

The Role of TRAP $\gamma$ /SSR3 in Preproinsulin Translocation Into the Endoplasmic Reticulum

Xu et al., Supplement

**Supplemental Figure S1: Preproinsulin synthesized in TRAP $\gamma$ /SSR3-KO 293T cells is not oxidized, whereas proinsulin, which enters the ER lumen, is oxidized.** Control 293T cells or TRAP $\gamma$ /SSR3-KO 293T cells were transfected to express recombinant WT human preproinsulin. At 48 h post-transfection, the cells were pulse-labeled, lysed, immunoprecipitated with anti-insulin, and the immunoprecipitates analyzed by SDS-PAGE under reducing conditions (DTT “+”) or nonreducing conditions (DTT “–”), followed by phosphorimaging.

**Supplemental Figure S2: TRAP $\gamma$ /SSR3 re-expression rescues proinsulin protein level in TRAP $\gamma$ /SSR3-KO 293T cells.** **A.** TRAP $\gamma$ /SSR3-KO 293T cells were transfected to express WT human preproinsulin plus plasmid encoding either empty vector (“EV”) or each individual subunit of the TRAP/SSR complex as indicated at the top of the Figure. At 48 h post-transfection, the cells were lysed and analyzed by reducing SDS-PAGE and immunoblotting with the antibodies indicated. **B.** Quantitation (mean  $\pm$  S.D.) of proinsulin and TRAP/SSR subunit protein levels from three independent experiments identical to that shown in panel A; \* $p < 0.05$ .

**Supplemental Figure S3. Effect of TRAP $\gamma$ /SSR3 deficiency on the mRNA levels encoding *INS* and other TRAP/SSR subunits.** INS1E cells were transfected with scrambled oligo (“Ctrl”) or TRAP $\gamma$ /SSR3-targeted siRNA for knockdown (“SSR3i”). **A.** At 72 h post-transfection, total insulin (*Ins1* + *Ins2*) mRNA levels were analyzed by rt-qPCR and quantitation (mean  $\pm$  S.D.) is shown from five independent experiments; \* $p < 0.05$ . **B.** At 72 h post-transfection, cells like those in panel A were preincubated in RPMI containing 2.5 mM glucose for 1.5 h followed by a 4 h incubation in 2.5, 11.1, or 25 mM glucose, respectively, as in Figure 4. Insulin (*Ins1* + *Ins2*) and TRAP/SSR complex subunit mRNA levels were analyzed by rt-qPCR and quantitation (mean  $\pm$  S.D.) is shown from three independent experiments; no significant acute glucose-dependent changes were seen for any mRNA.

**Supplemental Figure S4. TRAP $\gamma$ /SSR3 protein expression in the INS832/13  $\beta$ -cell line.** Immunofluorescence with anti-TRAP $\gamma$ /SSR3 (red) stains all INS832/13 cells. Nuclei were counter-stained with DAPI (blue); scale bar is indicated.

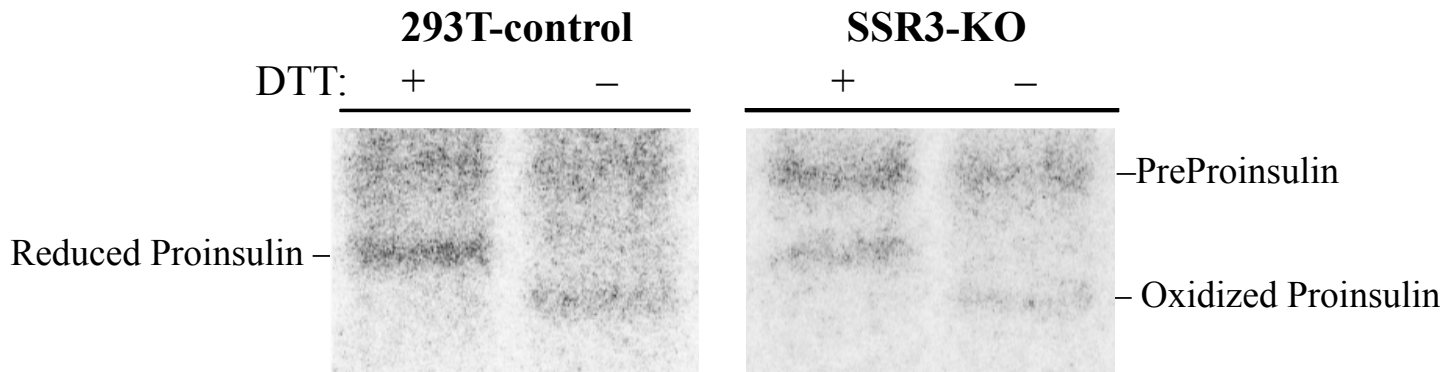
**Supplemental Figure S5. Expression of TRAP $\gamma$ /SSR3 restores proinsulin levels in SSR3-KD  $\beta$ -cells, but not in SSR1-KD  $\beta$ -cells.** INS832/13 cells were transfected with scrambled oligo (“Ctrl”) or TRAP $\alpha$ /SSR1-targeted siRNA (“SSR1-KD”) or TRAP $\gamma$ /SSR3-targeted siRNA (“SSR3-KD”). At 24 h post-transfection, cells were re-plated and transfected with empty vector (“–”) or plasmid encoding flag-

