Supplementary

The Transmembrane Protease Serine 2 (TMPRSS2) Non-Protease Domains Regulating Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Spike-Mediated Virus Entry

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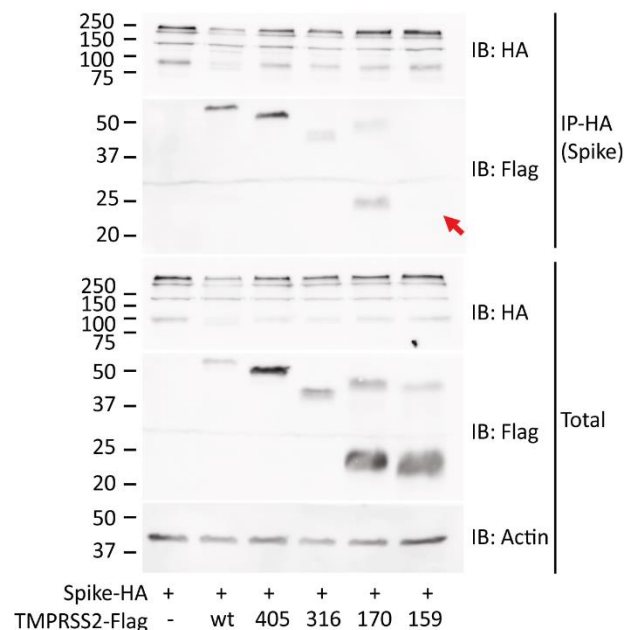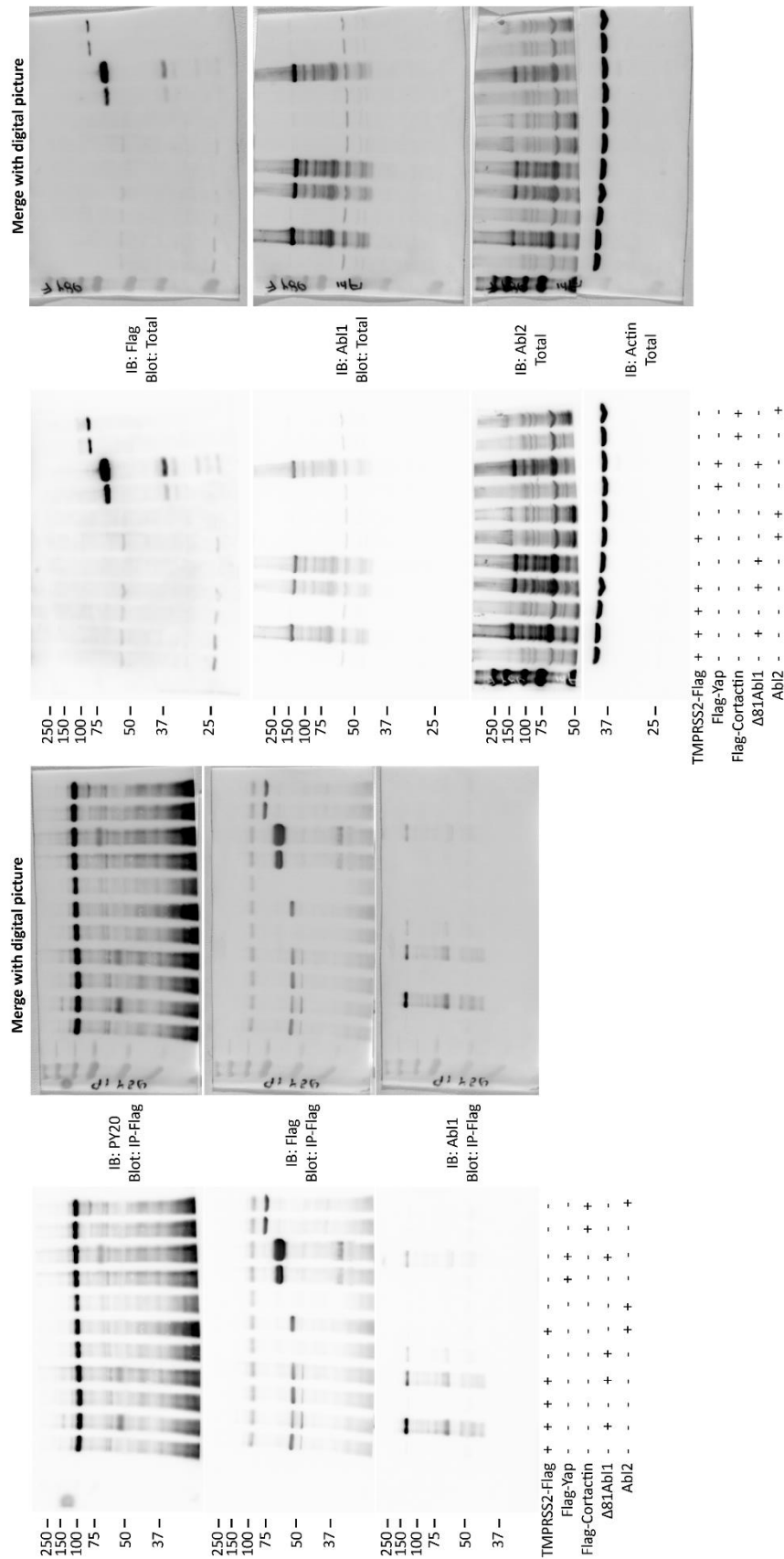
Supplementary Figures

Figure S1: The reciprocal experiment from WB Figure 3b Mapping the Spike-TMPRSS2 interacting region. HEK293T were transfected