Current Biology, Volume *32*

Supplemental Information

Determining protein polarization proteome-wide using physical dissection of individual *Stentor coeruleus* cells

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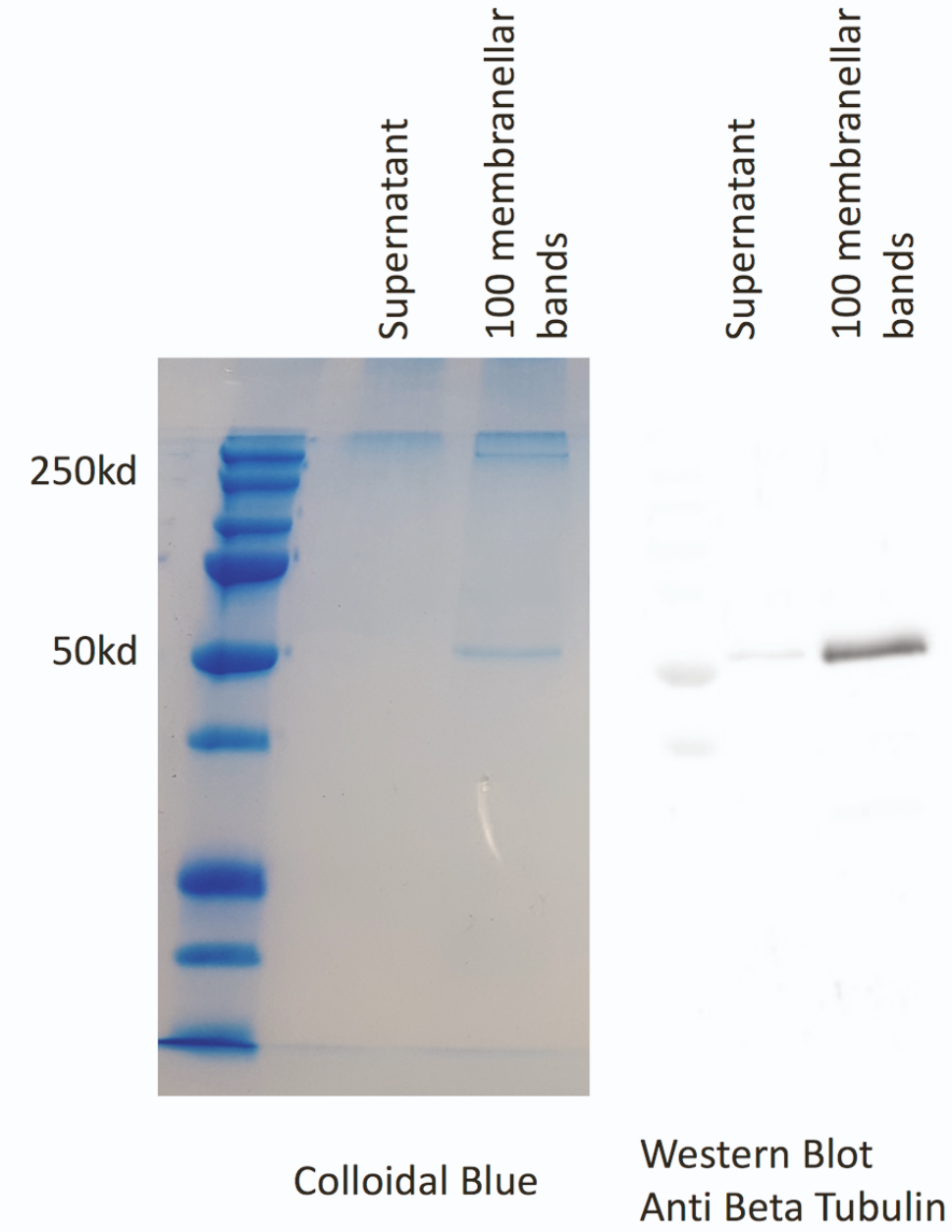

Supernatant
100 membranellar bands
Supernatant
100 membranellar bands
250kd
50kd
Colloidal Blue
Western Blot
Anti Beta Tubulin

Figure S1. Identifying most abundant proteins in MB fraction. Related to Figure 1.
isolated MB along with supernatant obtained during the MB collection. (B) Western blot for tubulin confirming the identity of the 55 kDa band. This figure relates to main text Figure 1 by providing additional validation of the proteomic analysis reported in that figure.

