Supplemental figures and movie legends

Supplemental figure S1. Boxplots showing distribution of lifetimes of randomly selected CCSs (CCPs and larger CCSs) are shown. n = 250 CCSs / condition, 5 cells.

Supplemental figure S2. (A) Fibronectin saturates coverslips at 50 μg/ml. Graph showing the integrated intensity (mean ± standard deviation) of fluorescent fibronectin-coated coverslips at concentrations shown. Data is derived from 4 coverslips, 10 regions / coverslip. (B-C) BSC1 CLC-EGFP cells express fibronectin shown by (B) Western blot of lysates from cells grown in serum-free medium ("lysate") or without cells ("no cells") and (C) indirect immunofluorescence (wide-field, epi-fluorescence imaging) in unpermeabilized cells expressing CLC-EGFP (left, green in merge) using anti-fibronectin antibody (center, red in merge) are shown. Scale bar = 10 μm.

Supplemental figure S3. (A) Graph shows uptake of HRP during a time-course of 30 minutes. (B) Bar graph shows the average HRP uptake / mg cell lysate of cells plated on fibronectin (FN)- or BSA-coated coverslips at 0 and 10 minutes of incubation at 37°C. n = 3 independent experiments performed in duplicate. This shows that there is no significant difference in HRP uptake between cells plated on fibronectin versus BSA. Cells were plated for 1-3 hours in serum-free medium.

Movie 1. Time-course of CLC-EGFP showing diverse structures in a BSC1 cell. Cell was plated on a coverslip for 48 hours in presence of serum-containing medium. IRM image shown to right.

Movie 2. Time-course of CLC-EGFP (pseudo-colored green) and mCherry-paxillin (pseudo-colored red), showing that the dynamics of clathrin structures in the vicinity of adhesions are

distinct from those distant from adhesion. Cell was plated on a coverslip for 48 hours in presence of serum-containing medium.

Movie 3. Time-course of CLC-EGFP in cell plated on fibronectin- (right) and BSA- (left) coated coverslip after ~3 hours incubation in serum-free medium.

Movie 4. Time-course of CLC-EGFP (left, pseudo-colored green in merge) and dynamin-mRFP (center, pseudo-colored red in merge) on BSA-coated coverslip after ~3 hours incubation in serum-free medium. Images in movie were processed with a gaussian kernel for presentation.

Movie 5. Time-course of CLC-EGFP on conA-coated coverslip. Cells were imaged after ~2 hours incubation in serum-free medium.

Movie 6. Time-course of CLC-EGFP in a cell plated on an uncoated coverslip for 48 hours in serum-free medium supplemented with control IgG (left) or anti-β1 integrin antibody (right).

