Supplementary Information for:

Dynamics of Drosophila Embryonic Patterning Network Perturbed In Space and Time Using Microfluidics

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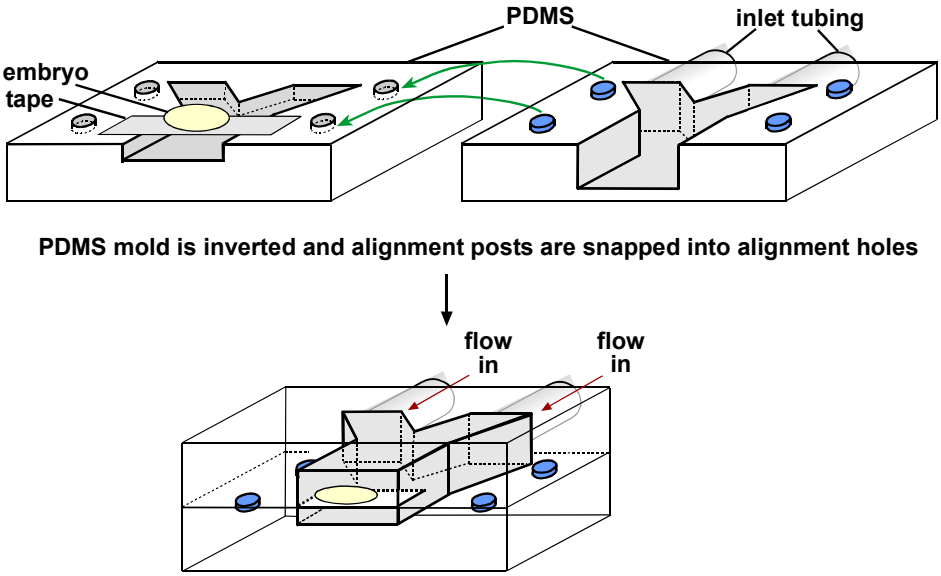
Fabrication and assembly of the microfluidic device. Two multilevel molds of different depths were fabricated using rapid prototyping in polydimethylsiloxane (PDMS) (Scheme S1). One mold was fabricated with a shallow channel (850 μm x 250 μm) and with alignment holes, while the other mold was fabricated with a deep channel (850 μm x 500 μm) and with alignment posts (illustration below). To perform the experiment, the embryo (~ 500 x 250 μm) was placed on double-sided tape on the mold with the shallow channel. The second mold with the deep channel was then inverted, placed on top of the first mold, and alignment posts were snapped into alignment holes (illustration below). The difference in the depth of the channels in the two molds allowed positioning of the embryo in the center of the cross-section of the main channel. This positioning was important to ensure that the embryo is completely enveloped by the flowing streams. The alignment posts and holes (Anderson, J. R. *et al.* Fabrication of topologically complex three-dimensional microfluidic systems in PDMS by rapid prototyping. *Anal. Chem.* **72**, 3158-3164 (2000)) allowed for rapid, precise assembly of the two molds without damage to the embryo. After assembly, the molds were clamped and the flow was started. The two molds could be

assembled and clamped in less than one minute, allowing for the embryo to be exposed to the flow as quickly as possible after fertilization.



Scheme S1

Characterization of flow and heat transport, and its effect on the embryo

Calculating the shear rate

Shear rate γ [s^{-1}] in the rectangular channel (Nataraja, N. M. & Lakshman, S. Laminar-Flow in Rectangular Ducts - Prediction of Velocity Profiles and Friction Factor. *Ind. J. Tech.* **10**, 435-438 (1973)) was calculated using

$$\gamma = U_m(n/b)((m+1)/m)((n+1)/n)$$

where U_m [cm s^{-1}] = 5 cm/s is the average velocity through the channel, and m = 2.64 and n = 2.10 are correction constants for a rectangular channel of the aspect ratio we used. Under these conditions, the shear rate at the embryo is ~ 700 s^{-1}. Viability of *Drosophila melanogaster* embryos exposed to flow of buffer has been demonstrated at higher shear

rates (higher flow velocities and smaller channel dimensions) (Furlong, E. E. M., Profitt, D. & Scott, M. P. Automated sorting of live transgenic embryos. *Nat. Biotechnol.* **19**, 153-156 (2001)).

