

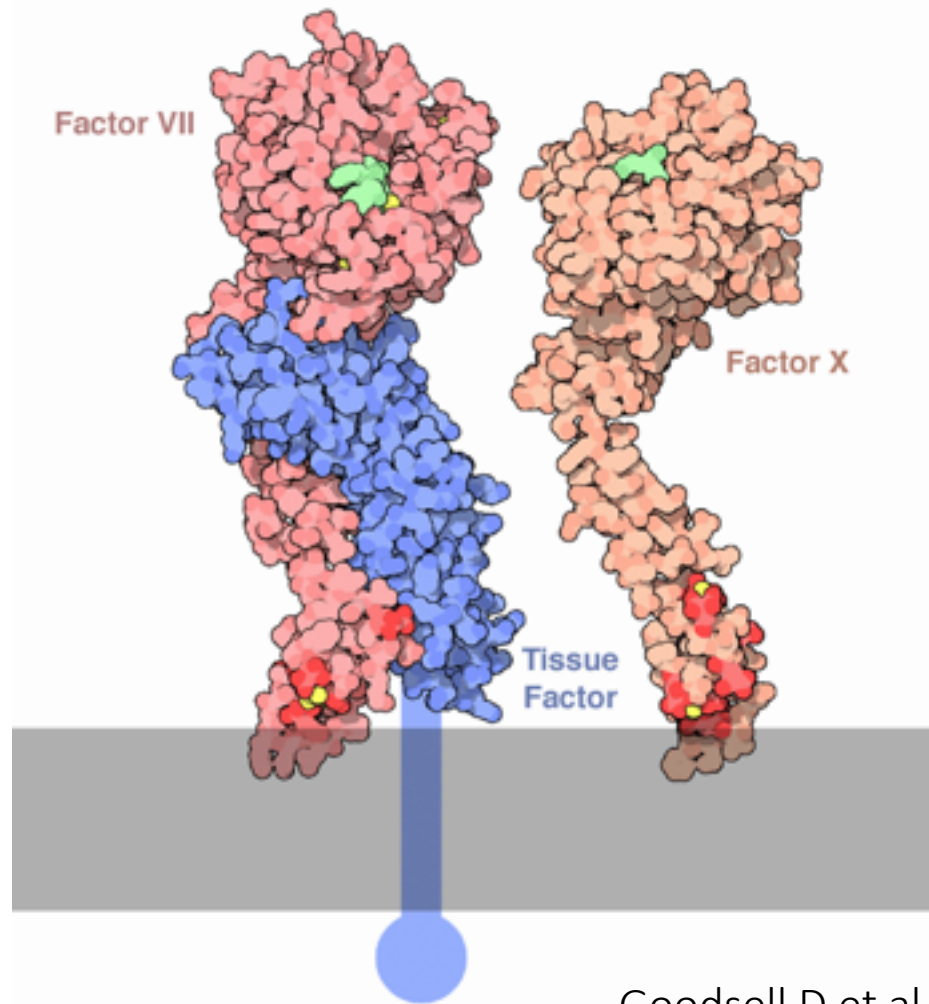
Tissue Factor/PAR2 signaling enhances the malignancy and radiation resistance of lung cancer brain metastases

Dusten Unruh, PhD

8th Annual Brain Metastases Research and
Emerging Therapy Conference



Tissue Factor (TF) is the primary physiologic trigger of blood clotting



Goodsell D et al., The Oncologist, 2006

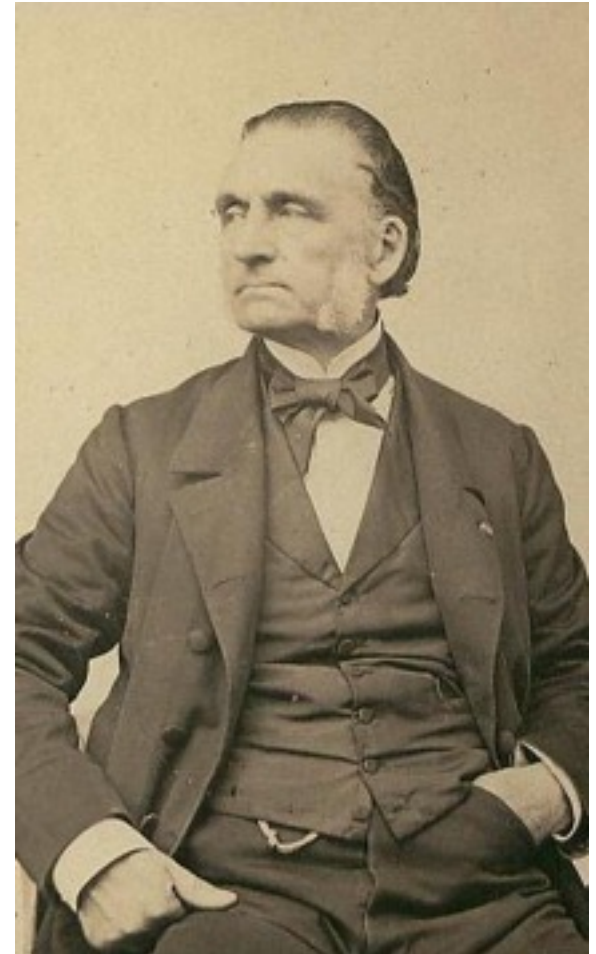
Cancer and thrombosis

Cancer patients have 4 to 6-fold increased risk of venous thromboembolism (VTE) compared to general population.¹

~20% of all new venous thromboembolic events are associated with cancer.²

“I am lost: the phlebitis that has just appeared tonight leaves me no doubt about the nature of my illness.”

Heit et al., Arch Intern Med, 2002



Armand Trousseau, 1865

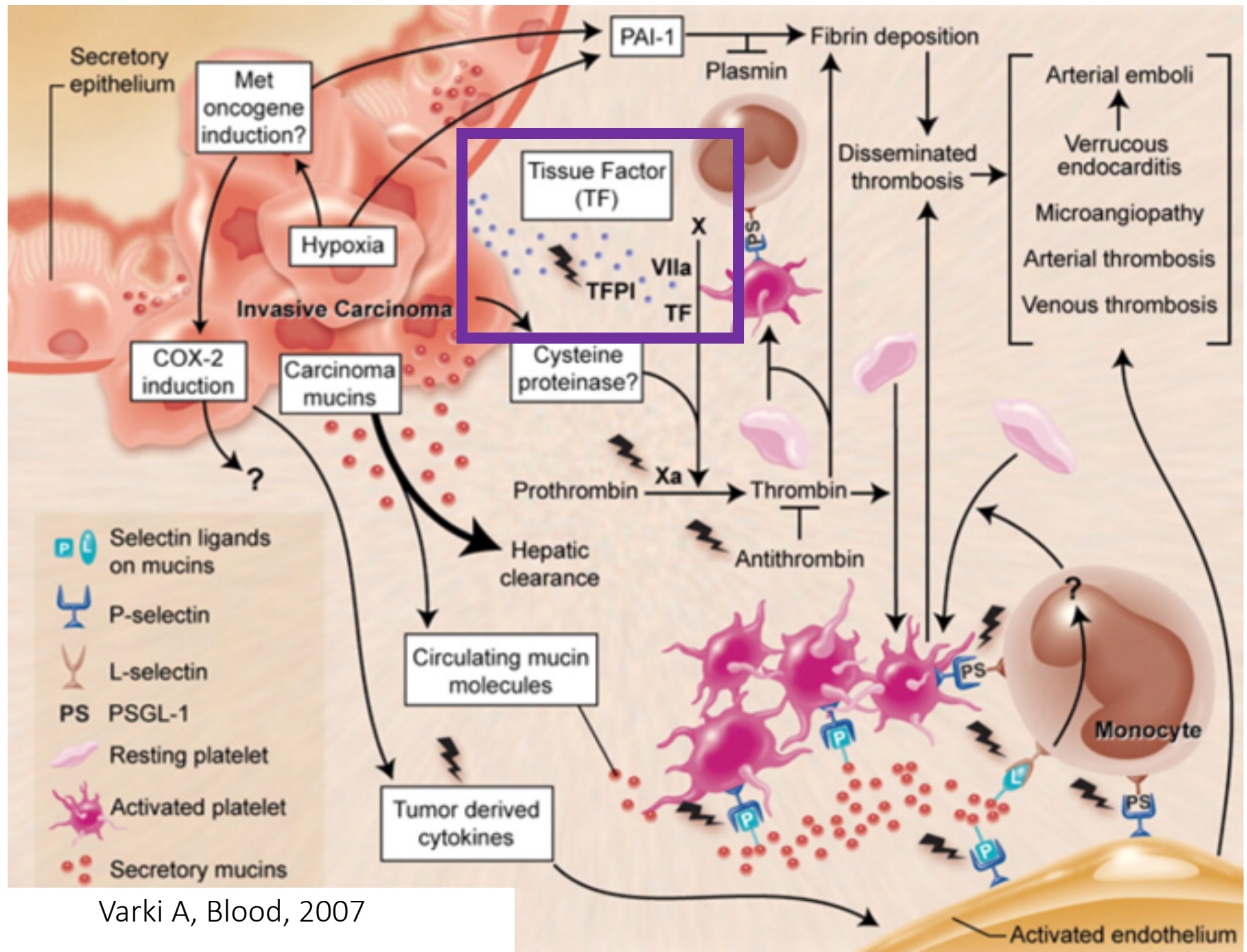
Venous thromboemboli (VTE)