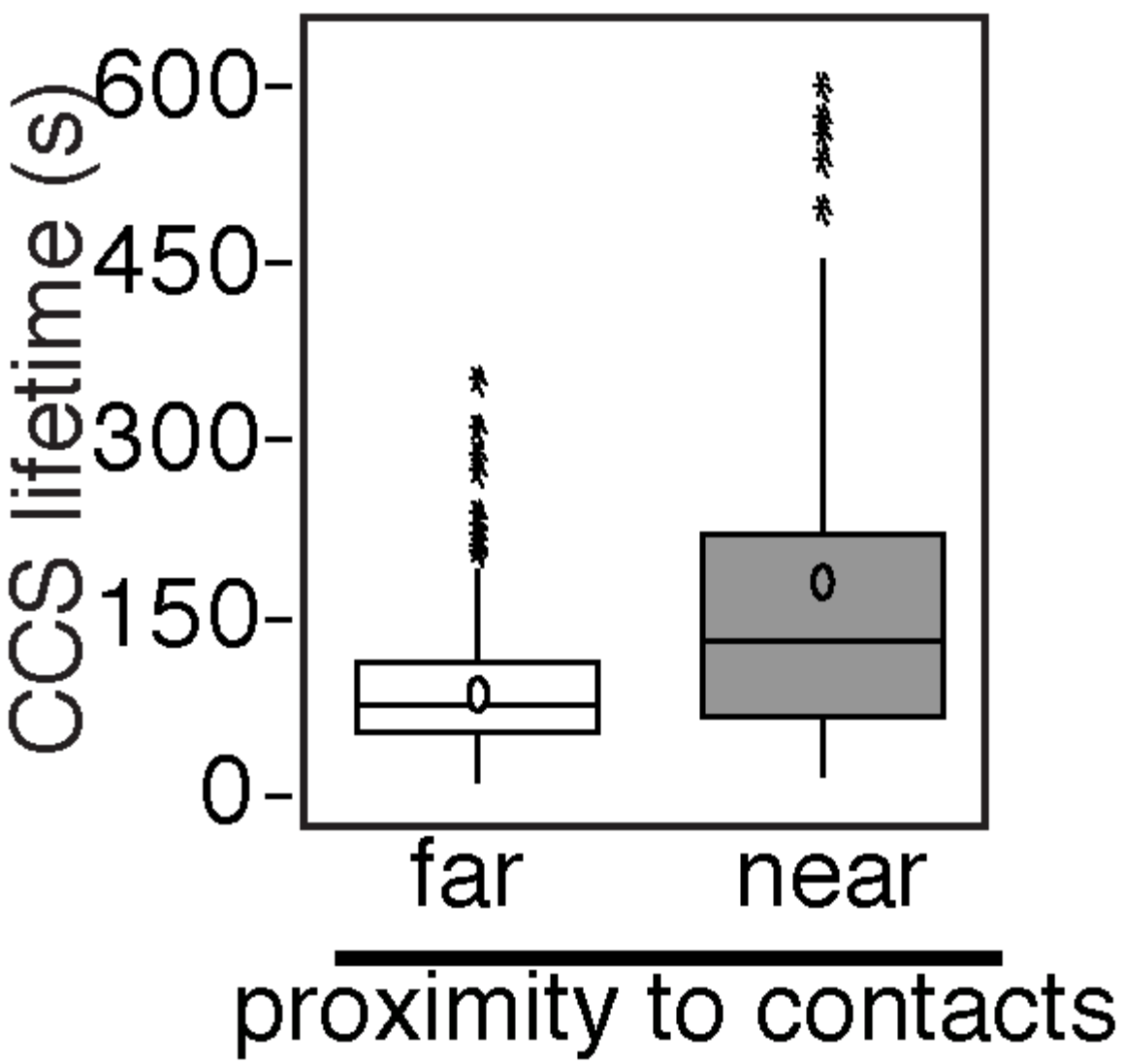
Movie 7. Time-course of CLC-EGFP in a cell plated on a fibronectin-coated coverslip, bathed in control IgG (left) or anti-β1 integrin inhibitory antibody (right) during adhesion. The cell was imaged after ~2 hour incubation in serum-free medium.

Movie 8. Time-course of CLC-EGFP (pseudo-colored green) and x-rhodamine actin (pseudo-colored red), showing that actin is recruited to CCPs during vesicle formation. Cells were plated on an uncoated coverslip for 48 hours in medium containing serum. Regions close to and far from the underlying substrate as determined by IRM imaging are outlined in magenta and yellow, respectively.

