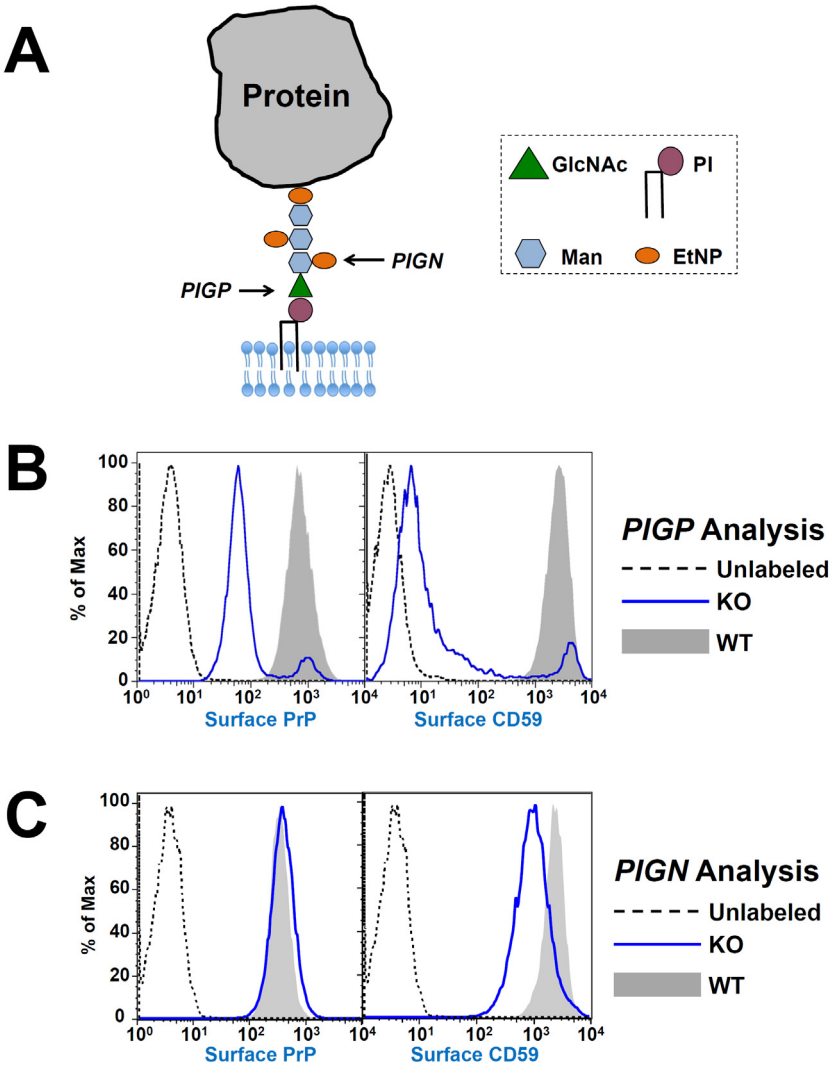

Figure S1. Effects of *PIGP* or *PIGN* knockout on the targeting of PrP and CD59, related to Figure 4. **(A)** Diagram of the GPI anchor depicting the modifications conferred by enzymes encoded by *PIGP* and *PIGN*. For clarity, only the relevant part of the GPI anchor is shown. EtNP: phosphoethanolamine; Man: mannose; PI: phosphatidylinositol; GlcNAc: N-acetylglucosamine. **(B)** *PIGP* was deleted from HAP1 cells using lentivirus-based CRISPR/Cas9. The pooled knockout cells were labeled with the indicated antibodies and analyzed by flow cytometry on a CyAn Analyzer. **(C)** *PIGN* was deleted from HAP1 cells using lentivirus-based CRISPR/Cas9 and knockout cells were pooled. The surface expression of PrP and CD59 was analyzed by flow cytometry. WT: wild type; KO: knockout.

