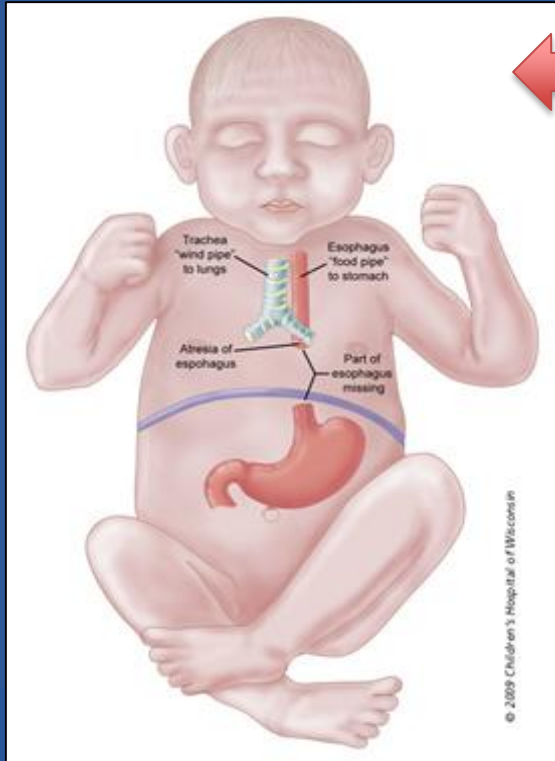


Molecular Mechanisms of Esophageal Epithelial Regeneration Following Repair of Surgical Defects with Acellular Silk Fibroin Grafts

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Esophageal Diseases and Prevalence



