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Supplemental information

**SARS-COV-2 spike binding to ACE2 in living
cells monitored by TR-FRET**

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SARS-COV-2 Spike binding to ACE2 in living cells monitored by TR-FRET

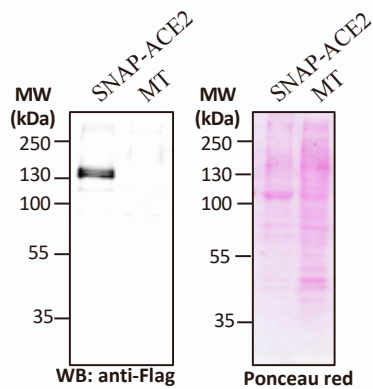


Figure S1 (related to Figure 1). Validation of SNAP-ACE2 expression in transfected HEK293 cells. Western blot of SNAP-ACE2 expression in HEK293 cells transfected with 1 μ g of vector versus mock-transfected (MT) cells. Primary antibody staining against FLAG-tag on SNAP-ACE2 construct; Ponceau red staining used as loading control.

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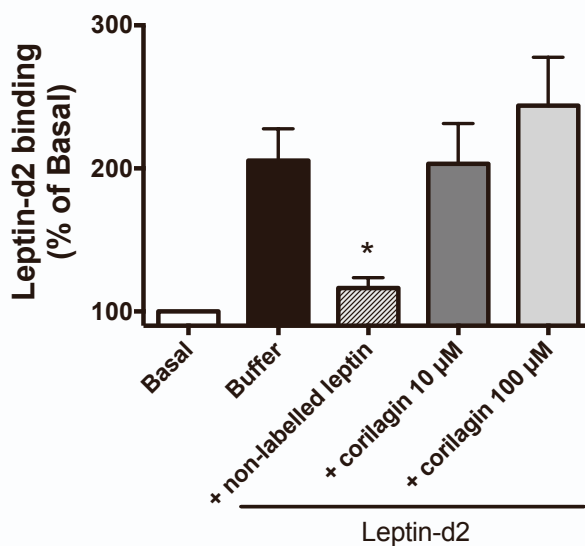


Figure S2 (related to Figure 2H). Counter-assay TR-FRET test of corilagin. Effect of corilagin (10 and 100 µM) on the TR-FRET signal resulted from Leptin-d2 (0.5 nM) binding to Lumi4-Tb-SNAP-LepR (leptin receptor) expression in HEK293 cells. Non-specific signal is defined in the presence of excess of non-labelled leptin (100 nM). Data are expressed as mean \pm SEM of 3 independent experiments, each performed in triplicate. “Leptin-d2 binding” corresponds to the TR-FRET ratio and is expressed as % of basal (absence of d2 ligand). * $p < 0.05$ by one-way ANOVA followed by Dunnett’s multiple comparisons test compared to the buffer-treated group.

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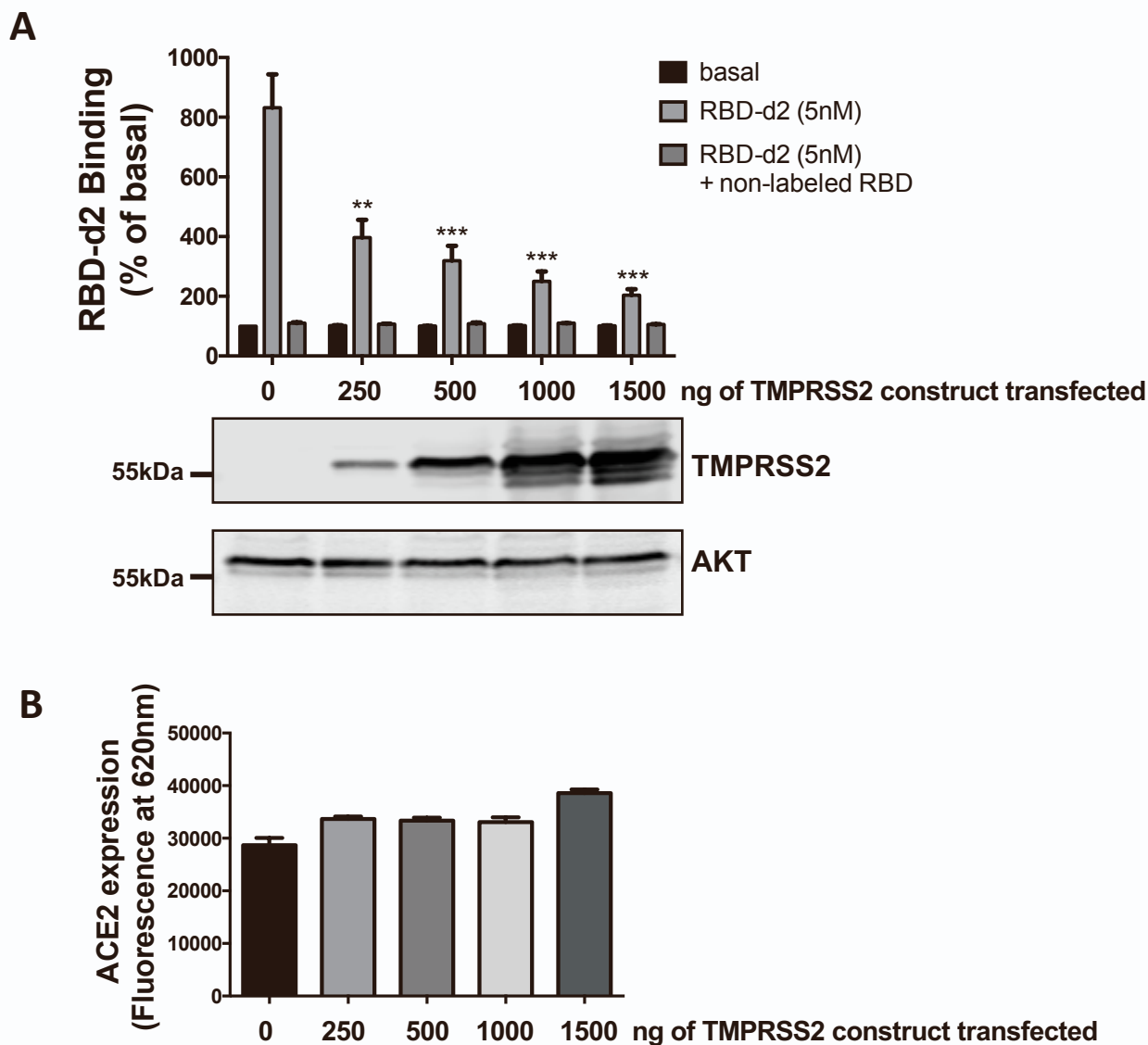


