SUPPLEMENTAL MATERIALS

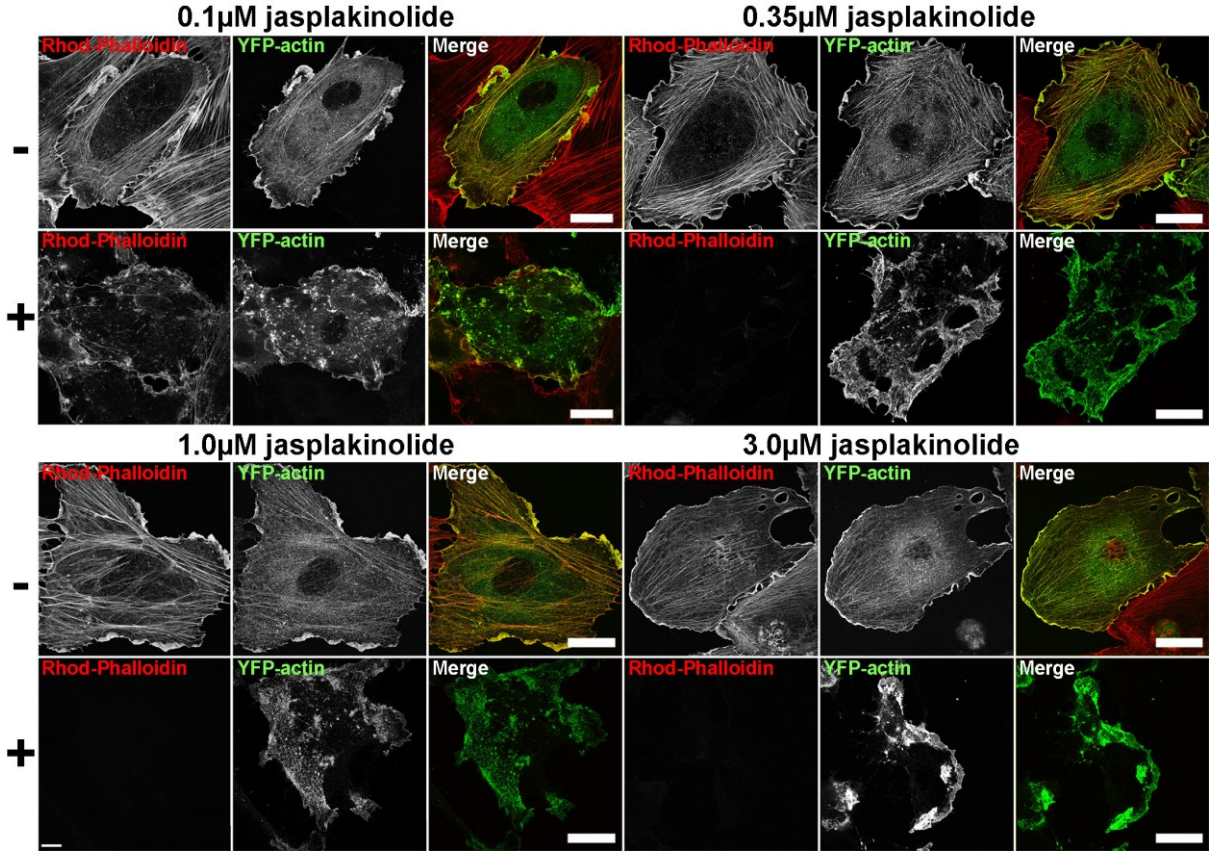


Fig. S1 Effect of jasplakinolide on actin cytoskeleton and cell morphology.

S1A: Images show the effect of a 20 minute incubation with vehicle (-) or 0.1 µM to 3.0 µM jasplakinolide (+), as indicated, on the actin cytoskeleton and cell morphology visualised by expression of YFP-actin (green in colour merged images). Competitive binding of jasplakinolide to actin is shown by the displacement of rhodamine-phalloidin staining (red in colour merge images). Scale bars are 10 µm.

