SUPPLEMENTARY MATERIAL (Gilbert *et al.,*)

Large-scale extract preparation and purification of HH-Rad9. Extracts were prepared from exponentially growing 80L fermentation cultures without irradiation as previously described (Green *et al.,* 1999). Extract preparation from large-scale UV irradiated cells was as follows. 80L of cells were grown to a density of $4x10^7$ cells/mL in a fermenter and harvested. The cell pellets (~ 750 gram) were washed in water and resuspended in 6L of saline (a cell density of $5.3x10^8$ cells/mL). Cells were UV irradiated at 800 J/m^2 in batches of 250 mL. Under these conditions the average dose per cell was adjudged, by comparision of the extent of Rad9 and Rad53 phosphorylation, to be equivalent to the dose received per cell when cells are irradiated with 30 to 50 J/m^2 at a density of $5x10^6$ cells/mL. Following irradiation the cells were harvested, washed in ice cold water and the cell pellets were piped into liquid nitrogen before preparation of the extracts as described previously (Green *et al.,* 1999). For g irradiation cells were harvested as above and resuspended in 1L of saline. The resuspended cells were irradiated using a ^{60}Co scource at a dose of 100 Gy and then treated as above for the UV irradiated cells. Clarified extracts were applied to a 300 mL heparin-sepharose column as previously described (Green *et al.,* 1999) but with the following exceptions. The heparin-sepharose column was washed with two column volumes of 1X lysis buffer containing 100 mM potassium acetate followed by elution of bound material with 1X lysis buffer containing 300 mM, 500 mM and finally 1M potassium acetate. The HH-Rad9 containing fractions were determined by western blotting and pooled. Pre-clearing with protein G beads, coupling of the 12CA5 monoclonal antibody to protein G, binding and HA peptide elution have been previously described (Green *et al.,* 1999). The HH-Rad9 peaks were again determined by western blotting, pooled, imidazole was added to a concentration of 2.5 mM and the pooled material from the 12CA5 immuno-affinity step was added to 100 mL Ni^{2+}-NTA agarose (Qiagen) previously pre-equilibrated in 1X lysis buffer containing 300mM potassium acetate, 2.5mM imidazole. Batch binding and elution from Ni^{2+}-NTA agarose has been described (Green *et al.,* 1999). Peak fractions were determined by western blotting, pooled and visualised either by silver or Coomassie staining of SDS-PAGE gels. Coomassie-stained protein bands were excised and identified by peptide mass fingerprinting using matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectroscopy (Erdjument-Bromage *et al.,* 1998).

