Beltman et al., http://www.jem.org/cgi/content/full/jem.20061278/DC1

SUPPLEMENTAL RESULTS

Periodic behavior of simulated T cells

Velocity fluctuations of our in silico T cells are comparable to those of real T cells (Fig. S2, A and B, top panels). Furthermore, Fourier and autocorrelation analyses of these brief time series (64 data points) result in a similar periodicity (Fig. S2, A and B, bottom two panels). In our model, periodic velocity fluctuations result from pausing due to random obstacles. We also studied T cell velocity fluctuations over long time periods and found no evidence of periodic behavior (Fig. S3 A). Hence, the large velocity fluctuations make it appear that there is a characteristic period when one analyzes brief time series. The analysis of long time series makes it clear that there is in fact no such characteristic period in the velocity fluctuations of our simulations. To see how easily stops caused by a true internal clock would be picked up by such an analysis, we modified the model and let the directional propensity of T cells be briefly turned off exactly every 2 min. In that case, the signal is not always picked up if one analyzes brief time series (Fig. S3 B). In contrast, periodic pauses are now convincingly shown in Fourier and autocorrelation analyses of long time series (Fig. S3 C). These findings indicate that the analysis of experimental velocity data performed thus far is insufficient to establish whether T cells have an internal clock that regulates velocity fluctuations over time. Furthermore, for this type of analysis, three-dimensional instead of two-dimensional, lateral velocities are required. Otherwise, the periodicity found in each cell differs markedly from that found with three-dimensional velocities (Fig. S4). Therefore, we performed new 2PM experiments with the aim to follow velocity fluctuations of individual T cells for long periods (see Results in main text).

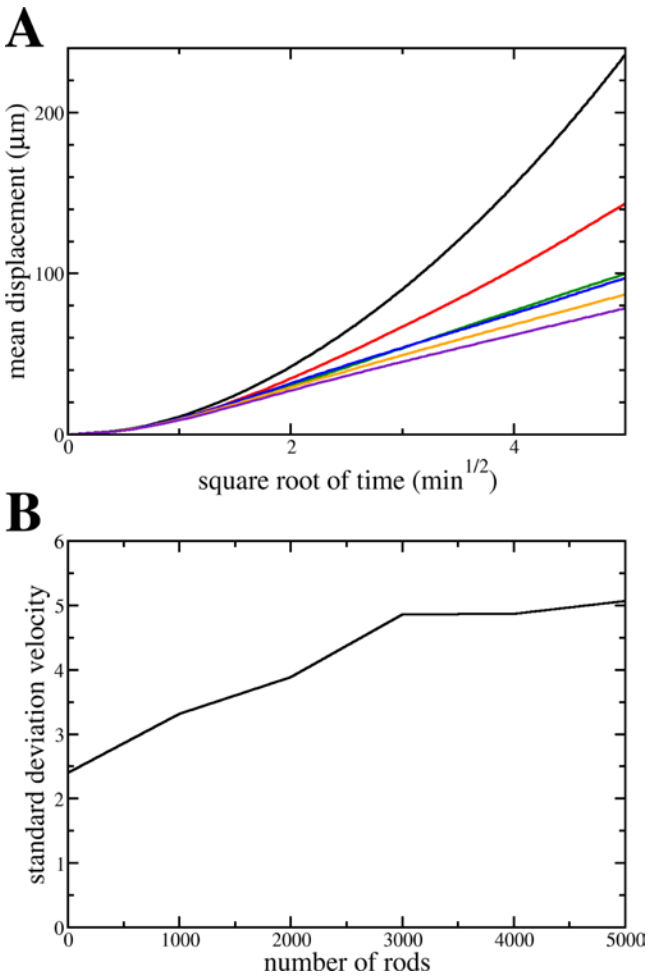

Figure S1. Effect of RN density on T cell displacement and velocity fluctuations. Mean displacement plot (A) and the standard deviation of T cell velocities (B) for different rod numbers. Note that the average velocity of T cells is approximately the same in these simulations, which we achieved by adjusting μ_{max} (black, no rods, 220 DCs, $\mu_{max} = 4 \times 106$; red, 1,000 rods, 195 DCs, $\mu_{max} = 4.5 \times 106$; green, 2,000 rods, 170 DCs, $\mu_{max} = 5 \times 106$; blue, 3,000 rods, 145 DCs, $\mu_{max} = 6 \times 106$; orange, 4,000 rods, 120 DCs, $\mu_{max} = 6 \times 106$; purple, 5,000 rods, 95 DCs, $\mu_{max} = 6 \times 106$).

