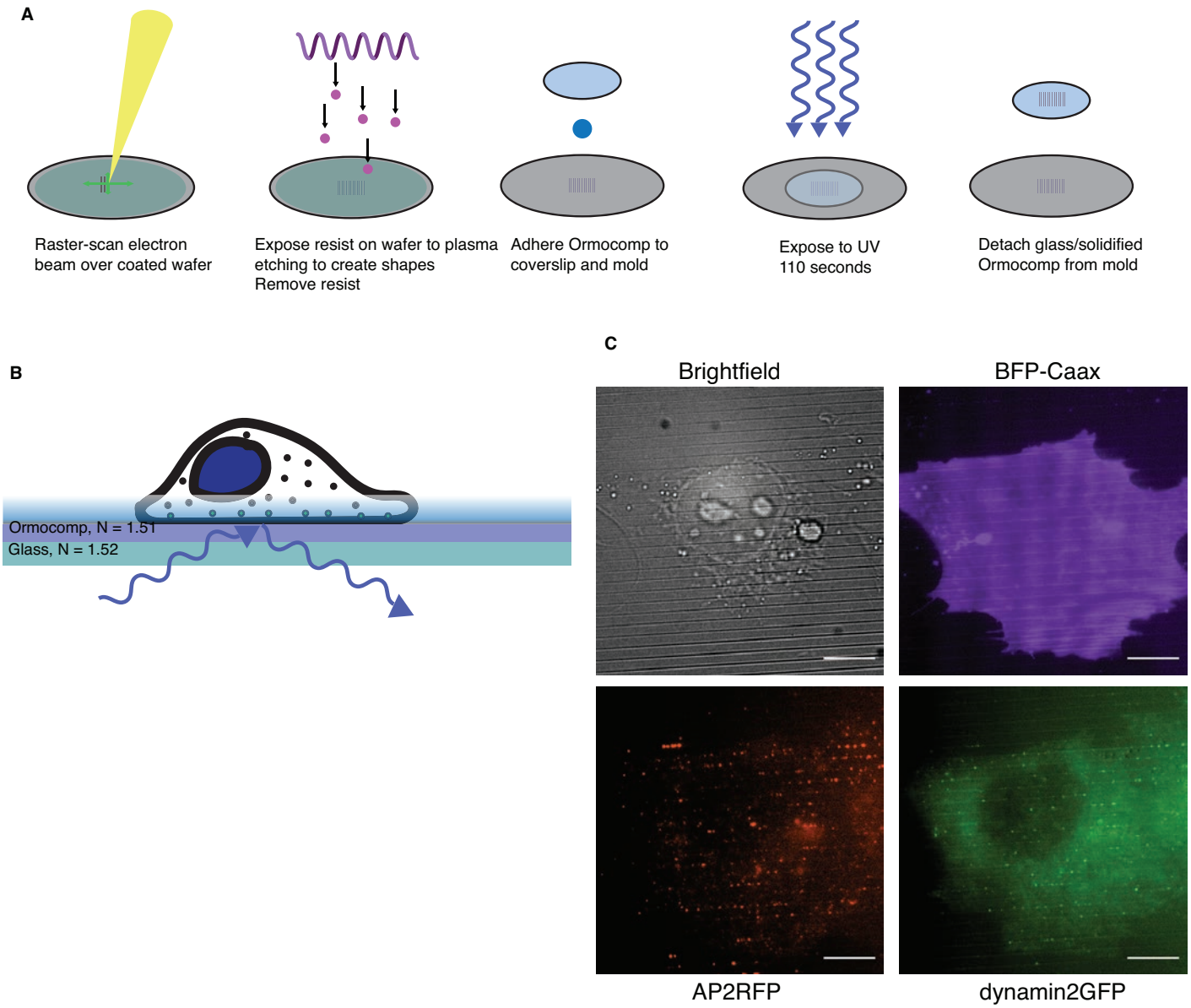

Fig. S1. UV-Nanoimprint Lithography creates glass-like substrates for induced curvature studies. (**A**) Method of silicon mold production and Ormocomp substrate manufacturing. (**B**) Ormocomp's refractive index allows for TIRF microscopy of live cells. (**C**) TIRF microscopy of a cell grown on 120-nanometer ridges expressing BFP-Caax, AP2RFP, and dynamin2GFP simultaneously.

