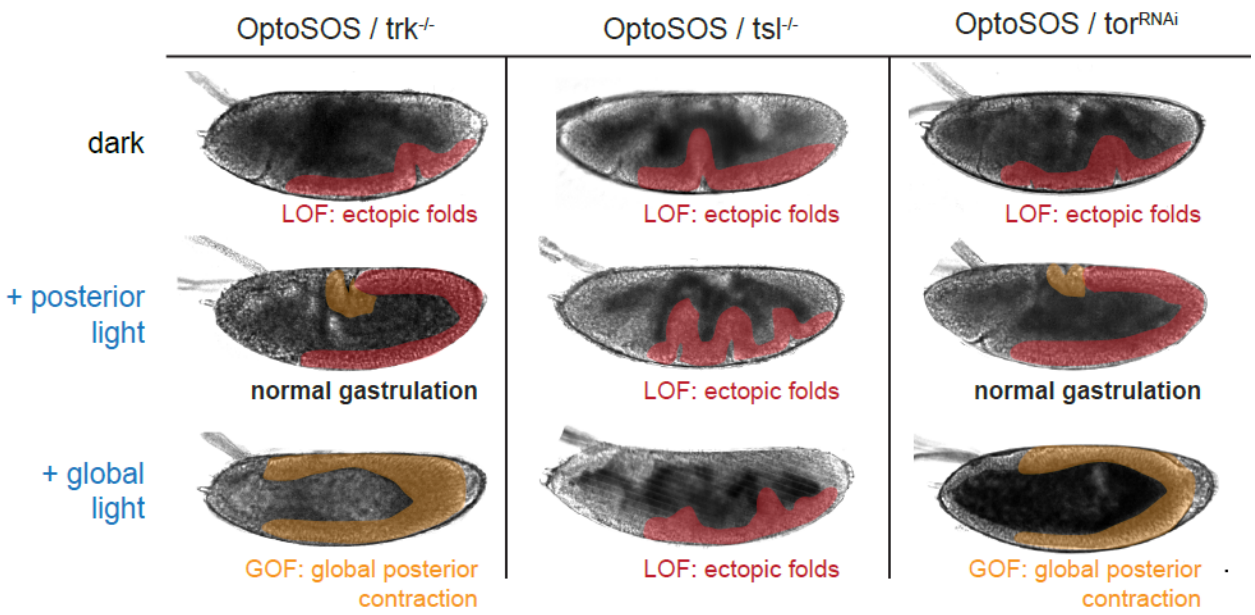

Figure S1: Characterizing optogenetic control in three Torso signaling pathway mutants. Related to Figure 1. Embryos were analyzed that expressed the OptoSOS system and were derived from mothers harboring loss-of-function Torso pathway alleles: homozygous *trk* (left), homozygous *tsl*, (middle) and 67;15>tor^{RNAi} (right). Each panel shows a representative embryo during gastrulation where the posterior midgut invagination (PMG invagination; yellow highlight) and elongating ventral mesoderm tissue (red highlight) are marked. Top row: all three mutants lead to loss-of-function phenotypes in the dark, including a failure to undergo PMG invagination and the appearance of ectopic folds along the embryo's ventral surface. Middle row: embryos were illuminated with a 0.6 sec of saturating 450 nm light every 30 sec, applied to the posterior-most 15% of the embryo. This stimulus rescues normal PMG invagination (yellow) and germ band elongation (red) in OptoSOS-*trk* and OptoSOS-tor^{RNAi} embryos. OptoSOS-*tsl* embryos still exhibit loss-of-function gastrulation phenotypes, including a lack of PMG invagination and ectopic folds. Bottom row: global illumination with 1 sec pulses of saturating 450 nm light every 30 sec leads to the large-scale formation of ectopic PMG and a massive contraction of the majority of the embryo in OptoSOS-*trk* and OptoSOS-tor^{RNAi} embryos but not in OptoSOS-*tsl* embryos. These data suggest that the *tsl* allele exhibits additional defects in tissue morphogenesis independently of its role in specifying terminal fates. All embryos in the figure are oriented with anterior to the left and ventral downward.

