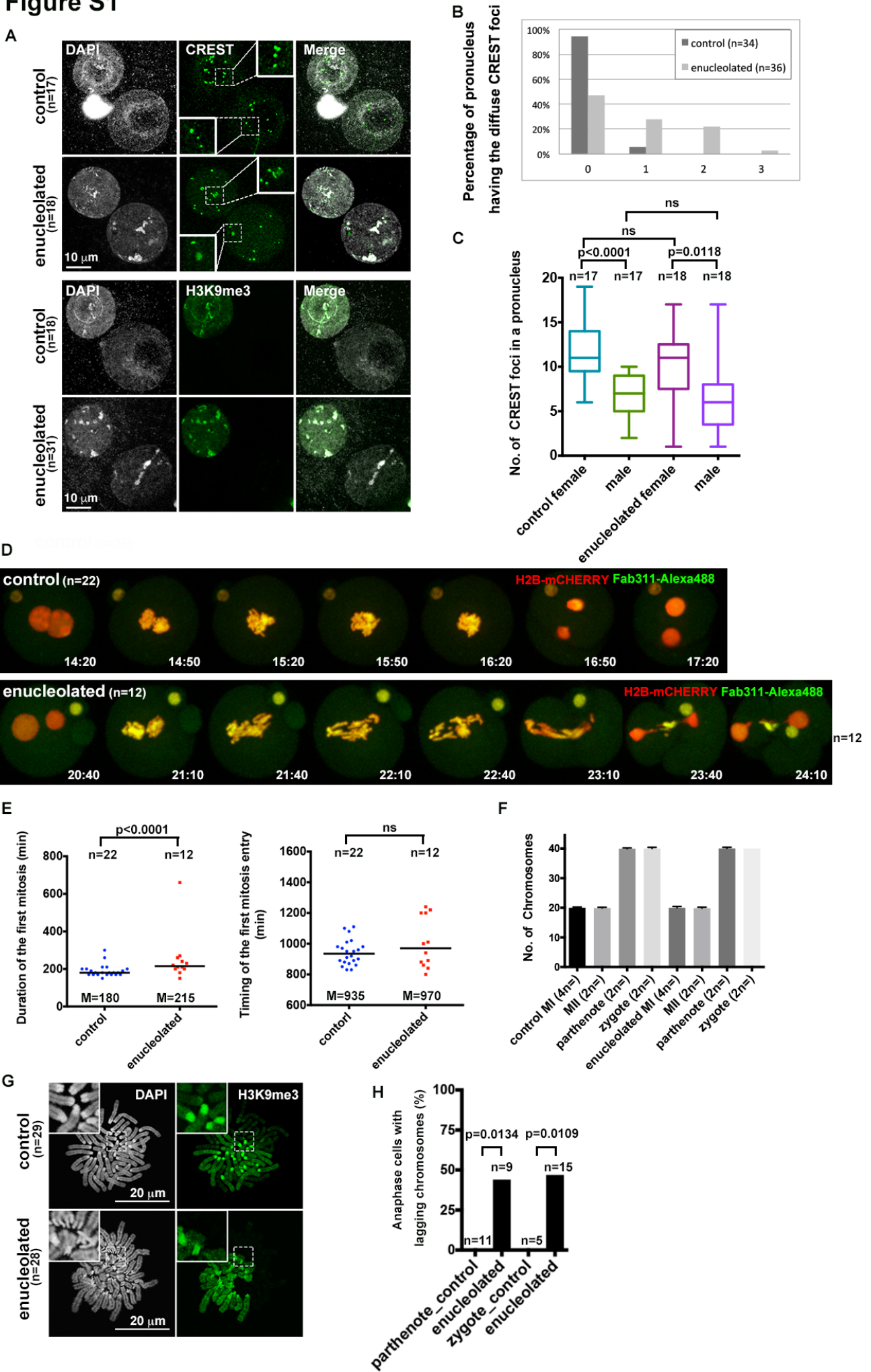# Figure S1

Figure S1

Loss of the oocyte nucleolus caused disorganization of higher chromatin in pronuclei and chromosome mis-segregation during the first mitosis in zygotes.

(A) Immunostaining using zygotes from enucleolated oocytes with the antibodies (green) shown in the panels. Insets show the diffuse CREST signals (>2 μm^2) in each pronucleus at 2-fold magnification. DNA was stained with DAPI (grey). Control, zygotes from nucleolus-reinjected oocytes. (B) Percentage of pronuclei having diffuse CREST signals in zygotes from control/enucleolated oocytes. The numbers on the x-axis of the graph indicate the number of diffuse signals in each pronucleus. (C) The number of CREST foci in each pronucleus of zygotes from control/enucleolated oocytes. The boxes show the median, 25th, and 75th percentiles, and the bars show the 10th and 90th percentiles. Two-tailed Mann-Whitney test. ns, not significant. (D) Representative stills from live cell imaging of zygotes from control/enucleolated oocytes. Chromosomes were labeled by H2B-mCHERRY (red) and chromosome condensation was visualized by Fab311-Alexa488 (green). The numbers in each panel show the time after sperm injection (hr:min). (E) The duration and entry timing of the first mitosis in zygotes are plotted. The numbers of zygotes measured in three independent experiments are indicated above the plots. Bars, median (M). Two-tailed Mann-Whitney test. (F) Representative images of chromosome spreads in zygotes stained with H3K9me3 antibody (green) and DAPI (grey). Insets in each panel are 2.5-fold magnifications of the regions shown in the dash-lined boxes. (G) The number of chromosomes in first meiosis (MI), second meiosis (MII), and parthenotes/zygotes from control/enucleolated oocytes. (n>10) Bars, SD. (H) The incidence rate of chromosome lagging during the first anaphase in parthenotes/zygotes from enucleolated oocytes. Two-tailed Chi-square test. (A-G, H) n, the numbers of parthenotes/zygotes measured in three independent experiments.
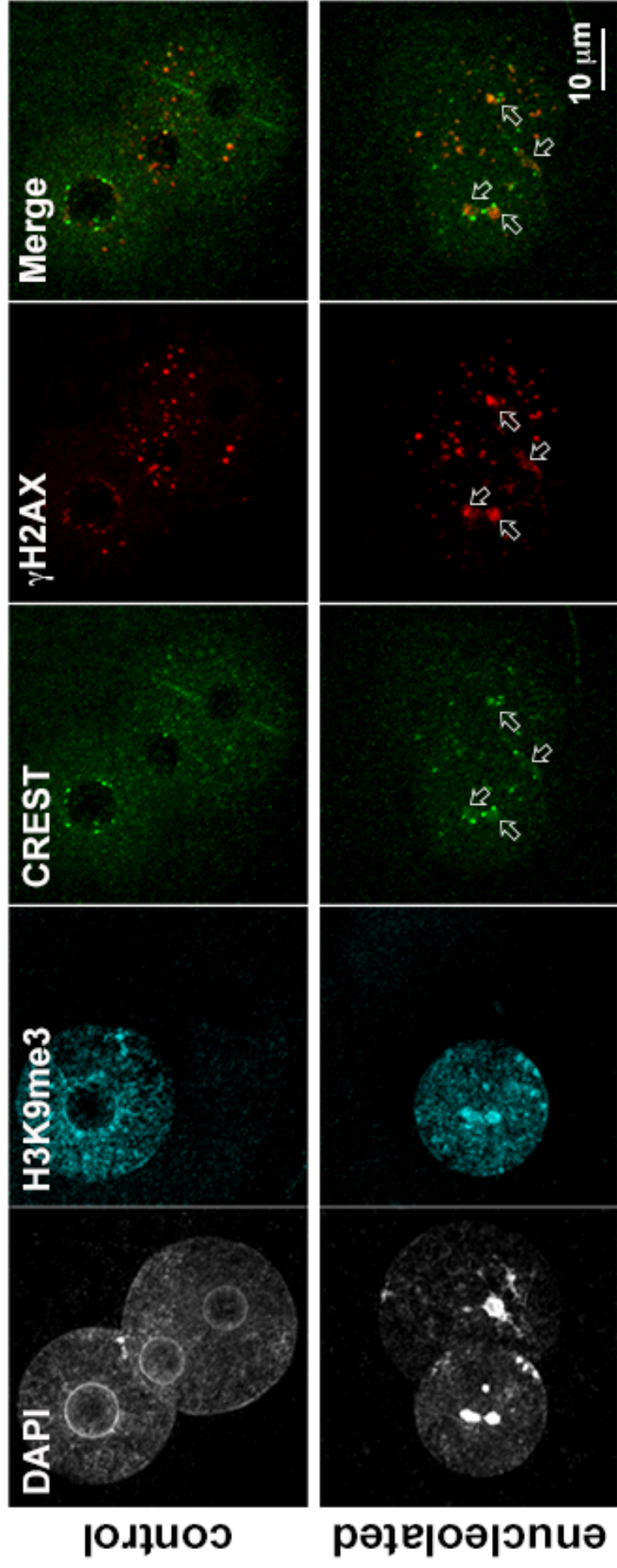
Figure S2

