Supplementary materials

Sel1L-Hrd1 ER-associated degradation maintains β-cell identity via TGFβ signaling

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Materials and methods

9 Supplementary figures with figure legends and one table

MATERIALS AND METHODS

Pulse labelling of islets. Around one hundred islets from wild-type and knockout mice were washed twice in prewarmed Met/Cys-deficient medium plus 1% BSA and 10 mM Hepes (pH 7.35). Islets were then metabolically labeled with ^{35}S-labeled amino acids in the same medium for 12 min. Labeled islets were briefly washed once with RPMI medium 1640 containing 10% FBS and either directly immersed in lysis buffer or chased for 1 hour or 2 hour at 37°C in RPMI medium 1640 (11.1 mM glucose plus 10% FBS). The media were collected, and the islets were lysed in RIPA buffer. Lysate aliquots were evaluated for trichloroacetic acid-precipitable radioactivity to normalize the immunoprecipitations.

Transfection and immunoprecipitation. HEK293T cells were transfected with plasmids within 16-22 hr after plating with lipofectamine 2000 and harvested around 24 hr after. HRD1-/- HEK cells were generated as previously described (1). For immunoprecipitation, cells were lysed in a buffer containing 150 mM NaCl, 50 mM Tris–HCl pH 7.5, 1 mM EDTA, 1% NP-40, protease inhibitors and phosphatase inhibitors, and 10 mM N-ethylmaleimide. A total of 1-2 mg protein lysates was incubated with antibody coated agarose beads overnight with gentle rocking at 4°C. Immuno-complexes were washed in a buffer containing 137 mM NaCl, 20 mM Tris–HCl pH 7.5, 2 mM EDTA, and 10% glycerol and eluted by boiling at 95°C for 5 min in SDS sample buffer.

In vitro glucose stimulated insulin secretion. Insulin secretion from isolated islets were monitored using batch incubation methods. 10 similar sized islets were pre-cultured at 37°C in 1X KRH (Alfa Aesar, #J67795AP) containing 0.5% BSA and 2 mM glucose for 1 hour. Islets were then incubated in 1X KRH containing 2 mM glucose and 16.7 mM glucose for 1 hour each and supernatant was collected to determine insulin secretion levels. Islets were then recovered and sonicated in acid-ethanol to obtain total insulin content. Insulin levels were determined using insulin ELISA kit (Crystal Chem #90080).

1. Sun S, Shi G, Sha H, Ji Y, Han X, Shu X, et al. IRE1a is an endogenous substrate of endoplasmic-reticulum-associated degradation. *Nat Cell Biol.* 2015;17(12):1546-55.

Table S1. Patient information for human pancreas donors.

	HPAP Identifier	Sex/Age	BMI	Medical History	HbA1c	GSIR
1	HPAP-001	M/47yo	32.2	T2DM 18 yrs	5.7	1.3
2	HPAP-007	F/65yo	42.6	T2DM 4 yrs	5.9	1.41
3	HPAP-010	F/42yo	36.8	T2DM 2yrs	6	1.11
4	HPAP-013	F/28yo	41.6	T2DM >5 yrs	6.3	1.46
5	ICRH114	F/57yo	32	No HX DIAB	5	1.35
6	ICRH120	66	22.5	No HX DIAB	NA	1.27
7	HPAP-006	46	19.1	No HX DIAB	5.3	1.41
8	HPAP-022	39	34.7	No HX DIAB	4.7	1.31

GSIR: glucose-stimulated insulin release.

SUPPLEMENTARY FIGURES AND FIGURE LEGENDS

Figure S1. Sel1L-Hrd1 expression in pancreatic islets. (A-B) ScRNA-seq analysis of mouse islets (7-week-old, males) showing the expression, expressed as unique molecular identifiers (UMI), of *Sel1L* and *Hrd1* in distinct islet cell populations. (C-D) Representative confocal images of Sel1L (C) and Hrd1 (D) with insulin on WT mouse pancreas sections.

