Microplate-based 3D Culture System to Screen for Hedgehog Pathway Gene: Environment Interactions that Cause Orofacial Clefting

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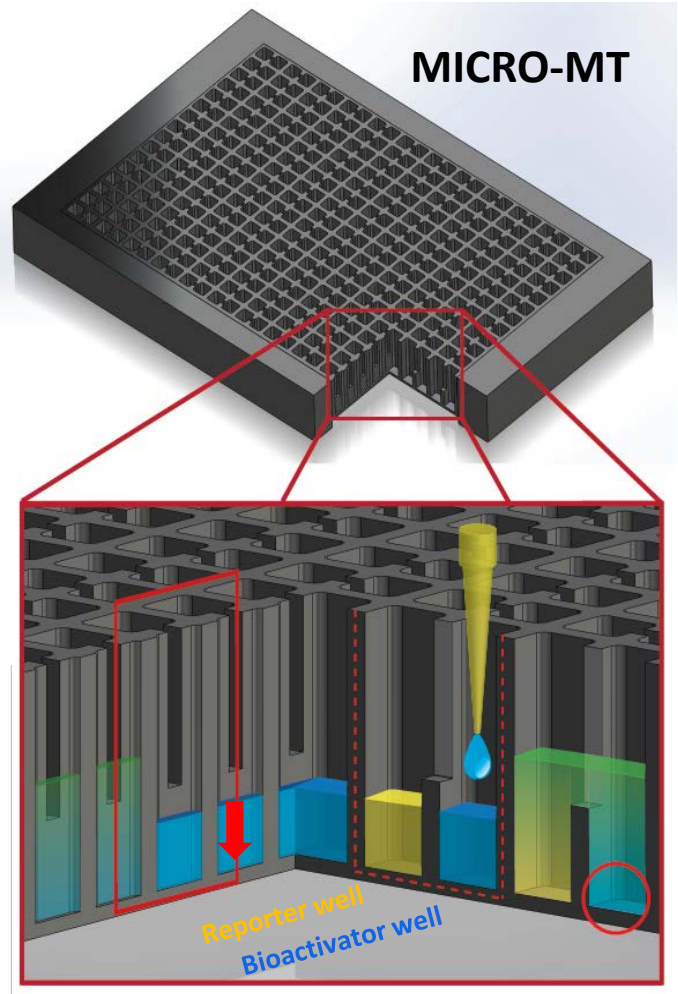
Conflict of Interest

Brian Johnson owns equity in Onexio Biosystems. A company that develops solutions for high-throughput toxicity testing.

TRANSFORM TOX TESTING CHALLENGE
INNOVATING FOR METABOLISM

ONEXIO



High-throughput co-culture
TTTC Semi-finalist-ongoing

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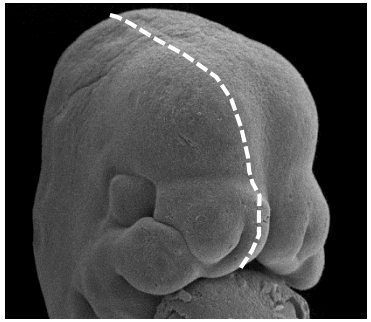
Gene-environment interactions in OFCs

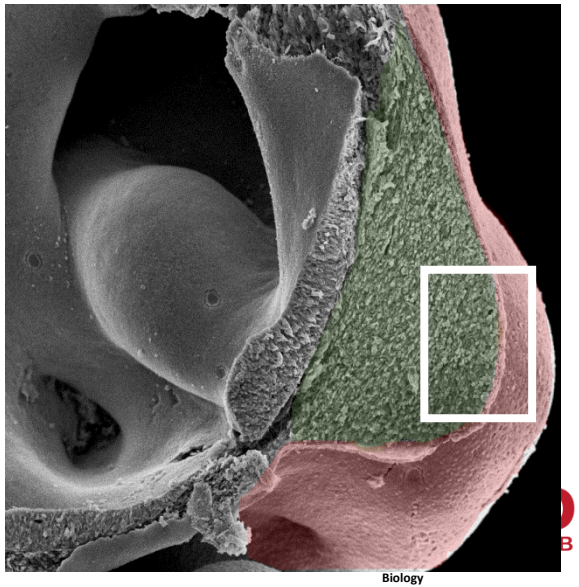
- Most birth defects are thought to result from a complex interaction of genetic and environmental factors
- OFC etiology
  - Several OFC genes have been identified but these do not act with Mendelian inheritance
  - Environmental factors have also been associated that elevated risk ratio
- Prevention strategies for OFCs have not been developed because our understanding of causative factors is inadequate

