Supporting Information

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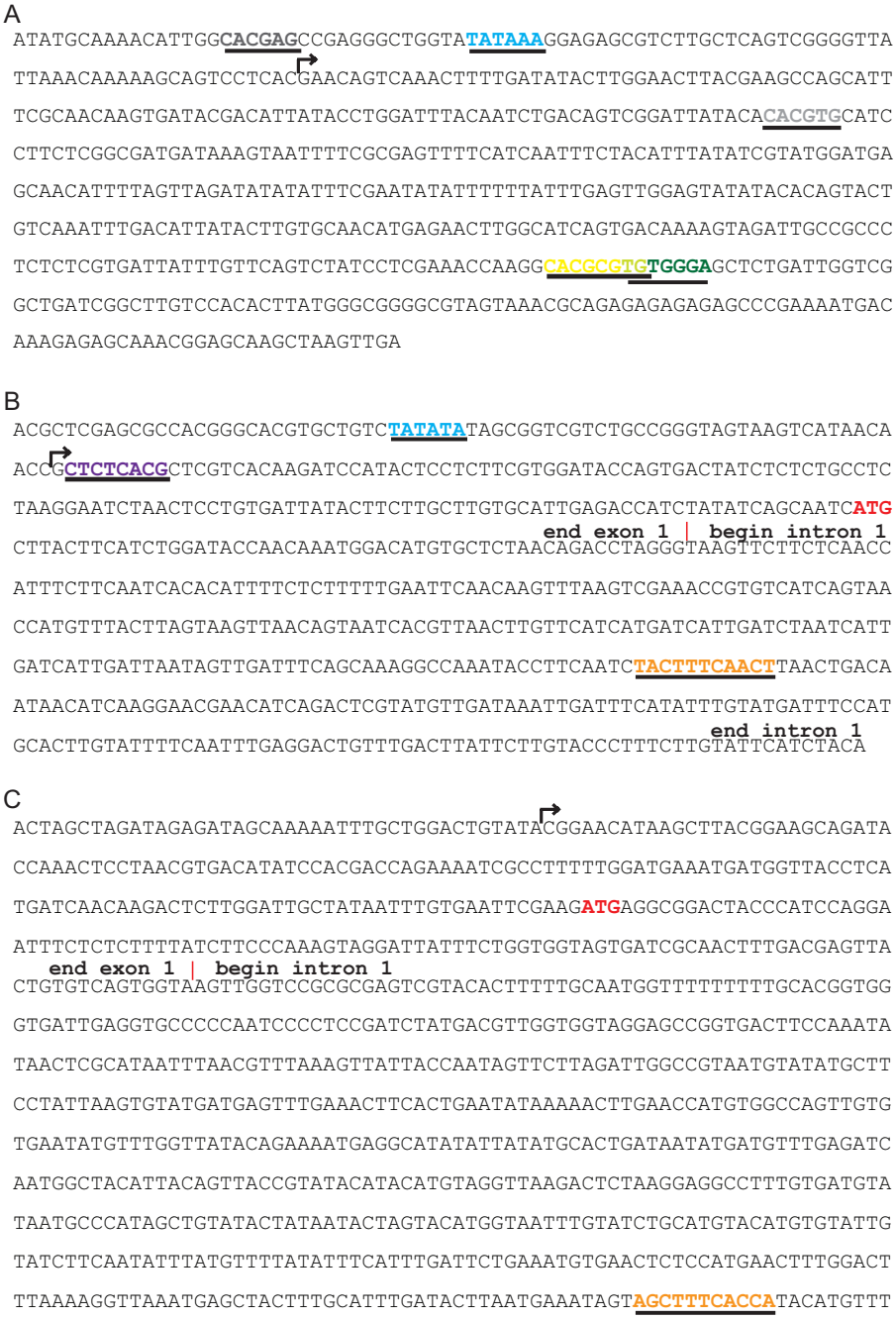

Fig. S1. Regulatory module sequences and features for *delta*, *hesC*, and *notch*. (*A*) The *delta* proximal module from −90 to +484 relative to start of transcription. N-box sequence, dark gray; E-box class B sequence, light gray; E-box class C sequence, yellow; Runx binding site, green; overlapping E-box class C sequence and Runx binding site, light green. (*B*) The *hesC* proximal region from −71 to end of first intron. Su(H) binding element, purple; Blimp target site in first intron, orange. (*C*) The notch locus from −41 to +860. Blimp target site in first intron, orange. Putative TATA-binding element, cyan; start of transcription, bent arrow; start of translation, red ATG. Red vertical bar marks exon1/intron1 boundary in (*B*) and (*C*).

