Supplementary data

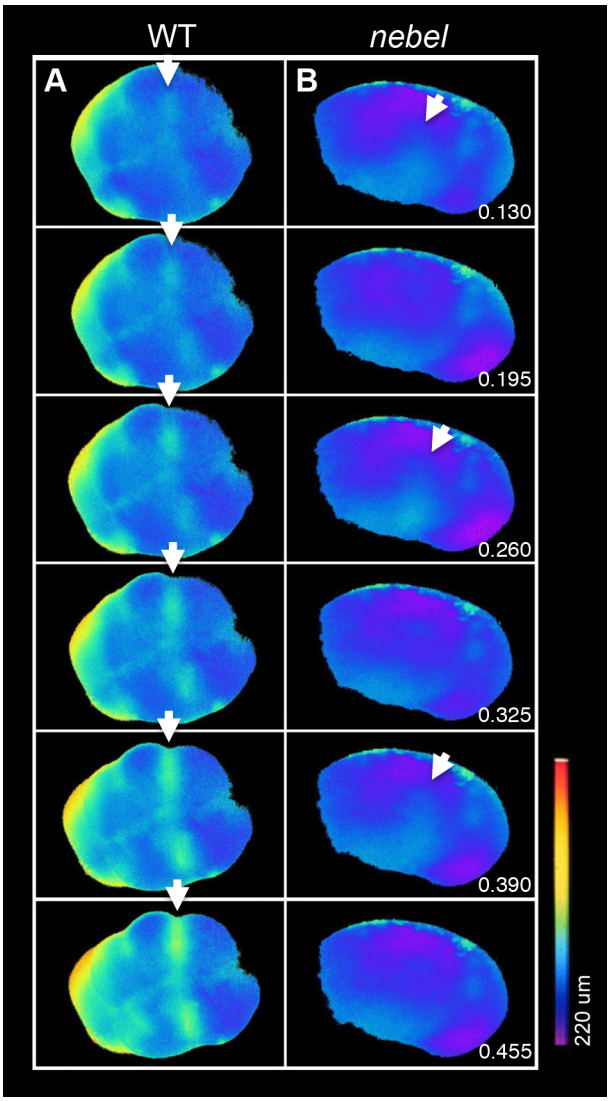


Fig S1. Intracellular calcium release in wild-type and *nebel* mutant embryos. (A,B) SCWs at the third cleavage furrow (arrow) visualized by injection of the ratiometric calcium-sensitive Fura-2 dye, as in Fig. 1A,B, showing a reduced yet repeated pattern of intracellular calcium release in *nebel* mutants (B). Details as in Fig. 1A,B.

