Fig. S1. Complex rearrangements at the pmar1 locus. A Family Relations II scatter plot of the genomic sequence surrounding the Strongylocentrotus purpuratus pmar1 gene against itself, using a 10-bp window and 100% identity threshold. The 45° line therefore plots the input sequences against each other: 45° lines parallel to this primary line indicate tandem duplication events, whereas 45° lines orthogonal to this indicate inverted duplications. Note the similarity between pmar1a and the pmar1b ORFs. These similar regions include noncoding sequence upstream of each gene. Together we term this the primary tandem duplication. (A secondary tandem duplication appears within the pmar1 intron.) We label the duplicated upstream region for each gene as DUR1 and DUR2. Note that two of the activation sites (one for Otx and one for Tcf) that we identified for pmar1a are not located in the duplicated region but instead in an indel immediately upstream of the pmar1a start of transcription; the hesC target site and a second Otx site are located within DUR1, but the sites themselves are not present in DUR2. The two pmar1 genes at this locus are flanked by unrelated genes. GA, regions of low complexity sequence rich in GA repeats; arrowheads, apparent indels within duplicated regions.
Fig. S2. High-density cDNA time courses for pmar1 and select genes downstream of the pmar1-hesC double-negative gate, measured by quantitative PCR. Embryos were cultured under standard conditions at 14 °C, and samples were taken at 20-min intervals with the exception of the earliest time points. Chart shows the averages of three separate cultures for transcripts numbers of pmar1, alx, delta, and gcm.
Fig. S3. Further examples of the effect of the pmart morpholino antisense oligonucleotide (MASO) and regulative recovery. (A) Control MASO at 24 h postfertilization. (B) Multiple embryos at the same stage but treated with the pmart MASO. Although most embryos show no skeletogenic mesenchyme ingression, others show some sign of ingression or even egression as in the last panel. (C) Embryos treated with the pmart MASO uniformly display recovery of SM ingression by 31 h. (D) Embryos treated with the pmart and blimp1 MASOs show no normal ingression at 24 h, not a phenotype associated with the blimp1 MASO alone [Smith J, Davidson EH (2008) Gene regulatory network subcircuit controlling a dynamic spatial pattern of signaling in the sea urchin embryo. Proc Natl Acad Sci USA 105:20089–20094]. (E) Embryos treated with the pmart and blimp1 MASOs show no recovery at 31 h. (F) Full recovery from the pmart MASO treatment: larvae with normal skeletons and pigment cells developing from zygotes injected with the pmart MASO.
Fig. S4. Further examples of embryos from the reengineering experiment of Fig. 2H.