

**Figure S1. EVL and deep cell movements are defective in MZalkbh4 and MZatrn embryos, while the cell number per embryo is not obviously affected.** (**A**) Whole-mount phalloidin and DAPI staining were used to observe the marginal positions of EVL and deep cells. White arrows indicate the marginal positions of deep cells; yellow arrows indicate the marginal positions of EVL cells. The mutant embryos were collected at both the same time point and comparable morphological stages compared with wild-type embryos at 75% epiboly stage. (**B**) The quantitative data of phalloidin-positive EVL cell numbers were derived from 3D images of the embryos in (A). Ne, the number of observed embryos. \*, p<0.01; n.s. indicates no significant difference. Scale bars 100 μm in (A).



Figure S2. Actomyosin band formation in the E-YSL and marginal EVL cells morphology are affected in Malkbh4 embryos. (A) phalloidin and anti-NMII co-staining showing the defects of actomyosin band formation and marginal EVL cells morphology in Malkbh4 embryos. (B) Quantitative measurements of actomyosin band widths in (A) at 75% epiboly stage. (C) Quantitative measurements of the marginal EVL cell length/width ratios in (A) at both 50% and 75% epiboly. Ne, the number of observed embryos; Nc, the number of observed cells. \*, p<0.01. Scale bar: 50  $\mu$ m in (A).



**Figure S3. E-YSL endocytosis is not affected in MZ***alkbh4* or MZ*atrn* mutant embryos. (A) Fluorescence images of live embryos showing endocytic vesicles in red in the E-YSL. Embryos at 50% and 75% epiboly stages were incubated in 10 mg/ml Rhodamine B isothiocyanate-Dextran for 30 minutes, and imaged by confocal microscope. (B) The quantitative data of fluorescence intensity measured by image J software are derived from (A). Ne, the number of observed embryos. n.s. indicates no significant difference. Scale bar: 100 μm in (A).



Figure S4. Microtubule arrays in MZ*alkbh4* and MZ*atrn* mutant embryos are not significantly altered. Confocal images of anti- $\alpha$ -tubulin and DAPI stained embryos at 75% epiboly stages. The mutant embryos were collected at both the same time point and comparable morphological stages compared with wild-type embryos. Scale bar: 100 µm.



Figure S5. Cell proliferation is not obviously affected in MZalkbh4 and MZatrn mutant embryo. (A) Confocal images of anti-pH3 and DAPI staining at 75% epiboly stage. Cell proliferation was not obviously affected in the mutants. (B) Mitotic indexes of (A) were characterized by the percentage of pH3-positive cell number to DAPI-positive cell number. Ne, the number of observed embryos. \*, p<0.01; n.s. indicates no significant difference. Scale bars 100  $\mu$ m in (A).