Supplemental Materials

Molecular Biology of the Cell

Tan *et al*.

Supplementary Figure S1. Intracellular diffusion increases with cell size in cell size mutant strains and at different temperatures

(**A**) Cell length and cytGEMs diffusion coefficients (mean ± SEM of cytGEMs trajectories per cell) plotted for individual cells for *wee1-50*, (**B**) wildtype, and (**C**) *cdc25-22* cells grown with the temperature shift protocol described in Figure 1A. Weighted linear regression (orange solid line) with 95% confidence interval (orange dashed lines) are shown (see Methods). Best-fit slopes are 0.007, 0.003, and 0.001 and N_{CELLS} = 160, 309, and 100 for (**A**), (**B**), and (**C**), respectively. (**D**) Cell length (mean ± STD of replicate experiments; $N_{CELLS} \geq 121$ per condition from at least 3 biological replicates) and (**E**) cytGEMs diffusion coefficients (mean ± STD of replicate experiments; $N_{GEMS} \geq 5135$ per condition from at least 3 biological replicates) for *wee1-50*, wildtype, and *cdc25-22* cells grown at the steady-state semi-permissive temperature 28°C. (**F**) Cell length (mean ± STD of replicate experiments; $N_{CELLS} \geq 200$ per condition from at least 3 biological replicates) and (**G**) cytGEMs diffusion coefficients (mean ± STD of replicate experiments; $N_{GEMS} \geq 5981$ per condition from at least 3 biological replicates) for *wee1-50*, wildtype, and *cdc25-22* cells grown at the steady-state permissive temperature 25°C. (**H**) CytGEMs diffusion coefficients (mean ± STD of replicate experiments) in wildtype and *cdc2-asM17* cells with varying conditions of 0.25% DMSO and 10μM 1-NM-PP1. (1-way ANOVA, * - $p < 0.05$, *** - $p < 0.001$, **** - $p < 0.0001$).

Supplementary Figure S2. Diffusion coefficients are comparable between large multinucleate cells and wildtype cells

(**A**) CytGEMs diffusion coefficients (mean ± STD of replicate experiments) in wildtype and *sid2-as* cells with varying conditions of 0.25% DMSO and 10μM 1-NM-PP1 (1-way ANOVA, $p > 0.05$). (**B**) CytGEMs diffusion coefficients (mean ± STD of replicate experiments; $N_{GEMS} \geq 7630$ per condition from 3 biological replicates) for wildtype and *cdc11-119* cells grown at permissive temperature 25°C overnight and shifted to the non-permissive temperature 36°C for 3 hr before imaging (Komogorv-Smirnov test, $p = 0.6$). **(C)** CytGEMs diffusion coefficients (mean ± STD of replicate experiments; $N_{GEMS} \geq 9782$ per condition from 3 biological replicates) for wildtype and *cdc11-119* cells grown at the steady-state permissive temperature 25°C (Komogorv-Smirnov test, $p = 0.6$).

