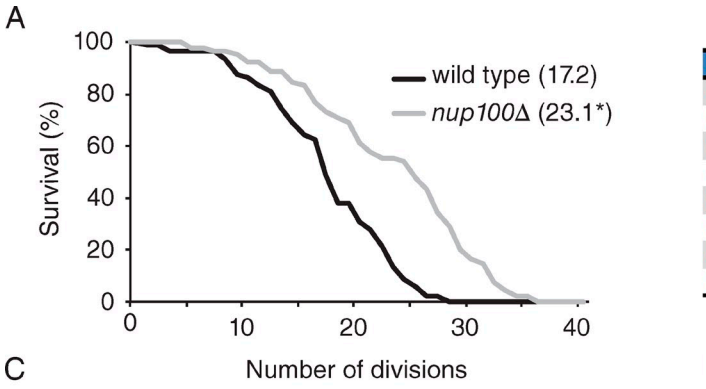
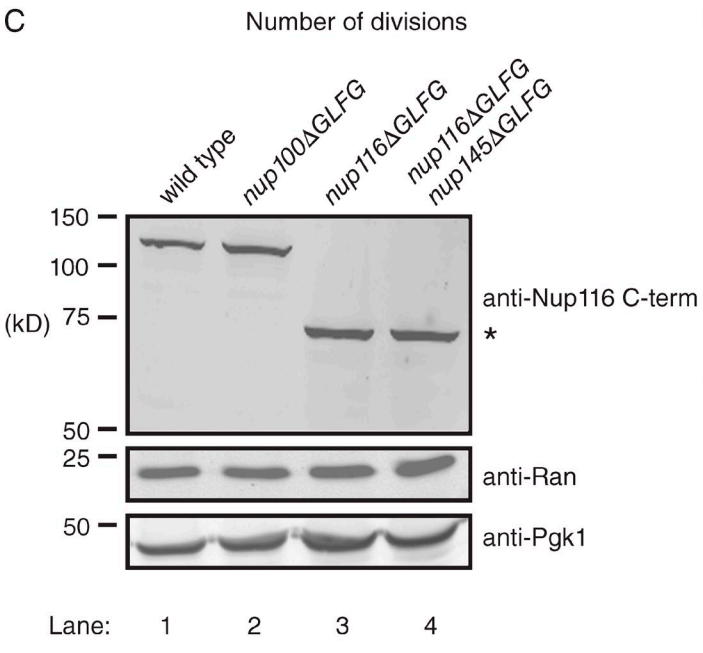
Lord et al., http://www.jcb.org/cgi/content/full/jcb.201412024/DC1



B

Strain	Doubling time	SEM
wild type	93.4	6.3
nup100ΔGLFG	99.2	15.1
nup116ΔGLFG	91.4	20.8
*nup116ΔGLFG nup145ΔGLFG**	117.3	5.5
wild type	81.3	4.6
*kap121-7**	118.2	5.6
wild type	95.1	7.9
*gsp1-322**	132.0	4.4



