Supplemental Information

Ku prevents permanent cell cycle arrest in *Ustilago maydis* by suppressing DNA damage signaling at telomeres

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SUPPLEMENTARY FIGURES

Figure S1

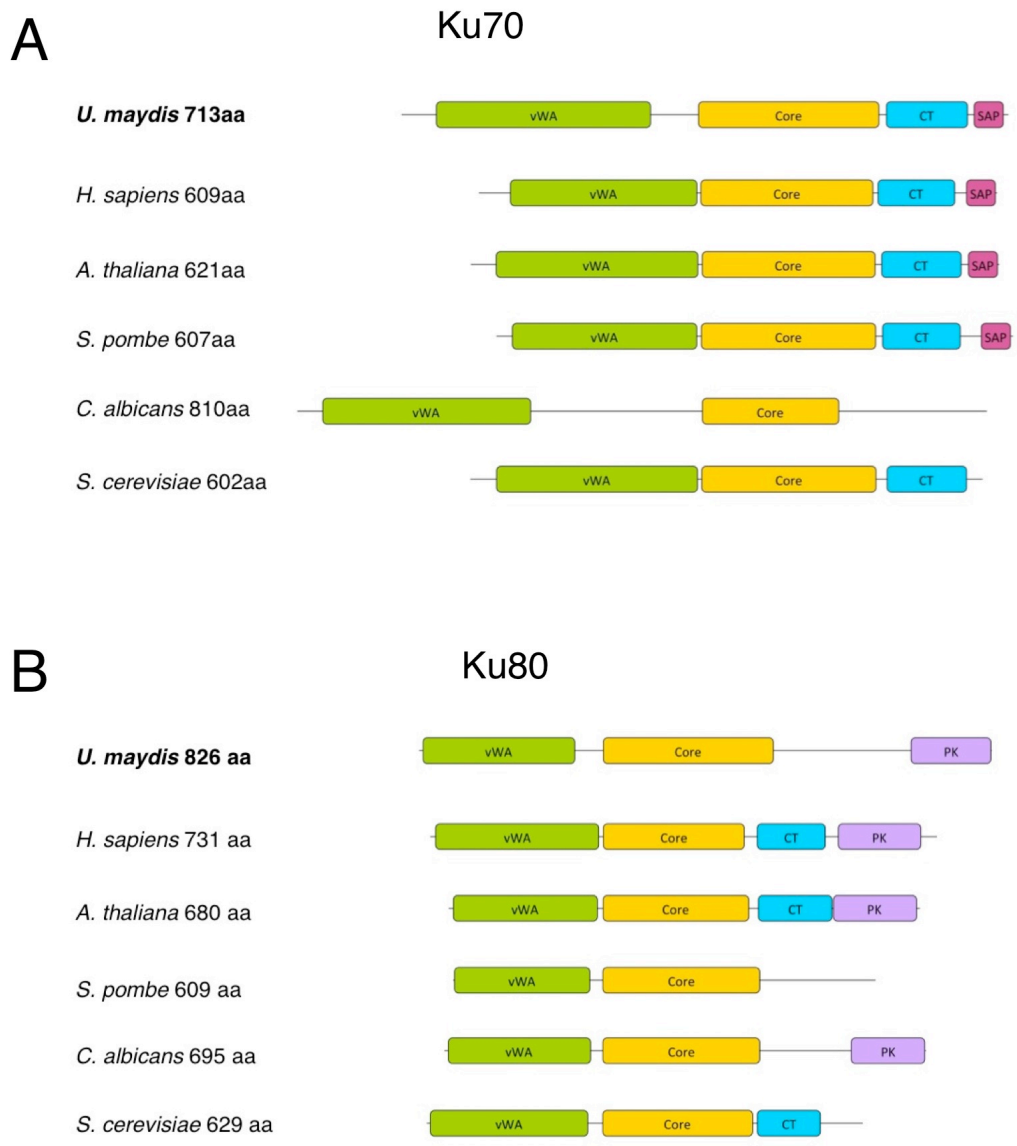


Figure S1. *U. maydis* Ku70 and Ku80 proteins.

A and B. The sizes and domain structures of Ku70 and Ku80 homologues in *U. maydis* and other species.

U. maydis Ku70 and Ku80 homologues were identified through a homology search using the BLAST program. Ku70 and Ku80 homologues in *S. cerevisiae*

and *S. pombe* were used as the queries. The search was done on the *U. maydis* database at the Munich Information Center for Protein Sequences, and subsequently the results were confirmed by performing a BLAST at the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The best hits from these searches were two loci that had previously been manually annotated as um05148 and um05756. Um05148 codes for a 713 amino acids protein with a predicted molecular weight of around 80 kD. Um05756 codes for a protein of 826 amino acids with a predicted molecular weight of around 92 kDa. Ku70 and Ku80 from different species share three common domains but possess distinct C-terminal domains. The shared domains are a N-terminal α/β domain or von Willebrand A domain (vWA) that is thought to be a protein-protein interaction site that binds to other repair factors; a central core (comprised of β-barrel) domain with a DNA binding activity that is dependent on heterodimer formation (Core); and a helical C-terminal arm which embraces the other subunit's core domain (CT) (1, 2). In some eukaryotes, Ku70 contains a SAP domain at the carboxy-terminus, named after three proteins containing this motif (SAF-A/B, Acinus and PIAS), which has been proposed to be a DNA binding domain (3). However, this domain is dispensable for the DNA-binding activity of the heterodimer. Alternatively, a role for SAP in pausing Ku at specific DNA sequences has been proposed. Some Ku80 homologs contain a carboxy-terminal extension that may bind the DNA-dependent protein kinase catalytic subunit (PK) (4).

1. Walker, J.R., Corpina, R.A., and Goldberg, J. (2001). Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. *Nature* **412,** 607-614.

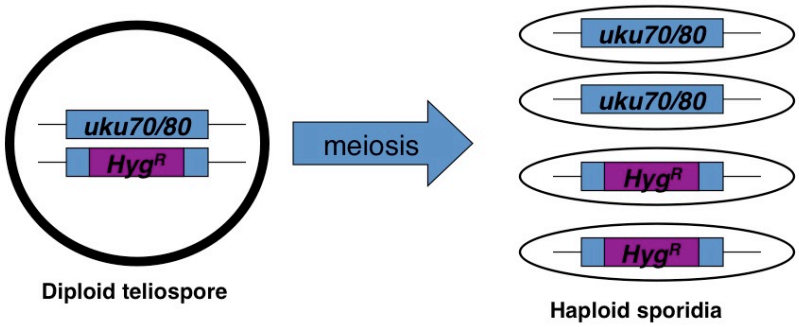
2. Wang, J., Dong, X., and Reeves, W.H. (1998). A model for Ku heterodimer assembly and interaction with DNA. Implications for the function of Ku antigen. *J Biol Chem* **273,** 31068-31074.

