The RNA m6A reader YTHDF2 is essential for the post-transcriptional regulation of the maternal transcriptome and oocyte competence.

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The RNA m\textsuperscript{6}A Reader YTHDF2 Is Essential for the Post-transcriptional Regulation of the Maternal Transcriptome and Oocyte Competence

Graphical Abstract

Highlights

- YTHDF2 deficiency is partially permissive in mice and required for female fertility
- YTHDF2 is maternally required for early zygotic development
- YTHDF2 post-transcriptionally regulates transcript dosage during oocyte maturation
- Maternal YTHDF2 is a key determinant of mammalian egg quality

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In Brief

In this issue of Molecular cell, Ivanova et al. report an in vivo function of the RNA m\textsuperscript{6}A reader YTHDF2 in the regulation of the maternal transcriptome during oocyte maturation and its importance for the establishment of competent oocytes that can sustain early embryo development.
The RNA m⁶A Reader YTHDF2 Is Essential for the Post-transcriptional Regulation of the Maternal Transcriptome and Oocyte Competence

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SUMMARY
YTHDF2 binds and destabilizes N⁶-methyadenosine (m⁶A)-modified mRNA. The extent to which this branch of m⁶A RNA-regulatory pathway functions in vivo and contributes to mammalian development remains unknown. Here we find that YTHDF2 deficiency is partially permissive in mice and results in female-specific infertility. Using conditional mutagenesis, we demonstrate that YTHDF2 is autonomously required within the germline to produce MII oocytes that are competent to sustain early zygotic development. Oocyte maturation is associated with a wave of maternal mRNA degradation, and the resulting relative changes to the MII transcriptome are integral to oocyte quality. The loss of YTHDF2 results in the failure to regulate transcript dosage of a cohort of genes during oocyte maturation, with enrichment observed for the YTHDF2-binding consensus and evidence of m⁶A in these upregulated genes. In summary, the m⁶A-reader YTHDF2 is an intrinsic determinant of mammalian oocyte competence and early zygotic development.

INTRODUCTION
RNA N⁶-methyadenosine (m⁶A) is the most abundant internal mRNA modification (Desroisiers et al., 1974; Perry, 1974) that is a key determinant of post-transcriptional mRNA regulation, with proven functions in mRNA processing, translation, and degradation (Fu et al., 2014). m⁶A is found enriched within the METTL3/14 methyltransferase RRACH (where R = G/A and H = A/C/U) consensus in the 3'UTR near the stop codon (Csepansy et al., 1990; Dominissini et al., 2012; Liu et al., 2014; Meyer et al., 2012; Ping et al., 2014; Schwartz et al., 2013; Wang et al., 2014b). The outcome of RNA methylation is instructed through changes in tertiary structure that recruit or displace defined RNA-binding proteins (Alarcón et al., 2015; Liu et al., 2015b) or by directly increasing the affinity of the binding site for YTH-domain-containing proteins (Theler et al., 2014). The mouse genome encodes five YTH domain-containing proteins, one nuclear (YTHDC1) (Xiao et al., 2016; Xu et al., 2014) and four normally cytoplasmic (YTHDF1–YTHDF3 and YTHDC2) (Shi et al., 2017; Tanabe et al., 2016; Wang et al., 2014a, 2015). YTHDC1 is required for early zebrafish embryonic development (Zhao et al., 2017). RNA m⁶A is required for mouse embryonic stem cell exit from pluripotency and preimplantation development (Batista et al., 2014; Geula et al., 2015; Wang et al., 2014b); however, the in vivo and developmental contribution of the m⁶A mRNA-YTHDF2-mediated mRNA destabilization pathway in mammals remains unknown.

Transcription and translation are uncoupled during defined stages of gametogenesis and early zygotic development; thus the regulation of gene expression occurs at the post-transcriptional level. In spermatogenesis, the lepto-zygotene stages of meiotic prophase as well as the latter part of spermiogenesis are transcriptionally inert (Monesi, 1964; Paronetto et al., 2011). Indeed, ALKBH5, an m⁶A RNA demethylase, is required for normal mouse spermatogenesis (Zheng et al., 2013). The maternal transcriptome is assembled during the growth phase of oogenesis, and is completed with the cessation of transcription in full-grown prophase I germinal vesicle (GV) oocytes (Eppig and Schroeder, 1989). Post-transcriptional regulation and utilization of the maternal transcriptome underpin meiotic maturation, fertilization, and early embryonic development (Bachvarova et al., 1985; Payton et al., 1988). Oocyte maturation is hormonally triggered and occurs just prior to ovulation when GV oocytes complete meiosis I and advance to metaphase II (MII) (Li and Albertini, 2013). In mice, these ovulated MI oocytes enter the oviduct, where they await fertilization. Oocyte maturation is
Figure 1. The RNA m6A Reader YTHDF2 Is Expressed in Multiple Tissues, in Mouse Fibroblasts, and in the Germline

(A) Schematic representation of the YTHDF2 protein, the Ythdf2^HA-Fl^ allele, and the HA-YTHDF2 fusion protein.

