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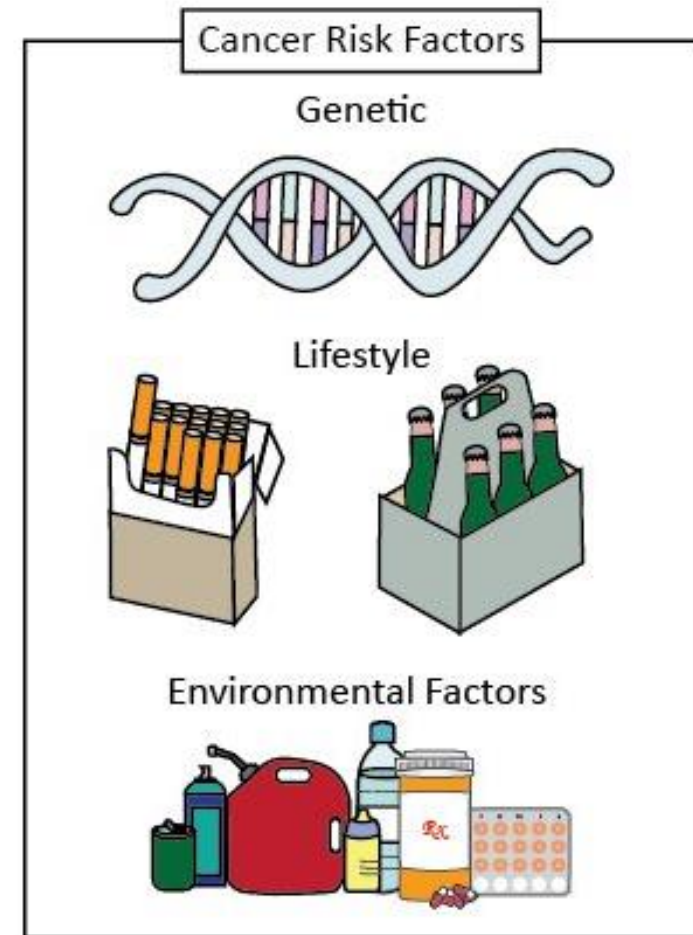
Utilizing an AOP-based approach in the development of a hormonally responsive organotypic breast model

Molly M. Morgan

Dr. David Beebe; MMB Lab

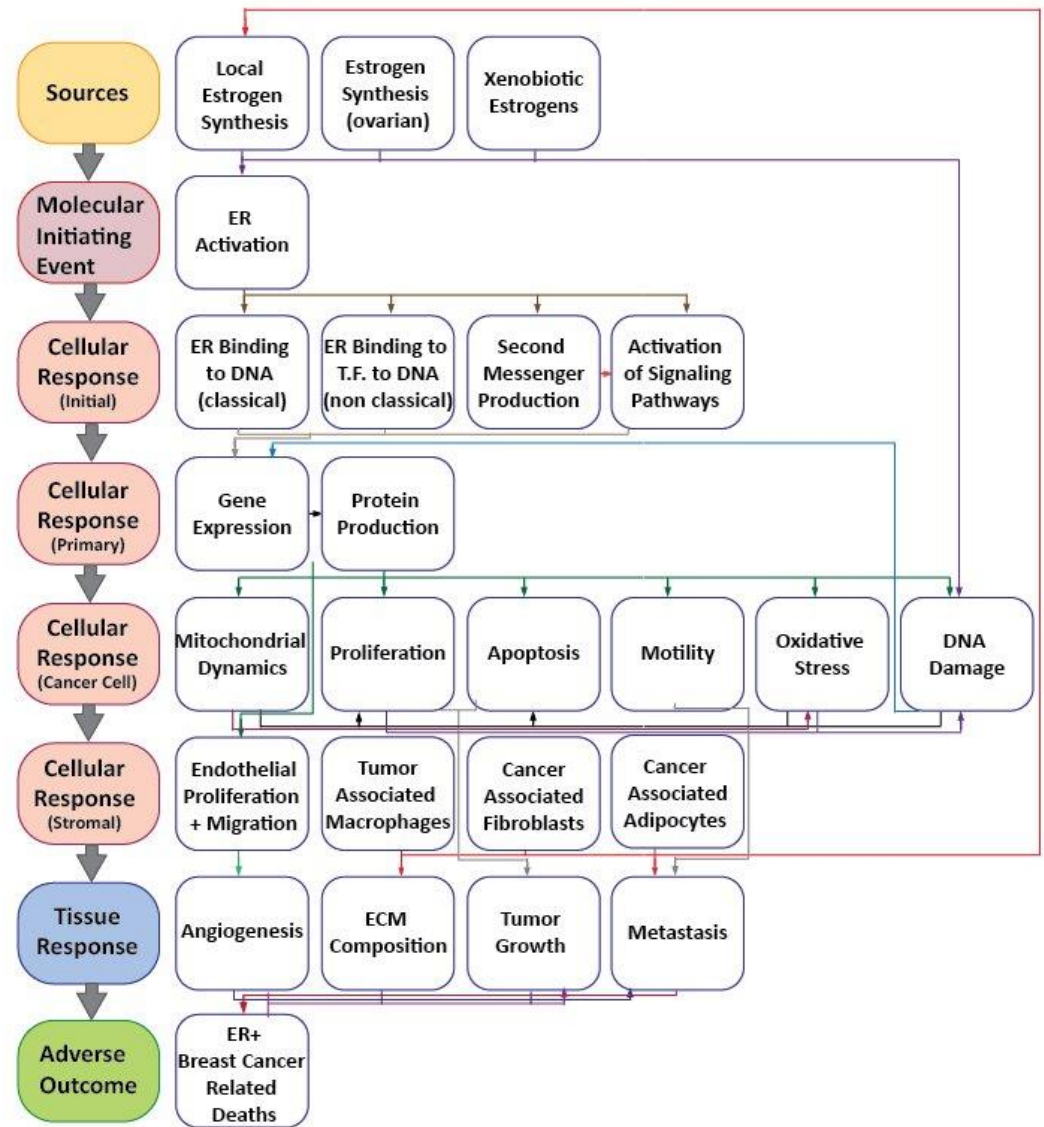
ER activation stimulates breast cancer progression

