

Fig. S1. Individual or single *fic1* phosphomutants are not temperature-sensitive. A-C) Ten-fold serial dilutions of the indicated strains were spotted on YE agar media and incubated at the indicated temperatures for 3-5 days.

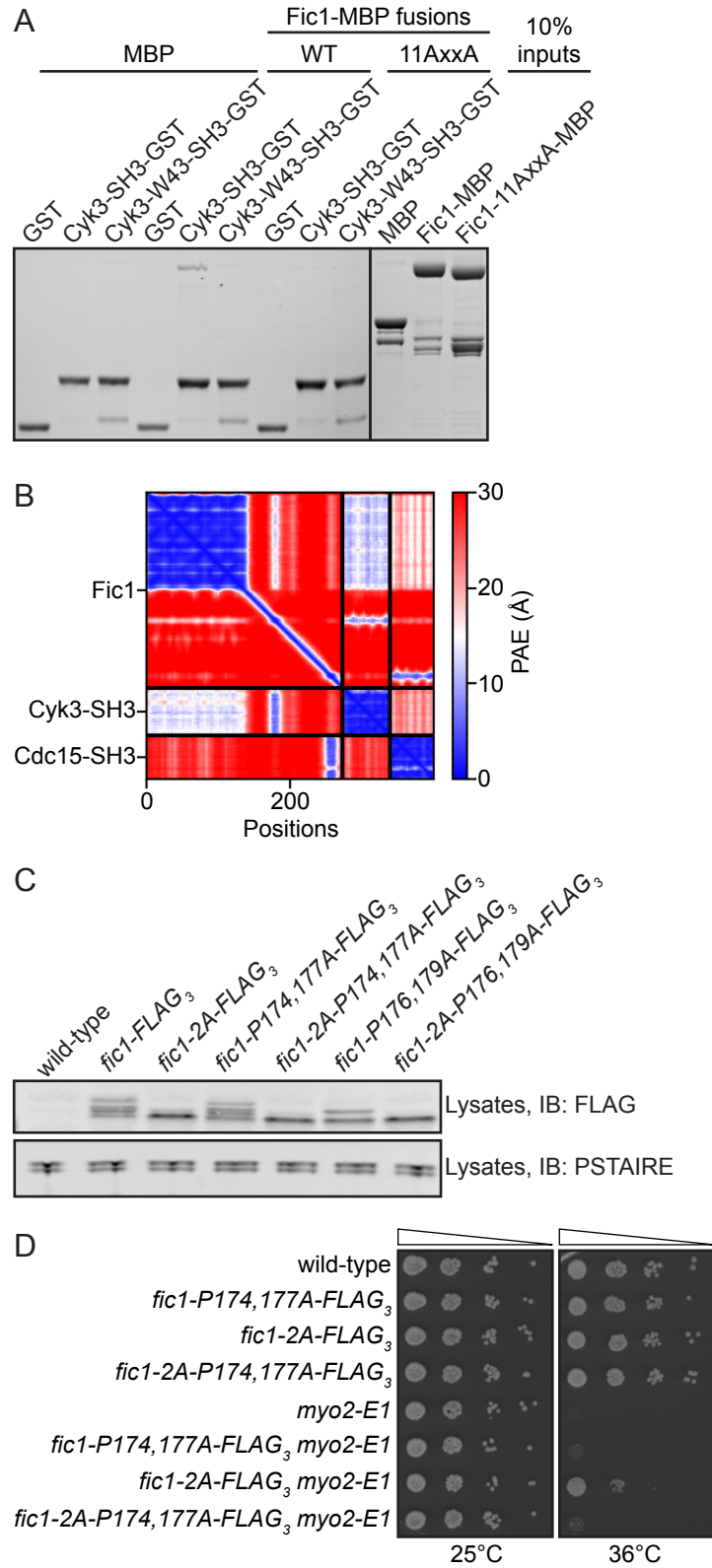


Fig. S2. P174,177 is required for *fic1-2A*'s suppression of *myo2-E1*. A) Coomassie-stained SDS-PAGE of *in vitro* binding assays using the indicated recombinant proteins. B) The predicted aligned error (PAE) map from the molecular modeling between Fic1, Cyk3-SH3, and Cdc15-SH3. C) Lysates from cells of the indicated genotypes were immunoblotted with anti-FLAG antibody to assess Fic1-FLAG₃ gel mobilities and anti-CDK (PSTAIRES) antibody as a loading control. D) Ten-fold serial dilutions of the indicated strains were spotted on YE agar media and incubated at the indicated 11 temperatures for 3-5 days.

