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Supplemental information

Inhibitors of VPS34 and fatty-acid metabolism suppress SARS-CoV-2 replication

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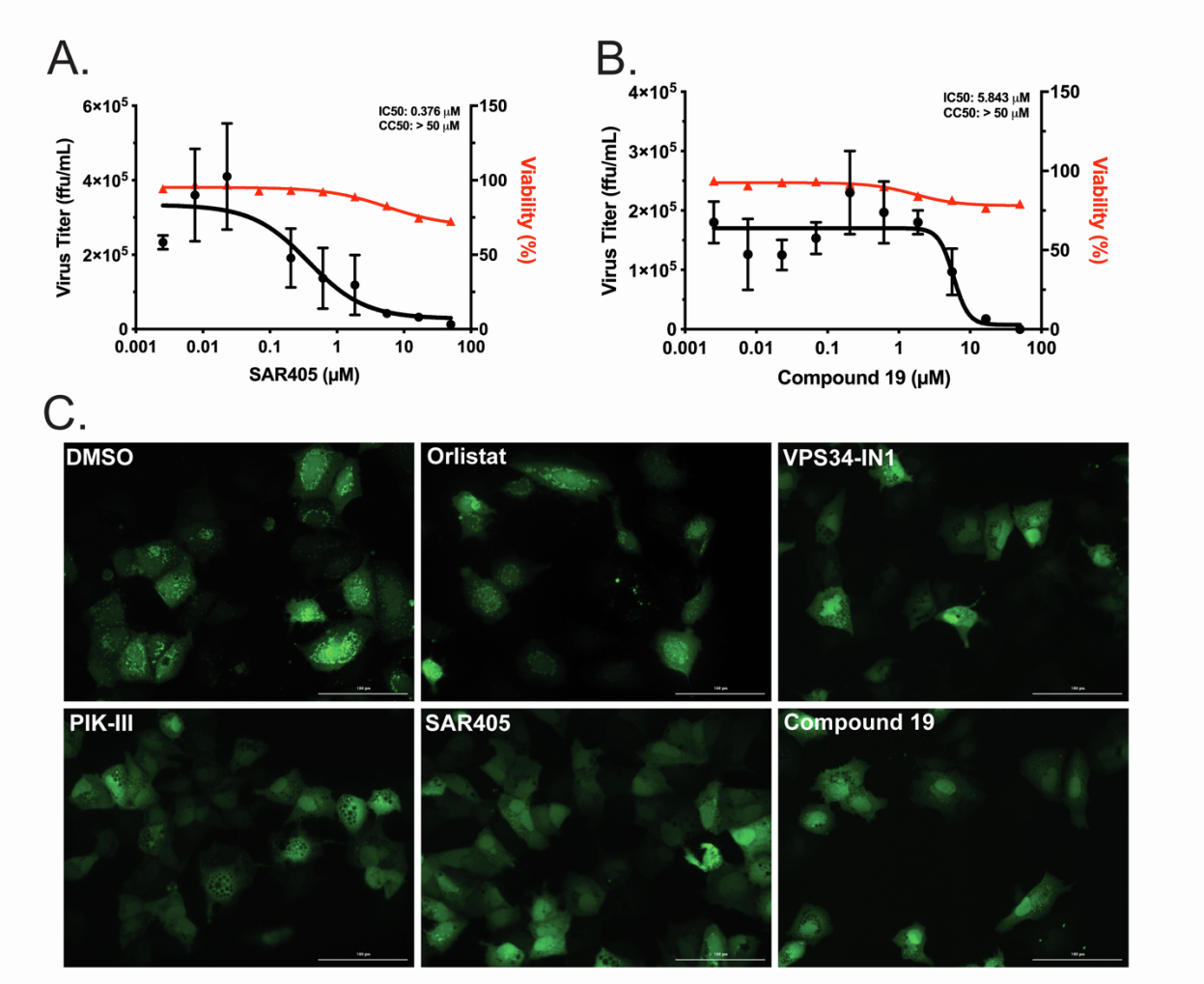
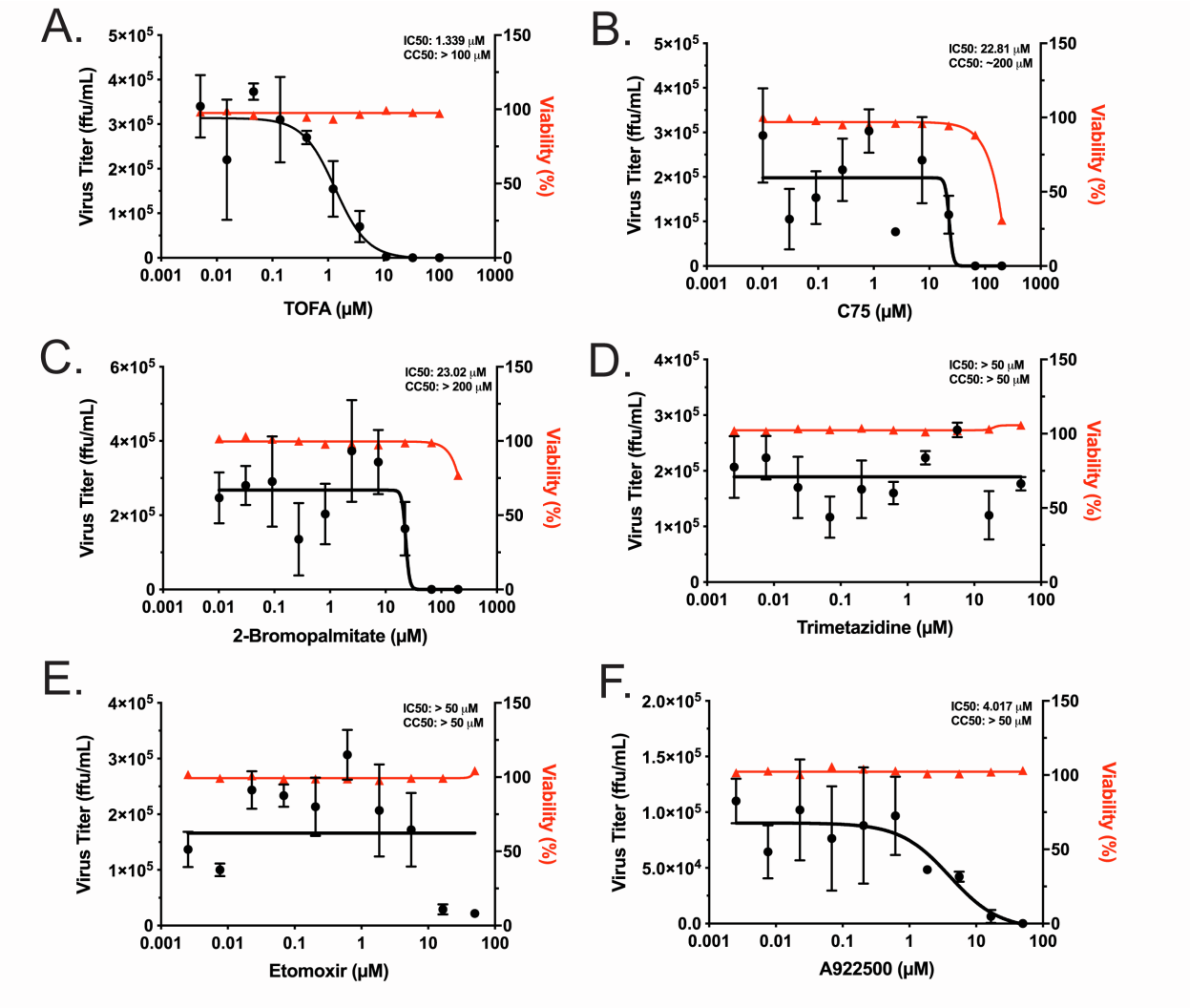
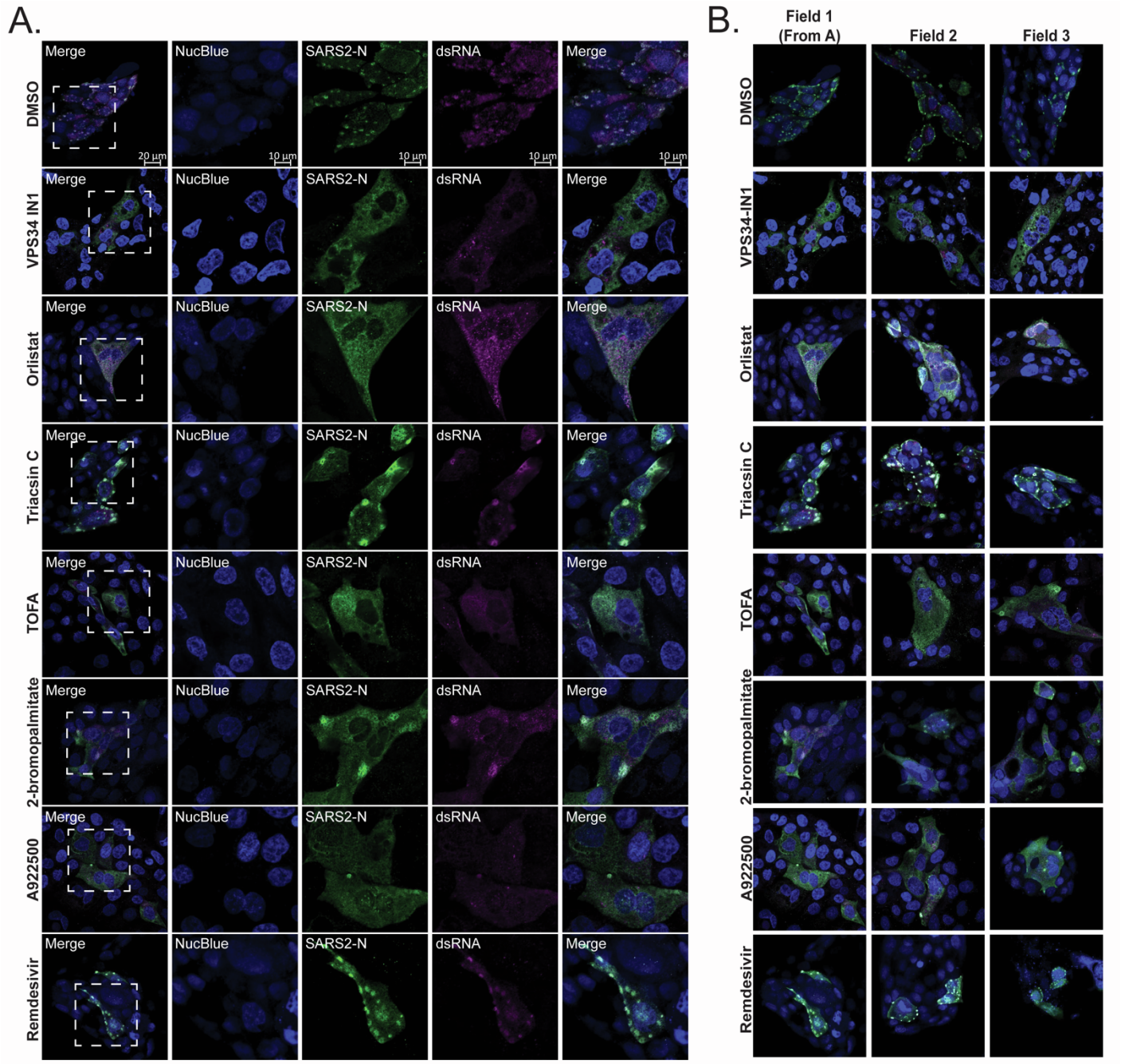
Supplemental Figures:



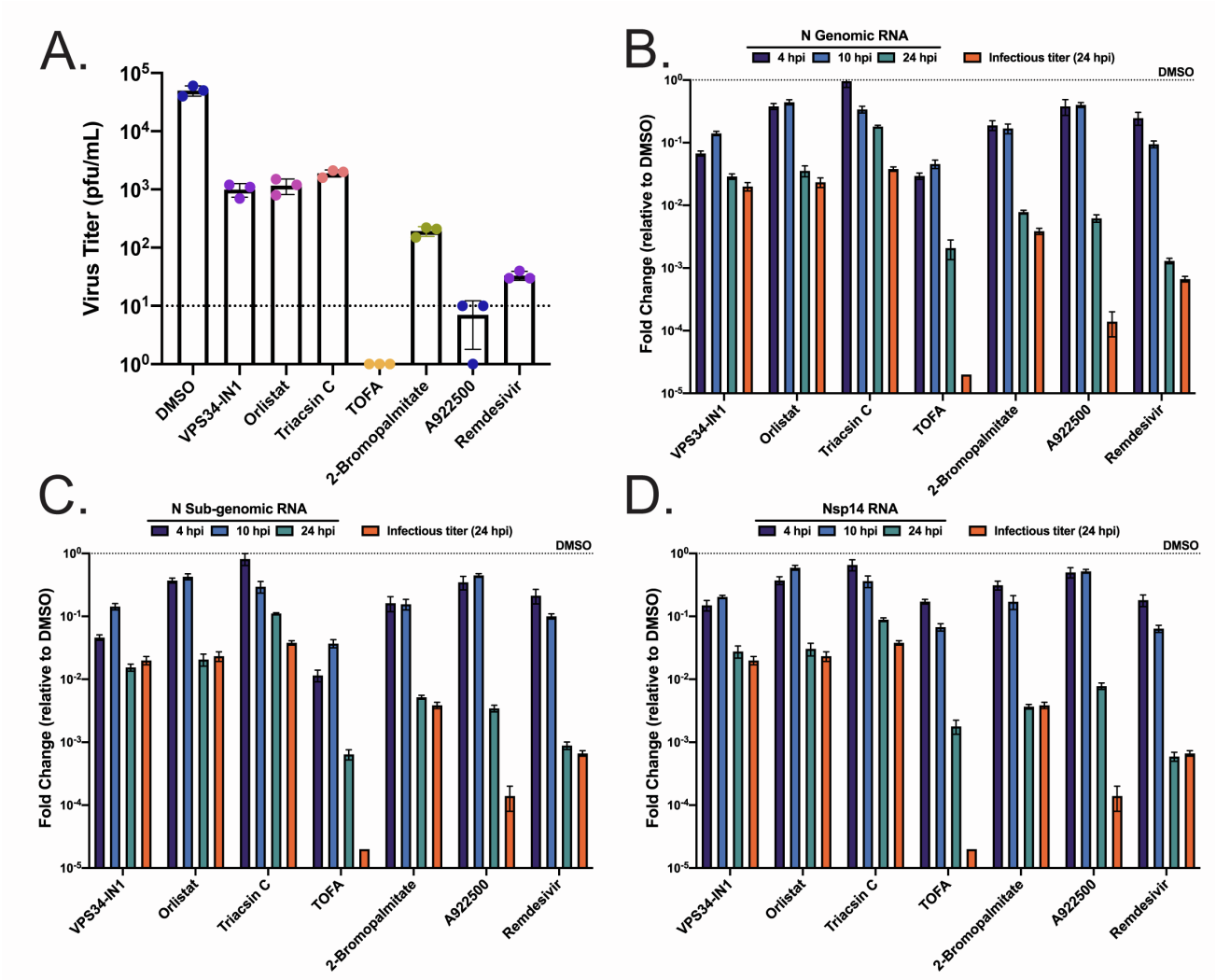
Supplementary Figure 1. VPS34 inhibitors actively block VPS34 activity. Related to Figure 5. The SARS-CoV-2 inhibitory activities of two additional VPS34 inhibitors (A) SAR405. (B) Compound 19 were tested. Calu-3 cells were seeded in 96-well format and grown to confluency. Cells were pre-treated with a series of 3-fold dilutions of the indicated compounds or DMSO for 1 hour, then infected with SARS-CoV-2 at an MOI of 0.01. Supernatants were collected at 48 hpi and infectious virus was quantified by focus forming assay on VeroE6 cells and presented as focus forming units (ffu)/mL. Cytotoxicity assays were performed in parallel and used to calculate percent viability as show in red (A). (C) To determine whether VPS34 inhibitors actively block VPS34 kinase activity, Huh7 cells were seeded in black walled 96-well plates and transfected with a 2X-FYVE-GFP plasmid. 24 hpt cells were treated with DMSO, VPS34-IN1 (2 μM), PIK-III (5 μM), SAR405 (5 μM), Compound 19 (5 μM), or Orlistat (200 μM). Cells were imaged to determine 2X-FYVE-GFP localization (B) using a BioTek Cytation 5. Scale bars represent 100 μM. Data is representative of the mean and standard error of three replicates.



