

Supporting Information

Analysis of Transient Migration Behavior of Natural Killer Cells Imaged *in vivo* and *in situ*

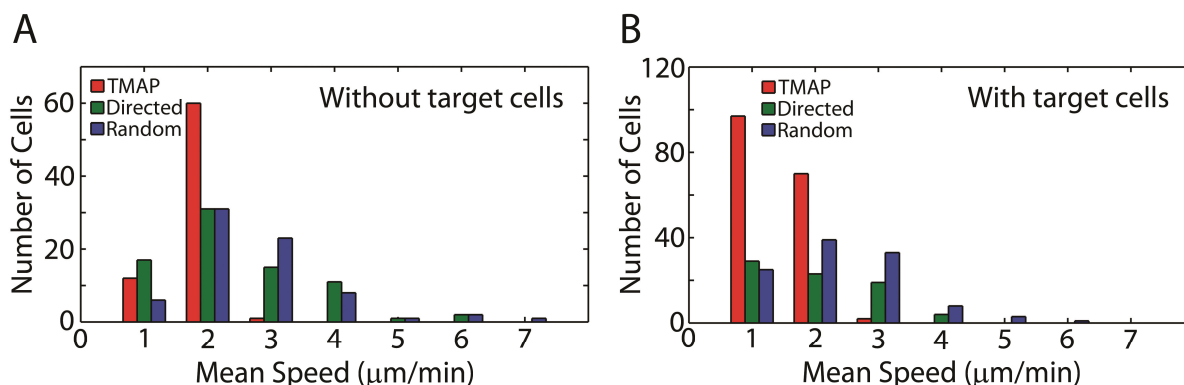


Figure S1. Mean speed of NK cells in different migration modes *in vitro*. Histograms of mean speeds of NK cells in TMAPs (red), periods of directed migration (green) and random movement (blue), for migration without (A) and with (B) target cells. The fastest migration was recorded when NK cells migrated in a random fashion (average mean speeds were 2.7 ± 0.1 μm/min without target cells and 2.3 ± 0.1 μm/min with target cells), followed by directed migration (average mean speeds were 2.4 ± 0.1 μm/min without target cells and 1.9 ± 0.1 μm/min with target cells) and TMAPs (average mean speeds were 1.6 ± 0.1 μm/min without target cells and 1.2 ± 0.1 μm/min with target cells).

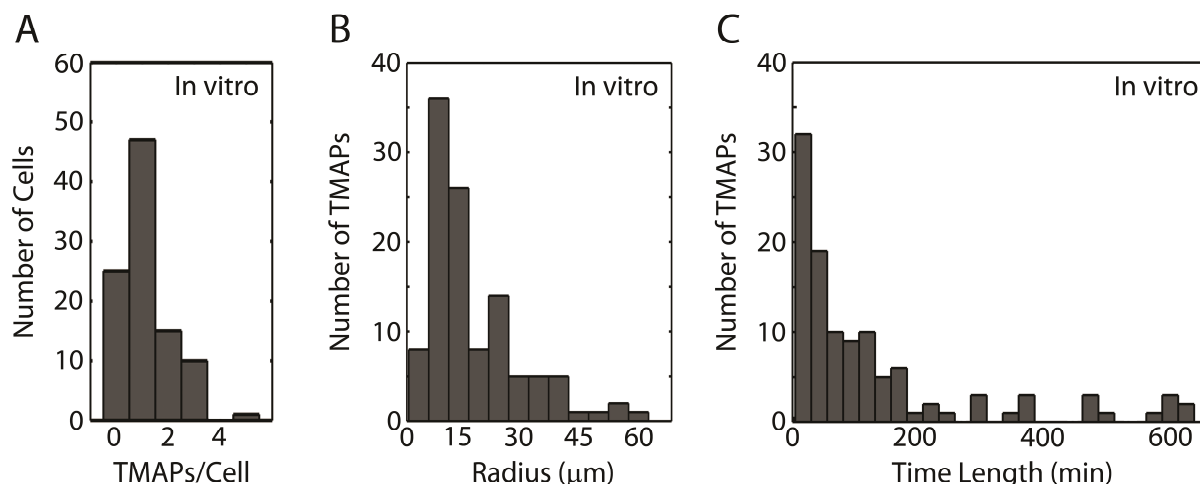


Figure S2. Properties of NK cell TMAPs *in vitro* without target cells. Histograms of the number of TMAPs/cell show that individual NK cells can move in and out of several TMAPs (A). The mean number of TMAPs formed for each NK cell was 1.1 ± 0.10 . Histogram showing the radius of individual TMAPs (B). The mean value was $R = 17.1 \pm 1.2$ μm. Histogram of the duration of individual TMAPs (C). The average duration of TMAPs was 142.8 ± 17.0 min.

