# The tumor microenvironment can vary with anatomical site to affect responses to therapy

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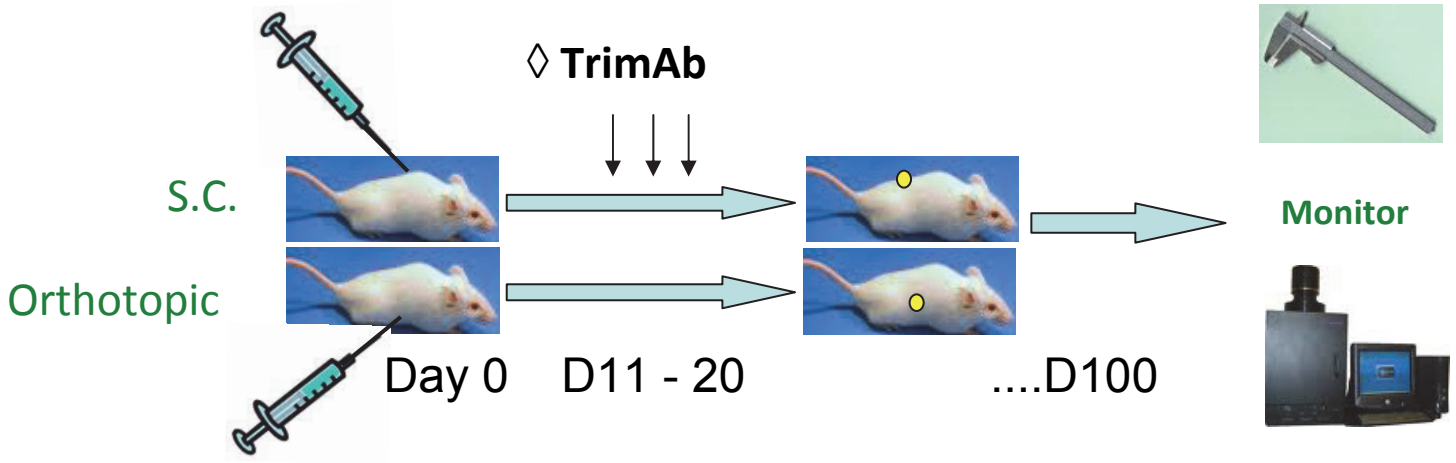
Presenter disclosure information:
No relationships or financial interests to disclose

# Part 1 - Immunotherapy is less effective against orthotopic tumors

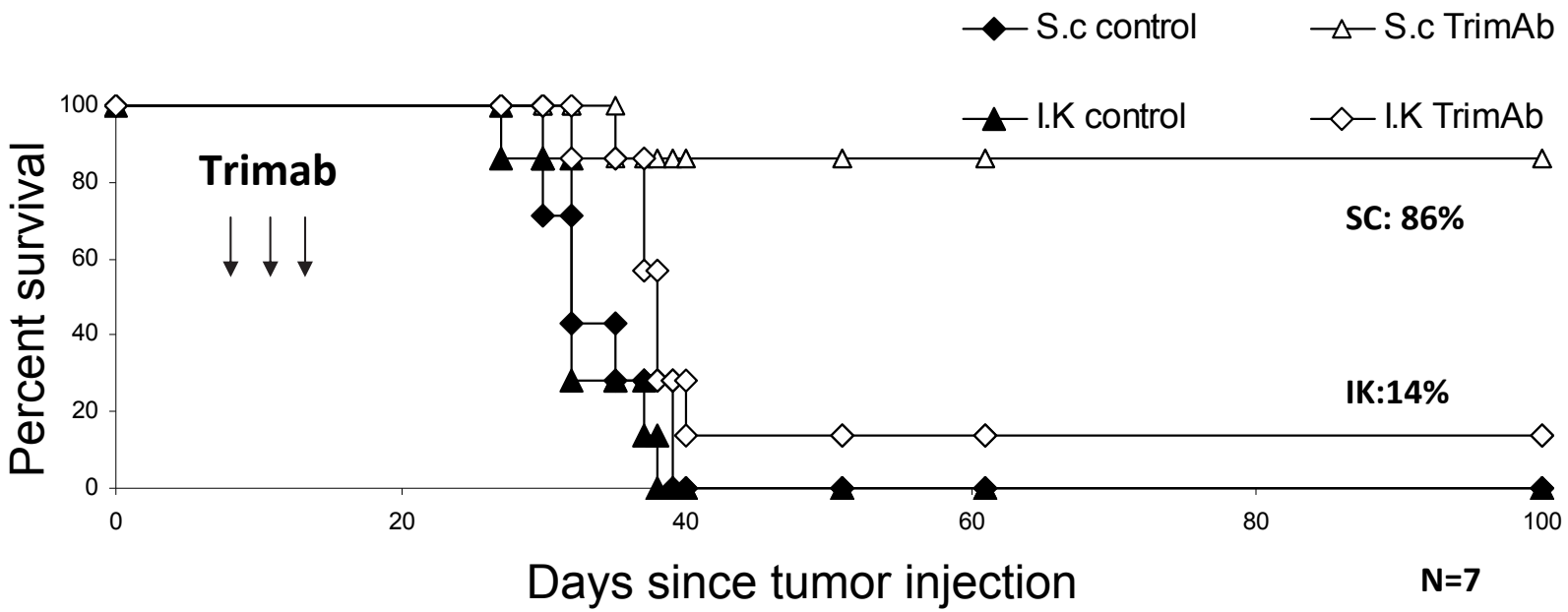
# Tumor models and therapy

- Tumors: Renca ($Ch^+Luc^+$), CT26, RM-1




- Three agonist antibodies (Trimab)
  - DR5 (apoptosis)
  - CD40 (antigen presentation)
  - CD137 (4-1BB, T cell activation)
- $CD8^+$ T cells and IFN-$\gamma$ are essential

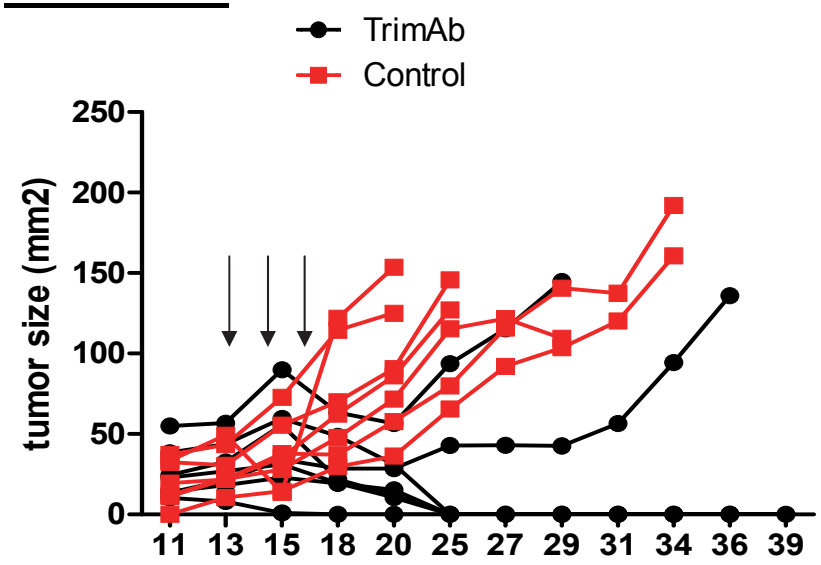
# Orthotopic renal tumors respond less than subcutaneous tumors to Trimab therapy



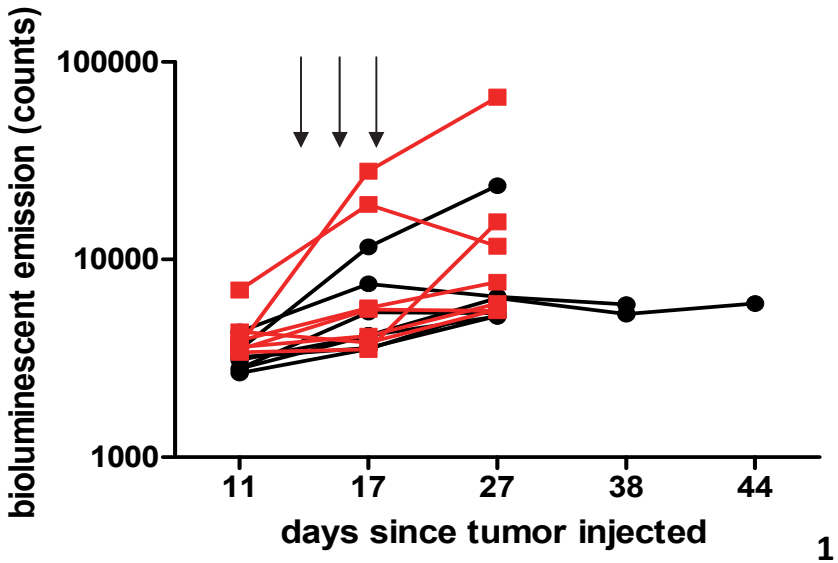

**1 experiment representative of 3**

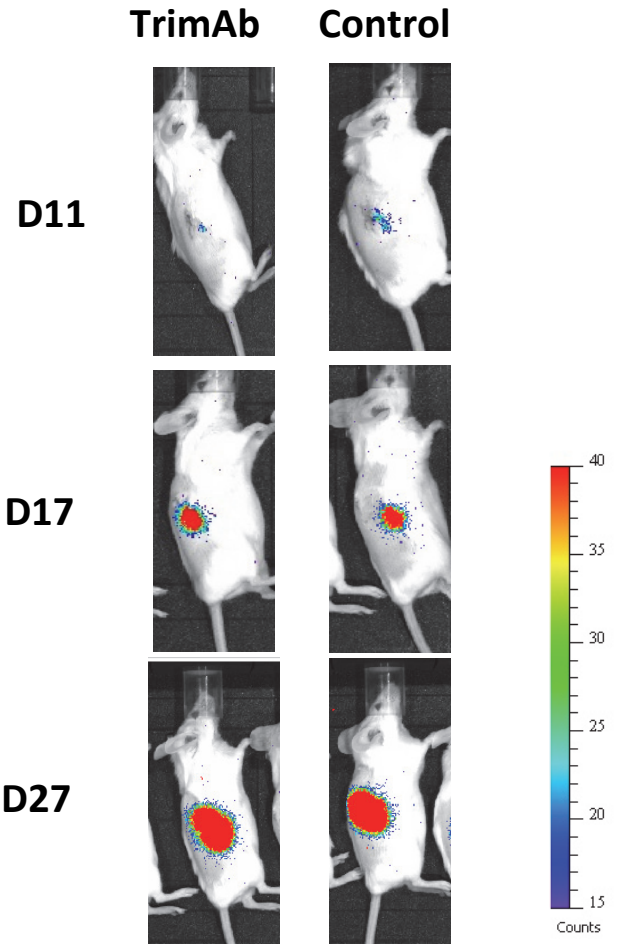
# Kidney tumors respond poorly to Trimab

