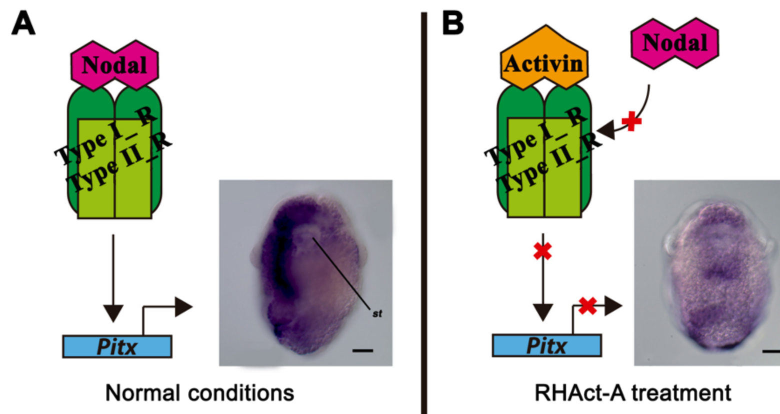
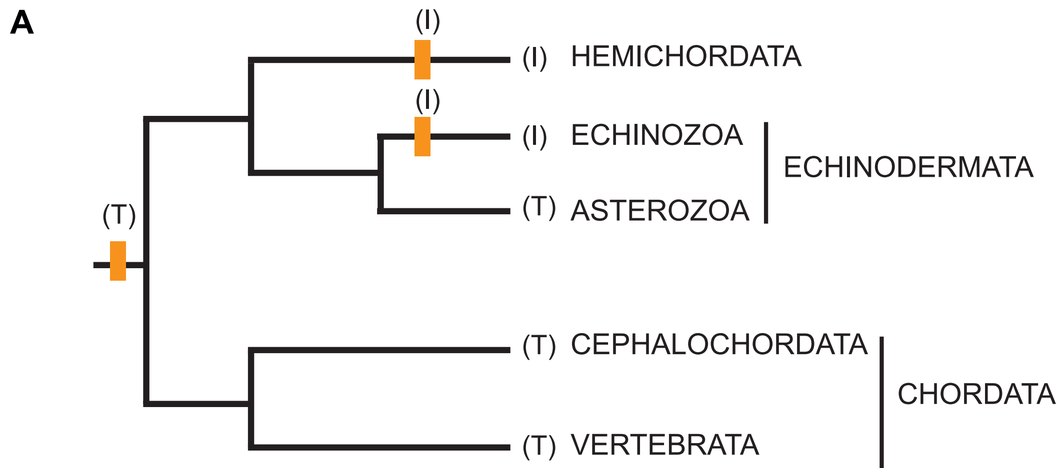
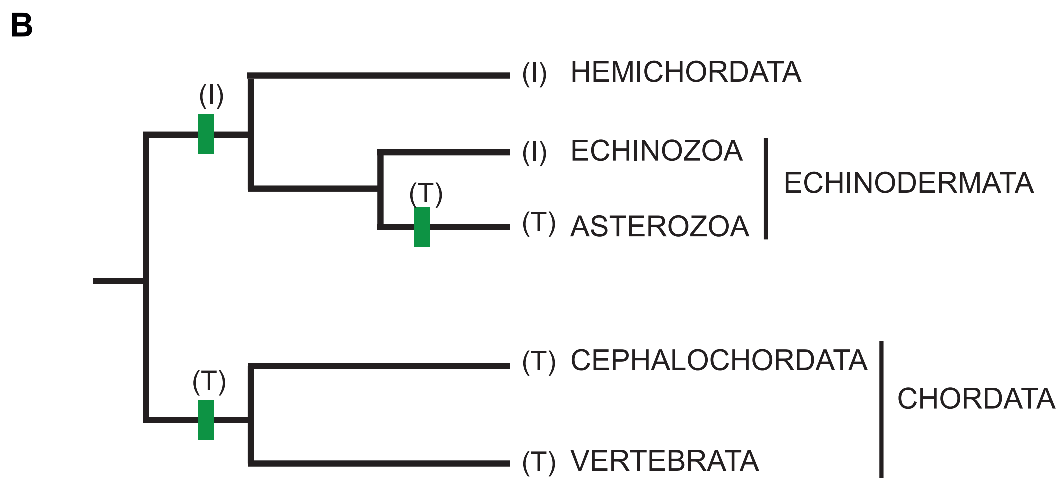
S1. Used and retrieved sequences for EGF-CFC in different organisms.
*** this study.**

