Supplemental Materials

Molecular Biology of the Cell

Wood *et al*.

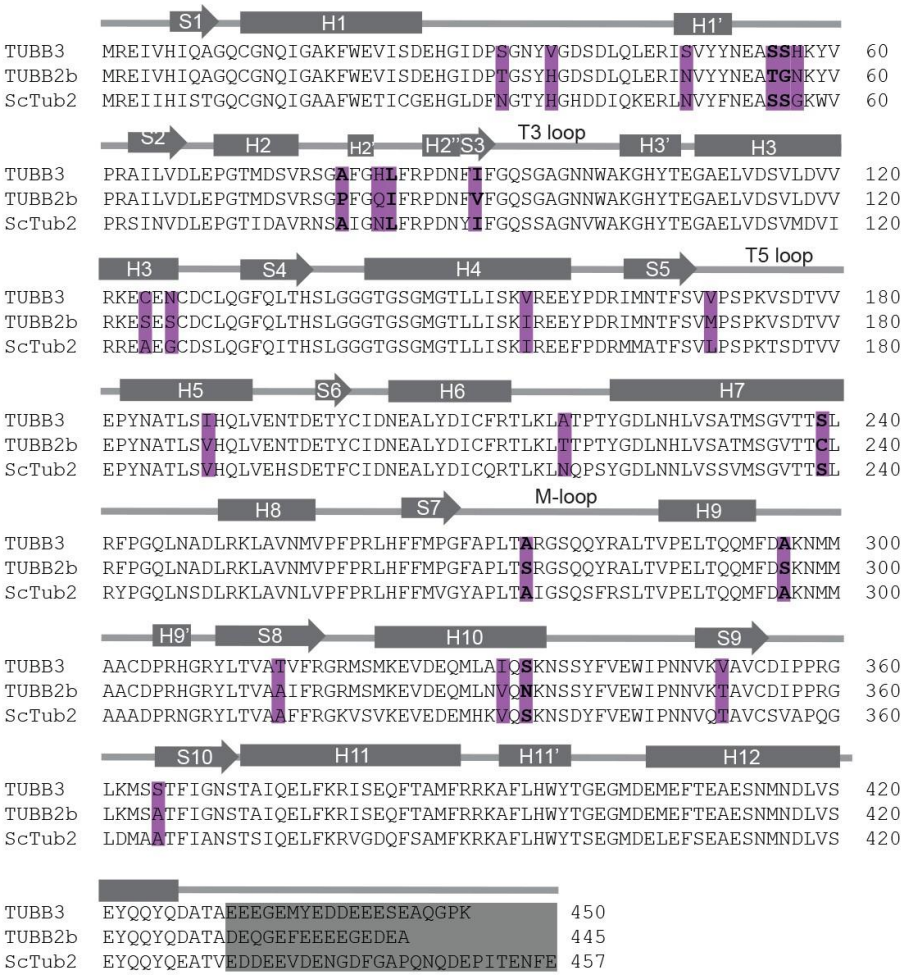

Figure S1
Alignment of the primary sequences of TUBB3, TUBB2B, and ScTub2. β3 variant residues are indicated by purple boxes and the divergent C-terminal region is highlighted in grey

