Supplemental Materials

Molecular Biology of the Cell

Okada et al.

Figure S1: PC1 explained the majority of the variability of the morphological changes induced by the drug treatments. Morphological data on cells treated with various drug concentrations were subjected to the first PCA, and PCs were extracted. Gray bars and red dots indicate the proportion of variance (left axis) and its cumulative value (right axis), respectively.

Figure S2: Independent cell morphology parameters induced by cell wall-affecting drugs. Independent parameters significantly correlated to PC1 in the first PCA (Table S1a–c) were extracted in the second PCA using morphological data from 122 replicated wild-type cells as a null distribution. (A) Proportion of variance in the second PCA. Solid and gray bars indicate the proportion of variance from the five replicated data on drug treated cells and from the 122 replicated data on wild-type cells, respectively. Red dots denote the cumulative proportion of variance for the drug treatment. Red dashed lines denote 0.6 of the cumulative proportion. (B) Dose–response of the independent features determined by the second PCA. PCs plotted for each drug treatment explained 60% of the variance in PC scores. Black dots and lines denote the PCs significantly correlated with at least one morphological parameter (Table S2a–c). Gray dots and lines indicate PCs not correlated with any morphological parameter.

Figure S3: Proportion of variance of PCs from the PCA of similar/dissimilar parameters. Color use is as in Figure S1.

Figure S4: Similar/dissimilar effects of drug treatment. Legend is as for Figure 5C.

Figure S5: Proportion of variance of the PCs from the PCA of *fks1* mutants. Color use is as in Figure S1.

Figure S6: Spa2–GFP localization among *fks1* mutants. (A) Cells were incubated with SD–U medium at 25ºC until the early log-phase followed by an additional incubation at 37ºC for 2 h. Cells were then harvested and observed without fixation. Bar = 5 μm. (B) In the specimens treated at 37ºC, Spa2–GFP localized cells were quantified from three independent experiments ($n > 300$), and the mean of the triplicates was plotted. Error bars indicate 1 S.D. *Significant difference ($p < 0.05$ by *t*-test after Bonferroni correction). *FKS1*, *fks1-1154*, and *fks1-1163* indicate YOC5041–5043, respectively.

Figure S7: Lethality of class II and III *fks1* mutations. Cells containing a plasmid (pYO916) harboring galactose-inducible or glucose-suppressible *FKS1* genes were cultured in liquid SGS–UT medium until the log phase and diluted serially to 1×10^6, 1×10^5, 1×10^4, 1×10^3, and 1×10^2 cells/ml. Suspensions (10 μl) were dropped onto SGS–UT or SD–UT plates and incubated at 25ºC for 3 days. Experiments were replicated at least twice, and representative results are shown. *FKS1*, *fks1-1154*, *fks1-1163*, *fks1-11631154*, and *fks1*Δ indicate YOC5003–5007, respectively.

