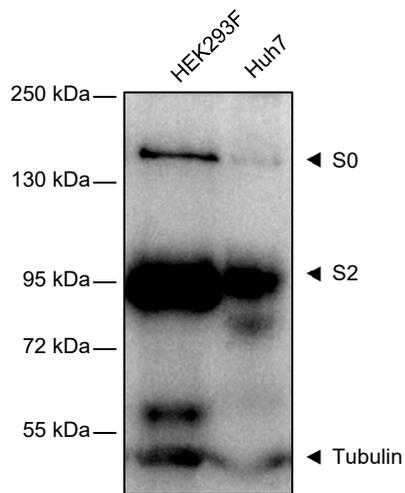
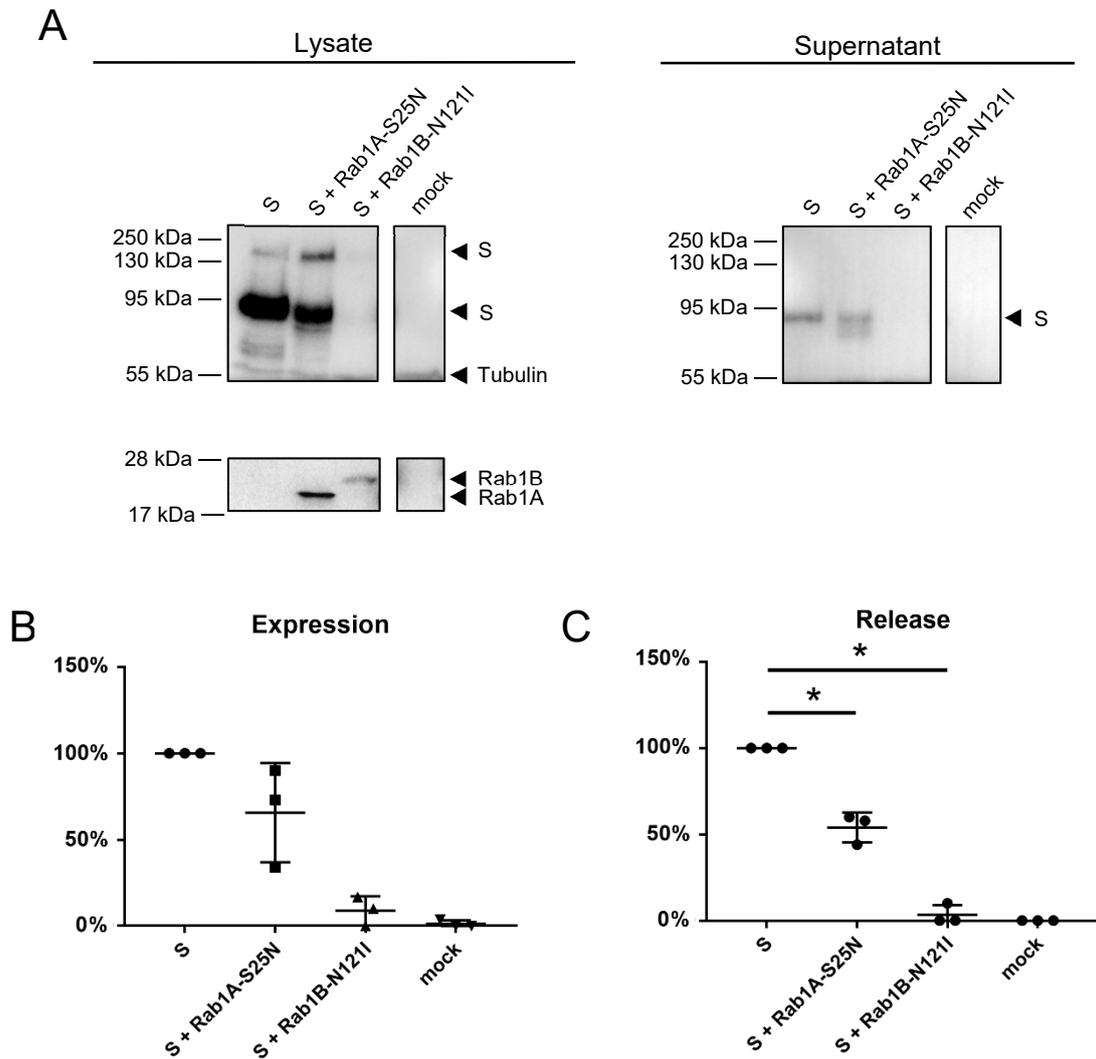


