

Figure S1. MS<sup>E</sup> spectrum of SWT7 derived HA glycopeptide, *m/z* 3858.62 [M+H]+ (Hex<sub>8</sub>HexNAc<sub>2</sub>)LYQNPTTYISVGTSTLNQR, containing site 4. The colors in the figure indicate the following: red-y ions; blue-b ions; green-y/b ions after neutral loss; gray-unassigned ions by the Biopharmalynx. Monosaccharide symbols: , N-acetylglucosamine; , mannose. Peptide fragments with glycosyl neutral loss are indicated.



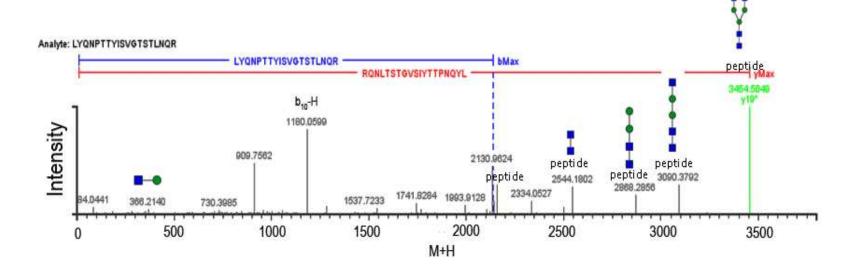


Figure S2. MS<sup>E</sup> spectrum of SWT7 derived HA glycopeptide, *m/z* 3454.50 [M+H]+ (Hex<sub>3</sub>HexNAc<sub>4</sub>)LYQNPTTYISVGTSTLNQR, containing site 4. The colors in the figure indicate the following: red, y ions; blue, b ions; green, y/b ions after neutral loss; gray-unassigned ions by the Biopharmalynx or manually assigned as indicated. Monosaccharide symbols: , N-acetylglucosamine;

• , mannose. Peptide fragments with glycosyl neutral loss are indicated.



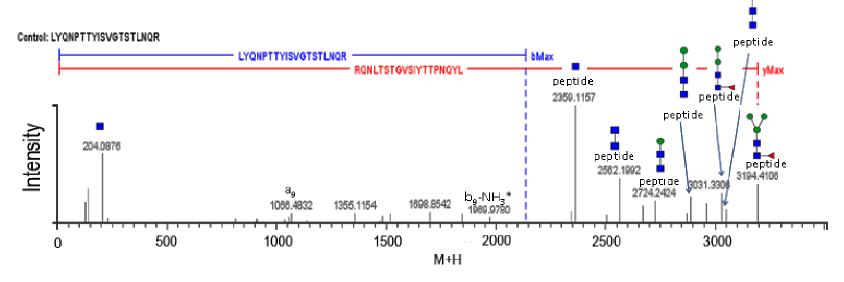


Figure S3. MS<sup>E</sup> spectrum of HighFive derived HA glycopeptide, *m/z* 3194.41 [M+H]+ (dHex<sub>1</sub>dHex<sub>3</sub>HexNAc<sub>2</sub>)LYQNPTTYISVGTSTLNQR, containing site 4. The colors in the figure indicate the following: red, y ions; blue, b ions; green, y/b ions after neutral loss; gray, unassigned ions by the Biopharmalynx or manually assigned as indicated.

Monosaccharide symbols: N-acetylglucosamine; , mannose; , fucose. Peptide fragments with glycosyl neutral loss are indicated.

