Figure S1

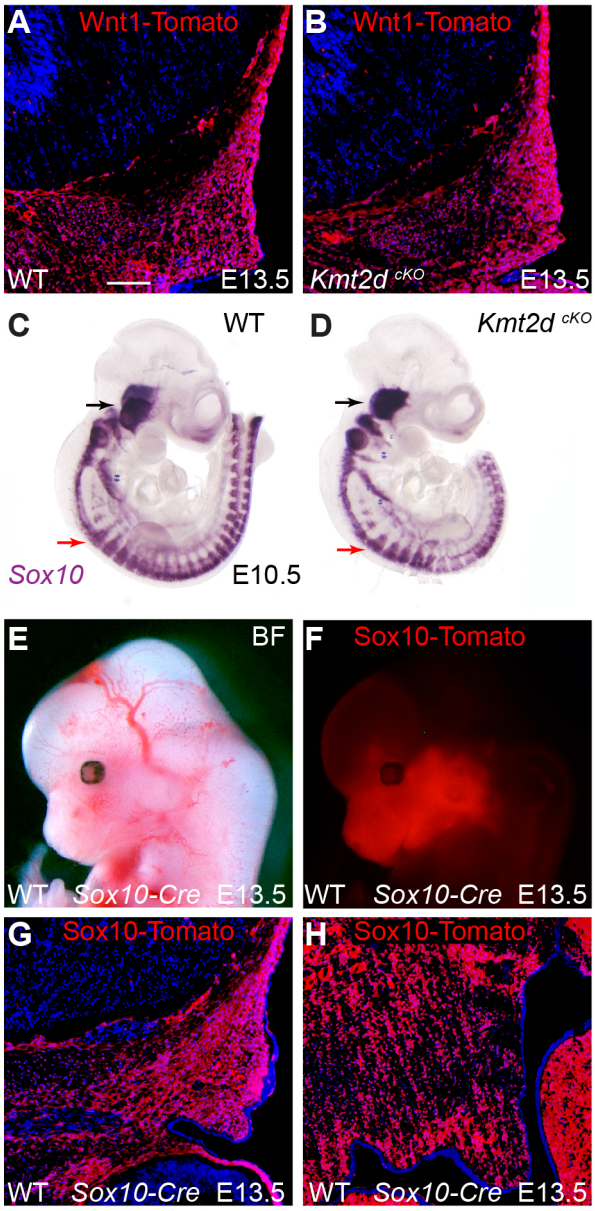

Figure S1: Localization of *Wnt1-Cre* and *Sox10-Cre* recombination domains and *Sox10* expression in *Kmt2d*^{cKO} embryos. (A-B) E13.5 WT and *Kmt2d*^{cKO} coronal sections of supraorbital frontal primordia indicating Wnt1-Cre driven tomato reporter fluorescence. White scale bar in A = 0.1 mm. (C-D) Whole mount RNA *in situ* hybridization for *Sox10* in WT and *Kmt2d*^{cKO} 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

