JCB

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>PGAP1-WT
ctgGGGCTGTGGGATGTCTTCTTCGGcttcgaggagaataagtgcagtatGAGCTACATGTTTGAGTACCCGGagt
>PGAP1-KO1-type1 (Δ47bp)
ctgGGGCTGTGGGATGTCTT.............................................ACCCGGagt
>PGAP1-KO1-type2 (Δ46bp)
ctgGGGCTGTGGGATGTCTT............................................TACCCGGagt

>SELT-WT
gagGCTTCTGCTGCTTCTCCTAGTGGcgg
>SELT-1-2-KO (+C)
gagGCTTCTGCTGCTTCTCCCTAGTGGcgg

>C18orf32-WT
cctGGAGCCATATATATACCCTCTGGttt
>C18orf32-KO4 (ΔC)
cctGGAGCCATATATATAC.CTCTGGttt

>SEC63-WT-exon2
gaaGGTGTATGTGGTATCGTTTACGGttattaaaaCCCCAGCCAAATATTATTCCTACagt
>SEC63-KO7-type1 (Δ22bp)
gaaGGTGTATGTGGTATCG......................CCAAATATTATTCCTACagt
>SEC63-KO7-type2 (ΔT in target1/ +62bp in target2)
gaaGGTGTATGTGGTATCGT.TACGGttattaaaaCCCCAGGAACTTGTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCAC
CACCCCGGTGAACACCAAATATTATTCCTACagt
>SEC63-KO7-type3 (ΔGC/+T in target1/ +183bp in target2)
gaaGGTGTATGTGGTATTTTTACGGttattaaaaCCCCAGGCGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCC
TTAAGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGGGAAAGAGACCTGCCGCAACCAAGAAGGCCGGC
CAGGCAAAAAAGAAAAAGTAAGAATTCCTAGAGCTCGCTGATCAGCCAAATATTATTCCTACagt

>CANX-WT
agcCCTTCCTGTTTGACACCAAGCCTctc
>CANX-KO8-type1(+T)
agcCCTTCCTTGTTTGACACCAAGCCTctc
>CANX-KO8-type2(ΔTGT)
agcCCTTCC.GTTTGACACCAAGCCTctc

>CLPTM1-WT
gaaCCTGCATGTGTACATCTCAGAGCacgagcactttacagacttcaACGCCACGTCGGCACTCTTCTGGgaa
>CLPTM1-KO3,4-12 (Δ54bp)
gaaCCTGCAT.....................................................TTCTGGgaa

>MOGS-WT
gccGTGGCCCCGGACCTCTTCTGGGGaacctaccgccctcacgtctacttcggcatgaagacCCGCAGCCCGAAGCCCCTCCTCAccg
>MOGS-KO1,2-2-type1 (ΔCT)
gccGTGGCCCCGGACCTCTT..GGGGaacctaccgccctcacgtctacttcggcatgaagacCCGCAGCCCGAAGCCCCTCCTCAccg
>MOGS-KO1,2-2-type2(ΔTT+CTG)
gccGTGGCCCCGGACCTCCTGCTGGGGaacctaccgccctcacgtctacttcggcatgaagacCCGCAGCCCGAAGCCCCTCCTCAccg

>GANAB-WT
ctgTACAACCCAATGGCCTTGTATGGgtctgtgcctgtgctcctggcACACAACCCTCATCGCGACTTGGgca
>GANAB-KO3,4-1-type1(+T)
ctgTACAACCCAATGGCCTTTGTATGGgtctgtgcctgtgctcctggcACACAACCCTCATCGCGACTTGGgca
>GANAB-KO3,4-1-type2(Δ26bp)
ctgTACAACCCTGTG.........................cctggcACACAACCCTCATCGCGACTTGGgca

>CALR-WT
cagCCAGGTGGAGTCCGGCTCCTTGGaagacgattgggacttcctgCCACCCAAGAAGATAAAGGATCCtga
>CALR-KO3,4-10-type1(Δ32bp)
cagCCAGGTGGAGTCCGGCT.............................AAGAAGATAAAGGATCCtga
>CALR-KO3,4-10-type2(Δ32bp+C)
cagCCAGGTGGAGTCCGGCT.............................CAAGAAGATAAAGGATCCtga

>CALR-WT
cagCCAGGTGGAGTCCGGCTCCTTGGaagacgattgggacttcctgCCACCCAAGAAGATAAAGGATCCtga
>CANX-CALR-DKO3-type1(Δ32bp)
cagCCAGGTGGAGTCCGGCT.............................AAGAAGATAAAGGATCCtga
>CANX-CALR-DKO3-type1(Δ34bp)
cagCCAGGTGGAGTCCGGCT...............................GAAGATAAAGGATCCtga

>STT3A-WT
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TCTAATCGATGCTGGAGGgtg
>STT3A-KO5 (Δ92bp)
ccaCCATCG.....................................................................................
............TGGAGGgtg

>STT3B-WT
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tattgatgatGTTTGCTGTCCACTGTACCTGGGtca
>STT3B-KO1 (Δ96bp)
agtGAGGAAACATGCAA.............................................................................
.........................CCTGGGtca
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Figure S1. **KO of candidate genes using the CRISPR-Cas9 system.** Candidate genes identified in the screening for GPI-APs resistance to PIPLC were knocked out in HEK293FF6 or HEK293 cells. Genomic DNA sequences of target gene regions from WT and KO cell lines are shown. Red letters indicate target sequences of guide RNA; underlined letters indicate the protospacer-adjacent motif sequence; blue letters indicate inserted nucleotides.