3. Aravind, L., and Koonin, E.V. (2000). SAP - a putative DNA-binding motif involved in chromosomal organization. *Trends Biochem Sci* **25,** 112-114.

4. Harris, R., Esposito, D., Sankar, A., Maman, J.D., Hinks, J.A., Pearl, L.H., and Driscoll, P.C. (2004). The 3D solution structure of the C-terminal region of Ku86 (Ku86CTR). *J Mol Biol* **335,** 573-582.

Figure S2

A



B

Spore relevant genotype	Number of spores analyzed	% of Hyg^R in the meiotic progeny
*a1/a2 b1/b2 uku70/uku70*Δ (HygR)	30	0
*a1/a2 b1/b2 uku80/uku80*Δ (HygR)	30	0

Figure S2. Meiotic analysis of the essentiality of *uku70* and *uku80* genes.

A. Scheme of the meiotic analysis in *U. maydis*. In the diploid strain FBD11, one of the two alleles of either *uku70* or *uku80* genes was disrupted and replaced with the hygromycin resistance cassette. These mutant strains were used to infect corn plants and the final product of the infection, i.e., teliospores were isolated. Teliospores are diploid and during their germination, meiosis is completed and haploid progeny cells are produced.

B. Results from the meiotic analysis. 30 teliospores isolated from corn plants infected with the indicated diploid strains were germinated and the percentage of hygromycin progeny from each was scored. We found no hygromycin resistant cells, indicating that both *uku70* and *uku80* are essential genes.

Figure S3

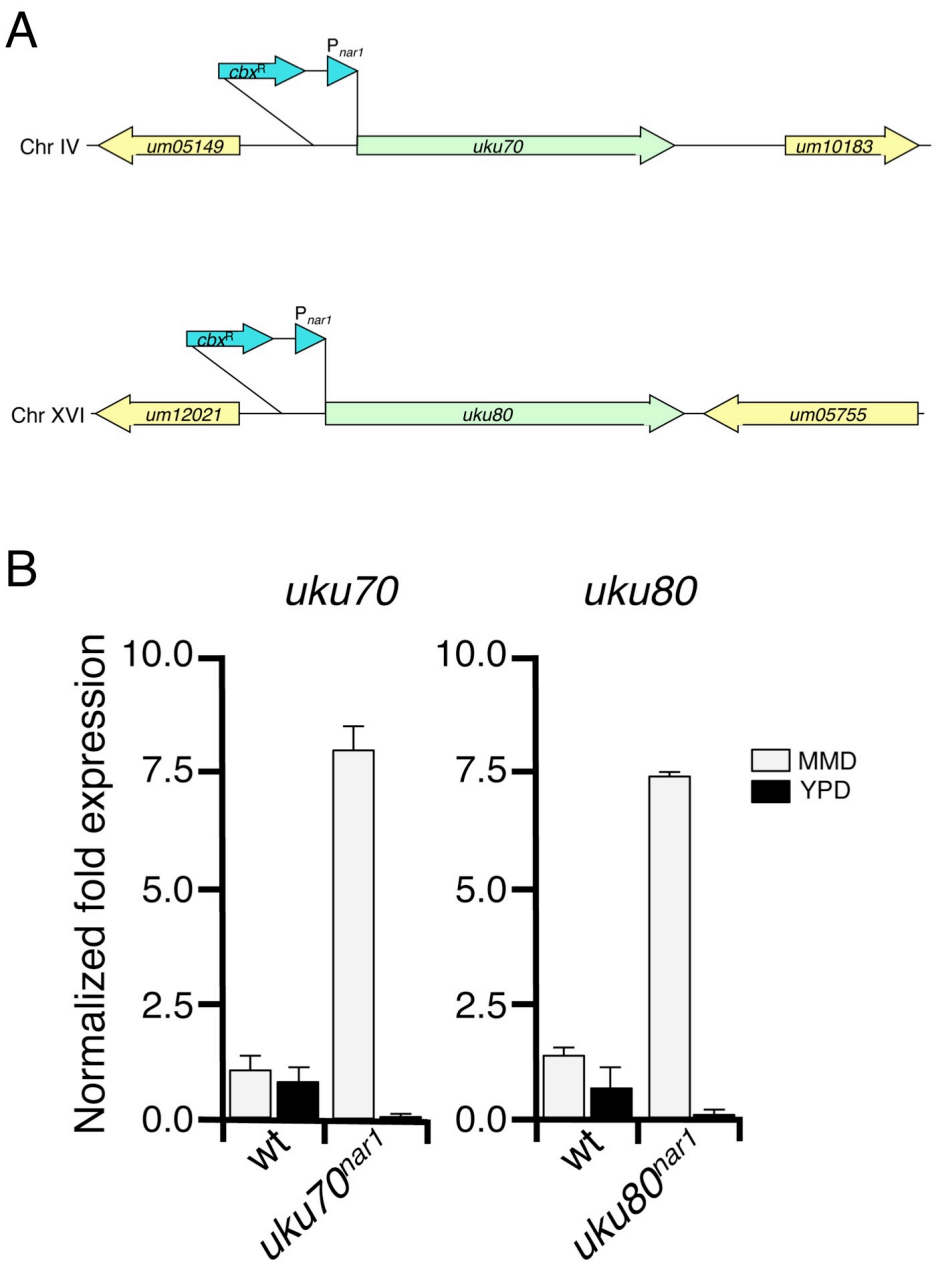


Figure S3. *uku70*^{nar1} and *uku80*^{nar1} conditional alleles

A. Schematics of the genomic structures of the conditional alleles *uku70*^{nar1} and *uku80*^{nar1}

B. Levels of *uku70 and uku80* mRNA in the respective conditional strains. The wild type FB1 and conditional strains were grown for 8 hours in permissive

(MMD) or restrictive conditions (YPD). Total RNA was extracted from each strain with acidic phenol solution. After extraction, the RNA was purified using the High Pure RNA Isolation Kit (Roche Diagnostics GmbH). For qRT-PCR, cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) employing 1 μg total RNA per sample. qRT-PCR was performed using the SsoAdvanced Universal SYBR Green Supermix (BioRad) in a CFX96 Real-Time PCR system (BioRad). Reaction conditions were as follows: 3 min 95ºC followed by 40 cycles of 10 sec 95ºC/10 sec 60ºC/30 sec 72ºC.

