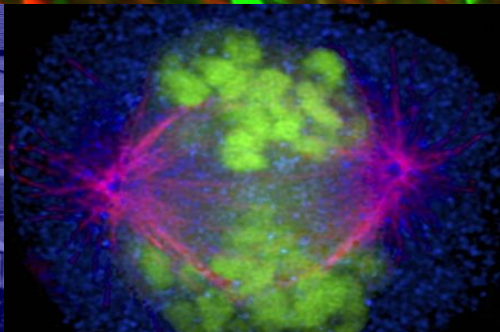


# Cancer associated fibroblast FAK regulates malignant cell metabolism

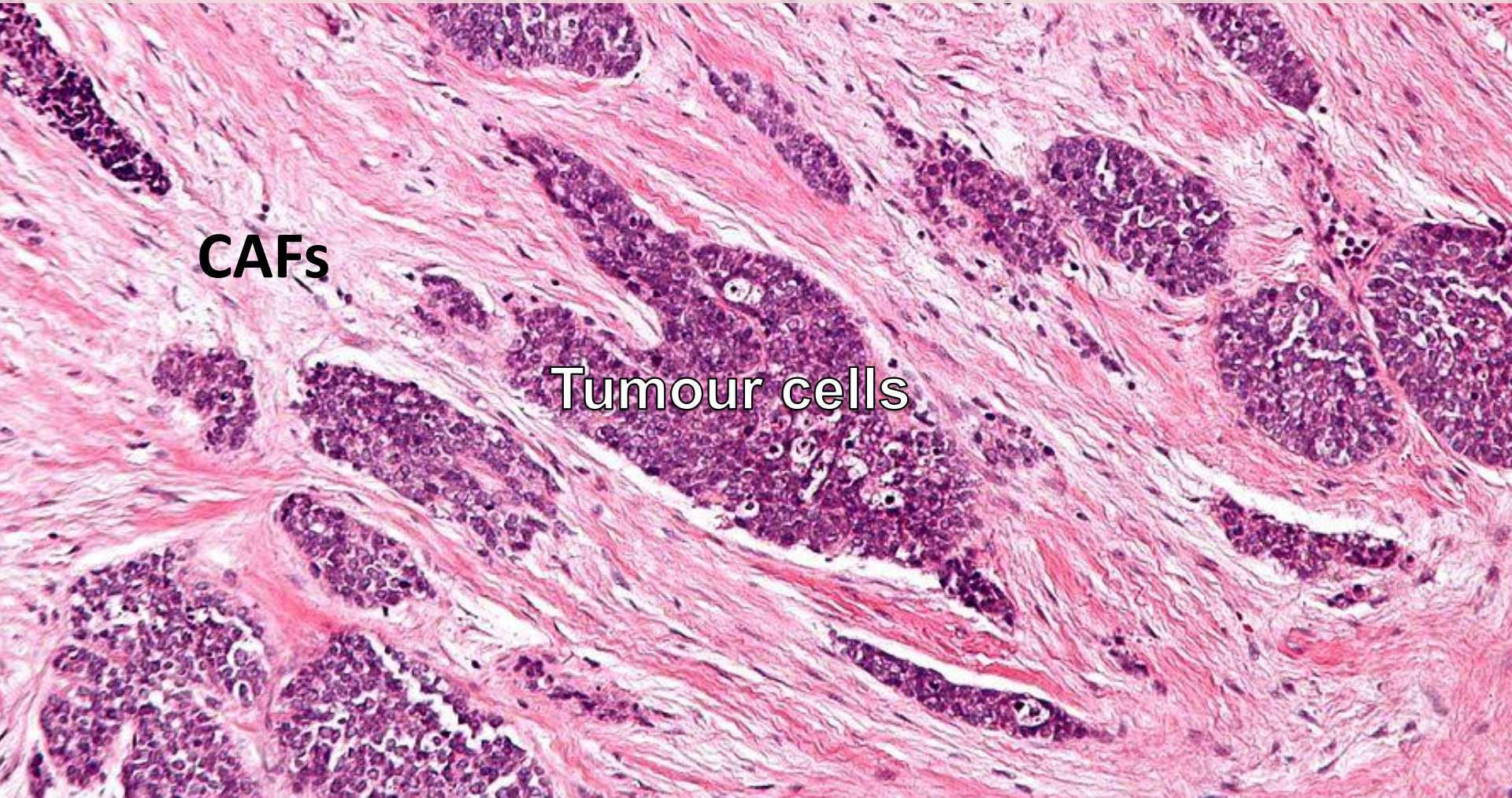
**Kairbaan Hodivala-Dilke**

Barts Cancer Institute,  
CR-UK Cancer Centre,  
Queen Mary University of London  
London, UK



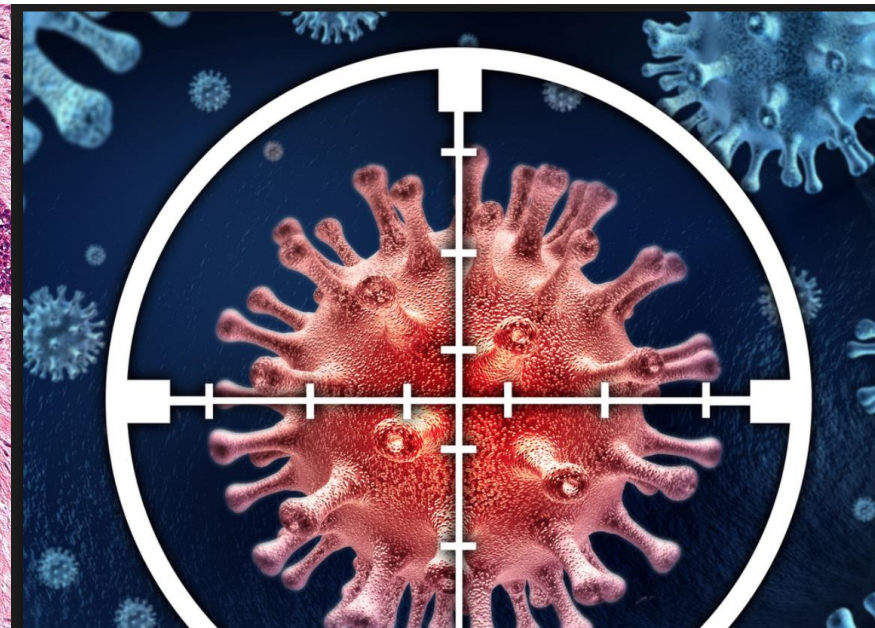
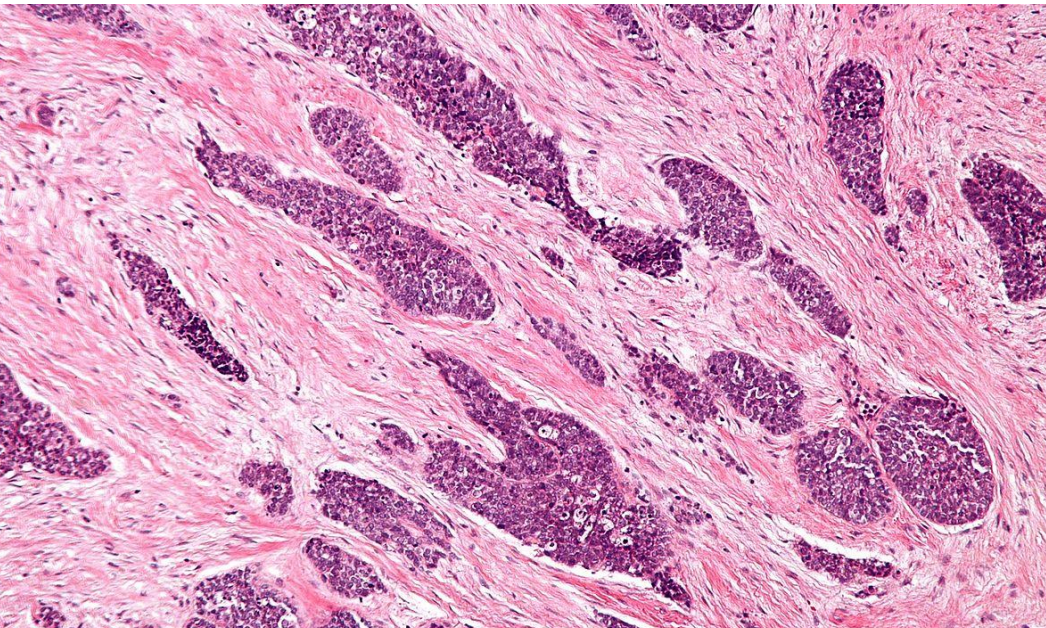
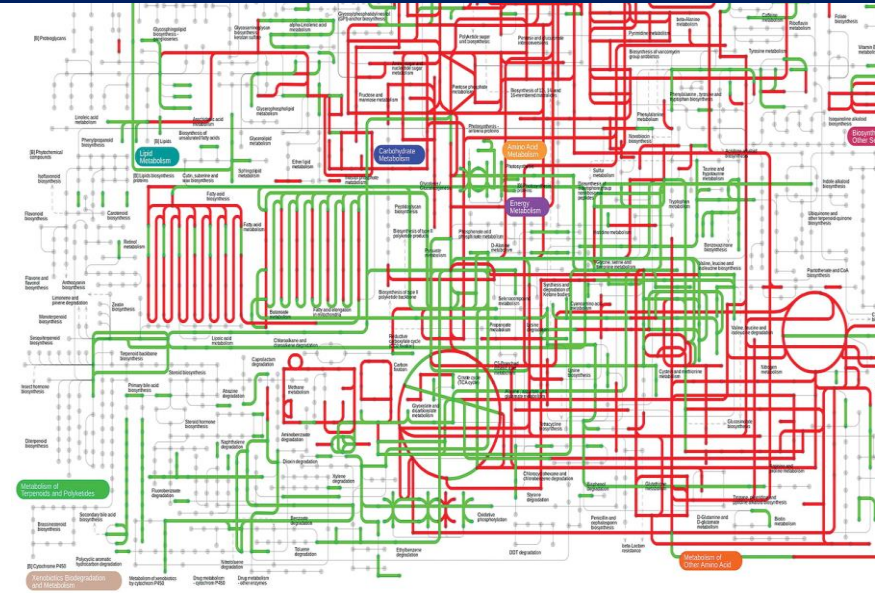
# Cancer progression *in vivo*

- Increased stromal responses
- Enhanced cancer associated fibroblast activity
- CAFs are regulators of cancer progression
- What regulates these regulators and how?