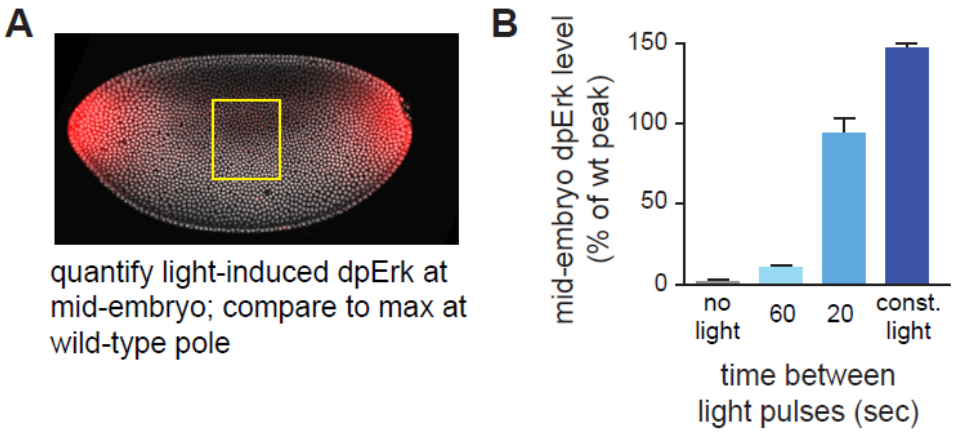

Figure S2: Titrating Erk phosphorylation using different light stimulus schedules. Related to Figure 2. (**A**) Nuclear cycle 12-14 OptoSOS embryos were illuminated for 1 hour under different stimulus conditions and then fixed and stained for doubly phosphorylated Erk (ppErk). The level of ppErk was quantified from a central embryonic region (yellow box) where endogenous terminal signaling would not contribute to Erk activity. As an internal standard, Erk phosphorylation was compared to the maximum level at the anterior pole of identically-stained wild-type embryos. A representative wild-type embryo exhibiting the normal terminal ppErk pattern is shown, reproduced from Figure 1A. Embryo is oriented with anterior to the left and ventral downward. (**B**) Quantification of ppErk levels in embryos stimulated with different cycles of saturating 450 nm light pulses: continuous light, a 1 sec pulse every 20 sec, a 1 sec pulse every 60 sec, or constant darkness. Illumination at 1 sec every 20 seconds led to ppErk levels comparable to the maximum level in the wild-type terminal pattern, whereas a 1 sec pulse every 60 sec drove steady-state ppErk levels at ~10% of the wild-type maximum. Error bars: standard deviation across spatial bins.

