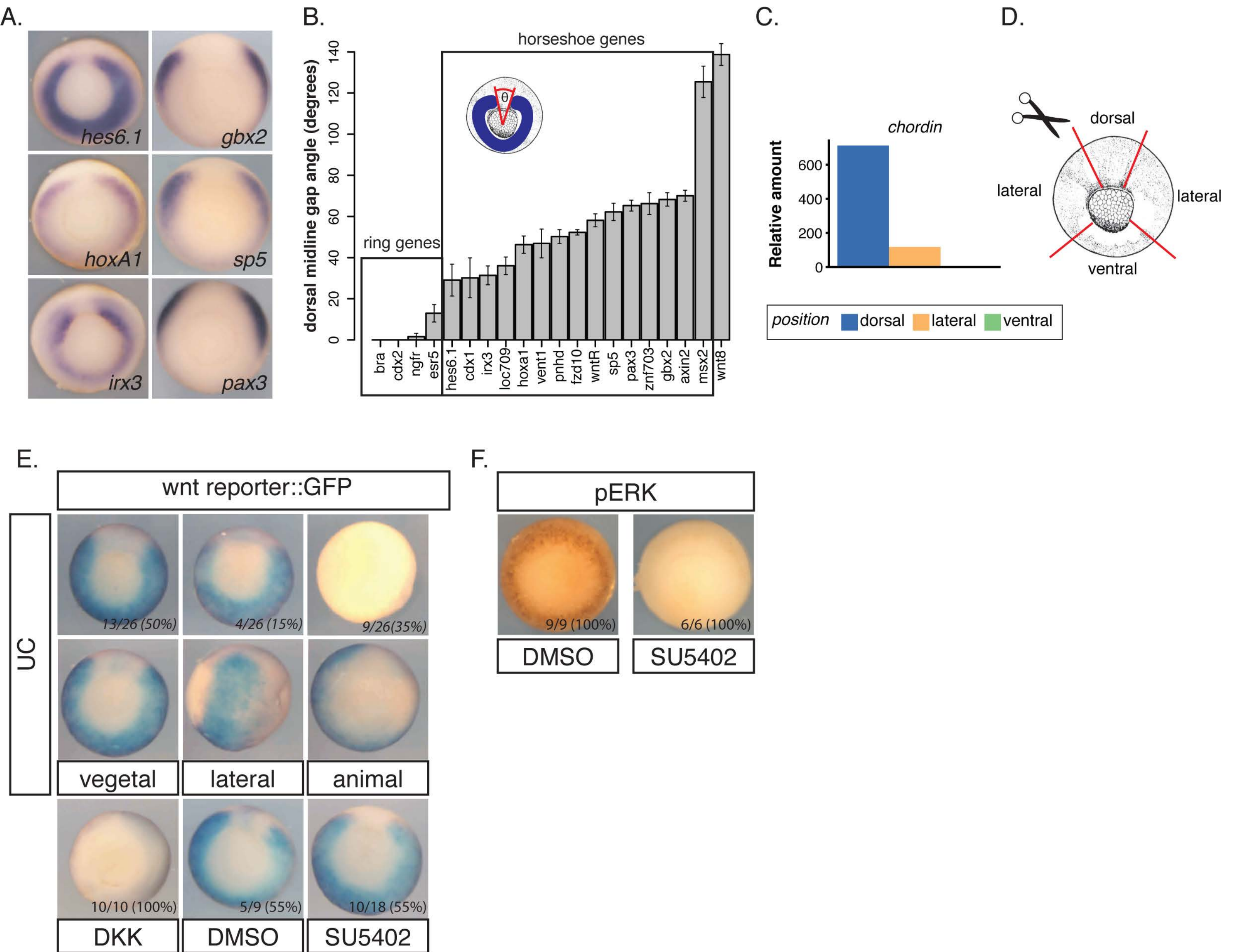
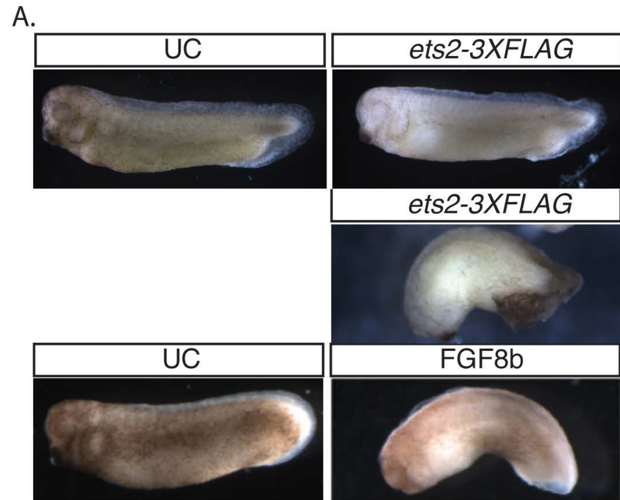
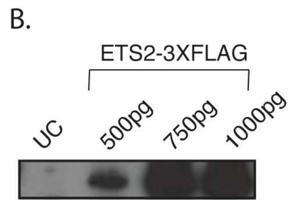