Atresia/TEF

1 in 4425 births

Strictures

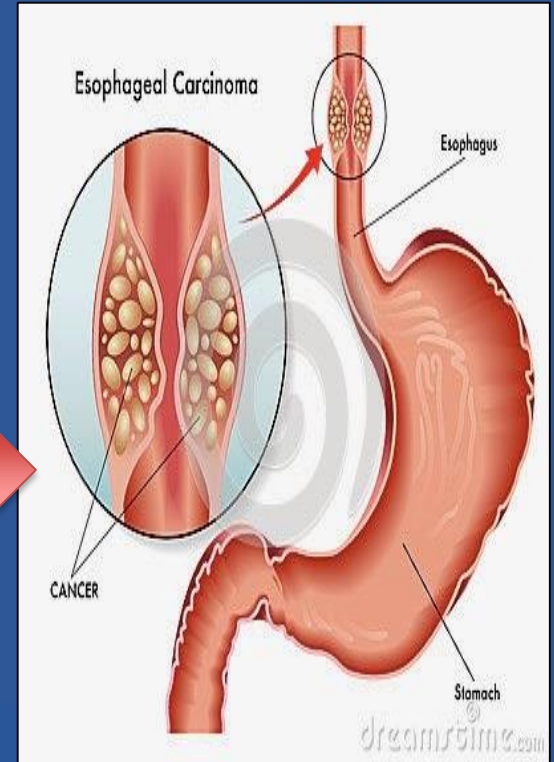
23% of patients
with reflux

Esophageal Cancer

6th leading cause of
cancer death

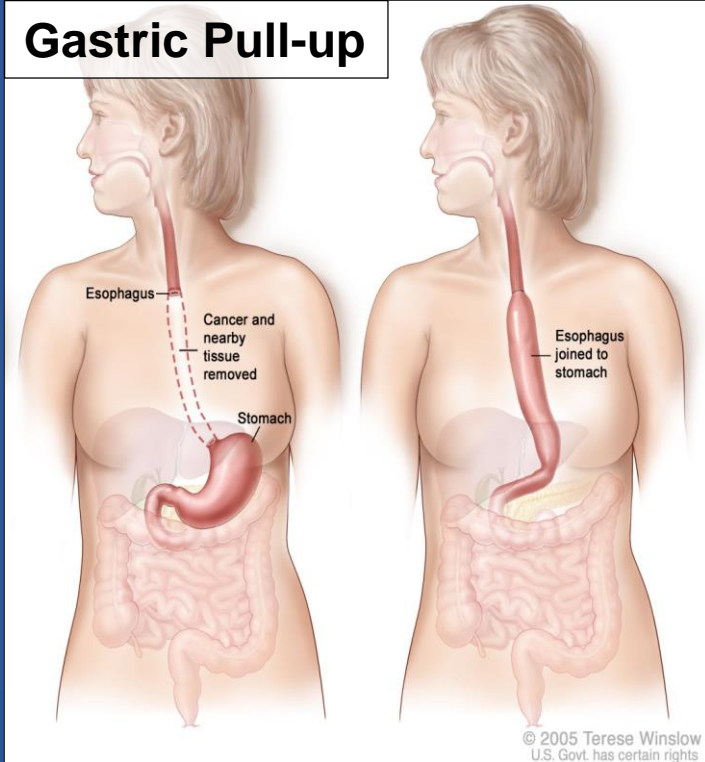
Barrett's Esophagus

20% of patients
with reflux

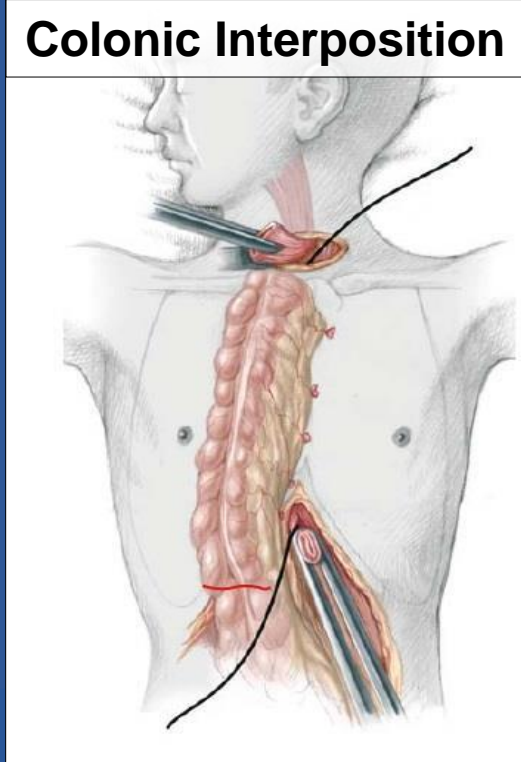


Current Treatment Options and Complications

Gastric Pull-up



Colonic Interposition



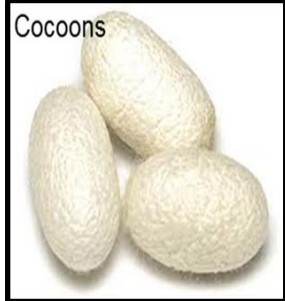
- **Anastomotic leakage (12-29%)**
- **Strictures (19-53%)**
- **Dysmotility and dysphagia (5-25%)**
- **Donor site morbidity (26-55%)**
- **Death (3-6%)**

~700 procedures/year in US

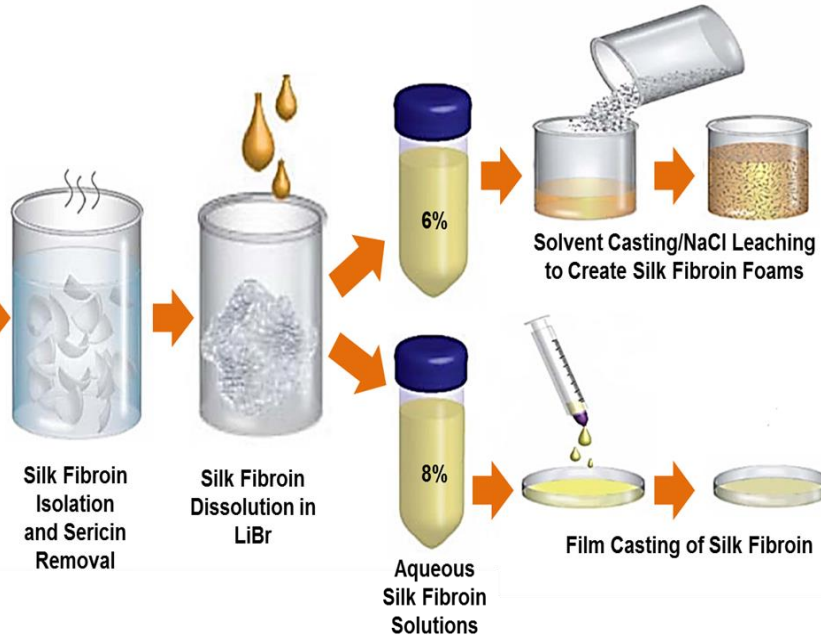
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Bi-Layer Silk Fibroin (BLSF) Grafts for Esophageal Tissue Reconstruction

