Brain-localized CD4 and CD8 T cells perform correlated random walks and not Levy walks

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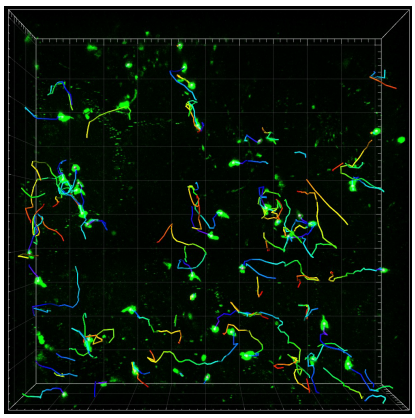
Supplemental Information

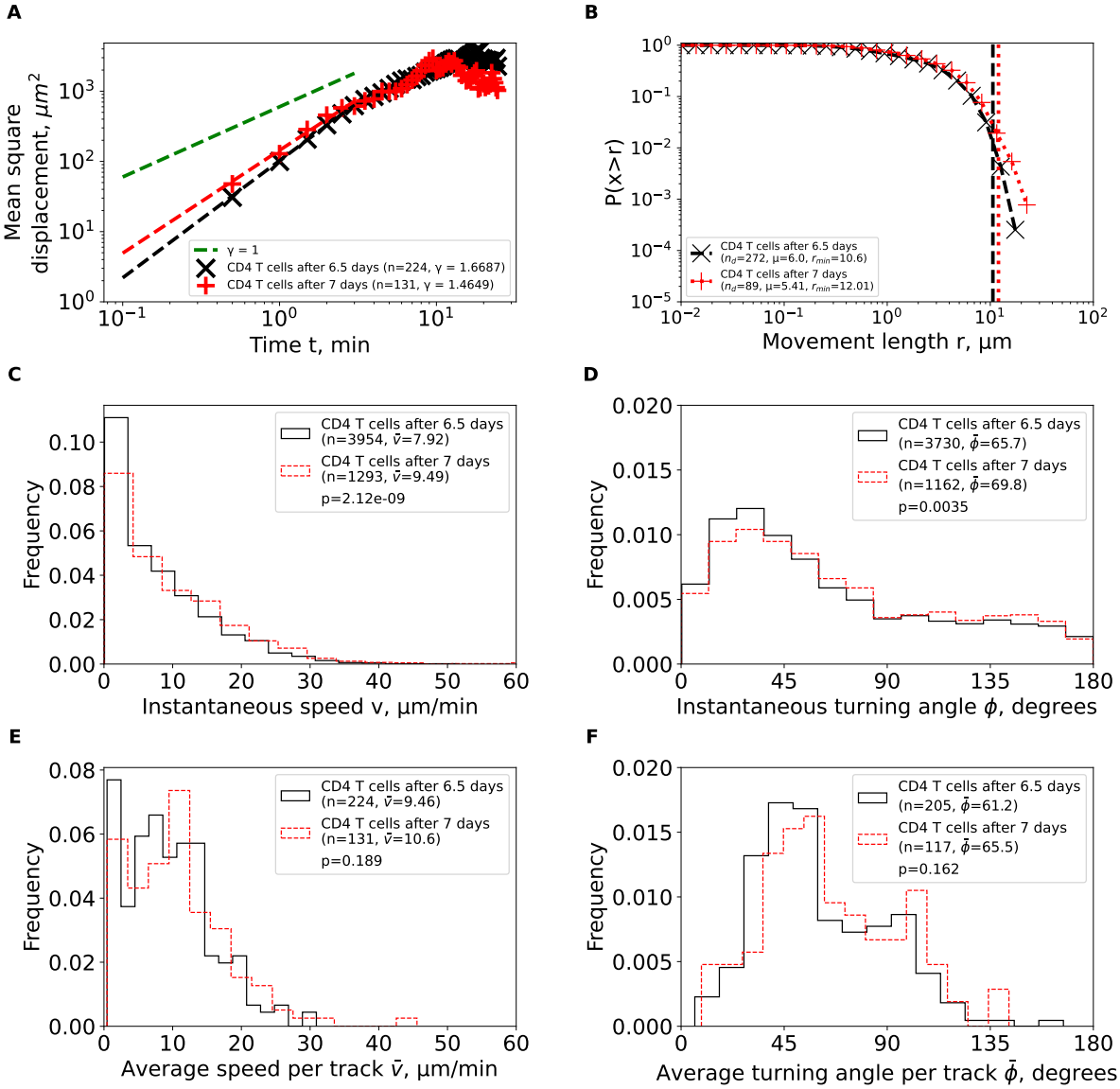
Additional details of tracing T cells using Imaris (ver 9.8.2). The algorithm used for tracing the relevant movies was carefully designed, in terms of parameters, to maximize data retention and accuracy. The option of "Segment only a Region of Interest" was deselected as the desired region of interest was the entire image and not a segment. The option of "Process entire Image finally" was selected as the desired region of interest was the entire image. The option of "Different Spot Sizes (Region Growing)" was deselected as the region of interest indicated no signs of T-cell growth or development. The option of "Track Spots (over time)" was selected as this allowed each T-cell traced to have a unique set of output measurements. The option of "Classify Spots" was deselected as information on this feature was not provided by the manual and therefore left out of the algorithm. The option of "Object-Object Statistics" was deselected as the information provided by this option such as "Shortest Distance To Spots" and "Shortest Distance To Surfaces" was not needed nor did it affect the final tracing results. By deselecting this feature, this sped up the tracing process.

