The 4th FaceBase Annual Meeting

FaceBase
A Resource For Craniofacial Researchers

Project updates on Functional Genomics, Imaging Analyses, and Rescue of Cleft Palate

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Bridget Samuels

U01 DE020065 NIDCR, NIH

National Institute of Dental
and Craniofacial Research

Center for
Craniofacial
Molecular
Biology

What did we propose to do and what have been accomplished

1. Global and specific gene expression analyses and 3D imaging study

   143 sets of microarray data uploaded to the hub

   87 hard and 104 soft tissue microCT images uploaded to the hub and how to use microCT images to study hard and soft tissue structures

   Development of a data presentation interface with FaceBase

2. High-throughput analysis of Tgf-β downstream target genes in our *Tgfbr* mutant models and to test whether manipulation of altered Tgf-β downstream signaling molecules offers the opportunity to rescue cleft palate in vivo.

   Discovery of an alternative Tgf-β signaling pathway and rescue of cleft palate in vivo (Iwata et al., *JCI*, 2012; Iwata et al., *Development*, 2013; Iwata et al., *JBC* 2011, 2012)

FaceBase

Search Msx1

Molecules:

- Growth factors
- Receptors
- Signaling molecules
- Transcription factors
- Intracellular molecules
- Extracellular molecules
- Plasma membrane molecules
- miRNA
- Enhancers

Tissues:

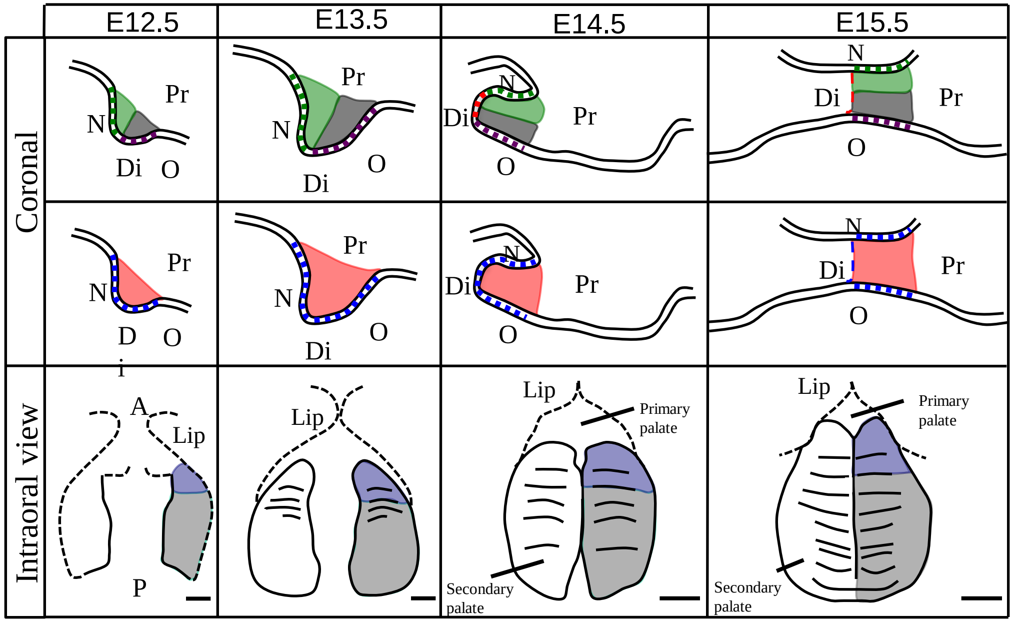
- **Epithelium**
  - Oral epithelium
  - Nasal epithelium
  - Midline epithelium
  - Basal epithelium
  - Peridermal cells
- **Mesenchyme**
  - Nasal region
  - Oral region
  - Anterior region
  - Posterior region
  - Palatal bone primordium
- **Muscles of the soft palate**
  - Levator
  - Tensor
  - Palatoglossus
  - Palatopharyngeous
  - Uvula

Species:

- Mouse
- Rat
- Human
- Other

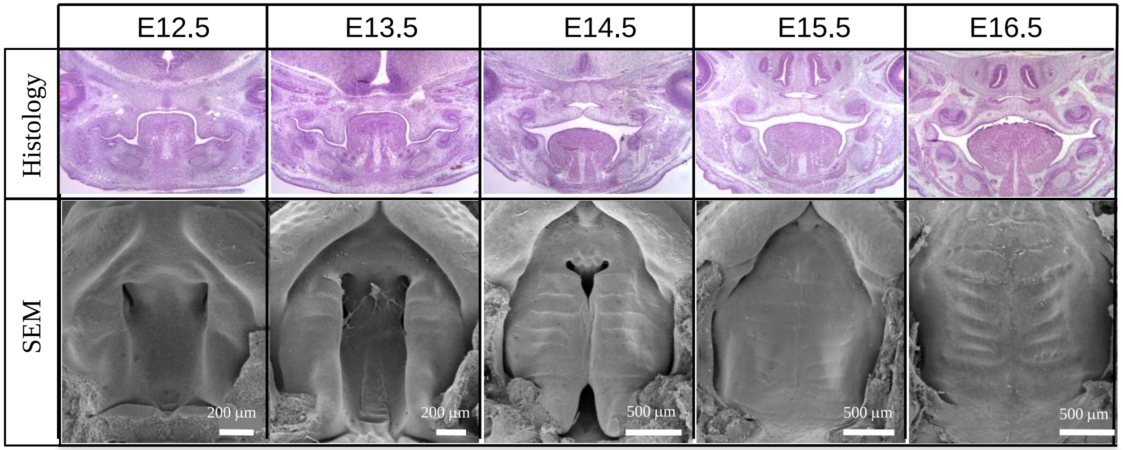
Gene expression in the secondary palate

Select the tissue of interest (click at the tissue of interest to get a list of the genes expressed in that tissue)

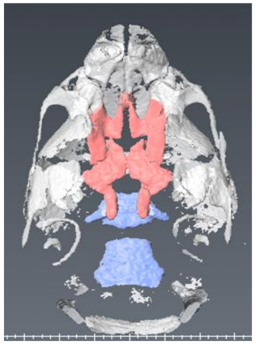


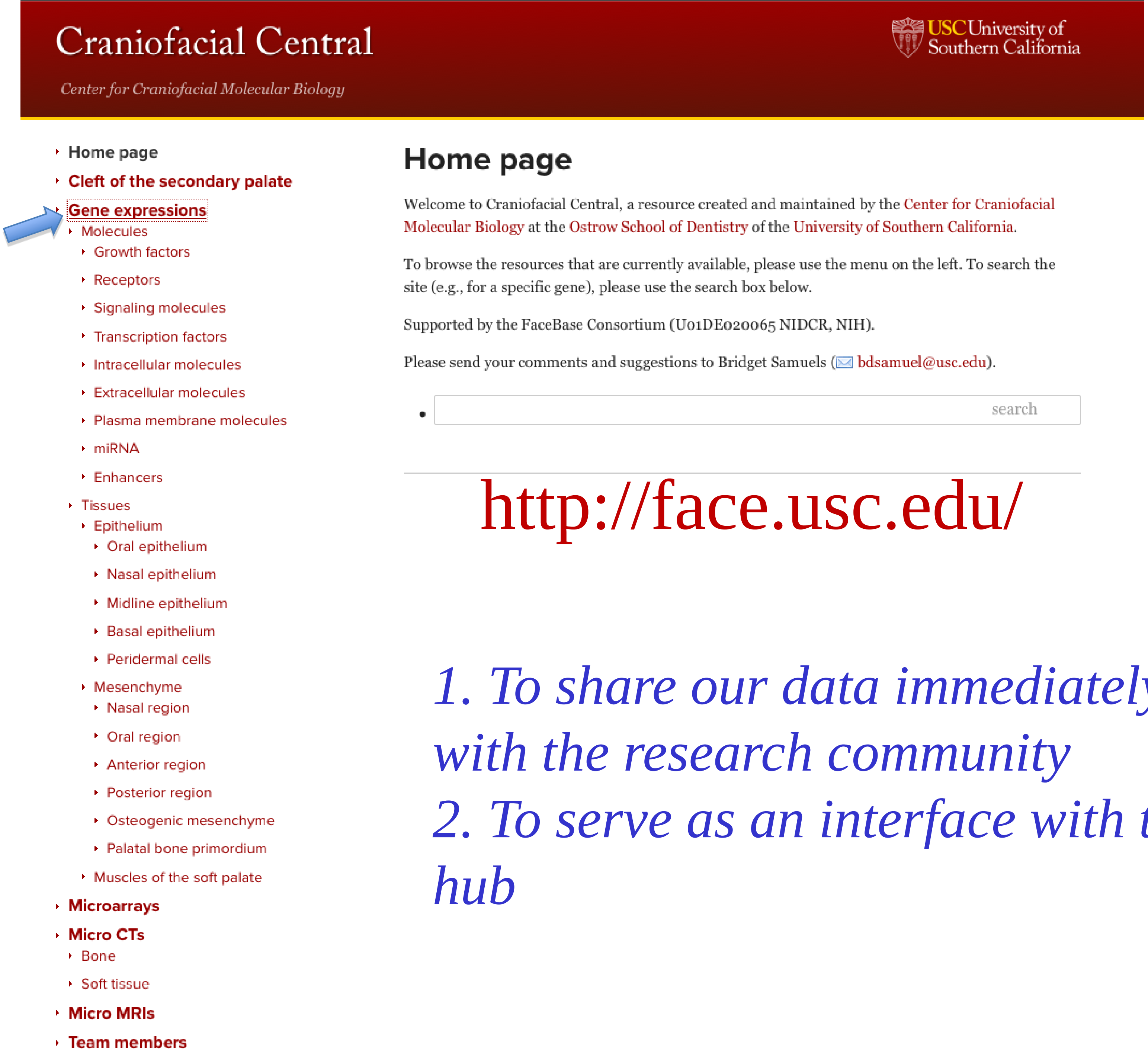
Color code:

- 🟩 Nasal mesenchyme
- ⬛ Oral mesenchyme
- 🟥 All mesenchyme
- 🟪 Anterior mesenchyme
- ⬜ Posterior mesenchyme
- 🟩🟩🟩🟩 Nasal epithelium
- 🟥🟥🟥🟥 Midline epithelium
- 🟪🟪🟪🟪 Oral epithelium
- 🟦🟦🟦🟦 All epithelium



3D images
µCT scans





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Home page

Welcome to Craniofacial Central, a resource created and maintained by the Center for Craniofacial Molecular Biology at the Ostrow School of Dentistry of the University of Southern California.

To browse the resources that are currently available, please use the menu on the left. To search the site (e.g., for a specific gene), please use the search box below.

Supported by the FaceBase Consortium (U01DE020065 NIDCR, NIH).

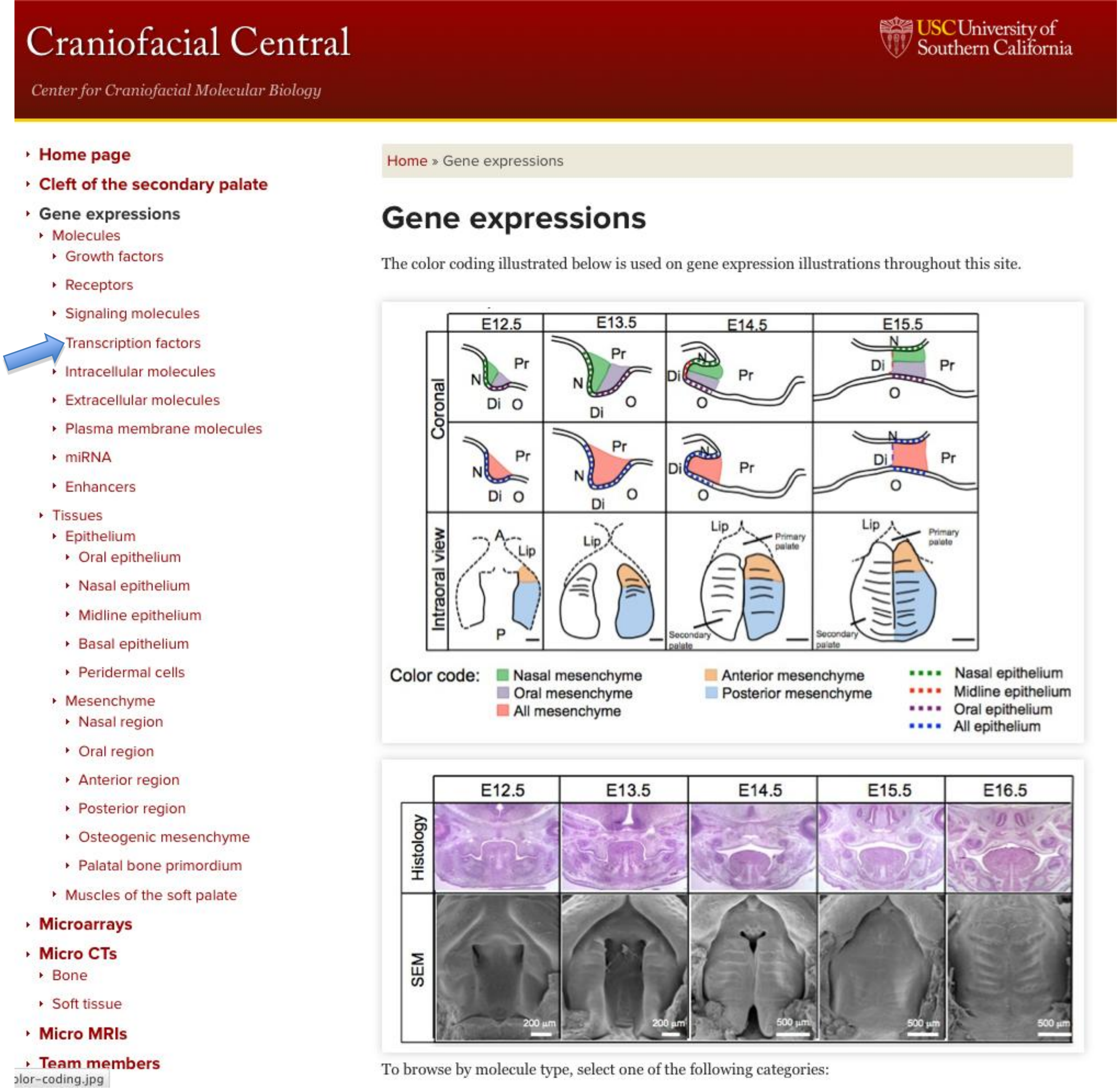
Please send your comments and suggestions to Bridget Samuels (✉ bdsamuel@usc.edu).

- search

http://face.usc.edu/

1. To share our data immediately with the research community

2. To serve as an interface with the hub



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olor-coding.jpg

Home » Gene expressions

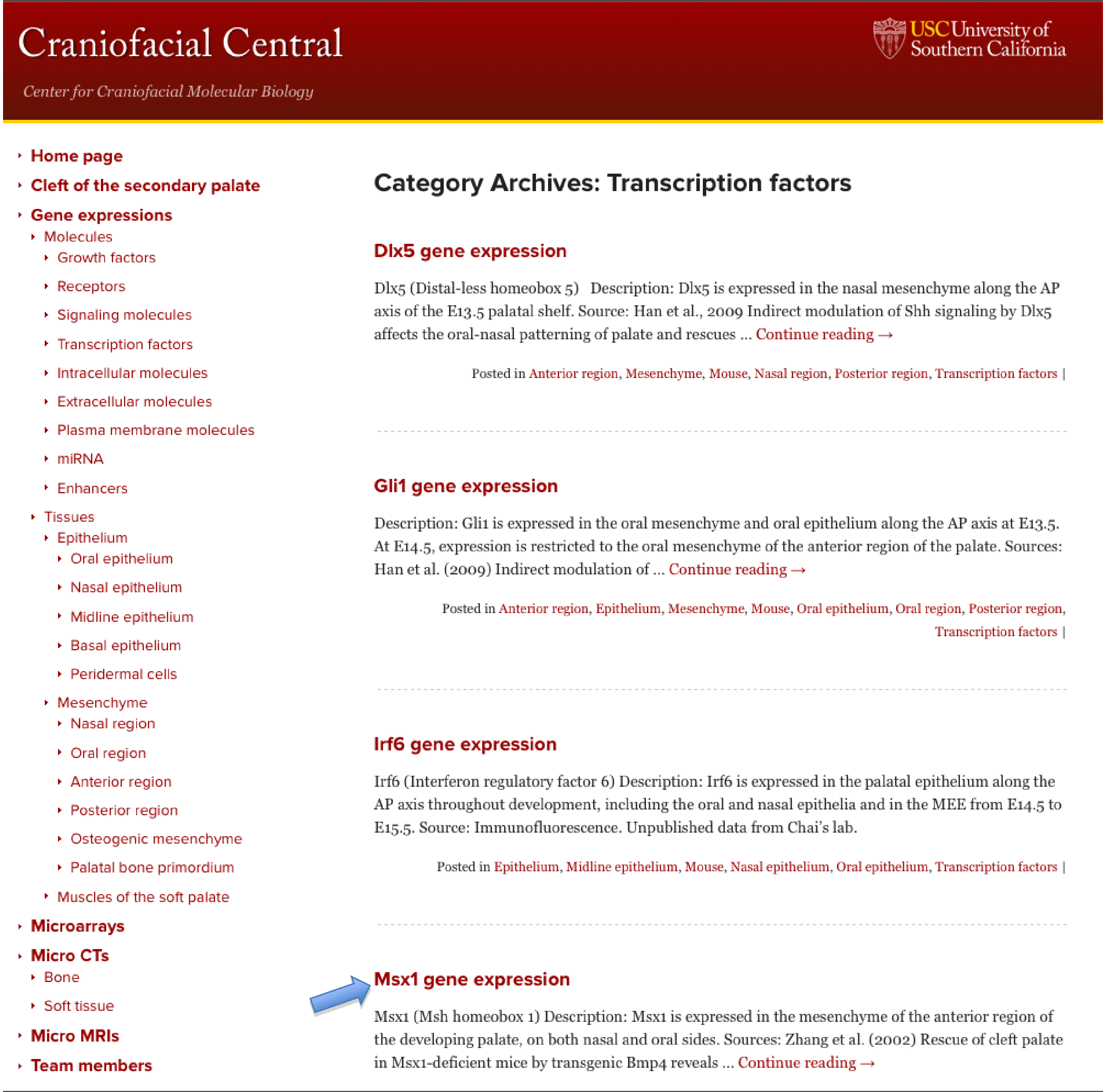
Gene expressions

The color coding illustrated below is used on gene expression illustrations throughout this site.

