Cell Host & Microbe, Volume *28*

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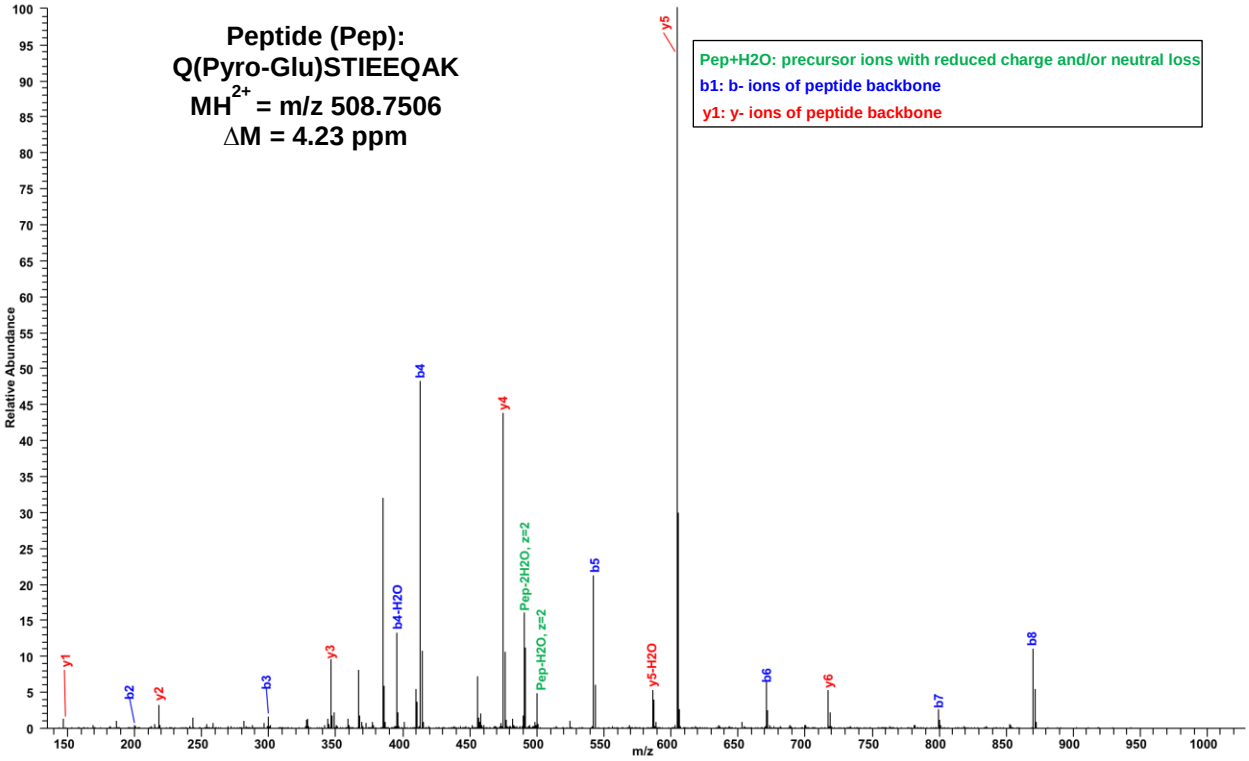
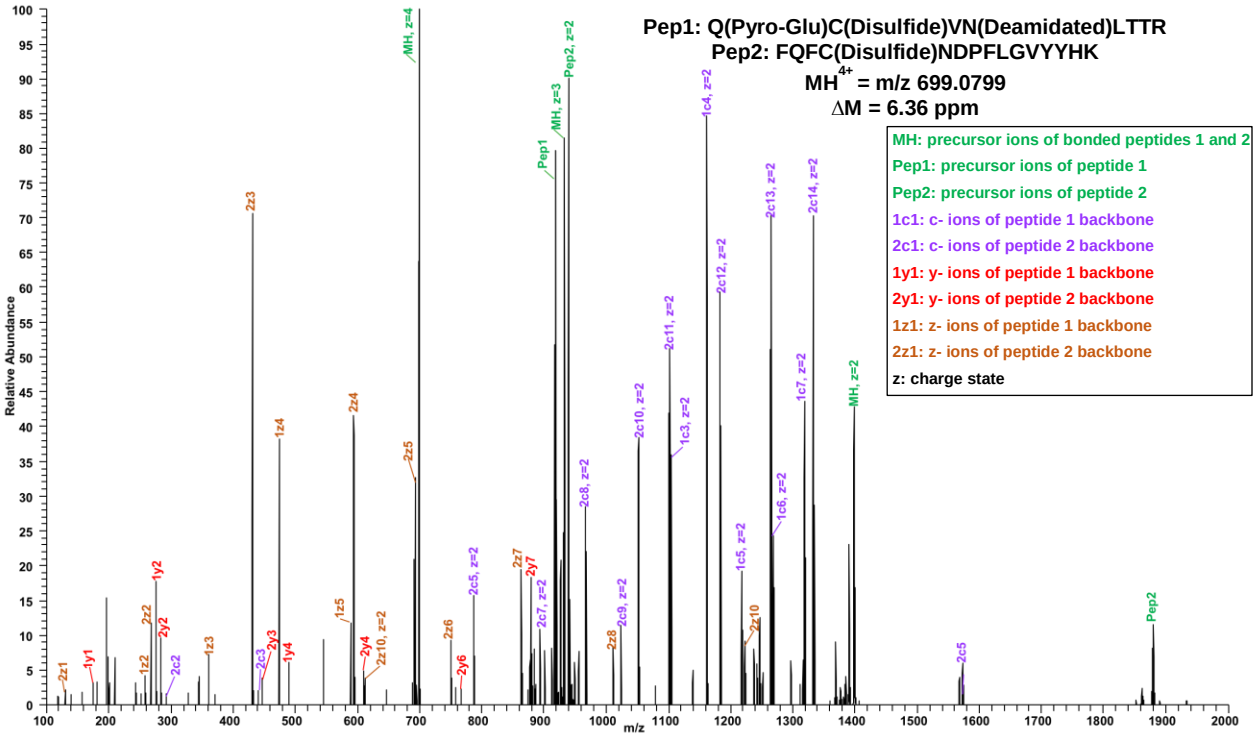

