

Leo Carlin (leo.carlin@glasgow.ac.uk)

Leukocyte Dynamics Group /

Beatson Advanced Imaging Resource

Pancreatic Cancer UK Discovery and Translational Research Forum

13 Jun 2022

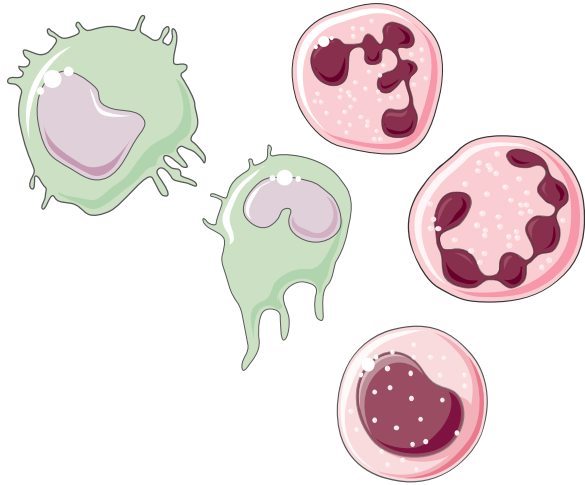


CANCER
RESEARCH
UK

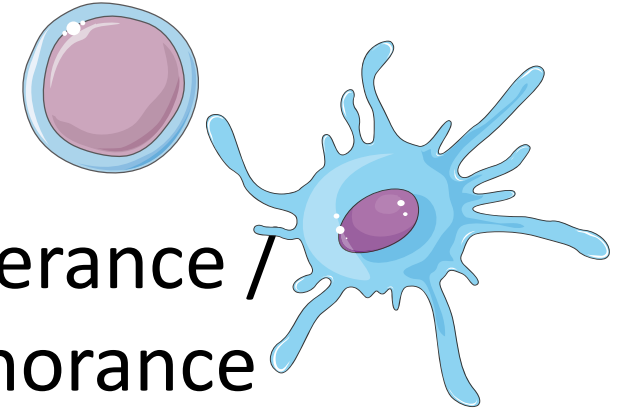
BEATSON
INSTITUTE

The immune system in cancer

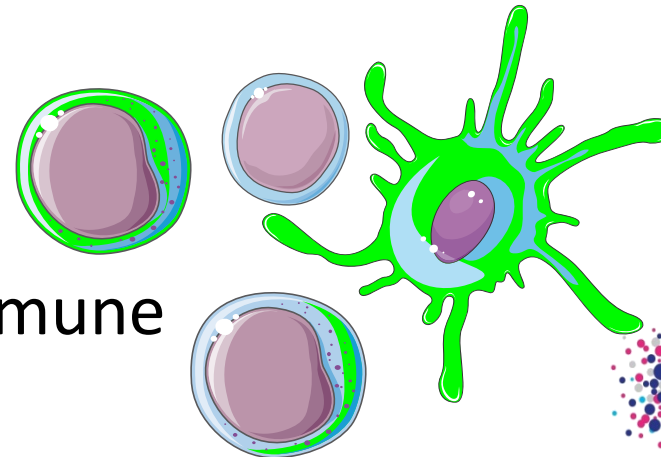
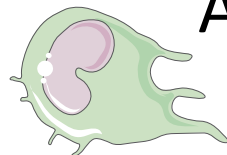
Tumour promoting
inflammation