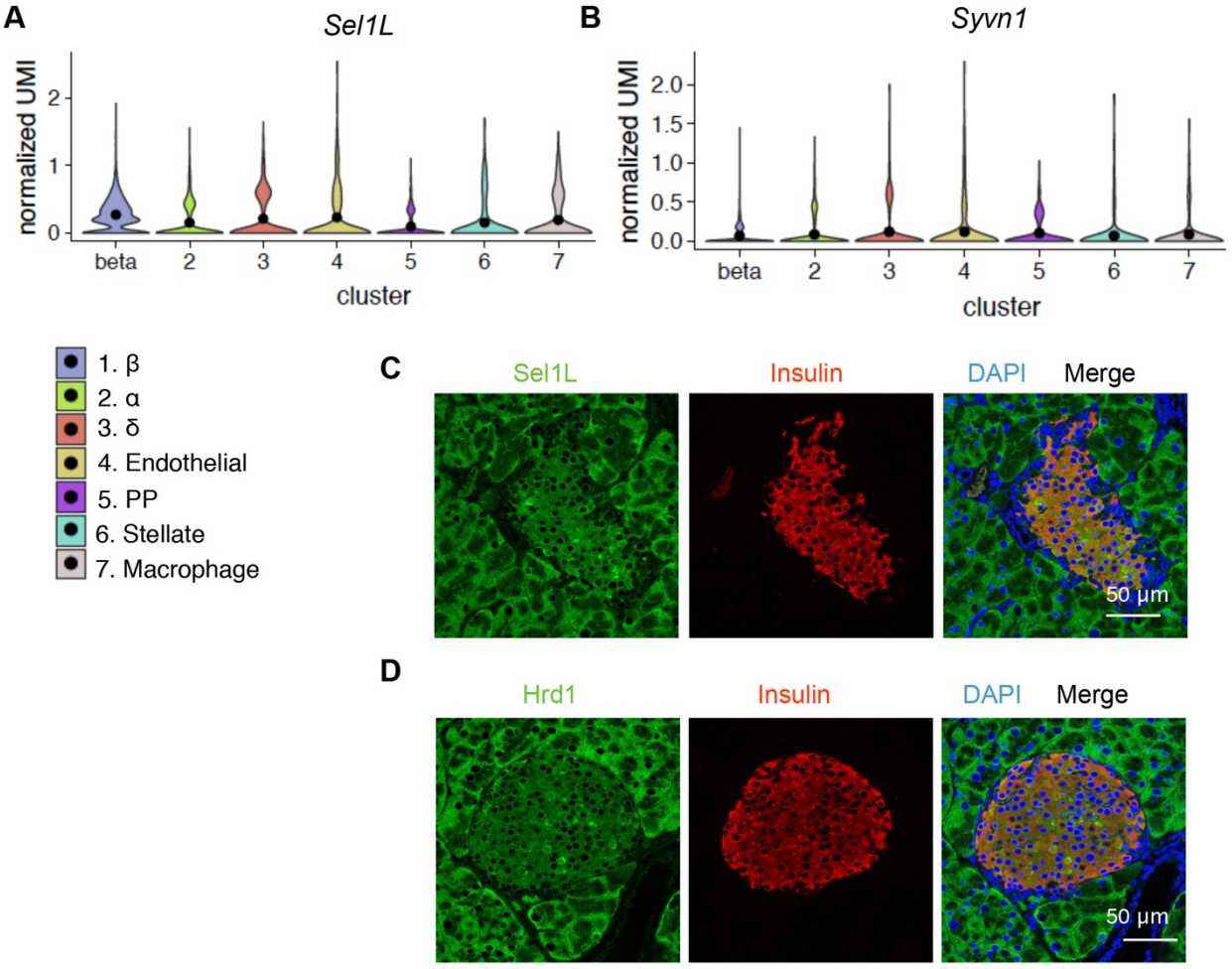

Figure S2. *Sel1L*^{Ins1} mice developed impaired glucose tolerance as early as 5 weeks, preceding *Atg7*^{Ins1} mice. Glucose tolerance test for *Sel1L*^{Ins1} (A, C) and *Atg7*^{Ins1} (B, D) at indicated ages (n=5-6 per group) with quantitation of area-under-curve shown on the right. Values, mean ± SEM; n.s., not significant; *, p<0.05; **, p<0.01 by unpaired Student's t-test.

