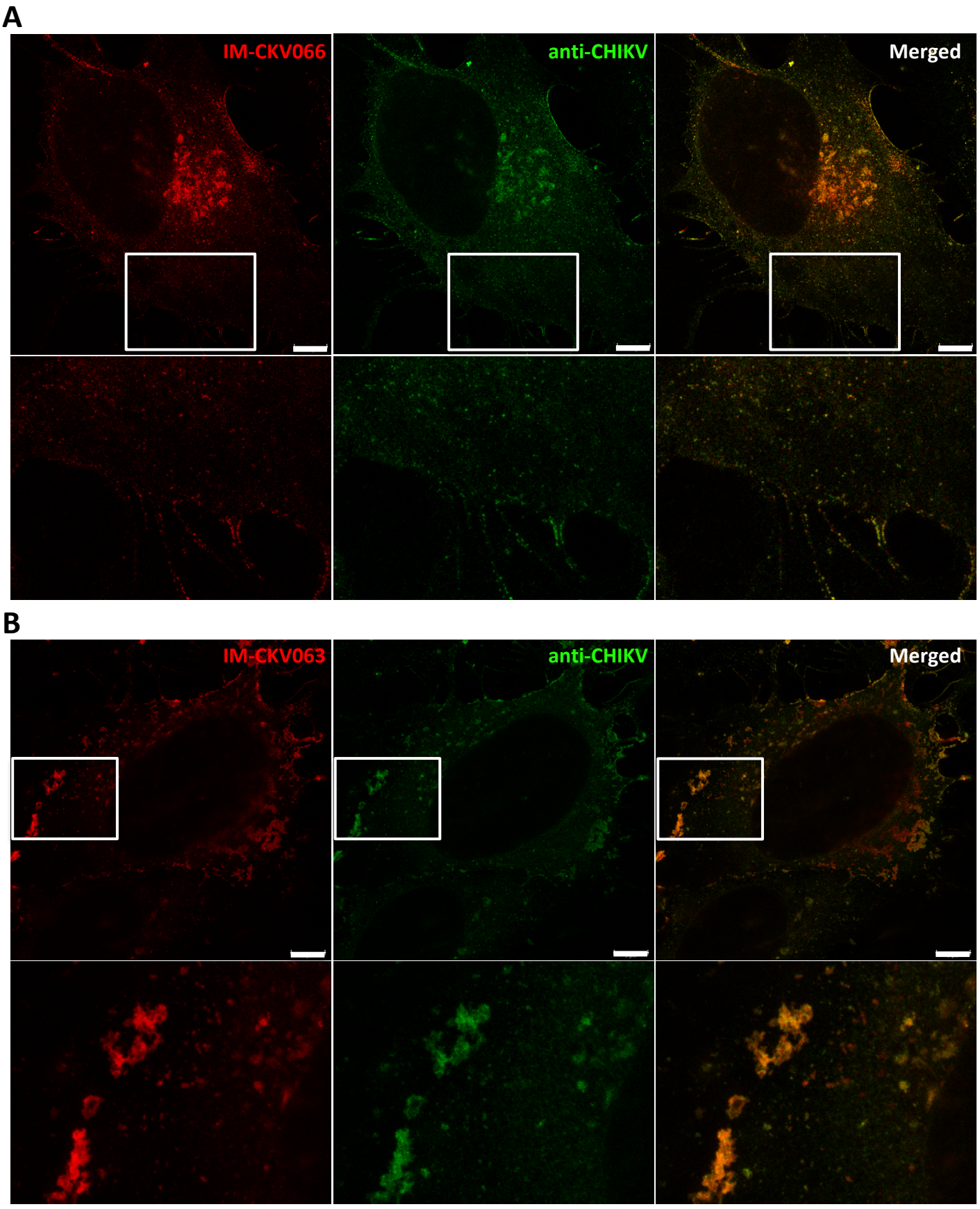
A
IM-CKV066
anti-CHIKV
Merged
B
IM-CKV063
anti-CHIKV
Merged

Figure S1. Anti-CHIKV E2 monoclonal antibodies co-localize with CHIKV structural proteins in CHIKV-infected cells (related to Figure 1). Representative STED super-resolution microscopy images of U2OS cells infected with CHIKV 181 before treatment with 10 µg/ml of **(A)** non-NAb IM-CKV066 or **(B)** NAb IM-CKV063. Cells were fixed and permeabilized, and probed with Alexa647-conjugated (red) anti-human antibody and rabbit anti-CHIKV followed by Alexa594-conjugated (green) anti-rabbit antibody. Zoomed-in views of the boxed areas are shown below each image. Scale bars: 10 µm.

