Bhattaram et al., http://www.jcb.org/cgi/content/full/jcb.201405098/DC1

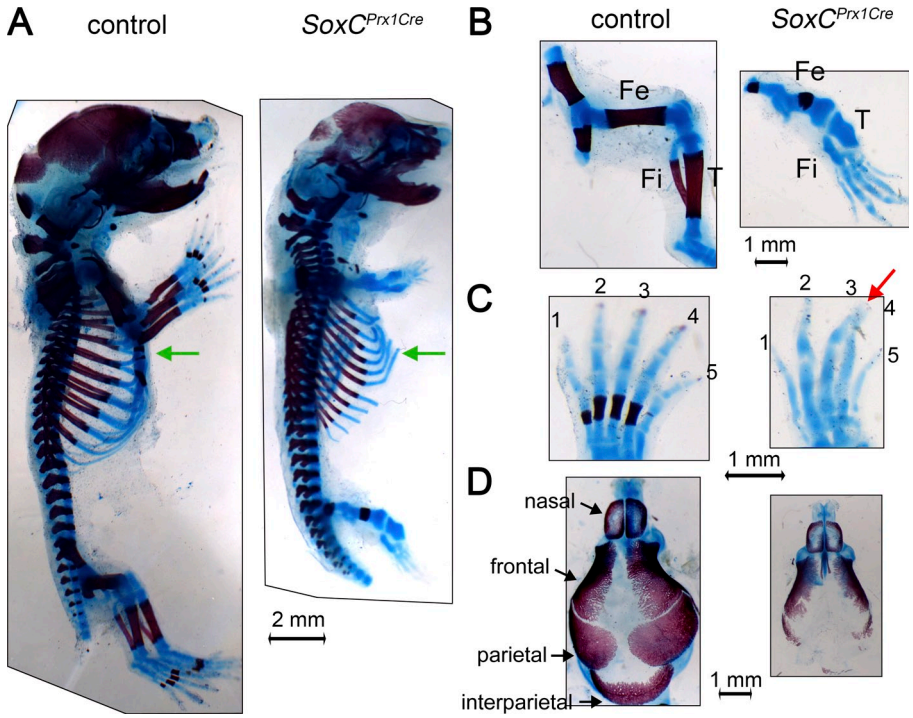

Figure S1. **Skeleton malformations in SOXC mutants.** Skeletons were stained with Alcian blue (nonmineralized cartilage) and Alizarin red (mineralized tissue). (A) Side views. (B and C) High-magnification views of hind limbs and hind paws, respectively. (D) Top views of heads. Fe, femur; Fi, fibula; T, tibia. Digits are numbered in the paws. The green arrow points to the sternum in the control and absence of sternum in the mutant. Red arrow, fusion of digits 3 and 4. Data were reproduced using more than three litters of control and mutant embryos.

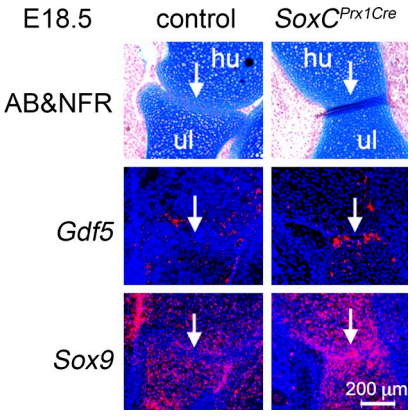

Figure S2. **SOXC genes secure the nonchondrocytic fate of joint cells.** Adjacent sections of elbow joints from E18.5 wild-type and *SoxC*^{Prx1Cre} forelimbs were stained with alcian blue and nuclear fast red (AB&NFR) or were hybridized with RNA probes (red signals) and counterstained with DAPI (blue dye), as indicated. hu, humerus; ul, ulna. Arrows, elbow joint. Data were reproduced three times using samples from different embryos.