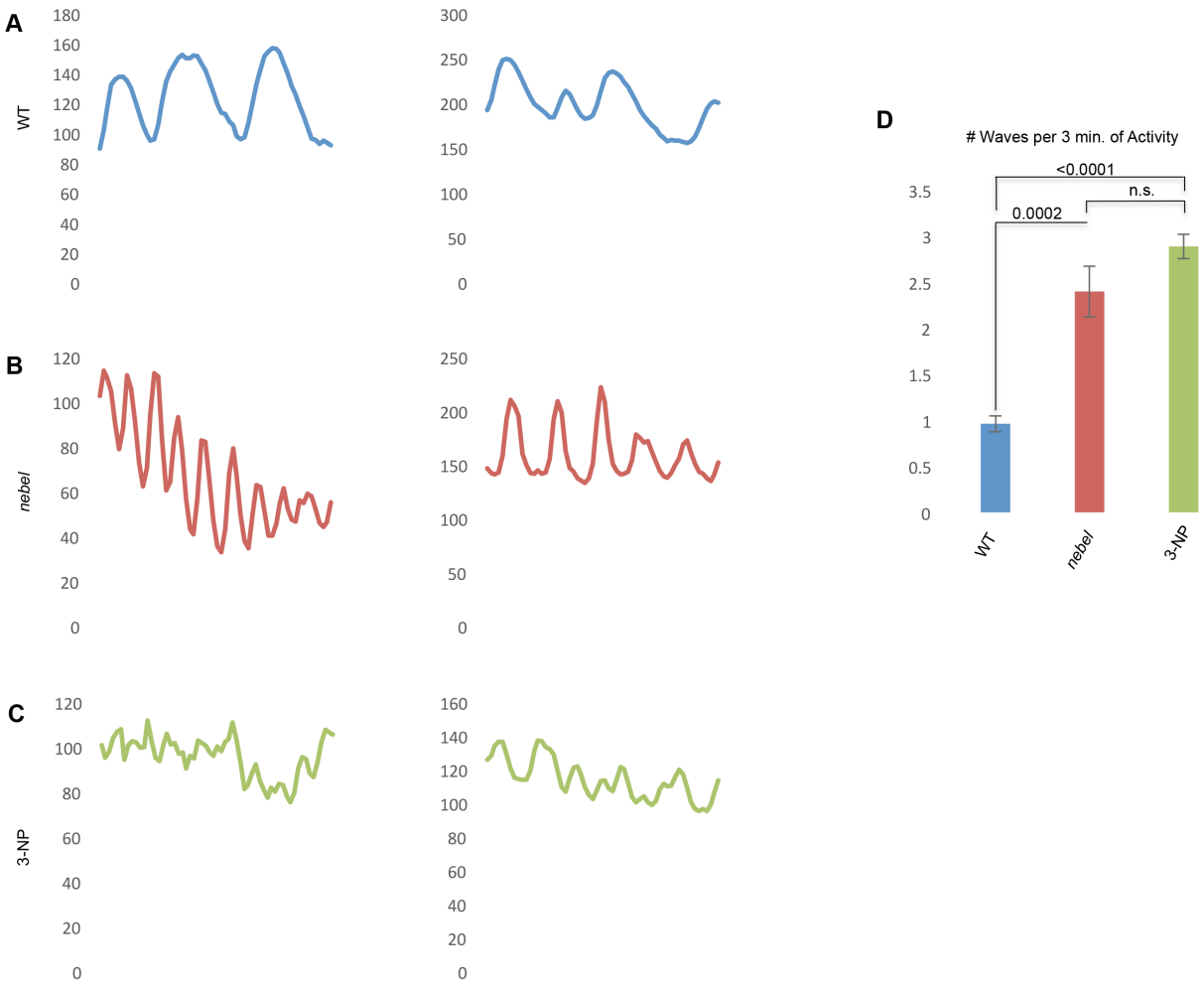


Fig. S2. *nebel* mutant embryos SCWs are more frequent than wild type embryos. (A-C) Representative profiles of calcium release along the furrow over a three minute period in wild type (A), *nebel* (B), and 3-NP treated (C) embryos, and average number of waves during a three minute period encompassing active waves (D). Data from 3 embryos in each case.

