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Supplemental Information

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Table S1. List of primers used in this study.

Primers	**Sequence (5'-3',)**
1F	ACCATGCGGAAGCAGAAGATCCAGGCC
1R	ATCTCGAAGGACGCGCGTTCG
2F	GATCATCGACTGAGGGACATG
2R	CCGCATGGTGGCGTAGTCATCGACGAC
Inter_CheY7_FwdBamHI	TGTACAGGATCCCGTCGACGACTCCAAGACCA
Inter_CheY7_RevBamHI	TGTACAGGATCCGACCGTCCATGCCCGGCATG

DelY7ChkFwd	CTGGTCCTTCGCCTATTCGCATC
DelY7ChkRev	AGCAGAGCAGCGTGACCCAGAG
pKNOCK_SK	CGCTCTAGAACTAGTGGATC
CheY7KpnI_CompFwd	TAGTCGTGGTACCGTGGCCAAGACCATTCTGAC
CheY7SacI_ CompRev	TAGTCGTGAGCTCTTACGGGCAGACCTTCTTCAC
CheY6KpnI_CompFwd	TAGTCGTGGTACCATGAAGATCCTTGTCGTCGAT
CheY6SacI_CompFwd	TAGTCGTGAGCTCCCCGCCGATGACGGCCTGGAT

Table S2 Number of tracks of free-swimming strains before and after post-processing.

Strains	**Before post-processing**	**After post-processing**
Sp7	1360	1317
Δche4	880	776
ΔcheA1	1034	844
ΔcheA4	1103	1029
ΔcheA1ΔcheA4	374	361
ΔcheY1	1104	1098
ΔcheY4	1192	1029
ΔcheY6	840	708
ΔcheY7	1226	1065

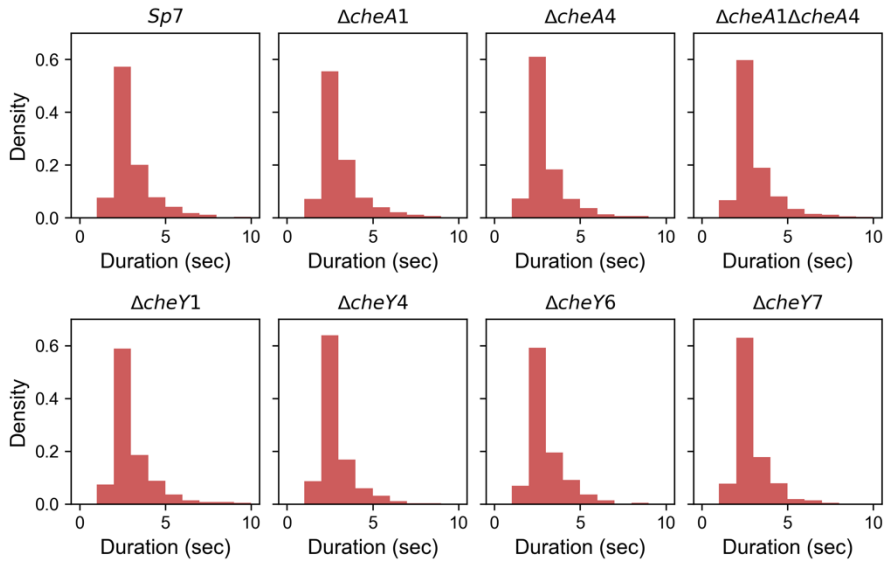

FIGURE S1 Distribution of duration for trajectories used in this study. No significant difference was observed among the strains in terms of the distributions of trajectory durations.