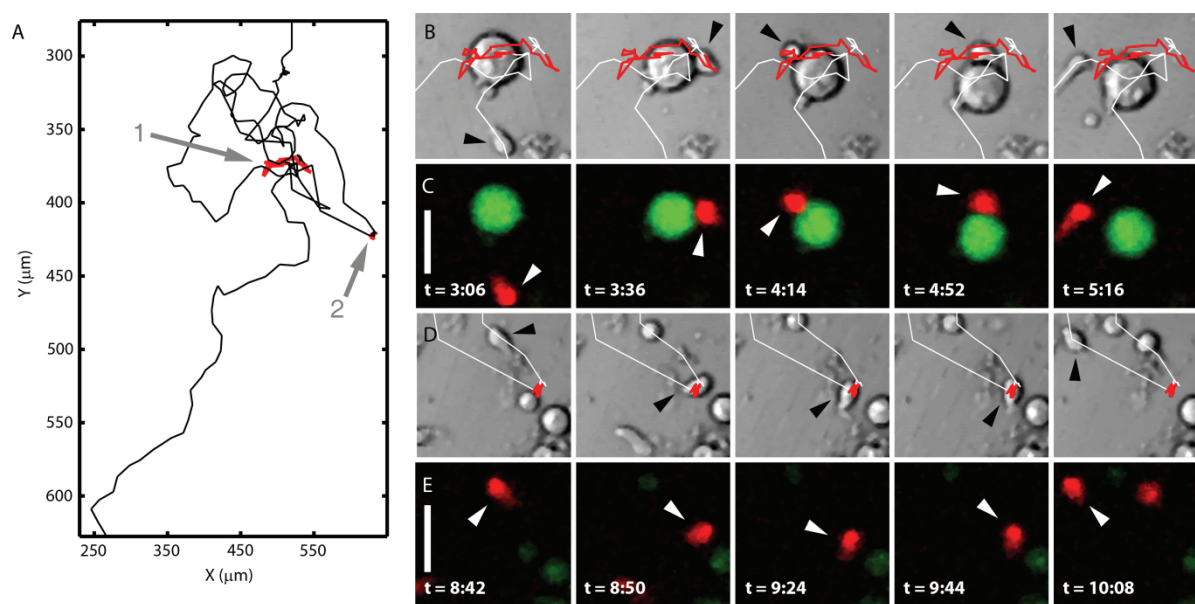


Figure S3. NK cells can form TMAPs during conjugation to target cells or spontaneously. A trajectory plotted (black line) for a single NK cell imaged *in vitro* (A). The NK cell forms one TMAP while in conjugate with a target cell (red line, grey arrow 1) and one TMAP independent of contact with other cells (red line, grey arrow 2). Snapshots from the time-lapse showing bright field (B, D) and fluorescence (C, E) of the cells involved in TMAP1 (B, C) and TMAP2 (D, E). The white lines in (B, D) show the trajectories immediately outside of the TMAP while the trajectories of the TMAPs are shown by red lines. Black and white arrowheads in the bright-field and fluorescence images, respectively, mark the position of the NK cell of interest. Indicated times are in the format hours: minutes. Scale bars, 20 μm.