Figure S4

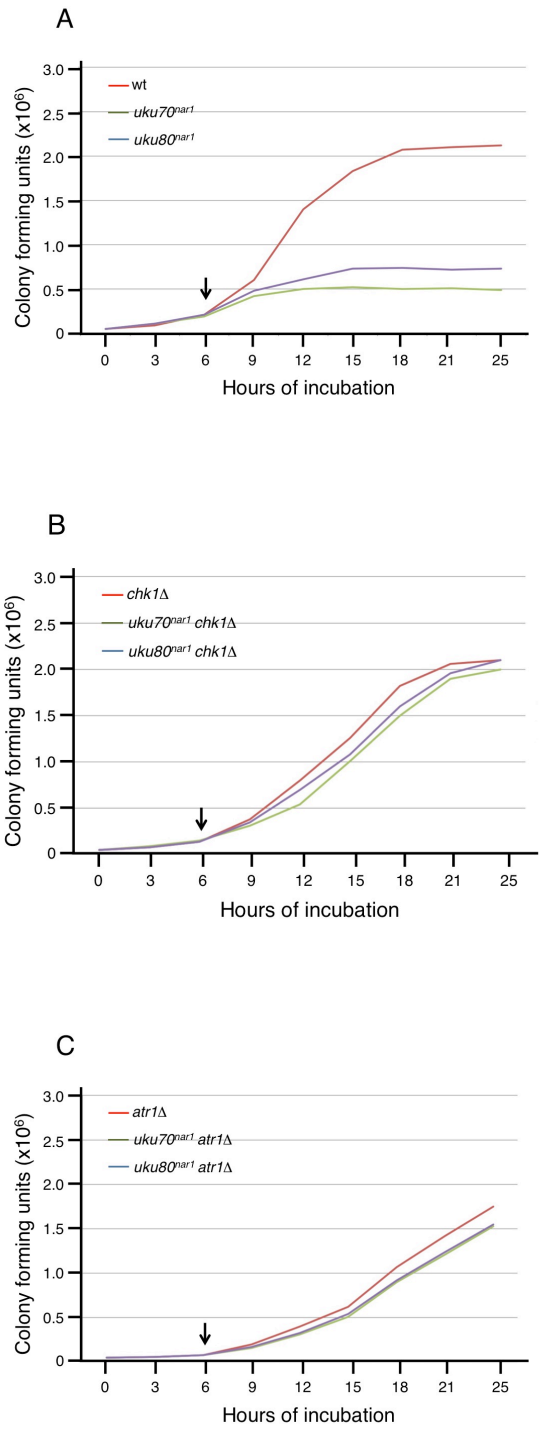


Figure S4. Disabling the DNA damage response suppressed the requirement of Ku for proliferation of *U. maydis* cells

A. Growth curves of FB1 (WT), UCS33 (*uku70*^{nar1}), and UCS30 (*uku80*^{nar1}) in liquid culture. Cells ($5x10^4$ cells /ml) were grown for 6 hours in $MMNO_3$ and then switched to YPD medium (repressive conditions for *nar1* expression). Samples of each culture were removed at the indicated time and plated on minimal medium plates to determine the number of viable cells at each time point (colony forming units). Arrow indicates the time point at which cultures were switched to YPD medium.

B. Growth curves for UMP122 (*chk1*Δ), UCS35 (*uku70*^{nar1} *chk1*Δ), and UCS39 (*uku80*^{nar1} *chk1*Δ) were determined as in A and plotted.

C. Growth curves for UCS1 (*atr1*Δ), UCS40 (*uku70*^{nar1} *atr1*Δ), and UCS44 (*uku80*^{nar1} *atr1*Δ) were determined as in A and plotted.

Figure S5

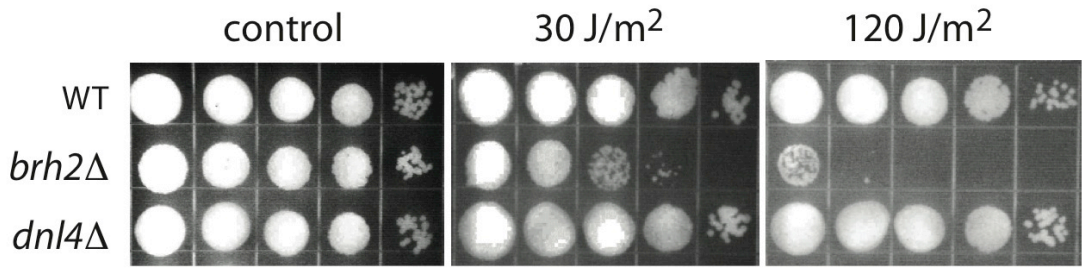


Figure S5. Ligase IV mutants are not defective in proliferation.

Control (wt) and ligase IV mutant (*dnl4*Δ) cells were spotted on solid medium and exposed to different doses of UV radiation. A strain carrying a deletion of *brh2*, encoding the BRCA2 homologue of *U. maydis* was used as the positive control.

Figure S6

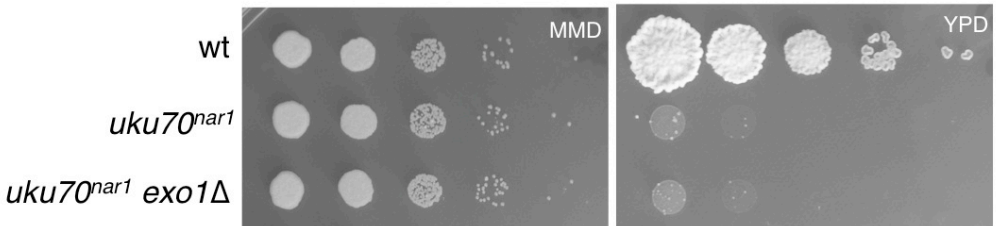


Figure S6. Deletion of *exo1* does not suppress the essentiality of *uku70*.

Serial tenfold dilutions of strain cultures carrying the indicated mutations were applied to solid rich medium (YPD) and minimal medium with nitrate (MMD). The YPD plates were incubated for 2 days and the nitrate plates for 4 days at 28ºC.

