

2015 FaceBase Annual Meeting



*Integrated research of functional genomics and
craniofacial morphogenesis*

PI: Yang Chai

University of Southern California

Team Members:

Carolina Parada

Alexandre Grimaldi

Thach-Vu Ho, Shery Park

Hu Zhao

Bridget Samuels

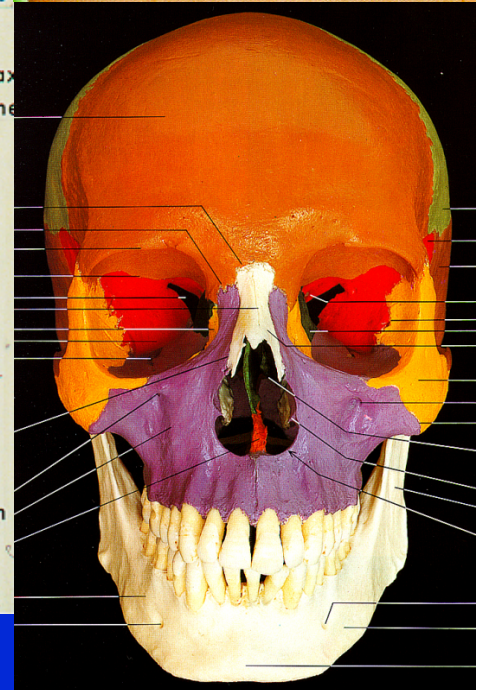
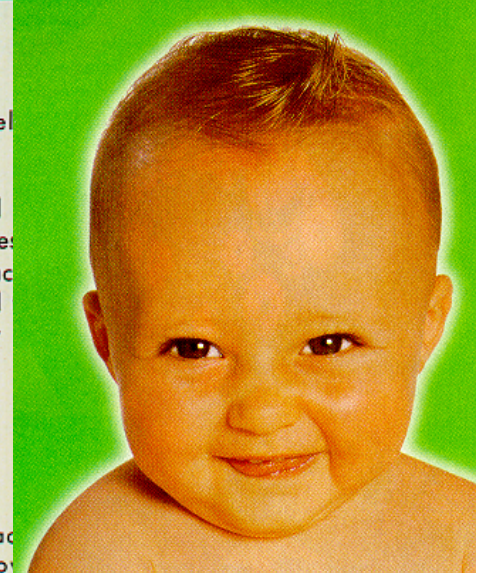
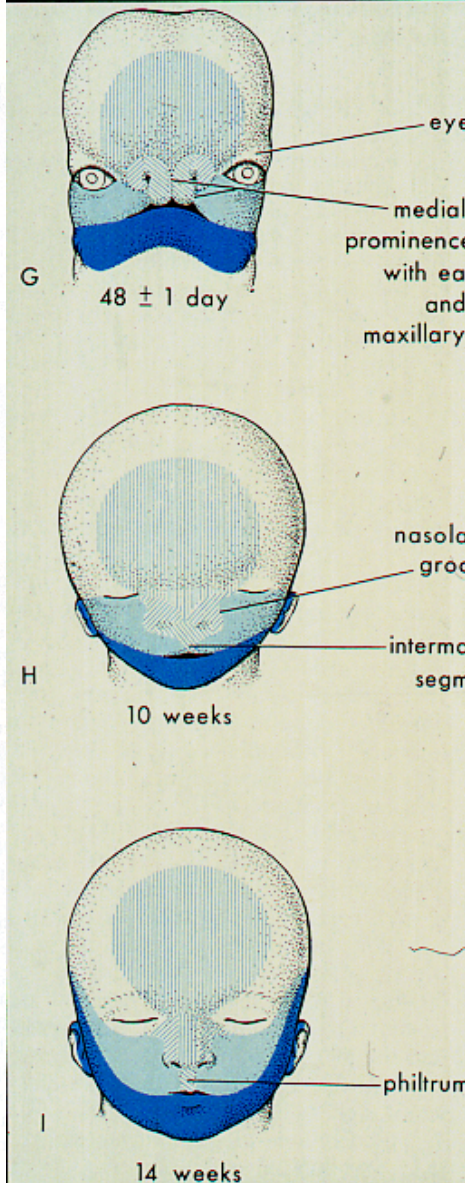
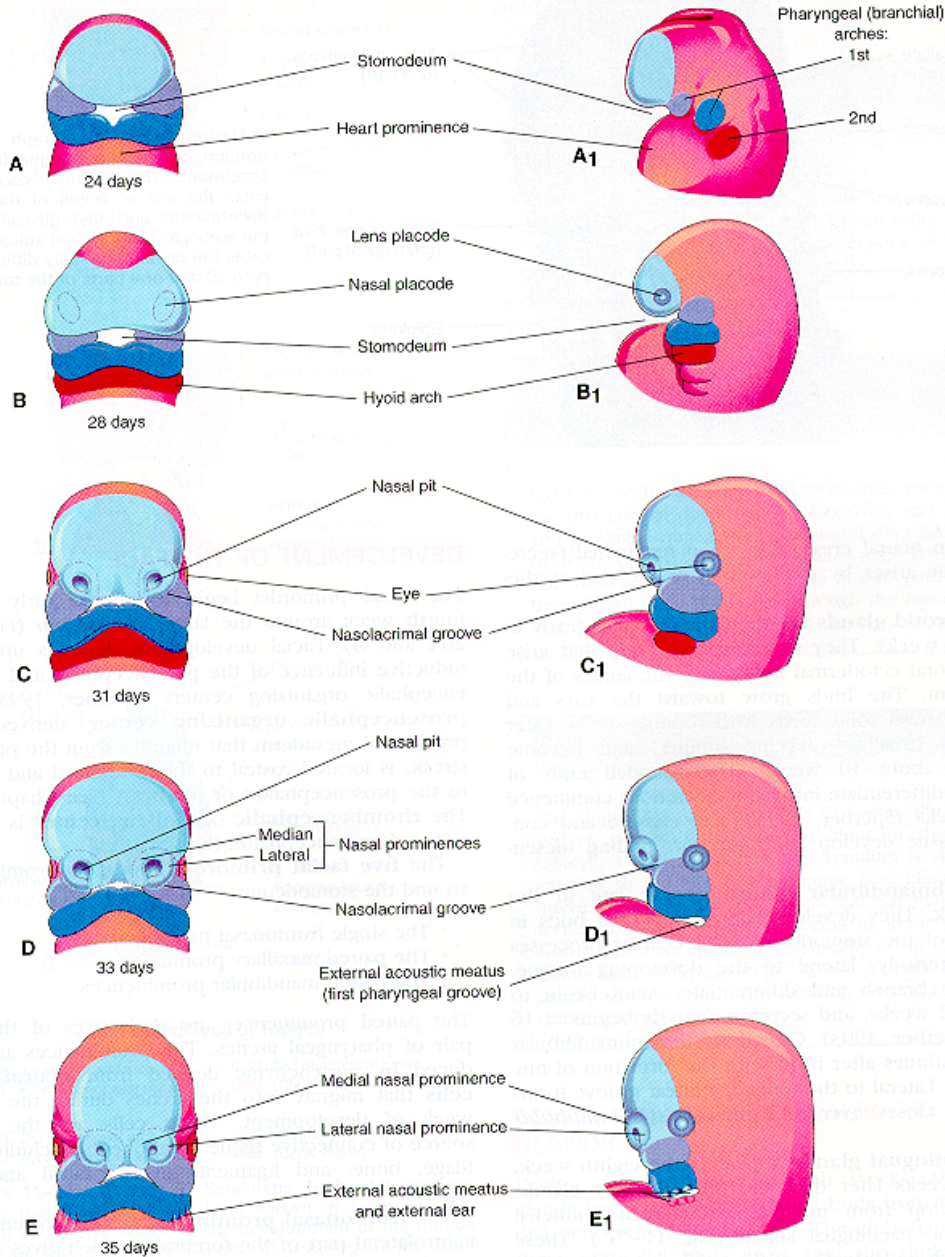
Pedro Sanchez

Paul Thomas (bioinformatics)

Jeremy Green (KCL)

U01 DE024421 NIDCR, NIH

Facial patterning and development – *Our face is our identity*



Specific Aims

1. Global and specific gene expression analyses and 3D imaging study
 - (1) General gene expression profiles during mandible development using microarray/RNA-Seq
 - (2) Specific gene expression, define sub-domains within the developing mandible, and correlate this information with cell fate analysis
 - (3) 3D imaging and ontology development
2. Gene expression, cell fate and 3D imaging during maxilla development