➢ **SC tumors**




➢ **IK tumors**

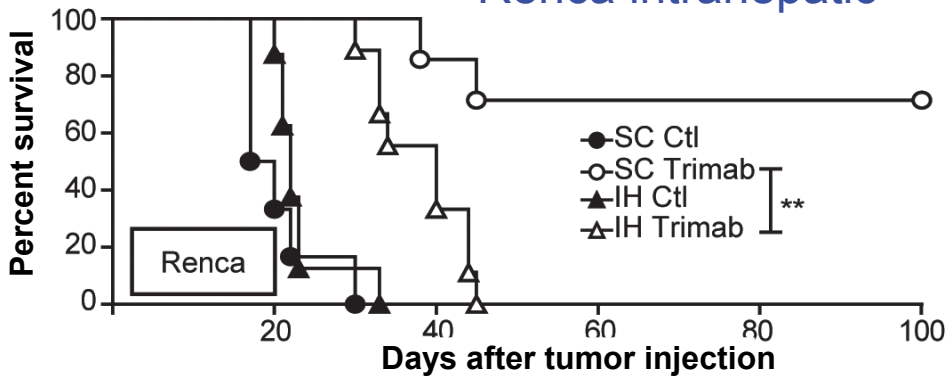


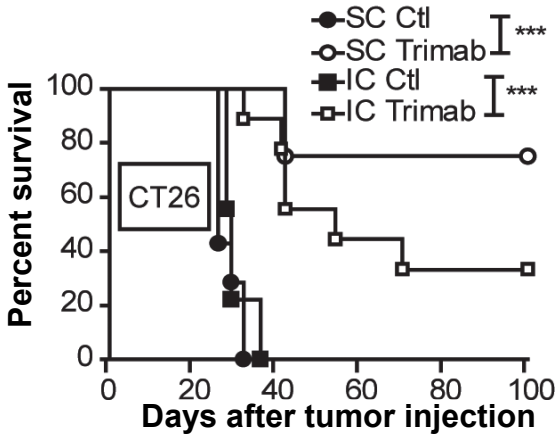

1 experiment representative of 3

# Differential responses to Trimab are also observed in other tumor models

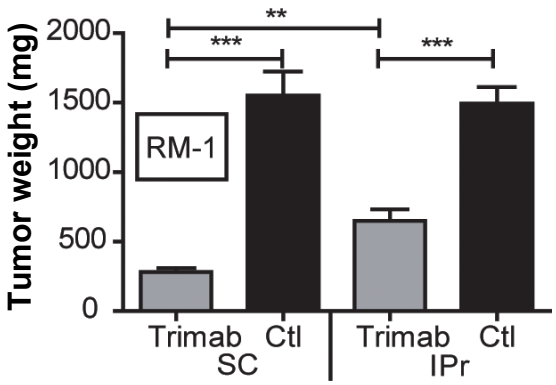
Renca intrahepatic




CT26 colon cancer




RM-1 prostate cancer

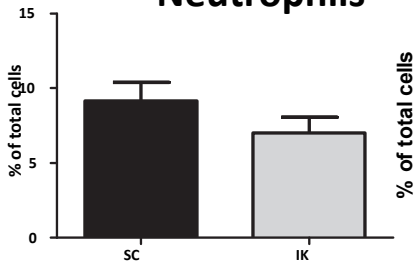


# Part 2 - The microenvironment of tumors varies with anatomical site

# No differences in frequency of immune cells in kidney tumors compared to subcutaneous tumors

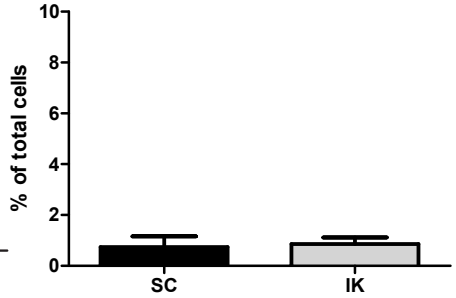
TCRβ$^-$/Ly6G$^+$/CD11b$^+$

**Neutrophils**



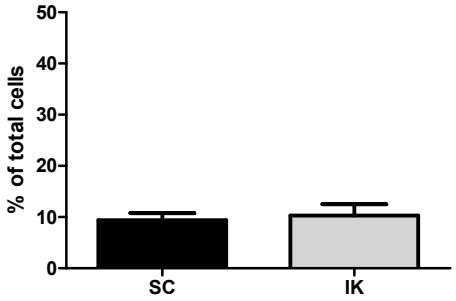

TCRβ$^-$/CD19$^+$

**B cells**



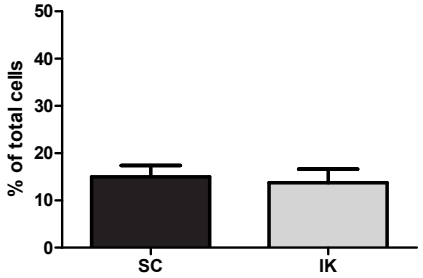

TCRβ$^-$/F4/80$^+$/CD11b$^+$

**Macrophages**



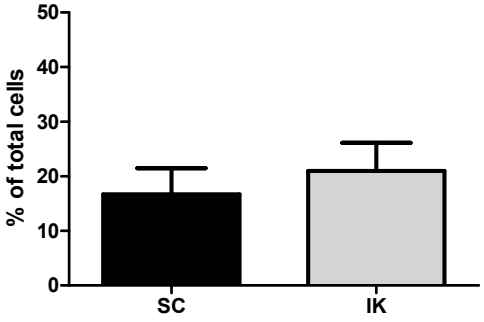

TCRβ$^-$/CD11c$^+$

**DCs**



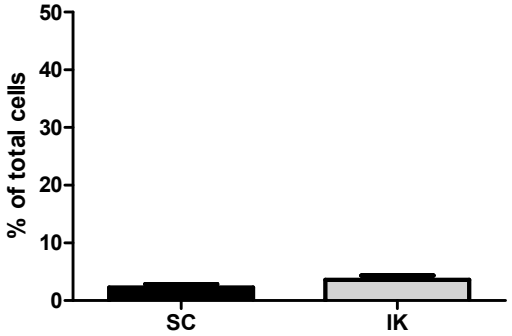

TCRβ$^+$/CD4$^+$

**CD4 Tcells**



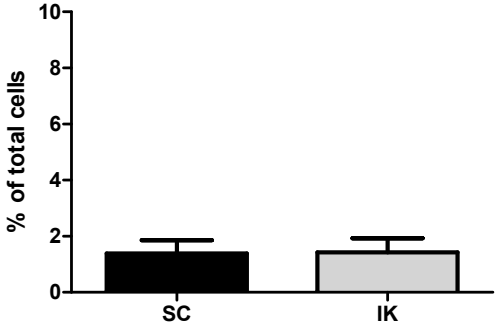

TCRβ$^+$/CD8$^+$

**CD8 T cells**



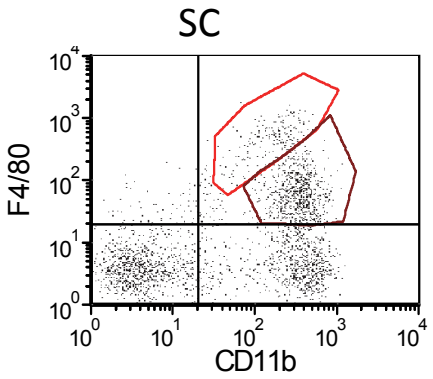

TCRβ$^+$/CD8$^-$/CD25$^+$/FR4$^{high}$

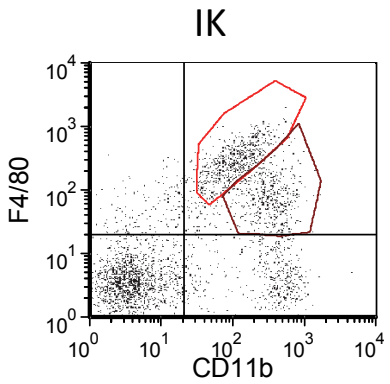
**TReg**

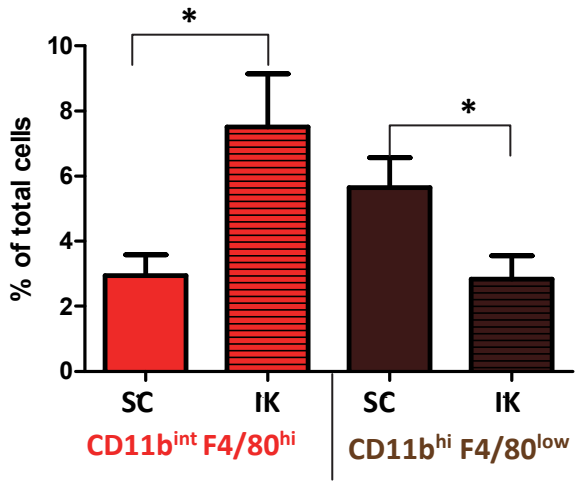



**4 independent experiments pooled**

# More F4/80$^{hi}$ macrophages are present in kidney tumors

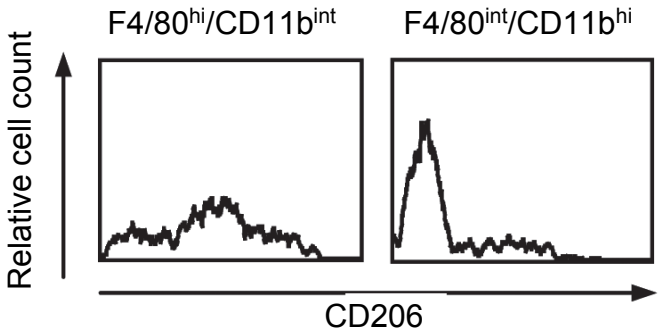


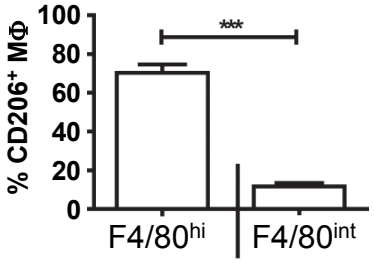




(>5 mice per group, > 5 expts)