with the indicated plasmids and IPed with HA-beads and WB were performed as above. Red arrow shows the expected location of the 1-159-TMPRSS2-Flag band within IP-Flag blot.
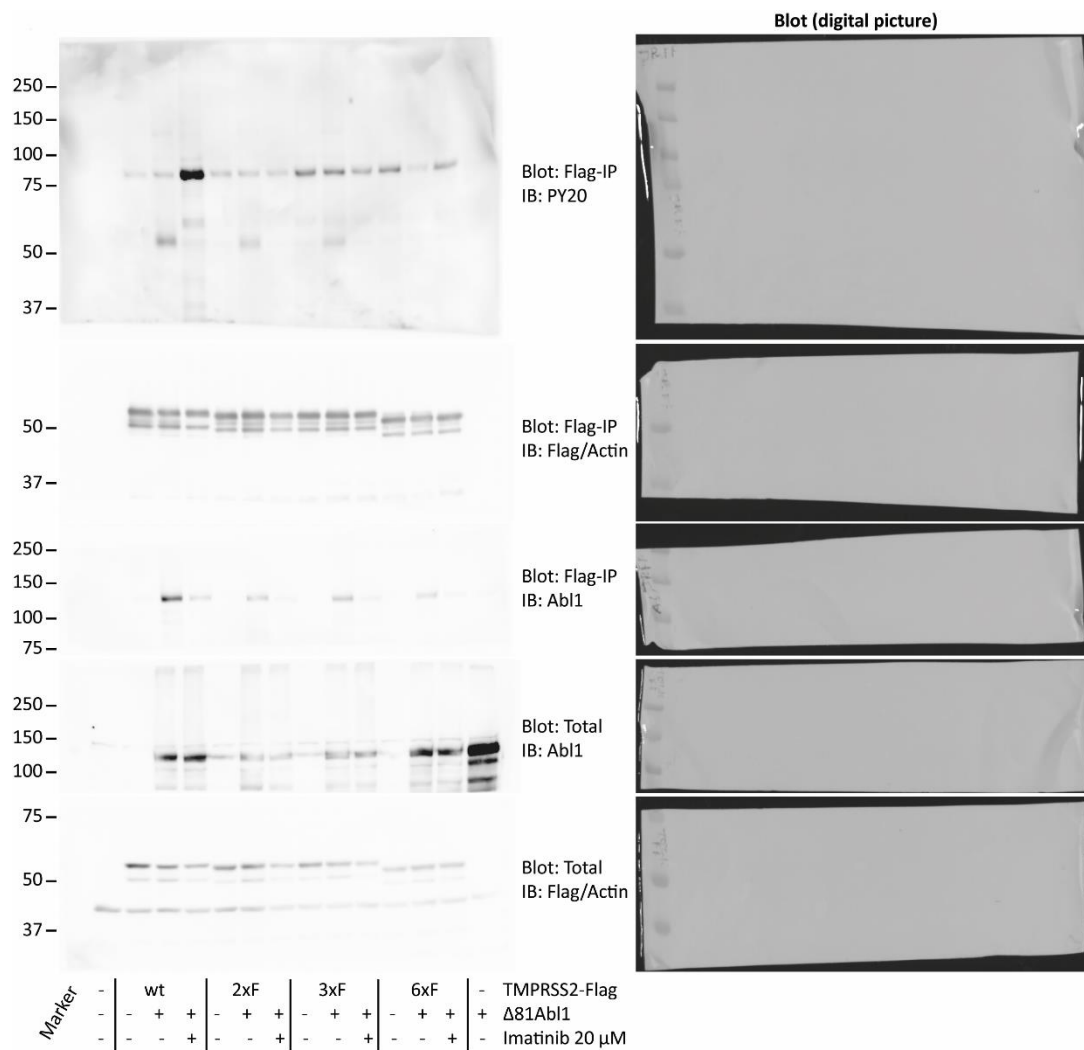
Figure S2: Original western blot image from Flag-IP Figure 1b. Blots were incubated and re-stained in following order for Flag-IP blot: 1) Anti-mouse-PY20, 2) Anti-mouse-Flag (TMPRSS2, Yap or Cortactin), 3) Anti-mouse-Abl1 and for