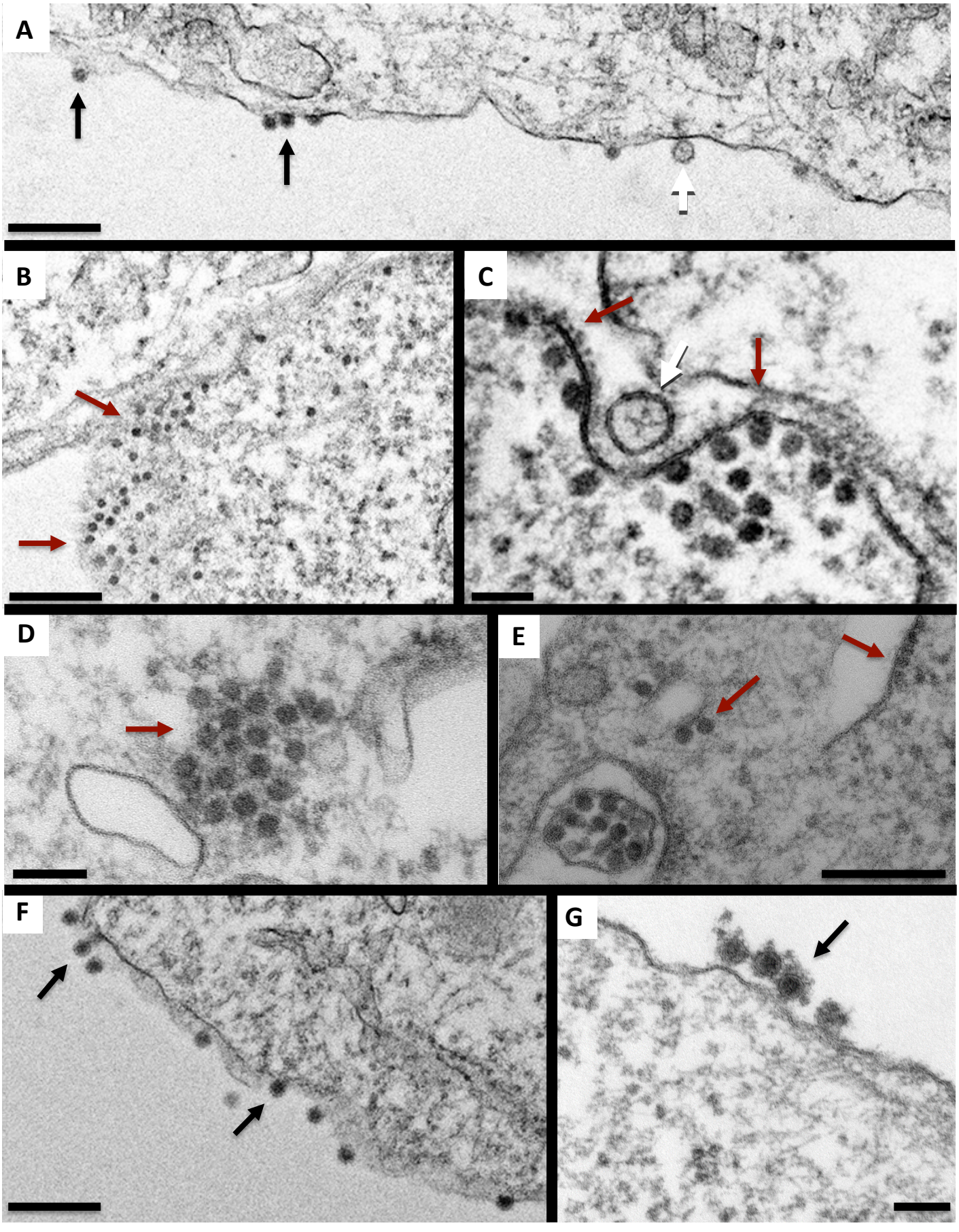
A
B
C
D
E
F
G

Figure S2. NAb treatment blocks CHIKV budding from infected cells by preventing membrane curvature formation around NCs (related to Figure 2). Representative thin-section TEM images of fixed RD cells infected with CHIKV and then cultured **(A)** in the absence of antibodies or in the presence of 2 μg/ml NAb **(B)** C9**, (C)** IM-CKV063, (**D**) CHK-9, (**E**) CHK-152 or non-NAb **(F)** IM-CKV066, (**G**) IM-CKV062. Virus budding sites are indicated with black arrows. Budding-arrested NCs are indicated with red arrows. Viral spherules are