Supporting Information

Sato *et al.* 10.1073/pnas.0807345105

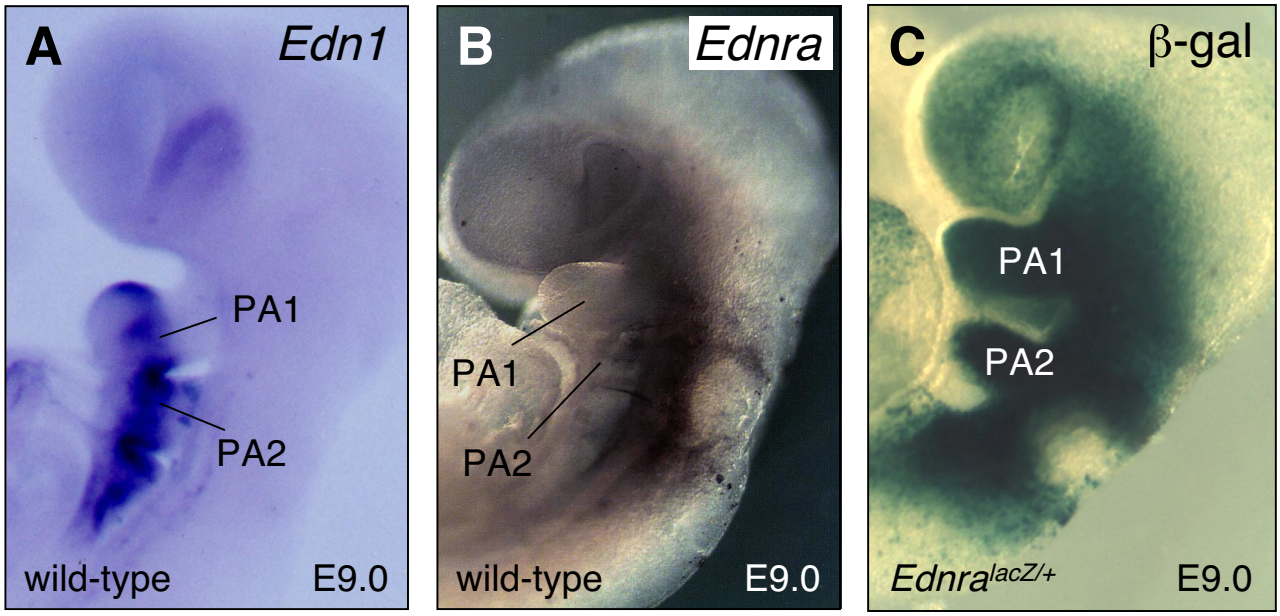

Fig. S1. Expression of *Edn1* and *Ednra* in the head and pharyngeal regions of E9.0 mouse embryos. (*A* and *B*) Whole-mount in situ hybridization for *Edn1* (*A*) and *Ednra* (*B*). (*C*) β-Galactosidase activity in *Ednra* $^{lacZ/+}$ embryos. The knocked-in *lacZ* expression mimics the endogenous *Ednra* expression shown in *B*.