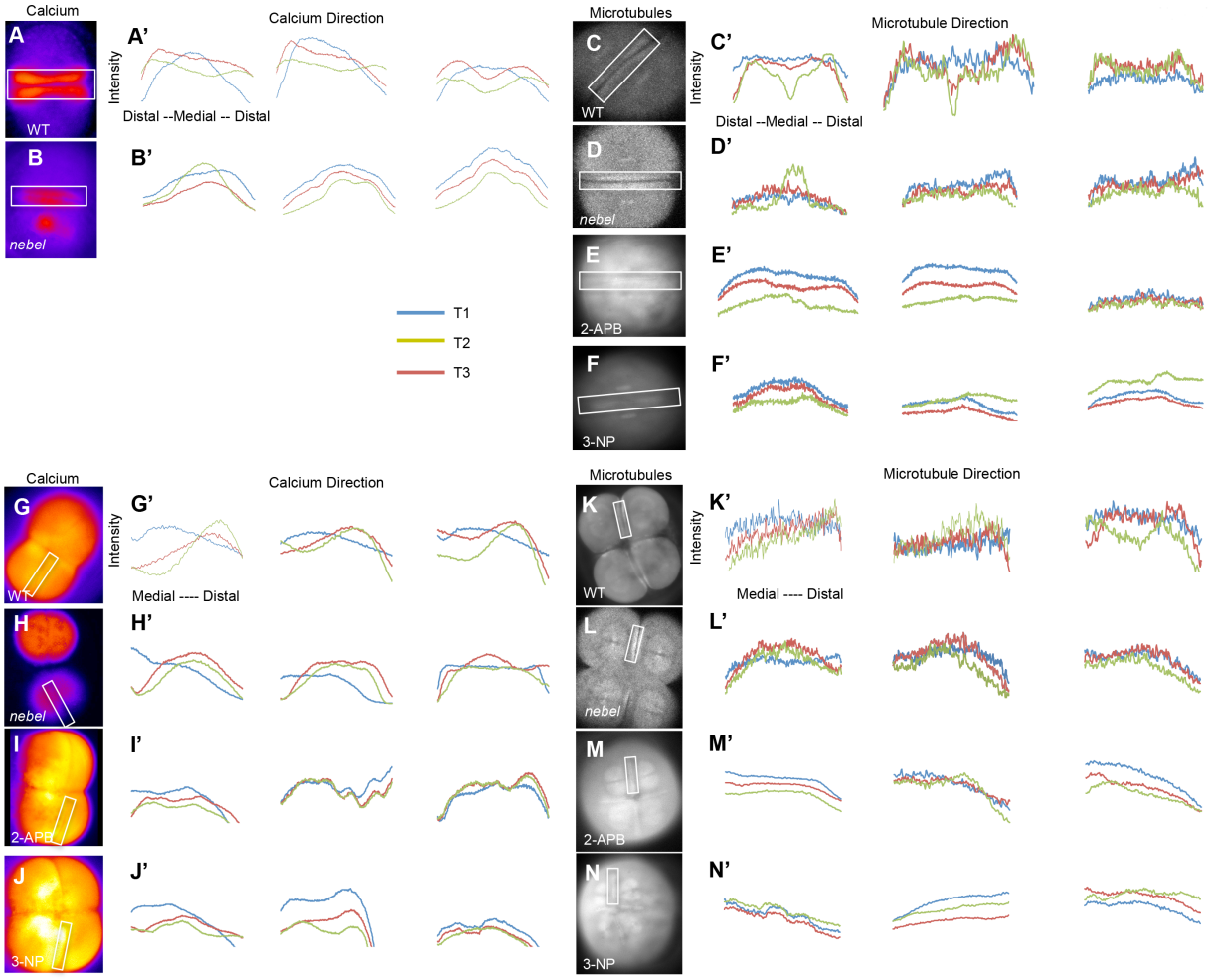


Fig. S3. Correlation of SCWs directionality and FMA movement. (A-B',G-J') Representative intensity profiles of SWCs (using injected OGB/Rhodamine (E-H') and the FMA (using the EMTB::EGFP transgene (C-F',K-N')) in the first and second furrows for three embryos and at three different time points during furrow formation (see Fig. 7 legend) in untreated wild-type (A,C,G,K) and *nebel* mutant (B,D,H,L) embryos, and 2-APB- (E,I,M) and 3-NP-treated (F,J,N) wild-type embryos. Boxes in the overview image indicate region for intensity scan analysis.

