2016 FaceBase Annual Meeting

FaceBase
A Resource For Craniofacial Researchers

Integrated research of functional genomics and craniofacial morphogenesis

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Zoe Johnson
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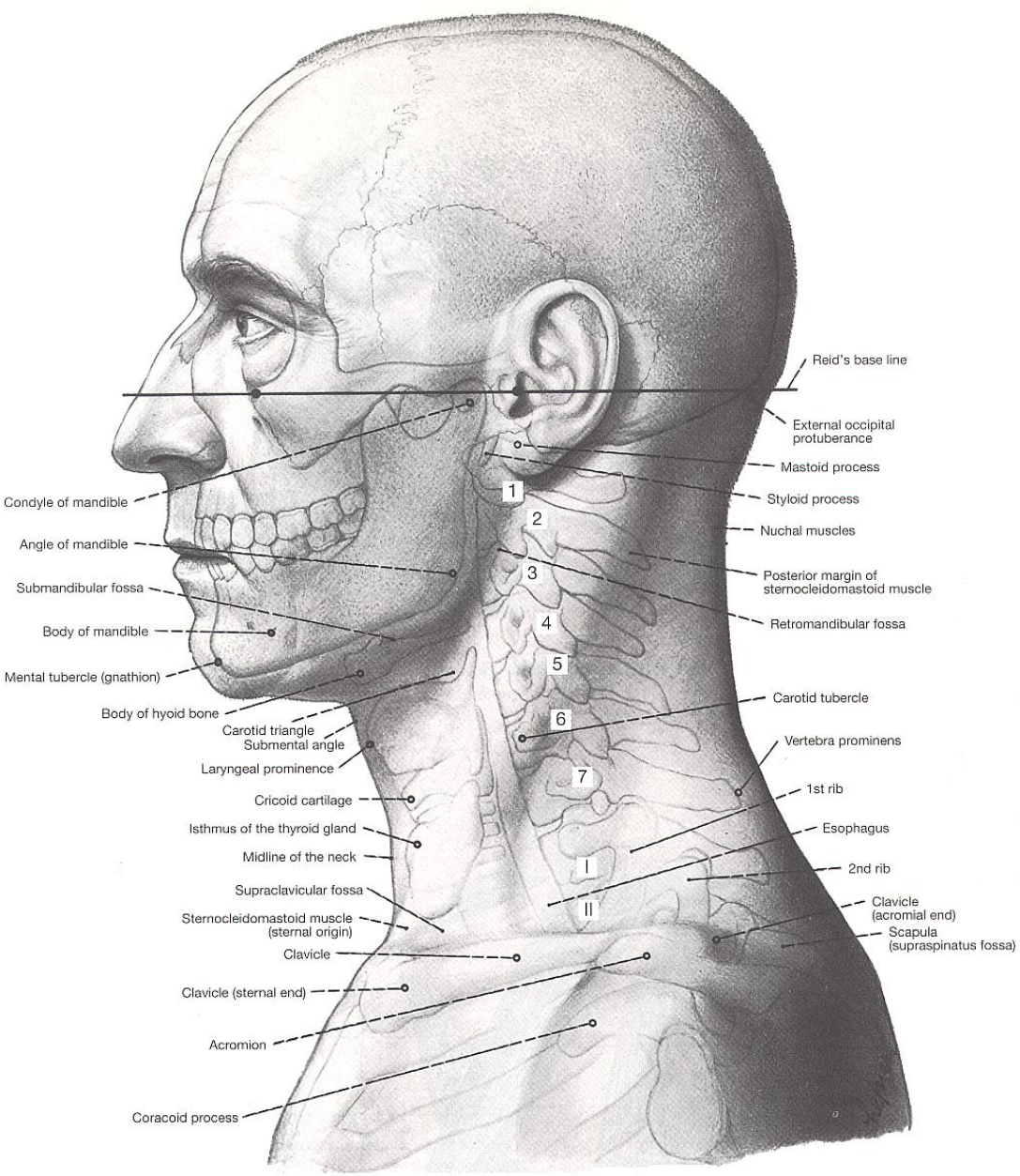
Pedro Sanchez
Paul Thomas (bioinformatics)
Jeremy Green (KCL)

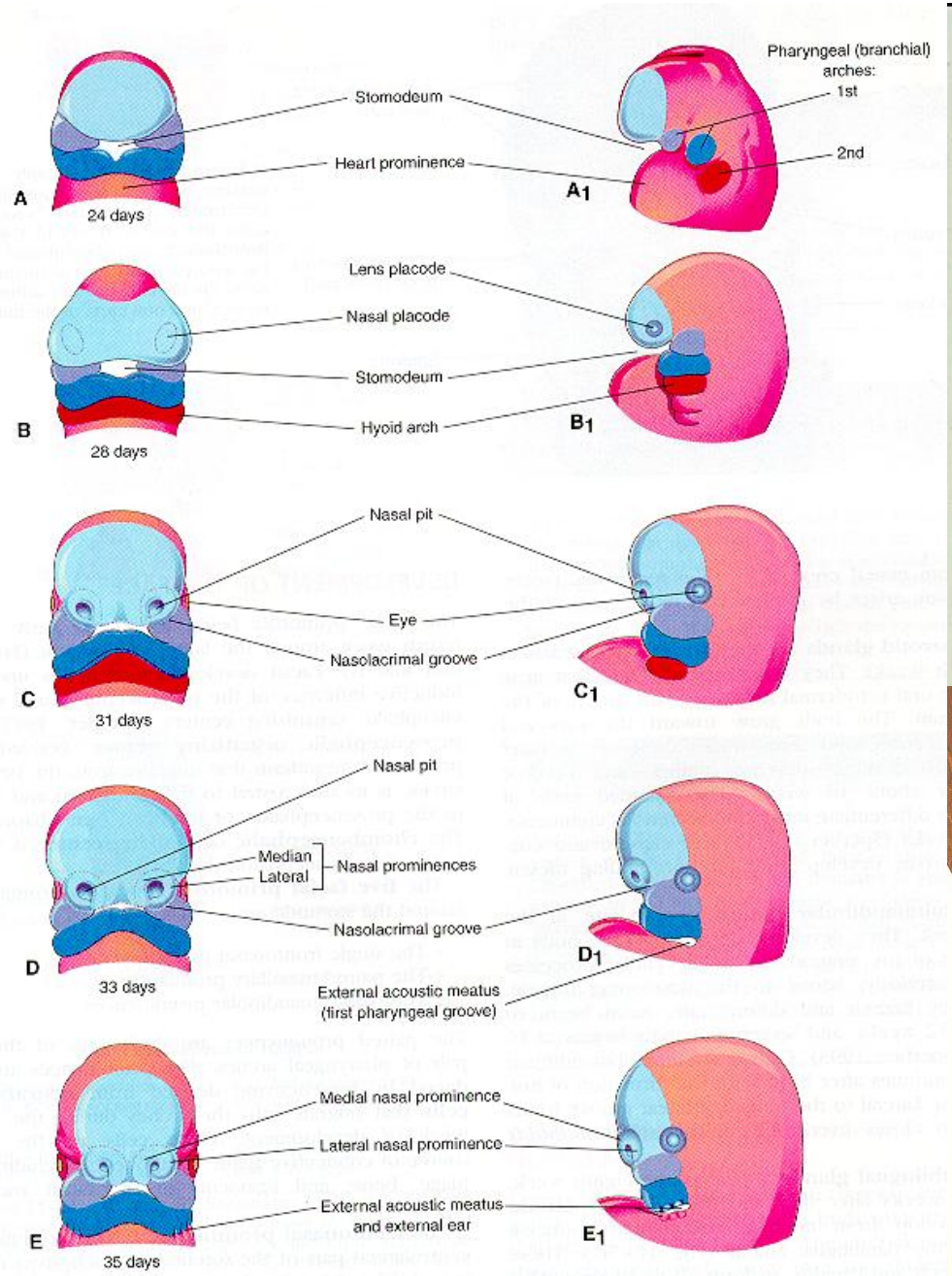
U01 DE024421 NIDCR, NIH

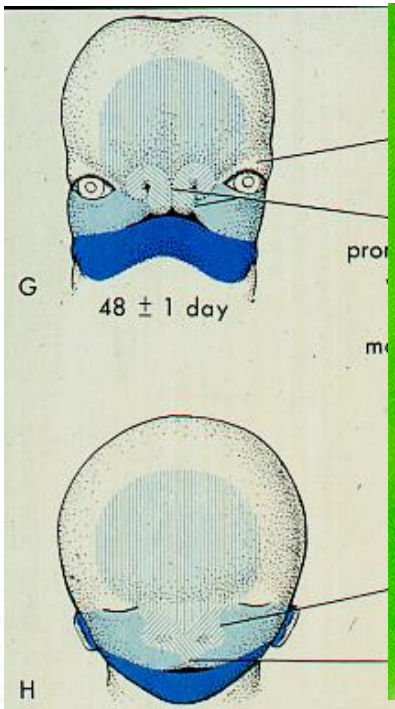
Our face is our identity

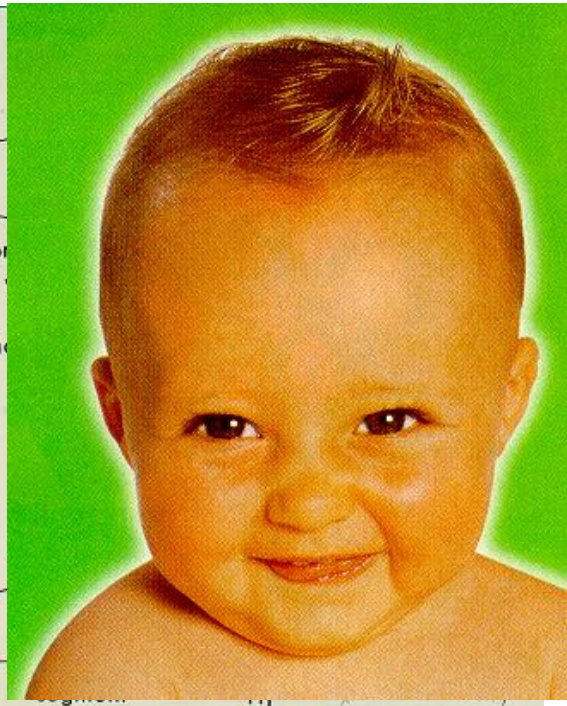
Craniofacial bones determine our facial appearance



