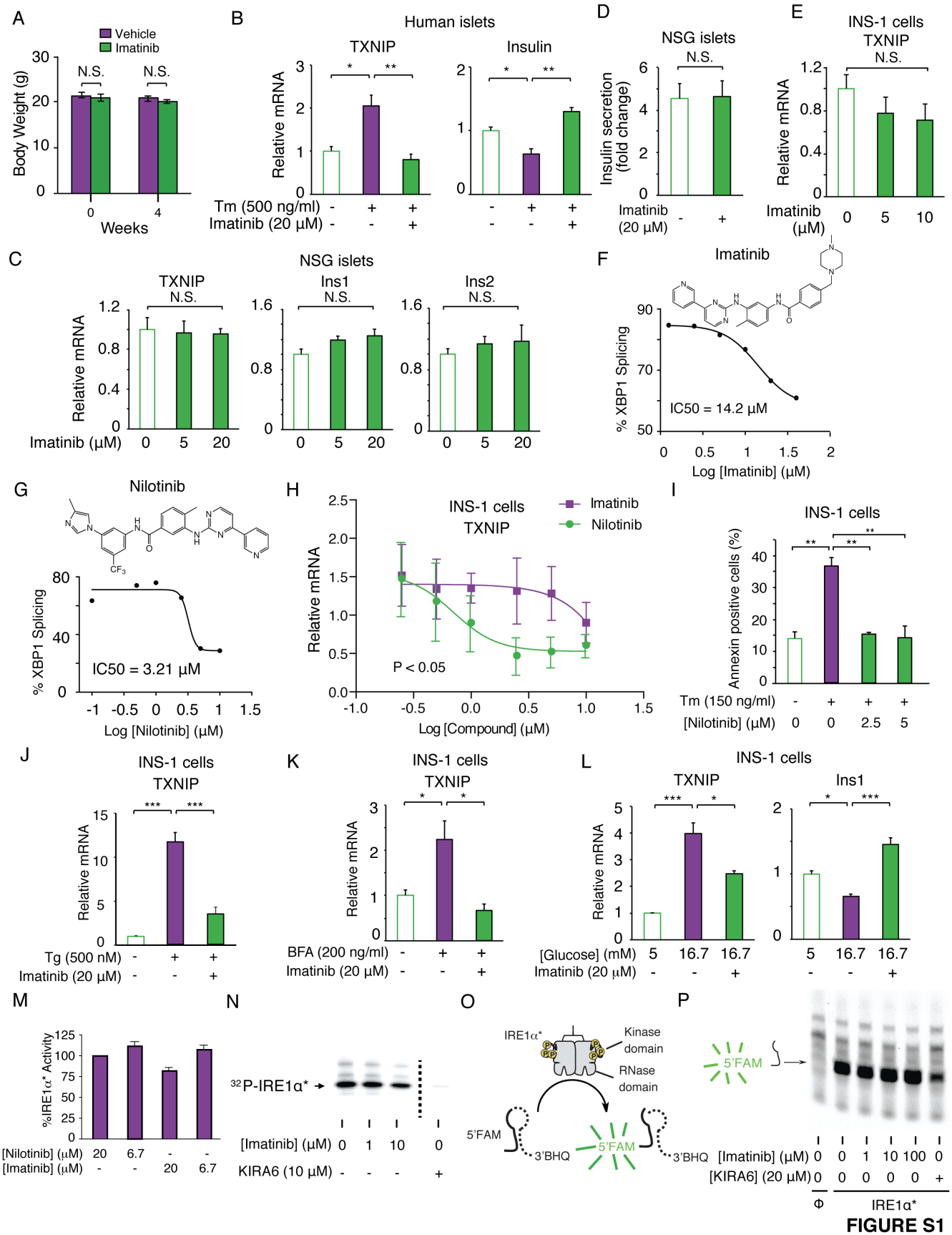
A
Vehicle
Imatinib
Body Weight (g)
N.S.
N.S.
Weeks
B
Human islets
TXNIP
Insulin
Relative mRNA
Tm (500 ng/ml)
Imatinib (20 μM)
C
NSG islets
TXNIP
Ins1
Ins2
N.S.
Relative mRNA
Imatinib (μM)
D
NSG islets
N.S.
Insulin secretion (fold change)
Imatinib (20 μM)
E
INS-1 cells
TXNIP
N.S.
Relative mRNA
Imatinib (μM)
F
Imatinib
% XBP1 Splicing
IC50 = 14.2 μM
Log [Imatinib] (μM)
G
Nilotinib
% XBP1 Splicing
IC50 = 3.21 μM
Log [Nilotinib] (μM)
H
INS-1 cells
TXNIP
Imatinib
Nilotinib
Relative mRNA
P < 0.05
Log [Compound] (μM)
I
INS-1 cells
Annexin positive cells (%)
Tm (150 ng/ml)
[Nilotinib] (μM)
J
INS-1 cells
TXNIP
Relative mRNA
Tg (500 nM)
Imatinib (20 μM)
K
INS-1 cells
TXNIP
Relative mRNA
BFA (200 ng/ml)
Imatinib (20 μM)
L
INS-1 cells
TXNIP
Ins1
Relative mRNA
[Glucose] (mM)
Imatinib (20 μM)
M
%IRE1α* Activity
[Nilotinib] (μM)
[Imatinib] (μM)
N
32P-IRE1α*
[Imatinib] (μM)
KIRA6 (10 μM)
O
IRE1α*
Kinase domain
RNase domain
5'FAM
3'BHQ
P
5'FAM
[Imatinib] (μM)
[KIRA6] (20 μM)
Φ
IRE1α*

FIGURE S1

Figure S1. Imatinib and nilotinib reduce ER stress-induced T-UPR endpoints to prevent β-cell death. Related to Figure 1 and 2.