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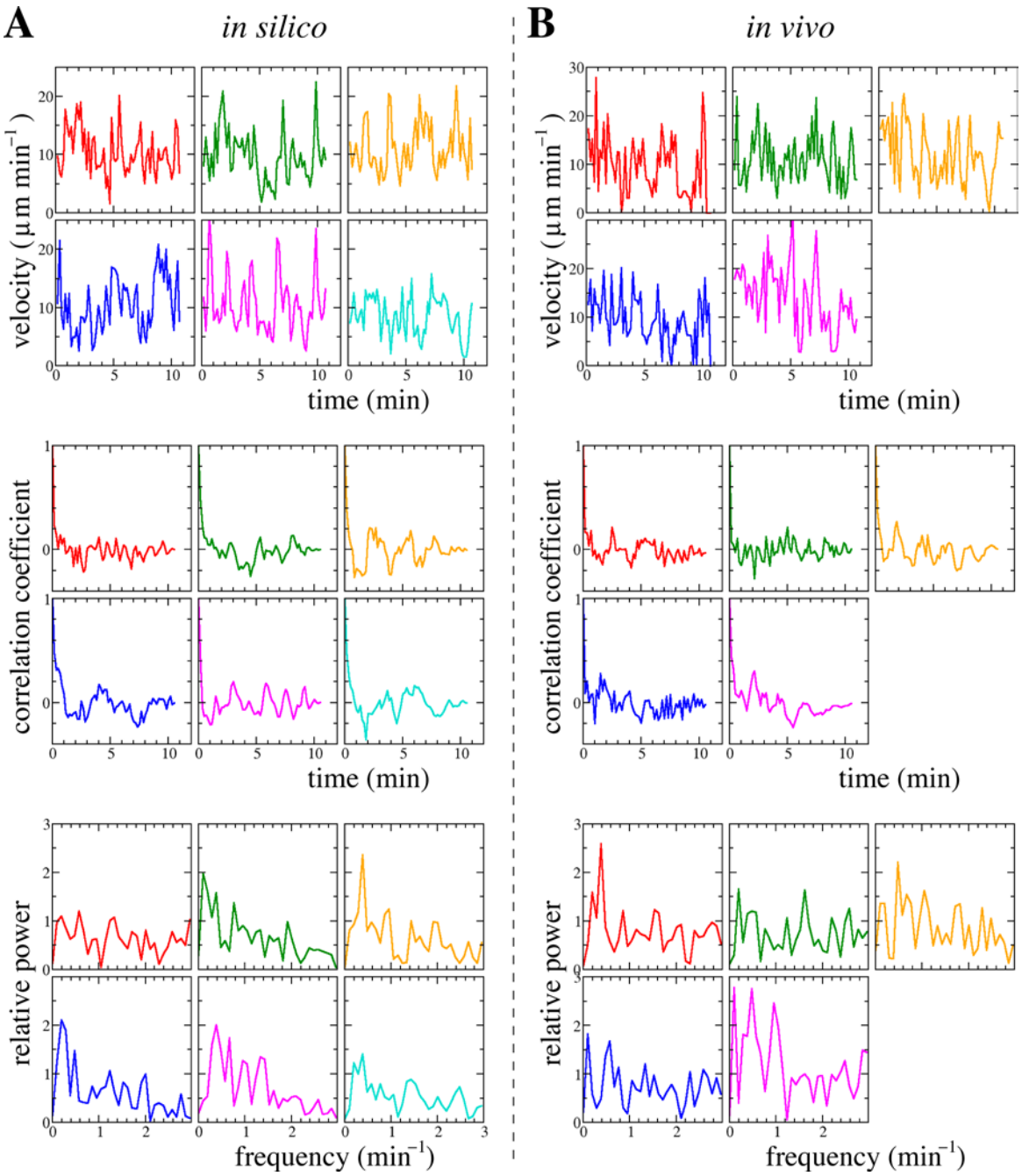

