Table S1 Plasmids used in this study

Plasmid	Relevant markers	Reference
JCB16	pFA6a-*KanMX6*	Longtine et. al (1998)
JCB31	pBG1805- *TPK2* (Gal1 promoter C-terminus 6XHIS-HA-3C-ZZ tag), 2µm *URA3*	Gelperin et al (2005)
JCB53	pFA6a-*HBH-hphMX4*	Tagwerker et. al (2006)
JCB58	*TPK1* in YEplac195, 2µm *URA3*	Pan et. al (1999)
JCB59	*TPK2* in YEplac195, 2µm *URA3*	Pan et. al (1999)
JCB60	*TPK3* in YEplac195, 2µm *URA3*	Pan et. al (1999)
JCB63	pRS426, 2µm *URA3*	Christiansonet.al (1992)
JCB65	*TA::MX4-natR* switcher cassette	A. Tong et. al (2005)
JCB83	pFA6a-*GFP(S65T)-His3MX6*	Longtine et. al (1998)
JCB153	*pGAL1-GST-TPK2* (K99R) in YEplac195	Pan et. al (2002)
JCB155	pC4- DASH Complex (*Spc34-6XHIS*)	Gestaut et. al (2008)
JCB352	*pHIS3p:mTurquoise2-Tub1+3' UTR::LEU2*	Markus et. al (2015)

Table S2 Yeast strains used in this study

Strain	Relevant genotype	Source or reference
JCY91[¥]	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Brachmann et al. (1998)
JCY124	Same as JCY91, *dam1-1::KanMX*[±]	Li et al. (2011)
JCY125	Same as JCY91, *dam1-11::KanMX*	Li et al. (2011)
JCY507*	MATα *DAM1-GFP*::*kanMX*	Mark Winey
JCY534	MATα *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1CFIII (CEN3L.YPH278) HIS3 SUP11*	Philip Hieter
JCY575	Same as JCY91, *dam1-1S257A::KanMX*	This Study
JCY588	MATa/α*pep4::HIS3/pep4::HIS3prb1∆1.6R/prb1∆1.6R can1/can1ura3-52/ura3-52 his3∆200/his3∆200 GAL1-GST-TPK2/TPK2*	Joseph Heitman
JCY611	Same as JCY534, *dam1-S31D::KanMX*	This Study
JCY612	Same as JCY534, *dam1-S31D::KanMX*	This Study
JCY613	Same as JCY534, *dam1-S31D::KanMX*	This Study
JCY986	Same as JCY91, *DAM1-HBH::HphMX4*	This Study
JCY988	Same as JCY91, *dam1-S31A-HBH::HphMX4*	This Study
JCY990	Same as JCY91, *dam1-S31A-GFP(S65T)::HIS3MX6*	This Study
JCY992	Same as JCY91, *dam1-S31D-GFP(S65T)::HISMX6*	This Study
JCY994	Same as JCY91, *dam1-S31A::KanMX*	This Study
JCY995	Same as JCY91, *dam1-S31A::NatMX*	This Study
JCY996	Same as JCY91, *dam1-S31D::KanMX*	This Study
JCY997	Same as JCY91, *dam1-S31D::KanMX*	This Study
JCY998	Same as JCY91, *dam1-1S31A::KanMX*	This Study
JCY999	Same as JCY91, *dam1-1S31D::KanMX*	This Study