- ~75% of breast cancer cases express estrogen receptor α (ER)
- ER ligand binding is promiscuous
- Highlights a need for a hormonally responsive organotypic breast model that can identify estrogenic compounds that increase breast cancer risk



Utilizing AOPs to build *in vitro* models: a 2 part approach

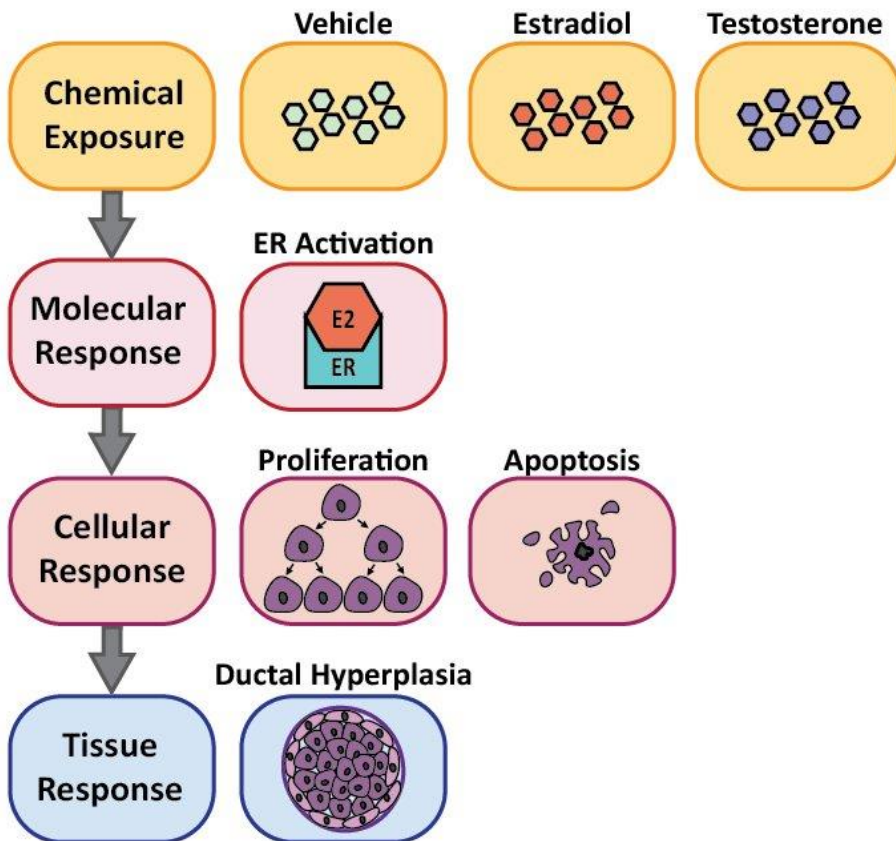
- We have developed the ER-mediated breast cancer AOP to facilitate model development
- We use the AOP to identify:
 - ER-driven readouts that lead to breast cancer
 - Cell types critical in disease progression



Morgan & Johnson et al., 2016

Part I: Develop ER-driven readouts on a molecular, cellular and tissue level

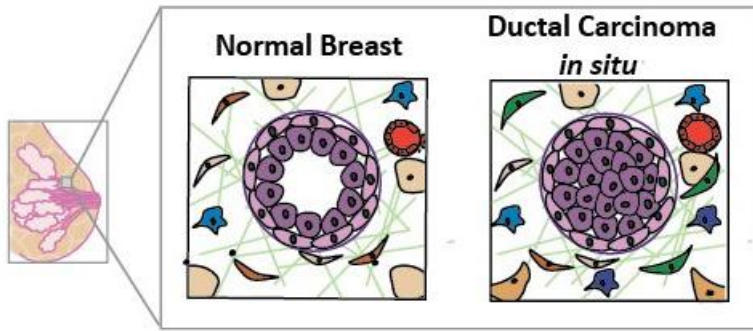
AOP-Based Readouts on a Molecular, Cellular and Tissue level



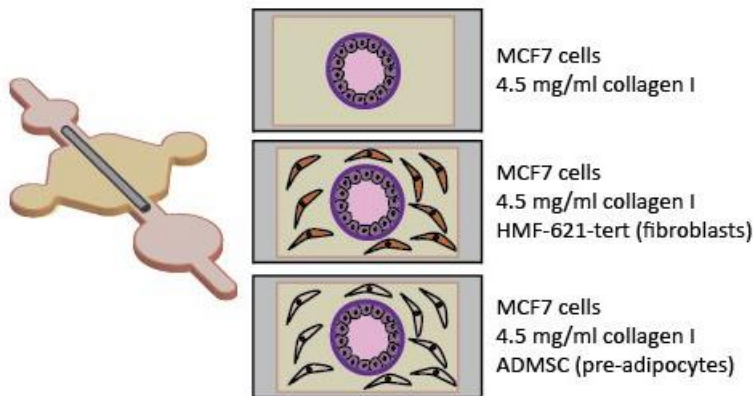
- **Current Approach:** Evaluates one response on a molecular or cellular level
- **Multiplexed Approach:** Evaluates ER-driven responses on a molecular, cellular and tissue level
- **Goal:** Identify chemicals that increase breast cancer risk through ER-dependent mechanisms as well as ER-independent mechanisms

