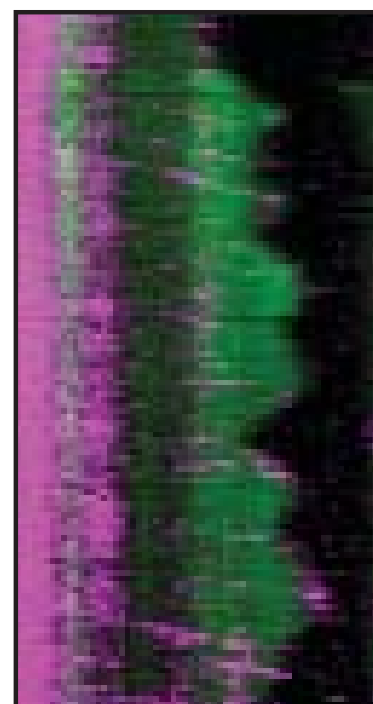
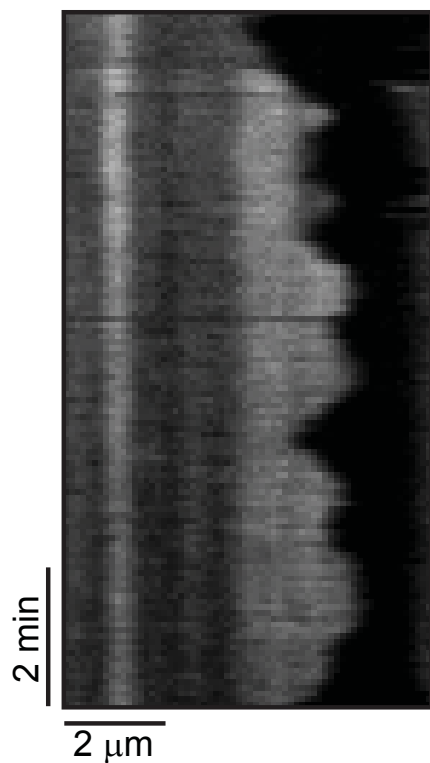


