

# **Investigating the differential response to immunotherapy of orthotopic tumors compared to subcutaneous tumors**

**Michael Kershaw**

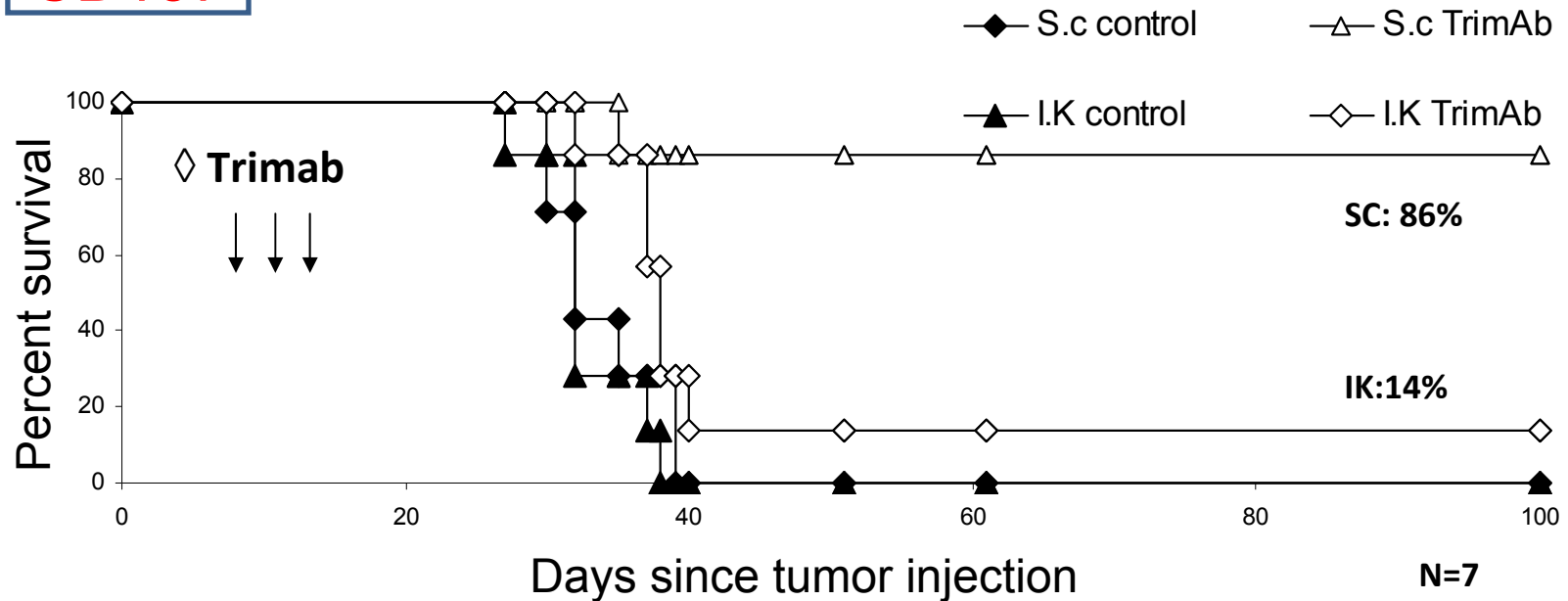
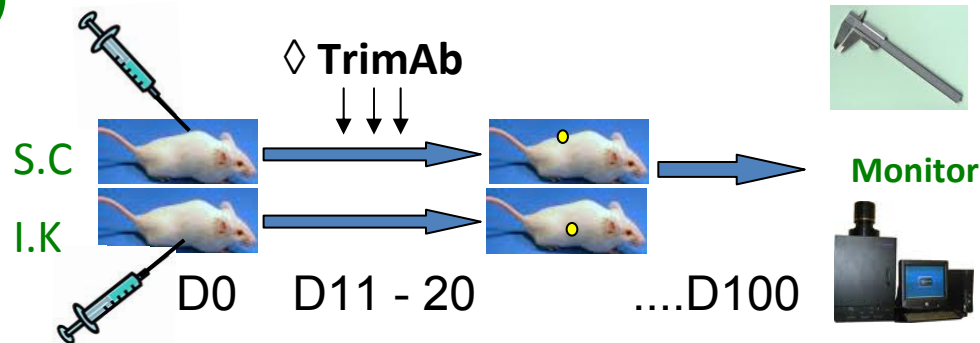
**Peter MacCallum Cancer Center, Melbourne,  
Australia**

The logo for Peter MacCallum Cancer Center, featuring the name "Peter Mac" in a blue cursive font and a stylized "iir" in red and blue block letters to the right.

