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Supplemental information

ACE2 interaction with cytoplasmic PDZ protein enhances SARS-CoV-2 invasion

Qiangmin Zhang, Julia Gefter, W. Bruce Sneddon, Tatyana Mamonova, and Peter A. Friedman

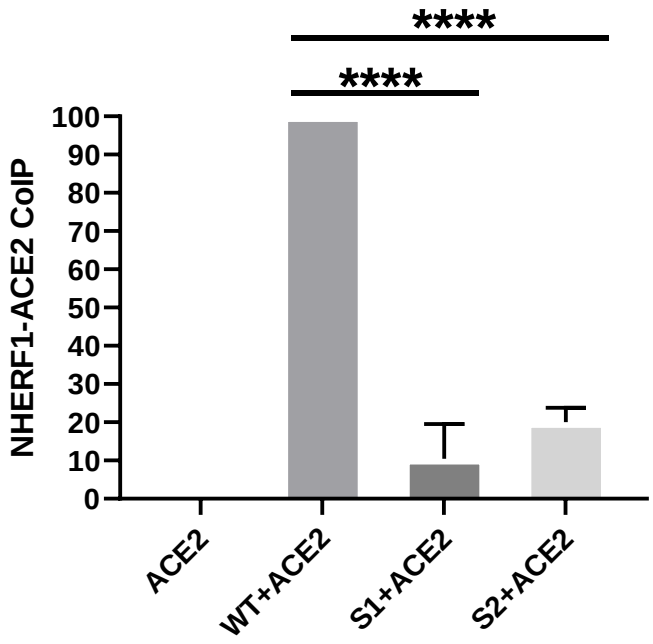

Figure S1. Quantification analysis of Figure 1D by ImageJ. Related to Figure 1D. Binding intensity of ACE2 to WT-NHERF1 was set to 100%. Relative binding of S1-NHERF1 or S2-NHERF1 was calculated and plotted using GraphPad 9. Data are means ± S.D. (error bars) of n=3 independent experiments (****, $p<0.0001$). Similar results were obtained as shown in Figure 1C.

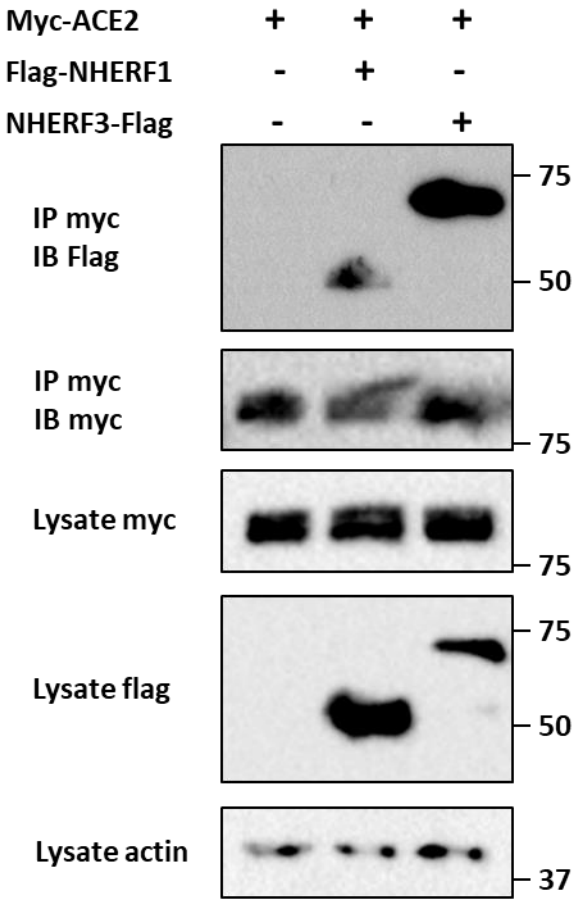

Figure S2. Coimmunoprecipitation analysis of NHERF3 binding to ACE2. Related to Figure 1B. Myc-ACE2, myc-ACE2 and Flag-NHERF1, or myc-ACE2 and NHERF3-flag were transfected into HEK293 GnTI$^-$ cells, respectively. The cell lysates were incubated with anti-Myc agarose beads followed by immunoblot analysis using anti-flag and anti-myc antibodies. Flag-NHERF1 binding to ACE2 was used as a positive control. β-actin served as a loading control. The molecular markers are indicated in kD on the right. Shown are representative blots of 3 independent experiments with similar results.