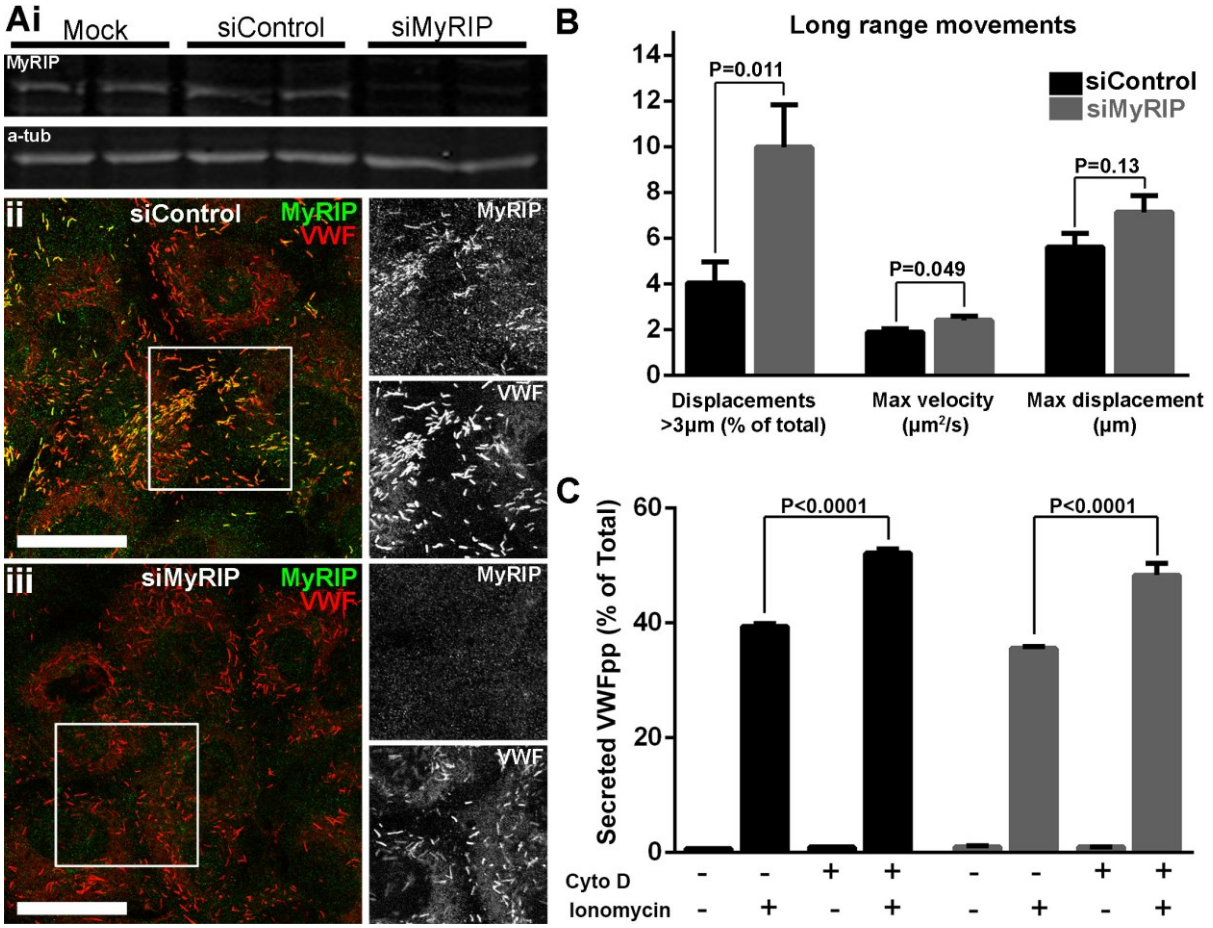


Fig. S2 Effect of MyRIP knockdown on WPB trafficking and VWFpp secretion.