the total blot: 1) Anti-mouse-Flag (TMPRSS2, Yap or Cortactin), 2) Anti-mouse-Abl1, 3) 2) Anti-mouse-Abl2 and 4) Anti-mouse-Actin

Figure S3: Original western blot image from Flag-IP Figure 1c. Blots were cut between lane 75 kDa and treated with indicated antibodies. The Flag-IP blot was cut after analysis of PY20 antibody treatment.
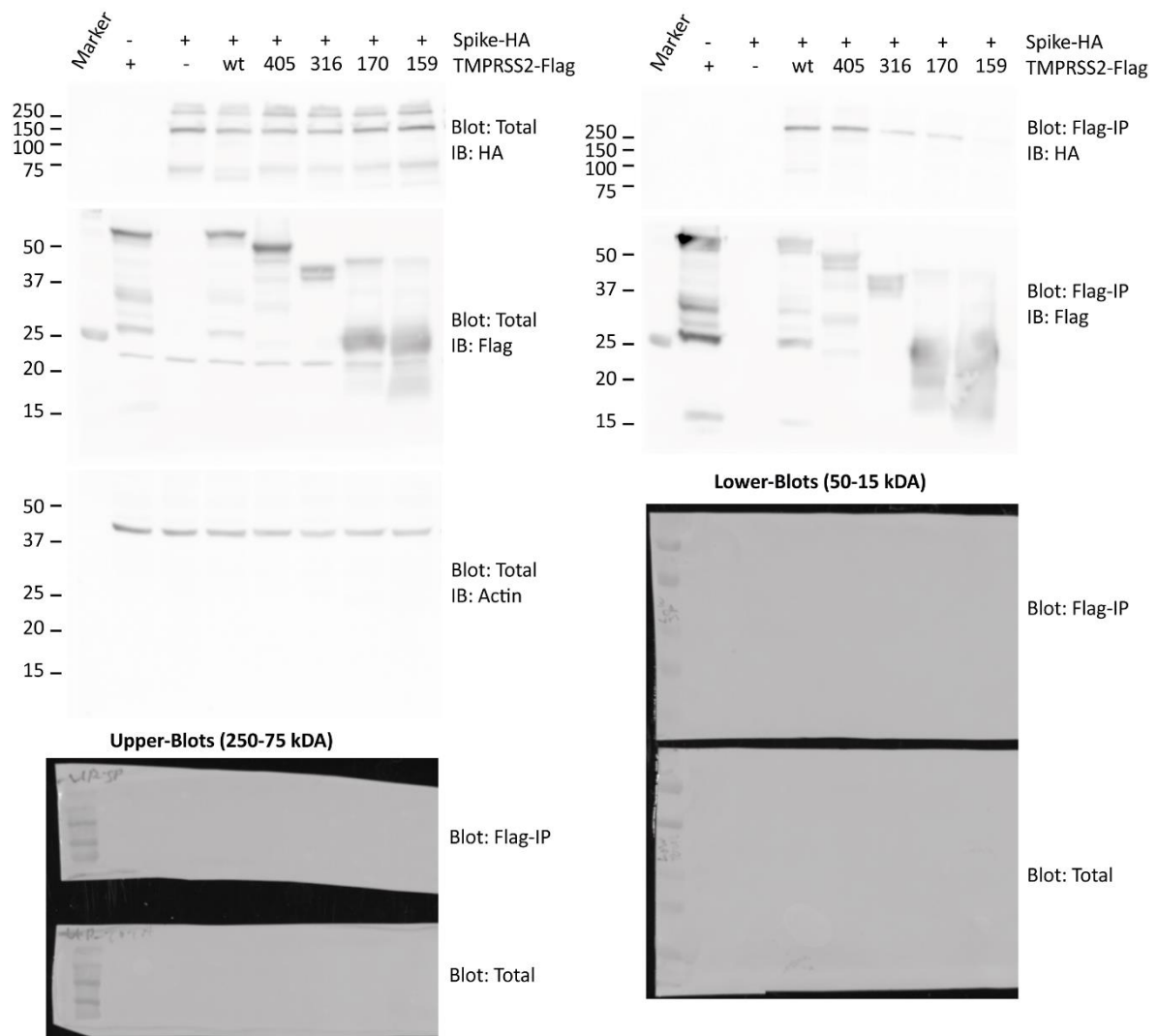
Figure S4: Original western blot image from Flag-IP Figure 3b. Blots were