

Figure S1. Tbx6 antibody-binding specificity. (A) Graphical representation of the full-length Tbx6 protein in wild-type embryos, and the truncated Tbx6 protein in *tbx6* mutants. All eight exons are depicted in different colors. The blue arrows represent the predicted T-box domain from amino acid 62 to 243. The sequence chosen to generate the Tbx6 antibody is highlighted in red, spanning the region from amino acid 327 to 545. (B) Representative example showing *tbx6* mRNA and protein expression patterns in wild type and in *tbx6* mutants at 90% epiboly. Immunolabeling using the Tbx6 antibody was followed by *in situ* hybridization using a *tbx6* probe and the Fast Red reaction (Roche) (C) Immunolabeling using Tbx6 (green) and tRFP (red fluorescent protein mKate2) antibodies. Animal poles of *tbx6* mutant embryos 4 hours post fertilization, injected at the 1-cell-stage with capped mRNAs (generated using the Tol2 kit and the mMessage kit, Ambion): *Ntla-T2A-mKate2CAAX*, *Tbx6-T2A-mKate2CAAX*, *Tbx6l-T2A-mKate2CAAX* or *Tbx16-T2A-mKate2CAAX*. The Tbx6 antibody only binds in embryos injected with *tbx6* mRNA. Embryos were imaged with a Zeiss LSM 780 and confocal z-stacks were flattened.

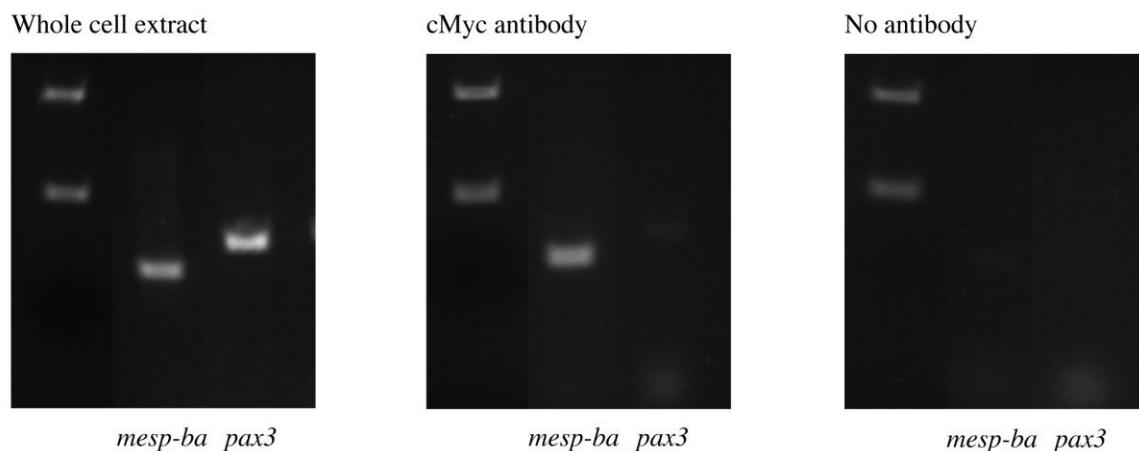


Figure S2. ChIP-PCR confirming Tbx6^{Myc} binding approximately 6700 bp upstream of the *mesp-ba* transcriptional start site (peak 16075430-16075735).
Tg(*hsp70l:tbx6^{myc}*) and wild-type embryos were heat-shocked, fixed and extracted as for ChIP-Seq. The extract was immunoprecipitated using the Myc epitope antibody (9B11, Cell Signaling Technology), as in Martins-Taylor et al. (2011). The DNA isolated by ChIP was amplified using the following primers: *mesp-ba* 5'-AATGGTAGTCAGGCAGAACAGTG-3', 5'-CTGCAGCAAGTTGCCTTACAG-3'; *pax3* 5'-AGTAGTATGCCCGGTCTTCTC-3', 5'-CCTGAGGGACAAACATC-3'. Specificity of Tbx6^{Myc} binding was confirmed, as DNA upstream of the *pax3* gene is not pulled down using a Myc antibody, serving as a negative control, whereas a no-Myc-antibody control fails to immunoprecipitate DNA from upstream both of *mesp-ba* and *pax3*. Peak 16082055-16082560 was confirmed by Kawamura et al. (2008), using the following primers: 5'-CAACAAACACAAAAAGCACACGTT-3', 5'-GGTGAAAGGAGGATGGAGGTTAT-3'.