### Supplementary Figure S1



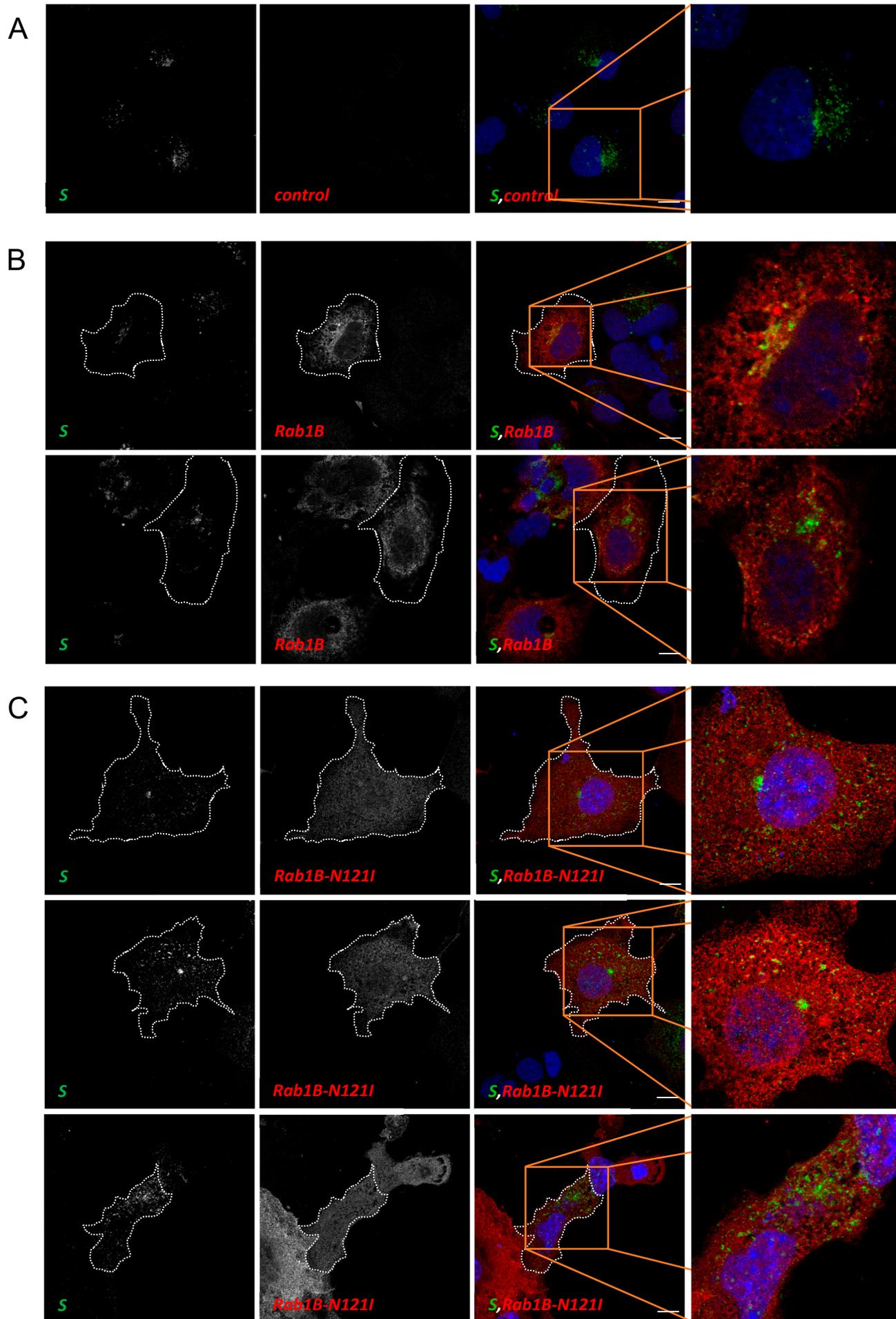
**Figure S1.** Cleavage of SARS-CoV-2 S can differ among different cell lines. Subconfluent HEK293F and HuH7 cells were transfected with 2  $\mu$ g C-terminally myc-tagged pCAGGS-S. Immunoblot was stained for S and tubulin.

