Supplemental Materials

Molecular Biology of the Cell

Yamagishi and Abe

Supplementary figure legends

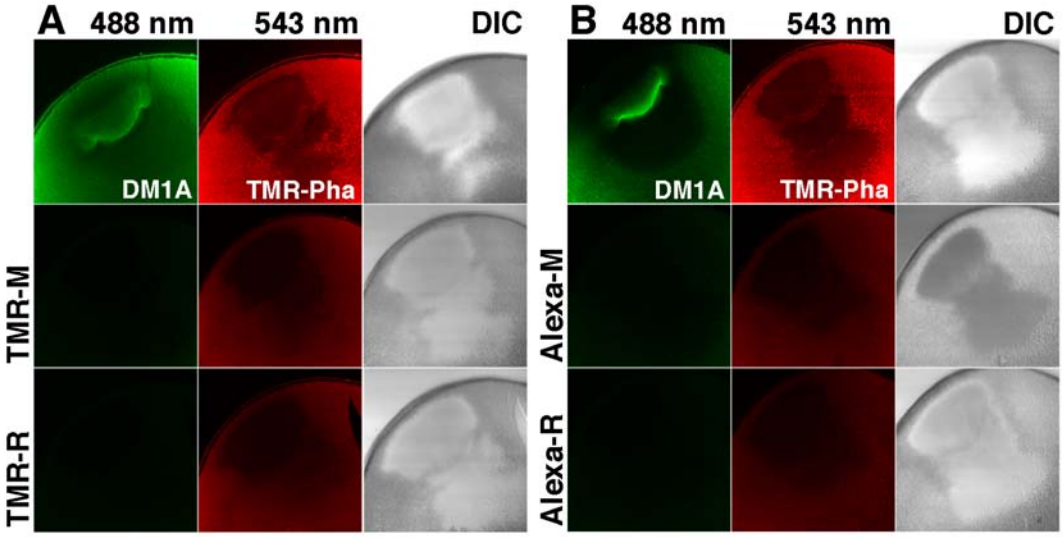

Fig. S1. Control staining of secondary antibodies. Serial sagittal cryosections of maturing oocytes immediately after GVBD were dually stained with anti-tubulin antibody (DM1A, excitation with 488 nm laser) and TMR-phalloidin (excitation with 543 nm laser) to show microtubule and actin structures (A and B, upper panels). (A) The serial sections were treated with TMR-labeled anti-mouse IgG alone (TMR-M, mid-panels) or TMR-labeled anti-rabbit IgG alone (TMR-R, lower panels), and then images were acquired by the respective laser channels. (B) The serial sections were treated with Alexa-488-labeled anti-mouse IgG alone (Alexa-M, mid-panels) or Alexa-488-labeled anti-rabbit IgG alone (Alexa-R, lower panels), and then images were acquired by the respective laser channels.

