Supplemental Data

Moss-Produced, Glycosylation-Optimized Human Factor H for Therapeutic Application in Complement Disorders

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SUPPLEMENTARY TABLE 1: Data are presented of a representative purification process. FHmoss was quantified by ELISA.

Step	Volume [ml]	FH [µg/ml]	FH [mg]	FH step recovery [%]	FH total recovery [%]
Starting material (*Physcomitrella* culture)	2000	9	18		
Concentration/Dialfiltration	354	38	13.5	75	
Phenyl pool	140	41	5.74	43	
Heparin load	1026	5	4.75	83	
Heparin pool	44	60	2.63	55	15
Heparin pool concentrated	0.25	9141	2.29	87	13
Heparin pool concentrated (0.22µm filtered)	0.18	8758	1.58	69	9

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MAFYKISSVF FIFCFFLIAL PFHSYAEDC NELPPRRNTE ILTGSWSDQTY
PEGTQAIYKC RPGYRSLGNV IMVCRKGEWV ALNPLRKCQK RPCGHPGDTP
FGTFTLTGGN VFEYGVKAVY TCNEGYQLLG EINYRECDTD GWTNDIPICE
VVKCLPVTAP ENGKIVSSAM EPDREYHFGQ AVRFVCNSGY KIEGDEEMHC
SDDGFWSKEK PKCVEISCKS PDVIDGSPIS QKIIYKENER FQYKCNMGYE
YSERGDAVCT ESGWRPLPSC EEKSCDNPYI PNGDYSPLRI KHRTGDEITY
QCRNGFYPAT RGNTAKCTST GWIPAPRCTL KPCDYPDIKH GGLYHENMRR
PYFPVAVGKY YSYYCDEHFE TPSGSYWDHI HCTQDGWSPA VPCLRKCYFP
YLENGYNQNY GRKFVQGKSI DVACHPGYALP KAQTTVTCM ENGWSPTPRC
IRVKTCSKSS IDIENGFISE SQYTYALKEK AKYQCKLGYV TADGETSGSI
TCGKDGWSAQ PTCIKSCDIP VFMNARTKND FTWFKLNDTL DYECHDGYES
NTGSTTGSIV CGYNGWSDLP ICYERECELP KIDVHLVPDR KKDQYKVGEV
LKFSCKPGFT IVGPNSVQCY HFGLSPDLPI CKEQVQSCGP PPELLNGNVK
EKTKEEYGHS EVVEYYCNPR FLMKGPNKIQ CVDGEWTTLP VCIVEESTCG
DIPELEHGWA QLSSPPYYYG DSVEFNCSES FTMIGHRSIT CIHGVWTQLP
QCVAIDKLKK CKSSNLIILE EHLKNKKEFD HNSNIRYRCR GKEGWIHTVC
INGRWDPEVN CSMAQIQLCP PPPQIPNSHN MTTTLNYRDG EKVSVLCQEN
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HGTKLSYTCE GGFRISEENE TTCYMGKWSS PPQCEGLPCK SPPEISHGVV
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CRDTSCVNPP TVQNAYIVSR QMSKYPSGER VRYQCRSPYE MFGDEEVMCL
NGNWTEPPQC KDSTGKCGPP PPIDNGDITS FPLSVYAPAS SVEYQCQNLY
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TGESVEFVCK RGYRLSSRSH TLRTTCWDGK LEYPTCAKR
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B

