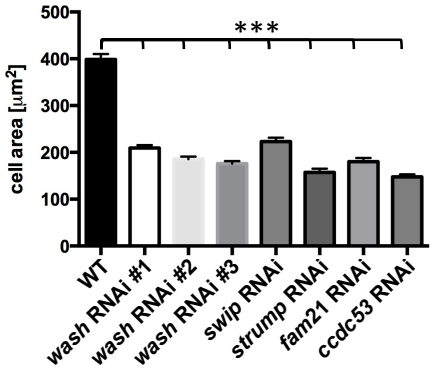
Supplementary material



Supplementary figure S1
WASH is associated in a pentameric complex whose stability depends on each member. When single complex members are knocked down by RNAi, macrophages are unable to spread properly, comparable to the *wash* mutant situation (n = 200 per genotype). *** = $p < 0.0001$, error bars represent SEM.

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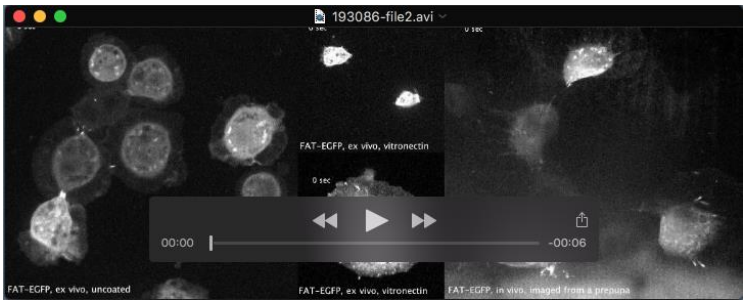
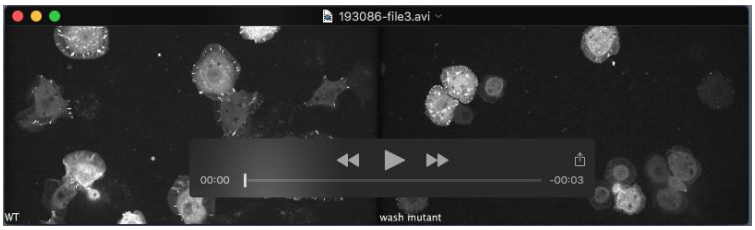


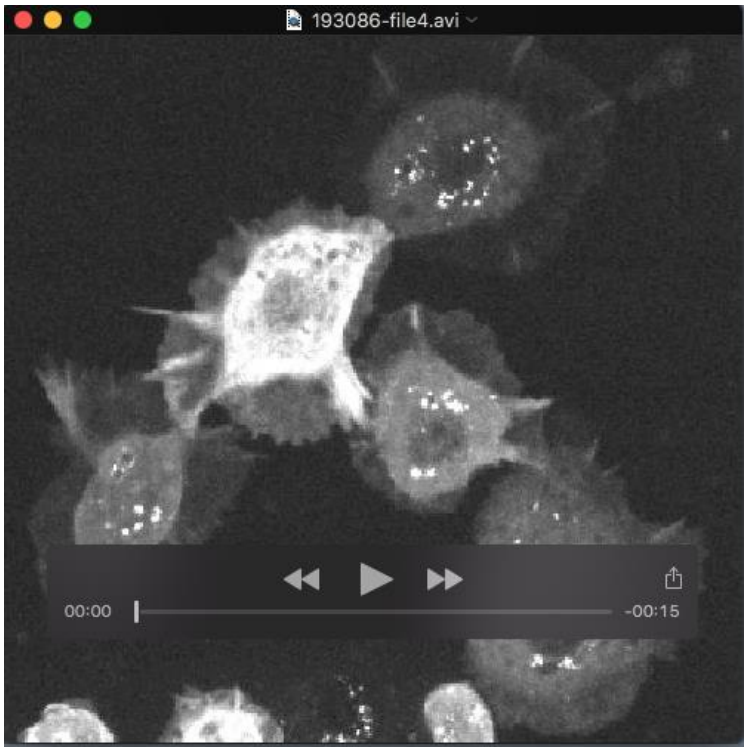
Supplementary Movie M1

Spinning disc microscopy time-lapse movie of macrophages expressing EGFP- FAT using the *hml*ΔGal4 driver *ex vivo* plated on coated and uncoated surfaces, as well as *in vivo* in a prepupa.



