

Figure S1. Ena remodels the lamellipod into fascin-decorated, actin-bundles

(a) control or *ena* macrophages expressing LifeAct-GFP and either Dia Δ Dad-GFP or Ena-GFP. Arrows highlight actin bundles. Scale bar =10 μ m.

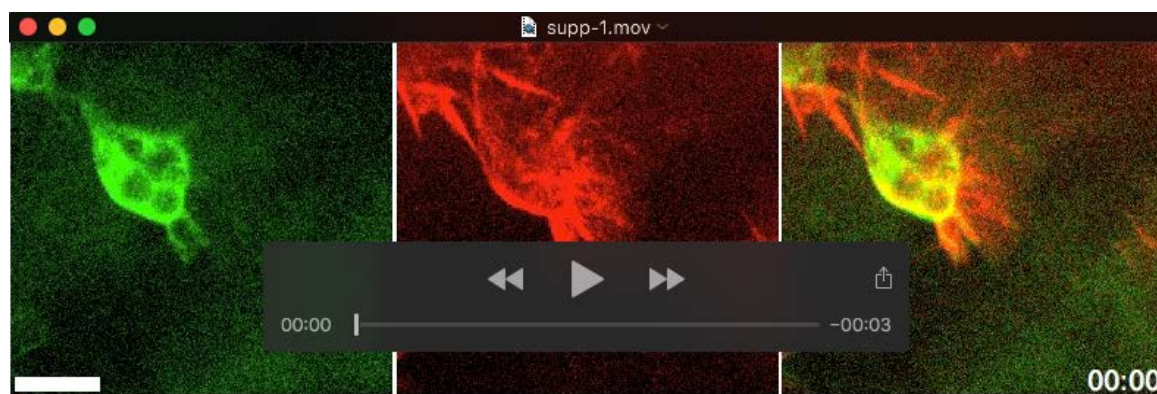
(b) Actin bundles / cell in control or *ena* macrophages expressing either Dia Δ DAD-GFP or Ena-GFP (*ena* =2.90 \pm 0.31, *ena*; Dia Δ DAD =1.58 \pm 1.16, *ena*; Ena =17.56 \pm 1.09, control =18.28 \pm 0.90, Dia Δ DAD =14.18 \pm 1.26, Ena =24.28 \pm 1.39 bundles / cell, mean \pm SEM, n \geq 10). Error bars are 95% CI and asterisks indicate statistical significance (ANOVA, p<0.05). ns =p>0.05.

(c) *scar* and *arp3* mutants expressing LifeAct-GFP (GREEN). Arrows highlight actin bundles / filopods. Scale bar =10 μ m.

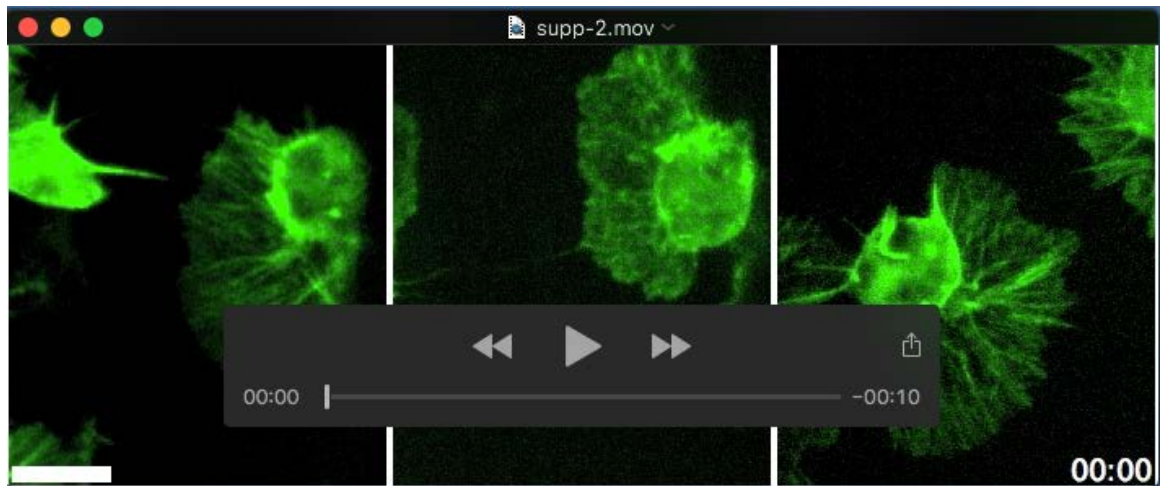
(d) Mean lamellipod area of control, *ena*, *scar* and *arp3* macrophages (control =411.68 \pm 17.20, *ena* =396.57 \pm 11.04, *scar* =194.41 \pm 39.64, *arp3* =56.05 \pm 16.91 μ m², mean \pm SEM, n \geq 12 cells / genotype).

