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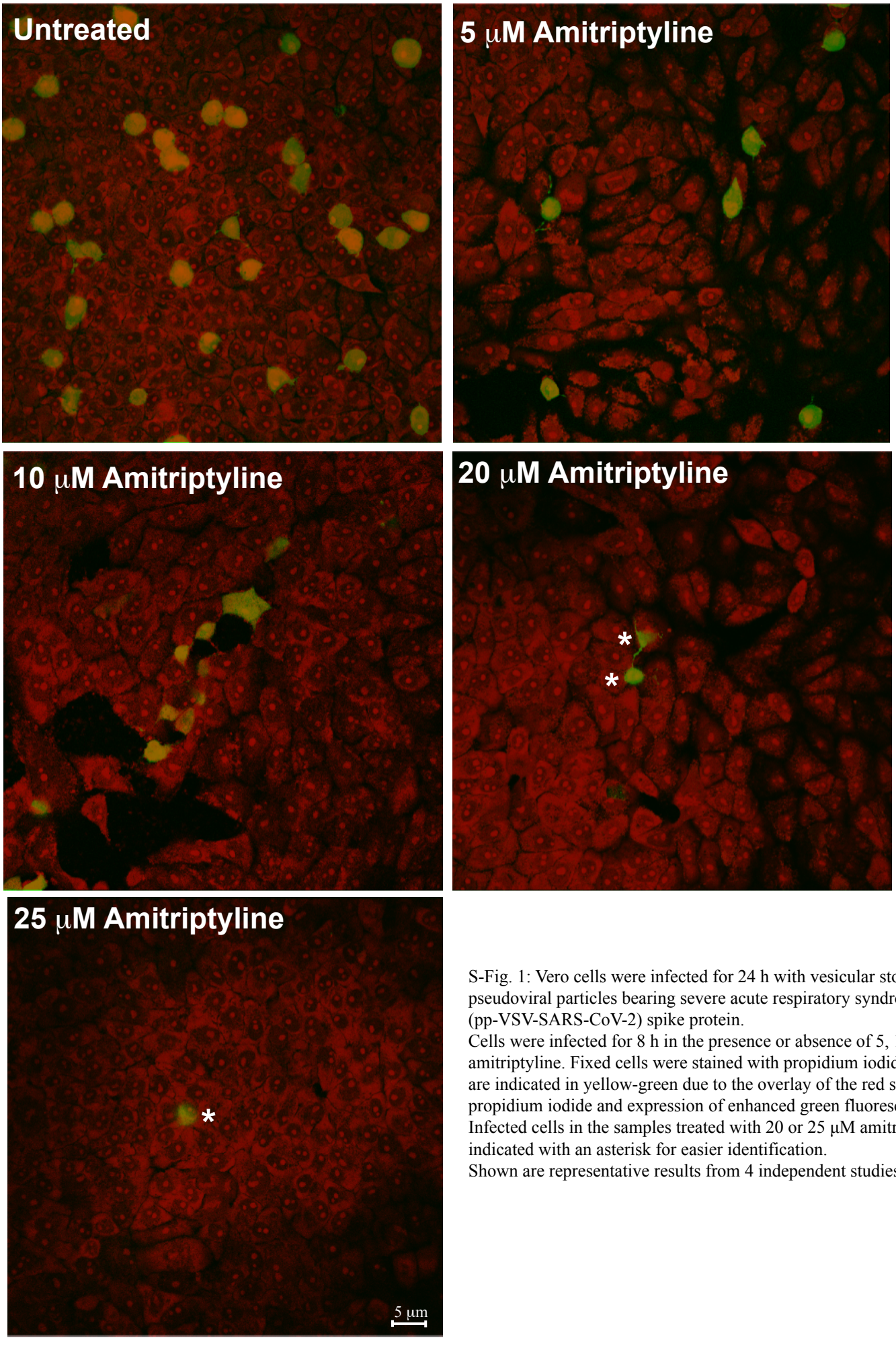
Supplemental Information

Pharmacological Inhibition of Acid Sphingomyelinase Prevents Uptake of SARS-CoV-2 by Epithelial Cells

