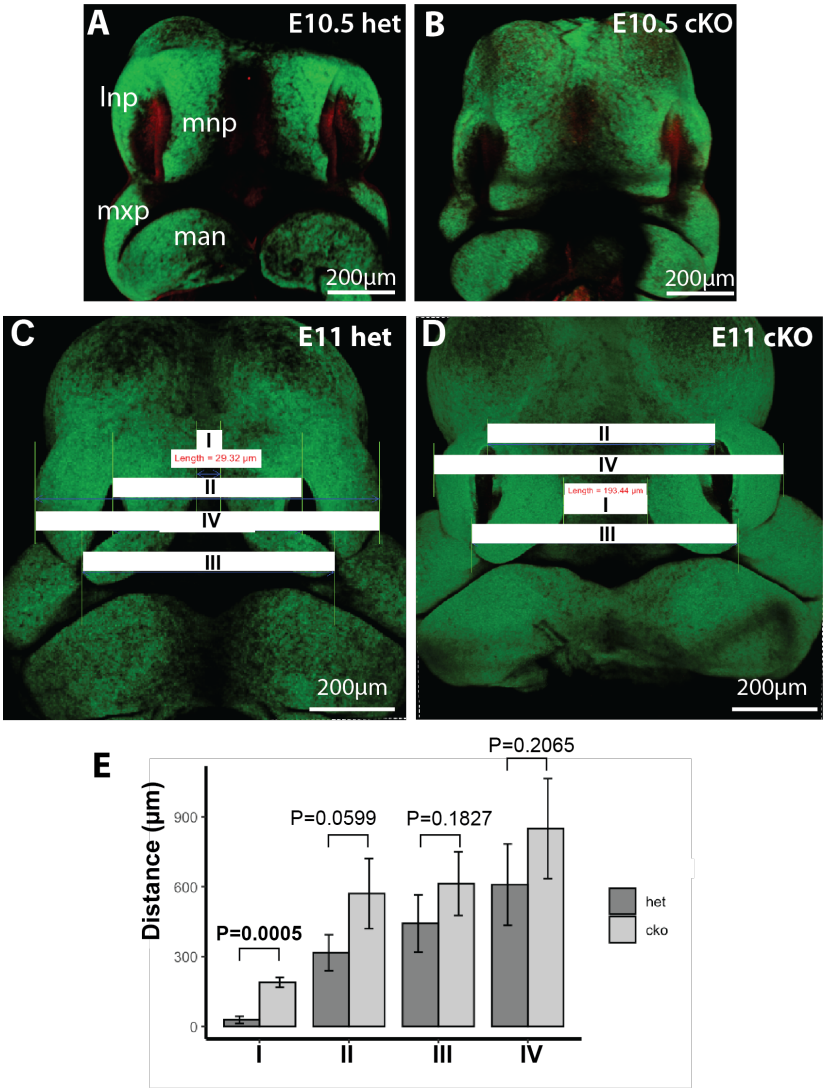

Fig. S1. Genetic fate mapping and midline gap quantification of *Pax3^{Cre};Wls^{flox}* midfacial primordia. (**A,B**) Front facial views of genetic fate mapping of the heterozygous and cKO embryos at E10.5. (**C,D**) Measurements and comparisons of facial distances of (I) the midline gap between MNPs, (II) between dorsal nasal tips, (III) between bilateral junction zones, and (IV) between the lateral edges of LNPs in the cKOs (n = 3) and littermate heterozygous controls (n = 3) at E11.0-E11.25. (**E**) Only the midline gap (I) is significantly increased in the cKOs (P = 0.0005, t-test). Other distances (II-IV) are also slightly increased in cKOs without statistical significance (P > 0.05).

