

Mechanisms coupling the thrombin/fibrinogen axis to cancer progression

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Division of Hematology

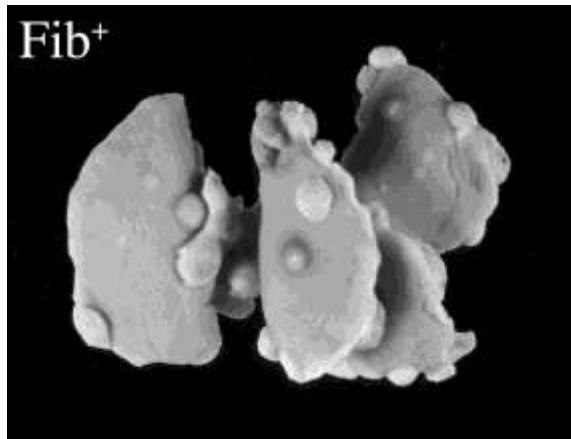
Cancer and Blood Diseases Institute

Hypothesis: The thrombin/fibrin(ogen) axis supports multiple aspects of cancer progression

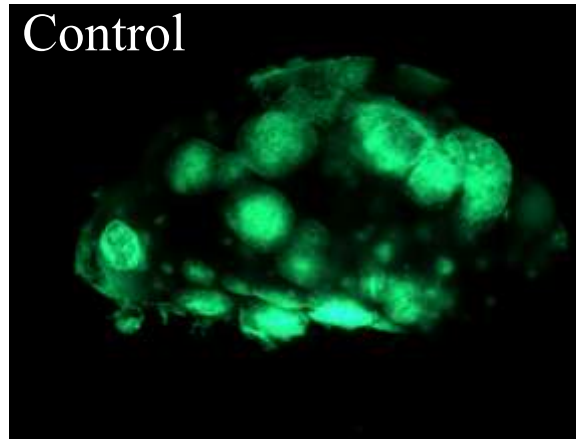
- Metastasis
- Tumorigenesis
- Tumor growth and stroma formation

The platelet/fibrin(ogen) axis promotes metastasis

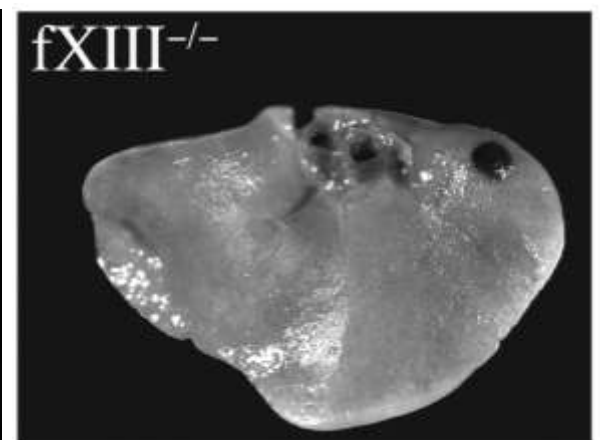
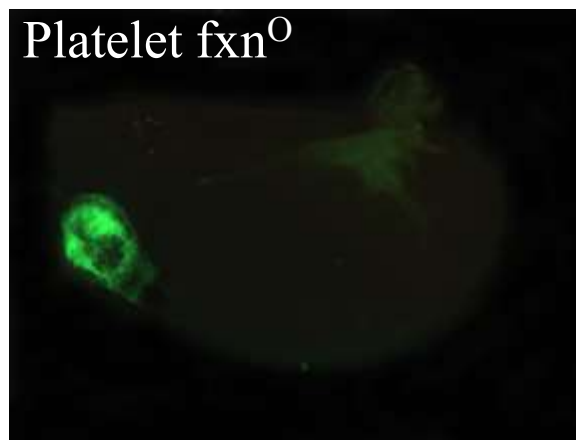
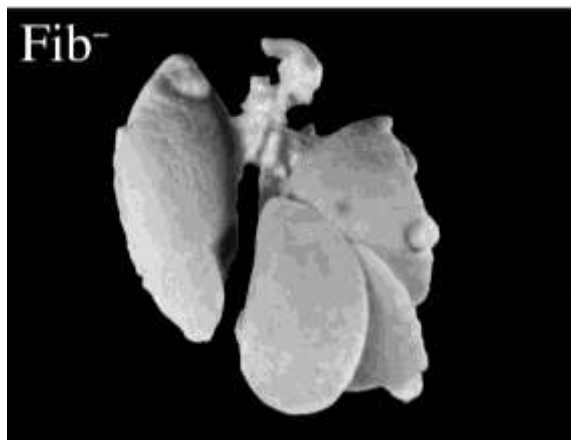
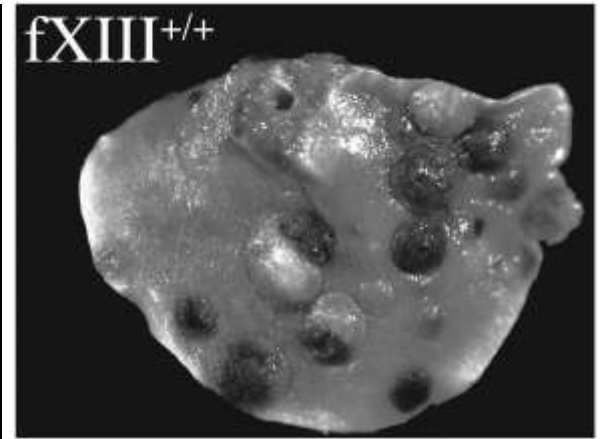
Fibrinogen



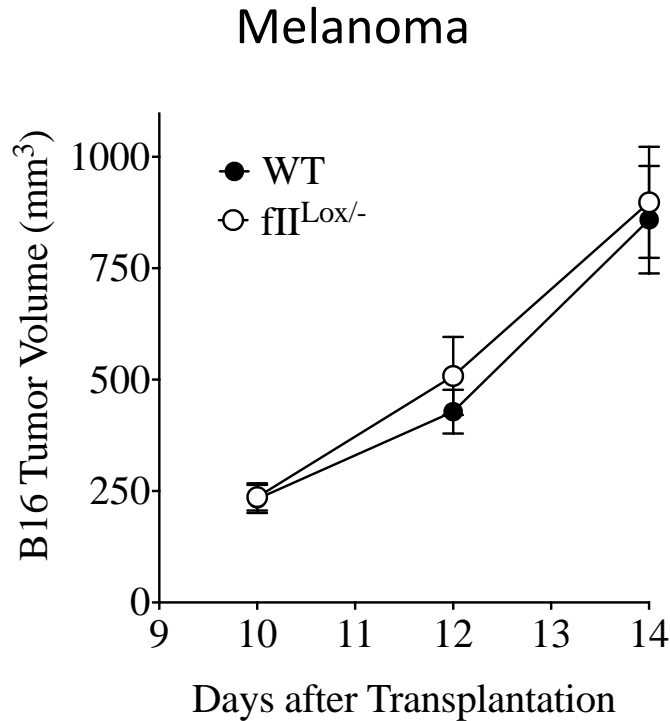
Platelet Function



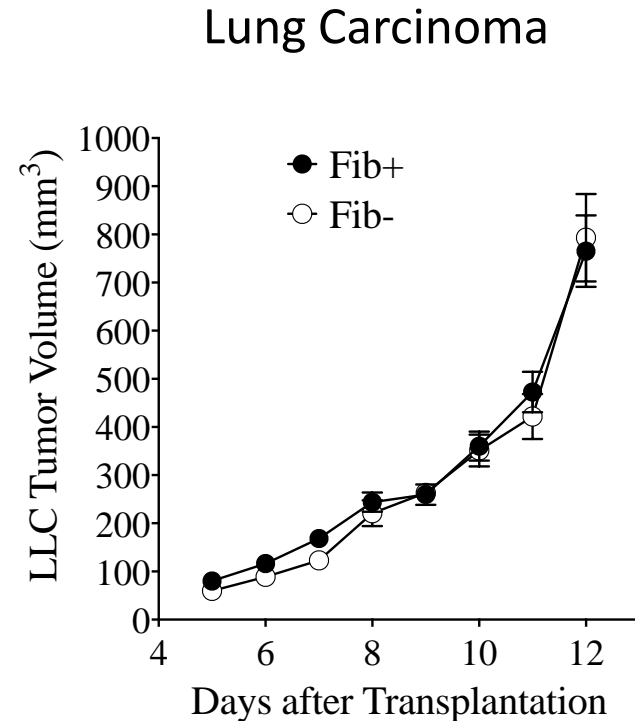
Factor XIII



Thrombin and downstream thrombin targets are not critically required for the growth of many tumor types.

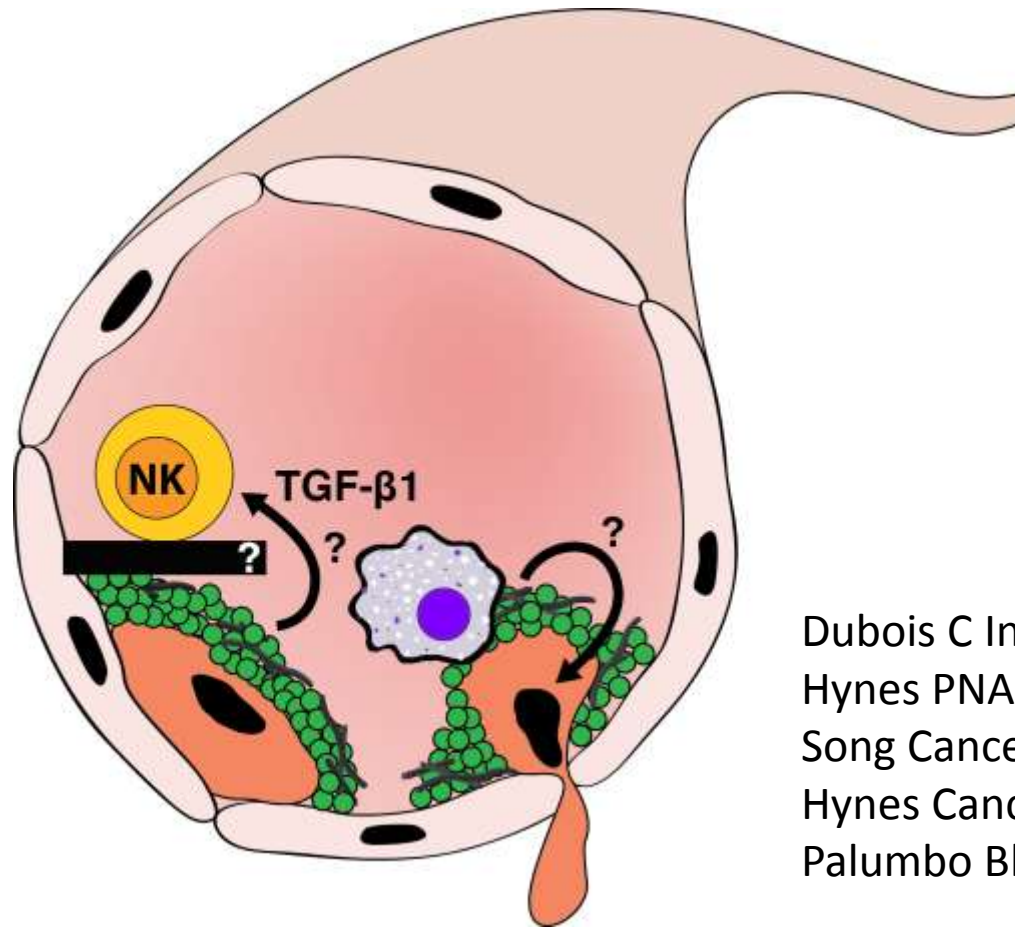


Prothrombin 10% of normal



Complete fibrinogen deficiency

Platelets and fibrin(ogen) support metastasis by impeding the clearance of newly formed micrometastases by natural killer cells, and promoting EMT.



Dubois C Int J Cancer 2015
Hynes PNAS 2014
Song Cancer Cell 2014
Hynes Cancer Cell 2011
Palumbo Blood 2007

What are the functional properties of fibrin(ogen) that support metastasis?

