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Supplemental Information

X-Linked *Huwe1* Is Essential for Oocyte Maturation and Preimplantation Embryo Development

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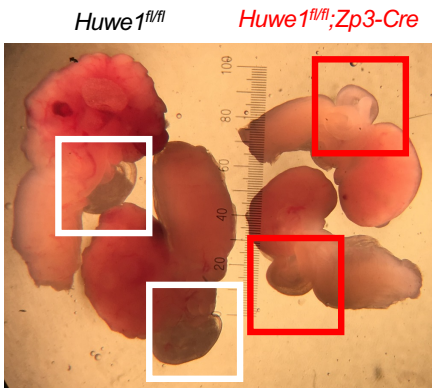
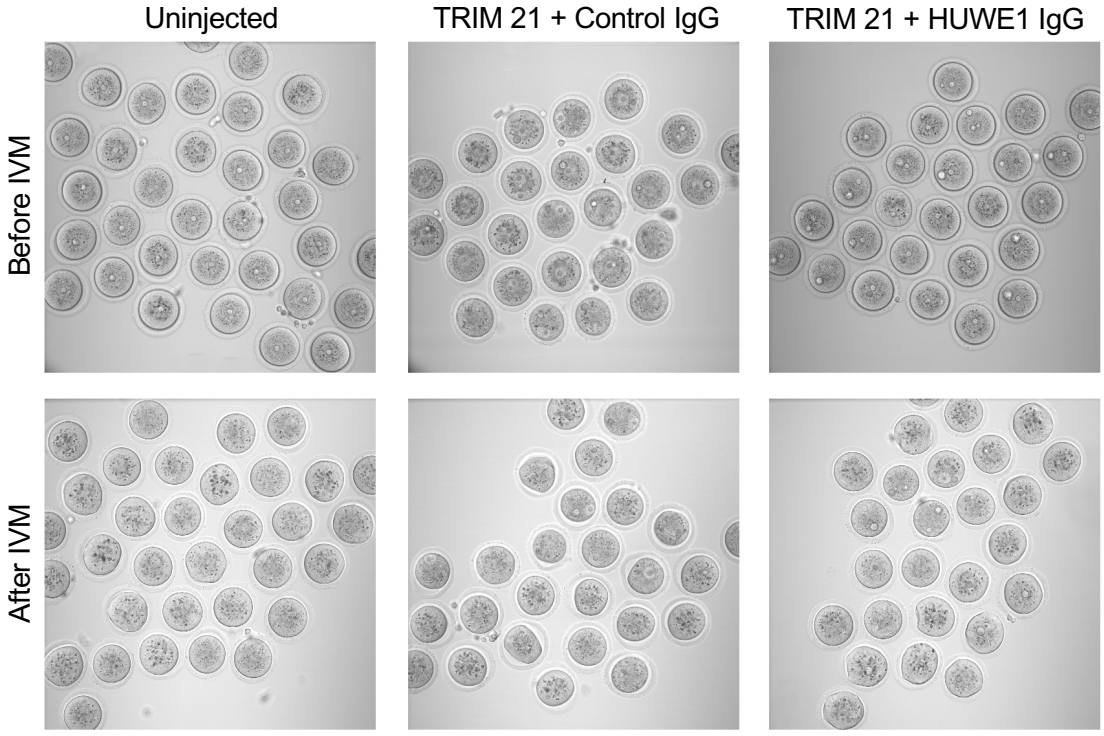
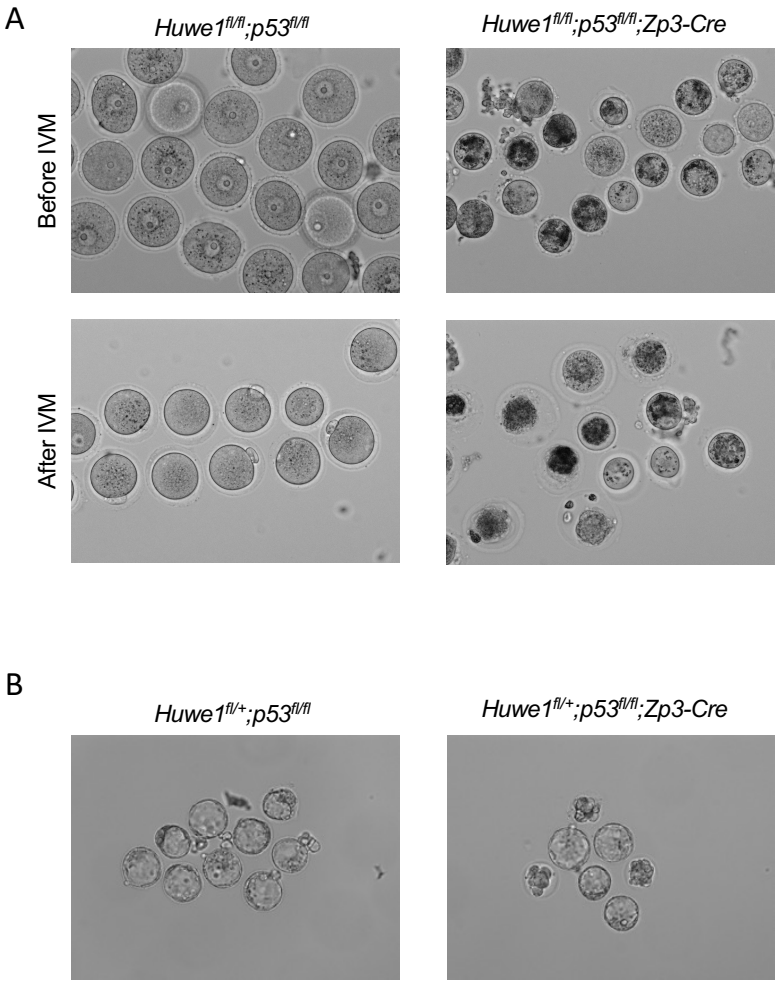
Eisa et al., Figure S1