deep venous thrombus

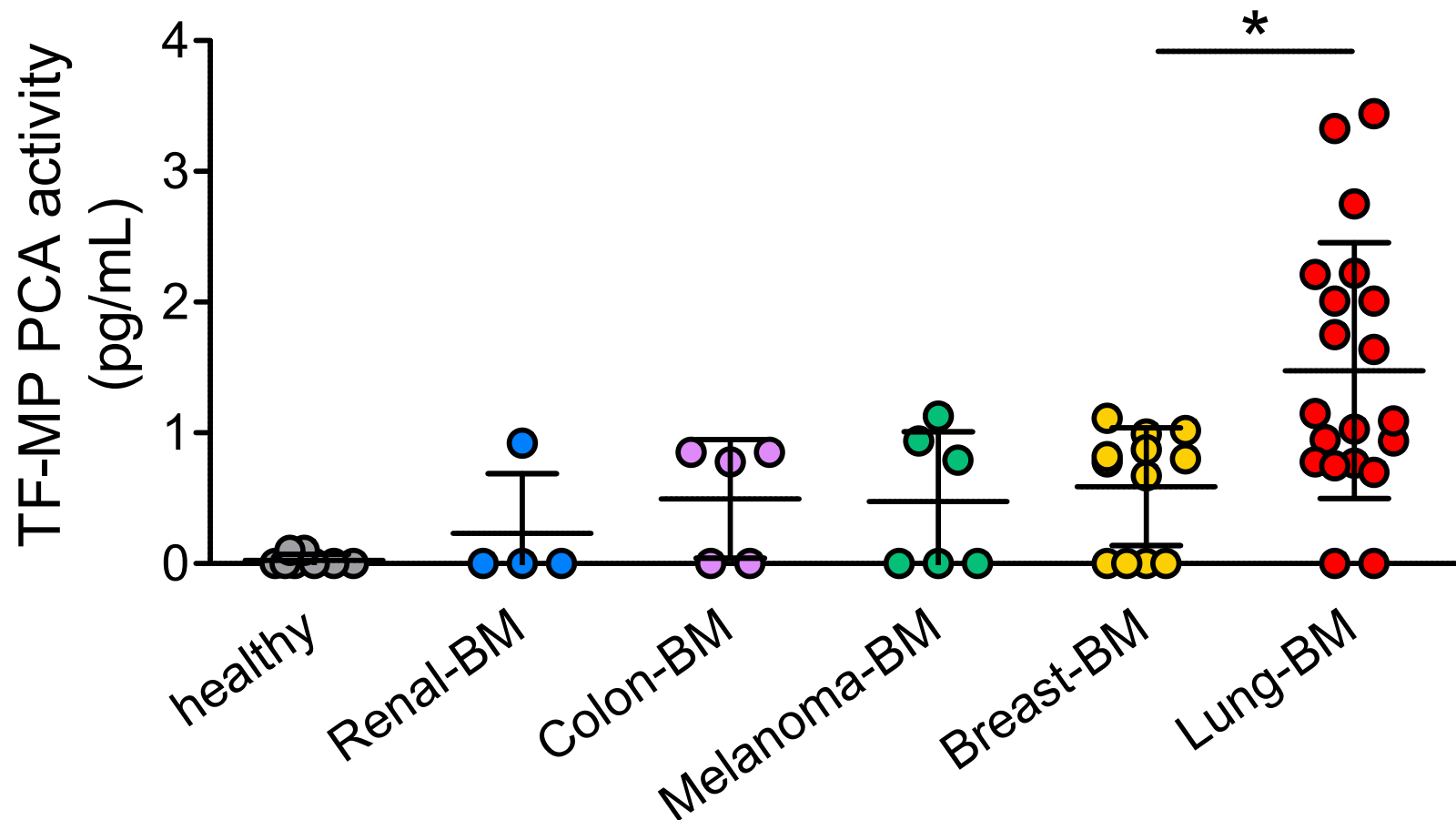


pulmonary embolus

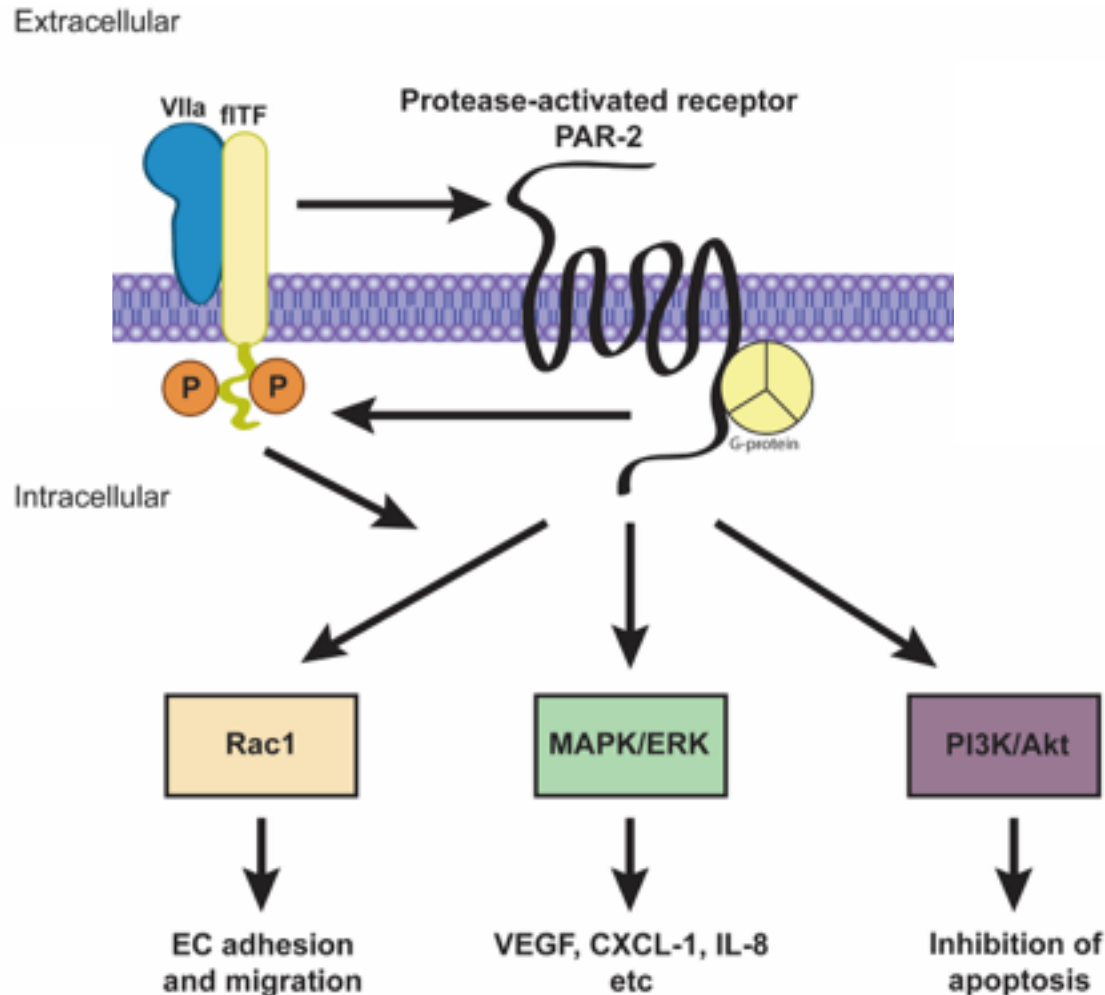


Varki A, Blood, 2007

Lung cancer brain metastases (BM) have elevated levels of circulating TF-microparticles



TF promotes malignancy through protease-activated receptor 2 (PAR-2)



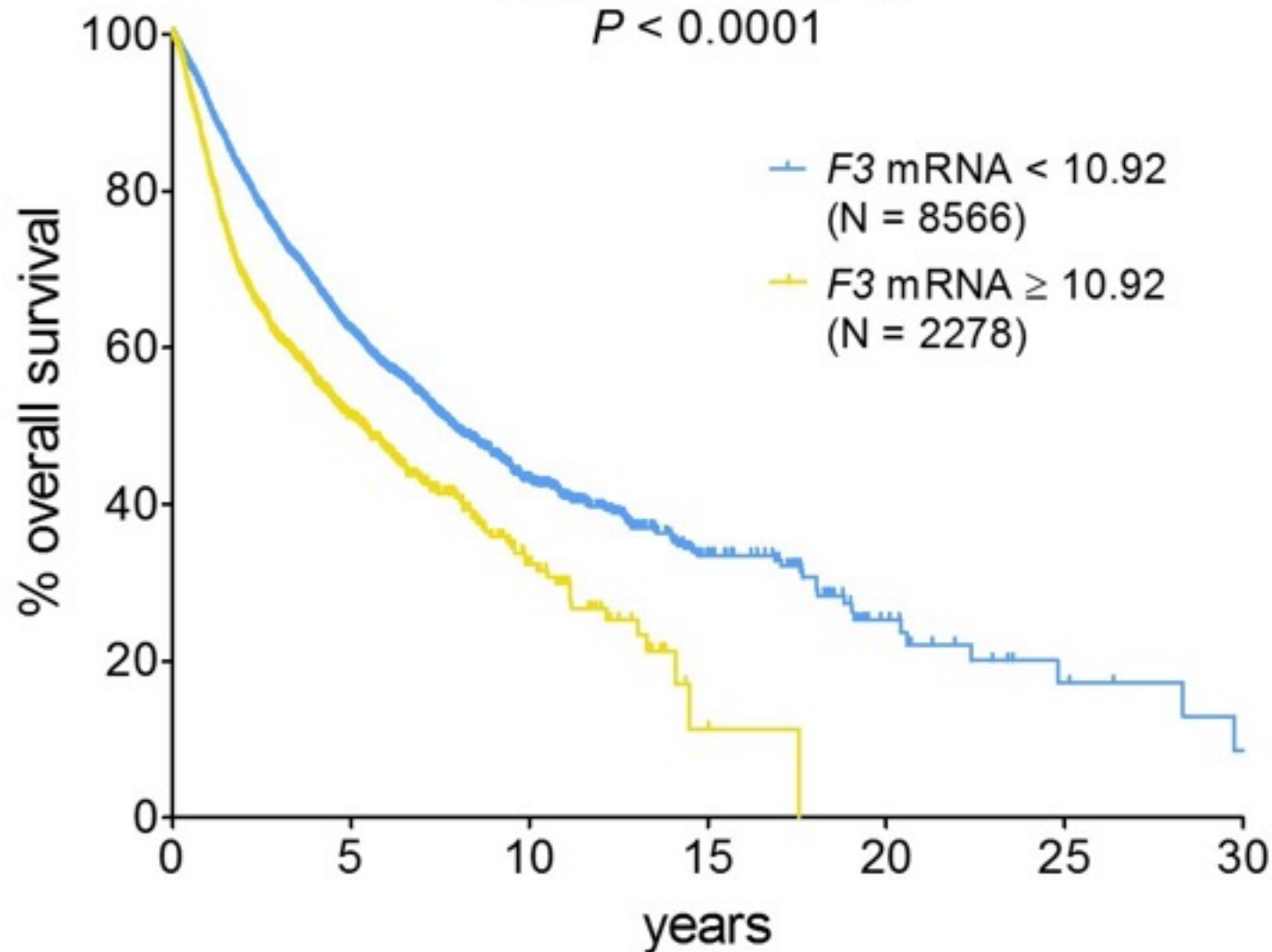
Cell survival, angiogenesis, and metastasis