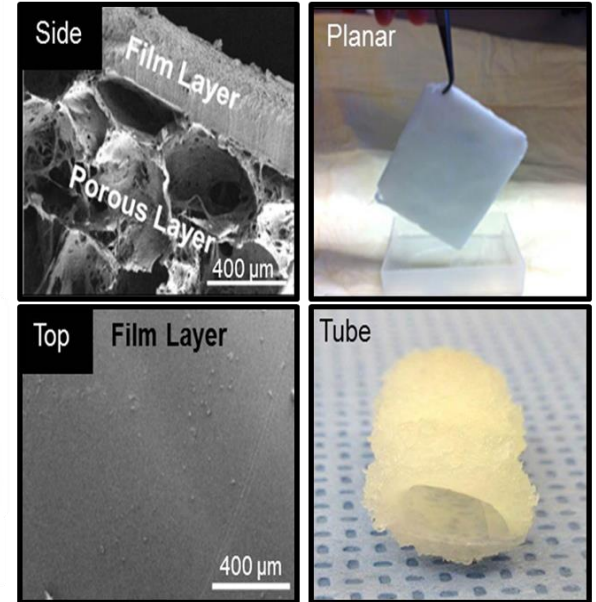
Silkworm Cocoons



Silk Processing and Graft Fabrication

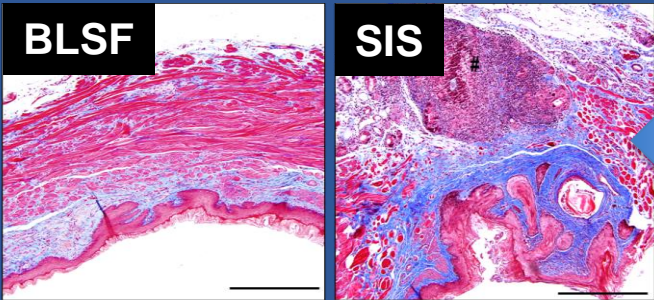


Bi-Layer Silk Fibroin Graft



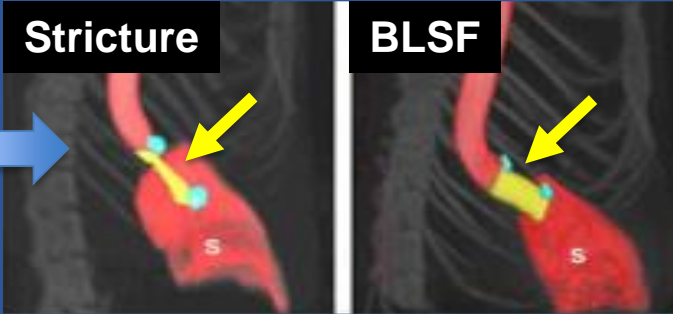
Performance of BLSF Grafts in Animal Models of Esophageal Repair

Onlay Esophagoplasty

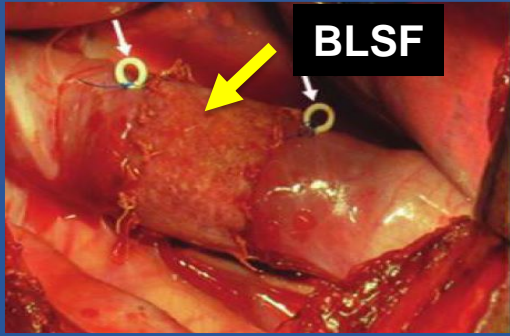


Rat Models
Algarrahi et al., 2015
Algarrahi et al., 2018a

Stricture Reconstruction



Tubular Esophagoplasty



Swine Models
Algarrahi et al., 2018b
Gundogdu et al., 2020

BLSF Repair- 3 months

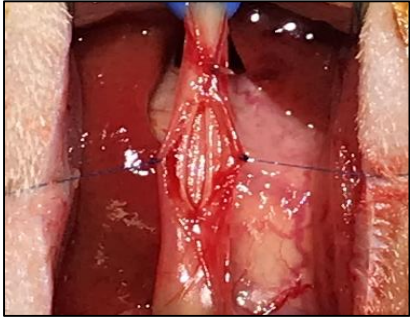


Goals and Experimental Design

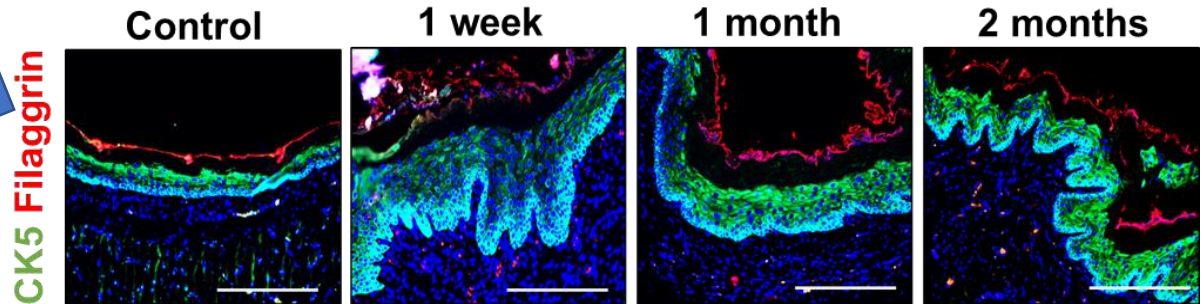
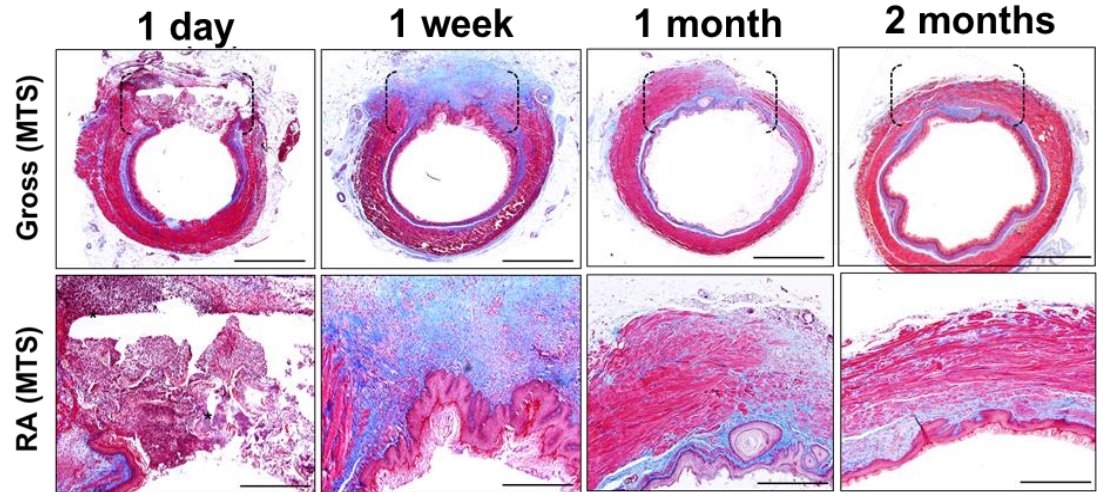
- Cell-free scaffolds rely on host progenitor cell populations to repopulate implant microenvironments and facilitate growth of de novo functional tissue.
- Intrinsic molecular mechanisms governing scaffold-mediated, constructive remodeling of esophageal defects are largely unknown.
- The main goals of our study were to (1) establish a temporal profile of the signaling cascades that occur during scaffold-mediated, constructive remodeling of the rat esophagus and (2) determine the significance and function of these pathways in neopithelial formation at graft sites.
- Mass spectrometry-based, quantitative proteomics and *in silico* Ingenuity pathway analysis were used to identify signal transduction clusters enriched during neopithelial formation. Pharmacologic inhibitor studies were employed to determine pathway involvement during regeneration.

