

591 **Supplementary Materials for**
592 **Bacterial motility patterns vary smoothly with spatial confinement**
593 **and disorder**

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597 **This PDF file includes:**

598 Figures S1 to S14

599 Captions for Movies S1 to S3

600 **Other Supplementary Materials for this manuscript:**

601 Movies S1 to S3

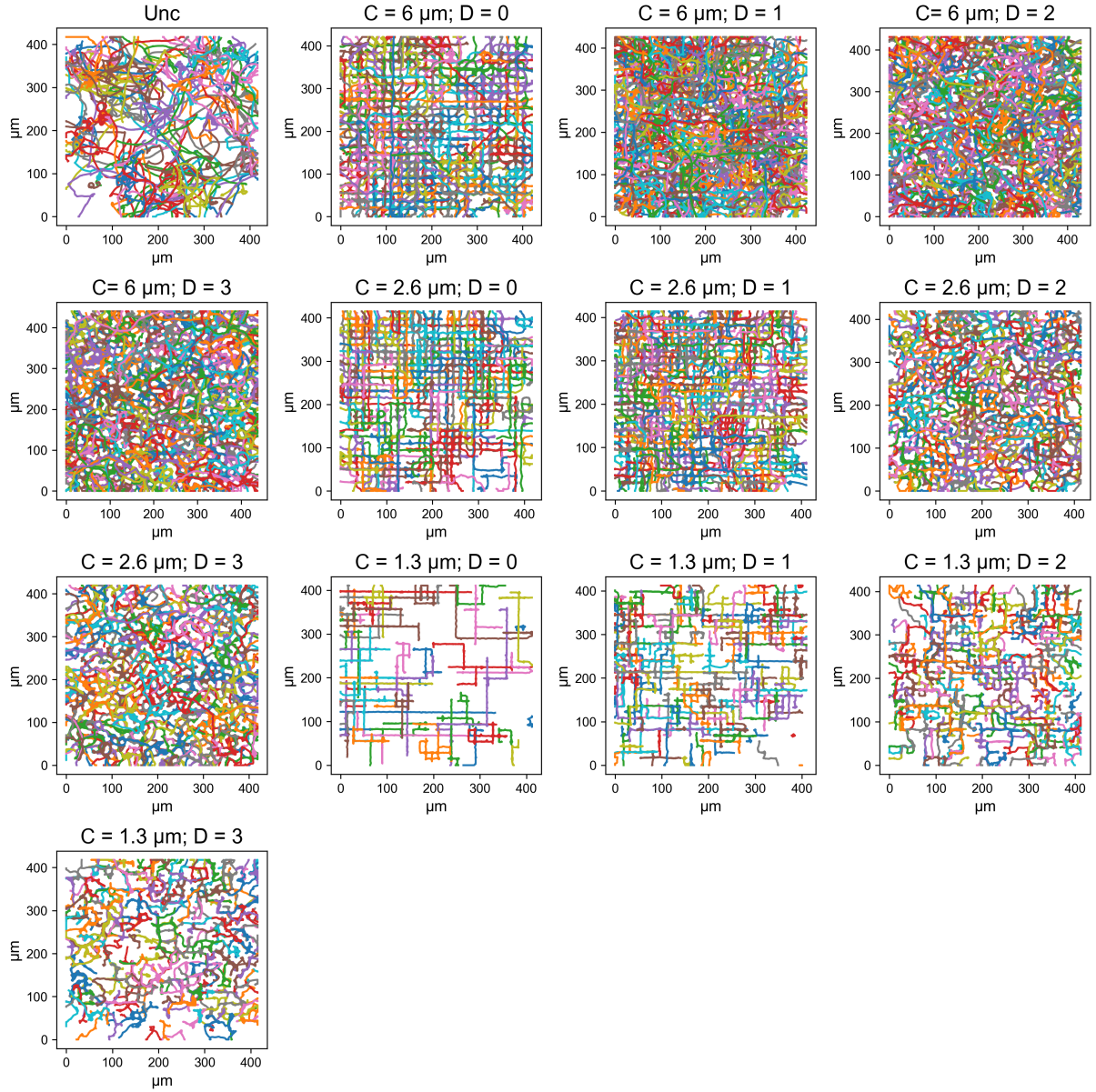


Figure S1: **Bacterial track overlays of all regions examined.** The label ‘unc’ denotes the condition ‘unconfined’. The colors of the trajectories are used to make bacterial tracks distinguishable.

