

Craniofacial Transcriptional Enhancers

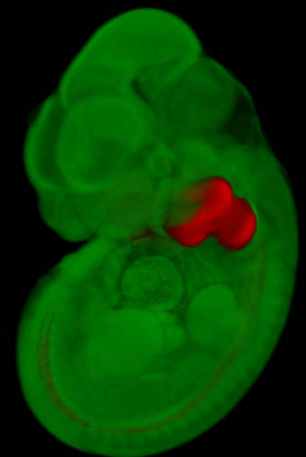


Project Update
Annual Investigator Meeting 2013
Iowa City

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](#)

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

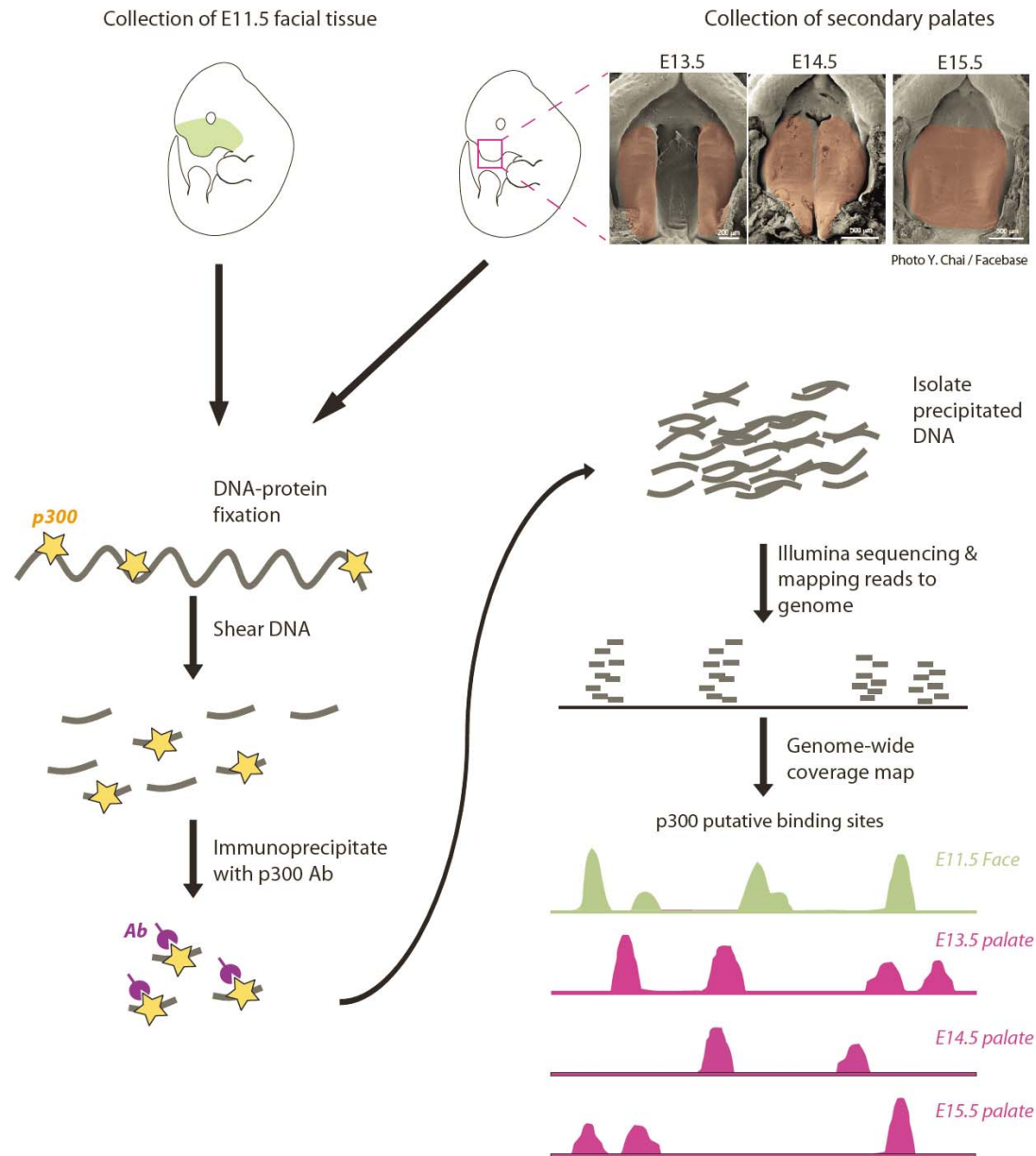
GOALS

Map craniofacial enhancers in the genome to understand their role in normal development, birth defects, and to interpret human genetic studies

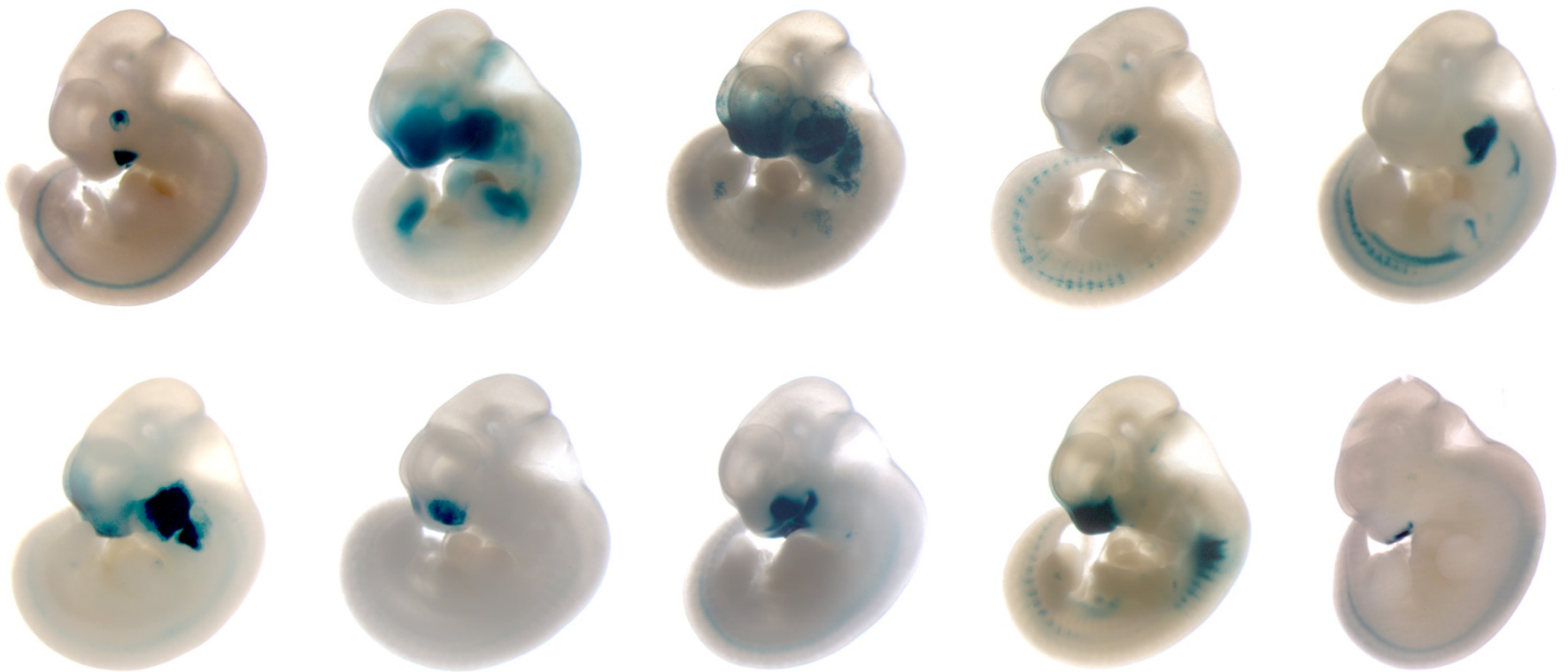
Study subsets in transgenic mice to understand their activities in detail