Figure S1

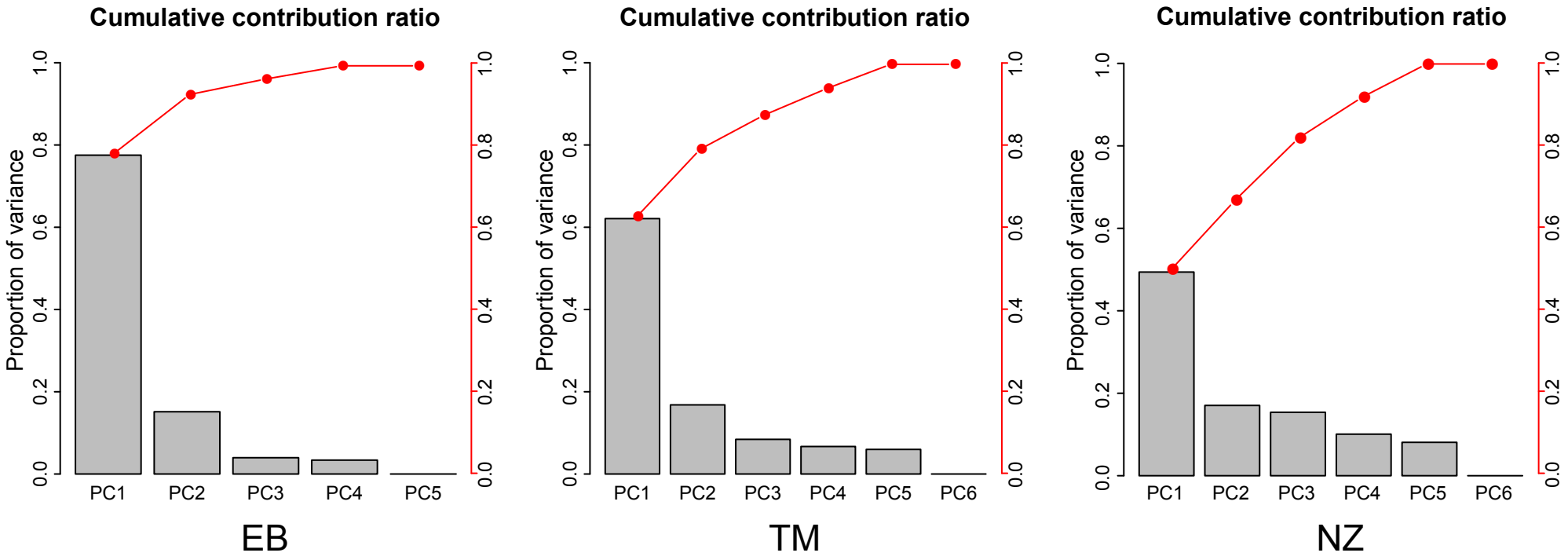

Cumulative contribution ratio
Proportion of variance
0.0
0.2
0.4
0.6
0.8
1.0
PC1
PC2
PC3
PC4
PC5
EB
Cumulative contribution ratio
Proportion of variance
PC1
PC2
PC3
PC4
PC5
PC6
TM
Cumulative contribution ratio
Proportion of variance
PC1
PC2
PC3
PC4
PC5
PC6
NZ