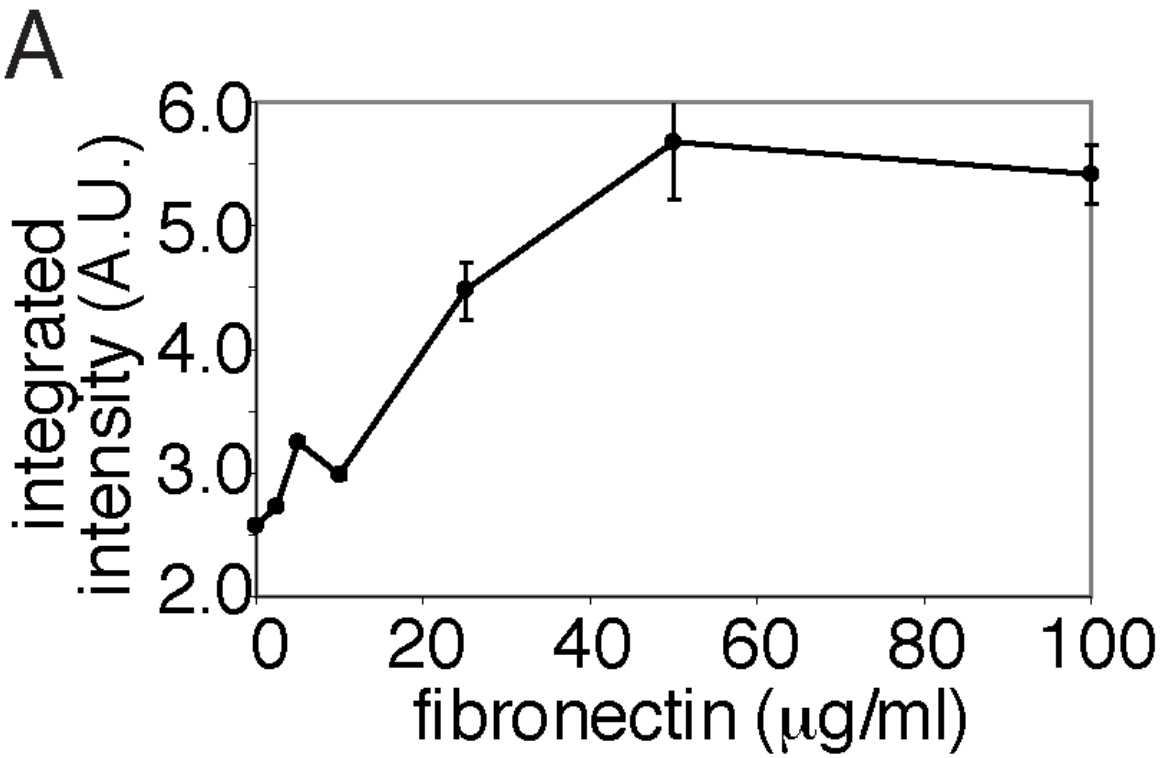
Movie 9. Time-course of CLC-EGFP (pseudo-colored green) and dynamin-mRFP (pseudo-colored red) in a cell perfused with 1 μM latrunculin B. Cells were plated on an uncoated coverslip in serum-containing medium for 48 hours before imaging.

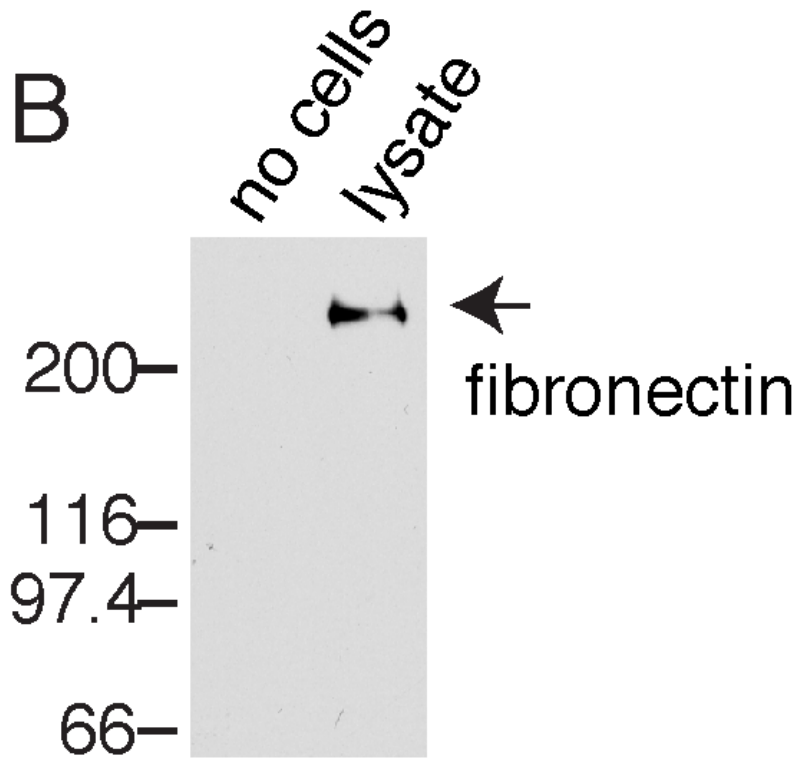
All time is shown as minutes : seconds. Calibrations bars = 5 μm. All movie images acquired by TIR-FM.