(Ai) Western blot showing depletion of endogenous MyRIP (top) using specific siRNA (siMyRIP) in HUVEC. Alpha-tubulin (α-tub) controls are shown below. (Aii-iii) Confocal immunofluorescence image of HUVEC labelled for endogenous MyRIP (green) and VWF (red) in cells transfected with siControl RNA (ii) or siMyRIP RNA (iii). Greyscale images are from regions indicated in the colour merged panels. Scale bars are 20 µm. (B) Parameters determined from detected trajectories of long range movements of WPBs in HUVEC expressing VWFpp-EGFP and treated with siControl (black) or siMyRIP (grey) RNAi (48 hours post transfection). Number of cells imaged and trajectories detected were: siControl, n=11 cells, 1470 trajectories; siMyRIP, n=12, 1324. Images were acquired at 30 frames per second and movements analysed in GMimPro at 10 frames per second. Comparisons by t-test. (C) Ionomycin (1µM) stimulated secretion of endogenous VWFpp in siControl (black) or siMyRIP (grey) treated cells. Cells were treated with vehicle (-; RM with 0.1% DMSO) or cytochalasin D (+; 1µM, 20 minutes) as indicated. Plots show data from one representative experiment of 3 separate experiments, each performed in triplicate (mean ±s.e.m.). ANOVA, Tukey multiple comparisons test.

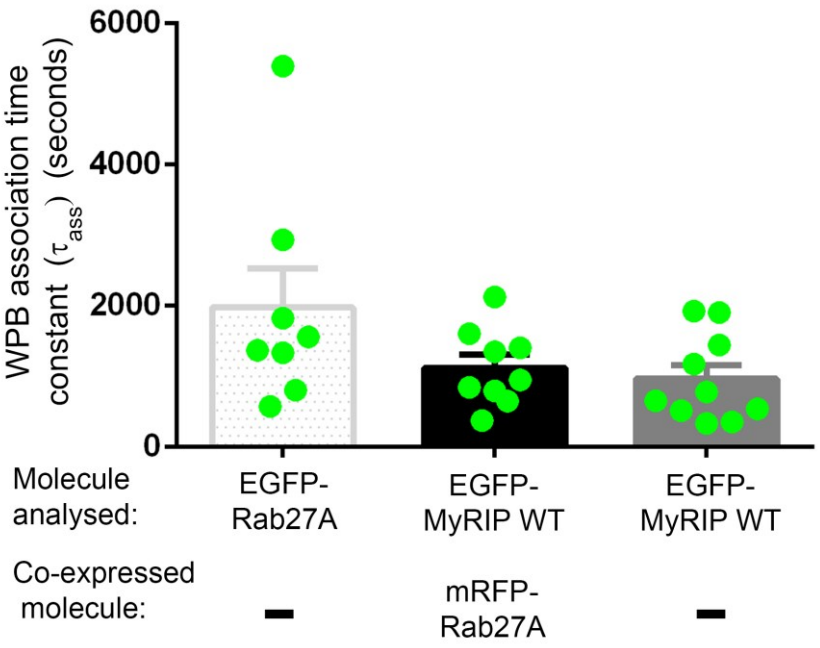
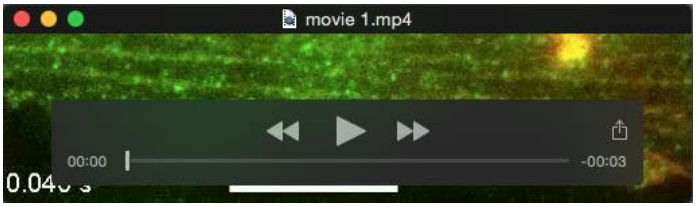
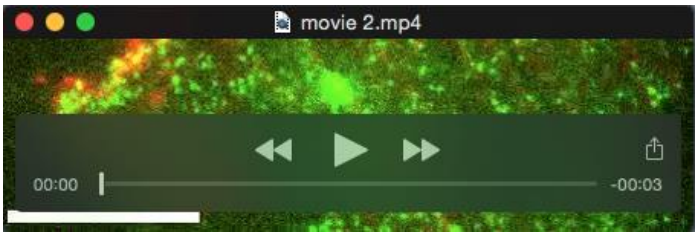


Fig. S3. Time constants for EGFP-MyRIP cycling onto WPBs determined by whole WPB bleaching.