Search

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

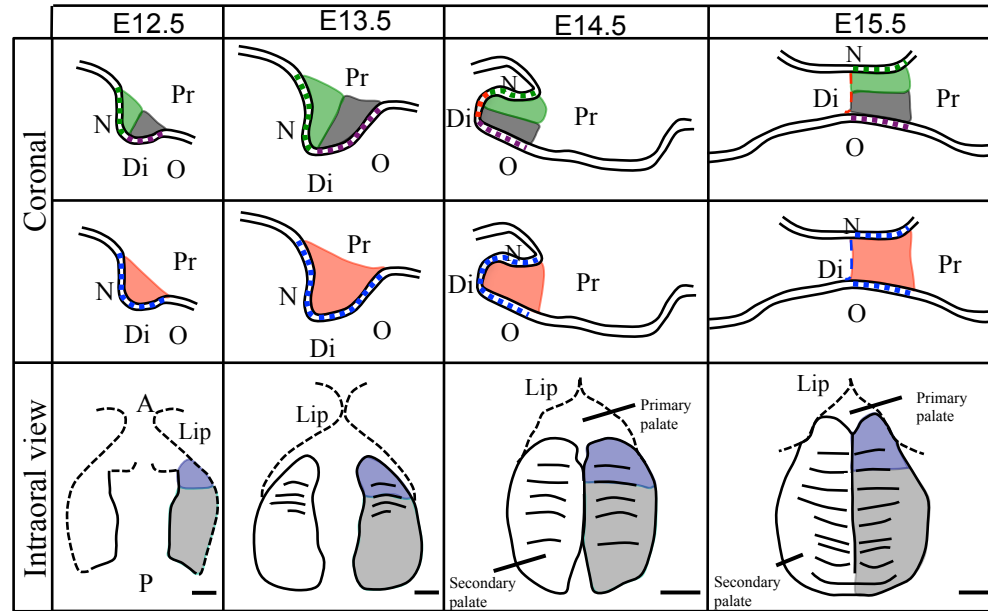
- [Levator](#)
- [Tensor](#)
- [Palatoglossus](#)
- [Palatopharyngeous](#)
- [Uvula](#)

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)

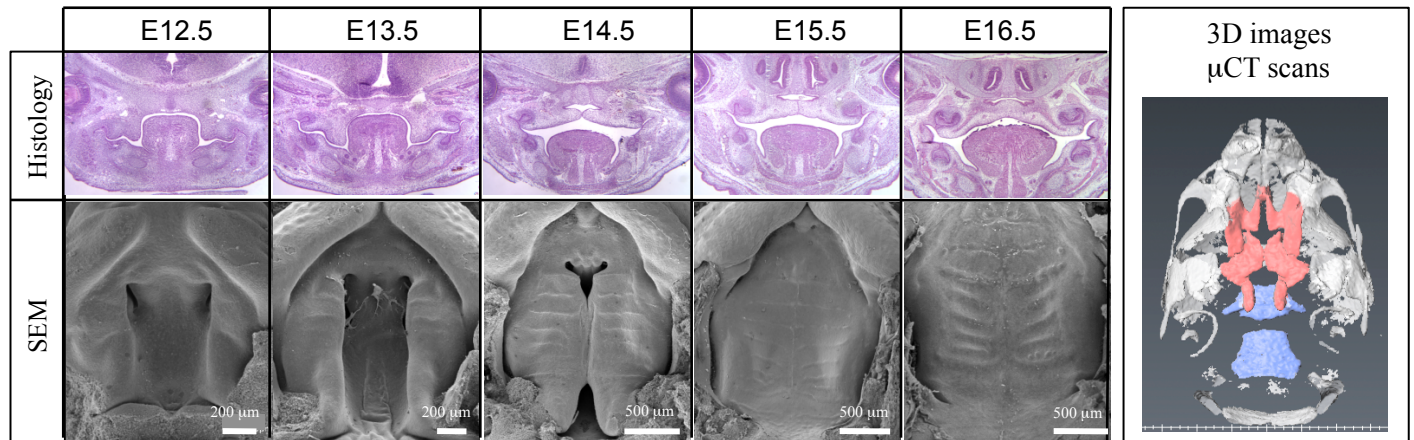


Color code:

- Nasal mesenchyme
- Oral mesenchyme
- All mesenchyme

- Anterior mesenchyme
- Posterior mesenchyme

- ⋯ Nasal epithelium
- ⋯ Midline epithelium
- ⋯ Oral epithelium
- ⋯ All epithelium



Mandible and Maxilla Developmental Defects



Treacher Collins syndrome

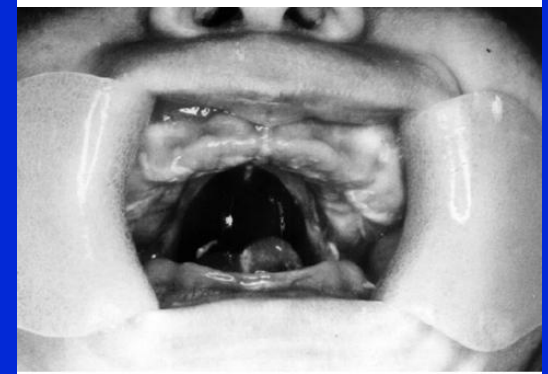
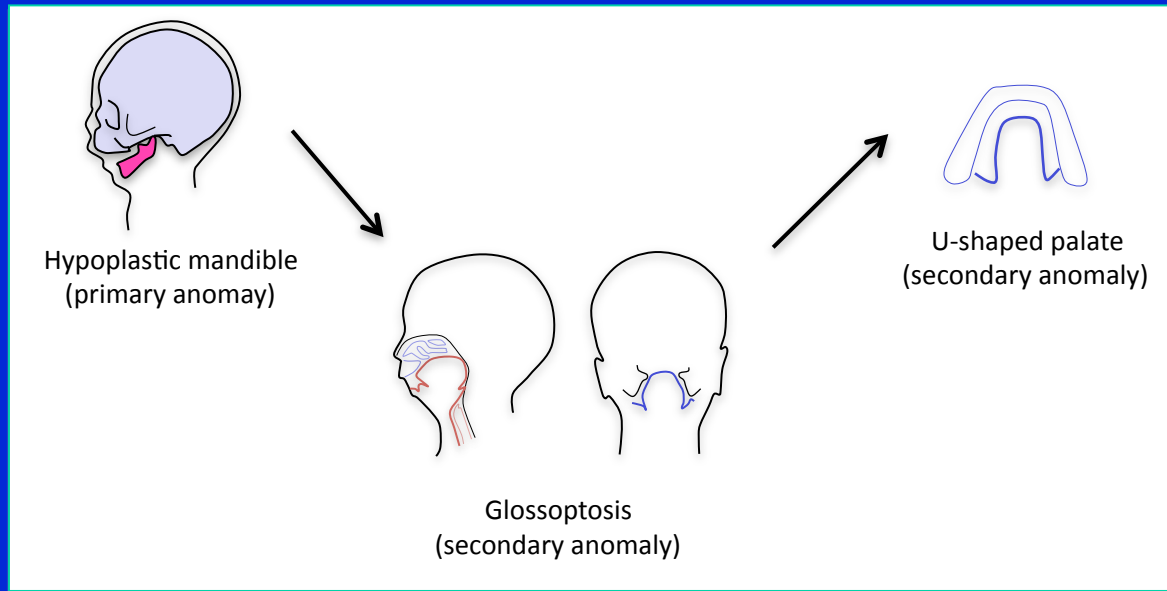
1/50,000



