Supplemental Materials

Molecular Biology of the Cell

He et al.

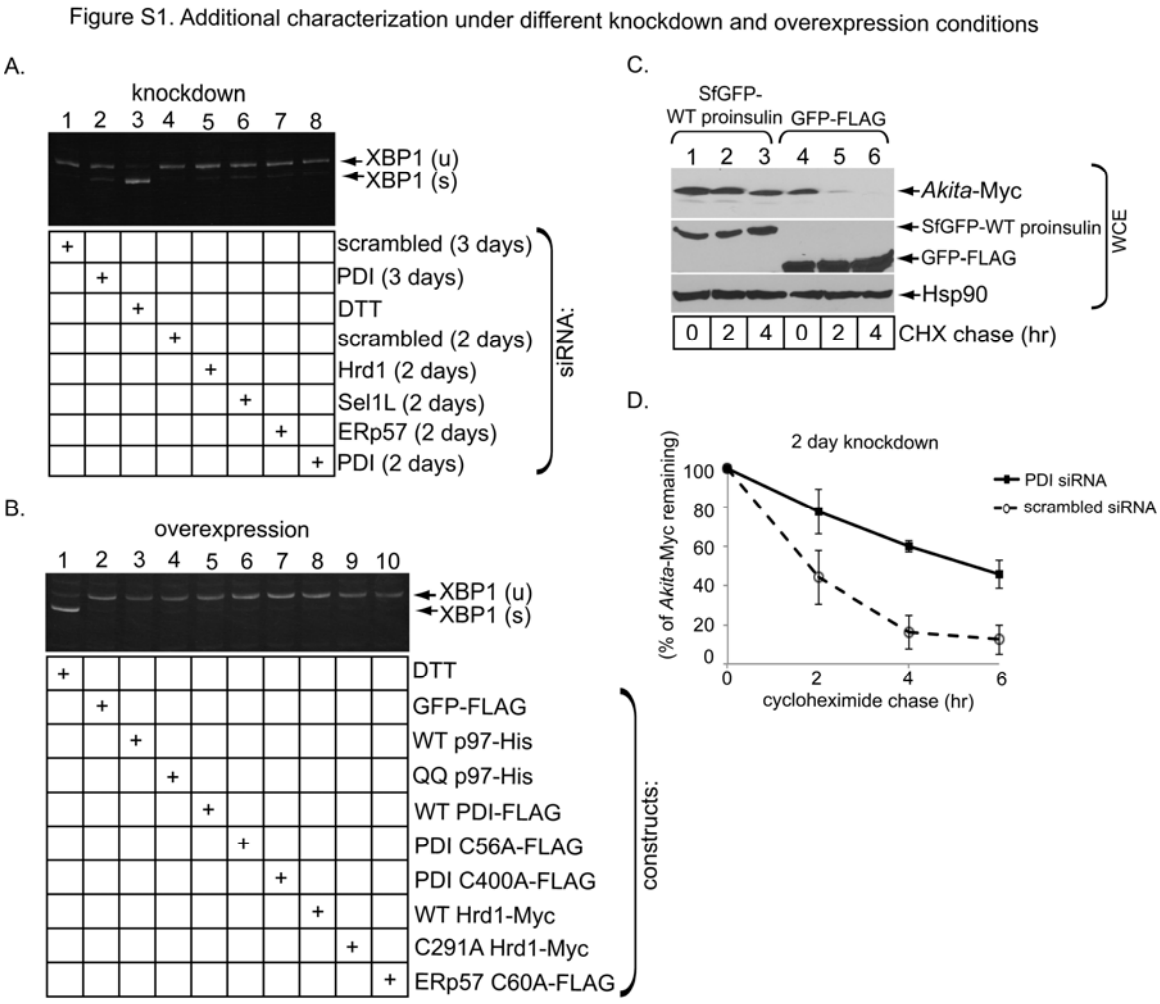

Figure S1. Additional characterization under different knockdown and overexpression conditions

A. Induction of XBP1 splicing was analyzed in cells transfected with the indicated siRNA for the indicated number of days. DTT-treated cells were used as a positive control. B. As in A, except cells were transfected with the indicated construct. C. As in Figure 1C, except cells were transfected with either SfGFP-WT proinsulin or GFP-FLAG. D. *Akita* degradation was analyzed as in Figure 3A, except cells were transfected with the PDI specific siRNA for only 2 days.