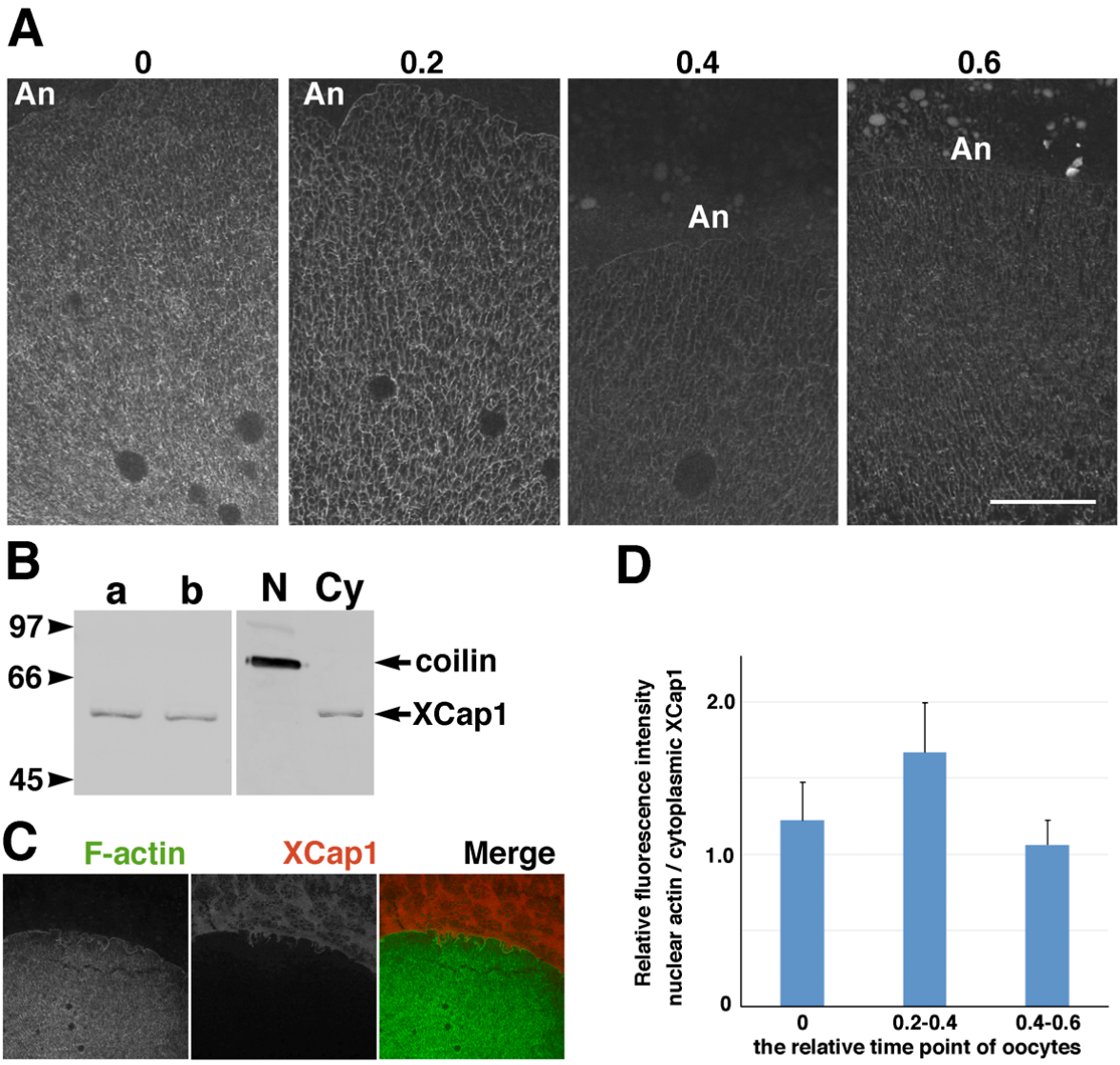

Fig. S2. Reorganization of intranuclear actin filaments during oocyte maturation until GVBD. (A) Fluorescent images of mid-sagittal cryosections of nuclei of maturing oocytes stained with Alexa 488-phalloidin. The relative time points until GVBD are indicated at the top of each panel. Bar, 50 μm. An, the margin of nuclei at the animal pole side. (B) The amount of XCap1 in immature (a) and GVBD stage (b) oocytes were examined by SDS-PAGE and immunoblotting. The antibody specifically and equally stained XCap1 in each lane. Two isolated nuclei (N) and one-fourth volume of an enucleated oocyte cytoplasm (Cy)were also subjected to SDS-PAGE and immunoblotting. XCap1 was detected in the cytoplasm fraction but scarcely present in the nuclear fraction. Coilin is used as a nuclear marker. (C) Confocal image of dual staining with Alexa-488-phalloidin and XCap1. The nucleus was scarcely stained by XCap1. (D) Changes in

relative fluorescence intensity of actin filaments normalized by the cytoplasmic XCap1 fluorescence intensity at the relative time point of 0 (n=3), 0.2 to less than 0.4 (n=8) and 0.4 to 0.7 (n=6). A total of 17 oocytes from 3 different females were examined.