**Craniofacial microsomia
(Goldenhar syndrome,
hemifacial microsomia,
Oculo-Auriculo-Vertebral
syndrome)**

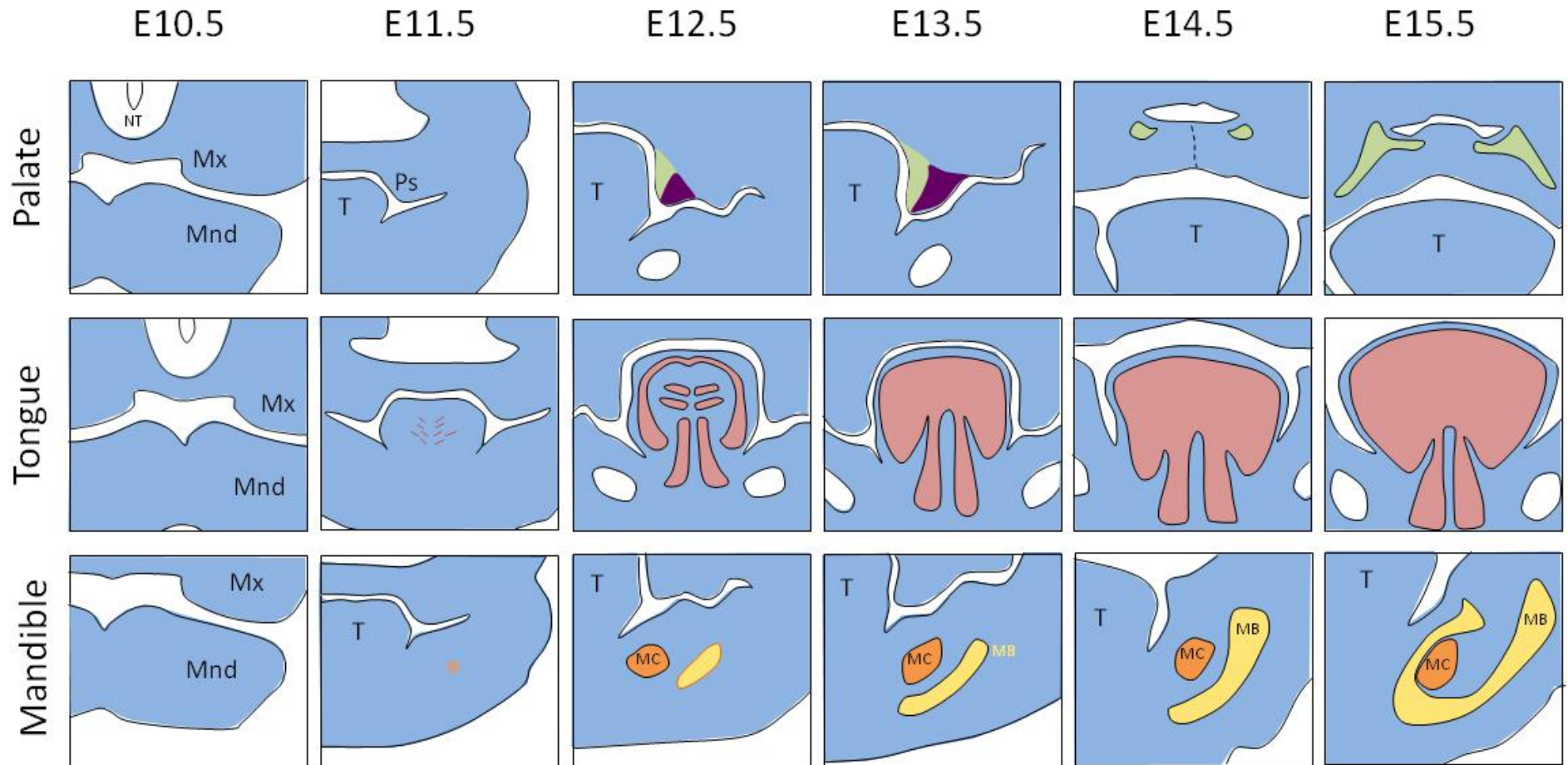
1/3500-25,000

Mandibular defects and craniofacial malformations



Pierre-Robin sequence

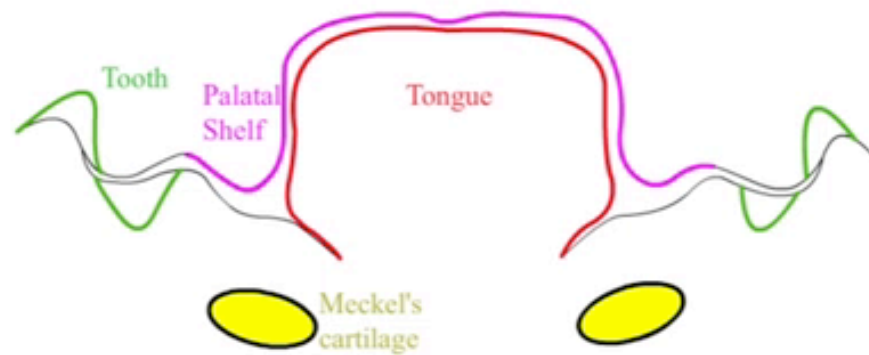
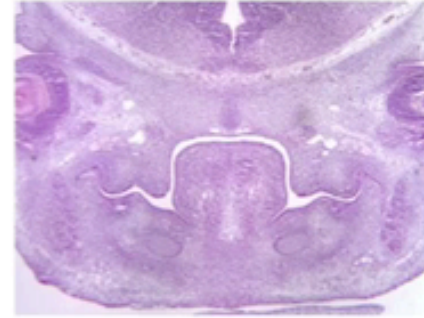
Integrated palate, tongue, and mandible development



cell fate, gene expression and 3D imaging analyses

Integrated palate, tongue, and mandible development

E12.5

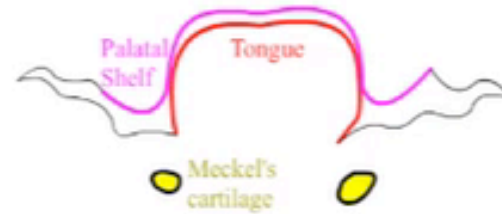


Cleft palate as a secondary defect of mandible malformation

E12.5



Control

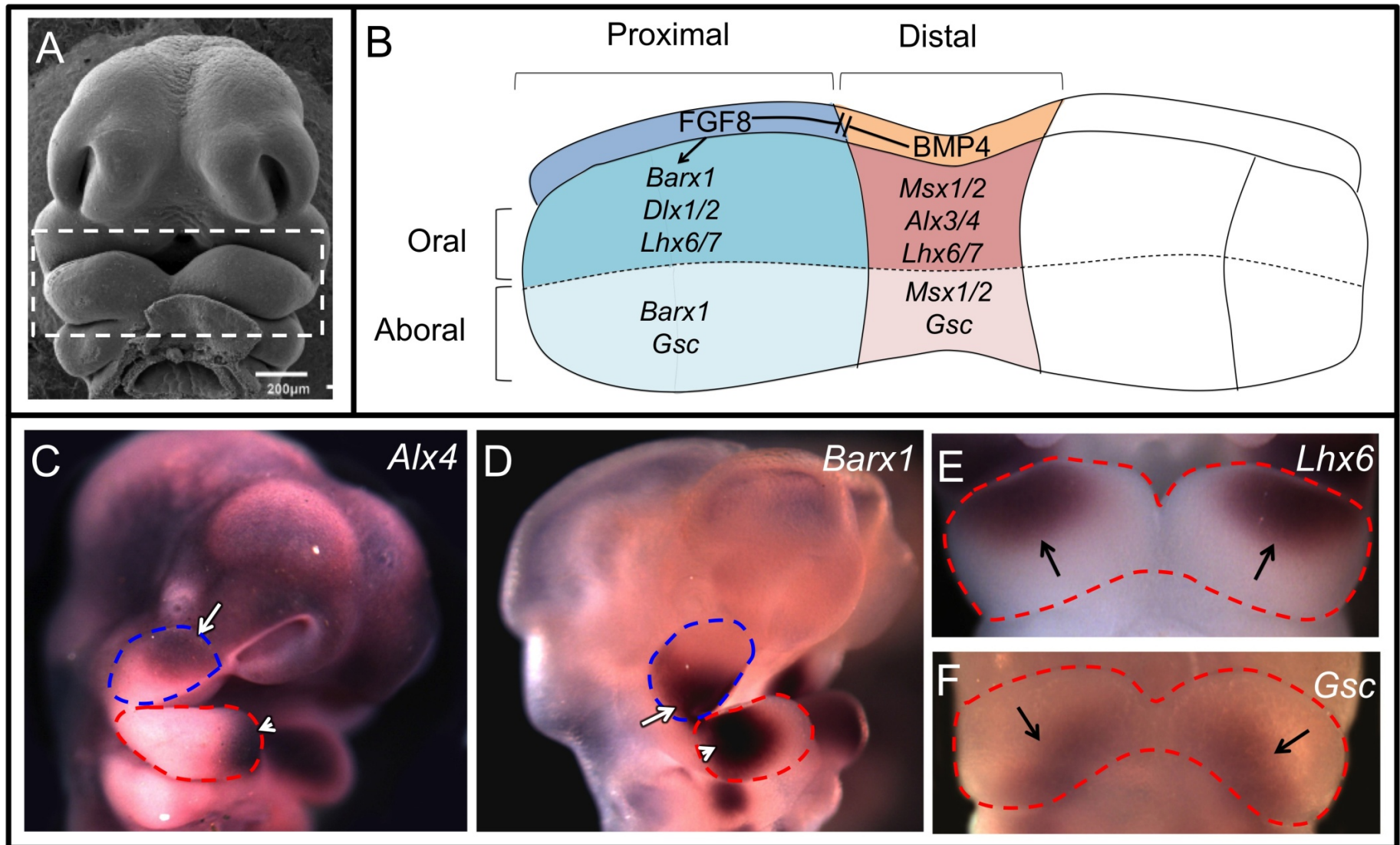


Wnt1-Cre;Erk2^{fl/fl}

See poster by Carolina Parada

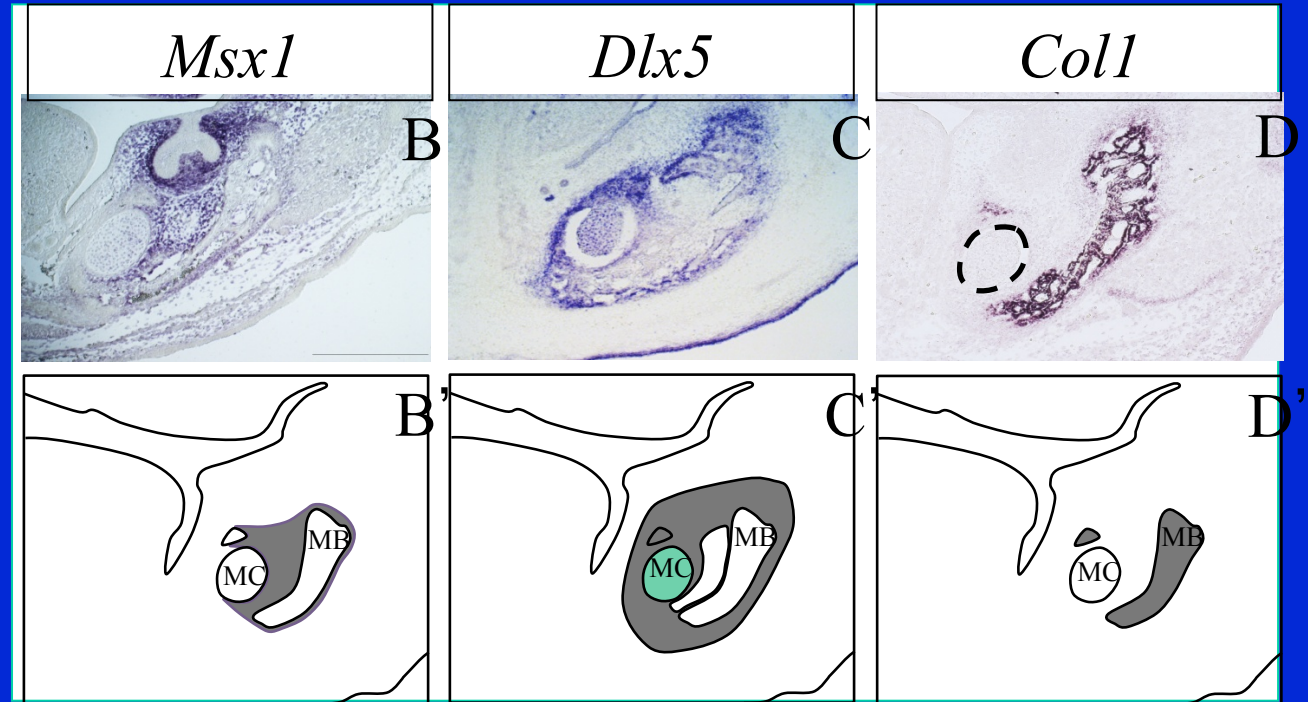
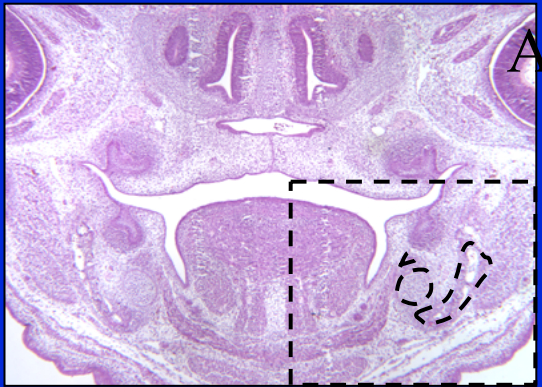
Gene expression and patterning of the branchial arch