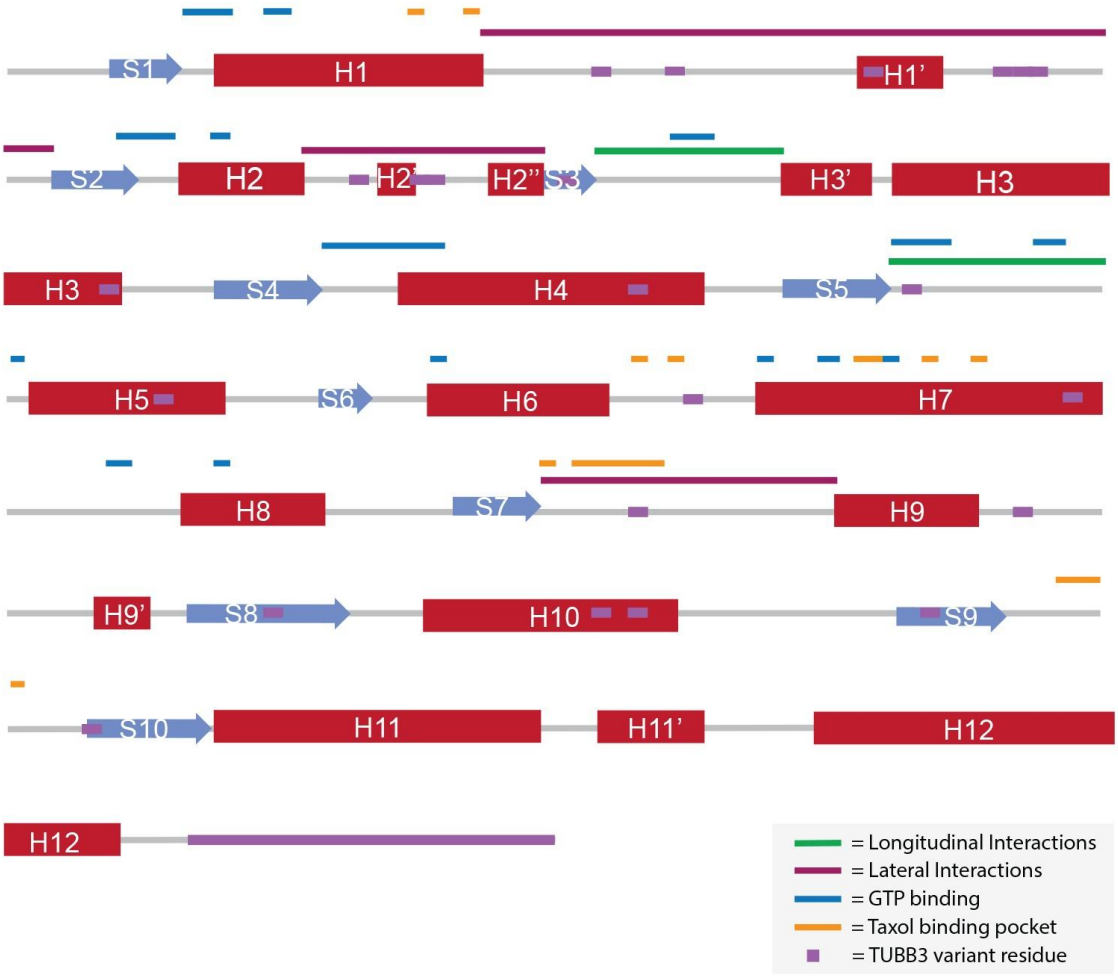

Figure S2.
Alignment of the β-tubulin secondary structure features with functional domains, indicated by color code. β3 variant residues are depicted by purple boxes overlaying the secondary structure features.

