Supplementary Materials and Methods

Screening of upstream gene candidates for larval skeleton formation in the cidaroid *P. baculosa* and collection of *pmar1/phb*-related genes from other echinoderms

For RNA-seq of *P. baculosa*, RNA was extracted using TRIzol Reagent (Life Technologies) and RNeasy Mini Kit (Qiagen). Paired-end libraries 151 bp in insert length were prepared, and libraries were sequenced on a NextSeq 500 platform (Illumina). Library preparation and sequencing were performed by Bioengineering Lab (Kanagawa, Japan). The short reads obtained were deposited in the Sequence Reads Archives of DDBJ (https://www.ddbj.nig.ac.jp/index-e.html) (accession number: DRA008358). The reads were trimmed and filtered by Trimmomatic (ver. 0.32) and NGS QC toolkit (ver. 2.3.3) (Bolger et al., 2014; Patel and Jain, 2012). De novo assembly was conducted with Trinity 2.2.0 (Grabherr et al., 2013). The FPKM values were calculated by RSEM with the Bowtie2 option (ver 1.2.18) (Li and Dewey, 2011).

We identified *pmar1* and *phb1* sequences of *E. tribuloides* using genomic and transcriptomic databases available in EchinoBase (http://www.echinobase.org/Echinobase/) (Kudtarkar and Cameron, 2017) by BLAST-based approach. For *pmar1*, we obtained two scaffolds (scaffolds 7904 and 10027) containing *pmar1*-like sequences from *E. tribuloide*s genome 1.0 database. Assuming that *pmar1* of this species also includes an intron in the same position with that of euechinoid *pmar1*, we manually predicted the *pmar1* sequences. The positions of exons for each *pmar1* sequence in these scaffolds are follows: for scaffold 7904, *pmar1-1* (31930–31736 and 30275–29771), *pmar1-2* (37174–36980 and 35956–35455), *pmar1-3* (43769–43575 and 42591–42093), *pmar1-4* (48119–48313 and 49773–50277), and *pmar1-L1* (40267–40067); for scaffold 10027, *pmar1-5* (8703–8509 and 7240–6736), *pmar1-6* (14248–14054 and 12559–12055), *pmar1-7* (17164–17358 and 18775–19282), and *pmar1-8* (24452–24643 and 26002–26503). For the *pmar1-L1*, start codon is substituted for the codon coding Y, and second exon was not found. The *E. tribuloides phb1* sequence (Locus_6652_Transcript_2/4_Confidence_0.750) was obtained from *E. tribuloide*s transcripts database of EchinoBase.

For the brittle star *A. kochii*, sea cucumber *A. japonicus*, and feather star *O. japonicus*, we searched the *pmar1/phb1*-related sequences using the BLAST-based approach on the transcript sequences assembled as mentioned above for *P. baculosa*. For the data of *A. japonicus* and feather star *O. japonicus*, trimming and filtering were not performed. The sequenced RNAs were derived from following developmental stages: for *A. kochii*, 4-cell (2 h), early blastula (4 h), mid-blastula (6 h), and hatched

blastula (8 h) stages; for *A. japonicus*, fertilized egg, 4-cell, morula, blastula, gastrula, late gastrula, early auricularia larva, and mid- auricularia larva stages; for *O. japonicus*, unfertilized egg, 2-cell, 8-cell, 32-cell, gastrula, hatched gastrula, early doliolaria larva, and doliolaria larva stages. The raw RNA-seq data of *A. kochii* was deposited in DDBJ (accession number: DRA008359). The RNA sequencing data of *A. japonicus* and *O. japonicus* were kindly provided by Naoki Irie.

For other echinoderms, we also surveyed *pmar1/phb1*-related sequences by BLAST search using *Pb-pmar1*, *phb1*, or *phb* sequences against for available genomic/transcriptomic data. The sequence of euechinoid *H. pulcherrimus phb1* (HPU_10182) was collected from HpBase (http://cell-innovation.nig.ac.jp/Hpul/) (Kinjo et al., 2018). The starfish *P. pectinifera phbA* and *phbB* were obtained from transcriptome data previously described in Kawai et al. (Kawai et al., 2016). For starfish *P. miniata*, *phbA* (gi|307020228|gb|HP094737.1|) was obtained from *Patiria miniata* RNA database of EchinoBase, whereas *phbB* was predicted from *Patiria miniata* contig_136381 (the position of homeodomain: 20733–20596 and 20052–20011) included in the *Patiria miniata* contigs 1.0 database of EchinoBase. The amplified sequences of *P. pectinifera phbA* and *phbB* were deposited in DDBJ (accession numbers: LC483153 and LC483154, respectively). The *A. planci phbA* and *phbB* (IDs: oki.14.227.t1 for *phbA* and oki.242.12.t1 for *phbB*) were collected from the cots_genemodels_nucl database of OIST Marine Genomics Unit (https://marinegenomics.oist.jp) (Hall et al., 2017). For sea cucumbers *Holothuria leucospilota* and *Parastichopus parvimensis*, *phb* sequences were collected from transcript assembly of Koga et al. (2016) and that obtained from EchinoBase (Pparv.xome11.fa; ID: Locus_3449_Transcript_32/68), respectively. After collection of *pmar1/phb* candidates, we performed molecular phylogenetic analysis as described below to identify each sequence.

Molecular phylogenetic analysis

Alignment of the homeodomain sequences (60 amino acids) was performed using SeaView software (Gouy et al., 2010). The best-fitting amino acid substitution model and maximum likelihood tree were inferred using RAxML software (ver. 8.2.0) (Stamatakis et al., 2008). The bootstrap values were calculated using 1,000 replicates. The aligned HD sequences used to construct the maximum likelihood tree of Figure 1 are shown in Data S2.