Prevention strategies

Epithelial-mesenchymal interactions drive outgrowth of the facial processes

- The tissues that form the upper lip and palate are primarily comprised of surface epithelium covering cranial neural crest-derived mesenchyme
- Outgrowth of the tissues that form the upper lip and palate is dependent upon dynamic molecular cross talk between the epithelium and mesenchyme
- Multiple pathways involved
  - WNT
  - BMP
  - FGF
  - Hedgehog (Hh)
    - SHH from the surface epithelium activates pathway activity in the adjacent mesenchyme

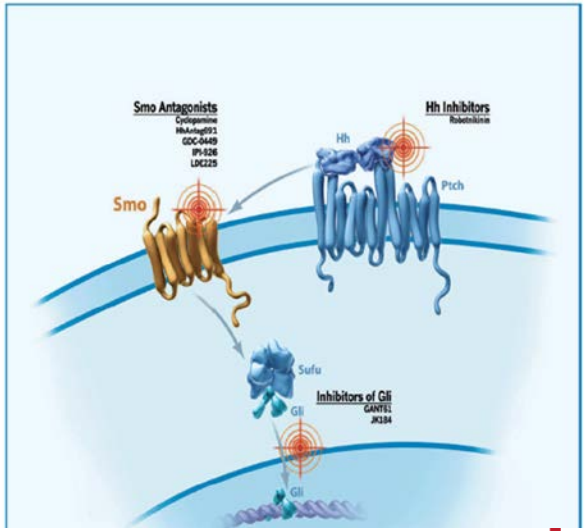




Genetic and environmental disruption of Hh signaling

- Mutations in core Hh pathway genes (eg. *SHH, GLI2*, and *PTC1*) as well as putative downstream targets (eg. *FOXF2*) have been linked by case studies to syndromic and non-syndromic OFCs

- The pathway is inherently sensitive to small molecule modulation by environmental compounds
  - Cyclopamine-like dietary alkaloids
  - Natural and synthetic pharmaceuticals
  - A common pesticide synergist