## LYQNPTTYISVGTSTLNQR

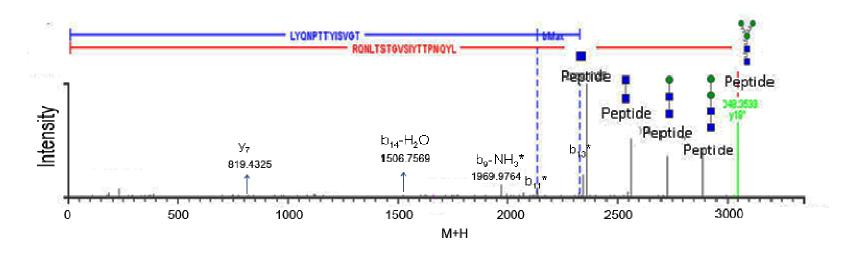


Figure S4. MS<sup>E</sup> spectrum of Sf9 derived HA glycopeptide, *m/z* 3048.35 [M+H]+ (Hex<sub>3</sub>HexNAc<sub>2</sub>)LYQNPTTYISVGTSTLNQR, containing site 4. The colors in the figure indicate the following: red, y ions; blue, b ions; green, y/b ions after neutral loss; gray, are unassigned ions by the Biopharmalynx or assigned as indicated. Monosaccharide symbols: , N-acetylglucosamine; , mannose. Peptide fragments with glycosyl neutral loss are indicated.

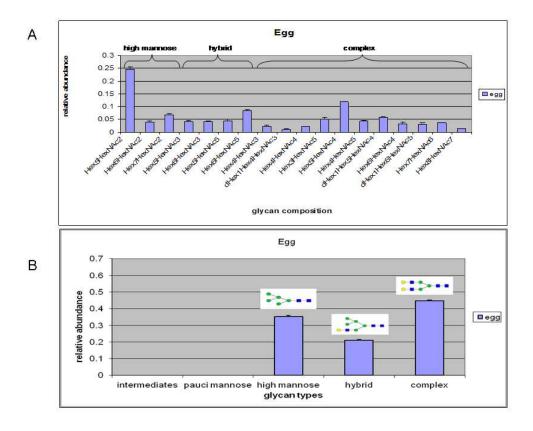


Figure S5. MALDI-TOF MS profile histogram of the egg derived HA released and permethylated glycans. Histograms are grouped by *N*-glycan subtype in A. The subtypes are summed in B for comparison. The inferred composition of the most abundant glycoforms within each sub-group is shown. The summed *N*-glycan subtypes from all cell substrates are shown as a three dimensional plot in

Figure S10. The inferred composition of the most abundant glycoforms within each subgroup is shown. Measurements were performed in triplicate and error bars represent one standard deviation.

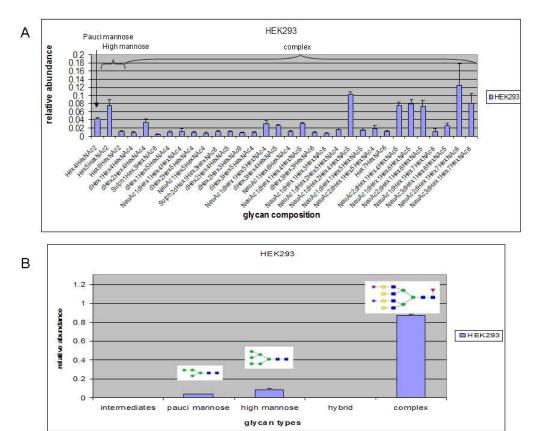


Figure S6. MALDI-TOF MS profile histogram of the HEK293 cell derived HA released and permethylated glycans. Histograms are grouped by *N*-glycan subtype in A. The subtypes are summed in B for comparison. The inferred composition of the most abundant glycoforms within each sub-group is shown. The summed *N*-glycan subtypes from all cell substrates are shown as a three dimensional plot in Figure S10. The inferred composition of the most abundant glycoforms within each sub-group is shown.

Measurements were performed in triplicate and error bars represent one standard deviation.