Fibrin(ogen) is a multifunctional provisional matrix protein

FIBRIN POLYMERIZATION

Fib^{AEK} – loss of the α chain thrombin cleavage site. Fibrinogen “locked” in the soluble state. Blood. 2015 Oct 22;126(17):2047-58

FIBRIN(OGEN)-LEUKOCYTE INTERACTIONS

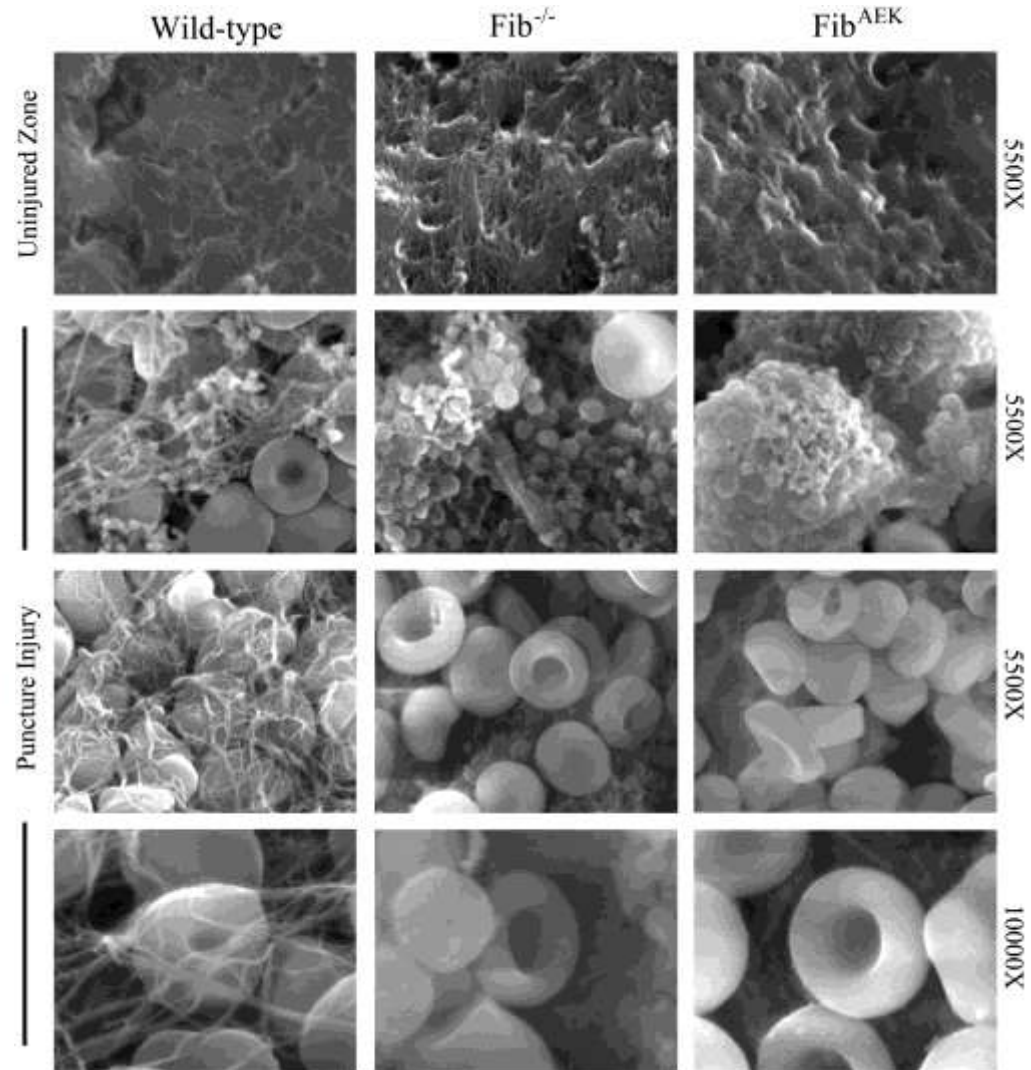
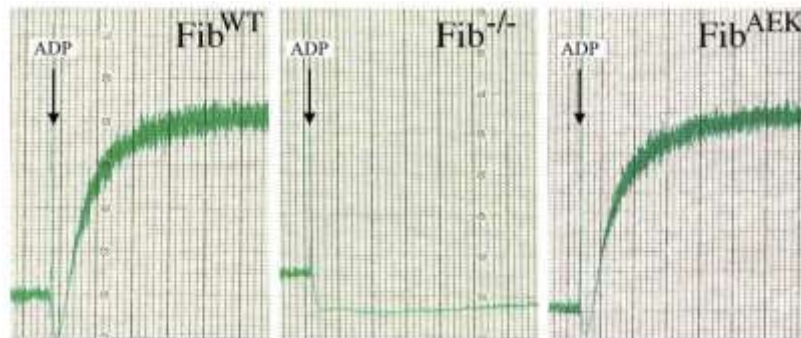
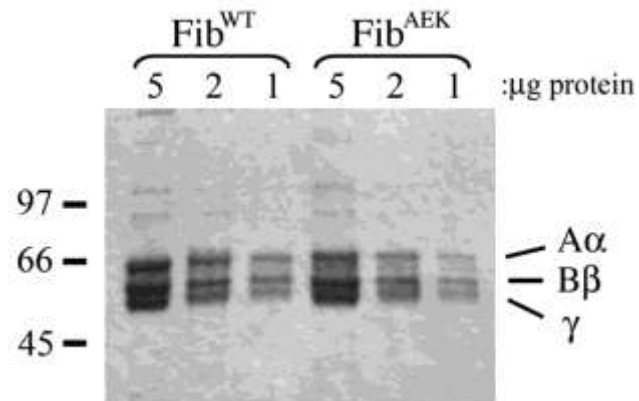
Fib $\gamma^{390-396A}$ – Loss of the γ chain binding motif for $\alpha_M\beta_2$. J Clin Invest. 2004 Jun;113(11):1596-606.

FIBRIN(OGEN)-MEDIATED PLATELET STABILIZATION

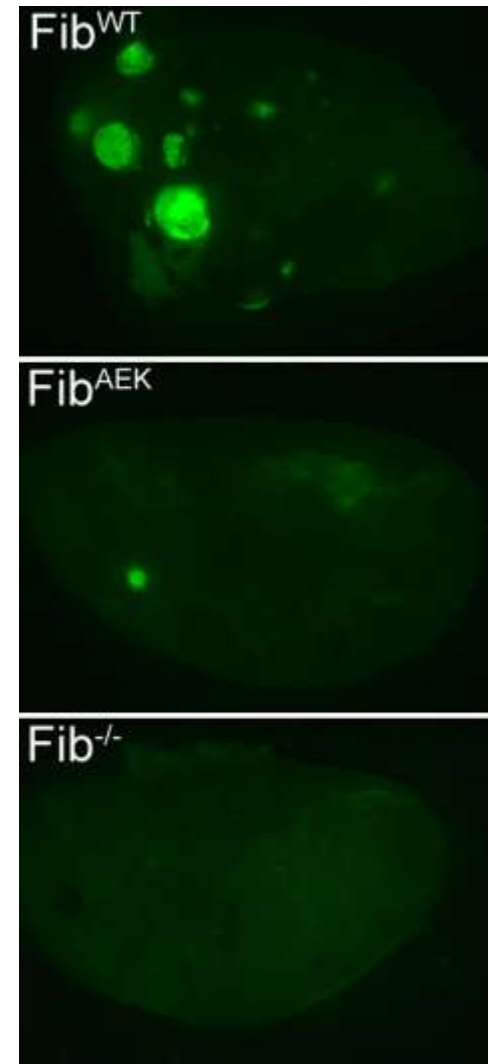
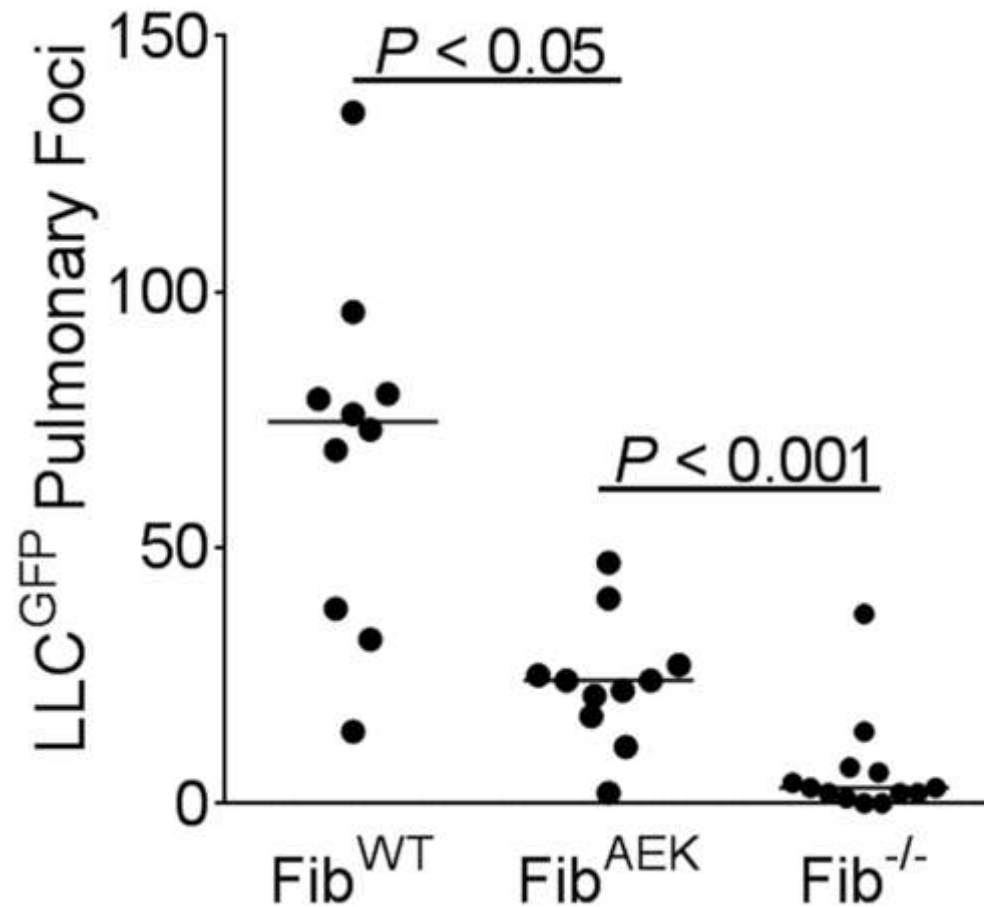
Fib $\gamma^{\Delta 5}$ – Deletion of the last 5 amino acids of the γ chain, resulting in loss of a key $\alpha_{IIb}\beta_3$ binding motif. EMBO J. 1996 Nov 1;15(21):5760-71

Is fibrin polymerization required for
metastasis?

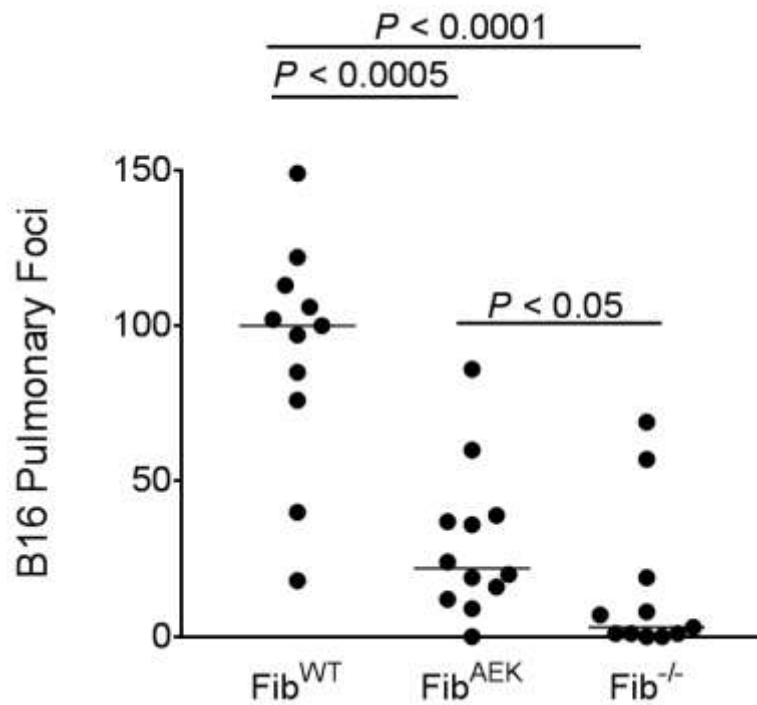
Fib^{AEK} Mice



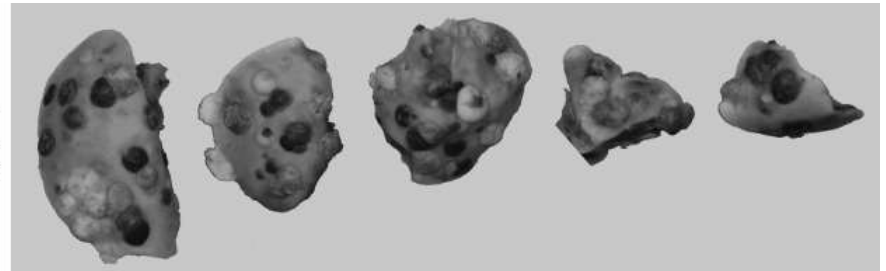
Fibrinogen retains significant potential to support metastasis in the absence of fibrin polymer formation



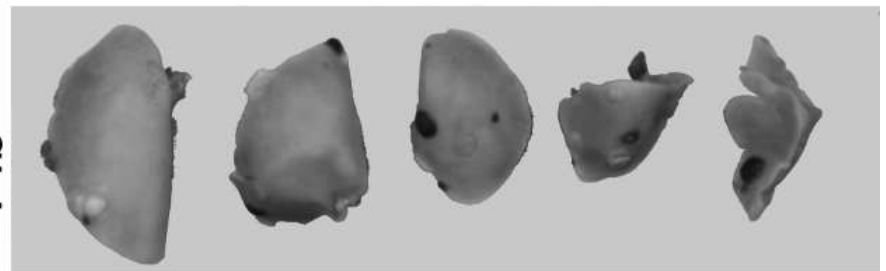
Fibrinogen retains significant potential to support metastasis in the absence of fibrin polymer formation



