GENETICS

Supporting Information
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The Mother Enrichment Program: A Genetic System for Facile Replicative Life Span Analysis in *Saccharomyces cerevisiae*

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FILE S1

SUPPORTING MATERIALS AND METHODS

Strain and plasmid construction:

Deletions of *sir2*Δ and *fob1*Δ were constructed by one-step gene replacement with drug resistance markers (GOLDSTEIN and MCCUSKER 1999) in the haploid MEP strains UCC5179 and UCC5181, which were subsequently mated to generate heterozygous deletions in diploid strains UCC8836 and UCC526, respectively. To allow mating of *sir2*Δ mutants, strains were transformed with pRS314-SIR2 (BEDALOV *et al.* 2001) before mating and the diploid strain was subsequently cured of the plasmid.

Construction of P_{scw11}-*cre-EBD78*:

The *GAL* promoter driving expression of a Cre-EBD fusion protein (CHENG *et al.* 2000) on plasmid pFvL113 was replaced by gap repair with a 1 Kb promoter region of *SCW11* (generated by PCR from p126SCW using oligonucleotides CreScwF and CreScwR) to create pDL01. Upon introduction of this plasmid into reporter strain UCC8612 carrying *loxP*-flanked *ADE2*, we found 100% of transformants had lost *ADE2* through Cre-mediated recombination, indicating high recombinase activity in the absence of estradiol. In order to create a version of Cre-EBD that displayed strict dependence on estradiol for activity, we used error-prone PCR mutagenesis to generate mutations within *cre-EBD* as described in (WILSON and KEEFE 2001) using oligonucleotides CreF and EbdR. These PCR products were co-transformed along with pDL01 (gapped by restriction digest with *Stu*I and *Spe*I) into UCC8612 to isolate candidate mutants by gap repair. Transformants that yielded unsectored white colonies on media lacking estradiol were patched to YEPD + 1 μM estradiol to screen for induction of recombinase activity. Candidates that gave robust induction based on color were recovered by plasmid rescue and further characterized. The lead candidate, pDL20/*cre-EBD78* displayed an *ADE2* recombination rate of 1.68 x 10^{-4} per cell division in the absence of estradiol. After a two hour exposure to 1 μM estradiol, *ADE2* was lost in ~53% of cells. Sequencing identified four missense mutations within the *cre* domain and an additional four within the *EBD* domain. The *cre-EBD78-NATMX* cassette was amplified by PCR with oligonucleotides HOpolyF and HOPolyR and subcloned into the *Eco*RI site of the HO-poly-HO vector (VOTH *et al.* 2001) generating pDL12. This vector was used for integration of *cre-EBD78-NATMX* at the *ho* locus after restriction digestion with *Not*I.

Construction of *loxP* target genes:

The 5' *loxP* site in *UBC9* was introduced by homologous recombination of a *loxP-KANMX-loxP* cassette generated from pUG6 (DELNERI *et al.* 2000) using oligonucleotides UBC9lox5F and UBC9lox5R in the diploid strain UCC8600. Excision of *KANMX* was induced with Cre-EBD expressed from pDL01, and strains were sporulated to verify viability

of the *loxP* allele and generate the haploid strain UCC8701. The 5' *loxP* site for *CDC20* was constructed as described above using oligonucleotides CDC20loxF and CDC20loxR to generate strain UCC8611.

To introduce 3' *loxP* sites, a double-stranded oligonucleotide (Bamlox1 + Bamlox2) containing the *loxP* sequence flanked by 4-bp single-stranded 5' overhangs was subcloned into the *Bam*HI site of pAG32 (GOLDSTEIN and MCCUSKER 1999) to generate pDL03(+) and pDL03(-) containing either *loxP* orientation. The *loxP-HPHMX* cassette from pDL03(-) was amplified using oligonucleotides CDC20lox3F and CDC20lox3R and integrated into UCC8611 by homologous recombination.

To introduce a different selectable marker along with the 3' *loxP* site at *UBC9*, a *loxP-LEU2* PCR product was amplified from pRS305 (SIKORSKI and HIETER 1989) with oligonucleotides RS+loxP-Not1 and RS-Not1 and subcloned into pDL03(-) by digestion with *Not*I to create pDL26(-). The *loxP-LEU2* cassette was integrated into UCC8701 by homologous recombination of a PCR product generated with oligonucleotides UBC9lox3F and UBC9lox3R using pDL26(-) as a template to generate strain UCC8697.