Make results community-accessible through FaceBase

Approach: Enhancer mapping by tissue-ChIP-seq



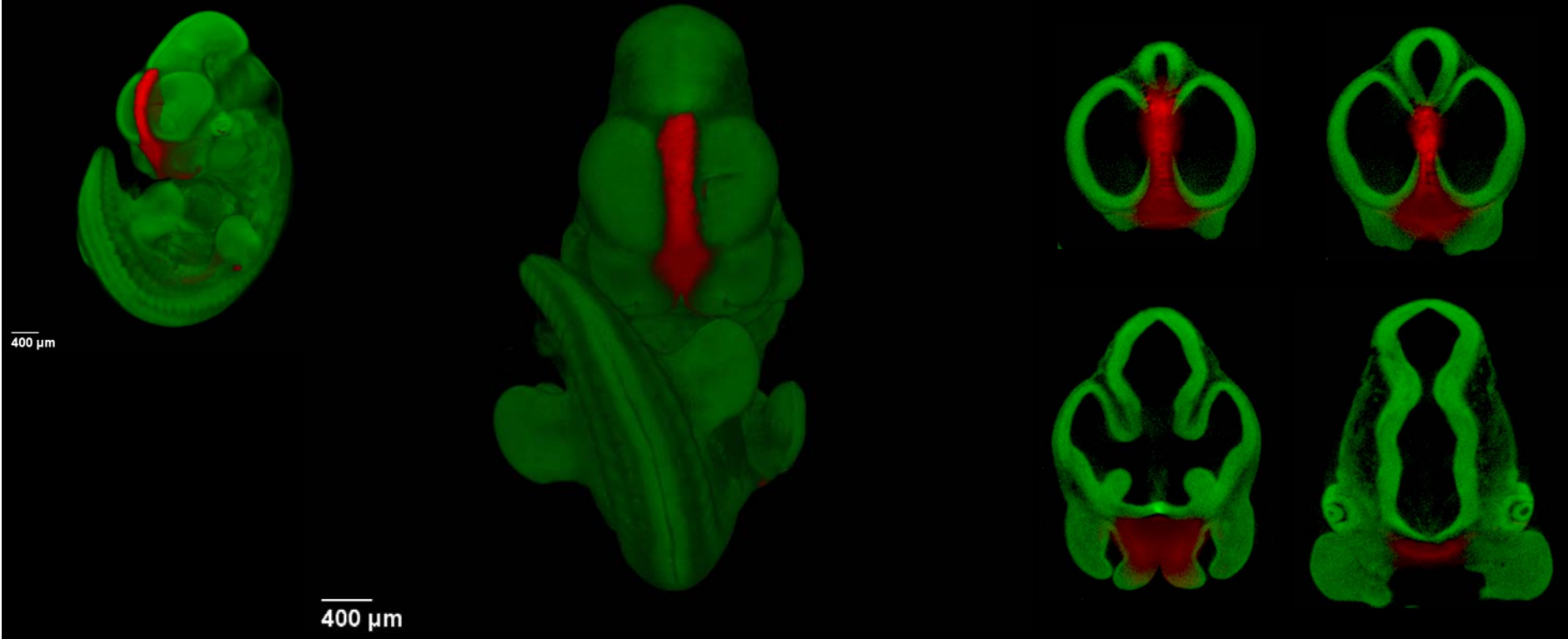
Transgenic Testing of Candidate Enhancers for Craniofacial *In Vivo* Activities



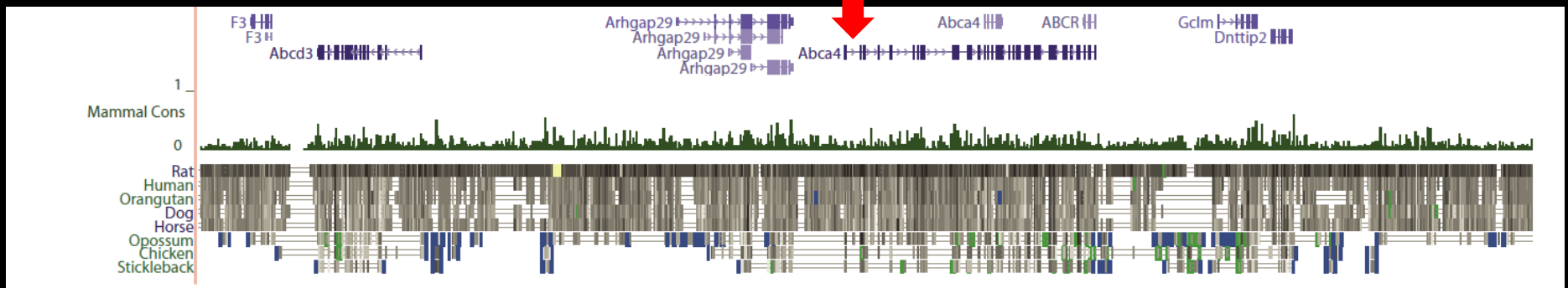
10 Example Enhancers (just one representative embryo each)



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



new tracks since
last meeting

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)

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)