(e) Colocalisation of LifeAct-mCherry (RED) with Dia-GFP (GREEN) at the residual lamellipodial bundles (arrows) in *ena* mutants. Scale bar =10 μ m.

(f) Number of fascin bundle coalescence events / min normalised to mean fascin bundle number of each genotype (control = 0.1634 \pm 0.014, Ena =0.206 \pm 0.010 events / cell, mean \pm SEM, n >20 cells / genotype). Error bars are 95% CI and asterisks indicate statistical significance vs. control mean (unpaired t test, p<0.05).

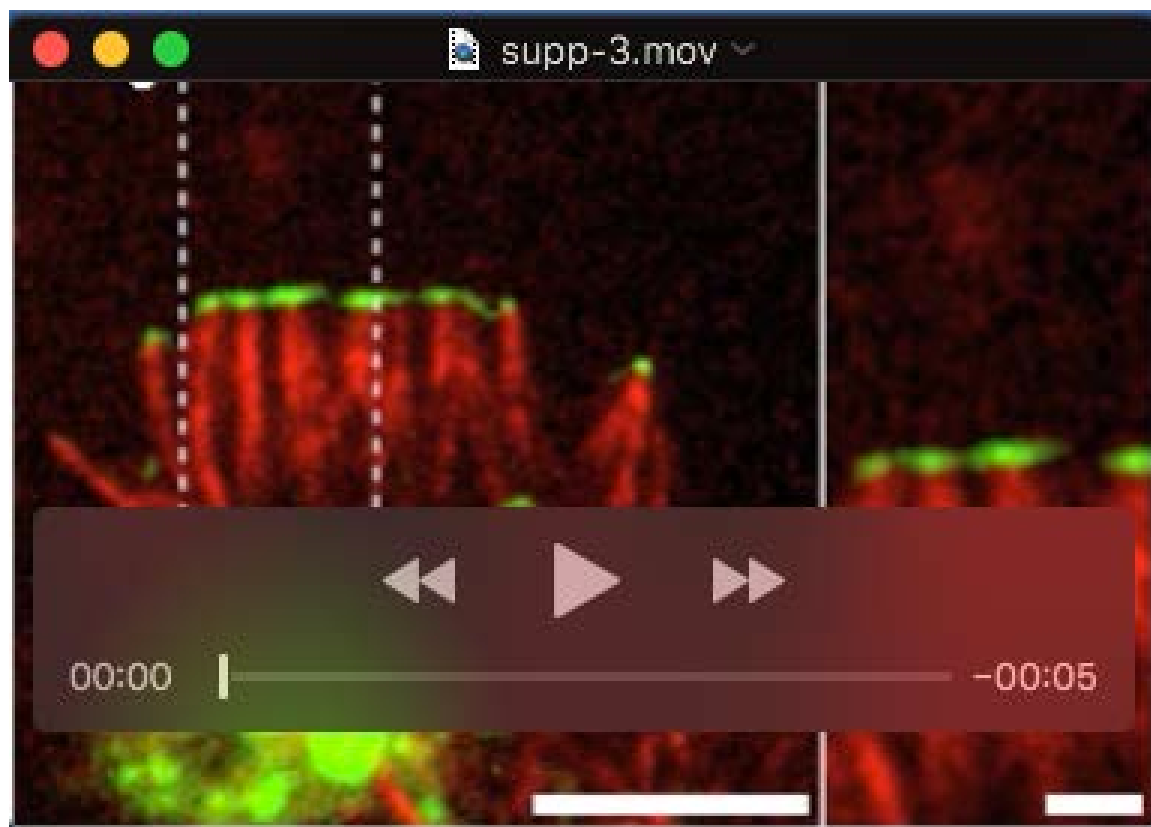


Movie 1. *In vivo* localisation of Dia in motile *Drosophila* macrophages. Full length Dia-GFP (GREEN) localises to rare actin bundles (LifeAct-mCherry, RED) within the lamellipod. Images were acquired with spinning disc confocal microscopy (Perkin Elmer Ultraview) every 30 s. The scale bar is 10 μ m. The movie frame rate is 4 frames/s.