Tolerance /
ignorance

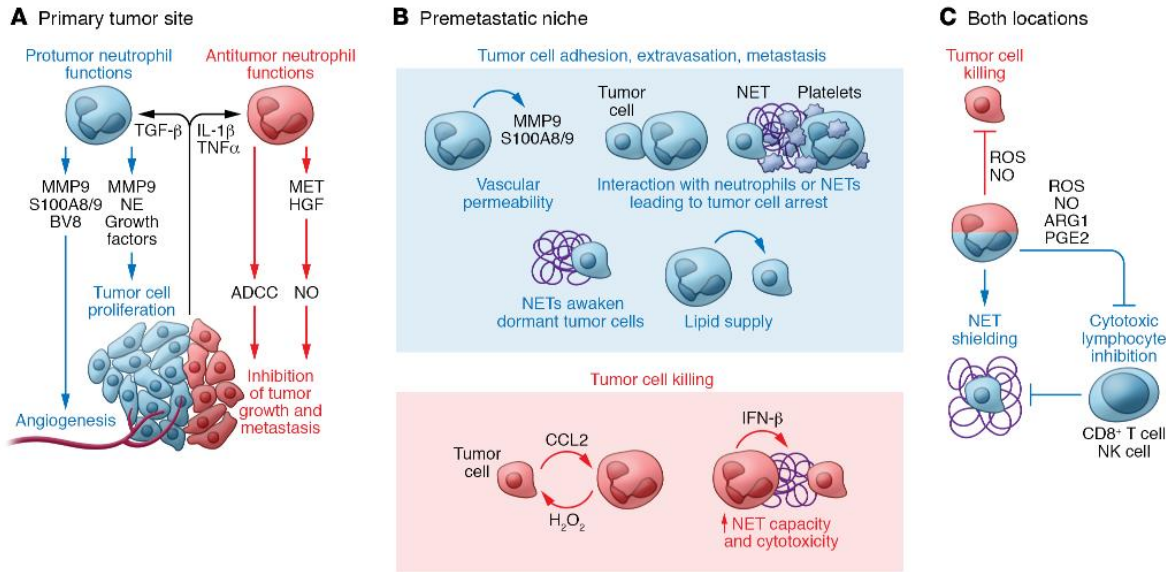


Anti-tumour immune
response



Neutrophils in Cancer

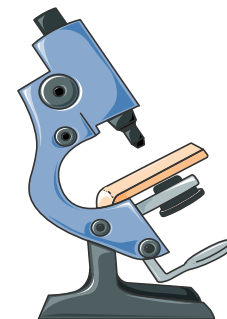
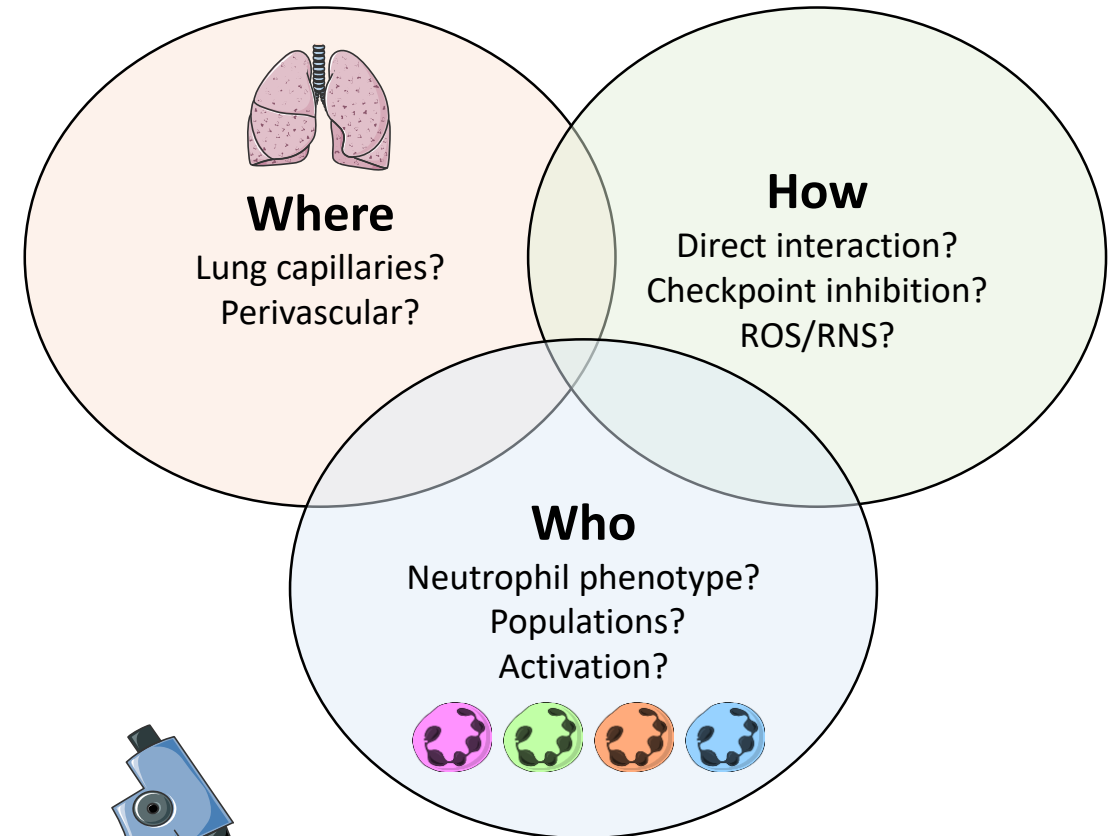
- Increased in many cancers
- Both pro and anti-tumour functions described
- **Are involved in metastasis to the lung**



McFarlane, Fercoq, Coffelt, Carlin JCI 2021

Pre-metastatic lung

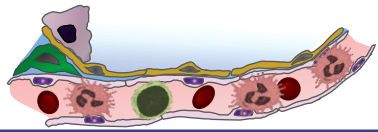
Neutrophil interactions *in situ*



Neutrophils in Health

- Not homogenous
- Versatile : acquire functional properties in tissues
- **Need to take local microenvironment into account**

- **3D multiplex imaging**
- **Live multiplex imaging**
- **Flow Cytometry**



Neutrophils in the (pre-)metastatic niche

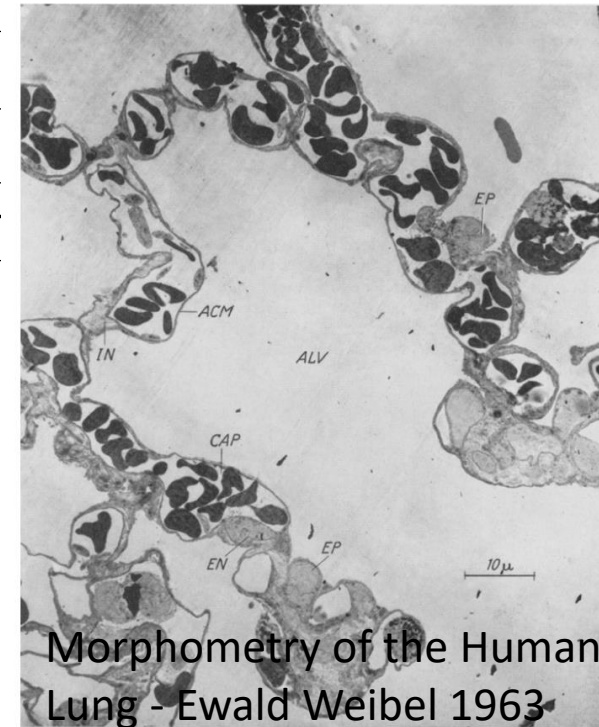
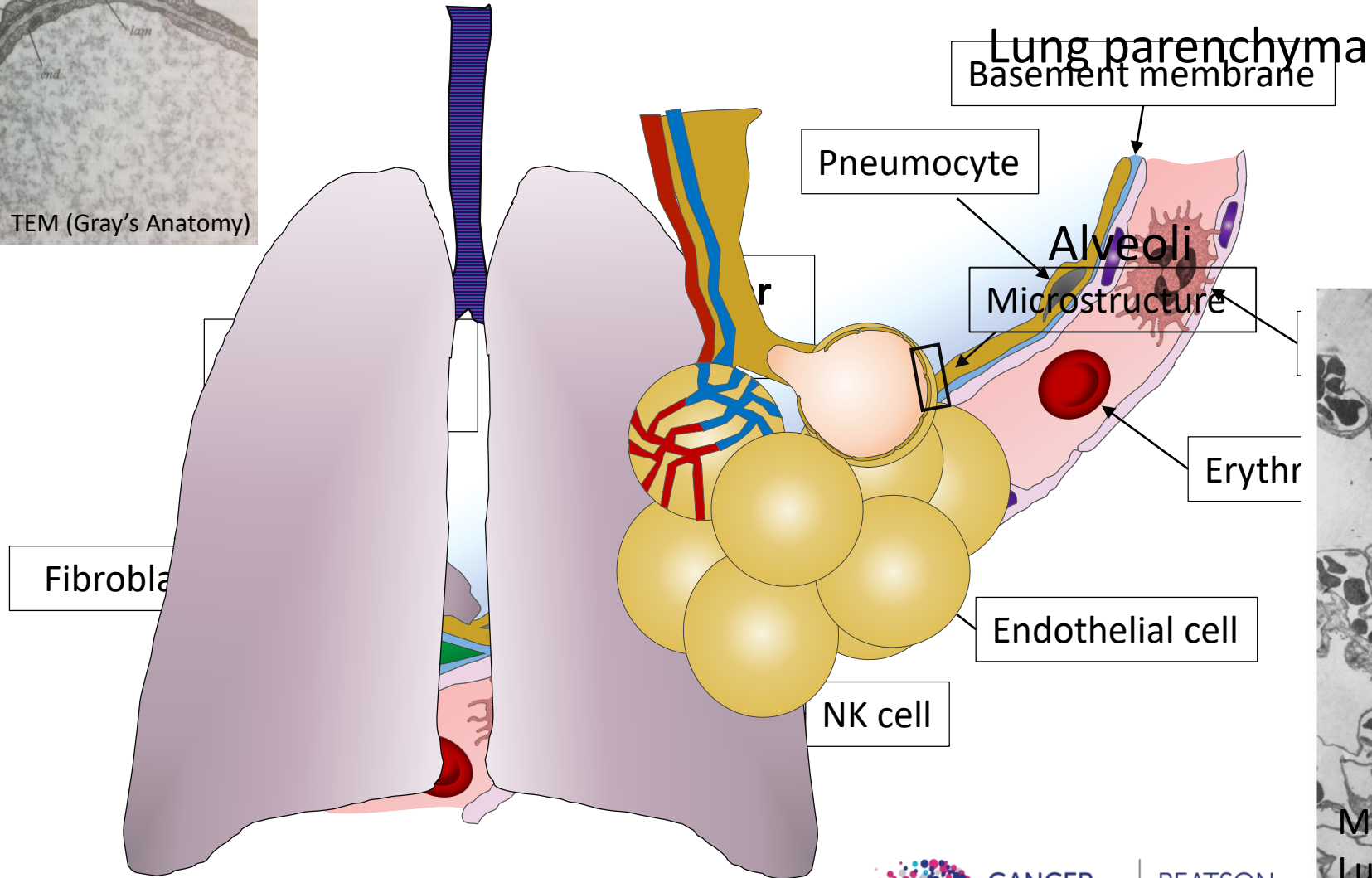
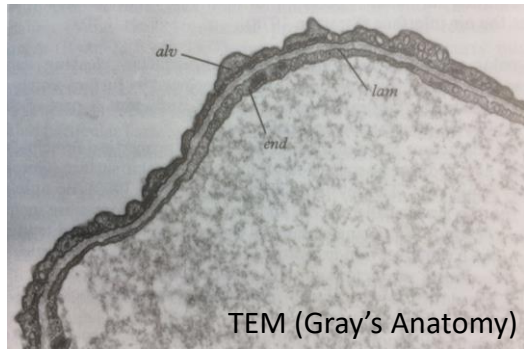
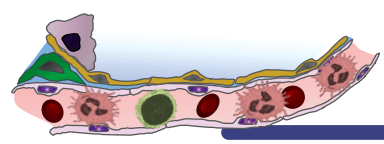


