Craniofacial Transcriptional Enhancers
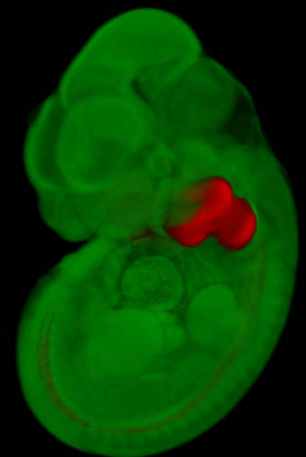
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Project Update
Annual Investigator Meeting 2013
Iowa City

Axel Visel

Genomics Division

Lawrence Berkeley National Laboratory

Visel Lab - Outline

Overview

Project Background
Data Available to Date – Summary

OPT Viewer

with Hub Team, Satya, et al.

Enhancer Cleft-Associated Variant testing

IRF6 enhancer Brazil mutation (with Brian Schutte et. al)

Enhancer Deletion Studies

with Benedikt Hallgrimsson

ERNEST ORLANDO LAWRENCE
BERKELEY NATIONAL LABORATORY

Project Overview

MOTIVATION

Non-coding sequences (distant-acting enhancers) play a role in many human traits and disease

They are difficult to study and characterize

GOALS

Map craniofacial enhancers in the genome to understand their role in normal development, birth defects, and to interpret human genetic studies
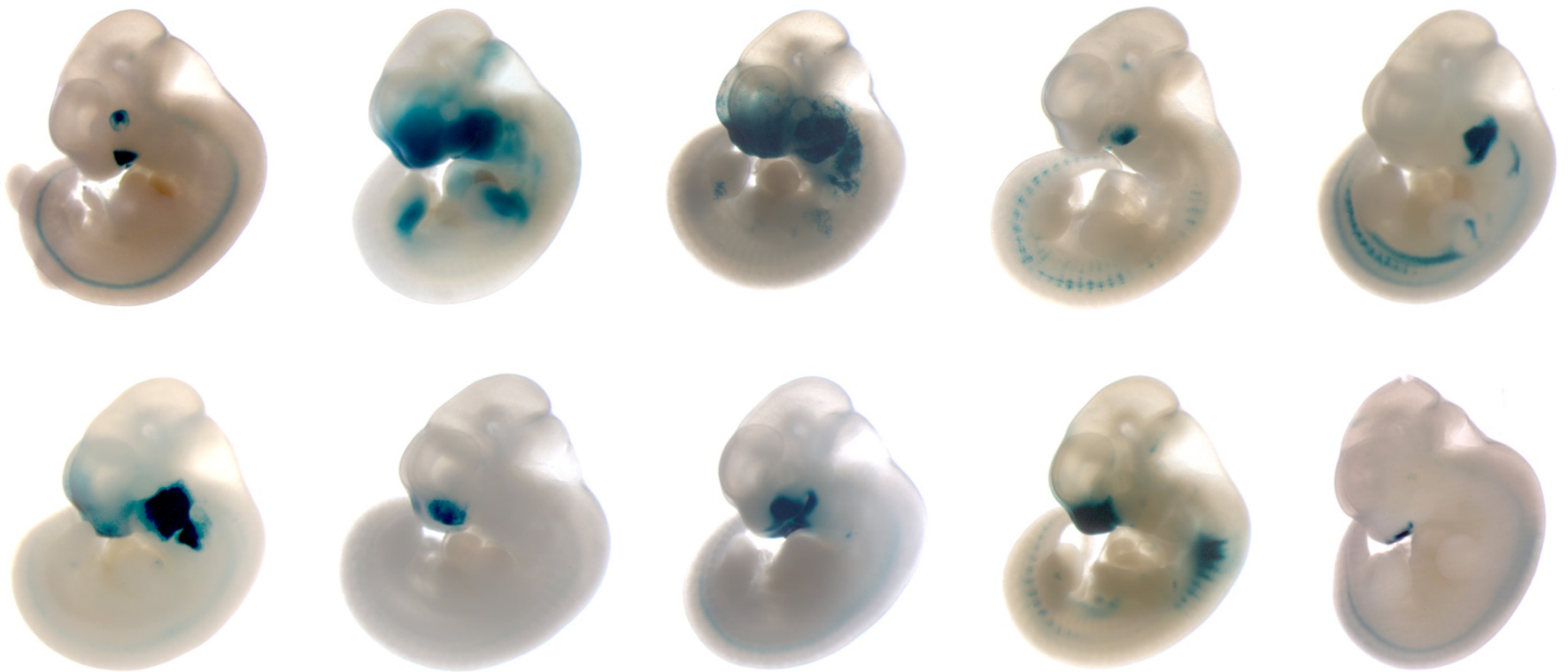
Study subsets in transgenic mice to understand their activities in detail

