Supporting Information

Figure S1

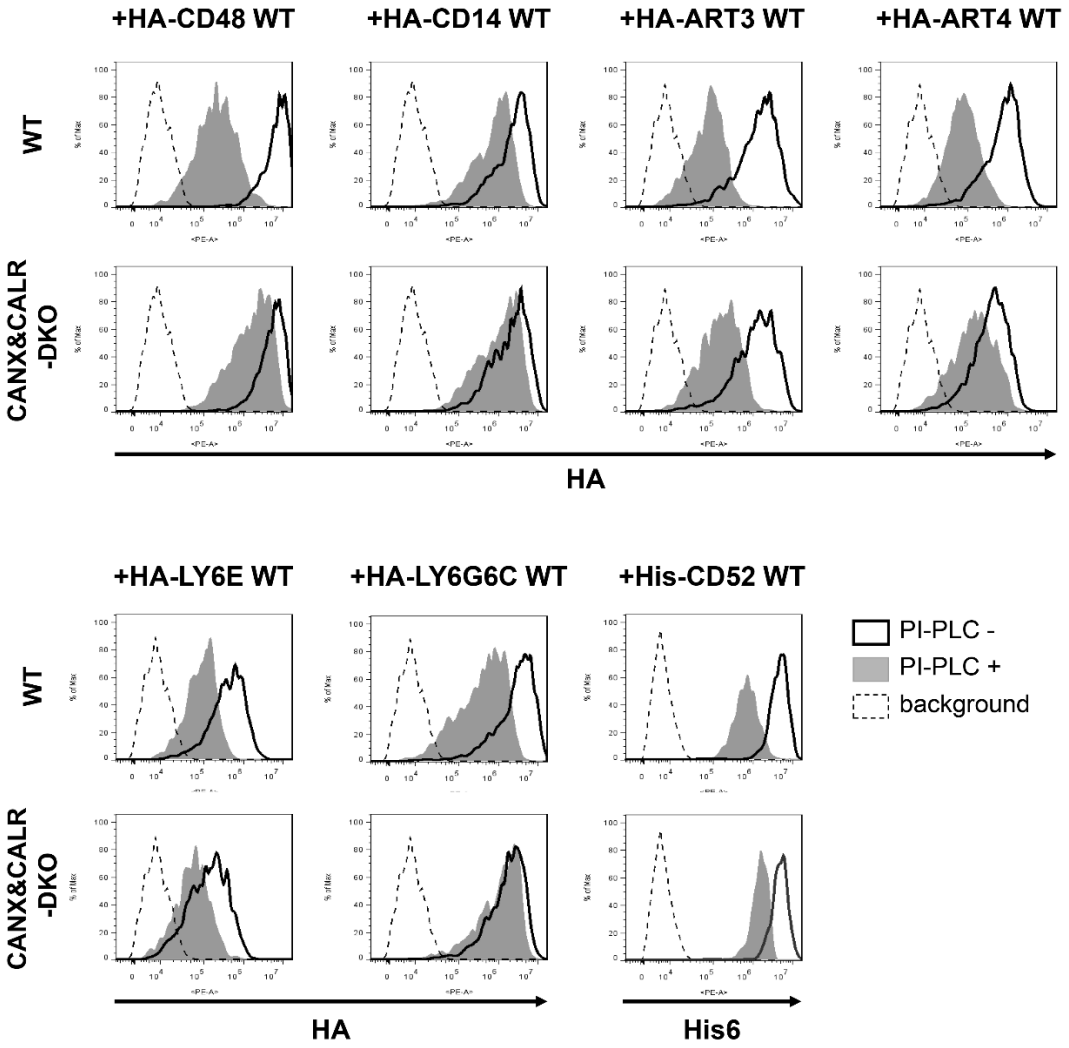

Figure S1. Calnexin/calreticulin-dependent GPI-inositol deacylation is commonly observed in N-glycosylated GPI-APs.