Calculating the Reynolds number

The dimensionless Reynolds number (*Re*) was calculated for the flow around the embryo using $Re = lU\rho/\mu$, where l is length [m], U is flow velocity [m/s], ρ is density of fluid [kg m^{-3}], and μ is viscosity of fluid [kg m^{-1} s^{-1}]. At a flow velocity of 5 cm/s, the calculated *Re* was 13. Laminar flow is present at $Re < 2000$ in simple geometries. While the overall flow is clearly laminar under the conditions used in experiments, at values of Re > 40 it is possible for localized eddies to exist. Such eddies could affect the temperature profile around the embryo, but we have not detected these eddies in the flow around the embryo.

Calculating thermal diffusivity

The thermal diffusivity D_T [m^2s^{-1}] was calculated through the egg shell and through the cytoplasm of the embryo using $D_T = k/\rho C_p$, where k is thermal conductivity [W $m^{-1}K^{-1}$], ρ is density [kg m^{-3}], and C_p is specific heat [J $kg^{-1}K^{-1}$]. The wax layer composing the eggshell was approximated by paraffin wax, where $k = 2.5\times10^{-3}$ kJ $m^{-1}s^{-1}K^{-1}$, ρ = 930 kg m^{-3}, and C_p = 2.1 kJ $kg^{-1}K^{-1}$. They cytoplasm was approximated by water, where $k = 6.0\times 10^{-3}$ kJ $m^{-1}s^{-1}K^{-1}$, ρ = 1000 kg m^{-3}, and C_p = 4.2 kJ $kg^{-1}K^{-1}$. The thermal diffusivity through the eggshell was calculated to be 1.3×10^5 μm^2s^{-1} and through the embryo was calculated to be 1.4×10^5 μm^2s^{-1}. The time scale t [s] of heat transport was approximated by $t = x^2 / 2D_T$, where x [μm] is distance over which heat transport is taking place. The time scale for heat

transfer through the 1.5 μm eggshell (Margaritis, L. H., Kafatos, F. C. & Petri, W. H. The eggshell of *Drosophila melanogaster* .1. Fine-Structure of the Layers and Regions of the Wild-Type Eggshell. *J. Cell Sci.* **43**, 1-35 (1980)) was calculated to be 0.9×10^{-5} s, and the time scale for heat transfer through the ~500 μm length of the embryo was calculated to be 0.9 s.

Oxygen content in buffer

To ensure normoxia of embryos placed in the device, the buffer used to flow around the embryo was saturated with air prior to the experiment, and the buffer was kept under slight excess pressure (~0.03 atm) of air. The solubility (χ) of O_2 in water is 2.5×10^{-5} M^{-1} at 20°C and 2.2×10^{-5} M^{-1} at 27°C, which is within the range of normoxia for *Drosophila*. (Furlong, E. E. M., Profitt, D. & Scott, M. P. Automated sorting of live transgenic embryos. *Nat. Biotechnol.* **19**, 153-156 (2001)).

Embryos developed in the microfluidic device for four hours hatched and survived to adulthood. Embryos developed in the device also showed normal staining patterns for both Even-skipped (Eve) and Hunchback (Hb).

Quantification of nuclear density in different regions of embryos exposed to a temperature step

The difference in nuclear density in different regions of DAPI stained embryos exposed to a temperature step was quantified using MetaMorph® Imaging System (Universal Imaging Corp). Images of DAPI stained embryos were analyzed as follows: a) the egg length (EL) along the antero-posterior (A-P) axis was determined by using the line function tool to measure from the anterior-most point of the embryo to the posterior-most

point of the embryo, b) square boxes were drawn in specific regions in the anterior and posterior halves of the embryo; the boxes were made to be either 18% EL in width for regions away from the poles of the embryo, or 9% EL in width for regions near the poles of the embryo. The 18% EL in width boxes were placed either 10% EL away from each pole or 20% EL away from each pole, and midway along the dorsal-ventral (D-V) axis. The 9% EL in width boxes were placed 5% EL away from each pole and midway along the D-V axis. The same analysis was done for control embryos developed at 25^{o}C on molasses plates and DAPI stained (Supplementary Figure 3 h-i). The number of nuclei were counted in regions of experimental embryos were compared to the number of nuclei counted in regions of control embryos to determine the cell cycle to which each experimental region is closest (Supplementary Figure 3 a-g).