## F4/80$^{hi}$ macrophages express CD206, mannose receptor

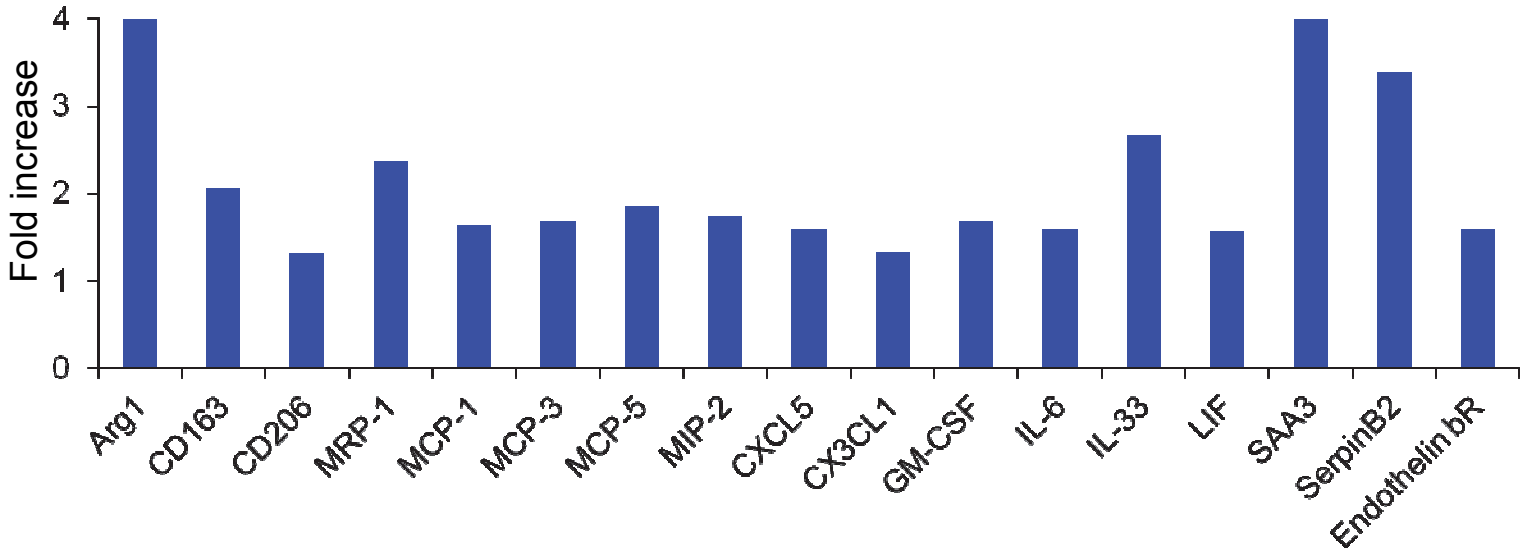


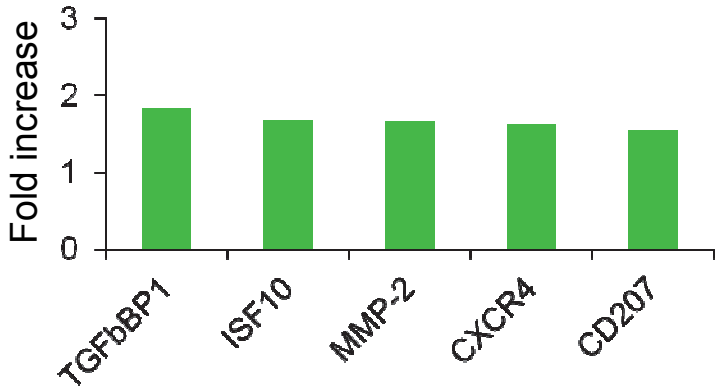

(5 mice per group, 3 expts)

# Immune gene expression differences between kidney and subcutaneous tumors (DNA microarray)

Upregulated in orthotopic renal tumors




Upregulated in S.C. tumors



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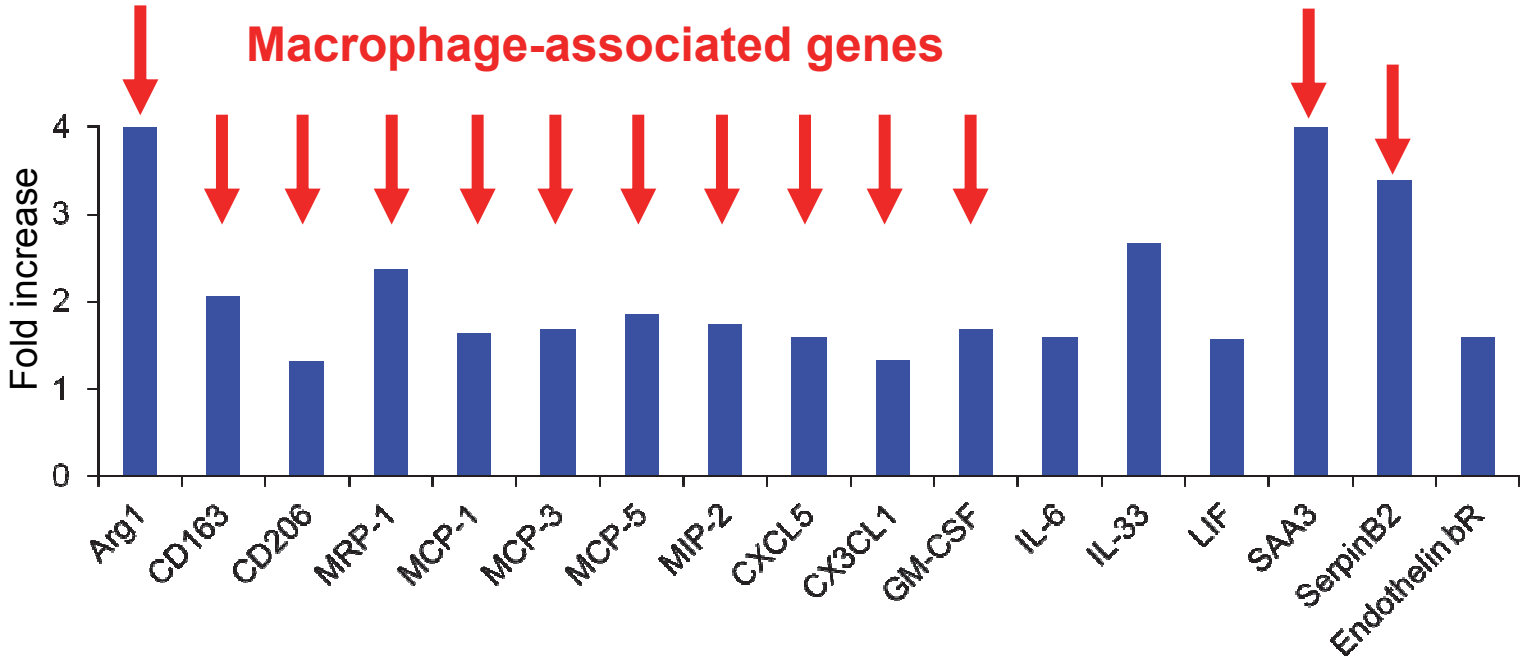


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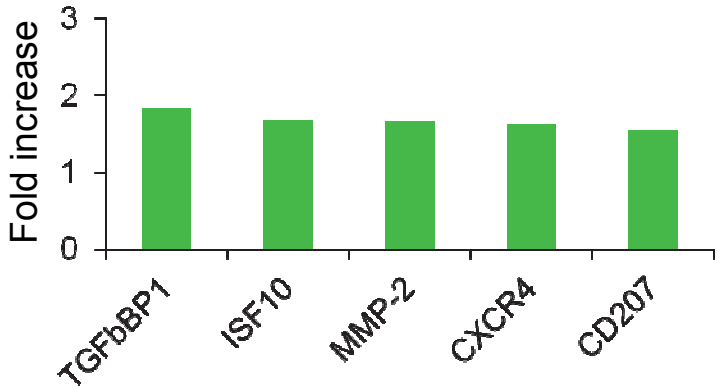

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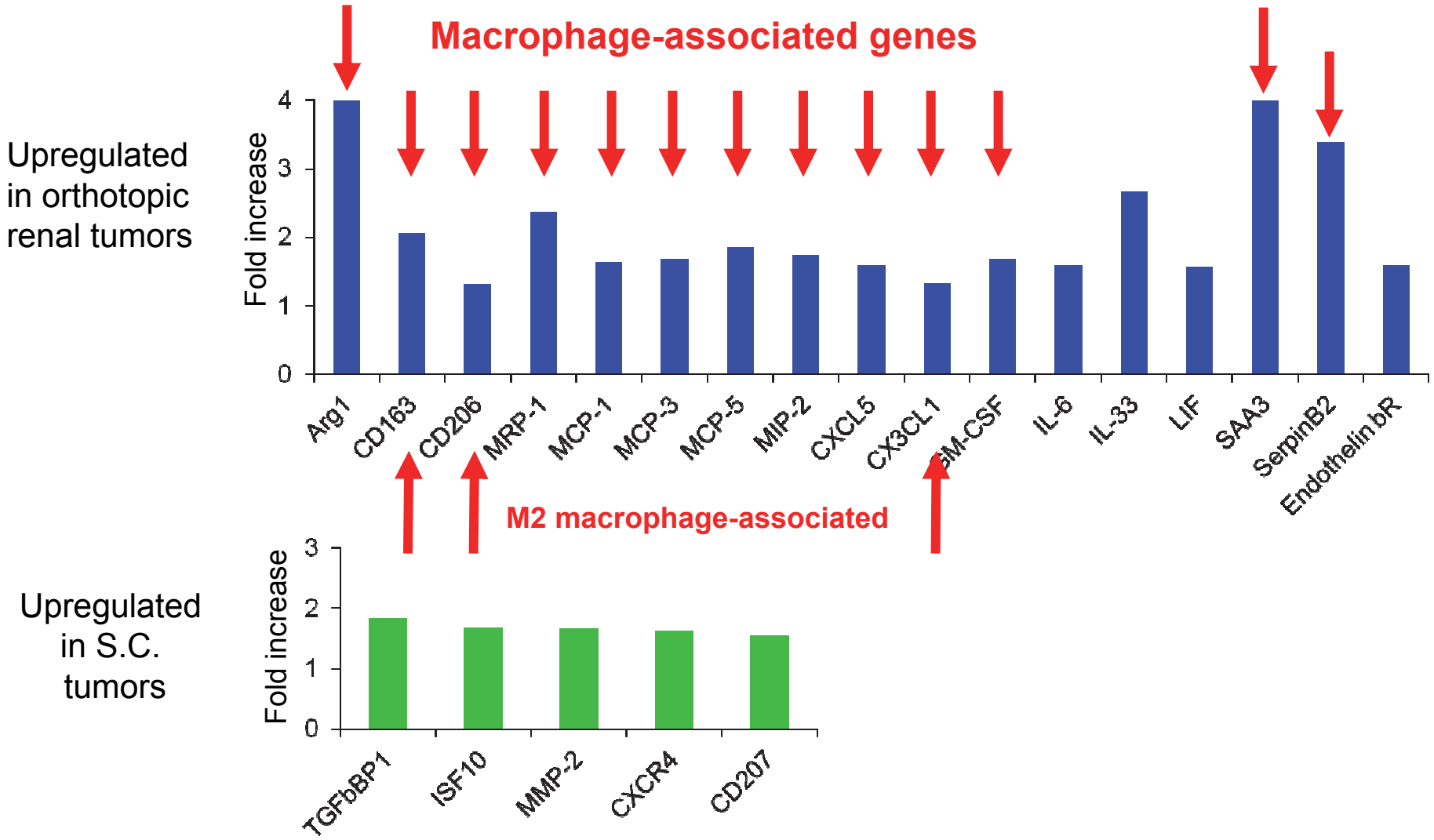