To construct the *CDC20-Intron* allele, an *HPHMX* cassette with no *loxP* site was introduced into UCC8611 by homologous recombination of a PCR product from pAG32 (GOLDSTEIN and MCCUSKER 1999) generated with oligonucleotides CDC20_hphF and CDC20_hphR to create UCC3813. A 5' *loxP-CDC20*-HPHMX cassette was PCR amplified from UCC3813 with oligonucleotides CDC20notF and CDC20notR and subcloned into the *Not*1 site of pRS313 (SIKORSKI and HIETER 1989) to create pEH5. An *ACT1* intron sequence containing a *loxP* site was amplified from pLND4 with oligonucleotides CDC20_ACT1_F and CDC20_ACT1_R and subcloned into the *Bst*EII site of pEH5 to create pEH6. A *Not*I-*Xho*I fragment from pEH6 containing the *loxP-CDC20-Intron-loxP-HPHMX* cassette was used to replace the *cdc20*Δ*::KANMX* allele by homologous recombination in UCC8723 to yield strain UCC8779. Plasmid pDL25 was constructed by amplifying *CDC20* from genomic DNA using oligonucleotides CDC20NotF and CDC20NotR. The PCR product was digested with *Not*I and subcloned into the *Not*I site of pRS316.

References:

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DELNERI, D., G. C. TOMLIN, J. L. WIXON, A. HUTTER, M. SEFTON *et al.*, 2000 Exploring redundancy in the yeast genome: an improved strategy for use of the cre-loxP system. Gene **252:** 127-135.

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VOTH, W. P., J. D. RICHARDS, J. M. SHAW and D. J. STILLMAN, 2001 Yeast vectors for integration at the HO locus. Nucleic Acids Research **29:** E59-59.

WILSON, D. S., and A. D. KEEFE, 2001 Random mutagenesis by PCR. Curr Protoc Mol Biol **Chapter 8:** Unit8 3.

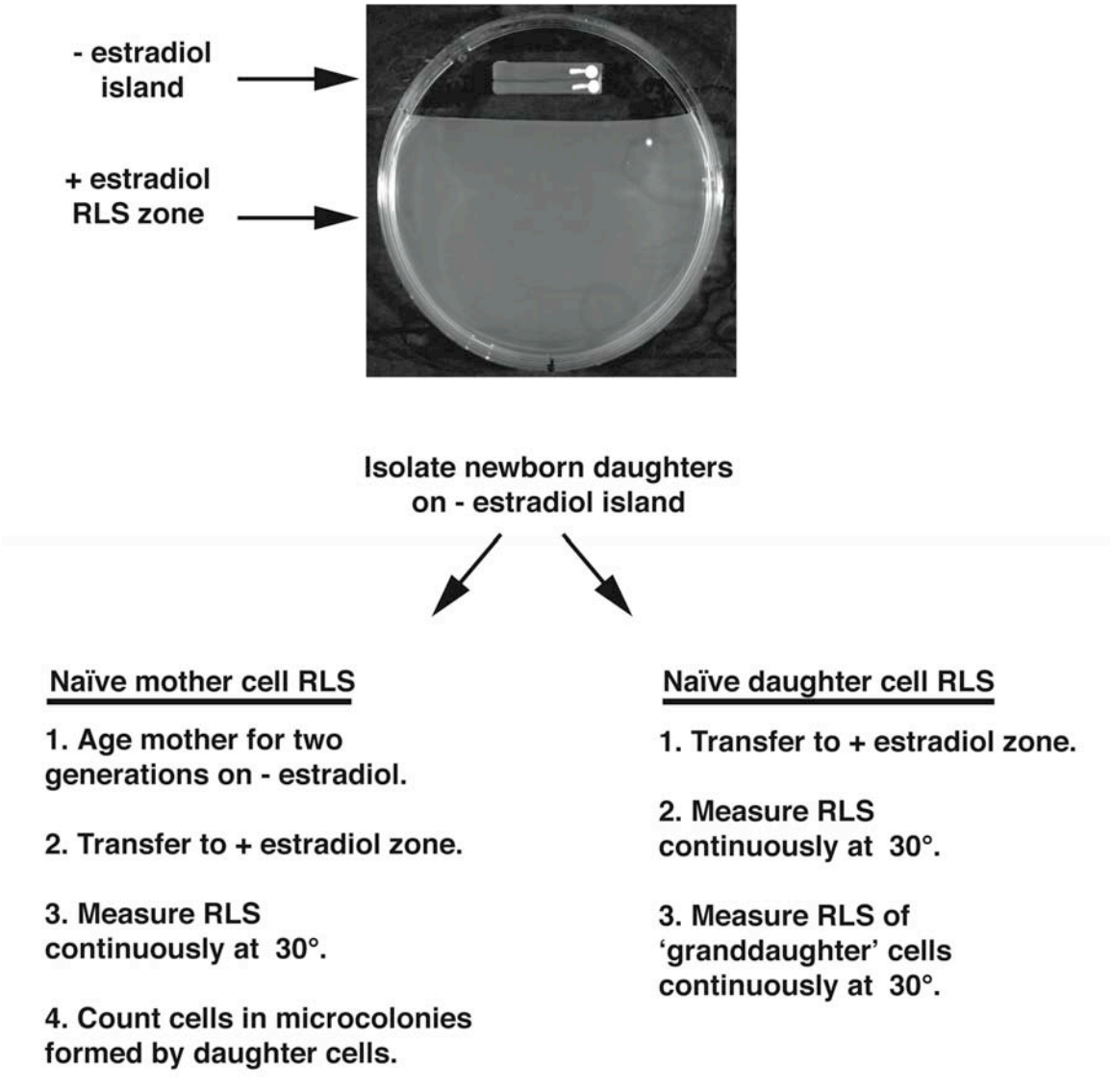

FIGURE S1.—Diagram of the experimental approach for measuring naïve mother and daughter RLS on media containing estradiol.