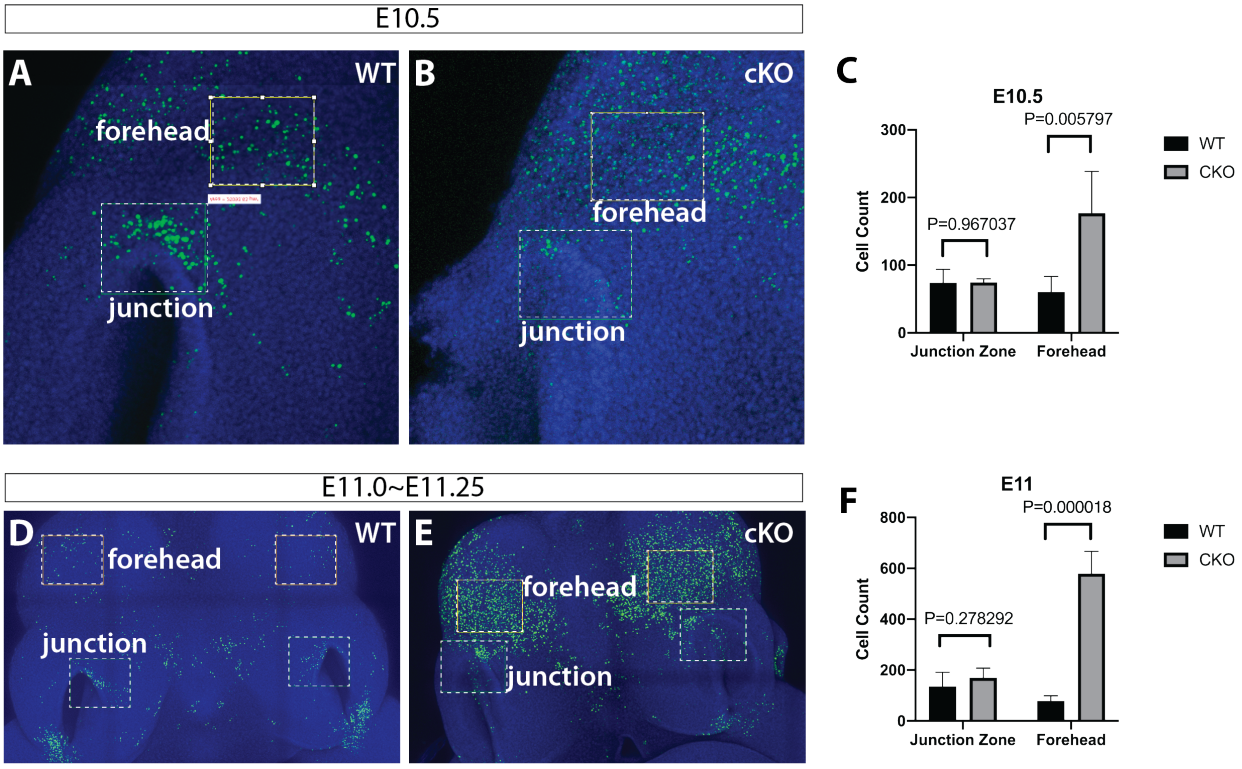

Fig. S2. Quantifications of apoptotic cells in the dorsal nasal primordial junction zones and forehead regions of the littermate control and Pax3Cre;Wls-cKO embryos during midfacial development. (**A,B**) TUNEL positive cells were automatically counted by ImageJ in the equally dashed-line squared areas covering the dorsal nasal primordial junction zones and forehead regions in the littermate control and cKO embryos at E10.5 (only right facial primordia were shown). (**C**) Bar graphs show no significant changes of TUNLE positive cells around the junction zones ($P > 0.05$), and they are increased 2.93 folds in the forehead regions ($P < 0.01$) of the cKOs (n = 4 in 2 cKOs) compared with the littermate wild-type (WT) controls (n = 6 in 3 embryos) at E10.5 (unpaired two-tailed Student's t-test). (**D,E**). TUNEL positive cells were counted in the equally dashed-line squared areas at E11.5. (**F**) Bar graphs show no significant changes of TUNLE positive cells around the junction zones ($P > 0.05$), and they are increased 7.42 folds in the forehead regions ($P = 0.000018$) of the cKOs (n = 6 in 3 mutants) compared with the littermate wild-type (WT) controls (n = 6 in 3 WT embryos) at E11.5 (two-tailed unpaired Student's t-test). $P < 0.05$ is considered statistically significant.

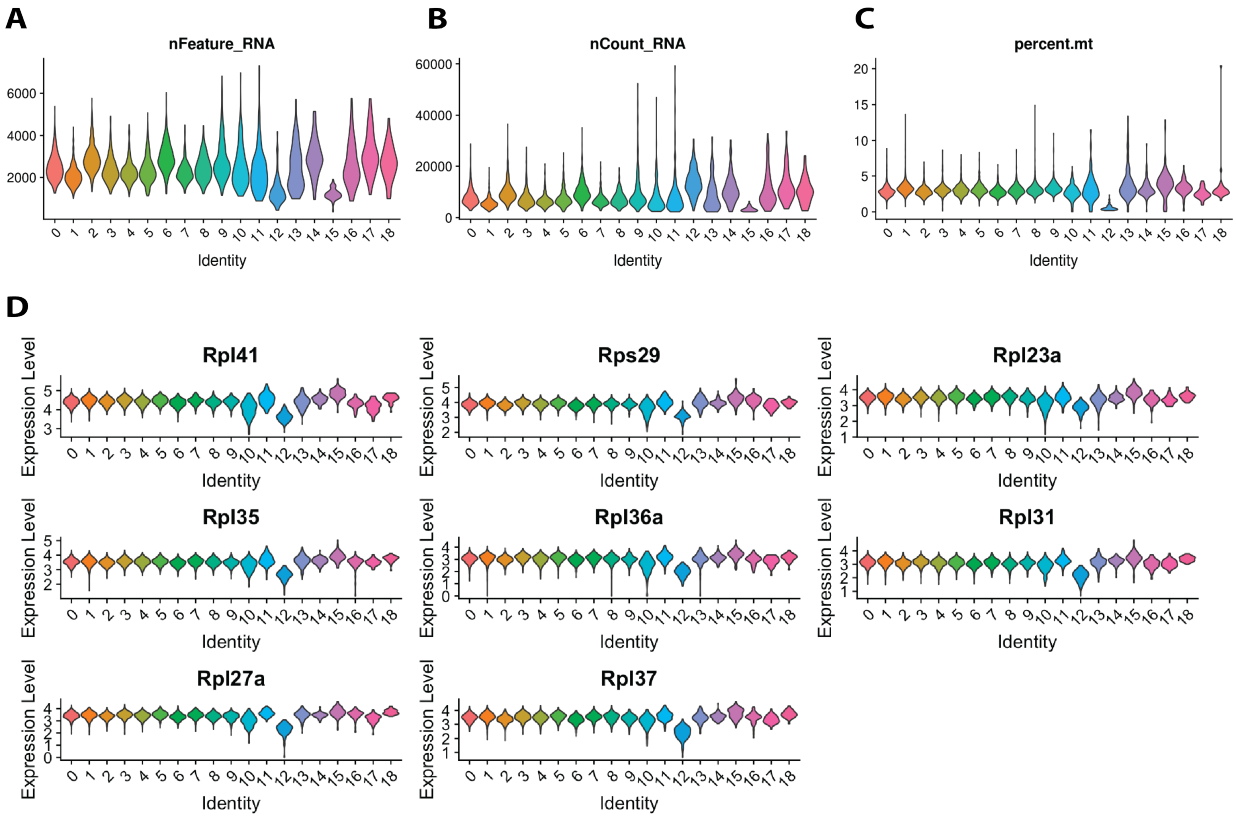

Fig. S3. Quality control (QC) for scRNA-seq data. (A-C) Violin plots show three quality control metrics, including the number of genes (nFeature_RNA) (A), number of unique molecular identifiers (UMIs or nCount_RNA) (B), and percentage of mitochondrial genes (percent.mt) (C) in individual clusters. (**D**) Violin plots of ribosomal genes in each cluster.

