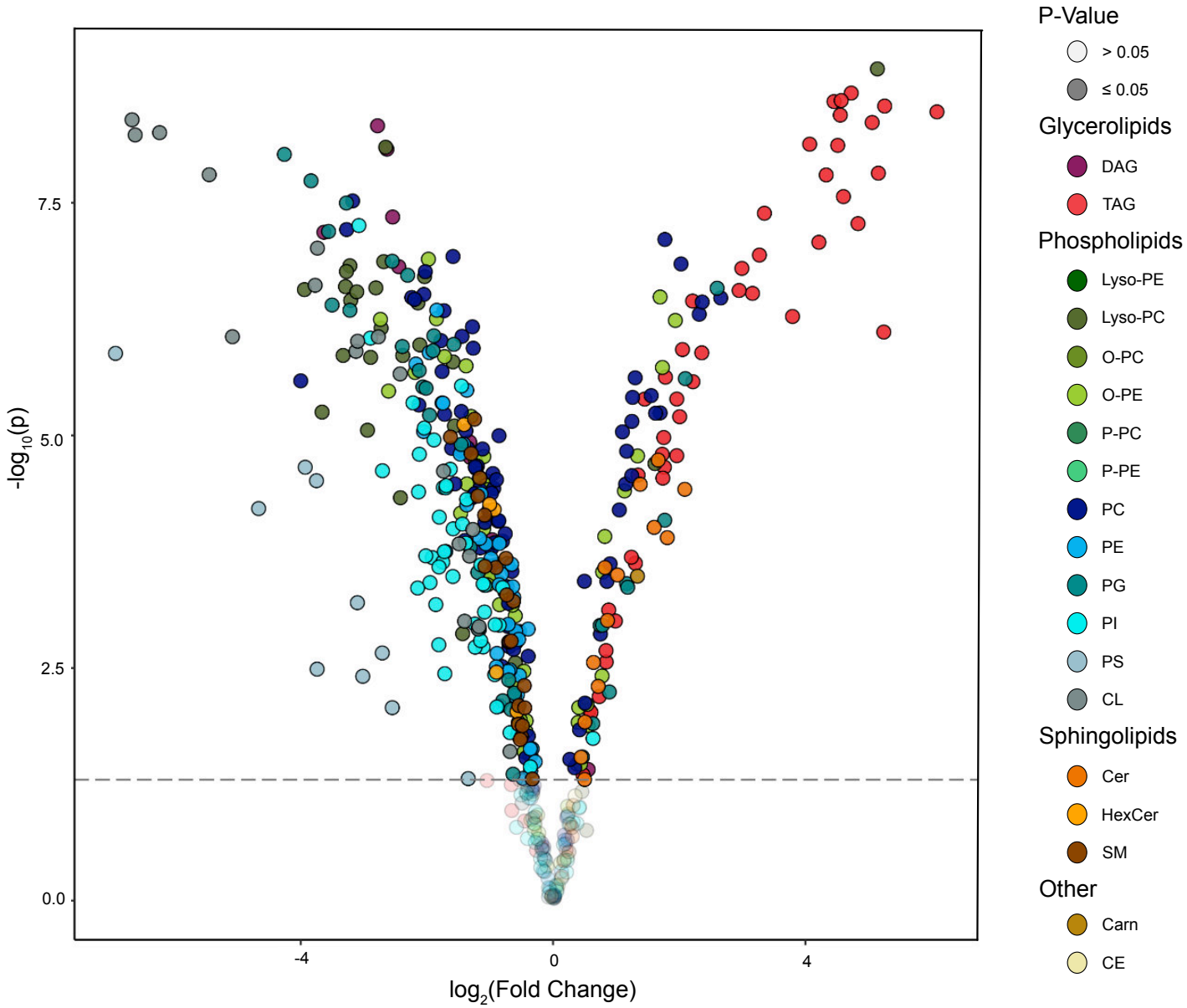


Fig. S1.