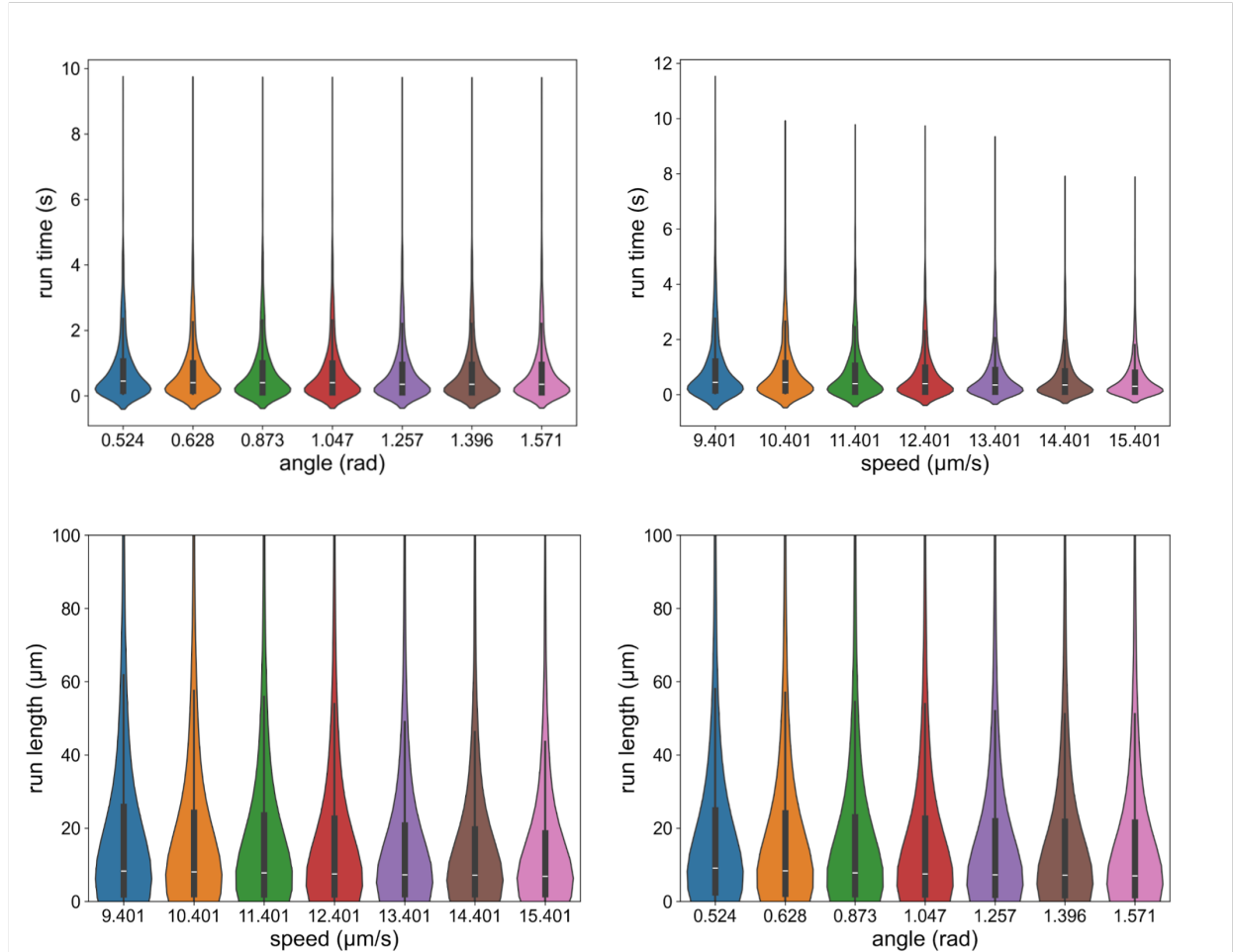


Figure S2: **Sensitivity checks of speed and angle thresholds.** The violin plots show the distributions of run times and run lengths across different speed and angle thresholds. The distributions are not sensitive to various thresholds tested.

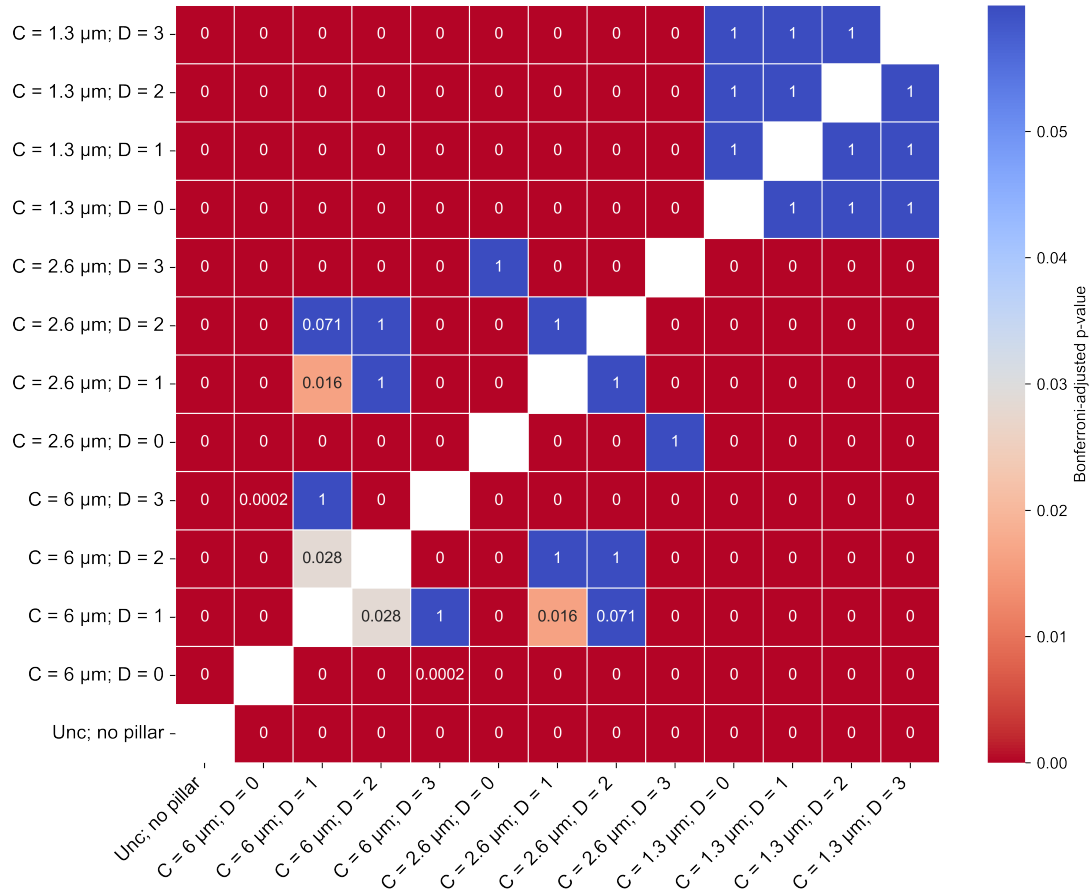


Figure S3: **Pairwise Mann-Whitney U test of swim durations between conditions.** P values are corrected by the Bonferroni method. The significance value is indicated by a color spectrum (red: very significant; gray: significant; blue: not significant).

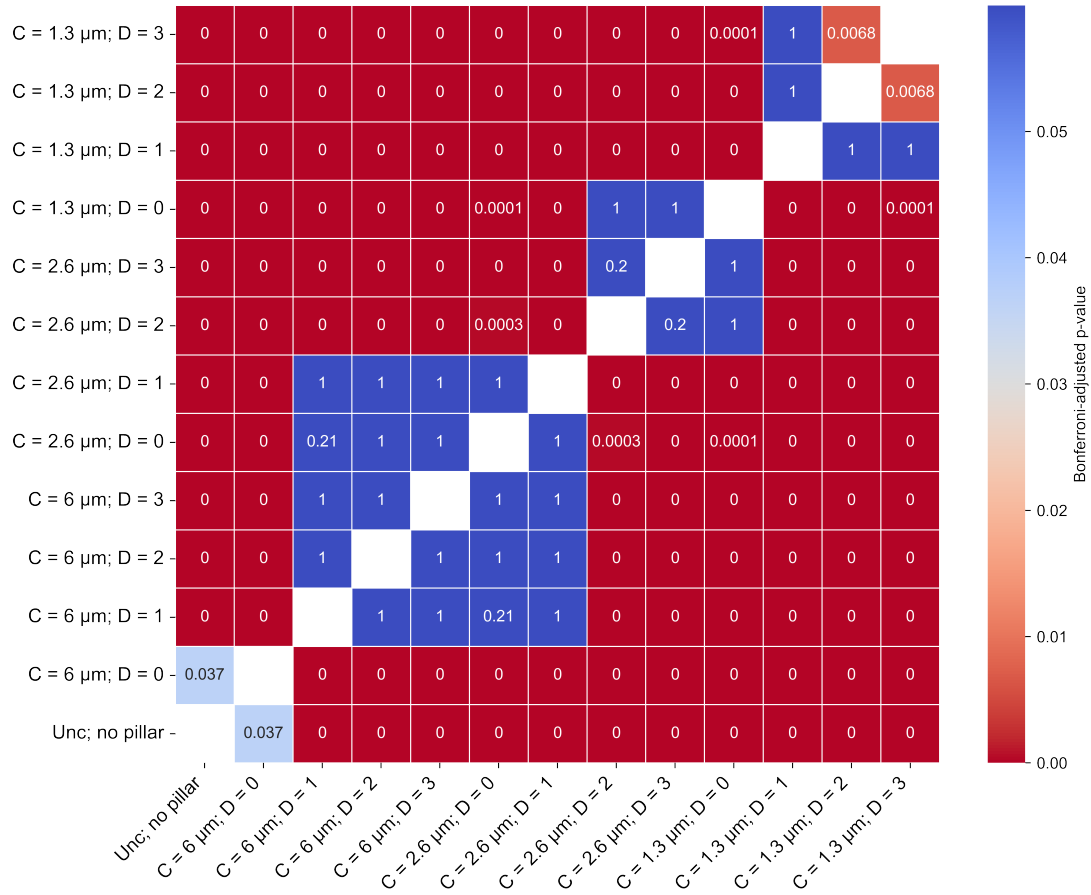


Figure S4: **Pairwise Mann-Whitney U test of stall durations between conditions.** P values are corrected by the Bonferroni method. The significance value is indicated by a color spectrum (red: very significant; gray: significant; blue: not significant).

