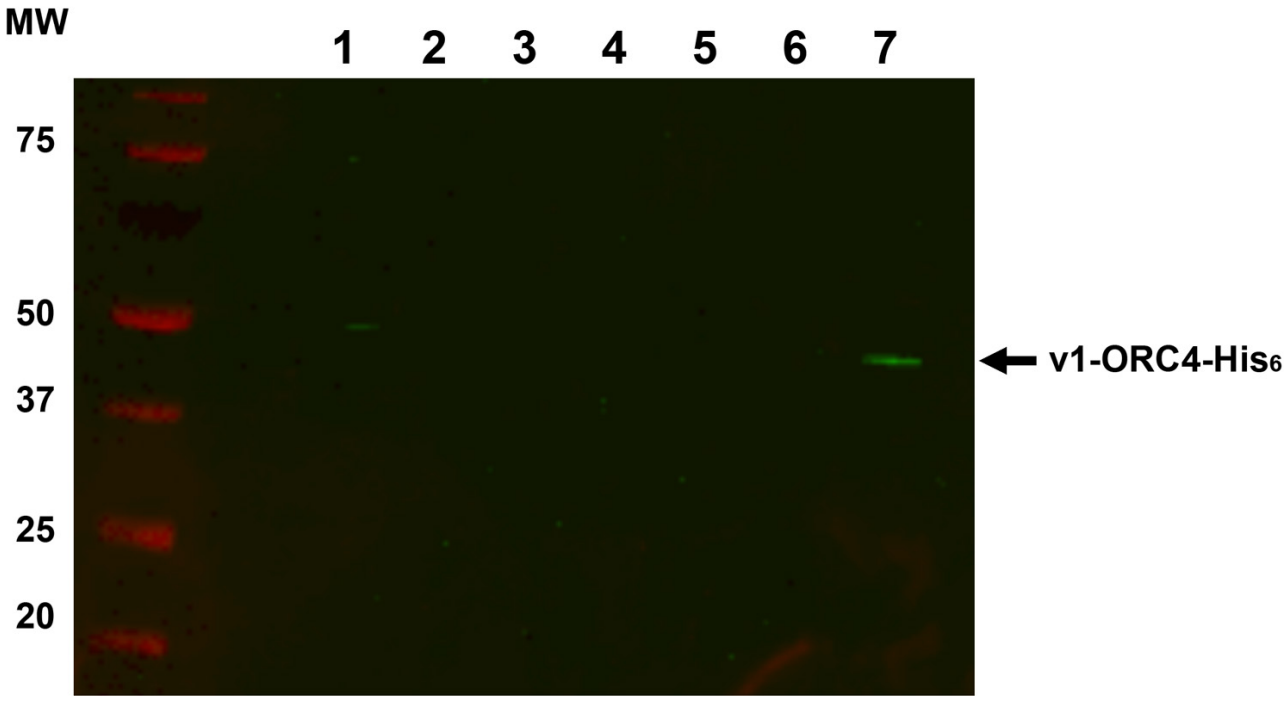
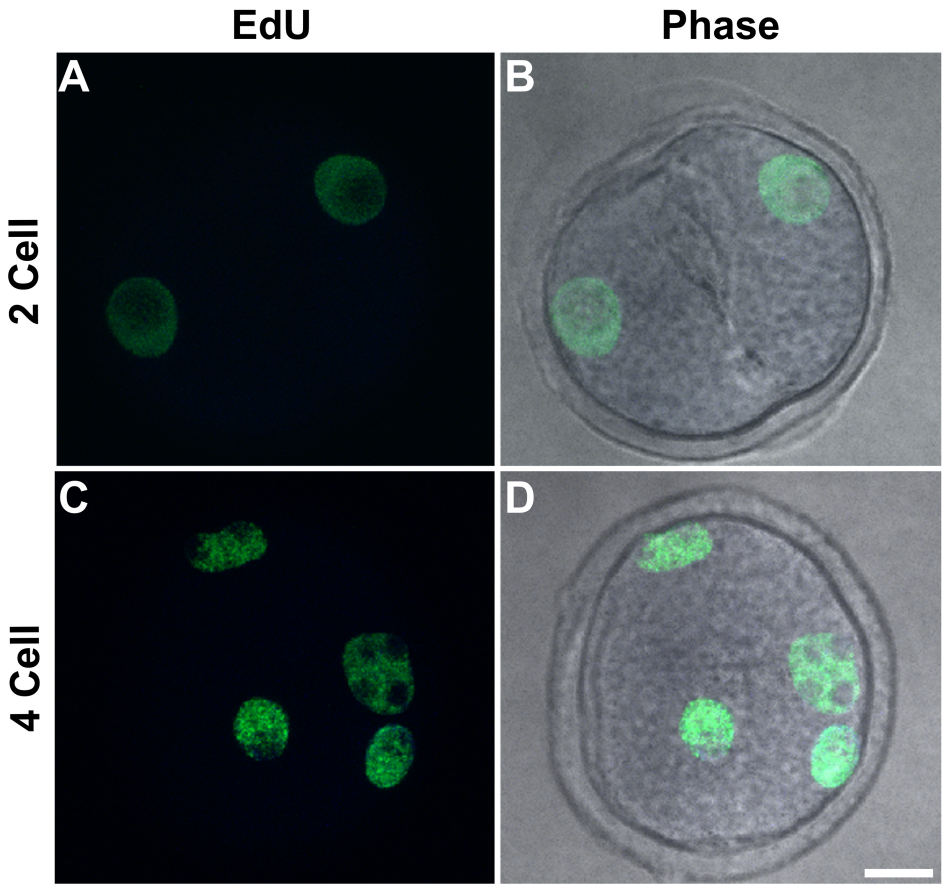
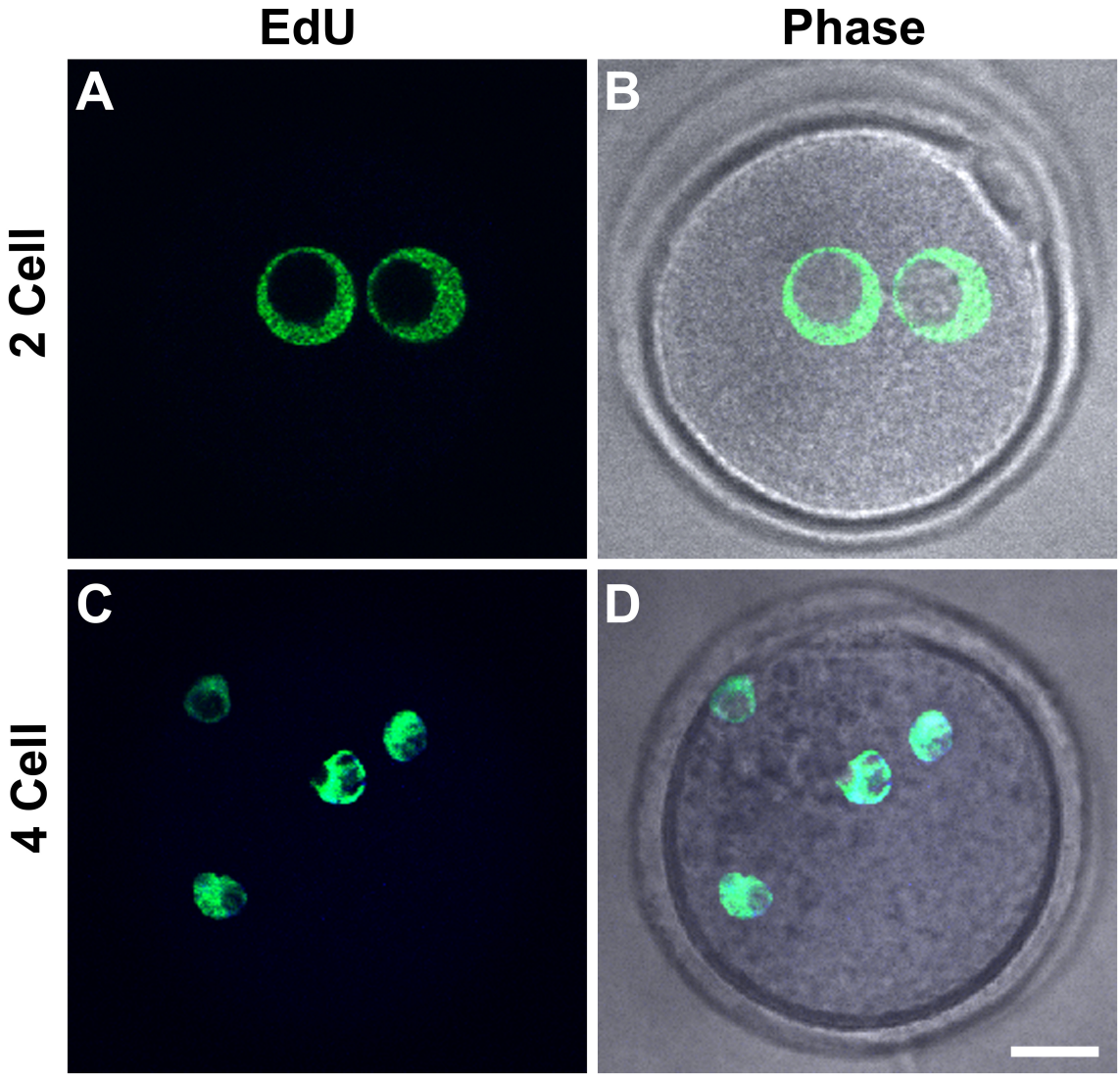
Supplement Figure 1: Confirmation of Antibody Recognition of ORC4. The *his-v1-mORC4* transcript was expressed in bacteria and the protein isolated on a nickel column, then by size exclusion chromatography (SEC). Fractions from the final SEC column are shown below. The isolated HIS-v1-ORC4 was analyzed by western blot using the antibody for ORC4 (C-15 from Santa Cruz Biotechnology) used in this study. The isolated HIS-v1-ORC4 was recognized by the antibody (lane 7). Lane 1, post induction fraction; lane 2, void volume (~90 mLs); lane 3, peak at 120 mLs; lane 4, peak at 130 mLs; lane 5, peak at 150 mLs; lane 6, peak 170 mLs; lane 7, 205 mLs (~ 47 kDa MW based on calibration of the column).