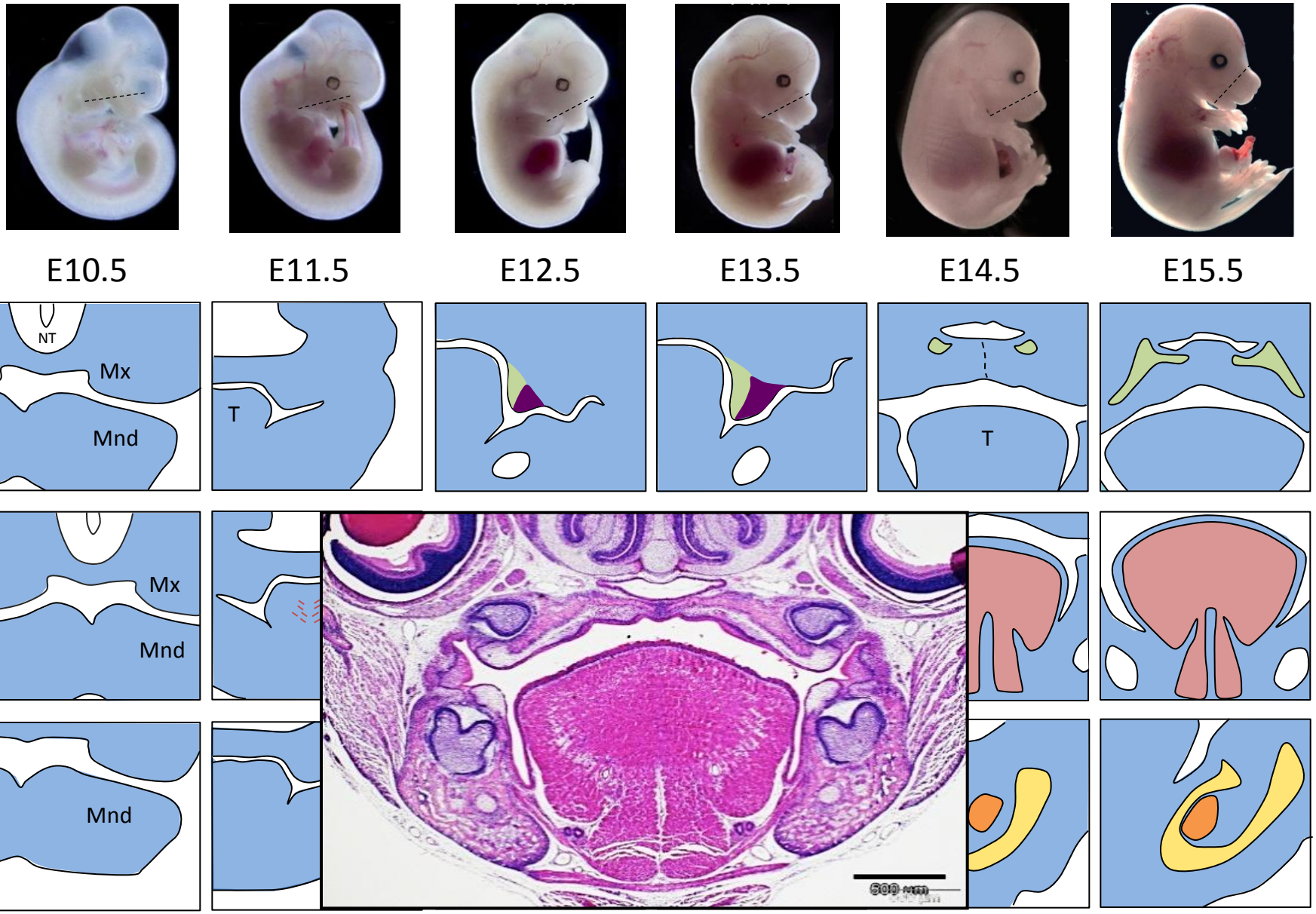
Different cellular contributions to facial development







Palate, tongue, and mandible development



Modified from Parada & Chai, FaceBase 2014

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

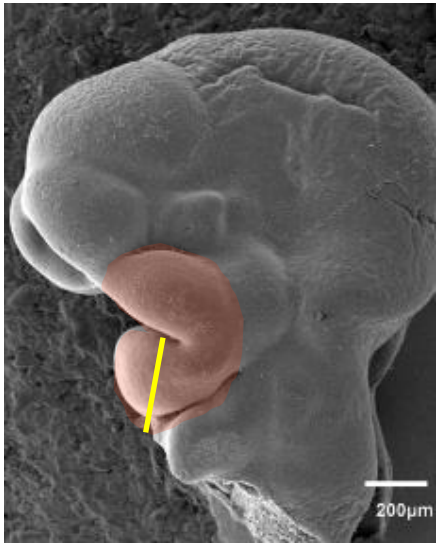
2. Gene expression, cell fate and 3D imaging during maxilla development

Our goals

1.To provide a foundation for the investigation of the molecular regulatory mechanisms of mandible/maxilla morphogenesis

2.To build collaborations for dental, oral, and craniofacial tissue/organ regeneration

Gene expression profile analyses



E10.5

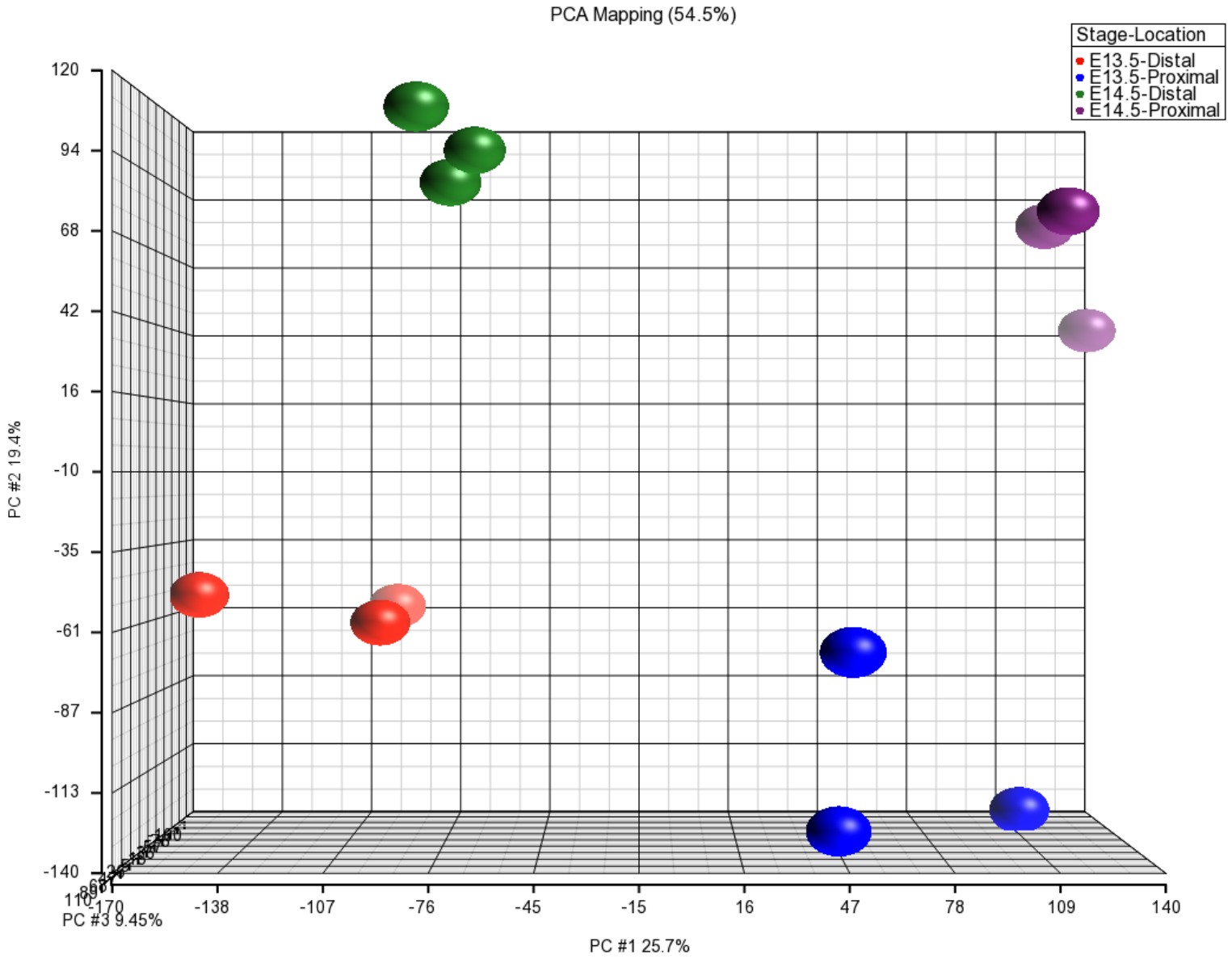
MicroArray for FaceBase2.0

	Mandible	
	Proximal	**Distal**
E10.5	3	3
E11.5	6	6
E12.5	3	3
	GSE67985	GSE67985
E13.5	3	3
	GSE67985	GSE67985
E14.5	3	3
	GSE67985	GSE67985

	Maxilla	
	Proximal	**Distal**
E10.5	3	3
E11.5	6	6
E12.5	3	3
	GSE67985	GSE67985
E13.5	3	3
	GSE67985	GSE67985
E14.5	3	3
	GSE67985	GSE67985

Queued for submission

Submitted to GEO # GSE67985



Principal Component Analysis shows good separation between mandibular sample groups

FaceBase Microarray Data

Select

- [x] E10.5_Mnd_D
- [ ] E11.5_Mnd_D
- [ ] E12.5_Mnd_D
- [ ] E13.5_Mnd_D
- [ ] E14.5_Mnd_D
- [ ] E10.5_Mnd_P
- [ ] E11.5_Mnd_P
- [ ] E12.5_Mnd_P
- [ ] E13.5_Mnd_P
- [ ] E14.5_Mnd_P
- [x] E10.5_Max_D
- [ ] E11.5_Max_D
- [ ] E12.5_Max_D
- [ ] E13.5_Max_D
- [ ] E14.5_Max_D
- [ ] E10.5_Max_P
- [ ] E11.5_Max_P
- [ ] E12.5_Max_P
- [ ] E13.5_Max_P
- [ ] E14.5_Max_P

Compare

- ( ) Distal vs. Proximal
- (•) Mandible vs. Maxilla
- ( ) E10.5 vs. E11.5
- ( ) E11.5 vs. E12.5
- ( ) E12.5 vs. E13.5
- ( ) E13.5 vs. E14.5

Absolute Fold Change Cutoff

1.5

Adjusted P-Value Cutoff

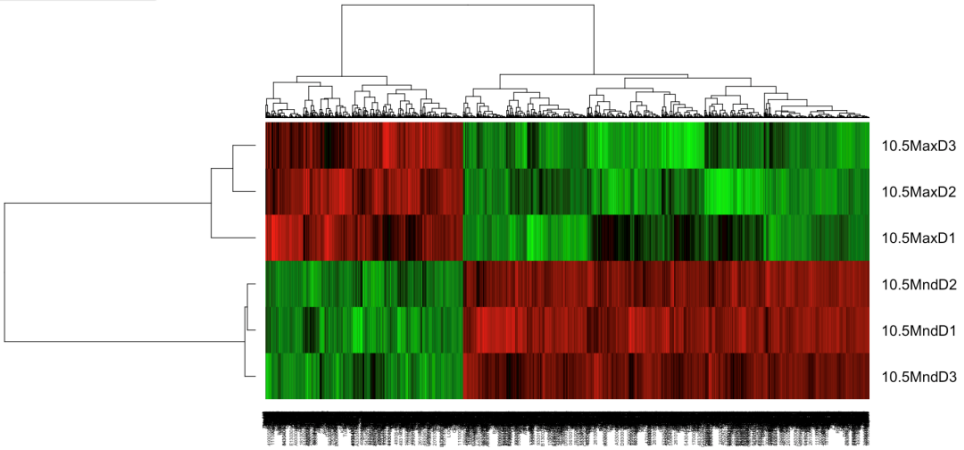
0.05

Max. Number of DE Probesets

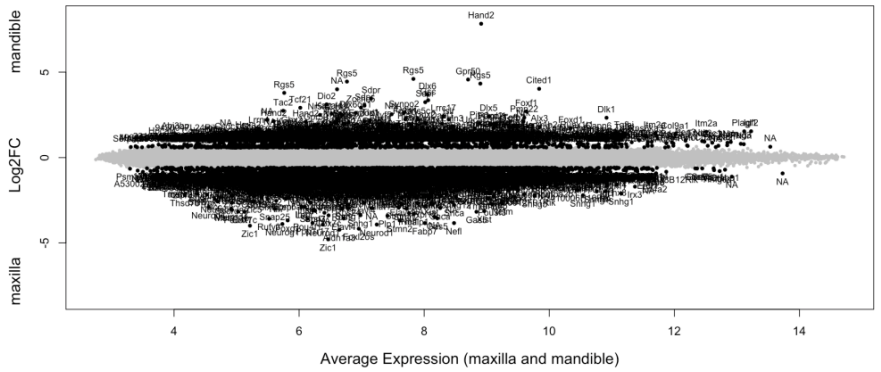
Inf

Submit (will take a few minutes the first time!)

