Online supplemental materials

3 supplemental figures and 5 movies.

Figure S1, related to Figure 1.
Figure S2, related to Figure 3.
Figure S3, related to Figure 4.
Movies S1-2, related to Figure 2.
Movies S3-5, related to Figure 3.

FIGURE S1

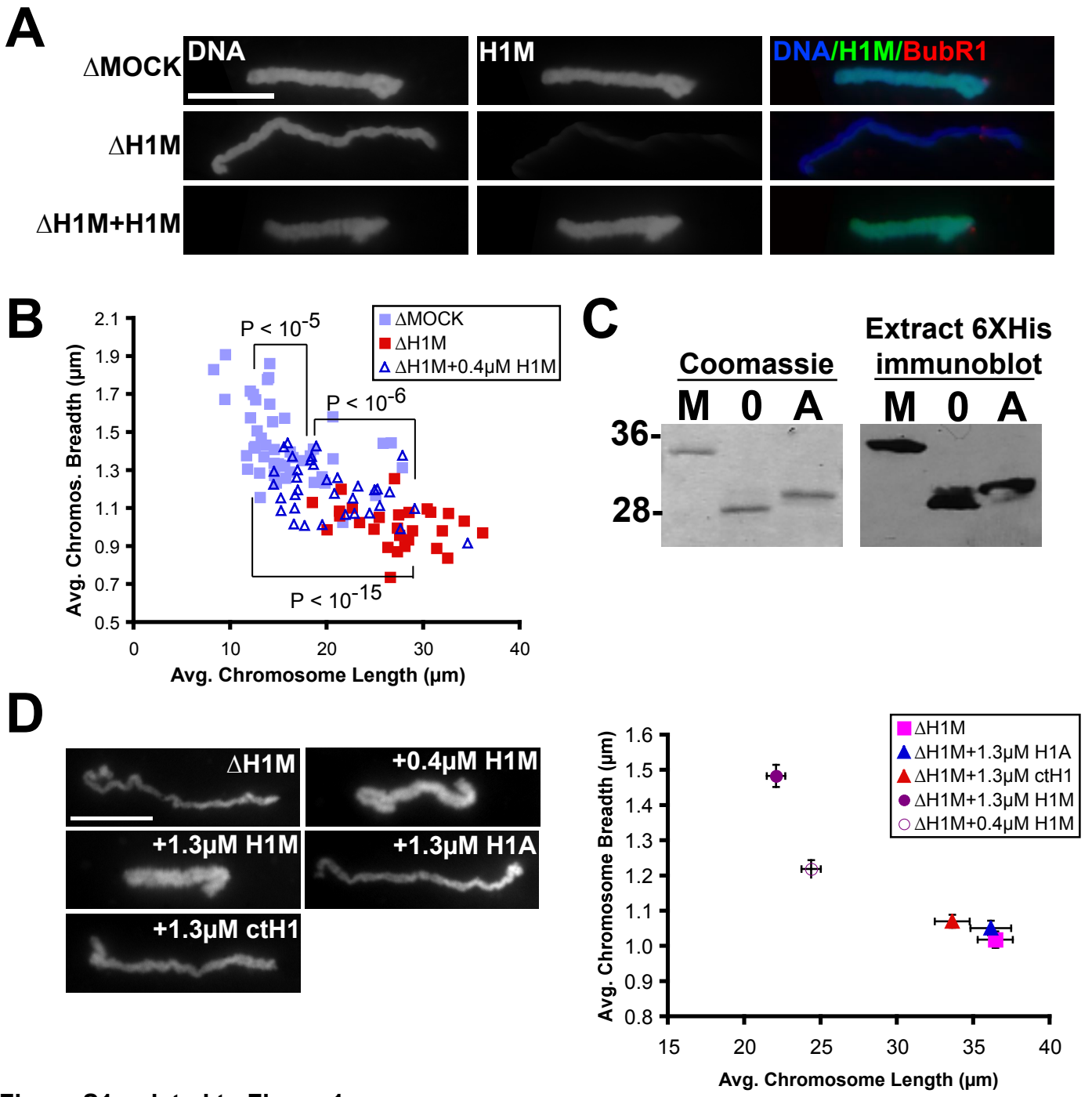

Figure S1, related to Figure 1.

