Supporting Information

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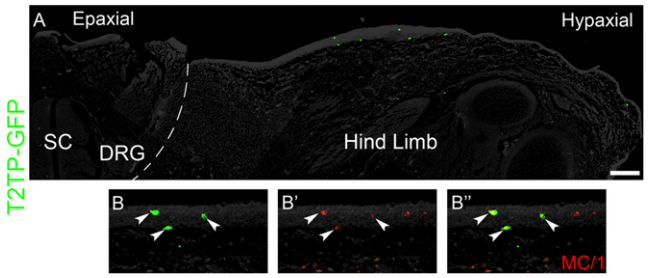

Fig. S1. Schwann cell progenitor (SCP)-derived melanocytes colonize the hypaxial domain. (*A–B″*) Transverse sections through the hindlimb region of the embryo presented in Fig. 2 *F–I*, showing restricted localization of GFP$^+$/MC/1$^+$ cells to the hypaxial limb domain with no crossing toward the epaxial region. Dotted line separates epaxial from hypaxial areas. (*B–B″*) High magnifications of a hypaxial region in *A* showing colocalization of the GFP lineage tracer with the melanocyte marker MC/1 (arrowheads). Methods: ventral to dorsal coelectroporation of pCAGG-T2TP and pT2K-EGFP was performed at embryonic day (E)2 to label SCPs that reach and associate with spinal nerves. Sixteen hours later, embryos were inspected to ensure that no residual GFP$^+$ cells remained in the NT (i.e., that the prospective late-emigrating, neural crest (NC)-derived melanocytes are unlabeled). At E6–E7, the localization of GFP$^+$ nerve-derived melanocytes cells was monitored (details in Fig.2). DRG, dorsal root ganglion; SC, spinal cord. (Scale bar, 40 μm in *A*; 20 μm in *B–B″*.)

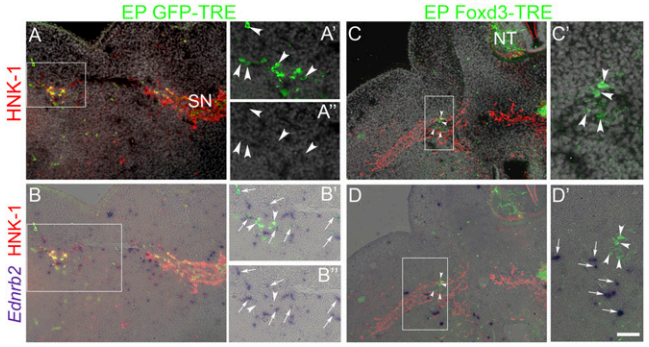

Fig. S2. Continuous expression of Foxd3 in SCP inhibits melanocyte development. (*A–D*) Transverse sections through E5.5 embryos electroporated at E2 (hindlimb level) with pCAGGS-rtTA2s-M2 together with either pBI-TRE-GFP (*A–B″*) or pBI-TRE-Foxd3 (*C–D′*). Doxycyclin was added 2 d later (see below for details and also legend to Fig. 3). In control embryos, GFP$^+$ cells are attached to the nerve, where they coexpress HNK-1 (*A*). Because HNK-1 is also expressed by the spinal nerve fibers, *A′* and *A″* show GFP$^+$ staining surrounding Hoechst$^+$ nuclei [i.e., corresponding to nerve-associated SCPs (arrowheads)]. In addition, control GFP$^+$ cells are distributed in the dermis and in the vicinity of nerve fibers, where many coexpress *Ednrb2* (arrows in *B′* and *B″*; arrowheads point to few GFP$^+$/*Ednrb2*- cells). In contrast, Foxd3$^+$ cells are absent from the dermis/epidermis. Instead they are only found in HNK-1–expressing SCPs associated with nerve fibers (arrowheads in *C* and *C′*), where they fail to up-regulate *Ednrb2* (arrowheads in *D* and *D′*). Arrows in *D′* represent *Ednrb2*$^+$ melanocytes that are negative for Foxd3-TRE. HL, hindlimb; NT, neural tube; SN, spinal nerve. In all panels, lateral is to the left. (Scale bar, 50 μm in *A–D*; 40 μm in *B* and *B′*; 30 μm in *A′*, *A″*, and *D′*; 20 μm in *C′*.)