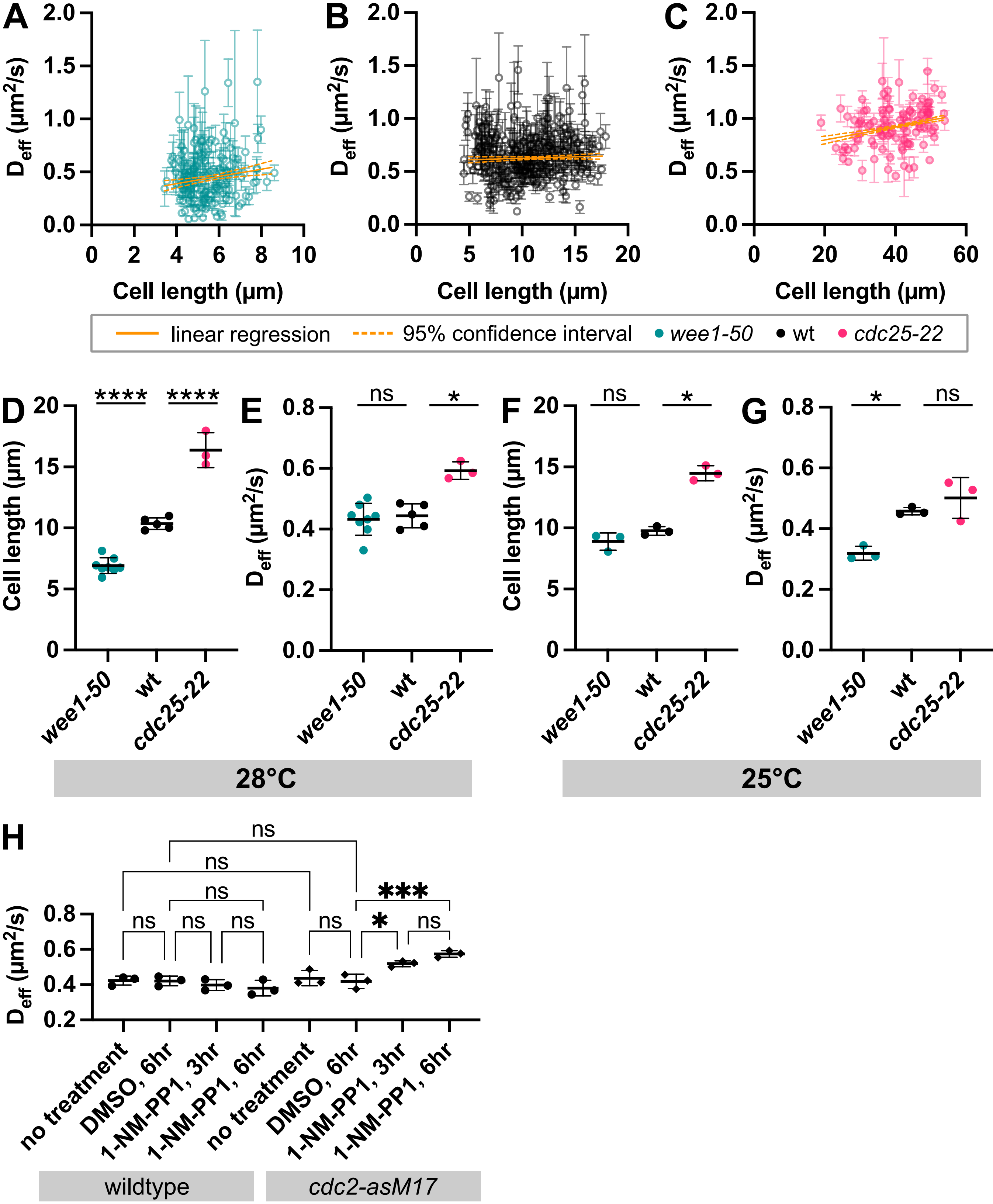
Supplementary Figure S3. Cell size scaling of cellular processes

(**A**) Effect of cell size on scaling of proteins implicated in ribosomal regulation, TORC1 pathways, stress pathway and trehalose regulation. Relative concentration of proteins in *S. pombe cdc25-*

22 and *wee1-50* strains grown with 6.5 hr shift to 36°C as described in Figures 1 and 3. Ratios depict ratios of *cdc25-22*/*wee1-50* proteomes. (**B**) Gene ontology analysis of sub- (red) and super-scaling (blue) proteins in comparison of *cdc25-22*/*wee1-50* proteomes as above.

Supplementary Figure S4. Cell size dependent proteome changes in additional data sets. Similar size-dependent effects on the proteomes were seen in comparisons of fission yeast cells grown in other conditions. (**A**) *cdc25-22* and wildtype cells were grown at the permissive temperature 25°C and shifted to 36°C for 6.5 hr before sample collection. Plot shows the average relative protein concentration ratios (*cdc25-22*/wildtype) of proteins were grouped by subcellular localization in two replicates. (**B**) *cdc25-22* and *wee1-50* cells were grown at semi-permissive temperature of 28°C at steady state. Average relative concentration ratios (*cdc25-22*/*wee1-50*) proteins were grouped by subcellular localization in two replicates.

