**Table S1: KO target sequences**

| | Target sequence | Oligonucleotide | Sequence | Oligonucleotide | Sequence | Species |
|---|---|---|---|---|---|---|
| SLC35A2-KO4 | GGTGGTTCCACCGCGGCGCCCGG | SLC35A2-KO4-F | caccGGTGGTTCCACCGCGGCGCC | SLC35A2-KO4-R | aaacGGCGCCGCGGTGGAACCACC | human |
| SLC35A2-KO1 | GTGGATCTACCGCTGCGGCCGGG | SLC35A2-KO1-F | caccGTGGATCTACCGCTGCGGCC | SLC35A2-KO1-R | aaacGGCCGCAGCGGTAGATCCAC | mouse |
| PIGS-KO1 | GCCGCGGTGGCCATTCTGCTGGG | PIGS-KO1-F | caccGCCGCGGTGGCCATTCTGCT | PIGS-KO1-R | aaacAGCAGAATGGCCACCGCGGC | hamaser |
| SLC35A2-KO2 | GTCATGGCTGAAGTGCTTAAAGG | SLC35A2-KO2-F | caccGTCATGGCTGAAGTGCTTAA | SLC35A2-KO2-R | aaacTTAAGCACTTCAGCCATGAC | hamaser |
| PGAP2-KO2 | GTCCCTGCTCTTCCACTTCAAGG | PGAP2-KO2-F | caccGTCCCTGCTCTTCCACTTCA | PGAP2-KO2-R | aaacTGAAGTGGAAGAGCAGGGAC | hamaser |
| PIGZ-KO1 | GATGGCAGCCAGGGTGATTTGGG | PIGZ-KO1-F | caccGATGGCAGCCAGGGTGATTT | PIGZ-KO1-R | aaacAAATCACCCTGGCTGCCATC | hamaser |

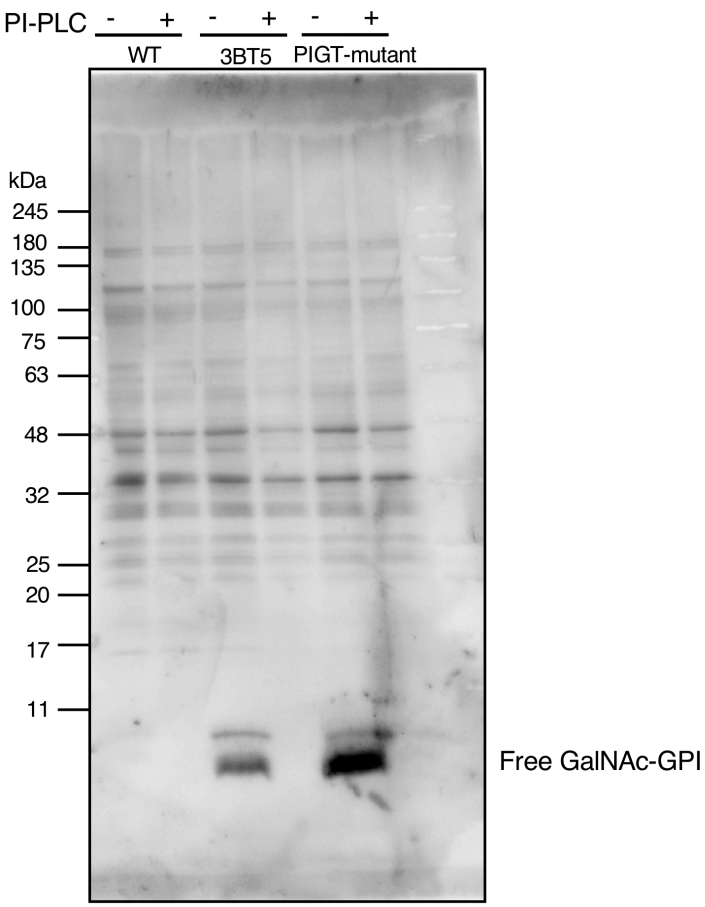


**Figure S1.** Unprocessed image of immunoblot using T5 mAb. Western blotting of free GPIs of 3B2A, 3BT5 and PIGT-mutant CHO cells. Lysates of WT (3B2A), 3BT5 and PIGT-mutant cells treated with or without PI-PLC were analyzed by western blotting with T5 mAb.