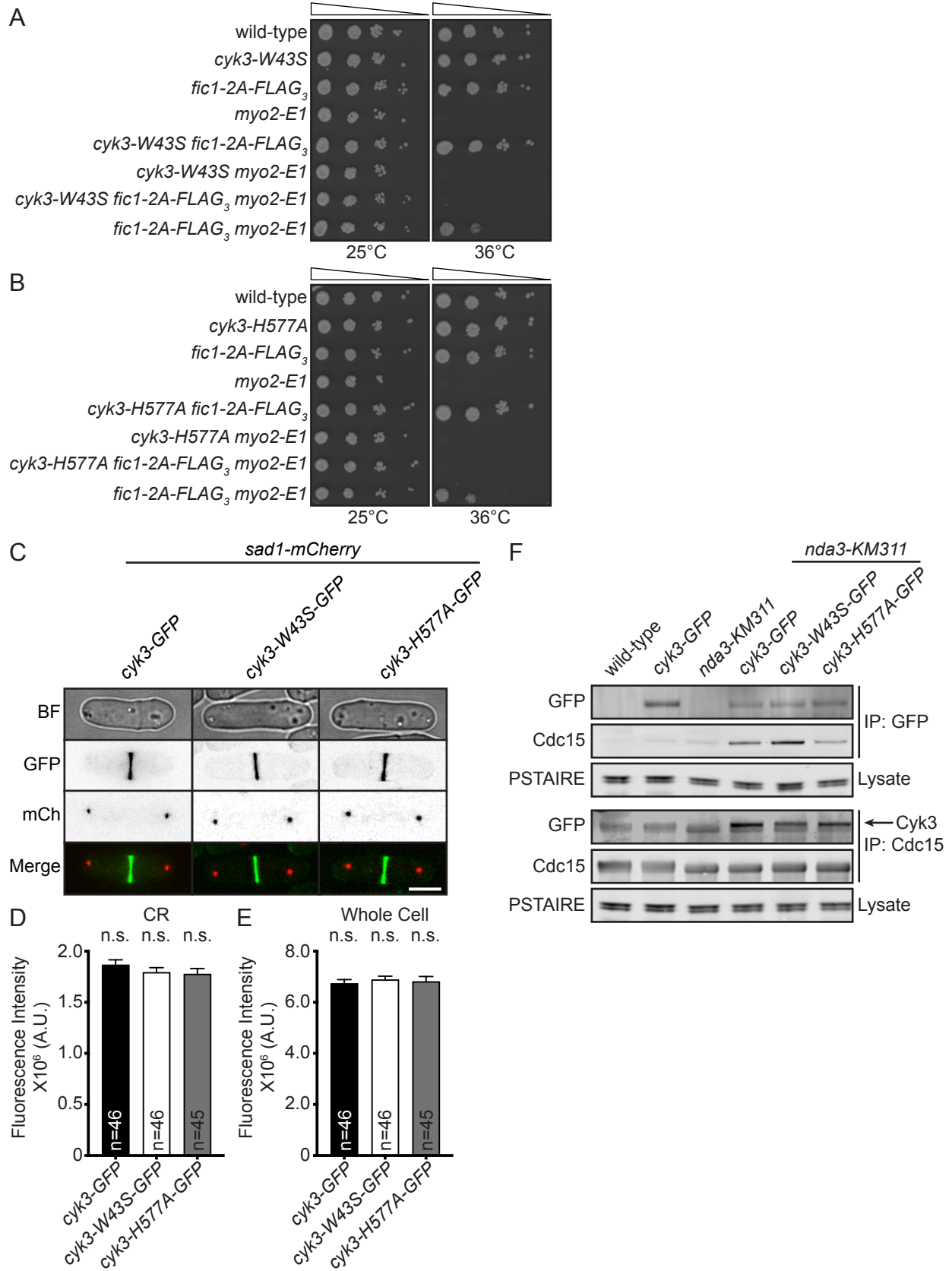


Fig. S3. Cyk3's SH3 and transglutaminase-like domain are required for *fic1-2A*'s suppression of *myo2-E1*. A and B) Ten-fold serial dilutions of the indicated strains were spotted on YE agar media and incubated at the indicated temperatures for 3-5 days. C) Live-cell bright field (BF), GFP, mCherry (mCh) and merged GFP/mCh images of cells of indicated genotypes during cytokinesis. Scale bar: 5 μ m. D and E) Quantification of CR (D) and whole cell (E) fluorescence intensities for cells of indicated genotypes. Data from three trials per genotype presented as mean \pm S.E.M. n.s., not significant, one-way ANOVA. F) Anti-GFP or anti-Cdc15 immunoprecipitates from cells of indicated genotypes were blotted with an anti-GFP or anti-Cdc15 antibody. Lysate samples were blotted with anti-CDK (PSTAIRE) as an input control for the immunoprecipitations. Arrow indicates Cyk3-GFP protein band.