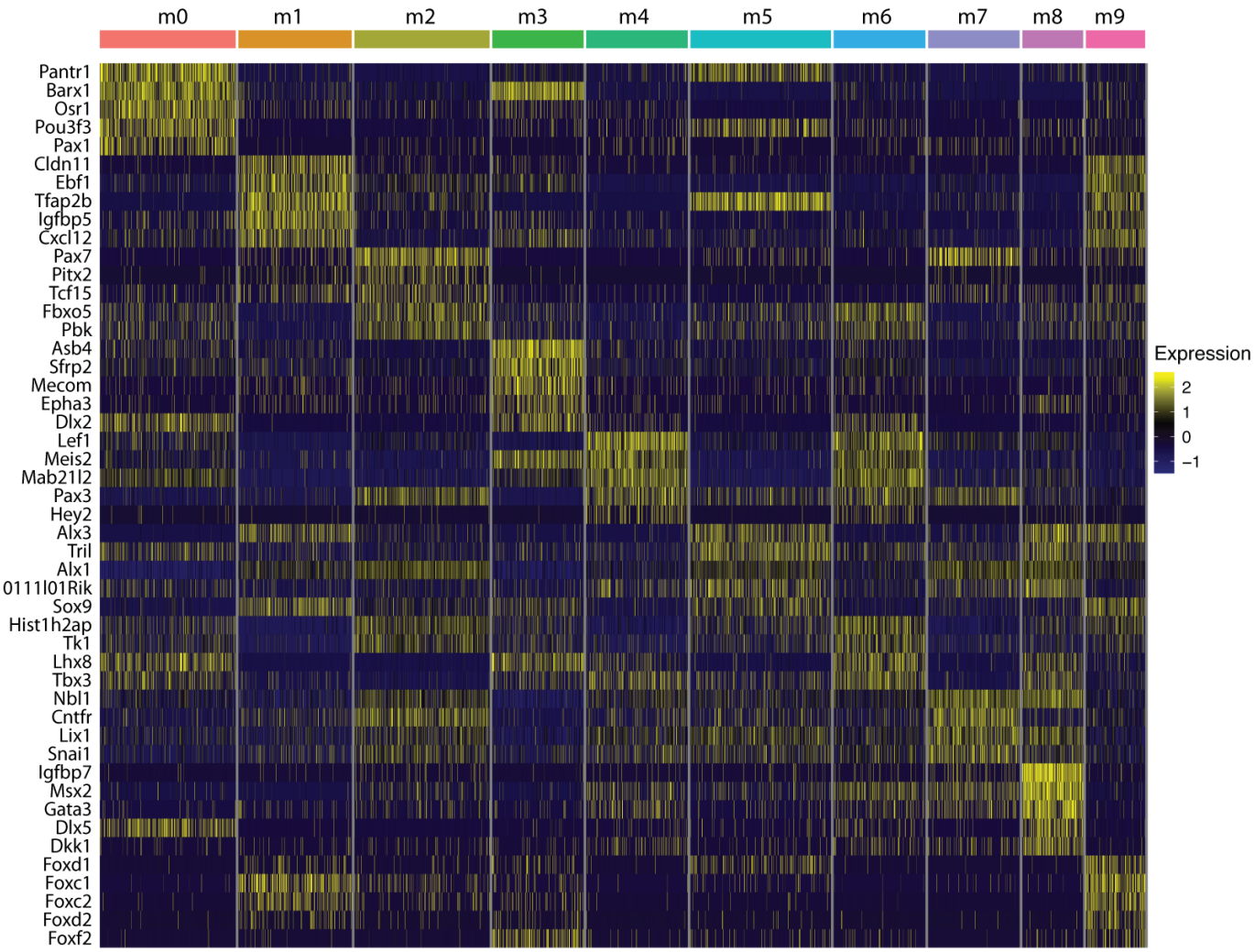

Fig. S4. Top five gene markers for each mesenchymal subpopulation. Heatmap shows expression of top five marker genes for 10 mesenchymal subpopulations labeled m0~m9 determined in E11.5 midfacial primordia.

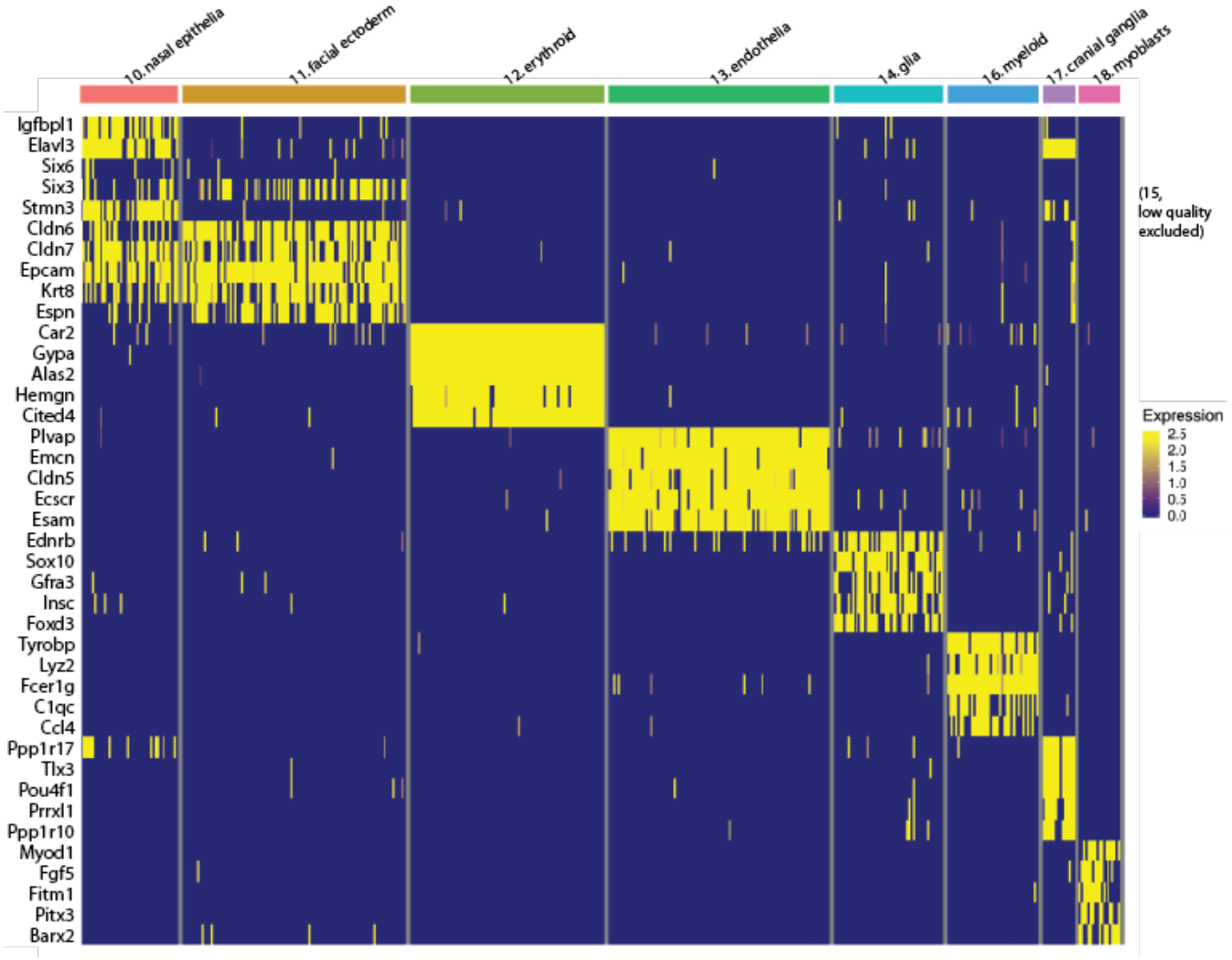

Fig. S5. Gene markers for non-mesenchymal cells. Heatmap shows expression of top five marker genes for 8 non-mesenchymal lineage cells of E11.5 midfacial primordia.