Fig. 66. Section of alveolus of rat lung, showing relation between alveolar air and capillary blood. The alveolo-capillary tissue layer is very thin ("membranous") over large areas (ACM), but is thicker where it contains alveolar epithelial (EP) and capillary endothelial cells (EN), or interstitial elements (IN). × 1350



BEATSON
INSTITUTE



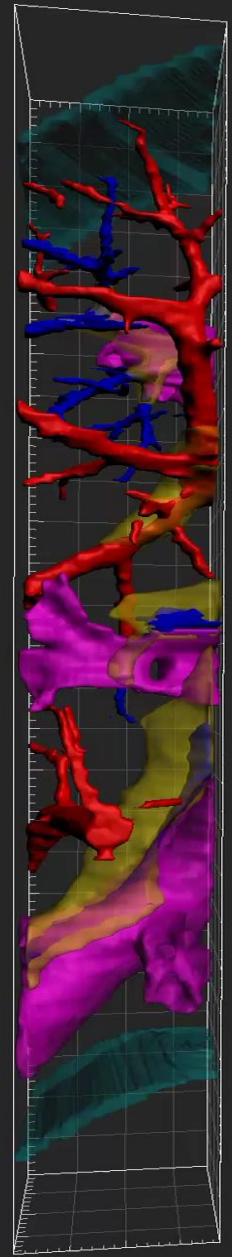
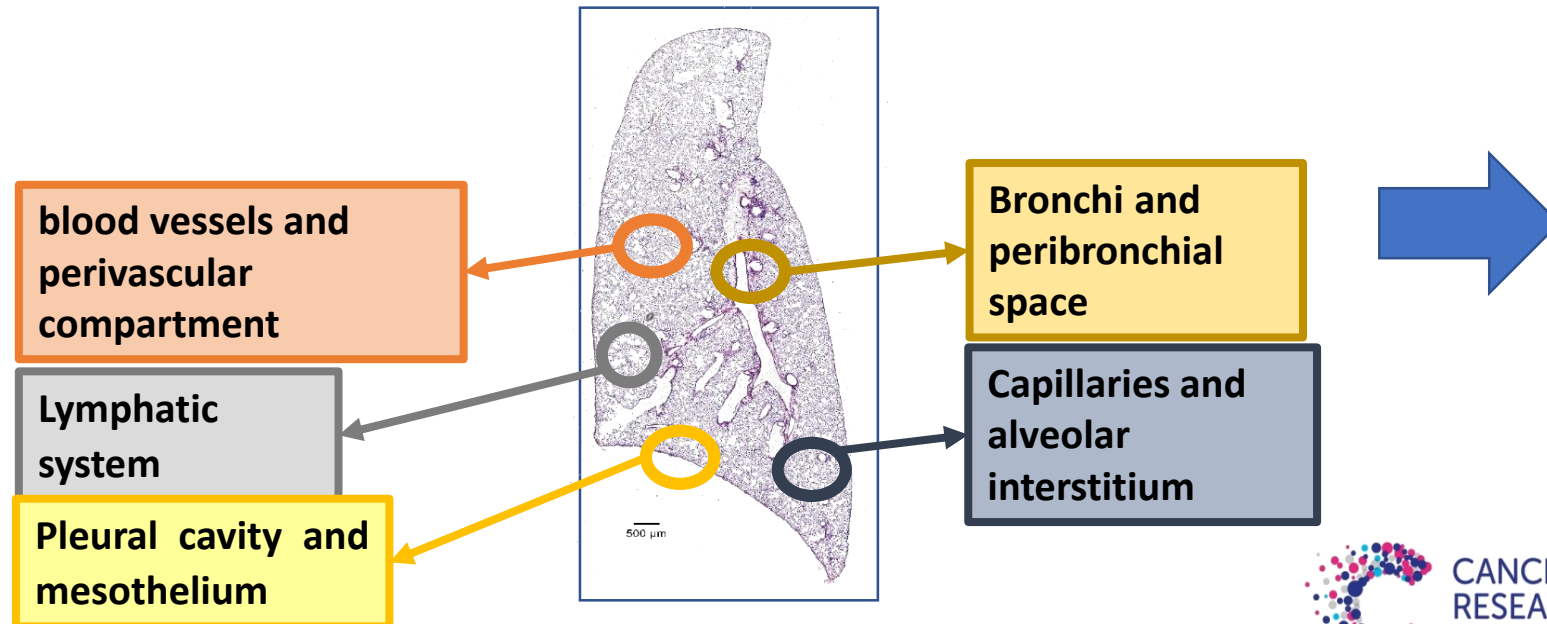
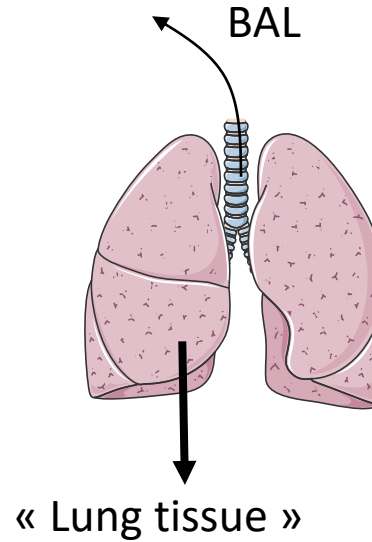
Spatiotemporal lung immunology

Many studies

- sampling of bronchoalveolar lavage (cells, cytokines...)
- dissociation of lung tissue (flow cytometry, transcripts...)
- more or less refined histological analysis.

