Table S1: KO target sequences

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|-------------------------------|--|-------------------------------|--|---------------------------------|--|--------------------|--|--|--|--|--|--|--|
| | Target sequence | Oligonucleotide | Sequence | Sequence | Species | | | | | | | | |
| SLC35A2- KO4 SLC35A2- | GGTGGTTCCACCGCGGCGCCCGG | SLC35A2- KO4-F SLC35A2- | caccGGTGGTTCCACCGCGGCGCC | SLC35A2- C KO4-R SLC35A2- | aaacGGCGCCGCGGTGGAACCACC | human | | | | | | | |
| KO1 PIGS-KO1 | GTGGATCTACCGCTGCGGCCGGG GCCGCGGTGGCCATTCTGCTGGG | KO1-F PIGS-KO1-F | caccGTGGATCTACCGCTGCGGCC caccGCCGCGGTGGCCATTCTGCT | C KO1-R C PIGS-KO1-R | aaacGGCCGCAGCGGTAGATCCAC aaacAGCAGAATGGCCACCGCGGC | mouse hamaser | | | | | | | |
| SLC35A2- KO2 | GTCATGGCTGAAGTGCTTAAAGC | SLC35A2- KO2-F | caccGTCATGGCTGAAGTGCTTA | SLC35A2- KO2-R | aaacTTAAGCACTTCAGCCATGAC | hamaser | | | | | | | |
| PGAP2-KO2 PIGZ-KO1 | GTCCCTGCTCTTCCACTTCAAGG GATGGCAGCCAGGGTGATTTGGC | PGAP2-KO2-F FIGZ-KO1-F | caccGTCCCTGCTCTTCCACTTCA caccGATGGCAGCCAGGGTGATT | PGAP2-KO2-R TPIGZ-KO1-R | aaacTGAAGTGGAAGAGCAGGGAC aaacAAATCACCCTGGCTGCCATC | hamaser 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 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.

| WТ | ATG/ M | ۹А <mark>G</mark> К | ATG M | i <mark>GC/</mark> A | AGC A | CAC | GGG [.] | TGA | | <u>GGC</u> N | <u>a</u> GT/ G | AGCO S | | AGC S | CTG L | CTTO | CGA R | GTC V | | GTG W | GTC C | ATCT |
|---------|-------------|------------------------|----------|-------------------------|------------------------|----------|-----------------------|----------|----------|-----------------|-------------------|-----------|-----------------------|------------|-----------------------|-------------|----------|----------|---------|----------|----------|-------------|
| | | | | | | | | | · | | | - | _ | - | - 1 | | | | | | | |
| PIGZ KO | ATG/ M | ۹А <mark>G</mark> К | ATG M | i <mark>GC/</mark> A | A <mark>GC</mark> A | CAC F |) { | | | | | | | | | | | | | | | |
| WT | CCTT L | rcco P | | GAC | TG(| GCT/ | ACA [.] Y | TAC I | ACC H | CAC P | GAT(| GAG | TTC ⁻ F | TTCC F | CAG [.] Q | TCA S | CCT P | GAG E | GT V | | GG | CAGA |
| | | | | | | | | | | | | | | | | | | | | | | |
| PIGZ KO | | | | - AC | TG | GCT | ACA [.] | ТАС | ACC | CAC | GAT | GAG | ттс | гтсо | CAG | TCA | сст | GAG | GT | САТ | GGG | CAGA |
| TIGE NO | | | | | L | А | Т | Y | Т | Q | М | S | S | S | S | Н | L | F | 8 3 | S | W | Q |
| WT | GGA D | CAT | CCT L | GG((| GTG G | TGC V | Q Q | GCT A | TCA S | CGC R | CCC P | CTG W | GGA E | ATT F | TTA(Y | CCC P | CAG S | CAA N | CT { | CCT S | GTC C | GCAC R T |
| PIGZ KO | GGA R | CAT T | CCT S | GG(W | GTG V | TGC C | AGC R | GCT L | TCA H | CGC C | àCCC à F | CTG | GGA | ATT N I | TTA | CCC(F F | CAG | CAA A | CT T | CCT P | GTC V | GCAC A |
| WT | TGT(V | GGT V | CTT F | CCC F | CC1 | IGC L | ГGA L | | | | | | | | | | | | | | | |
| PIGZ KO | TGTO L V | GGT V | CTT S | ccc s | CC1 P | rgc C | ГGA * | | | | | | | | | | | | | | | |

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.

PIGZ