GFP-Tub1

Kip3-TagRFP-T
Rhodamine-seeds

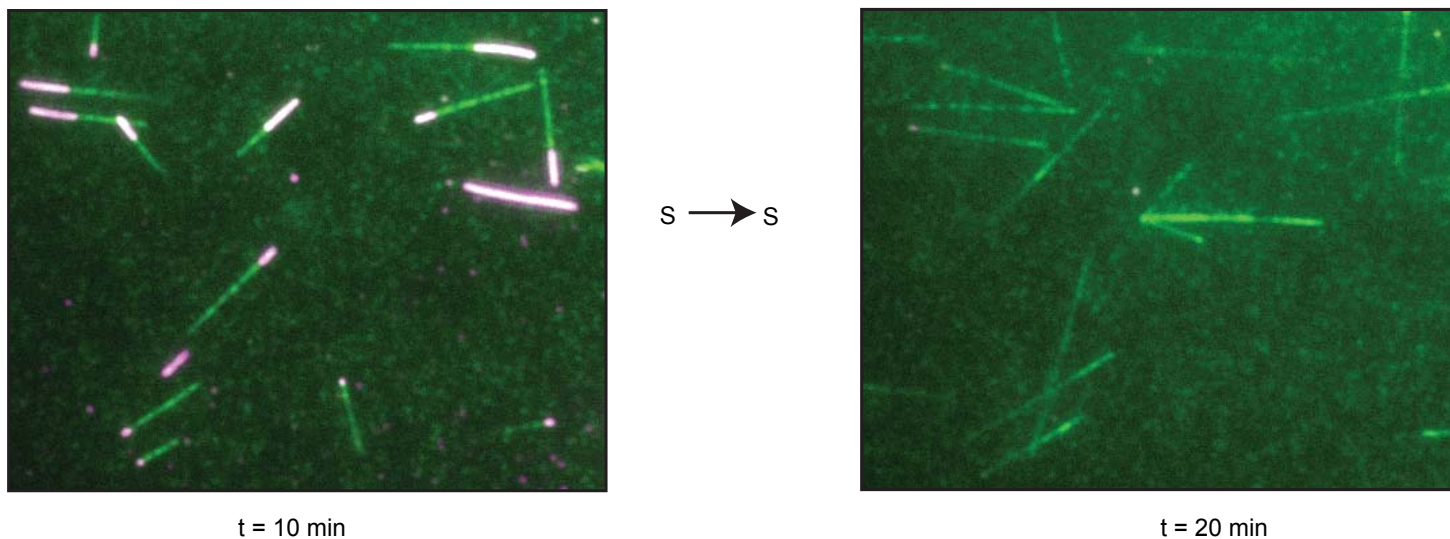
Kip3-TagRFP-T GFP-Tub1
Rhodamine-seeds



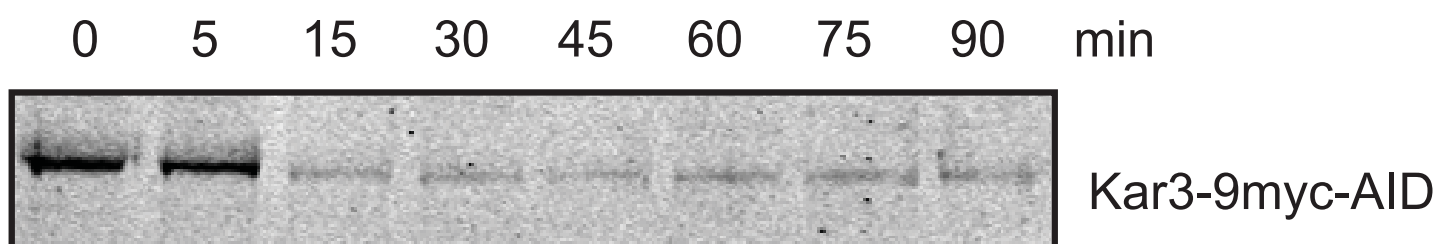
Supplemental Figure 1. Kymograph of Kip3-TagRFP-T along GFP-Tub1 MTs.

Motor proteins (magenta) will bind the MT (green) and move along the remaining length to the plus end.

Rhodamine-seeds GFP-Tub1



Supplemental Figure 2. Sequential Flow-through of S-phase Lysate. Fields of MTs and seeds in control experiments for lysate flow-through. MTs grew and were dynamic in lysate from S phase-arrested cells. More lysate was washed through the chamber and slightly interrupted the growth of existing MTs. After a short period, growth and dynamics resumed similar to those observed before the lysate replacement.