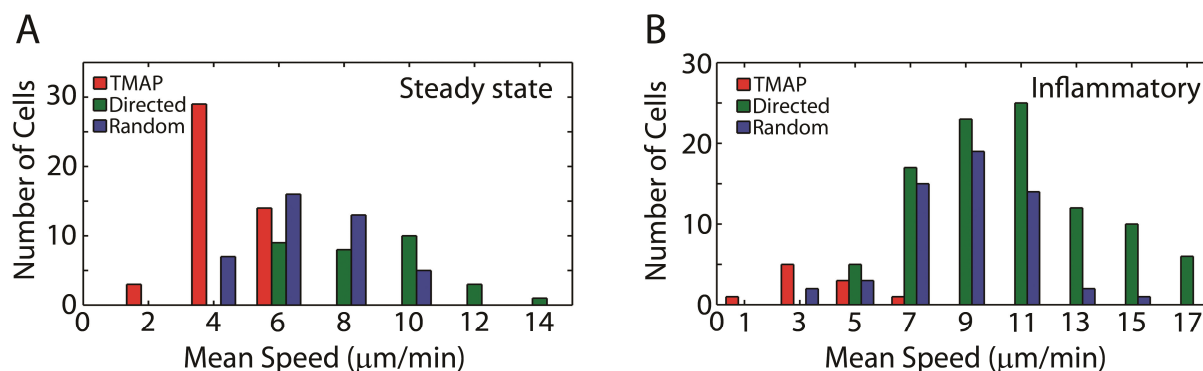


Figure S4. Mean speed of NK cells in different migration modes *in situ*. Histograms of mean speeds of NK cells in TMAPs (red), periods of directed migration (green) and random movement (blue), for migration under steady state (A) and inflammatory conditions (B). The fastest migration was recorded when NK cells migrated in a directed fashion (average mean speeds were 8.7 ± 0.6 μm/min at steady state and 10.6 ± 0.3 μm/min under inflammatory conditions), followed by random motion (average mean speeds were 6.5 ± 0.1 μm/min at steady state and 9.1 ± 0.3 μm/min under inflammatory conditions) and TMAPs (average mean speeds were 4.0 ± 0.2 μm/min at steady state and 4.7 ± 0.1 μm/min under inflammatory conditions).