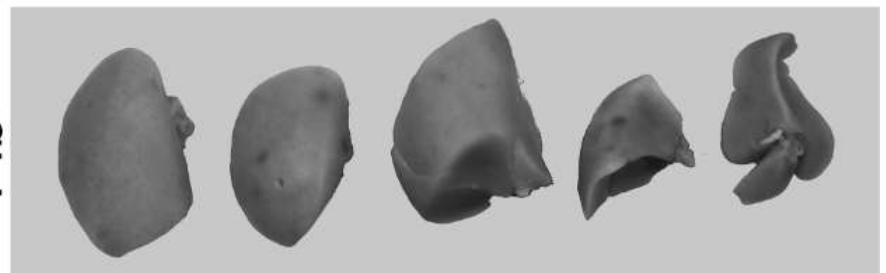
WT



Fib^{AEK}

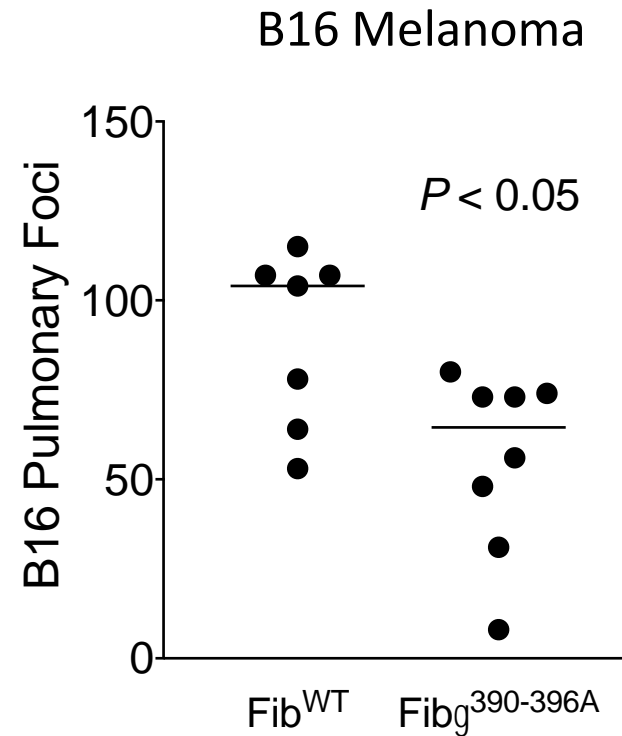
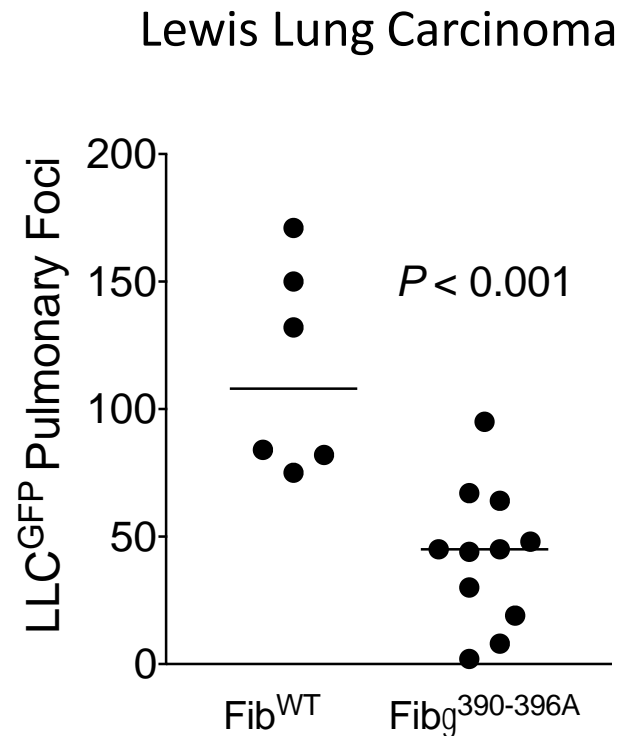


Fib^{-/-}

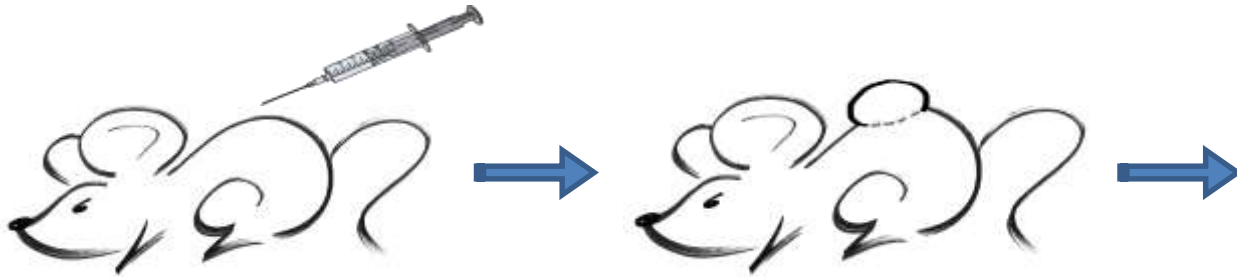


Does fibrin(ogen)-mediated $\alpha_M\beta_2$
engagement support metastasis?

Fibrinogen $\gamma^{390-396A}$ limits experimental metastases

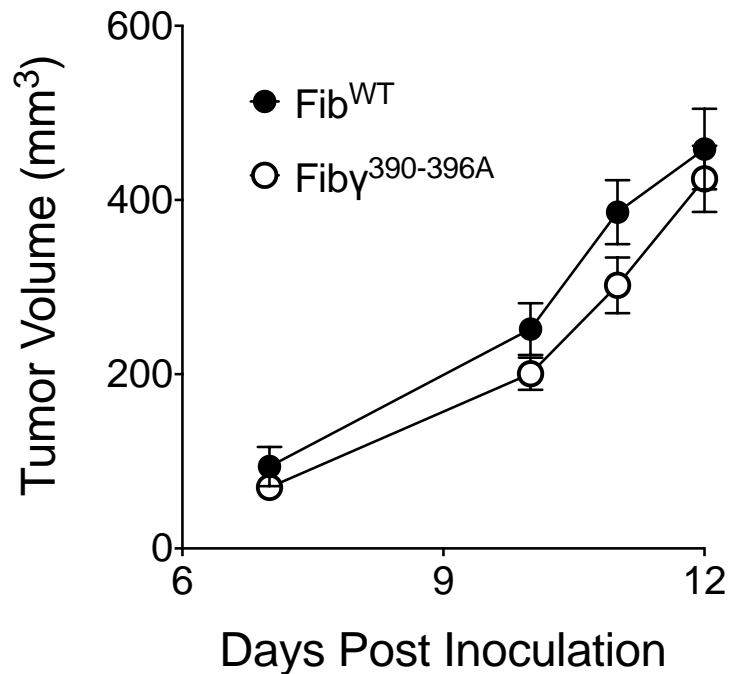


Spontaneous metastasis

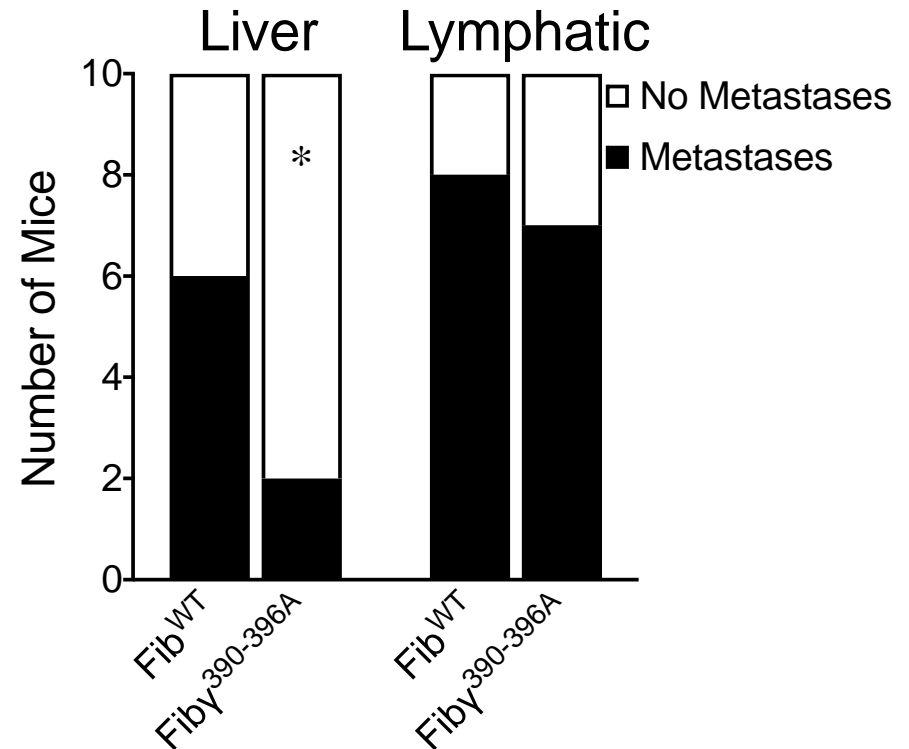
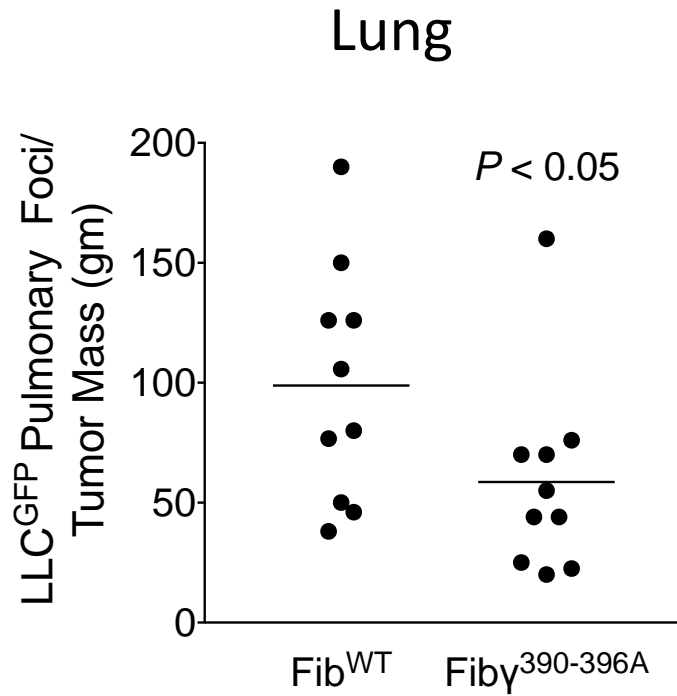


Evaluate metastasis
at multiple sites

Lewis Lung Carcinoma



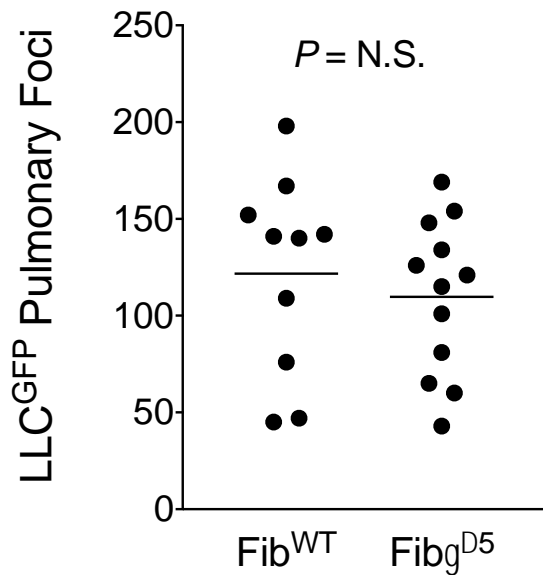
Fibrinogen $\gamma^{390-396A}$ limits spontaneous hematogenous metastasis



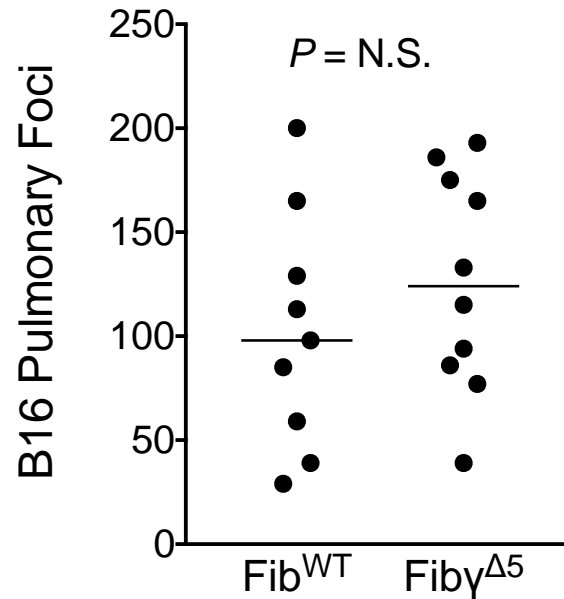
Do fibrin(ogen)-platelet interactions
mediated by $\alpha_{\text{IIb}}\beta_3$ support metastasis?