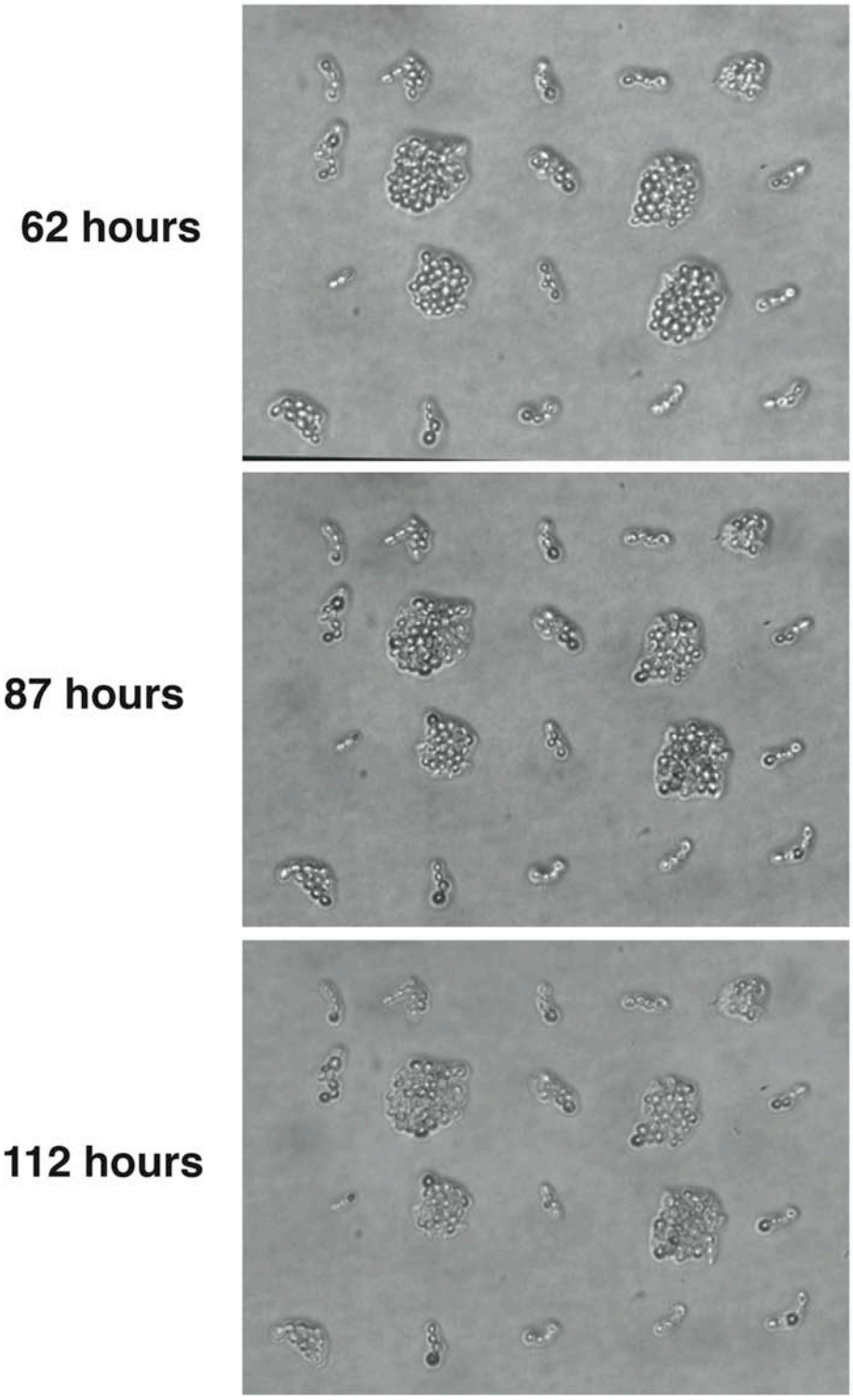

FIGURE S2.—Photographs of microcolonies formed by daughter cells born on estradiol during the life span measurement of naïve mother cells. Each photograph displays daughters #1-20 born to a single mother cell, arrayed from top left to bottom right. Times indicate hours after the mother was first placed on estradiol media (equivalent to 35, 60, and 85 hours after the birth of daughter #20). By 62 hours, each microcolony consisted of M-phase arrested cells. Microcolonies showed no change in cell number between 87 and 112 hours.