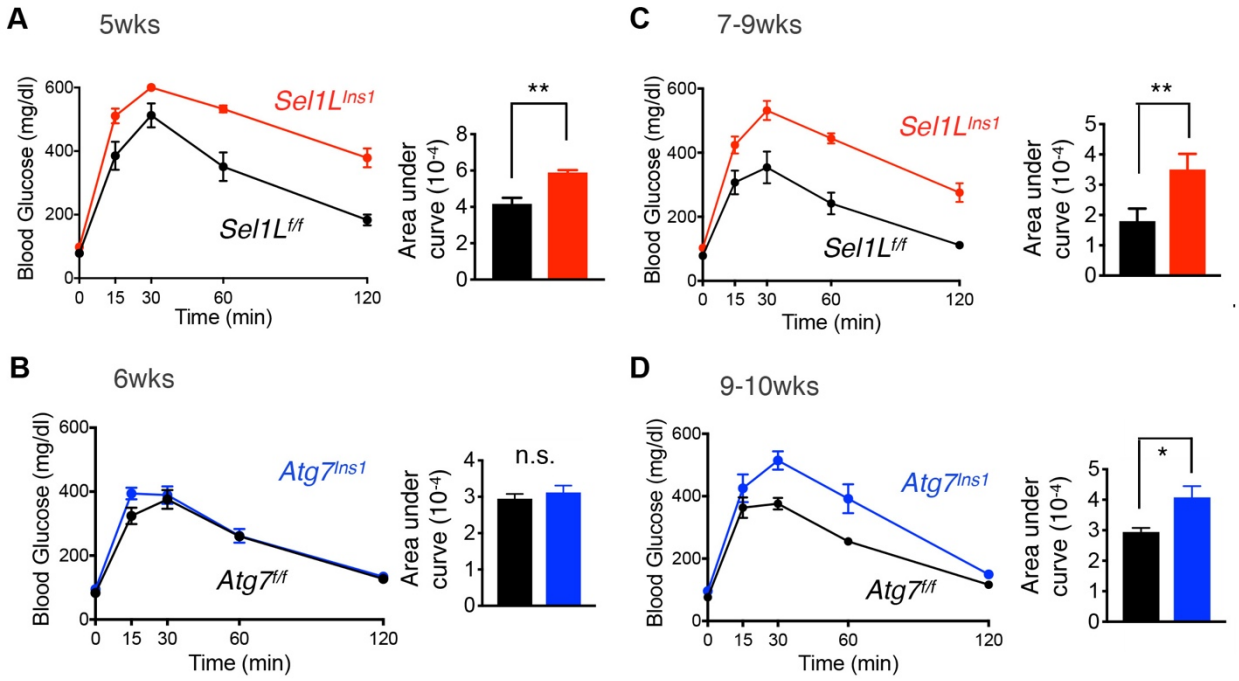

Figure S3. Histologically, peripheral tissues appeared largely normal in *Sel1L*^{Ins1} and *Atg7*^{Ins1} mice. Representative H&E images of (A) liver, (B) white (WAT) and (C) brown adipose tissue (BAT) obtained from 8-week-old WT, *Sel1L*^{Ins1} and *Atg7*^{Ins1} mice (n=2 per group).