Figure S2. Periodicity in in silico and real T cell velocity fluctuations for brief time series. Velocity fluctuations and accompanying autocorrelation and Fourier analyses of six simulated (A) and five real (B) T cells over a 640-s period. Experimental data from Miller et al. (Miller, M.J., S.H. Wei, M.D. Cahalan, and I. Parker. 2003. *Proc. Natl. Acad. Sci. USA.* 100:2604–2609) are replotted and reanalyzed. Experimental and simulated data have a similar periodicity in velocity fluctuations. In the model, this is caused by obstacles that T cells encounter on their path, resulting in brief pauses.

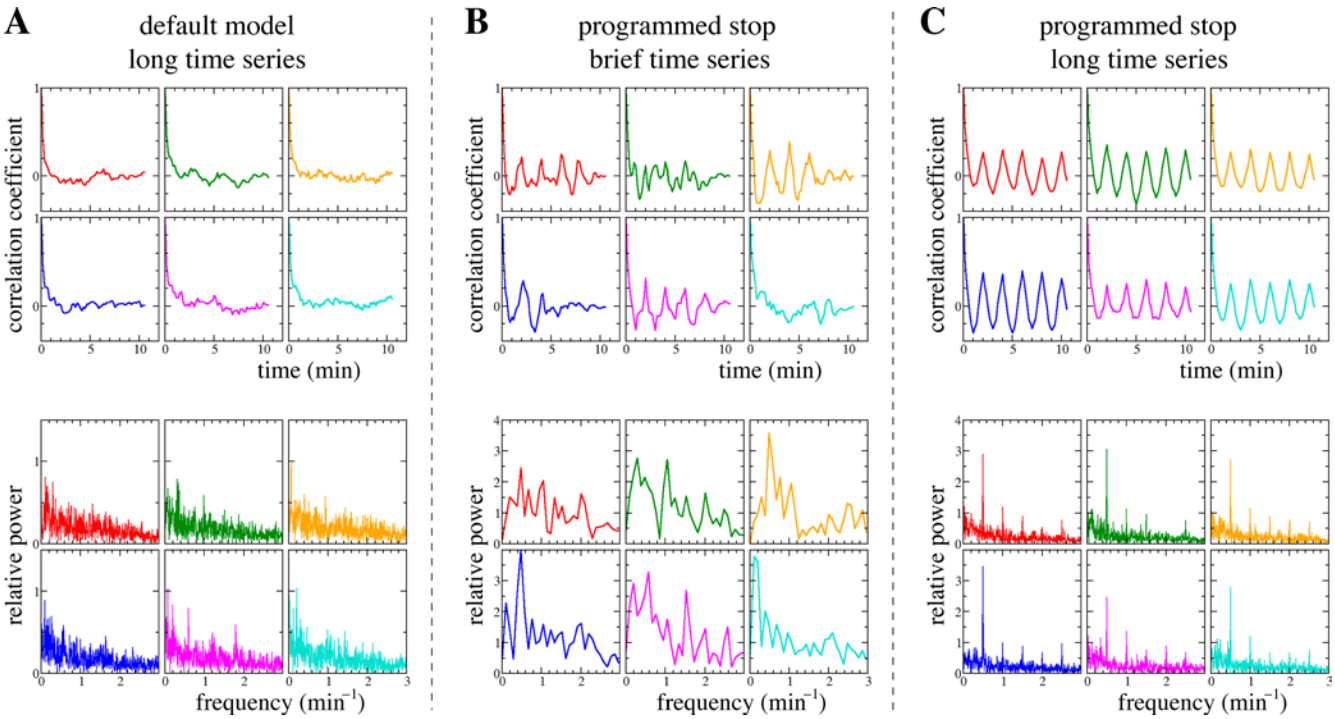

Figure S3. Periodicity in in silico T cell velocity fluctuations. (A) Autocorrelation and Fourier analyses of velocities of six simulated T cells over a long period (10,240 s). (B–C) Modified model ("programmed stop") with T cells' directional propensity put to 0 for 20 s each 2 minutes (phase is randomized such that this occurs asynchronously). Shown are autocorrelation and Fourier analyses of velocities of six T cells over a 640-s period (B) and a 10,240-s period (C). $\mu_{max} = 8.5 \times 106$. The difference in periodicity of velocity fluctuations between the models with and without programmed stop becomes clear for long time series.