Color code:

🟩 Nasal mesenchyme
🟪 Oral mesenchyme
🟥 All mesenchyme
🟧 Anterior mesenchyme
🟦 Posterior mesenchyme
▪▪▪▪ Nasal epithelium
▪▪▪▪ Midline epithelium
▪▪▪▪ Oral epithelium
▪▪▪▪ All epithelium

To browse by molecule type, select one of the following categories:



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Category Archives: Transcription factors

Dlx5 gene expression

Dlx5 (Distal-less homeobox 5) Description: Dlx5 is expressed in the nasal mesenchyme along the AP axis of the E13.5 palatal shelf. Source: Han et al., 2009 Indirect modulation of Shh signaling by Dlx5 affects the oral-nasal patterning of palate and rescues … Continue reading →

Posted in Anterior region, Mesenchyme, Mouse, Nasal region, Posterior region, Transcription factors |

Gli1 gene expression

Description: Gli1 is expressed in the oral mesenchyme and oral epithelium along the AP axis at E13.5. At E14.5, expression is restricted to the oral mesenchyme of the anterior region of the palate. Sources: Han et al. (2009) Indirect modulation of … Continue reading →

Posted in Anterior region, Epithelium, Mesenchyme, Mouse, Oral epithelium, Oral region, Posterior region, Transcription factors |

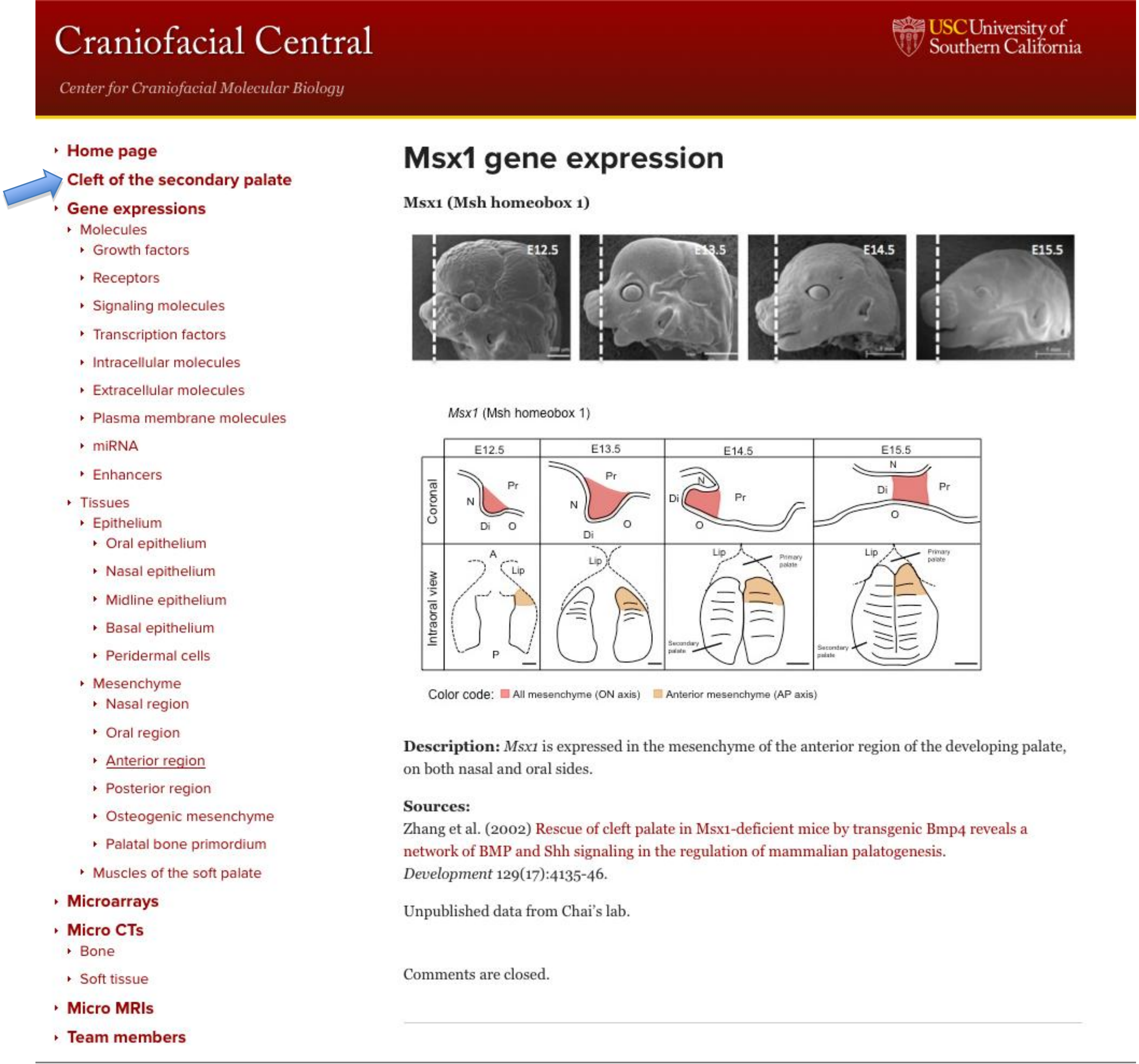
Irf6 gene expression

Irf6 (Interferon regulatory factor 6) Description: Irf6 is expressed in the palatal epithelium along the AP axis throughout development, including the oral and nasal epithelia and in the MEE from E14.5 to E15.5. Source: Immunofluorescence. Unpublished data from Chai's lab.

Posted in Epithelium, Midline epithelium, Mouse, Nasal epithelium, Oral epithelium, Transcription factors |

Msx1 gene expression

Msx1 (Msh homeobox 1) Description: Msx1 is expressed in the mesenchyme of the anterior region of the developing palate, on both nasal and oral sides. Sources: Zhang et al. (2002) Rescue of cleft palate in Msx1-deficient mice by transgenic Bmp4 reveals … Continue reading →



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Msx1 gene expression

Msx1 (Msh homeobox 1)

Msx1 (Msh homeobox 1)

Color code: 🟥 All mesenchyme (ON axis) 🟧 Anterior mesenchyme (AP axis)

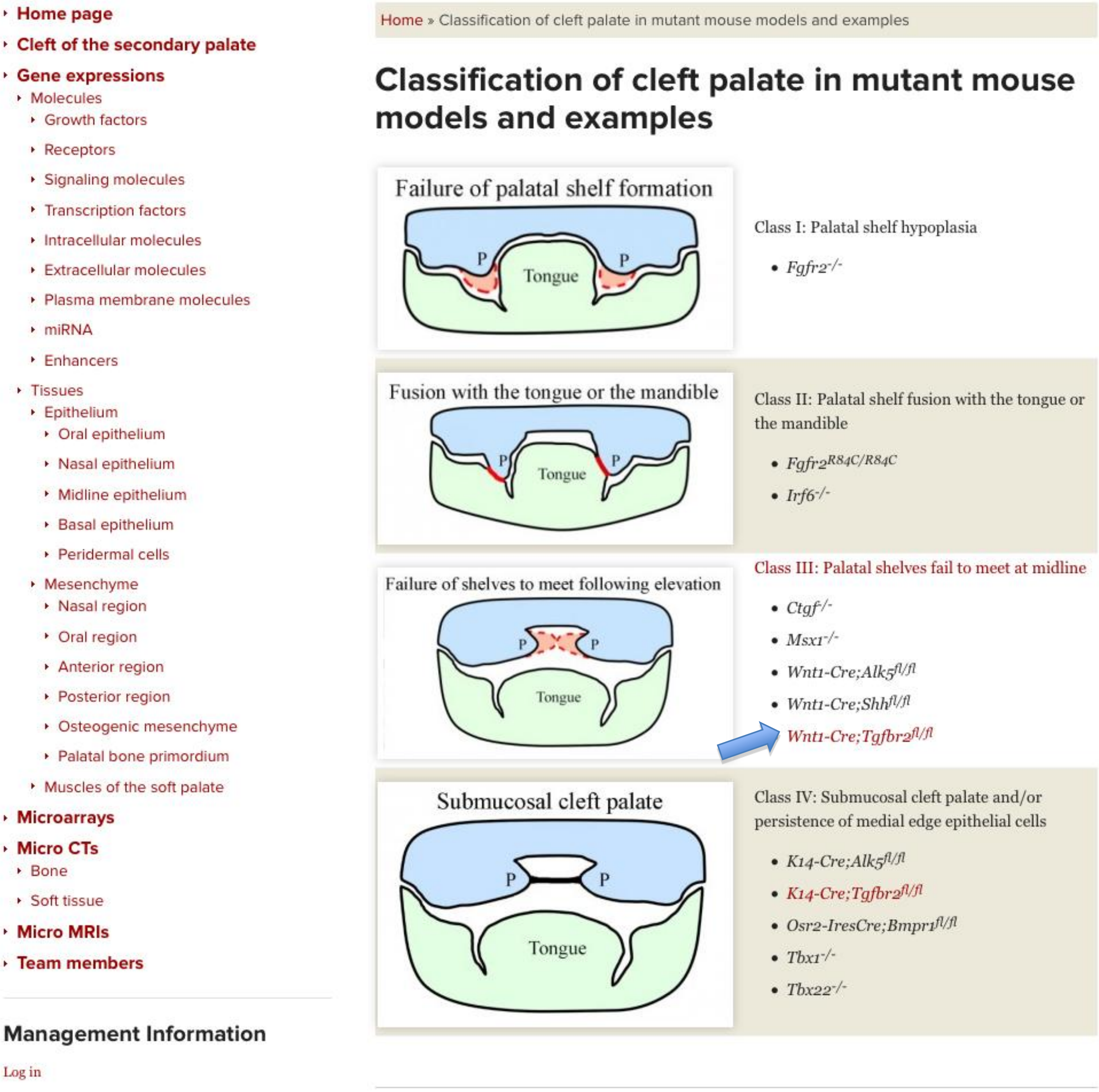
Description: *Msx1* is expressed in the mesenchyme of the anterior region of the developing palate, on both nasal and oral sides.

Sources:

Zhang et al. (2002) Rescue of cleft palate in Msx1-deficient mice by transgenic Bmp4 reveals a network of BMP and Shh signaling in the regulation of mammalian palatogenesis. *Development* 129(17):4135-46.

Unpublished data from Chai's lab.

Comments are closed.



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Management Information

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Classification of cleft palate in mutant mouse models and examples

Failure of palatal shelf formation

Class I: Palatal shelf hypoplasia

- *Fgfr2*$^{-/-}$

Fusion with the tongue or the mandible

Class II: Palatal shelf fusion with the tongue or the mandible

- *Fgfr2*$^{R84C/R84C}$
- *Irf6*$^{-/-}$

Failure of shelves to meet following elevation

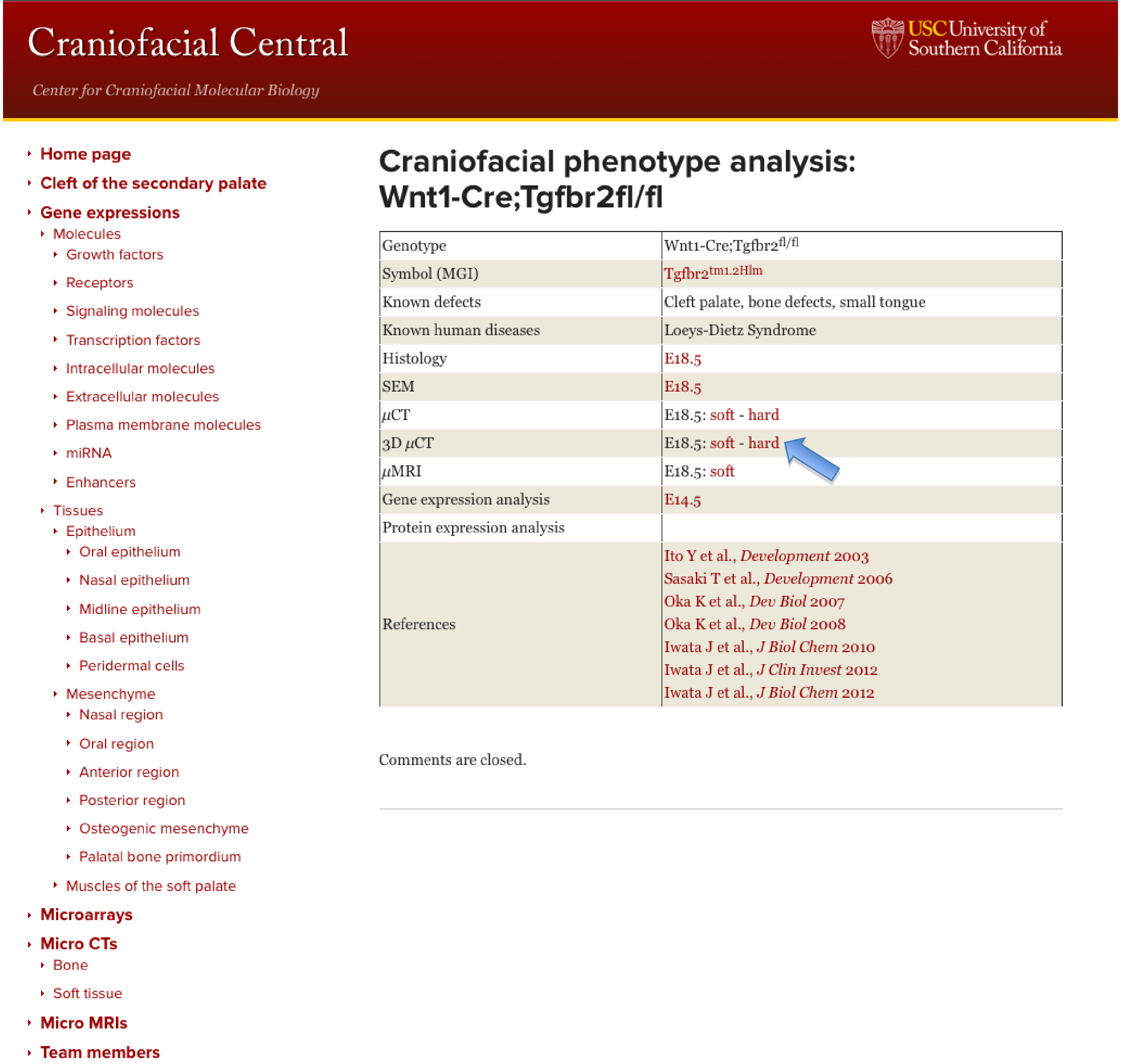
Class III: Palatal shelves fail to meet at midline

- *Ctgf*$^{-/-}$
- *Msx1*$^{-/-}$
- *Wnt1-Cre;Alk5*$^{fl/fl}$
- *Wnt1-Cre;Shh*$^{fl/fl}$
- *Wnt1-Cre;Tgfbr2*$^{fl/fl}$

Submucosal cleft palate

Class IV: Submucosal cleft palate and/or persistence of medial edge epithelial cells

- *K14-Cre;Alk5*$^{fl/fl}$
- *K14-Cre;Tgfbr2*$^{fl/fl}$
- *Osr2-IresCre;Bmpr1*$^{fl/fl}$
- *Tbx1*$^{-/-}$
- *Tbx22*$^{-/-}$



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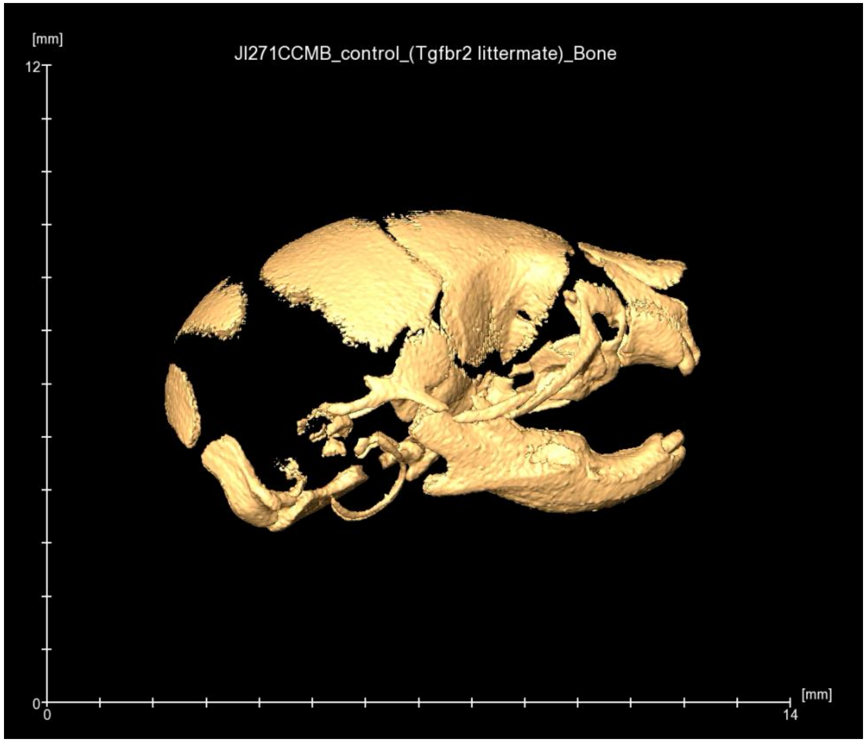
Craniofacial phenotype analysis: Wnt1-Cre;Tgfbr2fl/fl

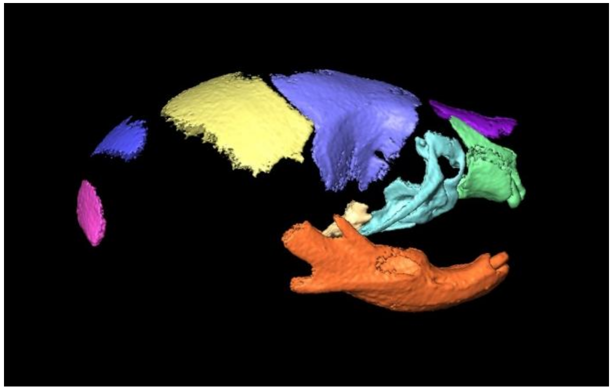
Genotype	Wnt1-Cre;Tgfbr2$^{fl/fl}$
Symbol (MGI)	Tgfbr2$^{tm1.2Hlm}$
Known defects	Cleft palate, bone defects, small tongue
Known human diseases	Loeys-Dietz Syndrome
Histology	E18.5
SEM	E18.5
μCT	E18.5: soft - hard
3D μCT	E18.5: soft - hard
μMRI	E18.5: soft
Gene expression analysis	E14.5
Protein expression analysis	
References	Ito Y et al., *Development* 2003 Sasaki T et al., *Development* 2006 Oka K et al., *Dev Biol* 2007 Oka K et al., *Dev Biol* 2008 Iwata J et al., *J Biol Chem* 2010 Iwata J et al., *J Clin Invest* 2012 Iwata J et al., *J Biol Chem* 2012

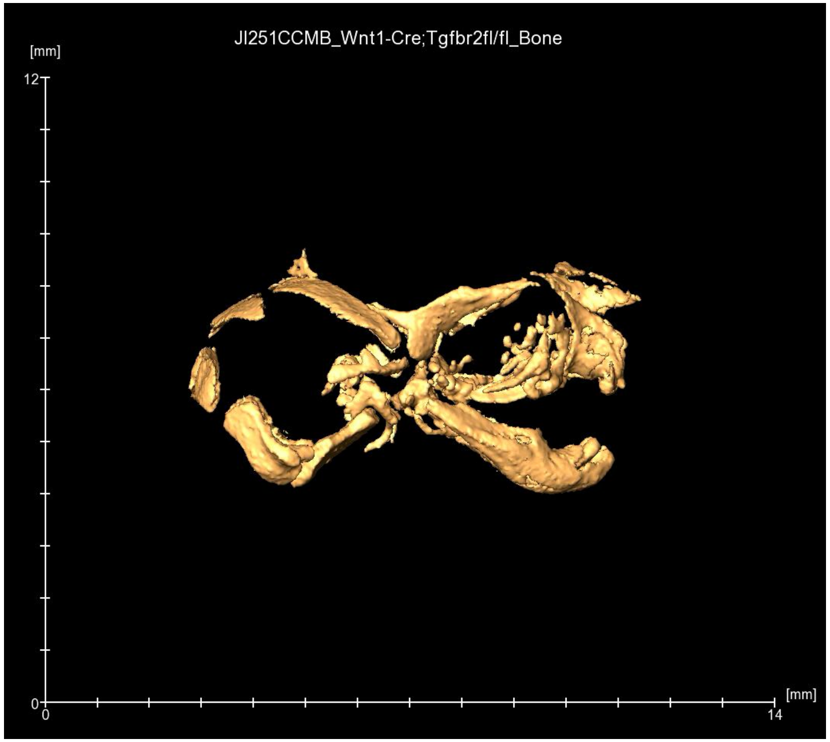
Comments are closed.