Figure S2

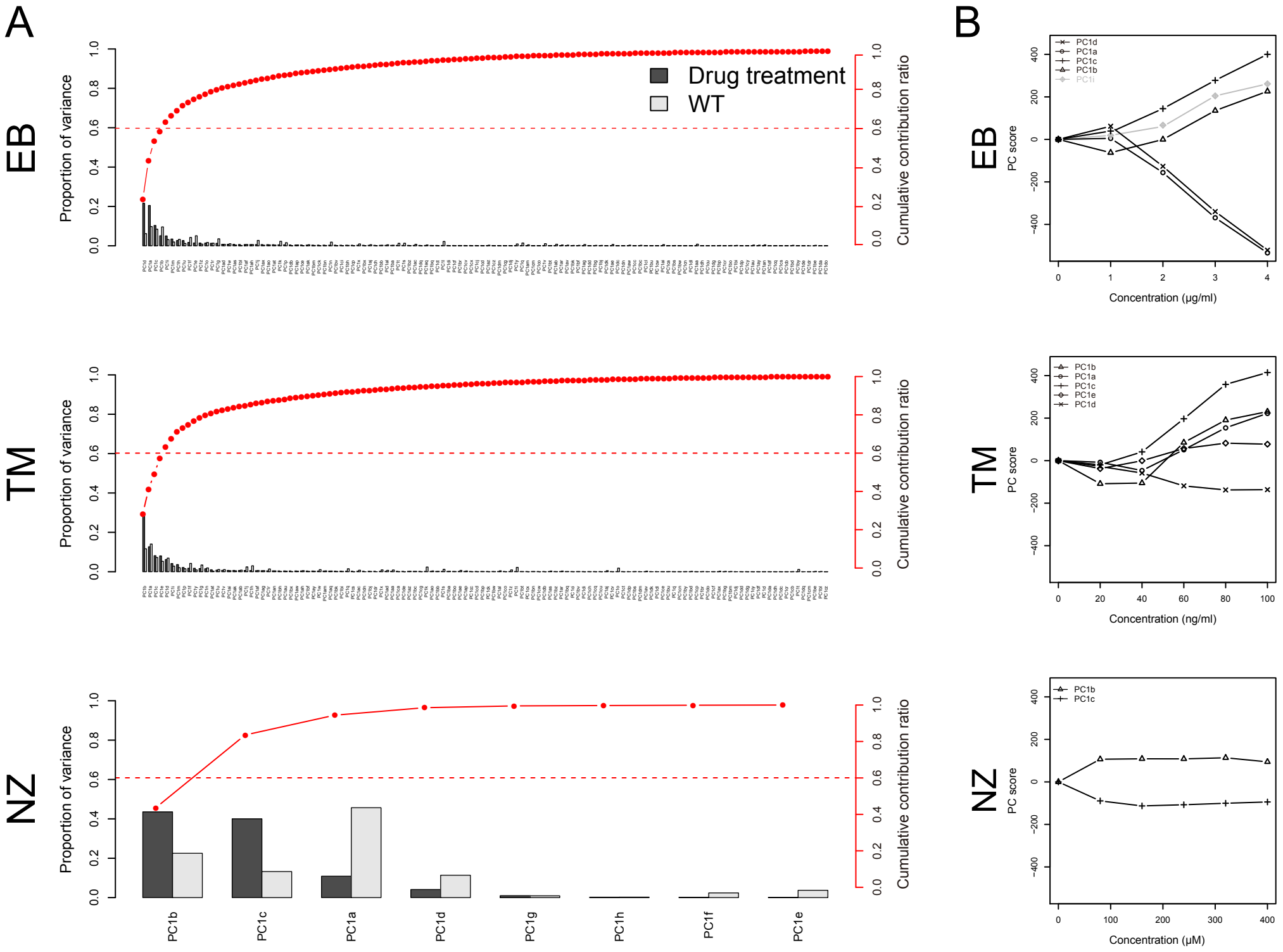

A
EB
TM
NZ
Proportion of variance
Cumulative contribution ratio
Drug treatment
WT
PC1b
PC1c
PC1a
PC1d
PC1g
PC1h
PC1f
PC1e
B
EB
TM
NZ
PC score
PC1d
PC1a
PC1c
PC1b
PC1i
PC1e
Concentration (μg/ml)
Concentration (ng/ml)
Concentration (μM)