SPECIES	ACCESION NUMBER
Novocrania anomala EGF_CFC_mRNA	OP006211*
Patella vulgata EGF_CFC_1_mRNA	OP006209*
Patella vulgata EGF_CFC_2_mRNA	OP006210*
*Lottia_gigantea*_EGF_CFC	Prot ID 233075
Crepidula fornicata EGF_CFC_mRNA	OP006207*
Owenia fusiformis EGF_CFC_mRNA	OP006212*
Biomphalaria glabrata EGF_CFC_mRNA	OP006208*
Spirobranchus lamarckii EGF_CFC_mRNA	OP006213*
*Branchiostoma_floridae*_EGF_CFC	XM_002207525
*Gallus_gallus*_EGF_CFC	NP_990031
*Monodelphis_domestica*_EG_CFC	ENSMODP0000003767
*Danio_rerio*_Oep	NP571167
*Takifugu_rubripes*_Oep	ENSTRUP00000013088
*Xenopus_laevis*_Xcr1	AAA83569
*Xenopus_laevis*_Xcr2	CAI15753
*Xenopus_laevis*_Xcr3	NP_001089135
*Xenopus_tropicalis*_Xcr1	ENSXETP00000020142
*Xenopus_tropicalis*_Xcr2	ENSXETP00000020145
*Xenopus_tropicalis*_Xcr3	NP_001078829
*Homo_sapiens*_Crc	NP_115934
*Mus_musculus*_Crc	NP_031711
*Loxodonta_africana*_Crc	ENSLAFP00000005045
*Erinaceus_europaeus*_Crc	ENSETEP00000015228
*Mus_musculus*_Cro	AAH52646
*Loxodonta_africana*_Cro	ENSLAFP00000007663
*Erinaceus_europaeus*_Cro	ENSEEUP00000004109
*Homo_sapiens*_Cro	NP_003203
*Homo_sapiens*_Cro3	P51864
*Strongylocentrotus_purpuratus*_EGF_CFC	XP_787941.2
*Lytechinus_variegatus*_EGF_CFC	XP_041475421.1
*Asterias_rubens*_EGF_CFC	XP_033636697.1
*Acanthaster_planci*_EGF_CFC	XP_022097255.1
*Patiria_miniata*_EGF_CFC	XP_038069138.1
*Ptychodera_flava*_EGF_CFC	AJS19017.1
*Saccoglossus_kowalevskii*_EGF_CFC	NP_001161522.1



S2. The addition of RHAct-A leads to the inactivation of the Nodal pathway, presumably by competition with the ligand NodalB for the receptors. A) Transcription of *Pitx* is activated by the signaling cascades transduced the NodalB binding to the receptor cluster. In preveliger larvae, *Pitx* is symmetrically expressed in the anterior region and is also asymmetrical on the right side (fig. 2C). B) RHAct-A presumably prevents NodalB from binding the receptor cluster, due to direct competition. Subsequently, no asymmetrical *Pitx* expression is promoted, as showed by *Pitx* whole mount *in situ* hybridization in treated embryos (fig. 3D); though such silencing only occurs in the asymmetrical territories, while its symmetrical activation signals remain unaffected. Scale corresponds to 30 μm.





S3. Two alternative hypotheses for the origin of the amino acid change in the position T88 (human) of the ancestral EGF-CFC sequences in Deuterostomia. A) Hypothesis 1 (orange): The change to a threonine occurred in the common ancestor of all deuterostomes and was subsequently modified in the stem lineages that lead to echinozoans and hemichordates (changing to an isoleucine). B) Hypothesis 2 (green): The change to a threonine occurred in the common ancestor of chordates and the presence of a threonine in asterozoans is the result of convergent evolution. The amino acid identity in position T88 (human) is shown in parenthesis for each main group of deuterostomes: I, isoleucine; T, threonine.

S4. Orthology analysis details. Orthology was determined by alignment of these candidates with the validated sequences from vertebrate representatives. Alignments were performed using ClustalX v2.1 (Larkin *et al.*, 2007), followed by refinement by eye, and trimmed in Mesquite v2.75 OSX (Maddison & Maddison, 2011) in order to select their main protein domains. All the potential EGF-CFC sequences retrieved in our searches contained the specific domains (EGF-like domain, CFC domain) (Fig 1E). The alignments of all these sequences were subject to coalescent-based Bayesian Inference (BI) phylogenetic analyses implemented in the software BEAST 1.8.3 (Drummond *et al.*, 2012). The WAG model (Whelan & Goldman, 2001) was selected as the best-fit model of protein evolution using ProtTest (Abascal *et al.*, 2005). Tree shown is the result of Bayesian analysis, run for 25,000,000 generations, and analyses run until the s.d. of split frequencies was below 0.01, with the first 25% of sampled trees discarded as ‘burn-in’.