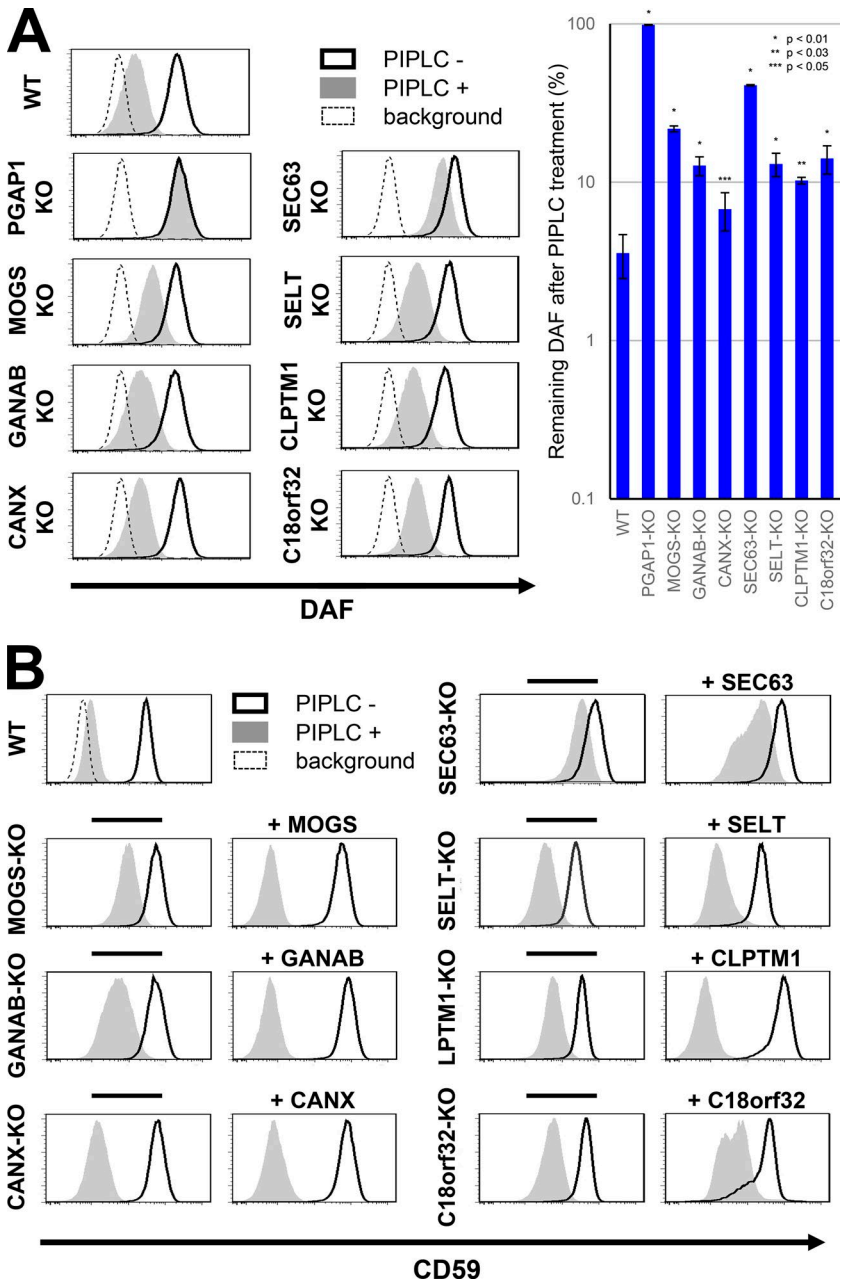


Figure S2. **PIPLC sensitivity of DAF in candidate gene KO cells and restoration of PIPLC sensitivity by expression of the responsible genes. (A)** KO cells were treated with or without PIPLC and then stained with an anti-DAF antibody and analyzed by flow cytometry. Shaded portions indicate cells treated with PIPLC, solid lines indicate cells without PIPLC treatment, and dashed lines indicate background. Percentages of DAF remaining after PIPLC treatment of the KO cell lines are plotted on the right. Values are means ± SEM of three independent measurements, with p-values (two-tailed Student's *t* test) shown above. **(B)** Gene KO cell lines were transfected with plasmids containing the respective cDNA genes. Cells were then treated with or without PIPLC, and surface CD59 was detected by flow cytometry. Shaded areas indicate cells treated with PIPLC, solid lines indicate cells without PIPLC treatment, and dashed lines indicate background.

