## Supplementary Information

## Comprehensive characterization of N- and O- glycosylation of SARS-CoV-2 human receptor angiotensin converting enzyme 2.

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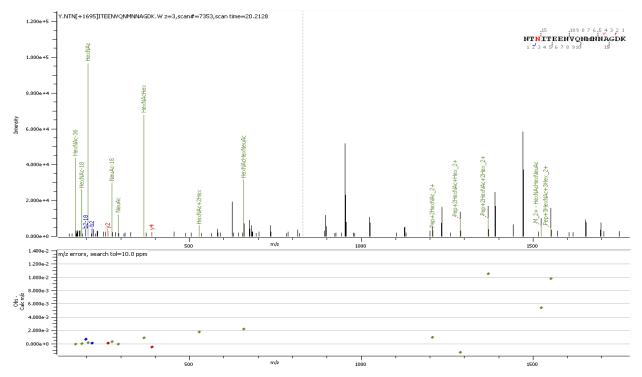
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Keywords: COVID-19 receptor, ACE2 glycosylation, SARS-CoV-2 receptor, ACE2 site mapping, hACE2 N-glycosylation.

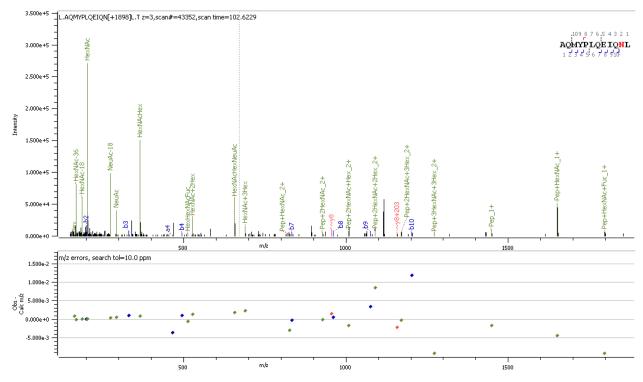
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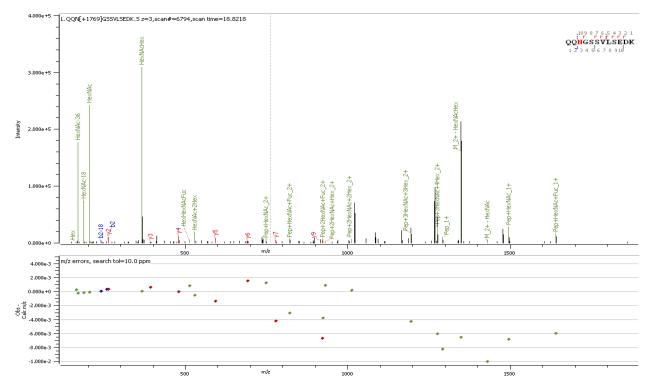
## **Glycopeptide Analysis:**



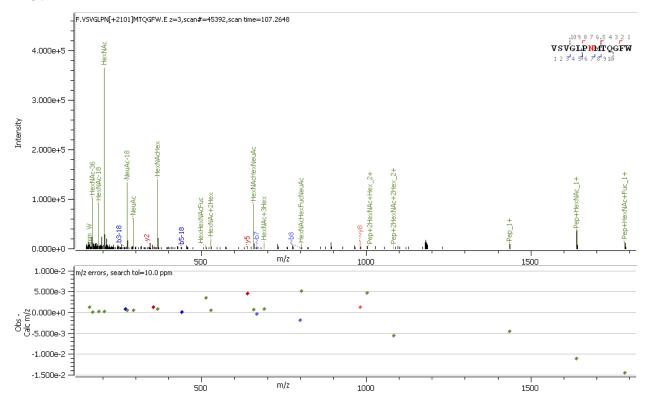
**Supp. Figure S1:** Representative HCD MS/MS spectrum of intact N-glycopeptide with assigned N-glycan (GlcNAc<sub>2</sub>Fuc<sub>1</sub>Man<sub>3</sub>GlcNAc<sub>1</sub>Gal<sub>1</sub>NeuAc<sub>1</sub>) at N53