(A) Imatinib (1.5 mg/mouse) or vehicle was administered daily by oral gavage for 4 weeks in pre-diabetic NOD mice. Body mass before and after treatment is shown. 4 mice per group. (B) qPCR of relative TXNIP and Insulin mRNA levels in human islets pretreated for 2 hr with ± imatinib, followed by ± Tm for 16 hr. (C) In the absence of ER stress agents, levels of relative indicated mRNAs, by qPCR, from NSG islets treated for 8 hr with indicated [imatinib] remained unchanged. (D) In the absence of ER stress agents, glucose-stimulated insulin secretion from NSG islets treated ± imatinib for 18 hr remained unchanged. [Glucose] was 2.5 mM or 28 mM for 30 min. Data shown as ratio of insulin levels at 28 mM over 2.5 mM glucose. (E) In the absence of ER stress agents, levels of relative TXNIP mRNA, by qPCR, in INS-1 cells treated for 6 hr with indicated [imatinib] remained unchanged. (F and G) % Spliced XBP1 in INS-1 cells pretreated with indicated [imatinib] (F) or [nilotinib] (G) for 4 hr, followed by 1 μg/ml Tm for 4 hr. (H) qPCR of relative TXNIP mRNA levels in INS-1 cells pretreated with indicated [imatinib] or [nilotinib] for 4 hr, followed by treatment with 1 μg/ml Tm for 8 hr. Two-way ANOVA was performed for statistical analysis. (I) Annexin V staining in INS-1 cells co-treated with Tm and indicated [nilotinib] for 48 hr. (J and K) qPCR of relative TXNIP mRNA levels in INS-1 cells pretreated with ± imatinib for 2 hr, followed by Tg (J) or BFA (K) for 8 hr. (L) qPCR for relative TXNIP and Ins1 mRNA levels in INS-1 cells co-incubated with either 5 mM or 16.7 mM glucose and ± imatinib for 72 hr. (M) Percent IRE1α* kinase activity in the presence of either nilotinib or imatinib at 20 μM and 6.7 μM relative to DMSO treated IRE1α*. Triplicated. (N) Autoradiogram showing the autophosphorylation of human WT IRE1α* under indicated [imatinib]. KIRA6 was used as a control. (O) 5' FAM-3' BHQ XBP1 mini-substrate used to measure RNase activity of IRE1α. (P) Urea-PAGE of XBP1 RNA mini-substrate products cleaved by IRE1α* incubated with indicated [imatinib] or

KIRA6. Bars; mean±SEM. Three independent biological samples were used for qPCR, insulin secretion, and Annexin V staining. P-values: $^{*} < 0.05$, $^{**} < 0.01$, $^{***} < 0.001$.

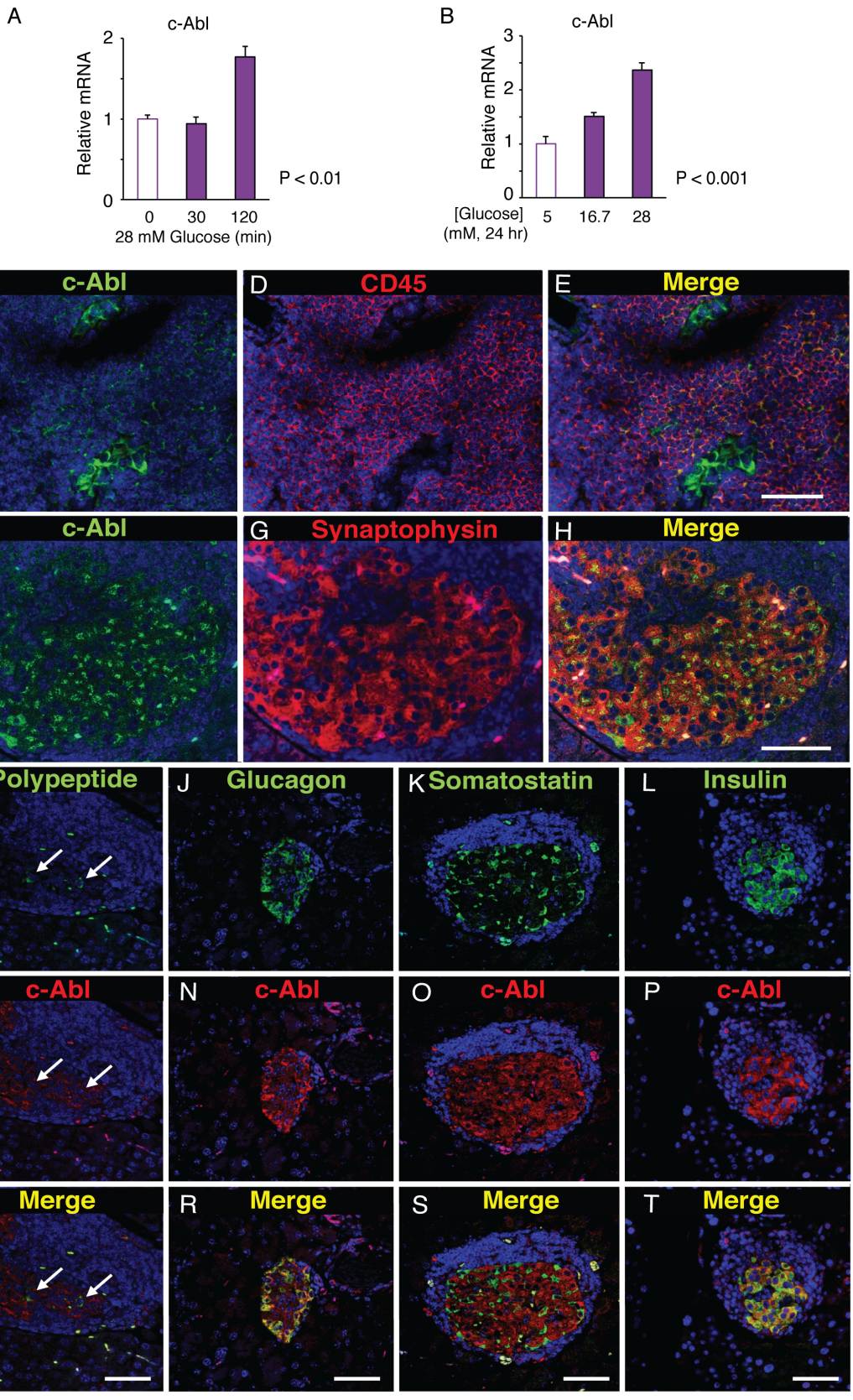
A
c-Abl
Relative mRNA
2
1
0
P < 0.01
0
30
120
28 mM Glucose (min)
B
c-Abl
Relative mRNA
3
2
1
0
P < 0.001
[Glucose]
(mM, 24 hr)
5
16.7
28
C c-Abl
D CD45
E Merge
F c-Abl
G Synaptophysin
H Merge
I Polypeptide
J Glucagon
K Somatostatin
L Insulin
M c-Abl
N c-Abl
O c-Abl
P c-Abl
Q Merge
R Merge
S Merge
T Merge

FIGURE S2

Figure S2. Endocrine cells are the predominant source of c-Abl in the NOD pancreas. Related to Figure 3.

