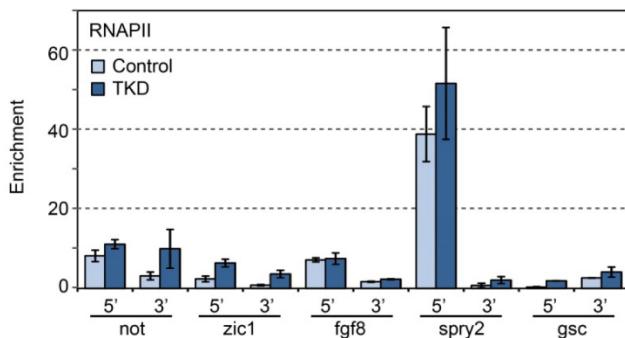
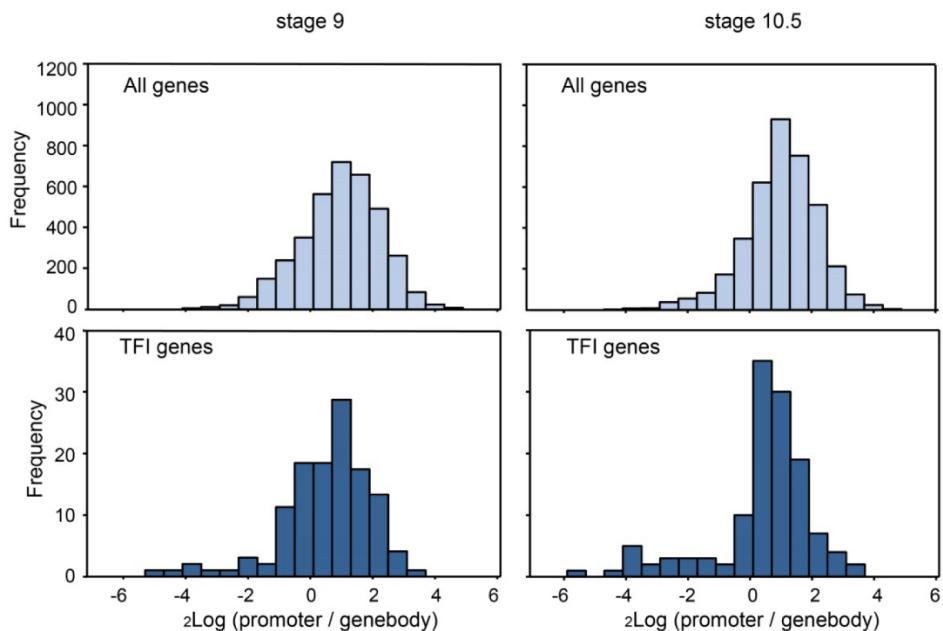