Whole-mount *in situ* hybridization (WMISH)

The fixation of embryos was performed as described previously (Yamazaki et al., 2010; Koga et al., 2010). The hybridization and staining were carried out according to the method of Morino et al. (2012). The concentrations of RNA probes in hybridization buffer were 100 ng/ml for *H. pulcherrimus* and *P. baculosa*, 250 ng/ml for *P. pectinifera*, *A. kochii*, and *A. japonicus* embryos. The primers used for amplification of cDNA fragments for RNA probes are shown in Table S2. For the other genes, we prepared RNA probes using DNA fragments described previously (Yamazaki et al., 2014; Koga et al., 2010).

Perturbation experiments

The mRNAs used in the overexpression experiments were synthesized from linearized plasmids using a mMESSAGE mMACHINE (Thermo Fisher Scientific). The mRNA was transcribed from a pBluescript RN3′ plasmid (Nishimura et al., 2004) containing the whole coding sequence or that fused to *Drosophila* engrailed repression region (EnR) or VP16AD which was cloned using In-Fusion HD Cloning Kit (Takara Bio). The primers used for preparing constructs are follows: *Pb-pmar1*-cds-F, 5′-AACTTTGGCAGATCTATGGCCGAATCGACA-3′; *Pb-pmar1*-cds-R, 5′-CAGATCCGCGGCCGCTCAGTGAAATCTGGT-3′; *Ppec-phbA*-cds-F, 5′-AACTTTGGCAGATCTATGCCTGCTAACGCT-3′; *Ppe-phbA*-cds-R, 5′-CAGATCCGCGGCCGCTCAGTGGCGAGCAAT-3′; *Ppe-phbB*-cds-F, 5′-AACTTTGGCAGATCTATGTCTTCTGCTGCC-3′; *Ppe-phbB*-cds-R, 5′-CAGATCCGCGGCCGCTTACATGTGATGGGA-3′; Enr-fusion-F, 5′-AACTTTGGCAGATCTATGGCCCTGGAGGAT-3′; Enr-*Ppe-phbA*-cds-fusion-R, 5′-AGCGTTAGCAGGCATCGAACCCAGAGCAGA-3′; Enr-*Ppe-phbB*-cds-fusion-R, 5′-GGCAGCAGAAGACATCGAACCCAGAGCAGA-3′; VP16AD-fusion-F, 5′-AACTTTGGCAGATCTAAGCTAGCGCCGCCA-3′; VP16AD-*Ppe-phbA*-fusion-cds-R, 5′-AGCGTTAGCAGGCATGAATTCCCCACCGTA-3′; VP16AD-*Ppe-phbB*-fusion-cds-R, 5′-GGCAGCAGAAGACATGAATTCCCCACCGTA-3′; RN3prime-BglII-fusion-R1, 5′-AGATCTGCCAAAGTTGAGCGTTTATTCTGA-3′; and RN3prime-NotI-fusion-F1, 5′-GCGGCCGCGGATCTGGTTACCACTAAACCA-3′. The *Hp-micro1* mRNA was synthesized from the construct described previously (Nishimura et al., 2004). The translational perturbations of *P. pectinifera phbA*, *phbB*, and *hesC* were performed using MOs purchased from GeneTools (Philomath, OR, USA). As a control, the MO for the homeobox gene from the limpet *Nipponacmea fuscoviridis*. The MO sequences were as follows: *Ppe-PhbA*-MO, 5′- AGCGTTAGCAGGCATGGTGTGAATA-3′; *Ppe-PhbB*-MO, 5′-ATACACGGCAGCAGAAGACATGGTT-3′; *Ppe-hesC*-MO-1, 5′-

TCATGCTGAAGATTGTCGAAGGACA-3'; *Ppe-hesC*-MO-2, 5'-AATTTGGCACGAGAGCCGTATTTAT-3'; and *Nf-PRD-V-A*-MO, 5'-GGATTTATAGCCTACCTGAGCAGAA-3'. The MO and mRNA were introduced into the embryos by microinjection according to the methods of Yamazaki et al. (2014) for echinoids and that of Saito et al. (2017) for the starfish. The mRNAs and MOs were diluted with 0.2 M KCl and the concentrations used for microinjection were 125 ng/µl for *Pb-pmar1* mRNA, 250 ng/µl for *Ppe-phbA* mRNA, 500 ng/µl for *Ppe-phbB* mRNA, 250 ng/µl for *Ppe-phbA-EnR*, 250 ng/µl for *Ppe-phbB-EnR*, 1000 ng/µl for *Ppe-phbA-VP16AD*, 1000 ng/µl for *Ppe-phbB-VP16AD*, 1 mM for starfish *phb* MOs, and 1–2 mM for starfish *hesC*. DAPT was used to inhibit Notch signaling (Erkenbrack et al., 2018). The embryos of *P. baculosa* and *P. pectinifera* were treated with 10 µM and 2–10 µM DAPT, respectively. The DAPT was added at the 2-cell stage in both embryos. All experiments were conducted in more than two batches.

Evaluation of mesenchyme cell and endoderm differentiation

Skeletogenic cell differentiation in echinoid embryos was evaluated using the P4 antibody, which recognizes the skeletogenic cell-specific glycoprotein in *H. pulcherrimus* (Shimizu et al., 1988). The embryos that had been fixed for WMISH analysis were used. As the secondary antibody, goat anti-mouse IgG (H+L) cross-adsorbed secondary antibody, Alexa Fluor 488 (diluted 1:200; Thermo Fisher Scientific) and the goat antibody against mouse IgGs (heavy and light chains) Hilyte Fluor 555-Labeled (diluted 1:200; AnaSpec) were used for *H. pulcherrimus* and *P. baculosa*, respectively. The total cell numbers of mesenchyme cells were counted in *P. baculosa* embryos fixed for WMISH analysis. In the starfish *P. pectinifera* embryos, MC5 antibody was used for visualization of mesenchyme cells. The fixation and staining were performed according to the method of Hamanaka et al. (2011). The goat anti-mouse IgG (H+L) cross-adsorbed secondary antibody, Alexa Fluor 488 (diluted 1:200; Thermo Fisher Scientific) was used as the secondary antibody. The staining of AP activity was performed by the method of Koga et al. (2010).