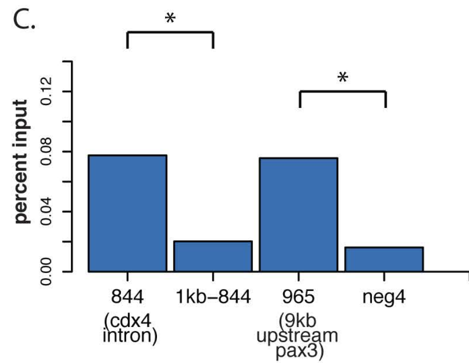
Figure S1

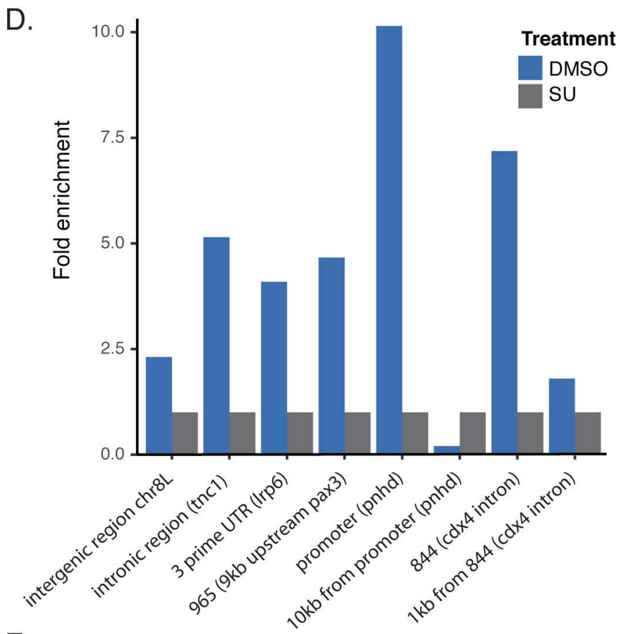
A. In situ hybridization of Wnt target genes at mid gastrula (stage 11.5). B. Angle of dorsal midline gap was mea-sured from the center of the blastopore. Error bars are standard error of the mean. C. qPCR data from 8 embryos dissected into dorsal, lateral, and ventral marginal zone pieces. Ef1a was used as an internal control and values were normalized to the ventral pieces. D. Schematic of dissections made at stage 11.5. E. RNA in situ hybridization for GFP reporter of Wnt activity. Activity is abolished with 100pg dkk injection. Activty of reporter does not appear to be affected by treatment with SU5402. F. pERK staining for activity of Fgf pathway. Activity is abolished as a result of incubation in 75uM SU5402.

