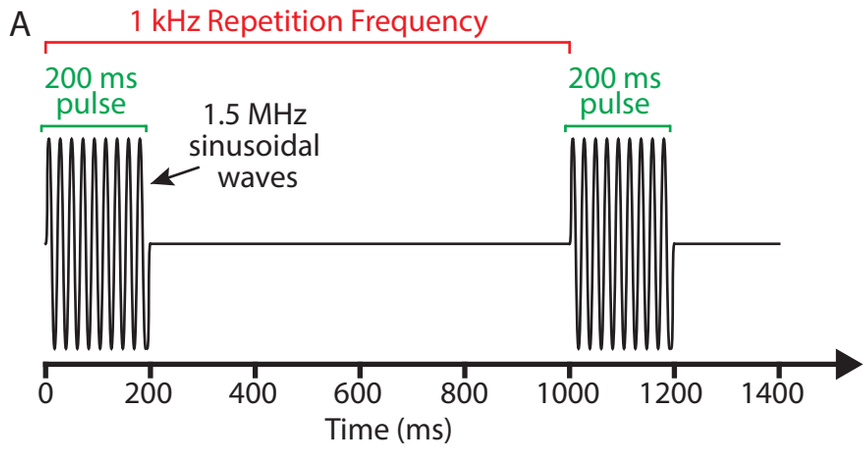
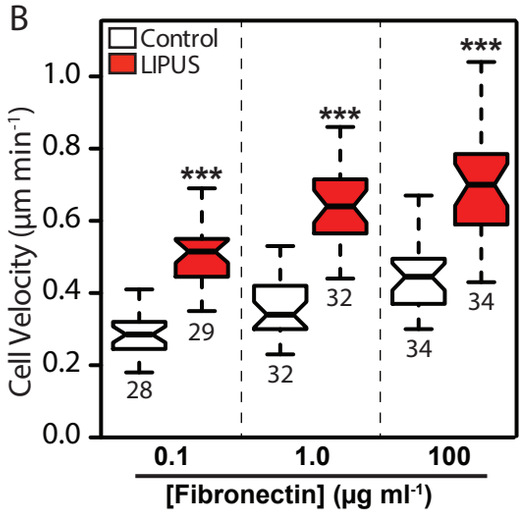
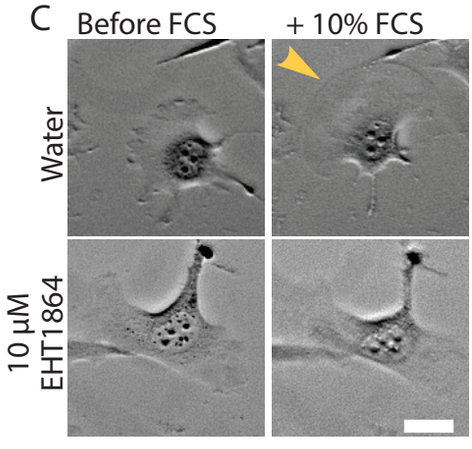
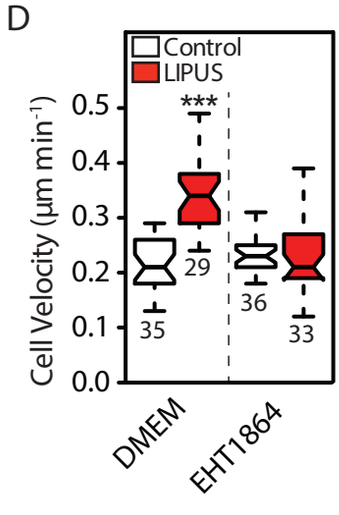