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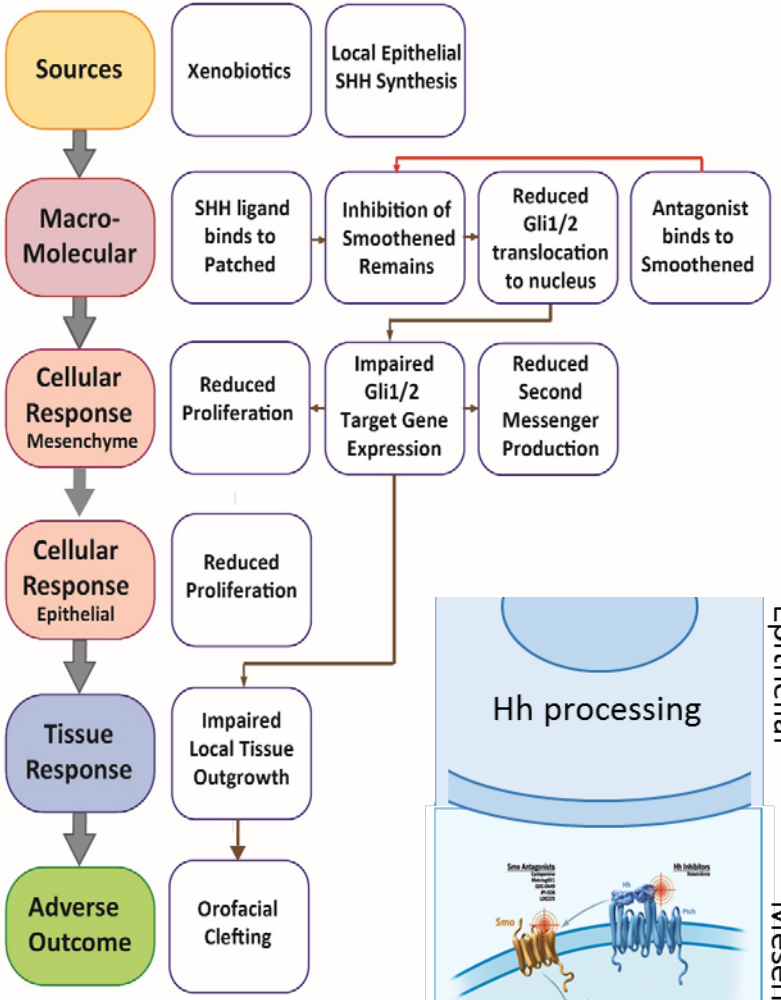
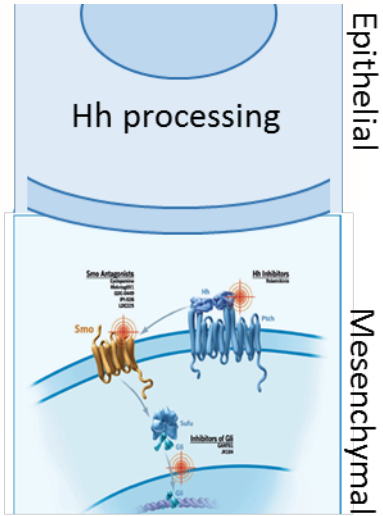
Building a Microphysiological Model for Hh Pathway Antagonism Leading to OFCs





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The AOP guides design ensuring incorporation of key molecular, cellular and tissue level components and endpoints

- Epithelial:Mesenchymal organization
- Directed Hh gradient and outgrowth
- Tissue fusion

- Low/Medium throughput compatible
  - Fluorescent and Luminescent readouts
  - Format/Material (PDMS, PS, etc.)
  - Compatible with high content imaging
  - Chemical sequestration (PS)

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Microphysiological Model Design