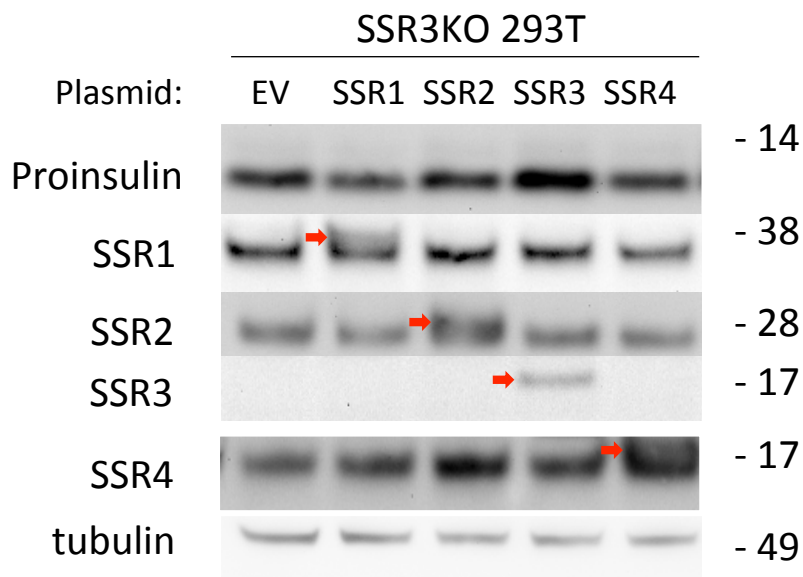
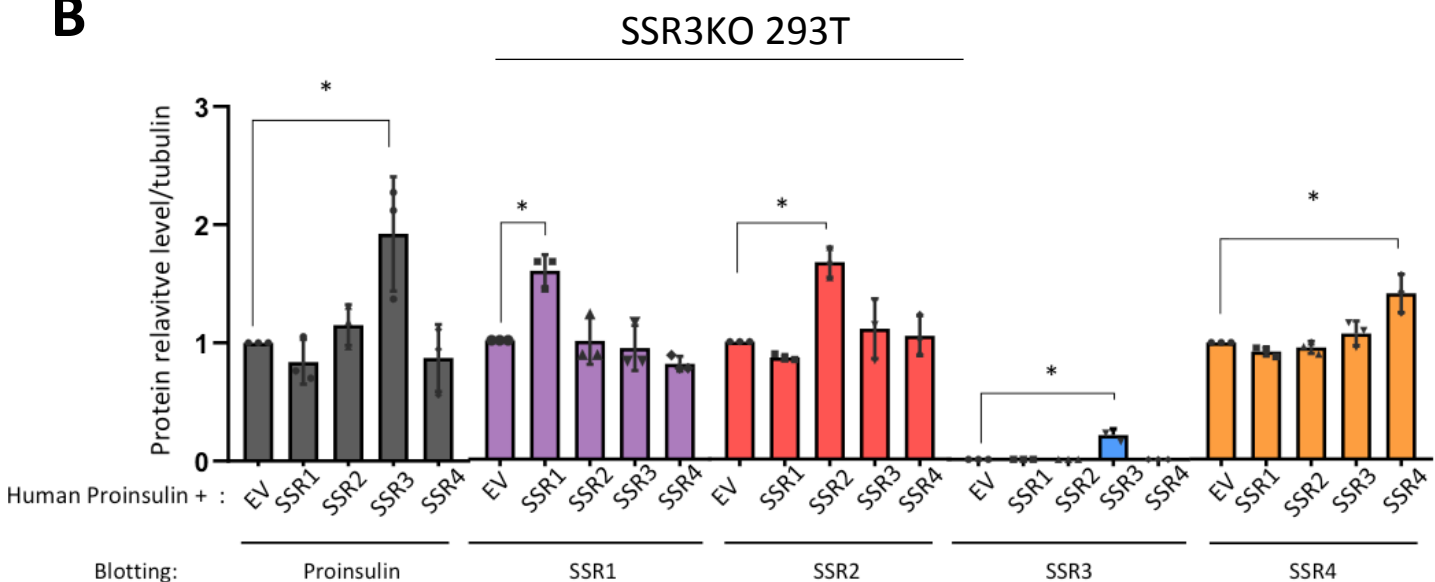
tagged TRAP $\gamma$ /SSR3 (“+”). After a further 48 h, cells were lysed and analyzed by reducing SDS-PAGE and immunoblotting with the indicated antibodies.

