Supplemental Information

Materials and Methods

**Plasmid construction.** Primers CTCFiF1 and CTCFiB1 were used to amplify a 697 bp fragment corresponding to bp 1619 to 2315 of CTCF (GenBank accession no. 29165753) with Ready-to-Go PCR beads (Amersham) from oocyte cDNA. PCR was performed for 1 cycle of 94°C for 2 min followed by 35 cycles of 15 s at 94°C, 10 s at 55°C, and 15 s at 72°C and 1 cycle of 72°C for 15 min. Primer sequences are listed in Table S1. The PCR product was cloned using the TOPO-TA Cloning Kit (Invitrogen) into the pCR2.1 vector to give pCiSR. The insert was excised with EcoRI and cloned into pBSIIKS (Stratagene), to give pCTCFi. The inverted repeat was generated by excising the fragment from pCTCFi with XhoI and XbaI and ligating into XhoI and XbaI digested pCiSR. The inverted repeat was then excised with SpeI, and ligated into pRNAi-Zp3-1 (I) that had been digested with XbaI, to give pMoZp3-dsCTCF.

**Transgenic mice.** All experiments were conducted with the approval of the Institutional Animal Care and Use Committee at the University of Pennsylvania. Transgenic animals were generated as previously described (2), with the following modifications. Animals were genotyped by PCR assay for EGFP. One hundred nanograms of tail DNA was PCR amplified in a 25 µl final volume using Ready-to-Go PCR beads and primers EGFPF2 and EGFPRI at a final concentration of 0.5 µM. PCR was performed for 1 cycle of 94°C for 2 min followed by 32 cycles of 15 s at 94°C, 10 s at 58°C, and 15 s at 72°C. Products were visualized on an ethidium bromide stained 2% agarose gel. Positive animals were
bred to C57BL/6J animals (The Jackson Laboratory). The transgenic lines were maintained by breeding transgenic males. For determination of litter size, N2 and later generation transgenic females and their non-transgenic sibblings were mated with C57BL/6J males for approximately 9 months. Transgenic males had expected litter sizes.

**Isolation of germinal vesicle stage oocytes.** Females, at least 6 weeks of age, were superovulated by injection of 7.5 IU of pregnant mare serum gonadotropin (Calbiochem). 44-48 h after injection, ovaries were removed and finely minced using a 20-guage needle. Individual oocytes were collected by mouth pipette and cumulus cells were mechanically removed. Oocytes were washed through several drops of medium to ensure they were free of cumulus cells. For isolation of RNA, oocytes were transferred to 100 µl of lysis buffer (Dynal). Oocytes used for bisulfite mutagenesis were transferred to a 1.5 ml tube with several microliters of PBS. For immunoflourescence, oocytes were fixed as described below.

**RNA isolation, reverse transcription, and PCR.** Poly-A⁺ mRNA was isolated from oocytes using the Dynabead RNA Isolation Kit (Dynal) according to manufacturers instructions and reverse transcribed for 60 min at 42°C followed by 10 min at 95°C. The following reaction conditions were used: 1X first-strand buffer (50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl₂; Invitrogen), 10 mM DTT (Invitrogen), 1 mM dNTP’s (Invitrogen), 25 ng/ml dT₁₂₋₁₈ (Amersham), 20 U RNaseOUT (Invitrogen), and 25 U SuperScript II (Invitrogen).
All PCR reactions were carried out using Ready-to-go-PCR beads in a final volume of 25µl and included 0.1 µCi of [α−32P]-labeled dCTP (NEN). The reaction conditions were such that the amount of product was in the linear region of semi-log plots of the amount of product versus cycle number (3). CTCF was amplified from 2.0 oocyte equivalents using a final concentration of 0.3 µM of each primer, CTCF5F1 and CTCF5R1. CTCF PCR was carried out for 1 cycle of 94°C for 2 min followed by 29 cycles of 15 s at 94°C, 10 s at 52°C, and 15 s at 72°C. GAPD was amplified from 1.0 oocyte equivalent using a final concentration of 0.5 µM of each primer, GAPDHF1 and GAPDHR1. GAPD PCR was carried out for 1 cycle of 94°C for 2 min followed by 28 cycles of 15 s at 94°C, 10 s at 60°C, and 15 s at 72°C. Primer sequences are described in Table S1. PCR products were resolved by 7% polyacrylamide gel electrophoresis and quantified using a Phosphorimager (Molecular Dynamics). Results were verified with real-time PCR on the Lightcycler instrument (Roche).

