Electronic Supplementary Material (ESI) for Integrative Biology.

Supplementary Figures:

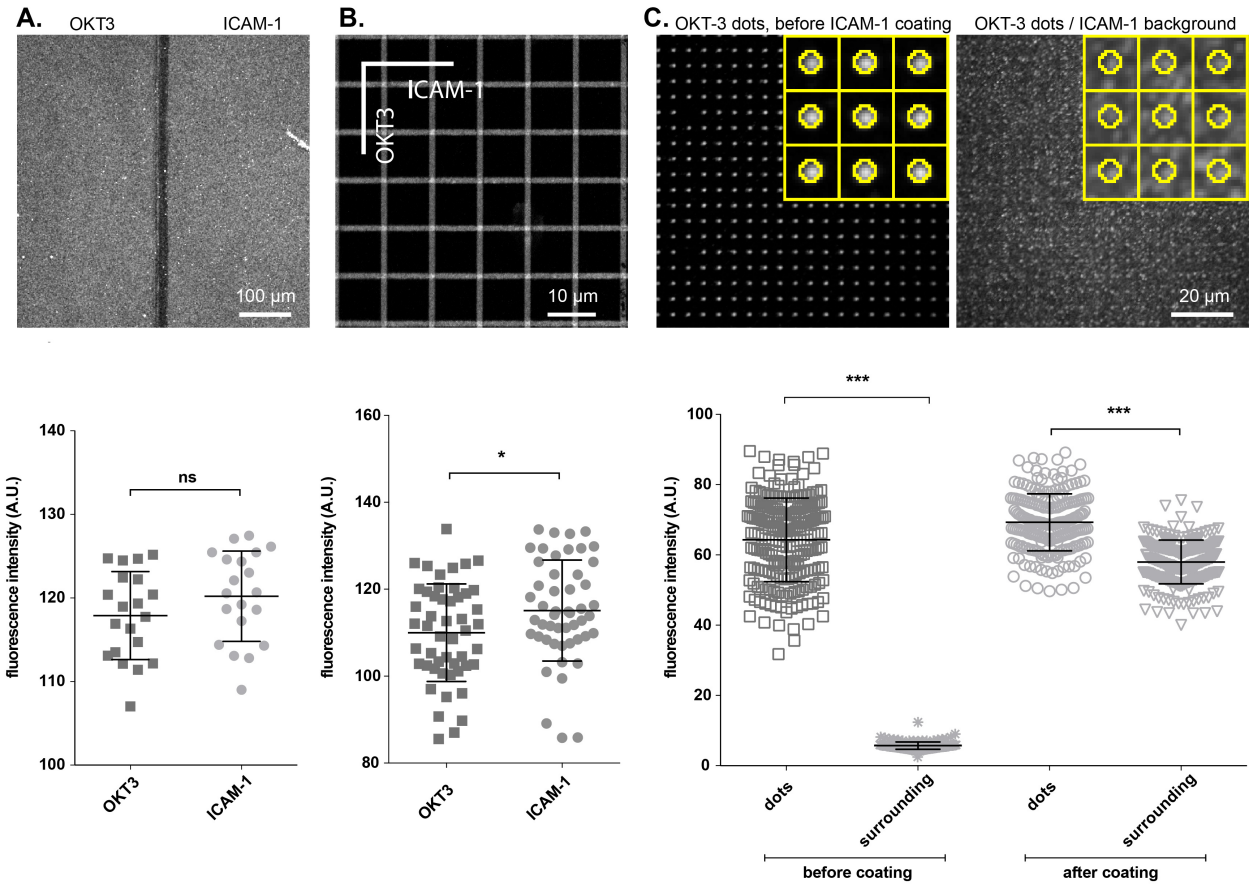

Figure S1. Comparison of antibody coating efficiencies. A) Direct-labelled Atto565-OKT3 and ICAM-1 bind with the same efficiency to plasma treated glass-coverslips. A hydrophobic pen was used to separate both antibodies. B) Comparison of micropatterning. A grid of Atto565-OKT3 and unlabelled anti-human Fab fragments was printed on the coverslip before blocking with BSA and incubation with Atto565-ICAM-1. C) Microprinted Atto565-OKT3 dots (left) were incubated with a solution of Atto565-ICAM-1 (right). There is a comparable, but slightly lower ICAM-1 density (see inset for measured areas), compared to the OKT3 dots. p values from student's t-test: $p<0.05$ (*); $p<0.0001$ (***); not significant (ns).

