

Distant Acting Enhancers in Craniofacial Development

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1 Background

- The shape of the human face and skull is highly heritable, but the genetic factors that contribute to normal variation in craniofacial morphology remain poorly defined.
- Arrays of non-coding elements modulate the expression of 'core craniofacial genes'. Variation within those elements could contribute to natural phenotypic variation of face morphology and represent risk factors for craniofacial birth defects.
- To identify craniofacial / palatal *in vivo* enhancers we have performed ChIP-seq on these tissues with the enhancer-associated protein p300. We validated the potential of identified bound regions as craniofacial enhancers through mouse transgenesis for a subset of them.
- To investigate the functional contribution of these enhancers to normal craniofacial development, we have selected 3 of them for knockout in mouse. 'Target' gene expression and skull morphology analyses of these mice are both consistent with a general role of *in vivo* enhancer in determining adult craniofacial morphology.

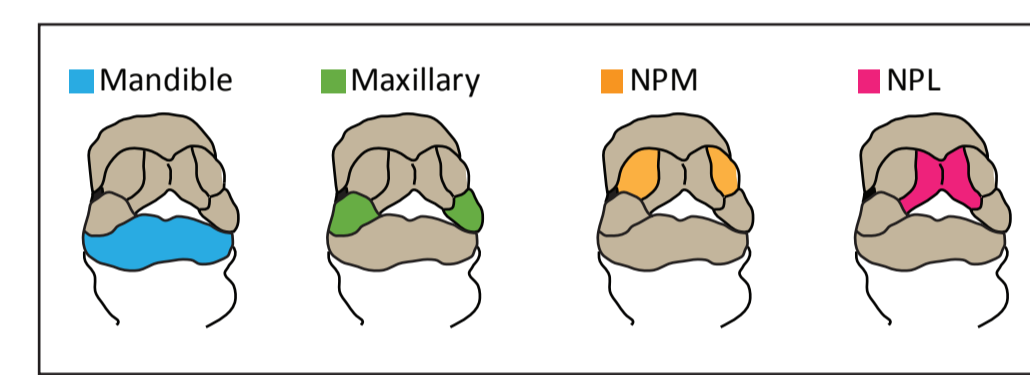
3 Craniofacial Enhancer knockouts

Selection criteria for enhancers to knockout

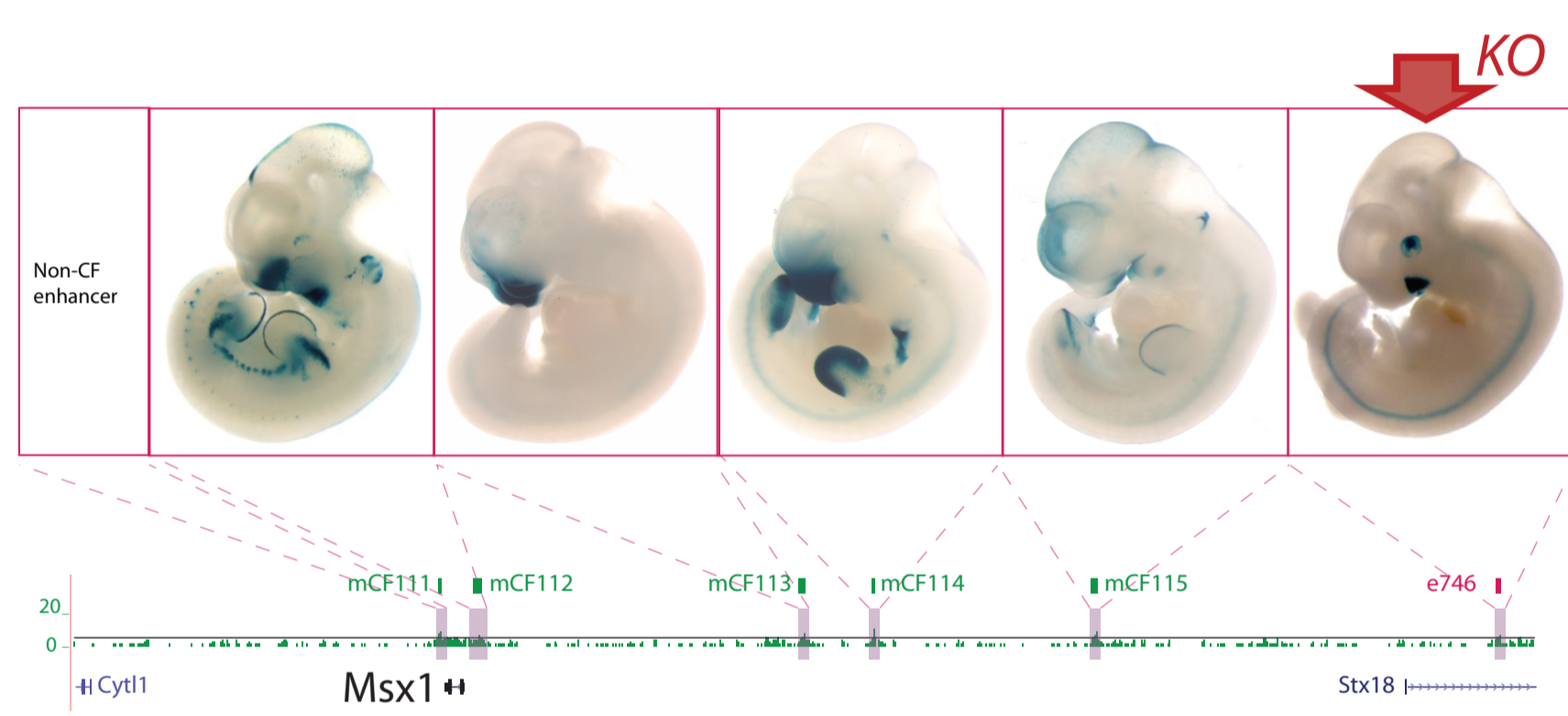
- Highly reproducible craniofacial enhancers
- Non-redundant *in vivo* activity with neighbouring enhancers
- Map within a craniofacial gene locus

