Supplemental Table I: Yeast strains used in this study

Strain	genotype	Ref
GA-180	*MATa, ade2-1 trp1-1 his3-11 his3-15 ura3-1 leu2-3 leu2-112, can1-100 (W303)*	
GA-2768	GA-180 *esc1::KanMx, yku70::HIS5*	This study
GA-2985	*sir3::TRP1 esc1::KanMx yku70::HIS5*	This study
GA-1998	*MATalpha trp1-1 his3-11 his3-15 ura3-1 leu2-3 leu2-112, hmr Aeb UASg hmr::URA3*	Chien et al. 1993
GA-2511	*GA-1998 lys2::*E*ADE2*I	This study
GA-2585	GA-2511 *yku70::HIS5*	This study
GA-2902	GA-2585 *esc1::KanX*	This study
GA-2886	GA-2511 *esc1::HIS5*	This study
GA-2586	GA-2511 *arp6::HIS3*	This study
GA-2587	GA-2511 *sas2::HIS3*	This study
GA-1997	GA-1998 *yku70::HIS5*	This study
GA-1053	*MATalpha ade2-1 trp1-1 his3-11 his3-15 ura3-1 leu2-3 leu2-112, can1-100 TelVIIL::URA3 lys2::HMLEI::ADE2*	Maillet et al. 2001)
GA-1055	GA-1053 *yku70::KanX*	Maillet et al. 2001
GA-148	GA-1055 *esc1::TRP1*	This study
GA-2411	*GA-1053 esc1::HIS5*	This study
GA-3213	GA-1055 *rif1::hph*	This study
GA-3214	GA-1053 *rif1::hph*	This study
GA-3215	GA-148 *rif1::hph*	This study
YG-342	*MATalpha ade2-1 trp1-1 his3-11 his3-15 ura3-1 leu2-3 leu2-112 can1-100 TelVIIL::URA3 rif1::HIS3 hdf1::LEU2*	Mishra and Shore, 1999
YG-345	*MATalpha ade2-1 trp1-1 his3-11 his3-15 ura3-1 leu2-3 leu2-112 can1-100 hmrΔA::TRP1 hdf1::LEU2 TelVIIL::URA3*	Mishra and Shore, 1999
GA-2142	YG-342 *esc1::KanX*	This study

Supplementary Figure legends:

Suppl. Fig. 1: *SIR1, SIR2, SIR3* and *SIR4* expression levels are not affected by deletion of *esc1* and *yku70.*

Transcript levels normalized to wild-type levels are shown for *SIR1, SIR2, SIR3* and *SIR4* in *wild-type, yku70 esc1, yku70 esc1 sir3* and *yku70 esc1 sir3* treated with splitomicin as indicated (see Methods).

Suppl. Fig. 2: Deletion of *esc1* increases the effect of *yku70* on gene repression.

A. Among the 137 genes down-regulated in *yku70* mutant 32 (23%) are further down-regulated in a *yku70 esc1* double mutant. **B.** Transcript levels normalized to wild-type

levels are shown for the different categories of *yku70* down-regulated genes presented in A, in wild-type, *esc1*, *yku70* and *esc1 yku70* strains as indicated. **C.** Among the 375 genes up-regulated in *yku70* mutant 19 (5%) are further up-regulated in the *yku70 esc1* double mutant while 160 are down-regulated (42%). **D.** Transcript levels normalized to wild-type levels are shown for the different categories of *yku70* up-regulated genes presented in B, in wild-type, *esc1*, *yku70* and *esc1 yku70* strains as indicated.

Suppl. Fig. 3: Distribution of expression changes in mutant vs wild-type cells for predicted targets of yeast transcription factors.

Shown are the mean log fold-change plus and minus one standard error (red bar), the median log fold-change (vertical black line), and the 25 and 75 percentiles (grey bar). These are grouped and labeled based on the transcription factor binding site found in the promoter region. ALL indicates the distribution of log fold-changes for all ORFs grouped together, serving as a control for specific subsets of genes. For the PAC and RRPE motifs separate distributions were determined for ORFs that are targets of both, of PAC only, and of RRPE only. Distributions are shown for all transcription factors that have significant changes (z-value larger than 2 or smaller than -2) in at least one of the mutants. Note the strong down-regulation of some of the targets of Mcm1 and Ste12/Dig1 in the triple mutant.