Figure S2

Loss of the oocyte nucleolus caused DNA damage especially at the diffused CREST signals of pronuclei in zygotes.

Immunostaining using zygotes from enucleolated oocytes 12 hr after fertilization with the antibodies shown in the panels. Arrows indicate the regions positive for γ-H2AX and diffused CREST signals. (control: n= 8, enucleolated: n=11)
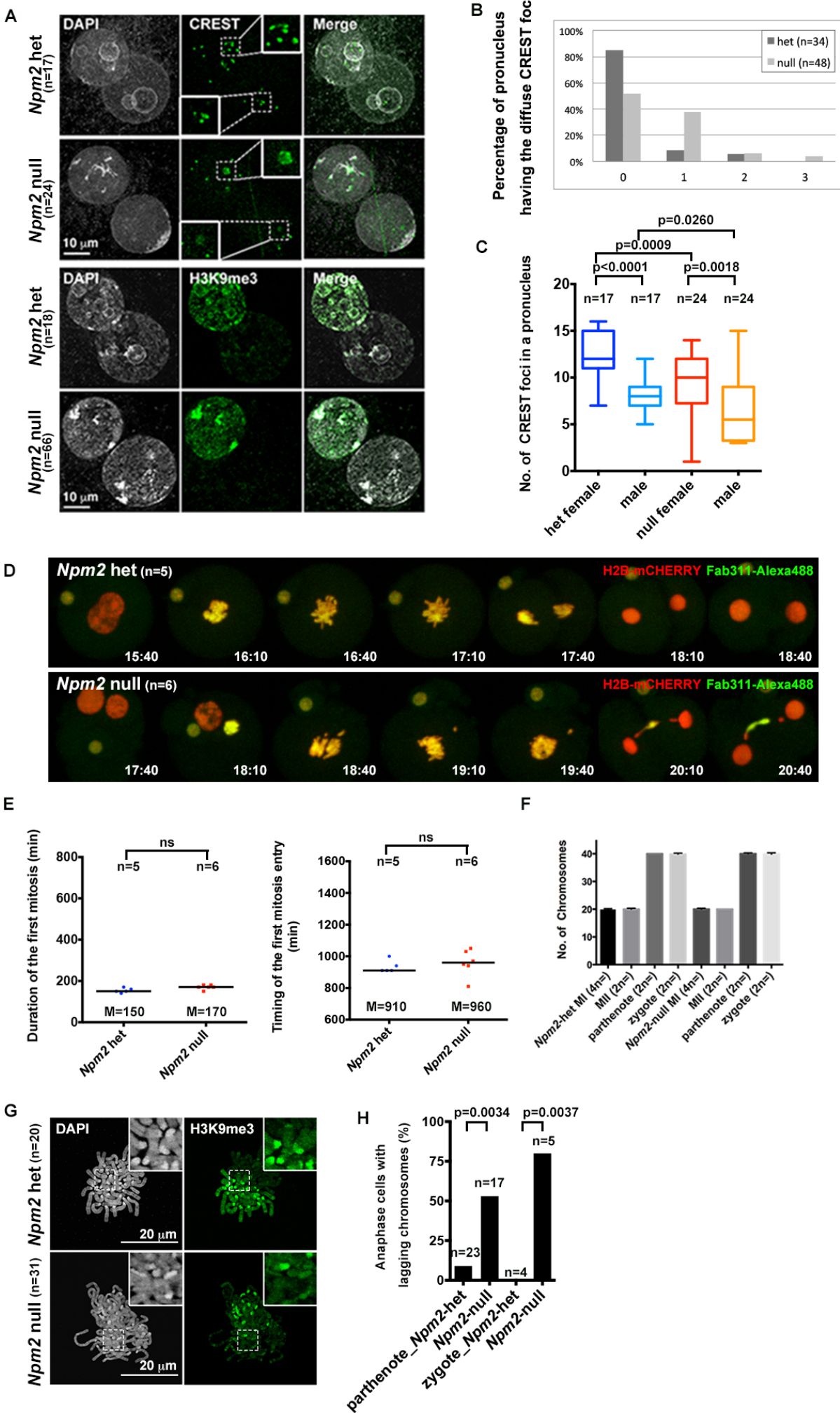
Figure S3