Volcano plot of individual lipid species detected by LC-ESI-MS/MS. Fold change is calculated relative to abundance in mock-infected controls. Lipid species are colored by class. Abbreviations: DAG = diacylglycerol; TAG = triacylglycerol; Lyso-PC = lysophosphatidylcholine; Lyso-PE = lysophosphatidylethanolamine; O-PC = phosphatidylcholine (ether linked); O-PE = phosphatidylethanolamine (ether linked); P-PC = phosphatidylcholine (plasmologen-linked); P-PE = phosphatidylethanolamine (plasmologen-linked); PC = phosphatidylcholine; PE = phosphatidylethanolamine; PG = phosphatidylglycerol; PI = phosphatidylinositol; PS = phosphatidylserine; CL = cardiolipin; Cer = ceramide; HexCer = hexosylceramide; SM = sphingomyelin; Carn = acylcarnitine; CE = cholesterol ester

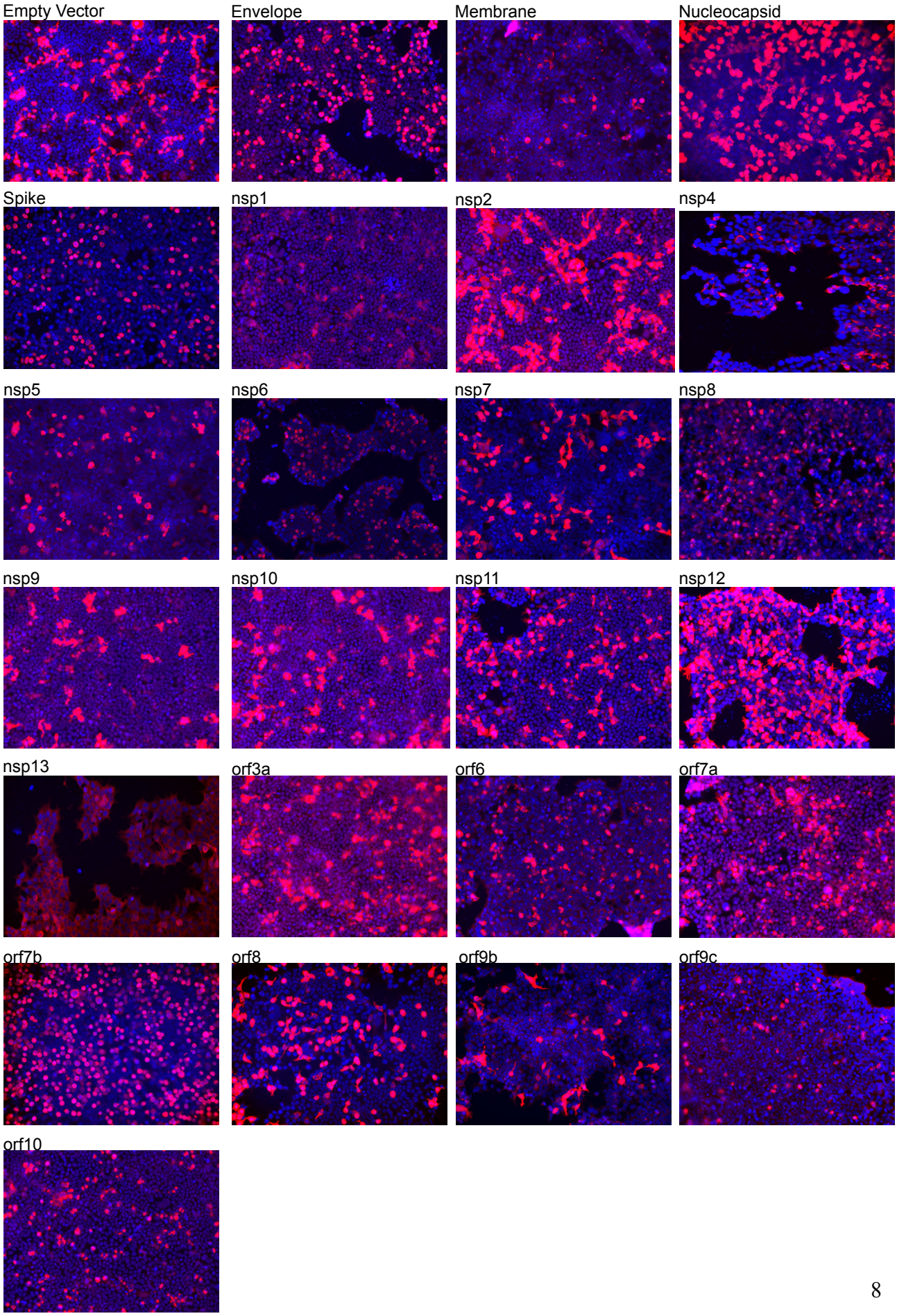
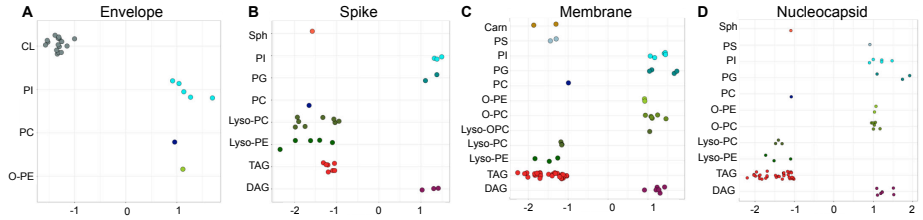
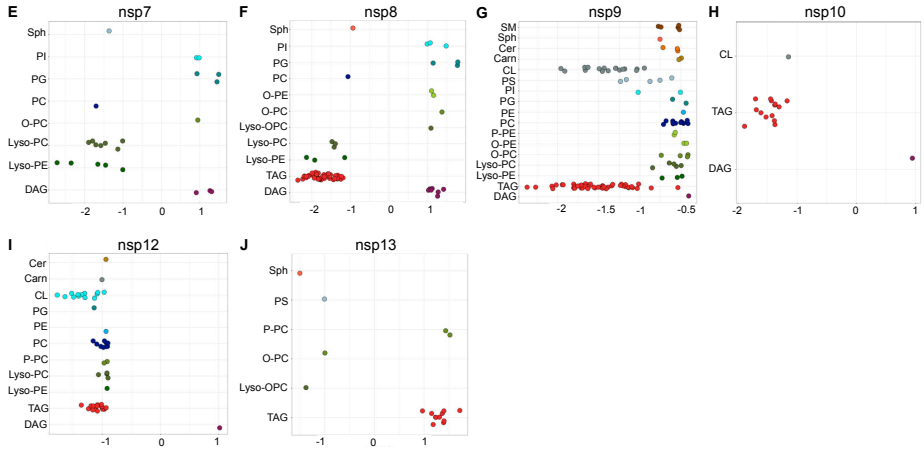