Peptide (Pep):
Q(Pyro-Glu)STIEEQAK
MH2+ = m/z 508.7506
ΔM = 4.23 ppm
Pep+H2O: precursor ions with reduced charge and/or neutral loss
b1: b- ions of peptide backbone
y1: y- ions of peptide backbone
Relative Abundance
m/z
y1
b2
y2
b3
y3
b4-H2O
b4
y4
Pep-2H2O, z=2
Pep-H2O, z=2
b5
y5-H2O
y5
b6
y6
b7
b8

Supplemental Figure S1. Defining N-terminus of ACE2 as pyro-glutamine at site Q0018.

Related to Figure 1. Representative HCD MS2 spectrum shown.

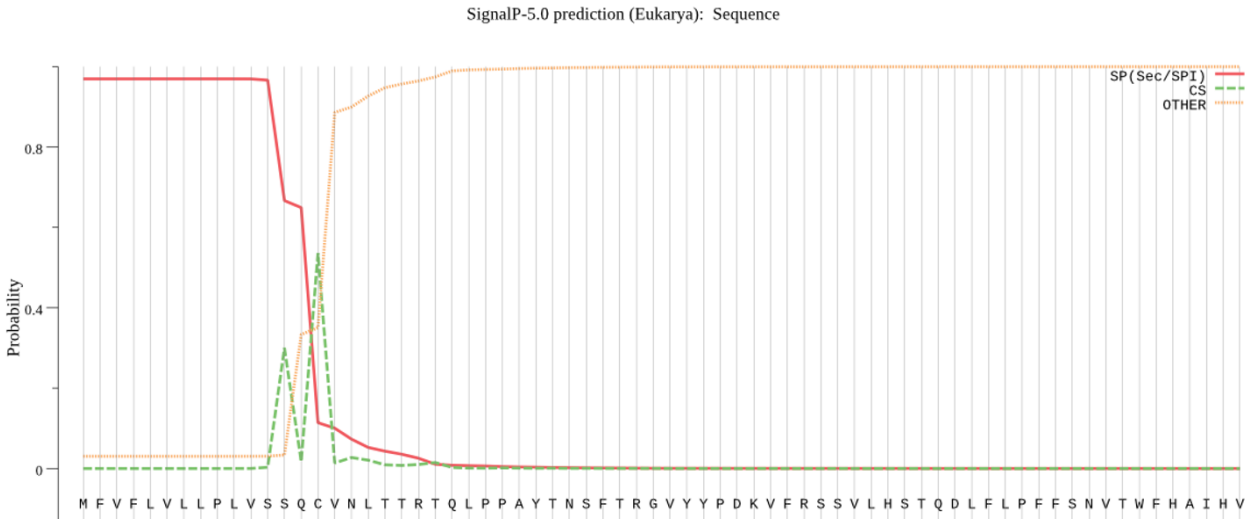

Pep1: Q(Pyro-Glu)C(Disulfide)VN(Deamidated)LTTR
Pep2: FQFC(Disulfide)NDPFLGVYYHK
MH4+ = m/z 699.0799
ΔM = 6.36 ppm
MH: precursor ions of bonded peptides 1 and 2
Pep1: precursor ions of peptide 1
Pep2: precursor ions of peptide 2
1c1: c- ions of peptide 1 backbone
2c1: c- ions of peptide 2 backbone
1y1: y- ions of peptide 1 backbone
2y1: y- ions of peptide 2 backbone
1z1: z- ions of peptide 1 backbone
2z1: z- ions of peptide 2 backbone
z: charge state
Relative Abundance
m/z

Supplemental Figure S2. Disulfide bond formed between Cysteines 0015 and 0136 of SARS-CoV-2 S.

Related to Figure 1. Representative EThcD MS2 spectrum shown.

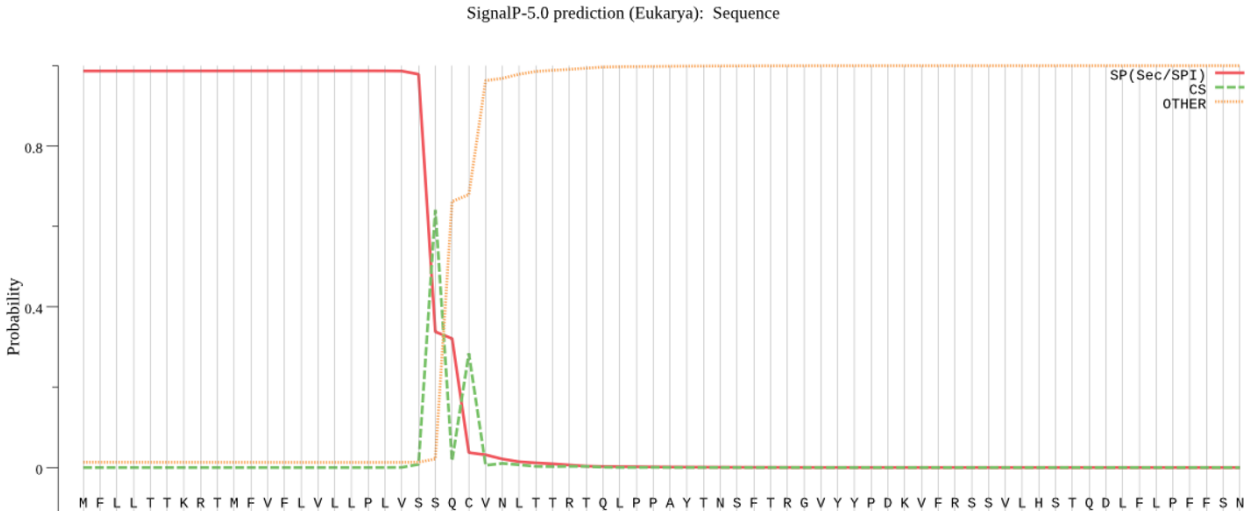
Utilized Start Met: MFVFLVLLPLVSSQCVNL…

Signal P: SSQC-VN (.54)