Elimination of the fibrinogen γ binding motif for $\alpha_{IIb}\beta_3$ (Fib $\gamma^{\Delta 5}$) has no impact on metastasis

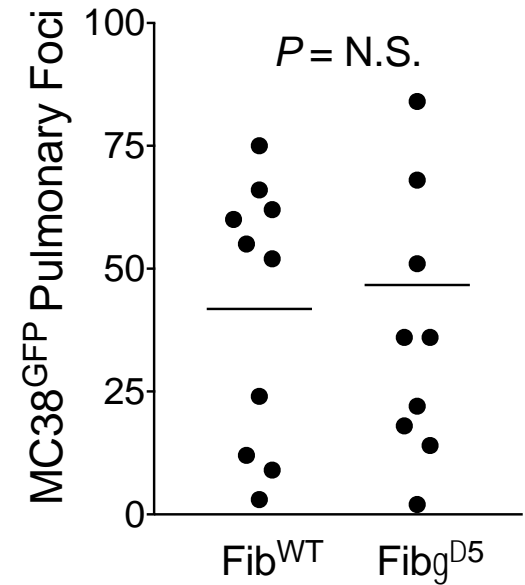
Lung Carcinoma



Melanoma



Colon Cancer



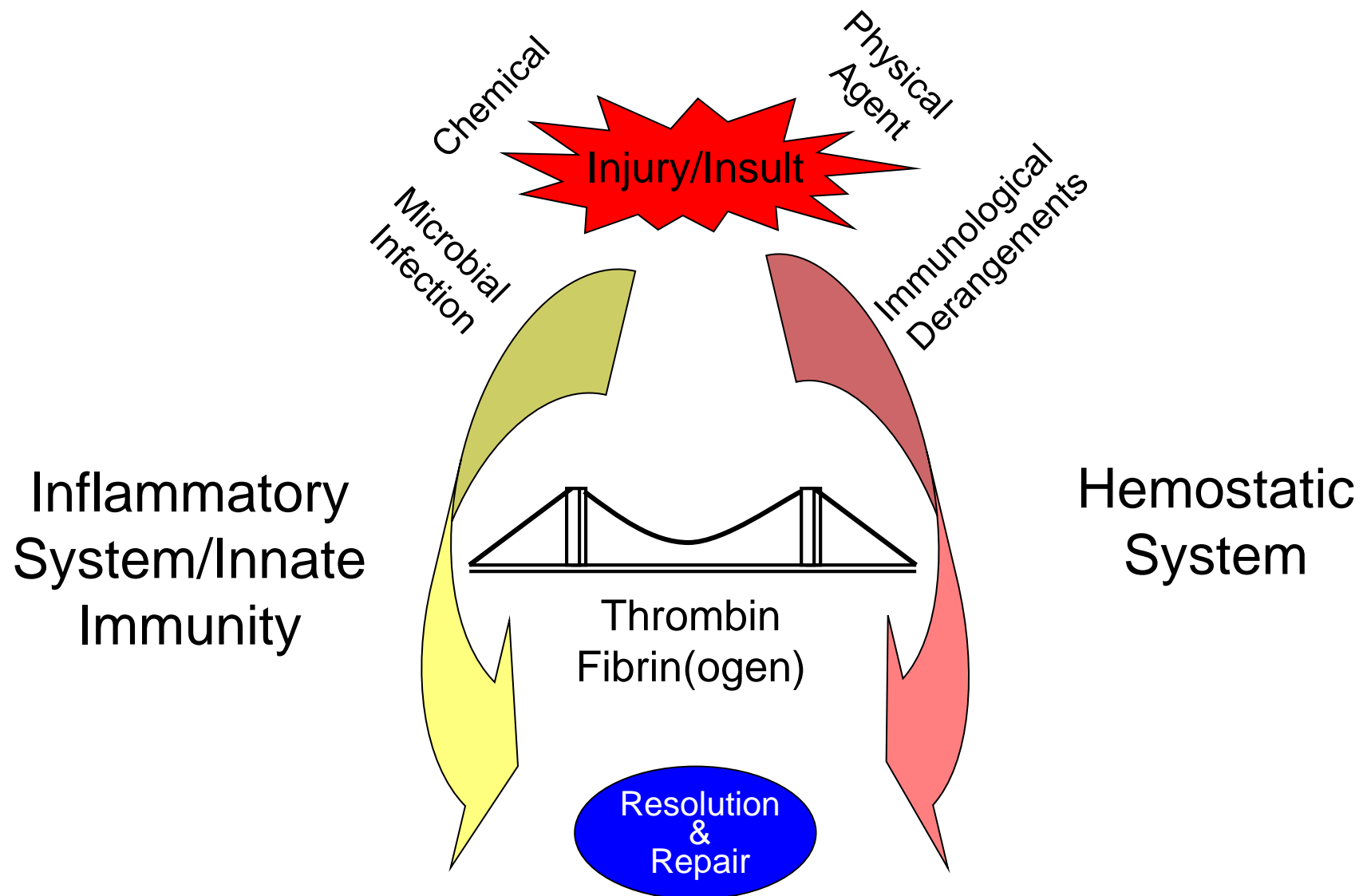
Summary of Part 1

- Thrombin-mediated fibrin polymerization promotes metastasis.
- Fibrinogen retains significant prometastatic capacity in the absence of thrombin-mediated polymerization.
- Fibrin(ogen)-leukocyte interactions mediated by $\alpha_M\beta_2$ appear to have a significant role in metastasis.
- The fibrinogen γ chain $\alpha_{IIb}\beta_3$ binding motif is dispensable for metastasis.

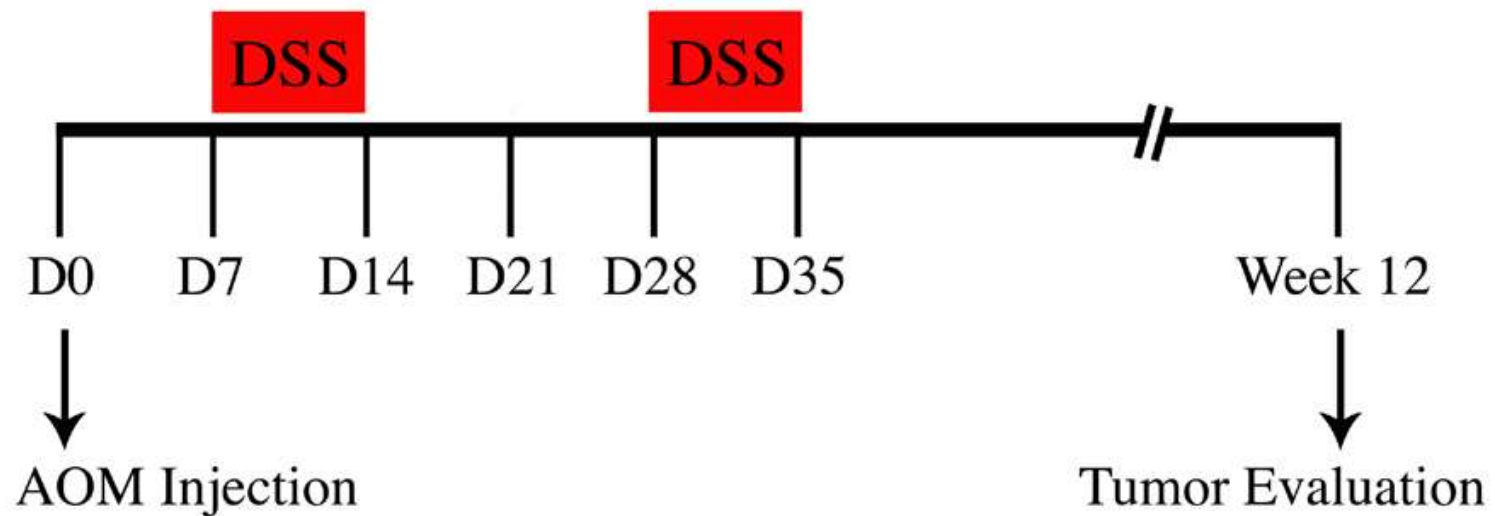
Are there contexts where the thrombin/fibrinogen axis supports tumorigenesis and tumor growth?

Colitis-associated colon cancer

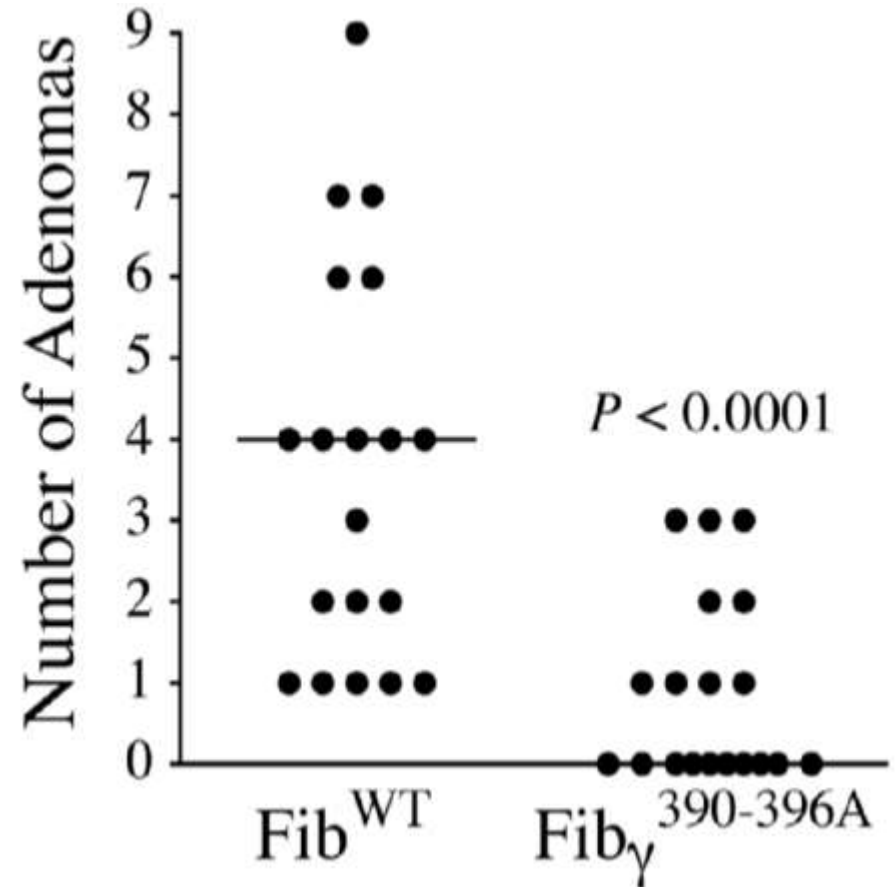
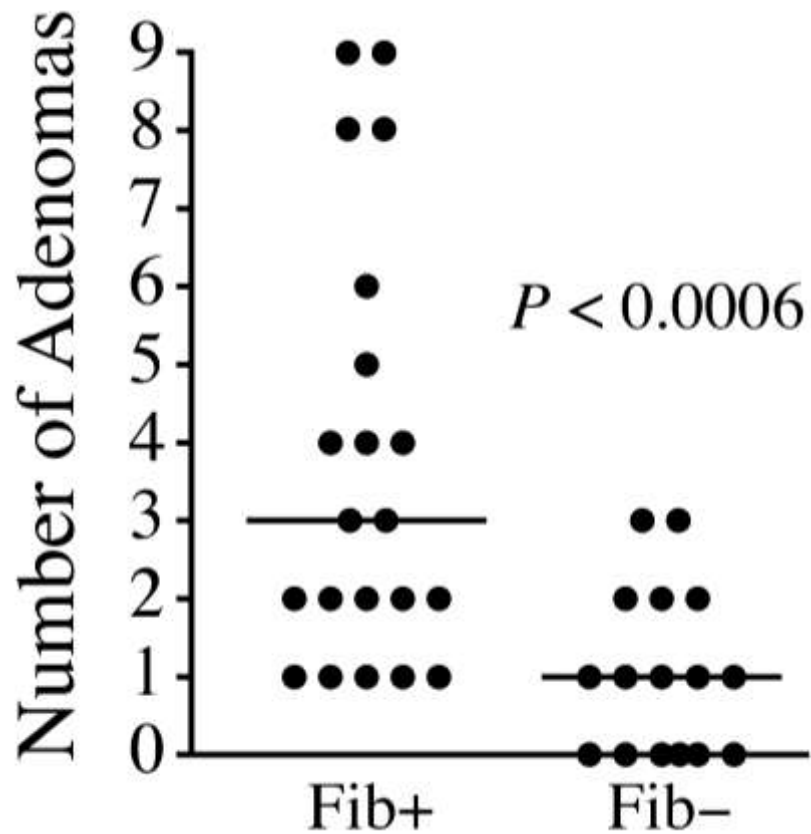
The coevolution of the inflammatory and hemostatic systems



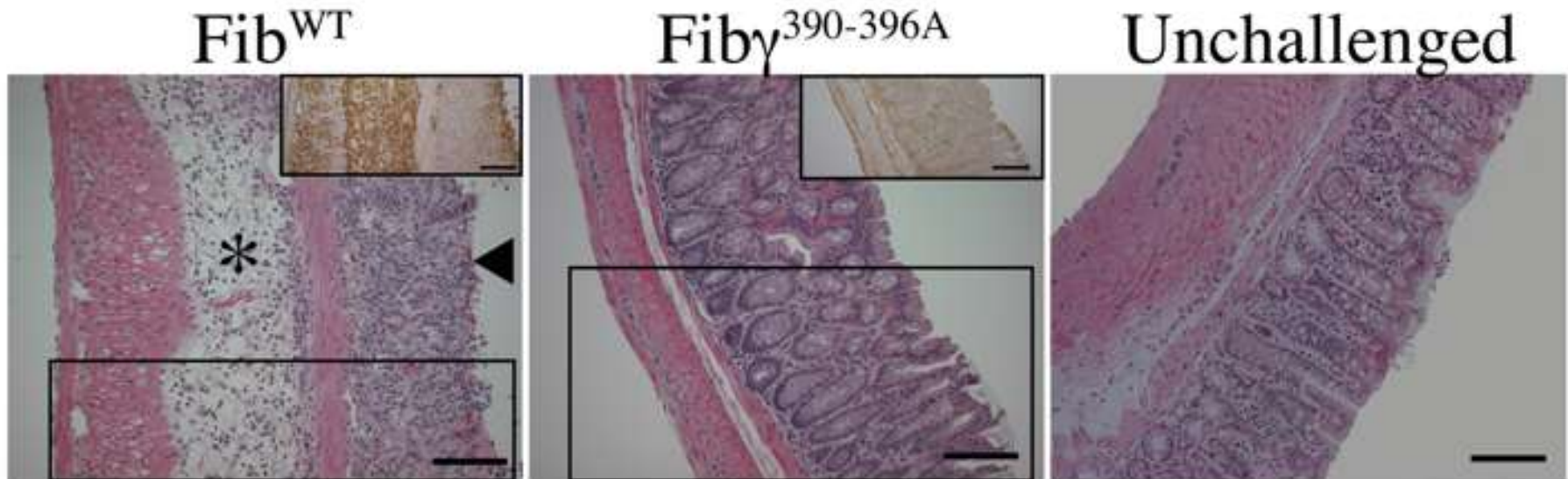
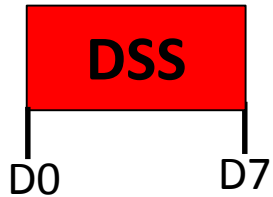
Induction of colitis-associated colon cancer (CAC) in mice



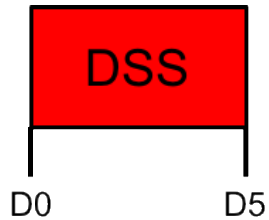
Fibrin matrices support colitis-associated adenoma formation via engagement of integrin $\alpha_M\beta_2$