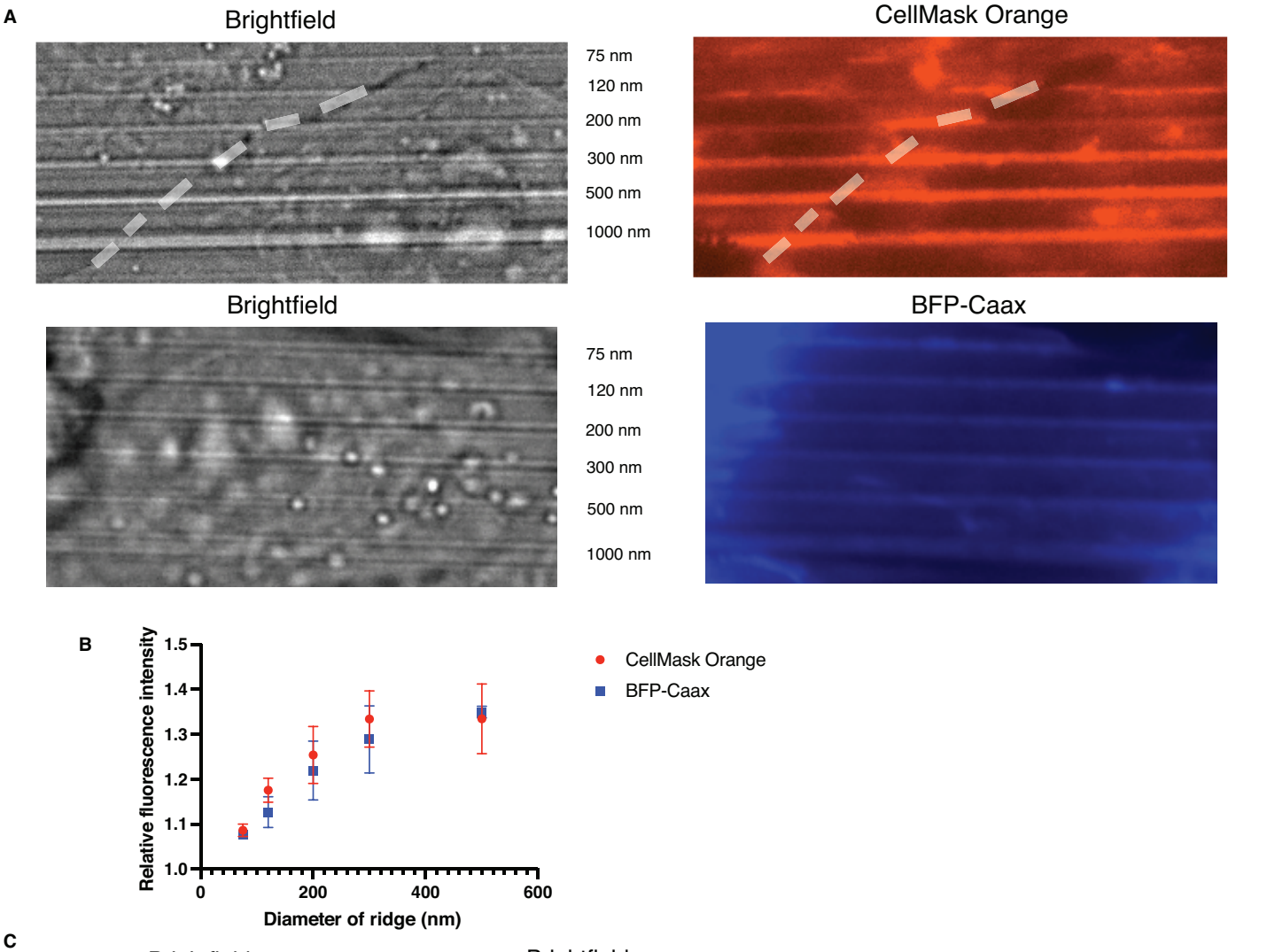
A
Brightfield
CellMask Orange
75 nm
120 nm
200 nm
300 nm
500 nm
1000 nm
Brightfield
BFP-Caax
75 nm
120 nm
200 nm
300 nm
500 nm
1000 nm
B
Relative fluorescence intensity
1.5
1.4
1.3
1.2
1.1
1.0
0
200
400
600
Diameter of ridge (nm)
CellMask Orange
BFP-Caax
C
Brightfield
Brightfield
AP2-GFP
dynamin2-RFP
D
AP2RFP/AP2GFP
Enrichment
4
2
0
0
200
400
600
Diameter of ridge (nm)
AP2-RFP
AP2-GFP