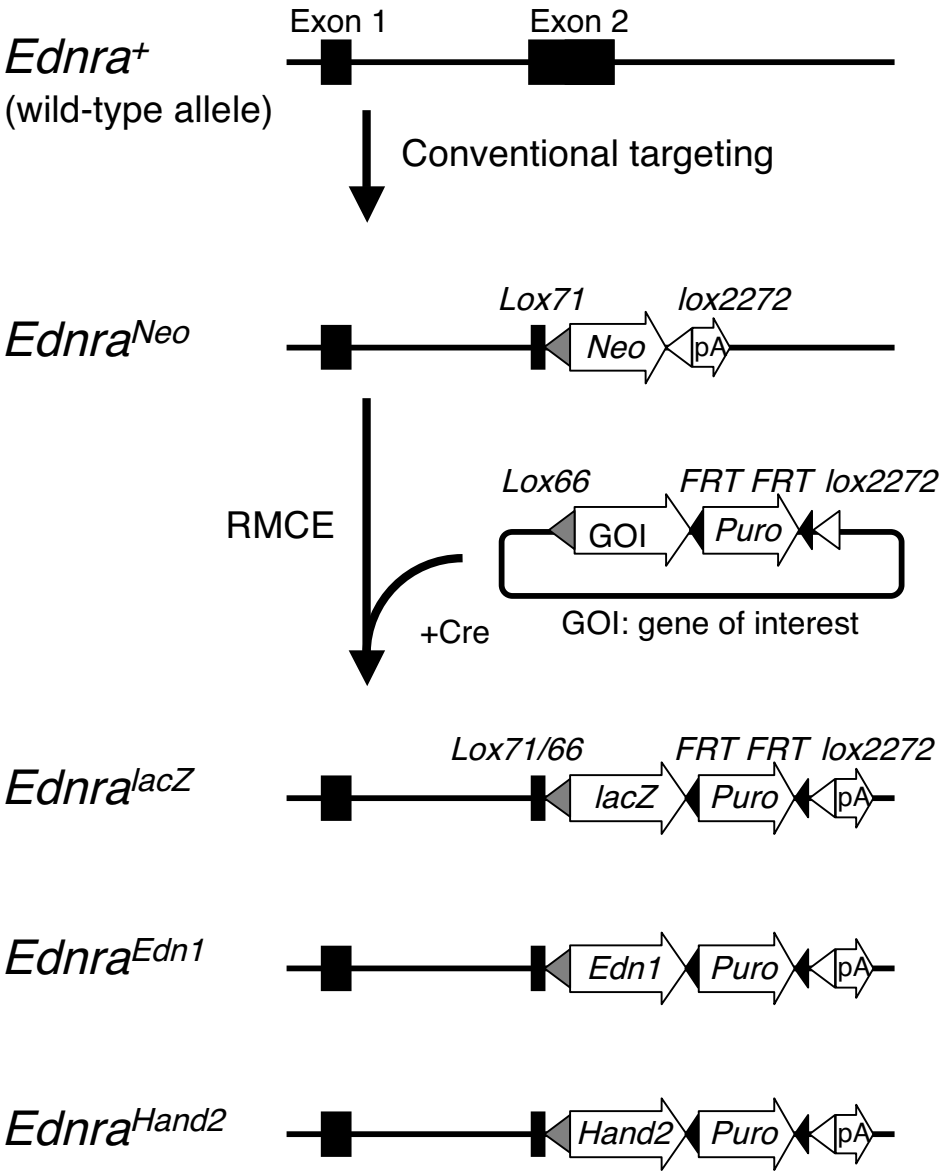

Fig. S2. Recombinase-mediated cassette exchange (RMCE) in the mouse *Ednra* gene. Wild-type allele (*Ednra*$^{+}$), first-step targeted allele (*Ednra*^{Neo}), *lacZ*-knocked-in allele (*Ednra*^{lacZ}), *Edn1*-knocked-in allele (*Ednra*^{Edn1}), *Hand2*-knocked-in allele (*Ednra*^{Hand2}), and knock-in vector are shown.