Embryos exposed to the temperature step of 17^{o}C/27^{o}C for 140 minutes displayed a difference in cell cycle between anterior and posterior halves of ~4x. The cool half and warm half were closest to a cycle 11 and cycle 13 control, respectively (Supplementary Figure 3 d-e). Embryos exposed to the temperature step of 20^{o}C/27^{o}C for 150 minutes displayed a difference in cell cycle between anterior and posterior halves of ~2x. The cool half and warm half were closest to cycle 11 and cycle 12, respectively (Supplementary Figure 3 f-g).

To determine age of embryos within Cycle 14, we analyzed the mid-plane of DAPI-stained nuclear images. During Cycle 14, embryos undergo cellularization. During phase 1 of cellularization at the beginning of Cycle 14, nuclei begin to elongate and furrow canals form between the nuclei. During phase 2, nuclei complete elongation. During phase 3, the furrow canals reach the base of the elongated nuclei, and during phase 4, cell wall forms

around the nuclei. (Mazumdar A., & Mazumdar M. How one becomes many: blastoderm cellularization in *Drosophila melanogaster*. *BioEssays* **24**, 1012-1022 (2002)). We measured the extent to which this process occurred to examine the age of embryos that correspond to the data graphed in Figure 4b. In experiments with anterior 27^{o}C/posterior 20^{o}C with the temperature step transiently reversed at 65 minutes (anterior 20^{o}C/posterior 27^{o}C) and reversed back at 100 minutes (anterior 27^{o}C/posterior 20^{o}C), the variability observed in the Hunchback boundary position is not correlated to age of the embryo.

Methods

Fly stocks: Wild-type Oregon R. *Drosophila* embryos were used for all experiments.

Experimental set-up

Embryos were collected on molasses plates for 2-5 minutes at 23°C, placed on Scotch® #667 double-sided tape, and assembled between two PDMS molds. The embryo was exposed to flow 7-10 minutes from the start of collection and kept in the flow for up to 240 minutes from the start of collection. The temperature of flowing streams was controlled by heating one stream and cooling one stream using a double chilling/heating plate (Echo therm™, Torrey Pines Scientific). Volumetric flow rate was controlled by syringe pumps (KdScientific).

Immunostaining of embryos

Embryos exposed to the temperature step were dechorionated on Scotch® #667 double-sided tape, fixed in 3% formaldehyde in PEM and immunostained as previously described using 2B8 mouse anti-Eve (Patel, N. H., Condron, B. G. & Zinn, K. Pair-rule expression patterns of Even-skipped are found in both short-germ and long-germ beetles.

Nature **367**, 429-434 (1994)), and IG10 mouse anti-Hb (Patel, N. H. *et al.* Grasshopper hunchback expression reveals conserved and novel aspects of axis formation and segmentation. *Development* **128**, 3459-3472 (2001)) monoclonal antibodies. AlexaFluor™ 488 goat anti-mouse IgG (H+L)-conjugated (Molecular Probes) was used as a secondary antibody. Embryos were counterstained with 0.1 μg/ml DAPI (4',6-diamidino-2-phenylindole) in 50% glycerol in 1x PBS buffer (Patel, N. H. *Methods in Cell Biology, Vol. 44. Drosophila melanogaster: Practical Uses in Cell Biology* (ed. Fyrberg, E.) (Academic Press, New York, 1994)).

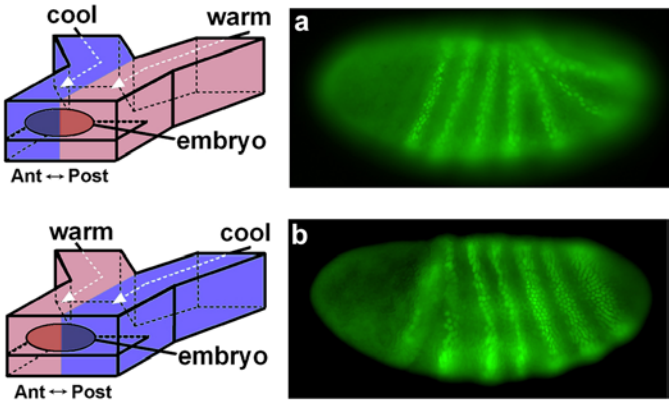
Imaging

Images were acquired using a Leica DM IRE2 inverted microscope with a 20x 0.7 NA objective and cooled CCD camera ORCA ERG 1394 (12-bit, 1344x1024 resolution) (Hamamatsu Photonics, K.K.). Lighting was provided by a Xe light source. MetaMorph® Imaging System (Universal Imaging Corp) was used to collect images at integration times from 30-300 ms. Images were processed using Adobe Photoshop.