Download heatmap



Download MA plot



Download table

	Probeset	Gene	Ave.Expr	Log2FC	FC	Adj.P	Description
1	1436041_at	Hand2	8.91	7.83	228.05	3.5e-06	heart and neural crest derivatives expressed transcript 2
2	1420940_x_at	Rgs5	7.83	4.61	24.34	5.0e-06	regulator of G-protein signaling 5
3	1455498_at	Gpr50	8.70	4.57	23.72	2.3e-03	G-protein-coupled receptor 50

Top 20 differentially expressed genes between distal region of mandible and maxilla at E10.5

Rank	Probeset	Gene	Avg. Expr.	Log2 FC	Fold change	Adj. P-Value	Description
1	1436041_at	Hand2	8.91	7.83	228.05	3.5e-06	heart and neural crest derivatives expressed transcript 2
2	1420940_x_at	Rgs5	7.83	4.61	24.34	5.0e-06	regulator of G-protein signaling 5
3	1455498_at	Gpr50	8.70	4.57	23.72	2.3e-03	G-protein-coupled receptor 50
4	1420941_at	Rgs5	6.76	4.45	21.82	6.5e-05	regulator of G-protein signaling 5
5	1417466_at	Rgs5	8.90	4.33	20.09	5.5e-06	regulator of G-protein signaling 5
6	1449031_at	Cited1	9.84	4.02	16.27	3.5e-06	Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1
7	1446812_at		6.61	4.00	15.96	2.5e-05	
8	1420942_s_at	Rgs5	5.76	3.79	13.80	2.5e-05	regulator of G-protein signaling 5
9	1452507_at	Dlx6	8.05	3.68	12.86	1.7e-03	distal-less homeobox 6
10	1416779_at	Sdpr	7.15	3.45	10.94	9.7e-04	serum deprivation response
11	1421412_at	Gsc	8.06	3.36	10.24	3.0e-03	goosecoid homeobox
12	1416778_at	Sdpr	8.02	3.24	9.46	6.3e-04	serum deprivation response
13	1418937_at	Dio2	6.44	3.11	8.65	7.5e-05	deiodinase, iodothyronine, type II
14	1443832_s_at	Sdpr	7.04	3.06	8.34	8.3e-05	serum deprivation response
15	1437355_at	Zcchc5	6.99	2.92	7.57	6.5e-05	zinc finger, CCHC domain containing 5
16	1417447_at	Tcf21	6.02	2.91	7.54	2.5e-04	transcription factor 21
17	1419411_at	Tac2	5.75	2.74	6.67	7.5e-05	tachykinin 2
18	1434939_at	Foxf1	9.63	2.70	6.51	8.3e-05	forkhead box F1
19	1435399_at	Synpo2	7.68	2.63	6.21	5.1e-03	synaptopodin 2
20	1438799_at	Dlx6os1	6.91	2.61	6.13	1.4e-04	distal-less homeobox 6, opposite strand 1

Validating our microarray data and generating new hypotheses to investigate the patterning of the branchial arch

Rank	Probeset	Gene	Avg. Expr.	Log2 FC	Fold change	Adj. P-Value	Description
1	1436041_at	Hand2	8.91	7.83	228.05	3.5e-06	heart and neural crest derivatives expressed transcript 2
2	1420940_x_at	Rgs5	7.83	4.61	24.34	5.0e-06	regulator of G-protein signaling 5
3	1455498_at	Gpr50	8.70	4.57	23.72	2.3e-03	G-protein-coupled receptor 50
4	1420941_at	Rgs5	6.76	4.45	21.82	6.5e-05	regulator of G-protein signaling 5
5	1417466_at	Rgs5	8.90	4.33	20.09	5.5e-06	regulator of G-protein signaling 5
6	1449031_at	Cited1	9.84	4.02	16.27	3.5e-06	Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1
7	1446812_at		6.61	4.00	15.96	2.5e-05	
8	1420942_s_at	Rgs5	5.76	3.79	13.80	2.5e-05	regulator of G-protein signaling 5
9	1452507_at	Dlx6	8.05	3.68	12.86	1.7e-03	distal-less homeobox 6
10	1416779_at	Sdpr	7.15	3.45	10.94	9.7e-04	serum deprivation response
11	1421412_at	Gsc	8.06	3.36	10.24	3.0e-03	goosecoid homeobox
12	1416778_at	Sdpr	8.02	3.24	9.46	6.3e-04	serum deprivation response
13	1418937_at	Dio2	6.44	3.11	8.65	7.5e-05	deiodinase, iodothyronine, type II
14	1443832_s_at	Sdpr	7.04	3.06	8.34	8.3e-05	serum deprivation response
15	1437355_at	Zcchc5	6.99	2.92	7.57	6.5e-05	zinc finger, CCHC domain containing 5
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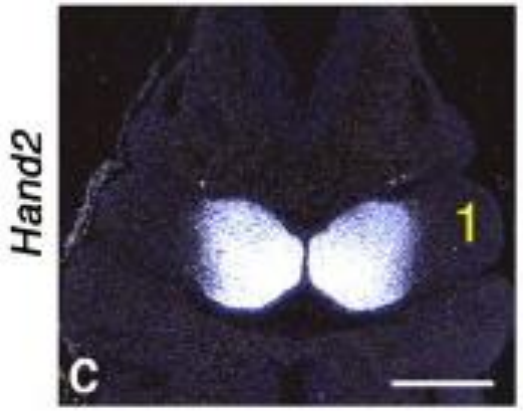
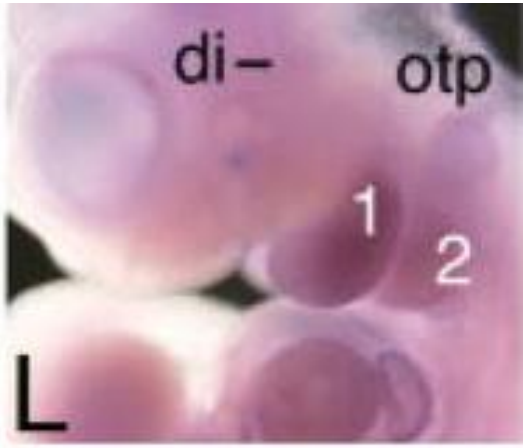
17	1419411_at	Tac2	5.75	2.74	6.67	7.5e-05	tachykinin 2
18	1434939_at	Foxf1	9.63	2.70	6.51	8.3e-05	forkhead box F1
19	1435399_at	Synpo2	7.68	2.63	6.21	5.1e-03	synaptopodin 2
20	1438799_at	Dlx6os1	6.91	2.61	6.13	1.4e-04	distal-less homeobox 6, opposite strand 1



Hand2 expression at E10.5
Barbosa et al., 2007 Dev. Biol.



Dlx6 expression at E10.5
Ruest et al., 2003, Development

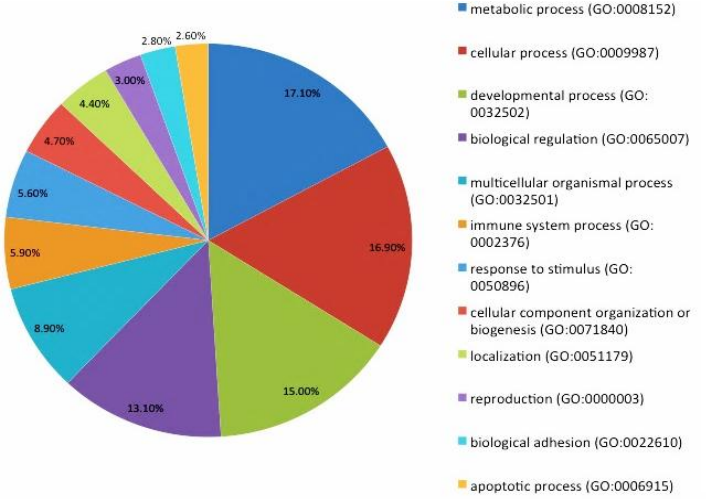
Gene expression profile along the distal-proximal axis in the developing mandible

