Supplemental material

Hornung et al., http://www.jcb.org/cgi/content/full/jcb.201403081/DC1

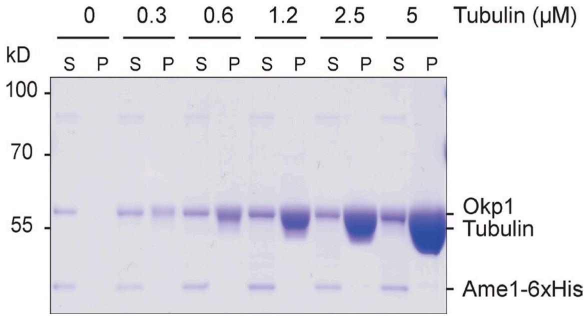

Figure S1. **Microtubule cosedimentation assay with recombinant AO complex.** S and P denote supernatant and pellet, respectively, after centrifugation with taxol-stabilized microtubules. Note that the AO complex is found exclusively in the supernatant fraction.

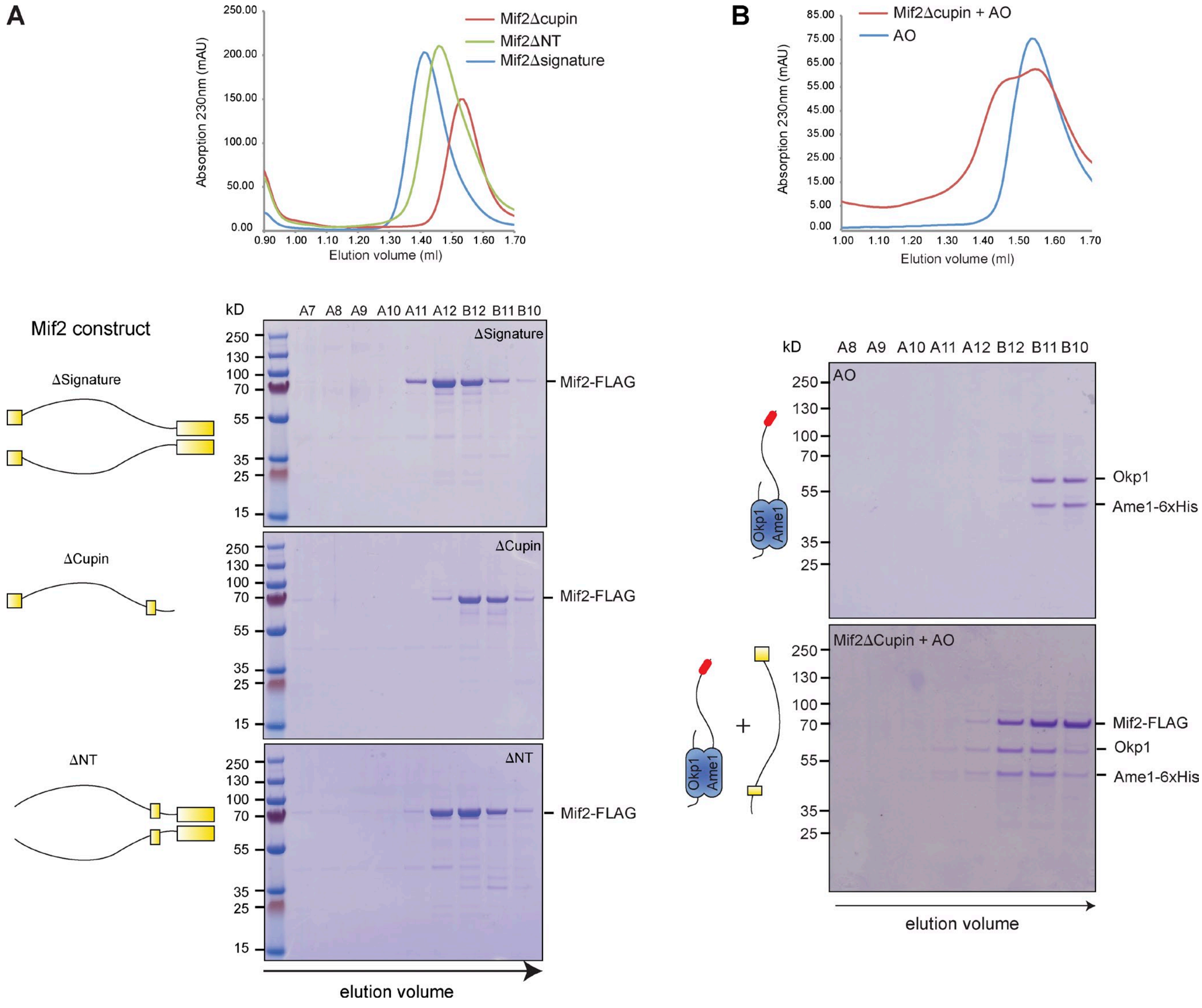

Figure S2. **Molecular requirements for Mif2 dimerization and AO binding.** (A) SEC experiment of different Mif2 constructs on a Superose 6 column. The depicted experiment is representative of *n* > 3 binding assays under identical conditions. (B) SEC of the AO complex alone (top) or in combination with the Mif2ΔCupin mutant (5 μM for each complex). Superose 6 PC 3.2/30 column was used for the experiment. The depicted experiment is representative of *n* > 3 binding assays under identical conditions. The same Ame1–Okp1 data are also presented in Fig. 7 B.