Make results community-accessible through FaceBase

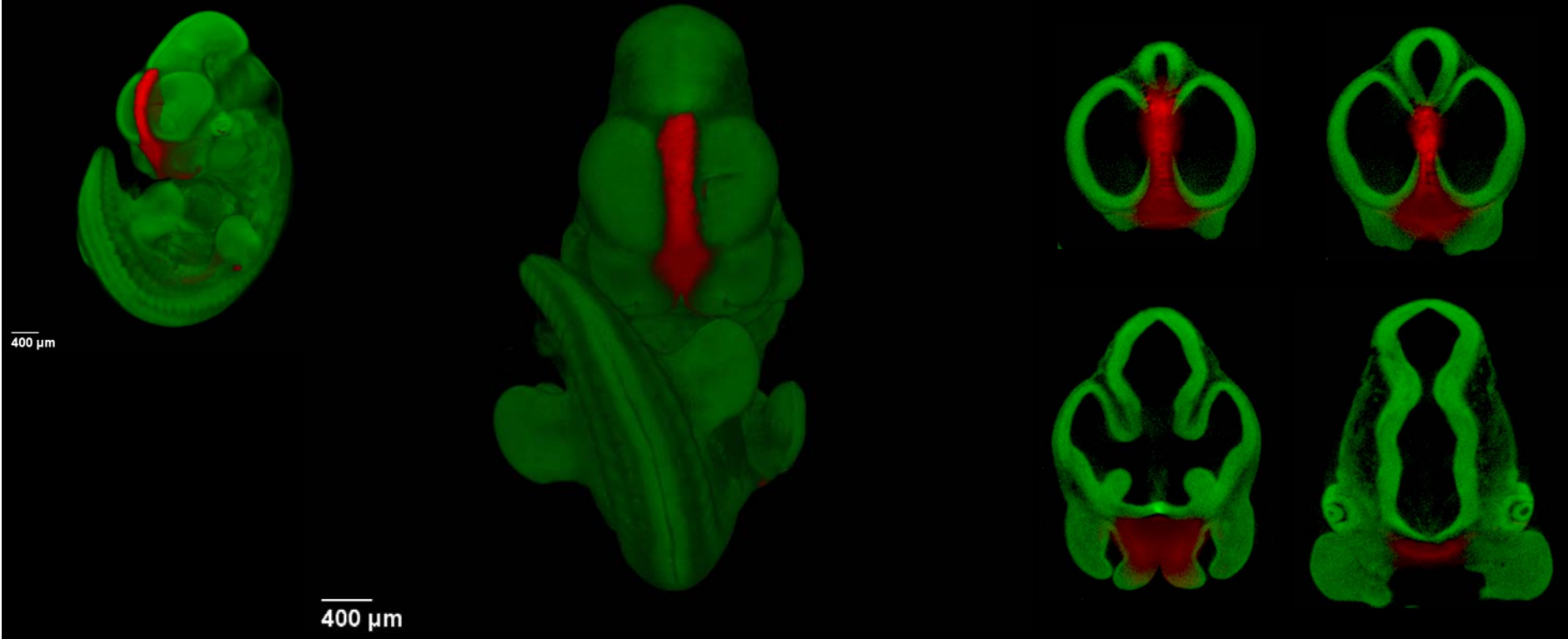# Approach: Enhancer mapping by tissue-ChIP-seq
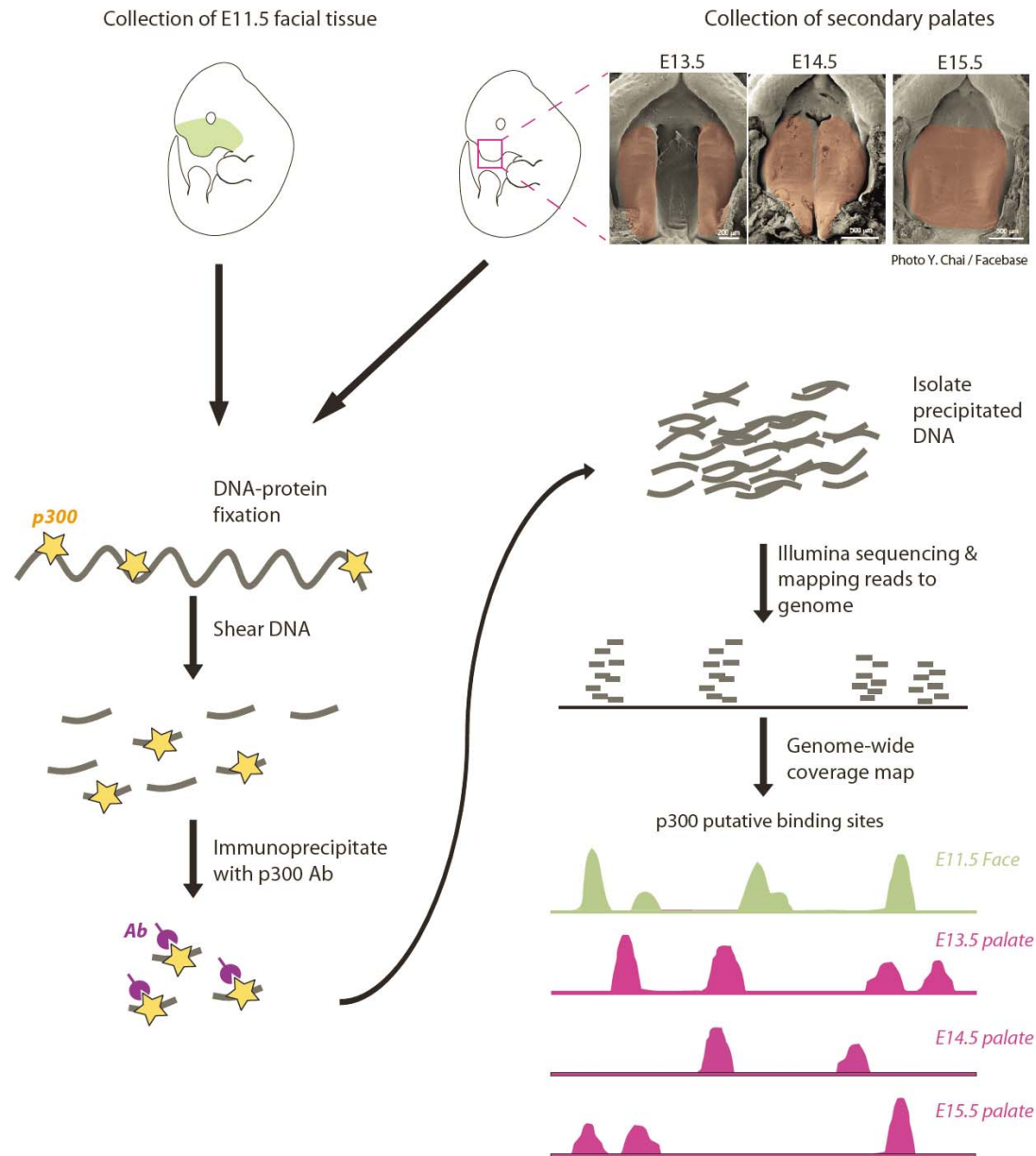
Transgenic Testing of Candidate Enhancers for Craniofacial *In Vivo* Activities
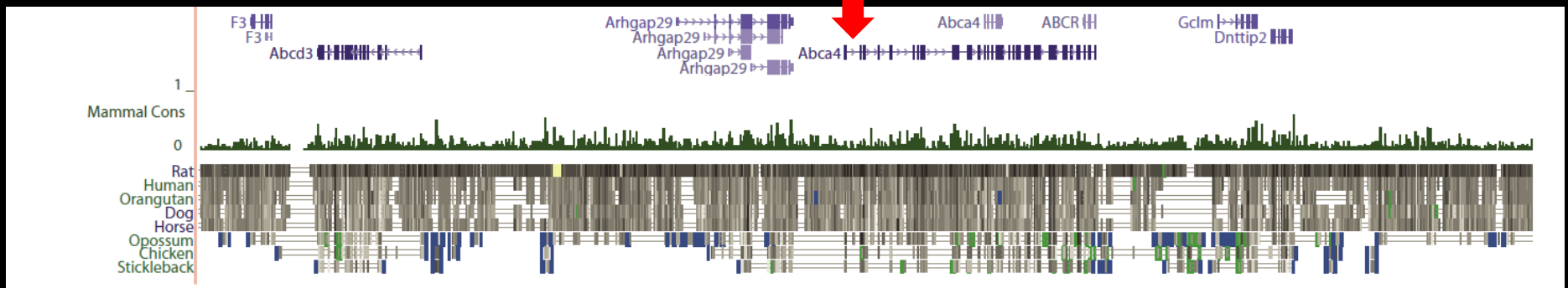