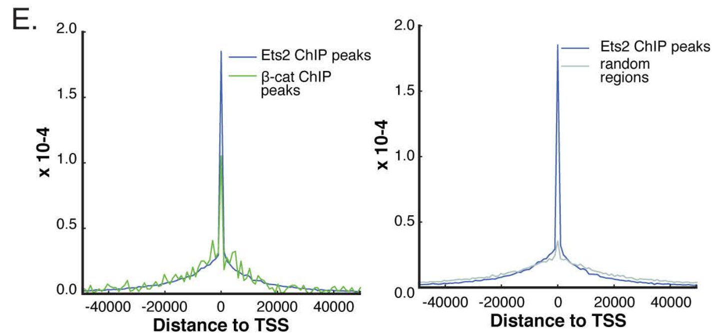
Development • Supplementary information

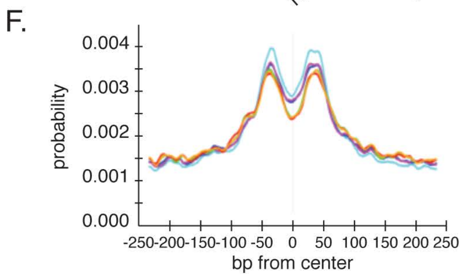

A.
UC
ets2-3XFLAG
ets2-3XFLAG
UC
FGF8b

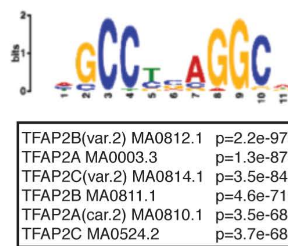

B.
ETS2-3XFLAG
UC
500pg
750pg
1000pg

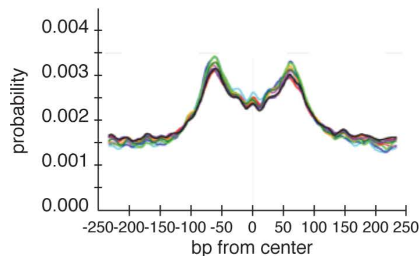

C.
percent input
844 (cdx4 intron)
1kb–844
965 (9kb upstream pax3)
neg4

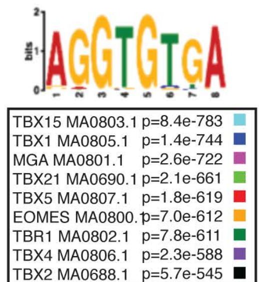

D.
Fold enrichment
Treatment
DMSO
SU
intergenic region chr8L
intronic region (tnc1)
3 prime UTR (lrp6)
965 (9kb upstream pax3)
promoter (pnhd)
10kb from promoter (pnhd)
844 (cdx4 intron)
1kb from 844 (cdx4 intron)


E.
Ets2 ChIP peaks
β-cat ChIP peaks
random regions
x 10-4
Distance to TSS


F.
probability
bp from center


TFAP2B(var.2) MA0812.1 p=2.2e-970
TFAP2A MA0003.3 p=1.3e-873
TFAP2C(var.2) MA0814.1 p=3.5e-847
TFAP2B MA0811.1 p=4.6e-710
TFAP2A(car.2) MA0810.1 p=3.5e-689
TFAP2C MA0524.2 p=3.7e-680


probability
bp from center


TBX15 MA0803.1 p=8.4e-783
TBX1 MA0805.1 p=1.4e-744
MGA MA0801.1 p=2.6e-722
TBX21 MA0690.1 p=2.1e-661
TBX5 MA0807.1 p=1.8e-619
EOMES MA0800.1 p=7.0e-612
TBR1 MA0802.1 p=7.8e-611
TBX4 MA0806.1 p=2.3e-588
TBX2 MA0688.1 p=5.7e-545