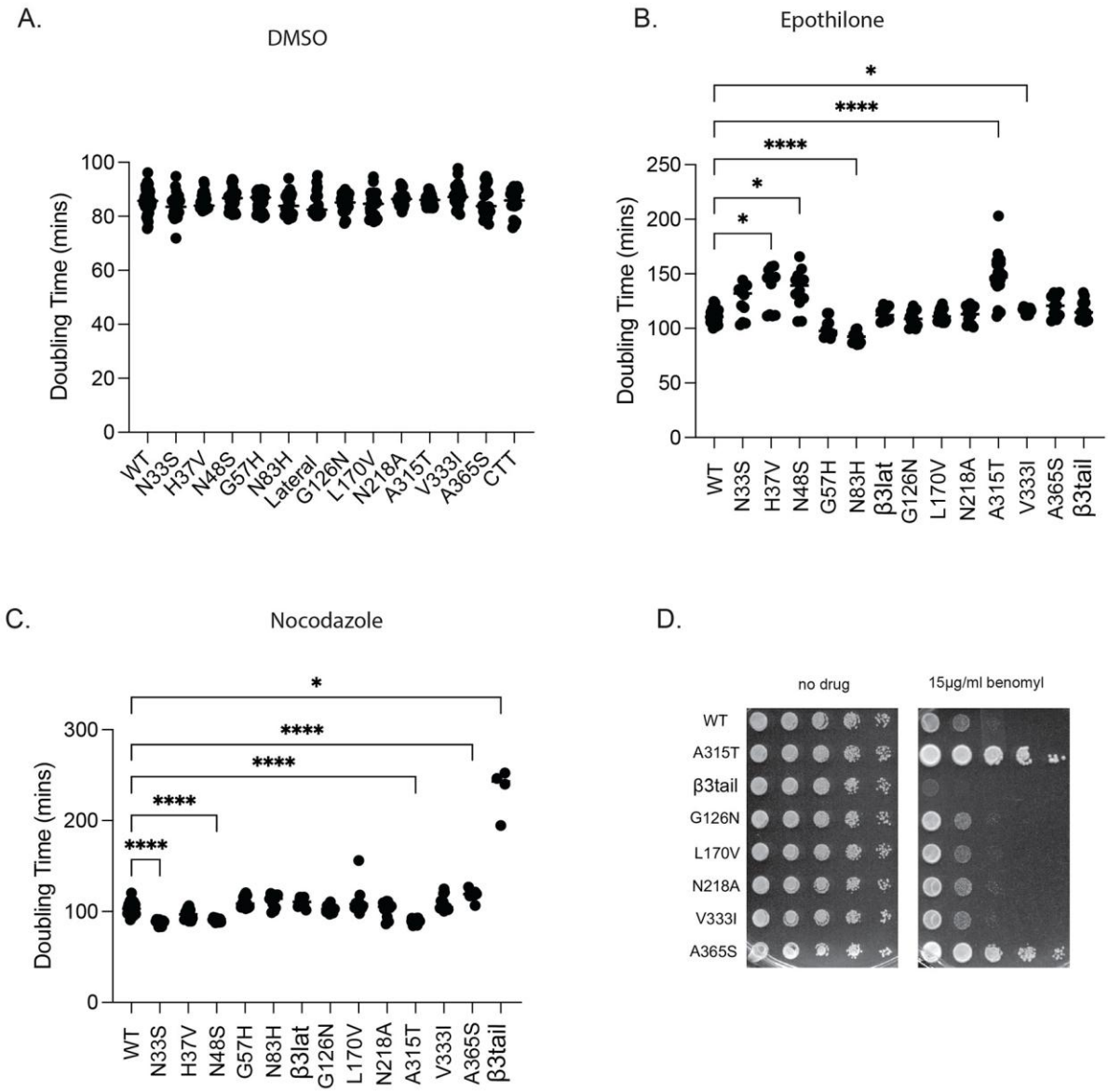

Figure S3.
(A) Liquid growth assay to determine the doubling time of yeast mutants expressing β3 variant residues. Doubling times are from triplicate experiments with biological replicates included in each experiment. No significant differences in doubling time were determined.
(B) Doubling time for the β3 variant residues in 15μM EpothiloneA.
(C) Doubling times for the β3 variant residues in 2.5μM Nocodazole.
(D) Serial dilution yeast cells expressing β3 variant residues indicated to the left of the plate. Cells were spotted to rich media or rich media supplemented with 15 μg/ml benomyl and grown for 2 days at 30C.

