Supplementary Figure Legends

Figure S1: Time-course of NE fusion (HMG1-GFP)

A cell expressing Htb2p-mRFP (ATY2835) was crossed with a cell expressing HMG1-GFP (ATY1528). Note that the appearance of HMG1-GFP signal in the *trans*-NE precedes its arrival in the *trans* cortical ER. Ch: chromatin.

Figure S2: FRAP for HMG1-GFP, GFP-Prm3p, GFP-Esc1p

Haploid cells were photobleached three times (arrows) and recovery of signal was then followed every 5 seconds. The cells expressed either HMG1-GFP (ATY1528), GFP-Esc1p (ATY1550) or GFP-Prm1p (ATY3236). The dark blue line indicates the area which was bleached, the pink line is from a nearby region of NE, and the yellow line is equidistant cortical ER (for HMG1-GFP).

Figure S3: Impact of DTT

A: Time course of inhibition of karyogamy by DTT.

Cells expressing Htb2p-mRFP (ATY2835, ATY2289) were used for a standard two-step assay. After recovery from the nocodazole plates, 96-99% had separate nuclei, 0-3% showed nuclear contact, and 1% had fused nuclei. They were then further incubated +/- 2mM DTT for 2, 3 or 4 hr and zygotes were classified as have separate nuclei, nuclei in contact with each other, or fused nuclei. DTT causes extensive inhibition of fusion and corresponding increases in images of contact.

B: The impact of DTT is not reduced by overexpression of Kar2p.

Two wt strains carrying pGAL-KAR2 were pregrown overnight in 2% galactose medium and crossed (ATY3253, AY3254), by comparison to the same parental strains carrying an empty plasmid (ATY3257, ATY3227). After preincubation on nocodazole-galactose plates, they were transferred to galactose medium with 0-5mM DTT and incubated for 2 hr. The percent of cells with separate nuclei, nuclei in contact, and fused nuclei was then determined. Note that the dose-response curve for DTT is not affected by the attempts at overexpression of Kar2p. All media included 2% raffinose and 0.1% fructose.

Figure S4: Representative FRAP evaluation of *cis-trans* diffusion of HMG1-GFP

A haploid cell (1), an early prezygote (2) and a later prezyogte (3) were studied. The prezyogtes are from crosses of HMG1-GFP-expressing cells (ATY1528) with non-fluorescent wt cells. In each of the three series, a small square area of NE was photobleached (white square). Recording was either from the same square (dark blue line), a second part of the same NE (pink square and line), and area from the *trans* NE (yellow square and line), or an irrelevant area of a bystander cell (light blue square and line). Images were collected every three seconds. Bleaching is indicated by the vertical arrows. Note the close *cis* coupling between the different areas of the NE and the lack of *trans* coupling even after dilation of the nexus.

Figure S5: Evaluation of the *cis*-first model of NE fusion.

As indicated in Figure S1, this model has the unattractive property of possibly allowing momentary leakage of nuclear content to the cytoplasm. It nevertheless appears topologically equivalent to mitotic NE breakdown in higher eukaryotes and half-open

mitosis, e.g. (Straube *et al.*, 2005). Moreover, even chromosomes can escape from the yeast nucleus during congression in *kar1-1* (Dutcher, 1981). We have taken first steps to evaluate this model by following a nucleoplasmic tracer (GFP-TBP) during karyogamy. We do not see evidence of its transient leakage to the cytoplasm during karyogamy. What is illustrated is a cross between cells expressing GFP-TBP1 (ATY2836 x ATY2289). It shows that the intense nuclear signal present before nuclear contact is not detectably released to the cytoplasm upon karyogamy. Control experiments show that at least 10 minutes are required for any cytoplasmic GFP-TBP to be extensively imported into the nucleus. N: nucleus, V: vacuole.

Figure S6: Impact of latrunculin A on congression

Crosses between ATY3629 (which expresses Spc42-mRFP, Tub1-GFP and Nup49-GFP) and ATY2289 (which expresses Htb2p-mRFP) were interrupted after two hrs and cells were then transferred to liquid medium containing 0.2 mM latrunculin A for 30-60 min before imaging. Note the erratic position of the SPBs (*) and the mis-orientation of the microtubule cables (arrows). The red images are included to make the tagged SPB visible (*). The lower pair includes a grazing section of the lower nucleus which does not make the NPCs visible.