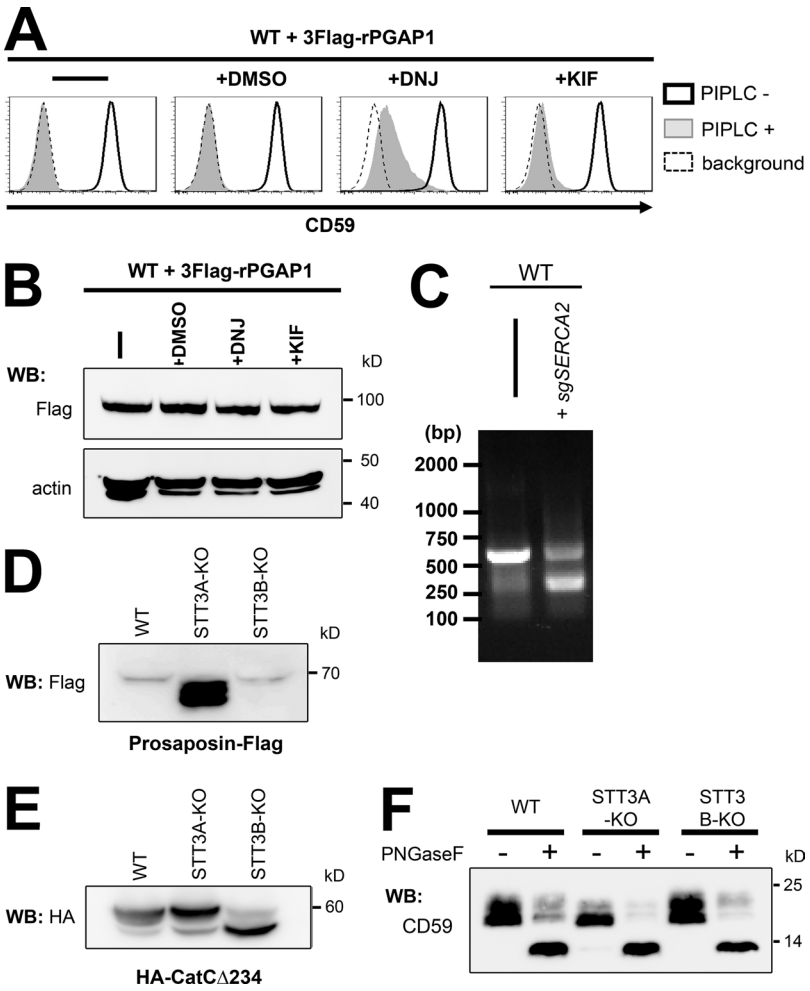
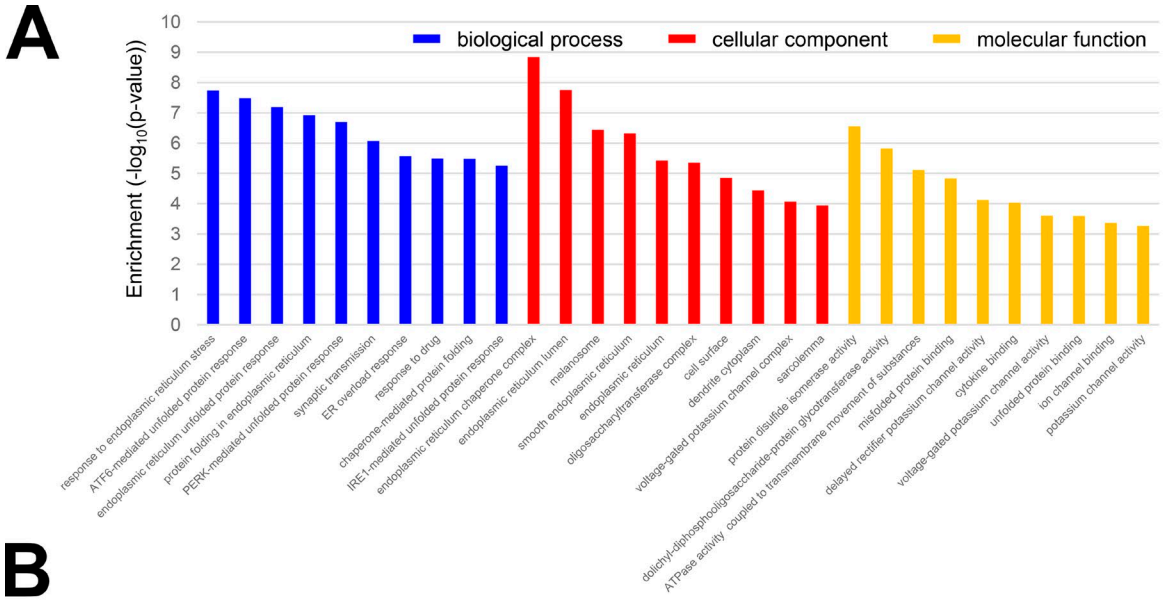