(B) Western blot using anti-HA and anti-α-tubulin antibodies on the indicated tissue and cell line lysates from Ythdf2^HA-Fl/HA-Fl^ and wild-type (WT).

(legend continued on next page)
accompanied by a wave of RNA degradation where approximately 20% of total maternal RNA is actively degraded (Bachanova et al., 1985; Ma et al., 2013; Payton et al., 1988; Su et al., 2007). This absolute reduction in cellular mRNA results in relative changes to transcript dosage in the MII transcriptome, where some transcripts are stabilized, are destabilized, or remain unchanged (Ma et al., 2013; Su et al., 2007). The MII transcriptome is a large determinant of oocyte competence (Li et al., 2010), and the mechanisms ensuring correct gene dosage achieved through meiotic maturation are not known.

RESULTS

YTHDF2 Is a Cytoplasmic Protein Expressed at All Stages of Mammalian Gametogenesis

To understand the in vivo function of YTHDF2 and m^6^A-mediated mRNA destabilization of transcripts, we generated an epitope-tagged and conditional allele of Ythdf2 (Ythdf2^HA-HA^) in mice (Figures 1A, S1A, and S1B). The N-terminal tagging of YTHDF2 with GFP-His6-FLAG-HA did not affect the function of the protein, as mice homozygous for Ythdf2^HA-HA^ were viable and fertile (Figure S1C, related to Figure 1). The HA-YTHDF2 protein was detectable by western blotting and was expressed in all tissues analyzed (Figure 1B). However, the respective tissues express differential amounts of YTHDF2, with tests displaying the highest expression (Figure 1B). We next sought to understand the expression of YTHDF2 at the cellular resolution in the germline during gametogenesis. YTHDF2 is expressed at all stages of spermatogenesis, with elevated expression observed in pachytene spermatocytes (Figures 1C). Folliculogenesis is the growth phase of oocyte development where the biomaterial and the maternal transcriptome required for oocyte competence are assembled (Eppig and Schroeder, 1989). This initiates when a clutch of primordial oocytes commence growth coincident with the expansion of surrounding somatic granulosa cells that collectively form the follicles with folliculogenesis, culminating in ovulation (Figure 1D) (Matzuk et al., 2002). At all stages of folliculogenesis, YTHDF2 is expressed both in the oocyte and in somatic granulosa cells (Figure 1E). YTHDF2 is also expressed during oocyte maturation, with abundant YTHDF2 detected in GV as well as in MII oocytes (Figure 1F). Both during spermatogenesis and folliculogenesis, YTHDF2 is cytoplasmic in both the germ and the somatic cells (Figures 1C, 1E, and 1F).

Ythdf2 Deficiency Is Partially Permissive in Mice and Results in Female-Specific Infertility

To understand the in vivo function of YTHDF2, we converted the conditional allele to a null allele (Ythdf2^−/−^) (Figures 2A, S1A, and S1B). We observed that Ythdf2^−/−^ mice are retrieved in sub-Mendelian ratios (Figure 2B), with approximately half of the expected Ythdf2^+/−^ mice observed at weaning. The loss of Ythdf2^−/−^ from heterozygous intercrosses increased as the allele was bred toward a C57Bl6 genetic background (Figure 2B). Nonetheless, the viable Ythdf2^−/−^ mice were indistinguishable from their heterozygous or wild-type littermates. We next determined the fertility status of both male and female Ythdf2^−/−^ mice by set-up crosses with wild-type mice. Despite the abundant YTHDF2 expression throughout spermatogenesis (Figure 1C), Ythdf2^−/−^ males were fertile (Figure 2C), with normal seminiferous tubule histology (Figure 2D). However, Ythdf2^−/−^ females were sterile (Figure 2E), with corpora lutea observed in the Ythdf2^−/−^ ovaries, indicating that ovulation had occurred (Figure 2F). In summary, YTHDF2 deletion is partially permissive in mice and results in female-specific infertility.

