

Zimniak et al., <http://www.jcb.org/cgi/content/full/jcb.200901036/DC1>

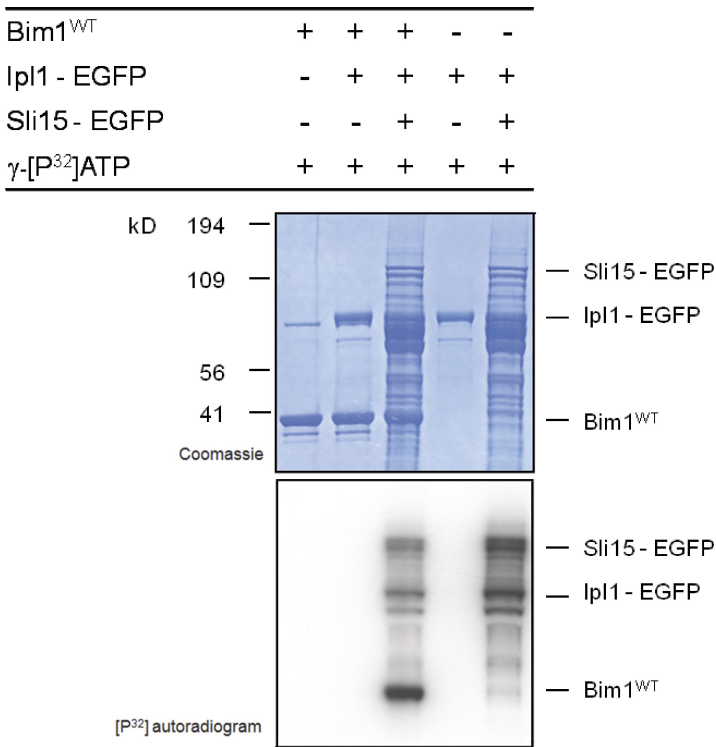


Figure S1. **Bim1p is phosphorylated by lpl1p only in the presence of the activating protein Sli15p.** Coomassie-stained gel and autoradiography of kinase assay using Bim1p in combination with lpl1-EGFP alone or after the addition of Sli15-EGFP. lpl1 kinase alone is unable to phosphorylate either Bim1p or itself, but upon the addition of Sli15-EGFP, autophosphorylation of lpl1p, phosphorylation of the activator Sli15p, and phosphorylation of Bim1p are detectable.

