Inventory of Supplemental Information

Supplemental Figures

Supplemental Tables

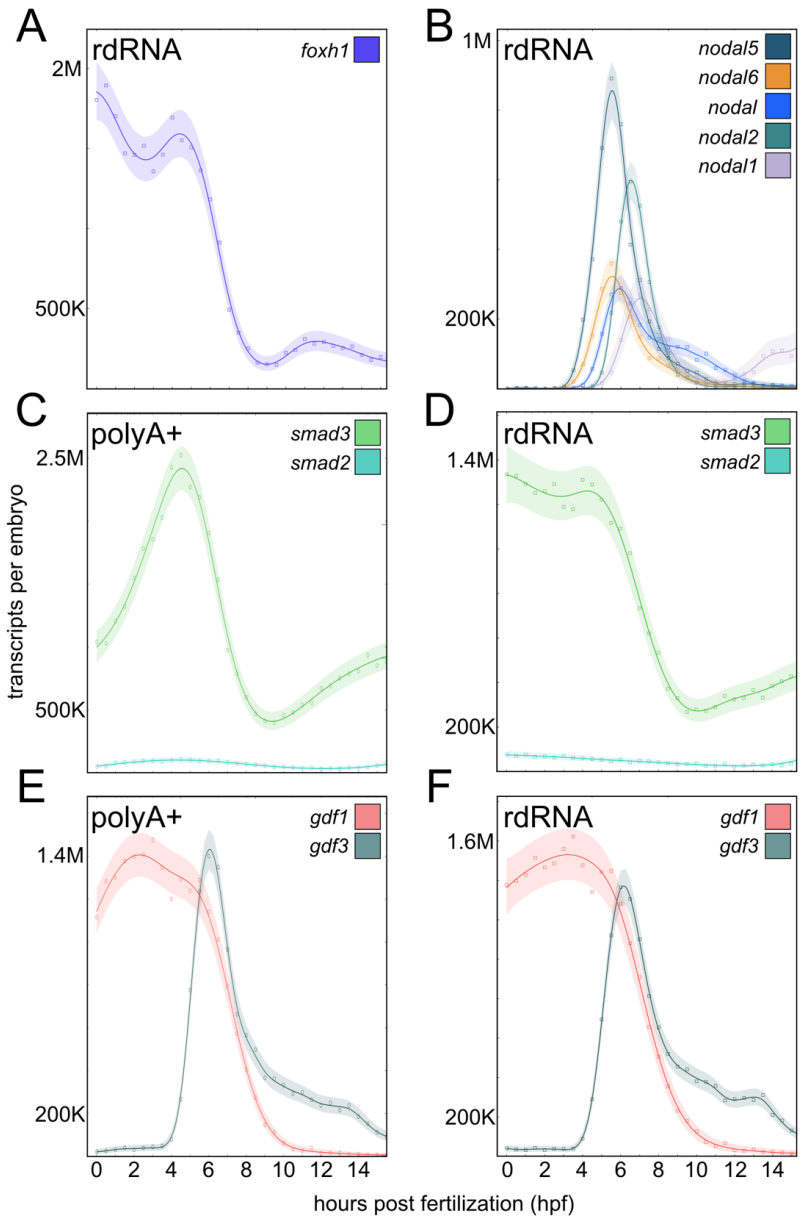

Figure S1. Expression patterns of TGFβ signaling components, related to Figure 1
(A) Ribosome-depleted RNA-seq (rdRNA-seq) reveals the maternal expression of *foxh1* (reproduced from inset in Figure 1A), and (B) zygotic expression of nodal ligands.
(C) PolyA+ RNA-seq and (D) rdRNA-seq confirm the maternal expression of *smad2* and *smad3*.
(E) PolyA+ RNA-seq and (F) rdRNA-seq expression patterns of TGFβ-related ligands *gdf1* (also known as *vg1*) and *gdf3* (also known as *derrière*) reveal maternal *gdf1* and zygotic transcription of *gdf3*. However, pSmad2/3 is not detected until stage 9 (Figure 1D). All transcriptomic analysis is from Owens et al. (2016), clutch A.

