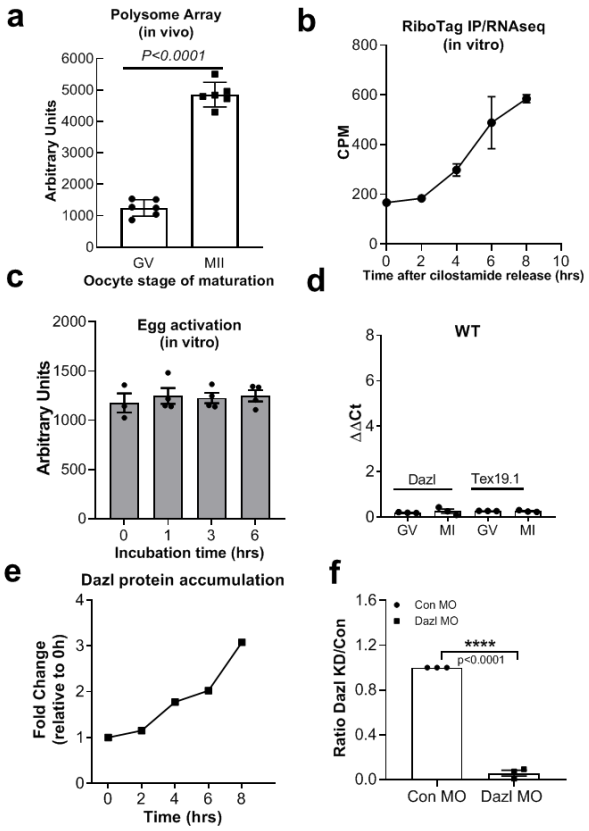
SUPPLEMENTARY INFORMATION

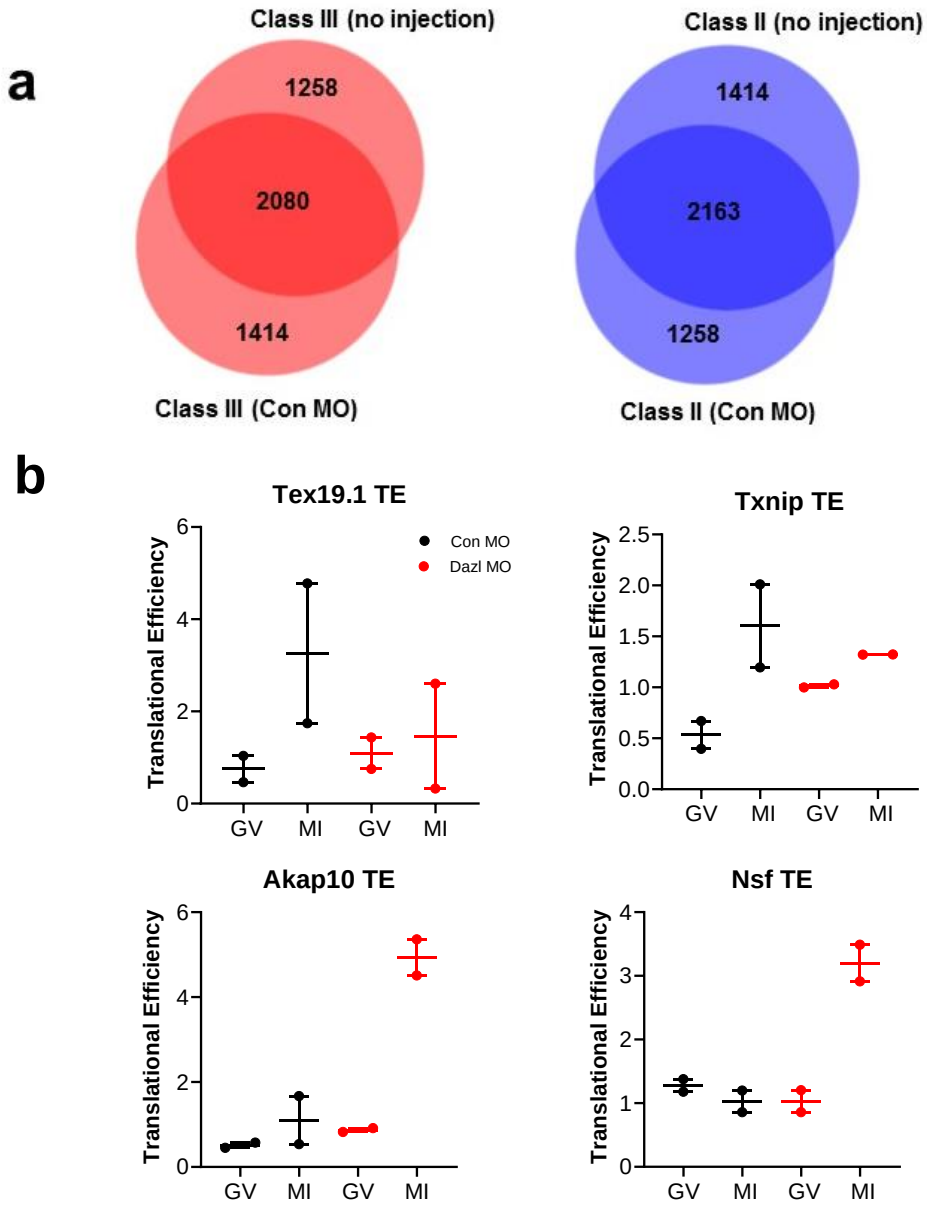
The RNA binding protein DAZL functions as repressor and activator of maternal mRNA translation during oocyte maturation