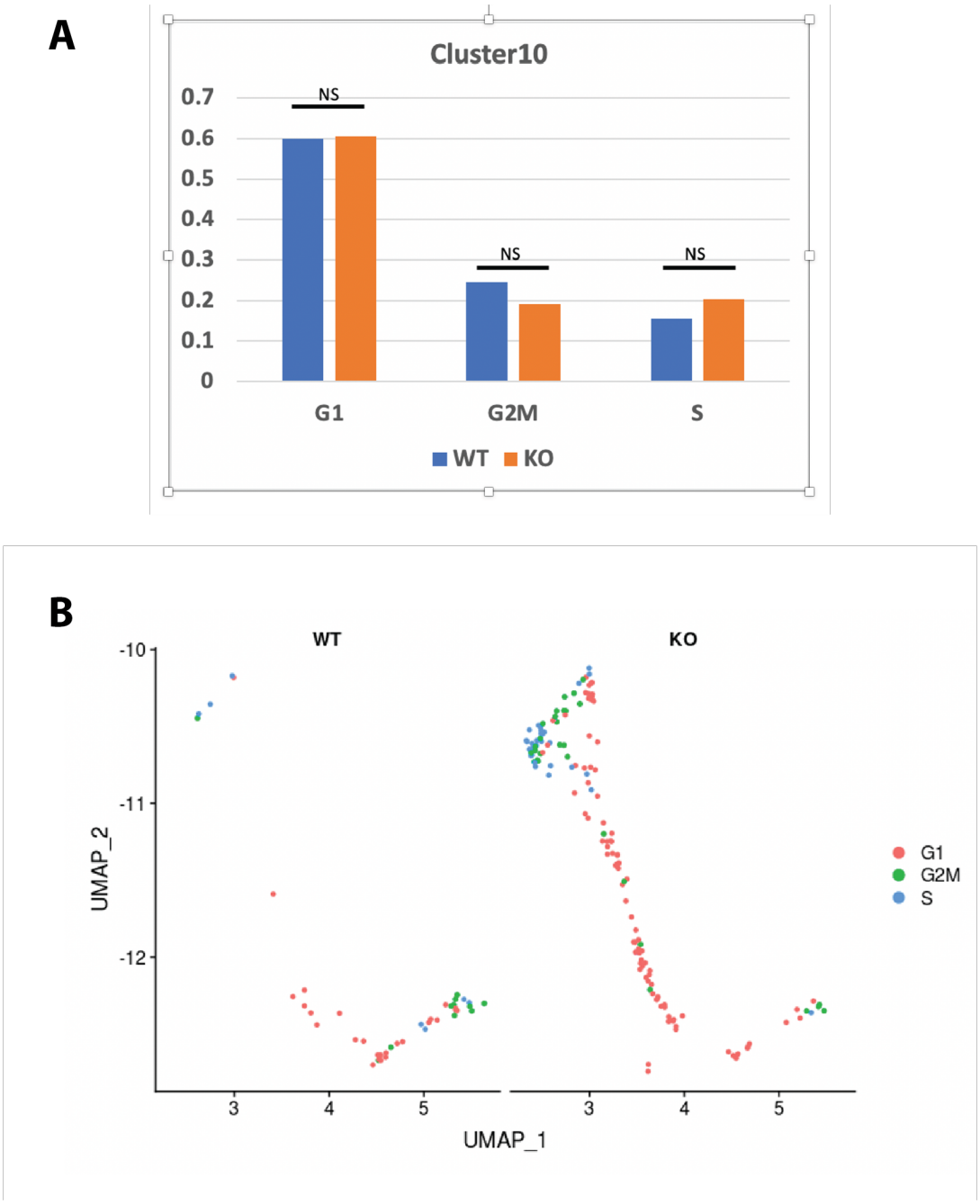

Fig. S6. Cell cycle analyses of cluster 10 (nasal epithelial cells). (**A**) Bar graphs show no significant (NS; Z-test) changes of G1, G2/M, and S phases of cluster 10 cells in Wls-cKOs. (**B**) UMAP shows significantly lower cell numbers in the wild-type (WT) control embryos compared to the cKO embryos, which is caused by a technical limitation of scRNA-seq preparation when individual cells were incorporated to liquid droplets from the WT midfacial primordia that contain much higher percentiles of mesenchymal cells than that in the cKO embryos, which reduces the chance of the much lower percentile of the WT nasal epithelial cells to be sequenced.