Part II: Integrate cellular components critical to chemical response

The mammary microenvironment is complex:
cancer progression involves many cell types

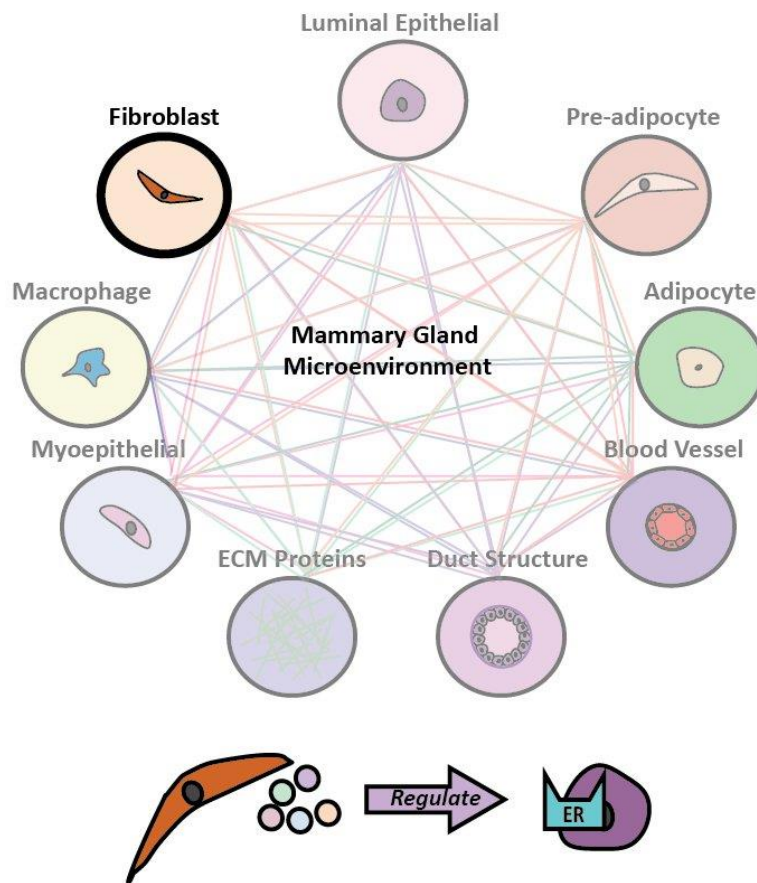


Reconstructing the mammary duct *in vitro*:
what cell types are critical to predicting chemical response?



- **Current Approach:** Evaluates chemicals on the cancer cell alone
- **Coculture Approach:** Evaluates chemicals on the cancer cell along with supportive cell types, because chemical toxicants could:
 - Alter stromal cell function
 - Disrupt stromal-epithelial interactions
 - Undergo stromal mediated metabolism
- **Goal:** Evaluate the need of stromal cells in ER+ breast cancer screening platforms

Are fibroblasts important when evaluating xenoestrogens?

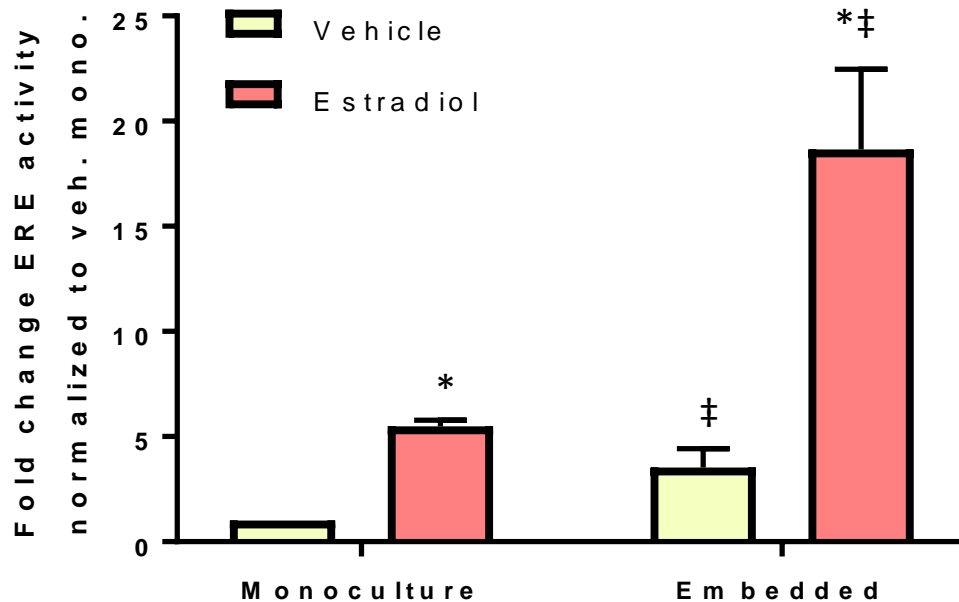
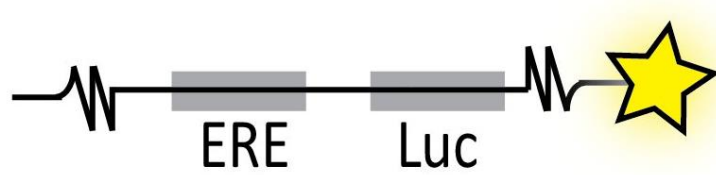


- Fibroblasts regulate ER expression in breast epithelial cells^{1,2}
- Is this significant when evaluating environmental chemicals?
 - Do fibroblasts modulate response to xenobiotics on a molecular, cellular and tissue level?