BUT

Lung is a complex organ composed of specialized microanatomy



KPC Pancreatic Cancer GEMMs



Highly metastatic KPC: Pdx1-Cre, Kras^{G12D/+}, p53^{R172H/+}

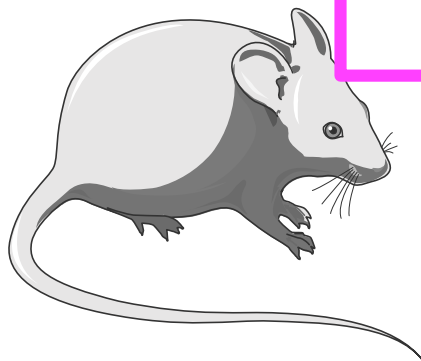
- Primary tumour 12-24 weeks
- Metastasis to liver / lung



Poorly metastatic KPflC: Pdx1-Cre, Kras^{G12D/+}, p53^{fl/+}

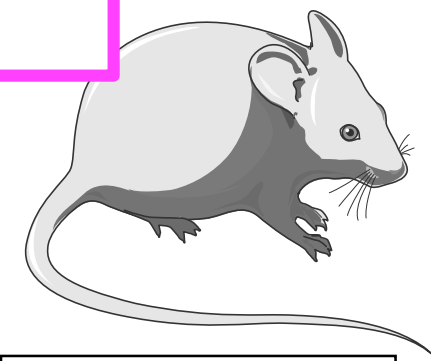
- Primary tumour 12-24 weeks

**Small palpable tumour
PRE-OVERT METASTASIS**



Control KC: Pdx1-Cre, Kras^{G12D/+}

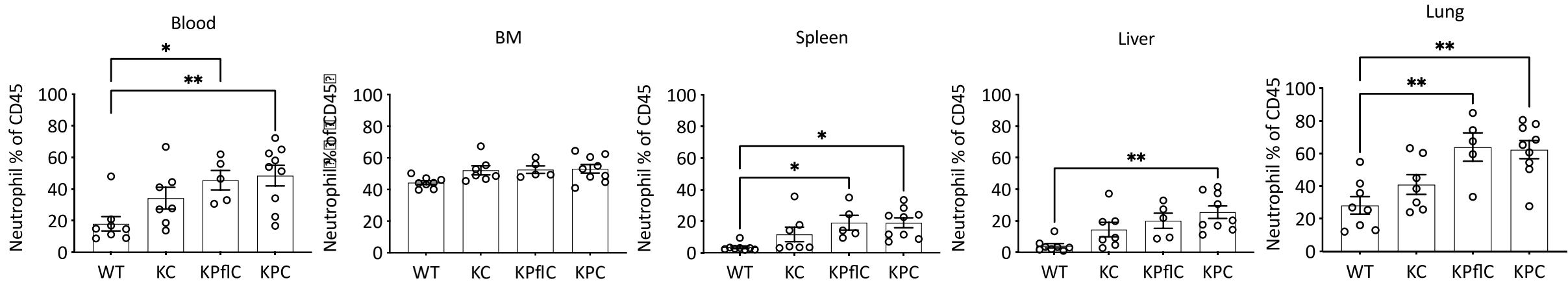
- No Primary tumour



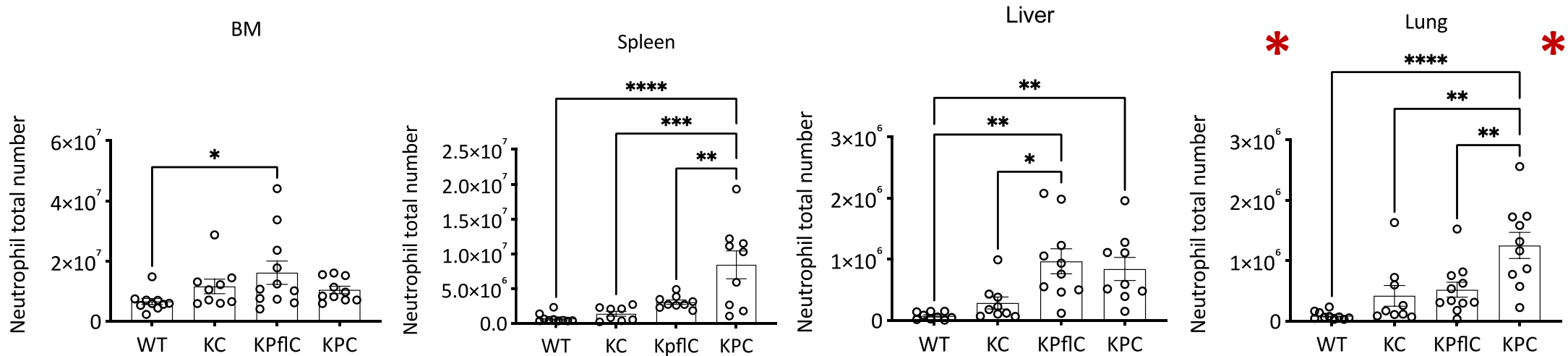
Control WT: Pdx1-Cre

Pulmonary neutrophils are increased in mutant p53 tumour bearing mice

Neutrophils % of leukocytes

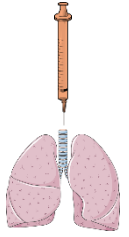


Neutrophil number



Neutrophils are similarly distributed regardless of metastatic potential

Inflate with agarose

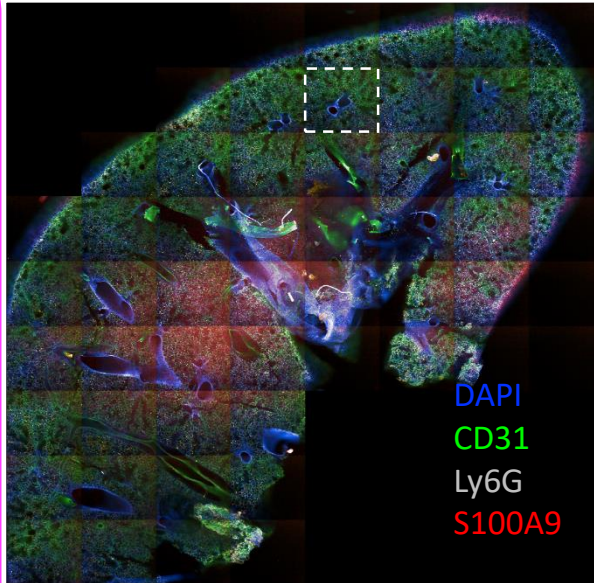


Fix lungs & slice

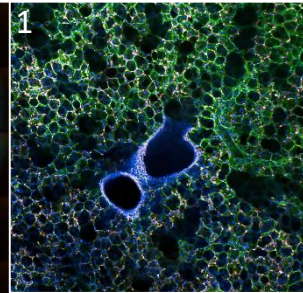
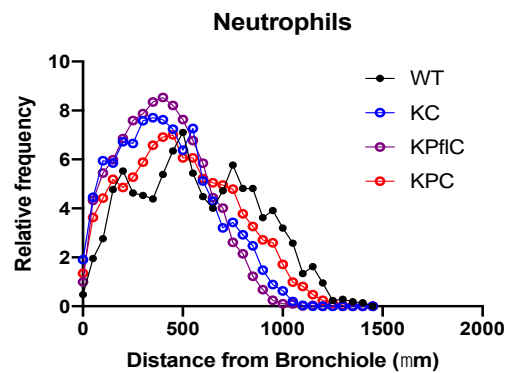


