Figure S1 Quantitation of ura4 loss.

RNA polymerase II transcription in the rDNA is repressed by silencing (Huang, 2002) and therefore ura4+ loss was confirmed by Southern blotting of representative colonies. The intensity of the band corresponding to the full-length ura4 sequence in the rDNA was compared to that of a truncated ura4-dS/E at the native locus. The upper band was absent in DNA from ura- colonies (-) but present in DNA from silenced colonies (±), which grew slowly on media lacking uracil, and unsilenced colonies (+). Colonies were analysed after 7 days on selective plates to distinguish between slow growth and marker loss. smc6-X has a silencing defect (Figure S3).
Figure S2 rDNA marker loss in \textit{rad22-d (RAD52)}

\textbf{A.} Deletion of \textit{rad22 (RAD52)} leads to very high marker loss (~50\%) from the rDNA and the levels are not significantly different in \textit{rad22-d smc6-X} or \textit{rad22-d swi6-d} double mutants. In \textit{S. pombe} the rDNA repeats are located at both ends of chromosome 3 and transcribed towards the telomeres (Yanagida et al., 1991). Recombination between rDNA repeats or proximal conserved regions may be required to maintain the telomeres of chromosome 3. Chromosome 3 is very heterogeneous in size in \textit{rad22-d} isolates (data not shown).
Figure S3 Silencing in the rDNA

RNA polymerase II transcribed genes are repressed in the rDNA. A strain containing \textit{LEU2}^+ and \textit{ura4}^+ markers integrated into a single rDNA repeat (\textit{smc6}^+, top row each panel) (Thon and Verhein-Hansen, 2000) shows reduced growth on plates lacking leucine or uracil but not on 5-FOA, which kills cells expressing \textit{ura4}, compared to complete media (YE). In contrast, \textit{swi6-d} strains (bottom row, each panel), defective in silencing, show reduced growth on 5-FOA and increased growth on plates lacking leucine or uracil. \textit{smc6-X} strains (six independent isolates shown, 1-6) also showed reduced silencing of \textit{LEU2} and \textit{ura4} (top panels). Silencing in \textit{smc6-74} strains, like \textit{smc6}^+ strains, was maintained epigenetically. Six independent isolates (1-6), three silenced (numbers: 2,3,4) and three unsilenced (numbers: 1, 5, 6) are shown. All the \textit{smc6-74} isolates showed similar rates of marker loss.
Irmisch Figure S4.

Figure S4 FACS profiles of mcm4-gfp and rad22-gfp strains after cdc25-22 block and release into HU.

mcm4-gfp and rad22-gfp strains in a cdc25-22 background were grown in parallel for 3.5 hrs at 36°C to arrest in G2. HU was added to 10mM and the cultures cooled to 25°C in iced water to release. Cultures were then incubated at 25°C and cell cycle progression monitored by flow cytometry. cdc25-22 blocks cells at G2/M by inhibiting Cdc2 but as inactive Cdc2 accumulates during the block when the cells are released activation of the large pool of Cdc2 causes an immediate and synchronous mitosis and this over-rides any cell cycle progression differences due to different genetic backgrounds. Consistently the two strains progressed into the HU block with similar kinetics.
Figure S5 Rpa-gfp levels are not altered in smc6 mutant strains

Western blot analysis of protein levels in Input samples from a representative ChIP experiment shows Rpa–gfp to be expressed at equivalent levels in all three strains, compared to a Cdc2 loading control. However, smc6-X but not smc6+ or smc6-74 has a slight growth defect with rpa-gfp and consistent with this both Rpa-GFP and control Cdc2 levels are reduced in smc6-X in untreated samples and in the ChIP analysis (Figure 5) the peak of Rpa loading occurs at 4hrs rather than 2.5hrs as in smc6+. (For ChIP experiments exponentially growing cells were diluted to 5x10^6/ml and cultured with or without HU. Equal volumes of cells were taken for analysis at 2.5 or 4 hrs +HU or at 4hrs –HU). Rpa may also be retained/reloaded at stalled forks in smc6-X due to aberrant processing but this has not been characterized further.
Figure S6 Brc1 over expression does not restore Rad52 chromatin association

Brc1, a protein containing six BRCT (named after Brca1 C-terminal domain) domains is an allele-specific high copy suppressor of the UV and MMS sensitivity of smc6-74 (Sheedy et al., 2005; Verkade et al., 1999). The sensitivity of smc6-74 but not smc6-X to HU can be rescued by over expression of brc1 and over expression of brc1 slightly sensitises smc6+ cells to HU (Ampatzidou et al., 2006). In order to avoid variation in plasmid copy number and Brc1 expression levels and to carry out Rad52 ChIP...
analysis under the same conditions as before, brc1 was over expressed at its genomic locus using the strong constitutive adh promoter (Broker and Bauml, 1989). A. Over expression of brc1 at its genomic locus increased the sensitivity of smc6+ (2) and smc6+ rad22-GFP (4) strains to HU. The sensitivity of smc6-74 rad22-GFP (6) was partially rescued in an adh-brc1 background but not up to rad22-GFP (4) sensitivity levels and the sensitivity of smc6-X rad22-GFP (8) was unaffected. B. ChIP analysis of Rad52 chromatin association. Log phase cultures of smc6+ and smc6-74 cells in a rad22-GFP background carrying either adh-brc1 or native brc1 were treated for four hours with 10mM HU (+HU) or left untreated (-HU). As expected in smc6+ cells containing native brc1 Rad52 chromatin association increased in HU at the rDNA and to a lesser extent at unique loci. In cells over expressing brc1 Rad52 association also increased in HU and at some loci (RFB, 17S and ars2004) slightly increased over levels without brc1 over expression. In smc6-74 cells containing native brc1 or adh-brc1 Rad52 levels did not increase to smc6+ levels in HU but Rad52 levels slightly increased in HU at the rDNA and ade6 loci when brc1 was over expressed. Thus over expression of brc1 in smc6-74 does not restore Rad52 chromatin association to smc6+ levels in HU. Thus, consistent with genetic analysis (Lee et al., 2007; Sheedy et al., 2005), suppression by Brc1 is a bypass of the defect in smc6-74. Bypass is dependent on a number of structure specific nucleases (Sheedy et al, 2005; Lee et al, 2007). Recruitment of nucleases to cleave an irreparably stalled fork could potentially enable Rad52 recruitment but this would not necessarily occur with the same timing and so may not be detectable in our assay.
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(Lehmann et al., 1995)  
(Verkade et al., 1999)  
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(Thon and Verhein-Hansen, 2000)  
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* independent isolates (h+ or h-)

In fission yeast, the Rad52 homologue is encoded by the *rad22* gene, Rad51 by *rhp51*, Rpa1 by *rad11*. 
References:


