

Blocking Tissue Factor signaling in breast cancer inhibits tumor metastasis

Betül Ünlü

Eindhoven Laboratory for Regenerative Medicine
The Netherlands



Breast cancer

2nd cause of death

Elevated Tissue Factor (TF)

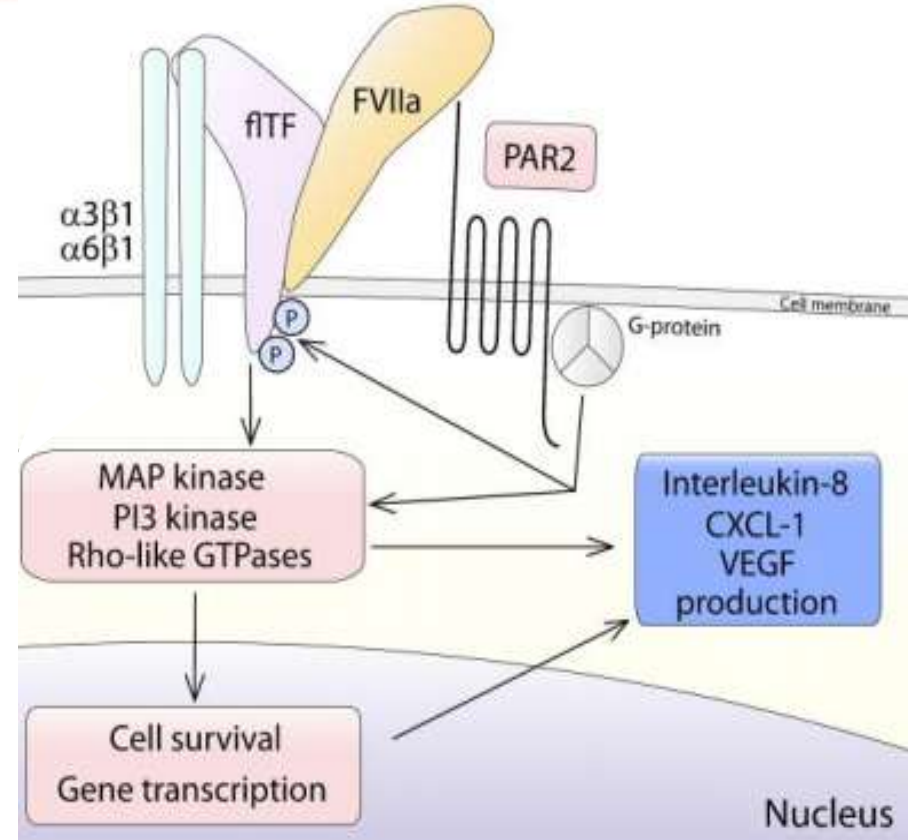
- Reduced survival
- Higher tumor grade
- Increased angiogenesis
- Increased invasive and metastatic behavior



TF signaling in breast cancer

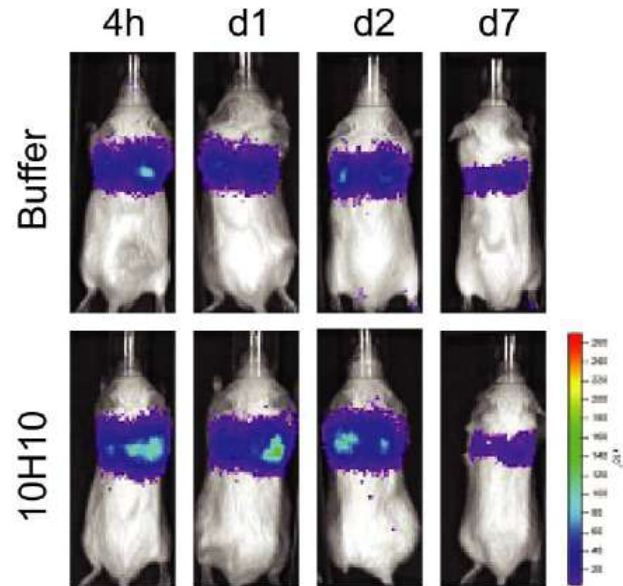
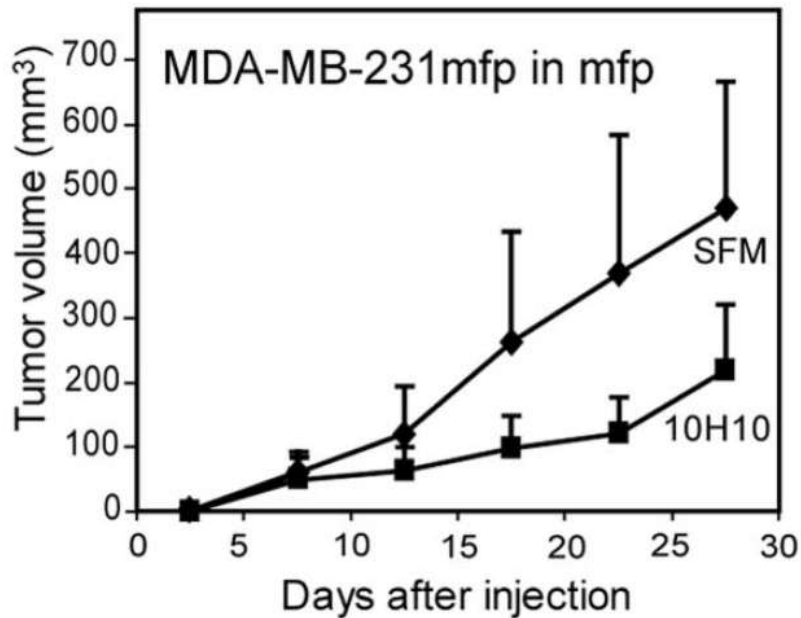
Two functional roles

1. **Signaling** to promote tumor growth
2. **Hemostasis** to promote survival of metastatic cells



