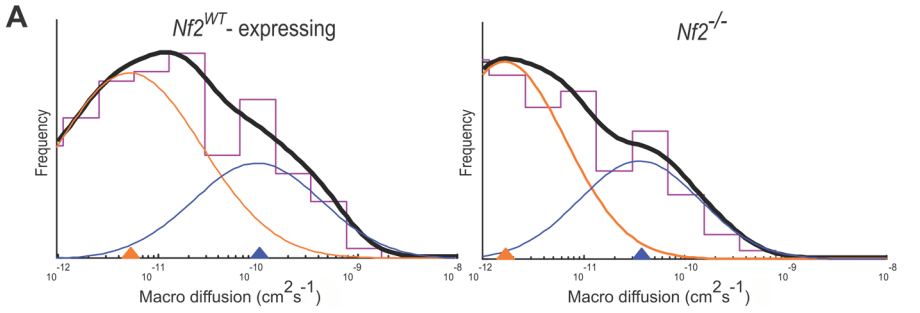
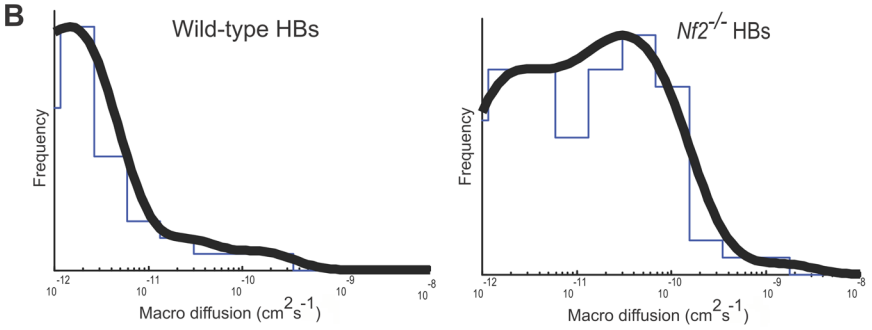
Chiasson-MacKenzie et al., http://www.jcb.org/cgi/content/full/jcb.201503081/DC1






| | | Calculated macro diffusion coefficients (x10$^{-12}$cm$^2$s$^{-1}$) | | |
|---|---|---|---|---|
| | | | Sub-populations | |
| | n= | Mean | Immobile/Confined | Freely Diffusing |
| non-confluent *Nf2*$^{WT}$-expressing LDCs | 135 | 13.0 +/- 1.2 | 5 (65%) | 95 (35%) |
| non-confluent *Nf2*$^{-/-}$ LDCs | 142 | 4.5 +/-3.8 | 2 (67%) | 36 (33%) |




| | n | Calculated macro diffusion coefficients (x10$^{-12}$cm$^2$s$^{-1}$) |
|---|---|---|
| Confluent Wild-type HBs | 61 | 1.2 +/- 0.1 |
| Confluent *Nf2*$^{-/-}$ HBs | 83 | 12.0 +/- 1.3 |

