Figure S1  Internal Mrc1 domains and DSGxxS sites do not affect protein stability. (A) Structural schematic of the Mrc1 protein. (B) Dia2-mediated degradation of Mrc1 is not dependent on the putative two-hybrid Dia2-interacting regions spanning residues 380-557, 701-800 of Mrc1. Cells were arrested in G1 by α-factor, released into YPD + 0.033% MMS for 40 minutes, and then released into YPD + 200μg/ml CHX. Protein samples were taken at indicated times. Pgk1 serves as a loading control. (C) The rate of checkpoint recovery is unaffected in mrc1 mutants lacking the putative Dia2-binding domains. Cells were treated as described in (B), except that CHX was not added. Samples from at the indicated time points were analyzed by flow cytometry. 1C and 2C indicate DNA content. (D-E) The DSGxxG phosphodegron does not play a role in the degradation of Mrc1 or S-phase checkpoint recovery. Samples were prepared as described in (B-C) and were analyzed for (D) Mrc1 stability and (E) DNA replication by flow cytometry.