TF blocking antibody 10H10

10H10 —| Signaling

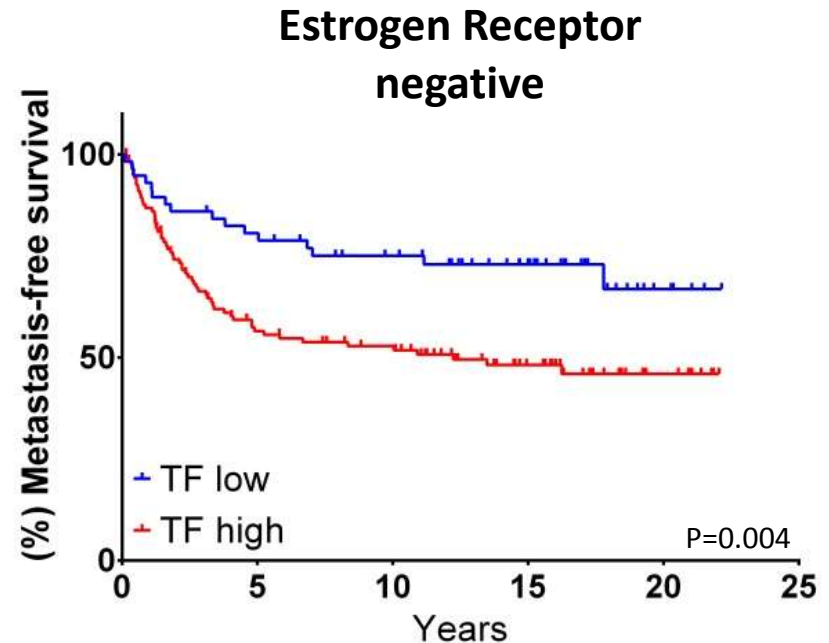
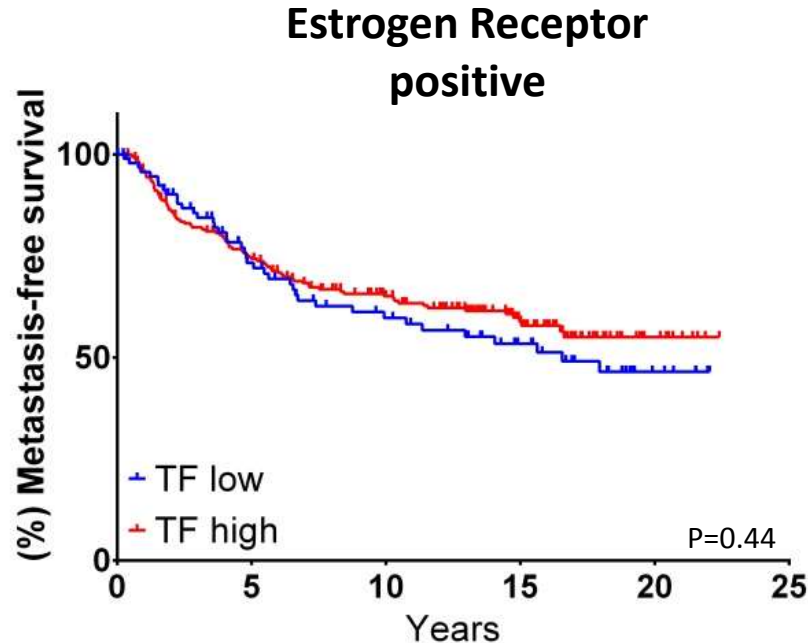


Aim

Investigate whether Tissue Factor signaling influences metastasis in breast cancer

Metastasis associates with TF in ER-negative patients

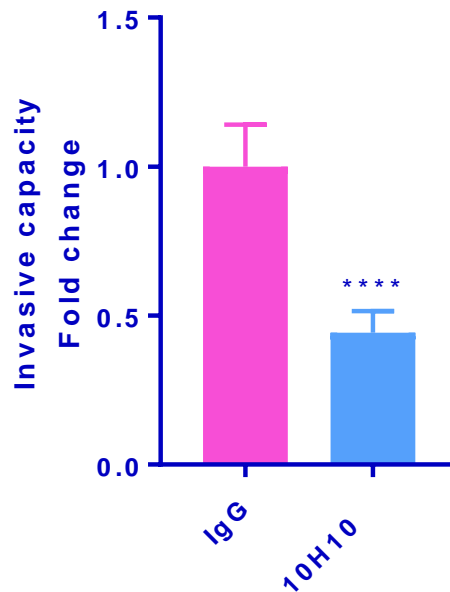
574 breast cancer specimens



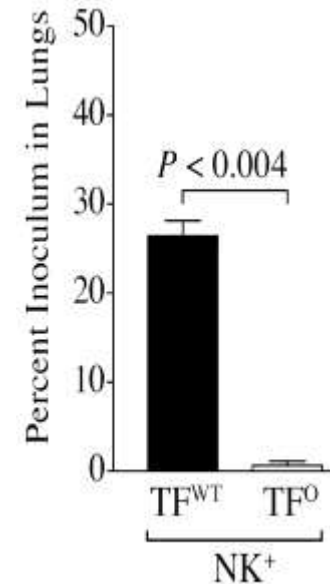
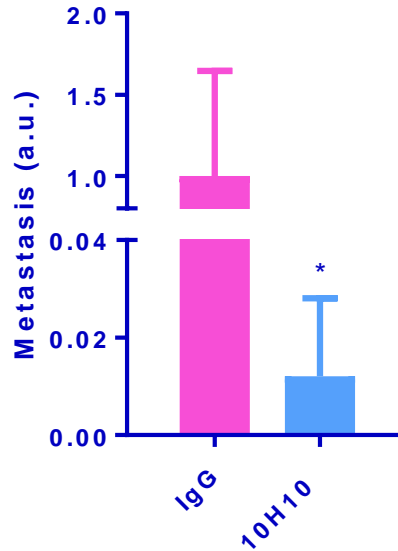
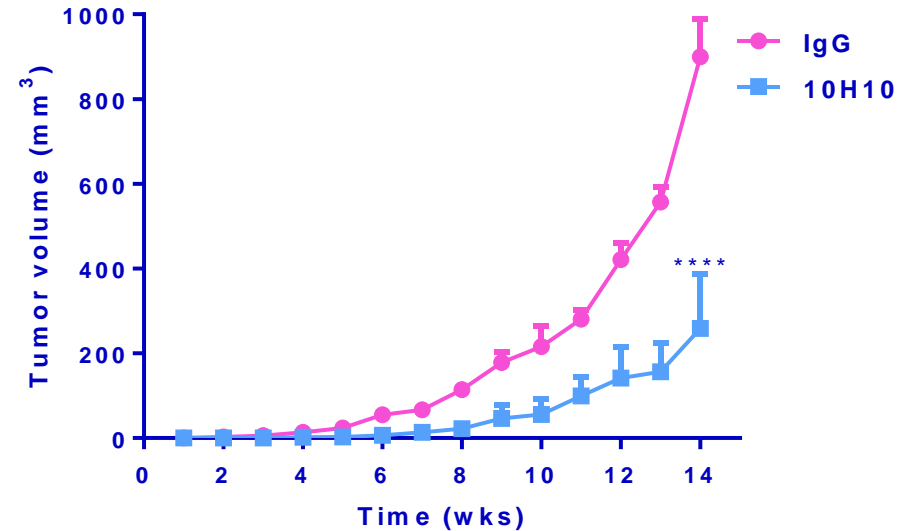
Invasion is inhibited by 10H10