References:

Dutcher, S.K. (1981). Internuclear transfer of genetic information in kar1-1/KAR1 heterokaryons in Saccharomyces cerevisiae. Mol Cell Biol *1*, 245-253.

Straube, A., Weber, I., and Steinberg, G. (2005). A novel mechanism of nuclear envelope break-down in a fungus: nuclear migration strips off the envelope. Embo J *24*, 1674-1685.

Table SI. Strains Used in this Study

Name	Relevant Genotype	Strain Origin	Plasmid Name
ATY1454	MAT a Spc42-GFP	IAY18, J. Kilmartin	
ATY1455	MAT α SPC42-CFP	J. Kilmartin, JK1659	
ATY1467	MAT a *kar2-1*	MS1111, M. Rose	
ATY1513	MAT α SIK1-mRFP	E. O'Shea	
ATY1515	MAT α SPC42-mRFP	E. O'Shea	
ATY1528	MAT α $HMG1_{1-702}$-GFP	SFNY1162, S. Ferro-Novick	
ATY1529	MAT a $HMG1_{1-702}$-GFP	SFNY1163, S. Ferro-Novick	
ATY1550	MAT α GAL-GFP-ESC1	YDZ49, R. Sternglanz	
ATY1713	MAT a *kar2-1* [pNUP49-GFP]	MS1111	AT635
ATY1774	MAT a SPC42-mRFP	This study, E. O'Shea	
ATY1812	MAT α SIK1-mRFP SPC42-mRFP	This study	
ATY1816	MAT a NUP49-GFP SPC42-mRFP	This study	
ATY1817	MAT a NUP49-GFP SPC42-mRFP	This study	
ATY1897	MAT a MID2-GFP NUP49-GFP SIK1-mRFP SPC42-mRFP	This study	
ATY1916	MAT α MID2-GFP NUP49-GFP SPC42-mRFP	This study	
ATY1917	MAT a SIK1-mRFP SPC42-mRFP	This study	
ATY1919	MAT a MID2-GFP SIK1-mRFP SPC42-mRFP	This study	
ATY1920	MAT α MID2-GFP SIK1-mRFP SPC42-mRFP	This study	
ATY2089	MAT a *cdc12-6*	E. Bi #743	
ATY2102	MAT a GAL-GFP-ESC1	YDZ49, R. Sternglanz	
ATY2138	MAT α *sec18-1* [pNUP49-GFP]	RSY271	AT735
ATY2226	MAT α NUP49-GFP	This study	
ATY2229	MAT a *cdc48-3 bub2Δ mad2Δ*	YZY36, Y. Zheng	AT986

	[pNUP49-GFP]		
ATY2632	MAT α *cdc48-3 bub2Δ mad2Δ* [pNUP49-GFP]	YZY36, Y. Zheng	AT986
ATY2289	MAT α HTB2-mRFP	W303	
ATY2404	MAT a [pNM22]	W303	AT948
ATY2524	MATa HTB2-mRFP leu2::TUB1-GFP		AT994, AT1180
ATY2538	MAT a *sec18-1* [pNUP49-GFP]	ATY2138, W303	AT986
ATY2549			
ATY2594	MAT a HTB2-mRFP [pNUP49-GFP]	ATY2835	AT986
ATY2673	MAT a HTB2-GFP	W303	
ATY2699	MAT a *esc1Δ mlp1Δ mlp2Δ*	YDZ237, R. Sternglanz	
ATY2782	MAT α *prm3Δ*	T. Lithgow	
ATY2784	MAT alpha *ndc10-1* HTB2-mRFP	W303, PWY611, J. KIlmartin	
ATY2823	MAT a [pMET25-GFP-PRM3]	W303	AT1143
ATY2835	MAT a HTB2-mRFP	W303	
ATY2836	MAT a [pGFP-TBP]	W303	AT622
ATY3004	MAT α SPC42-mRFP [pGAR1-GFP]	E. O'Shea	AT624
ATY3034	MAT a SPB42-mRFP leu2::TUB1-GFP		AT994
ATY3053	MAT a HTB2-mRFP [pRS316]	ATY2835	
ATY3129	MAT α [pCox4-dsRed]	W303	AT988
ATY3768	MAT a *ndc10-1* tetO (CENV) tetR-GFP	C. Antony T4734	