(A) qPCR for relative c-Abl mRNA levels in INS-1 cells treated with 5 mM glucose for 17 hr, and then treated with 28 mM glucose for the indicated time. One-way ANOVA with post-hoc test for trend was performed for statistical analysis. Biologically triplicated. (B) qPCR of relative c-Abl mRNA levels in INS-1 cells treated with indicated [glucose] for 24 hr. One-way ANOVA with post-hoc test for trend was performed for statistical analysis. Biologically triplicated. (C-E) Frozen sections of 12-week-old NOD pancreas were immunostained with antibodies specific for c-Abl (C, green) and CD45 (D, red). (F-H) Sections from formalin-fixed, 12-week-old NOD pancreas were stained with c-Abl (F, green) and synaptophysin (G, red) specific antibodies. (I-T) Identity of the endocrine cell expressing c-Abl was assessed by staining sections with antibodies specific for pancreatic polypeptide (I, green), glucagon (J, green), somatostatin (K, green) and insulin (L, green) and c-Abl (M-P, red). All images are representative of staining performed on 3 mice per cell-subset analyzed. Scale bar, 50 μm. Arrows in (I), (M) and (Q) indicate pancreatic polypeptide positive cells. Autofluorescence of red blood cells can be detected in the green and red channels, but not in the blue channel.

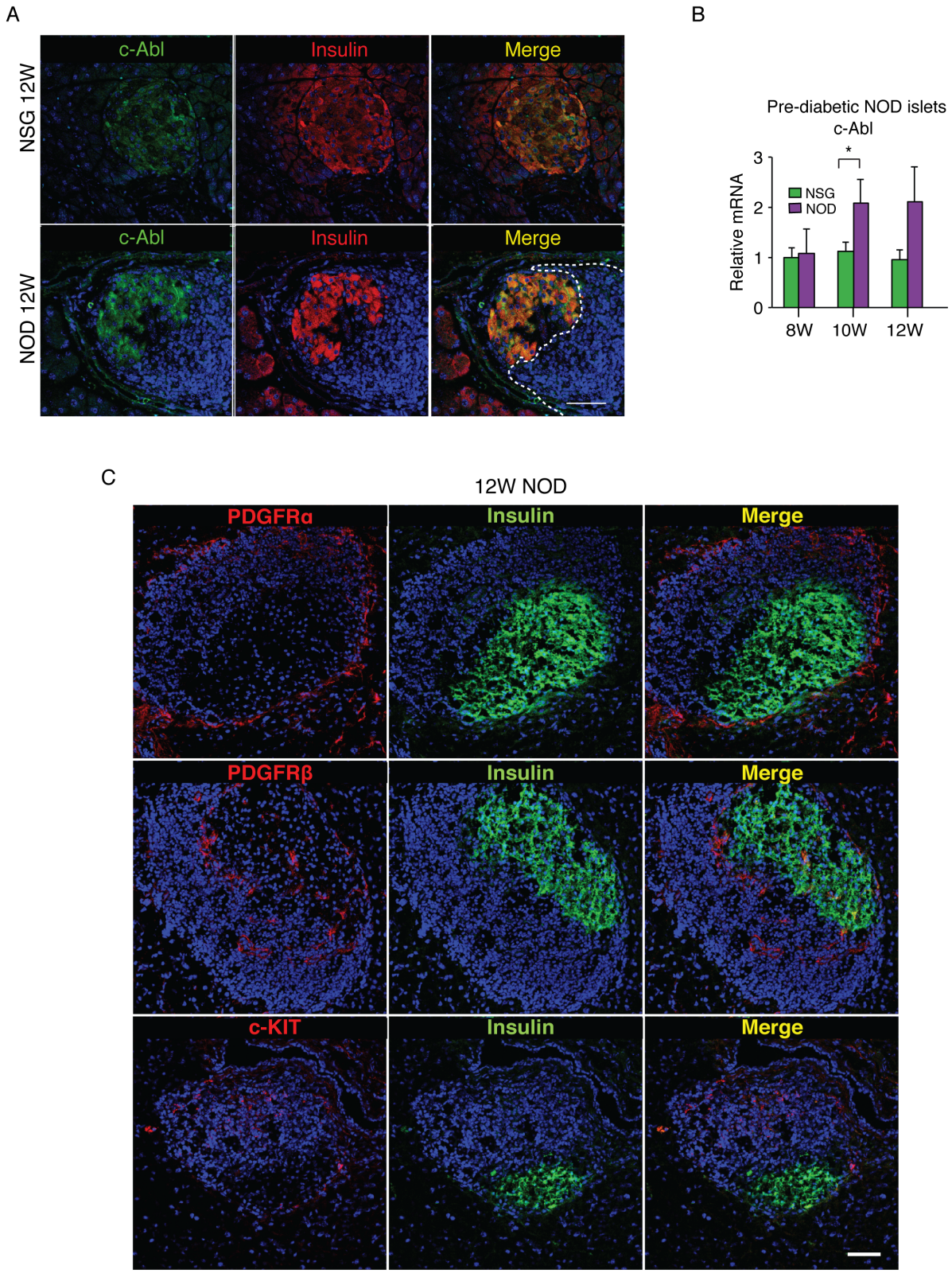
A
c-Abl
Insulin
Merge
NSG 12W
NOD 12W
B
Pre-diabetic NOD islets
c-Abl
Relative mRNA
NSG
NOD
8W
10W
12W
C
12W NOD
PDGFRα
PDGFRβ
c-KIT
Insulin
Merge

FIGURE S3

Figure S3. c-Abl is induced in NOD β-cells; PDGFR and c-KIT are not expressed in β-cells. Related to Figure 3.

(A) Anti-c-Abl (green) and anti-proinsulin (red) immunofluorescence staining of tissue sections prepared from pancreata isolated from 12-week-old NSG and NOD mice that were fasted overnight. Images are representative of 5 mice from each group. Infiltrating cells delineated by dashed outline. Scale bar, 50 μm. (B) qPCR for relative c-Abl mRNA levels in islets purified from pre-diabetic NOD and control NSG mice at the indicated ages. Two-way ANOVA followed by post-hoc Tukey's test was performed for statistical analysis. 3-7 mice per group. P-values: $^{*} < 0.05$. (C) Expression of the imatinib targets PDGFRα (top, red), PDGFRβ (middle, red) and c-KIT (bottom, red) in 12-week-old NOD pancreata was examined by immunofluorescence staining. Islets (anti-insulin staining) and nuclei (DAPI) are shown in green and blue, respectively.

