Appendixes

Figure A1. Labeling JUNV with non-neutralizing antibody LD05 does not affect JUNV infectivity. Vero cells were cooled down to 4℃ for 15 min and cold JUNV was added to the cells for 30 min. Then, inoculum was removed and indicated concentrations of the LD05 or GD01 antibodies, both specific for the GPC of JUNV, were added for an additional 30 min at 4℃. Cells were then washed and incubated for 16 h at 37℃ in virus media to allow infection of the bound viruses. Cells were then harvested, fixed, permeabilized and stained using the anti-Nucleoprotein (NP) antibody SA02 coupled to an Alexa Fluor 647 to detect infected cells by flow cytometry. The LD05 antibody (blue line) did not show any neutralizing capacity, while the GD01 antibody (red line) was potently neutralizing at concentrations from 5 µg ml^{-1}.

Figure A2. Efficiency of the actin-perturbating agents. Vero cells were transfected with LifeAct-RFP, re-plated on coverslips the following day and allowed to adhere for 24 h before treatment for 45 min with the indicated compounds. Cells were then fixed and mounted for microscopy analysis. Images were acquired using a spinning disk confocal microscope. Micrographs are representative of the LifeAct-RFP distribution for each condition. The dynamic range is shown in the upper right corner and the 10 µm scale bar in the lower left corner of each image.

Figure A3. Characterization of the $Cdc42^{-/-}$ monoclonal SUM159 cells. (A) Snapshots of wild type (WT), $Cdc42^{-/-}$ clone #3 or $Cdc42^{-/-}$ clone #11 SUM159 cells imaged using bright-field illumination and a 10X objective. (B) WT, $Cdc42^{-/-}$ clone #3 or $Cdc42^{-/-}$ clone #11 SUM159 cells were transfected with LifeAct-RFP, a fluorescent protein labeling polymerized actin. Two days after transfection, cells were plated on coverslips and imaged using a spinning disk confocal microscope and a 63X objective. The snapshots are representative of the organization of the actin network observed for each cell type.

Figure A4. Inhibition of JUNV entry using ML141. Vero cells were untreated or pre-treated for 15 min with 20 µM ML141 or 10 µM 17C9 and subsequently infected with JUNV in the presence or absence of the compounds for an additional hour. Cells were washed and incubated in virus media containing an anti-GPC neutralizing antibody for 16 h (as in Fig. 3A). Cells were fixed and stained with an anti-NP antibody coupled to an A647 and the percentage of infected cells was measured by flow cytometry.

Figure A5. Characterization of the EGFP-Rab5c$^{+/+}$ and Rab7a$^{+/-}$ SVG-A cells. (A-C) Wild type (WT), EGFP-Rab5c$^{+/+}$ or Rab7a$^{+/-}$ SVG-A cells were starved for 1 h and 45 min in α-MEM, then untreated (A) or treated with 50 µM ZCL278 (B) or 1 µM Jasp (C) for 15 min and then left untreated, or treated with 250 ng ml^{-1} EGF for two additional hours in the presence of the indicated compounds. Cells were subsequently fixed, permeabilized and stained with a mouse anti-EGFR antibody and a secondary anti-mouse IgG antibody coupled to an A647. The histogram represents A647 mean fluorescence intensity measured by flow cytometry and reflects the amount of EGFR per cell normalized by the "no EGF" control for each condition. Data corresponds to the mean ± s.d. of duplicates from at least 10,000 cells per condition.

Video legends

Video 1. Time-lapse imaging of SVG-A Rab5c-EGFP$^{+/+}$ cells acquired by high-resolution spinning disc confocal microscopy. The movie is the maximum projection of a 3D time series lasting 90 sec, made of a z-stack of 5 planes spaced 0.5 µm and imaged every 2 sec.

Video 2. Time-lapse imaging of SVG-A Rab7a-EGFP$^{+/-}$ cells acquired by high-resolution spinning disc confocal microscopy. The movie is the maximum projection of a 3D time series lasting 90 sec, made of a z-stack of 5 planes spaced 0.5 µm and imaged every 1.5 sec.