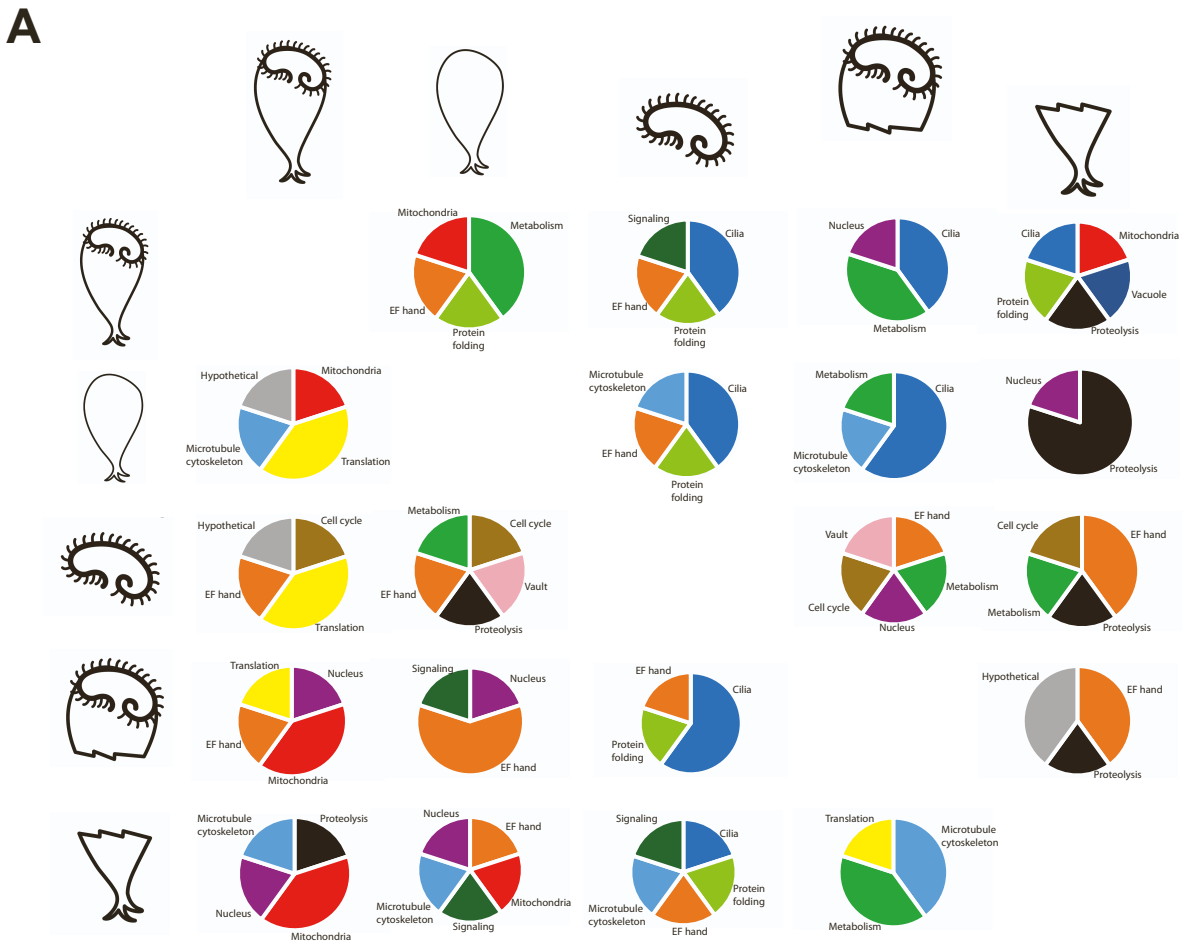
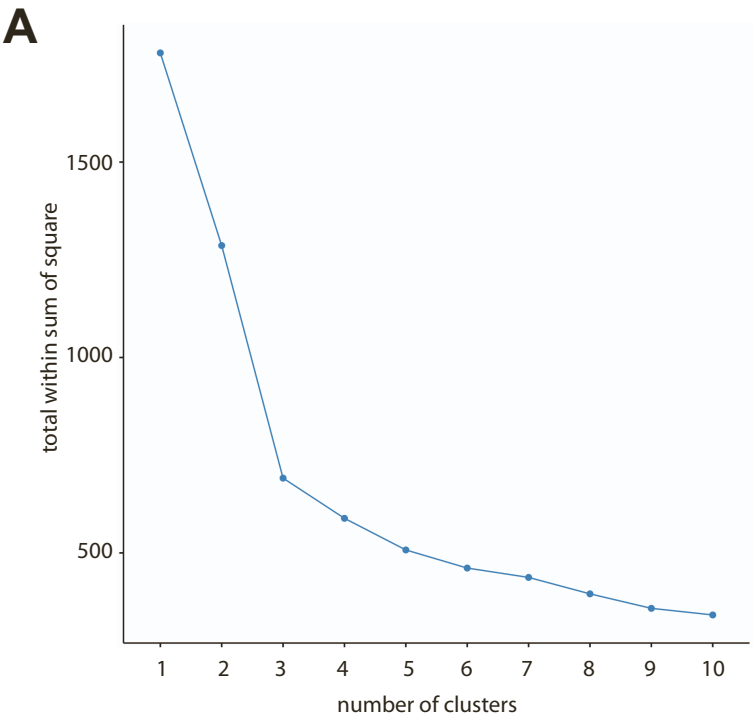
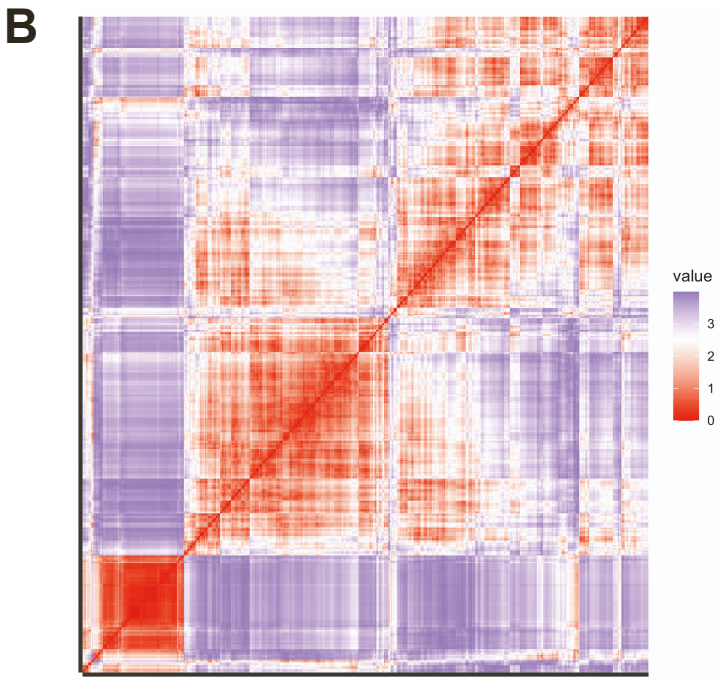