indicated with white arrows. Scale bars: 200 nm for **A**, **B**, **E** and **F**; 100 nm for **C** and **D**.

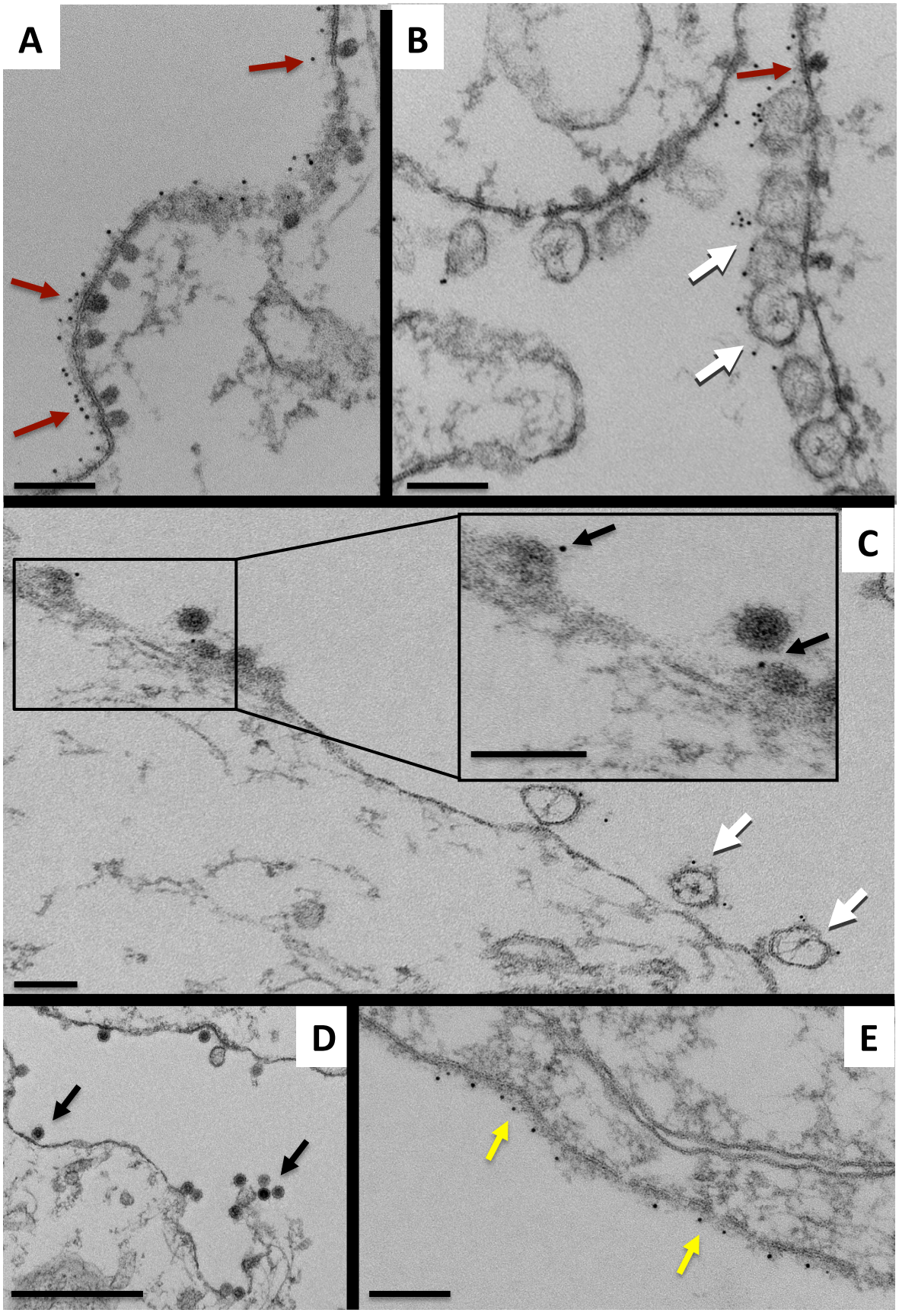
A
B
C
D
E

Figure S3. NAbs crosslink viral GP spikes at the outer leaflet of the plasma membrane of CHIKV-infected cells and block virus budding (related to Figures 2 & 5). Representative IEM images of fixed RD cells infected with CHIKV and cultured in the presence of 2 μg/ml **(A, B)** NAb C9 or **(C)** non-NAb IM-CKV066 or **(D)** in the absence of antibody. **(E)** Representative TEM image of fixed RD cells transfected with a CHIKV Env glycoprotein