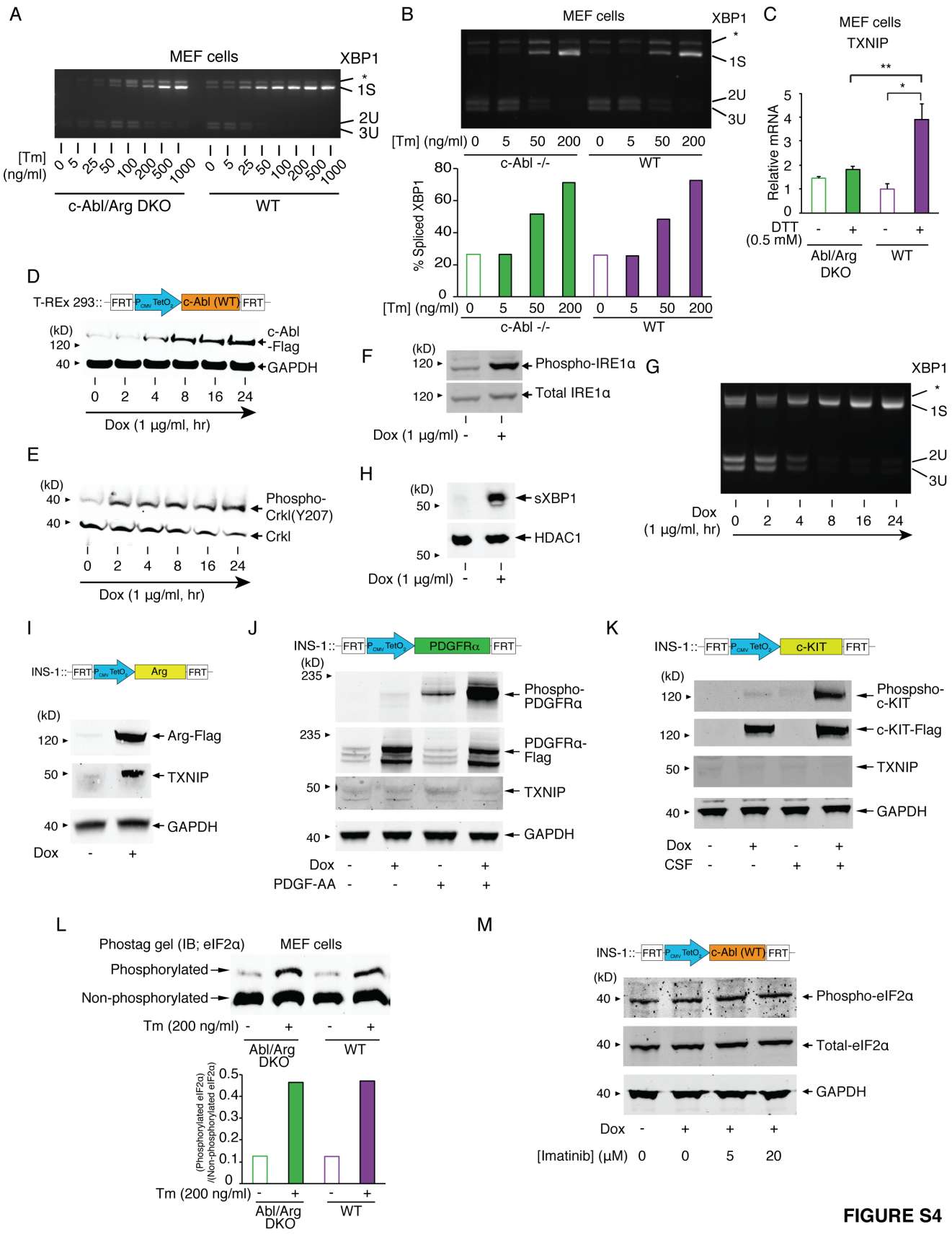
A
MEF cells
XBP1
*
1S
2U
3U
[Tm] (ng/ml)
0 5 25 50 100 200 500 1000
0 5 25 50 100 200 500 1000
c-Abl/Arg DKO
WT
B
MEF cells
XBP1
[Tm] (ng/ml) 0 5 50 200 0 5 50 200
c-Abl -/-
WT
% Spliced XBP1
C
MEF cells
TXNIP
Relative mRNA
DTT (0.5 mM)
Abl/Arg DKO
WT
D
T-REx 293::
FRT
c-Abl (WT)
(kD)
c-Abl -Flag
GAPDH
Dox (1 µg/ml, hr)
E
Phospho-Crkl(Y207)
Crkl
F
Phospho-IRE1α
Total IRE1α
Dox (1 µg/ml)
G
XBP1
H
sXBP1
HDAC1
I
INS-1::
Arg
Arg-Flag
TXNIP
GAPDH
J
PDGFRα
Phospho-PDGFRα
PDGFRα-Flag
PDGF-AA
K
c-KIT
Phospho-c-KIT
c-KIT-Flag
CSF
L
Phostag gel (IB; eIF2α)
MEF cells
Phosphorylated
Non-phosphorylated
Tm (200 ng/ml)
(Phosphorylated eIF2α) /(Non-phosphorylated eIF2α)
M
c-Abl (WT)
Phospho-eIF2α
Total-eIF2α
[Imatinib] (µM) 0 0 5 20

FIGURE S4

Figure S4. ABL family tyrosine kinases are necessary and sufficient for driving T-UPR-mediated apoptosis through IRE1α. Related to Figure 3.