Data sheet 1. Data of cell size dependent proteome changes. Data sheets present the proteomic data described in Fig. 4 and S4. "Rep" columns refer the protein concentration ratio for each protein in each of the two replicate experiments. "Rep peptide" columns refers to the number of peptides analyzed for each protein in each of two replicate experiments.


A
B
C
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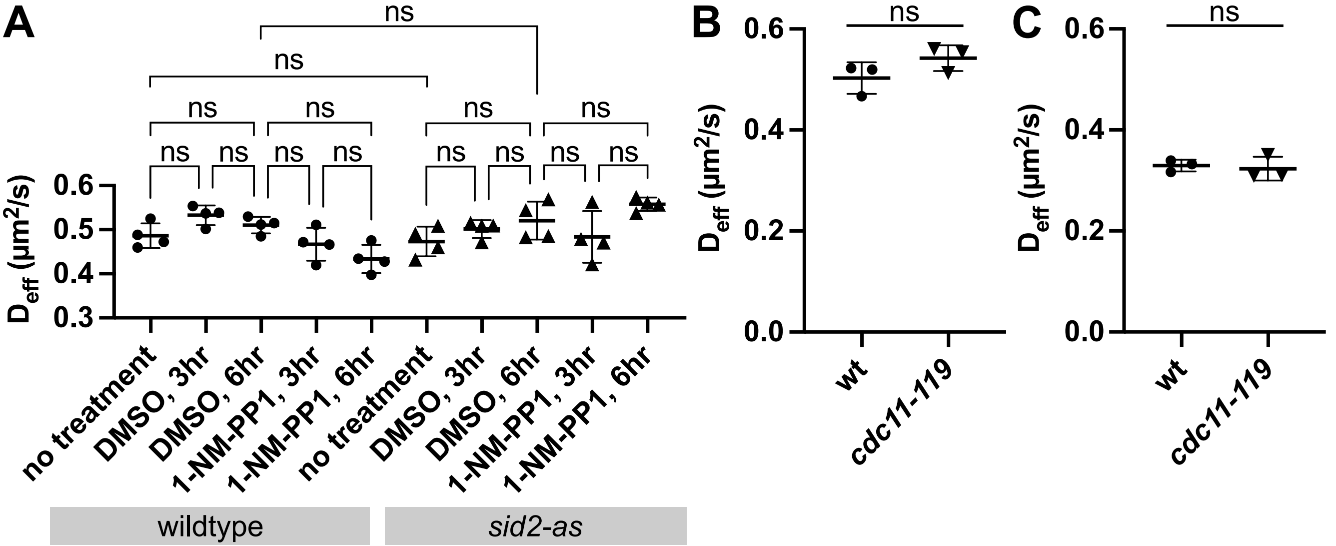
1.5
1.0
0.5
0.0
D_eff (μm²/s)
0 2 4 6 8 10