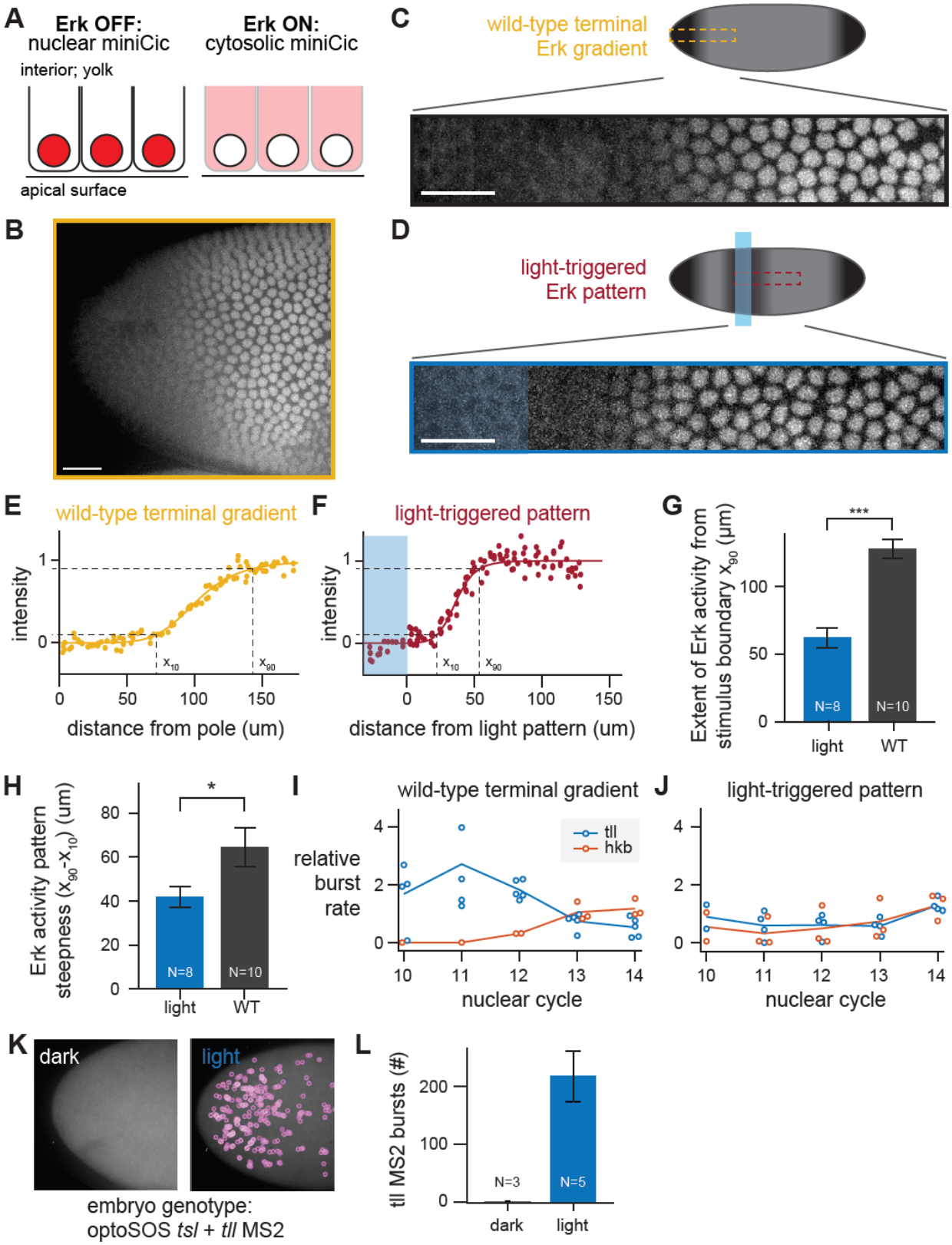
A
Erk OFF:
nuclear miniCic
Erk ON:
cytosolic miniCic
interior; yolk
apical surface
B
C
wild-type terminal
Erk gradient
D
light-triggered
Erk pattern
E
wild-type terminal gradient
intensity
distance from pole (um)
F
light-triggered pattern
intensity
distance from light pattern (um)
G
Extent of Erk activity from stimulus boundary x90 (µm)
***
N=8
N=10
light
WT
H
Erk activity pattern steepness (x90-x10) (um)
*
N=8
N=10
light
WT
I
wild-type terminal gradient
relative burst rate
tll
hkb
nuclear cycle
J
light-triggered pattern
nuclear cycle
K
dark
light
embryo genotype:
optoSOS tsl + tll MS2
L
tll MS2 bursts (#)
N=3
N=5
dark
light