expression vector only and then cultured in the presence of 2 μg/ml NAb C9 labeled with anti-human antibodies conjugated to 6-nm colloidal gold. Virus budding sites in are indicated with black arrows. Budding-arrested NCs are indicated with red arrows. Viral spherules are indicated with white arrows. Glycoproteins on the cell surface revealed by gold-conjugated antibodies are indicated with yellow arrows. Scale bars: 100 nm for **A**, **B**, **C**, and **E**; 500 nm for **D**.

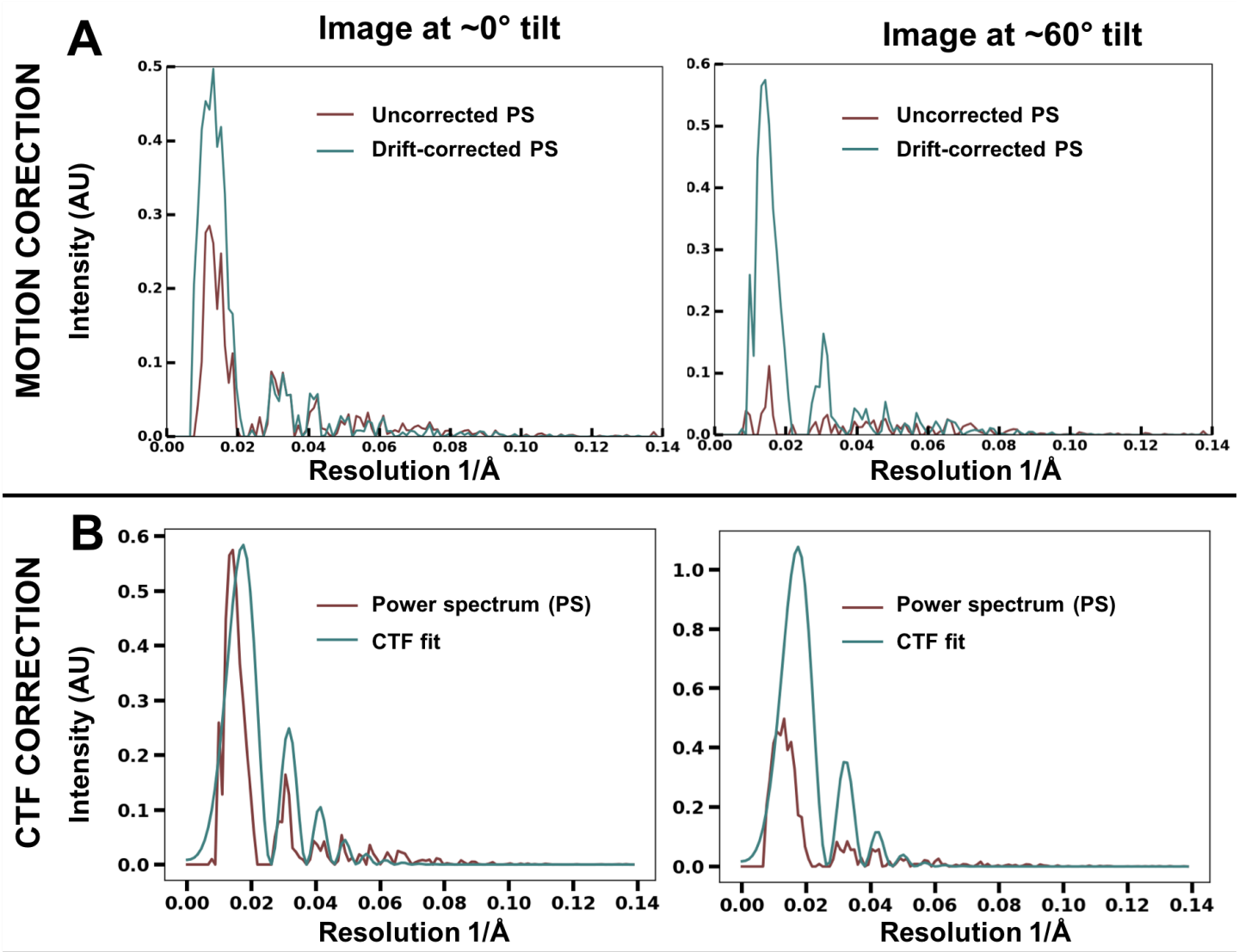
A
MOTION CORECTION
Image at ~0° tilt
Image at ~60° tilt
Intensity (AU)
