

SUPPLEMENTARY FIGURES

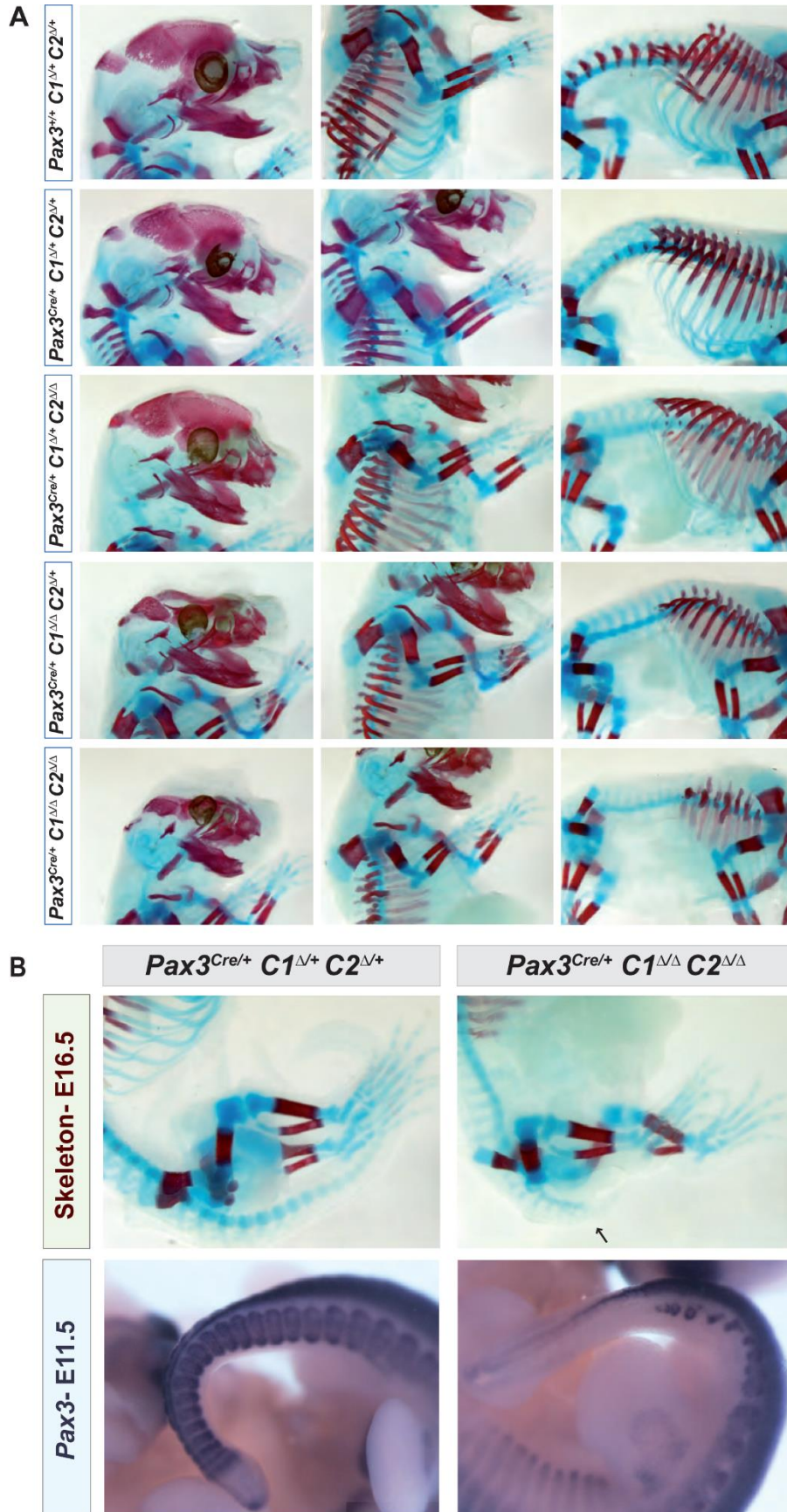


Figure S1. Skeletal defects and tail truncations in *Foxc1/c2* conditional mutant embryos