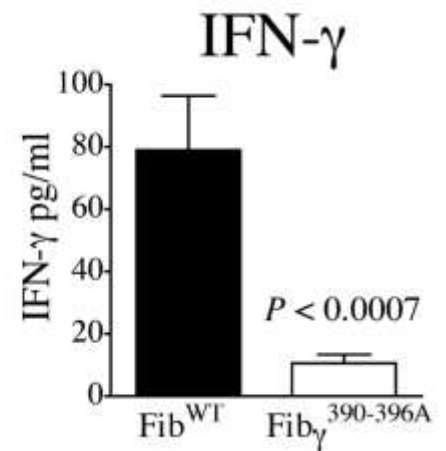
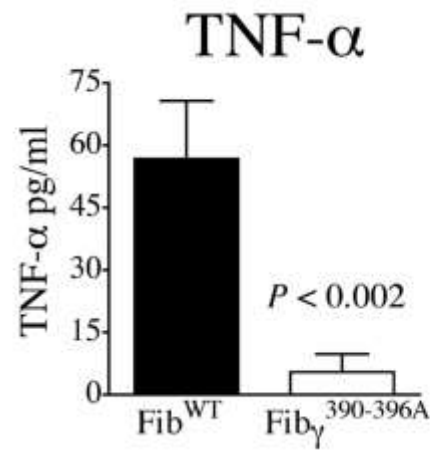
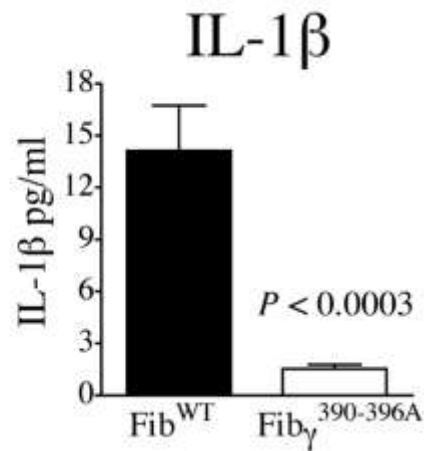
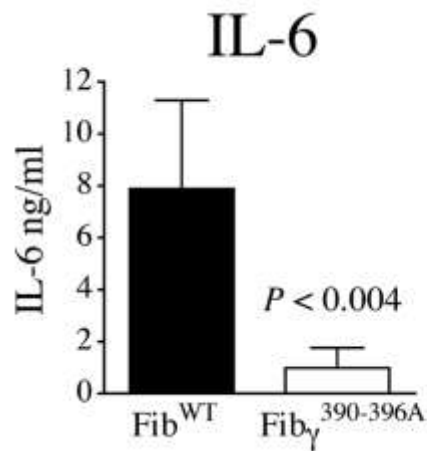
Fibrin- $\alpha_M\beta_2$ interactions promote early inflammatory events in colitis



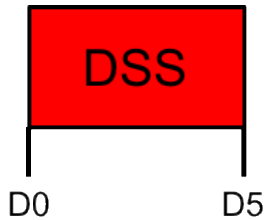
Fibrin-mediated engagement of $\alpha_M\beta_2$ leads to a cytokine-driven epithelial stress/proliferative response



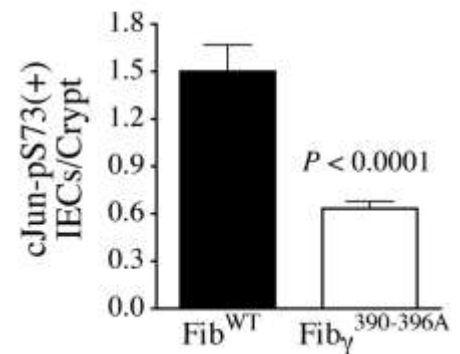
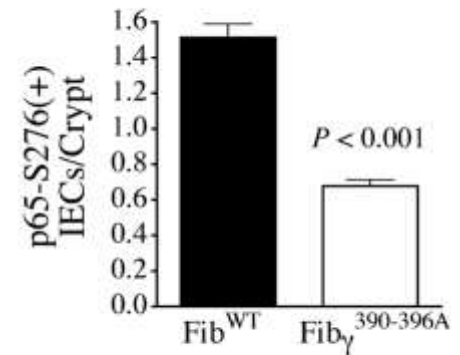
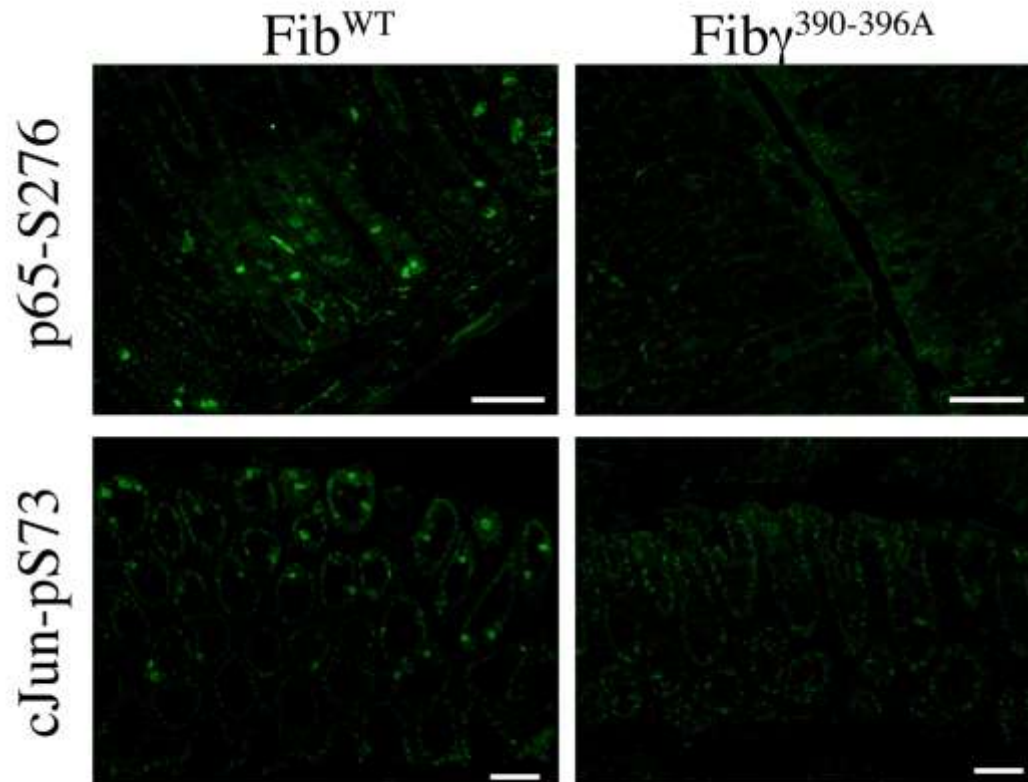
Prior to any significant epithelial damage



Fibrin-mediated engagement of $\alpha_M\beta_2$ leads to a cytokine-driven epithelial stress/proliferative response

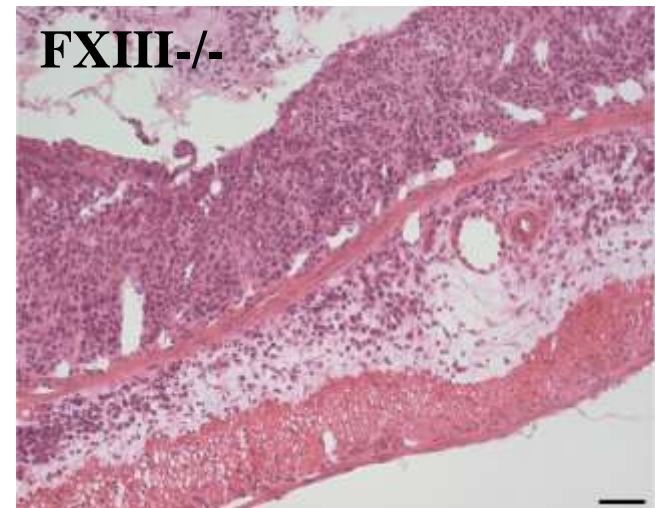
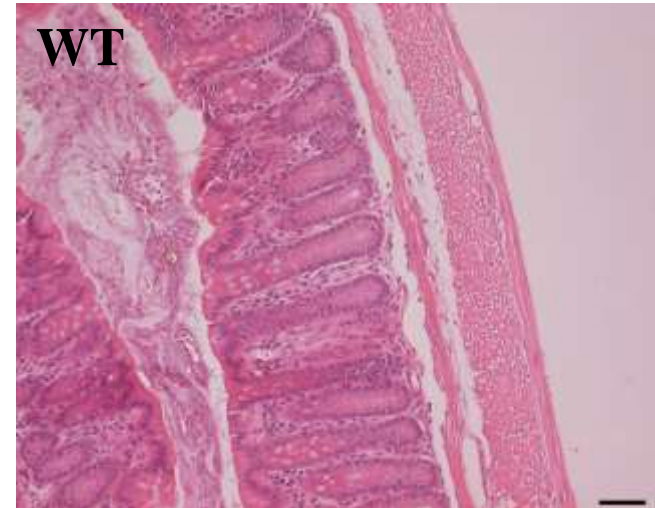
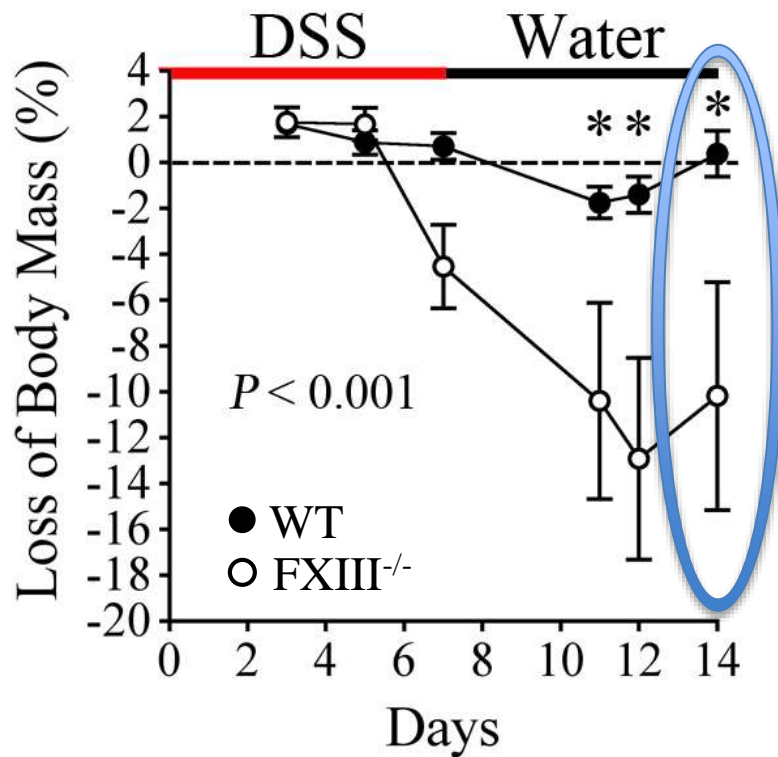


Prior to any significant epithelial damage

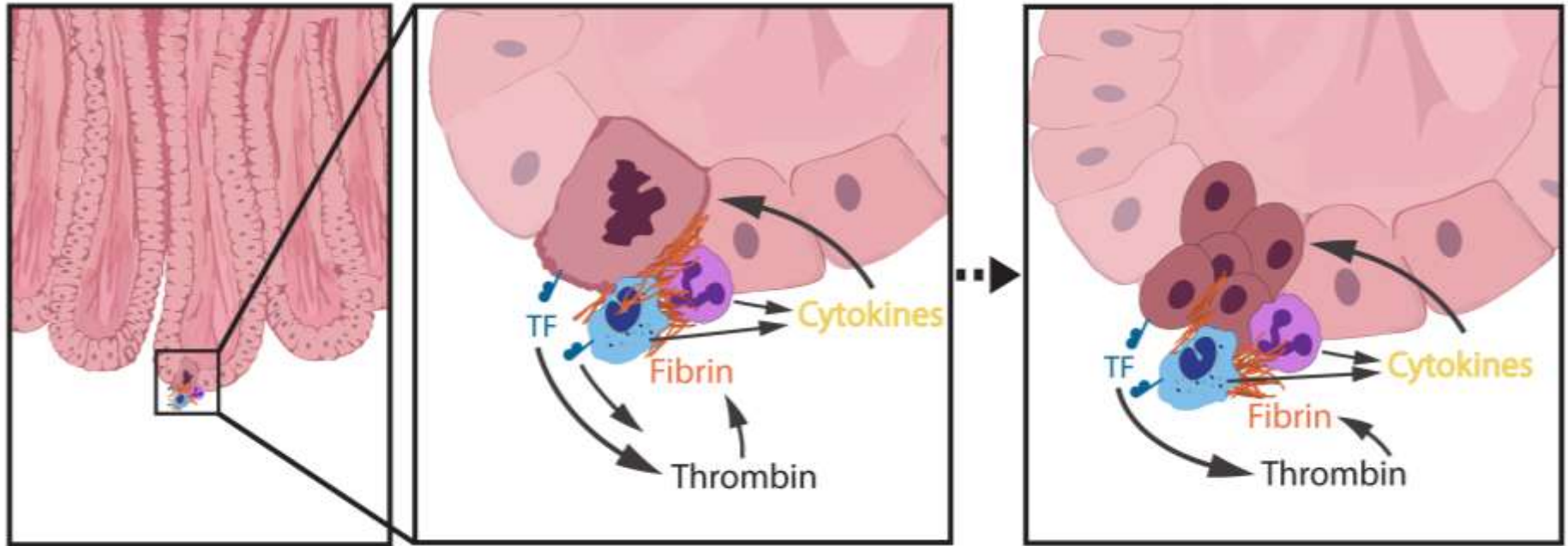


The potential of fibrinogen $\gamma^{390-396A}$ to limit DSS-induced colitis is not related to factor XIII

Factor XIII *promotes* intestinal healing following DSS colitis



Tumorigenesis

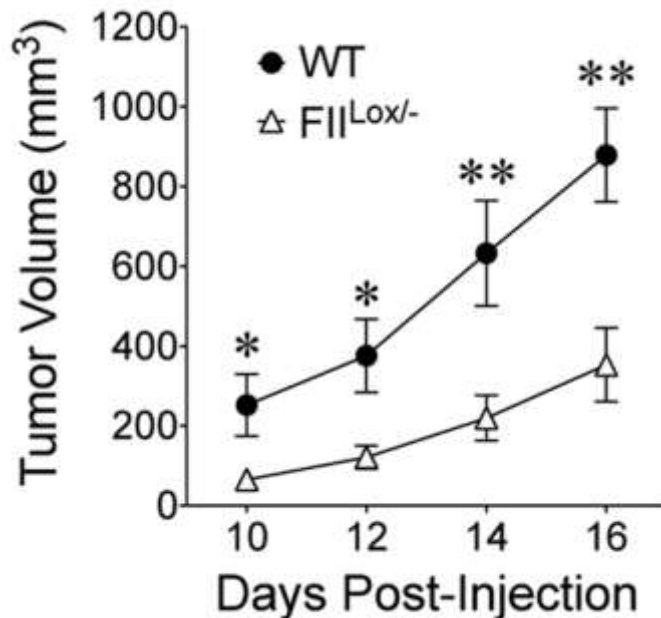


Fibrin(ogen)-leuckocyte interactions mediated by $\alpha_M\beta_2$ create an immunological milieu conducive to tumorigenesis.