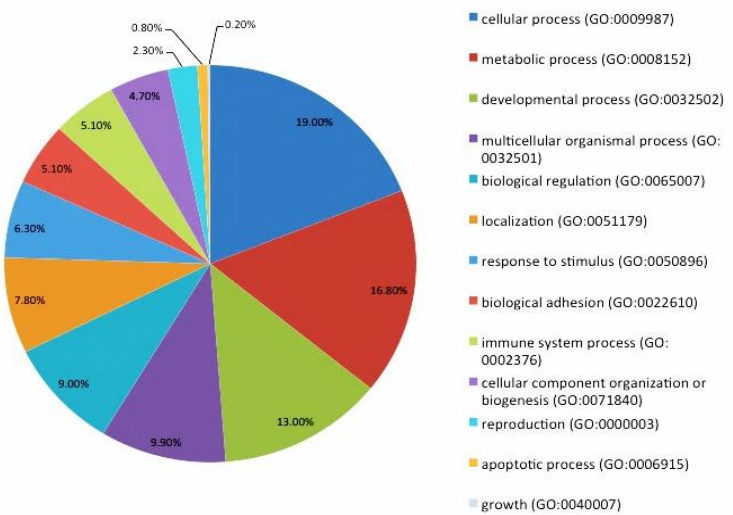
C

Downregulated genes

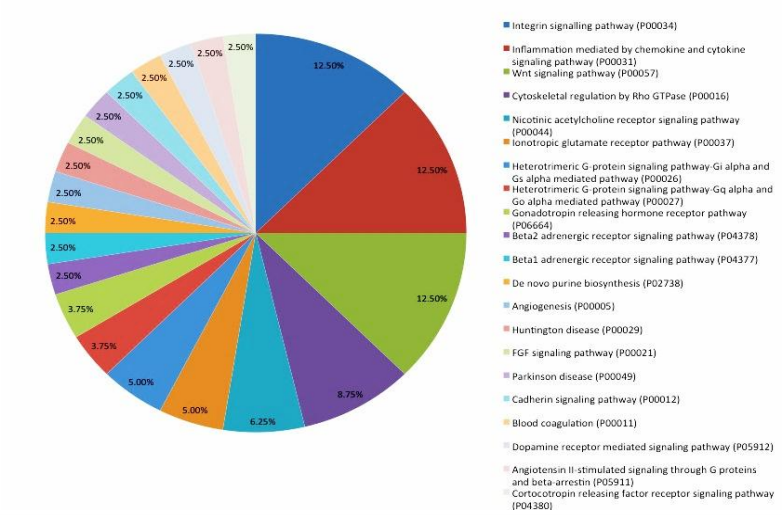
Upregulated genes

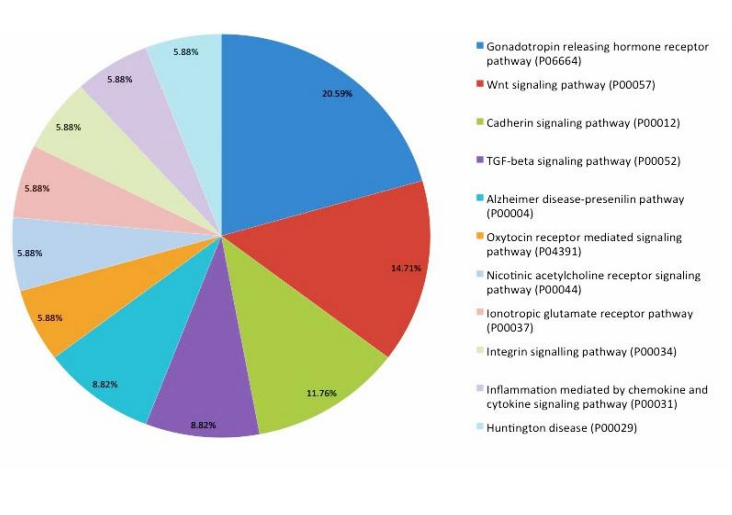
Biological processes





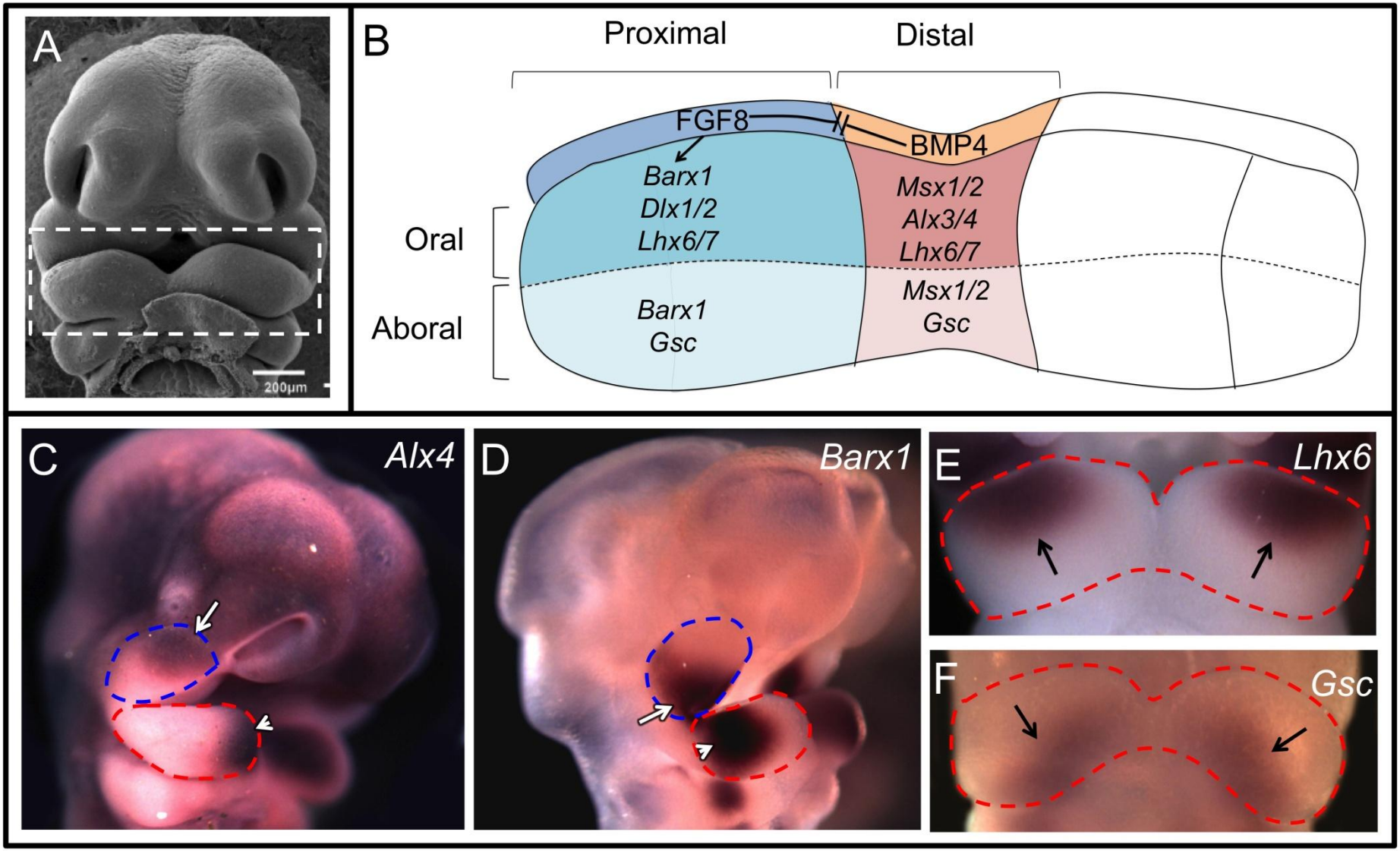
Pathways



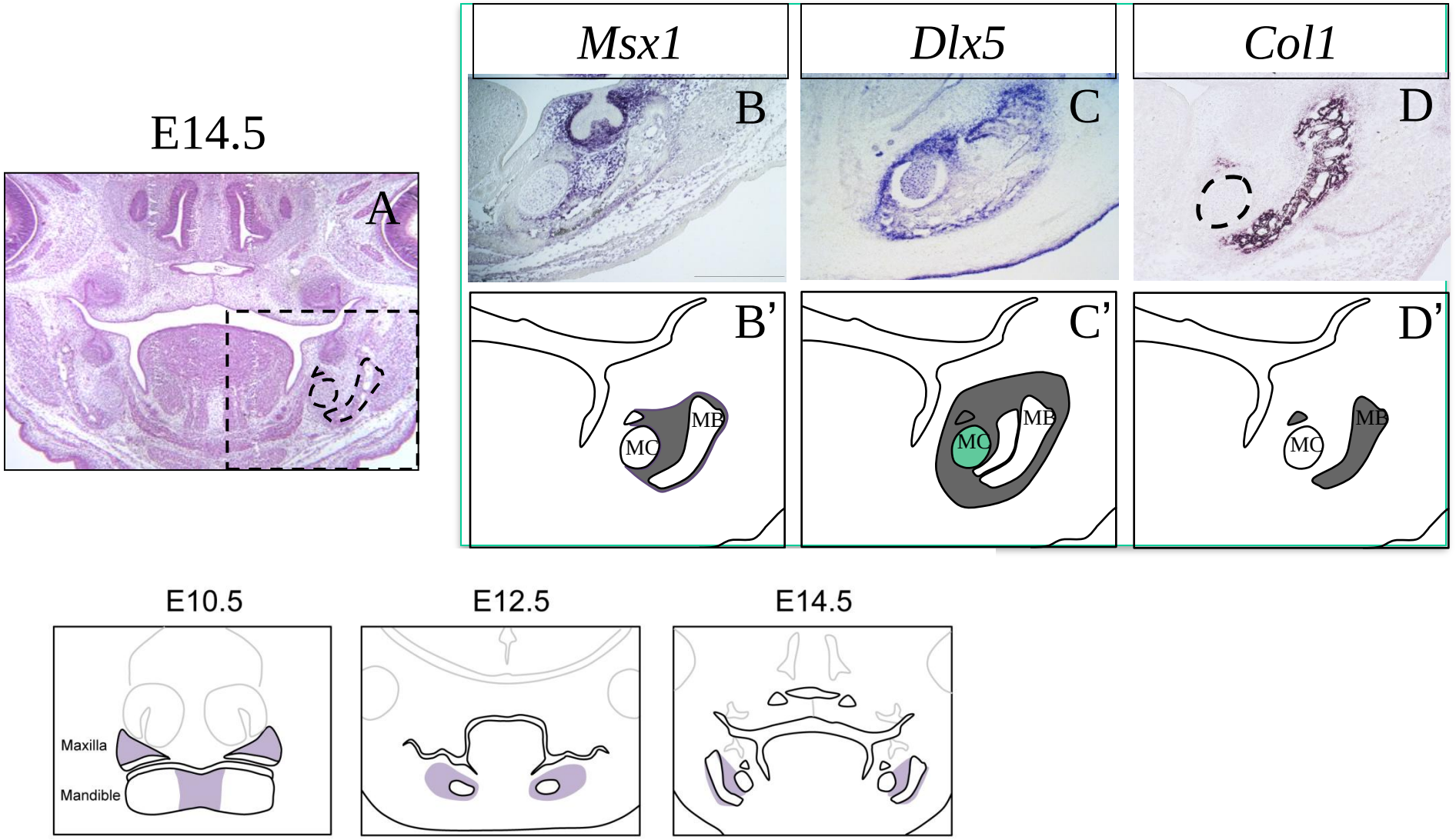


Gene expression and patterning of the branchial arch