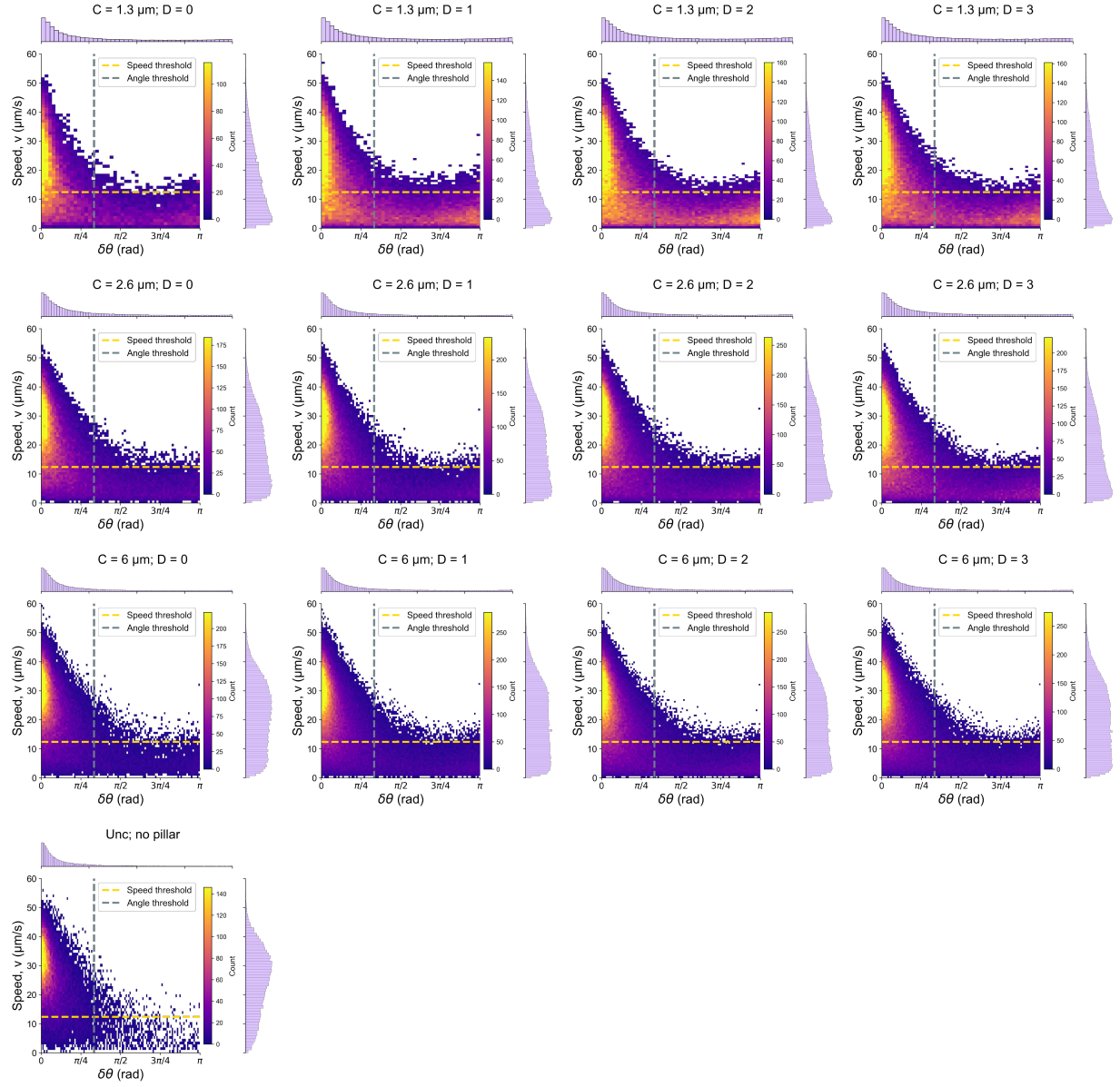


Figure S5: **Speed-angle jointplot of all regions.** The yellow dash line denotes speed threshold, and the gray dash line denotes velocity orientation angle threshold.