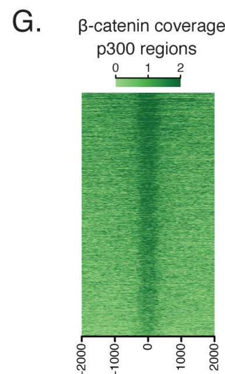
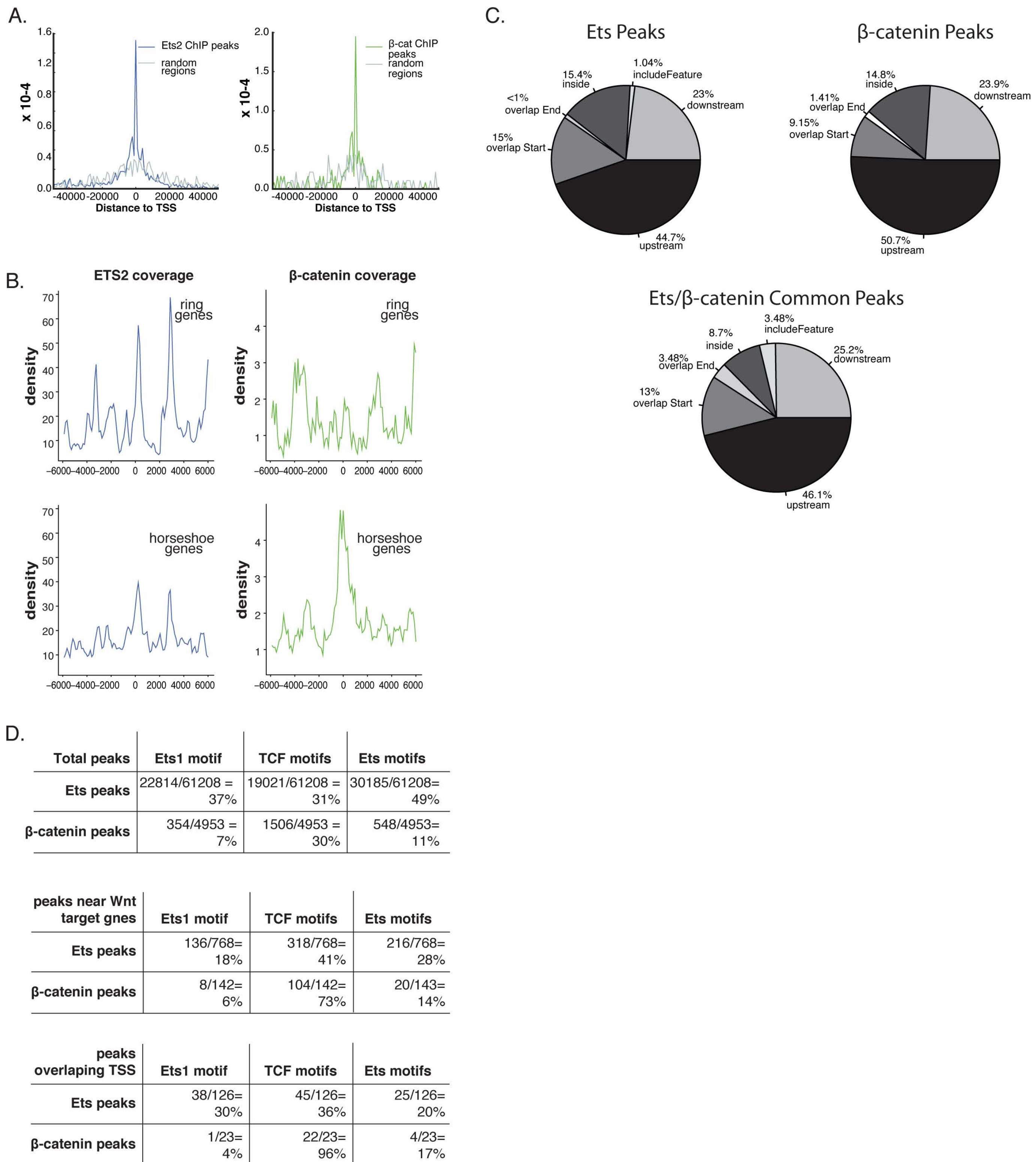

G.
β-catenin coverage
p300 regions

Figure S2

A. Phenotype of *ets2-3XFLAG* and *fgf8b* injected embryos. (Top *ets2-3XFLAG* injected with 500pg RNA and bottom *ets2-3XFLAG* injected with 1ng RNA). B. Western blot using FLAG antibody of lysates collected from embryos injected with different amounts of *ets2-3XFLAG*. C. ChIPqPCR results from *ets2-3XFLAG* injected embryos as a percent of input. Putative Ets binding regions were compared to regions 1kb away. *P<0.05 paired t-test. D. ChIPqPCR results using endogenous Ets2 antibody. Fold change of signal from embryos treated with SU5402 compared to DMSO is plotted. These are the results from a single experiment. E. Histogram of distance to transcriptional start site (TSS) of both Ets2 and -caten ChIP peaks compared to random regions. F. Using MEME-ChIP, TFAP2 family and TBX family motifs are enriched in Ets2 ChIPseq peaks. Graphs were generated by CentriMo showing the probability of finding a given motif in the Ets2 peak region. G. Heatmap of β-catenin ChIP sequencing coverage on p300 peaks.