Supplementary Figure 2. Effect of inhibitors of fatty acid metabolism on SARS-CoV-2. Related to Figure 6. Calu-3 cells were seeded in 96-well format and grown to confluency. Cells were pre-treated with a series of 3-fold dilutions of (A) TOFA, (B) C75, (C) 2-bromopalmitate, (D) Trimetazidine, (E) Etomoxir, (F) A922500 or DMSO for 1 hour, then infected with SARS-CoV-2 at an MOI of 0.01. Supernatants were collected at 48 hpi and infectious virus was quantified by focus forming assay on VeroE6 cells and presented as focus forming units ffu/mL (black). Cytotoxicity assays were performed in parallel and used to calculate percent viability (red). Data is representative of the mean and standard error of three replicates.



Supplementary Figure 3. Inhibition of VPS34 kinase activity and fatty acid metabolism affect formation of SARS-CoV-2 replication centers. Related to Figure 6. (A) Calu-3 cells were pre-seeded in 24-well format on fibronectin coated glass coverslips, allowed to grow to partial confluency, and infected at an MOI of 1. Inoculum was removed 2 hpi and replaced with media containing VPS34-IN1 (5 µM), Orlistat (500 µM), Triacsin C (5 µM), TOFA (50 µM), 2-bromopalmitate (50 µM), A922500 (30 µM), Remdesivir (1 µM), or DMSO. 24 hours post infection, cells were fixed and indirect immunofluorescence was performed using primary antibodies against SARS-CoV-2 N and dsRNA, and AlexaFluor488 and AlexaFluor647 conjugated secondaries, respectively. Cover slips were mounted using ProLong Glass Antifade mountant with NucBlue stain (ThermoFisher). Samples were imaged on Zeiss LSM800 Confocal and images were rendered in ZenBlue. Representative images are shown. (B) Additional fields of view of the experiment described in (A). The 20 µM scale bar shown for the DMSO sample in panel (A) applies to all images in this column and to the images in panel (B). The enlarged images in panel (A) correspond to the white hatched boxes in the first column. The 10 µM scale bars shown apply to their respective columns in panel (A).



Supplementary Figure 4. Mechanistic characterization of anti-SARS-CoV-2 activity on RNA synthesis. Related to Figure 6. Calu-3 cells were pre-seeded in 24-well format, allowed to grow to confluency, and infected at an MOI of 0.01. 2 hours post-infection cells were treated with VPS34-IN1 (5 μM), Orlistat (500 μM), Triacsin C (5 μM), TOFA (50 μM), 2-bromopalmitate (50 μM), A922500 (30 μM), Remdesivir (1 μM), or DMSO. At 4, 10, and 24 hpi total RNA was extracted from the cell monolayers, and 24 hpi supernatants were harvested for viral titers. Virus titers were determined by plaque assay (A). Levels of genomic RNA, subgenomic N RNA, and NSP14 RNA were quantified via qPCR (see materials and methods). Data are represented as fold change of RNA levels in infected compound treated samples versus infected DMSO treated samples (B-D). The virus titer at 24 hpi for compound treated cells is plotted alongside the qPCR data and presented as fold-change compared to titers from DMSO treated cells (orange bars). Data is representative of the mean and standard error of three replicates.