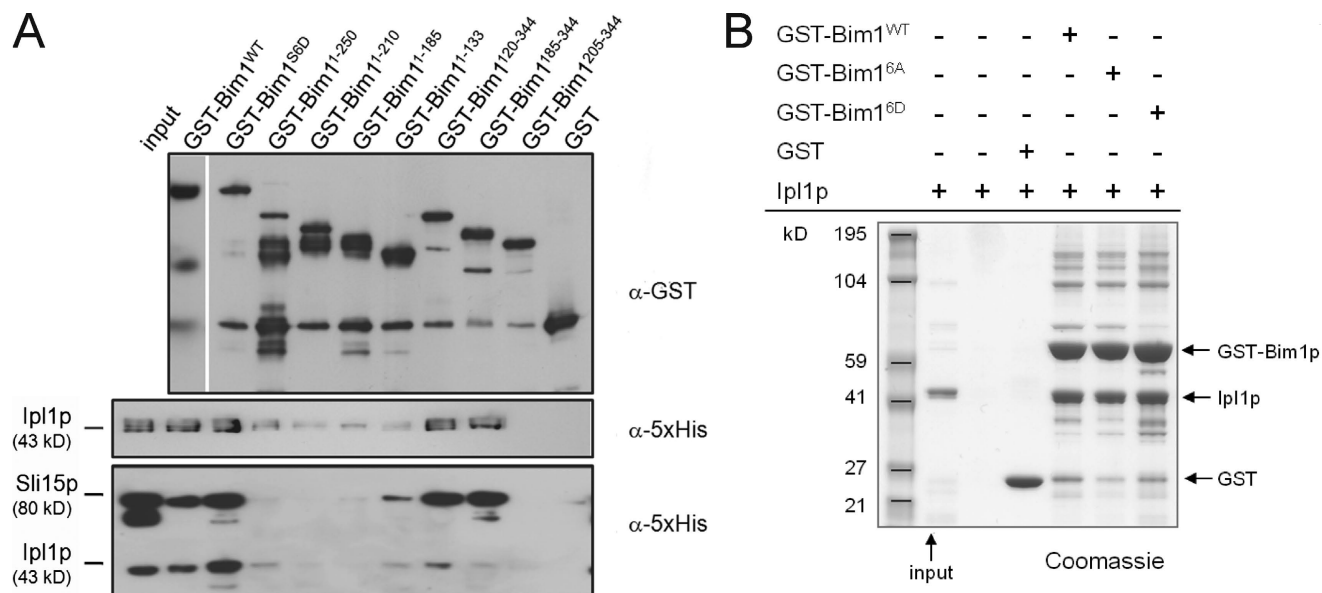


Figure S2. **Mapping the Bim1p interaction with the Ipl1-Sli15 complex and Ipl1p.** (A) Different N- and C-terminal truncations of Bim1p were expressed as GST fusion proteins in *E. coli* and used in pull-down assays either with His6-tagged recombinant Ipl1-Sli15 complex or with His6-tagged Ipl1p. Immobilized constructs were detected with an anti-GST antibody (top), and interacting proteins were detected with a 5x His antibody (bottom). The white line indicates that intervening lanes have been spliced out. (B) Coomassie-stained gel of the pull-down assay using GST-Bim1 phospho mutants and His6-tagged Ipl1p.