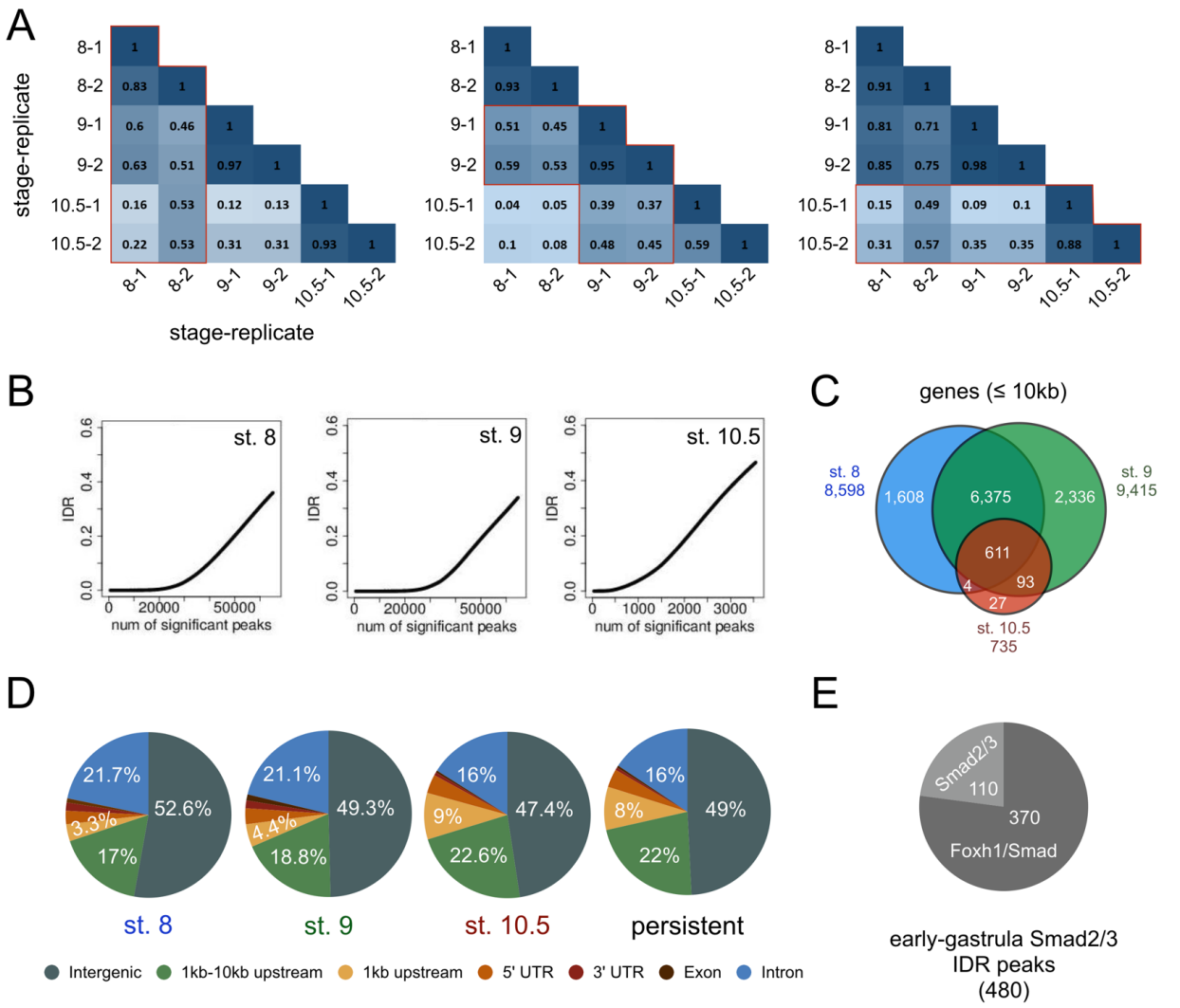

Figure S2. Characterization of the Foxh1 ChIP-seq time course, related to Figure 2
(A) Intra- and inter-stage pairwise Pearson correlation analyses comparing ChIP-seq signals of all MACS2-called peaks. The left-most panel shows comparisons, using stage 8 peaks, between all samples. The middle panel uses stage 9 peaks to compare between samples. And the right-most panel uses stage 10.5 peaks to compare between samples. Red boxed areas within each analysis highlight relevant comparisons using each stage's peaks. Intra-stage correlations were found to be significantly higher than inter-stage correlations. "-1" and "-2" refer to ChIP-seq dataset replicates 1 and 2 at each stage.
(B) Irreproducibility Discovery Rate (IDR) plots for two biological replicates at each stage investigated. These plots demonstrate the consistency of the two replicates. The transition between reproducible (low IDR value) and non-reproducible (high IDR value) peaks is observed.
(C) Venn diagram displaying the overlap of Foxh1-associated genes within 10kb at each of the three stages.