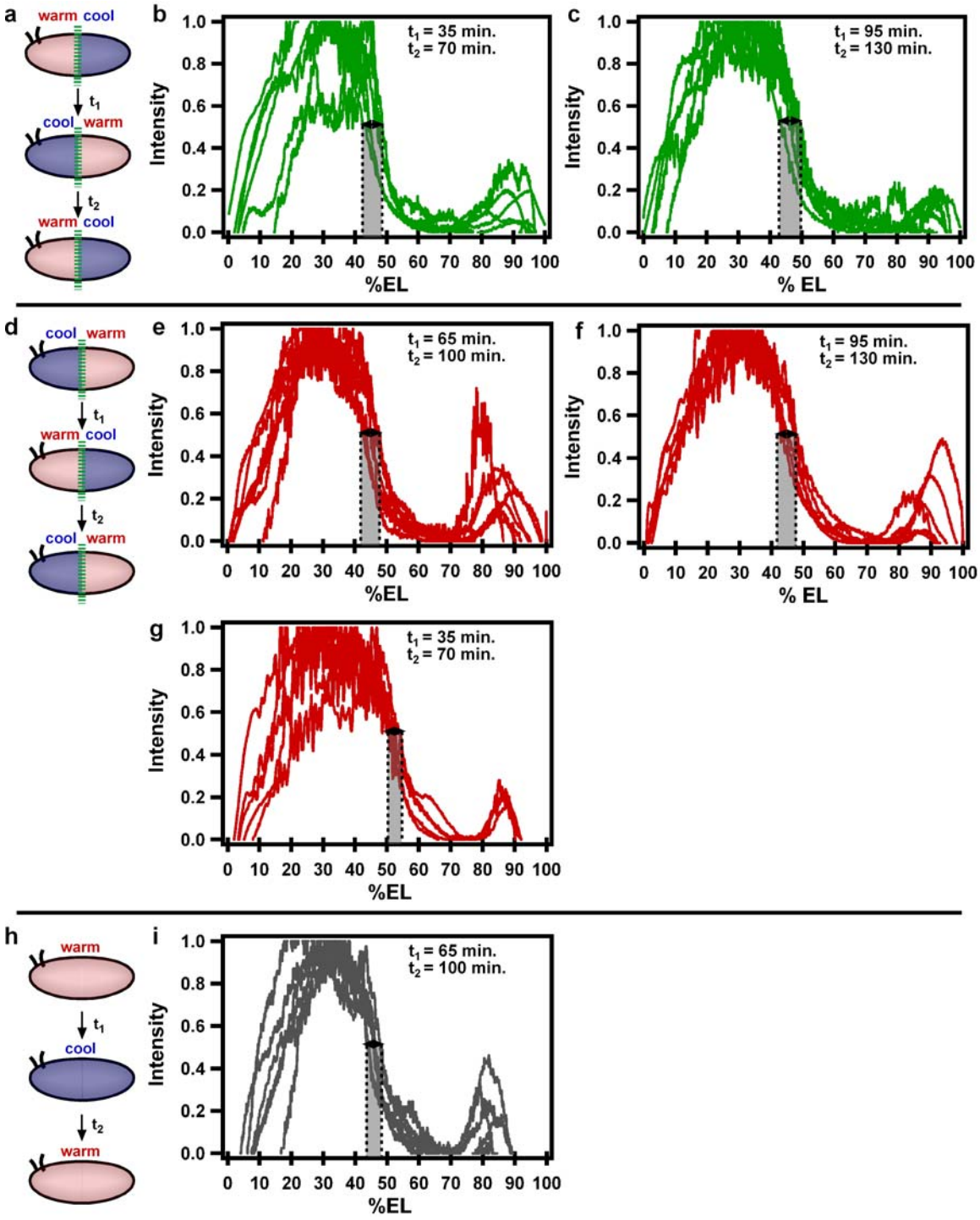
Image Analysis

Images were analyzed using MetaMorph® Imaging System (Universal Imaging Corp). The intensity profile of Eve and Hb expression was taken along the antero-posterior (A-P) axis, midway between the dorsoventral (D-V) axis, as it has been described previously (Myasnikova, E. *et al.* Registration of the expression patterns of Drosophila segmentation genes by two independent methods. *Bioinformatics* **17**, 3-12 (2001)). Position along the anterior-posterior axis was normalized for embryo length, and maximum and minimum intensities were normalized to one and zero, respectively. Positions of Eve

stripes along the antero-posterior axis were determined by determining positions of the maxima of the Eve intensity profiles.