Figure S3

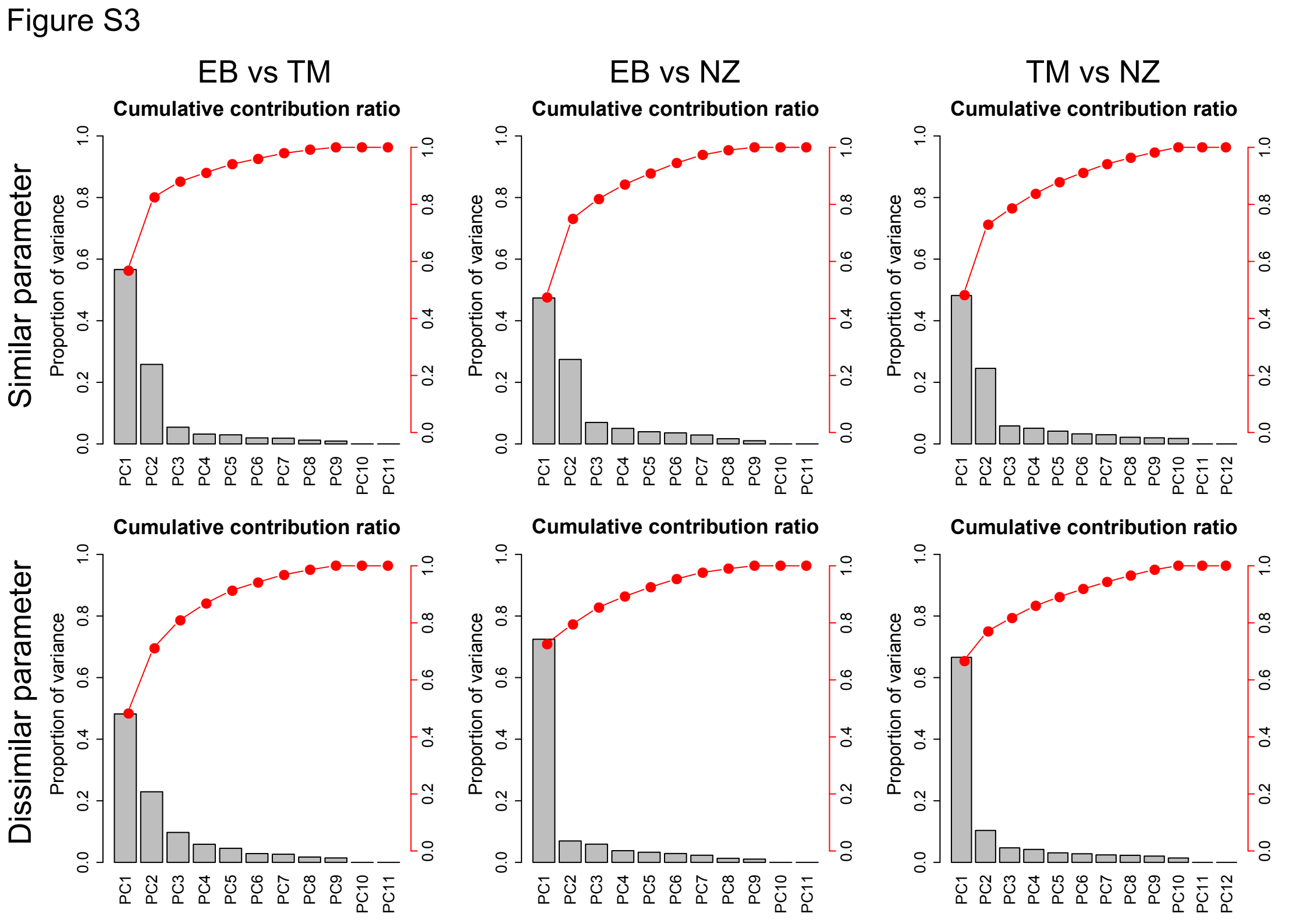

EB vs TM
EB vs NZ
TM vs NZ
Similar parameter
Dissimilar parameter
Cumulative contribution ratio
Proportion of variance
PC1
PC2
PC3
PC4
PC5
PC6
PC7
PC8
PC9
PC10
PC11
PC12
0.0
0.2
0.4
0.6
0.8
1.0