Fig. S2. Membrane enrichment on nanoridges increases with ridge size. (**A**) Representative TIRF microscopy images of live cells on variable-size nanoridges with CellMask Orange (top) or BFP-Caax (bottom), with gray dashed line indicating interface between two cells. (**B**) Fluorescence intensity increase of CellMask Orange and BFP-Caax over flat substrate (Mean ± SEM, n = 10 cells for CellMask Orange, 4 cells for BFP-Caax). (**C**) AP2GFP and DNM2RFP puncta, visualized by TIRF microscopy, demonstrating that they respond to induced curvature. (**D**) Comparison of enrichment score for AP2RFP and AP2GFP (Mean ± SD).

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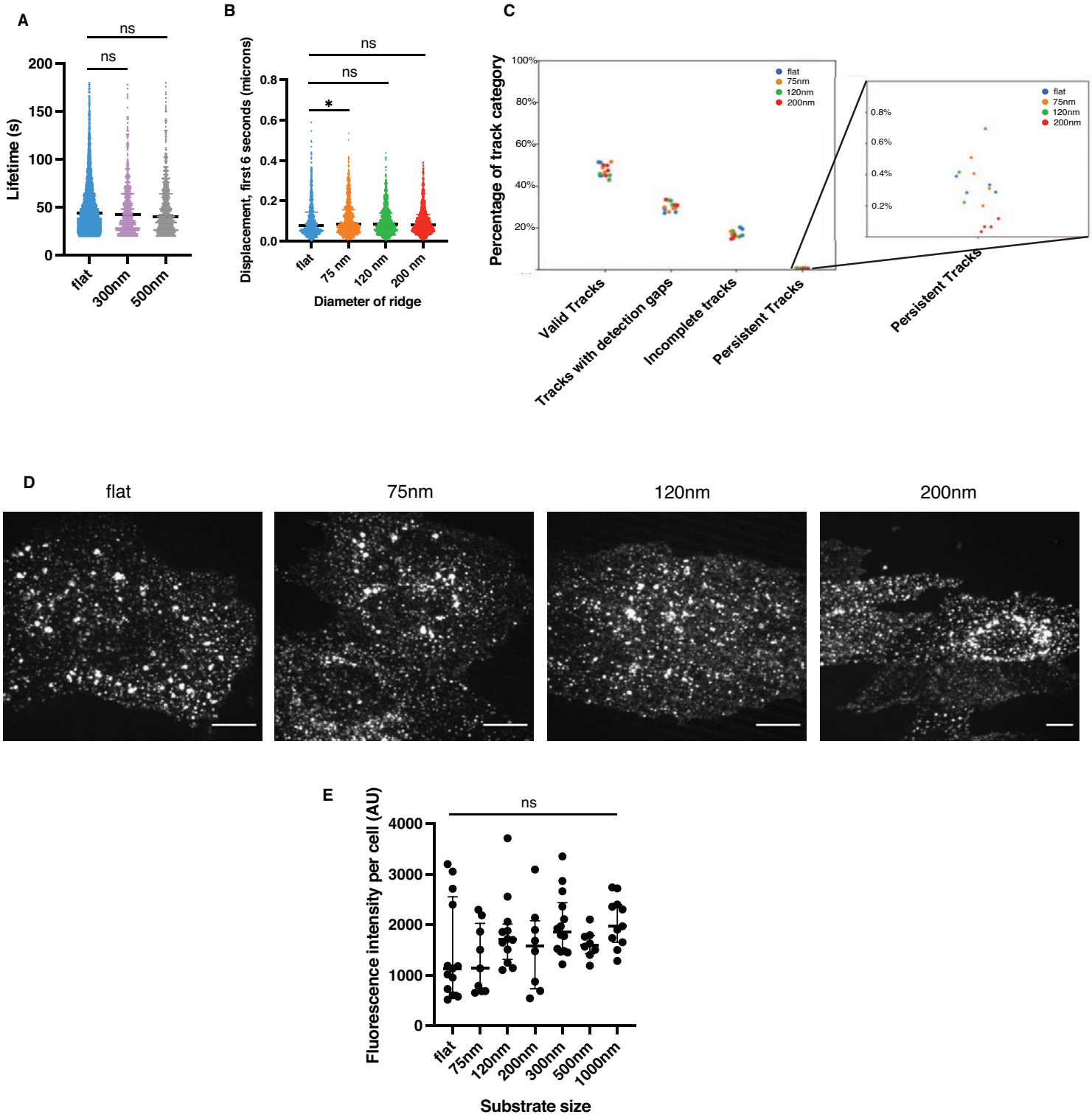