median survival 5.3 vs 7.9 years

HR = 1.7

95% CI = 1.6 to 1.9

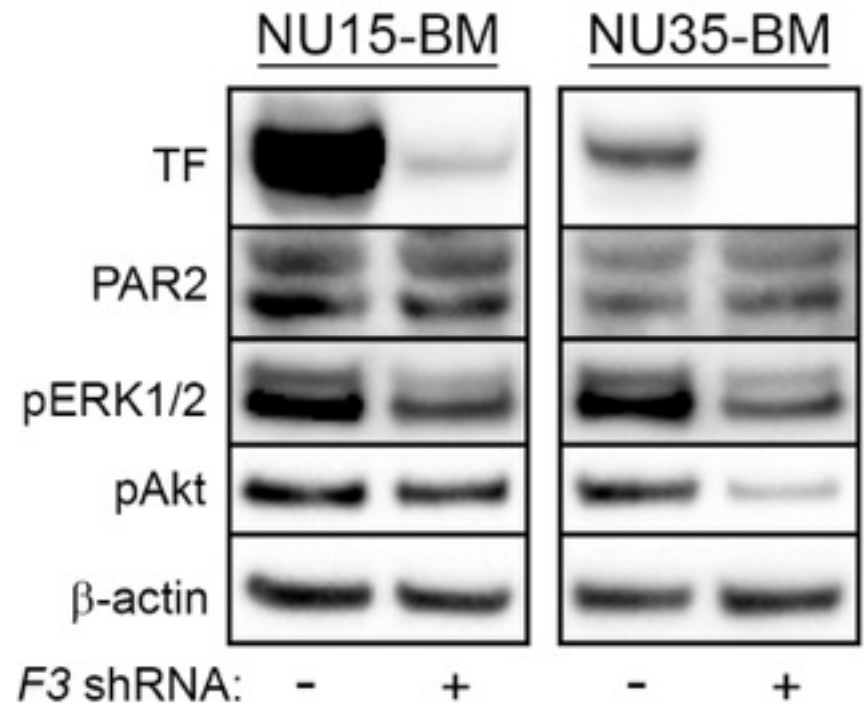
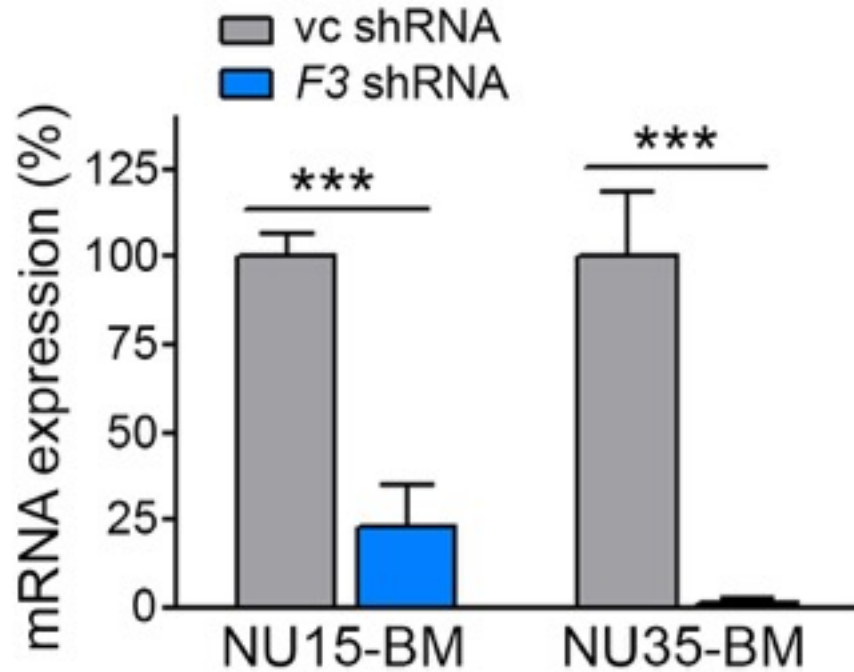
$P < 0.0001$



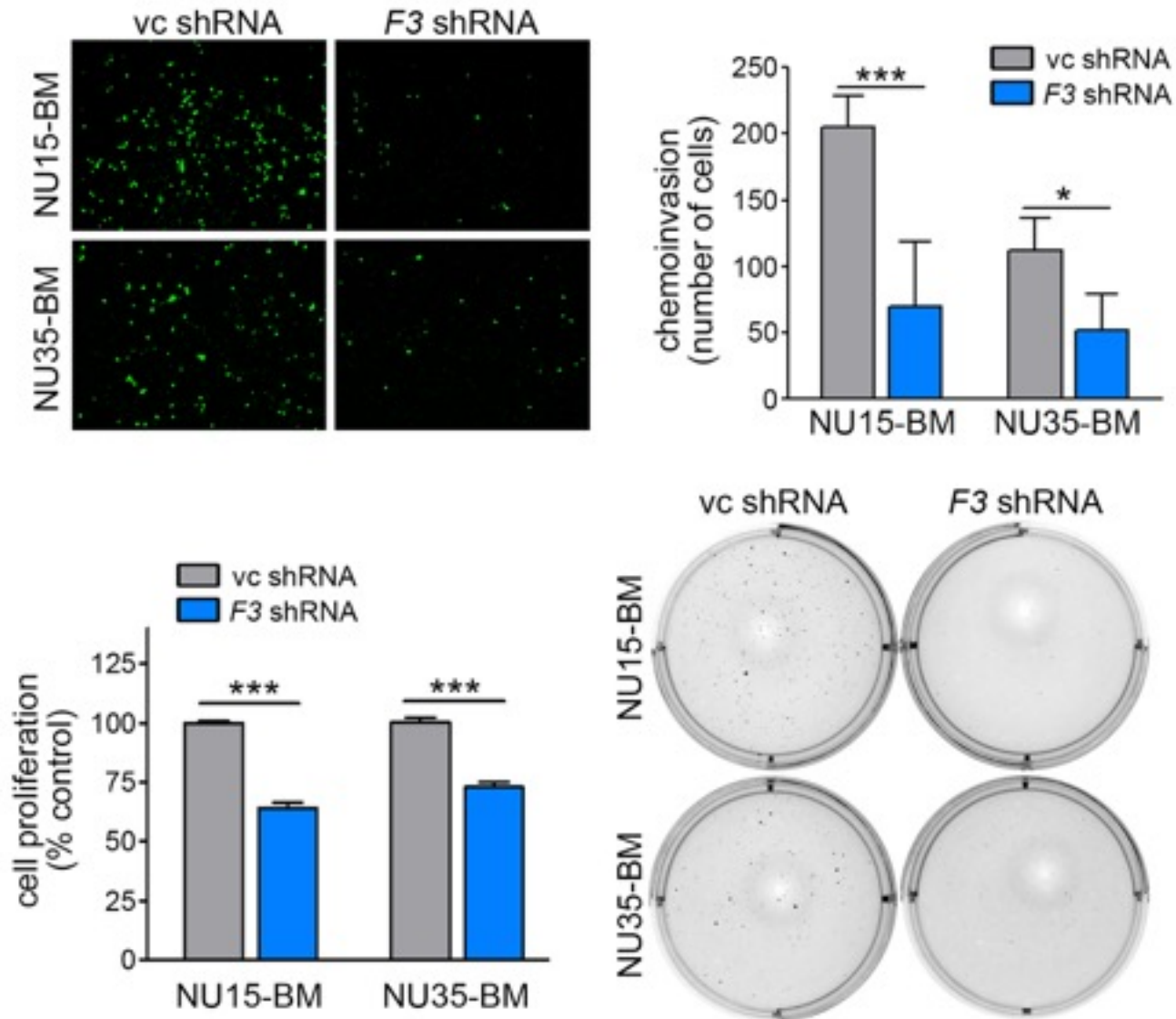
Two questions

1. Does TF-PAR2 signaling increase lung cancer brain metastatic malignancy?
2. Does inhibition of the TF-PAR2 signaling pathway reduce brain metastatic malignancy?

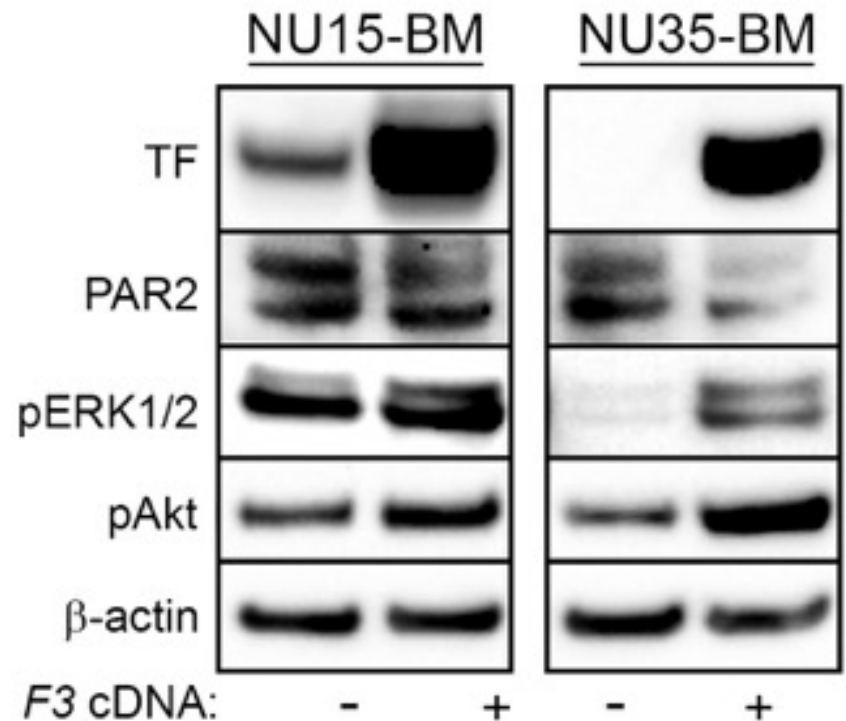
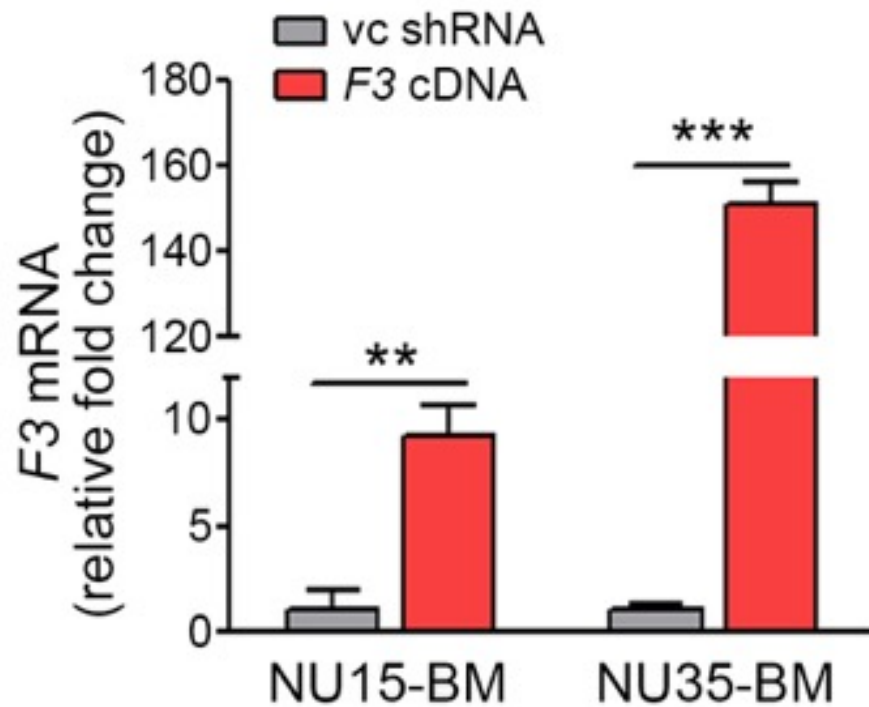
F3 shRNA reduces TF expression in lung cancer brain metastatic cells



