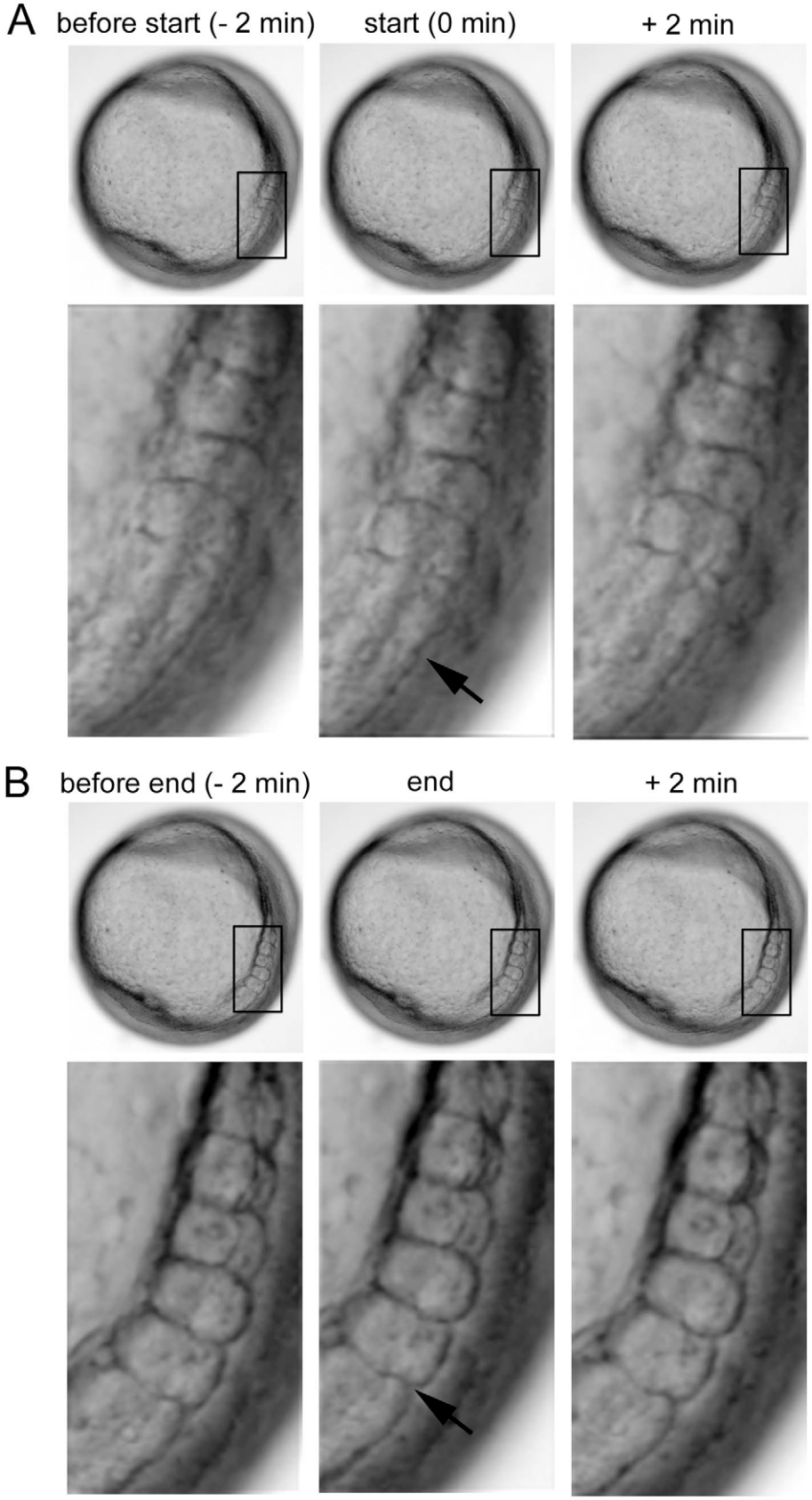



**Figure S1 Start and end of somite segmentation.** (A) Representative images of segmentation start. Lateral view, dorsal to the right. Lower panel: higher-magnification views of boxed areas. A furrow (arrow) appeared at the dorsal limit of the presomitic mesoderm at 0 min. Two min later, the furrow became clearer. (B) Representative images of segmentation end. Lower panel: higher-magnification views of boxed areas. The somite boundary (arrow) became clearer, and the somite and presomitic mesoderm were completely separated. Panels are frames of Movie 1.