JCY1000	Same as JCY534, *dam1-S31A::KanMX*	This Study
JCY1033	Same as JCY91, d*am1-S20DS31D::KanMX*	This Study
JCY1034	*bal1::LEU2 ARG+ ura3 ade1 his2 trp1 GFP-TUB1::URA3, SPC110-mCherry::hphMX*	M. Gardener
JCY1035	Same as JCY91, *Dam1-GFP(S65T)::HIS3MX6, TUB1-Turquoise (TUB1 3'UTR::LEU2)*	This Study
JCY1036	Same as JCY91, *dam1-S31A-GFP(S65T)::HIS3MX6, TUB1-Turquoise (TUB1 3'UTR::LEU2)*	This Study
JCY1037	Same as JCY91, *dam1-S31D-GFP(S65T)::HIS3MX6, TUB1-Turquoise (TUB1 3'UTR::LEU2)*	This Study
JCY1038	Same as JCY91, *dam1-1::KanMX, TUB1-Turquoise (TUB1 3'UTR::LEU2)*	This Study
JCY1039	Same as JCY91, *dam1-1S31A::KanMX, TUB1-Turquoise (TUB1 3'UTR::LEU2)*	This Study
JCY1040	Same as JCY91, *dam1-1S31D::KanMX, TUB1-Turquoise (TUB1 3'UTR::LEU2)*	This Study
JCY1041	Same as JCY91, *Dam1-GFP(S65T)::HIS3MX6, SPC110-mCherry::hphMX*	This Study
JCY1042	Same as JCY91, *dam1-S31A-GFP(S65T)::HIS3MX6, SPC110-mCherry::hphMX*	This Study
JCY1043	Same as JCY91, *dam1-S31D-GFP(S65T)::HIS3MX6, SPC110-mCherry::hphMX*	This Study
JCY1044	Same as JCY91, dam1-S20DS31D-GFP(S65T)::HIS3MX6	This Study
JCY1045	Same as JCY91, *dam1-S20DS31D-GFP(S65T)::HIS3MX6, SPC110-mCherry::hphMX*	This Study

¥ Derived from S288C
*Made in S228C background
‡*dam1-1* C111Y, Q61R, E160K

Table S3 Oligonucleotides used in this study

Primer	Sequence	Description
JCO53	CTGCAGCGAGGAGCCGTAAT	Reverse primer, binds to the TEF promoter region
JCO64	GCGTATAAGCTCAGCAATTGCACC	Forward primer, 64bp upstream of *DAM1* start codon
JCO65	CCGTTGTCCAGTTTCTTGTCAATTTGG	Reverse primer, 381bp downstream of *DAM1* stop codon
JCO76	AGCCCTCCAAGATAGGCAACAAC	Forward primer, 586bp downstream of *DAM1* start codon
JCO89	ACAGTAAACTGAGAAGAAAGGCCATCTTACATA CAATCAGA	Forward primer of *DAM1* that has a point mutation for S257A
JCO127	CTGTTGCAAAGAAAACTGAAAAAAAAATAAATA CAAGGCCCCCCTTCAGA cggatccccgggttaattaa	Forward primer of *DAM1* that also contains flanking sequence of pFA6a plasmid
JCO128	TAGCGATATATTTTGTGAGGAGGATAATTCTTT GGTTGGGTTGGGCGTAG gaattcgagctcgtttaaac	Reverse primer of *DAM1* that also contains flanking sequence of pFA6a vector
JCO130	GACCACAAGGTCTGCCACGGAATATCGTTTAT CCATTGGTAGCGCTCCGACTTCTAGAAGGTCG GCTATGGGTGAATCCTCATCCCTGAT	Forward primer of *DAM1* that has a point mutation for S31A and a silent mutation making XbaI restriction site
JCO147	GACCACAAGGTCTGCCACGGAATATCGTTTAT CCATTGGTAGCGCTCCGACTTCTAGAAGGTCG GATATGGGTGAATCCTCATCCCTGAT	Forward primer of *DAM1* that has a point mutation for S31D and a silent mutation making XbaI restriction site
JCO154	CTTTCTTCCTCCTTTTGCTTTAACCGTTGTCCA GTTTCTTGTCAATTTGGGAATTCGAGCTCGTTT AAAC	Reverse primer of *DAM1* that has flanking sequence of pFA6a vector. It binds to 401bp downstream of stop codon
JCO245	CTGCCACGGAATATCGATTAGCCATTGGTAGC GCTCCGACTTC	Forward primer of *DAM1* that has a point mutation for S20A and a silent mutation making ClaI restriction site
JCO246	CTGCCACGGAATATCGATTAGACATTGGTAGC GCTCCGACTTC	Forward primer of *DAM1* that has a point mutation for S20D and a silent mutation making ClaI restriction site

JCO269	TAGATAGTGTGAGCTCCTTGTAGAC	Forward primer, 240bp upstream of *SPC110* start codon
JCO270	CTAGGGAGATTAGCACACTACAATC	Reverse primer, 157bp downstream of *SPC110* stop codon