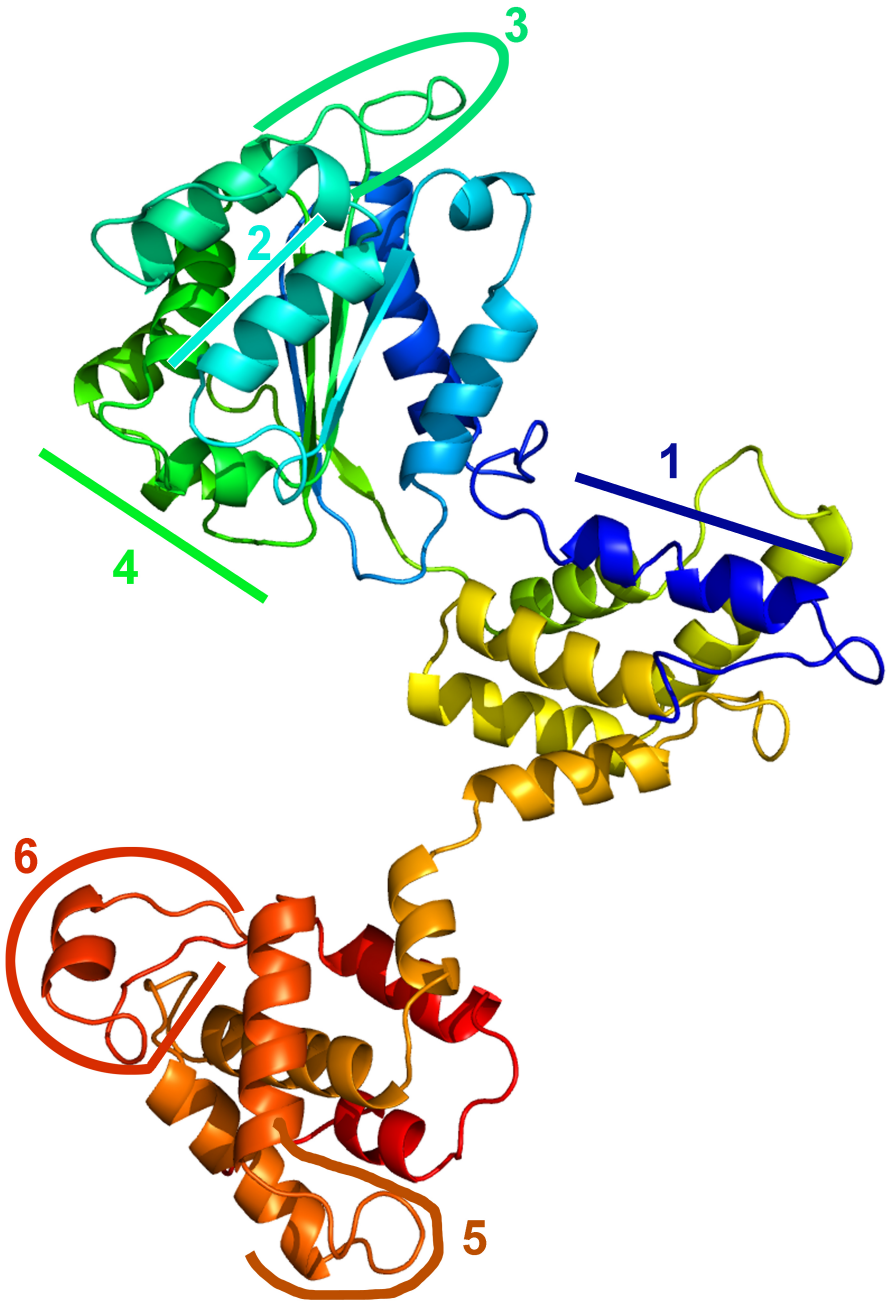
Supplementary Figure 2. BFA Treated Oocytes. MII oocytes were treated with 10 mM brefeldin (BFA) for two hours prior to activation. Oocytes were then placed in media that contained 10 mM $SrCl_2$ and incubated with 10 µm EdU, without BFA. After 8 hrs (A, B) or 24 hrs (C, D) the oocytes were fixed, and stained for the presence of EdU. (A-B) MII oocytes treated with BFA and allowed to develop for 8 hrs after activation divided into two equal-sized cells and replicated their DNA. (C-D) After 24 hours, the parthenogenetically activated oocytes divided into four cells, and continued DNA replication. Bar = 10 µm.



