Supporting Information

Coppola et al. 10.1073/pnas.0910213107

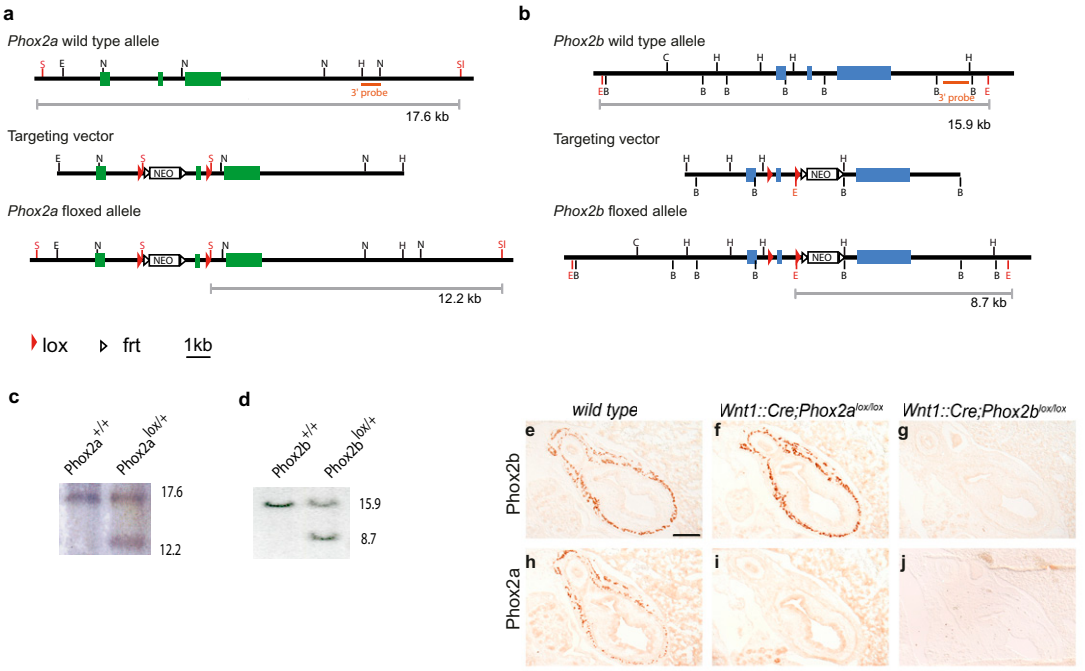

Fig. S1. Generation and validation of the conditional *Phox2a* and *Phox2b* knockout mice. (*A* and *B*) Schematics showing the *Phox2a* and *Phox2b* wild type alleles (*Top*), the targeting vectors (*Middle*), and the expected floxed alleles (*Bottom*). In both constructs, *lox* sites surround exon 2, which contains most of the homeodomain. B, BamHI; C, ClaI; E, EcoRI; H, HindIII; N, NcoI; S, Spe; SI, SalI. (*C* and *D*) Southern blot analysis of embryonic stem cell clones showing the correct targeting at the *Phox2a* (*C*) and *Phox2b* (*D*) loci, using the restriction enzymes and probes indicated in *A* and *B*. (*E–J*) Sagittal sections through the trunk of E13.5 embryos of the indicated genetic backgrounds, stained by immunohistochemistry with Phox2b (*E–G*) or Phox2a (*H–J*) to monitor recombination efficiency of the floxed *Phox2a* and *Phox2b* alleles by a *Wnt1::Cre* allele. In *Wnt1::Cre*, *Phox2a*$^{lox/lox}$ embryos the enteric neurons are devoid of Phox2a (*I*), but present (*F*), whereas in *Wnt1::Cre*, *Phox2b*$^{lox/lox}$ embryos they are absent (*G* and *J*) because of their dependency on *Phox2b* [Pattyn A, Morin X, Cremer H, Goridis C, Brunet J-F (1999) The homeobox gene *Phox2b* is essential for the development of autonomic neural crest derivatives. *Nature* 399:366–370.]. (Scale bar, 200 μm.)

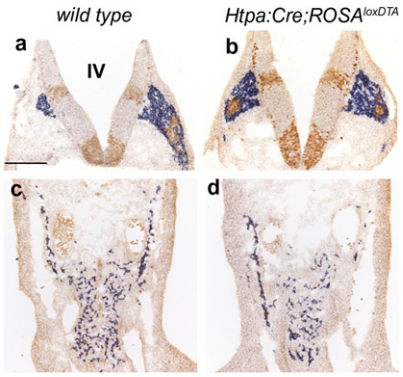

Fig. S2. Persistence of neural crest cells in *Htpa::Cre;Rosa*$^{lox\text{-}stop\text{-}lox\text{-}DTA}$ mutants. (*A–D*) Transverse sections through the head (*A* and *B*) or trunk (*C* and *D*) of E9.5 embryos in the indicated genetic background, hybridized with a *Sox10* probe (blue), followed by Phox2b immunohistochemistry (*orange*). Neural crest derived cells are present in the geniculate ganglion (*B*) and in the migrating enteric neurons (*D*) of *Htpa::Cre;Rosa*$^{lox\text{-}stop\text{-}lox\text{-}DTA}$ mutants. (Scale bar, 200 μm.)