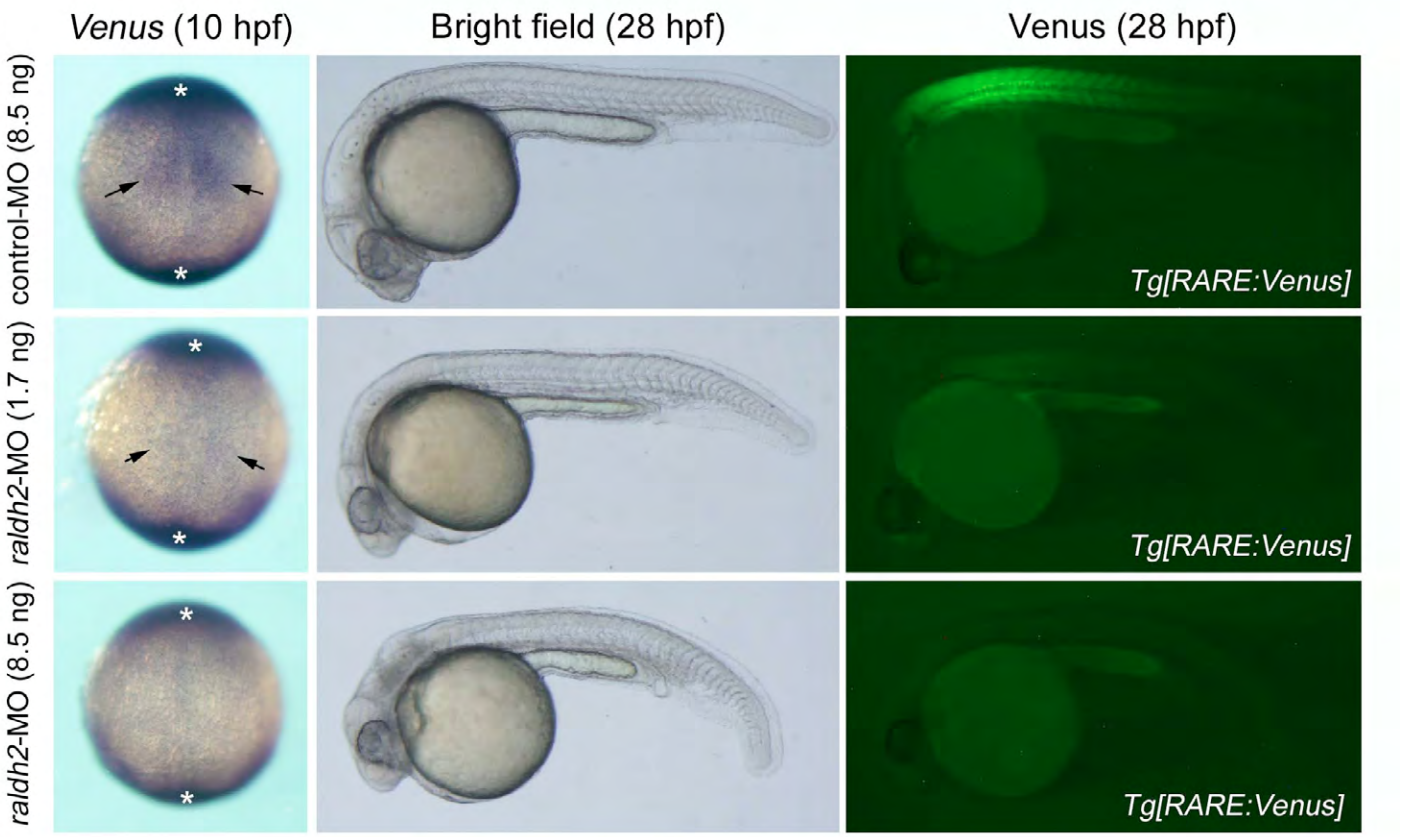



**Figure S2 Knockdown efficiency of *raldh2* evaluated by using *Tg[RARE:Venus]* embryos.** Left panel: Representative images of Venus expression in 10 hpf embryos injected with 8.5 ng control-MO (n = 42), 1.7 ng *raldh2*-MO (n = 34) or 8.5 ng *raldh2*-MO (n = 38). Dorsal view, anterior to the top. *in situ* signal marked by asterisk was considered as a background signal because similar signal could be detected in wildtype embryos and because *raldh2* is not expressed in these regions. Center and right panel: Representative images of Venus expression in 28 hpf embryos injected with 8.5 ng control-MO (n = 57), 1.7 ng *raldh2*-MO (n = 34) or 8.5 ng *raldh2*-MO (n = 41). Lateral view, anterior to the left. RA signal activity (Venus signals) could be seen at a part of paraxial mesoderm (arrow) in control embryos at 10 hpf, and at anterior