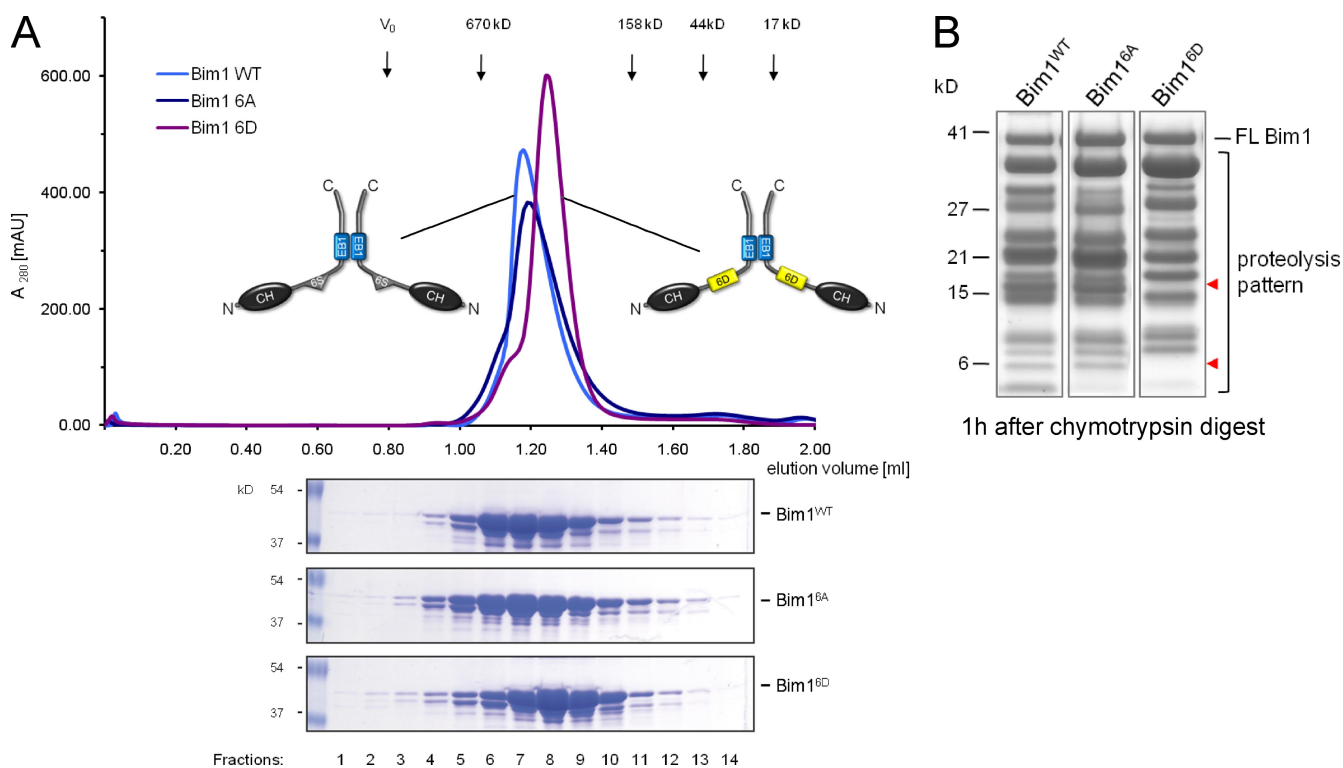


Figure S3. **Characterization of WT and Ipl1-phosphorylated Bim1p in vitro.** (A) SEC of different Bim1 variants on a Superdex 200 column. Coomassie-stained gels of the indicated fractions are shown below. Note the increased elution volume of the phosphomimicking Bim1^{6D} mutant. (B) Limited proteolysis of Bim1^{WT}, Bim1^{6A}, and Bim1^{6D} variants using chymotrypsin. A coomassie-stained gel showing products of limited proteolysis after 60 min is shown. Note the altered digestion pattern in the 6D mutant. Arrowheads point to altered proteolysis patterns of the Bim1^{6D} mutant. FL, full length.