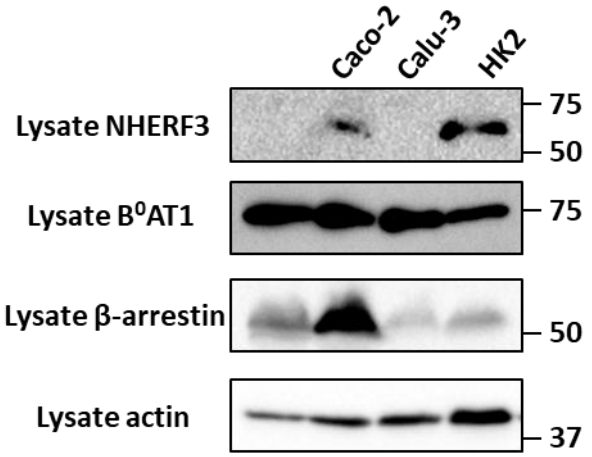

Figure S3. Expression profiling of endogenous NHERF3, B^0AT1, and β-arrestin in selected cell lines. Related to Figure 2A. Cell lysates containing equal amounts of total protein were loaded for immunoblotting analysis using anti-NHERF3, B^0AT1, β-arrestin and β-actin antibodies. β-actin was used as a loading control. The molecular markers are indicated in kD on the right. Shown are representative blots of 3 independent experiments with similar results.

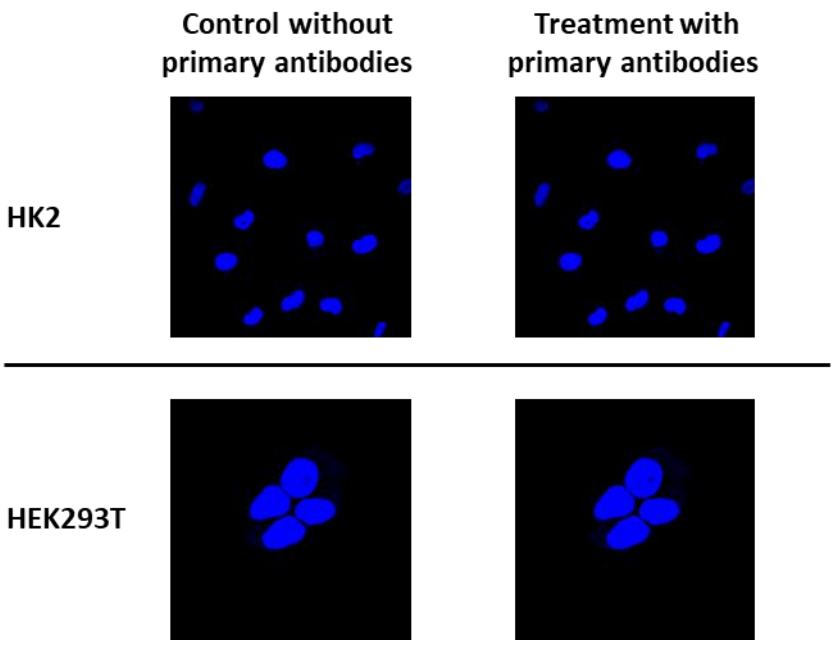

Figure S4. Proximity ligation assay to detect ACE2-NHERF1 interaction in HK2 or HEK293T cells. Related to Figure 2B. PLA signals (right) were not detected in either NHERF1-lacking HK2 or in ACE2-deficient HEK293T cells. Images are representative of ≥3 independent experiments.

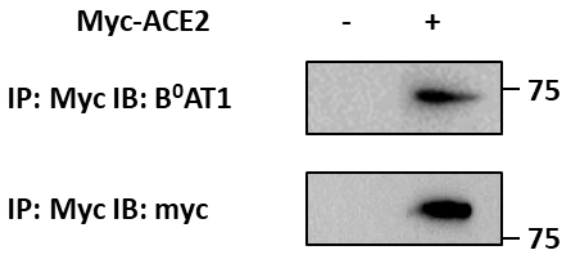

Figure S5. Endogenous B^0AT1 coprecipitates with overexpressed myc-ACE2 in HK2 cells Related to Figure 4B. Myc-ACE2 was transfected into HK2 cells. Untransfected cells were used as a negative control. The resulting cell lysates were incubated with anti-myc agarose beads followed by immunoblotting using anti-myc and anti-B^0AT1 antibodies. The molecular markers are indicated in kD on the right. Blots are representative of 3 independent experiments.