

Supporting Information

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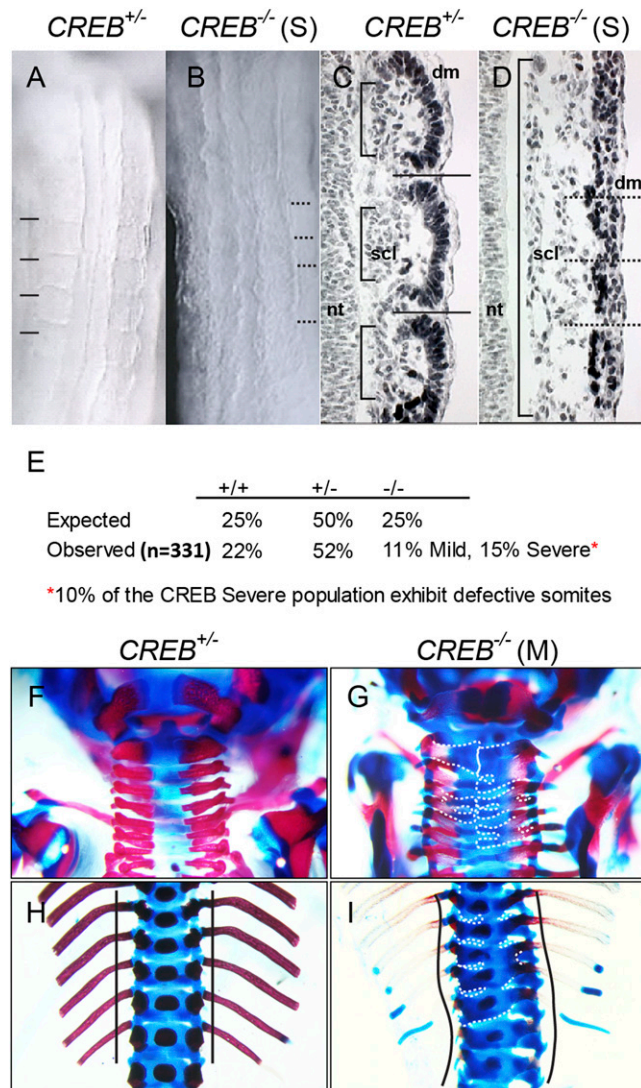


Fig. S1. Somitogenesis is defective in a small population of severe cAMP responsive element binding protein (*CREB*)-null embryos, whereas mild mutants exhibit sporadic skeletal fusions. (*A* and *B*) Bright-field images: (*A*) *CREB*^{+/-} embryonic day (E) 9.5 somites exhibit somite boundaries at regular intervals (lines); and (*B*) *CREB*^{-/-} severe (marked as "S") mutant somite boundaries appear to be lost (dotted lines). Posterior is to the top. (*C* and *D*) Horizontal sections of E9.5 embryos stained for Pax3. (*C*) *CREB*^{+/-} embryos exhibit normal arch-like dermomyotome structures, somite boundaries (lines), and compacted sclerotomes (brackets). (*D*) The epithelial structures of the dermomyotome are lost in *CREB*^{-/-} ("S") mutant; contributing to scattered sclerotomal cells (bracket) and a loss of somite boundaries (dotted lines). dm, dermomyotome; nt, neural tube; scl, sclerotome. (*E*) Table depicting expected Mendelian and observed percentages of a *CREB*^{+/-} intercross. (*F*–*I*) Alizarin red and Alcian blue-stained E18.5 skeletons. (*F* and *H*) *CREB*^{+/-} control (*G* and *I*) *CREB*^{-/-} mild (marked as "M") mutant. (*F* and *G*) are dorsal images of cervical vertebrae. (*H* and *I*) are ventral images of thoracic and lumbar regions. White dotted lines trace fusion events in neural arches (*G*) and vertebral bodies (*I*). Black lines show the curvature of the spine in mutants (*I*) compared with control (*H*).