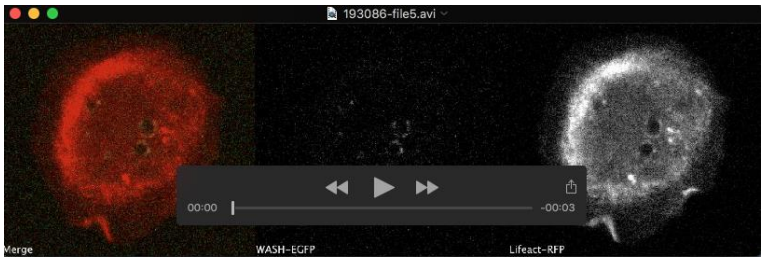
Supplementary Movie M2

Spinning disc microscopy time-lapse movie of *ex vivo* cultured wild type and *wash*Δ185* mutant macrophages expressing EGFP-FAT using the *hml*ΔGal4 driver.



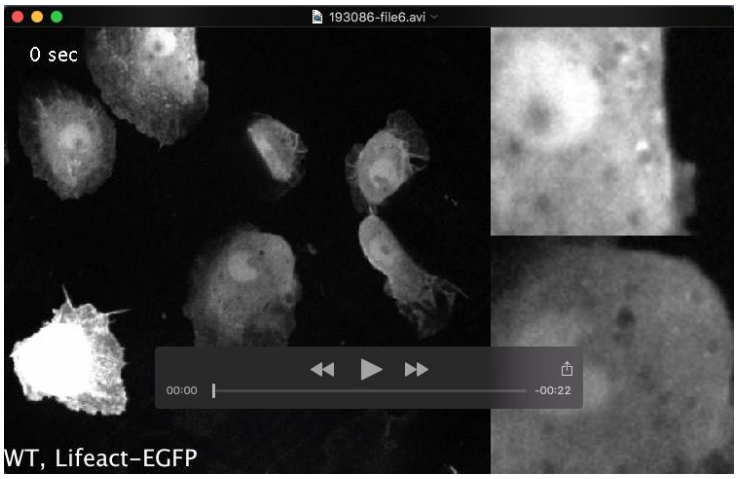
Supplementary Movie M3

Spinning disc microscopy time-lapse movie of *ex vivo* cultured macrophages expressing WASH-EGFP using the *hml*ΔGal4 driver.



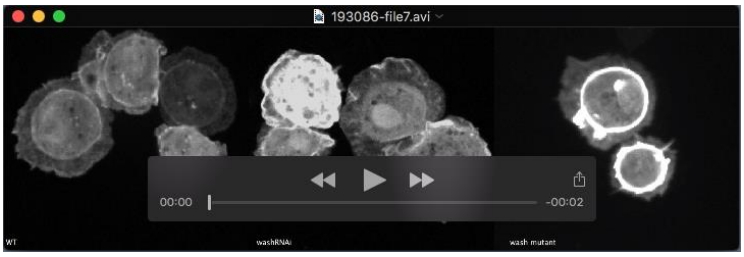
Supplementary Movie M4

Spinning disc microscopy time-lapse movie of *ex vivo* cultured macrophages expressing WASH-EGFP and Lifeact-RFP using the *hml*ΔGal4 driver.



Supplementary Movie M5

Spinning disc microscopy time-lapse movie of *ex vivo* cultured macrophages expressing Lifeact-EGFP using the *hml*ΔGal4 driver.



Supplementary Movie M6

Spinning disc microscopy time-lapse movie of *ex vivo* cultured (A) wild type (B) *wash*RNAi depleted and (C) *wash*Δ185* mutant macrophages expressing Lifeact-EGFP using the *hml*ΔGal4 driver.