**Supplemental Figure S6. Overexpression of TRAP $\alpha$ /SSR1 does not increase proinsulin levels in otherwise normal (control) INS-832/13 cells.** INS-832/13 cells were infected with a replication-deficient adenoviral vector driving the expression of human TRAP $\alpha$ /SSR1 at 50 PFU/cell, as indicated. After 48 hours post-infection, cell lysates were subjected to SDS-PAGE and immunoblotted with anti-proinsulin, anti-TRAP $\alpha$ /SSR1, and anti-tubulin (a loading control).

**Supplemental Figure S7. Effects of high glucose stimulation on proinsulin and TRAP $\gamma$ /SSR3 in human islets.** Quantitation (mean  $\pm$  S.D.) of proinsulin and TRAP $\gamma$ /SSR3 protein levels from three technical replicates of each condition in human islets including those shown in Figure 6G. CHX = cycloheximide (100  $\mu$ g/mL) during the 2 h high-glucose exposure; \*  $p < 0.05$ .

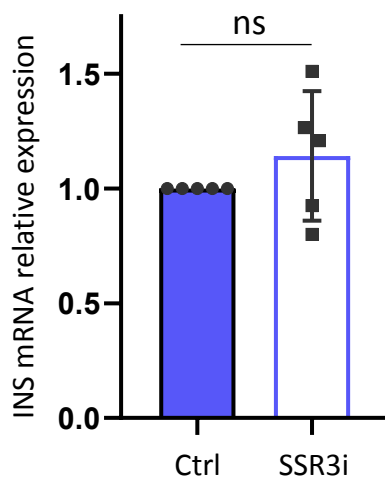
48 h post-transfection:  
10 min pulse-label with  $^{35}\text{S}$ -amino acids  
I.P. anti-insulin antibody; reducing/nonreducing SDS-PAGE



