Supp. fig.1. Antigen encounter triggers nucleation of actin foci and cellular symmetry. APS with indicated ligands were incubated with T cells for 2 min, fixed, stained with phalloidin-Alexa488 and imaged using SIM. The graph shows quantification of cell shape in cells.

Supp. fig.2. The aspect ratio (AR) reports on synaptic interface elongation associated with radial interface symmetry breaking. (a) Interference reflection microscopy (IRM) of mouse $CD4^+$ T cells (T cells) shows that significant changes in shape and motility of T cell contact interfaces can be recorded within a time span of 2 min, between their 'arrested' (sedentary) and 'motile' states. The motile or arrested cells were manually identified in the time-lapse images, 20 min post their initial contact with APS, and associated mean aspect ratios and speed were analyzed over a time span of 2min. The graph shows an average value of sped or AR spanning 2min. (b) Alteration in speed and shape measured at the population level during synapse breaking. Snapshots of T cells from time-lapse IRM imaging after seeding on APS, with overlaid center-of-mass tracks over time (in color). Shown at right are the speed and aspect ratios calculated within a 2 min window of observation at 5 min or 20 min post cell seeding; points are individual cells.

Supp. fig.3. (a-b) Image processing scheme to utilized to extract and quantify foci on per cell basis [1]. The images show 2D F-actin intensity as marked by phalloidin staining (top images), or a 3D view of spatial distribution of intensities in the phalloidin images (bottom images). To process the raw images for extracting foci intensities from overall F-actin signal, a Gaussian mask was generated by using a 1.6µm X 1.6µm rolling window, as optimized previously[1]. Subtraction of the mask image from the raw image generated a processed image that could be quantified to measure the average intensity contributed by the foci. Note that while this method reliably identifies the foci in raw images and reduces intensity contribution from the non-foci uniform lamellar area, the peripheral lamellipodial network still contributes a background of ~35% to the total foci intensity, regardless of the presence of profuse foci in arrested synapse, or their visible reduction in the motile phase.

Supp. fig.4. Calcium sequestration using BAPTA does not predispose cells to synapse breaking. T cells were incubated with APS for 5 min or for 20 min, along with vehicle control or BAPTA and EGTA in the last 10 min of incubation, fixed and imaged using SIM (b). Note that the BAPTA-treated cells retain symmetry more than the control cells, and display significantly more foci, even when they have comparable talin recruitment at the synapse.

Supp. fig.5. WASP overexpression rescues synapse breaking in T cells. (a) Western blotting of endogenous WASP and overexpressed GFP-WASP in T cells after 20min incubation on anti-CD3 coated substrates. The numbers in the graphs represent ratios of WASP:actin band intensities, normalized to the control lane. (b) TIRF imaging of wild type and GFP-WASP-overexpressing T cells incubated with anti-CD3/ICAM-1 substrates, for analysis of AR, actin, and pCasL levels. Fluorescence levels normalized to mean values at 5min; points represent data from individual cells.

Supp fig.6. Endogenous pCasL serves as a reliable mechanotransduction marker in T cells. (a) A schematic of mechanosensitive CasL phosphorylation in T cells. CasL is recruited to the signaling TCR clusters via LCK[2] via its Src kinase Binding domain (SB), and interacts with the F-actin cytoskeleton and adhesion complex binding proteins such as FAK via its SH3 domain[3]. Mechanical tension created due to polymerization and remodeling of F-actin at the foci leads to conformational changes in CasL, exposing tyrosine motifs in its substrate domain. These tyrosine residues are phosphorylated by the local Src-Family kinases[2], and could be immunolabelled to assess TCR-proximal actin cytoskeletal strains. (b, c) Colocalization index of foci and pCasL shows a high degree of association (left plot in c), and correlation with foci intensity per cell (right plot in c). (d) pCasL levels are sensitive to broad actin cytoskeletal perturbation. T cells from WT mice were treated with Latrunculin A (LatA), or left untreated, during incubation with substrate. Cells were subsequently fixed and processed for immunostaining and imaged using TIRF microscopy. ** =0.001 for pCasL. (e) WT T cells show reduced pCasL in polarized synapses. p <0.0001.

Supp. fig.7. Initial adhesion and spreading kinetics is comparable in $WASP^{-/-}$ and WT T cells activated on the antigenic surface, $WASP^{-/-}$ cells however show interface elongation at earlier time point (~3 min) than the WT cells. T cells were incubated with substrates on a temperature-controlled microscope stage, imaged live using TIRFM (Movie 15), and analyzed for spreading and shape elongation. (b) Further motility analysis of cells using IRM imaging (Movie 16).

Supp. fig.8. TCR engagement-induced phosphorylation of early signaling molecules Zap70 (a), SLP76, LAT (b), and the recruitment of CasL to the synapse in this setting (c).

Supp. fig.9. (a) SIM imaging of 2 min $WASP^{-/-}$ T cells synapses shows that these cells are able to initially generate radially symmetric ICAM-1 ring in their contact areas. Cells were incubated with lipid bilayers reconstituted with anti-CD3 and ICAM1-Cy5, fixed, stained for F-actin and talin and visualized using SIM. (b) Integrin hyperactivation does not rescue symmetry defects in $WASP^{-/-}$ T cells. WT or $WASP^{-/-}$ T cells were incubated with APS in the presence or absence of 0.5mM $MnCl_2$ for 5min, fixed and processed for talin, pCasL and F-actin visualization, and imaged using TIRFM.