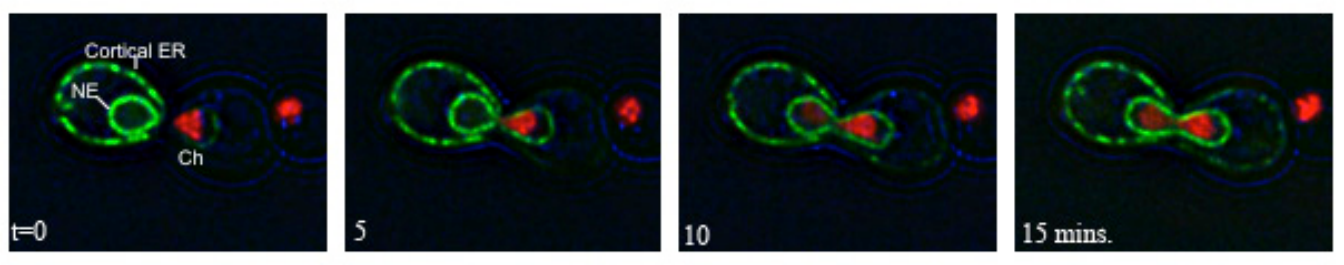
ATY3131	MAT α *prm3Δ* [pNUP49-GFP]	T. Lithgow	AT986
ATY3149	MAT α [pMET25-GFP-PRM3]	W303	AT1143
ATY3196	MAT α *ura3*::mRFP-HDEL	W303	AT1180
ATY3197	MAT a *kar2-1 ura3*::mRFP-HDEL	MS1111, M. Rose	AT1180
ATY3198	MAT a *kar1-1*	MY686, 8960-14B, M. Rose	
ATY3199	MAT a *kar1-1 ura3*::mRFP-HDEL	MY686, 8960-14B, M. Rose	AT1180
ATY3216	MAT a *prm3D ura3*::mRFP-HDEL	T. Lithgow	AT1180
ATY3220	MAT α [pGAR1-GFP]	W303	AT624
ATY3222	MAT a [pSEC63-GFP]	W303	AT1038
ATY3223	MAT a [pGFP-HDEL]	W303	AT1182
ATY3227	MAT a HTB2-mRFP [pRS316]	ATY2289	
ATY3236	MAT a [pMET-GFP-PRM3]	W303	AT1143
ATY3253	MAT a HTB2-mRFP [pGAL-KAR2]	ATY2289	AT1174
ATY3254	MAT α HTB2-mRFP [pGAL-KAR2]	ATY2835	AT1174
ATY3257	MAT a HTB2-mRFP [pRS316]	ATY2835	
ATY3277	MAT a *prm3Δ* $HMG1_{1-702}$-GFP	This study	
ATY3298	MAT α *prm3Δ* [pGAR1-GFP]	T. Lithgow	AT624
ATY3331	MAT a [pSEC63-mRFP]	W303	AT1187
ATY3342	MAT a *ura3*::mRFP-HDEL leu2::TUB1-GFP		
ATY3358	MAT α HTB2-mRFP [pNUP49-GFP]	ATY2289	AT986
ATY3359	MAT α [pNUP49-GFP]	W303	AT986
ATY3365	MAT α SPC42-CFP *ura3*::mRFP-HDEL	JK659, J. Kilmartin	AT1180
ATY3373	MAT a *kar1-1* [pGFP-HDEL]	MY686 (8960-14B)	AT1182
ATY3384	MAT α *sec18-1 ura3*::mRFP-HDEL	W303	
ATY3405	MAT a [pNUP49-GFP]	W303	AT986
ATY3456	MAT a *cdc12-6* HTB2-mRFP	ATY2089, ATY2289	