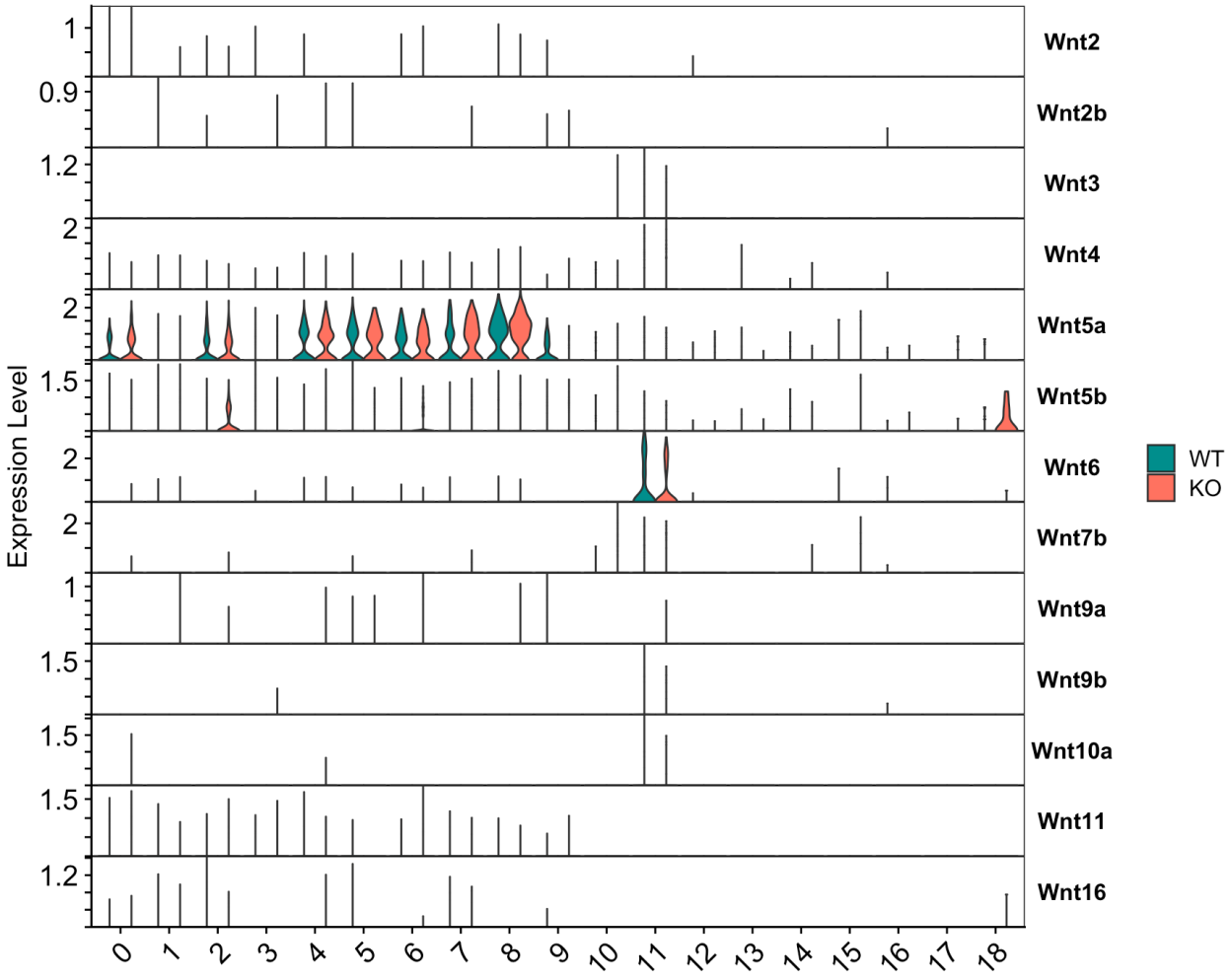

Fig. S7. Violin plots of 13 *Wnts* in various cell types of midfacial primordia at E11.5. *Wnt5a* is predominately expressed in midfacial mesenchymal cells (clusters 0-9) and Wnt6 is mainly expressed in the surface ectodermal cells (cluster 11). The rest of 6 *Wnts* are not expressed.