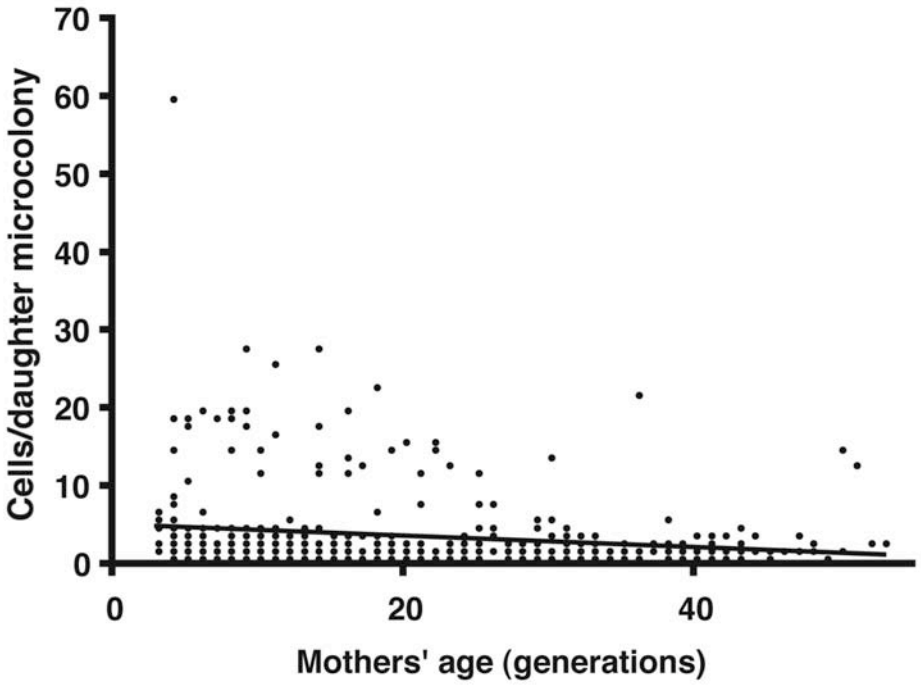

FIGURE S3.—A scatter plot comparing daughter's birth order (reflecting the age of the mother cell) to the number of cells in the daughter-cell microcolony (a measure of the daughter's proliferative capacity). The plot represents 472 daughter cells born on estradiol during the RLS analysis of naïve mothers. A best-fit line indicates negative correlation between mother's age and daughter's proliferative capacity.