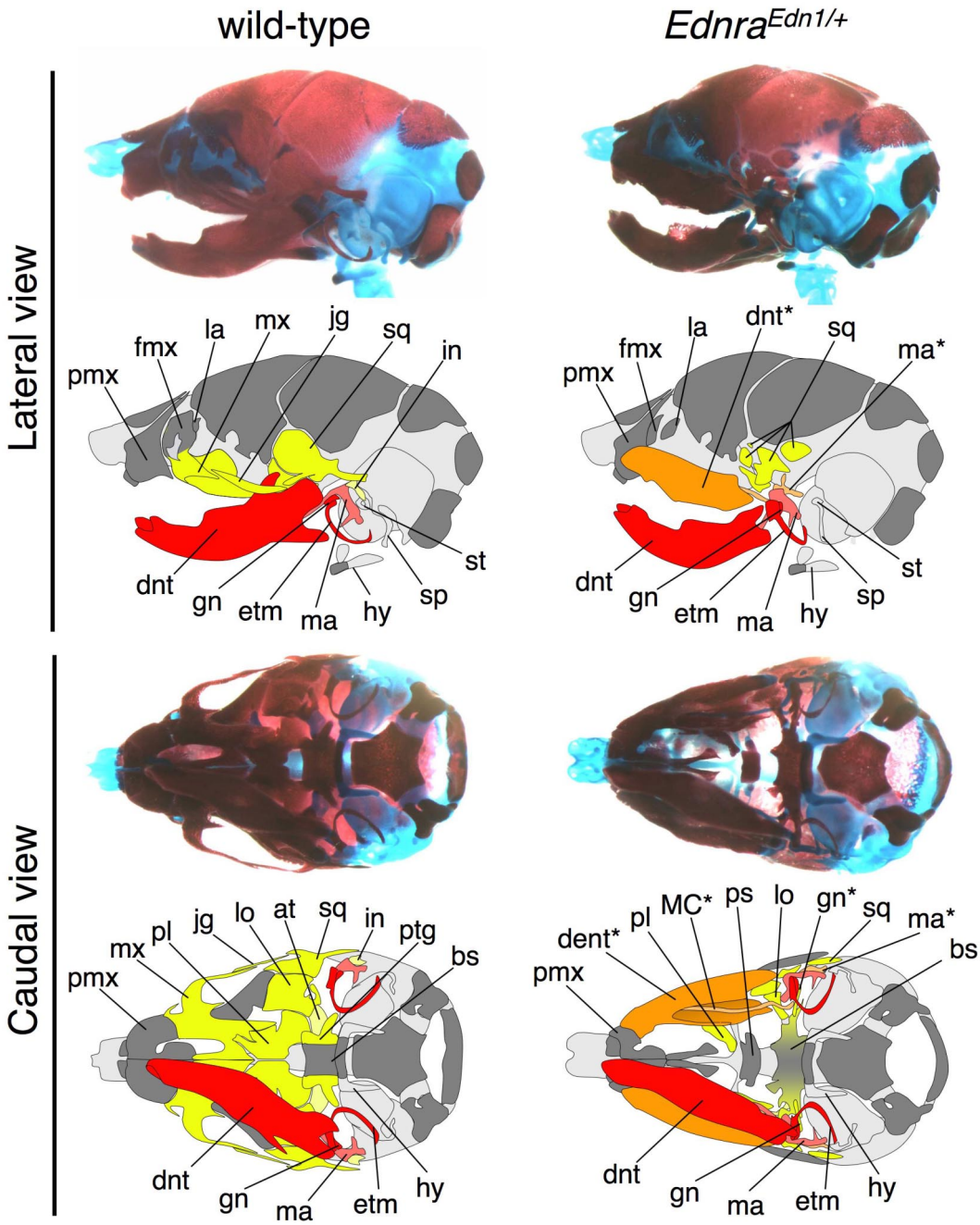

Fig. S3. Lateral and caudal views of E18.5 wild-type and *Ednra*$^{Edn1/+}$ craniofacial skeletons with schematic representations. The maxillary and mandibular elements are in yellow and red, respectively. Transformed parts of the maxillary structures are colored with orange. at, ala temporalis; bs, basisphenoid; dnt, dentary; etm, ectotympanic; fmx, frontal process of maxilla; gn, gonial; hy, hyoid; in, incus; jg, jugal; la, lacrimal; lo, lamina obturans; ma, malleus; MC, Meckel's cartilage; mx, maxilla; pl, palatine; pmx, premaxilla; ptg, pterygoid; sp, styloid process; sq, squamosal; st, stapes; *, ectopic structure.