Suppl. Fig. 4: Sir4 association at subtelomeric and internal loci in WT, *yku70* and *yku70 esc1* strains. detected by ChIP

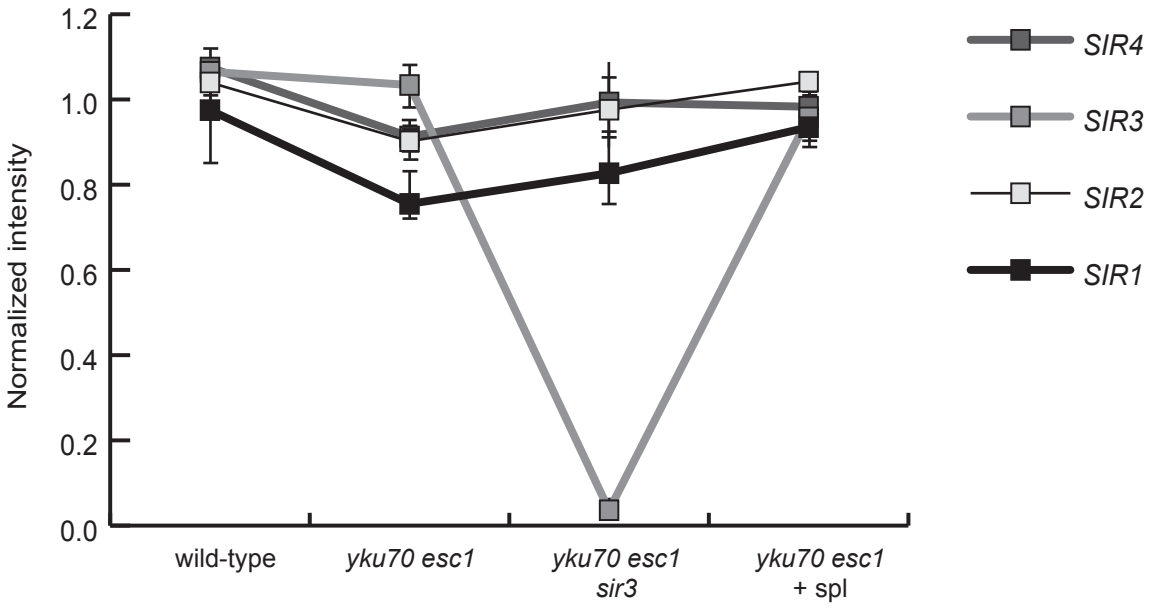
GA1053 (LM11), GA1055 (*yku70*) and GA148 (*yku70 esc1*) were grown overnight in 5 ml of YPAD. Next morning, cultures were diluted into 100 ml YPAD to a concentration of 1.5 x 10^6 cells/ml, and grown for an additional 2 to 3 generations. When the concentration reached approximately 5 x 10^6 cells/ml, cross-linking was performed with the final concentration of 1% formaldehyde for 15 min at 30°C. ChIP was performed as described (van Attikum et al 2004) on the indicated strains, using rabbit anti-Sir4 and rabbit anti-Myc (Santa Cruz) as a negative control (control Ab). For quantification, signals from each primer sets are normalized to a control signal from the genomic *FAB1* locus (a nontelomeric locus on Chr 6). The signals are also normalized to the input signal from each primer set. ChIP data are presented as the mean of multiple PCR runs +/- standard deviation from a representative experiment.

Suppl. Fig. 5: Promoters bearing Msn2, Abf1, PAC and RRPE motifs respond to released SIR complexes in a *set1 htz1* mutant

Distribution of expression changes (*htz1 set1* mutant vs wild-type and *htz1 set1 sir2* vs wild-type) are shown for predicted targets of selected yeast transcription factors. Raw data are from Venkatasubrahmanyam et al, 2008). Shown are the mean log fold-change plus and minus one standard error (red bar), the median log fold-change (vertical black line), and the 25 and 75 percentiles (grey bar). For reference the distribution of log fold-changes for all ORFs is also shown (All). For the PAC and RRPE motifs separate distributions were determined for ORFs that are targets of both, of PAC only, and of RRPE only.

Suppl. Fig. 6: Mutations affecting SIR spreading do not affect perinuclear silencing.