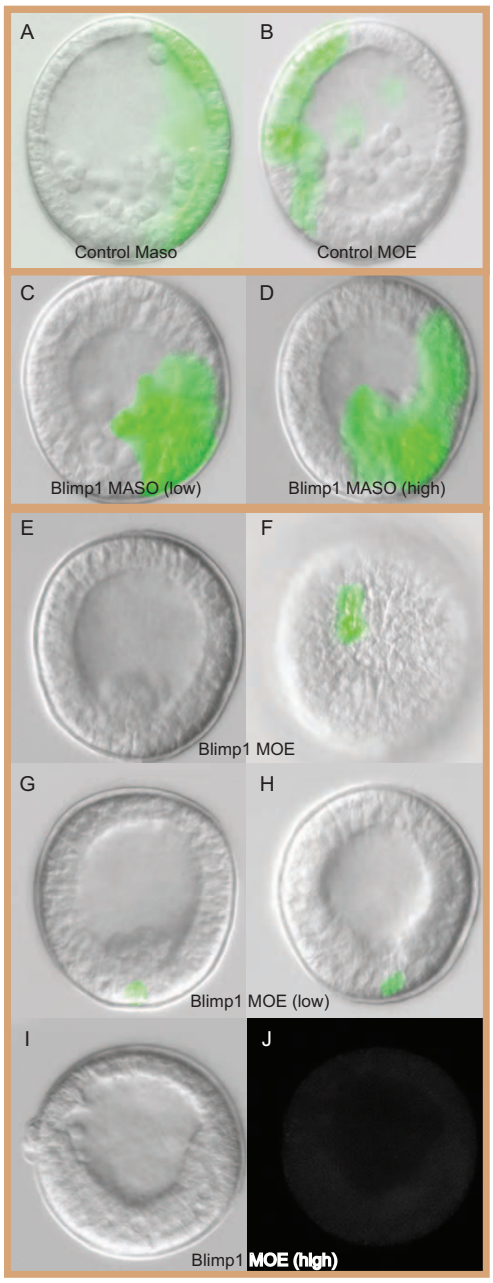

Fig. S2. HesC BAC:GFP reporter activity in response to Blimp1 MASO and mRNA overexpression (MOE) in 27 h mesenchyme blastula stage embryos; vegetal pole at bottom. (*A–D* and *F–H*) False-color overlay of fluorescent micrograph on differential interference contrast (DIC) image. (*A*) HesC BAC:GFP reporter activity in control MASO condition showing GFP expression in a large clone, including both ectoderm and presumptive endoderm, but no expression in the ingressed SM. (*B*) HesC BAC:GFP reporter in control MOE; similar to (*A*). (*C* and *D*) HesC BAC:GFP expression in different doses of Blimp1 MASO; both result in increase in fluorescence and ectopic appearance of reporter activity in mesoderm. The tremendous increase in fluorescence intensity results in pixel saturation in vegetal territories of the embryo, leaving ectodermal territories appearing unlabeled. (*E–H*) Effects of Blimp1 MOE. Embryos receiving low (10^4 copies) mRNA/embryo. (*I* and *J*) Embryos receiving high (5×10^4 copies) mRNA/embryo. (*E*) DIC image. (*F*) fluorescence overlay showing greatly reduced HesC BAC-GFP activity; a clone of 4–6 cells displays fluorescence, as seen in ≈40% of embryos. (*G* and *H*) Two additional embryos subjected to low concentration of Blimp1 MOE treatment display even more greatly reduced HesC BAC-GFP activity. Only slight fluorescence is detectable in what appears to be small micromere descendents, a result found in ≈50% of embryos in this condition. (*I* and *J*) Two images of an embryo subjected to high Blimp1 MOE treatment: (*I*) DIC image; (*J*) fluorescence image showing no detectable HesC BAC-GFP reporter activity.