Figure S3 (related to Figure 3B). Effect of TMPRSS2 on RBD binding to ACE2. **A)** Binding of RBD-d2 (5 nM) to Lumi4-Tb-labelled SNAP-ACE2 (250 ng of transfected cDNA) in HEK293 cells with and without co-expression of increasing amounts (in ng of transfected cDNA construct) of TMPRSS2 in HEK293 cells. Non-specific signal is defined in the presence of excess of non-labelled RBD (1 μ M). Data are expressed as mean \pm SEM of 3 independent experiments, each performed in triplicate. TR-FRET ratio is expressed as % of basal (absence of d2). ** $p < 0.01$; *** $p < 0,005$ by one-way ANOVA followed by Dunnett's multiple comparisons test compared to the group 0 (no TMPRSS2 transfection). Bottom: Western blot of TMPRSS2 expression in HEK293 cells transfected with the indicated ng of TMPRSS2 construct. Primary antibody staining against TMPRSS2, or AKT as protein loading control. **B)** Lumi4-Tb-labelled SNAP-ACE2 expression assessed by Tb fluorescence measurement (620 nm). Representative data expressed as mean \pm SD (n=3).

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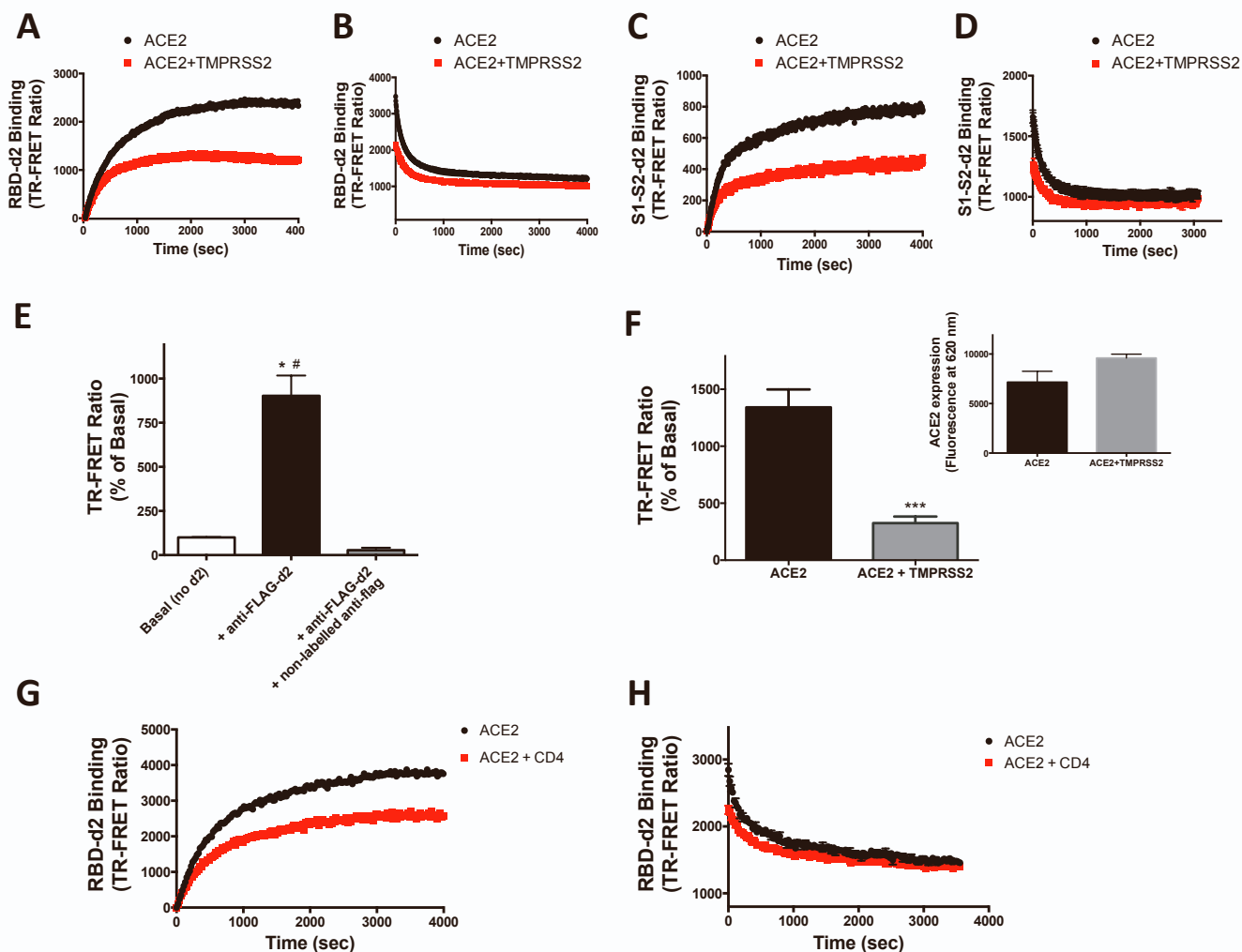


Figure S4 (related to Figure 3B-I). Effect of TMPRSS2 and CD4 on the kinetics of Spike protein binding to ACE2 and on ACE2 conformation. Representative curves of association (A,G) and dissociation (B,H) kinetics of RBD-d2 binding (5 nM) to Lumi4-Tb-SNAP-ACE2 in cells co-expressing or not TMPRSS2 (A-B; n=3) or CD4 (G-H; n=3). Dissociation was initiated by adding non-labelled RBD (1 μ M). Data are expressed as mean \pm SD of duplicates. C-D) Representative curves of association (C) and dissociation (D) kinetics of S1-S2-d2 binding (20 nM) to