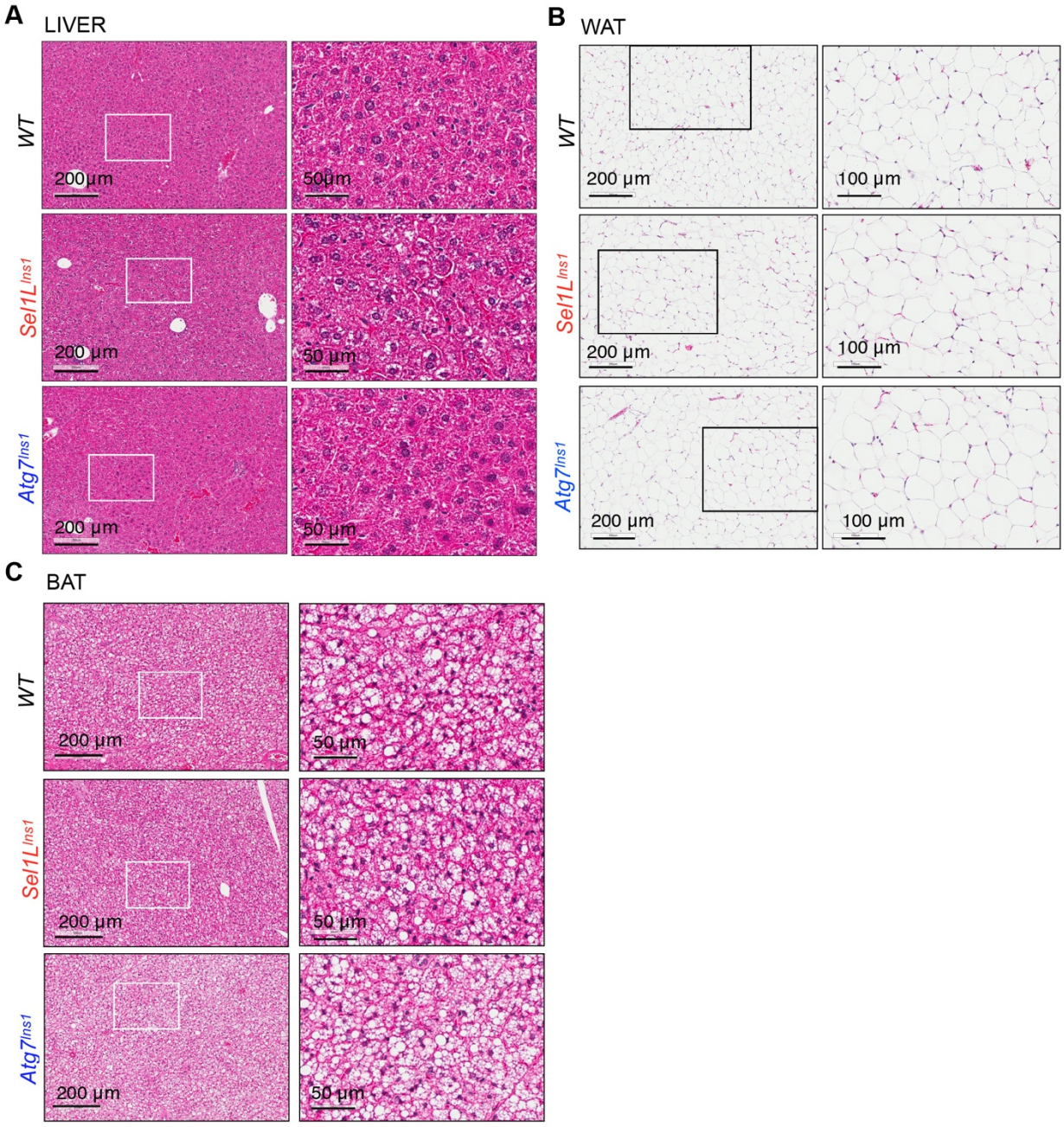

Figure S4. Loss of Sel1L had no effect on β proliferation, death, or size. Representative confocal images of Ki67 (A), TUNEL (B) (n=5-6 per genotype) and E-cadherin (C) (two independent repeats) staining in pancreatic sections.