Gene expression phenotypes (qPCR)

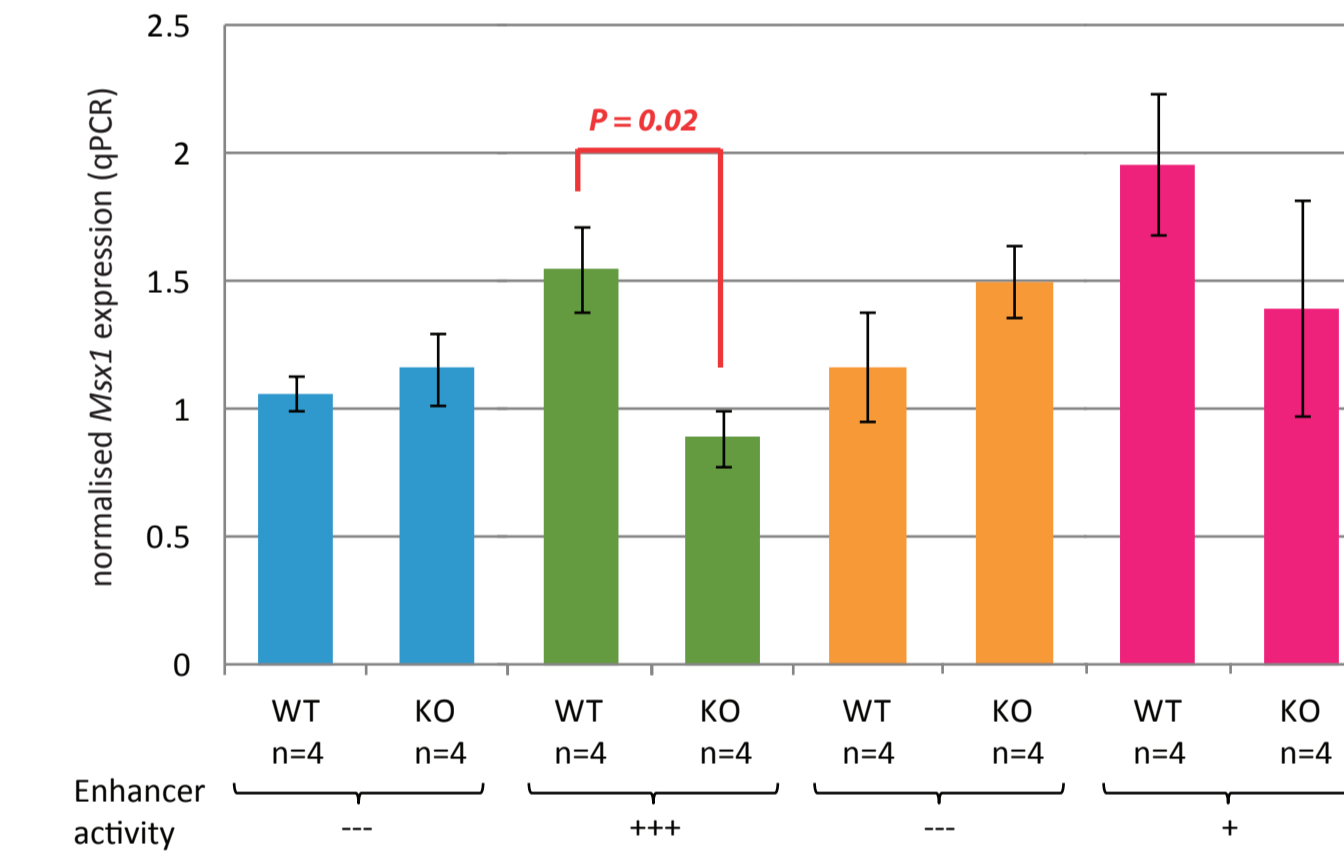
- Subregions of the faces were dissected for both WT and KO e11.5 mouse embryos (littermates)