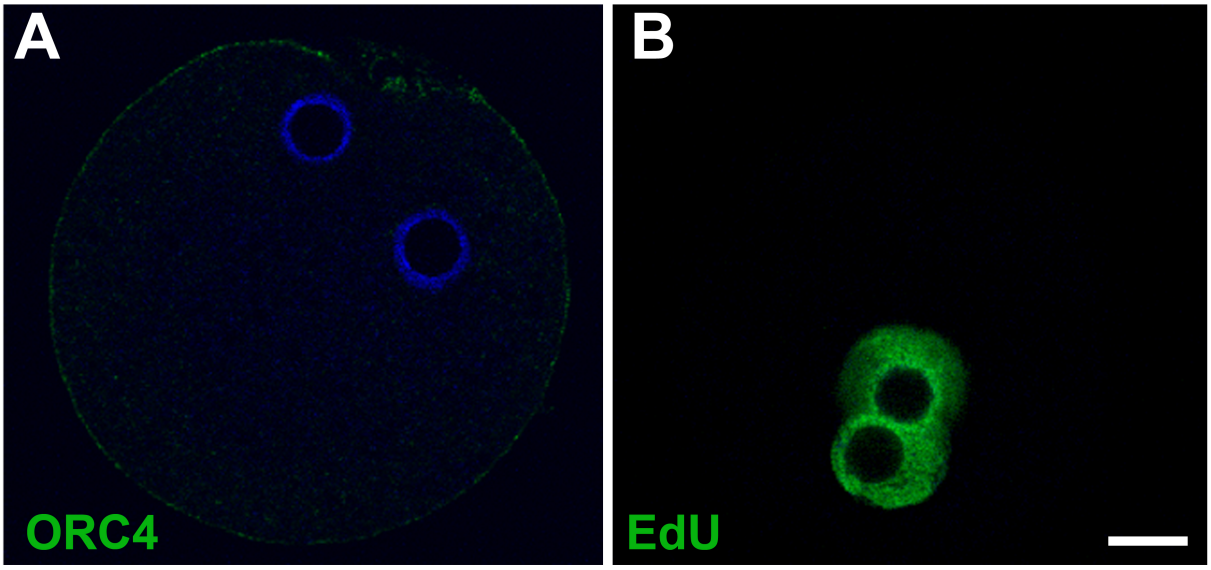
Supplemental Figure 3. Effect of Cytochalasin B on Polar Body Extrusion and ORC4 Cage Formation. MII oocytes were treated with Cytochalasin B (10 µg/ml) for 2 hrs, then activated with $SrCl_2$ in the presence of 10 µM EdU. The parthenogenetically activated oocytes were then incubated for up to 24 hrs, then stained for ORC4 and EdU incorporation. No ORC4 cages were formed (data not shown). At 8 hrs post activation, two pronuclei were formed inside the oocyte (A, B) and EdU staining indicated that DNA synthesis was not inhibited. By 24 hrs, the oocyte had four pronuclei that were all positive for EdU staining, suggesting that even though cytochalasin B inhibited polar body extrusion and oocyte cytokinesis, DNA synthesis and nuclear division was not. This supports the different roles of ORC4, one in DNA synthesis and one for DNA replication.