Figure S4

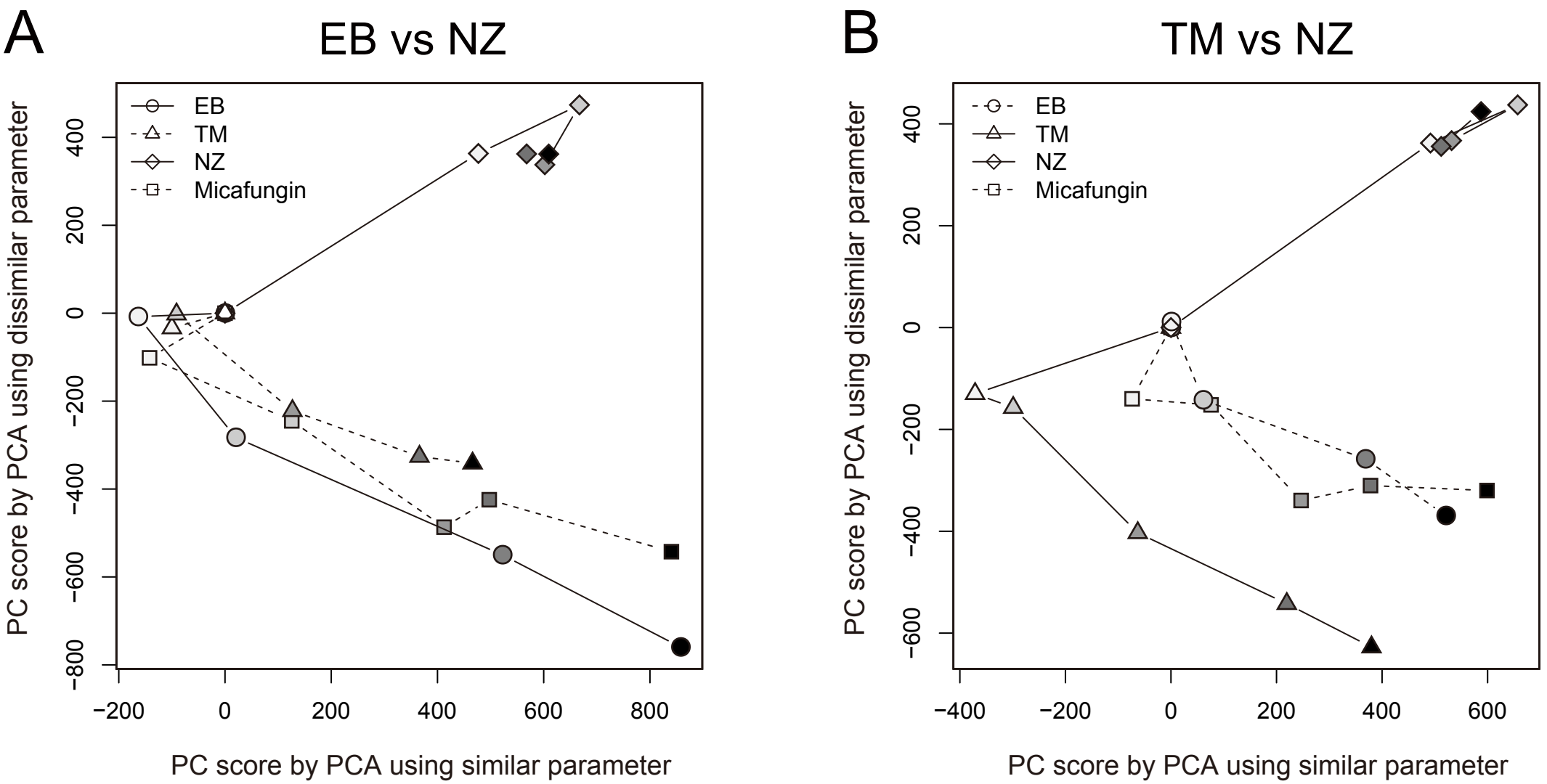

A
EB vs NZ
EB
TM
NZ
Micafungin
PC score by PCA using dissimilar parameter
PC score by PCA using similar parameter
−800
−600
−400
−200
0
200
400
−200
0
200
400
600
800
B
TM vs NZ
EB
TM
NZ
Micafungin
PC score by PCA using dissimilar parameter
PC score by PCA using similar parameter
−600
−400
−200
0
200
400
−400
−200
0
200
400
600

