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SUPPLEMENTARY MATERIAL

corresponding to:

Zygotic *VegT* is required for *Xenopus* paraxial mesoderm formation and regulated by Nodal signaling and Eomesodermin

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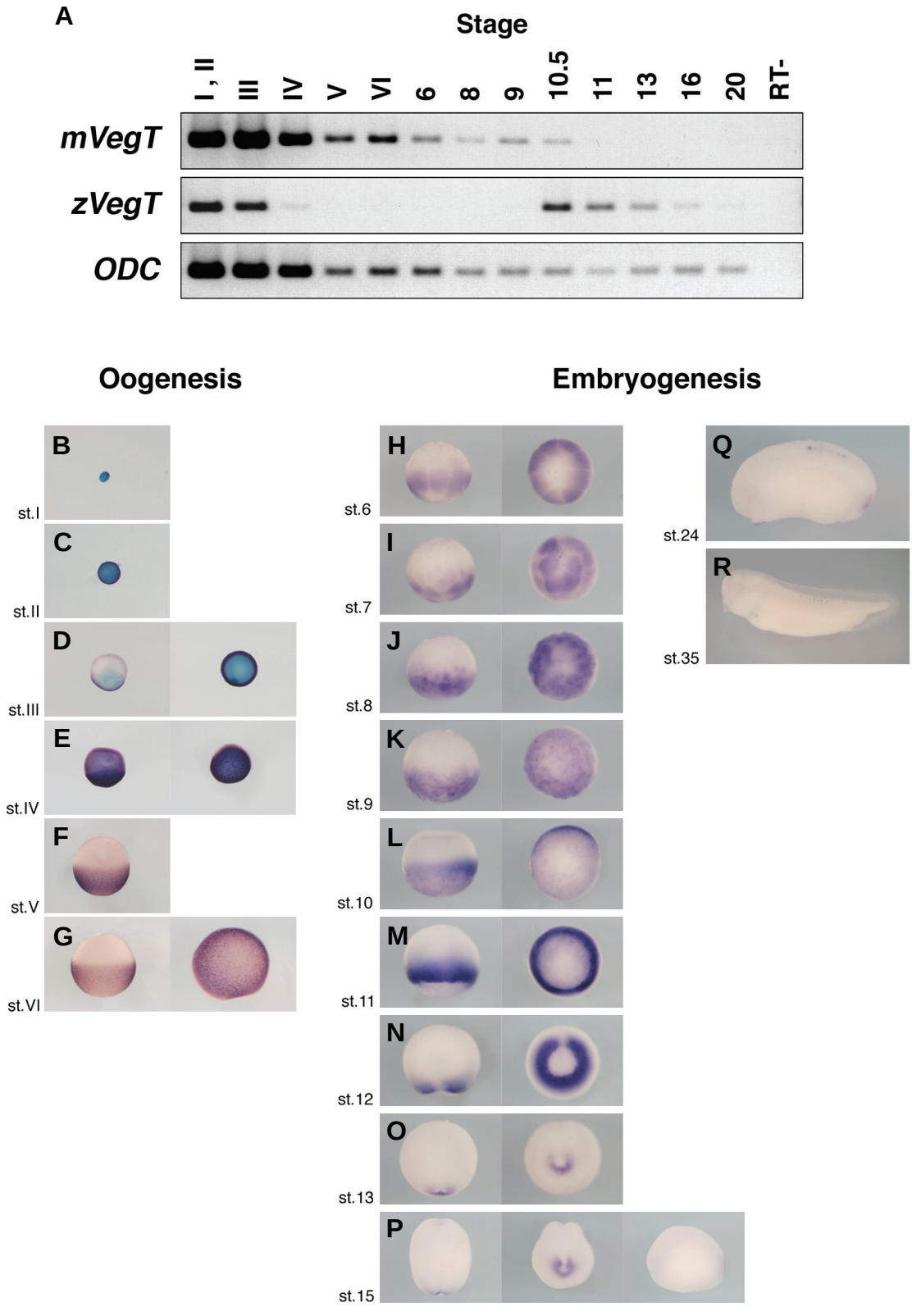
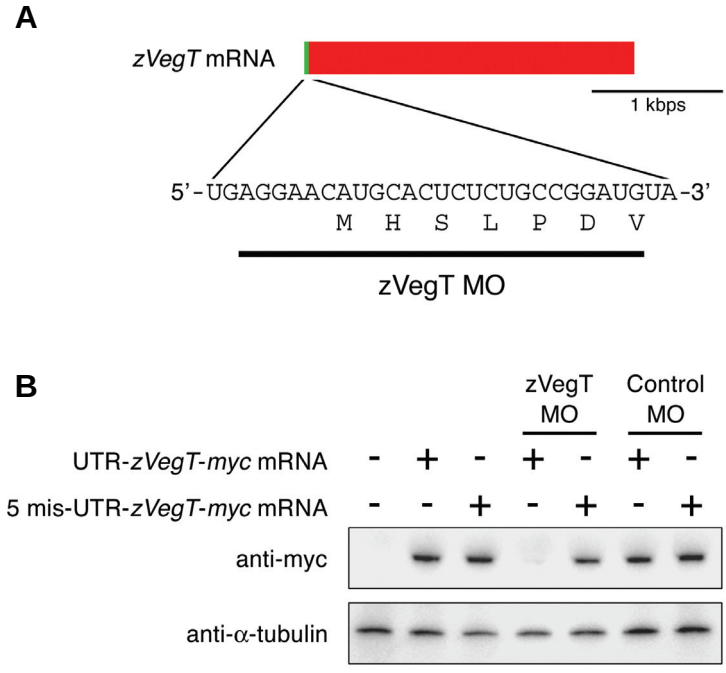
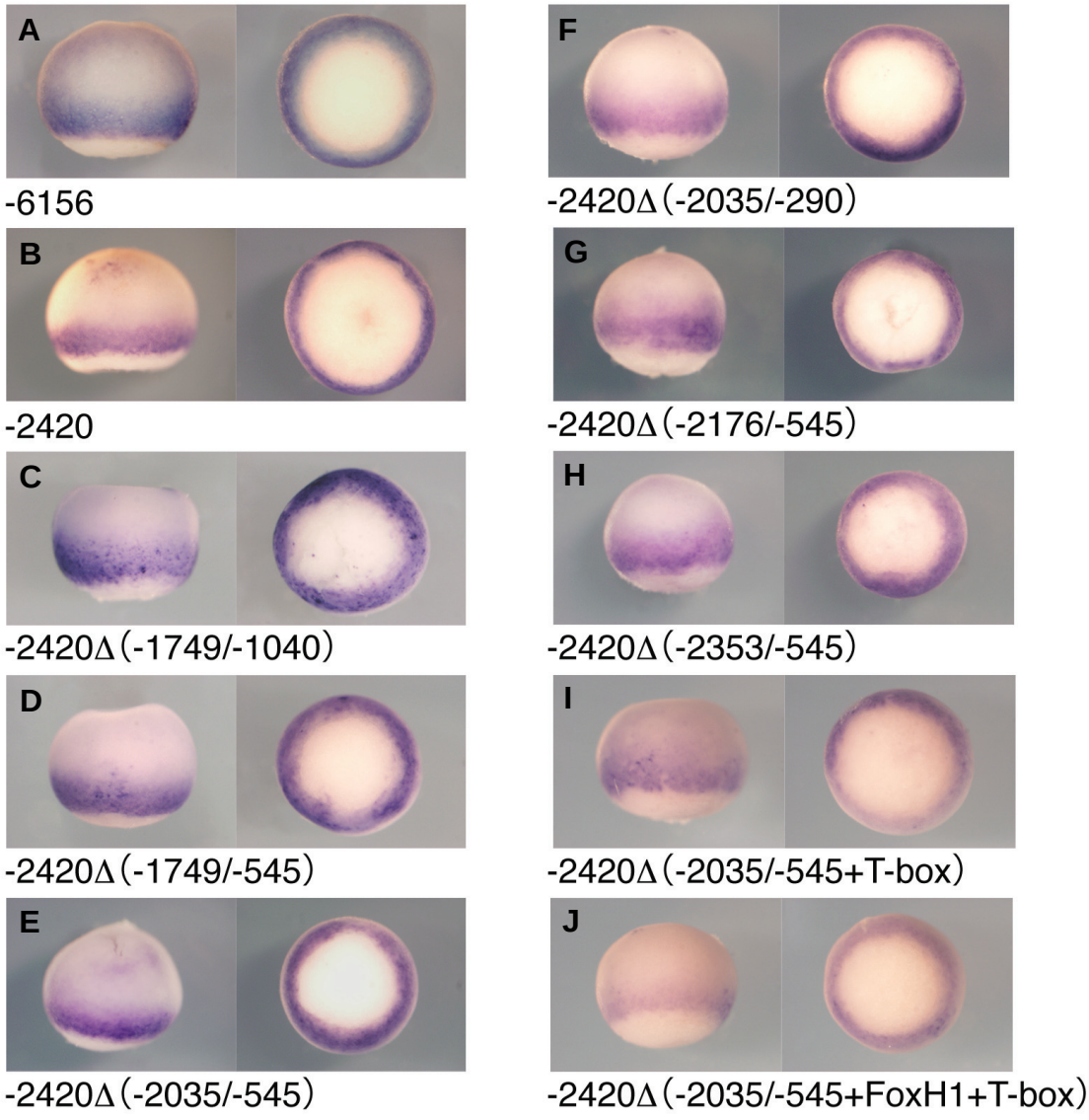


