

**Appendix for: “The E3 ubiquitin ligase adaptor KLHL8 targets ZAR1
to regulate maternal mRNA degradation in oocytes”**

Appendix Figure S1.....2

Appendix Figure S2.....3

Appendix Figure S3.....4

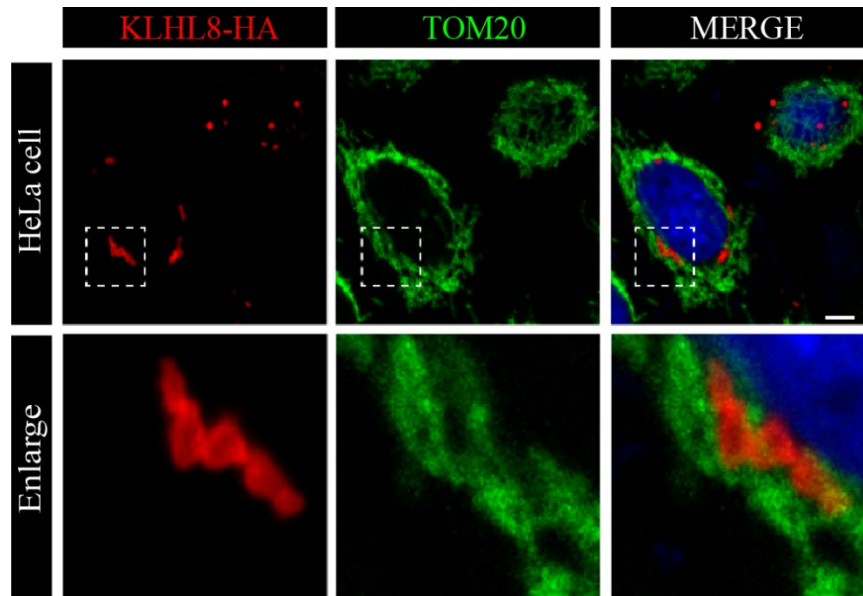
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Appendix Figure S6.....7

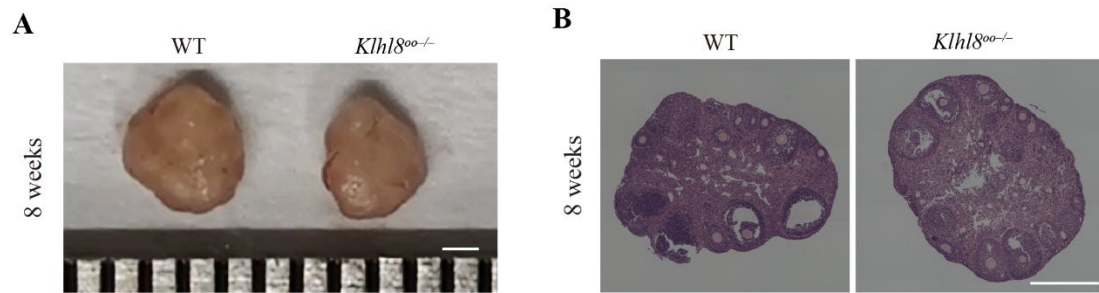
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Appendix Table S19



Appendix Figure S1. The localization of KLHL8 in HeLa cells.