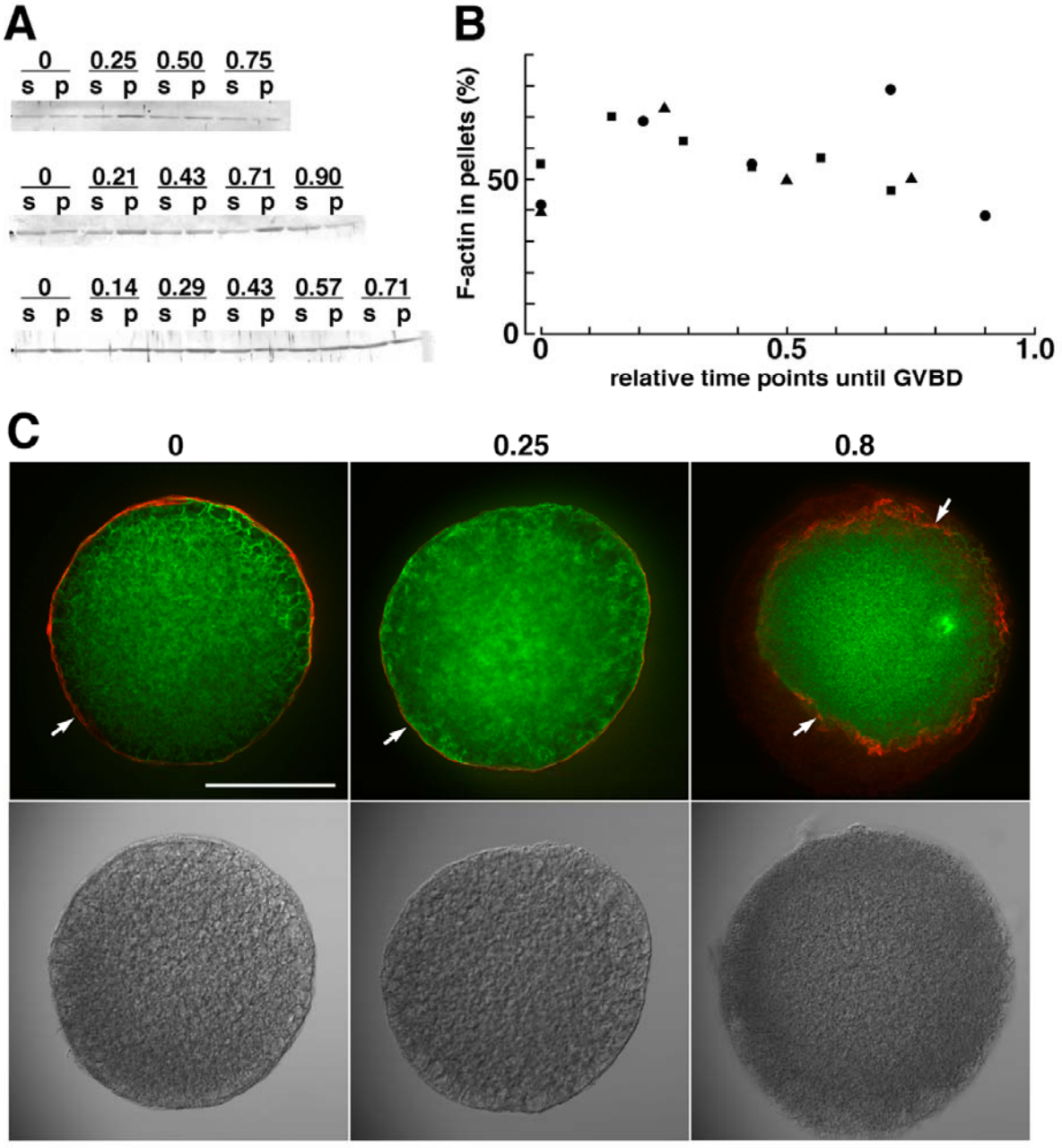

Fig. S3.(A) Pelleting assay of intranuclear actin filaments in maturing oocytes. Nuclei were isolated from oocytes at the relative time points as indicated at the top of each panel and ultracentrifuged. Supernatants (s) and pellets (p) were examined by SDS-PAGE andimmunoblotting with anti-actin antibody. Results from three independent experiments are shown. (B) The actin bands in (A) were

quantified by densitometry and percentages of precipitated actin(F-actin) at each relative time points are plotted. (C) Whole mount confocal images of isolated nuclei from immature oocytes (0) and from the oocytes at the relative time point of 0.25 or 0.8. They were dually stained with Alexa 488-phalloidin (green) and anti-lamin antibody (red). DIC images are also shown. Arrows indicate the nuclear periphery where lamin stained.