YTHDF2 Is Intrinsically and Maternally Required for Oocyte Competence

The female infertility in Ythdf2^−/−^ mice could arise from either germine- or somatic-related defects. Indeed, YTHDF2 is expressed both in the oocyte and in somatic granulosa cells during folliculogenesis (Figure 1E). The somatic cells support and transmit key instructive signals to the growing oocyte (Li and Albertini, 2013). We therefore employed conditional genetics to test the intrinsic and maternal oocyte function of YTHDF2. To this end, we combined the Zp3Cre allele that deletes in growing oocytes with our Ythdf2^HA-HA^ allele to generate experimental Ythdf2^HA-HA^; Zp3Cre Tg^+/+^, Zp3Cre Tg^+^ (Ythdf2^mCKO^) and control Ythdf2^HA-HA^; Zp3Cre Tg^+^ or Ythdf2^+/−^; Zp3Cre Tg^+^ (Ythdf2^CTL^) mice. This maternal conditional deletion (mCKO) strategy resulted in the oocyte-specific deletion of YTHDF2 without affecting its expression in somatic granulosa cells (Figure 3A). Crossing of Ythdf2^mCKO^ females with wild-type males revealed that maternal expression of YTHDF2 is intrinsically required for female fertility (Figure 3B). Histological analysis of Ythdf2^mCKO^ ovaries revealed the presence of corpora lutea, indicating that ovulation has occurred (Figure 3C). Hormone priming with pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) to induce oocyte growth and subsequent ovulation revealed that Ythdf2^mCKO^ females produce normal numbers of MII oocytes that have completed meiosis I and arrested at metaphase II properly (Figures 3D and 3E). We next sought to understand if Ythdf2^mCKO^ oocytes are competent to be fertilized. Ythdf2^CTL^ and Ythdf2^mCKO^ females were hormone primed and set up with wild-type males, and zygotes were collected 0.5 days later and examined for fertilization as evidenced by the progression to the 2 pronuclei (2PN) stage. A similar frequency of Ythdf2^mCKO^ and Ythdf2^CTL^ oocytes had reached the 2PN stage accompanied by the extrusion of the second polar body (Figure 3F); thus YTHDF2

(C) Top panel is a schematic representation of spermatogenesis. Below are shown confocal immunofluorescent images of testis sections stained with anti-HA antibody (green) and γ-H2AX (red) of the indicated spermatogenic cells from Ythdf2^+/+^ and Ythdf2^HA-HA-HA^ mice. Scale bar, 10 μm.
(D) Schematic representation of folliculogenesis and oocyte maturation. Abbreviations: GV, germinal vesicle oocyte; MII, metaphase II-arrested oocyte.
(E) Confocal immunofluorescent images of Ythdf2^+/+^ and Ythdf2^HA-HA-HA^ ovary sections stained with anti-HA antibody (green) and Hoechst (blue) for primordial, primary, secondary, early antral, and antral follicular stages. Scale bar, 30 μm. Top right corner square is a magnified image of the oocyte in the respective follicle.
(F) Confocal immunofluorescence images of GV and MII oocytes of wild-type and homozygous Ythdf2^HA-HA^ mice stained with anti-HA antibody (green) and Hoechst (blue) are shown as indicated. Scale bar, 20 μm.
See also Figure S1.
is not required for the process of fertilization per se. Harvesting zygotes at 2.5 days after priming and mating revealed that development is derailed at or prior to the two-cell stage in Ythdf2mCKO zygotes (Figure 3G). In comparison to Ythdf2CTL, fewer Ythdf2mCKO zygotes made two-cell stage embryos of normal morphology, with many of the Ythdf2mCKO two-cell embryos presenting various cytokinesis defects such as micronuclei and enucleated cells (Figure 3G). In summary, YTHDF2 is maternally required for early zygotic mouse development.

