Supplementary Figures

Legends to Supplementary Figures

Supplementary Figure 1. Phosphatase treatment of Mrc1 protein

Myc-tagged Mrc1 strain (SH2219) was treated with 12 mM HU for 4 hrs at 30°C and the whole cell lysate was prepared. The immunoprecipitate prepared by anti-Myc antibody was incubated with (lane 2) or without (lanes 1 and 3) lambda phosphatase in the phosphatase buffer and analyzed by Western blotting using the anti-Myc antibody. Incubation was at 30°C (lanes 1 and 2) or 0°C (lane 2).

Supplementary Figure 2. Kinase assays of endogenous Cds1 kinase activities

Cds1 kinase assays were conducted as described in "Experimental Procedures" using GST-Wee1[70] as a substrate. Extracts were prepared from non-tagged (YM71, lanes 2-4), Flag-tagged Hsk1 (MS337, lanes 5 and 6), *cds1*Δ (NI453, lanes 7 and 8) and *cds1*Δ Flag- tagged Hsk1 (MS113, lanes 9 and 10) strains. Cells were grown at 30°C and 12 mM HU was added (lanes 2, 3, 5, 7 and 9) or non-treated (lanes 4, 6, 8 and 10) and incubation was continued for 1.5 hr at 30°C. Lane 1, reaction without the extract. Upper, autoradiogram; lower, CBB staining.

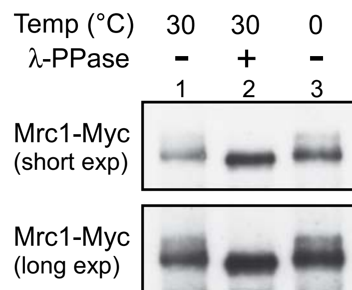
Supplementary Figure 3. Kinetic parameters for phosphorylation of Mrc1 protein by Hsk1 kinase

(A) Mrc1 protein was titrated in *in vitro* phosphorylation assays containing 0.05 μg of Hsk1+GST-Dfp1/Him1 complex. Incubation was for 15 min at 30°C, which is still within the linear range of the reaction. The amount of Mrc1 added was as follows. Lanes 1, 0 nM; lane 2, 0.18 nM; lane 3, 0.72 nM; lane 4, 1.8 nM; lane 5, 3.6 nM; lane 6,

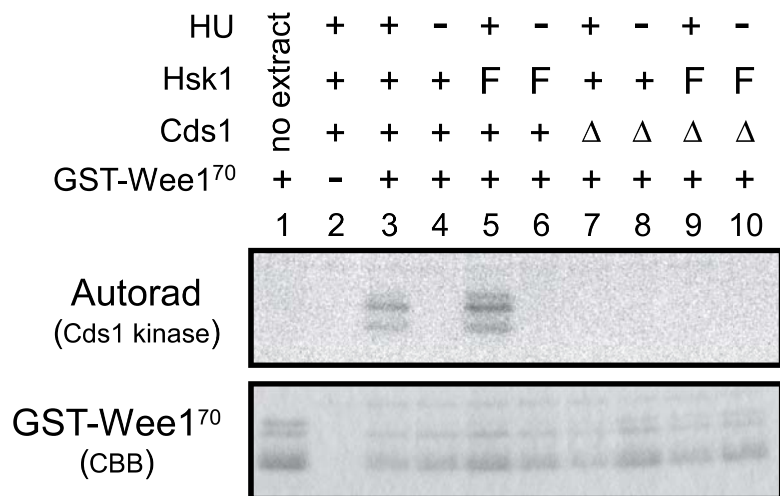
7.2 nM; lanes 7, 18 nM; lane 8, 36 nM; lane 9, 72 nM; lane 10, 18 nM. Left, autoradiogram; right, silver staining. (B) Quantification of the results. The Mrc1 protein bands were cut out from the dried gel and their radioactivity (cpm) was measured, and phosphate (pmole) incorporation per minute was calculated. Left, standard plot; right, the Lineweaver-Burk double reciprocal plot. Km and Vmax for the Mrc1 substrate were calculated to be 9.02 nM and 0.154 pmole/min, respectively.

Supplementary Figure 4. Prior phosphorylation of Mrc1 with Hsk1 does not affect the Rad3-mediated phosphorylation of the SQ/TQ sites of Mrc1.

