

# Hypoxic tumour cells induce myeloid-derived suppressor cell accumulation in metastatic target organs

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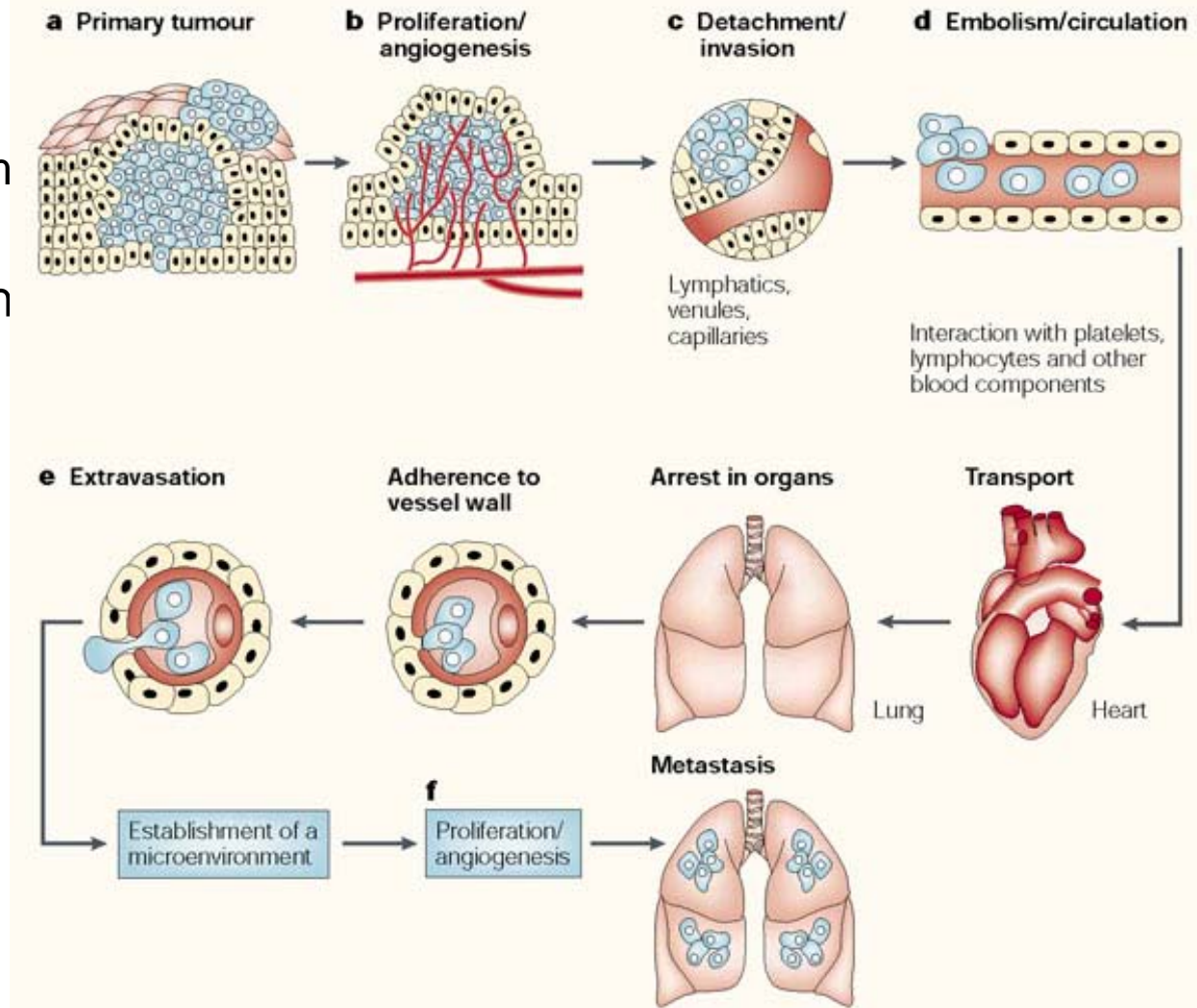


# Conflicts of interest

- None to declare

# Metastasis is a multi-step process

- Metastasis is remarkably inefficient
  - <math><0.01\%</math> of cells form macrometastases
- Migration and invasion
- Survival in the lymphatics or bloodstream
- Arrest, adhere, and/or extravasate in tissues
- Survival in new organ
  - micrometastases
- Colonization of new organ
  - macrometastases

