

## **Captions for supplementary datasets**

**Dataset S1. List of peptides identified with Orbitrap Fusion Lumos mass spectrometer for the three different S proteins.** Tabs with peptide groups, as well as PSMs are included for each different protein and digestion approach (i1 – monomeric RBD expressed in insect cells, i3 – ectodomain expressed in insect cells, h3m – ectodomain expressed in HEK 293F cells, Chy – chymotrypsin, GluTry – Glu-C followed by trypsin). Identified O-glycosites were validated by manual inspection of relevant spectra, and positions indicated at “Manual validation of PTM assignment” columns.

**Dataset S2. List of peptides identified with Q Exactive HF-X mass spectrometer for S protein expressed in HEK 293F cells.**

**Dataset S3. Quantification of O-glycosite occupancy for Orbitrap Fusion Lumos derived data for insect RBD.**

**Dataset S4. Quantification of O-glycosite occupancy for Orbitrap Fusion Lumos derived data for insect ectodomain.**

**Dataset S5. Quantification of O-glycosite occupancy for Orbitrap Fusion Lumos derived data for HEK 293F ectodomain.**

**Dataset S6. Quantification of O-glycosite occupancy for Q Exactive HF-X derived data.**

**Dataset S7. Examples of HCD MS2 spectra representing the different (glyco)peptides identified using the Q Exactive HF-X mass spectrometer.** The zipped file contains spectra of O-glycosylated peptides included in occupancy quantification (Figure 3b and Dataset S6) of data derived from the Q Exactive HF-X experiments. Examples of non-O-glycosylated peptide spectra are also included. The naming of the files follows the following format O-glycosite position-number-Y/N, where the number indicates the order number in Dataset S6, and the Y/N informs, whether the peptide is O-glycosylated (Y - yes), or not (N - no).