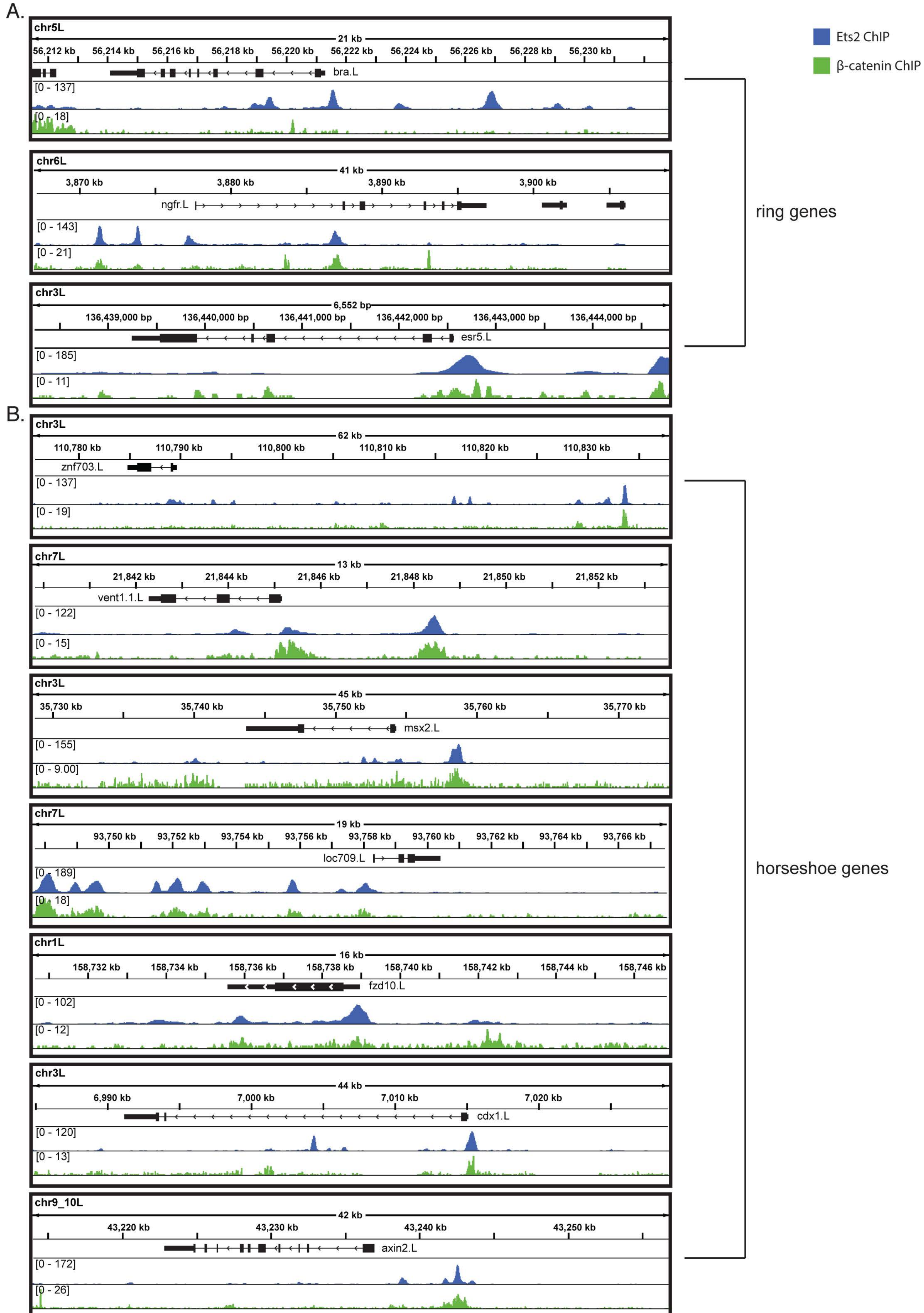
Total peaks	Ets1 motif	TCF motifs	Ets motifs
Ets peaks	22814/61208 = 37%	19021/61208 = 31%	30185/61208= 49%
β-catenin peaks	354/4953 = 7%	1506/4953 = 30%	548/4953= 11%

peaks near Wnt target gnes	Ets1 motif	TCF motifs	Ets motifs
Ets peaks	136/768= 18%	318/768= 41%	216/768= 28%
β-catenin peaks	8/142= 6%	104/142= 73%	20/143= 14%

peaks overlaping TSS	Ets1 motif	TCF motifs	Ets motifs
Ets peaks	38/126= 30%	45/126= 36%	25/126= 20%
β-catenin peaks	1/23= 4%	22/23= 96%	4/23= 17%

Figure S3

A. Histogram of distance to transcriptional start site (TSS) of both Ets2 and -caten ChIP peaks using subset of peaks near Wnt target genes compared to random regions. B. Wnt target genes were first separated based on having either a ring or horseshoe expression pattern. Then the coverage at +/- 6 kb from the TSS of each gene was calculated and plotted for both Ets2 and -catenin ChIP. C. Pie charts showing percentage of peaks (near Wnt target genes) overlapping with different genomic features. D. Percentage of peaks containing different motifs.