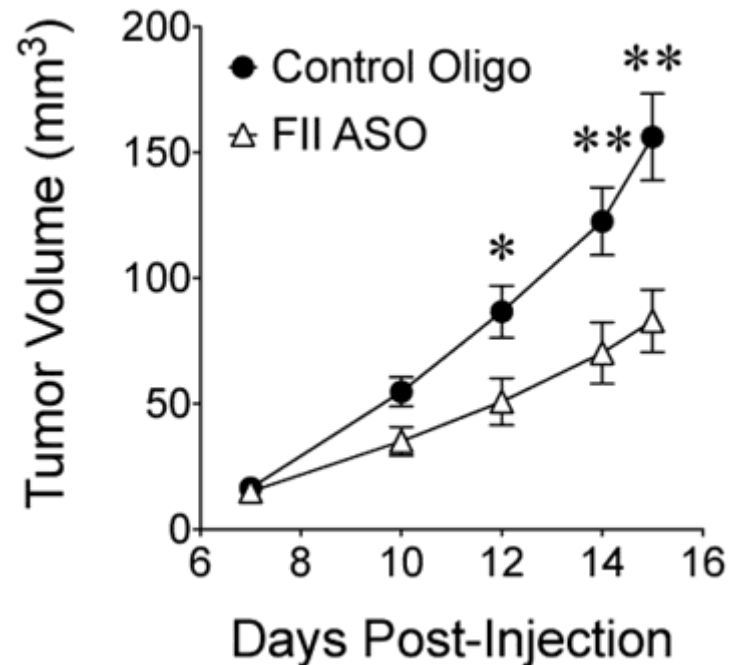
Does the thrombin/fibrin axis play a role in the growth of established colorectal carcinoma?

Thrombin promotes the growth of colonic adenocarcinoma in vivo

MC38 (murine-derived)



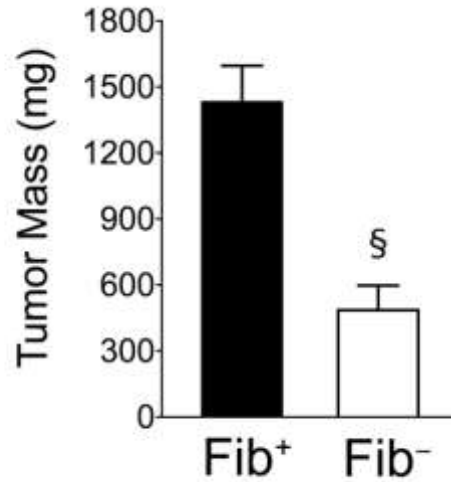
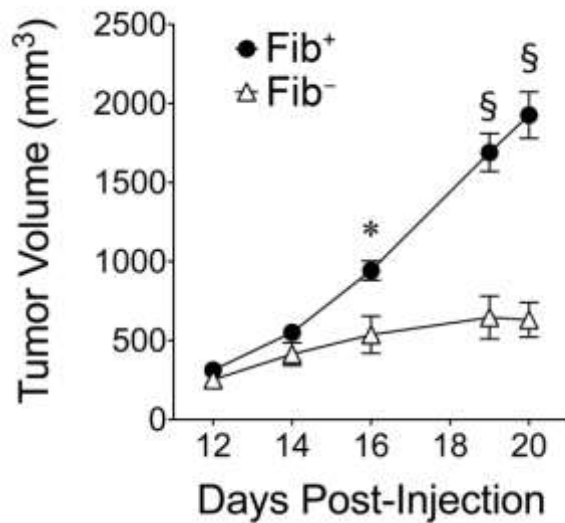
HCT116 (human-derived)



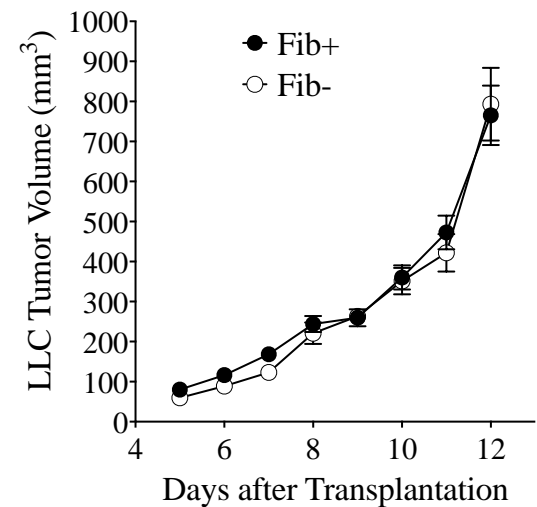
Prothrombin lowered to ~10% of normal

Fibrinogen is a major determinant of colonic adenocarcinoma growth

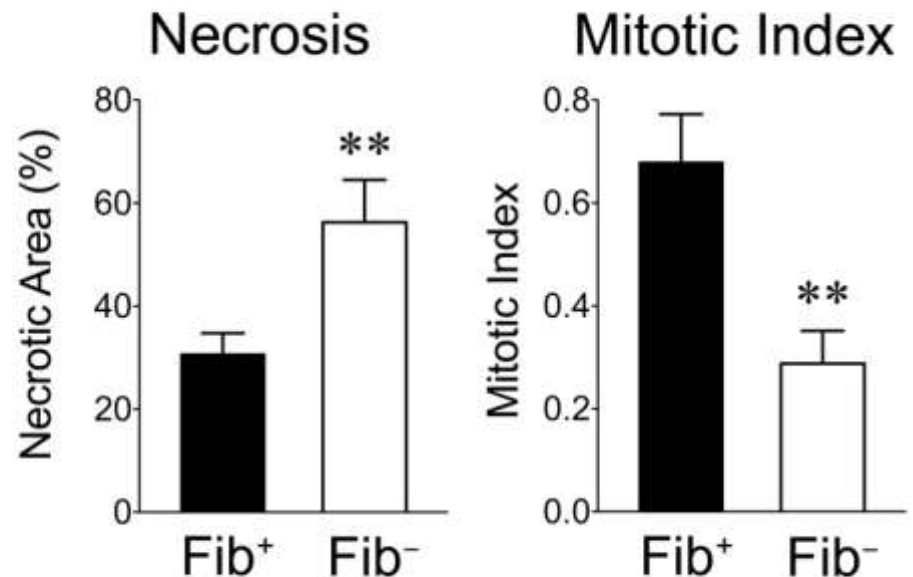
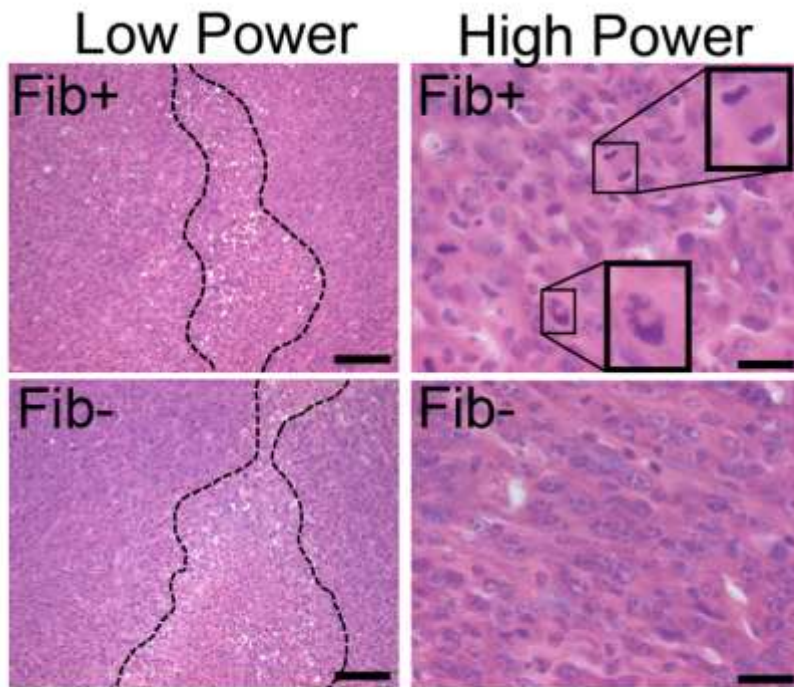
Colonic Adenocarcinoma



Lung Adenocarcinoma

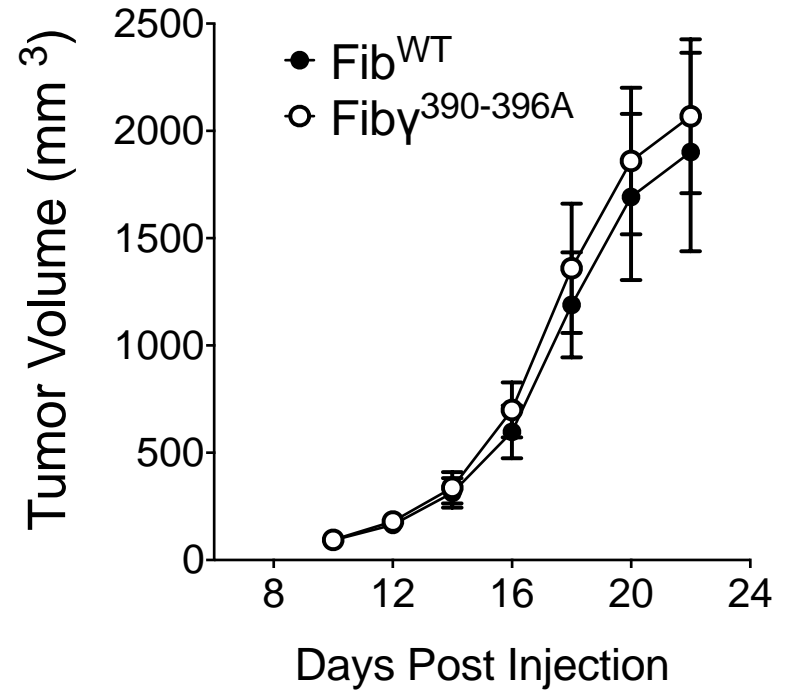
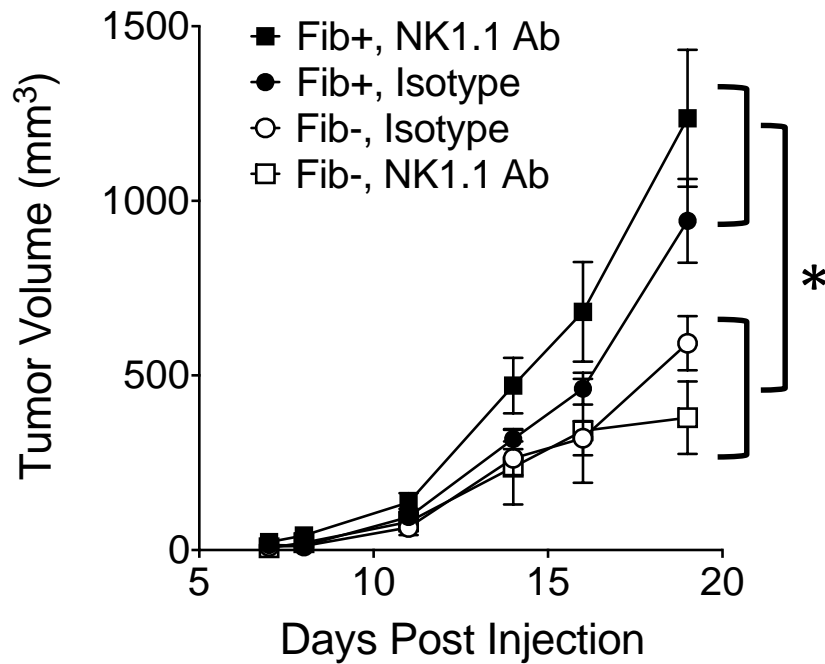


Loss of fibrinogen limits colon cancer proliferation and promotes necrosis



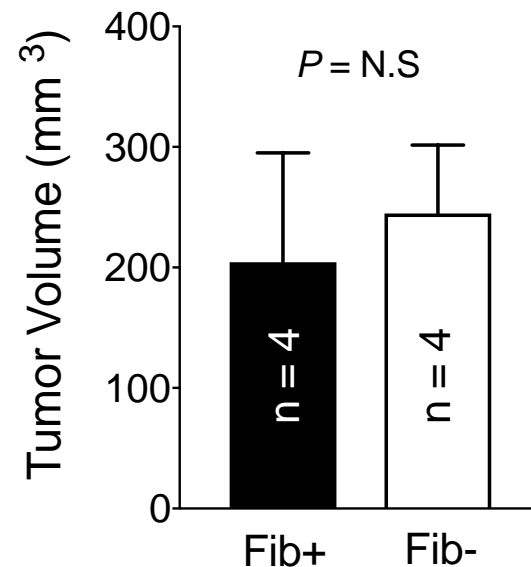
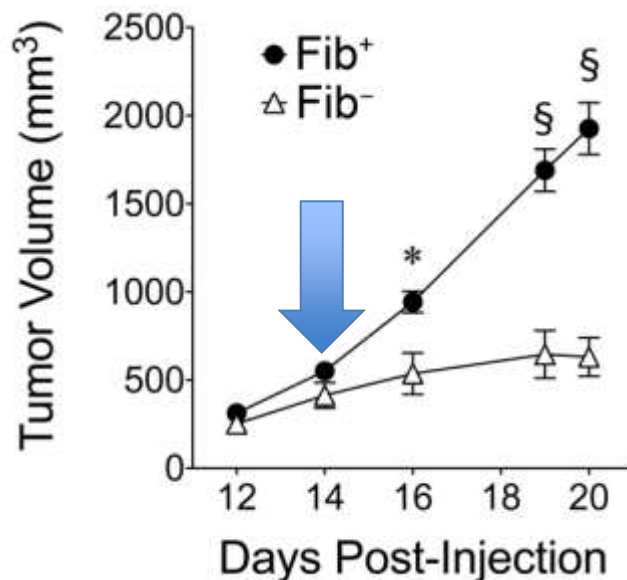
What are the mechanism(s) coupling fibrin(ogen) to colon cancer growth?