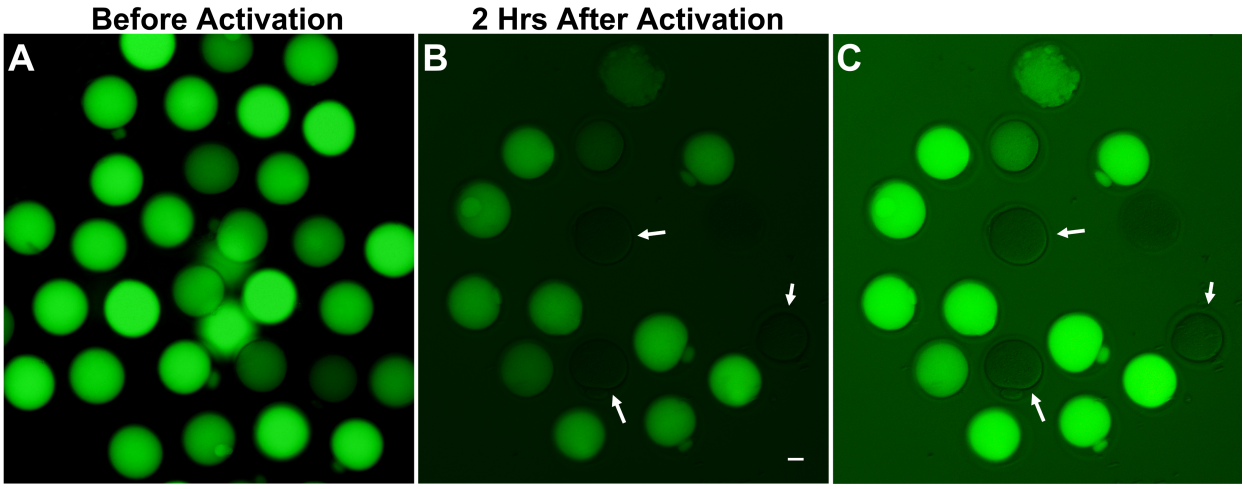
Supplemental Figure 4. Putative 3D Structure of Murine ORC4. Using the Phyre[2] software (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) we generated a predicted 3D model for the full length murine v1-ORC4 protein. The position of the six peptides used in this study are shown.