Cai-Rong Yang et al.



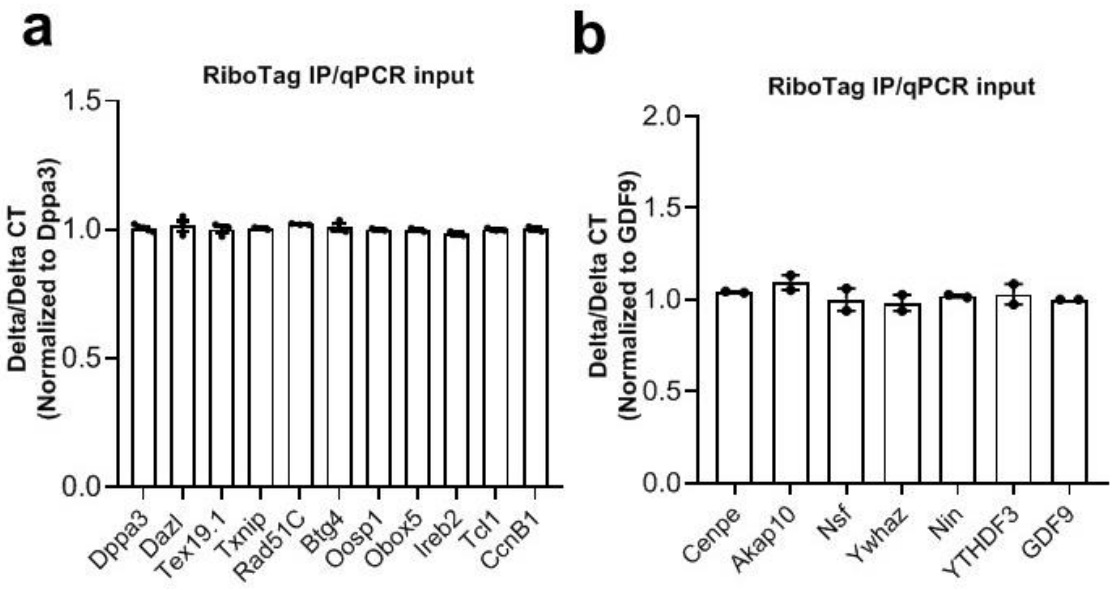
Supplementary Figure 1 Dazl mRNA translation is regulated during oocyte maturation.

