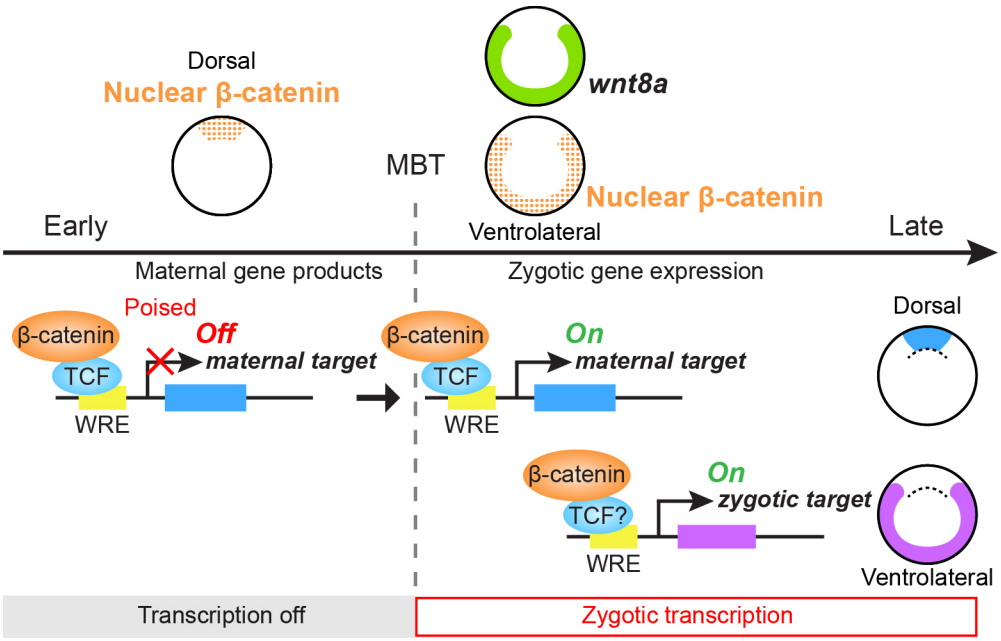

Fig. S1. Wnt/β-catenin target genes change during early development.
Embryonic development from early (left) to late (right) is illustrated. Maternal gene products (mRNA and protein) sustain the earliest embryonic development until Mid-Blastula Transition (MBT), when zygotic transcription is initiated to regulate further development. Maternal Wnt/β-catenin signalling localises β-catenin protein to nuclei of prospective dorsal cells (orange) (Schohl and Fagotto, 2002). After the MBT, *wnt8a* is expressed in ventral and lateral prospective mesoderm (green) (Christian et al., 1991) causing nuclear β-catenin localisation in ventrolateral cells (Schohl and Fagotto, 2002). Maternally regulated nuclear β-catenin initiates poised transcription in specific maternal Wnt/β-catenin target genes (blue), which are transcribed after MBT (Blythe et al., 2010) in dorsal mesoderm. *wnt8a*-regulated nuclear β-catenin is expected to initiate transcription of different specific zygotic Wnt/β-catenin target genes (violet) in ventrolateral embryonic cells.

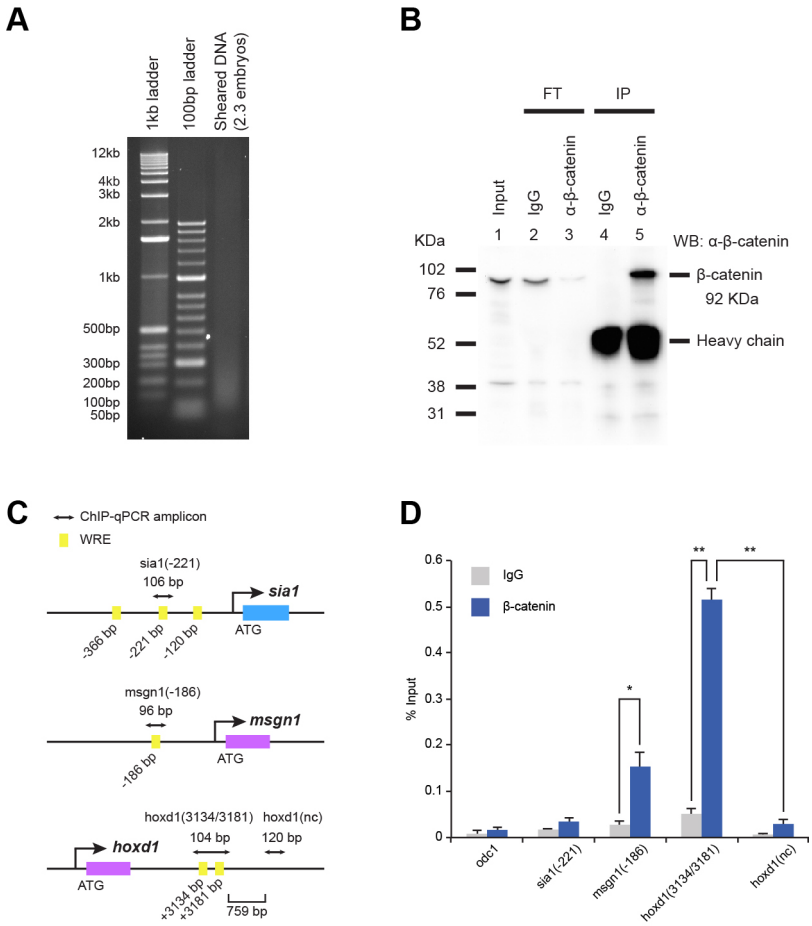

Fig. S2. β-catenin ChIP experimental condition.
(A) Chromatin shearing with optimised condition. Chromatin was extracted from 50 early gastrula embryos that were fixed for 30 minutes, and were sheared during 2.5 rounds of 10 cycles of 30 seconds ON/30 seconds OFF at high power setting using the Bioruptor Plus Instrument. After de-crosslinking and purification, the sheared DNA from 2.3 embryos was analysed by agarose gel electrophoresis. The sheared chromatin was enriched around 200 bp. (B) Immunoprecipitation for chromatin-associated β-catenin protein with optimised condition. Chromatin extract from approximately 42 embryos was incubated with 10 µg of either IgG (negative control) or β-catenin antibodies overnight at 4°C and subsequently precipitated with 50 µl of Dynabeads Protein G for 1 hour, followed by washing and de-crosslinking. Input chromatin extracts before antibody incubation (Input), flow-through supernatant after antibody incubation (FT), and immunoprecipitated samples following immunoprecipitation and elution (IP) were analysed for β-catenin protein by western blotting with β-catenin antibody. β-catenin protein was efficiently immunoprecipitated with β-catenin antibody (see lanes 3 and 5), while significant amount of β-catenin protein remained in supernatant after incubation with IgG (lane 2). Heavy chain indicates denatured IgG and β-catenin antibodies after heat incubation during elution. (C) Schematic diagrams of the *sia1*, *msgn1*, and *hoxd1* genomic loci. Known Wnt-response-elements (WREs, yellow boxes) are shown with the positions relative to the translation start site (ATG). The locations of amplicons (double-headed arrows) analysed by ChIP-qPCR are shown above the corresponding WREs. (D) Validation of β-catenin ChIP by qPCR. Co-immunoprecipitation of β-catenin protein with predicted genomic regions containing target WREs was

analysed by qPCR. Genomic regions of known WREs at the *msgn1* and *hoxd1* loci were detected at greater levels in β-catenin ChIP sample than in the IgG ChIP control sample. Negative control sites [*odc1* and *hoxd1*(nc)] and a WRE site of the maternal Wnt target *sia1* were not efficiently co-immunoprecipitated with β-catenin protein (at gastrula stage). Note that a significant difference in β-catenin ChIP recovery between *hoxd1* (3134/3181) and a genomic region only 759 bp downstream of it [*hoxd1*(nc)] demonstrates a high resolution of chromatin shearing. $*p < 0.01$; $**p < 0.00001$ (two-tailed Student's *t*-test). The error bars represent s.d. of three technical replicates.