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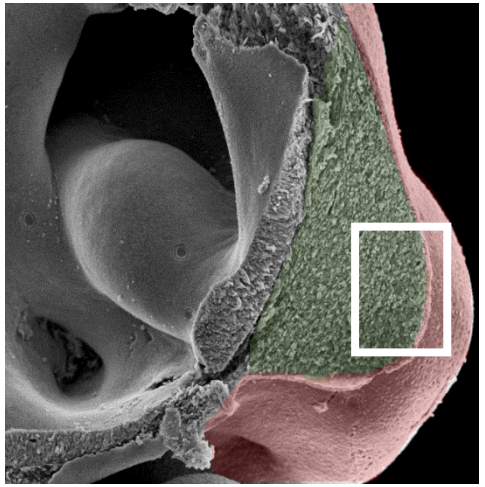
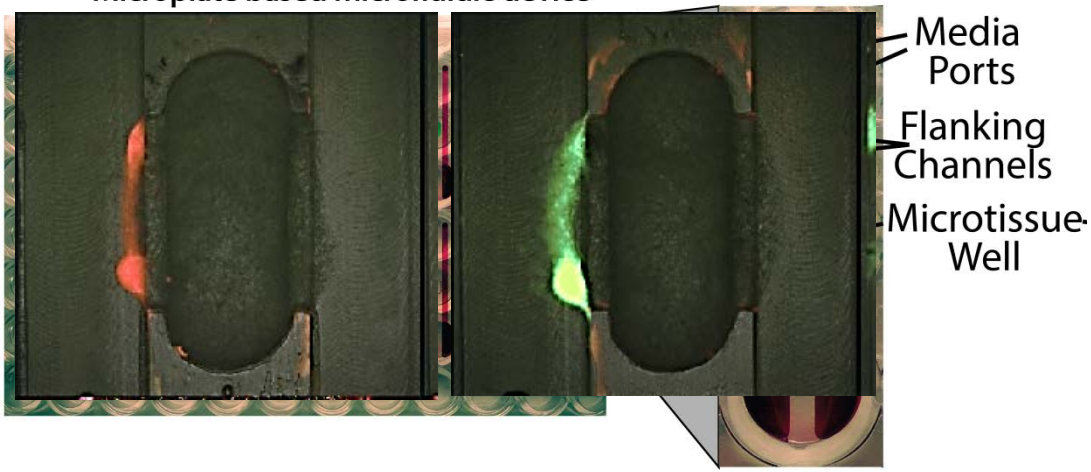
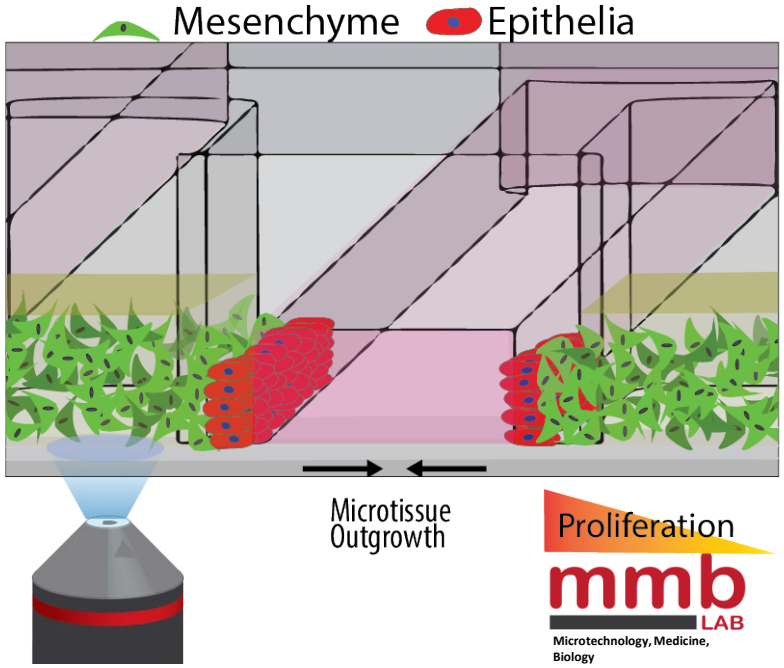