(A) Representative agarose gel of PstI-digested XBP1 cDNA amplicons in c-Abl/Arg DKO or WT MEF cells treated with the indicated [Tm] for 3 hr. Quantified % spliced XBP1 were in Figure 3D in triplicate. (B) Agarose gel of PstI-digested XBP1 cDNA amplicons from c-Abl -/- and WT MEF cells treated with indicated [Tm] for 3 hr. Quantification is shown at the bottom. (C) qPCR of relative TXNIP mRNA in c-Abl/Arg DKO or WT MEF cells treated with Dithiothreitol (DTT) for 3 hr. Two-way ANOVA followed by post-hoc Tukey's test was performed for statistical analysis. Biologically triplicated. Bars; mean±SEM. P-values: $^{*} < 0.05$, $^{**} < 0.01$. (D-F, H) Immunoblots for indicated proteins in T-REx 293 cells stably expressing WT c-Abl under Dox for indicated times. Nuclear protein extract used for (H). (G) XBP1 cDNA amplicons from T-REx 293 cells expressing WT c-Abl under Dox for indicated times. (I) Immunoblots of indicated proteins in INS-1 cells expressing Arg under ± 1 μg/ml Dox for 48 hr. (J) Immunoblots of indicated proteins in INS-1 cells expressing PDGFRα under ± 1 μg/ml Dox for 48 hr. Cells were treated with ± 2 ng/ml PDGF-AA for 30 min before lysates were prepared. (K) Immunoblots of indicated proteins in INS-1 cells expressing c-KIT under ± 1 μg/ml Dox for 48 hr. Cells were treated with ± 10 ng/ml SCF for 30 min before lysates were prepared. (L) Immunoblots of total eIF2α in c-Abl/Arg DKO or WT MEF cells treated with Tm for 3 hr. Lysates were separated using Phostag Gel. Quantification of phosphorylated-/non-phosphorylated-eIF2α signal intensity of blots is at the bottom. (M) Immunoblots of phospho- and total eIF2α in INS-1 cells expressing c-Abl (WT) under 1 μg /ml Dox ± indicated [imatinib] for 72 hr.

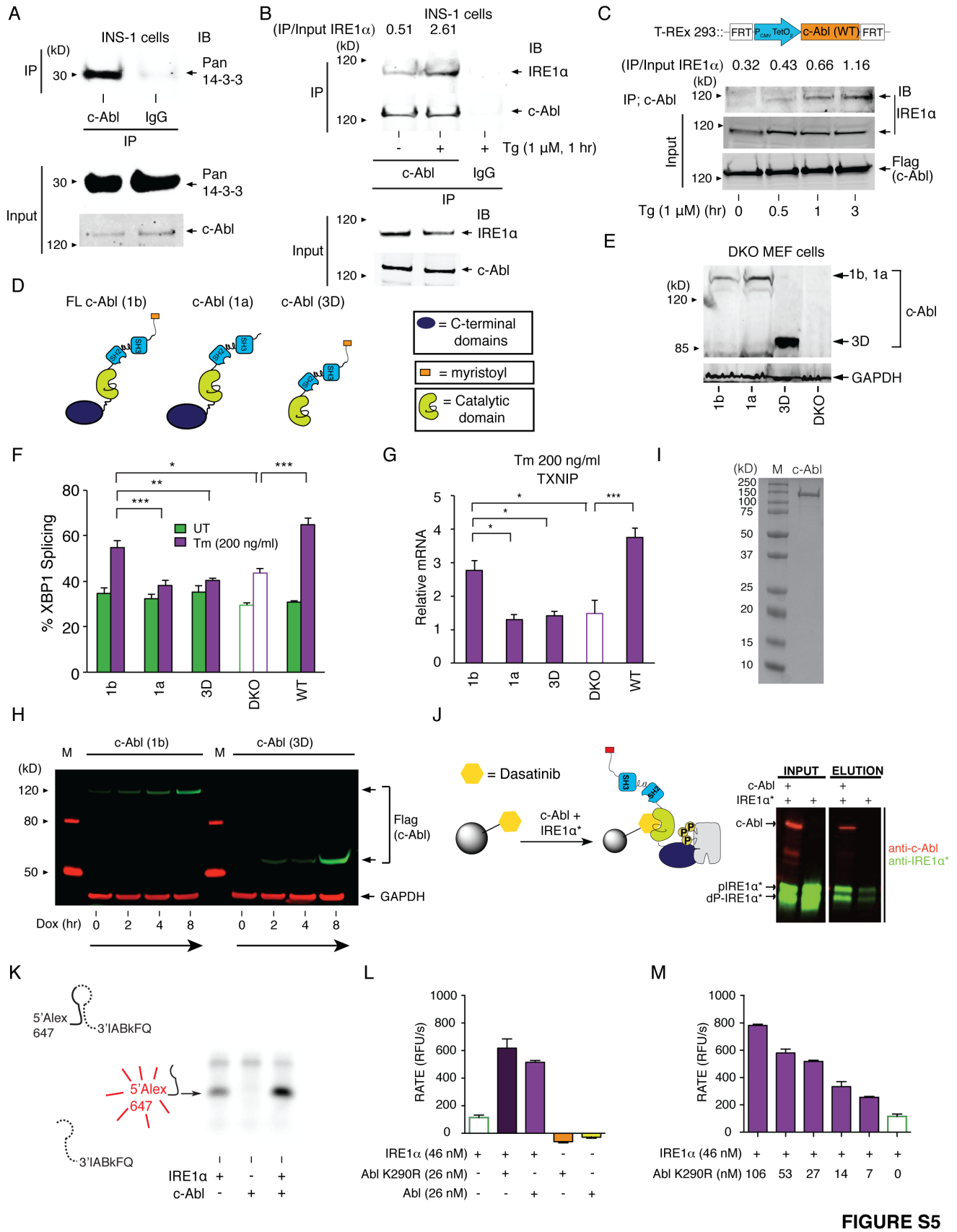
A
INS-1 cells
IB
IP
(kD)
30
Pan 14-3-3
c-Abl
IgG
IP
Input
30
Pan 14-3-3
120
c-Abl
B
INS-1 cells
(IP/Input IRE1α) 0.51 2.61
(kD)
120
IB
IRE1α
IP
120
c-Abl
- + + Tg (1 μM, 1 hr)
c-Abl IgG
IP
Input
120
IB
IRE1α
120
c-Abl
C
T-REx 293::
FRT
P_CMV TetO2
c-Abl (WT)
FRT
(IP/Input IRE1α) 0.32 0.43 0.66 1.16
(kD)
IP; c-Abl
120
IB
IRE1α
Input
120
120
Flag (c-Abl)
Tg (1 μM) (hr) 0 0.5 1 3
D
FL c-Abl (1b)
c-Abl (1a)
c-Abl (3D)
= C-terminal domains
= myristoyl
= Catalytic domain
E
DKO MEF cells
(kD)
120
1b, 1a
c-Abl
85
3D
GAPDH
1b 1a 3D DKO
F
% XBP1 Splicing
80
60
40
20
0
UT
Tm (200 ng/ml)
1b 1a 3D DKO WT
G
Tm 200 ng/ml
TXNIP
Relative mRNA
5
4
3
2
1
0
1b 1a 3D DKO WT
I
(kD)
M
c-Abl
250
150
100
75
50
37
25
20
15
10
H
M
c-Abl (1b)
M
c-Abl (3D)
(kD)
120
80
50
Flag (c-Abl)
GAPDH
Dox (hr) 0 2 4 8 0 2 4 8
J
= Dasatinib
c-Abl + IRE1α*
INPUT
ELUTION
c-Abl + +
IRE1α* + + + +
c-Abl
pIRE1α*
dP-IRE1α*
anti-c-Abl
anti-IRE1α*
K
5'Alex 647
3'IABkFQ
5'Alex 647
3'IABkFQ
IRE1α + - +
c-Abl - + +
L
RATE (RFU/s)
1000
800
600
400
200
0
IRE1α (46 nM) + + + - -
Abl K290R (26 nM) - + - + -
Abl (26 nM) - - + - +
M
RATE (RFU/s)
1000
800
600
400
200
0
IRE1α (46 nM) + + + + + +
Abl K290R (nM) 106 53 27 14 7 0