The "Source Channel" was changed to the channel containing the desired T-cells. The option of "Estimated XY Diameter" was set to 9.94 μm. We found that a value of 9.94 was a safe value as Imaris detected nearly all of the desired T-cells. The option of "Model PSF-elongation along the Z-axis" was deselected as a safe value for the Z diameter was not determined in order to prevent the loss of T-cells. The option of "Background Subtraction" was selected as this feature smooths the final image. The filter type was set to "Quality" as this allowed for manual control of the threshold value for which Imaris detected a T-cell. The threshold value was set to a low value (less than 100) as this ensured that nearly all of the T-cells were detected. The next set of options under the "Create", "Settings", and "Color" tab are left alone as the editing of T-cells and T-cell tracks was done after the completion of the algorithm. Features under "Settings" and "Color" are personal preference and do not affect the final tracing results.

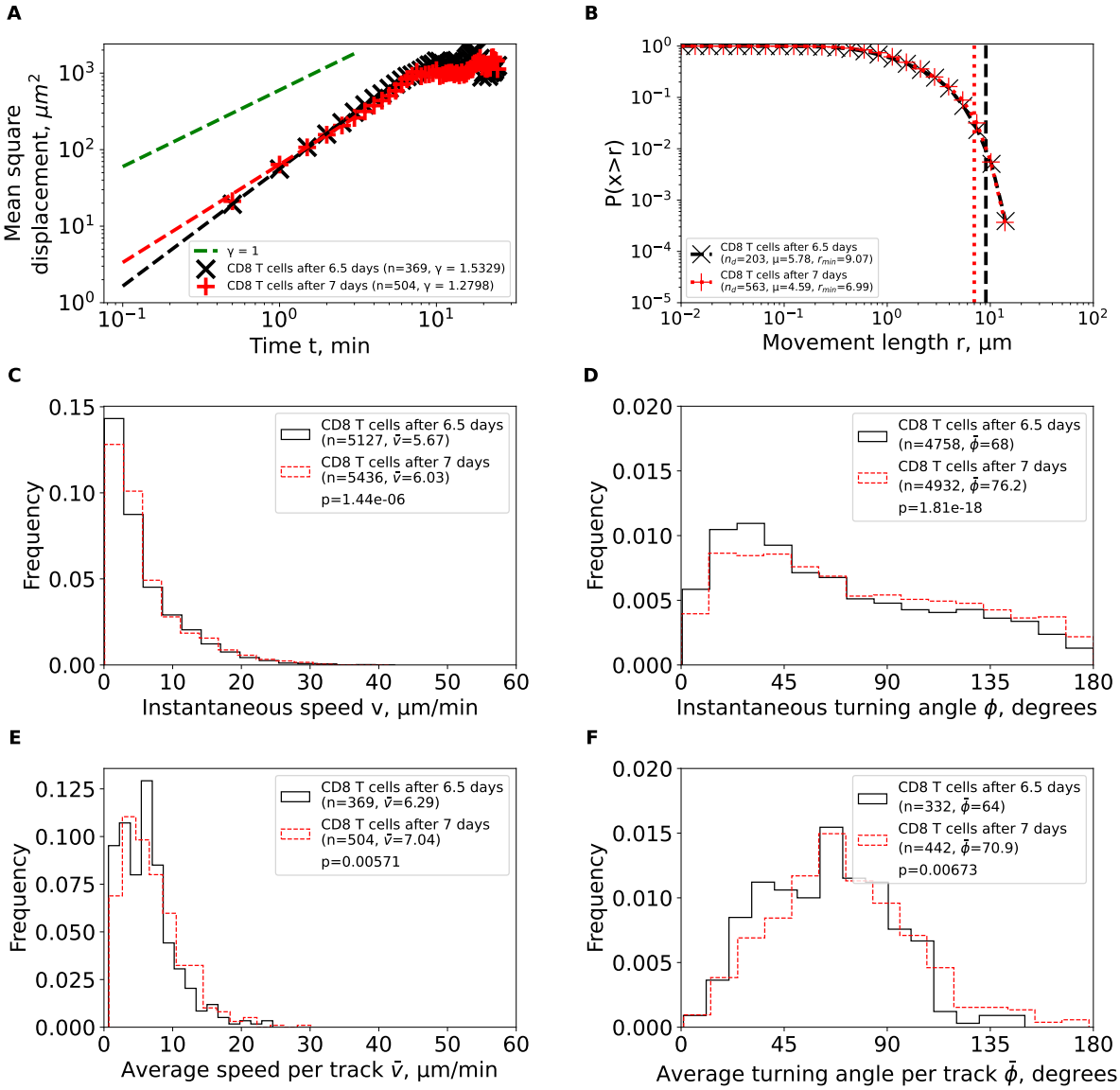
Afterwards, the "Tracking Algorithm" was set to "Autoregressive Motion" as this tracking algorithm best suited the data as the movement of T-cells were a continuous motion and not random. The next set of options were "Max Distance" and "Max Gap Size" which