

Fig. S1 Immuno-staining for  $\alpha$ -Tubulin

Wild-type embryos before gastrulation (A), and at middle (B), and late (C) stages of gastrulation were stained for  $\alpha$ -Tubulin. The focus was adjusted on the ventral surface of embryos. Some LM cells bearing a thin protruding structure (likely corresponding to an apical protrusion) are marked with asterisks. These thin structures were observed at the middle stage of gastrulation. Scale bar: 20 $\mu$ m.

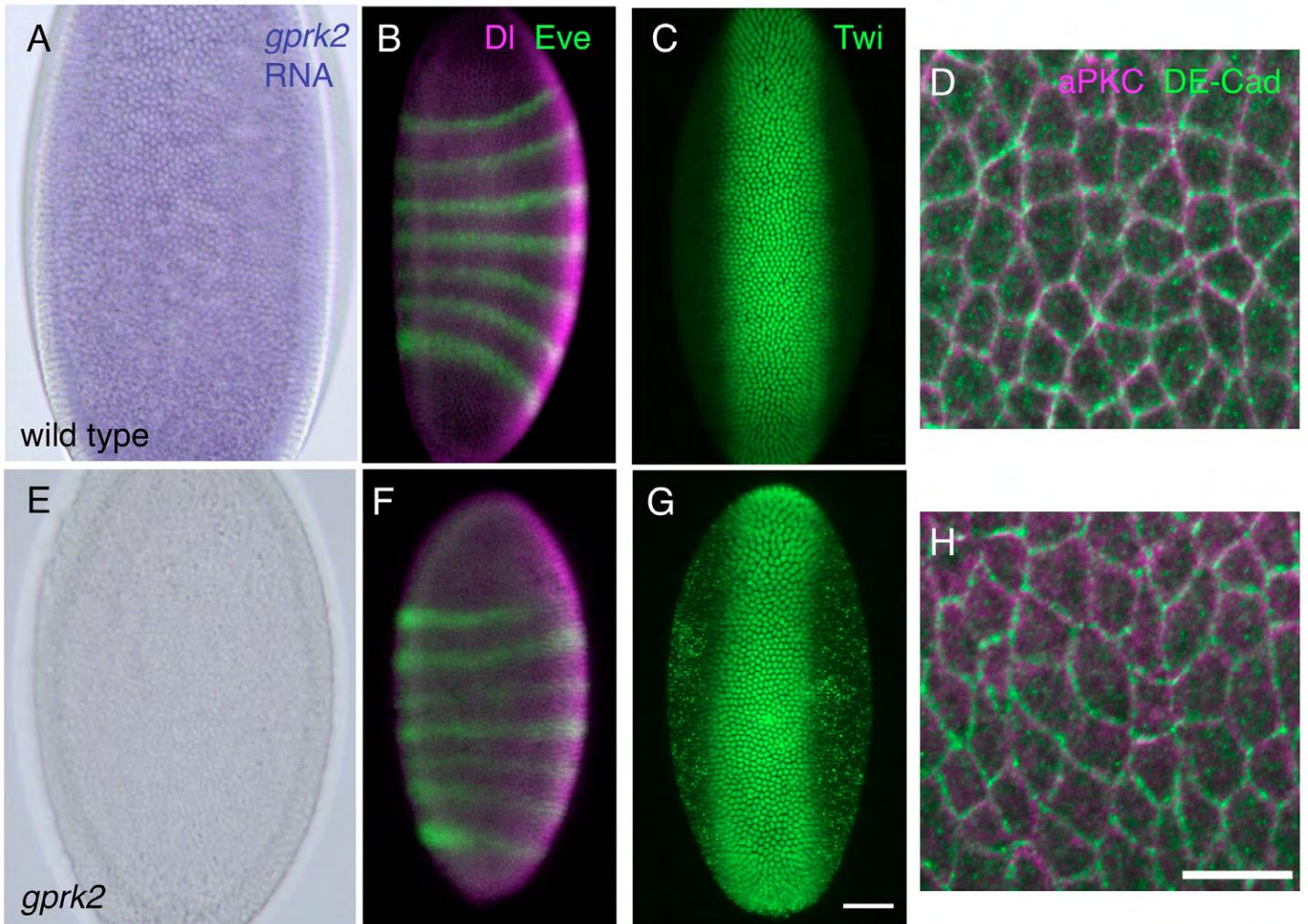


Fig. S2 Axial patterning and adherence junction in *Gprk2* mutant embryos  
 Wild-type embryos (A-D) and *Gprk2* mutant embryos (E-H) stained for *Gprk2* RNA (A, E), for DI and Eve proteins (B, F), for Twi protein (C, G) and for aPKC and DE-Cadherin proteins (D, H). *Gprk2* RNA is expressed uniformly in wild-type embryos, but is not expressed