Figure S3

Zygotes from *Npm2*-null oocytes also showed defects in higher-chromatin organization in pronuclei and chromosome mis-segregation during the first mitosis.

(A) Zygotes from *Npm2*-null oocytes stained with the antibodies shown in the panels (green) and with DAPI (grey). Insets show the diffuse CREST signals at 2-fold magnification. (B) Percentage of pronuclei having diffuse CREST signals in zygotes from *Npm2*-het/-null oocytes. (C) The number of CREST foci in each pronucleus of zygotes from *Npm2*-het/-null oocytes. Two-tailed Mann-Whitney test. ns, not significant. (D) Representative stills from live cell imaging of zygotes according to the method shown in Figure S1D. (E) Plots of the duration and entry timing of the first mitosis in zygotes. The numbers of zygotes measured in three independent experiments are indicated above the plots. Bars, median (M). Two-tailed Mann-Whitney test. (F) Representative images of chromosome spreads in zygotes stained with H3K9me3 antibody (green) and with DAPI (grey). Insets in each panel are 2.5-fold magnifications. (G) The number of chromosomes in first meiosis (MI), second meiosis (MII), and parthenotes/zygotes from *Npm2*-het/-null oocytes. (n>10) Bars, SD. (H) The incidence rate of chromosome lagging during the first anaphase in parthenotes/zygotes from *Npm2*-het/-null oocytes. Two-tailed Chi-square test. (A-G, H) n, the numbers of parthenotes/zygotes measured in three independent experiments.

Figure S4

A

	Npm2 het +eGfp 11 ug			*Npm2* null			
				+eGfp 11 ug	+Npm2 1.2 ug	+Npm2 11 ug	+Npm2 11 ug
No. of Oocytes	10	20	30	30	30	5	30