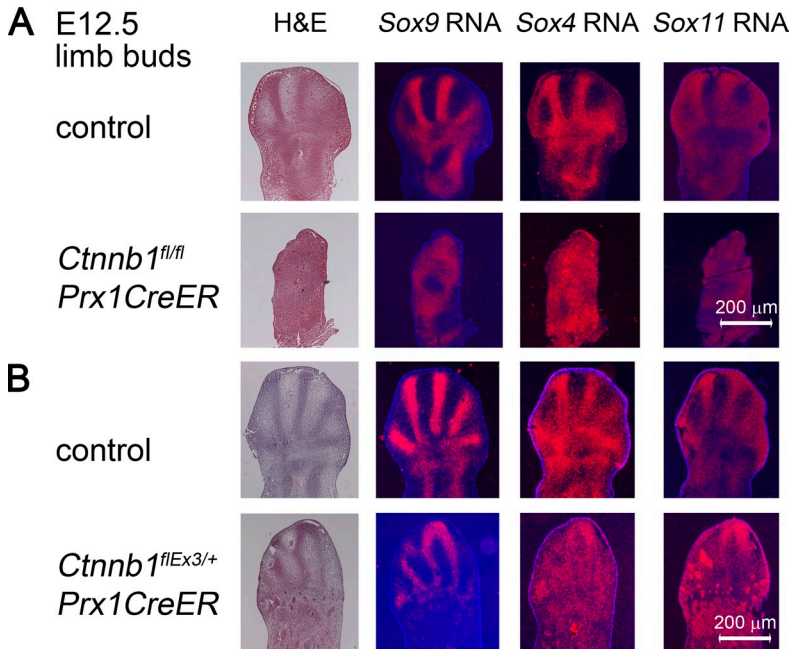

Figure S3. ***SoxC* expression in *Ctnnb1* mutant limbs.** (A and B) Limb bud sections from E12.5 control and *Ctnnb1*$^{fl/fl}$*Prx1CreER* (A) and *Ctnnb1*$^{flEx3/+}$ *Prx1CreER* (B) were stained with hematoxylin and eosin (H&E) or hybridized with RNA probes, as indicated. Data were reproduced using three pairs of control and mutant embryos.

