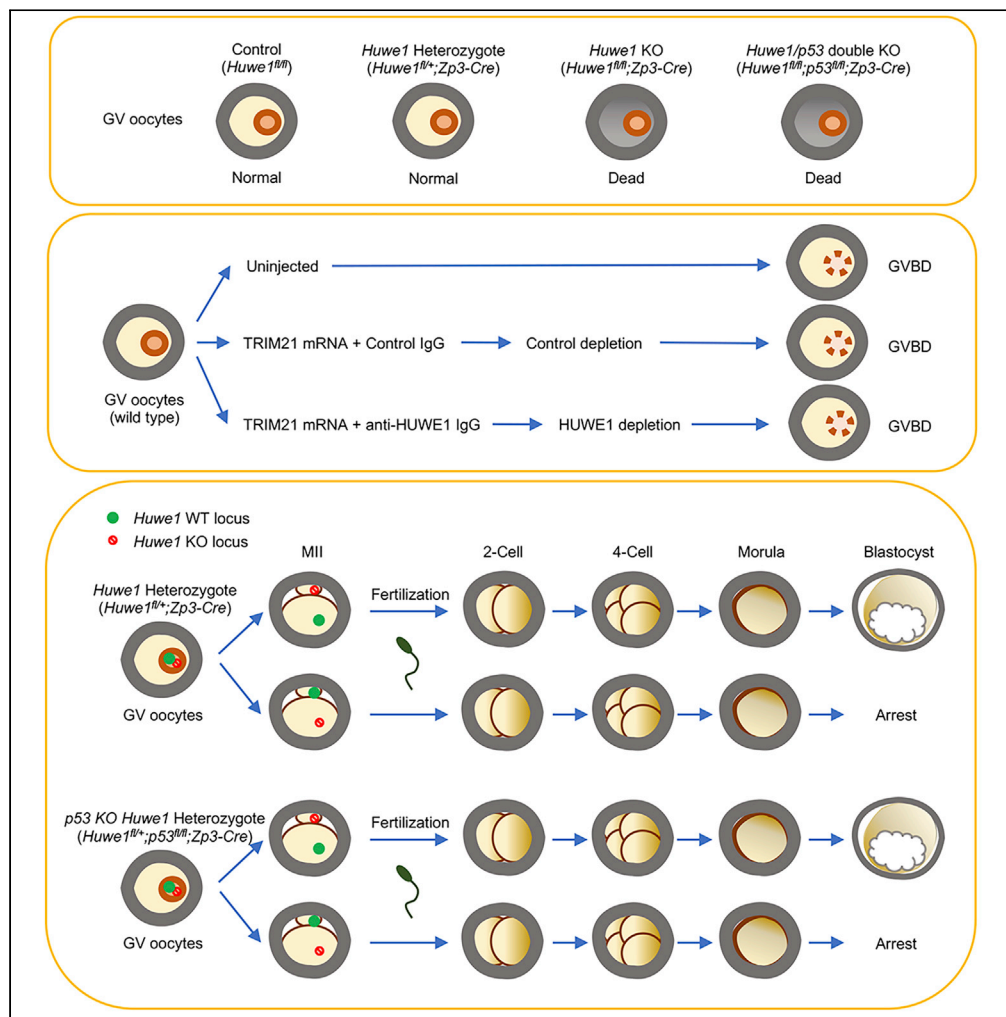# iScience

Article

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

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HIGHLIGHTS

Oocyte-specific *Huwe1* KO leads to female infertility in mice

Homozygous, but not heterozygous, *Huwe1* KO kills oocytes before the GV stage

Maternal *Huwe1* is also required for preimplantation embryonic development

Defects in oocyte and embryonic development of *Huwe1* KO mice are independent of p53

Article

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

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SUMMARY

HUWE1 is a HECT-domain ubiquitin E3 ligase expressed in various tissues. Although HUWE1 is known to promote degradation of the tumor suppressor p53, given a growing list of its substrates, *in vivo* functions of HUWE1 remain elusive. Here, we investigated the role of HUWE1 in the female reproductive system. Homozygous deletion of *Huwe1* in mouse oocytes of primary follicles caused oocyte death and female infertility, whereas acute depletion of HUWE1 protein by Trim-Away technology did not impact oocytes from antral follicles. Interestingly, oocytes from *Huwe1* heterozygous females matured and fertilized normally, but the majority of embryos that lacked maternal *Huwe1* were arrested at the morula stage after fertilization. Consequently, *Huwe1* heterozygous females only produced wild-type pups. Concomitant knockout of *p53* did not recover fertility of the *Huwe1* knockout females. These findings make HUWE1 a unique and critical maternal factor indispensable for maintaining the quality of oocytes and embryos.

INTRODUCTION

The development of mammalian oocytes is a tightly controlled cellular process. In adult mice and humans, the majority of oocytes are arrested at the diplotene stage of the first meiotic prophase, commonly referred to as the germinal vesicle (GV) stage. During an estrous cycle, a group of follicles is recruited to grow to their maximum size, while the follicle continues to increase the number of granulosa cells surrounding an oocyte. In response to the luteinizing hormone surge, an oocyte in a preovulatory follicle resumes meiosis. As females are born with a finite number of oocytes, and also because oocytes are single cells, even a single genetic mutation can lead to detrimental outcomes, such as oocyte death, abnormal fertilization, or embryonic lethality, which leads to infertility in females.

HUWE1 (also known as, ARF-BP1, MULE, and HectH9) is a HECT-domain ubiquitin E3 ligase widely expressed in normal tissues, and its gene, *Huwe1*, is located on the X chromosome in both mice and humans. HUWE1 was initially identified as an E3 ligase that targets the tumor suppressor p53 for degradation (Chen et al., 2005). Consistent with this finding, *Huwe1* RNAi knockdown or gene knockout (KO) results in p53 accumulation and the induction of apoptosis in certain tissues/cells (Chen et al., 2005; Hao et al., 2012; Kon et al., 2012; Wang et al., 2014). However, targeted deletion of *Huwe1* in other tissues, including keratinocytes, male germ cells, and hematopoietic progenitor cells, does not lead to noticeable p53 activation (Chen et al., 2016; Fok et al., 2017; Inoue et al., 2013; King et al., 2016), suggesting that the role of HUWE1 is complex and may be context dependent.