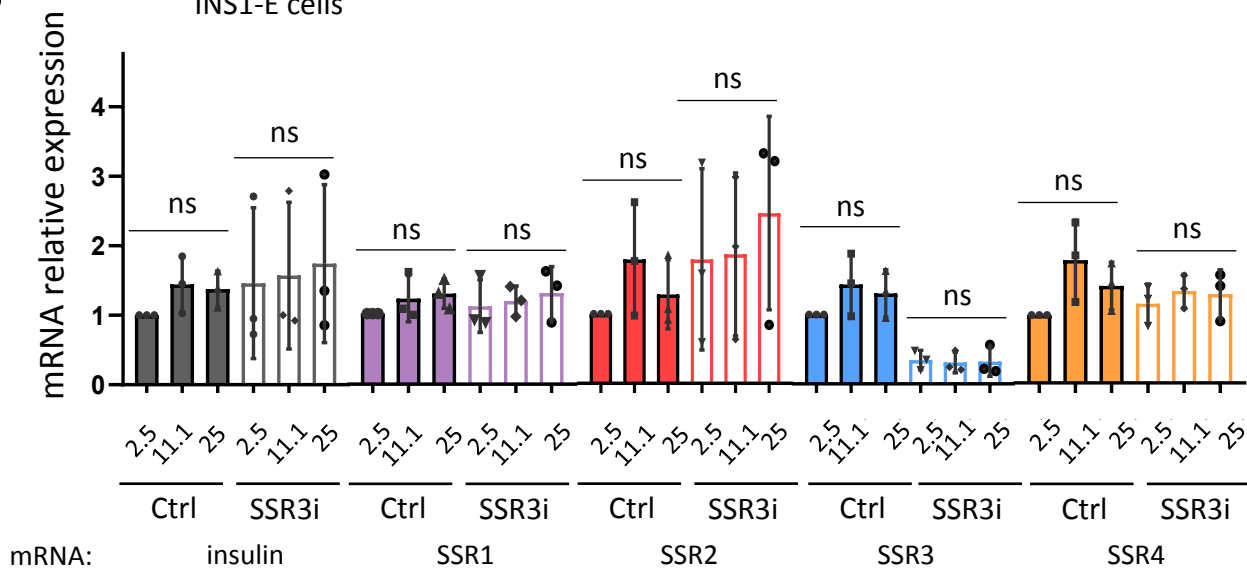
**A****B**

**A**

INS1-E cells

**B**

INS1-E cells



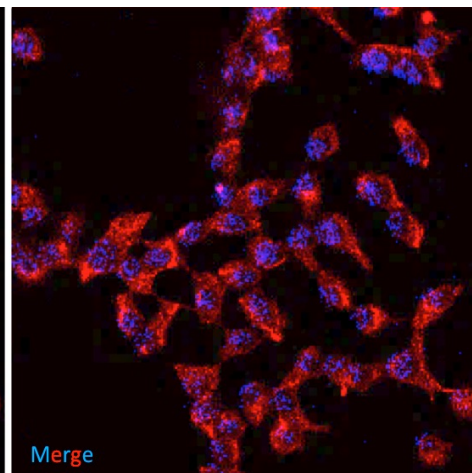
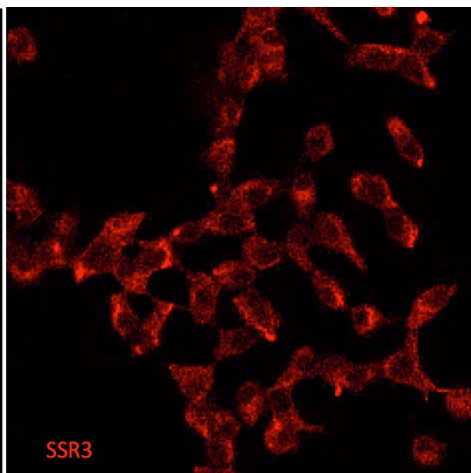
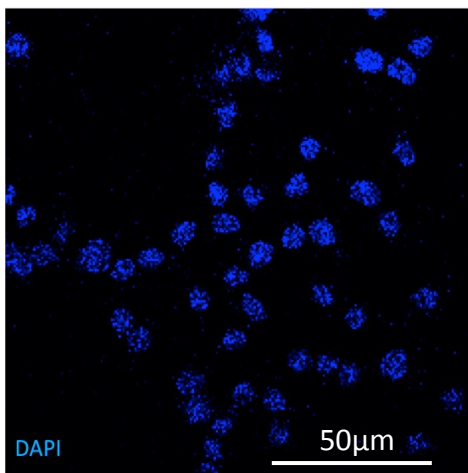
## INS-832/13 cells

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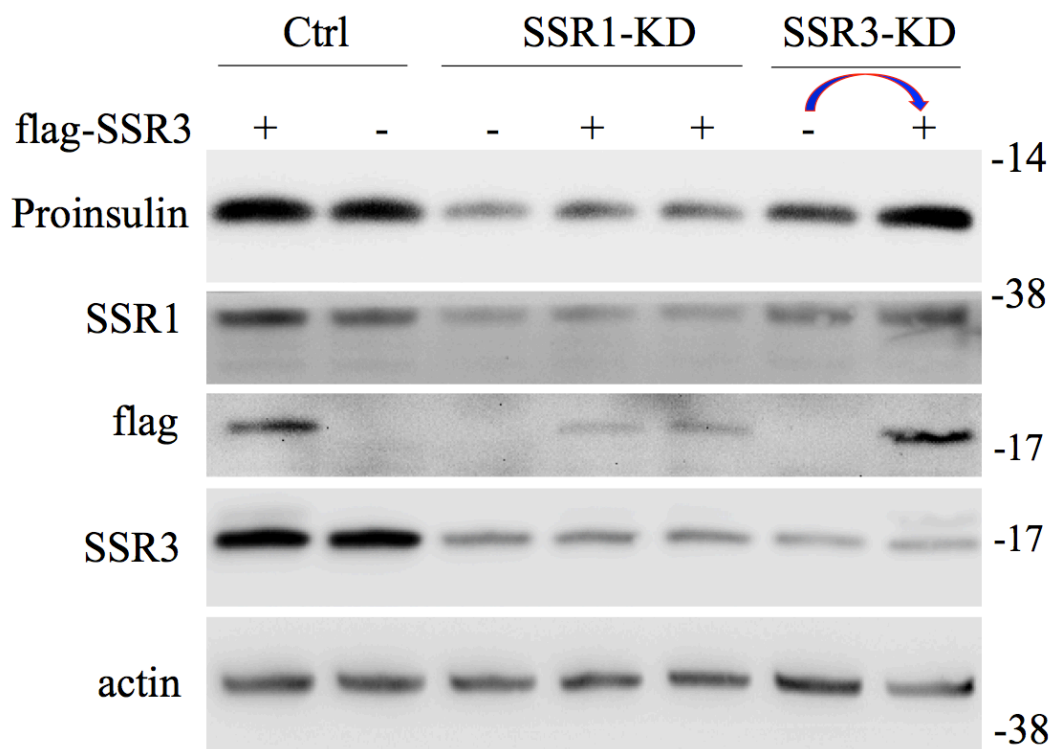
DAPI

Endogenous SSR3

Merge



INS832/13 cells (SSR1-KD, SSR3-KD, or Control)  
Transfected with empty vector or flag-tagged SSR3





INS1-832/13 cells