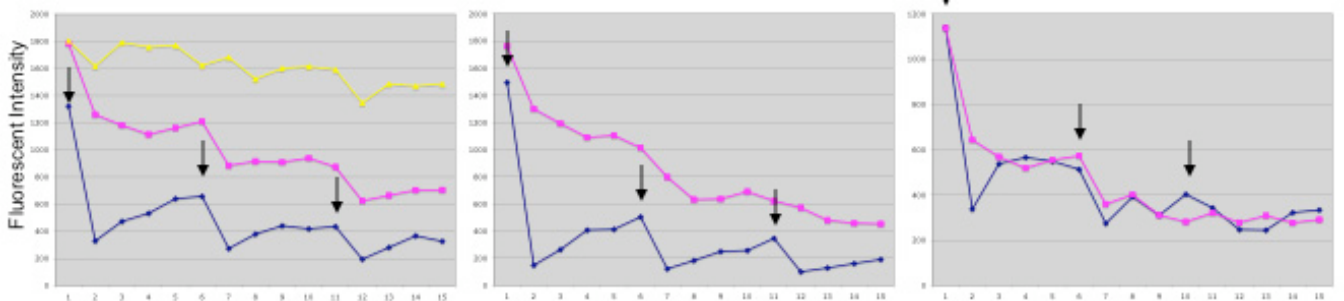
ATY3458	MAT α *cdc12-6* HTB2-mRFP	ATY2089, ATY2289	
ATY3474	MAT a *ire1Δ* HTB2-mRFP	This study, PW370 (P. Walter), ATY2289	
ATY3476	MAT α *ire1Δ* HTB2-mRFP	This study, PW370 (P. Walter), ATY2289	
ATY3525	MAT a HMG1-GFP SPC42-mRFP	This study	
ATY3528	MAT a HTB2-mRFP [pHAC1i]	ATY2835	AT1197
ATY3529	MAT a HTB2-mRFP [pHAC1i]	ATY2289	AT1197
ATY3629	MAT a SPC42-mRFP TUB1-GFP [pNUP49-GFP]	This study	AT986
ATY3871	MAT a *sec1-1 ura3*::GFP HTB2-mRFP	AFB138=SF263-1C, A. Franzusoff; ATY2289	AT1144
ATY3872	MAT α *sec1-1* HTB2-mRFP	AFB138=SF263-1C, A. Franzusoff; ATY2289	

Note: Isogenic pairs of strains were generated by transforming with pGAL-HO, inducing HO expression, plating on glucose medium, recovering colonies of the correct mating type, and eliminating the plasmid on 5'-FOA.

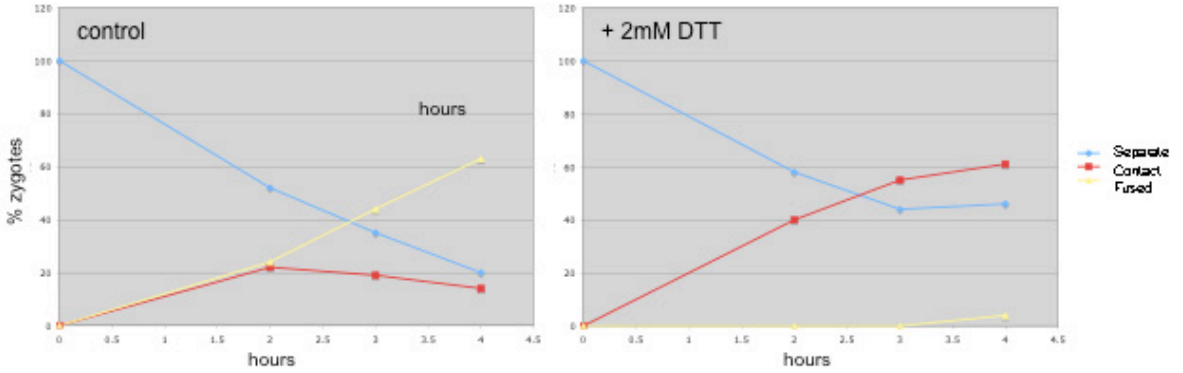
Table SII. Plasmids Used in this Study

Name	Original Name, Plasmid Type	Expression Unit	Source/Reference
AT161	pGAL-HO, CEN/URA3	GAL-HO	I. Herskowitz
AT622	pGFP-TBP, CEN/HIS3	GFP-TBP	A. Weil
AT624	ZUT3, CEN/URA3	GAR1-GFP	P.E. Gleizes
AT635	pUN100-LEU2-GFP-NUP49	NUP49-GFP	V. Doye
AT948	pNM22, CEN/URA3	MET25-HTB2-GFP	L. Pemberton
AT986	p251, CEN/URA3	NUP49-GFP	V. Doye
AT988	M376, CEN/URA3	COX4-dsRd	R. Jensen
AT994	BJ1351, LEU2, integ.	TUB1-GFP	J. Cooper
AT1011	BJ1333, URA3, integ.	TUB1-GFP	J. Cooper
AT1038	PJK59, CEN/URA3	SEC63-GFP	W. Prinz
AT1143	YCPyeGFP-PRM3, CEN/TRP1	MET25-GFP-PRM3 (full length)	T. Lithgow
AT1144	pEG220, URA3, integ.	GFP-SSO1 fusion	E. Grote
AT1174	pMR1341, CEN/URA3	GAL-KAR2	J. Brodsky/M. Rose
AT1180	pTi-kmRFP$_i$, TRP1, integ.	mRFP-HDEL	N. Dean
AT1182	pWP1055, CEN/URA3	GFP-HDEL	W. Prinz
AT1197	pPW1015=pJC835, CEN/HIS3	HAC1i	P. Walter
AT1037	pSV3, ARS/TRP1	lacO repeat in YRP17	S. Velmurugan