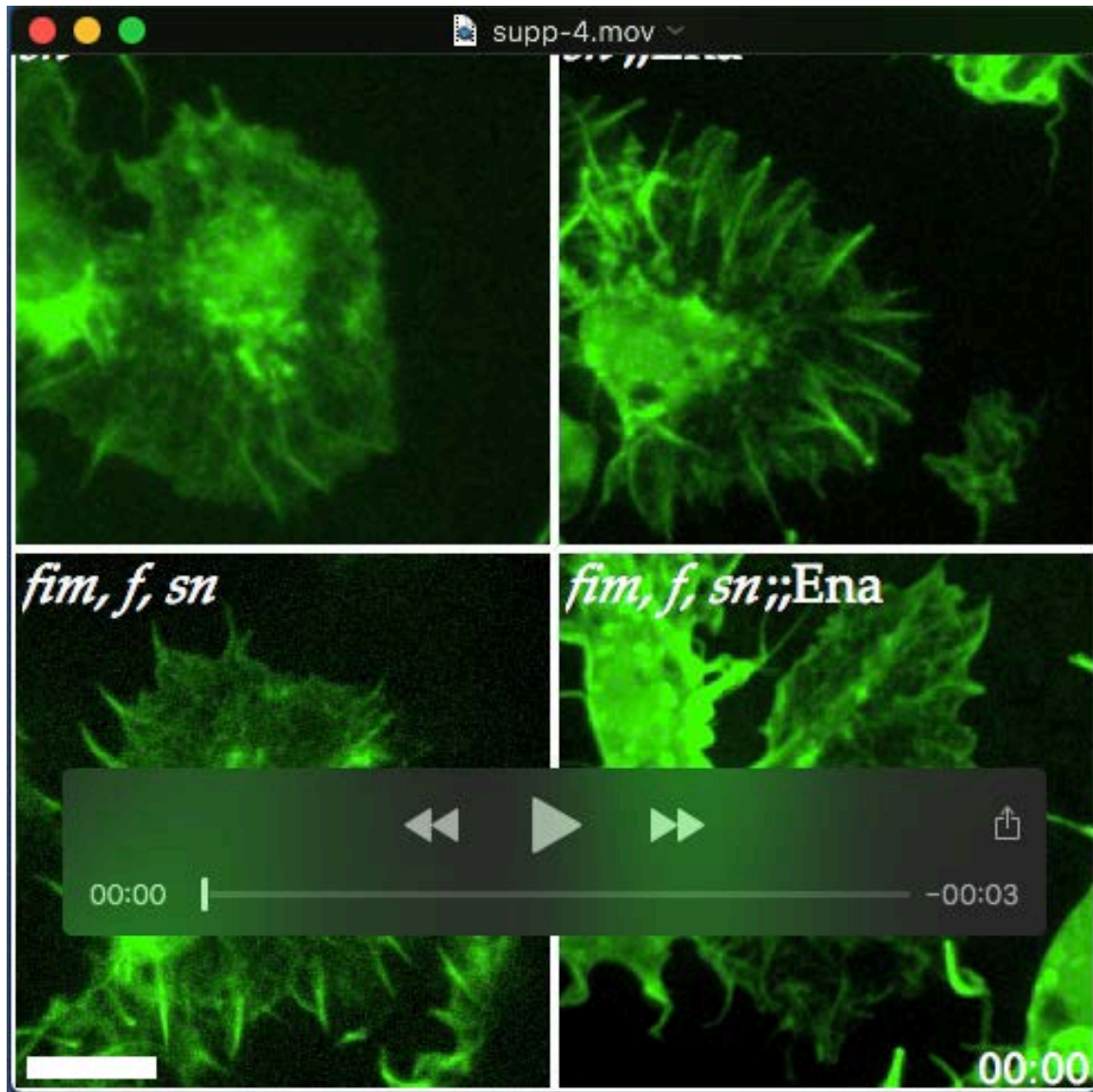


Movie 2. Loss of *ena* but not *dia* results in loss of nearly all lamellipodial bundles.