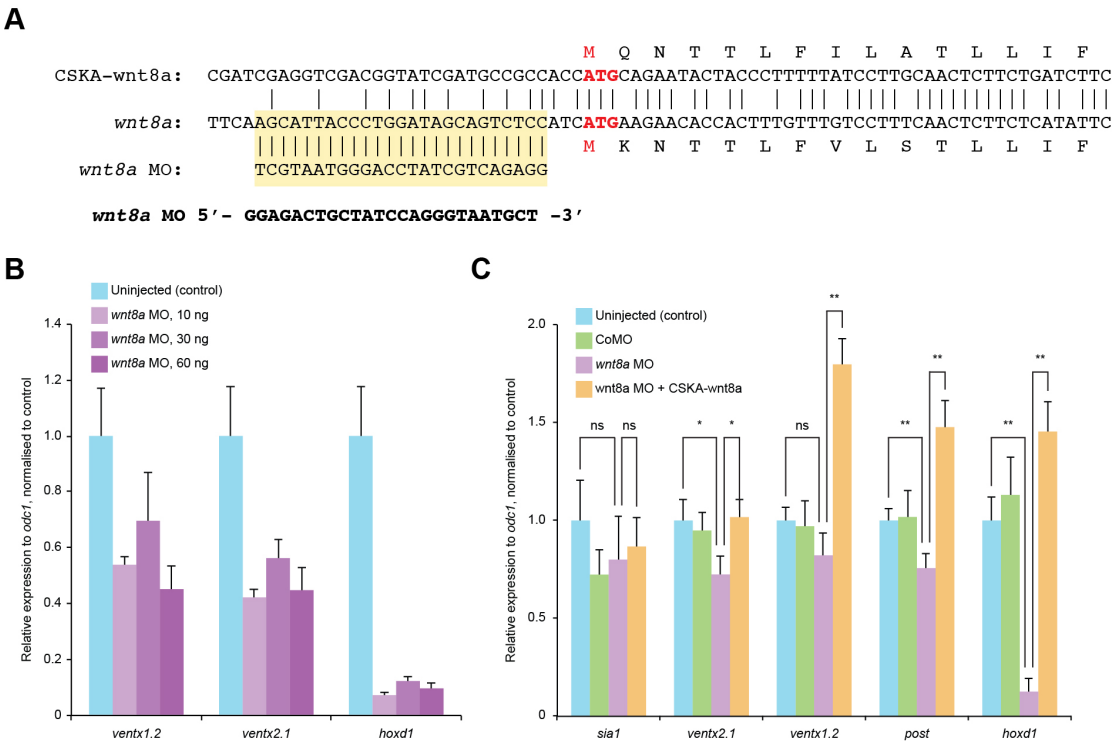

Fig. S3. RNA-seq experiment for identifying *wnt8a*-regulated genes.
(A) *wnt8a* MO and CSKA-wnt8a DNA construct. CSKA-wnt8a was created as a *wnt8a* MO-insensitive rescue DNA construct (see Supplementary Materials and Methods for details). Note that CSKA-wnt8a lacks nucleotide sequences targeted by previously validated *wnt8a* MO (Rana et al., 2006). A yellow box shows sequence homology between *wnt8a* MO and its target sequence of *wnt8a*. The start codons are shown in red. (B) Optimisation of *wnt8a* knockdown. Different amounts of *wnt8a* MO were injected into embryos at the two- to four-cell stage, and expression levels of known *wnt8a*-regulated genes were compared. Note that 10 ng of *wnt8a* MO is adequate to knockdown *wnt8a* activity resulting in downregulation of the known *wnt8a* target genes. Error bars represent s.d. of three technical replicates. (C) Validation of positive control *wnt8a*-regulated gene expression in samples for subsequent RNA-seq analysis. Embryos at the four-cell stage were injected at the marginal zone of both ventral blastomeres with either control MO (CoMO, 2.5 ng per blastomere), *wnt8a* MO (2.5 ng per blastomere), or a combination of *wnt8a* MO (2.5 ng per blastomere) and CSKA-wnt8a DNA (6.25 pg per blastomere). mRNA was extracted at the early gastrula stage and analysed by RT-qPCR. Expression levels were normalised to *odc1* and to uninjected control. Expression of known *wnt8a*-regulated genes such as *ventx2.1*, *ventx1.2*, *post*, and *hoxd1* decreased with *wnt8a* MO, and increased with *wnt8a* MO and CSKA-wnt8a DNA. In contrast, the known maternal Wnt target *sia1* did not show significant difference in gene expression in these experiments. $*p < 0.1$; $**p < 0.05$; ns, not significant ($p \geq 0.1$); two-tailed Student's *t*-test. Error bars represent s.e.m. of three biological replicates.