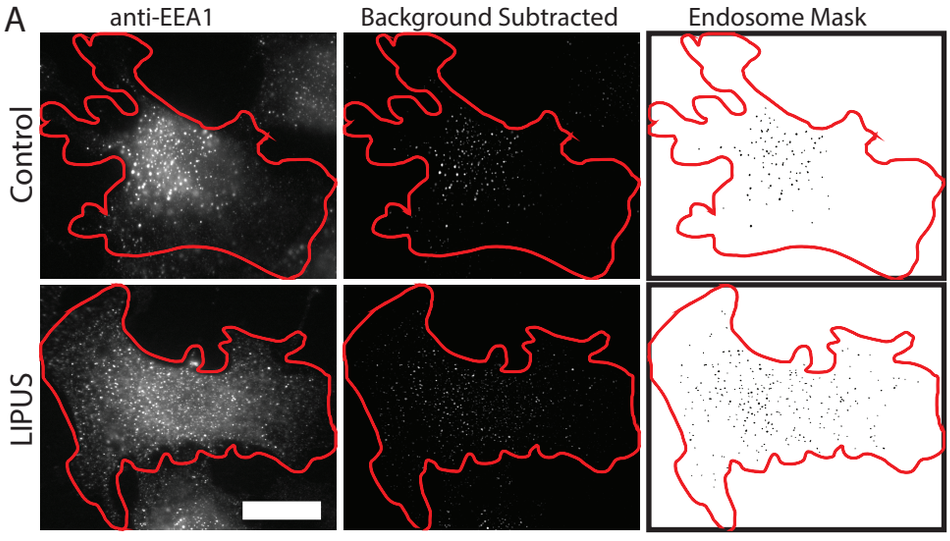


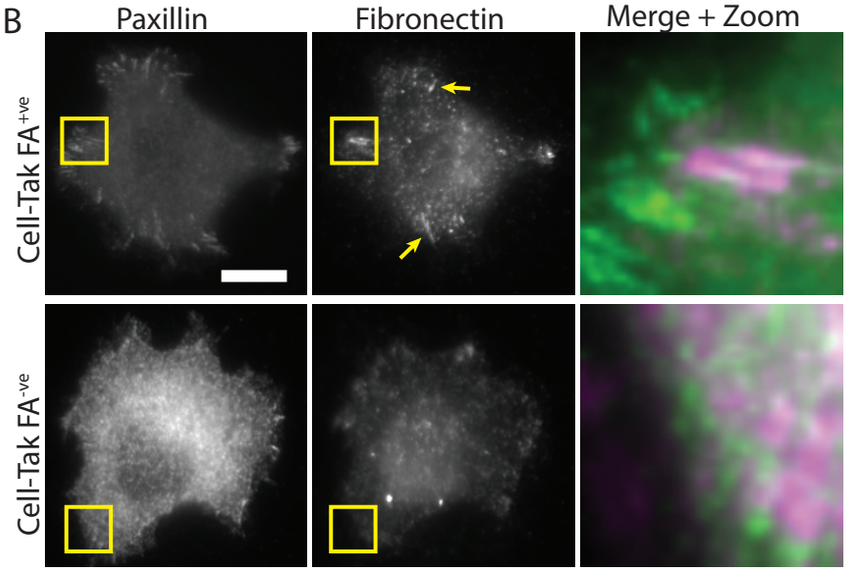


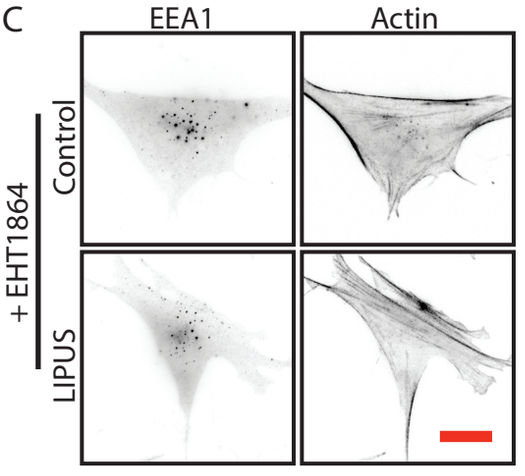


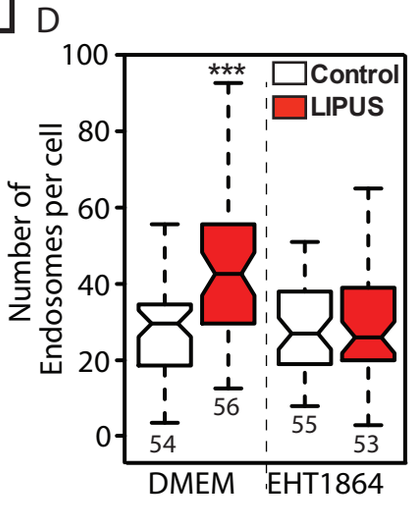
Supplemental Figure 1

A. Profile of the LIPUS signal. Cells are exposed to 200 ms pulses of 1.5 MHz sinusoidal waves. One 200 ms pulse is generated every second (a 1 kHz repetition frequency). LIPUS stimulation occurs for 20 minutes, before the signal is automatically switched off. **B**. LIPUS stimulation increases the migration speed of B16 cells on both low (0.1 or 1 μg ml^{-1}) and high (100 μg ml^{-1}) concentrations of fibronectin coating (results are representative of 3 independent experiments). **C**. B16 cells were serum-starved overnight and then incubated for 4 hours with either EHT1864 (10 μM), or an equivalent volume of water. Live-cell imaging of these cells shows that stimulation with 10% FCS induces lamellipodia formation in water-treated cells (top row, yellow arrow), but not in cells treated with EHT1864 (bottom row). Scale bar indicates 25 μm. **D**. Migration experiments were repeated in MC3T3 cells, treated with or without the Rac inhibitor EHT1864 (10 μM), in LIPUS-stimulated, or non-stimulated control cells. LIPUS stimulation increases the velocity of these cells by ~60%, in a Rac-dependent manner. Results are representative of 3 independent experiments, *** indicates $P < 0.001$ (ANOVA).