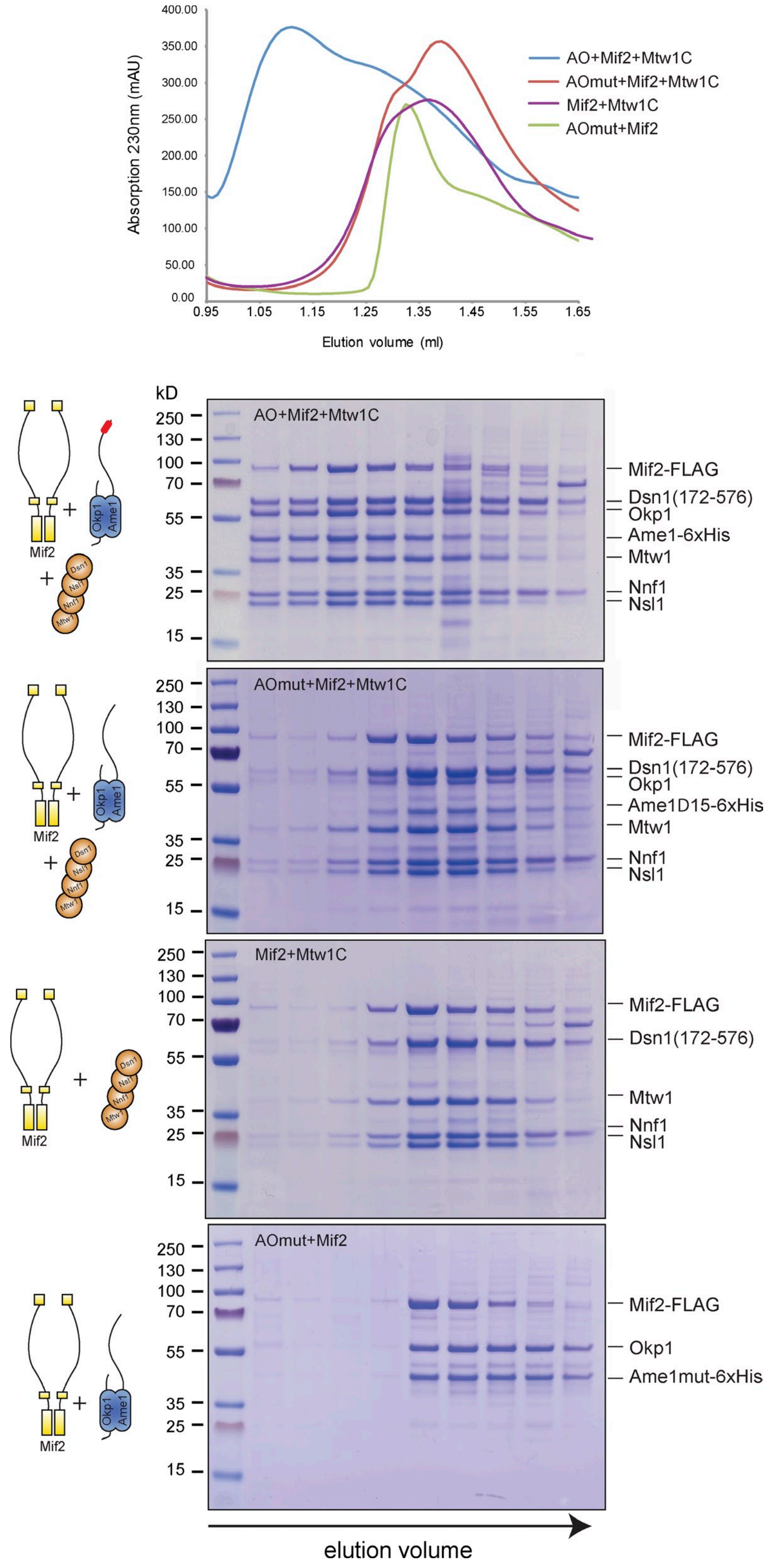

Figure S3. **Deletion of the Ame1 motif prevents the cooperative assembly of a large Mif2–AO–Mtw1C complex.** SEC experiment on a Superose 6 PC 3.2/30 column of full-length AO, Mtw1 complex, and Mif2 (top); the same combination with AO containing the Ame1$^{15\text{–}325}$ mutation (second row); the Mtw1 complex and Mif2 (third row); and AO with the Ame1$^{15\text{–}325}$ mutation and Mif2 (bottom). Note that formation of an early eluting Mif2–AO–Mtw1 complex depends on the Mtw1 binding motif in AO. Absorption profile corresponding to the gels is given at the top of the figure. The depicted experiment is representative of *n* > 3 binding assays under identical conditions. The same Mif2 + AO + Mtw1C data are also presented in Fig. 7 D.

Table S1. **Plasmids used in this study**

Number	Plasmid
PPH7	pST39-Mtw1/Nsl1/Nnf1-6×His/Dsn1
PPH13	pST39-Mtw1/Nsl1/Nnf1-6×His
PPH62	pET28-Dsn1$^{172–576}$
PPH173	pST39-Mtw1/Nnf1-6×His
PPH20	pST39-Mtw1/Nsl1-6×His/Dsn1
PPH35	pETDuett-Spc24-6×His/Spc25
PPH18	pACYCDuet1-Ndc80-6×His/Nuf2