Stain for neutrophil markers

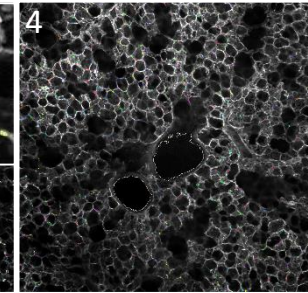
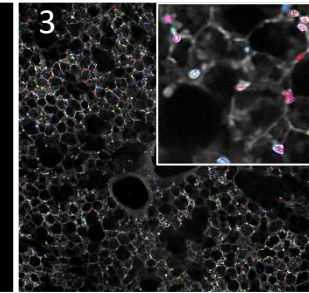
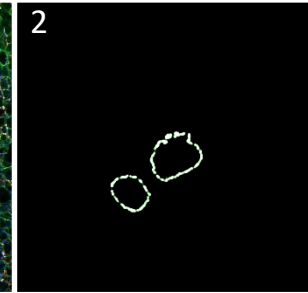
Scan using the Opera



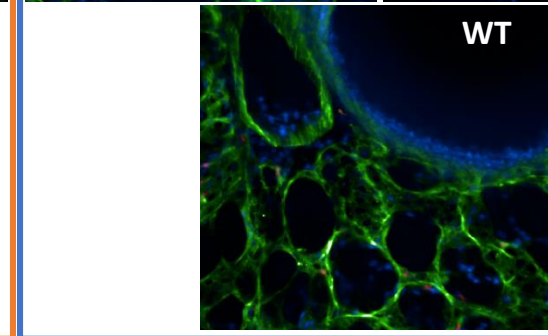
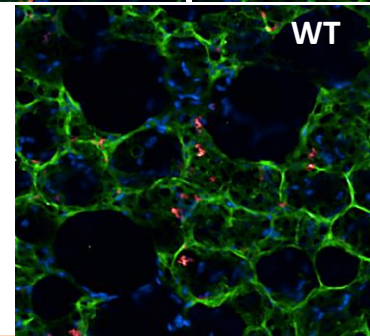
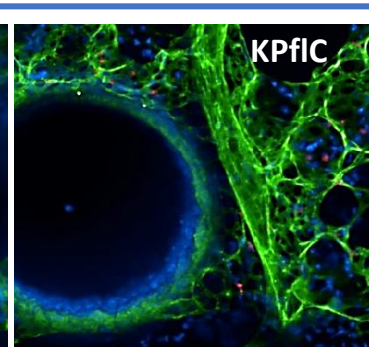
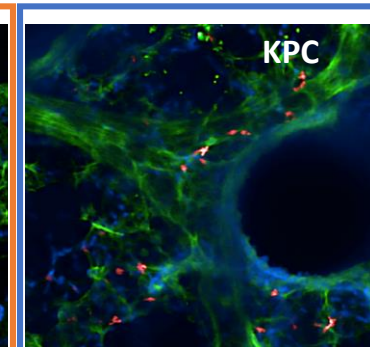
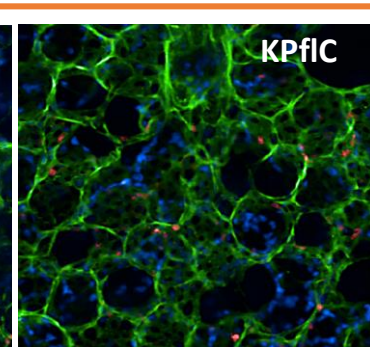
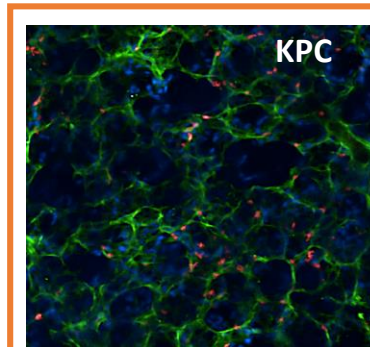
5