**YTHDF2 Post-transcriptionally Regulates Transcript Dosage during Meiotic Maturation**

The failure of Ythdf2mCKO oocytes to support early zygotic development could arise from the inability to process or utilize the transcriptome correctly. The utilization of the maternal transcriptome commences with the onset of oocyte maturation with an overall 20% reduction of cellular RNA in MII oocytes that results in relative changes to the MII transcriptome (Figures 4A and 4B) (Bachvarova et al., 1985; Flemr et al., 2010; Su et al., 2007). We decided to analyze the MII transcriptome, as this is the earliest stage where the maternal transcriptome is utilized, thus enabling us to determine the primary impact of YTHDF2 on the maternal transcriptome. Analysis of MII oocytes revealed deregulated gene expression within the Ythdf2mCKO transcriptome, with increased dosage of transcripts originating from 201 genes and decreased expression from 68 loci when applying a cut-off with a fold change greater or equal to two and a significance with a p value less than 0.05 (Figures 4C and S2). To exclude a function for YTHDF2 in the formation of the maternal transcriptome, we profiled gene expression in GV oocytes where transcription has ceased and the oocyte possesses a mature maternal transcriptome (Bachvarova et al., 1985; Paynton et al., 1988). This analysis revealed that Ythdf2mCKO GV oocytes contain a near normal transcriptome (Figure S3, related to Figure 4). In summary, the loss of YTHDF2 does not grossly impact oocyte growth or the formation of the maternal transcriptome but is required to instruct the correct gene dosage during oocyte maturation. The bias in deregulated gene expression in Ythdf2mCKO MII oocytes toward upregulation is consistent with the removal of a protein that potentiates RNA degradation. We next sought to understand what class of genes YTHDF2 regulates across oocyte maturation, whether those that are relatively stabilized, destabilized, or remain unchanged (Figure 4E). This analysis revealed that in the cohort of genes that are upregulated in Ythdf2mCKO MII oocytes, the majority (168/201) should remain relatively unchanged across oocyte maturation, and some (33/201) are destabilized (Figures 4E and S4). In summary, YTHDF2 function is intrinsically and maternally required to instruct the appropriate transcript dosage during oocyte maturation.

We next sought to understand if some of the upregulated transcripts could be direct targets of YTHDF2. GACU/A is the most common YTHDF2-binding motif identified from PAR-CLIP.
We therefore looked for enrichment of the GACU/A consensus ±400 bp around the stop codon in the top upregulated and downregulated genes as well as in genes whose dosage remains unchanged. To this end, we took the top 1,000 upregulated and downregulated transcripts; this selection is required for statistical power and corresponds to an approximate 1.4-fold change in expression levels. This analysis revealed a significant enrichment for the GACU/A motif only in upregulated genes in Ythdf2mCKO MII oocytes (Figure 4F). This enrichment was not observed around the 5' UTR (Figure 4F).

Figure 3. YTHDF2 Is Maternally Required for Oocyte Maturation and Early Zygotic Development

(A) Confocal immunofluorescence with anti-HA antibody (green) and Hoechst (blue) on ovary sections from homozygous Ythdf2HA-Fl and Ythdf2mCKO mice are shown. Primordial (P) and secondary (S) follicle stage are indicated. Scale bar, 30 μm.

(B) Number of pups born per plug from Ythdf2CTL and Ythdf2mCKO female mice. The number (n) of animals, mean, and SD are indicated (t test; ***p < 0.0001).

(C) PAS-stained ovary sections from Ythdf2CTL and Ythdf2mCKO females. CL indicates corpus luteum. Scale bar, 100 μm.

(D) Number of MII oocytes isolated from oviduct of hormone-primed Ythdf2CTL and Ythdf2mCKO female mice. The number (n) of animals, the mean, and SD are indicated (t test; n.s., p > 0.05).

(E) Percentage of normal MII oocytes isolated from hormone-primed Ythdf2CTL and Ythdf2mCKO females. The morphology of MII oocytes was assessed through immunofluorescent staining with anti-β-tubulin antibody (green) and Hoechst (blue). Representative images for Ythdf2CTL and Ythdf2mCKO MII oocytes are shown, with the zona pellucida indicated by a white dashed circle. Scale bar, 20 μm.

(F) Percentage of degenerated, sperm entry, and two pronuclei zygotes isolated from hormone-primed and stud male-mated Ythdf2CTL and Ythdf2mCKO female mice at embryonic day 0.5. The number (n) of zygotes assessed is indicated. Confocal immunofluorescent images of representative Ythdf2CTL and Ythdf2mCKO zygotes stained with Hoechst (blue) are shown, with the zona pellucida indicated by a white dashed circle. The female and male pronuclei are indicated with female and male signs, respectively; Pb denotes polar bodies. Scale bar, 20 μm.