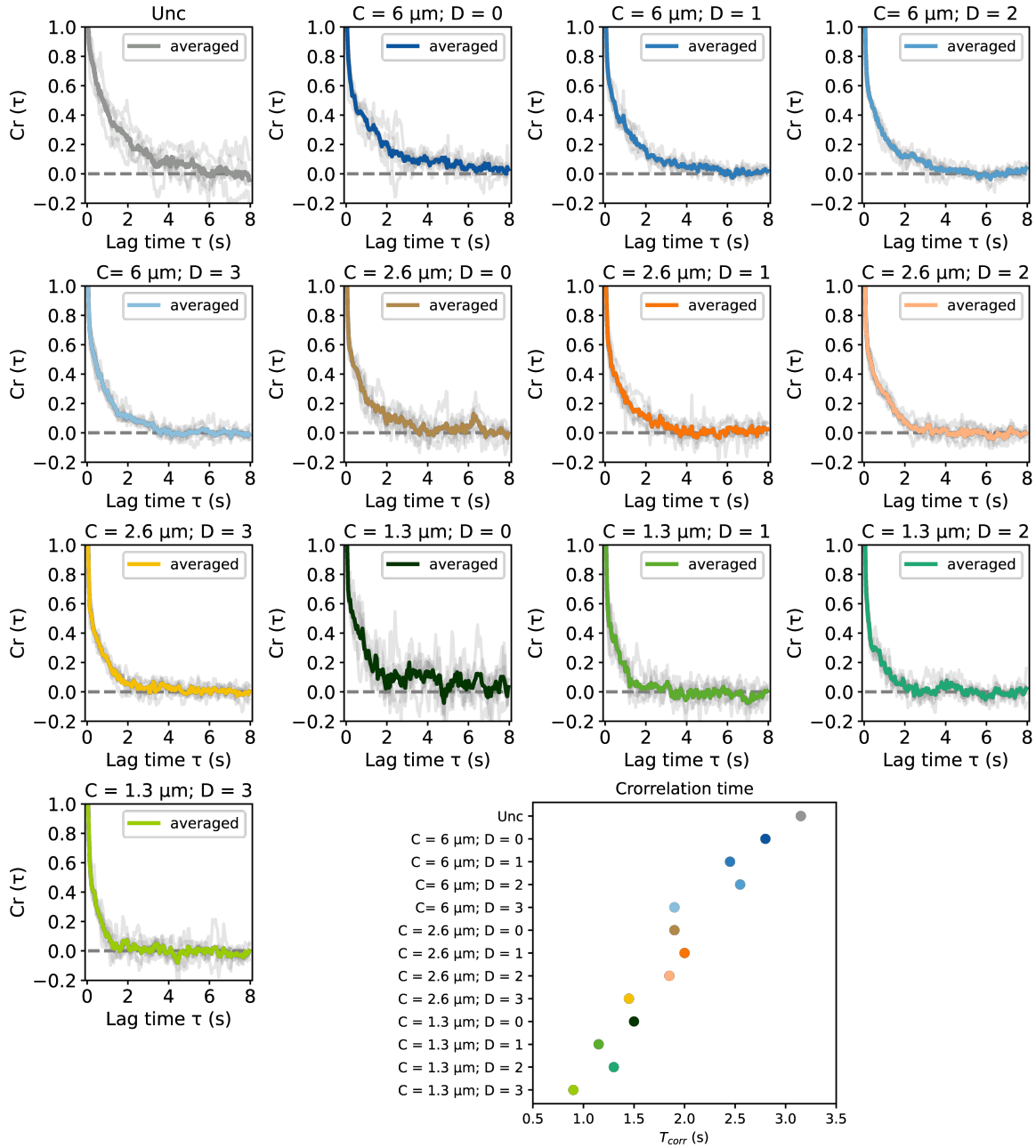


Figure S6: **Velocity autocorrelation function (VACF) plots and correlation times across conditions.** Subplot 1-13 show the VACF decay for 8 seconds. The subplot 14 (bottom) shows correlation times across conditions. The correlation time is determined by a cutoff threshold 0.1 using a sliding window method with the window size of 15 frames and 5 frames of overlap.

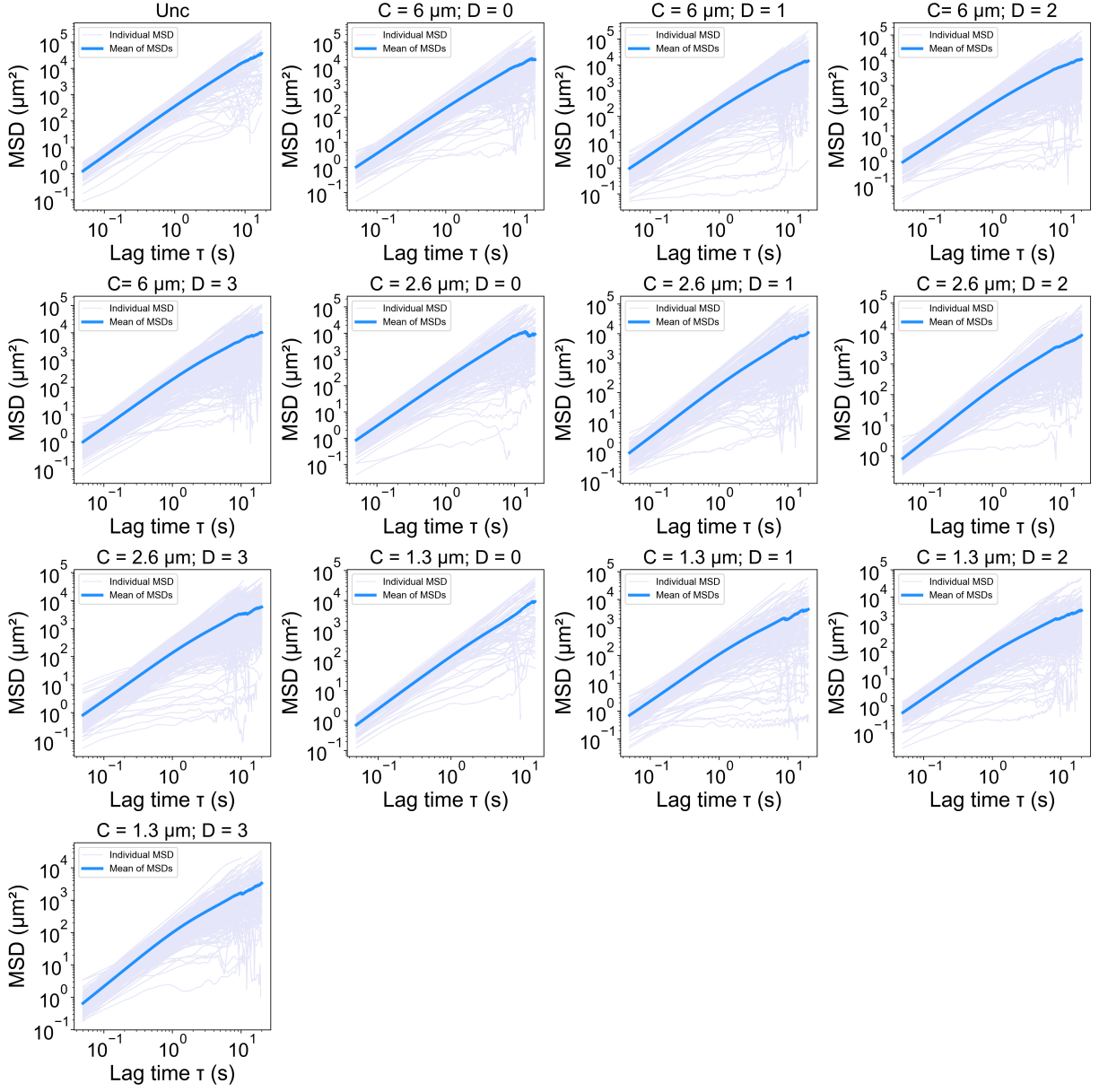


Figure S7: **MSD plots under all experimental conditions.** Individual MSDs of all regions are labeled in purple. The ensemble-average MSD of a region is labeled in blue.