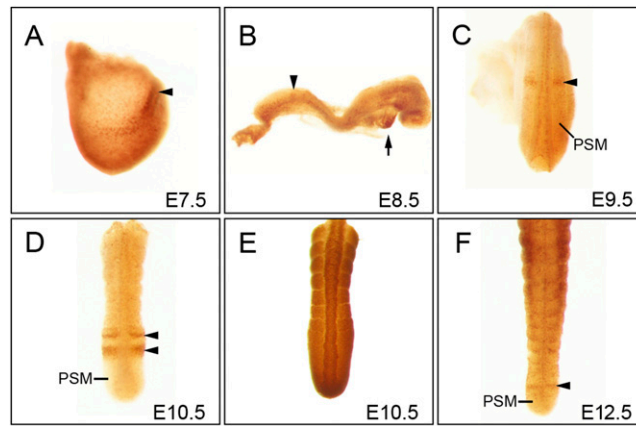


Fig. S2. Phosphorylated (P)-CREB is detected in the presomitic mesoderm (PSM) during most somite stages. (*A–D* and *F*) P-CREB whole-mount immunostaining of embryos during developmental stages E7.5 to E12.5. (*A* and *B*) Side view; anterior is to the right. (*C–F*) Dorsal view. (*A*) P-CREB was detected in the endocardial tube in E7.5 embryos (arrowhead). (*B–D* and *F*) P-CREB (arrowheads) was detected in the PSM as single or double stripes during somite stages E8.5 through E12.5. (*B*) P-CREB was detected in the heart at E8.5 (arrow). (*E*) CREB was ubiquitously expressed throughout the PSM and somites of an E10.5 mouse.

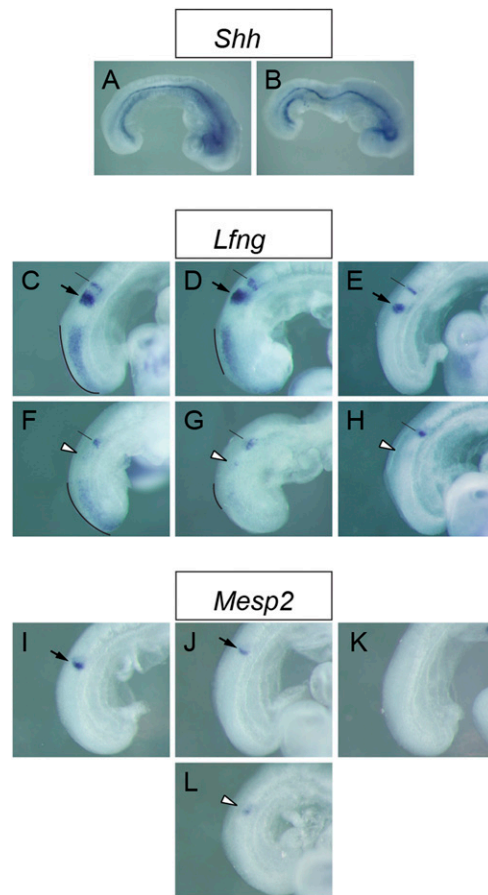


Fig. S6. Normal notochord patterning in mutants with defective Notch signaling and persistent *Mesp2* expression throughout segmentation. (A and B) *Shh* whole-mount in situ hybridization (WISH) analysis (A) *ROSA^{AC/AC}* (control) and (B) *ROSA^{AC/AC};T-Cre* (mutant). (C–H) *Lunatic fringe* (*Lfng*) WISH analysis in control (C–E) and mutants (F–H). *Lfng* phase-matched embryos are C and F, D and G, and E and H. Note *Lfng* is expression is initiated in the mutant posterior PSM (C and F, curved lines). An intermediate *Lfng* stripe is reduced in mutants (F–H, white arrowheads; $n = 6$) to controls (C–E, black arrows; $n = 7$). (F–H) Anterior most *Lfng* stripe (line) in mutants ($n = 5$) is indistinct compared with controls ($n = 6$; C–E). (I–L) *Mesp2* WISH analysis in controls (I–K): (I) a *Mesp2* broad stripe (arrow) was observed in controls ($n = 3$). (J) A narrow *Mesp2* stripe (arrow; $n = 2$). (K) Some controls did not exhibit any *Mesp2* expression ($n = 2$). (L) Mutants always expressed a single mosaic stripe (white arrowhead; $n = 5$).

Table S1. Down-regulated genes with full/half CRE-sites within the PSM CREB transcriptome