¹H. Brechbuhl et al., 2016

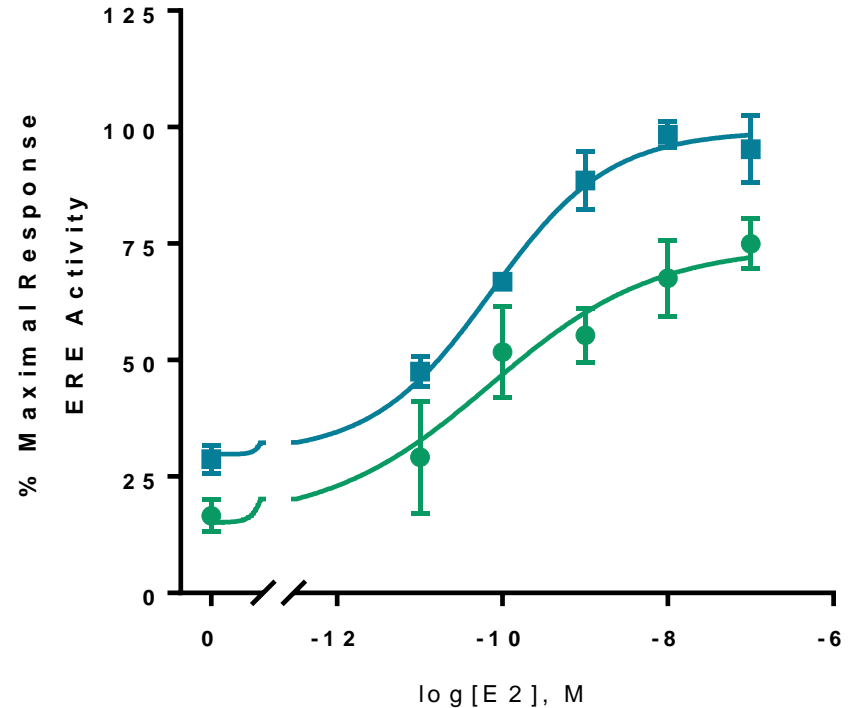
²J. Lang et al., 2013

ER transactivation is increased when fibroblasts are included in the matrix



* = vs. respective vehicle ($p < 0.05$)

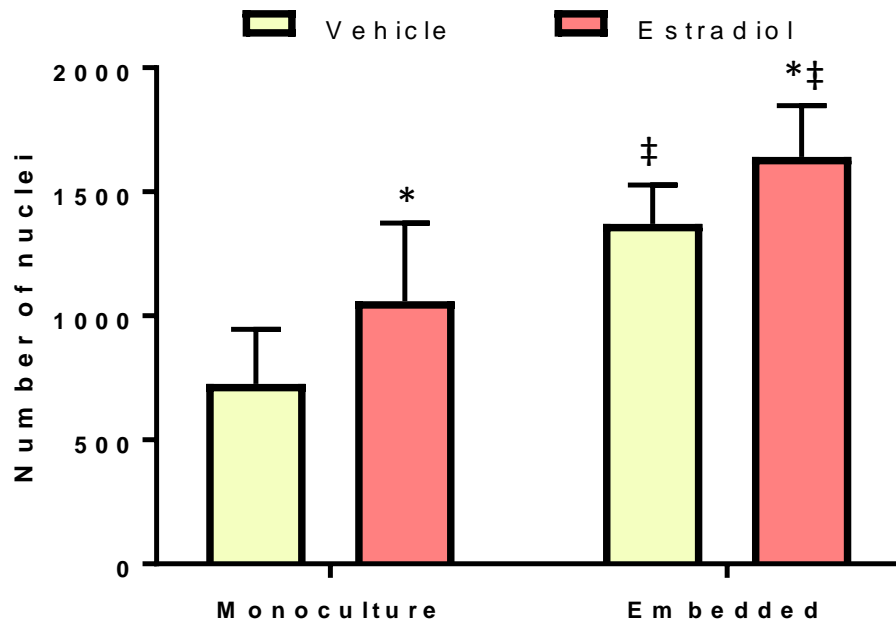
‡ = vs. respective monoculture ($p < 0.05$)



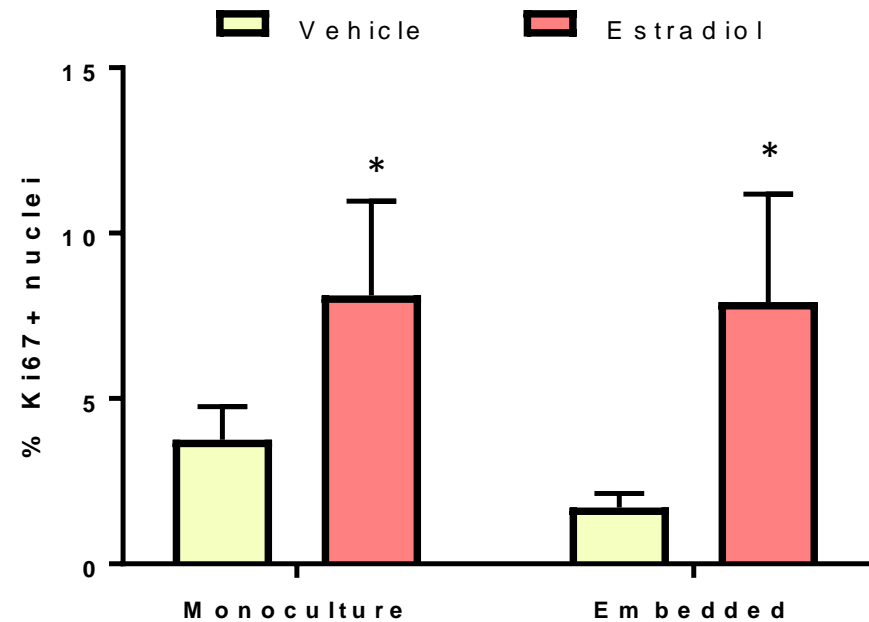
	EC50
● Monoculture	1.18e-10
■ Embedded	6.42e-11