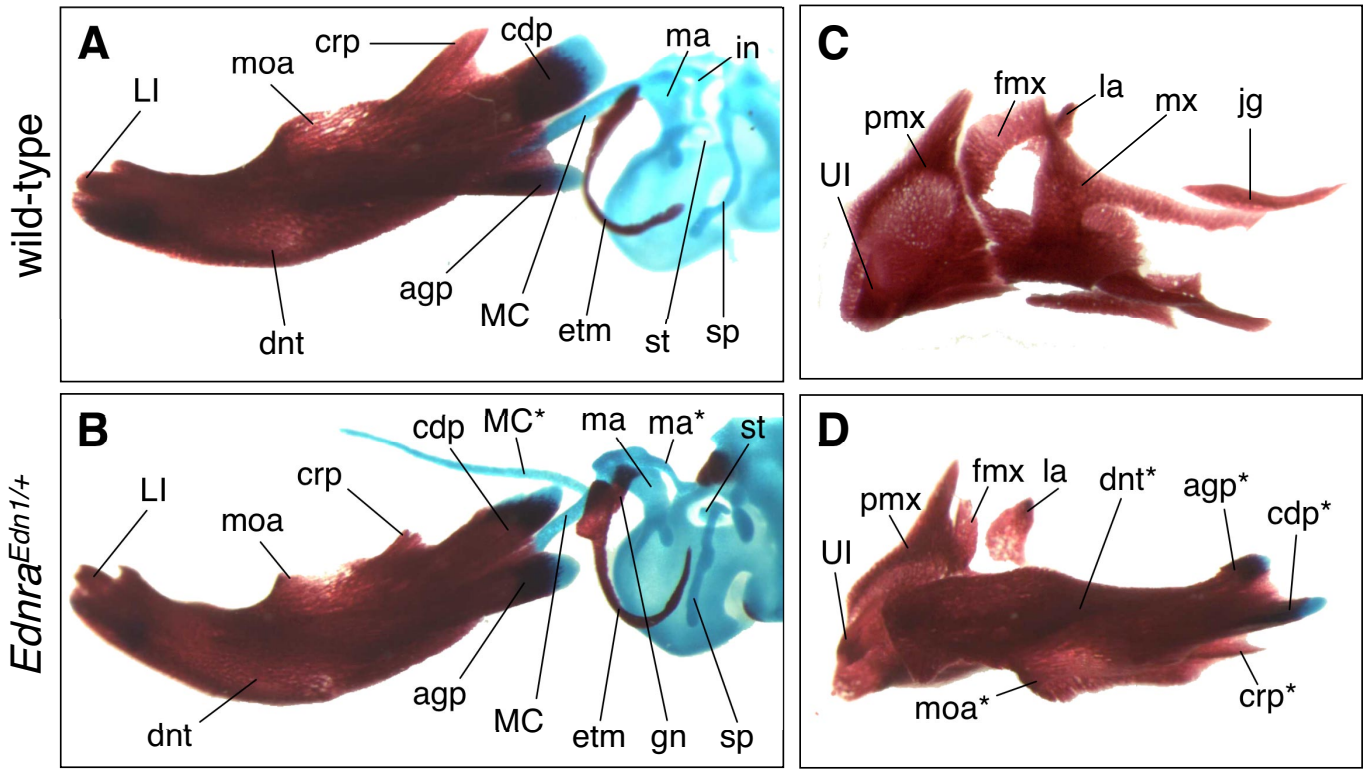

Fig. S4. Morphology of orthotopic and ectopic dentary bones and middle ear structures. (*A* and *B*) Orthotopic dentary bones and middle ear structures of E18.5 wild-type (*A*) and *Ednra*$^{Edn1/+}$ (*B*) mice. In *Ednra* $^{Edn1/+}$ mice, the coronoid and condylar processes are hypoplastic and the anterior part of the ectotympanic is hyperplastic. The stapes is fused to the styloid process in mutants. (*C* and *D*) Upper jaw components of E18.5 wild-type (*C*) and *Ednra*$^{Edn1/+}$ (*D*) mice. In the maxillary region of *Ednra* $^{Edn1/+}$ mice, a dentary-like bone with angular, condylar, and coronoid processes is formed. The tip of the ectopic dentary extends anteriorly and fuses to the premaxilla. agp, angular process, cdp, condylar process; crp, coronoid process; dnt, dentary; etm, ectotympanic; fmx, frontal process of maxilla; gn, gonial; in, incus; jg, jugal; la, lacrimal; LI, lower incisor; ma, malleus; MC, Meckel's cartilage; moa, molar alveolus; mx, maxilla; pmx, premaxilla; sp, styloid process; st, stapes; UI, upper incisor; *, ectopic structure.