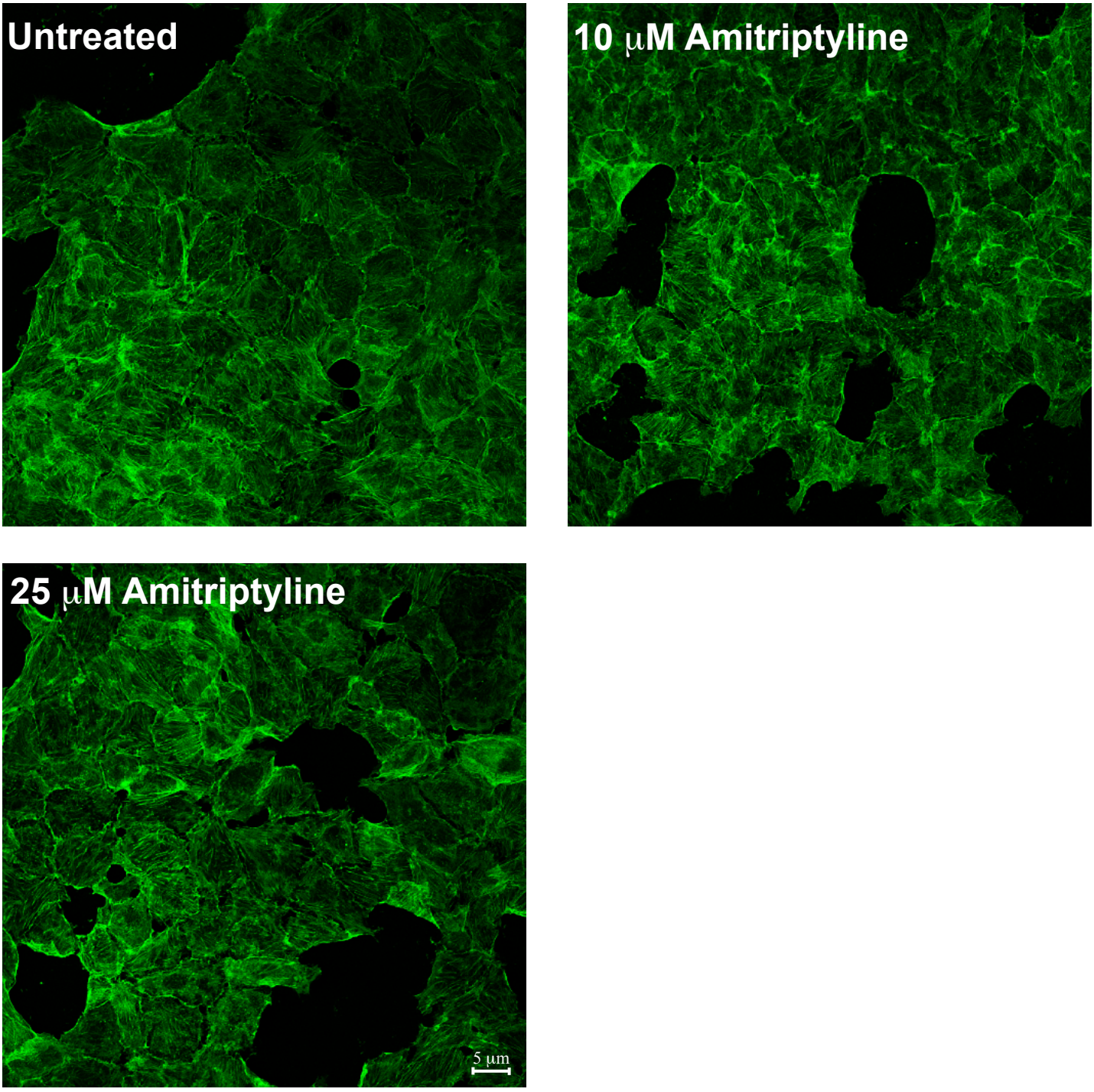
Alexander Carpinteiro, Michael J. Edwards, Markus Hoffmann, Georg Kochs, Barbara Gripp, Sebastian Weigang, Constantin Adams, Elisa Carpinteiro, Anne Gulbins, Simone Keitsch, Carolin Sehl, Matthias Soddemann, Barbara Wilker, Markus Kamler, Thomas Bertsch, Karl S. Lang, Sameer Patel, Gregory C. Wilson, Silke Walter, Hartmut Hengel, Stefan Pöhlmann, Philipp A. Lang, Johannes Kornhuber, Katrin Anne Becker, Syed A. Ahmad, Klaus Fassbender, and Erich Gulbins

Supplementary Fig. S1: Amitriptyline prevents infection with pp-VSV-SARS-CoV-2 spike. Related to Fig. 1A.



