**Supporting Information**

**Methods**

***Cryosections and Immunofluorescence***. Mouse ovaries were fixed for 48 h at 4°C in 4% paraformaldehyde. After fixing, they were placed in 15% sucrose in phosphate-buffered saline (PBS) overnight at 4°C and 30% sucrose in PBS, successively. The ovaries were then frozen in Tissue-Tec O.C.T. Compound at -60°C. Ovary sections (16 μm) were cut using a MICROM 500M, post-fixed with 4% formaldehyde, washed in PBS, permeabilized and blocked in 0.5% Triton-X 100 in 1% BSA- PBS. Primary antibody (HSF1 polyclonal, rabbit, gift from R.I. Morimoto) was used at 1:500 in 0.1% Triton-X 100 in 0.1%BSA-PBS and incubated overnight at 4°C. Secondary antibody directed against rabbit Ig (Goat anti-rabbit Alexa 546 diluted 1:500) was then applied for 1.5 hr at room temperature. TO-PRO-3 (Molecular Probes) was used at 1:200 in 0.1% Triton-X 100 in PBS for DNA staining. Sections were rinsed in PBS, mounted with Moewiol and analyzed with a Leica TCS SP-5 spectral confocal microscope.

**Figure legends**

**Fig. S1.** HSF1 is expressed during ovarian oogenesis. Immunostaining performed on frozen sections of adult ovary reveals HSF1 expression in oocytes (arrowheads) from primordial (*A*), primary (*B*), secondary (*C*) and antral follicles (*D*). Panels (*E*) and (*F*) represent experiments performed with HSF1-deficient ovary sections or without primary antibody, respectively. Each panel comprises immunodetection on the left and TO-PRO-3 staining on the right side. Bar = 10µm (*A, B, C*) and 50 µm (*D, E, F*).

**Fig. S2.** Spindle morphology in GVBD oocytes (*Hsf1*$^{-/-}$). I-X examples of the various spindle morphologies exhibited in *Hsf1*$^{-/-}$ oocytes 14 h after IBMX release (green: microtubules; blue: DNA). I –V spindles are not bipolar. VI-X spindles are almost or are completely bipolar.

***Table S1 – List of primers used in RT-qPCR and ChIP experiments***

| Gene symbol | Synonyms | Accession number | Sequences |
|---|---|---|---|
| Hspb1 | **Hsp25** | NM_013560 | F5'- CCAGACTGTTCAGACTTCCCAG-3'<br>R5'- ATCCCCTGAGGGCACACTTA-3' |
| Hspd1 | **Hsp60**, chaperonin | NM_010477 | F5'- GCCATGCTTGGAGATTTTGT-3'<br>R5'- CCATTCCAGGGTCCTTCTCT-3' |
| Hspa1b | **Hsp70.1**<br>Hsp70-1<br>hsp68<br>Hsp70<br>HSP70A1 | NM_010478 | F5'- TGTTCCAGTAGCCTGGGAAG 3'<br>R5'- CCACAAAACCTTAACATGGACA-3' |
| Hspa1a | **Hsp70.3**<br>Hsp70-3 | NM_010479 | F5'- TTCCAGTAGCCTGGGAAGAC -3'<br>R5'- CCACAAAACCTTAACATGGACA -3' |
| Hsp90aa1 | **Hsp90α**<br>86kDa,<br>89kDa,<br>hsp4,<br>Hsp86-1,<br>Hsp89,<br>Hsp90,<br>Hspca | NM_010480 | F5'- AAGGCAGAGGCTGACAAGA-3'<br>R5'- AGGGGAGGCATTTCTTCAGT-3' |
| Hsp90aa1 | **Hsp90α**<br>(ChIP) | NM_010480 | F5'- CTTGCGTTCGTTCTCCGC-3'<br>R5'- CGCACCAGGACACTGAAGC -3' |
| Hsp90ab1 | **Hsp90β**<br>90kDa,<br>Hsp84,<br>Hsp84-1,<br>Hsp90,<br>Hspcb | NM_008302 | F5'- AAGGCTAGGCAGACAAAA-3'<br>R5'- GGGATCTCATCAGGAACAGC-3' |
| Hsph1 | **Hsp105**<br>hsp-E7I,<br>Hsp105,<br>HSP110 | NM_013559 | F5'-TGAAATCAGAGCGAAGGTCA-3'<br>R5'-TTAGGGTGGCATTCACCATT-3' |
| Pou5f1 | **Oct4**<br>Oct-3,<br>Oct-3/4,<br>Otf-3,<br>Otf-4,<br>Otf3-rs7,<br>Otf3g,<br>(ChIP) | NM_013633 | F5'- CAG GCC GAG AGG GTG CA-3'<br>R5'- GTG GAA AGA CGG CTC ACC TA-3' |
| Rps16 | **S16**<br>ribosomal protein S16 | NM_013647 | F5'- AGGAGCGATTTGCTGGTGTGG-3'<br>R5'- GCTACCAGGGCCTTTGAGATGGA -3' |