Uncorrected PS
Drift-corrected PS
Resolution 1/Å
B
CTF CORRECTION
Power spectrum (PS)
CTF fit

Figure S4. Motion and CTF corrections (related to Figure 3-6). (A) Power spectra of images in cryoET tiltseries of CHIKV-infected cells at low (left) and high (right) tilt angles with and without motion-correction. **(B)** CTF fitting from power spectra of motion-corrected images in cryoET tiltseries of CHIKV-infected cells at low (left) and high (right) tilt angles.

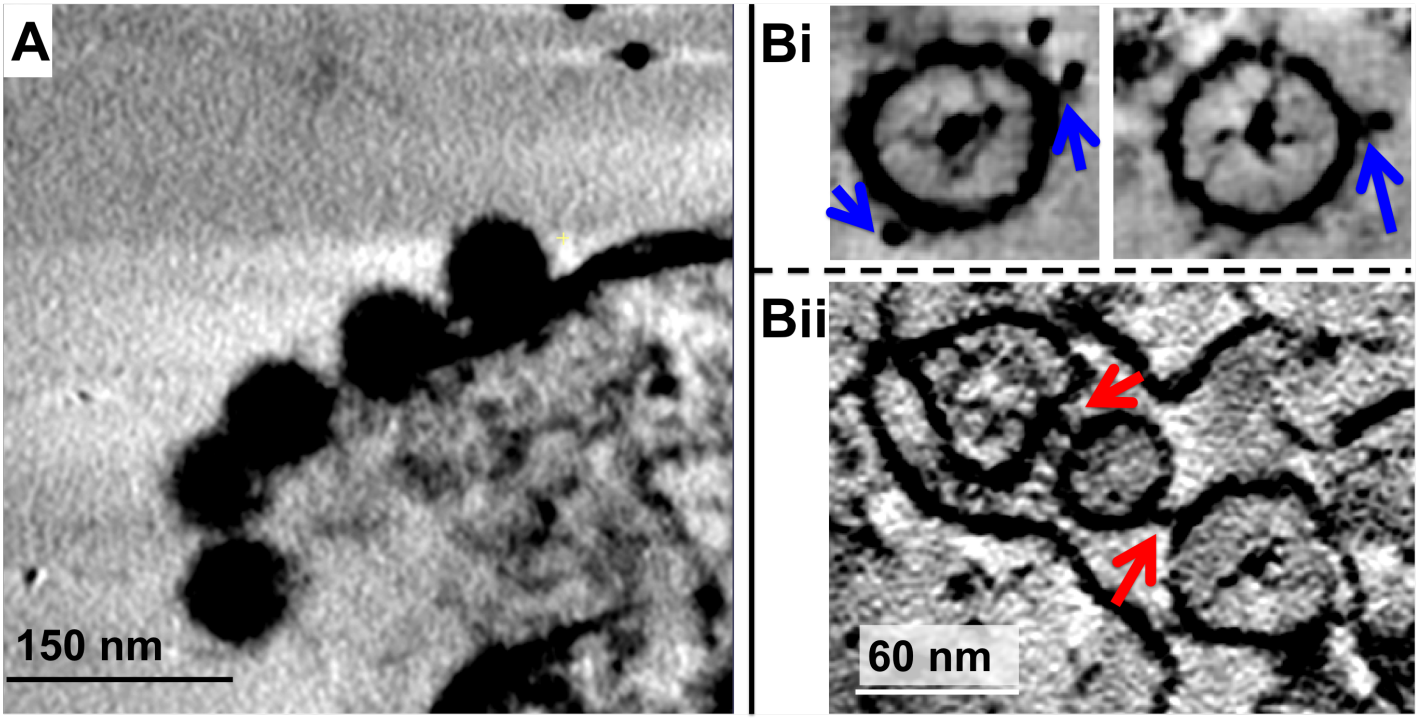
A
150 nm
Bi
Bii
60 nm