A. Scheme of the reporter genes used in this figure. **B.** Targeted silencing is monitored as in Fig. 1 in GA-2511 (WT), and derivatives carrying full deletions of either *yku70* (GA-2585), *arp6* (GA-2886), or *sas2* (GA-2902). **C.** Ectopic silencing is monitored in the same strains bearing at *lys2*::E-*ADE2*-I. Wild-type, *sas2* and *arp6* colonies are white colonies, indicative of *ADE2* expression, while *yku70* colonies are pink, indicative of *ADE2* silencing. **D.** Sir3 distribution in foci appears unaffected in *sas2* strains, as monitored by live imaging of Sir3-GFP. Similar results are obtained for *arp6* strain (data not shown). White lines represent the nuclear periphery of various individual yeast cells.



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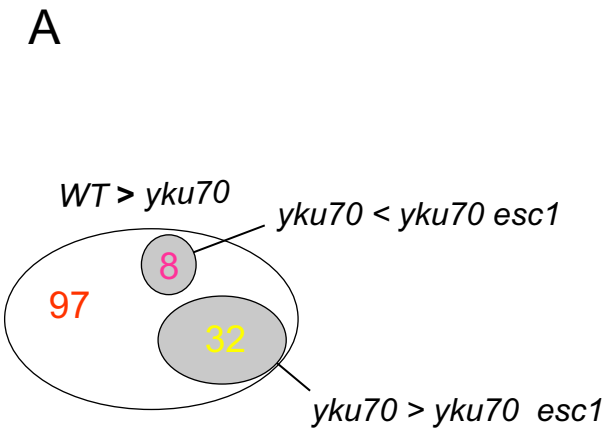
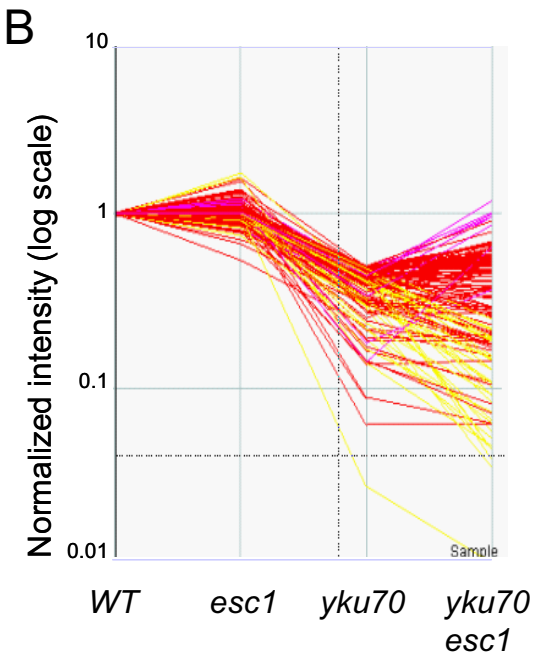
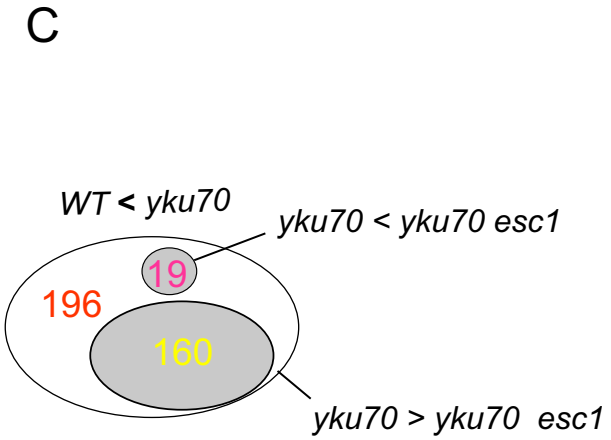
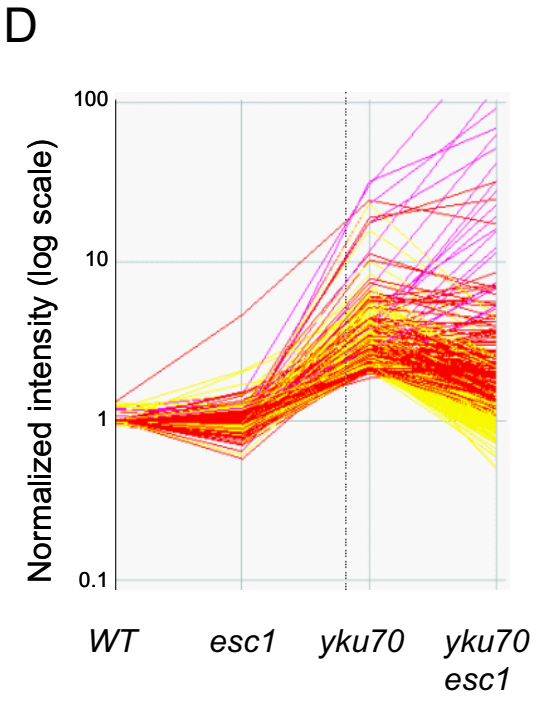
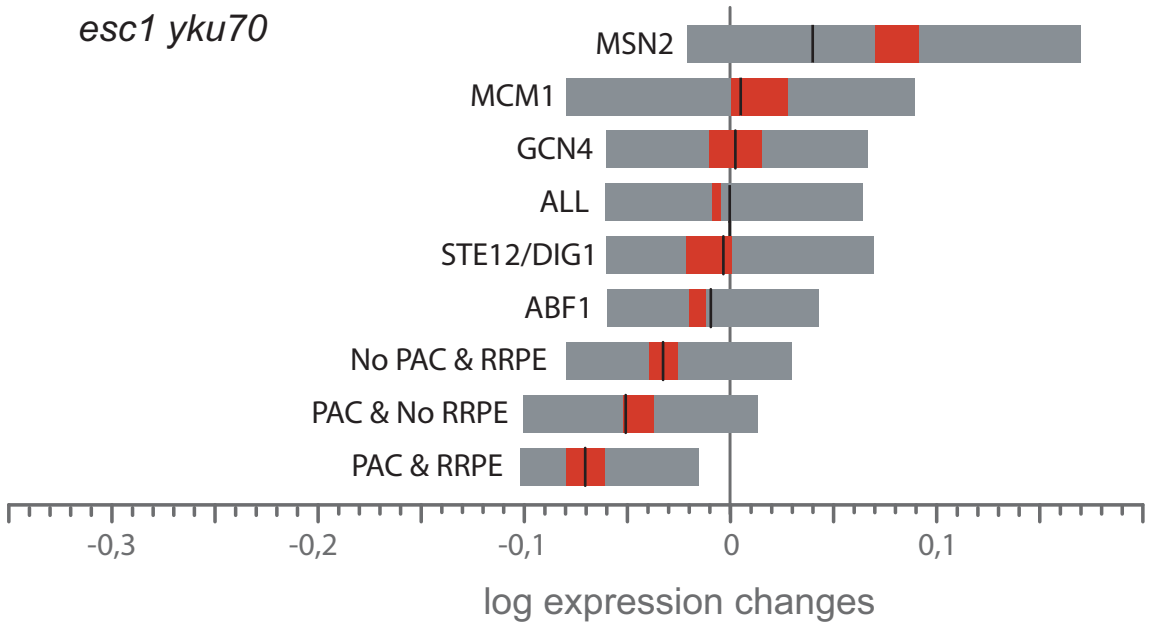