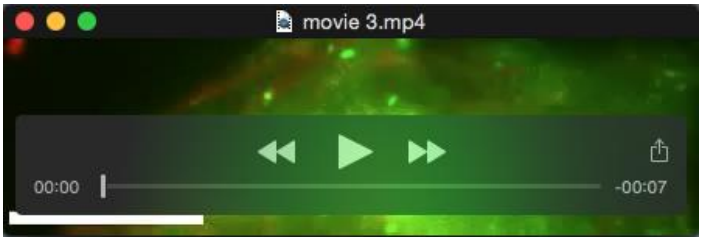
Fluorescence recovery after whole WPB bleaching is due to association of new fluorescent molecules with WPB. Average WPB association time constants (τ_{ass}) for EGFP-MyRIP (grey, 961.4±193.4s, n=10 WPBs, mean±SEM) or EGFP-MyRIP in the presence of co-expressed mRFP-Rab27A (black, 1120±181.0s, n=9) determined by whole WPB FRAP. Green circles show the individual data points. For comparison the mean WPB association time constant determined previously for EGFP-Rab27A (1970±550s, n=8), and adapted from Fig.3D of (Bierings et al., 2012), is shown in a pale grey spot hatch. Mean τ_{ass} for EGFP-MyRIP in the presence or absence of co-expressed mRFP-Rab27A, or for EGFP-Rab27A were not significantly different (ANOVA, Tukey multiple comparison test).



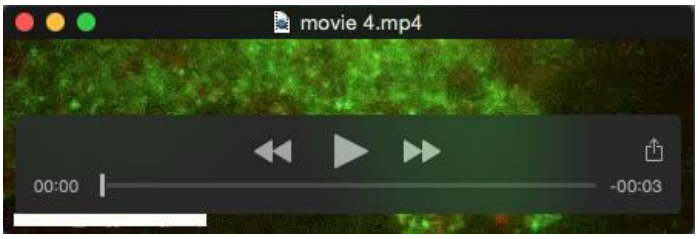
Movie 1. Single molecule imaging showing EGFP-MyRIP WT binding to actin.



Movie 2.. Single molecule imaging showing EGFP-MyRIP A751P binding to actin.



Movie 3. Single molecule imaging showing EGFP-MyRIP A4 not binding to actin.



Movie 4.. Single molecule imaging showing EGFP-MyRIP A751P A4 not binding to actin.

Movies show single-molecule TIRF imaging showing interactions of EGFP-MyRIP mutants with actin in live HUVEC.

Video clips (avi format, jpg compression in ImageJ (http://rsb.info.nih.gov/ij/)) of a sub-region of the live HUVEC co-expressing EGFP-MyRIP constructs (green) and TagRFP-actin (red). Videos show the dynamics of the EGFP fluorescence superimposed upon an image of the position of the actin cytoskeleton obtained by averaging between 401 and 900 consecutive frames of the TagRFP channel. Video SAi: MyRIP WT, Video SBi: MyRIP A751P, Video SCi: MyRIP 4A, Video SDi: MyRIP A751P 4A. Videos were acquired at 50 frames/second and time elapsed is shown. Scale bars are 10 µm. Discrete bright spots of EGFP fluorescence correspond to single EGFP fluorophores (Mashanov and Molloy, 2007). For MyRIP WT and MyRIP A751P many single EGFP fluorophores can be seen transiently localising to the actin cytoskeleton. For MyRIP 4A and MyRIP A751P 4A, single EGFP molecules do not associate with actin but remain cytosolic as a diffuse cloud of fast moving objects.