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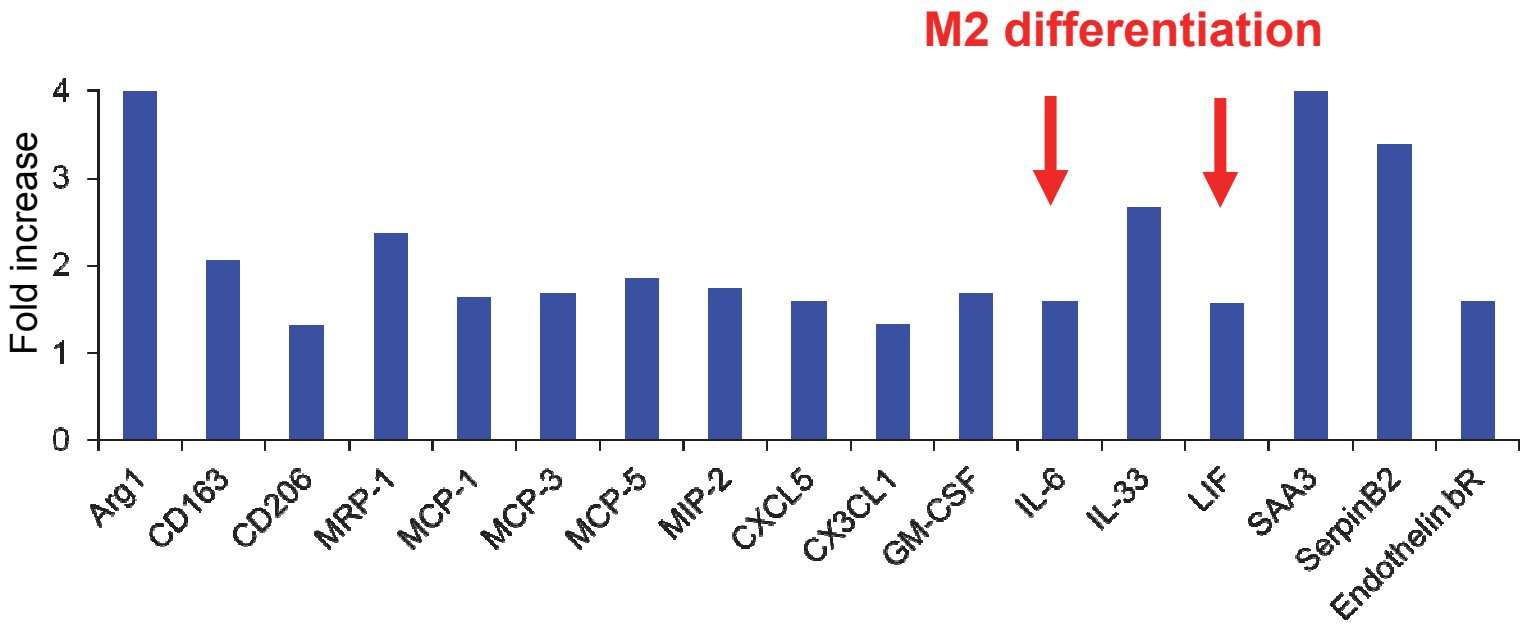


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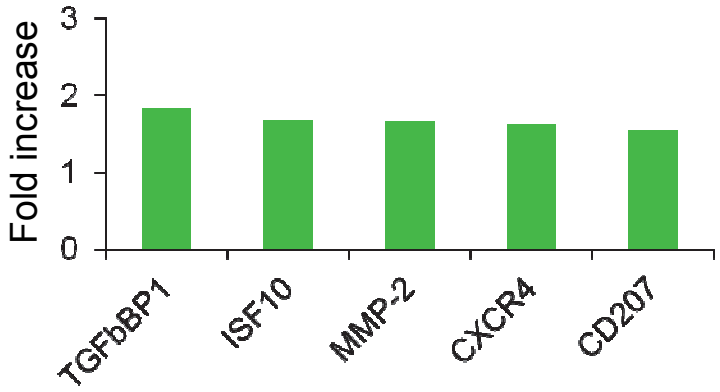

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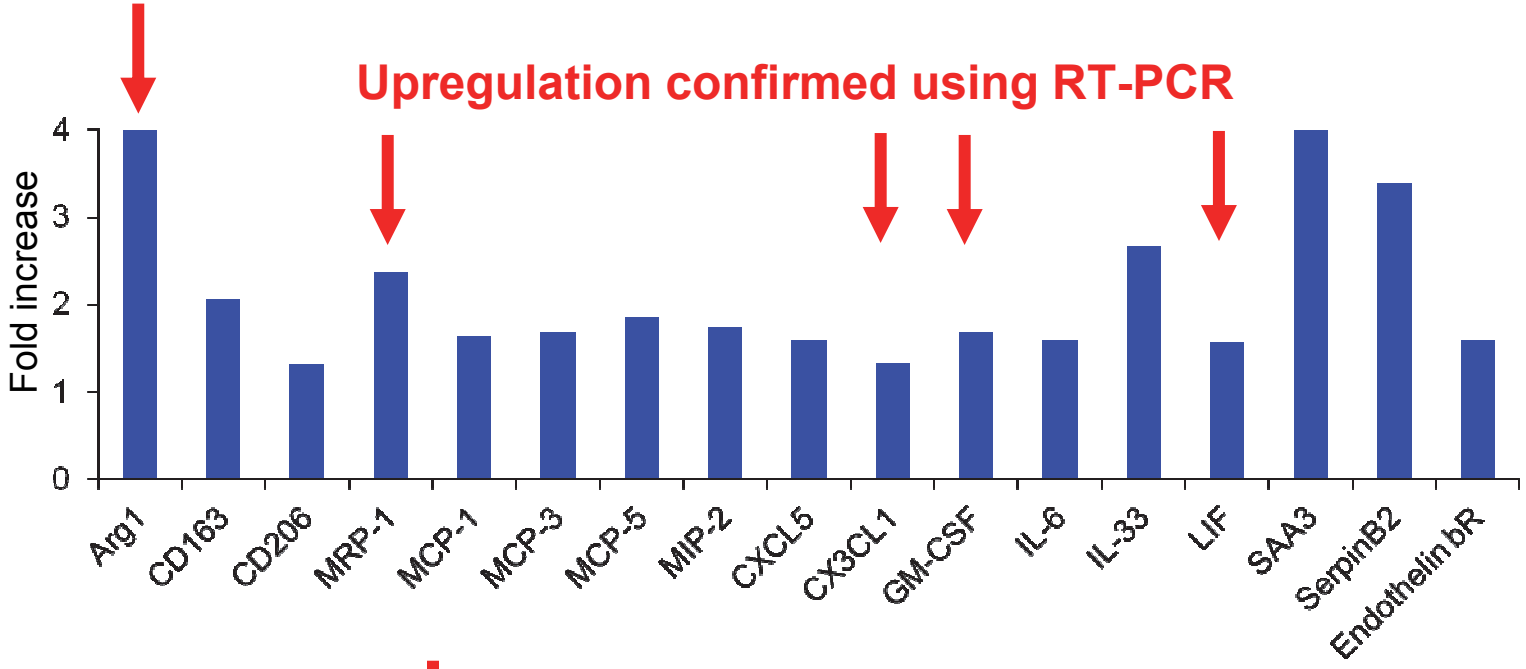
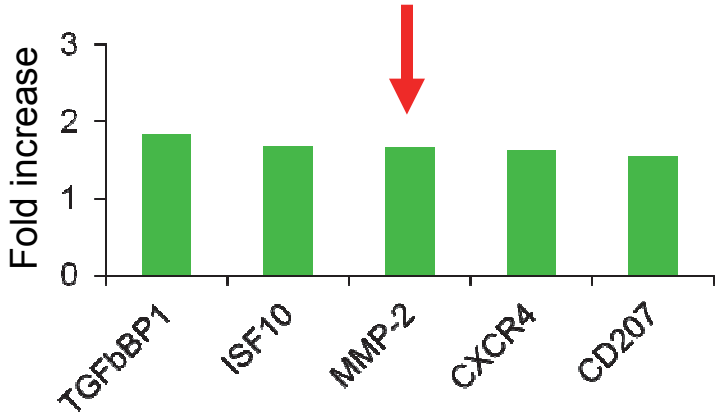