(D) The genomic distribution of Foxh1 peaks at each stage and persistent peaks. Not included in the percentages are the small number of peaks falling into the ‘downstream’ category (500bp downstream of a gene), which include 141 peaks for stage 8, 168 for stage 9, 6 for stage 10.5, and 1 for persistent peaks.
(E) Pie chart displaying the numbers of high confidence Smad2/3 ChIP-seq peaks that overlap Foxh1 peaks.

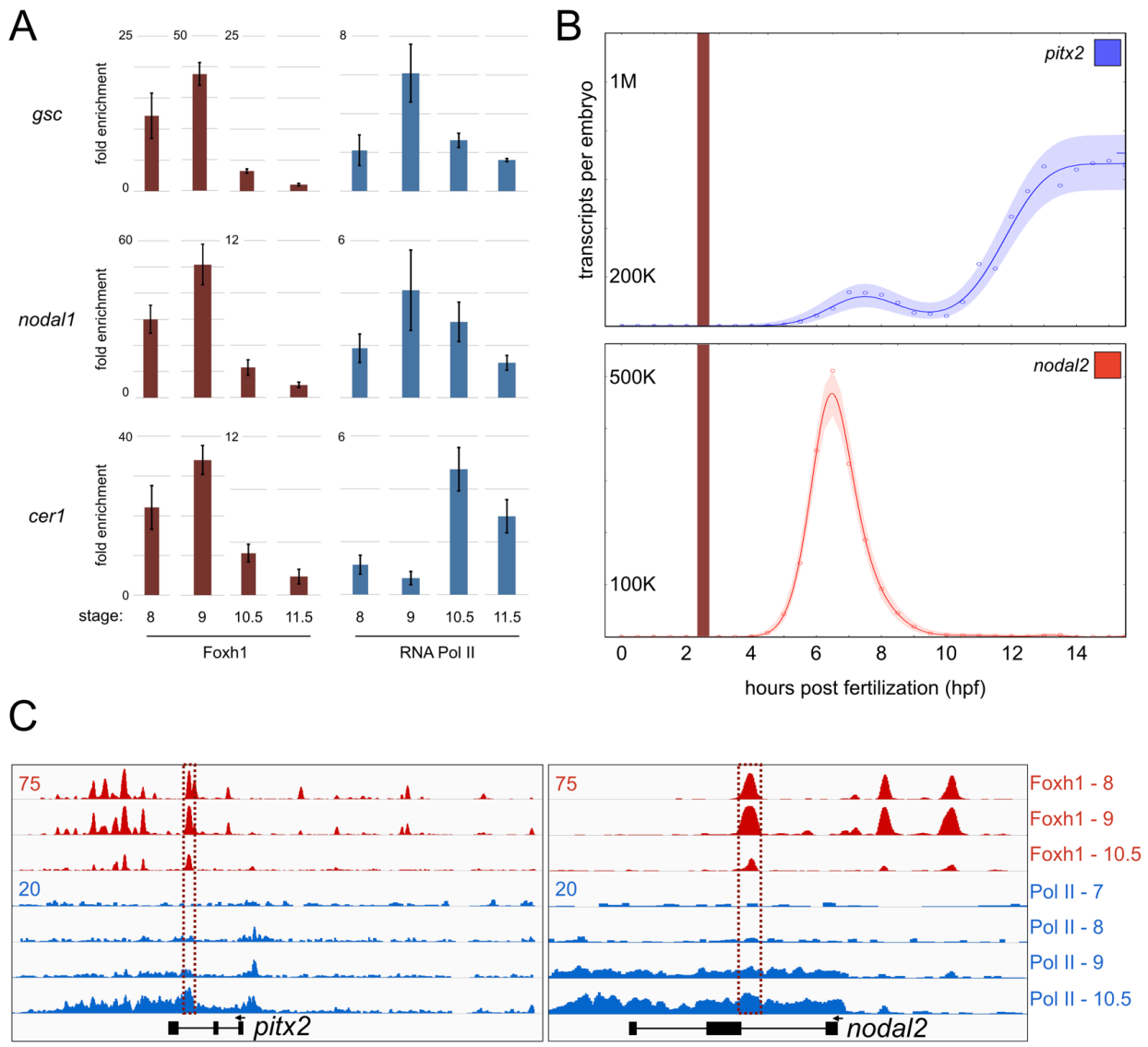

Figure S3. Foxh1 binding precedes the enrichment of RNA Polymerase II, related to Figure 5A

