Supplementary Materials

Molecular Biology of the Cell

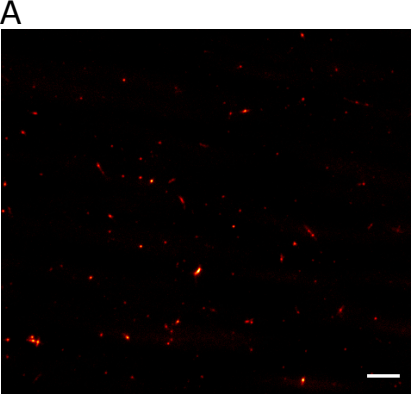
Nast-Kolb *et al*.

Supplemental Figure 1. A) No polymerization is visible, with no VASP incubated to the supported lipid bilayer (SLB) or a lipid monolayer (ML) (B). C) No polymerization visible with incubation of VASP to an SLB without Ni-NTA lipids. D) In bulk solution, 0.5 µM VASP forms loose thin actin bundles without profilin (Prof) or CP present. E) Ni-agarose beads incubated with 1 µM VASP displays outwards growing unbundled actin in the presence of profilin and CP. F) Confocal Z-stack shows the 2D nature of the bundles on the supported lipid bilayer. All scale bars are 5 µm.

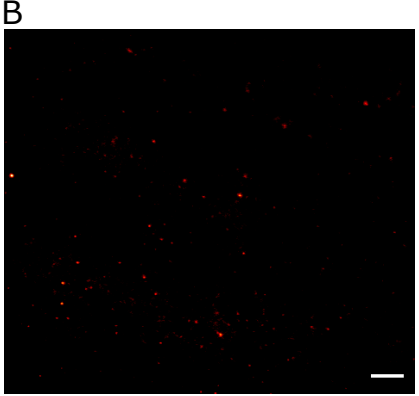
Supplemental Figure 2. Bundle network of actin filaments by 0.05 µM (A), 0.125 µM (B), 0.25 µM (C), 0.5 M (D), 1 µM (E) VASP incubated on a supported lipid bilayer. F) Actin intensity at the different VASP concentrations over time. G) Decoration of bilayer with actin at the shown VASP concentrations. H) Intensity of VASP on the bilayer at incubation of different concentrations of VASP on the bilayer. I) Histogram of actin bundle width distribution at the shown VASP concentrations with a log-normal fit to of the distribution. All scale bars are 5 µm.

Supplemental Figure 3. Actin bundle network at 0.25 µM VASP and varying actin concentrations of 0.5 µM (A), 1 µM (B), 2 µM (C), and 4 µM (D). E) Actin intensity over time at the different actin concentrations. F) Box plots of the bundle width and pore size showing the median, 25-75th percentile range as boxes and 1-99th percentile range as whiskers. All scale bars are 5 µm.

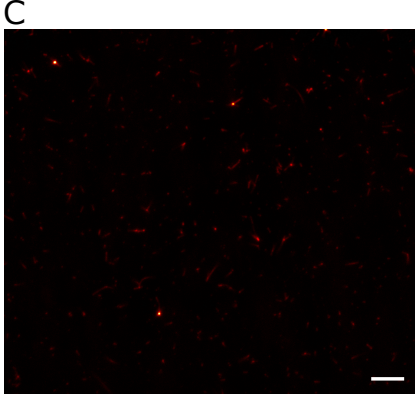
Supplemental Figure 4. A) Supported lipid bilayer (SLB) with 0.25 µM VASP and no CP during polymerization is more decorated with actin, but has smaller bundles than with CP present. B) On a monolayer (ML) without CP, actin polymerization, but no bundling is visible. C) Histogram of the actin bundle width distribution fitted with a log-normal distribution of VASP incubated to SLB or ML without CP. D) SLB with 0.25 µM VASP is being decorated with prepolymerized F-actin filaments in the polymerization mix. E) The corresponding color-coded intensity increase shows pulling down of preformed F-actin and no alignment. F) Box plots of the bundle width and pore size of the measurements with or without CP on an SLB or ML showing the median, 25-75th percentile range as boxes and 1-99th percentile range as whiskers. G) Before addition of actin, an enriched-phase of the labeled VASP can be observed on the bilayer from 0.25-2 µM of VASP incubated. H) Average area fraction of the VASP enriched phase increases with VASP concentration. I) Prepolymerized F-actin filaments are still bound to an ML and allow VASP localization to the actin filaments. All scale bars are 5 µm.