# Subcutaneous Renca tumors respond well to Trimab when compared to kidney tumors

Renca (Ch<sup>+</sup>/Luc<sup>+</sup>)

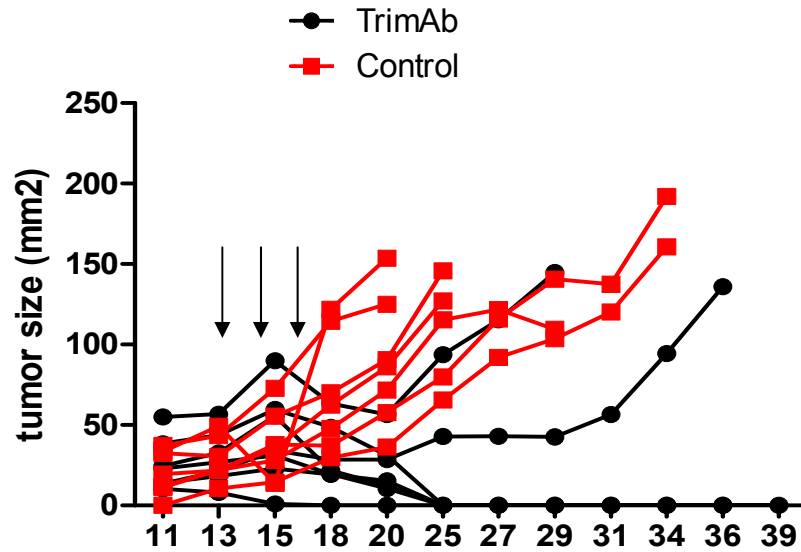
**Trimab**  
DR5  
CD40  
CD137



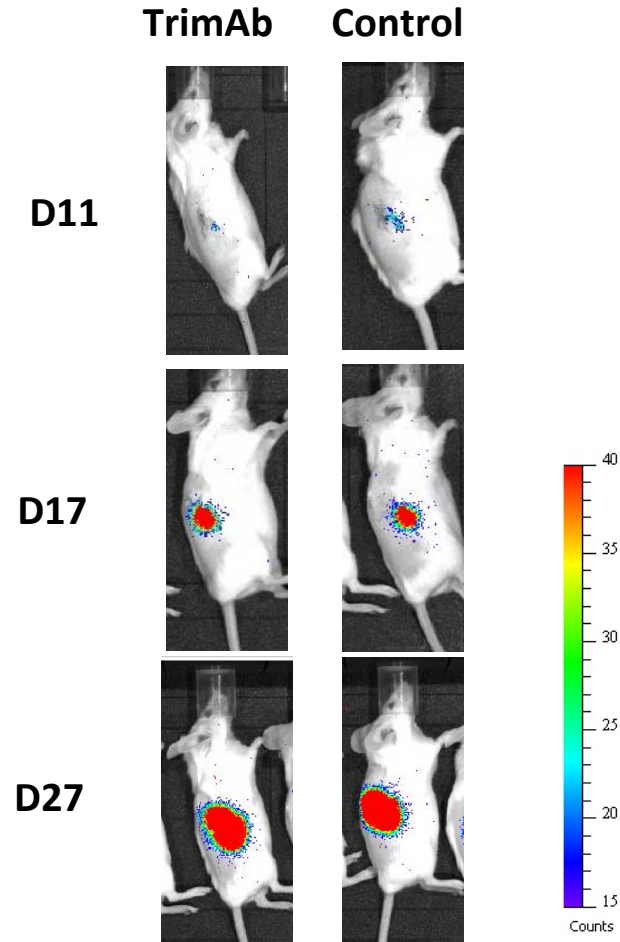
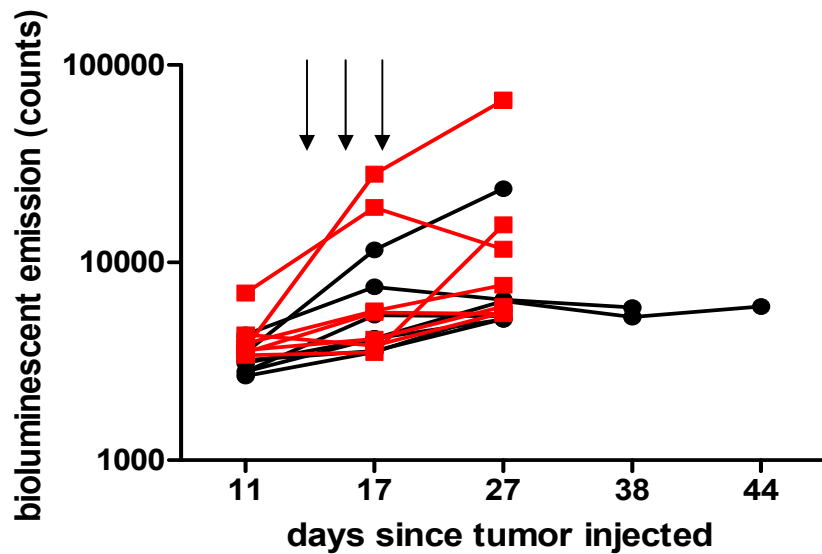
1 experiment representative of 3

