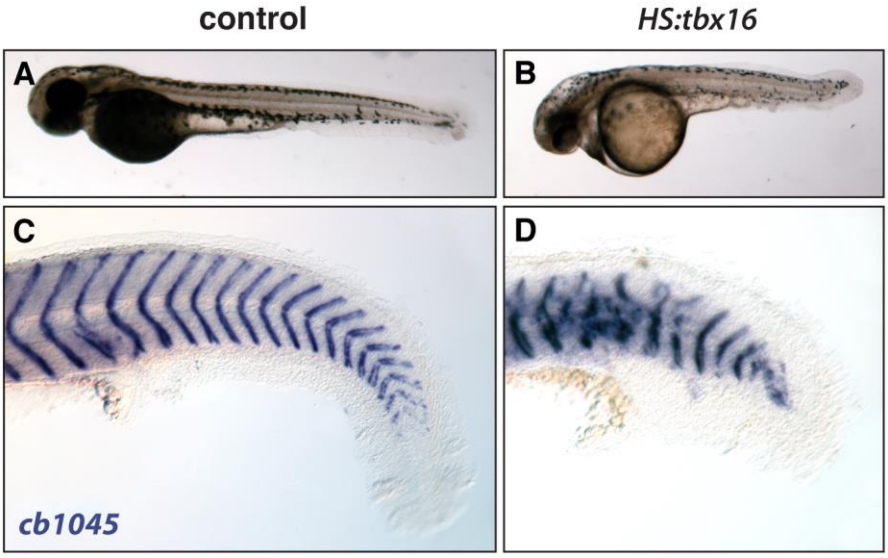

Figure S1. Ubiquitous *tbx16* expression causes organism-wide defects.

At 36 hpf, bright field images show that control siblings (A) are longer than embryos with the *HS:tbx16* transgene (B) after heat shock. In situ hybridization with *cb1045*, a probe that normally marks the somite boundaries in control siblings (C), shows severe disruptions in somite formation in *HS:tbx16* embryo (D). The left-most somite shown in C is the 14th somite and the left-most somite shown in D is the 13th somite. All embryos were heat shocked at the 12 ss.

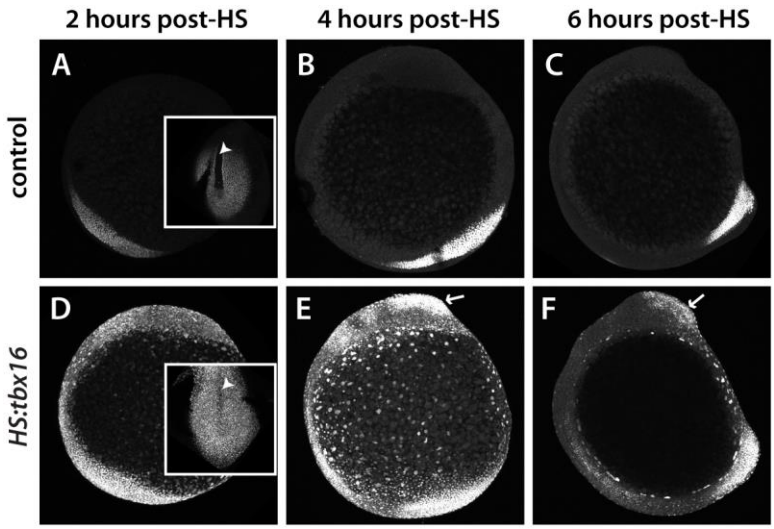

Figure S2. Ectopic Tbx16 is ubiquitous, labile and peaks at 4 hours post-heat shock.

In control (A-C) and *HS:tbx16* (D-F) embryos, immunofluorescence shows that Tbx16 protein levels have increased by 2 hours post-heat shock (A, D) with a peak at 4 hours post-heat shock (B, E), and that levels of Tbx16 have largely returned to normal by 6 hours post-heat shock (C, F). Inset in D shows ectopic Tbx16 in the notochord and neural tube (marked by arrowheads) when compared to the control inset in A (both dorsal views of the tailbud); arrows in E, F highlight the telencephalon, which retains ectopic Tbx16.

