Western Blotting
After boiling in protein loading buffer, cell lysates were separated on 4–12% NuPage gels (Invitrogen), transferred to PVDF membranes and probed with antibodies using standard techniques. WASp was detected with anti-WASp (clone B9 from Santa Cruz Biotechnology, Inc.) in 300.19 cell lysates. Actin was detected using anti-actin antibody (Sigma-Aldrich). Horseradish peroxidase–conjugated secondary antibody (GE Healthcare) was detected using SuperSignal West Pico chemiluminescent substrate system (Pierce Chemical Co.) and an Uvichemi documentation system (Uvitech). Similar techniques were used to detect anti-ezrin-radixin-moesin (ERM) and anti-phosphorylated ERM immunoblots (both anti-ERM antibodies purchased from Cell Signalling).

Scanning electron microscopy
300.19 cells or human PBMC were fixed in a Karnovsky mixture (3% glutaraldehyde and 1% paraformaldehyde in 0.08M sodium cacodylate buffered to pH 7.4 with 0.1M HCL), rinsed in 0.1M sodium cacodylate buffer and osmicated for 1 hour in 1% aqueous osmium tetroxide prior to dehydration in ascending alcohols (15 minutes in 50, 70, 90 and 3 ×100% ethanol). Cells were passed through 2 × 5 min changes of hexamethyldisilazane and then re-suspended in a 500ul volume. Pelleted cells were re-suspended by gentle trituration and applied directly to the tops of acetone cleaned aluminium stubs and air dried in a fume hood. Dried cells were sputter coated with gold-palladium using an Emitech K550 unit for viewing.

Immunogold labelling
300.19 cells were fixes with Karnovsky mixture (as for Scanning Electron microscopy – see above). After two rinses in 0.1M phosphate buffer pH 7.4, cells were placed in 1% aqueous osmium tetroxide for 1 h, rinsed three times in distilled water and dehydrated by sequential passage through 50%, 70% and 90% and finally three times in 100% ethanol (10 minutes per step). Coverslips were passed twice through 5 minute changes of HMDS (Hexamethyl disilazane, Sigma H4875) and air dried in a fume hood. Dry coverslips were mounted on 10mm JEOL stubs with conductive carbon tabs and Dag conductive paint, carbon string coated with an Emitech K950 coater prior to viewing.

Parallel Plate Flow chamber assay
Experiments were visualized using a Diaphot 300 inverted fluorescence microscope (Nikon) connected to a JVC TK-C1360B colour video camera and recorded on a Panasonic AG-6730 S-VHS video recorder (Microscope Service and Sales). 10 random fields were recorded for 15 s each using a × 10 objective (800 × 600 mm^2). Images were acquired into a video file (In Video PCI; Focus Enhancements, Campbell, CA) at 15 frames/s, and numbers of cells undergoing rolling and/or arrest, rolling velocity of individual cells, and mean velocity of the population were calculated using EML Motion Analysis software (Ed Marcus Laboratories, Boston, MA). For sLe^x immobilisation, the base of the parallel plate flow chamber was first incubated with 5 µg/ml NeutrAvidin™ (Pierce), dissolved in Ca^{2+}/Mg^{2+}-free PBS, for 30 minutes at room temperature. Multivalent sLe^x-PAA-biotin (Glycotech) dissolved in Ca^{2+}/Mg^{2+}-free PBS at 5ug/mL was added on to the plate for 2 hours at room temperature after washing off excess NeutrAvidin thoroughly with 5% BSA in PBS and blocking in the same buffer for 15 minutes at room temperature. After washing off the excess unbound sLe^x, the plates were blocked again for at least 1 hour with 5% BSA in PBS before flow experiment.

Interference reflection microscopy (IRM) measurements and calculation of contact point stability
Individual TIFF files were processed as follows: “Auto levels” were applied followed by a high pass filter (radius 10 pixels). “Auto levels” were reapplied followed by a median filter (radius 2 pixels) and a threshold (typically 90) was set so contact points appeared black. Images were inverted so that contacts appeared white on a black background. Using the “calculations” function, four successive frames were sequentially overlaid using the “add” blending function and an opacity of 100%. We thus obtained a composite image with 4 relevant grey levels (0=black, 63=dark grey, 127= light grey, 191=very light grey) which was inverted for further analysis. After inversion, dynamic adhesions, present in one or two of the four overlaid frames, appeared as light or very light grey pixels. Dark grey and black pixels represented increasingly stable adhesions present for three or four of the overlaid frames. Using the “histogram” function, we quantified the percentage of pixels corresponding to each grey level for individual cells. The proportion of stable adhesions was calculated as follows:
(pixels in level 0 + pixels in level 63)/Total pixels in all grey levels
Similarly, the proportion of unstable adhesions was calculated as follows:
(pixels in level 127 + pixels in level 191)/Total pixels in all grey levels
Obtained values were expressed as percentages and statistical significance assessed using the Student T-test test.