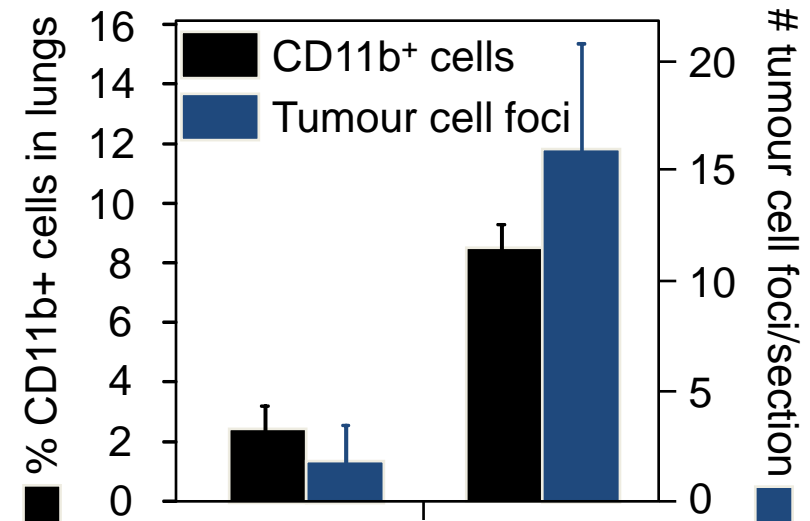
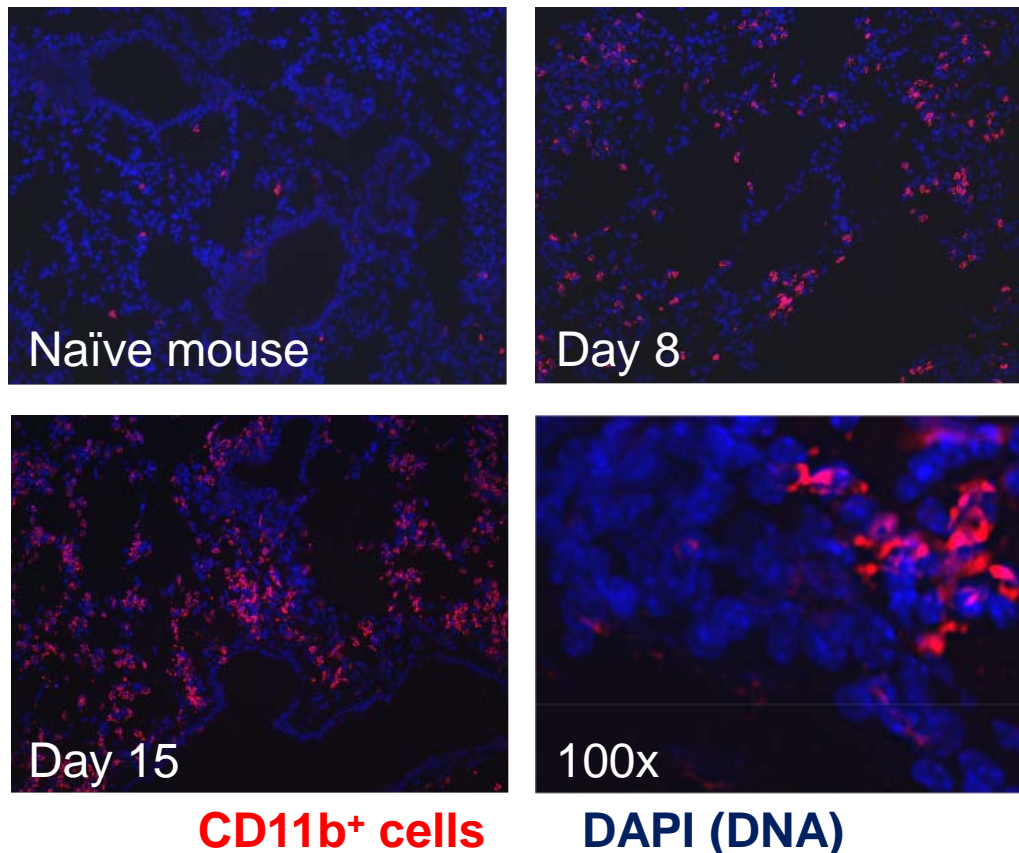


# Seed-and-soil hypothesis revisited

- “When a plant goes to seed, its seeds are carried in all directions; but they can only live and grow if they fall on congenial soil”  
Paget, *Lancet* (1889) 1:571-3
- Metastatic tumour cells (**seeds**) will only colonize “compatible” target organs (**soil**)
- Tumours can **manipulate** the soil in metastatic target organs
  - stimulate the accumulation of **bone marrow-derived cells** into “**pre-metastatic niches**”  
Kaplan *et al.*, *Nature*, 2005
- **Pre-metastatic niches are thought to be fertile regions of tissue that promote the invasion, proliferation, and/or survival of metastatic tumour cells**