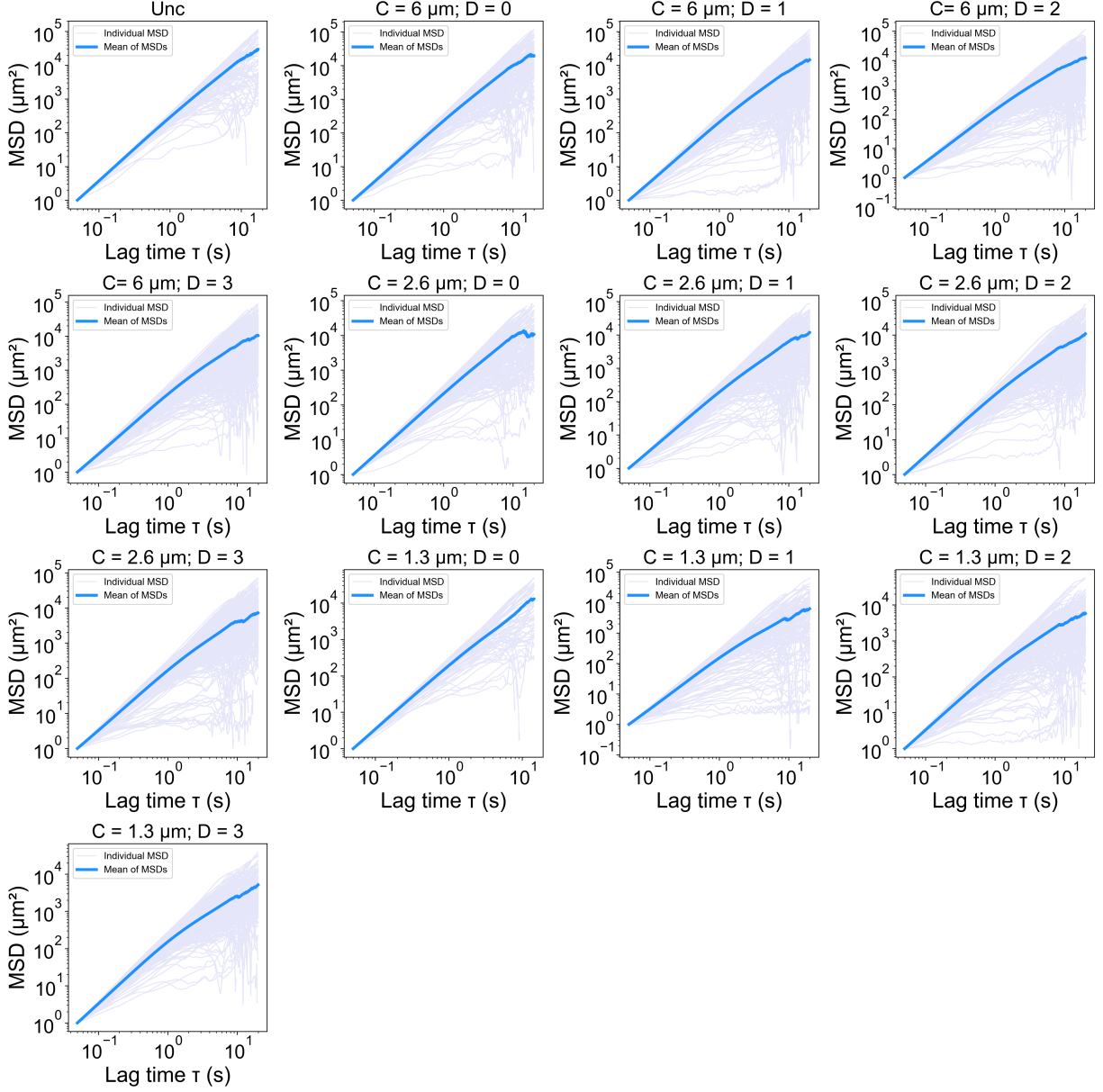


Figure S8: **Normalized MSD plots under all experimental conditions.** Normalized individual MSDs of all regions are labeled in purple. Normalized ensemble-average MSDs of specific regions are labeled in blue. Normalization is done by dividing the MSD value at each time point by the initial MSD value at the first time point.

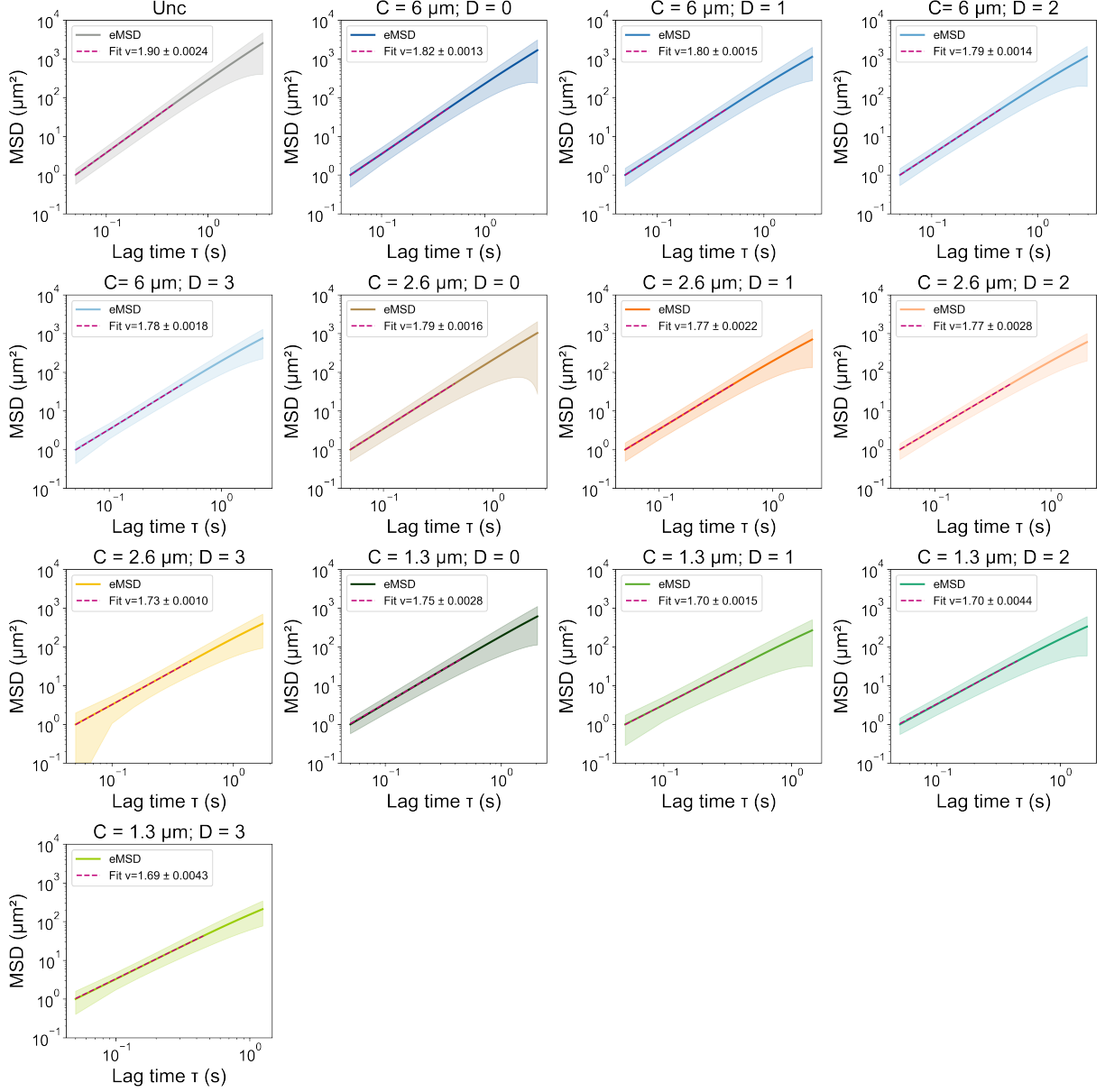


Figure S9: **Ensemble-average MSDs (eMSDs) and power-law fitting curves at early lag times for all conditions.** The bipolar bands indicate standard deviations (eMSDs $\pm$ std). The fit for early lag times are labeled in pink dashed lines by *Scipy* package.