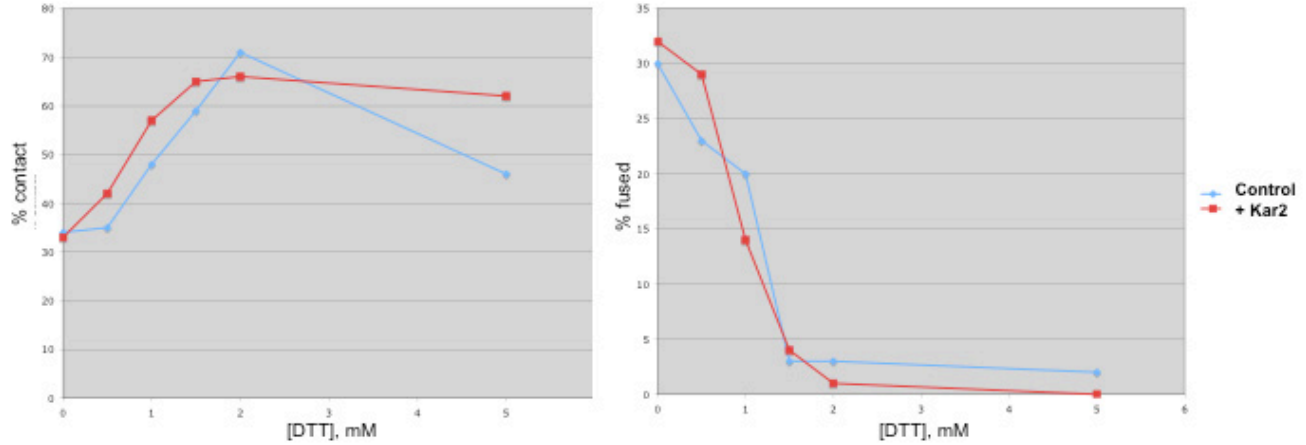

Cortical ER
NE
Ch
t=0
5
10
15 mins.