(a) The Dazl mRNA becomes associated with the polysome fraction at the GV-to-MII transition *in vivo*. Polysome array data described in previous publications were mined for the Dazl mRNA association with polysomes. The data are the Mean + SEM of six independent biological replicates. **(b)** Ribosome loading of *Dazl* mRNA is increased during in vitro oocyte maturation. After harvesting, oocytes were incubated *in vitro* for the times reported in the abscissa. At the end of the incubation, samples were processed for RiboTag IP/RNAseq as described in the 'Materials and Methods'. The data are the mean +/- range of duplicate samples for each time point. The details of the experiments are summarized in a paper in preparation. **(c)** Polysome associated transcript of *Dazl* is not changed upon egg activation in vitro. MII oocytes were treated *in vitro* Sr2+ for the indicated times. At the end of the incubation, cells were harvested and processed for polysome arrays as described. Each point is the Mean +/- SEM of three or four biological replicates. **(d)** RiboTag IP/qPCR of oocyte extracts from wild type mice. The mRNA recovered is at background levels and is similar in all the experimental groups. **(e)**Protein levels measurement for DAZL accumulation during oocyte maturation until 8 hrs (MI stage). **(f)** Measurement of protein levels for DAZL expression with DAZL-KO MO injection. Each group is the average and SEM of three biological replicates.



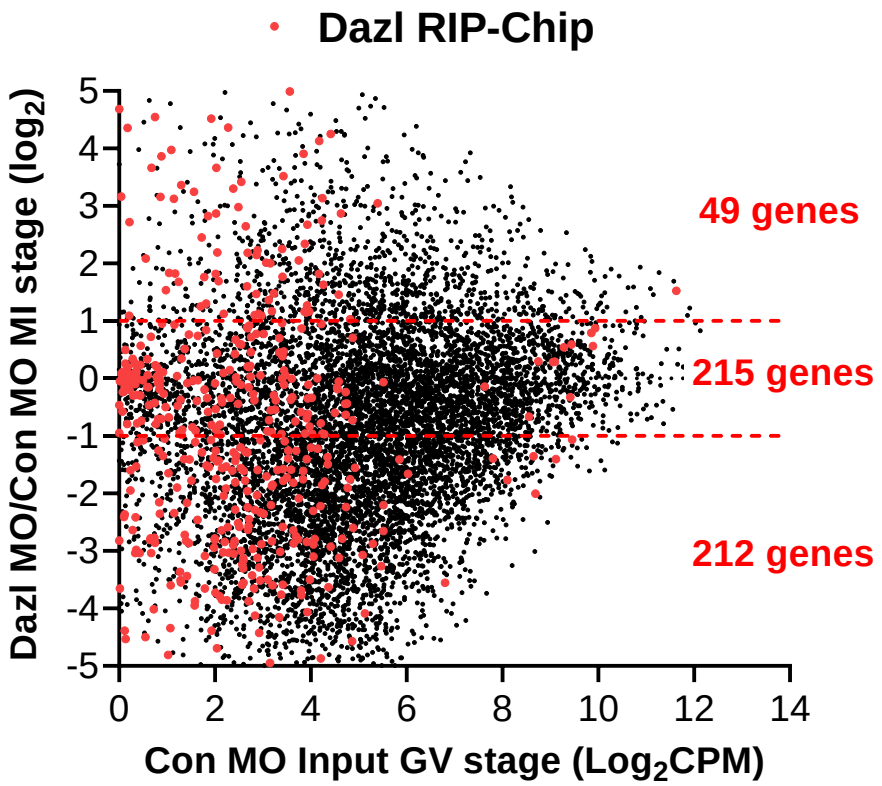
Supplementary Figure 2

(a) Comparison of the RiboTag IP/RNAseq data from CON-MO injected oocytes with RiboTag IP/RNASeq data for non-injected oocytes. Increased and decreased ribosome loading onto mRNAs at 6 hrs is compared. Qualitatively, the regulation of translation is comparable between the two experiments for the majority of transcripts. Quantitatively the data could not be directly compared because of batch effects. **(b)** Calculation of the translational efficiency (TE) for the candidate mRNAs reported in Fig. 2C. TE is calculated as the CPM of ribosome bound mRNA divided by the total mRNA levels expressed in CPM.