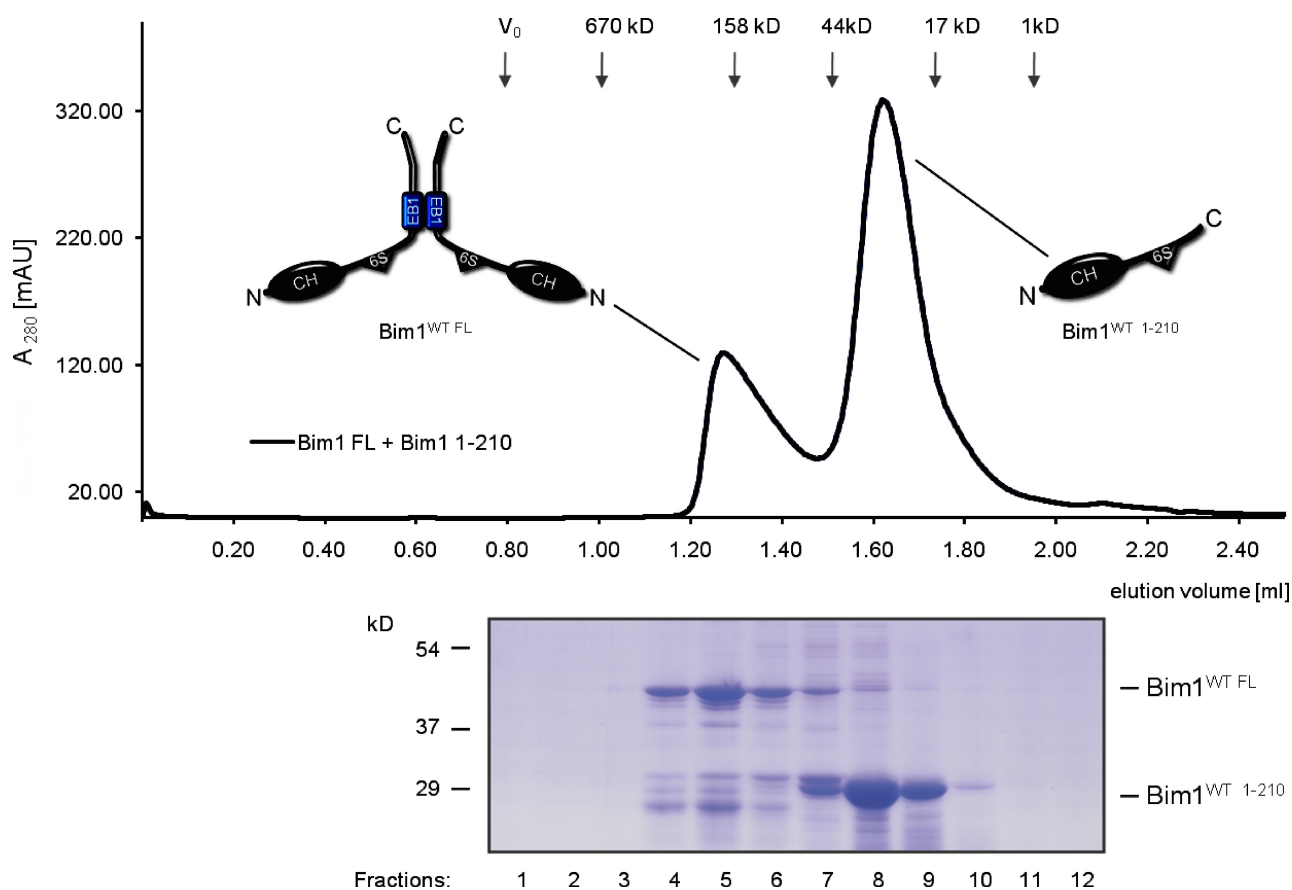


Figure S4. **Analytical SEC of full-length Bim1p and monomeric Bim1¹⁻²¹⁰**. Recombinant Bim1¹⁻³⁴⁴ and Bim1¹⁻²¹⁰ were combined and separated together on a Superdex 200 PC 3.2/30 column. Bim1 full-length (FL) elutes early, suggesting an elongated dimeric shape, and elimination of the last 134 residues of Bim1p (Bim1¹⁻²¹⁰) prevents dimerization. Note that these two variants elute separately, showing that they do not interact with each other.