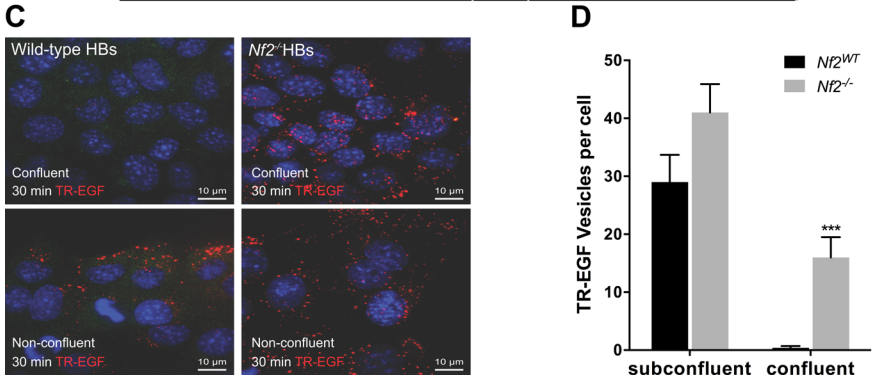



Figure S1. **Regulation of lateral mobility and internalization of EGFR by exogenous and endogenous Merlin is contact dependent.** (A) SPTM showing the mean lateral diffusivity of EGFR in nonconfluent *Nf2*$^{WT}$-expressing LDCs relative to *Nf2*$^{-/-}$ LDCs. Histograms (purple) show the relative frequency at which beads were observed (y axis) with a given $D_{macro}$ coefficient (x axis; log scale). Overlaid on each histogram is a two-Gaussian fit (orange and blue) and its sum (solid black). The underlying chart displays the log mean of $D_{macro}$ ± SEM as well as the mean of the two-Gaussian fit subpopulations. (B) Lateral mobility of EGFR in wild-type or *Nf2*$^{-/-}$ HBs. The underlying chart displays the log mean of $D_{macro}$ ± SEM. (C) Representative confocal images of internalized TR-EGF (red; 30 min after stimulation) in confluent or nonconfluent populations of wild-type or *Nf2*$^{-/-}$ HBs. Bars, 10 µm. (D) Quantification of TR-EGF internalization in confluent or nonconfluent wild-type or *Nf2*$^{-/-}$ HBs. Error bars indicate SEM. ***, P < 0.001 (Mann-Whitney test comparing wild-type to *Nf2*$^{-/-}$ populations). Data are representative of three experiments.

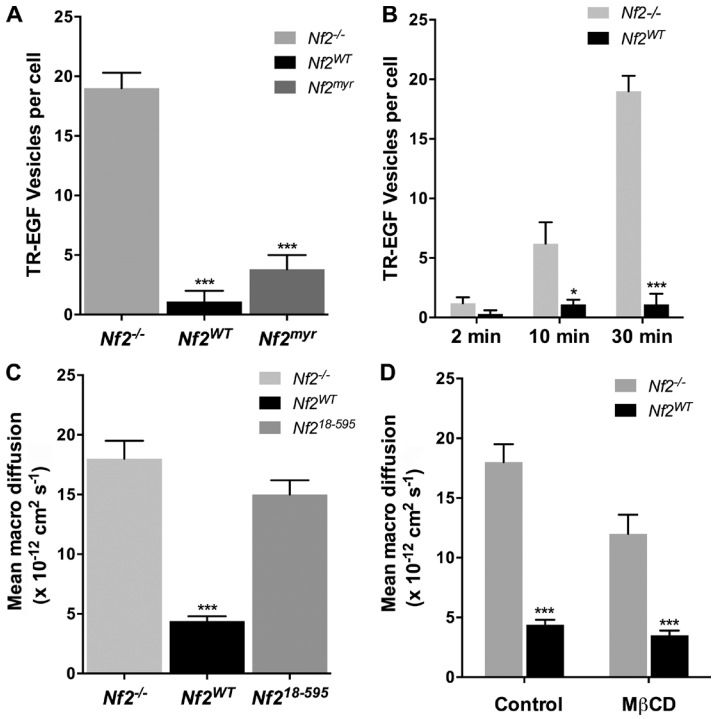