(A) Foxh1 and RNA pol II ChIP-qPCR validation of target genes *gsc*, *nodal1* and *cer1*. The Foxh1-bound enhancers interrogated are indicated in red boxes on genome browser tracks in Figure 5A. Pol II enrichment was analyzed at the TSS. Fold enrichment was calculated over the *eef1a1* background (not shown). See Table S8 for primer sequences.
(B) Expression patterns (Owens et al., 2016) of two additional Foxh1 pre-marked targets, *pitx2* and *nodal2*. The red line indicates Foxh1 binding at stage 6 (32-cells).
(C) Foxh1 (red tracks 1-3) and RNA pol II (blue tracks 4-7) ChIP-seq signal corresponding to additional Foxh1 target genes *pitx2* (scaffold_1:152,988,895-153,023,608) and *nodal2* (scaffold_3:5,679,413-5,690,764).

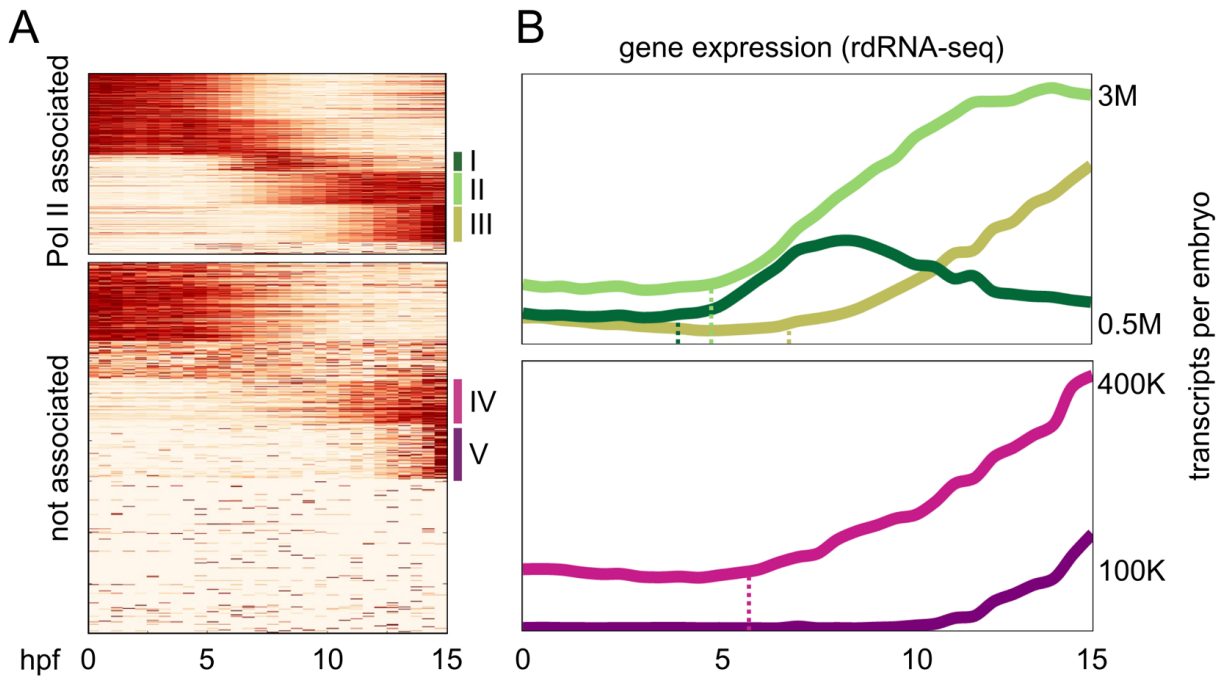

Figure S4. Gene expression patterns of Foxh1-bound genes, related to Figure 5D
(A) Gene expression heatmap of Foxh1-bound genes 'associated' (top) with or 'not associated' (bottom) with RNA pol II (reproduced from Figure 5D).
(B) Line plots showing the average expression pattern (in transcripts per embryo) of genes from each subcluster I-V. Transcripts per embryo values were obtained from rdRNA-seq profiling (Owens et al., 2016).