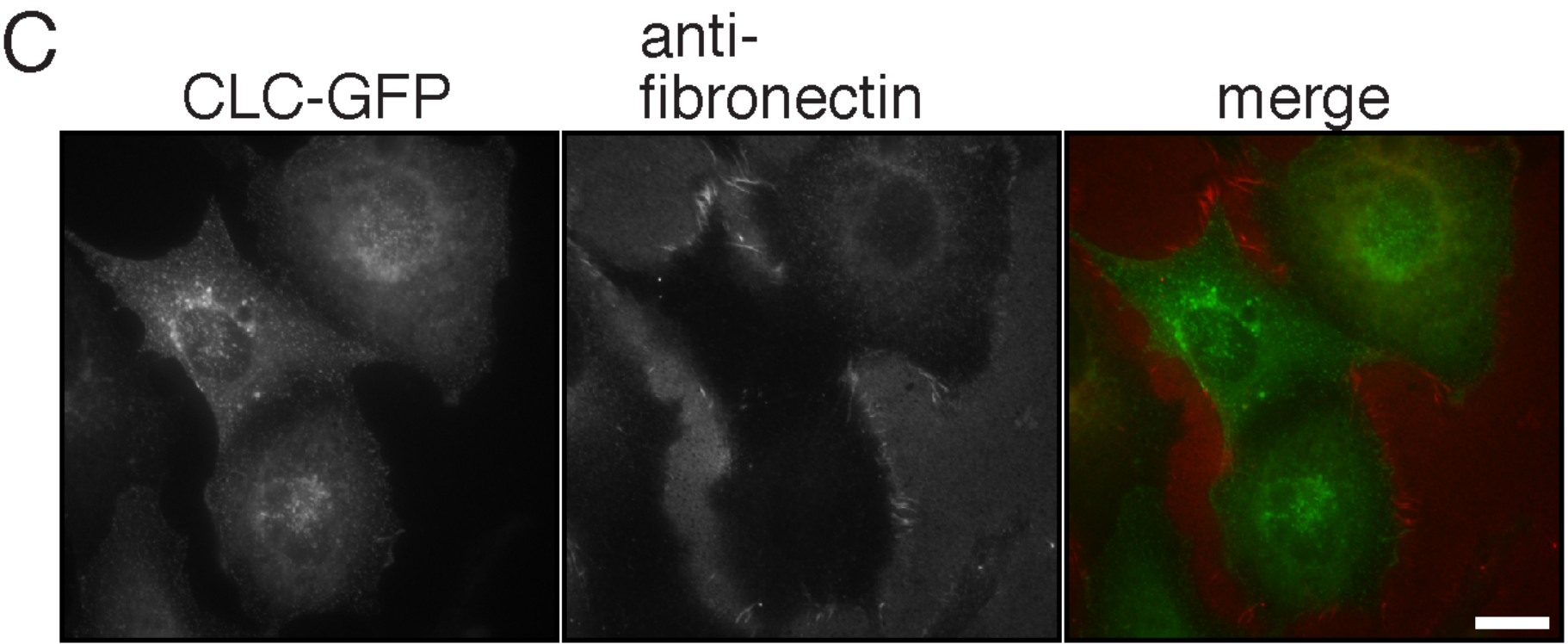
Batchelder and Yarar Supplemental figure S1.



Batchelder and Yarar
Supplemental figure S2.







Batchelder and Yarar.
Supplemental figure S3.