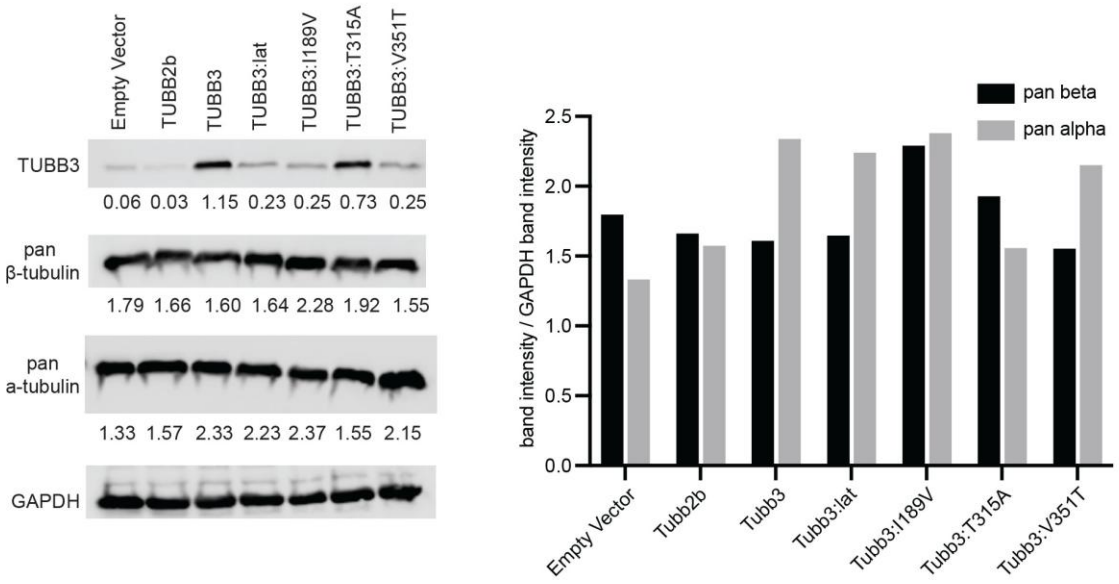

Figure S4.
a) Western blot of lysates from HeLa cells either with empty vector control or expressing the indicated β-tubulin. Blots are probed primary antibodies for β3-tubulin (Tuj1), pan β-tubulin (E7), pan α-tubulin (DM1A), or GAPDH. Values below the blots indicate band intensity divided by the GAPDH band intensity from the same lysate. B) Plot of band intensities divided by GAPDH band intensity from the same lysate.

Table S1- p-values compared to wild-type controls

	wild-type	wild-type	β3lat	β3lat	A315T	A315T	β3tail	β3tail
2.5 µM EpothiloneA	-	+	-	+	-	+	-	+
N	20	19	18	18	15	21	15	14
Mean Length (µm)	---	---	**<0.0001**	**<0.0001**	**0.0128**	**<0.0001**	**<0.0001**	0.1705
Coefficient of Variation	---	---	0.2022	**0.0004**	**0.0447**	0.0003	0.7346	0.1299
Polymerization Rate (µm/min)	---	---	0.1486	---	0.7983	---	0.3275	---
Depolymerization Rate (µm/min)	---	---	**0.0157**	---	0.8592	---	**0.0835**	---
Catastrophe Frequency (events per minute)	---	---	**0.0051**	---	0.12	---	0.424	---
Percent Paused	---	---	0.8073	---	0.5528	---	0.3311	---

Table S2. p-values compared to cells expressing TUBB3

	TUBB2b	TUBB3	TUBB3:lat	TUBB3:I189V	TUBB3:T315A	TUBB3:V351T
N	29	28	27	27	24	25
Growth Speed (µm/min)	**<0.0001**	---	**0.0029**	0.3988	0.9984	**<0.0001**
Comet Duration (seconds)	**<0.0001**	---	**<0.0001**	0.3407	0.1785	>0.9999
Growth Events / μm^2 / minute	0.3766	---	0.7495	**0.0323**	0.1891	0.3189

Table S3. Oligos used in this study

#	Name	Application	Sequence (5' to 3')	Species	Direction
1743	Tub2-N33S-forx	tub2 quikchange	tggtgagcacggtttggatttcagtgggacatatca	Yeast	Forward
1744	Tub2-N33S-rev	tub2 quikchange	tgatatgtcccactgaaatccaaaccgtgctcacca	Yeast	Reverse
886	tub2-H37V for	tub2 quikchange	cggtttggatttcaatgggacatatgttggccatgacgat	Yeast	Forward
887	tub2-H37V rev	tub2 quikchange	atcgtcatggccaacatatgtcccattgaaatccaaaccg	Yeast	Reverse
1745	Tub2-N48S-for	tub2 quikchange	cgatatccagaaggagagactgagcgtgtacttcaa	Yeast	Forward
1746	Tub2-N48S-rev	tub2 quikchange	ttgaagtacacgctcagtctctccttctggatatcg	Yeast	Reverse
888	tub2-G57H for	tub2 quikchange	gtgtacttcaacgaggcatcttctcataagtgggttccaagatctattaac	Yeast	Forward
889	tub2-G57H rev	tub2 quikchange	gttaatagatcttggaacccacttatgagaagatgcctcgttgaagtacac	Yeast	Reverse