Figure S7

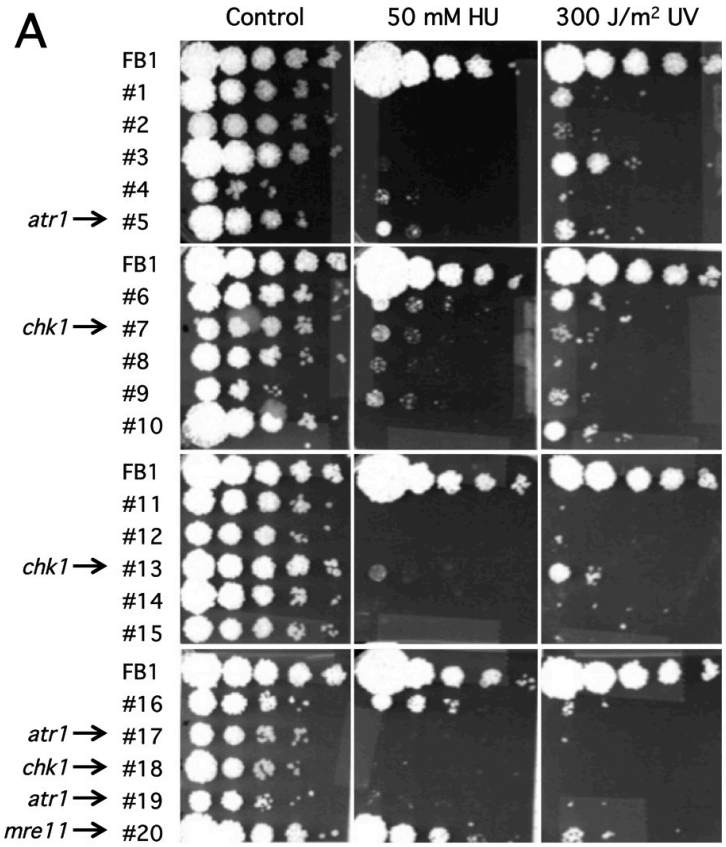
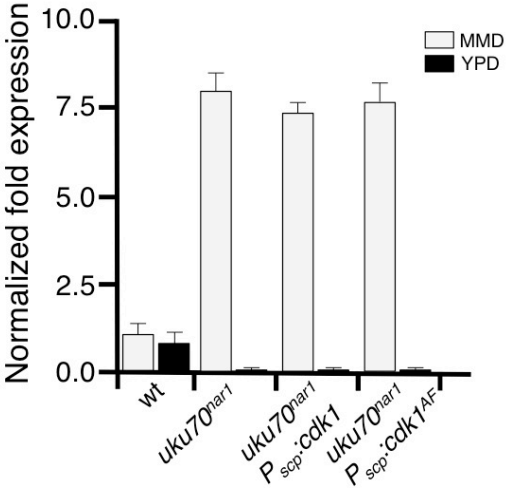


Figure S7. Genetic screen for suppressors of Ku complex essentiality.

UCS33 strain carrying the *uku70*^{nar1} allele was mutagenized using UV. 20 independent suppressors of Ku essentiality were spotted in restrictive conditions for *uku70*^{nar1} expression in plates containing 50 mM HU or YPD plates and irradiated with 300J/m2 of UV light. The majority of the isolated mutants showed hypersensitivity to at least one of these DNA damaging agents. Arrows on left side marked the strains in which plasmids carrying wild-type alleles of *chk1, atr1* or *mre11* were able to complement the DNA damage hypersensitive phenotype as well as the essentiality of Ku70.