10 Example Enhancers (just one representative embryo each)

Optical Projection Tomography (with D. FitzPatrick)

Enhancer
mCF121

Overview of Data Available (May 2013)

p300 ChIP-seq
- e11.5 - whole-face (on hub)
- e13.5 - 2nd palate (on hub)
- e13.5 - 2nd palate replicate (on hub)
- e14.5 - 2nd palate (on hub)
- e15.5 - 2nd palate – failed QC, repeat collection in progress

RNA-seq
- e11.5 - whole-face (on hub)
- e12.5 - 2nd palate (on hub)
- e13.5 - 2nd palate (on hub)
- e13.5 - 2nd palate replicate (on hub)
- e14.5 - 2nd palate (provided to hub, upload in progress)
- e15.5 - 2nd palate (provided to hub, upload in progress)

new tracks since last meeting

Transgenic experiments to date (e11.5/e13.5)

111 elements total tested (95/16)

↳ **63 were reproducible enhancers** (in some tissue, 59/4)

  ↳ **41 positive in craniofacial structures** (37/4)

    ↳ **40 OPT scanned** (37/3)

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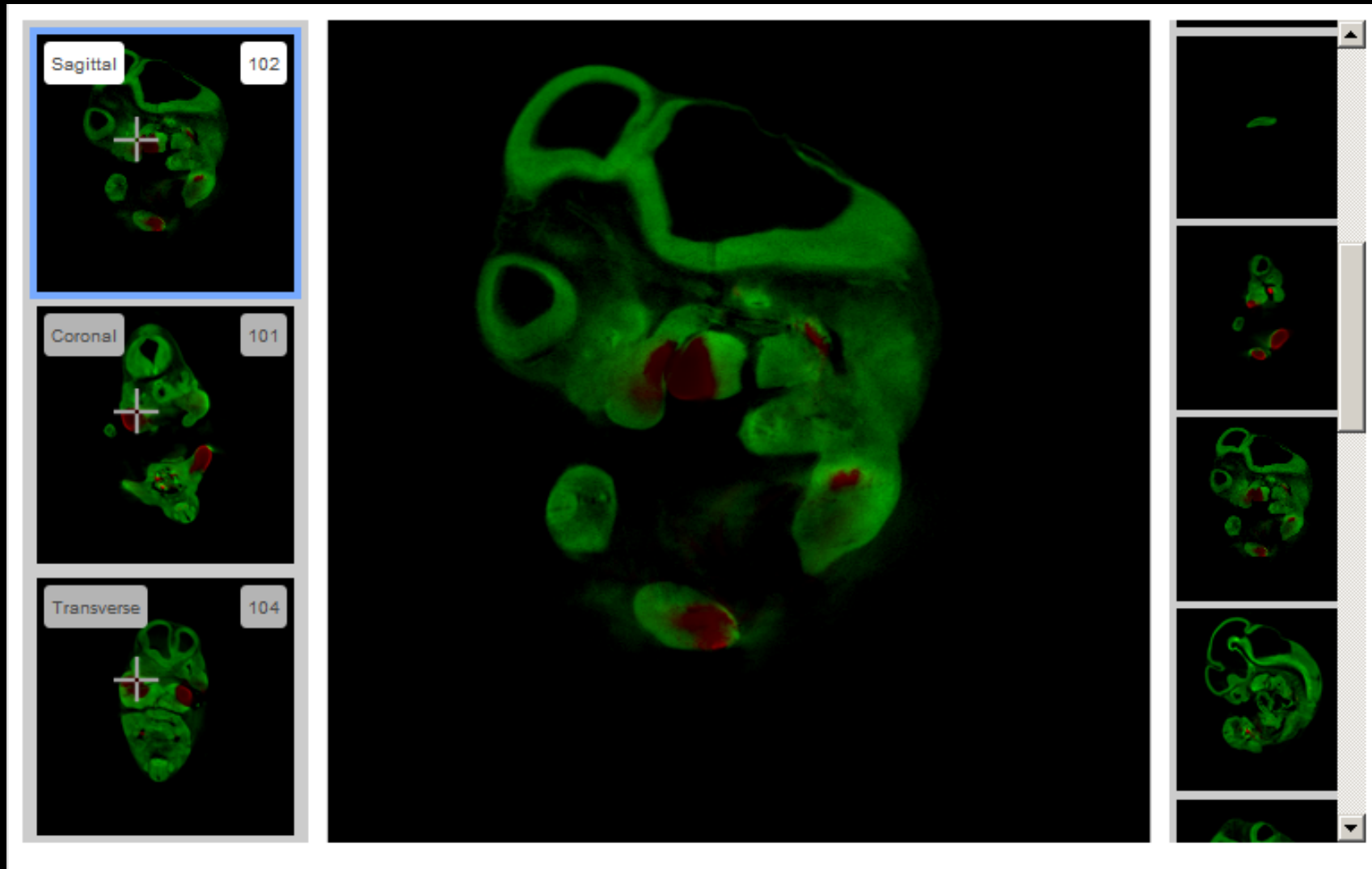BERKELEY NATIONAL LABORATORY

New Hub Feature: OPT Viewer

(with Jan Harkes, M. Satyanarayanan, Harry Hochheiser, Shiyi Shen)

New Hub Feature: OPT Viewer
(with Jan Harkes, M. Satyanarayanan, Harry Hochheiser, Shiyi Shen)

Example: mCF144

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Variant testing: IRF6 enhancer