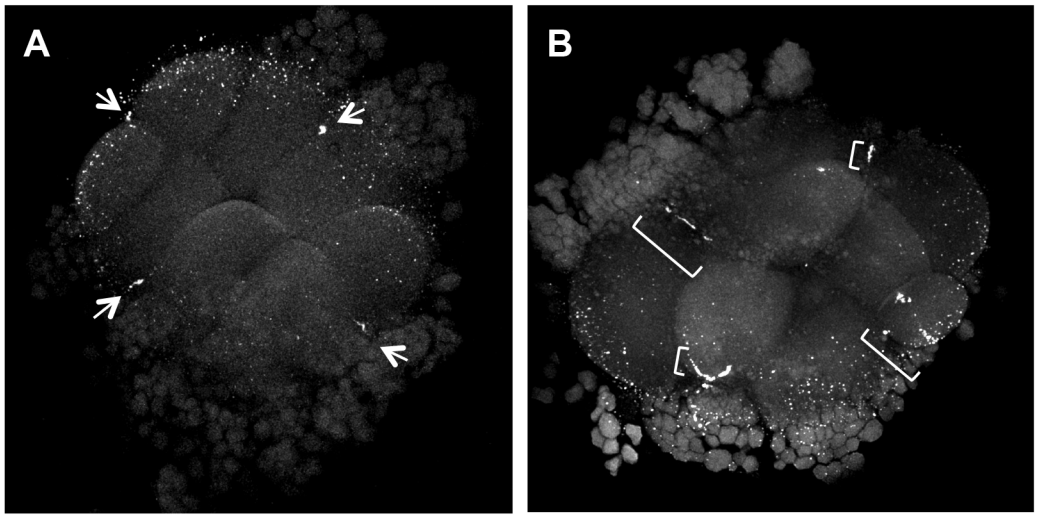
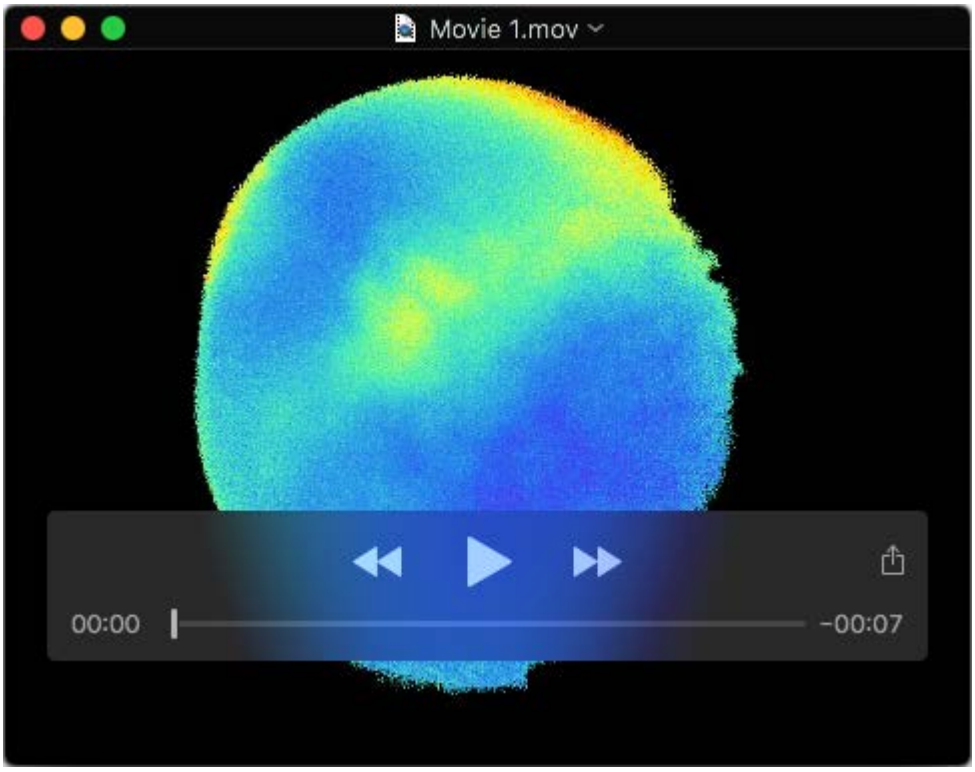
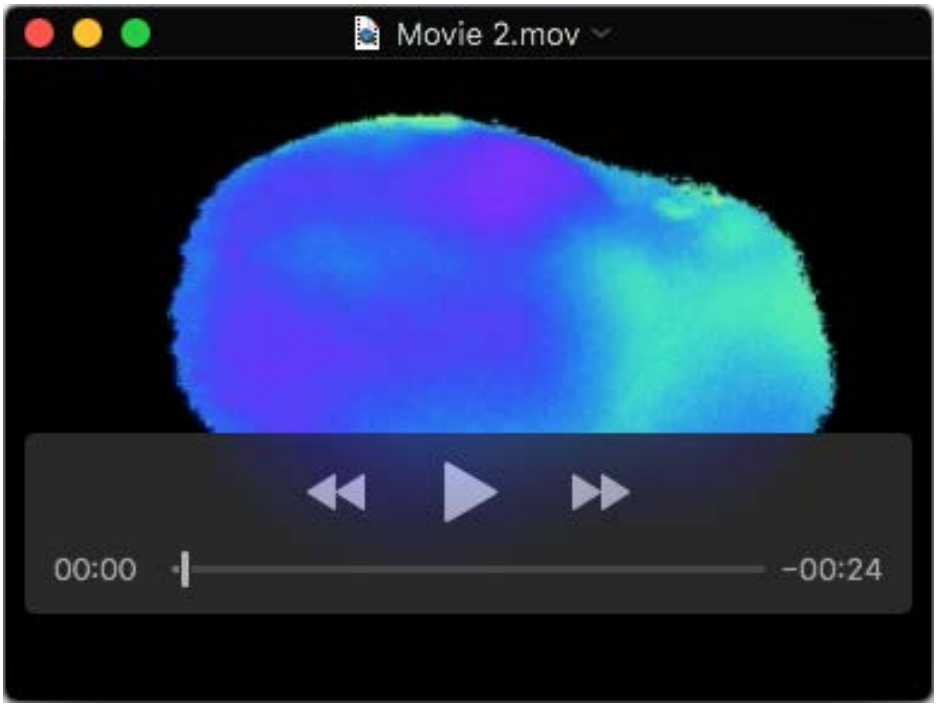