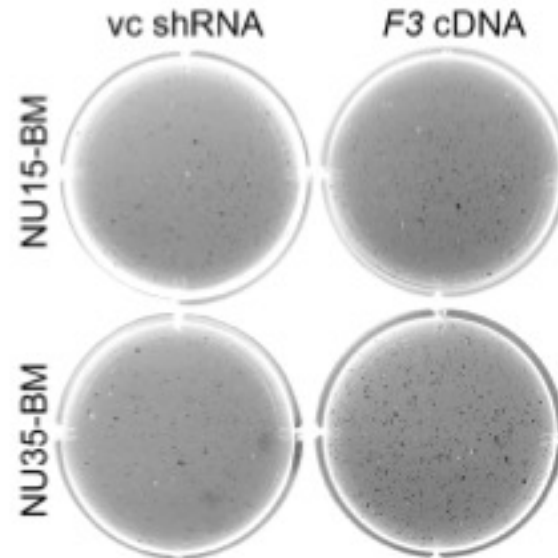
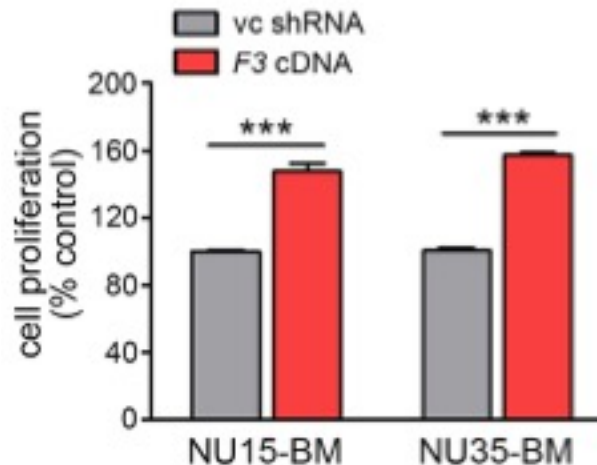
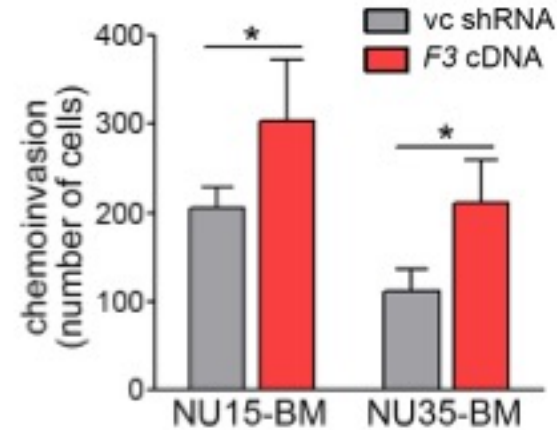
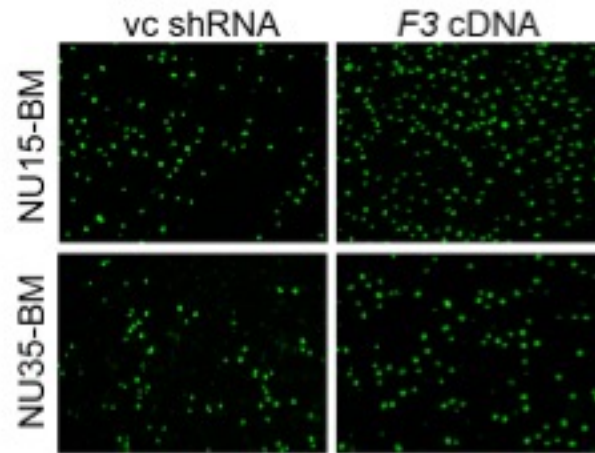
Tissue Factor knockdown reduces in vitro malignancy in Lung-BM cells



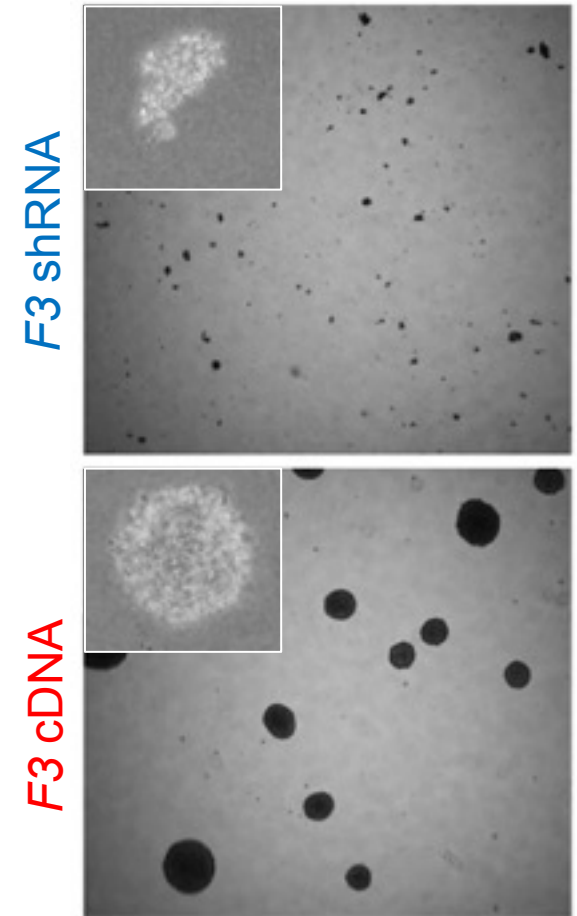
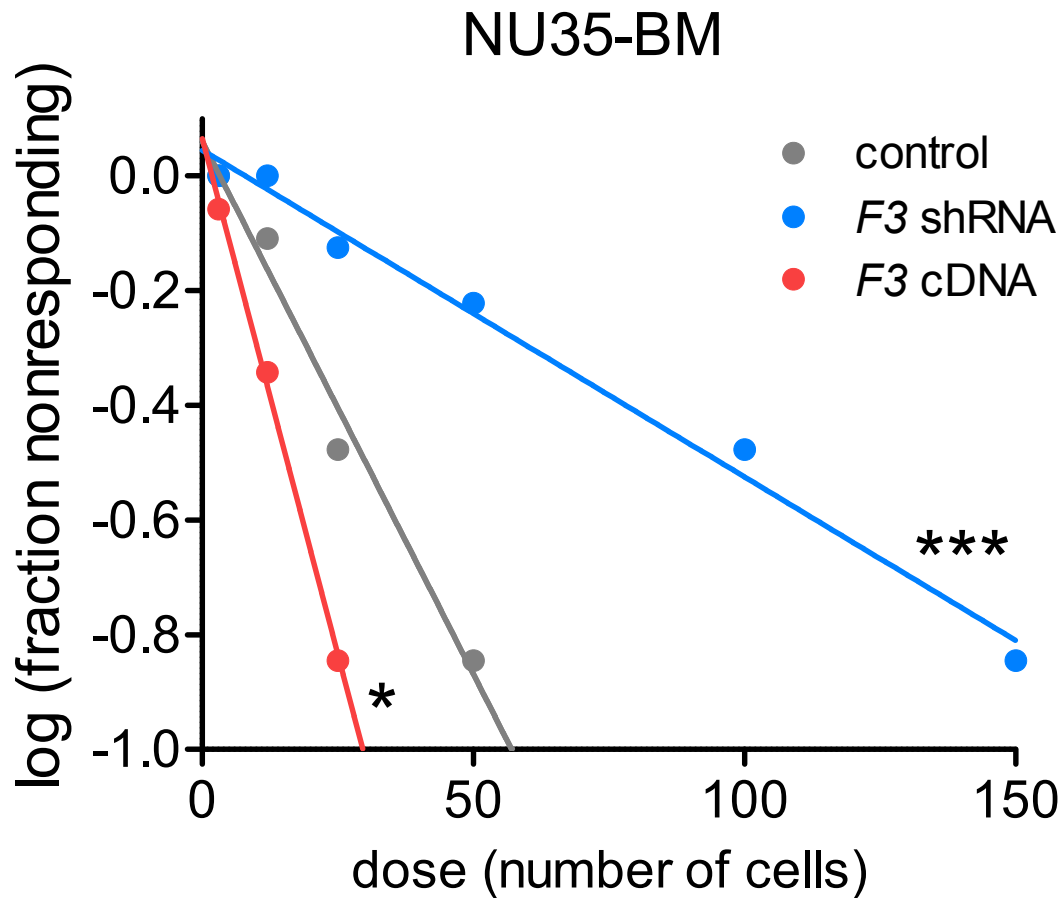
Tissue Factor overexpression increases Erk and Akt activation in Lung-BM cells



Tissue Factor over expression increases in vitro malignancy in Lung-BM cells



TF modulation alters tumor-initiating cell activity and tumorsphere morphology



F3 shRNA:



NU35-BM

CD133

CD44

β -actin

—

+

—

+



NU35-BM

CD133

CD44

β -actin

F3 cDNA:

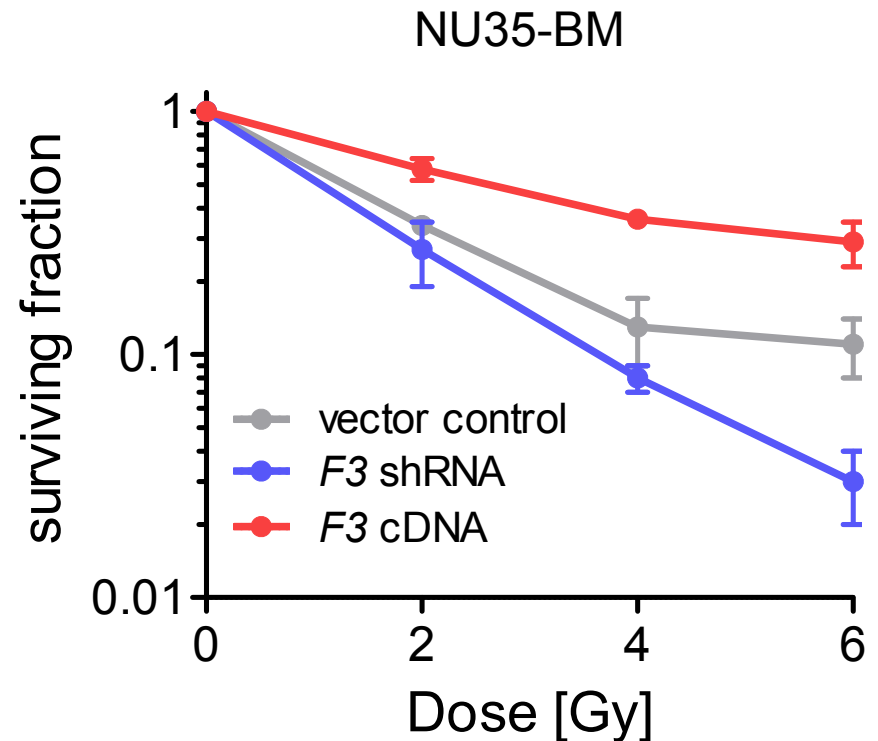
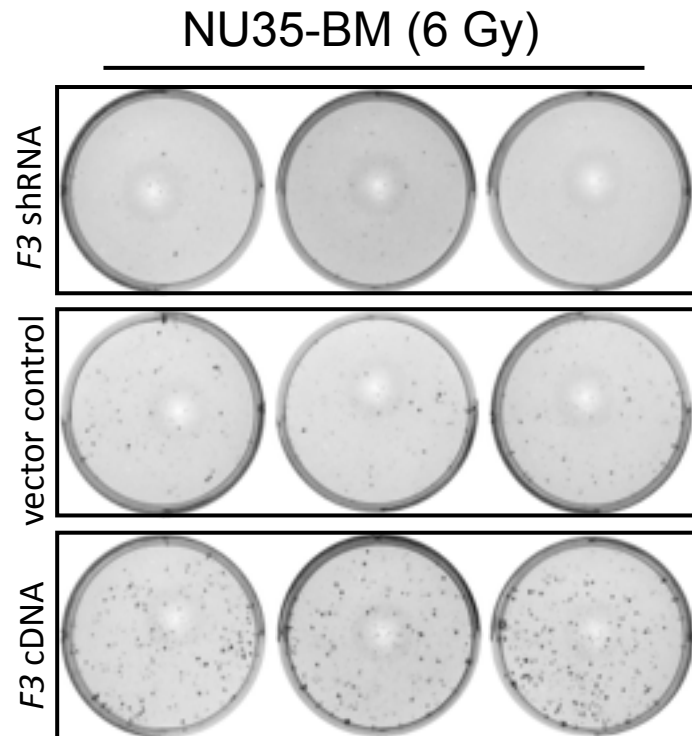
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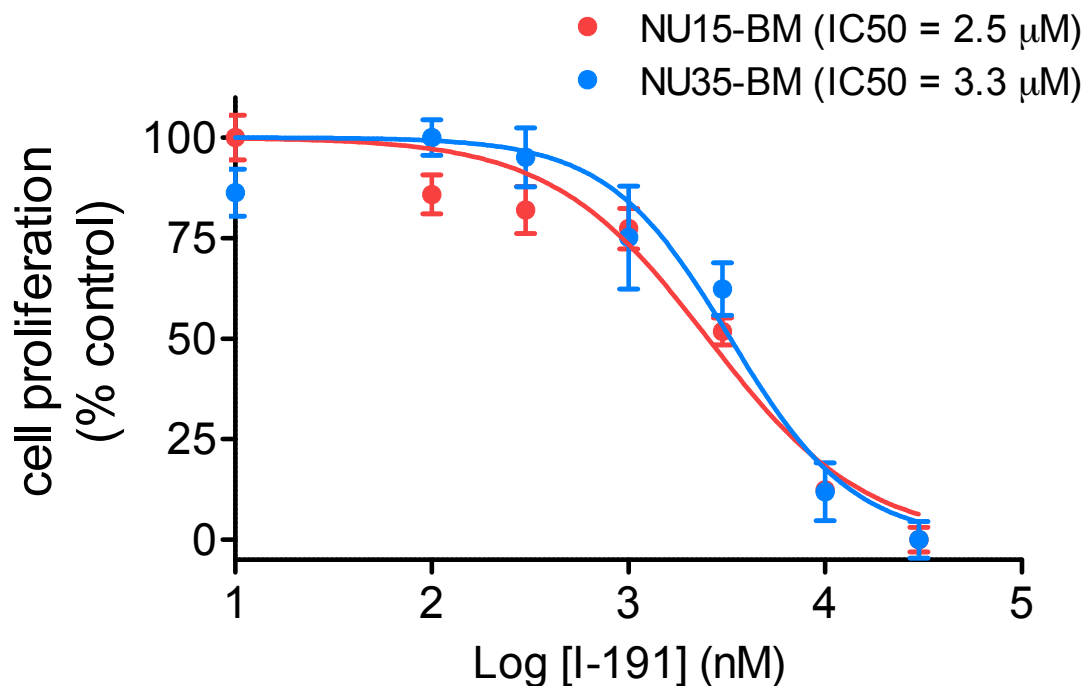
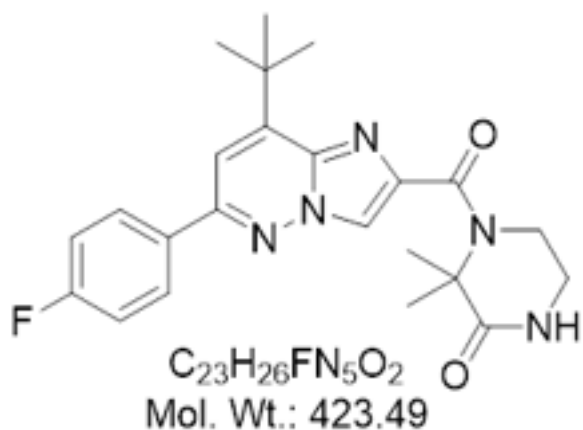
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TF signaling attenuates radiation-induced apoptosis