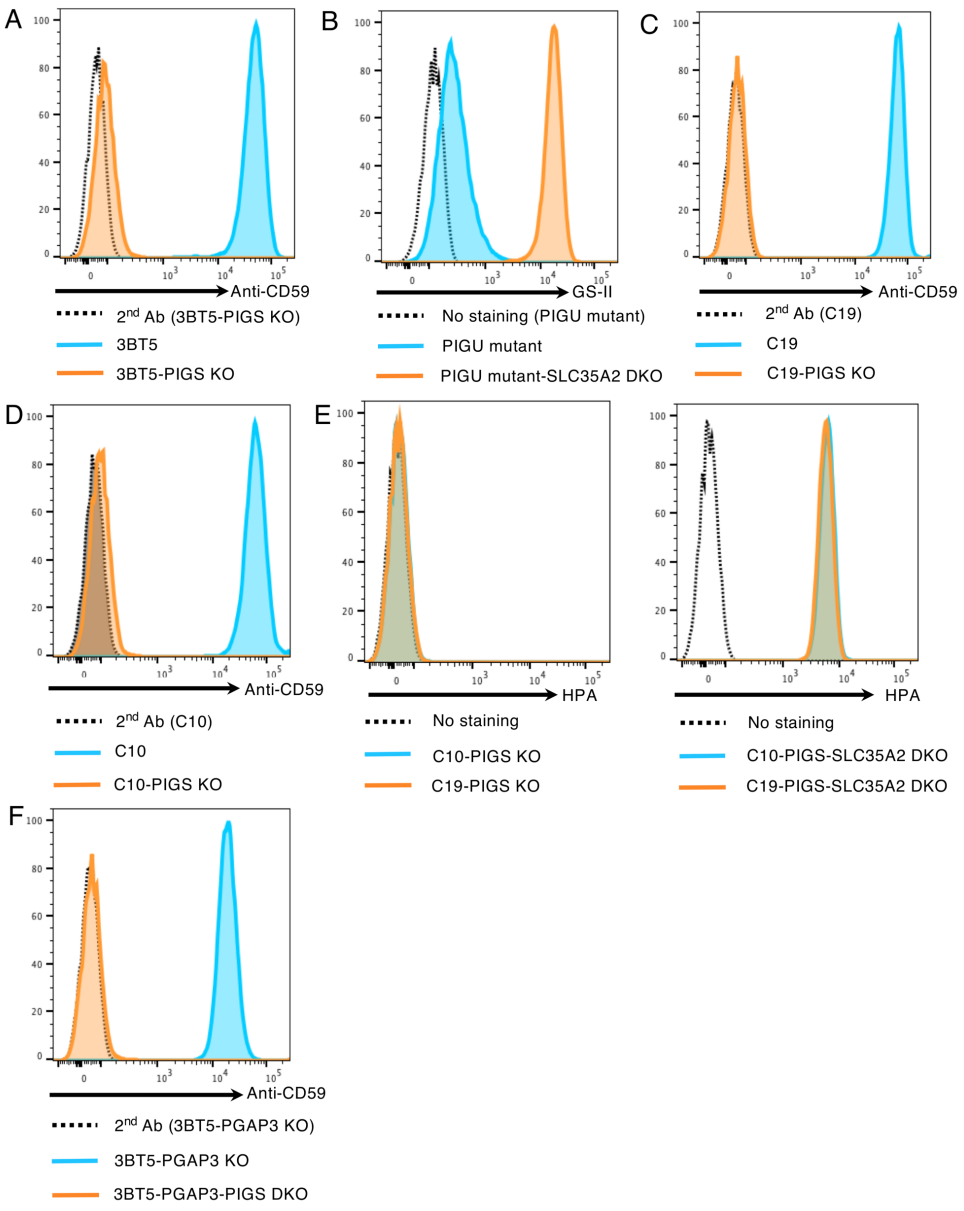


**Figure S2.** Confirmation of knockout (KO) of PIGS and SLC35A2. *(A)* Confirmation of KO of PIGS in CHO-3BT5 cells. 3BT5 and 3BT5-PIGS KO cells were stained with anti-CD59. *(B)* Confirmation of KO of SLC35A2 in PIGU-mutant CHO cells. PIGU-mutant and PIGU-mutant-SLC35A2 KO CHO cells were stained with lectin GS-II. *(C)* Confirmation of KO of PIGS in C19 cells. C19 and C19-PIGS KO cells were stained with anti-CD59. *(D)* Confirmation of KO of PIGS in C10 cells. C10 and C10-PIGS KO cells were stained with anti-CD59. *(E)* Confirmation of KO of SLC35A2 in C10-PIGS KO and C19-PIGS KO cells. C10-PIGS KO and C19-PIGS KO CHO cells (left), and C10-PIGS-SLC35A2 DKO and C19-PIGS-SLC35A2 DKO CHO cells (right) were stained with lectin HPA, which binds to GalNAc exposed *O*-glycan. *(F)* Confirmation of KO of PIGS in 3BT5-PGAP3 KO cells. 3BT5-PGAP3 KO and 3BT5-PGAP3-PIGS DKO cells were stained with anti-CD59.

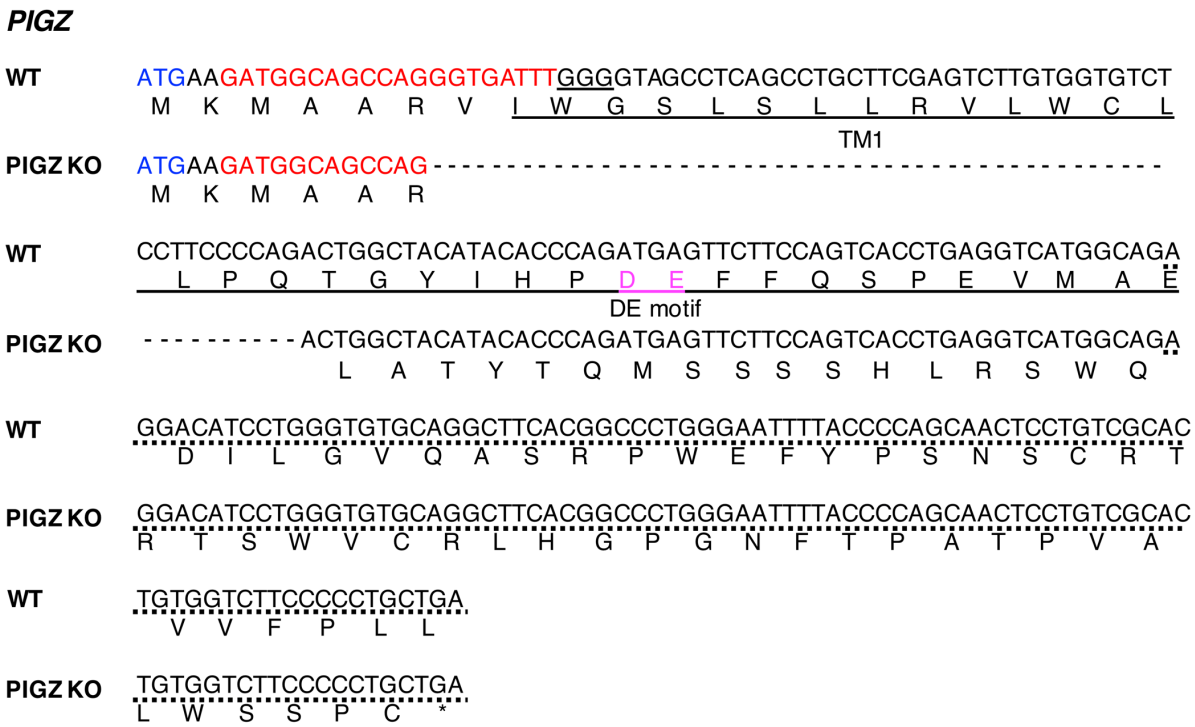


**Figure S3.** Confirmation of KO of PIGZ by Sanger sequencing. Nucleotide and translated amino acid sequences are aligned for wild-type PIGZ (WT) and gene-edited PIZG (PIGZ KO). Nucleotide sequences are shown from the initiation ATG codon (blue). Twenty nucleotides (red) located 3-nucleotide downstream of the initiation codon are targeted by gRNA. PAM sequence (GGG) is underlined. Sanger sequencing confirmed a deletion of 55 base pairs (dashed region) from within the gRNA target sequence, which caused a frame-shift leading to generation of a premature termination codon (asterisk). A putative catalytic site, DE motif (magenta), within the first transmembrane domain (TM1; underlined) was lost due to the frame-shift. Nucleotides with or without dotted underline are in exon 3 and exon 2, respectively. Exon 2 sequences were determined by Sanger method whereas exon 3 sequence was from the NCBI data base.