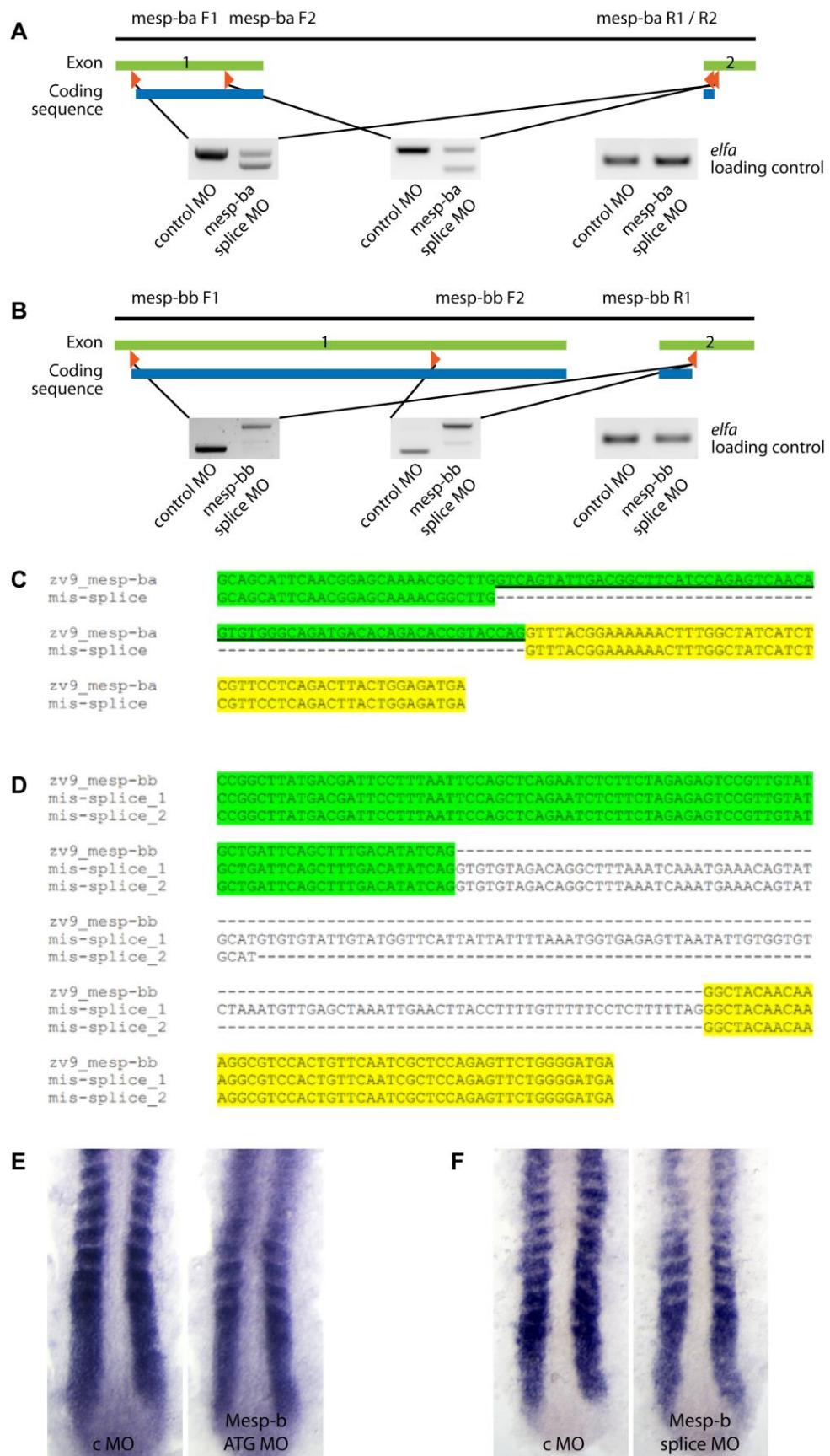


Figure S3. Mesp-b MO validation. (A,B) Mesp-ba (A) and Mesp-bb (B) splice morpholino transcript schematics and PCR primers are shown on top, the RT-PCR of

injected embryos is shown below. (C) Green highlight - 3' part of Zv9 *mesp-ba* exon 1; Yellow highlight - Zv9 *mesp-ba* exon 2 (until stop codon, excluding 3' UTR); Underlined - Sequence missing from *mesp-ba* mis-splice product. (D) Green highlight - 3' part of Zv9 *mesp-bb* exon 1; Yellow highlight - Zv9 *mesp-bb* exon 2 (until stop codon, excluding 3' UTR). Mis-splice_1 is the larger product, mis-splice_2 the smaller product shown in (B). (E,F) In situ hybridization for *meox1* mRNA in control and Mesp-b MO-treated embryos. Mesp-b ATG MOs and Mesp-b splice MOs cause similar reduction of *meox1* expression in maturing somites.