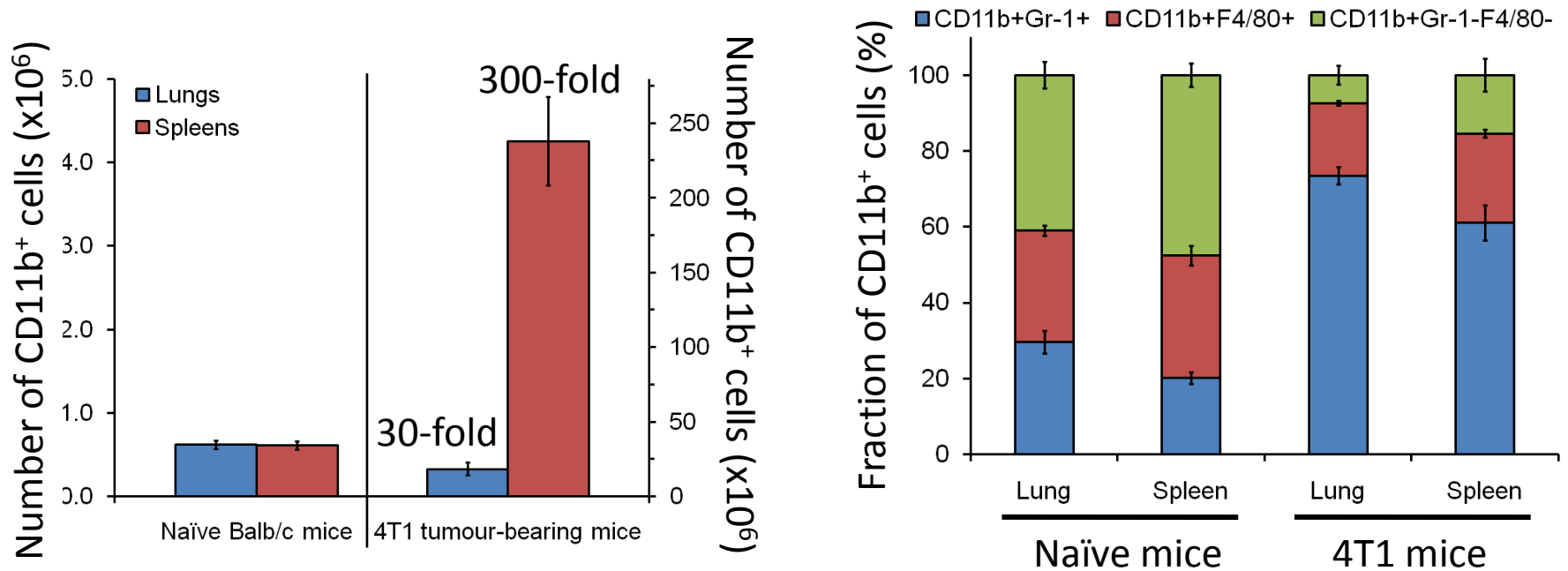
# CD11b+ cells in the pre-metastatic niche

- Cells expressing **CD11b** accumulate in metastatic target organs of mice bearing human breast or murine mammary tumours
- CD11b+ cells can include myeloid cells (neutrophils, monocytes, macrophages), natural killer cells, and a subset of B cells



