Supplementary Materials

Molecular Biology of the Cell

Soh *et al*.

Supplemental figure 1. Cilia are intracellularly connected by BBs and BBs are positioned at variable angles along the cell cortex. (A) WT BB connections at 30°C. BB connections were quantified from FIB-SEM volume projections. Percentages indicate the observed frequency of BB connections. SF (n = 26). pcMTs (n = 25). tMTs (n = 27). (B) Long SFs of ciliated BBs (bottom BB) may interact with the pcMTs of both the unciliated BB (middle BB; SF not shown) and the anterior BB that is two BB units away (top BB). BB, red. SF, green. pcMT, white. Distances between SF and pcMTs at positions 1 and 2 are indicated in yellow. n = 3 BBs. Scale bar, 200 nm. (C) EM image depicting unciliated BB (top) and ciliated BB (bottom) positioning at the cell cortex. Cell cortex (green dashed line) and BB distal and proximal ends (red dashed lines) are marked to reflect BB positioning relative to the cell cortex. Scale bars, 200 nm. (D) *Left panel*: Schematic illustrates the analysis methods to quantify BB positioning along the cell cortex. Reference axes across the distal end of three consecutive BBs (method 1) and across peaks along the cell cortex (method 2) were used. *Right panel*: Quantification of BB angle using both methods. BBs are docked at variable angles along the cell cortex. n = 5 cells. Unciliated BBs: 10. Ciliated BBs: 13. (E) Correlation between cilium position along the power stroke axis and the BB position along the cell cortex quantified using analysis methods 1 and 2. Cilium position along the power stroke axis is defined by measuring the angle from 1.5 μm – 2.0 μm up the cilium relative to the reference axes.

Supplemental figure 2. Immobilization of live *Tetrahymena* cells using DIPULL microscopy. (A) Phagocytosis of iron particles by non-dividing and dividing cells. Scale bar, 10 μm. (B) Percentage of cells with iron uptake after exposure to different concentrations of iron particles. Quantitation was performed at 30 min post iron feeding. n = 150 – 250 cells. (C) Area (2-dimensional) of phagocytosed iron particles when cells were fed with varying concentrations of iron particles for 30 min. n = 30 – 50 cells. (D) Growth rate of *Tetrahymena* cells is not inhibited by the range of iron concentrations tested. (E and F) In the presence of the applied magnetic field used in DIPULL microscopy, *Tetrahymena* cells' swim trajectory is not affected but they swim slightly faster. Student's t-test. n = 90 cells. (G) Quantification of *Tetrahymena* cell width to determine microfluidic chamber width for long-term live cell imaging. n = 179 cells. Chamber width of 27 μm was determined to accommodate most cells. (H) DIPULL does not inhibit *Tetrahymena* ciliary beat frequency. Free-swimming n = 23 cells. Immobilized n = 47 cells. Mann-Whitney test. Mean±SD. (I) Quantification of WT and *disA-1* cilia length. n = 18 cells (3 cilia each). Student's t-test. Mean±SD. (J) Imaging setup

and microfluidic chamber design.