(G) Percentage of degenerated, abnormal two-cell, two-cell, three- to four-cell, and six- to eight-cell zygotes isolated from stud male-mated hormone-primed Ythdf2CTL and Ythdf2mCKO female mice at embryonic day 2.5. The number of zygotes assessed is indicated. Confocal immunofluorescent images of representative Ythdf2mCKO arrested abnormal two-cell zygotes (Panels II–IV) stained with anti-β-tubulin (green) and Hoechst (blue) are shown. Scale bar, 20 μm.
Figure 4. YTHDF2 Regulates Maternal Transcript Dosage during Oocyte Maturation

(A) Schematic representation of oocyte maturation with a graph indicating the approximate levels of maternal RNA and transcription. Abbreviations: GV, germinal vesicle oocyte; GVBD, germinal vesicle break down oocyte; MI, metaphase I oocyte; and MII, metaphase II-arrested oocyte.

(B) Expression scatterplot showing the relative average expression of transcripts from GV and MII oocytes. In red are highlighted the genes that are significantly changed (p < 0.05) with a fold difference greater or equal to 2 between GV and MII oocytes. Analysis was done on biological three to four replicas.

(C) Expression scatterplot showing the relative average expression of transcripts between Ythdf2CTL and Ythdf2mCKO MII oocytes. Significantly deregulated (p < 0.05) genes with a fold change greater than or equal to 2 are shown in red. Analysis was done on biological triplicates.

(D) Gene ontology analysis for the upregulated genes in Ythdf2mCKO MII oocytes; the top ten most significant processes identified are shown.

(E) Expression scatterplot showing the relationship between transcriptome changes during oocyte maturation (shown on the y axis) and changes in Ythdf2mCKO with respect to Ythdf2CTL MII oocytes (shown on the x axis). The significantly upregulated and downregulated genes in Ythdf2mCKO MII oocytes are shown in red and blue points, respectively. The unchanged transcripts in oocyte maturation and in Ythdf2mCKO versus Ythdf2CTL MII oocytes are indicated by horizontal and vertical dashed red lines, respectively. The number of genes in the respective gates is indicated.

(F) Graph representation of Loess smoothed sum of YTHDF2-binding motif GACU/A occurrences ±400 nt around the stop codon (left panel) and around the start codon (right panel). Data are shown for the top 1,000 most-upregulated transcripts (red), 1,000 downregulated transcripts (blue), and 1,000 transcripts whose expression remained unchanged (green) in Ythdf2mCKO MII oocytes. Only enrichment of the upregulated genes compared to unchanged genes around the stop codon is statistically significant (p < 0.05).

(G) Prevalence of m6A peaks from public mouse datasets around the gene bodies of upregulated, downregulated, and unchanged transcripts in Ythdf2mCKO MII oocytes. Gene bodies are scaled to the same length in each case. Only enrichment of the upregulated genes compared to unchanged genes around the stop codon is statistically significant (p < 0.05).

See also Figures S2, S3, and S4.
where YTHDF2 is known not to occupy under steady-state non-stress conditions (Wang et al., 2014a). The limiting amount of RNA that can be isolated from oocytes excludes the possibility of performing m^6A-seq to determine if the consensus-containing upregulated genes in Ythdf2^mCKO MII oocytes are methylated. We therefore sought to determine if we can find evidence for their methylation in other mouse tissues using the MeT-DB database (Liu et al., 2015a). We found a stark enrichment for m^6A adjacent to the stop codon in the transcripts that are upregulated in Ythdf2^mCKO MII oocytes (Figure 4G). In summary, we find that the upregulated transcripts in the Ythdf2^mCKO MII oocytes are enriched for the m^6A/YTHDF2 consensus motif, and for some we find evidence for their m^6A methylation in other cell types.