S5. Primers used to clone the different fragments and complete CDS of *C. fornicata egf-cfc* and NodalB, and *D. rerio oep*

PRIMER NAME	SEQUENCE
Criptocrepi-F-2	GGGCGAAGGCAGGATGTAACTT
Criptocrepi-F-2-IN	ATGTAACTTGTGTCGCTGCTTTG
CriptoCrepi-F3	GAAGTTTTACGGGCGCTACTGTGA
CriptoCrepi-F3-in	GCGCTACTGTGAGTATGAGCTGTC
CriptoCrepi-R3	TGACACGAGTGTCCATGAGGTCCA
CriptoCrepi-R3-in	CCATGAGGTCCAGAGTATCGTAAC
CriptoCrepi-R4	TTCTATTATGTATACGCATATTCC
CriptoCrepi-R4-in	TACGCATATTCCACTTCCAGTTTT
CriptoCrepi-R5	AGTTATGTGCATAGGCAGCATGGT
CriptoCrepi-R5-in	ATCTTTAAGTGTAGTTATGTGCAT
CriptoCrepi-F4	GACGTGTCGTCTGTCAGGGGTCTG
CriptoCrepi-F4-in	GTCAGGGGTCTGATCACGCTGGTC
CriptoCrepiBamHI-F	ggggatccATGGACGTGTCGTCTGTCAGGGGT
CriptoCrepiXhoI-R	CCCCTCGAGTGACACGAGTGTCCATGAGGTCCA
CR_L56T_F	GTCGTGCTGCCTGAACGGGGGCACGTGTGTGCTGG
CR_L56T_R	TTGGAGGGTCTAGGGTTCAAGTCTCCCAGCCTG
CR_L56A_F	GTCGTGCTGCCTGAACGGGGGCGCGTGTGTGCTG
CR_L56A_R	TTGGAGGGTCTAGGGTTCAAGTCTCCCAGCGTCTTC
CR_DEGF_F	AAGCCATGTGGTCCTGTGCCTCATGGCAGCTGGGC
CR_DEGF_R	GGGTTTGGGGTTCAAGTCTCCCAGCGTCTTCTTCG
NodalCrepi_F2	GTGGGTCATAATAAACACAGGTCT
NodalCrepi_F2_in	AAACACAGGTCTCGTGACAAGAGA
NodalCrepi_R2	CAGCTATCATATTTTCGTGGTGGC
NodalCrepi_R2_in	TTTCGTGGTGGCGAACCACAATCT
NodalB Crepi_F3	ACTTGACCGTGGTAGGTCAAGACT
NodalB Crepi_F3_in	TAGGTCAAGACTCAGATGTCCAGT
NodalB Crepi_R3	CTACTGGTCCTAGAGGTAGTAGAT
NodalB Crepi_R3_in	GAGGTAGTAGATGCTCTCTTGTCA
NodalB_Crepi_DCS_F	GCCCCCAAGCACGAAAAATGCCAGATGTAC
NodalB_Crepi_DCS_	ACGTTTAGATTCCCTACTGGTCCTAGAGG
NodalB Crepi_F4	CACAAGAGGTGATGTCTCACAGAG
NodalB Crepi_F4in	TGTCTCACAGAGAGGTCTCTAGGT
NodalB Crepi_R4	GTGCACACATGGACGACAATCACC
NodalB Crepi_R4in	ACGACAATCACCTACACCCACACT
NodalB _Crepi_EcoI_F	GGGGAATTCATGCCATTATTTTCGGCAGCC
NodalB _Crepi_SalI_R	CCCGTCGACCCTACACCCACACTCATCAGC
Oep_ZF_F	GGCCAGCGGAATGACGAGTCAAC
Oep_ZF_R	AAACTCATTTACAGCAGGCGGTG
Oep_ZF_BI_F	ggggatccATGACGAGTCAACTGTTCGGGT
Oep_ZF_SI_R	CCCGTCGACCAGCAGGCGGTGTAAAATAAAAGTG

S6. Construct details and microinjection protocol. The *nodal2-ΔCS* construct lacks 13 amino acids [**RKNR**NNKKK**RWYR**], which potentially includes the cleavage site domain. This fragment contains two potential sites, according to the consensus RXXR. A previous publication suggests a redundancy between two close sites, hence a deletion containing both was designed (Eimon & Harland, 2002). The EGF-CFC_ΔEGF construct lacks 29 amino acids corresponding to the EGF-like domain [CCLNGGLCVLDSFCHCPKKFYGRYCEYKP]. A mutation was introduced to the EGF-CFC_L56T construct, changing the Leucine at position 56 (bolded) inside the EGF-like domain to Threonine [CCLNGG**L**CVLDSFCHCPKKFYGRYCEYKP]. The mRNA was prepared by PCR amplification of the construct, with SP6 and T3 primers and Phusion High Fidelity DNA polymerase (New England, BioLabs). The purified product was used as a template for the transcription reaction using the mMessage mMachine SP6 RNA transcription kit (AM1340, Ambion, Austin, TX), as previously described (Henry *et al.,* 2010). The same protocol was followed for *oep* mRNA, amplified from cDNA generated from 24 hours post-fertilization *D. rerio* embryos. Fertilized eggs of *C. fornicata* and *D. rerio* were pressure microinjected. The device used for the experiments on *C. fornicata* is described in Truchado-Garcia *et al.*, 2018 (Truchado-Garcia M, Harland RM, Abrams MJ, unpublished method, https://www.biorxiv.org/content/10.1101/376657v1.full), and a semi-dry technique (pretri-dish and a glass slide) was used to hold zebrafish embryos.