Figure S8

A



B

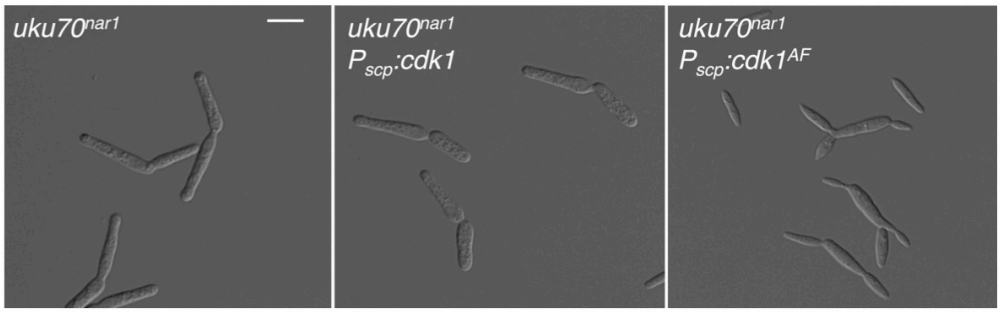


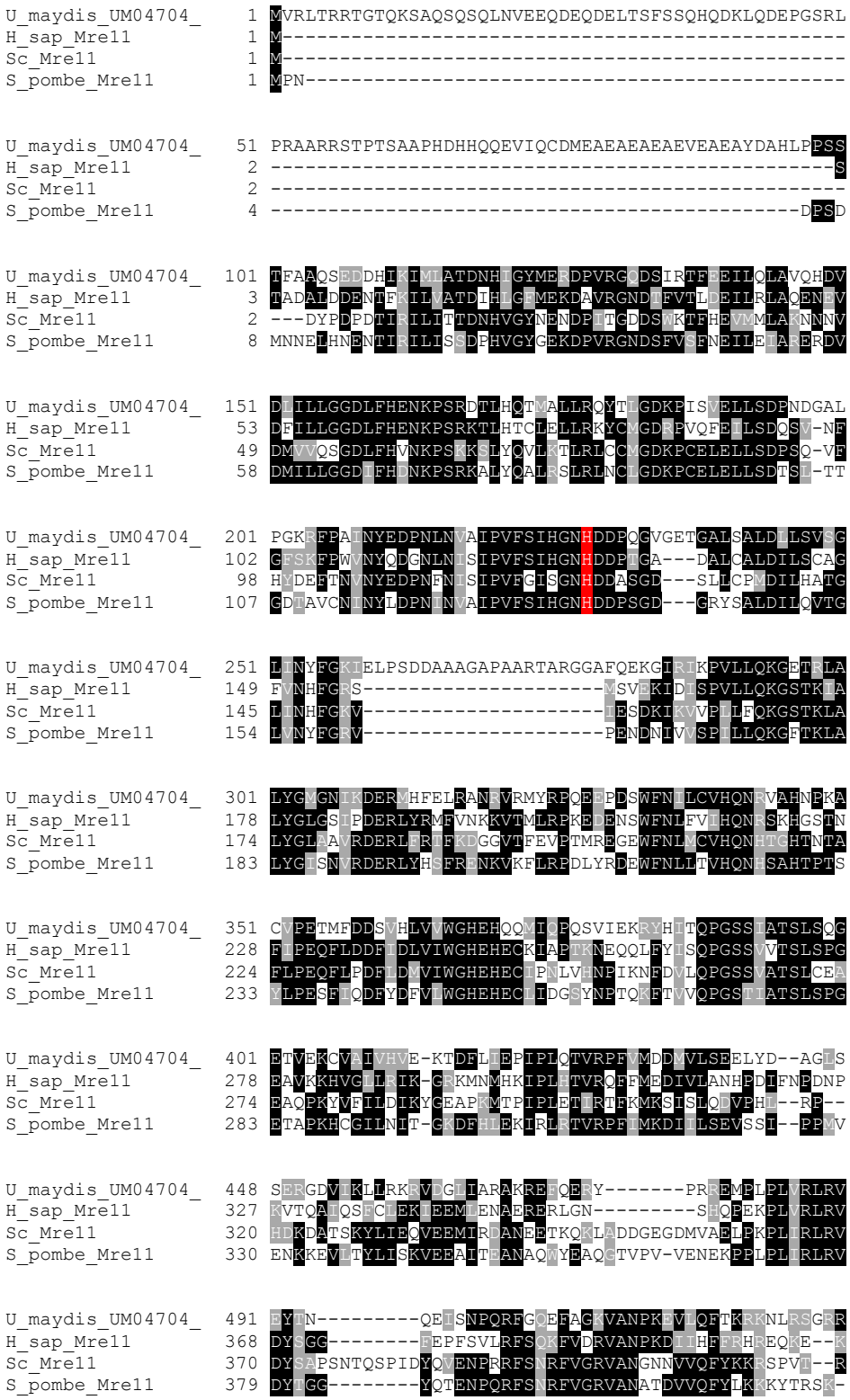
Figure S8, Related to Figure 3

A. The levels of *uku70* mRNA in the respective conditional strains. The wild type FB1 and the conditional strains were grown for 8 hours in permissive (MMD) or restrictive conditions (YPD). Total RNA from each strain was extracted, and the *uku70* RNA level quantified as explained in Fig. S3.

B. Morphology of *uku70^{nar1}* (UCS33), *uku70^{nar1} P$_{scp}$:cdk1* (UMP221) and *uku70^{nar1} P$_{scp}$:cdk1^{AF}* (UMP222) cells incubated for 8 hours in restrictive conditions (YPD). All cells were shown at the same magnification, Bar: 15 μm.

The presence of smaller, non-elongated cells in UMP222 is consistent with suppression of the G2 arrest phenotype.

Figure S9



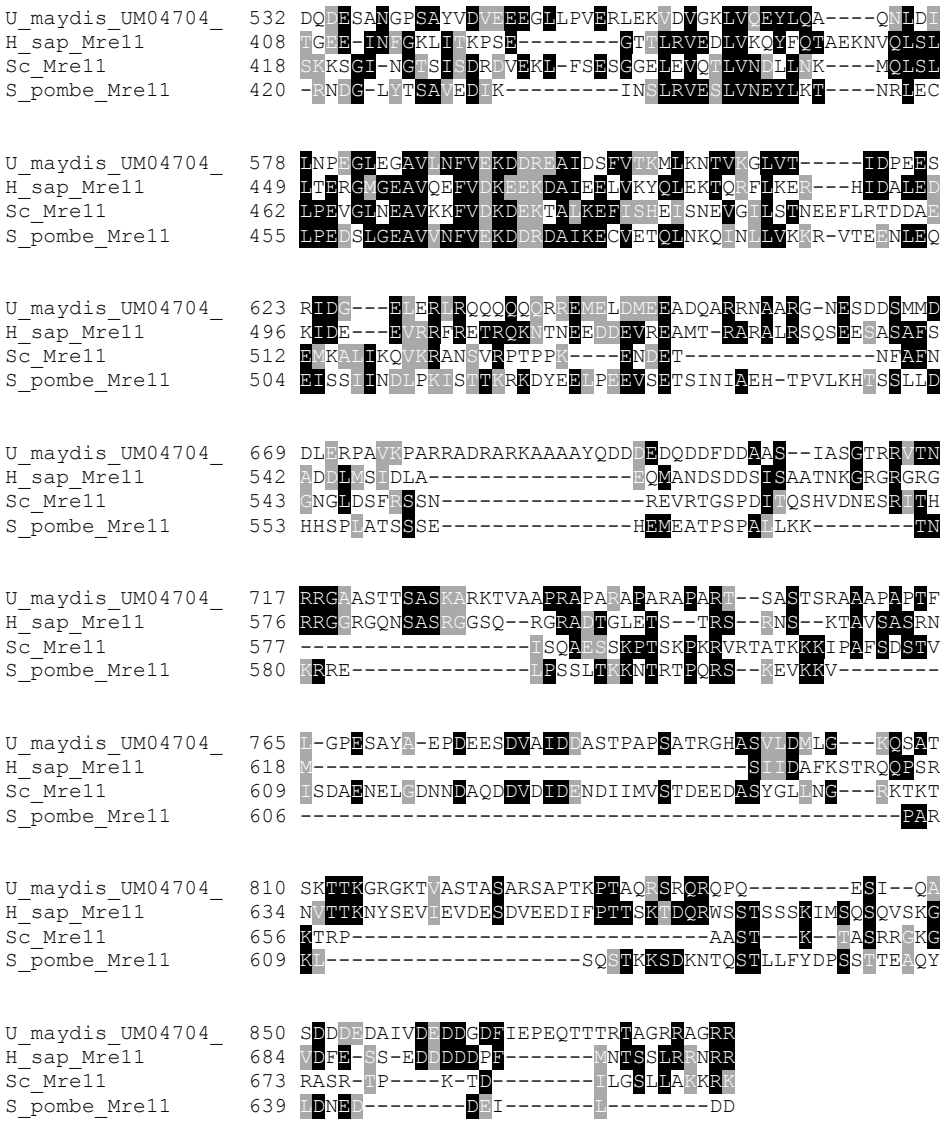


Figure S9. *U. maydis* Mre11

A multiple sequence alignment of Mre11 from *S. cerevisiae*, *S. pombe* and humans with the hypothetical *U. maydis* UM04704 protein. Identical amino acids are indicated in black boxes while conservative changes are indicated in gray boxes. The conserved histidine residue in nuclease motif III is highlighted in a red box.