# No differences in tumor growth rate between SC and IK

## SC tumors



## IK tumors



1 experiment representative of 3

**AIM: Determine the reasons behind the differential responses to immunotherapy of tumors in different locations**

**Immune related differences:**

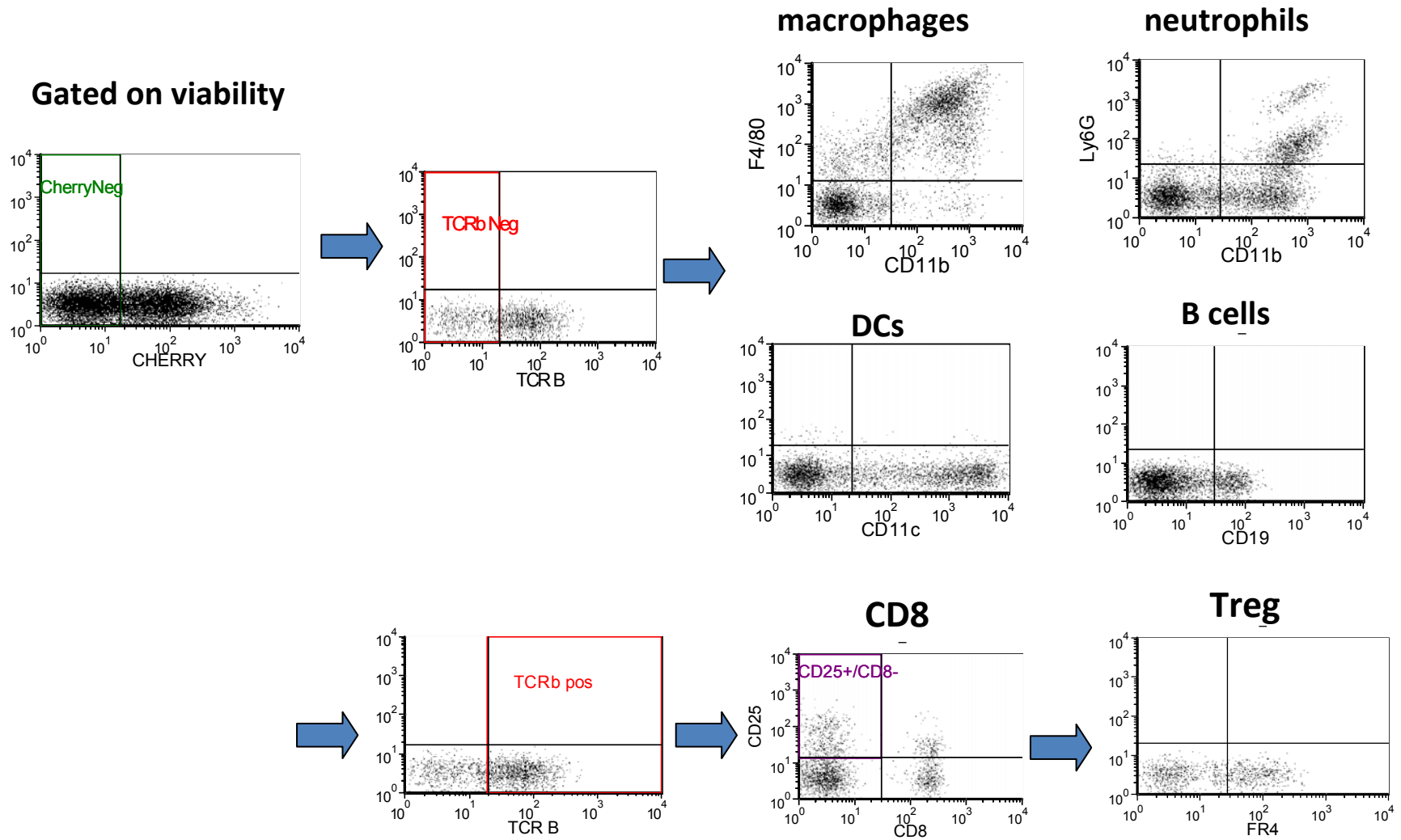
cells and molecules of the tumor microenvironment before treatment