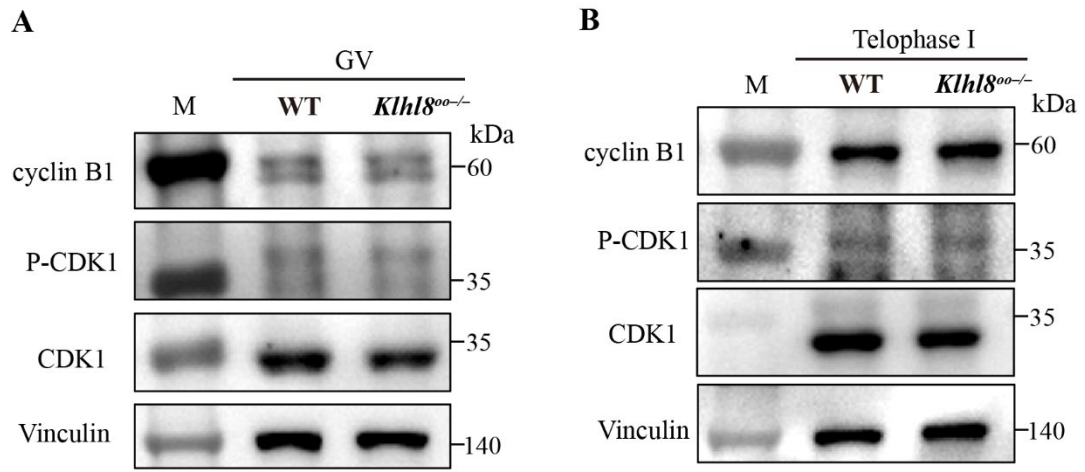
Representative immunofluorescence images of KLHL8 and TOM20 in HeLa cells. Red, KLHL8-HA; Green, TOM20. Scale bar, 5 μ m.



Appendix Figure S2. The ovary size and histology of WT and *Khlh8*^{oo-/-} mice.

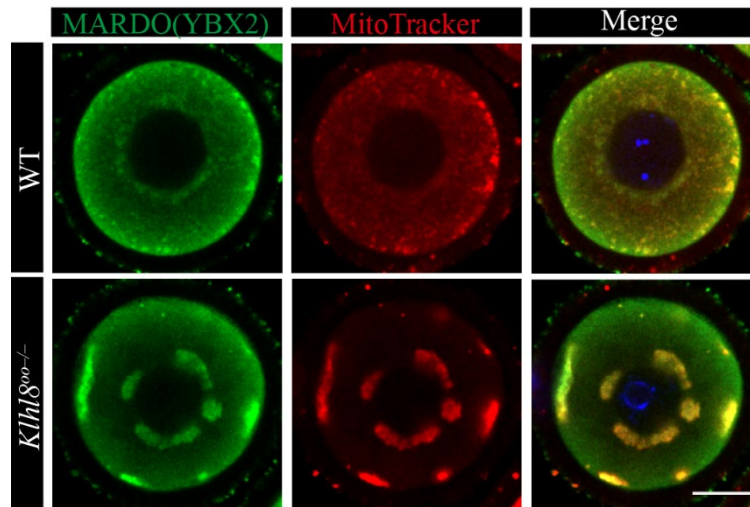
(A) Morphology of ovaries from WT and *Khlh8*^{oo-/-} mice at 8 weeks. Scale bar = 0.5 mm.

(B) Hematoxylin staining of ovary sections from WT and *Khlh8*^{oo-/-} mice at 8 weeks. Scale bar, 1 mm.



Appendix Figure S3. Activity of MPF in WT and *Klh18^{oo/-}* oocytes.