2008 Nature Genetics (Rahimov et. al)
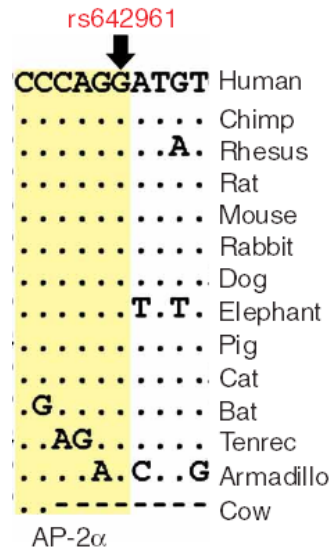
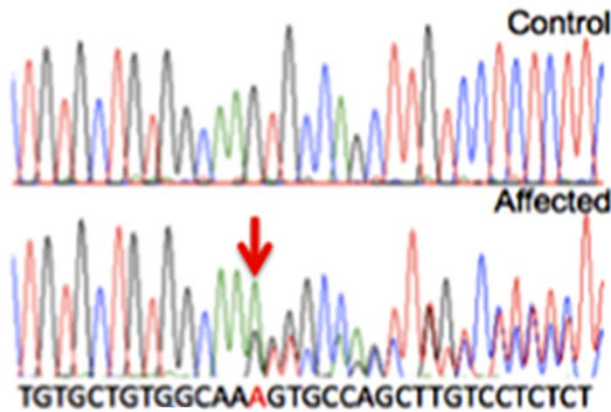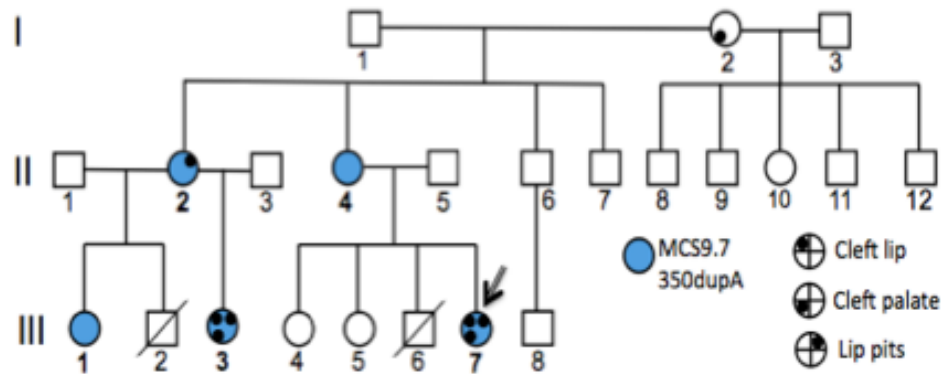SNP disrupts conserved AP-2α binding site (EMSA)
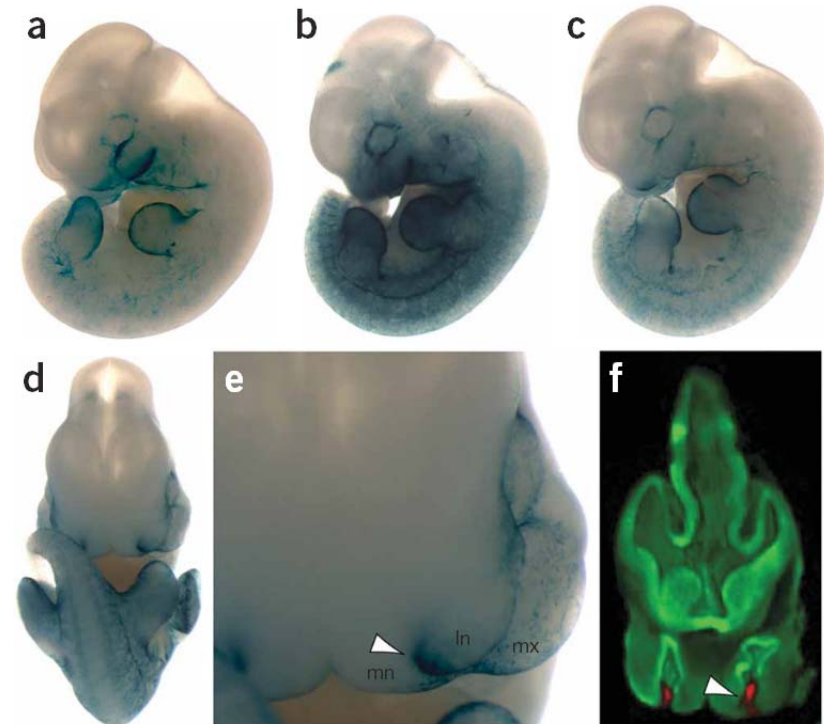
MCS-9.7 enhancer of IRF6

Revisiting the MCS-9.7 enhancer of IRF6
(with Walid Fakhouri, Fedik Rahimov, Renata de Lima, Temis Felix, Jeff Murray, Brian Schutte)

Pedigree (Brazil)

- Van der Woude Syndrome

- No IRF6 coding mutations

- sequencing identifies a new variant in IRF6 MCS-9.7 enhancer

- insertion *350dupA* abolishes activity in cell culture

- *350dupA* disrupts a p63 binding site in vitro

- also disrupts p63 binding at a 2nd site ~60bp away (presumptive gain-of-function, see Brian for details)

Question:
IN VIVO Effects of *350dupA* ???

A new variant that disrupts MCS-9.7 in vivo activity

(with Walid Fakhouri, Fedik Rahimov, Renata de Lima, Temis Felix, Jeff Murray, Brian Schutte)