in *Gprk2* mutant embryos (A, E). Ventral views (A, C, E, G) and lateral views (B, F) are shown. The staining of scattered particles in G is non-specific background. High-magnification views of lateral ectodermal cells focused on the sub-apical section indicate cortical localization of aPKC and DE-Cadherin (D, H: anterior is toward left). Scale bars: (G) 50  $\mu$ m, (H) 20  $\mu$ m.

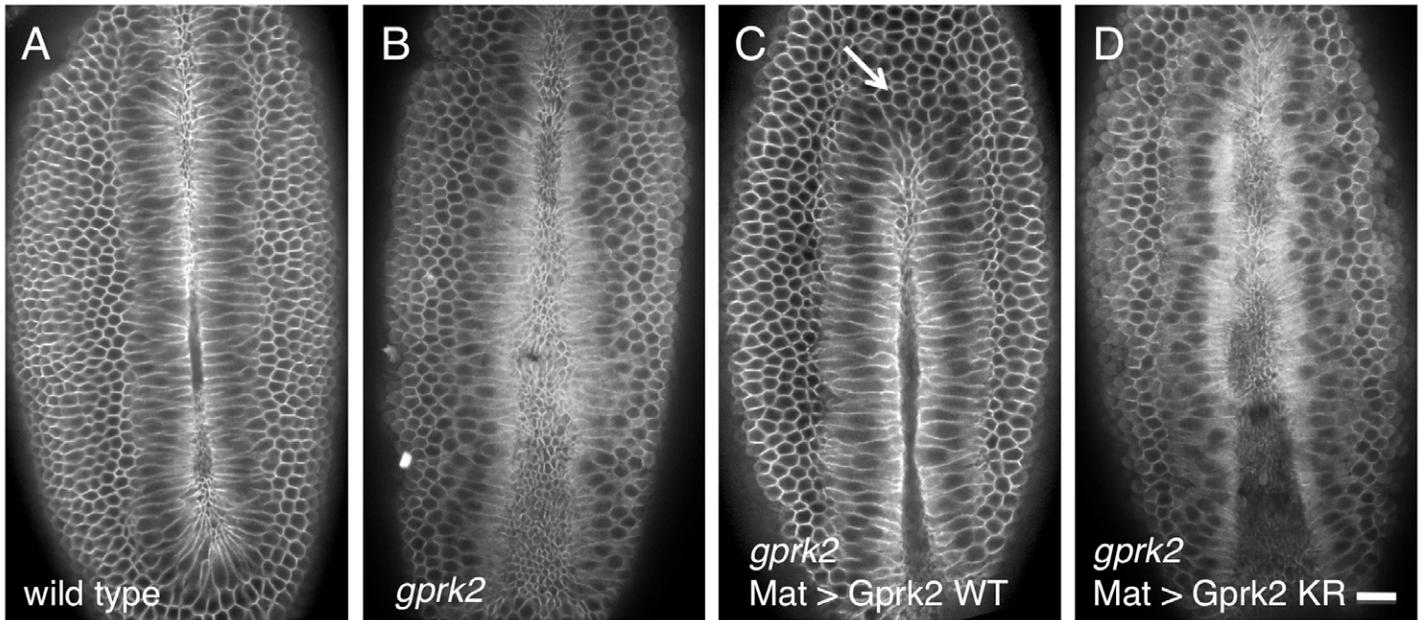


Fig. S3 Rescue experiment for *Gprk2* mutant  
(A) Wild-type embryo, (B) *Gprk2* mutant embryo, (C) *Gprk2* mutant embryo with Maternal-Gal4-driven *Gprk2* (wild-type) expression, (D) *Gprk2* mutant embryo with Maternal-Gal4-driven *Gprk2* (K338R) expression. Embryos were stained for Dlg protein. (C) The wild-type *Gprk2* transgene partially blocked apical constriction (arrow). Scale bar: 20  $\mu\text{m}$ .