Supplemental figure 3. BB connections support ciliary waveform and coordination. (A) Schematic illustrates the power stroke trajectories of two adjacent cilia imaged from the top of the cell. BB orientation is determined based on the cilium's power stroke axis between adjacent cilia. If adjacent cilia are oriented, the power stroke angular difference is close to zero. Conversely, disoriented adjacent cilia would have large power stroke angular differences. Bar: 1 μm. (B) Quantification of ciliary beat frequency using cilia that were imaged from the top of the cell. *disA-1* ciliary beat frequency is comparable to WT cilia. WT: n = 11 cells (25 cilia). *disA-1*: n = 13 cells (32 cilia). Mann-Whitney test. Mean±SD. (C) Oriented and disoriented cilia display comparable ciliary beat frequencies. *disA-1*: n = 38 cells (72 oriented cilia; 41 disoriented cilia). Mann-Whitney test. Mean±SD. (D) Net curvature difference and statistical comparison between WT and *disA-1* cilia during the power stroke. *disA-1* cilia are more bent in the medial and proximal region between cilium position 60° - 75° (black dotted outline and pink outline). Absolute values of curvature difference are indicated (white: zero curvature difference; red: large curvature difference). Black indicates regions of the cilium where the curvature difference is statistically significant. Student's t-test. P value < 0.01. (E) Net curvature difference and statistical comparison between WT and *disA-1* cilia during the recovery stroke. *disA-1* cilia are slightly more bent at cilium positions 75°, and 120-135° (black dotted outline and pink outline). Absolute values of curvature difference are indicated (white: zero curvature difference; red: large curvature difference). Black indicates regions of the cilium where the curvature difference is statistically significant. Student's t-test. P value < 0.01. (F) Ciliary power stroke forces were estimated using resistive force theory. WT cilia exert more power stroke force per stroke than *disA-1* cilia (9 cilia, 6 ciliary beat cycles each, 9 cells). Data bin size is 15°. (G) Ciliary recovery stroke forces were estimated using resistive force theory. WT cilia exert comparable recovery stroke force per stroke relative to *disA-1* cilia (9 cilia, 6 ciliary beat cycles each, 9 cells). Data bin size is 15°. (H) Quantification of WT and *disA-1* phase differences. WT: n = 11 cells (13 cilia pairs). *disA-1*: n = 13 cells (19 cilia pairs). Mann-Whitney test; F-test for variance comparison. Mean±SD (I) Individual power stroke waveforms of WT and *disA-1* cilia. Representative examples of WT and *disA-1* power stroke waveforms showing three consecutive beat cycles. *disA-1* cilia display variable power stroke waveforms. Imaging frame intervals are indicated (msec). Scale bar, 2 μm. (J) Individual recovery stroke waveforms of WT and *disA-1* cilia. Representative examples of WT and *disA-1* recovery stroke waveforms showing three consecutive beat cycles. Imaging frame intervals are indicated (msec). Scale bar, 2 μm.