New Hub Feature: OPT Viewer

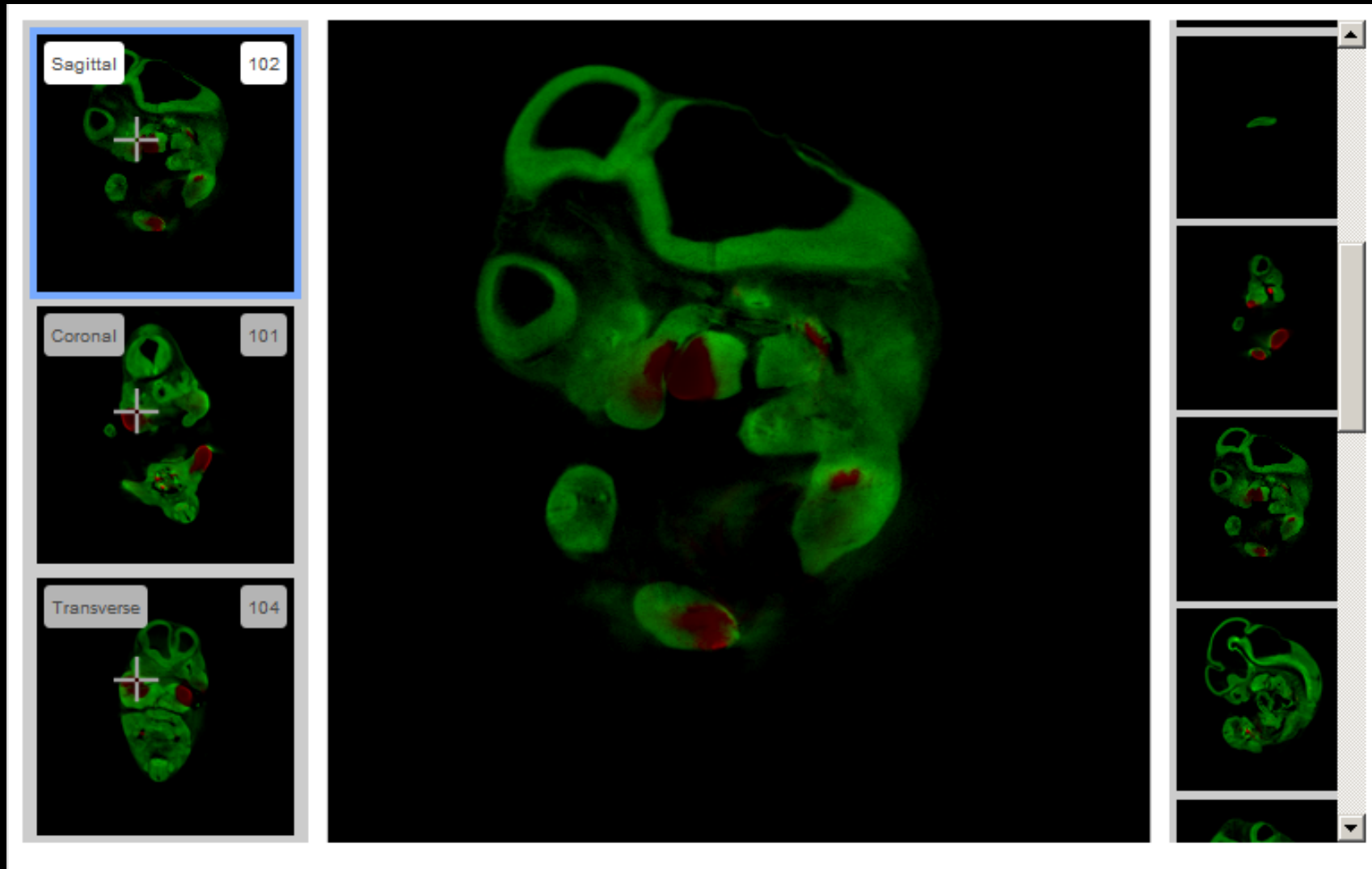
(with Jan Harkes, M. Satyanarayanan, Harry Hochheiser, Shiyi Shen)

The screenshot shows the FaceBase website interface. At the top left is the FaceBase logo. To the right, it says "Hello, Catia Attanasio" and "Log out". Below the logo is a navigation menu with items: About, Data, Tools, Resources, Community, Publications, and Biorepository. A search bar is located to the right of the navigation menu. The main content area is divided into several sections:

- A Resource For Craniofacial Researchers**: A large blue banner with text describing the FaceBase Consortium.
- NEWS & EVENTS**: A section with a date "JAN 29 2013" and text "2013 FaceBase annual meeting to be held in Iowa City".
- DATA**: A section with three sub-sections: HUMAN (with a 3D model of a human face), MOUSE (with a 3D model of a mouse face), and ZEBRAFISH (with a fluorescence microscopy image).
- RESEARCH HIGHLIGHT**: A section with gene names [Msx1](#), [Tgfr2](#), and [Irf6](#). Below this is a partial view of a research highlight card for [Msx1](#), including a description: "Msx1 is expressed in the mesenchyme of the anterior..." and a "More Details" link.
- WHAT'S NEW**: A section listing new features:
 - [FishFace](#): An atlas of zebrafish craniofacial development
 - [OCDM](#): Ontology of Craniofacial Development and Malformation
 - [3D Facial Norms Database](#): Normative facial images
 - [CranloGUI](#): An interactive... of 3d facial images
 - [Optical Projection Tomography Viewer](#): This tool allows you to view optical projection tomography(OPT) images. This item is circled in red.

