SUPPLEMENTARY INFORMATION
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Chemokines and integrins independently tune actin flow and substrate friction during intranodal migration of T cells

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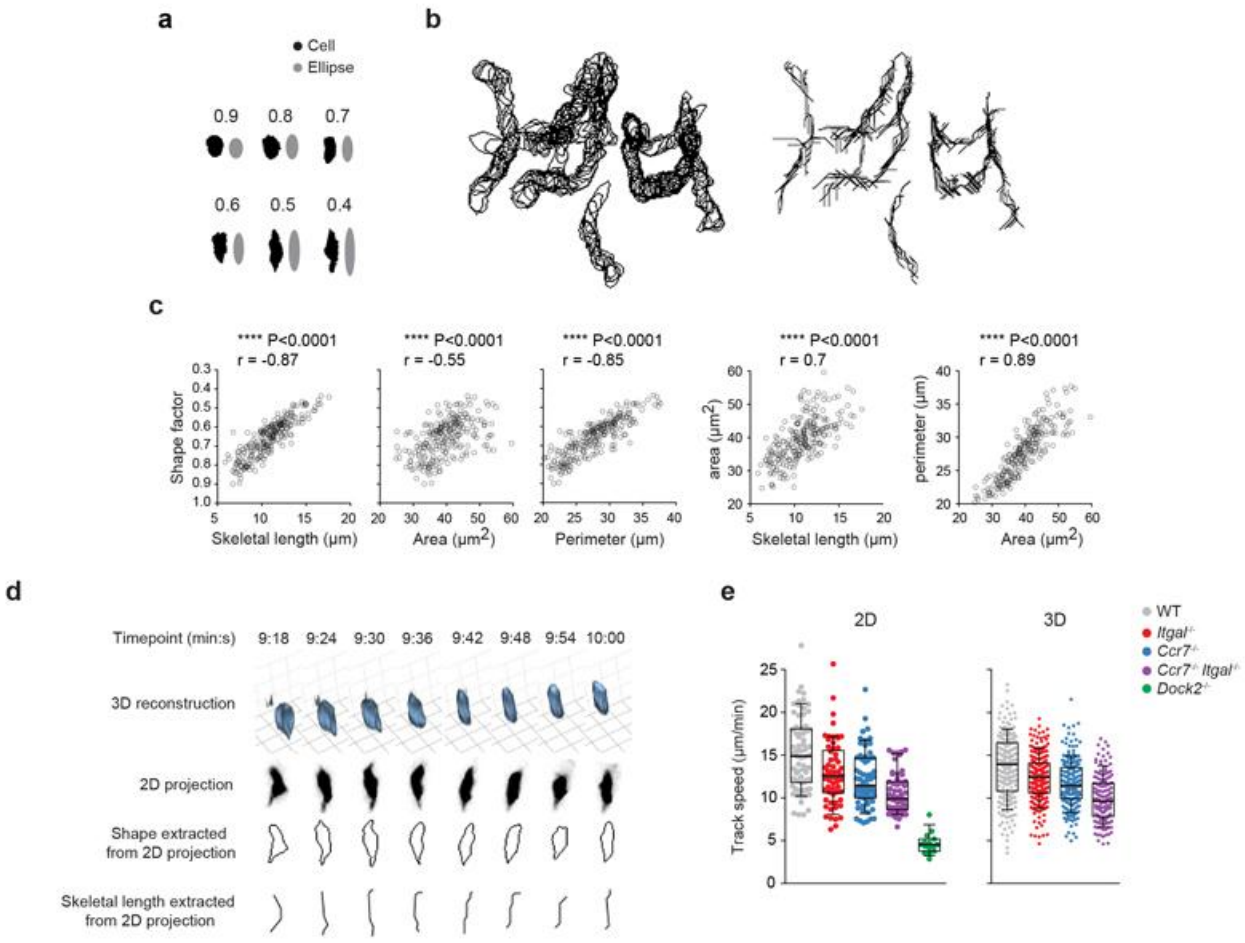
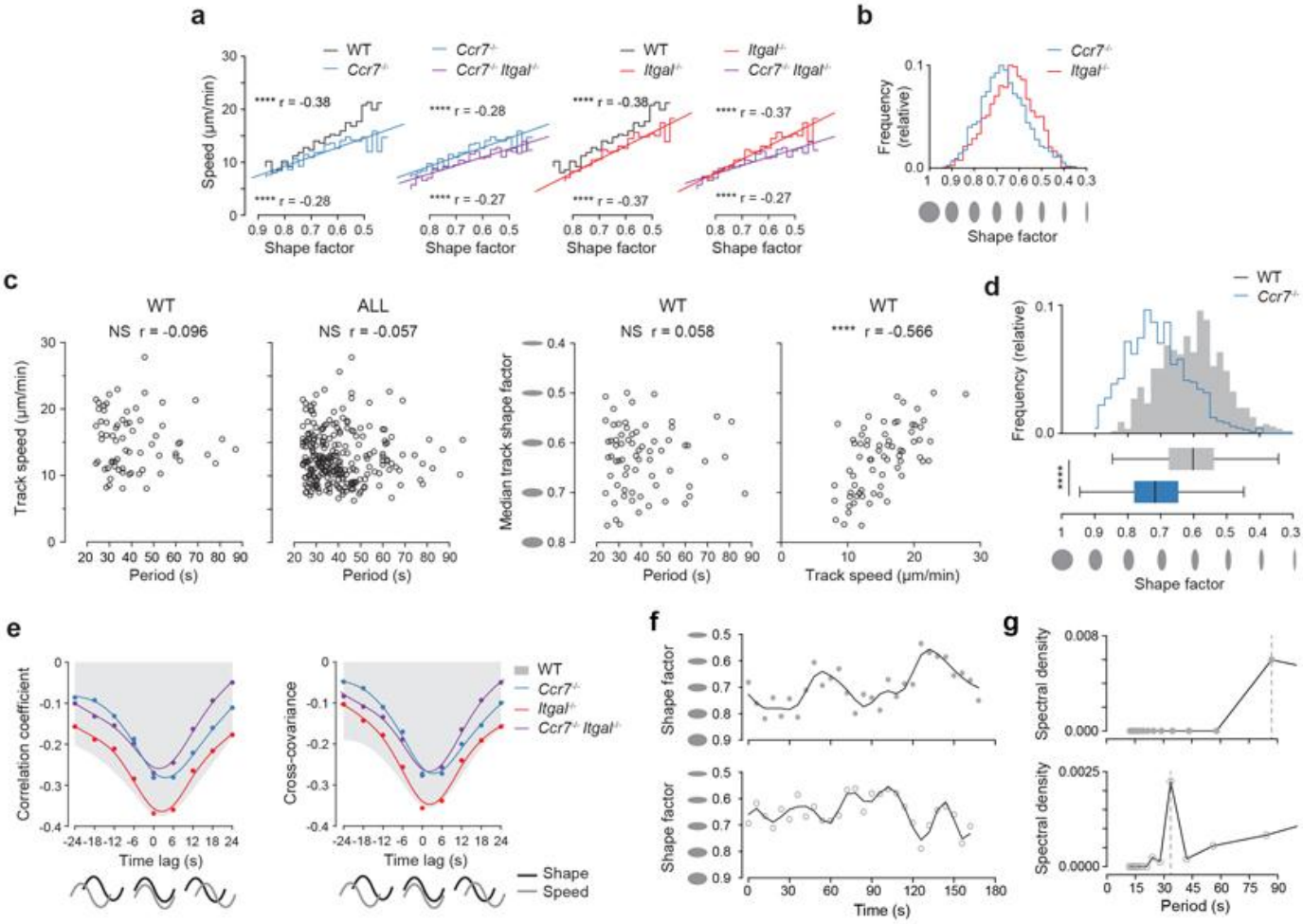