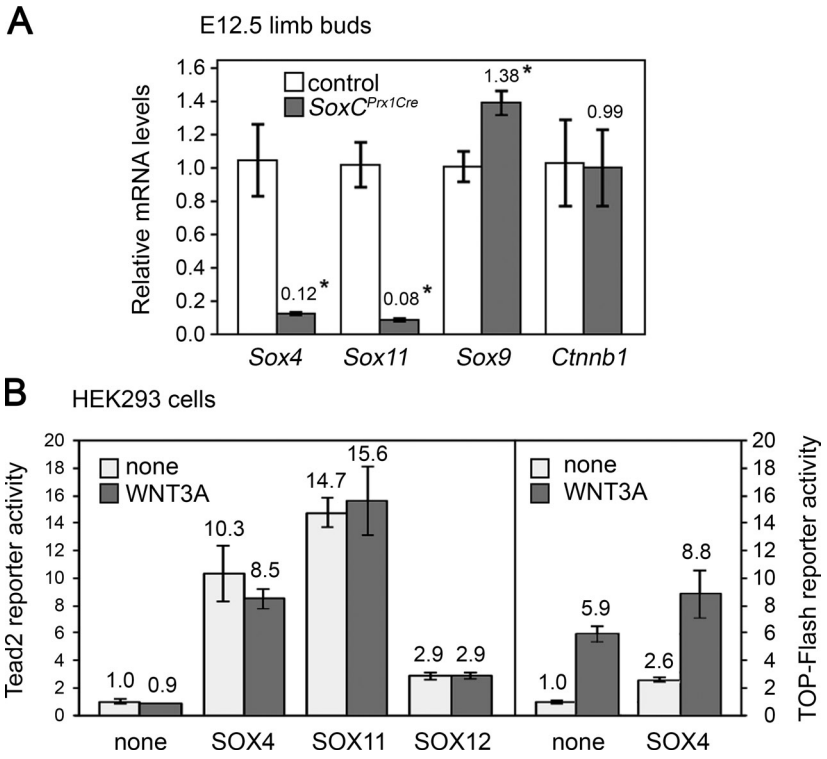

Figure S4. **Test of functional interaction between SOXC and β-catenin.** (A) *Sox4*, *Sox11*, *Sox9*, and *Ctnnb1* mRNA levels measured by qRT-PCR relative to *Gapdh* RNA level in limb buds from E12.5 control and *SoxC*^{Prx1Cre} littermates. Each assay was performed using control and mutant embryos from three distinct litters. Data are presented as mean values with standard deviation. *, $P < 0.05$. (B) SOXC-dependent Tead2 or β-catenin–dependent TOP-Flash reporter activities in HEK293 cells transfected with SOXC expression plasmids for 24 h. 20% WNT3A medium was added for the last 6 h. Normalized reporter activities are presented as mean values with standard deviation of triplicates in a representative experiment. TOP-Flash values prove that WNT3A medium was active. All data were reproduced in at least three independent experiments. Each panel shows the results of one representative experiment.

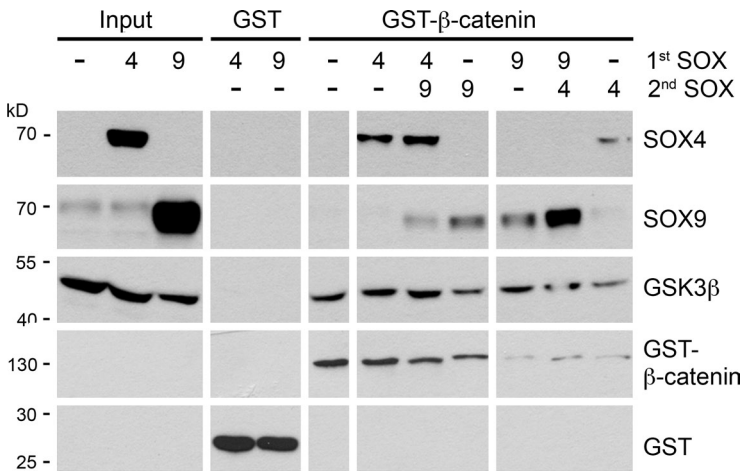

Figure S5. **Physical interaction of SOX proteins with β-catenin.** An in vitro GST pull-down assay was performed according to GST Protein Interaction Pull-Down kit (Thermo Fisher Scientific). In brief, 100 ng of purified GST protein (Sigma-Aldrich) or N-terminal GST–β-catenin protein (Novus Biologicals) was bound to glutathione agarose beads for 2 h and was subsequently incubated for 16 h (first SOX) and then for 4 h (second SOX) with extracts from HEK293 cells expressing no exogenous protein (−), 3FLAG-SOX4 (4), or 3FLAG-SOX9 (9). Inputs and eluted proteins were detected by Western blotting. Western blots are shown for a representative experiment. Vertical white lines were added in blot pictures to indicate that the order of lanes was rearranged for clarity of presentation. The data are representative of those obtained in two independent experiments.

Table S1. **Frequency of syndactyly in *SoxC/Ctnnb1* partial and compound mutants**

Phenotype	*Ctnnb1*		*SoxC*		*Ctnnb1/SoxC* compound mutants
	Controls	Partial mutants	Controls	Partial mutants	
Soft-tissue fusions in toes 3 and 4	0/6	0/6	0/9	2/9	9/11[a]
Cartilage fusions in toes 3 and 4	0/6	0/6	0/9	0/9	6/11[a]