Microplate based microfluidic device





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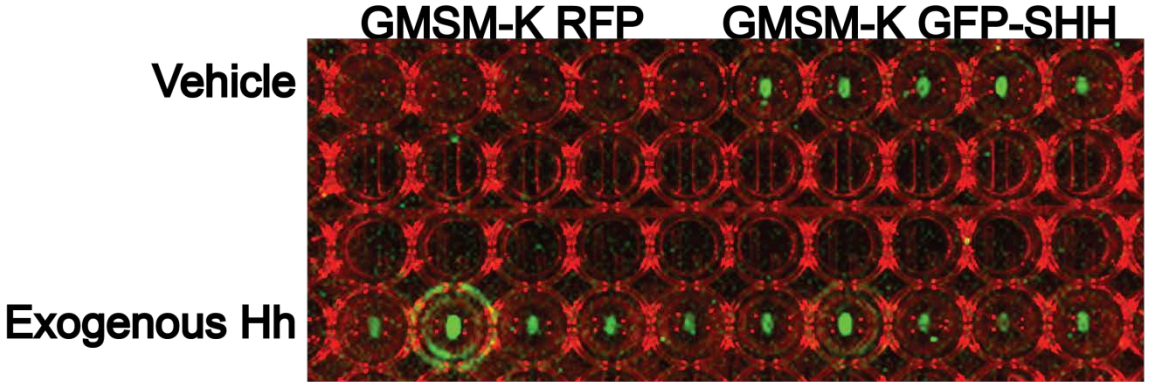
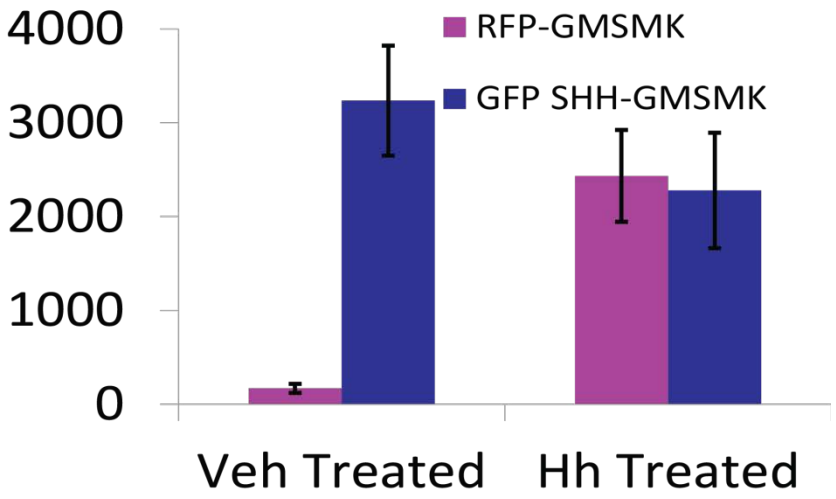
Exogenous and Endogenous Hh Pathway Response





- Live cell luciferase activity.
  - Red Brightfield
  - Green Luminescence
- Exogenous Shh activates Gli driven luciferase in 3T3 cells.