Figure S2. PDI trap mutants selectively capture *Akita* and block its degradation.

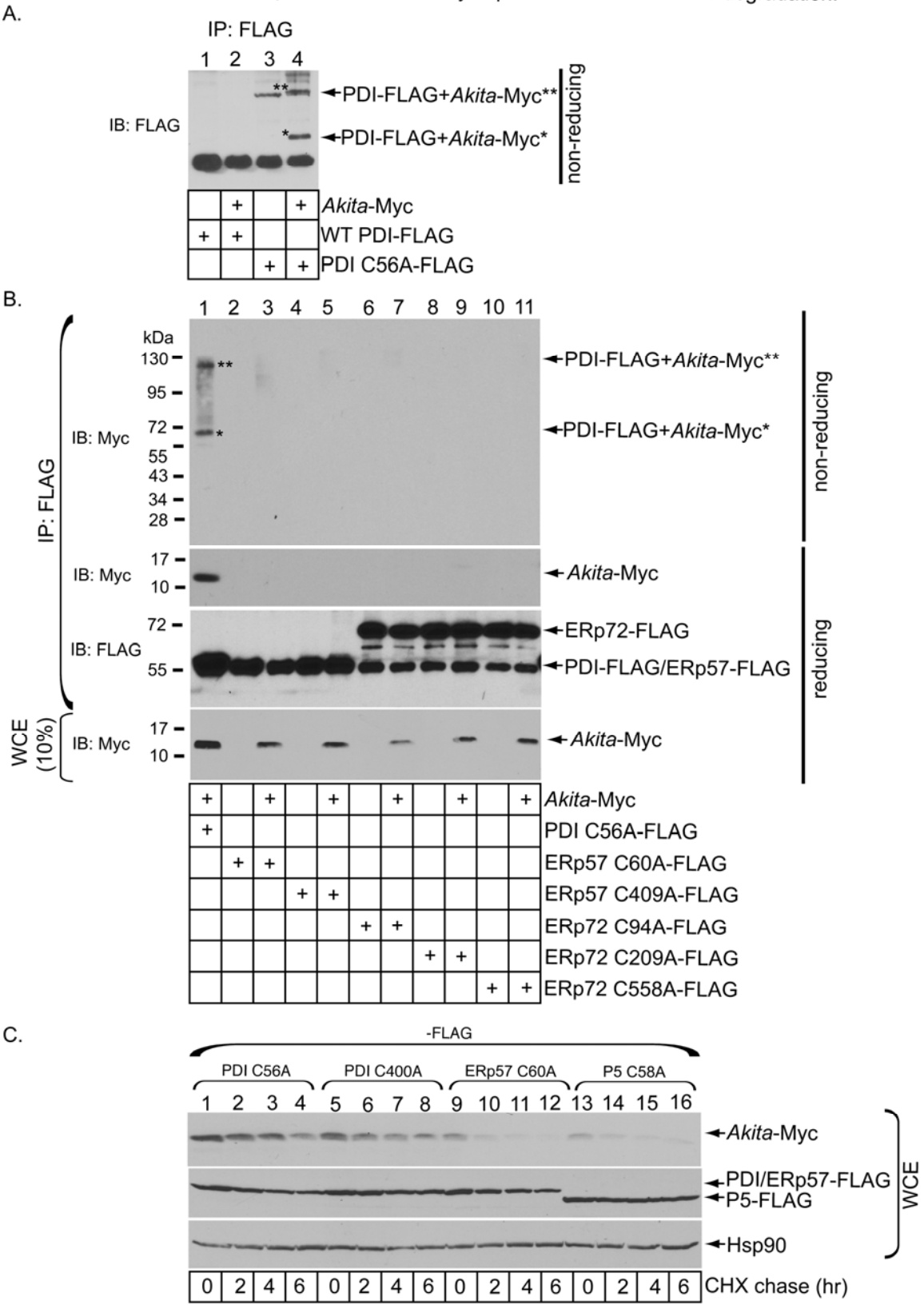

Figure S2. PDI trap mutants selectively capture *Akita* and block its degradation.