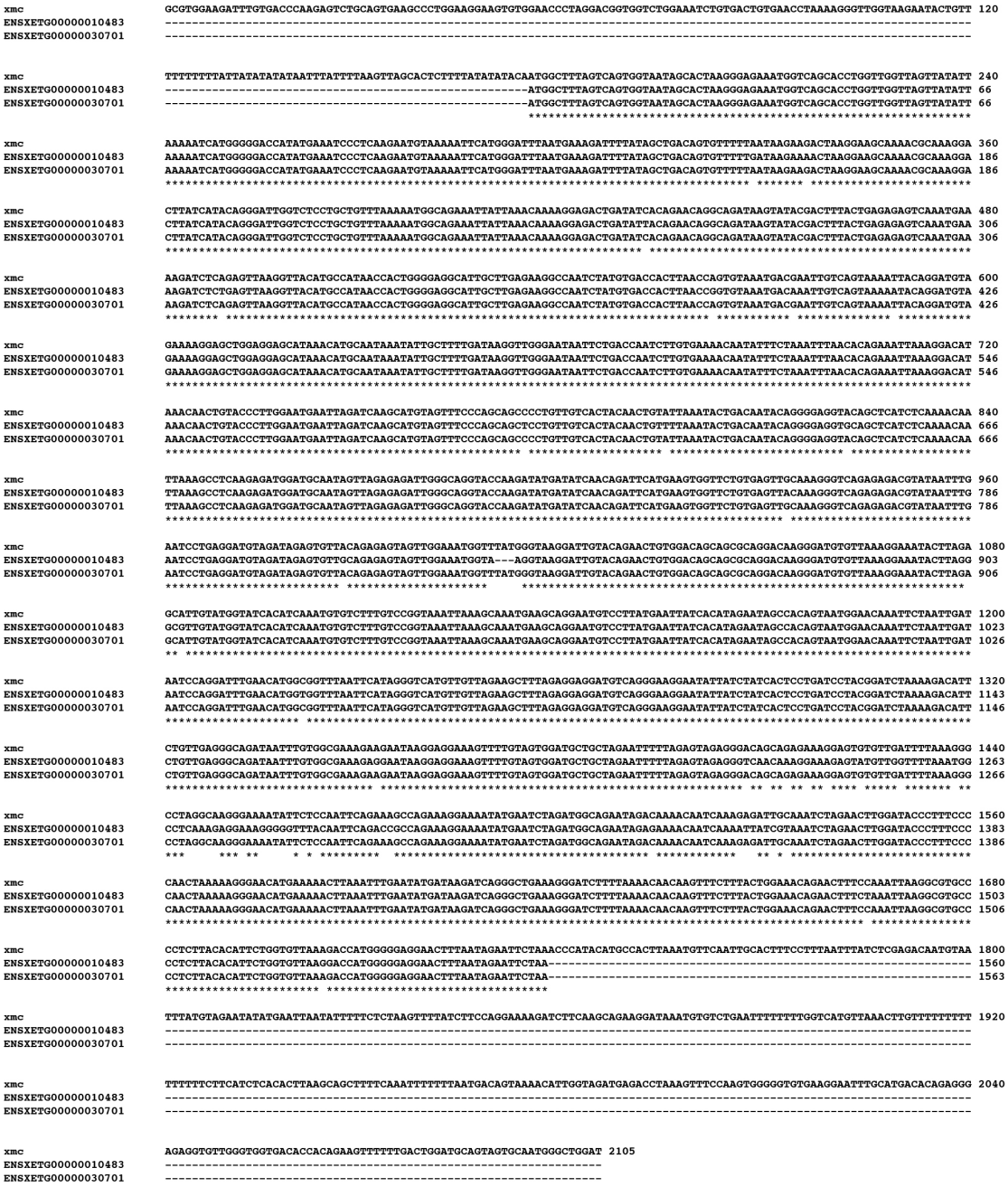

Fig. S4. Sequence similarity of *xmc* genes.
ClustalW2-based multiple sequence alignment of DNA sequences of the *Xenopus tropicalis* homologue of *Xenopus laevis marginal coil* (*xmc*) [GenBank: XM_002944874.1] and of two *xmc*-like genes (*ENSXETG00000010483* and *ENSXETG00000030701*). *ENSXETG00000010483* and *ENSXETG00000030701* show 97% and 98% identities with the *Xenopus tropicalis xmc* gene, respectively.

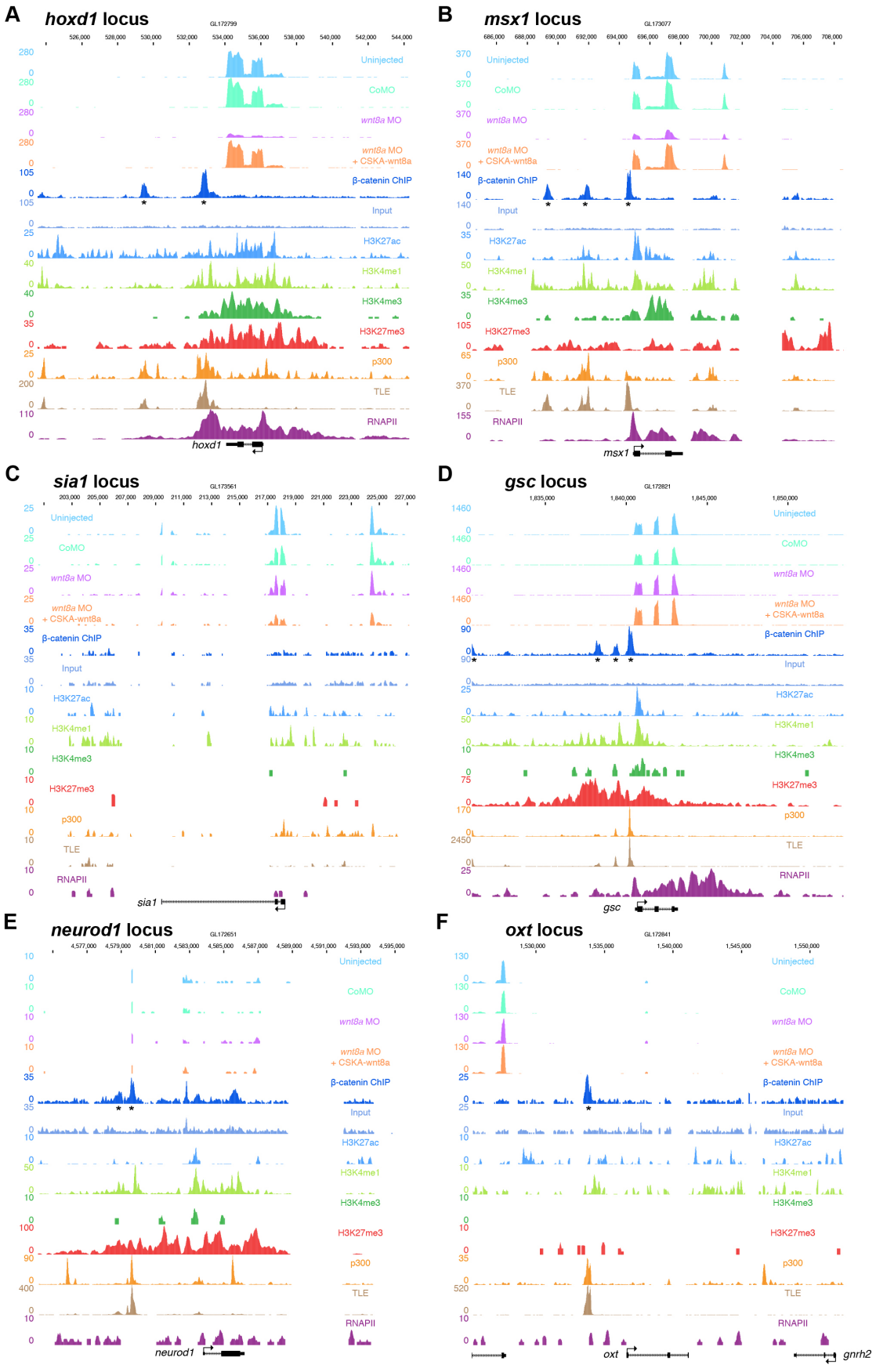

Fig. S5. Genome browser representation of ChIP-seq and RNA-seq data in exemplary genes.

Genomic loci of *wnt8a*-regulated target genes *hoxd1* (A), *msx1* (B), maternal Wnt/β-catenin target *sia1* (C), maternal Wnt regulated gene *gsc* (D), non-*wnt8a*-regulated gene *neurod1* (E) and transcriptionally silent gene *oxt* (F) are visualised as indicated with RNA transcript profiles of four experimental samples from RNA-seq analysis (Uninjected, CoMO-, *wnt8a* MO-, and *wnt8a* MO + CSKA-wnt8a DNA-injected), β-catenin ChIP-seq data (β-catenin ChIP and Input), ChIP-seq data of histone marks (H3K27ac, H3K4me1, H3K4me3, and H3K27me3) and RNA polymerase II (RNAPII), and of transcriptional co-factors (p300 and TLE). *denotes β-peak position identified by peak calling with the IDR method. Note β-peaks correlate with the histone mark and transcriptional co-factor peaks. *hoxd1* and *msx1* show RNA transcript profiles that correlate with RNA-seq experimental conditions where they are expressed at low levels in *wnt8a* MO but expressed in the re-instatement *wnt8a* MO + CSKA-wnt8a at comparable levels to the Uninjected and CoMO controls. There is no β-peak in the proximal promoter of the *sia1* locus, which has been shown to contain functional TCF/LEF-binding sequences mediating response to maternal Wnt/β-catenin signalling. The *gsc* locus contains multiple β-peaks that correlate with p300 and TLE peaks. The *neurod1* and *oxt* loci associate with two β-peaks (-4 kb and -5kb regions) and one β-peak (-3 kb region), respectively, but mRNA expression levels are low and remain unaffected by change in Wnt8a activity.

