Supporting Information

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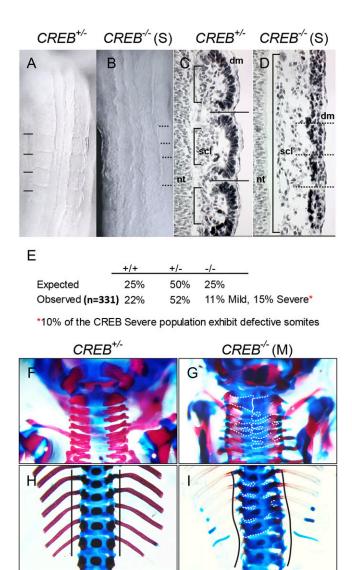


Fig. 51. Somitogenesis is defective in a small population of severe cAMP responsive element binding protein (*CREB*)-null embryos, whereas mild mutants exhibit sporadic skeletal fusions. (*A* and *B*) Bright-field images: (*A*) *CREB^{+/-}* embryonic day (E) 9.5 somites exhibit somite boundaries at regular 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 at 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^{+/-}* 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 (*J*). Black lines show the curvature of the spine in mutants (*J*) compared with control (*H*).

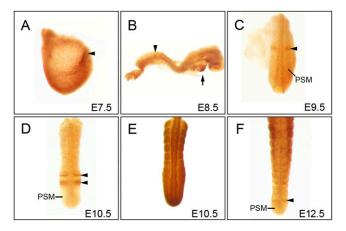


Fig. 52. Phosphorylated (P)-CREB is detected in the presomitic mesoderm (PSM) during most somite stages. (*A*–*D* and *F*) P-CREB whole-mount immunostaining of embryos during developmental stages E7.5 to E12.5. (*A* and *B*) Side view; anterior is to the right. (*C*–*F*) Dorsal view. (*A*) P-CREB was 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.

DNAS Nd

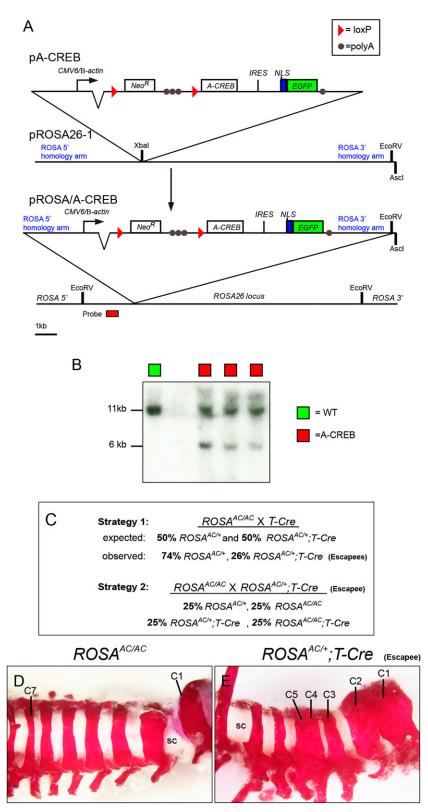


Fig. S3. Generation of *Acidic-CREB* (*A-CREB*) mice. (*A*) A 7-kb pA-CREB construct was generated and digested with Xbal, followed by insertion into pROSA26-1 to create pROSA/A-CREB. The following targeting construct was linearized with Ascl and integrated into the *ROSA26* locus by homologous recombination. Red triangles are loxP sites and gray circles are polyA signals. The red rectangle signifies a probe used for Southern blot analysis. Note the diagram is not drawn to scale. (*B*) Verification of germ-line transmission by Southern blot analysis of genomic DNA digested with EcoRV and probed for a WT 11-kb band and an A-CREB 6-kb band. Green square, WT; red square, *A-CREB* mutant. (*C*) Strategy 1: generate *ROSA^{AC/+}; T-Cre* male (escapees) from a *ROSA^{AC/AC}* (control) and *T-Cre* cross. The resulting escapees (26%) then underwent a successive round of breeding (Strategy 2) with *ROSA^{AC/AC}* to generate embryos with either one or two copies of *A-CREB* with *T-Cre*. (*D* and *E*) Alizarin red staining of adult males. Anterior is to the right. (*D*) Control. (*E*) Cervical fusions (C1–C2; C3–C5) in escapee. Sc, spinal cord.

