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Supplemental information

Regulation of RNA localization during oocyte maturation by dynamic RNA-ER association and remodeling of the ER

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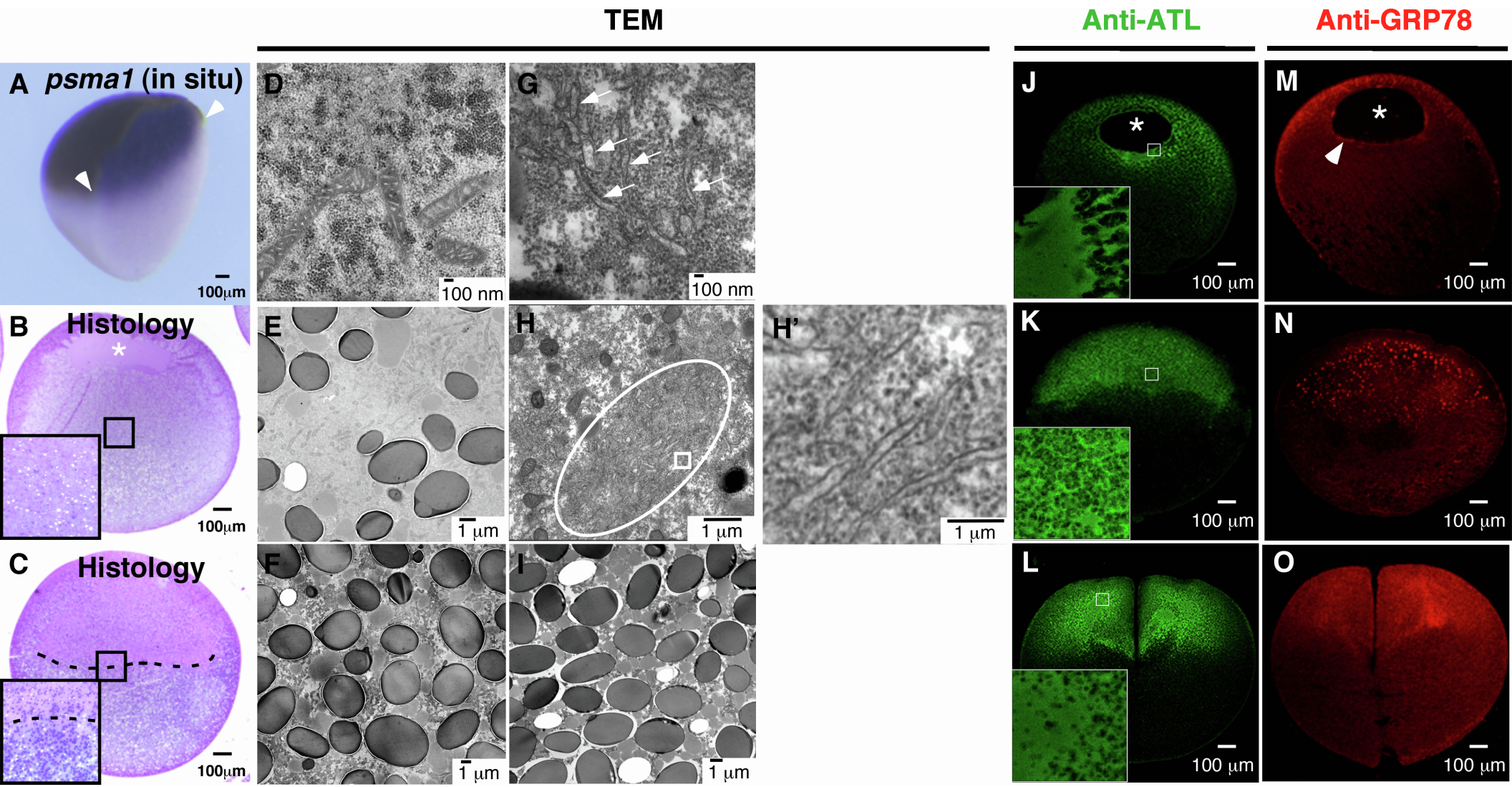
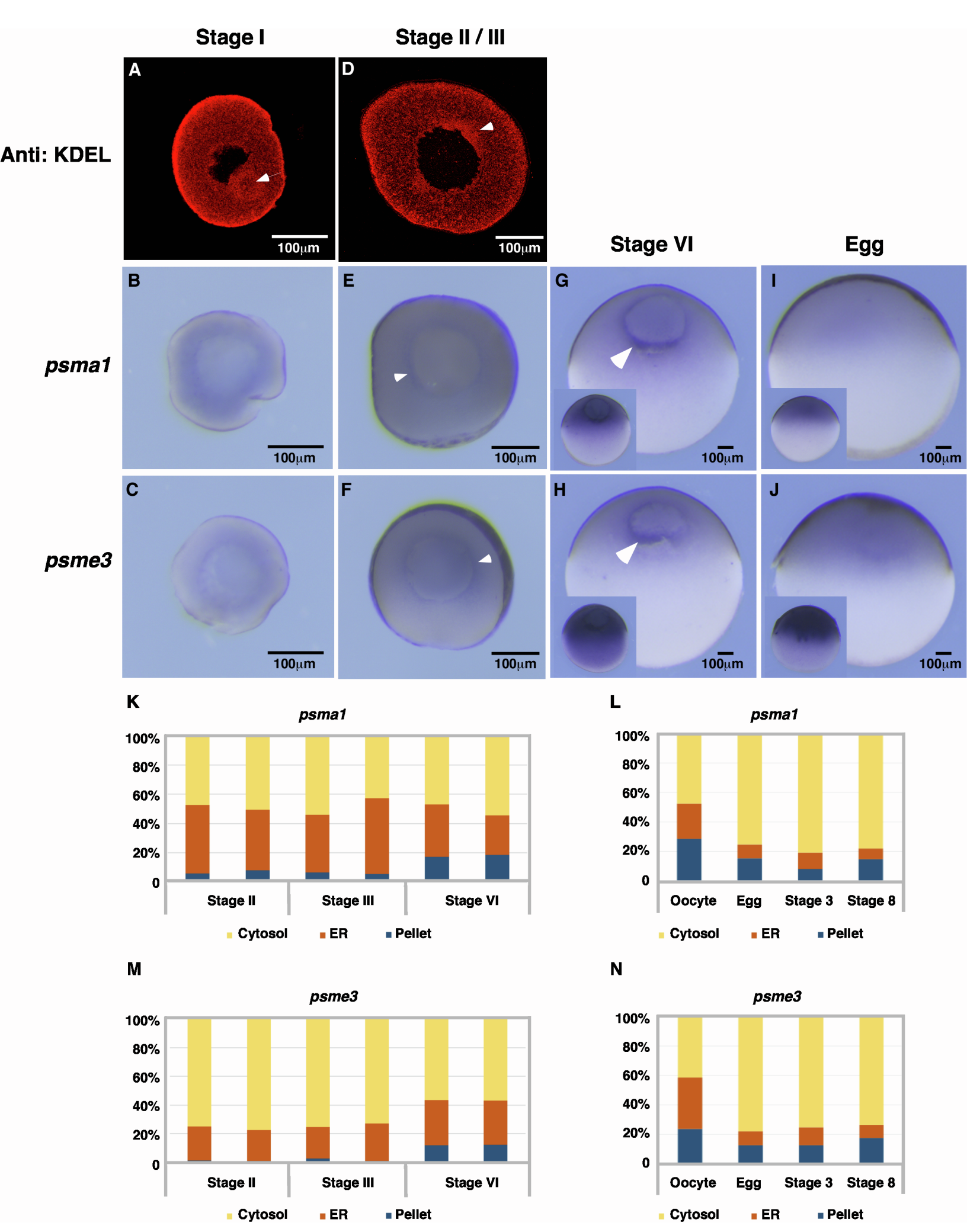
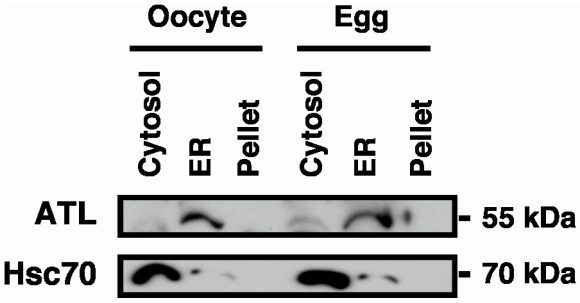