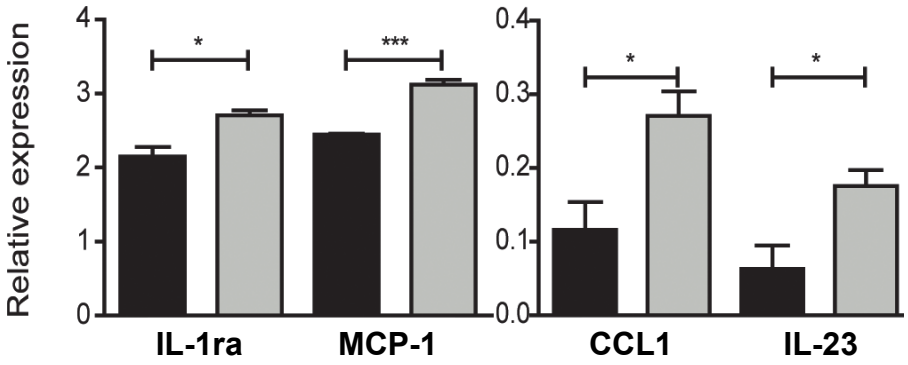

Upregulated in S.C. tumors

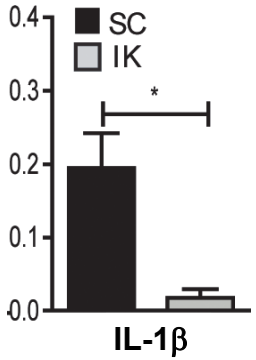


# Differences in protein expression determined using protein array

Higher in kidney tumors

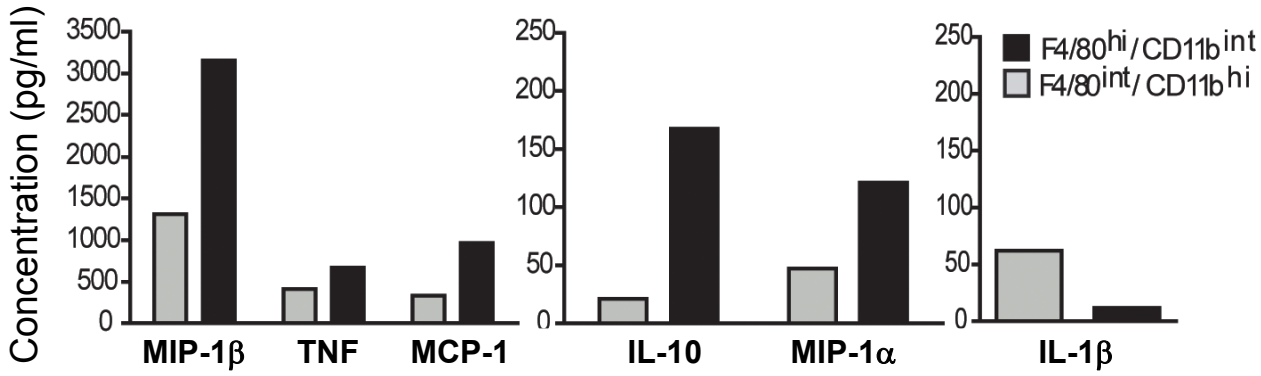
Higher in SC tumors



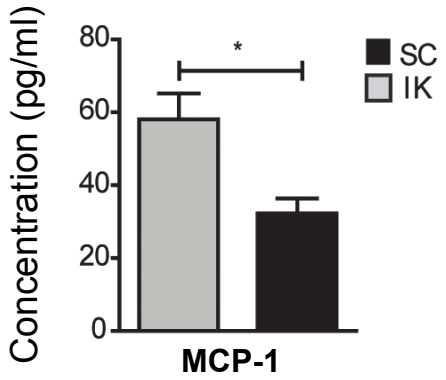

N = 3

# Cytokines and chemokines secreted by macrophages isolated from tumors



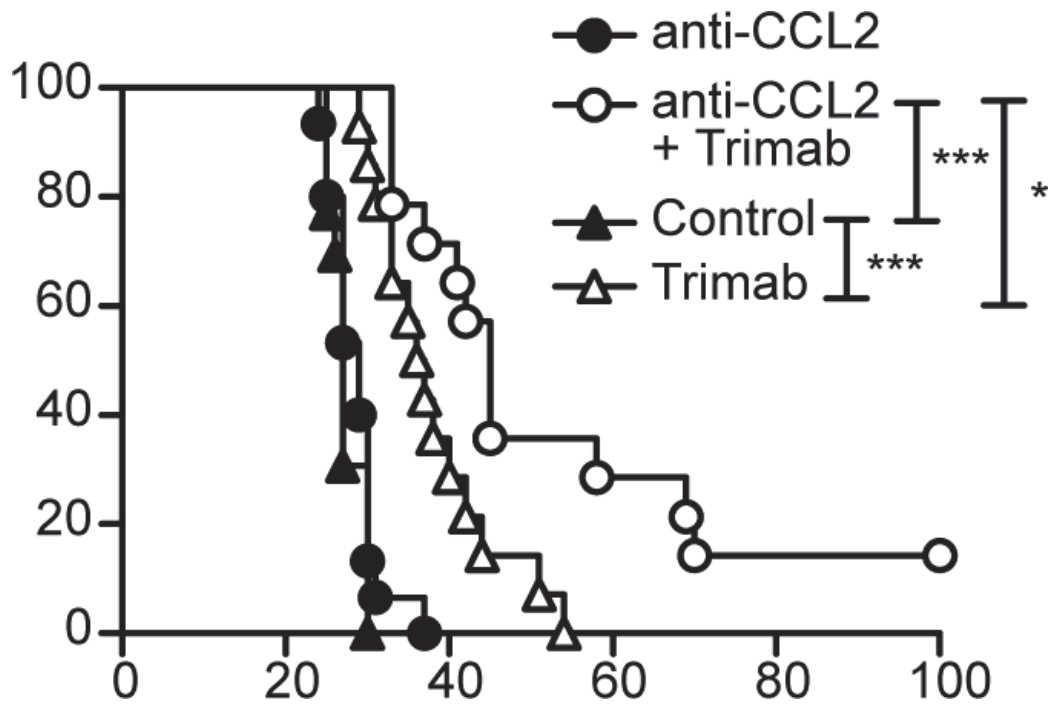

2 expts pooled

# Higher level of CCL2 is present in the serum of mice bearing kidney tumors



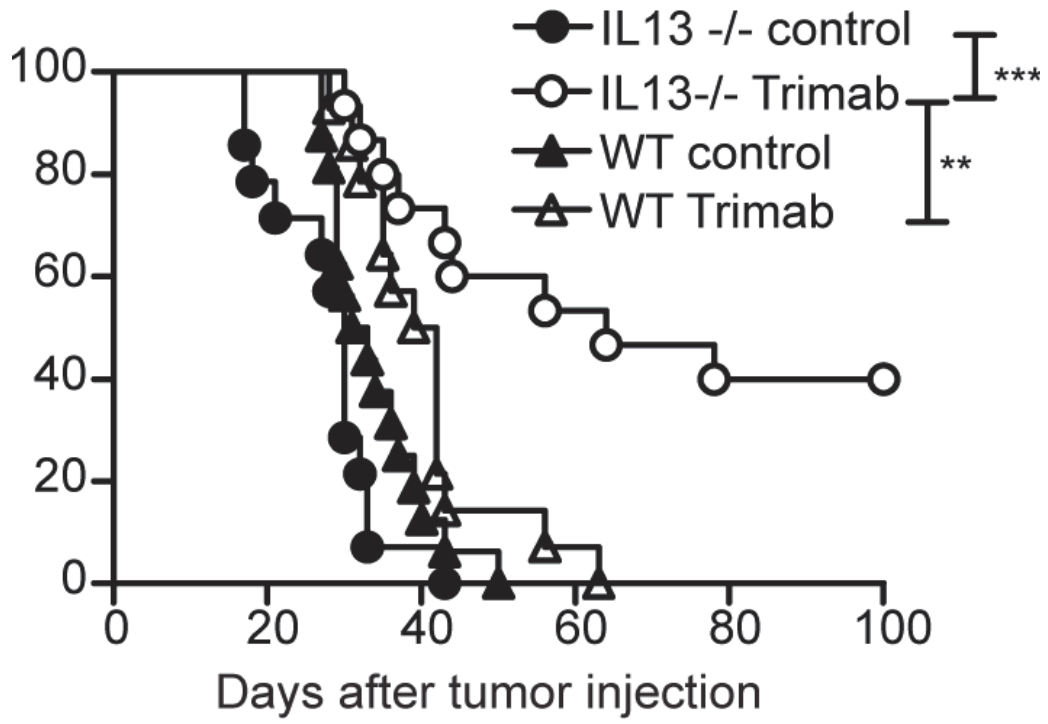

N = 5

# Trimab works better against kidney tumors when CCL2 is neutralized




2 experiments pooled
N = 13-14 per group

# Trimab works better against kidney tumors in mice deficient in IL-13




2 experiments

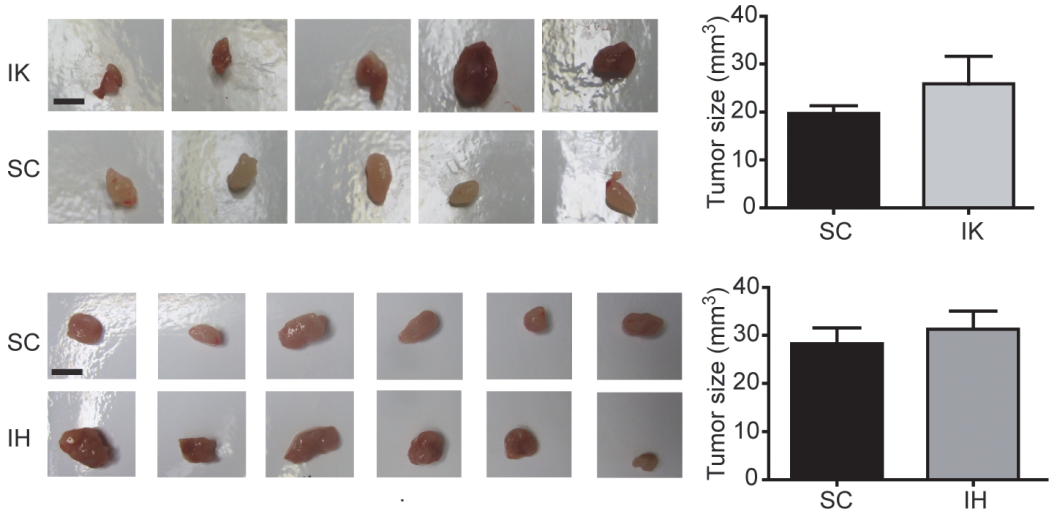
# Summary Part 2 – markers of M2 macrophages and Th2 cells are associated with orthotopic tumors