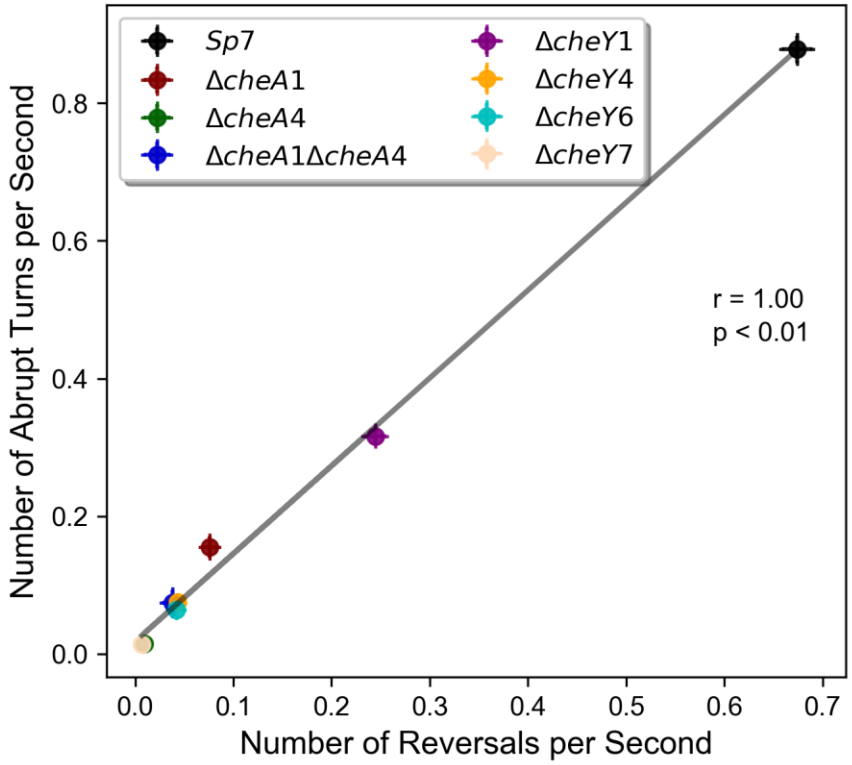

FIGURE S2 - Correlation of abrupt turns per sec vs reversals per sec for all the chemotaxis strains used in this study. A very strong correlation is seen with a Pearson correlation coefficient, r, of 1 (p-value < 0.01).

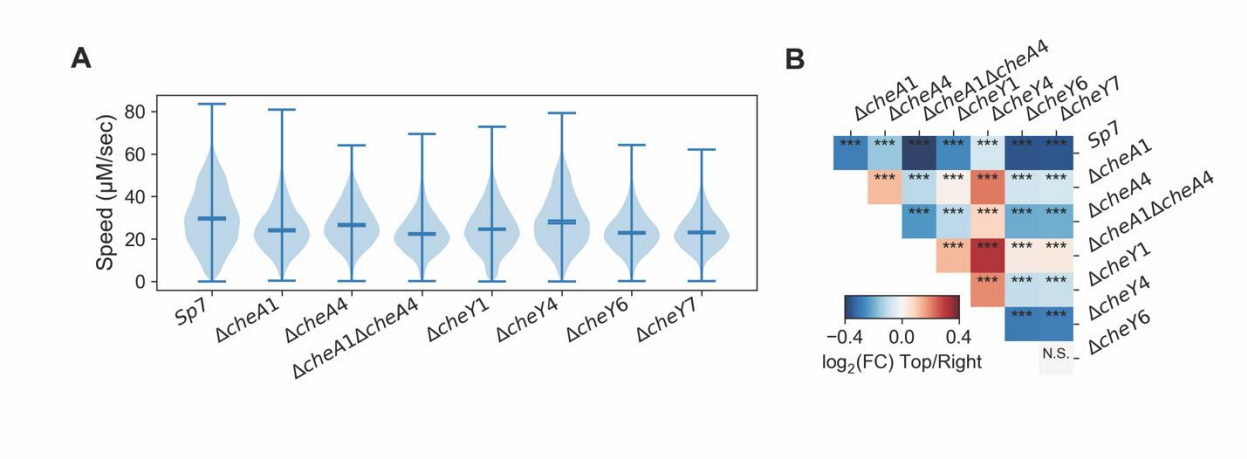

FIGURE S3 A) Distribution of speed of all strains used in this study. The distributions shown here are the instantaneous speeds for all frames of all trajectories in various strains. The top part of the bar is maximum instantaneous speed while the bottom bar is minimum instantaneous speed. The middle bar represents mean. B) Summary matrix of significance among different strains used in this study. Significance of the changes among strains were also tested using pairwise t-test. FC denotes fold change, *** denotes statistically significant differences at the $p < 0.001$ level, NS denotes no significance.