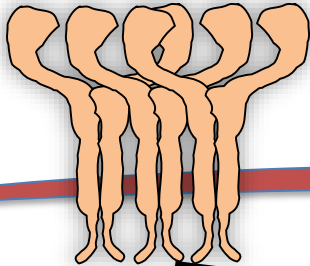
# Understanding the cellular and molecular control of cancer metabolism

- Intrinsic oncogenic and epigenetic changes have been related to changes in malignant cell metabolism.
- Targeting cancer metabolism for cancer treatment.
- *In vivo* evidence dissecting the stromal contribution of the molecular control of malignant cell metabolism with matched oncogene and tumour suppressor gene profiles still required.

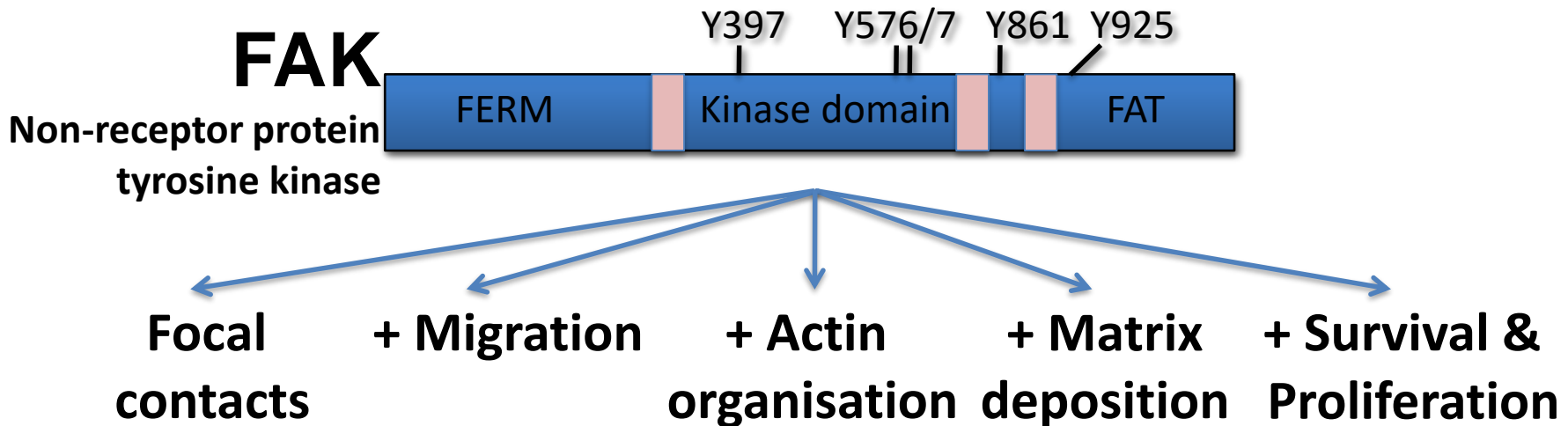
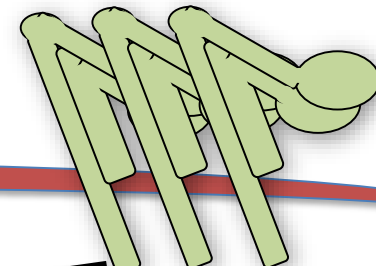


# Focal Adhesion Kinase: Point of converging signals

**Growth factor receptors**



**Integrins**



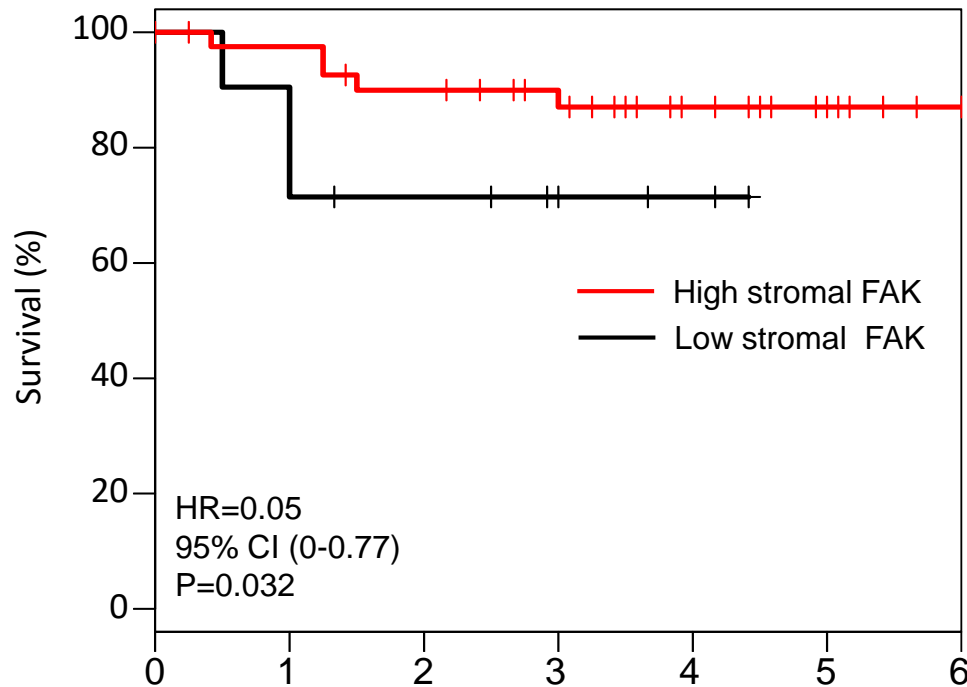
=expected role of FAK in fibroblast activation *in vitro*....  
*but what happens in vivo?*

## Hypothesis

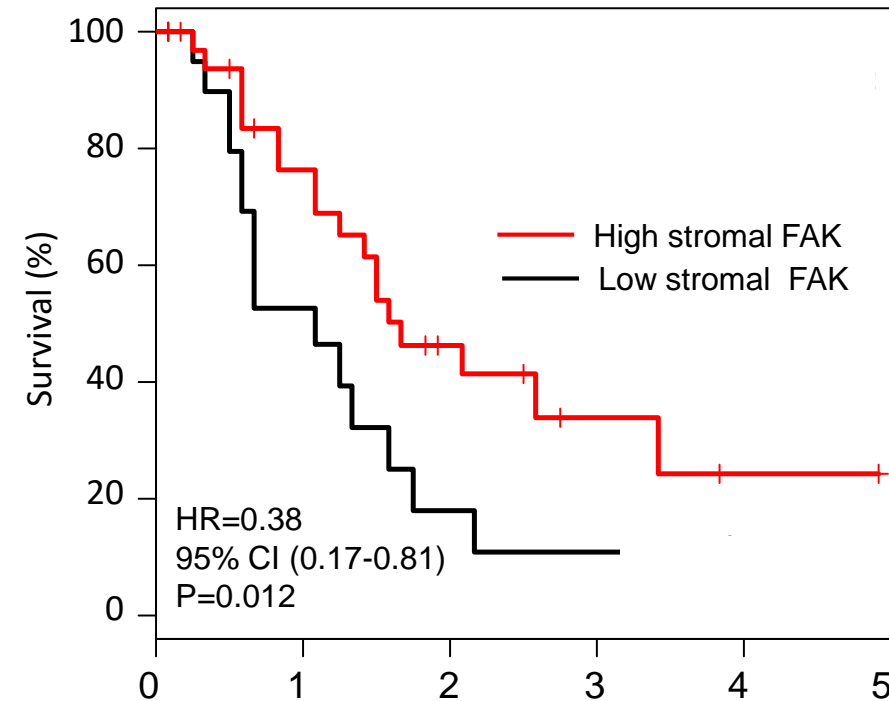
**Loss of CAF-FAK will reduce fibroblast activation,  
thus reduce tumour growth *in vivo***