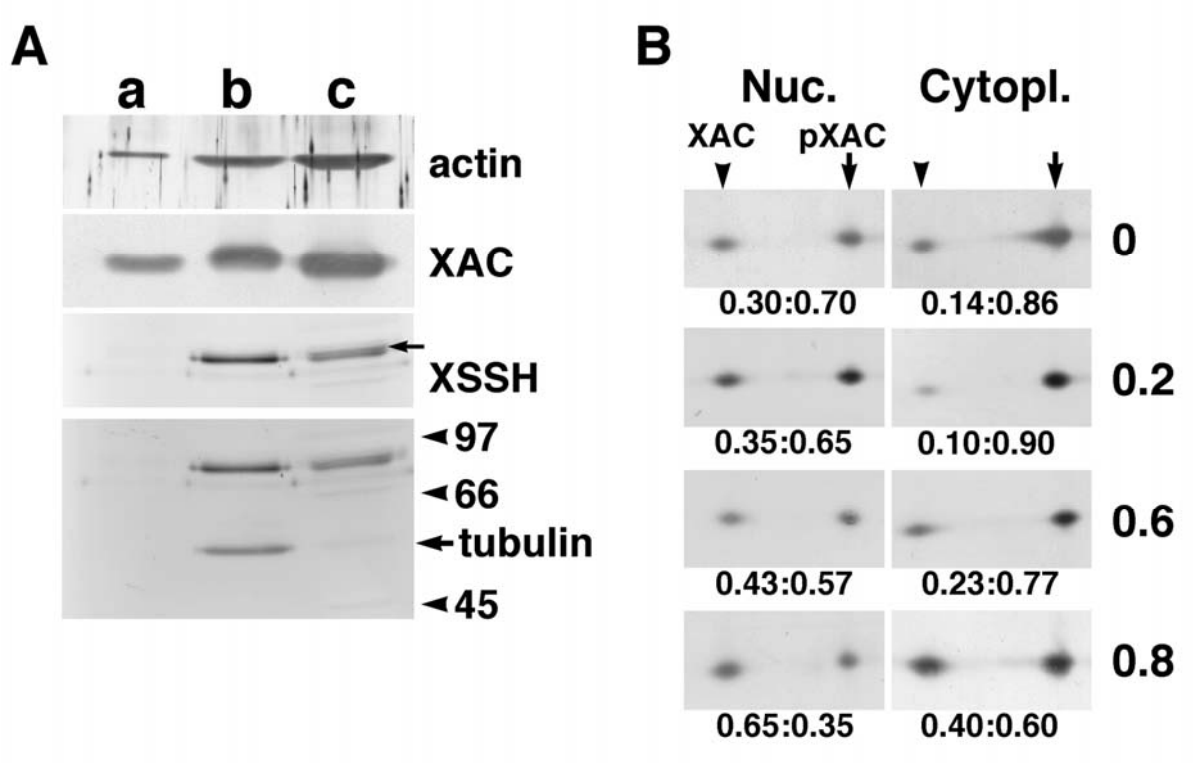

Fig. S4. (A) Relative amounts of actin, XAC and XSSH in the nucleus and cytoplasm of full-grown stage VI oocytes. One-fourth volume of a single nucleus (a) and an enucleated oocyte (b), and five isolated nuclei (c) were subjected to SDS-PAGE and immunoblotting with each antibody. Since the sizes of an oocyte and a nucleus are 1.2 mm and 0.4-0.5 mm in diameter, respectively, the volume of cytoplasm is 14 to 27 times as large as that of nucleus. One-fourth volume of cytoplasm and five nuclei were applied to lanes b and c, respectively. Therefore, the concentrations of actin and XAC in the nuclei are almost the same as those in the cytoplasm, as the blotting bands of both proteins are rather dense in lane c. In contrast, XSSH is not so accumulated in the nuclei, as judged by the band intensity between lanes b and c.The membrane stained with anti-XSSH antibody was reprobed by anti-tubulin antibody as a cytoplasmic marker (lower panel).(B) Time-course changes in phosphorylation levels of XAC during oocyte maturation. Four samples of 2D-immunoblots of

XAC in isolated nuclei(left panel, Nuc.) and enucleated oocytes (right panel, Cytopl.) at the relative time points indicated on the right side. The spots of XAC and pXAC are represented by arrowheads and arrows, respectively. The ratio of XAC to pXAC is indicated under each panel.