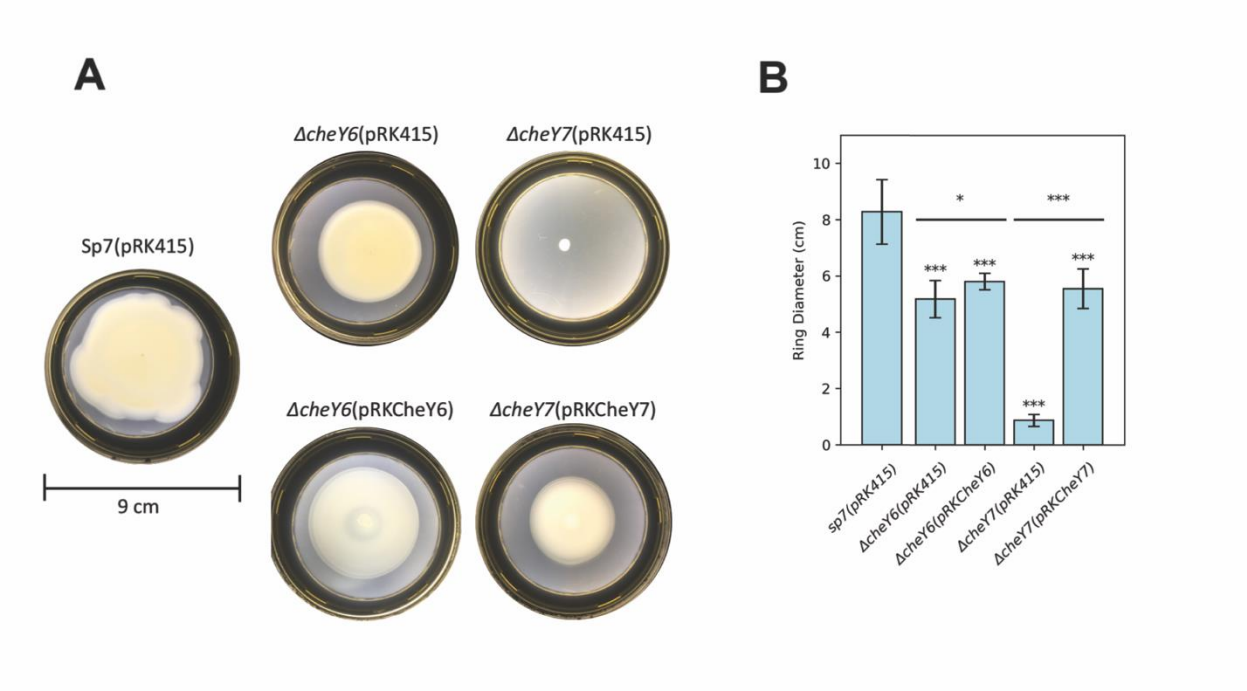

FIGURE S4 Complementation of different CheY homologs. A) Representative image of a swim plate assay done in triplicate. B) Bar graph showing ring diameter for various strains. Pairwise t test was used to test significant difference between strains. * denotes statistically significant differences at the $p < 0.05$ level, *** denotes statistically significant differences at the $p < 0.001$ level.

List of Supporting Movies

MOVIE S1: Video of a tethered Sp7 (Wild type) single cell.

MOVIE S2: Video of a tethered Δ*cheY4* single cell

MOVIE S3: Video of a tethered Δ*cheY7* single cell