# Low levels of stromal FAK correlate with poorer prognosis in human breast and pancreatic cancer

Human breast cancer  
Finak et al., stromal laser capture

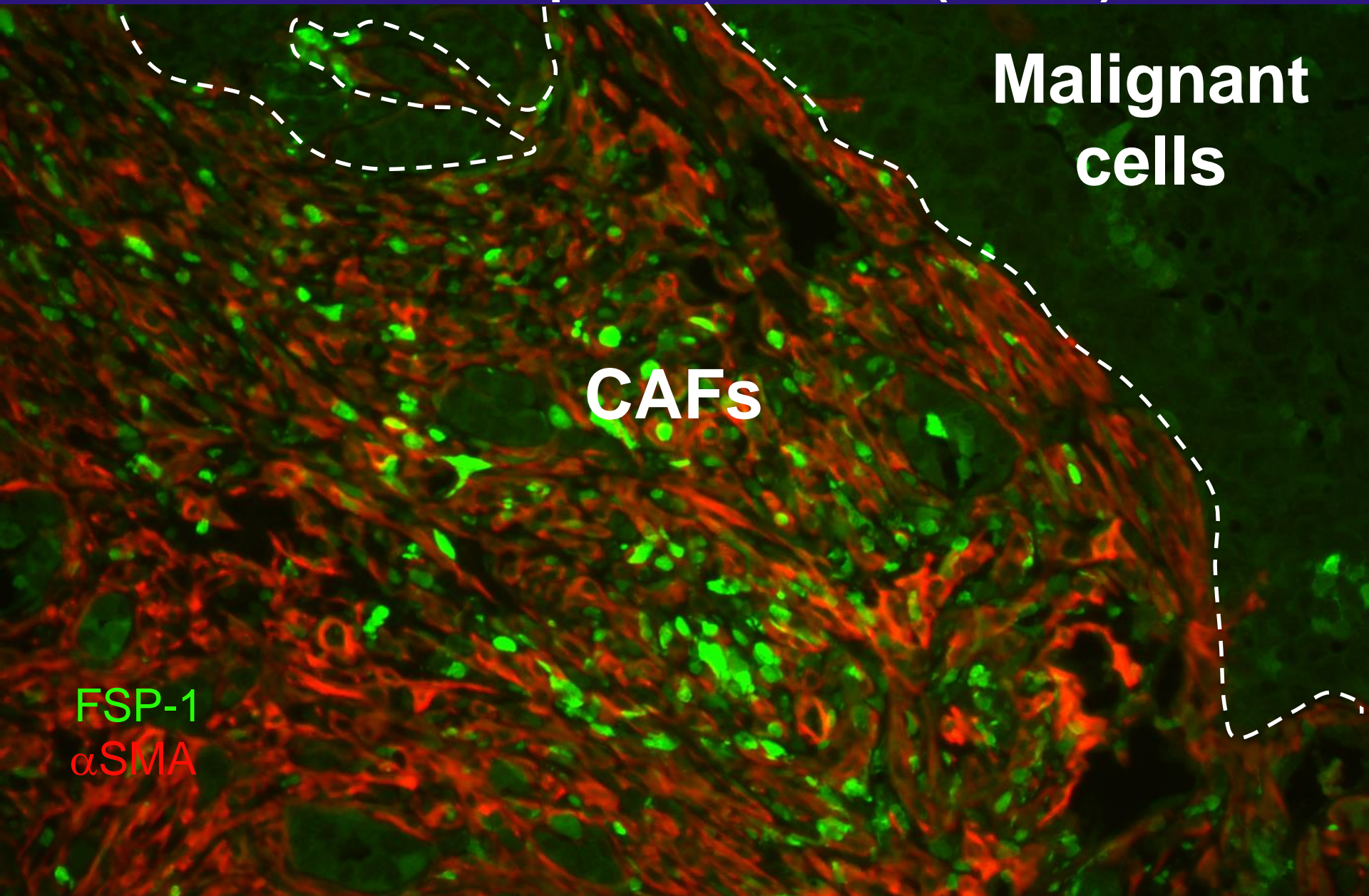


Human pancreatic cancer  
Stratford et al., activated stroma samples



Require a model the CAF FAK-loss in vivo

# A subpopulation of CAFs express Fibroblast Specific Protein (FSP-1)



**Malignant  
cells**

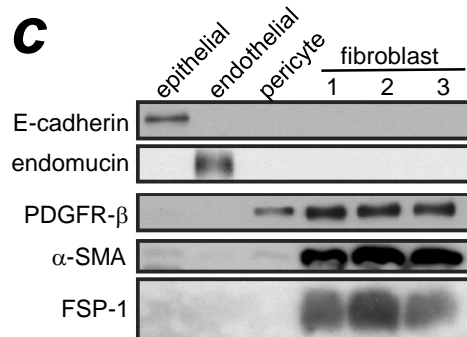
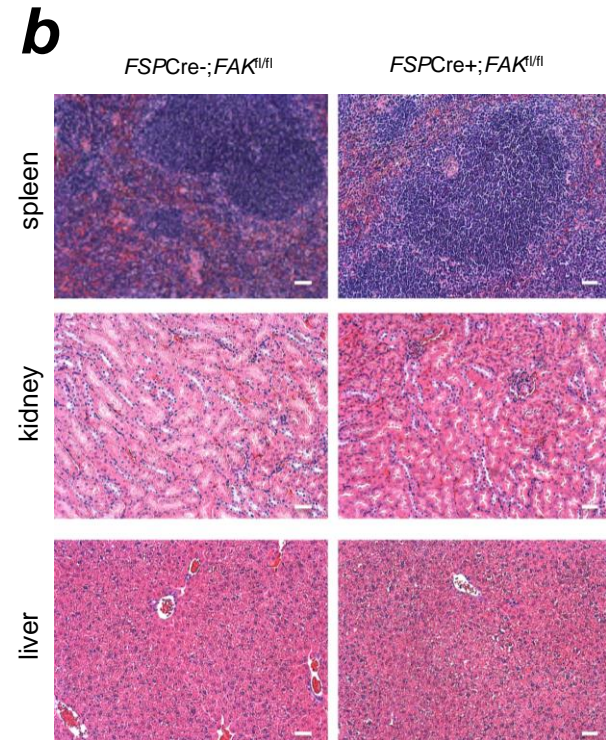
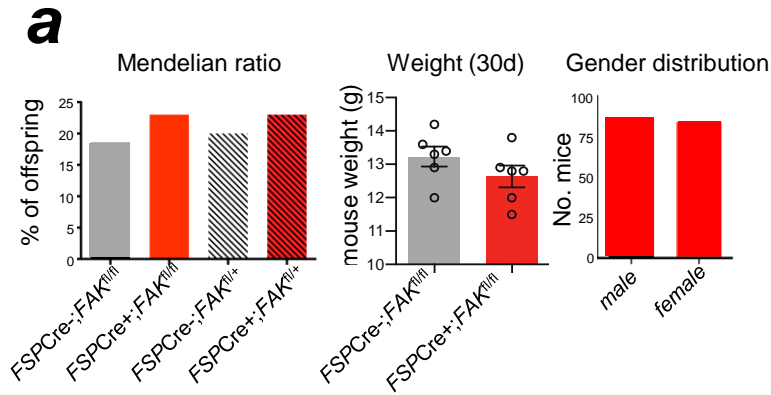
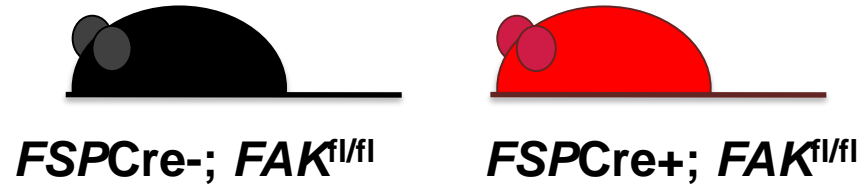
**CAFs**

**FSP-1**  
 **$\alpha$ SMA**

# Targeting cancer associated fibroblast FAK

## Generation of *FSP-Cre; FAK<sup>fl/fl</sup>* mice

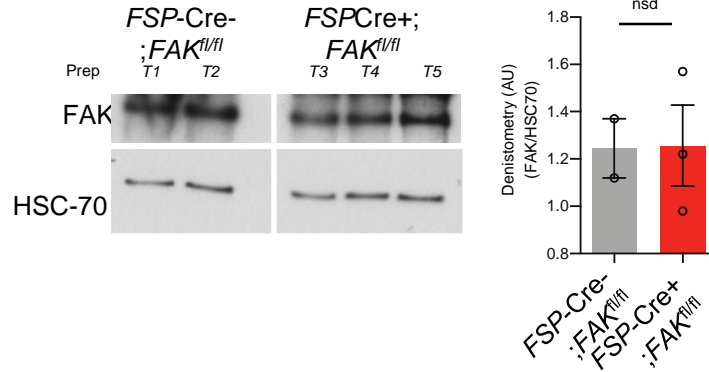
### No apparent gross defects



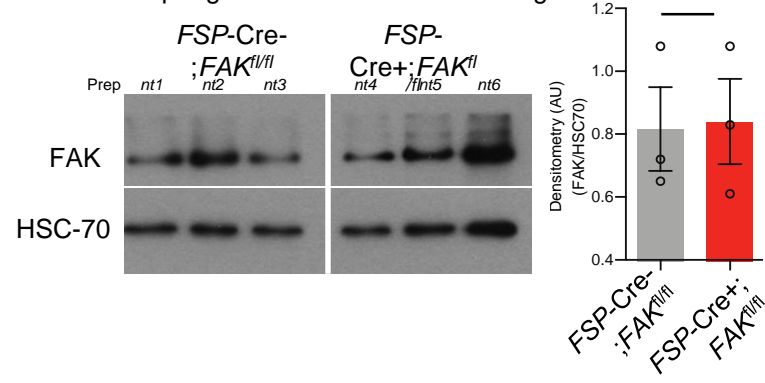
# Targeting cancer associated fibroblast FAK

## Generation of FSP-Cre; FAK<sup>fl/fl</sup> mice

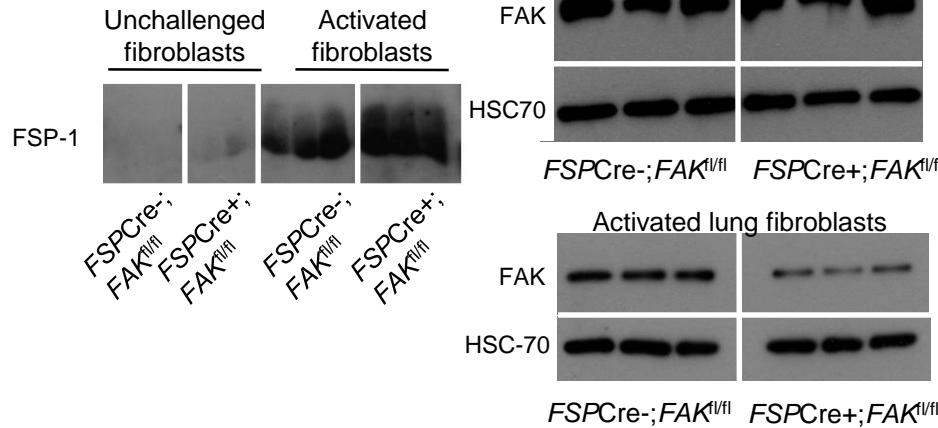
Macrophages from tumour bearing mice



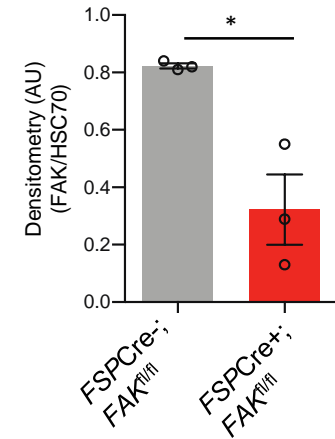
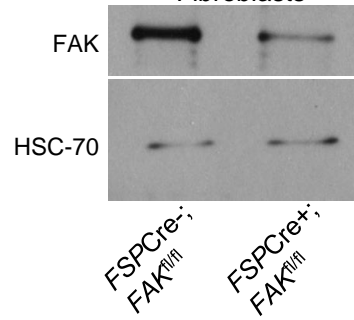
Macrophages from non-tumour bearing mice