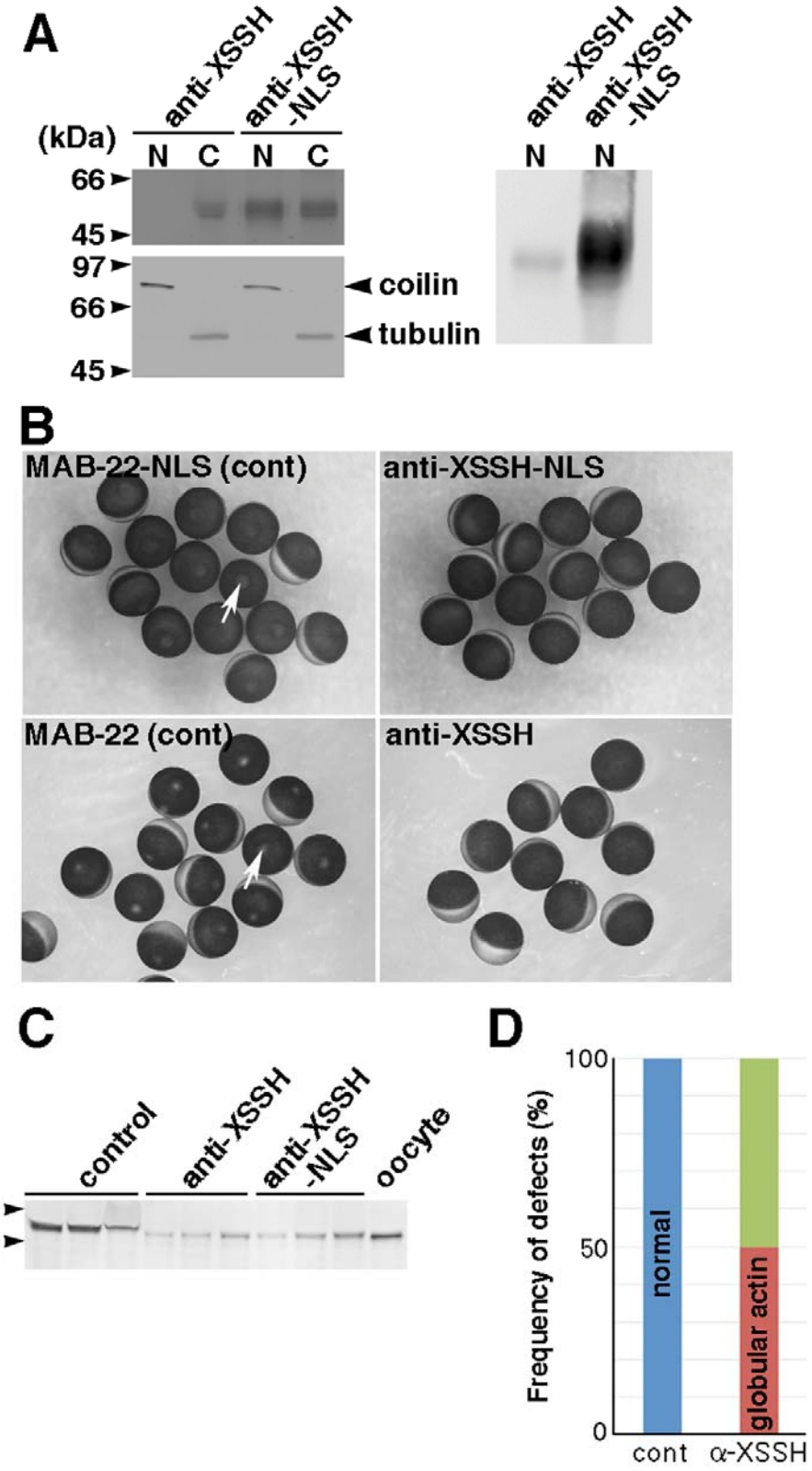

Fig. S5.(A) Nuclear accumulation of the NLS-conjugated anti-XSSH IgG. Oocytes were injected with purified anti-XSSH IgG (anti-XSSH) or NLS-conjugated anti-XSSH IgG (anti-XSSH-NLS) and incubated for 12 h. Then, nuclei were isolated from the injected oocytes. Asingle isolated nucleus (N) and one-fourth volume of the resultant enucleated oocyte (C) were subjected to SDS-PAGE followed by immunoblotting with anti-rabbit secondary antibody to detect the heavy chain of injected IgG (left upper panel). Internal controls are indicated (left lower panel) by immunoblotting with anti-coilin (nuclear marker) and anti-tubulin (cytoplasmic marker) antibodies for the same samples. To compare the amount of IgG in the nuclei, one isolated nucleus from the oocytes injected with the respective IgG was applied to each lane (right panel). The NLS-conjugated IgG is successfully accumulated into the nucleus. Notably, the heavy chain of anti-XSSH IgG-NLS shows slower mobility than that of anti-XSSH IgG because of NLS-peptide conjugation.(B) *Xenopus* oocytes were injected with purified MAB-22 IgG conjugated with NLS peptide (control), anti-XSSH-NLS, MAB-22 IgG (control) and anti-XSSH IgG. The concentration of IgG was 10 mg/ml. Following progesterone treatment, WMS formation (indicated by arrows) was examined. MAB-22 IgG- or MAB-22 IgG-NLS-injected oocytes exhibited WMS formation, while either anti-XSSH-NLS or anti-XSSH-injected oocytes suppressed the formation of WMS. (C) Effects of anti-XSSH antibody on phosphorylation of XSSH in oocytes. Oocytes were injected with MAB-22-IgG (control; 3 samples), anti-XSSH IgG (3 samples) or anti-XSSH-NLS (3 samples) and examined at 3 h after GVBD. Control and antibody-injected oocytes were homogenized with PBS and centrifuged at low-speed. The supernatants were subjected to SDS-PAGE and immunoblotting with guinea pig anti-XSSH antibody. A sample on the right is untreated immature oocytes as controls to determine mobility of nonphosphorylated XSSH. Injection of anti-XSSH IgG and anti-XSSH-NLS decreased the amount of XSSH and partially inhibited full phosphorylation of XSSH during oocyte maturation. (D) Effects of anti-XSSH antibody on the formation of globular actin filament structure and assembly of MTOC-TMA. All antibody-injected oocytes (n=12) which suppressed WMS formation showed defects in MTOC-TMA assembly (light green), and among them, a half oocytes formed intranuclear globular actin filaments (red).