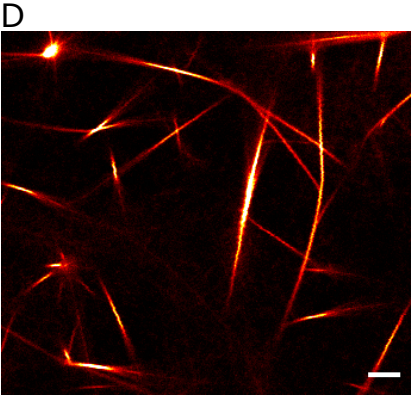
no VASP - SLB



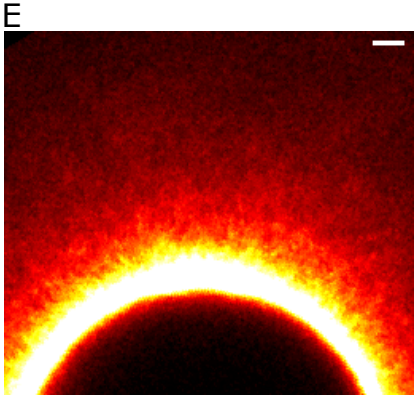
no VASP - ML



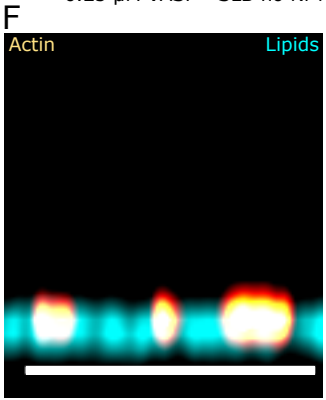
0.25 µM VASP - SLB no Ni-NTA



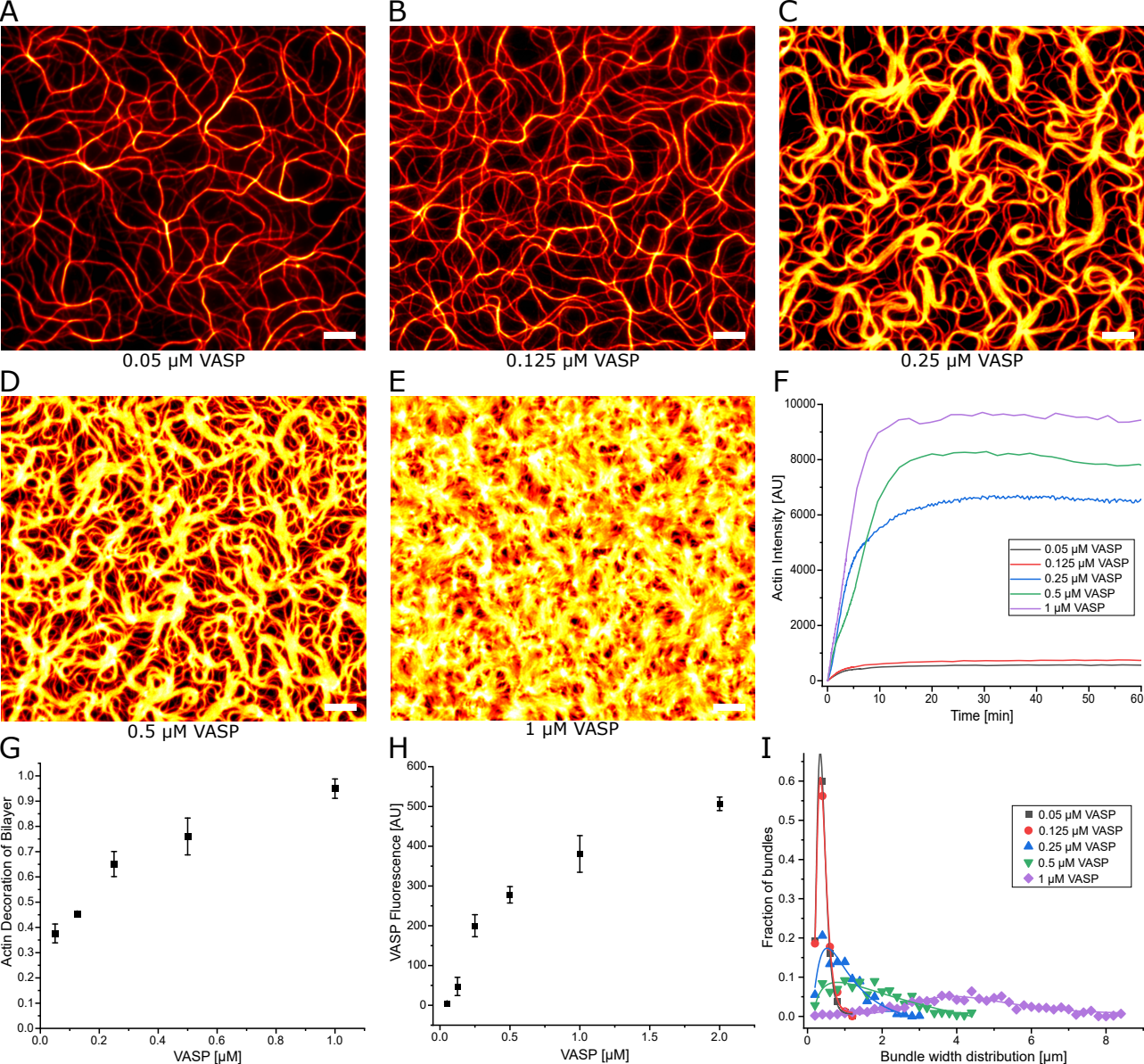
0.5 µM VASP in bulk - no Prof,CP

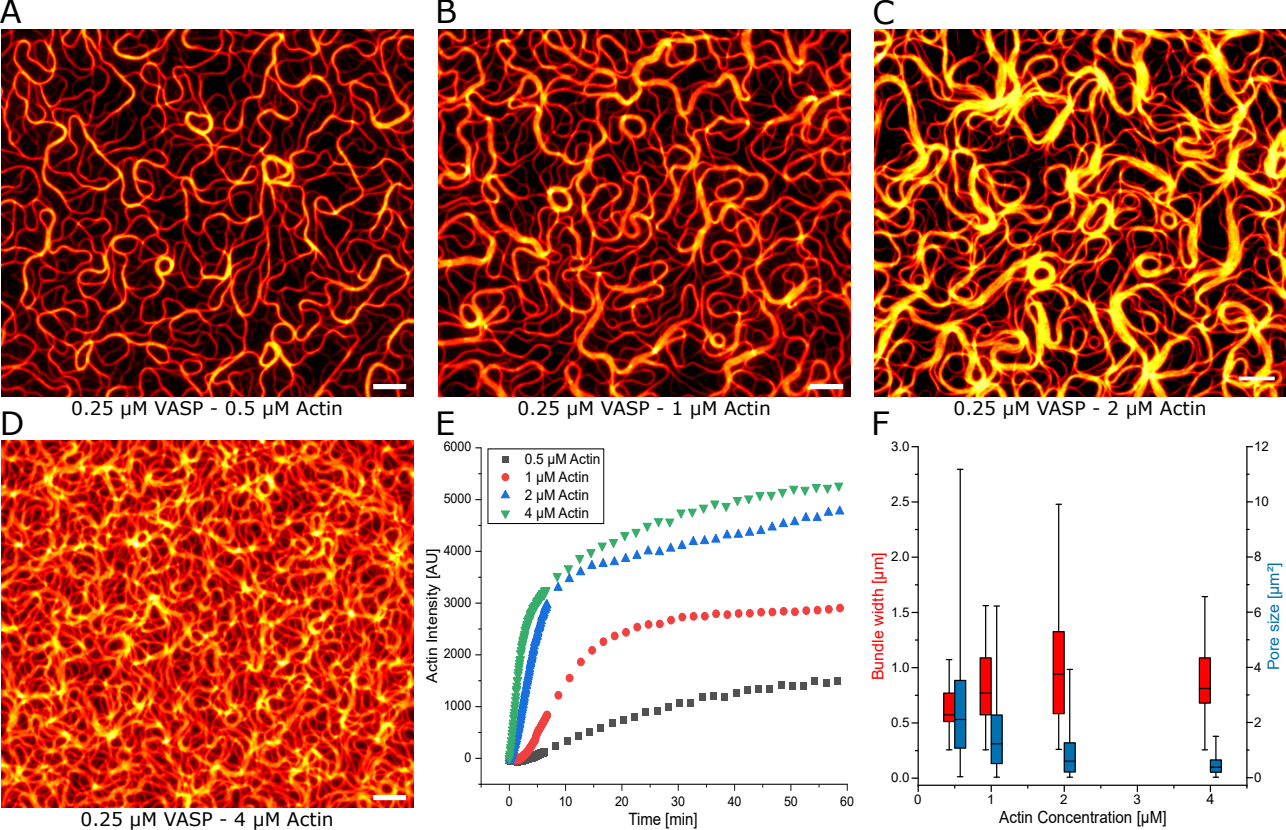


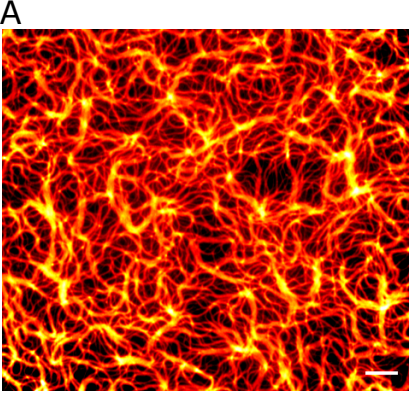
