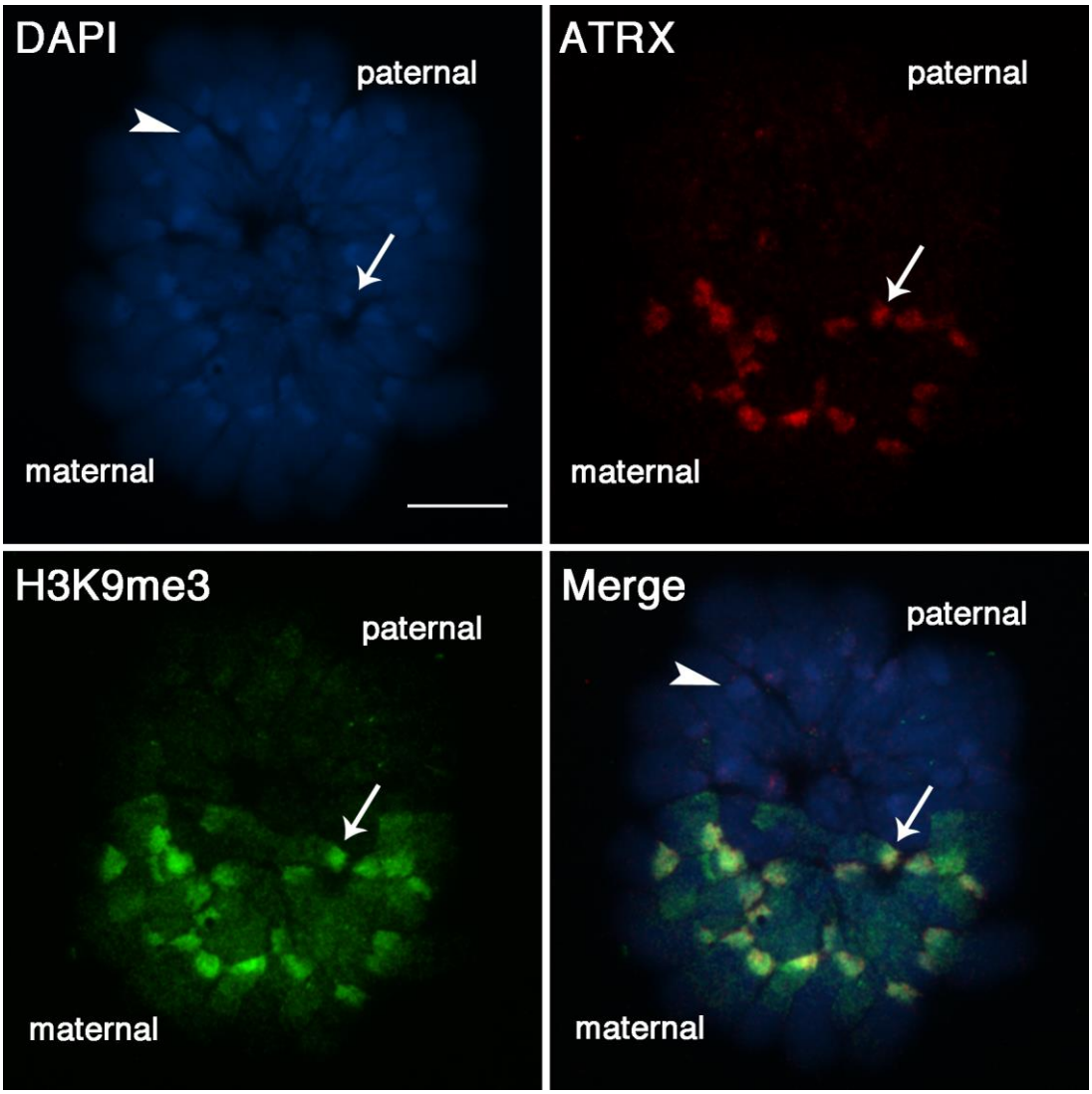
Supplemental Materials and Methods:

Antibodies used for chromosome immunofluorescence

The following antibodies were used for immunofluorescence staining: rabbit anti-ATRX (H-300 and D-5) antibodies (Santa Cruz, 1:200), mouse monoclonal anti-ATRX antibody (Baumann et al., 2010) (1:5), rabbit and mouse anti-DAXX (M-112 and H-7) antibodies (Santa Cruz; 1:100), human anti-CREST antiserum (Cortex Biochem, Inc.; 1:500), rabbit anti-CENP-C (Pluta AF, 1996)1:1000), mouse anti-phospho-Histone H2A.X (Ser139) (EMD Millipore, 1:400), mouse anti-phospho-Histone H3S10 (EMD Millipore, 1:1000), mouse anti-Aurora kinase B (AIM-1) (BD Biosciences, 1:100) and mouse anti-BrdU antibody (Roche; 2µg/ml). Surface spread chromosomes were maintained overnight in PBS containing 1 mg/ml BSA and 0.01% Triton X 100 at 4°C, before detection of primary antibodies with appropriate 488- or 555-coupled Alexa Fluor secondary antibodies (Molecular Probes) at a dilution of 1:1000 for 1 h at room temperature. Samples were counterstained and mounted using Vectashield containing DAPI (4', 6-diamidino-2-phenylindole; Vector Laboratories, Inc. Burlingame, CA). Following image acquisition, slides were processed for FISH analyses.

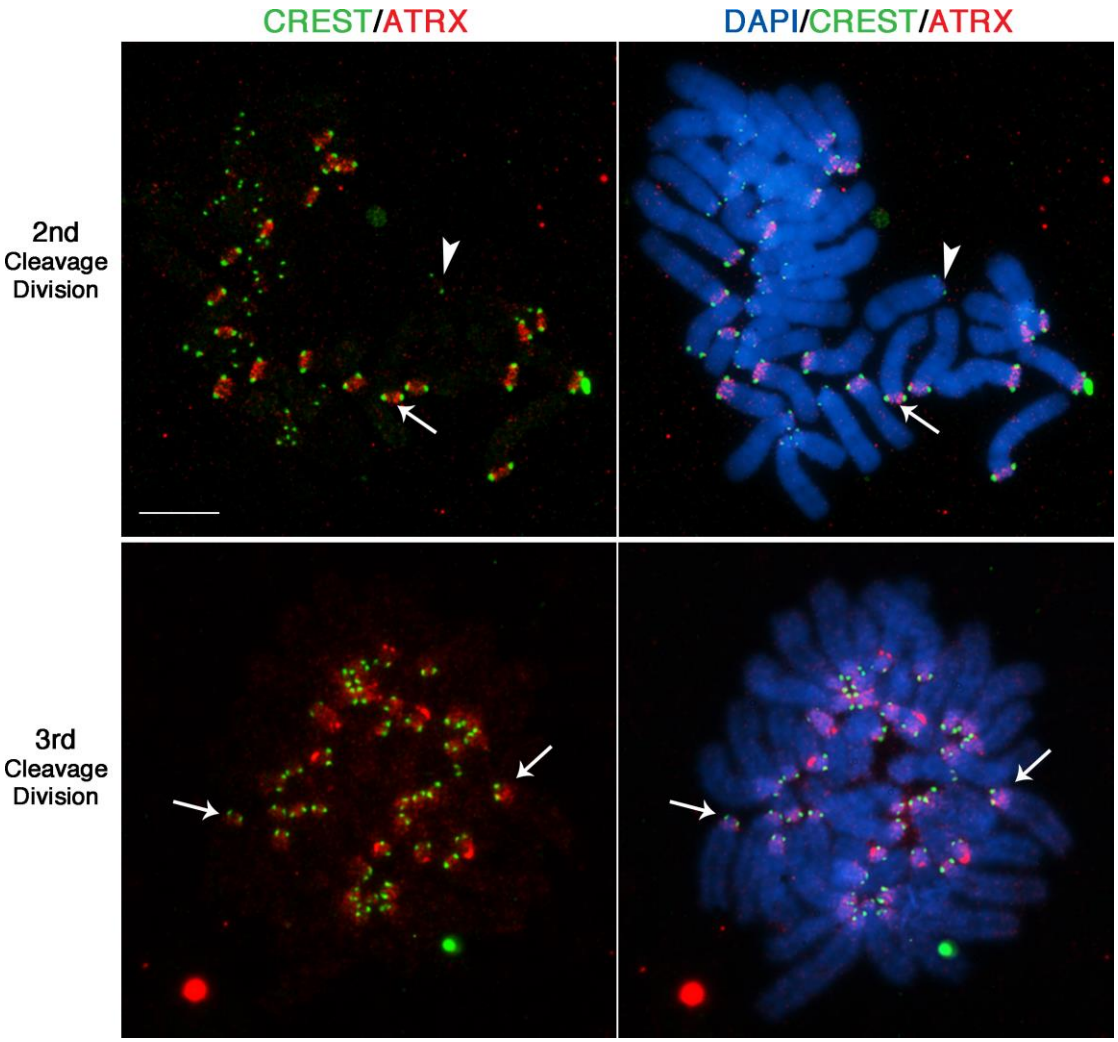
For whole mount analyses, pronuclear stage zygotes and parthenotes were fixed at indicated hours post-fertilization (hpf) using a solution containing 2% paraformaldehyde and 0.1% TX-100 in PBS. To obtain metaphase-stage zygotes, fertilized embryos were cultured for 18 to 20 hpf. For high-resolution chromosome analyses, zygotes and cleavage stage embryos were surface spread following removal of the zona pellucida as described (Baumann et al., 2010). Sister chromatid exchanges were detected following exposure of zygote stage embryos to 5-bromo-2’deoxyuridine (250 µM; Sigma) during the first cell cycle (6-24 hpf). After over