The role of fibrin(ogen) in colon cancer growth appears to be independent of NK cells and $\alpha_M\beta_2$ engagement

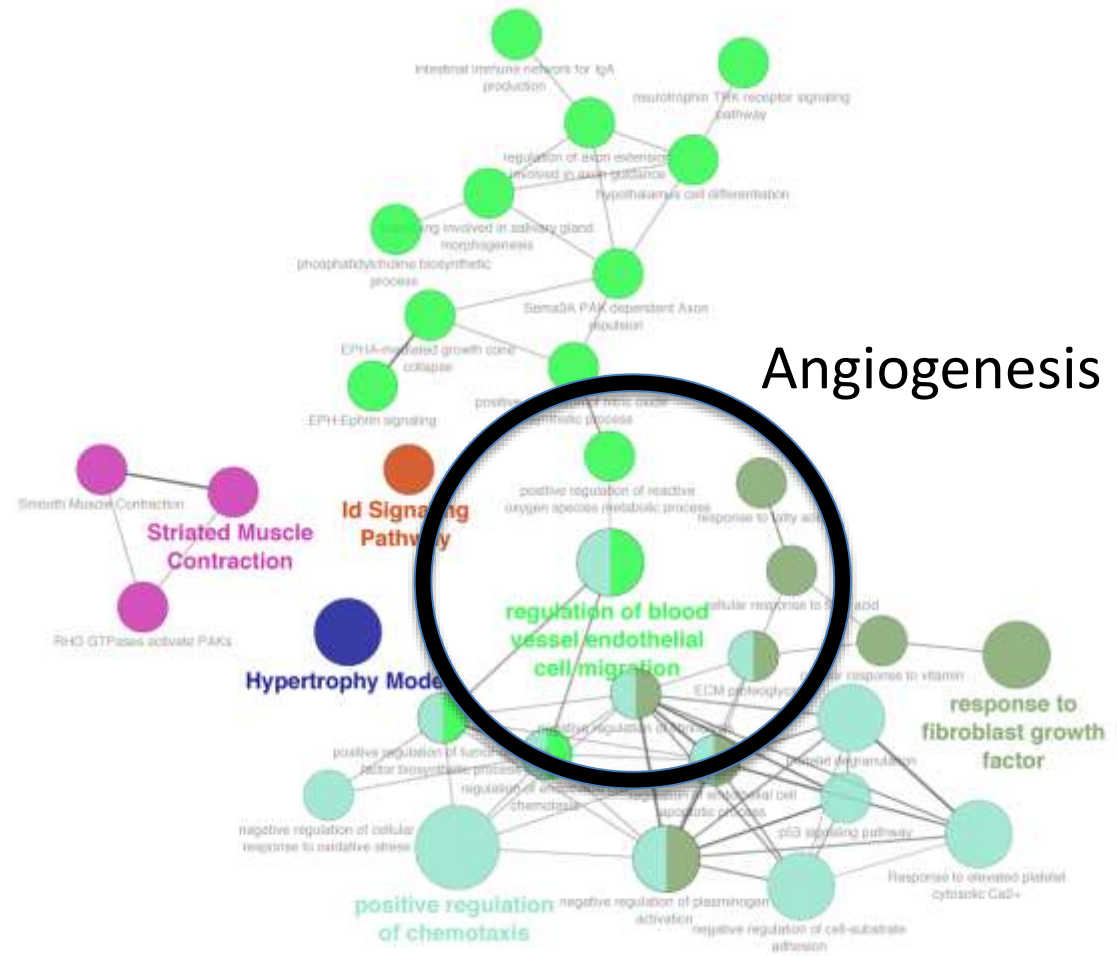
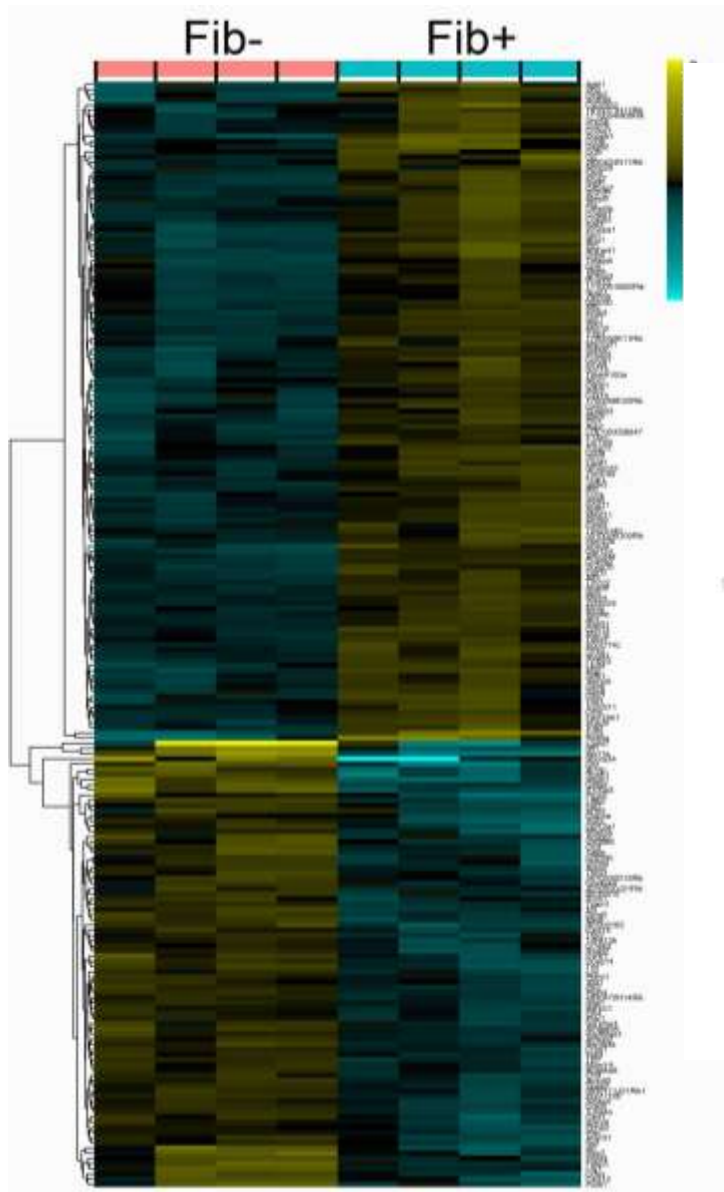


An unbiased approach

- Harvested tumors at an early time point, before there was a clear fibrinogen-dependent difference in tumor growth.
- Analyzed the samples by RNAseq.

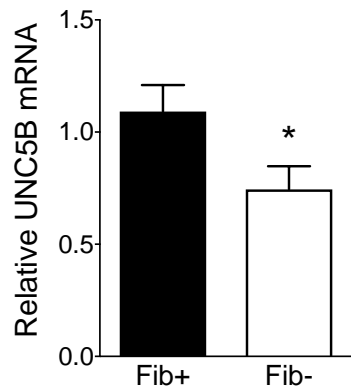


An unbiased assessment suggests fibrin(ogen) impacts angiogenesis in this experimental setting

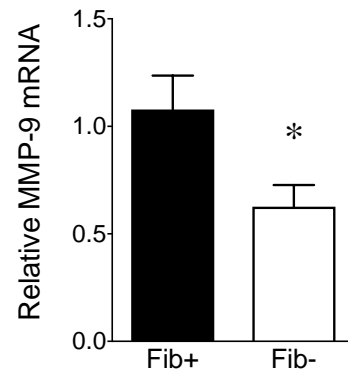


Fibrin(ogen) promotes the expression of multiple genes associated with angiogenesis and the inflammatory response