(A) Skeletal preparations of control $Pax3^{+/+};Foxc1^{flox/+};Foxc2^{flox/+}$ ($Pax3^{+/+} C1^{\Delta/+} C2^{\Delta/+}$), double heterozygous $Pax3^{Cre/+};Foxc1^{flox/+};Foxc2^{flox/+}$ ($Pax3^{Cre/+} C1^{\Delta/+} C2^{\Delta/+}$), *Foxc2* mutant $Pax3^{Cre/+};Foxc1^{flox/+};Foxc2^{flox/flox}$ ($Pax3^{Cre/+} C1^{\Delta/+} C2^{\Delta/\Delta}$), *Foxc1* mutant $Pax3^{Cre/+};Foxc1^{flox/flox};Foxc2^{flox/+}$ ($Pax3^{Cre/+} C1^{\Delta/\Delta} C2^{\Delta/+}$), and double conditional mutant ($Pax3^{Cre/+}; Foxc1^{flox/flox}; Foxc2^{flox/flox}$ ($Pax3^{Cre/+} C1^{\Delta/\Delta} C2^{\Delta/\Delta}$) embryos, at E16.5, reveal major defects in the absence of *Foxc1* and/or *Foxc2* in the cranial skeleton (neural crest derived), ribs and vertebrae (somite derived). Alcian blue stains non-mineralized cartilage, while Alizarin red stains mineralized bone and cartilage. **(B)** Upper panels of skeletal preparations show the absence (arrow) of posterior vertebrae (after the hindlimb) in the tail of double conditional mutant embryos ($Pax3^{Cre/+} C1^{\Delta/\Delta} C2^{\Delta/\Delta}$) compared to heterozygote controls ($Pax3^{Cre/+} C1^{\Delta/+} C2^{\Delta/+}$), at E16.5. Lower panels show the tail region of E11.5 embryos after whole mount *Pax3* *in situ* hybridisation, demonstrating the lack of posterior somites, marked by *Pax3* expression in the heterozygote control.