A. As in 4A, except WT PDI-FLAG was used. B. As in 4A, except the indicated ERp57 or ERp72 trap mutant constructs were used. B. As in 2A, except the indicated PDI, ERp57, or P5 trap mutant construct was used.

Figure S3. The B7 cysteine residue in *Akita* does not preferentially form a disulfide bond with the PDI C56A mutant.

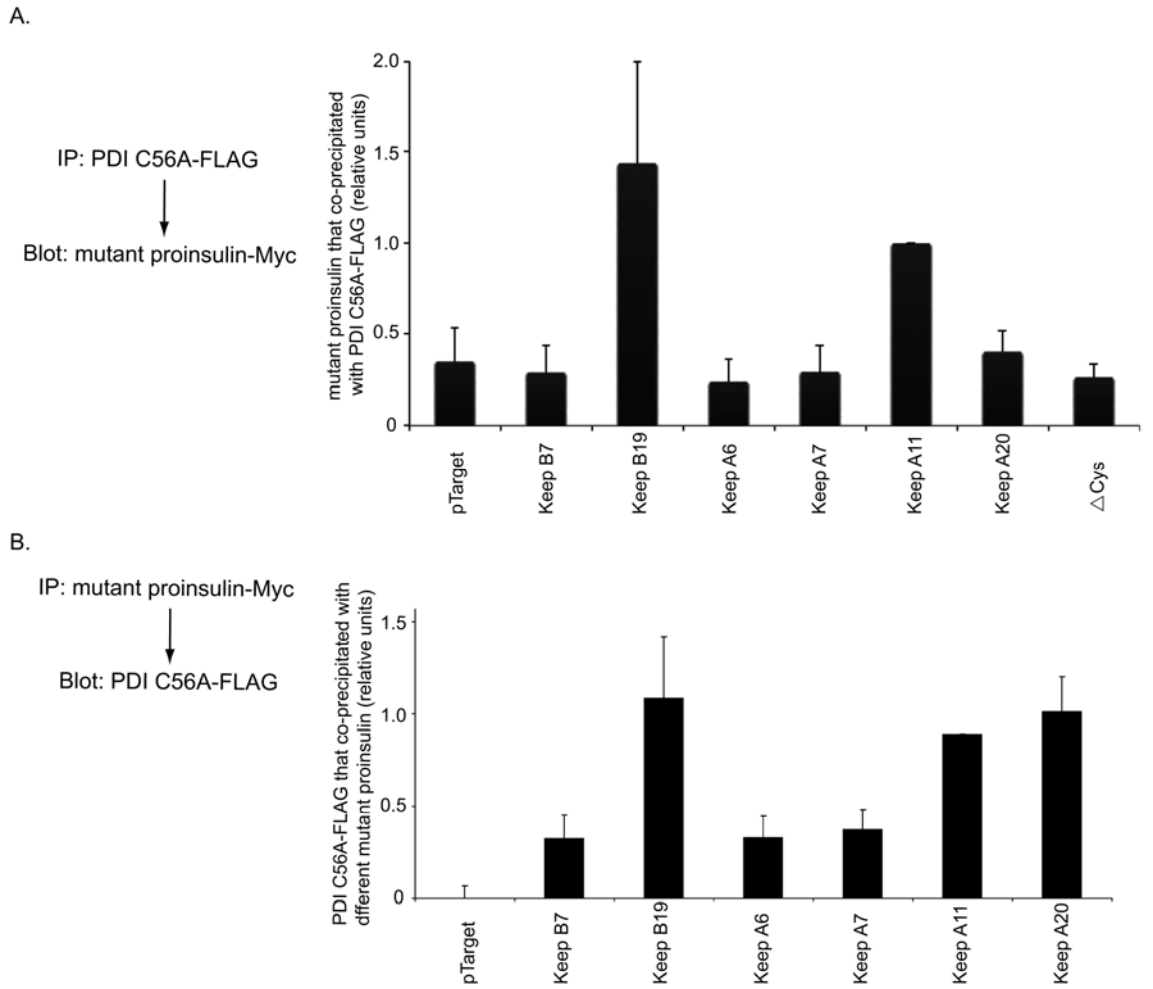

Figure S3. The B7 cysteine residue in *Akita* does not preferentially form a disulfide bond with PDI.

A. The level of mutant proinsulin-Myc in 3H was quantified by ImageJ (NIH). B. 293T cells expressing the indicated Myc-tagged mutant proinsulins were co-transfected with PDI C56A-FLAG. The cells were lysed and immunoprecipitated with anti-Myc (9E10) antibody, analyzed