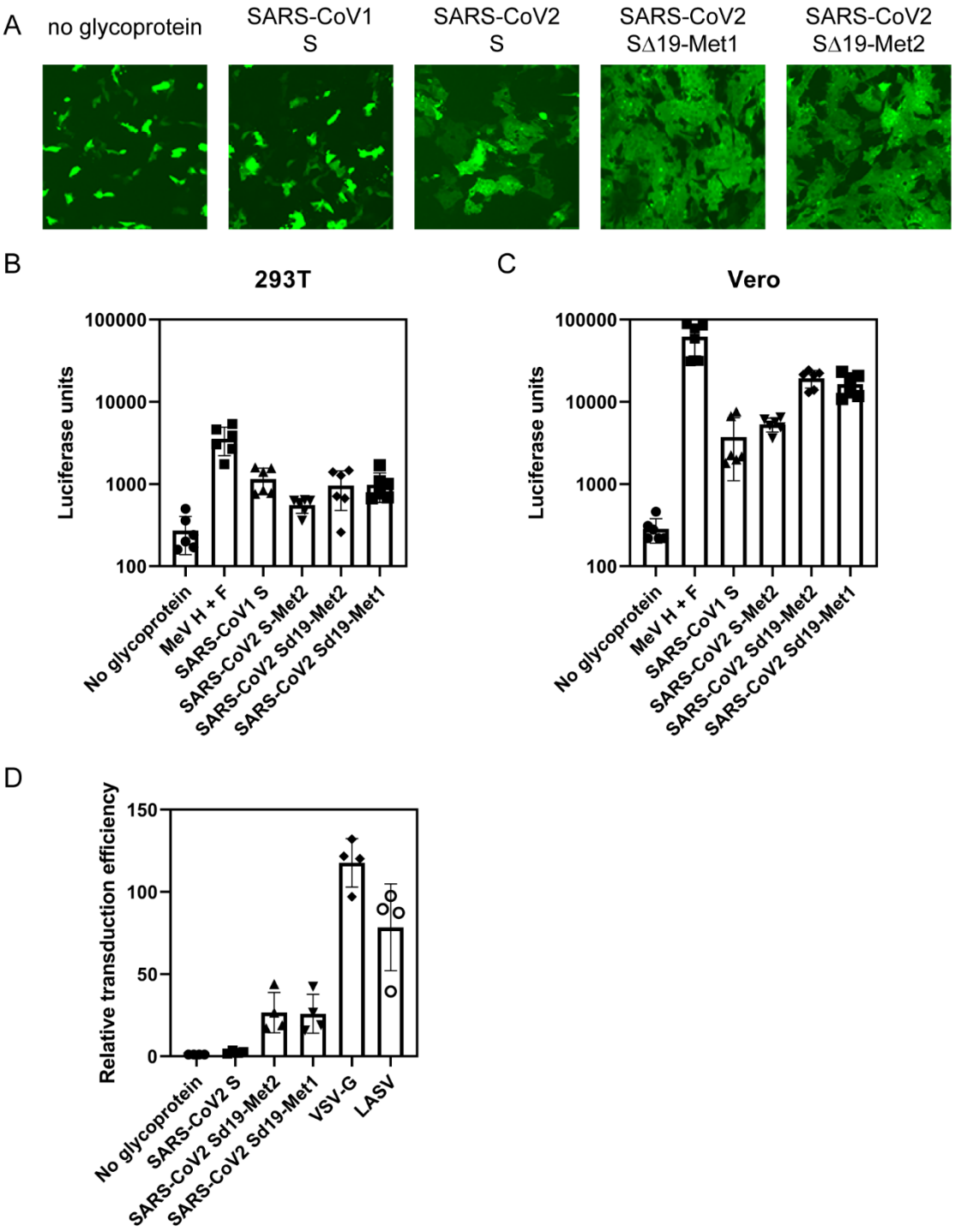
Upstream Met: MFLLTTKRT**M**FVFLVLLPLVSSQCVNL…

Signal P: SS-QCVN (.64)