Ctnnb1$^{fl/fl}$*Prx1CreER* males were bred with wild-type females to obtain control (no *Prx1CreER*) and *Ctnnb1*$^{fl/+}$*Prx1CreER* (partial mutant) littermates. *Sox4*$^{f/fl}$*11*$^{fl/fl}$*12*$^{-/-}$ *Prx1CreER* males were bred with Sox*11*$^{fl/f}$*Ctnnb1*$^{fl/+}$ females to obtain control, *Sox4*$^{fl/+}$*11*$^{fl/fl}$*12*$^{+/-}$*Prx1CreER* (*SoxC* partial mutant) and *Sox4*$^{fl/+}$*11*$^{fl/fl}$*12*$^{+/-}$*Ctnnb1*$^{fl/+}$ *Prx1CreER* (*SoxC/Ctnnb1* compound mutant) littermates. Pregnant females were injected with tamoxifen at gestation day 10.5, and embryos were analyzed at E14.5.

[a]$P < 0.05$, for mutants compared to controls or partial mutants, calculated using a two-tailed Fisher's exact test.

Table S2. **RNA probe plasmids**

Gene	Plasmid
Ccnd1	Mouse *Ccnd1* cDNA sequence cloned in pCR4-TOPO vector (Invitrogen). Forward primer, 5′-T CACAGCGGTAGGGATGAAATAG-3′; reverse primer, 5′-CTCTCAGACATGGCCCTAAAC-3′
Gdf5	Dy et al., 2010
HoxD13	Hérault et al., 1996
Lef1	Mouse *Lef1* cDNA sequence cloned in pCR4-TOPO vector (Invitrogen). Forward primer, 5′-TGGCATCCCTCATCCAGCTATTGT-3′; reverse primer, 5′-AAGAGGTGGCAGTGACTGTGTCTT-3′
Msx1	Hill et al., 1989
Sox4	Dy et al., 2008
Sox11	Dy et al., 2008
Sox12	Dy et al., 2008
Sox9	Dy et al., 2010
Tgfbr2	Lawler et al., 1994
Wnt4	Mouse *Wnt4* cDNA sequence cloned in pCR4-TOPO vector (Invitrogen). Forward primer, 5′-GCAGATGTGCAAACGGAACCTTGA-3′; reverse primer, 5′-ATGTGGCTTGAACTGTGCATTCCG-3′
Wnt9a	Später et al., 2006

Table S3. **Antibodies used in this study**

Target	Antibody type	Assay	Dilution	Source, catalog no.
Axin1	Rabbit, polyclonal	Western blot	1:2,000	Cell Signaling Technology, 4691
α-Tubulin	Mouse, monoclonal	Western blot	1:5,000	Abcam, ab7291
Axin1	Mouse, monoclonal	IP	5 µg/IP	EMD Millipore, 2087
β-Actin	Mouse, monoclonal	Western blot	1:5,000	Santa Cruz Biotechnology, Inc., sc-47778 (c4)
β-Catenin	Mouse, monoclonal	Western blot; immunostaining	1:1,000; 1:200	BD, 610153
pβ-Catenin (S33/37T41)	Rabbit, polyclonal	Western blot	1:500	Cell Signaling Technology, 9561
pβ-Catenin (S45)	Rabbit, polyclonal	Western blot	1:500	Cell Signaling Technology, 9564
Fibronectin	Mouse, monoclonal	Immunostaining	1:500	Life Technologies, 132600
FLAG	Mouse, monoclonal, HRP conjugated	Western blot	1:1,000	Sigma-Aldrich, A8952
GAPDH	Mouse, HRP conjugated	Western blot	1:5,000	Sigma-Aldrich, G9295
GST	Mouse, monoclonal	Western blot	1:2,000	Santa Cruz Biotechnology, Inc., sc-374171
GSK3β	Rabbit, polyclonal	Western blot	1:1,000	Cell Signaling Technology, 9315
HDAC1	Rabbit, polyclonal	Western blot	1:2,000	Biovision, 3601
IgG, rabbit	Goat, HRP-conjugated	Western blot	1:5,000	Bio-Rad Laboratories, 170-6515
IgG, rabbit	Goat, Alexa Fluor 594 conjugated	Immunostaining	1:200	Life Technologies, A-11037
IgG, mouse	Goat, HRP conjugated	Western blot	1:2,000	Bio-Rad Laboratories, 170-6516
IgG, mouse	Nonimmune	IP	5 µg/IP	EMD Millipore, 12-371
IgG, mouse	Goat, Alexa Fluor 488 conjugated	Immunostaining	1:200	Life Technologies, A11017
Na^+/K^+ ATPase	Rabbit, polyclonal	Western blot	1:1,000	Abcam, ab7671
LRP6	Rabbit, polyclonal	Western blot	1:1,000	Cell Signaling Technology, 2568
pLRP6	Rabbit, polyclonal	Western blot	1:1,000	Cell Signaling Technology, 3395
SOX9	Rabbit, polyclonal	Western blot; immunostaining	1:2,000; 1:200	EMD Millipore, AB5535