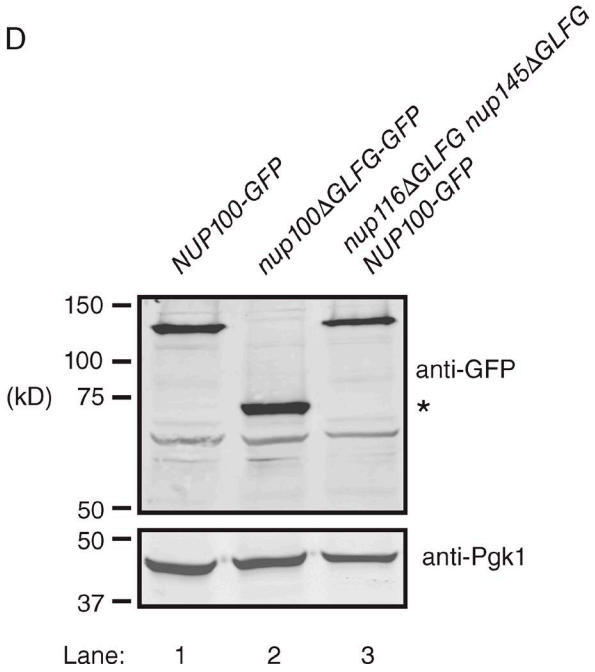


Figure S1. **Nup100 and Nup116 levels are not altered in *ΔFG* strains.** (A) Survival curves for wild-type and *nup100Δ* BY4741 cells. The mean RLSs are listed next to each strain name; *, $P < 0.0001$ when the curve is compared with wild type using a log-rank test ($n \geq 75$ cells). Mean RLSs are listed in parentheses. (B) Doubling times for the listed strains. Error bars represent SEM; *, $P < 0.05$ when compared with wild type using a two-tailed Student's *t* test. (C) Lysates from the listed strains were immunoblotted using anti-Ran, anti-Pgk1, and anti-Nup116 C-terminal antibodies. The asterisk is used to show the relative mobility of Nup116ΔGLFG. (D) Lysates from the listed *NUP100-GFP::HIS3* strains were immunoblotted using anti-Pgk1 and anti-GFP C-terminal antibodies. The asterisk is used to show the relative mobility of Nup100ΔGLFG-GFP.