A ***de novo*** **motif discovery with 10,009 β-peaks from 5,009 non-*wnt8a*-regulated genes**

MEME				DREME			
Motif logo	E-value	Sites	TF	Motif logo	E-value	Sites	TF
	1.8e-1845	2,614	AP2		2.1e-205	2,525	SOX
	2.7e-470	784	ZNF		1.9e-186	5,220	FOX
	2.9e-577	3,835	SOX		3.3e-206	1,487	AP2
	5.5e-445	1,115	ZIC		4.8e-099	1,902	POU/Oct-4
	1.5e-210	554	TBX		3.6e-079	3,650	SOX
	3.9e-169	2,409	POU/Oct-4		1.0e-061	463	TBX
	2.7e-147	696	FOX		1.2e-053	1,073	ZIC
	1.5e-071	211	Eve		6.8e-036	1,152	TCF/LEF
	6.1e-098	387	TCF/LEF		1.2e-026	515	ZNF
	1.0e-039	73	NR4A2		7.4e-026	377	AP2
	3.4e-005	105	Homeobox		1.1e-025	782	TCF/LEF

B ***de novo*** **motif discovery with 624 β-peaks from 179 Wnt8a/β-catenin target genes**

MEME				DREME			
Motif logo	E-value	Sites	TF	Motif logo	E-value	Sites	TF
	1.9e-066	143	AP2		5.4e-014	166	POU/Oct-4
	3.3e-033	43	ZNF		4.2e-011	158	SOX
	2.2e-029	80	TBX		1.2e-006	82	TBX
	2.3e-026	170	SOX		1.6e-007	93	AP2
	6.4e-010	63	FOX		6.0e-005	178	FOX
	2.5e-007	65	No hit		1.1e-002	57	Homeobox
	1.3e-003	77	SOX/FOX		1.8e-002	39	Homeobox
					3.8e-002	31	TCF/LEF

Fig. S6. ***De novo*** **motif discovery on β-peaks in comparing non-*wn8a*- and *wnt8a*-regulated genes.**

De novo motif search was performed on β-peaks of 5,009 non-*wnt8a* regulated genes (A) and 179 *wnt8a*-regulated genes (B), using the MEME-ChIP software with MEME and DREME search functions. *De novo* motif logo, e-value, the number of sites containing the motif, and transcription factor names having similar target motif are indicated. Top eleven motifs are shown for non-*wnt8a*-regulated genes.

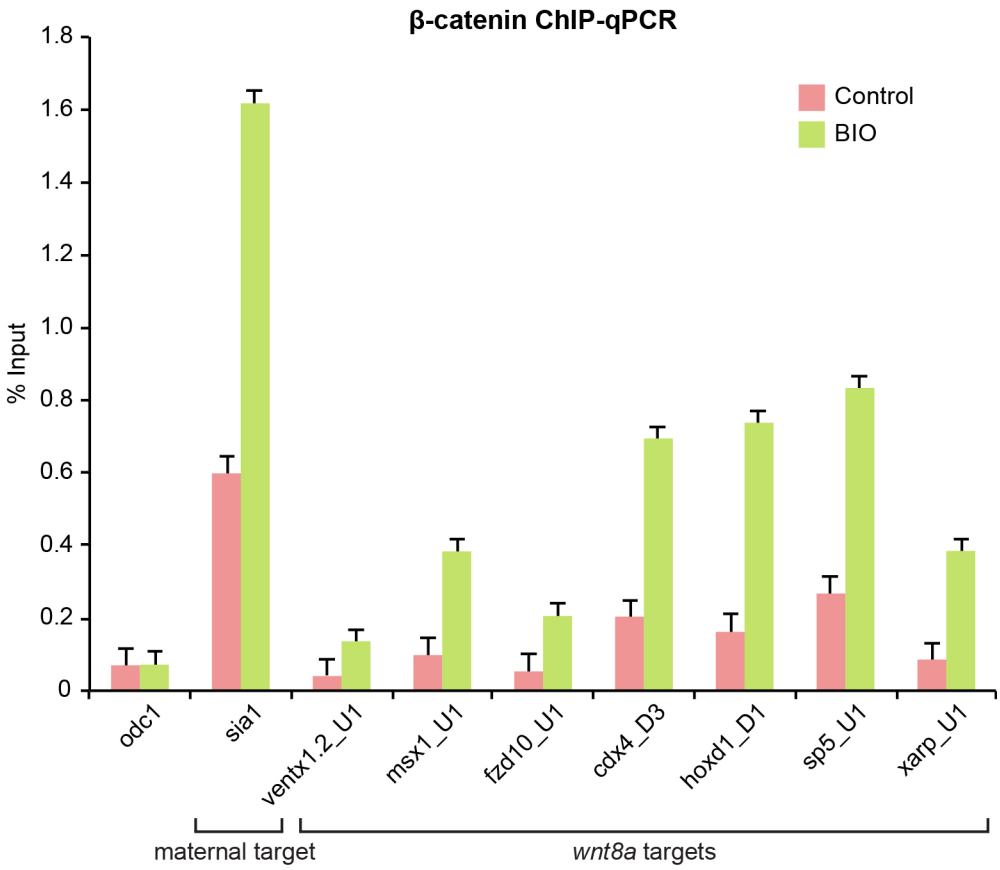

Fig. S7. Maternal Wnt signalling regulates β-catenin recruitment to both maternal Wnt target and zygotic *wnt8a* target loci.
In order to experimentally activate maternal Wnt/β-catenin signalling, embryos were treated with the glycogen synthase kinase-3 inhibitor BIO at the four-cell stage until the 1000-cell stage, when the embryos were collected for β-catenin ChIP analysis to compare β-catenin occupancy levels with untreated control embryos. Enhanced activity of maternal Wnt signalling with BIO increased β-catenin binding levels at both maternal Wnt target *sia1* and zygotic *wnt8a* target gene loci. Error bars represent s.d. of two biological replicates.