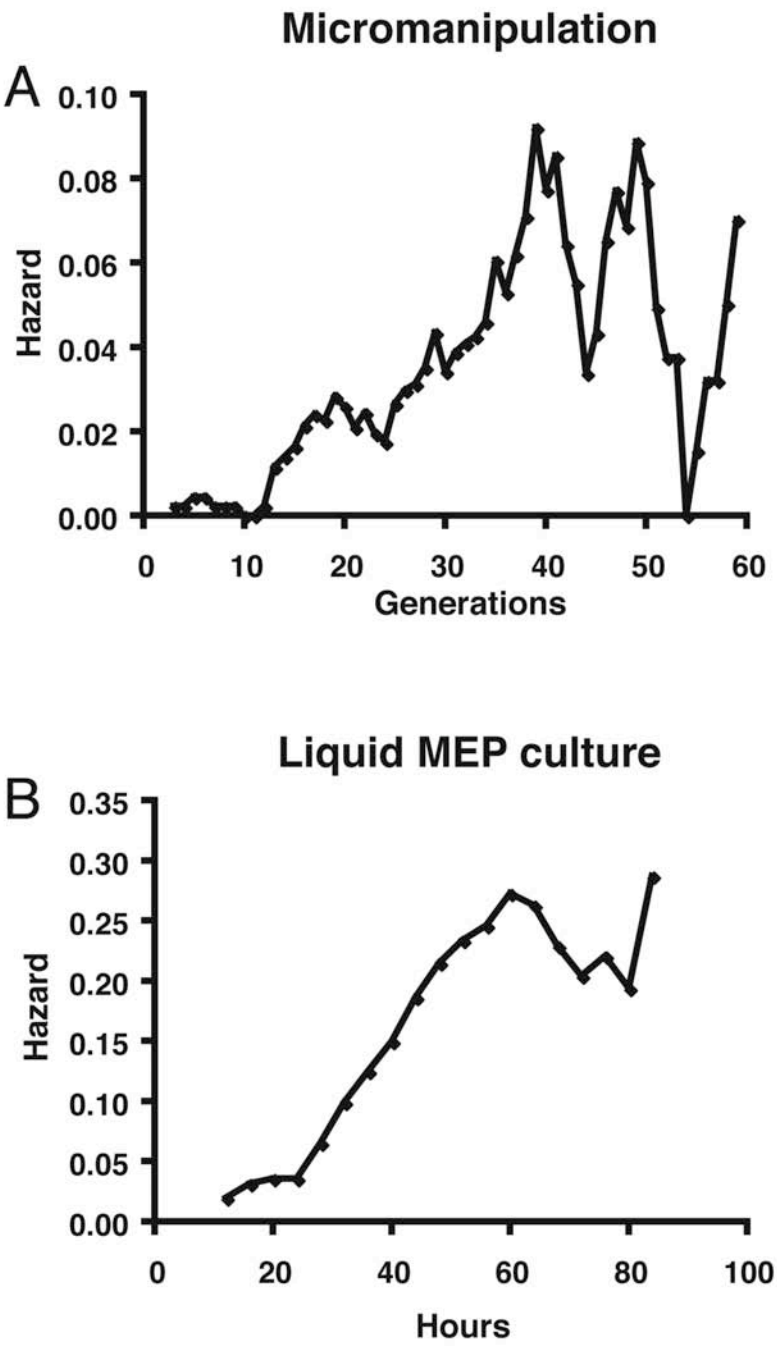

FIGURE S4.—(A) Hazard rate plot (# deaths/# individuals at risk) of UCC5185 RLS measured on estradiol media by micromanipulation, smoothed over an average of five neighbors. Hazard plots of wild type yeast populations typically display a low hazard rate at young ages, followed by an exponentially increasing rate. At very old ages, the small number of individuals observed results in large variations in hazard rate. (B) Hazard rate plot of UCC8848 viability measured in liquid estradiol media, smoothed over an average of five neighbors. To allow smoothing, intermediate values were estimated for four hour intervals based on a linear change in viability between sampling points.