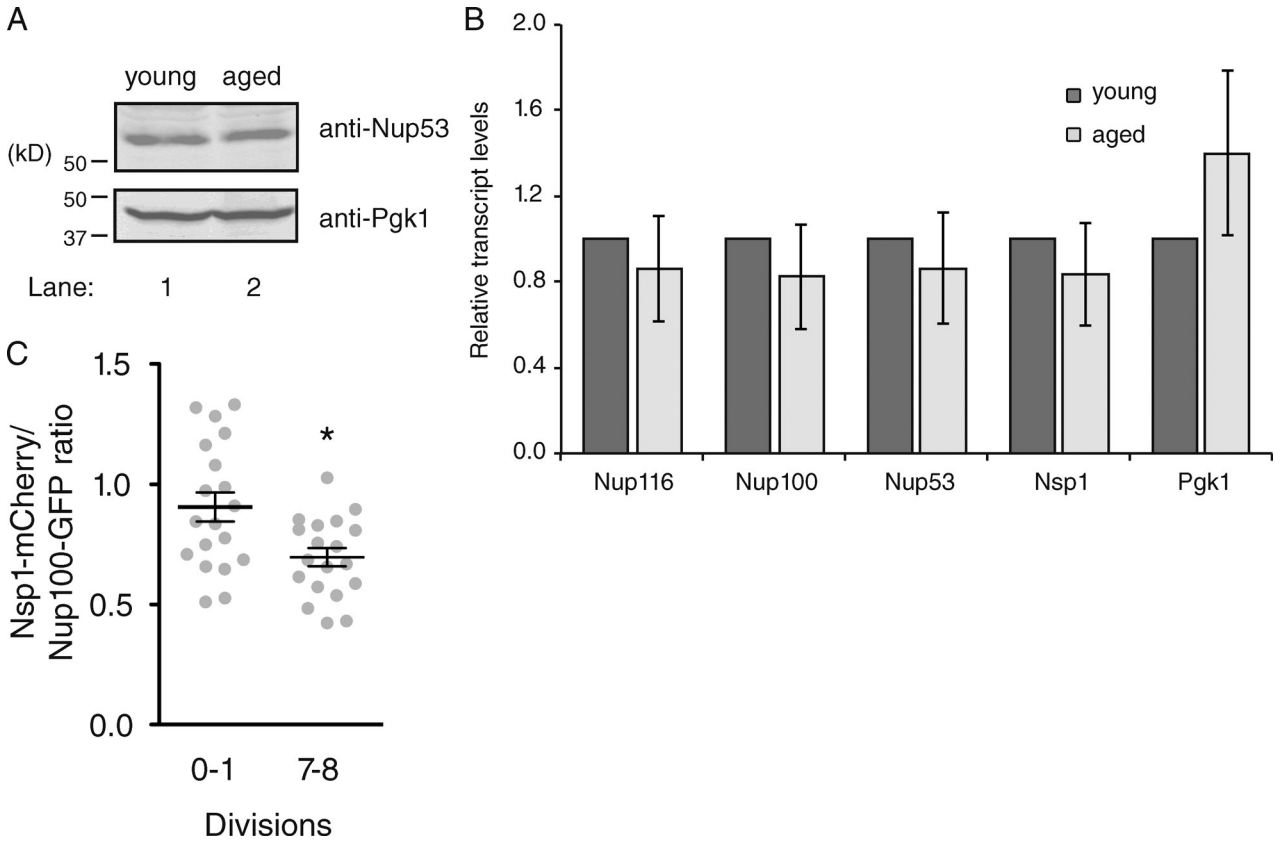


Figure S2. **NPCs are altered during replicative aging.** (A) Enriched mother (~6–9 divisions) and young (~0–1 divisions) wild-type cells were lysed, and immunoblotting was conducted using anti-Nup53 and anti-Pgk1. (B) Relative levels of the listed transcripts were measured by quantitative RT-PCR using RNA derived from young (~0–1 divisions) or enriched mothers (~6–9 divisions). Error bars represent SEM from three independent experiments, each of which had three replicates per transcript. (C) Relative levels of Nup100-GFP and Nsp1-mCherry fluorescence at the nuclear rim in 0–1 or 7–8 division cells. *, $P < 0.04$ when the datasets were compared using a two-tailed Student's *t* test ($n \geq 20$ cells throughout three separate experiments). Error bars represent SEM.