**Differences in intrinsic tumor qualities:**

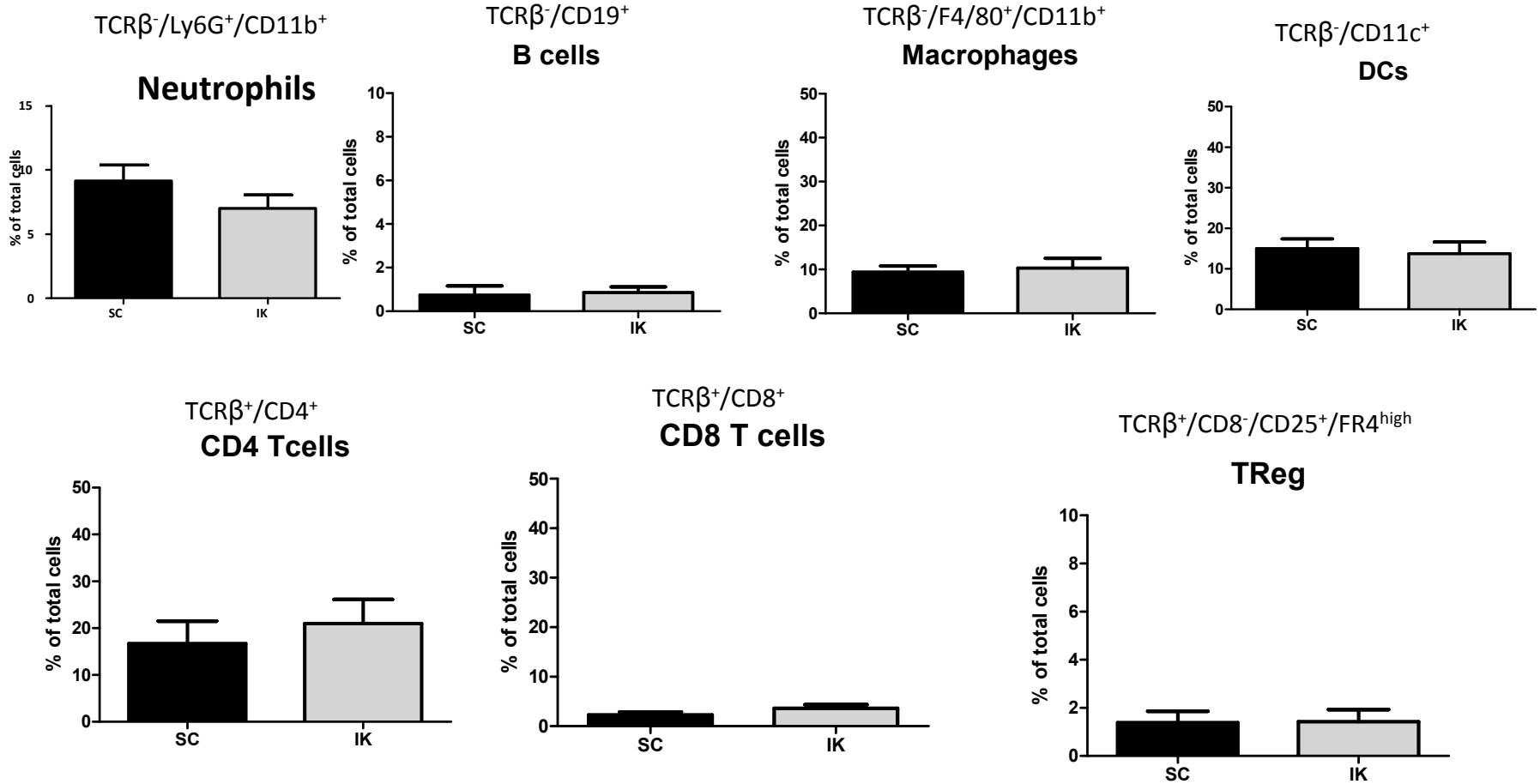
resistance to apoptosis, MHC expression, morphological/structural differences

