#### **1** Supplementary Figure Legends

Figure S1



2

### 3 Figure S1. AurkB and AurkC siRNA knock-down efficiency confirmation in preimplantation

### 4 embryos.

- 5 (A) Schematic view of AurkB and AurkC siRNA microinjection and Q-PCR confirmation of
  6 knocking-down efficiency in preimplantation mouse embryos.
- 7 (B) Relative genes expression analysis. SiRNAs (Control, siNC, siAurkB1, siAurkB2, siAurkC1,
- 8 siAurkC2, siAurkC3) were injected at 1-cell sate, early morula stage (8-cell stage) embryos
- 9 were collected, lysed and subjected to RT-QPCR analysis. The level of AurkA, B and C in the
- 10 siControl injected embryos was considered "1", the bar and whiskers indicate means and SEM, 11 \*\*p < 0.01.
- 12 (C) Q-PCR analysis of AurkB and AurkC expression level before and after mRNA injection.
- 13 mRNAs were injected at zygote stage, at early 4-cell stage, embryos were collected, lysed and
- subjected to RT-QPCR analysis. The level of AurkB in wild type embryos was considered "1",
- 15 the bar and whiskers indicate means and SEM, \*\*p < 0.01.
- 16 (D) Early and late division percentage of injected cells in the experiment of Figure 1E-H were 17 counted, the bar and whiskers indicate means and SD, \*p < 0.01, \*\*p < 0.001.

## Figure S2





# Figure S2. Immunostaining of H3K9me3 and HP1β with AurkB and AurkC overexpression during 4-cell stage.

- 21 (A)H3K4me3 and H3S10P immunostaining of morula stage embryos in Control (n = 18),
- 22 AurkB-OE (n = 26), AurkC-OE (n = 36) groups. DNA (blue), H2B-GFP (Marker, yellow),
- 23 H3K9me3 (red), HP1 $\beta$  (green). Scale bars, 20  $\mu$ m.
- 24 (B) Bar graph quantification of relative fluorescence intensity of H3K9me3 in injected and
- 25 non-injected cells of Control (n = 18), AurkB-OE (n = 26), AurkC-OE (n = 36) groups. The bar
- and whiskers indicate means and SD, \*\*\*p < 0.001.
- 27 (C) Bar graph quantification of relative fluorescence intensity of HP1β in injected and non-injected
- cells of Control (n = 18), AurkB-OE (n = 26), AurkC-OE (n = 36) groups. The bar and whiskers
  indicate means and SD.







# Figure S3. Immunostaining of H3K3me3 and H3S10P with AurkB and AurkC overexpression during morula stage.

- 33 (A) H3K4me3 and H3S10P immunostaining of morula stage embryos in Control (n = 24),
- 34 AurkB-OE (n = 28), AurkC-OE (n = 26) groups. DNA (blue), H2B-GFP (Marker, yellow),
- H3K4me3 (red), H3S10P (green). Scale bars, 20 μm.
- 36 (B) Bar graph quantification of relative fluorescence intensity of H3K4me3 in injected and
- 37 non-injected cells of Control (n=24), AurkB-OE (n=28), AurkC-OE (n=26) groups. The bar
- 38 and whiskers indicate means and SD.
- 39 (C) Bar graph quantification of relative fluorescence intensity of H3S10P in injected and
- 40 non-injected cells of Control (n=24), AurkB-OE (n = 28), AurkC-OE (n = 26) groups. The bar
- 41 and whiskers indicate means and SD, \*p < 0.05, \*\*\*p < 0.001.

## Figure S4





#### 43 Figure S4. Images of Oct4-paGFP fluorescence decay after photoactivation (FDAP) assay.

- 44 (A) A 2-cell stage embryo with Oct4-paGFP and H2B-mCherry expression before and after
- 45 photoactivation, H2B-Cherry (Marker, red), Oct4-paGFP (green), 3D projections (max. proj.)
- 46 of merged images are shown in the right panels. Scale bars, 20 μm.
- 47 (B) Relative fluorescence intensity of Oct4-paGFP and H2B-mCherry before and after
- 48 photoactivation, the bar and whiskers indicate means and SD, \*\*\*p < 0.001.
- 49 (C) The same 4-cell stage embryo with Oct4-paGFP and H2B-mCherry expression in different
- 50 focus planes. Scale bar, 20 μm.