A
Mitochondria
Metabolism
EF hand
Protein folding
Signaling
Cilia
EF hand
Protein folding
Nucleus
Cilia
Metabolism
Cilia
Mitochondria
Protein folding
Vacuole
Proteolysis
Hypothetical
Mitochondria
Microtubule cytoskeleton
Translation
Microtubule cytoskeleton
Cilia
EF hand
Protein folding
Metabolism
Cilia
Microtubule cytoskeleton
Nucleus
Proteolysis
Hypothetical
Cell cycle
EF hand
Translation
Metabolism
Cell cycle
EF hand
Vault
Proteolysis
EF hand
Vault
Cell cycle
Metabolism
Nucleus
Cell cycle
EF hand
Metabolism
Proteolysis
Translation
Nucleus
EF hand
Mitochondria
Signaling
Nucleus
EF hand
EF hand
Cilia
Protein folding
Hypothetical
EF hand
Proteolysis
Microtubule cytoskeleton
Proteolysis
Nucleus
Mitochondria
Nucleus
EF hand
Microtubule cytoskeleton
Mitochondria
Signaling
Signaling
Cilia
Protein folding
Microtubule cytoskeleton
EF hand
Translation
Microtubule cytoskeleton
Metabolism

Figure S2. **Pairwise comparison between samples. Related to Figure 2.** Each pie chart represents the proteins most enriched in each *Stentor* sample compared to each other sample, regardless of total quantity. Color scheme is the same as in Figure 2C. This figure relates to main text Figure 2 by providing an alternative analysis of differences between cell fractions.