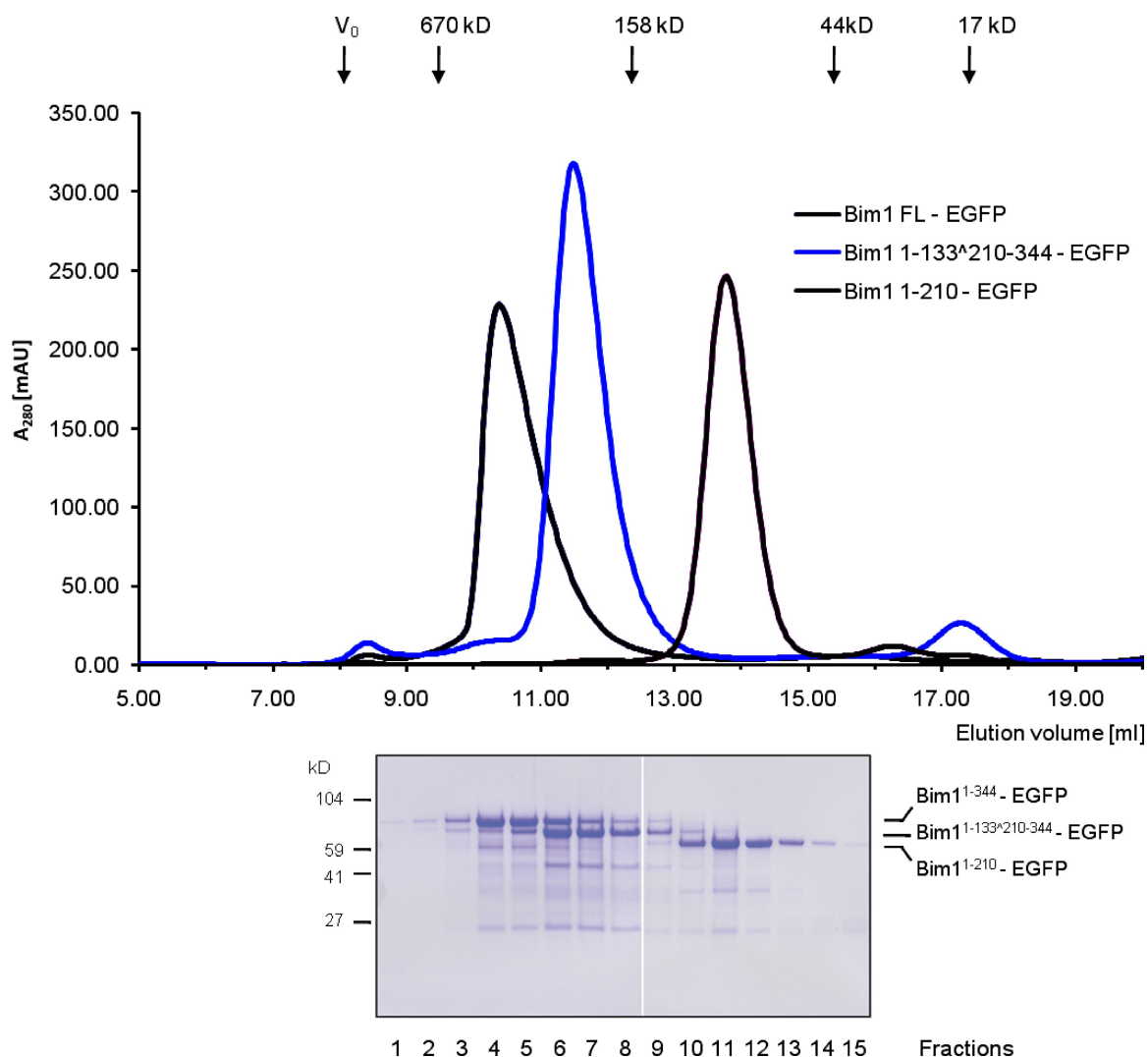
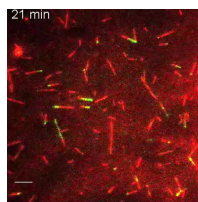
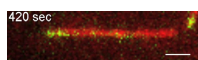


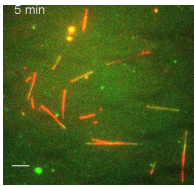
Figure S5. **Analytical SEC of the full-length Bim1-EGFP, Bim1^{1-133^210-344}-EGFP, and Bim1¹⁻²¹⁰-EGFP.** Elution profiles and Coomassie-stained gel of the indicated proteins separated individually on a Superdex 200 PC 3.2/30 column. Elimination of the Bim1 linker region shifts the elution profile toward a smaller molecular mass but does not change its dimeric properties. The white line indicates that this blot is a composite of two gels. FL, full length.



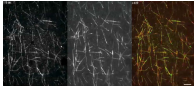
Video 1. **Autonomous MT plus end tracking of Bim1-EGFP in vitro.** Dynamic MT growth was induced from stable GMPCPP seeds in the presence of rhodamine-labeled tubulin (red) and 70 nM Bim1-EGFP (green). Dynamic MT polymerization and Bim1 plus end tracking was visualized using two-color TIRF microscopy. Images were recorded every 5 s. A kymograph from this video is shown in Fig. 5 C (left). The video is shown at 30 frames/s. Bar, 6 μ m.



