

Supplemental Tables and Figures for

Interrogation of γ -tubulin alleles using high-resolution fitness measurements reveals a distinct cytoplasmic function in spindle alignment

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Supplemental Tables

Supplemental Table I. Yeast strains used in this study

Yeast Strain	Genotype	Notes	Reference
YV838, YV856, YV2175, YV2176	<i>SPC42-cerulean-HYGB</i> ; <i>MATa</i> ; <i>his3Δ1</i> ; <i>leu2Δ0</i> ; <i>ura3Δ0</i> ; <i>met15Δ0</i>	wild-type; isolate 1, 2, 3, 4	Nazarova, et al., 2013 and this study.
YV2275, YV2276, YV2464	<i>tub4-Y362E-NAT</i> ; <i>SPC42-cerulean-HYGB</i> ; <i>MATa</i> ; <i>his3Δ1</i> ; <i>leu2Δ0</i> ; <i>ura3Δ0</i> ; <i>met15Δ0</i>	γ-tub-Y362E; isolate 1, 2, 3	This study.
YV2098, YV2361, YV2362	<i>tub4-Y445D-NAT</i> ; <i>SPC42-cerulean-HYGB</i> ; <i>MATa</i> ; <i>his3Δ1</i> ; <i>leu2Δ0</i> ; <i>ura3Δ0</i> ; <i>met15Δ0</i>	γ-tub-Y445D; isolate 1, 2, 3	This study.
YV2230, YV2231, YV2232	<i>kar9Δ::KAN</i> ; <i>SPC42-cerulean-HYGB</i> ; <i>MATa</i> ; <i>his3Δ1</i> ; <i>leu2Δ0</i> ; <i>ura3Δ0</i> ; <i>met15Δ0</i>	kar9Δ; isolate 1, 2, 3	This study.
YV2235, YV2236, YV2237	<i>dyn1Δ::KAN</i> ; <i>SPC42-cerulean-HYGB</i> ; <i>MATa</i> ; <i>his3Δ1</i> ; <i>leu2Δ0</i> ; <i>ura3Δ0</i> ; <i>met15Δ0</i>	dyn1Δ; isolate 1, 2, 3	This study.
YV2331, YV2332, YV2333	<i>tub4-Y362E-NAT</i> ; <i>kar9Δ::KAN</i> ; <i>SPC42-cerulean-HYGB</i> ; <i>MATa</i> ; <i>his3Δ1</i> ; <i>leu2Δ0</i> ; <i>ura3Δ0</i> ; <i>met15Δ0</i>	γ-tub-Y362E; kar9Δ; isolate 1, 2, 3	This study.
YV2492, YV2493	<i>tub4-Y362E-NAT</i> ; <i>dyn1Δ::KAN</i> ; <i>SPC42-cerulean-HYGB</i> ; <i>MATa</i> ; <i>his3Δ1</i> ; <i>leu2Δ0</i> ; <i>ura3Δ0</i> ; <i>met15Δ0</i>	γ-tub-Y362E; dyn1Δ; isolate 1, 2	This study.
YV2500, YV2501, YV2502	<i>HIS3::venus-TUB1</i> ; <i>SPC42-cerulean-HYGB</i> ; <i>MATa</i> ; <i>his3Δ1</i> ; <i>leu2Δ0</i> ; <i>ura3Δ0</i> ; <i>met15Δ0</i>	wild-type; isolate 1, 2, 3	This study.
YV2509, YV2510, YV2511	<i>tub4-Y362E-NAT</i> ; <i>HIS3::venus-TUB1</i> ; <i>SPC42-cerulean-HYGB</i> ; <i>MATa</i> ; <i>his3Δ1</i> ; <i>leu2Δ0</i> ; <i>ura3Δ0</i> ; <i>met15Δ0</i>	γ-tub-Y362E; isolate 1, 2, 3	This study.

Supplemental Table II. Plasmids used in this study

Plasmid	Description	Reference
pJV0372	pBKS- <i>tub4</i> -NAT	This study.
pJV0373	pBKS- <i>tub4</i> -Y362E-NAT	This study.
pJV0374	pBKS- <i>tub4</i> -Y445D-NAT	This study.
Addgene #50656	pHIS3p:Venus-Tub1+3'UTR::HIS3	Markus et al., 2015