890	tub2-N83H for	tub2 quikchange	gcaattctgccatcgggcatttgtttagacctgac	Yeast	Forward
891	tub2-N83H rev	tub2 quikchange	gtcaggtctaaacaaatgcccgatggcagaattgc	Yeast	Reverse
1883	Tub2-G126N-for	Gibson Assembly	atgttattagacgagaggccgaaaattgcgactcccttcaaggtt	Yeast	Forward
1884	Tub2-G126N-rev	Gibson Assembly	aaccttgaagggagtcgcaattttcggcctctcgtctaataacat	Yeast	Reverse
1885	Tub2-I155V-for	tub2 quikchange	acgcttttgatctcgaaggttagggaagagtttcctg	Yeast	Forward
1886	Tub2-I155V-rev	tub2 quikchange	caggaaactcttccctaaccttcgagatcaaaagcg	Yeast	Reverse
1887	Tub2-L170V-for	Gibson Assembly	ccaccttctccgtcgtgccctctccgaag	Yeast	Forward
1888	Tub2-L170V-rev	Gibson Assembly	cttcggagagggcacgacggagaaggtgg	Yeast	Reverse
1889	Tub2-V189I-for	tub2 quikchange	catacaatgccacgttgtctatacaccaattggtagaacactc	Yeast	Forward
1890	Tub2-V189I-rev	tub2 quikchange	gagtgttctaccaattggtgtatagacaacgtggcattgtatg	Yeast	Reverse
1891	Tub2-N218A-for	tub2 quikchange	catctgtcaaaggaccttaaagttggctcaaccttcttatggagatttgaac	Yeast	Forward
1892	Tub2-N218A-rev	tub2 quikchange	gttcaaatctccataagaaggttgagccaactttaaggtcctttgacagatg	Yeast	Reverse
1893	Tub2-A315T-for	tub2 quikchange	ggtagataccttaccgttgcaaccttctttagaggtaaag	Yeast	Forward
1894	Tub2-A315T-rev	tub2 quikchange	ctttacctctaaagaaggttgcaacggtaaggtatctacc	Yeast	Reverse
1895	Tub2-V3331-for	tub2 quikchange	ggaggtggaagatgaaatgcataaaatacaatctaaaaactcagactatttc	Yeast	Forward
1896	Tub2-V333I-rev	tub2 quikchange	gaaatagtctgagtttttagattgtattttatgcatttcatcttccacctcc	Yeast	Reverse
1897	Tub2-T351V-for	tub2 quikchange	ggaatggatccccaacaatgtgcaagttgctgtgtgttctg	Yeast	Forward
1898	Tub2-T351V-rev	tub2 quikchange	cagaacacacagcaacttgcacattgttggggatccattcc	Yeast	Reverse
1899	Tub2-A365S-for	tub2 quikchange	ctcaaggtttggacatggctagtactttcattgctaactcca	Yeast	Forward
1900	Tub2-A365S-rev	tub2 quikchange	tggagttagcaatgaaagtactagccatgtccaaaccttgag	Yeast	Reverse
1865	Tub2-N33S-H37V-for	tub2 quikchange	cgtcatggccgacatatgtcccactgaaatccaaaccgtgctcac	Yeast	Forward
1866	Tub2-N33S-H37V-rev	tub2 quikchange	gtgagcacggtttggatttcagtgggacatatgtcggccatgacg	Yeast	Reverse
2146	Hs-Tubb3-I189V-rev	Tubb3 quikchange	ccagctggtggacggacagcgtggc	Human	reverse
2147	Hs-Tubb3-I189V-for	Tubb3 quikchange	gccacgctgtccgtccaccagctgg	Human	forward
2148	Hs-Tubb3-T315A-rev	Tubb3 quikchange	cccggaacacggcggccaccgtcag	Human	reverse
2149	Hs-Tubb3-T315A-for	Tubb3 quikchange	ctgacggtggccgccgtgttccggg	Human	forward
2150	Hs-Tubb3-V351T-rev	Tubb3 quikchange	gatgtcacacacggccgtcttcacgttgttgggg	Human	reverse
2151	Hs-Tubb3-V351T-for	Tubb3 quikchange	ccccaacaacgtgaagacggccgtgtgtgacatc	Human	forward
462	TUB2 Ftest -300	screening	GATATCCTCC TCAAAACTGG TG	Yeast	Forward
802	Tub2+427 TRP Rev	screening	CATGTTGTAATCTTCTTTCTTTGGAAGCAGCAGTCCGAGCTC GTTTAAACCTCCTTACGC	Yeast	reverse