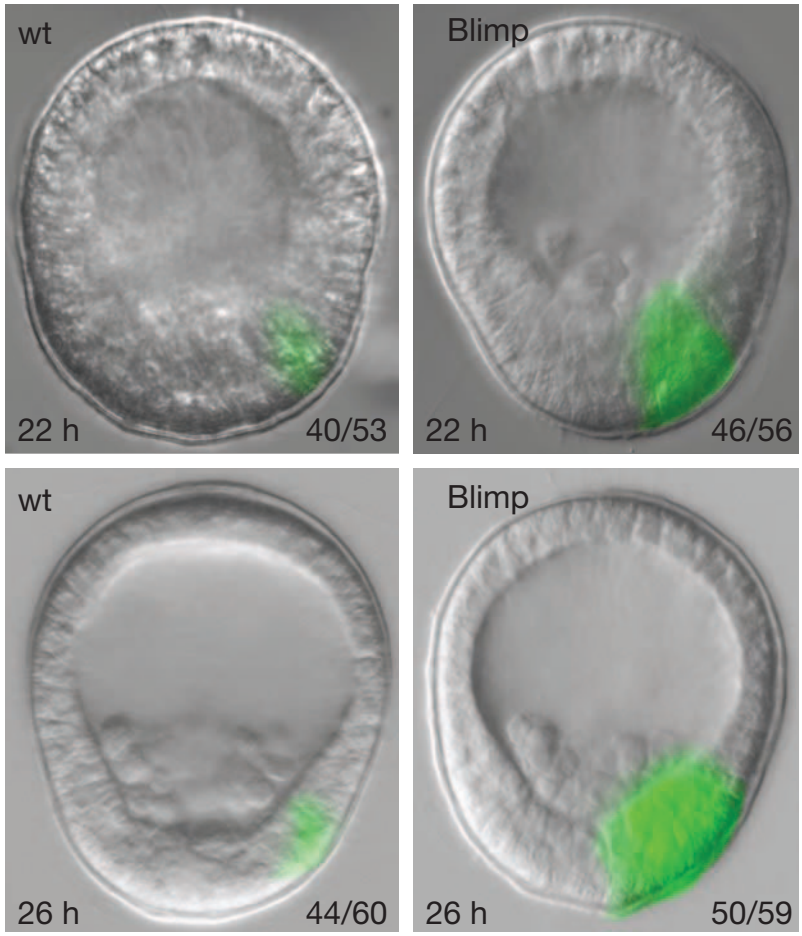

Fig. S3. Notch BAC:GFP reporter assays. The reporter activity for wild-type Notch BAC:GFP reporters or Notch BAC:GFP reporters with Blimp target site in first intron disrupted was assessed at 22 h and 26 h postfertilization as indicated. Disruption of Blimp site results in *notch* reporter activity remaining in the NSM in a majority of embryos tested. Quantities indicate proportion of all GFP+ embryos having the dominant pattern of expression (as shown) for each reporter.