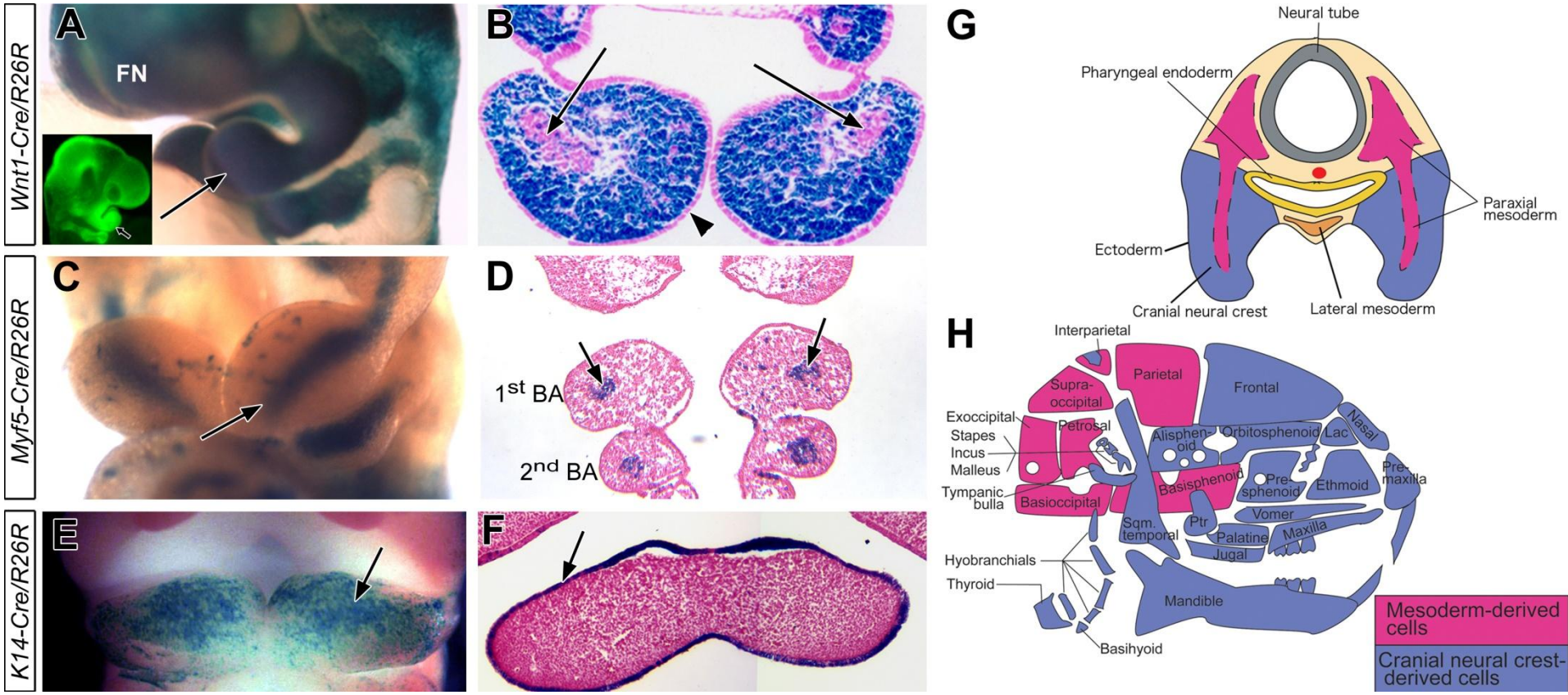
Early patterning of the maxillary and mandibular arches



Molecular identity of the proximal region of the developing mandible



Genetic cell lineage analyses



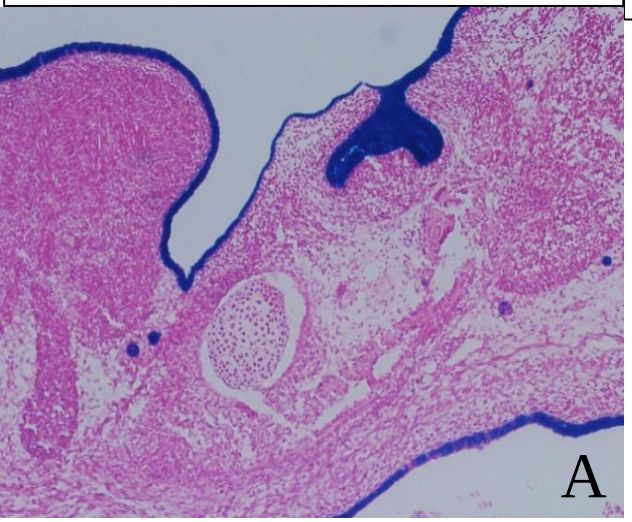
E10.5

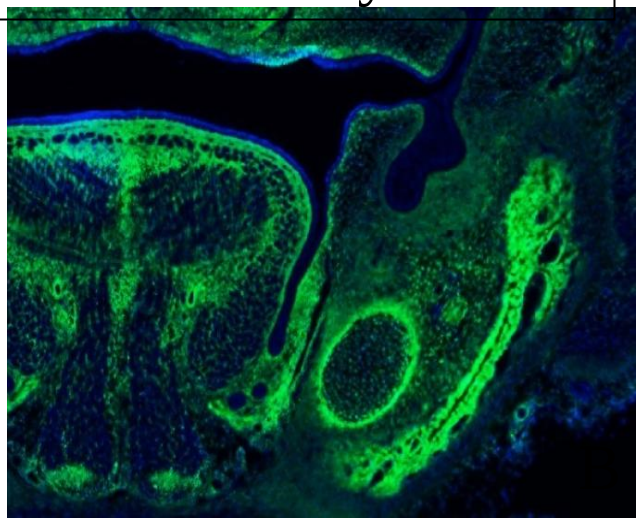
Cell lineage analysis during mandible development