Gene symbol	MGI database object name	Fold change down	CRE-site
<i>Ripply2</i> ***	Ripply2 homologue (zebrafish)	3.036	Half, conserved
<i>Gng4</i>	Guanine nucleotide binding protein (G protein), γ 4	2.629	Half, conserved
<i>Rps27</i>	Ribosomal protein S27	2.611	Half, conserved
<i>Pcdh8</i> *	Protocadherin 8	2.597	Half, conserved
<i>Zfp61</i>	Zinc finger protein 61	2.357	Half, nonconserved
<i>Cnn2</i>	Cytochrome P450, family 51	2.273	Half, nonconserved
<i>Hoxd1</i> *	Homeobox D1	2.157	Half, nonconserved
<i>Prodh</i>	Proline dehydrogenase	2.148	Half, nonconserved
<i>Ube2j1</i>	Ubiquitin-conjugating enzyme E2, J1	2.083	Half, conserved
<i>Narg2</i>	NMDA receptor-regulated gene 2	2.071	Full and half conserved
<i>Sc5d</i>	Sterol-C5-desaturase (fungal ERG3, δ -5-desaturase)	2.007	Half, conserved
<i>Brca2</i>	Breast cancer 2	2.006	Full and half conserved
<i>Ranbp6</i>	RAN binding protein 6	2.006	Full and half conserved
<i>H2-T10 III H2-T22</i>	None	1.994	Half, nonconserved
<i>Siah2</i>	Seven in absentia 2	1.994	Half, conserved
<i>BC022687</i>	cDNA sequence BC022687	1.907	Full and half conserved
<i>Rrm1</i>	Ribonucleotide reductase M1	1.890	Half, conserved
<i>Kcnk6</i>	Potassium inwardly rectifying channel, subfamily K, member 6	1.881	Half, nonconserved
<i>Foxf1a</i>	Forkhead box F1a	1.876	Half, conserved
<i>Ms4a4d</i>	Membrane-spanning 4-domains, subfamily A, member 4D	1.867	Half, nonconserved
<i>Rce1</i>	RCE1 homologue, prenyl protein peptidase (<i>Saccharomyces cerevisiae</i>)	1.860	Full and half conserved
<i>Trim9</i>	Tripartite motif-containing 9	1.845	Half, nonconserved
<i>Bcap29</i>	B-cell receptor associated protein 29	1.844	Half, nonconserved
<i>Pop4</i>	Processing of precursor 4, ribonuclease P/MRP family	1.834	Full and half conserved
<i>Dkk1</i> **	Dickkopf homologue 1 (<i>Xenopus laevis</i>)	1.823	Half, nonconserved
<i>Kdr</i>	Kinase insert domain protein receptor	1.822	Half, nonconserved
<i>Nek1</i>	NIMA (never in mitosis gene a)-related expressed kinase 1	1.811	Full and half conserved
<i>Cacna2d3</i>	Calcium channel, voltage-dependent, α 2/ δ subunit 3	1.798	Half, nonconserved
<i>Ulk2</i>	Unc-51 like kinase 2 (<i>Caenorhabditis elegans</i>)	1.792	Half, conserved
<i>LOC100047504</i>	None	1.780	Half, nonconserved
<i>Hhex</i>	Hematopoietically expressed homeobox	1.776	Half, conserved
<i>Jph3</i>	Junctophilin 3	1.760	Half, nonconserved
<i>Guk1</i>	Guanylate kinase 1	1.758	Half, nonconserved
<i>Fli1</i>	Friend leukemia integration 1	1.751	Half, conserved
<i>Ssca1</i>	Sjogren syndrome/scleroderma autoantigen 1	1.749	Half, conserved
<i>Adar</i>	Adenosine deaminase, RNA-specific	1.748	Half, nonconserved
<i>Tbx6</i> ***	T-box 6	1.748	Half, conserved
<i>Ldlr</i>	LDL receptor	1.746	Half, nonconserved
<i>Ndufb2</i>	NADH dehydrogenase (ubiquinone) 1 β subcomplex, 2	1.728	Full and half conserved
<i>Gpm</i>	Glycerol-3-phosphate acyltransferase, mitochondrial	1.723	Half, conserved
<i>Tal1</i>	T-cell acute lymphocytic leukemia 1	1.717	Half, nonconserved
<i>Nphp1</i>	Nephronophthisis 1 (juvenile) homologue (human)	1.714	Half, conserved
<i>Slc25a16</i>	Solute carrier family 25 (mitochondrial carrier, Graves disease)	1.700	Half, conserved
<i>Pdgfd</i>	PDGF, D polypeptide	1.698	Half, conserved
<i>Dll1</i> *	δ -like 1 (<i>Drosophila</i>)	1.695	Half, conserved
<i>Nup12</i>	Nucleoporin like 2	1.695	Full, conserved
<i>B230219D22Rik</i>	RIKEN cDNA B230219D22 gene	1.681	Half, nonconserved
<i>Prrx1</i>	Paired related homeobox 1	1.674	Half, conserved
<i>Zdhhc3</i>	Zinc finger, DHHC domain containing 3	1.666	Half, nonconserved
<i>Snrk</i>	SNF-related kinase	1.664	Half, nonconserved
<i>Ikbkb</i>	Inhibitor of κ B kinase β	1.658	Full, conserved
<i>Sox17</i>	SRY-box containing gene 17	1.655	Half, nonconserved
<i>Stk16</i>	Serine/threonine kinase 16	1.636	Half, conserved
<i>Mrpl4</i>	Mitochondrial ribosomal protein L4	1.630	Half, nonconserved
<i>Sbsn</i>	Suprabasin	1.621	Half, nonconserved
<i>Tm6sf1</i>	Transmembrane 6 superfamily member 1	1.621	Half, nonconserved
<i>Gaa</i>	Glucosidase, α , acid	1.618	Half, nonconserved
<i>Mrpl55</i>	Mitochondrial ribosomal protein L55	1.614	Half, conserved
<i>Ddx28</i>	DEAD (Asp-Glu-Ala-Asp) box polypeptide 28	1.613	Full, conserved
<i>Timm44</i>	Translocase of inner mitochondrial membrane 44	1.600	Half, conserved
<i>Panx1</i>	Pannexin 1	1.591	Half, conserved
<i>Hmgcs1</i>	3-Hydroxy-3-methylglutaryl-CoA synthase 1	1.590	Half, nonconserved
<i>Syt13</i>	Synaptotagmin XIII	1.584	Half, conserved