Dynamic microCT data for analysis of skull development and malformations

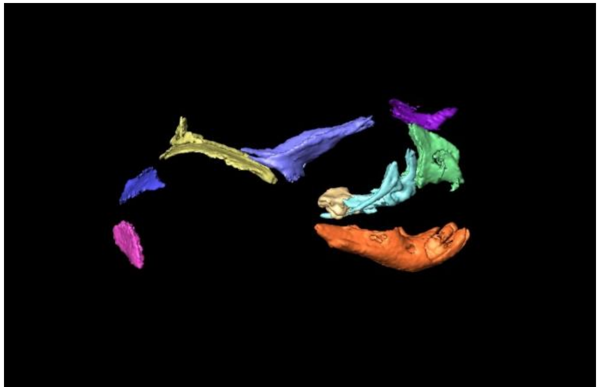


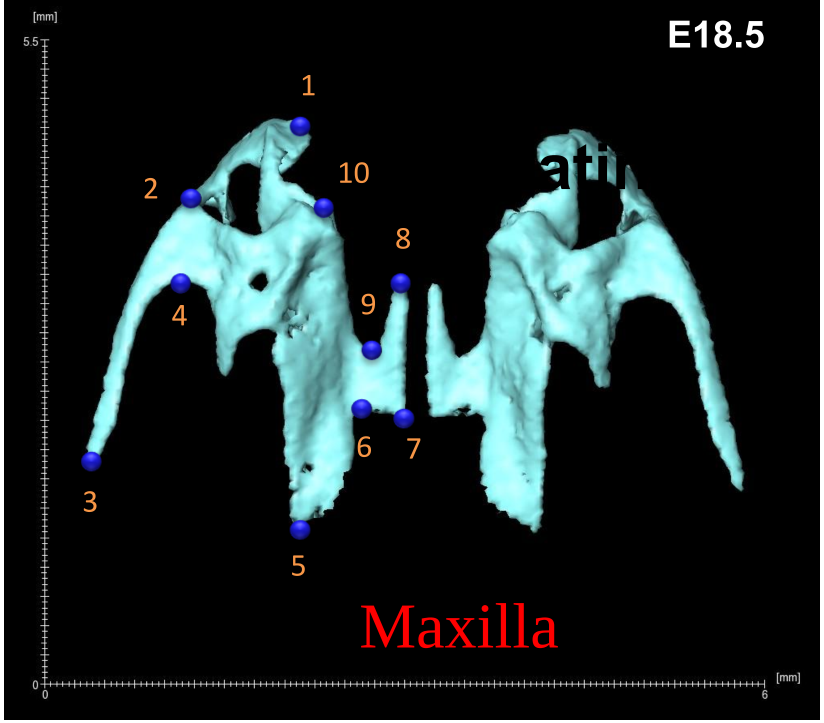
Control_E18.5



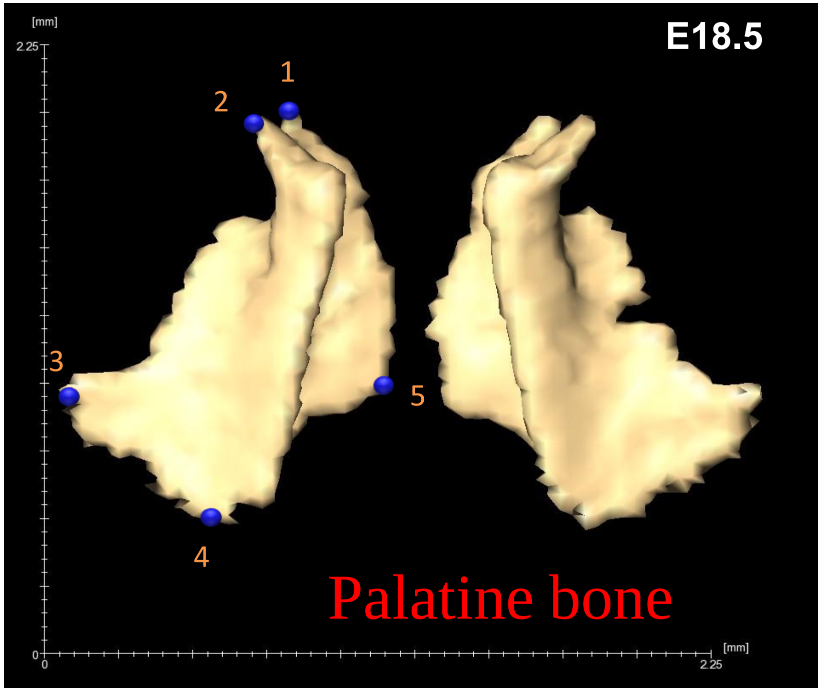


Wnt1-Cre;Tgfbr2$^{fl/fl}$_E18.5

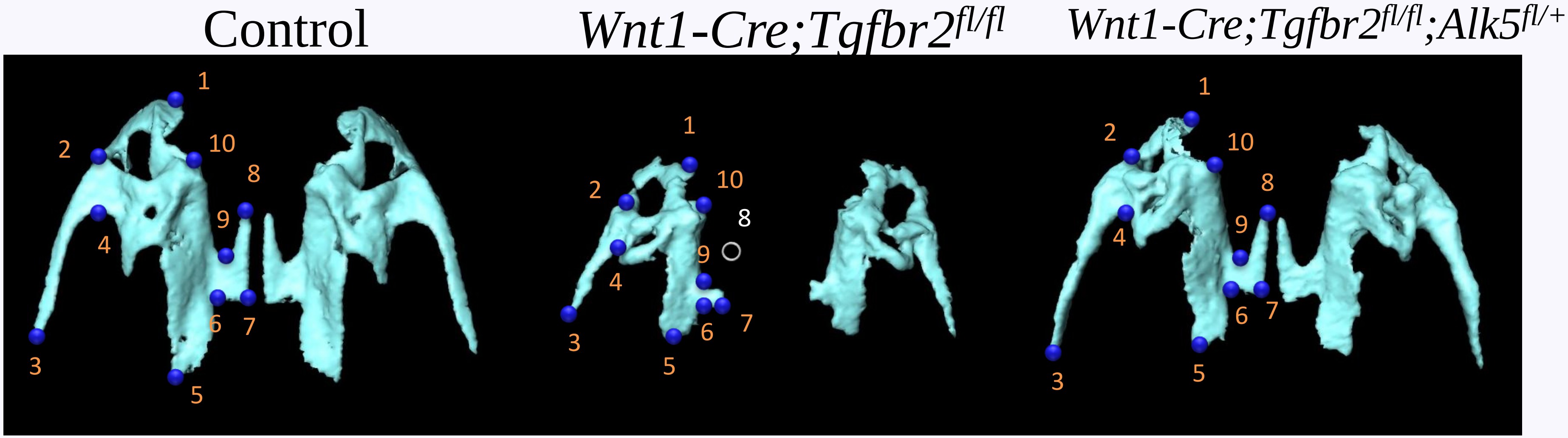




1. Anterior point of maxilla
2. Lateral point of premaxillary-maxillary suture
3. Tip of zygomatic process of maxilla
4. Anterior-medial point to zygomatic process
5. Posterior point of maxilla
6. Posterior-lateral point of the palatal process of maxilla
7. Posterior-medial point of the palatal process of maxilla
8. Anterior-medial point of palatal process of maxilla
9. Anterior-lateral point of the palatal process of maxilla
10. Medial point of premaxillary-maxillary suture



1. Anterior point of palatine bone
2. Anterior point of the ridge of palatine bone
3. Lateral point of the pyramidal process of palatine bone
4. Posterior point of the pyramidal process of palatine bone
5. Posterior-medial point of the horizontal plate of palatine bone


Control
Wnt1-Cre;Tgfbr2$^{fl/fl}$
Wnt1-Cre;Tgfbr2$^{fl/fl}$;Alk5$^{fl/+}$
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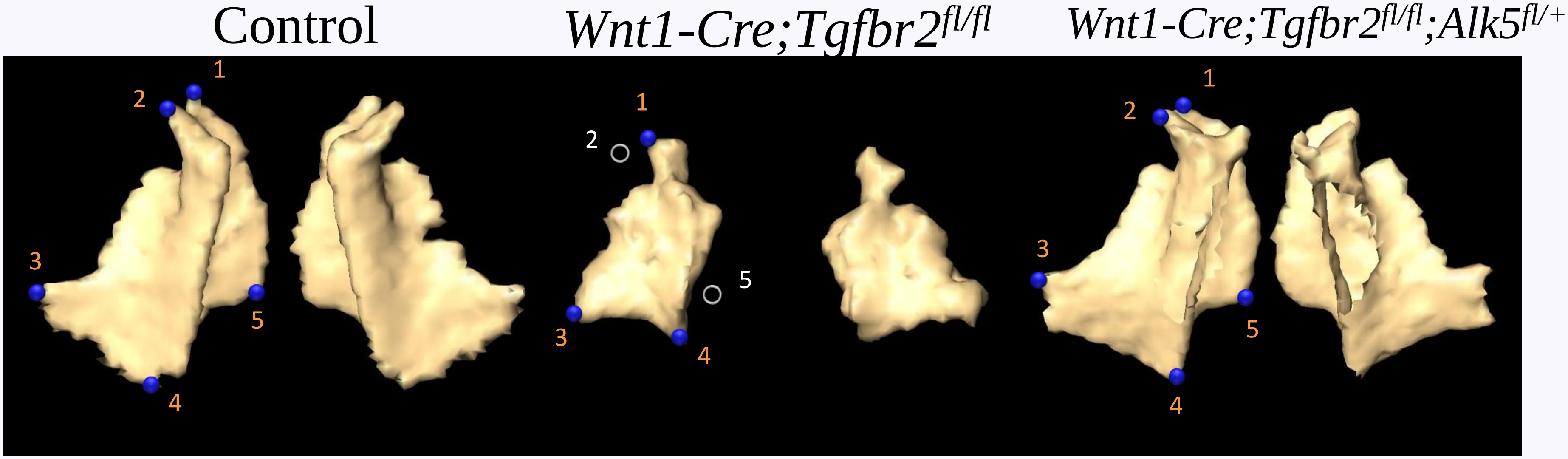
Control
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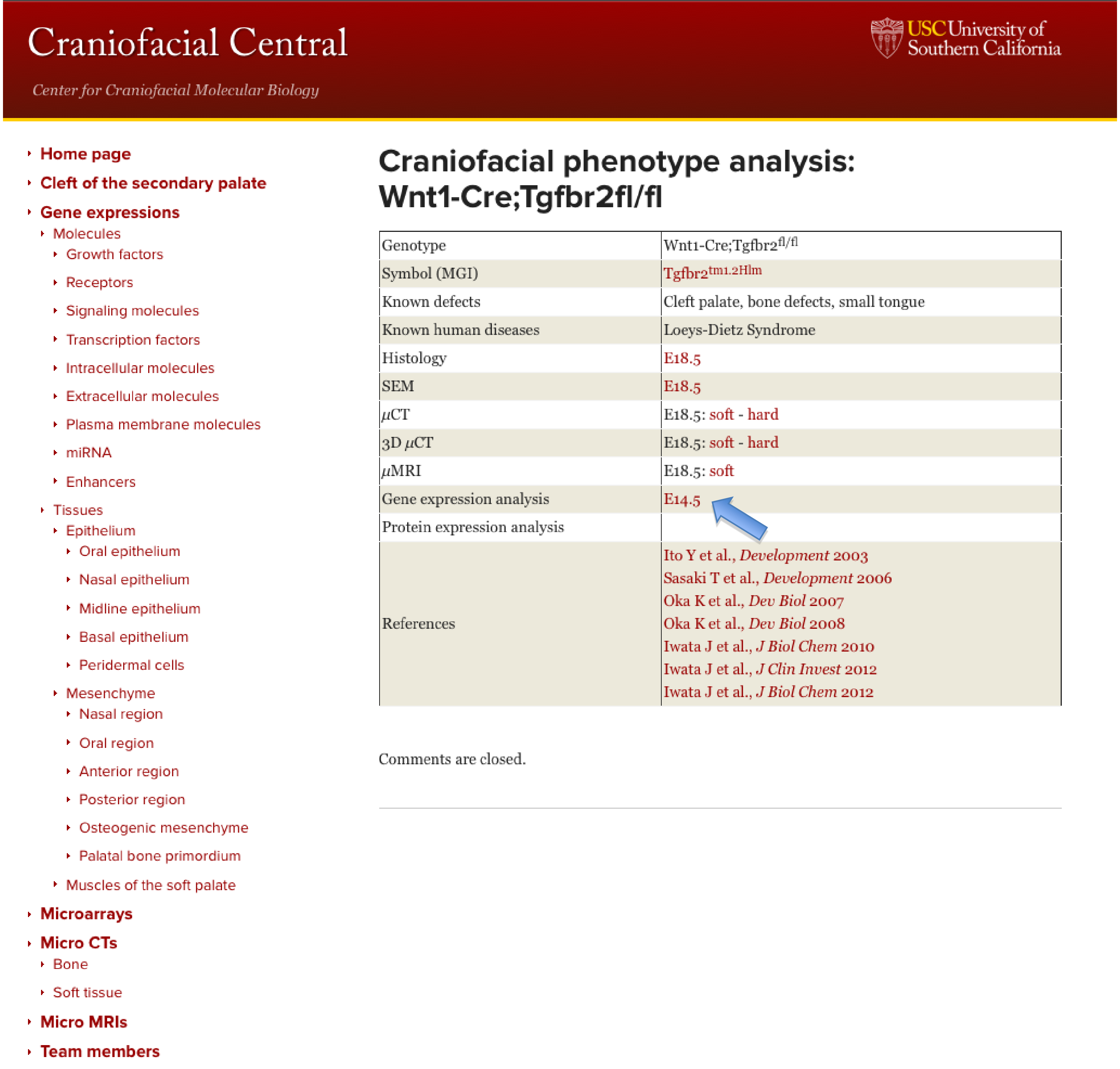

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Microarray: E14.5 Tgfbr2 Palate

Whole-genome transcriptome profiling by microarray of palatal tissue of E14.5 murine embryos with and without a conditional inactivation of *Tgfbr2* in the cranial neural crest

See a heatmap of the top 10 most differentially expressed genes between mutant and control samples for the following signaling pathways:

- BMP/TGF-Beta signaling pathway
- FGF signaling pathway
- Hedgehog signaling pathway
- Wnt (canonical) signaling pathway

See an interactive pie chart of the enriched Gene Ontology (GO) biological processes for:

- Genes upregulated in Wnt1-Cre;Tgfbr2$^{fl/fl}$ mutant mice
- Genes downregulated in Wnt1-Cre;Tgfbr2$^{fl/fl}$ mutant mice

Download the original CEL files here.

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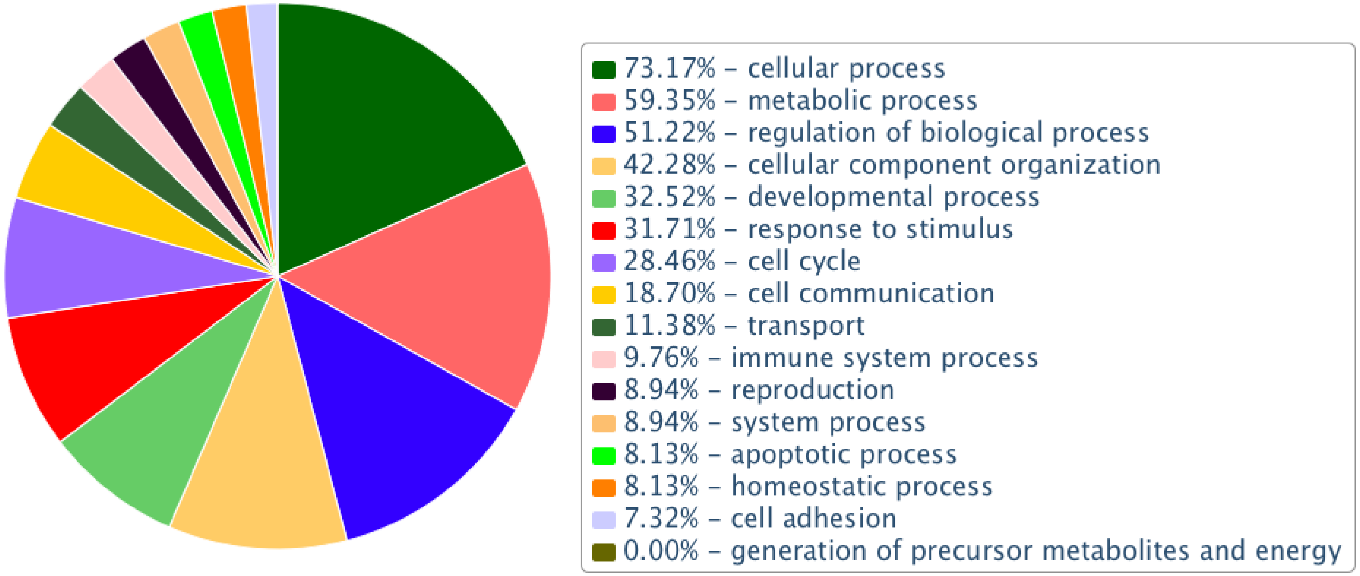
Functional Categories Enriched With Genes Downregulated in Wnt1-Cre;Tgfbr2 fl/fl mutant

Relative Proportions of Category Representation



Highcharts.com

Click on a sector to see a list of genes in that category.

Based on the HighCharts JavaScript library.

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Functional Categories Enriched With Genes Downregulated in Wnt1-Cre;Tgfbr2 fl/fl mutant

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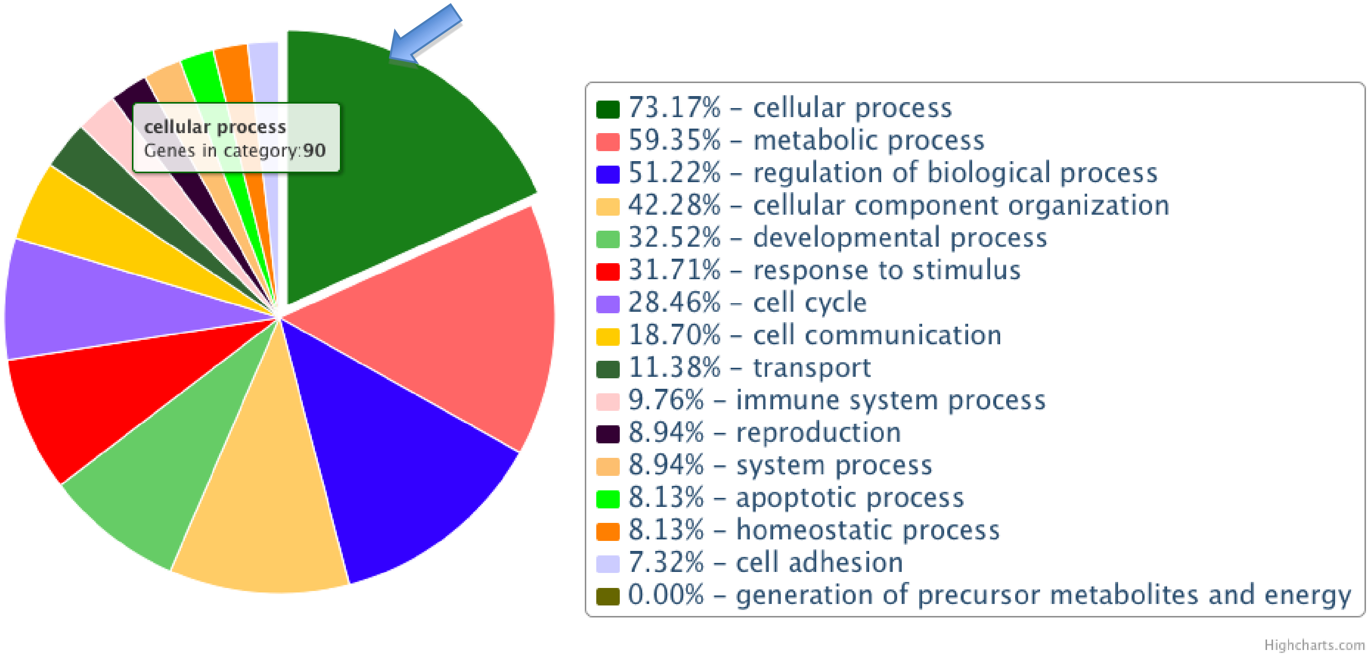

Click on a sector to see a list of genes in that category.

Based on the HighCharts JavaScript library.

