Supporting Information

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SI Materials and Methods

Plasmid Constructions. pBSAA is a versatile cloning shuttle vector. pBSAA was constructed by introducing an AscI site into either end of the multiple cloning site (MCS) of pBluescript KS(−) (Stratagene).

pBSSA-AR is a *Hydra* transformation vector. pBSSA was constructed by introducing an AscI site into the 5′-most side of SacI of the MCS of pBluescript KS(−). The *Hydra actin* promoter and *RFP* gene was cloned from pHyVec5 (gift of R. Steele, Department of Biological Chemistry, University of California Irvine, Irvine, CA, GenBank accession no. DQ344484) into pBSSA, which resulted in pBSSA-AR.

pBSSA-AR-hsp.mini-EGFP is a *Hydra* enhancer detector vector. For the heterologous reporter construct, the core promoter (−122 to −50 bp) of the *Hydra hsp70.1* gene (1) was amplified from genomic DNA and cloned upstream of EGFP in pBSSA-AR, generating pBSSA-AR-hsp.mini-EGFP.

The HyWnt3FL-EGFP reporter gene was cloned a PstI-SpeI fragment from hoT G-HyWnt3FL-EGFP into PstI and XbaI digested pBSAA, which resulted in pBSAA-HyWnt3FL-EGFP. The deletion and mutation constructs of HyWnt3FL were generated by PCR and/or restriction digest by using pBSAA-HyWnt3FL-EGFP and subsequently cloned into the AscI site of pBSSA-AR or the MCS of pBSSA-AR-hsp.mini-EGFP. pBSSA-AR-HyWnt3FL was generated by cloning a HyWnt3FL-EGFP fragment into pBSSA-AR. Primer sequences used for the constructs are provided in Table S6.

For Dl−2129/−1517, Dl−1494/−985, Dl−1201/−604, and Dl−842/−406, each fragment was amplified by PCR with specific primers, digested with XbaI, and relegated.

For −985, the fragment was amplified as a fragment containing EGFP by PCR, digested with XbaI and NcoI, and cloned into SpeI and NcoI-digested pBSSA-AR-HyWnt3FL.

For −985/−479, HyWnt3act, and −758/−386, each fragment was amplified by PCR, cut with SalI and BamHI, and cloned into the corresponding sites of pBSSA-AR-hsp.mini-EGFP.

−2149/−386 was amplified by PCR, cut with KpnI and BamHI, and cloned into the corresponding sites of pBSSA-AR-hsp.mini-EGFP.

HyWnt3prox was constructed by digestion of Dl−842/−406 with SpeI and XbaI.

Act-Wnt3prox was generated by introducing the *Hydra actin* promoter (−1,300 to −221 bp), forming KpnI-XbaI fragment into HyWnt3prox digested with KpnI and XbaI.

The Act-hsp.mini was generated by introducing the HyActin 5′ sequence as a XhoI-XbaI fragment into SalI and XbaI sites of the pBSSA-AR-hsp.mini-EGFP.

For mTCFall, mTCF1, mTCF2, and mTCF3, TCF-binding sites were mutated by using site-directed mutagenesis. Base substitutions (indicated in Table S6 in lowercase) were A to C or T to G (or vise versa). Subsequent cloning followed the −985/−386 construction.

For Hyβ-catenin-EGFP, 5′ (2,520 bp) and 3′ (598 bp) flanking regions of *Hyβ-catenin* were amplified from genomic DNA by PCR and cloned upstream and downstream of EGFP of pBSAA containing EGFP, respectively. Subsequently, *Hyβ-catenin* cDNA was cloned downstream of the *Hyβ-catenin* 5′ flanking sequence and upstream of the EGFP.

1. Gellner K, Praetzel G, Bosch TC (1992) Cloning and expression of a heat-inducible hsp70 gene in two species of Hydra which differ in their stress response. *Eur J Biochem* 210: 683–691.

Fig. S1. New vector systems for *Hydra* transgenesis. (*A*) Schematic diagram of *HyActin-RFP::HyWnt3FL-EGFP*. *HyActin-RFP* was used as a transformation marker to detect transgenic cells with a new vector system in which a reporter gene, *HyWnt3FL-EGFP*, can be cloned at the AscI site of the vector carrying a *HyActin* driving *RFP* reporter gene expression and analyzed for its activity in transgenic *Hydra*. (*B*–*D*) Transgenic animals carrying the *HyActin-RFP::HyWnt3-EGFP*. *HyActin-RFP* is expressed ubiquitously (*B*) whereas *HyWnt3FL-EGFP* is localized to the head (*C*) (*n* = 11). (*E*–*H*) A functional heterologous *Hyhsp70* core promoter in transgenic *Hydra*. (*E*) Schematic diagram of reporter constructs containing the *HyWnt3* −2149/−386 regulatory sequence with or without *Hyhsp70* core promoter (hsp.mini). (*F*–*H*) Reporter activities driven by −2149/−386.hsp (+), −2149/−386.hsp (−), or hsp.mini. EGFP expression (green) was detectable only in combination with the *Hyhsp70* core promoter (*F*). Transformation marker RFP (magenta) was expressed ubiquitously in the transgenic animals.

Fig. S2. *cis*-Regulatory activity of *HyWnt3* reporter constructs. (*A–Z*) EGFP expression (green) in the hypostome of transgenic animals with the reporter constructs HyWnt3FL (*A* and *B*), Dl−2129/−1517 (*C* and *D*), Dl−1494/−985 (*E* and *F*), Dl−1201/−604 (*G* and *H*), Dl−842/−406 (*I* and *J*), −985 (K and *L*), HyWnt3act (*M* and *N*), −985/−479 (*O* and *P*), −758/−386 (*Q* and *R*), HyWnt3FL(+hsp.mini) (*S* and *T*), −2149/−386 (*U* and *V*), HyWnt3prox (*W* and *X*), and Act-Wnt3prox (*Y* and *Z*). Ectodermal lines are shown in *A*, *C*, *E*, *G*, *I*, *K*, *M*, *O*, *Q*, *S*, *U*, *W*, and *Y*, and endodermal lines are in *B*, *D*, *F*, *H*, *J*, *L*, *N*, *P*, *R*, *T*, *V*, *X*, and *Z*. Expression of the *HyActin-RFP* transformation marker is shown in magenta. (Scale bar: 100 μm.)

Fig. S3. The *Hydra Gata* gene homologue is expressed in epithelial cells in the body column. (*A–C*) *Hydra Gata* was cloned (Table S7) and its mRNA (GenBank accession no. XM002155219; *Hydra* genome browser model no. Hma2.212479) is distributed in epithelial cells in the body column. (Scale bars: *A*, 100 μm; *B*, 45 μm; *C*, 15 μm.)

Other Supporting Information Files

Table S1 (DOC)
Table S2 (DOC)
Table S3 (DOC)
Table S4 (DOC)
Table S5 (DOC)
Table S6 (DOC)
Table S7 (DOC)