cut between lane 75 kDa and the upper blots were treated with Anti-mouse-HA (TMPRSS2) antibodies while lower blots was treated with Anti-rabbit-Flag (Spike) and two days later with Anti-mouse-Actin antibodies.
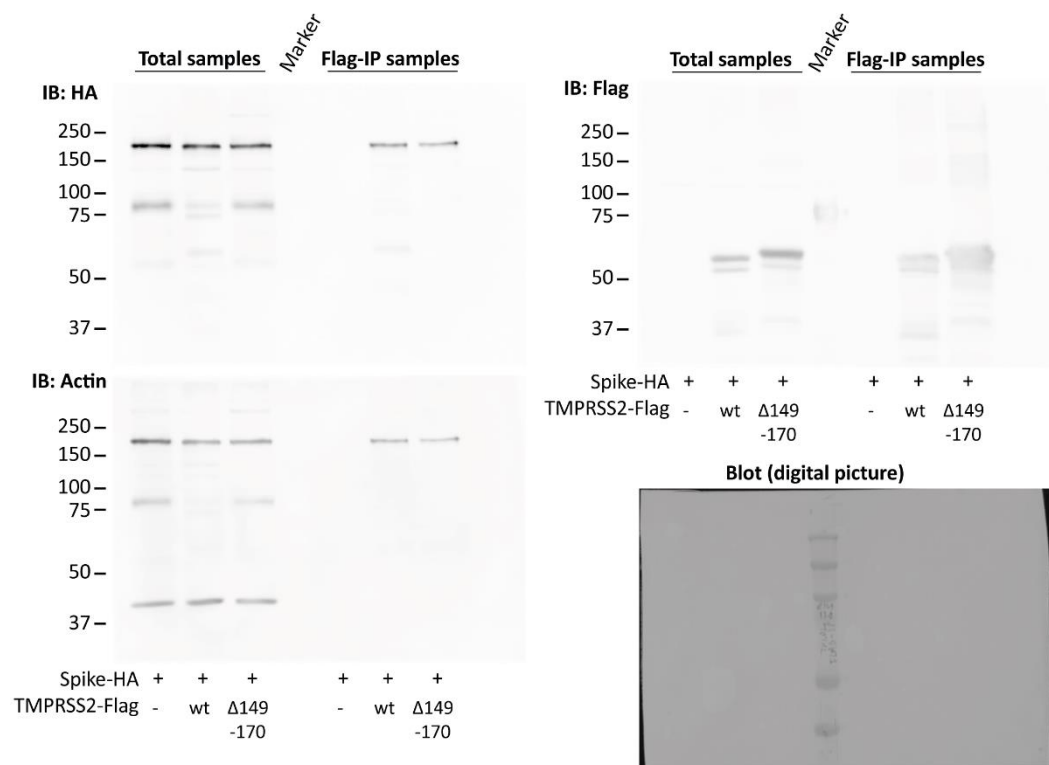
Figure S5: Original western blot image from Flag-IP Figure 3d. Blot was incubated and re-stained in following order: 1) Anti-mouse-HA (Spike), 2) Anti-rabbit-Flag (TMPRSS2) and 3) Anti-mouse-Actin.
