Supplementary Figure S1. The specificity of anti-H3K9me2 and anti-H3K27me3 evaluated by ELISA. Microtiter plates coated with the peptides were incubated with 3-fold dilutions of each antibody, starting from 1:100 dilution of a hybridoma culture supernatant. After incubation with peroxidase-conjugated secondary antibody and washing, the colorimetric signal of tetramethylbenzidine was detected by measuring the absorbance at 405 nm (Abs) using a plate reader.
Supplementary Figure S2. Localization of histone H3 modifications in living cells probed with the specific Fabs. HeLa cells expressing H2B-mRFP were loaded with Alexa488-labeled Fabs, or were fixed and stained with Alexa488-labeled IgG. Confocal images are shown. Bar, 10 μm.
Supplementary Figure S3. FRAP.
The relative intensity of bleached area for the indicated Fabs is plotted (averages of > 10 cells). The data of FabH3K4me2 is represented from Figure 2 for comparison. The mobility of FabH3K9me1, FabH3K4me1 and FabH3K4me3 is faster than FabH3K4me2.
Supplementary Figure S4. SDS-PAGE analysis of Fab.
To prepare Fabs, histone H3 modification-specific monoclonal antibodies were digested with immobilized papain (Pierce Fab Preparation Kit; lanes 1-6) or ficin (Pierce Mouse IgG1 Fab and F(ab')2 Preparation Kit; lanes 7-15), and Fc was removed using protein A-agarose. IgG and Fab fractions (1 μg) were separated in a 10-20% gradient SDS-polyacrylamide gel and stained with Coomassie. The sizes of prestained markers (M) are indicated on the right. In IgG fractions, the heavy and light chains (HC and LC, respectively) are observed (lanes 1, 3, 5, 7, 9, 11 and 13); the sizes of both HC and LC vary because of the different length of variable regions. In most Fab fractions (lanes 2, 4, 8, 10 and 12), new single major bands corresponding to the heavy chain Fab portions are observed (e.g., closed arrowhead). The light chain remains intact after the protease digestion (e.g., open arrowheads). In H3K9me3 Fab fractions, extra bands at ~9 kDa are observed by digestion either with papain (lane 6) or ficin at a standard (lane 14) and milder (lane 15) conditions, indicating that H3K9me3 IgG heavy chain contains protease-sensitive sites.
Supplementary Figure S5. FRAP analysis using a reaction-diffusion model. HeLa cells were loaded with FabH3K9ac-Cy3 and FabH3K9me2-488, and a 2 μm spot was bleached. The temporal evolution of the radial intensity profile of the bleach spot (see cartoon inset) was then fit using a reaction-diffusion model. From each fit, the average diffusion coefficient and binding association and residence times of Fabs were quantified, from which the bound/free fractions of Fabs were calculated (ref. 28). To quantify total binding, the pure-diffusion coefficient of each Fab was measured in the cytoplasm.

(A) Sample FRAP image sequences for FabH3K9ac-Cy3 are shown, along with a sample fit (solid colored lines) to the extracted data (colored shapes) below. From lowest to highest, data/lines correspond to times 0.38, 0.76, 1.14, 1.52, 1.90, 3.79, 7.58, and 27.67 s post-bleach. According to these fits, the bound/free fraction, t_on (s), and t_off (s) of FabH3K9ac-Cy3 is 12.2, 6.1, and 7.3, respectively, in the cell without TSA and 41.2, 4.0, and 9.0, respectively, with TSA.

(B) Same as (A), but for FabH3K9me2-488. According to these fits, the bound/free fraction of FabH3K9me2-488 is 7.6 in the cell without TSA and 5.2 with TSA. Bar, 10 μm.
Supplementary Figure S6. Distribution of FabH3K27me3 in living mouse preimplantation embryos.
Time-lapse images were acquired for ICSI (A) and SCNT (B) embryos injected with FabH3K27me3-488. Typical images at the stages of zygote, the first anaphase and 2-cell are shown. Bar, 10 μm.
Supplementary Figure S7. Monitoring the levels of histone H3K9ac and H3K27ac in living mouse preimplantation embryos. Time-lapse images were acquired for IVF embryos injected with FabH3K9ac-488 and FabH3K27ac-Cy3 in the absence or presence of TSA. Typical images are shown. The intensity ratio of nucleus to cytoplasm was measured and the averages (n > 10) are plotted. Bar, 10 μm.
Legends to Supplementary Movies

**Movie 1.** IgGH3K9ac enters into the nucleus after cell division.
IgGH3K9ac-488 was loaded into the cytoplasm of HeLa cells. Phase-contrast (left) and fluorescence (right) images were captured every 30 min using an EM-CCD (iXon+; Andor) equipped with an inverted microscope (Ti-E; Nikon) with a Plan-Apochromat VC 100× NA 1.4 oil immersion objective lens. The time after the Fab loading (h:min) is indicated. Cytoplasmically loaded IgG was excluded from the nucleus until the breakdown of nuclear membrane in mitosis. Some still images are shown in Figure 1B. The display rate is 1.5 frames/s.