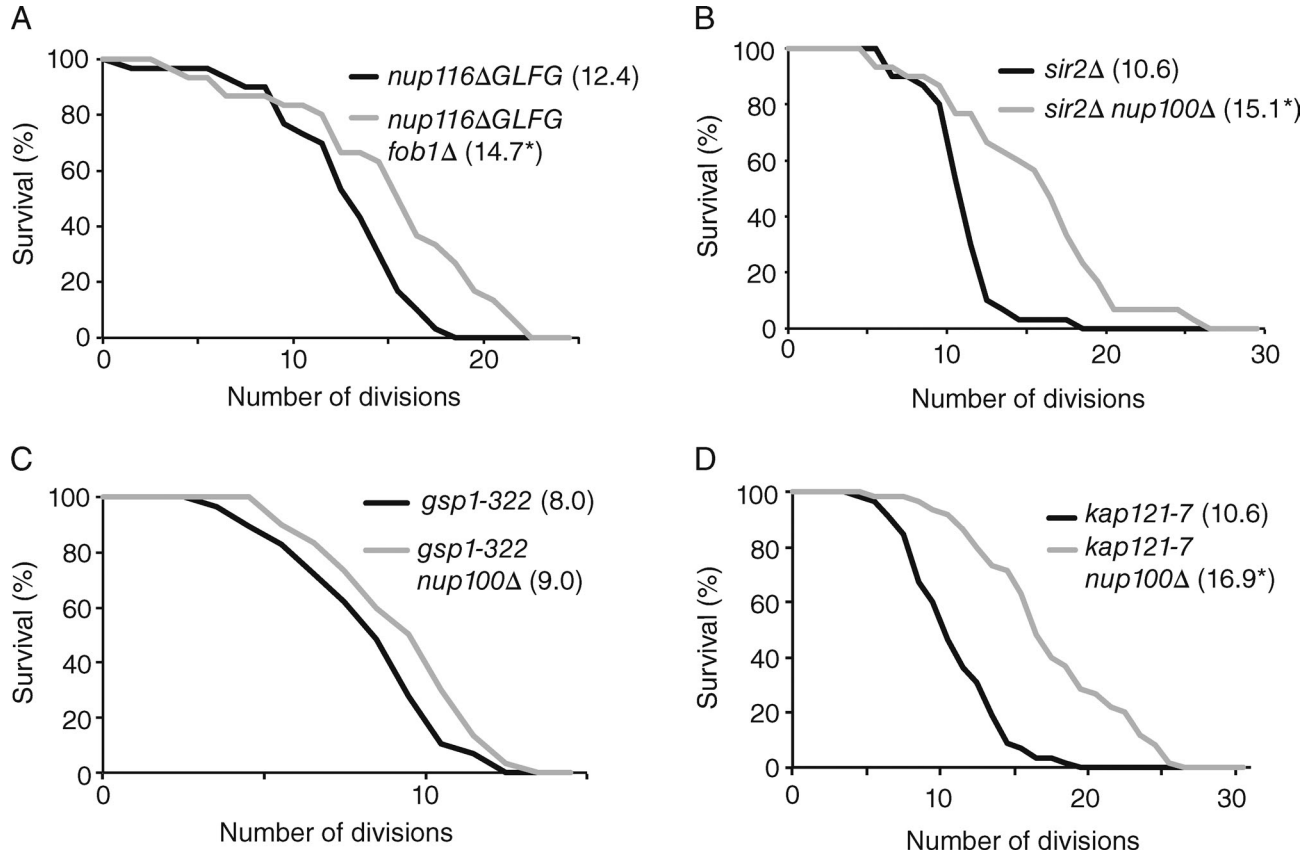


Figure S3. **Epistasis analysis of NPC mutants.** (A and B) RLSs for the listed strains were measured at 30°C on glucose plates. *, $P < 0.05$ when the curves on the same graph are compared using a log-rank test with $n \geq 30$ cells. (C) Survival curves for *gsp1-322* and *gsp1-322 nup100Δ* mutants. (D) Survival curves for *kap121-7* and *nup100Δ kap121-7* cells were determined on 0.1 M KCl plates at 30°C; *, $P < 0.05$ when curves were compared using a log-rank test ($n \geq 30$ cells). Mean RLSs are listed in parentheses.