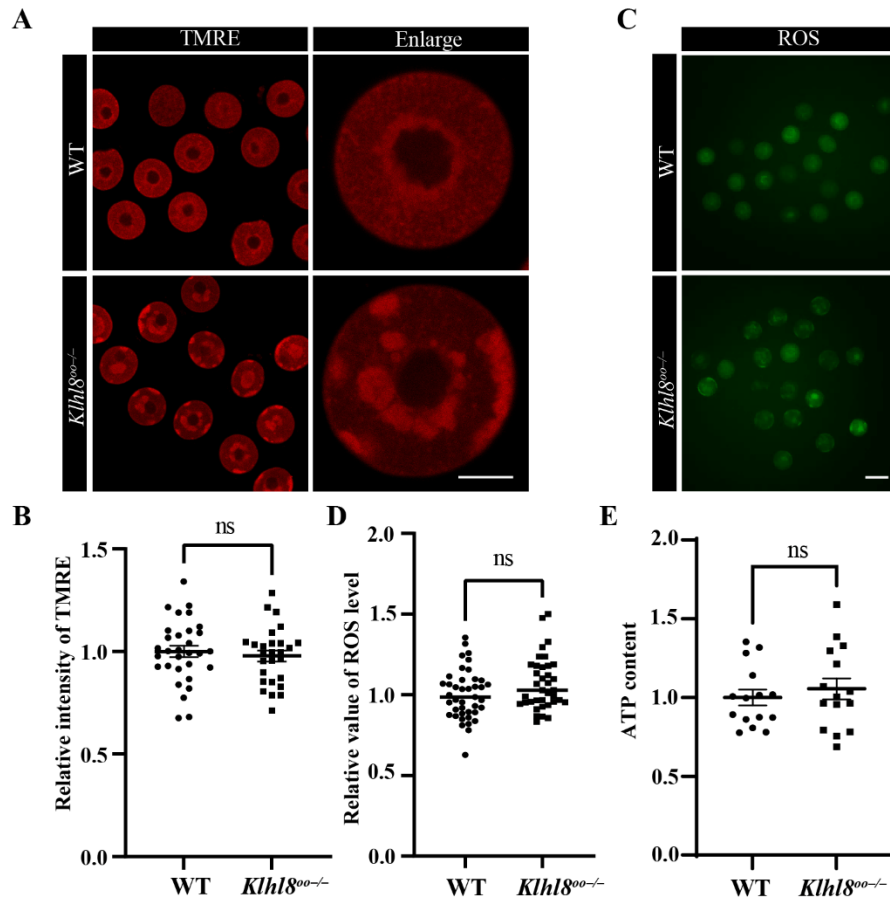
(A) Activity of MPF in WT and *Klh18^{oo/-}* oocytes at GV stage. **(B)** Activity of MPF in WT and *Klh18^{oo/-}* oocytes at Telophase I (the final stage of meiosis I) stage.



Appendix Figure S4. *Klh18* deletion causes MARDO coalescence in oocytes.

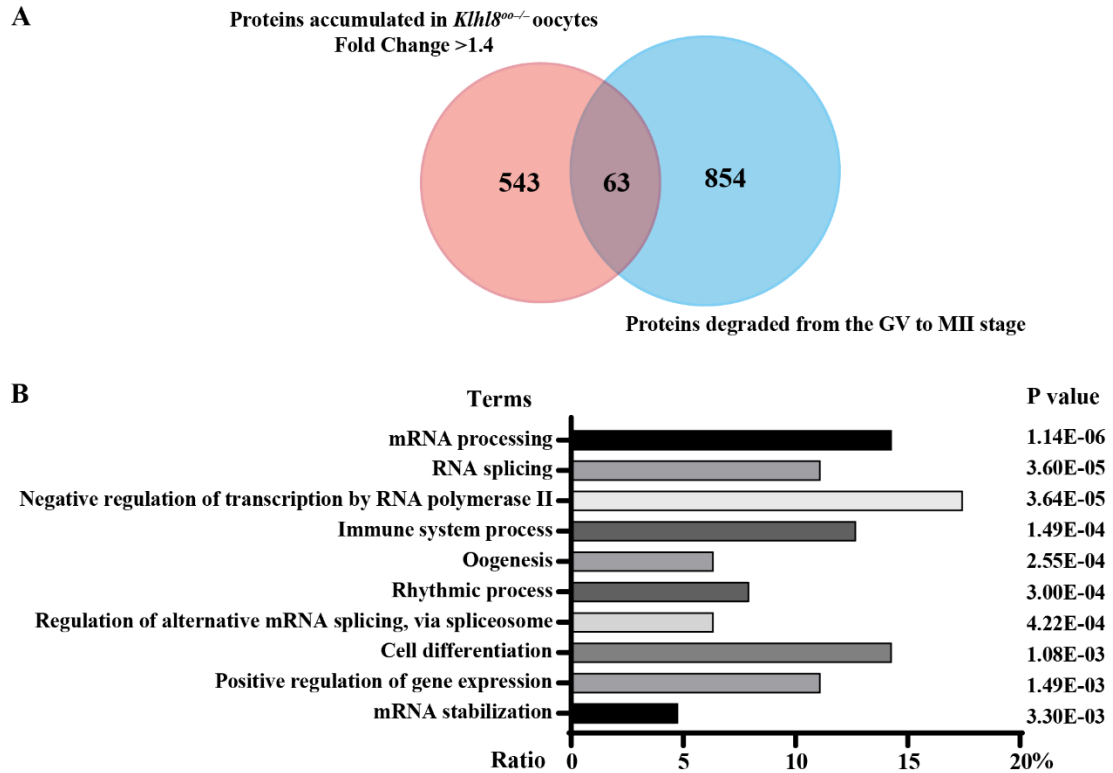
Representative immunofluorescence images of GV oocytes collected from WT and *Klh18^{oo-/-}* mice.