can be found under "Parameters". These values were not tampered with as the values were automatically generated by Imaris and may vary from data set to data set. The option of "Fill gaps with all detected objects" was deselected to prevent the possibility of Imaris merging two independent T-cells into one T-cell resulting in error. The option of "Filter Type" was set to "Track Duration" as this allowed for manual control and the filtering of certain tracks with an "x" duration. The "Track Duration" value was set to a value less than 100 in order to prevent the loss of potential T-cell tracks. The option "Classification" and "Event Setup" were not added to the algorithm as information on this feature was not explained in the manual and therefore left out of the algorithm.



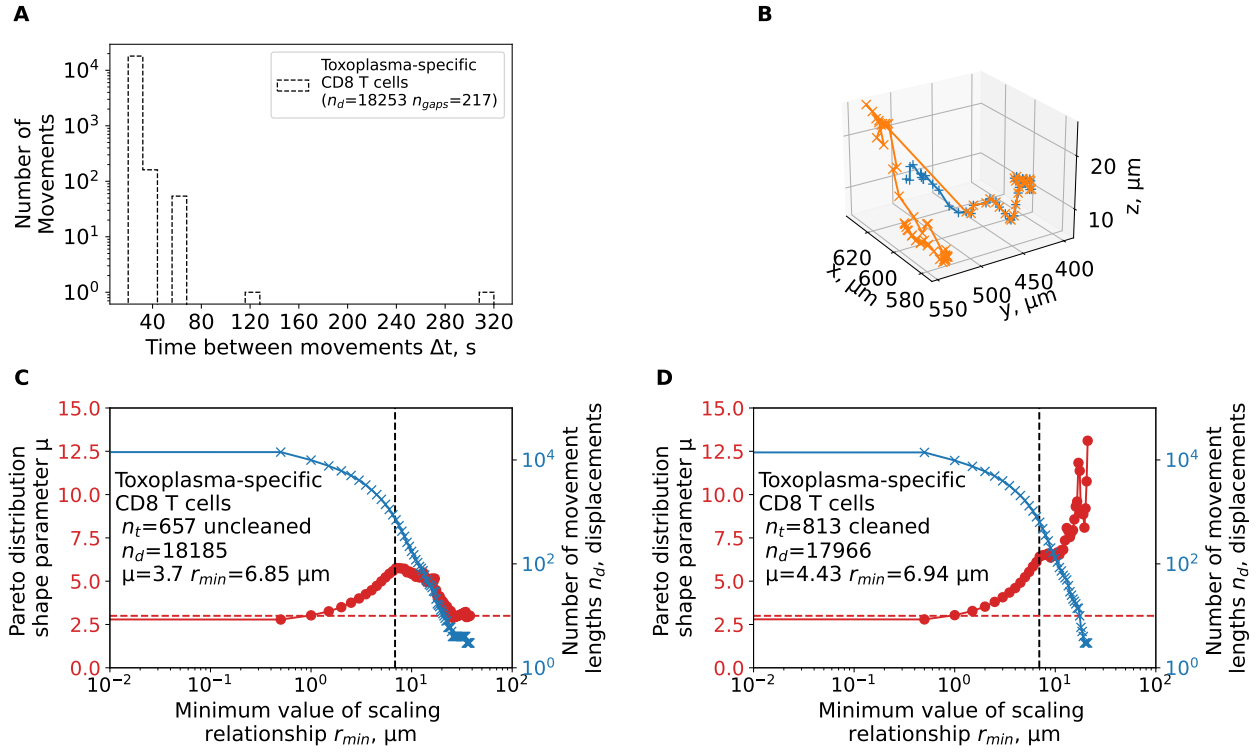
Supplemental Movie S1: Example of movement of brain-localized Plasmoidum-specific CD4 and CD8 T cells at different times after *Plasmodium berghei ANKA* **(PbA) infection**. Experiments were performed as described in **Figure 1** in 5 individual mice. In the movie red are CD4 T cells and green are CD8 T cells. Bar scale is 50 μm. The volume of $512 \times 512 \times 44$ μm was scanned approximately every 30 sec (exact imaging frequency varied by the movie, see Materials and methods for more detail).