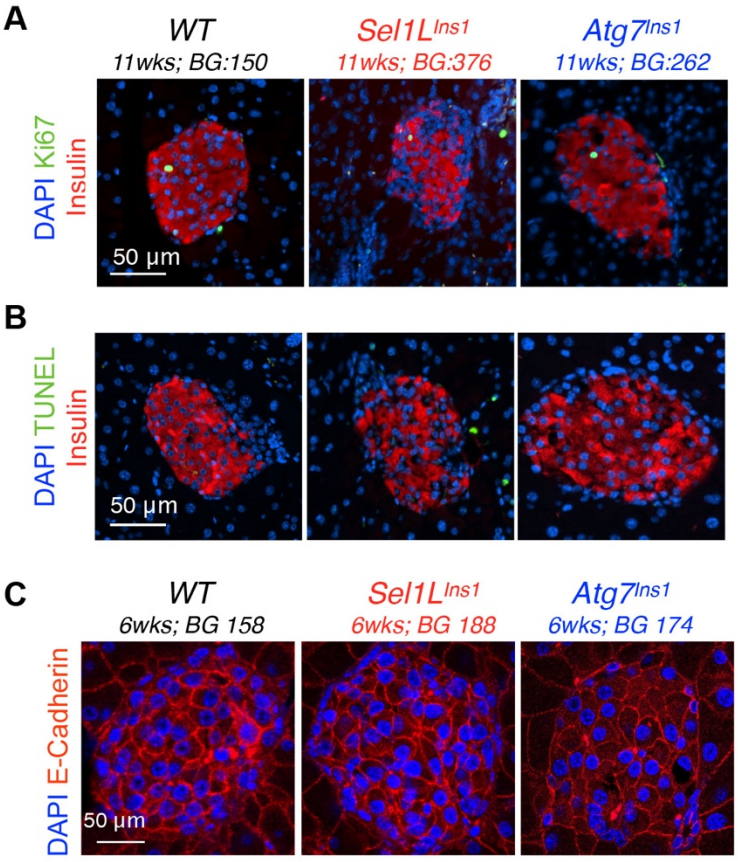

Figure S5. Loss of Sel1L did not impair proinsulin maturation and insulin secretion. (A) Western blot of *Sel1L*^{Ins1} and *Atg7*^{Ins1} islets (n=2 mice per group). Tubulin, a loading control. (B) Phos-tag-based western blot analysis of Ire1α phosphorylation in *WT* and *Sel1L*^{Ins1} islets (n=2 mice per group). (C) RT-PCR analyses of percent of spliced *Xbp1s* to unspliced *Xbp1u* mRNA in islets (n=2 mice per group). Tunicamycin (Tm)-injected liver, a positive control. L32, a loading control. (D) Pulse chase analysis of islets isolated from 7-week old mice (2 independent repeats). (E) Representative immunofluorescence images of BiP and proinsulin (n=3 mice each) in pancreatic sections. (F) Total insulin content in isolated primary islets from *WT* and *Sel1L*^{Ins1} mice (6 weeks). (G-H) GSIS in *WT* and *Sel1L*^{Ins1} islets in medium containing low (2.8 mM) or high (16.7 mM) glucose. Secretion shown before (G) and after normalization to total content (H). Values, mean ± SEM. n.s., not significant; *, p<0.05; ****, p<0.0001 by unpaired Student's t-test.