**DISCUSSION**

The loss of METTL3 demonstrated an indispensable function for RNA m^6A in embryonic stem cell exit from pluripotency and pre-implantation development (Batista et al., 2014; Geula et al., 2015; Wang et al., 2014b). Here we report the deletion of a mammalian m^6A reader and identify the physiological importance of the m^6A-YTHDF2-mediated mRNA destabilization pathway in mice. We identify and characterize an essential role for YTHDF2 in the female germline. We also find that loss of YTHDF2 is partially permissive in mice, indicating that YTHDF2 has other important developmental functions outside of the germline. The presence of Ythdf2^-/- viable adult mice indicates that this branch of the RNA m^6A regulatory pathway is not essential for mice under normal conditions. However, given that YTHDF2 has been shown to be involved in the cellular heat-shock response (Zhou et al., 2015), it remains to be seen if YTHDF2 function is required for appropriately respond to various physiological and environmental perturbations. The fact that Ythdf2^-/- mice show a genetic background effect indicates that mRNA m^6A sites could constitute a basis for modifier alleles. If this is the case, it would demonstrate the power of mRNA m^6A sites as potential modifiers of development and disease. This could be especially relevant to human development and disease given the increased frequency of RNA m^6A observed in human versus mouse cell lines (Dominisini et al., 2012). While the mouse m^6A demethylase ALKBH5 is required for normal spermatogenesis (Zheng et al., 2013), we demonstrate that the YTHDF2-mediated destabilization of m^6A-containing transcripts is dispensable for male gametogenesis, at least on a mixed genetic background. In yeast RNA, m^6A is specific to meiosis (Clancy et al., 2002); the loss of Ime4, an ortholog of METTL3, or the YTH-domain containing m^6A reader Mrb1, affects meiotic progression (Schwartz et al., 2013; Shah and Clancy, 1992). In mice, YTHDF2 does not regulate the dosage of meiosis-specific genes (Figure 4D), with meiosis I and likely II being completed normally (Figure 3E) in Ythdf2^mCKO oocytes. Ythdf2^-/- male mice are fertile, with no impact observed in spermatogenesis (Figures 2C and 2D). In summary, the YTHDF2-mediated regulatory RNA m^6A pathway is not important for mouse meiosis.

The maternal loss of zebrafish Ythdf2 has a modest impact on zygotic development, whereas maternal or zygotic Ythdf2 depletion profoundly impairs embryogenesis (Zhao et al., 2017). Here we show a defining maternal function for YTHDF2 in regulating transcript dosage across oocyte maturation which is essential for generating MII oocytes that are competent to sustain early zygotic development. The loss of YTHDF2 during oocyte maturation results in the deregulation of approximately 270 genes, leading to an arrest prior to or at the two-cell stage, with various cytokinesis defects observed in the two-cell embryos (Figure 3G). The maternal transcriptome is essential for the first mitotic division (Clift and Schuh, 2013). We posit that the deregulated gene expression in Ythdf2^mCKO oocytes poisons the maternal transcriptome, rendering it incompetent to support the mitotic division. We have shown that YTHDF2 is expressed throughout oocyte growth and from GV through to MII oocytes but that the phenotype only arises after meiotic resumption (Figures 3E–3G). Furthermore, YTHDF2 does not majorly impact on the formation of the maternal transcriptome (Figure S3, related to Figure 4), although, present throughout oocyte growth, these observations beg the question as to why YTHDF2-mediated mRNA degradation is only active upon meiotic maturation. In somatic cells, YTHDF2 has been shown to function through the recruitment of deadenylases and subsequent mRNA decapping (Du et al., 2016; Wang et al., 2014a). RNA degradation factors are downregulated during oocyte growth, favoring the accumulation of RNA and the building of the maternal transcriptome (Flemr et al., 2010). During meiotic maturation, the deadenylase CNOT7 and the decapping enzymes DCP1A and DCP2 are translated from maternal transcripts that enable the resumption of active RNA degradation pathways (Ma et al., 2013, 2015). We believe this reactivation of RNA degradation machinery enables YTHDF2 to directly degrade the bound transcripts selectively during meiotic maturation. In summary, we demonstrate that the m^6A reader YTHDF2 is an essential regulator of the mammalian maternal transcriptome and egg quality.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **CONTACT FOR REAGENT AND RESOURCE TABLE**
- **METHOD DETAILS**
  - Generation of alleles and mice in this study
  - Southern blotting
  - Western blotting
  - Oocyte and zygote collection
  - Immunofluorescence
  - Histology
  - Oocytes mRNA expression analysis
  - Statistics
  - Motif analysis
  - m^6A peaks dataset analysis
  - qRT-PCR
- **DATA AND SOFTWARE AVAILABILITY**

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes four figures and one table and can be found with this article at http://dx.doi.org/10.1016/j.molcel.2017.08.003.
AUTHOR CONTRIBUTIONS

I.I. contributed to the design, execution, and analysis of most of the experiments. D.O. conceived this study. C.M. designed, generated, and validated the Ythdf2−/− allele and qRT-PCR experiments. M.D.G. and C.A. contributed to histology and imaging experiments. I.I., C.C., J.M., and M.M. performed the bioinformatic transcriptome analysis. P.N.M. aided in analysis of mouse zygotes. A.J.E. performed the consensus enrichment analysis and oversaw all bioinformatic analyses performed. D.O. and A.J.E. supervised this study. I.I., A.J.E., and D.O. wrote the final version of the manuscript.