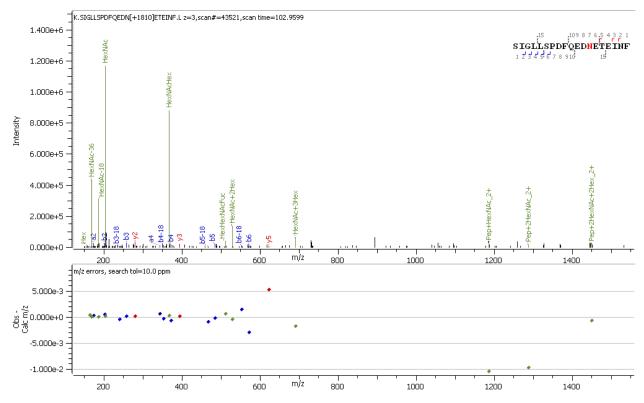
**Supp. Figure S2:** Representative HCD MS/MS spectrum of intact N-glycopeptide with assigned N-glycan (GlcNAc<sub>2</sub>Fuc<sub>1</sub>Man<sub>3</sub>GlcNAc<sub>2</sub>Gal<sub>1</sub>NeuAc<sub>1</sub>) at N90



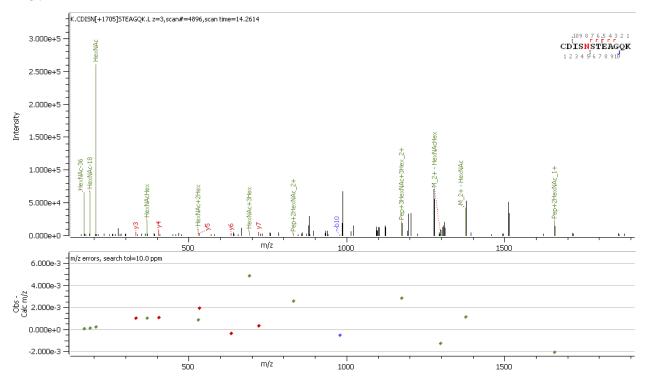
**Supp. Figure S3:** Representative HCD MS/MS spectrum of intact N-glycopeptide with assigned N-glycan (GlcNAc<sub>2</sub>Fuc<sub>1</sub>Man<sub>3</sub>GlcNAc<sub>2</sub>Gal<sub>2</sub>) at N103



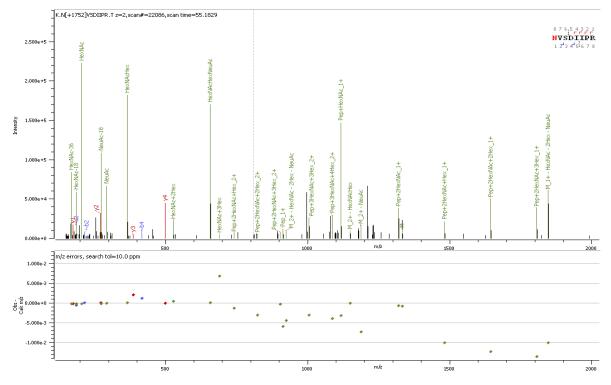
**Supp. Figure S4:** Representative HCD MS/MS spectrum of intact N-glycopeptide with assigned N-glycan (GlcNAc<sub>2</sub>Fuc<sub>1</sub>Man<sub>3</sub>GlcNAc<sub>3</sub>Gal<sub>1</sub>NeuAc<sub>1</sub>) at N322