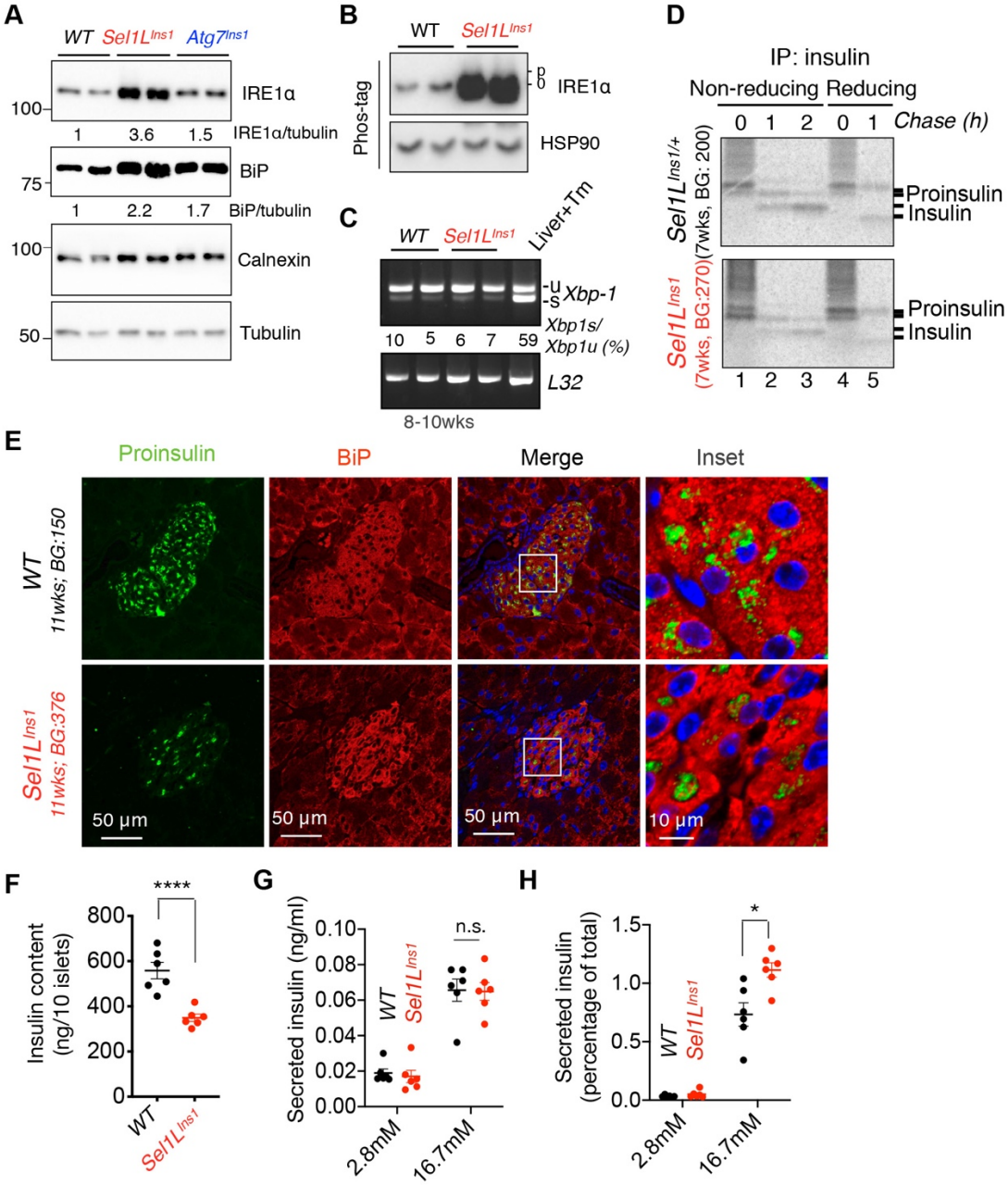

Figure S6. Single cell (sc)RNA-Seq analysis of primary islets identifies seven major clusters. (A-B) Identification of major clusters based on reference markers.