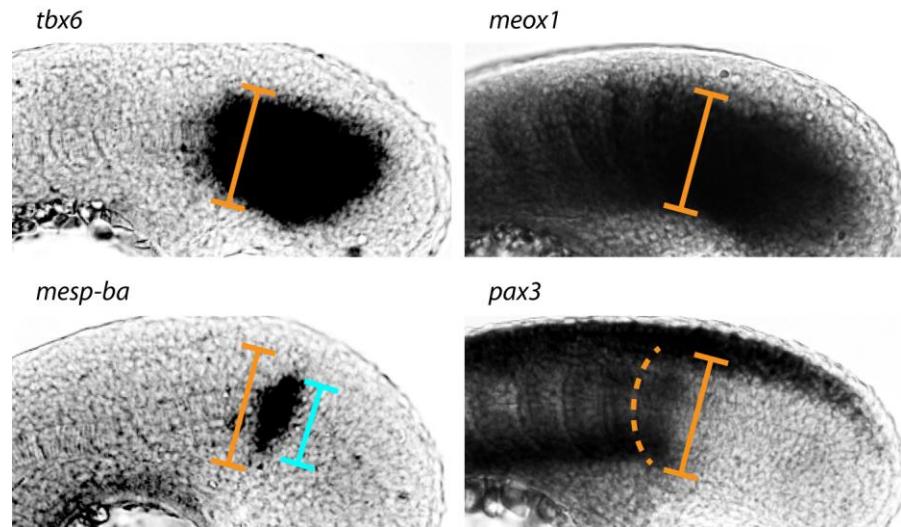


Figure S4. Expression of *tbx6*, *meox1*, *mesp-ba* and *pax3* mRNA. Expression of *tbx6*, *meox1* and *pax3* spans the entire dorsal-ventral extent of the paraxial mesoderm (orange brackets). *mesp-ba* mRNA is highly expressed in the central region (blue bracket) but not detectable in the peripheral portions of the presomitic paraxial mesoderm. Dashed line indicates most recently formed somite boundary.

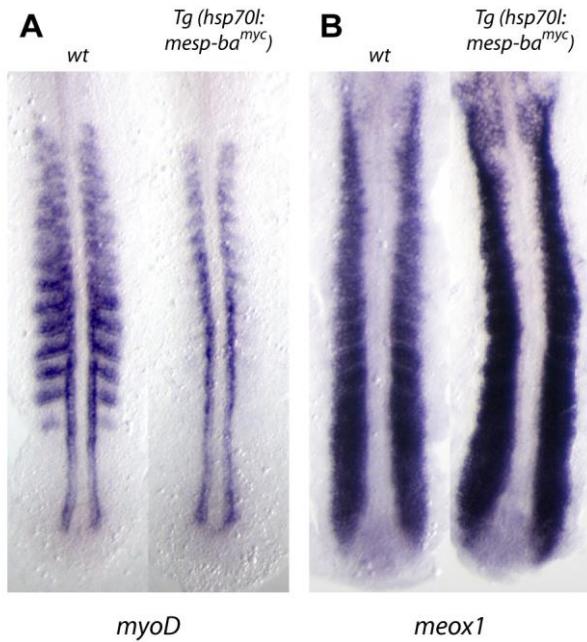


Figure S5. Ubiquitous Mesp-ba expression immediately changes *myoD* and *meox1* mRNA levels. *In situ* hybridization for *myoD* (C) and *meox1* (D) in wild-type (left side) and *Tg(hsp70l:mesp-ba^{myc})* embryos (right side) 15 minutes after the end of a 1-h heat shock. *myoD* is downregulated and *meox1* is upregulated in the lateral paraxial mesoderm.