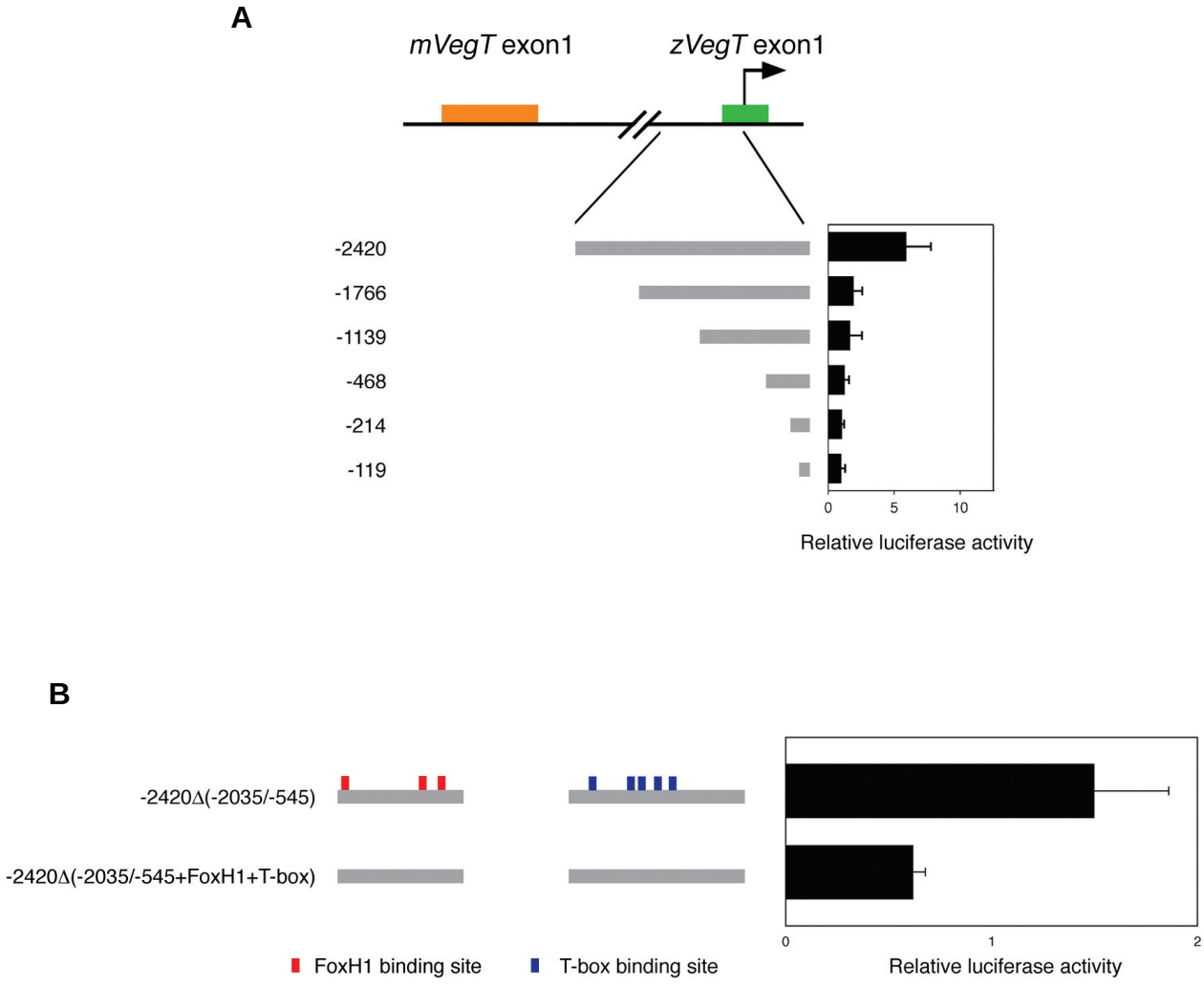
Supplementary Fig. S1. Temporal and spatial gene expression patterns of *VegT* during oogenesis and embryogenesis of *X. tropicalis*. (A) *Temporal expression was analyzed by semi-quantitative RT-PCR. Primer pairs specific for* X. tropicalis ODC *were described previously (Haramoto* et al.*, 2004). The primers designed for* X. tropicalis mVegT *are as follows: 5′-ATGAGAAACTGCTGTCAGGAACAC-3′ and 5′-TGAAACCTGGGCTTGTAGCG-3′.* **(B-R)** *Spatial expression was analyzed by WISH. Roman figure shows stage of oogenesis.*



