

**Figure S5.** A) A549 cells were transiently transfected with GFP-tagged constructs and mCherry. Cells were visualized 24 hours after transfection and all images were taken with identical fluorescence gain settings. Scale bar: 100 µm. B) Fluorescent intensities across 3 fields per conditions were measured with ImageJ and displayed as mean ± standard error. Staining for poly-A mRNA in C) A549 and D) Calu3 cells revealed SARS-CoV-2 ORF6 Met58Ala-expressing cells do not accumulate mRNA in the nuclei, consistent with the results of the mCherry co-transfection assay. White arrows indicate transfected cells. Scale bar: 5 µm. \* p < 0.05; \*\* p < 0.01.