In this study, we investigated the role of HUWE1 in the development of oocytes and preimplantation embryos by using oocyte-specific *Huwe1* and *p53* KO mouse models. We also employed Trim-Away technology to promote acute degradation of HUWE1 protein in GV-stage oocytes. Our results uncover critical, p53-independent roles of HUWE1 in the female reproductive system.

RESULTS

Oocyte-Specific *Huwe1* KO Mice Are Completely Infertile

To investigate the role of HUWE1 in the female reproductive system, we employed a *Cre-loxP* strategy to achieve oocyte-specific disruption of the *Huwe1* gene from primary follicle stage. We generated oocyte-specific *Huwe1*

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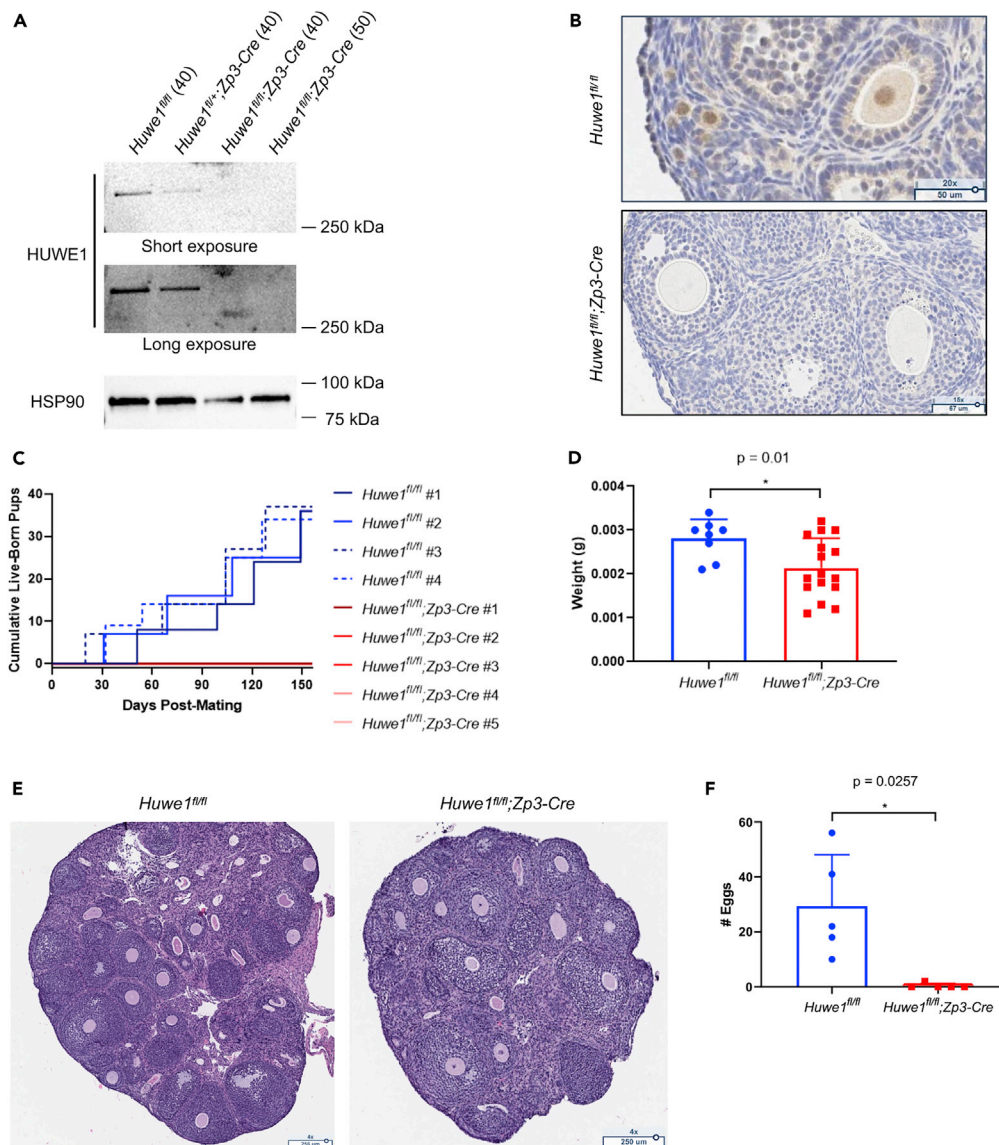
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Figure 1. *Huwe1* KO Females are Completely Infertile

(A) Western blot performed with the indicated antibodies for whole GV-stage oocytes collected from control (*Huwe1$^{fl/fl}$*), *Huwe1* heterozygous (*Huwe1$^{fl/+}$;Zp3-Cre*), and *Huwe1* KO (*Huwe1$^{fl/fl}$;Zp3-Cre*) mice. The numbers of oocytes loaded in each lane were indicated in the parentheses.

(B) Representative images of ovaries from 3-week-old control (*Huwe1$^{fl/fl}$*) and *Huwe1* KO (*Huwe1$^{fl/fl}$;Zp3-Cre*) mice stained with the indicated antibodies.

(C) The cumulative number of live-born pups when 4 control (*Huwe1$^{fl/fl}$* #1–4) and 5 *Huwe1* KO (*Huwe1$^{fl/fl}$;Zp3-Cre* #1–5) females were bred with wild-type males, respectively.

(D) The average weight of single ovaries collected from 3-week-old control (*Huwe1$^{fl/fl}$*) and *Huwe1* KO (*Huwe1$^{fl/fl}$;Zp3-Cre*) mice.

(E) Hematoxylin and eosin stain of ovaries collected from control (*Huwe1$^{fl/fl}$*) and *Huwe1* KO (*Huwe1$^{fl/fl}$;Zp3-Cre*) mice.

(F) The average number of eggs collected from control (*Huwe1$^{fl/fl}$*) and *Huwe1* KO (*Huwe1$^{fl/fl}$;Zp3-Cre*) mice after superovulation. In (D) and (F), statistical significance was assessed using Student's t test. The error bars represent the standard deviation.