Rat Onlay Esophagoplasty Model with BLSF Grafts

Surgical Patch Defect

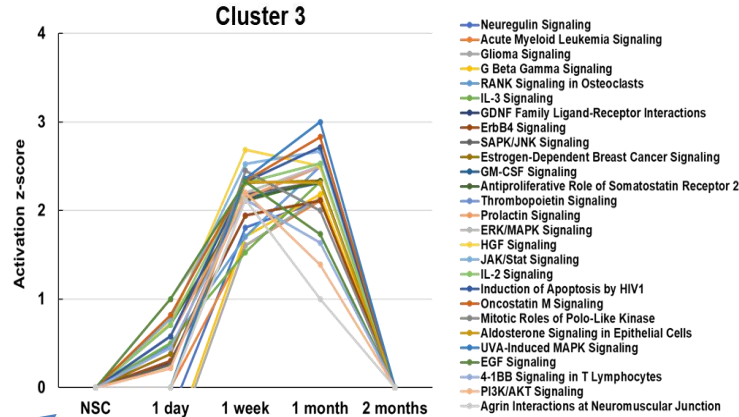
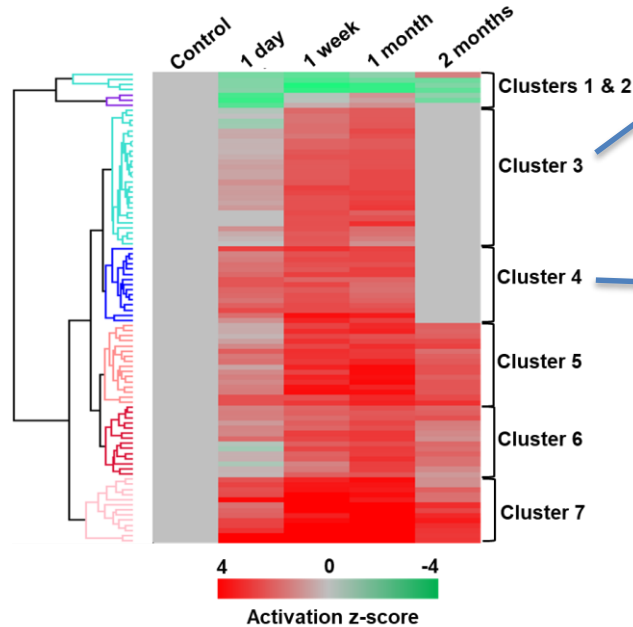


BLSF Implant

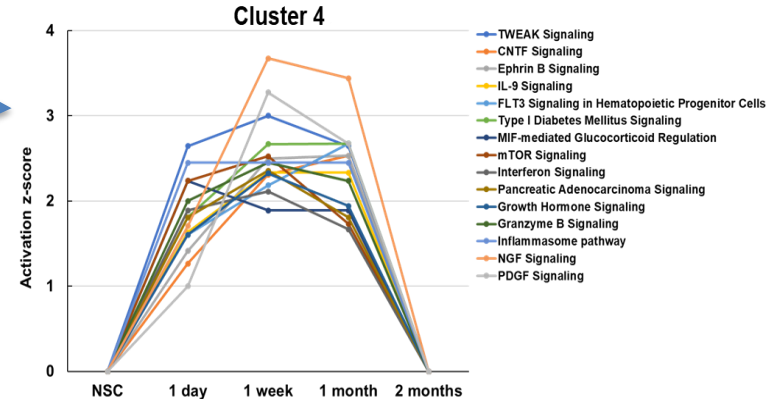


Proteomics Results

- 5,682 proteins; false discovery rate of 1%
- 4,150 proteins present in all samples
- Differentially expressed proteins (DEP):
q-values < 0.05; log2foldΔ > 0.348
- 340 pathways identified, p-value < 0.05 and
-2 > z-score > 2 at one timepoint/controls