HA-tagged or His6-tagged exogenous GPI-APs were expressed in WT and CANX&CALR-DKO cells, and their PI-PLC sensitivity was analyzed. The pME-HA-GPI-AP plasmid was transfected together with pME-BFP into WT and CANX&CALR-DKO cells. The plasmid pLIB2-His6-GPI-AP-IRES-BFP was transfected into HEK293T packaging cells, and retroviral vectors were infected into WT and CANX&CALR-DKO cells. Three days after transfection, cells were treated with or without PI-PLC. Anti-HA or anti-His6 were used as the primary antibodies for staining the surface proteins, which were analyzed by flow cytometry. The cells showing the same BFP intensities were gated. The values are shown in Figure 1C.

Figure S2

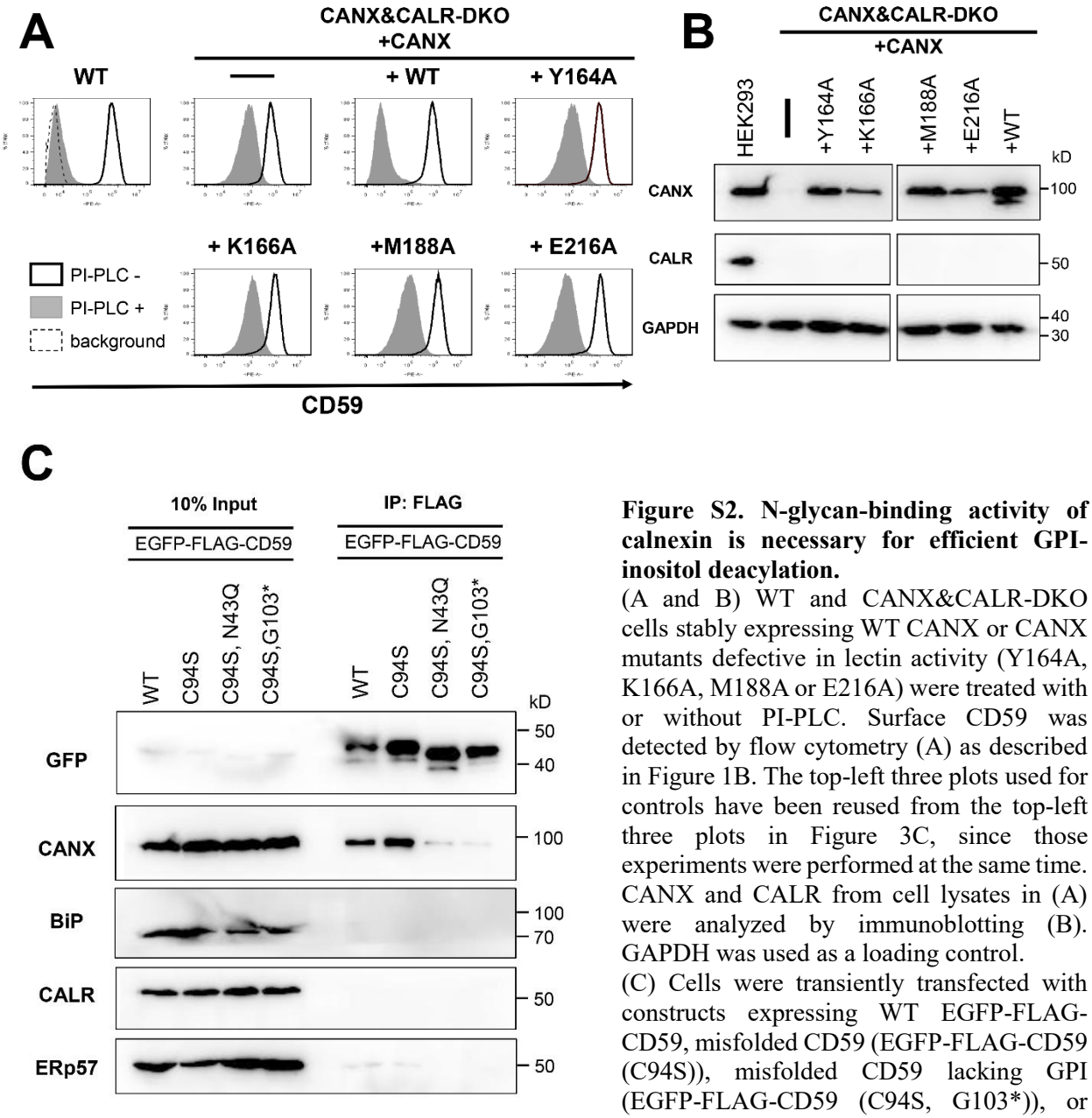

Figure S2. N-glycan-binding activity of calnexin is necessary for efficient GPI-inositol deacylation.