1829	pRS GA F	Gibson Assembly	AGACGGTCACAGCTTGTCTG		forward
1830	pRS GA R	Gibson Assembly	GCTTACAGACAAGCTGTGAC		reverse
1831	pJM74 GA F	Gibson Assembly	GACTCACTATAGGGCGAATTG		forward
1832	pJM74 GA R	Gibson Assembly	GTATTACAATTCACTGGCC		reverse

Table S4. Plasmids used in this study

Plasmid ID	Plasmid Name	Source
pJM0091/pSK1050	pUC19-LEU2::GFP-TUB1	Song and Lee, 2001
pJM0093/pAFS125	pTUB1-GFP-TUB1-URA3	Straight et al., 1997
pJM385	UAS-TUB2-UTR-Trp1	This study
pJM576	UAS-tub2(H37V)-UTR-Trp1	This study
pJM733	Tubb3 cDNA (IOH3755)	ThermoFisher
pJM734	Tubb2b cDNA (IOH3691)	ThermoFisher
pJM453	UAS-tub2(G57H)-UTR-Trp1	This study
pJM454	UAS-tub2(N83H)-UTR-Trp1	This study
pJM795	pCIG2-IRES-GFP-Macf18	This study
pJM832	pCIG2-Tubb2b-IRES-Macf18-GFP	This study
pJM861	pCIG2-Tubb3-IRES-Macf18-GFP	This study
pJM857	UAS-tub2(N33S)-UTR-Trp1	This study
pJM858	UAS-tub2(N48S)-UTR-Trp1	This study
pJM892	UAS-tub2(A315T)-UTR-Trp1 Iso 1	This study
pJM894	UAS-tub2(V333I)-UTR-Trp1 Iso 1	This study
pJM896	UAS-tub2(T351V)-UTR-Trp1 Iso 1	This study
pJM898	UAS-tub2(A365S)-UTR-Trp1 Iso 1	This study
pJM915	UAS-tub2(V189I)-UTR-Trp1 Iso 2	This study
pJM908	UAS-tub2(N218A)-UTR-Trp1 Iso 1	This study
pJM962	UAS-tub2(G126N)-UTR-Trp1 Iso 1	This study
pJM963	UAS-tub2(L170V)-UTR-Trp1 Iso 1	This study
pJM987	Tubb3-Tubb2lateral_pUC57-Kan	Genscript
pJM988	Tubb2b-Tubb3lateral_pUC57-Kan	Genscript
pJM939	UAS-tub2(N33S, H37V, N48H, G57H, N83H, G126N, I155V, V189I, N218A, A315T, V333I, T351V, A365S)-UTR-Trp1 Iso 2	This study
pJM795	pCIG2-IRES-GFP-Macf18	This study
pJM832	pCIG2-Tubb2b-IRES-GFP-Macf18	This study
pJM861	pCIG2-Tubb3-IRES- GFP-Macf18	This study
pJM992	pCIG2-Tubb3::Tubb2lateral-IRES- GFP-Macf18	This study
pJM993	pCIG2-Tubb3-I189V-IRES- GFP-Macf18	This study
pJM994	pCIG2-Tubb3-T315A-IRES- GFP-Macf18	This study
pJM995	pCIG2-Tubb3-V351T-IRES- GFP-Macf18	This study