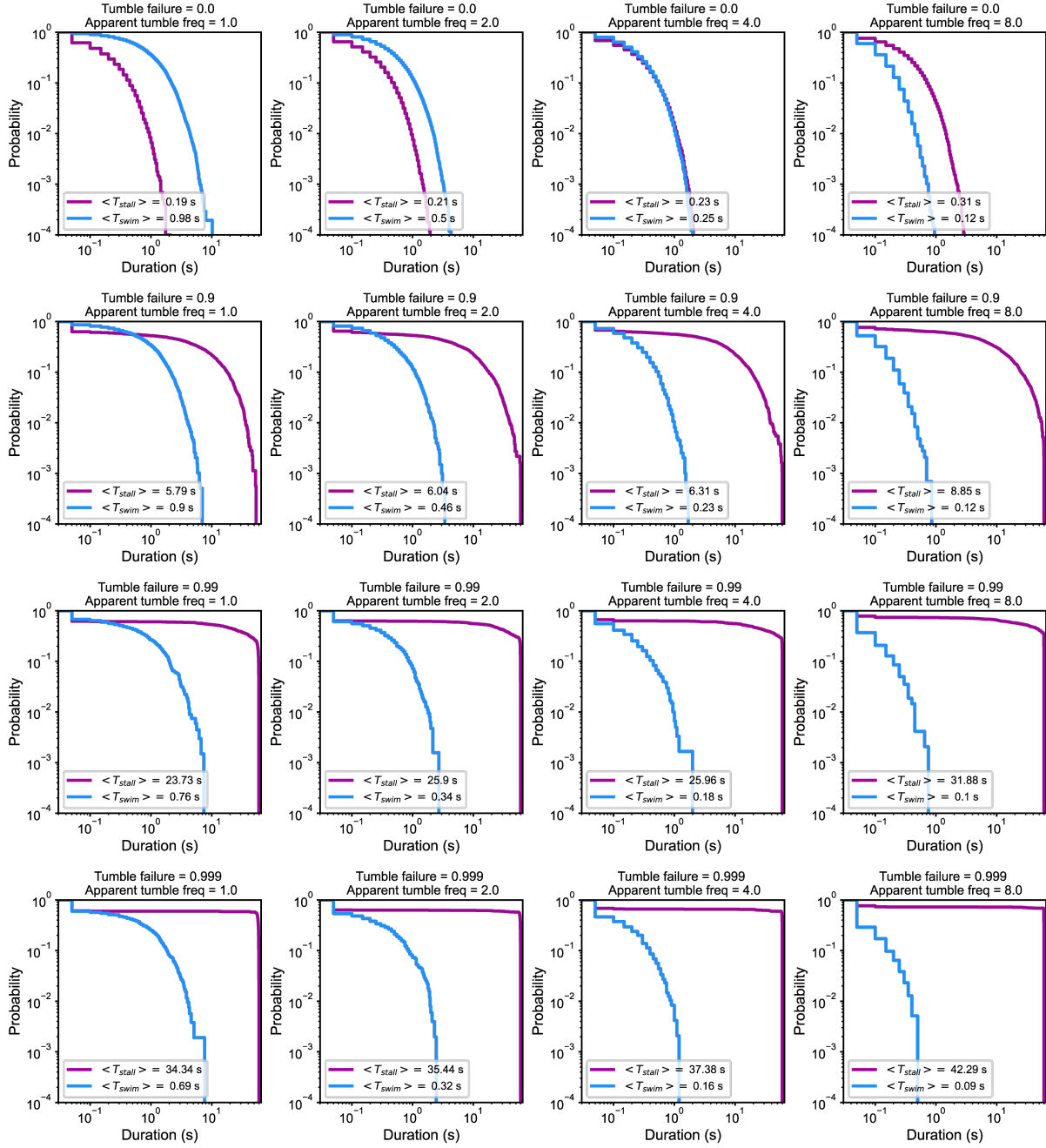


Figure S10: **Complementary cumulative distribution function (CCDF) plots of stall and swim durations under all simulated conditions.** In each subplot, CCDF for stall duration is labeled in magenta and CCDF for swim duration is labeled in blue. Each subplot has mean stall and swim durations indicated in the inset.

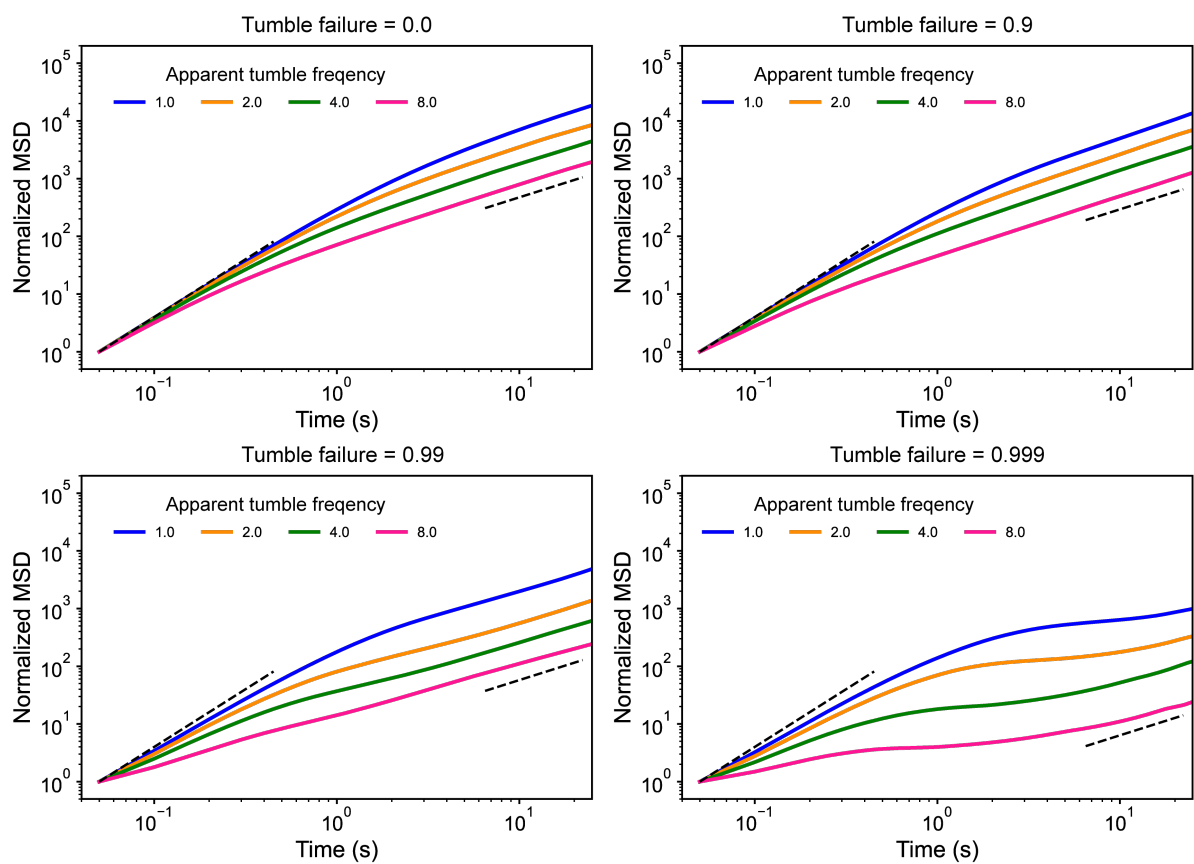


Figure S11: **Ensemble-average MSDs varying apparent tumble frequency.** Each subplot has a fixed tumble failure probability: 0, 0.9, 0.99, and 0.999, respectively.