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### STAR METHODS

#### KEY RESOURCES TABLE

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<td>Mouse: Ythdf2HA-HFR</td>
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<td><strong>Software and Algorithms</strong></td>
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<td>Gorilla</td>
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CONTACT FOR REAGENT AND RESOURCE TABLE

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Dónal O’Carroll (donal.ocarroll@ed.ac.uk).

METHOD DETAILS

Generation of alleles and mice in this study
For the Ythdf2\textsuperscript{HA-F3} allele, a GFP-prediction-His6-Flag-HA-HA epitope tag was inserted after the endogenous starting initiation ATG codon in exon 1 of Ythdf2. In addition, twoloxP sites were placed flanking exon 2. The targeting construct was genetically modified so that it contained homology arms and FRT sites flanking a neomycin cassette 3’ of exon 1. Southern blotting of EcoRV-digested DNA extracted from ESC-derived clones with exogenous 5’ probe was used for the validation of homologous recombinants. The wild-type Ythdf2 locus generates a ~9 kb DNA fragment, whereas the integration of the secondloxP site introduced an additional EcoRV site, thus decreasing the size of the EcoRV DNA fragment to 8 kb in the targeted allele. Flip-mediated recombination removed the FRT flanked neomycin cassette and generated the Ythdf2\textsuperscript{HA-F3} allele that can be identified by the 5’ probe as a 6 kb EcoRV DNA fragment. Cre-mediated deletion of theloxP flanked exon 2 resulted in 5.5 kb EcoRV DNA fragment, that can be identified by the 5’ probe and validate the Ythdf2\textsuperscript{−/−} allele. The Zp3Cre Tg (de Vries et al., 2000) allele was also used in this study for the generation of Ythdf2\textsuperscript{mCKO} female mice. Fertility analysis with male and female mice was backcrossed six times toward the C57BL/6 genetic background. All mice used in this study were on mixed or C57BL/6 genetic background and were bred and maintained in EMBL Mouse Biology Unit, Monterotondo, and subsequently at the Centre for Regenerative Medicine, Edinburgh. All procedures were done in accordance to the current Italian legislation (Art. 9, 27. Jan 1992, nu116) under license from the Italian health ministry or the UK Home Office regulations, respectively.

Southern blotting
The forward 5’-GCAGGTGACCTCTTCAGAAG-3’ and reverse 3’-CCAGTCCCTGTAGATTTTAGAG-5’ primers were used to generate an exogenous 5’ probe for detection of the targeted, Ythdf2\textsuperscript{HA-F3} and Ythdf2\textsuperscript{−/−} alleles. Genomic DNA was restriction digested and run on a 0.8% agarose gel. The DNA fragments were then transferred to an Amersham Hybond-XL membrane (GE Healthcare) through alkaline solution (0.4 M NaOH, 1.5 M NaCl) overnight. The membrane was neutralized in 2X SSC solution, UV-crosslinked with 150 mJ/cm\textsuperscript{2} and incubated in prehybridization solution (0.5 M Na\textsubscript{2}HPO\textsubscript{4}, 1 mM EDTA, 5% SDS, 3% BSA) for 2 hr at 65°C. DNA probe was synthesized with Random Primers DNA Labeling System (Thermo Fisher Scientific) in accordance to manufacturer’s protocol and was hybridized with the membrane overnight at 65°C. The membrane was washed in 40 mM Na\textsubscript{2}HPO\textsubscript{4}, 1 mM EDTA, 5% SDS and exposed on a phosphor screen (Fujifilm).

Western blotting
Protein extracts were prepared with dounce homogenizer in lysis buffer (50 mM Tris pH 7.8, 150 mM NaCl, 0.4% NP-40, 2 mM MgCl\textsubscript{2}, 1 mM DTT) supplemented with proteinase inhibitors. Extracts were collected after centrifugation at 14000 g for 10 min at 4°C, resolved on 7.5% SDS-PAGE gel and transferred on Immobilon-P membrane (Millipore) via wet transfer overnight. Membranes were blocked in 5% milk-PBST (PBS with 0.1% Tween20) and probed with anti-HA (Covance, MMS-101P, 1:1000) and anti-alpha-tubulin antibody (Sigma-Aldrich, T9026, 1:1000) for 4 hr at room temperature. Membrane was washed in PBST and incubated with appropriate horseradish peroxidase-coupled secondary antibody (Amersham) in 5% milk-PBST for 1 hr. Proteins were detected with ECL Western Blotting Detection Reagent (Amersham) and acquired on a ChemiDoc XRS system (BioRad).