Figure S2. **EGFR immobilization and inhibition of internalization occurs at the plasma membrane independently of sterol-rich membrane microdomains.** (A) TR-EGF internalization in confluent LDCs expressing a c-src myristoylation sequence for membrane localization (*Nf2*$^{myr}$) relative to *Nf2*$^{-/-}$ or *Nf2*$^{WT}$-expressing LDCs. Error bars indicate SEM. ***, P < 0.001 (one-way ANOVA, comparing each *Nf2*-expressing group to the *Nf2*$^{-/-}$ group). (B) Time-dependent internalization of TR-EGF in *Nf2*$^{-/-}$ or *Nf2*$^{WT}$-expressing LDCs. Internalization was measured after 2 min, 10 min, or 30 min of stimulation with TR-EGF. *, P < 0.05; ***, P < 0.001 (unpaired *t* test comparing *Nf2*$^{WT}$ to *Nf2*$^{-/-}$ at each time point). (C) Average lateral mobility of EGFR in *Nf2*$^{-/-}$, *Nf2*$^{WT}$, or *Nf2*$^{18-595}$, which cannot localize to the cortical actin cytoskeleton. Error bars indicate SEM. ***, P < 0.001 (one-way ANOVA, comparing each *Nf2*-expressing group to the *Nf2*$^{-/-}$ group). (D) Mean lateral mobility of EGFR in *Nf2*$^{-/-}$ or *Nf2*$^{WT}$-expressing LDCs treated with MβCD (10 mM) to disrupt membrane cholesterol. Error bars indicate SEM. P > 0.05 (unpaired *t* test comparing control to MβCD-treated populations).