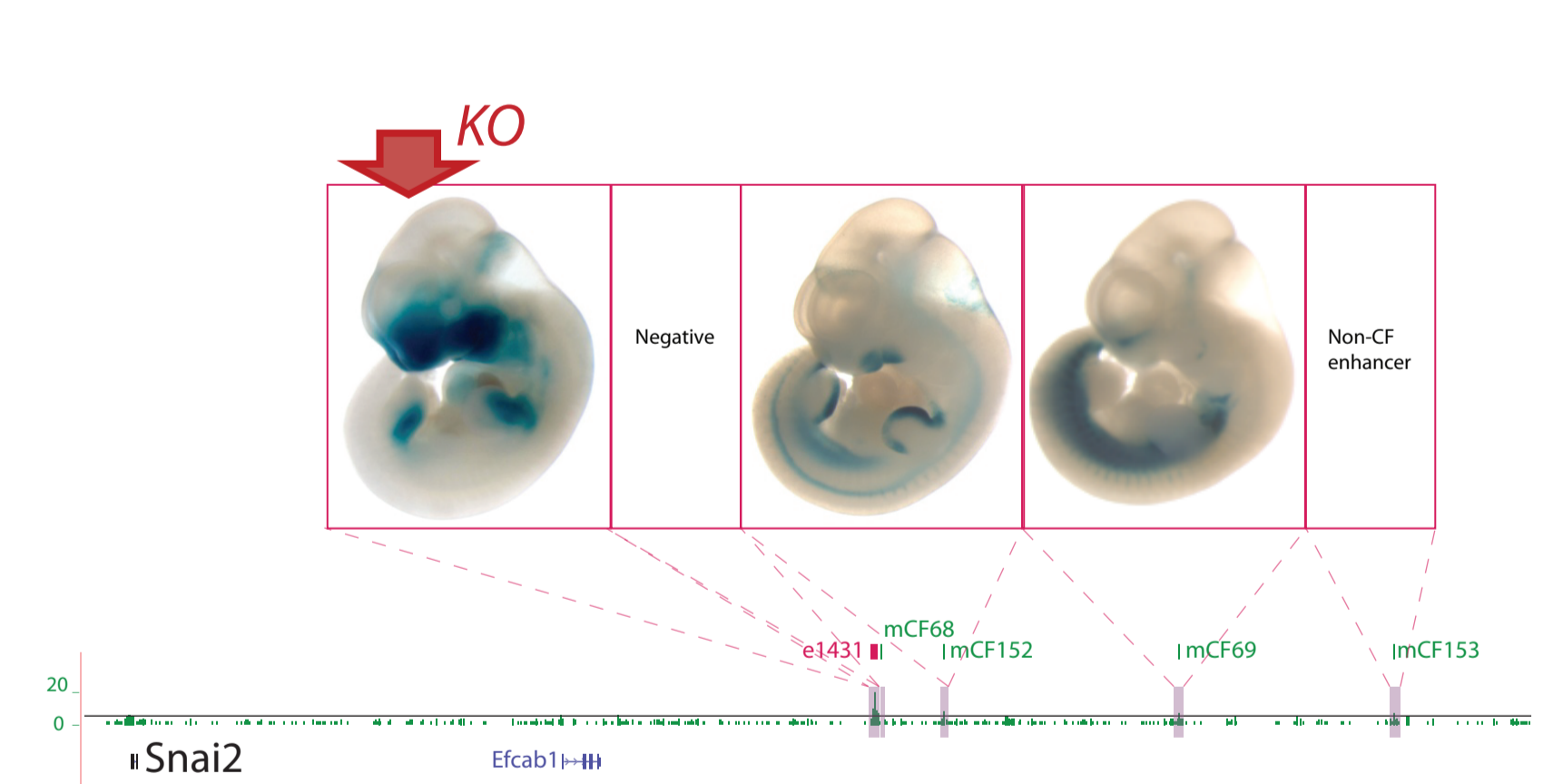
1) Enhancer 746 - *Msx1* locus



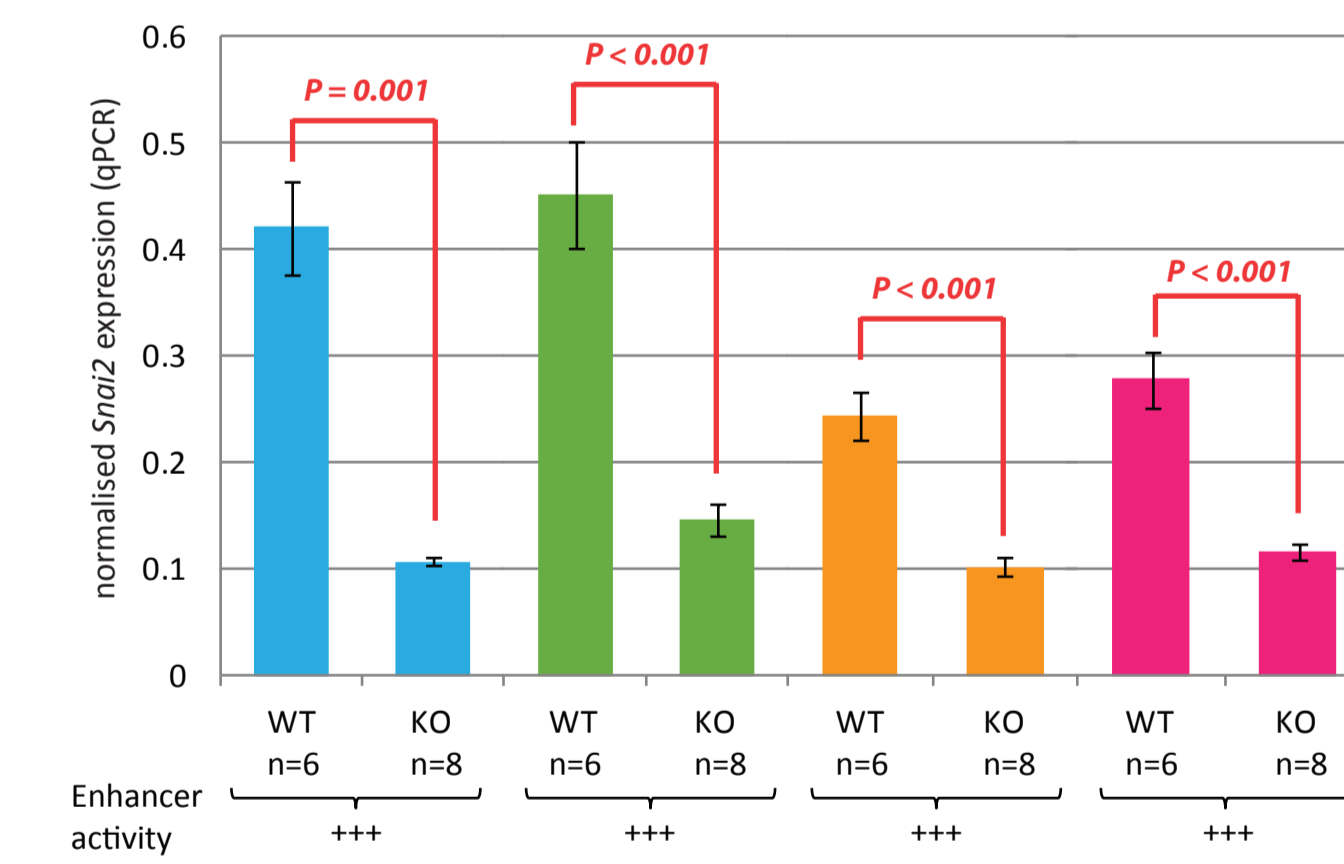
Msx1 expression in e746 WT vs KO *



2) Enhancer 1431 - *Snai2* locus

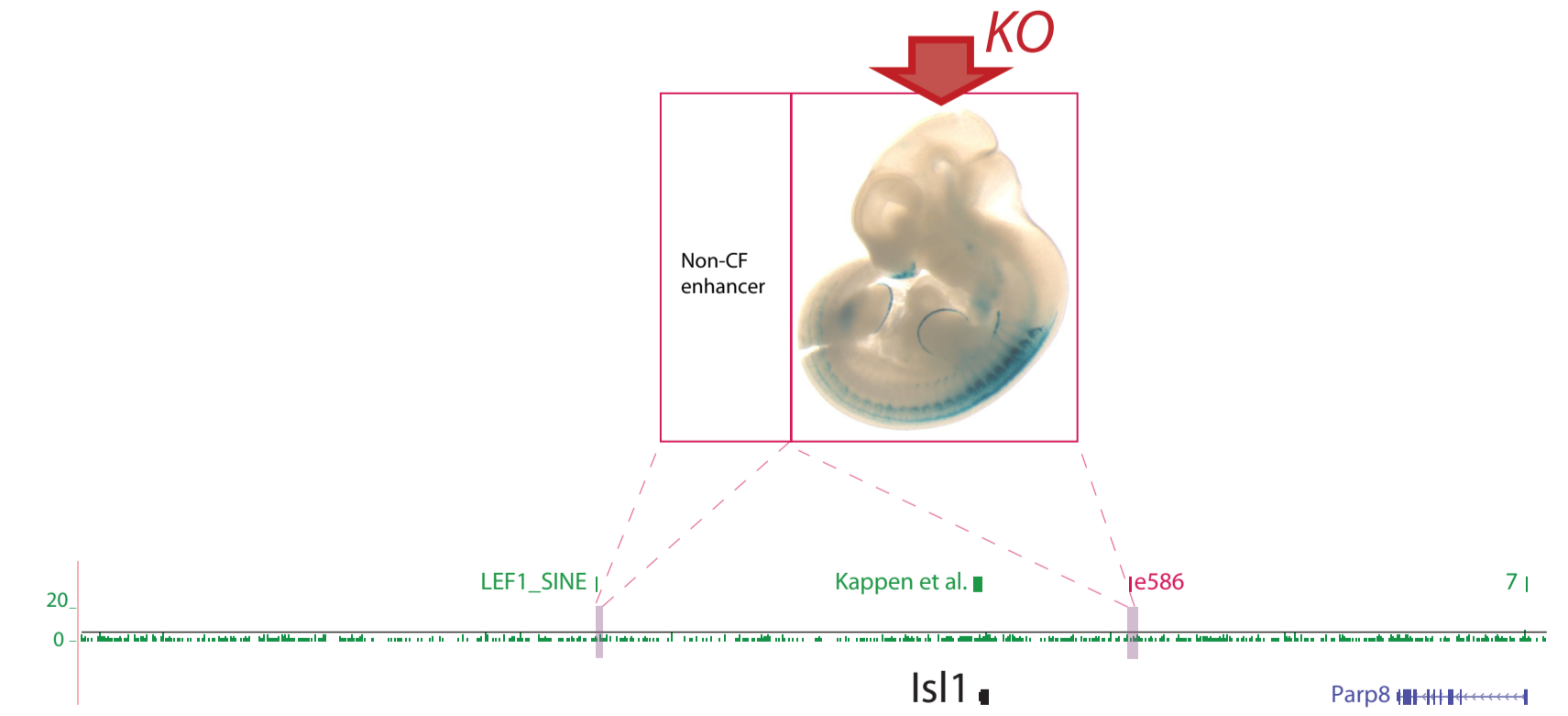


Snai2 expression in e1431 WT vs KO *



* Shown is the relative expression of the target gene within each individual (samples not pooled). Errors bars (SEM) show inter-individual expression variations.