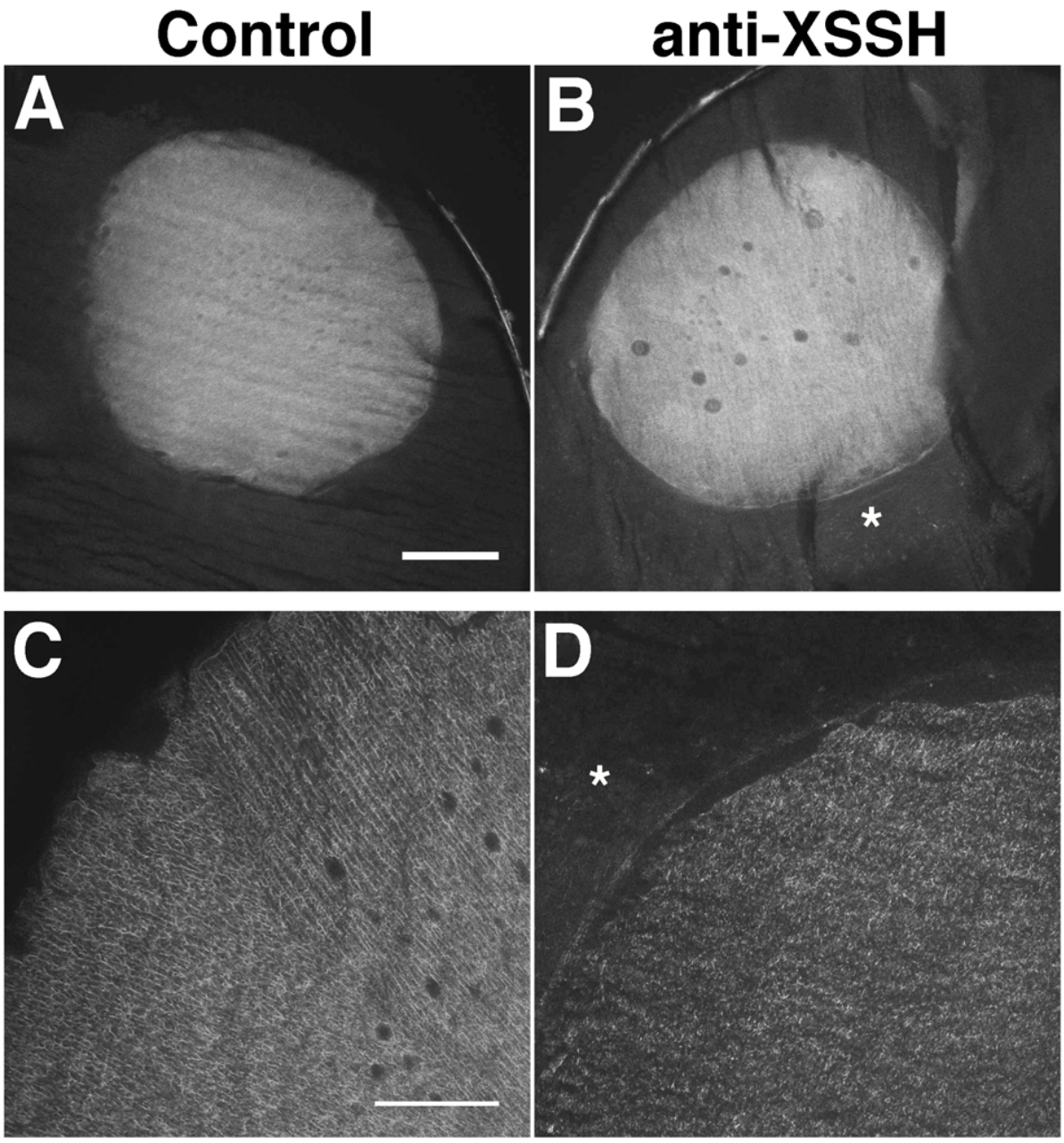

Fig. S6. Effects of anti-XSSH antibody injection on immature oocytes. Mid-sagittal sections of control (A and C) and antibody-injected (B and D) immature oocytes were stained with Alexa-488-phalloidin. Asterisks represent the cytoplasm stained more brightly in antibody-injected oocytes. Bars in A and C, 100 and 50 μm, respectively.