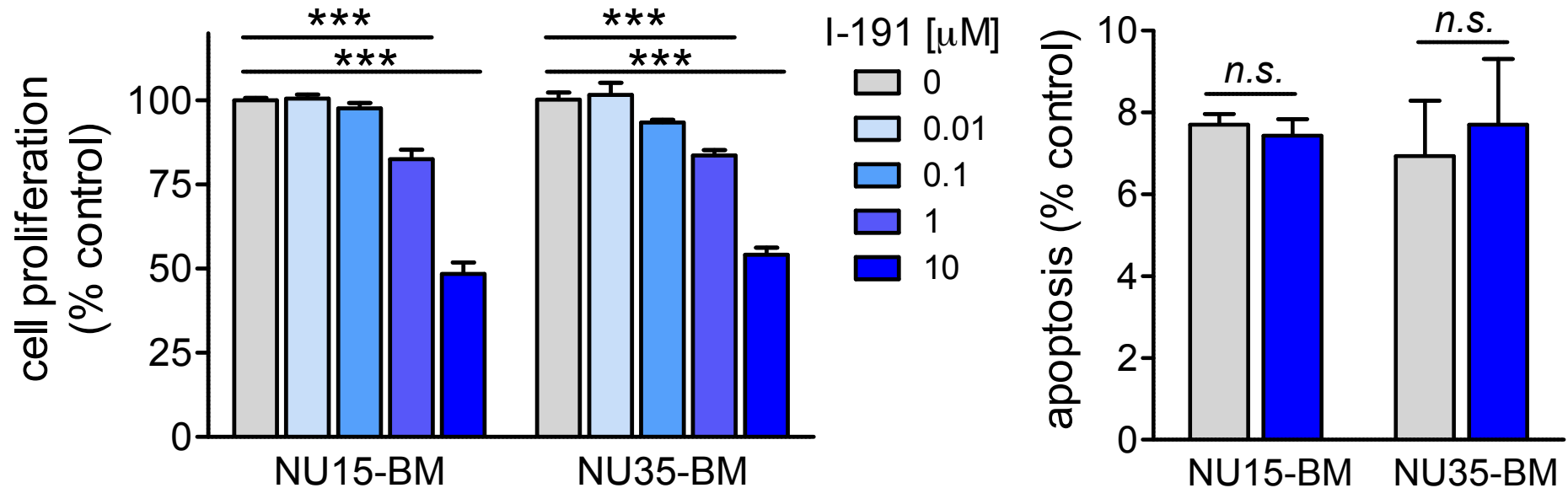


Effect of novel PAR2 antagonist (I-191) on Lung-BM cell proliferation

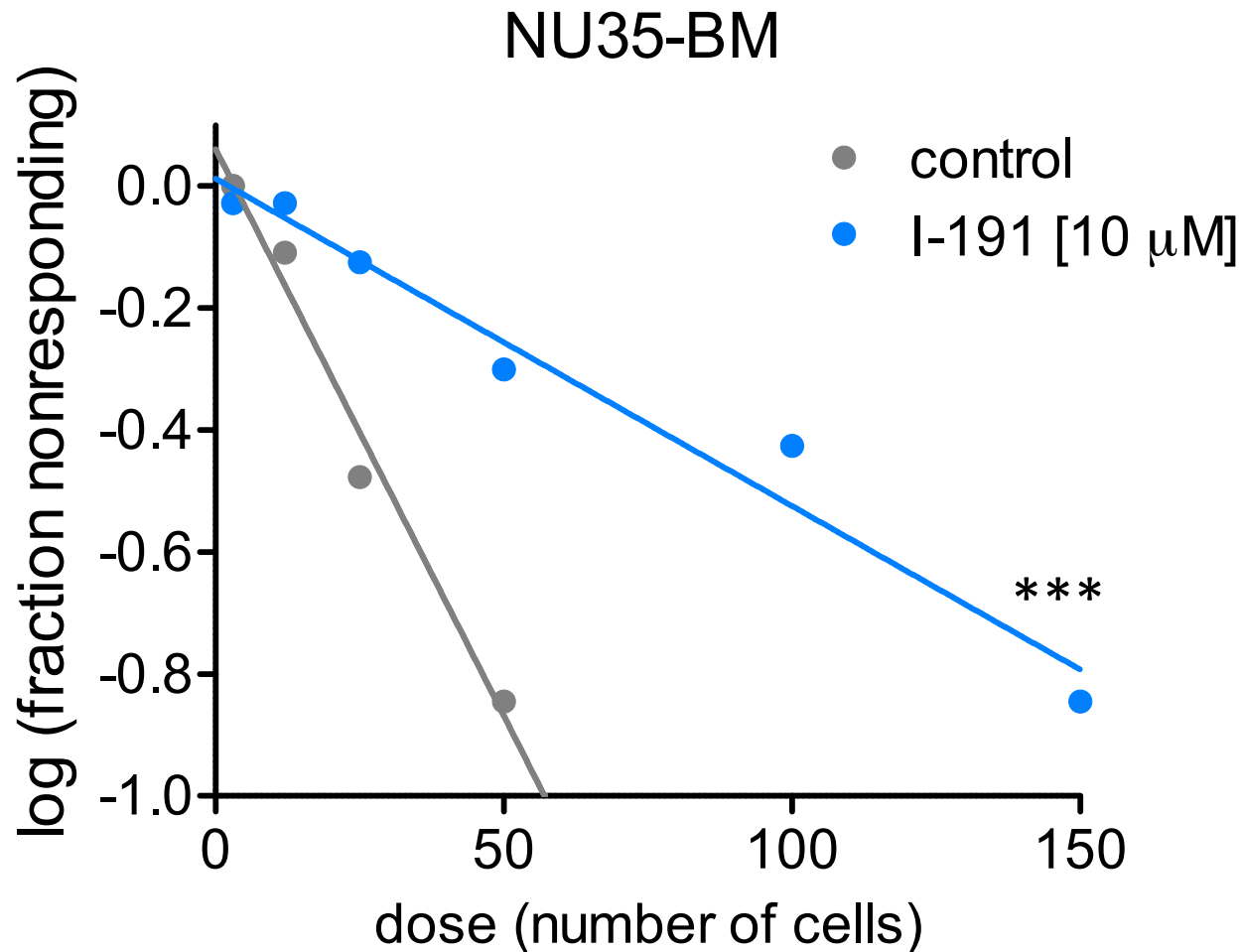
PAR2 antagonist (I-191)



I-191 blocks cell proliferation but doesn't increase apoptosis

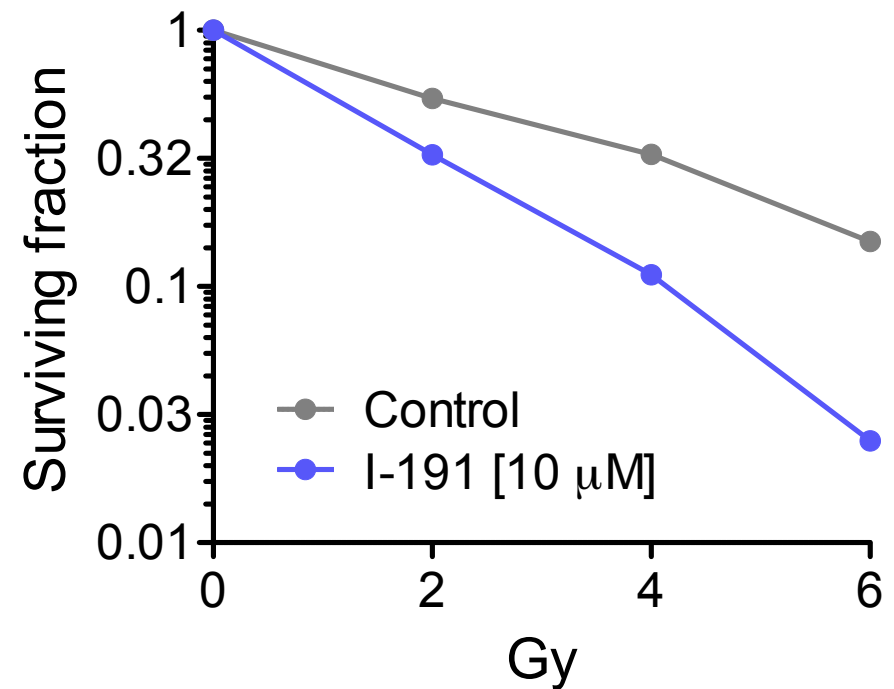
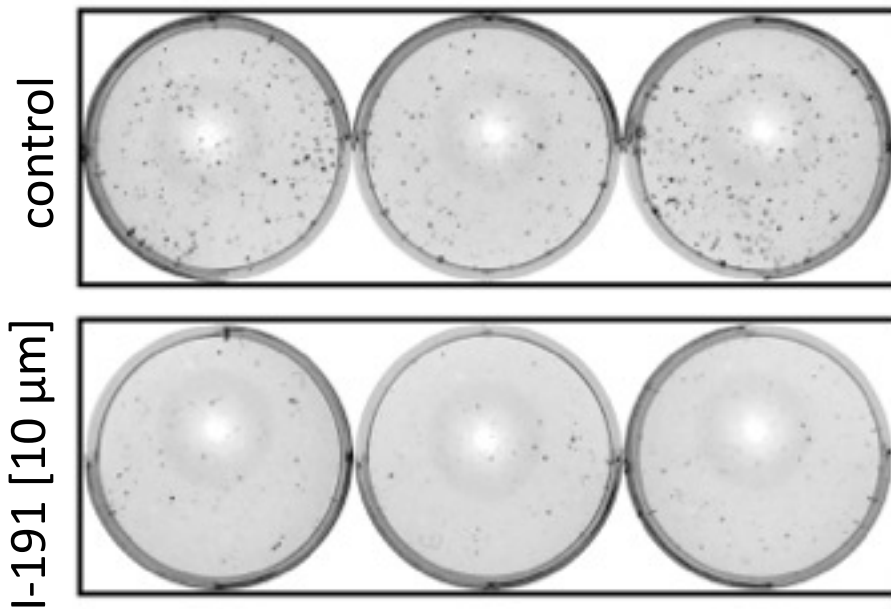


TF modulation alters tumor-initiating cell capacity and tumorsphere formation

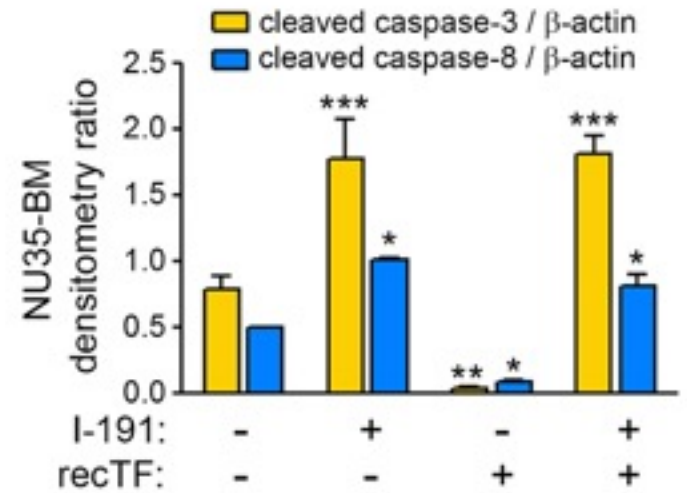
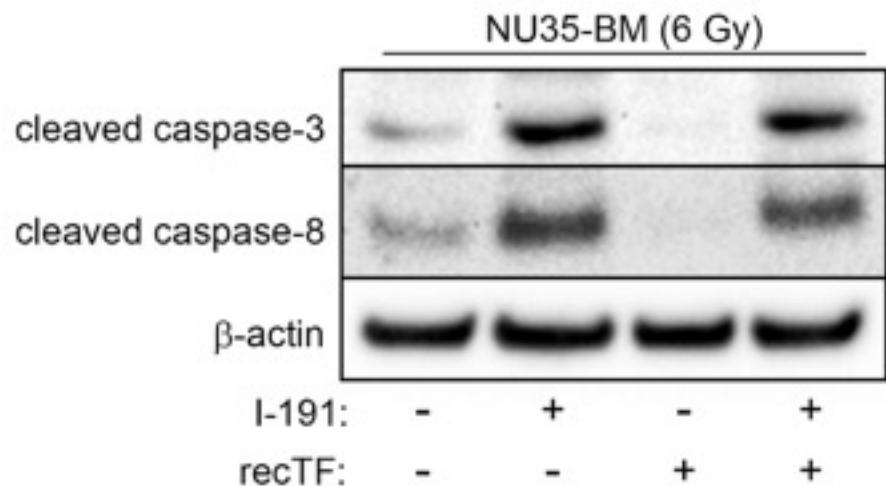
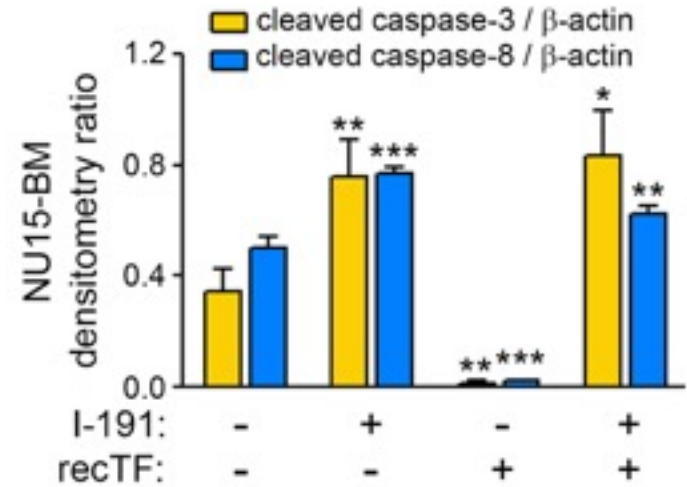
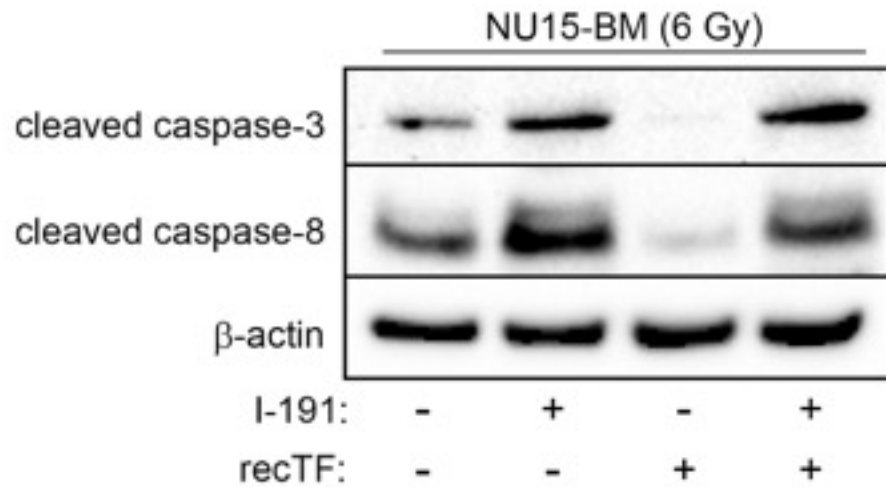