Table S1. Cont.

Gene symbol	MGI database object name	Fold change down	CRE-site
<i>Bag2</i>	BCL2-associated athanogene 2	1.581	Half, conserved
<i>Sc4mol</i>	Sterol-C4-methyl oxidase-like	1.581	Half, nonconserved
<i>Insig1</i>	Insulin induced gene 1	1.575	Half, conserved
<i>Clcn4-2</i>	Chloride channel 4-2	1.573	Full and half nonconserved
<i>Ppp1r14a</i>	Protein phosphatase 1, regulatory (inhibitor) subunit 14A	1.570	Half, nonconserved
<i>Abca1</i>	ATP-binding cassette, subfamily A (ABC1), member 1	1.563	Half, nonconserved
<i>Smarca1</i>	SWI/SNF related, matrix associated, subfamily a, member 1	1.561	Half, conserved
<i>Olfm1</i>	Olfactomedin 1	1.534	Half
<i>Enpp2</i>	Ectonucleotide pyrophosphatase/phosphodiesterase 2	1.525	Half, nonconserved
<i>Kif18a</i>	Kinesin family member 18A	1.520	Half, nonconserved
<i>Nfatc4</i>	Nuclear factor of activated T cells, cytoplasmic, calcineurin 4	1.505	Half, conserved
<i>Gas5 // Snord47</i>	None	1.503	Full, conserved
<i>Rnps1</i>	RNA binding protein S1	1.503	Half, conserved
<i>Gpc1</i>	Glypican 1	1.500	Half, nonconserved

Conserved CRE-sites are defined by conservation among human, mouse, and rat genomes. Nonconserved CRE-sites are only observed in one or two mammalian genomes. Gray columns indicate segmentation genes. CRE, cAMP responsive element.

*Notch signaling, **Wnt signaling, ***both.

Table S2. PCR primer sets used in this study

Set	Primer
<i>CREB^{+/-}</i>	Forward primer: 5'-AAG CGC CAT TCG CCA TTC AGG C-3' Reverse primer: 5'-GAT GTA CAA ACA TAC CAG ATC CGC-3' LacZ primer: 5'-CAC AGA ACC TAC TGT TAG CAG ATG-3'
<i>T-Cre</i>	Forward primer 5'-ATT TGC CTG CAT TAC CGG TC-3' Reverse primer 5'-ATC AAC GTT TTC TTT TCG G-3'
<i>ROSA^{ACIAC}</i>	ROSA forward primer: 5'-GGA GCG GGA GAA ATG GAT ATG-3' ROSA reverse primer: 5'-AAA GTC GCT CTG AGT TGT TAT-3' A-CREB/CMV reverse primer: 5'-CTC CAT ATA TGG GCT ATG AAC TAA TGA-3'
<i>Dll-1 + CRE-site</i>	Forward primer: 5'-TGG GCC CTC CCA ATA AAC TCA TTT-3' Reverse primer: 5'-TCC GGC ACA TGT TTG AAA GAC TC-3'
<i>Dll-1 - CRE-site</i>	Forward primer: 5'-GGC TGT TAC AAA GGG CTC CTA A-3' Reverse primer: 5'-GGC AAG TCA TGA TTG AGG TGA G-3'
<i>Ripply2 + CRE-site</i>	Forward primer: 5'-TGT GCC AGA CAT GCA ACA AG-3' Reverse primer: 5'-GGG CTG CAA TGA CGT GAC TA-3'
<i>Ripply2 - CRE-site</i>	Forward primer: 5'-CAC AGC TCC CGT TCT GTG AA-3' Reverse primer: 5'-TTC CAA AGG CCA GAG GCT AC-3'