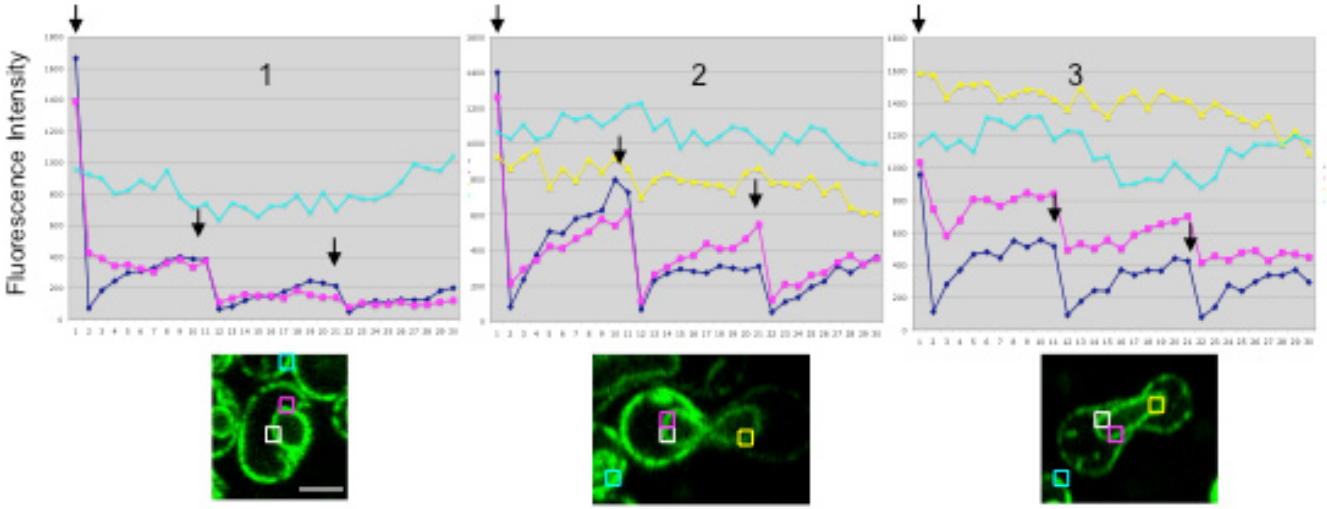

Fluorescent Intensity
HMG1-GFP
GFP-ESC1
GFP-PRM3

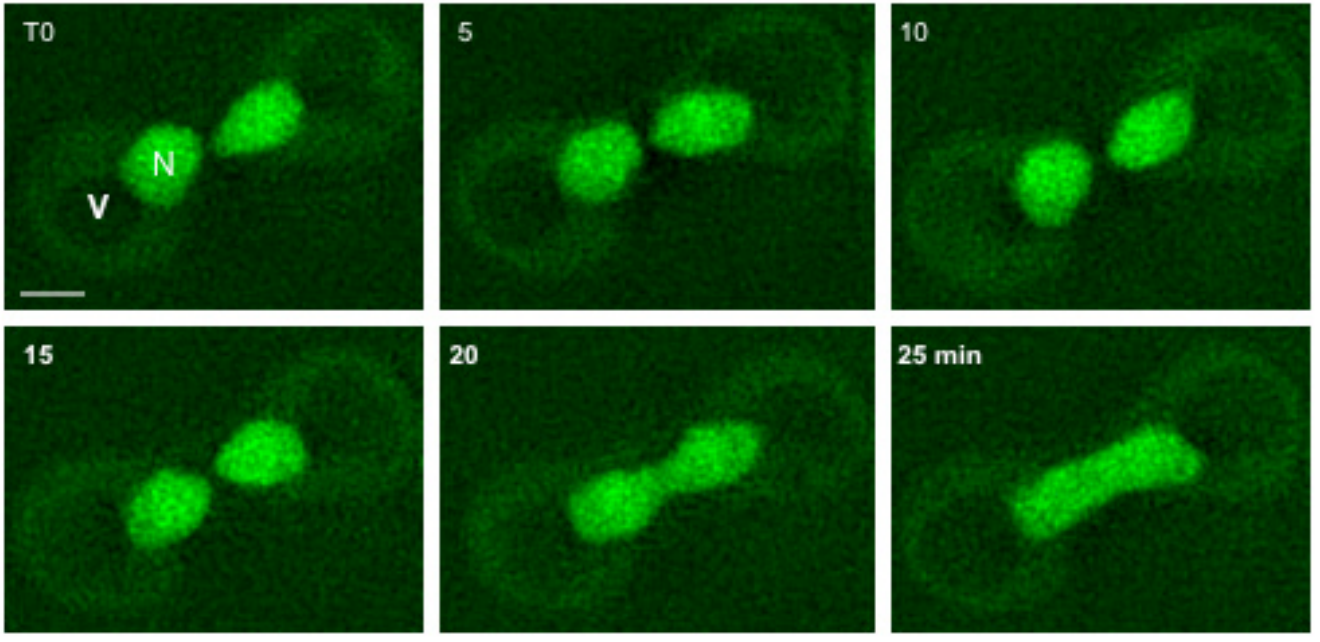
S3A