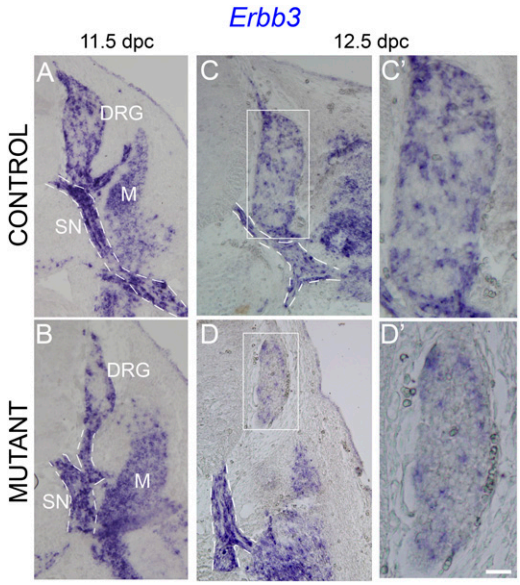

Fig. S3. Expression of *Erbb3* in control and Foxd3 mutants. Transverse sections showing expression of *Erbb3* mRNA in control (*A*, *C*, and *C′*) and FoxD3 mutant (*B*, *D*, and *D′*) embryos at 11.5 and 12.5 days post-coitum (dpc). A strong signal is apparent in SCPs lining the spinal nerves in all cases (nerves delimited by dotted white lines). In contrast, mutant DRG exhibit reduced *Erbb3* expression that is mainly localized to cells in the ganglion periphery; in controls, *Erbb3*-positive cells are distributed throughout the DRG. Note that overall DRG size is slightly reduced in mutants. (*C′* and *D′*) Higher magnifications of DRG in *C* and *D*, respectively. *Erbb3* is also transcribed in skeletal muscle (M). (Scale bar, 90 μm in *A*; 80 μm in *B*; 60 μm in *C* and *D*; 30 μm in *C′* and *D′*.)

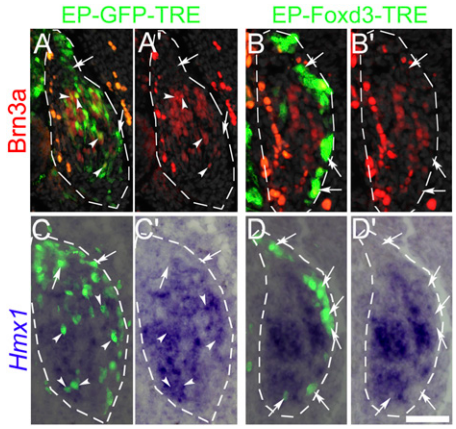

Fig. S4. Conditional Foxd3 misexpression in DRG inhibits sensory neurogenesis. Expression of the sensory neuron markers Brn3a and *Hmx1* in E4 DRG, electroporated with pCAGGS-rtTA2s-M2 along with either pBI-TRE-GFP (control; *A*, *A′*, *C*, and *C′*) or pBI-TRE-Foxd3 (*B*, *B′*, *D*, and *D′*) at E2, and injected with doxycyclin at E3 (details in legend to Fig. 5). Note homogeneous localization of control GFP^+ cells throughout the DRG (*A* and *C*), contrasting with the restricted distribution of Foxd3/GFP^+ cells to the ganglion periphery (*B* and *D*). Arrowheads in *A* and *C* point to neurons coexpressing control GFP and Brn3a or *Hmx1*. No such double-positive cells were detected in Foxd3-treated ganglia (arrows in *A*–*D′*). (Scale bar, 30 μm.)

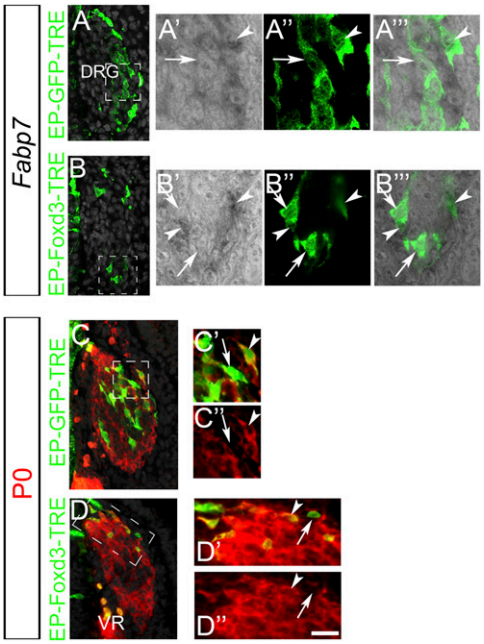

Fig. S5. Sustained Foxd3 activity in DRG is compatible with glial development. Electroporation of control GFP (*A* and *C*) or conditional misexpression of Foxd3 in DRG (*B* and *D*). pCAGGS-rtTA2s-M2 together with either pBI-TRE-GFP or pBI-TRE-Foxd3 were electroporated into hemitubes at E2. Doxycyclin was added 32 h after electroporation when NC cells already formed DRG, and analysis performed 24 h later. (*A* and *B*) Costaining of GFP with *Fabp7*. (*C* and *D*) Costaining of GFP with melyn protein zero (P0). Arrowheads point to double-positive glia cells and arrows to GFP^{+}/*Fabp7* or P0-negative progenitors. (*A′–A‴*, *B′–B‴*, *C′–C″*, and *D′–D″*) Higher magnifications of the boxed areas in *A–D*, respectively. (Scale bar, 60 μm in *A*, *C*, and *D*; 50 μm in *B*; 20 μm in *A′–A‴* and *B′–B‴*; 25 μm *C′–C″*; 30 μm in *D′–D″*.)