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MAFYKISSVF FIFCFFLIAL PFHSYAEDC NELPPRRNTE ILTGSWSDQTY
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FGTFTLTGGN VFEYGVKAVY TCNEGYQLLG EINYRECDTD GWTNDIPICE
VVKCLPVTAP ENGKIVSSAM EPDREYHFGQ AVRFVCNSGY KIEGDEEMHC
SDDGFWSKEK PKCVEISCKS PDVIDGSPIS QKIIYKENER FQYKCNMGYE
YSERGDAVCT ESGWRPLPSC EEKSCDNPYI PNGDYSPLRI KHRTGDEITY
QCRNGFYPAT RGNTAKCTST GWIPAPRCTL KPCDYPDIKH GGLYHENMRR
PYFPVAVGKY YSYYCDEHFE TPSGSYWDHI HCTQDGWSPA VPCLRKCYFP
YLENGYNQNY GRKFVQGKSI DVACHPGYALP KAQTTVTCM ENGWSPTPRC
IRVKTCSKSS IDIENGFISE SQYTYALKEK AKYQCKLGYV TADGETSGSI
TCGKDGWSAQ PTCIKSCDIP VFMNARTKND FTWFKLNDTL DYECHDGYES
NTGSTTGSIV CGYNGWSDLP ICYERECELP KIDVHLVPDR KKDQYKVGEV
LKFSCKPGFT IVGPNSVQCY HFGLSPDLPI CKEQVQSCGP PPELLNGNVK
EKTKEEYGHS EVVEYYCNPR FLMKGPNKIQ CVDGEWTTLP VCIVEESTCG
DIPELEHGWA QLSSPPYYYG DSVEFNCSES FTMIGHRSIT CIHGVWTQLP
QCVAIDKLKK CKSSNLIILE EHLKNKKEFD HNSNIRYRCR GKEGWIHTVC
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PSFENAIPMG EKKDVYKAGE QVTYTCATYY KMDGASNVTC INSRWTGRPT
CRDTSCVNPP TVQNAYIVSR QMSKYPSGER VRYQCRSPYE MFGDEEVMCL
NGNWTEPPQC KDSTGKCGPP PPIDNGDITS FPLSVYAPAS SVEYQCQNLY