Development • Supplementary information

Figure S4
A/B. Genome-browser view of Ets and β-catenin coverage around ring genes (A) and horseshoe genes (B).

Development • Supplementary information

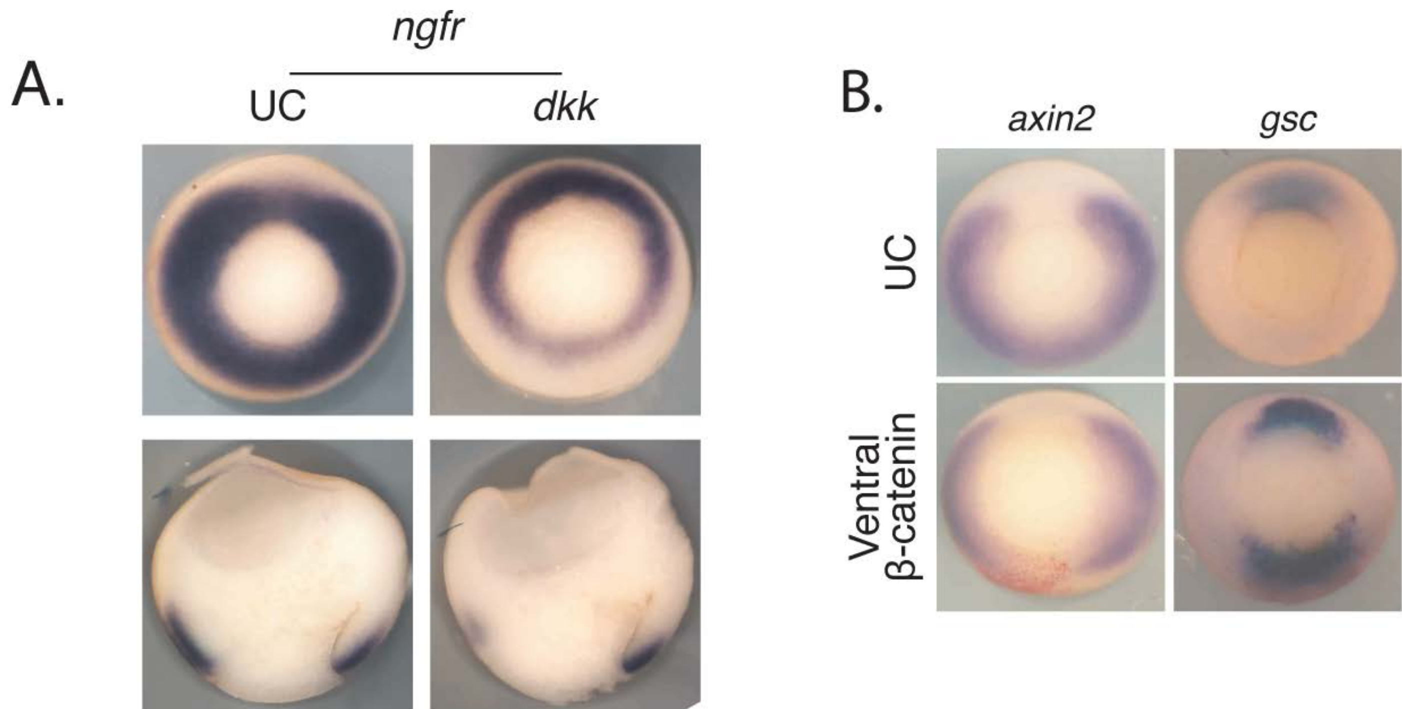


Figure S5
A. In situ hybridization on gastrula stage embryos injected with 100 pg dKK in all 4 blastomeres of a 4-cell staged embryo. B. In situ hybridization on gastrula stage embryos injected in both ventral blastomeres at the 4-cell stage with β-catenin and β-gal tracer.