Figure S3. **Increased PIPLC resistance of GPI-APs with DNJ treatment and chronic ER stress. (A)** HEK293FF6 cells stably expressing Flag-tagged rat PGAP1 were treated with or without DNJ or KIF for 8 d. Then, cells were treated with or without PIPLC, and surface CD59 was detected by flow cytometry. **(B)** Western blotting (WB) analysis of PGAP1 to assess its stability after DNJ treatment. Actin was used as the loading control. **(C)** WT HEK293FF6 cells were transfected with plasmids to knock out SERCA2 (sgSERCA2). After transfection, plasmid-positive cells were sorted and cultured for >10 d, and genomic DNA was extracted from the cell population. The KO region was amplified using primer sets for assessing SERCA2-KO and analyzed by agarose gel electrophoresis. The size of designed DNA fragments for SERCA2 is 570 bp for WT HEK293 cells. With SERCA2 correctly deleted by the CRISPR-Cas9 system, the size would become 302 bp. **(D and E)** Plasmids expressing STT3A-specific substrate Flag-tagged prosaposin (D) or STT3B-specific substrate HA-tagged CatCΔ234 (E) were transiently transfected into WT, STT3A-KO, and STT3B-KO cells. Glycosylation status of proteins was analyzed by Western blotting. **(F)** The lysates prepared from HEK293, STT3A-KO, and STT3B-KO cells were treated with or without PNGase F. Glycosylation status of CD59 was analyzed by Western blotting.



B

ID	3309	811	7494	9601	5611	10130	51726	56605	1649	9709	821	2923	9451	201595	6184	488	23071	2673	
Gene_name	HSPA5	CALR	XBP1	PDIA4	DNAJC3	PDIA6	DNAJB11	ERO1LB	DDIT3	HERPUD1	CANX	PDIA3	EIF2AK3	STT3B	RPN1	ATP2A2	ERP44	GFPT1	
Fold_Change	3.037	3.474	2.598	1.991	2.205	1.965	1.838	2.002	2.150	1.812	1.765	1.520	1.589	1.383	1.353	1.4599	1.404	1.4402	
KEGG:hsa04141	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				Protein processing in endoplasmic reticulum
GO:0034976			+	+		+			+	+		+	+			+	+		response to endoplasmic reticulum stress
GO:0036500	+	+	+						+										ATF6-mediated unfolded protein response
GO:0030968	+	+	+		+	+	+		+	+			+					+	endoplasmic reticulum unfolded protein response
GO:0034975	+	+									+	+							protein folding in endoplasmic reticulum
GO:0036499	+								+	+			+						PERK-mediated unfolded protein response
GO:0036498	+		+		+	+	+											+	IRE1-mediated unfolded protein response

