Supporting Information

Mitchell and Boeke 10.1073/pnas.1414399111

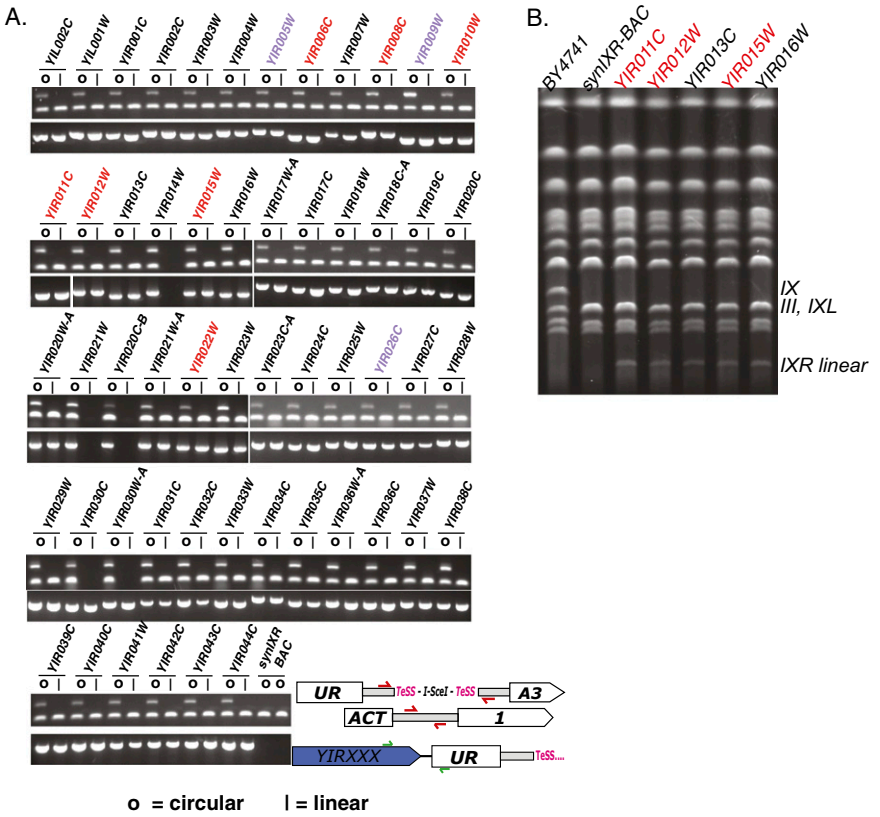

Fig. S1. PCR analysis of panel of *synIXR* BAC permutable strains. (*A*) PCR confirmation of integration and circular/linear status. All permutations were tested, pre- and postlinearization (circular and linear, respectively), by PCR using two primer sets. First, integration was confirmed using a gene-specific primer in combination with a primer that annealed within the telomerator (green arrows; lower gel). These primers confirmed the presence of the telomerator cassette at each position whether in the linear or circular conformation of *synIXR* in all strains. Second, a primer pair designed to anneal to the *ACT1* intron (red arrows) was used. This primer pair amplified a product from the endogenous *ACT1* intron at its native locus on chromosome six (upper gel, lower band), as well as from an intact telomerator cassette (upper gel, upper band). Following growth in galactose, absence of the latter band is consistent with linearization. (*B*) Pulsed field gel showing separation of full-length chromosomes isolated from five *synIXR* linear derivative strains (3′ of *YIR011C, YIR012W, YIR013C, YIR015W,* and *YIR016W*). Compared to a wild-type strain encoding a native chromosome *IX* (BY4741) or the parental strain encoding a circular *synIXR* (*synIXR BAC*), only the linear derivatives display a fast migrating band, consistent with linearization.