KO mice by crossing *Huwe1* flox (*Huwe1$^{fl/fl(y)}$*) mice, in which *Huwe1* exon 11 is flanked by *loxP* sites (Kon et al., 2012), to oocyte-specific Cre-expressing mice (*Zp3-Cre*). Western blot showed complete depletion of HUWE1 protein in oocytes collected from *Huwe1$^{fl/fl}$;Zp3-Cre* mice (Figure 1A). Immunohistochemistry also confirmed the absence of HUWE1 protein in oocytes of KO mice, whereas HUWE1 protein predominantly localized in the

nucleus of oocytes in control mice (Figure 1B). When control (*Huwe1^fl/fl^*) and *Huwe1* KO (*Huwe1^fl/fl^;Zp3-Cre*) females were bred with wild-type males, there were no pups obtained from KO females (Figure 1C). Ovaries from *Huwe1* KO mice were significantly lighter (Figure 1D) compared with those from control mice. Histological analysis of ovaries showed that ovaries from *Huwe1* KO mice contained fewer numbers of follicles at 4 weeks of age (Figure 1E). When superovulation was induced with Equine Chorionic Gonadotropin (eCG) and Human Chorionic Gonadotropin (hCG) injections, KO mice at 8 weeks of age ovulated nearly no eggs, whereas control mice ovulated an average of 29.4 eggs per female (Figures 1F and S1).

Huwe1 KO Oocytes Fail to Mature

The failure in superovulation prompted us to analyze oocytes in *Huwe1* KO mice. GV-stage oocytes were retrieved from ovaries from control (*Huwe1^fl/fl^*) and *Huwe1* KO (*Huwe1^fl/fl^;Zp3-Cre*) mice following eCG administration. The total number of oocytes retrieved from ovaries was not statistically different between *Huwe1* KO mice and littermate control mice (Figure 2A). However, many of the oocytes collected from *Huwe1* KO mice showed morphological abnormalities, such as the lack of a clear GV and the presence of dark spots in the cytoplasm (Figures 2B and 2C). Accordingly, upon *in vitro* maturation (IVM), the vast majority of *Huwe1* KO oocytes did not complete GV breakdown (GVBD) and none of the KO oocytes extruded the first polar body (Figure 2D).

Interestingly, compared with control mice, *Huwe1* heterozygous mice (*Huwe1^fl/+^;Zp3-Cre*) also produced significantly fewer GV oocytes (Figure 2A). However, unlike *Huwe1* KO oocytes, oocytes collected from *Huwe1* heterozygous mice were morphologically indistinguishable from control oocytes (Figure 2B) and successfully matured *in vitro* (Figures 2C and 2D), indicating that one copy of the *Huwe1* gene is sufficient to support oocyte maturation. It should be noted that compared with control oocytes, approximately 50% less Huwe1 protein was detected in *Huwe1* heterozygous oocytes (Figure 1A).

As our results showed that *Huwe1* KO oocytes were already dead at or before the GV stage, we next investigated the impact of acute deletion of HUWE1 protein in GV-stage oocytes. Toward this end, we used the "Trim-Away" technology to force degradation of HUWE1 protein (Clift et al., 2018, 2017). In brief, wild-type mouse oocytes were co-injected with mRNA encoding TRIM21 and control IgG or an anti-HUWE1 antibody (Figure 2E). Western blot analysis confirmed that a substantial reduction of HUWE1 protein was induced by the anti-HUWE1 antibody (Figure 2F). Interestingly, the depletion of HUWE1 protein did not affect the morphology of the oocytes (Figure S2). Moreover, oocytes underwent GVBD normally despite HUWE1 depletion (Figures 2G and S2). Taken together, these results suggest that HUWE1 is required before GV formation.

Maternal HUWE1 Is Also Required for Early Embryogenesis

The aforementioned studies showed that one copy of the *Huwe1* gene is sufficient to support full oocyte development (Figure 3A). Therefore, *Huwe1* heterozygous mice (*Huwe1^fl/+^;Zp3-Cre*) were fertile when bred with a wild-type male. However, all the offspring derived from the heterozygous mice carried the *Huwe1* wild-type allele, but not the *Huwe1* flox allele (Figure 3B). Moreover, the number of offspring from *Huwe1* heterozygous mice (3.9 per female) was approximately half of that from control (*Huwe1^+/+^;Zp3-Cre*) female mice (7.4 per female) (Figure 3C). These results suggest that in addition to oocyte death, the lack of maternal *Huwe1* would result in embryonic lethality. Thus, we next performed *in vitro* fertilization (IVF) to investigate how the lack of *Huwe1* impacts fertilization and early embryonic development. *Huwe1* heterozygous mice (*Huwe1^fl/+^;Zp3-Cre*) responded normally to the superovulation treatment, although the average number of ovulated eggs was slightly smaller than that of control mice (*Huwe1^fl/+^*) (Figure 3D). Likewise, eggs from *Huwe1* heterozygous mice fertilized and developed to the 4-cell stage at a similar rate to eggs from control mice (Figures 3E and 3F). Interestingly, whereas 82.7% fertilized control eggs developed to blastocysts by 72 h post-IVF, only 41.7% eggs from *Huwe1* heterozygous mice reached the blastocyst stage (Figures 3F and 3G). At 72 h post-IVF, 43.5% eggs from *Huwe1* heterozygous mice were still at the morula stage, whereas only 17.3% control eggs remained at this stage (Figures 3F and 3G). It is expected that 50% eggs derived from *Huwe1* heterozygous mice carry the *Huwe1* KO locus (Figure 3A). Importantly, it was confirmed by PCR that the majority of the morulae (23/30) carried the *Huwe1* KO locus. In contrast, 6 of 20 blastocysts were positive for *Huwe1* deletion. These results indicated that the lack of maternal *Huwe1* impairs the development of preimplantation embryos.