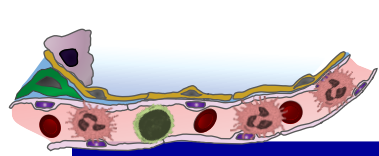
Parenchyma



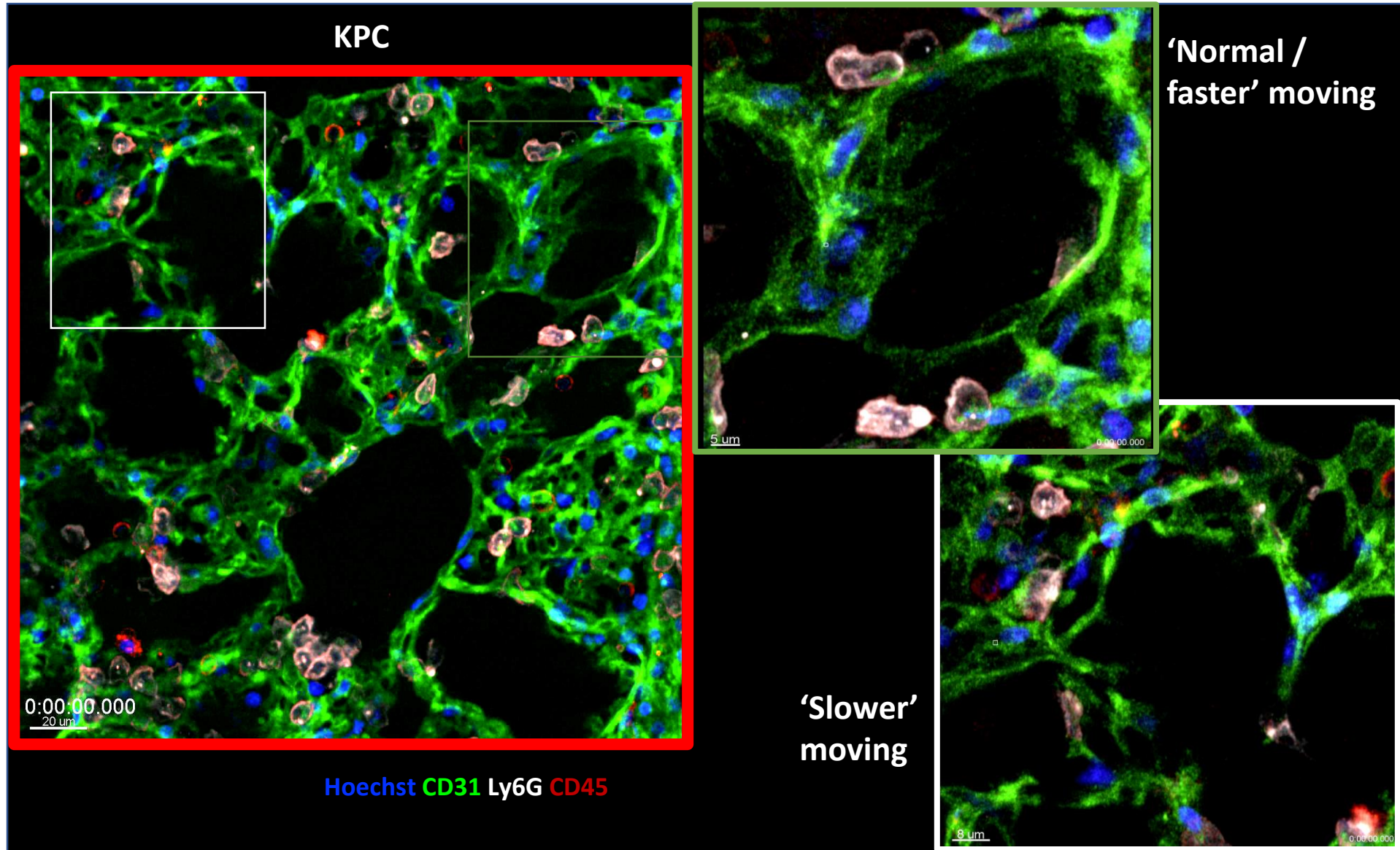
Perivascular

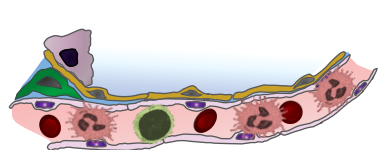


Unpublished, Please don't post

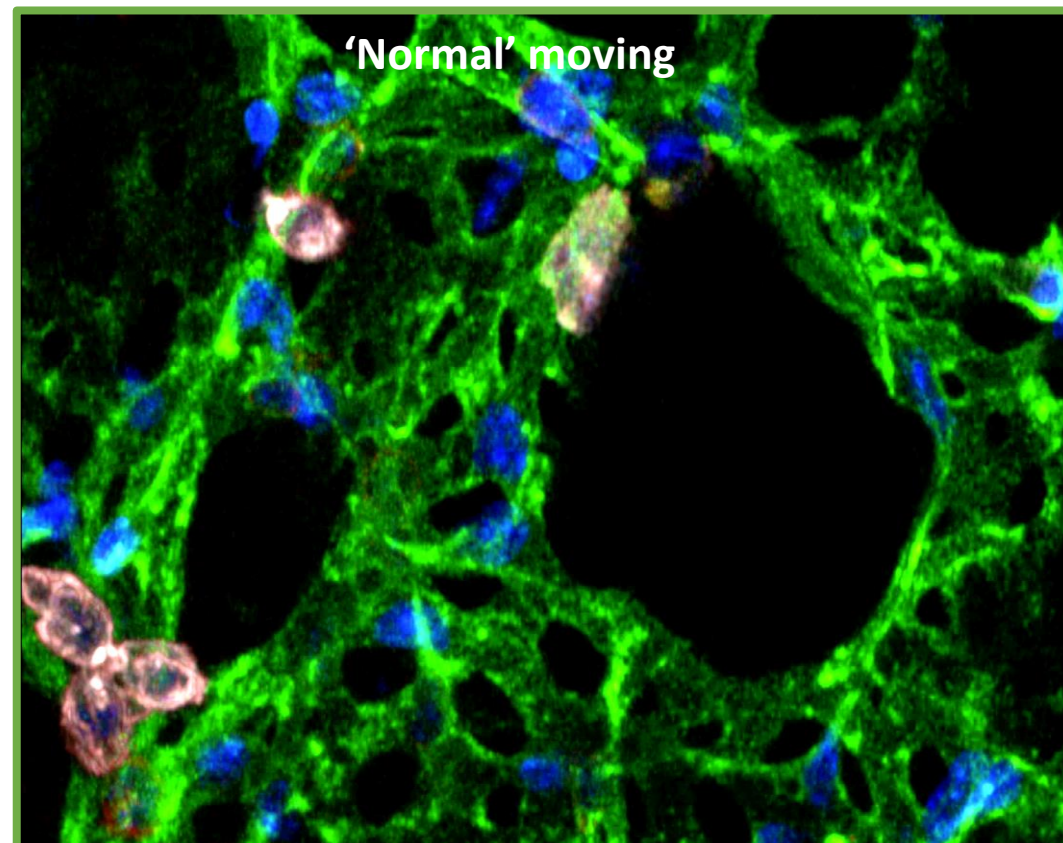
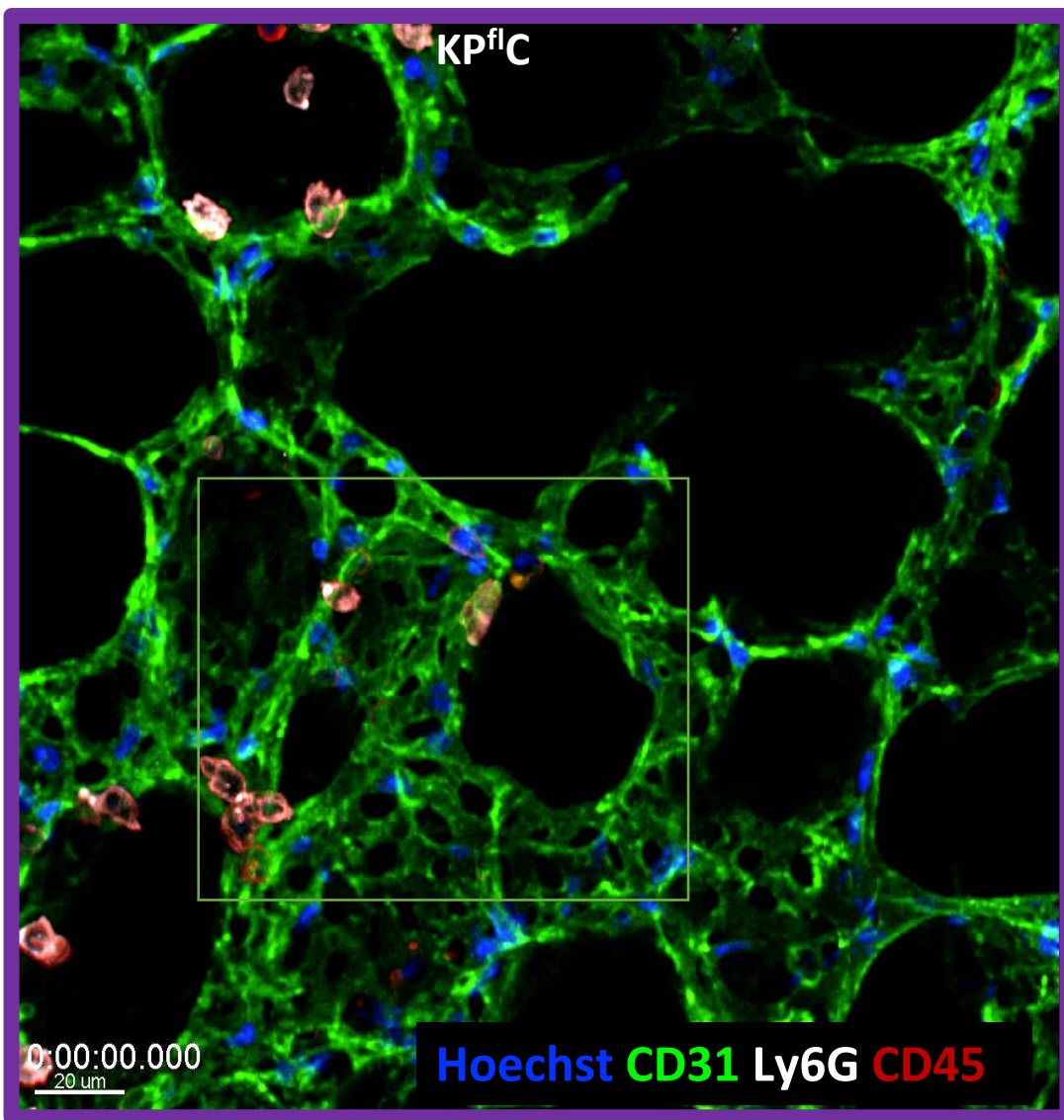