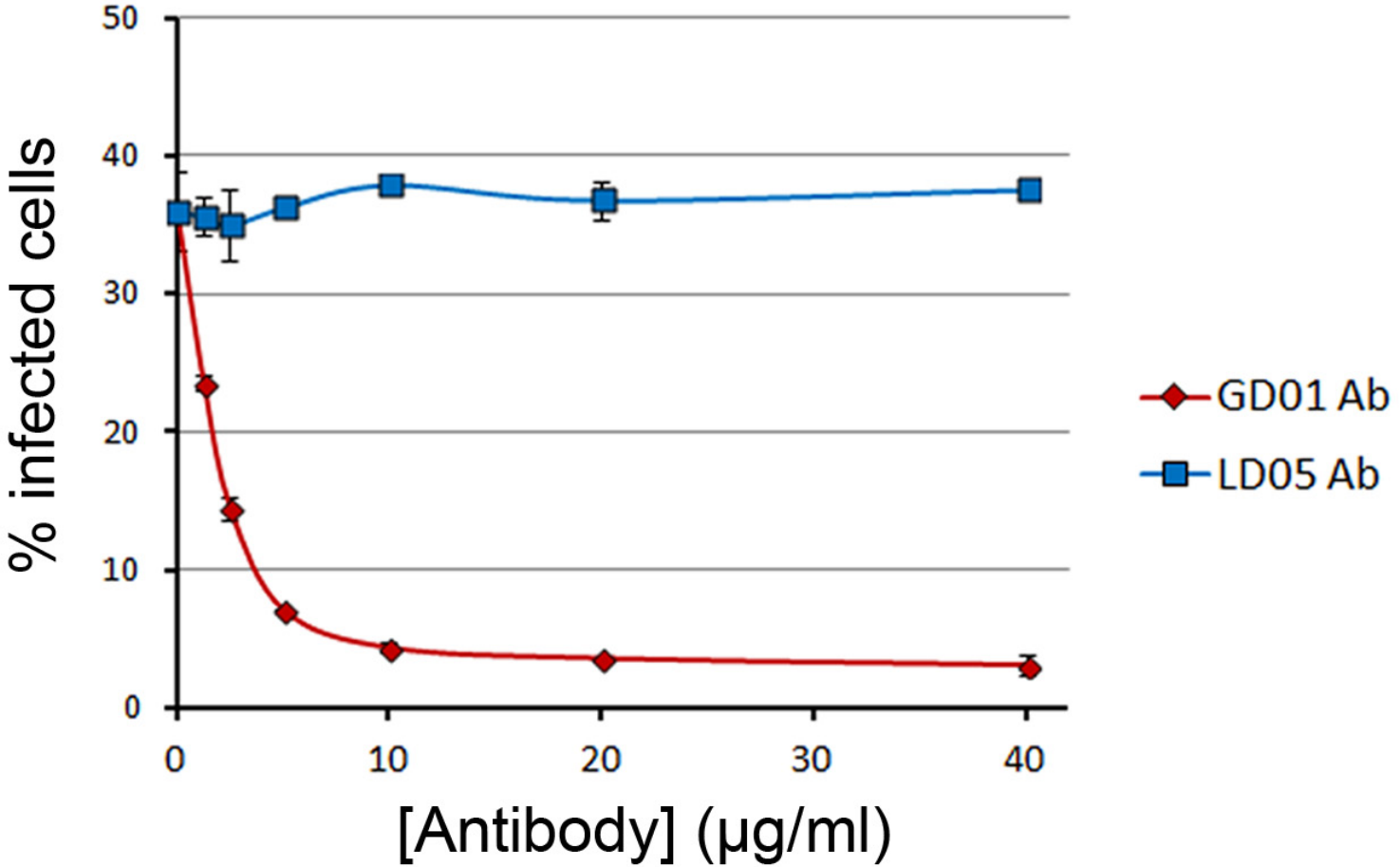

50
40
30
20
10
0
% infected cells
0
10
20
30
40
[Antibody] (µg/ml)
GD01 Ab
LD05 Ab

Figure A1

Polymerized actin network (LifeAct-RFP)