(A and B) WT and CANX&CALR-DKO cells stably expressing WT CANX or CANX mutants defective in lectin activity (Y164A, K166A, M188A or E216A) were treated with or without PI-PLC. Surface CD59 was detected by flow cytometry (A) as described in Figure 1B. The top-left three plots used for controls have been reused from the top-left three plots in Figure 3C, since those experiments were performed at the same time. CANX and CALR from cell lysates in (A) were analyzed by immunoblotting (B). GAPDH was used as a loading control.

(C) Cells were transiently transfected with constructs expressing WT EGFP-FLAG-CD59, misfolded CD59 (EGFP-FLAG-CD59 (C94S)), misfolded CD59 lacking GPI (EGFP-FLAG-CD59 (C94S, G103*)), or misfolded CD59 lacking an N-glycan (EGFP-FLAG-CD59 (C94S, N43Q)) and then were lysed with lysis buffer containing 0.5% CHAPS. The lysates were then subjected to immunoprecipitation (IP) with anti-FLAG affinity gel, and after washing, the precipitated proteins were released by the addition of SDS sample buffer. The input (10% of total protein) and immunoprecipitated fractions were analyzed by immunoblotting with the indicated antibodies. It is noted that EGFP-FLAG-CD59 (C94S, G103*) was not immunoprecipitated with calreticulin, whereas it was previously shown they are weak co-immunoprecipitated (22). The difference might come from the weak interaction.