α-NPM2

α-TUB

B

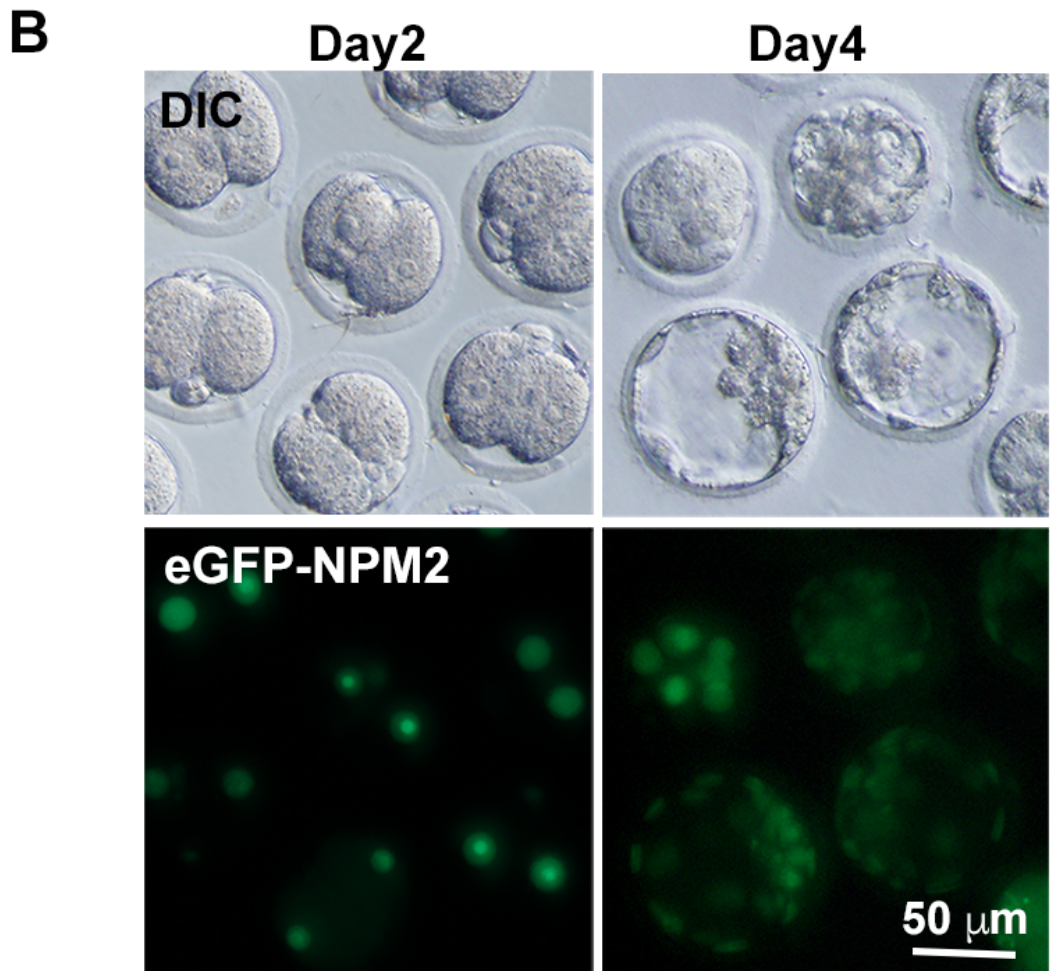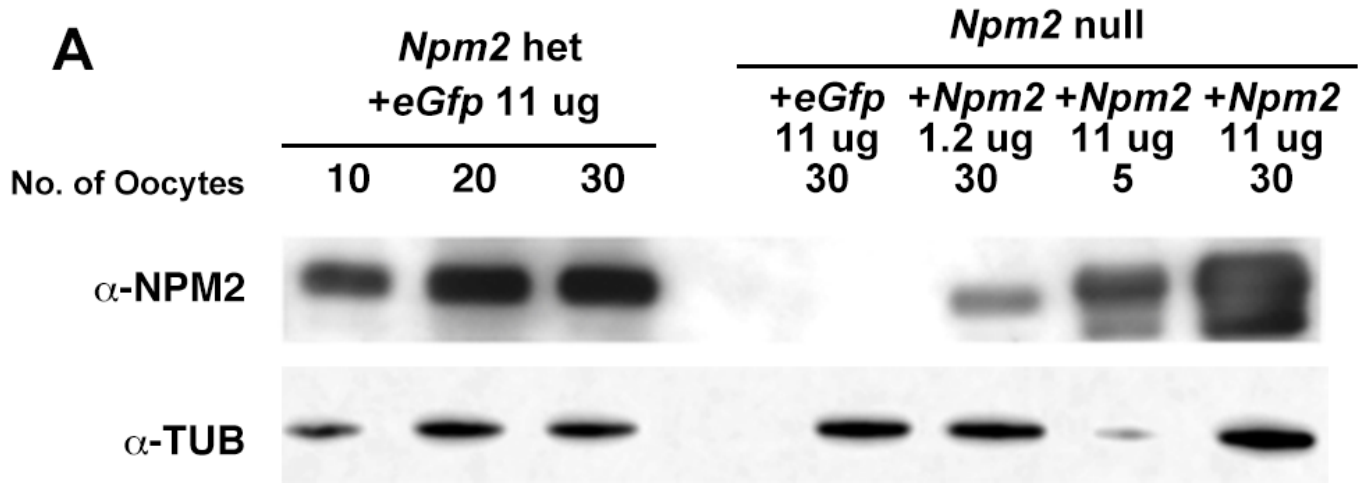Day2   Day4