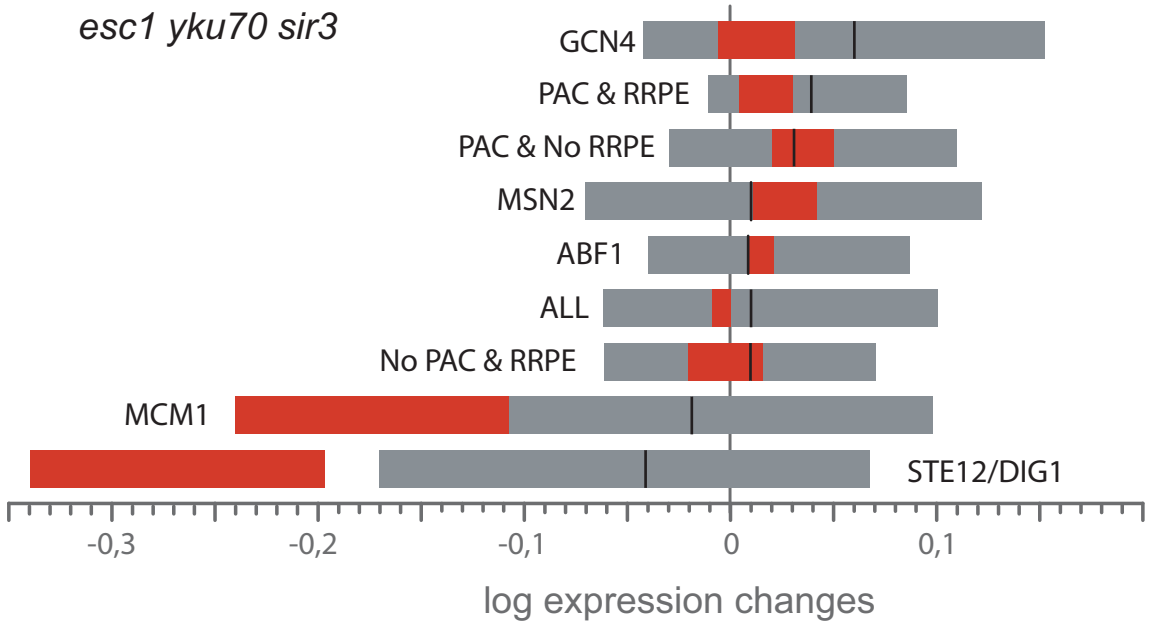






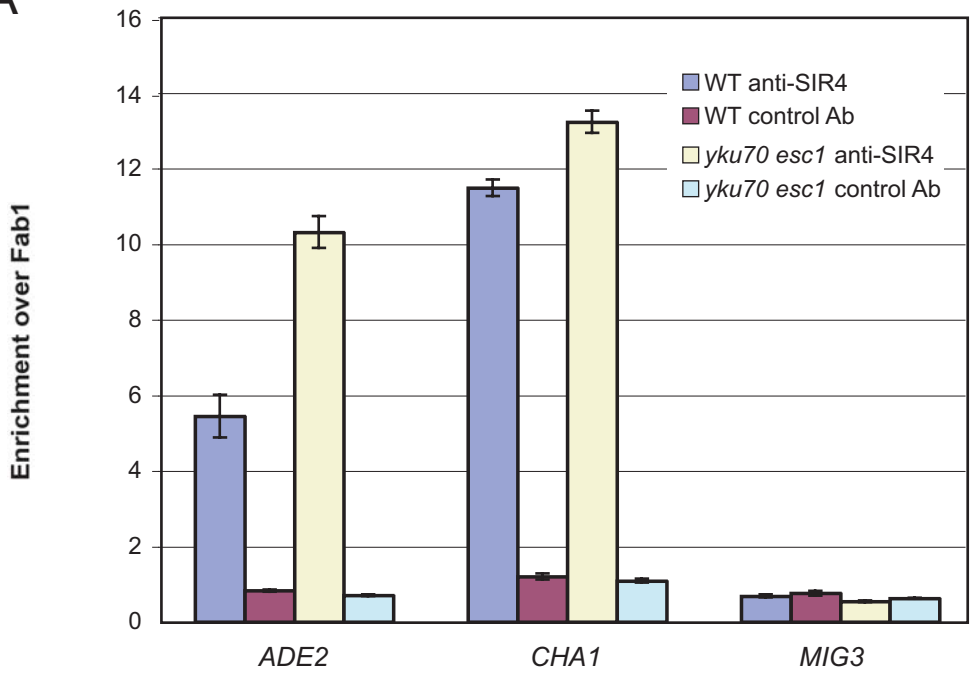
Taddei *et al*. Supplementary Figure 2





Taddei *et al.*, Supplementary Figure 3

