

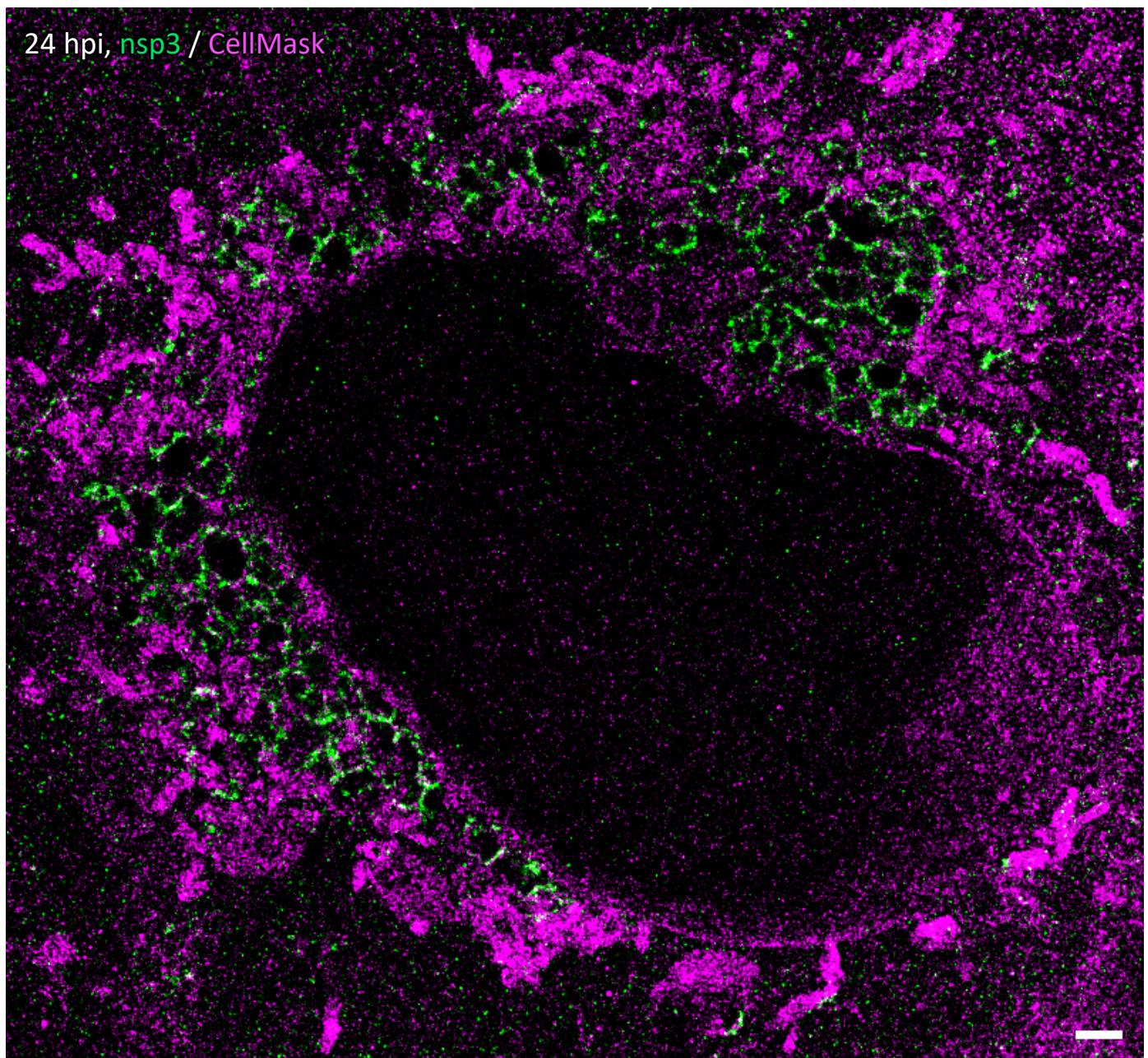
Supplementary figures

Nanoscale cellular organization of viral RNA and proteins in SARS-CoV-2 replication organelles