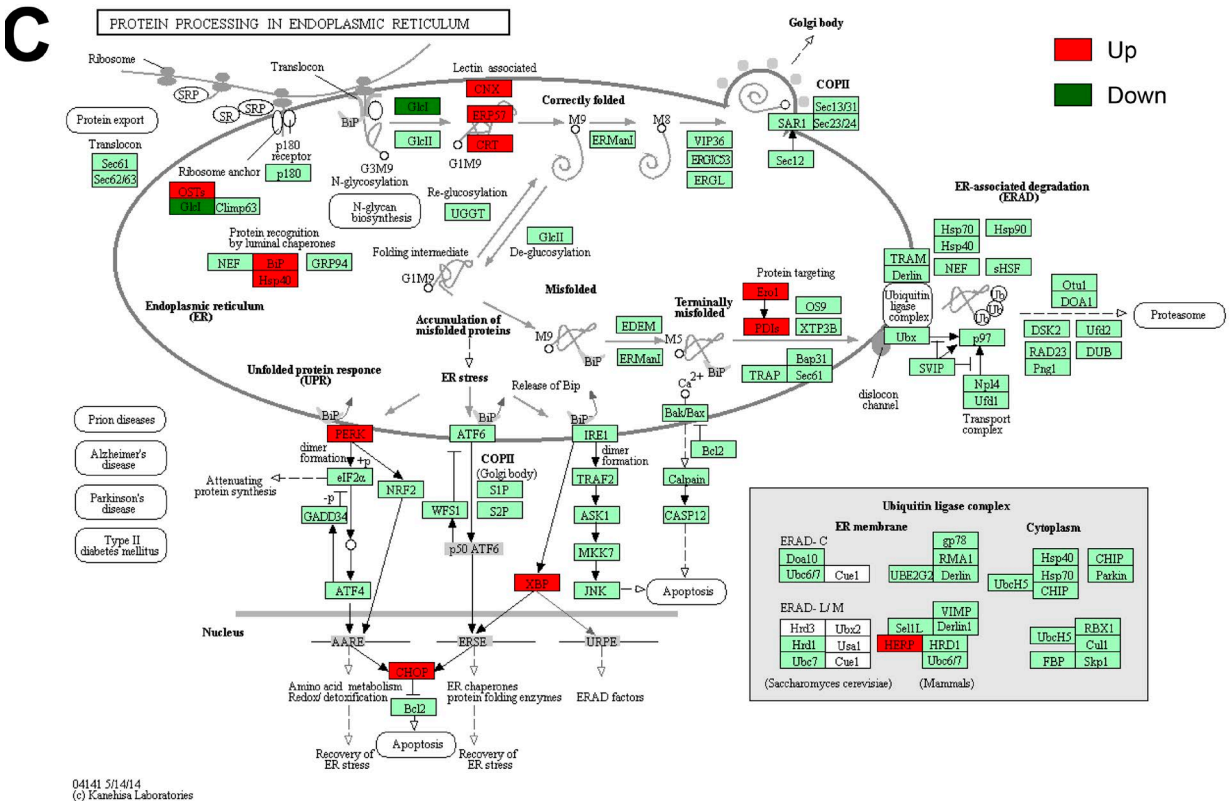


Figure S4. **ER stress occurred in MOGS-KO cells. (A)** RNA-seq analysis in WT and MOGS-KO HEK293FF6 cells (see also Table S5). A set of genes that were up-regulated in MOGS-KO cells were analyzed using Gene Ontology enrichment analysis. The significant shared Gene Ontology (GO) terms are shown as bar plots. Blue indicates biological processes, red indicates cellular components, and orange indicates molecular function. **(B and C)** Protein processing in the ER in the KEGG pathway was significantly altered in MOGS-KO cells. Among genes up-regulated in MOGS-KO cells, genes categorized in "hsa04141: Protein processing in endoplasmic reticulum" in the KEGG pathway and other genes related to the UPR in Gene Ontology terms were listed (B). In the genes in "Protein processing in endoplasmic reticulum" in the KEGG pathway, genes altered expression in MOGS-KO cells were shown in red (up-regulation) or dark green (down-regulation) boxes (C).