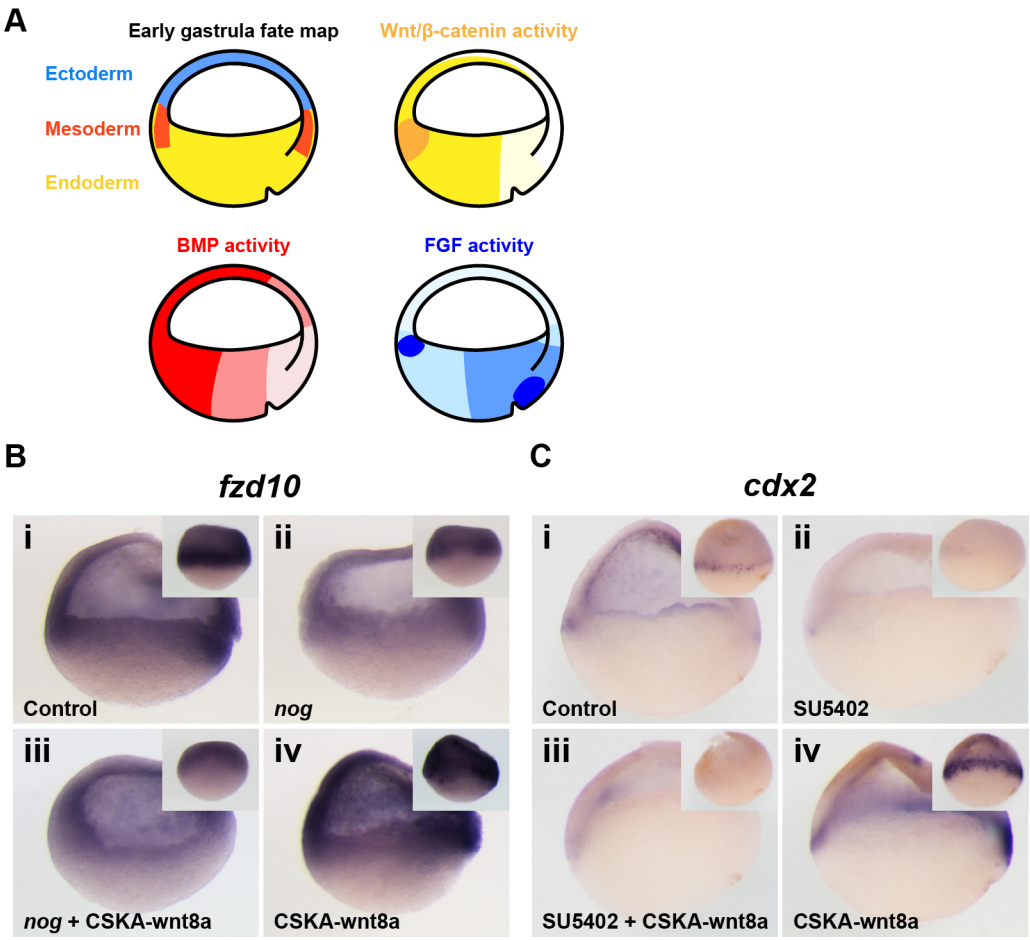

Fig. S8. BMP or FGF signalling is required for context-specific regulation of *wnt8a* target gene expression.
(A) Schematic diagrams of fate map in early gastrula and of Wnt/β-catenin, BMP, and FGF signalling pathway activities (Schohl and Fagotto, 2002). Regions with higher pathway activities are shown in darker colours. (B,C) *In situ* hybridisation shows expression of *fzd10* (B) and *cdx2* (C) in sagittal sections and lateral views (insets) of control uninjected, untreated embryos and experimentally manipulated embryos as indicated, with dorsal right.

Table S1. β-peaks identified in β-catenin ChIP-seq data.
Sheet 1: 68,045 peaks called by MACS2 (version 2.0.10)(p-value cutoff of 0.01) using β-catenin ChIP-seq data. "fold-change" was used for IDR analysis. Sheet 2: 60,888 peaks called by SPP (version 1.10.1)(FDR cutoff of 0.1) using β-catenin ChIP-seq data. "signalValue" was used for IDR analysis. Sheet 3: 10,638 β-catenin ChIP-seq peaks (β-peaks) identified by the irreproducible discovery rate (IDR) method (IDR threshold of 0.01) using the MACS2 and SPP peaks. The position of each β-peak ("peakname") is indicated by "scaffold", "matchstart" and "matchend", with "summit" of the peak. The nearest genes of β-peaks were annotated using distanceToNearest function (rtracklayer version 1.2.26 and GenomicRanges version 1.12.5). The nearest genes are indicated by "gene" and "gene name" with their start ("genestart") and end ("geneend") positions, and direction of gene-encoding ("genestrand") on the scaffold. "genedistance" indicates the distance of a β-peak from the transcriptional start site of the nearest gene. "IDR" shows IDR of each β-peak.

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Table S2. Identification of *wnt8a*-regulated genes from RNA-seq analysis data.
Sheet 1: 14 *wnt8a*-regulated target genes (shortlisted). The identified *wnt8a* targets are indicated with their "Gene ID", "Gene Name", and "Gene Function" using the Xenbase annotation. "Fold change" and "FDR" indicate those values of "*wnt8a* knockdown" experiments (compared CoMO control) or of "Wnt8a re-instatement" experiments (compared *wnt8a* MO) obtained from GLM analysis. Sheet 2: gene list from GLM analysis of *wnt8a* knockdown, including Uninjected, CoMO, and *wnt8a* MO samples (FDR < 0.1). "Down" and "Up" indicate decreased and increased expression of corresponding gene in *wnt8a* MO against CoMO condition, respectively. Sheet 3: gene list from GLM analysis of Wnt8a re-instatement, including Uninjected, CoMO, *wnt8a* MO, and *wnt8a* MO + CSKA-wnt8a samples (FDR < 0.1). "Down" and "Up" indicate negative and positive regulation of expression of corresponding gene in *wnt8a* MO + CSKA-wnt8a against *wnt8a* MO condition, respectively. Sheet 4: pair-wise analysis of Uninjected and CoMO (FDR < 0.1). "Down" and "Up" indicate downregulation and upregulation of corresponding gene in CoMO against Uninjected condition, respectively. These genes affected by CoMO injection were excluded from analysis identifying *wnt8a*-regulated genes.

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Table S3. β-peaks of *wnt8a*-regulated genes.
Sheet 1: a list of 179 genes that are positively regulated by Wnt8a signalling and that are associated with β-peaks. The 179 *wnt8a*-positively regulated genes contain 624 β-peaks (see also Table S1 for details). Sheet 2: a list of β-peaks associated with 13 shortlisted Wnt8a/β-catenin target genes. The β-peaks were named as U (upstream) or D (downstream) plus a number corresponding to the position of the peak relative to the transcriptional start site.

Click here to Download Table S3

Table S4. β-peaks of maternal Wnt-regulated genes.
A list of β-peaks associated with genes that are transcriptionally regulated by maternal Wnt signalling (see text for detail). The β-peaks were named as U (upstream) or D (downstream) plus a number corresponding to the position of the peak relative to the transcriptional start site.

Click here to Download Table S4

Supplementary Materials and Methods:

Embryo experiments

Xenopus tropicalis (Gray) were purchased from Nasco (http://www.enasco.com/xenopus/) and the European Xenopus Resource Centre (EXRC)(http://www.port.ac.uk/research/exrc/). Adult female and male *X. tropicalis* were primed with 10 units of human chorionic gonadotropin (HCG) 12 hours before boosting. 5 hours before embryo collection, female and male frogs were boosted with 200 units and 100 units of HCG, respectively. For natural mating, single pairs of female and male frogs were placed together, and embryos were harvested approximately every 1 hour after the onset of egg laying. For *in vitro* fertilisation, testes were dissected from euthanized male frogs and kept in 1x Modified Barth's Saline (MBS) + 0.1% BSA on ice. The testes were subsequently minced and used to fertilise eggs. Fertilised embryos were dejellied in 3% cysteine/0.1x Marc's Modified Ringer (MMR), washed with 0.1x MMR, and kept at 18°C until they developed to desired stages. Injection experiments were performed by microinjecting the following reagents (MOs, mRNA, and DNA) into the marginal zone of two ventral blastomeres of four-cell-stage embryos for the RNA-seq, and into the marginal zone of each blastomere of embryos at the two- to four-cell stage for other experiments:

- CoMO and *wnt8a* MO, 2.5 ng per blastomere: 5 ng per embryo for the RNA-seq analysis (Fig. 2A; Fig. S3C) and 10 ng per embryo for the other experiments;
- CSKA-wnt8a DNA, 6.25 pg per blastomere: 12.5 pg per embryo for the RNA-seq analysis (Fig. 2A; Fig. S3C) and 25 pg per embryo for the re-instatement experiments (Fig. 2C) and 100 pg for the overexpression experiments (Figs. 4C,D, 5;
- *wnt8a* mRNA, 40 pg per embryo;
- *axin* mRNA, 2 ng per embryo;
- *nog* mRNA, 500 pg per embryo.