Figure S5

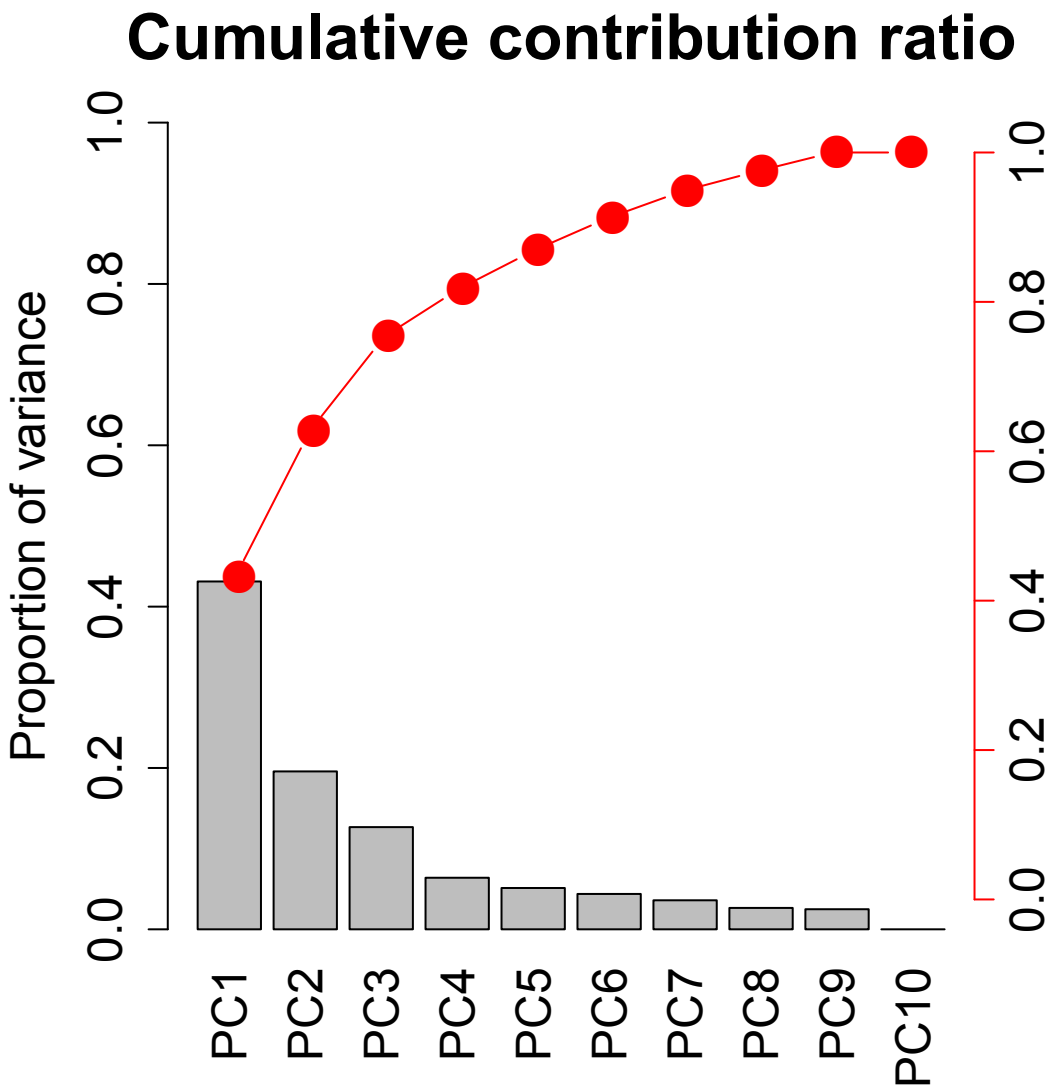

Cumulative contribution ratio
Proportion of variance
0.0
0.2
0.4
0.6
0.8
1.0
PC1
PC2
PC3
PC4
PC5
PC6
PC7
PC8
PC9
PC10