3) Enhancer 586 - *Isl1* locus



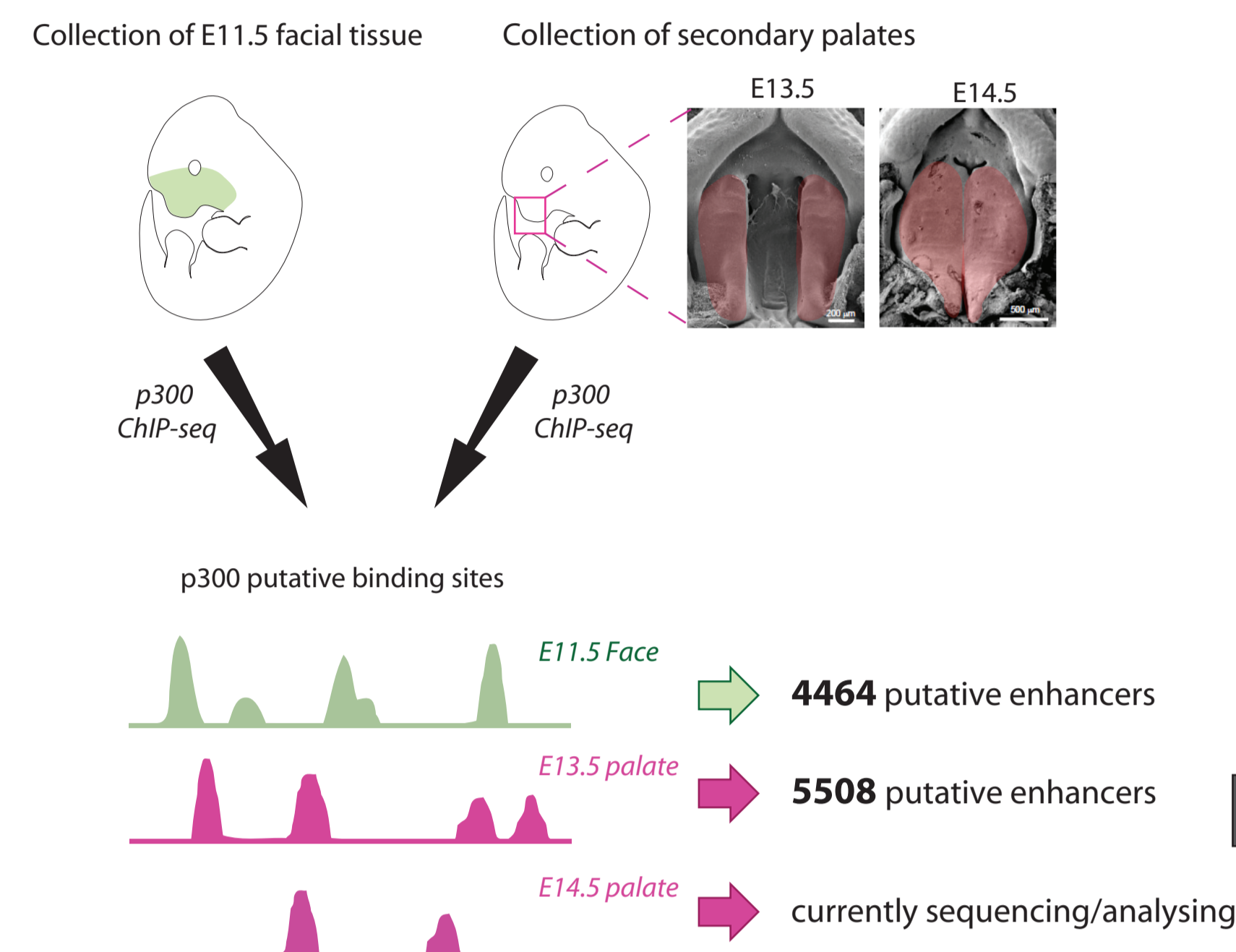
Isl1 expression in e586 WT vs KO
Not significantly different in any substructures