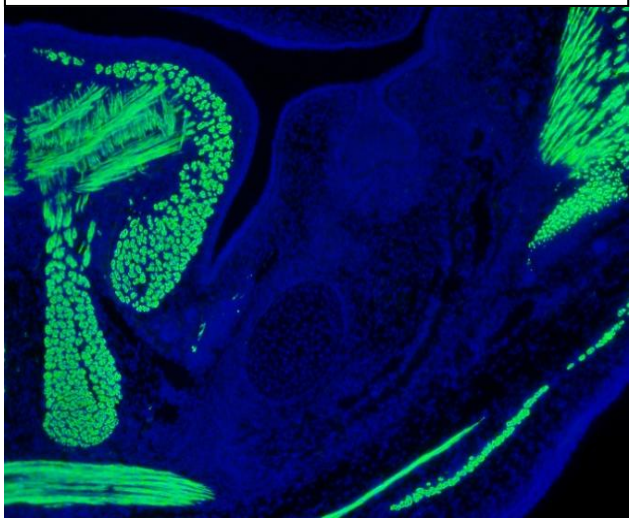
Epithelium

CNCC-derived mesenchyme

Myogenic cells







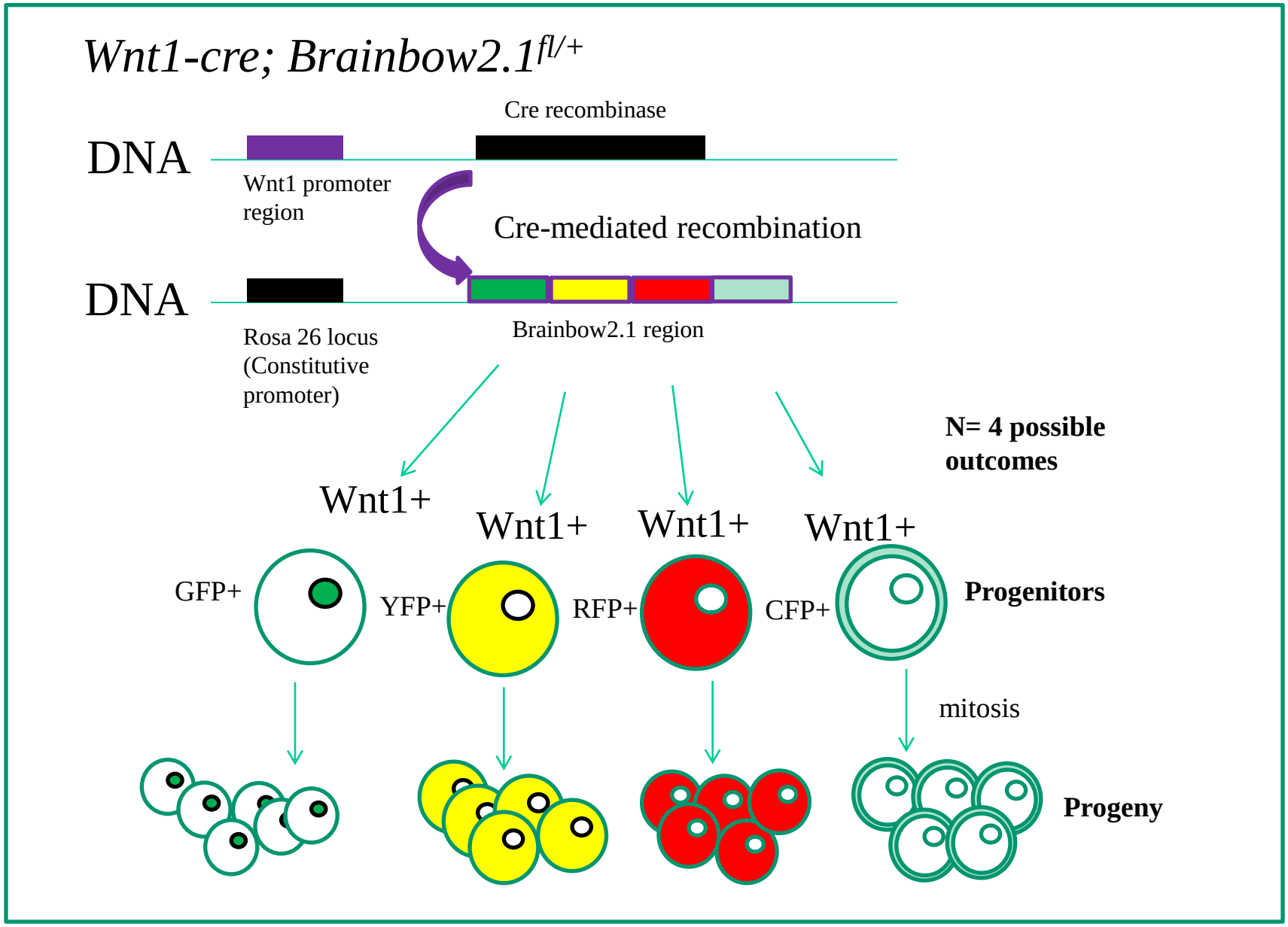
K14-Cre;R26R

Wnt1-Cre;ZsGreen

Myf5-Cre;ZsGreen

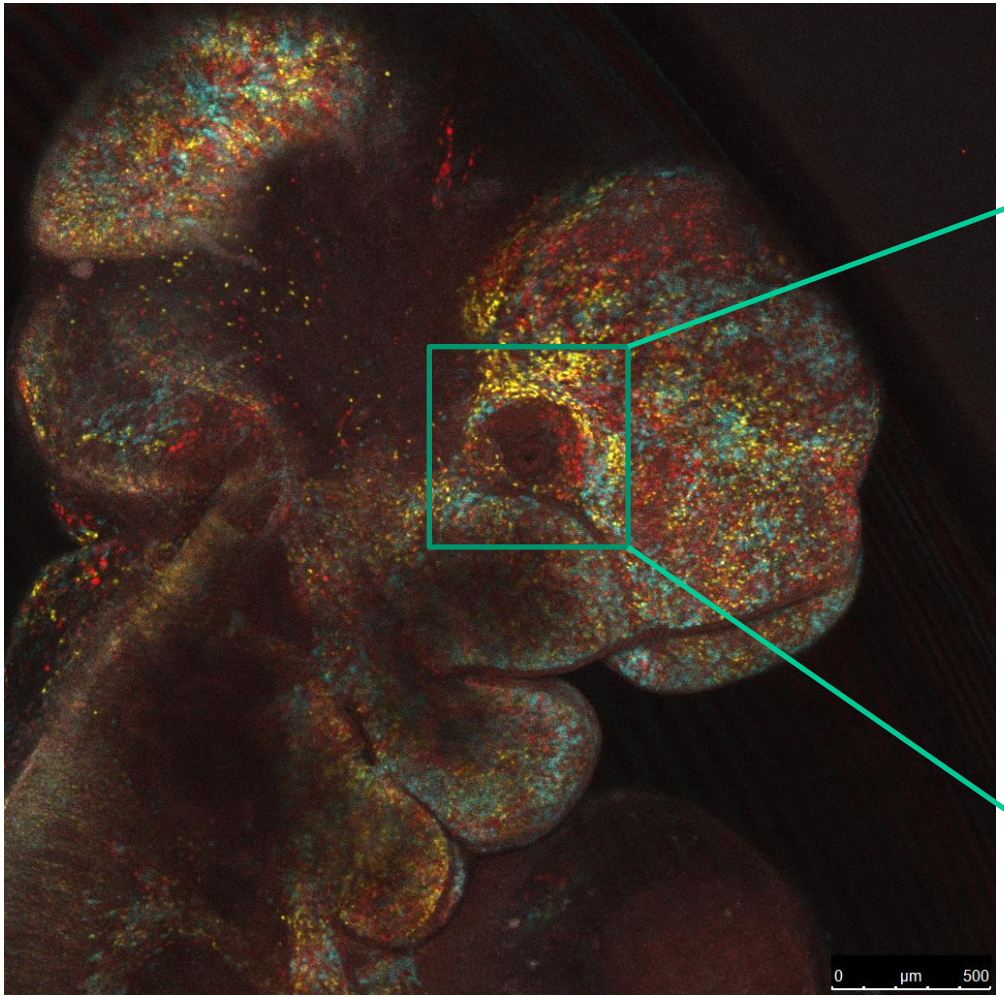
E14.5

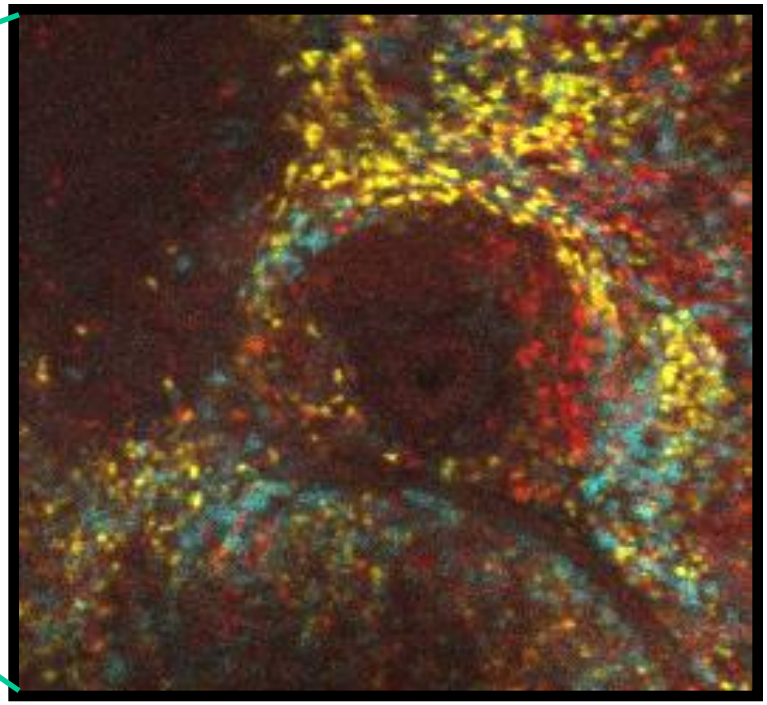
Cell lineage tracing using Brainbow mice: an opportunity to trace a single progenitor cell and its progeny through tissue-specific multicolor stochastic recombination



E10.5

Wnt1-cre; Brainbow2.1$^{fl/+}$





N= 4 in *Brainbow2.1$^{fl/+}$*

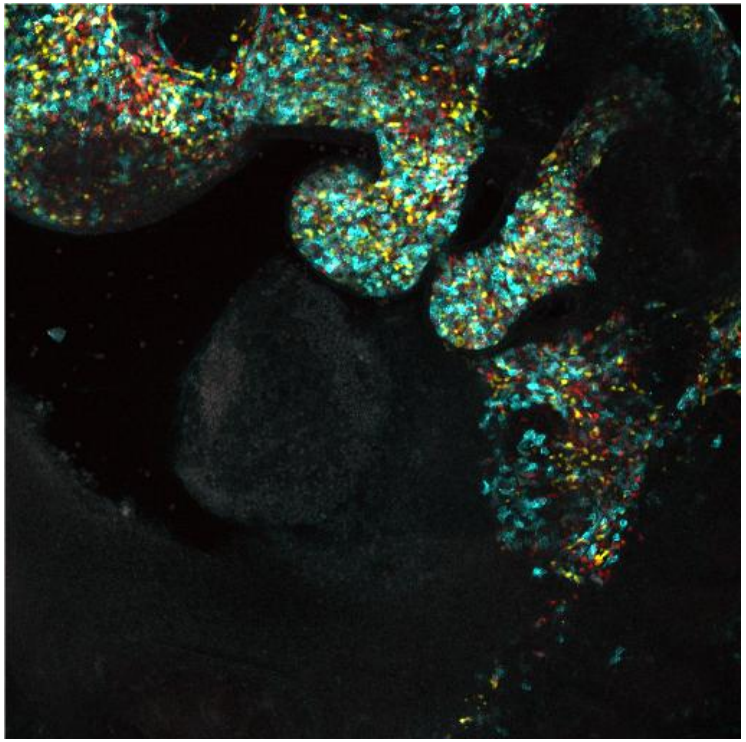
p= probability that 2 adjacent cells of the same color derive from the same progenitor

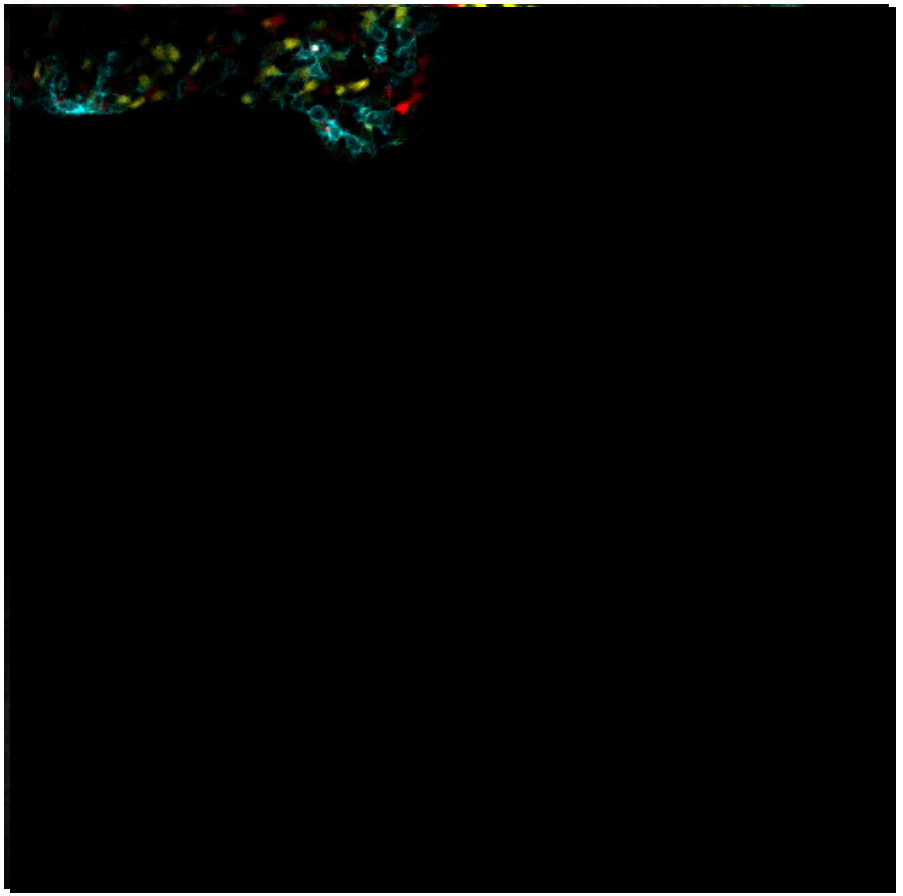
If n=High then p=High
If n=Low then p= Low