Fig. S4. Rho inhibition results in defects in germ plasm RNP distal compaction. At a time (70 mpf) when germ plasm masses are highly compacted in distal furrow regions (A, arrows,12/12 furrows), embryos injected with the Rho inhibitor C3 exoenzyme show expanded RNP domains along the furrow (B, brackets, compacted in 4/20 furrows), indicative of defects in germ plasm distal compaction.



Movie 1. Calcium image analysis of an early stage wild-type embryo using ratiometric calcium-sensitive Fura-2 dye. Imaging was initiated at the 2-cell stage to insure that imaged embryos were fertilized. Images in the movie correspond to embryo in Fig. 1A (second cleavage furrow), and Fig. S1A (third cleavage furrow) after a 90^{o}C counterclockwise rotation. The presented movie is representative of movies in three analyzed wild-type embryos. See Methods, Fig. 2 legend and Fig. S1 legend for details.



Movie 2. Calcium image analysis of an early stage *nebel* mutant embryo using ratiometric calcium-sensitive Fura-2 dye. Imaging was initiated at the 2-cell stage to insure that imaged embryos were fertilized. Images in the movie correspond to embryo in Fig. 1B (second cleavage furrow), and Fig. S1B (third cleavage furrow). The presented movie is representative of movies in six analyzed *nebel* mutant embryos. See Methods, Fig. 2 legend and Fig. S1 legend for details.

Table S1. Primers for end-point genotyping.