by reducing SDS-PAGE, and immunoblotted using the appropriate antibodies. The level of PDI C56A-FLAG was quantified using ImageJ (NIH).

Figure S4. Autophagy inhibition does not prevent loss of *Akita* dimer and trimer in PDI-depleted cells.

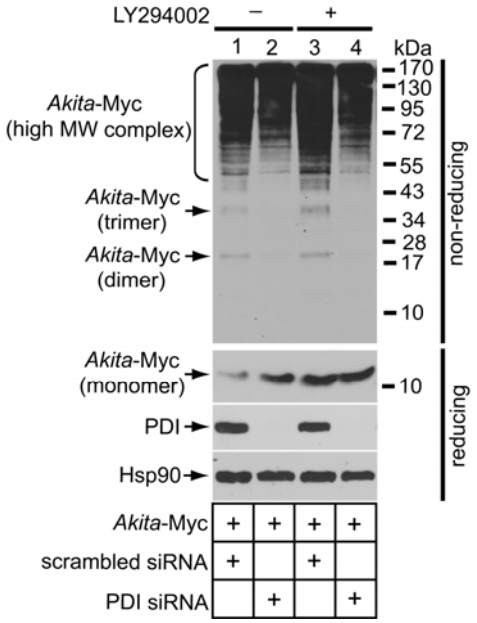

Figure S4. Autophagy inhibition does not prevent loss of *Akita* dimer and trimer in PDI-depleted cells.

As in Figure 4B, except cells were treated with 20 μM of the autophagy inhibitor LY294002 for 2 h instead of MG132.

Figure S5. DTT reduces *Akita*.

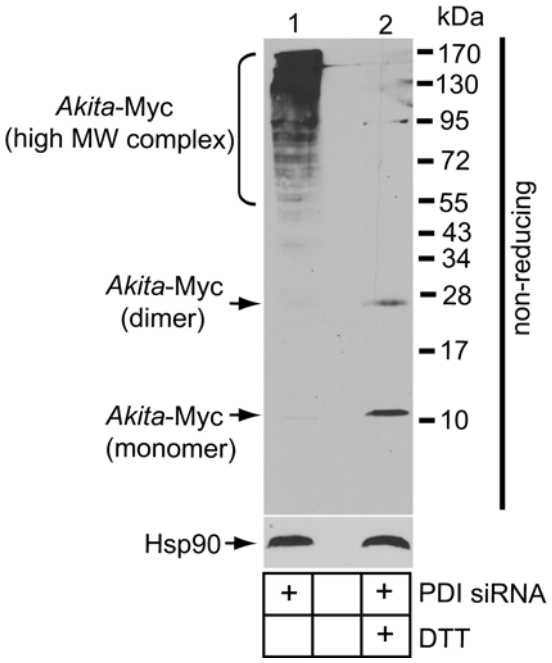

Figure S5. DTT reduces *Akita.*

A cell extract derived from PDI-depleted cells was treated with or without DTT, and analyzed by non-reducing SDS-PAGE.