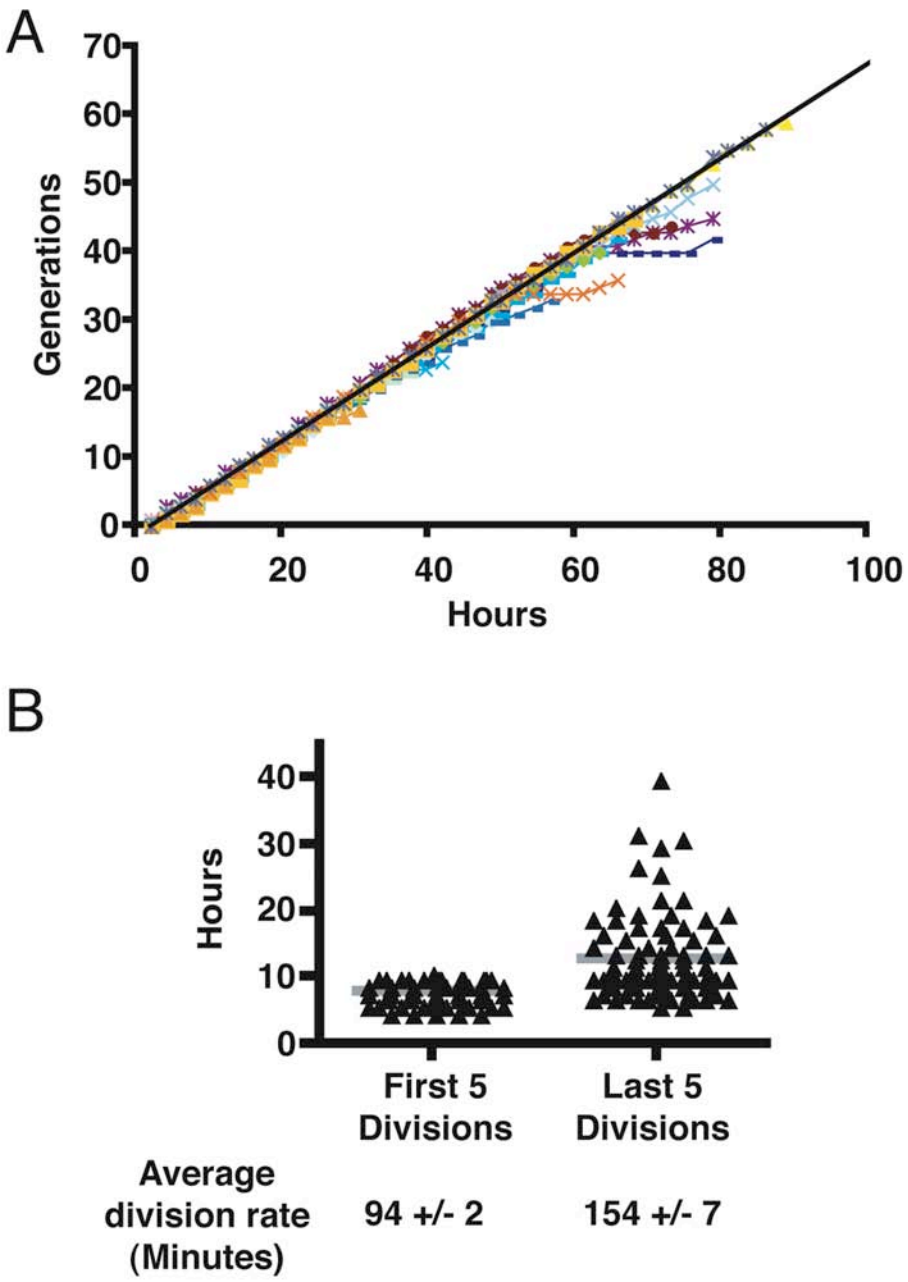

FIGURE S5.—(A) Division rates of 22 individual mother cells (UCC5185) during RLS measurement on estradiol media. A linear best-fit trendline (black, R=0.9992) based on the first 20 generations indicates a constant division rate of 87 minutes. Individual mothers approaching the end of their RLS often fall away from the trendline, indicating prolonged cell division times. (B) Time required for individual mothers to complete their first five divisions, and last five divisions before senescence. Each group represents a combined analysis of four pedigrees of UCC5185 (two on YEPD, two on estradiol media, n=99). Crossbars indicate the mean value of each group. The average division rate in minutes represents the mean value divided by five divisions.

TABLE S1

Oligonucleotides used in this study

Name	Sequence
CreScwF	AGCTCTAGTACGGATTAGAAGCCGCCGAGCGGGTGACAGCTTAATTACTTGTGTCTTG
CreScwR	TTAACACTCAGATAATGGTTTTAAGTAAAGTGTACAGGATGATGGTTTTCTATTAGAT
Bamlox1	GATCCTCTAGAATAACTTCGTATAATGTATGCTATACGAAGTTATG
Bamlox2	GATCCATAACTTCGTATAGCATACATTATACGAAGTTATTCTAGAG
RS+loxP-Not1	GAAAAAAGCGGCCGCCATAACTTCGTATAGCATACATTATACGAAGTTATGATTGTACT GAGAGTGCACC
RS-Not1	AAGGAAAAAAGCGGCCGCCTGTGCGGTATTTCACACCG
CreF	CCTGTACACTTTACTTAAAACC
EbdR	CACGAAATGTTCAGCACTAC
HOPolyF	CGAGGAATTCCATGATTACGCCAAGCGCGC
HOPolyR	CGAGGAATTCGCAGATTGTACTGAGAGTGC
UBC9lox5F	AAGGTAAGTAGTAGTTTTCCTCCTTTTATGCTTACATTGCATAGGCCACTAGTGGATCTG
UBC9lox5R	TTGTTAGTATACCGCTGGATGAAATTGTGTATGCCTACCAGCTGAAGCTTCGTACGC
UBC9lox3F	ATCTTTCCCATTCTTCCTCCTTTTGTACTTTATTTAACTAGGTGACACTATAGAAC
UBC9lox3R	TAATGTTATTTTGCTCTATTTTTGTTACACAACGACATAATACGACTCACTATAGG
CDC20loxF	ATTGGAAAGAAACCCAAAAATATAGAAATCGTCCATTCGCATAGGCCACTAGTGGATCTG
CDC20loxR	TAGTCTTCTTTGTAATACTTGTCTTTTGATATTTCTGCACCAGCTGAAGCTTCGTACGC
CDC20lox3F	TTAAAGAACCCACACACCACACGCGCGAGAACTGGGAGGGTAGGTGACACTATAGAAC
CDC20lox3R	TTCTTCCAAGGCCTAAATTTTGTTATTTGCTAAATTTCTCAATACGACTCACTATAGG
CDC20_hphF	TTAAAGAACCCACACACCACACGCGCGAGAACTGGGAGGGCACCCGGCCAGCGACATGGA
CDC20_hphR	TTCTTCCAAGGCCTAAATTTTGTTATTTGCTAAATTTCTCATAGGCCACTAGTGGATCTG
CDC20notF	TCACGCGGCCGCGTACCCTACATACTACATATTTC
CDC20notR	TCACGCGGCCGCTTCCTTATCGATTCTACAGC
CDC20_ACT1_F	CCAGAAAGCTCTAGAGATAAGGGAAATGCAGCAATTAGCGGTATGTTCTAGCGCTTGCAC
CDC20_ACT1_R	AGCTTTGTTGGGGACGCAATAGAAAGTACAGAACGGTTACCTAAACATATAATATAGCAA

TABLE S2

Yeast strains used in this study.