Figure S3. Precise spatial patterning of Erk activity using light. Related to Figure 3. (**A**) A live-cell fluorescent biosensor of Erk activity (miniCic) was used to quantify the spatial distribution of Erk activity in living embryos. The miniCic biosensor is localized to the nucleus in the absence of Erk activity (left panel) and is phosphorylated and exported from the nucleus upon Erk activation (right panel). (**B**) Nuclear miniCic intensity imaged at the termini of wild-type nuclear cycle 14 (NC14) embryos, with a representative embryo shown oriented with its posterior pole to the left. A gradient of nuclear miniCic is formed, reflecting the endogenous gradient of Erk activity at the poles. Scale bar: 20 μm. (**C-D**) Comparison of the endogenous terminal pattern (in **C**) to an all-or-none light stimulus of 0.6 sec pulses of saturating 450 nm light delivered every 30 sec within the boxed region (in **D**). Representative miniCic fluorescence images from NC14 OptoSOS/miniCic embryos are shown, zoomed in from the indicated illumination regions (dashed boxes). Scale bars: 25 μm. (**E-F**) miniCic nuclear intensity was quantified from the embryo images in **C-D** as a function of position from the embryo pole (yellow curve) or the edge of the all-or-none light pattern (red curves). Best-fit Hill functions (solid lines) are shown. (**G-H**) Quantification of the length of gradient (in **G**) and gradient steepness (in **H**) for embryos stimulated as in **C**-**D**. '*' indicates $p < 0.05$ using Student's t test; '***' indicates $p < 0.01$ using Student's t test. The number of identically-stimulated embryos is shown for each bar. (**I-J**) Quantification of *tll* and *hkb* bursts over time for the embryos stimulated and analyzed in **Figure 3**. Points indicate the number of bursts per minute in each nuclear cycle, normalized to the average number of bursts across all nuclear cycles, for each embryo. Lines indicate the mean across multiple embryos; see **Table S2** for the number of embryos in each condition. (**K**) Still images OptoSOS-*tsl* embryos expressing MCP-mCherry and the *tll* upstream regulatory sequence driving MS2 stem-loops, incubated in the absence of 450 nm light or in response to an all-or-none light stimulus of 0.6 sec pulses of saturating 450 nm light delivered every 30 sec. Images are maximum intensity projected across all z-planes and time points. Representative embryos are oriented with posterior poles to the left. (**L**) Quantification of the number of transcriptional foci observed in embryos prepared as in **K**. The number of identically-stimulated embryos is shown for each bar.