# Cytometry gating for immune cells in tumors

Before treatment (D12 after tumor cell injection)

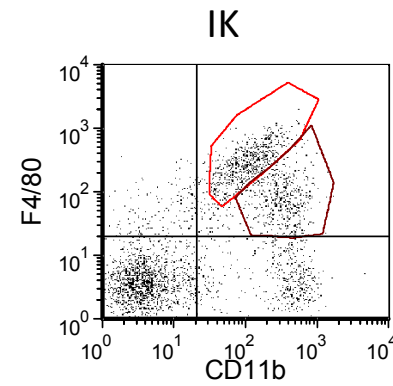
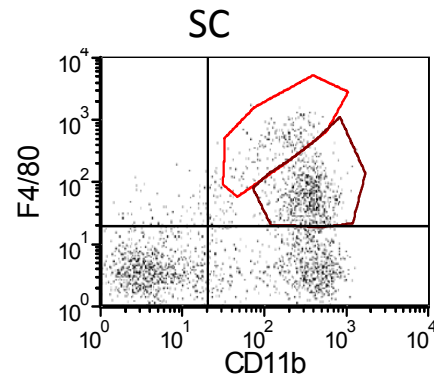
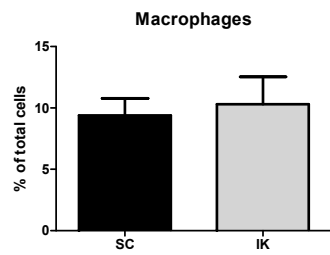