Supplementary Figure 1

Two-photon intravital microscopy and analysis showing morphodynamical parameters of T cells migrating in LN parenchyma after transfer into wild-type mice.

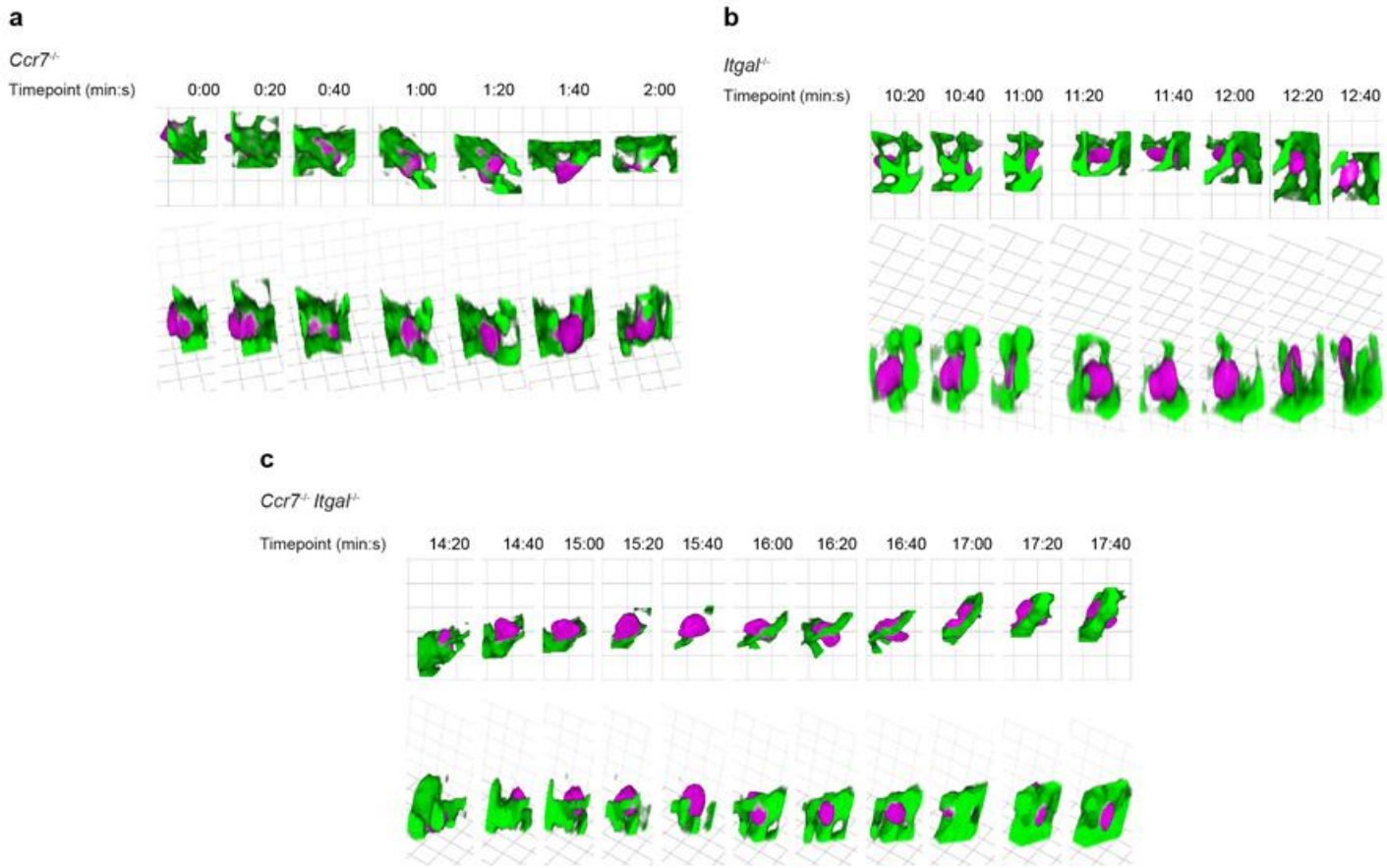
(a) Examples of actual cell shapes and idealized ellipses of a given shape factor. **(b)** Comparison of cell shape (left) and skeletal length (right) of WT T cells on an example dataset. **(c)** 2D morphometrical parameters of WT T cells from sample dataset as in **b**, Spearman correlation coefficient (r) as indicated, **** P<0.0001, n = 226 shapes. **(d)** Example 3D reconstruction, 2D projection and morphometrical parameters from a track as in **Fig. 1b**. **(e)** Speed of primary naïve T cells migrating in LN parenchyma as determined by 2D versus 3D tracking. Left, T cells tracked from 2D videos as in **Fig. 1** and **Fig. 2**, 6 s intervals, minimum track span 2 min. Dots represent individual cell tracks. WT n=69 cells, *Ccr7*$^{-/-}$ n=66 cells, *Itgal*$^{-/-}$ n=61 cells, *Ccr7*$^{-/-}$ *Itgal*$^{-/-}$ n=55 cells, *Dock2*$^{-/-}$ n=15 cells. Right, tracks from independent experiments analyzed in 3D, tracked at 20 s intervals, minimum track span 7 min. Dots show individual cell tracks. WT n=227 cells, CCR7$^{-/-}$ n=217 cells, LFA-1$^{-/-}$ n=245 cells, CCR7$^{-/-}$ LFA-1$^{-/-}$ n=155 cells, whiskers represent Tukey fences.