Fibroblasts increase total epithelial cell number, but have no effect on proliferation

Cell Number

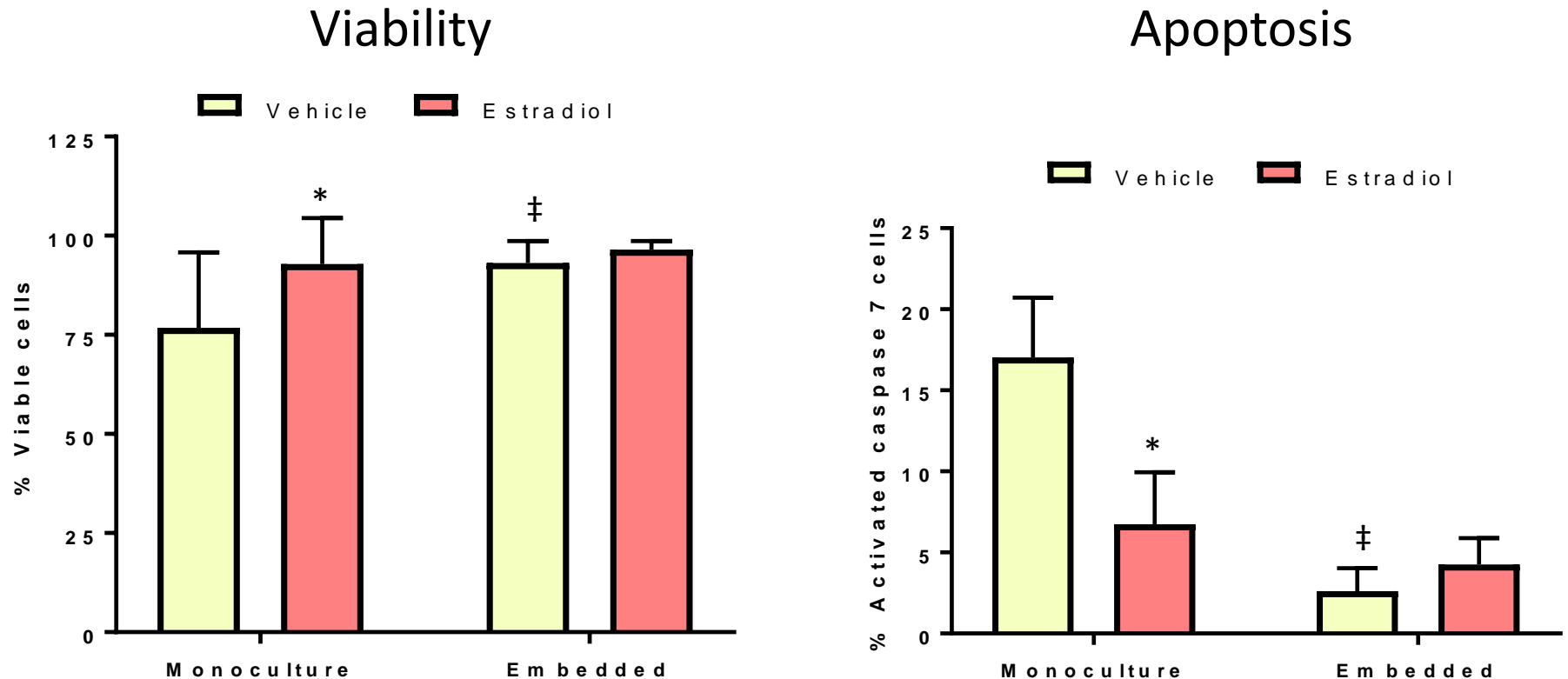


Proliferation



* = vs. respective vehicle (p < 0.05)
‡ = vs. respective monoculture (p < 0.05)

In the presence of stromal cells, epithelial cell viability is increased and apoptosis is decreased



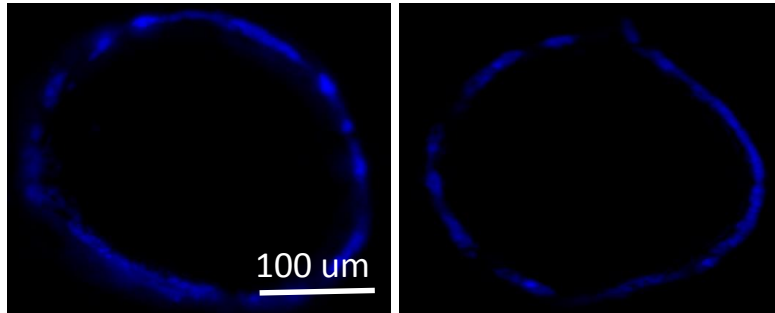
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‡ = vs. respective monoculture ($p < 0.05$)