Figure S10

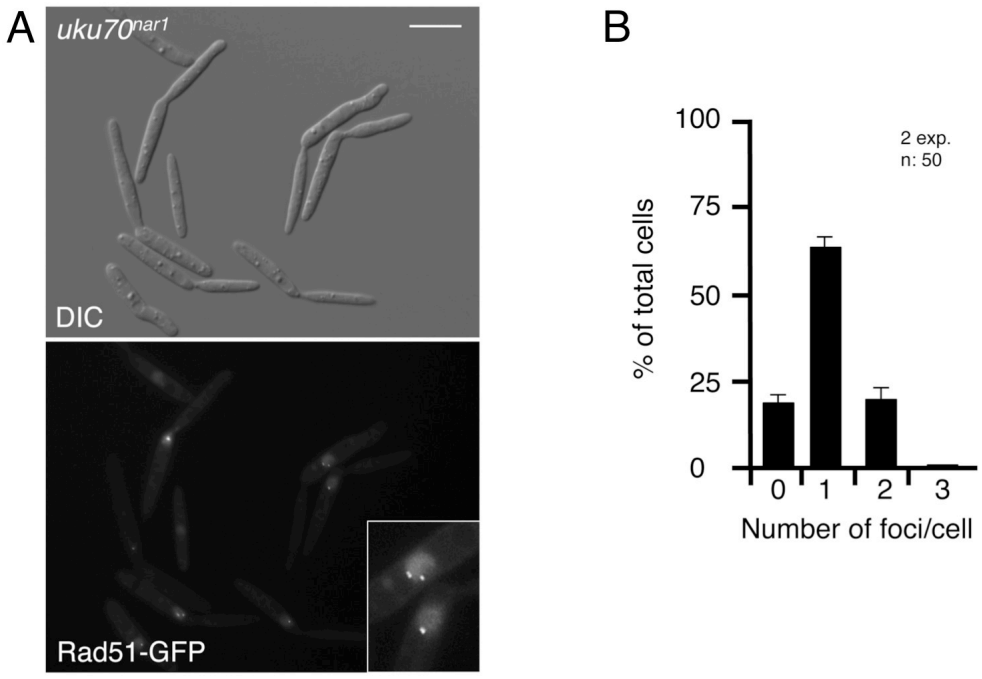


Figure S10. Formation of Rad51-GFP foci in Ku70-depelted cells.

A. Images of UCS33 cells carrying a Rad51-GFP fusion as well as the *uku70*^{nar1} allele after 8h of incubation in restrictive conditions for *uku70*^{nar1} expression. Bar: 10 μm. Inset shows a magnification of selected nuclei.

B. Quantification of cells showing punctate Rad51-GFP foci.

Figure S11

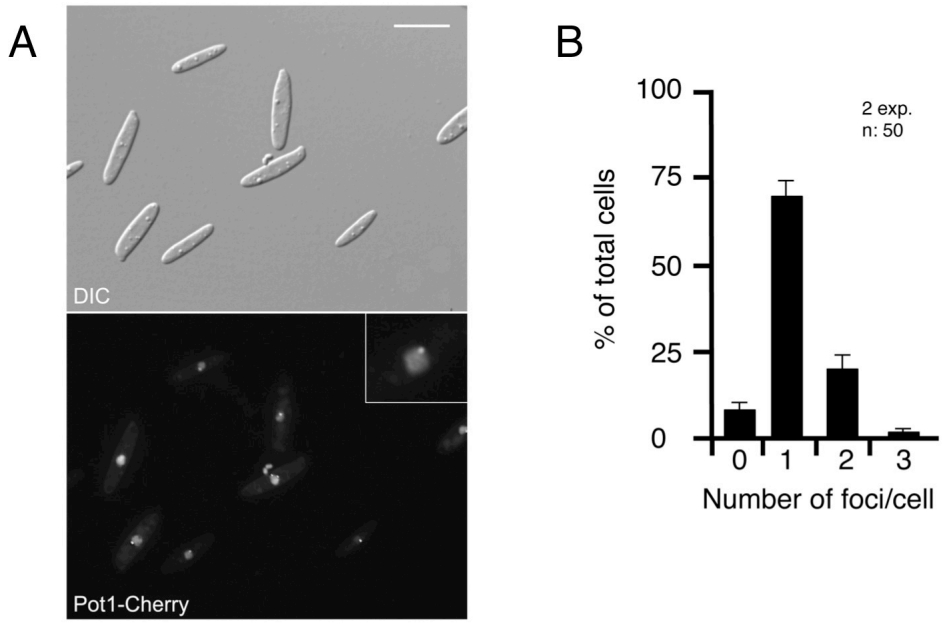


Figure S11. Pot1-cherry as a telomeric marker in *U. maydis*.

A. Images of UMP192 cells carrying a Pot1-cherry fusion grown until the mid-exponential phase in YPD. Bar: 10 μm. Inset shows a magnification of a selected nucleus.