I-191 treatment increases the effectiveness of radiotherapy in Lung-BMs

NU35-BM (6 Gy)



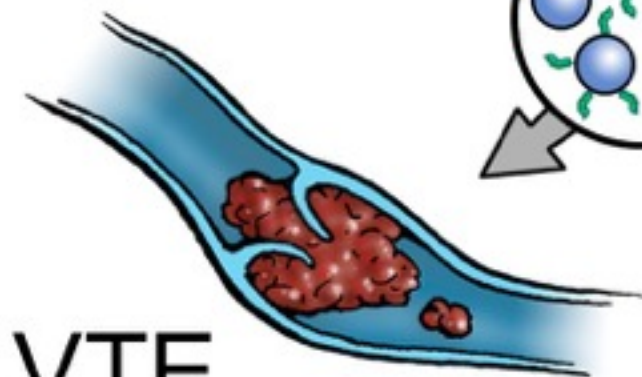
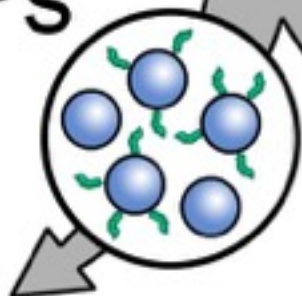
I-191 increases radiation-induced apoptosis in lung-BMs



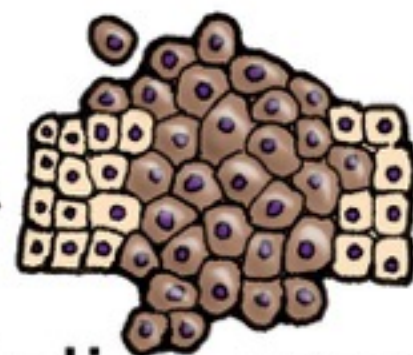
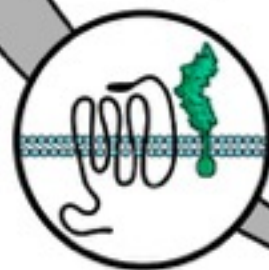
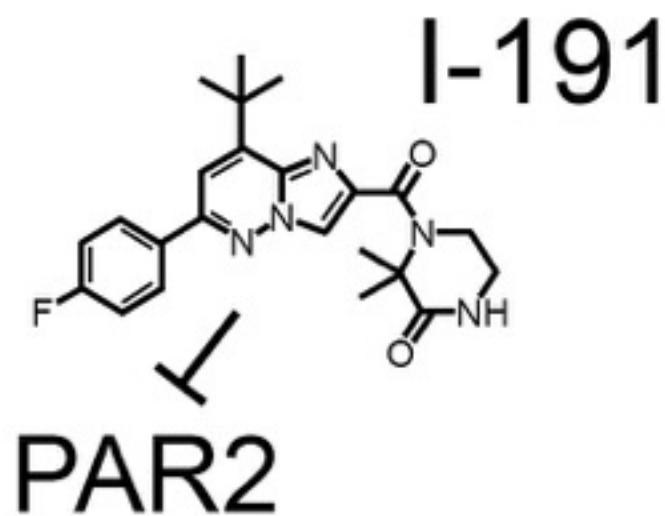
Tissue
Factor



TF-MPs



VTE



Malignancy

Acknowledgements

Craig Horbinski, PhD, MD – Mentor

David James, PhD – lung-met PDX models

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- Katy McCortney (NSTB manager)
- Alicia Steffens (histotechnician)
- Rodrigo Javier (NSTB technician)

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Craig Horbinski,
M.D., Ph.D.
(Primary mentor)

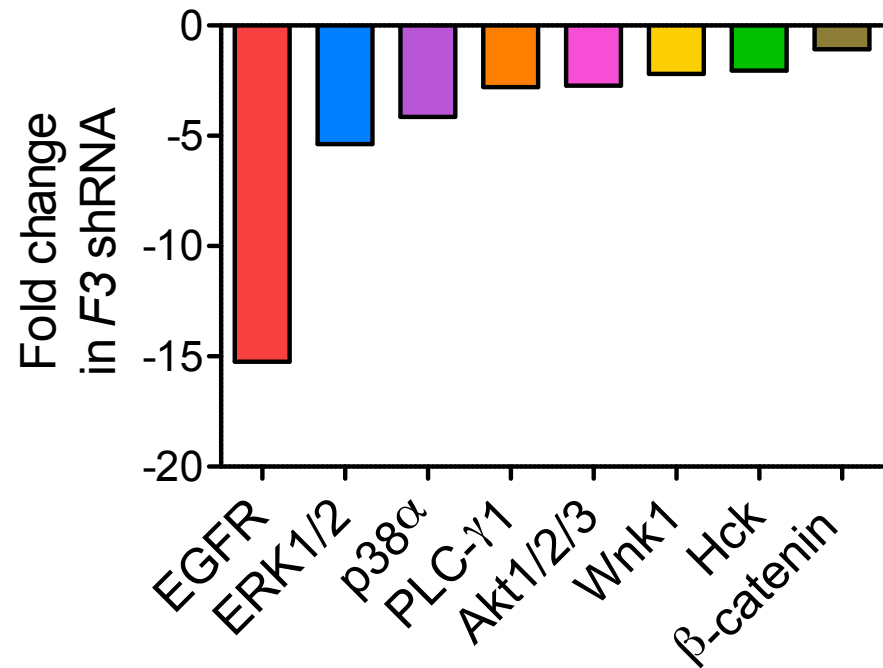
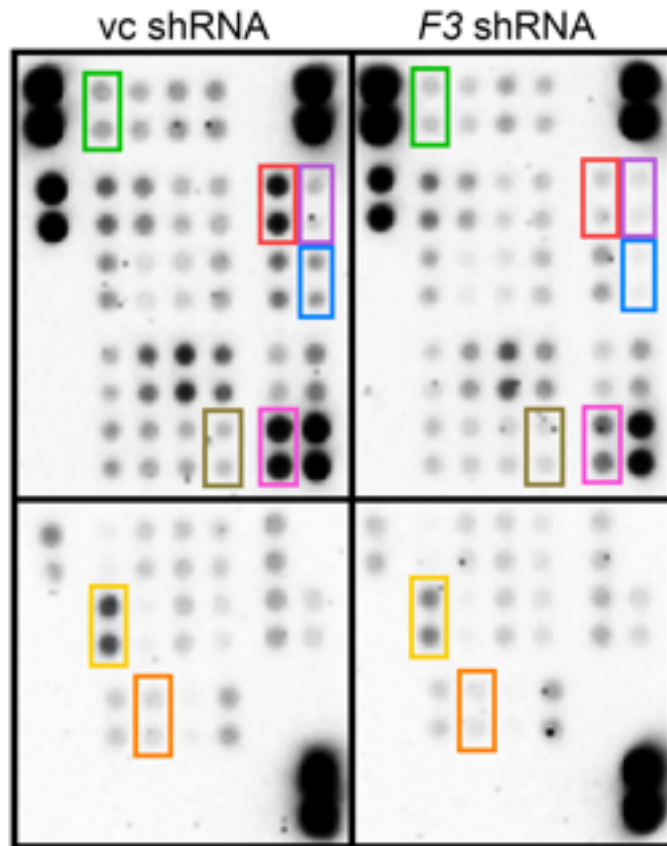


David James, Ph.D.
(PDX models)

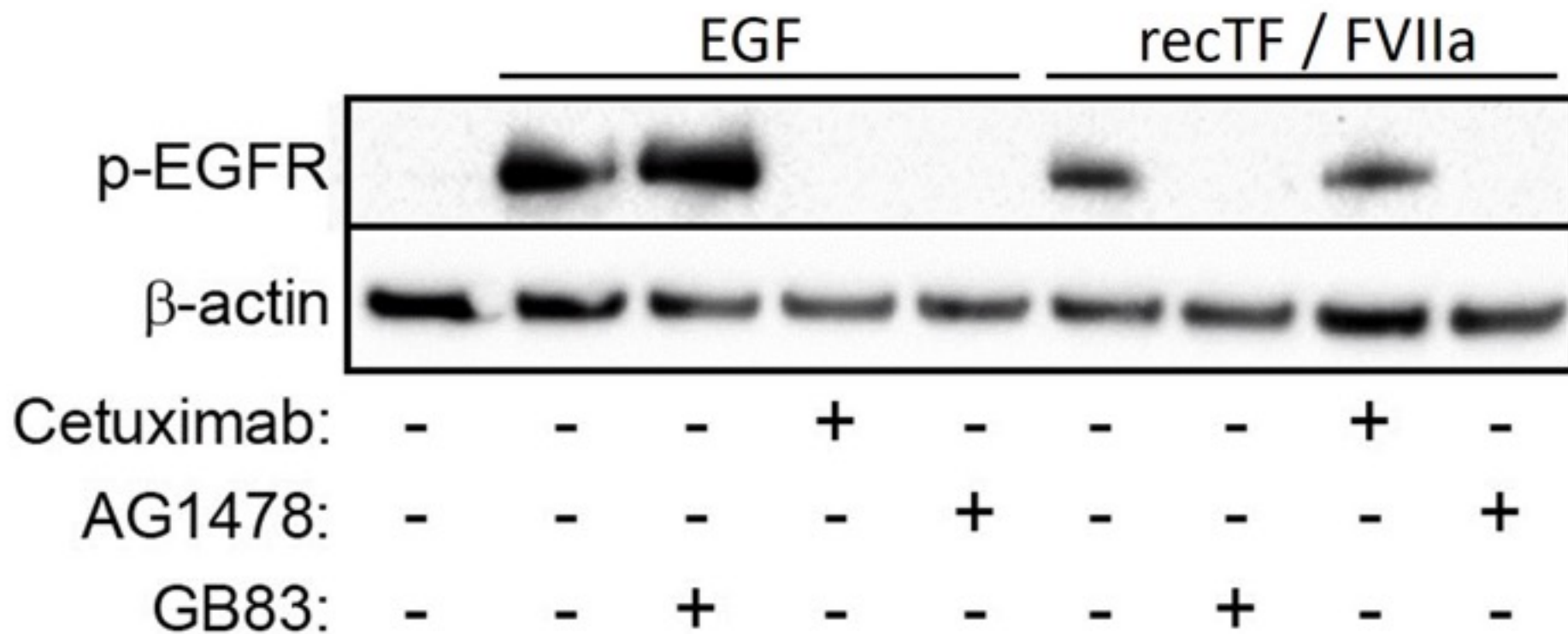
Questions?