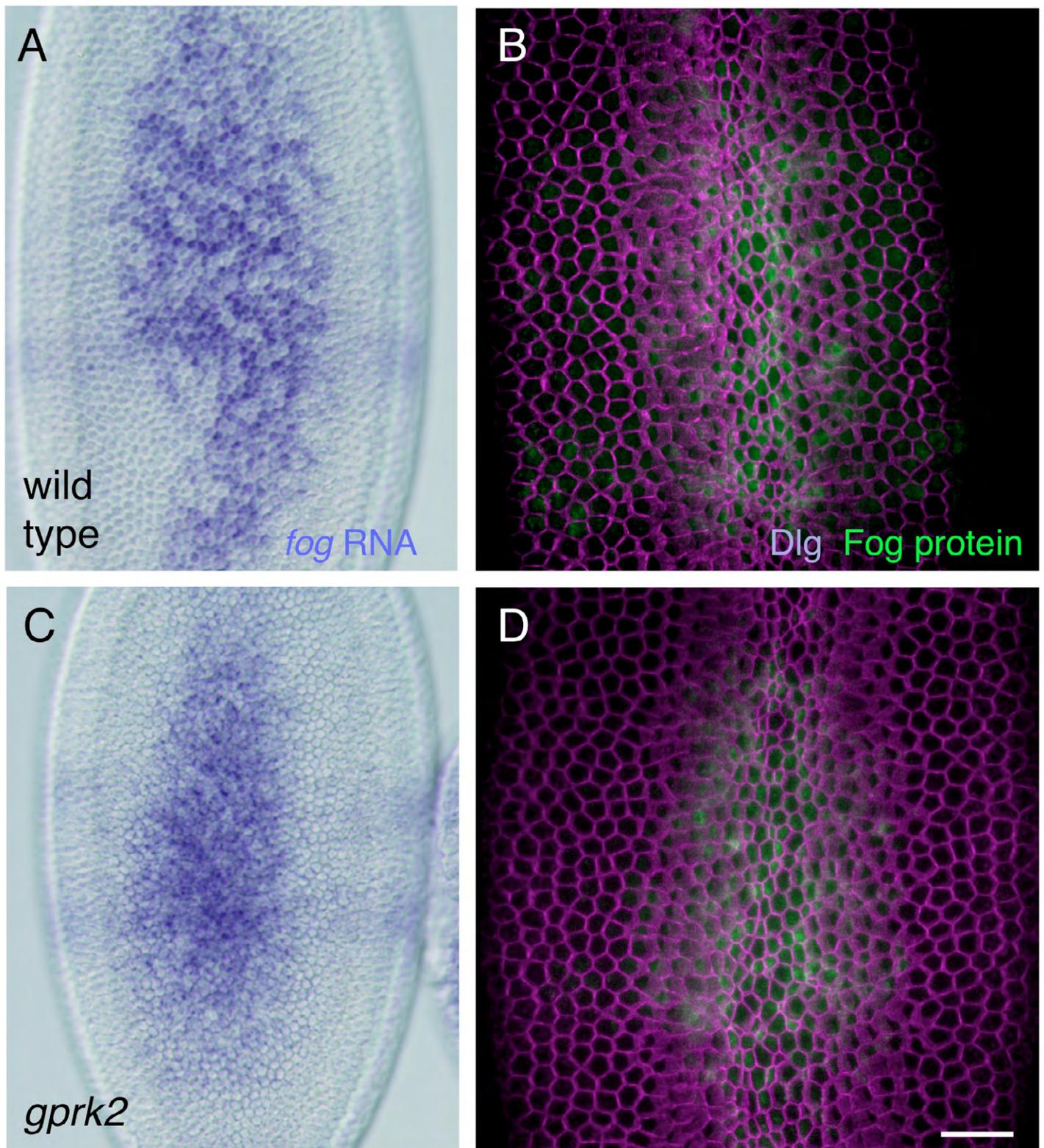


Fig. S4 Fog expression in *Gprk2* mutant embryos

(A, C) *fog* RNA expression was detected by Dig-AP staining in wild-type (A) and *Gprk2* mutant (C) embryos. (B, D) Wild-type (B) and *Gprk2* mutant (D) embryos were stained for Dlg and Fog proteins. Fog protein is expressed in a similar pattern in wild-type (B) and *Gprk2* mutant (D). Scale bar: 20  $\mu$ m.