Roles of *Huwe1* in Oogenesis and Embryonic Development Are p53 Independent

Among the known key substrates of HUWE1 is p53, which regulates cell death. Oocyte-specific deletion of MDM2, another major E3 ligase for p53, results in female infertility due to oocyte death caused by
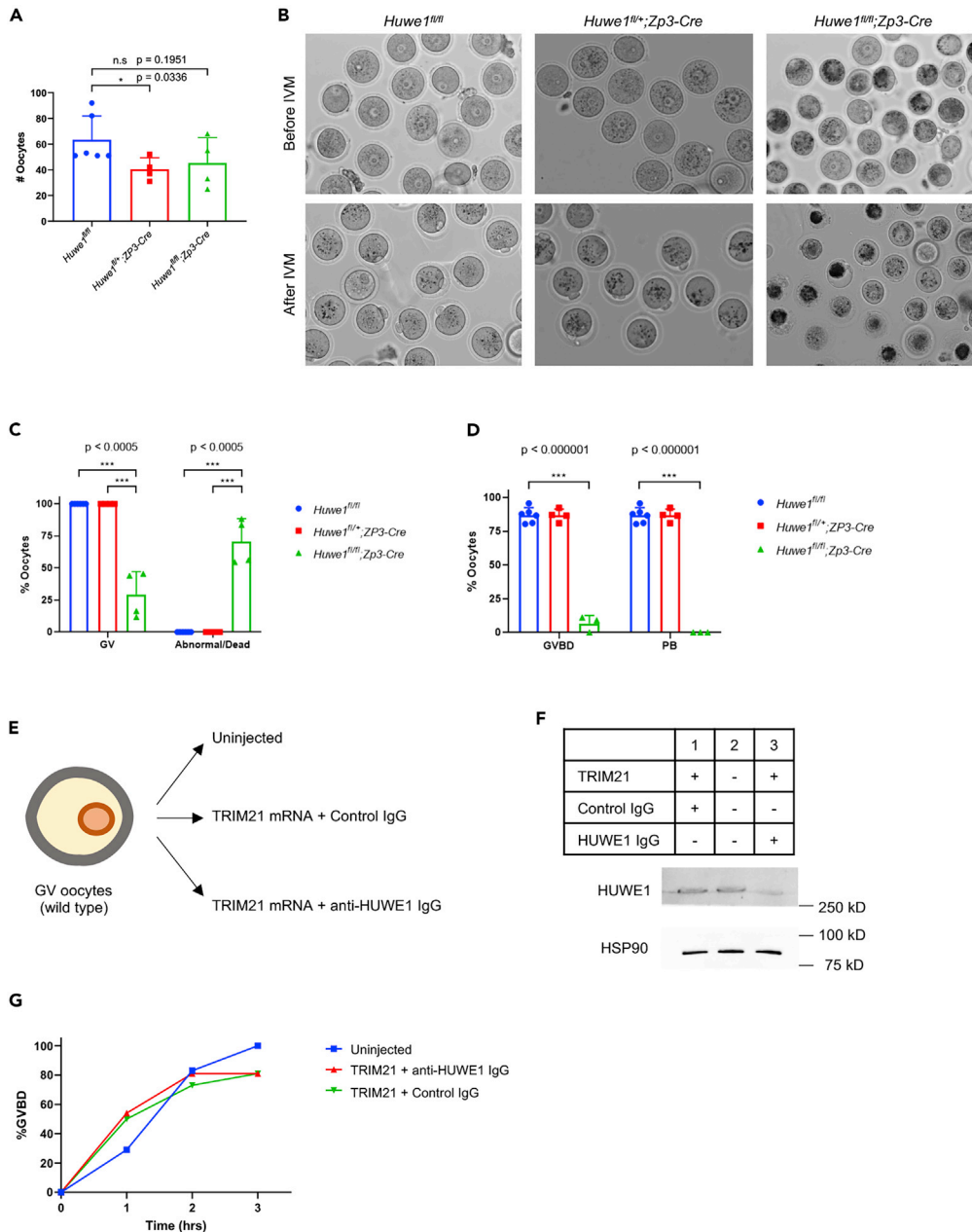
Figure 2. *Huwe1* KO Oocytes Fail IVM

(A) The number of oocytes collected from antral follicles of control (*Huwe1^fl/fl^*), *Huwe1* heterozygous (*Huwe1^fl/+^;Zp3-Cre*), and *Huwe1* KO (*Huwe1^fl/fl^;Zp3-Cre*) mice.

(B) Representative images of oocytes collected from control (*Huwe1^fl/fl^*), *Huwe1* heterozygous (*Huwe1^fl/+^;Zp3-Cre*), and *Huwe1* KO (*Huwe1^fl/fl^;Zp3-Cre*) mice before and after *in vitro* maturation (IVM).

(C) The percentage of morphologically normal GV-stage oocytes and abnormal oocytes collected from antral follicles of control (*Huwe1^fl/fl^*), *Huwe1* heterozygous (*Huwe1^fl/+^;Zp3-Cre*), and *Huwe1* KO (*Huwe1^fl/fl^;Zp3-Cre*) mice.

(D) The percentage of GV-stage oocytes that underwent GVBD or extruded the first polar body (PB) after *in vitro* maturation. In (A), (C), and (D), statistical significance was assessed using Student's t test. The error bars represent the standard deviation.

(E) Scheme of the Trim-Away method.

(F) Western blot performed with the indicated antibodies for whole GV-stage oocytes after Trim-Away.