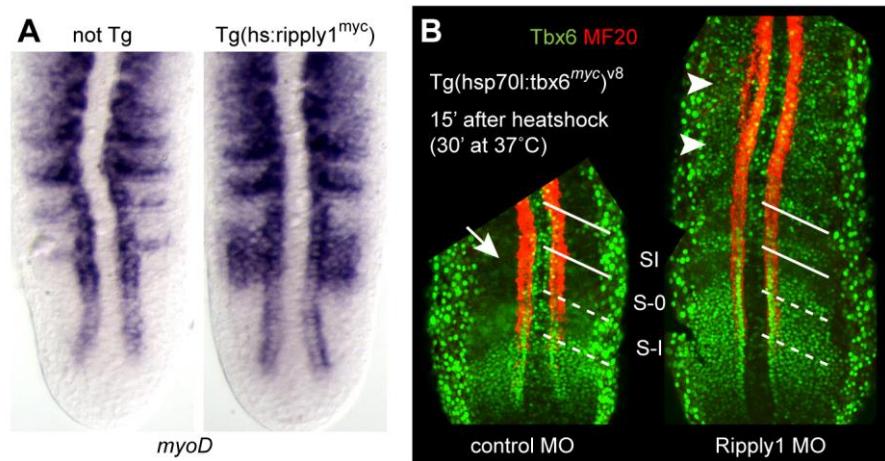


Figure S6. Rippoly1 regulates Tbx6 function. (A) *In situ* hybridization for *myoD* in wild-type embryos and *Tg(hsp70l:rippoly1^{myc})* siblings after a series of three heat shocks (30 minutes each, followed by 30-minute breaks). Ubiquitous expression of Rippoly1 leads to an increase of *myoD* expression in the anterior presomitic mesoderm. (B) *Tg(hsp70l:tbx6^{myc})^{v8}* embryos, injected with control (left) or Rippoly1 MOs (right); heat-shocked at the 10S stage for 30 minutes and fixed 15 minutes after the end of heatshock treatment. Immunolabeling shows expression of Tbx6 (green) and myosin heavy chain (MF20, red). Immediately after ubiquitous Tbx6^{MyC} expression, Tbx6 protein is cleared from the paraxial mesoderm in control but not in Rippoly1 MO-treated embryos.