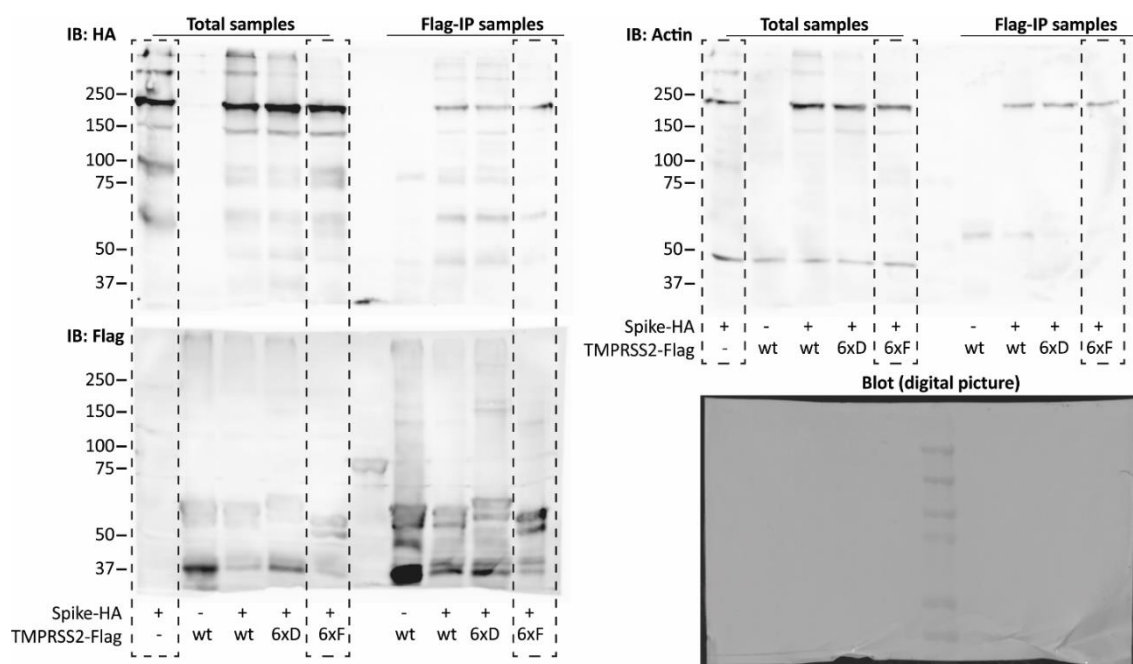

Figure S6: Original western blot image from Flag-IP Figure 4a. Dashed box shows excluded lanes from figure 4a in main text. Blots were incubated and re-stained in following order: 1) Anti-mouse-HA (Spike), 2) Anti-rabbit-Flag (TMPRSS2) and 3) Anti-mouse-Actin.