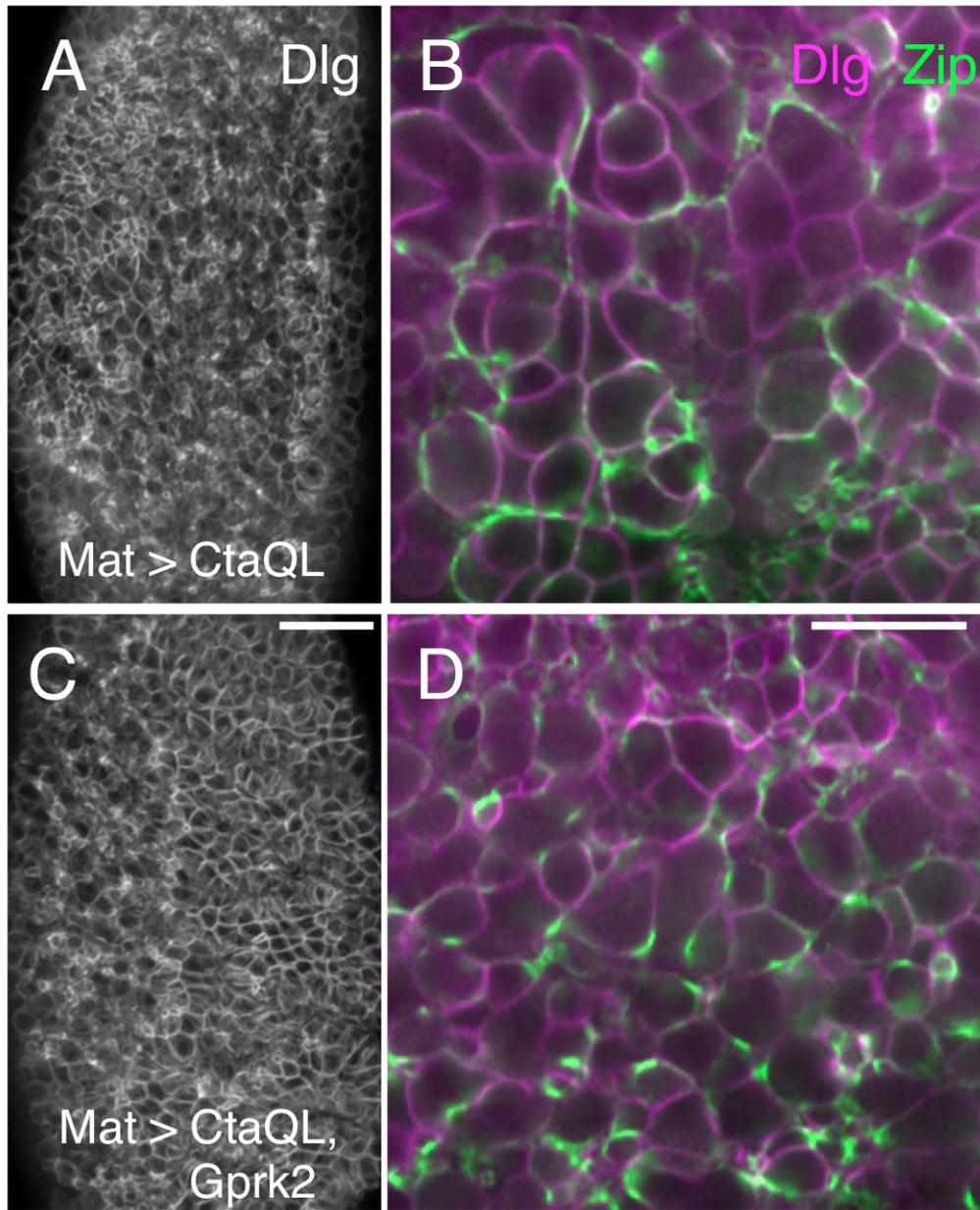


Fig. S5 Over-expression of Cta Q303L and Gprk2

(A, C) Embryos at late gastrulation stage were stained for Dlg protein. (B, D) High-magnification views of the apical surface of lateral ectodermal cells stained for Dlg and Zip proteins. Anterior is toward left and dorsal is toward the top. Genotypes of the embryos were Maternal-Gal4, UAS-Cta Q303L (A, B), and Maternal-Gal4, UAS-Cta Q303L, UAS-Gprk2 (C, D). Scale bars: (C) 20  $\mu\text{m}$ , (D) 10  $\mu\text{m}$ .



Movie 1 Time-lapse movie of apical surface of gastrulae

Movie of ventral view of a living Moe::GFP embryo. Focus was adjusted on the surface (left) and 9  $\mu\text{m}$  depth (right) of the same embryo. Time 0 was taken at the start of observation. Anterior is toward the upper left.