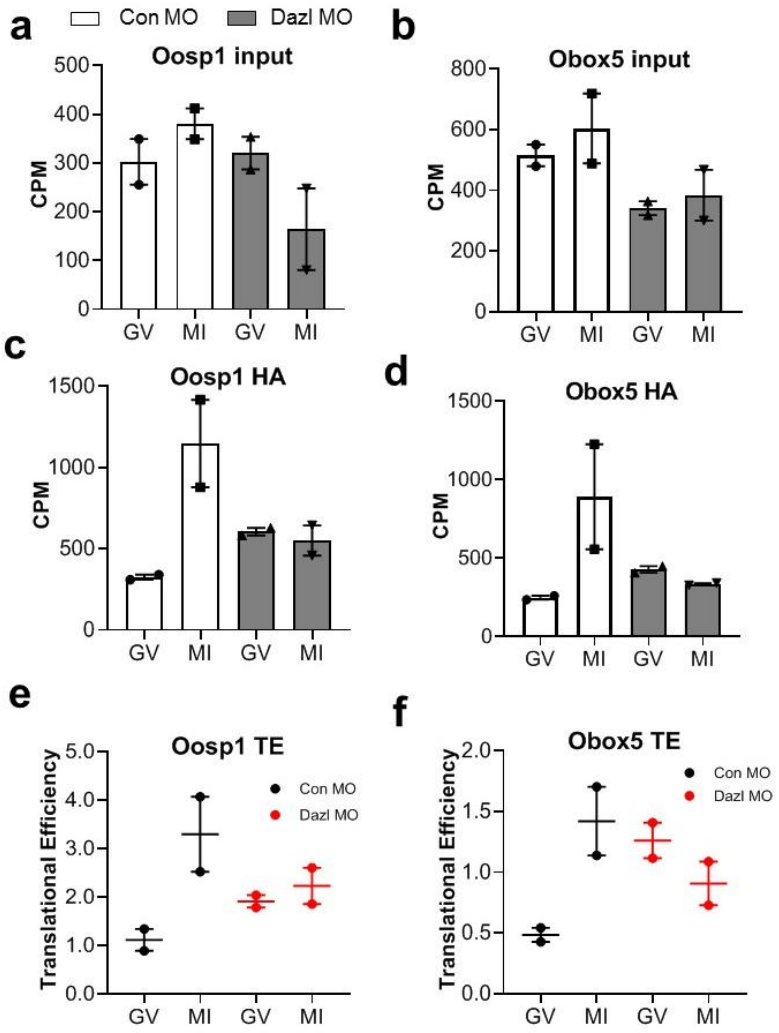
Supplementary Figure 3

Total mRNA levels of the representative Dazl targets reported in Figure 3. GV stage oocytes from wild type or Dazl$^{+/-}$ mice were injected with CON-MO or DAZL-MO. After overnight preincubation with 2 μM milrinone, oocytes were cultured in inhibitor-free medium for maturation. Oocytes were collected at 6 hrs for qPCR analysis of total transcript levels. The samples are the same as those reported in Figure 3.