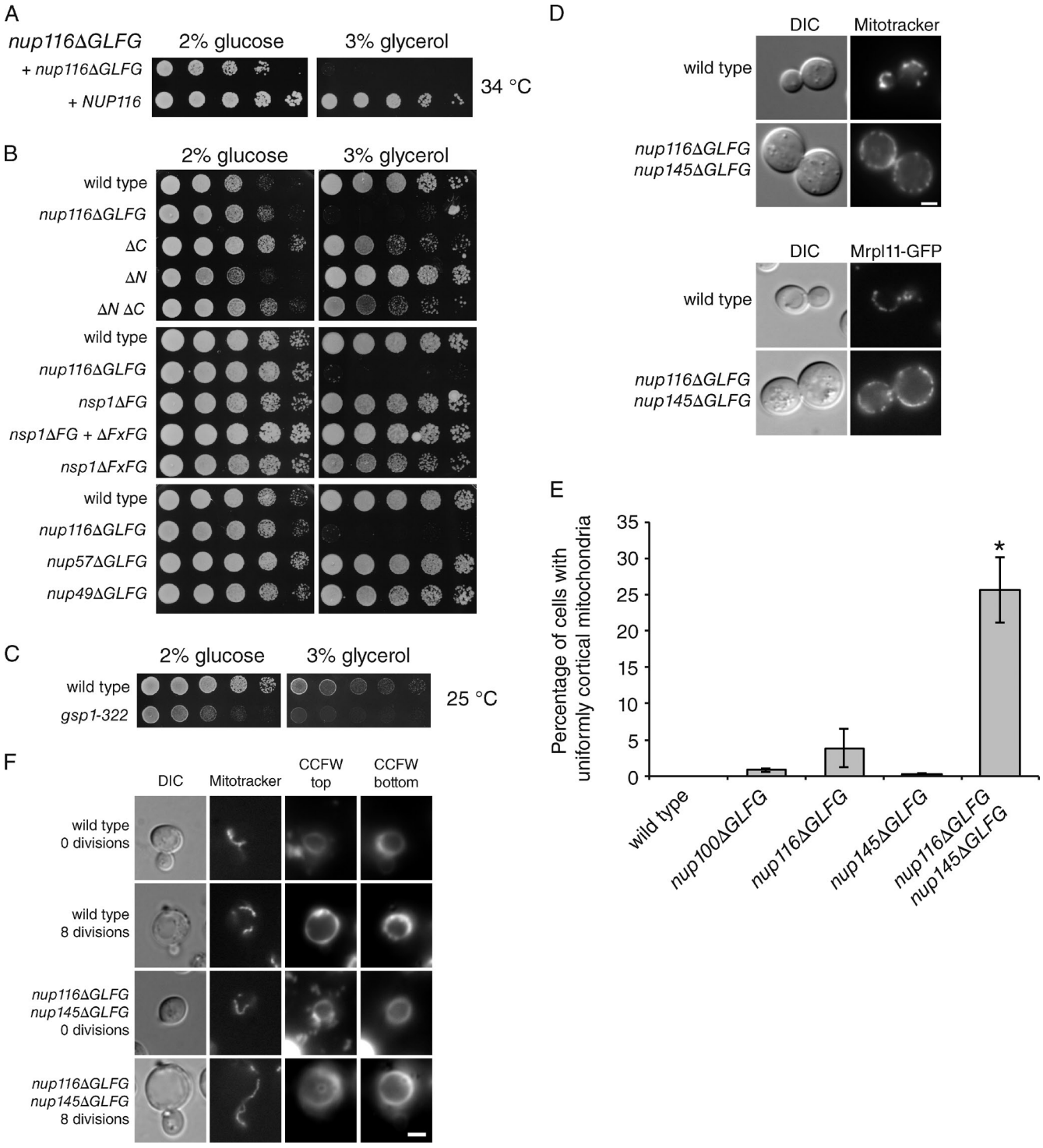


Figure S4. **Mitochondrial function is regulated by NUP116.** (A) *nup116ΔGLFG* cells were transformed with pRS314 vectors containing *nup116ΔGLFG* or *NUP116*. The strains were then serially diluted onto SC-Trp glucose or glycerol and incubated at 34°C until grown as shown. (B) The listed *ΔFG* strains were serially diluted on glucose or glycerol and incubated at 37°C until grown as shown. The *ΔC* strain lacks FG regions of the cytoplasmic-facing Nup42 and Nup159, the *ΔN* strain lacks FG regions of the nuclear-oriented Nup1, Nup2, and Nup60, and the *ΔNΔC* strain combines these mutations. (C) Wild-type or *gsp1-322* cells were serially diluted onto glucose or glycerol and incubated at 25°C until grown as shown. (D) Single wild-type or *nup116ΔGLFG nup145ΔGLFG* cells stained using MitoTracker red CMXRos (top) or expressing endogenously tagged Mrpl11-GFP (bottom) were visualized using fluorescence microscopy at 25°C. Bar, 2.5 µm. (E) Quantification of the percentage of cells exhibiting a uniformly cortical mitochondrial localization when stained with MitoTracker red CMXRos at 25°C. *, $P < 0.05$ when compared with wild-type cells using Student's *t* test; error bars represent SEM for three independent experiments ($n \geq 100$ cells per experiment). (F) Representative images of zero or eight division Mrpl11-GFP cells stained with CCFW and visualized using fluorescence microscopy. CCFW images show the top and bottom of cells for visualization of most bud scars. DIC, differential interference contrast. Bar, 2.5 µm.