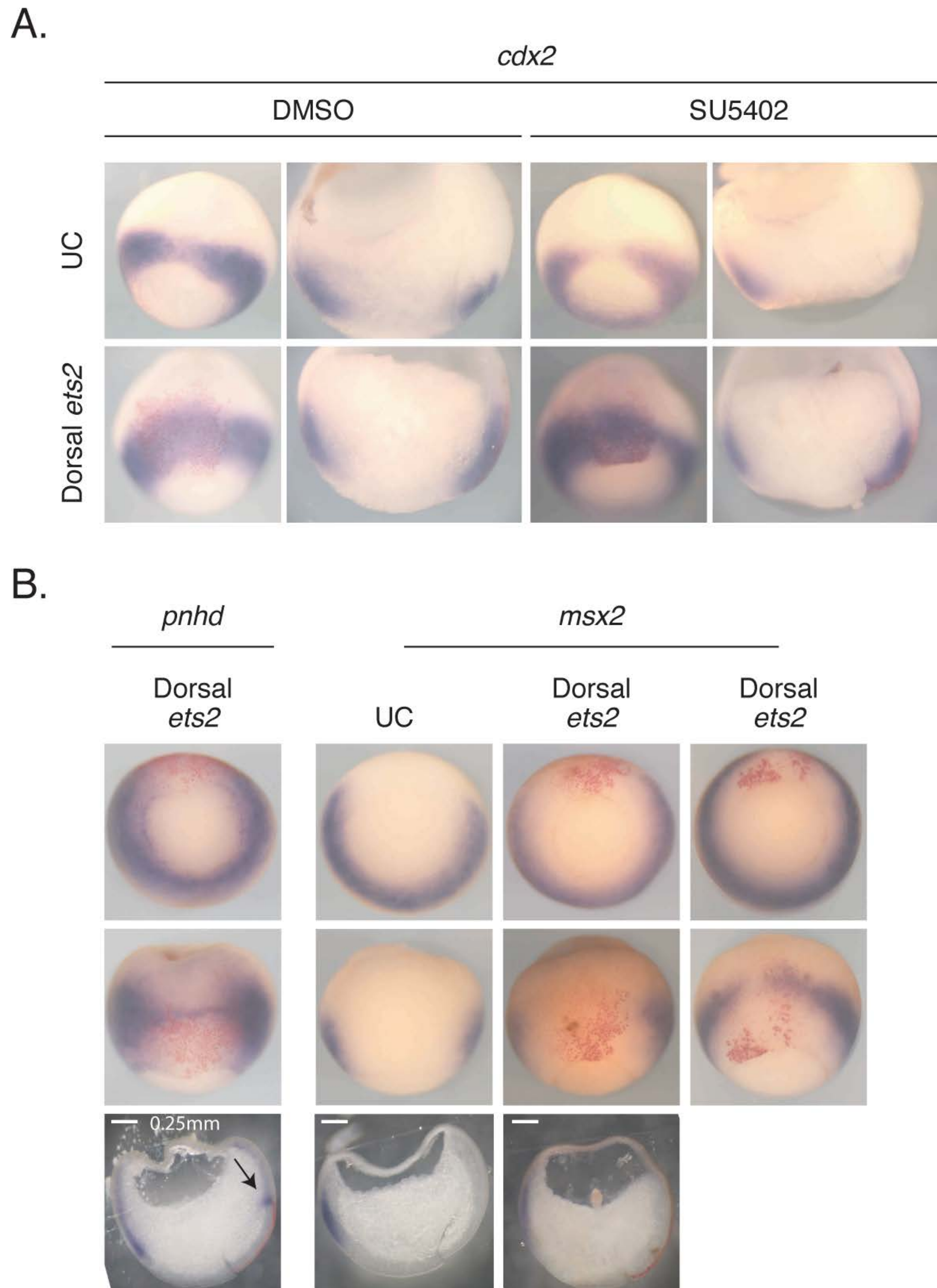


Figure S6
A. In situ hybridization on gastrula stage embryos injected with ets2 mRNA and -gal tracer in the two dorsal blastomeres at the 4-cell stage. Embryos were further cultured in either DMSO or SU5402. Sections are of whole embryos pictured and made by bisecting embryo. B. In situ hybridization on gastrula stage embryos injected with ets2 mRNA and -gal tracer in the two dorsal blastomeres at the 4-cell stage. Sections are of whole embryos pictured and made with the vibratome.