Ductal hyperplasia is more prominent and seen at sooner time points in coculture

3 day exposure

Vehicle

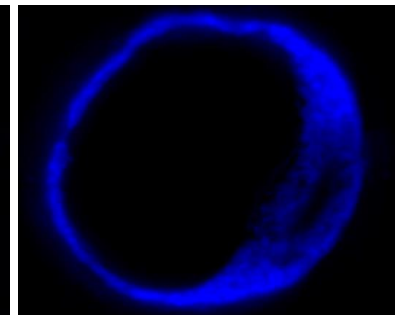
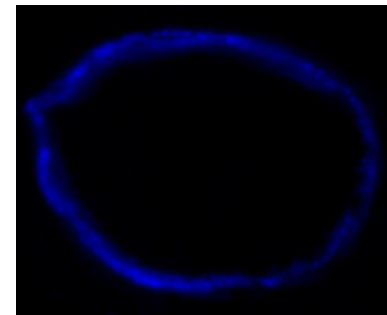
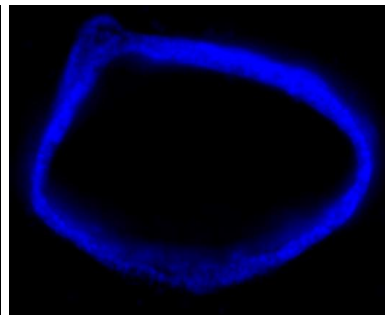
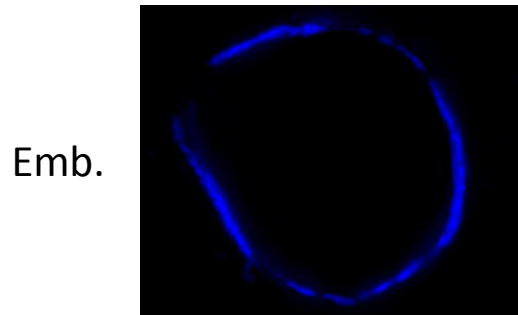
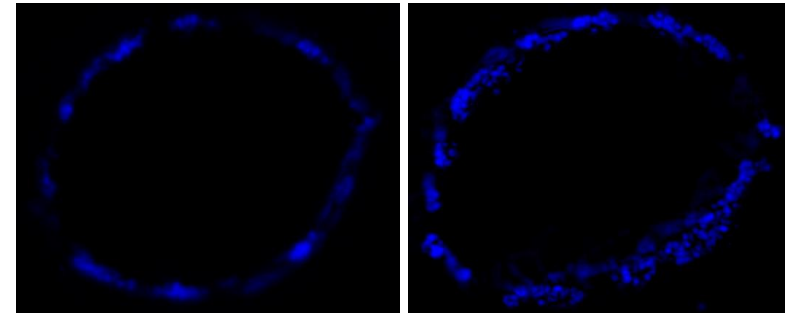
100 nM E2