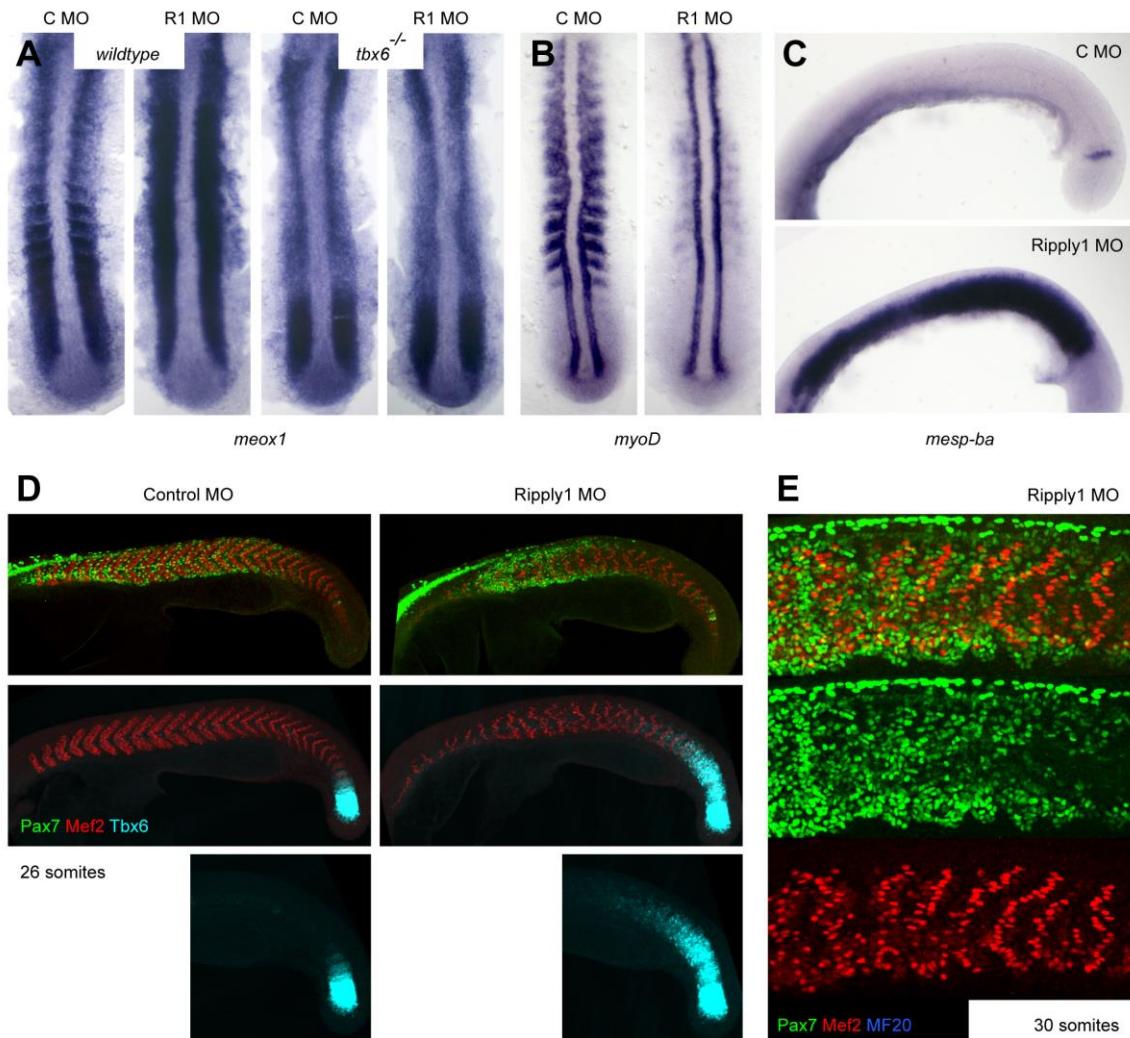


Figure S7. Knockdown of Ripply1 promotes dermomyotome development at the expense of primary fast myotome primary fast muscle formation but delays maturation. (A-C) *In situ* hybridization for *meox1* (A), *myoD* (B) and *mesp-ba* (C) in control MO and Ripply1 MO-injected embryos during segmentation. Note that Ripply1 MO treatment shows no effects in *tbx6* mutants. (D,E) Immunolabeling in Ripply1 MO- and control MO-treated embryos. (D) Comparison of Pax7 (green) and Tbx6 expression (cyan) in embryos at the 26S stage. Myonuclei are labeled with Mef2 (red). (E) Ripply1 morphant showing that the onset of Pax7 expression (green) spatially correlates with the appearance of the first primary fast myotome cells (Mef2, faint red). Slow fiber nuclei express Mef2 (bright) before the maturation of the dermomyotome.

Table S1. ChIP-Seq. Tables listing the positions of significant peaks identified in duplicate anti-Myc immunoprecipitation experiments from *Tg(hsp70l:tbx6^{myc})* embryos 1 h after heat shock treatment (see Fig. 1F), and corresponding gene positions.

Chromosome	Peak start	Peak stop	Peak size	Proximal gene
chr7	16074838	16075110	273	<i>mespba;mespaa</i>
chr7	16075430	16075735	306	<i>mespba;mespaa</i>
chr7	16077395	16077604	210	<i>mespba;mespaa</i>
chr7	16082055	16082560	506	<i>mespaa;mespba</i>
chr21	36933621	36934092	472	<i>mespbb;mespab</i>
chr21	36935687	36936177	491	<i>mespbb;mespab</i>
chr21	36936989	36937357	369	<i>mespbb;mespab</i>
chr21	36951345	36951918	574	<i>mespbb;mespab</i>
chr21	36952555	36952924	370	<i>mespbb;mespab</i>
chr21	36958013	36958556	544	<i>mespbb;mespab</i>
chr21	36960812	36961218	407	<i>mespbb;mespab</i>
chr25	11386856	11387147	292	<i>rippy1</i>
chr25	11386856	11387147	292	<i>rippy1</i>
chr25	11389407	11389883	477	<i>rippy1</i>
chr25	11393143	11393773	631	<i>rippy1</i>
chr25	11396259	11396835	577	<i>rippy1</i>
chr25	11398508	11398877	370	<i>rippy1</i>
chr25	11400599	11401158	560	<i>rippy1</i>

Gene name	Chromosome	Gene start	Gene stop	Strand
<i>mespaa</i>	chr7	16098653	16101163	1
<i>mespba</i>	chr7	16082277	16085567	1
<i>mespab</i>	chr25	11382068	11383326	-1
<i>mespbb</i>	chr25	11392554	11393558	-1
<i>rippy1</i>	chr21	36930713	36937075	-1

Table S2. Location of potential T-boxes within genomic regions represented in Figure 1F.

All Tboxes of >50% of the maximum possible score relative to the motif identified in this study are shown (see Materials and Methods). Whether each sequence is located under a ChIP-seq peak is indicated.

