Supporting Information

Miletich et al. 10.1073/pnas.1112801108

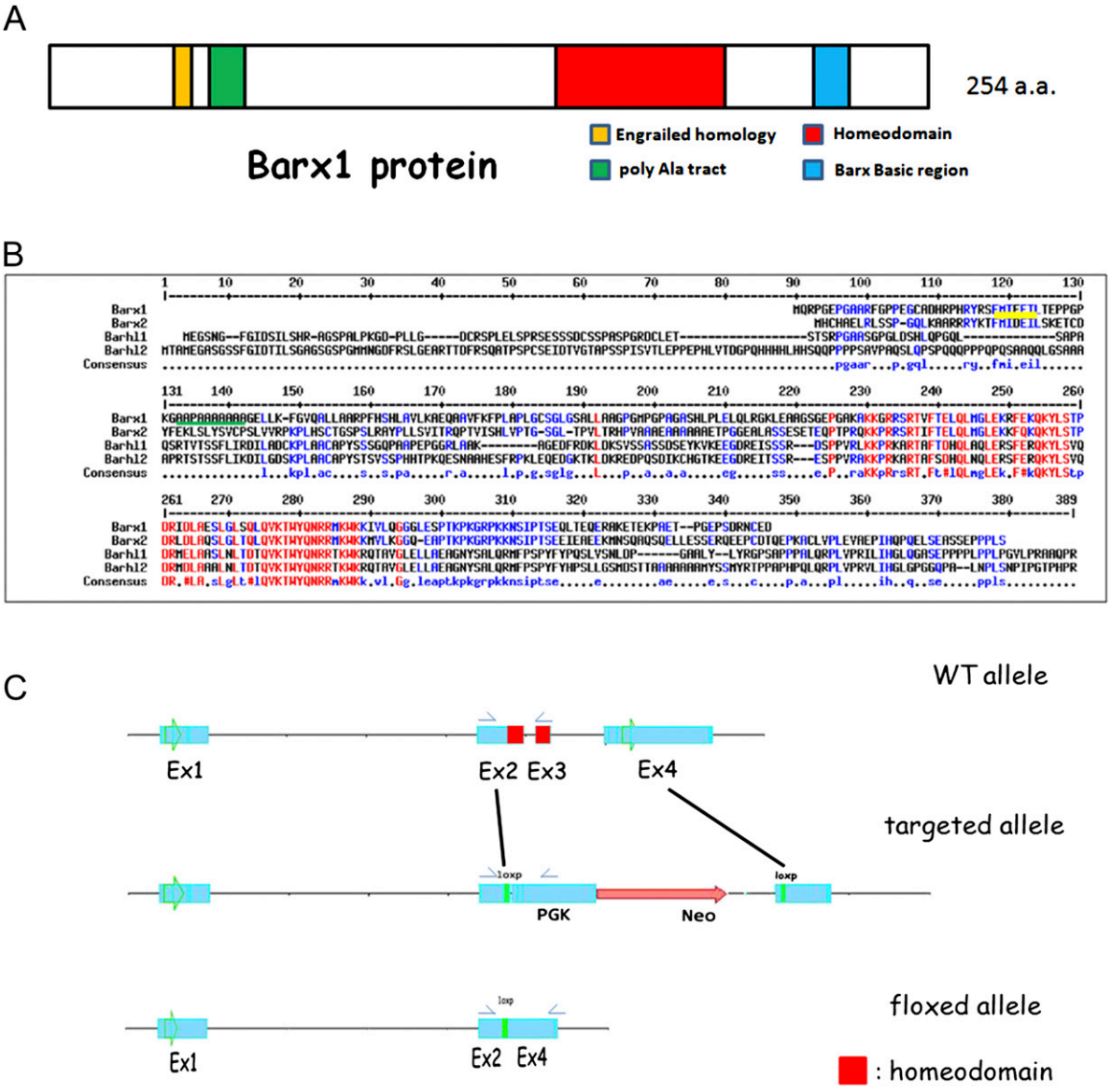

Fig. S1. Generation of *Barx1* knockout mice. (*A*) Schematic representation of Barx1 protein showing localization of conserved domains. (*B*) Homeodomain of Barx1 is 95.2% identical to Barx2 and 72.6% to Barhl1 and Barhl2. There are other homologous regions between Barx1 and Barx2 such as the Engrailed homology domain and the Barx basic region (BBR). (*C*) *Barx1* locus was targeted with a PGK-Neo cassette to replace exon 2–exon 4 of the gene in a 129Sv BAC clone, leading to the deletion of the whole homeodomain and the BBR. To verify that the targeted mutation resulted in a null allele, we used a riboprobe that recognized the 3′ end of the nontargeted Barx1-encoding region to detect any mRNA expression during embryo development. mRNA-encoding *Barx1* homeodomain and BBR was no longer expressed in the *Barx1* mutant mice at E10.5. *Barx1* mutants were recovered from the intercross matings at the expected Mendelian ratio until birth and all *Barx1* KO pups died shortly after birth due to a fully penetrant cleft palate phenotype. To eliminate the possibility that the neo cassette in the targeted allele might interfere with Barx1 function, we crossed the mice with β-actin–Cre mice. The resulting $Barx1^{-/-}/Neo^{-/-}$ animals were indistinguishable from $Barx1^{-/-}/Neo^{+/-}$.

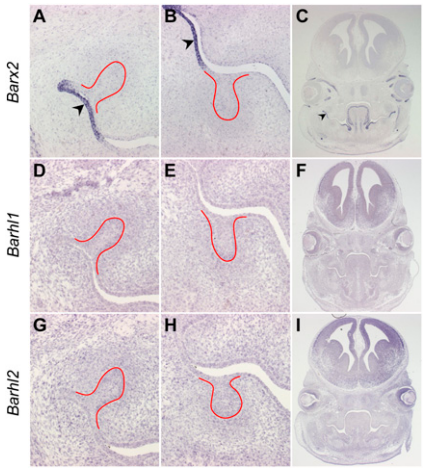

Fig. S2. Expression of *Barx2*, *Barhl1*, and *Barhl2* at E13.5 in molar tooth buds and other craniofacial structures at the level of the first molars. In situ hybridization for *Barx2* (*A*–*C*), *Barhl1* (*D*–*F*), and *Barhl2* (*G*–*I*) on frontal sections of E13.5 WT heads. (*A*) *Barx2* is not expressed in upper (*A*) and lower (*B*) molar tooth buds, although it is expressed in the oral epithelium flanking the lower first molar on the buccal side (*A*–*C*, arrowhead). Only background staining can be observed at the level of the first molars with *Barhl1* (*D* and *E*) and *Barhl2* (*G* and *H*) riboprobes. However, expression of *Barhl1* and *Barhl2* can be detected in the brain (*F* and *I*) as previously reported (1, 2). Upper and lower first molar tooth buds are outlined in red.

1. Bulfone A, et al. (2000) Barhl1, a gene belonging to a new subfamily of mammalian homeobox genes, is expressed in migrating neurons of the CNS. *Hum Mol Genet* 9:1443–1452.
2. Mo Z, Li S, Yang X, Xiang M (2004) Role of the Barhl2 homeobox gene in the specification of glycinergic amacrine cells. *Development* 131:1607–1618.