

Supplementary material

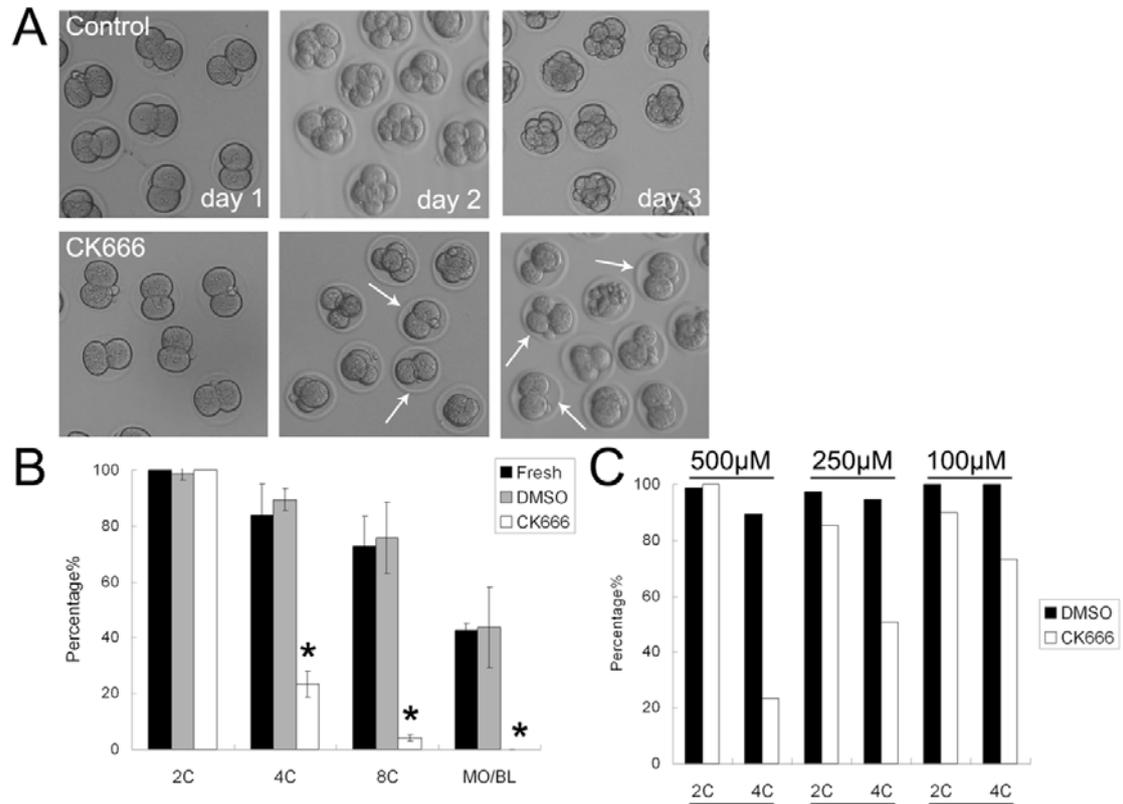


Fig. S1. Effects of CK666 treatment on ICR mouse embryo early cleavage. (A) Embryos failed to develop to the 4-cell stage after treatment with CK666. (B) Embryo developmental stages after CK666 treatment. (C) Effect of CK666 concentration on ICR mouse embryo development. * $P < 0.05$.

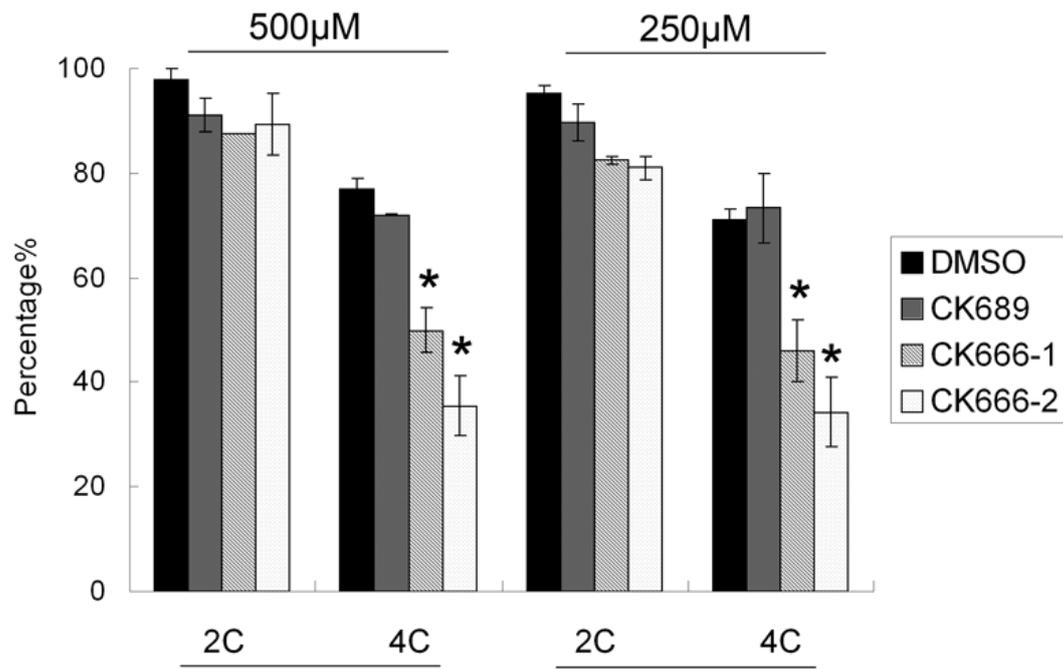


Fig. S2. Effect of DMSO, CK689, gift CK666 (CK666-1) and commercial CK666 (CK666-2) on ICR mouse embryo development.