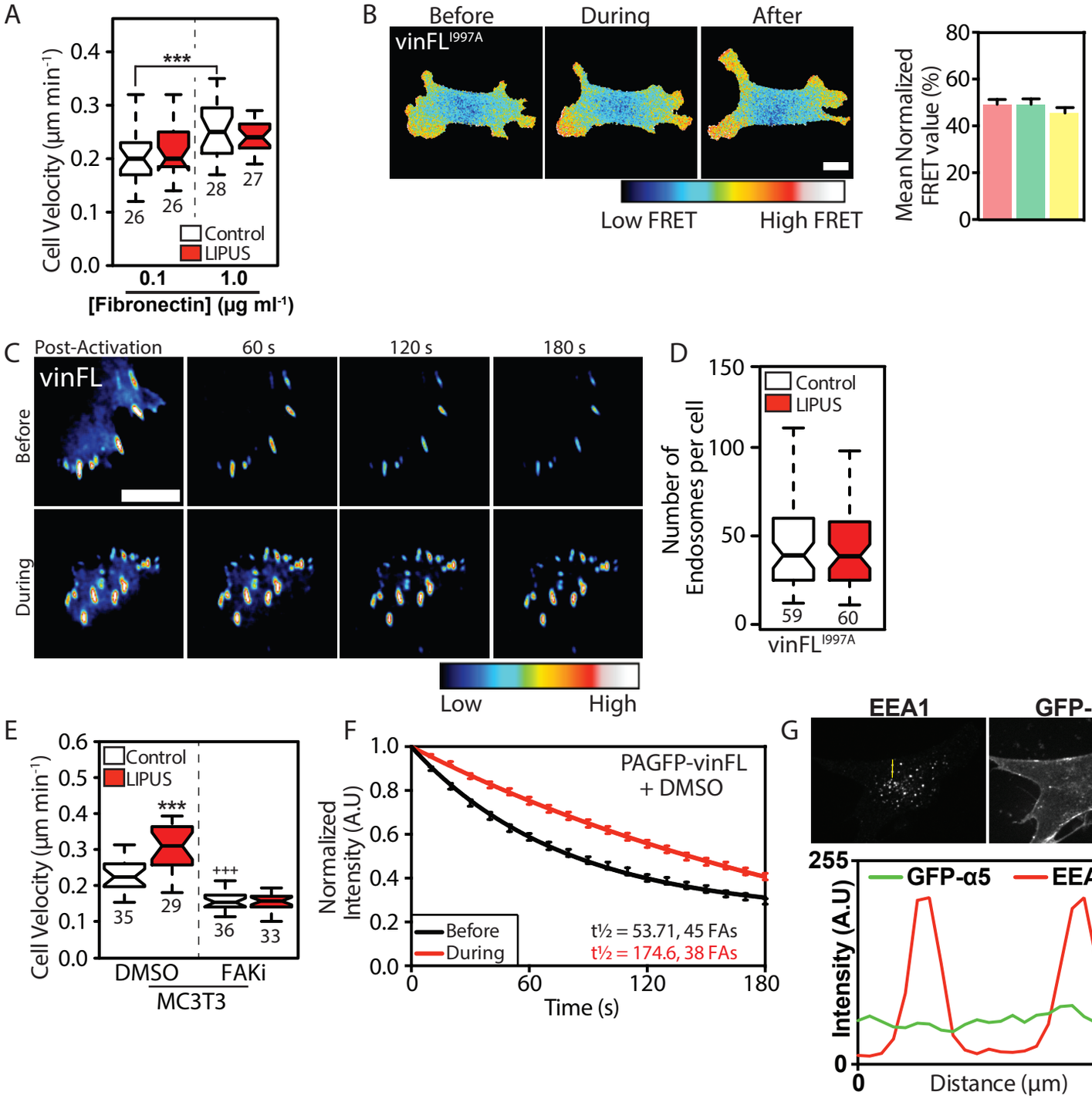






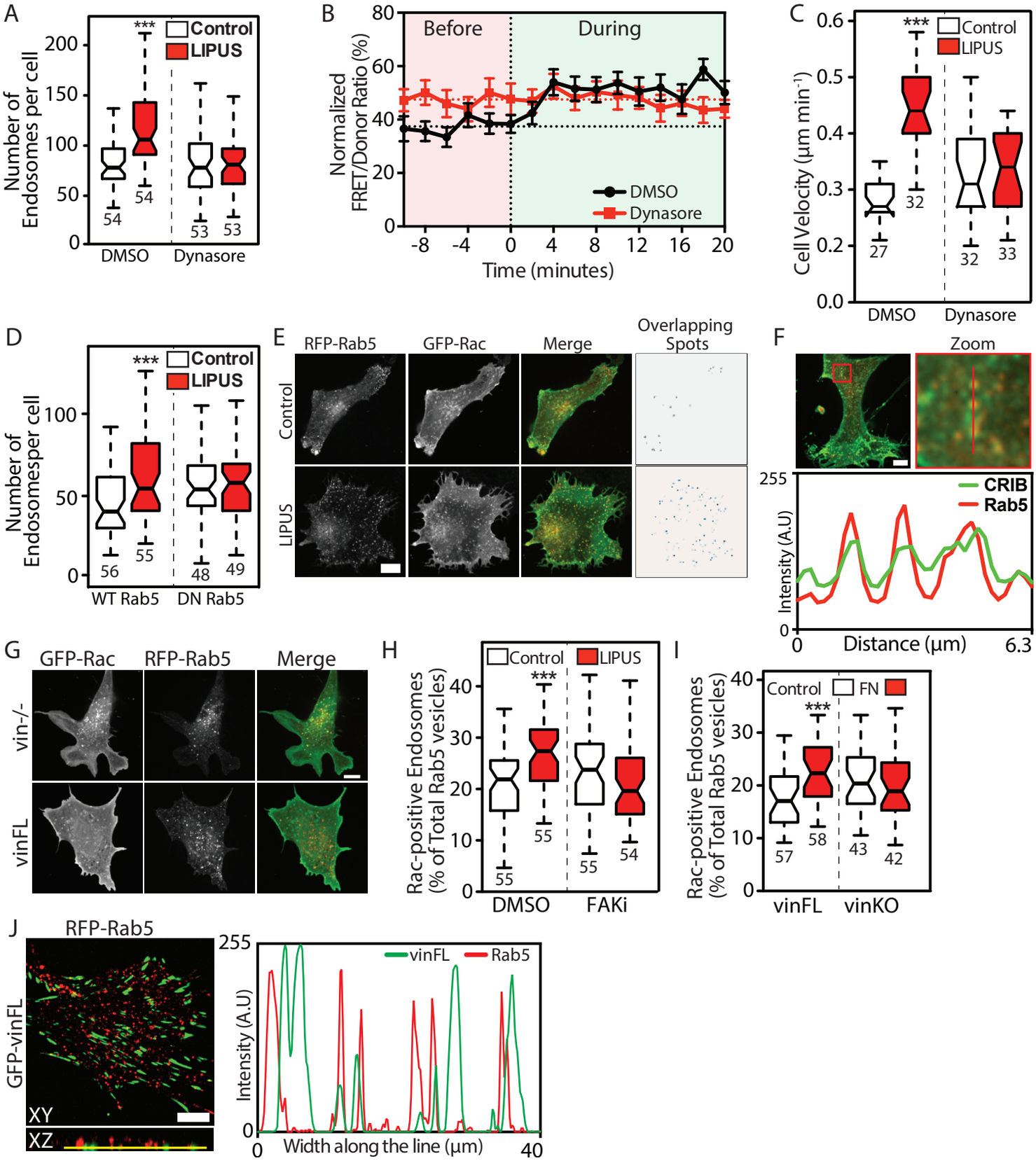
Supplemental Figure 2

A. For endosome quantification, cells were fixed and stained with an antibody against Early Endosome Antigen 1 (EEA1). ImageJ was used to quantify the number of EEA1-positive endosomes: a region of interest was drawn around the cell (top row), followed by background subtraction (middle row) and subsequent thresholding and particle analysis (lower row). Scale bar indicates 25 μm. **B**. Immunofluorescence images of B16 cells on Cell-Tak stained for paxillin and fibronectin. Cells that have begun to form adhesions also have fibronectin streaks (indicated by yellow arrows), indicating that adhesion formation occurs due to deposition of ECM proteins. Scale bar indicates 10 μm. **C**. Immunofluorescence images of vin-/- MEFs rescued with vinFL, showing Early Endosome Antigen 1 (EEA1) and actin in inverted colours, treated with the Rac inhibitor EHT1864 (10 μM) for 4 hours, either with or without LIPUS stimulation. **D**. Note that there is no difference in the number of endosomes between Control and LIPUS-treated cells after pre-treating with the Rac inhibitor (*** indicates $p < 0.001$ (ANOVA), n numbers are pooled from 3 independent experiments).



