Supplemental FIGURE S1

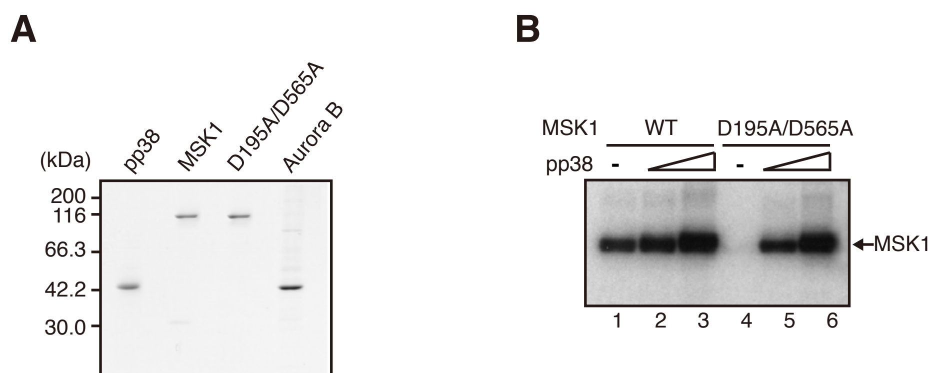

FIGURE S1. **Purified kinases for chromatin kinase assays.** *A*, the p38 kinase was co-expressed with constitutively active MKK6 in E. coli to obtain its active form, pp38 whereas recombinant MSK1, its inactive mutant, D195A/D565A, and Aurora B were expressed in insect cells. The expressed kinases were purified to near homogeneity and separated by SDS-PAGE. The gel was stained with CBB. In D195A/D565A, aspartates at residues 195 and 565 were mutated to an alanine to inactivate both NTKD and CTKD activities. *B*, wild-type MSK1 and D195A/D565A (0.5 μg each) were phosphorylated by 2.5 ng (*lanes 2* and *5*) or 25 ng (*lanes 3* and *6*) of pp38 in 35 μM [γ-^{32}P]ATP. The phosphorylated proteins were detected by autoradiography. The position of MSK1 is indicated on the right. In all the subsequent experiments using MSK1 or its derivative mutants, ~4.2 ng of pp38 was added per 100 ng of MSK1 to fully activate MSK1 purified from insect cells.

