Figure S1: Localization of Wnt1-Cre and Sox10-Cre recombination domains and Sox10 expression in Kmt2dc^KO embryos. (A-B) E13.5 WT and Kmt2dc^KO coronal sections of supraorbital frontal primordia indicating Wnt1-Cre driven tomato reporter fluorescence. White scale bar in A = 0.1 mm. (C-D) Whole mount RNA in situ hybridization for Sox10 in WT and Kmt2dc^KO E10.5 embryos. Black arrows indicate post-migratory NCC localization to trigeminal ganglia and red arrows depict dorsal root ganglia. (E-F) Bright field (BF) and tomato fluorescent images of NCC lineage tracing in WT E13.5 Sox10-Cre embryos. (G-H) Coronal sections through WT E13.5 Sox10-Cre supraorbital ridge (G) and palatal shelf (H) indicating efficient tomato reporter activity in NCCs from these regions.
Figure S2: Expression patterns of *Kmt2d, Utx,* and *Kdm6b* in NCCs. (A-E) Expression in supraorbital frontal primordia. White scale bars in A and B = 0.1 mm. *Kmt2d* expression was analyzed by RNA *in situ* hybridization (A). UTX immunofluorescence was performed (B) with *Utx<sup>cko</sup>* tissue serving as negative control (C). *Kdm6b* expression was analyzed by X-Gal staining of a heterozygous *Kdm6b* gene trap (*Kdm6b<sup>+/-GT</sup>*) that fuses the transcript with β-galactosidase (D). WT sections served as control for the β-galactosidase assay (E). Black and white arrows in A-E denote osteoblasts while red arrows denote chondrocytes. (F-J) Expression patterns of *Kmt2d, Utx,* and *Kdm6b* in coronal palatal shelves with staining as indicated in parts A-E. Black arrows highlight osteoblast differentiation, red arrows depict subepithelial mesenchymal expression, and the white asterisk illustrated expression in the distal palatal tip. (K-N) Expression patterns of *Utx* and *Kdm6b* in sagittal presphenoid regions of the P1 cranial base with staining as indicated in parts B-E. White and black scale bars in K and M = 0.2 mm.
Figure S3

A RUNX2/COL2
B RUNX2/COL2
C RUNX2/COL2
D RUNX2/COL2
E SOX9
F OSX
G BrdU
H BrdU
I BrdU cells/area (10^4 foci/mm²)
J cCASP3
K cCASP3
Figure S3: Cellular phenotypes in Kmt2dcKO supraorbital frontal primordia. (A-B) Osteoblast (RUNX2+) or chondrocyte (COL2+) domains in E15.5 WT or Kmt2dcKO frontal coronal sections. White scale bar in A = 0.2 mm. (C-D) Pre-osteoblast (RUNX2+) or pre-chondrocytes (COL2+) in E12.5 WT or Kmt2dcKO coronal sections of frontal primordia. White scale bar in C = 0.1 mm. (E) SOX9+ pre-chondrocytes in E12.5 WT coronal sections of frontal primordia. (F) E12.5 pre-osteoblasts do not express OSX. (G-H) E12.5 WT and Kmt2dcKO embryos were labeled with BrdU for 2 hours and detected by immunofluorescence. (I) E12.5 BrdU positive cells were counted from osteoblast regions (identified in parts C-D) and normalized to area scored. N ≥ 4 supraorbital osteoblast domains averaged from 3 sections spaced 60 microns apart. (J-K) Apoptotic cells were identified in E12.5 WT or Kmt2dcKO frontal primordia by immunofluorescence for active cleaved Caspase 3.
Figure S4: Cellular phenotypes in Kmt2dcKO palatal shelves. (A) qRT-PCR verification of genes identified in E14.25 Kmt2dcKO palatal shelf RNA-seq. (B) The area of the distal palatal shelf (PaS) tip indicated in Figure 5J-K was measured. N = 3 sets of palatal shelf distal tip measurements averaged from 2 anterior-central sections spaced 80 microns apart. (C) E14.25 WT and Kmt2dcKO embryos were labeled with BrdU for 2 hours and detected by immunofluorescence (D-E). White scale bar in D = 0.1 mm. BrdU positive cells were counted from the distal palatal tip (white asterisks) and plotted normalized to area scored (C). N = 4 sets of palatal shelf distal tip measurements averaged from 2 anterior-central sections spaced 80 microns apart. (F-G) Apoptotic cells were identified in E142.5 WT or Kmt2dcKO distal palatal extensions (white asterisks) by immunofluorescence for active cleaved Caspase 3. (H-I) Picosirius red staining of E12.5 WT and Kmt2dcKO palatal outgrowths. Dashed line indicates region measured in part K. Black scale bar in H = 0.1 mm. (J-K) Measured area (J) and length (K) of the Kmt2dcKO palatal outgrowth indicated in parts H-I. Anterior to middle palatal sections were scored and quantified. N ≥ 7 sets of palatal shelf outgrowth measurements scored and quantified from anterior-central sections demonstrating the largest measurements. (L-M) E12.5 WT and Kmt2dcKO embryos were labeled with BrdU for 2 hours and detected by immunofluorescence in palatal outgrowths (white asterisks). White scale bar in L = 0.1 mm. (N-O) Apoptotic cells were identified in E12.5 WT or Kmt2dcKO palatal outgrowths by immunofluorescence for active cleaved Caspase 3. (P) BrdU positive cells were counted from the E12.5 anterior palatal outgrowths (white asterisks in parts L-M) and plotted normalized to area scored. N = 4 palatal shelf outgrowth measurements averaged from 2 anterior-central sections spaced 60 microns apart.
Figure S5: \textit{Kmt2d}^{cS10KO} cranial base quantitation. (A) Basisphenoid (B-sphenoid) and presphenoid (P-sphenoid) bone lengths were imaged and measured from whole mount alizarin red and alcian blue skeletal preparations in P1 WT and \textit{Kmt2d}^{cS10KO} pups. N ≥ 4.
Table S1: Differential gene expression analysis for RNA-seq on E14.25 WT and Kmt2d<sup>cKO</sup> palatal shelves. Sheet 1: Significantly downregulated genes (FDR < 0.05) in Kmt2d<sup>cKO</sup> palatal shelves. Sheet 2: Significantly upregulated genes (FDR < 0.05) in Kmt2d<sup>cKO</sup> palatal shelves. Sheet 3: Kmt2d<sup>cKO</sup> downregulated genes from sheet 1 with elevated WT expression (RPKM > 2) and greater fold change (Kmt2d<sup>cKO</sup> logFC < -1). Sheet 4: Extracellular matrix components identified by MSigDB or IPA pathway analysis that were downregulated in Kmt2d<sup>cKO</sup> palatal shelves.

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Table S2: Genotyping and qRT-PCR primers used in this study.

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