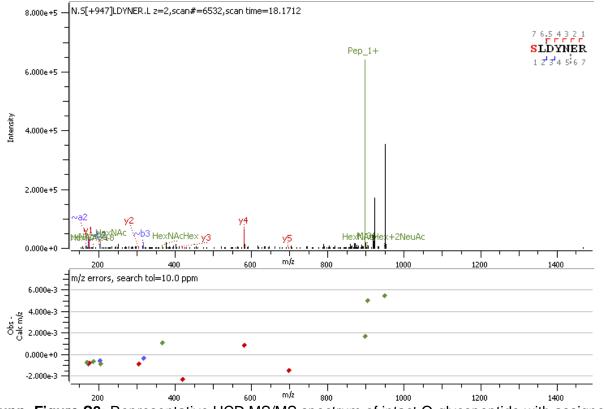
**Supp. Figure S5:.** Representative HCD MS/MS spectrum of intact N-glycopeptide with assigned N-glycan (GlcNAc<sub>2</sub>Fuc<sub>1</sub>Man<sub>3</sub>GlcNAc<sub>3</sub>Gal<sub>1</sub>) at N432



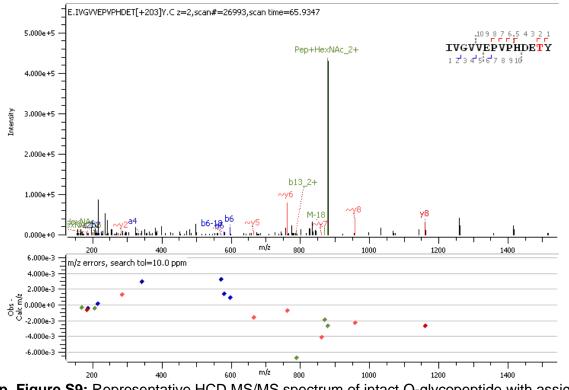
**Supp. Figure S6:** Representative HCD MS/MS spectrum of intact N-glycopeptide with assigned N-glycan (GlcNAc<sub>2</sub>Man<sub>3</sub>GlcNAc<sub>4</sub>) at N546



**Supp. Figure S7:** Representative HCD MS/MS spectrum of intact N-glycopeptide with assigned N-glycan (GlcNAc<sub>2</sub>Man<sub>3</sub>GlcNAc<sub>2</sub>Gal<sub>1</sub>NeuAc<sub>1</sub>) at N690

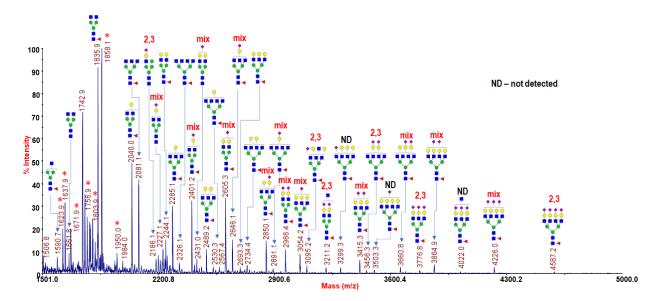


**Supp. Figure S8:** Representative HCD MS/MS spectrum of intact O-glycopeptide with assigned O-glycan (GalNAcGalNeuAc<sub>2</sub>) at O155.

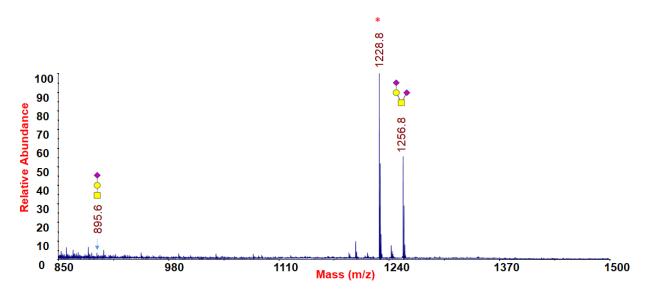


**Supp. Figure S9:** Representative HCD MS/MS spectrum of intact O-glycopeptide with assigned O-glycan (HexNAc) at O496.

**Glycomics Analysis:** 



**Supp. Figure S10:** MALDI-MS spectrum showing the permethylated N-glycans released from hACE2 by PNGase F along with sialic acid linkages (determined by ESI-MS<sup>n</sup>).



Supp. Figure S11: MALDI-MS spectrum showing the permethylated O-glycans released from hACE2 by  $\beta$ -elimination.