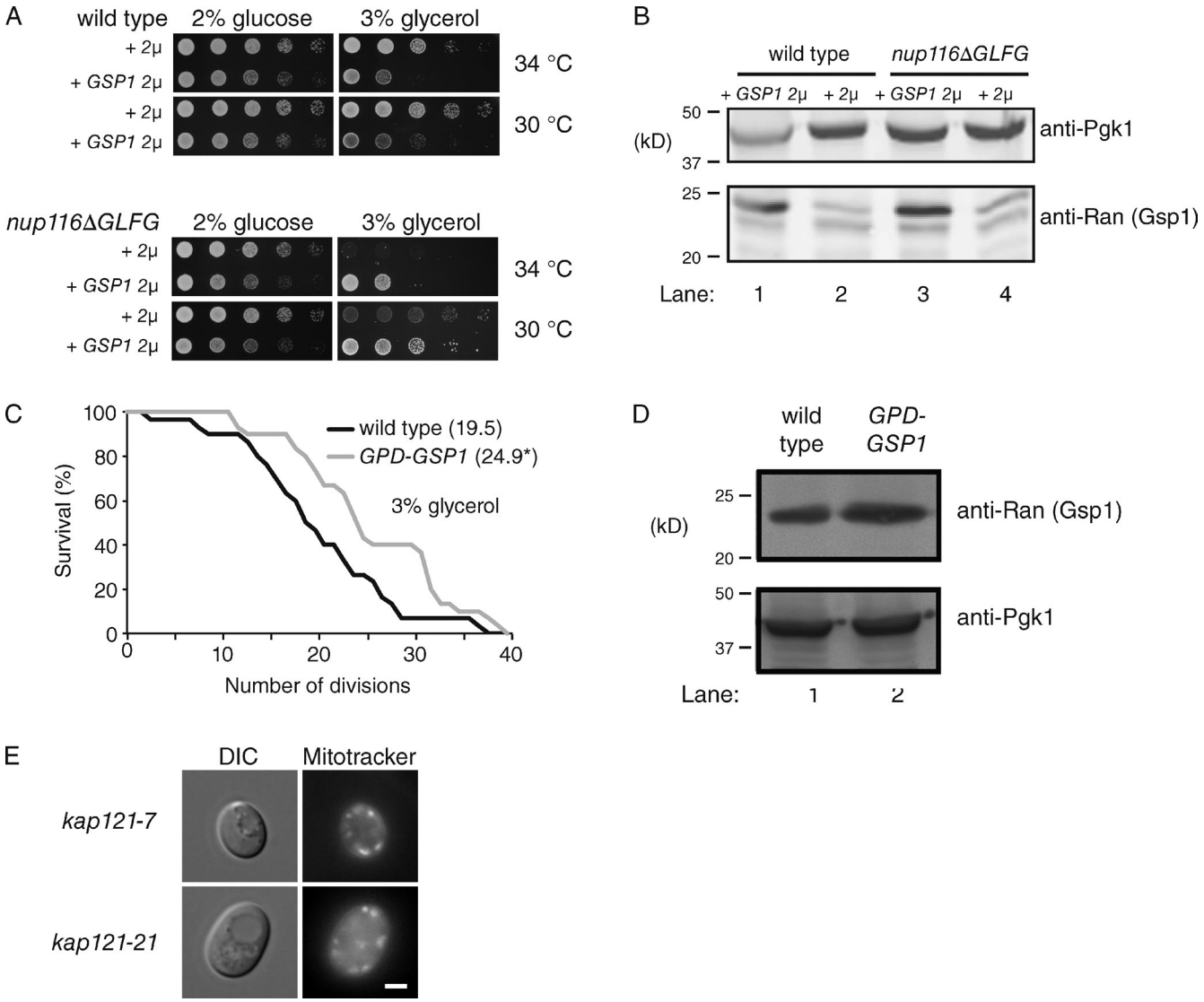


Figure S5. **Ran and Kap121 are required for RLS.** (A) Wild-type and *nup116ΔGLFG* cells transformed with an empty 2μ vector or a 2μ vector containing *GSP1* were serially diluted onto glucose or glycerol at 30 and 34°C until grown as shown. (B) Lysates of strains in A were immunoblotted using anti-Pgk1 and anti–mouse Ran antibodies. Anti–mouse Ran is able to detect yeast Gsp1 as a result of the high identity (80%) between these proteins. (C) Survival curves for the listed strains were determined on glycerol plates at 30°C. *, $P < 0.02$ when the curves are compared using a log-rank test with $n = 30$ cells. (D) Lysates from the strains in C were immunoblotted with anti–mouse Ran and anti-Pgk1. (E) *kap121-7* and *kap121-21* cells stained with MitoTracker red CMXRos were visualized using fluorescence microscopy. Brightness and contrast settings were adjusted on images shown in Fig. 6 B using ImageJ to better visualize MitoTracker signal. DIC, differential interference contrast. Bar, 2.5 μm.

