

## Supplementary Information

**Figure S1. Phylogenetic analysis of the ETS transcription factor complement in *Nematostella vectensis*.** The *Nematostella* gene sequences described in this paper were translated and protein domains identified using SMART (89) (90) (<http://smart.embl-heidelberg.de/>). They were aligned with orthologous sequences (obtained by database searches using BlastP (<http://www.ncbi.nlm.nih.gov/BLAST/>) in MacVector using the ClustalW tool and corrected by hand. A neighbor joining phylogenetic analysis was performed using the phylogenetic tree tool in MacVector. Abbreviations correspond to the following species: **hs** - *Homo sapiens*, **mm** - *Mus musculus*, **dr** - *Danio rerio*, **dm** - *Drosophila melanogaster*, **Nv** - *Nematostella vectensis*,

**Figure S2. Overexpression of NvERG:Venus can reverse effects of Mo-NvERG.** Overexpression of (A) MO-NvERG, (B) NvERG:Venus alone or (C) in the presence of MO-NvERG shows that MO-NvERG has no effect on NvERG:Venus translation. The red color in (A) reflects the dextran that was co-injected with MO-NvERG. The red channel has been omitted in B,C for a better visualization of nuclear NvERG:Venus. (D, E, F) Morphological effects of injecting (D) MO-NvERG, (E) NvERG:Venus alone or (F) in the presence of MO-NvERG showing the capacity of NvERG:Venus to partially revert the effects of MO-NvERG, showing that MO-NvERG specifically targets endogenous *Nverg* in injected embryos. **In order to verify the specificity of the NvERG:Venus rescue, we also co-injected MO-NvERG with dnTomatoNLS mRNA (nuclear staining in D) and observed that the Mo-NvERG phenotype was not rescued simply by mRNA injection (H).**

## Figure S3. Gene expression analyzed by qPCR

(A) endomesodermal, (B) salt-and-pepper and (C) ectodermal genes as represented in Figure 2, 3. High-density gene expression profiles represented by charts for all genes expressed at the blastula stage (24hpf) and/or gastrula stage (48hpf) analyzed in this study. Y-axis indicates the relative fold change compared to unfertilized eggs. X-axis indicates developmental time in hours

post fertilization. Gene names as indicated in the top left corner and the Cp value in unfertilized eggs is indicated in the top right corner of each panel that was used to determine the presence of maternal transcripts in Figure 4 (Cp>34.00). Cp corresponds to the crossing point (also known as Ct (cycle threshold) value).

**Figure S4. Molecular phenotype analysis of NvERG-DB injected embryos on genes expressed in the central domain.** Changes in gene expression of ten NvERG downstream targets (see Fig. 5,6) in NvERG-DB injected embryos compared to control embryos and analyzed by qPCR. Changes in gene expression are indicated as relative fold changes compared to MO-CTRL injected control embryos ( $\bar{x} \pm \text{sem}$ , n = 3 per gene). The grey bar indicates no significant change in gene expression (-1.5,1.5). Stars below the bars indicate significant variation.

**Figure S5. Updated GRN framework orchestrating embryonic development in the cnidarian *N. vectensis*.**

Enhanced Biotapestry diagram (85) of the gene regulatory network describing the gene deployment at 24 hpf and regulatory interactions of endomesodermal, ectodermal and neuronal genes identified in previous studies (24,31,35,55,71). No assumption on whether these interactions are direct or indirect is made. Solid lines indicate functional evidence obtained by qPCR as well as *in situ* hybridization, dashed lines indicate evidence obtained only by qPCR. The colored boxes represent the spatial domains as described in Figure 4A. Genes inactivated by repression in a given territory are represented in light grey. Controversial results (24,73) about the role of cWnt/TCF signaling on *NvsnailA* expression is indicated by a red dashed arrow. This diagram also contains non-connected genes that are positioned within the GRN based on their expression information obtained by qPCR and *in situ* hybridization. The colored boxes represent the spatial domains as described in Figure 4A.

**Figure S6.** GRN framework for body wall endomesoderm, pharynx (endomesoderm and ectoderm), mouth, body wall ectoderm, sub-apical and apical domain including components of the *Nematostella* nervous system at the end of gastrulation (48hpf). Biotapestry diagram as indicated in Figure 7. The colored boxes represent the spatial domains as described in Figure 4B. Included functional data have been obtained from (23,32,35,37,60). No assumption on whether these interactions are direct or indirect is made.

### **Supplementary Tables**

**Table S1.** Selection of genes upregulated after 1-azakenpaullone (ectopic cWNT activation) treatments. (See (28) for more information)

**Table S2.** Primer pairs used in this study for qPCR analysis

**Table S3.** Primer pairs used in this study for gene cloning

**Table S4.** Selection of genes upregulated after UO126 (inhibition of MEK/ERK signaling) treatments. (See (35) for more information)

**Table S5.** Selection of genes downregulated after UO126 (inhibition of MEK/ERK signaling) treatments. (See (35) for more information)