B. Quantification of cells showing Pot1-cherry foci.

Figure S12

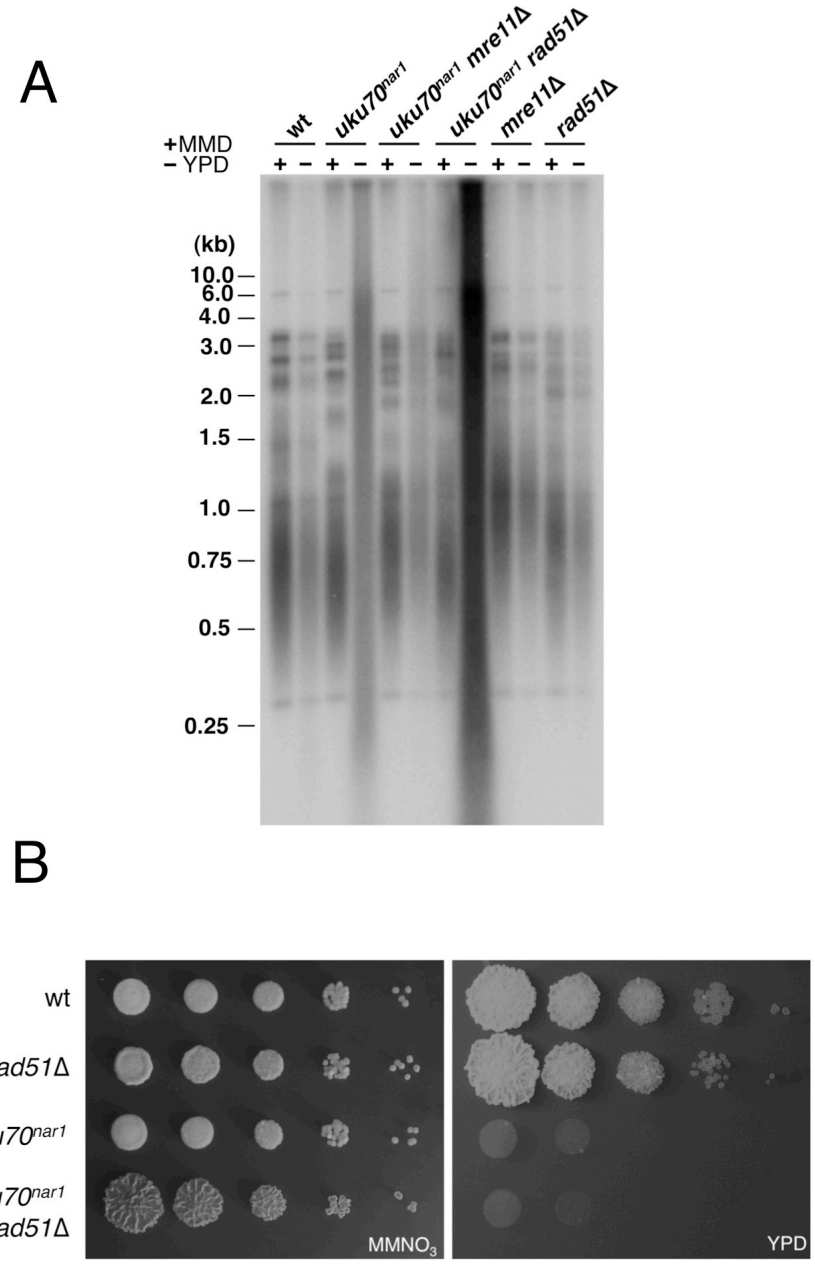


Figure S12. Rad51 does not suppress the essentiality of Ku70

A. DNAs from the indicated strains (FB1 (WT), UCS33 (*uku70^{nar1}*), UMP218 (*uku70^{nar1} mre11*Δ), UMP215 (*uku70^{nar1} rad51*Δ), UMP219 (*mre11*Δ), and

UMP214 (*rad51*Δ)) grown in restrictive (-,YPD) or permissive (+, MMD) conditions for 18 h were isolated, digested with *Pst*I, and hybridized with a radioactively labeled telomere-specific probe.

B. Serial tenfold dilutions of FB1 (WT), UMP214 (*rad51*Δ), UCS33 (*uku70*^{nar1}), and UMP215 (*uku70*^{nar1} *rad51*Δ) cultures were applied to solid rich medium (YPD) and minimal medium with nitrate (MMD). The YPD plates were incubated for 2 days and the nitrate plates for 4 days at 28ºC.

Figure S13

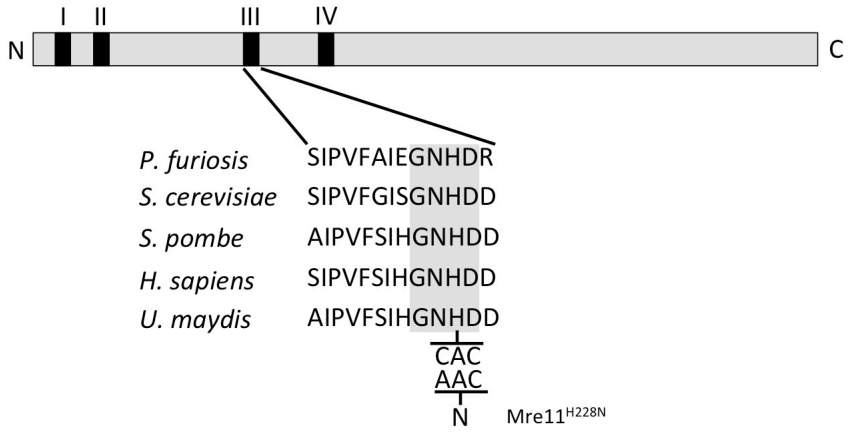


Figure S13. Alignment of the Mre11 nuclease motif IIIs in five different homologues.

Invariant residues are highlighted. The histidine-to-asparagine change (H228N) is depicted below the active-site histidine.

SUPPLEMENTARY TABLES

Table S1. *U. maydis* strains used in this study

Strain	Relevant genotype	Source
FB1	*a1 b1*	(1)
UCS30	*a1 b1 uku80^{nar1}*	this work
UCS33	*a1 b1 uku70^{nar1}*	this work
UMP132	*a1 b1 cut11-cherry*	(2)
UCS37	*a1 b1 uku80^{nar1} cut11-cherry*	this work
UCS34	*a1 b1 uku70^{nar1} cut11-cherry*	this work
UMP122	*a1b1 chk1*Δ	(3)
UCS39	*a1 b1 uku80^{nar1} chk1*Δ	this work
UCS35	*a1 b1 uku70^{nar1} chk1*Δ	this work
UCS1	*a1b1 atr1*Δ	(4)
UCS44	*a1 b1 uku80^{nar1} atr1*Δ	this work
UCS40	*a1 b1 uku70^{nar1} atr1*Δ	this work
UMP111	*a1 b1 chk1-3GFP*	(3)
UCS43	*a1 b1 uku80^{nar1} chk1-3GFP*	this work
UCS42	*a1 b1 uku70^{nar1} chk1-3GFP*	this work
UMP124	*a1 b1 chk1-3MYC*	(3)
UMP231	*a1 b1 uku70^{nar1} chk1-3MYC*	this work
UMP221	*a1 b1 uku70^{nar1} P$_{scp}$:cdk1*	this work
UMP222	*a1 b1 uku70^{nar1} P$_{scp}$:cdk1^{AF}*	this work
UMP210	*a1b1 rec1*Δ	this work
UMP219	*a1b1 mre11*Δ	this work
UMP220	*a1b1 uku70^{nar1} rec1*Δ	this work
UMP218	*a1b1 uku70^{nar1} mre11*Δ	this work