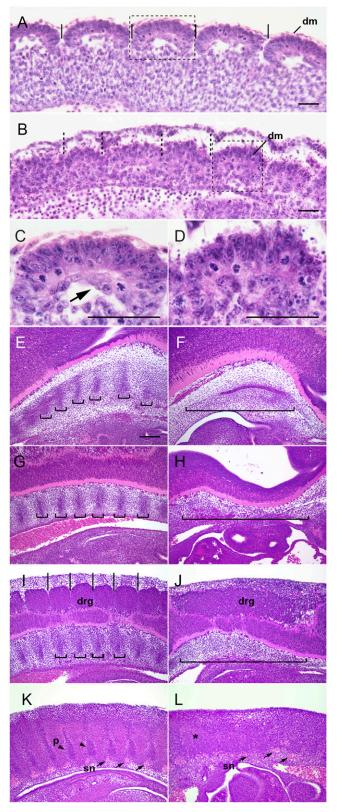


Fig. 54. Mutant somites fail to organize the sclerotome and the peripheral nervous system (PNS). (A-L) H&E-stained sagittal sections (right is anterior in all images). (A) Vertical lines indicate somite boundaries of an E9.5 $ROSA^{AC/AC}$ embryo (control). (B) Dotted lines mark perturbations of somite boundaries in an E9.5 $ROSA^{AC/AC}$; *T-Cre* embryo (mutant). dm, dermomyotome. (Magnification of 20× in A and B.) (C) Magnified rectangular area of control (A). (D) Magnified rectangular area in mutant (B). (Magnification of 100× in C and D.) Note greater myotomal mass (arrow) in control (C) than in mutant (D). (E and G) Sclerotomal condensations (brackets) form in the cervical and thoracic regions, respectively, in E11.5 control. (*I* and *K*) PNS organization of control is normal. (*I*) Dorsal root ganglia (drg) are segmented (lines), along with sclerotomal condensations (brackets). (K) Pedicle anlagen (marked as "P" with arrowheads) and Legend continued on following page

spinal neurons (sn; arrows) display segmented organization. (*F* and *H*) The sclerotome is unsegmented (wide brackets) in the cervical and thoracic areas, respectively, in E11.5 mutant. (*J* and *L*) Defective PNS patterning in mutant. (*J*) Note dorsal root ganglia are fused along the thoracic region. In addition, the sclerotome is unsegmented (bracket). (*L*) Note spinal nerves are disorganized (arrows). Pedicle primordia are indistinct and mixed with the myotome (asterisk). (Scale bars: 50 μ M.)

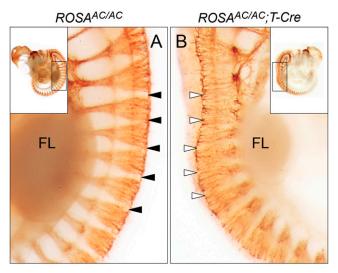


Fig. S5. Segmented PNS organization is lost in *ROSA*^{AC/AC}; *T*-Cre mutants. (*A* and *B*) Analysis of PNS organization and spinal nerve projections at the thoracic level by whole-mount staining for neurofilament on E11.5 *ROSA*^{AC/AC} (control) and *ROSA*^{AC/AC}; *T*-Cre (mutant) embryos (FL, forelimb). (*Insets*) Magnified regions. (*B*) White arrowheads indicate spinal neuron invasion in the entire somite in mutant compared with the neat triangular bundles (black arrowheads) found at the same axial level in control (*A*).