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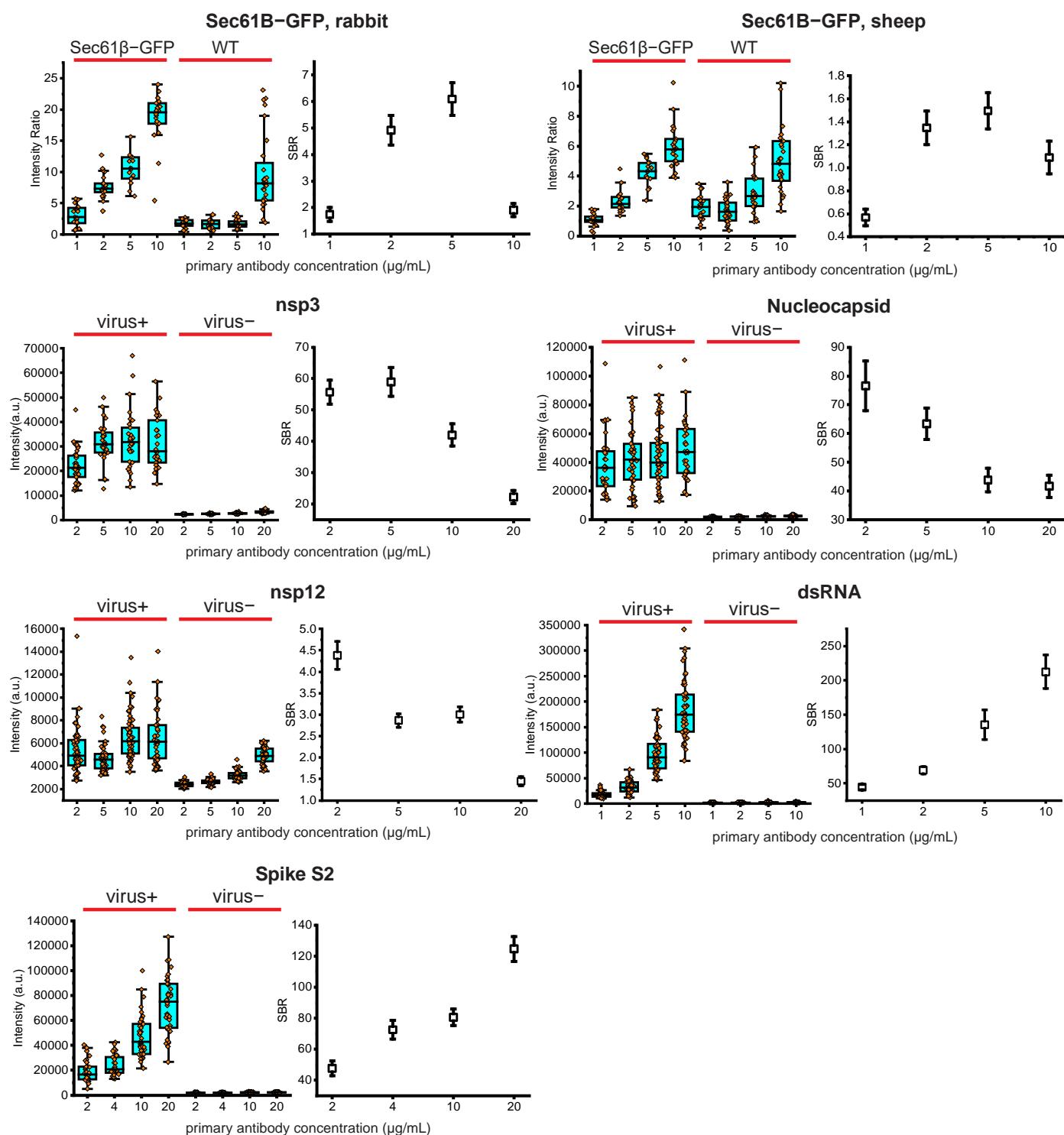
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