Figure S5. TEM tomography of fixed CHIKV-infected cells (related to Figure 2, 3 & 6). RD cells were infected with CHIKV before being cultured **(A)** in the absence or **(B)** in the presence of 2 µg/ml C9. Cells were fixed at 18 h post infection and labeled with anti-human antibodies conjugated to 6-nm colloidal gold before imaging with EM tomography. **(A)** A re-projection from a ~115 nm thick slice of a selected region of a representative tomogram showing a cluster of five co-releasing virions. **(Bi)** Selected sub-tomographic slices showing viral spherules labeled with gold particles. **(Bii)** A selected sub-tomographic slice showing densities connecting neighboring spherules.

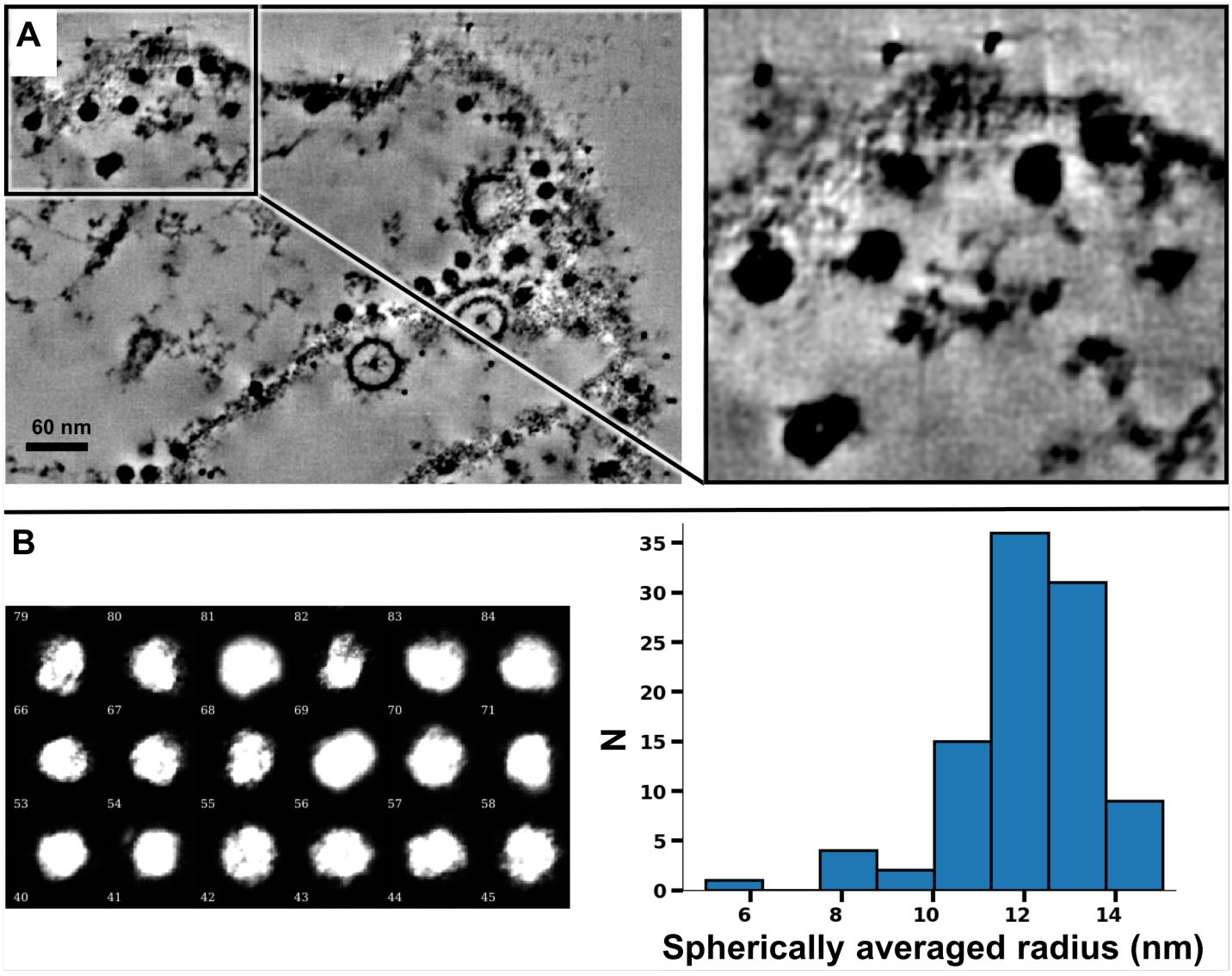
A
60 nm
B
79
80
81
82
83
84
66
67
68
69
70
71
53
54
55
56
57
58
40
41
42
43
44
45
N
35
30
25
20
15
10
5
0
6
8
10
12
14
Spherically averaged radius (nm)

Figure S6. IEM tomography of fixed CHIKV-infected cells treated with NAb C9 labeled with anti-human antibodies conjugated to 6-nm colloidal gold (related to Figures 2 & 4). (A) A ~3 nm thick tomographic slice of a representative tomogram, and a zoomed-in view to highlight arrested NCLPs. **(B)** Re-projections of representative NCLP subtomograms and histogram showing their size distribution.