MDA-231-MFP cells

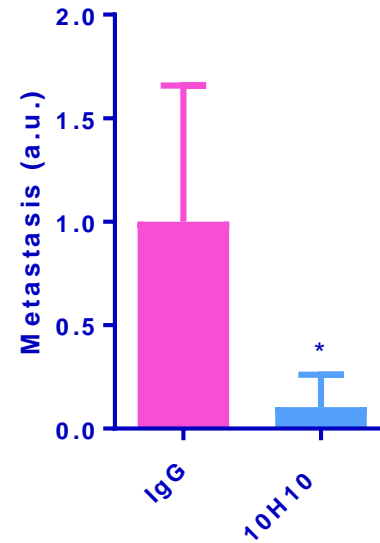
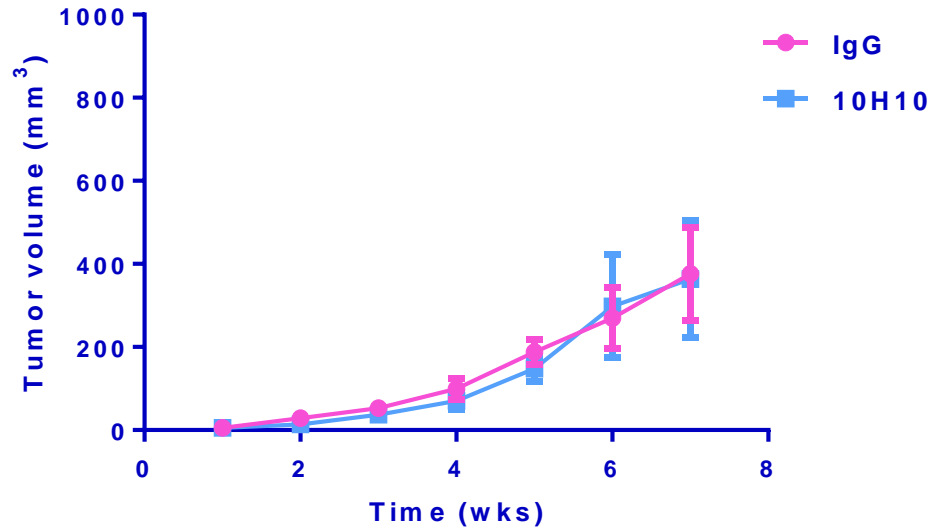
- Highly aggressive subclone
- Triple negative breast cancer



10H10 reduces metastasis in an NK cell-dependent mouse model

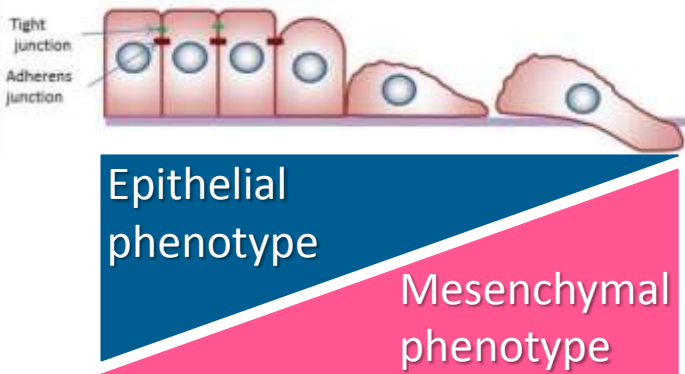


10H10 reduces metastasis in an NK cell-independent mouse model

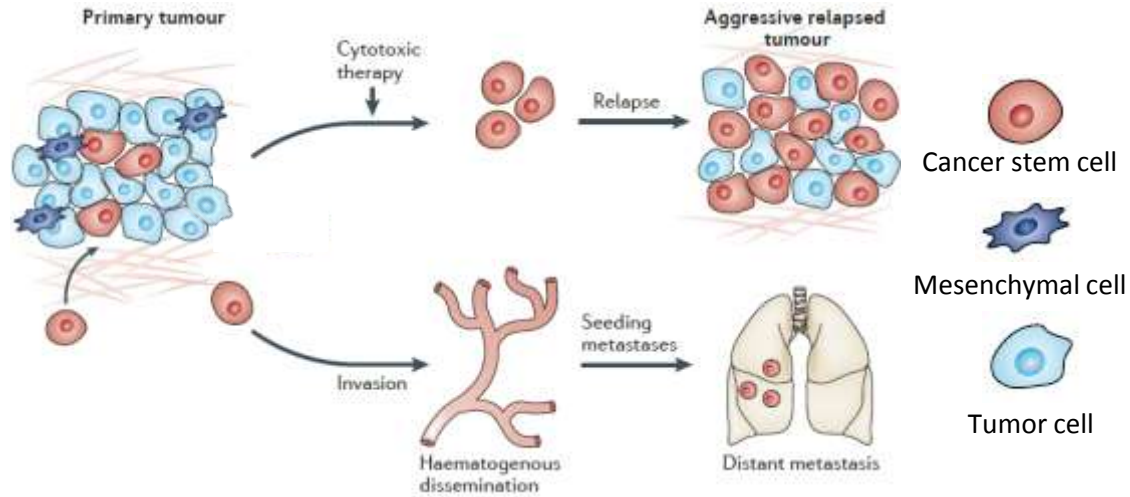


Molecular mechanism of metastasis

Epithelial to Mesenchymal Transition (EMT)



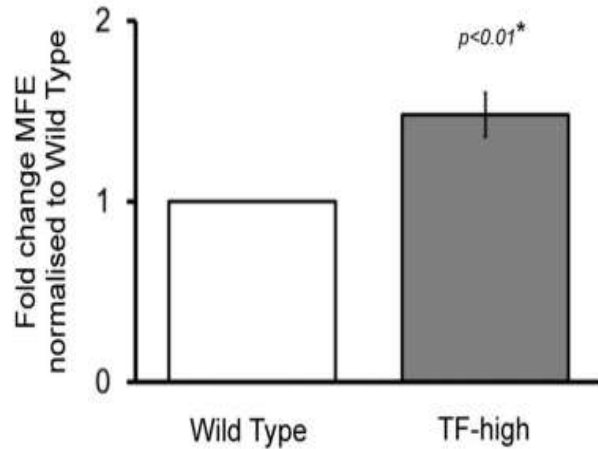
Cancer stem cells (CSCs)