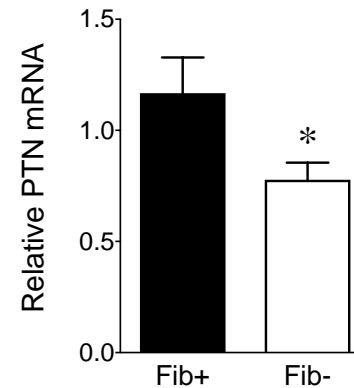
Netrin-1 Receptor



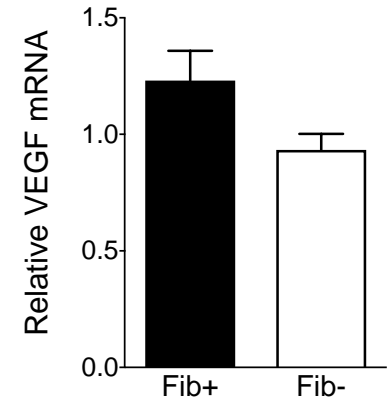
MMP-9



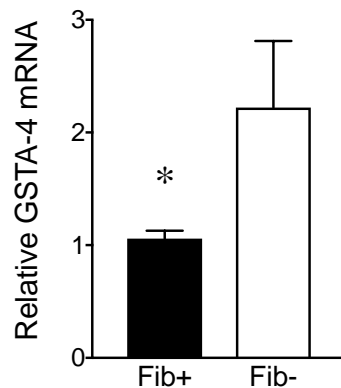
Pleiotrophin



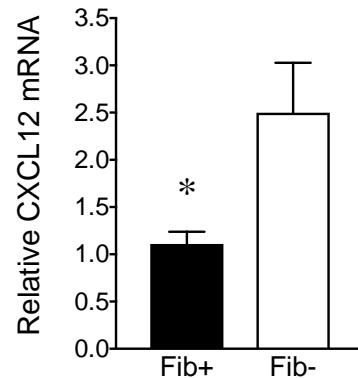
VEGF-A



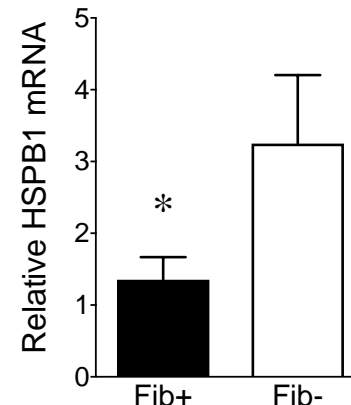
Glutathione S-transferase



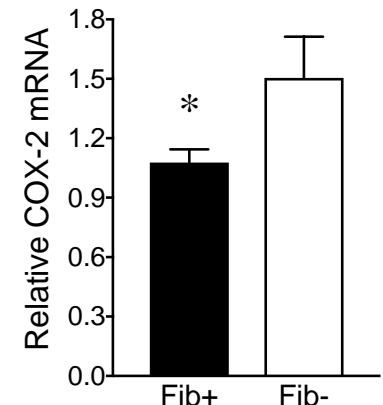
CXCL12



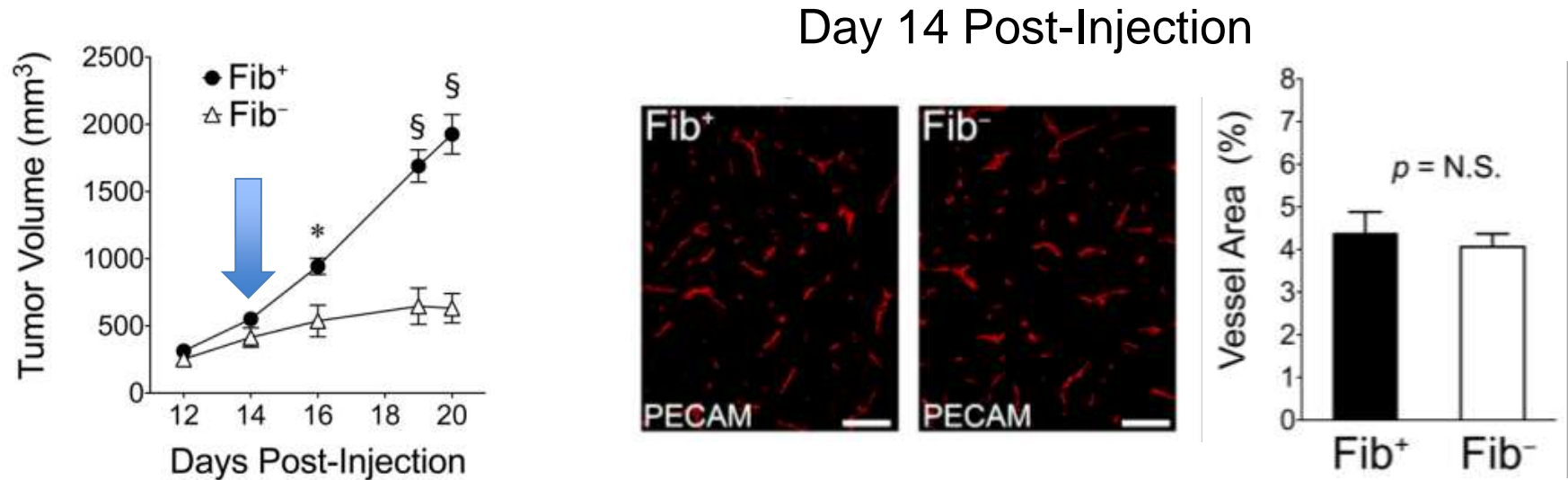
Heat Shock Protein B1



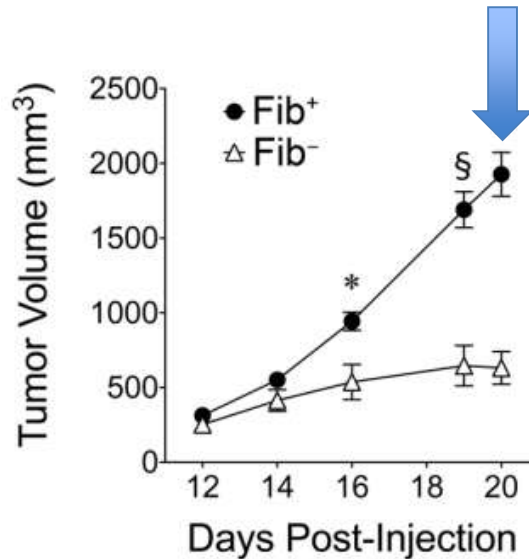
COX-2



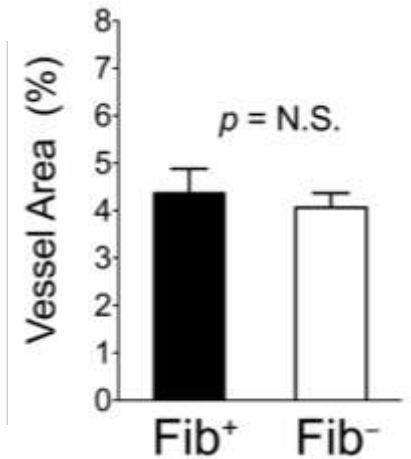
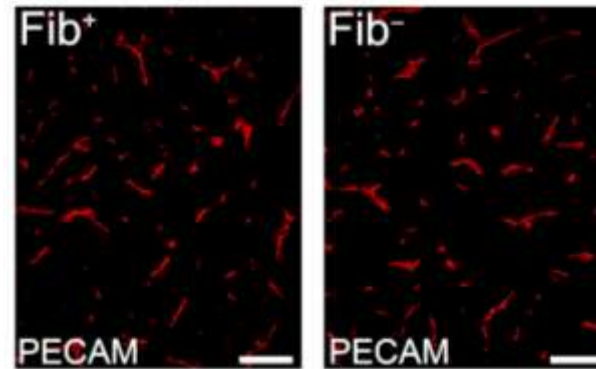
Vessel density is not fibrin(ogen) dependent early in colon cancer growth



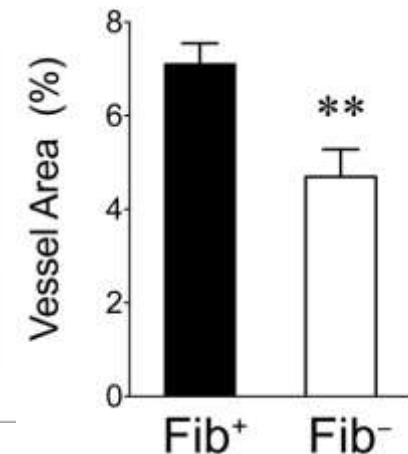
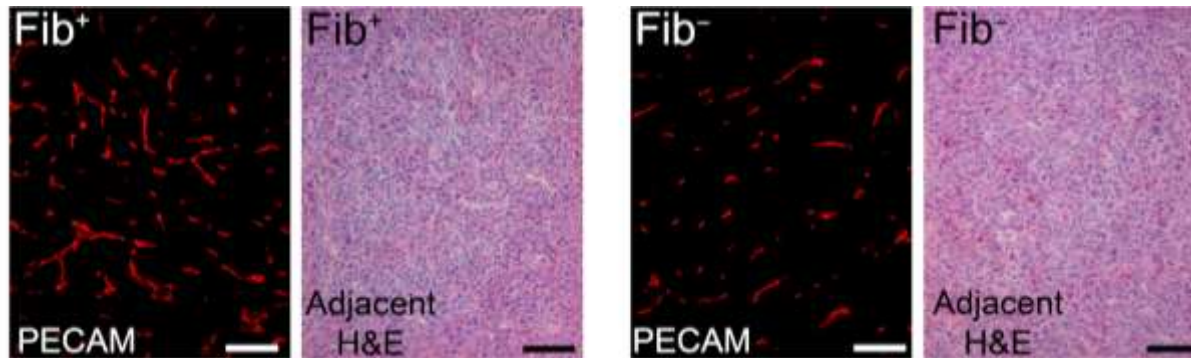
Fibrinogen deficiency results in a diminished tumor vascularity over time



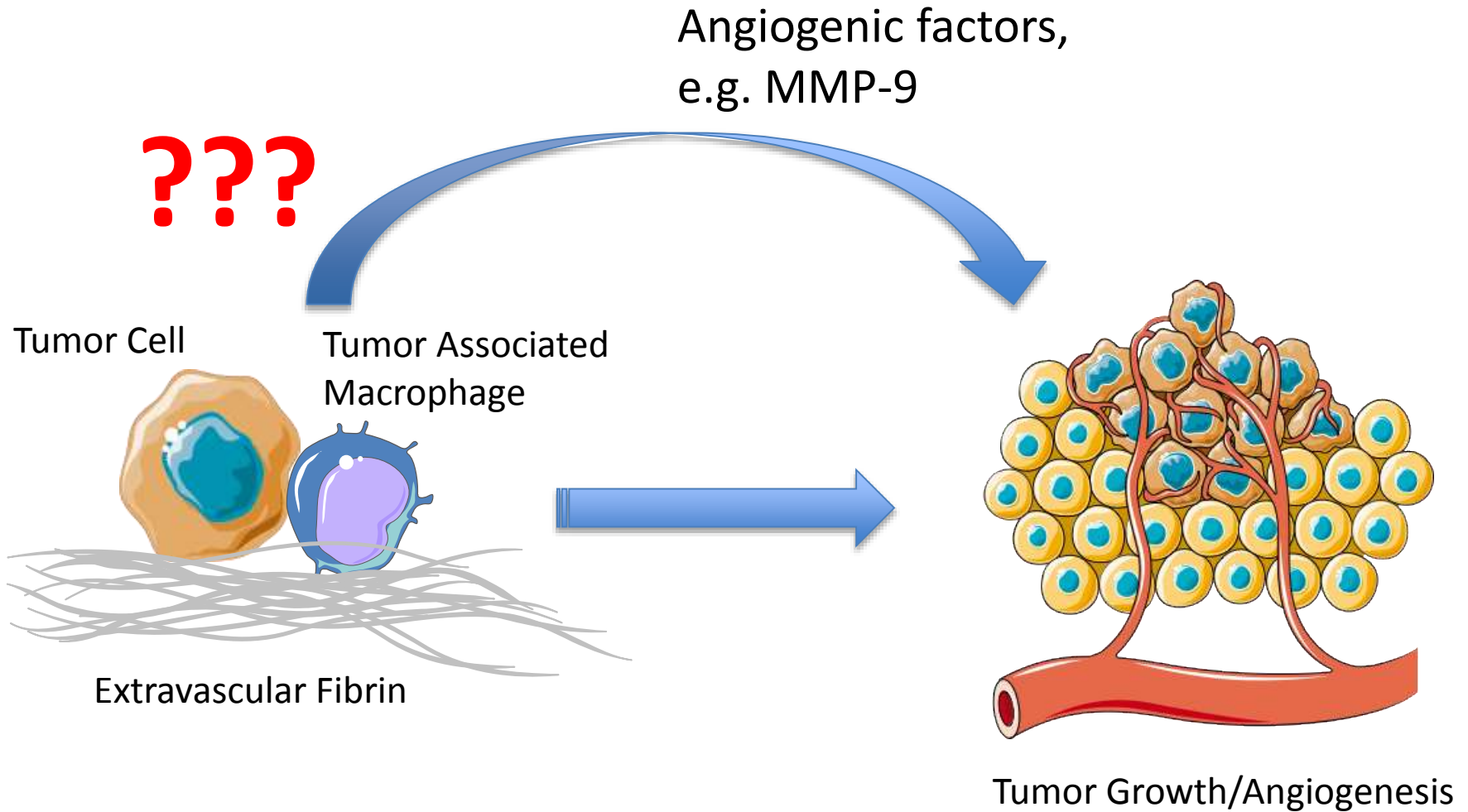
Day 14 Post-Injection



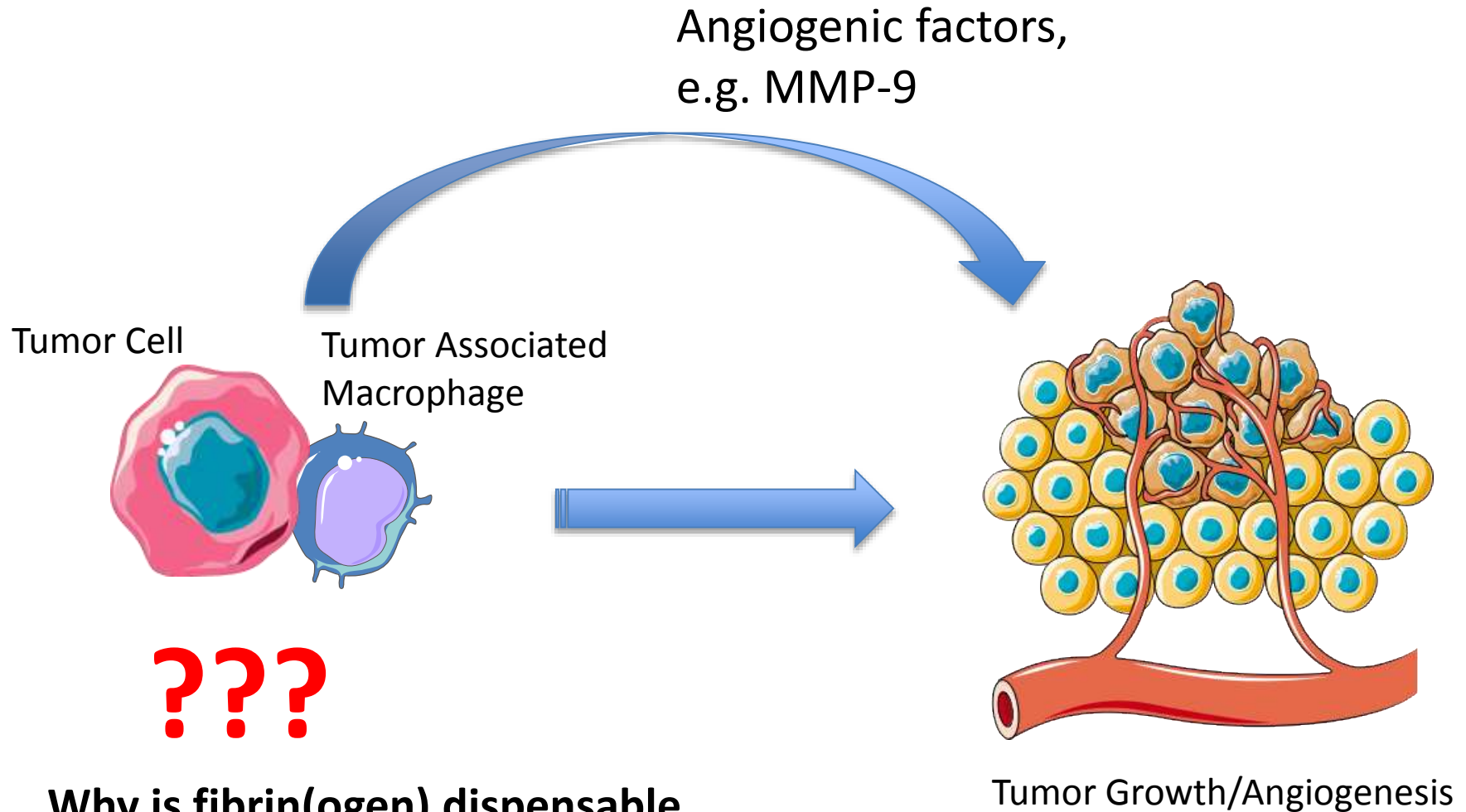
Day 20 Post-Injection



Tumor Growth and Stroma Formation



Tumor Growth and Stroma Formation



**Why is fibrin(ogen) dispensable
for the growth of some tumors?**

Acknowledgements

Palumbo Lab

Brian Turpin
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Kris Steinbrecher

Infectious Disease

Joe Qualls

DHC Bioinformatics

Rebekah Karns

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Alexey Revenko

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The potential for the thrombin/fibrinogen axis to drive tumor growth is not limited to colon cancer

C57BL/6-derived Prostate Carcinoma