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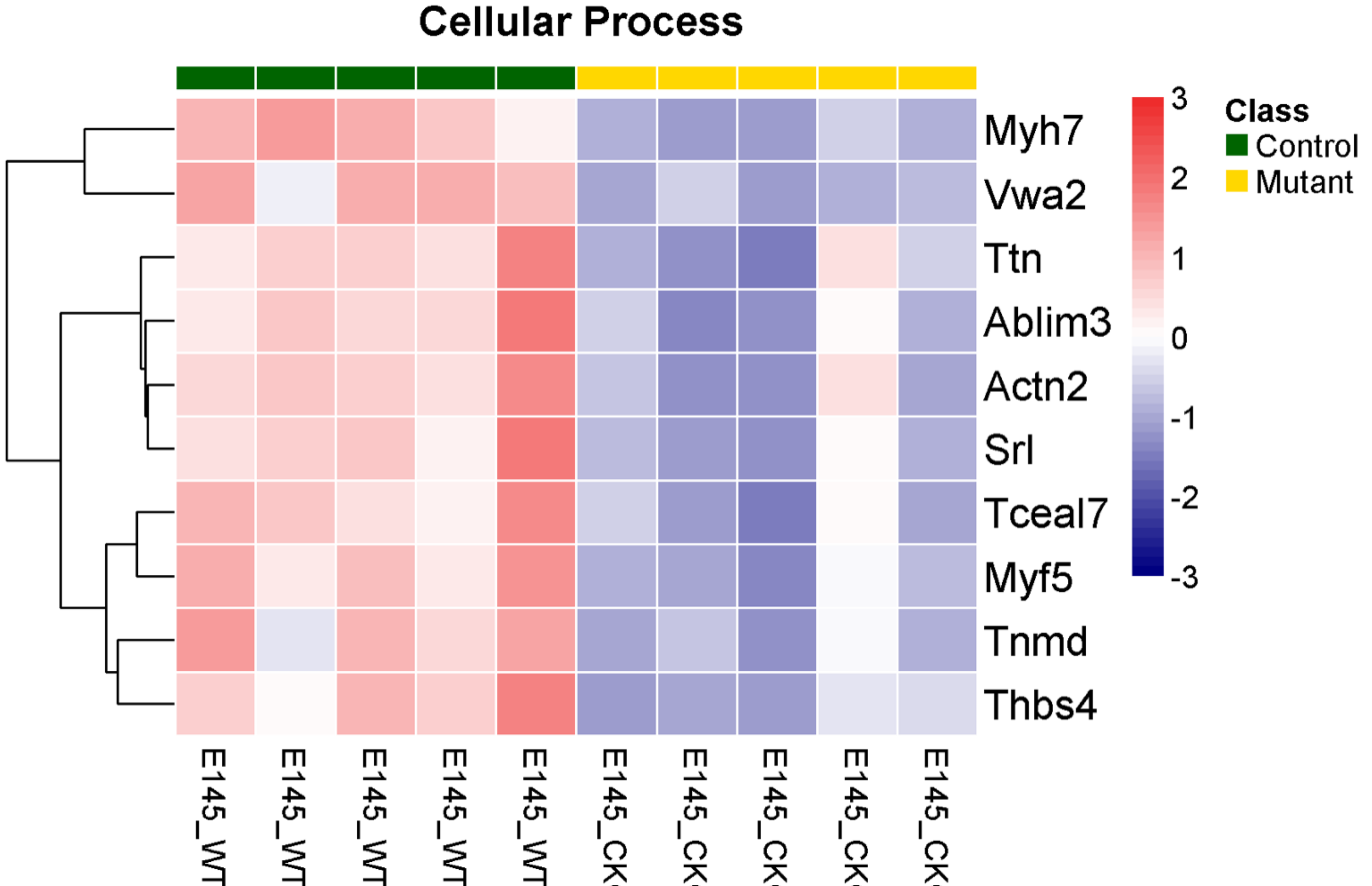
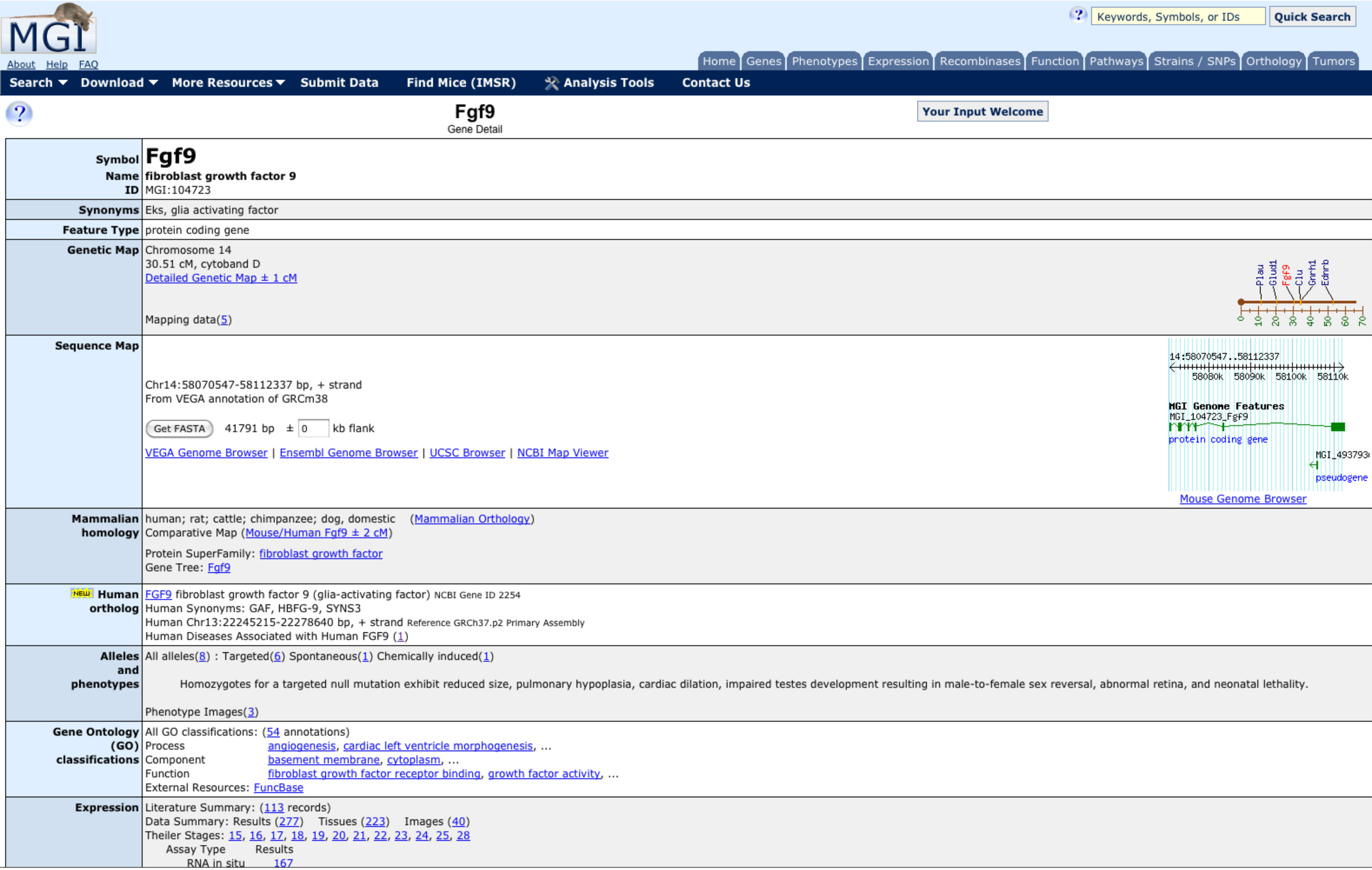
GO Term (GO ID)	Genes Annotated to the GO Term	GO Term Usage in Gene List	Genome Frequency of Use
cellular process (GO:0009987)	4632434I11Rik , Ablim3 , Actn2 , Adcy8 , Angptl1 , Anp32a , Birc5 , Bub1 , Bub1b , Calca , Car2 , Ccdc99 , Ccna2 , Ccnb1 , Cd55 , Cdc25c , Cdc45 , Cdc6 , Cdca3 , Cdca5 , Cdh4 , Cdkn3 , Cenph , Crym , Cyp26b1 , D2Ertd750e , Depdc1b , Dscc1 , Dusp9 , Eme1 , Epha4 , Esco2 , Etv5 , Exo1 , Fabp4 , Fbln5 , Fbxo5 , Fen1 , Fgf9 , [illegible] , Gas2 , Gins1 , Gmnn , Gsg2 , H2afv , Hfe2 , Hist1h3a , Hmga2 , Hmgb2 , Irx2 , Kif11 , Kif20b , Kif22 , [illegible] , Lars2 , [illegible] , Mtm1 , Myf5 , Myh6 , Myh7 , Nasp , Ncapg , Ndc80 , Nptx1 , Pde1a , Pf4 , Pitx2 , Rad51ap1 , Rasl11b , Rrm2 , Sgol1 , Ska1 , Slc40a1 , Smc6 , Smyd1 , Spc24 , Spc25 , Spsb4 , Srl , Tacc3 , Tceal7 , Tgfbr2 , Thbs4 , Tk1 , Tnfsf11 , Tnmd , Traip , Ttn , Tyms , Vwa2	90 of 123 genes, 73.17 %	13370 of 25000 annotated genes, 53.48 %

Fgf9



Cellular Process

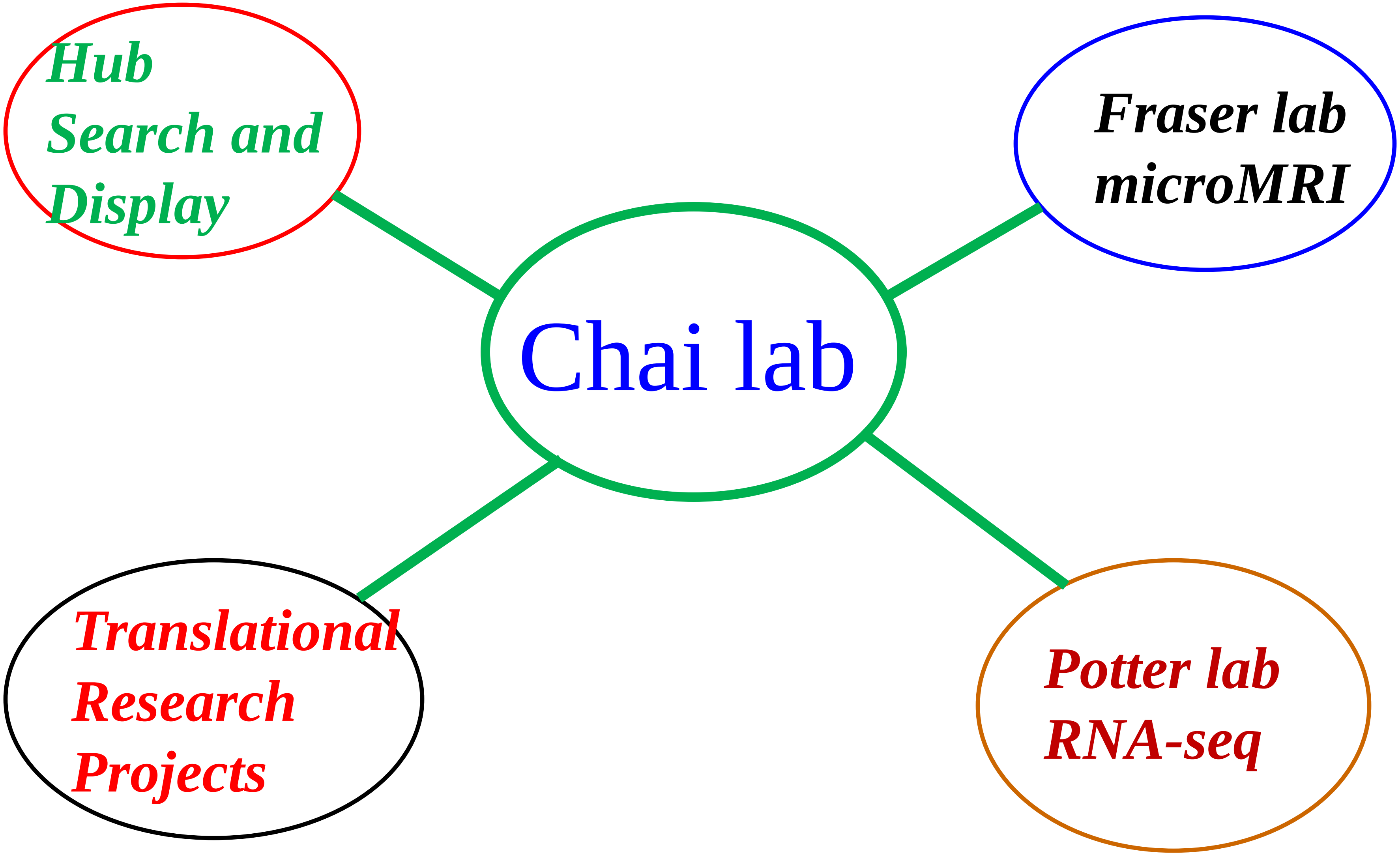


Fgf9

Gene Detail

Symbol **Name** **ID**	**Fgf9** **fibroblast growth factor 9** MGI:104723
Synonyms	Eks, glia activating factor
Feature Type	protein coding gene
Genetic Map	Chromosome 14 30.51 cM, cytoband D Detailed Genetic Map ± 1 cM Mapping data(5)
Sequence Map	Chr14:58070547-58112337 bp, + strand From VEGA annotation of GRCm38 Get FASTA 41791 bp ± 0 kb flank VEGA Genome Browser | Ensembl Genome Browser | UCSC Browser | NCBI Map Viewer
Mammalian homology	human; rat; cattle; chimpanzee; dog, domestic (Mammalian Orthology) Comparative Map (Mouse/Human Fgf9 ± 2 cM) Protein SuperFamily: fibroblast growth factor Gene Tree: Fgf9
Human ortholog	FGF9 fibroblast growth factor 9 (glia-activating factor) NCBI Gene ID 2254 Human Synonyms: GAF, HBFG-9, SYNS3 Human Chr13:22245215-22278640 bp, + strand Reference GRCh37.p2 Primary Assembly Human Diseases Associated with Human FGF9 (1)
Alleles and phenotypes	All alleles(8) : Targeted(6) Spontaneous(1) Chemically induced(1) Homozygotes for a targeted null mutation exhibit reduced size, pulmonary hypoplasia, cardiac dilation, impaired testes development resulting in male-to-female sex reversal, abnormal retina, and neonatal lethality. Phenotype Images(3)
Gene Ontology (GO) classifications	All GO classifications: (54 annotations) Process angiogenesis, cardiac left ventricle morphogenesis, ... Component basement membrane, cytoplasm, ... Function fibroblast growth factor receptor binding, growth factor activity, ... External Resources: FuncBase
Expression	Literature Summary: (113 records) Data Summary: Results (277) Tissues (223) Images (40) Theiler Stages: 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 28 Assay Type Results RNA in situ 167

Collaborations



Chai lab

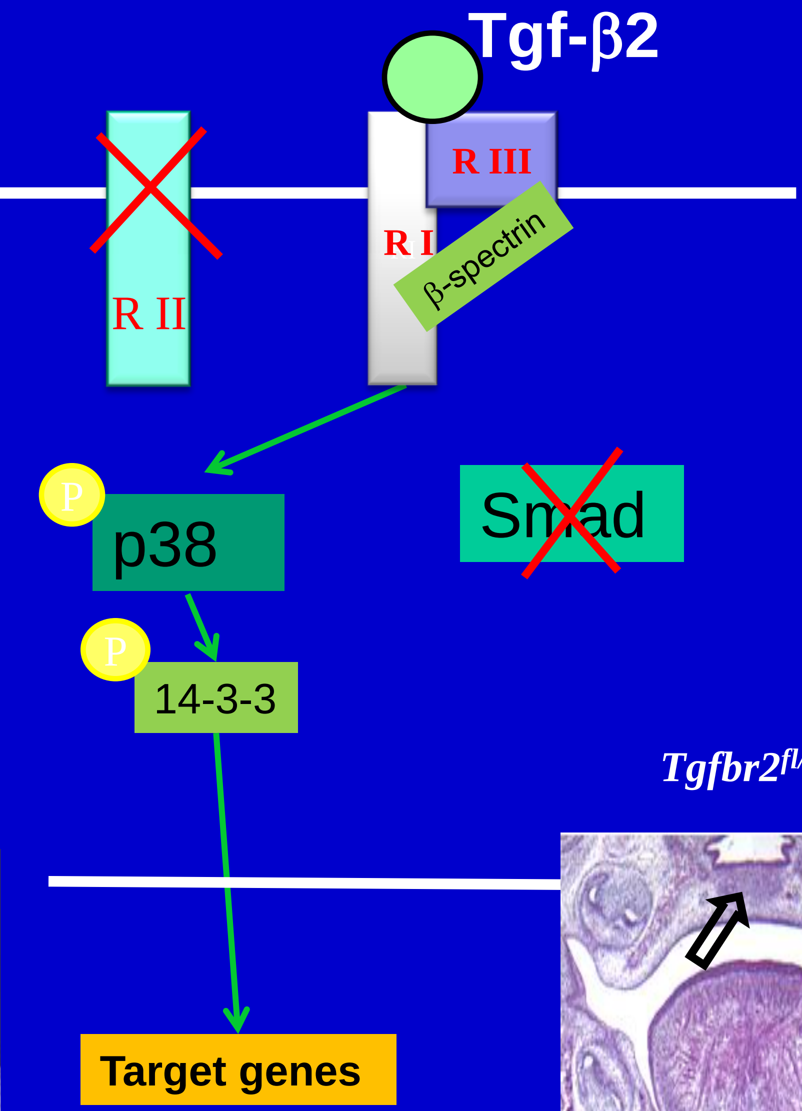
Hub Search and Display

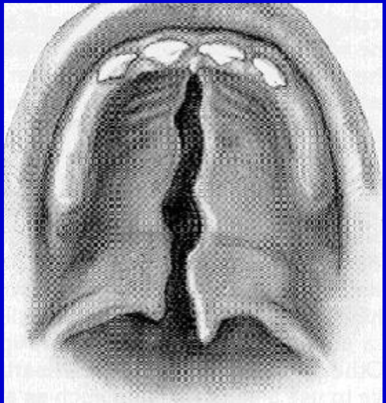
Fraser lab microMRI

Translational Research Projects

Potter lab RNA-seq

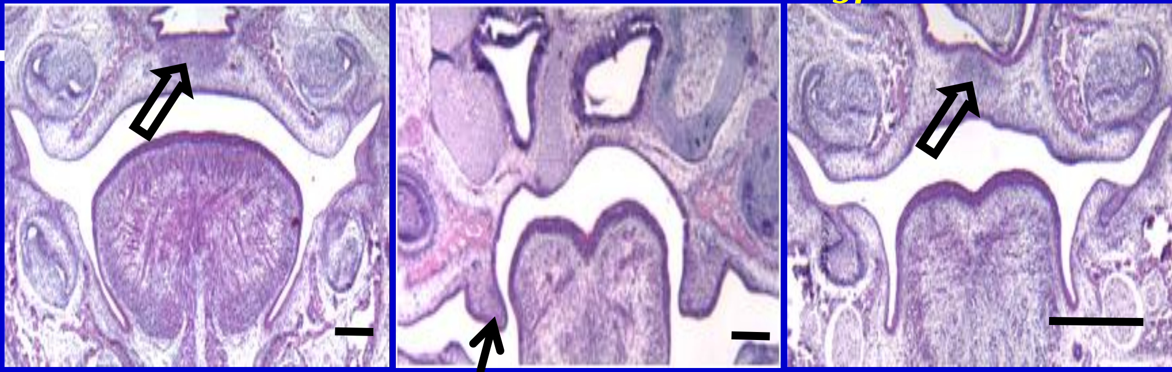
Discovery of a novel TGF-β signaling mechanism





Cleft of the secondary palate

Wnt1-Cre;Tgfbr2$^{fl/fl}$



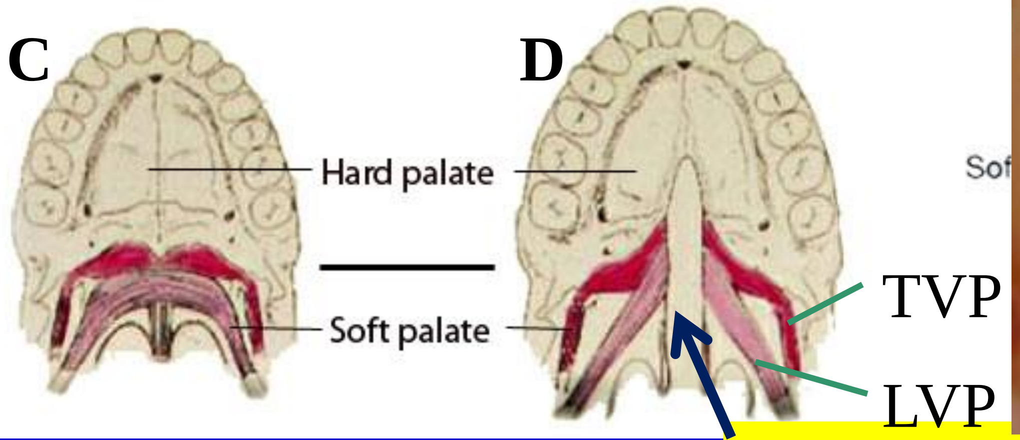
Tgfbr2$^{fl/fl}$ *Wnt1-Cre:Tgfbr2*$^{fl/fl}$ *Wnt1-Cre;Tgfbr2*$^{fl/fl}$*; Tgfb2*$^{+/-}$

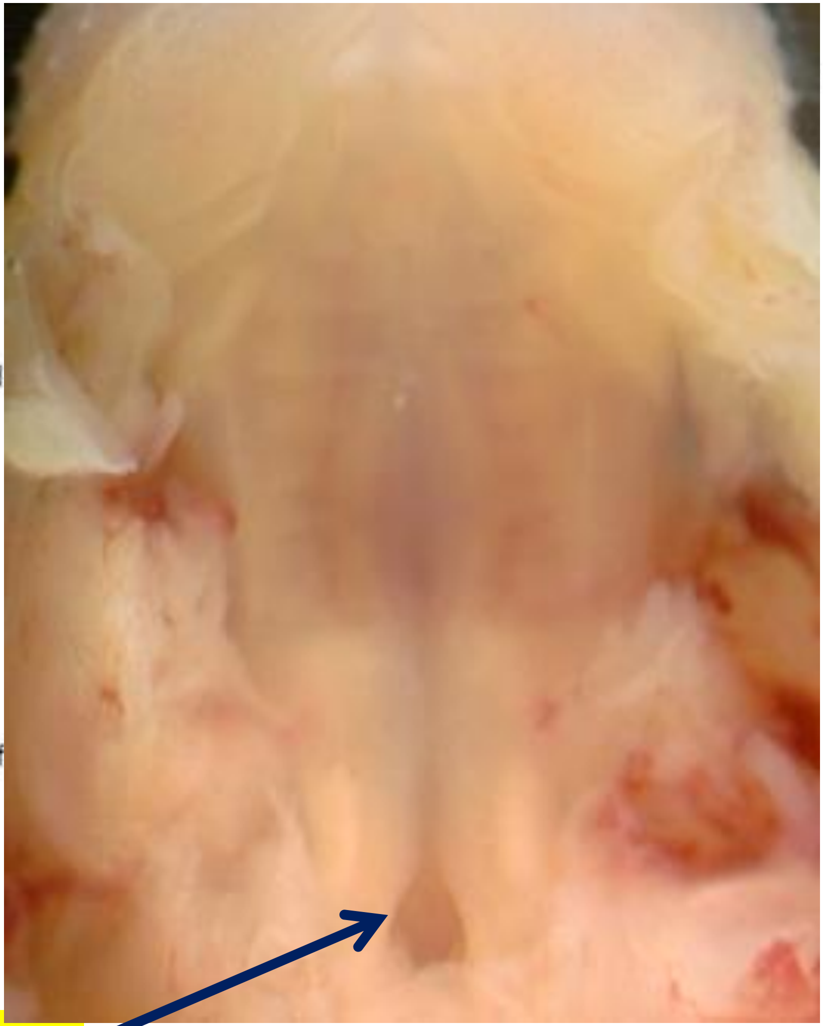
Iwata et al., JCI 2012

TGF-β-mediated epithelial-mesenchymal interactions and palatal muscle development