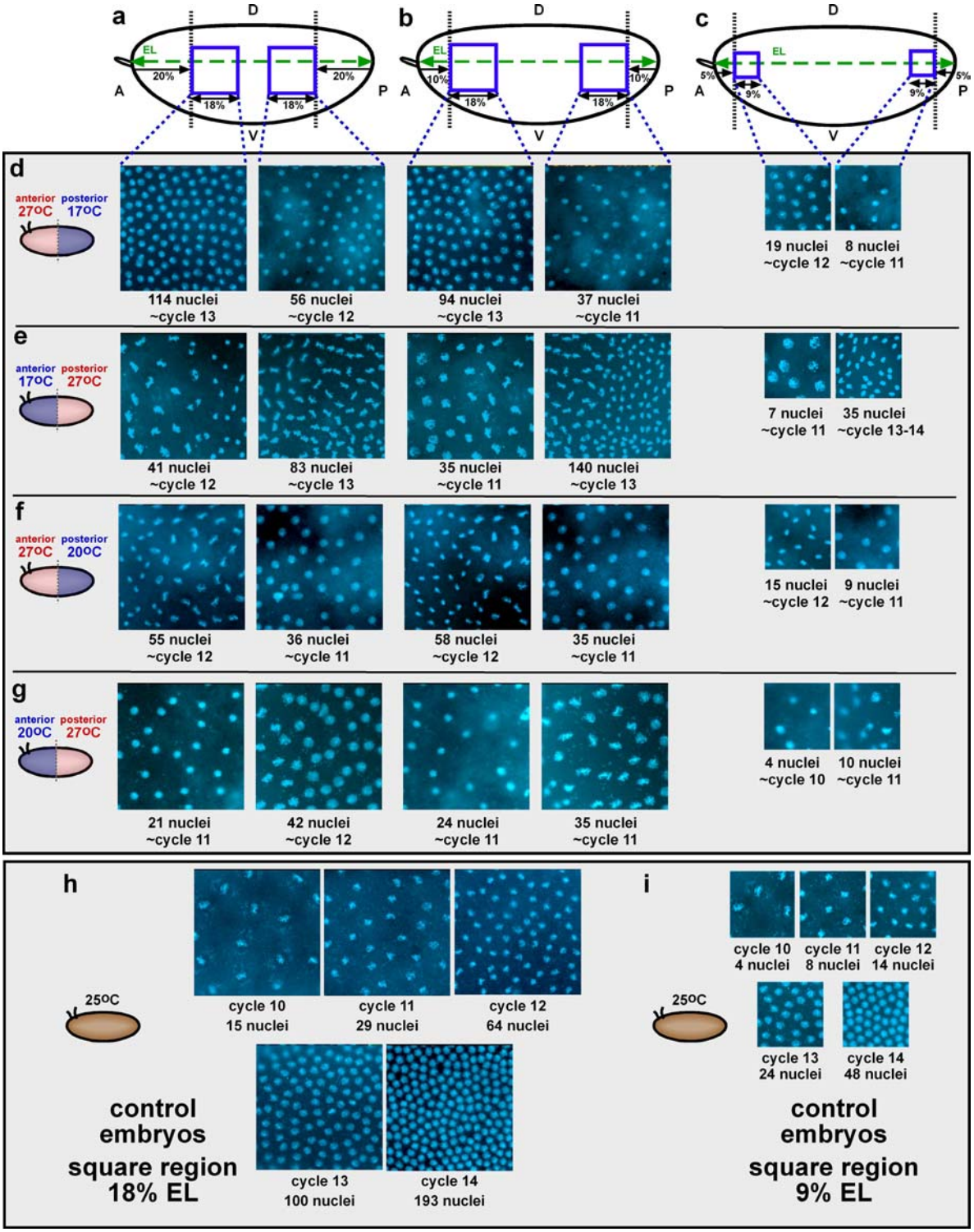


Supplementary Figure 1 Embryos continue to be affected by the temperature step of 20°C/27°C after cycle 14. **a,** The posterior half of the embryo (warm) gastrulated before the anterior half of the embryo (cool). **b,** The anterior half of the embryo (warm) gastrulated before the posterior half of the embryo (cool).



Supplementary Figure 2 a, Schematic of embryos exposed to a time-dependent temperature step with anterior half at 27°C and posterior half at 20°C with

exception of a brief time frame from t_1 to t_2 in which the temperature step was reversed (anterior 20°C/posterior 27°C). **b**, Hb intensity profiles where t_1 = 35 minutes, t_2 = 70 minutes. The Hb boundary was variable over 7% EL (42 – 49%EL). **c,** Hb intensity profiles where t_1 = 95 minutes, t_2 = 130 minutes. The Hb boundary was variable over 7% EL (43 – 50%EL). **d**, Schematic of embryos exposed to a time-dependent temperature step with anterior half 20°C and posterior half at 27°C with exception of a brief time frame from t_1 to t_2 in which the temperature step was reversed (anterior 27°C/posterior 20°C). **e**, Hb intensity profiles where t_1 = 65 minutes, t_2 = 100 minutes. The Hb boundary was variable over 5% EL (43 – 48%EL). **f**, Hb intensity profiles where t_1 = 95 minutes, t_2 = 130 minutes. The Hb boundary was variable over 6% EL (43 – 49%EL). **g**, Hb intensity profiles where t_1 = 35 minutes, t_2 = 70 minutes. The Hb boundary was variable over 4% EL (51 – 55%EL), with a slight posterior bias. **h**, Schematic of embryos exposed to a uniform change in temperature. The entire embryo developed at 27°C with exception of a brief time frame from t_1 to t_2 in which the temperature over the entire embryo was cooled (20°C). **i**, Hb intensity profiles where t_1 = 65 minutes, t_2 = 100 minutes. The Hb boundary was variable over 4% EL (from 44 – 48%EL).