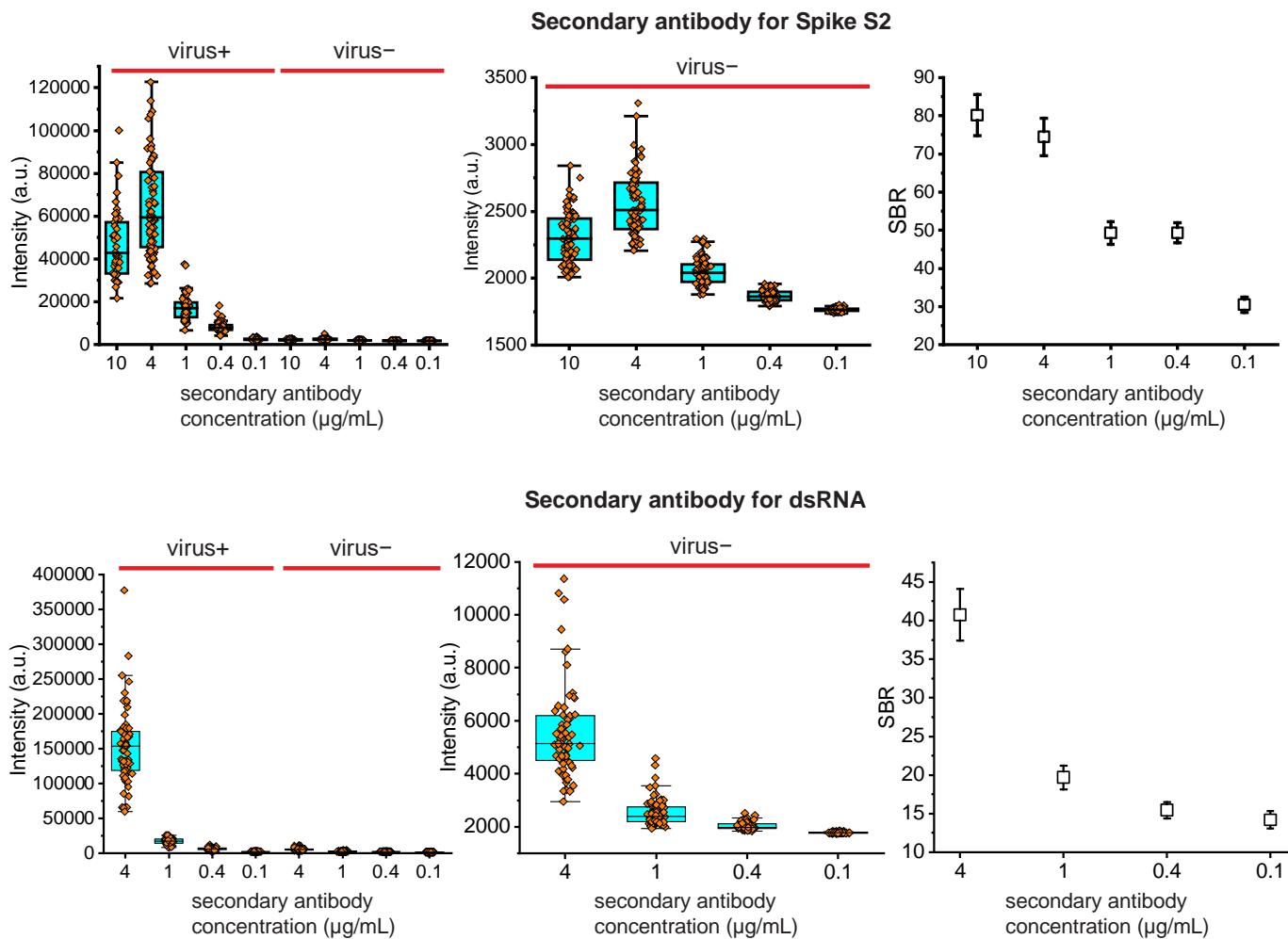
Supplementary Fig. S1. Nanoscale colocalization of nsp3 with membranes at 24 hpi.