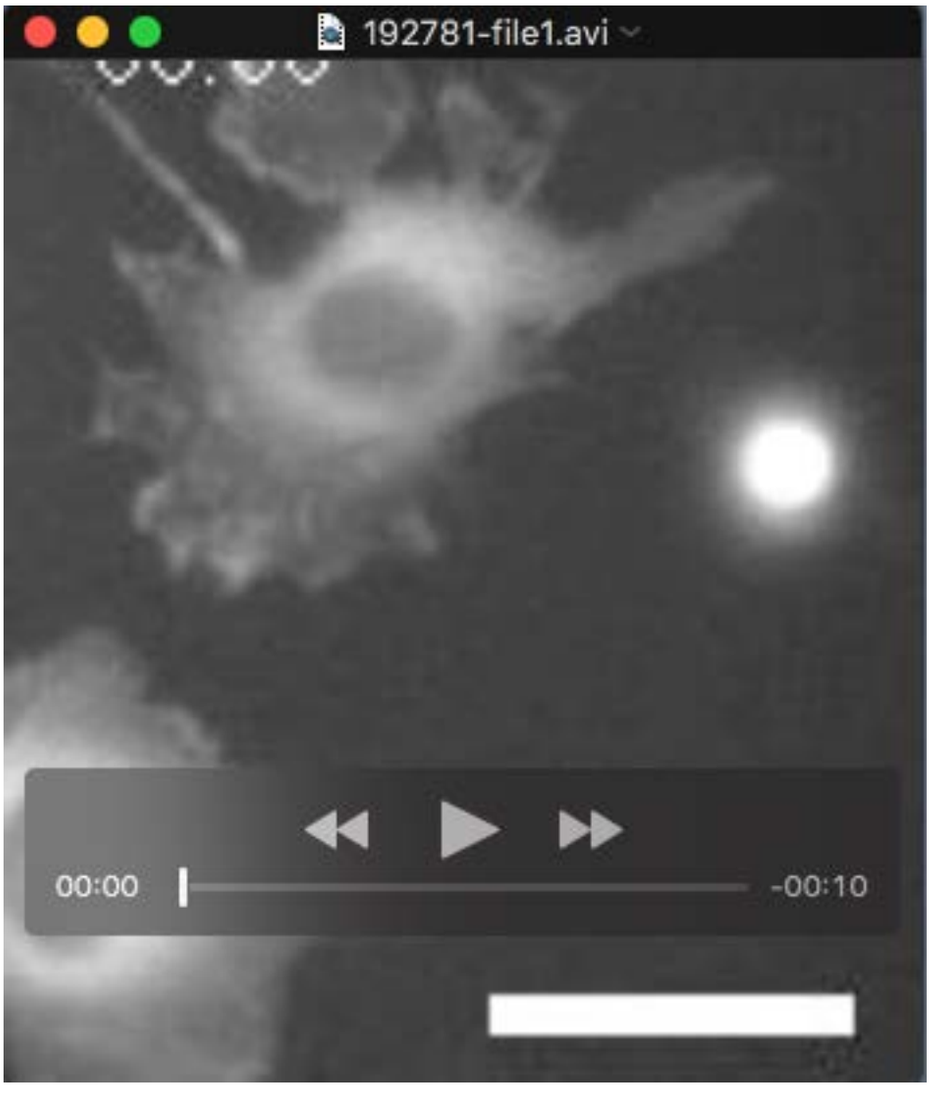
Supplemental Figure 3

A. LIPUS stimulation does not affect the migration speed of vin-/- MEFs even on very low concentrations of fibronectin (results are representative of 3 independent experiments). **B**. Representative images from Raichu Rac FRET experiments in vin-/- MEFs co-expressing vinculin^{I997A}, scale bar indicates 10 μm. **C.** Representative confocal images from Fluorescence Loss After Photoactivation (FLAP) experiments, pseudocoloured for intensity (scale bar indicates 10 μm), of NIH3T3 cells co-expressing mCherry zyxin (a FA marker) and PAGFP-vinculin. Note the loss of fluorescence from the photoactivated regions is quicker in non-stimulated cells ('Before') than in cells undergoing LIPUS stimulation ('During'). **D**. Vin-/- MEFs expressing vinculin^{I997A} show no increase in the number of early endosomes after LIPUS stimulation (indicated number of measurements (n) is pooled from 3 independent experiments). **E**. The increased migration of MC3T3 cells after LIPUS stimulation is also blocked by pre-treatment for 1 hour with FAKi (3 μM). Results are representative of 3 independent experiments, *** indicates $P < 0.001$ (ANOVA). **F**. FLAP curves with t½ values for DMSO-treated cells (control for FAKi-treated cells). **G**. Immunofluorescence images of vin-/- MEFs co-expressing CFP-vinculin and GFP-α5 integrin cultured on collagen-coated glass overnight, before fixation after LIPUS stimulation and staining for EEA1. Note the lack of co-localization between GFP-α5 and EEA1 in these cells on collagen.



Supplemental Figure 4

A. Quantification of EEA1-positive vesicles in Control and LIPUS-stimulated cells pre-treated with either 80 μM Dynasore or with an equivalent volume of DMSO. N numbers are pooled from 3 independent experiments. **B**. Raichu Rac FRET measurements from 28 DMSO-treated and 30 Dynasore-treated cells (n numbers are pooled from 3 independent experiments), error bars show s.e.m. Vertical bar indicates when LIPUS stimulation began; horizontal bar shows the baseline (calculated as the mean of 6 pre-LIPUS values.). **C**. Quantification of cell motility, tracked