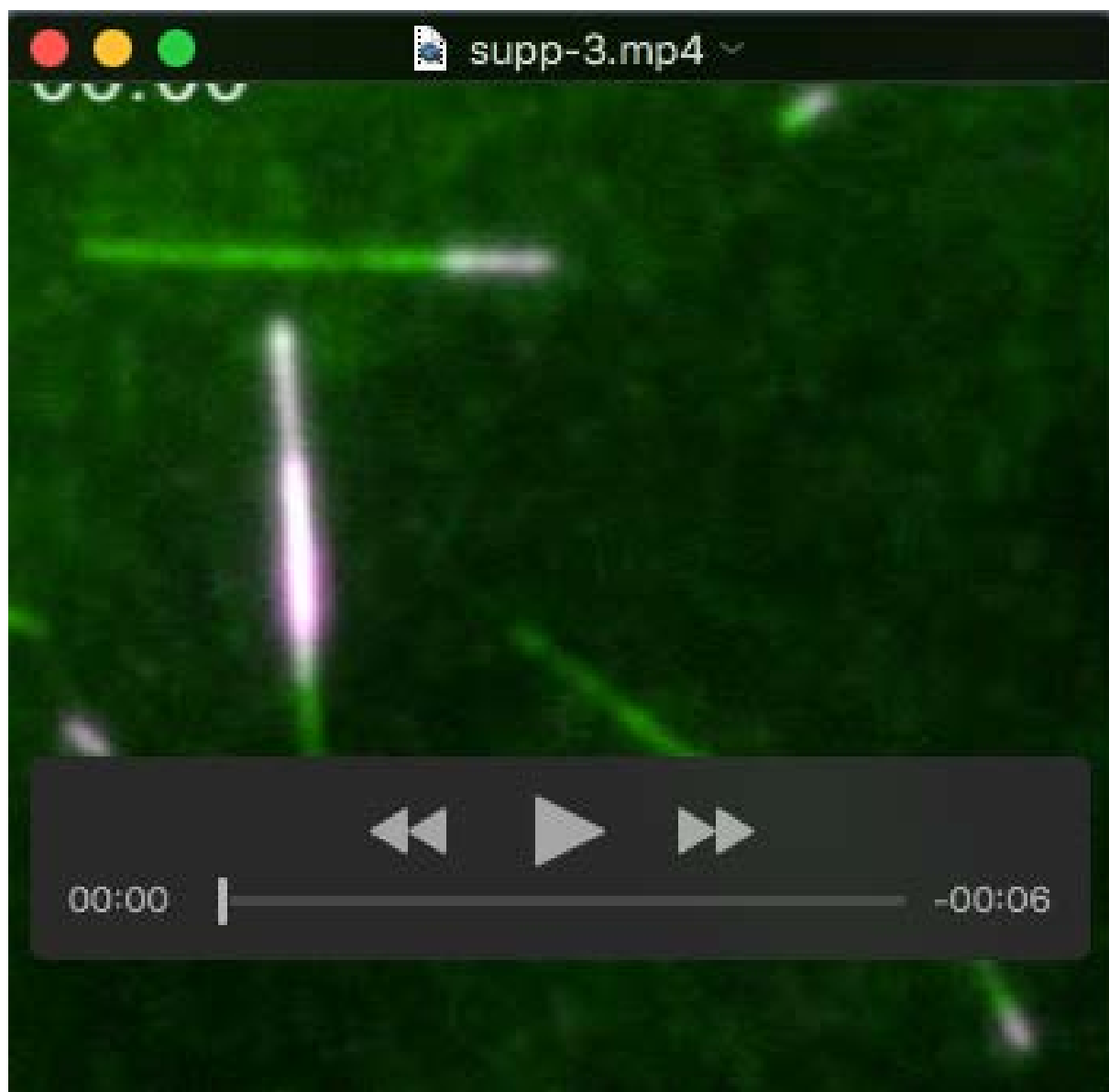
Supplemental Figure 3. Depletion of Kar3 from Cells. Western blot of protein lysate from fixed *KAR3-9myc-AID* cells treated with 250 μ M 3-indole acetic acid. Cells were harvested every 15 minutes and fixed.