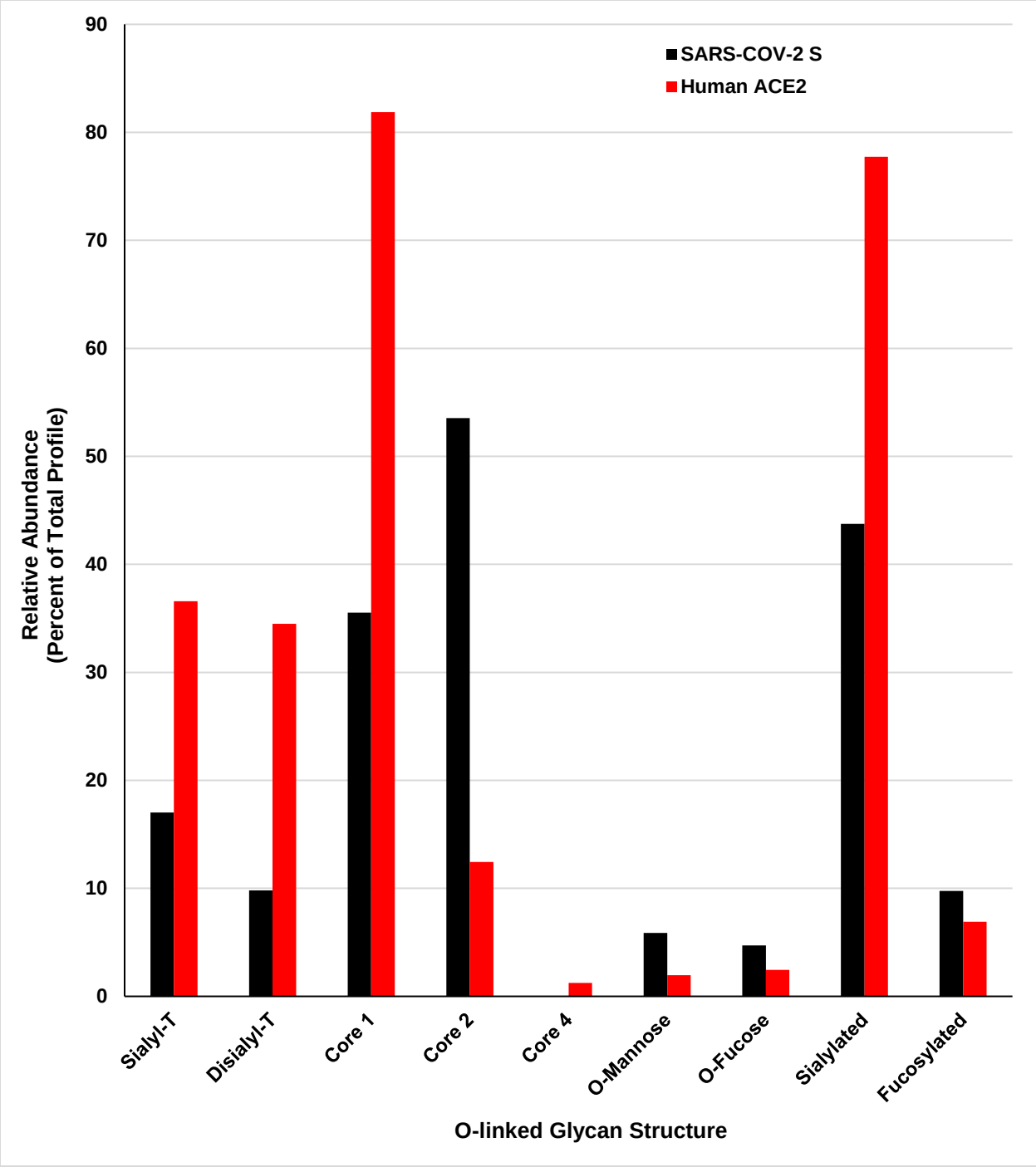
Supplemental Figure S3. Signal P prediction of two different start methionines for SARS-CoV-2 S.

Related to Figure 1.


A
no glycoprotein
SARS-CoV1 S
SARS-CoV2 S
SARS-CoV2 SΔ19-Met1
SARS-CoV2 SΔ19-Met2
B
293T
Luciferase units
100000
10000
1000
100
No glycoprotein
MeV H + F
SARS-CoV1 S
SARS-CoV2 S-Met2
SARS-CoV2 Sd19-Met2
SARS-CoV2 Sd19-Met1
C
Vero
Luciferase units
100000
10000
1000
100
No glycoprotein
MeV H + F
SARS-CoV1 S
SARS-CoV2 S-Met2
SARS-CoV2 Sd19-Met2
SARS-CoV2 Sd19-Met1
D
Relative transduction efficiency
150
100
50
0
No glycoprotein
SARS-CoV2 S
SARS-CoV2 Sd19-Met2
SARS-CoV2 Sd19-Met1
VSV-G
LASV