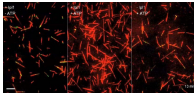
Video 2. **Plus end association of Bim1-EGFP on an individual dynamic MT.** GMPCPP MT seeds initiating MT growth were observed in the presence of rhodamine-labeled tubulin (red) and 70 nM Bim1-EGFP (green). TIRF images were recorded at 5-s intervals. A single MT from the experiment is shown. The video is shown at 20 frames/s. Bar, 4 μ m.



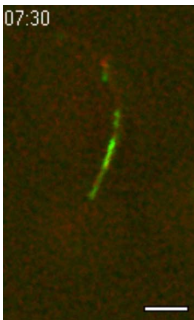
Video 3. **The monomeric Bim1¹⁻²¹⁰-EGFP shows weak MT lattice binding.** MT growth is induced from stable GMPCPP MT seeds in the presence of rhodamine-labeled tubulin (red) and 1 μ M Bim1¹⁻²¹⁰-EGFP (green). TIRF images were recorded every 5 s, showing reduced Bim1¹⁻²¹⁰-EGFP association with MTs. A kymograph from this video is shown in Fig. 5 C (right). The video is shown at 25 frames/s. Bar, 6 μ m.



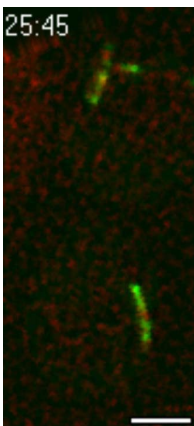
Video 4. **Ipl1-Sli15 phosphorylation removes Bim1-Alexa Fluor 488 from stable MTs.** Taxol-stabilized, rhodamine-labeled MTs (middle) were adhered to the coverslip, followed by incubation with Bim1-Alexa Fluor 488 and wash out of unbound protein. Under these conditions, Bim1p binds all along the MT lattice. Subsequently, reaction buffer containing 0.5 μ M of the Ipl1-Sli15 complex was introduced into the flow chamber. TIRF images were recorded at 2-s intervals, and after \sim 100 s, 0.5 mM ATP was injected into reaction chamber. The Bim1-Alexa Fluor 488 signal (left) is rapidly disappearing from MTs. The video is shown at 10 frames/s. Bar, 8 μ m.



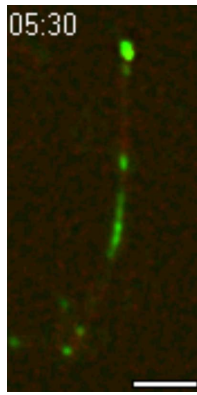
Video 5. **Ipl1-Sli15 phosphorylation removes Bim1-EGFP from dynamic MT plus ends.** MT growth is induced from stable GMPCPP seeds in the presence of rhodamine-labeled tubulin (red), 70 nM Bim1-EGFP (green), 1 μ M of unlabeled Ipl1-Sli15 complex, and 0.5 mM ATP, as indicated at the top of the panels. The Bim1-EGFP signal is rapidly lost from MT tips in the presence of the kinase complex and ATP (middle). TIRF images were recorded at 5-s intervals. The video is shown at 30 frames/s. Bar, 6 μ m.



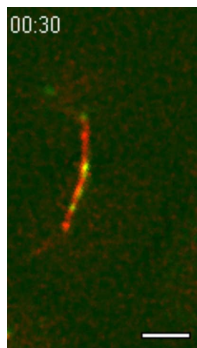
Video 6. **Dynamic localization of Bim1^{WT}-3x GFP on the anaphase spindle.** Bim1 was C-terminally tagged with 3x GFP and visualized together with mCherry-Tub1 by live cell Deltavision microscopy. z stacks (eight stacks 0.35 μ m apart) were acquired at 15-s intervals, deconvoluted, and projected into 2D images. Note the decreasing Bim1-3x GFP (green) signal at the spindle midzone before the spindle disassembly (red). The video is shown at 10 frames/s. Bar, 2 μ m.