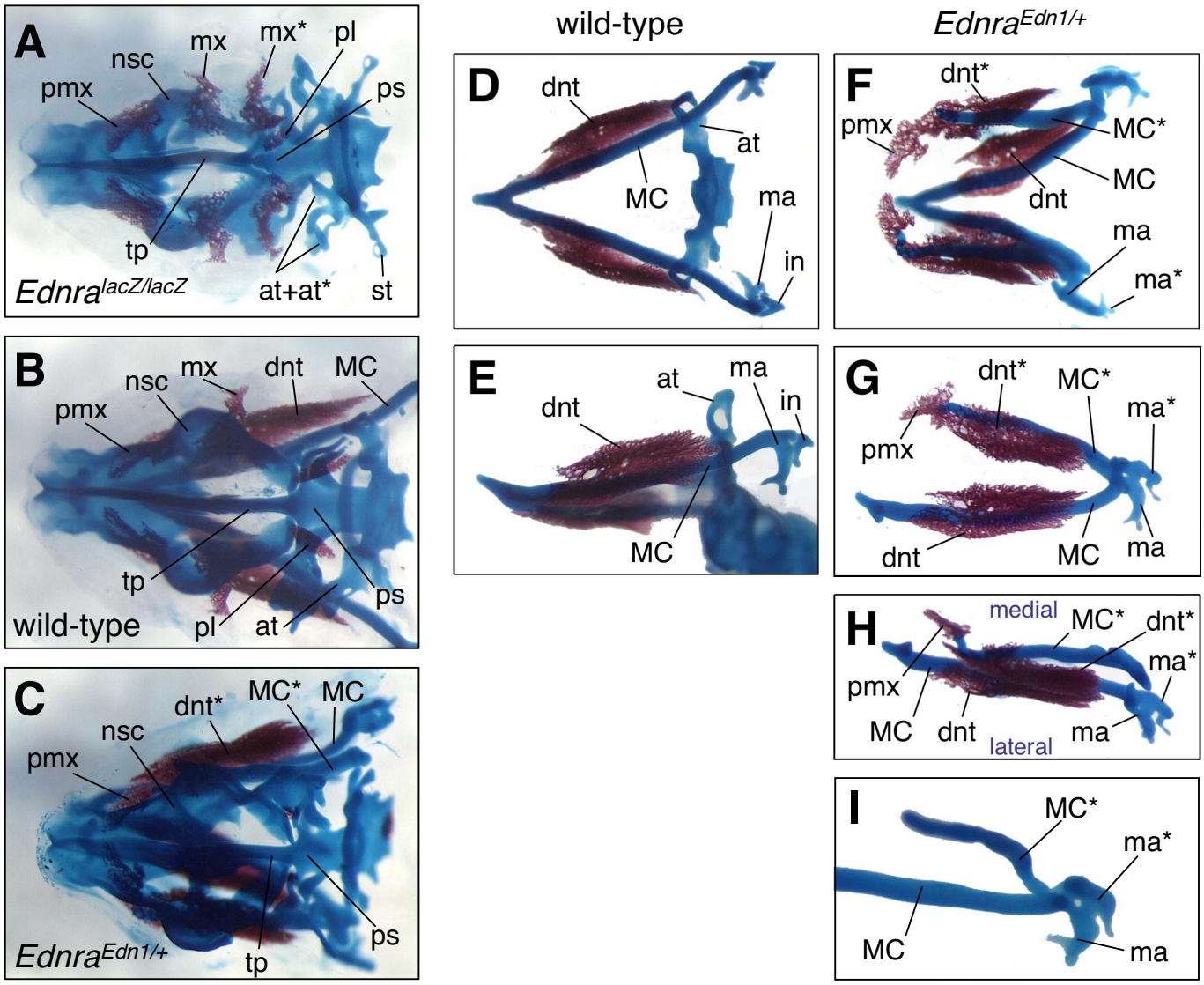

Fig. S5. Skeletal components of E15.5 wild-type and *Ednra*^{Edn1} jaws. (*A–C*) Cranial views of the nasomaxillary region in E15.5 *Ednra*$^{lacZ/lacZ}$ (*A*), wild-type (*B*), and *Ednra*$^{Edn1/+}$ (*C*) mice. Mandibular-to-maxillary and maxillary-to-mandibular transformations are observed in *Ednra*$^{lacZ/lacZ}$ and *Ednra*$^{Edn1/+}$ mutants, respectively. Midline structures such as the nasal capsule and the trabecular plate are well formed in both mutants. (*D–I*) PA1-derived skeletons of wild-type (*D* and *E*) and *Ednra*$^{Edn1/+}$ (*F–I*) mice. The duplicated MC extends beyond the ectopic dentary bone forming laterally to it, as in case with their orthotopic counterparts. The ectopic MC is discontinuous to the transformed incus-malleus in some cases (*H*), but is continuous to it in other cases (*I*). at, ala temporalis; dnt, dentary; in, incus; ma, malleus; MC, Meckel's cartilage; mx, maxilla; nsc, nasal capsule; pl, palatine; pmx, premaxilla; ps, presphenoid; tp, trabecular plate; *, ectopic structure.