Chromosome	Region start	Region end	Tbox start	Tbox end	Tbox strand	% of max. score	Sequence	Under ChIP-seq peak
7	16069000	16104000	16070479	16070486	+	59.2	TCACACAC	
7	16069000	16104000	16070660	16070667	-	54.8	TCACAGCA	
7	16069000	16104000	16071406	16071413	-	54.8	TCACAGCA	
7	16069000	16104000	16071516	16071523	-	61.5	TCACACTA	
7	16069000	16104000	16071869	16071876	-	83.3	TCACACTT	
7	16069000	16104000	16072717	16072724	-	57.3	TGACACCA	
7	16069000	16104000	16074474	16074481	+	54.8	TCACAGCA	
7	16069000	16104000	16075011	16075018	+	100.0	TCACACCT	*
7	16069000	16104000	16075046	16075053	-	100.0	TCACACCT	*
7	16069000	16104000	16075312	16075319	-	100.0	TCACACCT	
7	16069000	16104000	16075592	16075599	+	61.5	TCACACTA	*
7	16069000	16104000	16077272	16077279	-	72.0	TCACATCT	
7	16069000	16104000	16077486	16077493	-	59.0	TCACACAA	*
7	16069000	16104000	16077497	16077504	+	100.0	TCACACCT	*
7	16069000	16104000	16077744	16077751	-	55.4	TCACATT	
7	16069000	16104000	16078528	16078535	+	60.5	ACACACTT	
7	16069000	16104000	16078566	16078573	+	56.4	AGACACCT	
7	16069000	16104000	16078600	16078607	+	58.1	ACACACAT	
7	16069000	16104000	16078629	16078636	+	58.1	ACACACAT	
7	16069000	16104000	16079062	16079069	-	60.0	TCACAGTT	
7	16069000	16104000	16079666	16079673	+	83.3	TCACACTT	
7	16069000	16104000	16080777	16080784	+	55.4	TCACATT	
7	16069000	16104000	16082207	16082214	+	100.0	TCACACCT	*
7	16069000	16104000	16082307	16082314	-	79.2	TGACACCT	*
7	16069000	16104000	16082310	16082317	+	55.2	TGTCACCT	*
7	16069000	16104000	16082740	16082747	+	52.6	TCTCAGCT	
7	16069000	16104000	16083275	16083282	+	55.3	ACACACCA	
7	16069000	16104000	16083386	16083393	-	60.1	TGACACAT	
7	16069000	16104000	16084350	16084357	-	58.1	ACACACAT	
7	16069000	16104000	16084624	16084631	+	55.4	TCACATT	
7	16069000	16104000	16084808	16084815	-	79.2	TGACACCT	
7	16069000	16104000	16084871	16084878	+	77.2	ACACACCT	
7	16069000	16104000	16084924	16084931	-	60.5	ACACACTT	
7	16069000	16104000	16085135	16085142	+	51.3	TGACATCT	

25	11374000	11409000	11405827	11405834	-	100.0	TCACACCT	
25	11374000	11409000	11406440	11406447	-	55.4	TCACATT	
25	11374000	11409000	11406938	11406945	-	57.3	TGACACCA	

Table S3. List of primers, antibodies and RNA probes

Morpholino sequences	
Ripply1	5'-CATCGTCACTGTGTTTCTGTTTG-3' (Kawamura et al., 2005)
Mesp-ba ATG	5'-TCGGTTCTGCTTGAGGTTGCATG-3' (Kawamura et al 2005)
Mesp-bb ATG	5'-CGTCCA TTCTGTGTGGTTGGAGA TT-3'
Mesp-ba splice	5'-TAACTTAACATAACCTGGTACGGTGT-3'
Mesp-bb splice	5'-TTAAAGCCTGTCTACACACCTGAT-3'
Standard control	5'-CCTCCTACCTCAGTTACAATTATA-3'
p53 MO	5'-GCGCCATTGCTTGCAAGAATTG-3' (Robu et al., 2007)

Primary antibodies	
Pax7, MF20, F59, cMyc	1:10, DSHB
Mef2	1:100, Santa Cruz
b-cat	1:1000, Sigma
Tbx6	1:250

Secondary antibodies	
Alexa-conjugated	1:800, Invitrogen

RNA probes	
<i>meox</i>	(Neyt et al., 2000)
<i>mesp-ba</i>	(Sawada et al., 2000)
<i>mesp-bb</i>	(Cutty et al., 2012)
<i>myf5</i>	(Coutelle et al., 2001)
<i>myoD</i>	(Weinberg et al., 1996)
<i>pax3, pax7</i>	(Seo et al., 1998)
<i>ripply1, tbx6</i>	(Kawahara et al., 2005)