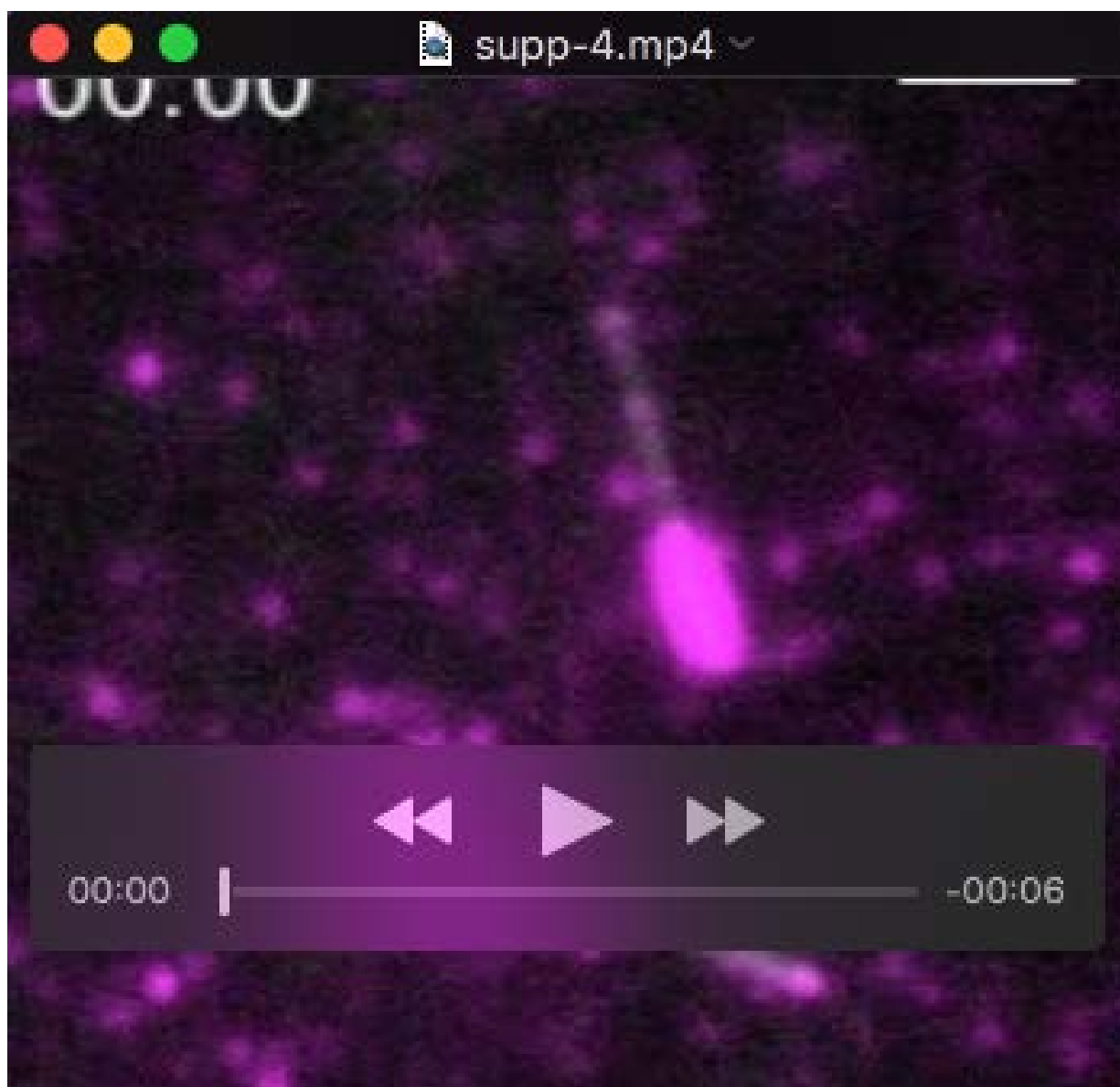
Supplemental Movie 1. MTs in lysate from S phase-arrested cells. GFP-Tub1 (green) and rhodamine-labeled seeds (magenta). Corresponds to kymographs in Figure 1A. Scale bar is 2 μm . Playback is 20fps.



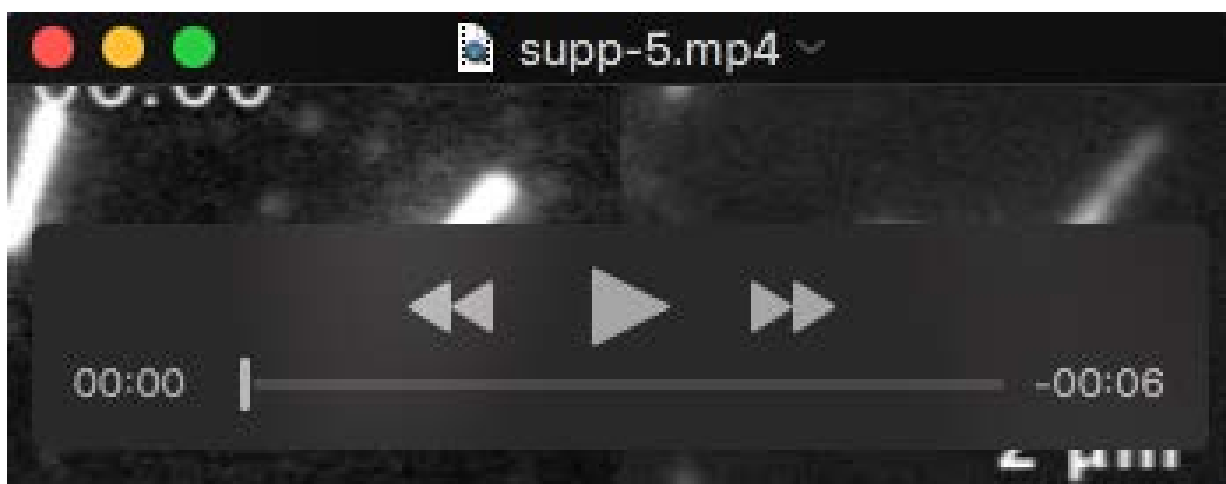
Supplemental Movie 2. MTs in lysate from metaphase-arrested cells. GFP-Tub1 (green) and rhodamine-labeled seeds (magenta). Corresponds to kymographs in Figure 1A. Scale bar is 2 μ m. Playback is 20fps.