DIC

eGFP-NPM2

50 μm

Figure S4

The early embryonic development of embryos from *Npm2*-null oocytes was rescued by NPM2 in a concentration-dependent manner.

(A) NPM2 protein expression level in *Npm2*-null oocytes rescued by various concentrations of *Npm2*-mRNAs. The oocyte number in each sample is indicated above the blots and alpha-TUBULIN (TUB) was used as a loading control (n=3). (B) *eGfp-Npm2* mRNA injected into MII oocytes was expressed through to the blastocyst stage. DIC, differential interference contrast.
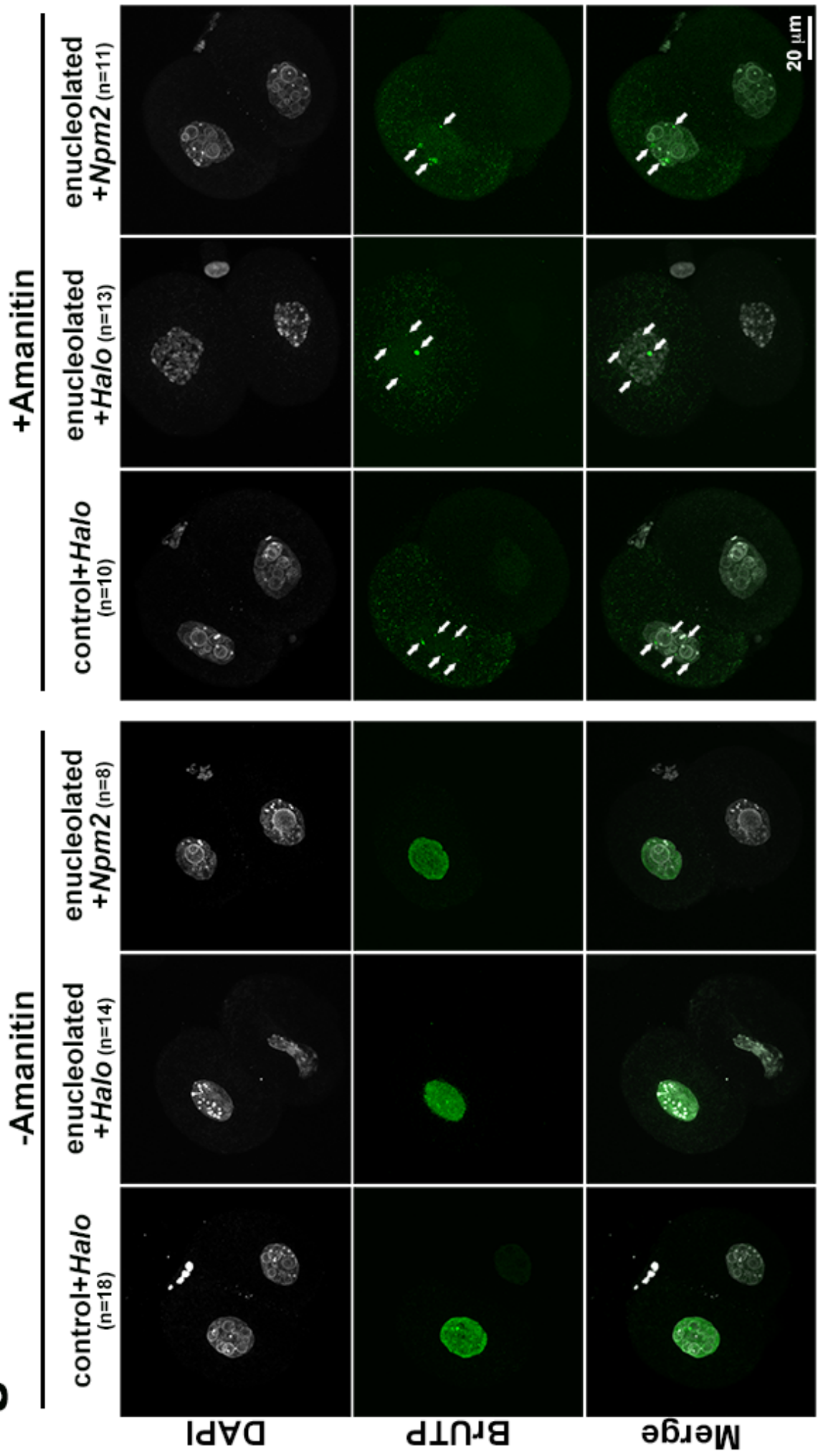
Figure S5