Movie 2 Dynamics of apical protrusions

High magnification views of Movie 1. Focus was adjusted on the surface (left) and 9  $\mu\text{m}$  depth (right) of the same area. While LM cells moved toward mid-ventral (the lower left), apical protrusions dynamically changed their morphology.



Movie 3 Analysis of cell movements

An example of tracking of cell movements of a Moe::GFP embryo. Focus was adjusted at 7  $\mu\text{m}$  depth. Anterior is toward the upper left. The cell vertexes chosen for tracking are marked with colored circles.



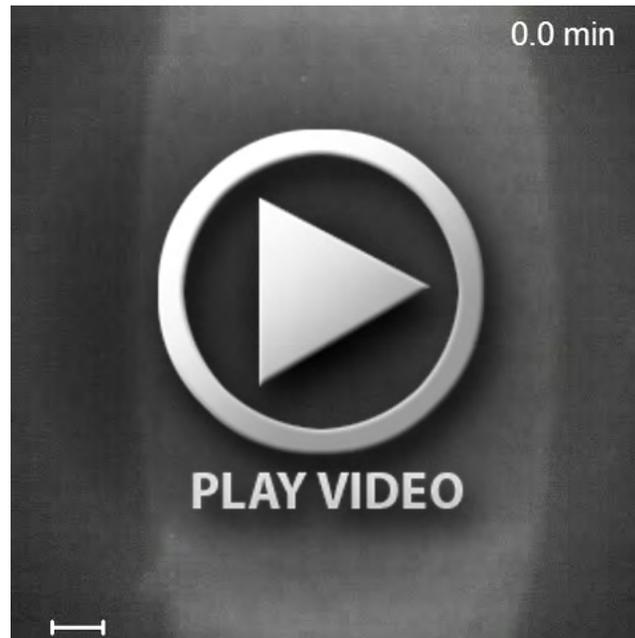
Movie 4 Time-lapse movie of Moe::GFP embryo

Movie of ventral view of a living Moe::GFP embryo. Five stacked images along the Z-axis (4  $\mu\text{m}$  depth) were merged by maximum intensity projection. Anterior is toward the top.



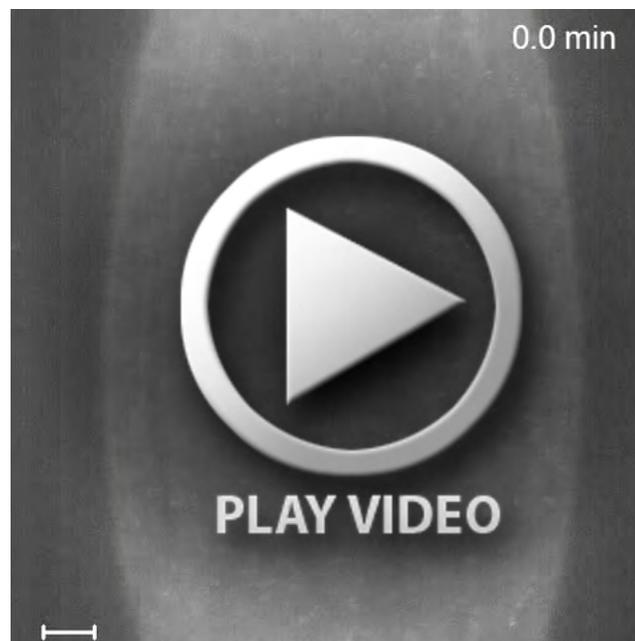
Movie 5 Time-lapse movie of *gprk2* mutant embryo with Moe::GFP

Movie of ventral views of a living *gprk2* mutant embryo with Moe::GFP. Five stacked images along the Z-axis (4  $\mu\text{m}$  depth) were merged by maximum intensity projection. Anterior is toward the bottom. Images of Fig. 4C were rotated 180°.



Movie 6 Time-lapse movie of Sqh::GFP embryo

Movie of ventral view of a living *gprk2* heterozygote embryo with Sqh::GFP as a control. Ten stacked images along the Z-axis (9  $\mu\text{m}$  depth) were merged by maximum intensity projection. Anterior is toward the top.



Movie 7 Time-lapse movie of *gprk2* mutant embryo with Sqh::GFP

Movie of ventral views of a living *gprk2* homozygote mutant embryo with Sqh::GFP. Ten stacked images along the Z-axis (9  $\mu\text{m}$  depth) were merged by maximum intensity projection. Anterior is toward the top.