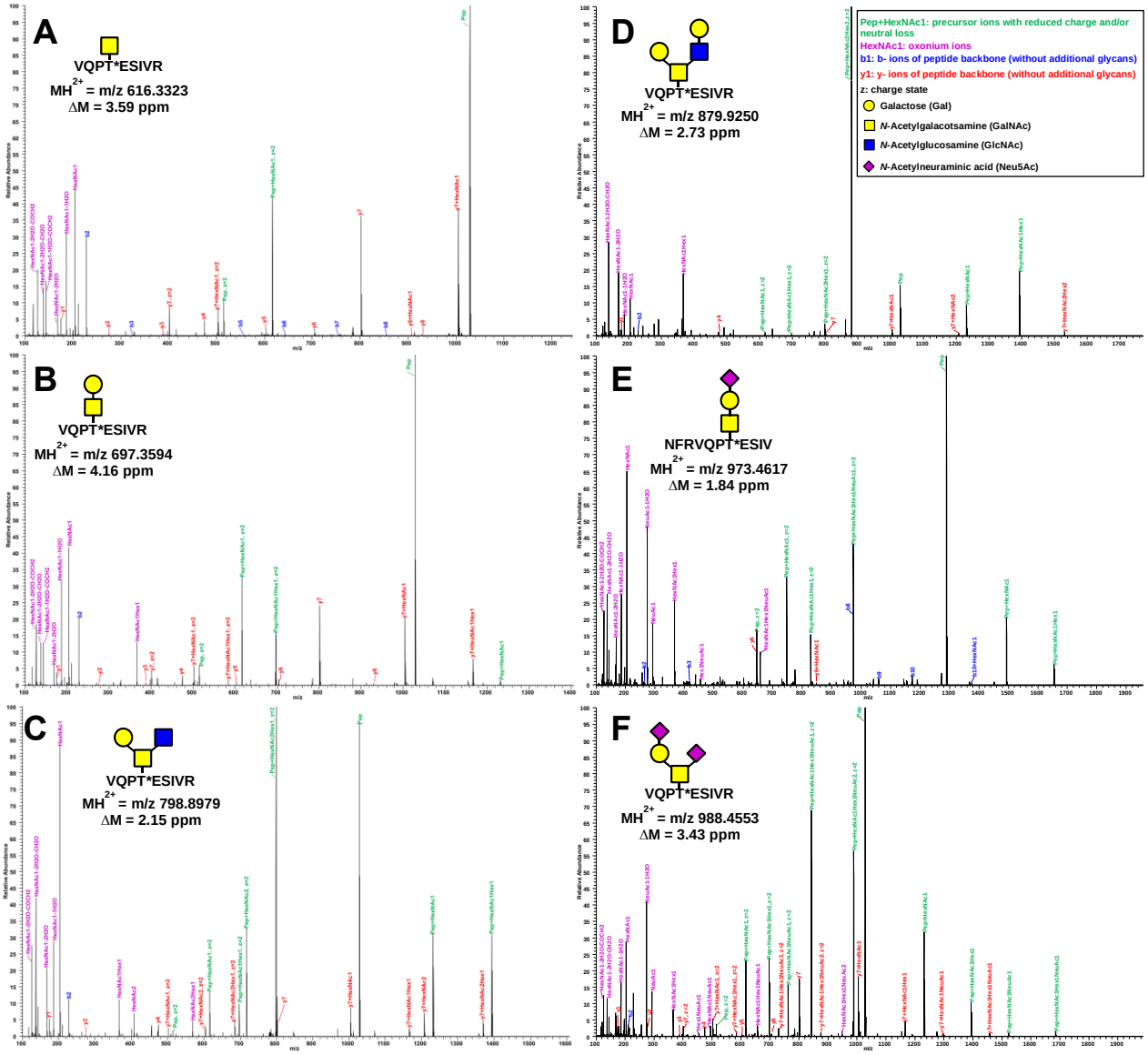
Supplemental Figure S4. Functional characterization of various S constructs in Pseudovirus.

Related to Figure 1. **(A)** Syncytia produced by SARS-CoV-2 S constructs in VeroE6 cells co-transfected with a GFP plasmid to visualize cell-to-cell fusion. Quantification of fusion using a luciferase complementation assay in 293T **(B)** or VeroE6 cells **(C)**. **(D)** Transduction efficiency in Vero E6 cells of ppVSV-GFP particles coated in the indicated glycoprotein. Results suggest that start methionine does not alter fusion or efficiency.


SARS-COV-2 S
Human ACE2
Relative Abundance
(Percent of Total Profile)
90
80
70
60
50
40
30
20
10
0
Sialyl-T
Disialyl-T
Core 1
Core 2
Core 4
O-Mannose
O-Fucose
Sialylated
Fucosylated
O-linked Glycan Structure

Supplemental Figure S5. Detection of O-linked glycans released from SARS-CoV-2 S and human ACE2.

Related to Figure 4. The detected O-glycans were categorized based on their structures and types. Relative abundance (prevalence) of each species is calculated based on peak intensity in full MS.


A
VQPT*ESIVR
MH2+ = m/z 616.3323
ΔM = 3.59 ppm
B
VQPT*ESIVR
MH2+ = m/z 697.3594
ΔM = 4.16 ppm
C
VQPT*ESIVR
MH2+ = m/z 798.8979
ΔM = 2.15 ppm
D
VQPT*ESIVR
MH2+ = m/z 879.9250
ΔM = 2.73 ppm
E
NFRVQPT*ESIV
MH2+ = m/z 973.4617
ΔM = 1.84 ppm
F
VQPT*ESIVR
MH2+ = m/z 988.4553
ΔM = 3.43 ppm
Pep+HexNAc1: precursor ions with reduced charge and/or neutral loss
HexNAc1: oxonium ions
b1: b- ions of peptide backbone (without additional glycans)
y1: y- ions of peptide backbone (without additional glycans)
z: charge state
Galactose (Gal)
N-Acetylgalacotsamine (GalNAc)
N-Acetylglucosamine (GlcNAc)
N-Acetylneuraminic acid (Neu5Ac)

Supplemental Figure S6. O-linked glycans detected at site T0323 of SARS-CoV-2 S.