Strain	Genotype
UCC526	*MAT**a**/MATα ade2::hisG/ade2::hisG his3/his3 leu2/leu2 LYS2/lys2 ura3Δ0/ura3Δ0 trp1Δ63/trp1Δ63 MET15/met15Δ:: ADE2 hoΔ::P_{SCW11}-cre-EBD78-NATMX/hoΔ::P_{SCW11}-cre-EBD78-NATMX loxP-UBC9-loxP-LEU2/loxP-UBC9-loxP-LEU2 loxP-CDC20-Intron-loxP-HPHMX/loxP-CDC20-Intron-loxP-HPHMX fob1Δ::KANMX/fob1Δ::KANMX*
UCC3813	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 loxP-CDC20-HPHMX*
UCC5179	*MAT**a** ade2::hisG his3 leu2 lys2 ura3Δ0 trp1Δ63 hoΔ::P_{SCW11}-cre-EBD78-NATMX loxP-UBC9-loxP-LEU2 loxP-CDC20-Intron-loxP-HPHMX*
UCC5181	*MATα ade2::hisG his3 leu2 trp1Δ63 ura3Δ0 met15Δ::ADE2 hoΔ::P_{SCW11}-cre-EBD78-NATMX loxP-UBC9-loxP-LEU2 loxP-CDC20-Intron-loxP-HPHMX*
UCC5185	*MAT**a**/MATα ade2::hisG/ade2::hisG his3/his3 leu2/leu2 LYS2/lys2 ura3Δ0/ura3Δ0 trp1Δ63/trp1Δ63 MET15/met15Δ::ADE2 hoΔ::P_{SCW11}-cre-EBD78-NATMX/hoΔ::P_{SCW11}-cre-EBD78-NATMX loxP-UBC9-loxP-LEU2/loxP-UBC9-loxP-LEU2 loxP-CDC20-Intron-loxP-HPHMX/loxP-CDC20-Intron-loxP-HPHMX*
UCC8600	*MAT**a**/MATα ADE2/ade2Δ::hisG his3Δ1/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0/lys2Δ0 ura3Δ0/ura3Δ0 TRP1/trp1Δ63 MET15/met15Δ0 CAN1/can1Δ::pMFA1-HIS3, pMFα1-LEU2*
UCC8611	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 loxP-CDC20*
UCC8612	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 can1Δ::pMFa1-HIS3, pMFα1-LEU2 loxP-ADE2-loxP-HPHMX rga1*
UCC8650	*MAT**a** his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 hoΔ::P_{SCW11}-cre-EBD78-NATMX*
UCC8697	*MATα his3Δ200 leu2Δ0 lys2Δ0 ura3Δ0 loxP-UBC9-loxP-LEU2*
UCC8701	*MATα his3Δ200 leu2Δ0 lys2Δ0 ura3Δ0 loxP-UBC9*
UCC8723	*MAT**a** his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 cdc20Δ::KANMX [pDL25]*
UCC8740	*MAT**a** his3Δ1 leu2Δ0 ura3Δ0 hoΔ:: P_{SCW11}-cre-EBD78-NATMX loxP-UBC9-loxP-LEU2*
UCC8779	*MAT**a** his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 loxP-CDC20-Intron-loxP-HPHMX*
UCC8836	*MAT**a**/MATα ade2::hisG/ade2::hisG his3/his3 leu2/leu2 LYS2/lys2 ura3Δ0/ura3Δ0 trp1Δ63/trp1Δ63 MET15/met15Δ::ADE2 hoΔ::P_{SCW11}-cre-EBD78-NATMX/hoΔ::P_{SCW11}-cre-EBD78-NATMX loxP-UBC9-loxP-LEU2/loxP-UBC9-loxP-LEU2 loxP-CDC20-Intron-loxP-HPHMX/loxP-CDC20-Intron-loxP-HPHMX sir2Δ::KANMX/sir2Δ::KANMX*
UCC8848	*MAT**a**/α ADE2/ade2::hisG his3/his3 leu2/leu2 LYS2/lys2Δ0 trp1Δ63/ trp1Δ63 ura3Δ0/ura3Δ0 hoΔ::P_{SCW11}-cre-EBD78-NATMX/hoΔ::P_{SCW11}-cre-EBD78-NATMX loxP-CDC20-Intron-loxP-HPHMX/loxP-CDC20-Intron-loxP-HPHMX loxP-UBC9-loxP-LEU2/loxP-UBC9-loxP-LEU2*
UCC8849	*MAT**a**/MATα ade2::hisG/ade2::hisG his3/his3 leu2/leu2 LYS2/lys2 ura3Δ0/ura3Δ0 trp1Δ63/trp1Δ63 met15Δ::ADE2 /met15Δ::ADE2 hoΔ::P_{SCW11}-cre-EBD78-NATMX/hoΔ::P_{SCW11}-cre-EBD78-NATMX loxP-UBC9-loxP-LEU2/loxP-UBC9-loxP-LEU2 loxP-CDC20-Intron-loxP-HPHMX/loxP-CDC20-Intron-loxP-HPHMX sir2Δ::KANMX/sir2Δ::KANMX*
UCC8850	*MAT**a**/MATα ade2::hisG/ade2::hisG his3/his3 leu2/leu2 LYS2/lys2 ura3Δ0/ura3Δ0 trp1Δ63/trp1Δ63 met15Δ::ADE2/ met15Δ::ADE2 hoΔ::P_{SCW11}-cre-EBD78-NATMX/hoΔ::P_{SCW11}-*