FIGURE S5

Figure S5. Co-localization of c-Abl with IRE1α at the ER membrane drives T-UPR endpoints through a scaffolding effect. Related to Figure 4.

(A) Immunoprecipitation (IP) of c-Abl with blotting for pan 14-3-3 and c-Abl in INS-1 cells. (B) IP of endogenous c-Abl with blotting for endogenous IRE1α and c-Abl in INS-1 cells with ± Tg for 1 hr. (C) IP of c-Abl with blotting for IRE1α and c-Abl in T-REx 293 cells expressing c-Abl treated with 1 μg/ml Dox for 24 hr, followed by Tg for the indicated times. (D) Structures of human FL c-Abl1b, c-Abl1a, and c-Abl(3D). (E) Immunoblots of c-Abl in DKO and DKO MEF cells overexpressing c-Abl (1b, 1a, or 3D)-mCherry (C-terminal tagged). (F) Percent XBP1 splicing of RNA from c-Abl/Arg DKO MEF cells overexpressing c-Abl (1b, 1a, or 3D)-mCherry, c-Abl/Arg DKO, or wild type (WT) MEF cells treated with Tm for 3 hr. Two-way ANOVA followed by post-hoc Tukey's test performed for statistical analysis. Biologically triplicated. (G) qPCR for TXNIP mRNA expressed as a ratio relative to untreated cells in the samples of (F). One-way ANOVA followed by post-hoc Tukey's test was performed for statistical analysis. Biologically triplicated. (H) Immunoblots for indicated proteins in T-REx 293 cells expressing human c-Abl1b or c-Abl(3D) under 1 μg/ml Dox for the indicated time. M; protein marker. (I) A coomassie blue-stained gel of the purified c-Abl used in IRE1α* auto-phosphorylation experiments. M; protein marker. (J) (Left) A direct interaction between purified IRE1α* and purified c-Abl was probed with a pull-down experiment using an immobilized, Abl-selective ATP-competitive inhibitor–Dasatinib. (Right) Immunoblots for total IRE1α and c-Abl. IRE1α* and c-Abl (lane 1) or IRE1α* alone (lane 2) were added to immobilized Dasatinib beads. Input shows protein components prior to addition to immobilized Dasatinib. Retained proteins after serial washed with buffer were eluted using SDS. (K) RNase activity of IRE1α* (200 ng) in the presence or absence of c-Abl (50 ng) in 15 μl incubation volume. The assay was performed for 60 min. The cleaved XBP1 RNA mini-substrate was separated by urea PAGE. (L and M) The rates of XBP1 mini-substrate

cleavage by IRE1α* in the absence or presence of either c-Abl or indicated concentration of c-Abl K290R. Triplicated. Bars; mean±SEM. P-values: $^{*} < 0.05, ^{**} < 0.01, ^{***} < 0.001$.

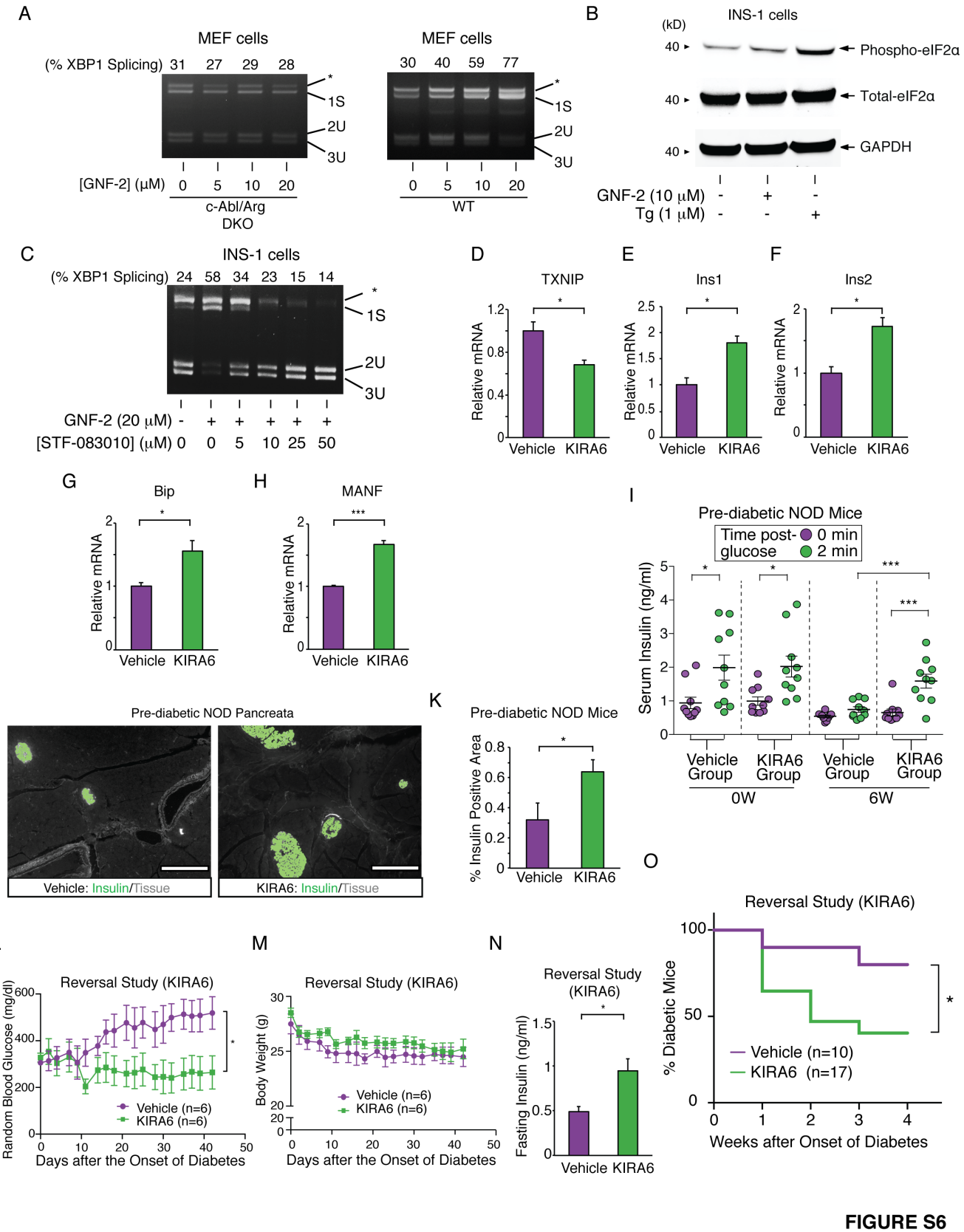
A
MEF cells
(% XBP1 Splicing) 31 27 29 28
1S
2U
3U
[GNF-2] (μM) 0 5 10 20
c-Abl/Arg DKO
MEF cells
30 40 59 77
0 5 10 20
WT
B
INS-1 cells
(kD)
40
Phospho-eIF2α
Total-eIF2α
GAPDH
GNF-2 (10 μM) - + -
Tg (1 μM) - - +
C
INS-1 cells
(% XBP1 Splicing) 24 58 34 23 15 14
GNF-2 (20 μM) - + + + + +
[STF-083010] (μM) 0 0 5 10 25 50
D
TXNIP
Relative mRNA
Vehicle KIRA6
E
Ins1
F
Ins2
G
Bip
H
MANF
I
Pre-diabetic NOD Mice
Time post-glucose 0 min 2 min
Serum Insulin (ng/ml)
Vehicle Group
KIRA6 Group
0W
6W
J
Pre-diabetic NOD Pancreata
Vehicle: Insulin/Tissue
KIRA6: Insulin/Tissue
K
Pre-diabetic NOD Mice
% Insulin Positive Area
L
Reversal Study (KIRA6)
Random Blood Glucose (mg/dl)
Vehicle (n=6)
KIRA6 (n=6)
Days after the Onset of Diabetes
M
Reversal Study (KIRA6)
Body Weight (g)
N
Reversal Study (KIRA6)
Fasting Insulin (ng/ml)
O
Reversal Study (KIRA6)
% Diabetic Mice
Vehicle (n=10)
KIRA6 (n=17)
Weeks after Onset of Diabetes