Table S1. *S. pombe* strains used in this study

Figure 1		
KGY246	<i>ade6-M210 ura4-D18 leu1-32 h⁻</i>	Lab Stock
KGY11856	<i>fic1-T178A, S241A-FLAG₃:kan^R ade6-M210 ura4-D18 leu1-32 h⁺</i>	Lab Stock
KGY11861	<i>fic1-T178D, S241D-FLAG₃:kan^R ade6-M210 ura4-D18 leu1-32 h⁺</i>	Lab Stock
KGY2971	<i>myo2-E1 ade6-M216 leu1-32 ura4-D18 h⁺</i>	Lab Stock
KGY1018-2	<i>myo2-E1 fic1-T178A, S241A-FLAG₃:kan^R ade6-M210 leu1-32 ura4-D18 h⁻</i>	This Study
KGY15368	<i>myo2-E1 fic1-T178D, S241D-FLAG₃:kan^R ade6-M210 leu1-32 ura4-D18 h⁺</i>	This Study
KGY6008	<i>fic1::ura4⁺ leu1-32 ura4-D18 ade6-M21X h⁺</i>	Lab Stock
KGY6665	<i>fic1::ura4⁺ myo2-E1 leu1-32 ura4-D18 ade6-M21X h⁺</i>	Lab Stock
KGY5305-2	<i>sid4-GFP:kan^R rlc1-mNeonGreen:hyg^R ade6-M210 ura4-D18 leu1-32 h⁺</i>	This Study
KGY5659-2	<i>fic1::ura4⁺ sid4-GFP:kan^R rlc1-mNeonGreen:hyg^R ade6-M21X ura4-D18 leu1-32 h⁻</i>	This Study
KGY5602-2	<i>fic1-T178A, S241A-FLAG₃:kan^R sid4-GFP:kan^R rlc1-mNeonGreen:hyg^R ade6-M210 ura4-D18 leu1-32 h⁺</i>	This Study
KGY5334-2	<i>fic1-T178D, S241D-FLAG₃:kan^R sid4-GFP:kan^R rlc1-mNeonGreen:hyg^R ade6-M210 ura4-D18 leu1-32 h⁺</i>	This Study
Figure 2		
KGY2152-2	<i>act1:LactC2-GFP:nat^R sad1-GFP:kan^R ade6-M210 ura4-D18 leu1-32 h⁺</i>	(Curto et al., 2014)
KGY4102-2	<i>fic1-T178A, S241A-FLAG₃:kan^R act1:LactC2-GFP:nat^R sad1-GFP:kan^R ade6-M210 ura4-D18 leu1-32 h⁺</i>	This Study
KGY3655-2	<i>myo2-E1 act1:LactC2-GFP:nat^R sad1-GFP:kan^R ade6-M210 ura4-D18 leu1-32 h⁺</i>	This Study
KGY4138-2	<i>fic1-T178A, S241A-FLAG₃:kan^R myo2-E1 act1:LactC2-GFP:nat^R sad1-GFP:kan^R ade6-M210 ura4-D18 leu1-32 h⁺</i>	This Study
Figure 3		
KGY11876	<i>fic1-P257A-FLAG₃:kan^R leu1-32 ura4-D18 ade6-M21X h⁺</i>	Lab stock
KGY11934	<i>fic1-T178A, S241A, P257A-FLAG₃:kan^R leu1-32 ura4-D18 ade6-M21X h⁺</i>	This Study
KGY3373-2	<i>fic1-P257A-FLAG₃:kan^R myo2-E1 leu1-32 ura4-D18 ade6-M21X h⁻</i>	This Study
KGY19546	<i>fic1-T178A, S241A, P257A-FLAG₃:kan^R myo2-E1 leu1-32 ura4-D18 ade6-M21X h⁻</i>	This Study
KGY7490	<i>cyk3::ura⁺ ade6-M21X leu1-32 ura4-D18 h⁻</i>	Lab stock
KGY15446	<i>cyk3::ura4⁺ fic1-T178, S241A-FLAG₃:kan^R ade6-M21X leu1-32 ura4-D18 h⁺</i>	This Study
KGY7635	<i>cyk3::ura4⁺ myo2-E1 ade6-M21X leu1-32 ura4-D18 h⁻</i>	This Study