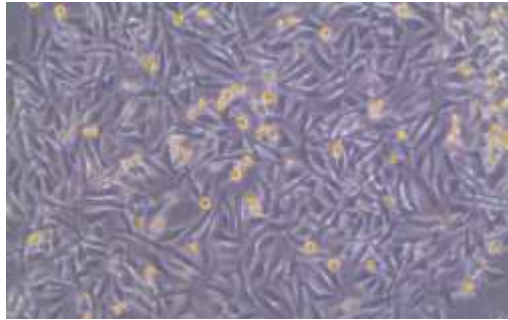
Molecular mechanism of metastasis

MCF7

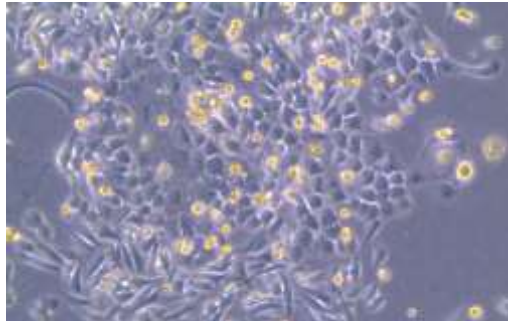
Mammosphere formation



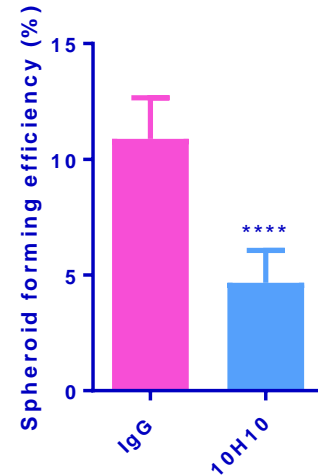
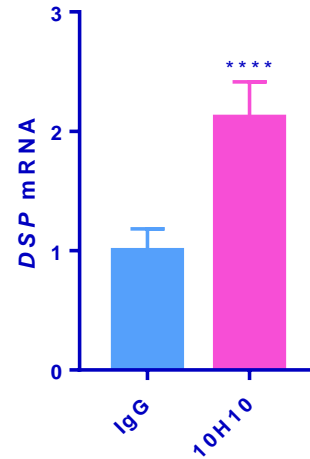
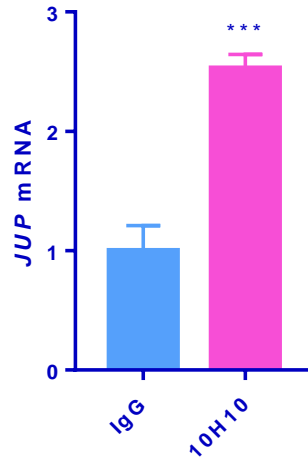
Permanent changes *ex vivo* with 10H10



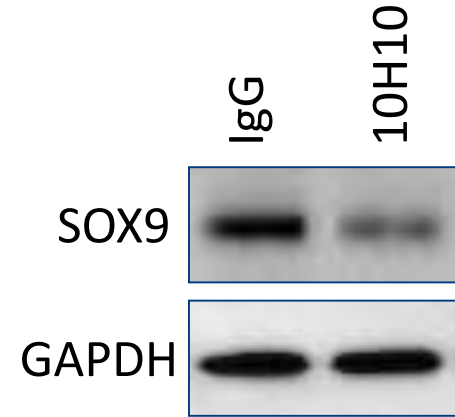
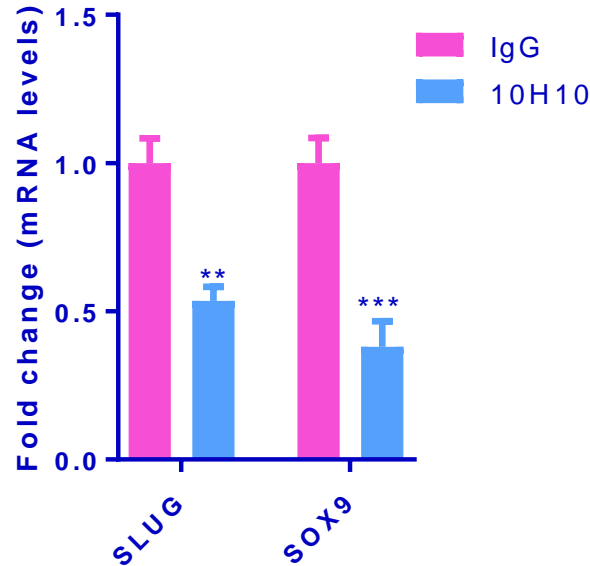
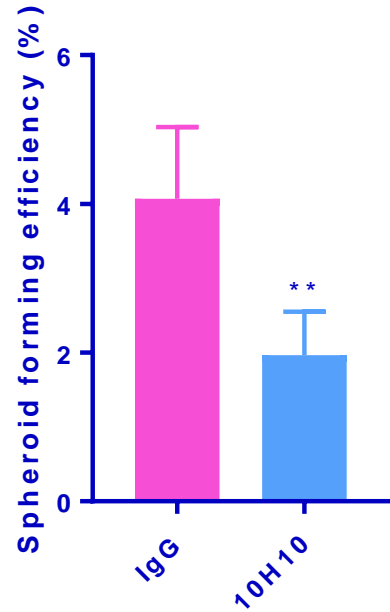
IgG: mesenchymal



10H10: epithelial



Inhibition of TF signaling results in reduced cancer stem cell features *in vitro*