Supplementary Figure 2

Two-photon intravital microscopy showing morphodynamical parameters of wild-type, *Ccr7*$^{-/-}$, *Itgal*$^{-/-}$ and *Ccr7*$^{-/-}$; *Itgal*$^{-/-}$ T cells migrating in LN parenchyma after transfer into wild-type mice.

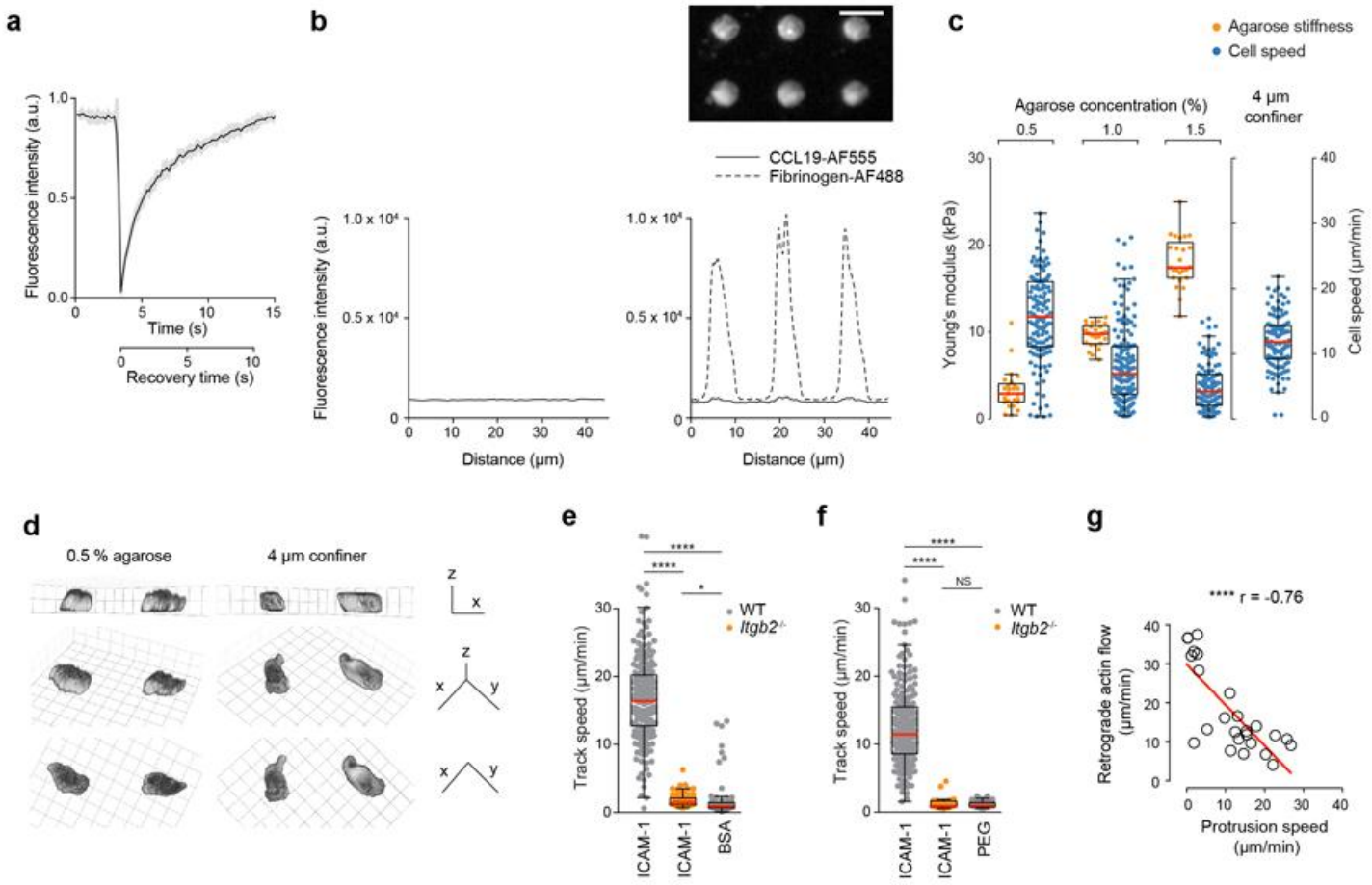
(a) Cell speed – shape correlation as in **Fig. 2a**, **e** and **f**. Lines show linear correlation fit, stairs show median values in respective bins. Spearman correlation coefficients (r) as indicated. **** P<0.0001. **(b)** Cell shape frequency distribution of *Ccr7*$^{-/-}$ and *Itgal*$^{-/-}$ T cells as in **Fig. 2b**. **(c)** Relationship between periodicity of cell shape, track mean speed and track median cell shape of WT and all (WT, *Ccr7*$^{-/-}$, *Itgal*$^{-/-}$ and *Ccr7*$^{-/-}$ *Itgal*$^{-/-}$) T cells analyzed. Dots show individual tracks. WT n=69 tracks, all n=251 tracks. Spearman correlation coefficient (r) as indicated, NS = not significant, **** P<0.0001. **(d)** Shape frequency distribution of WT and *Ccr7*$^{-/-}$ T cells co-transferred in WT mice and analyzed from movies where both cell phenotypes were present. WT n=553 shapes, *Ccr7*$^{-/-}$ n=726 shapes. Box plots correspond to distribution plots, whiskers represent Tukey fences. Mann-Whitney test, **** P<0.0001. **(e)** Temporal shift of speed - shape correlation and cross-covariance of speed and shape factor of WT, *Ccr7*$^{-/-}$, *Itgal*$^{-/-}$ and *Ccr7*$^{-/-}$ *Itgal*$^{-/-}$ T cells. Dots indicate correlation coefficient or cross-covariance values. Curves show spline interpolation. **(f)** Evolution of cell shape in time illustrated on two example cell tracks. Dots and circles show actual measurements, curves show Fourier smoothing. **(g)** Spectral analysis of respective cell tracks from **f**.