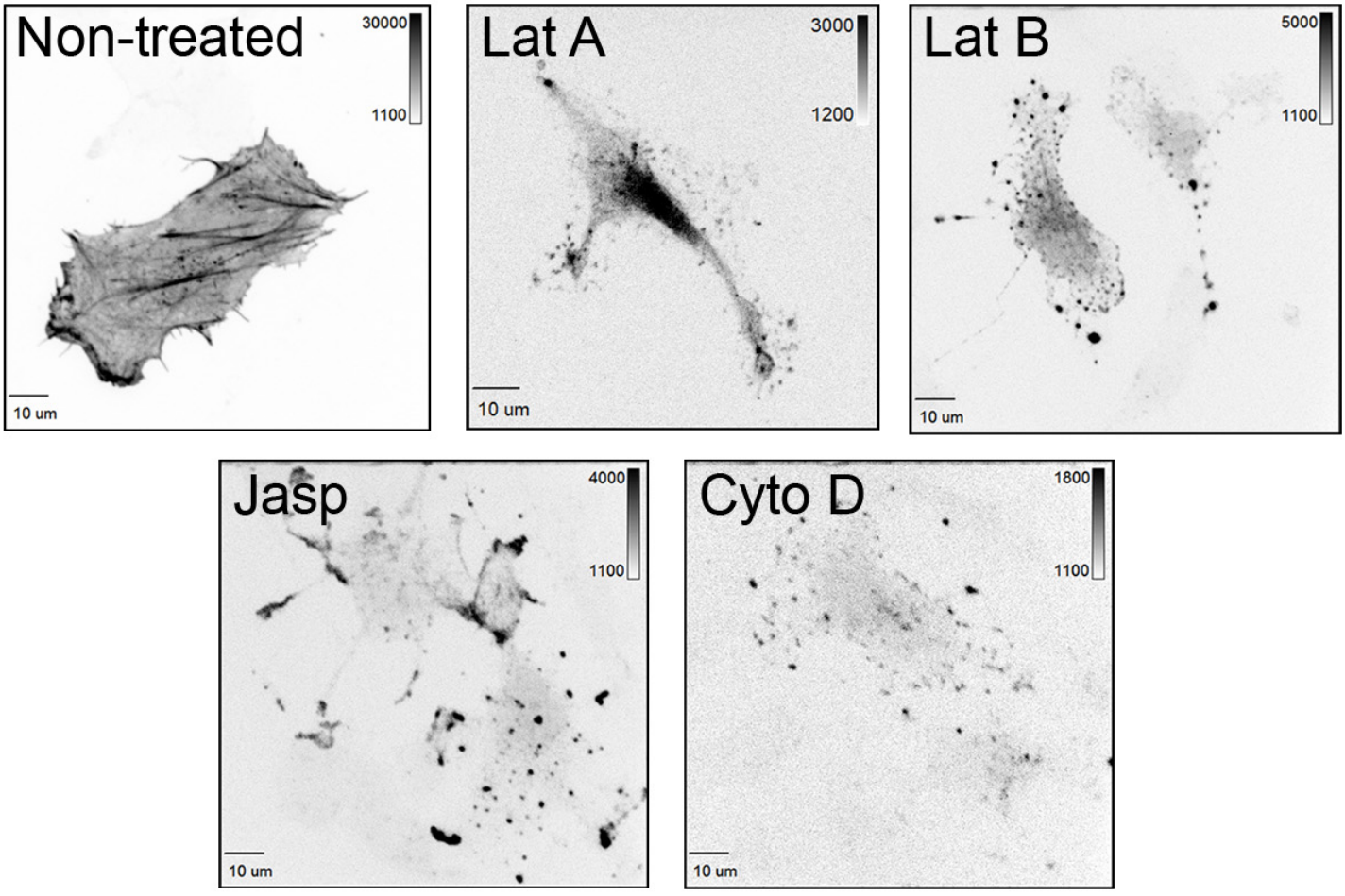


Figure A2

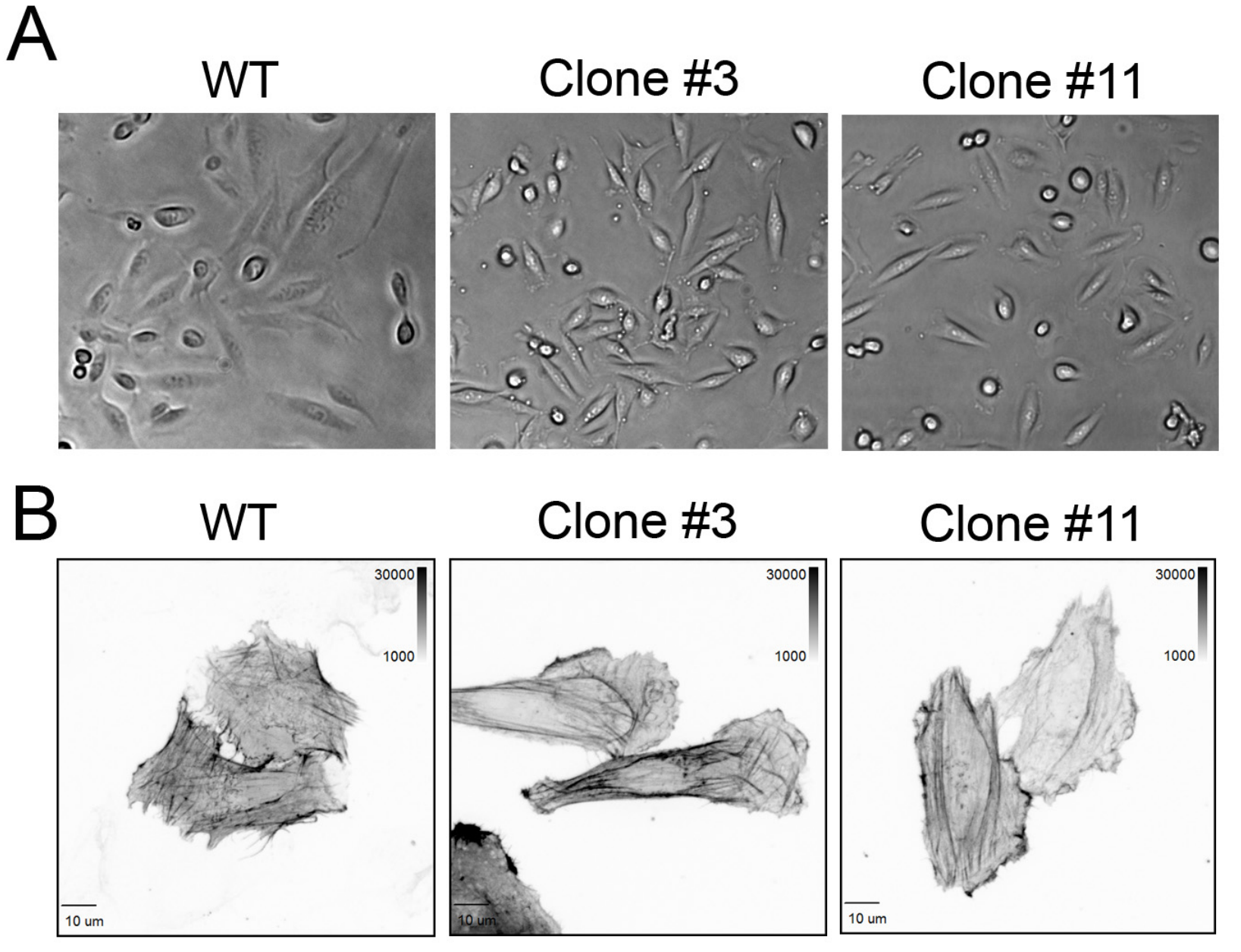

A
WT
Clone #3
Clone #11
B
WT
Clone #3
Clone #11
30000
1000
10 um
30000
1000
10 um
30000
1000
10 um