TF expression associates with breast cancer stem cell marker ALDH1

CSC in clinical specimens

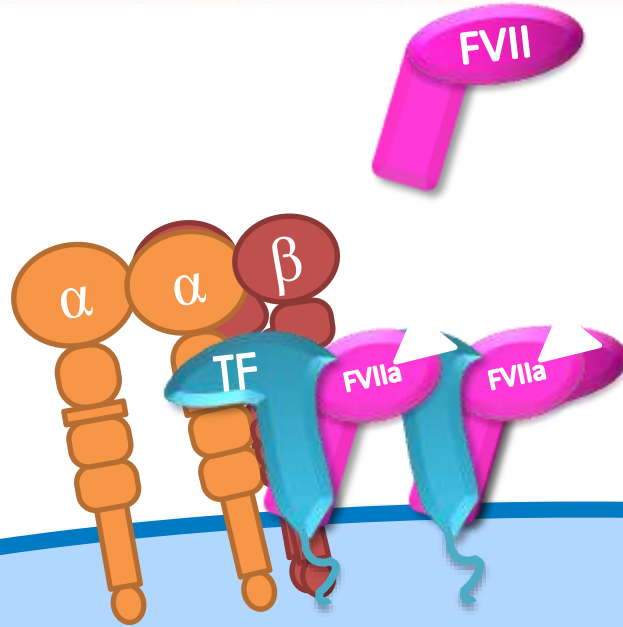
	TF negative		TF positive		
	N	%	N	%	P
ALDH1 -	74	50.7	105	34.1	0.001
ALDH1 +	72	49.3	203	65.9	

TF and $\beta 1$ -integrin complexes

Integrins:

- Heterodimers of α - and β -subunits
- Bind extra-cellular matrix
- Migration and apoptosis