Cleft soft palate

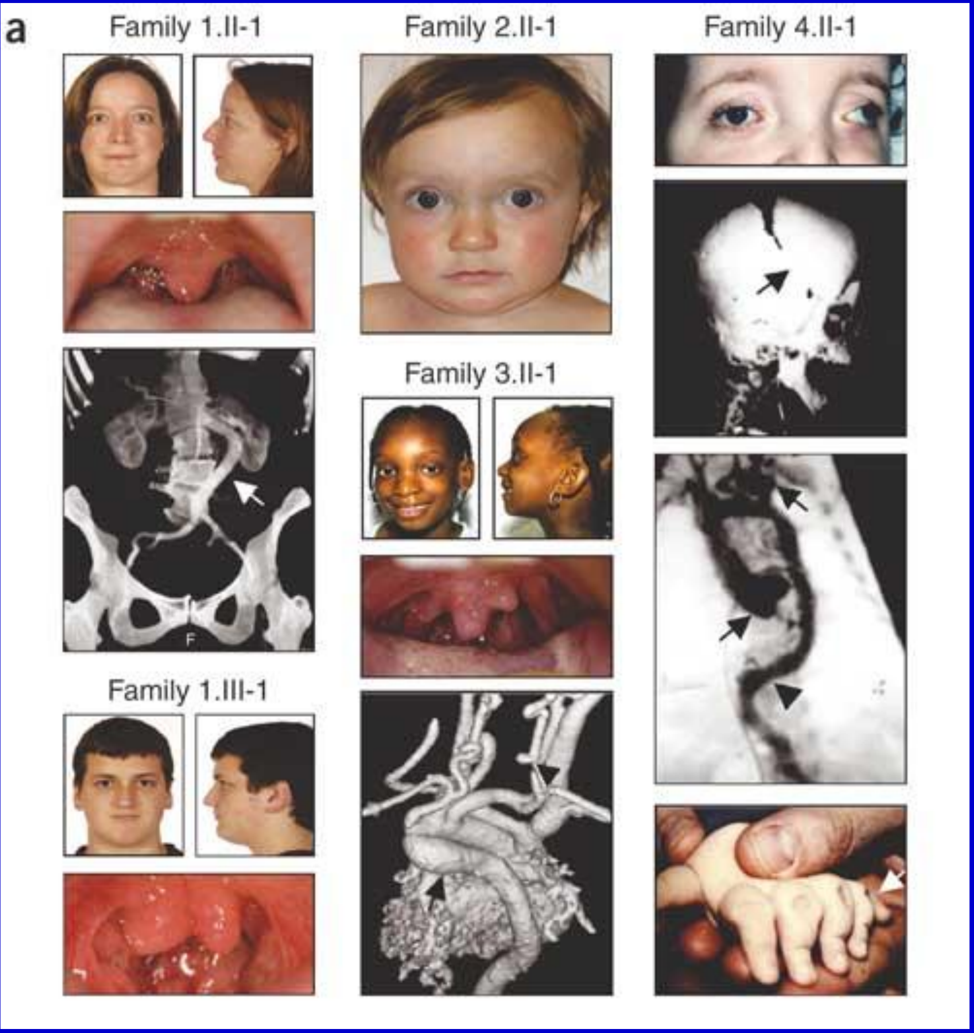
K14-Cre;Tgfbr2$^{fl/fl}$





Cleft palate

TGF-β signaling mechanism in patients with TGF-β receptor mutations



Loeys-Dietz syndrome

(*TGF-β RII* or *TGF-β RI* mutations)

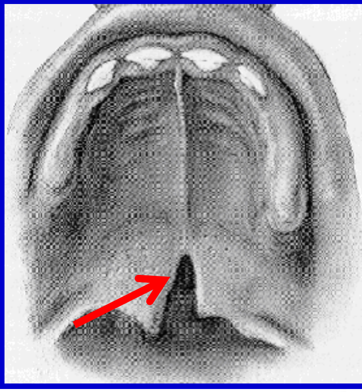
(Marfan’s syndrome type II)

What is the TGF-β signaling mechanism in patients with TGF-β RII mutations?

TGF-β receptor mutations and cleft palate

Loeys et al., 2005, Nature Genetics 37, 275-281

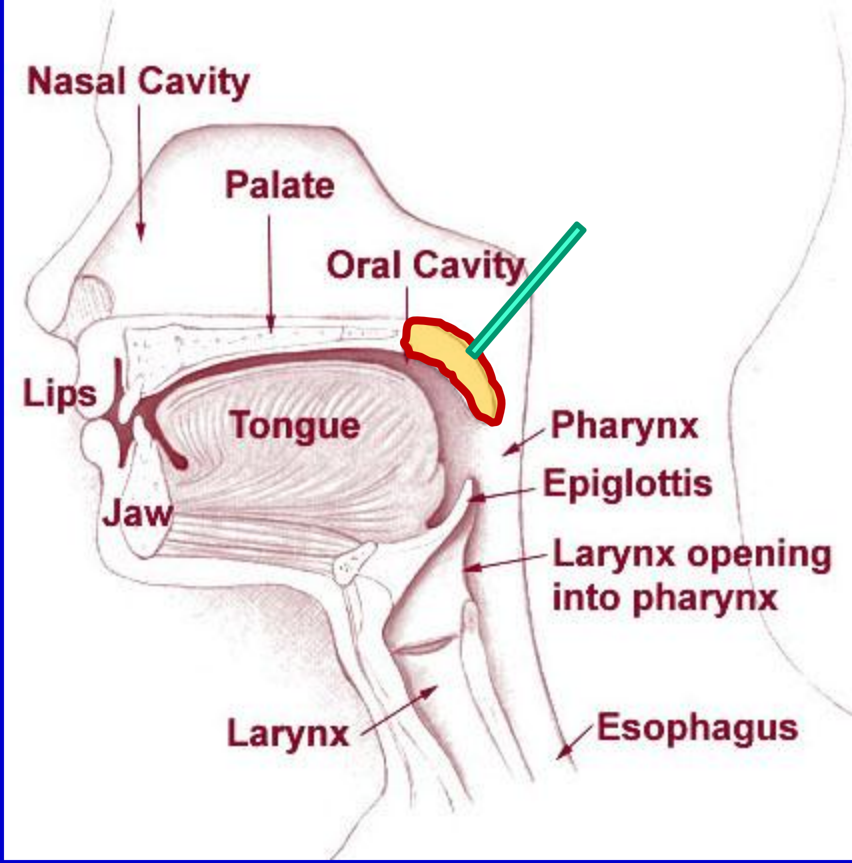
Why is the soft palate important?



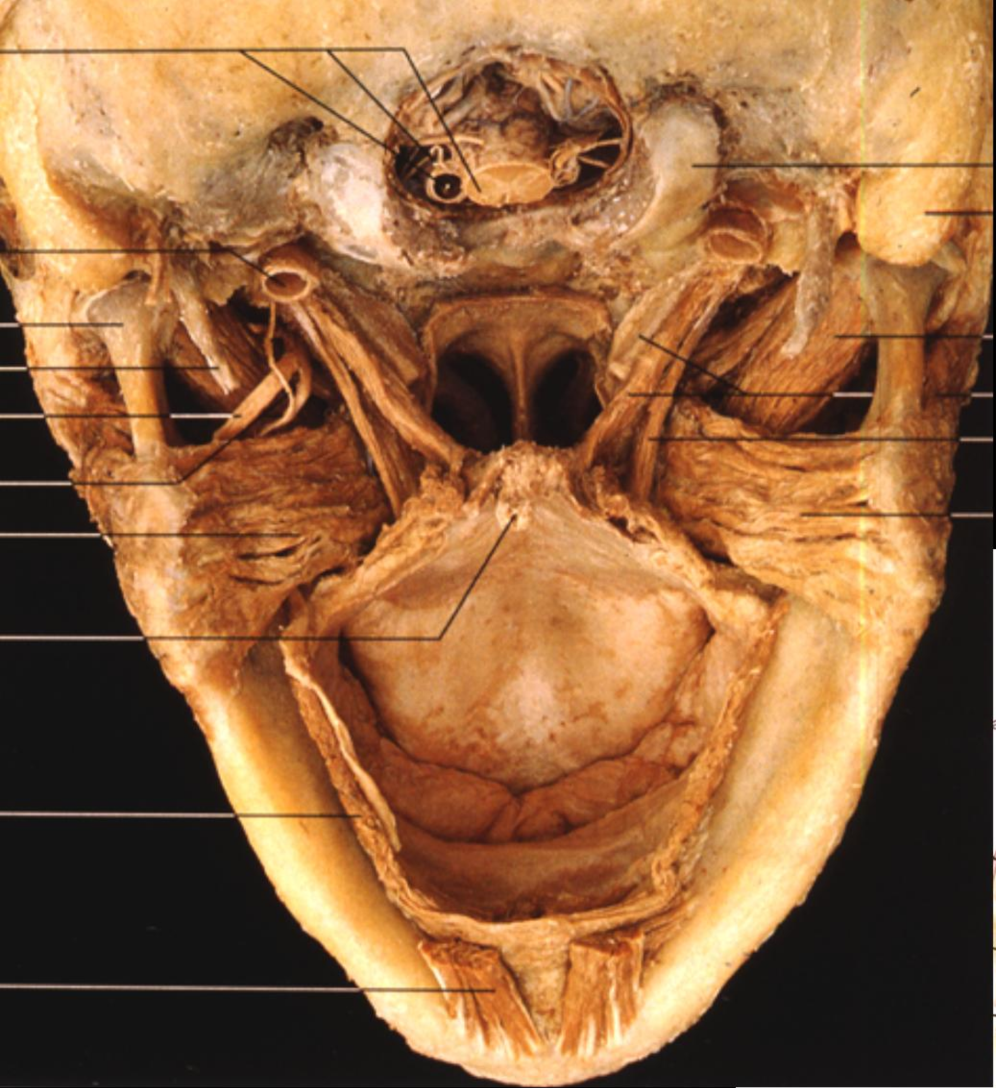
- Speech
- Hearing
- Swallowing

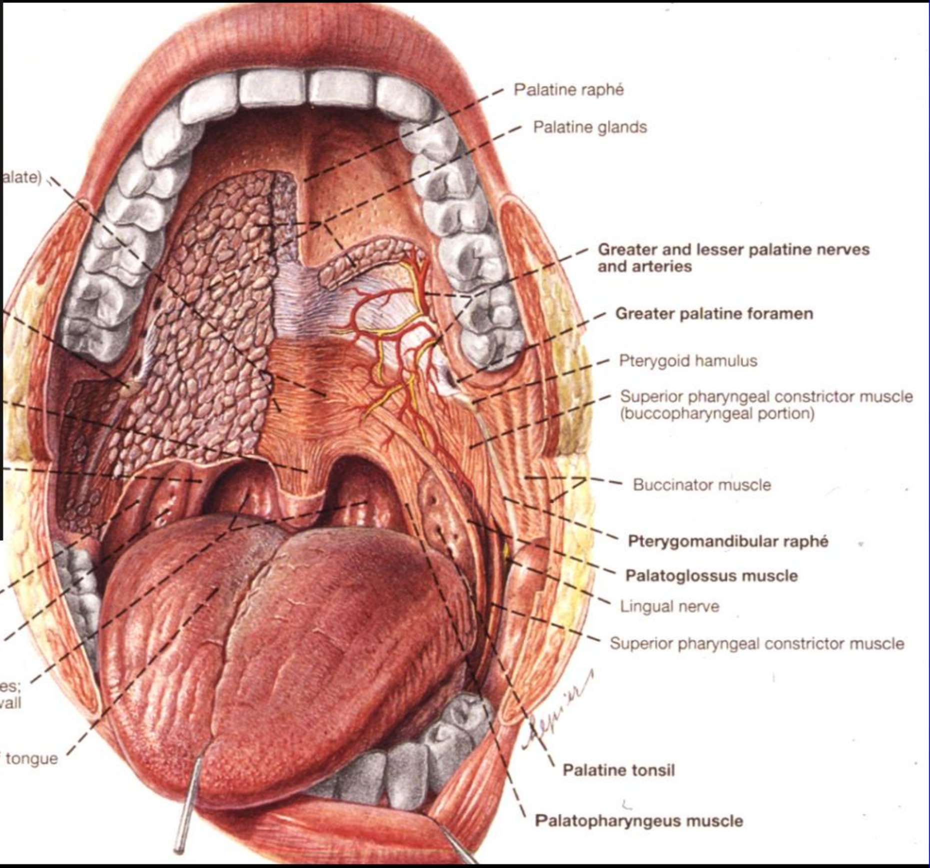
Cleft soft palate,

risk for speech problems, middle ear infection, and difficulties in swallowing



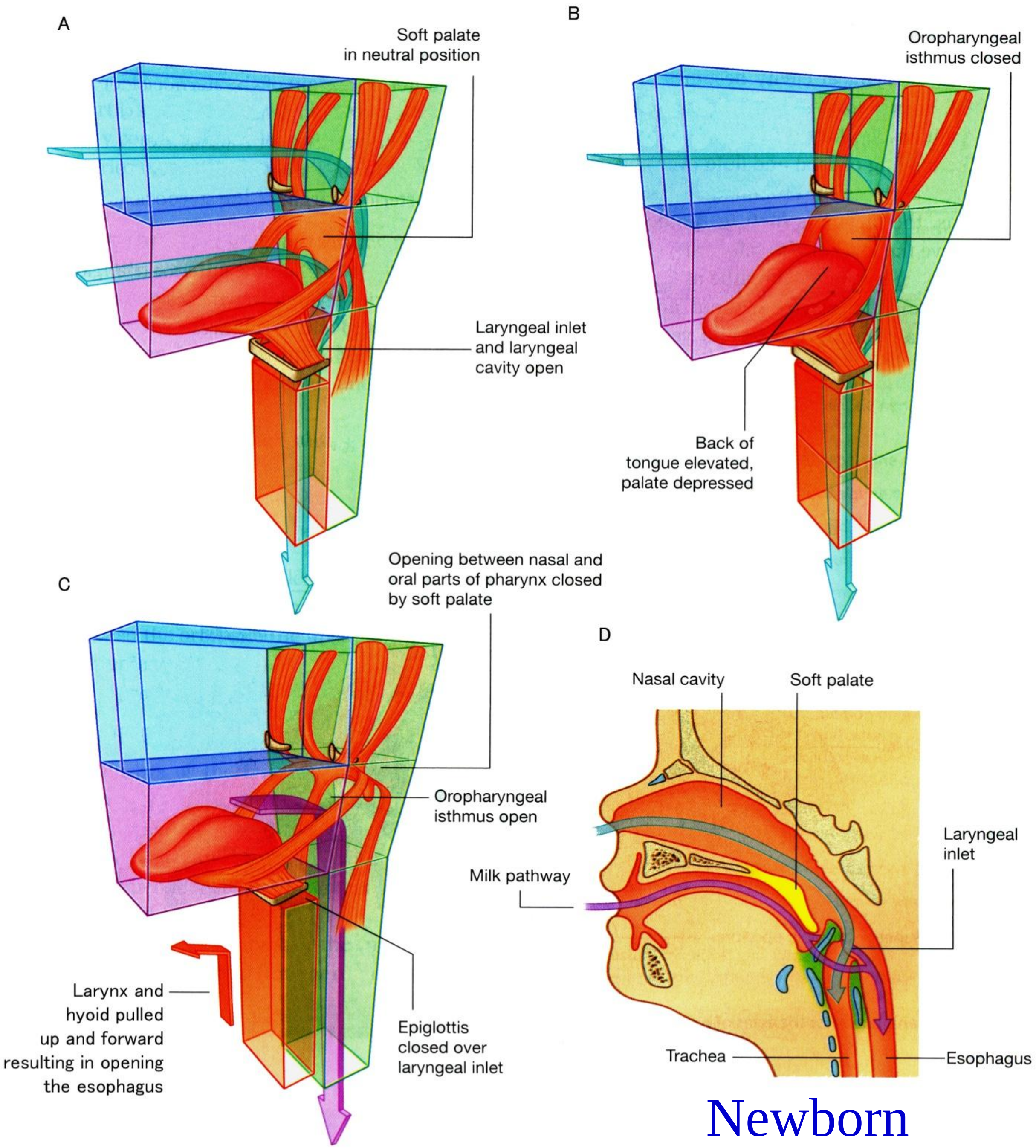
Functional Significance of Soft Palate Muscles





Functional separation of the digestive and respiratory passages

The Soft Palate

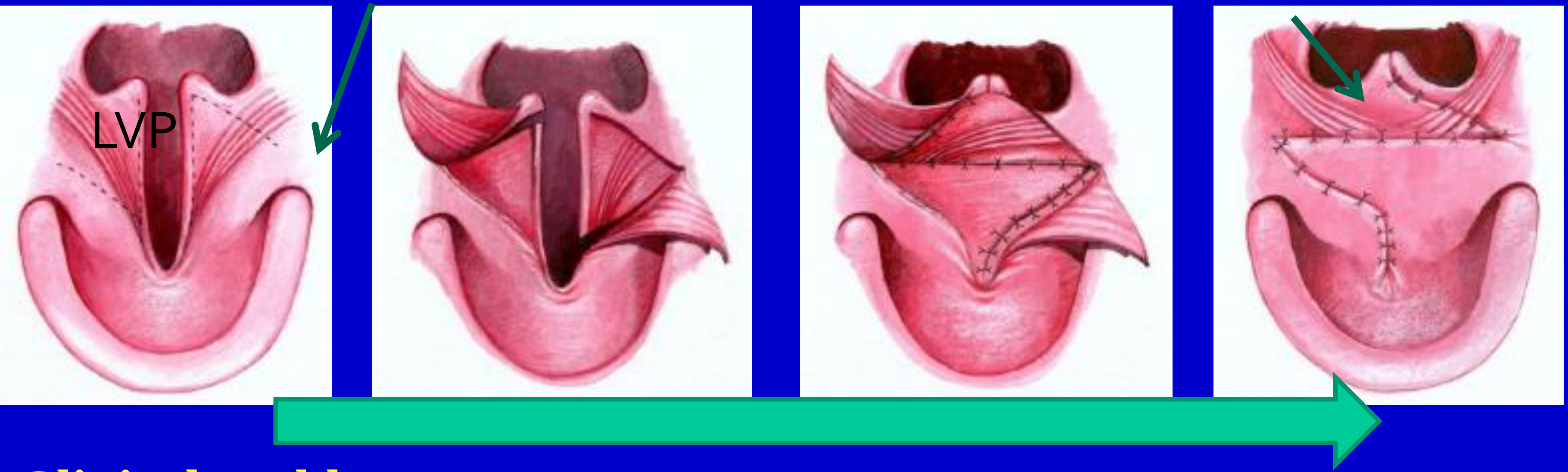


Newborn

Repair of cleft soft palate

Abnormal orientation of LVP muscle

Surgical correction of the orientation of LVP muscle



<u>Clinical problems</u>:

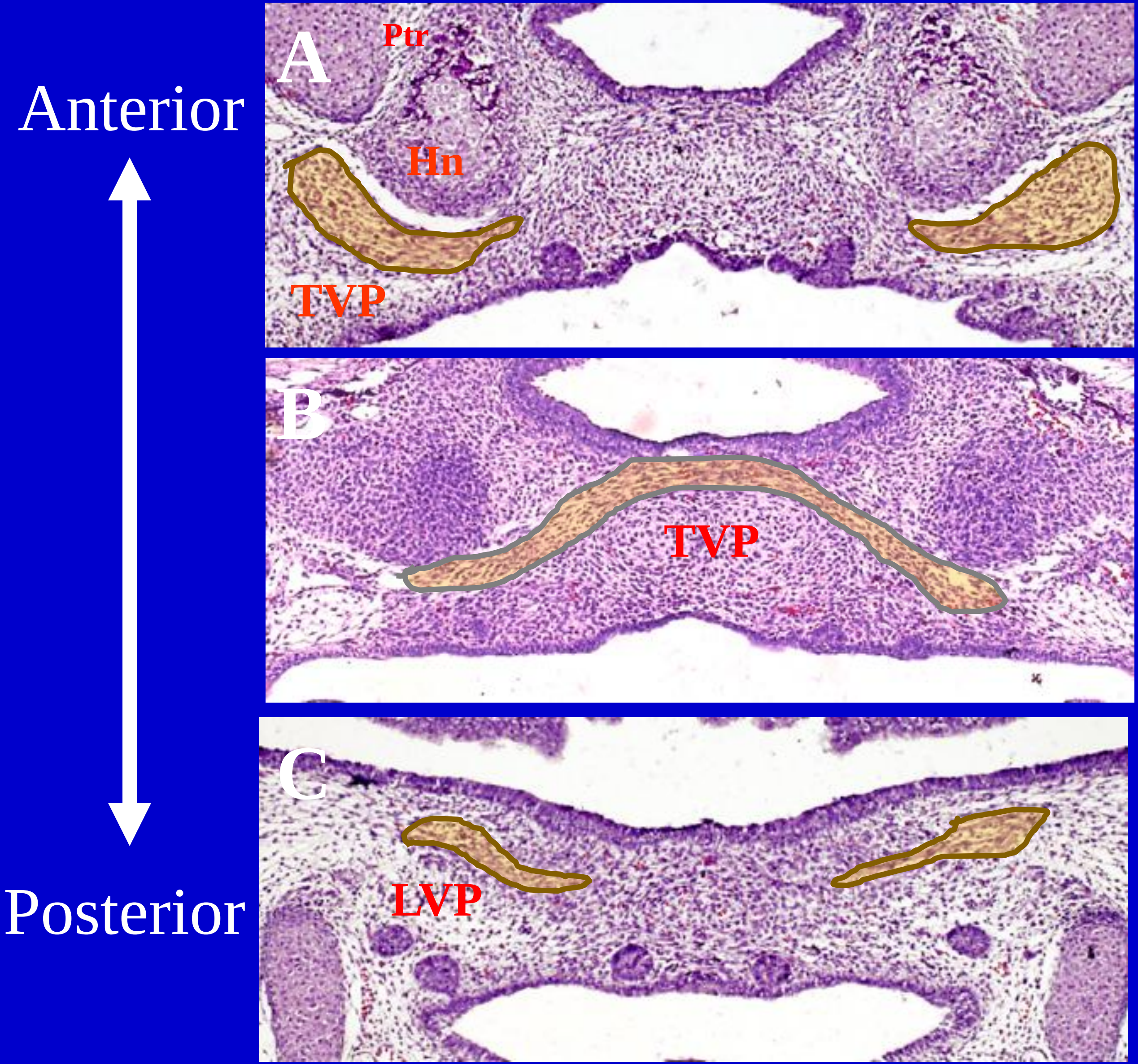
Following the surgical correction of mis-oriented LVP muscle, these patients still have difficulty with precise pronunciation. **Why?**

1. Intrinsic functional defects in soft palate muscles (fast/slow fibers)?

2. Decreased volume of muscles?

3. Molecular regulation of muscles in soft palate is still defective

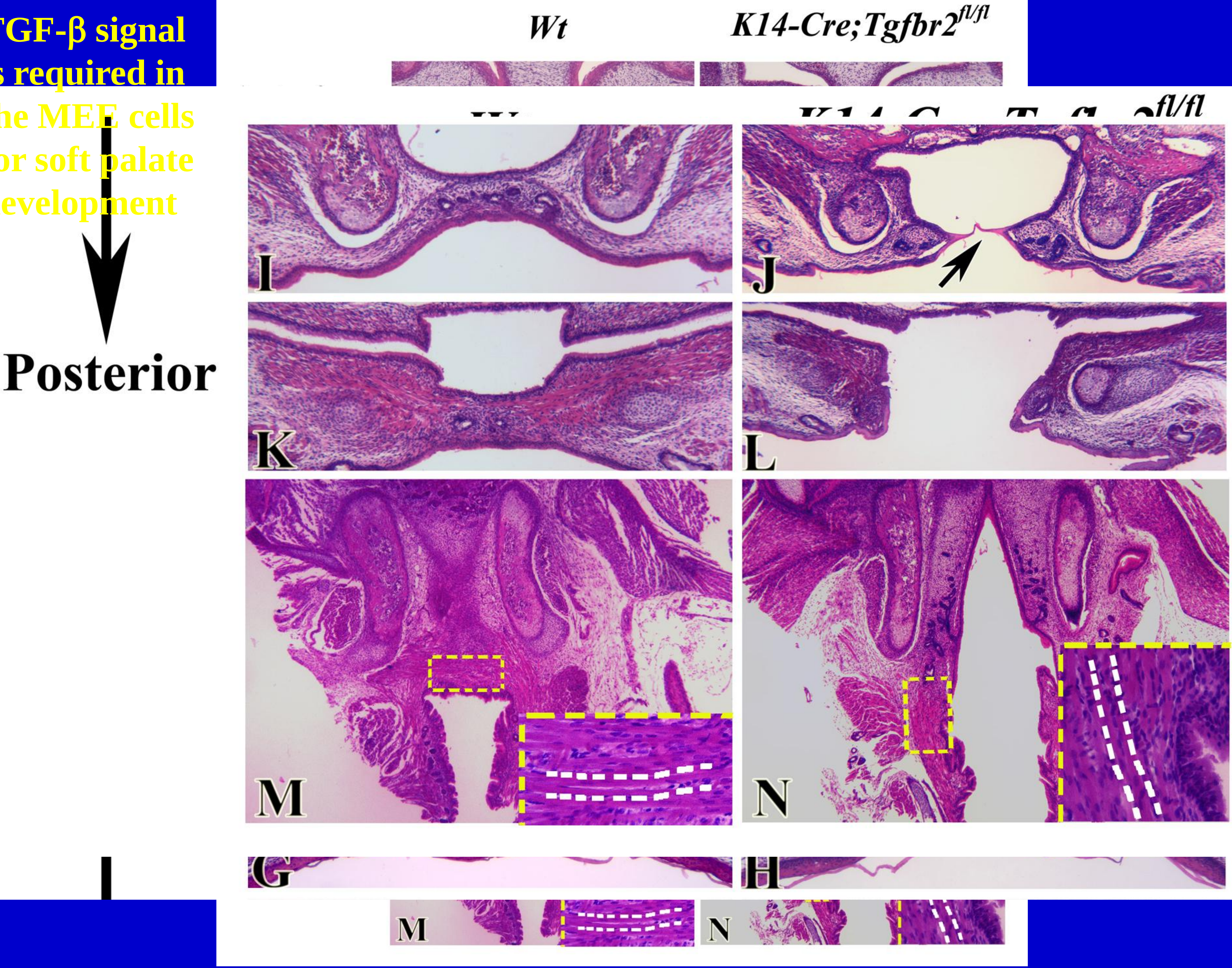
Soft palate muscles



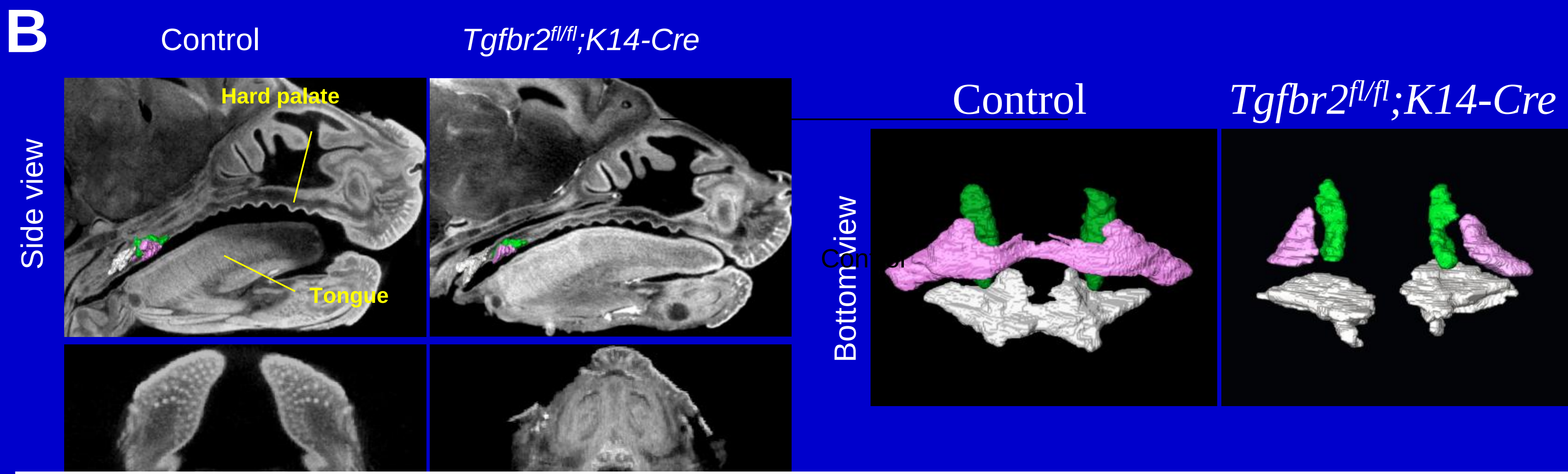
E15.5

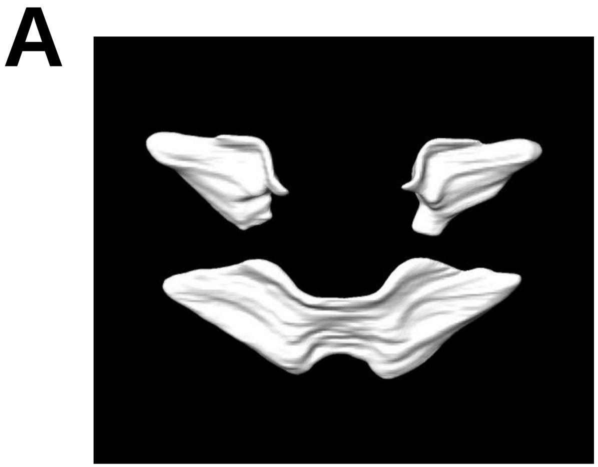
Ptr; Pterygoid plate
Hn; Hamular notch
TVP; Tensor veli palatini muscle
LVP; Levator veli palatini muscle

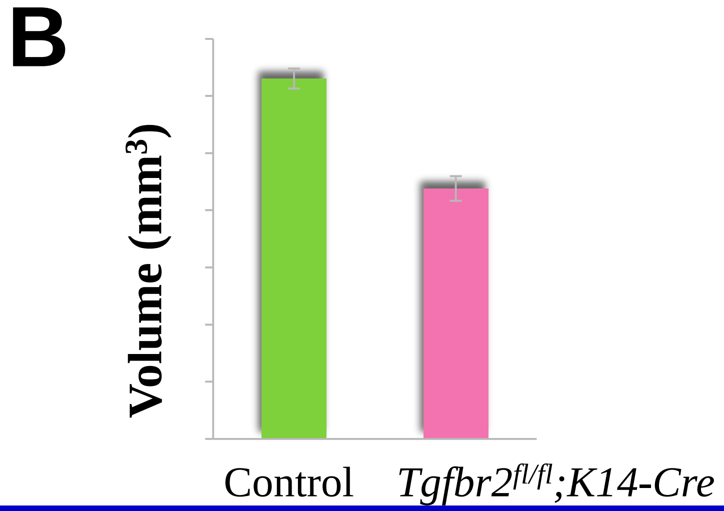
TGF-β signal is required in the MEE cells for soft palate development



Muscles of the soft palate analyzed by microCT

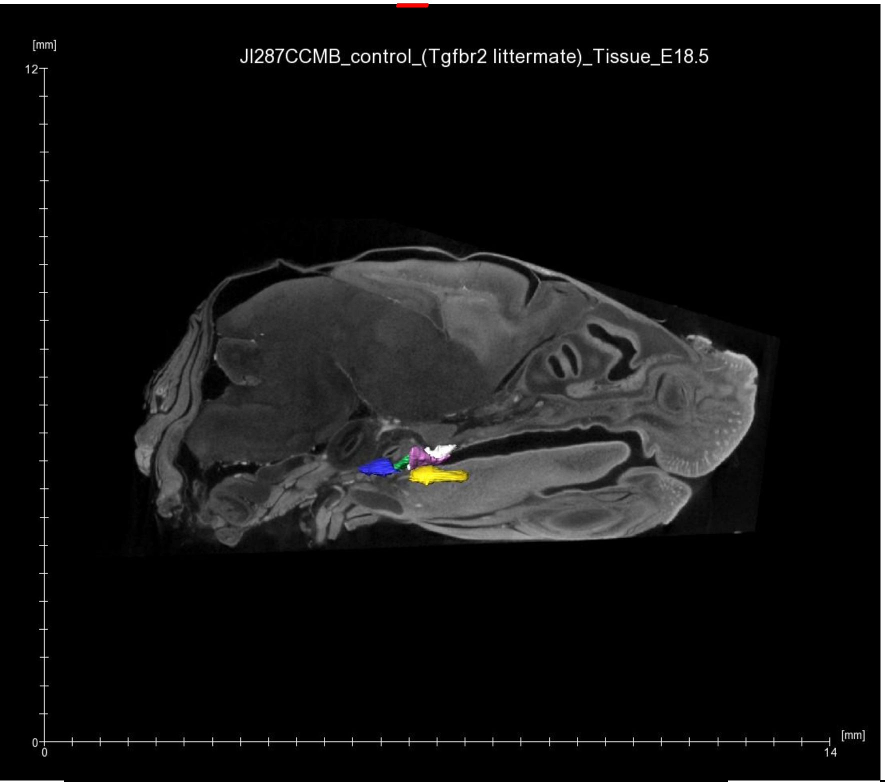


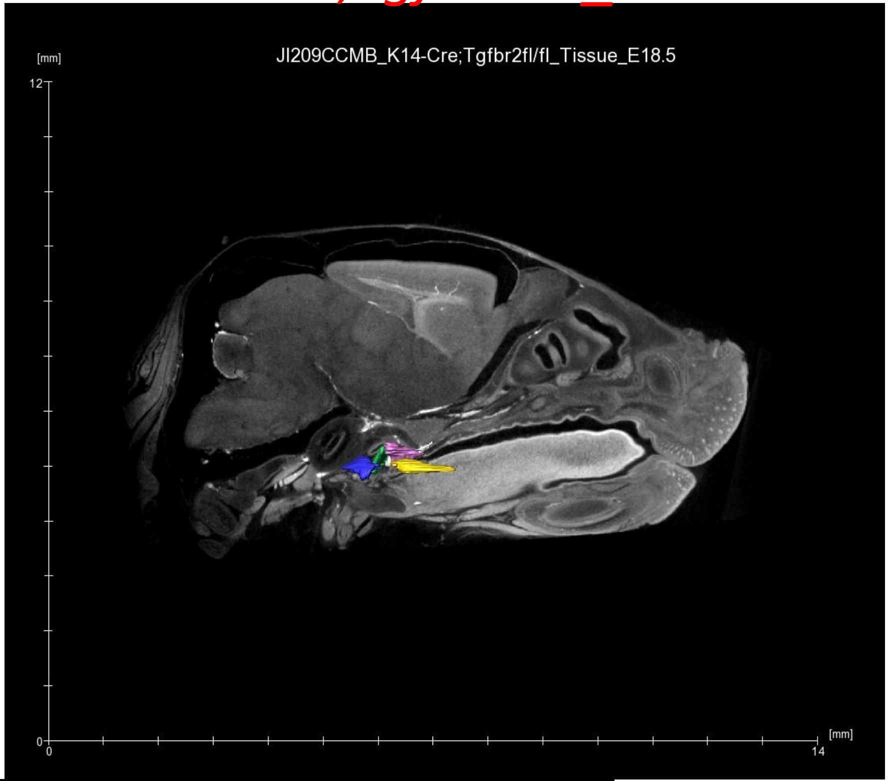


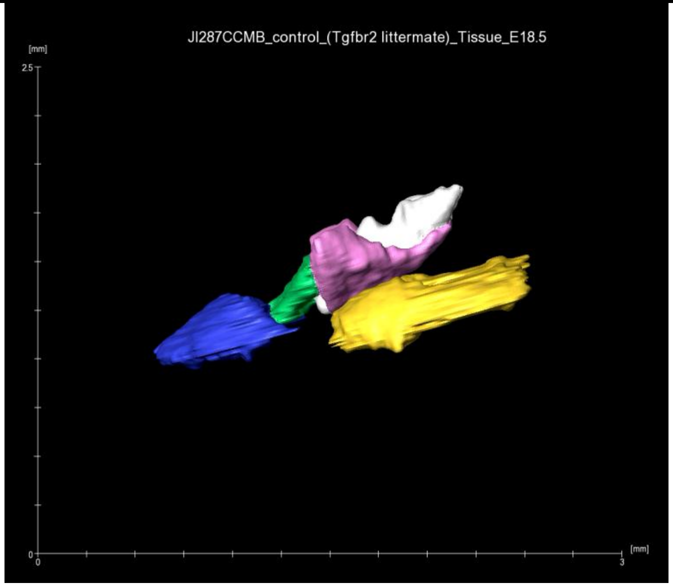


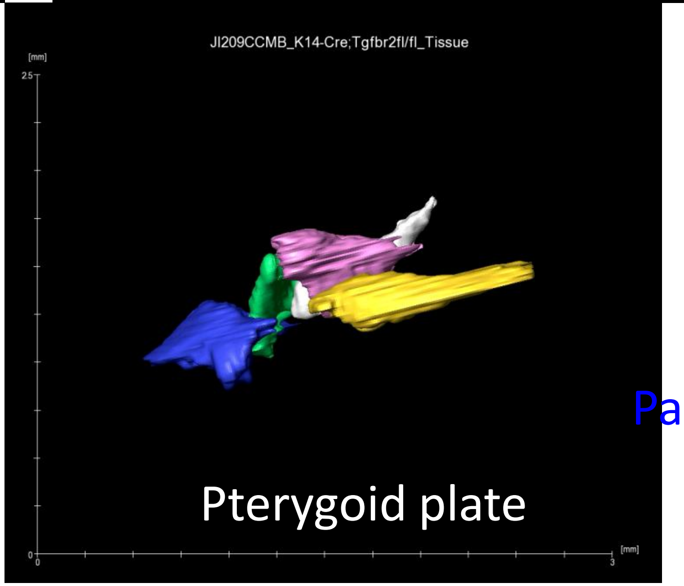
Control_E18.5

K14-Cre;Tgfbr2$^{fl/fl}$_E18.5









Tensor veli palatini

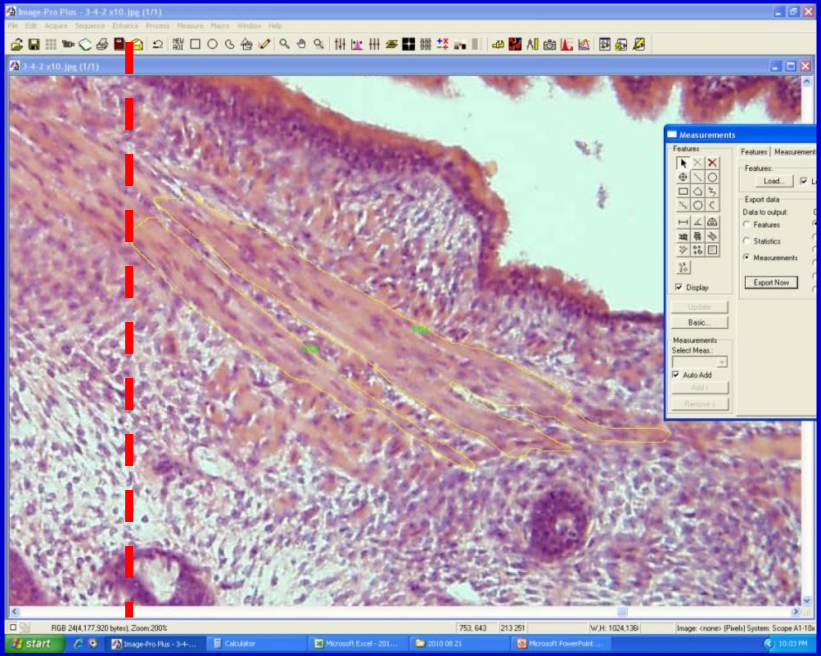
Levator veli palatini

Palatoglossus

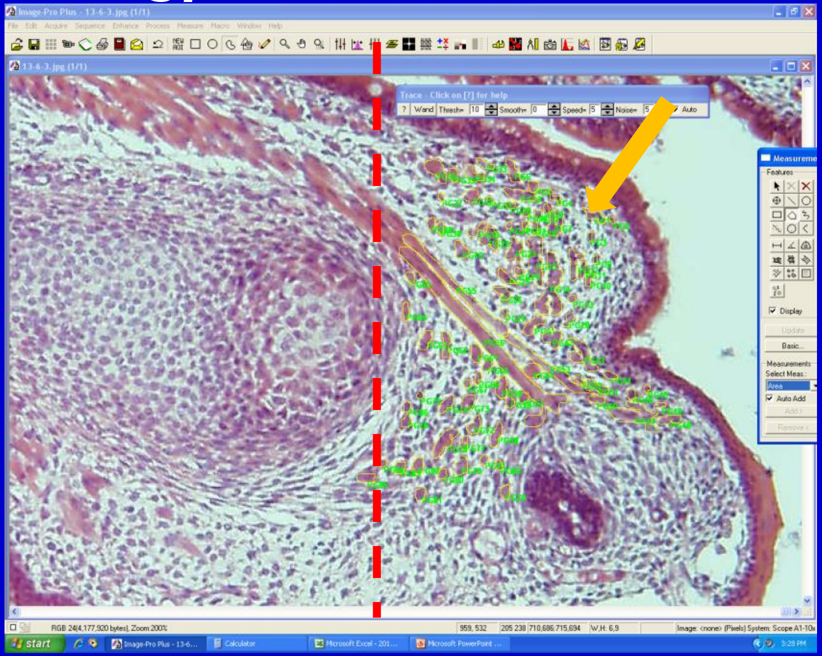
Palatopharyngeus

Muscle volume in *Tgfbr2$^{fl/fl}$;K14-Cre* mice

Control



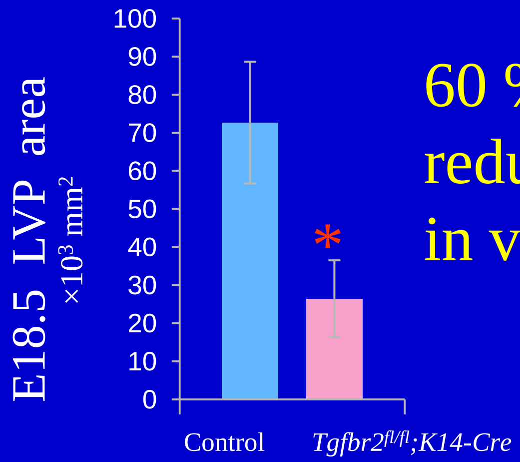
Tgfbr2$^{fl/fl}$;K14-Cre



Serial coronal sections for the LVP muscle (E18.5)