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TGESVEFVCK RGYRLSSRSH TLRTTCWDGK LEYPTCAKR
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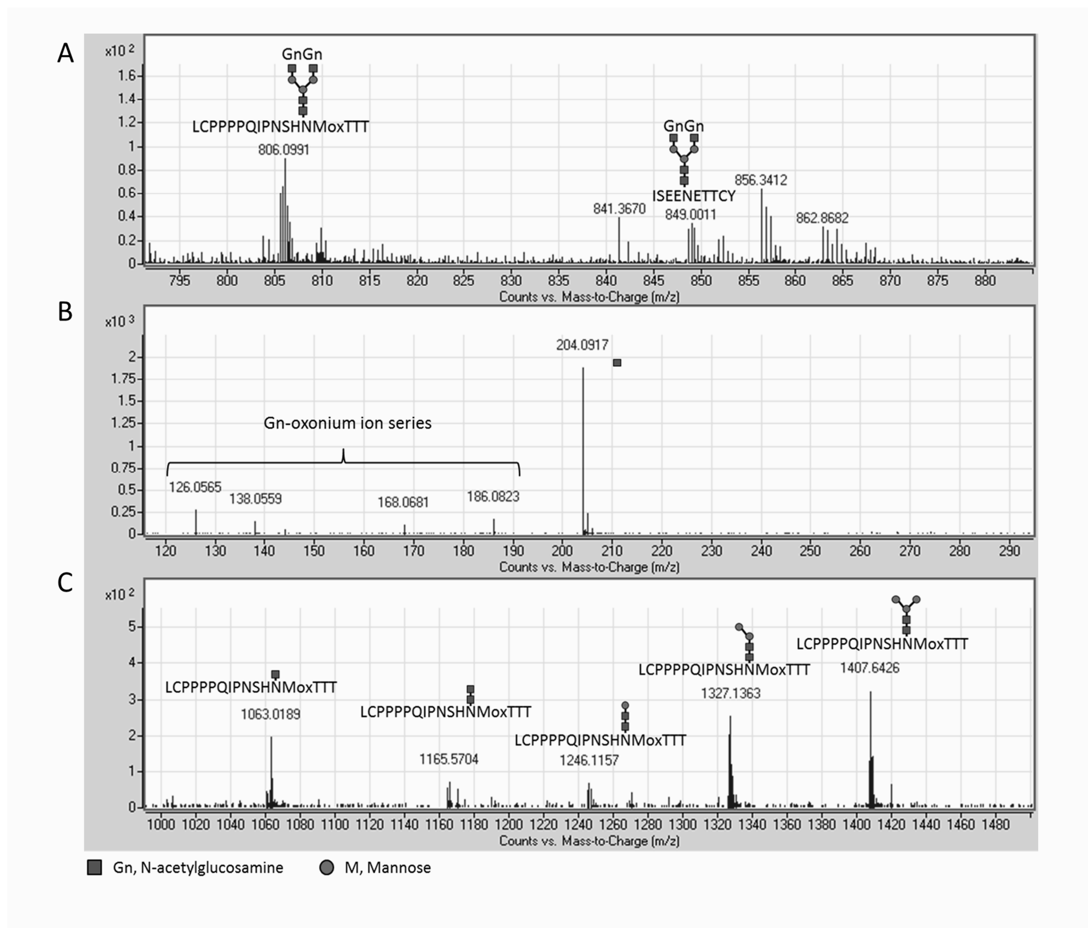
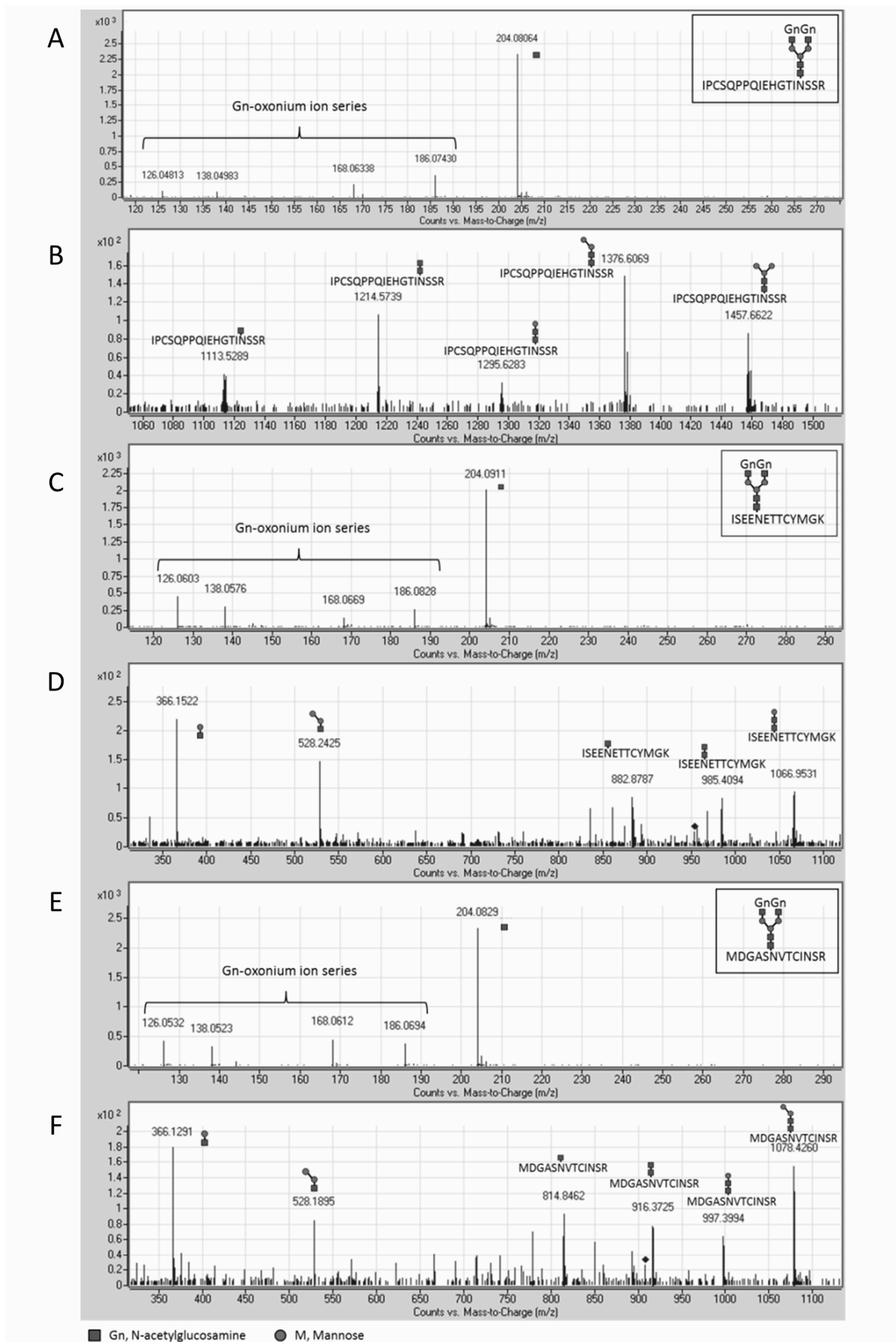
Supplementary Figure 1. Mass spectrometric detected sequence coverage of FHmoss. Shown is the mature FH sequence (black) fused to the *ToH1*-signal peptide (grey). Identified peptides are highlighted, N-glycosylation sites, including the deamidated site Asn199→Asp199, are underlined. FHmoss from the upper and the lower FH band was separately digested with trypsin, thermolysin or chymotrypsin, respectively and analyzed by nano LC-MS/MS on a Q-TOF instrument. The analyses of the raw data were performed with Mascot Distiller V2.4 and glycosylated peptides were identified manually. The highlighted peptides were identified with high confidence at a false discovery level of 0% on the protein as well as on the peptide level according to Protein- and PeptideProphet™ filtering with Scaffold4 software with a sequence coverage of 74 % for the upper FH band (A) and a sequence coverage of 57 % for the lower FH band (B).