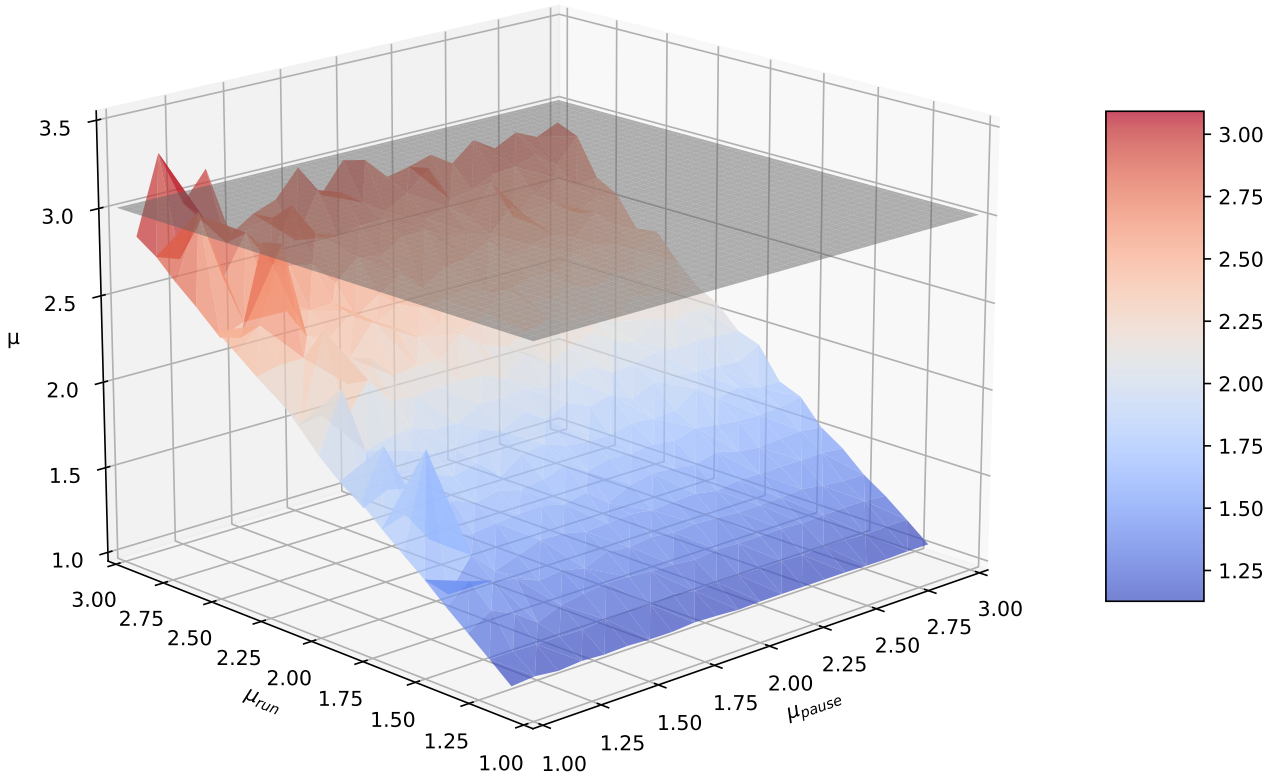
Supplemental Figure S1: Brain-localized Plasmodium-specific CD4 T cells have higher instantaneous speed and higher turning angle at later times of PbA infection. The data from **Figure 3** we analyzed trajectories of CD4 T cells separately for 6.5 or 7 days since PbA infection. Other notations are the same as in **Figure 3**.