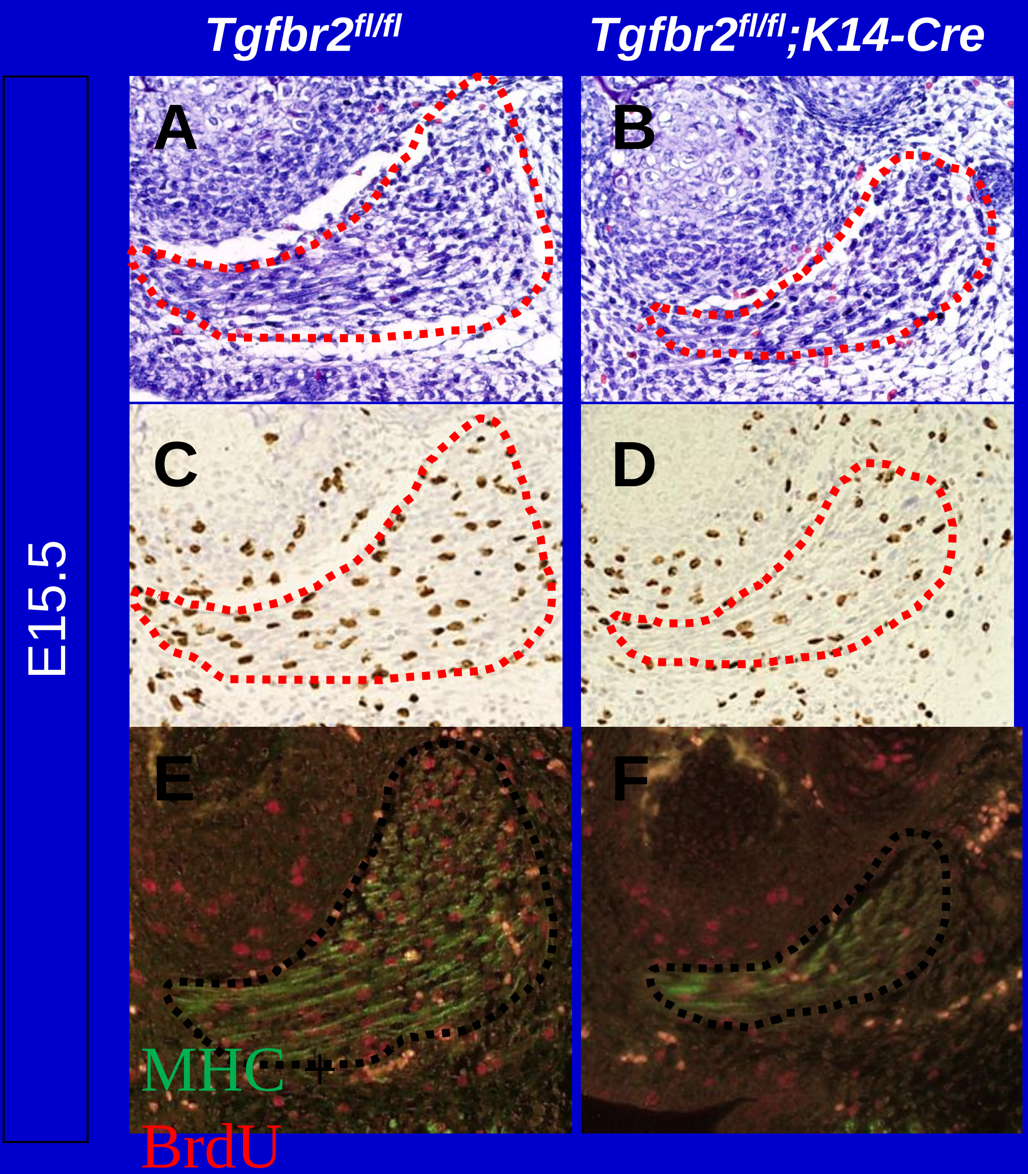
Isolation of muscle fibers on each section

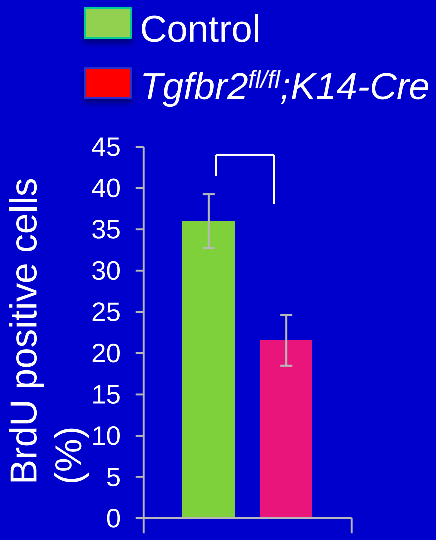
Volume analysis



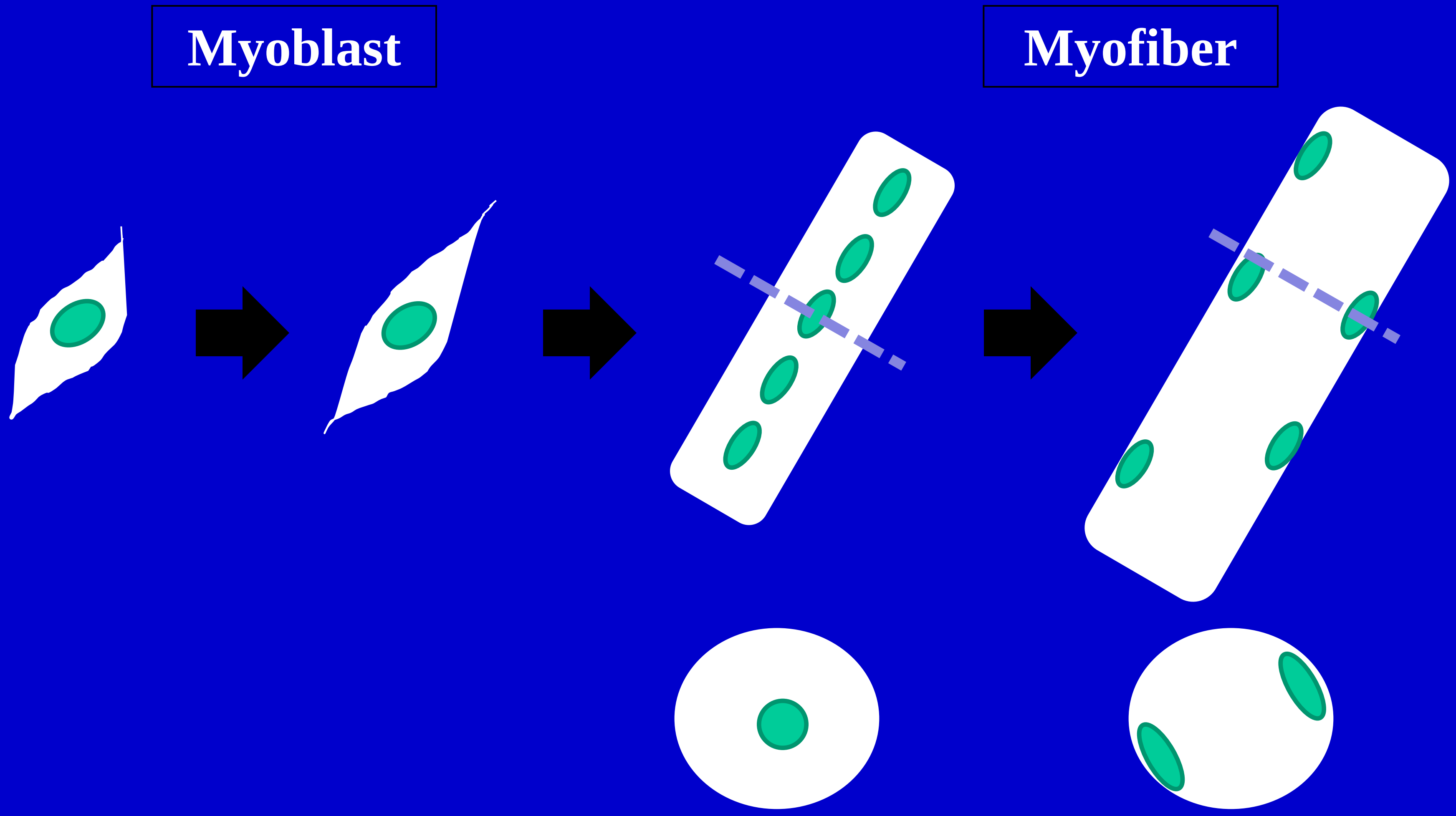
60 % reduction in volume

Cell proliferation defect in the muscles of *Tgfbr2*$^{fl/fl}$*;K14-Cre* mice





Muscle development



Myoblast

Myofiber

Progenitors

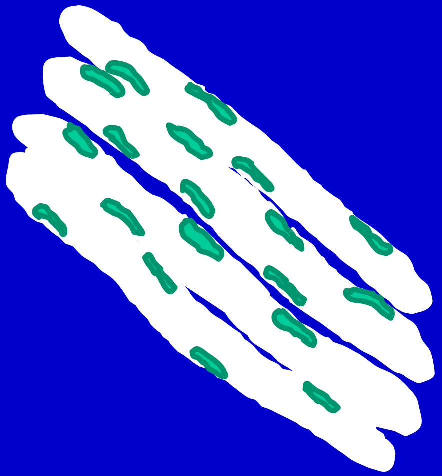
Commitment to differentiation

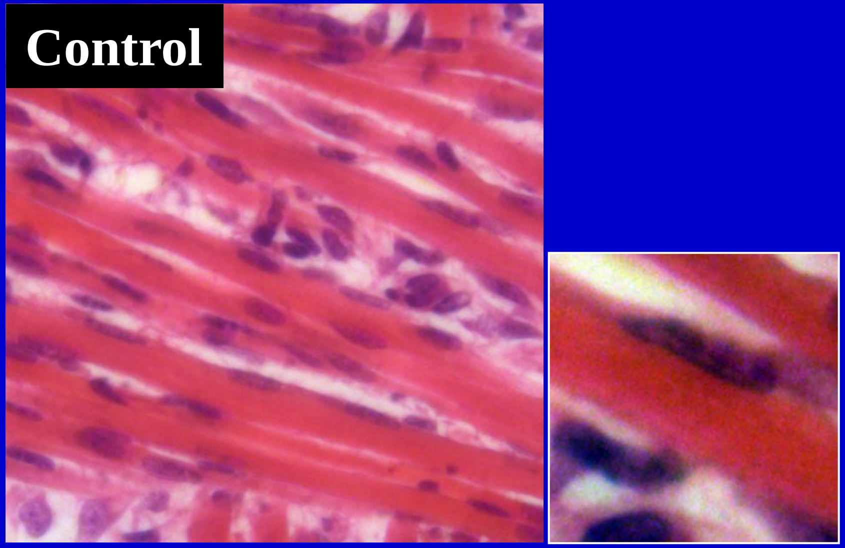
Fusion into myotube

Maturation of myofiber

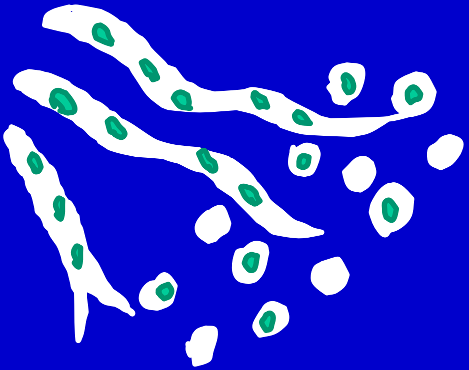
Muscle development defects in *Tgfbr2* mutant model

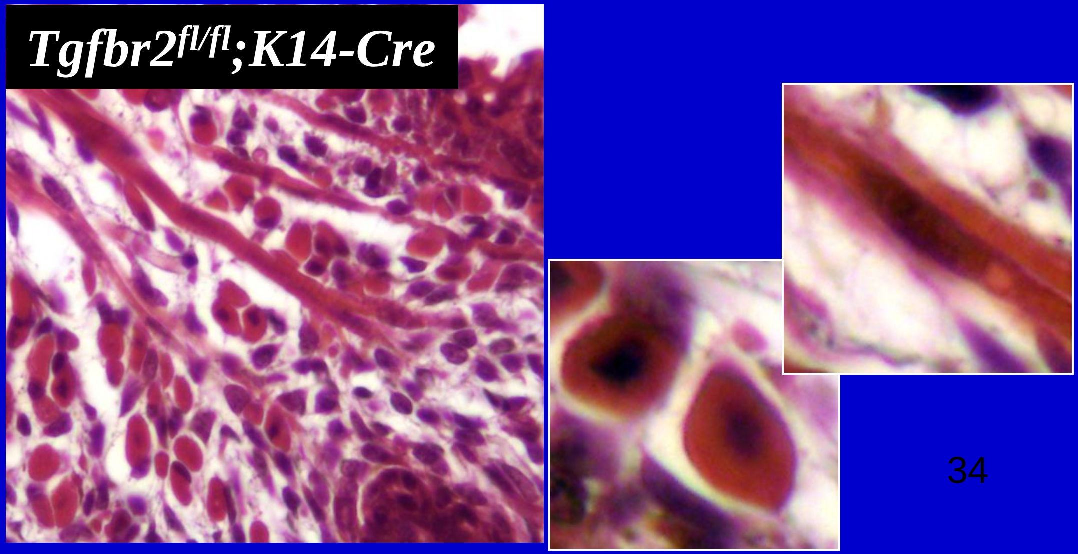
Control





Cleft soft palate

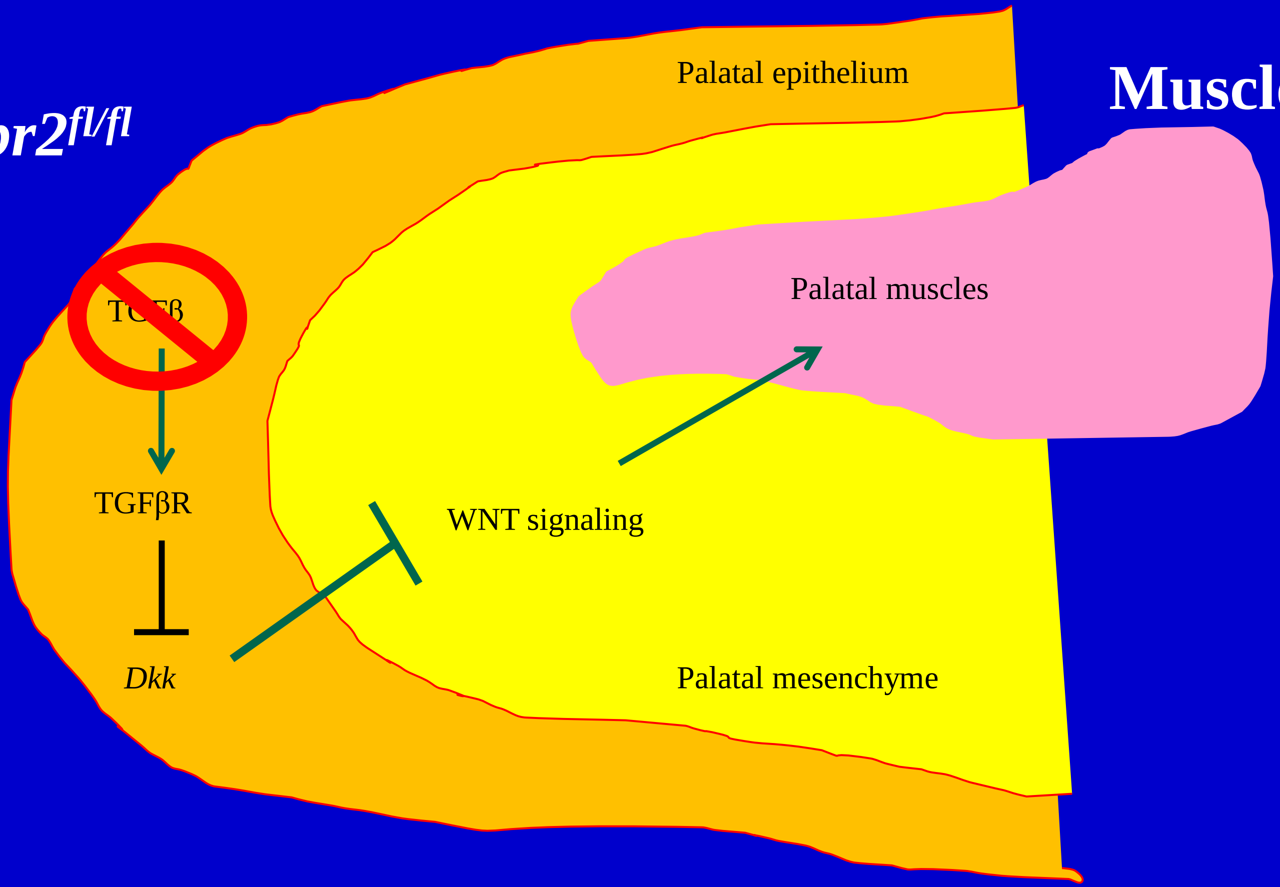




How does TGF-β mediated tissue-tissue interaction control muscle development in the soft palate?