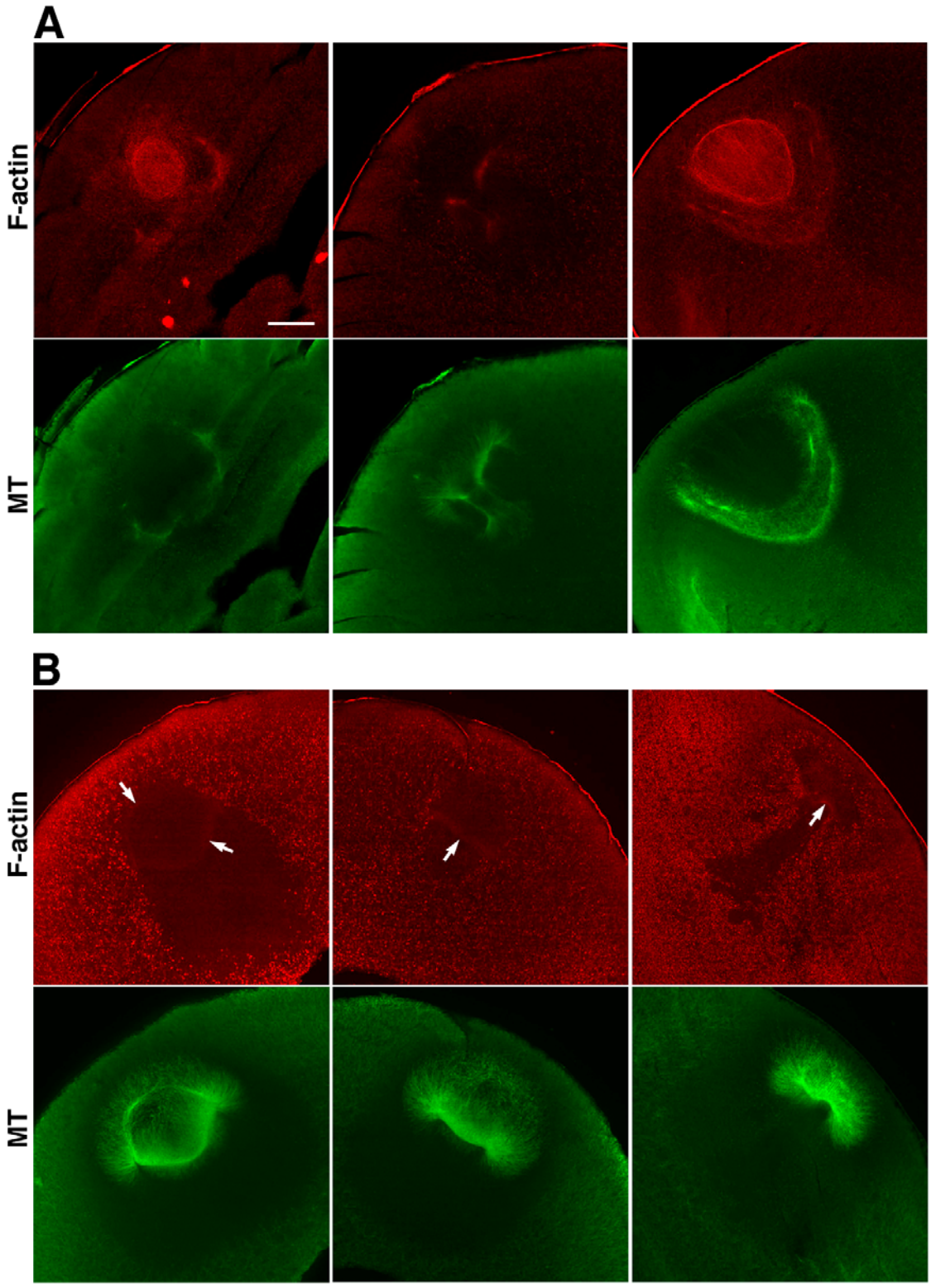

Fig. S7. Three different examples of anti-XSSH antibody- (A) and S3A cofilin-injected oocytes (B) dually stained with TMR-phalloidin (F-actin) and anti-tubulin antibody (MT). Arrows in (B) represent faint actin staining at the base of MTOC-TMA.The arrow at the animal pole side in left

panel shows atypical actin staining where microtubules also accumulated. Bar, 100 μm