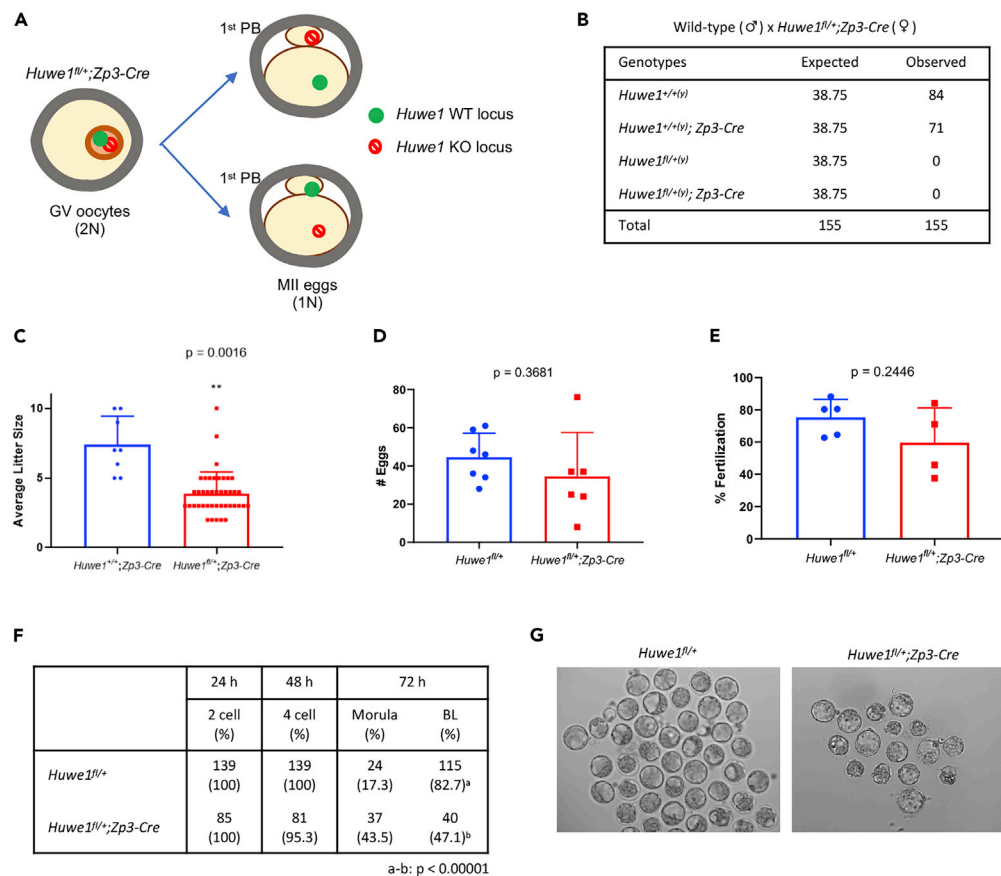(G) The percentage of oocytes undergoing GVBD over time after Trim-Away.

Figure 3. The Lack of Maternal *Huwe1* Leads to Impaired Blastocyst Formation *In Vitro*

(A) Schematic of the transition from the GV stage to the second stage of meiosis (MII) in oocytes carrying a *Huwe1* KO locus.

(B) Analysis of offspring from the cross between *Huwe1* heterozygous (*Huwe1$^{fl/+}$;Zp3-Cre*) females and wild-type males.

(C) The average litter size of control (*Huwe1$^{+/+}$;Zp3-Cre*) and *Huwe1* heterozygous (*Huwe1$^{fl/+}$;Zp3-Cre*) mice when crossed with wild-type males.

(D) The number of MII eggs collected from control (*Huwe1$^{fl/+}$*) and *Huwe1* heterozygous (*Huwe1$^{fl/+}$;Zp3-Cre*) mice after superovulation.

(E) The *in vitro* fertilization rates of eggs from control (*Huwe1$^{fl/+}$*) and *Huwe1* heterozygous (*Huwe1$^{fl/+}$;Zp3-Cre*) mice with wild-type mouse sperm. In (C) and (D), statistical significance was assessed using Student's t test. The error bars represent the standard deviation.

(F) Analysis of preimplantation embryo development after IVF. Statistical significance in the blastocyst (BL) formation was assessed using chi-square test (a-b: $p < 0.00001$).

(G) Representative images of preimplantation embryo 72 h after IVF.

abundant p53 (Livera et al., 2016). Hence, we examined whether co-deletion of *p53* could rescue the infertility in *Huwe1*-deficient female mice. Interestingly, oocyte-specific *Huwe1/p53* double KO female mice (*Huwe1$^{fl/fl}$;p53$^{fl/fl}$;Zp3-Cre*) were still completely infertile (Figure 4A) and did not release any eggs upon superovulation (Figure 4B). When the number of total GV-stage oocytes in ovaries was analyzed after eCG administration, there were no significant differences between control and double KO mice (Figure 4C). However, similar to what was observed in *Huwe1* single KO mice (Figure 2B), more than 70% oocytes from double KO mice were found to be abnormal or dead (Figures 4D and S3A). Upon IVM, very few double KO oocytes underwent GVBD and none of them extruded the first polar body (Figures 4E and S3A).