10 day exposure

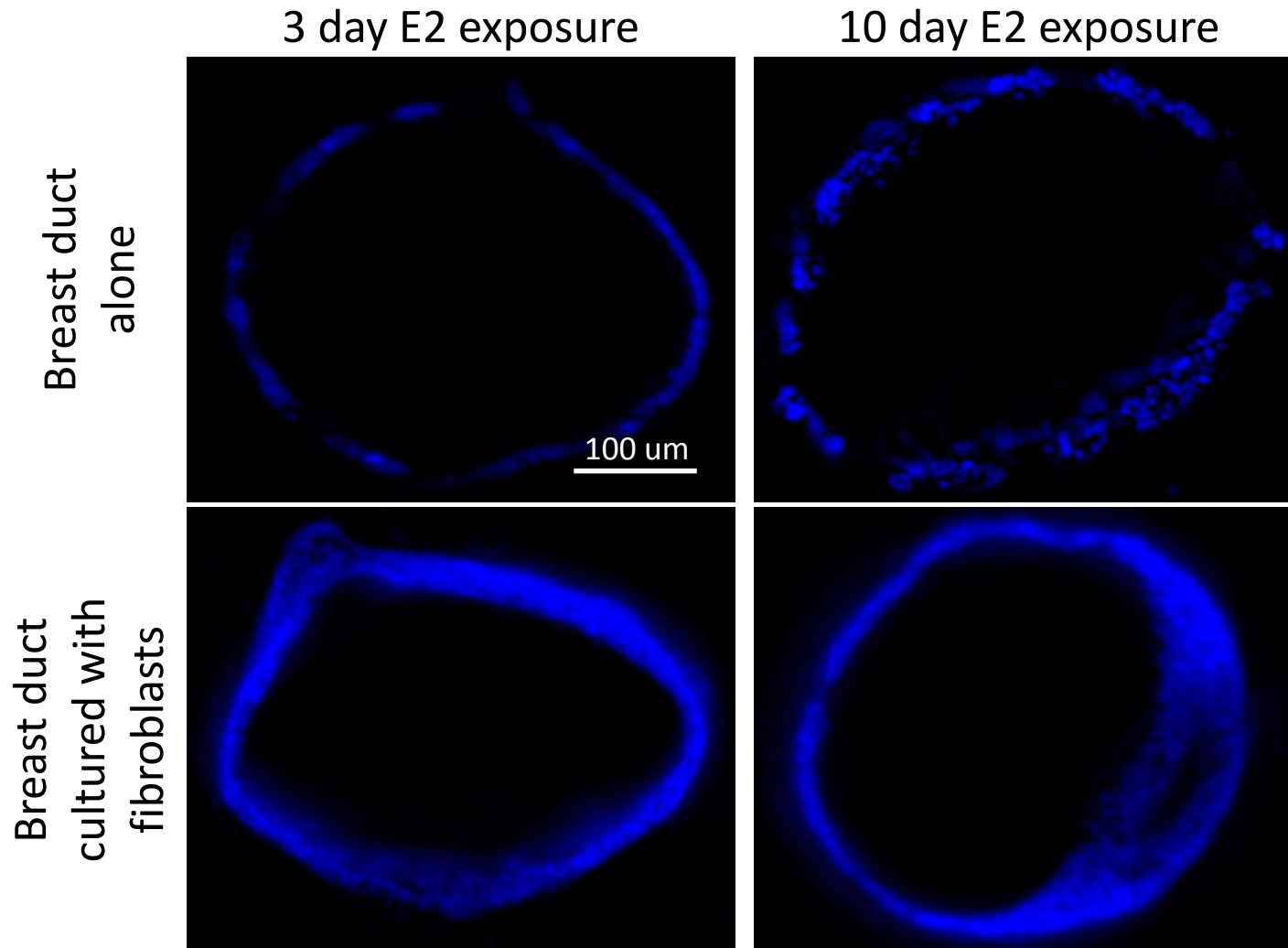
Vehicle

100 nM E2

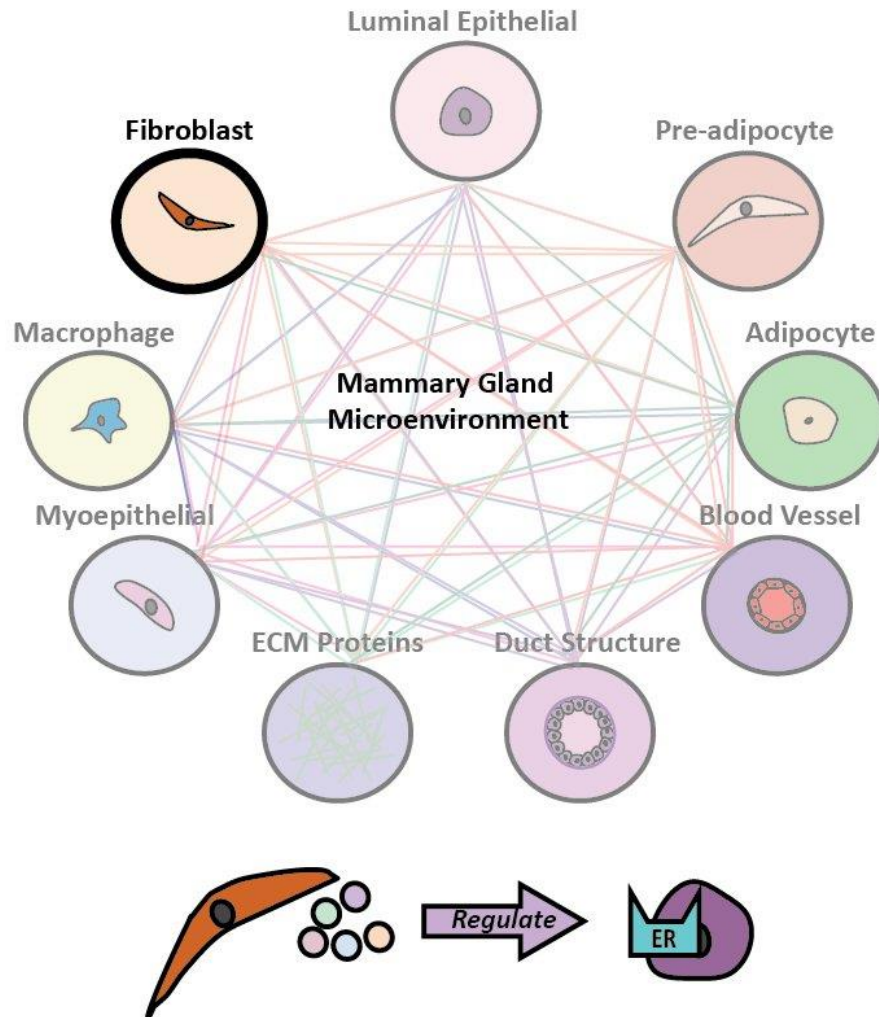


Nuclei

Ductal hyperplasia is more prominent and seen at sooner time points in coculture



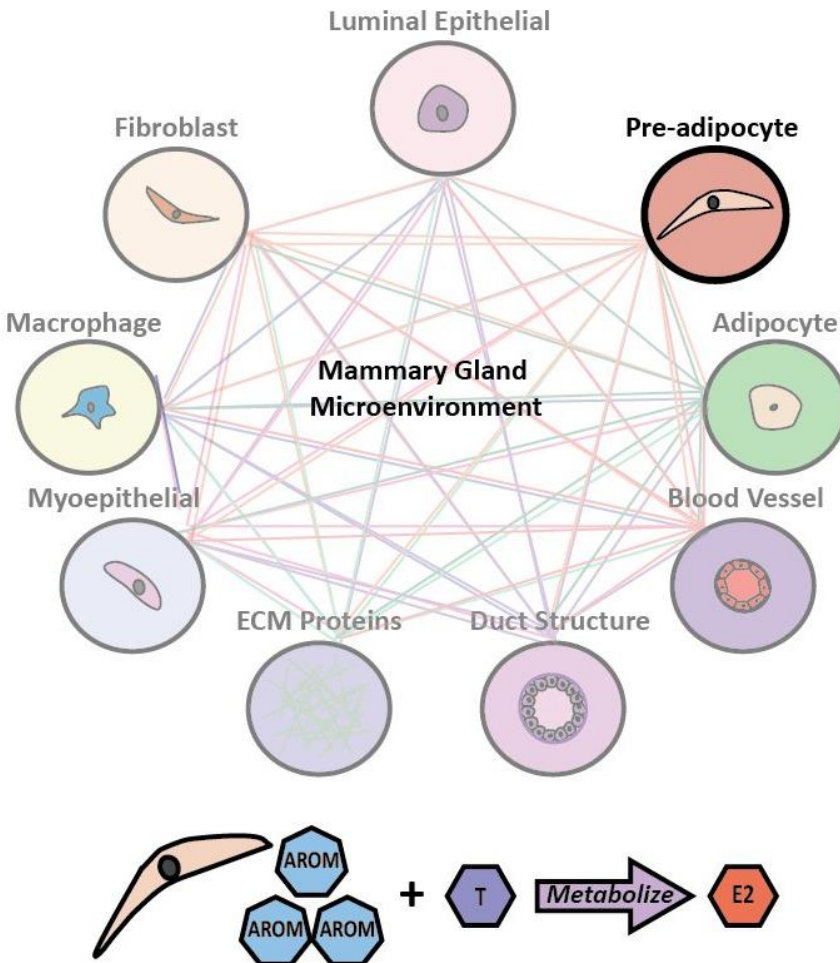
Summary: Fibroblasts modulate epithelial response to ER ligands