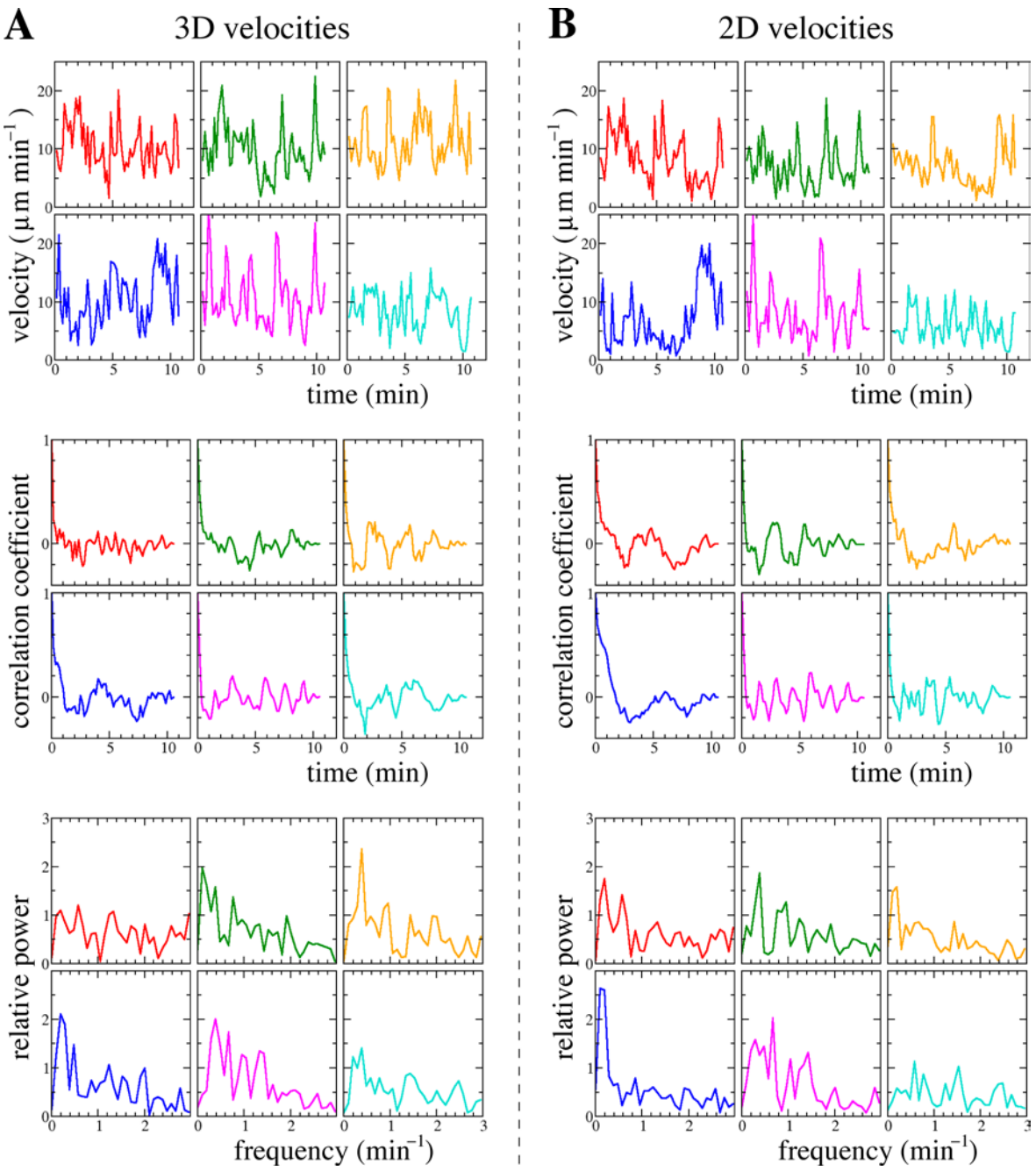

Figure S4. Comparison of periodicity in T cell velocity fluctuations for two- and three-dimensional velocities. (A–B) Velocity fluctuations and accompanying autocorrelation and Fourier analyses of six simulated T cells over a 640-s period. Either three-dimensional velocities (A) or the two-dimensional lateral velocity components of the same cells (B) are used. When the velocity component in the z-direction is not taken into account, the observed period with which each cell seems to stop changes dramatically. This can be seen from a comparison per individual T cell (each small panel shows results for one T cell).