somites and eyes in control embryos at 28 hpf (upper panels). The activity was detected weakly in embryos injected with 1.7 ng *raldh2*-MO (middle panels), but not in embryos injected with 8.5 ng *raldh2*-MO (lower panels).

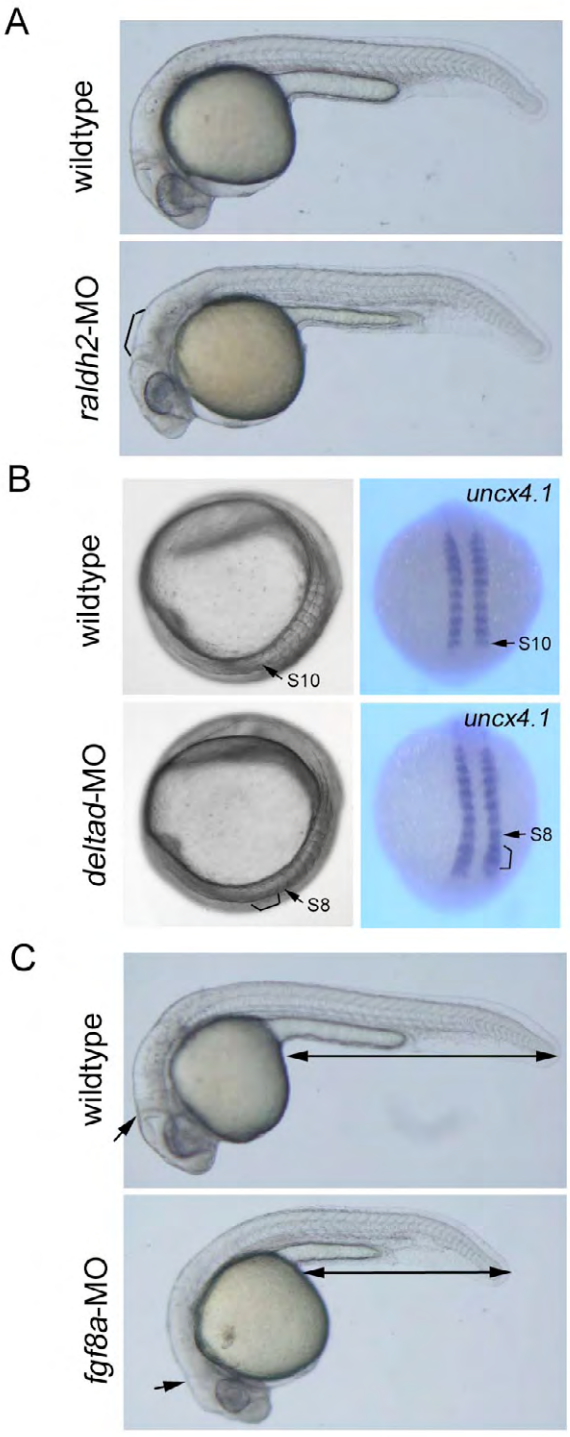



**Figure S3 Evaluation of MO activity after time-lapse imaging.** (A) *raldh2* morphants. Embryos (24 – 30 hpf) showing defects in hindbrain (bracket) and trunk somites were selected and used to further analyses. (B) *deltad* morphants. Embryos (14 hpf) which phenocopy *after eight* (*deltad* mutants) were selected. In these embryos, segmentation defects could be seen around S8 – 10 (bracket). (C) *fgf8a* morphants. Embryos (24 – 30 hpf) that phenocopy *ace* (*fgf8a* mutants) were selected. Defects in midbrain-hindbrain boundary (arrow) and tail elongation (double arrow) could be seen in phenocopied embryos.

