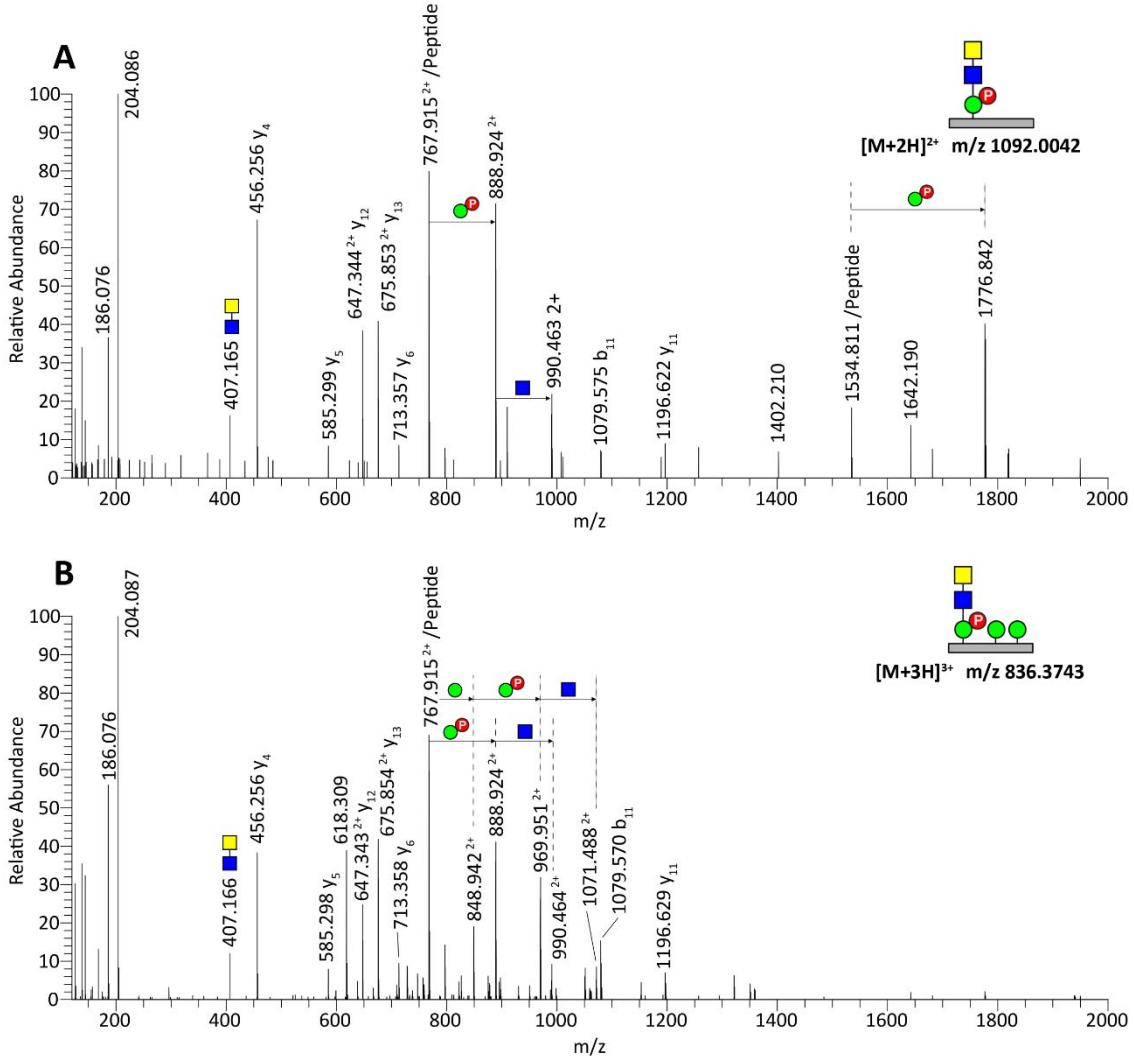
Supplemental Figs. 1-4

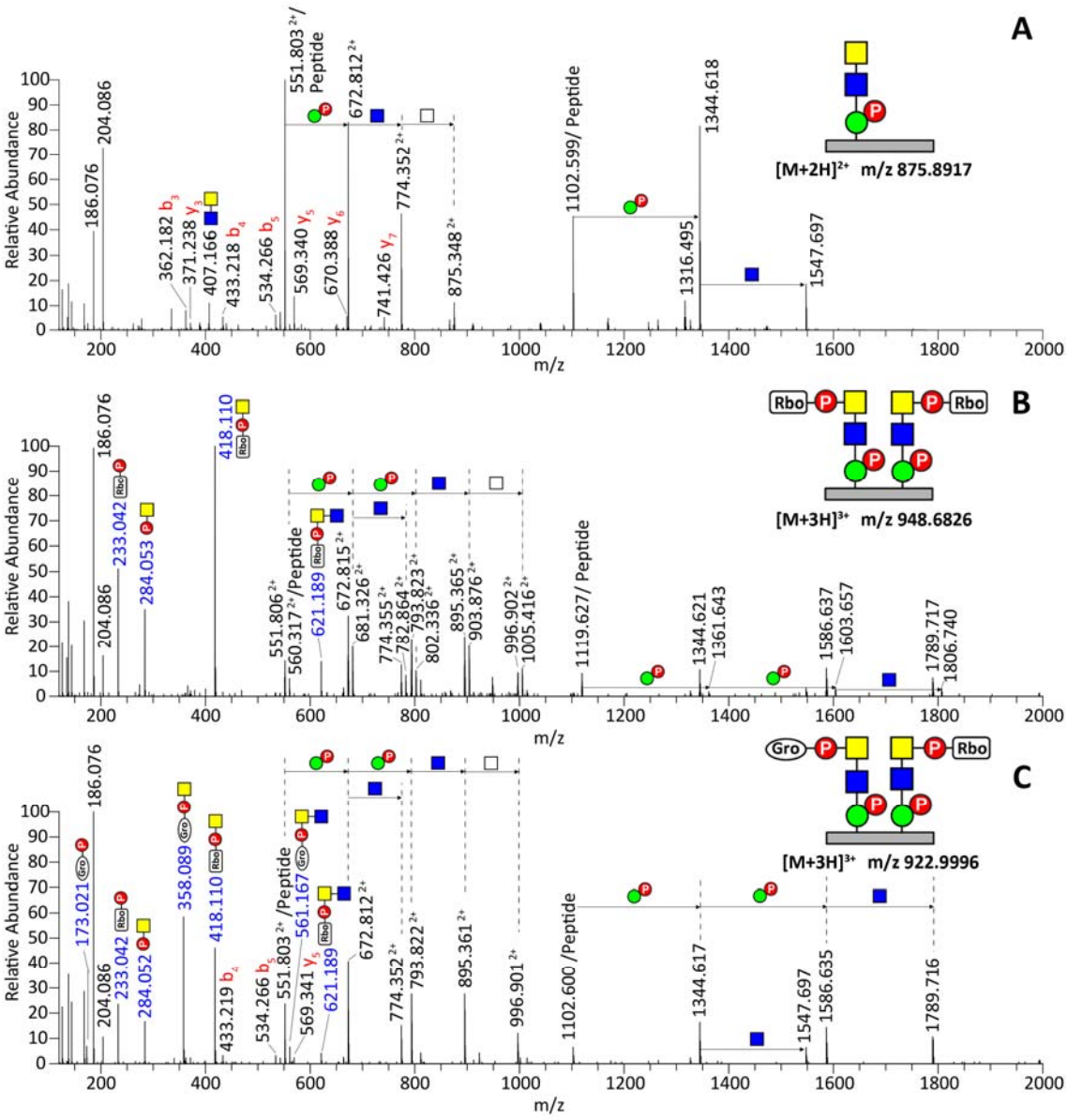
MRMSVGLSLL LPLWGRTFLL LLSVVMAQSH WPSEPSEAVR DWENQLEASM
HSVLSDLHEA VPTVVGIPDG TAVVGRSFRV TIPTDLIASS GDIIKVSAAG
KEALPSWLHW DSQSHTLEGL PLDTDKGVHY ISVSATRLGA NGSHIPQTSS
VFSIEVYPED HSELQSVRTA SPDPGEVVSS ACAADEPVTV LTVILDADLT
KMTPKQRIDL LHRMRSFSEV ELHNMKLVPV VNNRLFDMSA FMAGPGNAKK
VVENGALLSW KLGCSLNQNS VPDIHGVEAP AREGAMSAQL GYPVVGWHIA
NKKPPLPKRV RRQIHATPTP VRAIGPPTTA IQEPPSRIVP TPTSPAIAPP
TETMAPPVRD PVPGKPTVTI RTRRALEPKS CDKTHTCPPC PAPELLGGPS
VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT
KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN
NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK
SLSLSPGK

Supplemental Fig. S1 | Entire amino acid sequences of the N-domain (green), mucin domain (black), and Fc region (orange). The possible sites for displaying the laminin-binding glycans are shown in red. T322 was substituted with arginine shown in blue.