HGF Signaling
EGF Signaling
PI3K/AKT Signaling
MAPK Signaling

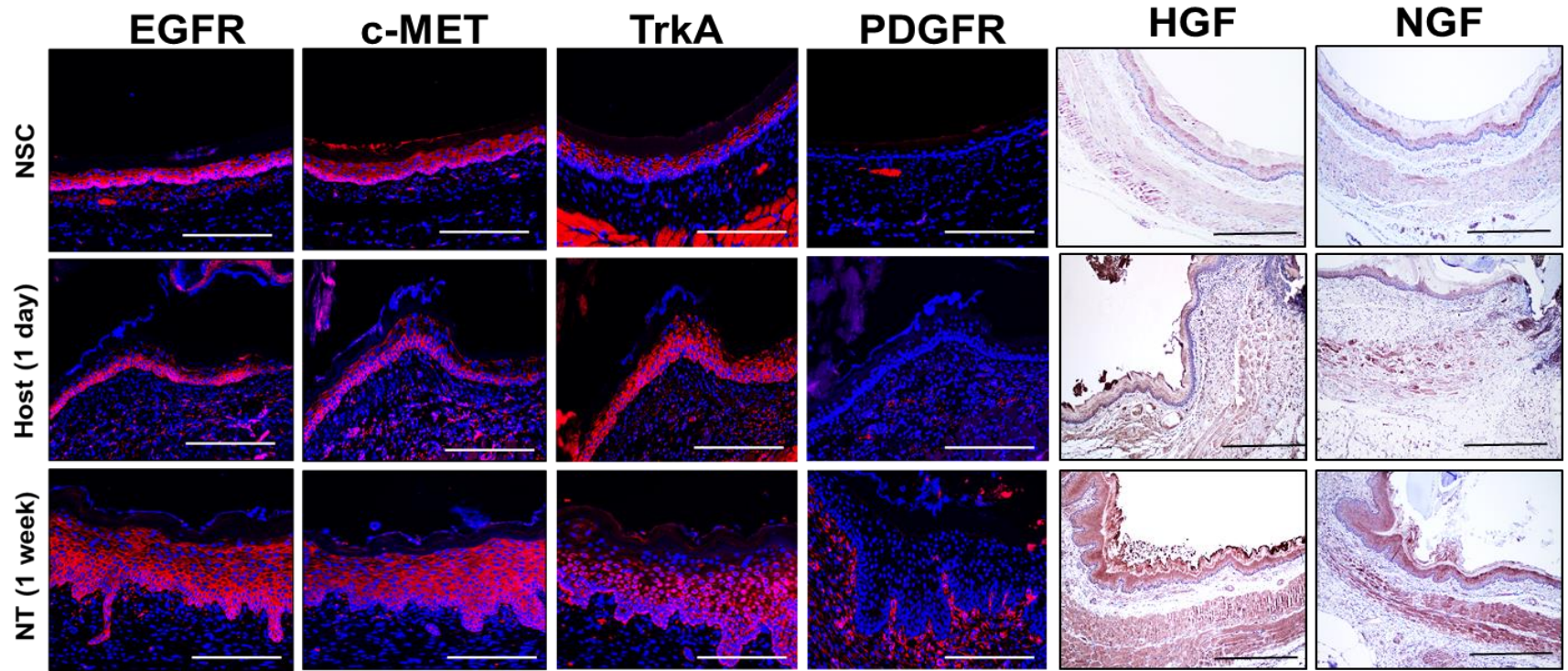


NGF Signaling
PDGF Signaling

N=4 rats per group

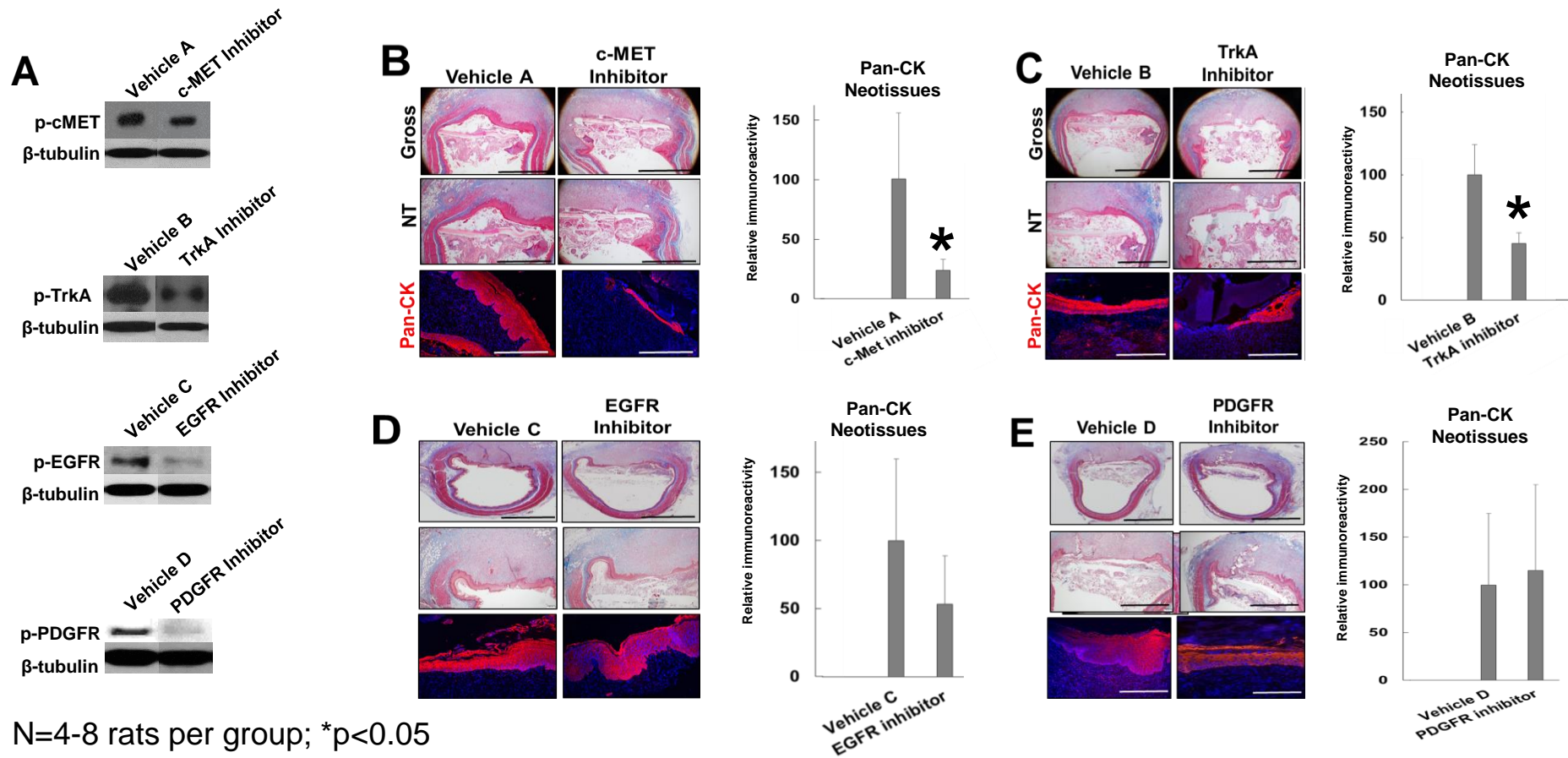
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Remodeling Esophageal Epithelium expresses EGFR, c-MET, and TrkA Receptors as well as HGF and NGF Ligands