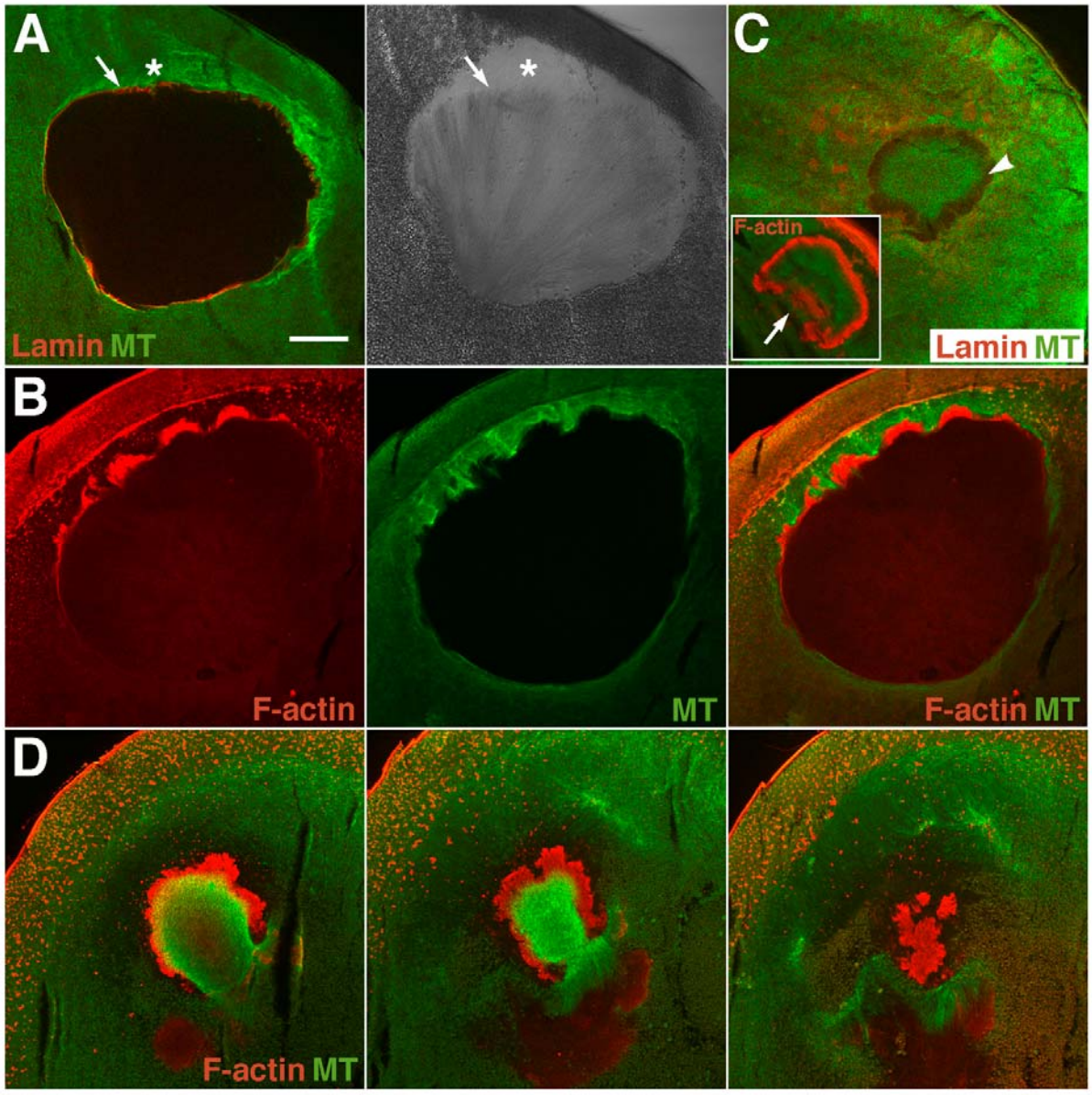

Fig. S8. Effects of 10 μM jasplakinolide on immature (A and B) and matured (C and D) oocytes. Mid-sagittal sections of immature oocytes treated with jasplakinolide for 3 h were dually stained with anti-lamin(red) and anti-tubulin (green) (A, merged and DIC image) or TMR-phalloidin (red) and anti-tubulin (green) (B). Strong F-actin staining located under the cell membrane and nuclear envelope is characteristic of this treatment. Arrows and asterisks in A represent the position of nuclear envelope and the yolk-free region, respectively. Immediately after GVBD (C), dual staining with anti-lamin and anti-tubulin antibodies revealed that nuclei of jasplakinolide-treated oocytes

shrink with the disassembly of lamin and microtubules locate at the center region of nucleoplasm exclusively from the nuclear periphery (arrowhead).Dual staining with TMR-phalloidin (red) and anti-tubulin antibody (green) showed the nuclear periphery to be accumulated with actin filaments (inset in C). In addition, the peripheral actin filaments often disassembled from the vegetal side (inset in C, arrow). Serial sections of later GVBD stage oocytes revealed that microtubules (green) accumulated into the nucleoplasm from the vegetal side where actin filaments (red) had disassembled (D). At least 6 oocytes from 3 different females were examined in each staining. Bar, 100 μm.