PPH60	pST39-Ctf19/Mcm21/Okp1/Ame6×His
PPH64	pST39-Ctf19/Mcm21/Okp1/Ame6×FLAG
PPH71	pST39-Ctf19-6×His/Mcm21
PPH74	pST39-Okp1-Ame1-6×His
PPH117	pCOLA-Iml3/Chl4-FLAG
PPH123	pET28-6×His-Mif2
PPH108	pST39-Mtw1/Nsl1/Nnf1/6×His-Dsn1
PPH96	pST39-Mtw1/Nnf1-6×His/Ame1-FLAG
PH174	pST39-Okp1/Ame1$^{15–324}$-6×His
PPH112	pRS316-Ame1^{WT}
PPH111	pRS316-Ame1$^{15–324}$
PPH145	pRS306- Ame1^{WT}-6×-FLAG
PPH146	pRS306- Ame1$^{15–324}$-6×-FLAG
PPH156	pRS306-Ame1^{WT}-EGFP
PPH157	pRS306-Ame1$^{15–324}$-EGFP
PPH160	pRS306-Ame1^{R3D}-EGFP
PPH170	pRS306-Ame1^{R10D}-EGFP
PPH168	pRS306-Ame1^{R12D}-EGFP
PPH169	pRS306-Ame1^{R10A}-EGFP
PPH167	pRS306-Ame1^{R12A}-EGFP
PPH172	pGEX6P1-Ame1$^{1–30}$
PPH171	pGEX6P1-Ame1$^{1–114}$
pSW181	Mif2-Flag in pFL
pSW183	Mif2-ΔSignature-FLAG in pFL
pSW184	Mif2-ΔCupin-FLAG in pFL
pSW186	Mif2-ΔNT-FLAG in pFL
pSW189	Strep-Dsn1 in pFL with Mtw1, Nnf1, Nsl1
pSW190	Mif2-Flag + Mtw1 complex untagged in pFL
pSW192	Mif2 + −250 wild-type XhoI–Not1 into pRS306
pSW194	Mif2-ΔSignature in pRS306
pSW193	Mif2-ΔNT in pRS306
pSW195	Mif2-ΔCupin in pRS306