Table S5. Yeast strains used in this study

Yeast Strain	Genotype	Source
yJM1837	*MATa* ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	Bi, E., Pringle, J. R., 1996
yJM533	MATa PDR1::pdr1-DBD-CYC8::LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM2306	MATa PDR1::pdr1-DBD-CYC8::LEU2 Tub1-GFP-URA ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5250	MATa tub2-N33S-TRP ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5282	MATa tub2-N33S-TRP PDR1::pdr1-DBD-CYC8::LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5283	MATa tub2-N33S-TRP PDR1::pdr1-DBD-CYC8::LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM3864	MATα tub2-H37V-TRP ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5721	MATα tub2-H37V-TRP PDR1::pdr1-DBD-CYC8::LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5722	MATα tub2-H37V-TRP PDR1::pdr1-DBD-CYC8::LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5253	tub2-N48S-TRP ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5556	MATα tub2-N48S-TRP PDR1::pdr1-DBD-CYC8::LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5558	MATα tub2-N48S-TRP PDR1::pdr1-DBD-CYC8::LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM3004	MATa tub2-G57H-TRP, ura3-52 lys2-801 leu2-Δ his3-Δ200 trp1-Δ63	This study
yJM3084	MATα tub2-G57H-TRP, Pdr1-dbd-CYC8-LEU ura3-52 lys2-801 leu2-Δ his3-Δ200 trp1-Δ63	This study
yJM3085	MATa tub2-G57H-TRP, Pdr1-dbd-CYC8-LEU ura3-52 lys2-801 leu2-Δ his3-Δ200 trp1-Δ63	This study
yJM3010	MATα tub2-N83H-TRP, ura3-52 lys2-801 leu2-Δ his3-Δ200 trp1-Δ63	This study
yJM3087	MATa tub2-N83H-TRP, Pdr1-dbd-CYC8-LEU ura3-52 lys2-801 leu2-Δ his3-Δ200 trp1-Δ63	This study
yJM3088	MATα tub2-N83H-TRP, Pdr1-dbd-CYC8-LEU ura3-52 lys2-801 leu2-Δ his3-Δ200 trp1-Δ63	This study
yJM5257	MATa tub2-N33S, H37V, N48S, G57H, N83H-TRP ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5303	MATa tub2-N33S, H37V, N48S, G57H, N83H-TRP PDR1::pdr1-DBD-CYC8-LEU ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5304	MATα tub2-N33S, H37V, N48S, G57H, N83H-TRP PDR1::pdr1-DBD-CYC8-LEU ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5300	MATa tub2-N33S, H37V, N48S, G57H, N83H-TRP Tub1-GFP-URA PDR1::pdr1-DBD-CYC8-LEU ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5731	MATα tub2-G126N-TRP, ura3-52 lys2-801 leu2-Δ his3-Δ200 trp1-Δ63	This study
yJM5706	MATa tub2-G126N-TRP PDR1::pdr1-DBD-CYC8::LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5707	MATa tub2-G126N-TRP PDR1::pdr1-DBD-CYC8::LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5733	MATα tub2-L170V-TRP, ura3-52 lys2-801 leu2-Δ his3-Δ200 trp1-Δ63	This study
yJM5751	MATα tub2-L170V-TRP, PDR1::pdr1-DBD-CYC8-LEU2 ura3-52 lys2-801 leu2-Δ his3-Δ200 trp1-Δ63	This study