Video 7. **The Bim1^{6A}-3x GFP phospho mutant persists on the disassembling spindle.** The Bim1^{6A}-3x GFP phospho mutant and mCherry-Tub1 were visualized by live cell Deltavision microscopy. z stacks (eight stacks 0.35 μ m apart) were acquired at 15-s intervals, deconvoluted, and projected into 2D images. Note the prominent Bim1^{6A}-3x GFP (green) signals remaining on the MTs after spindle disassembly (red). The video is shown at 10 frames/s. Bar, 2 μ m.



Video 8. **The Bim1^{6A}-3x GFP phospho mutant persists on the disassembling spindle.** The Bim1^{6A}-3x GFP phospho mutant and mCherry-Tub1 were visualized by live cell Deltavision microscopy. z stacks (eight stacks 0.35 μ M apart) were acquired at 15-s intervals, deconvoluted, and projected into 2D images. Note the example of polymerization and spindle bending. The video is shown at 10 frames/s. Bar, 2 μ m.



Video 9. **The Bim1^{6D}-3x GFP phospho mutant localizes weakly to the midzone and disassembles efficiently.** The Bim1^{6D}-3x GFP phospho mutant and mCherry-Tub1 were visualized by live cell Deltavision microscopy. z stacks (eight stacks 0.35 μ M apart) were acquired at 15-s intervals, deconvoluted, and projected into 2D images. The video is shown at 10 frames/s. Bar, 2 μ m.

Table S1. **Yeast strains used in this study**

Strain number	Genotype
SWY 167	<i>Mat a, leu2, ura3-52, trp1, prb1-1122, pep4-3, pre1-451, Bim1-S-Tag-TEV-ZZ::KanMX</i>
TZY 39	<i>Mat α, his3Δ200, leu2-Δ1, ura3-52, ade2-101, lys2-801, TUB1-LYS2, bim1Δ::URA3, BIM1-3x GFP::HISMX5</i>
TZY 40	<i>Mat α, his3Δ200, leu2-Δ1, ura3-52, ade2-101, lys2-801, TUB1-LYS2, bim1Δ::URA3, bim1^{6A}-3x GFP::HISMX5</i>
TZY 41	<i>Mat α, his3Δ200, leu2-Δ1, ura3-52, ade2-101, lys2-801, TUB1-LYS2, bim1Δ::URA3, bim1^{6D}-3x GFP::HISMX5</i>
TZY 114	<i>Mat a, his3Δ200, leu2-3,112, lys2-801, trp1-1, BIM1^{WT}::KanMX, GFP-Tub1::URA</i>
TZY 115	<i>Mat a, his3Δ200, leu2-3,112, lys2-801, trp1-1, bim1^{6A}::KanMX, GFP-Tub1::URA</i>
TZY 116	<i>Mat a, his3Δ200, leu2-3,112, lys2-801, trp1-1, bim1^{6D}::KanMX, GFP-Tub1::URA</i>
TZY 143	<i>Mat α, his3Δ200, leu2-3,112, lys2-801, trp1-1, bim1Δ::KanMX, mCherry-Tub1::URA, BIM1^{WT}-3x GFP::HISMX5</i>
TZY 144	<i>Mat α, his3Δ200, leu2-3,112, lys2-801, trp1-1, bim1Δ::KanMX, mCherry-Tub1::URA, bim1^{6A}-3x GFP::HISMX5</i>
TZY 145	<i>Mat α, his3Δ200, leu2-3,112, lys2-801, trp1-1, bim1Δ::KanMX, mCherry-Tub1::URA, bim1^{6D}-3x GFP::HISMX5</i>
TZY 146	<i>Mat a, his3Δ200, leu2-3,112, lys2-801, trp1-1, bim1Δ::KanMX, GFP-Tub1::URA</i>