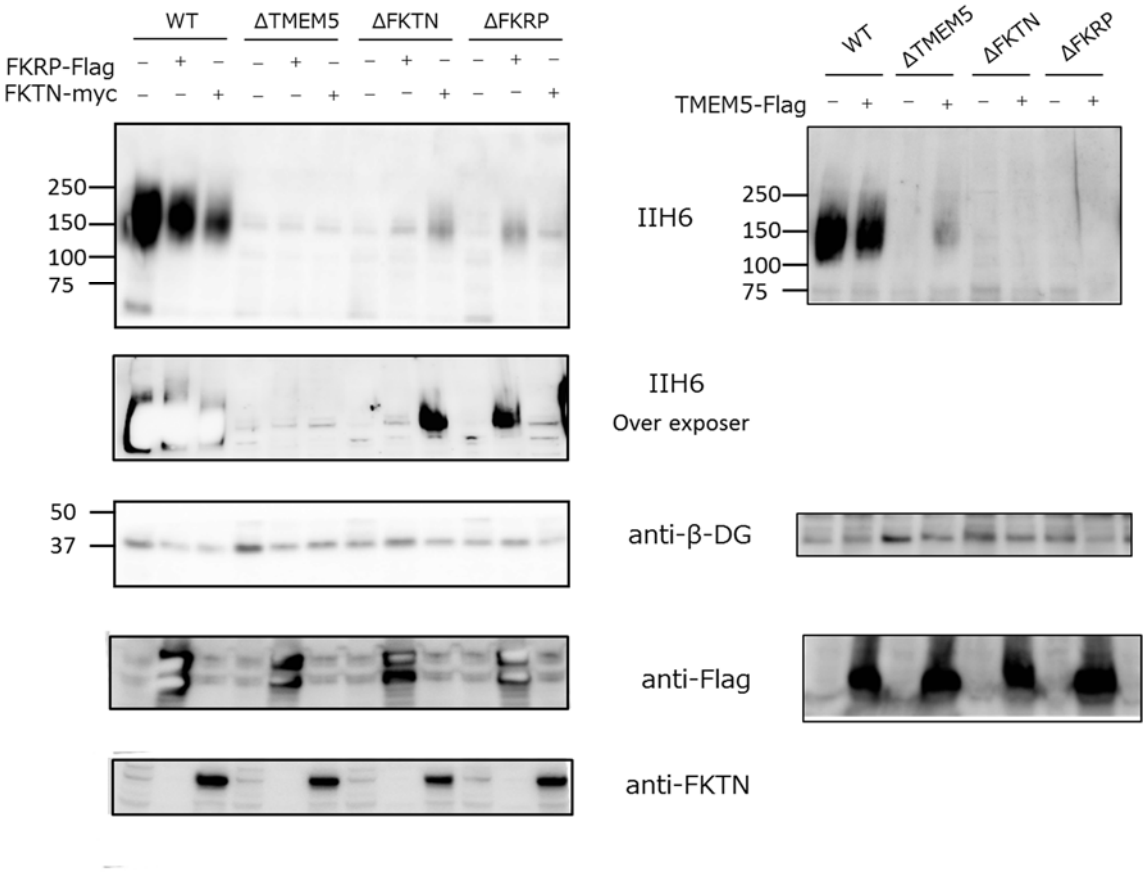
.

Supplemental Fig. S2 | Representative HCD MS^2 spectra of tryptic αDG373(T322R)-Fc glycopeptides, ^{323}AIGPPTTAIQEPPSR^{337}, carrying at least a phosphorylated core M3 with and without additional *O*-glycans. The presence of the core M3 is defined by detecting i) a $HexNAc_2$ oxonium ion at *m/z* 407.166 and ii) a phospho-Hex increment from the singly and/or doubly charged peptide core at *m/z* 1534.811 and/or 767.915^{2+}, respectively. Assignment and annotation are similar to those described for Fig. 2.



Supplemental Fig. S3 | HCD MS^2 spectra of tryptic αDG373(T322R)-Fc glycopeptides carrying only one phosphorylated core M3 (A), two phosphorylated core M3 moieties with two RboP (B), and two phosphorylated core M3 moieties with one RboP + one GroP (C). As shown in Fig. 2 and listed in Table S1, the presence of 1-2 phosphorylated core M3 along with other mucin-type *O*-glycans, with and without a single RboP or GroP substituent, was common on the ^{313}pyrQIHATPTPVR^{322} peptide derived from αDG373(T322R)-Fc expressed in HEK293T cells. These glycoforms were also commonly found on the same tryptic ^{313}pyrQIHATPTPVR^{322} peptide core derived from αDG373(T322R)-Fc expressed in HCT116 and its various mutants (Table S1, Fig. 7), which also produced a higher proportion of non-pyroglutamylated ^{313}QIHATPTPVR^{322} glycopeptides (peptide core at 560.317^{2+} or 1119.512^{1+}). In general, glycoforms found on the latter could also be identified

on the pyroglutamylated peptide counterparts, except for the rare combination of two RboP substituents detected only on non-pyroglutamylated peptide derived from HCT116 *Δ*FKRP mutant (B). No fragment ion could be found to support their occurrence as tandem RboP. Instead, these are most likely single RboP substituents on each of the phosphorylated core M3 structures. Similarly, the glycoforms with the combination of single RboP and single GroP could only be detected on the pyroglutamylated peptide derived from HCT116 *Δ*FKRP mutant, with no evidence for their occurrence in tandem (C). The assignment and annotation are similar to those described for Fig. 2.



Supplemental Fig. S4 | FKTN, FKRP, and TMEM5 are not functionally compensating one another. Wild-type HCT116 and its mutants (*Δ*FKTN, *Δ*FKRP, and *Δ*TMEM5) were transfected with or without FKTN-myc, FKRP-Flag, and TMEM-Flag expression vectors. WGA-enriched cell lysates were subjected to immunoblot analysis using IIH6 and anti-β-DG antibodies. Cell lysates were analyzed for the expression of FKTN-myc, FKRP-Flag, and TMEM-Flag by Western blots using anti-FKTN, anti-Flag, and anti-Flag antibodies.