New Hub Feature: OPT Viewer

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



Variant testing: IRF6 enhancer

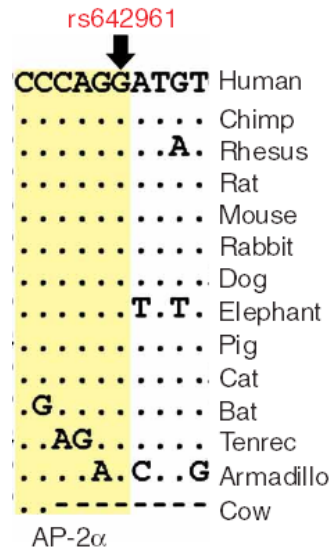
LETTERS

nature genetics

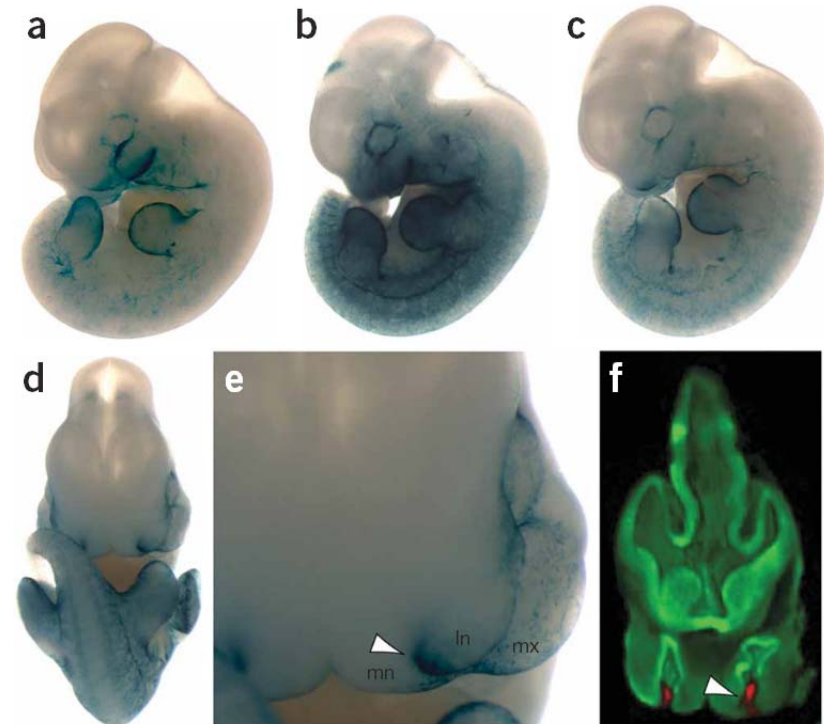
Disruption of an AP-2 α binding site in an *IRF6* enhancer is associated with cleft lip

Fedik Rahimov¹, Mary L Marazita², Axel Visel³, Margaret E Cooper², Michael J Hitchler⁴, Michele Rubini⁵, Frederick E Domann⁴, Manika Govil², Kaare Christensen⁶, Camille Bille⁶, Mads Melbye⁷, Astanand Jugessur⁸, Rolv T Lie⁸, Allen J Wilcox⁹, David R Fitzpatrick¹⁰, Eric D Green¹¹, NISC Comparative Sequencing Program¹¹, Peter A Mossey¹², Julian Little¹³, Regine P Steegers-Theunissen¹⁴, Len A Pennacchio³, Brian C Schutte¹ & Jeffrey C Murray¹

2008 Nature Genetics (Rahimov et. al)



SNP disrupts conserved AP-2 α binding site (EMSA)



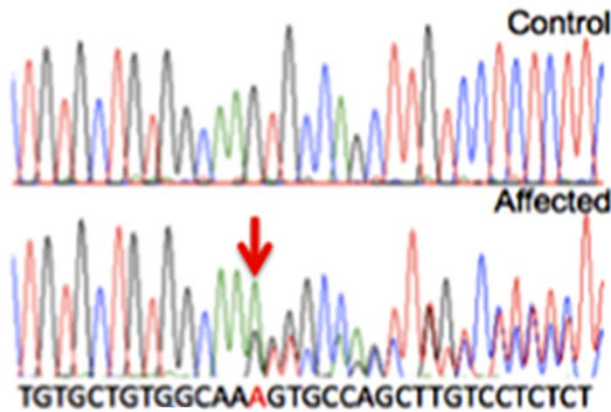
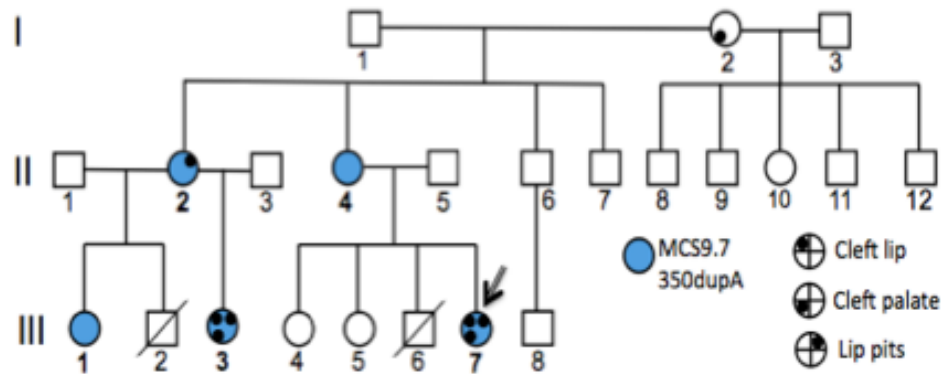
MCS-9.7 enhancer of *IRF6*