Figure S1

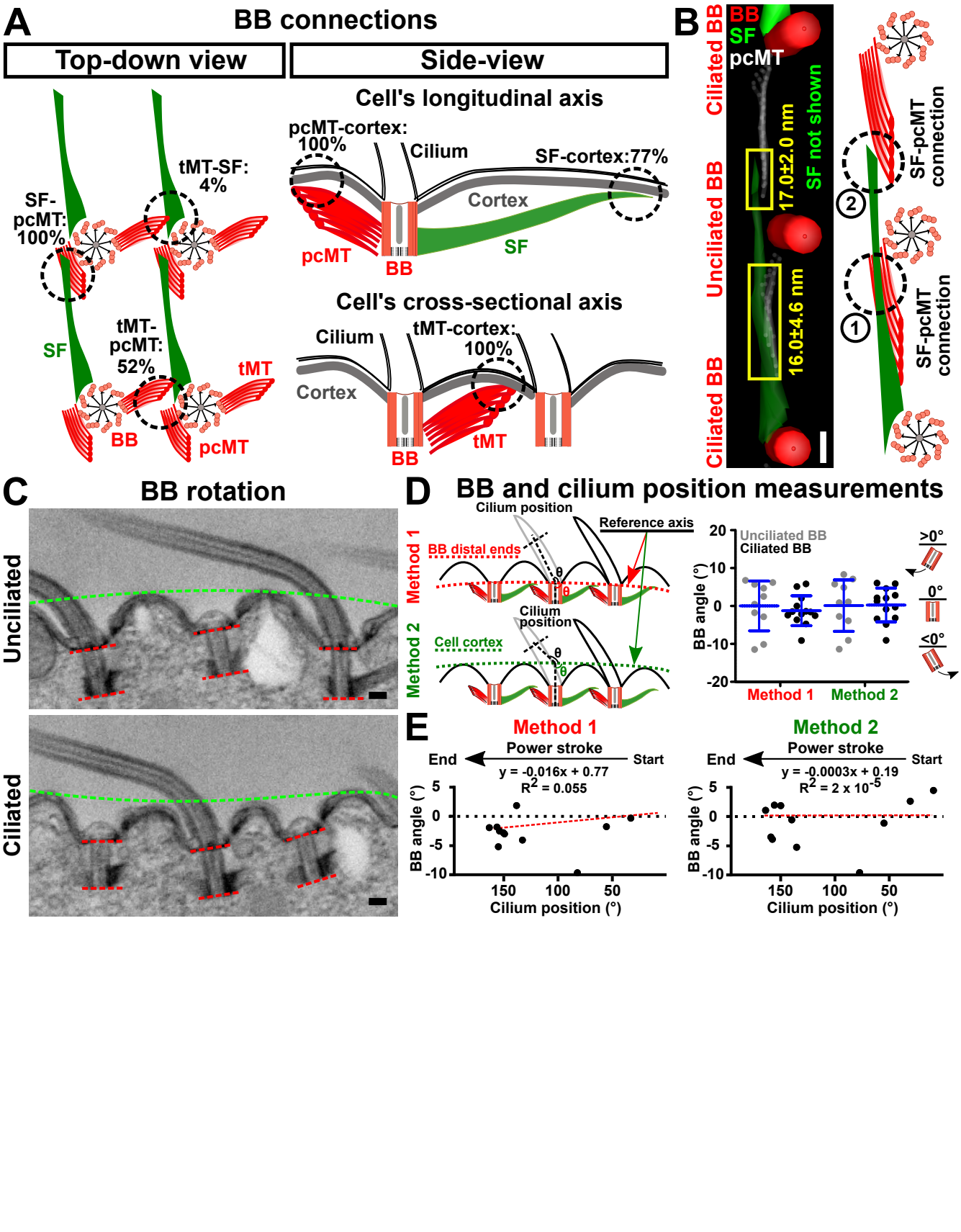

A
BB connections
Top-down view
Side-view
SF-pcMT: 100%
tMT-SF: 4%
SF
tMT-pcMT: 52%
tMT
BB
pcMT
Cell's longitudinal axis
pcMT-cortex: 100%
Cilium
SF-cortex:77%
Cortex
pcMT
BB
SF
Cell's cross-sectional axis
Cilium
tMT-cortex: 100%
Cortex
BB
tMT
B
Ciliated BB
Unciliated BB
Ciliated BB
BB
SF
pcMT
17.0±2.0 nm
16.0±4.6 nm
SF not shown
SF-pcMT connection
SF-pcMT connection
2
1
C
BB rotation
Unciliated
Ciliated
D
BB and cilium position measurements
Cilium position
Reference axis
Method 1
BB distal ends
θ
Cilium position
Method 2
Cell cortex
Unciliated BB
Ciliated BB
BB angle (°)
Method 1
Method 2
>0°
0°
<0°
E
Method 1
Power stroke
End
Start
y = -0.016x + 0.77
R2 = 0.055
BB angle (°)
Cilium position (°)
Method 2
Power stroke
End
Start
y = -0.0003x + 0.19
R2 = 2 x 10-5
BB angle (°)
Cilium position (°)