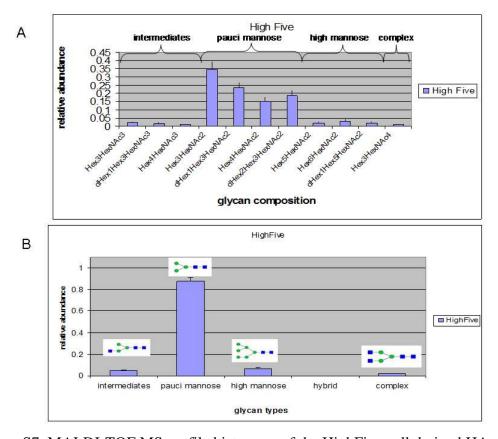


Figure S7. MALDI-TOF MS profile histogram of the HighFive cell derived HA released and permethylated glycans. Histograms are grouped by *N*-glycan subtype in A. The subtypes are summed in B for comparison. The inferred composition of the most abundant glycoforms within each sub-group is shown. The summed *N*-glycan subtypes from all cell substrates are shown as a three dimensional plot in Figure S10. Measurements were performed in triplicate and error bars represent one standard deviation.

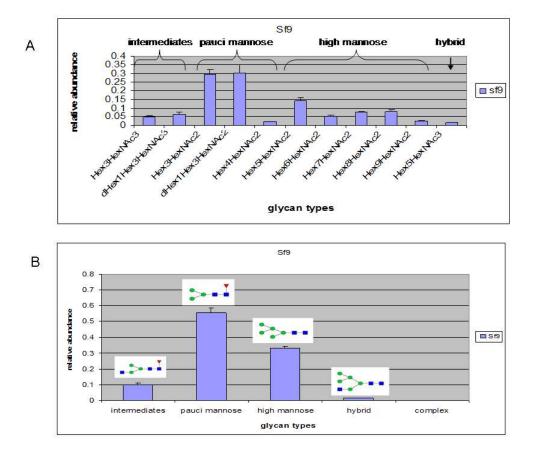


Figure S8. MALDI-TOF MS profile histogram of the Sf9 cell derived HA released and permethylated glycans. Histograms are grouped by *N*-glycan subtype in A. The subtypes are summed in B for comparison. The inferred composition of the most abundant glycoforms within each sub-group is shown. The summed *N*-glycan subtypes from all cell substrates are shown as a three dimensional plot in Figure S10. Measurements were performed in triplicate and error bars represent one standard deviation.

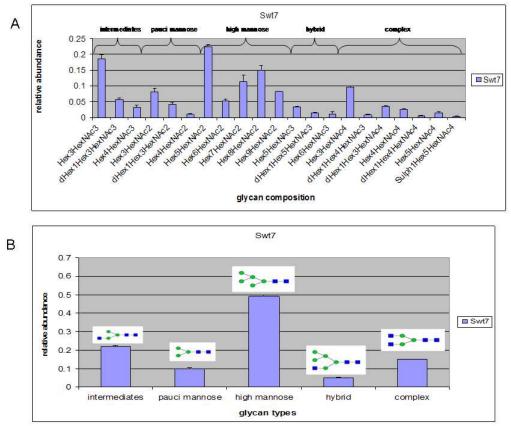


Figure S9. MALDI-TOF MS profile histogram of the SWT7 cell derived HA released and permethylated glycans. Histograms are grouped by *N*-glycan subtype in A. The subtypes are summed in B for comparison. The inferred composition of the most abundant glycoforms within each sub-group is shown. The summed *N*-glycan subtypes from all cell substrates are shown as a three dimensional plot in Figure S10. Measurements were performed in triplicate and error bars represent one standard deviation.

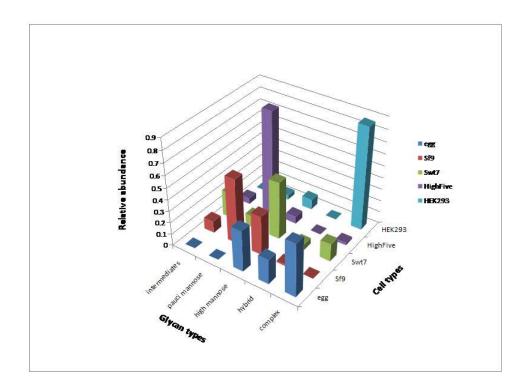


Figure S10. *N*-glycan abundances within subgroups. *N*-glycans present in intermediate, pauci mannose, high mannose, hybrid and complex subgroups are shown for HAs derived from each cell platform.