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

Strain	Genotype	Source	Description
arx1Δ	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 arx1Δ::KAN*	Giaever et al., 2002	
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Giaever et al., 2002	Wild-type S288C strain used with deletion library strains
fob1Δ	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 fob1Δ::KAN*	Giaever et al., 2002	
kap123Δ	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 kap123Δ::KAN*	Giaever et al., 2002	
kap95-L63A	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 kap95-L63A*		BY4741 background
N43-6C-GSP1	*MATa gsp1Δ::HIS3::GSP1::LEU2 ade2 leu2 trp1 ura3*	Oki et al., 1998	Wild-type strain for *gsp1-322* mutant
N43-6c-gsp1-322	*MATa gsp1Δ::HIS3::gsp1-322::LEU2 ade2 leu2 trp1 ura3*	Oki et al., 1998	
Nsp1-GFP	*MATa nsp1-GFP:HIS3 his3Δ0 leu2Δ0 met15Δ0 ura3Δ0*	Huh et al., 2003	
nup100Δ	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 nup100Δ::KAN*	Giaever et al., 2002	
PSY1213	*MATa pse1-7 (kap121-7) ura3-52 trp1Δ63 leu2Δ1*	Seedorf and Silver, 1997	
PSY1214	*MATa pse1-21 (kap121-21) ura3-52 trp1Δ63 leu2Δ1*	Seedorf and Silver, 1997	
PSY580	*MATa ura3-52 trp1Δ63 leu2Δ1*	Winston et al., 1995	Wild-type strain for PSY1213 and 1214
rpl23aΔ	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 rpl23aΔ::KAN*	Giaever et al., 2002	
SWY127	*MATa nup116-5::HIS3 ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100* +pSW131	Iovine et al., 1995	
SWY2284	*MATα ura3-1 his3-11,15 trp1-1 leu2-3,112*	Strawn et al., 2004	Wild-type W303 strain used with *ΔFG* mutants
SWY2708	*MATa trp1-1 ura3-1 leu2-3,112 his3-11,15 HA-LoxP-nup49ΔGLFG*	Strawn et al., 2004	
SWY2724	*MATa lys2 ura3-1 leu2-3,112 his3-11,15 HA-LoxP-nsp1ΔFXFG*	Strawn et al., 2004	
SWY2734	*MATα trp1-1 ura3-1 leu2-3,112 his3-11,15 HA-LoxP-nup42ΔFG myc-LoxP-nup159ΔFG*	Strawn et al., 2004	
SWY2752	*MATα trp1-1 ura3-1 leu2-3,112 his3-11,15 myc-LoxP-nup57ΔGLFG*	Strawn et al., 2004	
SWY2762	*MATα trp1-1 ura3-1 leu2-3,112 his3-11,15 HA-LoxP-nup100ΔGLFG*	Strawn et al., 2004	
SWY2791	*MATα trp1-1 ura3-1 leu2-3,112 his3-11,15 T7-LoxP-nup116ΔGLFG*	Strawn et al., 2004	
SWY2813	*MATα lys2 ura3-1 leu2-3,112 his3-11,15 Flag-LoxP-nsp1ΔFG*	Strawn et al., 2004	
SWY2867	*MATα lys2 ura3-1 leu2-3,112 his3-11,15 myc-LoxP-nup145ΔGLFG*	Strawn et al., 2004	
SWY2896	*MATa lys2 ura3-1 leu2-3,112 his3-11,15 myc-LoxP-nup2ΔFXFG T7-LoxP-nup1ΔFXFG myc-LoxP-nup60ΔFXFG*	Strawn et al., 2004	
SWY2916	*MATa trp1-1 ura3-1 leu2-3,112 his3-11,15 myc-LoxP-nup145ΔGLFG T7-LoxP-nup116ΔGLFG*	Strawn et al., 2004	
SWY2919	*MATa lys2 ura3-1 leu2-3,112 his3-11,15 Flag-LoxP-nsp1ΔFXFG-ΔFG*	Strawn et al., 2004	
SWY2971	*MATα trp1-1 ura3-1 leu2-3,112 his3-11,15 HA–LoxP-nup42ΔFG myc-LoxP-nup159ΔFG T7-LoxP-nup1ΔFXFG myc-LoxP-nup2ΔFXFG myc-LoxP-nup60ΔFXFG*	Strawn et al., 2004	
SWY2973	*MATa ura3-1 leu2-3,112 his3-11,15 HA–LoxP-nup100ΔGLFG myc-LoxP-nup145ΔGLFG*	Strawn et al., 2004	
SWY4232	*MATa lys2 ura3-1 leu2-3,112 his3-11,15 nup100Δ::KAN*	This study	
SWY5678	*MATa ura3-1 his3-11,15 leu2-3,112 lys2 htb2-mCherry:HIS3*	This study	
SWY5680	*MATa ura3-1 his3-11,15 leu2-3,112 lys2 T7-LoxP-nup116ΔGLFG htb2-mCherry:HIS3*	This study	
SWY5724	*MATα ura3-1 his3-11,15 trp1-1 leu2-3,112 nup188Δ::KAN*	This study	
SWY5725	*MATα ura3-1 his3-11,15 trp1-1 leu2-3,112 nup188Δ::KAN htb2-mCherry:HIS3*	This study	
SWY5728	*MATα trp1-1 ura3-1 leu2-3,112 his3-11,15 HA-LoxP-nup100ΔGLFG htb2-mCherry:HIS3*	This study	
SWY5836	*MATα ura3-1 his3-11,15 trp1-1 leu2-3,112 mrpl11-GFP:HIS3*	This study	
SWY5837	*MATα trp1-1 ura3-1 leu2-3,112 his3-11,15 HA-LoxP-nup100ΔGLFG mrpl11-GFP:HIS3*	This study	
SWY5838	*MATα trp1-1 ura3-1 leu2-3,112 his3-11,15 T7-LoxP-nup116ΔGLFG mrpl11-GFP:HIS3*	This study	
SWY5839	*MATα lys2 ura3-1 leu2-3,112 his3-11,15 myc-LoxP-nup145ΔGLFG mrpl11-GFP:HIS3*	This study	
SWY5840	*MATa trp1-1 ura3-1 leu2-3,112 his3-11,15 myc-LoxP-nup145ΔGLFG T7-LoxP-nup116ΔGLFG mrpl11-GFP:HIS3*	This study	
SWY5874	*MATa trp1-1 ura3-1 leu2-3,112 his3-11,15 myc-LoxP-nup145ΔLFG T7-LoxP-nup116ΔGLFG htb2-mCherry:HIS3*	This study	
SWY5909	*MATa trp1-1 ura3-1 leu2-3,112 his3-11,15 myc-LoxP-nup145ΔGLFG T7-LoxP-nup116ΔGLFG sir2Δ::KAN*	This study	