Eisa et al., Figure S2



Eisa et al., Figure S3



Supplementary Figure Legends

Figure S1. *Huwe1* KO females fail to superovulate, Related to Figure 1. The ovary and a part of the uterus including the oviduct were isolated from the mice 14 hours following hCG administration. The ampullae are indicated by white (control) and red (*Huwe1* KO) rectangles. Note that oocyte/cumulus complexes are detected inside the ampullae of control mice.

Figure S2. Acute depletion of HUWE1 protein in GV oocytes does not affect *in vitro* maturation, Related to Figure 2. Representative images of oocytes before and after *in vitro* maturation (IVM) following the Trim-Away experiments.

Figure S3. Concomitant deletion of *p53* does not rescue the phenotypes of *Huwe1* KO oocytes/eggs, Related to Figure 4. (A) Representative images of oocytes collected from control (*Huwe1*$^{fl/fl}$*;p53*$^{fl/fl}$) and *Huwe1/p53* double KO (*Huwe1*$^{fl/fl}$*;p53*$^{fl/fl}$*;Zp3-Cre*) mice before and after *in vitro* maturation (IVM). **(B)** Representative images of preimplantation embryo 72 hours after *in vitro* fertilization (IVF).

Transparent Methods

Mice

Huwe1$^{fl/fl(y)}$ mice were described previously (Kon et al., 2012). *Zp3-Cre* (C57BL/6-Tg(Zp3-cre)93Knw/J, Stock No: 003651) and *p53*$^{fl/fl}$ (B6.129P2-Trp53^{tm1Brn}/J, Stock No: 008462) mice were obtained from the Jackson Laboratory. To obtain control (*Huwe1*$^{fl/fl}$), oocyte-specific Huwe1 heterozygous (*Huwe1*$^{fl/+}$*;Zp3-Cre*), and KO mice (*Huwe1*$^{fl/fl}$*;Zp3-Cre*) as littermates, *Huwe1*$^{fl/+}$ or *Huwe1*$^{fl/fl}$ female mice were bred with *Huwe1*$^{fl/y}$ male mice that carried one copy of the *Zp3-Cre* allele. Likewise, *Huwe1*$^{fl/fl}$*;p53*$^{fl/fl}$ female mice were bred with *Huwe1*$^{fl/y}$*;p53*$^{fl/fl}$ male mice with one copy of the *Zp3-Cre* allele to produce control (*Huwe1*$^{fl/fl}$*;p53*$^{fl/fl}$) and oocyte-specific *Huwe1/p53* double KO (*Huwe1*$^{fl/fl}$*;p53*$^{fl/fl}$*;Zp3-Cre*) mice as littermates. All the animal experiments were approved by Kent State University Institutional Animal Care and Use Committee (IACUC) in accordance with the NIH and National Research Council's publication "Guide for Care and Use of Laboratory Animals."