Early patterning of the maxillary and mandibular arches



Molecular identity of the proximal region of the developing mandible

E14.5

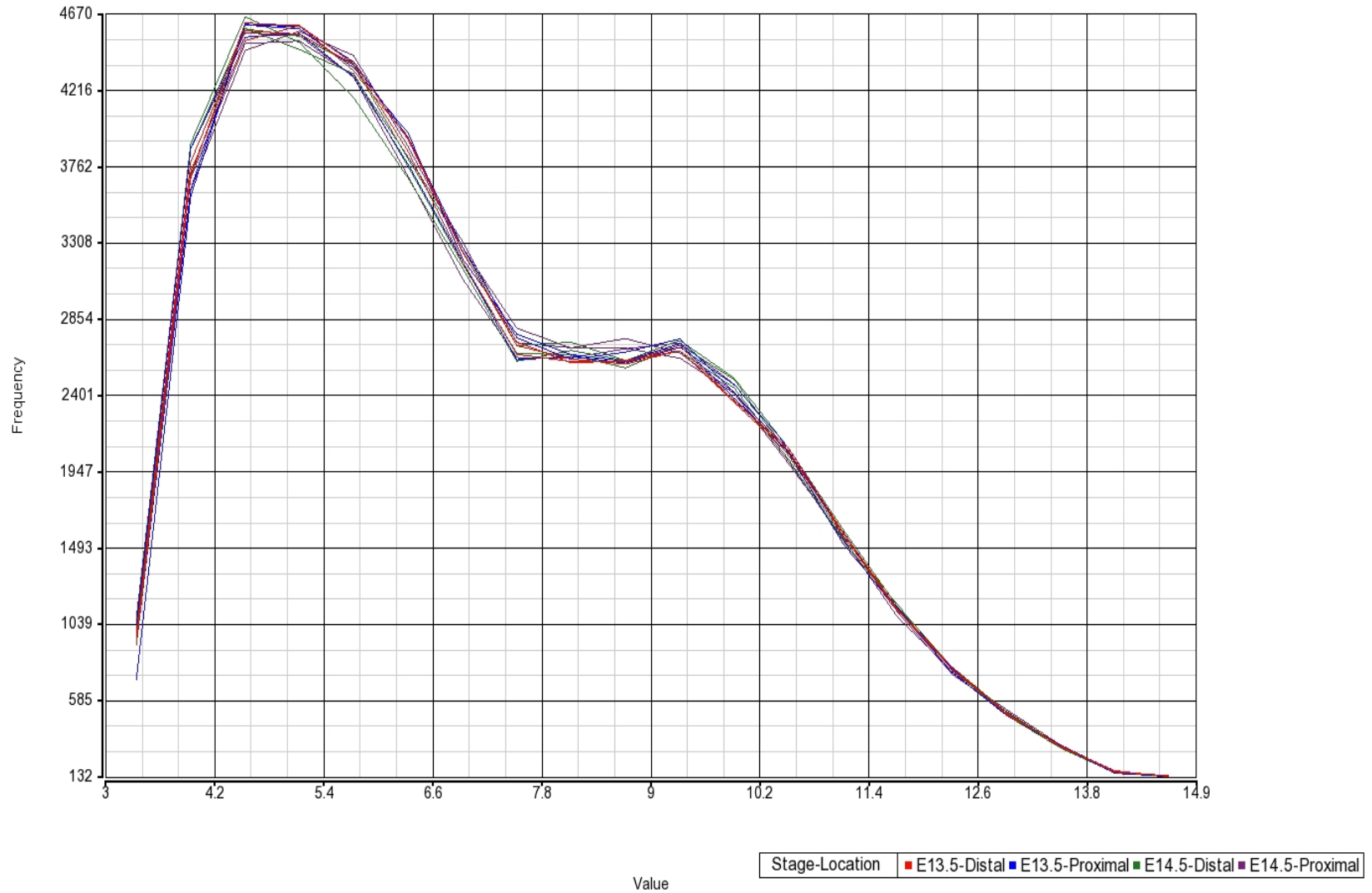


Gene expression profile analyses

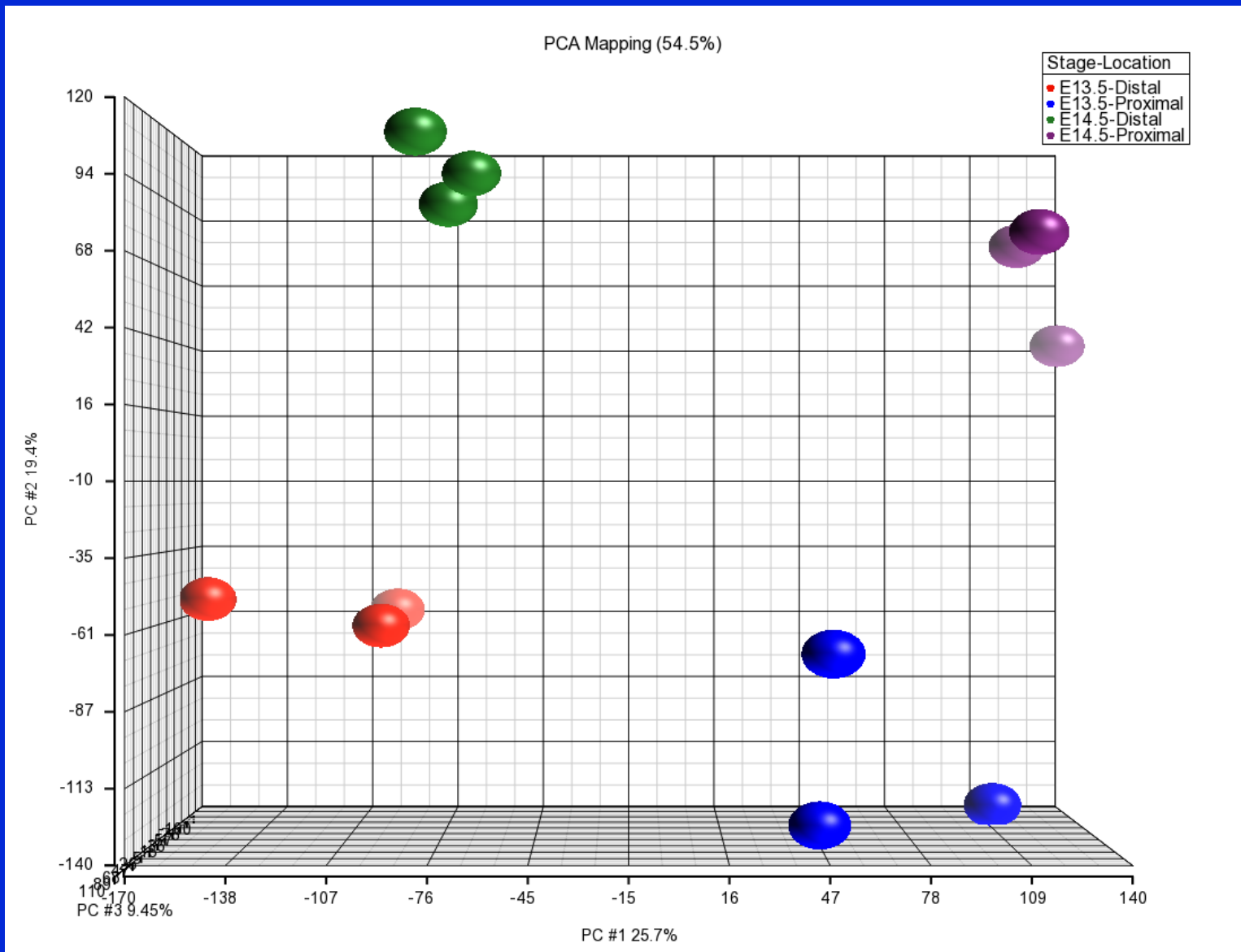
No. Samples	Scan Date	Location	Stage
3	11/20/2014	Distal	E13.5
3	11/20/2014	Distal	E14.5
3	11/20/2014	Proximal	E13.5
3	11/20/2014	Proximal	E14.5