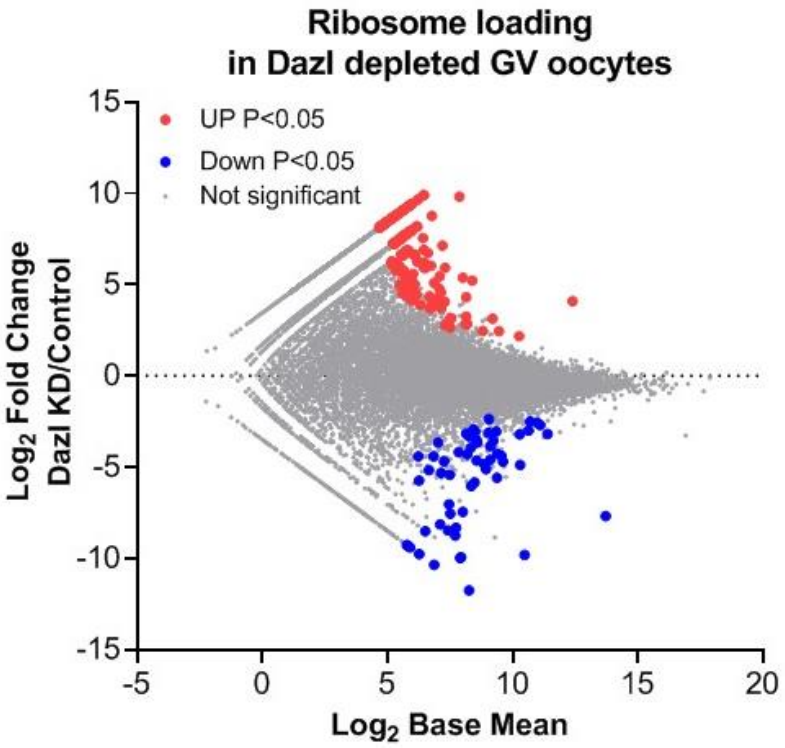
Supplementary Figure 4

Comparison of genes whose ribosome loading is affected by DAZL depletion and their interaction with DAZL protein. The plot reports the ratio of log2 fold changes of RiboTag IP/RNASeq between the DAZL-MO and CON-MO. The genes marked in red are specifically immunoprecipitated (P<0.05; >1.5 over IgG) by a DAZL antibody in the DAZL RIP-Chip experiment. The dashed red lines mark the 2fold change threshold.



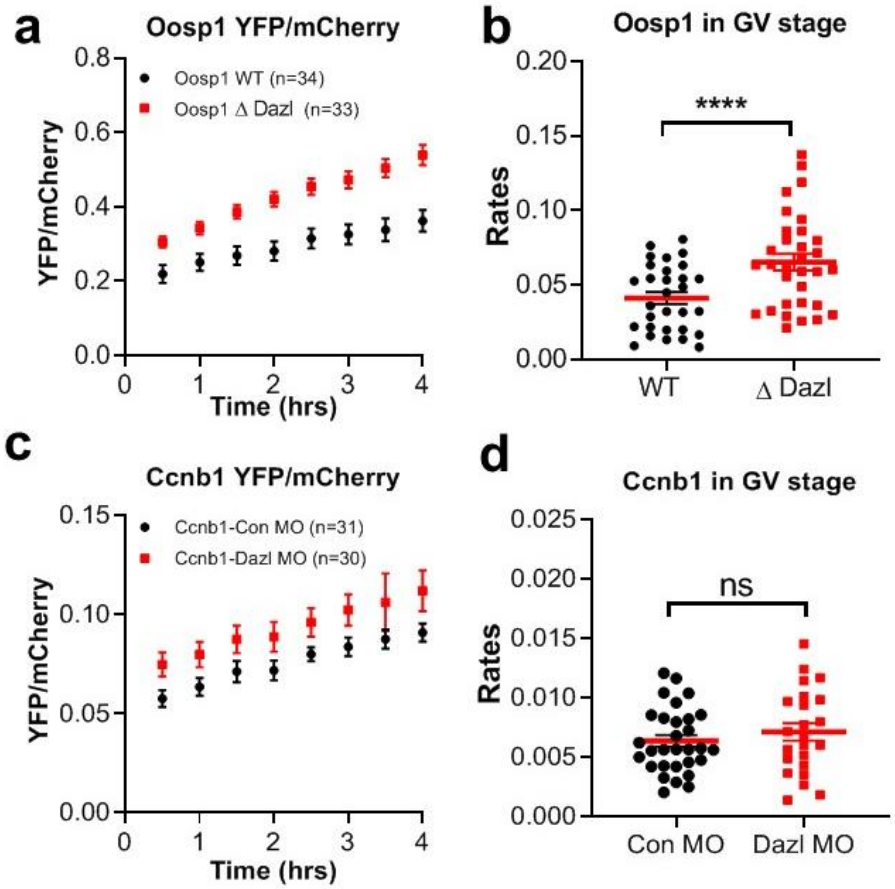
Supplementary Figure 5

RiboTag IP/RNASeq data and TE calculation for the *Oosp1* and *Obox5* mRNAs. The data are calculated as described above. Notice the loss of translational activation in oocytes depleted of DAZL. This is due to increased levels of translation during GV-arrested and decreased activation during MI.



