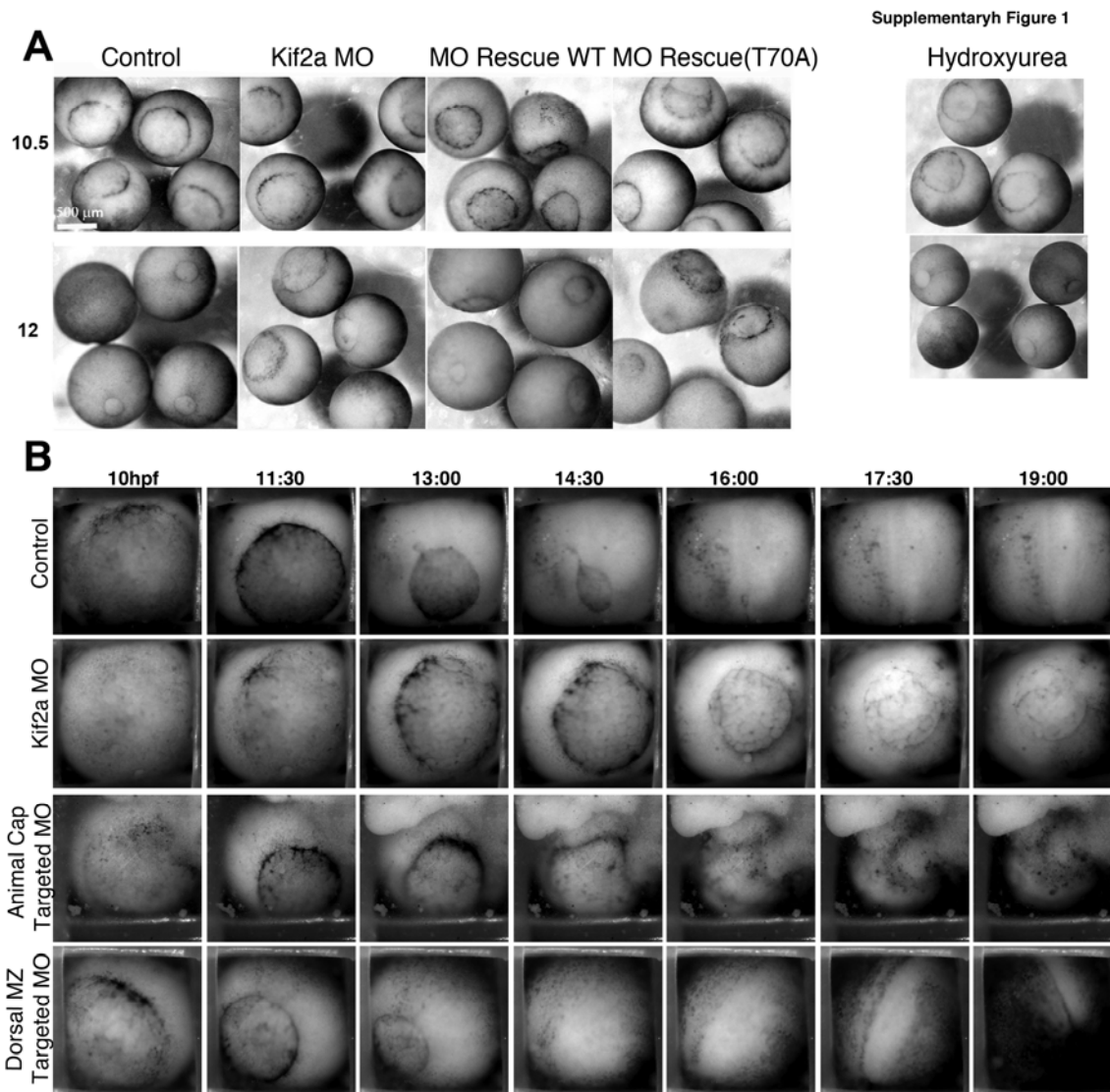


# Supplemental Materials

*Molecular Biology of the Cell*

Eagleson et al.