KGY15443	<i>cyk3::ura4⁺ fic1-T178,S241A-FLAG₃:kan^R myo2-E1 ade6-M21X leu1-32 ura4-D18 h⁺</i>	This Study
KGY3873-2	<i>cps1-191 fic1-FLAG₃:kan^R chs2-V5₃:hyg^R lys1-131 ade6-M21X ura4-D18 leu1-32 h⁻</i>	This Study
Figure 4		
KGY6365-2	<i>chs2::kan^R ade6-M210 ura4-D18 leu1-32 h⁻</i>	This Study
KGY6580-2	<i>chs2::kan^R fic1-T178A,S241A-FLAG₃:kan^R ade6-M210 ura4-D18 leu1-32 h⁻</i>	This Study
KGY6570-2	<i>chs2::kan^R myo2-E1 ade6-M210 ura4-D18 leu1-32 h⁺</i>	This Study
KGY6571-2	<i>chs2::kan^R fic1-T178A,S241A-FLAG₃:kan^R myo2-E1 ade6-M210 ura4-D18 leu1-32 h⁺</i>	This Study
KGY6434-2	<i>fic1::ura4⁺ chs2::kan^R leu1-32 ura4-D18 ade6-M21X h⁺</i>	This Study
KGY15954	<i>fic1::ura4⁺ cyk3::ura4⁺ leu1-32 ura4-D18 ade6-M21X h⁻</i>	Lab stock
KGY6930-2	<i>chs2::kan^R cyk3::ura4⁺ ade6-M21X leu1-32 ura4-D18 h⁻</i>	This Study
KGY7101-2	<i>chs2::kan^R cyk3::ura4⁺ fic1::ura4⁺ ade6-M21X leu1-32 ura4-D18 h⁻</i>	This Study
Supplemental Figures		
Figure S1		
KGY441	<i>cdc4-8 ade6-M210 ura4-D18 h⁺</i>	Lab Stock
KGY15363	<i>fic1-T178A,S241A-FLAG₃:kan^R cdc4-8 ade6-M21X ura4-D18 h⁻</i>	This Stock
KGY15364	<i>fic1-T178A,S241D-FLAG₃:kan^R cdc4-8 ade6-M21X ura4-D18 h⁺</i>	This Stock
KGY1105	<i>sid2-250 ade6-M21X ura4-D18 leu1-32 h⁻</i>	Lab Stock
KGY15374	<i>fic1-T178,S241A-FLAG₃:kan^R sid2-250 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This Study
KGY15375	<i>fic1-T178,S241D-FLAG₃:kan^R sid2-250 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This Study
KGY748	<i>cdc12-299 h⁻</i>	Lab Stock
KGY15365	<i>fic1-T178,S241A-FLAG₃:kan^R cdc12-299 leu1-32 ura4-D18 h⁺</i>	This Study
KGY15366	<i>fic1-T178,S241D-FLAG₃:kan^R cdc12-299 299 leu1-32 ura4-D18 h⁺</i>	This Study
KGY17207	<i>cps1-191 ura4-D18 leu1-32 ade6-M210 lys1-131 h⁺</i>	Lab Stock
KGY17771	<i>cps1-191 fic1-T178A,S241A-FLAG₃:kan^R ura4-D18 ade6-M21X h⁻</i>	This Study
KGY17773	<i>cps1-191 fic1-T178D,S241D-FLAG₃:kan^R ura4-D18 ade6-M21X h⁻</i>	This Study
KGY11106	<i>cwg1-1 leu1-32 h⁻</i>	Lab Stock
KGY17681	<i>cwg1-1 fic1-T178A,S241A-FLAG₃:kan^R ade6-M21X ura4-D18 leu1-32 h⁻</i>	This Study
KGY17683	<i>cwg1-1 (bgs4 ts) fic1-T178D,S241D-FLAG₃:kan^R ade6-M21X ura4-D18 leu1-32 h⁻</i>	This Study
KGY19522	<i>fic1-T178A-FLAG₃:kan^R ade6-M210 ura4-D18 leu1-32 h⁺</i>	Lab Stock