Neutrophils move differently in KPC PCLS

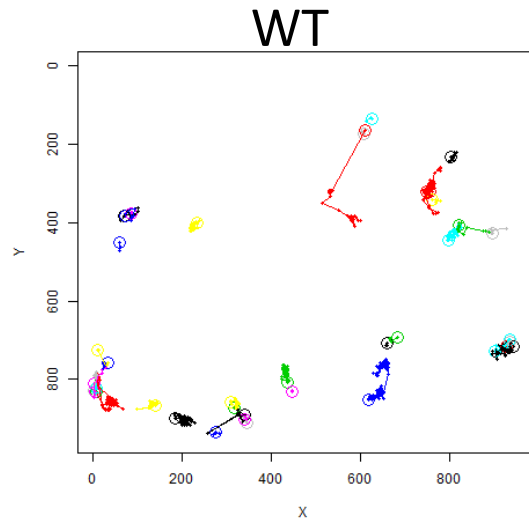




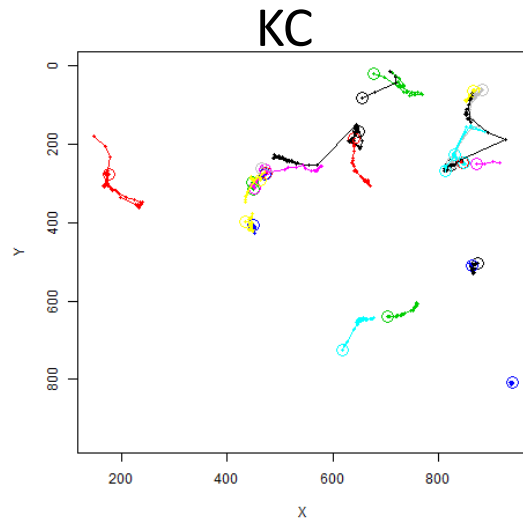
'Lazy' population is absent from KP^{fl}C



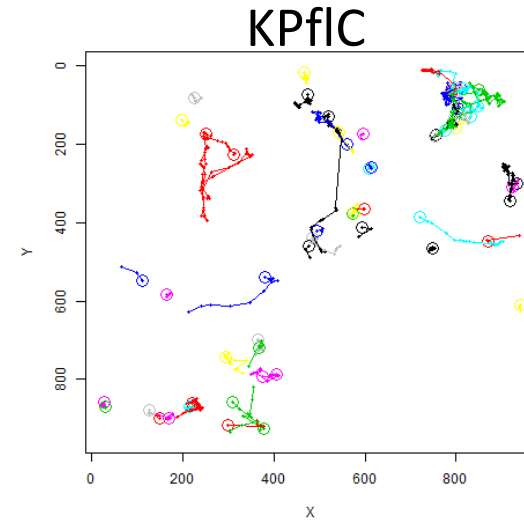
Increased number of slow moving / stationary neutrophils in KPC lungs