Lumi4-Tb-SNAP-ACE2 in cells co-expressing or not TMPRSS2 (n=3). Dissociation was initiated by adding non-labelled S1-S2 (200 nM). Data are expressed as mean \pm SD of duplicates. E) Intramolecular TR-FRET sensor of ACE2 conformation based on TR-FRET signal between Lumi4-Tb-SNAP-ACE2 and d2-labelled anti-FLAG antibody (2 μ g/mL). Non-specific signal is defined in the presence of excess of non-labelled anti-FLAG antibody (20 μ g/mL). Data are expressed as mean \pm SEM of 3 independent experiments. * p <0,001 (vs. Basal, one-way ANOVA); # p <0,001 (vs. anti-FLAG-d2 + non-labelled anti-FLAG, one-way ANOVA). F) TR-FRET signal between Lumi4-Tb-SNAP-ACE2 and d2-labelled anti-FLAG antibody in cells expressing only SNAP-ACE2 or co-expressing SNAP-ACE2 and TMPRSS2. Data are expressed as mean \pm SEM of 3 independent experiments; *** p <0,001, Student t test. Insert: Lumi4-Tb-labelled SNAP-ACE2 expression assessed by Tb fluorescence measurement (620 nm). Representative data expressed as mean \pm SD.

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Table S1 (related to Figure 1). Binding constants of SARS-CoV-2 Spike binding to ACE2 in vitro reported in the literature.

Technique	K_{on} ($10^5 \text{ M}^{-1} \text{ s}^{-1}$)	K_{off} (10^{-3} s^{-1})	Kinetically derived $K_d = K_{off}/K_{on}$ (nM)	Reference
SPR	1.88	2.76	14.7	Wrapp et al.
SPR	1.75	7.75	44.2	Shang et al.
SPR	0.40	3.80	94.6	Wang et al.
BLI	2.80	6.0	22.0	Chan et al.
BLI	1.40	0.16	1.2	Walls et al.
BLI			20.4	Glasgow et al.
SPR	3.6	9	26	Toelzer et al.
Mean	2.0 ± 1.1	4.9 ± 3.3	33.8 ± 33.0	

Mean values were derived from the published studies. SPR, surface plasmon resonance; BLI, biolayer interferometry.