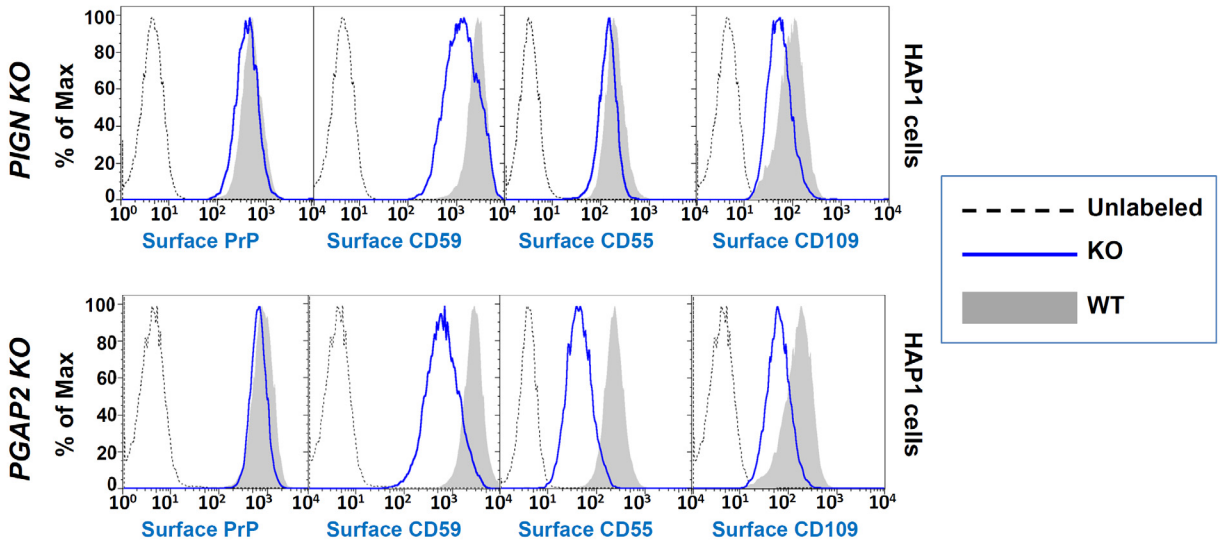

Figure S2. Effects of *PIGN* or *PGAP2* knockout on the surface expression of GPI-APs, related to Figure 4. *PIGN* knockout cells were prepared as described in Fig. S1. *PGAP2* was deleted from HAP1 cells using lentivirus-based CRISPR/Cas9 and individual knockout clones were isolated. WT and knockout cells were labeled with the indicated antibodies and analyzed by flow cytometry on a CyAn Analyzer.