A
1500
1000
500
total within sum of square
1
2
3
4
5
6
7
8
9
10
number of clusters


B
value
3
2
1
0

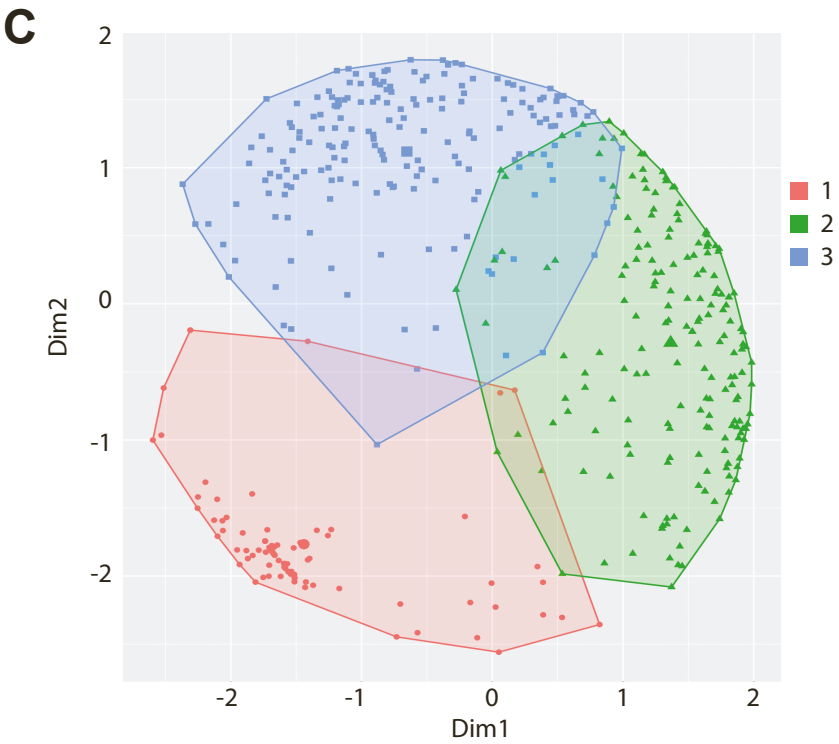

C
2
1
0
-1
-2
Dim2
-2
-1
0
1
2
Dim1
1
2
3

Figure S3. **Clustering of proteomic data. Related to Figure 4.** (A) Nbclust plot showing clear inflection clusters. (B) Distance map showing three blocks of correlation. (C) Cluster diagram from same assignment as used to produce the heatmap in Figure 4A, indicating that cluster 1 is well separated from clusters 2 and 3. This figure relates to Figure 4 by providing evidence to support the use of three clusters in generating the heatmap in Figure 4A.