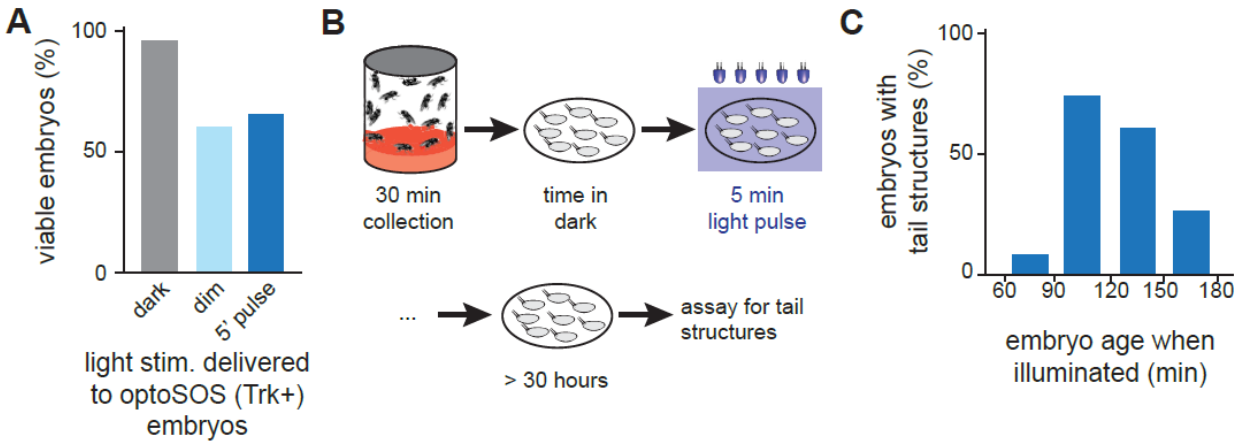

Figure S4: Programming of tail structures at low Erk levels. Related to Figure 4. (**A**) Assessing the effects of short-duration and low-intensity illumination on OptoSOS embryos that still exhibit normal endogenous terminal signaling. Embryos were incubated in the dark, stimulated globally with a 1 sec light pulse delivered every 120 sec ("dim"), or incubated under a single 5 min bolus of constant, saturating 450 nm light ("5' pulse"), and viability was assessed. Under these conditions, a majority of larvae still hatch and appear normal, indicating that the light intensity used to rescue tail structures is below the threshold for eliciting strong gain-of-function phenotypes. "Dim" light indicates 1 sec pulses of saturating 450 nm light every 120 sec, expected to lead to Erk activity less than 10% of maximal levels (see **Figure S2**). (**B**) Schematic of experiment to define the time window during which tail structures are specified. OptoSOS-*trk* embryos were collected over a 30 min period, incubated in the dark for varying amounts of time, and then globally illuminated with a 5 min light pulse. Cuticle preparations were then used to assess the formation of tail structures at the end of embryogenesis. (**C**) The fraction of embryos with normal tail structures was defined in each 30 min collection window, with most embryos harboring tails when stimulated in a 1 h window, 90-150 min after fertilization.