one representative embryo each
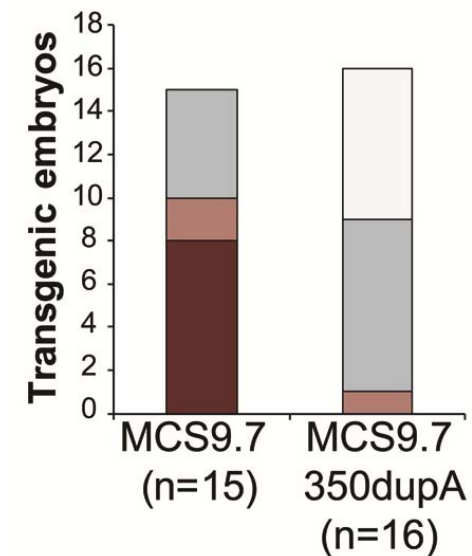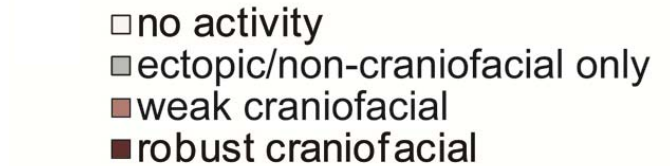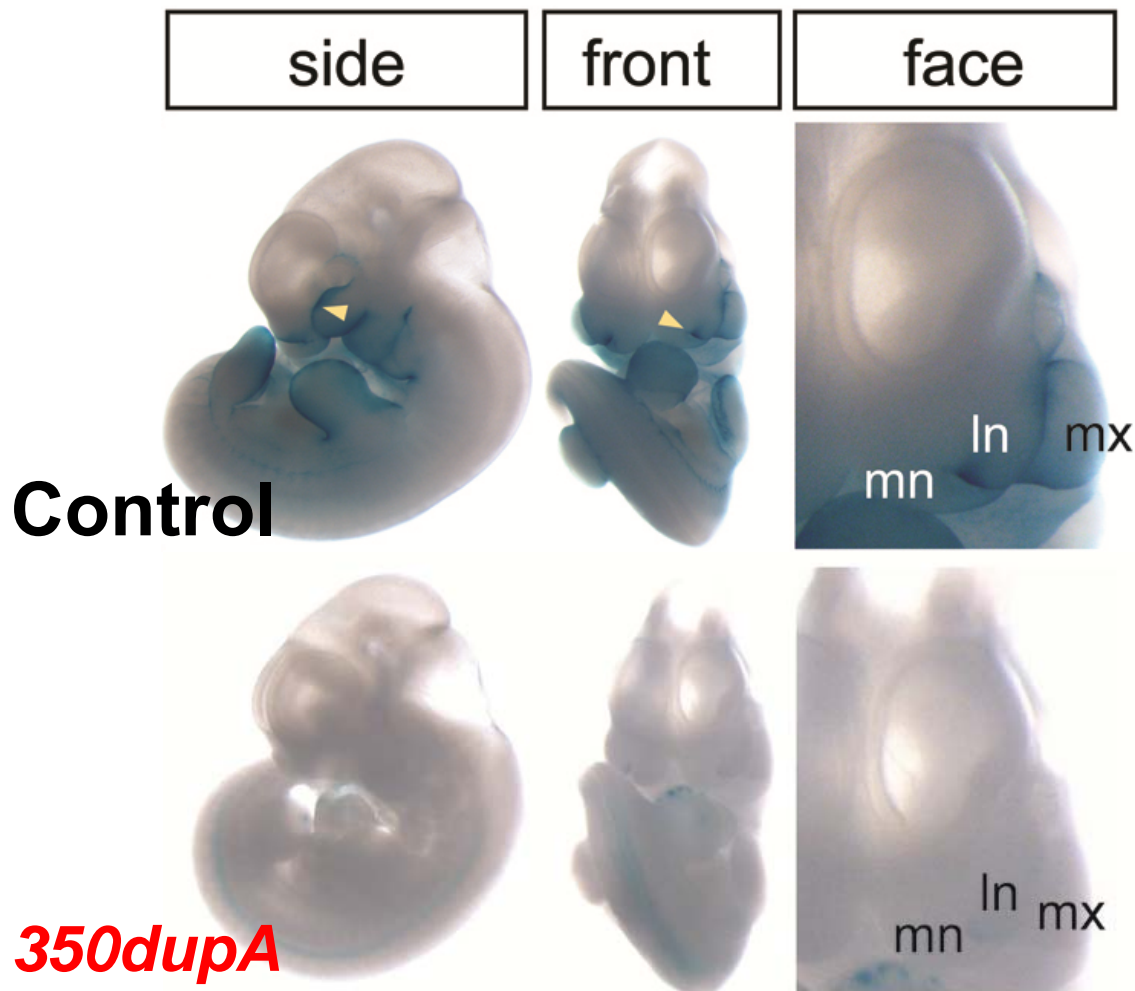
quantitative assessment

Fakhouri et al., *in preparation*

Research project: *In vivo* function of craniofacial enhancers

Human face morphology is largely genetically determined – e.g. illustrated by monozygotic twins

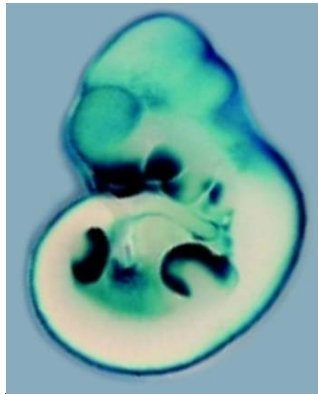**But what are the genetic drivers of normal variation?**

Model:

- core sets of developmental genes

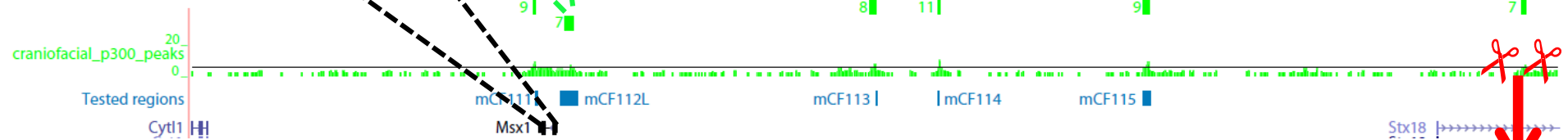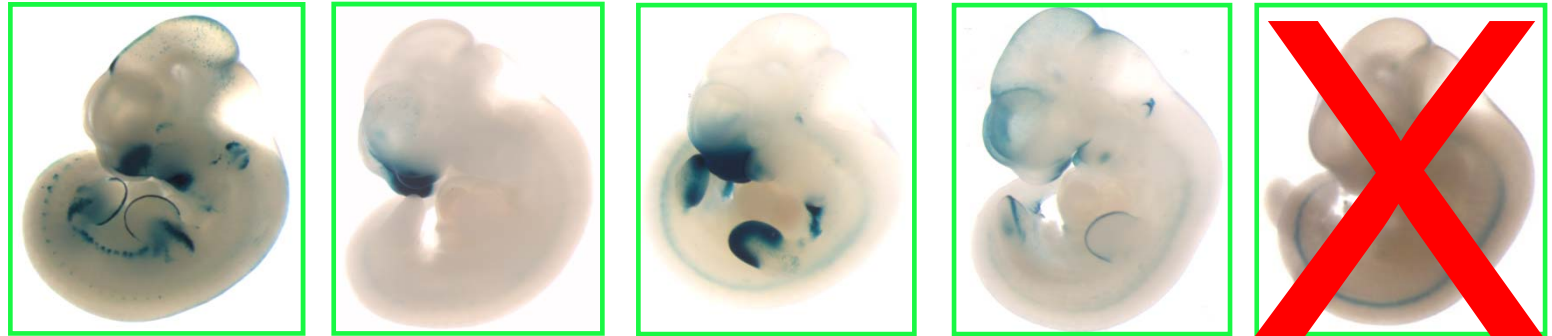- fine tuned by arrays of distant-acting enhancers

Dissecting the Regulatory Landscapes of Craniofacial Genes

(shown here: *Msx1*)

Msx1 expression
(Coudert et al 2005)