Unchallenged fibroblasts

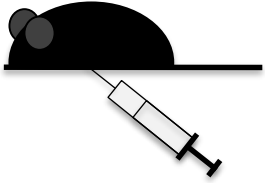


Cancer Associated Fibroblasts

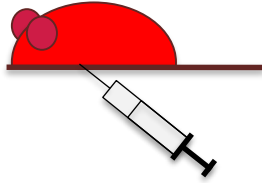


# CAF-FAK deletion enhances tumour growth

*FSPCre-; FAK<sup>fl/fl</sup>*

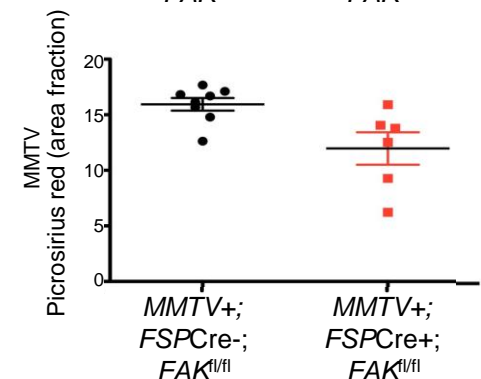
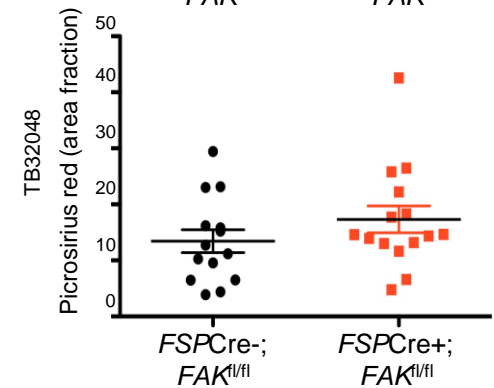
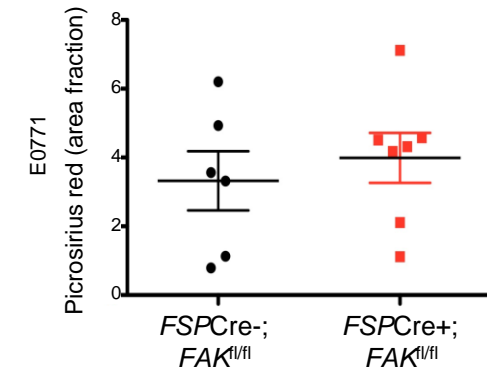
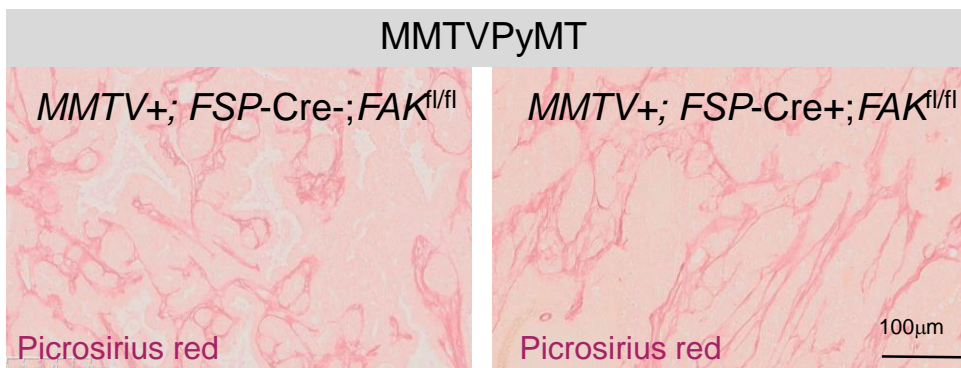
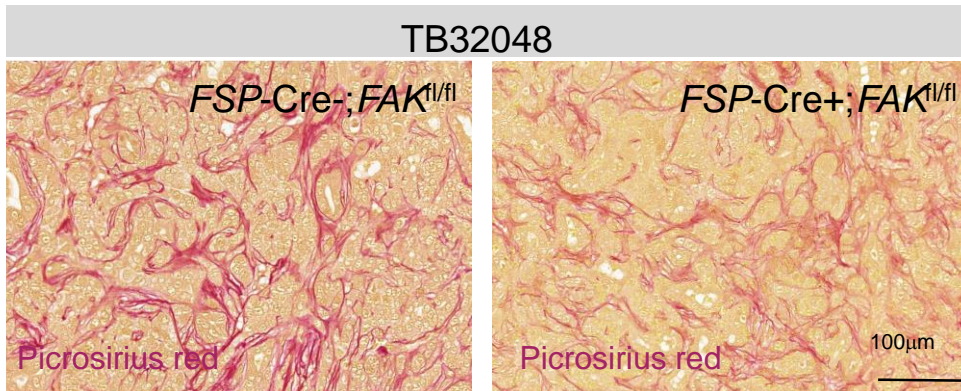
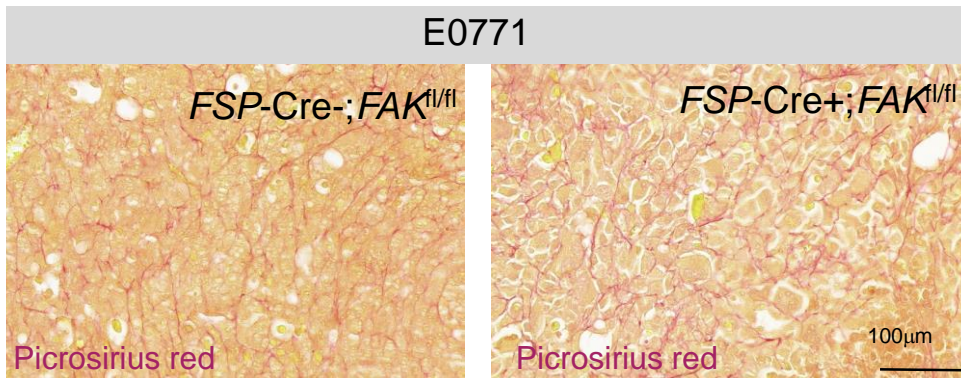


*FSPCre+; FAK<sup>fl/fl</sup>*

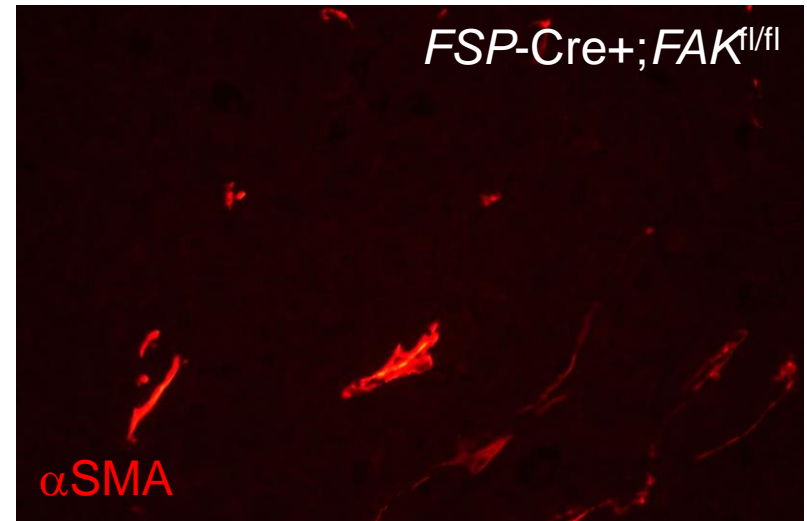
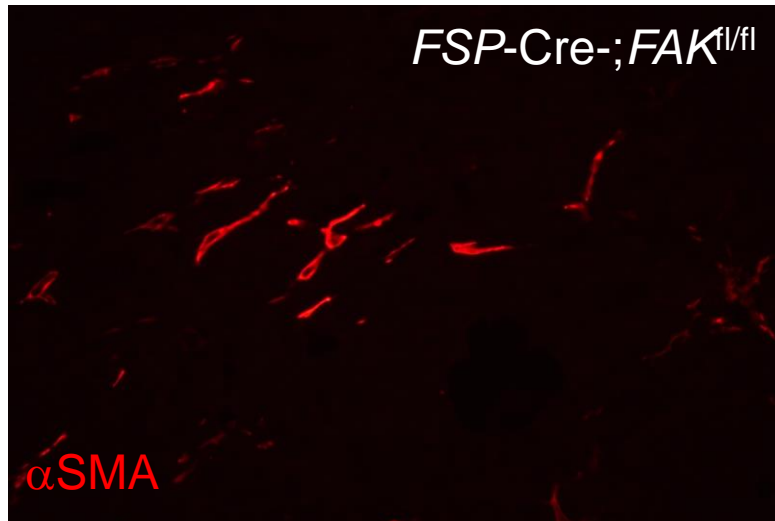


E0771 orthotopic  
breast cancer

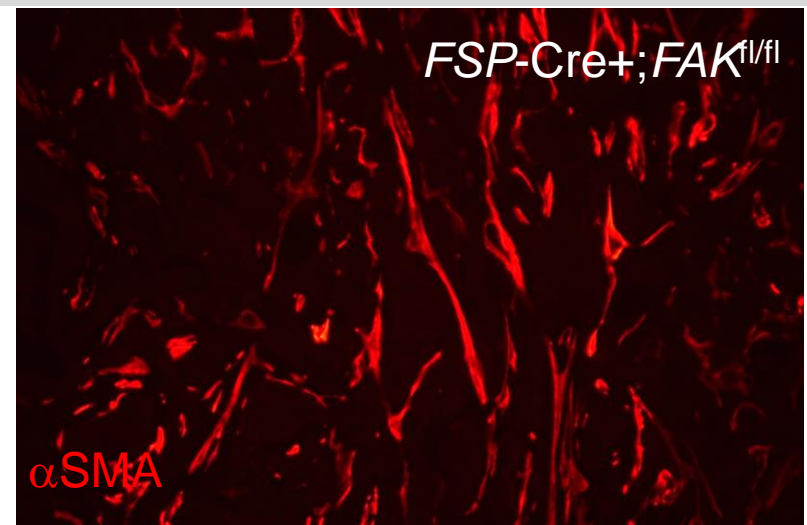
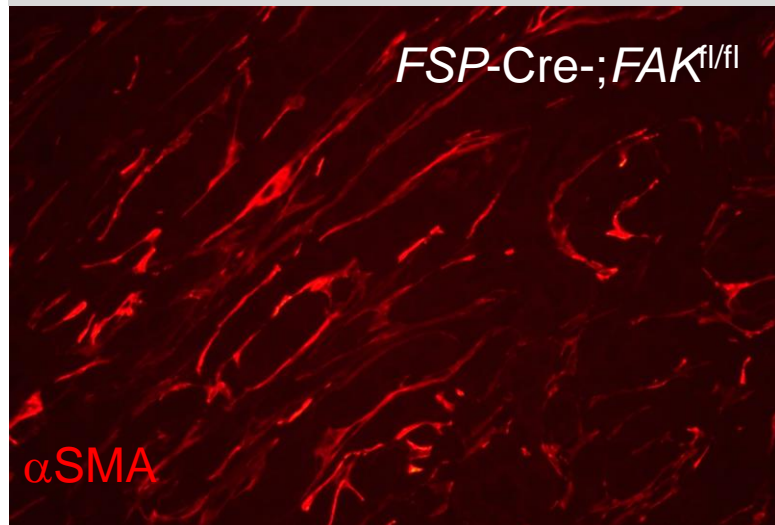
# Loss of CAF-FAK does not affect desmoplasia