- Endogenous SHH secreted by overlaid GMSM-K cells activates Gli driven luciferase in 3T3 cells.
- Combination indicates max responsiveness

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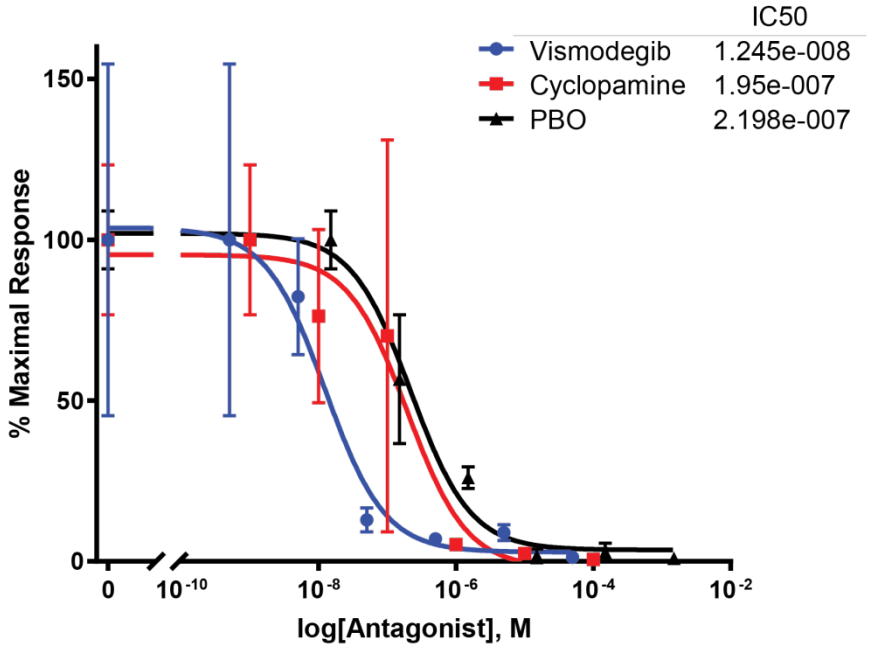
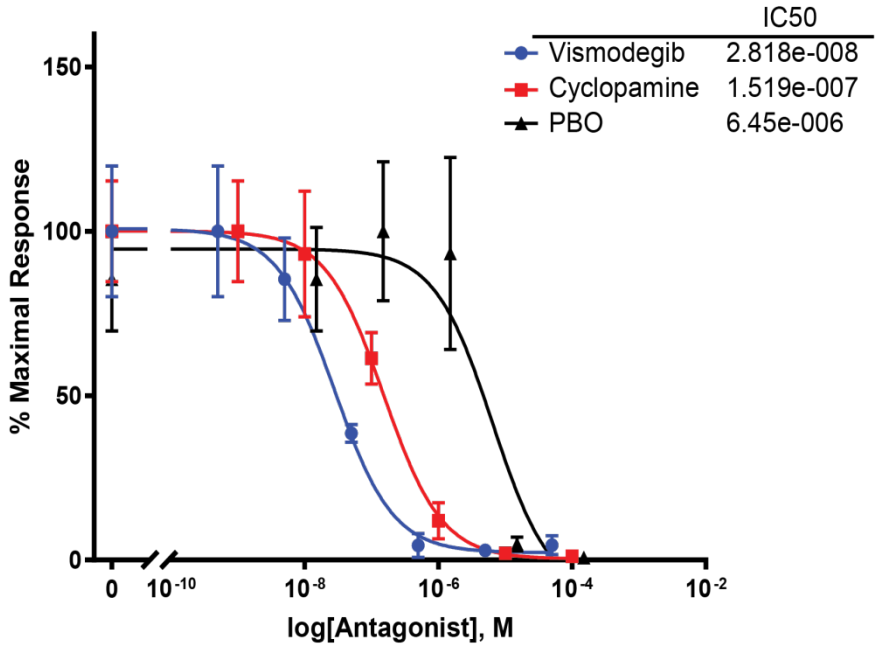
Antagonism of Endogenous Hh Pathway Response