We next examined how the concomitant deletion of *p53* affected impaired blastocyst formation of eggs from *Huwe1* heterozygous mice. Interestingly, deletion of *p53* did not impact the survival of embryos that lacked maternal *Huwe1*, as *Huwe1* heterozygous *p53* KO females (*Huwe1$^{fl/+}$;p53$^{fl/fl}$;Zp3-Cre*) only produced pups with wild-type *Huwe1* allele(s) (Figure 4F). As seen in *Huwe1* heterozygous females (Figure 3B),
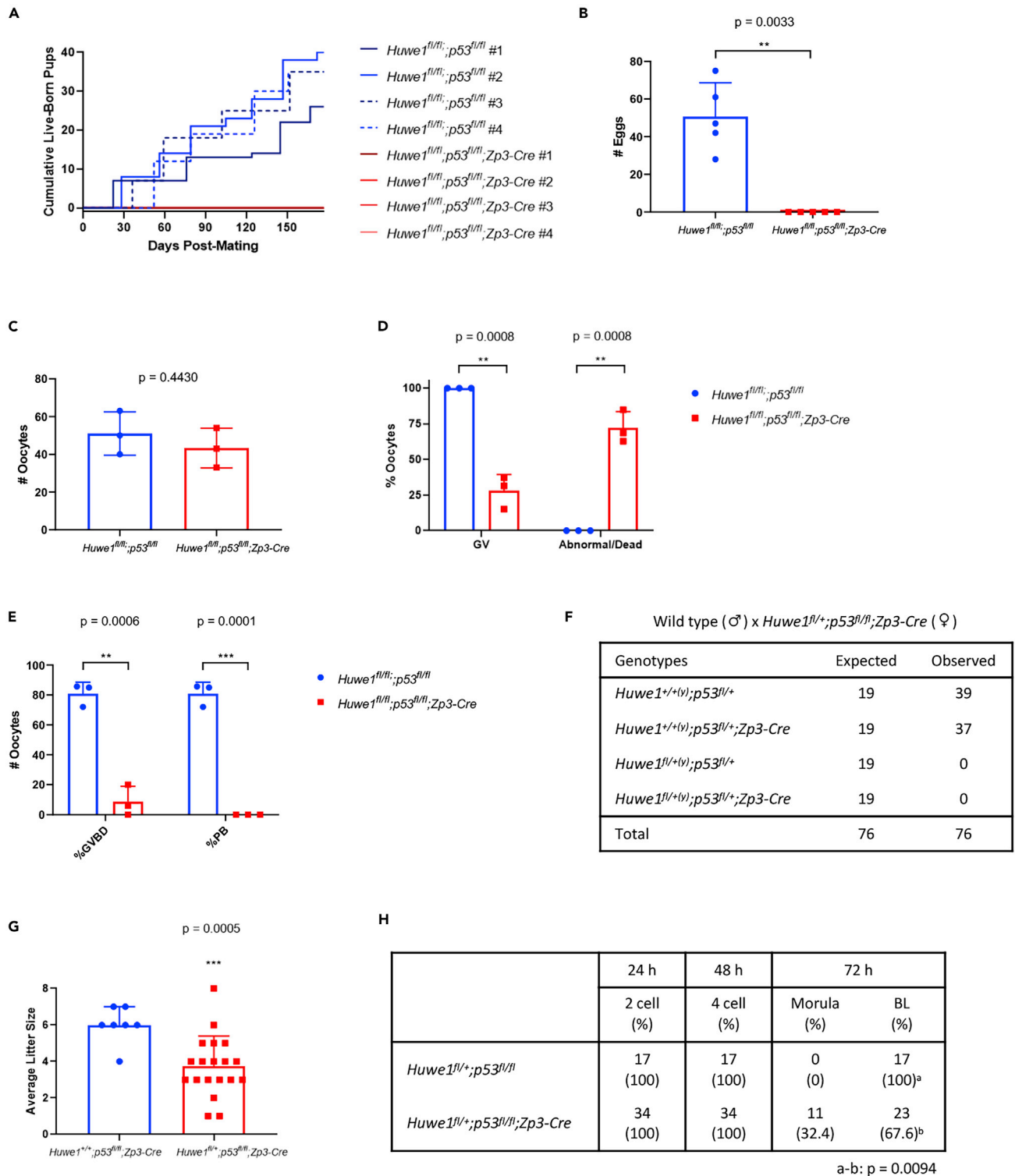
Figure 4. Concomitant Deletion of *p53* Does Not Recover the Fertility of Huwe1 KO Females

(A) The cumulative number of live-born pups when 4 control (*Huwe1$^{fl/fl}$;p53$^{fl/fl}$*, #1–4) and 4 *Huwe1/p53* double KO (*Huwe1$^{fl/fl}$;p53$^{fl/fl}$;Zp3-Cre*, #1–4) females were bred with wild-type males, respectively.

(B) The average number of oocytes released from control (*Huwe1$^{fl/fl}$;p53$^{fl/fl}$*) and *Huwe1* KO (*Huwe1$^{fl/fl}$;p53$^{fl/fl}$;Zp3-Cre*) mice following superovulation.

(C) The number of oocytes collected from antral follicles of control (*Huwe1$^{fl/fl}$;p53$^{fl/fl}$*) and *Huwe1* KO (*Huwe1$^{fl/fl}$;p53$^{fl/fl}$;Zp3-Cre*) mice.

Figure 4. Continued

(D) The percentage of morphologically normal GV-stage oocytes and abnormal oocytes collected from antral follicles of control (*Huwe1*$^{fl/fl}$;*p53*$^{fl/fl}$) and *Huwe1* KO (*Huwe1*$^{fl/fl}$;*p53*$^{fl/fl}$;*Zp3-Cre*) mice.

(E) The percentage of GV-stage oocytes that underwent GVBD or extruded the first polar body (PB) after *in vitro* maturation.

(F) Analysis of offspring from the cross between *p53* KO *Huwe1* heterozygous (*Huwe1*$^{fl/+}$;*p53*$^{fl/fl}$;*Zp3-Cre*) females and wild-type males.

(G) The average litter size of *p53* KO (*Huwe1*$^{+/+}$;*p53*$^{fl/fl}$;*Zp3-Cre*) females and *p53* KO *Huwe1* heterozygous (*Huwe1*$^{fl/+}$;*p53*$^{fl/fl}$;*Zp3-Cre*) females when crossed with wild-type males. In (B), (C), (D), (E), and (G), statistical significance was assessed using Student's t test. The error bars represent the standard deviation.

(H) Analysis of preimplantation embryo development after IVF. Statistical significance in the blastocyst (BL) formation was assessed using Fisher's exact test (a-b: $p = 0.0094$).

even after concomitant deletion of p53, the average litter size of *Huwe1* heterozygous females (3.7 per female) was still significantly smaller than that of control mice (6.0 per female) (Figure 4G). IVF experiments showed that eggs from *Huwe1* heterozygous *p53* KO females (*Huwe1*$^{fl/+}$;*p53*$^{fl/fl}$;*Zp3-Cre*) developed to blastocysts at a significantly lower rate than eggs from control females (*Huwe1*$^{fl/+}$;*p53*$^{fl/fl}$) (Figures 4H and S3B). These results indicate that the functions of HUWE1 in oogenesis and embryonic development are independent of p53.

DISCUSSION

In the present study, we demonstrated that *Huwe1* KO leads to complete infertility due to death of GV-stage oocytes. We also showed that although one copy of *Huwe1* is sufficient for oocytes to undergo maturation, ovulation, and fertilization, the lack of maternal *Huwe1* still hampers the subsequent development and results in early embryonic lethality. Together, our results indicate that HUWE1 is a critical maternal factor that plays important roles in both oocyte maturation and preimplantation embryo development.