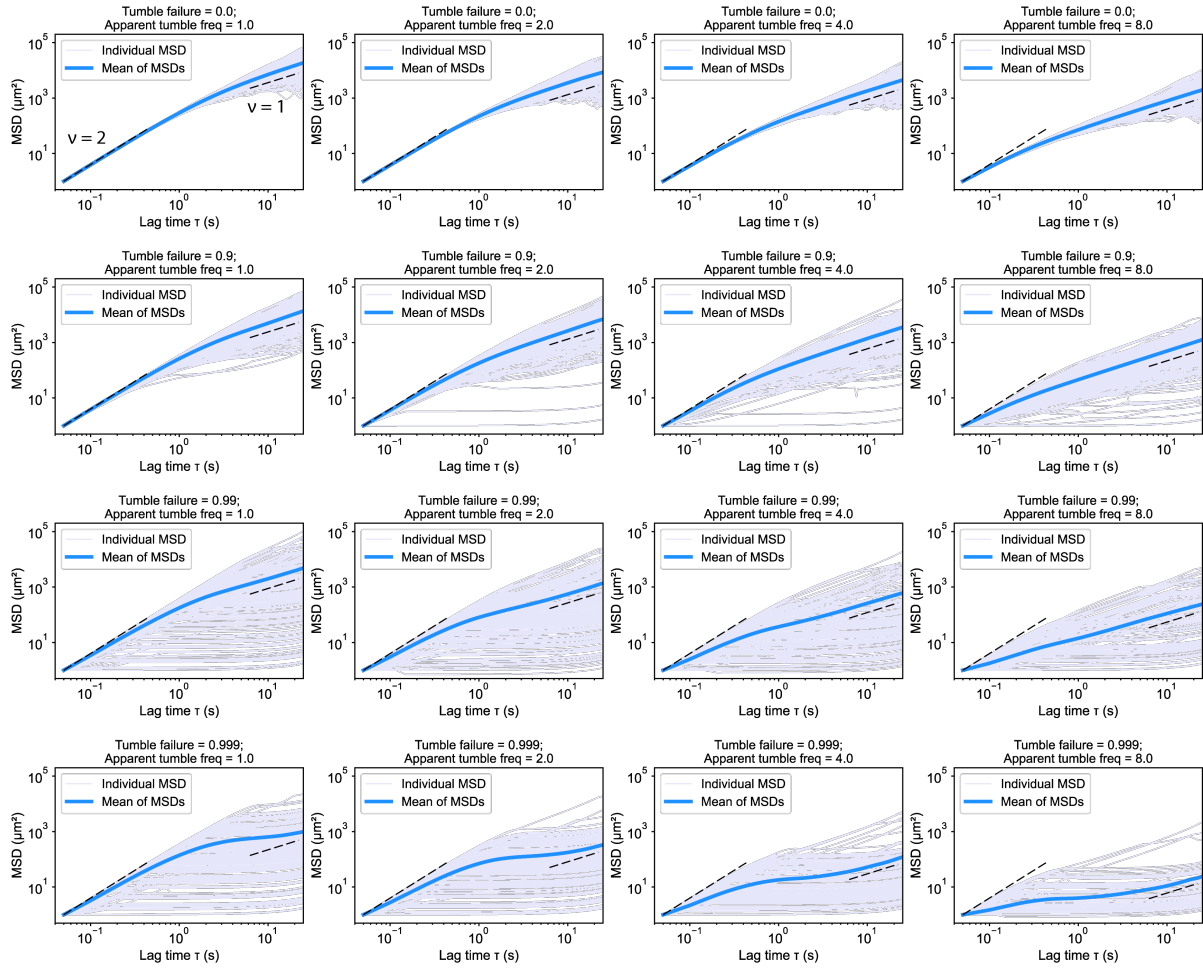


Figure S12: **Simulated MSD plots of all conditions.** Individual MSDs of all regions are labeled in purple. The ensemble-average MSD is labeled in blue in a subplot.