# Loss of CAF-FAK does not affect desmoplasia

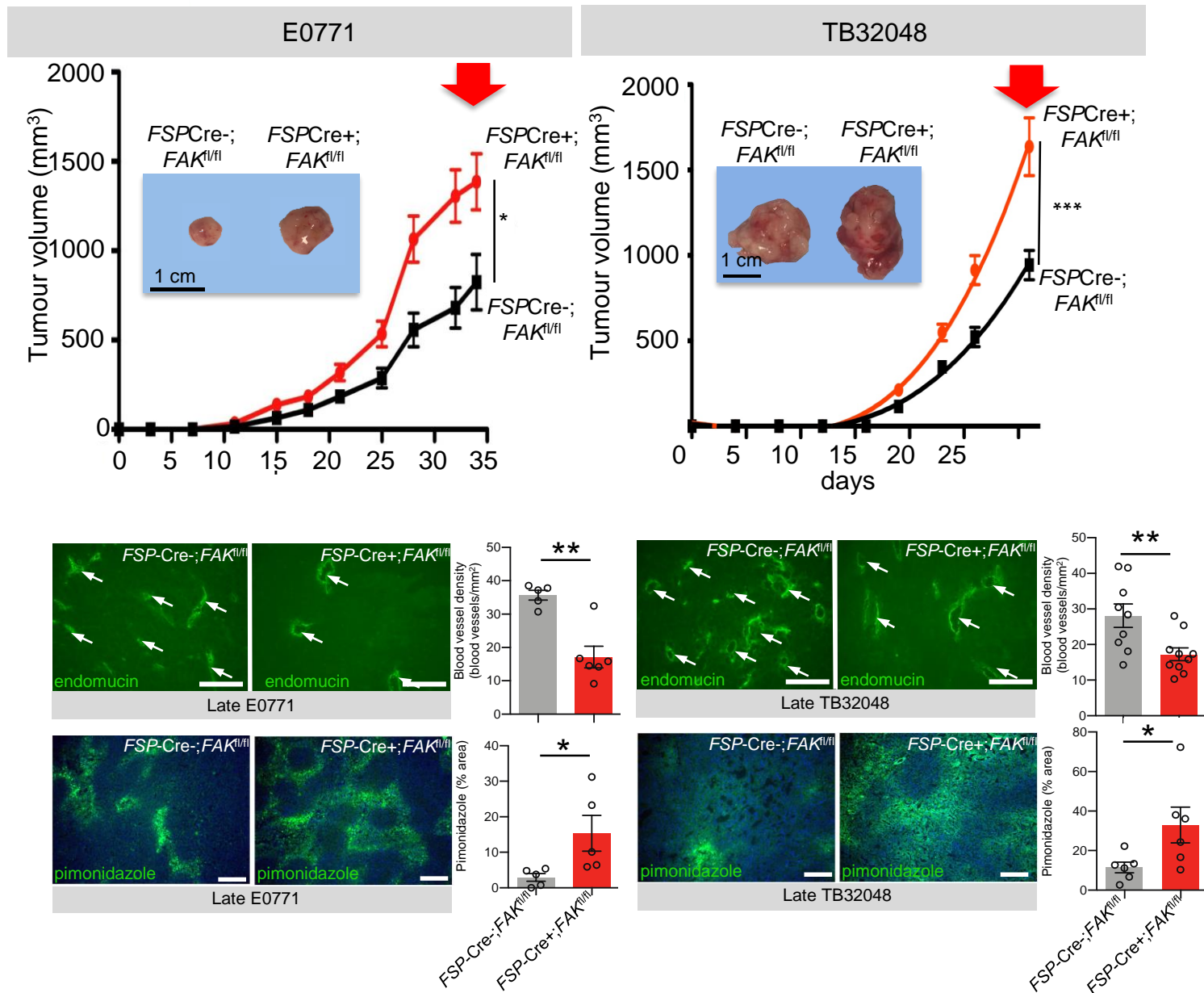


E0771

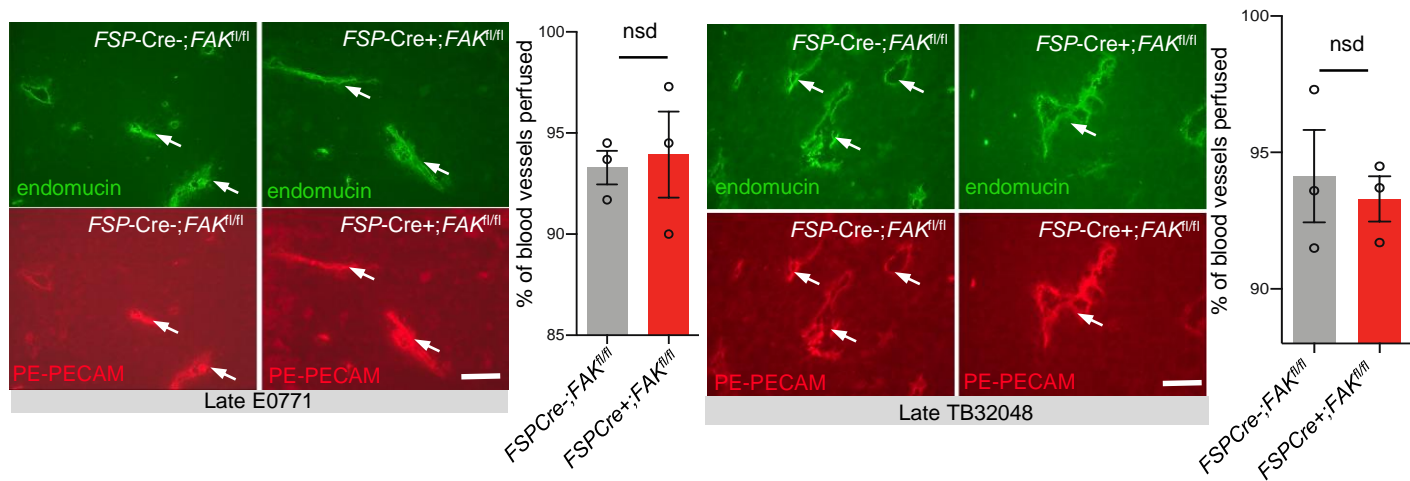


TB32048

# Enhanced tumour growth in *FSPC*Cre+;*FAK*<sup>fl/fl</sup> mice is associated with reduced blood vessel density



# No change in tumour blood vessel perfusion in *FSPCre+;FAK<sup>fl/fl</sup>* mice



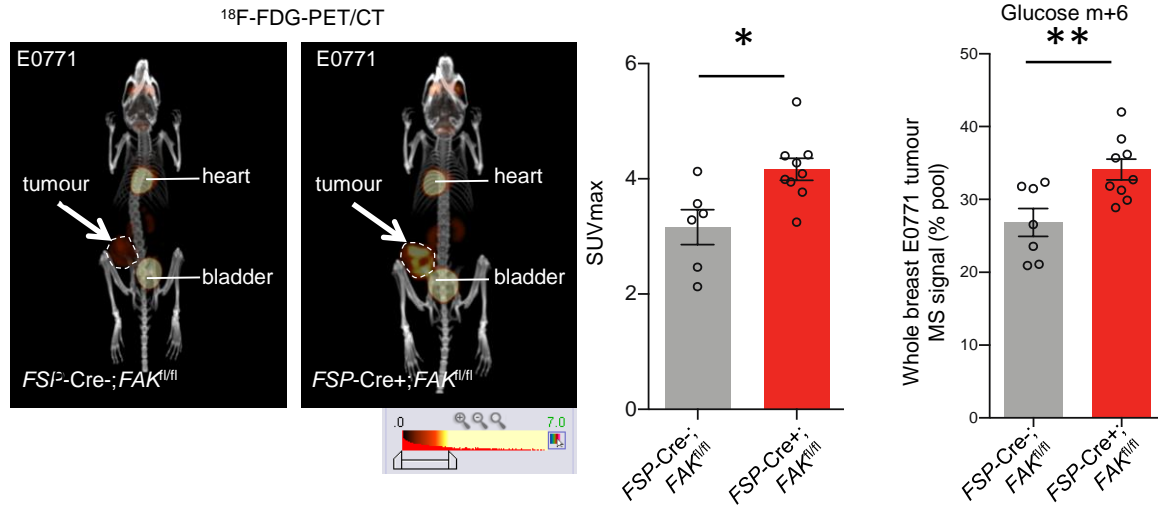


# Summary (1)

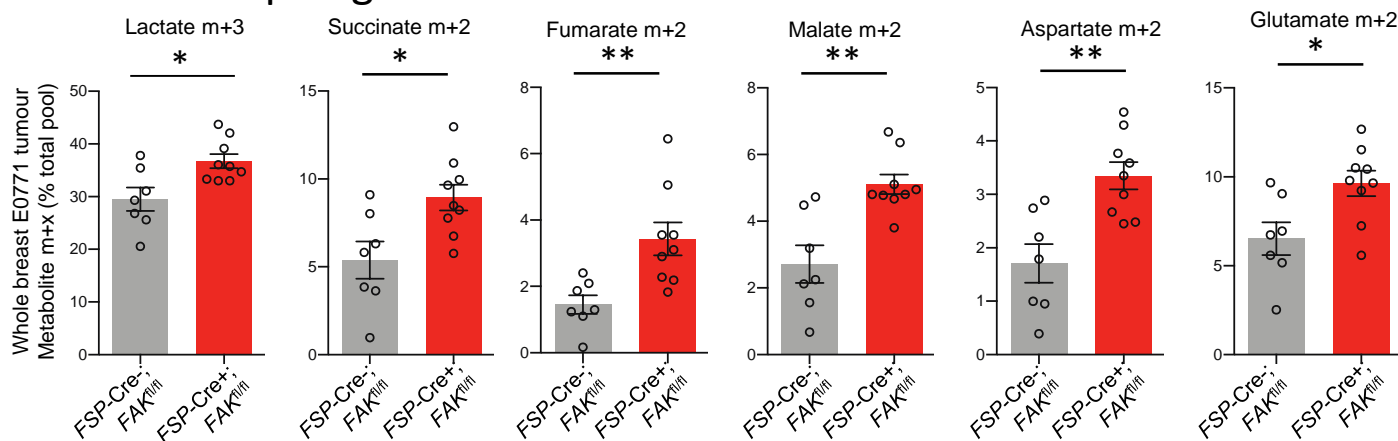
1. Reduced stromal FAK levels correlate with poor prognosis in human breast and pancreatic cancers
2. Loss of CAF FAK in *FSPCCre+;FAK<sup>fl/fl</sup>* mice show enhanced tumour growth
3. Tumours in *FSPCCre+;FAK<sup>fl/fl</sup>* mice grow with reduced angiogenesis – suggests alternative strategy for malignant cell growth?