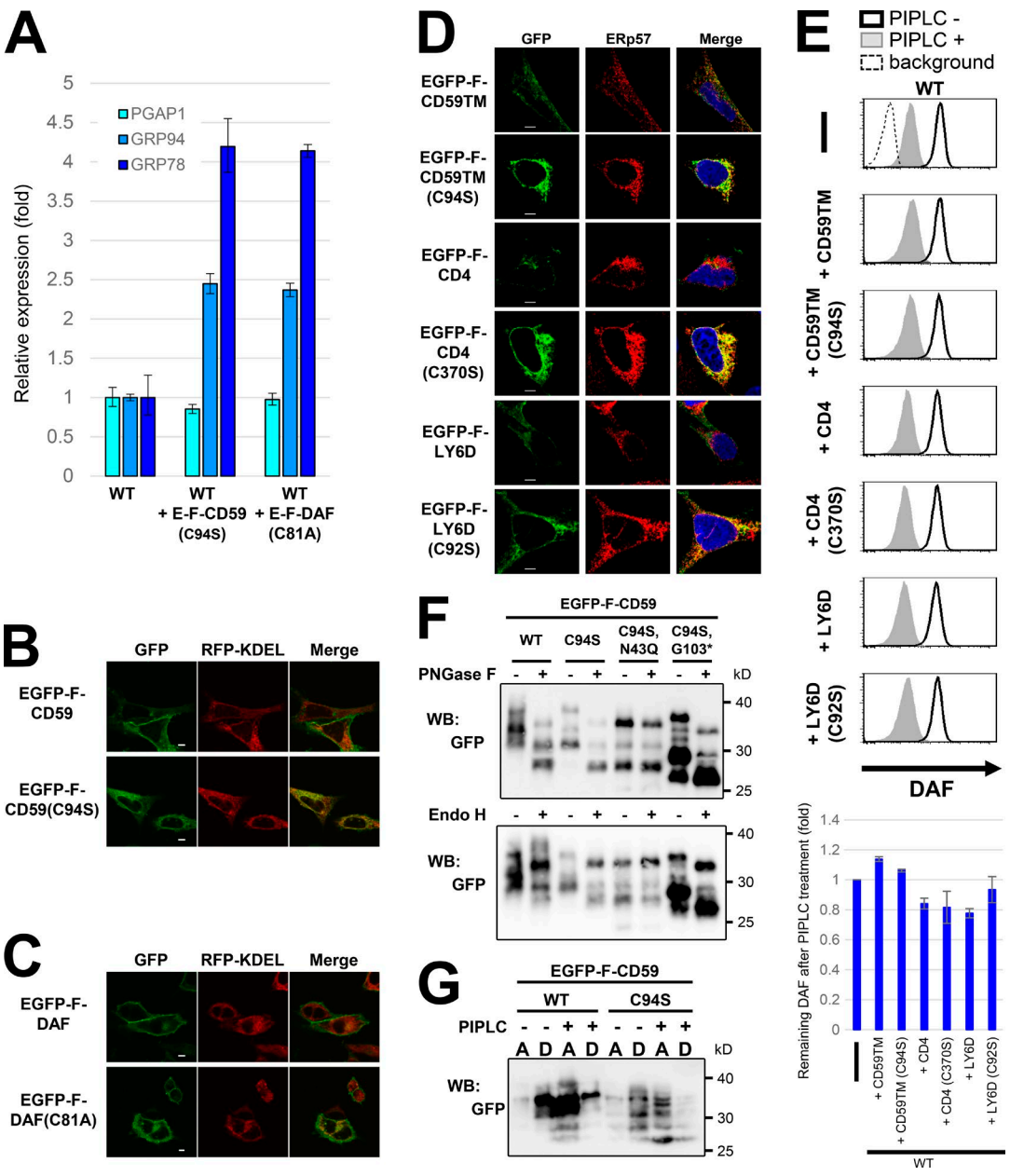


Figure S5. **Localization of EGFP-Flag-tagged GPI-APs and their misfolded forms. (A)** Quantitative RT-PCR analysis of PGAP1, GRP78, and GRP94 mRNA levels in HEK293FF6 WT cells and HEK293FF6 cells stably expressing mutant CD59 (C94S) or mutant DAF (C81A). GAPDH levels were used to normalize the data. The bars represent relative quantification (RQ) values ± RQmax and RQmin (error bars) from triplicate samples. **(B)** EGFP-Flag–tagged WT CD59 (EGFP-F–CD59) or mutant CD59 (EGFP-F–CD59 [C94S]) together with an ER marker RFP-KDEL were transiently expressed in HEK293FF6 cells. Cell images were obtained by confocal microscopy. **(C)** EGFP-Flag–tagged WT DAF (EGFP-F–DAF) or mutant DAF (EGFP-F–DAF [C81A]) together with an ER marker RFP-KDEL were transiently expressed in HEK293FF6 cells. Cell images were obtained by confocal microscopy. **(D)** EGFP-Flag–tagged (EGFP-F–) WT CD59TM, mutant CD59TM (C94S), WT CD4, mutant CD4 (C370S), WT LY6D, or mutant LY6D (C92S) were stably expressed in HEK293FF6 cells. ERp57 was stained as an ER marker. Cell images were obtained by confocal microscopy. DAPI staining was shown as blue in merged images. Bars, 5 μm. **(E)** HEK293FF6 cells stably expressing WT EGFP-Flag–tagged (EGFP-F–) WT CD59TM, mutant CD59TM (C94S), WT CD4, mutant CD4 (C370S), WT LY6D, or mutant LY6D (C92S) were treated with or without PIPLC. After treatments, cells were stained for endogenous DAF and analyzed by flow cytometry. DAF levels remaining after PIPLC treatment are plotted below. The value for remaining DAF in WT cells without any exogenous expression was set to 1. Relative values were calculated and are represented as means ± SEM from three independent experiments. **(F)** The EGFP-F–CD59 proteins were treated with PNGase F to analyze the *N*-glycosylation (top). CD59 has one *N*-glycosylation site. By the PNGase treatment, all the bands except CD59 (C94S and N43Q) were shifted. The shifted bands of the WT and C94S CD59 became the same migration with CD59 (C94S and N43Q), suggesting that all the bands possess one *N*-glycan. The EGFP-F–CD59 proteins were treated with EndoH to determine the glycan structures. The bands of the WT CD59 were still resistant, whereas those of the CD59 (C94S) were sensitive, similar to PNGase treatment, suggesting that some differences of the bands between the WT and misfolded CD59 proteins were caused by the *N*-glycan structures. **(G)** EGFP-F–CD59 and mutants were analyzed using Triton X-114 and PIPLC. If proteins are GPI-anchored, they are fractionated in detergent phase (D) by Triton X-114 partition but moved into aqueous phase (A) after PIPLC treatment. Most bands of misfolded CD59 were also fractionated into detergent phase and moved into aqueous phase, similar to WT CD59. The results suggest that misfolded CD59 is also modified with GPI. It is noted that the band pattern differences between figures were caused by the different transfection conditions: transient (Fig. 7 A) and stable (Figs. 8 E and S5, F and G) expression of the EGFP-F–CD59 proteins. The multiple bands would not be the proteolytic products. CD59 has potential *O*-glycosylation sites (UniProt), which might result in the multiple bands. WB, Western blot.