Figure S2:

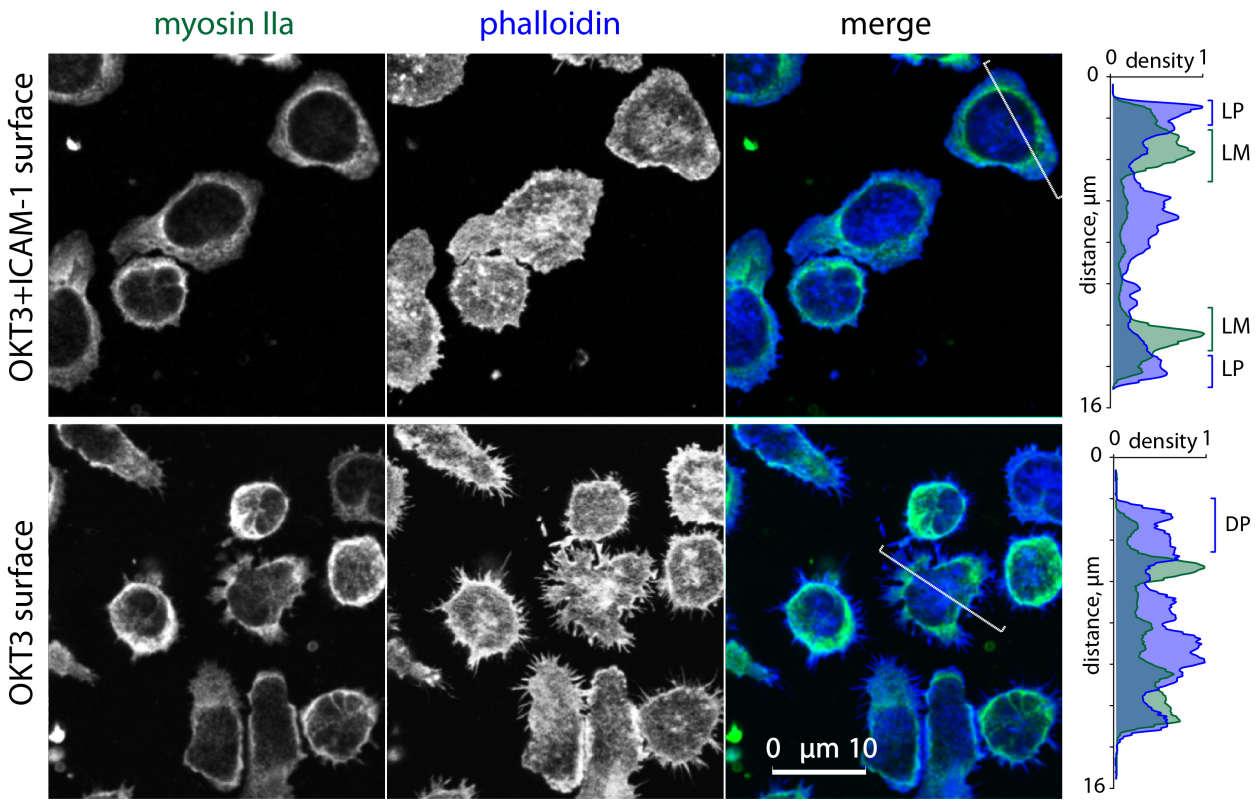

Figure S2. Primary T-cells develop a lamellipodium (LP) and lamellum (LM) on OKT3+ICAM-1 surfaces, but only dendritic protrusions (DP) on OKT3 alone. Density profiles for phalloidin (blue) and myosin IIa (green) are shown on the right.