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Supplementary Tables

Table S1. FPKM values for *alx1* and candidate upstream regulatory genes during early developmental stages of *P. baculosa*.

		FPKM value				
Gene name	DNA-binding motif	2 h	4 h	6 h	10 h	14 h
alx1	Homeobox	0.05	0	0.08	11.54	21.62
pmar1	Homeobox	0.44	1.96	11.04	2.44	0.2
z137	zf-C2H2	0.18	0.58	1.44	9.05	3.66
thr_1	zf-C4	6.98	7.3	6.06	59.71	7.12
hypp_2098	Hairy_orange	0.43	0.2	0.95	8.2	7.53
smarce1	HMG_box	1.18	0.89	1.84	9.56	9.51
hesD	HLH	1.56	0.67	1.05	8.69	13.69
foxO	Forkhead	1.26	1.96	2.42	18.66	14.03
hbn	Homeobox	0	0.03	2.28	8.12	16.53
nkx2-1	Homeobox	0.55	0.35	1	6.45	17.84
runt	Runt	3.37	2.87	3.03	34.01	24.12
crebzf	bZIP_1	1.87	1.54	7.74	20.12	26.08
elfA	Ets	0.29	0.16	5.68	33.03	28.44
l3mbt	zf-C2HC	0	1.68	1.41	10.3	28.44
emx	Homeobox	0.74	0.62	4.8	30.64	29.08
awh	Homeobox	0	0.14	40.56	40.76	31.18
sp5	zf-C2H2	0	0.21	9.43	32.26	32.11
nfat	RHD_DNA_bind	1.89	2.43	4.07	20	36.88
lim1	Homeobox	1.18	0.93	1.39	40.8	40.09
bra	T-box	0	0.26	0.65	39.95	41.2
ets4	Ets	9.68	10.79	162.38	79.23	41.9
z246-like1	zf-C2H2	0	0.58	2.81	8.96	42.4
nfe2	bZIP_1	5.43	3.31	8.78	53.81	45.2
cebpaL	bZIP_1	0	0.77	7.85	11.07	74.22
krlL-like1	zf-C2H2	14.52	92.6	221.1	266.66	75.22
z246-like2	zf-C2H2	1.33	0.55	1.74	15	81.68

eve	Homeobox	0.21	4.59	69.66	44.45	84.81
z185	zf-C2H2	6.3	7.59	13.31	111.69	85
krl-like2	zf-C2H2	7.58	44.23	156.88	225.43	85.42
hesA	HLH	0.08	0.34	15.74	64.09	95.84
soxC	HMG_box	0.06	0.05	8.9	80.89	106.31
rxr	zf-C2H2	0.27	0.27	3.44	84.8	110.15
foxA	Forkhead	0.1	0.13	1.67	36.32	133.94
myc	HLH	5.55	3.4	10.7	56.88	154.9
klf2/4	zf-C2H2	0.52	3.29	119.92	295.57	181.89
creb3l3	bZIP_2	8.53	4.19	8.31	140.11	188.55
atf4L	bZIP_1	3.96	2.48	15.7	186.79	459.34

Table S2. Primers used for cDNA fragment amplifications for RNA probes.

Animals	Species	Gene name	Primer name	Sequence 5'–3'
Cidaroid	*P. baculosa*	*pmar1*	F	ATGGCCGAATCGACATCGTCTTTTCAACCC
			R	TCAGTGAAATCTGGTTTCATTGGTCGTCGC
		delta	F	AAGAACCAGGGGACTGCATTTGCAAAGACG
			T3-R	ATTAACCCTCACTAAAGGGAAGTTGCAAGTAACCT GACGG
		foxA	F	ATGGCAAATAGCGCCATGATTTCACCCAAG
			T3-R	ATTAACCCTCACTAAAGGGACAGCGCGTGATTTGTT TGTG
		z137	F	ATGTCCTAGAATAAGTGGTGCCTTGCCCAA
			T3-R	ATTAACCCTCACTAAAGGGATAGCTTCATCGTCCGG CAAT
		thr_1	F	ATGTCTAAGTTCAAATTTGGAGAGCCACCT
			T3-R	ATTAACCCTCACTAAAGGGAGGTGTCTTTGGTCTCG TTTC
		hypp_2098	F	ATGGATTCTTGCCAGAGGCACGTTTCTGCT
			T3-R	ATTAACCCTCACTAAAGGGATTATTCTTGGGCAGGT CTCA
		smarce1	F	ATGTCTAGTCAGAATCGACGCAGCACAAGC
			T3-R	ATTAACCCTCACTAAAGGGAGATGGTTTCCAAGGA GTAAT
		hesD	F	ATGACAACATCACCTCCCGTGGACATGGCT
			T3-R	ATTAACCCTCACTAAAGGGATTACCATGGTCTCCAA ACAG
		foxO	F	ATGGTTGATATCGATCCCGATTTTGAGCCA
			R	TCAGTGCACCCAACTTGGAGCAGCCATCGT
		hbn	F	ATGGCAGCTTTGATAGCAACAACACCCAGA
			T3-R	ATTAACCCTCACTAAAGGGAATCCGATAACGTTGG GGAAT
		nkx2-1	F	ACCGACATCCTAACCCCTCTAGAAGAAAGC
			T3-R	ATTAACCCTCACTAAAGGGAGACTGAGGCCGATGC CCGAG