# Enhanced metabolic activity and glucose uptake in early size-matched tumours grown in *FSP-Cre+; FAK<sup>fl/fl</sup>* mice

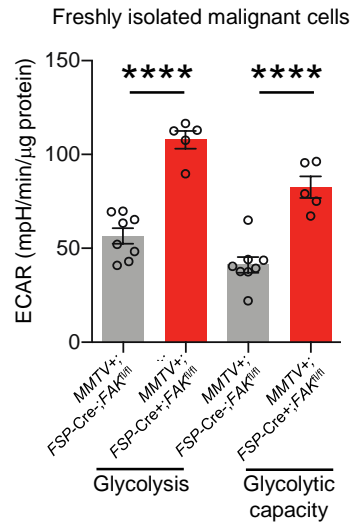
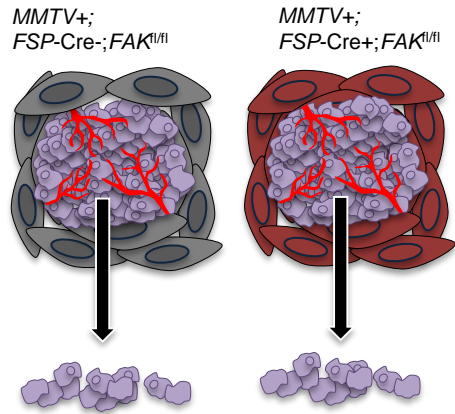
## Increased glucose uptake in size matched tumours



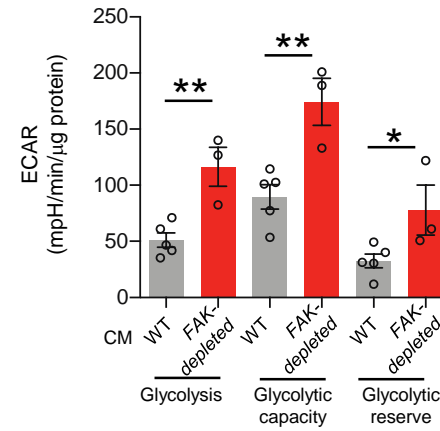
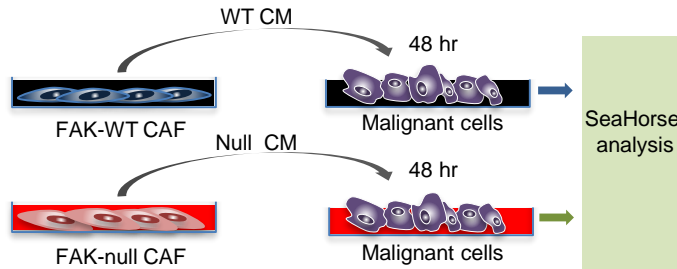
## LC-MS whole tumours: Increased contribution to TCA cycle, increased % labelled isotopologues



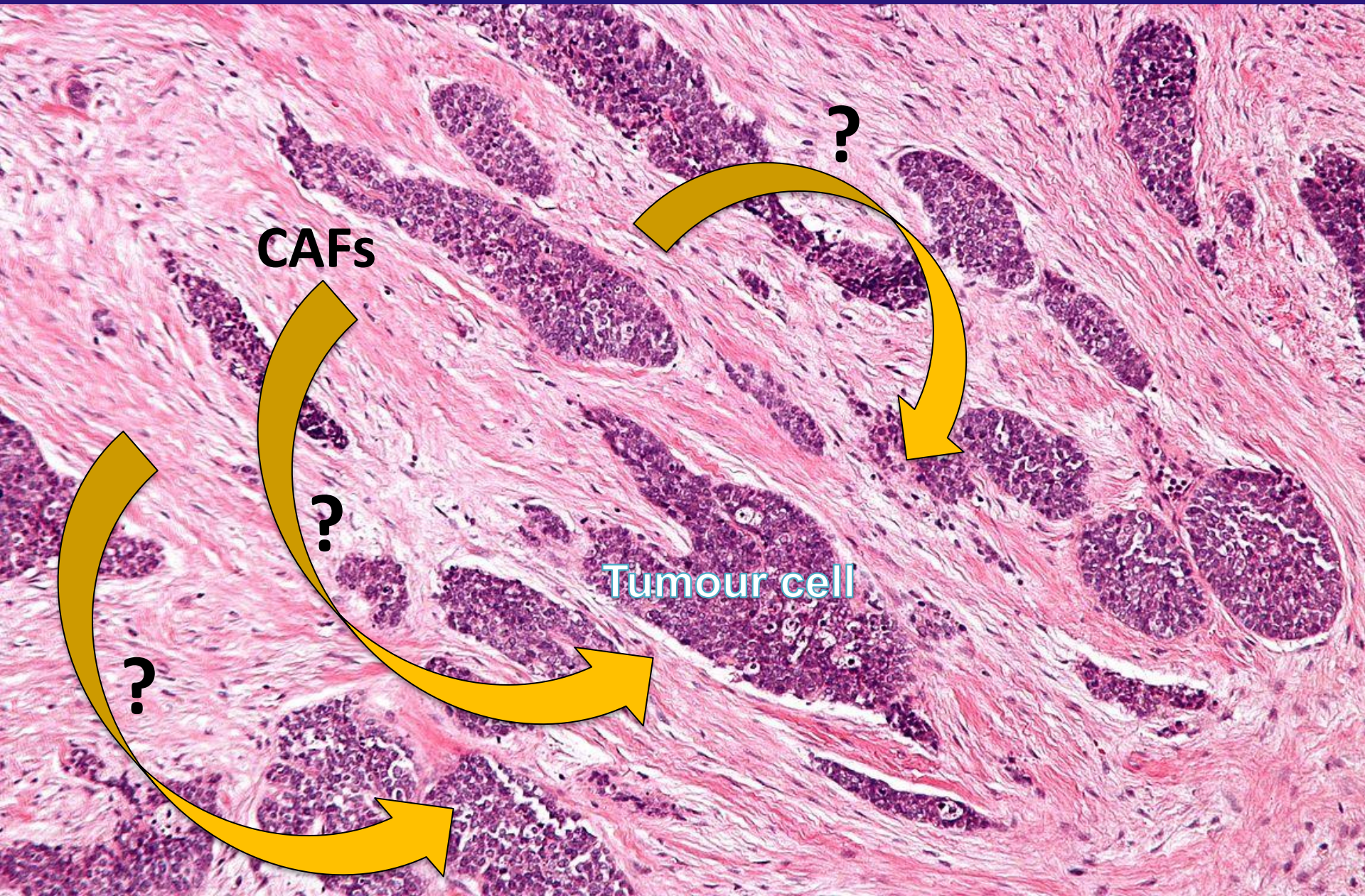
# Freshly isolated malignant cells show elevated glycolysis and glycolytic capacity that is dependent on CAF interaction



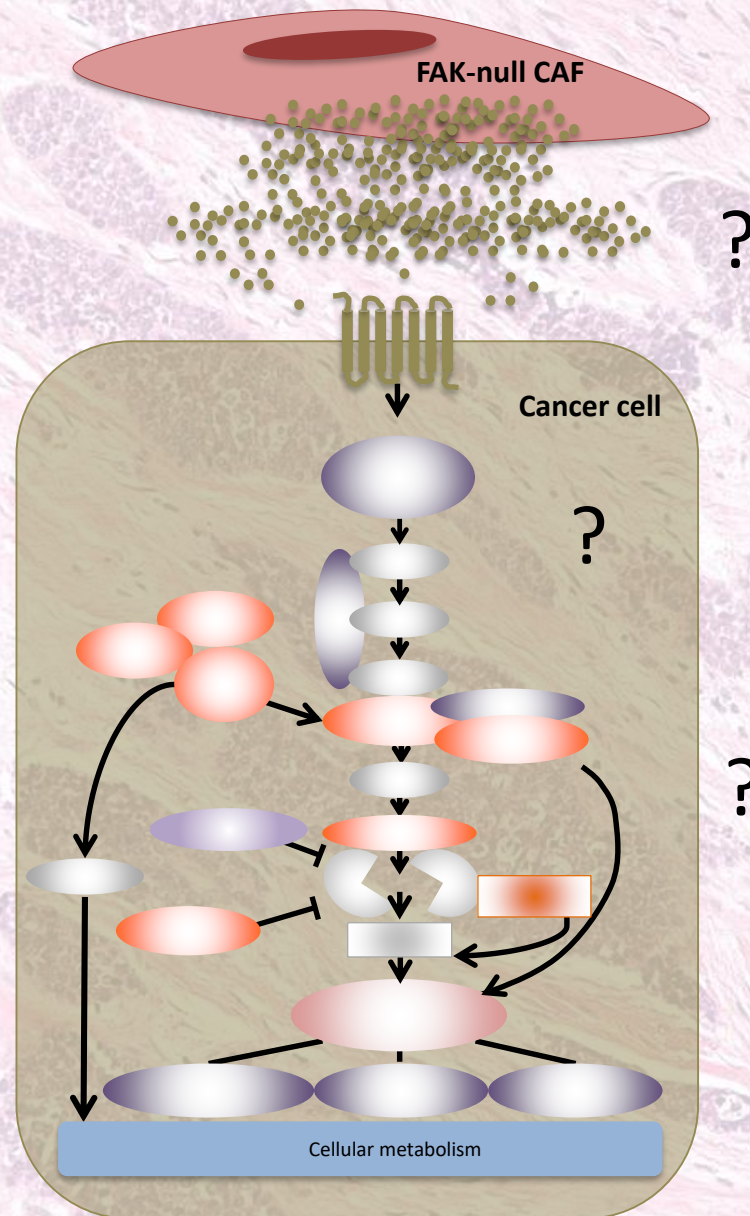
# Evidence for non-metabolite paracrine effect of CAF-FAK-deficiency on malignant cell metabolism



# How do FAK-null CAFs affect tumour cell metabolism?

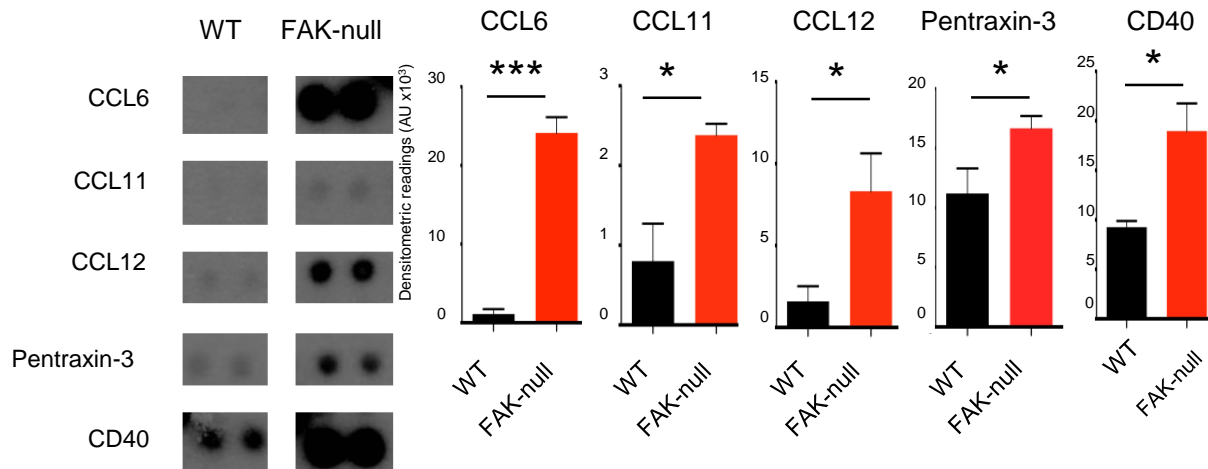
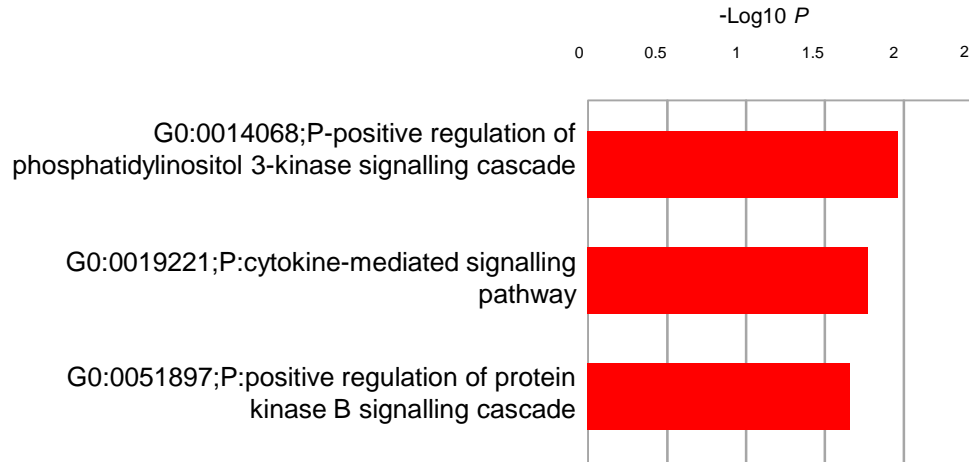