SR image of nsp3 and membranes as labelled by CellMask in SARS-CoV-2 infected cells at 24 hpi. Nsp3 forms a network-like pattern in the perinuclear region that colocalizes with the CellMask pattern. Scale bar, 1 μ m.



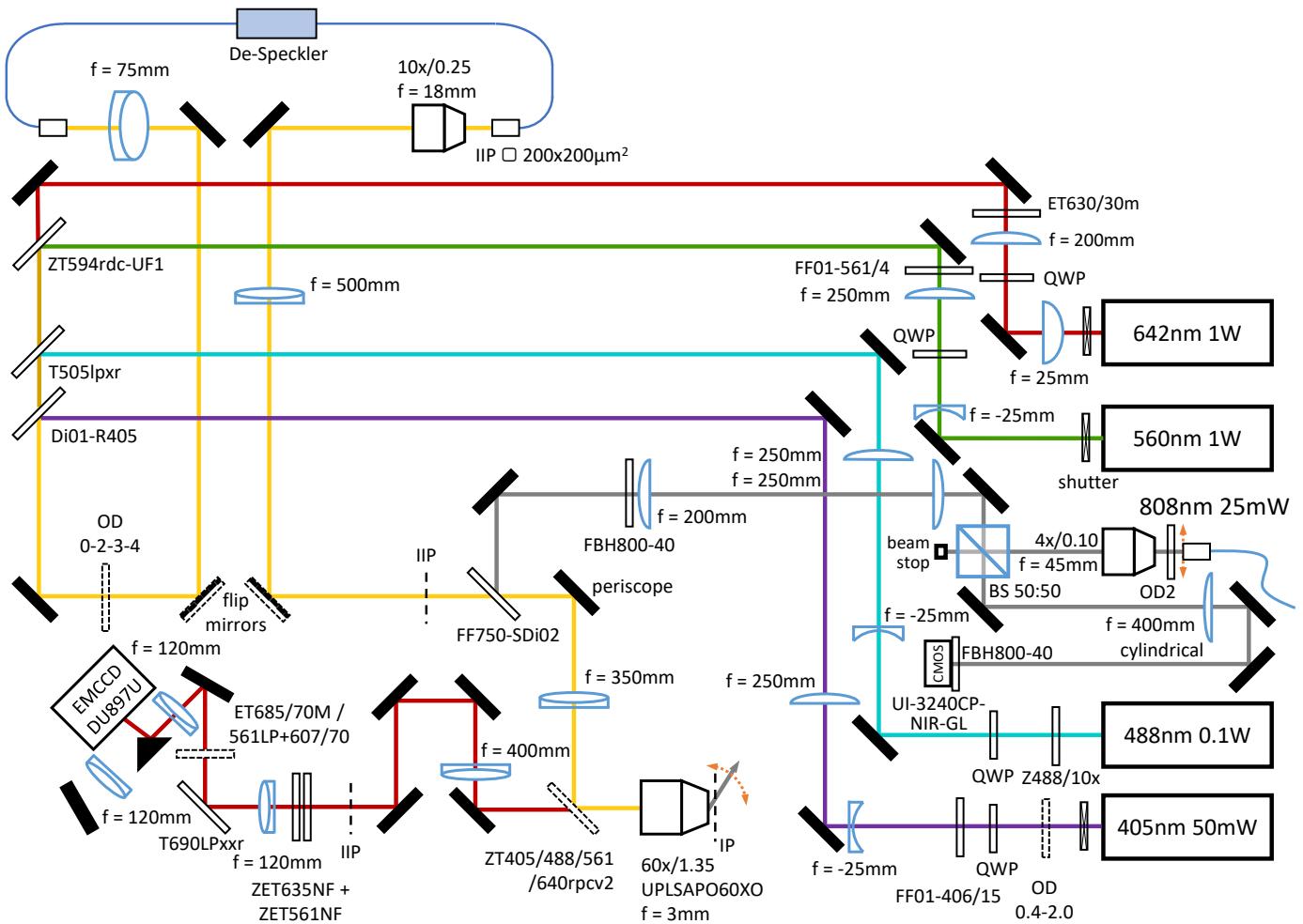
Supplementary Fig. S2. Optimization of primary antibody concentrations.

The concentration of primary antibodies was optimized to minimize the background or to maximize the signal to background ratio (SBR) between SARS-CoV-2 infected and non-infected cells or between cells expressing Sec61β-GFP and WT cells (see Methods)



Supplementary Fig. S3. Optimization of secondary antibody concentrations.

The concentration of secondary antibodies was optimized to minimize the background or to maximize the SBR between SARS-CoV-2 infected and non-infected cells (see Methods).



Supplementary Fig. S4. Path diagram of SR microscope used in this study.

Black-filled icons: mirrors; thin empty rectangles: dichroic or neutral density filters; dashed rectangles: movable or motorized components; boxes: cameras or lasers; bent lines: optical fiber; icons with blue edges: lenses or a beam splitter cube; QWP: quarter-wave plate; IP: image plane; IIP: intermediate image plane; BS: beam splitter; OD: optical density. Optics are shown for producing a second image on the EMCCD, but the second path was not used in this study. The gray lines denote the 808 nm beam in the focus lock apparatus.