Figure S2

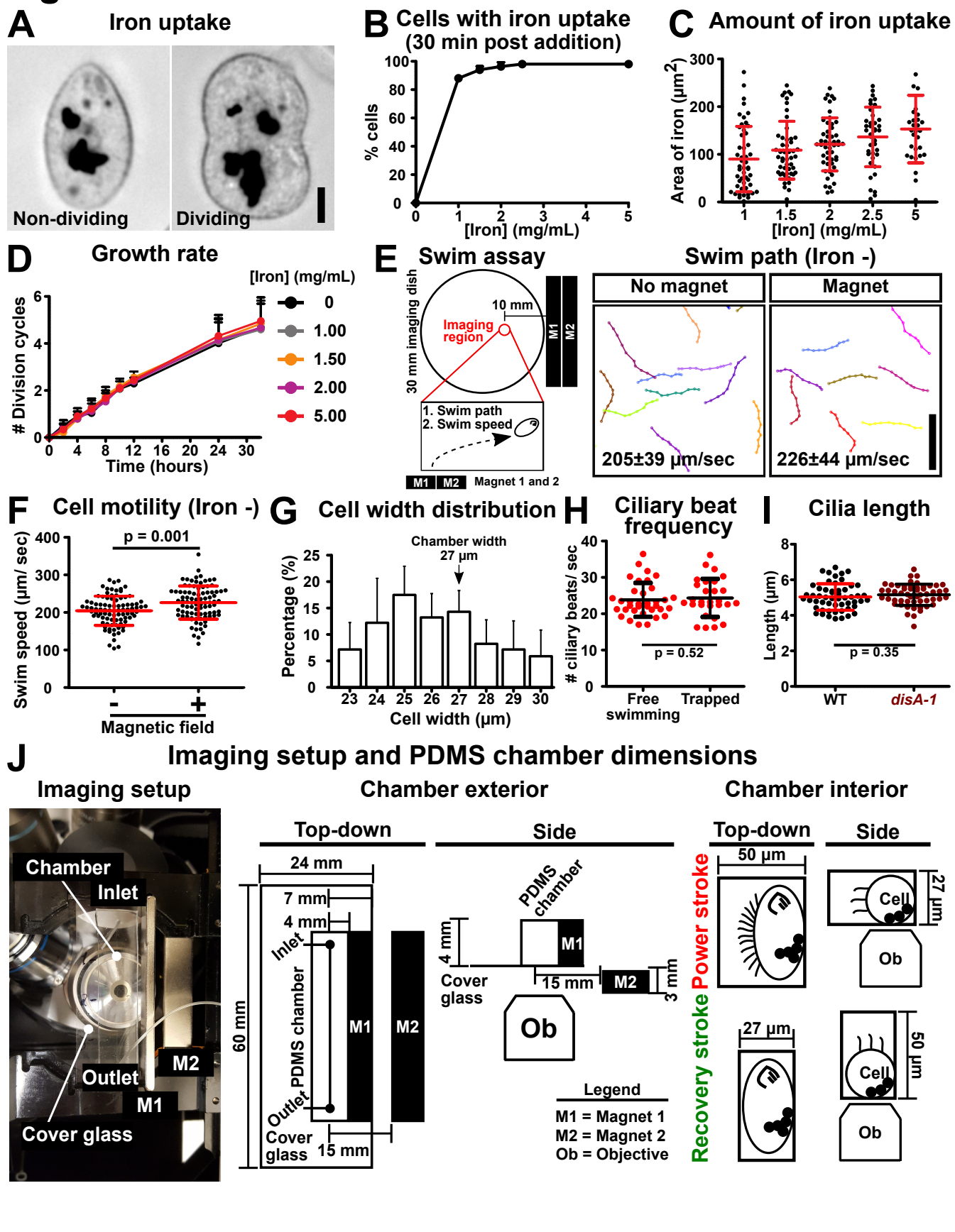
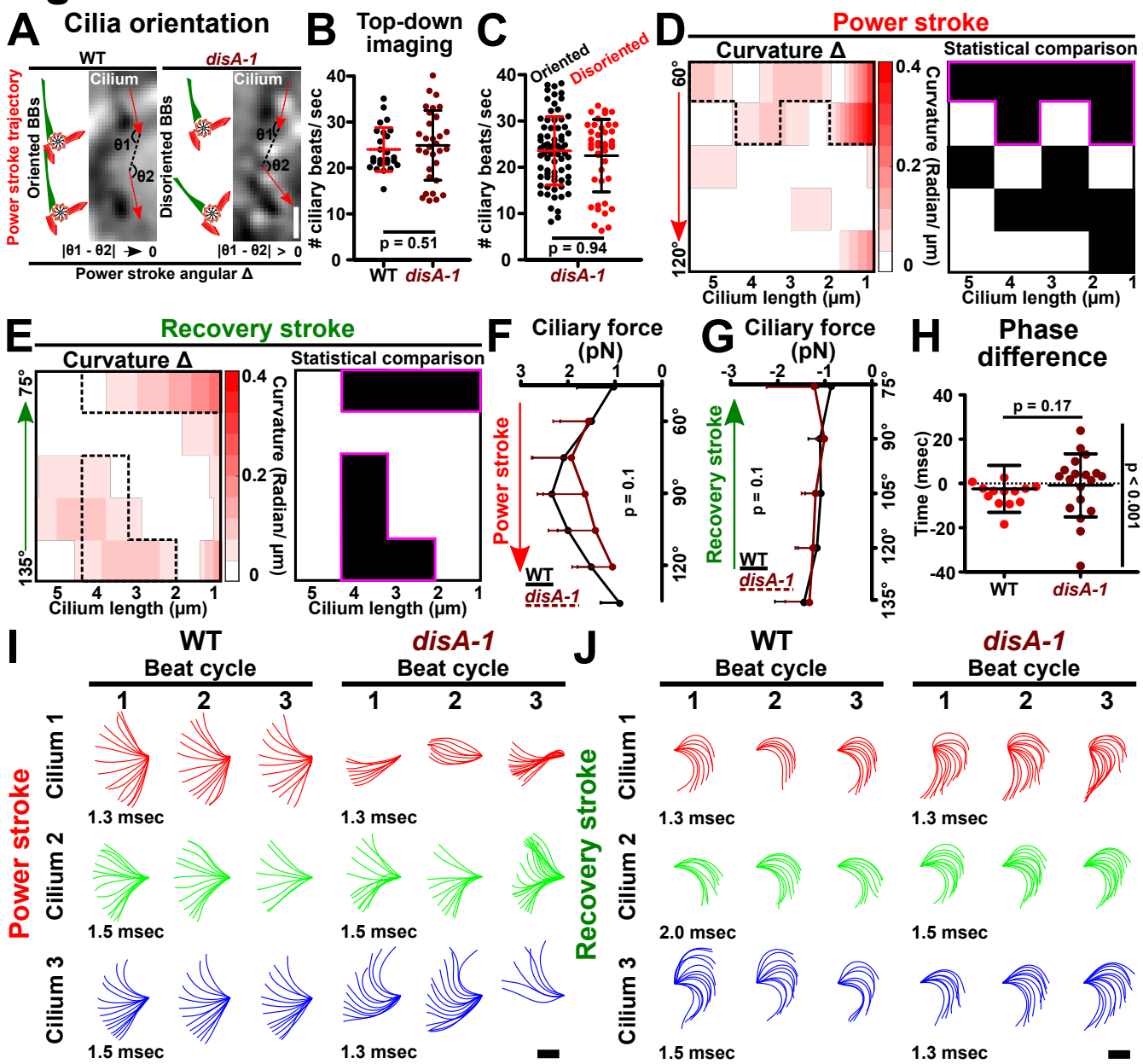