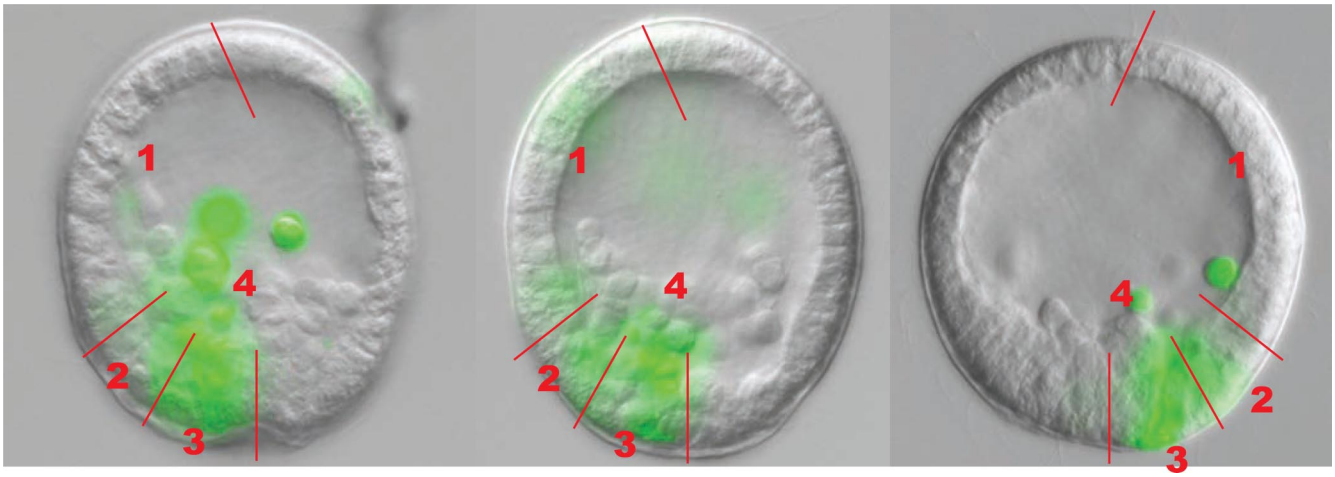

Fig. S4. Additional embryos, equivalent to that shown in Fig. 4*B*, harboring the *hesC* reporter construct with a mutation of the Blimp1 binding site located in intron 1 of the *hesC* locus. Disruption of the Blimp1 site caused expression of the *hesC* reporter to remain strong in the NSM territory (zone 3) and in the SM (zone 4). This contrasts with the expression pattern of native *hesC* reporter (Fig. 4*A*). These results demonstrate that Blimp1 is responsible for the persistent extinction of *hesC* transcription in both of these mesodermal territories. Embryos were oriented identically, and the pattern shown in red was superimposed on them as in Fig. 4. Tabulated data are shown in Table 2.

Table S1. Primers used for reporter constructs

Reporter	Primer ID	Primer sequence
Delta 5 kb	Delta 1	5′-GAACTTTGATTGGCGGTGTT
	Delta 2	5′-CTATCGGCATGCAGCAAAAT
Delta proximal module	Delta 5	5′-GAAGGCACGCGAACTAATGT
	GFP KanR (specific to cassette used for BAC GFP knockin)	5′-accggatctagataactgat
Delta HesC site disruption	D del H4.3	5′-CGAAACCAAGGACATATTGTGGGAGCTCTGATTGGTCGGCTGAT
	D del H4.4	5′-GCTCCCACAATATGTCCTTGGTTTCGAGGATAGACTGAAC
HesC 10 kb	HesC3	5′-GTGTCCCAAAAGGGCTCATA
	HesC4	5′-TATCGGTCAGGCGGAAATAG
HesC Blimp site disruption	Hes delB1.1	5′-CAGTTACTCGTCCCTGCGATTGAAGGTATTTGGCCTTTGCTG
	Hes delB1.2	5′-TCAATCGCAGGGACGAGTAACTGACAATAACATCAAGGAACG
HesC Su(H) site disruption	Hes delS1.1	5′-ACGAGCGGTATCGCGGTTGTTATGACTTACTACCCGGC
	Hes delS1.2	5′-GTCATAACAACCGCGATACCGCTCGTCACAAGATCCATACTCCTCT

Table S2. QPCR results of mRNA overexpression experiments on *hesC* transcript levels

Gene overexpressed	Time sample collected, h	*hesC*Δ Δ Ct	Gene Δ Δ Ct
FoxA	12	−0.25	+11.14
GataE	12	−0.38	+17.90
Hox11/13b	12	−0.04	+7.84
Prox	12	−0.25	N/D
SoxC	12	−0.29	+8.24
Z13/Krl	12	−0.14	N/D
FoxA	17	+0.01	+6.90
GataE	17	+0.29	+8.45
Hox11/13b	17	+0.61	+6.52
Prox	17	−0.02	N/D
SoxC	17	+0.07	+8.28
Z13/Krl	17	−0.05	N/D

N/D, no data. A ± 1.6 cycle difference is considered the threshold of significance.