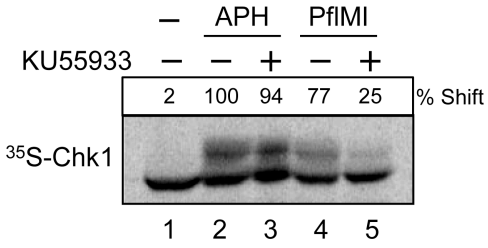

Figure S1. Inhibition of ATM does not block aphidicolin-induced activation of Chk1 in egg extracts. Egg extracts were incubated in the absence (lane 1) or presence of either aphidicolin (lanes 2 and 3) or PflMI (lanes 4 and 5). In addition, extracts were treated with the ATM inhibitor KU55933 (lanes 3 and 5) or solvent alone (lanes 1, 2, and 4). Nuclear fractions were analyzed by phosphorimaging to assess phosphorylation-dependent shifting of Chk1. Numbers above each lane depict quantitation of phosphorylation relative to aphidicolin-treated extracts lacking KU55933.

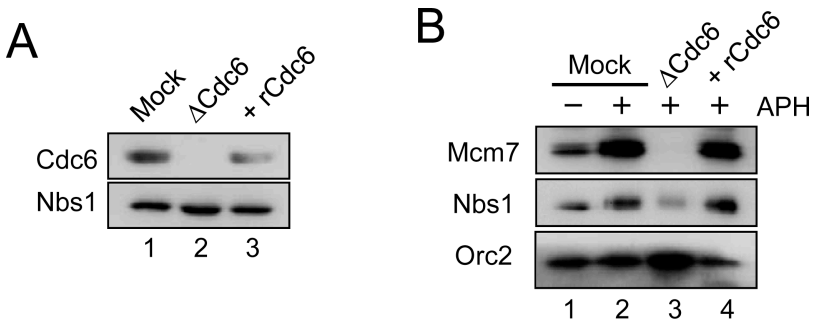

Figure S2. Depletion of Cdc6 compromises binding of Nbs1 to chromatin. (A) Egg extracts were mock-depleted with control antibodies (lane 1) or immunodepleted with anti-Cdc6 antibodies (lanes 2 and 3). Recombinant Cdc6 protein (rCdc6) was added back to the extract in lane 3. Extracts were immunoblotted for the indicated proteins. (B) The extracts from panel A were incubated in the absence (lane 1) or presence of aphidicolin (lane 2-4). Chromatin fractions from the extracts were immunoblotted for the indicated proteins.

