# 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 $1.68 \times 10^{-4}$ 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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