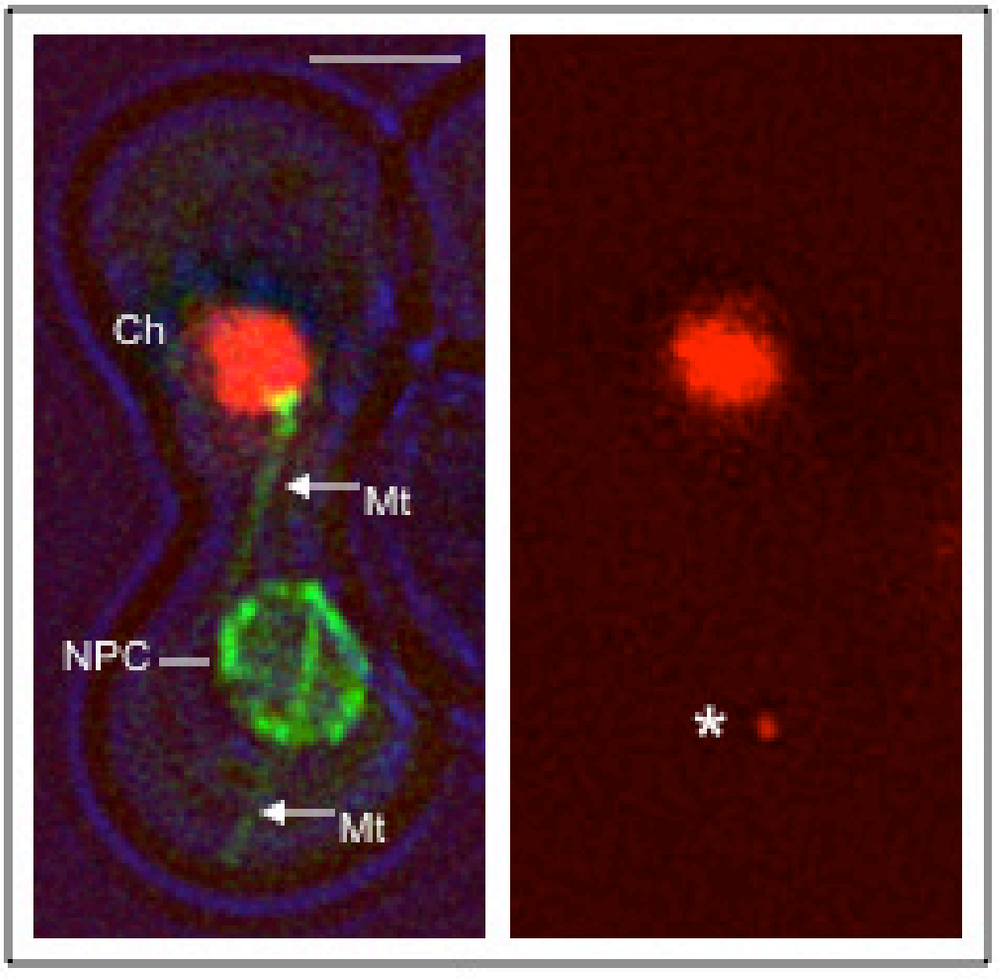

control
hours
% zygotes
hours
+ 2mM DTT
hours
Separate
Contact
Fused

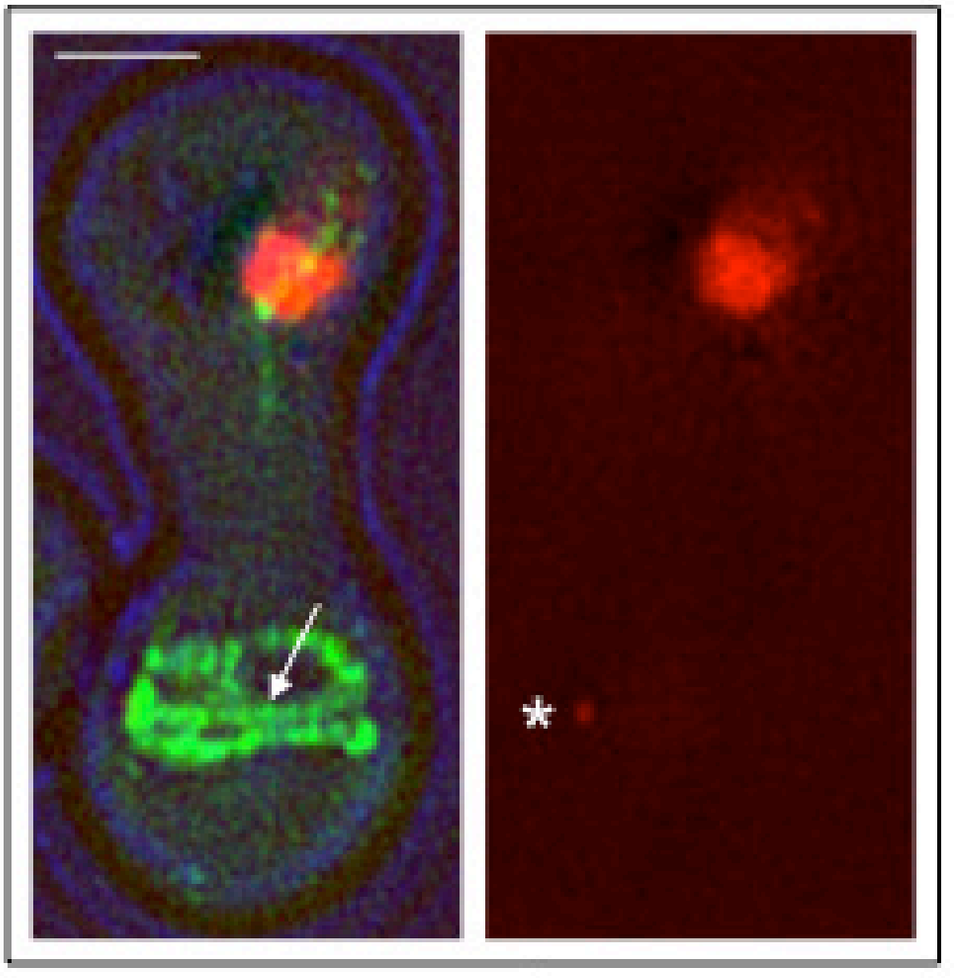
S3B