Table S2. **Yeast strains used in this study**

Strain	Genotype
SWY536	MAT a, tor1-1, fpr1::loxP-Leu2-loxP, RPL13A-2xFKBP12::lox-TRP1-loxP, Ame1-FRB::KanMX
YPH 18	MAT a, tor1-1, fpr1::loxP-Leu2-loxP, RPL13A-2xFKBP12::lox-TRP1-loxP, Ame1-FRB::KanMX, pRS316-Ame1::URA
YPH 19	MAT a, tor1-1, fpr1::loxP-Leu2-loxP, RPL13A-2xFKBP12::lox-TRP1-loxP, Ame1-FRB::KanMX, pRS316-Ame1$^{15–324}$::URA
YPH 24	MAT a, tor1-1, fpr1::loxP-Leu2-loxP, RPL13A-2xFKBP12::lox-TRP1-loxP, Ame1-FRB::KanMX, Ame1-6×FLAG::URA
YPH 25	MAT a, tor1-1, fpr1::loxP-Leu2-loxP, RPL13A-2xFKBP12::lox-TRP1-loxP, Ame1-FRB::KanMX, Ame1$^{15–324}$6×FLAG::URA
YPH 26	MATa/alpha, leu2-3,112/leu2-3,112, ura3-52/ura3-52, ade2-1/ADE2, lys2/LYS, CNN1/cnn1Δ::HIS3, Ame1::URA3
YPH 27	MATa/alpha, leu2-3,112/leu2-3,112, ura3-52/ura3-52, ade2-1/ADE2, lys2/LYS, CNN1/cnn1Δ::HIS3, Ame1(15–324)::URA3
YPH 29	MAT a, tor1-1, fpr1::loxP-Leu2-loxP, RPL13A-2xFKBP12::lox-TRP1-loxP, Ame1-FRB::KanMX, Ame1-6×FLAG::URA, Pds1-HA::natNT2
YPH 30	MAT a, tor1-1, fpr1::loxP-Leu2-loxP, RPL13A-2xFKBP12::lox-TRP1-loxP, Ame1-FRB::KanMX, Ame1$^{15–324}$6×FLAG::URA, Pds1-HA::natNT2
YPH 31	MAT a, tor1-1, fpr1::loxP-Leu2-loxP, RPL13A-2xFKBP12::lox-TRP1-loxP, Ame1-FRB::KanMX, Ame1-EGFP::URA
YPH 32	MAT a, tor1-1, fpr1::loxP-Leu2-loxP, RPL13A-2xFKBP12::lox-TRP1-loxP, Ame1-FRB::KanMX, Ame1-EGFP$^{15–324}$::URA
YPH 33	MAT a, tor1-1, fpr1::loxP-Leu2-loxP, RPL13A-2xFKBP12::lox-TRP1-loxP, Ame1-FRB::KanMX, Ame1-EGFP::URA, Mif2-mCherry::His
YPH 34	MAT a, tor1-1, fpr1::loxP-Leu2-loxP, RPL13A-2xFKBP12::lox-TRP1-loxP, Ame1-FRB::KanMX, Ame1-EGFP$^{15–324}$::URA, Mif2-mCherry::His
YPH 35	MAT a, tor1-1, fpr1::loxP-Leu2-loxP, RPL13A-2xFKBP12::lox-TRP1-loxP, Ame1-FRB::KanMX, Ame1-EGFP::URA, Mtw1-mCherry::His
YPH 37	MAT a, tor1-1, fpr1::loxP-Leu2-loxP, RPL13A-2xFKBP12::lox-TRP1-loxP, Ame1-FRB::KanMX, Ame1-EGFP$^{15–324}$::URA, Mtw1-mCherry::His