UMP223	*a1 b1 uku70^{nar1} rec1Δ chk1-3GFP*	this work
UMP224	*a1 b1 uku70^{nar1} mre11Δ chk1-3GFP*	this work
UMP228	*a1 b1 uku70^{nar1} mre11Δ chk1-3MYC*	this work
UMP186	*a1 b1 rad51-GFP*	(2)
UCS33	*a1 b1 uku70^{nar1} rad51-GFP*	this work
UCS41	*a1 b1 uku80^{nar1} rad51-GFP*	this work
UMP192	*a1 b1 pot1-cherry*	this work
UCS57	*a1 b1 rad51-GFP pot1-cherry*	this work
UCS45	*a1 b1 uku70^{nar1} rad51-GFP pot1-cherry*	this work
UCS46	*a1 b1 uku80^{nar1} rad51-GFP pot1-cherry*	this work
UMP214	*a1b1 rad51Δ*	this work
UMP215	*a1b1 uku70^{nar1} rad51Δ*	this work
UMP235	*a1b1 uku70^{nar1} mre11Δ* cbx:mre11	this work
UMP236	*a1b1 uku70^{nar1} mre11Δ* cbx:mre11^{H228N}	this work
UMP237	*a1b1 uku70^{nar1} mre11Δ* cbx:mre11 *chk1-3MYC*	this work
UMP238	*a1b1 uku70^{nar1} mre11Δ* cbx:mre11^{H228N} *chk1-3MYC*	this work
UMP230	*a1 b1 mre11-GFP pot1-cherry*	this work
UMP231	*a1 b1 uku70^{nar1} mre11-GFP pot1-cherry*	this work
UMP234	*a1 b1 uku70^{nar1} mre11-GFP pot1-cherry chk1Δ*	this work
UMP232	*a1 b1 mre11-3HA*	this work
UMP233	*a1 b1 uku70^{nar1} mre11-3HA*	this work
UMP279	*a1 a2 b1 b2 uku70 uku70Δ*	this work
UMP280	*a1 a2 b1 b2 uku80 uku80Δ*	this work
UMP281	*a1b1 uku70^{nar1} exo1Δ*	this work

1. Banuett, F., and Herskowitz, I. (1989). Different a alleles of *Ustilago maydis* are necessary for maintenance of filamentous growth but not for meiosis. *Proc Natl Acad Sci U S A* **86,** 5878-5882.

2. Mielnichuk, N., Sgarlata, C., and Perez-Martin, J. (2009). A role for the DNA-damage checkpoint kinase Chk1 in the virulence program of the fungus *Ustilago maydis*. *J Cell Sci* **122,** 4130-4140.

3. Perez-Martin, J. (2009). DNA-damage response in the basidiomycete fungus *Ustilago maydis* relies in a sole Chk1-like kinase. *DNA Repair* ***8,*** 720-731.

4. de Sena-Tomas, C., Fernandez-Alvarez, A., Holloman, W.K., and Perez-Martin, J. (2011). The DNA damage response signaling cascade regulates proliferation of the phytopathogenic fungus *Ustilago maydis* in planta. *Plant Cell* **23,** 1654-1665.

Table S2. Oligonucleotide primers used in this study

Name	Sequence 5'-3'
uku80 conditional allele	
Ku80-2	TTAATTAAAGCGACACAGAGGCCAATAATGGTC
Ku80-3	GAATTCATGCGGATGTGGCGGCCATGACAAGGG
Ku80-4	CATATGAGTGTCGAATCCAACACGCTCACGCTC
Ku80-5	TTAATTAACGGGGGCTACAGGTGTCGAGGTGGG
uku70 conditional allele	
Ku70-2	TTAATTAACGGGCAAACCTTGCAGCCTCAACCT
Ku70-3	CAATTGAATACCGCACAAGTTGGAGTATGTGGC
Ku70-4	CATATGCCCAAGGCTTACTTTGTCAACAAGCGC
Ku70-5	TTAATTAACACAACACGTTTGGGTGTCTCGCGC
mre11 deletion allele	
Mre11-2	GCTTAATTAATATTTGCCTGTTGTCTGTGCGTTGAGAACG
Mre11-3	GGTGGCCATCTAGGCCTCGCTTGCTCGCACGAAATCAAACTAGATA
Mre11-4	GGTGGCCTGAGTGGCCGATTCAGCGAGTCGGCCAAGATGGTGGAGA
Mre11-5	GCTTAATTAAAATATCCAGCTGGCTTCGACATTCGACCAA
rec1 deletion allele	
Rec1-2	GCTTAATTAAGCTGGAACTCCACTCTGCTCTAGCTC
Rec1-3	GGTGGCCATCTAGGCCGGCATGCTGACGGTGGCGTCAACTGG

Rec1-4 ATAGGCCTGAGTGGCCTTGCGCAATCGCCGCTGAAGTTGATC

Rec1-5 GGTTAATTAATCGAGTTGGCCTTCTTGTCTGCTGCA

rad51 deletion allele

Rad51-2 GCTTAATTAACATGGCTTCACCCCGCGGCTCTCCCT

Rad51-3 GGTGGCCATCTAGGCCAGAGTGTCGGAAGGACAGTTTTACAGGTT

Rad51-4 ATAGGCCTGAGTGGCCGTCATCCTGCTCCTACTCTTGCTCGCAGC

Rad51-5 GCTTAATTAAGGTCGCCTCTAGGTGAAGCTTGTTGC

exo1 deletion allele

Exo1-2 CTAGGTCTCGCCTGCGTTTAAACAAGATCAAGCGAATGTCAGCGAT

Exo1-3 CTAGGTCTCCAGGCCGGCGGTGCAGGCCGAAGGCGTTACAAG

Exo1-4 TAGGGTCTCCGGCCCTGAAGGGTCTGCAGGTAATAGGCAGC

Exo1-5 CTAGGTCTCGCTGCGTTTAAACTGGACAGAGGGAAGATGGAGAAA

pot1-cherry allele

Pot1-2 TTAATTAAGACAGGAGACATCATCCGCATCCAA

Pot1-3 GGTGGCCGCGTTGGCCAATAGATCGTGTTCGTCAGATAGAACGTT

Pot1-4 ATAGGCCTGAGTGGCCAGACCCGAGGATGAATAACATTCCAGTTC

Pot1-5 TTAATTAAGGTGGCCTCTCGAACCGCCCGAGAA

mre11-3GFP allele

Mre11-GFP1 GCGACCAGGACGAGAGCGCCAACGGACCAA

Mre11-GFP2 GCTTAATTAAGAGGAAGGCCTGCTGCCGGTCGAACG

Mre11-GFP3	GGTGGCCGCGTTGGCCCGCCGTCCAGCCCGTCGCCCAGCAGTCCG
Mre11-GFP4	ATAGGCCTGAGTGGCCAACGTGGAACACGAACCGCGCTGCGCAAC

mre11-nd allele

Mre11-1	AGCTGATTCGTGAAATCGTGAATCC
Mre11nd-1	GTCTCTCCGACACCTTGGGGATCGTCGTTATTGCCGTGGATCGAA
Mre11nd-2	TTCGATCCACGGCAATAACGACGATCCCCAAGGTGTCGGAGAGAC
Mre11-6	CGTCCAACTTGCAAGAAGCCGCAGC