A



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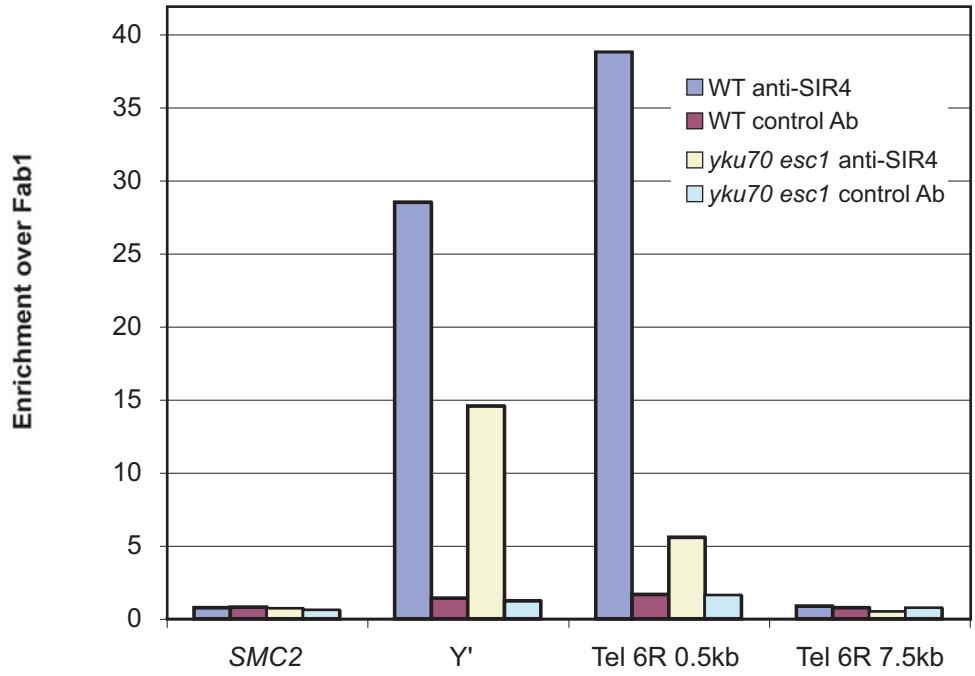
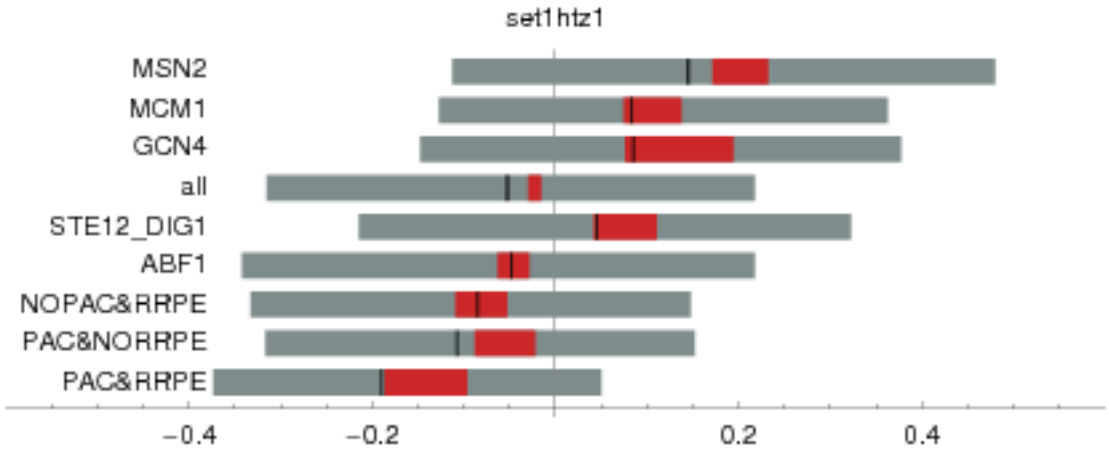