YPH 41	MAT a, tor1-1, fpr1::loxP-Leu2-loxP, RPL13A-2xFKBP12::lox-TRP1-loxP, Ame1-FRB::KanMX, Ame1-R3D, R10D, R12D -6×FLAG::URA
YPH 43	MAT a, tor1-1, fpr1::loxP-Leu2-loxP, RPL13A-2xFKBP12::lox-TRP1-loxP, Ame1-FRB::KanMX, Ame1-EGFP-R3A::URA
YPH 44	MAT a, tor1-1, fpr1::loxP-Leu2-loxP, RPL13A-2xFKBP12::lox-TRP1-loxP, Ame1-FRB::KanMX, Ame1-EGFP-R10D::URA
YPH 45	MAT a, tor1-1, fpr1::loxP-Leu2-loxP, RPL13A-2xFKBP12::lox-TRP1-loxP, Ame1-FRB::KanMX, Ame1-EGFP-R12A::URA
YPH 46	MAT a, tor1-1, fpr1::loxP-Leu2-loxP, RPL13A-2xFKBP12::lox-TRP1-loxP, Ame1-FRB::KanMX, Ame1-EGFP-R12D::URA
SWY903	Mat a, lys2-801, leu2-3,112, trp1, mif2Δ::HIS3, ura3-52::Mif2-WT::URA3
SWY905	Mat a, lys2-801, leu2-3,112, mif2Δ::HIS3, ura3-52::Mif2-ΔN::URA3
SWY907	Mat a, ade2-1, leu2-3,112, mif2Δ::HIS3, ura3-52::Mif2-ΔCupin::URA3
SWY909	Mat alpha, leu2-3,112, lys2-801, mif2Δ::HIS3, ura3-52::Mif2-ΔN::URA3, Mtw1-GFP::KanMX
SWY383	MAT a; leu2, ura3-52, trp1, prb1-1122, pep4-3, pre1-451, Ame1-6×His-6×Flag::KanMX
SWY397	MATa/alpha, leu2-3,112/leu2-3,112, ura3-52/ura3-52, ade2-1/ADE2, lys2/LYS, Ame1/ame1Δ::HIS3
SWY911	Mat alpha, ura3-52, leu2-3,112, lys2-801, Mtw1-GFP::KanMX, Spc42-RFP::HIS3
SWY909	Mat alpha, leu2-3,112, lys2-801, mif2Δ::HIS3, ura3-52::Mif2-ΔN::URA3, Mtw1-GFP::KanMX
SWY918	Mat a, leu2-3,112, lys2-801, mif2Δ::HIS3, ura3-52::Mif2-ΔN::URA3, cnn1Δ::KanMX
SWY922	Mat a, his3Δ200, Ame1-FRB::KanMX, tor1-1, tetR-GFP::LEU2, fpr1 Δ::LEU2, RPL13-FKBP12::TRP1, tetOx112::URA3
SWY923	Mat a, his3Δ200, Ame1-FRB::KanMX, tor1-1, tetR-GFP::LEU2, fpr1Δ::LEU2, RPL13-FKBP12::TRP1, tetOx112::URA3 + pCEN(Ame1-WT)::HIS
SWY924	Mat a, his3Δ200, Ame1-FRB::KanMX, tor1-1, tetR-GFP::LEU2, fpr1Δ::LEU2, RPL13-FKBP12::TRP1, tetOx112::URA3 + pCEN(Ame1-15–324)::HIS

Table S3 provides a comprehensive list of all interprotein cross-links found by the MS analysis of the Mif2–AO–Mtw1 complex.