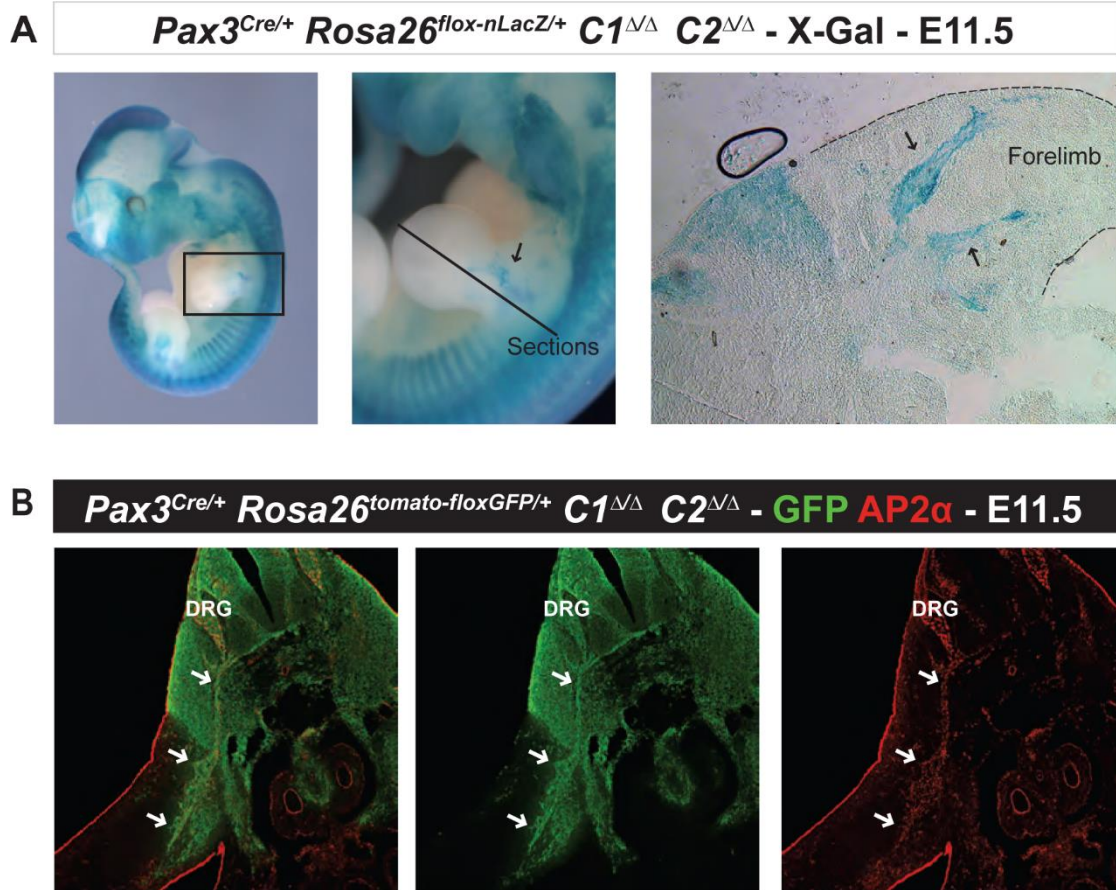


Figure S2. Neural crest derivatives in the forelimb

(A) X-Gal staining of a *Pax3^{Cre/+};Rosa26^{flox-nLacZ};Foxc1^{flox/flox};Foxc2^{flox/flox}* (*Pax3^{Cre/+} Rosa26^{flox-nLacZ/+} C1^{Δ/Δ} C2^{Δ/Δ}*) embryo at E11.5 showing the labelled structure in the proximal forelimb derived from *Pax3* expressing progenitors indicated by arrows in the close up and the section. (B) Immunostaining, with antibodies to GFP and AP2 α that marks neural crest cells, of a section at forelimb level of a *Pax3^{Cre/+};Rosa26^{tomato-floxGFP/+};Foxc1^{flox/flox};Foxc2^{flox/flox}* (*Pax3^{Cre/+} Rosa26^{tomato-floxGFP/+} C1^{Δ/Δ} C2^{Δ/Δ}*) embryo at E11.5 showing neural crest cells in the dorsal root ganglia (DRG) and extending into the forelimb (arrow). These cells contribute to the sympathetic nervous system in the limbs.

SUPPLEMENTARY MATERIALS AND METHODS

Table S1. Primary antibodies

(IF, Immunofluorescence on section; Wh, Whole mount immunofluorescence; DSHB, *Developmental Studies Hybridoma Bank*)

Antibodies	Application	Source	Dilution
Monoclonal mouse Anti- Pax3	IF	DSHB (Pax3-c)	1/250
Monoclonal mouse Anti- MF20	IF	DSHB (MF-20-c)	1/250
Polyclonal rabbit Anti- Myogenin (M-225)	IF	Santa Cruz (sc-576) #J2813	1/250
Polyclonal rabbit Anti- MyoD (C-20)	IF	Santa Cruz (sc-304) #D2709	1/250
Monoclonal mouse Anti- Myosin (Skeletal, Fast) Alkaline Phosphatase Conjugate	Wh IF	Sigma (C6198) #051M4773	1/1000
Monoclonal rat Anti- CD31 (Pecam-1)	IF	BD Pharmingen (550274) #2243973	1/250
Polyclonal rabbit Anti- Myf5 (C-20)	IF	SantaCruz (sc-302) # H1407	1/250
Monoclonal mouse Anti- AP-2 alpha	IF	DSHB (5E4)	1/250
Polyclonal rabbit Anti- Zo-1	IF	Invitrogen (61-7300) #636050A	1/150
Polyclonal rabbit Anti- Lbx1	IF	Gift of Dr. C.Birchmeier	1/5000
Polyclonal chicken Anti- GFP	IF	Life Technologies (A10262) #1602788	1/500

DSHB: Developmental Studies Hybridoma Bank

All antibodies were used on control sections at the same time as the experiment on mutant sections, as shown in the figures.

Table S2. RT-qPCR primer sequences

Gene Name	Forward	Reverse
<i>Foxc1</i>	AGAGCCAAATGGAATGGAAC	ATTCTGTTCGCTGGTGTGAG
<i>Foxc2</i>	GCAACCCAACAGCAAACCTTC	GACGGCGTAGCTCGATAGG
<i>Gapdh</i>	GGCAAAGTGGAGATTGTTGC	AATTTGCCGTGAGTGGAGTC
<i>Lbx1</i>	CTCGCCAGCAAGACCTTTA	AAAGCGTTTCTCCAACCTCGT
<i>Flk1</i>	GTCGACATAGCCTCCACTGTTT	GTGATGTACACGATGCCATGCT