Oocyte and zygote collection
For the collection of GV oocytes, 3-8 weeks old females were injected with 10 U of pregnant mare serum gonadotropin (PMSG) (Henry Schein). After 44-48 hr GV oocytes were collected through puncturing the ovarian follicles in M2 media (Sigma-Aldrich). Subsequently, GV oocytes were released from the somatic cells via manual mechanical separation.

For the collection of MII oocytes, 3-8 weeks old females were injected with 10 U of PMSG and after 46-48 hr with 10 U of human chorionic gonadotropin (hCG) (Intervet). MII oocytes were isolated from the oviduct of the hormone-stimulated females 14 hr after the hCG injection. MII oocytes were cleaned from the somatic cells with hyaluronidase (Sigma-Aldrich) in M2 media.

For the collection of zygotes, PMSG and hCG stimulated females (as described above) were set up with a stud male immediately after the last injection. Zygotes were isolated from the oviduct of plugged females 0.5 and 2.5 days after the hCG injection. The collected 0.5 day zygotes were briefly cleaned from the somatic cells with hyaluronidase in M2 media.
Total RNA was isolated from 50-90 GV and MII oocytes with QIAzol lysis reagent (QIAGEN) following the manufacturer instructions.

Oocytes mRNA expression analysis

stained with periodic acid Schiff reagent (Sigma-Aldrich) and Hematoxylin (Sigma-Aldrich) as per manufacturer’s instructions.

For RNA expression analysis, robust multi-array average (RMA) was used for the raw data normalization and limma package to determine differential expression (Ritchie et al., 2015). Moderate t-statistics was done with adjusted p values. Gene ontology analysis was done with Gene ontology enrichment analysis and visualization tool Gorilla (Eden et al., 2009).

Motif analysis

Affymetrix identifiers were mapped to Ensembl transcripts using Biomart (32). Biomart was then used to obtain 400 nt 5’ and 3’ of both the start and stop codon. Sequences obtained that were shorter than 400 nt were padded to this length with the addition of ’N’s. Each transcript may only be present once in the Affymetrix gene list. Where multiple transcripts are present, the one with the highest absolute fold-change is retained. Only one isomir is retained when multiple transcripts have the same sequence. Motif occurrences were directly computed using Perl regular expression matching for “GCA[UA]" for sequences assigned to the top 1000 most upregulated, downregulated and for 1000 transcripts from the center of the gene list. A matrix of 1000x800 elements is hence obtained for each of the three sets, indicating which sequence and at which nucleotides motifs occur. This matrix is column summed and plotted using a cubic spline smoothing function smooth.spline from R/Bioconductor. Statistics for the motif enrichment was done with a hypergeometric test.

m6A peaks dataset analysis

Public m6A peak data was obtained from MeT-DB (http://compgenomics.utsa.edu/methylation/) for 12 mouse (mm9) samples. Peaks were translated into BED files with enrichment scores, lifted-over to mm10 via UCSC liftover, sorted, filtered and overlapping regions condensed into bigwig files. These bigwig files were provided to the DeepTools package v1.5.9.1 (ComputeMatrix and Heatmapper) (Ramírez et al., 2014) to explore peak enrichments around gene-bodies from the start to the stop codon with 400 nt either side. The Ensembl GTF corresponding to GRCm38 version 79 was used for gene body coordinates. Statistics for the m6A peak enrichment was done with two-sample t test.
qRT-PCR
Total RNA from 50-80 MII oocytes per biological replicate was reverse-transcribed using SuperScript IV and random hexamers (both Invitrogen) according to manufacturer’s instructions. qRT-PCR was performed using the LightCycler 480 SYBR Green I Master mix (Roche), and samples were run in technical triplicates on a Roche LightCycler 480 instrument. Ct values were normalized against the internal controls Gapdh, Sod1 and Bmp15. Fold differences in expression levels were calculated according to the $2^{-\Delta\Delta Ct}$ method.

DATA AND SOFTWARE AVAILABILITY

Original images, immunoblots and radiography can be found in Mendeley Data (http://dx.doi.org/10.17632/zb7zyfghg3.1).