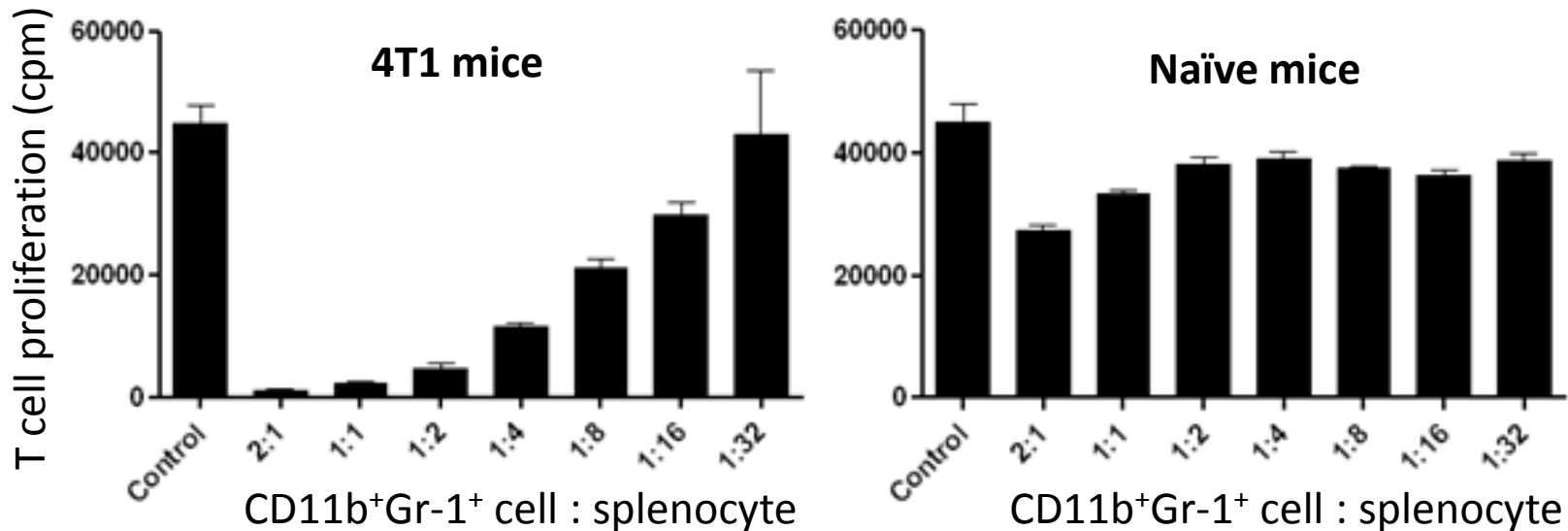
- Induction of CD11b+ cells in the lungs increases growth of metastatic tumour cell foci

# CD11b+Gr-1+ cells



- The majority of CD11b<sup>+</sup> cells that accumulate in the lungs and spleen with time after 4T1 tumour implant co-express Gr-1
- **CD11b<sup>+</sup>Gr-1<sup>+</sup> cells are a heterogeneous mixture of immature myeloid cells**
- CD11b<sup>+</sup>Gr-1<sup>+</sup> cells may include myeloid-derived suppressor cells (**MDSCs**)

# Myeloid-derived suppressor cells (MDSCs)



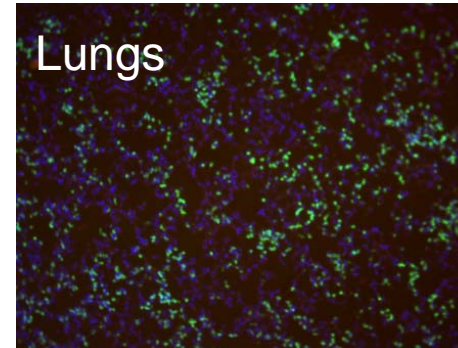
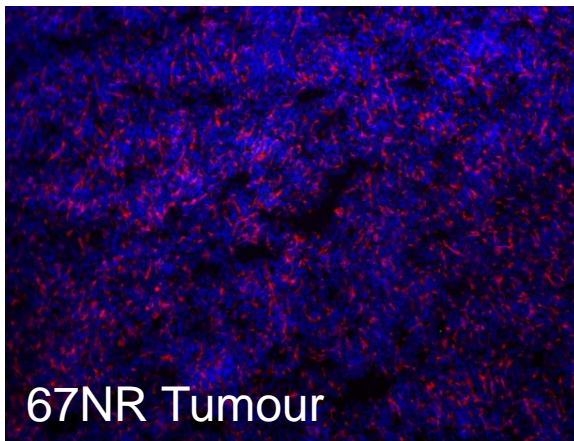
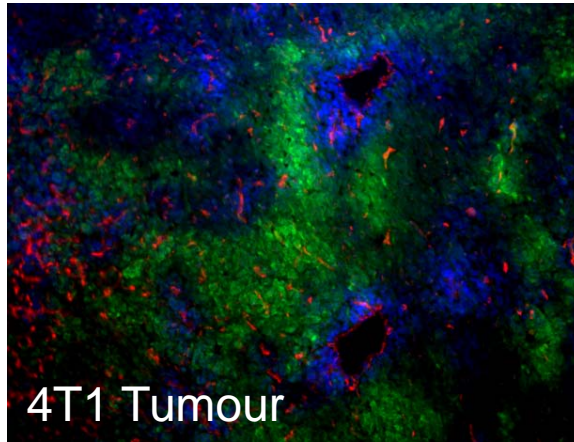
- CD11b<sup>+</sup>Gr-1<sup>+</sup> cells in the spleen and lungs of 4T1 tumour-bearing mice are immunosuppressive MDSCs
- CD11b<sup>+</sup>Gr-1<sup>+</sup> cells in the spleen and lungs of naïve mice have minimal immunosuppressive function
- Inhibition of immunosuppressive function to decrease metastatic growth?