## Supplementary Figure S2



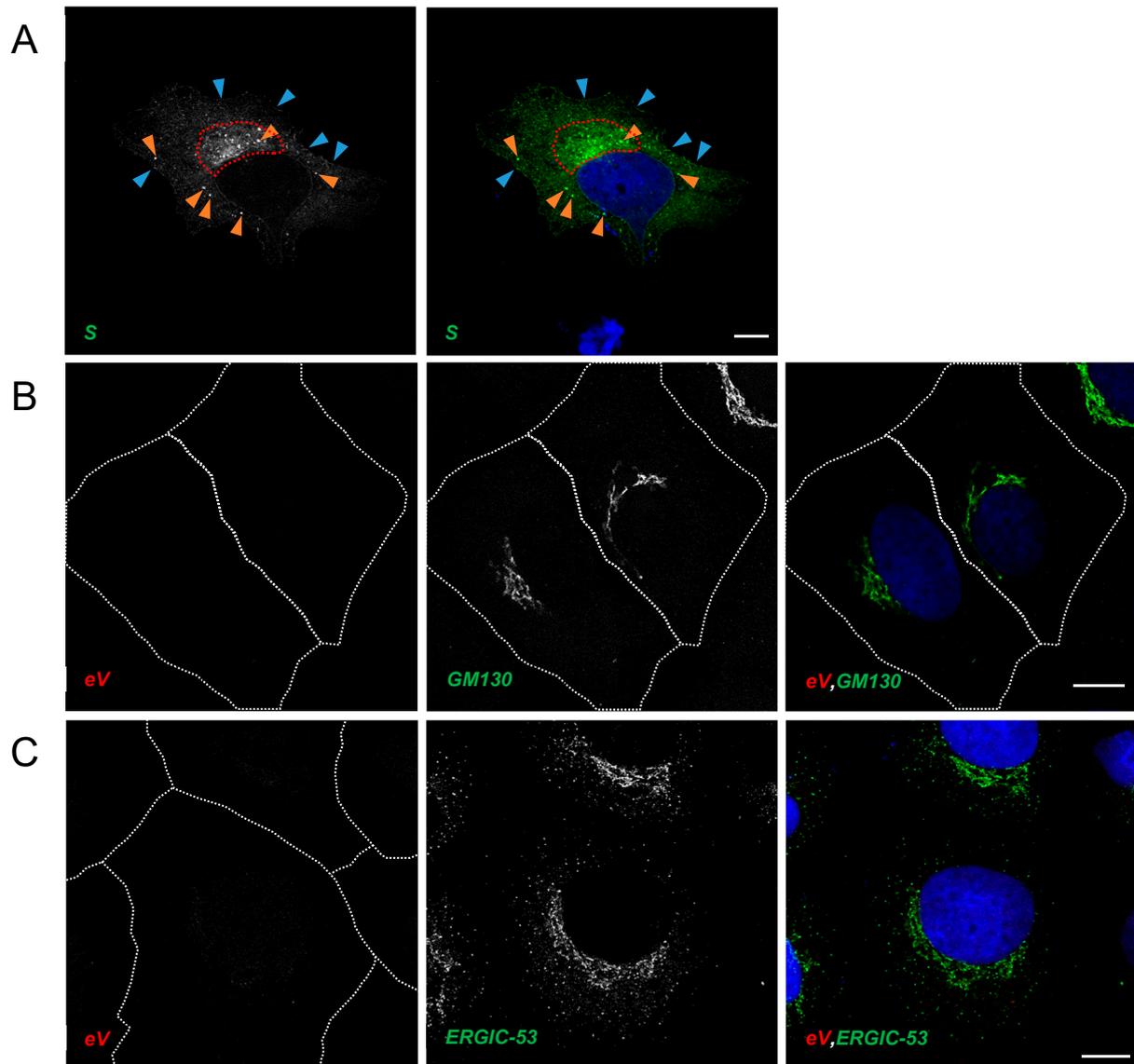
**Figure S2.** Rab1A and Rab1B are involved in release of SARS-CoV-2 S. (A) Subconfluent HuH7 cells were transfected with 1  $\mu$ g pCAGGS-S and and 3  $\mu$ g pCMV-Rab1A-myc, pCMV-Rab1B-myc, the corresponding DN mutants or empty vector pCAGGS as a control, respectively. Cell lysates and supernatant were harvested 24 hpt. Released S protein was isolated by ultracentrifugation and resuspended in SDS sample buffer. Immunoblots were stained for S protein, Rab1A/Rab1B (anti-myc) and tubulin. (B) Levels of S in the lysate were quantified and normalized to tubulin. (C) Levels of released S was quantified and normalized to S in the lysates. The values in the graphs represent triplicates from three independent experiments. Statistical analysis was performed using Mann-Whitney test in R-Studio v.1.3.1073. \*:  $p < 0.05$

Supplementary Figure S3



**Figure S3.** Inhibition of Rab1B leads to redistribution of SARS-CoV-2 S in infected HEK293<sup>ACE2</sup> cells. Subconfluent HEK293<sup>ACE2</sup> cells were infected with SARS-CoV-2. Then, cells were transfected with 1000 ng (A) empty vector pCAGGS (control), (B) pCMV-Rab1B-myc or (C) pCMV-Rab1B-N121I-myc, respectively. Next, 24 hpt cells were fixed and stained for SARS-CoV-2 S (green), Rab1B (anti-myc, red) and nuclei (DAPI). Magnified region shows the difference in distribution of S. Scale bars: 10  $\mu$ m. Data representative for a duplicates of three independent experiments.

Supplementary Figure S4



**Figure S4.** Control Panels of single-expressed S and endogenous GM130 and ERGIC-53 in HuH7 cells. Subconfluent HuH7 cells were transfected with (A) 500 ng pCAGGS-S or (B, C) 750 ng empty vector (eV) pCAGGS. Next, 24 hpt cells were fixed and stained for nuclei (DAPI), (A) SARS-CoV-2 S (green), and (B, C) Rab1B (anti-myc, red) as well as (B) endogenous GM130, or (C) endogenous ERGIC-53, respectively. (A) i) Encircled are marks perinuclear accumulation, ii) orange arrows demonstrate vesicle-like structures and iii) blue arrows mark localization at the plasma membrane. Scale bars: 10  $\mu\text{m}$ . Data representative of three independent experiments.