

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Structure and manipulation of Hmx1 gene sequences. (A) Native structure of Hmx1 gene and mRNA, location of the *dmbo* mutation, and predicted structure of the truncated gene product. The expression domain selected for antibody generation (Methods) lies N-terminal to the conserved homeodomain and includes the predicted *dmbo* gene product. (B) Sequence comparison of native Hmx1 mRNA and codon-optimized sequence used for antibody generation. The location of the Hmx1 real-time PCR genotyping oligonucleotides are shown with red arrows (Methods).

Figure S2. Expression of Hmx1 in the statoacoustic ganglion and the superior ganglion of IX-X nerve. Sagittal sections of an E13.5 embryo are shown; A, C, and E show progressively more medial sections. (A-D) Immunofluorescence for Brn3a, which is widely expressed in sensory neurons, and Hmx1 show that Hmx1 is expressed in a discrete region of the lateral part of the statoacoustic ganglion (B, arrow). Hmx1 is also expressed in the superior ganglion of the IX-X complex in a scattered distribution that resembles that of the DRG. The inferior component of the IX-X ganglion complex (petrosal and/or nodose ganglion, circles in B,D) contains a small population of Brn3a⁺ neurons but no Hmx1⁺ cells. (E) Immunofluorescence reveals that Hmx1 is expressed in a subset of neurons in the sympathetic chain and stellate ganglia. (F) In situ hybridization data from the Allen Embryonic Atlas for Hmx1, Islet1 (sensory+sympathetic), and TH (sympathetic) confirms expression of Hmx1 in autonomic neurons. C2, DRG of spinal segment C2; Ctx, cerebral cortex (telencephalon); Inf IX/X, inferior ganglion of the IX/X ganglion complex; IO, inferior olive; P, pons; SAG, statoacoustic ganglion complex; SC, superior colliculus; SG, superior ganglion (of the IX/X ganglion

complex); Stel, stellate ganglion; Symp, sympathetic chain ganglia; TG, trigeminal ganglion; Scale A,C 400 μ M; B,D 100 μ M.

Figure S3. Trigeminal neurogenesis and subtype specification in $Hmx1^{dm/dm}$ embryos.

Sagittal sections of an E13.5 embryo are shown. Brn3a is employed here as a general marker of sensory neurogenesis, and TrkA and TrkC mark the nociceptor and proprioceptor subclasses, respectively. Hmx1 expression is restricted to the mnTG. (A,B) Brn3a and TrkA expression are unchanged in TG of an $Hmx1^{dm/dm}$ embryo compared to control. (C,D) TrkC expression is unchanged in the $Hmx1^{dm/dm}$ TG. HB, hindbrain; mand, mandibular branch of trigeminal nerve; max, maxillary branch of trigeminal nerve; mnTG, mandibular lobe of trigeminal ganglion; mxTG, maxillary lobe of trigeminal ganglion. Scale 100 μ M.

Figure S4. Expression of progenitor/glial marker Sox10 in trigeminal of $Hmx1^{dm/dm}$

embryos. Sagittal sections of an E13.5 embryo are shown. (A,B) Sox10 is expressed uniformly in glial precursors in the TG at this stage, and appears unchanged in $Hmx1^{dm/dm}$ embryos. Mand, mandibular branch of trigeminal nerve; max, maxillary branch of trigeminal nerve; mnTG, mandibular lobe of trigeminal ganglion; mxTG, maxillary lobe of trigeminal ganglion. Scale 100 μ M.

Figure S5. Marked loss of geniculate neurons at E14.5 in $Hmx1^{dm/dm}$ embryos.

Horizontal sections of E14.5 $Hmx1^{dm/dm}$ and control embryos were stained for Brn3a and β galactosidase expressed from a $Brn3a^{tlz}$ reporter allele. Brn3a immunoreactive nuclei were counted in each 16 μ M section. (A) Cell counts in the domain of the geniculate ganglion normally expressing

Hmx1. (B-G) Examples of the geniculate at various levels from dorsal (B,C) to ventral (F,G). Asterisks in (B,C) indicates the viscerosensory Hmx1-negative region of the geniculate ganglion. The weakly Brn3a⁺ neurons dispersed in this region were not counted. n7, facial nerve.

Video 1. OPT imaging of the sensory innervation of the head in E14.5 Hmx1^{dm/dm} and control embryos. Embryos bearing a Brn3a^{tlz} reporter allele were hemisected and stained for β galactosidase activity, cleared, and imaged through 400 rotational positions. This video is a composite generated from images through 360 degrees of rotation. R,C,D,V, rostral, caudal, dorsal, ventral. DRG, dorsal root ganglion; GG, geniculate ganglion; M, midbrain; mand, mandibular branch, trigeminal nerve; max, maxillary branch, trigeminal nerve; P, pons; pa, posterior auricular nerve; SC, spinal cord; SG, superior ganglion (of IX/X ganglion complex); TG, trigeminal ganglion.