# MDSC accumulation is related to poorly oxygenated (hypoxic) cells in the primary tumour

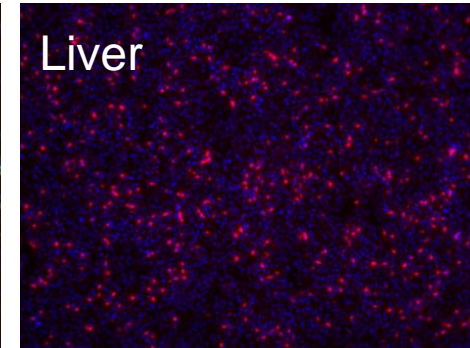
**CD31  
(vasculature)**

**Pimonidazole  
(hypoxic  
cells)**

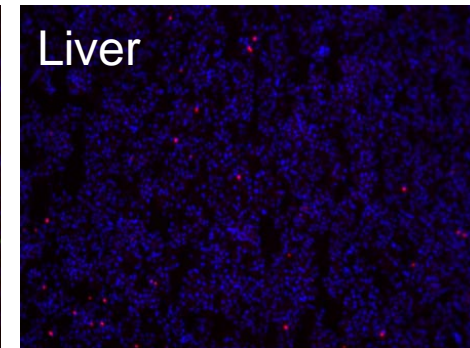
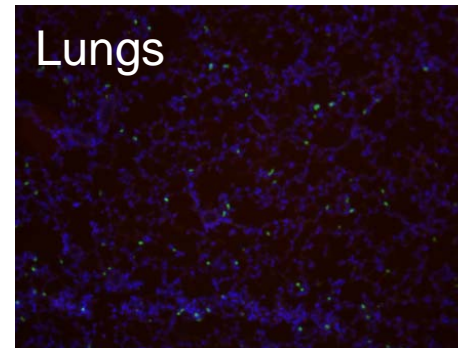
**Hoechst  
33342  
(perfusion)**



**CD11b<sup>+</sup> cells  
DAPI (DNA)**

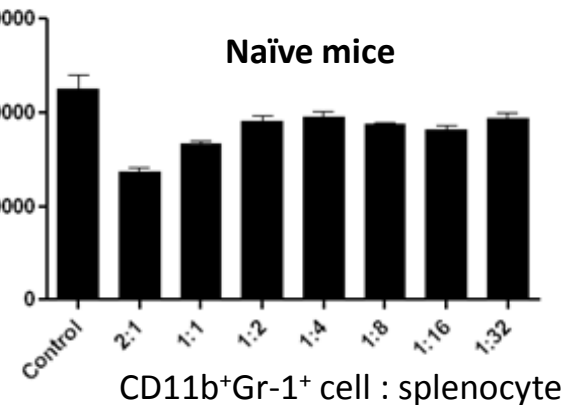
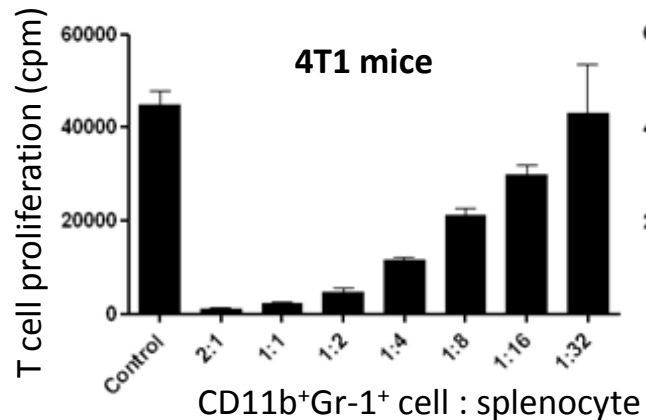
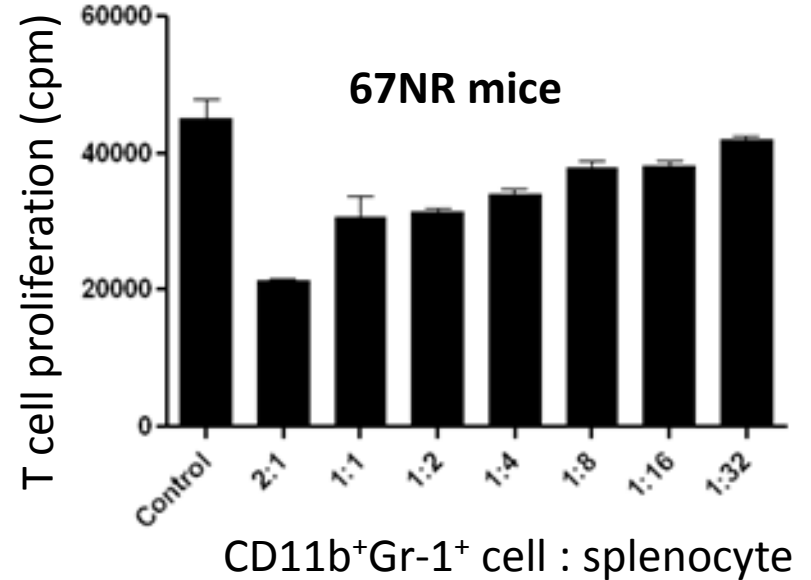
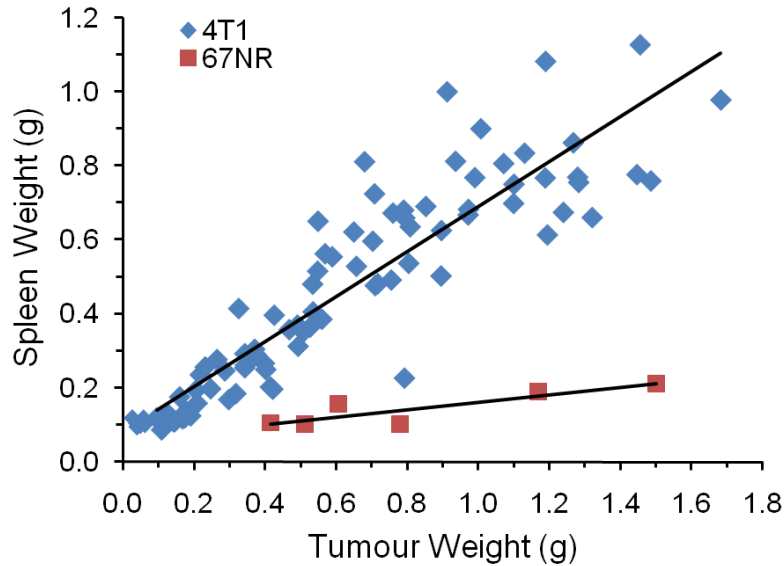


