and other remnants are cleaned by placing the embryo in Ringer's solution or PBS and embryos were cultured ventral side up on the solidified culture medium. Embryos were incubated on either a Nikon Eclipse Ti (mercury bulb UV excitation; Fig. S5), Zeiss Axiozoom V16 (HXP 200C LED excitation; Fig. S6) or Zeiss LSM880 (confocal laser; Fig. S7) at 37ºC, with images taken of embryos between every 10-30 minutes, depending on imaging set-up.

Determining the duration of topically applied TAT-Cre Recombinase activity via Affi-Gel Blue Beads in ex ovo EC culture

To examine the duration that TAT-Cre Recombinase is active if applied topically via an Affi-Gel Blue bead, we undertook the following experiments *ex ovo* in EC culture (as described above). In EC *ex ovo* culture, eight TAT-Cre Recombinase soaked beads were placed in anterior to posterior sequence on endoderm, either side of the midline. Beads on the right side were left in place for the duration of the experiment, while beads on the left side were removed after 30 seconds, 1 hour, 2 hours and 4 hours and visualised on a Zeiss AxioZoom V16 microscope after 24 hours of incubation.

Supplementary References

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