FIGURE S6

Figure S6. Effect of GNF-2 on IRE1α signaling; and effect of KIRA6 on NOD mice. Related to Figure 5 and 6.

(A) Agarose gel of PstI-digested XBP1 cDNA amplicons in c-Abl/Arg DKO or wild type (WT) MEF cells treated with the indicated [GNF-2] for 1 hr. (B) Immunoblots for indicated proteins in INS-1 cells treated with or without GNF-2 or Tg for 2 hr. (C) Agarose gel of PstI-digested XBP1 cDNA amplicons in INS-1 cells pretreated with indicated [STF-083010] for 2 hr, followed by GNF-2 for 1 hr. (D-H) qPCR of indicated mRNA levels in islets of female NOD mice injected with either KIRA6 (5 mg/kg body weight) (n=3) or vehicle (n=3) b.i.d. starting at 10 weeks of age. Injections were continued for 7 days after which islets were harvested. (I) First-phase insulin response in pre-diabetic NOD mice before (8 weeks of age, 0W) and after 6 weeks (6W) of i.p. injection with either vehicle or KIRA6 b.i.d. (n=10 per group). Serum insulin levels were quantified by an insulin-specific ELISA. Two-way ANOVA followed by post-hoc Tukey's test was used for statistical analysis. Each symbol denotes an individual mouse. (J and K) Immunofluorescent staining of insulin and quantitation of the insulin-positive area in pancreatic sections prepared from NOD mice treated with vehicle or KIRA6 (5 mg/kg) b.i.d. for 6 weeks starting at 8 weeks of age (n=5 per group). (J) Representative images of the insulin staining (green) and the tissue sectional area (grey). Scale bar, 200 μm. (L and M) Random blood glucose measurement (L) and body weight (M) of female NOD mice injected with either KIRA6 (5 mg/kg) (n=6) or vehicle (n=6) b.i.d. starting at the onset of diabetes. Injections were continued for 42 days. Two-way ANOVA was used for difference between groups. (N) Percentages of diabetic NOD mice for KIRA6-treated (n=17) and vehicle-treated (n=10) group. KIRA6 or vehicle treatment was initiated at the onset of hyperglycemia (blood glucose >250 mg/dl). Log-rank test was performed for statistical analysis. Data are cumulative of two independent experiments. (O) Fasting serum insulin levels in

NOD mice 8 weeks after treatment with KIRA6 or vehicle initiated at diabetes onset. n=5 per group. Bars; mean±SEM. n; number of mice. P-values: $^{*} < 0.05$, $^{***} < 0.001$.