- Downloaded from CHLA on 11/25/2014
- All samples run on Affymetrix GeneChip Mouse Genome 430 2.0
- Data imported into Partek Genomics Suite 6.6
- Preliminary analysis completed on 12/9/2014

All Rows of 1



Consistent distribution of probeset intensities across all samples



Principal Component Analysis shows good separation between sample groups

Analyses

Partek ANOVA and Ingenuity Pathway Analysis (IPA)

1. Distal vs Proximal in Stage E13.5
 2. Distal vs Proximal in Stage E14.5
 3. Stage 13.5 vs Stage E14.5 in Distal
 4. Stage 13.5 vs Stage E14.5 in Proximal
- Genes from each ANOVA with fold change >1.5 or <-1.5 & FDR-adjusted $p < 0.05$ were carried forward to each respective IPA
 - All other default settings used

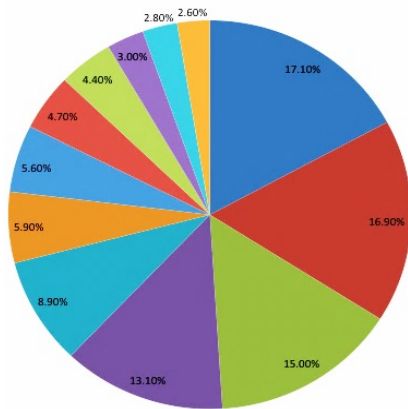
Gene expression profile along the Distal-Proximal axis in the developing mandible

C

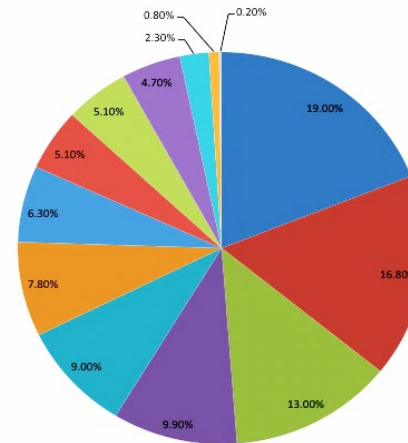
Downregulated genes

Upregulated genes

Biological processes

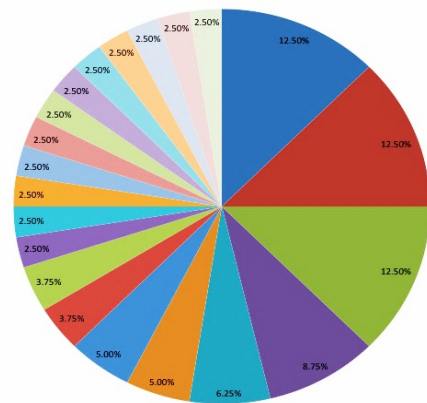


- metabolic process (GO:0008152)
- cellular process (GO:0009987)
- developmental process (GO:0032502)
- biological regulation (GO:0065007)
- multicellular organismal process (GO:0032501)
- immune system process (GO:0002376)
- response to stimulus (GO:0050896)
- cellular component organization or biogenesis (GO:0071840)
- localization (GO:0051179)
- reproduction (GO:0000003)
- biological adhesion (GO:0022610)
- apoptotic process (GO:0006915)

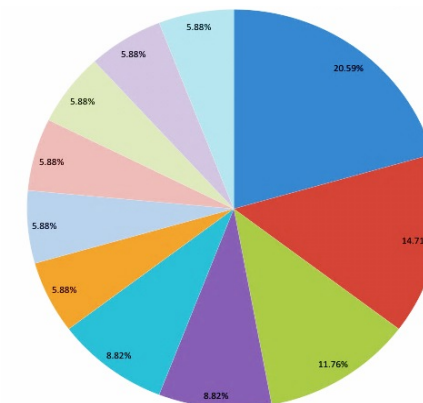


- cellular process (GO:0009987)
- metabolic process (GO:0008152)
- developmental process (GO:0032502)
- multicellular organismal process (GO:0032501)
- biological regulation (GO:0065007)
- localization (GO:0051179)
- response to stimulus (GO:0050896)
- biological adhesion (GO:0022610)
- immune system process (GO:0002376)
- cellular component organization or biogenesis (GO:0071840)
- reproduction (GO:0000003)
- apoptotic process (GO:0006915)
- growth (GO:0040007)

Pathways



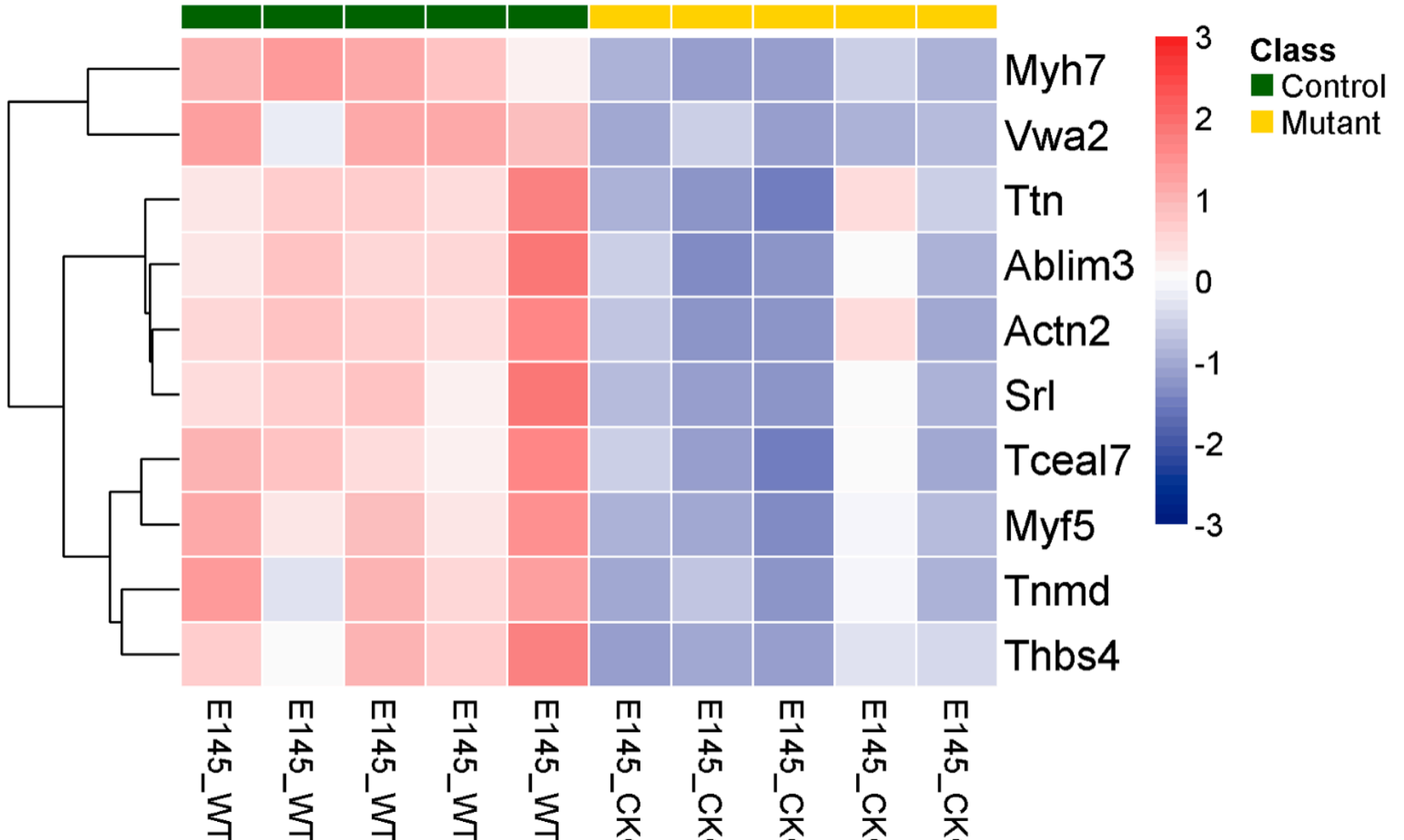
- Integrin signalling pathway (P00034)
- Inflammation mediated by chemokine and cytokine signalling pathway (P00031)
- Wnt signalling pathway (P00057)
- Cytoskeletal regulation by Rho GTPase (P00016)
- Nicotinic acetylcholine receptor signaling pathway (P00044)
- Ionotropic glutamate receptor pathway (P00037)
- Heterotrimeric G-protein signaling pathway-Gi alpha and Gs alpha mediated pathway (P00026)
- Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway (P00027)
- Gonadotropin releasing hormone receptor pathway (P06664)
- Beta2 adrenergic receptor signaling pathway (P04378)
- Beta1 adrenergic receptor signaling pathway (P04377)
- De novo purine biosynthesis (P02738)
- Angiogenesis (P00005)
- Huntington disease (P00029)
- FGF signaling pathway (P00021)
- Parkinson disease (P00049)
- Cadherin signaling pathway (P00012)
- Blood coagulation (P00011)
- Dopamine receptor mediated signaling pathway (P05912)
- Angiotensin II-stimulated signaling through G proteins and beta-arrestin (P05911)
- Corticotropin releasing factor receptor signaling pathway (P04380)