Table S4 Mass spectrometry analysis of *in vitro* kinase reactions with TPKs and the Dam1 complex

TPK1

	S20	S31	S257	S265	S292
Sum of area for all peptides	1163353647	473827230	692964114	35434359	850317047
Sum of area for all phosphorylated peptides	4416337	189476867	680729723	326215	0
% Phosphorylated peptides	0.38	**39.9**	**98.2**	0.92	0

TPK2

	S20	S31	S257	S265	S292
Sum of area for all peptides	956148127	459557535	511556947	7149586	743184641
Sum of area for all phosphorylated peptides	2316054	139153211	478776059	93598	0
% Phosphorylated peptides	0.24	**30.3**	**93.6**	0.31	0

No Kinase

	S20	S31	S257	S265	S292
Sum of area for all peptides	956936102	648129051	859270598	2213956	912500283
Sum of area for all phosphorylated peptides	224169	68557	2193516	0	0
% Phosphorylated peptides	0.02	0.01	0.26	0	0

Peptides analyzed are those that contain the indicated serines. Area = ion current chromatographic peak area.

Figure S1

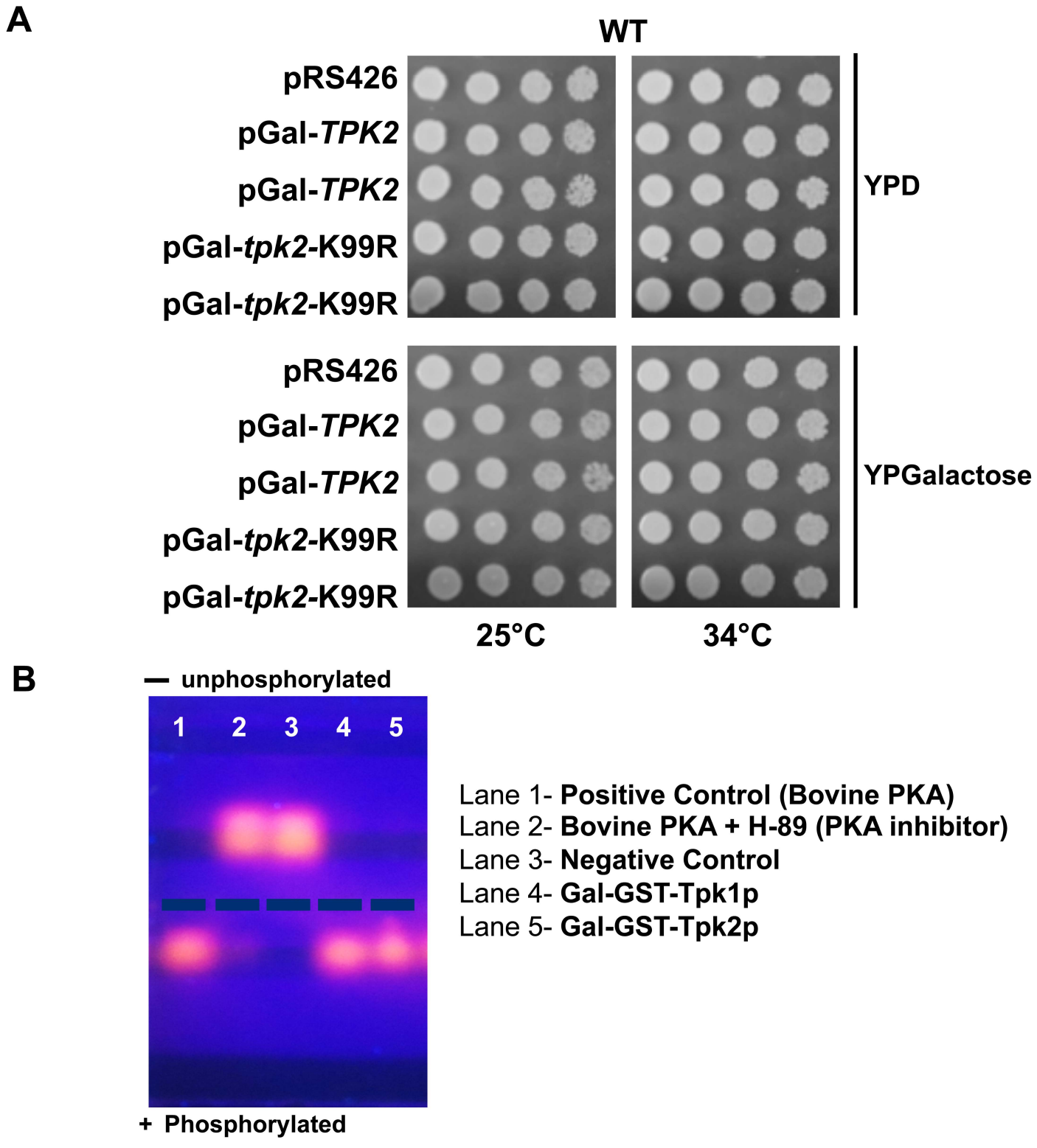

Figure S1. Wild-type cells do not display growth defects when *TPK2* is overexpressed and GST-Tpks purified from yeast are highly active. (A) BY4741 (WT) cells carrying empty vector (pRS426) or wild-type *TPK2* (JCB31) or a catalytically inactive *tpk2*-K99R mutant under the control of a galactose inducible promoter (JCB153). Serial five-fold dilutions of the indicated strains were spotted on YP 2% glucose (YPD) or YP 2% galactose (YPG) at 25°C and 34°C. Cells were grown for 4 days and imaged. (B) Measuring PKA activity of purified GST-Tpk1p and GST-Tpk2p using a PepTag kinase assay. Cells carrying GST-Tpk1p and GST-Tpk2p under the control of a galactose inducible promoter were grown overnight in YPRaffinose. Next day fresh cultures were started and induced with YPGalactose. Whole cell extracts were prepared followed by affinity purification of GST-Tpk1p and GST-Tpk2p with glutathione beads. Bovine PKA was used as a positive control. H-89 and reactions without any kinase were used as negative controls.