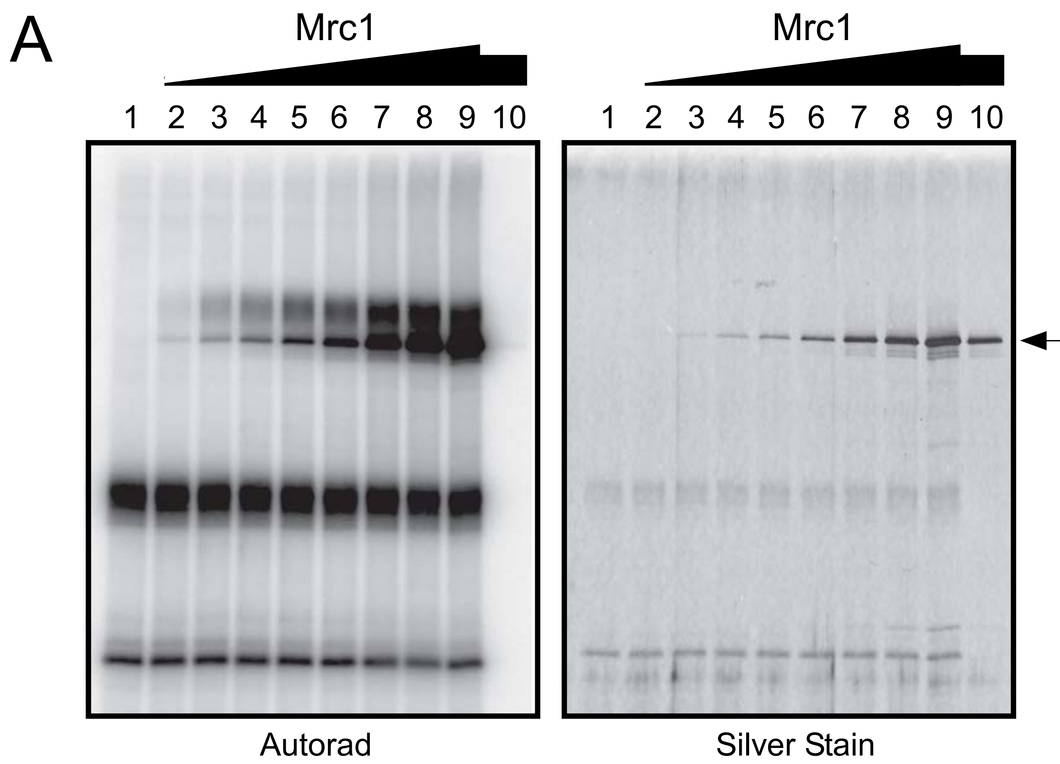
(A) Mrc1 protein was first incubated with beads-conjugated Hsk1(WT, wild-type)-Dfp1/Him1 or Hsk1(KD, kinase dead-Dfp1/Him1) in the presence of cold ATP (1mM) for 30 min (1μl of 5μl). Beads were removed and supernatants were incubated with immunoprecipitates of Rad3-myc beads (HU-treated or HU-nontreated), or control beads from non-tagged strain in the presence [γ-^{32}P] ATP. Products were analyzed by SDS-PAGE (7.5% polyacrylamide gel). (B) Mrc1 was co-overepressed in bacterial together with Hsk1-Dfp1/Him1 (lanes 1-3 and 4-6; wild-type or kinase-dead, or respectively) or overexpressed alone (lanes 7-9) and Mrc1 was purified. The purified Mrc1 was used for kinase assays with no kinase (lanes 1, 4 and 7), with Rad3-beads, and Cds1 protein (lanes 7-9). The products were run on SDS-PAGE, which was blotted with the antibody against the phosphorylated SQ/TQ.