SU5402

SU5402 (Sigma, SML0443) stock was made in DMSO and freshly diluted to 50 µM in 0.1x MMR before use.

BIO

BIO (Tocris Bioscience, 3194) stock was made in DMSO and freshly diluted to 20 µM in 0.1x MMR before use.

pCSKA-wnt8a construct

pCSKA-wnt8a plasmid DNA construct was created by introducing nucleotide substitutions and Kozak consensus sequence into the *Xenopus laevis* (Daudin) *wnt8a* (*Xwnt8*) gene. A *Xwnt8* DNA fragment was PCR-amplified from pCSKA-Xwnt-8 (Christian and Moon, 1993) as a template, using the following forward and reverse primers (restriction enzyme sites are underlined and Kozak consensus sequence is italic and bold): 5'-CCATCGAT***GCCGCCACC***ATGCAGAATACTACCCTTTTTATCCTTGCAAC TCTTCTG-3' and 5'-GGAATTCTCATCTCCGGTGGCCTC-3'. The resulting PCR product was digested with ClaI and EcoRI and subcloned into pCSKA digested with

ClaI and EcoRI, which resulted in the rescue construct carrying *wnt8a* coding sequence that is not recognised by the *wnt8a* MO.

ChIP

Embryos were harvested at the developmental stage of interest and fixed at room temperature with 1% formaldehyde in phosphate-buffered saline (PBS) for 45 minutes (1000-cell stage embryos) or for 30 minutes (early gastrulae). Immediately after fixation, the embryos were incubated with 125 mM glycine/PBS for 10 minutes and washed three times with ice-cold PBS for 5 minutes. Batches of 50 embryos were snap-frozen in liquid nitrogen and stored at -80°C for future use. For the following method, all solutions and samples were kept on ice. RIPA buffer (50 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% IGEPAL CA-630, 0.25% Sodium deoxycholate, 0.1% SDS, 0.5 mM DTT) supplemented with Protease Inhibitor Cocktail (Sigma, P8340) was added to frozen embryos. Embryos were thawed on ice for 10-15 minutes, homogenised, and then kept on ice for 10 minutes. After re-homogenisation, the embryo extracts were transferred to TPX microtubes (Diagenode) and sonicated during 2.5 rounds of 10 cycles with 30 seconds ON/30 seconds OFF at high power setting using the Bioruptor Plus Instrument (Diagenode). The sonicated samples were centrifuged at 14,000 rpm for 10 minutes at 4°C, and the supernatant was transferred to a 1.5ml tube for subsequent use for ChIP and input samples. A small aliquot of the supernatant was used for checking chromatin shearing. The input samples were stored at -20°C for later usage. The supernatant for ChIP were incubated for 1 hour at 4°C with Dynabeads Protein G (Life technologies) that had been blocked with 5% BSA/PBS for 1 hour at 4°C. After snap-spin, the supernatant was transferred to a 1.5ml safe-lock tube and incubated with antibodies (2 ug) overnight at 4°C. On the following day, chromatin was precipitated with 5% BSA/PBS-blocked Dynabeads Protein G for 1 hour at 4°C and then the beads were successively washed with ChIP buffer 1 (20 mM Tris pH 8.0, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS), ChIP buffer 2 (20 mM Tris pH 8.0, 500 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS), ChIP buffer 3 (10 mM Tris pH 8.0, 250 mM LiCl, 1 mM EDTA, 1% IGEPAL CA-630, 1% Sodium deoxycholate), ChIP buffer 4 (10 mM Tris pH 8.0, 1 mM EDTA) for 5 minutes each. Chromatin was eluted from the beads with elution buffer (50 mM Tris pH 8.0, 10 mM EDTA, 1% SDS) for 20 minutes in a thermoshaker (65°C, 900 rpm). At this stage, the frozen input samples were supplemented with elution buffer. ChIP and input samples were incubated with RNase A at 37°C for 30 minutes. The samples were then added with NaCl and incubated for over 16 hours in a thermoshaker (65°C, 900 rpm). The samples were further treated with proteinase K for 2 hours in a thermoshaker (65°C, 900 rpm). Eventually, the de-crosslinked DNA fragments were purified with phenol:chloroform:isoamylalcohol and precipitated in ethanol for qPCR or using MinElute Reaction Cleanup Kit (QIAGEN) for sequencing.

ChIP-seq

Three independent β-catenin ChIP experiments were performed as described above, and sheared chromatin was collected from approximately 750 early gastrula embryos (stage 10.25) in total. Each ChIP DNA and input control DNA was purified using MinElute Reaction Cleanup Kit (QIAGEN) and pooled to one sample. The purified DNA was quantified using Qubit dsDNA HS Assay Kits (Life technologies) by Qubit 2.0 Fluorometer (Life technologies). Two Illmina TrueSeq ChIP libraries were constructed from the ChIP DNA and the input control DNA samples and sequenced

using 50 bp single-end reads by Illumina HiSeq 2500 at The Genome Analysis Centre (TGAC, Norwich, UK). After quality control using FasQC, sequenced reads were mapped to the *X. tropicalis* genome assembly JGI 4.2/xenTro3 using bwa (version 0.7.5a) (Li and Durbin, 2009). Multi-mapped reads were filtered with samtools version 1.1.19 (Li et al., 2009). In order to focus our analysis on a set of highly reliable peaks we performed peak calling using two different methods [SPP (version 1.10.1)(Kharchenko et al., 2008) and MACS2 (version 2.0.10.20120913)(Zhang et al., 2008)] and then used IDR (Li et al., 2011) to identify the peaks, which were reproducible using the two methods. This approach has an advantage over using the overlap of the peaks obtained using the two methods since IDR quantitatively assesses when the f ndings are no longer consistent across replicates. The ranked list of peaks for both methods was generated with a low confidence threshold, which is necessary for IDR to separate signal from noise. We used $p \leq 0.01$ for MACS2 and FDR ≤ 0.1 for SPP resulting in 68,045 and 60,088 peaks respectively, which include both high and low quality peaks. These were used as input for IDR with threshold of 0.01 and resulted in 10,638 peaks. Peaks were assigned to closest genes using distanceToNearest function (rtracklayer version 1.2.26 and GenomicRanges v 1.12.5). For visualising heat maps, in addition to β-catenin ChIP-seq data, ChIP-seq data were used of H3K27ac, H3K4me1, p300 and TLE (all of these from *X. tropicalis* early gastrula whole embryos at stage 10.5) from Yasuoka et al. (2014) and of H3K4me3 and H3K27me3 (each of these from *X. tropicalis* gastrula whole embryos at stage 11-12) from Akkers et al. (2012). The coverage for each base was divided by the total number of all bases of ChIP-seq data, and then normalised by multiplying a million. Using the normalised ChIP-seq data, a heatmap data matrix files were generated using HOMER (Heinz et al., 2010), clustered using Cluster 3.0 with *k*-means clustering (*k*=10)(de Hoon et al., 2004), and visualised using Java Treeview (version 1.1.6r4)(Saldanha, 2004). Histograms of β-catenin occupancy level around the peak summit were generated using HOMER with bin size in 100 bp and visualised in Excel. For *de novo* motif discovery, DNA sequences of 100 bp regions centred on the peak summit were analysed using MEME-ChIP with MEME and DREME algorithms coupled with CentriMo and Tomtom algorithms to find the motif width from 5 to 10 nucleotides (Ma et al., 2014). Genome browser representation files were generated by converting ChIP-seq data to bigWig format. This was done using genomeCoverageBed from bedtools v 2.17.0 to generate a bed file then UCSC bedGraphToBigWig to convert the bed to bigWig format.