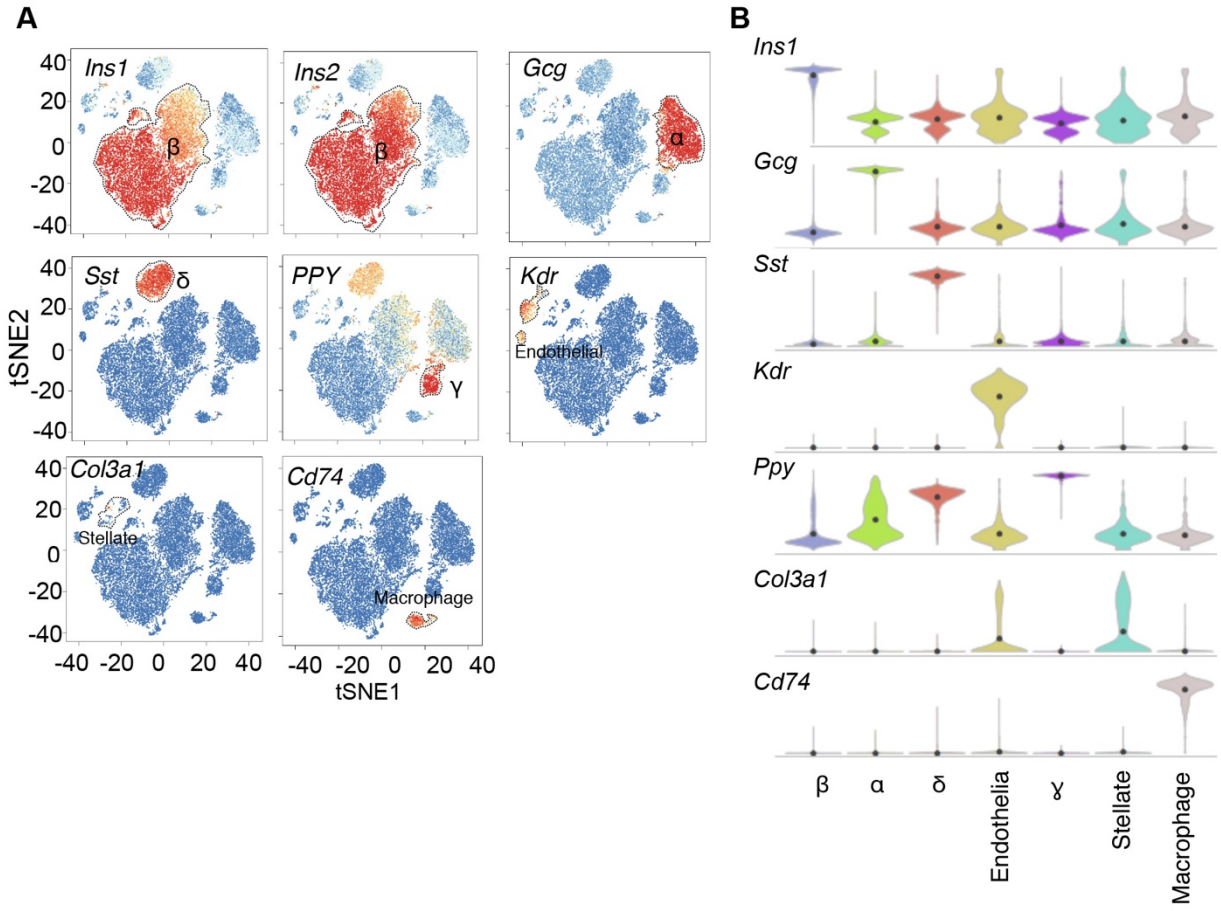

Figure S7. *Sel1L* deficiency reduced Ucn3 expression in β-cells. Representative confocal images of Ucn3 and insulin in pancreatic sections (n=2 mice each for each genotype).

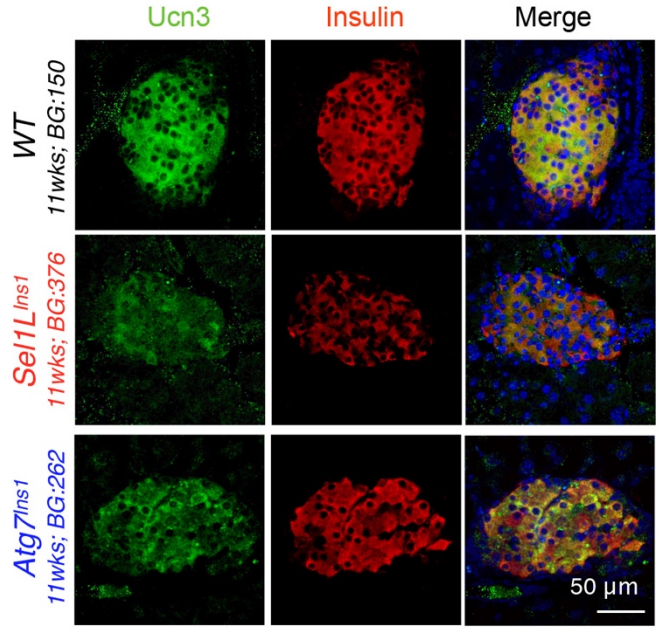

Figure S8. Sel1L-Hrd1 ERAD regulated TGFβ signaling in β cells. (A) GSEA analysis of top upregulated pathways from microarray analysis. (B) Heatmap representation of a set of genes associated with TGFβ signaling in β cell clusters. (C) Western blot of His-immunoprecipitates of His-TGFβR1-transfected HEK293T cells expressing a combination of plasmids as indicated, showing ubiquitination of TGFβ receptor I (TGFβRI). C2A Hrd1, an Hrd1 E3-ligase dead mutant. Two independent repeats.