Figure S3:

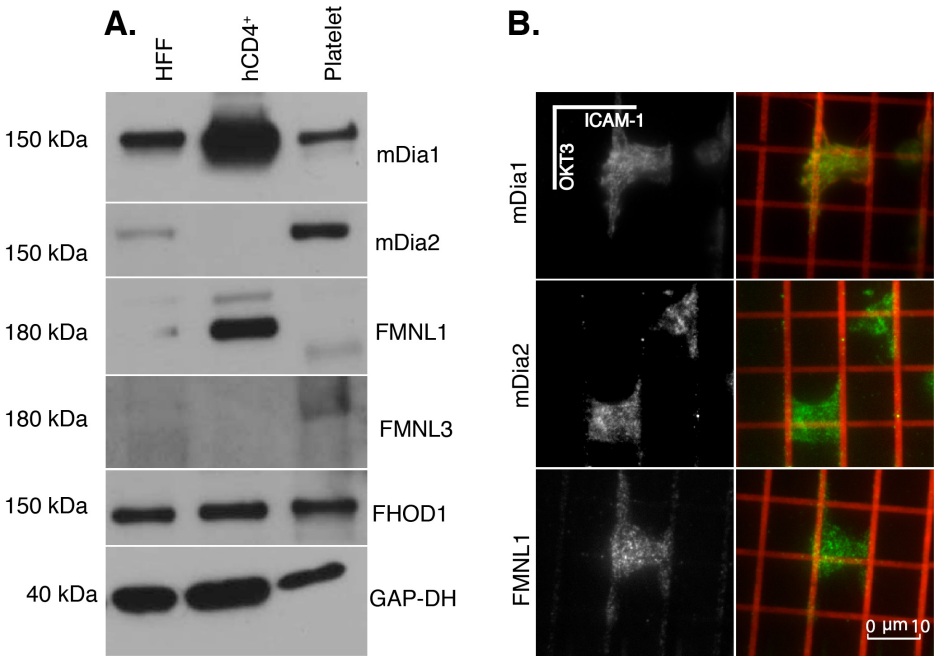

Figure S3. Formin expression and localization in human CD4+ cells. A) FMNL1, mDia1 and FHOD1 are highly expressed in CD4+ T-cells, compared to human foreskin fibroblasts (HFF) or human Platelets. B) FMNL1, mDia1 and mDia2 displayed no clear localization towards the microprinted ligands (OKT3 and ICAM-1).

Supplementary Movie Legends:

Movie 1: Jurkat cell spreading on OKT3 + ICAM-1 labelled PDMS pillars. Jurkat cells spread out and form flat protrusions on OKT3 + ICAM-1 pillar arrays (height = 1.3 µm, diameter = 0.5 µm, distance from centre-to-centre = 1 µm). The pillar displacement analysis indicates initial outwards displacements (red arrows), followed by contractile inwards displacements (green arrows). The movie was taken at 1 frame per second (fps) and is displayed at 30 fps.

Movie 2: Jurkat cell spreading on OKT3 labelled PDMS pillars. On OKT3 labelled pillar arrays (height = 1.3 µm, diameter = 0.5 µm, distance from centre-to-centre = 1

μm), cells display shorter and smaller contractile inwards pillar displacements, when compared to OKT3 + ICAM-1 pillars (Movie 1). Presumably due to the lack of traction forces, cells retract after the initial spreading. The movie was taken at 1 frame per second (fps) and is displayed at 30 fps. Red arrows indicate pillars that were moved outwards and green arrows indicate pillars that were displaced inwards.