Empty Vector
Envelope
Membrane
Nucleocapsid
Spike
nsp1
nsp2
nsp4
nsp5
nsp6
nsp7
nsp8
nsp9
nsp10
nsp11
nsp12
nsp13
orf3a
orf6
orf7a
orf7b
orf8
orf9b
orf9c
orf10

Fig. S2.
Images of HEK293T cells transfected with each viral protein taken on a BZ-X700 all-in-one fluorescent microscope (Keyence) at 20x resolution. Red is anti-Strep-tag immunostaining; blue is DAPI.

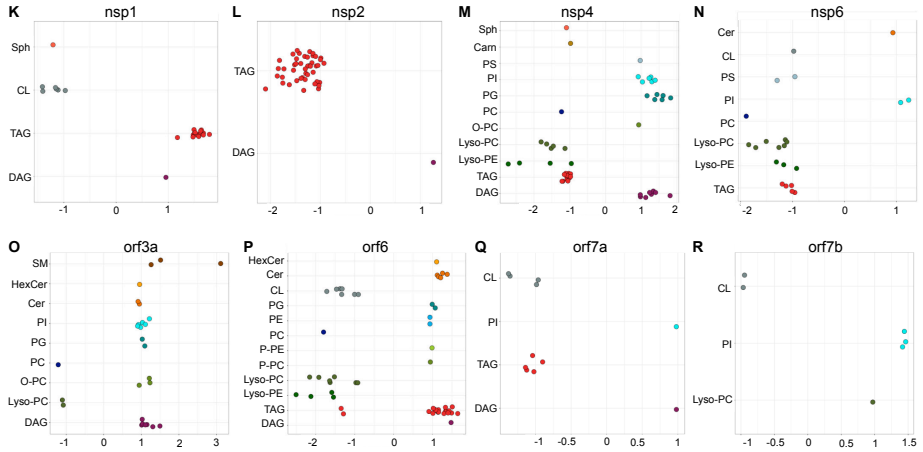
Structural Proteins



RNA-Binding Proteins



Membrane-Binding Proteins



Other

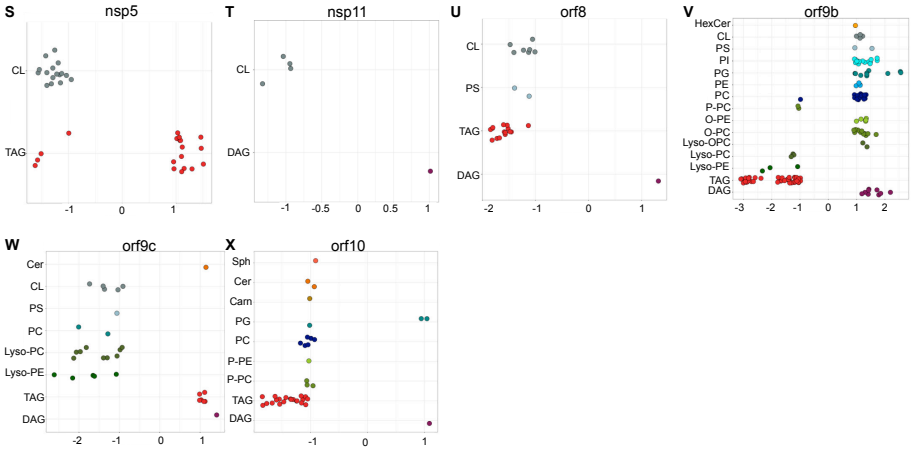


Fig. S3.

For each viral protein, the lipids that changed significantly relative to empty vector were selected. To identify the most influenced pathways, only lipids with a log2(fold change) greater than 0.9 or less than 0.9 were plotted. Log2(fold change) relative to empty vector for each individual lipid species is shown.
(A-D) Structural proteins
(E-J) RNA-binding proteins
(K-R) Membrane-binding proteins
(S-X) Other proteins