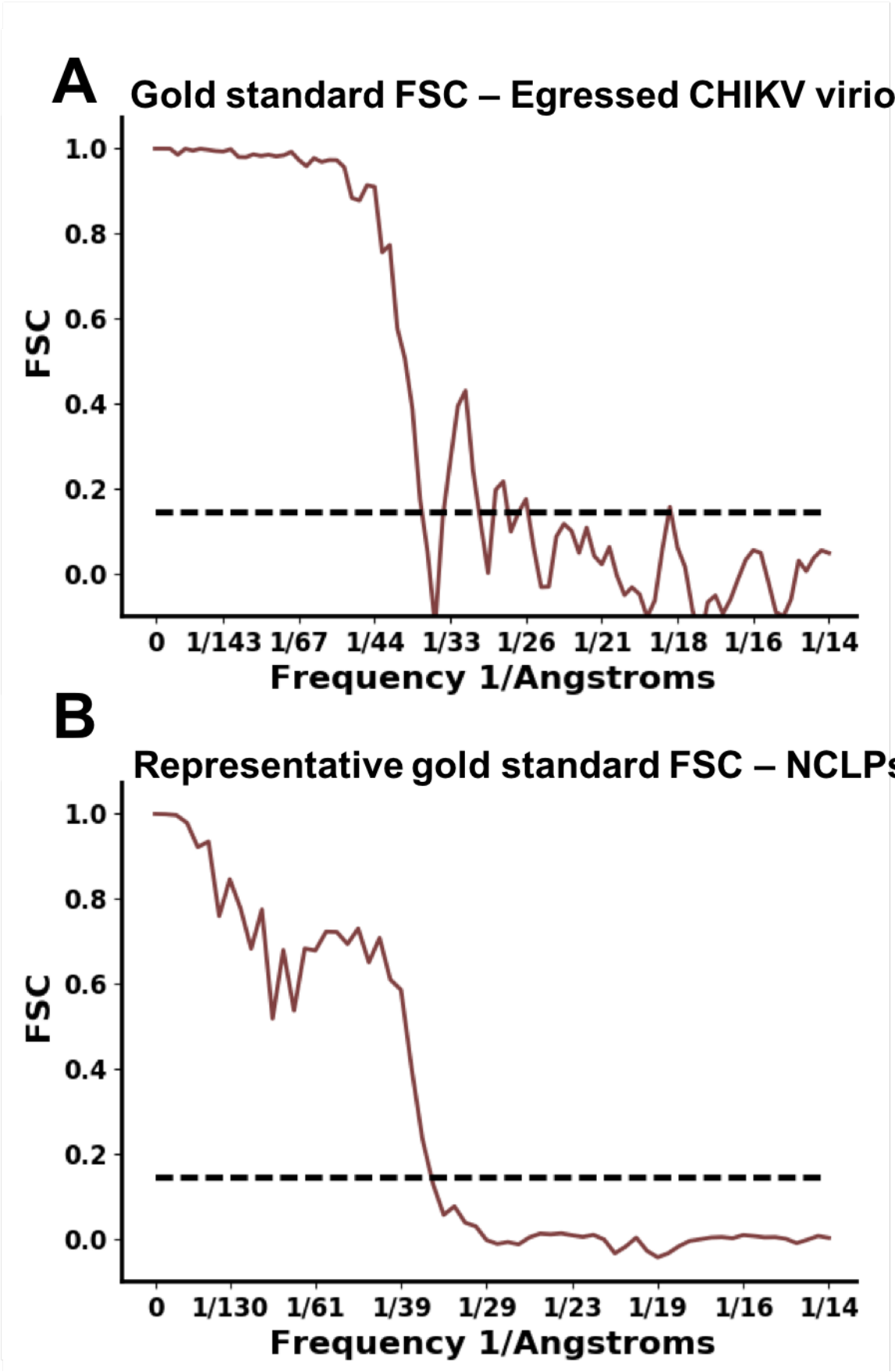
A
Gold standard FSC – Egressed CHIKV virion
FSC
1.0
0.8
0.6
0.4
0.2
0.0
0
1/143
1/67
1/44
1/33
1/26
1/21
1/18
1/16
1/14
Frequency 1/Angstroms
B
Representative gold standard FSC – NCLPs
FSC
1.0
0.8
0.6
0.4
0.2
0.0
0
1/130
1/61
1/39
1/29
1/23
1/19
1/16
1/14
Frequency 1/Angstroms

Figure S7. Gold-standard FSC curves for (A) the final subtomogram average of released CHIKV virions and (B) a representative subtomogram average of NCLPs showing the resolution cutoff at the FSC = 0.143 criterion (related to Figures 3 & 4).