- Kidney tumors
  - $F4/80^{Hi}$
  - CD206
  - CD163
  - MCP-1
  - MCP-3
  - MCP-5
  - MIP-1$\alpha$
  - MIP-1$\beta$
  - IL-10
  - IL-13
  - IL-23
- Subcutaneous tumors
  - IL-1$\beta$
  - MMP2
  - CD207

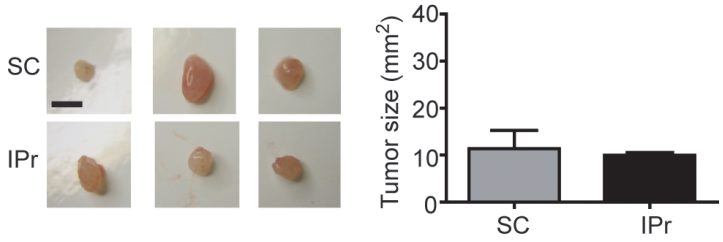
# Part 3 - Tumors in different sites differ intrinsically

# Orthotopic tumors are similar in size to subcutaneous tumors

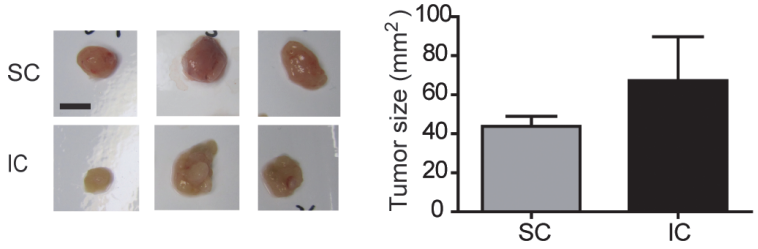
Renca
(kidney and liver)




RM-1 prostate

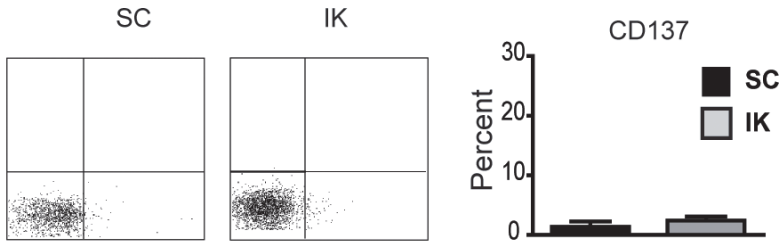



CT26 cecum

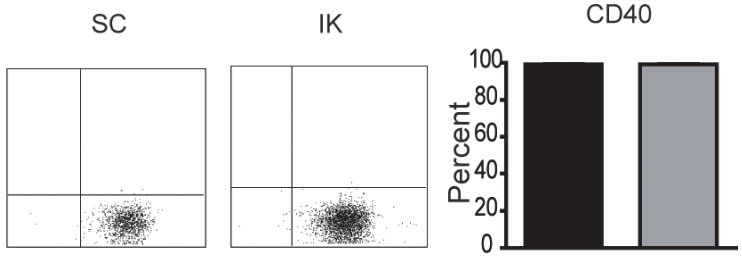


# Subcutaneous and orthotopic kidney tumor cells express similar levels of Trimab target molecules

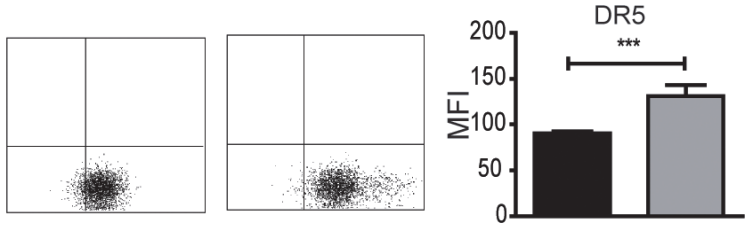
CD137




CD40

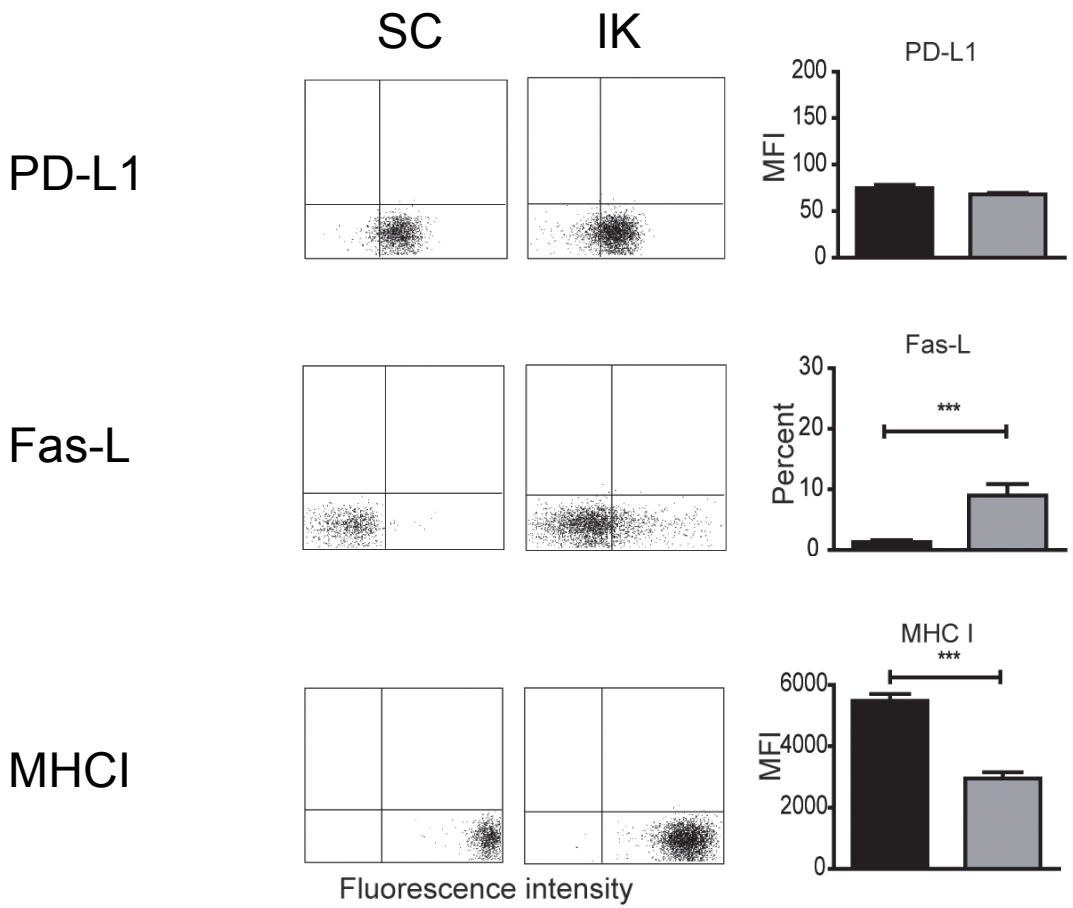



DR5



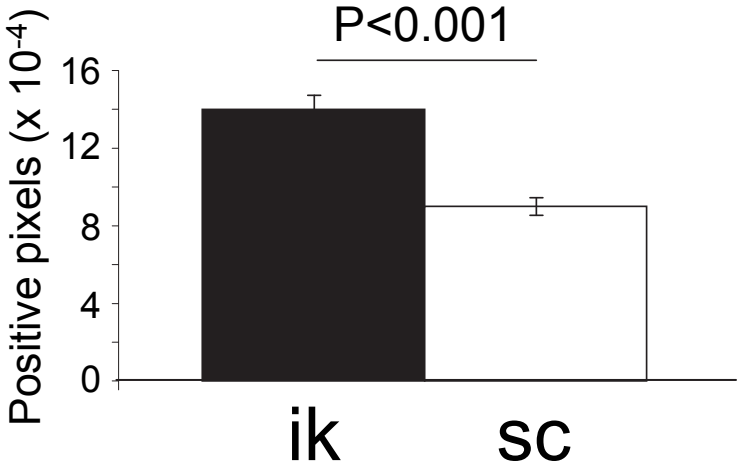

2 experiments
N = 10 per group

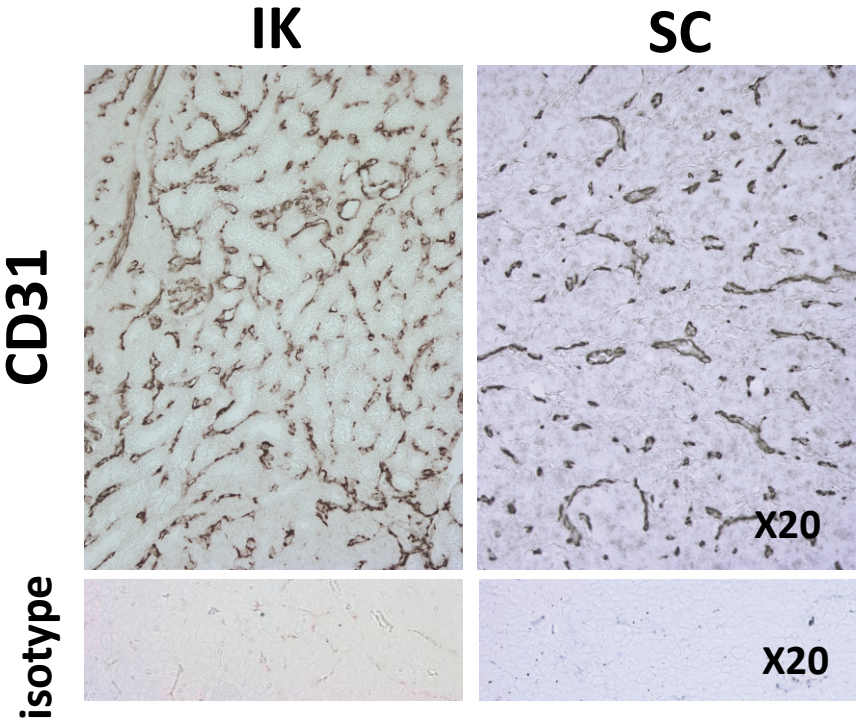
# Expression of some immune markers varies on SC and IK tumor cells