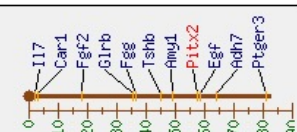
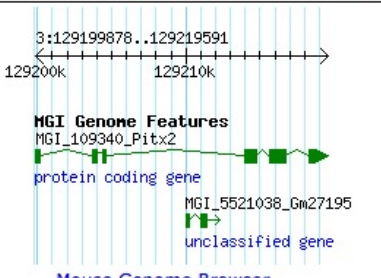
- Gonadotropin releasing hormone receptor pathway (P06664)
- Wnt signalling pathway (P00057)
- Cadherin signaling pathway (P00012)
- TGF-beta signaling pathway (P00052)
- Alzheimer disease-presenilin pathway (P00004)
- Oxytocin receptor mediated signaling pathway (P04391)
- Nicotinic acetylcholine receptor signaling pathway (P00044)
- Ionotropic glutamate receptor pathway (P00037)
- Integrin signalling pathway (P00034)
- Inflammation mediated by chemokine and cytokine signaling pathway (P00031)
- Huntington disease (P00029)

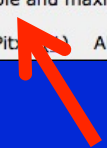
GO Term (GO ID)	Genes Annotated to the GO Term	GO Term Usage in Gene List	Genome Frequency of Use
cellular process (GO:0009987)	4632434I11Rik, Ablim3, Actn2, Adcy8, Angptl1, Anp32a, Birc5, Bub1, Bub1b, Calca, Car2, Ccdc99, Ccna2, Ccnb1, Cd55, Cdc25c, Cdc45, Cdc6, Cdca3, Cdca5, Cdh4, Cdkn3, Cenph, Crym, Cyp26b1, D2Erd750e, Depdc1b, Dscc1, Dusp9, Eme1, Epha4, Esco2, Etv5, Exo1, Fabp4, Fbln5, Fbxo5, Fen1, Fgf9, Fli1, Gas2, Gins1, Gmnn, Gsg2, H2afv, Hfe2, Hist1h3a, Hmga2, Hmgb2, Hnf1b, Kif11, Kif20b, Kif22, Kif23, Lars2, Limd1, Mtm1, Myf5, Myh6, Myh7, Nasp, Ncapg, Ndc80, Nptx1, Pde1a, Pfkfb3, Plac1, Rad51ap1, Rasi11b, Rrm2, Sgol1, Ska1, Slc40a1, Smc6, Smyd1, Spc24, Spc25, Spsb4, Srl, Tace1, Tceal7, Tgfbr2, Thbs4, Tk1, Tnfrsf11, Tnmd, Traip, Ttn, Tyms, Ywa2	90 of 123 genes, 73.17 %	13370 of 25000 annotated genes, 53.48 %

Cellular Process

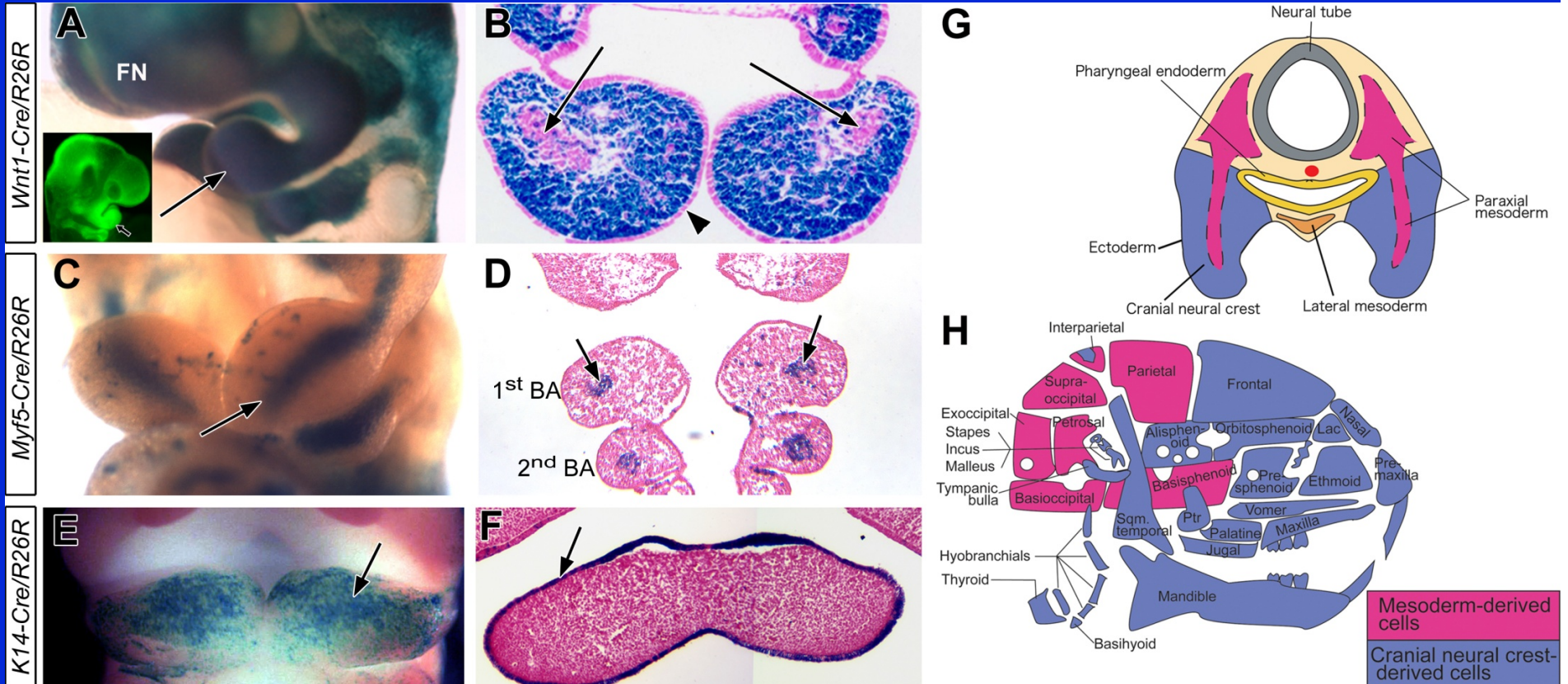


Pitx2 Gene Detail Your Input Welcome

Symbol	Pitx2	
Name	paired-like homeodomain transcription factor 2	
ID	MGI:109340	
Synonyms	Brx1, Brx1a, Brx1b, Munc30, Otlx2, Pitx2a, Pitx2b, Pitx2c, Ptx2, Rieg, solurshin	
Feature Type	protein coding gene	
Genetic Map	Chromosome 3 57.84 cM Detailed Genetic Map ± 1 cM Mapping data(5)	
Sequence Map	Chr3:129199878-129219591 bp, + strand From VEGA annotation of GRCm38 <input type="button" value="Get FASTA"/> 19714 bp ± 0 kb flank VEGA Genome Browser Ensembl Genome Browser UCSC Browser NCBI Map Viewer	 Mouse Genome Browser
Vertebrate homology	HomoloGene:55454 Vertebrate Homology Class 1 human; 1 mouse; 1 rat; 1 chimpanzee; 1 rhesus macaque; 1 cattle; 1 dog; 1 chicken; 1 western clawed frog; 1 zebrafish Protein SuperFamily: homeobox protein, Pitx/Unc30 types Gene Tree: Pitx2	
Human homologs	Human Homolog PITX2, paired-like homeodomain 2 NCBI Gene ID 5308 neXtProt AC NX_Q99697 Human Synonyms ARP1, Brx1, IDG2, IGDS, IGDS2, IHG2, IRID2, Otlx2, PTX2, RGS, RIEG, RIEG1, RS Human Chr (Location) 4q25; chr4:110617423-110642123 (-) <i>GRCh38</i> Disease Associations (4) Diseases Associated with Human PITX2	
Mutations, alleles, and phenotypes	All mutations/alleles(18) : Targeted(18) Incidental mutations (data from Mutagenetix , APF) Homozygotes for targeted mutations show failed ventral body wall closure, right pulmonary isomerism, septal and valve defects, absent ocular muscles, arrested pituitary and tooth development, optic nerve, mandible and maxilla defects, and embryonic death. Human Diseases Modeled Using Mouse Pitx2 Alleles Annotated to Human Diseases(6)	