In Vitro *Maturation (IVM)*

IVM was performed as previously described (Eisa et al., 2019). Female mice were injected with 5 IU of equine chorionic gonadotropin (eCG; Sigma-Aldrich) to stimulate follicle growth. 44-48 hours after eCG administration, the ovaries were collected and oocytes were retrieved by puncturing antral follicles with a 26-gauge needle. Cumulus cell-enclosed oocytes were isolated into MEMα containing 0.1 mg/mL dibutyryl cAMP (dbcAMP; Sigma-Aldrich), and the cumulus cells were removed by repeated pipetting though a small-bore glass needle. After the removal of cumulus cells, oocytes were washed and subjected to overnight culture in MEMα supplemented with 26 μg/ml pyruvate and Penicillin/Streptomycin in a humidified incubator containing 5% CO_2/95% air.

In Vitro *Fertilization (IVF)*

IVF was performed as previously described (Eisa et al., 2019). Eggs at the MII stage were collected from the ampulla of 8-12-week-old female mice after superovulation by injection of 5 IU of eCG, followed 48 hours later by 5 IU of human chorionic gonadotropin (hCG; Sigma-Aldrich). Eggs were harvested 14 hours post-hCG into HTF medium. Sperm were collected from the *cauda epididymis* and *vas deferens* of 3-5-month-old male mice into HTF medium (Millipore: EmbryoMax® Human Tubal Fluid). The sperm were capacitated at 37°C in an atmosphere of 5% CO_2/95% air for 1 hour. After capacitation, 10-15 µl of the sperm suspension was added to the drop containing eggs/cumulus complexes, which was further incubated for 4 hours for IVF at 37° and 5% CO_2. After IVF, eggs were washed twice and cultured in HTF at 37°C and 5% CO_2.

Trim-Away

HUWE1 antibody (ab70161, Abcam) was concentrated in PBS and combined with RNA encoding HA-tagged TRIM21 for a final concentration of 1 mg/ml antibody and 0.3 mg/ml RNA. 0.05% NP-40 was added to reduce stickiness of the antibody. 1 mg/ml rabbit IgG (dialyzed in PBS) combined with RNA encoding TRIM21 was used as a control. 10 pl was injected into oocytes. Oocytes were injected in HEPES-buffered MEMα containing 10 µM milrinone and were transferred to bicarbonate-buffered MEMα containing milrinone for overnight incubation in a humidified incubator containing 5% CO_2/95% air. The next morning, oocytes were washed out of milrinone and GVBD was scored every hour. Oocytes were photographed before and 3 hours after the removal of milrinone and harvested for Western blot.

Western blot

Oocytes were directly collected into a tube with Laemmli SDS sample buffer. Proteins were separated by 4-15% polyacrylamide gels (BioRad) and transferred onto PVDF membranes (Millipore). After blocking in 3% BSA for 30 min at room temperature, membranes were incubated with primary antibodies overnight at 4°C with gentle agitation. Membranes were developed using

Pierce™ ECL (Thermo Scientific) and iBright CL750 Imaging System (Invitrogen). The following antibodies were used for Western blot: anti-HUWE1 (A300-486A, Bethyl) and anti-HSP90 (4877, Cell Signaling).

Supplemental References

Eisa, A.A., De, S., Detwiler, A., Gilker, E., Ignatious, A.C., Vijayaraghavan, S., Kline, D., 2019. YWHA (14-3-3) protein isoforms and their interactions with CDC25B phosphatase in mouse oogenesis and oocyte maturation. Bmc Dev Biol 19, 20. https://doi.org/10.1186/s12861-019-0200-1