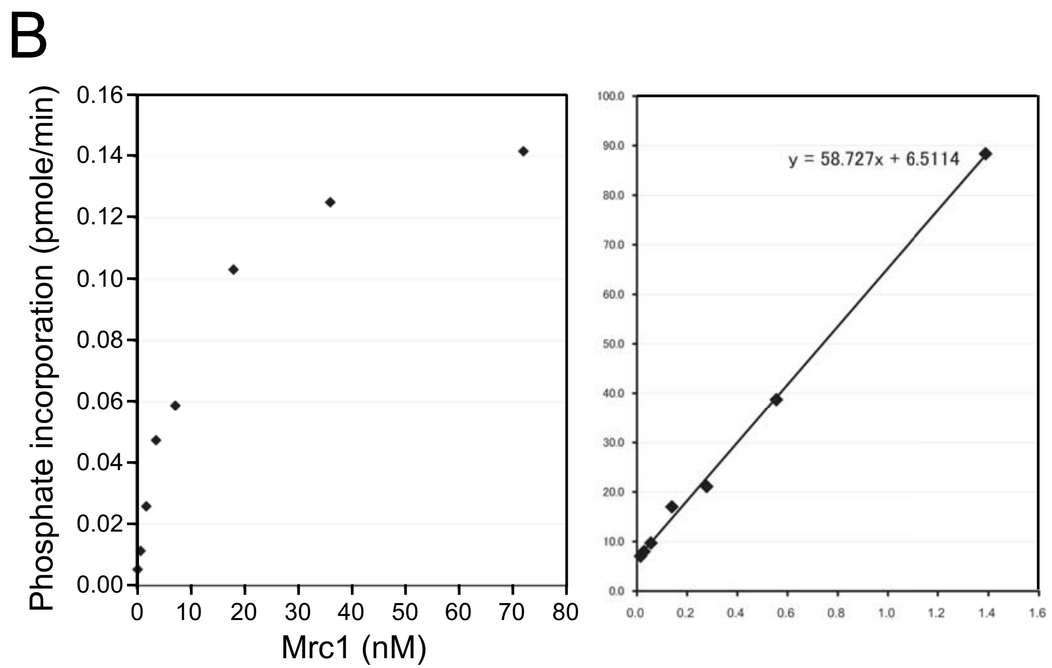


Supplementary Figure 1 - Matsumoto *et al*.

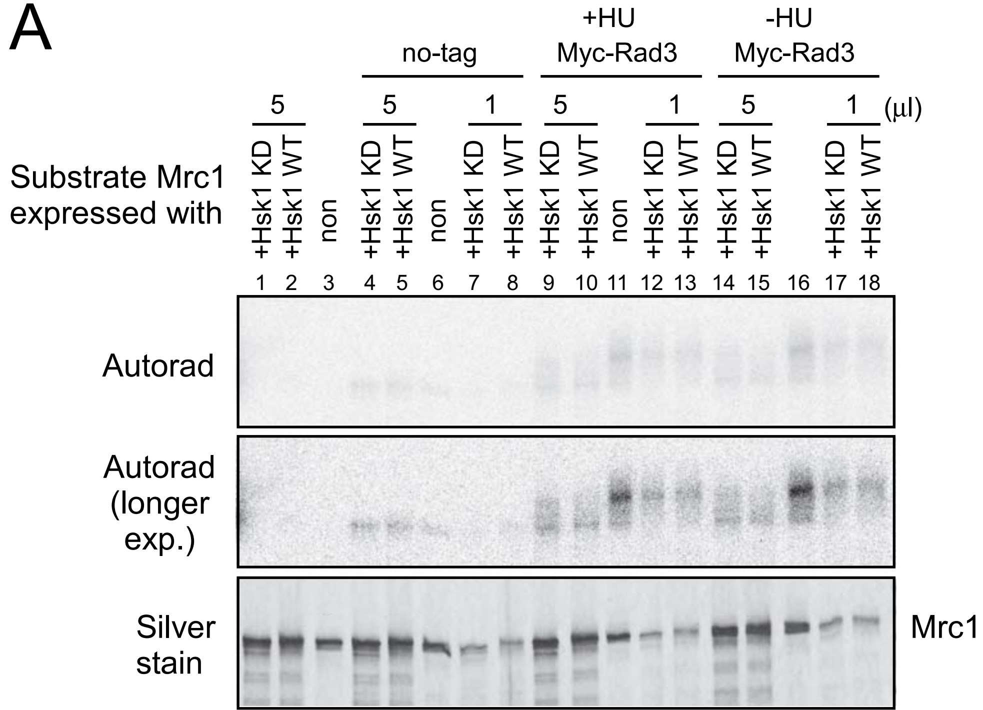


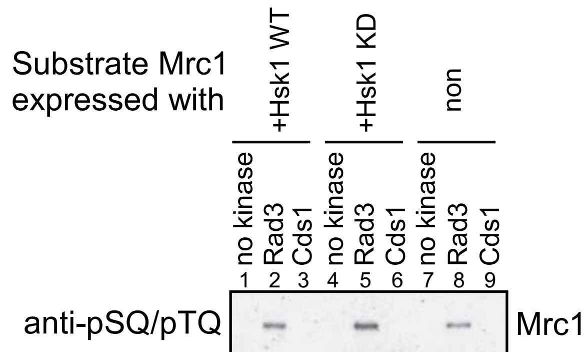
Supplementary Figure 2 - Matsumoto *et al.*





Supplemental Figure 3 - Matsumoto *et al.*





Supplemental Figure 4 - Matsumoto *et al.*