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



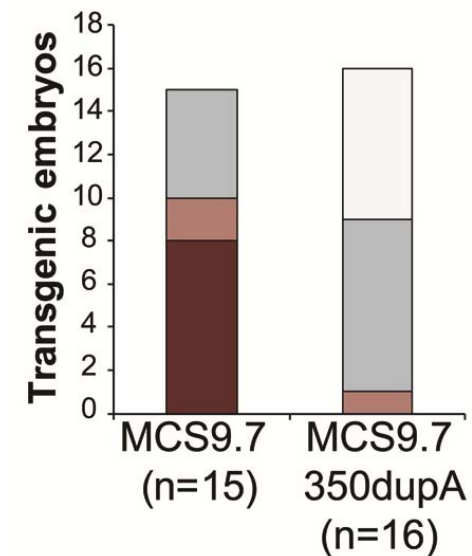
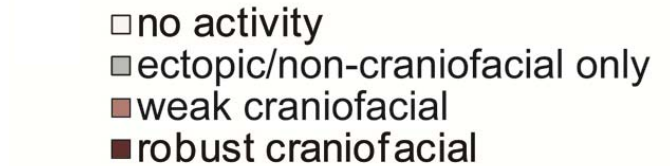
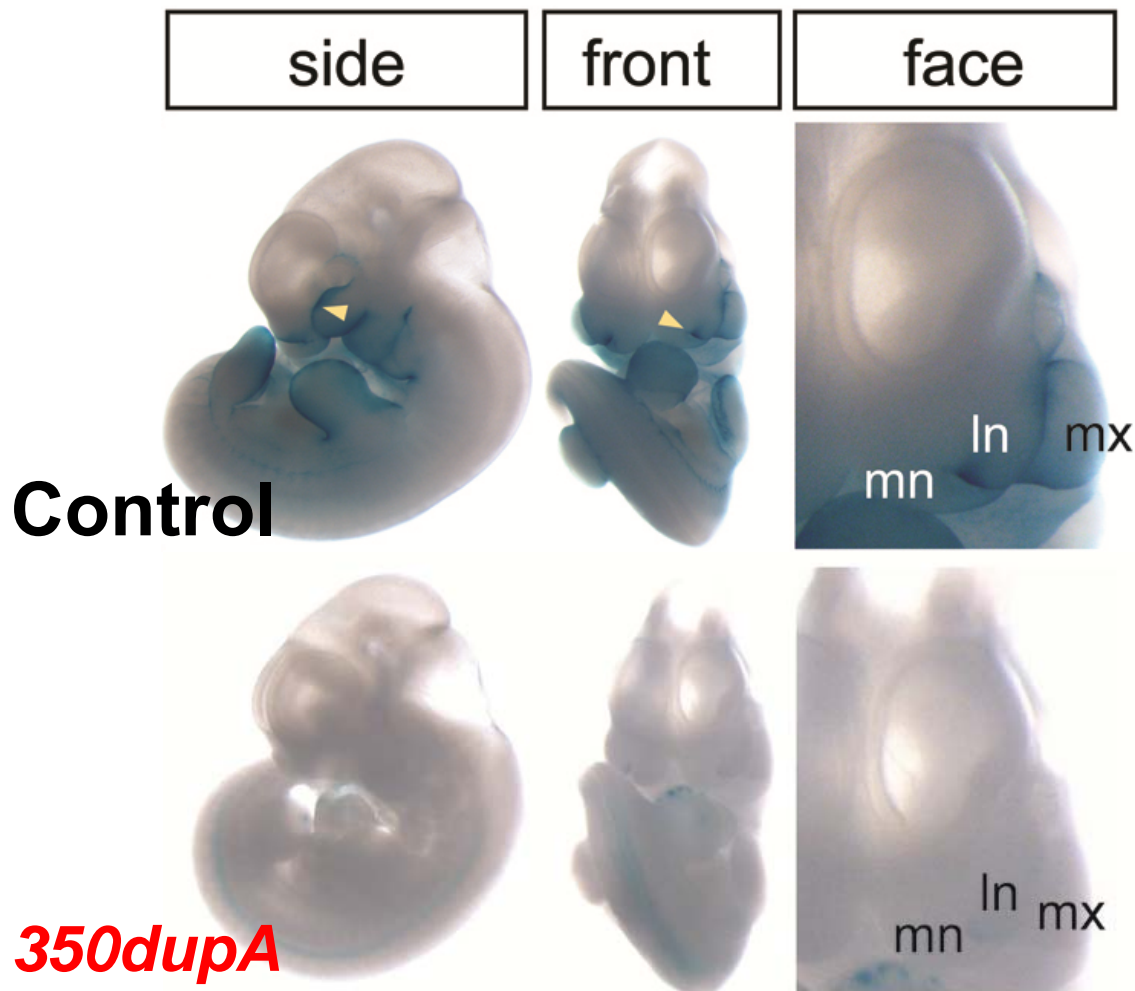
- sequencing identifies a new variant in IRF6 MCS-9.7 enhancer
- insertion **350dupA** abolishes activity in cell culture
- **350dupA** disrupts a p63 binding site in vitro
- also disrupts p63 binding at a 2nd site ~60bp away (presumptive gain-of-function, see Brian for details)

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)



quantitative assessment

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?

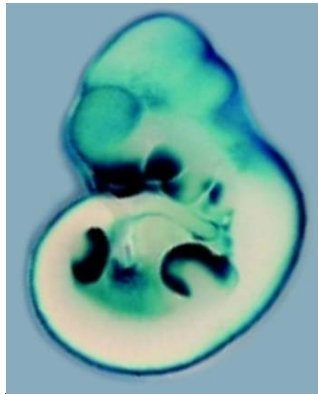
Model:

- core sets of developmental genes
- fine tuned by arrays of distant-acting enhancers

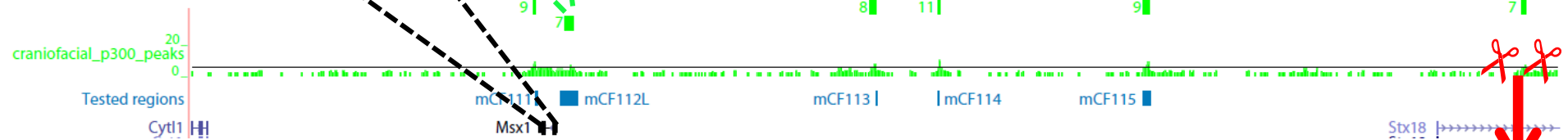
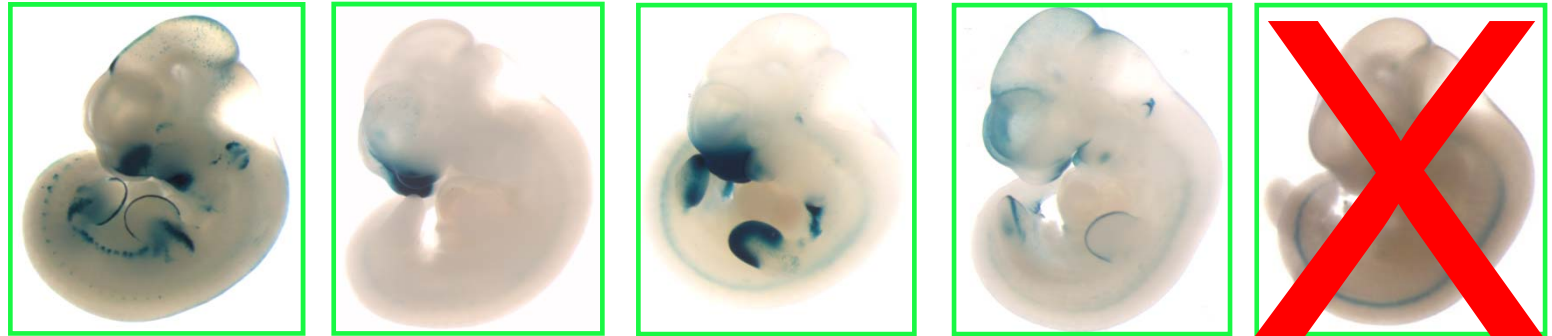
Dissecting the Regulatory Landscapes of Craniofacial Genes

(shown here: *Msx1*)

Msx1 expression
(Coudert et al 2005)