Supplemental Movie 3. MTs in lysate from anaphase-arrested cells. GFP-Tub1 (green) and rhodamine-labeled seeds (magenta). Corresponds to kymographs in Figure 1A. Scale bar is 2 μm . Playback is 20fps.



Supplemental Movie 4. Association of Bim1 with MTs in lysate. A zoomed-in view of Bim1-TagRFP-T (magenta) associated with MTs (green) along their length and accumulated at the plus ends from lysate of cells arrested in S phase. Rhodamine-labeled seeds are also in magenta at the minus ends. Corresponds to kymographs in Figure 3A. Scale bar is 2 μ m. Playback is 20fps.



Supplemental Movie 5. Translocation of Kip3 along MTs in Lysate. Kip3-TagRFP-T (traveling dots) and rhodamine-labeled seeds (bright bars) on the left and GFP-tubulin MTs on the right from lysate of cells arrested in metaphase. Kip3-TagRFP-T puncta bound along the MT and moved towards the plus end. Corresponds to kymographs in Figure S1. Scale bar is 2 μm . Playback is 20fps.

Supplementary Table 1. Strains Used in this Study

Strain Name	Genotype
DDY3435	<i>MATα</i> , <i>lys2-801</i> , <i>his3Δ-200</i> , <i>leu2-3, 112</i> , <i>ura3-52::GFP-TUB1::URA3</i>
DDY5662	<i>MATα</i> , <i>lys2-801</i> , <i>his3Δ-200</i> , <i>leu2-3, 112</i> , <i>ura3-52::GFP-TUB1::URA3</i> , <i>cdc28-4</i>
DDY5663	<i>MATα</i> , <i>lys2-801</i> , <i>his3Δ-200</i> , <i>leu2-3, 112</i> , <i>ura3-52::GFP-TUB1::URA3</i> , <i>cdc7-1</i>
DDY5664	<i>MATα</i> , <i>lys2-801</i> , <i>his3Δ-200</i> , <i>leu2-3, 112</i> , <i>ura3-52::GFP-TUB1::URA3</i> , <i>cdc23-1</i>
DDY5665	<i>MATα</i> , <i>lys2-801</i> , <i>his3Δ-200</i> , <i>leu2-3, 112</i> , <i>ura3-52::GFP-TUB1::URA3</i> , <i>cdc15-2</i>
DDY5666	<i>MATα</i> , <i>lys2-801</i> , <i>his3Δ-200</i> , <i>leu2-3, 112</i> , <i>ura3-52::GFP-TUB1::URA3</i> , <i>BIM1-TagRFP-T::kanMX</i> , <i>cdc7-1</i>
DDY5667	<i>MATα</i> , <i>lys2-801</i> , <i>his3Δ-200</i> , <i>leu2-3, 112</i> , <i>ura3-52::GFP-TUB1::URA3</i> , <i>KIP3-TagRFP-T::HIS3MX</i> , <i>cdc23-1</i>
DDY5668	<i>MATα</i> , <i>lys2-801</i> , <i>his3Δ-200</i> , <i>leu2-3, 112</i> , <i>kip3Δ::kanMX</i> , <i>ura3-52::GFP-TUB1::URA3</i>
DDY5669	<i>MATα</i> , <i>lys2-801</i> , <i>his3Δ-200</i> , <i>leu2-3, 112</i> , <i>kip3Δ::kanMX</i> , <i>ura3-52::GFP-TUB1::URA3</i> , <i>cdc28-4</i>
DDY5670	<i>MATα</i> , <i>lys2-801</i> , <i>his3Δ-200</i> , <i>leu2-3, 112</i> , <i>kip3Δ::kanMX</i> , <i>ura3-52::GFP-TUB1::URA3</i> , <i>cdc7-1</i>
DDY5671	<i>MATα</i> , <i>lys2-801</i> , <i>his3Δ-200</i> , <i>leu2-3, 112</i> , <i>kip3Δ::kanMX</i> , <i>ura3-52::GFP-TUB1::URA3</i> , <i>cdc23-1</i>
DDY5672	<i>MATα</i> , <i>lys2-801</i> , <i>his3Δ-200</i> , <i>leu2-3, 112</i> , <i>kip3Δ::kanMX</i> , <i>ura3-52::GFP-TUB1::URA3</i> , <i>cdc15-2</i>
DDY5673	<i>MATα</i> , <i>lys2-801</i> , <i>his3Δ-200</i> , <i>leu2-3, 112</i> , <i>KAR3-9myc-AID::HIS3</i> , <i>TIR1::LEU2</i> , <i>ura3-52::GFP-TUB1::URA3</i>
DDY5674	<i>MATα</i> , <i>lys2-801</i> , <i>his3Δ-200</i> , <i>leu2-3, 112</i> , <i>KAR3-9myc-AID::HIS3</i> , <i>TIR1::LEU2</i> , <i>ura3-52::GFP-TUB1::URA3</i> , <i>cdc28-4</i>

