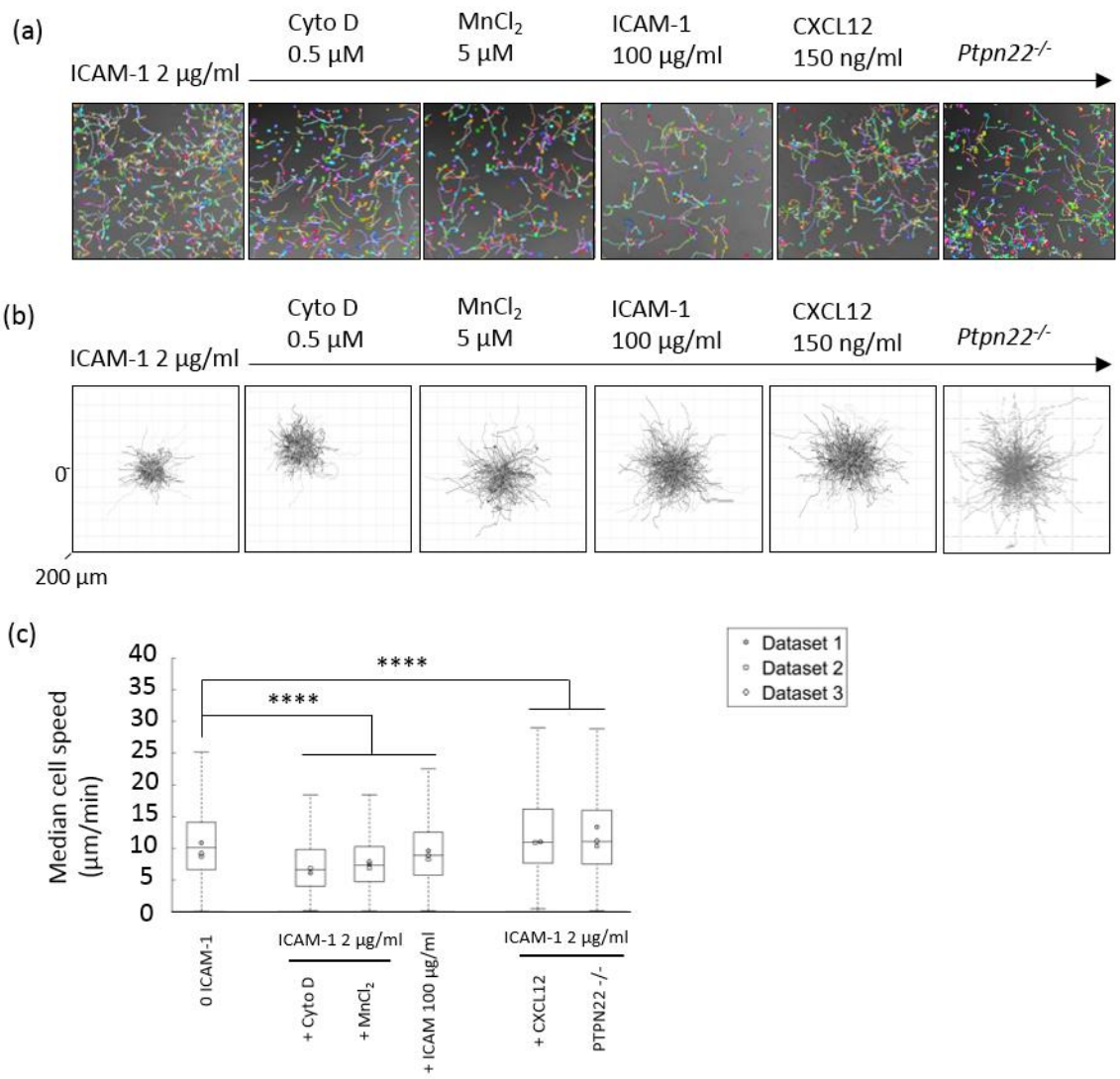

Figure S1: T cells imaged and automatically tracked during migration display a random walk on ICAM-1 coated glass. a) Representative graphs of track segments generated and quantitated in bespoke ICY software during a 10 min time lapse movie of T-blasts migrating on glass coated with murine recombinant ICAM-1 (2ug/ml) and in the presence of $MnCl_2$ 5 µM; Cytochalasin D (CytoD) 0.5 µM, a raised concentration of ICAM-1 on the surface (100 µg/ml); CXCL12 (150 ng/ml) or using T cells deficient for PTPN22. b) Directionality is represented here whereby track segments have been organised by of point of origin in these spider graphs. This clearly illustrates the maintained random walk nature of T-blast migration in these assays. C) Boxplot showing pooled median cell speed from the tracks derived from cell from 3 mice. Individual median value from each mouse shown as symbols denoted dataset 1, 2 and 3. N = 500 cells per condition from 3 mice. Kruskal Wallis and Dunn's post hoc testing was used to compare the cell speeds of hundreds of cells. P****<0.00001.