TEM
Anti-ATL
Anti-GRP78
A psma1 (in situ)
100μm
B Histology
100μm
C Histology
100μm
D
100 nm
G
100 nm
E
1 μm
H
1 μm
H'
1 μm
F
1 μm
I
1 μm
J
100 μm
M
100 μm
K
100 μm
N
100 μm
L
100 μm
O
100 μm

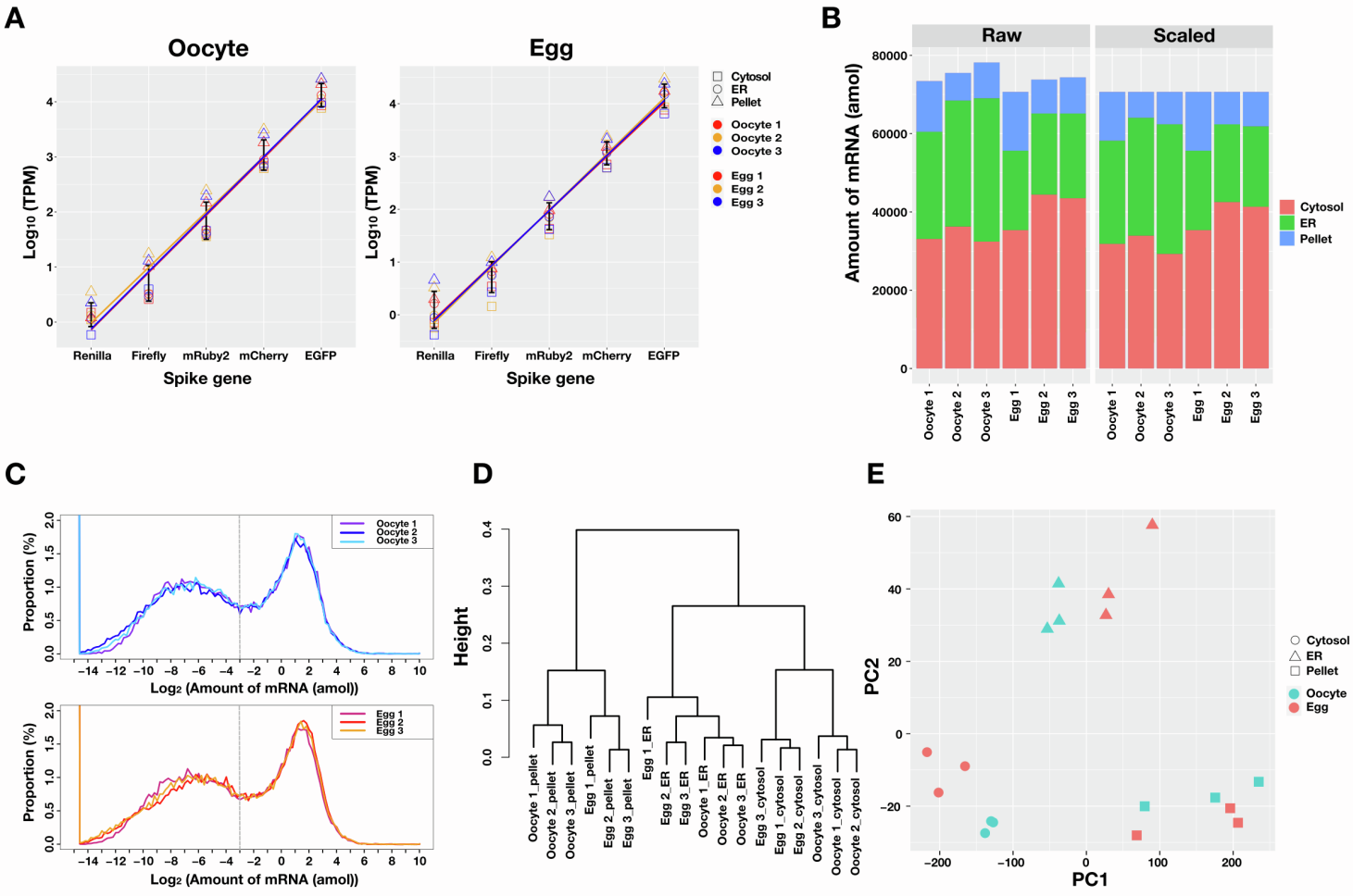
S-Fig 1. ER dynamics during the OET. Related to Figure 2. A. *In situ* hybridization of a hemi-sectioned egg, showing the expression of *psma1*. White arrowheads marked the lower edge of the pigmented area on the surface of the animal hemisphere. **B** and **C.** Histology of a fully-grown oocyte (**B**) and egg (**C**). Dashed lines mark the upper border of the heavy yolk platelet-rich territory in the egg. Inserted at the lower right corner are high-magnification images of the boxed areas in the equatorial region. **D** – **I**. Representative TEM images from the animal (**D, E, G, H**) and vegetal (**F, I**) hemispheres of an oocyte (**D, E, F**) and an egg (**G, H, I**). White arrows (**G**) and circle (**H**) highlight a small cluster of tubular ER and a dense ER patch in the animal hemisphere of an egg, respectively. **H'** is an enlarged image of the boxed area in **H**. **J – O**. Immunofluorescence staining of pan-Atlastin (**J, K, L**) and GRP78 (**M, N, O**) in oocytes (**J, M**), eggs (**K, N**), and 2-cell stage embryos (**L, O**). The position of GV in J and M was marked by "*". White arrowheads in M point to dense ER patches associated with GV.