RNA-seq

X. tropicalis embryos were injected into both ventral blastomeres at the four-cell stage. Total RNA was extracted as described in Lee-Liu et al. (2012) from stage 10.25 uninjected embryos and embryos that had been injected with MOs (2.5 ng per blastomere) or together with pCSKA-wnt8a (6.25 pg per blastomere). The quality of total RNA was assessed using Agilent 2100 Bioanalyzer whether the RNA integrity number was 7 or higher. Illumina TruSeq RNA libraries were constructed from 12 total RNA samples (biological triplicates of each experimental conditions: uninjected, CoMO-injected, *wnt8a*MO-injected, *wnt8a*MO and pCSKA-wnt8a-coinjected). The libraries were sequenced using 100 bp paired-end reads on Illumina HiSeq 2000 at TGAC. Sequenced reads were checked for base qualities, trimmed where 20% of the bases were below quality score 20, and filtered to exclude sequences that were shorter than 20 bp using Fastx (Version 0.0.13). Sequences were aligned to the *X. tropicalis* genome JGI 4.2/xenTro3 with gsnap (Wu and Watanabe, 2005) with parameters -B 4

-E 100 -N 1. Aligned reads were counted using HTSeq (version 0.5.4p2) with parameters -m intersection-strict -s no -a 20 and further differential gene expression analysis was carried out using DESeq2 (version 1.0.19)(Love et al., 2014) with defaults. To identify genes that are differentially expressed in *wnt8a* knockdown or Wnt8a re-instatement, we used generalised linear model (GLM) analysis as follows. Differentially expressed genes for *wnt8a* knockdown were obtained using a GLM with explanatory variables "CoMO control", "*wnt8a* knockdown" and tested against the null hypotheses where the "*wnt8a* knockdown" variable was excluded. Differentially expressed genes for Wnt8a re-instatement were obtained using a GLM with explanatory variables "CoMO control", "*wnt8a* knockdown", "Wnt8a re-instatement" and tested against the null hypotheses where the "Wnt8a re-instatement" variable was excluded. DESeq2 was used to normalise the counts by size factor, estimate dispersions and perform Wald test on a negative binomial model. The p-values were adjusted for multiple testing with the Benjamini-Hochberg procedure using DESeq2's default setting. RNA-seq profiles was displayed on the UCSC genome browser as described above for ChIP-seq data.

RT-qPCR primers

odc1:	F:	TTTGGTGCCACCCTTAAAAC
	R:	CCCATGTCAAAGACACATCG
hoxa1:	F:	ACCAACTTCACCACCAAACAGC
	R:	AGAGCAGCAGCAATTTCTACCC
hoxd1:	F:	CAAGTATCTCACCAGGGCAAG
	R:	GAGTTTTTACGCAGATACTGGATG
sp5:	F:	ACTCAGATTGCTGCACTACTGC
	R:	ACCACTGGAAGTTTGGCAGTTG
msgn1:	F:	AACCTTCCATGACAGTCCAACG
	R:	AAATTGTTGCGCAGGGTGTG
cdx2:	F:	AATCTGGGGCTTTCGGAGAGAC
	R:	ATTTTGGCCAGTCTGAGTCTGC
msx1:	F:	TTTAGGTTTGGGGAGCTTGGC
	R:	AACGCAAACAGACAGTGCTG
cdx4:	F:	TTTGTCTCACACAGCTGCCAAC
	R:	AAGTCGATTGCACGGTTTTCCC
fzd10:	F:	ATTTAGCAGCCTGGGCAATTCC
	R:	ATTGACATCCATGCTGCCAACG
xarp:	F:	ACGCTTTTCCGCATGTACTTGG
	R:	TTGATGTCTTGGGTTCCAACGG
xmcl1:	F:	ACAATTCAGACCGCCAGAAAGG

	R:	TGGGGAAAGGGTATCCAAGTTC
xmcl2:	F:	ACTTGGATACCCTTTCCCCAAC
	R:	AAGAGGGGCACGCCTTAATTTG
ventx1.2:	F:	GGATTCTCTATTGACCTCATTC
	R:	CTTTCTCCTTGGTATCTCCTTG
gbx2.2:	F:	TGCATTCTGTTCCAGCCTTGC
	R:	TTTTCAAAGGCCCCATGCAGAC
neurog1:	F:	AGTAAAGAATGACGCCGTGCTG
	R:	TTGTGCATTCGGTTCCTTTCCC
atp12a:	F:	AAGCAATTGCACGGTGTGTAGG
	R:	TTCACCACACAAGCACATGC
sia1:	F:	TTGACCCCCTAGTCAACAGC
	R:	ACCAGCGGCCTCTTACATT
nodal3.1:	F:	AGGAAGGTGGACATGTTTGTGG
	R:	GCATCGTCCGTCTCATTCAGTGG
gsc:	F:	GTTGCACGTACAGACGCCTA
	R:	TAAGGGAGCATTTGGTGAGG
chrd:	F:	TGAAGCAGTGGGATTCTAGAGG
	R:	GGCAGGATTTAGAGTTGCTTC
nog:	F:	AGGTTTTGGCCTCGCTATGTG
	R:	TGGCAGCTTTGCAAACCATG
fst:	F:	AAGAAGAACAAGCCGAGGTGTG
	R:	TTTGCCATCTATTCCGCACACG
frzb:	F:	AACGCTCACTGTGCTTCATGTG
	R:	AATGGCATTGGCTTGAGTGC
wnt8a:	F:	CTGCAGTGATAATGCAGAATTTG
	R:	TGCAAGTCTTCCTGCTTCATTG
ventx2.1:	F:	GGCTTCTGAGAGACGGAAAC
	R:	TTGTATTTCATCCTGCGGTTC
post:	F:	AAGCAAGGTGGGATACAGTGAG
	R:	CTGATTGGGGGCTAAAGAGAG

ChIP-qPCR primers

odc1	F:	GTGCACGCCTGAATTCTTTCT
	R:	GGCTCAGCAATGATGGTCACT
ventx1.2_U1	F:	GCCCATTCTGATAGCTATTATCCA
	R:	AGTTGTGTGTACACAAAGCCTATG
msx1_U1	F:	CGCTCCTATTAAACCGGCTTAGC
	R:	GCTCTTGTTGTTGACTCGCTTC
msx1_U2	F:	TGCGAGTTAACCTCCTCAATGG
	R:	GCGCCTGCATTGCTAATTGC
msgn1_U1 (-186)	F:	GACCAGTCCATTTTCCATGTTGA
	R:	GGCCCTTTTATACAGACCTGCTAA
neurog1_D1	F:	GAAGCTGAAACAAGCAAGCC
	R:	TTACGGGCAGCCAATCACAG
cdx4_D3	F:	GCTCATTGTCTTCTCCTAGCTCAG
	R:	TCCATCTCCCTTTGATCCTTCC
cdx2_U1	F:	AGGGGGTCTTTGTTCTTCCTTG
	R:	AGGGGCAGATGTATAGGCACTG
hoxd1_D1 (3134/3181)	F:	TGTTGTAGATGCTGATGCTTATCG
	R:	AACAGAAAATCAAAGGCTTGCA
hoxa1_D1	F:	TCTAAAGAAACACGGCGGAGTC
	R:	TAAGCCGTGCCACCATTTAC
gbx2.2_D3	F:	TCCTCTCCAGGCAACAATTAGG
	R:	ACAACCTCTTCCTGCACTGTTC
sp5_U1	F:	AAGTTTGCCGCTGCCCAATC
	R:	CACTCCATGAGGGCTTTGTACATTC
xmcl1_D1	F:	CATCAAACAGTATCCAGCCCATTG
	R:	AGAGGGAGAGGTGTTGGATGTTG
fzd10_U1	F:	AGTGCCACAATCCCACACTTTC
	R:	ACAGTGAGCAATAACGGCCAAG
fzd10_U2	F:	AACCAAACAGACCCAACGTG
	R:	AAAAAGAGCTCAGGGGTCCATC
xarp_U1	F:	CCCCCTCTGGTTAAGAAAAAGAG
	R:	GGGATGAAAGGAATAGCTGCTG
sia1 (-221)	F:	AAGATCAAGGGAACCAGGTG
	R:	TTGCACCCTACAAACATGGG