runt	F	ATGCATATTACTGAAGTTGATCACCACCTC
	T3-R	ATTAACCCTCACTAAAGGGATCAGTACGGCCGCCA CACGT
crebzf	F	ATGTGCTTGGACATTTACAAAACTACAGAT
	T3-R	ATTAACCCTCACTAAAGGGACGAGTTGAATGTAGC TTGGG
elfA	F	TCTGGATTTACTGCTCGAAGCAGCTAATGC
	T3-R	ATTAACCCTCACTAAAGGGATCAACTGCAGTCAATT TCGC
l3mbt	F	TGTCCCACCCTGGGCTGCAGTGGTATAGGC
	T3-R	ATTAACCCTCACTAAAGGGAAGTATCAATATTGCGT TGTA
emx	F	ATGTCGGCTGTGGACTTATTTCGGCCGCCT
	T3-R	ATTAACCCTCACTAAAGGGATCAATCATTTTCTCCTT GCT
awh	F	AGCTGTATACCACGATGAGCCAGGTCAAAC
	R	CAGCCCGGCATTTTATCGCAGAGGCAGTAG
sp5	F	ACAGCTTCCAGAAAGTAACCCTGGCGAACT
	T3-R	ATTAACCCTCACTAAAGGGATTCCGAAGTTACGTCA ACGA
nfat	F	TCACGGGCAAATTTCGTCGGAAGGACTCTT
	T3-R	ATTAACCCTCACTAAAGGGACTTACCAGGCTCTGG GATAT
lim1	F	GTGCAGTTGTGCGCGGGCTGCGACCGGCCG
	T3-R	ATTAACCCTCACTAAAGGGACCACACGCCTTCGGTT AGTT
bra	F	ATGCCAGCAATGAGCGCGGAAGCTATGAGA
	T3-R	ATTAACCCTCACTAAAGGGACTGACGAGATGCTGA TGCGG
ets4	F	ATGTTTTCGACCGGCGAAGTTTGCATTGTT
	T3-R	ATTAACCCTCACTAAAGGGATCAGCAGGGGTGTAC GAATT
z246-like1	F	ATGCGGCAAAGGTATGCGAGAACTCTGGAG
	T3-R	ATTAACCCTCACTAAAGGGATATGTCCAGATATGTG GTGG

nfe2	F	ATGATGGATTACGATTTTGCTGAAATGGGC
	T3-R	ATTAACCCTCACTAAAGGGACTACATTGATTGCTCG TCAC
cebpaL	F	ATGGACTTTTATTCAGATACAGTTTCACGA
	R	TTACTCGAGGGCTTTTTCAAGTTTCACTGG
krlL-like1	F	GTAACACAAGTGATGACTTCAAACCATCAT
	R	CGGTGTGCGTACGACGATGTTTGGTCAGCG
z246-like2	F	AGCCTTCGAATTTCAAGGCTGCTACTTTCA
	T3-R	ATTAACCCTCACTAAAGGGATTATATGTCCAGATAT GTGG
eve	F	ATGGAAAGAGGTTTCACCATGTTACCAACG
	T3-R	ATTAACCCTCACTAAAGGGAAAAGTTTCCTGGAATC GGCA
z185	F	ATGGCGTCATCTGAAGAAGCAAGTGTGAAG
	T3-R	ATTAACCCTCACTAAAGGGACCGACATGGTCTGCTC ACGT
krl-like2	F	GCAGACGACATCGCGTCACTTGCCAGTTGT
	R	CGGTGTGCGTACGACGATGTTTGATCAGCG
hesA	F	ATGCCTAACTGTGAAGTATACCACACCAAG
	R	TTATTCGTGGTGGCTCCATGGTCGCCAGAC
soxC	F	ATGGTTCCTCAAACCCTTACGAACGGCCTG
	T3-R	ATTAACCCTCACTAAAGGGATTAATTCGAATATGCC GAAA
rxr	F	AGCGGTATGGCTTACTGTCAACCCCTGGAC
	T3-R	ATTAACCCTCACTAAAGGGATCAAATCGCCATCATG GGCA
myc	F	ATGGCTCATATTTTGGATACGGACTTCGA
	T3-R	ATTAACCCTCACTAAAGGGATCACTTTAAAGACTCC AACT
klf2/4	F	ATGGCAGCTACCTTAGCAGAGGTGAACCAA
	T3-R	ATTAACCCTCACTAAAGGGATCACATGTGACGTTTC ATGT
creb3l3	F	CAGAATATTCGAGAGTGGAGACACGGATTTC
	T3-R	ATTAACCCTCACTAAAGGGAAGTTGAGTTGGCTTT

				GATTG
		atf4L	F	ATGAGCCTGGAAGACTTGCAGATCCTGTCG
			T3-R	ATTAACCCTCACTAAAGGGAACCAAATGTACTTCAACCAT
Sea cucumber	*A. japonicus*	*phb*	F	ATGCCAGCAAGTTGTGTGACATTATCCTCT
			T3-R	ATTAACCCTCACTAAAGGGAGGCATCGGGGCACCATGACG
Brittle star	*A. kochii*	*phbA*	F	ATGCCTGCTAATGTCACGATGTCTTCTGTA
			R	TTATACGGCAATCTTCAGAGGTTGACTCGC
		phbB	F	ATGTCAGTGCCTATTTCCAATCGTCGTCGT
			R	CTACAAGTACATGGAAATCAAATCTTCAAT
		phbC	F	ATGGCGTCCTCCTCAACACCAATCACGCCT
			R	TTAGAGATGTCTTCCACTGATCATGTTTGG
		phbE	F	ATGGAATATTACAACAGGCAGAATCTTGCA
			R	TTACTGGAAAACATCCATACTCTGTTTGAC
		foxQ2	F	ATGGCAATTTTTGACTCACCTGAGAAAAGA
			T3-R	ATTAACCCTCACTAAAGGGATCAATTGGCAGGATACATAG
Starfish	*P. pectinifera*	*phbA*	F	ATGCCTGCTAACGCTATGGACAGCACCGTG
			T3-R	ATTAACCCTCACTAAAGGGATCAGTGGCGAGCAATGGCCAGAA
		phbB	F	ATGTCTTCTGCTGCCGTGTATCTTGAGG
			T3-R	ATTAACCCTCACTAAAGGGATTACATGTGATGGGAAGTTG
		hesC	F	ATGATTGCCAACATGATGCACAGCACTGCA
			R	CCAGGGTCTCCAGACATTCTCCTGGGGGAC
		delta	F	ATGGGTCGGTTACGGCTCTCCTGGAGATAT
			R	GGCGGTAAGCTGGCACTCAGTCAGCTCAGT
		foxA	F	ATGGCAAACAGCGCCATGATCTCGCCCAAG
			T3-R	ATTAACCCTCACTAAAGGGACATGTTGTGGTTCGCTGTGG