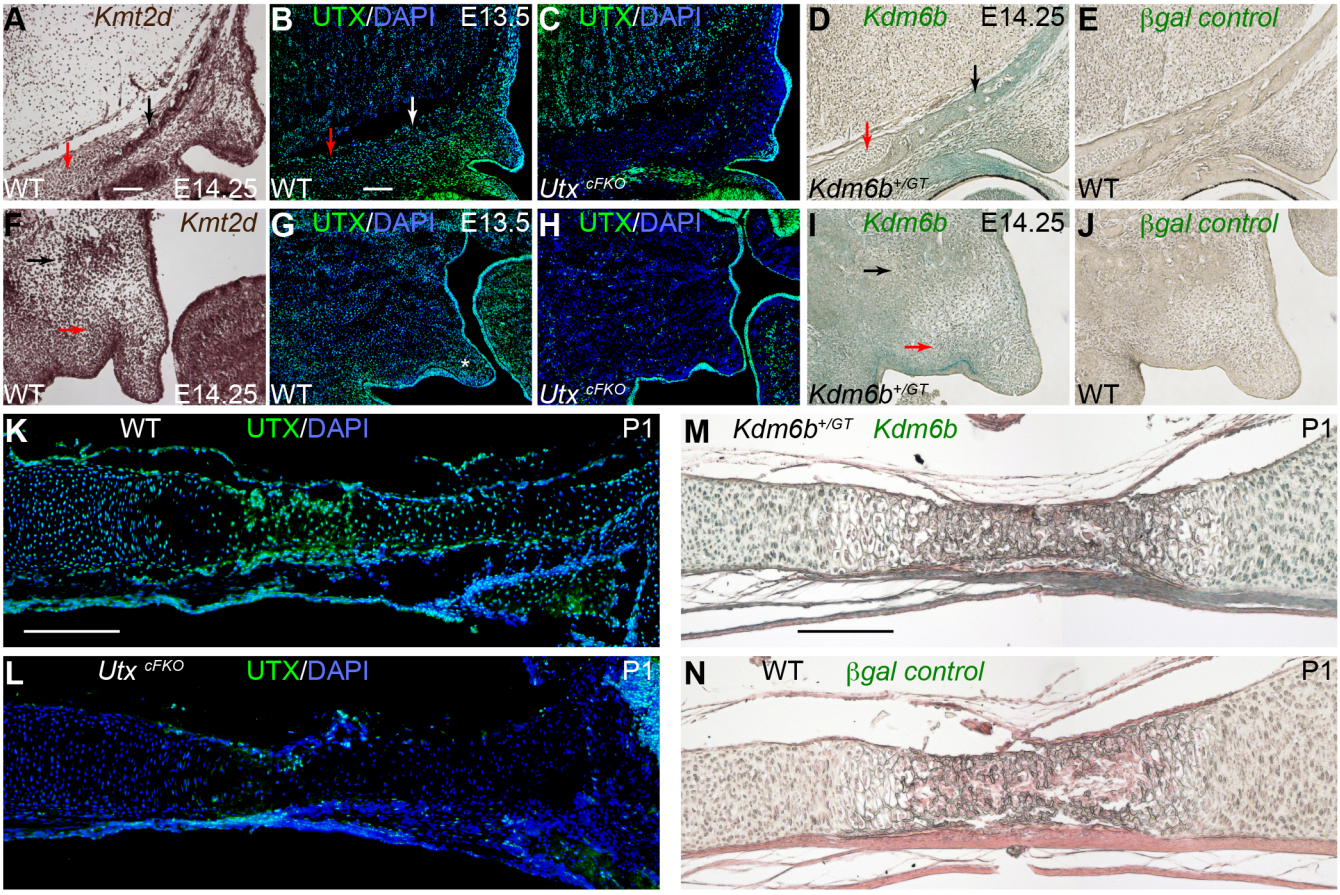

Figure S2: Expression patterns of *Kmt2d*, *Utx*, and *Kdm6b* in NCCs. (A-E) Expression in supraorbital frontal primordia. White scale bars in A and B = 0.1 mm. *Kmt2d* expression was analyzed by RNA *in situ* hybridization (A). UTX immunofluorescence was performed (B) with Utx^{cFKO} tissue serving as negative control (C). *Kdm6b* expression was analyzed by X-Gal staining of a heterozygous *Kdm6b* gene trap ($Kdm6b^{+/GT}$) 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

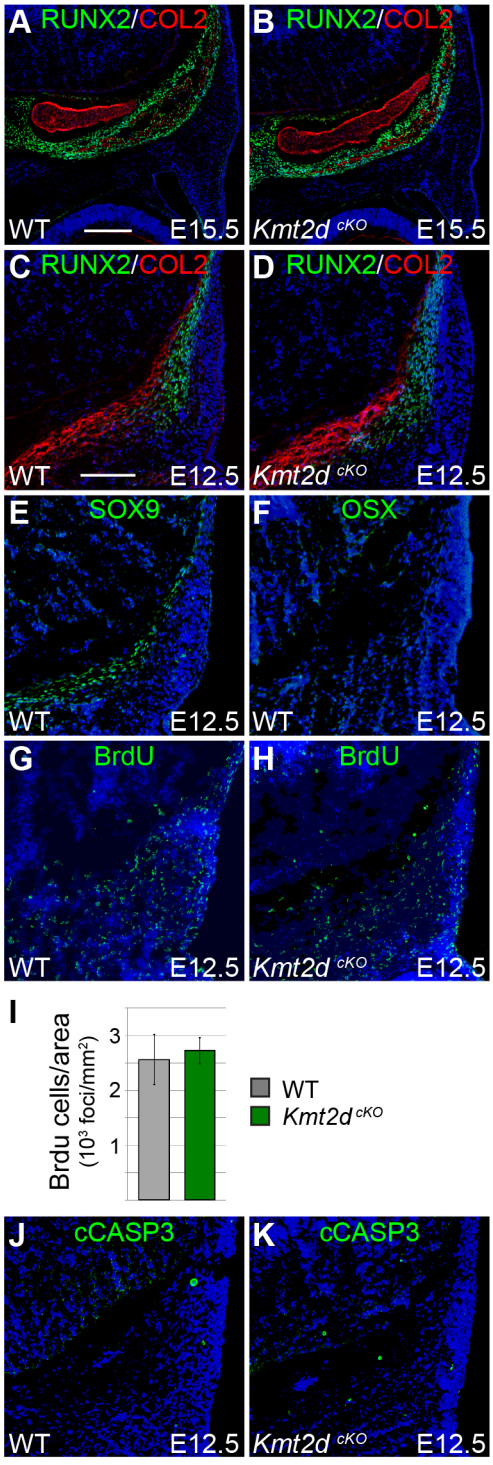

Figure S3: Cellular phenotypes in *Kmt2d*^{cKO} supraorbital frontal primordia. (A-B) Osteoblast (RUNX2+) or chondrocyte (COL2+) domains in E15.5 WT or *Kmt2d*^{cKO} frontal coronal sections. White scale bar in A = 0.2 mm. (C-D) Pre-osteoblast (RUNX2+) or pre-chondrocytes (COL2+) in E12.5 WT or *Kmt2d*^{cKO} 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 *Kmt2d*^{cKO} 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 *Kmt2d*^{cKO} frontal primordia by immunofluorescence for active cleaved Caspase 3.