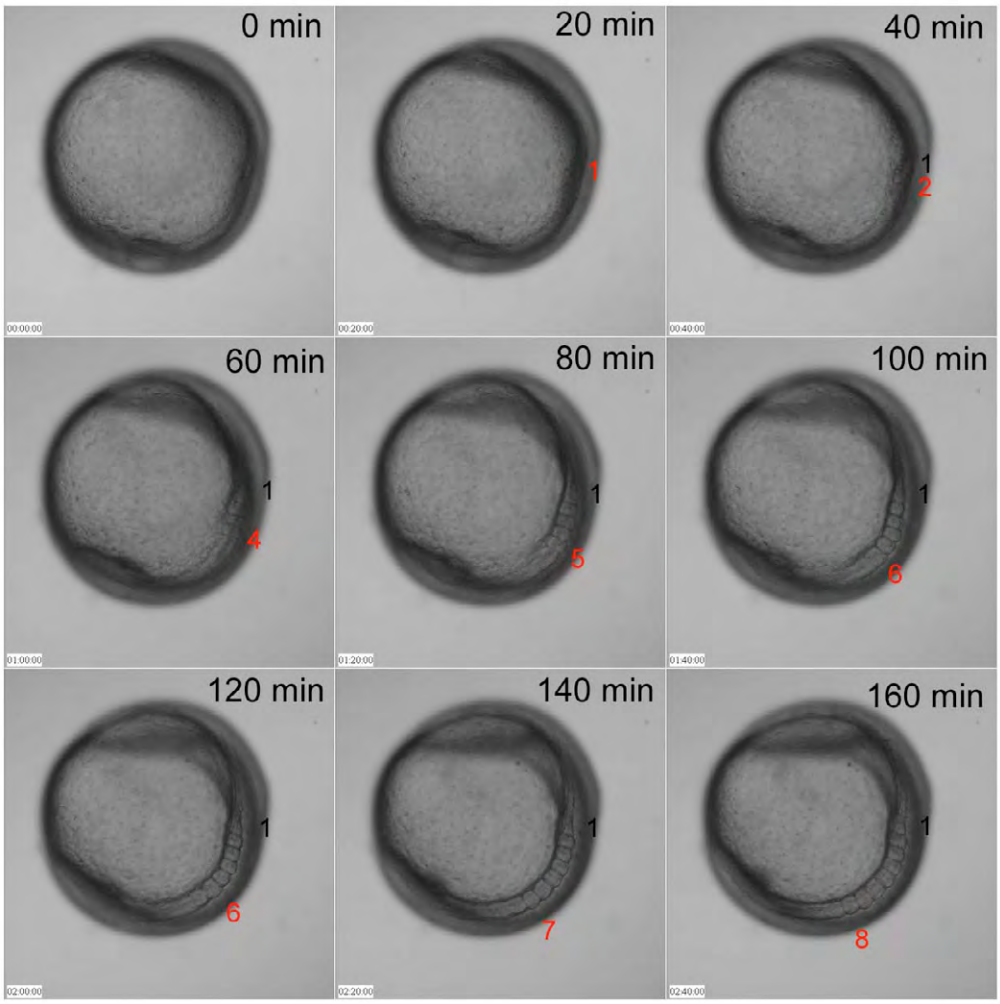



**Figure S4 A difference between anterior and posterior somitogenesis.** Time-lapse images every 20 min are displayed. The first somite (numbered 1) and the newest somite at each time point (red) are indicated. Panels are frames of Movie 1.