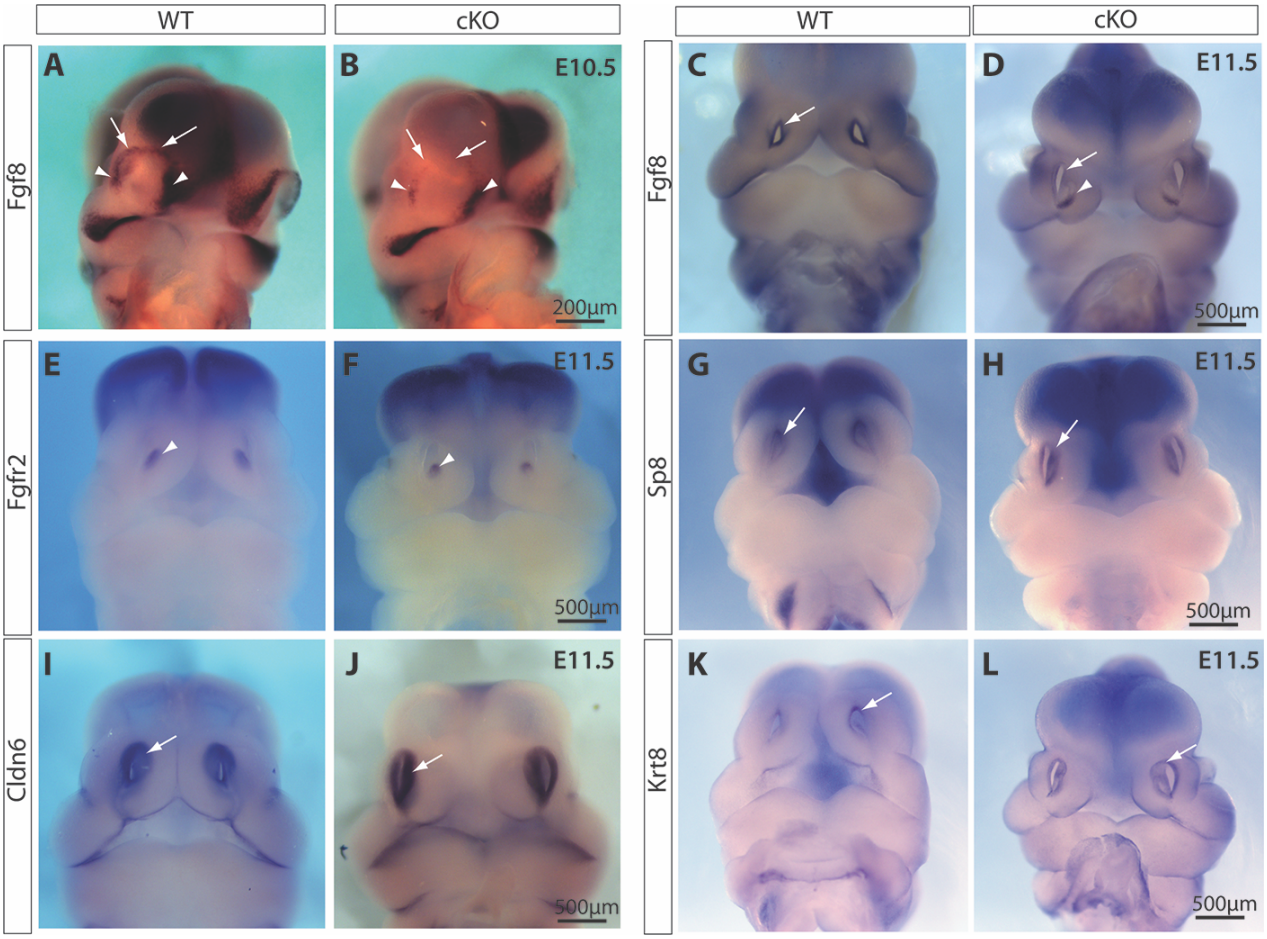

Fig. S8. Non-neural nasal epithelial and surface ectodermal cell markers in WT and Wls-cKO midfacial primordia. (**A-D**) Altered *Fgf8* expression patterns in the distal nasal epithelia of Wls-cKOs at E10.5 and E11.5. Arrows indicate the diminished and arrowheads indicate the conserved expression domains of *Fgf8* in the cKOs. (**E,F**) *Fgfr2* expression (arrowheads) is not altered in the nasal epithelia adjacent to mutant MNPs. (**G,H**) *Sp8* expression is not altered in the mutant nasal pit. (**I,J**) *Cldn6* expression is relatively conserved in the mutant nasal epithelia and surface ectoderm. (**K,L**) *Krt8* expression is relatively conserved in the mutant.

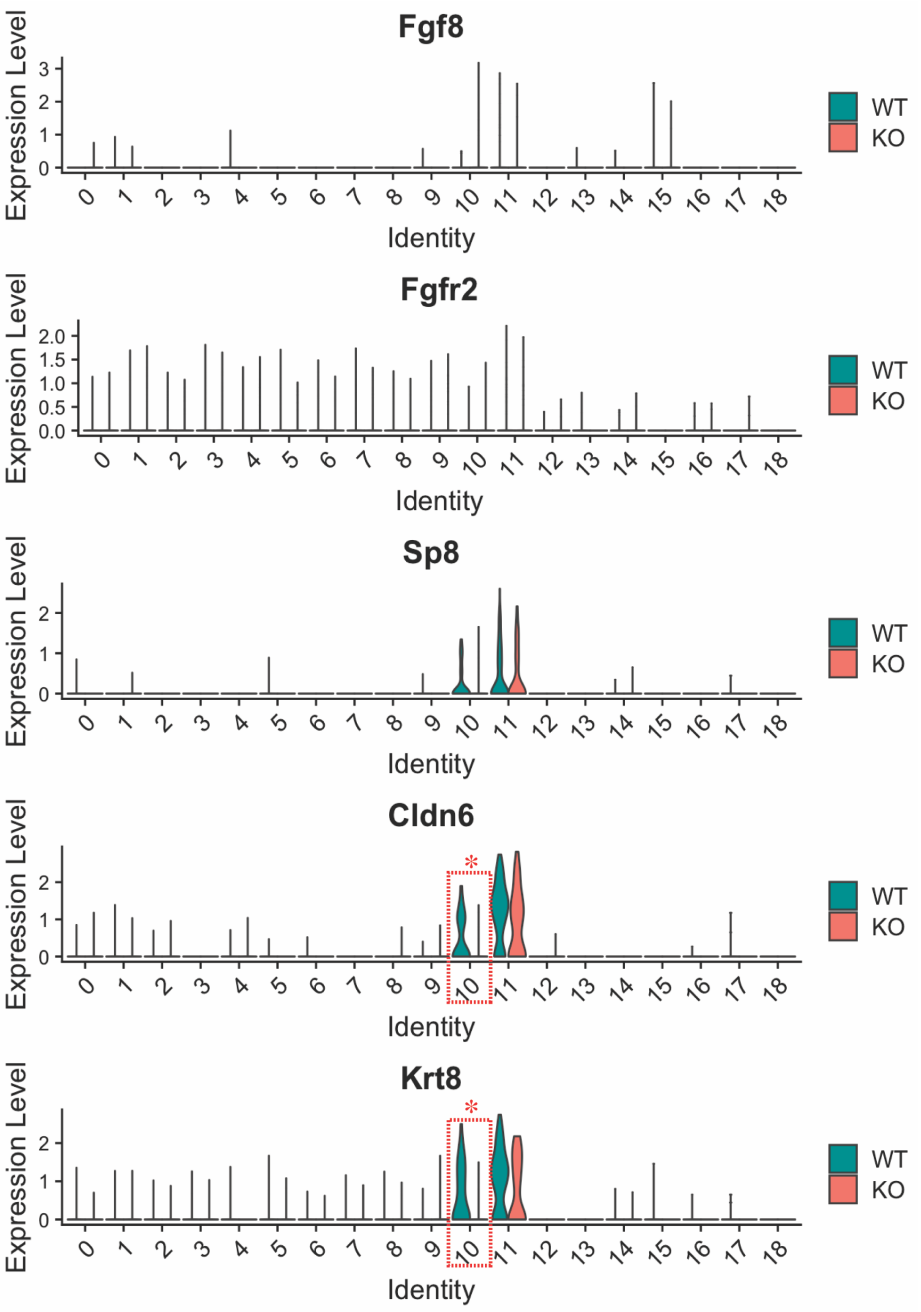

Fig. S9. Violin plots of *Fgf8* and related non-neural epithelial genes in WT and Wls-cKO midfacial primordia at E11.5. *Fgf8* is mainly expressed clusters 10, 11, and 15, but it is not determined as a DEG by scRNA-seq analysis. *Fgfr2* is widely detected in both mesenchymal and epithelial cells. *Sp8* is detected predominately in epithelial clusters 10 and 11. Cldn6 and Krt8 are high in 10 and 11, and both diminished in cluster 10. *, P_adjust value < 0.05, bimod test.