Stage I
Stage II / III
Stage VI
Egg
Anti: KDEL
psma1
psme3
A
D
B
E
G
I
C
F
H
J
100μm
K
psma1
L
psma1
M
psme3
N
psme3
100%
80%
60%
40%
20%
0
Stage II
Stage III
Stage VI
Oocyte
Egg
Stage 3
Stage 8
Cytosol
ER
Pellet

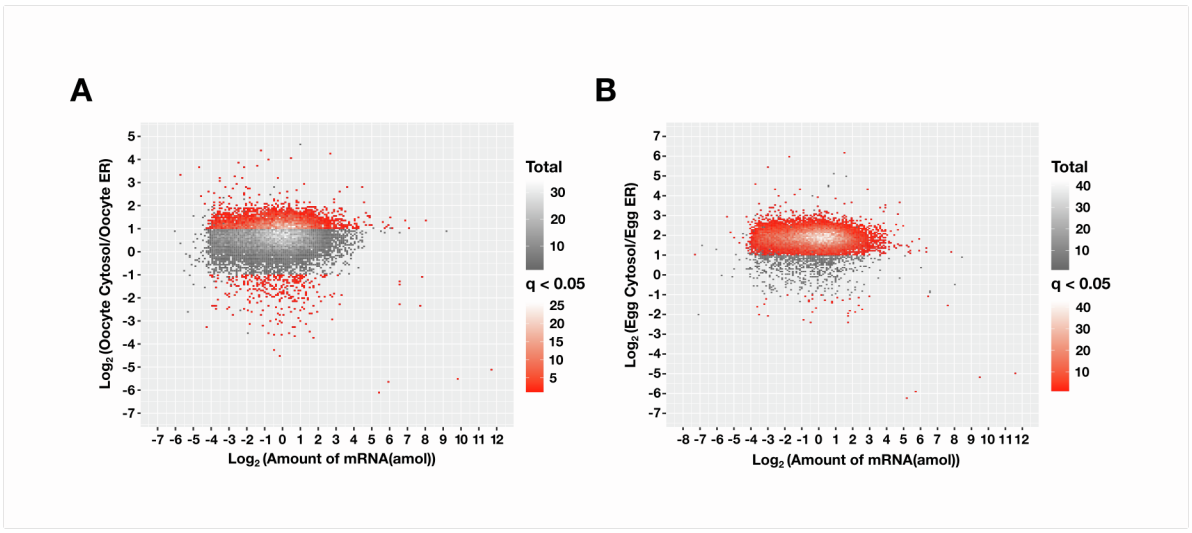
S-Fig 2. Association between proteasome mRNAs and the ER during oogenesis and early development. Related to Figure 3. A and **D.** Immunofluorescence staining of KDEL shows the distribution of the ER in stage I (**A**) and stage II/III (**D**) oocytes. **B, C, E, F-J**. *In situ* hybridization of hemi-sectioned oocytes, showing the expression of *psma1* and *psme3* in stage I (**B** and **C**), stage II/III (**E** and **F**), stage VI (**G** and **H**) oocytes, and mature eggs (**I** and **J**). All these samples were stained in BM-purple for 3 hours. Inserts at the lower left corner in **G, H, I, and J** are samples being stained in BM-purple overnight. **K-N.** Fractionation RT-qPCR results show the percentage distribution of *psma1* (**K** and **L**)*,* and *psme3* (**M** and **N**) across the cytosolic, ER, and pellet fractions during oogenesis (**K** and **M**) and early development (**L** and **N**).