Supplemental Figure S2: Brain-localized Plasmodium-specific CD8 T cells have higher speeds and higher turning angle at later times of PbA infection. The data from **Figure 3** we analyzed trajectories of CD8 T cells separately for 6.5 or 7 days since PbA infection. Other notations are the same as in **Figure 3**.



Supplemental Figure S3: Artifacts in the track data have likely generated an appearance of Levy walks for brain-localized *Toxoplasma gondii*-specific CD8 T cells. We have reanalyzed track data from a previous publication [2] and calculated the shape parameter of the Pareto distribution using tail analysis of the movement length displacement data. **A**: The time between sequential movements in the data. **B**: a single large displacement for a trajectory yep.3829 observed in the data. **C-D**: We estimated the shape parameter μ of the Pareto distribution (eqn. (3), see Materials and Methods for details) by tail analysis of the movement length distributions for uncleaned (original) data (**C**) or when cell tracks were cleaned/split to allow for sequential timeframes for all trajectories (**D**) . We also performed the analysis when we adjusted the tail cutoff value $r_{\min}$ (minimum value of the scale parameter) to different values denoted on the x-axis.



Supplemental Figure S4: Tail analysis of movement lengths with python package `powerlaw` is a robust method to detect Levy walks. Generalized Levy walkers were generated for $1 < \mu_{run} \leq 3$ and $1 \leq \mu_{pause} \leq 3$, in 0.1 increments per parameter with $r_{min} = 1$ for all Pareto distributions generated (see Materials and Methods for more detail). For each μ_{run} and μ_{pause} combination, 100 generalized Levy walkers were simulated for 100 steps. Then, the shape parameter μ of the tail of the movement length distribution was estimated using `powerlaw` package in `python`.

Cell population	# of displ	**Shape k**	**Location θ**	**Scale σ**	**Mean**	**Variance**
All Plasmodium-specific CD4 T cells	5257	-0.08	0.01	4.47	4.15	14.84
All Plasmodium-specific CD8 T cells	10563	-0.06	0.03	3.07	2.93	7.5
Plasmodium-specific CD4 T cells at 6.5 days	3954	-0.09	0.04	4.28	3.97	13.03
Plasmodium-specific CD4 T cells at 7 days	1293	-0.08	0.01	5.11	4.74	19.26
Plasmodium-specific CD8 T cells at 6.5 days	5127	-0.04	0.03	2.93	2.84	7.24
Plasmodium-specific CD8 T cells at 7 days	5436	-0.07	0.05	3.17	3.02	7.71
All Toxoplasma-specific CD8 T cells	18036	0.09	0.02	1.79	1.97	4.6
Simulated Brownian walkers	10000	0.15	1	0.27	1.32	0.14
Simulated CRW walkers	10000	0.15	1	0.27	1.32	0.14
Simulated Levy walkers	10000	0.72	1	0.65	3.32	nan
Simulated Generalized Levy walkers	1881	0.65	1	0.67	2.9	nan
Simulated Bullet motion walkers	10000	2.01	1	1.97	∞	nan

Supplemental Table S1: Estimated parameters of the generalized Pareto distribution fitted to the movement length data. We fitted the generalized Pareto distribution (eqn. (2)) using likelihood method (eqn. (4)) to different subsets of data including data from simulations. Using estimated parameters we also calculated the mean and variance of the distribution (see eqn. (2)). For every dataset we list the number of displacements used for fitting the GP distribution to the data, and "nan" stands for not-available. We typically simulated movements of 100 cells for 100 movements (with 10 sec for each movement).