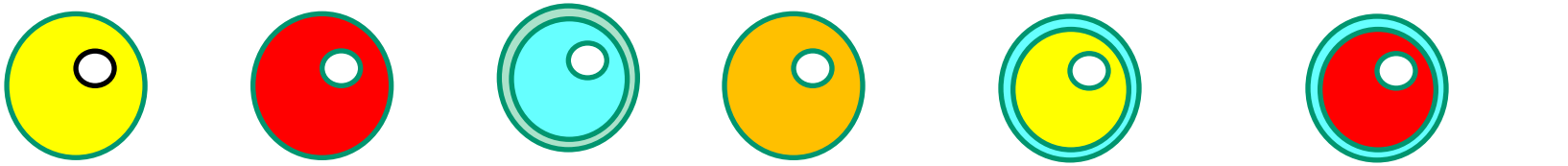
n=number of possible recombination

Wnt1-cre; Brainbow2.1$^{fl/fl}$

E9.5

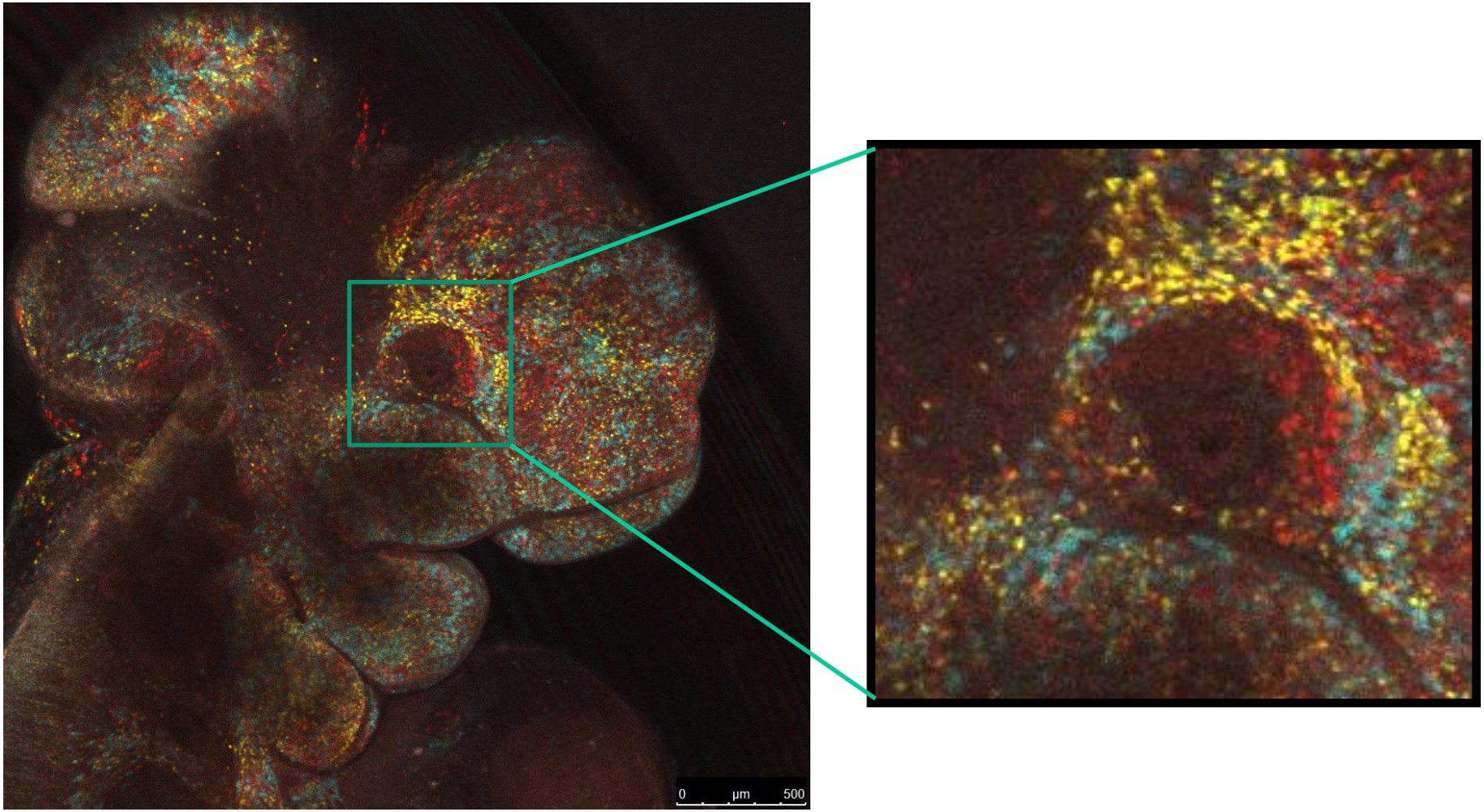






Superficial structure: the eye

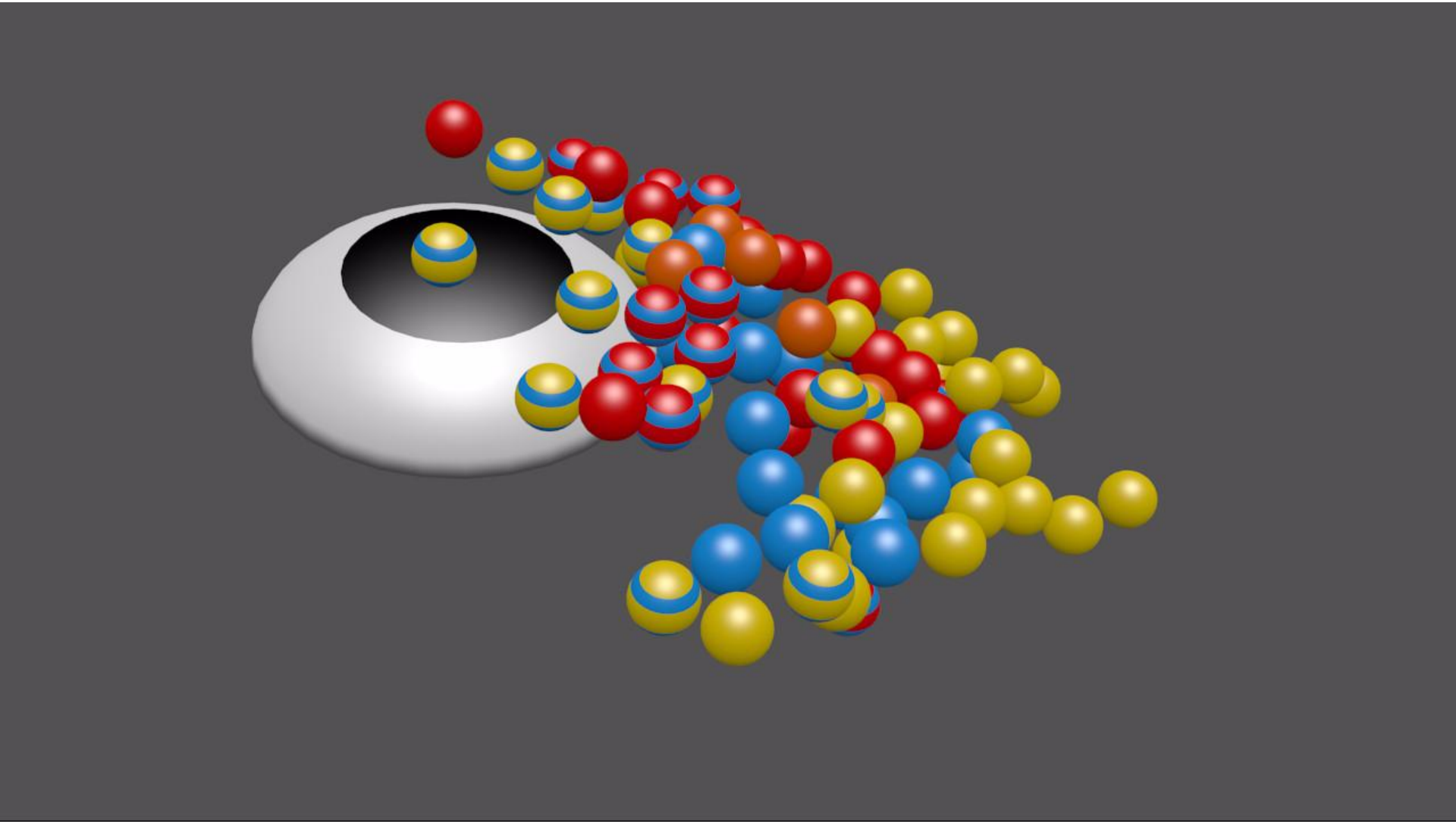
E10.5



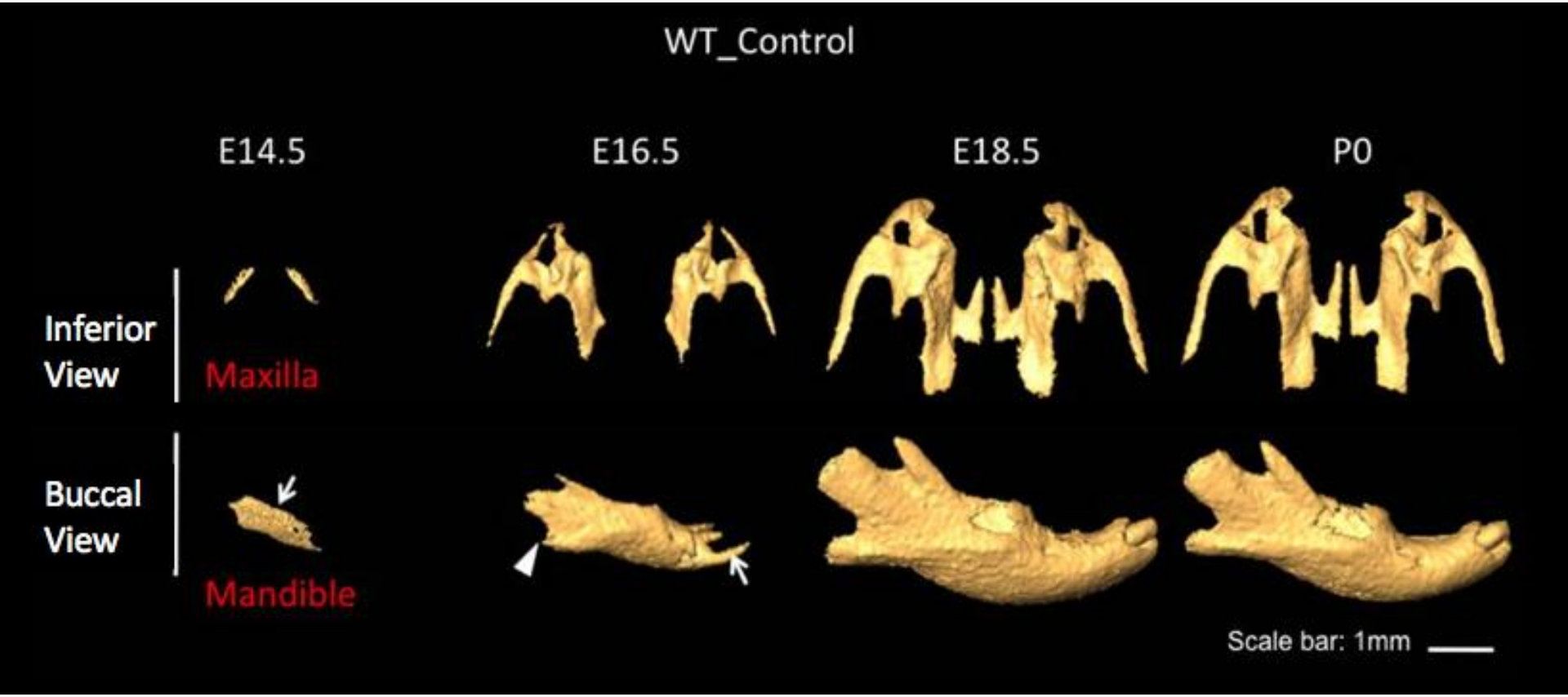
Same progenitor?