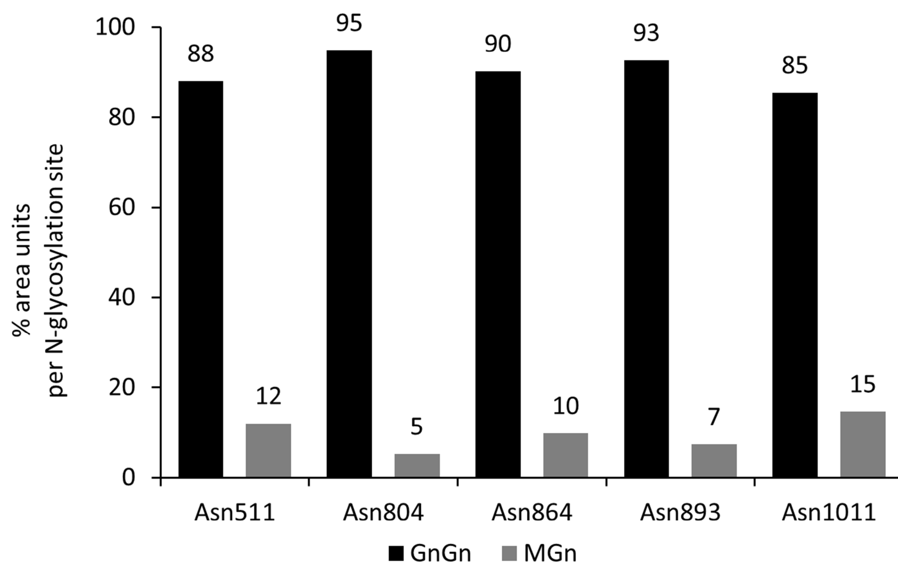


Supplementary Figure 2. Summed MS1 scans showing the glycosylated Asn804- and Asn893 glycosylation sites of FHmoss (A) and the proof of the Asn804 glycopeptide identity on MS2-level (B and C). FHmoss was digested with thermolysin and analyzed by nano LC-MS/MS on a Q-TOF instrument. (A) Among the identified peptides, the glycopeptide 792-808 (LCPPPPQIPNSHNMTTT, glycosylation site Asn804) with an oxidized methionin (Mox) and the glycopeptide 889-901 (ISEENETTCY, glycosylation site Asn893) were identified in a GnGn-glycosylated manner. (B and C) The glycopeptide identity of the GnGn-LCPPPPQIPNSHNMTTT glycopeptide could be proven on MS2 level by the identification of the N-glycan reporter ion N-acetylglucosamine (Gn, m/z= 204.0872) and its oxonium ions with m/z= 186.0766, 168.0661, 138.0555 and 126.0555 (B) and by the detection of m/z-values of product ions consisting of fragmented glycan structures attached to the intact peptide (C). Shown m/z values correspond to the most abundant isotope peak for each peptide. The monoisotopic masses for the identified glycopeptides are listed in table 1.