Movie 3: FHOD1 shRNA transfected Jurkat cell spreading on OKT3 + ICAM-1 labelled PDMS pillars. Jurkat cells were transfected with FHOD1 shRNA plasmids for 3 days for an efficient knock down (see supplementary figure 1) and spread on OKT3 + ICAM-1 pillar arrays (height = 1.3 μm, diameter = 0.5 μm, distance from centre-to-centre = 1 μm). Similar to control cells on OKT3 pillars (Movie 2), traction forces are smaller, when compared to control cells on OKT3 + ICAM-1 pillars (Movie 1). The movie was taken at 1 frame per second (fps) and is displayed at 30 fps. Red arrows indicate pillars that were moved outwards and green arrows indicate pillars that were displaced inwards.

Movie 4: smiFH2 treated Jurkat cell spreading on OKT3 + ICAM-1 labelled PDMS pillars. Jurkat cells were treated with the pan-formin inhibitor smiFH2 and spread on OKT3 + ICAM-1 labelled pillar arrays (height = 1.3 μm, diameter = 0.5 μm, distance from centre-to-centre = 1 μm). Similar to FHOD1 knockdown (Movie 3), smiFH2 treatment reverts the traction force profile on OKT3 + ICAM-1 pillars to what is observed on pillars, coated with OKT3 alone (Movie 2). The movie was taken at 1 frame per second (fps) and is displayed at 30 fps. Red arrows indicate pillars that were moved outwards and green arrows indicate pillars that were displaced inwards.

Movie 5: Bifurcation in primary CD4$^+$ cells. GFP-actin expressing primary CD4+ cells were spread on a pattern of OKT3 dots over ICAM-1 background (1μm dots, 5 μm pitch). Engagement of an OKT3 dot is immediately followed by strong actin nucleation and accelerated actin network extension, cantered on the OKT3 dot. GFP-actin is displayed in green and OKT3 is displayed in red. The moment of bifurcation is indicated in the movie. The movie was taken at 1 frame every 4 seconds and is displayed at 10fold speed (40 frames per second).

Movie 6: Bifurcation in Jurkat cells. GFP-actin expressing Jurkat cells were spread on a pattern of OKT3 dots over ICAM-1 background (1μm dots, 5 μm pitch). Jurkat cells display comparable bifurcation sequences to primary CD4+ cells (Movie 5). OKT3 feature engagement is followed by actin nucleation and OKT3 centric actin network extension. GFP-actin is displayed in green and OKT3 is displayed in red. The moments of bifurcation are indicated in the movie. The movie was taken at 1 frame every 4 seconds and is displayed at 10fold speed (40 frames per second).

Movie 7: Actin vortex formation in GFP-actin expressing Jurkat cells.
GFP-actin expressing Jurkat cells were spread on a pattern of OKT3 dots over ICAM-1 background (1μm dots, 5 μm pitch). Actin nucleation bursts at OKT3 features within the central area of the cell-substrate interface are frequently associated with appearance of rotating actin vortices unfolding from the TCR foci, indicating different actin dynamics over the OKT3 and ICAM-1 areas. GFP-actin is displayed in green and OKT3 is

displayed in red. The movie was taken at 1 frame every 2 seconds and is displayed at 10 fold speed (20 frames per second).

Movie 8: Calyculin A driven cell collapse is accompanied by formation of tethers between the cell body and the OKT3 dots. GFP-actin expressing Jurkat cells were spread on a pattern of OKT3 dots over ICAM-1 background (1μm dots, 5 μm pitch) and treated with Calyculin A. The moment of Calyculin A addition is indicated in the movie. GFP-actin is displayed in green and OKT3 is displayed in red. The movie was taken at 1 frame every seconds and is displayed at 20 fold speed (20 frames per second).

Movie 9: F-actin polymerization bursts at the apices of a trident OKT3 pattern. Multi-apical adhesive features are known to concentrate mechanical tension at the tips of the pattern. Jurkat cells spread on trident pattern of OKT3 over ICAM-1 background display repeated actin nucleation at the apices (marked with arrowheads), followed by actin network extension. GFP-actin is displayed in green and OKT3 is displayed in red. The movie was taken at 1 frame every seconds and is displayed at 20 fold speed (20 frames per second).