Figure S2

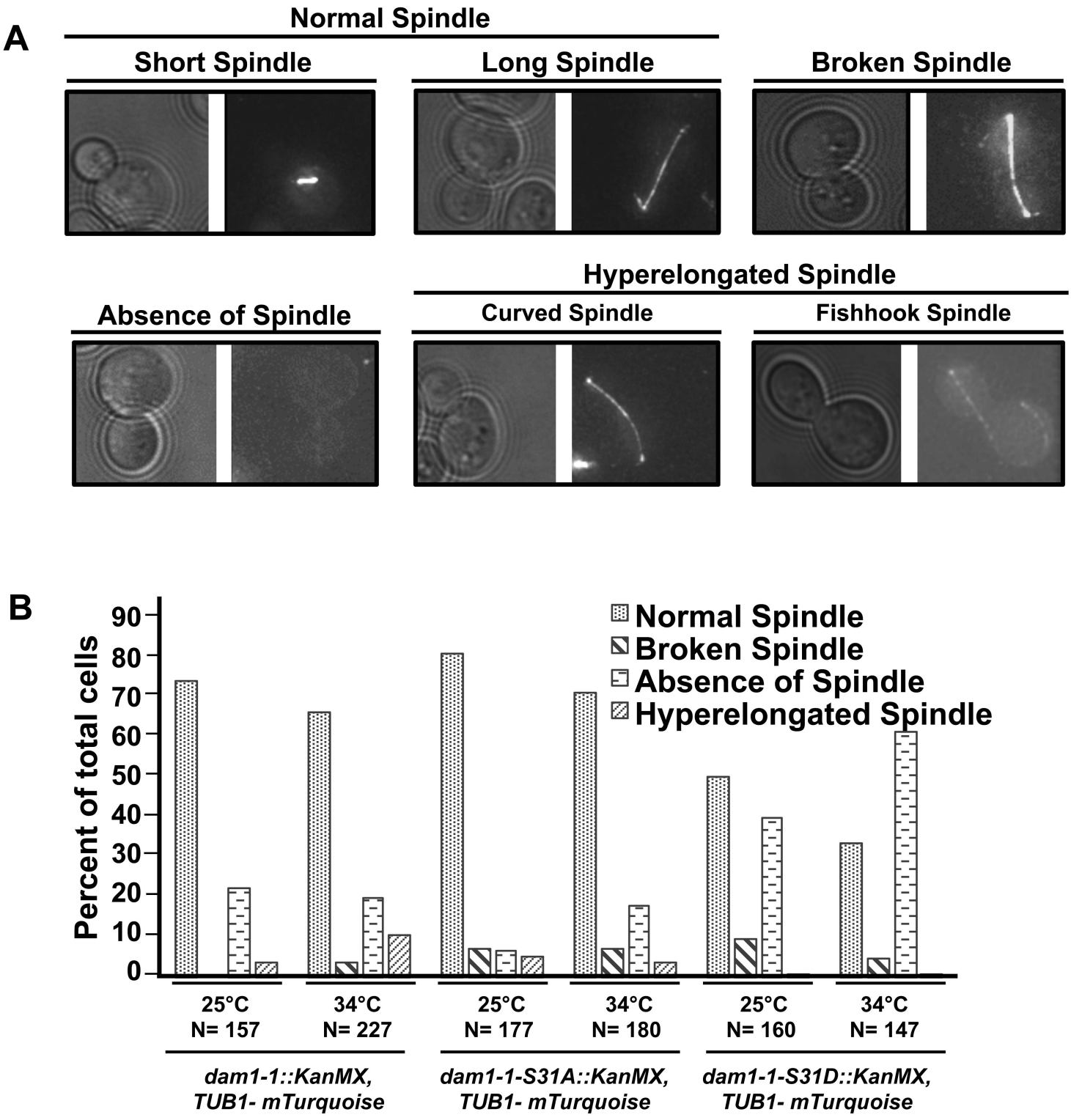

Figure S2. Phospho-mimetic *dam1-1*-S31D mutant is associated with increased defects in spindle morphology. (A) *dam1-1*, *TUB1-mTurquoise* (JCY1038), *dam1-1*-S31A, *TUB1-mTurquoise* (JCY1039) and *dam1-1*-S31D, *TUB1-mTurquoise* (JCY1040) were freshly grown in SC media from a 1:10 dilution of overnight cultures either at 25ºC or 34ºC for ~ 4 hr. Z-stacks were taken with a DeltaVision Elite microscope as described in methods. Cells were analyzed based upon four major categories which include (1) normal spindle (either short or long spindles), (2) broken spindle, (3) absence of spindle and (4) hyperelongated spindle (either curved spindle or fishhook spindle). (B) Percent of cells in different categories are plotted. Number of cells counted are indicated by (N).