Supplemental FIGURE S2

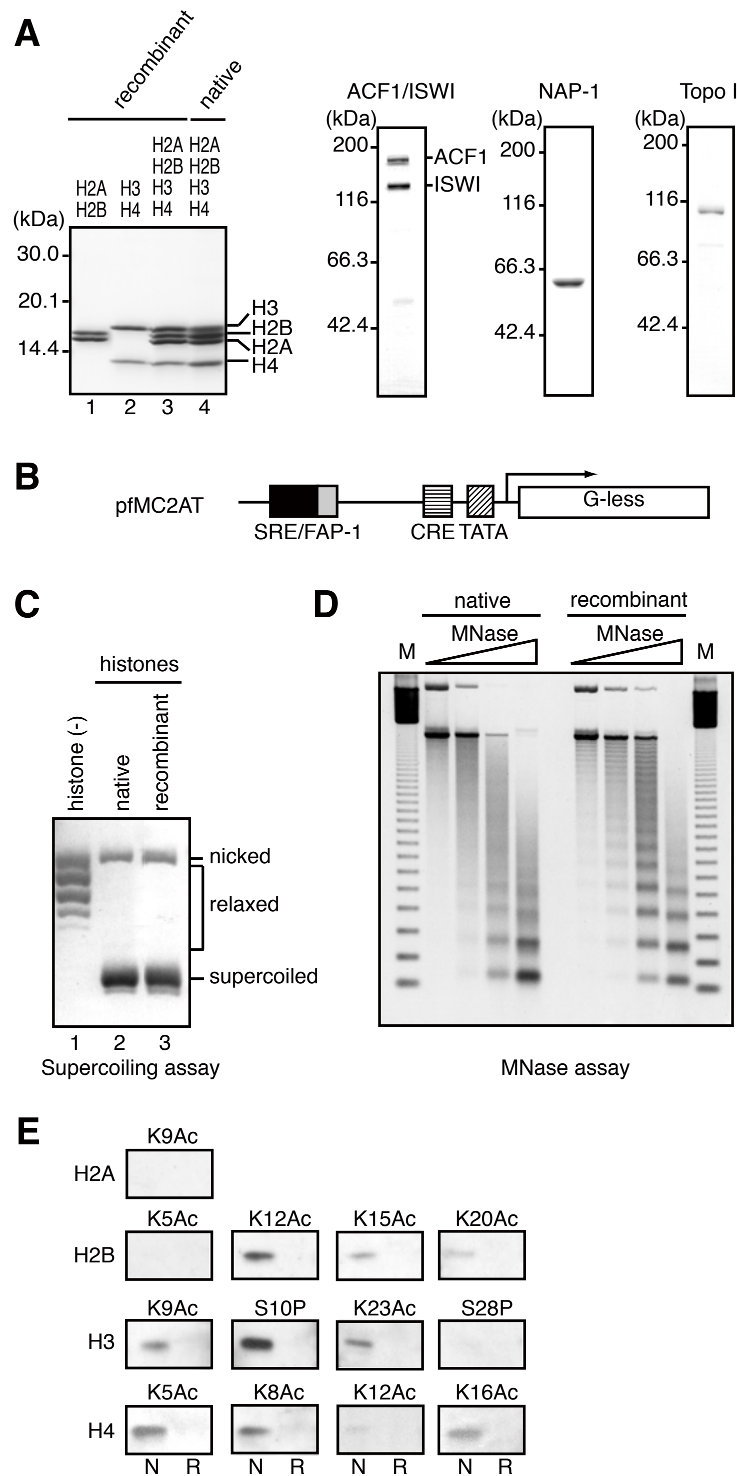

FIGURE S2. Nucleosomes assembled on the c-*fos* promoter with recombinant histones. *A*, purified histones and chromatin assembly factors. Recombinant histones H2A and H2B (*lane1*), histones H3 and H4 (*lane 2*), mixture of histones H2A, H2B, H3 and H4 (*lane 3*) as well as HeLa cell-derived core histones (*lane 4*) were analyzed by SDS-PAGE. The purified chromatin assembly factors including Drosophila ACF1/ISWI, human NAP-1, and human Topo I were analyzed by SDS-PAGE. *B*, the 3,420-bp plasmid (pfMC2AT) includes the c-*fos* promoter (-11~-402) and a G-less cassette. The positions of the SRE, FAP-1, CRE and the TATA box are indicated. *C*, the relaxed plasmid (*lane 1*) was assembled into chromatin with recombinant (*lane 2*) or HeLa cell-derived core histones (*lane 3*), and the degree of superhelicity was analyzed by electrophoresis on an agarose gel. The positions of nicked, relaxed and supercoiled plasmids are indicated. *D*, micrococcal nuclease (*MNase*) digestion assays. The assembled chromatins were partially digested at two different concentrations of MNase, and the digested plasmid DNA was analyzed by electrophoresis on an agarose gel. The marker lane (*M*) is a 123-bp DNA ladder. *E*, analyses of posttranslational modifications of recombinant or HeLa cell-derived histones. Histone modifications were analysed by immunoblot using antibodies that recognize specifically modified residues within histones. HeLa cell-derived native histones (*N*) or recombinant histones (*R*) were tested for acetylation (*Ac*) and phosphorylation (*P*) of the indicated residues. *K9Ac*, for example, indicates the acetylated lysine 9.

Supplemental FIGURE S3

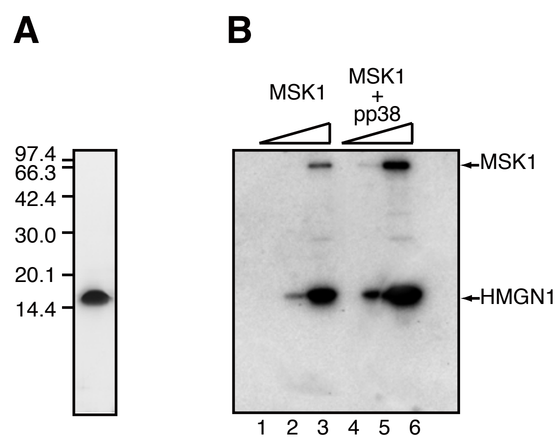

FIGURE S3. Phosphorylation of HMGN1 by MSK1. *A*, purified recombinant HMGN1 (500 ng) was analyzed by SDS-PAGE. *B*, HMGN1 (500 ng) was phosphorylated by MSK1 alone (*lanes 1-3*) or MSK1 and pp38 (*lanes 4-6*). The reactions contained 5 ng (*lanes 1* and *4*), 50 ng (*lanes 2* and *5*) and 500 ng (*lanes 3* and *6*) of MSK1, together with 0.25 ng (*lane 4*), 2.5 ng (*lane 5*) and 25 ng (*lane 6*) of pp38. The phosphorylation was detected by autoradiography, and the positions of HMGN1 and phosphorylated MSK1 are indicated on the right.

Supplemental FIGURE S4

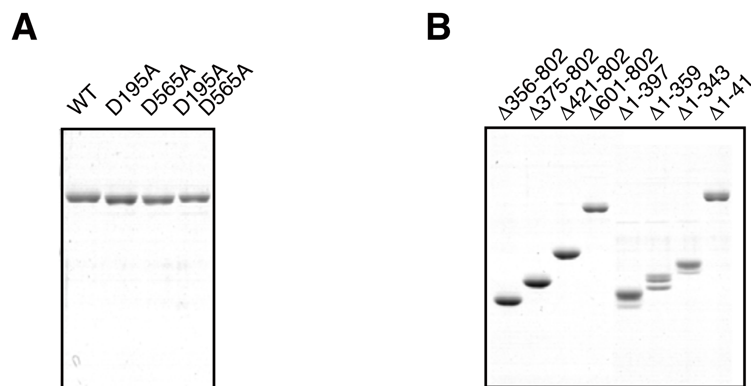

FIGURE S4. Purified MSK1 and its mutants. *A*, D195A, D565A and D195A/D565A have an alanine in place of an aspartate at residue(s) 195, 565 or both 195 and 565, respectively. Purified recombinant MSK1 mutants were analyzed by SDS-PAGE. *B*, a series of N-terminal and C-terminal deletion mutants were purified and analyzed by SDS-PAGE.