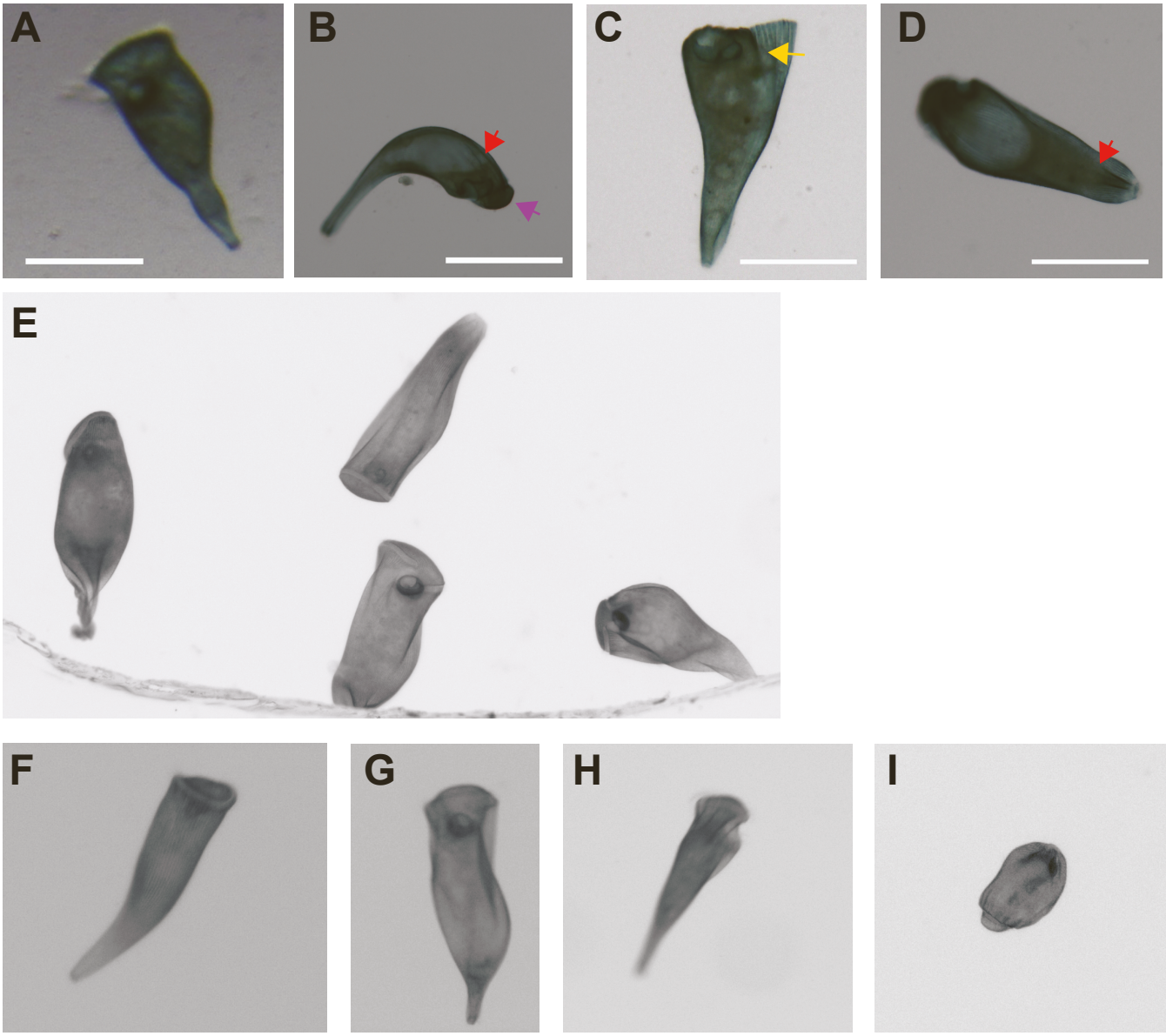

A
B
C
D
E
F
G
H
I
J
fraction of cells showing phenotype
1.0
0.75
0.5
0.25
0
Day 4
Day 5
Day 6
Day 7
time since initiation of RNAi
surface folds
MB deformation
extruded frontal field

Figure S4. Calyculin A treatment recapitulates phenotype of PP2a RNAi. Related to Figure 4(A) Untreated cell showing normal morphology. (B-D) Calyculin A treated cell showing surface folds (red arrow), protruding frontal field (purple arrow), and membranellar bands that fail to close into a ring (yellow arrow) comparable to phenotypes seen with PP2a RNAi in Figure 4. (E) cells treated phenotype. (F) cell at day 4 of treatment showing normal-looking morphology. (G) cell at day 4 showing surface folds. (H) cell at day 5 showing surface folds combined with deformed MB. (I) cell at day 6 showing surface folds and extruded frontal field. (J) Proportion of cells showing each phenotype as RNAi progresses. This figure relates to Figure 4 by providing additional information about the possible function of PP2a.