DDY5675	<i>MATα, lys2-801, his3Δ-200, leu2-3, 112, KAR3-9myc-AID::HIS3, TIR1::LEU2, ura3-52::GFP-TUB1::URA3, cdc7-1</i>
DDY5676	<i>MATα, lys2-801, his3Δ-200, leu2-3, 112, KAR3-9myc-AID::HIS3, TIR1::LEU2, ura3-52::GFP-TUB1::URA3, cdc23-1</i>
DDY5677	<i>MATα, lys2-801, his3Δ-200, leu2-3, 112, KAR3-9myc-AID::HIS3, TIR1::LEU2, ura3-52::GFP-TUB1::URA3, cdc15-2</i>
DDY5678	<i>MATα, lys2-801, his3Δ-200, leu2-3, 112, KAR3-9myc-AID::HIS3, TIR1::LEU2, ura3-52::GFP-TUB1::URA3, kip3Δ::kanMX</i>
DDY5679	<i>MATα, lys2-801, his3Δ-200, leu2-3, 112, KAR3-9myc-AID::HIS3, TIR1::LEU2, ura3-52::GFP-TUB1::URA3, kip3Δ::kanMX, cdc28-4</i>
DDY5680	<i>MATα, lys2-801, his3Δ-200, leu2-3, 112, KAR3-9myc-AID::HIS3, TIR1::LEU2, ura3-52::GFP-TUB1::URA3, kip3Δ::kanMX, cdc7-1</i>
DDY5681	<i>MATα, lys2-801, his3Δ-200, leu2-3, 112, KAR3-9myc-AID::HIS3, TIR1::LEU2, ura3-52::GFP-TUB1::URA3, kip3Δ::kanMX, cdc15-2</i>
DDY5682	<i>MATα, lys2-801, his3Δ-200, leu2-3, 112, ura3-52::GFP-TUB1::URA3, cik1Δ::HIS3</i>
DDY5683	<i>MATα, lys2-801, his3Δ-200, leu2-3, 112, ura3-52::GFP-TUB1::URA3, vik1Δ::HIS3</i>

All strains were constructed for this study, except for DDY3435 (source: Drubin/Barnes lab). All strains are S288C background.