- When fibroblasts are included in the matrix surrounding the breast duct, we found:
 - **Molecular level:** an increase in base ERE activity, maximal response, and a two-fold reduction in EC50
 - **Cellular level:** an increase in total cell number and duct viability, and a decrease in apoptosis
 - **Tissue level:** an increase in estrogen-induced hyperplasia

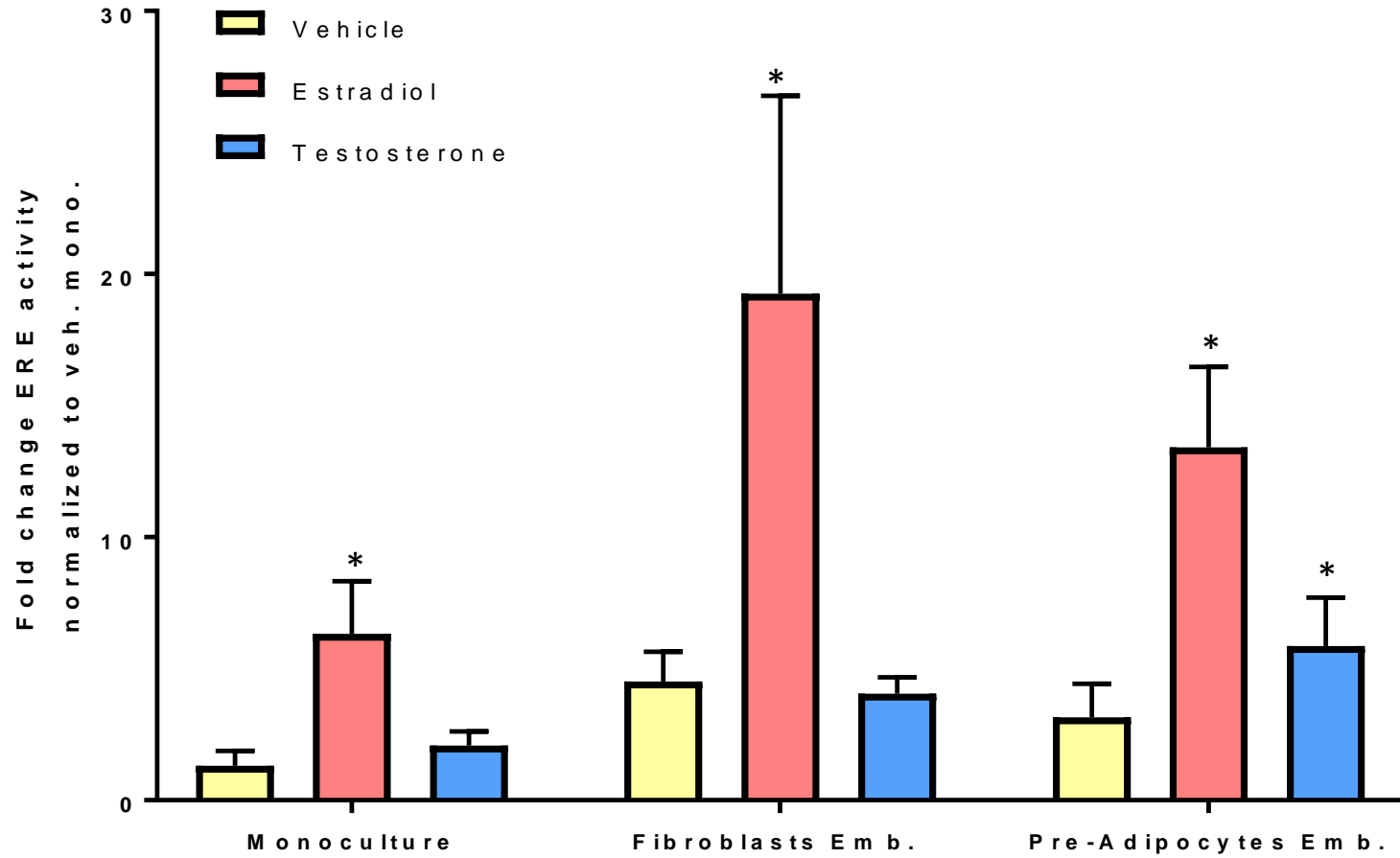
Are pre-adipocytes important when evaluating xenoestrogens?

- In the breast, pre-adipocytes produce the enzyme aromatase^{1,2}
- Aromatase metabolizes T → E2
- Is this significant when evaluating environmental chemicals?
- Do pre-adipocytes modulate response to xenobiotics on a molecular, cellular and tissue level?



¹A. Bielli et al., 2014
²W. Miller et al., 1998

Testosterone induces ER activation when pre-adipocytes are included in matrix



* = vs. respective vehicle (p<0.05)

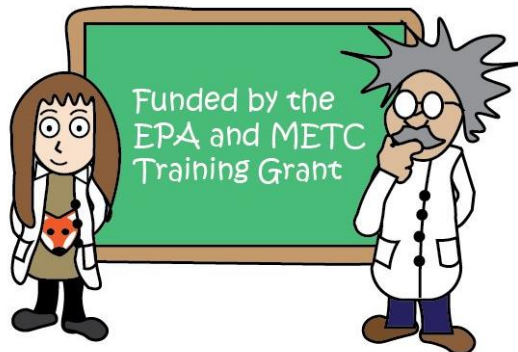
Future directions

- While stromal cells influence epithelial cell behavior and estrogenic response, are they **needed** when evaluating xenoestrogens?
 - ✓ Complexity v. simplicity
- Highlights a need to conduct some preliminary screens to evaluate relevance of our complex system
 - ✓ How do our hits match the hits of validated *in vitro/in vivo* assays?

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