nodal3.1	F:	ATAGCTTCAATGTACCACAGTGCA
	R:	AGAGTCTGGCAGGTCCCTG
gsc_U1	F:	ACCATTTCTTACCCAGAGAAACG
	R:	TCCTTGCTCTCAATCCCAATCC
nog_D1	F:	TTGGCAATCTCTCCTCTGATGTCC
	R:	AGGGGCCATTCAAAAGGTGTC
chrd_U1	F:	TGGGACTAGCGCAGGATTTATAGG
	R:	ACTCATCAACTCCCAGAGTGAGTG
fst_D2	F:	ACAGGACCAGTGTAGGTAAACG
	R:	AAATTGGCCGACCCTTTCACAC
frzb_U1	F:	AAATCCACAGGAGGGACGTTTC
	R:	AGCCCAGAGATACAAGAGTGTCAC
hoxd1(nc)	F:	GTACCACATAGCAACCAATCAG
	R:	GGCTGCATGCATGGCAAATC

cDNA plasmid clone for *in situ* hybridisation

cDNA was amplified by PCR using primers described below, from first-strand cDNA synthesized from mRNA extracted from *X. tropicalis* gastrula embryos. The amplified DNA fragments were subclonced into pGEM-T vector (Promega). For making digoxigenin-labelled antisense RNA probes, the following restriction enzymes (RE) and RNA polymerases (RNA pol) were used.

Gene	Plasmid	Primer	RE	RNA pol
wnt8a	pYNX22	CCATCGATTGGCTGAGGATACTGTTCAAGCATTAC CCATCGATGTCTCCGGTGGTGGCCTCTGTTCTTC	ApaI	SP6
hoxd1	pYNX27	CACGTGACCGCCACTCTATATTAGG CTAGCTGTGAGTCTTTATACTTAAACGTCC	NcoI	SP6
sp5	pYNX36	AGGGGAGGCTACCTCACTAACTG AGTATGAAAACAAGGTATCCTCTCCAAG	NotI	T7
msgn1	pYNX35	GAAGCTCCTGGTTGGAACCATTTAG ATATACACAAACCATGGGGTATTTACAG	NotI	T7
cdx2	pYNX39	ACAGGATTATGCAGCTAGCTGGCAC ATTGCCGACCCGAACAATGTGCAC	SphI	SP6
msx1	pYNX44	TTCCCAGCTCGGATATCTCTGTATG CATACAATCCCTTCCAAAGGGATTATTG	NcoI	SP6
cdx4	pYNX56	GTAGCATCAAGGCACCGGCCTAAC GCAGTGCCCCAGACATAAGGATTTAC	NotI	T7
fzd10	pYNX43	CTGGAGCAAGGATGACAAGAAGTTTG ACCTTAGCATGCAGTCTCTGGTTTG	NotI	T7
xarp	HAR-199	(obtained from EXRC)	EcoRI	T7
xmcl1	pYNX34	ATGGCTTTAGTCAGTGGTAATAGCAC GGGCACGCCTTAATTTGGAAAGTTC	NcoI	SP6
ventx1.2	HAR-56	(obtained from EXRC)	EcoRI	T7

Luciferase reporter constructs and assay

Genomic fragments of β-peaks were amplified from *X. tropicalis* genomic DNA by PCR using primers described below and cloned into pGEM-T (Promega). Luciferase reporter constructs for β-peaks in the proximal regions were created by introducing a genomic fragment of the β-peak region into the pGL4.10 vector (Promega). For β-peaks in distant regions, corresponding genomic fragments were subclonced into pβ-actin-luc, which contains a chicken *β-actin* basal promoter in front of the luciferase reporter gene. pβ-actin-luc was generated by subcloning a chicken *β-actin* basal promoter as a SmaI-HindIII fragment from pBSSK2+ βEGFP (Ogino et al., 2008) into the EcoRV and HindIII sites of pGL4.10. Reporter plasmid DNA (40 pg per embryo) was injected together with pRL-CMV (40 pg per embryo, Promega) into the marginal zone of both dorsal and ventral blastomeres at the two- to four-cell stage. Embryos were collected at the early gastrula stage (stage 10.25) and assayed for luciferase activity. Primers used for cloning are as follows (restriction enzyme sites are underlined with their names on the right side):

msx1-U1-luc	GGAAGATCTAGCAGATTTATTTATATGGATAACAGG	BglII
	CCCAAGCTTACAGAGATATCCGAGCTGGGAA	HindIII
fzd10-U1-luc	CCGCTCGAGACACAAAATACACAACAGTGAGC	XhoI
	CCCAAGCTTGCCCGCAGCCCAACTCG	HindIII
ventx1.2-U1-luc	CGGGATCCATGGGATTCAGTGCCGGCCAATG	BamHI
	CCCAAGCTTCTGAAGGGAAACCTGCTCTGG	HindIII
sp5-U1-luc	GGAAGATCTTACAGTGTGTGGCCACCTTAG	BglII
	CCCAAGCTTAGTCCAGCTCCTACAGGTGC	HindIII
cdx4-U1-luc	GGAAGATCTGGTTGGGTAGTTGTTAGTGGATG	BglII
	CCCAAGCTTTCCTAGGCGAGATCCTTGGTG	HindIII
hoxd1_D1-luc	CTAGCTAGCGGCCAATTGAATGAAGGA	NheI
	CCGCTCGAGACAAAATGTCACTGATAGGA	XhoI
hoxd1_D2-luc	CTAGCTAGCGGCTAATCAGAGCTCACTTGAAC	NheI
	CCGCTCGAGTTACAGACACGTTAATGCAATTATC	XhoI
msx1-U2-luc	CTAGCTAGCGGTTGGAAAGCAGCAAAGCTTTG	NheI
	CCGCTCGAGAAAGTGGAGAGTGGTGCATGAAG	XhoI
cdx4p_D1-2-luc	CTAGCTAGCTATGCCTGCATTTTGTCATCAATG	NheI
	CCGCTCGAGTGCCACTCTTATTACCATACCTG	XhoI
cdx4p_D3-luc	CTAGCTAGCGAGGACAGTAATTATGCCTTATAC	NheI
	CCGCTCGAGTTAAACATGACTGAGCATTTGTATG	XhoI
cdx2p_U1-2-luc	CTAGCTAGCTGACTCCATTAGGGCATATTCTG	NheI
	CCGCTCGAGTTTGCTAAATACAAGTGCTATACAG	XhoI
hoxa1_D1-luc	CTAGCTAGCTGCGCCAACGTTTCGTTTTTATTC	NheI
	GCGTCGACATTTTTGTGATACAGTATGGAACTG	SalI

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