Figure S3

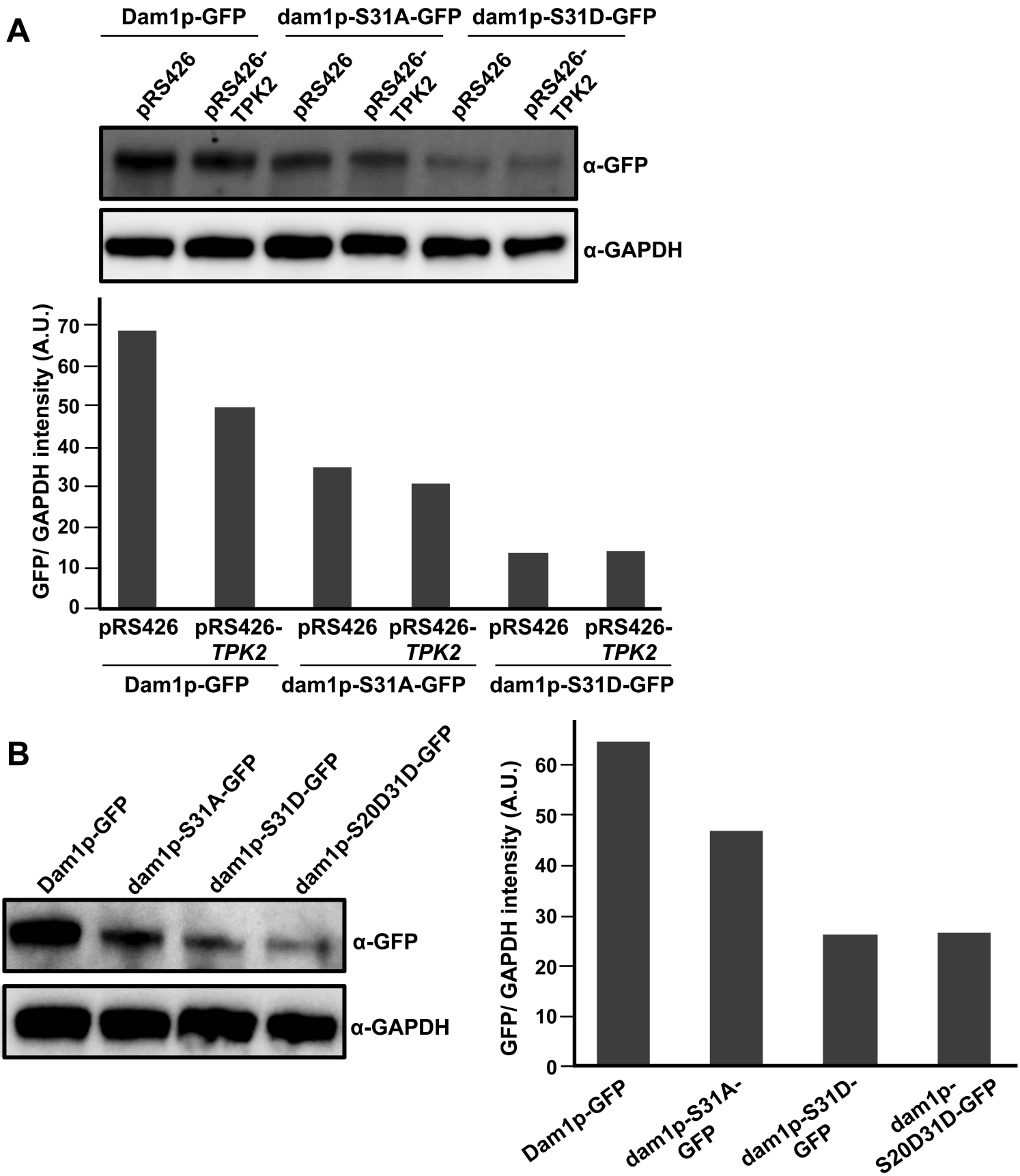

Figure S3. Western analysis of total wild-type Dam1p-GFP and Dam1p-GFP phospho-mutants. Whole cell extracts from the following strains were run on SDS-PAGE and blotted for total GFP and GAPDH: (A) *Dam1*-GFP (JCY507), *dam1*-S31A (JCY990), *dam1*-S31D (JCY992) carrying empty vector pRS426 or pRS426-*TPK2*. (B) *Dam1*-GFP (JCY507), *dam1*-S31A (JCY990), *dam1*-S31D (JCY992), and *dam1*-S20DS31D (JCY1045). Blots were subjected to analysis by ImageJ software to quantify GFP and GAPDH levels and the ratio of GFP/GAPDH intensity plotted (A and B, right). Note that levels of dam1p-31D-GFP and dam1p-20D31D-GFP mutants are strongly reduced and dam1p-S31A-GFP is also reduced but to a lesser extent, compare to wild-type Dam1p-GFP.