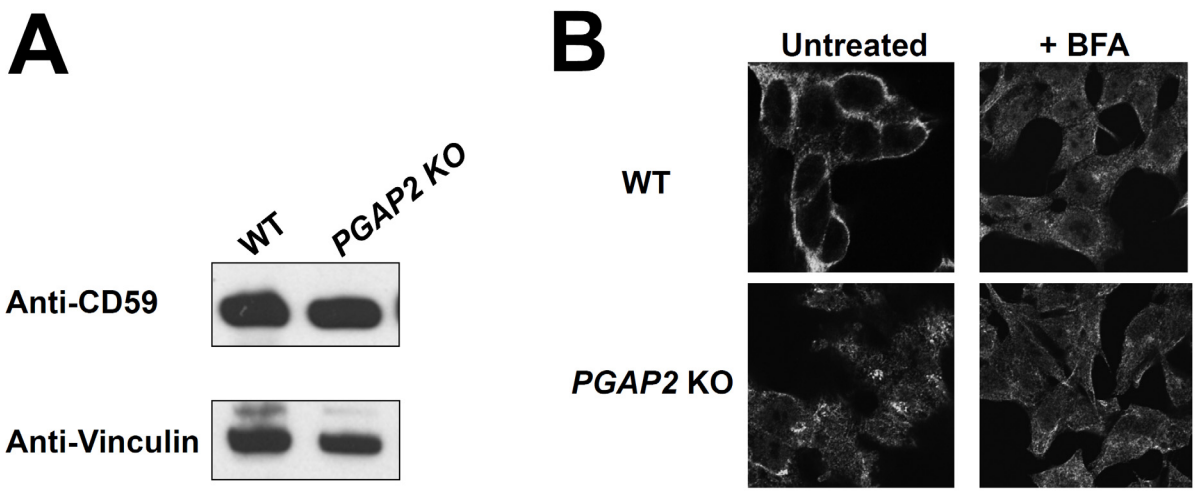

Figure S3. Effects of *PGAP2* knockout on CD59 total protein levels and localization, related to Figure 4. (A) Whole cell lysates were prepared from WT and *PGAP2* knockout cells and endogenous levels of CD59 in the cells were measured by immunoblotting. **(B)** Localization of endogenous CD59 was determined by immunostaining in WT and *PGAP2* knockout HAP1 cells. The cells were either untreated or treated with 5 μg/mL Brefeldin A (BFA) for two hours prior to fixation and staining. The images were acquired on a Carl Zeiss 510 confocal microscope.

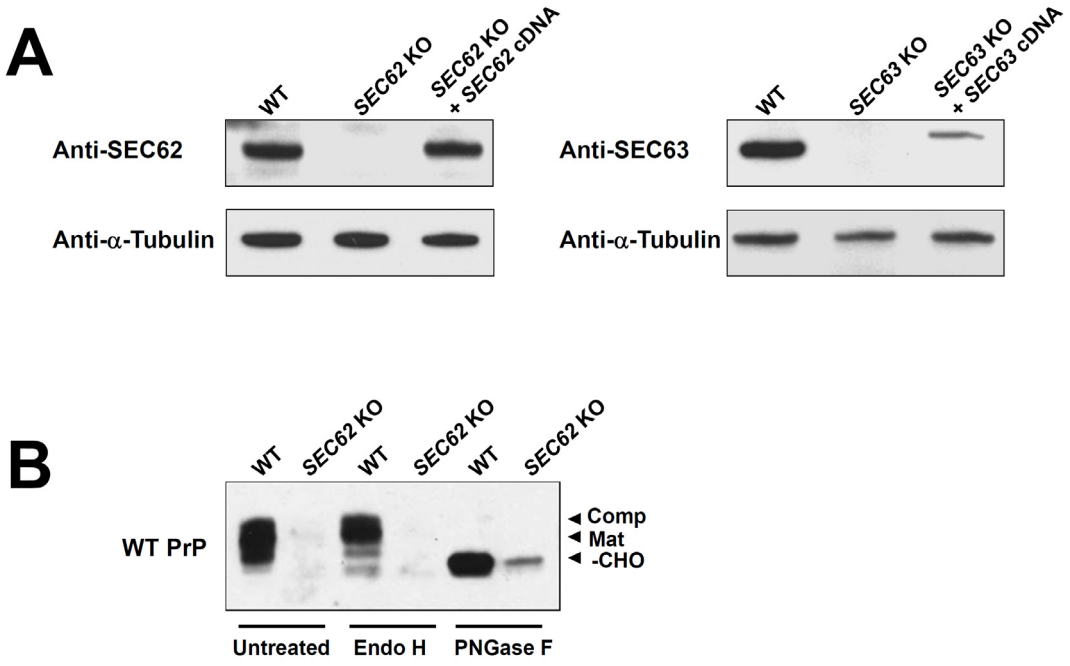

Figure S4. Analysis of *SEC62* and *SEC63* knockout cells, related to Figures 5 and 6. (A) Immunoblots showing the expression of *SEC62* and *SEC63*. *SEC62* and *SEC63* were individually deleted from HAP1 cells by pX330-based CRISPR/Cas9 and individual knockout clones were isolated. To rescue gene expression, plasmids encoding SEC62 or SEC63 were transiently transfected in the knockout cells. **(B)** Immunoblot showing the expression of HA-tagged PrP in WT and *SEC62* knockout cells. The samples were either untreated or treated with Endo H or PNGase F for 30 minutes prior to immunoblotting analysis. –CHO: the cytosolic/immature form; Mat: the fully glycosylated mature form; Comp: the fully glycosylated complexed form.