1 µM VASP - Ni-agarose beads



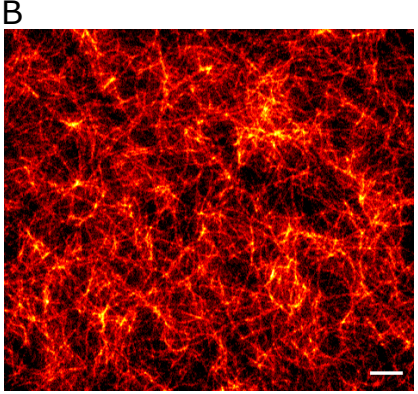
0.25 µM VASP - SLB XZ image


A
B
C
0.05 µM VASP
0.125 µM VASP
0.25 µM VASP
D
E
F
0.5 µM VASP
1 µM VASP
Actin Intensity [AU]
Time [min]
0.05 µM VASP
0.125 µM VASP
0.25 µM VASP
0.5 µM VASP
1 µM VASP
G
H
I
Actin Decoration of Bilayer
VASP [µM]
VASP Fluorescence [AU]
VASP [µM]
Fraction of bundles
Bundle width distribution [µm]

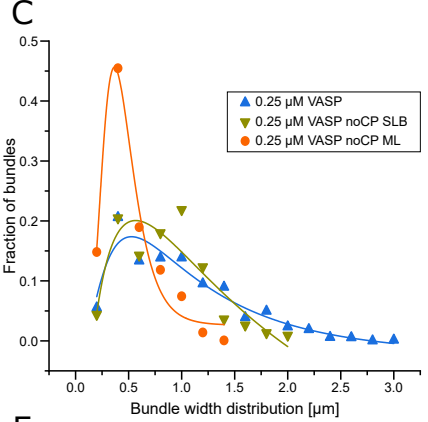

A
B
C
0.25 µM VASP - 0.5 µM Actin
0.25 µM VASP - 1 µM Actin
0.25 µM VASP - 2 µM Actin
D
E
F
0.25 µM VASP - 4 µM Actin
0.5 µM Actin
1 µM Actin
2 µM Actin
4 µM Actin
Actin Intensity [AU]
Time [min]
Bundle width [µm]
Pore size [µm²]
Actin Concentration [µM]