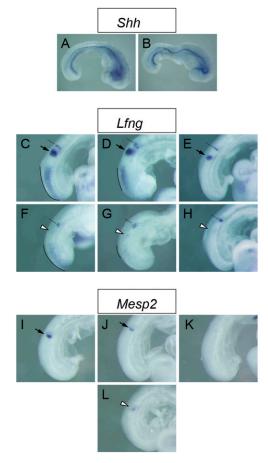


Fig. S6. Normal notochord patterning in mutants with defective Notch signaling and persistent *Mesp2* expression throughout segmentation. (A and B) Shh whole-mount in situ hybridization (WISH) analysis (A) $ROSA^{ACIAC}$ (control) and (B) $ROSA^{ACIAC}$, *T*-Cre (mutant). (*C*-*H*) *Lunatic fringe* (*Lfng*) 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

PNAS PNAS

Gene symbol	MGI database object name	Fold change down	CRE-site
Ripply2***	Ripply2 homologue (zebrafish)	3.036	Half, conserved
Gng4	Guanine nucleotide binding protein (G protein), γ 4	2.629	Half, conserved
Rps27	Ribosomal protein S27	2.611	Half, conserved
Pcdh8 [*]	Protocadherin 8	2.597	Half, conserved
Zfp61	Zinc finger protein 61	2.357	Half, nonconserved
Cnn2	Cytochrome P450, family 51	2.273	Half, nonconserved
Hoxd1 [*]	Homeobox D1	2.157	Half, nonconserved
Prodh	Proline dehydrogenase	2.148	Half, nonconserved
Ube2j1	Ubiquitin-conjugating enzyme E2, J1	2.083	Half, conserved
Varg2	NMDA receptor-regulated gene 2	2.071	Full and half conserved
Sc5d	Sterol-C5-desaturase (fungal ERG3, δ -5-desaturase)	2.007	Half, conserved
Brca2	Breast cancer 2	2.006	Full and half conserved
Ranbp6	RAN binding protein 6	2.006	Full and half conserved
H2-T10 /// H2-T22	None	1.994	Half, nonconserved
Siah2	Seven in absentia 2	1.994	Half, conserved
BC022687	cDNA sequence BC022687	1.907	Full and half conserved
Rrm1	Ribonucleotide reductase M1	1.890	Half, conserved
(cnk6	Potassium inwardly rectifying channel, subfamily K, member 6	1.881	Half, nonconserved
oxf1a	Forkhead box F1a	1.876	Half, conserved
As4a4d	Membrane-spanning 4-domains, subfamily A, member 4D	1.867	Half, nonconserved
Rce1	RCE1 homologue, prenyl protein peptidase (Saccharomyces cerevisiae)	1.860	Full and half conserved
Trim9	Tripartite motif-containing 9	1.845	Half, nonconserved
Bcap29	B-cell receptor associated protein 29	1.843	Half, nonconserved
•	Processing of precursor 4, ribonuclease P/MRP family	1.834	•
Pop4			Full and half conserved
Okk1**	Dickkopf homologue 1 (Xenopus laevis)	1.823	Half, nonconserved
Kdr	Kinase insert domain protein receptor	1.822	Half, nonconserved
Vek1	NIMA (never in mitosis gene a)-related expressed kinase 1	1.811	Full and half conserved
Cacna2d3	Calcium channel, voltage-dependent, $\alpha 2/\delta$ subunit 3	1.798	Half, nonconserved
Jlk2	Unc-51 like kinase 2 (<i>Caenorhabditis elegans</i>)	1.792	Half, conserved
.OC100047504	None	1.780	Half, nonconserved
Hhex	Hematopoietically expressed homeobox	1.776	Half, conserved
Iph3	Junctophilin 3	1.760	Half, nonconserved
Guk1	Guanylate kinase 1	1.758	Half, nonconserved
Fli1	Friend leukemia integration 1	1.751	Half, conserved