Oocyte
Egg
Cytosol
ER
Pellet
Cytosol
ER
Pellet
ATL
55 kDa
Hsc70
70 kDa

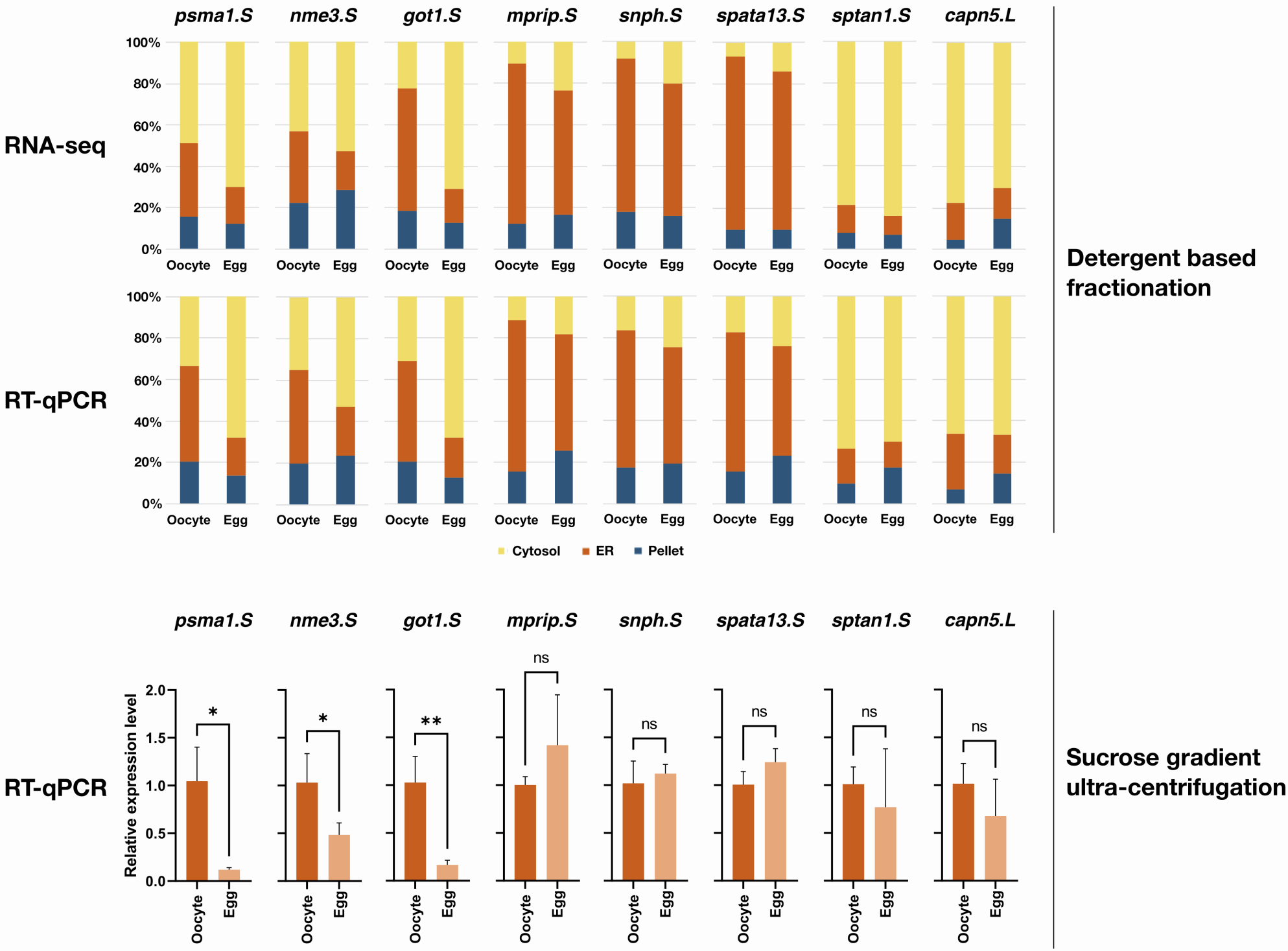
S-Fig 3. Western blot shows efficient fractionation of *Xenopus* oocytes and eggs. Related to Figure 3. Fractionated samples were subjected to western blot analysis, using anti-pan-Atlastin and anti-Hsc70 antibodies. Results show that Atlastin and Hsc70 were enriched in the ER and cytosolic fractions, respectively**.**


A
Oocyte
Egg
Log10 (TPM)
Renilla
Firefly
mRuby2
mCherry
EGFP
Spike gene
Cytosol
ER
Pellet
Oocyte 1
Oocyte 2
Oocyte 3
Egg 1
Egg 2
Egg 3
B
Raw
Scaled
Amount of mRNA (amol)
C
Proportion (%)
Log2 (Amount of mRNA (amol))
D
Height
E
PC1
PC2
F
Distribution
Oocyte 1
Oocyte 2
Oocyte 3
Egg 1
Egg 2
Egg 3