We showed that the deletion of the *Huwe1* gene at the primary follicle stage, when Cre recombinase is expressed under the *Zp3* promoter (Lan et al., 2004), killed the vast majority of oocytes before they reached the antral follicle stage (Figures 2B–2D). Interestingly, one copy of *Huwe1* is sufficient to protect oocytes during this process (Figures 2B–2D). Moreover, the acute depletion of HUWE1 protein in the GV-stage oocytes isolated from antral follicles did not affect GVBD (Figures 2G and S2). These results suggest that either HUWE1 is indispensable only for the early oocyte maturation process or the loss of *Huwe1* in the primary follicle stage leads to the accumulation of HUWE1 substrates over time, which causes oocyte death at a later stage of folliculogenesis. Interestingly, it is reported that HUWE1 is required for the transition from gonocytes to spermatogonia, as well as for the maintenance of spermatogonia (Bose et al., 2017; Fok et al., 2017). Thus, these results indicate that the requirement of HUWE1 is shared in both spermatogenesis and oogenesis.

Previously, it was shown that small interfering RNA (siRNA)-mediated *Huwe1* knockdown in fertilized mouse eggs caused apoptosis and resulted in poor embryonic development *in vitro* (Chen et al., 2016). It is interesting that siRNA-mediated HUWE1 depletion only affected the late stage (i.e., blastocyst formation) of preimplantation embryos *in vitro* (Chen et al., 2016). Our results support this previous observation as the loss of one copy of maternal *Huwe1* mainly impacted the transition from morulae to blastocysts (Figure 3F). Although it is tempting to speculate that the majority of embryos that lack the maternal Huwe1 die before implantation, this needs to be tested by directly analyzing early post-implantation embryos in the uterus. It is interesting to note that another earlier study demonstrated that when *Huwe1*$^{fl/+}$ female mice were crossed with *Rosa26-Cre* homozygote males, *Huwe1* KO embryos (*Huwe1*$^{fl/y}$;*Rosa26-Cre*) survived until E14.5 when they died due to hemorrhage (Kon et al., 2012). This may be ascribed to a different Cre system used in the study. The *Zp3-Cre* system deletes *Huwe1* specifically in oocytes of primary follicles, whereas sperm-delivered Cre recombinase may not be fully expressed until the 2-cell stage or later, which would result in genetic mosaics of *Huwe1* in the embryos. Regardless, our finding is consistent with the results from these earlier studies, highlighting the critical role of HUWE1 during embryogenesis.

p53 is a transcription factor that can induce apoptosis and cell-cycle arrest in response to cytotoxic or genotoxic stimuli. The ubiquitin E3 ligase MDM2 is the major inhibitor of p53 (Wade et al., 2010). *Mdm2* KO induces embryonic lethality in mice due to fatal p53 activation (Jones et al., 1995; Montes de Oca Luna et al., 1995). Moreover, oocyte-specific *Mdm2* KO mice are infertile because of oocyte death in the early phase of folliculogenesis (Livera et al., 2016). Importantly, the infertility can be rescued by co-KO of *p53*, indicating that death in *Mdm2* KO oocytes is ascribed to lethal p53 activation (Livera et al., 2016). Similar to MDM2, HUWE1 is also a p53-targeting E3 ligase (Chen et al., 2005). However, in the present study, we determined that co-depletion of *p53* does not recover the fertility of *Huwe1* KO female mice, demonstrating that defects in oocyte maturation and embryonic

development of the KO female mice are independent of p53. The role of HUWE1 in p53 regulation appears to be complex and may change during development or depending on stress signals or cell types. It is likely that the infertile phenotype of *Huwe1* KO female mice is secondary to the upregulation of a HUWE1 substrate other than p53. Indeed, HUWE1 ubiquitinates a wide range of cellular substrates, including MYC and MCL1 (Cassidy et al., 2020; Chen et al., 2005; Herold et al., 2008; Zhao et al., 2008; Zhong et al., 2005). It is suggested that the "net effect" of *Huwe1* depletion is determined by the availability of its substrates as well as the function of each substrate. In this regard, the major substrate of HUWE1 that causes defects in oocyte maturation and embryogenesis remains to be determined.

Limitations of the Study

This study demonstrated that the ubiquitin E3 ligase HUWE1 is essential for oocyte maturation and preimplantation embryo development. The specific substrate(s) of HUWE1 that regulates these cellular processes remains to be identified.

Resource Availability

Lead Contact

Further information and requests should be directed to and will be fulfilled by the Lead Contact, Manabu Kurokawa (mkurokaw@kent.edu).

Materials Availability

New unique reagents were not generated in this study.

Data and Code Availability

The data in this study are available from the corresponding author upon request.

METHODS

All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101523.