## Supplemental Figures

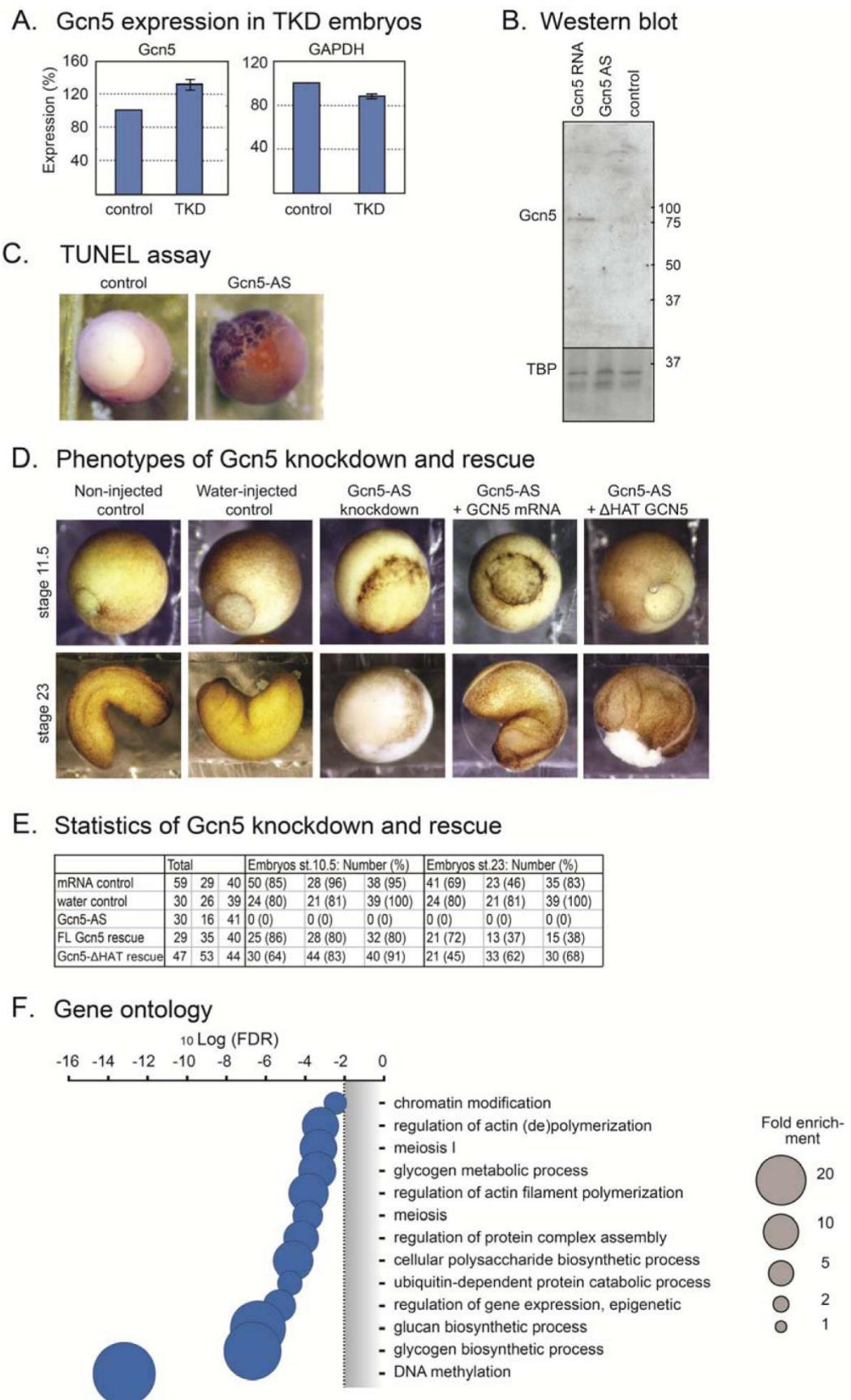
### A. RNAPII recruitment in TKD embryos



### B. RNAPII pausing index



**Supplemental Figure S1.** **(A)** Recruitment of RNA polymerase II (RNAPII) to TFI genes determined by ChIP-qPCR using an antibody recognizing the (unphosphorylated) carboxy-terminal domain (CTD). 5' and 3' ends of TBP family-insensitive (TFI) genes were analyzed. Signals obtained from control (light blue) and TKD (dark blue) embryos indicate RNAPII enrichment relative to a negative control region. **(B)** RNAPII pausing index of TFI orthologs in *X.tropicalis* compared to other genes using RNAPII ChIP-seq data (van Heeringen et al., 2014).



**Supplemental Figure S2.**

**(A)** Transcript levels of *gcn5* and *gapdh* transcript levels in TBP, TLF, TBP2 triple knockdown (TKD) embryos and control embryos, as determined by RT-qPCR.

**(B)** Reactivity of C26A10 antibody with *Xenopus* Gcn5 in embryos injected with 1 ng of *gcn5* mRNA, Gcn5-AS oligonucleotide and in control embryos (weak signal). No cross-reactive bands are observed. TBP is shown as control. For validation of TBP, TBP2 and RNAPII antibodies see

(Akhtar and Veenstra, 2009; Jallow et al., 2004; Veenstra et al., 1999).