Integrin dependent TF signaling



EMT
Migration
CSC



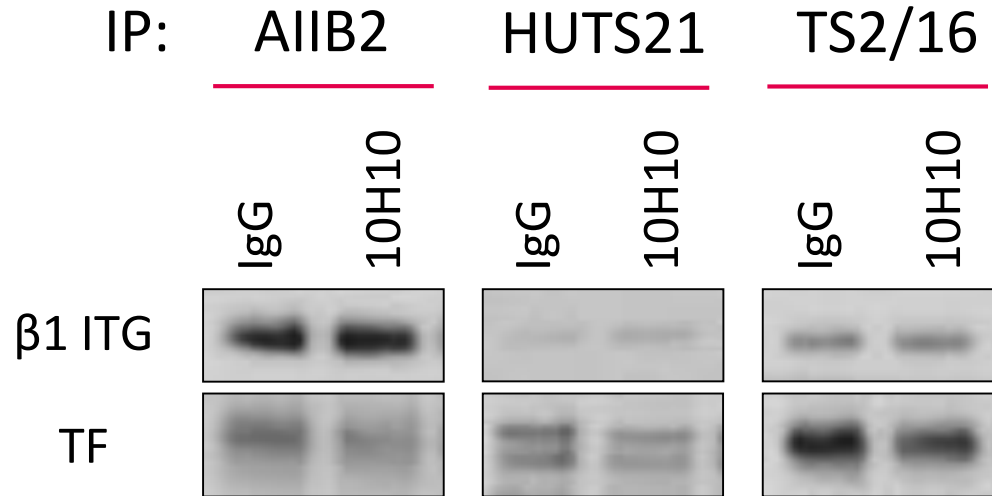
Angiogenesis
Proliferation

Inhibition of TF signaling affects $\beta 1$ -integrin

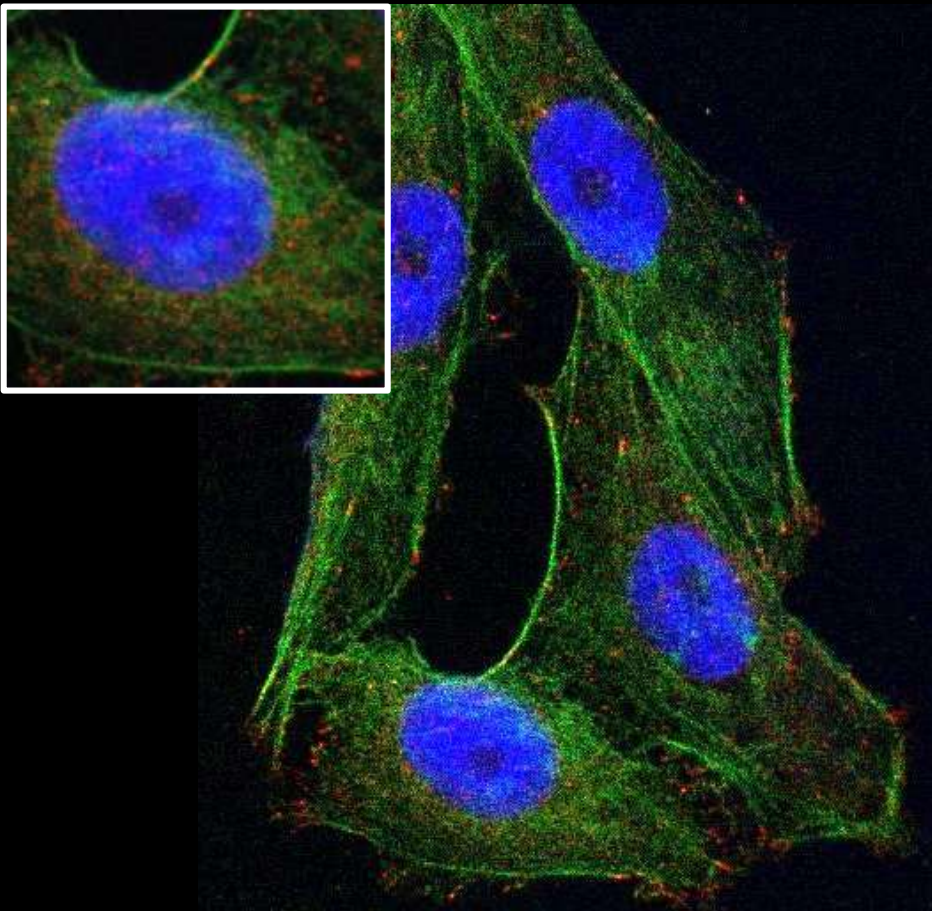
Recognizes

Active: AIIB2, HUTS21

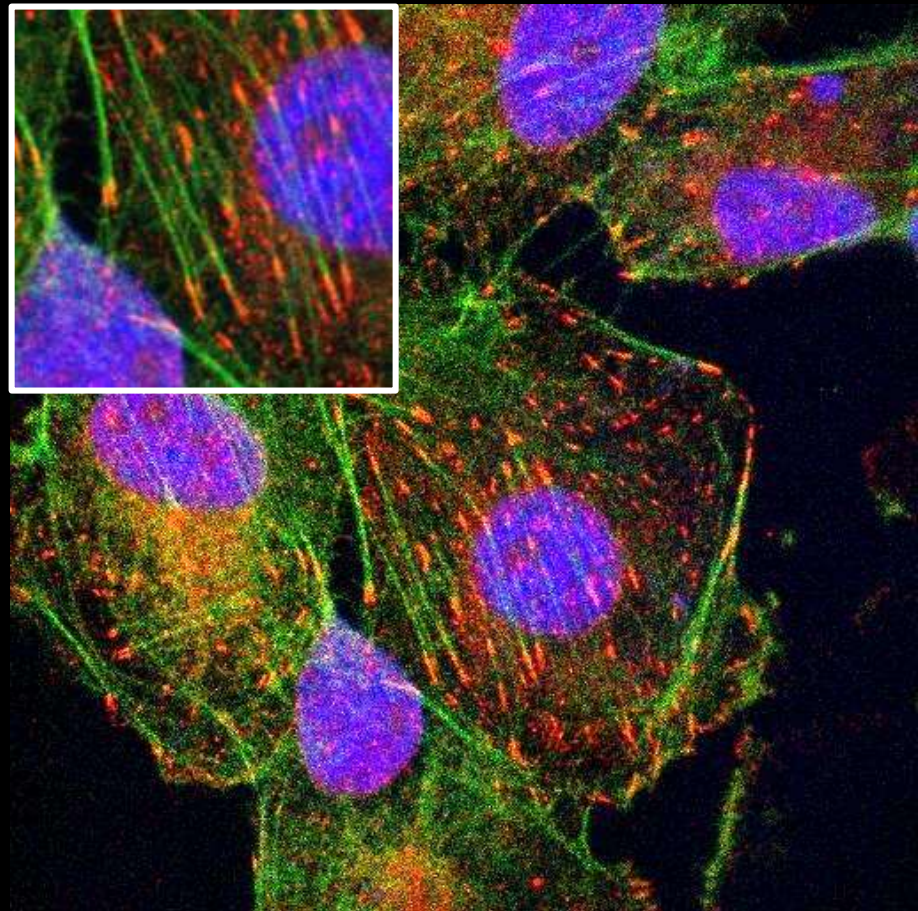
Inactive: TS2/16



FAK Actin Nucleus

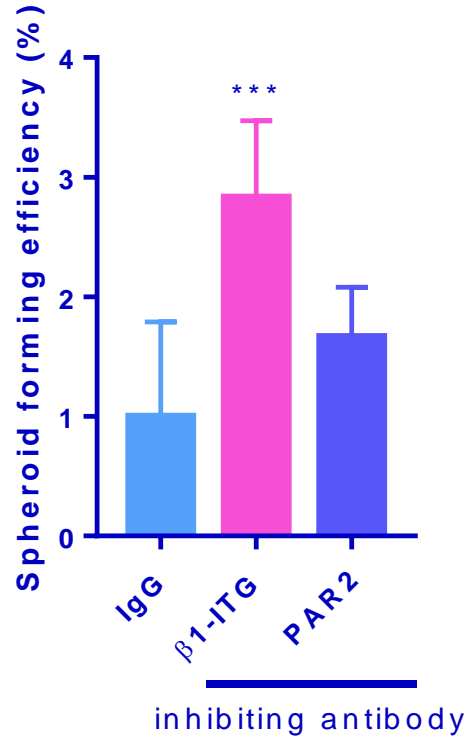


IgG



10H10

Active β 1-integrin reduces cancer stemness

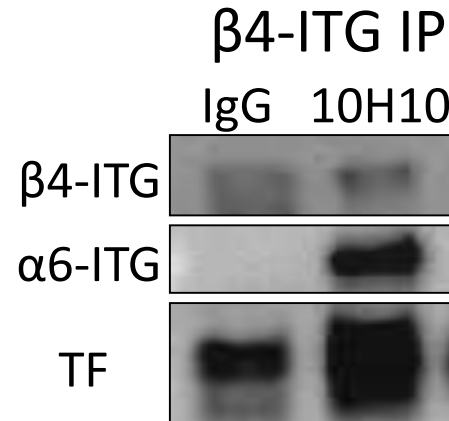
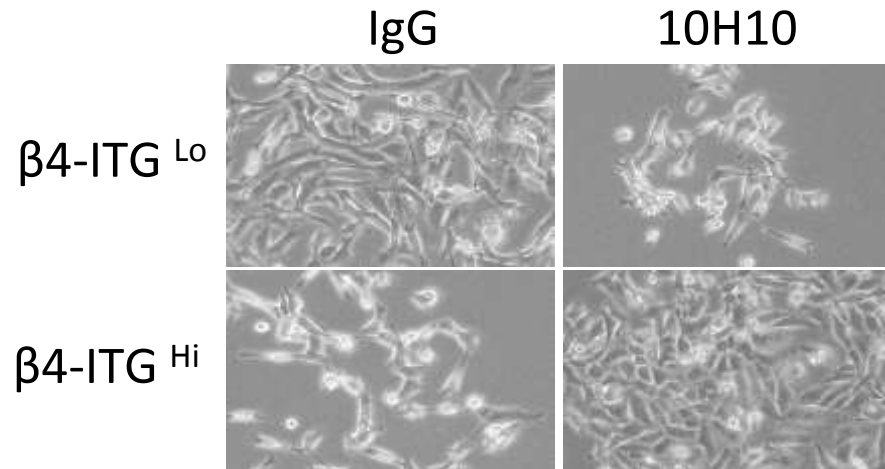