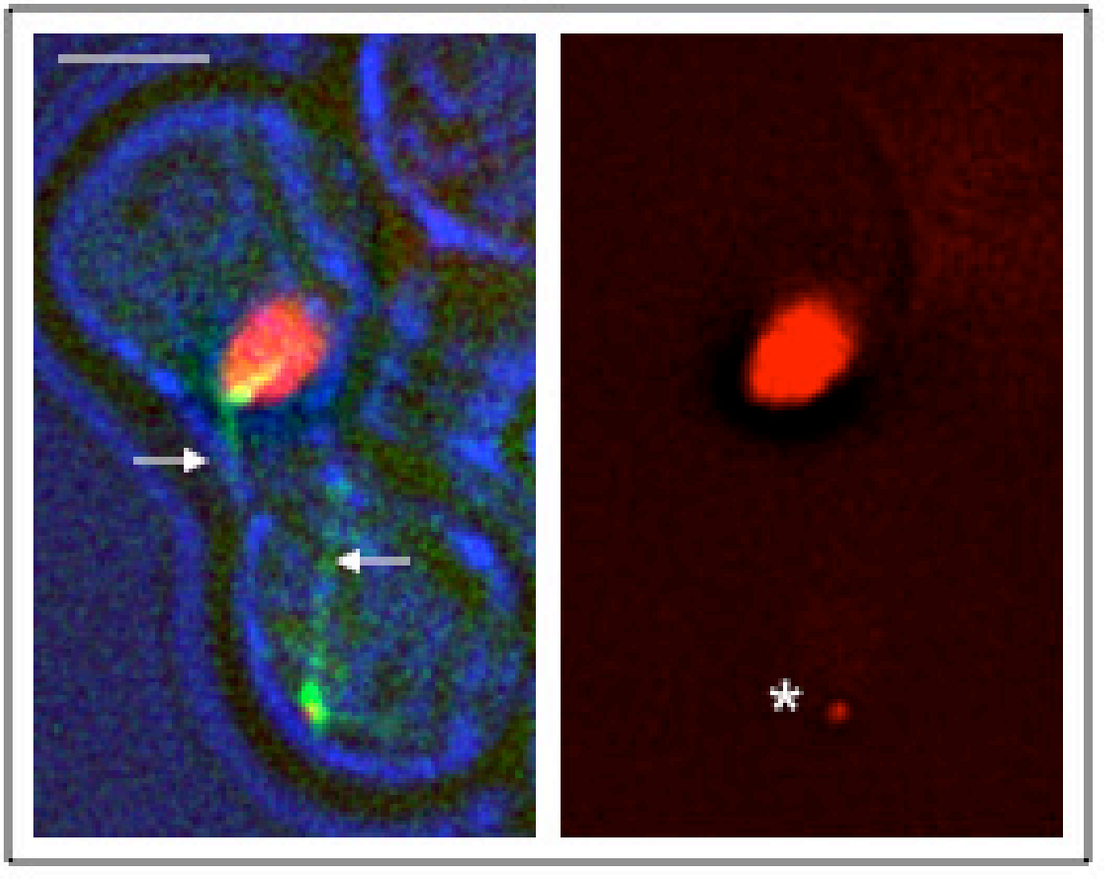

% contact
[DTT], mM
% fused
[DTT], mM
Control
+ Kar2


Fluorescence Intensity
1
2
3


T0
N
V
5
10
15
20
25 min


Ch
Mt
NPC
Mt
*


*


*