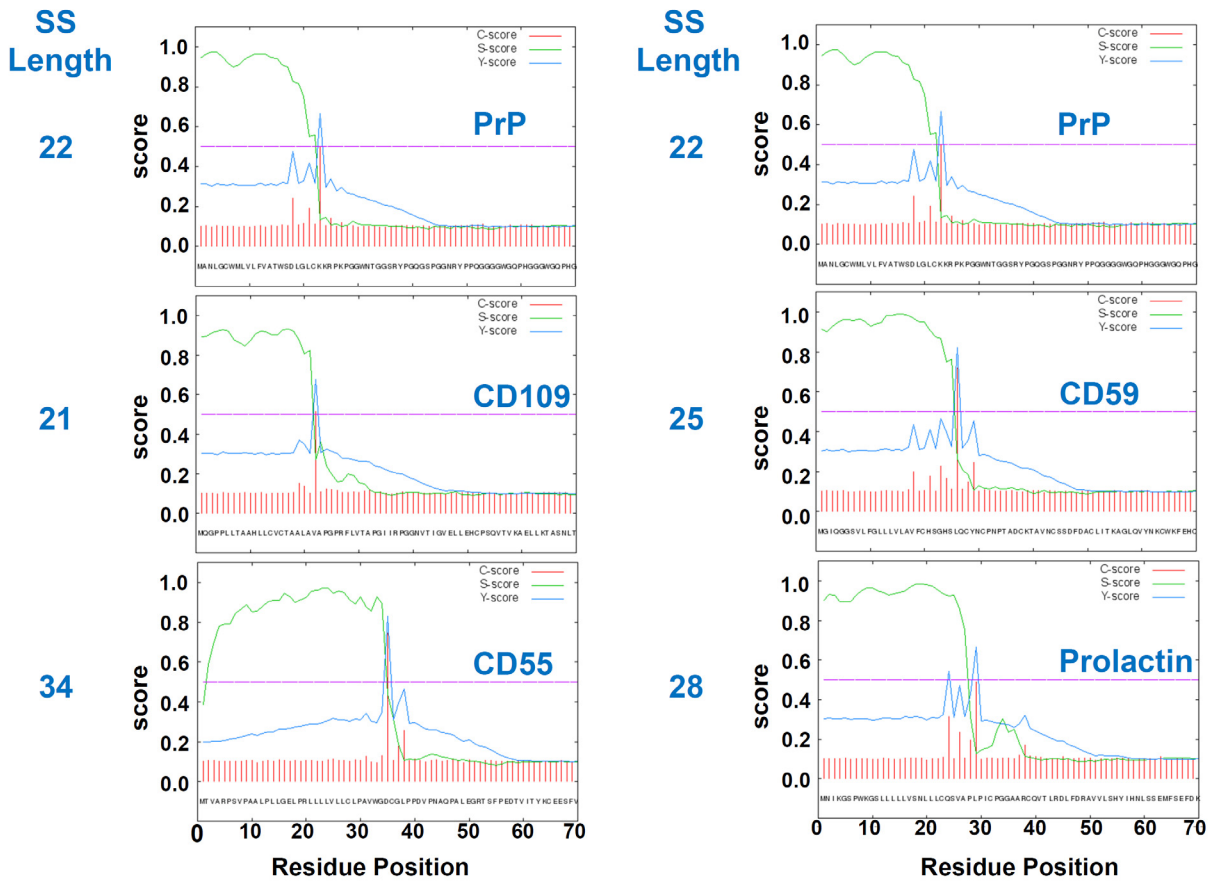

Figure S5. Analysis of the signal sequences of GPI-APs, related to Figure 6. The N-terminal signal sequences of the indicated GPI-APs were identified and analyzed using the SignalP 4.1 server (*http://www.cbs.dtu.dk/services/SignalP*). The X axis shows the residue positions of the proteins. The Y axis indicates the raw cleavage score (C-score), signal peptide score (S-score), and combined cleavage site score (Y-score) generated by SignalP4.1.

Table S1. Gene-trap insertions in the haploid genetic screens, related to Figures 2 and 3.