- Hh antagonists Vismodegib, Cyclopamine and Piperonyl Butoxide.
- Microtissues dosed for 3 days show dose response.
- Plates fully recovered Hh activity after treatment.
- Microtissues re-dosed in reverse at 21 days show similar dose-response curves

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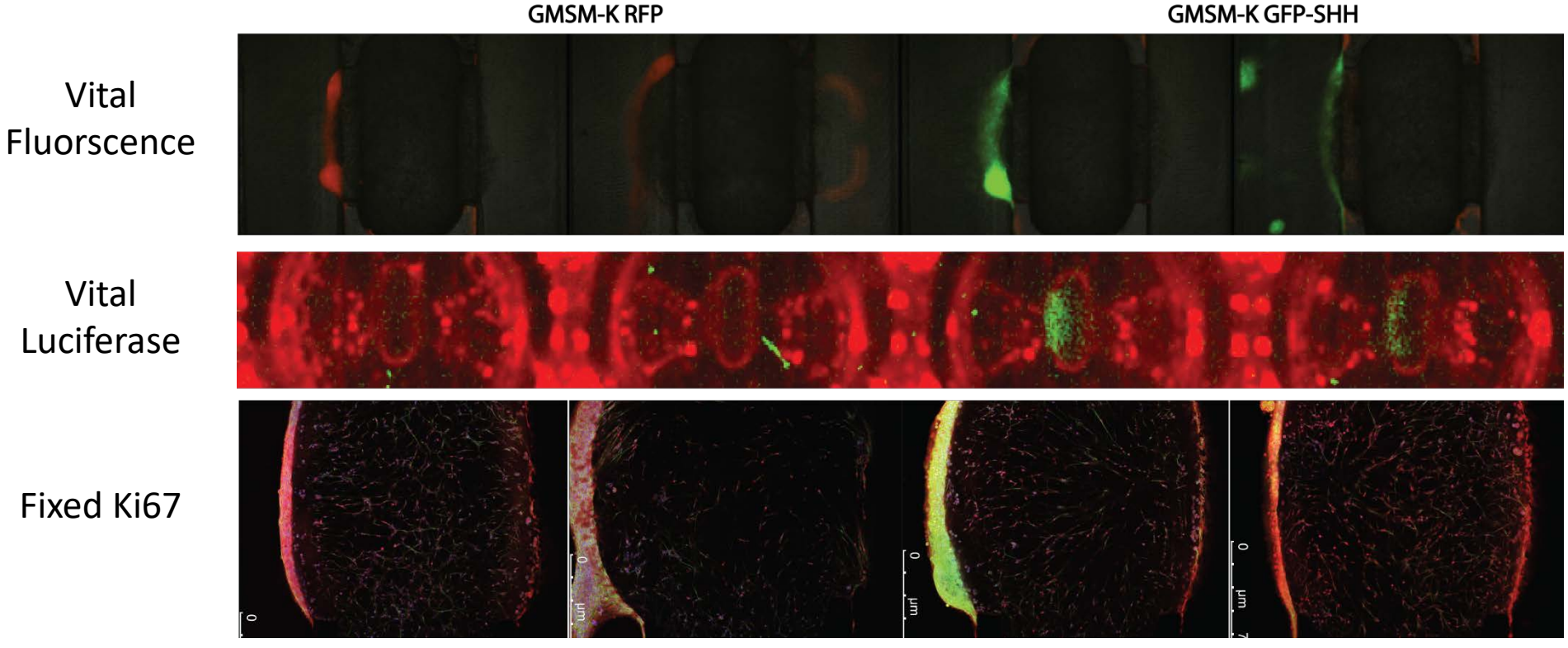
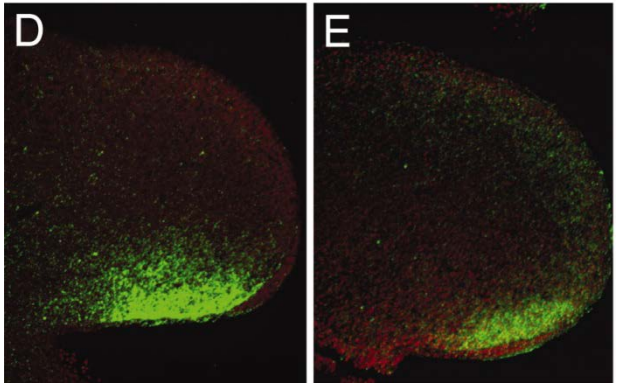
Matched High-Content Imaging



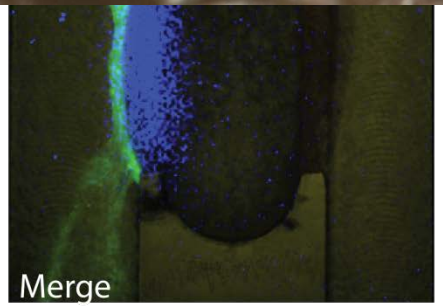
Antagonism of Endogenous Hh Pathway Activity

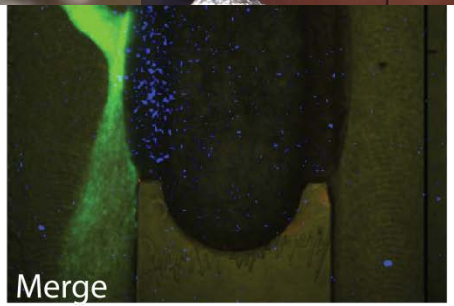
Hedgehog gradients drive developmental patterning throughout the body (shown - hind limb bud E10.5)