yJM5752	MATa tub2-L170V-TRP, PDR1::pdr1-DBD-CYC8-LEU2 ura3-52 lys2-801 leu2-Δ his3-Δ200 trp1-Δ63	This study
yJM5479	MATα tub2-N218A-TRP ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5517	MATα tub2-N218A-TRP PDR1::pdr1-DBD-CYC8::LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5548	MATa tub2-N218A-TRP PDR1::pdr1-DBD-CYC8::LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5799	MATa tub2-A315T-TRP ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5446	MATa tub2-A315T-TRP PDR1::pdr1-DBD-CYC8::LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5447	MATα tub2-A315T-TRP PDR1::pdr1-DBD-CYC8::LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5793	MATa tub2-A315T-TRP Tub1-GFP-URA PDR1::pdr1-DBD-CYC8::LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5482	MATα tub2-V333I-TRP ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5520	MATα tub2-V333I-TRP PDR1::pdr1-DBD-CYC8::LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5737	MATa tub2-V333I-TRP PDR1::pdr1-DBD-CYC8::LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5796	MATα tub2-A365S-trp ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5450	MATa tub2-A365S-TRP PDR1::pdr1-DBD-CYC8-LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5451	MATα tub2-A365S-TRP PDR1::pdr1-DBD-CYC8-LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM1518	MATα Tub2-423^TUBB3::TRP ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	Fees et al. 2016
yJM2395	MATα Tub2-423^TUBB3::TRP PDR1::pdr1-DBD-CYC8::LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	Fees et al. 2016
yJM2396	MATa Tub2-423^TUBB3::TRP PDR1::pdr1-DBD-CYC8::LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM5546	MATa Tub2-423^TUBB3::TRP PDR1::pdr1-DBD-CYC8::LEU2 Tub1-GFP-URA ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJM2435	MATA/α tub2-TRP/TUB2 ura3-52/ura3-52 lys2-801/lys2-801 leu2-Δ1/leu2-Δ1 his3-Δ200/his3-Δ200 trp1-Δ63 /trp1-Δ63	This study
yJM5596	MATA/α tub2-TRP/TUB2 Tub1-GFP-URA Tub1-GFP-LEU ura3-52/ura3-52 lys2-801/lys2-801 leu2-Δ1/leu2-Δ1 his3-Δ200/his3-Δ200 trp1-Δ63 /trp1-Δ63	This study
yJM5704	MATA/α TUB2/tub2::Tubb3 full mimic-trp ura3-52/ura3-52 lys2-801/lys2-801 leu2-Δ1/leu2-Δ1his3-Δ200/his3-Δ200 trp1-Δ63 /trp1-Δ63	This study
yJM5827	MATA/α TUB2/tub2::Tubb3 Full mimic-trp Tub1-GFP-URA Tub1-GFP-LEU ura3-52/ura3-52 lys2-801/lys2-801 leu2-Δ1/leu2-Δ1 his3-Δ200/his3-Δ200 trp1-Δ63 /trp1-Δ63	This study
yJM5463	MATA/α TUB2/Tub2-423::TUBB3-trp Tub1-GFP-URA ura3-52/ura3-52 lys2-801/lys2-801 leu2-Δ1/leu2-Δ1 his3-Δ200/his3-Δ200 trp1-Δ63 /trp1-Δ63	This study
yJM5600	MATA/α TUB2/tub2-N33S, H37V, N48S, G57H, N83H, G126N, I155V, T218A, A315T, V333I, T351V, A365S-TRP Tub1-GFP-URA Tub1-GFP-LEU ura3-52/ura3-52 lys2-801/lys2-801 leu2-Δ1/leu2-Δ1 his3-Δ200/his3-Δ200 trp1-Δ63 /trp1-Δ63	This study
yJM1517	MATA/α Tub2-423^TUBB3::TRP/TUB2 ura3-52/ura3-52 lys2-801/lys2-801 leu2-Δ1/leu2-Δ1 his3-Δ200/his3-Δ200 trp1-Δ63 /trp1-Δ63	This study
yJM5598	MATA/α TUB2/Tub2-423::TUBB3-trp Tub1-GFP-URA Tub1-GFP-LEU ura3-52/ura3-52 lys2-801/lys2-801 leu2-Δ1/leu2-Δ1 his3-Δ200/his3-Δ200 trp1-Δ63 /trp1-Δ63	Park et. Al, 2021
yJM2065	MATA/α tub2::CaUra3/Tub2 ura3-52/ura3-52 lys2-801/lys2-801 leu2-Δ1/leu2-Δ1 his3-Δ200/his3-Δ200 trp1-Δ63 /trp1-Δ63	Park et al., 2021