**Immunofluorescence.** Oocytes were fixed in 2.0% paraformaldehyde, pH 7.5, for 20 min at room temperature, washed 2-3x in 3 mg/ml PVP in PBS (PBS/PVP) and stored at 4°C in PBS/PVP until staining. For staining, oocytes were permeabilized for 15 min in freshly prepared PBS containing 0.1% Triton-X100, then blocked for 1 h in fresh 20µg/ml donkey anti-mouse F(ab) fragment/0.1% IgG-free BSA (Jackson ImmunoResearch)/0.01% Tween-20/PBS. Oocytes were then incubated for 1 h with monoclonal anti-human CTCF antibody (Pharmingen) diluted 1:3 in blocking solution. After four 15 min washes in 0.1% IgG-free BSA/0.01% Tween-20/PBS, oocytes were transferred to a 1:200 dilution of donkey Cy3-conjugated anti-mouse IgG (Jackson
ImmunoResearch) and 2 µg/ml DAPI (Sigma) in blocking solution for 60 min. Samples were washed three times for 15 min. Finally, oocytes were mounted on a glass slide and visualized with a confocal microscope (Leica). All incubations were at room temperature unless otherwise noted.

**Bisulfite mutagenesis.** Pools of 15-80 oocytes, free of cumulus cells, were embedded in approximately 10 µl of molten 2% low-melting point SeaPlaque agarose (BMA), and treated as previously described (4). For PCR, 5-10 oocyte equivalents of the mutagenized DNA were used for each reaction with the previously described primers and conditions (5, 6). The regions from 1304-1726 bp (U19619) and from 504-812 bp (M12347) were analyzed for the *H19* DMD and *skeletal α-actin*, respectively. PCR products were cloned using the TOPO-TA cloning kit and sequenced. Six clones were sequenced for each PCR reaction. The CpG content in the analyzed fragments is similar: 16/400 bp (*H19*) and 13/310 bp (*actin*).

**In vitro fertilization.** Metaphase II (MII) eggs were collected from the oviducts of superovulated female mice and placed in Whitten’s medium containing 10% fetal bovine serum (FBS) (7). *In vitro* fertilization was performed as previously described (8) with the following modifications. Briefly, sperm were collected from 8-10 week-old B6SJLF1/J male mice (Jackson Laboratories) by placing the caudae epididymides and vas deferens from a single mouse in a 900-µl drop of Whitten’s medium supplemented with 15 mg/ml lipid-rich bovine serum albumin (BSA, Life Technologies) under mineral oil; sperm were allowed to swim out for 10-15 min, and then the tissue was removed from the medium.
Sperm were capacitated at 37°C in an atmosphere of 5% CO₂, 5% O₂, 90% N₂ for 1.5-2 h. Eggs were washed free of FBS and placed in 100-µl drops of Whitten’s containing 15 mg/ml BSA. Sperm were added to these drops at a final concentration of 500,000/ml. Sperm and eggs were incubated for 3 h at 37°C in an atmosphere of 5% CO₂, 5% O₂, 90% N₂. Eggs were removed from the fertilization drop, washed through 5 drops of CZB medium and cultured in CZB up to the two-cell stage. Fertilization was evaluated by the formation of both male and female pronuclei ~7-8 h post-insemination. Two-cell stage embryos were cultured to the blastocyst stage in KSOM (9).

Statistical analysis. Statistical values for each data set were determined by the two-tailed Student’s T-test, unequal variance.
Fig. S1.
Fig. S3.
**Table S1.** Sequence of primers

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Legends for Supplemental Figures

Fig. S1. (A) Design of the transgenic construct used in this study. A 697 bp fragment of the CTCF cDNA (GenBank accession no. 29165753, nucleotide 1619 to 2315) was cloned as an inverted repeat and inserted into the XbaI site as described in Materials and Methods. Expression is driven by the oocyte-specific Zp3 promoter. (B) The five pedigrees represent the transgenic lines analyzed. Below the line number, the N0 generation identifies the sex of the founder animal, with boxes corresponding to males and circles designating females. Transgenic mice are represented by half filled symbols.

Fig. S2. CTCF protein levels in non-transgenic and transgenic oocytes. CTCF was visualized with anti-CTCF monoclonal antibody and Cy3-conjugated anti-mouse IgG (A, C, E, G). DAPI was used as a counterstain to visualize DNA in all samples (B, D, F, H, J). (A, B) Non-transgenic oocytes show CTCF localization to the germinal vesicle (GV, indicated by white arrow heads) of oocytes and to the nuclei of cumulus cells (denoted by asterisks). CTCF protein in lines 21 (C, D), 1 (E, F), and 56 (G, H) are shown. Primary antibody was omitted in the non-transgenic oocytes shown in (I, J). Specificity of CTCF targeting in oocytes is confirmed by CTCF positive staining in the cumulus cells. The scale bar represents 40µm.

Fig. S3. Methylation pattern of oocytes from lines 21 and 56. Each line denotes an individual strand of DNA with unfilled and filled circles corresponding to unmethylated and methylated CpGs, respectively. The number of strands observed with a given
methylation profile (if greater than one) is indicated to the left of each line. Black bars represent CTCF binding sites.
Supplemental References