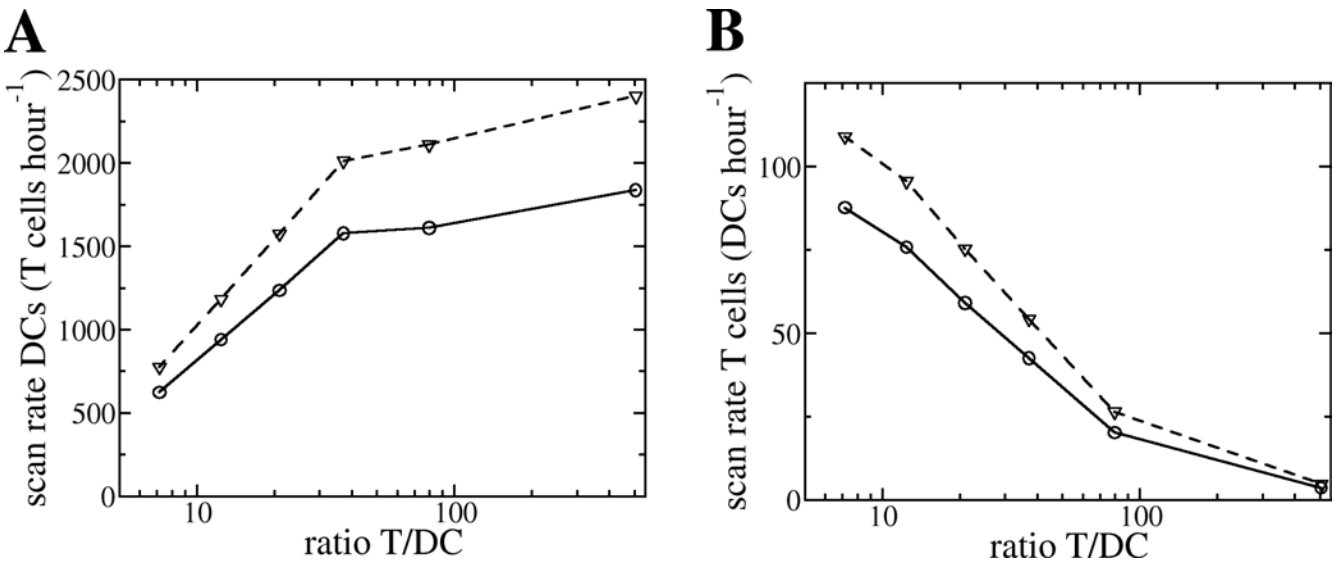

Figure S5. T/DC ratio and scanning rate. Scanning rates of T cells by DCs (A) and vice versa (B) for simulations with a different T cell/DC ratio. The T/DC ratio is changed while keeping the ECM density approximately constant, i.e., the T cell and DC number are changed simultaneously (parameters of different simulations: 235 DCs, 1,680 T cells; 190 DCs, 2,355 T cells; 145 DCs, 3,030 T cells; 100 DCs, 3,705 T cells; 55 DCs, 4,380 T cells; 10 DCs, 5,055 T cells).