Supplementary Figure 3

Two-photon intravital microscopy showing interactions of T cells with LN stroma after transfer of fluorescently labeled T cells into bone marrow chimeric mice expressing GFP in the LN stroma.

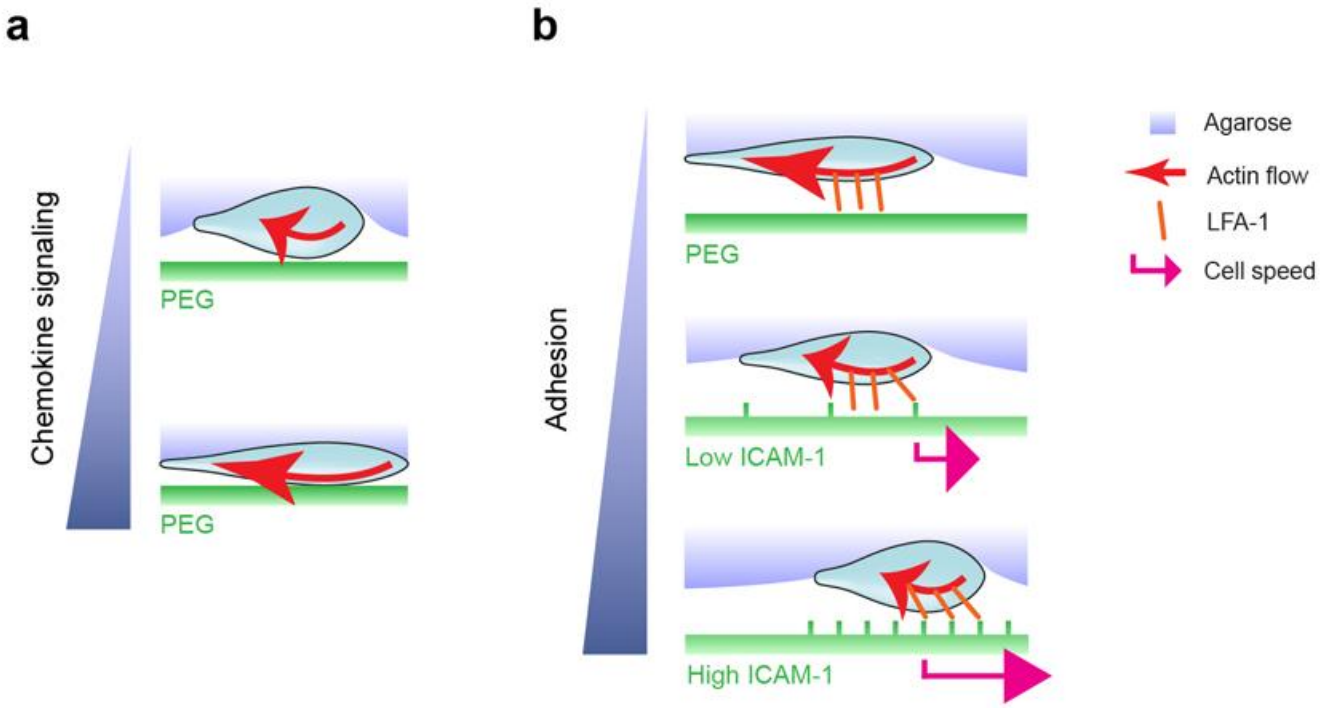
(**a**) Example *Ccr7*$^{-/-}$ T cell interacting with local areas of LN stroma. Top view and upper left view (below) on a time-lapse sequence of a 3D reconstitution. **(b)** *Itgal*$^{-/-}$ T cell interacting with local areas of LN stroma. Top view and bottom up view (below) on a time-lapse sequence of a 3D reconstitution. **(c)** *Ccr7*$^{-/-}$ *Itgal*$^{-/-}$ T cell interacting with local areas of LN stroma (green). Top view and upper right view (below) on a time-lapse sequence of a 3D reconstitution. **a-c** represent examples of data as described in **Fig. 3**. In **a-c**, side of one square is equivalent to 6.3 µm.