over 16 hours in cells pre-treated with either 80 μM Dynasore or an equivalent volume of DMSO. Results are representative of 3 independent repeats. **D**. LIPUS stimulation increases the number of early endosomes in cells expressing WT Rab5, but not in cells expressing DN Rab5. *** indicates $p < 0.001$ (ANOVA); data are pooled from 3 independent experiments. **E**. Confocal images of B16 cells (either LIPUS-stimulated or non-stimulated) expressing RFP-Rab5 and GFP-Rac. Images were analysed using the JaCoP plugin for ImageJ to quantify the number of overlapping spots between the two channels. Scale bar indicates 10 μm. **F**. Confocal image of a B16 cell expressing the CFP-CRIB-YFP probe and RFP-Rab5, with a line profile taken from the indicated magnified region. Note the colocalization between the two channels. **G**. Representative images of vin-/- MEFs with or without vinFL, co-expressing GFP-Rac and RFP-Rab5. Note the increased number of Rab5-positive vesicles also positive for GFP-Rac (quantification in Figure 7D) Scale bar indicates 10 μm. **H**. Increased Rac-Rab5 co-localisation in LIPUS-stimulated cells is blocked by pre-treatment with 3 μM FAKi; n numbers are pooled from 3 independent experiments. **I**. Rac-Rab5 co-localisation can also be increased by stimulation with soluble fibronectin (10 μg/ml); n numbers are pooled from 3 independent experiments, *** indicates $p < 0.001$ (t-test). **J**. Confocal microscopy of vin-/- MEFs expressing RFP-Rab5 and GFP-vinculin reveals a number of Rab5-positive vesicles are present in proximity to FAs. Image shows the XY plane and the XZ plane, with the line profile taken from the XZ plane. Scale bar indicates 10 μm.



Supplemental Movie 1. LIPUS stimulation induces actin-rich Circular Dorsal Ruffles.

Two-hour movie from live-cell imaging of a B16 GFP-actin cell, recorded in 60 s intervals. An ethanol-sterilised LIPUS transducer is placed in the media throughout the movie, allowing only fluorescence imaging. LIPUS is started at the end of the first hour, which rapidly leads to rearrangement of the actin cytoskeleton into a circular dorsal ruffle. Scale bar indicates 20 μm.