(A) Replicated mitotic chromosomes from mock- and H1M-depleted extracts. Paired sisters are visible along the length of the chromosomes and paired kinetochores (BubR1) are frequently observed. Addition of 1 μM H1M restores morphology and anti-H1M immunofluorescence.

(B) Partial rescue of H1M-depleted chromosome morphology by 0.4 μM H1M. To illustrate the normal variability among chromosome dimensions, three conditions from Figure 1B are shown as a scatter plot of individual chromosome morphologies instead of averages. Also shown are P-values (unpaired t-test) for length differences between the populations.

(C) Coomassie-stained SDS-PAGE gel of recombinant, 6XHistidine-tagged X. tropicalis H1M, H10, and H1A proteins (M, 0, A) and corresponding anti-6XHistidine immunoblot of egg extract supplemented with each of the proteins.

(D) Representative images (left) and morphological quantification (right) of mitotic chromosomes assembled in H1M-depleted extracts. 1.3 μM recombinant Xenopus H1A or calf thymus H1 (ctH1) fails to rescue the phenotype. Scale bars, 10 μm; error bars, standard error.

FIGURE S2

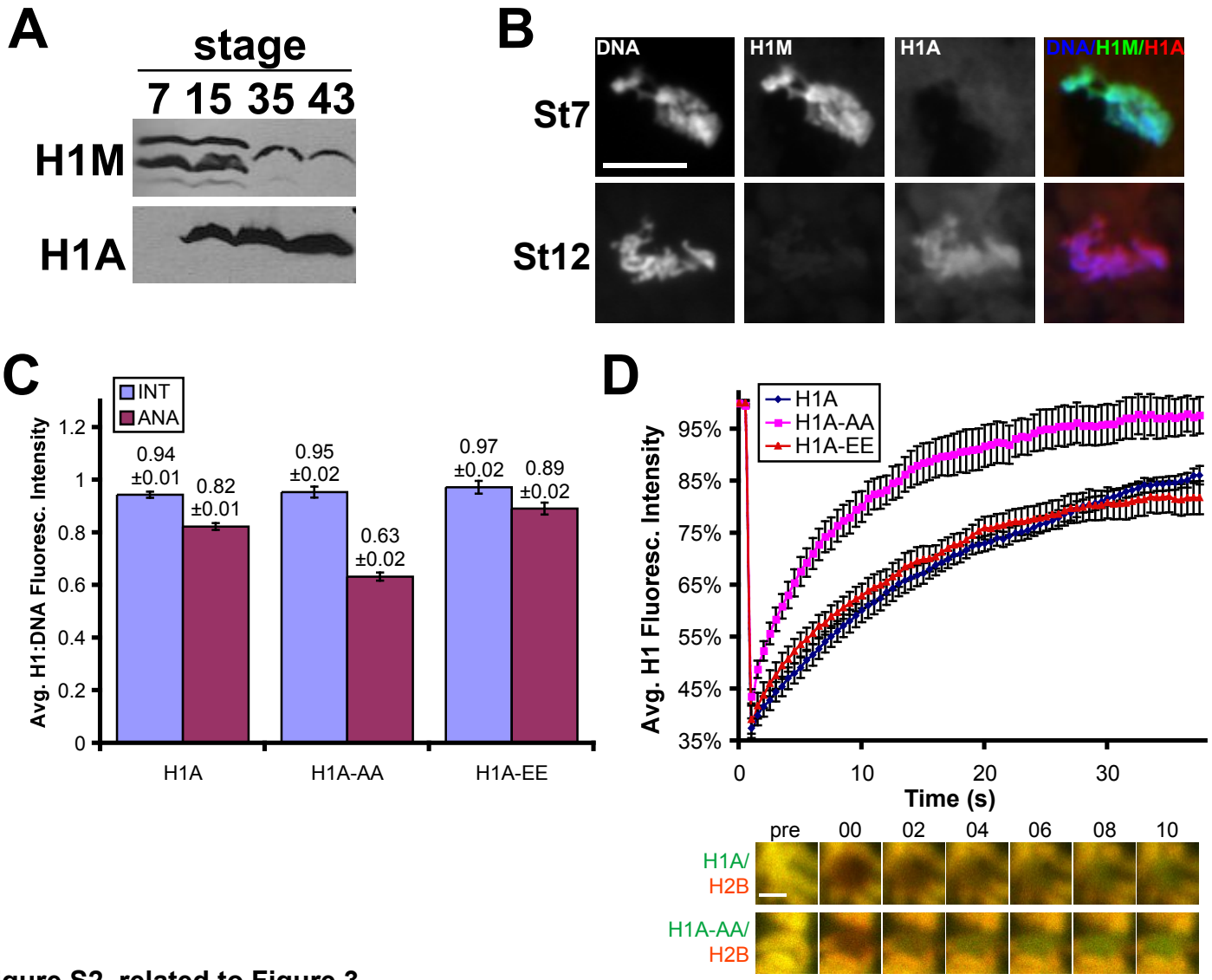

Figure S2, related to Figure 3.