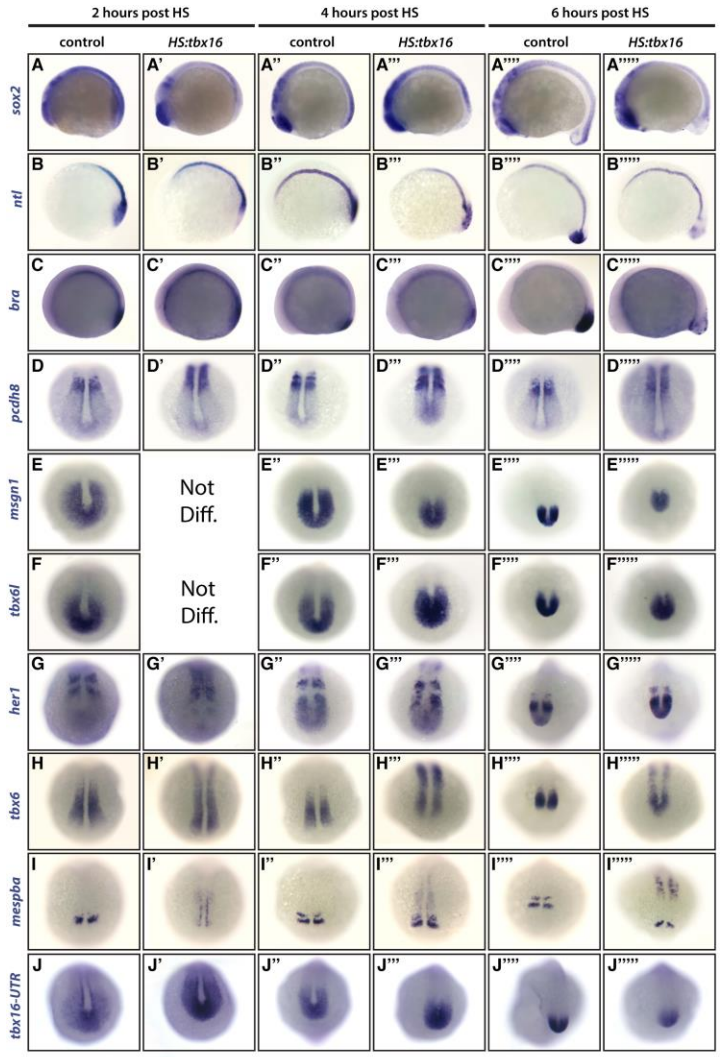

Figure S3. Gene expression after heat shock activation of *HS:tbx16*.

Whole mount in situ hybridization data used for the summary of changes to genes after the expression of ectopic *tbx16* (shown in Figure 2A). Because of the large number of embryos screened for this analysis, transgenic embryos were not separated from their nontransgenic siblings prior to in situ hybridization. A minimum of 30 embryos was used for each condition. In all cases except where noted as Not Diff. (not different), approximately 50% of the embryos matched previously published wild-type expression patterns and 50% showed the altered pattern. See Table S1 for quantification of the results.

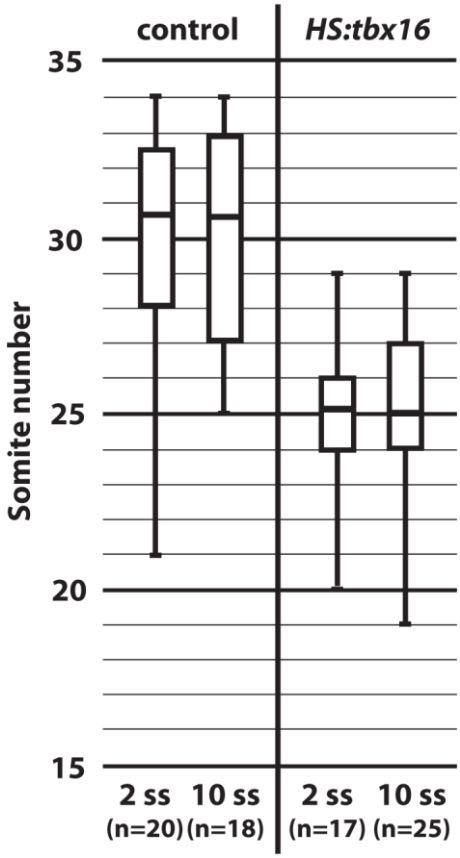

Figure S4. The timing of heat shock does not determine the timing of the cell exit driven by *HS:tbx16*.