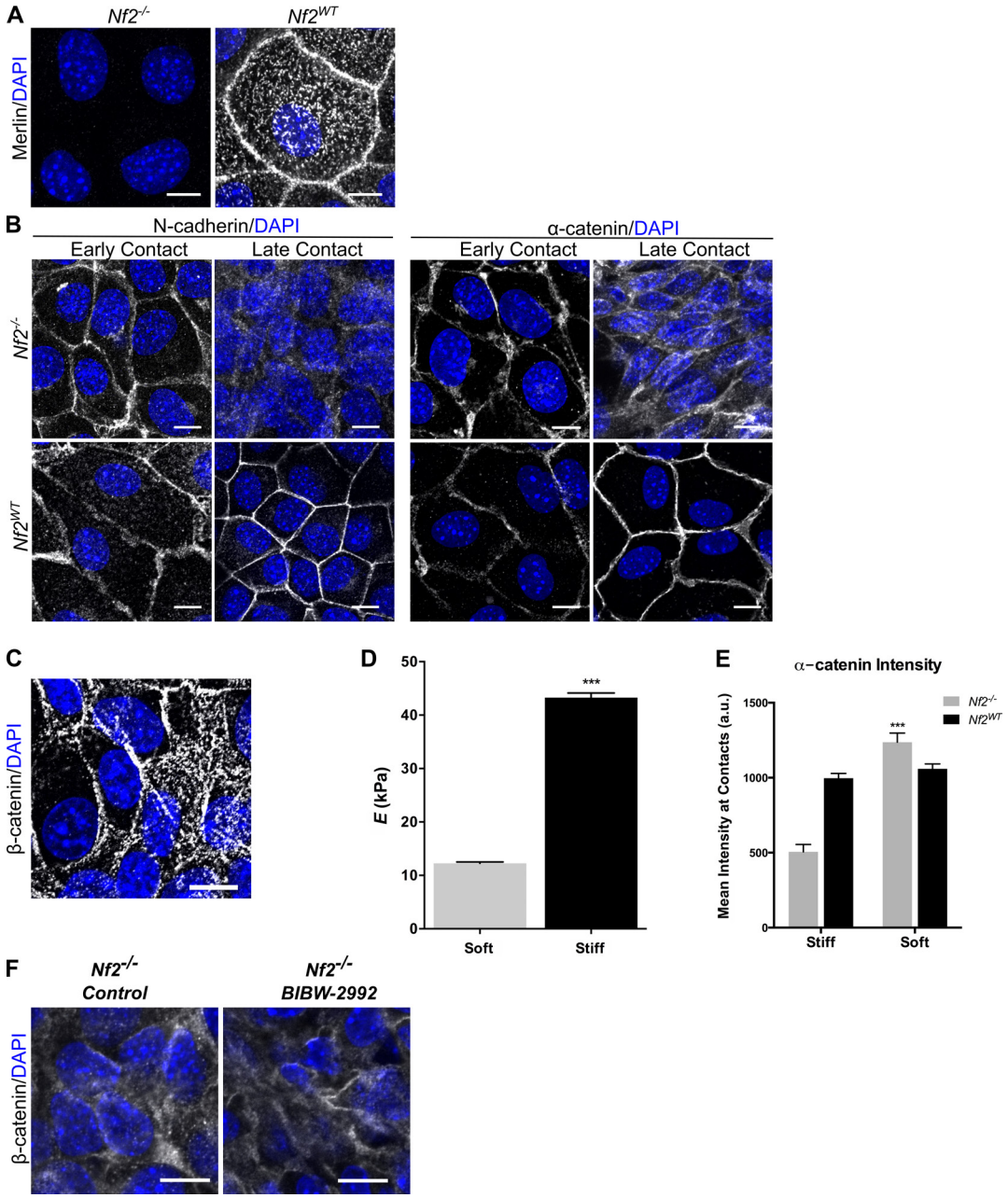



Figure S3. **Localization of Merlin and AJ proteins in *Nf2*$^{-/-}$ and *Nf2*$^{WT}$-expressing LDCs.** (A) Confocal images showing Merlin localization in *Nf2*$^{-/-}$ and *Nf2*$^{WT}$-expressing LDCs. (B) Confocal images showing localization of N-cadherin or α-catenin in *Nf2*$^{-/-}$ or *Nf2*$^{WT}$-expressing LDCs at early (left) or late (right) stages of confluence. (C) Confocal images demonstrating that β-catenin localizes similarly to vinculin in *Nf2*$^{-/-}$ LDCs. (D) The elastic modulus (*E*) of polyacrylamide hydrogels was measured using a rheometer as described in the Materials and methods section Polyacrylamide gel fabrication . . .. (E) Measurement of junctional α-catenin intensity in *Nf2*$^{-/-}$ or *Nf2*$^{WT}$-expressing LDCs cultured on stiff (40 kPa) and soft (12 kPa) polyacrylamide hydrogels. (F) Confocal images of *Nf2*$^{-/-}$ LDCs stained for β-catenin after treatment with the irreversible ErbB inhibitor BIBW-2992. Bars, 10 µm. DAPI staining of nuclei is shown in blue. (D and E) Error bars indicate SEM. ***, $P < 0.001$ (Mann-Whitney test). a.u., arbitrary units.

