***Preparation of stimulatory surfaces***

Uniformly coated stimulatory surfaces, micro-contact printed arrays of stimulatory spots and bilayers were prepared essentially as previously described (Mayya et. al, Cell Reports, 2018). Briefly, Labtek 8-well chambers (Nunc) were used as previously described for uniformly coated stimulatory surfaces with immobilized CCL21, ICAM1 and OKT3. Micro-contact printing technology was used to obtain repeating arrays of circular, stimulatory 'spots' with immobilized, Alexa Fluor 647 conjugated OKT3 or 2C11 on coverslips. The primary difference in this study is that the coverslips for micro-contact printing were cleaned in 30% 7X Cleaning Solution (MP Biomedicals) on a hot plate (set for 205 °C) for 30-40 minutes, followed by extensive rinsing under flowing deionized water. The assembled sticky-Slide $VI^{0.4}$ (Ibidi) channels were then coated sequentially with CCL21 and ICAM1. Each Ibidi sticky-Slide $VI^{0.4}$ channel contains ~63,000 spots that are 10 µm wide and 30 µm apart and ~22,800 spots that are 20 µm wide and 50 µm apart. Supported Lipid Bilayers (SLBs) presenting fluorescent-dye conjugated ICAM1 (200 molecules/$\mu m^2$) and UCHT1 Fab` (varied density, produced in-house) were also assembled in sticky-Slide $VI^{0.4}$ channels.

***Quantification of fraction of cells forming IS or IK on SLB***

Those fixed cells with >50% of the area under attachment as per the IRM channel and having a 'circle-like' attachment footprint were counted as having formed IS or IK. Cells with no or <50% area under attachment or discontinuous or highly elongated or highly irregular or fan-shaped attachment footprint were disregarded while counting cells with IS or IK. These criteria rule out highly motile cells in low adhesive state. These criteria also ensure that both IS and IK are counted together without distinguishing them and thus allow for counting all cells that have passed through the phase of immature IS. Manual counts were corrected for less than 100% purity of naïve and memory subsets, as assessed by flow cytometry, and also for deviation from 1:1 ratio when mixed together after differential labelling. Finally, fraction of cells is obtained based on the expected number of all cells in the field when introduced at the same density (1.5 million/ml). This was done to prevent overestimation of fraction of cells forming IS or IK, as non-attached cells tend to get washed away (or displaced) during fixation and subsequent washes.

***Quantification of fraction of cells forming IS or IK on coated surfaces***

Attached segments of tracks from live imaging were selected first, for which we calculated arrest coefficient with the threshold speed of 3 μm/min, implying arrest or deceleration due to IS or IK formation. Any attached segment with arrest coefficient of >0.4 was selected to have led to IS or IK formation in the cell for a significant period during the observation. These relaxed criteria for deceleration allow for inclusion of all cells that have passed through the phase of immature IS. Average number of all cells in the field over the duration of the time-lapse (typically ~50) was used to get the fraction of cells with IS or IK.

***Calculation of on-rate of attachment, encounter rate and arrest efficiency on stimulatory spots***

DIC images with binary masks, both for attachment and location of spots, were used to select cells arrested and attached to spots. Plotting number of arrested cells on spots over time gives the attachment curve. The slope of attachment curve over the first ~40 minutes was used for naïve cells whereas data for the first ~15 minutes was used for the rate of attachment (on-rate) of memory cells. Many intervening data points were often removed in the case of naïve cells as there were no additional arrest events and this improved the regression coefficient ($R^2$) of linear fitting to be >0.85. The slopes were normalized as per the relative number of cells to spots. The normalization also assumed 1 cell arresting on 10 μm spots and 4 cells arresting on 20 μm spots, which is the typical scenario. Non-responding cells were manually identified and disregarded during normalization, based on non-polarized or shrunken appearance and Brownian motion.

Portions of tracks wherein a cell is on a spot, based on fluorescence value associated with the track-positions, are identified as encounter events. Total number of such ‘clipped’ tracks gives the total number of encounter events. All encounter events during the first 90-120 minutes for naïve cells and 45-60 minutes for memory cells were tallied and divided by the duration of time to calculate encounter rate. Arrest efficiency is defined as the number of separate arrest events on spots over all encounters (transient and durable) with spots for all the cells in the field within a certain period of time. Apart from the presence of a spot beneath a cell for at least 4 minutes, additional criterion of attachment is also included to define arrest events on spots.