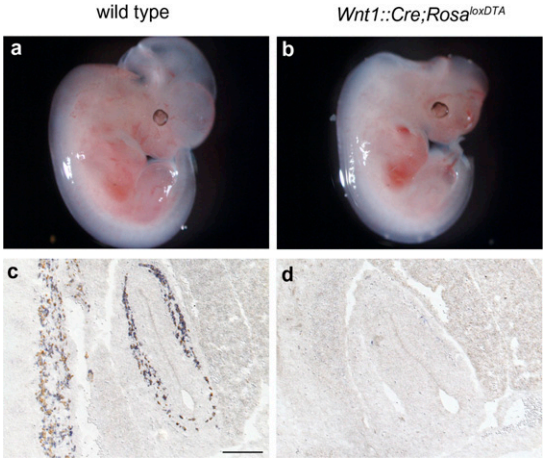

Fig. S3. Altered morphology and absence of neural crest derived neurons in *Wnt1::Cre;Rosa*$^{lox\text{-}stop\text{-}lox\text{-}DTA}$ embryos. (*A* and *B*) Control and *Wnt1::Cre; Rosa*$^{lox\text{-}stop\text{-}lox\text{-}DTA}$ embryos at E11.5, showing the abnormal morphology of the head region in *Wnt1::Cre; Rosa*$^{lox\text{-}stop\text{-}lox\text{-}DTA}$ mutants. (*C* and *D*) Sagittal sections of E11.5 of wild type and *Wnt1::Cre; Rosa*$^{lox\text{-}stop\text{-}lox\text{-}DTA}$ embryos at trunk level, hybridized with a *Sox10* probe (blue), followed by Phox2b immunohistochemistry (orange). No neural crest-derived cell is found in *Wnt1::Cre;Rosa*$^{lox\text{-}stop\text{-}lox\text{-}DTA}$ mutants. (Scale bar, 200 μm.)

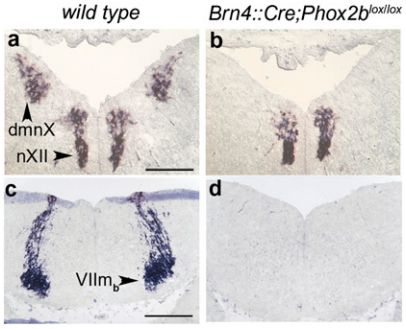

Fig. S4. Branchial and visceral motoneurons neurons are missing in *Brn4::Cre;Phox2b*$^{lox/lox}$ embryos. (*A–D*) Transverse sections through the hindbrain of wild-type and *Brn4::Cre;Phox2b*$^{lox/lox}$ embryos at E13.5 hybridized with a *Peripherin* probe showing that the dorsal motor nucleus of the vagus nerve (dnmX) (*A* and *B*) and the facial branchiomotor neurons (VIIm$_b$) (*C* and *D*) are missing in the mutant, just like in *Phox2b* knockouts [Pattyn A, Morin X, Cremer H, Goridis C, Brunet J-F (1999) The homeobox gene *Phox2b* is essential for the development of autonomic neural crest derivatives. *Nature* 399:366–370.]. The motor nucleus of the hypoglossal nerve (nXII), made up of somatic motoneurons, never expresses *Phox2b* and is consequently not affected. [Scale bar: (*A* and *B*) 100 μm; (*C* and *D*) 200 μm.]

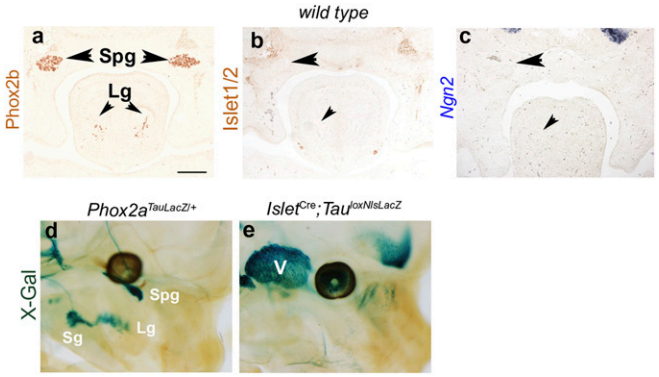

Fig. S5. Neither *Islet1* nor *Ngn2* expression is detected in parasympathetic ganglia. (*A*–*C*) Transverse sections through the head of E13.5 wild-type embryos, stained with immunohistochemistry with *Phox2b* (*A*) or *Islet1/2* (*B*) or hybridized with a *Ngn2* probe (*C*). The black arrowheads in *B* and *C* point to the unstained Spg and Lg ganglia. (*D* and *E*) X-gal staining on whole E13.5 embryos in the indicated genetic backgrounds. Expression of *LacZ* from the *Phox2a* locus reveals the Spg and S/Lg (*D*); no LacZ activity is detected in parasympathetic ganglia when reporter expression is controlled by the *Islet1* promoter (*E*). Lg: lingual ganglion; Sg: submandibular ganglion; Spg: sphenopalatine ganglion; V: trigeminal ganglion. (Scale bar, 200 μm.)

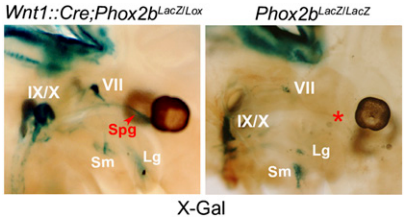

Fig. S6. Spg precursors are transiently detected in *Wnt1::Cre;Phox2b*$^{LacZ/lox}$ but not in *Phox2b*$^{LacZ/LacZ}$ mutants. X-gal staining on whole E12.5 embryos in the indicated genetic background. The red asterisk indicates the absence of the Spg precursors in *Phox2b*$^{LacZ/LacZ}$ embryos. Note that the VII and the IX/X ganglia are also affected in this mutant, but they are spared in *Wnt1::Cre; Phox2b*$^{LacZ/Lox}$ because of removal of *Phox2b* in neural crest cells only. Lg: lingual ganglion; Sg: submandibular ganglion; Spg: sphenopalatine ganglion; VII: geniculate ganglion; IX/X: petrose/nodose ganglionic complex.