2 experiments
N = 10 per group

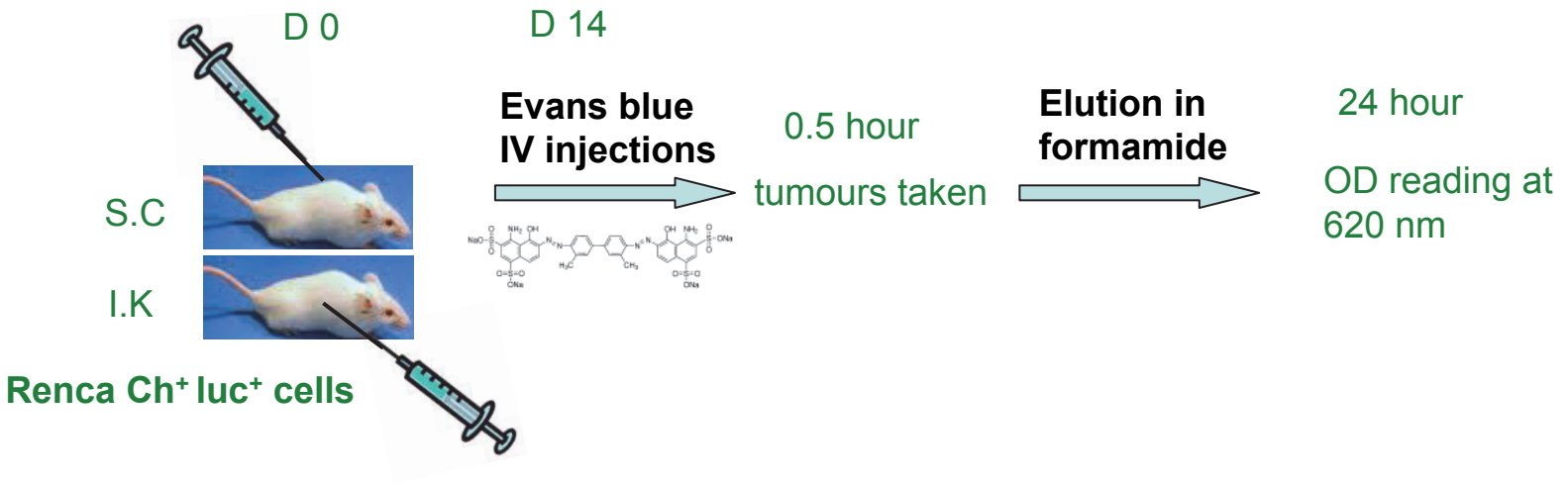
# Kidney tumors are more highly vascularized

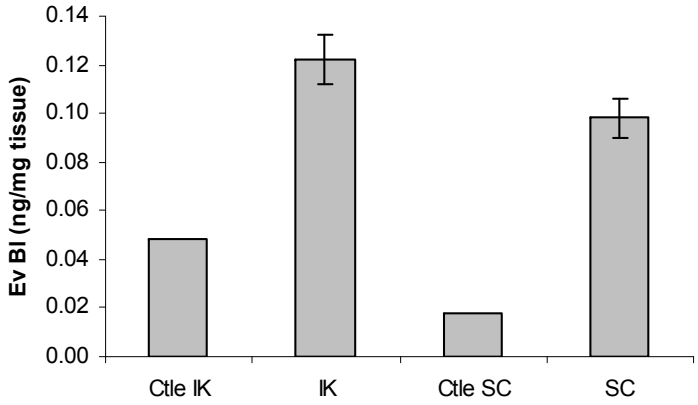



(5 tumors, 10 fields/tumor)

# Similar vascular permeability in SC and IK tumors

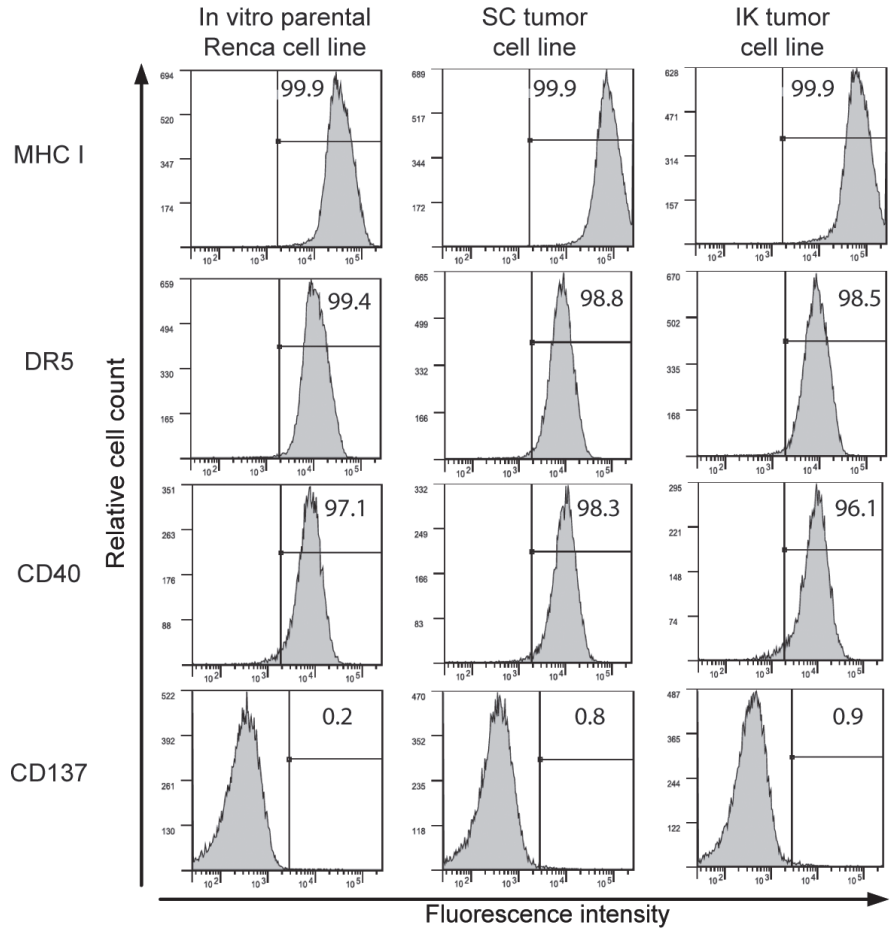



**1 experiment of 3**

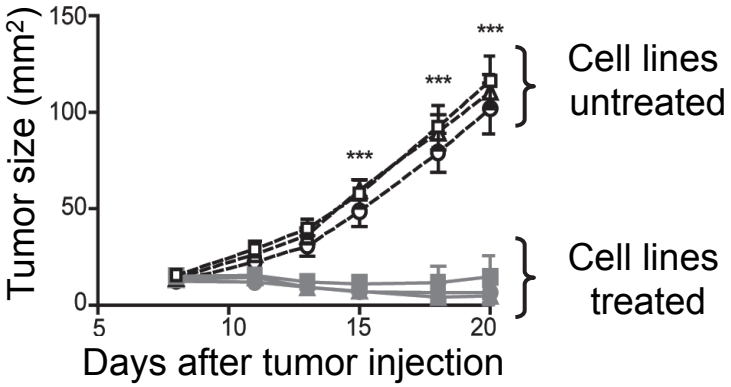
# Part 4 - The tissue can instruct the development of the tumor microenvironment

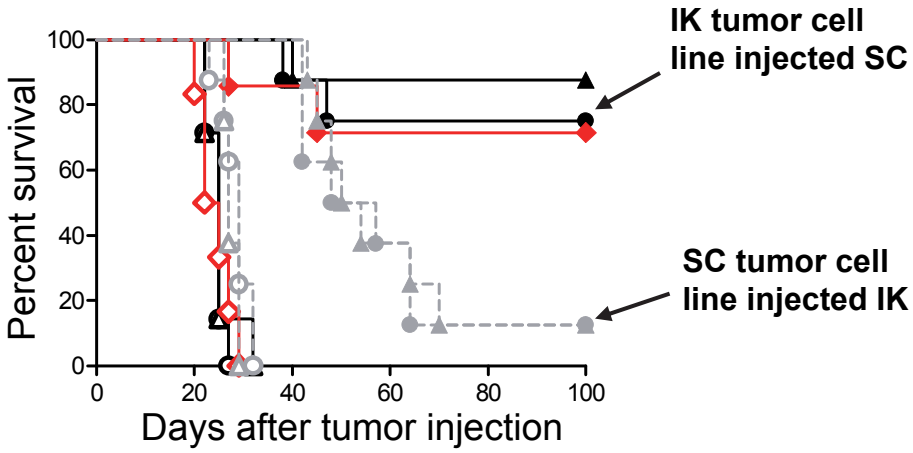
# Kidney and SC tumor cells have a similar phenotype following culture ex vivo



# Cell line from kidney tumors, when injected SC, responds to Trimab

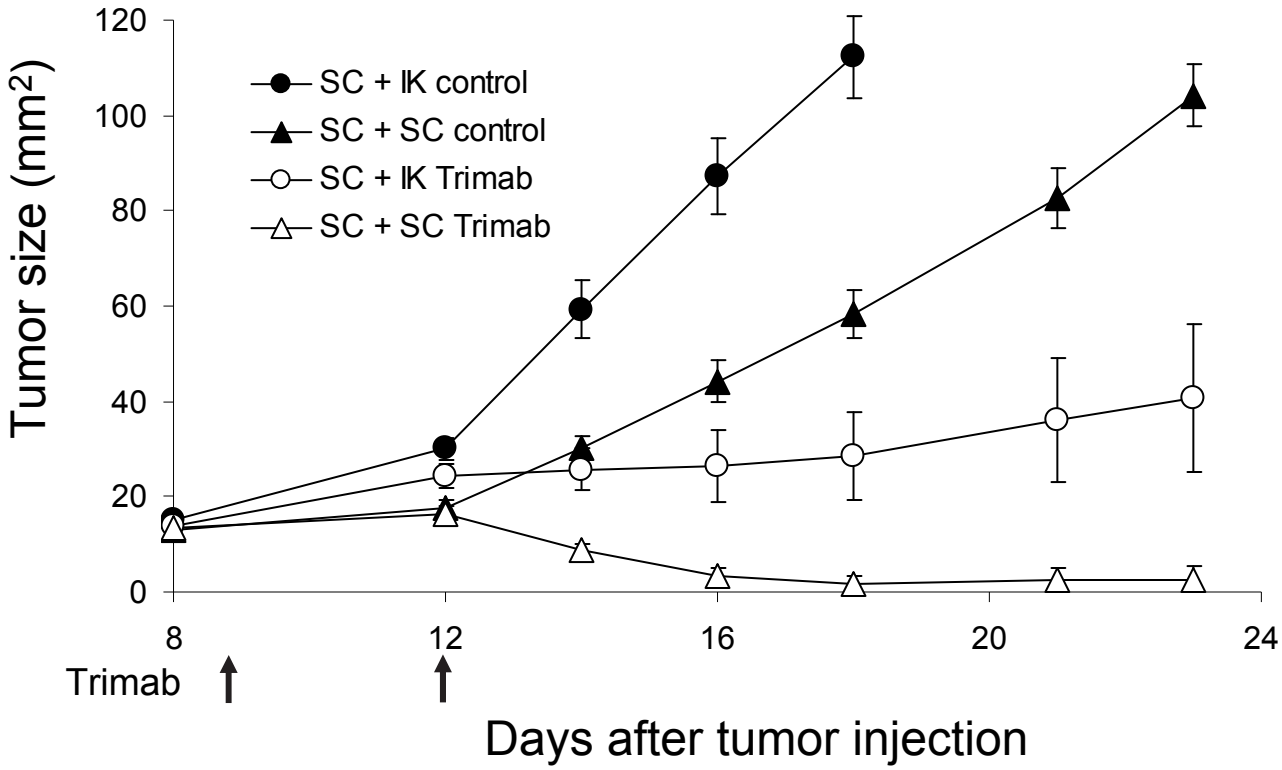
(cross over experiment)