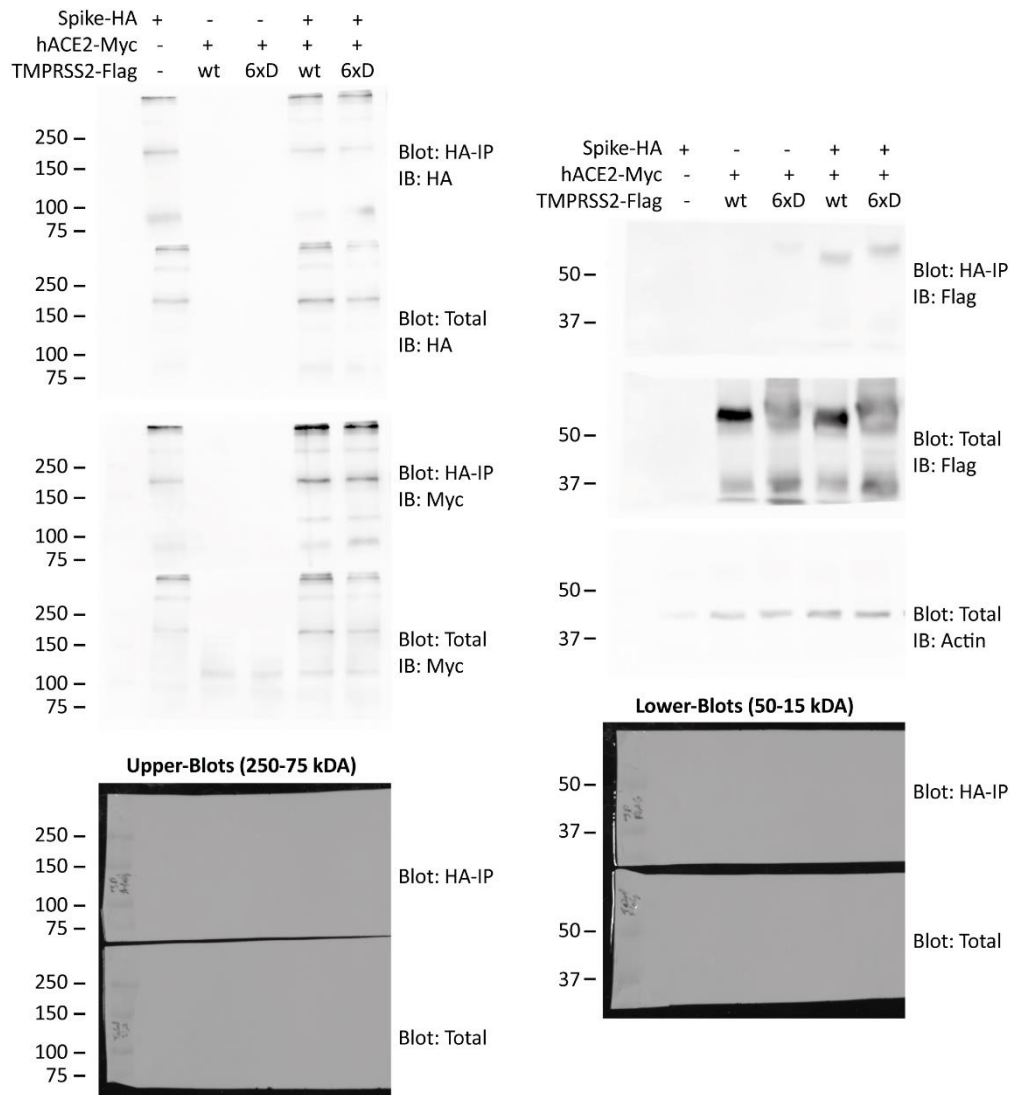
Figure S7: Original western blot image from Flag-IP Figure 4c. Blots were cut between lane 75 kDa and the upper blots were treated with Anti-mouse-HA (Spike) and anti-Myc (hACE2) antibodies while lower blots was treated with Anti-rabbit-Flag (TMPRSS2) and with Anti-mouse-Actin antibodies. Result was verified with an additional experiment.