0 5 10 15 20
0 20 40 60
Cell length (μm)
linear regression
95% confidence interval
wee1-50
wt
cdc25-22
D
E
F
G
20
15
10
5
0
Cell length (μm)
0.8
0.6
0.4
0.2
0.0
****
****
ns
*
wee1-50
wt
cdc25-22
28°C
25°C
H
ns
***
no treatment
DMSO, 6hr
1-NM-PP1, 3hr
1-NM-PP1, 6hr
wildtype
cdc2-asM17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B
C
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D_{eff} (µm^2/s)
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0.5
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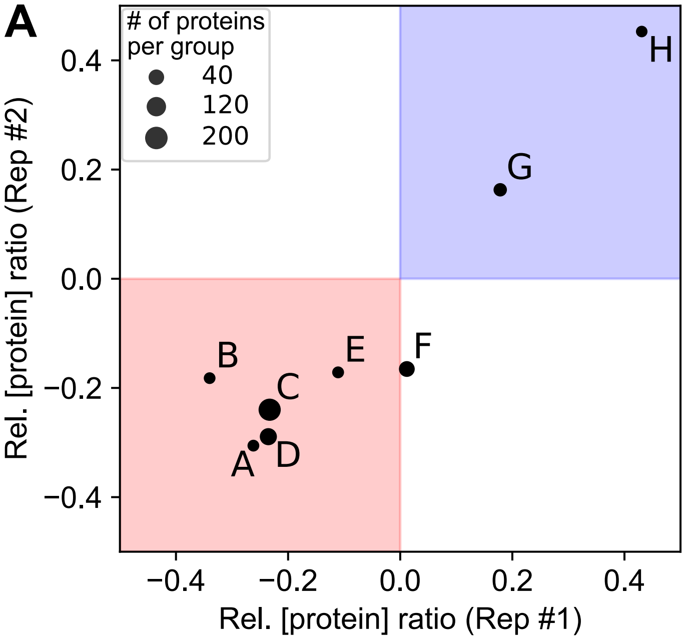
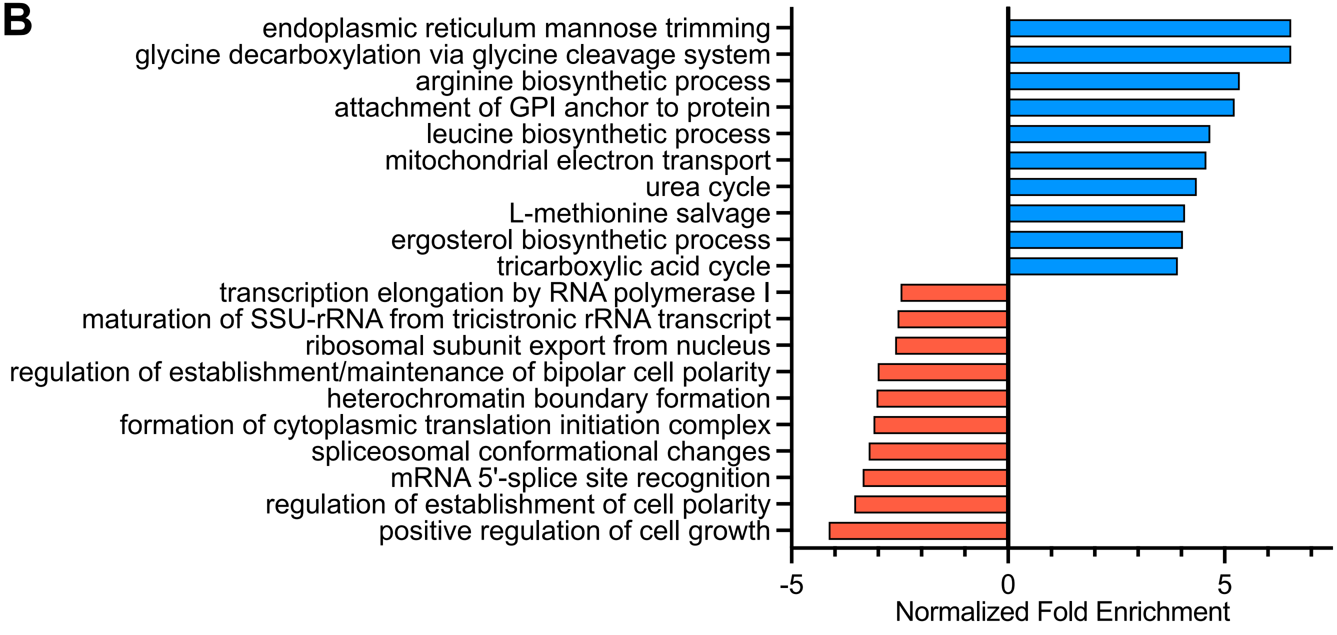
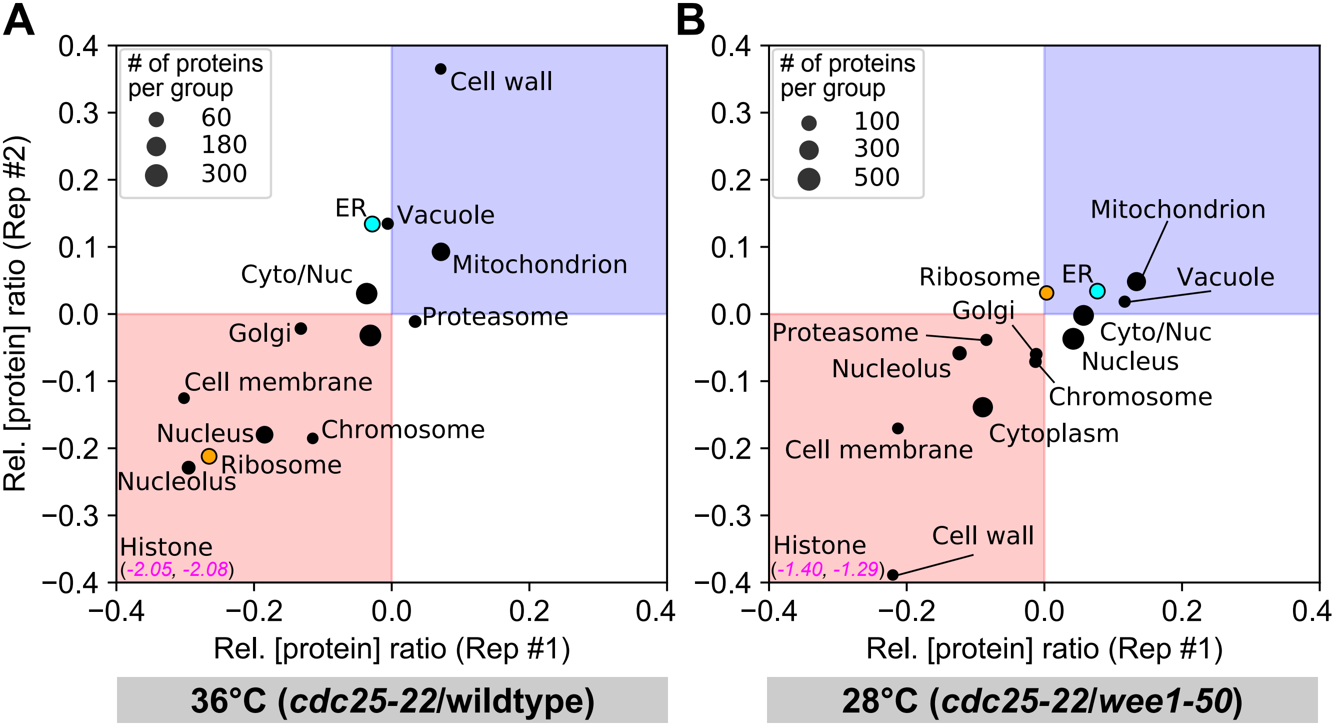
1-NM-PP1, 6hr
wildtype
sid2-as
wt
cdc11-119



	Annotation
A	TORC1 complex
B	TORC2 complex
C	Ribosome biogenesis
D	Downregulated genes in *sfp1*∆ (Tai et al.)
E	Trehalose biosynthetic process
F	Upregulated CESR genes (Chen et al.)
G	Upregulated genes in *tor2-ts6* in (Wei et al.)
H	Trehalose catabolic process





36°C (*cdc25-22*/wildtype)

28°C (*cdc25-22*/*wee1-50*)