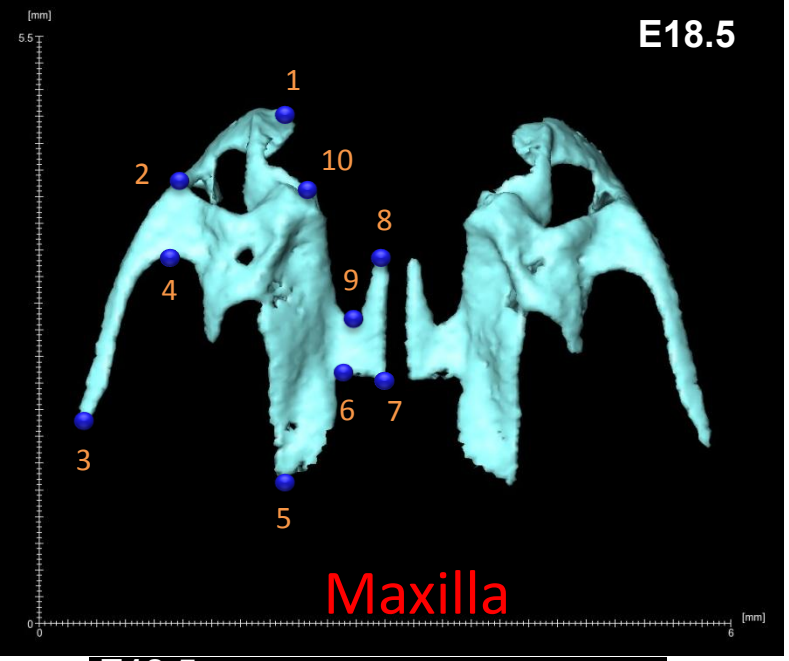
E9.5

Superficial structure: cells above the eye

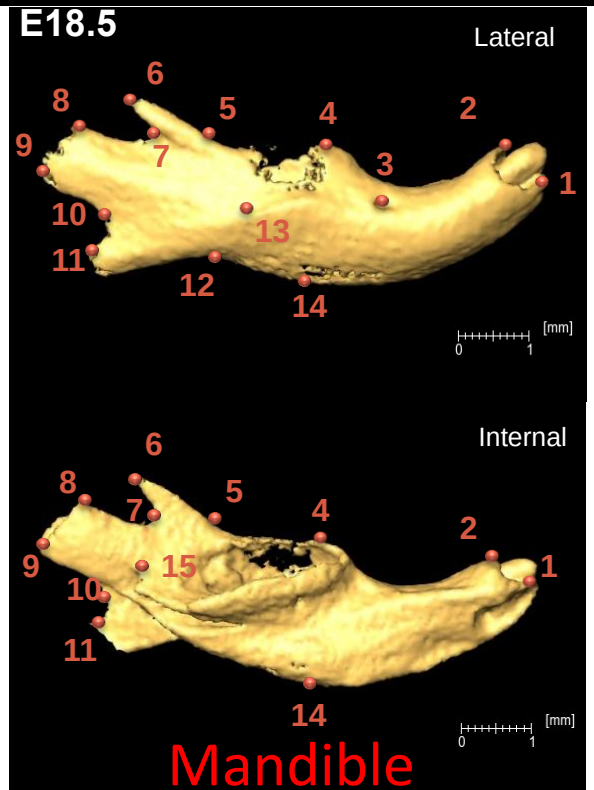


Morphogenesis of maxilla and mandible



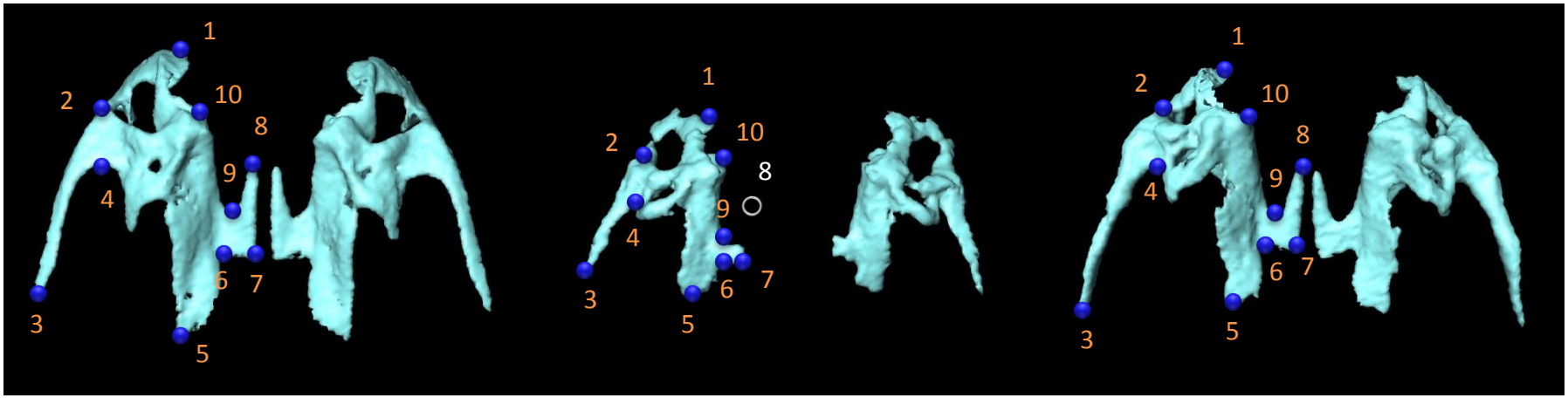


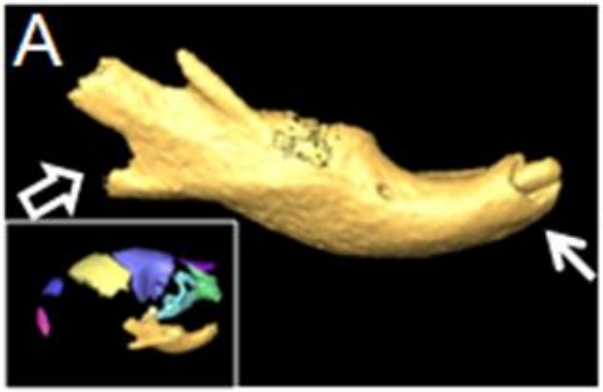
1. Anterior point of maxilla
2. Lateral inferior intersection of frontal and zygomatic process
3. Junction point of zygoma with zygomatic process
4. Anterio-medial point to zygomatic process
5. Posterior point of maxilla
6. Posterior-lateral point of the palatine process
7. Posterior-medial point of 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

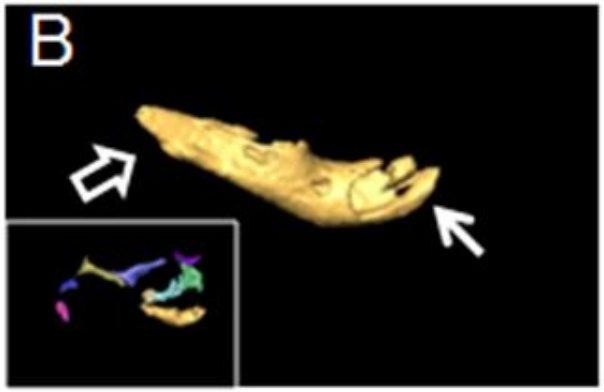


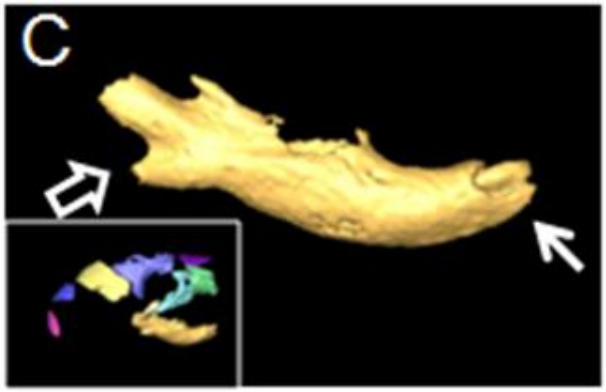
1. Most anterior point of mandible
2. Anterior-superior point of mandible
3. Mental foramen
4. Molar alveolus of dentary
5. Anterior junction of mandibular ramus and body
6. Superior tip of coronary process
7. Most inferior point of mandibular notch
8. Anterior point of condylar process
9. Posterior 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

Control *Wnt1Cre;Tgfbr2$^{fl/fl}$* *Wn1Cre;Tgfbr2$^{fl/fl}$;Tgfbr1$^{fl/+}$*





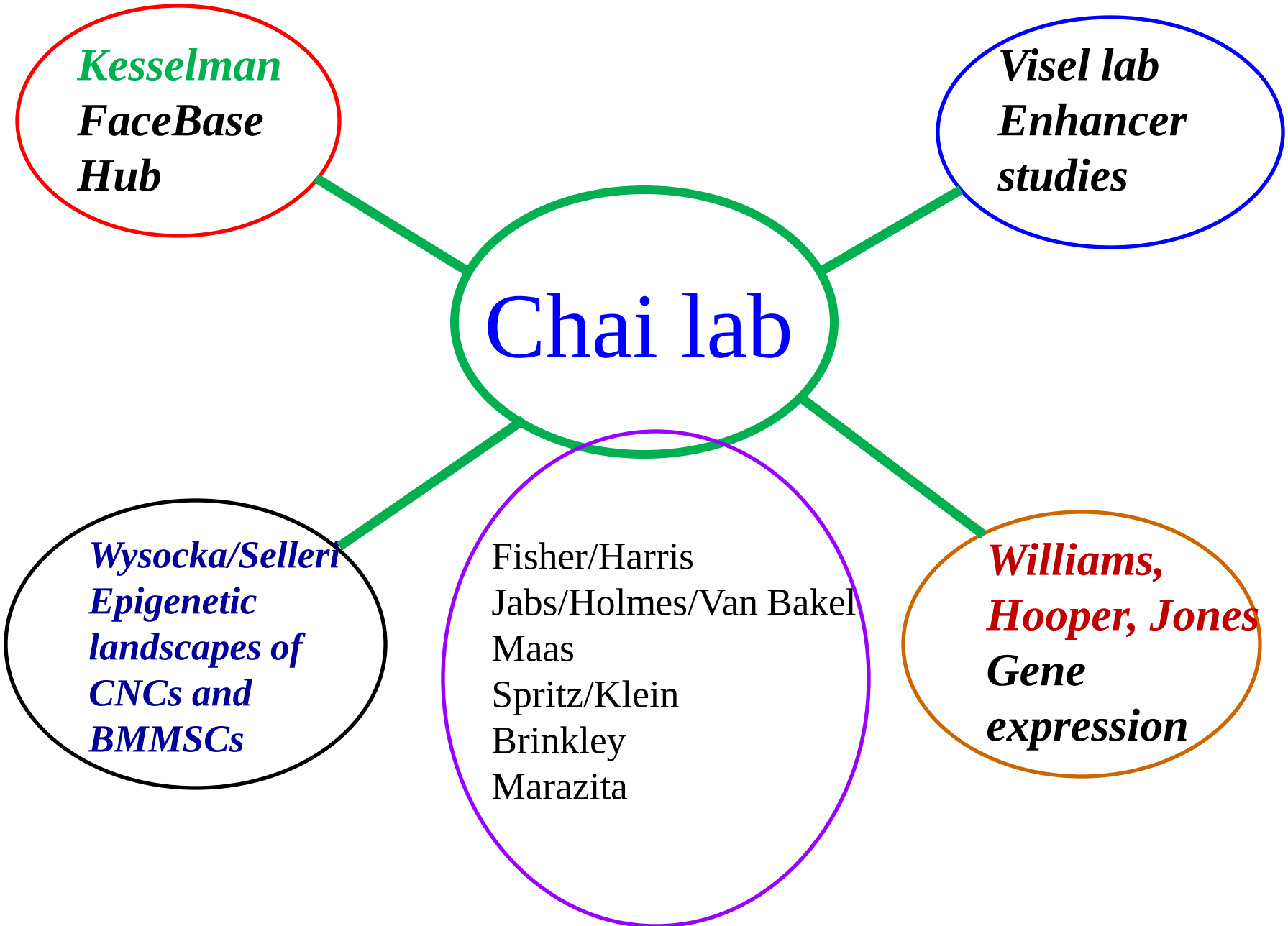




OUR MILESTONES

MILESTONES	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**
SA 1-1: Microarrays (mandibles)	50 control arrays		75 mutant arrays		
SA 1-1: In situ analysis (mandible)	8 genes	7 genes	7 genes	7 genes	2 genes
SA 1-2: Cell lineage tracing (mandible)	24 controls				
SA 1-2: 3D imaging (mandible)	12 controls		36 mutants		
SA 2-1: Microarrays (maxillas)	10 control arrays		20 mutant arrays		
SA 2-1: In situ analysis (maxilla)	7 genes	7 genes	7 genes		
SA 2-2: Cell lineage tracing (maxilla)	same as in SA1-2				
SA 2-2: 3D imaging (maxilla)	12 controls		36 mutants		
SA 2-2: Heat maps (mandible + maxilla)	4 controls	12 mutants			

Collaborations and interactions



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