night incubation, 2-cell embryos were washed and subsequently cultured in fresh KSOM media supplemented with 1 mg/ml BSA for 12 h before addition of Colchicine (100 nM).



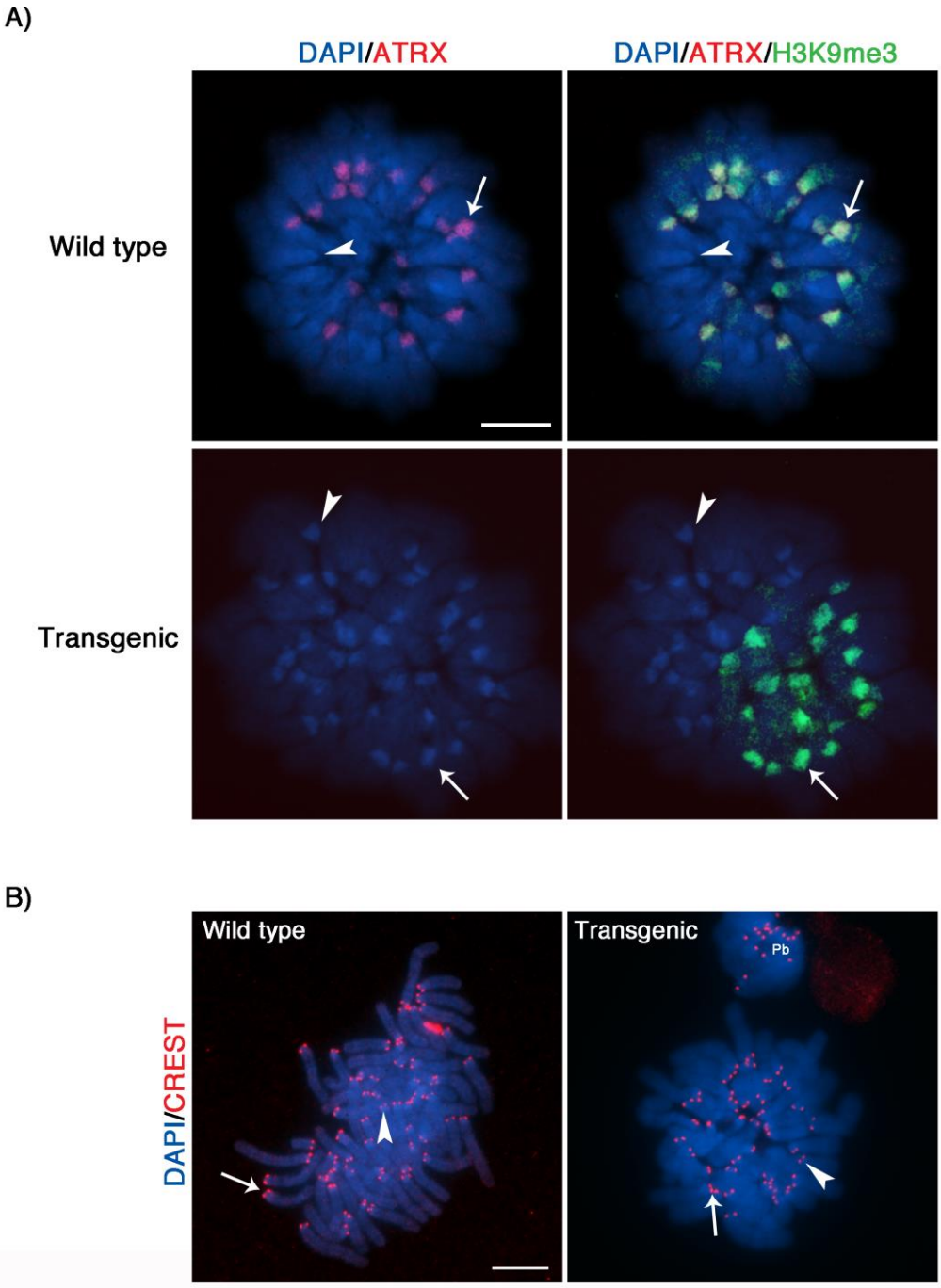
Supplemental Figure 1. ATRX contributes to the epigenetic asymmetry of the maternal genome in the zygote stage embryo. Representative chromosome spread from a wild type embryo during the first mitosis. ATRX (red) is present at pericentric