Supplementary Figure 3 a-c, Schematic representations of method of region selection for quantitative determination of number of nuclei in different regions of

DAPI stained embryos. Regions were taken at different points along the antero-posterior (A-P) axis to assess the effect of the temperature step close to the boundary of the two streams and at the poles of the embryo. **a**, Nuclei were counted in square boxes 18% egg length (EL) in width, positioned 20% EL in from each pole of the embryo along the A-P axis and centered at the midpoint along the dorsal-ventral (D-V) axis. **b**, Nuclei were counted in square boxes 18% EL in width positioned 10% EL from each pole of the embryo along the A-P axis and centered at the midpoint along the D-V axis. **c**, Nuclei were counted in square boxes 9% EL in width positioned 5% EL from each pole of the embryo along the A-P axis and centered at the midpoint along the D-V axis. The boxes for quantification of nuclear density in these regions were made smaller because of their proximity to the pole of the embryo. **d-e**, Quantification of nuclear density and approximate cell cycle of embryos exposed to a temperature step of 20°C/27°C for 140 minutes. **d**, Anterior half 27°C, posterior half 20°C. **e**, Anterior half 20°C, posterior half 27°C. **f-g**, Quantification of nuclear density and approximate cell cycle of embryos exposed to a temperature step of 17°C/27°C for 150 minutes. **f**, Anterior half 27°C, posterior half 17°C. **g**, Anterior half 17°C, posterior half 27°C. **h-i**, Regions of control embryos developed at 25°C on molasses plates (cycles 10-14). **h**, Nuclei were counted in square boxes 18% EL in width made 20% EL from each pole of the embryo along the A-P axis and centered at the midpoint along the D-V axis. **i**, Nuclei were counted in square boxes 9% EL in width made 5% EL from each pole of the embryo along the A-P axis and centered at the midpoint along the D-V axis.