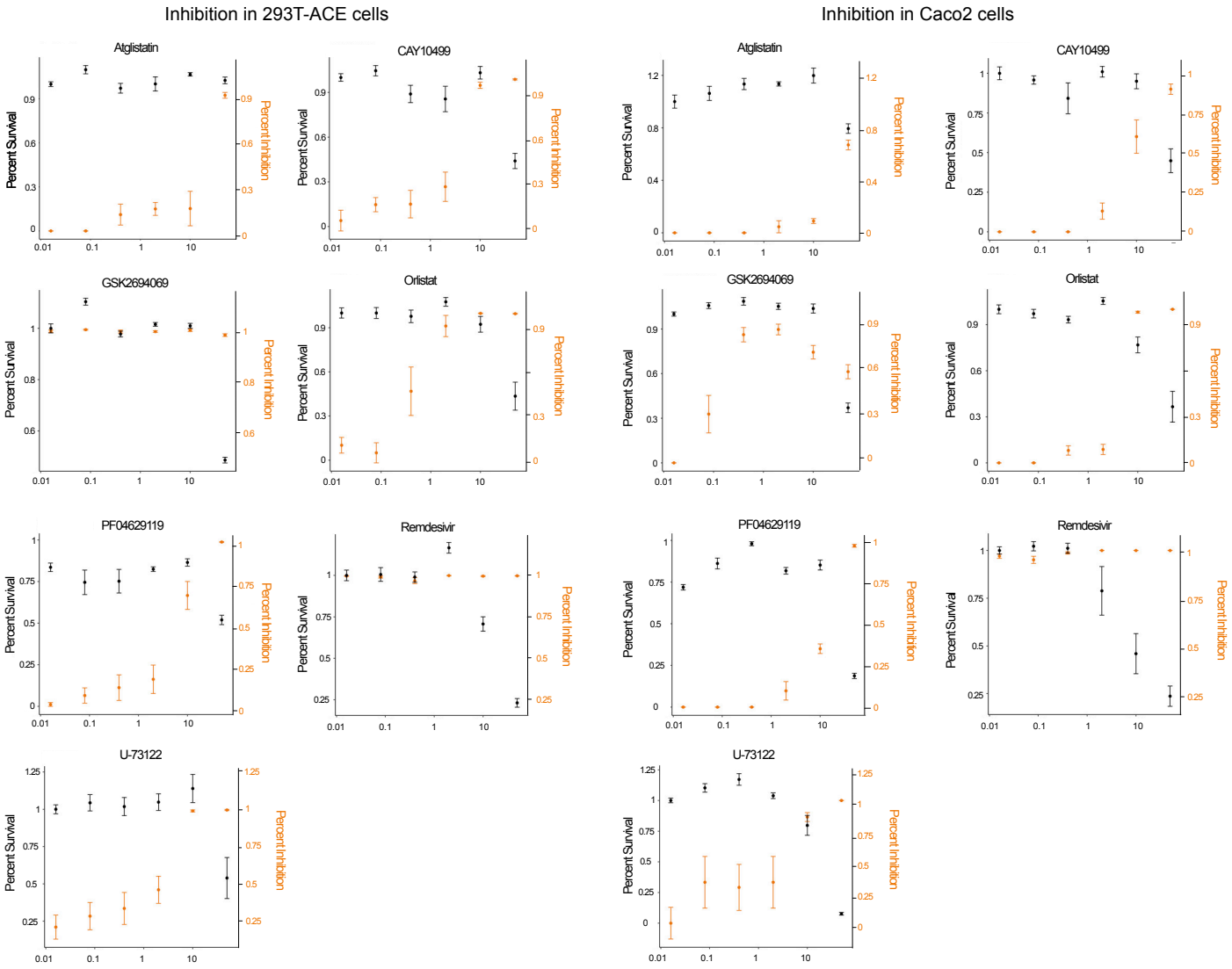


Fig. S4.
Percent inhibition/percent survival curves for each compound in 293T-Ace2 cells or Caco2 cells. Percent survival was calculated by dividing the resazurin fluorescence in drug-treated wells by the resazurin fluorescence in vehicle-treated wells. Percent inhibition was calculated by dividing the number of foci in drug-treated wells by the number of foci in vehicle-treated wells. Error bars represent standard error, calculated from three independent experiments.