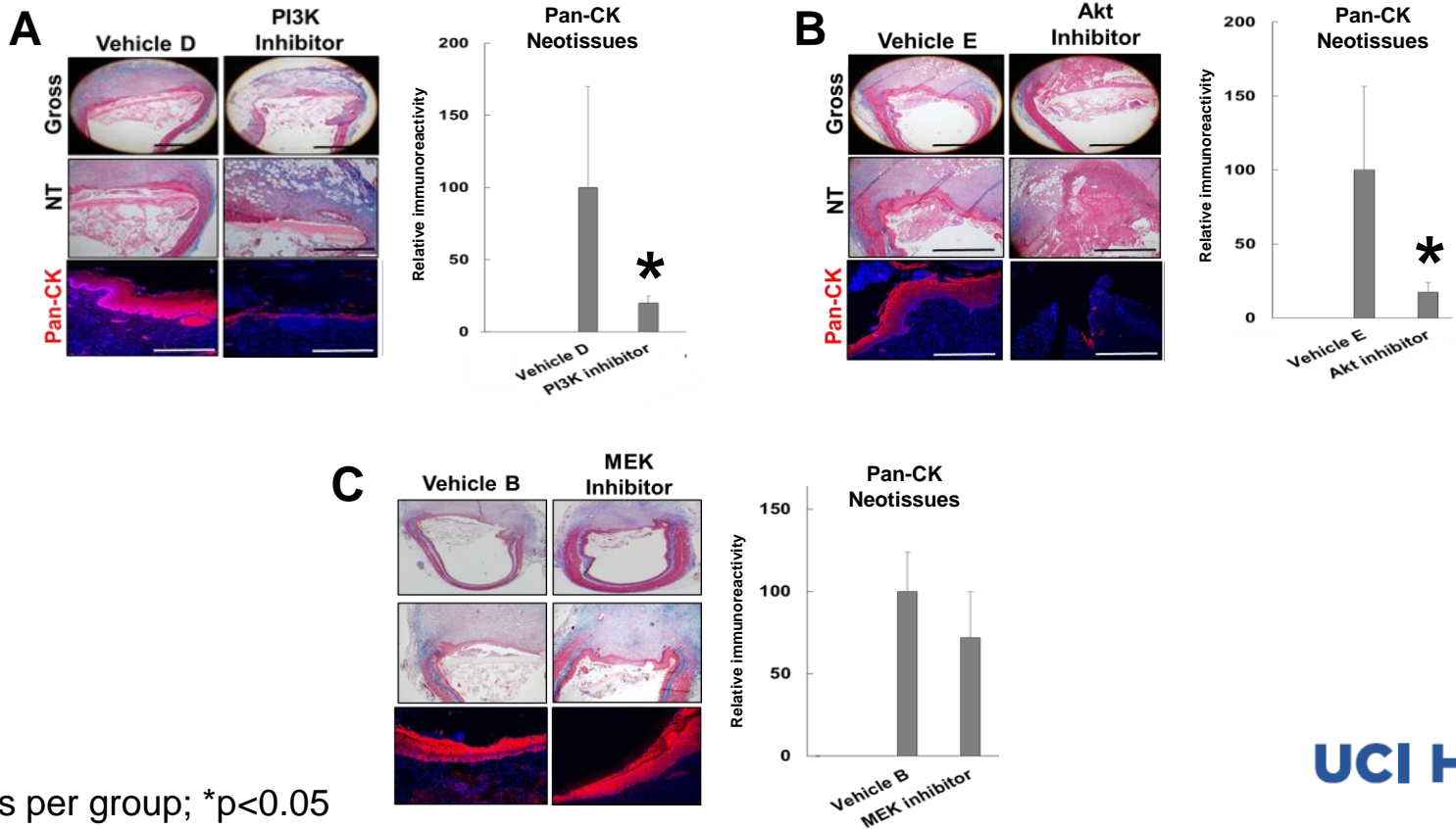


N=4-8 rats per group

Esophageal Epithelial Regeneration is Dependent on c-MET and TrkA Activation, but not EGFR or PDGFR Activation