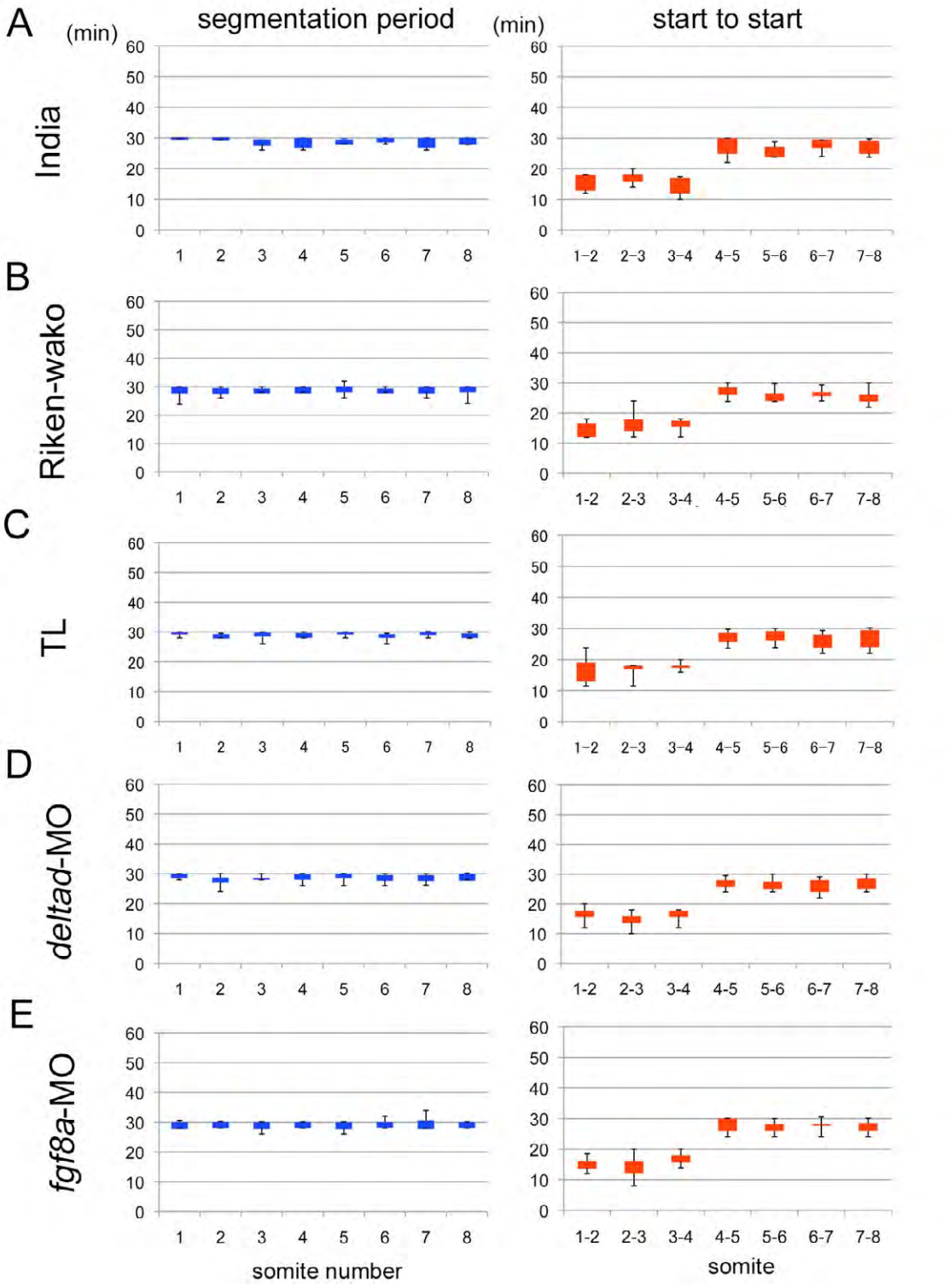



**Figure S5 The AP difference occurs in several wildtype strains and in *deltad* and *fgf8a* morphants.** Left panel: segmentation period; right panel: start to start. (A) India (n = 8). (B) Riken-wako (n = 8). (C) TL (n = 7). (D) *deltad* morphants (n = 7). (E) *fgf8a* morphants (n = 9).