Enhancers near Msx1 drive different parts of the mRNA expression pattern
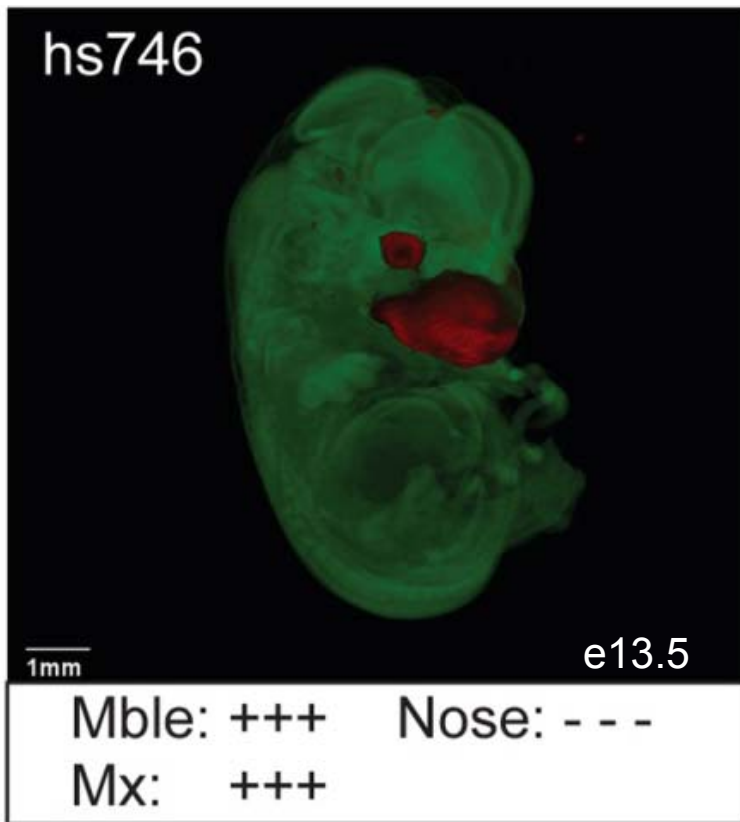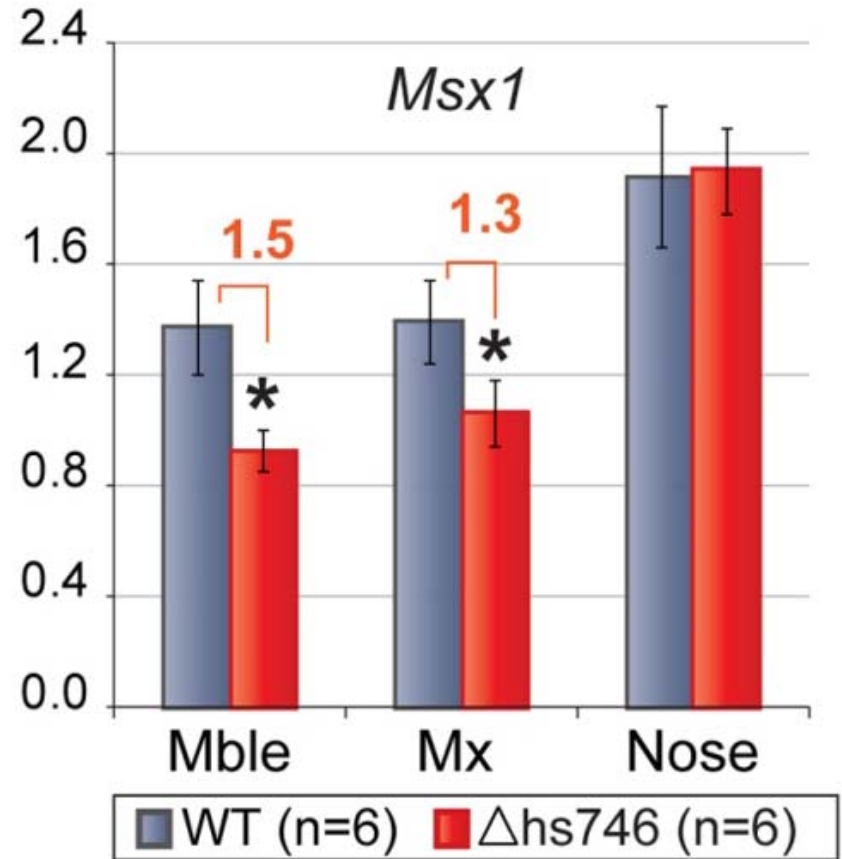
Delete just the enhancer
(not the gene!)
from mouse genome

100 kb

Enhancer activity is required for region-specific activity
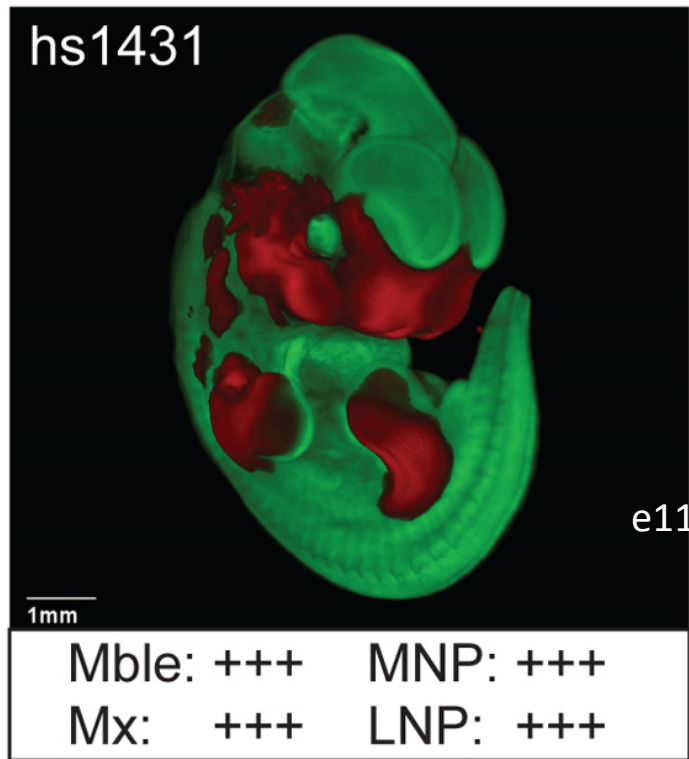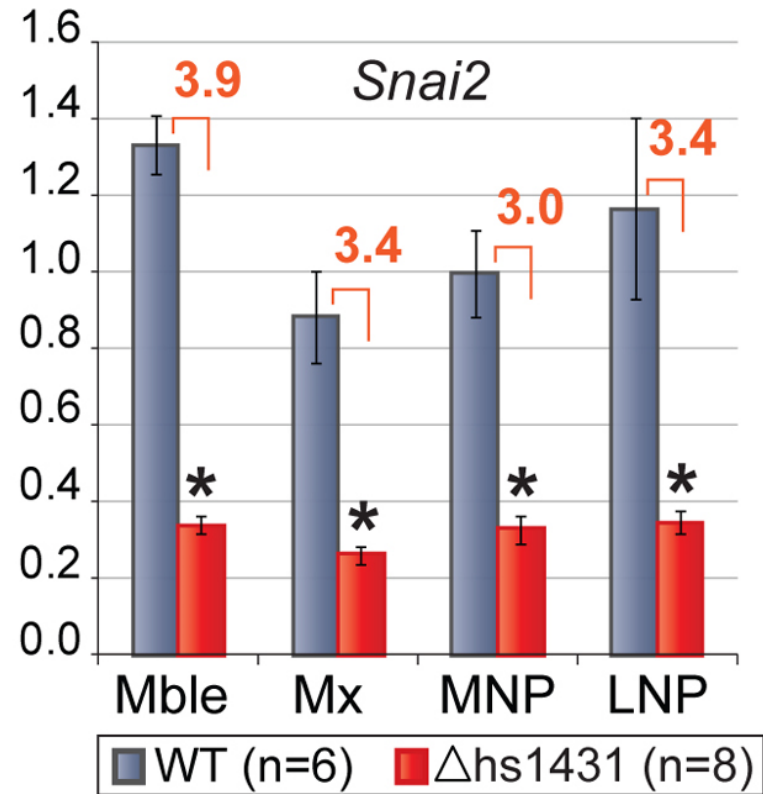
hs746 (*Msx1*) activity at e13.5