5 Conclusions

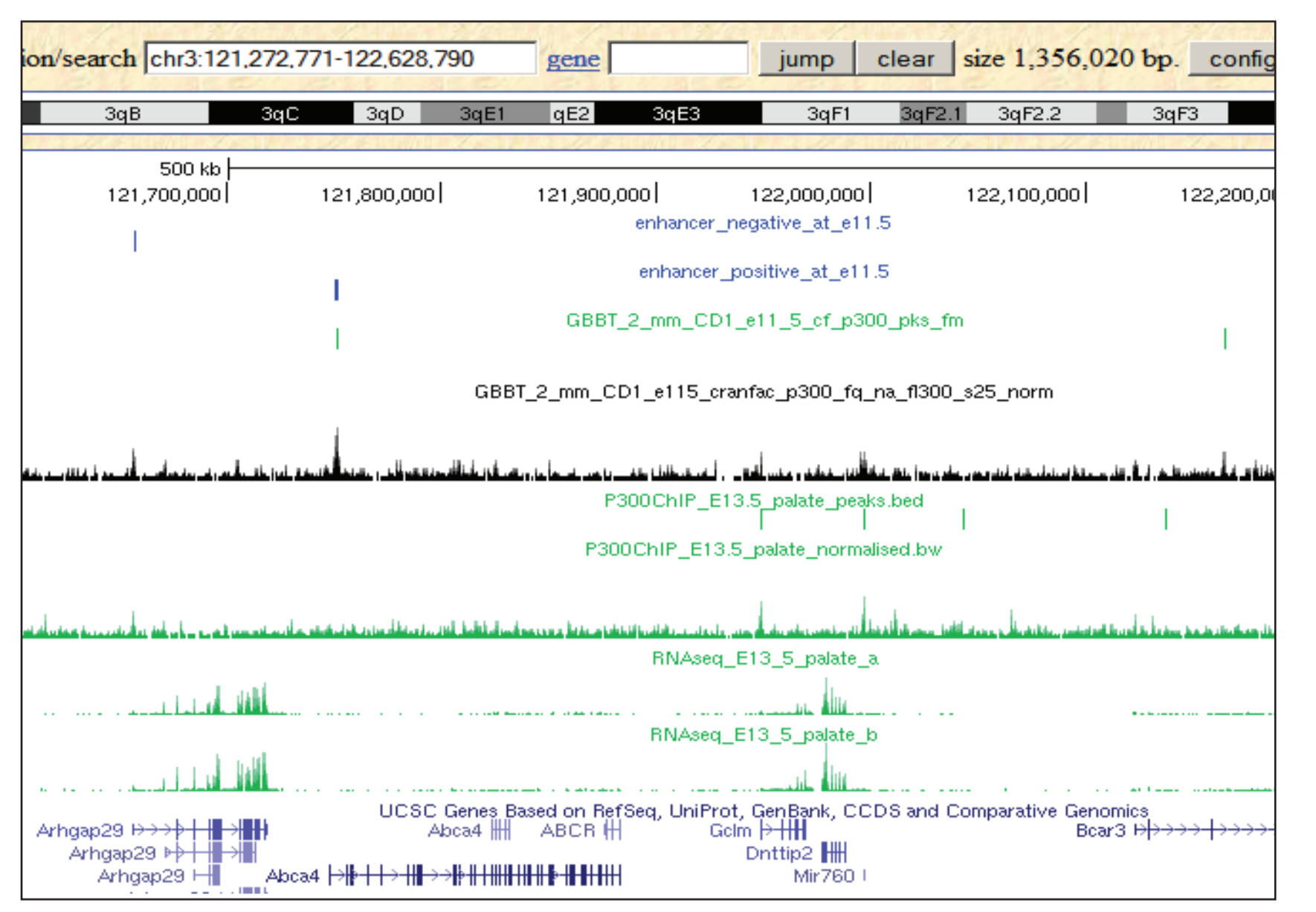
- Using p300 ChIP-seq in e11.5 faces and e13.5 secondary palates we identified thousands of distant-acting enhancers that likely orchestrate gene expression during craniofacial/palate development.
- In vivo* dissection of the regulatory landscape of craniofacial genes reveals the complexity of enhancer arrays involved in their regulation.
- In vivo* functional characterization of a subset of craniofacial enhancers demonstrates that enhancers can contribute to normal phenotypic variation and support the notion that they likely contribute to pathological aberrations of craniofacial morphology.

2 Facebase Update - Data available on the Hub

P300 ChIP-seq (& matched RNA-seq)