Quantification of the most posterior somite in a 36 hpf embryo occupied by a fluorescently labeled cell with fiber-like morphology when embryos were heat shocked at the 2 ss and the 10 ss. Somitic distribution of *HS:tbx16* expressing cells were significantly different from controls at each time point (Mann Whitney U, $P < 0.05$).

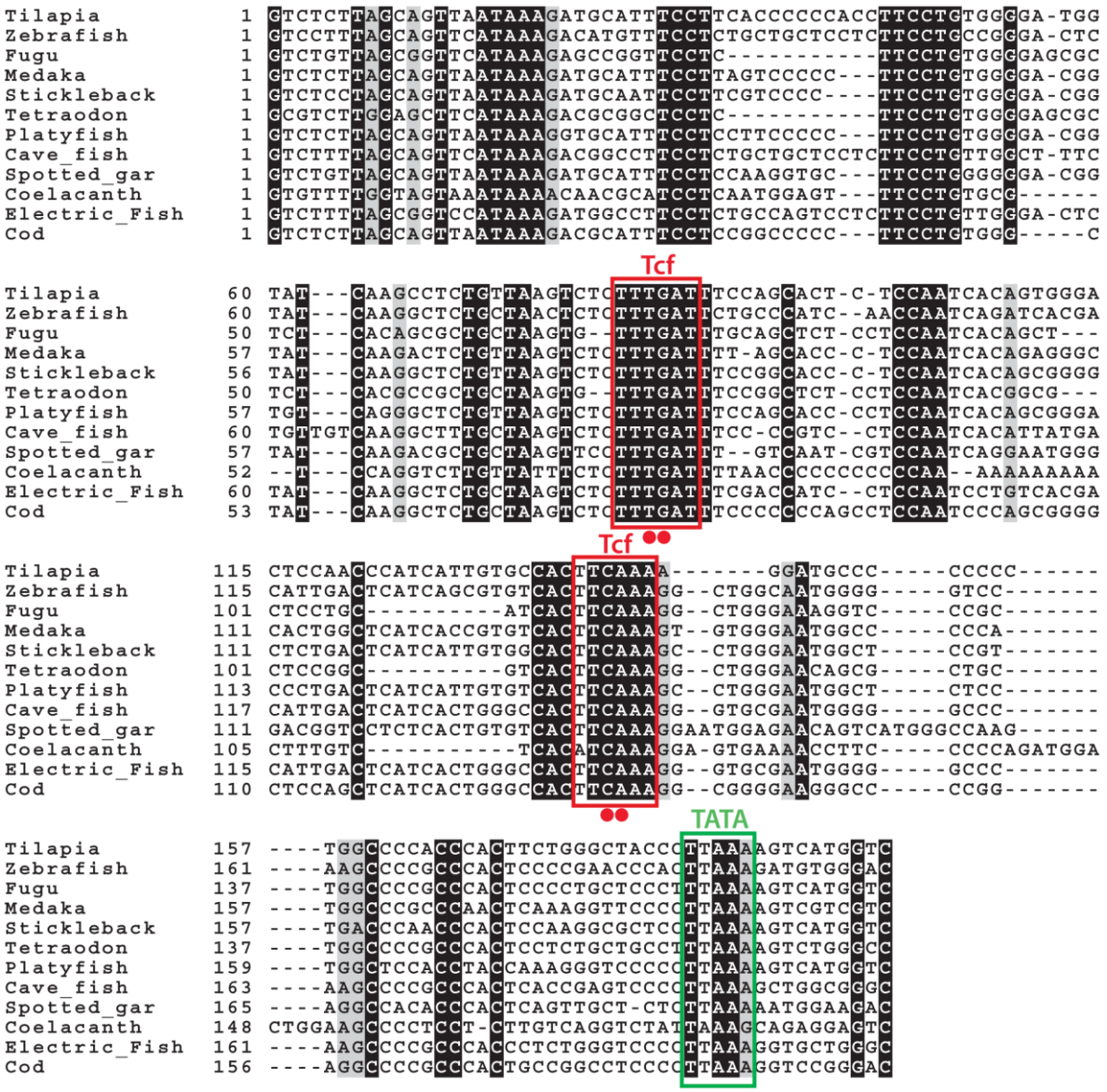

Figure S5. A conserved region in the *tbx16* promoter contains Tcf sites.