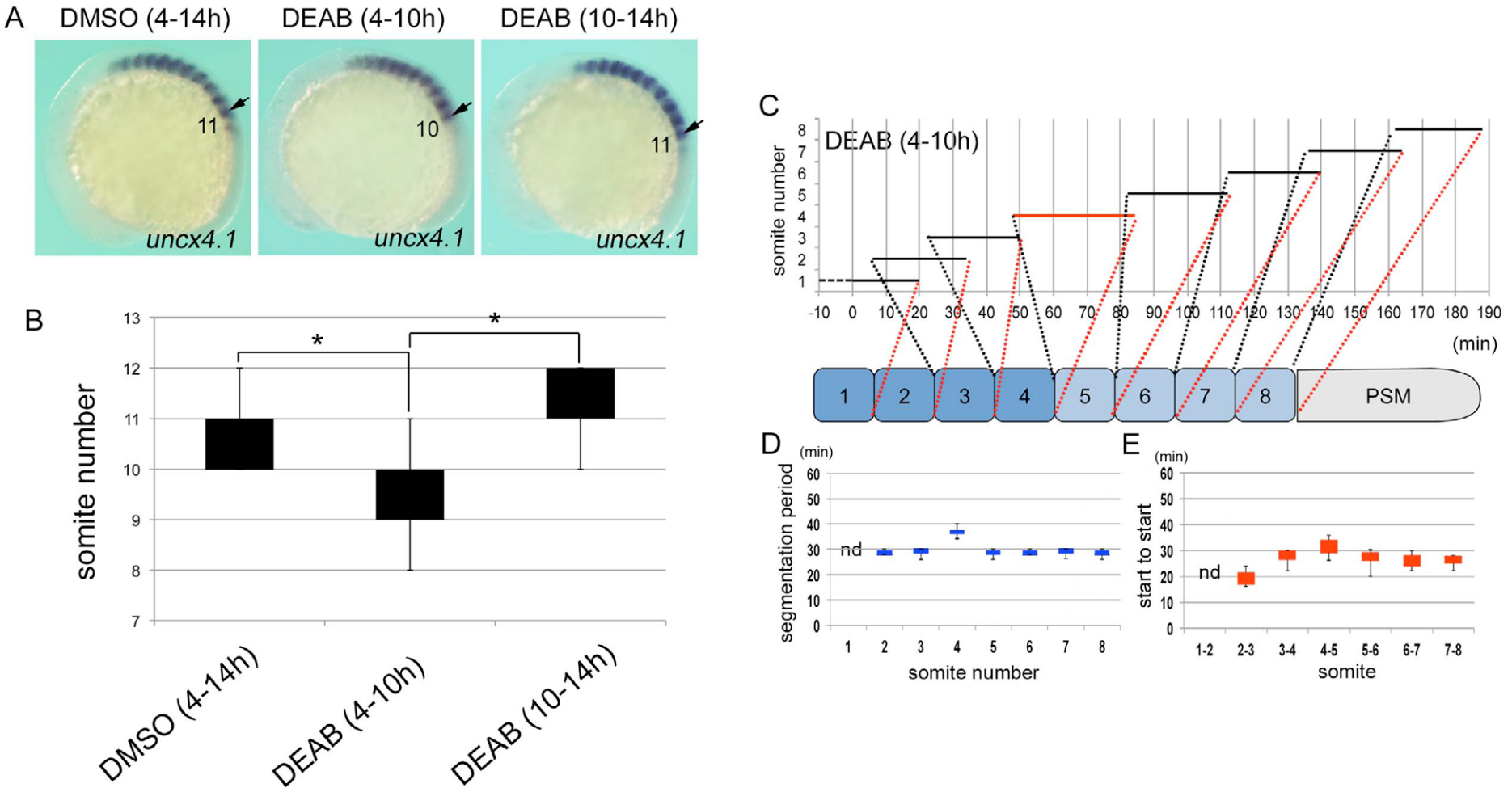



**Figure S6 Inhibition of RA synthesis during gastrulation results in loss of a somite.** (A) Representative images of *uncx4.1* expression in embryos treated with DMSO (4 – 14 hpf) (left), DEAB (4 – 10 hpf) (center) or DEAB (10 – 14 hpf) (right). Lateral view, anterior to the left. Arrows marked the position at SI. (B) Box and whisker plots of somite number in embryos treated with DMSO (4 – 14 hpf) (n = 34), DEAB (4 – 10 hpf) (n = 29) or DEAB (10 – 14 hpf) (n = 25). Statistically significant difference (asterisk, $P < 0.05$) could be seen in DMSO versus DEAB (4 – 10 hpf), and DEAB (4 – 10 hpf) versus DEAB (10 – 14 hpf). (C) Time-lapse data for somitogenesis in an embryo treated with DEAB (4 – 10 hpf) (see Movie 4, 4-1). (D, E) Box and whisker plots of segmentation period (D) or start to start (E) for embryos treated with DEAB during 4 – 10 hpf (n = 8). Results from statistical analyses are shown in Table S1 – 4.