Sssca1	Sjogren syndrome/scleroderma autoantigen 1	1.749	Half, conserved
Adar	Adenosine deaminase, RNA-specific	1.748	Half, nonconserved
Tbx6***	T-box 6	1.748	Half, conserved
ldlr	LDL receptor	1.746	Half, nonconserved
Vdufb2	NADH dehydrogenase (ubiquinone) 1 β subcomplex, 2	1.728	Full and half conserved
Gpam	Glycerol-3-phosphate acyltransferase, mitochondrial	1.723	Half, conserved
Tal1	T-cell acute lymphocytic leukemia 1	1.717	Half, nonconserved
Vphp1	Nephronophthisis 1 (juvenile) homologue (human)	1.714	Half, conserved
5lc25a16	Solute carrier family 25 (mitochondrial carrier, Graves disease	1.700	Half, conserved
Pdgfd	PDGF, D polypeptide	1.698	Half, conserved
D//1*	δ-like 1 (Drosophila)	1.695	Half, conserved
Vupl2	Nucleoporin like 2	1.695	Full, conserved
3230219D22Rik	RIKEN cDNA B230219D22 gene	1.681	Half, nonconserved
Prrx1	Paired related homeobox 1	1.674	Half, conserved
Zdhhc3	Zinc finger, DHHC domain containing 3	1.666	Half, nonconserved
Snrk	SNF-related kinase	1.664	Half, nonconserved
kbkb	Inhibitor of κB kinase β	1.658	Full, conserved
50x17	SRY-box containing gene 17	1.655	Half, nonconserved
Stk16	Serine/threonine kinase 16	1.636	Half, conserved
Mrpl4	Mitochondrial ribosomal protein L4	1.630	Half, nonconserved
ibsn	Suprabasin	1.621	Half, nonconserved
Tm6sf1	Transmembrane 6 superfamily member 1	1.621	Half, nonconserved
Gaa	Glucosidase, α, acid	1.618	Half, nonconserved
	Mitochondrial ribosomal protein L55	1.614	Half, conserved
Mrpl55	•		
Ddx28	DEAD (Asp-Glu-Ala-Asp) box polypeptide 28	1.613	Full, conserved
Timm44	Translocase of inner mitochondrial membrane 44	1.600	Half, conserved
Panx1	Pannexin 1	1.591	Half, conserved
Hmgcs1	3-Hydroxy-3-methylglutaryl-CoA synthase 1	1.590	Half, nonconserved
Syt13	Synaptotagmin XIII	1.584	Half, conserved

Table S1. Cont.

PNAS PNAS

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

Set	Primer
CREB ^{+/-}	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'
T-Cre	Forward primer 5'-ATT TGC CTG CAT TAC CGG TC-3'
	Reverse primer 5'-ATC AAC GTT TTC TTT TCG G-3'
ROSA ^{ACIAC}	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'
Dll-1 + CRE-site	Forward primer: 5'-TGG GCC CTC CCA ATA AAC TCA TTT-3'
	Reverse primer: 5'-TCC GGC ACA TGT TTG AAA GAC TC-3'
Dll-1 – CRE-site	Forward primer: 5'-GGC TGT TAC AAA GGG CTC CTA A-3'
	Reverse primer: 5'-GGC AAG TCA TGA TTG AGG TGA G-3'
Ripply2 + CRE-site	Forward primer: 5'-TGT GCC AGA CAT GCA ACA AG-3'
	Reverse primer: 5'-GGG CTG CAA TGA CGT GAC TA-3'
Ripply2 – CRE-site	Forward primer: 5'-CAC AGC TCC CGT TCT GTG AA-3'
	Reverse primer: 5'-TTC CAA AGG CCA GAG GCT AC-3'

Table S2.	PCR	primer	sets	used	in	this	study	1
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