**CD11b<sup>+</sup> cells  
DAPI (DNA)**

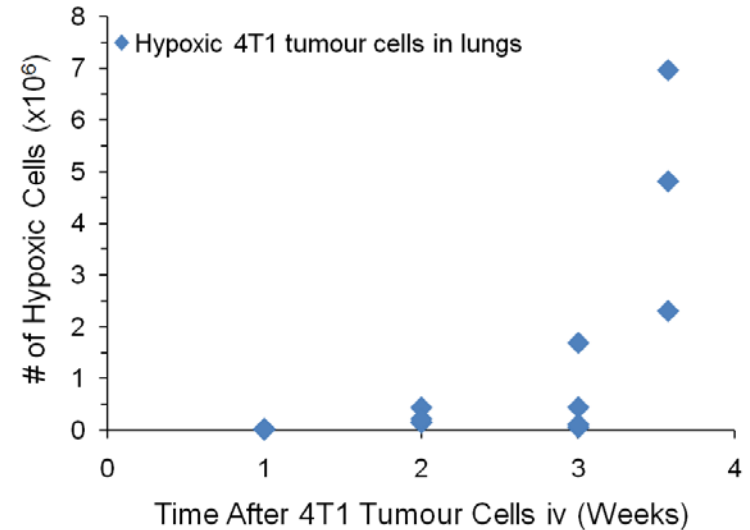
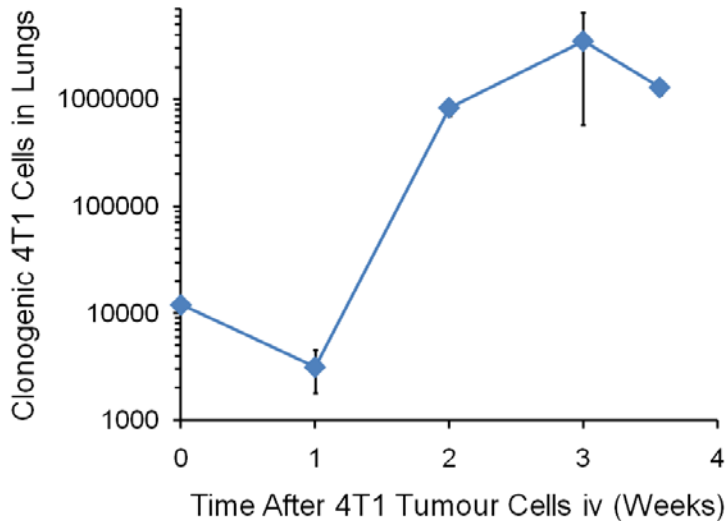