**(C)** TUNEL assay for the detection of apoptosis in control and Gcn5-AS-injected embryos.

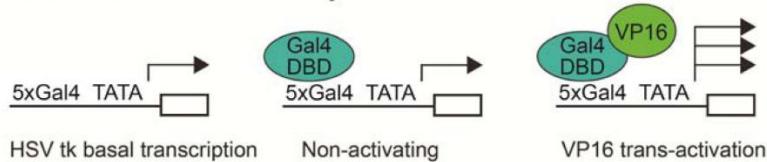
**(D)** Morphology of non-injected and water-injected controls, Gcn5-AS-injected and rescue (Gcn5-AS rescued with co-injected full length human *GCN5* mRNA or *GCN5-ΔHAT* mRNA encoding a truncated GCN5 protein lacking the histone acetyltransferase domain) embryos are shown. Photos were taken at stages 11.5 and 23 (controls).

**(E)** Summary of survival

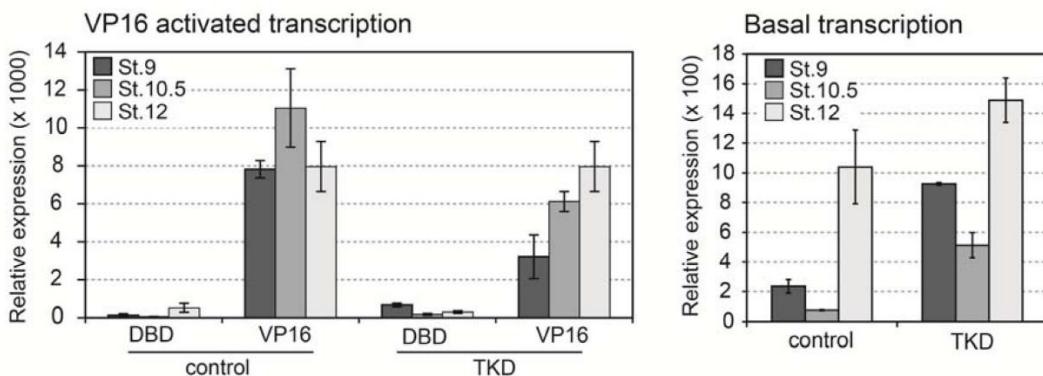
(normal development) statistics after embryo collection at stage 10.5 and 23. Gcn5-AS embryos were rescued by full length human GCN5 mRNA at a rate of 80-86% at stage 10.5 and 38-72% at stage 23. Embryos rescued with GCN5-ΔHAT exhibited 64-91% rescue efficiency at stage 10.5 and 21-30% at stage 23.

**(F)** Gene ontology analysis of decreased transcripts in Gcn5-AS embryos.

### A. VP16 activation assay



### B. Basal and activated transcription in TKD embryos

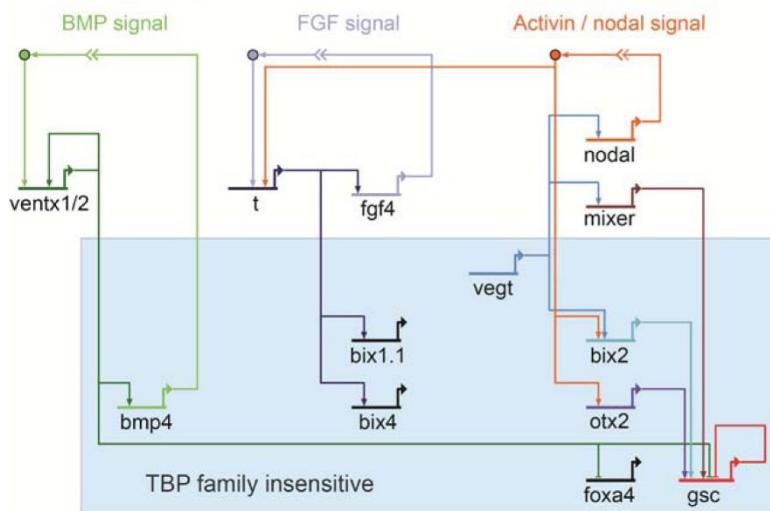


**Supplemental Figure S3.** VP16 transcription activation assay combined with TBP family loss-of-function experiments. See Fig. 5A for ChIP analysis in the assay system.