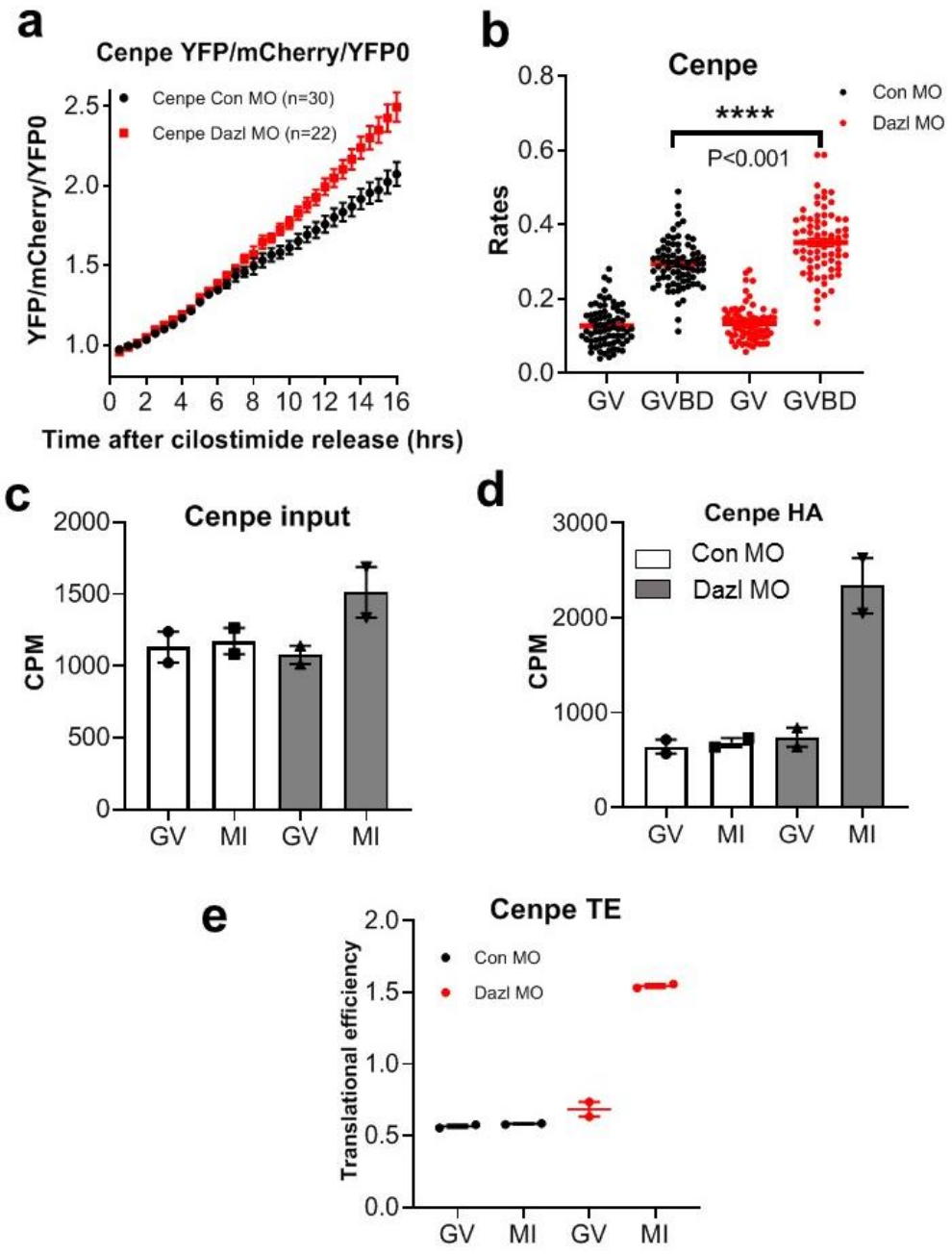
Supplementary Figure 6.

Effect of DAZL depletion on ribosome loading of mRNAs in GV-arrested oocytes. The ribosome loading of 153 transcripts was significantly increased in GV oocytes depleted of DAZL whereas 69 transcripts were decreased (no fold change cutoff, FDR < 0.05).



Supplementary Figure 7. Oosp1 accumulation during overnight incubation

Mutation of the DAZL-binding element causes an increase in translation rate of the *YFP-Oosp1* reporter in GV-arrested oocytes. Oocytes were injected with the YFP reporter fused wild type or mutated DAZL-binding element 3'UTR of *Oosp1*. After 3 hrs of preincubation for recovery, the oocytes were maintained in GV with cilostamide and florescence monitored by time lapse microscopy. The number of oocytes analyzed in each group is reported in the figure.