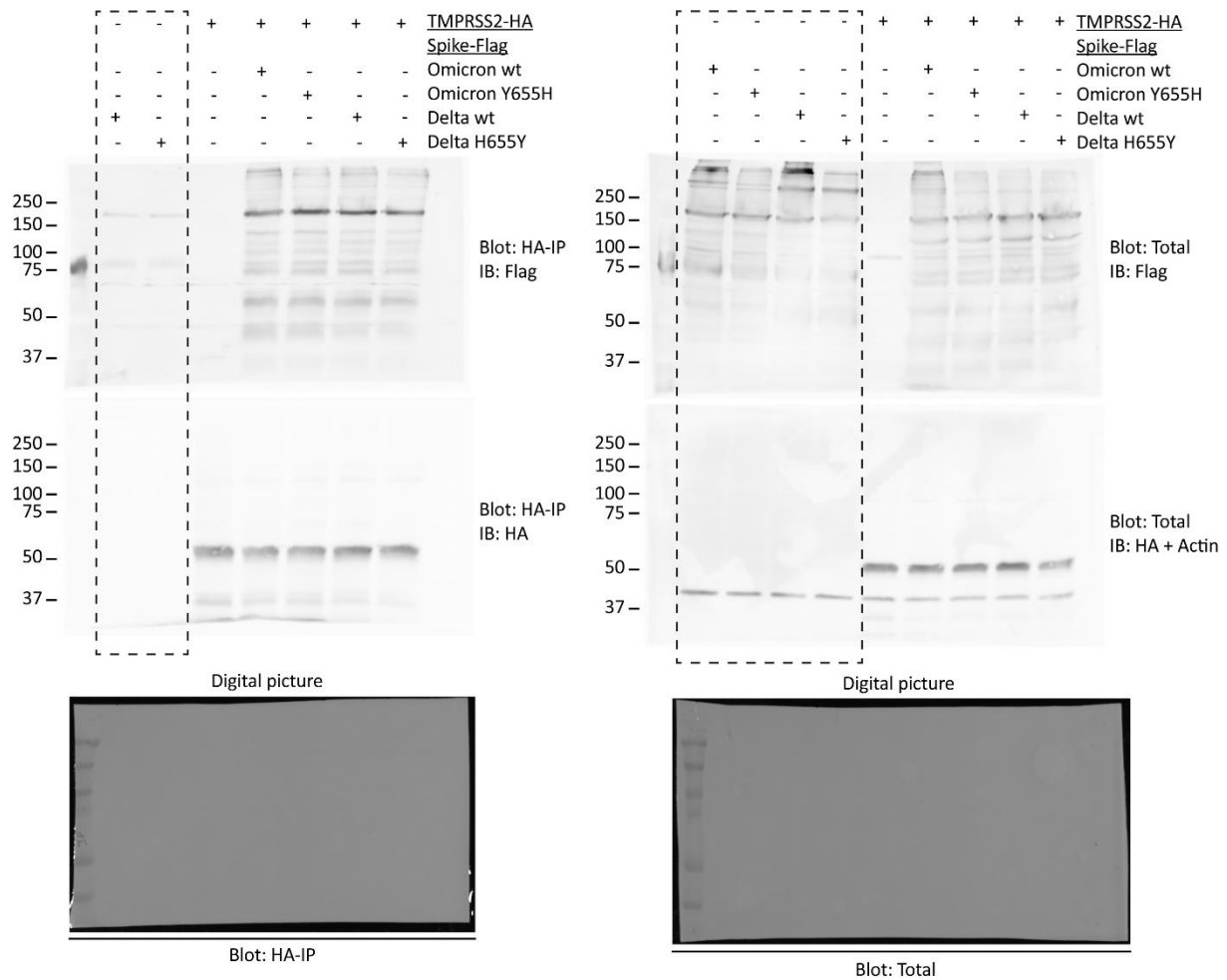
Figure S8: Original western blot image from Flag-IP Figure 5b. Dashed box shows excluded lanes from figure 5b in main text. Blots were incubated and re-stained in following order: 1) Anti-rabbit-Flag (Spike), 2) Anti-mouse-HA (TMPRSS2) + Anti-mouse-Actin.

Supplementary Table

Supplement Table S1: List of Primer used for cloning of described constructs.