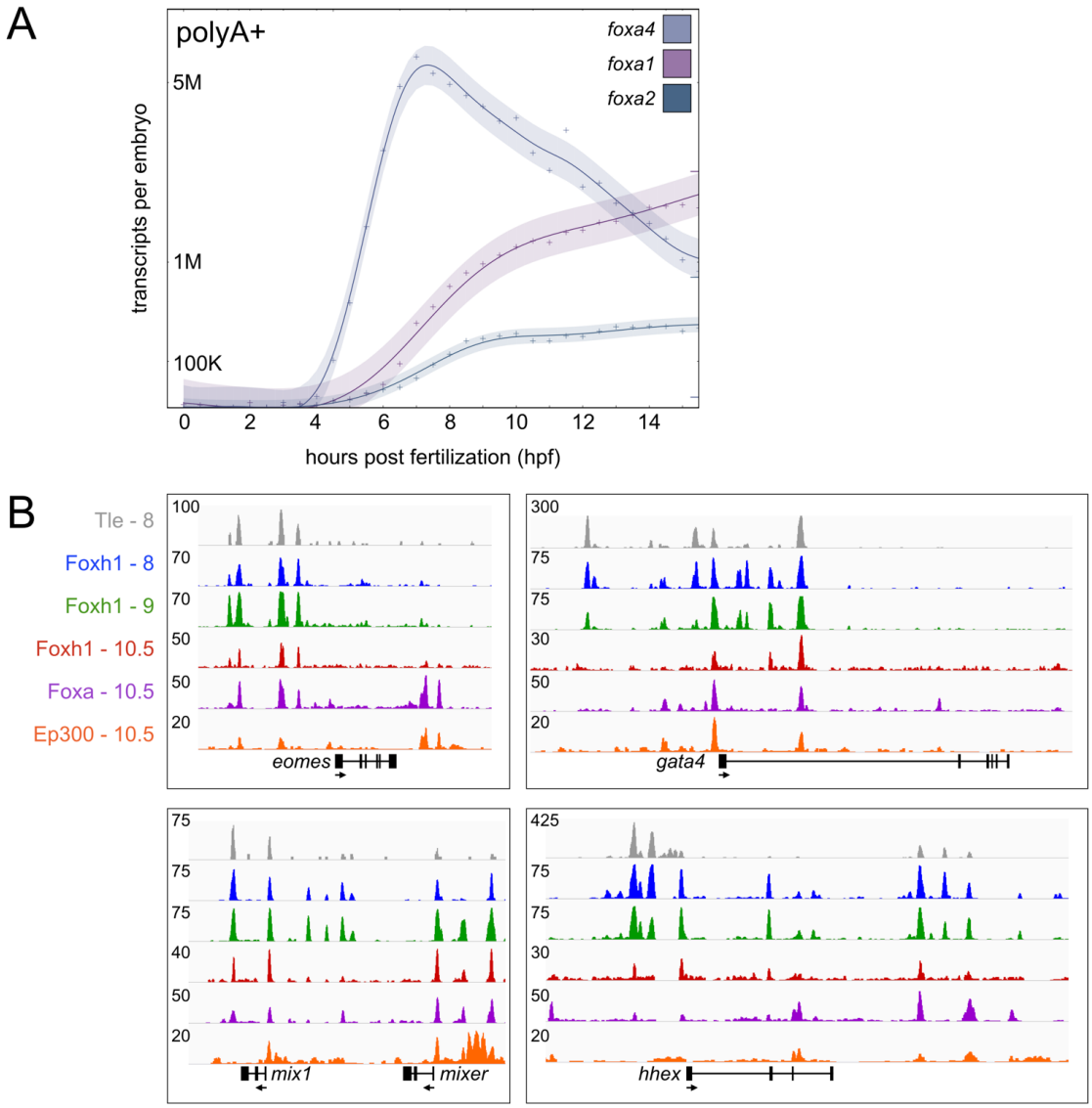

Figure S5. Zygotic Foxa co-binds Foxh1 marked regions, related to Figure 6A
(A) The three *Xenopus foxa* TFs are zygotically expressed (Owens et al., 2016). *foxa4* is the earliest and most abundantly expressed during the time period shown.
(B) Genome browser visualization of Foxh1 (grey; stages 8, 9, and 10.5) and Foxa (purple; stage 10.5) overlapping peaks at the mesendodermal genes *eomes* (scaffold_6:97,439,727-97,463,462), *gata4* (scaffold_5b:1,527,163-1,564,825), *hhex* (scaffold_7:31,972,378-32,001,902), and *mix1* and *mixer* (scaffold_5:118,168,334-118,191,571).