Taddei et al, Supplementary Figure 4

A



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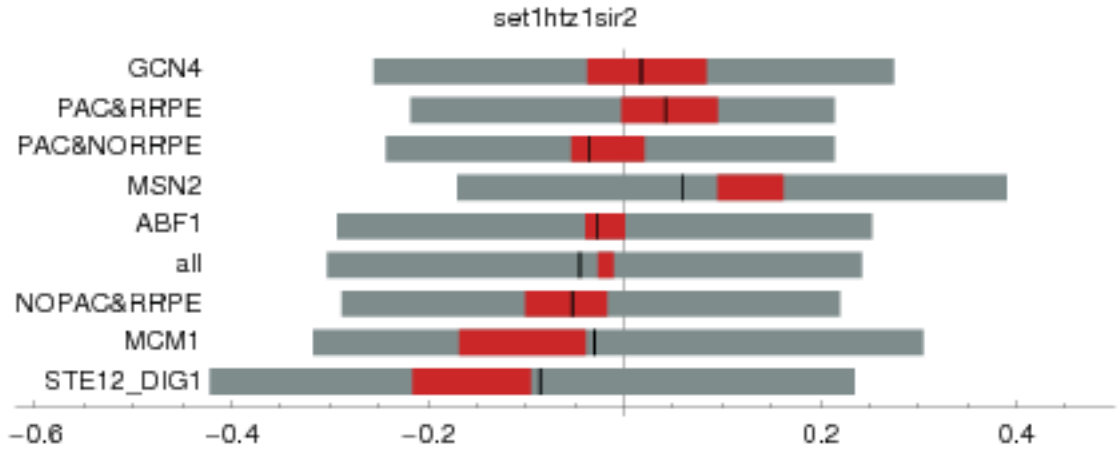
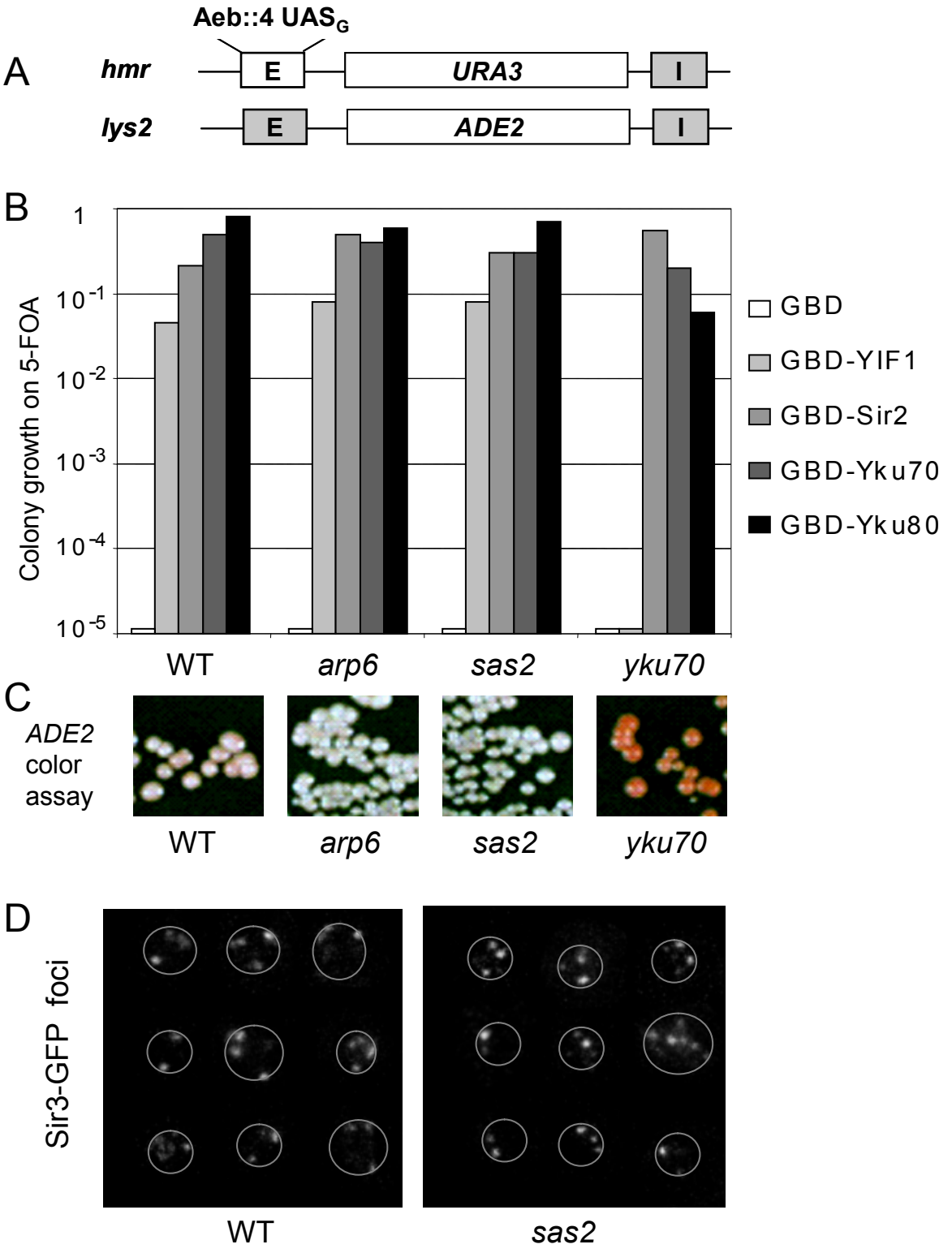


Taddei et al, Supplementary Figure 5



Taddei *et al*. Supplementary figure 6