Green, MARDO (YBX2); Red, mitochondria (MitoTracker). Scale bar, 20 μ m.



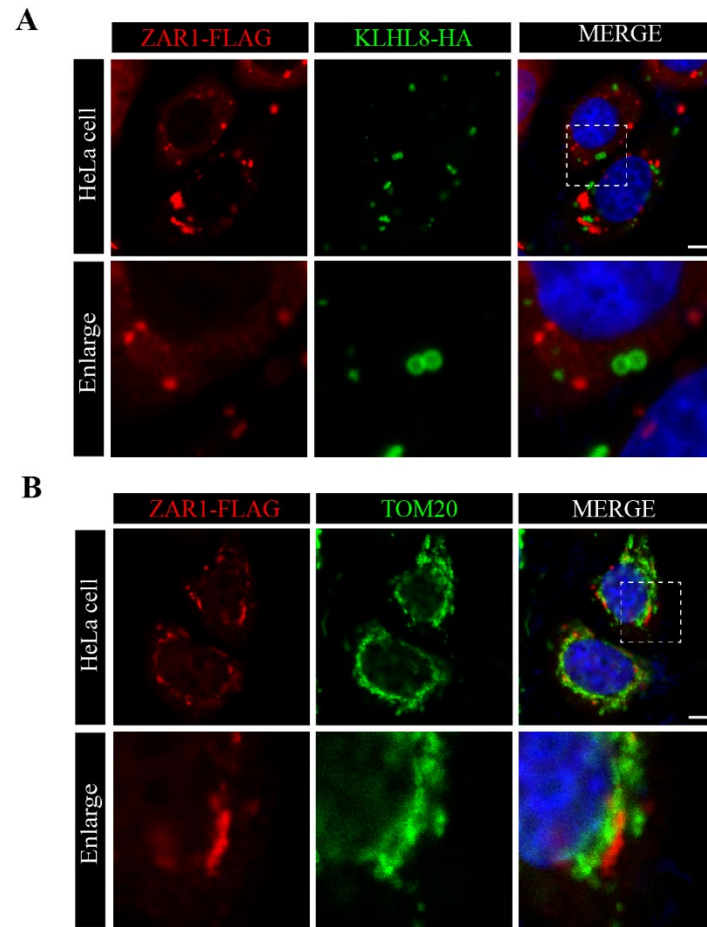
Appendix Figure S5. Detection of mitochondrial function in WT and *Khlh8*^{oo-/-} oocytes.

(A) Representative immunofluorescence images of MMP level in WT and *Khlh8*^{oo-/-} oocytes. Scale bar, 20 μ m. (B) Quantification of MMP fluorescence intensity in WT and *Khlh8*^{oo-/-} oocytes. Each point represents one sample (WT, n = 35; *Khlh8*^{oo-/-}, n = 33). (C) Representative immunofluorescence images of ROS level in WT and *Khlh8*^{oo-/-} oocytes. Scale bar, 100 μ m. (D) Quantification of ROS fluorescence intensity in WT and *Khlh8*^{oo-/-} oocytes (WT, n = 60; *Khlh8*^{oo-/-}, n = 60). (E) The ATP content in WT and *Khlh8*^{oo-/-} oocytes. Each point represents a sample (WT, n = 20; *Khlh8*^{oo-/-}, n = 20). Data information: The fluorescence intensity was quantified by Image J software. In (B, D-E), data are presented as mean $\pm$ SEM. Statistical significance was determined using an unpaired two-tailed t test: ns = not significant.