# What is changing in CAFs and how does this affect malignant cell metabolism?

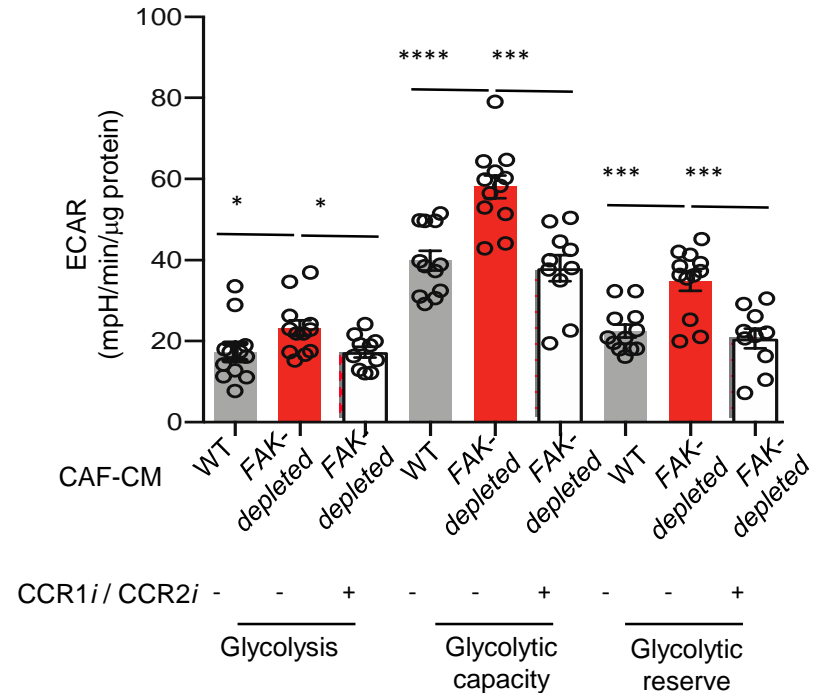
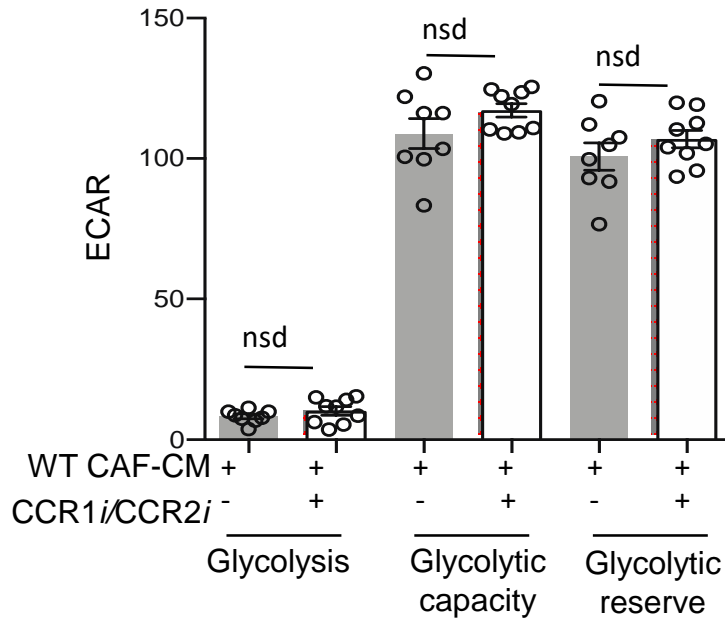
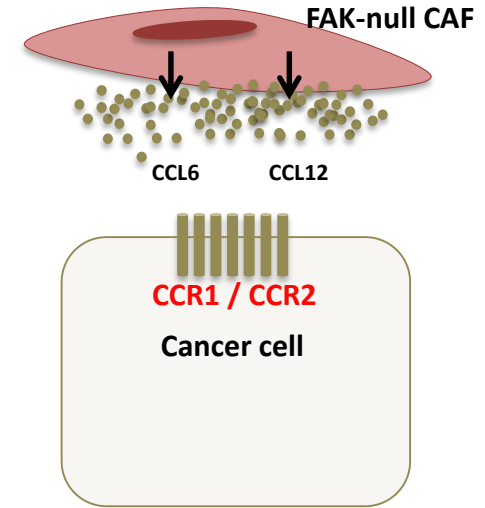
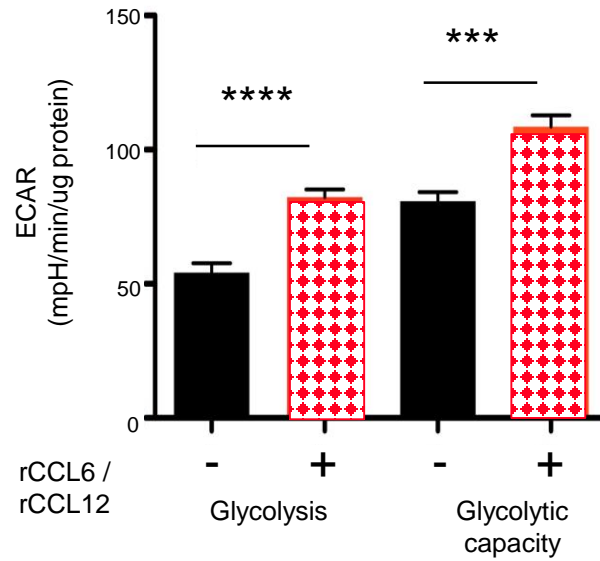