Related to Figure 4. Representative Step-HCD spectra shown for 6 glycoforms.

A

```
CoV-1R MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTQDLFL   60
CoV-2I MFVFLVLLPLVS-SQCVNLTTRTQL-PPAYT--NSFTRGVYYPDKVFRSSVLHSTQDLFL   56
       **:**:.*.*.* *.....**...: .*.**  .*..*******:.***..*:.******
                  ——VAR1——
CoV-1R PFYSNVTGFHTI-----NHT--FGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQS  113
CoV-2I PFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQS  116
       **:****.**.*     *.*  *.***:**.**:***:*****:.:***:**:*:::*:**
                                          ————VAR2————
CoV-1R VIIINNSTNVVIRACNFELCDNPFFAV----SKPMGTQTHTMIFDNAFNCTFEYISDAFS  169
CoV-2I LLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFL  176
       ::*:**:*****:.*.*:.*::**..*    :.....::...::.:*.******:*..*.

CoV-1R LDVSEKSGNFKHLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNTLKPIFKLPLGINIT  229
CoV-2I MDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINIT  236
       :*:..*.****:********.**:..:*..:.**::*****.**:.*:*:..**:*****
             ———VAR3———
CoV-1R NFRAIL----TAFSPAQDI--WGTSAAAYFVGYLKPTTFMLKYDENGTITDAVDCSQNPL  283
CoV-2I RFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPL  296
       .*:.:*    :..:*....  *...****:****:*.**:***:***********:.:**

CoV-1R AELKCSVKSFEIDKGIYQTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERK  343
CoV-2I SETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRK  356
       :*.**:.:***.::***********.*:...*******************:*.*****.**

CoV-1R KISNCVADYSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTG  403
CoV-2I RISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTG  416
       :**************..*********.********:********:.:**:**********

CoV-1R VIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPFERDISNVPFSPD  463
CoV-2I KIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAG  476
       .************.***:***:.*:*:*:...*****.**..*...*:*******...:...

CoV-1R GKPCT-PPALNCYWPLNDYGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKLSTDLIKN  522
CoV-2I STPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKN  536
       ..**. ....***:**..***..*.*:***************:*********.**:*:**

CoV-1R QCVNFNFNGLTGTGVLTPSSKRFQPFQQFGRDVSDFTDSVRDPKTSEILDISPCAFGGVS  582
CoV-2I KCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVS  596
       :***************.*:*:*.********:.:*.**:****:*.*****:**:*****

CoV-1R VITPGTNASSEVAVLYQDVNCTDVSTAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAEHV  642
CoV-2I VITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHV  656
       *******.*::***********:*..*********.**:****:*****:**********
                                          VAR4
CoV-1R DTSYECDIPIGAGICASYHTVSLL----R 667
CoV-2I NNSYECDIPIGAGICASYQTQTNSPGGSG 685
       :.***************.*.:..    .
```