Appendix Figure S6. Overlap analysis of proteins who accumulated in *Klh18^{oo-/-}* oocytes and degraded in the transition from the GV to MII stage.

(A) Venn diagrams showing the overlap proteins who accumulated in *Klh18^{oo-/-}* GV oocytes and degraded in the transition from the GV to MII stage. The latter data were from a previous study (Sun *et al*, 2023). (B) Gene ontology (GO) enrichment analysis of the genes coding shared proteins.



Appendix Figure S7. The localization of KLHL8 in ZAR1-overexpressed HeLa cells.

(A) Representative immunofluorescence images of ZAR1 and KLHL8 in HeLa cells. Red, ZAR1-FLAG; Green, KLHL8-HA. Scale bar, 5 μ m. (B) Representative immunofluorescence images of ZAR1 and TOM20 in HeLa cells. Red, ZAR1-FLAG; Green, TOM20. Scale bar, 5 μ m.

Appendix Table S1. List of primers used in PCR and qRT-PCR.

Primer name	Sequence	Purpose
<i>Klhl8</i> -F	CGAATCTGACCAATGGAGCACAG	qRT-PCR
<i>Klhl8</i> -R	GGTGATACCTCTCCACACTGGA	qRT-PCR
<i>Zar1</i> -F	CGGCTACTATCACTGCAAGGAC	qRT-PCR
<i>Zar1</i> -R	GACAGGTGATGTCCTCCACTCT	qRT-PCR
<i>Dazl</i> -F	TGACGTGGATGTGCAGAAGATAG	qRT-PCR
<i>Dazl</i> -R	CAAAGGACGTGGCTGCACATGA	qRT-PCR
<i>Fgf8</i> -F	TTGGAAGCAGAGTCCGAGTTCG	qRT-PCR
<i>Fgf8</i> -R	GCCGTGTAGTTGTTCTCCAGCA	qRT-PCR
<i>Ftdc2</i> -F	ATCCTGCTAGCCCTAAAGGCTG	qRT-PCR
<i>Ftdc2</i> -R	GAGAAAGTTGACTTTCCAGATACTC	qRT-PCR
<i>Khdc3</i> -F	ACTGTACGTGGAACCTCGGCTA	qRT-PCR
<i>Khdc3</i> -R	CCAAATCTCAGCTCGCCTTTCAG	qRT-PCR
<i>Zp2</i> -F	CTCATCACACGCTATCTGCGCA	qRT-PCR
<i>Zp2</i> -R	TCCTCAGGGATGCTCCATTGTC	qRT-PCR
<i>Padi6</i> -F	CTGAGCGAGAAGAGCAAAGTGC	qRT-PCR
<i>Padi6</i> -R	ATGACACCGTCTTGTGAGGAGC	qRT-PCR
<i>Lhx8</i> -F	AGCACAGTTCGCTCAGGACAAC	qRT-PCR
<i>Lhx8</i> -R	GCTGAGGAAGAATGGTTGGGAC	qRT-PCR
<i>Tcp1</i> -F	TTTGTGGAAGCTGGTGCCATGG	qRT-PCR
<i>Tcp1</i> -R	CTCTTCCGCTTGTCCCAACATC	qRT-PCR
<i>Paip2</i> -F	GAGGATAATCCATTTGCAGAGTAC	qRT-PCR
<i>Paip2</i> -R	AGCATTTCTTGGAACAGCGTTCA	qRT-PCR
<i>Klhl8</i> -F2	ATTGCATGACTACTGTGTAGTTCC	Mouse genotype identification
<i>Klhl8</i> -R2	TTCATCTCCTAAGTTAGCCACACG	Mouse genotype identification
<i>ZP3</i> -F	CAGATGAGGTTTGAGGCCACAG	Mouse genotype identification
<i>ZP3</i> -R	GCGAACATCTTCAGGTTCTGC	Mouse genotype identification
Oligo (dT)	Cy3-TTTTTTTTTTTTTTTTTTTTTT	mRNA-FISH