**Movie 2.** FabH3K9ac enters into the nucleus immediately.
FabH3K9ac-488 was loaded into the cytoplasm of HeLa cells. Phase-contrast (left) and fluorescence (right) images were captured every 5 min as in Movie 1. The time after the Fab loading (h:min) is indicated. Cytoplasmically loaded Fab diffused into the nucleus within minutes and became concentrated in the nucleus. Some still images are shown in Figure 1C. The display rate is 1.5 frames/s.

**Movie 3.** FabH3K9ac-loaded HeLa cells divide normally.
FabH3K9ac-488 was loaded into HeLa cells. Phase-contrast and fluorescence images were captured every 30 min as in Movie 1. The elapsed time from the start of acquisition (h:min) is indicated. FabH3K9ac-loaded and imaged cells went through a few cell divisions. Merged images are shown. Some still images are shown in Figure 1E. The display rate is 4.2 frames/s.

**Movie 4.** An inactive X chromosome replicates without dynamic repositioning in the nucleus.
FabH3K27me3-488 and PCNA-Cy3 were loaded into hTERT-RPE1 cells. Phase-contrast and fluorescence images were captured every 1 h as in Movie 1. The elapsed time from the start of acquisition (h:min) is indicated. Merged images of FabH3K27-488 (green) and PCNA-Cy3 (red) are shown. Some still images are shown in Figure 3E. The display rate is 2 frames/s.

**Movie 5.** Effect of TSA on FabH3K27ac nuclear concentration.
FabH3K27ac-488 was loaded into U2OS cells. Fluorescence images were captured every 15 min as in Movie 1. TSA (1 μM) was added at time 0, and the
time before and after TSA addition is indicated (h:min). Pseudocolor images are shown. Some still images are shown in Figure 4B. The display rate is 1.5 frames/s.

**Movie 6.** Effect of TSA on FabH3K9ac and FabH3K9me2. FabH3K9ac-Cy5, FabH3K9me2-488, and PCNA-Cy3 were loaded into HeLa cells. Fluorescence images were captured every 15 min as in Movie 1. TSA (3.3 μM) was added at time 0, and the time before and after TSA addition is indicated (h:min). FabH3K9me2 (top left), FabH3K9ac (top right), PCNA (bottom left), and merged images (bottom right) are shown. Some still images are shown in Figure 4E. The display rate is 2 frames/s.

**Movie 7.** Reversibility of FabH3K9ac and FabH3K9me2 levels after TSA removal. FabH3K9ac-Cy3 and FabH3K9me2-488 were loaded into HeLa cells. Fluorescence images were captured every 30 min as in Movie 1. TSA (3.3 μM) was added at time 0, and 3 h later TSA was removed by washing 4 times with TSA-free medium. Cells were further incubated for 3 h in the absence of TSA. The time before and after TSA addition is indicated (h:min). FabH3K9ac (top left) and FabH3K9me2 (top right) are shown with phase-contrast (bottom right) and in merged images (bottom left; FabH3K9ac and FabH3K9me2 are shown in red and green, respectively). Some still images are shown in Figure 4I. The display rate is 2 frames/s.

**Movie 8.** FabH3K9ac in mouse IVF preimplantation embryos. Mouse embryos were injected with H2B-mRFP mRNA and FabH3K9ac-488. Images of 51 focal planes (2-μm intervals) were captured at 15-min intervals using an inverted microscope (IX-71) with a UPlan-Apochromat 20× (NA = 0.8) oil immersion objective lens. Maximum projections of FabH3K9ac (top left) and H2B-mRF (top right) are shown with bright-field (BF; bottom left) and in merged images (bottom right; FabH3K9ac and H2B-mRFP are shown in green and red, respectively). The elapsed time from the start of acquisition (h:min) is indicated. H2B-mRFP represents the distribution of chromatin. Some still images are shown in Figure 6C. The display rate is 10 frames/s.

**Movie 9.** FabH3K27ac in mouse IVF preimplantation embryos.
Mouse embryos were injected with H2B-mRFP mRNA and FabH3K27ac-488. Images were captured as in Movie 8. Maximum projections of FabH3K27ac (top left) and H2B-mRFP (top right) are shown with bright-field (BF; bottom left) and in merged images (bottom right; FabH3K27ac and H2B-mRFP are shown in green and red, respectively). The elapsed time from the start of acquisition (h:min) is indicated. H2B-mRFP represents the distribution of chromatin. Some still images are shown in Figure 6E. The display rate is 10 frames/s.