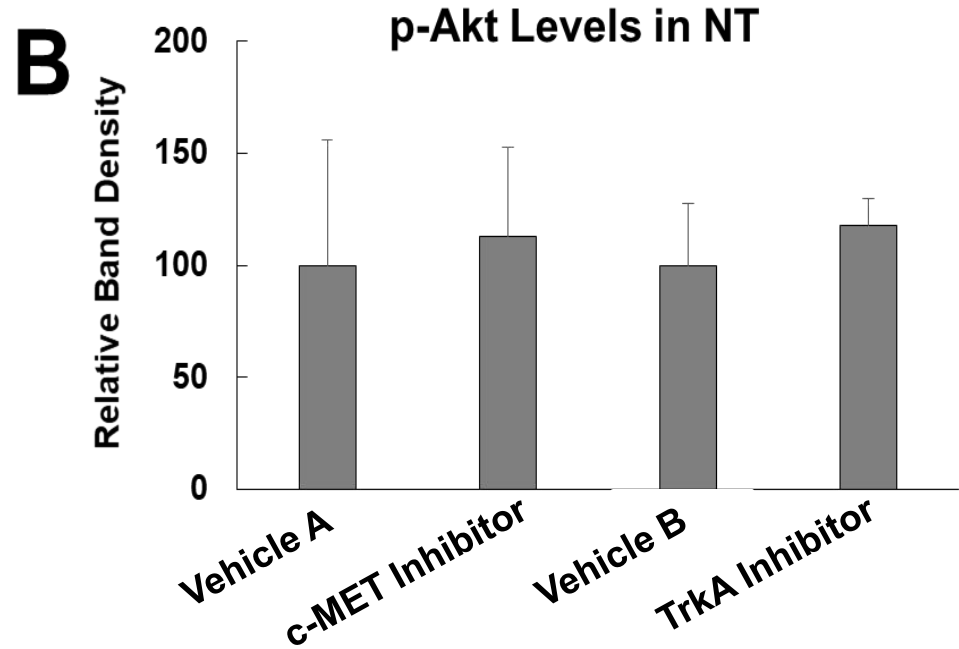
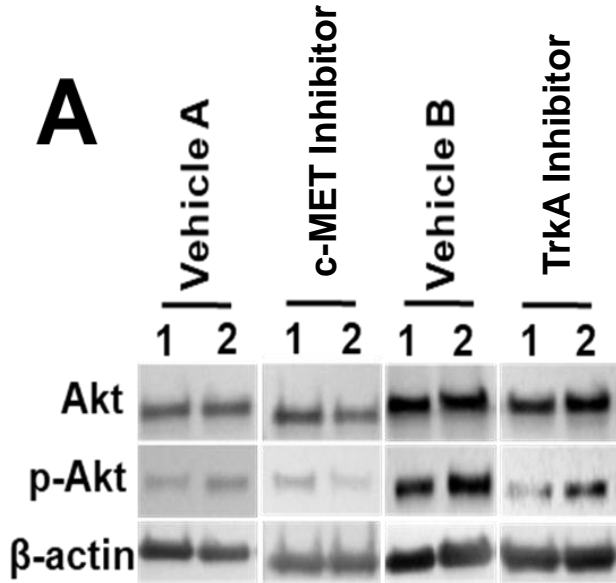


Esophageal Epithelial Regeneration is Dependent on PI3K and Akt activation, but is MEK Independent