- 4T1 and 67NR tumour cell lines were originally derived from the same spontaneous tumour
- Very different metastatic propensities

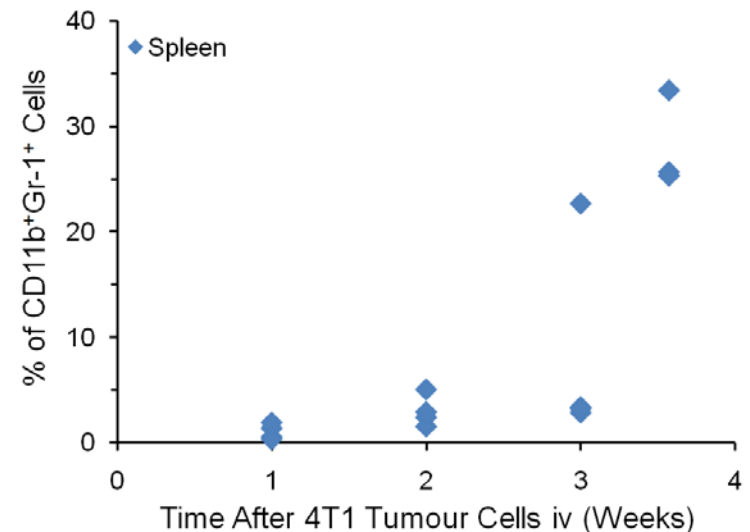
# 67NR tumours do not induce MDSC expansion or accumulation in tissues



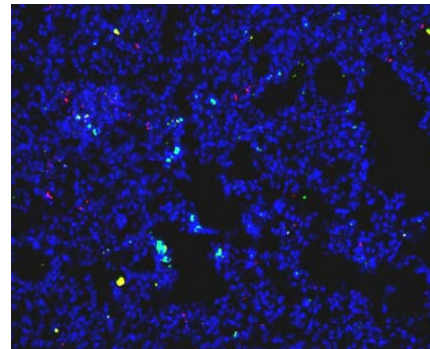
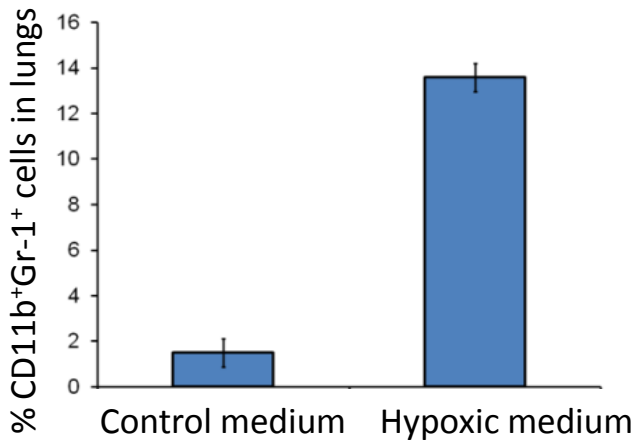
# MDSC accumulation with hypoxia



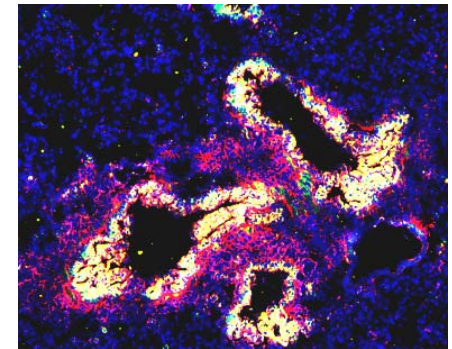
- Hypoxia develops in 4T1 tumour cell foci 3-3.5 weeks after iv tumour cell injection
- MDSCs increase in spleen and lungs after substantial numbers of tumour cells have become **hypoxic**
- High numbers of (normoxic) 4T1 tumour cells are **insufficient** to induce MDSCs
- Targeting hypoxic tumour cells to decrease metastatic growth?