Enhancers near *Msx1* drive different parts of the mRNA expression pattern

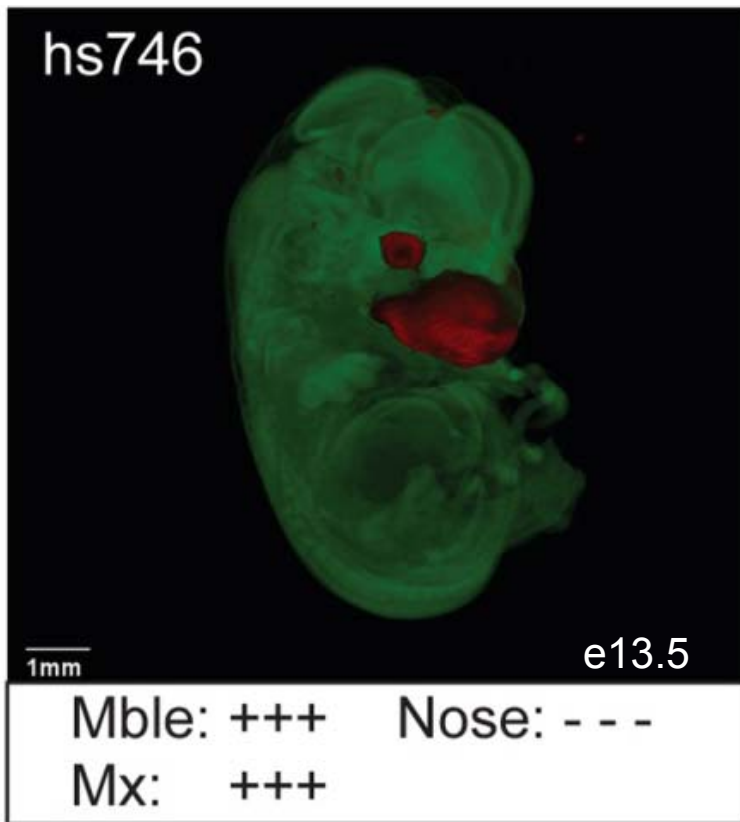


Delete just the enhancer
(not the gene!)
from mouse genome

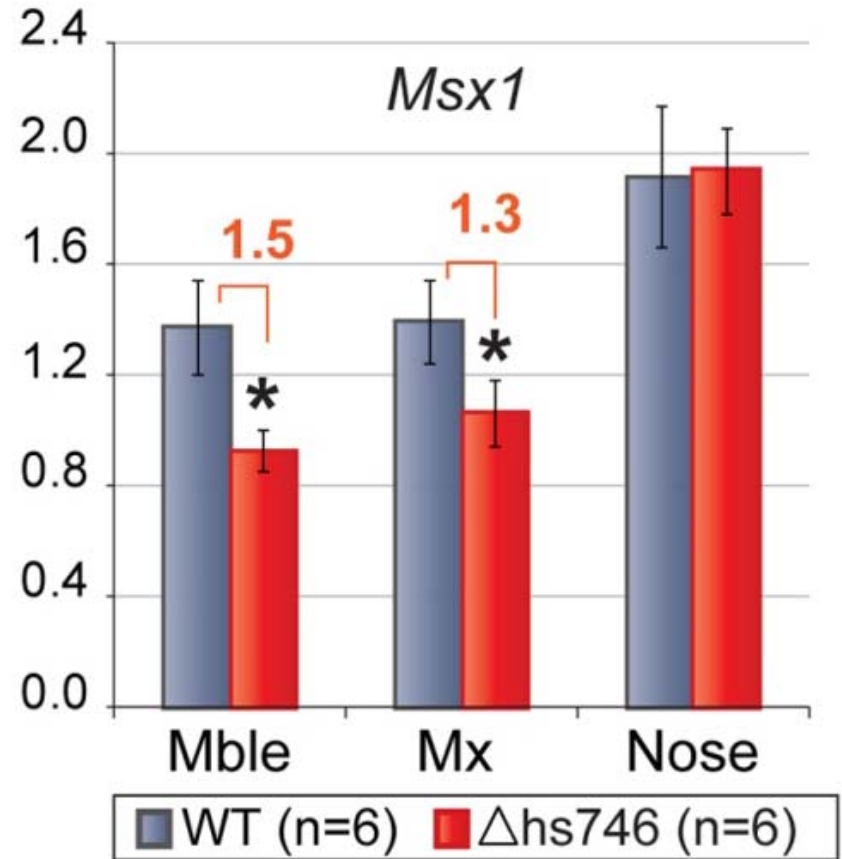
100 kb

Enhancer activity is required for region-specific activity

hs746 (*Msx1*) activity at e13.5

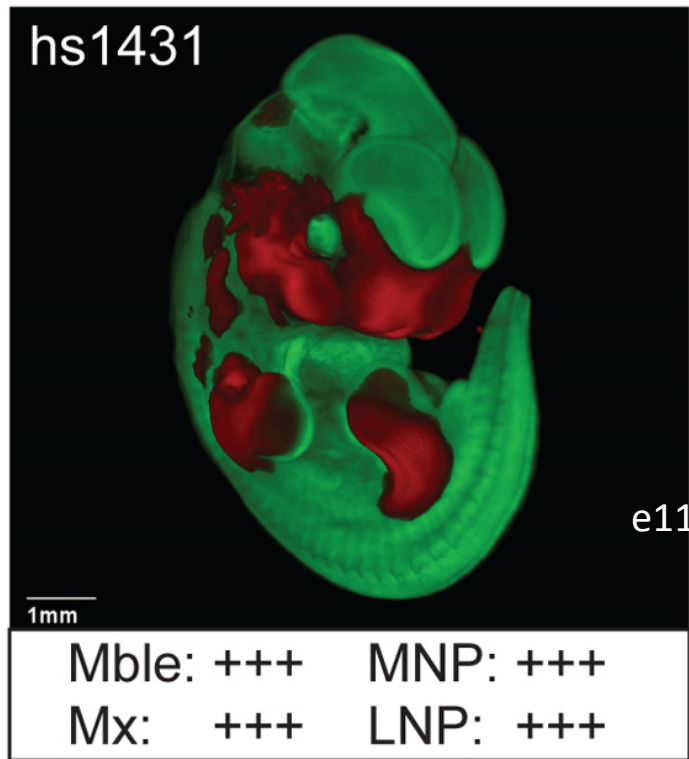