Supplementary Figures

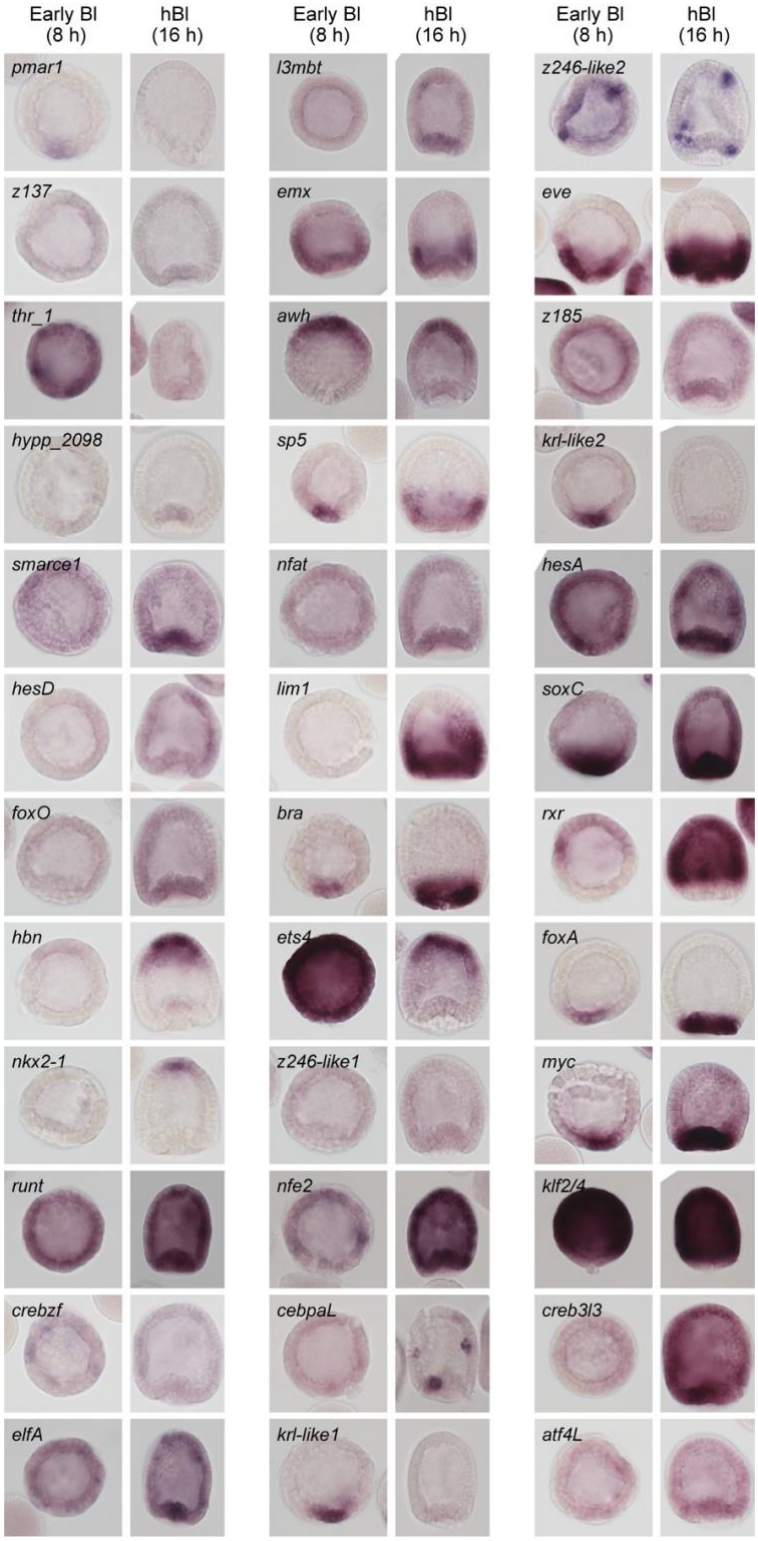

Fig. S1. Expression patterns of early expressing candidates of upstream regulatory genes in *P. baculosa* embryos.

Expression was examined at the early blastula (8 h) and hatched blastula (hBl) stages (16 h) of *P. baculosa* embryos by WMISH. The putative gene names are shown in the upper left corner of each image.