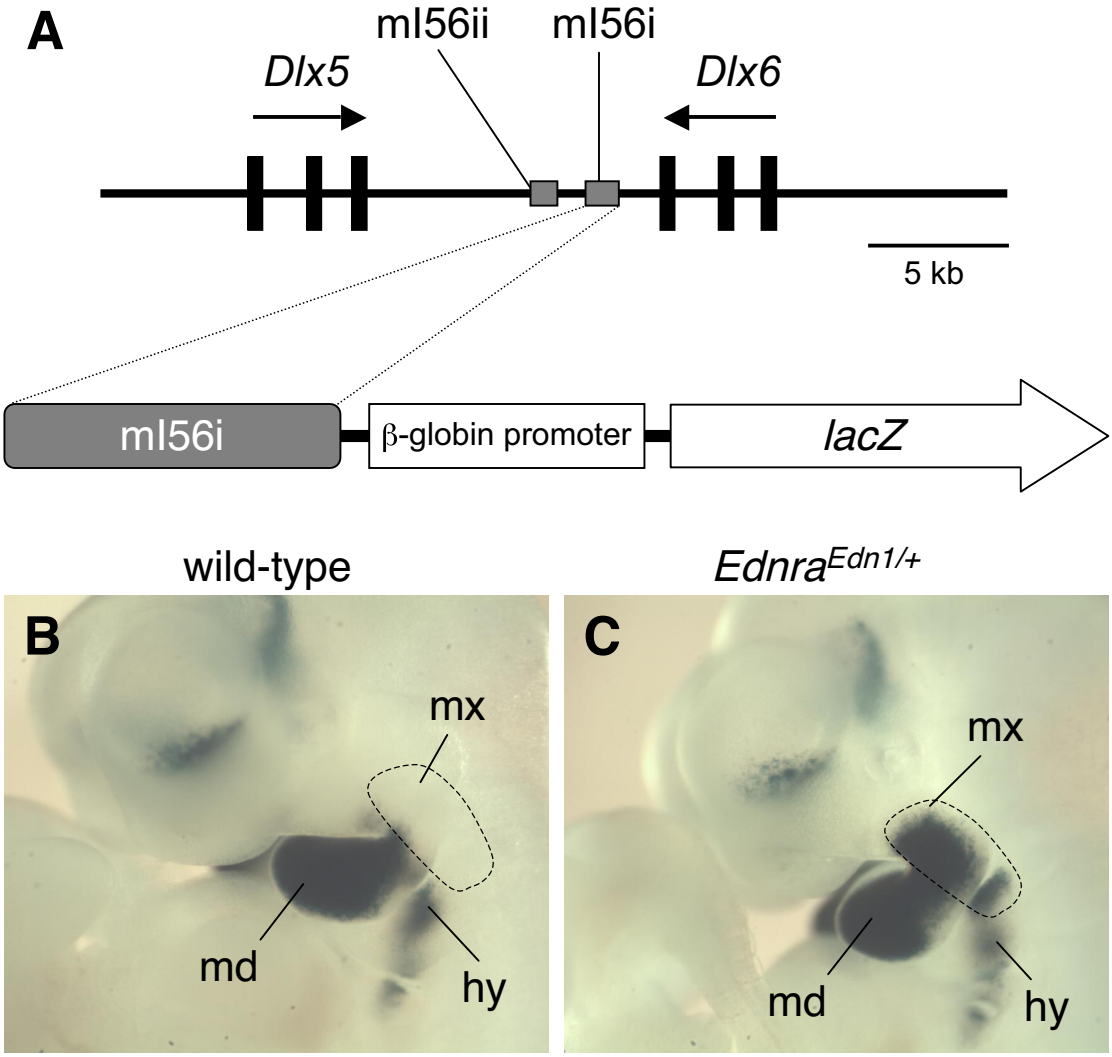

Fig. S6. Effect of *Edn1* knock-in on the activity of the *mI56i* enhancer in the *Dlx5*/*Dlx6* intergenic region. (*A*) Structures of the mouse *Dlx5*/*Dlx6* locus and the *mI56i-lacZ* transgene (*2*). (*B* and *C*) Expression of the *mI56i-lacZ* transgene in E10 $Ednra^{+/+}$, *mI56i-lacZ* (*B*) and $Ednra^{Edn1/+}$, and *mI56i-lacZ* (*C*) embryos. *LacZ* expression extended to the dorsal region of PA1 and PA2 in $Ednra^{Edn1/+}$; *mI56i-lacZ* embryos (encircled by broken line). hy, hyoid arch; md, mandibular arch; mx, maxillary arch.