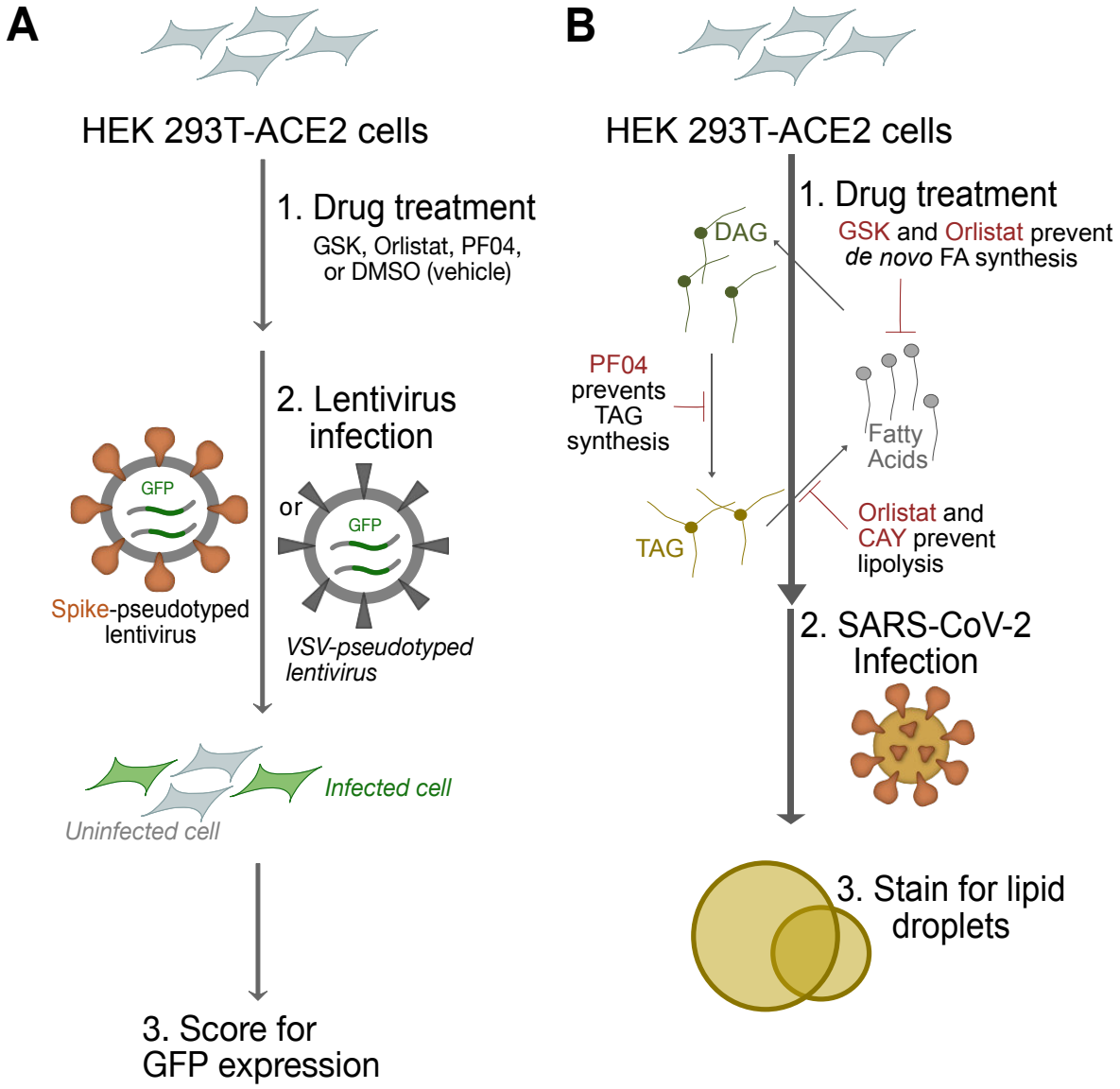


Fig. S5.
(**A**) Spike-mediated SARS-CoV-2 entry assay using Spike-pseudotyped lentivirus: experimental scheme. HEK293T-ACE2 cells are treated overnight with 10 µM of selected inhibitors then infected with lentiviruses coated expressing the SARS-CoV-2 Spike protein (or the VSV G protein as a control) and bearing a GFP construct. Successfully infected cells express GFP; GFP fluorescence (normalized to DAPI) is used to quantify Spike-mediated viral uptake (**B**) Experimental design to test the effect of glycerolipid biosynthesis inhibition on SARS-CoV-2 induced lipid droplet formation. HEK293T-ACE2 cells were treated overnight with 10 µM of selected inhibitors and then infected with SARS-CoV-2 (MOI = 1) and fixed after 48 hours. Lipid droplets and infected cells were vidsualized with BODIPY 493/503 and anti-dsRNA immunofluorescence, respectively.