Table S4. **qRT-PCR primers**

Gene	Primer	Sequence
mCtnnb1	Forward	5′-GATGTAGAGACAGCTCGTTGT-3′
	Reverse	5′-GGCGTAGAACAGTACAGAATCC-3′
mGapdh	Forward	5′-GCACAGTCAAGGCCGAGAAT-3′
	Reverse	5′-GCCTTCTCCATGGTGGTGAA-3′
mSox4	Forward	5′-GCCTCCATCTTCGTACAACC-3′
	Reverse	5′-AGTGAAGCGCGTCTACCTGT-3′
mSox11	Forward	5′-ATCAAGCGGCCCATGAAC-3′
	Reverse	5′-TGCCCAGCCTCTTGGAGAT-3′
mSox9	Forward	5′-TCCACGAAGGGTCTCTTCTC-3′
	Reverse	5′-AGGAAGCTGGCAGACCAGTA-3′

Table S5. **Reporter and expression plasmids**

Plasmid	Description
FLAG-SOX4	Mouse SOX4 coding sequence cloned in frame with a N-terminal FLAG peptide in the pcDNA3.1 mammalian expression plasmid (Dy et al., 2008)
FLAG-SOX11	Mouse SOX11 coding sequence cloned in frame with a N-terminal FLAG peptide in pcDNA3.1 (Dy et al., 2008)
FLAG-SOX12	Mouse SOX12 coding sequence cloned in frame with a N-terminal FLAG peptide in pcDNA3.1 (Dy et al., 2008)
β-Catenin–FLAG	*Xenopus laevis* β-Catenin coding sequence cloned in frame with two copies of a C-terminal FLAG peptide in the pCS2 mammalian expression plasmid (Lee et al., 2001)
Stabilized β-catenin–FLAG	*Xenopus* β-catenin coding sequence with S33A, S37A, and T45A point mutations, cloned in frame with two copies of a C-terminal FLAG peptide in pCS2 plasmid (Lee et al., 2001)
3FLAG-SOX4	Mouse SOX4 coding sequence cloned in frame with an N-terminal 3-copy-FLAG-linker peptide in pcDNA3.1
3FLAG-SOX9	Human SOX9 coding sequence cloned in frame with an N-terminal 3-copy-FLAG-linker peptide in pcDNA3.1
3FLAG-SOX11	Mouse SOX11 coding sequence cloned in frame with an N-terminal 3-copy-FLAG-linker peptide in pcDNA3.1
TOP-Flash	Eight copies of TCF/LEF binding sites cloned upstream of the firefly luciferase coding sequence in the pTA-Luc vector (Veeman et al., 2003)
Tead2 reporter	Mouse *Tead2* promoter and intron-1 sequence containing SOXC protein binding sites, cloned upstream of the firefly luciferase coding sequence in the pA3-Luc vector (Bhattaram et al, 2010)
pSV2βgal	*Escherichia coli lacZ* gene driven by SV40 regulatory elements (Promega)
pmaxGFP	GFP gene driven by the cytomegalovirus promoter (Lonza)

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