KGY19523	<i>fic1-S241A-FLAG₃:kan^R ade6-M210 ura4-D18 leu1-32 h⁺</i>	Lab Stock
KGY7615-2	<i>fic1-T178A-FLAG₃:kan^R myo2-E1 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This Study
KGY7628-2	<i>fic1-S241A-FLAG₃:kan^R myo2-E1 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This Study
KGY6288	<i>fic1-FLAG₃:kan^R ade6-M216 leu1-32 ura4-D18 h⁻</i>	Lab stock
KGY11908	<i>fic1-T178D-FLAG₃:kan^R ade6-M210 ura4-D18 leu1-32 h⁺</i>	Lab stock
KGY11854	<i>fic1-S241D-FLAG₃:kan^R leu1-32 ura4-D18 ade6-M21X h⁺</i>	Lab stock
Figure S2		
KGY4705-2	<i>fic1-P174,177A-FLAG₃:kan^R leu1-32 ura4-D18 ade6-M21X h⁺</i>	This Study
KGY4774-2	<i>fic1-T178A,S241A-P174,177A-FLAG₃:kan^R leu1-32 ura4-D18 ade6-M21X h⁺</i>	This Study
KGY4706-2	<i>fic1-P176,179A-FLAG₃:kan^R leu1-32 ura4-D18 ade6-M21X h⁺</i>	This Study
KGY4708-2	<i>fic1-T178A,S241A-P176,179A-FLAG₃:kan^R leu1-32 ura4-D18 ade6-M21X h⁺</i>	This Study
KGY4717-2	<i>fic1-P174,177A-FLAG₃:kan^R myo2-E1 leu1-32 ura4-D18 ade6-M21X h⁺</i>	This Study
KGY4852-2	<i>fic1-T178A,S241A-P174,177A-FLAG₃:kan^R myo2-E1 leu1-32 ura4-D18 ade6-M21X h⁻</i>	This Study
Figure S3		
KGY899-2	<i>cyk3-W43S ade6-M21X leu1-32 ura4-D18 h⁻</i>	This Study
KGY2086-2	<i>cyk3-W43S fic1-T178A,S241A-FLAG₃:kan^R ade6-M21X leu1-32 ura4-D18 h⁺</i>	This Study
KGY2141-2	<i>cyk3-W43S myo2-E1 ade6-M21X leu1-32 ura4-D18 h⁻</i>	This Study
KGY2505-3	<i>cyk3-W43S fic1-T178A,S241A-FLAG₃:kan^R myo2-E1 ade6-M21X leu1-32 ura4-D18 h⁻</i>	This Study
KGY1886-2	<i>cyk3-H577A ade6-M21X leu1-32 ura4-D18 h⁻</i>	This Study
KGY2120-2	<i>cyk3-H577A fic1-T178A,S241A-FLAG₃:kan^R ade6-M21X leu1-32 ura4-D18 h⁺</i>	This Study
KGY2161-2	<i>cyk3-H577A myo2-E1 ade6-M21X leu1-32 ura4-D18 h⁻</i>	This Study
KGY2559-2	<i>cyk3-H577A fic1-T178A,S241A-FLAG₃:kan^R myo2-E1 ade6-M21X leu1-32 ura4-D18 h⁻</i>	This Study
KGY6897	<i>cyk3-FLAG₃:kan^R ade6-M21X leu1-32 ura4-D18 h⁻</i>	Lab Stock
KGY2460-2	<i>cyk3-GFP:kan^R sad1-mCherry:nat^R ade6-M210 ura4-D18 leu1-32 h⁻</i>	This Study
KGY2362-2	<i>cyk3-W43S-GFP:kan^R sad1-mCherry:nat^R ade6-M21X leu1-32 ura4-D18 h⁻</i>	This Study
KGY2364-2	<i>cyk3-H577A-GFP:kan^R sad1-mCherry:nat^R ade6-M21X leu1-32 ura4-D18 h⁻</i>	This Study
KGY2202-3	<i>cyk3-GFP:kan^R ade6-M210 ura4-D18 leu1-32 h⁻</i>	Lab Stock
KGY56	<i>nda3-km311 leu1-32 h⁺</i>	Lab Stock

KGY2890-2	<i>cyk3-GFP:kan^R nda3-km311 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This Study
KGY2895-2	<i>cyk3-W43S-GFP:kan^R nda3-km311 ura4-D18 leu1-32 h⁻</i>	This Study
KGY2892-2	<i>cyk3-H577A-GFP:kan^R nda3-km311 ade6-M210 ura4-D18 leu1-32 h⁻</i>	This Study