Table S1. **Oligonucleotides used for KO construction**

Primer name	Sequence (5′–3′)
sgPGAP1-1F	CACCGGGCTGTGGGATGTCTTCTT
sgPGAP1-1R	AAACAAGAAGACATCCCACAGCCC
sgPGAP1-2F	CACCGAGCTACATGTTTGAGTACC
sgPGAP1-2R	AAACGGTACTCAAACATGTAGCTC
sgSELT-F	CACCGCTTCTGCTGCTTCTCCTAG
sgSELT-R	AAACCTAGGAGAAGCAGCAGAAGC
sgC18orf32-F	CACCGGAGCCATATATATACCCTC
sgC18orf32-R	AAACGAGGGTATATATATGGCTCC
sgCANX-3F	CACCAGGCTTGGTGTCAAACAGGA
sgCANX-3R	AAACTCCTGTTTGACACCAAGCCT
sgMOGS-1F	CACCGTGGCCCCGGACCTCTTCTG
sgMOGS-1R	AAACCAGAAGAGGTCCGGGGCCAC
sgMOGS-2F	CACCTGAGGAGGGGCTTCGGGCTG
sgMOGS-2R	AAACCAGCCCGAAGCCCCTCCTCA
sgGANAB-3F	CACCTACAACCCAATGGCCTTGTA
sgGANAB-3R	AAACTACAAGGCCATTGGGTTGTA
sgGANAB-4F	CACCACACAACCCTCATCGCGACT
sgGANAB-4R	AAACAGTCGCGATGAGGGTTGTGT
sgCLPTM1-3F	CACCGCTCTGAGATGTACACATGC
sgCLPTM1-3R	AAACGCATGTGTACATCTCAGAGC
sgCLPTM1-4F	CACCACGCCACGTCGGCACTCTTC
sgCLPTM1-4R	AAACGAAGAGTGCCGACGTGGCGT
sgSEC63-1F	CACCGGTGTATGTGGTATCGTTTA
sgSEC63-1R	AAACTAAACGATACCACATACACC
sgSEC63-2F	CACCGTAGGAATAATATTTGGCTG
sgSEC63-2R	AAACCAGCCAAATATTATTCCTAC
sgCALR-3F	CACCCCAGGTGGAGTCCGGCTCCT
sgCALR-3R	AAACAGGAGCCGGACTCCACCTGG
sgCALR-4F	CACCGGATCCTTTATCTTCTTGGG
sgCALR-4R	AAACCCCAAGAAGATAAAGGATCC
sgALG6-1F	CACCTGAGTCTTGGCTTTGCTTTG
sgALG6-1R	AAACCAAAGCAAAGCCAAGACTCA
sgALG6-2F	CACCAGCAAAATGCCAGTGACCCT
sgALG6-2R	AAACAGGGTCACTGGCATTTTGCT
sgALG8-1F	CACCCATTTCTTGATCAAAATATT
sgALG8-1R	AAACAATATTTTGATCAAGAAATG
sgALG8-2F	CACCGAGTACATCCATAAAGATGA
sgALG8-2R	AAACTCATCTTTATGGATGTACTC
sgSTT3A-1F	CACCGGTAAGGTGGTACGTGACGA
sgSTT3A-1R	AAACTCGTCACGTACCACCTTACC
sgSTT3A-2F	CACCGCCTCTCTAATCGATGCTGG
sgSTT3A-2R	AAACCCAGCATCGATTAGAGAGGC
sgSTT3B-1F	CACCGTTTGCTGTCCACTGTACCT
sgSTT3B-1R	AAACAGGTACAGTGGACAGCAAAC
sgSTT3B-2F	CACCGAGGAAACATGCAACTGAAC
sgSTT3B-2R	AAACGTTCAGTTGCATGTTTCCTC
sgSERCA2-1F	CACCGCAAATAAGGGAGATGACTT
sgSERCA2-1R	AAACAAGTCATCTCCCTTATTTGC
sgSERCA2-2F	CACCGTTATCTGCTCAGACAAGAC
sgSERCA2-2R	AAACGTCTTGTCTGAGCAGATAAC