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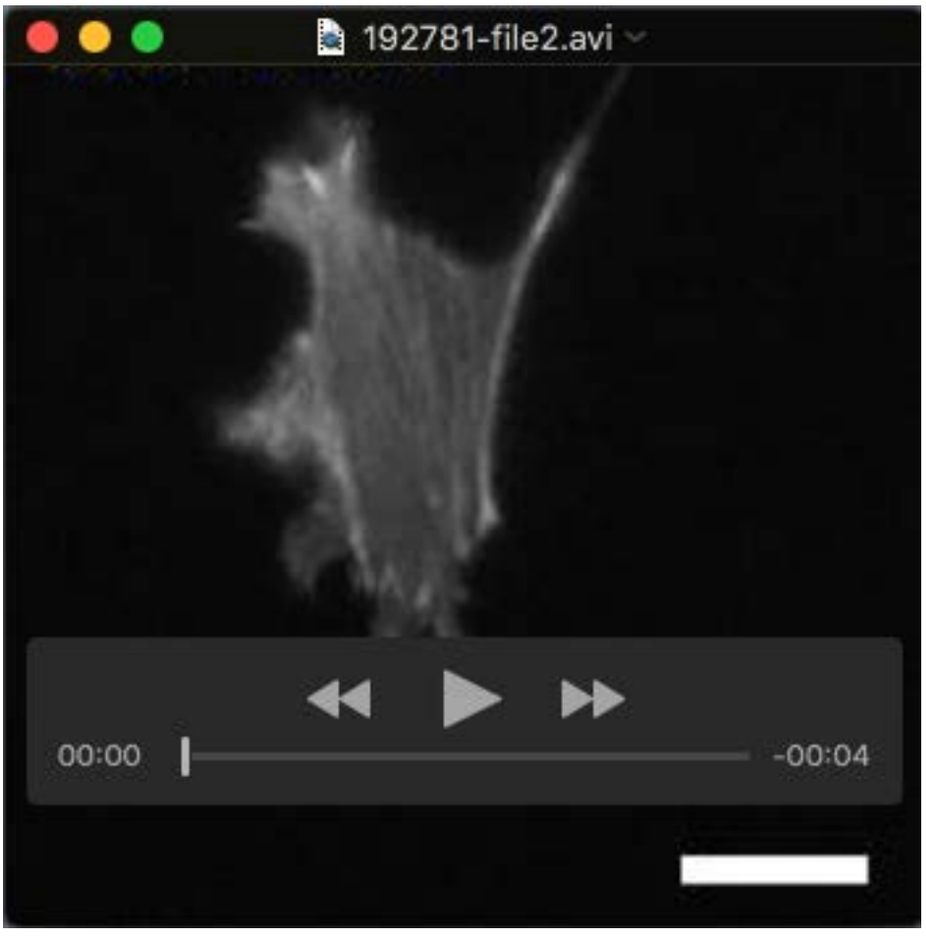
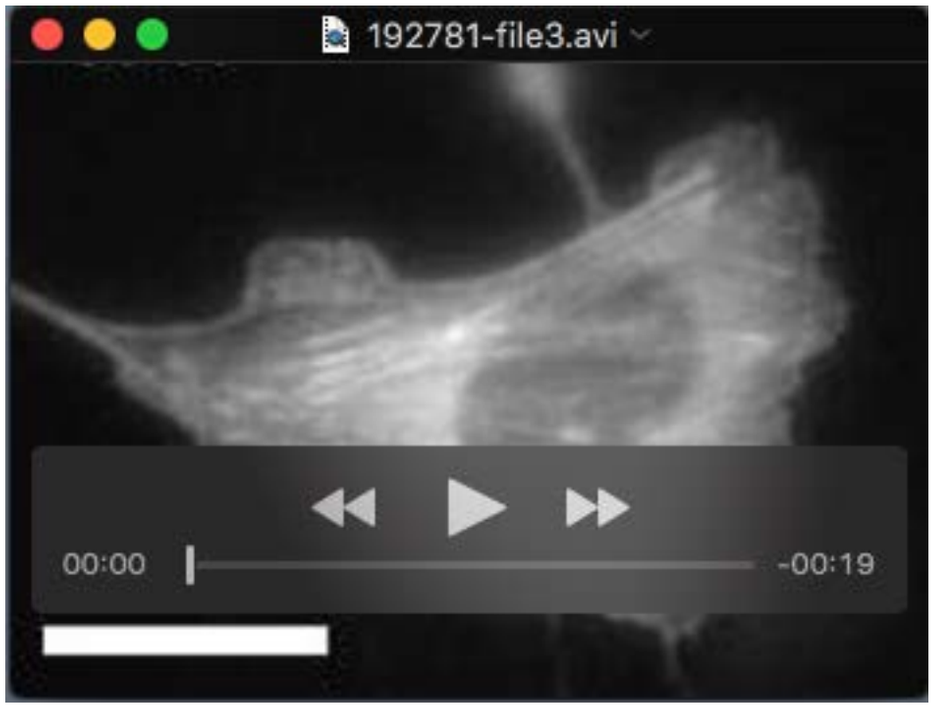