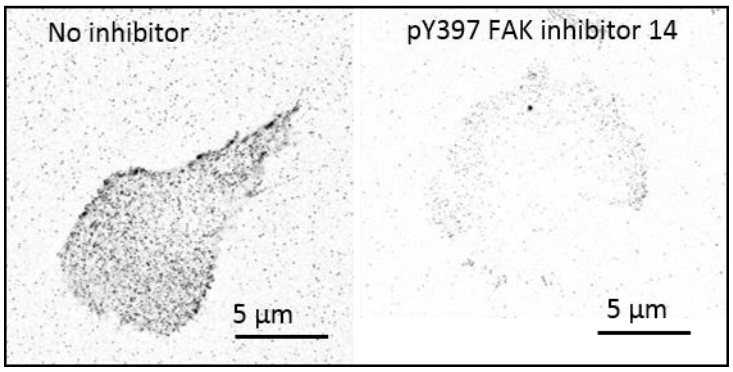

Figure S2: Validation of pY397 FAK antibody specificity. Inhibitor 14 was used to block the phosphorylation of FAK in T-blasts migrating on ICAM-1 (2ug/ml) coated glass which were treated with 5 µg/ml (17.6 uM) of inhibitor for 10 minutes, fixed, permeablised and stained with pY397 FAK antibody conjugated to alexa fluor 647 then imaged by dSTORM microscopy. Quantification shows a specific reduction of pY397 FAK with inhibitor 14 compared to control. N = 16 cells.