Figure S3

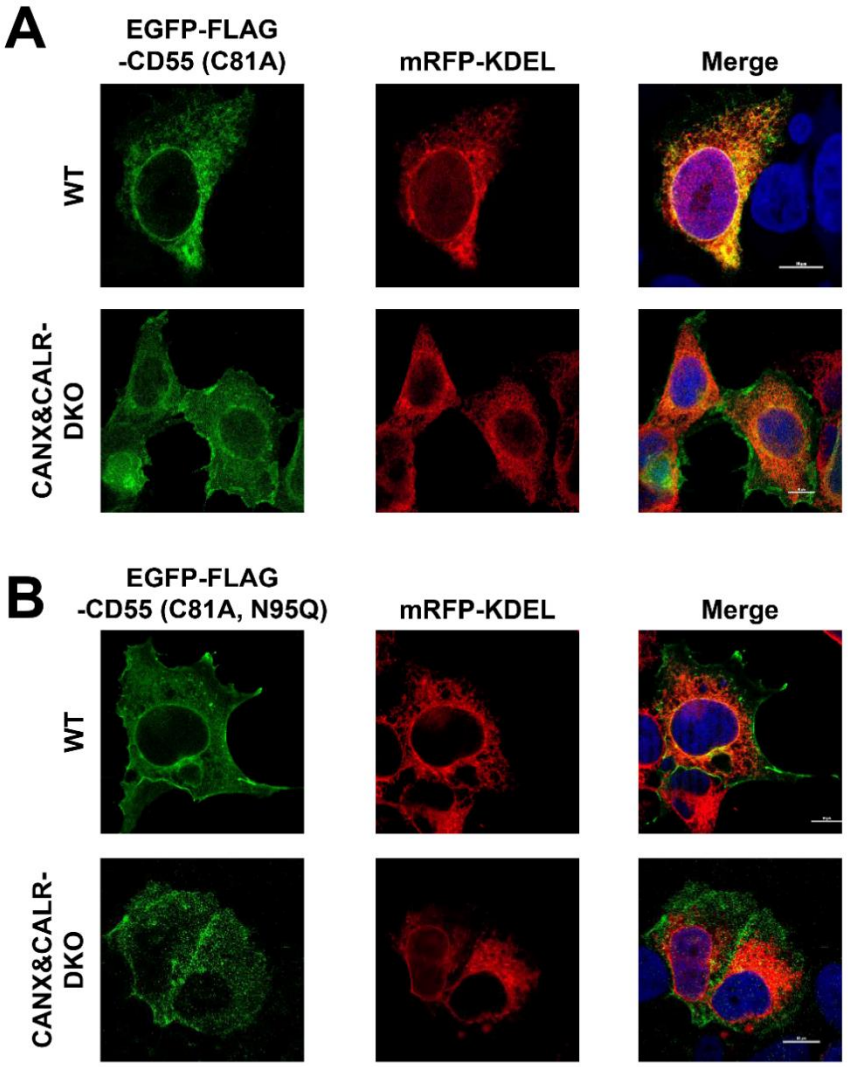

Figure S3. Localization of misfolded GPI-APs in CANX&CALR-DKO cells.
(A and B) EGFP-FLAG-CD55 (C81A) or EGFP-FLAG-CD55 (C81A, N95Q) constructs were transiently transfected together with mRFP-KDEL into WT and CANX&CALR-DKO cells. The images were obtained using confocal microscopy at 3 days after transfection. DAPI staining is shown as blue in merged images. Scale bar, 10 μm.
(C and D) EGFP-FLAG-CD59 (C94S) and mRFP-KDEL constructs were transiently transfected into CANX&CALR-DKO cells carrying CANX mutants defective in N-glycan binding activity (Y164A, K166A, M188A or E216A) or defective in ERp57 binding (W342A&D343A, D343A&E351A or D347A&E351A). Confocal images were obtained at 3 days after transfection. Scale bar, 10 μm.
(E) The GPI attachment signal of CD59 (C94S) was replaced with the transmembrane sequence of CD46 to generate a transmembrane form of misfolded CD59 (C94S) (EGFP-FLAG-CD59 (C94S) TM) and was co-transfected with mRFP-KDEL into WT and CANX&CALR-DKO cells. The localization of EGFP-FLAG-CD59 (C94S) TM was imaged by confocal microscopy. Scale bar, 10 μm.