Supplementary Figure 8

Injection of the *YFP-CenpE* reporter construct recapitulates the effect of DAZL depletion on ribosome loading of the endogenous *CenpE* mRNA. **(a and b)** Accumulation of the YFP-CenpE reporter during oocyte maturation and measurement of rates of translation in individual injected oocytes. **(c and d)** RiboTag IP/RNASeq data for the endogenous *CenpE* mRNAs. Each point is the average and range of two biological replicates.

Supplementary Table 1

Name	Primers
Akap10 FW	AGTCATCAGATTCCCACCGAC
Akap10 REV	TGGCTTCTGTAATTGGTATTGGC
Cenpe FW	TGTCTGTGTTCGTGTGCGAC
Cenpe REV	AAGATTTACCCCCATCGCTCT
Ywhaz FW	AAAGGCAGGGCGTCATTCAG
Ywhaz REV	ACGGGGTTTCCTCCAATCAC
Nin FW	AGAACTCTATCCAGTGAGGAGC
Nin REV	TAGTGGCTCAAGCACTGTCAC
Dazl FW	GGATGAAACCGAAATCAGGA
Dazl REV	ATAGCCCTTCGACACACCAG
Oosp1 FW	TCTCTGGGGTTTGATCTTCAGC
Oosp1REV	CGTAGGCCTGATCCTTAGATGG
Obox5 FW	AGGGGATATCATGTTGGAGCC
Obox5 REV	GTTCCTTGCCGGTTCTTGAG
Tex19.1 FW	GGCTGTACCATCTTGTCCA
Tex19.1 REV	TCCTCTTCCTCTTCCTCCTC
Txnip FW	TGGACGACTCTCAAGACAGC
Txnip REV	CATTTCCTGCAGGCTCACTG
Tcl1 FW	TCGGAGTCCAACGATGAATAACC
Tcl1 REV	CTTCTTGGAGCCCAGTGTAGAG
Btg4 FW	TCGATCCCTATGAGGTGTGC
Btg4 REV	CCTGCTGCAGCTTTCTTCATC
Rad51C FW	TCAAGCTTTGCTTGTTCCATTA
Rad 51C REV	TATCGTAGACTCCTTCTGGCTTG
Ireb2 FW	TCAATGTACCTAAACTTGGCGG
Ireb2 REV	AAGGGCACTTCAACATTGCTC
Gdf9 FW	CAAACCCAGCAGAAGTCAC
Gdf9 REV	TTAAACAGCAGGTCCACCA
Ccnb1 FW	AAGGTGCCTGTGTGTGAACC
Ccnb1 REV	GTCAGCCCCATCATCTGCG
Dppa3 FW	GACCCAATGAAGGACCCTGAA
Dppa3 REV	GCTTGACACCGGGGTTTAG
Zp3 FW	TTTCGGCATTTCAAGTCCC
Zp3 REV	GGTGATGTAGAGCGTATTTCTG
Oosp1 3'UTR FW	CATCACCATTGAatggtctggtgatttcttatctcc
Oosp1 3'UTR REV	GCGGGTTTAAACttagacacggcactaatggg
Oosp1 YFP FW	gtgccgtgtctaaGTTTAAACCCGCTGATCAGCCTC
Oosp1 YFP REV	tcaccagaccatTCAATGGTGATGGTGATGATGAC
Obox5 3'UTR FW	CATCACCATTGAcatatcagatgactggcttac
Obox5 3'UTR REV	GCGGGTTTAAACaaagaaatttaaatttactattttctcc
Obox5 YFP FW	ttaaatttctttGTTTAAACCCGCTGATC

Obox5 YFP REV	tcatctgatatgTCAATGGTGATGGTGATG
Cenpe 3'UTR FW	CATCACCATTGAatgcccctgtcccgtc
Cenpe 3'UTR REV	AGCGGGTTTAAACccttcaagacctttattcttcgc
Cenpe YFP FW	aaaggtcttgaaggGTTTAAACCCGCTGATCAGCCTC
Cenpe YFP REV	ggacaggggcatTCAATGGTGATGGTGATGATGAC