Supplementary Figure 4

Characterization of the under-agarose assay.

(a) FRAP recovery of CCL19-AF555 in the agarose block. Grey areas indicate SD, n=11 bleaches. Data show one experiment. **(b)** CCL19 immobilization on surfaces. Fluorescent signal from UV-ablated areas on PEG-coated slides incubated with 100nM CCL19-AF555 (left). Subsequently, slides were incubated with 5µg/mL fibrinogen-AF488 (Right). Image on top shows UV-ablated pattern highlighted with fibrinogen-AF488, bar indicates 10 µm. Data show one experiment. **(c)** Agarose stiffness interpolated with cell speed as a function of agarose concentration. Agarose Young's modulus was determined by atomic force microscopy, 25 measurements per condition were taken in a 5 x 5 array. Primary WT T cells were migrating on dishes coated with 2 µg/mL ICAM-1 under agarose supplemented with 10 nM CCL19. 0.5% n=126 cells, 1% n=159 cells, 1.5% n=111 cells. Right, tracks speeds of cells migrating under 4 µm high PDMS confiner, n=112 cells. Dots indicate individual cell tracks or individual AFM measurement, whiskers represent Tukey fences. Data show one experiment. **(d)** Representative 3D reconstructions of cells under 0.5% agarose or 4 µm high PDMS confiner. Cells were placed on PEG coated surfaces in the presence of 10 nM CCL19. Side of one square is equivalent to 5.2 µm. **(e)** Speeds of WT and integrin β2-deficient T cells on 10% BSA or ICAM-1 (2 µg/mL) blocked with 10% BSA. Cells were placed under agarose supplemented with 5% BSA and 10 nM CCL19. WT on ICAM-1 n=210 cells, *Itgb2*$^{-/-}$ on ICAM-1 n=108 cells, WT on BSA n=95 cells. **(f)** Speed of WT and integrin β2-deficient T cells on ICAM-1 immobilized on biotinylated PEG or on biotinylated PEG only. Cells were placed under agarose supplemented with 5% BSA and 10 nM CCL19. WT on ICAM-1 n=229 cells, *Itgb2*$^{-/-}$ on ICAM-1 n=25 cells, WT on PEG n=88 cells. In **e** and **f** dots indicate individual cell tracks, whiskers represent Tukey fences. Kruskal-Wallis test, NS = not significant, * P<0.05, **** P<0.0001. Data represent pool of two experiments. **(g)** Correlation of retrograde actin flow and cell protrusion speed of T cells migrating on 10 ng/mL ICAM-1 blocked with BSA as in **Fig. 7c**. Cells were confined under agarose supplemented with 5% BSA with 10 nM CCL19. Data represent pool of three experiments. n=26 cells, r = spearman correlation coefficient, **** P<0.0001.



Supplementary Figure 5

Scheme of T cell shape and speed determination.

(a) T cell elongation is given by speed of cortical actin flow. High actin flow speed correlates with increase in cell elongation (below). **(b)** In absence of integrin engagement T cells can not migrate and actin flow speed and cell elongation are at maximum. Force transmission of actin flow through LFA-1 engagement with ICAM-1 leads to actin flow deceleration, cell speed increase and cell rounding (below).