Figure S4

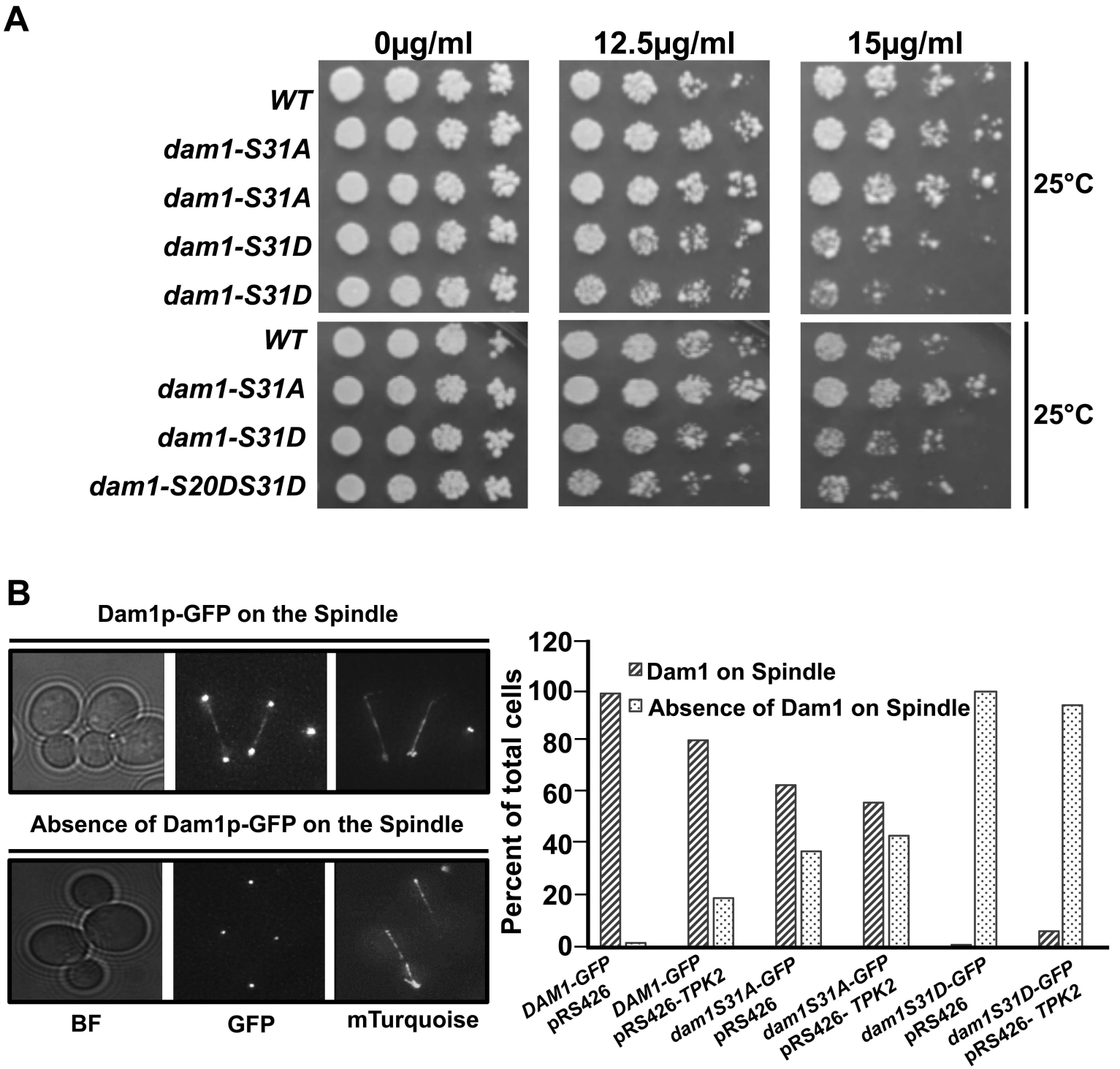

Figure S4. Sensitivity to benomyl and localization of Dam1p to spindle microtubules. (A) The double phospho-mimetic mutant *dam1*-S20D31D (JCY1033) displays greater sensitivity to benomyl compared to wild-type (JCY91) or *dam1*-S31A (JCY994, JCY995) cells but behaves similarly to the phospho-mimetic *dam1*-S31D (JCY996, JCY997) cells. Five-fold serial dilutions of the indicated strains were spotted onto YPD plates with or without benomyl at the indicated concentrations and incubated at 25ºC for 3-4 days. (B) Dam1p-GFP is localized to spindles and when high copy *TPK2* is present, there is a reduction of spindle localization. In contrast, Dam1p spindle localization is strongly reduced in the phospho-mimetic dam1p-S31D and in to a lesser extent in the non-phosphorylatable dam1p-S31A mutant. Indicated strains were freshly grown in SC-URA media with or without *TPK2* for 4hr at 34ºC. Images were taken with DeltaVision Elite microscope as described in methods. GFP colocalization with mTurquoise labeled spindles were counted as Dam1p on the spindle. BF = bright field, GFP = green fluorescent protein