Supplementary Fig. S2. zVegT MO specifically inhibits the translation of *zVegT*. (A) *zVegT MO was designed to bind the translational start region of* X. tropicalis zVegT. **(B)** *1 ng of UTR-*zVegT-myc *mRNA or 5mis-UTR-*zVegT-myc *mRNA was coinjected with 40 ng of zVegT MO or Control MO into both blastomeres at the two-cell stage in* X. laevis *embryos. The injected embryos were cultured until stage 9 for Western blot analysis. To generate pCS2-UTR-zVegT-myc and pCS2-5mis-UTR-zVegT-myc, the UTR and coding regions of* X. tropicalis zVegT *were amplified by PCR using different primer pairs and subcloned into the pCS2-MT vector, respectively. The construct pCS2-5mis-UTR-zVegT-myc contains five silent mutations in the MO-binding region. These mutations were generated by PCR using the following primers: forward, 5'-GAAgATGCAtTCaCTtCCGGAcG-3' and reverse, 5'-CCAACAGCTGTATGGAAAGAGAG-3' (mutated bases are shown in lower case letters). Cloning products were verified by sequence analysis. For Western blot analysis, the zVegT protein with 6myc-epitope tags was detected using the anti-c-myc (9E10) HRP-conjugated monoclonal antibody (Santa Cruz Biotechnology). The internal control, α-tubulin, was detected using the monoclonal anti-α-tubulin antibody (mouse IgG1 isotype) (Sigma). Anti-mouse IgG, HRP-linked antibody (Cell Signaling Technology, Inc.) was used as the secondary antibody. zVegT MO inhibited translation from UTR-*zVegT-myc *mRNA, but not from 5mis-UTR-* zVegT-myc *mRNA.*



Supplementary Fig. S3. *X. laevis* transgenic embryos expressed the *EGFP* reporter gene in the marginal region. *Reporter* EGFP *expression was detected by WISH (Figs. 3A and 5A,B). Panels at the left and right indicate lateral and vegetal views with dorsal side up, respectively. All embryos show equivalent expression patterns for endogenous* zVegT.



Supplementary Fig. S4. Transcriptional activity of several DNA reporter constructs in *X. laevis* embryos. *To generated firefly luciferase reporter constructs, -2420, -1766, -1139, -468, -214, -119, -2420Δ(-2035/-545), and -2420Δ(-2035/-545+FoxH1+T-box), the 5' upstream regions were amplified by PCR and inserted into the pGL3-basic vector (Promega). Schematic diagrams representing the* cis*-regulatory region of each construct are shown on the left of the graphs. The FoxH1 and T-box binding sites are indicated as red and blue boxes, respectively. Reporter activity was measured by the Dual-Luciferase Reporter Assay System (Promega). Each experiment was repeated at least three times in duplicate. Specific pGL3-zVegT promoter constructs (50 pg) were injected along with the* Renilla *luciferase construct pRL-TK (50 pg) into the marginal zone of both blastomeres at the two-cell stage, and embryos were harvested at stage 11.*