

**FIG S5** Reovirus transcripts differentially localize in viral factories at late timepoints

- post-adsorption. HEK293T cells were adsorbed with reovirus strain T3D at an MOI of 20
- 4 PFU/cell, incubated for 9, 24, and 48 h, fixed, stained using RNA FISH probes specific

- 5 for σNS mRNA (white) or σ3 mRNA (yellow), μNS-specific antiserum (magenta), and
- 6 DAPI (blue), and imaged using confocal microscopy. Bar, 5 μm. The percentage of
- 7 cytoplasmic (B, white bars) σNS-mRNA and (B, yellow bars) σ3-mRNA FISH signals in
- 8 reovirus factories was quantified by dividing the sum of FISH signal in reovirus factories
- 9 by the sum of cytoplasmic FISH signal. Individual data points represent single cells.
- 10 Percentage values that differ significantly from those obtained from 9 h post-adsorption
- by one-way analysis of variance (ANOVA) with Dunnett's multiple-comparison test are
- 12 shown (\*, *P* < 0.0332; \*\*\*, *P* < 0.0002; \*\*\*\*, *P* < 0.0001).

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