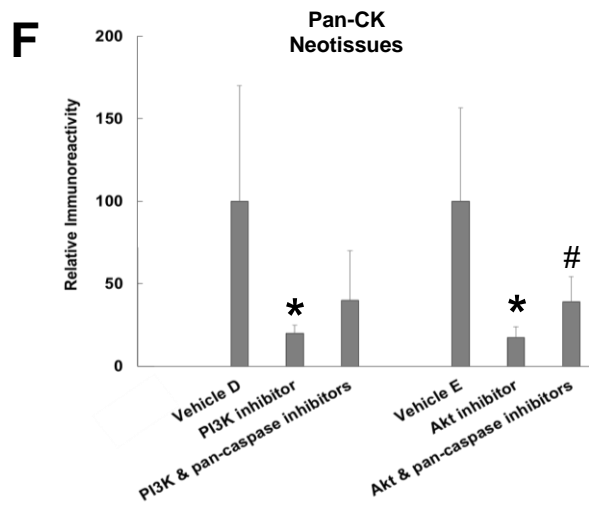
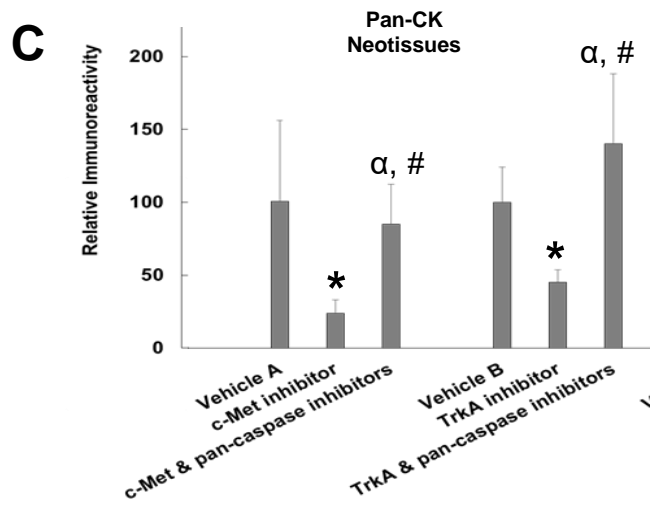
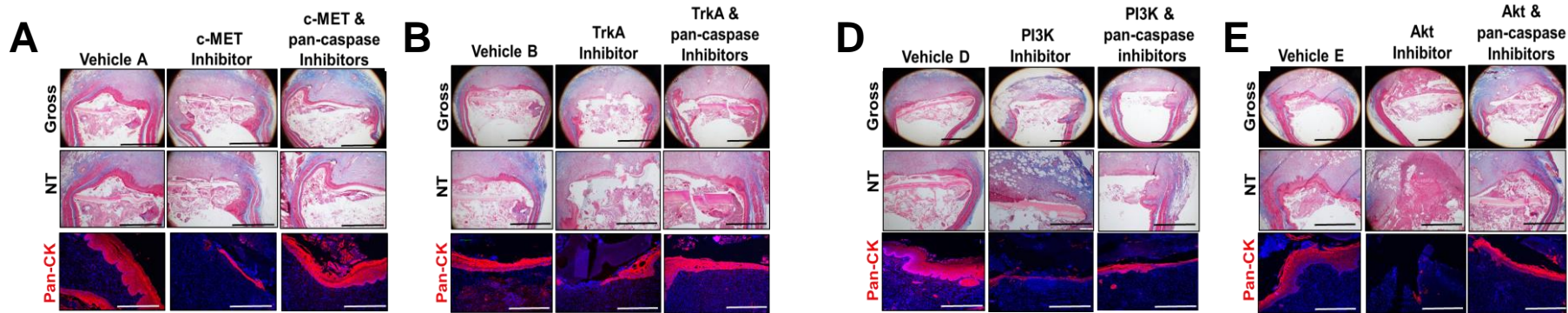
N=4-8 rats per group; *p<0.05

c-MET and TrkA Signaling Promotes Esophageal Epithelial Regeneration in an Akt Independent Manner



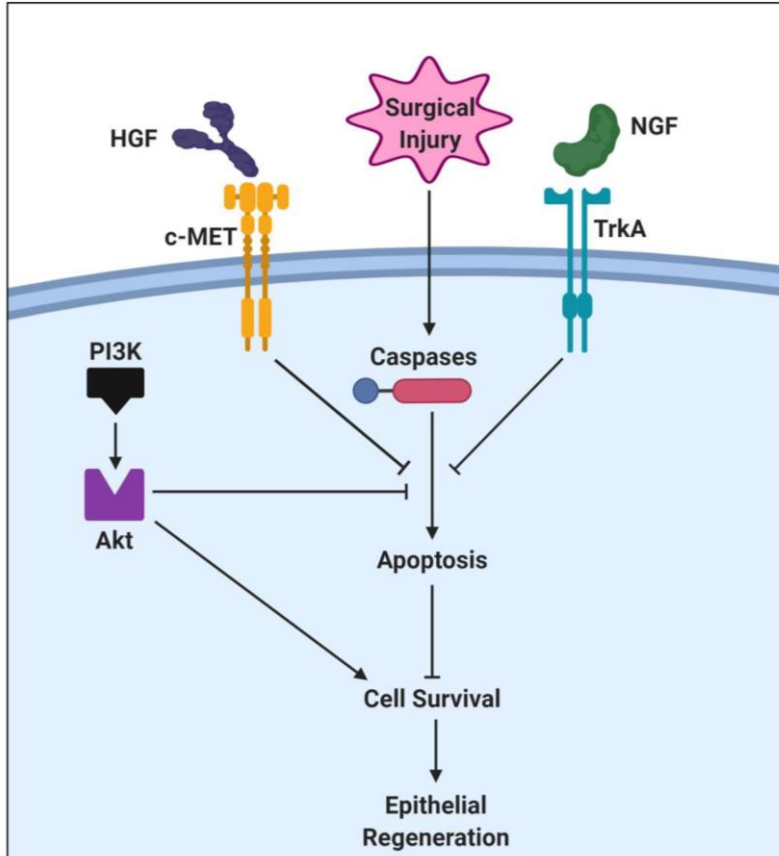
N=3-4 rats per group

Pan-Caspase Inhibition Rescues Esophageal Epithelial Regeneration Following Inhibition of c-MET, TrkA, PI3K/Akt Activation



N=4-8 rats per group;
 *p<0.05 relative to vehicle
 #p<0.05 relative to inhibitor
 α p>0.05 relative to vehicle

Model and Summary



- Following surgical injury, c-MET and TrkA receptors are activated in host epithelial cells via binding of HGF and NGF ligands, respectively.
- These pathways lead to inhibition of pan-caspase activity in the neoeplithelium which mitigates apoptosis and encourages epithelial survival allowing for epithelial regeneration.
- In parallel, surgical injury also activates PI3K which leads to phosphorylation of Akt that is capable of exerting pro-survival stimuli in the neoeplithelium partly through pan-caspase inhibition.

Acknowledgements

Project Team

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