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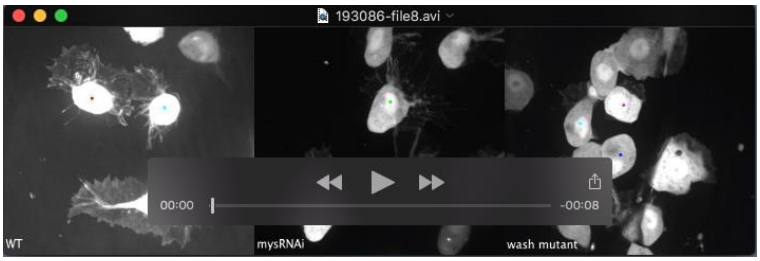

Supplementary Movie M7

Spinning disc microscopy video of migrating (A) wild type, (B) βPS-integrin knockdown and (C) *wash*Δ185* mutant macrophages expressing a EGFP transgene imaged from a living prepupa (2 h APF). Migratory tracks of individual cells are indicated (colored, jagged lines).

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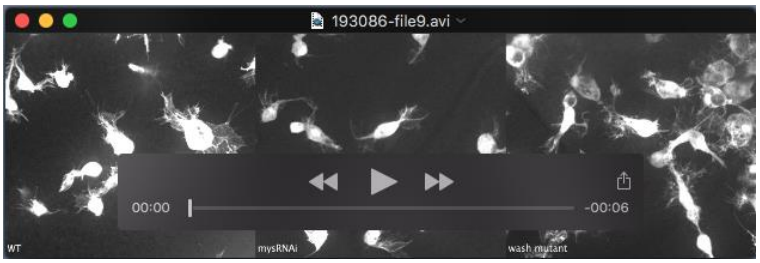

Supplementary Movie M8

Spinning disc microscopy video of migrating (A) wild type, (B) βPS-integrin knockdown and (C) *wash*Δ185* mutant macrophages expressing a EGFP transgene imaged from a pupal wing (17 h APF) upon laser-induced cell ablation.

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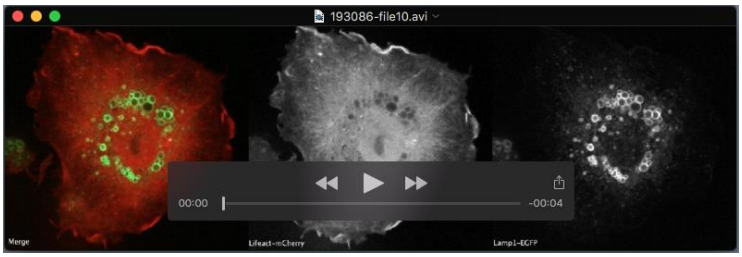
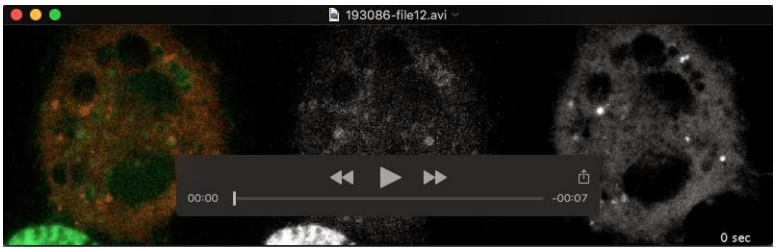


Supplementary Movie M9

Spinning disc microscopy time-lapse movie of cultured S2R^{+} cells transfected with Lifeact-mCherry and Lamp1-EGFP.

Supplementary Movie M10

Spinning disc microscopy time-lapse movie of cultured S2R^{+} cells transfected with WASH-mCherry and Lamp1-EGFP.



Supplementary Movie M11

Spinning disc microscopy time-lapse movie of cultured S2R^{+} cells transfected with WASH-mCherry and Vha55-EGFP.

Supplementary Movie M12

Spinning disc microscopy time-lapse movie of an *ex vivo* cultured macrophage expressing Lifeact-EGFP using the *hml*ΔGal4 driver phagocytosing pHrodo-conjugated *E. coli*

Supplementary Movie M13

Spinning disc microscopy time-lapse movie of an *ex vivo* cultured macrophage expressing WASH-EGFP (green) and phagocytosing pHrodo (magenta). WASH-EGFP is recruited to phagolysosomes with internalized *E. coli* pHrodo particles upon acidification.

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