A
ns
ns
Lifetime (s)
200
150
100
50
0
flat
300nm
500nm
B
ns
ns
*
Displacement, first 6 seconds (microns)
0.8
0.6
0.4
0.2
0.0
flat
75 nm
120 nm
200 nm
Diameter of ridge
C
Percentage of track category
100%
80%
60%
40%
20%
flat
75nm
120nm
200nm
0.8%
0.6%
0.4%
0.2%
Valid Tracks
Tracks with detection gaps
Incomplete tracks
Persistent Tracks
Persistent Tracks
D
flat
75nm
120nm
200nm
E
ns
Fluorescence intensity per cell (AU)
4000
3000
2000
1000
0
flat
75nm
120nm
200nm
300nm
500nm
1000nm
Substrate size

Fig. S3. Substrate diameter does not affect percentage of bona fide endocytic sites or uptake of fluorescent transferrin. (**A**) Lifetime of bona fide CCPs from flat, 300nm, and 500nm substrates (Mean ± interquartile range, $n > 800$ tracks per condition, from 3-4 cells per condition, *P* Student's t test). (**B**) Displacement in the first 6 seconds of detection for CCPs from flat, 75nm, 120nm, and 200nm substrates (Mean ± interquartile range, *U* Mann-Whitney test). (**C**) Left: Percentage of each class of classified CCP from 3-4 cells grown on each size of substrate. Right: Zoom view of percentage of persistent tracks. (**D**) Representative maximum intensity projection images of fixed cells, imaged by confocal microscopy, with AlexaFluor-647 tagged transferrin taken up by cells on different size nanoridges. (**E**) Quantification of (**D**) (Mean ± SD, *P* two-way ANOVA).