hs746

1mm

e13.5

Mble: +++   Nose: - - -
Mx: +++

Δhs746 expression phenotype

Msx1

1.5   1.3

*   *

Mble   Mx   Nose

WT (n=6)   Δhs746 (n=6)

Attanasio et al., *in preparation*

Enhancer activity is required for region-specific activity

hs1431 (*Snai2*) activity

Δhs1431 expression phenotype

hs1431

1mm
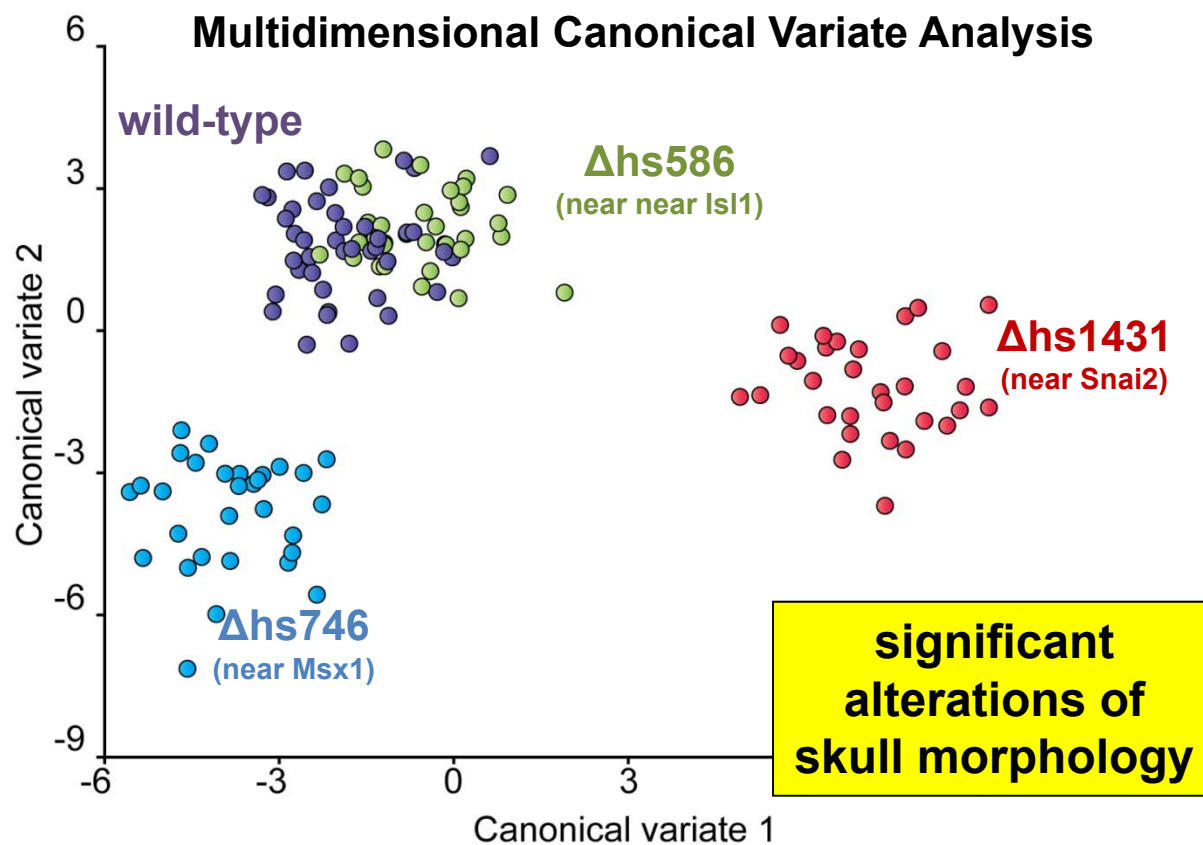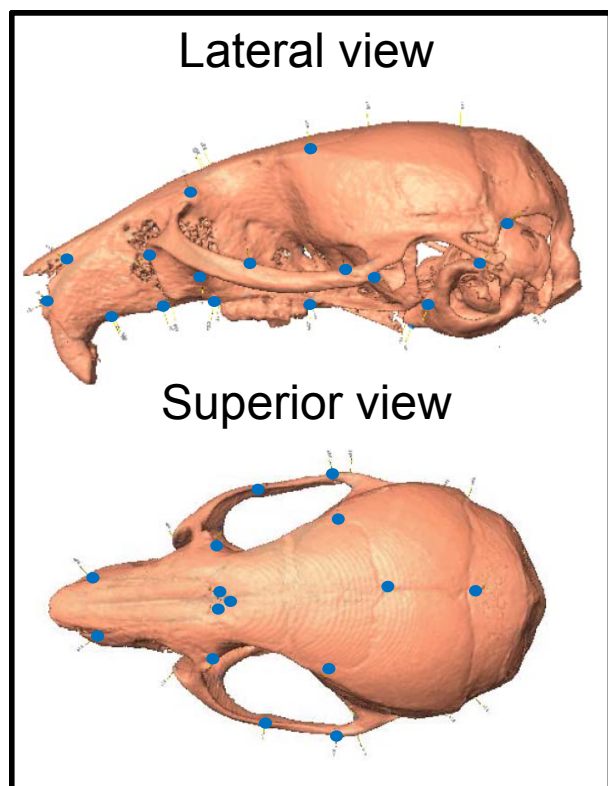
| Mble: +++ | MNP: +++ |
| Mx: +++ | LNP: +++ |

e11

Snai2

3.9 3.4 3.0 3.4

WT (n=6)   Δhs1431 (n=8)