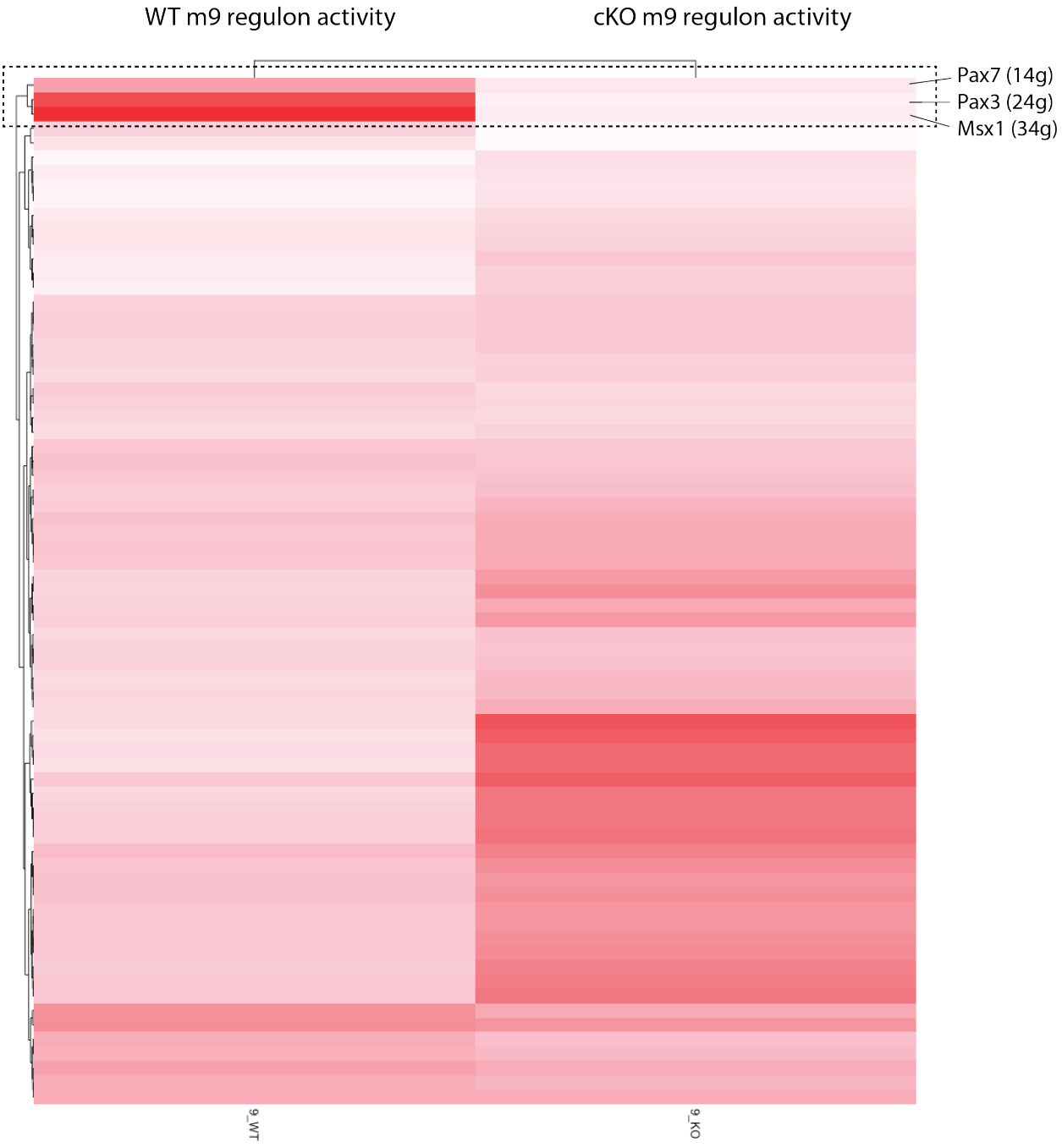

Fig. S10. Heatmap of regulon activities in WT and Wls-deficient m9 mesenchymal cells. A subcluster (dashed square) with high regulon activities consists of Msx1, Pax3, and Pax7 in the WT, but they show little activities in the mutant. The number of predicted target genes is labeled after each regulon. For instance, Msx1 (34g) means that Msx1 regulates 34 target genes (see details in Fig. 11B).

Table S1. Sequencing information.

Samples (E11.5 mouse midfacial primordia)	**Littermate control**	***Pax3^{Cre};Wls-cKO***
Estimated Number of Cells	4,284	3,441
Mean Reads per Cell	37,778	63,081
Median Genes per Cell	2,241	2,489
Number of Reads	161,841,670	217,063,706
Sequencing Saturation	65.80%	78.40%
Reads Mapped to Genome	91.50%	92.50%
Reads Mapped Confidently to Intronic Regions	15.10%	15.80%
Reads Mapped Confidently to Exonic Regions	70.20%	70.60%
Total Genes Detected	17,903	17,981
Median UMI Counts per Cell	6,685	7,475

Table S2. Gene ontology relevant to midfacial development. m0~m9, mesenchymal clusters 0~9; ne, nasal epithelia (cluster 10); se, surface ectoderm (cluster 11). *, The bottom two rows are not specifically relevant to midfacial development.