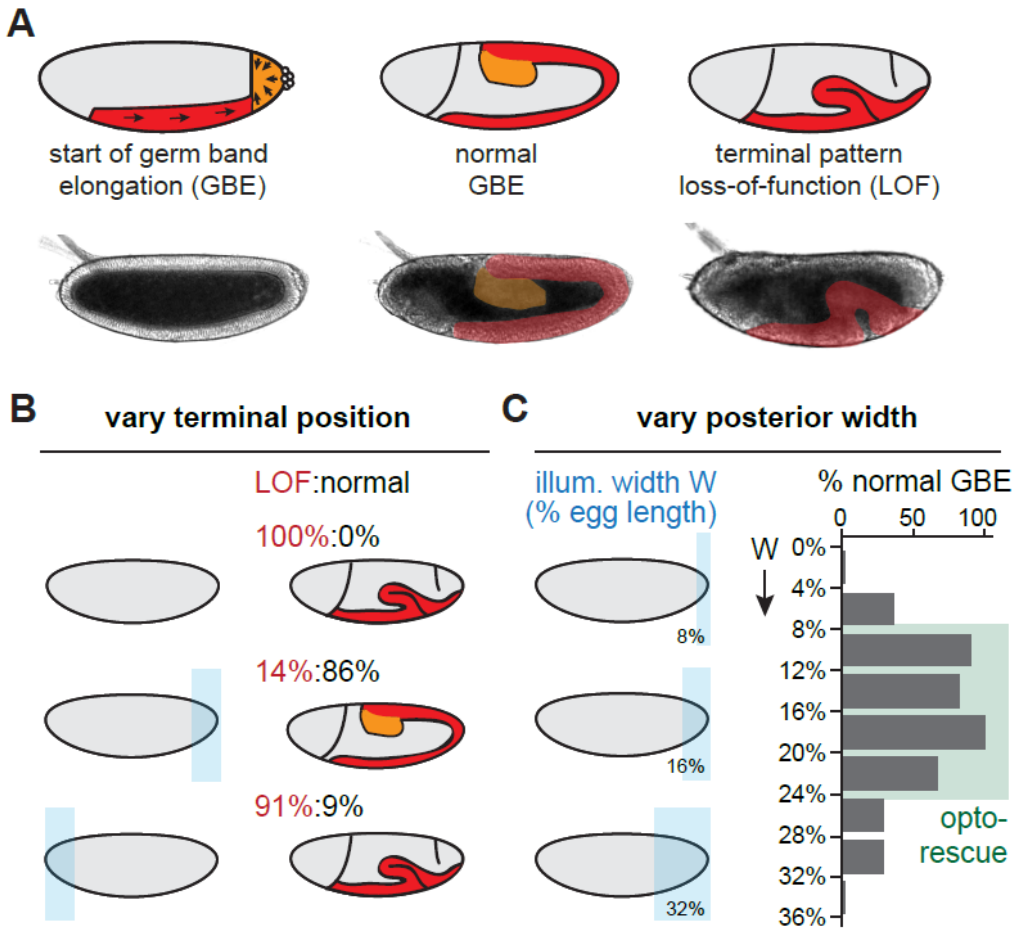

Figure S5. The lower limit of spatial signaling required for tissue morphogenesis. Related to Figure 5. (**A**) Schematic of posterior tissue movements during germ band elongation (GBE), with representative images for each phenotype shown below. Left: During germ band elongation, cells migrate to the ventral surface (red highlight) and intercalate, while the posterior endoderm (orange highlight) constricts and internalizes. Middle: This results in the large-scale movement of posterior tissue along the dorsal surface towards the head. Right: In the absence of terminal signaling, germ band elongation is blocked, leading to buckling of ventral tissue. All embryos are oriented with anterior to the left and ventral downward. (**B-C**) Optogenetic dissection of the spatial requirements for terminal signaling in gastrulation. All embryos were stimulated with a 90 min overall stimulus of 0.6 sec pulses of saturating 450 nm light delivered every 30 sec to the indicated spatial regions. "Normal" gastrulation was defined as successful posterior invagination and a germ band whose length was within the 95% confidence interval obtained from 27 wild-type embryos. A loss-of-function ("LOF") embryo was defined by a lack of posterior invagination and germ band elongation. (**B**) Gastrulation was scored for embryos that were kept in the dark or illuminated at either the anterior or posterior pole and monitored by differential interference contrast (DIC) imaging. (**C**) Gastrulation was scored for embryos that were illuminated with different posterior pattern widths, from 0-36% of the embryo's length. Illumination widths that produce normal gastrulation in a majority of embryos are marked with a green box. For **B-C**, normal gastrulation is defined by presence of posterior invagination and a germ band that extends to a length within the wild-type 95% confidence interval.

stimulus duration	tail structures	proper segmentation	gastrulation movements	head structures	hatching
dark	33/204 (16%)	3/109 (2.7%)	0/10 (0%)	0/5 (0%)	0/200 (0%)
5 min	**G: 66/90 (73%)**	P: 21/172 (12.2%)	ND	ND	ND
15-30 min	**P: 9/9 (100%)**	**P: 9/9 (100%)**	P: 4/69 (5.7%)	A: 0/8 (0%)	ND
45-90 min	**7/7 (100%)**	**6/7 (86%)**	**P: 39/40 (97.5%)**	**A: 13/15 (87%)**	**9/31 (29%)**

Table S1: Quantification of embryo phenotypes from optogenetic experiments and dark-incubated controls. Related to Figures 2 and 4. Each entry represents the total number of embryos whose structures could be assessed under light stimulation of the duration indicated. In each entry case, the location of illumination is noted (P = posterior; A = anterior; G = global). All anterior and posterior light stimuli consisted of 0.6 sec pulses of saturating 450 nm light delivered every 30 sec; global illumination was delivered using 1 sec pulses of saturating 450 nm light every 30 sec. Red entries indicate a failure to rescue (normal phenotype less than 20%), whereas green bolded entries indicate successful rescue. Data is taken from multiple experiments described in Figure 2 and Figure 4.

Figure	# of embryos per condition
2A	15
2B	7
2C	22
2D	31, 200
3C,D	6, 3, 5, 5
4A	204, 90, 68
4B	109, 172, 9
4C	8, 43, 26, 7, 4, 30
5B	70 (WT = 27)
5C	82 (WT = 27)
S2B	52, 28, 26, 10
S3G,H	8, 10
S3I,J	6, 3, 5, 5
S3L	3, 5
S4A	88, 21, 117
S4C	102, 90, 81, 139
S5B	10, 44, 34
S5C	2, 11, 20, 17, 4, 3, 7, 7, 3 (WT = 27)

Table S2: Number of embryos quantified in each experiment. Related to Figures 2-5 and S2-S5. Comma-separated numbers indicate the number of embryos quantified in each experimental condition, from left to right as indicated in the corresponding figure.