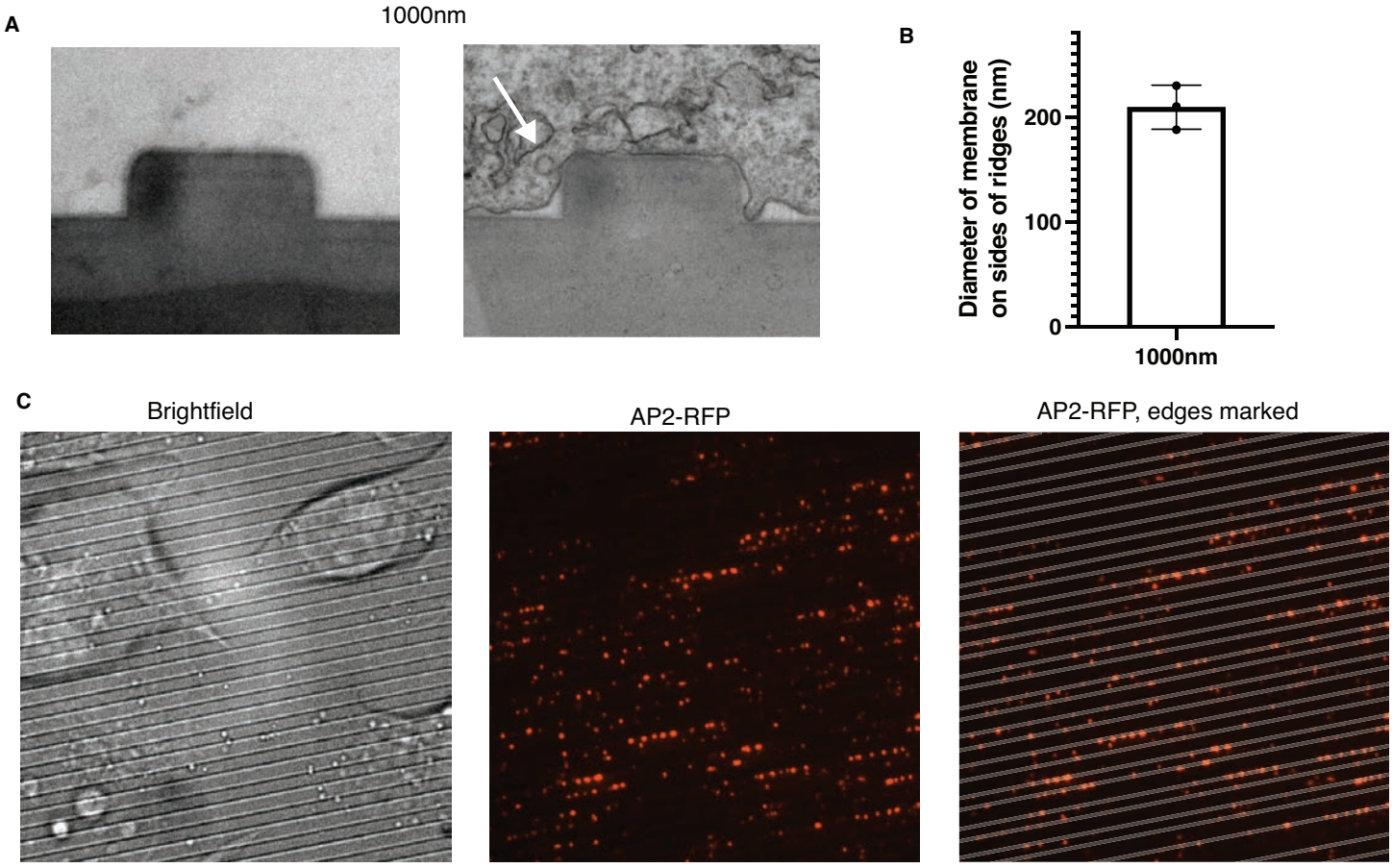
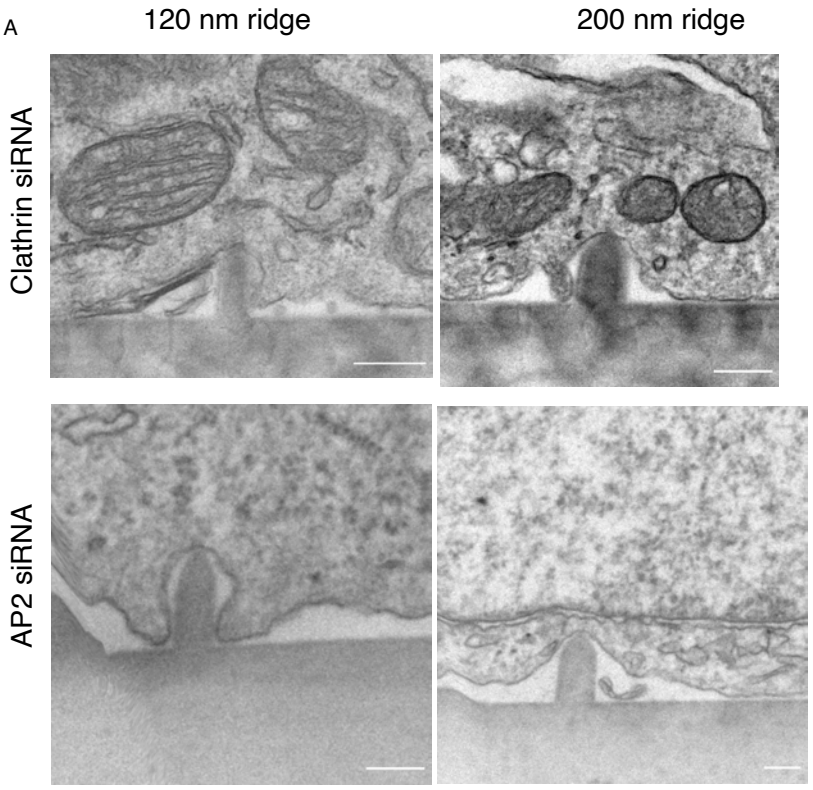
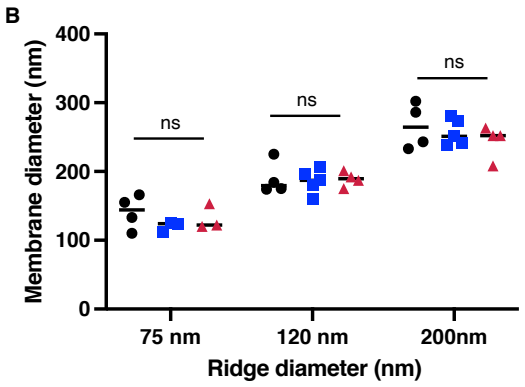
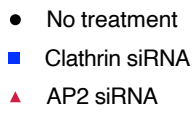