	cre-EBD78-NATMX loxP-UBC9-loxP-LEU2/loxP-UBC9-loxP-LEU2 loxP-CDC20-Intron-loxP-HPHMX/loxP-CDC20-Intron-loxP-HPHMX fob1Δ::KANMX/fob1Δ::KANMX
UCC8852	*MAT**a** his3 leu2 lys2Δ trp1Δ63 ura3Δ0 hoΔ::P_{SCW11}-cre-EBD78-NATMX loxP-CDC20-Intron-loxP-HPHMX*
UCC8853	*MAT**a** his3 leu2 lys2Δ ura3Δ0 hoΔ::P_{SCW11}-cre-EBD78-NATMX loxP-CDC20-Intron-loxP-HPHMX loxP-UBC9-loxP-LEU2*
UCC8857	*MAT**a**/MATα ADE2/ade2::hisG his3Δ1/his3 leu2Δ0/leu2 LYS2/lys2D0 TRP1/trp1Δ63 ura3D0/ura3D0 hoΔ::P_{SCW11}-cre-EBD78-NATMX/hoΔ::P_{SCW11}-cre-EBD78-NATMX*
UCC8858	*MAT**a**/MATα his3Δ1/his3 leu2Δ0/leu2 ura3Δ0/ura3Δ0 hoΔ::P_{SCW11}-cre-EBD78-NATMX/hoΔ::P_{SCW11}-cre-EBD78-NATMX loxP-UBC9-loxP-LEU2/loxP-UBC9-loxP-LEU2*
UCC8859	*MAT**a**/MATα his3/his3 leu2/leu2 LYS2/lys2Δ TRP1/trp1Δ63 ura3Δ0/ura3Δ0 hoΔ::P_{SCW11}-cre-EBD78-NATMX/hoΔ::P_{SCW11}-cre-EBD78-NATMX loxP-CDC20-Intron-loxP-HPHMX/loxP-CDC20-Intron-loxP-HPHMX*
UCC8860	*MAT**a**/MATα ADE2/ade2::hisG his3/his3 leu2/leu2 LYS2/lys2 ura3Δ0/ura3Δ0 TRP1/trp1Δ63 MET15/met15Δ::ADE2 hoΔ::P_{SCW11}-cre-EBD78-NATMX/hoΔ::P_{SCW11}-cre-EBD78-NATMX loxP-CDC20-Intron-loxP-HPHMX/loxP-CDC20-Intron-loxP-HPHMX loxP-UBC9-loxP-LEU2/loxP-UBC9-loxP-LEU2*
UCC8861	*MAT**a**/MATα ade2::hisG/ade2::hisG his3/his3 leu2/leu2 LYS2/lys2 ura3Δ0/ura3Δ0 trp1Δ63/trp1Δ63 met15Δ::ADE2/ met15Δ::ADE2 hoΔ::P_{SCW11}-cre-EBD78-NATMX/hoΔ::P_{SCW11}-cre-EBD78-NATMX loxP-UBC9-loxP-LEU2/loxP-UBC9-loxP-LEU2 loxP-CDC20-Intron-loxP-HPHMX/loxP-CDC20-Intron-loxP-HPHMX*
UCC8863	*MAT**a**/MATα ADE2/ade2::hisG his3/his3 leu2/leu2 LYS2/lys2Δ ura3Δ0/ura3Δ0 trp1Δ63/trp1Δ63 hoΔ::SCW11pr-cre-EBD78-NatMX/hoΔ::SCW11pr-cre-EBD78-NatMX loxP-CDC20-Intron-loxP-HPHMX/loxP-CDC20-Intron-loxP-HPHMX loxP-UBC9-loxP-LEU2/loxP-UBC9-loxP-LEU2 fob1Δ::KANMX/fob1Δ::KANMX*

TABLE S3

Plasmids used in this study

Plasmid	Source	Notes
pDL01	This work	P_{SCW11}-*cre-EBD-NATMX* CEN ori ampR
pDL03(-)	This work	*loxP-HPHMX* CEN ori ampR
pDL12	This work	*cre-EBD78-NATMX* in the HO-poly-HO integration vector
pDL20	This work	P_{SCW11}-*cre-EBD78-NATMX* CEN ori ampR
pDL25	This work	*CDC20* subcloned into *Not*1 site of pRS316
pDL26	This work	*loxP-LEU2* CEN ori ampR
pEH5	This work	*loxP-CDC20-HPHMX HIS3* CEN ori ampR
pEH6	This work	*loxP-CDC20-Intron-loxP-HPHMX HIS3* CEN ori ampR
pLND4	Lazar Dimitrov	*loxP* site inserted into *ACT1* intron on plasmid pRX1 (CHENG *et al.*)[a]
pFvL113	Fred van Leeuwen	*HIS3* from pSH62 (CHENG *et al.*)[a] replaced with *NATMX*
p126SCW	Daniel Lockshon	P_{SCW11}-*GFP HIS3* CEN ori ampR

(a) CHENG, T. H., C. R. CHANG, P. JOY, S. YABLOK and M. R. GARTENBERG, 2000 Controlling gene expression in yeast by inducible site-specific recombination. Nucleic Acids Research **28:** E108.