Gene	Sequence
<i>Hsp70.1</i>	F: TACATGTCTACACTGCCACCCTGT R: AGAAGCCCAGAATCCATTAGCCCT
<i>Eif-1A</i>	F: ACATCAGCACTCGCACAAAGATGC R: TGCAAGCAGGAAACAGGAAATGGG
<i>Zar1</i>	F: CTACTATCACTGCAAGGACTGCAA R: AGGTGATGTCCTCCACTCTGTAAG
<i>Npm2</i>	F: GAAGAGGAAGATGATGAGGATGAG R: CTTGTCTCTAACGCTGGCTCTTAT
<i>Mater</i>	F: AAGGCTCTTACCTCAGTCCTTT R: CAACAGCTGGTGATTTTCTATCA
<i>Jmy</i>	F: TTCAAATTACAAGCCGTGCACCCG R: AGCTGCCTTCTGGACCTTACTGA
<i>Wave2</i>	F: AACTCCATGCTGTGCATGTTTCCC R: TCTATTTGGAAGGACCACTGCCCT
<i>GAPDH</i>	F: ACACATTGGGGGTAGGAACA R: AACTTTGGCATTGTGGAAGG

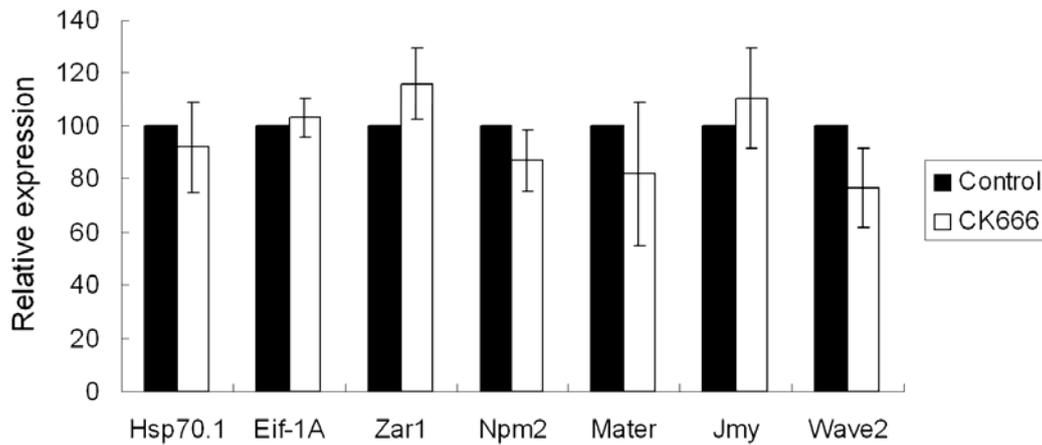


Fig. S3. Expression of the zygotic genome activation (ZGA) genes *Hsp70.1* and *Eif-1A*, the maternal genes *Zar1*, *Npm2* and *Mater*, and the nucleation-promoting factor (NPFs) genes *Jmy* and *Wave2* after CK666 treatment. There were no significant differences compared with the control group.

Table 1. Effect of CK666 concentration on BDF1 mouse embryo development

		500 μM (%)	250 μM (%)	100 μM (%)
8-cell	Control	84.6 \pm 7.6	94.7 \pm 3.2	86.4 \pm 10.7
	CK666	16.7 \pm 16.7	69.5 \pm 4.7	77.3 \pm 9.2
MO/BL	Control	60 \pm 14.1	85.7 \pm 7.3	79.2 \pm 5.5
	CK666	4.2 \pm 4.2	42.9 \pm 12.1	58.3 \pm 8.7

Table 2. Effect of CK666 concentration on ICR mouse embryo development

		500 μM (%)	250 μM (%)	100 μM (%)
2-cell	Control	98.7	97.4	100
	CK666	100	83.5	90
4-cell	Control	89.5	94.6	100
	CK666	23.4	50.6	73.1

Table 3. Effect of DMSO, CK689, gift CK666 (CK666-1) and commercial CK666 (CK666-2) on ICR mouse embryo development

		500 μM (%)	250 μM (%)
2-cell	DMSO	97.9 \pm 2.1	95.3 \pm 1.4
	CK689	91.2 \pm 3.2	89.7 \pm 3.6
	CK666-1	87.5 \pm 0	82.5 \pm 0.8
	CK666-2	89.4 \pm 5.8	81 \pm 2.3
4-cell	DMSO	77.1 \pm 2.1	71.1 \pm 2.2
	CK689	72.1 \pm 0.1	73.4 \pm 6.6
	CK666-1	50 \pm 4.2	46 \pm 6.0
	CK666-2	35.5 \pm 5.7	34.3 \pm 6.7