Supplemental Figure 5. Oocytes Injected with Peptide 10 Progress to 2 PN and Replicate Their DNA. MII oocytes were injected with peptide 10 and then parthenogenetically activated with $SrCl_2$, and incubated with 10 µm EdU. At 8 hrs post activation, the oocytes were fixed and stained for ORC4 (A) or EdU (B). A significant number of oocytes did not extrude the second polar body and had two pronuclei, instead (SFig. 1-2, E&F, box). These oocytes had detectable levels of ORC4 near the oolemma, but did not form ORC4 cages (A). Even though the ORC4 cage was inhibited, these oocytes still replicated their DNA (B). Bar = 10 µm.



Supplemental Figure 6. Murine Ooctyes Express Injected mRNA. To test our ability to inject mRNA into oocytes, we prepared mRNA for the green fluorescent protein, GFP, and injected this into MII oocytes 30 min before parthenogenetic activation with $SrCl^2$. (A) At 30 min post injection, and before activation, fluorescent images were taken of live oocytes. Most oocytes expressed the GFP protein. (B) By 2 hrs post activation, most oocytes were still expressing GFP, though some had stopped (arrows). (C) The same image as (B) but shown with enhanced brightness to more clearly show the oocytes that had stopped expressing GFP. The images in A and B are shown at the same intensity.



Supplemental Table 1. Fisher's Test for Peptides. The number of 2 PN embryos that resulted from peptide injection were compared by the Fisher's test, and the p values are shown. In the second column (Exps.) the experiments for each group were compared with each other. At least two experiments were conducted for each group. In the other columns, the total results from each peptide was compared with each other. Significant values (shown in green) were $p < 0.05$. Insignificant values are shown in red.

	Exps.	Peptides				
Peptide		1	6	9	10	14
1	0.2391					
6	1.0000	0.0004				
9	1.0000	0.0001	1.0000			
10	1.0000	0.0612	0.0141	0.0003		
14	0.5005	0.0001	1.0000	0.5053	0.0013	
16	1.0000	0.0001	1.0000	0.2274	0.0115	1.0000

Supplemental Table 2. Fisher's Test for mRNA ORC4 Fragments. The number of 2 PN embryos that resulted from mRNA injection were compared by the Fisher's test, and the p values are shown. In the second column (Exps.) the experiments for each group were compared with each other. At least two experiments were conducted for each group. In the other columns, the total results from each ORC4 fragment was compared with each other. Significant values (shown in green) were $p < 0.05$. Insignificant values are shown in red.

	Exps	ORC4 Fragments				
ORC4 Frag		**v2-ORC4**	**Pep. 1 sm**	**Pep. 1 Lg**	**Pep. 10 sm**	**Pep 10 Lg**
v2-ORC4	0.8408					
Peptide 1 sm	0.2100	0.8887				
Peptide 1 Lg	0.4623	0.8910	1.0000			
Peptide 10 sm	0.5408	0.1927	0.2028	0.1022		
Peptide 10 Lg	1.0000	1.0000	0.7777	0.8972	0.2033	
Peptide 16	1.0000	0.0001	0.0001	0.0001	0.0002	0.0001

Supplemental Table 3. Fisher's Test for Comparison of Embryos with injection of Peptides vs mRNA for ORC4 Fragments. The results of the number of 2PN embryos that resulted from peptide injection was compared with those of injection with mRNA for ORC4 fragments. All experiments were performed twice. Significant values (shown in green) were $p < 0.05$. Insignificant values are shown in red.

	ORC4 Fragments Injected as mRNA					
Peptides	**v2-ORC4**	**Peptide 1**	**Peptide 1 Lg**	**Peptide 10**	**Peptide 10 Lg**	**Peptide 16**
1	0.7267	0.5994	0.6136	0.5833	0.7305	0.0002
6	0.0001	0.0001	0.0001	0.0009	0.0001	0.3315
9	0.0001	0.0001	0.0001	0.0001	0.0001	0.0718
10	0.0063	0.0022	0.0016	0.1070	0.0050	0.0239
14	0.0001	0.0001	0.0001	0.0001	0.0001	0.4469
16	0.0001	0.0001	0.0001	0.0001	0.0001	0.7029