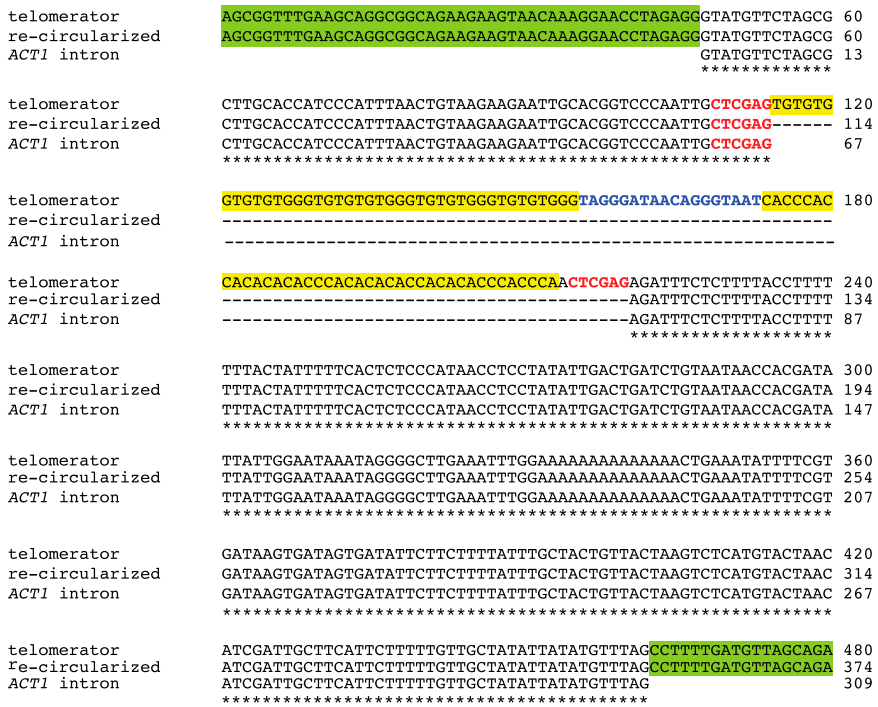

Fig. S2. Sequence alignment comparing telomerator to recircularized *synIXR* BAC and the native *ACT1* intron. Blue, recognition sequence for the I-*Sce*I homing endonuclease; green, exonic sequence; red, *Xho*I recognition sequences used for cloning; yellow, telomere seed sequences (TeSSs).

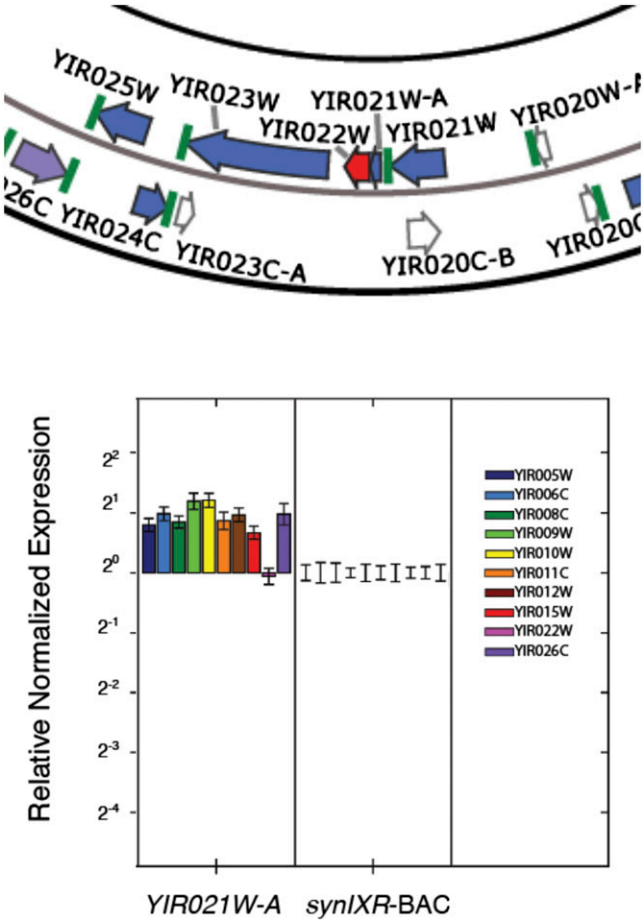

Fig. S3. Linearizing 3′ of *YIR021W*. Integration of the telomerator 3′ of *YIR021W-A* should interrupt the promoter of the essential gene *YIR022W*. qPCR analysis reveals a twofold increase in expression of essential genes and genes required for fast growth except for *YIR022W* in an Foa^R/Ura- clone post-linearization 3′ of *YIR021W-A*. We predict that integration of the telomerator 3′ of *YIR021W-A* is only tolerated if *synIXR BAC* is present in a second copy, which can compensate for the loss of expression of *YIR022W* upon integration.

Table S1. qPCR primer sequences

Primer name	Sequence
YIR005W-For	GAA TTC TTA GCC CGC ATC AAT C
YIR005W-Rev	ACT GTC AAT ATG TCA CCT TCT GT
YIR006C-For	GCA ACT GGC TTT GTC AAC TC
YIR006C-Rev	GCC TGA TCT TGG GCA GTA ATA
YIR008C-For	CCC AAG CAA GAC TCT CAC ATA A
YIR008C-Rev	CCA CAT CCA GCT TCG GAT AAA
YIR009W-For	CAC TGT GGC TGA ACC AGT AA
YIR009W-Rev	TAC TCG CAG TCG TTG CAT ATT
YIR010W-For	TGC GAT AGA TTG GGA AGA TGA G
YIR010W-Rev	CAG TTC CGT GTC ATC GTA GTT
YIR011C-For	ACG AGG CAT GGA ACT AAT GAA
YIR011C-Rev	TGA CTC TCG TAT GTA CGC TTT G
YIR012W-For	GAT ACA AGT GCA TGG AGA GTA AGA
YIR012W-Rev	CCA TTG ATA CAC GAT GCG AAT AAG
YIR015W-For	GAA AGC ACA CGG AGG AAA GA
YIR015W-Rev	GGT AAG CGG ATA TTT GGT AGA GG
YIR022W-For	GTG TAT GAG GTC GAA GGG AAA
YIR022W-Rev	CTT GTC CGC GTG ATT GTT ATG
YIR026C-For	CAC CGC AGT GAG ATG TAA GAA
YIR026C-Rev	GAA GTG TCC CTC TGA TGA TTC C
TAF10-For	GTA GTG GAT GAT GGG AGT GAA A
TAF10-Rev	TTA CTG CAT CGG GAA TGA TAG G
UBC6-For	AAC CAC CGG CTA TCA GAA TG
UBC6-Rev	TTC CAA GTA TCA GGG TGG TAA TC