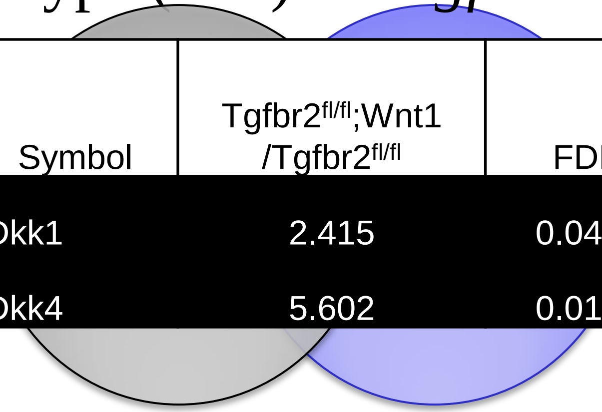
K14-Cre;Tgfbr2$^{fl/fl}$

Muscle Defects



Microarray analysis

1.5-fold, <5% false discovery rate (FDR)



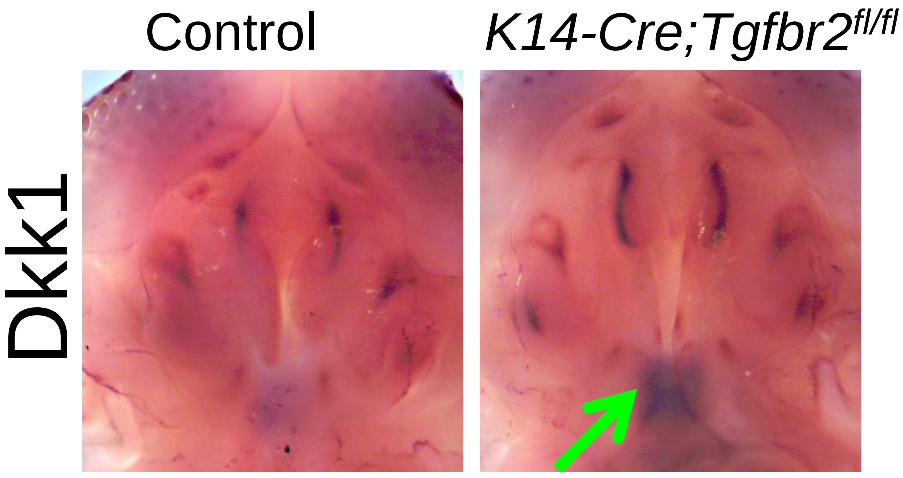
Wild-type (WT) *Tgfbr2*$^{fl/fl}$*;K14-Cre* (CKO)

AFFY_ID	Symbol	Tgfbr2$^{fl/fl}$;Wnt1 /Tgfbr2$^{fl/fl}$	FDR
1420360_at	Dkk1	2.415	0.0448
1425447_at	Dkk4	5.602	0.0103

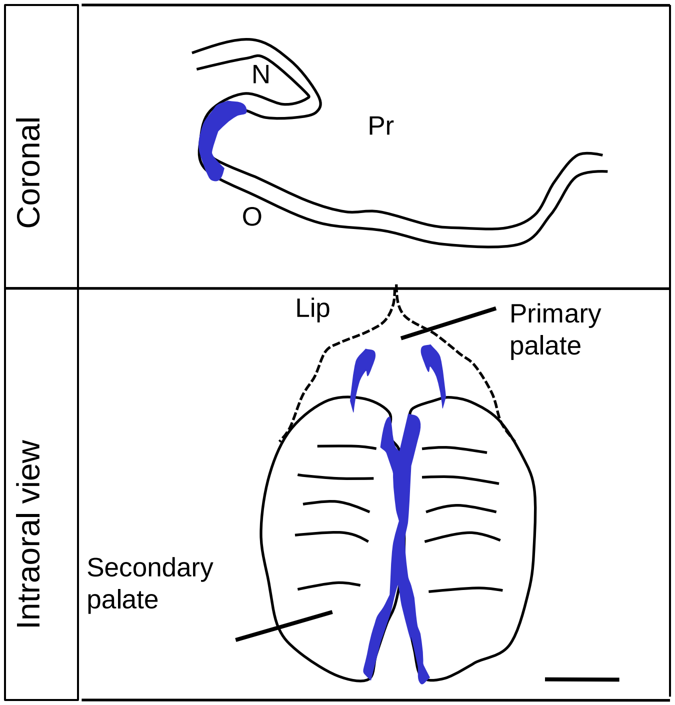
E15.5 Posterior palate

549 probe sets representing transcripts that were differentially expressed

(WT Post vs. CKO Post)

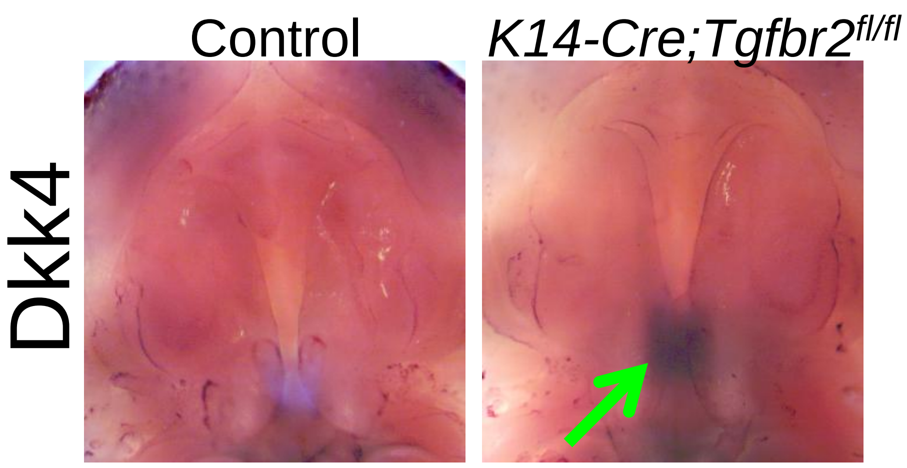


E14.5

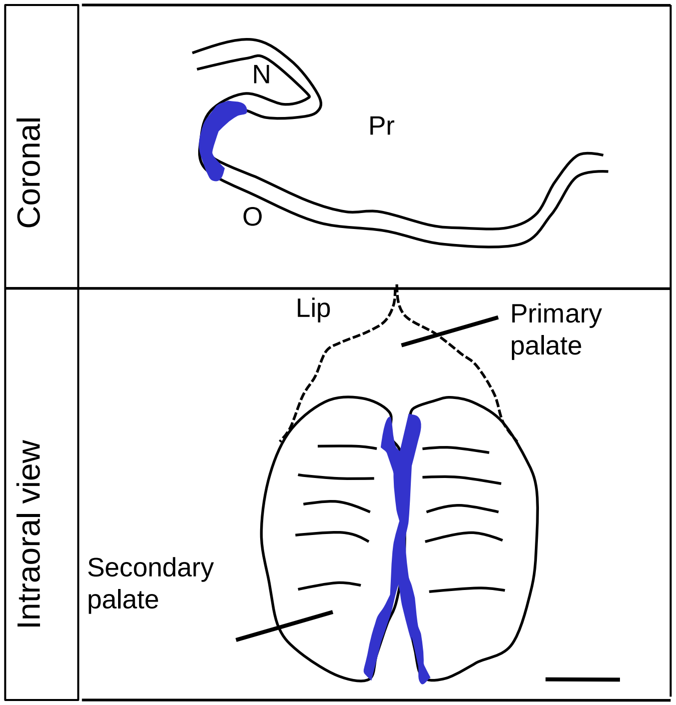


Color code: 🟥MEE

Dkk1



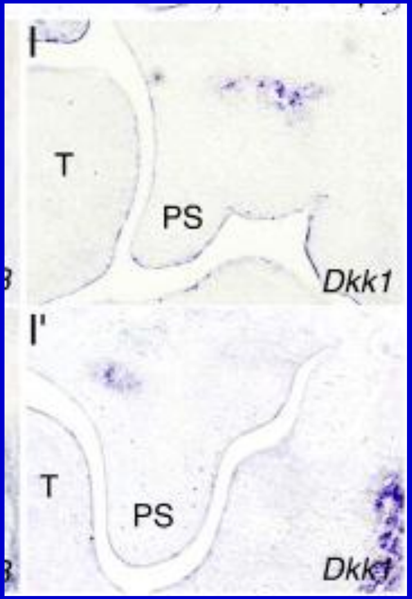
E14.5



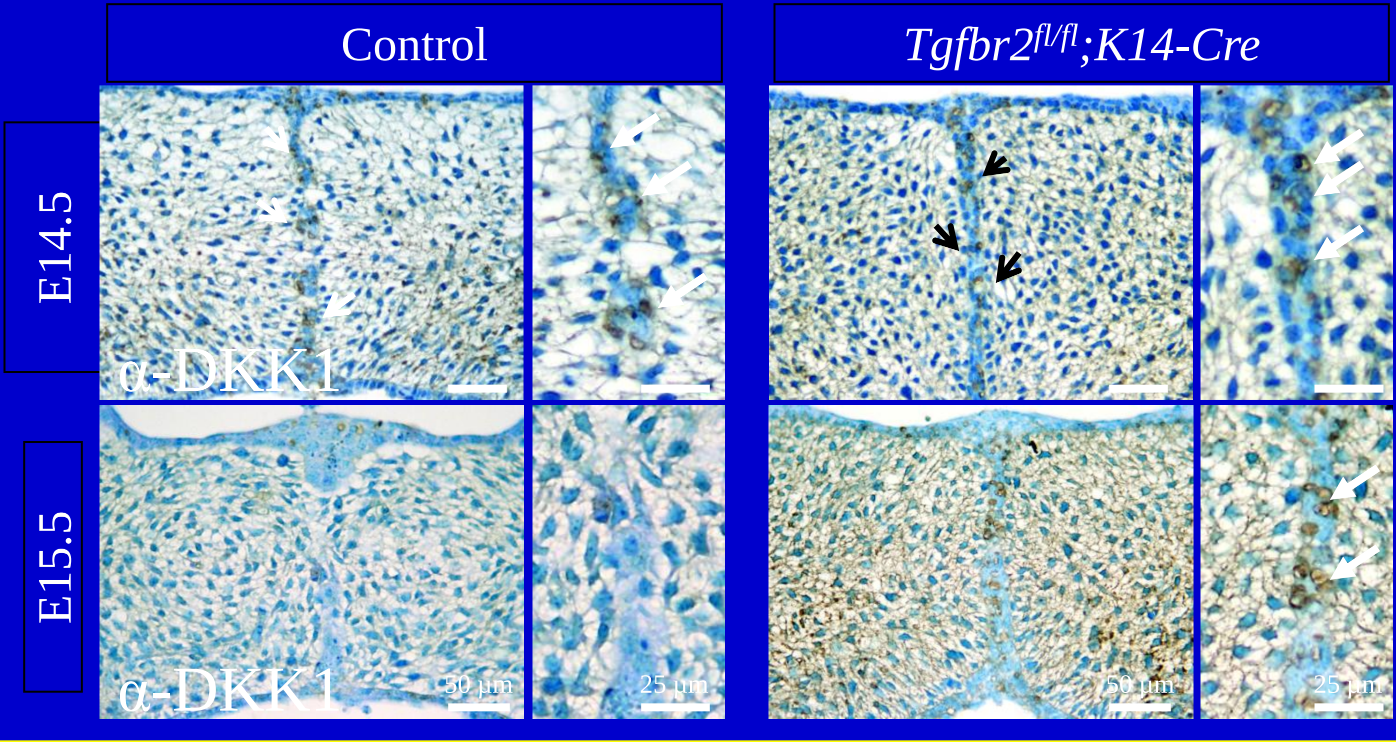
Color code: 🟥MEE

Dkk4

Identification of TGF-β downstream molecules altered in the soft palate of *Tgfbr2$^{fl/fl}$;K14-Cre* mice

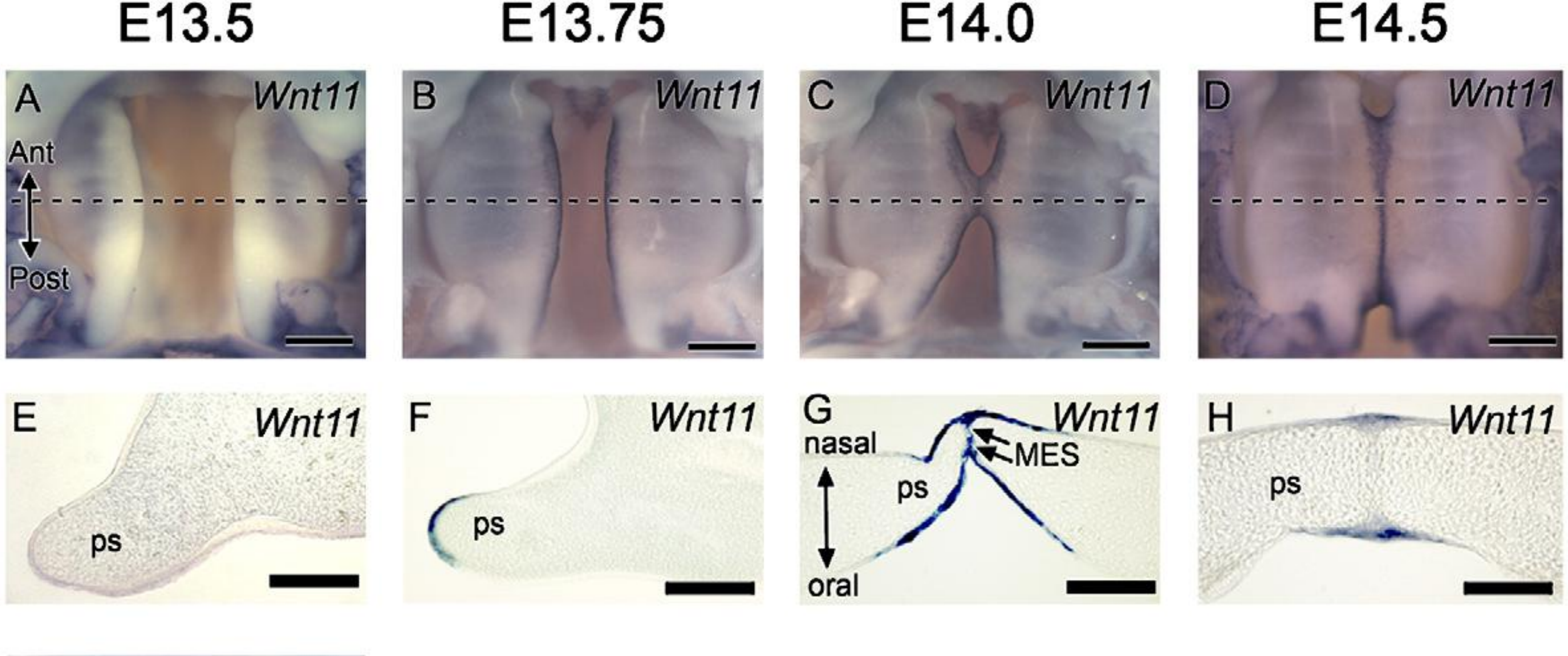


Developmental Biology 350 (2011) 511–519



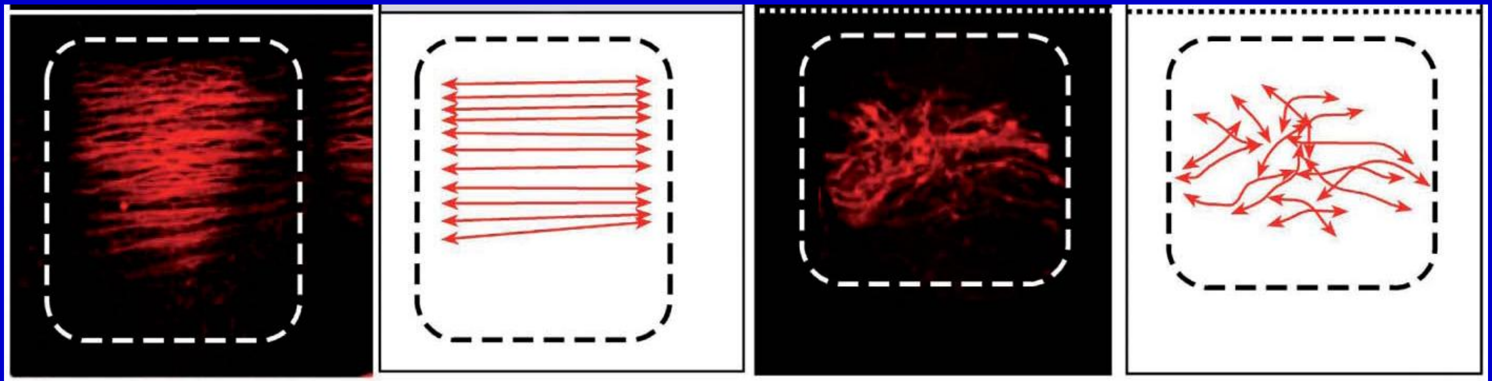
In *K14Cre;Tgfbr2$^{fl/fl}$* mice, there is persistence of MEE cells. There is also persistence of DKK1 expression in the MEE and palatal mesenchyme.

Wnt11 expression in the palate



Developmental Biology 314, 341–350, 2008

WNT11 acts as a directional cue to organize the elongation of early muscle fibers *Nature, 29, 589-593, 2009*

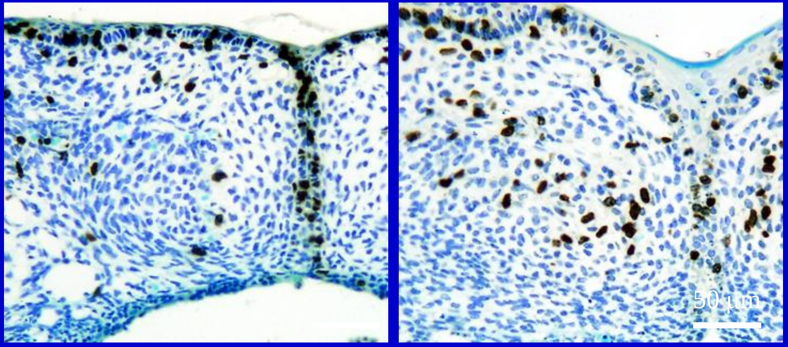


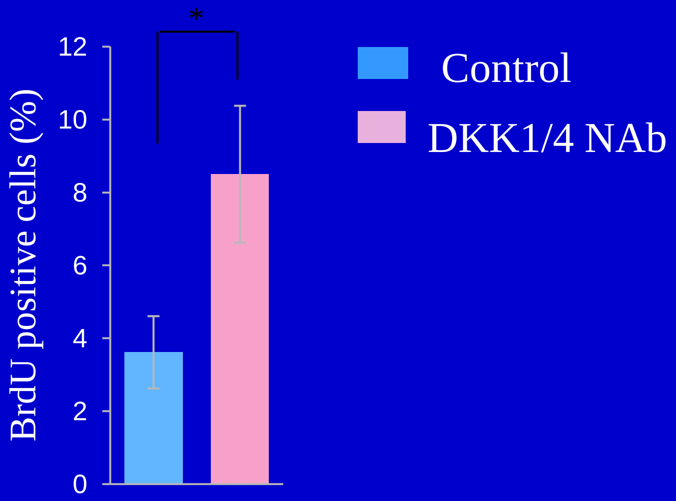
Rescue of the cell proliferation defect in the soft palate of *Tgfbr2*$^{fl/fl}$*;K14-Cre* mice

A

Tgfbr2$^{fl/fl}$*;K14-Cre*

Control DKK1/4 NAb



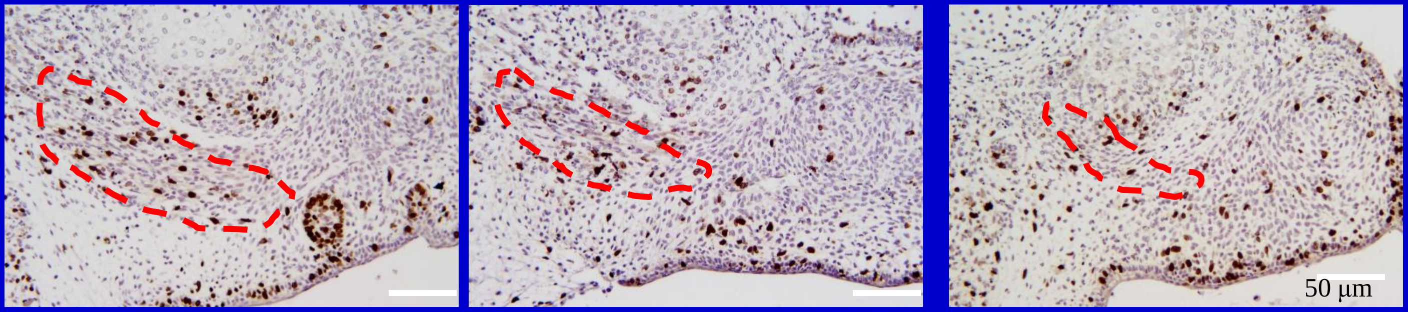


B

Tgfbr2$^{fl/fl}$

Tgfbr2$^{fl/fl}$*;K14-Cre*

Control end-IWR1 Control



LOPAC Library Screen Summary Statistics

Parameters	**Assays**	
	CellTiter-Glo Luminescence	CellTiter-Fluor Fluorescence
1536-well plates	7 (+2 DMSO)	7 (+2 DMSO)
Compounds tested	1280	1280
Points per titration	7	7
Data points	8,960	8,960
B:I	13.6 ± 2.6	3.5 ± 0.6
Output Signal	37700 ± 6800 RLU	595000 ± 98000 FLU
CV	17.9 ± 11.2	25.5 ± 12.4
Z' factor	0.45 ± 0.14	-0.70 ± 0.34
Control condition 1	Double Cell number	Double Cell number
Control compound	FGF9	FGF9
Control IC_{50} (μM)	N/A	N/A

Summary

1. **Loss of *Tgfbr2* in the palatal epithelium results in myogenic progenitor cell proliferation, differentiation and muscle fiber orientation defects. There is reduced soft palate muscle mass in *Tgfbr2*$^{fl/fl}$*;K14-Cre* mice.**

2. ***Dkk1* and *Dkk4*, negative regulators of WNT/β-catenin signaling, are up-regulated in *Tgfbr2*$^{fl/fl}$*;K14-Cre* mice and may be responsible for disrupting epithelial-mesenchymal interactions and causing muscle defects in the soft palate.**

3. ***Tgfbr2*$^{fl/fl}$*;K14-Cre* mice can serve as an animal model to investigate tissue-tissue interactions in regulating palatal muscle formation.**

Center for
Craniofacial
Molecular
Biology

Acknowledgements

CCMB USC

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