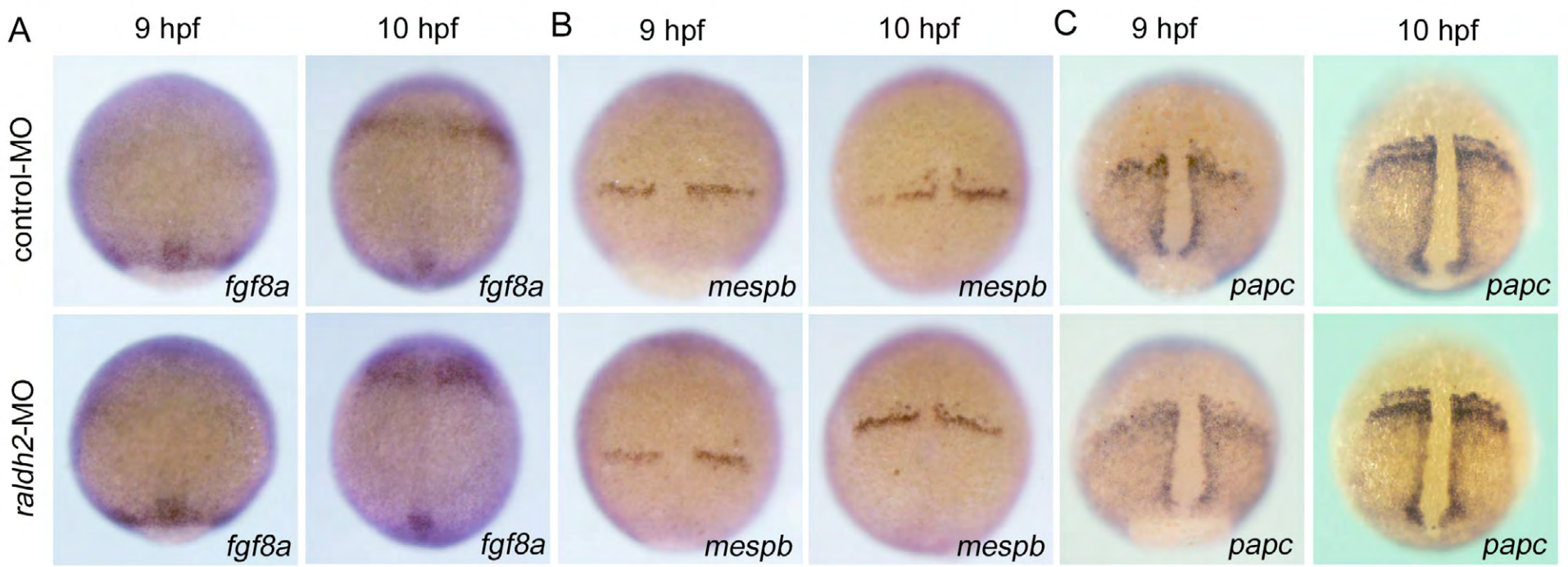
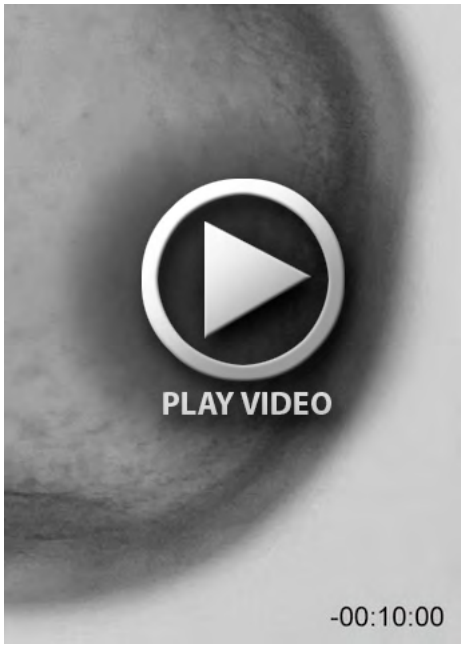



**Figure S7 *raldh2* knockdown does not affect expression of *fgf8a*, *mespb* or *papc*.** Expression of *fgf8a* (A), *mespb* (B) or *papc* (C) in control (upper) or *raldh2* morphants (lower) at 9 (left) or 10 hpf (right).