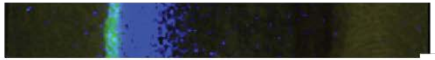


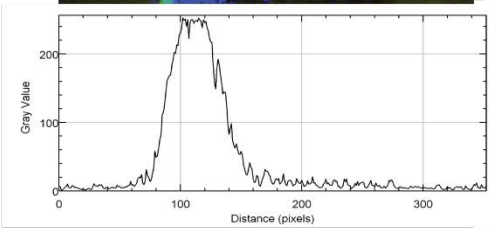
Yina Li et al. PNAS 2006;103:6548-6553

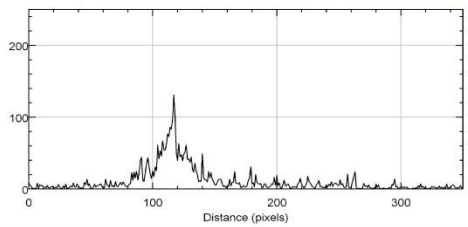
Gli
Luciferase
Epithelia
Stroma











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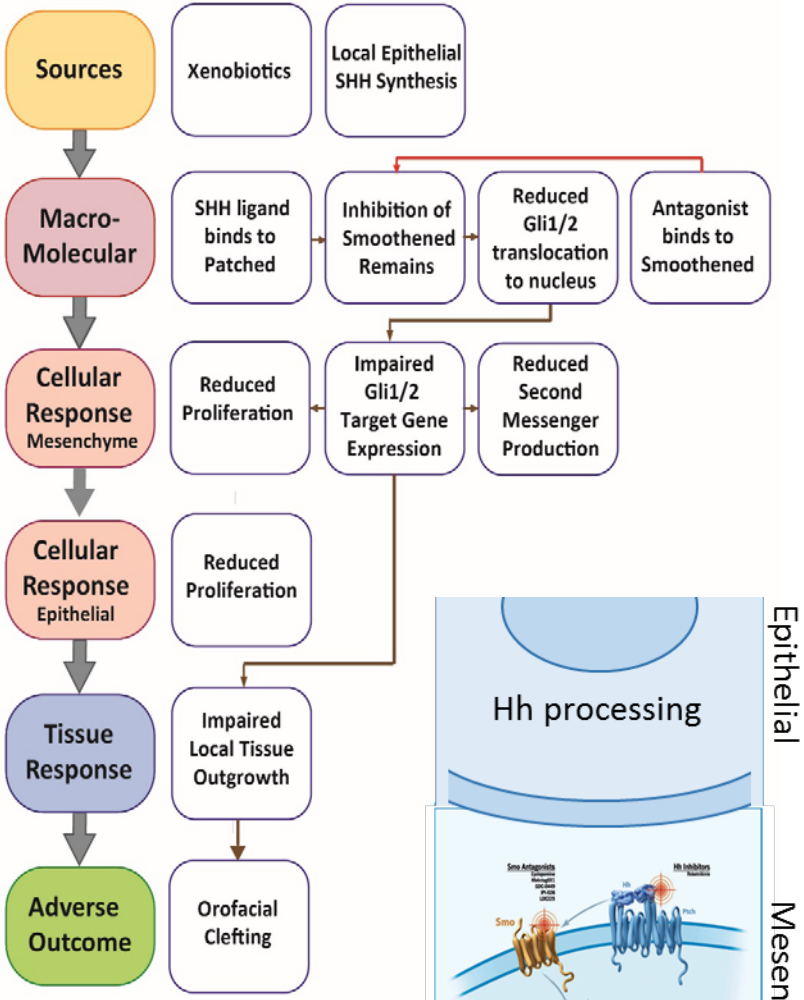

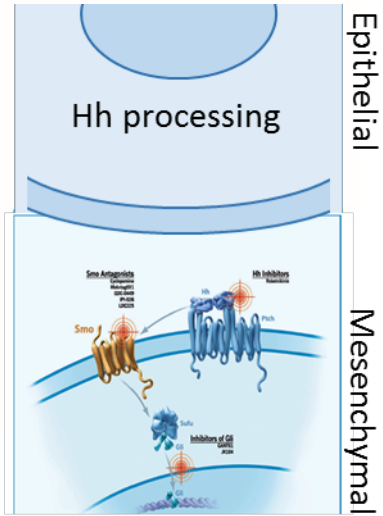

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Summary

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  - RO3, K99 pending

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