A
Iron uptake
Non-dividing
Dividing
B
Cells with iron uptake
(30 min post addition)
% cells
[Iron] (mg/mL)
C
Amount of iron uptake
Area of iron (μm²)
[Iron] (mg/mL)
D
Growth rate
[Iron] (mg/mL)
0
1.00
1.50
2.00
5.00
Division cycles
Time (hours)
E
Swim assay
30 mm imaging dish
10 mm
Imaging region
M1
M2
1. Swim path
2. Swim speed
Magnet 1 and 2
Swim path (Iron -)
No magnet
Magnet
205±39 μm/sec
226±44 μm/sec
F
Cell motility (Iron -)
p = 0.001
Swim speed (μm/ sec)
Magnetic field
G
Cell width distribution
Chamber width
27 μm
Percentage (%)
Cell width (μm)
H
Ciliary beat frequency
# ciliary beats/ sec
p = 0.52
Free swimming
Trapped
I
Cilia length
Length (μm)
p = 0.35
WT
disA-1
J
Imaging setup and PDMS chamber dimensions
Imaging setup
Chamber
Inlet
Outlet
M1
M2
Cover glass
Chamber exterior
Top-down
Side
24 mm
7 mm
4 mm
Inlet
PDMS chamber
Outlet
Cover glass
15 mm
60 mm
M1
M2
PDMS chamber
4 mm
Cover glass
15 mm
3 mm
Ob
Legend
M1 = Magnet 1
M2 = Magnet 2
Ob = Objective
Chamber interior
Top-down
Side
Power stroke
Recovery stroke
50 μm
27 μm
Cell
Ob
27 μm
50 μm
Cell
Ob

Figure S3


A
Cilia orientation
WT
disA-1
Cilium
Power stroke trajectory
Oriented BBs
Disoriented BBs
θ1
θ2
|θ1 - θ2| → 0
|θ1 - θ2| > 0
Power stroke angular Δ
B
Top-down imaging
# ciliary beats/ sec
p = 0.51
WT
disA-1
C
Oriented
Disoriented
# ciliary beats/ sec
p = 0.94
disA-1
D
Power stroke
Curvature Δ
Statistical comparison
Curvature (Radian/ μm)
Cilium length (μm)
E
Recovery stroke
Curvature Δ
Statistical comparison
Curvature (Radian/ μm)
Cilium length (μm)
F
Ciliary force (pN)
Power stroke
p = 0.1
WT
disA-1
G
Ciliary force (pN)
Recovery stroke
p = 0.1
WT
disA-1
H
Phase difference
p = 0.17
p < 0.001
Time (msec)
WT
disA-1
I
WT
disA-1
Beat cycle
Power stroke
Cilium 1
Cilium 2
Cilium 3
1.3 msec
1.5 msec
1.3 msec
1.5 msec
J
WT
disA-1
Beat cycle
Recovery stroke
Cilium 1
Cilium 2
Cilium 3
1.3 msec
2.0 msec
1.5 msec
1.5 msec
1.3 msec