



Figure 3-figure supplement 1. A GFP-complementation based assay for assessing coronavirus fusogenicity. **(A)** HEK-293T cells expressing an empty vector or S protein together with GFP-11 tagged beta actin and a BFP containing a nuclear localization signal were added to cells stably expressing GFP1-10. Fusion of these two cell types allowed GFP-complementation in cells expressing a nuclear BFP, facilitating easy quantification of nuclei per syncytial cell. Unfused cells only expressed BFP in the nucleus. Fusion with VeroE6 GFP1-10 cells 18 hours after addition of the fusogenic HEK-293T is shown as an example. **(B, C and D)** Full well scans of the complemented GFP signal 18 hours after addition of the fusogenic HEK-293T cells to Calu-3 GFP1-10 **(B)**, VeroE6 GFP1-10 **(C)** and VeroE6-TMPRSS2 GFP1-10 **(D)** cells are shown. Dashed areas are enlarged next to each well. Scale bars indicate 50 μ m. **(E and F)** Fusogenicity of SARS-CoV-2 S and SARS-CoV S was assessed after 18 hours by measuring the sum of all GFP+ pixels per well in VeroE6 cells **(E)** and VeroE6 TMPRSS2 cells **(F)**. Statistical analysis was performed by one-way ANOVA on SARS-CoV-2 S-mediated fusion compared with SARS-CoV S. * $p < 0.05$. **(G)** Fold change in total GFP+ pixels by TMPRSS2 overexpression in VeroE6 cells.