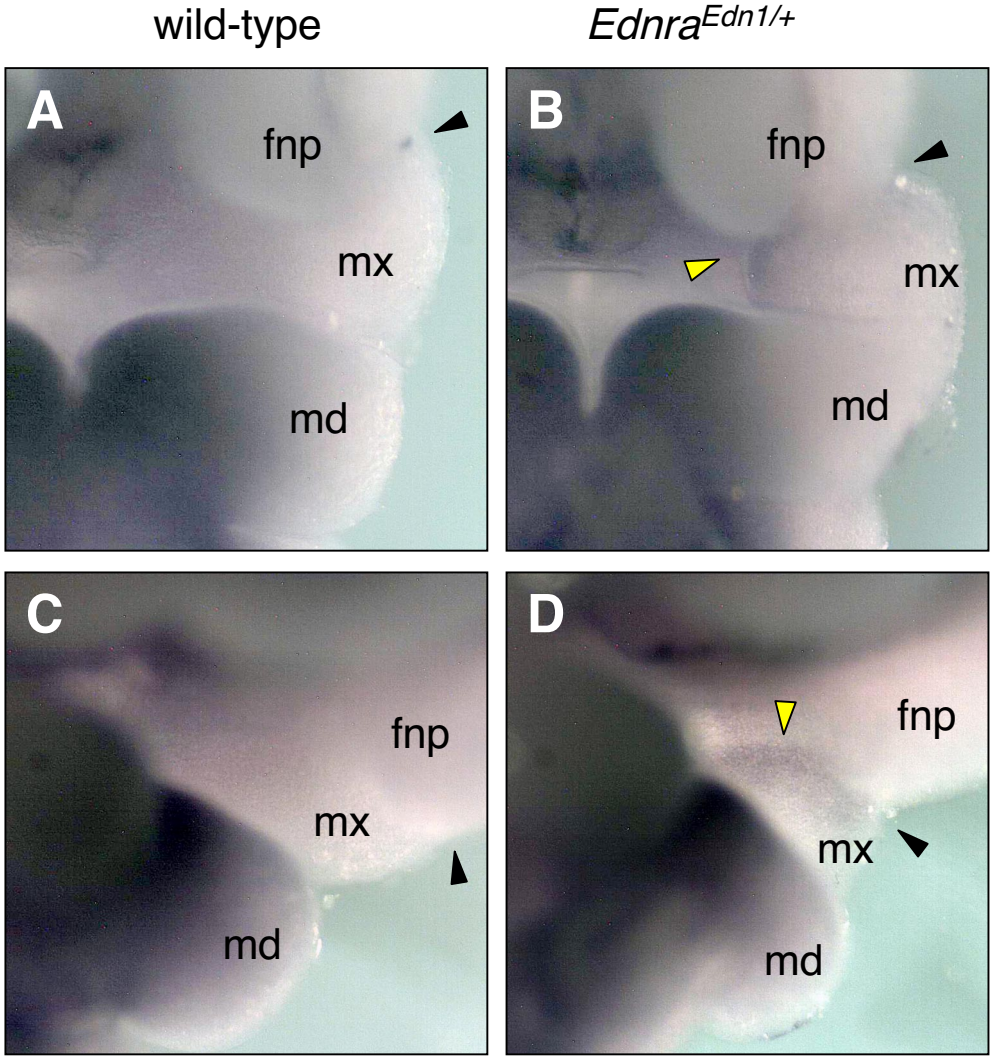

Fig. S7. High magnification of *Hand2* expression in E10.5 PA1. Ventral (*A* and *B*) and medial (*C* and *D*) views of whole-mount in situ hybridization for *Hand2* in E10.5 wild-type (*A* and *C*) and $Ednra^{Edn1}$ (*B* and *D*) embryos. Ectopic *Hand2* expression in the $Ednra^{Edn1}$ maxillary arch is observed along the nasolacrimal groove (black arrowheads) with the highest intensity in the medial region abutting the frontonasal process (yellow arrowheads). fnp, frontonasal process; md, mandibular arch; mx, maxillary arch.

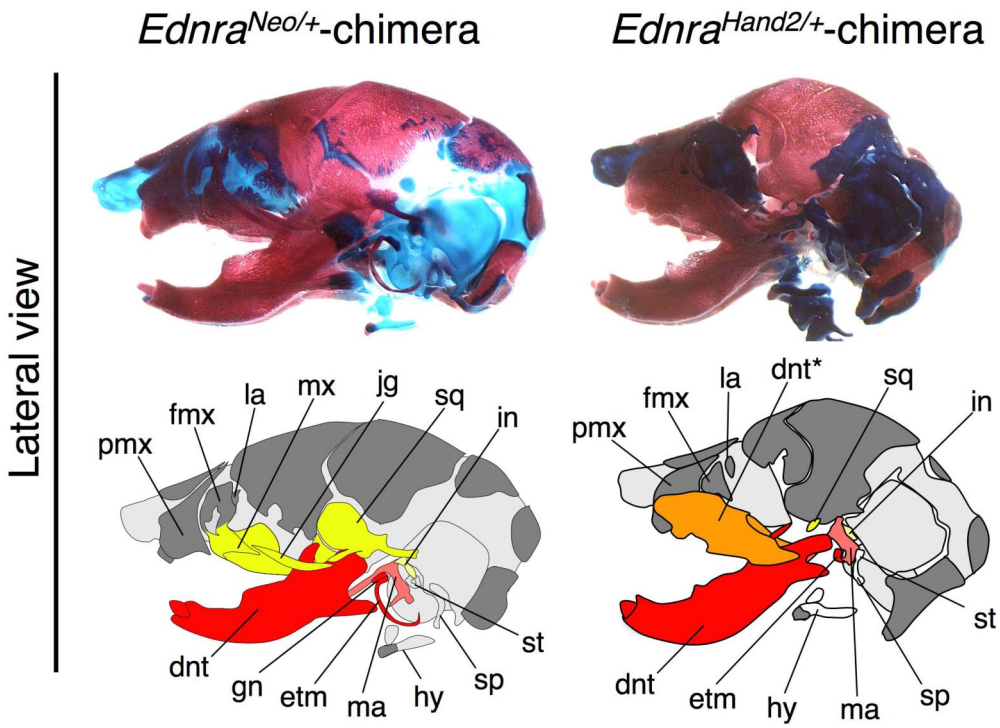

Fig. S8. Lateral views of the craniofacial skeletons of E18.5 *Ednra*$^{Neo/+}$ ES cell-derived and *Ednra*$^{Hand2/+}$ ES cell-derived chimeric mice with schematic representations. The maxillary and mandibular elements are in yellow and red, respectively. Transformed parts of the maxillary structures are colored with orange. dnt, dentary; etm, ectotympanic; fmx, frontal process of maxilla; gn, gonial; hy, hyoid; in, incus; jg, jugal; la, lacrimal; ma, malleus; mx, maxilla; pmx, premaxilla; sp, styloid process; sq, squamosal; st, stapes; *, ectopic structure.