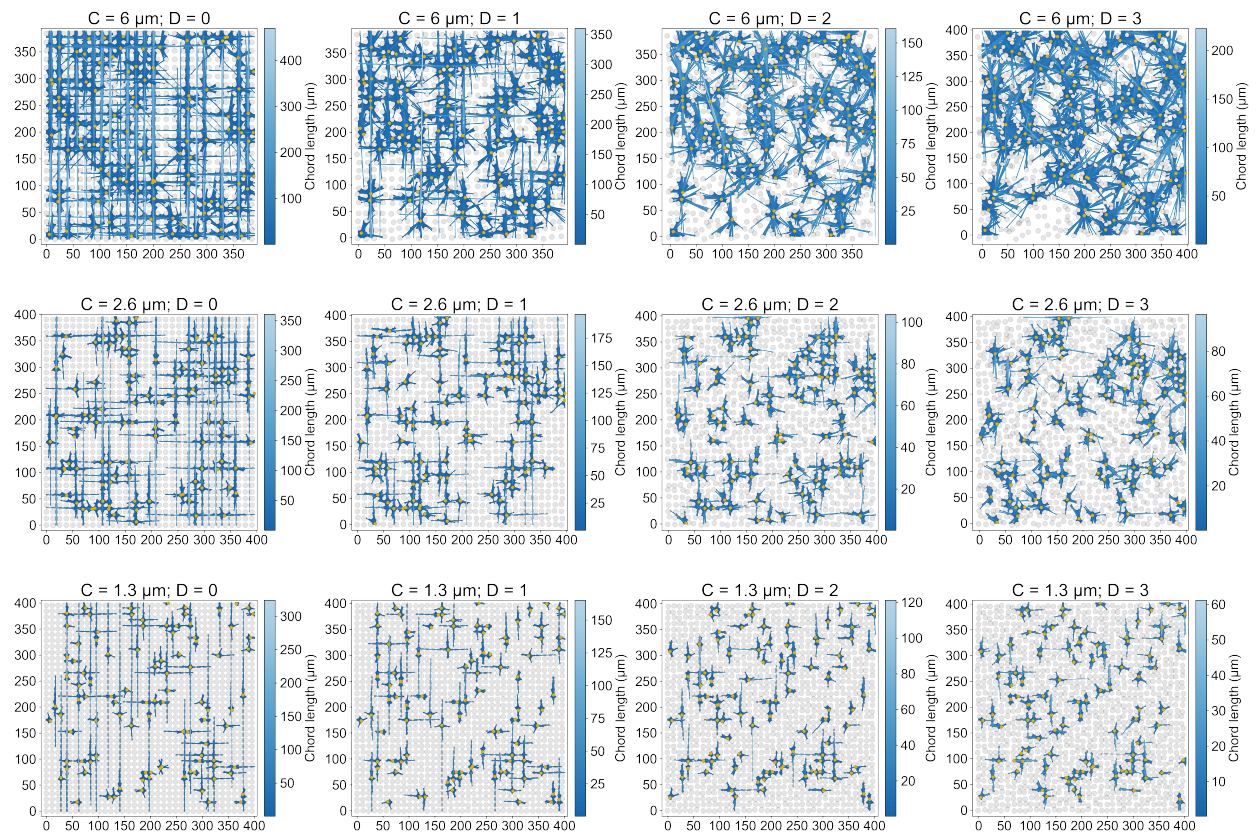


Figure S13: **Chord length simulations of all regions.** Every simulated region has one hundred origins indicated by yellow circles and each origin has one hundred chords. The color bars show the range of chord lengths.

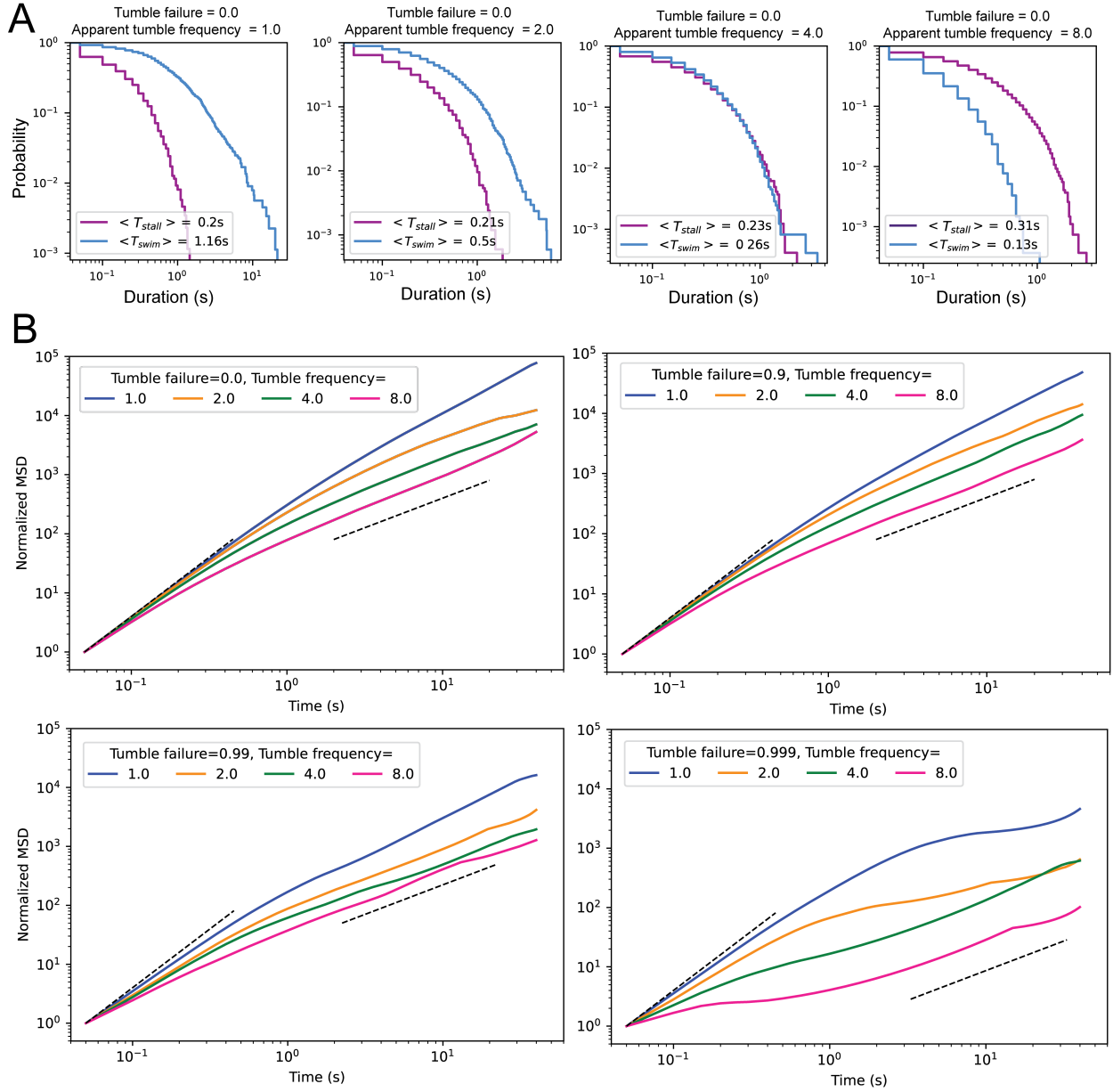


Figure S14: Robustness of the simulation to the deviation of swim durations from a stochastic process. (A) Complementary cumulative distribution function (CCDF) plots of stall and swim durations in simulations with time-correlated tumble initiation. Simulations incorporate temporal fluctuations in the tumble initiation rate modeled as an Ornstein-Uhlenbeck process ($t_{corr} = 20s$; $\sigma = 0.2$). Resulting swim duration distributions (blue) exhibit heavy tails relative to a pure exponential, while stall durations (purple) remain exponential. This behavior is consistent with rate variability affecting run termination but not tumble exit dynamics [11]. (B) Ensemble-average MSDs varying apparent tumble frequency in simulations with time-correlated tumble initiation. Each subplot has a fixed tumble failure probability: 0.0, 0.9, 0.99, and 0.999, respectively.

Supplementary Movies

Caption for Movie S1. Fluorescently labeled *E. coli* HCB 33 explore the microfluidic region $C = 6 \mu m$, $D = 0$ in real time. The movie frame rate is 50 frames per second. The pillars in the background are not visible as a result of the transparency of the PDMS-based microfluidics and fluorescence microscopy.

Caption for Movie S2. Fluorescently labeled *E. coli* HCB 33 explore the microfluidic region $C = 1.3 \mu m$, $D = 0$ in real time. The movie frame rate is 50 frames per second. The pillars in the background are not visible as a result of the transparency of the PDMS-based microfluidics and fluorescence microscopy.

Caption for Movie S3. Fluorescently labeled *E. coli* HCB 33 explore the microfluidic region $C = 1.3 \mu m$, $D = 3$ in real time. The movie frame rate is 50 frames per second. The pillars in the background are not visible as a result of the transparency of the PDMS-based microfluidics and fluorescence microscopy.