Primer Name	5'-> 3'	Use
nebel Forward	CTGGCACAAGTGAATGTCAAAC	genotyping
nebel Reverse	CCCTCTGTCCAATCAGAGAGTA	genotyping
nebel MUT Probe	/56FAM/CTA+GGC+T+T+TC+TC+CA/3IABkFQ/	genotyping
nebel WT Probe	/5HEX/TA+GGC+T+C+TCTC+CA/3IABkFQ/	genotyping

Table S2. Defects in *nebel* mutant embryos affect primarily furrow maturation, not initiation.

Furrow initiation [a,b]				
	normal	No initiation both furrows	no initiation one furrow	Total affected
WT	1.0	0	0	0
nebel/+	1.0	0	0	0
nebel/nebel	0.98	0.01	0.01	0.02
Furrow maturation[a.c]				
	Normal	No adhesion	Regression	Total affected
WT	0.998	0.002	0	0.002
nebel/+	0.82	0.15	0.03	0.18
nebel/nebel	0.10	0.61	0.29	0.90

a. Embryos were transferred to embryonic medium pre-cooled to 22.5°C within 10 mpf, sorted for symmetric cleavage during the first cell cycle to select for fertilized zygotes, and scored for defects in both the initiation and completion of cytokinesis. The same set of embryos was used to score both processes, and the number of scored embryos and different females from which they were derived is indicated. Mutant embryos showed furrow maturation defects at high frequencies, while furrow initiation was largely unaffected in these embryos. Number of embryos subsequently tested for both traits: WT (AB strain): 490 (5 females); *nebel* / + : 507 (6 females); *nebel/nebel*: 255 (4 females).

b. Lack of furrow clefting in the second cell cycle, as observed at the beginning of the 4-cell stage (50 mpf), was used as an indicator of failed furrow initiation. Observation of the second furrow was chosen as an assay because symmetric cleavage at the first cell cycle allowed to readily select for properly fertilized eggs and the large size of blastomeres of the second cell cycle facilitates the observation of furrow indentation.

c. Lack of the formation of an interblastomere adhesive septum at the site corresponding to the first cleavage furrow was used as an indicator of failed furrow maturation. Affected embryos showed reduced adhesion (rounded appearance of blastomeres) or furrow regression. Cytokinesis in the zebrafish cleavage-stage embryo undergoes a process of maturation that encompasses several cell cycles (Urven et al, 2006, J. Cell Sci 119, 4342-4352). Observation of completion of the first furrow was chosen because it completes maturation soon after the initiation of the second furrow, allowing us to determine both defects in the same embryos within a minimal time window. In addition, the relative elongation of blastomeres along the plane perpendicular to the first furrow cleavage facilitates the observation of cell rounding, associated with defective adhesion, at the first furrow.

Table S3. Injection of calcium induced pseudocleavages in water-activated *nebel* mutant embryos.

	no pseudo-cleavage	pseudo-cleavage	lysed after injection[c]	Total response[d]	n
WT, uninjected	**0.27**	**0.73**	**n.a.**	**n.a.**	**113**
***nebel*, uninjected**	**0.94**	**0.06**	**n.a.**	**n.a.**	**125**
***nebel*, 0.1 mM $CaCl_2$[b]**	**0.39**	**0.42**	**0.19**	**0.61**	**111**
***nebel*, 0.2 mM $CaCl_2$[a]**	**0.23**	**0.54**	**0.23**	**0.77**	**115**
***nebel*, 0.1 mM KCl**	**0.94**	**0.06**	**0**	**0.06**	**123**

a. Compilation of at least two separate experiments per catergory, which led to similar results

b. Solution also contained 0.1 M KCl to prevent damage to the eggs (Gilmour et al. 2002).

c. Lysis after injection is also associated with $CaCl_2$ injection and may reflect rapid exocytosis of internal vesicles, which rupture the plasma membrane of the egg.

d. Total fraction of injected eggs exhibiting pseudocleavage or lysis.