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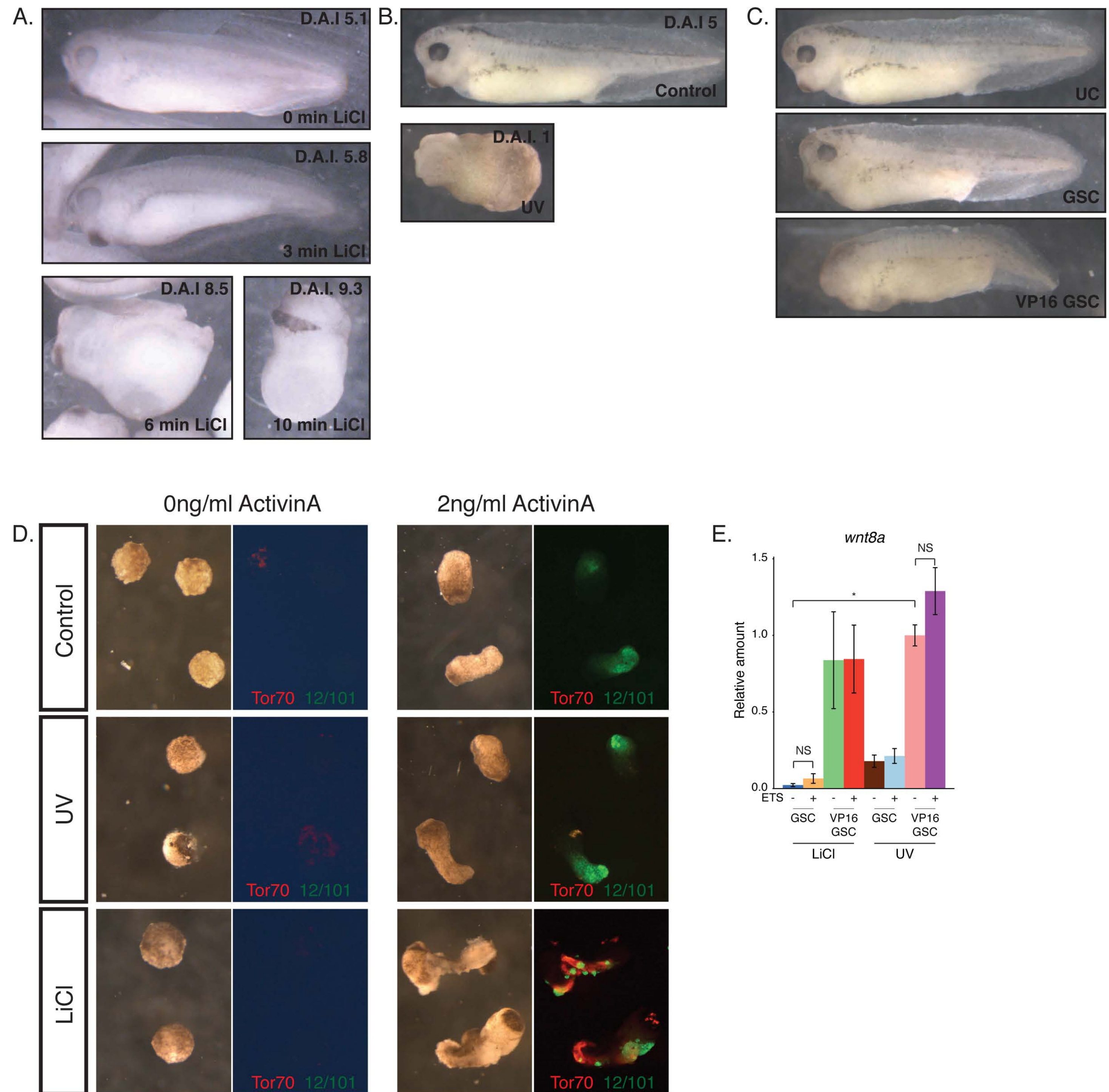

Figure S7
A. Phenotype of embryos dorsalized with 0.3M LiCl at the 8-16 cell stage for indicated time. B. Phenotype of embryo ventralized with UV treatment with Auto Crosslink setting using the UV Stratalinker 1800 (see materials and methods). C. Phenotype of embryos injected with either gsc or vp16gsc. D. Brightfiled and fluorescent images of animal caps immunostained with Tor70 and 12/101. E. qPCR from triplicate experiments showing expression of wnt8 from dorsal and ventral mesodermal caps with or without the addition of ets2 mRNA. * P<0.05, students t-test.

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