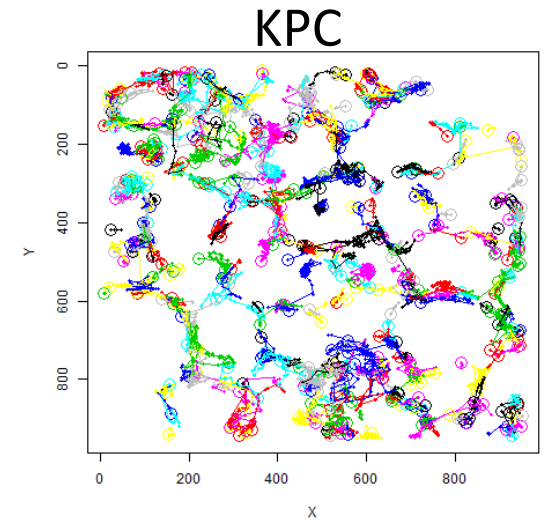
Slow moving



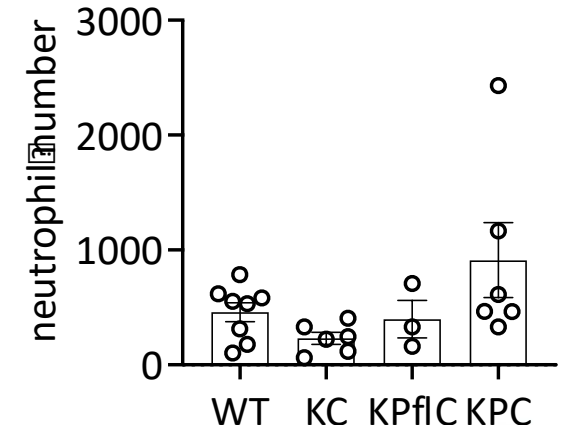
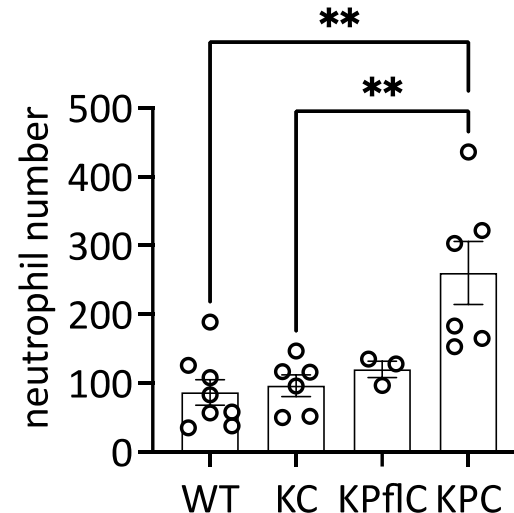
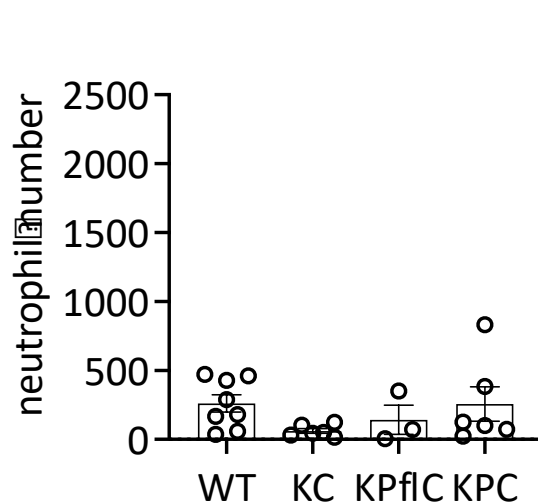
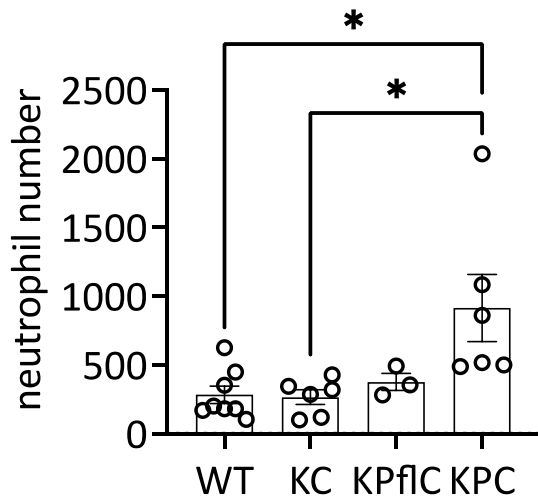
Fast moving



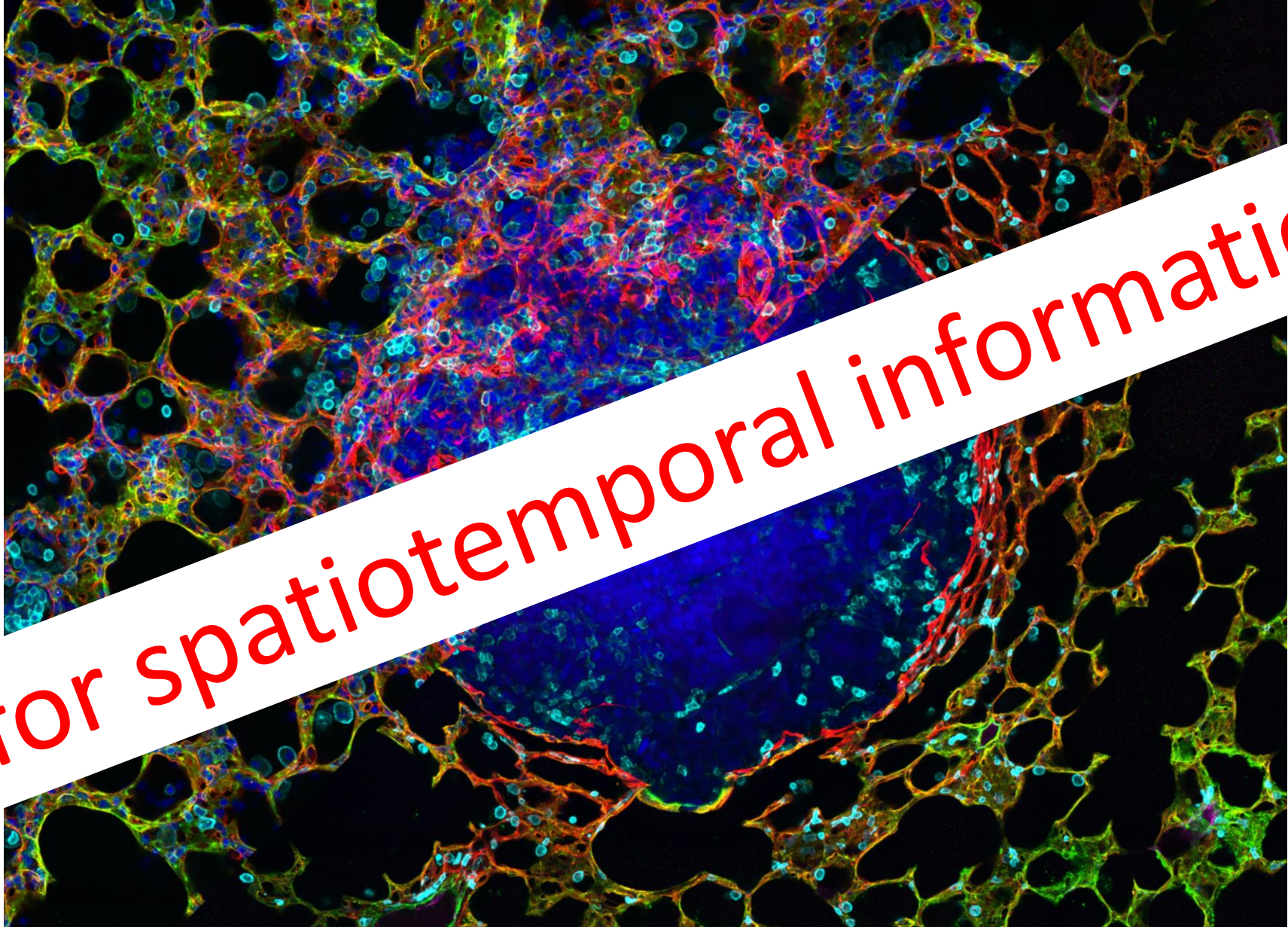
Short distance



Long distance



Vascularisation pattern may influence the immune microenvironment when metastases are established



Need for spatiotemporal information

Acknowledgements

Leukocyte Dynamics Group

Fred Fercoq
Amanda McFarlane
Ximena Raffo-Iraolagoitia
Marco De Donatis

Alumni

John Mackey
Judith Secklehner
Juho Vuononvirta
Katia De Filippo
Sophie Bruce (UoG MSci)
Dide Reijmer (Erasmus Student 2018)

Beatson Advanced Imaging Resource (BAIR)

Ewan McGhee
Lynn McGarry
Nikki Paul
Peter Thomason

Flow Cytometry

Tom Gilbey
Yi-Hsia Liu

BRU and Histology Beatson Core Facilities

Collaborations:

Seth Coffelt
Jim Norman
Jen Morton
Karen Byth
Daniel Murphy
Sara Zanivan
Owen Sansom
Tom Bird
Ed Roberts
Jack Leslie, Erik Gil, Derek Mann (Newcastle)



Imperial College
London



BREAST
CANCER
NOW The research
& care charity