Supp. fig.10. Intracellular calcium flux in not enough to revert asymmetry in $WASP^{-/-}$ cells. T cells from WT or $WASP^{-/-}$ mice were incubated with APS in the presence of DMSO or 1µM Thapsigargin (Thapsi) for 5'. The cells were then fixed and processed for talin, pCasL and F-actin visualization. Note that while Thapsigargin treatment is unable to restore symmetry in $WASP^{-/-}$ cells (a, b), it induces downregulation of talin in both WT and $WASP^{-/-}$ T cells (quantification in b).

Supp. fig.11. (a-c) Exogenous expression of WASP in mouse $WASP^{-/-}$ T cells using lentiviral transduction reconstitutes WASP (a), foci, pCasL levels and synapse symmetry (b-c).

Supp. fig.12. (a) pCasL-enriched actin foci in antigen-specific cell-cell conjugate setting. BMDCs loaded with OTII peptide were incubated with mouse WT or $WASP^{-/-}$ OTII transgenic $CD4^+$T cells for 5', fixed and processed for SIM imaging. The image shows maximum intensity projection from 2µm depth of the synaptic area of a single T cell, marked by a white box. The graph on the right shows the intensity profiles of F-actin and pCasL across a single foci, outlined in the adjacent image in the 'WT' case.

(b) Representative Total Internal Reflection Fluorescence microscopy (TIRF) Images of primary human $CD4^+$ T cells isolated from healthy individuals or WAS patients and activated for 5' on APS. Graph on the right shows quantification of actin foci, pCASL, and cell AR normalized to values of control healthy individual cells.

Supp. fig.13. Transient reduction of WASP levels in human cells elicits defects comparable to those observed in murine T cells genetically deficient of WASP. (a) Human $CD4^+$ T cells transduced with control lentivirus or with

lentivirus delivering WASP shRNA (as described in {Kumari, 2015 #434}, seeded onto superantigen-loaded HUVEC cells for 5', then fixed and imaged using SIM superresolution imaging.

(b) Foci polymerization role of WASP underlies its mechanical tension-generating activity. Human $CD4^{+}$T cells were transfected with human WT WASP-GFP, WASPΔC or with WASP shRNA (shR)-transducing lentiviral particles. The data shoes that the WASP shR and WASPΔC reduce foci and pCasL at the synapse to a similar extent. The remaining foci in the cells in the shR and WASPΔC are contributed by APC cytoskeletal features underneath the synapse, which are quantified along with foci by our foci extraction algorithm outlined in Supp. fig. 3.

Movies:

Supplementary Movie 1. Six different examples of cells breaking their sedentary primary contacts and showing interface shape elongation and a shift in motility, imaged using IRM, indicate that rapid shape transitions can be quantified with 2 min of time duration.

Supplementary Movie 2. IRM live imaging of T cells using reveals a significant shift away from the primary synapse site within 20 min of APS encounter. The images were negatively contrasted to identify cell positions and better highlight individual cell boundaries using automated cell outlining (object identification) routine in ImageJ. The residual material left by the T cells on the primary contact site is reminiscent of membrane fragments, as described in[5].

Supplementary Movie 3. LLSM live imaging of mouse T cell synapse expressing LifeAct-GFP, during transition to the motile phase.

Supplementary Movie 4. Another example of LLSM live imaging of LifeAct-GFP expressing T cell synapse, as described above.

Supplementary Movie 5. LifeAct-GFP expressing WT T cells reveal differential dynamics of lamella vs. the foci. Cells were allowed to attach to the APS for 5min (t=0 in the movie), and then imaged using TIRFM.

Supplementary Movie 6. LifeAct-GFP expressing WASP-/- T cells show the lack of foci and lamellar symmetry breaking. Cells were allowed to attach to the APS for 5min (t=0 in the movie), and then imaged using TIRFM.

Supplementary Movie 7. TIRF imaging of WT and $WASP^{-/-}$ T cells expressing LifeAct-GFP (pseudocolored green) and membrane targeted Td-Tomato (pseudocolored red), during initial adhesion to the APS.

Supplementary Movie 8-9. WASP$^{-/-}$ cells break contact symmetry faster than the WT T cells. T cells isolated from WT or WASP$^{-/-}$ mice were imaged live using IRM for 10 min at 3 frames/min. The images were negatively contrasted for automated boundary identification (Movie 8; see 'Methods') and tracked for the center of mass movement. Bottom panels represent the cell traces overlaid in top panels. Movie 9 shows WT and WASP$^{-/-}$ T cell synaptic interfaces, acquired using IRM imaging at higher magnification.

Supplementary Movie 10. LLSM live imaging of WASP$^{-/-}$ T cell synapse expressing LifeAct-GFP.

Supplementary Movie 11-14. Simulations showing evolution of F-actin network in the WT (whole stable synapse, Movie 11; magnified view of cytoskeletal dynamics around foci, Movie 12), WASP$^{-/-}$ (predisposed to breaking, Movie 13), and a WT synapse transitioning into polarized state (Movie 14), soon after the initial antigen encounter and spreading. Each movie represents a synapse as a rectangular simulation space, based on the scheme presented in Fig. 4a. Note that in Movie 14, WT synapse loses established symmetry the instant WASP is removed from the simulation.

Supplementary Movie 15. LLSM imaging of cells treated with CK666 show a loss of foci and altered actin dynamics in the synapse similar to that in the case of WASP$^{-/-}$ cells.

Supplementary Movie 16. Simulation showing the effect of localized myosin perturbation on F-actin network connectivity on the tension within the synapse. The simulation scheme described in Fig. 4a was used to create F-actin distribution in synapse, where a perturbation in myosinII was introduced in a rectangular shaped subsynaptic region, shortly after the synapse was established (corresponding to the image shown in Fig. 5h).

Supplementary Movie 17, 18 Control (Movie 17) or Azidoblebb.-treated (Movie 18) cells, corresponding to the images shown in Fig. 4h (photoactivable inhibition of myosinII).

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