Figure S6

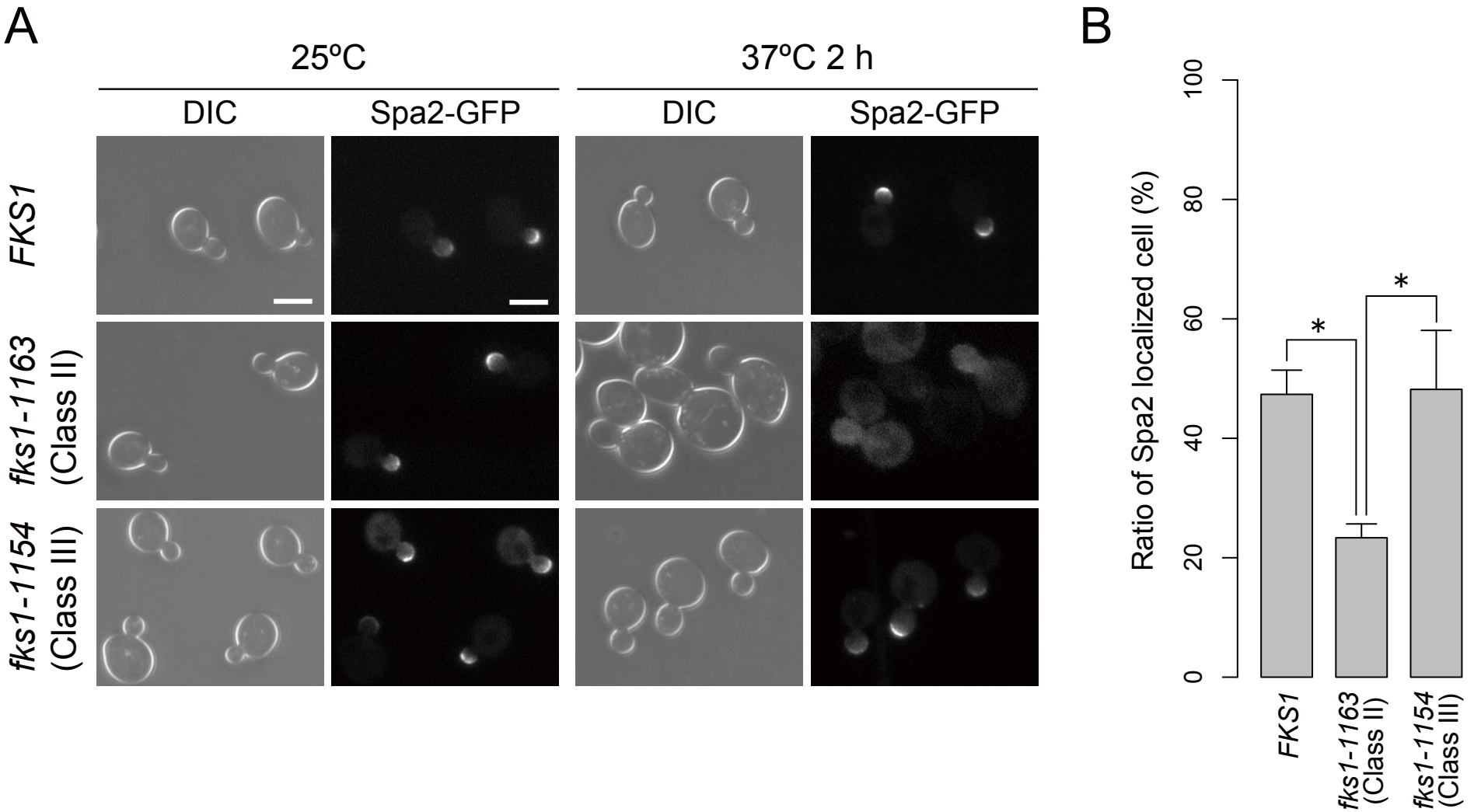
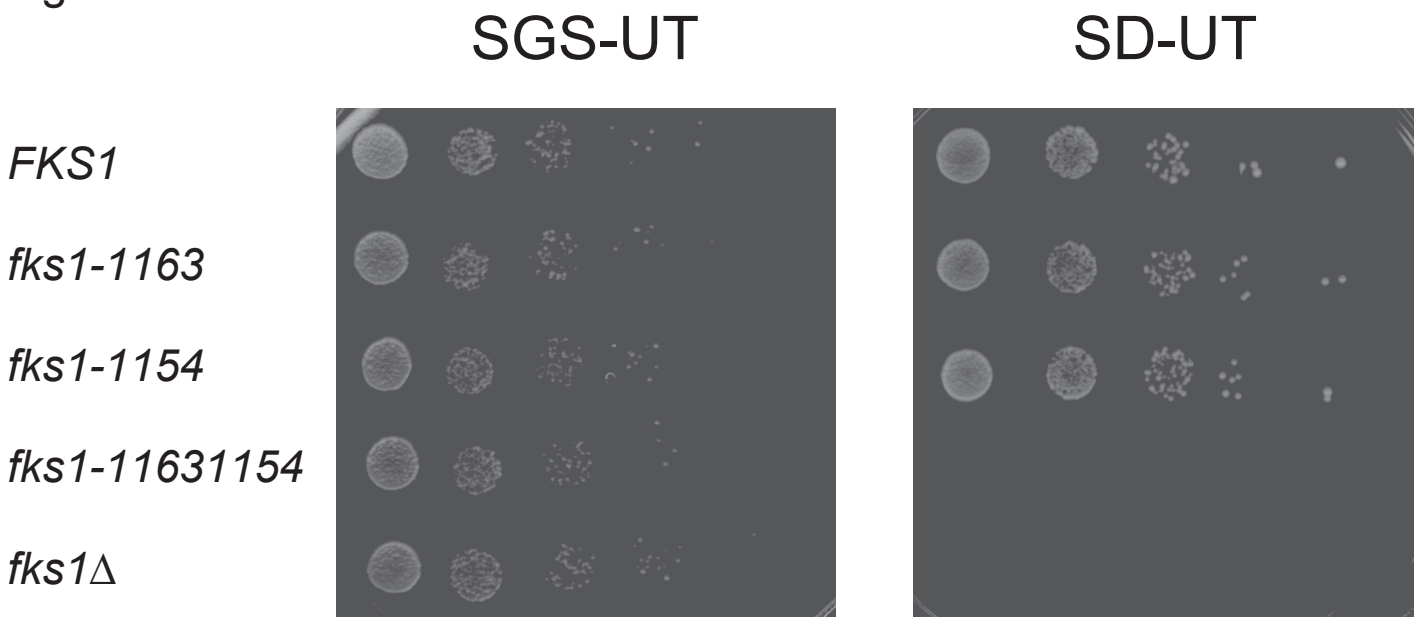

A
25ºC
37ºC 2 h
DIC
Spa2-GFP
DIC
Spa2-GFP
FKS1
fks1-1163 (Class II)
fks1-1154 (Class III)
B
Ratio of Spa2 localized cell (%)
0
20
40
60
80
100
*
*
FKS1
fks1-1163 (Class II)
fks1-1154 (Class III)

Figure S7



Supplemental reference list

Brachmann, C. B., Davies, A., Cost, G. J., Caputo, E., Li, J., Hieter, P., and Boeke, J. D. (1998). Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast *14*, 115–132.

Sekiya-Kawasaki, M., Abe, M., Saka, A., Watanabe, D., Kono, K., Minemura-Asakawa, M., Ishihara, S., Watanabe, T., and Ohya, Y. (2002). Dissection of upstream regulatory components of the Rho1p effector, 1,3-beta-glucan synthase, in *Saccharomyces cerevisiae*. Genetics *162*, 663–676.

Sikorski, R. S., and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics *122*, 19–27.