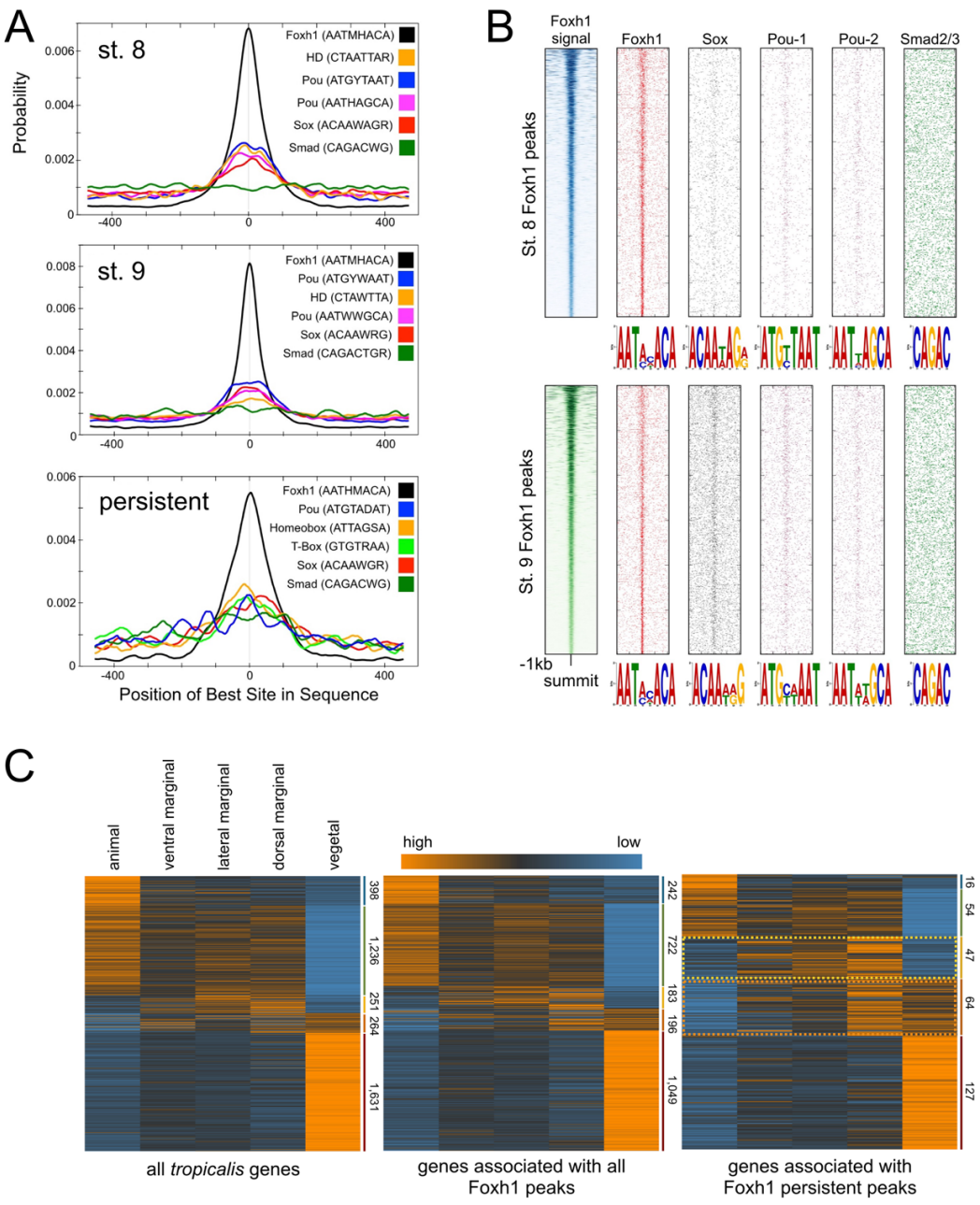

Figure S6. Foxh1 persistent peaks mark mesendoderm CRMs, related to Figure 6B-C

(A) Positional distribution analysis (CentriMo) of selected motifs under the Foxh1 peaks. Stage 8 and 9 CentriMo is displayed with a window setting of 50, and persistent peaks with a window setting of 80. Motifs analyzed were discovered *de novo* using DREME.