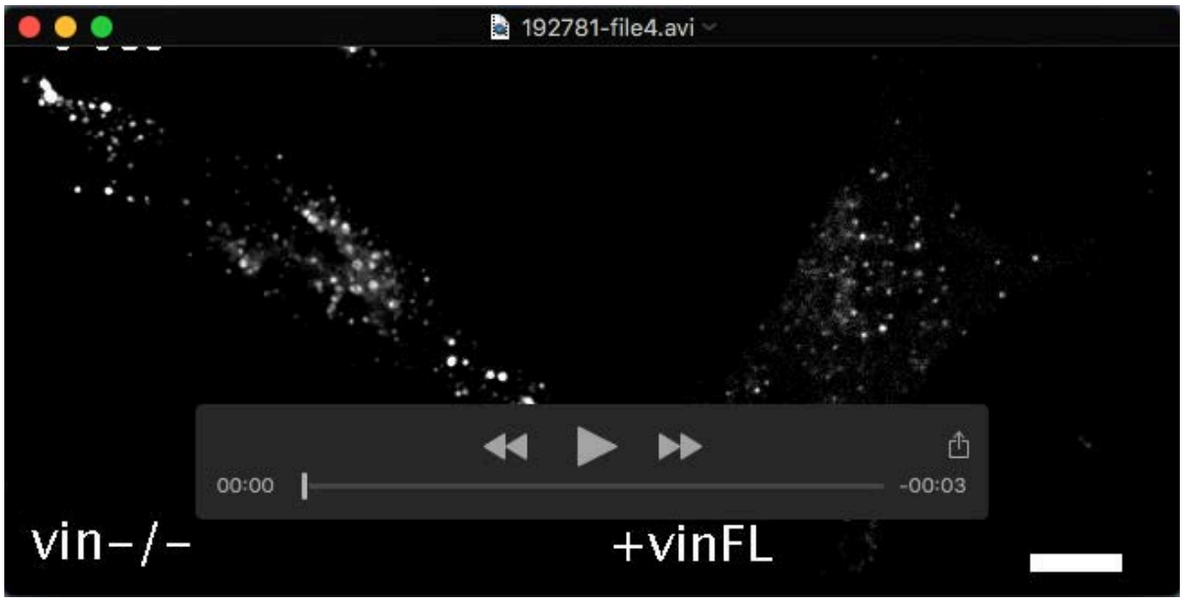
Supplemental Movie 2. LIPUS stimulation induces membrane protrusion formation in MEFs.

One-hour movie from live-cell imaging of a Mouse Embryonic Fibroblast (MEF) expressing Life-Act, imaged in 60 s intervals. The first 20 minutes (indicated adjacent to the time-stamp) are before LIPUS, the second 20 minutes are 'during'; the final 20 minutes are 'after'. Arrows indicate membrane protrusions, which were quantified by manual counting. Scale bar indicates 10 μm.



Supplemental Movie 3. LIPUS stimulation of serum-starved cells induces actin 'comet-tail' formation

Two-hour movie from live-cell imaging of a B16 GFP-actin cell, previously serum-starved for 24 hours. LIPUS is started at the end of the first hour, which rapidly induces the formation of actin 'comet-tails'. Scale bar indicates 15 μm.



Supplemental Movie 4. Movie of Rab5-positive vesicles in vin-/- MEFs, and in vinFL-rescued cells

Images were acquired on a spinning-disk confocal microscope every second for 1 minute. Left cell is a vin-/- MEFs, right cell is a vin-/- MEF expressing GFP-vinFL (not shown), both are expressing RFP-Rab5. Note the faster movement of vesicles in the vin-/- MEF, compared to the cell rescued with vinFL. Scale bar indicates 10 μm.

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