NSTB ID	Diagnosis	Mutations		
NU00194	Poorly differentiated Metastatic Carcinoma (Lung)	CK7 positive		
NU15		CK20 negative		
		TTF-1 negative		
		Napsin A negative		
		P63 negative		
		ER negative		
		PR negative		
		BRST-2 negative		
		Mammaglobin negative		
		EGFR negative		
		KRAS c.34G>T (G12C) mutation		
NU00302	Metastatic Adenocarcinoma (lung)	CK7 positive		
NU35		CK20 negative		
		TTF-1 negative		
		Napsin A focal weakly positive		
		EGFR negative		
		KRAS negative at codons 12, 13, 61, 117, and 146		
		Gain of ALK - positive		

TF knockdown suppresses multiple signaling pathways



TF-PAR2 activates EGFR on the *intracellular* side



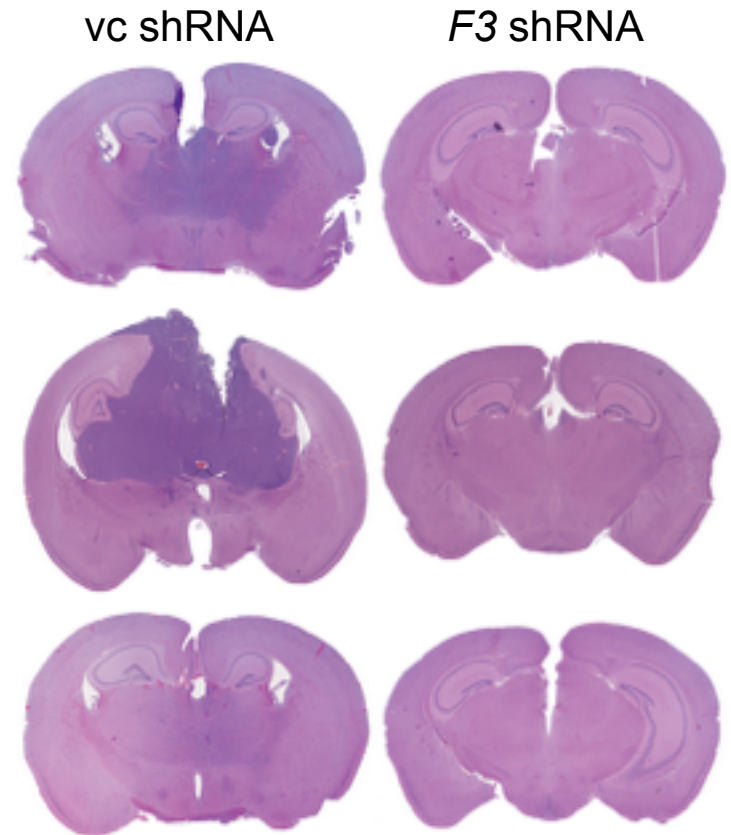
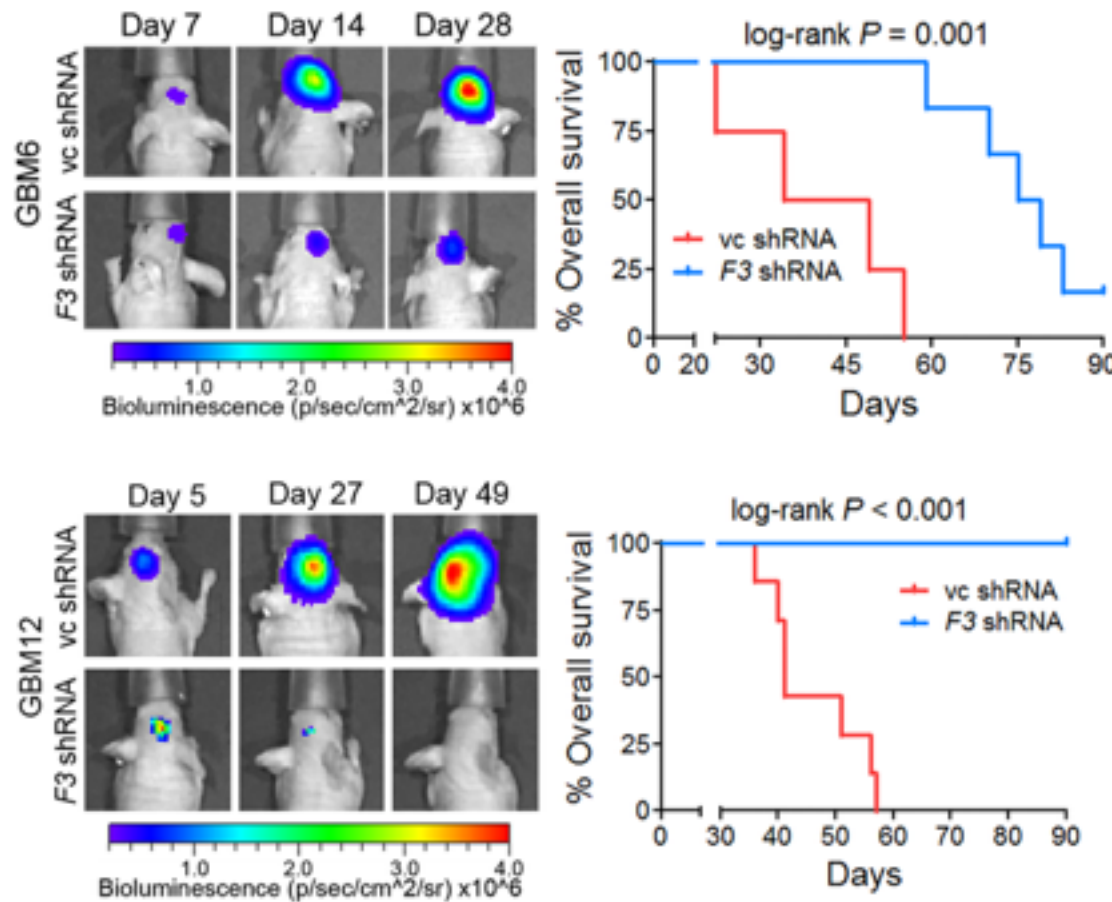
Cetuximab = extracellular EGFR antagonist

AG1478 = intracellular EGFR antagonist

GB83 = PAR2 inhibitor

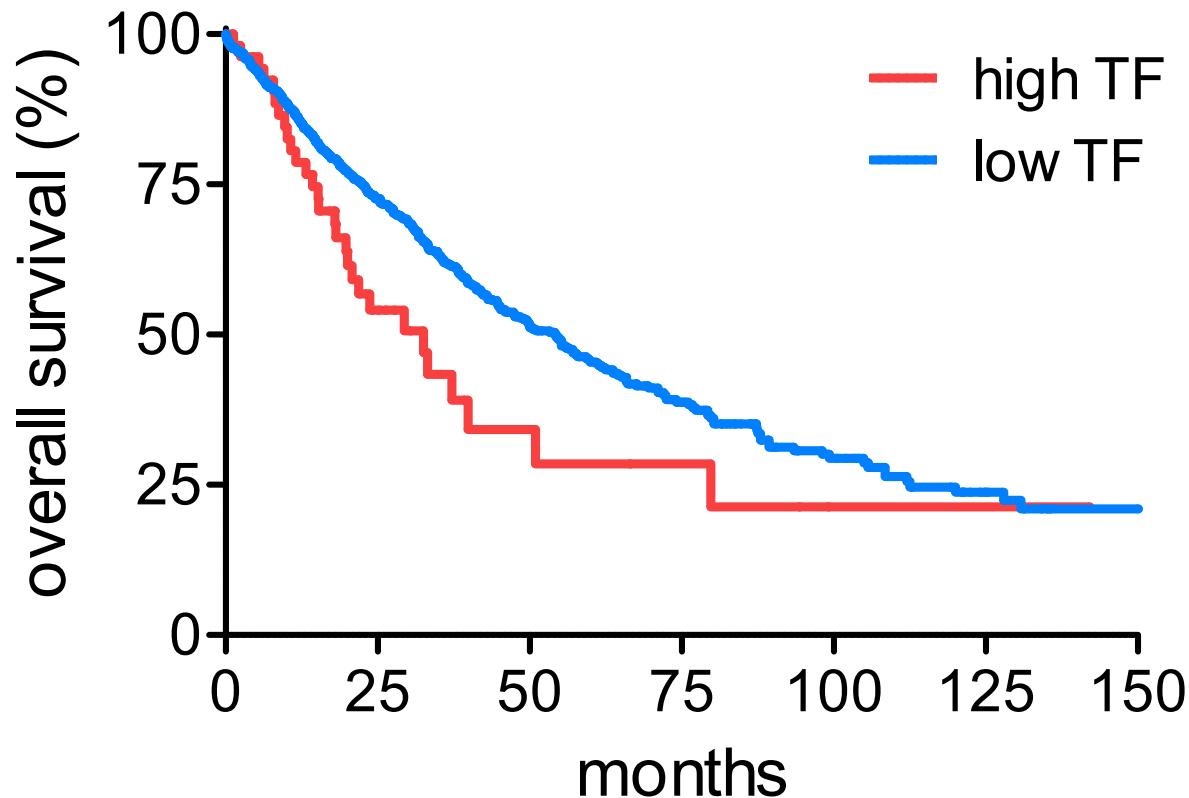
Unruh et al., Clin Cancer Res, 2018

Tissue Factor knockdown reduces the *in vivo* growth of gliomas



Low TF expression correlates with a better prognosis in lung cancer patients

LUNG TCGA dataset
median survival 32.53 vs. 54.40
log-rank $P = 0.0179$



Brain cancers are highly prone to VTE

Table 1 Rates of DVT/PE in different malignancies

Site	Rate of DVT/PE per 10 000 patients
Head/neck	16
Bladder	22
Breast	22
Oesophagus	43
Uterus	44
Cervix	49
Prostate	55
Lung	61
Rectal	62
Liver	69
Colon	76
Leukaemia	81
Renal	84
Stomach	85
Lymphoma	96
Pancreas	110
Brain	117
Ovary	120