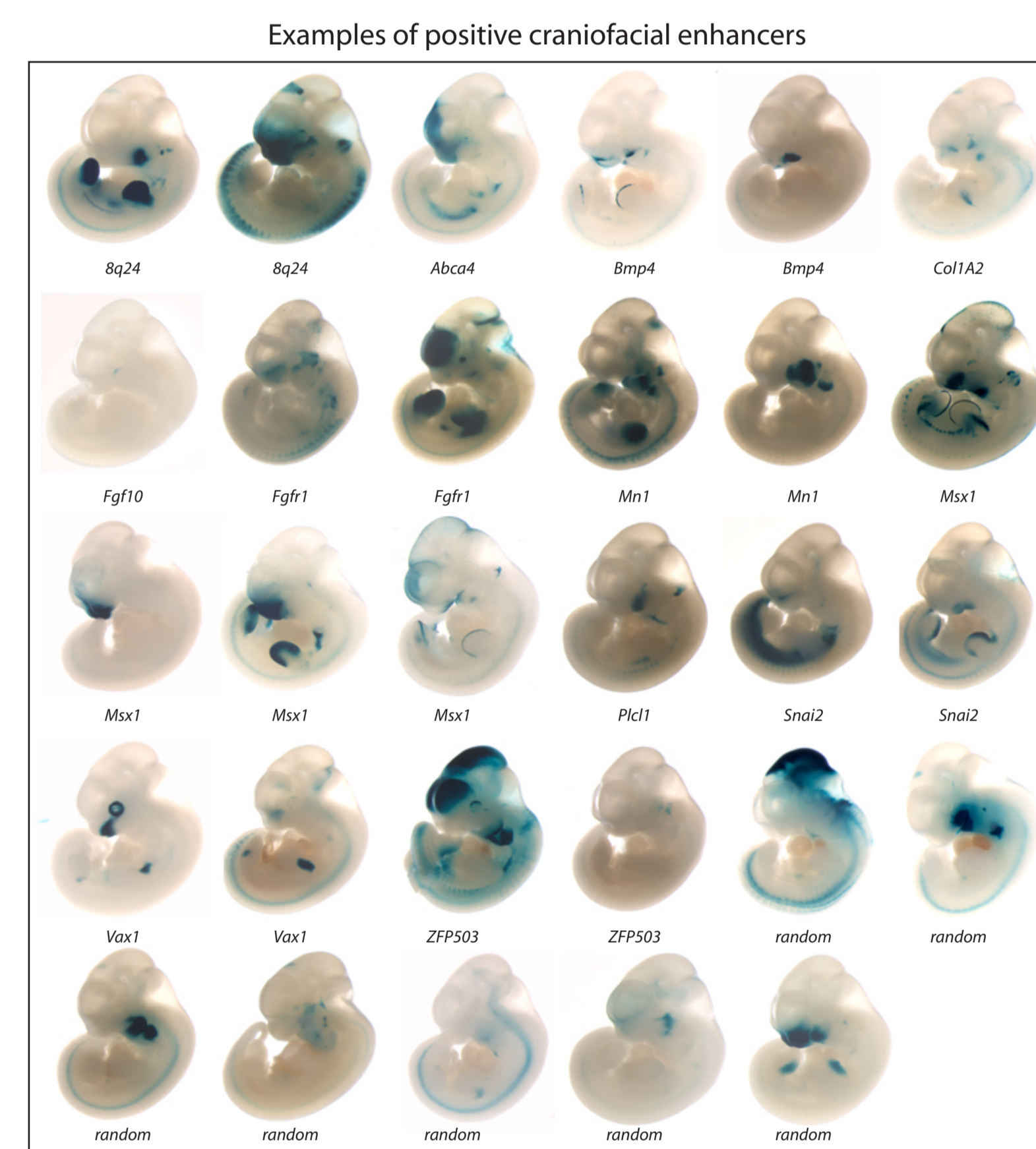


Snapshot of ChIP-seq and RNA-seq tracks currently available on the FaceBase Hub



In vivo transgenesis

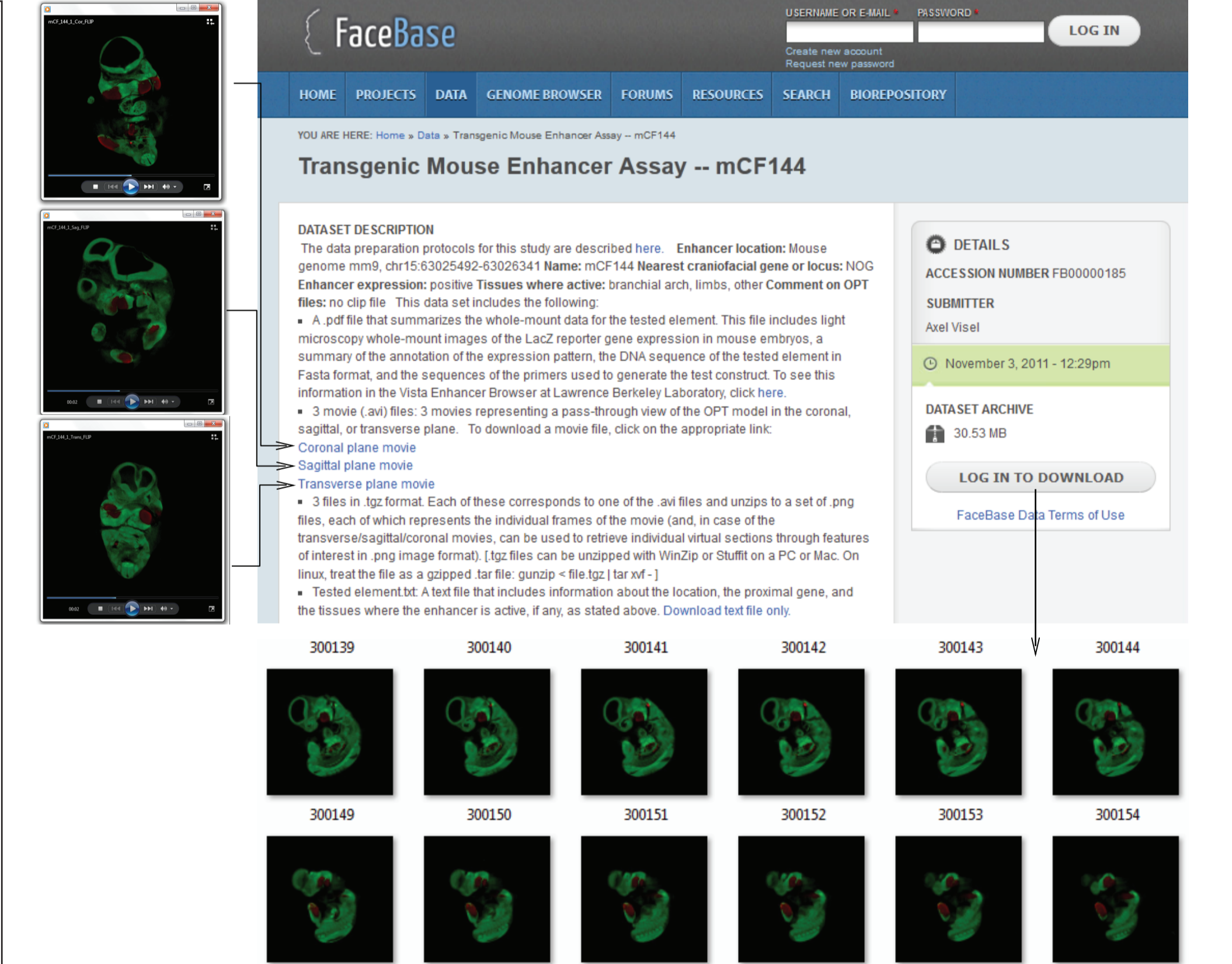
- 90 craniofacial putative enhancers tested