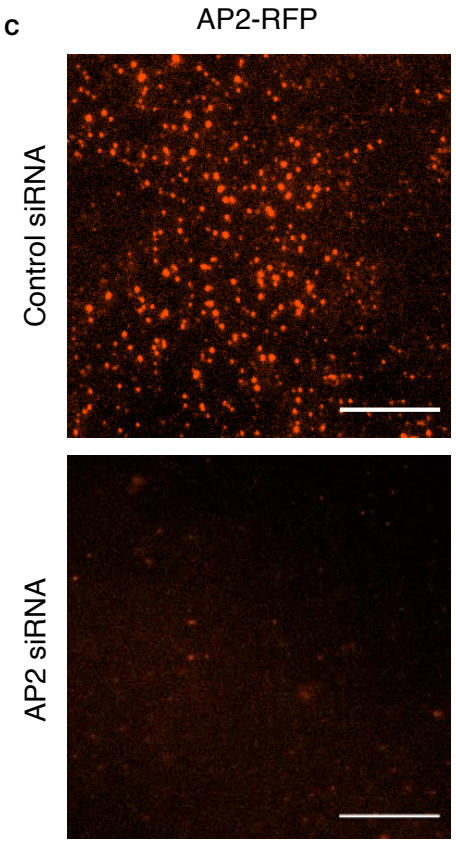
Fig. S4. 1000-nanometer ridges induce curvature on the side, but not the top, of the ridge.