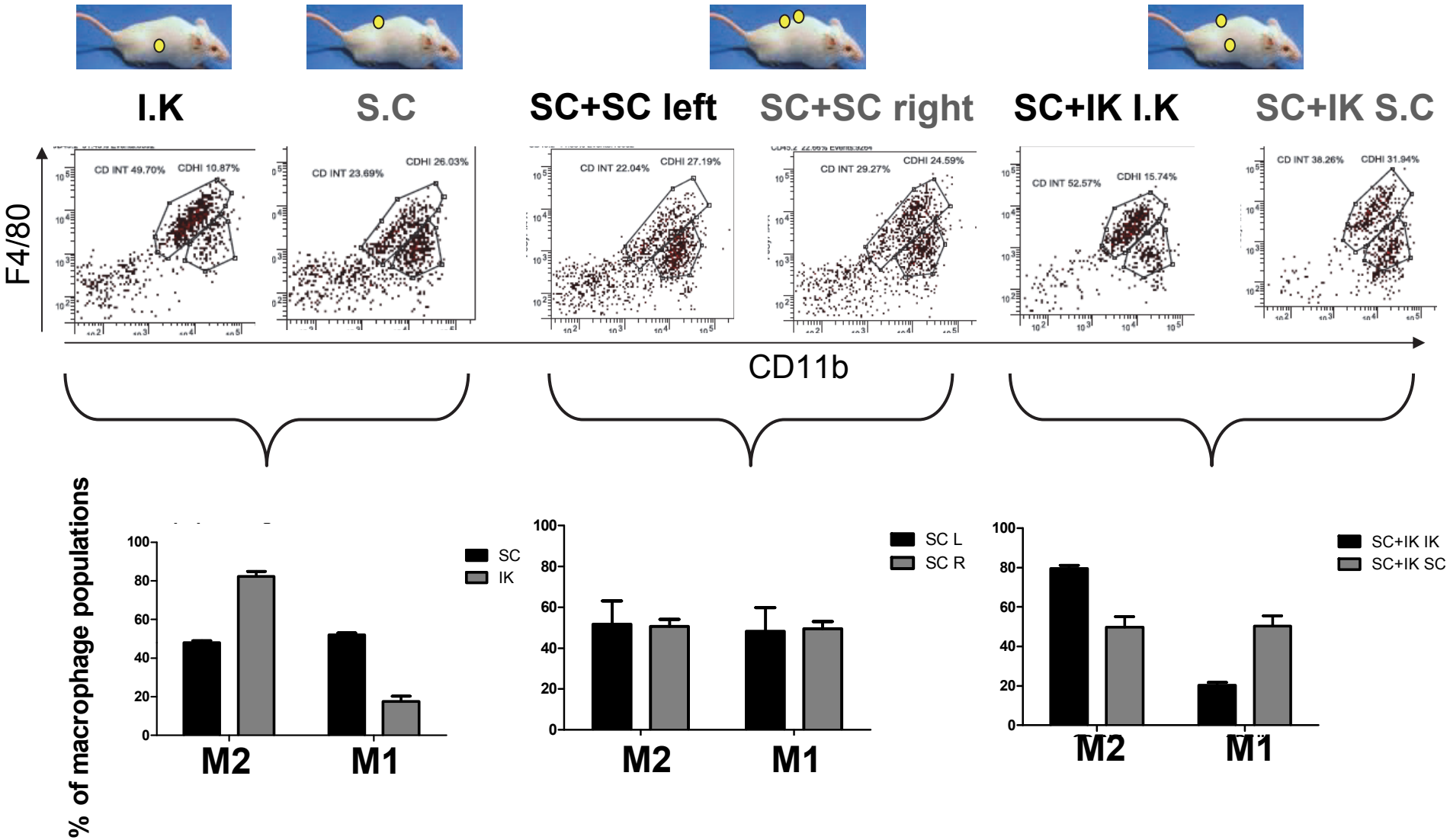
# Part 5 - Orthotopic tumors can affect the response of subcutaneous tumors to immunotherapy

# SC tumors respond less to therapy when IK tumors are present



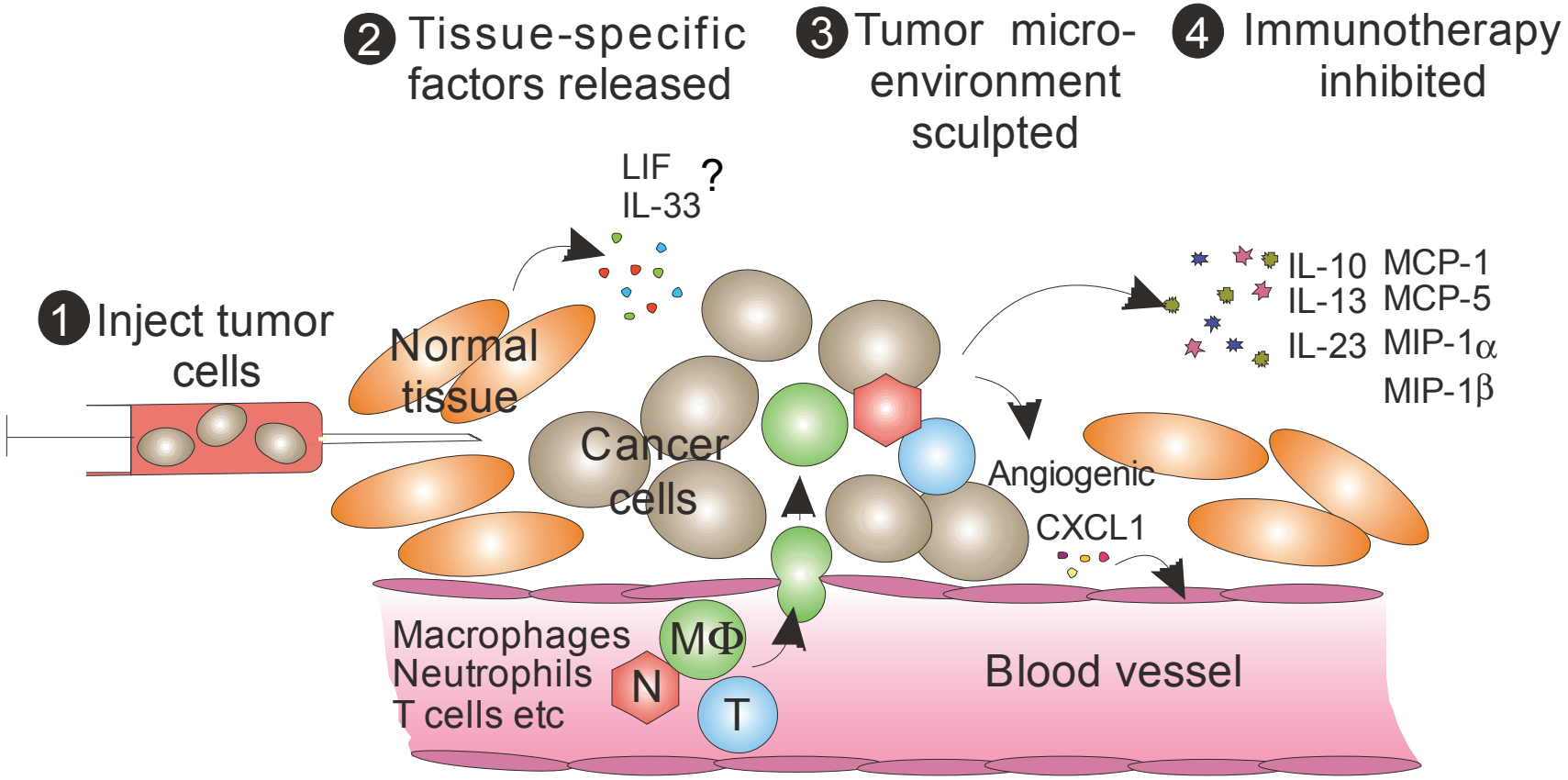

n = 9-11
2 expts

# Similar macrophage profile in mice bearing 1 or 2 tumours



# Points to make

- Immunotherapy is less effective against orthotopic tumors
- The microenvironment of tumors varies with anatomical site
- Tumors in different sites differ intrinsically
- The tissue can instruct the development of the tumor microenvironment
- Orthotopic tumors can affect the response of subcutaneous tumors to immunotherapy


2 Tissue-specific factors released
3 Tumor micro-environment sculpted
4 Immunotherapy inhibited
LIF
IL-33
?
1 Inject tumor cells
Normal tissue
Cancer cells
IL-10
IL-13
IL-23
MCP-1
MCP-5
MIP-1α
MIP-1β
Angiogenic
CXCL1
Macrophages
Neutrophils
T cells etc
MΦ
N
T
Blood vessel

# Implications

- Tumor model is important when investigating immunotherapies
- Tumor location needs to be considered when deciding best therapy
- Identification of tissue instructing factors may enable early intervention strategies
- Removing immunosuppressive tumors may permit responses of other tumors to immunotherapy

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