Figure S5

Late 2-cell embryos from enucleolated oocytes resumed nascent transcription of total RNA and r RNA.

Immunostaining using parthenotes from enucleolated oocytes and from enucleolated oocytes injected with *Npm2* mRNA 38-40 hr after artificial activation with the antibodies shown in the panels. The panels under –Amanitin shows nascent transcripts of total RNA, and those under +Amanitin shows nascent transcripts of rRNA (arrows). n, the numbers of embryos measured in two independent experiments.
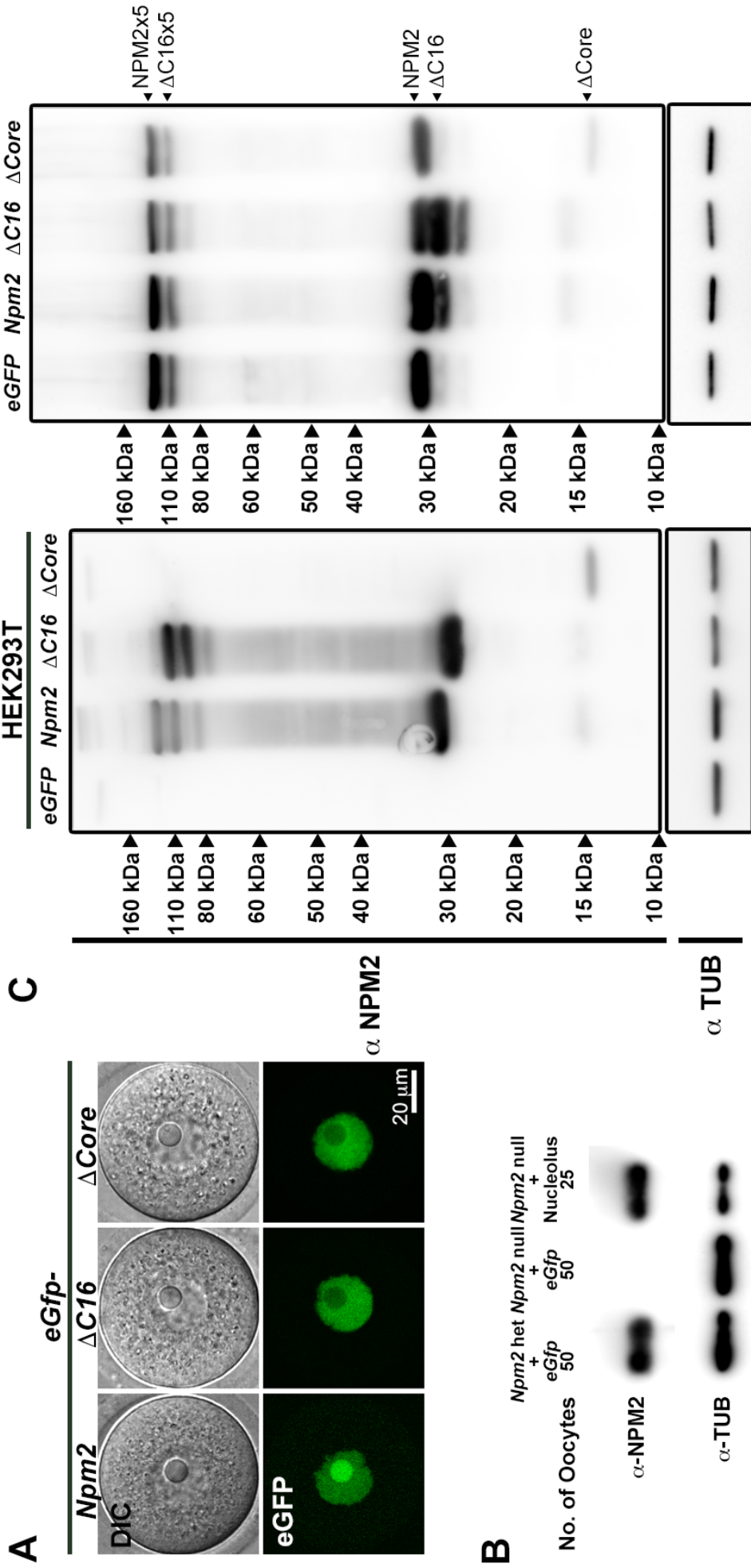
Figure S6