A sequence alignment of the *tbx16* promoter from multiple fish species shows a conserved region of approximately 150 bp. Black highlighted bases are completely conserved, and grey highlighted bases are mostly conserved. Two Tcf consensus elements are boxed in red, and a predicted TATA sequence is boxed in green. Below the Tcf sites are dots indicating the bases mutated in zebrafish *tbx16* to make *tbx16-1.2ΔTCF*.

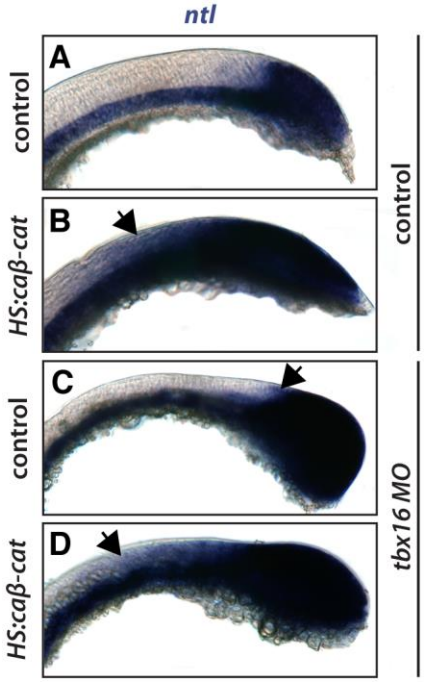

Figure S6. Wnt signaling regulates *ntl* independently of *tbx16*.

Expression of *ntl* is expanded in *HS:ca*β*-catenin* when compared to control embryos (A, B; 17 control and 15 transgenic); with *tbx16 MO, ntl* is similarly expanded in *HS:ca*β*-catenin* when compared to control embryos (C, D; 17 control and 26 transgenic).

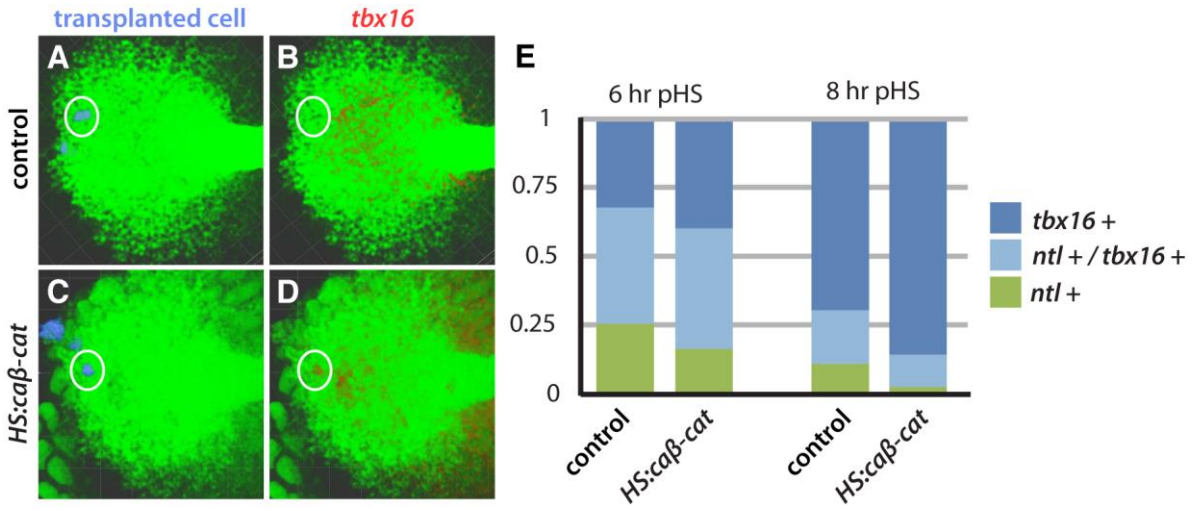

Figure S7. Transplanted *HS:caβ-catenin* cells activate *tbx16* cell-autonomously.