Δ hs746 expression phenotype

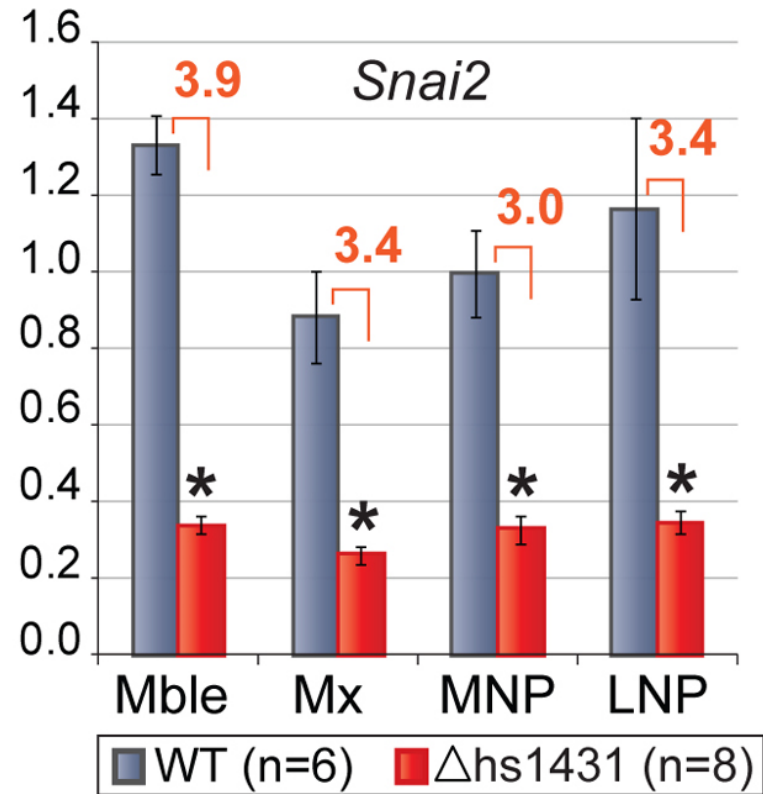


Enhancer activity is required for region-specific activity

hs1431 (*Snai2*) activity



Δ hs1431 expression phenotype

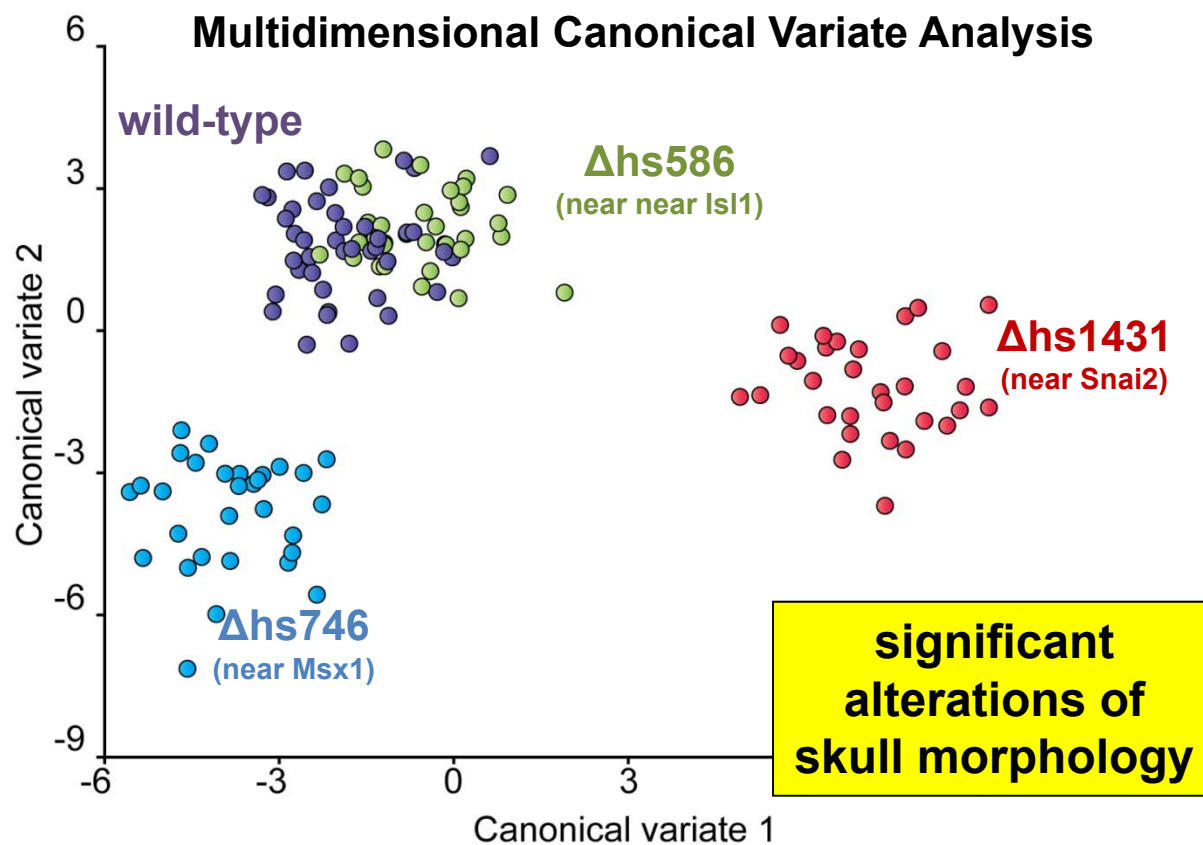
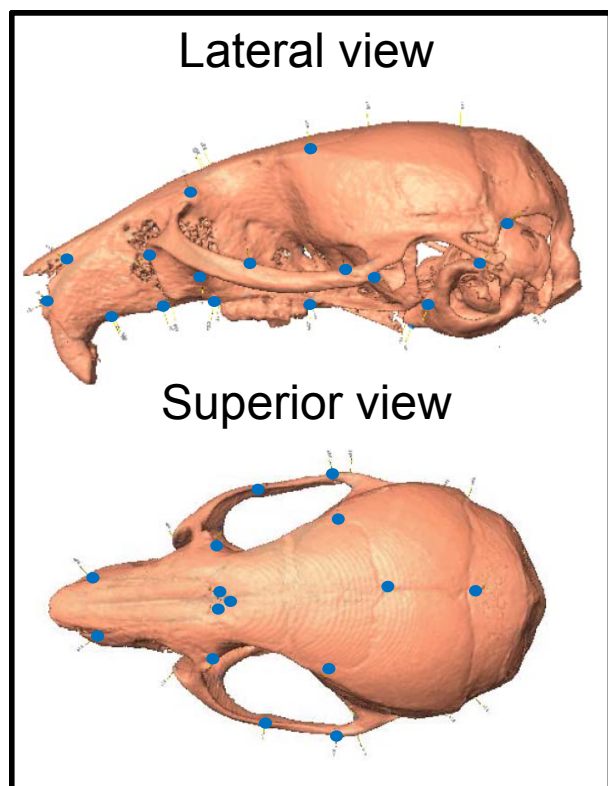


Enhancer deletion results in down-regulation

Craniofacial morphometric analysis (micro-CT scan)