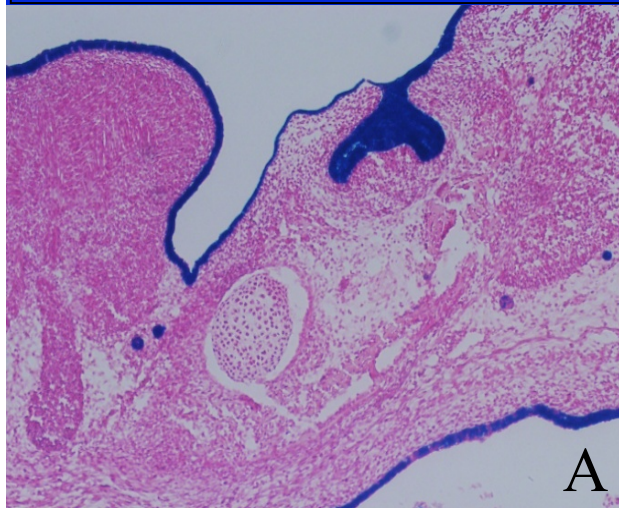
Genetic cell lineage analyses



E10.5

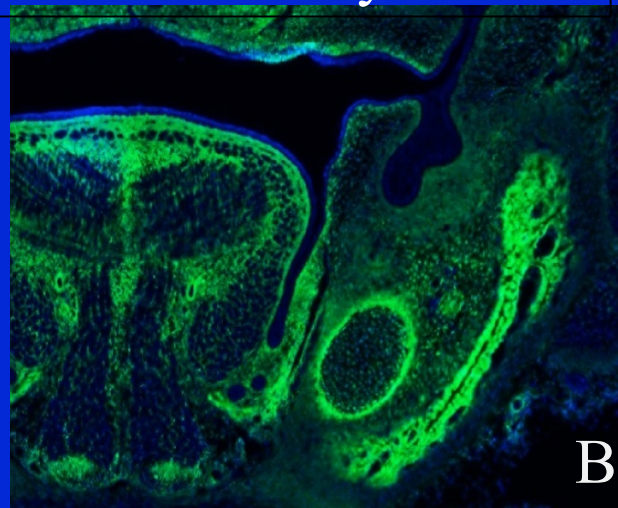
Cell lineage analysis during mandible development

Epithelium



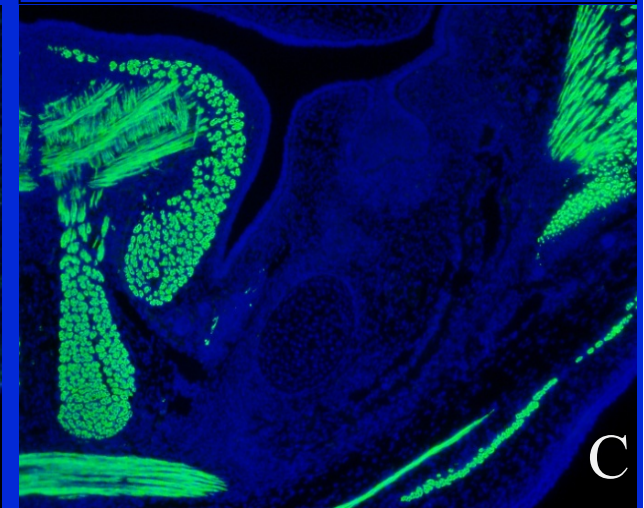
K14-Cre;R26R

CNCC-derived
mesenchyme



Wnt1-Cre;ZsGreen

Myogenic cells

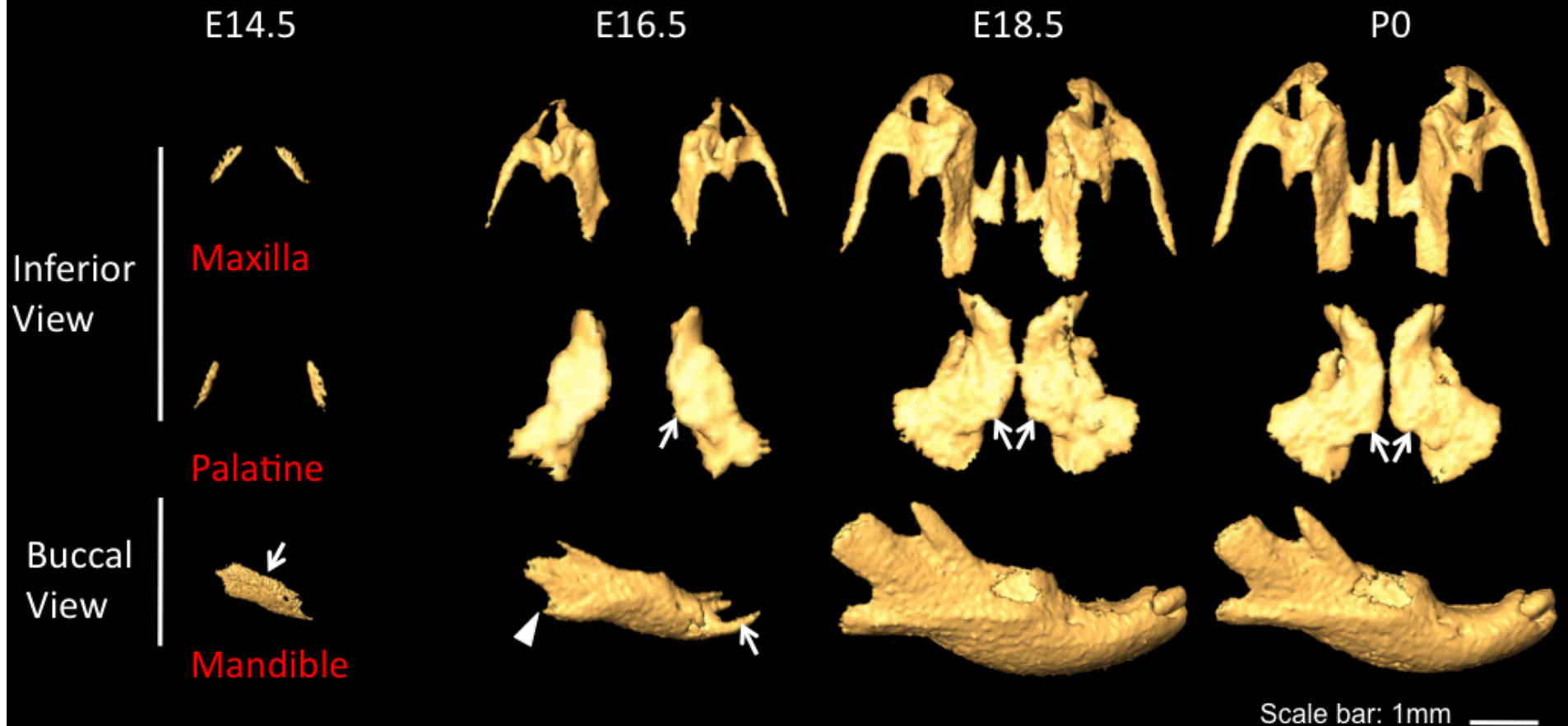


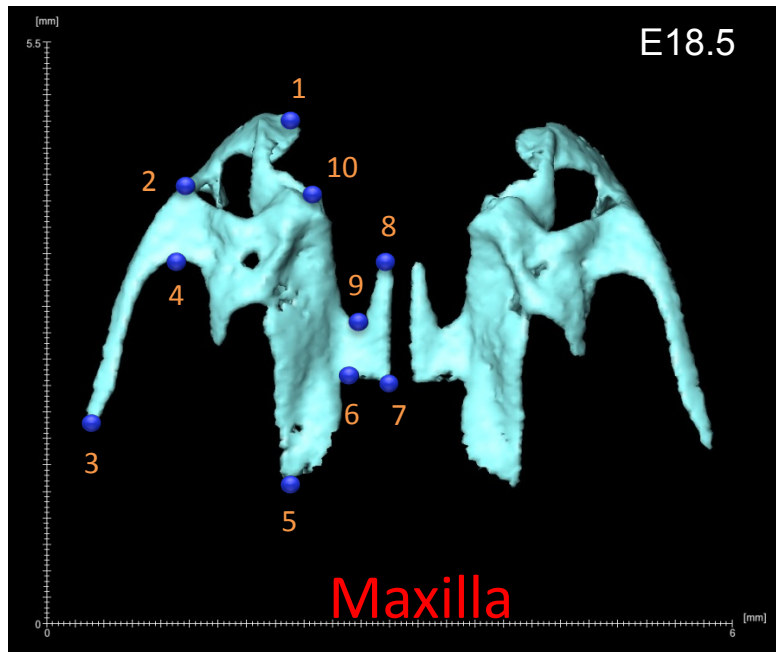
Myf5-Cre;ZsGreen

E14.5

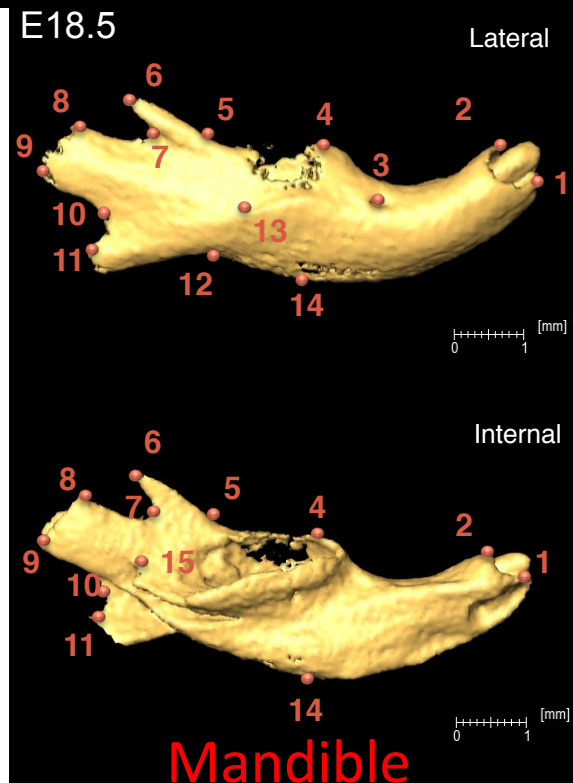
Morphogenesis of craniofacial bones

WT_Control





1. Anterior point of maxilla
2. Lateral inferior intersection of frontal and zygomatic process
3. Junction point of zygoma with zygomatic process
4. Anterio-medial point to zygomatic process
5. Posterior point of maxilla
6. Posterior-lateral point of the palatine process
7. Posterior-medial point of the palatine process
8. Most Anterior-medial point of palatine process
9. Anterior-lateral point of palatine process
10. Medial point of premaxillary-maxillary suture