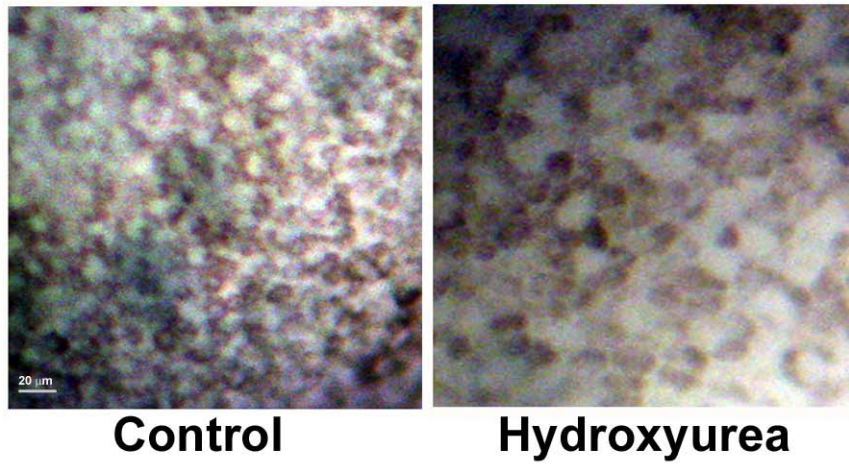


**Figure S1. Targeted Injection of Kif2aMO shows defect in epiboly, not cell division responsible for failure of gastrulation.**

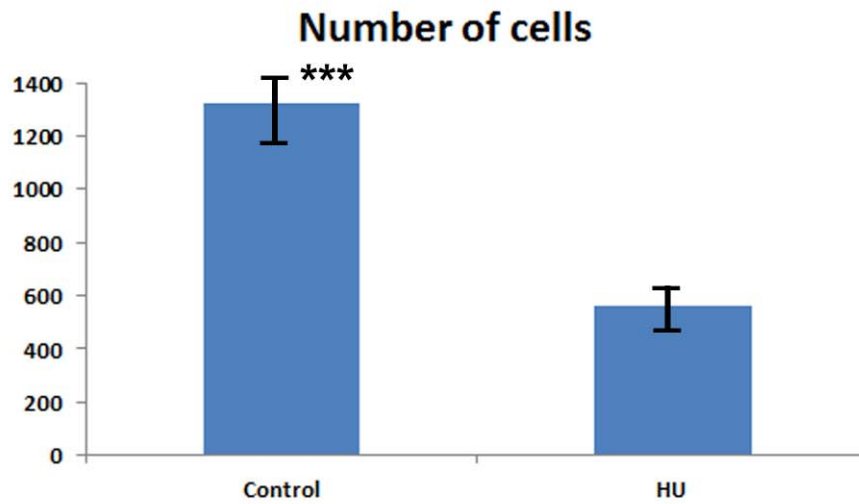
(A) One cell embryos were injected with either water (control), Kif2a morpholino (MO), the MO +plus RNA encoding human (h) Kif2a (MO WT RNA), MO plus RNA encoding hKif2a(T70A) mutant (MO (T70 RNA) or treated with Hydroxyurea and aphidicolin at stage 9 (Hydroxyurea). Embryos were allowed to develop to the indicated stage and the blastopore lips were imaged to follow the extent of gastrulation. The MO disrupts the completion of gastrulation at stage 12, which is rescued by hKif2a RNA but not hKif2a(T70A). Incubation of embryos starting at stage 9 in aphidicolin (150  $\mu$ M) and hydroxyurea (20 mM) does not prevent embryos from completing gastrulation (to stage 12) indicating that a lack of cell division is not the main factor in preventing the completion of gastrulation within the MO-injected group. Note that Hydroxyurea treated embryos are differently scaled than the other embryos. (B) Low-light timeslapse of blastopore closure from St. 10 to St. 14, of embryos injected at two-cell stage with water (Control), Kif2aMO (20nL at 1mg/mL per cell), or at 16-cell stage targeted to the animal cap (AC Target) (A tier: 2nL of [1mg/mL] in each of four cells) or dorsal marginal zone

(MZ target) (B and C tier, two dorsal cells-2nL of [1mg/mL] in each of four cells). Still frames shown of blastopore closure progress every 90min after St. 10. Note that an adjacent embryo died in the "Animal Cap targeted MO" generating the material that can be seen coming in from the top.