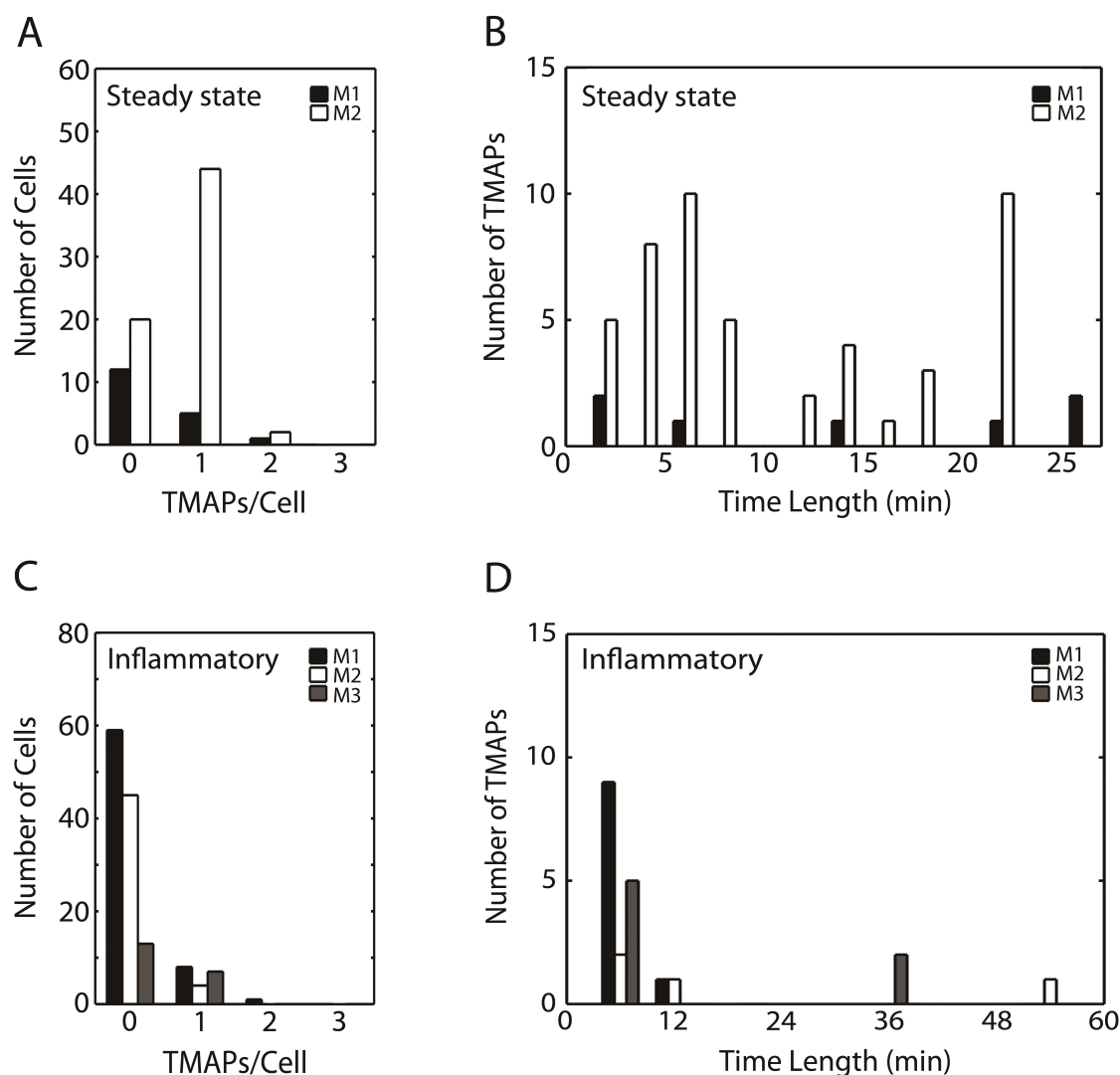


Figure S5. Properties of TMAPs detected *in situ*. The same data as presented in figure 8 of the main manuscript but separated into each individual experiment. M1-M3 shown in the legends refer to the individual movies. For steady state the total imaging times were, 25 min 22 sec (M1), 21 min 59 sec (M2) and for inflammatory conditions, 35 min 44 sec (M1), 60 min 05 sec (M2) and 56 min 24 sec (M3).

Supporting movie1. NK cells (red) killing a tumor target cell (green). At the start of the movie the NK cell is directly on top of the target cell seen as a yellow spot overlapping the green target cells. NK cell mediated killing is seen as an abrupt leakage of the calcein dye at $t=2:56:00$ (hrs:min:sec) followed by blebbing and other morphological changes of the target cell. Experimental conditions as described in materials and methods.

Supporting movie2. NK cells (red) and DCs (green) migrating in a mouse lymph node under steady state conditions. Imaging procedure as described in the Material and methods. Here, images were acquired every 18.6 s and the total imaging time was 21 min 59 s. The movie is shown in 10 frames per second.

Table S1. Parameters measured for transient migration behavior of NK cells *in vitro* and *in situ*

Condition	Number of cells	Mean speed (μm/min)	Mean speed in TMAPs (μm/min)	Mean speed in directed migration (μm/min)	Mean speed in random movement (μm/min)	Mean radius of TMAPs (μm)	Mean duration of TMAPs (min)	Number of TMAPs per Cell	Fraction of time in TMAPs (%)	Fraction of time in directed migration (%)	Fraction of time in random movement (%)
<i>In vitro</i> without target cells	98	2.41±0.10	1.56±0.04	2.37±0.13	2.68±0.12	17.07±1.20	142.82±17.02	1.14±0.10	34.78	20.73	44.49
		*	*	*	*	NS	*	*			
<i>In vitro</i> with target cells	175	1.60±0.06	1.20±0.03	1.91±0.10	2.28±0.09	18.20±0.71	241.43±13.34	1.67±0.07	69.50	8.90	21.60
<i>In situ</i> steady state	84	6.00±0.25	3.97±0.23	8.71±0.63	6.52±0.14	4.85±0.40	10.35±1.13	0.65±0.06	40.82	23.92	35.26
		*	*	*	*	*	*	*			
<i>In situ</i> inflammatory	137	9.32±0.24	4.72±0.13	10.59±0.32	9.09±0.28	3.27±0.57	8.42±3.43	0.15±0.03	5.55	56.01	38.44

Measurements are presented as mean±SEM. * Indicates a statistically significant difference between mean values. NS indicates no statistically significant difference between the mean values. No statistical comparison was made between *in vivo* and *in vitro* data. Statistical significance was calculated using Mann-Whitney Wilcoxon unpaired test. P<0.05 was considered significant.