**Supplementary Figure Legends**

**S-Figure 1.** Experimental flowchart for the profiling and quantitation of N- and O-glycans in membrane fractions of hESCs.

**S-Figure 2.** MALDI-FT ICR mass spectra of glycans released from membrane fraction of hESCs under the presence of EDTA and SDS. Only polymer peaks with non-carbon isotopic distribution were observed instead of glycan peaks.

**S-Figure 3.** SDS-PAGE electrophoresis of membrane fractions followed by Western blotting. The membrane fractions contained minimal contaminants from the ER, nucleus, and cytosol.

**S-Figure 4.** Gene Ontology analysis of the H1 stem cell membrane fraction using DAVID.

**S-Figure 5.** Structure elucidation of N-glycans found on hESC membrane by tandem mass spectrometry using IRMPD. (A) A high mannose N-glycan (m/z 2068). (B) A complex (m/z 2013).

**S-Figure 6.** Representative MALDI-FTICR mass spectra of O-glycans found in undifferentiated hESCs membrane in the positive detection ion mode. O-glycans are marked with a filled circle (●).

**S-Figure 7.** Extracted ion chromatogram of high mannose type glycans found in H1 hESC. Left panel shows GlcNAc$_2$Man$_7$ (Man7) isomers and right panel shows GlcNAc$_2$Man$_8$ (Man8) isomers.

**S-Figure 8.** (A) Analytical reproducibility on the same sample process. Triplicate MS analyses were performed for each sample. Three experiments were plotted against each other and the respective correlation coefficient R is inset. (B) Biological reproducibility of hESC surface glycan. Bio-triplicates of HSF-6 hESCs were independently prepared and surface glycans were released and compared for the reproducibility. Only glycan peaks detected by MALDI-FTICR MS were
used for scatter plots and the respective correlation coefficient R is inset. NAPI represents normalized absolute peak intensity.

**S-Figure 9.** Cell surface glycan analysis using flow cytometry. To determine the presence of glycans, live hESCs labeled with antibody against SSEA-4 (stem cell marker), propidium iodide (P.I., to detect dead cells), lectins Con A (to detect N-glycans), GNA (to detect terminal high mannose), and Jacalin (JAC; to detect O-linked glycans) conjugated to FITC. The mean fluorescence intensity (M.F.I.) represents the quantification of live hESCs (P.I. negative and SSEA-4 positive) labeled with different concentrations (5, 10, 20 40 μg/ml) of lectins with or without inhibitory control (“+ I”).
Supplementary Figure 1.
Supplementary Figure 2.

\[ \Delta m = 160 \]

\[ \Delta m = 2 \]
Supplementary Figure 3.

<table>
<thead>
<tr>
<th>Whole cell lysate (control)</th>
<th>Membrane fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>150 kD —</td>
<td>CD49b (plasma membrane)</td>
</tr>
<tr>
<td>78 —</td>
<td>BiP/GRP78 (endoplasmic reticulum)</td>
</tr>
<tr>
<td>62 —</td>
<td>Nuclear pore complex (nucleus)</td>
</tr>
<tr>
<td>50 —</td>
<td>α- tubulin (cytosol)</td>
</tr>
</tbody>
</table>

Supplementary Figure 4.
Supplementary Figure 5.

A

B
contamination by N-glycans
Supplementary Figure 7.
Supplementary Figure 8.

A

1 vs 2 replicates

1 vs 3 replicates

2 vs 3 replicates

B

\( r = 0.96 \)

\( r = 0.96 \)

\( r = 0.97 \)

1 vs 2 replicates

1 vs 3 replicates

2 vs 3 replicates

\( \mu \) and \( \varphi \)
Supplementary Figure 9.