Optical Projection Tomography

- OPT scans are available for every identified craniofacial enhancer

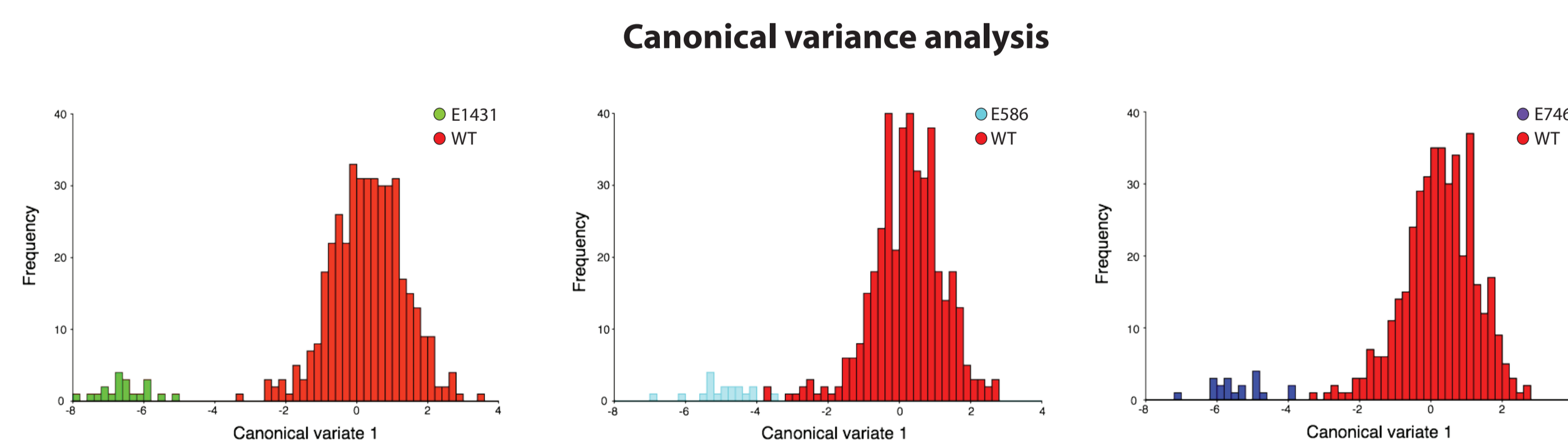
FaceBase snapshot with a representative example of the available imbedded OPT data



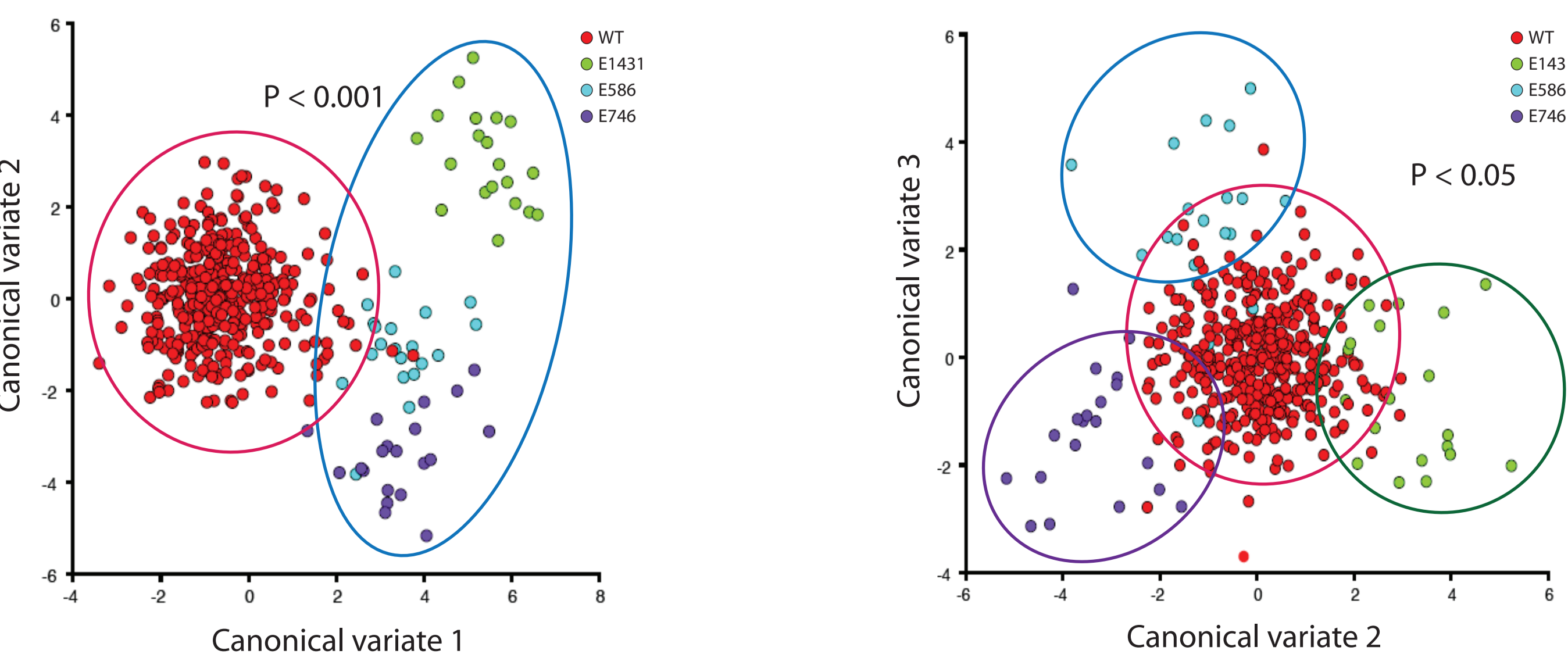
4 Micro-CT scans

Morphometric analysis of WT and KO adult skulls (8 weeks old)

- 20 adult KO mice / genotype
- 60 matched WT (littermates) with same genetic background (C57/Sv129)
- Additional matched WT (non-littermates) with same genetic background
- Analysis of 50 hallmarks



All 3 KO lines significantly differs from WT



CAV1: 66% of observed variance
CAV2: 24% of observed variance
CAV3: 9% of observed variance