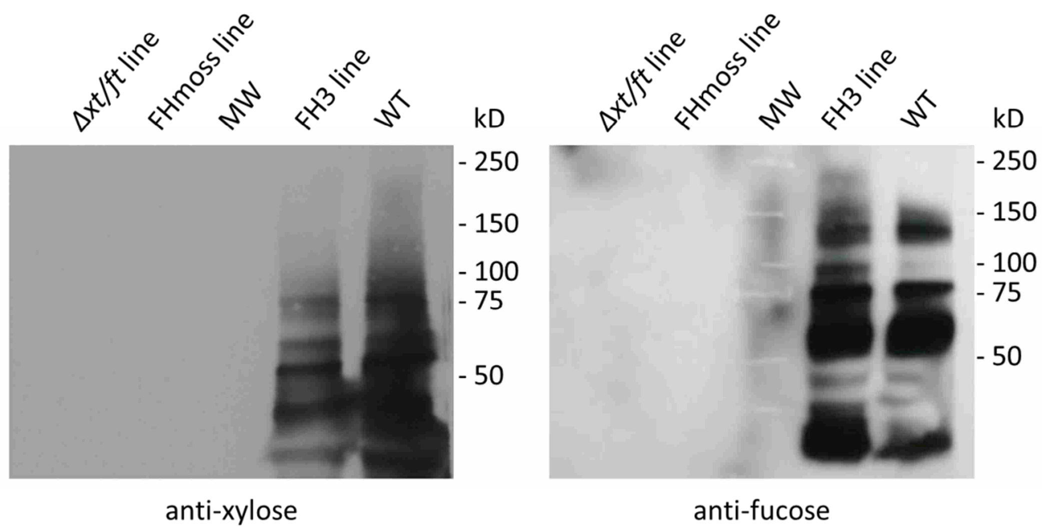


Supplementary Figure 3. MS2 spectra of detected N-glycosylation sites of FHmoss. FHmoss was tryptically digested and analyzed by nano LC-MS/MS on a Q-TOF instrument. Among the identified peptides, the glycopeptides 850-867 (IPCSQPPQIEHGTINSSR, glycosylation site Asn864) (A and B), 889-901 (ISEENETTCYMGK, glycosylation site Asn893) (C and D) and 1006-1018 (MDGASNVTCINSR, glycosylation site Asn1011) (E, F) were identified and proven by

MS2 spectra inspection. For each glycopeptide the presence of the N-glycan reporter ion N-acetylglucosamine (Gn, m/z= 204.0872) and its oxonium ions with m/z= 186.0766, 168.0661, 138.0555 and 126.0555 could be shown on MS2 level (A, C and E). Further, also N-glycan product ions consisting of fragmented glycan structures and glycan fragments attached to the intact peptide could be identified (B, D and F). Shown m/z values correspond to the most abundant isotope peak for each peptide. The monoisotopic masses for the identified glycopeptides are listed in table 1.



Supplementary Figure 4. Relative quantification of N-glycans identified on FHmoss. FHmoss was digested with trypsin, thermolysin and chymotrypsin, respectively and analyzed by nano LC-MS/MS on a Q-TOF instrument. Relative quantification is based in peak area integration of extracted ion chromatograms (EICs) for every on MS1 and/or in MS2 level identified N-glycan form at the respective glycosylation site.



Supplementary Figure 5. FHmoss is devoid of plant-specific xylose and fucose. Western blots of total protein extracts of FHmoss-producing line, in which fucosyl- and xylosyltransferases have been knocked-out, and previous generation plant FH3[38], with intact glycosyltransferases, and the respective parental plants *Δxt/ft* and WT. (A) Western blot using antibodies specifically recognizing β1,2-linked xylose. (B) Western blot using antibodies specificallyrecognizing α1,3-linked fucose.