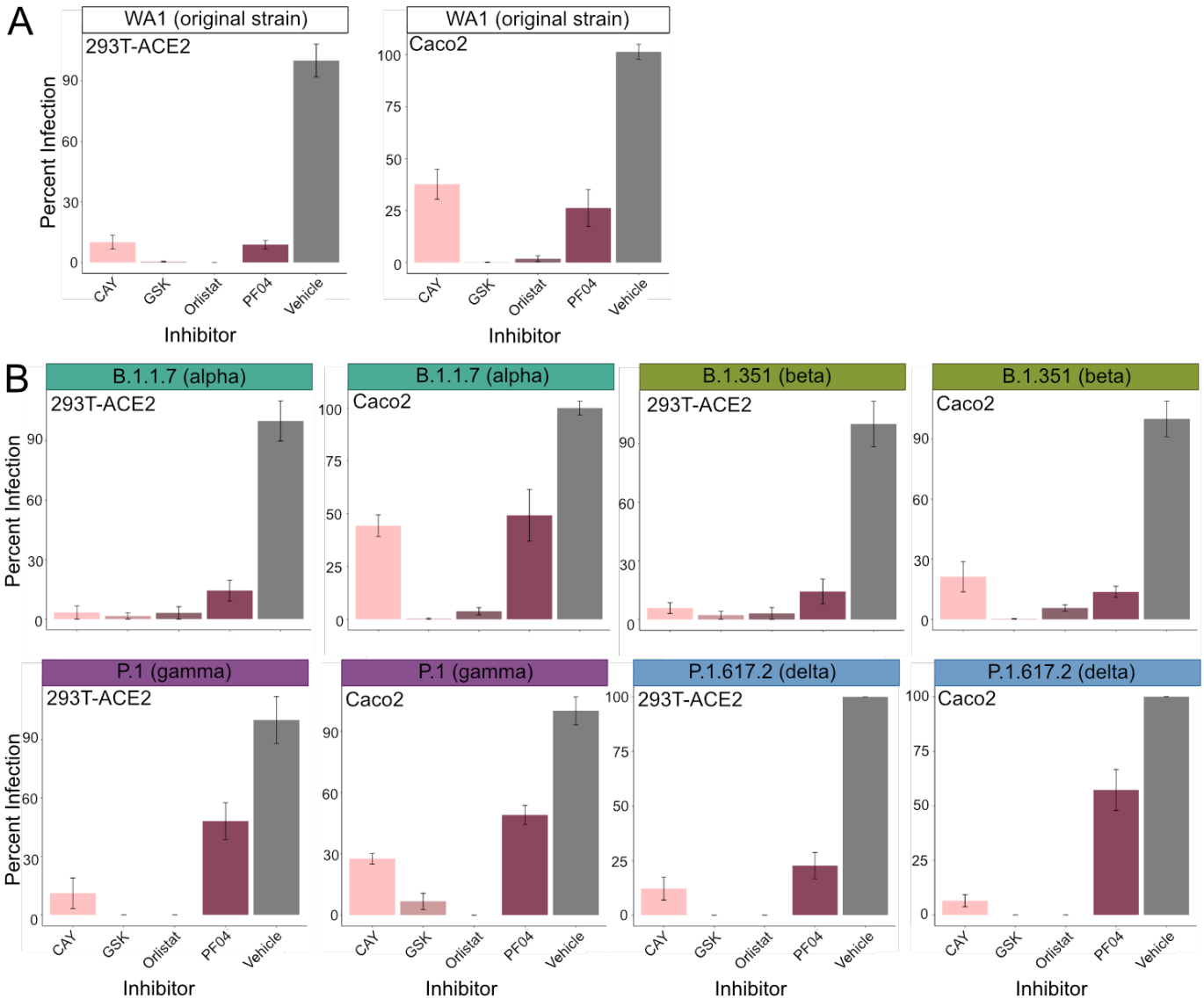


Fig. S6.

(**A**) Inhibition of the original SARS-CoV-2 strain by four inhibitors of glycerolipid biosynthesis, each at 10 µM overnight prior to a 48-hour infection (MOI = 0.1). Data are from three independent experiments; data are mean ± SE. (**B**) Inhibition of four variants of concern by four inhibitors of glycerolipid biosynthesis, each at 10 µM overnight prior to a 48-hour infection (MOI = 0.1). Data are from three independent experiments; data are mean ± SE.

Viral Protein	Plasmid ID	Amount of DNA (μg)
nsp1	A01	30
nsp2	A02	30
nsp4	A05	30
nsp5	A06	30
nsp7	A09	20
nsp8	A10	30
nsp9	A11	40
nsp10	A12	35
nsp11	B01	30
nsp12	B02	45
nsp13	B03	35
S	B07	30
orf3a	B08	30
E	B10	30
M	B11	20
orf6	B12	35
orf7a	C01	35
orf7b	C02	30
orf8	C03	30
N	C04	10
orf9b	C05	30
orf9c	C06	35
orf10	C07	45
Empty Vector	PLVX	35

Table S1.

Amounts of DNA used in viral protein transfection, related to Figure 2 and Figure S2.

Data S1. (separate file)
Fold changes and p-values for each lipid observed in the live virus infection experiment

Data S2. (separate file)
Fold changes and p-values for each lipid observed in the viral protein transfection experiment