Inhibition of TF signaling affects β 4-integrin

β 4-ITG^{Lo} : mesenchymal

β 4-ITG^{Hi} : epithelial

		IgG	10H10
		(%)	(%)
β 1-integrin	+	30.5	36.5
	-	69.5	63.5



Conclusion

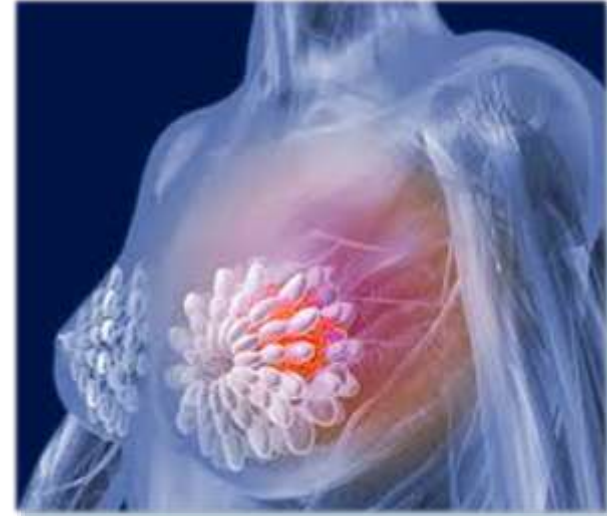
TF associates with

- Metastasis in ER-negative tumors
- CSC marker ALDH1

Inhibition of TF signaling reduces

- *SLUG* and *SOX9*
- Primary tumor-resident cancer stem cells
- Metastasis

TF signaling affects integrin-complexes



Conclusion

TF associates with

- Metastasis in ER-negative tumors
- CSC marker ALDH1

Inhibition of TF signaling reduces

- *SLUG* and *SOX9*
- Primary tumor-resident cancer stem cells
- Metastasis

TF signaling affects integrin-complexes

TF signaling plays a role in early onset of metastasis by affecting CSC behavior



B. Kocatürk

E. Blok

R.F.P van den Akker

J.T. Buijs

E.H. Laghmani

M.Y. Kapteijn

C. Kroone

P.J.K. Kuppen

A.M. da Rocha Rondon

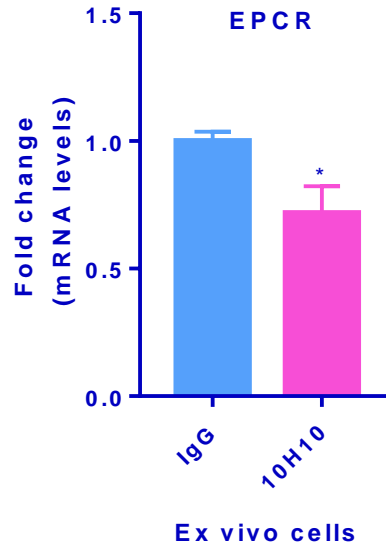
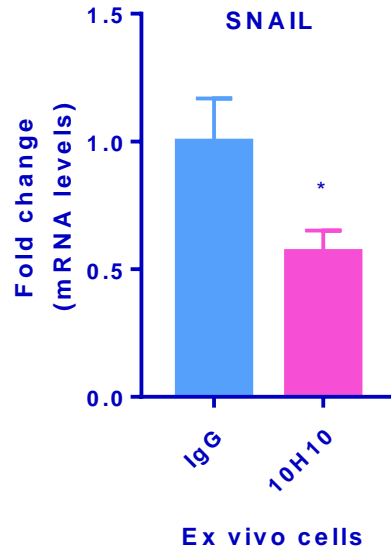
N. Swier

H.H. Versteeg

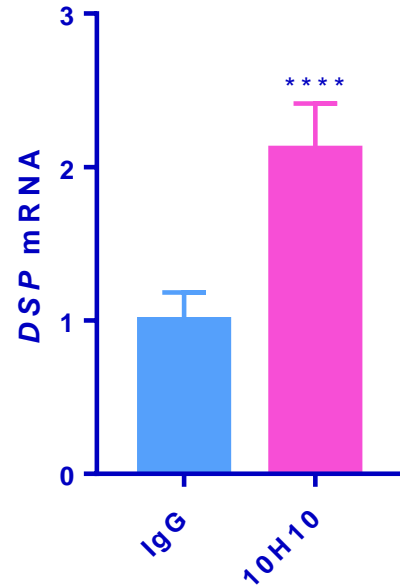
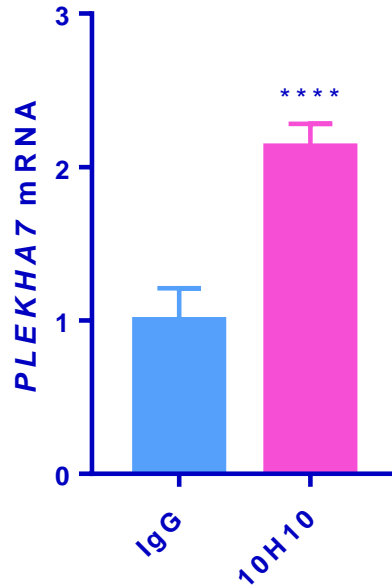
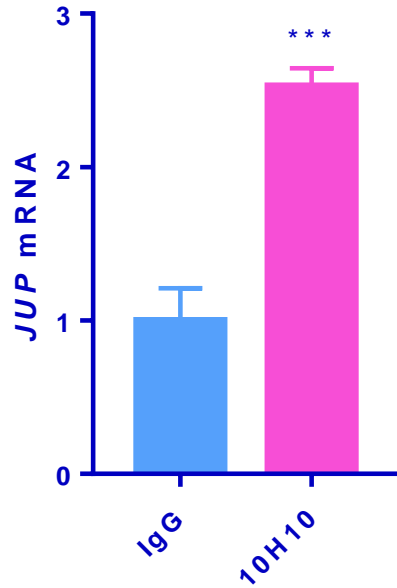
W. Ruf