B

```
AAP13441.1         ... LTSGSDLDRCTTFDDVQA--PNYTQHTSSMRGVYYPD ... FLPFYSNVTGFHTI-----NHT--FGNPVI ...
AGZ48831.1         ... LVSSYTIEKCLDFDDRT--PPANTQFLSSHRGVYYPD ... FLPFDSNVTRFITFGLN-------FDNPII ...
AVP78031.1         ... LVNS----QCVNLTGRTPLNPNYTN--SSQRGVYYPD ... FLPFYSNVSWYYSLTTNNA-ATKRTDNPIL ...
AVP78042.1         ... LVNS----QC-DLTGRTPLNPNYTN--SSQRGVYYPD ... FLPFYSNVSWYYSLTTNNA-ATKRTDNPIL ...
QIA48641.1         ... LVSS----QCVNLTTRTGIPPGYTN--SSTRGVYYPD ... FLPFFSNVTWFNTI--TYQGGSKKFDNPVL ...
QIA48614.1         ... LVSS----QCVNLTTRTGIPPGYTN--SSTRGVYYPD ... FLPFFSNVTWFNTI--NYQGGFKKFDNPVL ...
QIQ54048.1         ... LVSS----QCVNLTTRTGIPPGYTN--SSTRGVYYPD ... FLPFFSNVTWFNTIHLNYQGGFKKFDNPVL ...
QIA48623.1         ... LVSS----QCVNLTTRTGIQPGYTN--SSTRGVYYPD ... FLPFFSNVTWFNTI--NYQGGFKKFDNPVL ...
QIA48632.1         ... LVSS----QCVNLTTRTGIPPGYTN--SSTRGVYYPD ... FLPFFSNVTWFNTI--NYQGGFKKFDNPVL ...
YP_009724390.1     ... LVSS----QCVNLTTRTQLPPAYTN--SFTRGVYYPD ... FLPFFSNVTWFHAIHVSGTNGTKRFDNPVL ...
QHR63300.2         ... LVSS----QCVNLTTRTQLPPAYTN--SSTRGVYYPD ... FLPFFSNVTWFHAIHVSGTNGIKRFDNPVL ...
                       *...    .*  :       *  *:  *  *******     **** ***: : ::           .**::

AAP13441.1         ... FGSTMNNKSQSVIIINNSTNVVIRAC ... PFFAVSKPMGTQTHTM----IFDNAFNCTFEYIS ...
AGZ48831.1         ... FGSTMNNKSQSVIIMNNSTNLVIRAC ... PFF--VVLKSNNTQ-IPSY-IFNNAFNCTFEYVS ...
AVP78031.1         ... FGTTLDNTSQSLLIVNNATNVIIKVC ... PYLSGYYH-NNKTWSIREFAVYSSYANCTFEYVS ...
AVP78042.1         ... FGTTLDNTSQSLLIVNNATNVIIKVC ... PYLSGYYH-NNKTWSIREFAVYSFYANCTFEYVS ...
QIA48641.1         ... FGTTLDARTQSLLIVNNATNVVIKVC ... PFLGVYYHNNNKTWVENEFRVYSSANNCTFEYIS ...
QIA48614.1         ... FGTTLDARTQSLLIVNNATNVVIKVC ... PFLGVYYHNNNKTWVENEFRVYSSANNCTFEYIS ...
QIQ54048.1         ... FGTTLDARTQSLLIVNNATNVVIKVC ... PFLGVYYHNNNKTWVENEFRVYSSANNCTFEYIS ...
QIA48623.1         ... FGTTLDARTQSLLIVNNATNVVIKVC ... PFLGVYYHNNNKTWVENEFRVYSSANNCTFEYIS ...
QIA48632.1         ... FGTTLDARTQSLLIVNNATNVVIKVC ... PFLGVYYHNNNKTWVENEFRVYSSANNCTFEYIS ...
YP_009724390.1     ... FGTTLDSKTQSLLIVNNATNVVIKVC ... PFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVS ...
QHR63300.2         ... FGTTLDSKTQSLLIVNNATNVVIKVC ... PFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVS ...
                       **:*::  :**::*:**:*::*..*     *::       . ::.      ::.   ******:*

AAP13441.1         ... FSLDVSEKSGNFKHLREFVF ... LPLGINITNFRAIL ...
AGZ48831.1         ... FNLDLGEKPGNFKDLREFVF ... LPLGINITNFRTLL ...
AVP78031.1         ... FMLNISGNGGLFNTLREFVF ... LPVSINITKFRTLL ...
AVP78042.1         ... FMLNISGNGGLFNTLREFVF ... LPVSINITKFRTLL ...
QIA48641.1         ... FLMDLEGKQGNFKNLREFVF ... LPIGINITRFQTLL ...
QIA48614.1         ... FLMDLEGKQGNFKNLREFVF ... LPIGINITRFQTLL ...
QIQ54048.1         ... FLMDLEGKQGNFKNLREFVF ... LPIGINITRFQTLL ...
QIA48623.1         ... FLMDLEGKQGNFKNLREFVF ... LPIGINITRFQTLL ...
QIA48632.1         ... FLMDLEGKQGNFKNLREFVF ... LPIGINITRFQTLL ...
YP_009724390.1     ... FLMDLEGKQGNFKNLREFVF ... LPIGINITRFQTLL ...
QHR63300.2         ... FLMDLEGKQGNFKNLREFVF ... LPIGINITRFQTLL ...
                       * :::  : * *: ******     **:.****.*.::* ...
```

Supplemental Figure S7. Sequence alignments of SARS-CoV-1 and SARS-CoV-2 S variants as well as alignment of multiple S proteins from related coronaviruses.

Related to Figure 4.