heterochromatin domains in the maternal chromosome complement (white arrow) where it is specifically co-localized with histone H3 tri-methylated at lysine 9 (H3K9me3; green) a bonafide epigenetic mark of the maternal genome. Lack of transcriptionally repressive chromatin marks in the paternal chromosome complement (arrowhead), results in epigenetic asymmetry between parental genomes in the zygote stage embryo. Scale Bar=10μM.



Supplemental Figure 2. Post-meiotic kinetochore asymmetry and epigenetic asymmetry at pericentric heterochromatin persist at the second mitotic division in the mouse cleavage stage embryo. Upper panel: Metaphase spread of a wild type embryo during the second mitotic division showing ATRX staining (red) at pericentric heterochromatin domains only on chromosomes of maternal origin (arrow). Note the presence of kinetochore size asymmetry resulting in larger CREST signals detected in chromosomes that exhibit ATRX staining. Paternal chromosomes (arrowhead) lack ATRX staining and consistently exhibit reduced CREST signals. **Lower panel:** Both kinetochore size asymmetry and epigenetic asymmetry are lost at

the third cleavage division. Representative metaphase spread of a wild type embryo during the third mitosis. ATRX (red) is now detected at pericentric heterochromatin domains of all chromosomes. Notably, differences in kinetochore size as determined by CREST signals are no longer detectable at this stage. Loss of both kinetochore asymmetry and epigenetic asymmetry render both parental chromosome complements indistinguishable by the third cleavage division. Data from three independent experimental replicates. Scale Bar=10μM.



Supplemental Figure 3. ATRX is not required for maintenance of kinetochore size asymmetry in the cleavage stage embryo. A) Metaphase spread of a wild type, zygote stage embryo at the first mitosis. ATRX (red) is found co-localized with H3K9me3 (green) at pericentric heterochromatin domains in the maternal

chromosome complement (arrow). Transgenic ATRX deficient embryos exhibit only H3K9me3 staining (green) at pericentric heterochromatin of maternal chromosomes. **B)** The kinetochore size asymmetry observed between parental genomes of the wild type embryo as determined by CREST signals (red) is maintained during the first mitosis in ATRX deficient embryos. Data from three independent experimental replicates. Scale Bar=10μM.

References to Supplementary Materials and Methods

Baumann, C., Viveiros, M. M. and De La Fuente, R. (2010). Loss of Maternal ATRX Results in Centromere Instability and Aneuploidy in the Mammalian Oocyte and Pre-Implantation Embryo. *PLoS Genet* **6**, e1001137.

Pluta AF, E. W. (1996). Specific interaction between human kinetochore protein CENP-C and a nucleolar transcriptional regulator. *J Biol Chem.* **271**, 18767-18774.