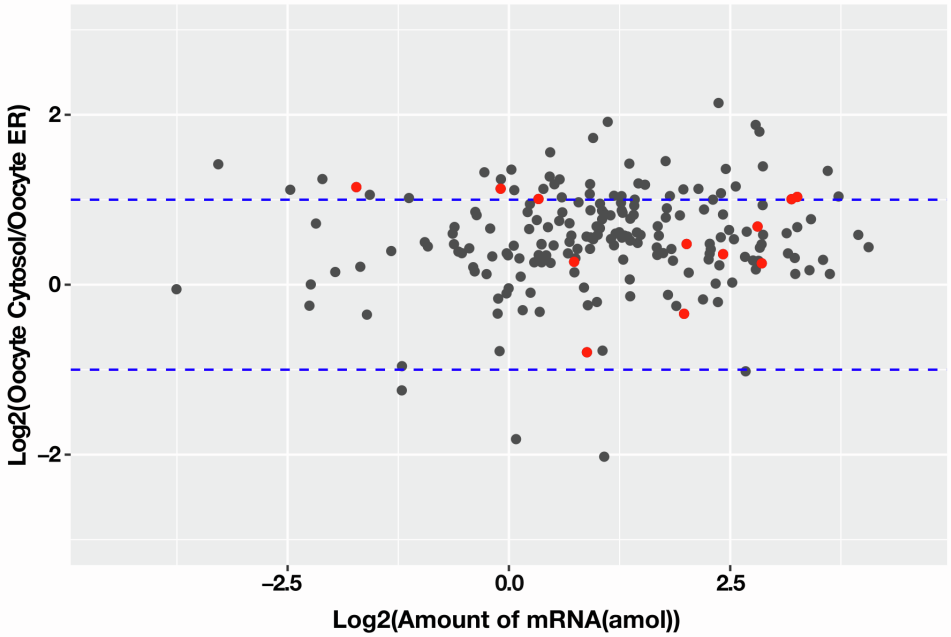
S-Fig 4. Transcriptomic quantification in the cell fractionation for the mRNA-ER association analysis during oocyte maturation. Related to Figure 4. A. The graph shows the linear correlation of the RNA-seq estimated TPM values and the actual input amount of five spike-in RNAs in the oocyte and mature egg. The linear regression of them was used to convert the TPM to the absolute atto-moles (amol). **B.** The bar graph shows the distribution of total RNAs in each cytosolic, ER, and pellet fraction between oocyte and mature egg. We normalized the total amount of RNAs for each sample (we used the same number of eggs and oocytes) presented on the right side. **C.** Histogram shows the distribution of the mRNA absolute amount in the oocyte (blue series) and mature egg (red series). The dashed line indicates 0.125 amol, and transcripts less than 0.125 amol were filtered out. A total of 17,811 transcripts were used for further analysis. **D-E.** Hierarchical clustering dendrogram **(D)** and principal component analysis (PCA) plot **(E)** of three biological replicates in the oocyte and mature egg, respectively. **F.** Histogram shows the distribution of the proportion of transcripts in each cytosolic (pink), ER (blue), and pellet (grey) fraction in the oocyte (top) and mature egg (bottom).