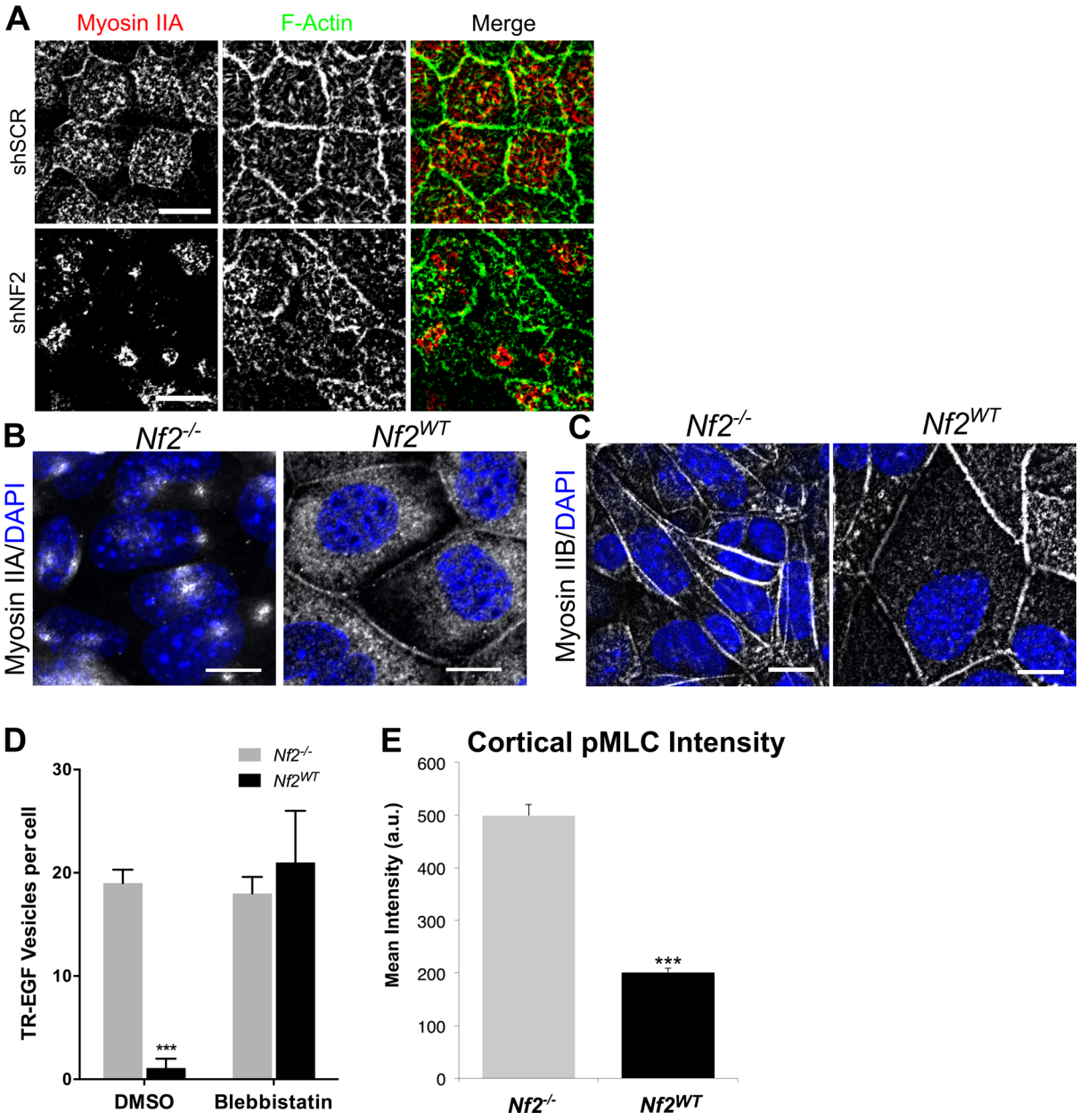



Figure S4. **LDCs exhibit MyoIIA coalescence and Myosin-dependent regulation of TR-EGF internalization.** (A) Confocal images showing the cortical distribution of MyoIIA (red) and F-actin (green) in control (shSCR) and shNF2-expressing Caco2 monolayers. (B) Confocal images showing localization of MyoIIA in *Nf2*$^{-/-}$ or *Nf2*$^{WT}$-expressing LDCs. (C) Confocal images showing localization of Myosin IIB in *Nf2*$^{-/-}$ or *Nf2*$^{WT}$-expressing LDCs. (D) TR-EGF internalization of EGFR in 100-µM blebbistatin–treated *Nf2*$^{-/-}$ or *Nf2*$^{WT}$-expressing LDCs. (E) Quantification of activated myosin regulatory light chain intensity in *Nf2*$^{-/-}$ and *Nf2*$^{WT}$-expressing LDCs. (D and E) Error bars indicate SEM. ***, $P < 0.001$ (unpaired *t* test). DAPI staining of nuclei is shown in blue. Bars, 10 µm. a.u., arbitrary units.