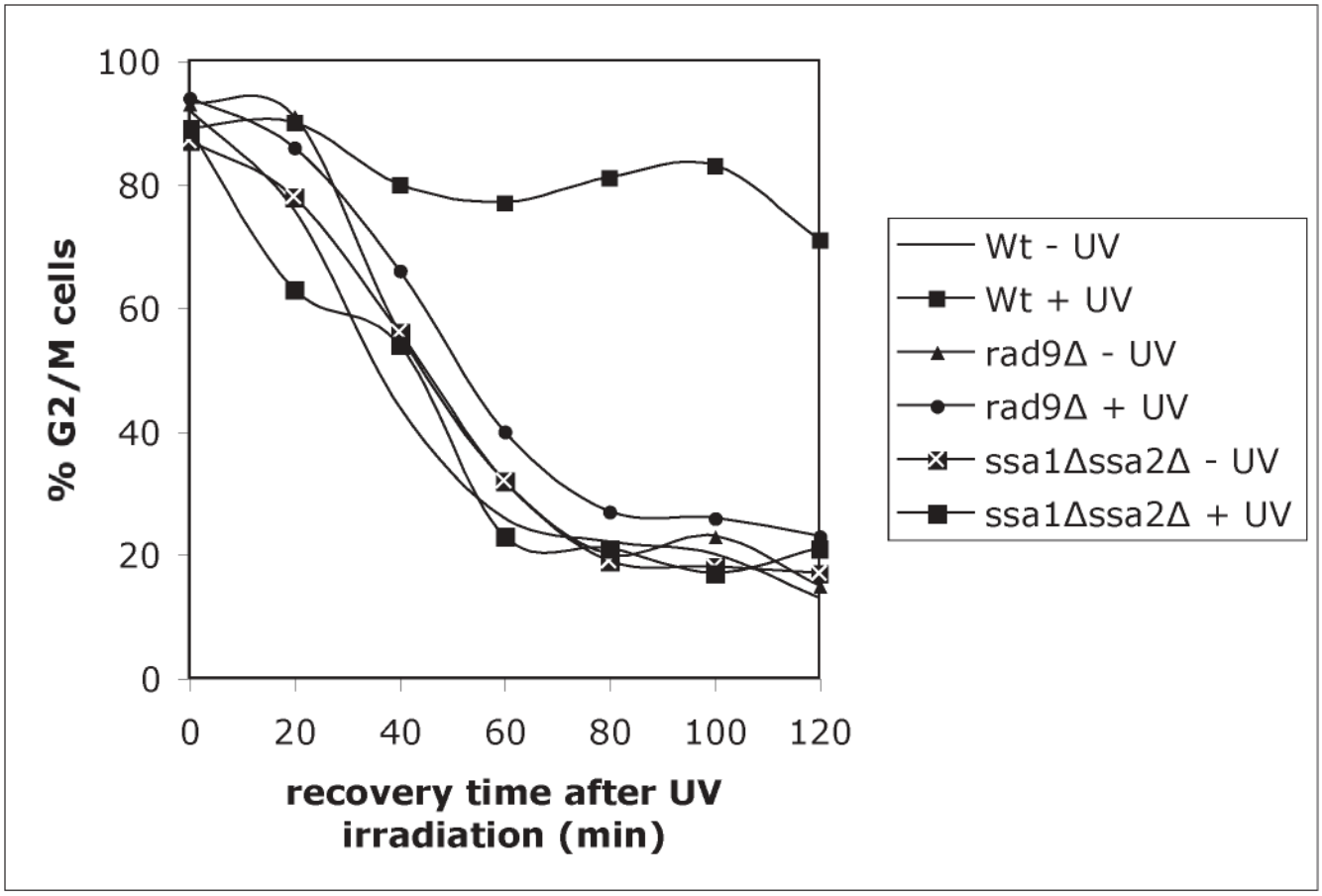
Hydrodynamic analyses. Superose 6 gel filtration and 20-35% glycerol gradient sedimentation analyses have been described previously (Gilbert *et al.,* 2001). The standard proteins used were (masses, stokes radii and sedimentation coefficients respectively): T - Thyroglobulin (670kDa, 8.5nm, $18x10^{-13}$sec), F - Ferritin (440kDa, 6.1nm, $65x10^{-13}$sec), Ca - Catalase (232kDa, 5.2nm, $11.3x10^{-13}$sec), A - Aldolase (158kDa, 4.8nm, $7.4x10^{-13}$sec), B - Bovine serum albumin (67kDa, 3.6nm, $4.3x10^{-13}$sec), O - Ovalbumin (44kDa, 3.1nm, $3.7x10^{-13}$ sec), Ch - Chymotrypsinogen (25kDa, 2.1nm, sedimentation coefficient not known). DB – dextran blue (~2,000 kDa).

REFERENCES

Erdjument-Bromage, H. *et al*. (1998) Examination of micro-tip reversed-phase liquid chromatographic extraction of peptide pools for mass spectrometric analysis. *J. Chromatogr. A.,* **826**,167-181.