Table S2. **Oligonucleotide primers used in this study**

Primer name	Sequence (5′–3′)
CANX-F	AAAAGAATTCCACCATGGAAGGGAAGTGGTTGCTGT
CANX-R	AAAAGCGGCCGCTCACTCTCTTCGTGGCTTTCTGTT
MOGS-F	AAAAGCGGCCGCCACCATGGCTCGGGGCGAGCGG
MOGS-R	AAAACTCGAGTCAGTAGTCTTCAGCCATGGCCAG
GANAB-F	AAAAGAATTCCACCatggcggcggtagcggcagt
GANAB-R	AAAAGCGGCCGCTTATCGCAGGTGAATACTCCAATC
SELT-F	AAAAGAATTCCACCatgaggcttctgctgcttctccta
SELT-R	AAAAGCGGCCGCGCCAGCACTGAAACACTATCTTGCA
CLPTM1-F	AAAAGAATTCCACCATGGCGGCGGCGCAGGAG
CLPTM1-R	AAAAGCGGCCGCCTAATCCTTTTTCTTGTCCTCTGCTGGC
C18orf32-F	AAAAGTCGACGAATTCcaccATGGTGTGCATTCCTTGTATCGT
C18orf32-R	AAAAACGCGTGCGGCCGCAATGATGGGGTCCTTTAGGAAAATT
SEC63-F	AAAACTCGAGACCACCATGGCCGGGCAGCAGTTCCA
SEC63-R	AAAAGCGGCCGCCTAGTCATCATCTTCTTCTT
CALR-F	AAAAGAATTCCACCATGCTGCTATCCGTGCCGCT
CALR-R	AAAAGCGGCCGCCTACAGCTCGTCCTTGGCCTG
CD59ss-mEGFP-F	AAAAGAATTCCACCATGGGAATCCAAGGAGGGTC
CD59ss-mEGFP-R	AAAAGCGGCCGCCTACAGCTCGTCCTTCTTGTACAGCTCGTCCATGC
CANX(Y164A)-F	GTTTTAGAAAGCAGTTTCACAGCGGCACCACCACATTCTATTCC
CANX(Y164A)-R	GGAATAGAATGTGGTGGTGCCGCTGTGAAACTGCTTTCTAAAAC
CANX(E216A)-F	CTGGCCTCTTAGCATGTTTTGCTTCATAGATACCCGTTTTG
CANX(E216A)-R	CAAAACGGGTATCTATGAAGCAAAACATGCTAAGAGGCCAG
DAF(C81A)-F1	CGACGATGACAAGCTTGACTGTGGCCTTCCCCC
DAF(C81A)-R1	TTAAGGGCGATCACTGAGTCCTTCTC
DAF(C81A)-F2	AGTGATCGCCCTTAAGGGCAGTCAATG
DAF(C81A)-R2	ACTAGTCTAGCGGCCGCCTAAGTCAGCAAGCCCATGGTTA
CD59(C94S)-F1	CGACGATGACAAGCTTCTCGAGCTTCAGTGCTACAACT
CD59(C94S)-R1	AAAGTTACTCAGGTCCTTCTTGCAGC
CD59(C94S)-F2	GACCTGAGTAACTTTAACGAACAGCTT
CD59(C94S)-R2	ACTAGTCTAGCGGCCGCTTAGGGATGAAGGCTCCA
CD59(N43Q)-F	GCATCAAAATCAGATGAACACTGGACGGCTGTTTTGCAGTCAG
CD59(N43Q)-R	CTGACTGCAAAACAGCCGTCCAGTGTTCATCTGATTTTGATGC
CD59(G103*)-F	CTGATAAGGATGTCCCCTAATTCTCGAGCTGTTCGTTAAAGTTACTCAGG
CD59(G103*)-R	CCTGAGTAACTTTAACGAACAGCTCGAGAATTAGGGGACATCCTTATCAG
rPGAP1(N234Q)-F	CAGCTACAGAAAGTGTGGTCAACTGTATGTGCCGAGCATTTAAAATCC
rPGAP1(N234Q)-R	GGATTTTAAATGCTCGGCACATACAGTTGACCACACTTTCTGTAGCTG
rPGAP1(N234Q)-R	CACCGTTATCTGCTCAGACAAGAC
rPGAP1(N234Q)-R	AAACGTCTTGTCTGAGCAGATAAC
rPGAP1(N363Q)-F	GCAAAGTAAATCTTATCAGATTCCTGATAAGCCACATAGGACCATCTGGA
rPGAP1(N363Q)-R	TCCAGATGGTCCTATGTGGCTTATCAGGAATCTGATAAGATTTACTTTGC
rPGAP1(N402Q)-F	GCACATGGAAGTGCTCTGTATGCAGCCAAAAATCCAGCTATTTG
rPGAP1(N402Q)-R	CAAATAGCTGGATTTTTGGCTGCATACAGAGCACTTCCATGTGC
rPGAP1(N485Q)-F	TCTTCCAGGAAGGCCATAATACAGACGAGCGGCCG
rPGAP1(N485Q)-R	CGGCCGCTCGTCTGTATTATGGCCTTCCTGGAAGA
rPGAP1(N558Q)-F	CCACATGGCTGTCCTGTTCTGGCTGAGCAACATGAAGCTT
rPGAP1(N558Q)-R	AAGCTTCATGTTGCTCAGCCAGAACAGGACAGCCATGTGG

F, forward; R, reverse

Table S3 is a separate Excel file showing enrichment of gene-trap insertions in the populations resistant to PIPLC.

Table S4 is a separate Excel file showing differential expression of genes in MOGS-KO cells.

Table S5 is a separate Excel file showing a list of GPI-APs and their potential *N*-glycan sites.