(B) Heatmaps displaying the positions of select motifs within a 2kb window surrounding the summit of the stage 8 and stage 9 Foxh1 peaks. The position weight matrix of the

motif searched is shown below the heatmap. All heatmaps are sorted based on the Foxh1 ChIP-seq signal strength at that indicated stage.
(C) Localized gene expression was identified from mRNA-seq performed on dissected early gastrula embryos (Blitz et al., in press) (see Supplemental Experimental Procedures). Expression levels of the localized transcripts (all *X. tropicalis* genes, genes associated with all Foxh1 peaks, and genes associated with persistent peaks) in each tissue type (vertical) were displayed as z-scores. K-means clustering was then used to generate 5 clusters (horizontal). These clusters, indicated with colored bars to the right of each panel (using the same color scheme as in Figure 6E) represent, from top to bottom, genes expressed in the following patterns: 1) animal (high) to vegetal (low) (blue), 2) animal to marginal graded (green), 3) mesoderm-specific (yellow), 4) dorsal-mesoderm and endoderm (orange), and 5) endoderm-specific (red). The number of genes in each category are indicated. Regions indicated by dotted boxes in right-most panel highlight two clusters that are statistically over-represented in the genes with persistent peaks compared to all genes with Foxh1 peaks, suggesting that genes with Foxh1 persistent peaks are more likely to be expressed in dorsal mesoderm and endoderm. Heatmaps were converted to bar plots for Figure 6E.

Target	Forward	Reverse
pitx2 enhancer	ACATCTGTGCGTCTCAGTGG	ACGATCTGCAATGTCCAACA
cer1 enhancer (-2kb), Figure 2E	CCAAACCCTGACAGCAAGAT	CAAGCGGTGTGTGGATTACA
cer1 enhancer (-2kb), Figure 3F and Figure 5B	CCTGCTAATTTGGCTCAACC	AAAGGCTTCACCAGCAAGAG
nodal1 enhancer	TGAAGTGCCGTTCAGATACAG	ATAGCACCCACCAACCTCAA
nodal2 enhancer (Foxh1/Sox CRM) (st10.5-peak9406)	GGCATGTTTAGCAACATTTGG	CCAGCCCTTTTGTTAATAGCC
gsc enhancer (PE) (Foxh1/Sox CRM) (st10.5-peak24451)	ATTGTCCCTTGAGCTGTTGG	CGGAGCTAAAGGGGTTAAACA
mix1 enhancer (Foxh1/Sox CRM) (st10.5-peak16835)	GGAGAGAGGGGCAGACTAGC	CCACAAAGCCACAAAGGAAT
sebox enhancer (Foxh1/Sox CRM) (st10.5-peak6180)	GGAAGTCCTGAGCCACTC	CCAAAAGAGCGTCTGACA
mespb enhancer (Foxh1/Sox CRM) (st10.5-peak9832)	GGCTGCTGGAACATCAAATA	GGAGGCTAATGAGCAGCAGT
mixer enhancer (Foxh1/Sox CRM) (st10.5-peak16838)	CCAGGTAGACCCCATTGTTC	ATTGTTTTGGCCCCTAATCC
snai1 enhancer (Foxh1/Sox CRM) (st10.5-peak4291)	TGGGCAGCCTATTAACACCT	TTTGGCTGTAAAGGGCAGAC
eomes enhancer (Foxh1/Sox CRM) (st10.5-peak18942)	TGCCTAGGCTTCAATCAACA	TATCCTTGGGGCTTTTTGTG
pcdh8.2 enhancer (Foxh1/Sox CRM) (st10.5-peak7264)	GGCATTCTTGCATGGTAGGT	ATTTCAGCCACCGAATCAAC
nodal1 promoter	TGCTGTCAGAAATGCCATCC	CAGGGGAATGCTCTGTTTGT
gsc promoter	TCCTCTGCTGGGTAGTGAGT	CACACATGGGAGAGCACTG
cer1 promoter	CCTTGCAATGATTCTGAGCA	CTGCAGTTGACAAAGAAATGC
eef1a1 (negative) (+2kb)	CCAGATGCAAGTGGTCAAGA	GCTACAACCCAGCGACTGTT

Table S8. ChIP-qPCR primer sequences, related to STAR Methods