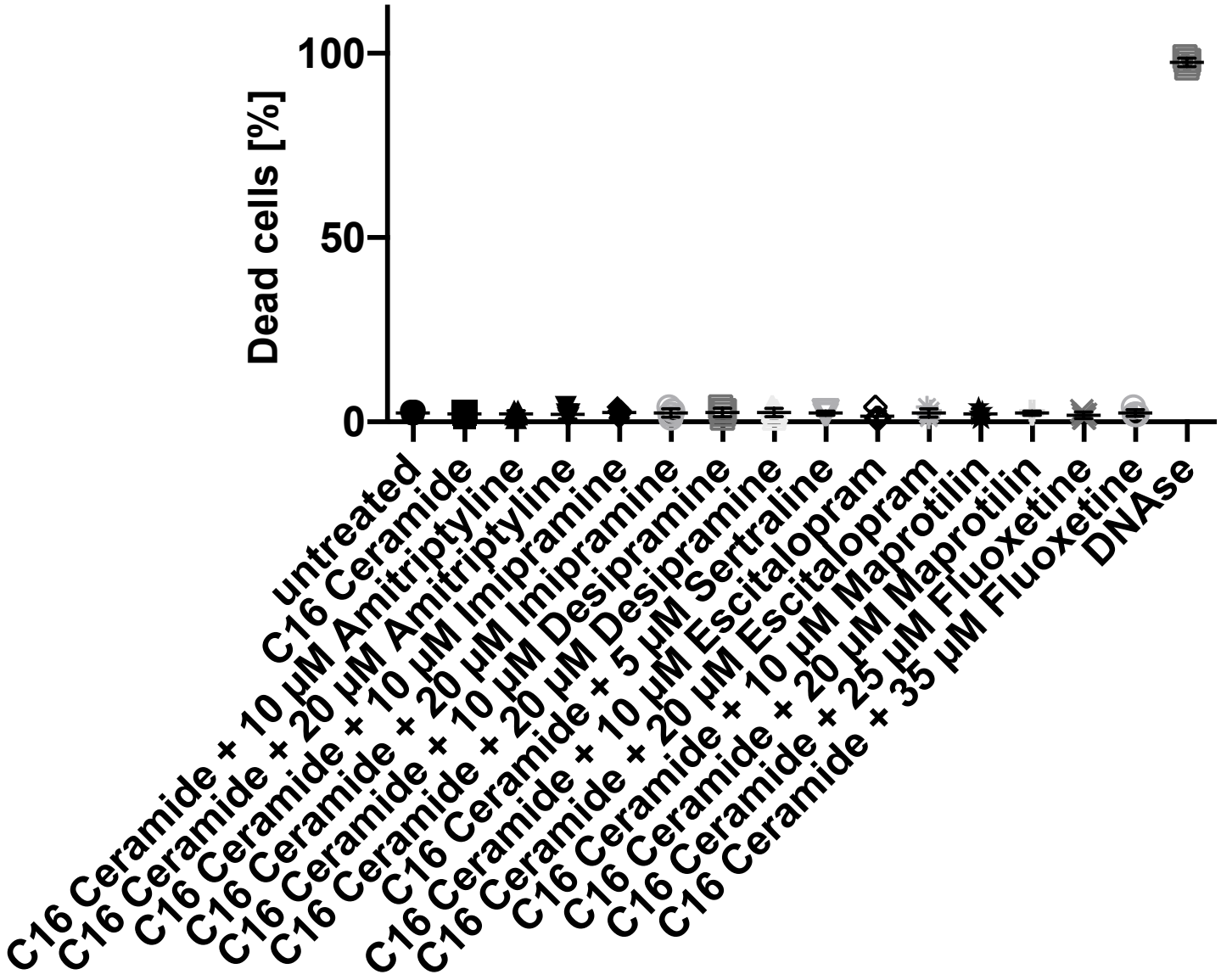
S-Fig. 1: Vero cells were infected for 24 h with vesicular stomatitis virus (VSV) pseudoviral particles bearing severe acute respiratory syndrome coronavirus 2 (pp-VSV-SARS-CoV-2) spike protein.
Cells were infected for 8 h in the presence or absence of 5, 10, 20, or 25 μM amitriptyline. Fixed cells were stained with propidium iodide. Infected cells are indicated in yellow-green due to the overlay of the red staining from propidium iodide and expression of enhanced green fluorescent protein (eGFP). Infected cells in the samples treated with 20 or 25 μM amitriptyline are indicated with an asterisk for easier identification.
Shown are representative results from 4 independent studies. Related to Fig. 1A.

Supplementary Fig. S2: Treatment with Amitriptyline does not change the cytoskeleton. Related to Fig. 1C and 1D.



S-Fig. 2: Vero cells were grown on glass coverslips, treated with 10 or 25 μM amitriptyline for 24 h, washed, fixed, washed, permeabilized with 0.1% Triton X-100 and stained with FITC-Phalloidin. Cells were then analyzed by confocal microscopy on a Leica TCS SL confocal microscope . Shown are representative results from 4 independent studies. Related to Fig. 1C and 1D.

Supplementary Fig. S3: Addition of exogenous C16 ceramide does not kill cells. Related to Figure 4C and 5C.



S-Fig. 3: Cells were treated with 10 or 20 µM amitriptyline, 10 or 20 µM imipramine, 10 or 20 µM desipramine, 5 µM sertraline, 10 or 20 µM escitalopram, or 10 or 20 µM maprotiline or 25 or 35 µM fluoxetine for 4 h and 10 µM C16 ceramide (Avanti Polar Lipids; # 860516) was added as described in the method section. Cell death was analyzed by TUNEL assay (Roche) following exactly the protocol of the vendor. As positive control cells were permeabilized and treated with DNase prior to TUNEL staining. TUNEL staining was analyzed by flow cytometry. Given is the mean ± SD (n=5) of the percentage of TUNEL positive cells.
Related to Fig. 4C and 5C.