AUTHOR CONTRIBUTIONS

Conception and Design, M.K.; Development of Methodology, A.A.E., S.B., N.K., W.G., and L.M.M.; Acquisition of Data, A.A.E., S.B., K.J.C., E.M.M., W.W.F., W.W., and L.M.M.; Analysis and Interpretation of Data, A.A.E., S.B., K.J.C., E.M.M., W.W.F., W.W., L.M.M., and M.K.; Writing of the Manuscript; A.A.E., S.B., K.J.C., and M.K.; Administrative, Technical, or Material Support, S.D., W.W., N.K., W.G., L.M.M., and S.V.; Study Supervision: M.K.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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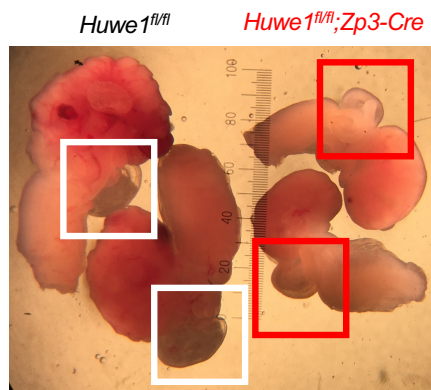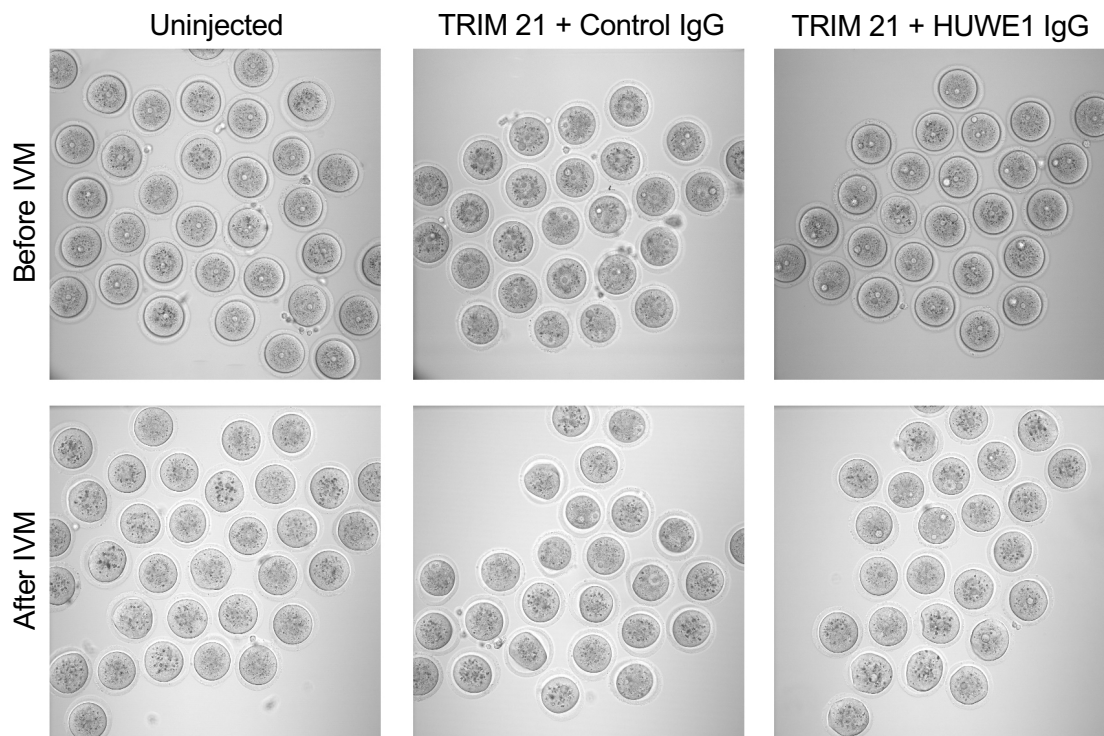
Supplemental Information

X-Linked *Huwe1* Is Essential for Oocyte

Maturation and Preimplantation
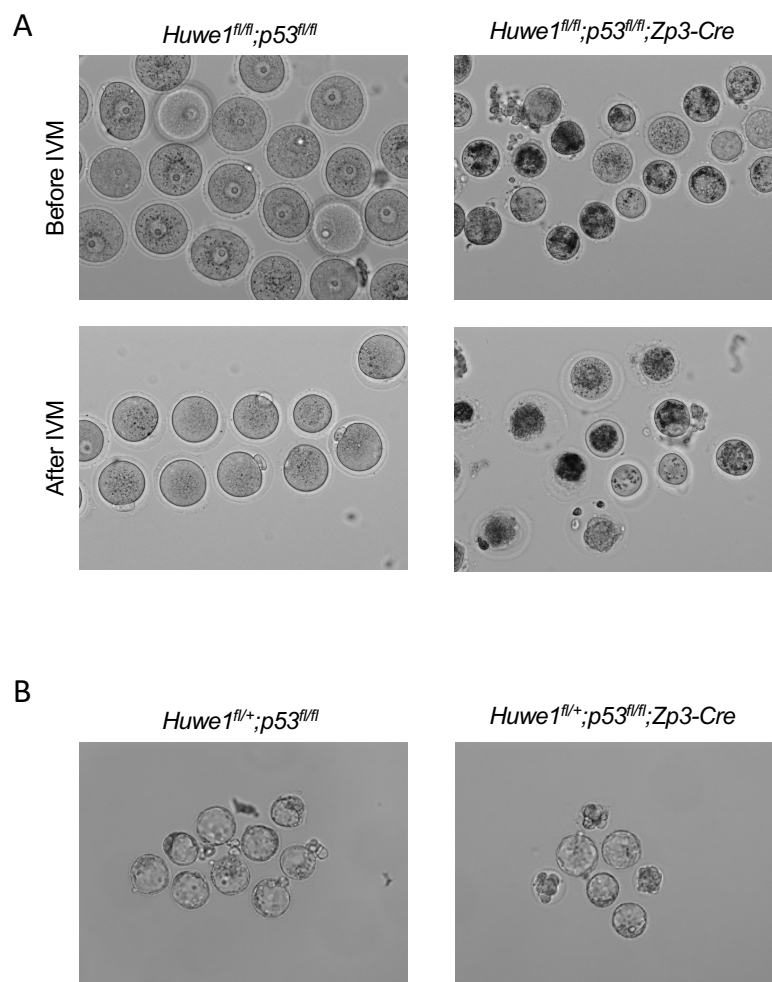
Embryo Development

Alaa A. Eisa, Scott Bang, Katherine J. Crawford, Emily M. Murphy, William W. Feng, Souvik Dey, Wendy Wells, Ning Kon, Wei Gu, Lisa M. Mehlmann, Srinivasan Vijayaraghavan, and Manabu Kurokawa

Eisa et al., Figure S1

Huwe1fl/fl   *Huwe1fl/fl;Zp3-Cre*

	Uninjected	TRIM 21 + Control IgG	TRIM 21 + HUWE1 IgG

Before IVM

After IVM

A

$Huwe1^{fl/fl};p53^{fl/fl}$ $Huwe1^{fl/fl};p53^{fl/fl};Zp3\text{-}Cre$

Before IVM

After IVM

B

$Huwe1^{fl/+};p53^{fl/fl}$ $Huwe1^{fl/+};p53^{fl/fl};Zp3\text{-}Cre$

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^{TM} 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

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