Figure S3

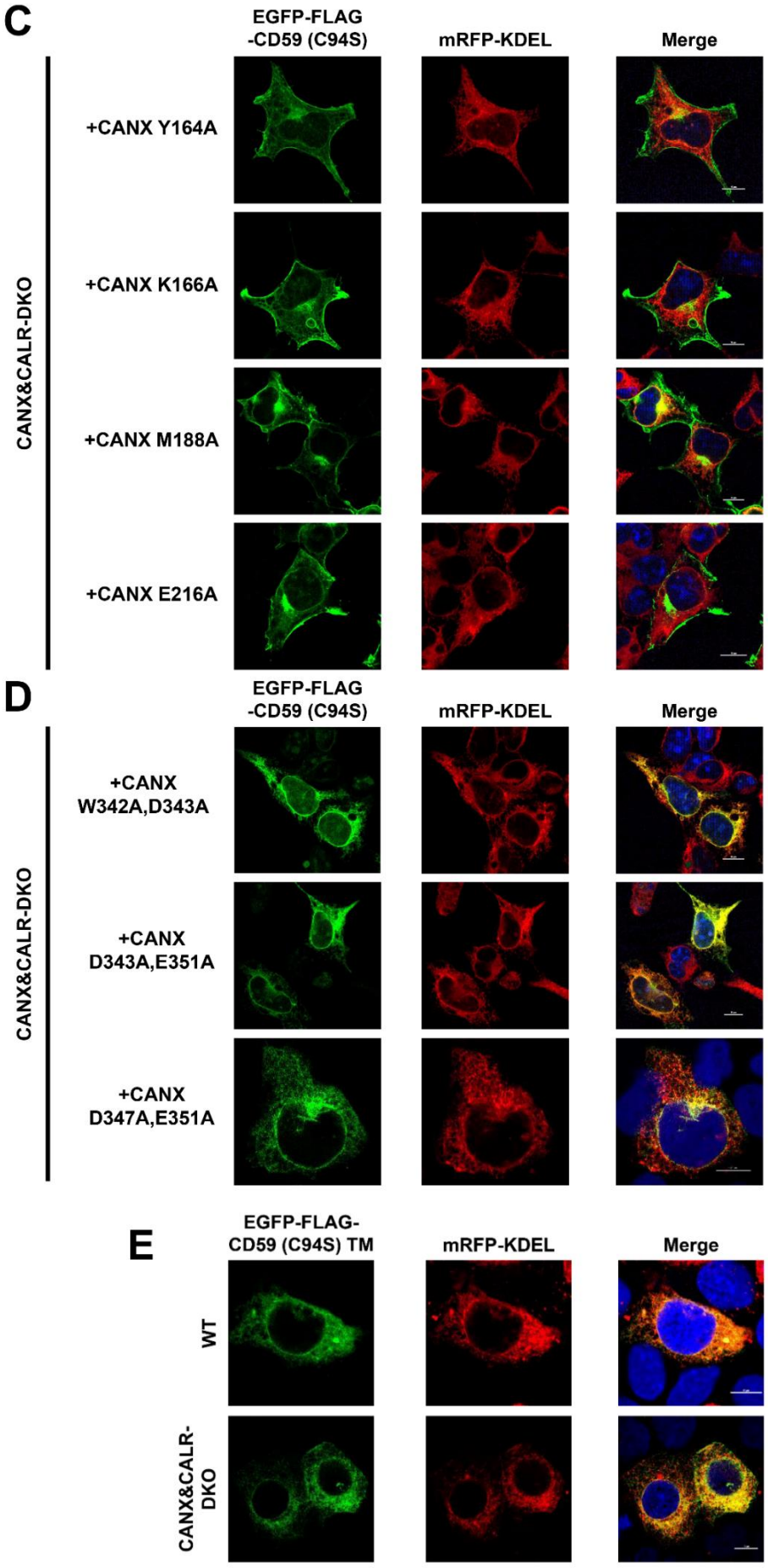
C
EGFP-FLAG -CD59 (C94S)
mRFP-KDEL
Merge
CANX&CALR-DKO
+CANX Y164A
+CANX K166A
+CANX M188A
+CANX E216A
D
EGFP-FLAG -CD59 (C94S)
mRFP-KDEL
Merge
CANX&CALR-DKO
+CANX W342A,D343A
+CANX D343A,E351A
+CANX D347A,E351A
E
EGFP-FLAG-CD59 (C94S) TM
mRFP-KDEL
Merge
WT
CANX&CALR-DKO

Figure S4

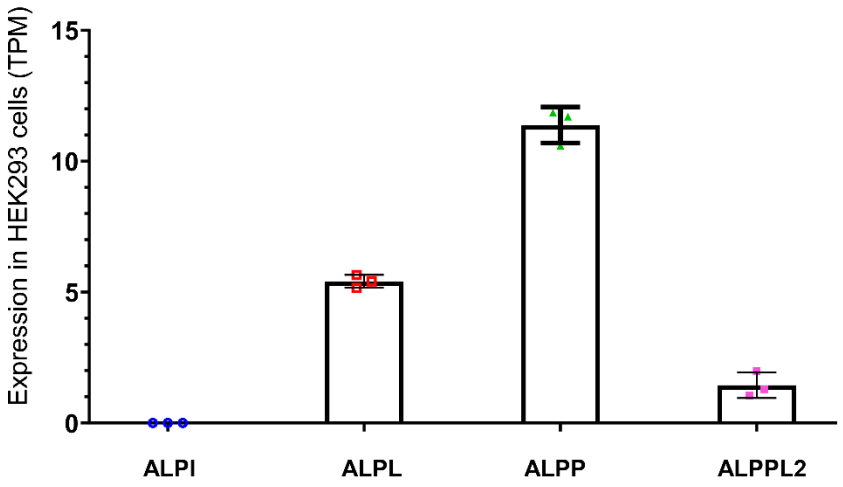

Figure S4. Expression of genes encoding alkaline phosphatases in HEK293 cells.
The RNA-seq data of HEK293 cells (Huang et al., manuscript in preparation) were used to assess gene expression. Transcription per million (TPM) values are presented as the means ± SD of three independent measurements. ALPI, intestinal-type alkaline phosphatase; ALPL, tissue-nonspecific alkaline phosphatase; ALPP, placental type alkaline phosphatase; ALPPL2, germ cell type alkaline phosphatase.