Name	Sequence
TMPRSS2_Fw_NheI	AATTGCTAGCGGGCCCACCATGGCTTTGAACTCAGGGTCACC
TMPRSS2_Rv_Flag_XbaI	AATTTCTAGATTACTTGTCGTCATCGTCTTTGTAGTCGCCGTCTGCC CTCATTTGTCGATA
TMPRSS2_Rv_HA-tag_XbaI	AATTTCTAGATTAtgcataatccggaacatcatacggataGCCGTCTGCCCT CATTTGTCGATA
TMPRSS2-1347_Rv_Flag_XbaI	CCAATTTCTAGATTACTTGTCGTCATCGTCTTTGTAGTCCTTCGAA GT GACCAGAGG
TMPRSS2-1215_Rv_Flag_XbaI	CCAATTTCTAGATTACTTGTCGTCATCGTCTTTGTAGTCAATGAgA AG CAccTTGGC
TMPRSS2-1083_Rv_Flag_XbaI	CCAATTTCTAGATTACTTGTCGTCATCGTCTTTGTAGTCCACTAGG TC GTTGAAAGT
TMPRSS2-948_Rv_Flag_XbaI	CCAATTTCTAGATTACTTGTCGTCATCGTCTTTGTAGTCTCTCAAA AT CCCCGCAAA
TMPRSS2-513_Rv_Flag_XbaI	CCAATTTCTAGATTACTTGTCGTCATCGTCTTTGTAGTCAGGGTGC C AGGACTTCCT
TMPRSS2-477_Rv_Flag_XbaI	GGGCCCTCTAGATTACTTGTCGTCATCGTCTTTGTAGTCCTGAAG G ATGAAGTTTGG
TMPRSS2-444_Rv_Flag_XbaI	GGGCCCTCTAGATTACTTGTCGTCATCGTCTTTGTAGTCACACCGA T TCTCGTCCTC
TMPRSS2-411_Rv_Flag_XbaI	CCAATTTCTAGATTACTTGTCGTCATCGTCTTTGTAGTCTGACACG C CATCACACCA
TMPRSS2_dAA149-170_Fw	GGACGAGAATCGGTGTGTGTGCCAAGACGACT
TMPRSS2_dAA149-170_Rv	AGTCGTCTTGGCACACACACCGATTCTCGTCC
TMPRSS2_Rv-YY-FF_P-dead	CACGGGGGACGGGaAGaACTGAGCCGGATG
TMPRSS2_Fw-YY-FF_P-dead	CATCCGGCTCAGTtCTtCCCGTCCCCCGTG
TMPRSS2_Rv-YY-DD_P-mim	ACGGGGGACGGGTcGTcCTGAGCCGGATGCAC
TMPRSS2_Fw-YY-DD_P-mim	TGCATCCGGCTCAGgACgACCCGTCCCCCG
TMPRSS2-Y52_Fw-Y-F	CCCGTGCCCCAGTtCGCCCCGAGGGTCCTG

TMPRSS2-Y52_Rv-Y-F	GACCCTCGGGGCGaACTGGGGCACGGGGGA
6xf-TMPRSS2-Fw_NheI	CTATAGgctagcGGGCCCACCATGGCTTTGAACTCAGGGTCACCAC CAGCTATTGGACCTTtCTtTGAAAACCATGGA
6xf-TMPRSS2-Rv	GACCCTCGGGGCGaACTGGGGCACGGGGGACGGGAAGAACTGA GCCGGATGCACCTCGaAGACAGTGGGGAC
6xd-TMPRSS2-Fw_NheI	CTATAGgctagcGGGCCCACCATGGCTTTGAACTCAGGGTCACCAC CAGCTATTGGACCTgACgATGAAAACCATGGA
6xd-TMPRSS2-Rv	GACCCTCGGGGCGTcCTGGGGCACGGGGGACGGGTcGTcCTGAG C CGGATGCACCTCGTcGACAGTGGGGAC
Spike_Fw_NheI_pcDNA3. 1	aagctggctagcatgttcgtgttcct
Spike- Flag_Rv_XhoI_pcDNA3.1	ATGCCCctcgagTCActtgtcgtcatcgtctttgtagtctgtatagtGcagtttgacg cccttc