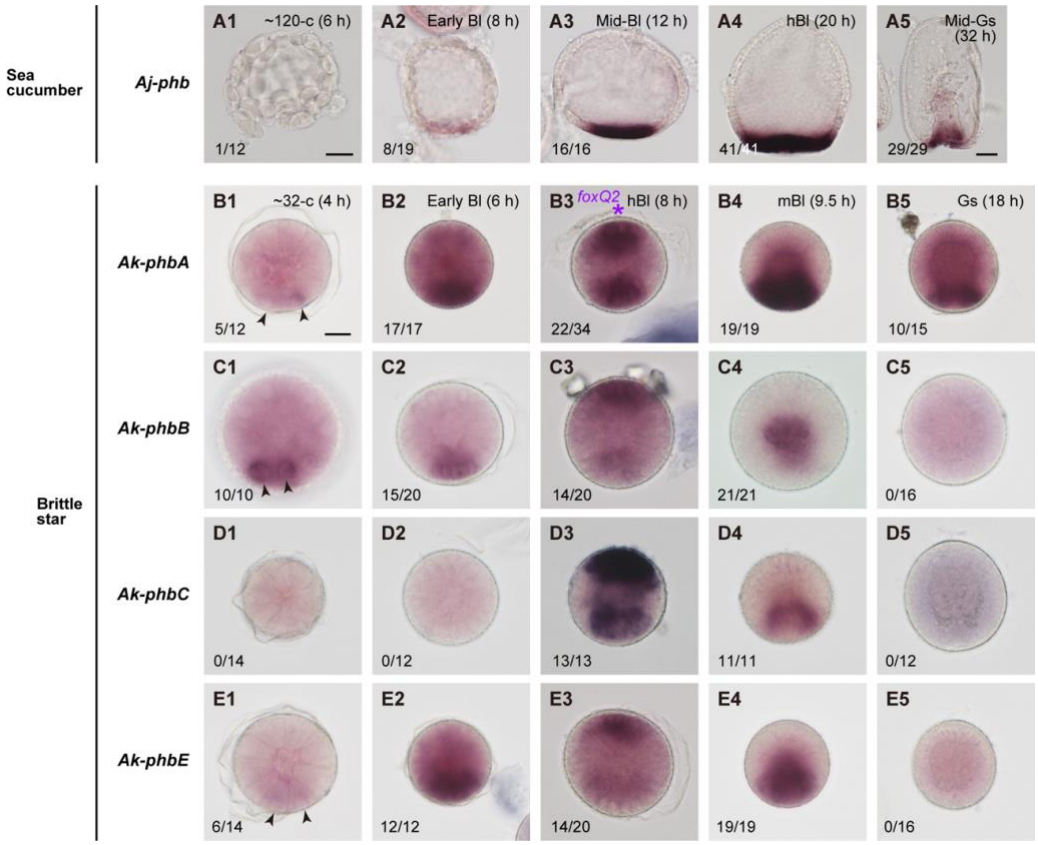

Fig. S2. Expression of *phb* genes until the gastrula stage in sea cucumber and brittle star embryos.

Expression patterns of *phb* genes were observed by WMISH. (A1–A5) Expression of *A. japonicus phb*. *Aj-phb* expression was detected from early blastula (8 h) at the vegetal pole. In the embryos at the mid-gastrula stage, the signal was detected in the region encircling the blastopore (A5). (B-E) Expression of *A. kochii phbA* (B1–B5), *phbB* (C1–C5), *phbC* (D1–D5), *phbE* (E1–E5). (B3, C3, D3, E3) The WMISH embryos using a mixture of RNA probes for each *phb* and *foxQ2* (animal pole marker). Although the spatial expression patterns of brittle star *phb* genes were slightly different each other, all genes were expressed at the vegetal pole. The expression of *phbA*, *phbB*, and *phbE* was detected in several blastomeres at the ~32-cell stage (indicated by arrowheads). At this stage, 4–8 cells per embryo expressed *phbB*, which exhibited the strongest signal (mean±SD=6.43±1.51; n=7). By contrast, no *phbC* expression was detected until the early blastula stage (6 h). *PhbA* continued to be expressed until the gastrula stage, whereas expression of the other genes disappeared until gastrula stage. (F, G) The numbers shown in the lower left corner of each image indicate the numbers of embryos showing WMISH signals in the examined embryos in one or two batches. Scale bar indicates 50 µm.

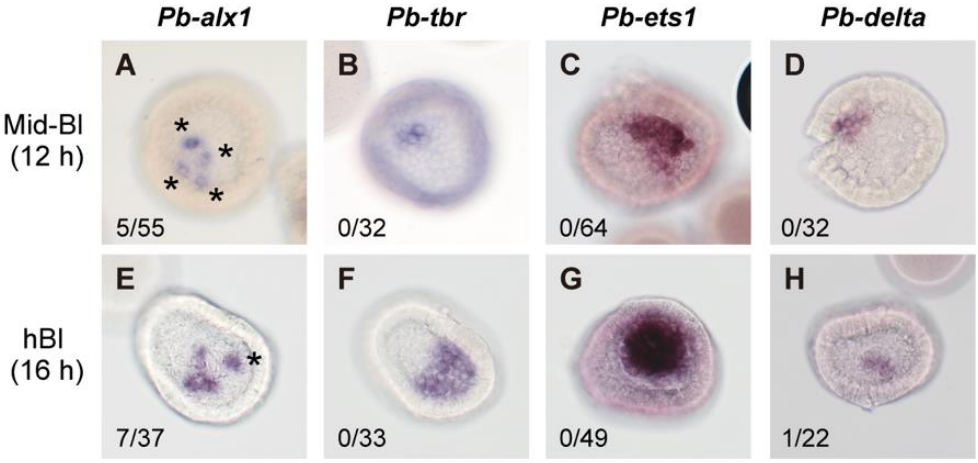

Fig. S3. Characteristic expression of *alx1* during the early developmental stages of *P. baculosa*.

Expression of *alx1* (A, E), *tbr* (B, F), *ets1* (C, G), and *delta* (D, H) during blastula stages in the cidaroid *P. baculosa*. All images are of vegetal views. (A–D) Mid-blastula (mid-Bl) stage (12 h). (E–H) Hatched blastula (hBl) stage. The number shown in the lower left corner of each image indicates the number of embryos showing patchy expression (asterisks in A and E) in the examined embryos. The numbers of embryos are obtained from experiments in two batches. Scale bar indicates 50 µm.