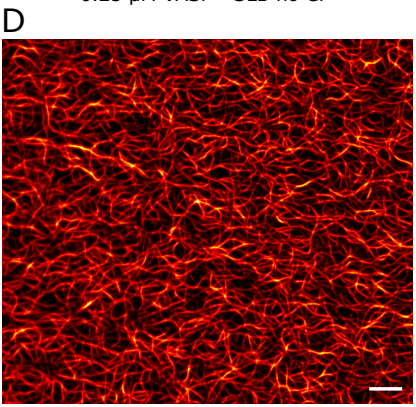


0.25 µM VASP - SLB no CP

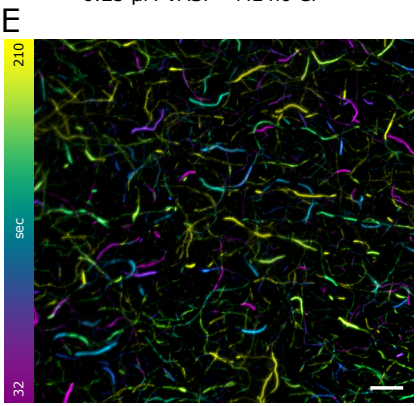


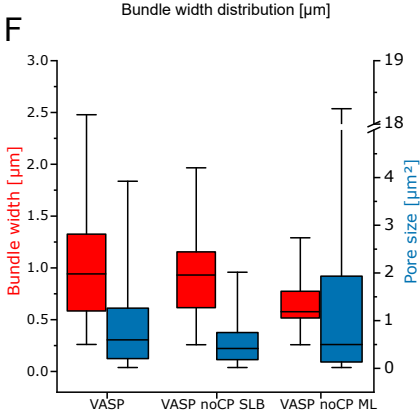
0.25 µM VASP - ML no CP

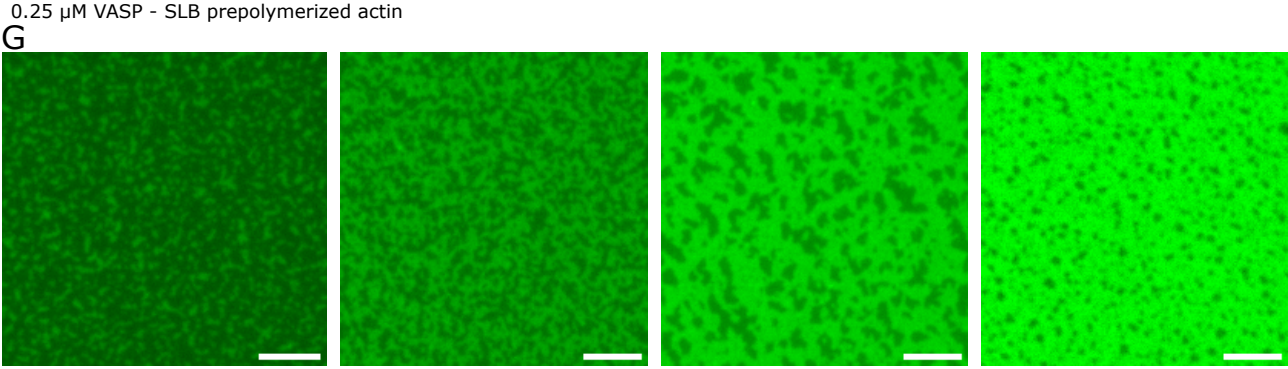




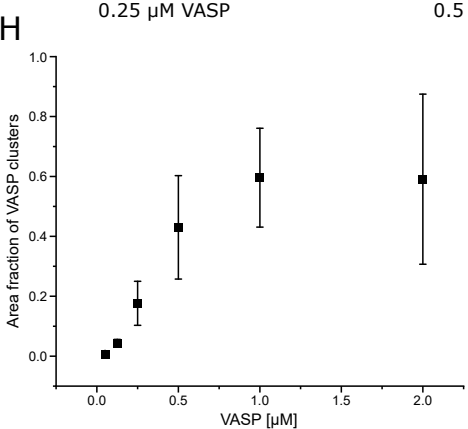
0.25 µM VASP - SLB prepolymerized actin

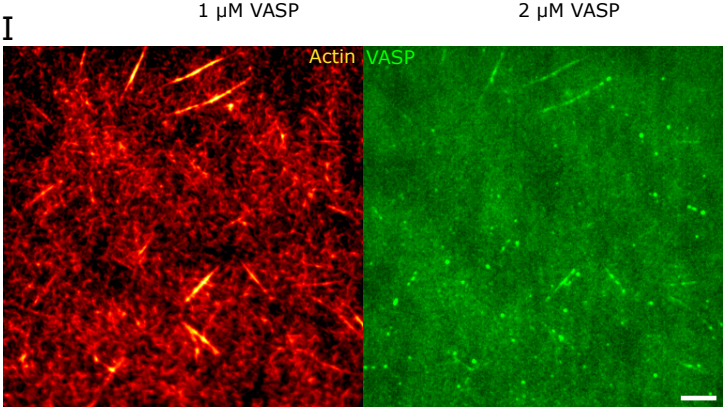






0.25 µM VASP | 0.5 µM VASP | 1 µM VASP | 2 µM VASP





0.25 µM VASP - ML prepolymerized actin