

## Materials and Methods

**Transgenic mice:** All the animal work was performed at the University of California, Berkeley, and the University of California, Los Angeles, and approved by the Animal Care and Use Committee of each university. Sox10-Cre mice were generated in Dr. Andrew S. McCallion's laboratory,<sup>1</sup> and maintained by breeding male Sox10-Cre mice and female wild-type C57BL/6J (#000664) mice. Wnt1-Cre mice (#022501) and Rosa-LoxP-RFP mice (#007909) were purchased from The Jackson Laboratory. Sox10-Cre/Rosa-LoxP-RFP and Wnt1-Cre/Rosa-LoxP-RFP mice were generated by breeding male Cre and female Rosa-LoxP-RFP mice. PCR genotyping was performed using the primers against Cre and Rosa-LoxP-RFP, according to the protocols of The Jackson Laboratory. Six male Sox10-Cre/Rosa-LoxP-RFP mice of two months old and three Wnt1-Cre/Rosa-LoxP-RFP mice of one month old were used for histological analysis of different tissues and organs.

**Histological analysis:** The mouse tissues and organs were fixed in 4% paraformaldehyde for half hour at room temperature, transferred to 15% and 30% sucrose overnight in cold room, and then embedded in OCT for cryosection. For immunostaining, the tissue sections were permeabilized in 0.5% Triton X-100 (Sigma) for 5 minutes, blocked with 5% donkey serum (Jackson Immuno Research Labs) for 1 hour, incubated in primary antibodies for 2 hours at room temperature or overnight at cold room. After primary antibody incubation, the samples were washed with 0.1% Triton for 3 times, incubated with corresponding secondary antibodies (Molecular Probes) for 2 hours and 4',6-diamidino-2-phenylindole (DAPI) for 30 minutes. The slides were washed, mounted by Fluoromount (Southern Biotech) and imaged by confocal microscopy (Prairie, Zeiss LSM710 and Leica SP5). The following primary antibodies were used: CD31 (1:100, ab28364, abcam), ACTA2 (1:100, ab32575, abcam), NG2 (1:100, AB5320, EMD Millipore), MYH11 (1:100, BT-562, Biomedical Technologies), Sox10 (1:30, sc-17342, Santa Cruz Biotech).

## References:

1. Stine ZE, Huynh JL, Loftus SK, Gorkin DU, Salmasi AH, Novak T, Purves T, Miller RA, Antonellis A, Gearhart JP, Pavan WJ, McCallion AS. Oligodendroglial and pan-neural crest expression of cre recombinase directed by sox10 enhancer. *Genesis*. 2009;47:765-770