Supplemental data

Suppl. Fig. 1. Treatment of HMW1 with PNGaseF. 25 μg samples of protein were incubated with or without 2 units of PNGaseF, and aliquots were resolved on an SDS-PAGE gel and stained with Coomassie blue. Fetuin was used as a positive control. Lane 1, fetuin; lane 2, fetuin plus PNGaseF; lane 3, HMW1; lane 4, HMW1 plus PNGase F.

Suppl. Fig. 2. Mass spectrum of the doubly charged modified peptide INITK. Panel A shows the acquired mass spectrum of the INITK peptide, and panel B shows the theoretical isotopic distribution of the INITK peptide.

Suppl. Fig. 3. GC-MS and GC-MS/MS data of the hexoses derived from the glycopeptide NVTNNNITSHK. Panel A shows the total ion chromatogram, and panels B and C show the GC-MS/MS spectrum of the glucose and mannose peaks, respectively. In the total ion chromatogram, the large peaks marked X are probably byproducts of the hydrolysis rather than carbohydrate peaks, since they do not match with any of the available standards.

Suppl. Fig. 4. HMW1 sequence. Modification sites are circled. Partial sequences that were not identified are underlined. The HMW1 sequence lacking the signal peptide starts with a proline at position 442.

Suppl. Fig. 5. The graphics and tables show all MS/MS spectra that carry at least one 162 modification. To indicate the location of the modification, the amino acid carrying the modification has been underlined in the case of a single modification and underlined and bolded for a double modification. The tables list the theoretical fragment ion masses (calculated with MS-Product in ProteinProspector version 4.27.2 basic). Note that tables do not contain fragment ion masses involving neutral losses. The Roepstorff nomenclature for peptide fragment ions is used (a-, b-, y-ions with or without water/ammonia loss). Internal fragment ions are shown and a charge state up to +3 is considered. One hexose modification on an Asn site is defined as “u” in the sequence and has the elemental composition of C10 H16 N2 O7. Two hexose modifications on one Asn site are defined as “v” (C16 H26 N2 O12).
The order of peptides is the same as in Table 1 in the published article.