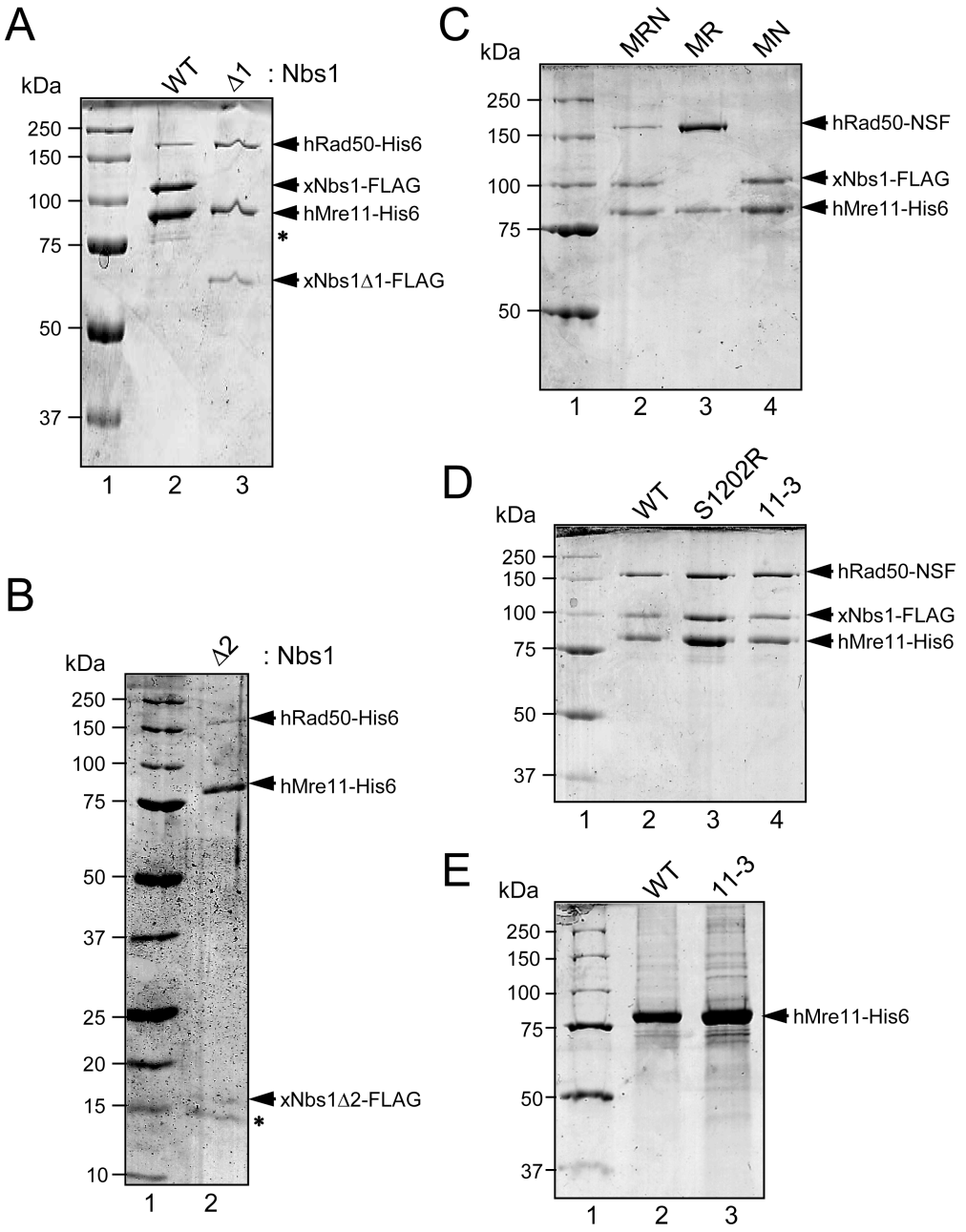

Figure S3. Characterization of various recombinant MRN proteins. (A-E) Sf9 insect cells were infected with one or more baculoviruses encoding recombinant versions of the indicated MRN subunits. Proteins were purified as described in Materials and Methods. Samples were processed for SDS-PAGE and staining with Coomassie Brilliant Blue. Various subunits are denoted with an arrow. Non-specific bands are denoted with an asterisk.

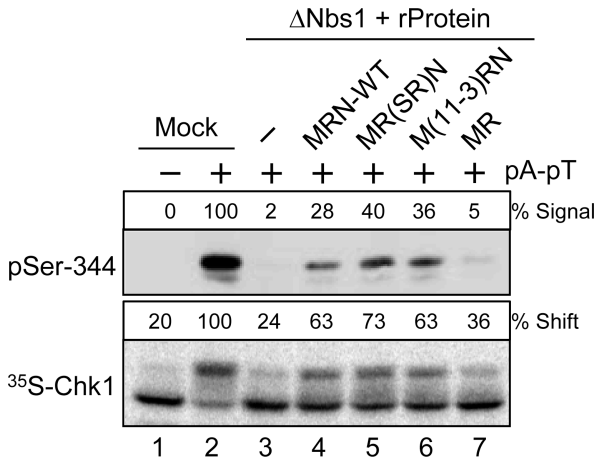

Figure S4. Response of various reconstituted egg extracts to pA-pT. (A) The mock-depleted (lanes 1 and 2) or Nbs1-depleted extracts (lanes 3-7) from Figure 5A were supplemented with the following: control buffer (lanes 1-3); recombinant MRN complexes containing all wild-type subunits (lane 4), a Rad50-SR mutant subunit (lane 5), or an Mre11-3 mutant subunit (lane 6); and dimeric MR complex (lane 7). Extracts were incubated with ^{35}S-Chk1 in the absence (lane 1) or presence of pA-pT (lanes 2-7). Extracts were processed for immunoblotting with anti-pSer-344 Chk1 antibodies (top) or for phosphorimaging to detect radiolabeled Chk1 (bottom). The numbers above each lane denote quantitation of phosphorylation relative to mock-depleted extracts containing pA-pT.