**A**

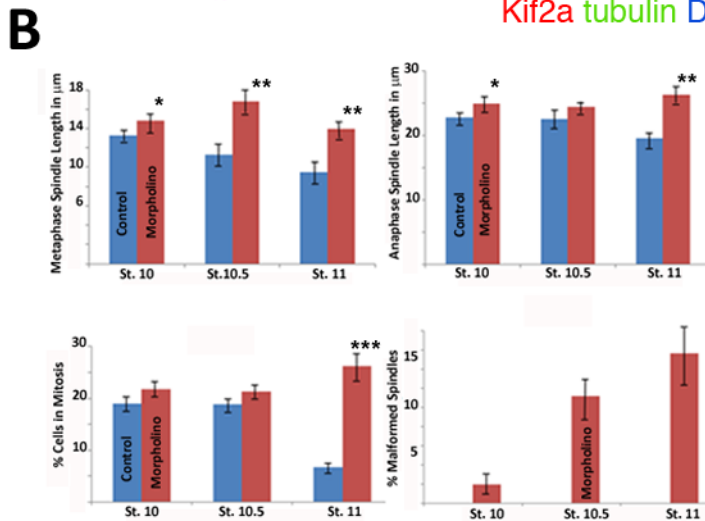
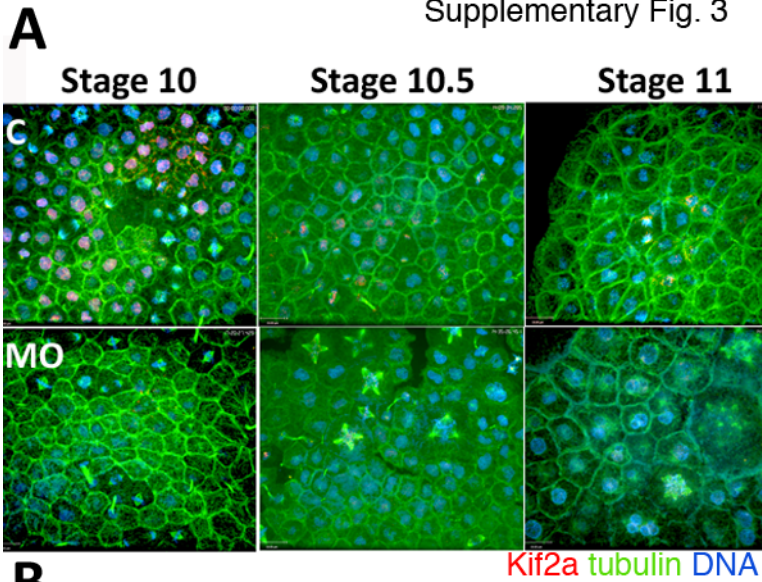


**B**



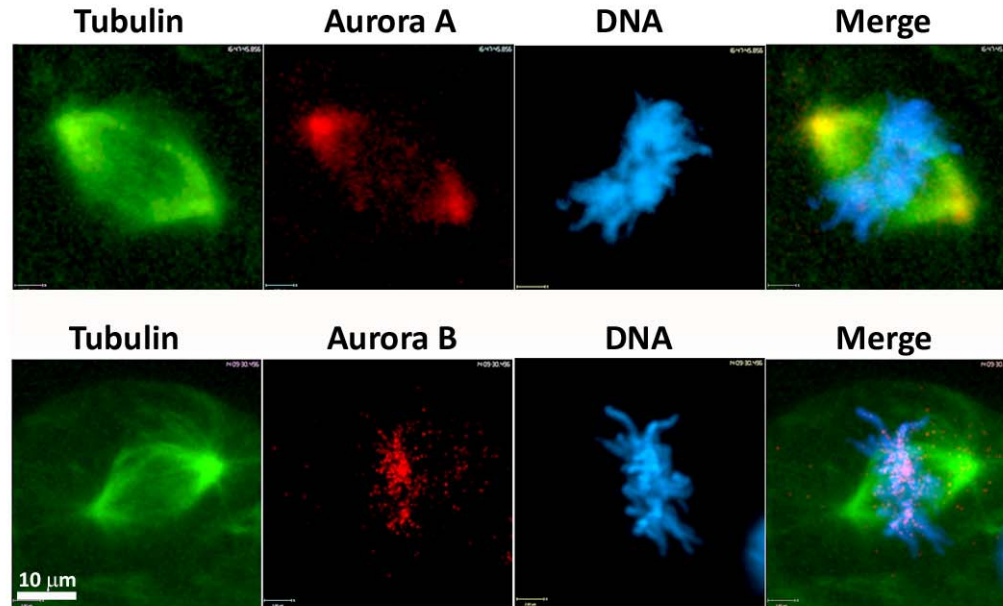
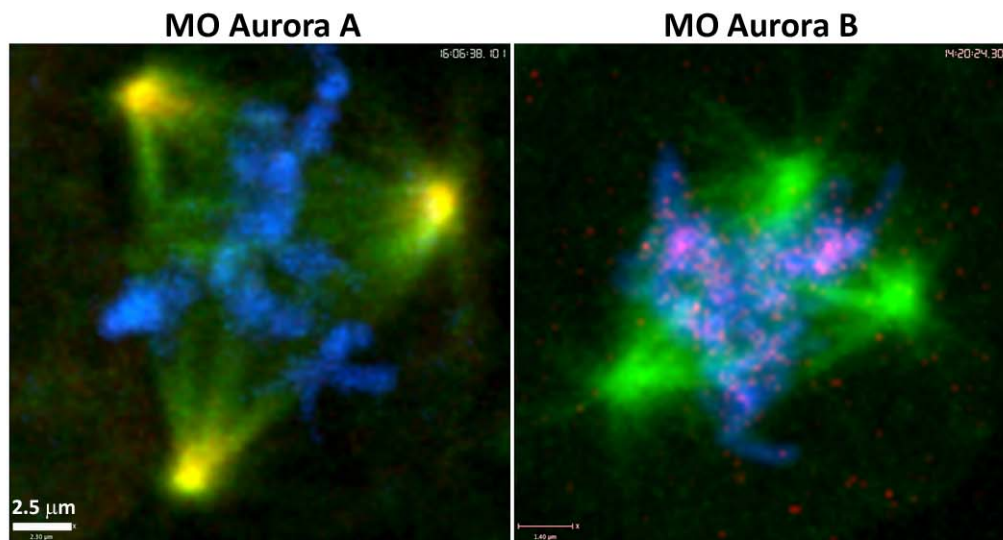
**Figure S2. Embryos treated with Hydroxyurea and aphidicolin fewer and larger cells than controls indicating that the treatment prevented cellular proliferation.** Embryos were imaged at identical magnification (A) and the number of cells in a field were quantified (B).