1. Most anterior point of mandible
2. Anterior-superior point of mandible
3. Mental foramen
4. Molar alveolus of dentary
5. Anterior junction of mandibular ramus and body
6. Superior tip of coronary process
7. Most inferior point of mandibular notch
8. Anterior point of condylar process
9. Posterior point of condylar process
10. Superior point of angular process
11. Secondary cartilage of angular process
12. Inferior junction of mandibular ramus and body
13. Midpoint of external oblique ridge
14. Inferior point of mandibular body
15. Mandibular foramen

3D interactive imaging of the craniofacial complex

The screenshot shows the homepage of the Craniofacial Central website. The browser address bar displays 'https://face.usc.edu'. The page header includes the site title 'Craniofacial Central' and the affiliation 'USC University of Southern California' with its logo. Below the header is a navigation menu with categories such as 'Home page', 'Cleft of the secondary palate', 'Gene expressions', 'Microarrays', 'Micro CTs', 'Micro MRIs', 'SEM', and 'Team members'. The main content area features a 'Home page' section with a welcome message, a search instruction, and a search box. A 'FaceBase' logo is also present, identifying it as a resource for craniofacial researchers.

← → ↻ 🏠 <https://face.usc.edu> ⚙️ ⭐ 🔍 🌐 📄 🗨️ ☰

Craniofacial Central

Center for Craniofacial Molecular Biology

USC University of Southern California

- ▶ Home page
- ▶ Cleft of the secondary palate
- ▶ Gene expressions
 - ▶ Molecules
 - ▶ Growth factors
 - ▶ Receptors
 - ▶ Signaling molecules
 - ▶ Transcription factors
 - ▶ Intracellular molecules
 - ▶ Extracellular molecules
 - ▶ Plasma membrane molecules
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 - ▶ Mesenchyme
 - ▶ Nasal region
 - ▶ Oral region
 - ▶ Anterior region
 - ▶ Posterior region
 - ▶ Osteogenic mesenchyme
 - ▶ Palatal bone primordium
 - ▶ Muscles of the soft palate
- ▶ Microarrays
- ▶ Micro CTs
 - ▶ Individual bones
 - ▶ Interactive 3D analysis
- ▶ Soft tissue
- ▶ Whole skull
- ▶ Micro MRIs
- ▶ SEM
- ▶ Team members

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).

FaceBase
A Resource For Craniofacial Researchers

OUR MILESTONES


MILESTONES

SA 1-1: Microarrays (mandibles)
 SA 1-1: In situ analysis (mandible)
 SA 1-2: Cell lineage tracing (mandible)
 SA 1-2: 3D imaging (mandible)
 SA 2-1: Microarrays (maxillas)
 SA 2-1: In situ analysis (maxilla)
 SA 2-2: Cell lineage tracing (maxilla)
 SA 2-2: 3D imaging (maxilla)
 SA 2-2: Heat maps (mandible + maxilla)

<u>Year 1</u>	<u>Year 2</u>	<u>Year 3</u>	<u>Year 4</u>	<u>Year 5</u>
50 control arrays		75 mutant arrays		
8 genes	7 genes	7 genes	7 genes	2 genes
24 controls				
12 controls		36 mutants		
10 control arrays		20 mutant arrays		
7 genes	7 genes	7 genes		
same as in SA1-2				
12 controls		36 mutants		
4				
controls	12 mutants			

Collaborations and interactions

1. The FaceBase hub
2. Williams, Hooper, Jones (gene expression)
3. Visel lab (enhancer studies)
4. Spritz, Ophir (dysmorphic facial development)
5. Jabs, Holmes, van Bakel (suture gene expression, genetic cell lineage)
6. Brinkley (ontology)
7. Others



Center for
Craniofacial
Molecular
Biology

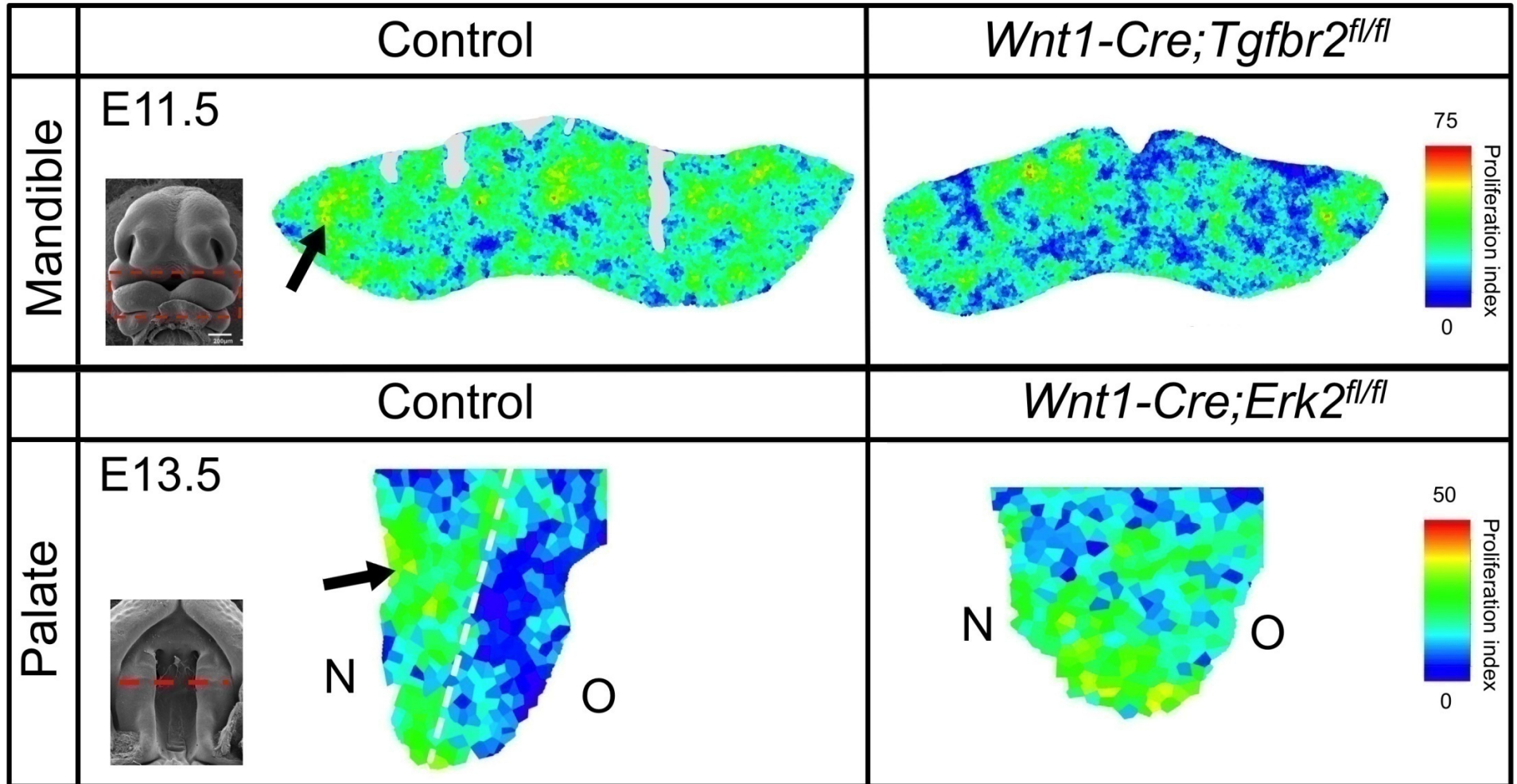
Acknowledgements

Supported by NIDCR, NIH

Carolina Parada, Alexandre Grimaldi, Pedro Sanchez, Thach-Vu Ho, Bridget Samuels, Shery Park, Jingyuan Li, Yang Liu, Julie Mayo, Hu Zhao, Mark Urata, Jifan Feng, and Patricia Sarrion.

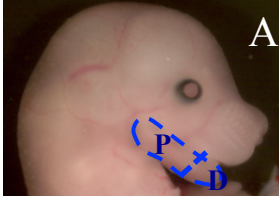


Heat map on cell proliferation activity



Yang Chai and Jeremy Green (Kings College London)

Gene expression profile along the Distal-Proximal axis in the developing mandible



**Top 5
downregulated**

Rank	D vs P in E14.5	Fold change	<i>p</i> value
1	Oc90	-37.9093	5.89E-05
2	Matn1	-34.3355	0.0003732 18
3	Myl2	-24.7842	0.0001036 52
4	Zic3	-20.5493	0.0002684 16
5	Crh	-17.7517	7.55E-06
3	Dynap	17.2311	5.73E-06
4	Tmprss11a	14.3198	2.47E-06
5	Krt6b	12.5041	1.71E-05

Top 5 upregulated