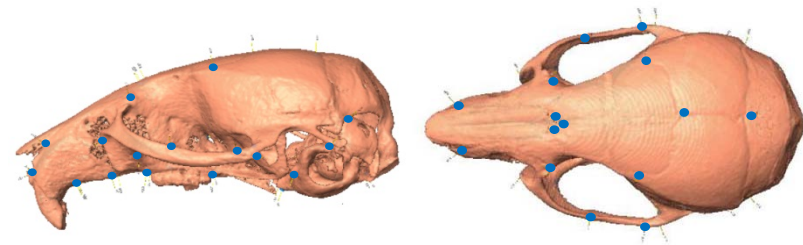
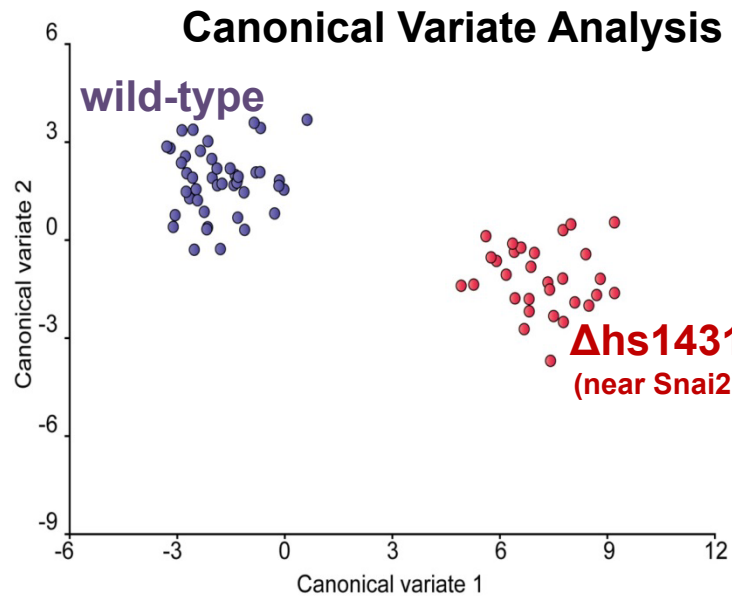
With Benedikt Hallgrímsson Lab

Compare adult KO skulls to matched WT control skulls

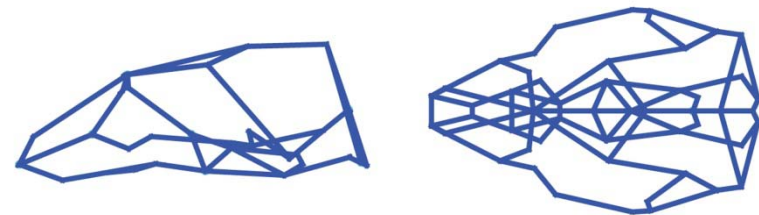


Morphological changes are not limited face region

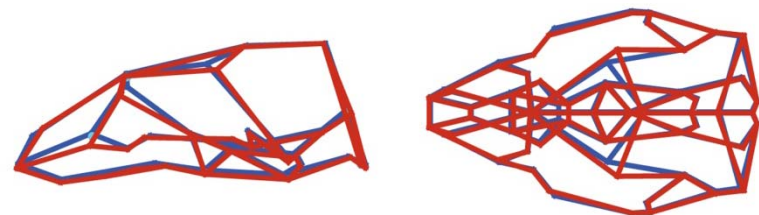
With B. Hallgrimsson Lab



Wild type wireframe



Knockout wireframe super-imposed

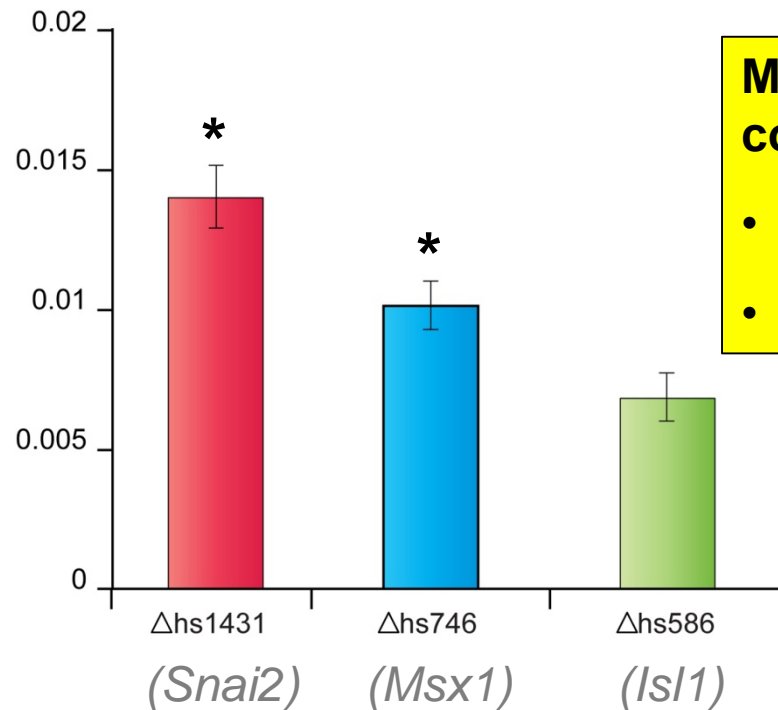


- increased facial length
- increased anterior cranium width
- shortened cranial base

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

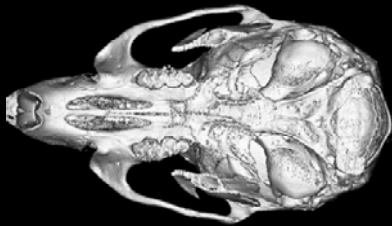
Enhancer KO studies - Conclusions



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

Acknowledgments

**Lawrence Berkeley National Lab
and DOE Joint Genome Institute**

Catia Attanasio, Alex Nord, Matt Blow

**Len Pennacchio
Eddy Rubin**

Mouse Transgenics
Malak Shoukry
Jennifer Akiyama
Veena Afzal
Amy Holt
Ingrid Plaijzer-Frick
Roya Hosseini

Next-Gen Sequencing
Tao Zhang
Feng Chen
Crystal Wright
Enhancer Browser
Inna Dubchak

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Robert Cornell, Mike Dixon, David FitzPatrick, Benedikt Hallgrímsson, Rulang Jiang, Michael Lovett, Mary Marazita, Jeff Murray, Stephen Murray, Bing Ren, John Rubenstein, Brian Schutte

**NIDCR
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NHGRI**

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