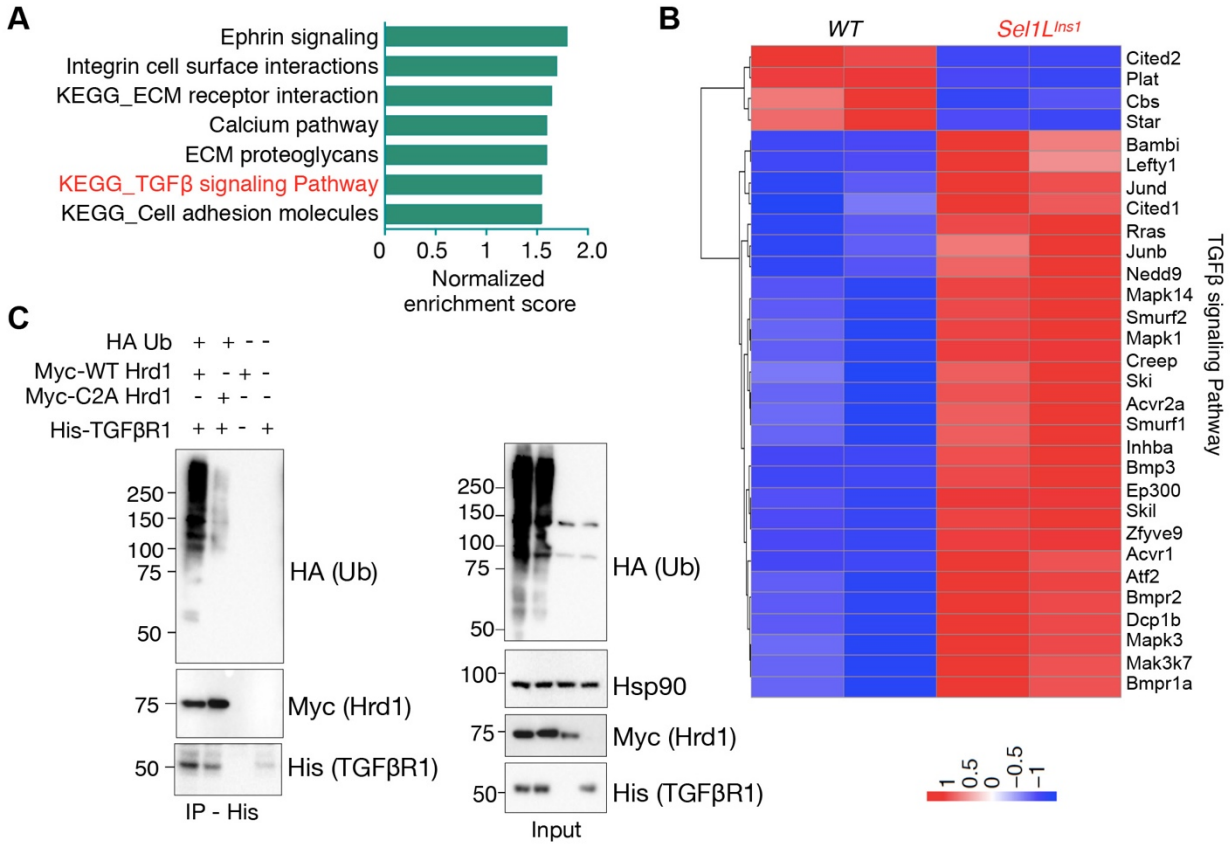

Figure S9. Inhibition of TGFβ signaling rescued MafA expression in *Sel1L*$^{-/-}$ islets. (A) Western blot analysis of total and phosphorylated Smad2/3 in primary islets treated with DMSO or 10 μM TGFβRI for 24 hr (two independent repeats). (B) Representative immunofluorescence images of MafA in dispersed primary islets treated with DMSO or 10 μM TGFβRI for 24 hr (two independent repeats).