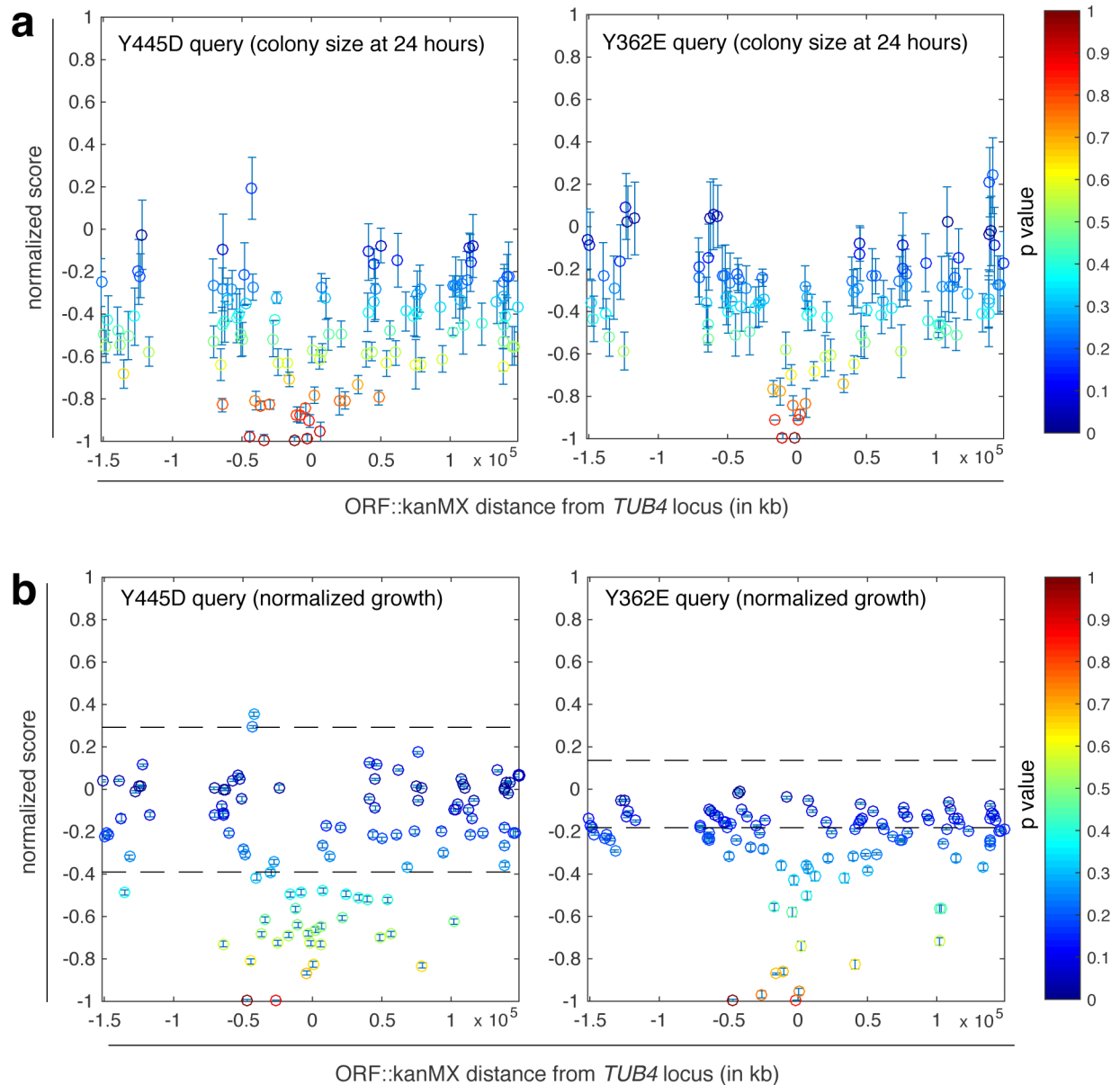


Figure S1 | Growth rates reflect strain fitness independently of initial conditions. Linkage group analysis for Y445D and Y362E alleles based on colony size (a) and measured growth rates (b). A linkage group extending ~150 kb (50 cM) on either side of the *TUB4* locus is detected for the Y445D and Y362E alleles using both methods. Colony sizes at 24 hours have a large variance that increases with distance. This variance arises from initial conditions and experimental procedures, and must be corrected in post-processing. In contrast, measured growth rates for double mutants have increased precision and accuracy as a consequence of relatively uniform growth rates across replicates and duplicates.

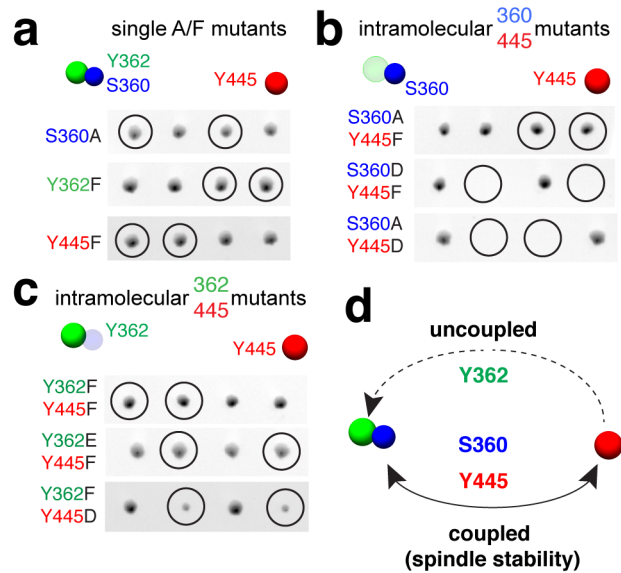


Figure S2 | Y362E is a separation of function allele. Meiotic outcomes of (a) single phosphor-inhibiting mutants and intramolecular phosphor-mutants between (b) S360 and Y445 and (c) Y362 and Y445. While individual phosphor-inhibiting mutations S360A, Y362F and Y445F have no detectable growth phenotype, lethality is observed when combined with phosphor-mimetic mutations S360D or Y445D. Intramolecular co-lethality suggests functional coupling between S360 and Y445. A weak growth defect is observed between the Y362F and Y445D mutant. (d) residues S360 and Y445 contribute to spindle stability, while Y362 contributes to spindle positioning.