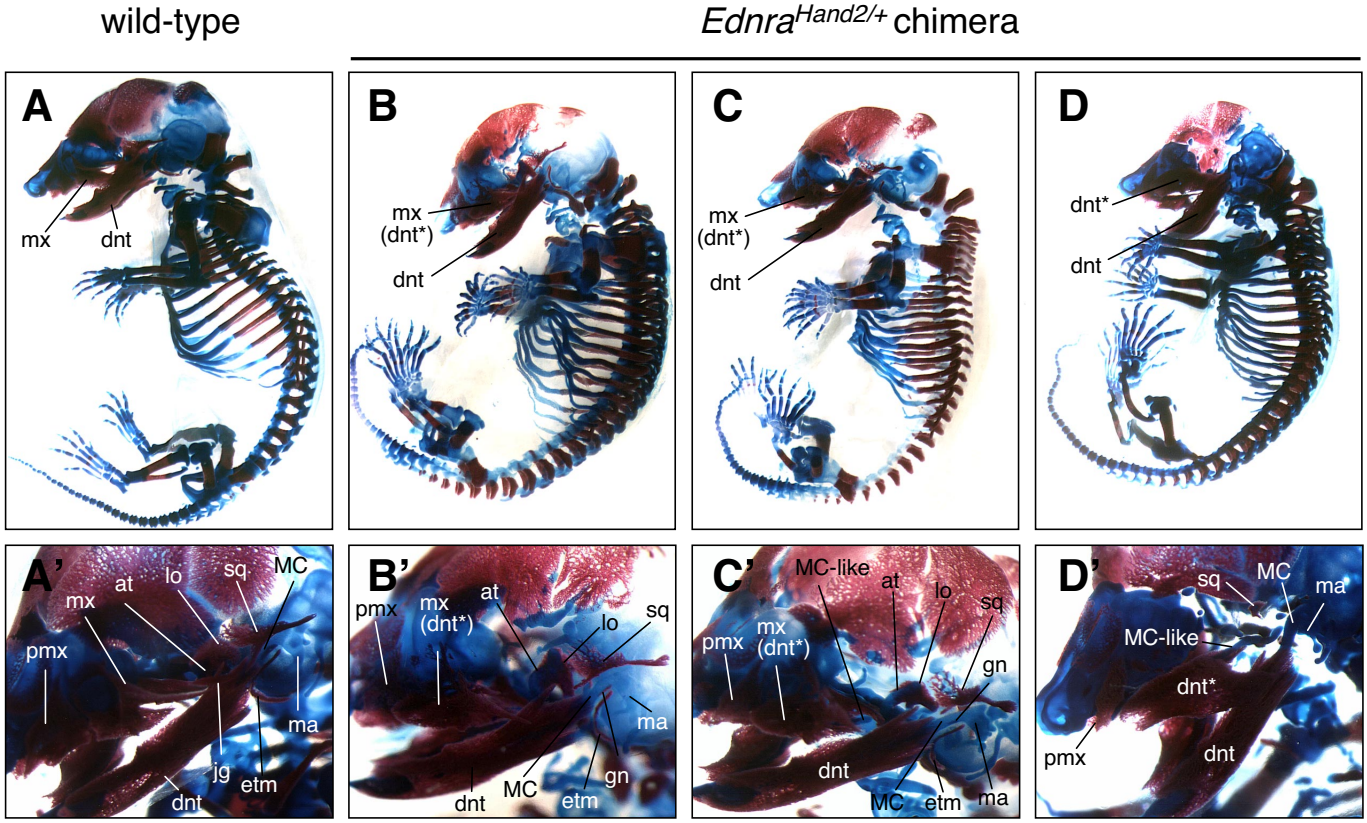

Fig. S9. Skeletal abnormalities of *Ednra*$^{Hand2/+}$ ES cell-derived chimeras at E16.5. (*A* and *A′*–*D* and *D′*) Representative bone and cartilage morphology of E16.5 wild-type (*A* and *A′*) and *Ednra*$^{Hand2/+}$ chimeric (*B* and *B′*–*D* and *D′*) mice. *Ednra*$^{Hand2/+}$ chimeras demonstrate craniofacial abnormalities and mirror-image polydactyly (*B*–*D*). Some mutant chimeras with polydactyly show transformation of the maxilla into dentary-like morphology without MC formation (*B′*). Others exhibit ectopic formation of MC-like rod-shaped cartilage in the maxilla with (*C′*) or without (*D′*) the ala temporalis. In severely affected chimeras, the gonial, ectotympanic, squamosal, and lamina obturans were deformed (*C*) or almost missing (*D*). at, ala temporalis; dnt, dentary; etm, ectotympanic; gn, gonial; in, incus; jg, jugal; lo, lamina obturans; ma, malleus; MC, Meckel's cartilage; mx, maxilla; pmx, premaxilla; sp, styloid process; sq, squamosal; st, stapes; ⋆, ectopic structure.