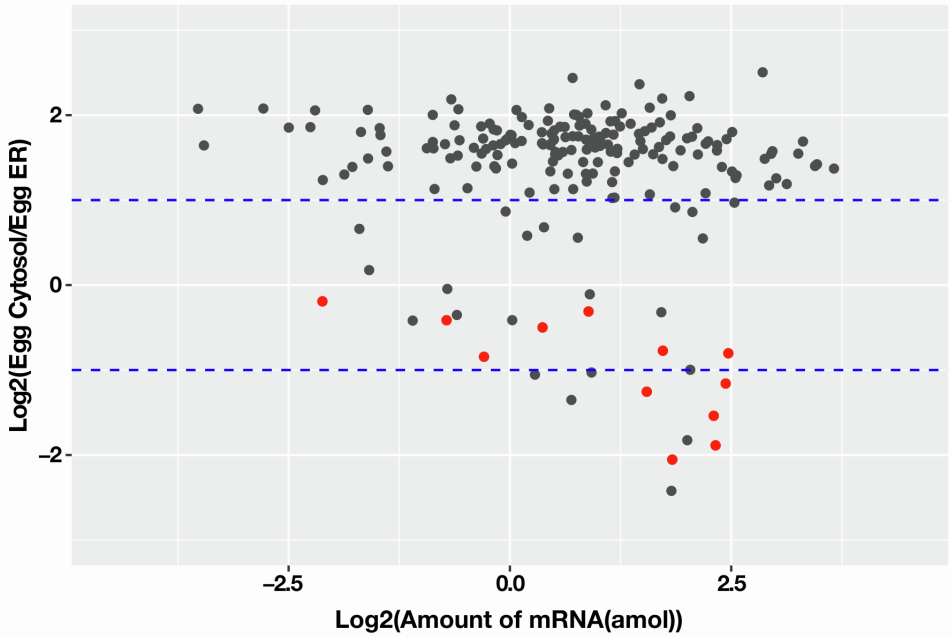

A
B
Log2 (Oocyte Cytosol/Oocyte ER)
Log2 (Amount of mRNA(amol))
Total
q < 0.05
Log2 (Egg Cytosol/Egg ER)
Log2 (Amount of mRNA(amol))
Total
q < 0.05

S-Fig 5. Down-regulation of the mRNA-ER association during oocyte maturation. Related to Figure 4. A. B. MA plots show the difference in the ratio of the cytosol to ER between the oocyte **(A)** and mature egg **(B)**. The x-axis indicates the amount of RNAs, and the y-axis indicates the ratio of transcripts between the cytosol and ER. A differentially localized gene (red) was defined as a q-value less than 0.05 and cytosol to ER ratio difference greater than 2 (Grey - total 17,811 transcripts). 4,179 and 432 transcripts in the oocyte are significantly biased to the cytosol and ER, respectively. However, these numbers were more skewed in the mature egg, as 16,491 transcripts in the cytosol and 37 in the ER were differentially localized.


psma1.S
nme3.S
got1.S
mprip.S
snph.S
spata13.S
sptan1.S
capn5.L
RNA-seq
RT-qPCR
100%
80%
60%
40%
20%
0%
Oocyte
Egg
Cytosol
ER
Pellet
Detergent based fractionation
RT-qPCR
Relative expression level
2.0
1.5
1.0
0.5
0.0
*
**
ns
Sucrose gradient ultra-centrifugation

S-Fig 6. Validation of fractionation RNA-seq. Related to Figure 4. The percentage distribution of *psma1, nme3.S got1.S, mprip.S*, *snph.S*, *spata13.S, sptan1.S,* and *capn5.L* across the cytosolic, ER, and pellet fractions were calculated using the RNA-seq data and plotted into graphs in the upper panel. The middle panel is RT-qPCR validation of the distribution of the above transcripts using RNAs purified from the detergent-based fractionation. The lower panel is RT-qPCR validation of the ER association of the above transcripts using RNAs purified from microsomes. Purification of microsomes was carried out using the standard sucrose density gradient ultra-centrifugation protocol. Among all transcripts being tested, only *psma1.S, nme3.S,* and *got1.S* show a decreased ER association after oocyte maturation. RT-qPCR was performed on biologically independent triplicated samples and Two-tailed Students' *t*-tests were performed. *$p<0.05$; **$p<0.01$.


Log2(Oocyte Cytosol/Oocyte ER)
2
0
−2
−2.5
0.0
2.5
Log2(Amount of mRNA(amol))


Log2(Egg Cytosol/Egg ER)
2
0
−2
−2.5
0.0
2.5
Log2(Amount of mRNA(amol))

S-Fig 7. The majority of transcripts exhibiting an increase in the polysome fraction during oocyte maturation are released from the ER into the cytosol. Related to Figure 4. Transcripts showing a significant increase in the polysome fraction during oocyte maturation are plotted in the MA plots to show the difference in the ratio of the cytosol to ER in the oocyte **(left)** and mature egg **(right)**. The x-axis indicates the amount of RNAs, and the y-axis indicates the ratio of transcripts between the cytosol and ER. Transcripts being significantly released from the ER into the cytosol are shown in black. Red dots represent transcripts whose ER association remains unchanged.