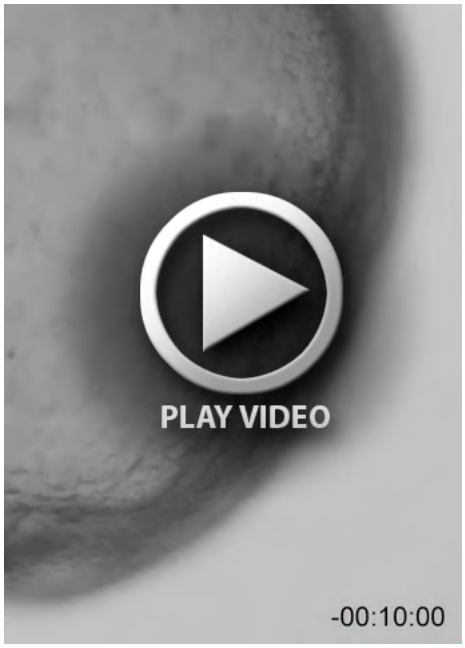

**Movie 1A.** This movie shows the lateral view of a wildtype embryo. The time-lapse covers the period of 10 – 13 hpf.



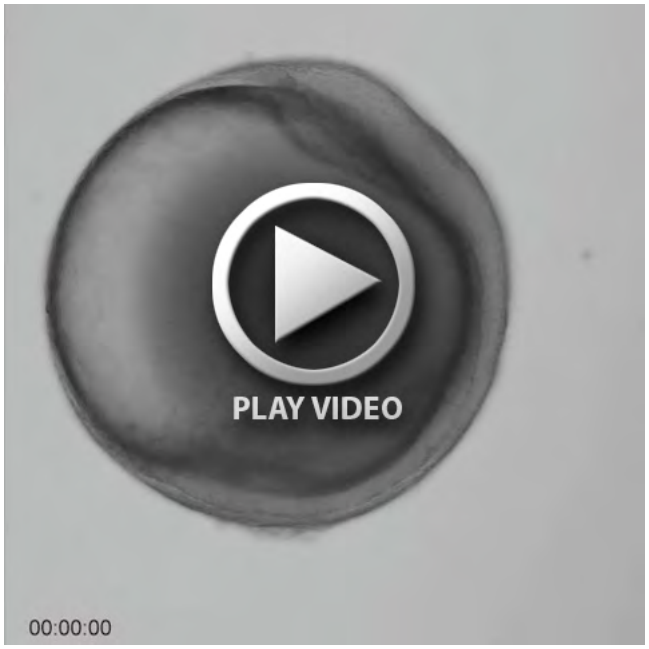

**Movie 1B.** Higher-magnification views of somite areas in Movie 1A. Black and red arrows indicate start and end of morphological segmentation.


**Movie 2.** This movie shows the lateral view of a wildtype embryo. The time-lapse covers the period of 13 – 16 hpf.


**Movie 3A.** This movie shows the lateral view of a wildtype embryo injected with *raldh2*-MO (a *raldh2* morphant). The time-lapse covers the period of 10 – 13 hpf.



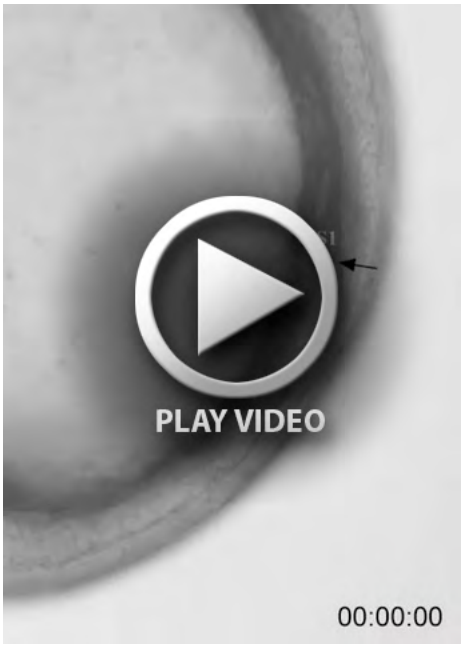

**Movie 3B.** Higher-magnification views of somite areas in Movie 3A. Black and red arrows indicate start and end of morphological segmentation.




**Movie 4A.** This movie shows the lateral view of a wildtype embryo treated with DEAB (4 – 10 hpf). The time-lapse covers the period of 10 – 13 hpf.


**Movie 4B.** Higher-magnification views of somite areas in Movie 4A. Black and red arrows indicate start and end of morphological segmentation.


**Movie 5A.** This movie shows the lateral view of a *raldh2* morphant treated with RA (4 – 10 hpf). The time-lapse covers the period of 10 – 13 hpf.




**Movie 5B.** Higher-magnification views of somite areas in Movie 5A. Black and red arrows indicate start and end of morphological segmentation.

**Table S1.**

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**Table S2.**

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**Table S6.**

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**Table S7.**

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**Table S8.**

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