Table S1. **Yeast strains used in this study** *(Continued)*

Strain	Genotype	Source	Description
SWY5912	*his3Δ1 leu2Δ0 ura3Δ0 fob1Δ::KAN nup100Δ::KAN*	This study	BY4741 background
SWY5913	*MATα ura3-1 his3-11,15 trp1-1 leu2-3,112 nup100-GFP:HIS3*	This study	
SWY5914	*MATα trp1-1 ura3-1 leu2-3,112 his3-11,15 HA-LoxP-nup100ΔGLFG-GFP:HIS3*	This study	
SWY5915	*MATa trp1-1 ura3-1 leu2-3,112 his3-11,15 myc-LoxP-nup145ΔGLFG T7-LoxP-nup116ΔGLFG nup100-GFP:HIS3*	This study	
SWY6012	*MATa pse1-7 (kap121-7) ura3-52 trp1Δ63 leu2Δ1 flo11Δ::KAN*	This study	
SWY6013	*MATa pse1-21 (kap121-21) ura3-52 trp1Δ63 leu2Δ1 flo11Δ::KAN*	This study	
SWY6014	*MATa ura3-52 trp1Δ63 leu2Δ1 flo11Δ::KAN*	This study	
SWY6044	*MATα ura3-1 his3-11,15 trp1-1 leu2-3,112 sir2Δ::KAN*	This study	
SWY6169	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 GPD-GSP1:URA3*	This study	BY4741 background
SWY6170	*MATα ura3-1 his3-11,15 trp1-1 leu2-3,112 sir2Δ::KAN nup100Δ::TRP1*	This study	
SWY6236	*MATa gsp1Δ::HIS3::gsp1-322::LEU2 ade2 leu2 trp1 ura3 nup100Δ::TRP1*	This study	
SWY6237	*MATa pse1-7 (kap121-7) ura3-52 trp1Δ63 leu2Δ1 nup100Δ::TRP1*	This study	
SWY6238	*MATα ura3-1 his3-11,15 trp1-1 leu2-3,112 nup100-GFP:HIS3 nsp1-mCherry:KAN*	This study	
SWY6239	*nup116ΔGLFG fob1Δ::KAN*	This study	

A list of yeast strains as well as their origins. Descriptions are provided for some strains that provide context about their use.

Table S2. **Plasmids used in this study**

Plasmid	Description	Source
GFP-4PrA	GFP-4PrA cloned into pRS415 (*LEU2 CEN* single copy) under *TPI1* promoter	This study
pGAD-GFP	SV40 NLS fused to GFP cloned into a *LEU2 2μ* plasmid under *ADH1* promoter	Shulga et al., 1996
pRS426	*URA3 2μ* (multicopy) empty vector	
pSpo12$_{76–130}$-GFP	Spo12 NLS fused to GFP cloned into *LEU2 2μ* multicopy plasmid under *TPI1* promoter	Chaves and Blobel, 2001
pSW173	*nup116ΔGLFG* cloned into pRS314 (*TRP1 CEN* single copy)	Iovine et al., 1995
pSW176	*NUP116* cloned into pRS314 (*TRP1 CEN* single copy)	Iovine et al., 1995
pSW273	nup116SFxFG$_{Nsp1}$ cloned into pRS314 (*TRP1 CEN* single copy)	Iovine et al., 1995
pSW287	nup116SGLFG$_{Nup100}$ cloned into pRS314 (*TRP1 CEN* single copy)	Iovine et al., 1995
pSW326	nup116SGLFG$_{Nup116}$ cloned into pRS314 (*TRP1 CEN* single copy)	Iovine et al., 1995
pSW4070	*GSP1* cloned (*URA3 2 μ* multicopy) into under its own promoter	This study
Rpl25NLS-EGFP	Rpl25 NLS fused to GFP cloned into pYX242 (*LEU2 2μ* multicopy) under *TPI1* promoter	Timney et al., 2006

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