Figure S4

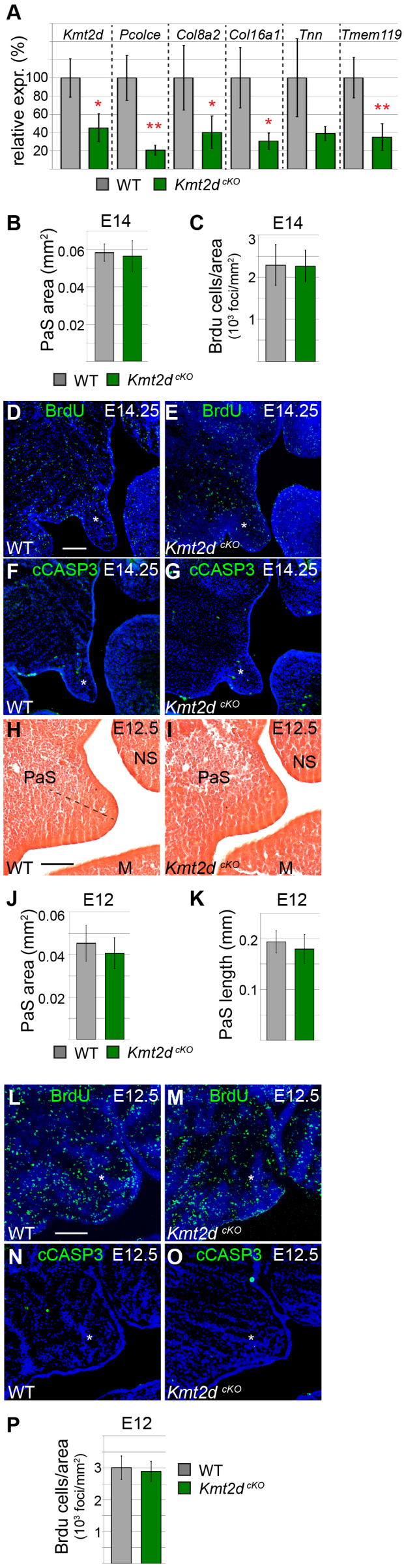

Figure S4: Cellular phenotypes in *Kmt2d*^{cKO} palatal shelves. (A) qRT-PCR verification of genes identified in E14.25 *Kmt2d*^{cKO} palatal shelf RNA-seq. (B) The area of the distal palatal shelf (PaS) tip indicated in Figure 5J-K was measured. N = 3 sets of palatal shelf distal tip measurements averaged from 2 anterior-central sections spaced 80 microns apart. (C) E14.25 WT and *Kmt2d*^{cKO} 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 *Kmt2d*^{cKO} distal palatal extensions (white asterisks) by immunofluorescence for active cleaved Caspase 3. (H-I) Picrosirius red staining of E12.5 WT and *Kmt2d*^{cKO} 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 *Kmt2d*^{cKO} 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 *Kmt2d*^{cKO} 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 *Kmt2d*^{cKO} 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

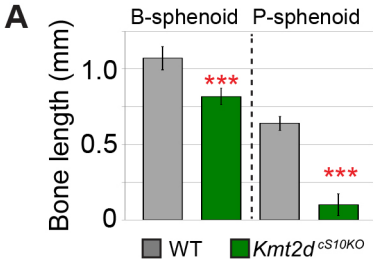

Figure S5: *Kmt2d*^{cS10KO} cranial base quantitation. (A) Basisphenoid (B-sphenoid) and presphenoid (P-sphenoid) bone lengths were imaged and measured from whole mount alizarin red and alcian blue skeletal preparations in P1 WT and *Kmt2d*^{cS10KO} pups. N ≥ 4.

Table S1: Differential gene expression analysis for RNA-seq on E14.25 WT and *Kmt2d*^{cKO} palatal shelves. Sheet 1: Significantly downregulated genes (FDR < 0.05) in *Kmt2d*^{cKO} palatal shelves. Sheet 2: Significantly upregulated genes (FDR < 0.05) in *Kmt2d*^{cKO} palatal shelves. Sheet 3: *Kmt2d*^{cKO} downregulated genes from sheet 1 with elevated WT expression (RPKM > 2) and greater fold change (*Kmt2d*^{cKO} logFC < -1). Sheet 4: Extracellular matrix components identified by MSigDB or IPA pathway analysis that were downregulated in *Kmt2d*^{cKO} palatal shelves.

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

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