(A) Anti-H1M and anti-H1A Western blots of whole embryos during X. laevis development. Embryos were extracted in RIPA buffer and the soluble fraction was resuspended in SDS-PAGE sample buffer. Transcription initiates at stage 8.

(B) H1M and H1A immunofluorescence in somatic cells. Embryos were paraformaldehyde-fixed, cryosectioned, and stained using isoform-specific antibodies. Prior to the mid-blastula transition (St7), only H1M localizes to mitotic chromosomes. In gastrulae (St12), H1A localizes brightly while H1M appears reduced due to dilution of the maternal pool. Bar, 10 μm.

(C) Absolute H1:DNA fluorescence intensities of H1A-GFP wild-type versus non-phosphorylatable (AA), or phosphomimetic (EE) mutants expressed in embryos from Figure 3D. A significant difference between H1A-AA and the other two conditions is observed during anaphase (ANA) but not interphase (INT). Values are ± standard error.

(D) Fluorescence recovery after photobleaching (FRAP) curves for H1A wild-type, non-phosphorylatable (AA), and phosphomimetic (EE) mutant. The AA mutant recovers much more rapidly and to a greater final extent than wild-type or EE, indicating a shorter residence time and larger mobile fraction. Representative time-lapse images for H1A-GFP and H1A-AA-GFP are shown at bottom (Bar, 1 μm). Five or more FRAP curves were averaged for each timepoint and plotted. Error bars, standard error.

