Supplemental Figure 1. ORC1-3 and ORC5 are adjacent to the chromosomes at anaphase II. This is an expanded copy of Fig. 1 in which the fluorescent images are separated. ICSI generated zygotes were fixed and stained for ORC1 (A-D), ORC2 (E-H), ORC3 (I-L) and ORC5 (M-P) and visualized by confocal microscopy. All four subunits localize to the area between separating maternal chromosomes in anaphase II. The decondensing sperm nucleus had no visible staining in any oocyte. Insets in (E-H) and (M-P) represent areas in different confocal planes than the maternal chromatin. Sp, decondensing sperm nucleus. All images are shown at the same magnification, bar =  $10 \mu m$ .



Supplemental Figure 2. ORC1, ORC3 and ORC5 are at the spindle poles in zygotic metaphase. ICSI generated zygotes at metaphase were fixed and stained for ORC1 (A-D), ORC3 (E-H), or ORC5 (I-L) and visualized by confocal microscopy. All three subunits localize to the spindle poles, similar to ORC2 (ref. [15]). All images are shown at the same magnification, bar = 10  $\mu$ m.



Supplemental Figure 3. ORC1 surrounds the chromosomes at zygotic anaphase. ICSI generated zygotes at metaphase were fixed and stained for ORC1. This is a single plane of a confocal microscope image. All images are shown at the same magnification, bar = 10  $\mu$ m.



**Supplemental Figure 4. ORC6 in the first zygotic cell cycle.** This is an expanded copy of Fig. 2 in which the fluorescent images are separated. ICSI generated zygotes were fixed and stained for ORC6 at zygotic G1 (A-D), zygotic telophase (E-H), and 2-cell G1 and visualized by confocal microscopy. Inset in (I-L) is the area in different confocal plane from the main image that contained the second nucleus. PB, second polar body. All images are shown at the same magnification, bar = 10  $\mu$ m.



Supplemental Figure 5. ORC4 associates briefly with the decondensing sperm nucleus and the maternal chromatin just after fertilization. This is a companion figure to Fig. 3F-J showing two additional examples of transient ORC4 binding to all the zygotic chromatin. ICSI generated zygotes were fixed and stained for ORC4 at anaphase II, 2 hrs after fertilization, and visualized by confocal microscopy. Sp, decondensing sperm nucleus; M PN, maternal pronuclei. All images are shown at the same magnification, bar = 10  $\mu$ m.



Supplemental Figure 6. ORC2 localization during oocyte maturation. GV oocytes were isolated and cultured in vitro, then stained for ORC2 and visualized by confocal microscopy. ORC2 localizes at the spindle poles in metaphase I (A-D) and between the separating chromosomes at anaphase I (E-H). This is the same behavior as that of ORC2 at anaphase II (Fig. 1B and ref [15]). All images are shown at the same magnification, bar = 10  $\mu$ m.



Supplemental Figure 7. ORC4 translocates to the second polar body nucleus but not to the fist at zygotic anaphase/telophase. ICSI generated zygotes were fixed and stained for ORC4 at zygotic telophase, and visualized by confocal microscopy. Three zygotes are shown, each with ORC4 localized to the cytoplasm of the first polar body (1<sup>st</sup> PB) and in the nucleus of the second polar body (2<sup>nd</sup> PB). Insets in (A-D) show the 2<sup>nd</sup> PB in a different confocal plane from the rest of the image. Insets in (I-L) is the area in different confocal plane from the main image that contained the second set of chromosomes. All images are shown at the same magnification, bar = 10  $\mu$ m.