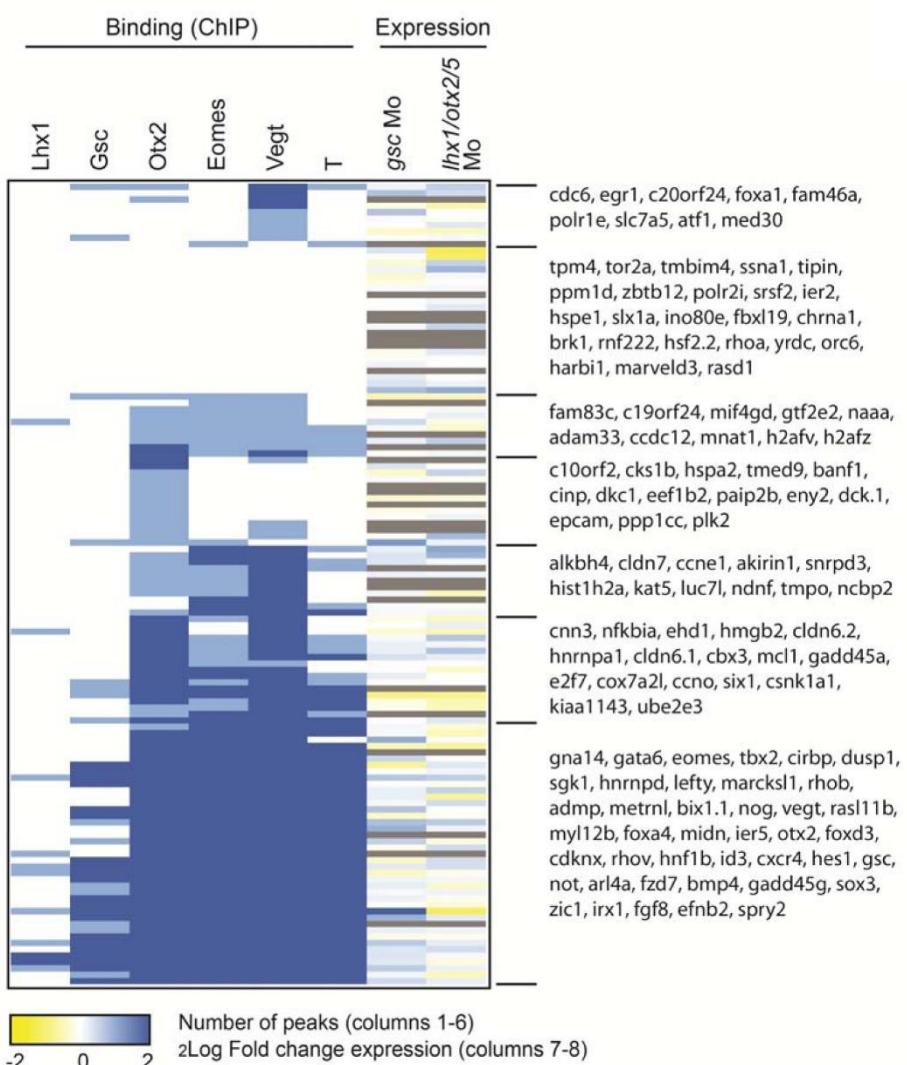
**(A)** Schematic overview of VP16 assay.

**(B)** Transcription in non-activating and VP16-activated conditions (left panel) and basal levels of transcription (right panel) are compared in TBP family triple knockdown (TKD) and control embryos. Embryos were staged according to the morphology of control embryos. Stages of 9, 10.5 and 12 were collected for analysis.

### A. Mesoderm specification network



### B. Recruitment of T-box and Homeobox factors to TFI genes



**Supplemental Figure S4.**

**(A)** The subset of the mesoderm specification network (Koide et al., 2005) as it relates to TFI genes (blue box) and BMP, FGF and Activin/nodal signaling.

**(B)** Hierarchical clustering of TFI genomic interaction network as determined by assigning ChIP-seq peaks of Lhx1, Gsc, Otx2, Eomes, Vegt and T (Xbra) to genic regions (Methods). In addition, expression changes in *gsc* and *lhx1/otx2/otx5* morphant (Mo) embryos are shown (Yasuoka et al., 2014). Color intensity reflects number of peaks in the locus (ChIP, 0, 1 or >=2 peaks) or Log<sub>2</sub> fold expression change (RNA-seq, yellow decreased, blue increased, grey not determined). The genes shown to the right represent all named *X. tropicalis* TFI genes.

Supplemental Data

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