	m0	m1	m2	m3		m4	m5		m6	m7	m8	m9		ne	se
	down	down	down	up	down	down	up	down	down	down	up	up	down	down	up
face development							Pax9, Dlx5, Zfand5						Msx1, Cdk2ap1, Wnt5a		
nose development							Six1, Dlx5							Gng8, Dlx5, Six1, Smchd1	
roof of mouth development							Inhba, Foxf2, Dlx5, Osr2, Satb2, Tbx3, Alx1						Alx1, Prrx1, Msx1, Sox11, Tbx2, Anp32b, Acvr2b, Wnt5a, Gas1		
cell fate commitment							Six1, Tfap2c, Satb2, Tbx3, Ptch1	Pax3, Gap43, Hes1					Prrx1, Pax3, Tbx2,Pax7, Id2, Wnt5a, Bcl11b, Eya1, Gas1	Gap43, Ebf2, Nrg1, Neurog1, Olig1, Six1, Myt1l	
pattern specification process							Foxf1, Six1, Satb2, Tbx3, Gpc3, Alx1, Ptch1, Pcsk5, Gja1, Zeb2	Pax3, Irx3, Nbl1, Wls, Hes1					Wls, Alx1, Pax3, Irx3, Msx1, Tbx2, Pax7, Acvr2b, Wnt5a, Eya1, Gas1		
neural crest cell development								Pax3, Sox11, Hes1					Alx1, Pax3, Twist1, Sox11		
mesoderm development							Foxf1,Inhba,Tead2, Tbx3, Gja1						Wls, Irx3, Acvr2b, Wnt5a, Eya1		
mesenchyme development							Foxf1, Six1, Frzb, Epha3, Tead2, Alx1, Gja1, Trim28, Zeb2	Pax3, Sox11, Hes1				Foxc2, Foxd1, Pdcd4, Anxa6,Glipr2	Alx1, Pax3, Msx1, Twist1, Smad7, Sox11, Tbx2, Wnt5a		
mesenchymal cell differentiation							Frzb, Epha3, Alx1, Gja1, Trim28, Zeb2	Pax3, Sox11, Hes1				Foxc2, Pdcd4, Anxa6, Glipr2	Alx1, Pax3, Msx1, Twist1, Smad7, Sox11, Wnt5a		
mesenchymal cell proliferation			Prrx2, Msx1				Foxf1, Six1, Gpc3				Hmgb1, Six1	Foxp1, Foxp2, Gpc3	Prrx1, Prrx2, Msx1, Wnt5a, Gas1		Hmgb1
bone development							Dlx5, Osr2, Serpinh1, Mmp14, Gja1					Sparc, Col2a1, Serpinh1, Anxa6			
osteoblast differentiation				Cbfb, Id2, Sfrp2			Dlk1, Dlx5, Satb2, Ptch1,Gja1						Id1, Id3, Twist1, Sox11, Erh, Acvr2b, Id2		
osteoblast proliferation							Osr2, Fbln5								
cartilage development			Prrx2, Msx1				Frzb, Osr2, Serpinh1, Satb2					Col2a1, Serpinh1, Anxa6, Col11a1	Prrx1, Prrx2, Msx1, Smad7, Pax7, Wnt5a		
connective tissue development												Col2a1, Foxc2, Serpinh1, Foxd1, Anxa6, Col11a1	Prrx1, Prrx2, Msx1, Smad7, Pax7, Id2, Wnt5a		
muscle tissue development							Six1, Fos, Ppp3ca, Tbx3, Plagl1, Gja1, Zfand5				Id2, Six1, Fos, Ddx5	Myl6, Tpm1, Foxp1, Foxp2, Foxc2, Col11a1, Col3a1	Pax3, Twist1, Smad7, Sox11, Tbx2, Pax7, Id2,Wnt5a, Eya1		
canonical Wnt signaling pathway							Frzb, Rspo3, Dlx5, Sox4, Gpc3								
non-canonical Wnt signaling pathway				Gpc3, Sfrp2			Frzb, Rspo3, Gpc3								
cellular aldehyde metabolic process	Aldoa, Tpi1				Aldoa, Tpi1	Aldoa, Tpi1			Aldoa, Tpi1						
cytoplasmic translation		Rpl8, Rps2, Rpl29, Rpsa	Rpl8, Rpsa, Rps2					Rpl8, Rps2, Rpsa		Rps2, Rpl8			Rpl8, Rps2, Rps23, Rpl18a,Rpl10a, Rpsa, Rpl19, Rpl18, Eif5, Pkm		

Table S3. List of primers used for quantification of specific gene expression in this study.

Genes	Forward Primer	Reverse Primer
Alx1	TTACCAAGGACGGACAGCTA	CATGCATGACGTAACCACAG
Alx3	CGTACTGCCCAGAACTGACA	ATGCCCTCTGGAGACATGAG
Alx4	ACACATGGGCAGCCTGTTTG	TGCTTGAGGTCTTGCGGTCT
Dlx5	CTGGTGACTGTGGCGAGTTA	CAGAAGAGTCCCAAGCATCC
Dlx6	CTCAATACCTGGCCCTTCC	AGAGCGCTTATTCTGAAACCAT
Gapdh	CAACGACCCCTTCATTGACC	GGTCTCGCTCCTGGAAGATG
Id1	TTCTCAGGATCATGAAGGTCGCCA	TTTGCTCCGACAGACCAAGTACCA
Id2	TGCCCAATGTAAGCAGACTTTGCC	ACAGCATTCAGTAGGCTCGTGTCA
Lef1	TCACTGTCAGGCGACACTTC	TGAGGCTTCACGTGCATTAG
Msx1	AAGATGCTCTGGTGAAGGCCGAAA	CTTGCGGTTGGTCTTGTGCTT
Msx2	ATACAGGAGCCCGGCAGATACT	AACTTGCGCTCCAAGGCTAGAA
Pax3	TGCCCTCAGTGAGTTCTATCAGC	GCTAAACCAGACCTGCACTCGGGC
Pax7	CCGTGTTTCCCATGGTTGTG	GAGCACTCGGCTAATCGAAC
Pdgfra	GAGGATAAGCTGAAGGACTGGGAAGG	TACTGGAACCTGTCTCGATGGCACT
Tfap2a	CGCTCCTGGGCGGAGTA	CCTATCTTGTCCAGTTTTTCTCTTAAAGA
Tfap2b	GGCTTCTTGGGAGGAATGTCAG	CCTTCTACCAGTGAGGTGAGTAACG
Twist1	AGTCTGAACACTCGTTTGTGTCCC	ATGCCTTTCCTGTCAGTGGCTGAT
Wls	TTTCCAAATCGTTGCCTTTCTG	TGGTTCTTACGGACATCCAC
Wnt5a	AGGAGTTCGTGGACGCTAGA	ACTTCTCCTTGAGGGCATCG
Wnt9b	AAGTACAGCACCAAGTTCCTC	CACTTGCAGGTTGTTCTCAG

Table S4. List of primers used for ChIP PCR assays in this study.

Gene	Forward primer (5'→3')	Reverse primer (5'→3')	Size (bp)
Wnt5a_BS1	GGGAGTGAACCGATCTTTGA	CTTCGGCTCTTTTCCTAAGC	152
Wnt5a_BS2	GCGGGGGTTAGTTTGTGAAC	CCGAAGGAAAAGTTGTTTGG	159
Kif26b_BS1	GTATTCTGCCCCCTCCTTTC	CGGGGTGTCATTTTTCATTT	259
Kif26b_BS2	CGGATTCTCTAGACCAAAAATAAA	TCCCCAAATTAAGGTGTAAATTC	179
Kif26b_BS3	CAGCCCAGGCTAAGTCAAAG	GTTTGCATGGGTCTGTTTCC	154
Smad7_BS1	GTGCACAGAATTCGAGGAGA	CTACAGTGGCTCCCGAGTGT	254
Smad7_BS2	CTGAATCCAGGGTCTCTAAGGA	AGGACAGTCATGAAACTTACGG	220
Smad7_BS3	CTTCCCGGGTTGCTTTAGA	CCATTCCCCTTCCACACTAA	240
Smad7_BS4	CACCTACTTGCCCCATCTGT	GCCACCACTGCTCTGCTAGT	256
Smad7_BS5	ACCATAAAGCACCAGCCATT	CAGATGGGGCAAGTAGGTGT	243
Smad7_BS6	CTGCACCGTGATTAGGGTTT	GGCTCTTCTTTCGCCTCAC	237