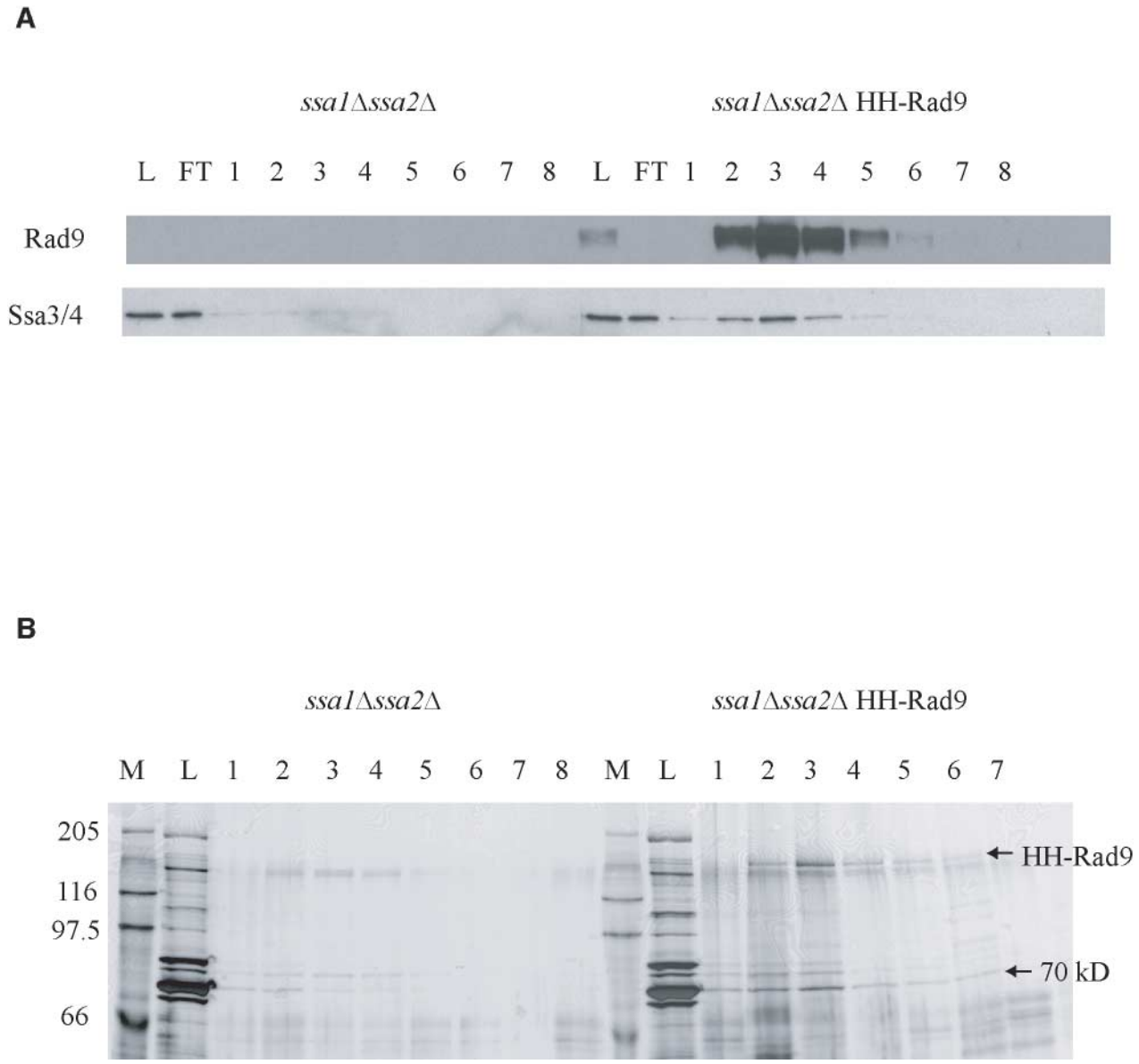
Gilbert, C.S. *et al*. (2001) Budding yeast is an ATP-dependent activating machine. *Molec. Cell,* **8**, 129-136

Green, C.M. *et al.* (1999) A novel Rad24 checkpoint complex closely related to Replication Factor C. *Curr Biol*., **17**,39-42.



Supplementary Figure 1: The *SSA1* and *SSA2* genes are required for normal G2/M regulation after DNA damage.

Wildtype (Wt), *rad9*Δ and *ssa1*Δ*ssa2*Δ strains were blocked by nocodazole in the G2/M phase of the cell cyle, irradiated with UV (50 J/m^2) and released into the cell cycle by addition of pre-warmed media. The percentage of cells in G2/M was scored by DAPI staining and examining cellular and nuclear morphologies.



Supplementary Figure 2: Ssa3/Ssa4 proteins co-purify with HH-Rad9 in the absence of Ssa1 and Ssa2

(a) Rad9 and Ssa3/4-specific western blot. Crude cell extract from undamaged *ssa1Δssa2Δ* cells either containing untagged Rad9 (left panel) or *ssa1Δssa2Δ* cells containing HH-Rad9 (right panel) were bound to 12CA5 beads and peptide eluted. The eluant was bound to Ni^{2+}-NTA-agarose and eluted with imidazole. Ssa3/Ssa4 co-purify with HH-Rad9, the peak elution for both is fraction 3. When Rad9 is untagged Ssa3/Ssa4 do not co-purify with Rad9. **(b)** Silver stained 6.5% SDS-PAGE gel of the Ni^{2+}-NTA-agarose elutions shown in panel (a). HH-Rad9 is indicated. The relative migration position of the co-purifying Ssa3/4 (70 kD) polypeptides is also indicated. L (Load); FT (Flow-through); M (Marker).