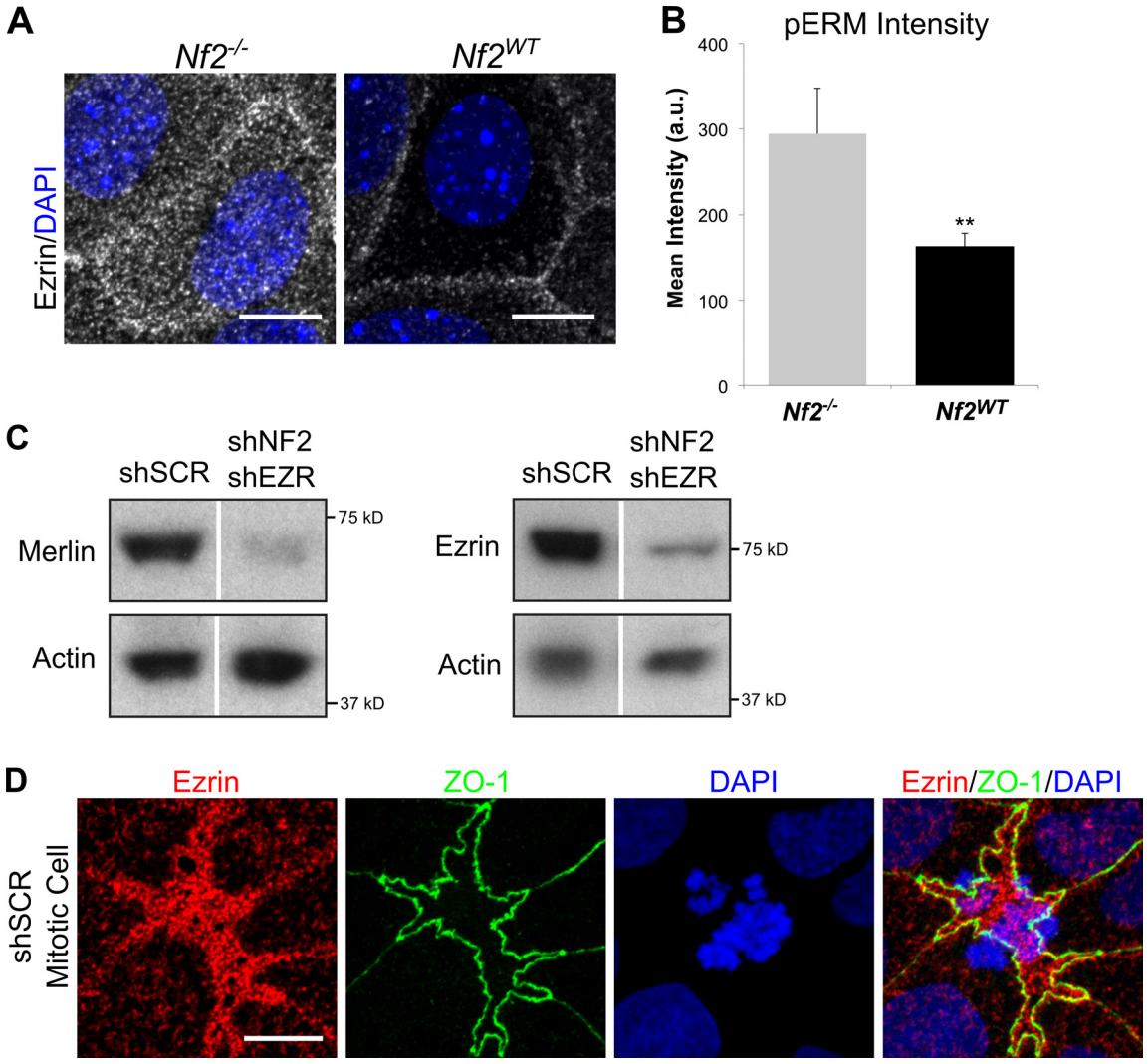



Figure S5. **Increased cortical Ezrin drives apical contractility.** (A) Confocal images showing localization of Ezrin in *Nf2*$^{-/-}$ and *Nf2*$^{WT}$-expressing LDCs. DAPI staining of nuclei is shown in blue. (B) Quantification of pERM intensity in *Nf2*$^{-/-}$ and *Nf2*$^{WT}$-expressing LDCs labeled with an anti-pERM antibody. Error bars indicate SEM ($n$ = 10 cells). (C) Immunoblot confirming loss of Merlin and Ezrin in Caco2 cells expressing shNF2 and shEZR. Bands are from nonadjacent lanes on the same gel. (D) Confocal images depict the levels and distribution of cortical Ezrin (red) and ZO-1 (green) in mitotic control Caco2 cells. **, $P < 0.01$ (unpaired $t$ test). Nuclei are labeled with DAPI. Data are representative of at least two experiments. Bars, 10 µm. a.u., arbitrary units.

**Table S1 is provided as an Excel file and shows the $D_{macro}$ coefficients for EGFR under all experimental conditions tested**

**Table S2 is provided as an Excel file and shows all of the data for TR-EGF internalization under all experimental conditions tested**

**Popall is provided as a custom MATLAB program and loads the dataset and analyzes micro, macro, and a diffusion**

**PopfunL is provided as a custom MATLAB program and carries out population analysis of SPTM results using log normal distribution analysis**

**PopfunN is provided as a custom MATLAB program and carries out population analysis of SPTM results using normal distribution analysis**

**Sptload is provided as a custom MATLAB program and reads all SPTM files and ensures the frame rates match**

**Sptworkup is provided as a custom MATLAB program and loads all trajectories into an array and analyzes them using MSD and subconfinement algorithms**