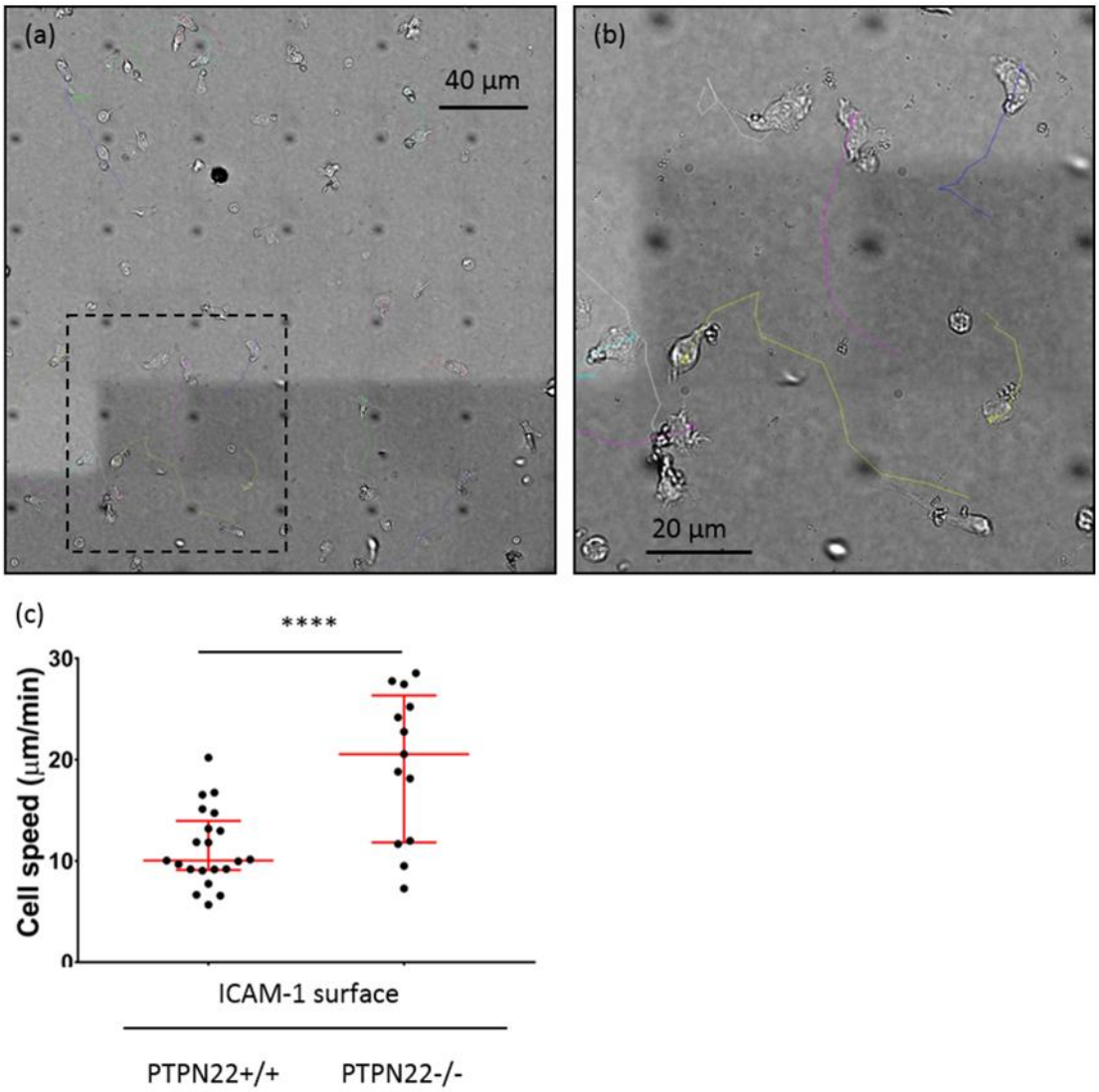

Figure S3: T-cells migrating with differential speeds prior to fixation and staining directly on the microscope were imaged to correlate speed and individual cell fluorescent quantification a) A large FOV was attained using 36 small 100 x fields of view stitched together computationally (Nikon elements software) b) zoomed image showing migrating cells were tracked to attain cell speeds, up to the point of fixation on the stage. c) quantitation of cell speeds show that PTPN22-/- cells move faster on average. P****<0.0001.

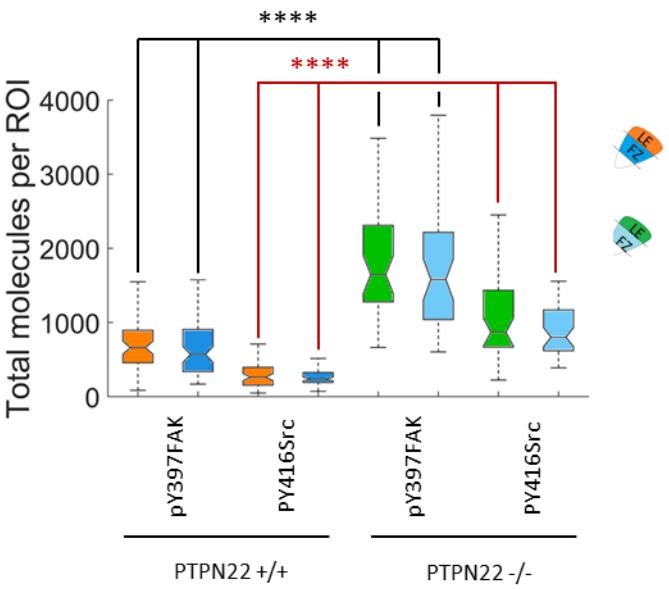

Figure S4: The total number of phosphorylated SFK and FAK molecules are increased in PTPN22 deficient cells. Individual cells were sequentially stained and imaged by madSTORM. The total number of molecules of pY397 FAK and pY416 Src family kinases in the same cell per 2 μm² ROI were counted. ****p < 0.0001. Kruskal Wallis and Dunn's post hoc testing was used to compare the clustering of each molecule, in each zone, in PTPN22 positive and negative cells.

	Cyto D	Mn	ICAM 100	CXCL12	PTPN KO
Size LE	10	7	25	32	33
Size FZ	9	0	17	22	23
Molecules LE	35	6	35	29	24
Molecules FZ	25	-5	15	15	5

< 10	
11 to 20	
> 20	

Table S1: Simple heat map to show percentage changes in the size and number of molecules per cluster compared to untreated cells.