***Table S2 – Antibodies used in Western blot (WB) and immunostaining (IS) experiments***

| **Protein Symbol** | **Antibody name (species, type)** | **Dilution** | **Source** |
|---|---|---|---|
| AKT | Anti-PKB<br>(rabbit polyclonal) | 1:400 | KAP-PK004<br>Stressgen Bioreagents |
| CDK1 | Cdc2-p34<br>(mouse monoclonal) | 1:200 | Sc-54<br>Santa Cruz biotechnology |
| ERK 1/2 | p44/42 MAP kinase<br>(rabbit polyclonal) | 1:1000 | #9102<br>Cell signaling technology |
| ERK 1/2-P | Phospho-p44/42 MAP kinase<br>(Thr202/Tyr204)<br>(rabbit polyclonal) | 1:1000 | #9101<br>Cell signaling technology |
| HSF1 | HSF1<br>(rabbit polyclonal) | 1:500 | gift from Dr R Morimoto |
| Hsp90α | Heat shock protein 86<br>(rabbit polyclonal) | 1:1000<br>1:200 (IS) | PA3-013<br>Affinity BioReagents (ABR) |
| Hsp90β | Heat shock protein 84<br>(rabbit polyclonal) | 1:1000<br>1:200 (IS) | PA3-012<br>Affinity BioReagents (ABR) |
| PLK1 | Mouse anti-Plk<br>(mouse monoclonal : PL6-2) | Mouse<br>1:500 | 33-1700<br>Zymed Laboratories<br>Invitrogen immunodetection |
| αTUBULIN | Anti-α-Tubulin<br>(mouse monoclonal : DM1A) | 1:1000 (WB) | T 9026<br>SIGMA |
| αTUBULIN<br>FITC conjugate | Anti-α-Tubulin<br>(mouse monoclonal : DM1A) | 1:100 (IS) | F 2168<br>SIGMA |

***Table S3- Timing of meiotic progression in Hsp90-depleted (Hsf1$^{-/-}$) and Hsp90-inhibited (17AAG) oocytes.***

| | Effect of Hsp90 depletion | | Effect of Hsp90 inhibition (without preincubation) | |
|---|---|---|---|---|
| | *Hsf1*$^{+/+}$ | *Hsf1*$^{-/-}$ | *Hsf1*$^{+/+}$ (DMSO) | *Hsf1*$^{+/+}$ (17AAG) |
| Oocytes (n) | 90 | 95 | 24 | 44 |
| GV – GVBD[a] (min) | 69.5±8.65 | 285±26 | 105±16 | 92.7±5.04 |
| GV – GVBD[b] (min) | 58±4.9 | 156±18.2 | ND | ND |
| GVBD – PBE1 (min) | 727±20.9 | 746±36.5 | 554±41.12 | 680±18.2 |

[a] Timing calculated from the entire group of experimental oocytes. [b] Timing calculated from the group of experimental oocytes that had completed meiosis I and extruded the 1$^{st}$ polar body (PBE1).

Figure S1

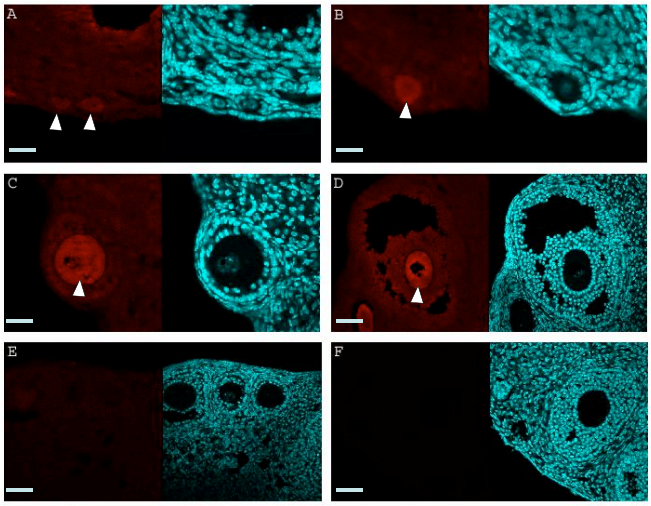

Figure S2