Figure A3

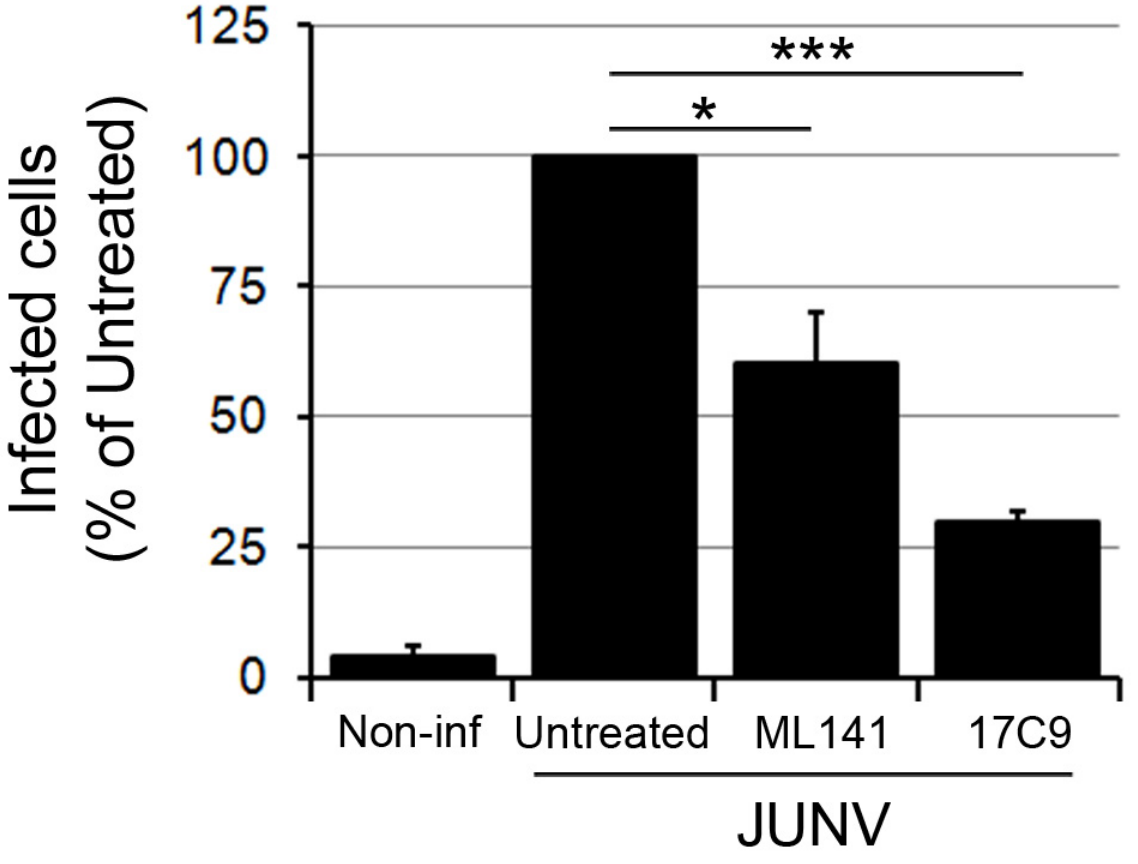
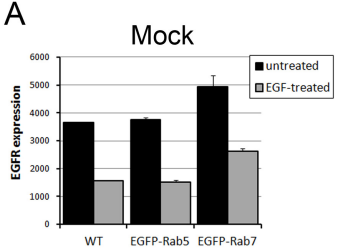
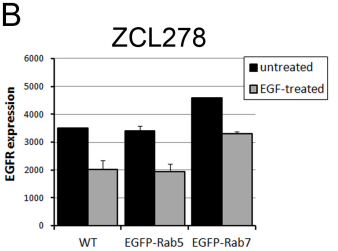

Infected cells
(% of Untreated)
125
100
75
50
25
0
***
*
Non-inf
Untreated
ML141
17C9
JUNV

Figure A4


A
Mock
EGFR expression
6000
5000
4000
3000
2000
1000
0
untreated
EGF-treated
WT
EGFP-Rab5
EGFP-Rab7


B
ZCL278
EGFR expression
6000
5000
4000
3000
2000
1000
0
untreated
EGF-treated
WT
EGFP-Rab5
EGFP-Rab7

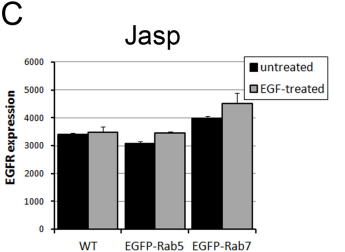

C
Jasp
EGFR expression
6000
5000
4000
3000
2000
1000
0
untreated
EGF-treated
WT
EGFP-Rab5
EGFP-Rab7

Figure A5