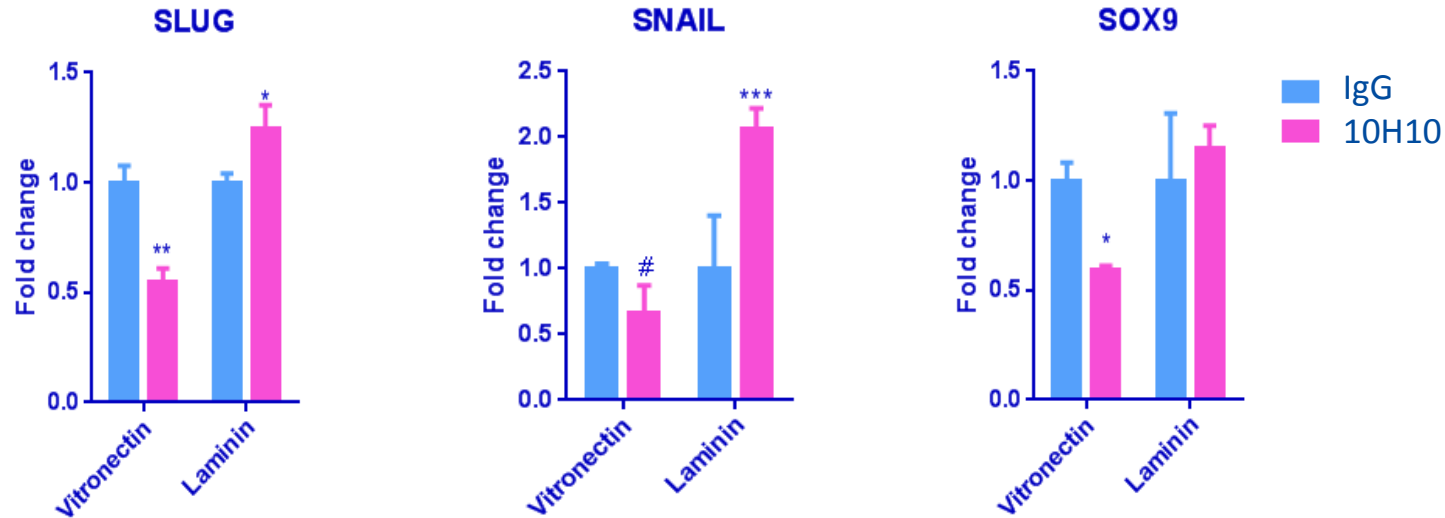
Ex vivo cells: mRNA expression levels



Ex vivo cells: Adherent junction mRNA expression levels

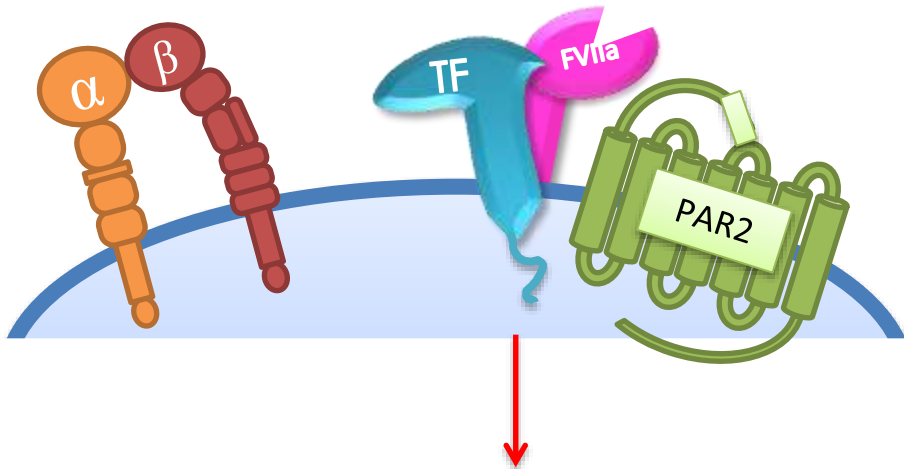


ECM as TF signaling modulator



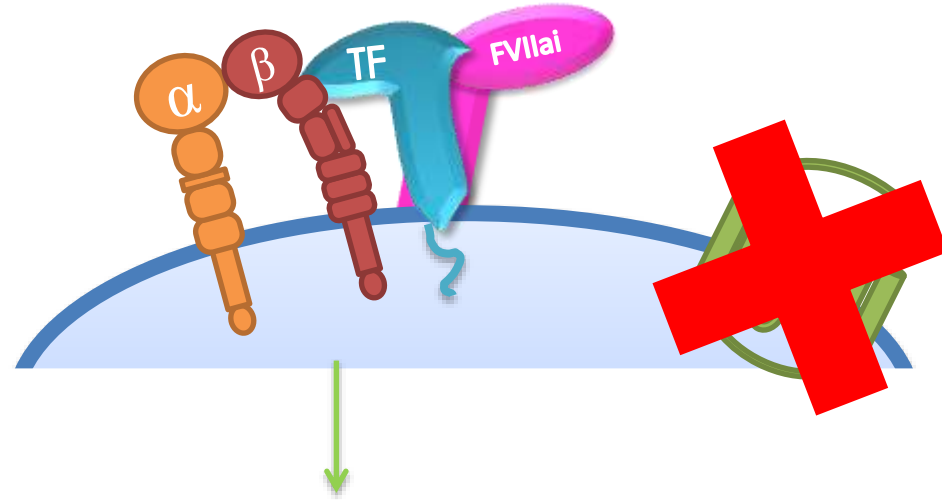
Distinguish between PAR-2 and β 1-integrin dependent TF signaling

Recombinant FVIIa



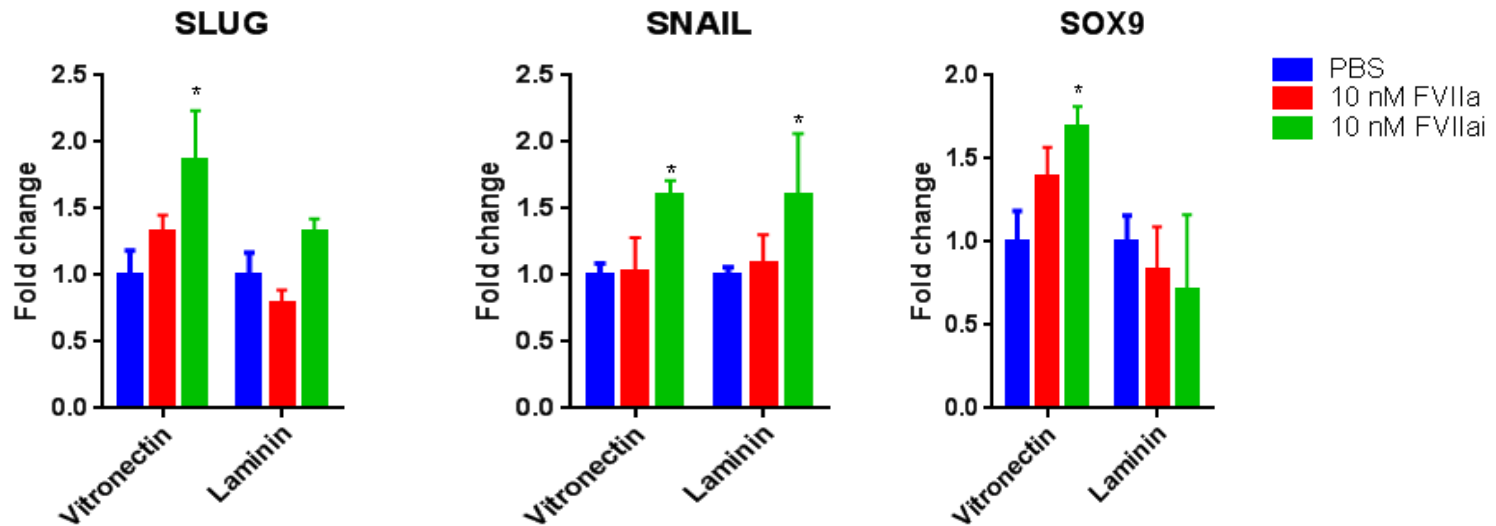
(Mainly) **PAR2**-dependent signaling

Active site-inhibited FVIIai



β 1 integrin-dependent signaling

ECM as TF signaling modulator



ECM as TF signaling modulator