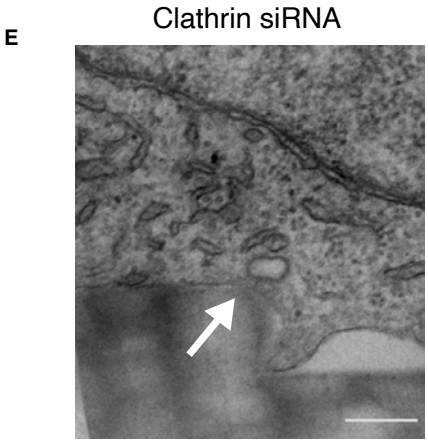
(**A**) Representative TEM images of the 1000-nanometer ridge (left) and a cell grown on the ridge (right). (**B**) Average diameter of the induced curvature on the edge of the 1000-nm ridge (Mean ± SD). (**C**) Representative TIRF microscopy images of live cells grown on 1000-nanometer ridge with AP2-RFP localizing to the edges of the ridge.


A
120 nm ridge
200 nm ridge
Clathrin siRNA
AP2 siRNA


B
Membrane diameter (nm)
400
300
200
100
0
ns
ns
ns
75 nm
120 nm
200nm
Ridge diameter (nm)


No treatment
Clathrin siRNA
AP2 siRNA


C
AP2-RFP
Control siRNA
AP2 siRNA


E
Clathrin siRNA

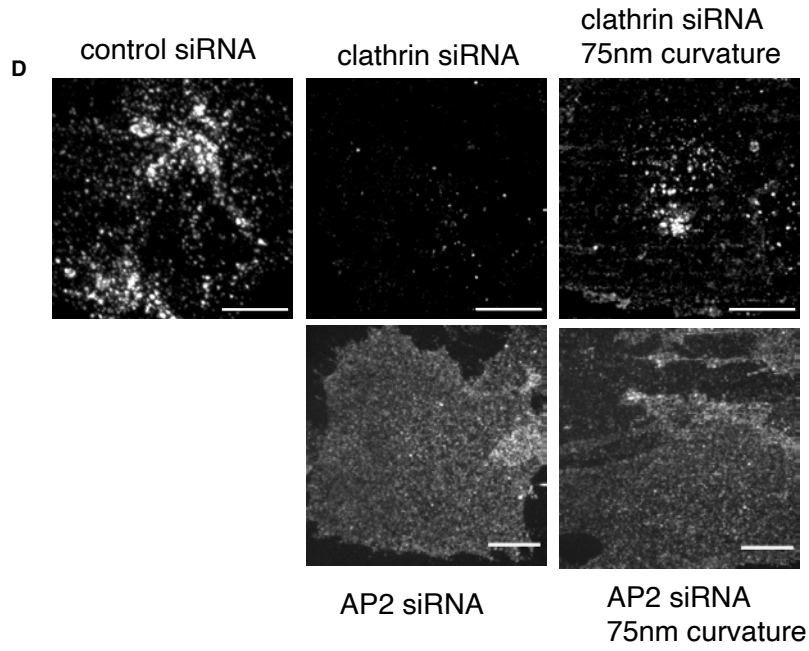

D
control siRNA
clathrin siRNA
clathrin siRNA
75nm curvature
AP2 siRNA
AP2 siRNA
75nm curvature

Fig. S5. Clathrin/AP2 knockdown do not affect membrane wrapping on substrates. (**A**) Representative TEM images of clathrin (top) and AP2 (bottom) siRNA-treated cells. (**B**) Quantification of membrane diameter in (**A**) (Mean ± SD, *P* Student's t test). (**C**) AP2 siRNA significantly reduces the number of puncta of AP2RFP visible by TIRF microscopy. (**D**) Maximum intensity projections of fluorescent transferrin uptake assay from cells treated with siRNAs to clathrin (top) or AP2 (bottom) and exposed to flat or 75-nm substrates, imaged by confocal microscopy. (**E**) An electron-clear, membrane-enclosed but clathrin-negative structure visible in thin-section TEM of clathrin knockdown cells with induced curvature. (**F**) Quantification of fluorescence intensity per cell of AP2 siRNA-treated cells with or without induced curvature (Mean ± SD, *P* Student's t test)