# Elevated cytokine mediated signalling and cytokine levels in FAK-null CAFs

## Phosphoproteomics: Most enriched signalling pathways in FAK-null CAFs



# CCL6 and CCL12 regulation of malignant cell metabolism

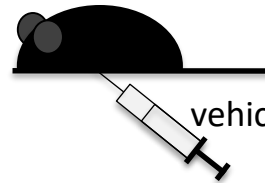


# Inhibition of CCR1 and CCR2 inhibit the *enhanced* tumour metabolism and growth *in vivo*

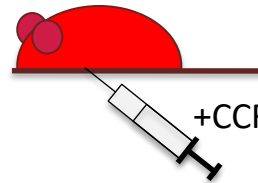
E0771 orthotopic breast cancer

FSPCre-; FAK<sup>fl/fl</sup>

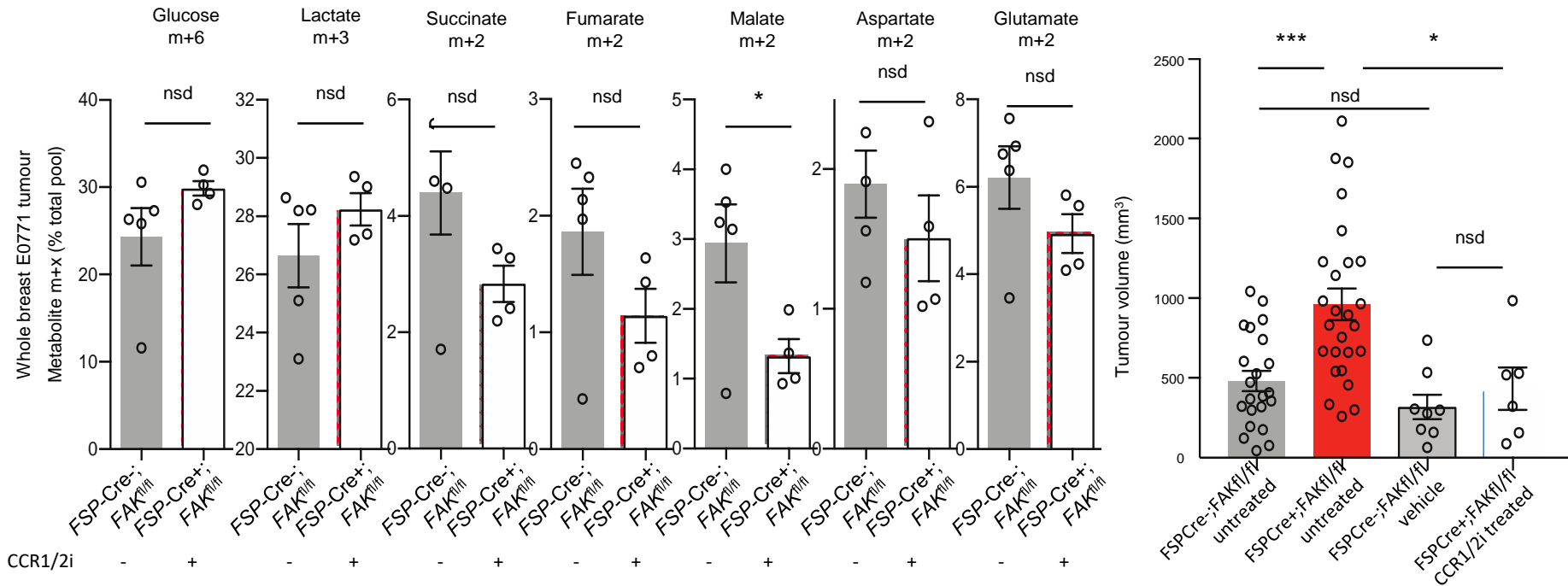
FSPCre+; FAK<sup>fl/fl</sup>



vehicle



+CCR1i/CCR2i

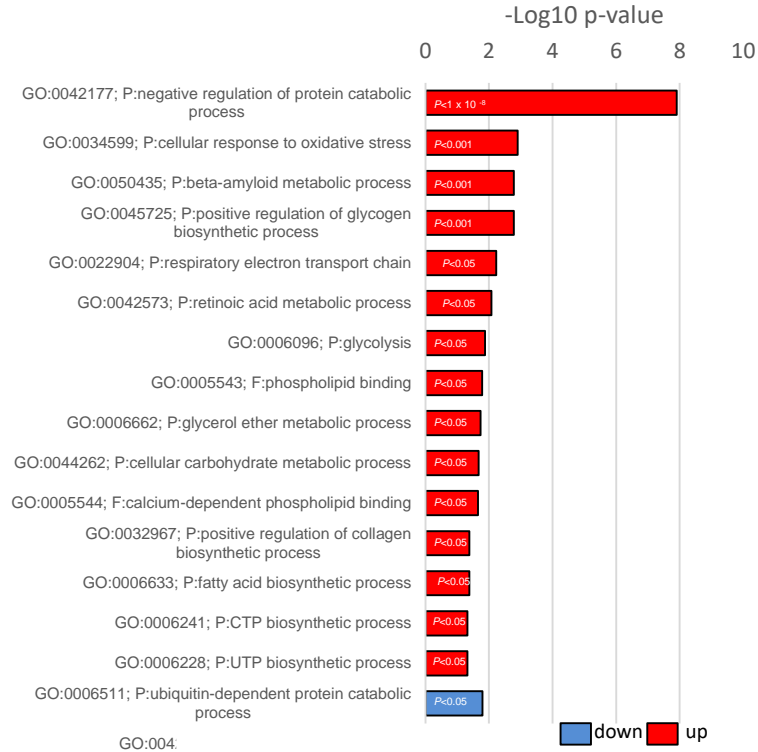




# Enhanced metabolic signaling pathways in mouse malignant cells incubated with FAK-null CAF CM

## Mouse malignant cells

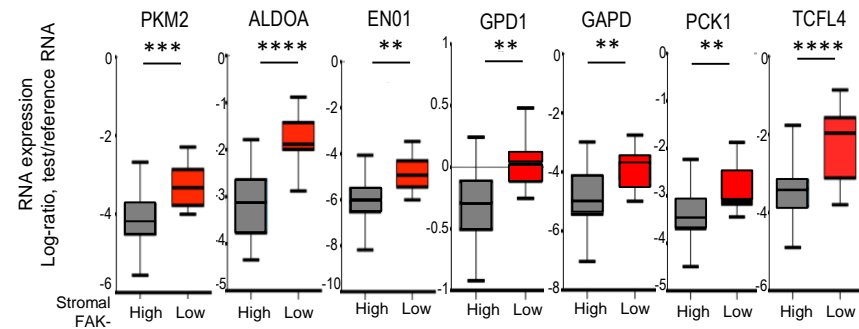
Most significantly changed metabolic pathways in malignant cells incubated with CM from FAK-null CAFs



## Mouse malignant cells

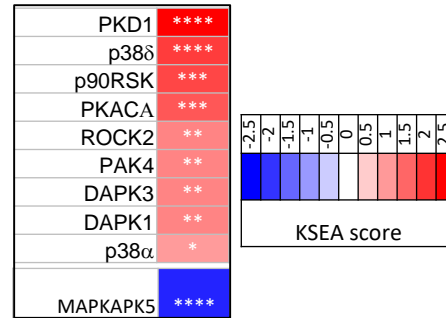
| Glucose metabolism                                     |     |
|--|-----|
| Pyruvate kinase PKM                                    | *   |
| Fructose-bisphosphate aldolase A                       | *   |
| Fructose-bisphosphate aldolase C                       | *   |
| Alpha-enolase  | *   |
| Beta-enolase   | *   |
| Glyceraldehyde-3-phosphate dehydrogenase               | *   |
| L-lactate dehydrogenase A chain                        | *   |
| L-lactate dehydrogenase B chain                        | **  |
| Pyruvate dehydrogenase E1 component subunit alpha,     | *   |
| 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2 | **  |
| Phosphoglycerate kinase 2                              | **  |
| Triosephosphate isomerase                              | **  |
| Glucose-6-phosphate 1-dehydrogenase X                  | *** |
| 6-phosphogluconate dehydrogenase, decarboxylating      | *** |

## Similarities with human malignant cells

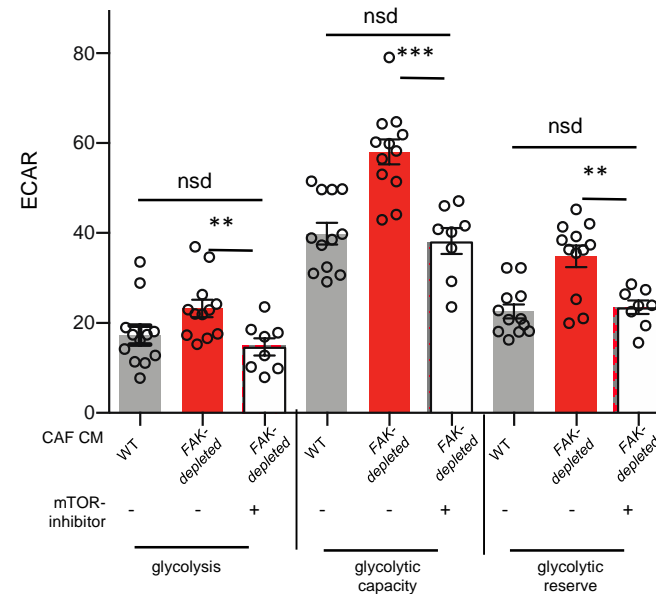
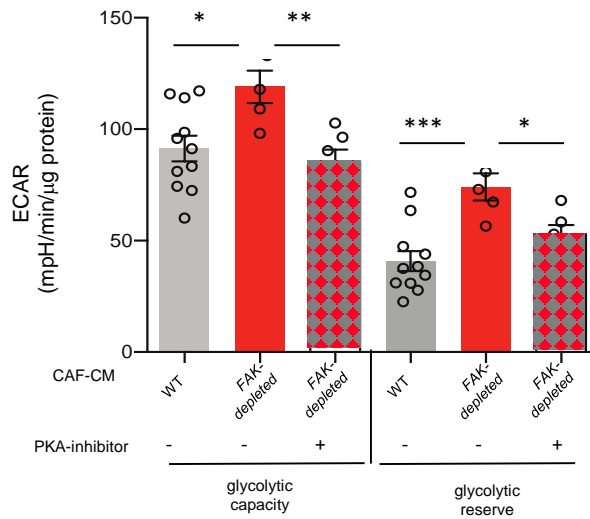
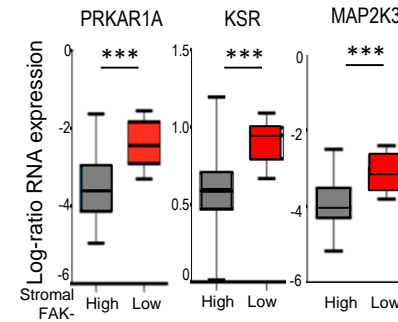


# Elevated PKA and mTOR signaling is important in enhanced metabolism of malignant cells exposed to FAK-null CAF CM

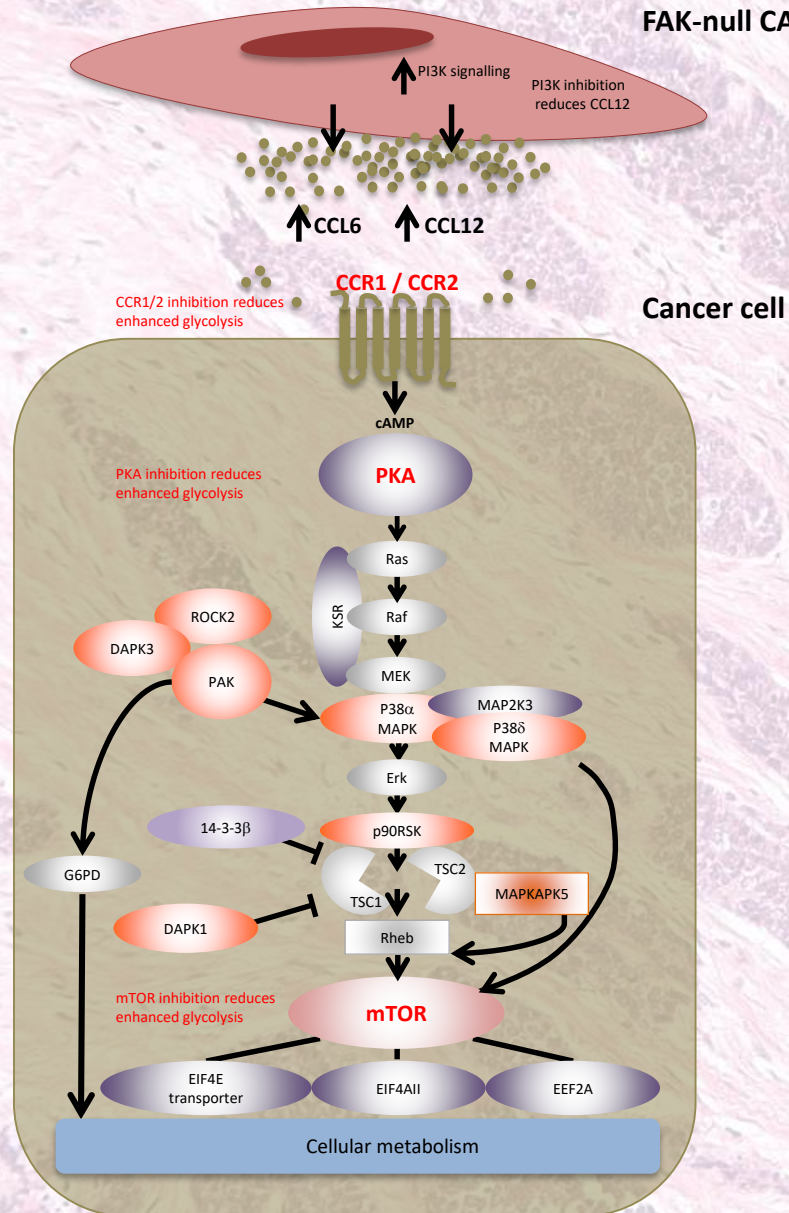
Mouse malignant cells



human malignant cells



# Overall summary



## Loss of CAF-FAK:

- Does not affect tumour desmoplasia *in vivo*
- Reduced angiogenesis
- Elevates CAF CCL6 and CCL12 expression

## In tumour cells:

- Enhanced signaling downstream of CCR1/CCR2
- Elevated requirement for PKA and mTOR signaling in enhanced glycolysis
- Correlation of enhanced metabolic pathways in mouse tumour cells exposed to FAK-null CAF CM and patient data with low stromal FAK expression
- Enhanced tumour growth
- *Evidence for CAF-FAK regulation of tumour cell metabolism.*
- *Extrinsic control of tumour cell metabolism*
- *Give an example that levels of CAF-FAK affect tumour cell metabolism*
- *Implications for targeting tumour cell metabolism*

# Acknowledgments

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