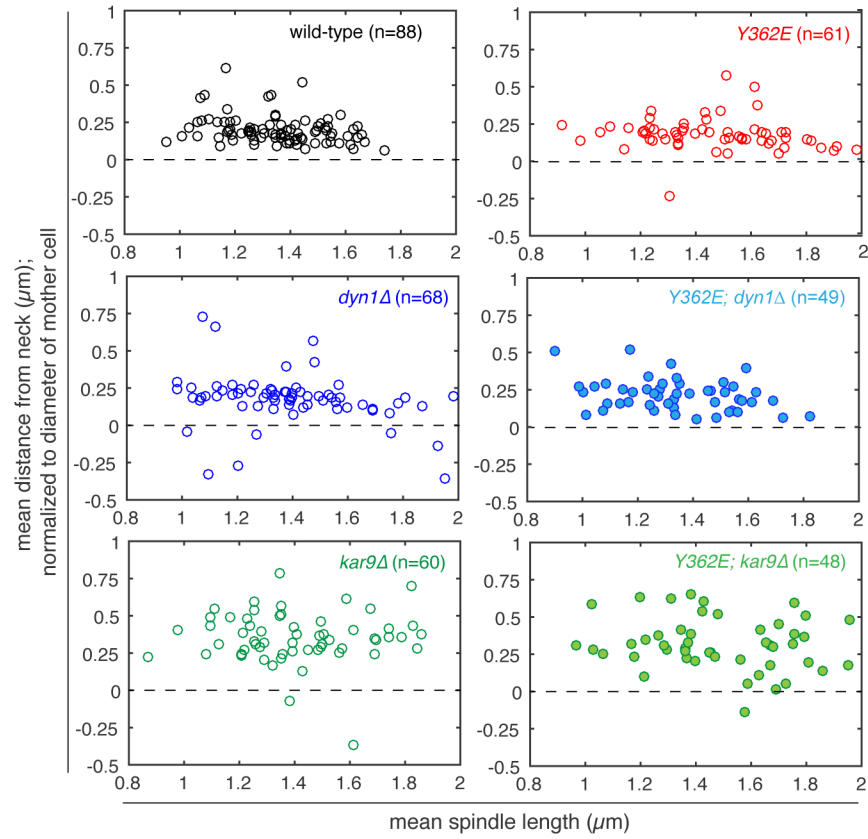


Figure S3 | γ Tub-Y362E does not perturb spindle positioning at the bud neck. Distance from the proximal pole to the bud neck, normalized to the initial length of the mother cell, plotted as a function of mean spindle length for wild-type cells and γ tub-Y362E, kar9 Δ , dyn1 Δ single mutants and γ -tubY362E; kar9 Δ and γ tub-Y362E; dyn1 Δ double mutants.

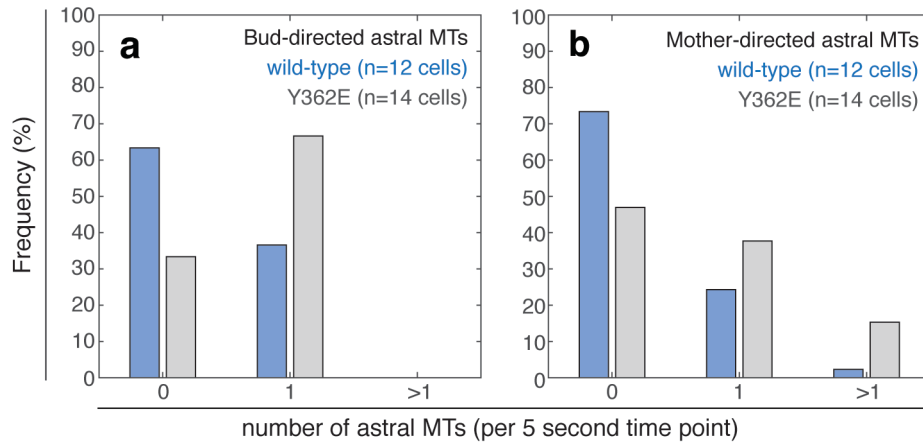


Figure S4 | A single astral MT enters the bud in both wild-type and γ tub-Y362E cells. The number of bud-directed astral MTs never exceeds one per 5 second time point in both wild-type (n = 12 cells) and γ tub-Y362E (n = 14 cells). Frequency of time points in which cells had 0, 1, or >1 (a) bud-directed or (b) mother-directed astral MT(s).