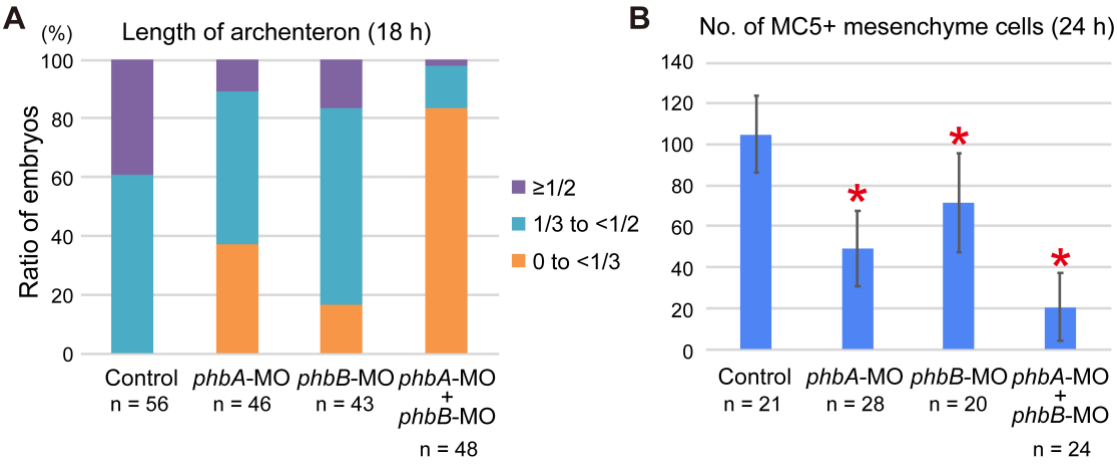

Fig. S4. Observations of archenteron and mesenchyme cell formation in the starfish *P. pectinifera* embryos injected with morpholino antisense-oligos of *Ppe-PhbA* and *Ppe-phbB*. (A) The ratio of embryos with various archenteron lengths (<1/3, ≥1/3 to <1/2, and ≥1/2 of embryo height) at 18 h. (B) The average numbers of mesenchyme cells expressing MC5 antigen were examined at 24 h. Data are mean±SD. The results of (A) and (B) were obtained from two batches. Red asterisks indicate the significant decrease when compared with control embryos ($p<0.05$, Mann–Whitney U test).

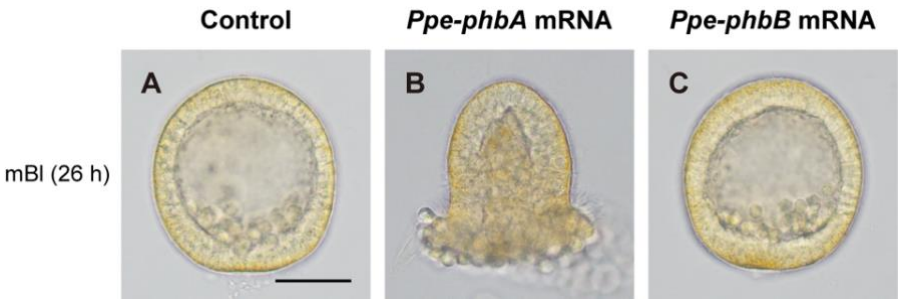

Figure S5. Overexpression of starfish Phbs in the euechinoid *H. pulcherrimus* embryos.

(A) Control embryos injected with 0.2 M KCl. (B) Embryos overexpressing Ppe-PhbA, and (C) embryos overexpressing Ppe-PhbB at the mesenchyme blastula stage (mBl, 26 h). *Ppe-phbA* mRNA-injected embryos formed more skeletogenic cells, although animal blastomeres did not develop into skeletogenic cells, in contrast to sea urchin Pmar1 overexpression. The scale bar indicates 50 µm.