FIGURE S3

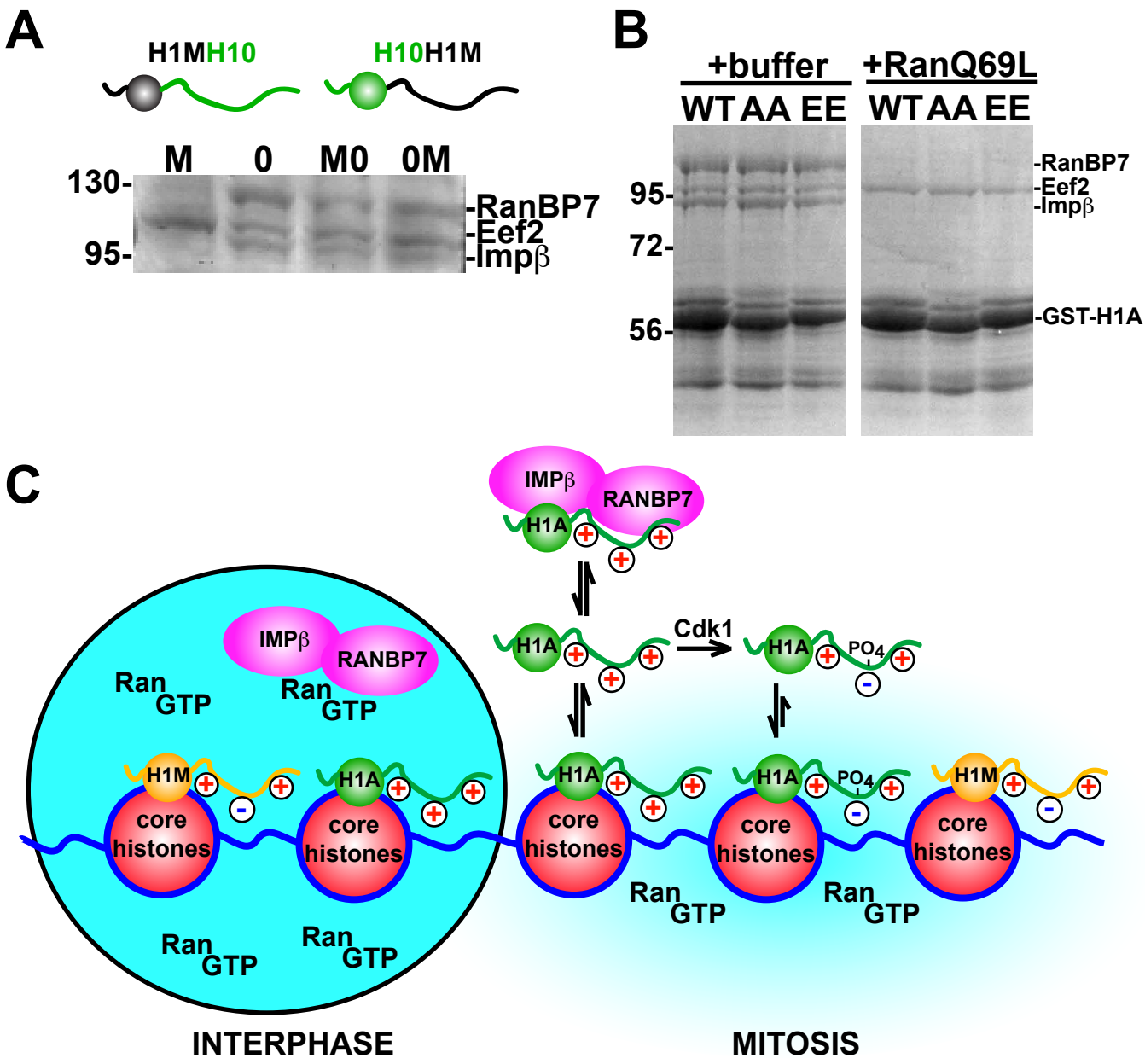

Figure S3, related to Figure 4.

(A) Top: Domain structure of H10/H1M chimeras shown in schematic. Bottom: Coomassie-stained gel showing associated protein profiles for H1M, H10, and H1M/H10 chimeric GST fusion proteins retrieved from mitotic egg extracts. Both chimeras showed higher affinity for importin β/RanBP7 than H1M. Substitution of chimeras for H1M in extracts gave inconsistent results in chromosome morphology and binding assays (not shown).

(B) GST-fusion pull-downs of wild-type (WT) H1A or mutants in which consensus phosphorylation serines have been replaced with alanines (AA) or glutamic acids (EE). All interact with importin β/RanBP7 in a RanGTP-regulated manner. The lowest band represents a minor degradation product of the GST-tagged proteins.

(C) Model of linker histone function at interphase (left) and mitosis (right). Unlike H1M (orange), somatic H1 (green) binds a heterodimer of importin β/RanBP7 (pink). In interphase, high concentrations of RanGTP (light blue) cause release from the heterodimer and promote chromatin binding. At mitosis, cytoplasmic H1A exists in an equilibrium between importin- and chromatin-associated forms, dependent on RanGTP as well as multiple phosphorylation by Cdk1.