(A, C) Transplanted cells in the progenitor population are identified in blue. Cell-autonomous expression of *tbx16* is not apparent in control cells (B) but is apparent in cells with *HS:ca*β-*catenin* (D). (E) Quantification of all transplanted cells within the tailbud from the same experiment as shown in A-D. At both 6 and 8 hrs post-heat shock, cells expressing *caβ-catenin* were less likely to express *ntl* and more likely to express *tbx16* than control transplanted cells. At 6 hours post-heat shock, 151 cells were analyzed from 16 embryos (control) and 190 cells were scored from 19 embryos (*HS:ca*β-*catenin*); the distribution of control cells was different from *HS:ca*β-*catenin* cells (chi-square; P=0.052). At 8 hours post-heat shock, 131 cells were analyzed from 15 embryos (control) and 99 cells were scored from 14 embryos (*HS:ca*β-*catenin*); the distribution of control cells was significantly different from *HS:ca*β-*catenin* cells (chi-square; P<0.01).

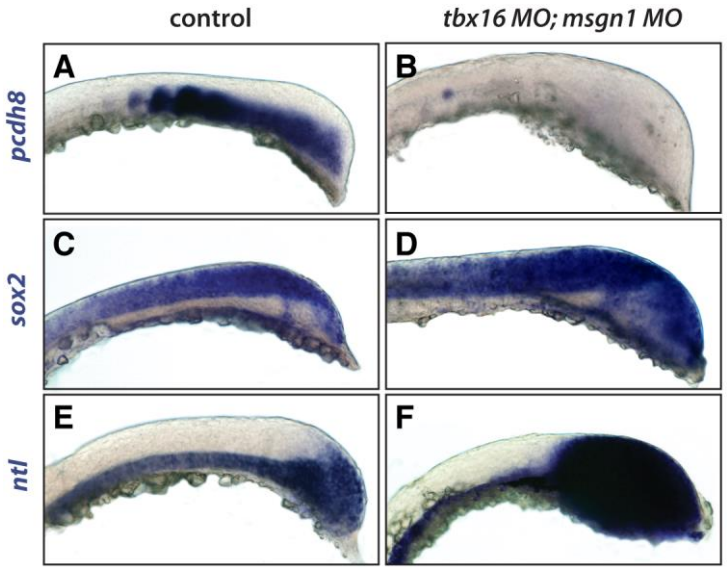

Figure S8. *sox2* and *ntl* increase when Tbx16 and Msgn1 are depleted.

Whole mount in situ hybridization shows a decrease in *pcdh8* expression in embryos (A, B; 13 controls and 12 morphants) injected with *tbx16 MO;msgn1 MO* (B). *sox2* expression (C, D; 11 controls and 13 morphants) and *ntl* expression (E, F; 16 controls and 10 morphants) increase in embryos injected with *tbx16 MO;msgn1 MO* (D, F).

Table S1: Summary of the number of embryos analyzed

		# of control embryos			# of transgenic embryos		
Transgene	**Transcript analyzed**	**2 hr**	**4 hr**	**6 hr**	**2 hr**	**4 hr**	**6 hr**
HS:tbx16	***sox2***	17	18	15	19	19	21
	ntl	22	18	20	18	22	21
	bra	17	19	25	13	14	23
	msgn1	ND (37)	16	22	ND (37)	16	19
	tbx6l	ND (36)	23	20	ND (36)	16	17
	pcdh8	18	18	17	22	22	23
	her1	19	24	20	17	16	19
	tbx6	21	20	22	19	19	18
	mespba	21	25	20	19	17	16
	tbx16-UTR	13	18	18	10	15	16
	wnt3a		20			22	
	Tbx16 protein (Immuno Fluor)	3	4	5	5	3	3
HS:tbx16-EnR	***tbx6l***		22			12	
	pcdh8		22			16	
	her1		23			17	
	sox2		28			13	
	ntl		30			16	
	wnt3a		23			13	
HS:caβ-catenin	***tbx16/ntl* (Fluor In Situ)**		4			6	
	tbx16-1.2			11			10
	tbx16-1.2ΔTCF			7			22
HS:tbx16	***cb1045* (33 hr pHS)**	15			6		