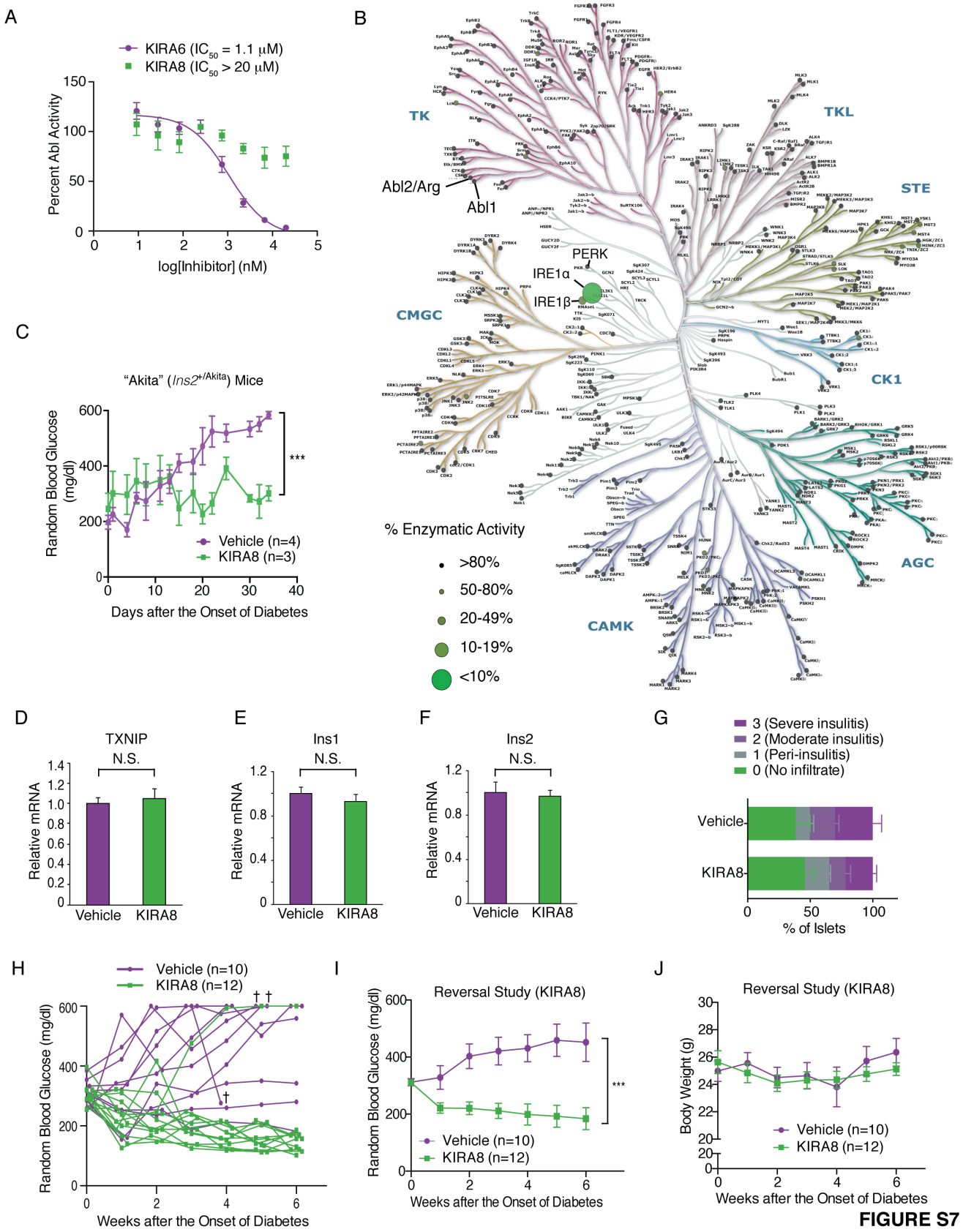
A
KIRA6 (IC50 = 1.1 μM)
KIRA8 (IC50 > 20 μM)
Percent Abl Activity
log[Inhibitor] (nM)
B
TK
TKL
STE
CMGC
CK1
AGC
CAMK
Abl2/Arg
Abl1
PERK
IRE1α
IRE1β
% Enzymatic Activity
>80%
50-80%
20-49%
10-19%
<10%
C
"Akita" (Ins2+/Akita) Mice
Random Blood Glucose (mg/dl)
Vehicle (n=4)
KIRA8 (n=3)
Days after the Onset of Diabetes
D
TXNIP
N.S.
Relative mRNA
Vehicle
KIRA8
E
Ins1
N.S.
F
Ins2
N.S.
G
3 (Severe insulitis)
2 (Moderate insulitis)
1 (Peri-insulitis)
0 (No infiltrate)
% of Islets
H
Vehicle (n=10)
KIRA8 (n=12)
Weeks after the Onset of Diabetes
I
Reversal Study (KIRA8)
J
Body Weight (g)

FIGURE S7

Figure S7. Mono-selective KIRA8 spares β-cells and reverse established diabetes in NOD mice. Related to Figure 6.

(A) Percent Abl(3D) activity in the presence of indicated [KIRA6] (IC50 = 1.1 μM) and [KIRA8] (IC50 > 20 μM). IC50 values were determined in the presence of 1 mM ATP. (B) Selectivity of KIRA8 against 365 kinases; 1 μM KIRA8 was tested for inhibition of each kinase in duplicate. (C) Random blood glucose levels of male *Ins2*$^{+/Akita}$ mice i.p., injected daily for 35 days with KIRA8 (50 mg/kg) (n=3) or vehicle (n=4) starting at P21 (i.e., day 1). Statistical analysis; repeated measures two-way ANOVA. (D-F) qPCR of indicated mRNA levels in islets of C57BL/6 mice injected with either 50 mg/kg KIRA8 or vehicle, i.p., daily, starting at 10 weeks of age. Injections were continued for 7 days after which islets were harvested. n=3 per group. (G) Quantification of the severity of insulitis reported as the percentage of islets with a given score. Pre-diabetic NOD mice were analyzed after 6 weeks of i.p. injection with vehicle or KIRA8 (50 mg/kg), daily, starting at 10 weeks of age. At least 60 islets were scored per mouse. n=4 per group. (H-J) Random blood glucose measurement (H and I) and body weight (J) of female NOD mice from survival curves in Figure 6Q (n=12 for KIRA8, n=10 for vehicle group). Injections continued for 6 weeks. (H): Individual measurements. 3 mice in vehicle group with blood glucose levels greater than 600 mg/dl died at 4-5 weeks (shown as †). (I and J): Mean±SEM. Two-way ANOVA was used for statistical analysis in (I) and (J). Bars; mean±SEM. n; number of mice. P-values: $^{***} < 0.001$.

	$T_{1/2}$	T_{max}	C_{max}	$AUC_{(0-t)}$	Plasma level at 4 hr	Plasma level at 6 hr
	hr	hr	ng/ml	ng/ml*hr	ng/ml	ng/ml
30 mg/kg, i.p.	3.49	0.25	3285	9679	598	337

Table S2. Pharmacokinetic profile of KIRA8 in ICR mice after single intraperitoneal injection. Related to Figure 6 and S7.

The pharmacokinetic profile of KIRA8 was determined in male ICR mice i.p. dosed at 30 mg/kg. Based on these PK data, daily i.p. doses were chosen for the Akita and NOD model.

	Vehicle (n=10)	Imatinib (n=10)	Vehicle (n=10)	KIRA6 (n=17)	Vehicle (n=10)	KIRA8 (n=12)
% Diabetic mice	90	20	80	41.2	90	8.3

Table S3. Summary of the effects of small molecules on new-onset diabetes in NOD mice. Related to Figure 1, 6, and S6.

Percent diabetic mice at 4 weeks after treatment with indicated small molecules used in the reversal studies.