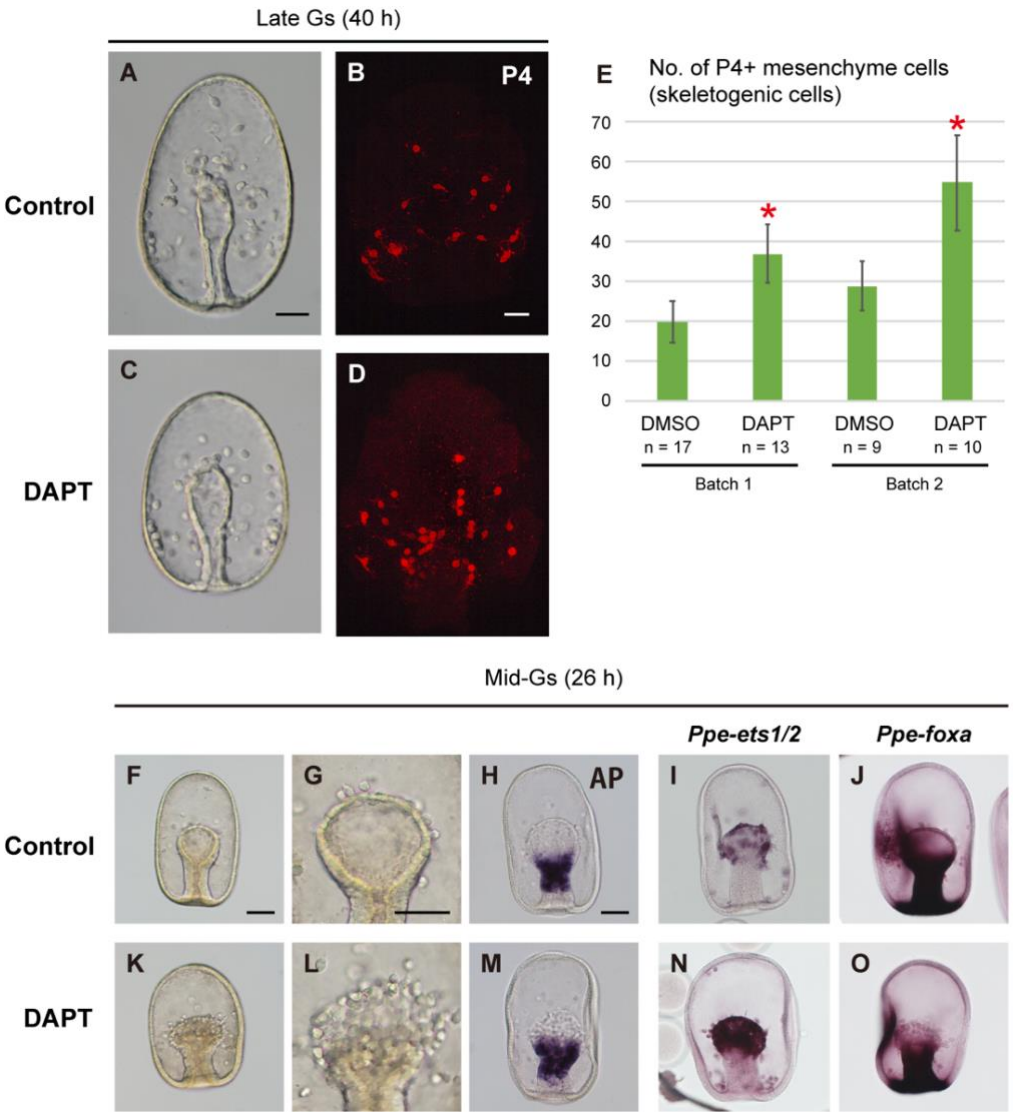

Figure S6. DAPT-treated cidaroid and starfish embryos at the gastrula stage.
(A–E) Observation of the morphology at the late gastrula stage (40 h) of *P. baculosa* embryos. (A, B) Control embryos treated with DMSO. (C, D) DAPT-treated embryos. (B, D) Fluorescence images of embryos examined by immunohistochemistry using the skeletogenic cell-specific P4 antibody. (E) The numbers of P4-expressing mesenchyme cells (presumptive skeletogenic cells) were counted in the experimental embryos of two batches. Data are mean±SD. Red asterisks indicate significant differences between control and treatment embryos ($p<0.05$, Mann–Whitney U test). The number of P4-expressing cells increased in DAPT-treated embryos. (F–O) Observations in *P. pectinifera* embryos. (F–J) Control embryos. (K–O) DAPT-treated embryos. (F, G, K, L) Living embryos. (H, M) Embryos stained for AP activity. (I, J, N, O) WMISH embryos hybridized with *ets1/2* (I, N) and *foxA* (J, O) probes. When mesenchyme cells began to

ingress from upper part of archenteron in control embryos (F, G), a large part of the upper archenteron developed into globular mesenchyme cells in DAPT-treated embryos (K, L). The lower part of archenteron showed AP activity in control (H) and DAPT-treated embryos (M). The *ets1/2* signal is stronger in DAPT-treated gastrulae (N) compared to control gastrulae (I). No difference in the expression of *foxA*, which is expressed in the endoderm and stomodeum regions, was observed between control (J) and experimental embryos (O). The scale bar represents 50 µm.

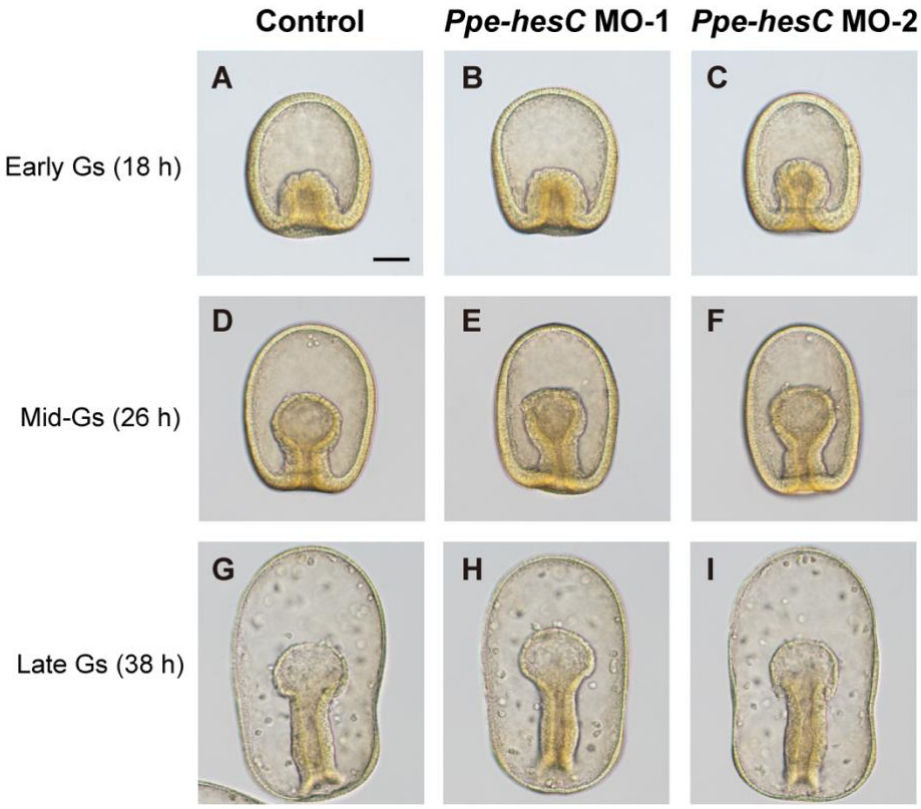

Figure S7. Knockdown experiments of starfish *hesC*.

(A, D, G) Control starfish *P. pectinifera* embryos. (B, E, H) Embryos injected with *Ppe-hesC* MO-1. (C, F, I) Embryos injected with *Ppe-hesC* MO-2. Embryos were observed at the early gastrula (A–C), mid-gastrula (D–F), and late gastrula (G–I) stages. No visible defects in the endomesodermal tissues were observed in the knockdown embryos. The scale bar indicates 50 µm.

Supplementary Data

Data S1

The assembled reads for early regulatory gene candidates obtained from the cidaroid *P. baculosa*.

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Data S2

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