Supplementary Fig. 3



**Figure S3. Confocal micrograph images of control and Kif 2a MO-injected *X. laevis* animal caps fixed at stages 10, 10.5 and 11 of development.**

The animal caps were immunostained for Kif 2a (red) and  $\alpha$ - tubulin (green) and stained for DNA (blue). There is a gradual accumulation of multipolar spindles with development within the Kif 2a morphant group. Higher magnification images of Fig 3.

**A****B**

**Figure S4. The localization of Aurora kinases the spindles of animal caps.** Confocal micrographs of stage 10.5 control animal cap cells were processed for immunofluorescence with the indicated Aurora kinase antibodies (red) and  $\alpha$ -tubulin antibodies (green). DNA is stained in blue. (B) Aurora kinases are not mislocalized in Kif2a morphant embryos.

Video - Supplementary Movie 1A  
Control Gastrulating embryo (Refers to figure 2)

Video - Supplementary Movie 1B  
Gastrulation defects after depleting Kif2a (Refers to figure 2)

Video - Supplementary Movie 1C  
Gastrulation defects after depleting Kif2a are rescued by connection of human Kif2a RNA (Refers to figure 2)

Video - Supplementary Movie 1D  
Gastrulation defects after depleting Kif2a are rescued by connection of human Kif2b RNA (Refers to figure 2)

Video - Supplementary Movie 2A  
Epibody movements of a control injected embryo (Refers to figure 2)

Video - Supplementary Movie 2B  
Epibody movements of a Kif2a MO injected embryo (Refers to figure 2)

Video - Supplementary Movie 3  
Gastrulation of the indicated embryos after removal of the animal cap tissue (Refers to figure 3)

Video - Supplementary Movie 4A  
Control animal cap mitosis (Refers to figure 6)

Video - Supplementary Movie 4B  
Kif2a MO injected animal cap mitosis (Refers to figure 6)

Video - Supplementary Movie 4C  
Kif2a MO injected animal cap mitosis rescued by coinjection with human Kif2a RNA (Refers to figure 6)

Video - Supplementary Movie 4D  
Kif2a MO injected animal cap mitosis rescued by coinjection with human Kif2b RNA (Refers to figure 6)

Video - Supplementary Movie 5A  
Control injected embryo gastrulation (Refers to Supplementary Figure 1)

Video - Supplementary Movie 5B  
Gastrulation of embryos after Kif2a MO was injected in the entire embryo (Refers to Supplementary Figure 1)

Video - Supplementary Movie 5C

Gastrulation of embryos after Kif2a MO was injected specifically in cells fated to develop into the animal cap (Refers to Supplementary Figure 1)

Video - Supplementary Movie 5D

Gastrulation of embryos after Kif2a MO was injected specifically in cells fated to develop into the marginal zone (Refers to Supplementary Figure 1)