# Hypoxia-induced secreted proteins



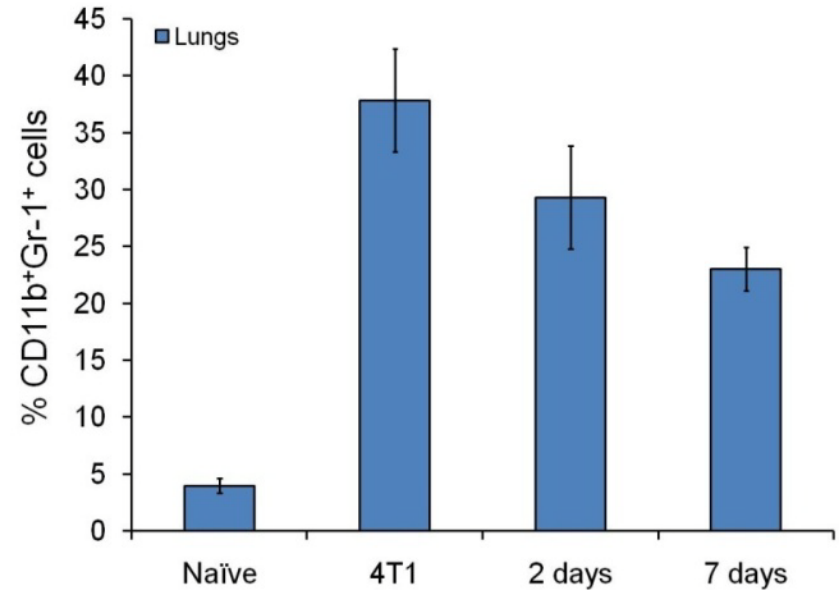
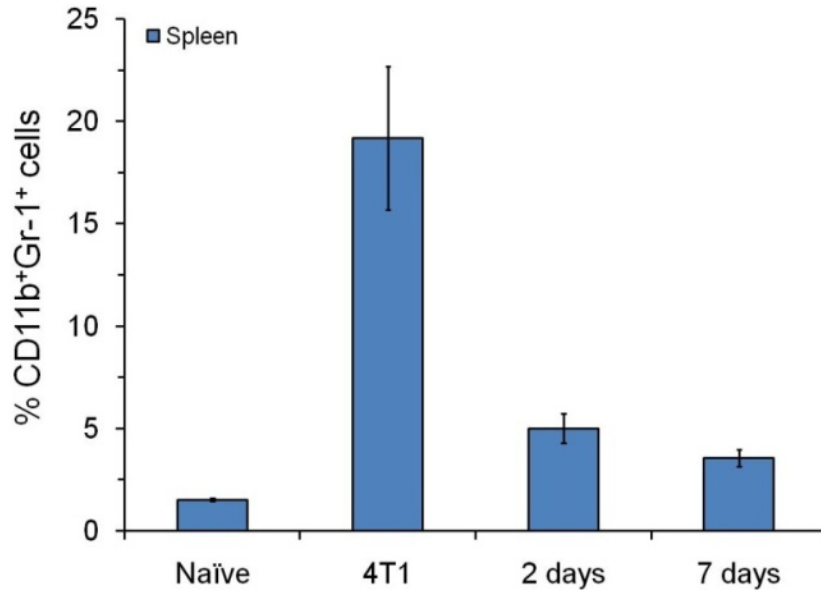
Normoxic medium



Hypoxic medium

- MDSC accumulation can be induced by injections of conditioned medium from hypoxic tumour cells
- Proteomics approach to identify and quantify proteins secreted by hypoxic tumour cells (identified **129** for 4T1 cells)
- Fibronectin                      Kaplan *et al.*, *Nature*, 2005; Erler, Bennewith *et al.*, *Cancer Cell*, 2009
- Lysyl oxidase like-4                      Wong *et al.*, *Proc Natl Acad USA*, 2011
- Matrix metalloproteinase-9                      Hiratsuka *et al.*, *Cancer Cell*, 2002
- Therapeutic targets to decrease MDSC accumulation and decrease metastatic growth?

# CD11b<sup>+</sup>Gr-1<sup>+</sup> cell stability



- 4T1 tumours excised 2 weeks after implantation
- CD11b<sup>+</sup>Gr-1<sup>+</sup> cells decrease rapidly in spleen
  - splenomegaly is rapidly reversed
- CD11b<sup>+</sup>Gr-1<sup>+</sup> cells are more stable in lungs
- CD11b<sup>+</sup>Gr-1<sup>+</sup> cells may remain in metastatic target organs for extended periods of time

# Conclusions

- Immunosuppressive MDSCs accumulate in spleens, lungs, and livers of mice with metastatic 4T1 tumours
  - MDSC do not accumulate in mice with non-metastatic 67NR tumours
- MDSC accumulation requires hypoxic tumour cells
- MDSC accumulation can be initiated by conditioned medium from hypoxic tumour cells
- MDSCs may remain in metastatic target organs for extended periods of time after accumulation
  - May pre-dispose tissues to subsequent metastatic colonization
- **We are currently developing therapeutic strategies to inhibit MDSC accumulation and function in metastatic target organs to decrease metastatic tumour growth**