Figure S6

Expression of NPM2 truncation mutants disrupted pentamer formation of endogenous NPM2

(A) Localization of NPM2 truncated mutants in oocytes at the germinal vesicle stage. DIC, differential interference contrast. (B) NPM2 protein expression level in *Npm2*-het oocytes. Half of *Npm2*-null oocytes injected with nucleoli from wild oocytes showed a level of NPM2 expression similar to that of *Npm2*-het oocytes. The oocyte number in each sample is indicated above the blots and TUB was used as a loading control (n=3). (C) NPM2 truncation mutants were expressed in HEK293T cells transiently, and in oocytes by mRNA injection. Each lane was loaded with 5 μg of total proteins from each group that expressed the indicated plasmids in HEK293T cells or 40 oocytes injected with the indicated mRNAs. The oocyte number in each sample is indicated above the blots and TUB was used as a loading control (n=3).

Table S1. The average duration and entry timing of the first mitosis in parthenotes/zygotes from control/enucleolated and *Npm2*-het/-null oocytes

Experimental Group	Average Duration of 1stM (mean±SD)	Average Timing of 1stM Entry (mean±SD)
control Sr^{2+}	176.6±43.04[a]	932.9±73.95[a]
enucleolated Sr^{2+}	235.6±90.76[b]	1085±195.3[b]
Npm2-het Sr^{2+}	183.3±38.46[a]	865.0±30.00[a]
Npm2-null Sr^{2+}	192.5±46.34[a]	897.5±47.31[a]
control ICSI	190.9±33.08[a]	942.7±83.11[a]
enucleolated ICSI	252.5±132.5[b]	1007±165.4[a]
Npm2-het ICSI	154.0±11.40[a]	934.0±39.12[a]
Npm2-null ICSI	170.0±10.95[a]	958.3±84.95[a]

Values with different superscript within the same line are significantly different by two-tailed Mann-Whitney test ($p<0.05$).

Table S2. The list of proteins identified from nucleoli isolated from mouse GV-oocytes

Click here to Download Table S2

Table S3. Developmental potency of embryos from *Npm2*-het/-null oocytes injected with *Npm2* truncated mutant mRNAs

		eGfp	Npm2	ΔC16
		11 µg/µl	11µg/µl	11µg/µl
Npm2 het	No of Embryos Transferred	40	40	40
	No of Pups Obtained	18[a]	21[a]	0
Npm2 null	No of Embryos Transferred	42	61	38
	No of Pups Obtained	0	33[a]	0

Values with different superscript within the same raw are significantly different by chi-square test (p<0.05).