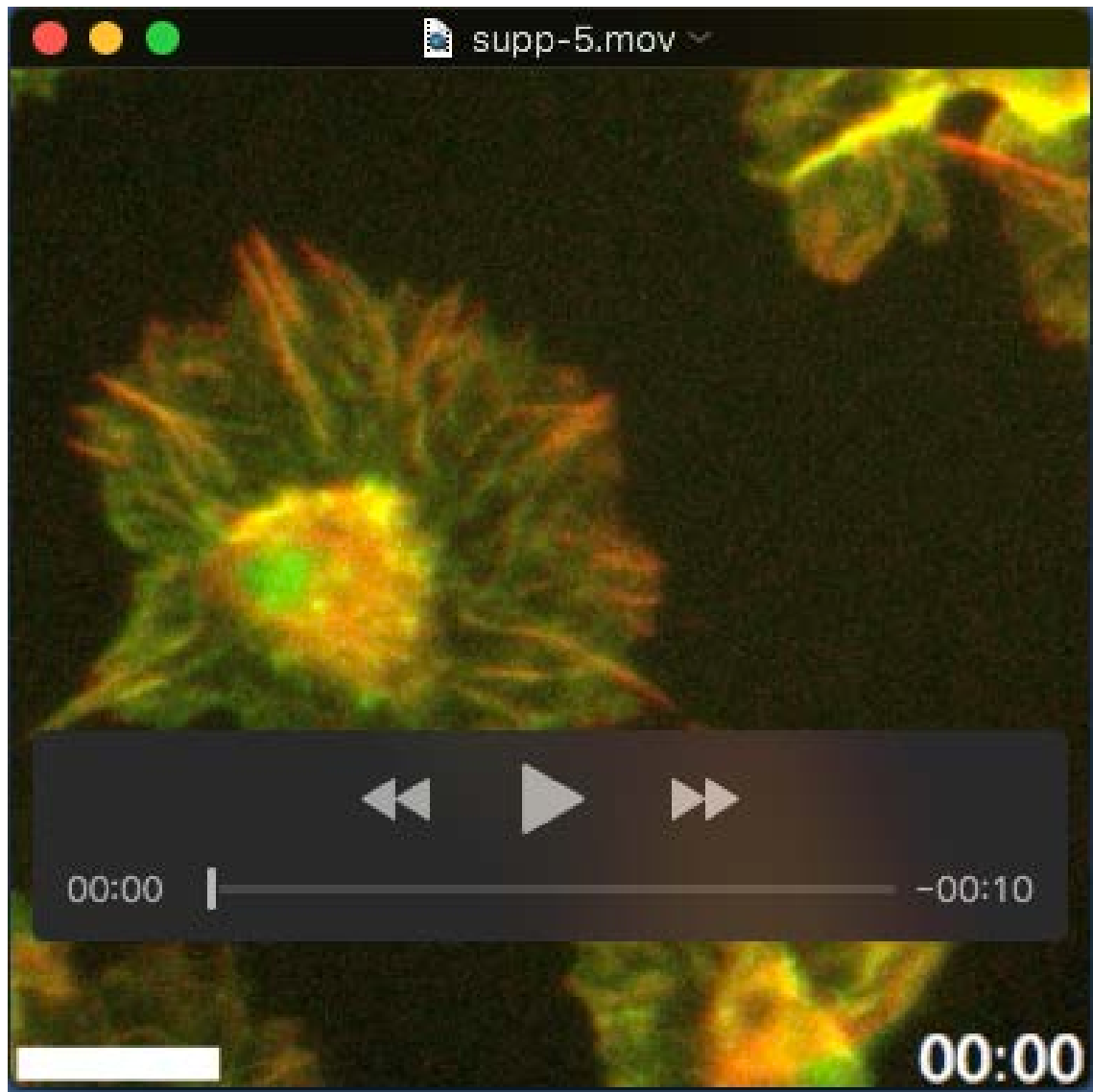
Lamellipodial bundling in control, *ena* and *dia* M/Z macrophages expressing LifeAct-GFP (GREEN). A rare, probing *ena* actin bundle/filopod is shown. Images were acquired with spinning disc confocal microscopy (Perkin Elmer Ultraview) every 30 s. The scale bar is 10 μ m. The movie frame rate is 4 frames/s.



Movie 3. Ena mediates coalescence of fascin bundles. Examples of Ena-GFP (GREEN) capped fascin bundles (Fascin-mCherry, RED) coalescing within a protruding lamellipod. Dashed box is enlarged in left-hand panel. Images were acquired with spinning disc confocal microscopy (Perkin Elmer Ultraview) every 6 s. Time is in seconds and the scale bars = $10/2 \mu\text{m}$. The movie frame rate is 4 frames/s.



Movie 4. Ena over-expression utilises fimbrin and/or forked to restore lamellipodial bundling in absence of fascin. Actin bundling (LifeAct-GFP, GREEN) in *sn* or *fim, f, sn* triple mutants \pm Ena over-expression. Images were acquired with spinning disc confocal microscopy (Perkin Elmer Ultraview) every 30 s. The scale bar is 10 μ m. The movie frame rate is 4 frames/s.



Movie 5. Fimbrin compensates for loss of fascin to maintain lamellipodial bundling. Fimbrin-mCherry (RED) decorates actin bundles (LifeAct-GFP, GREEN) within the lamellipod of *sn* mutant macrophages. Images were acquired with spinning disc confocal microscopy (Perkin Elmer Ultraview) every 30 s. The scale bar is 10 μ m. The movie frame rate is 4 frames/s.