Enhancer deletion results in down-regulation

Attanasio et al., *in preparation*

Craniofacial morphometric analysis (micro-CT scan)
With Benedikt Hallgrimsson Lab
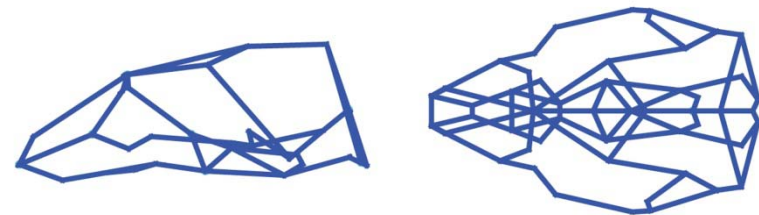
Compare adult KO skulls to matched WT control skulls

Attanasio et al., *in preparation*

Morphological changes are not limited face region

With B. Hallgrimsson Lab

Canonical Variate Analysis

wild-type

Δhs1431
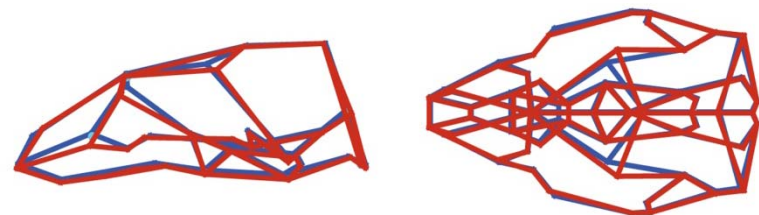(near Snai2)
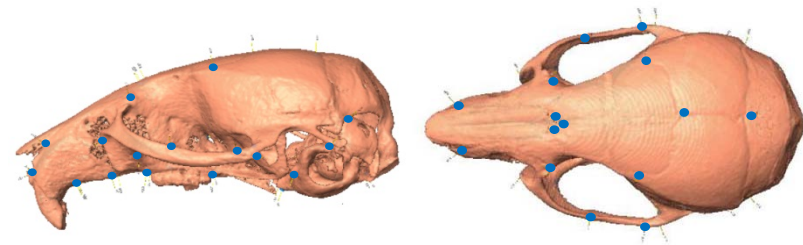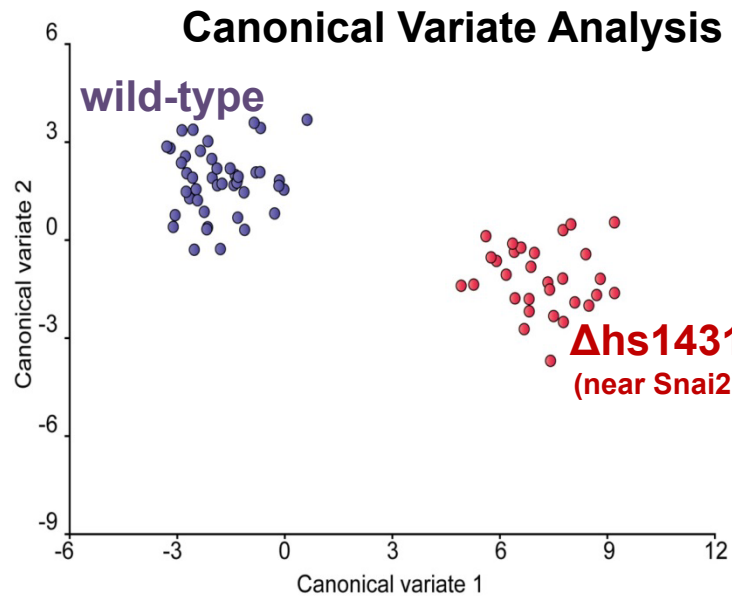
Wild type wireframe

Knockout wireframe super-imposed

- increased facial length
- increased anterior cranium width
- shortened cranial base

Attanasio et al., *in preparation*

Craniofacial morphometric analysis (micro-CT scan)
With B. Hallgrimsson Lab

Magnitudes of shape difference between null mice and wild-type
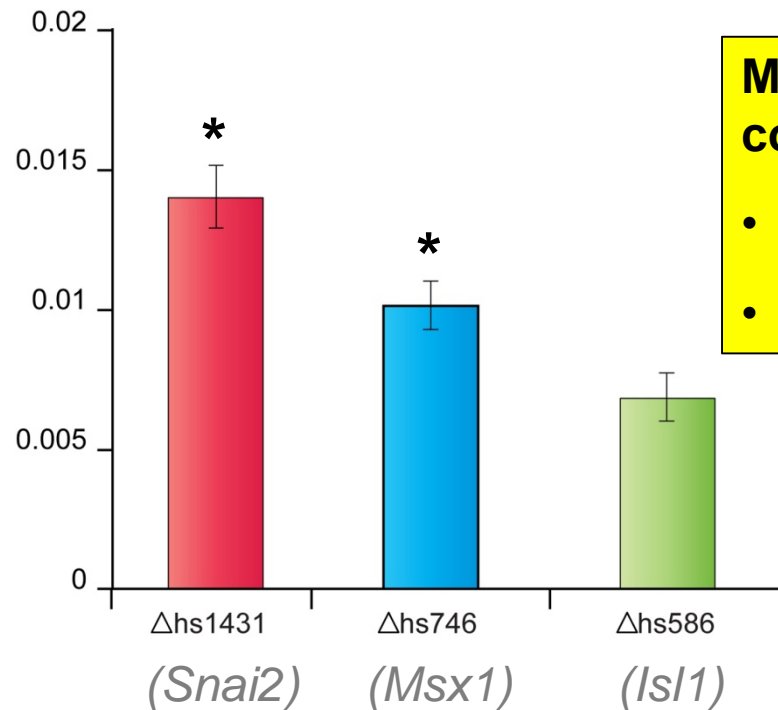
Magnitude of shape differences correlates with:

- **Magnitude of expression phenotype**

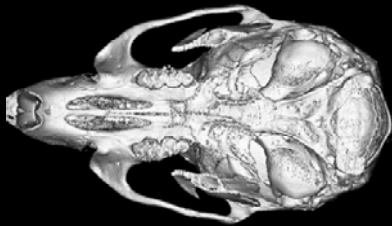- **Spatial extent of enhancer activity**

Enhancer KO studies - Conclusions

At least two out of three KOs affect craniofacial shape

Magnitude of shape differences broadly correlates with expression phenotypes

Findings consistent with a model in which craniofacial morphology is fine-tuned by enhancers

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Acknowledgments

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