# No differences in frequency of immune cells in kidney tumors compared to subcutaneous tumors



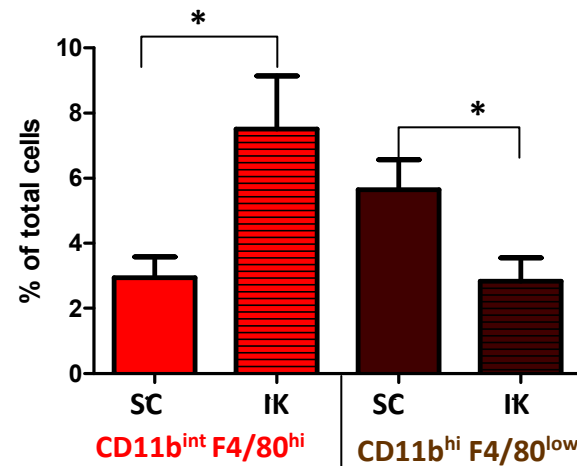
4 independent experiments pooled

# Differences in macrophage profile between SC/IK tumors



CD11b<sup>int</sup> F4/80<sup>hi</sup>

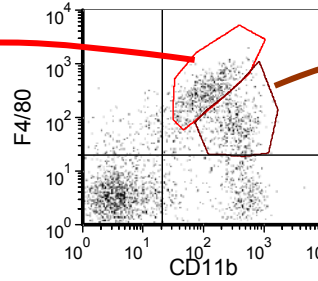
CD11b<sup>hi</sup> F4/80<sup>low</sup>



3 independent experiments pooled

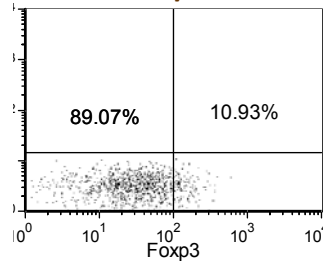
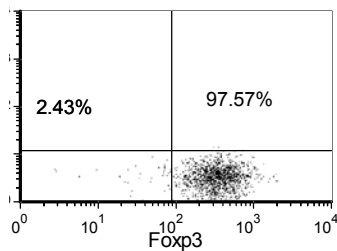
# F4/80<sup>hi</sup>CD11b<sup>int</sup> macrophages express FoxP3 and the mannose receptor (CD206)

IK

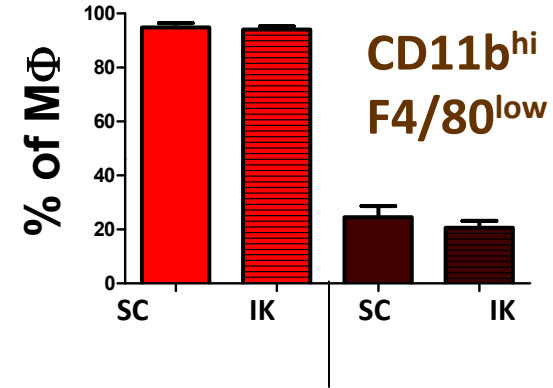


CD11b<sup>int</sup> F4/80<sup>hi</sup>

CD11b<sup>hi</sup> F4/80<sup>low</sup>



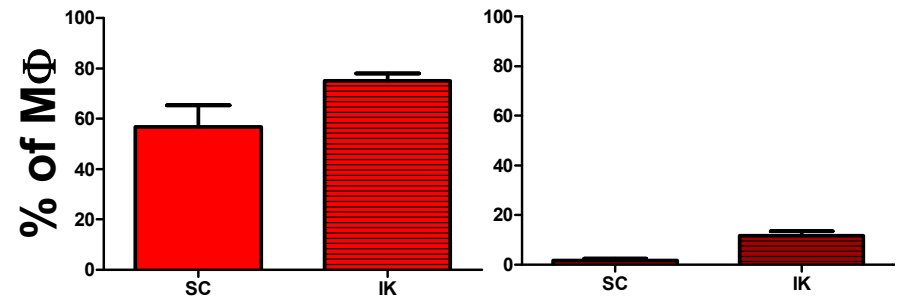
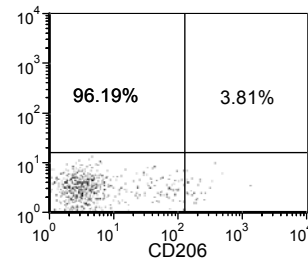
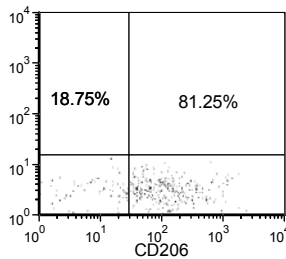
CD11b<sup>int</sup>  
F4/80<sup>hi</sup>



CD11b<sup>hi</sup>  
F4/80<sup>low</sup>

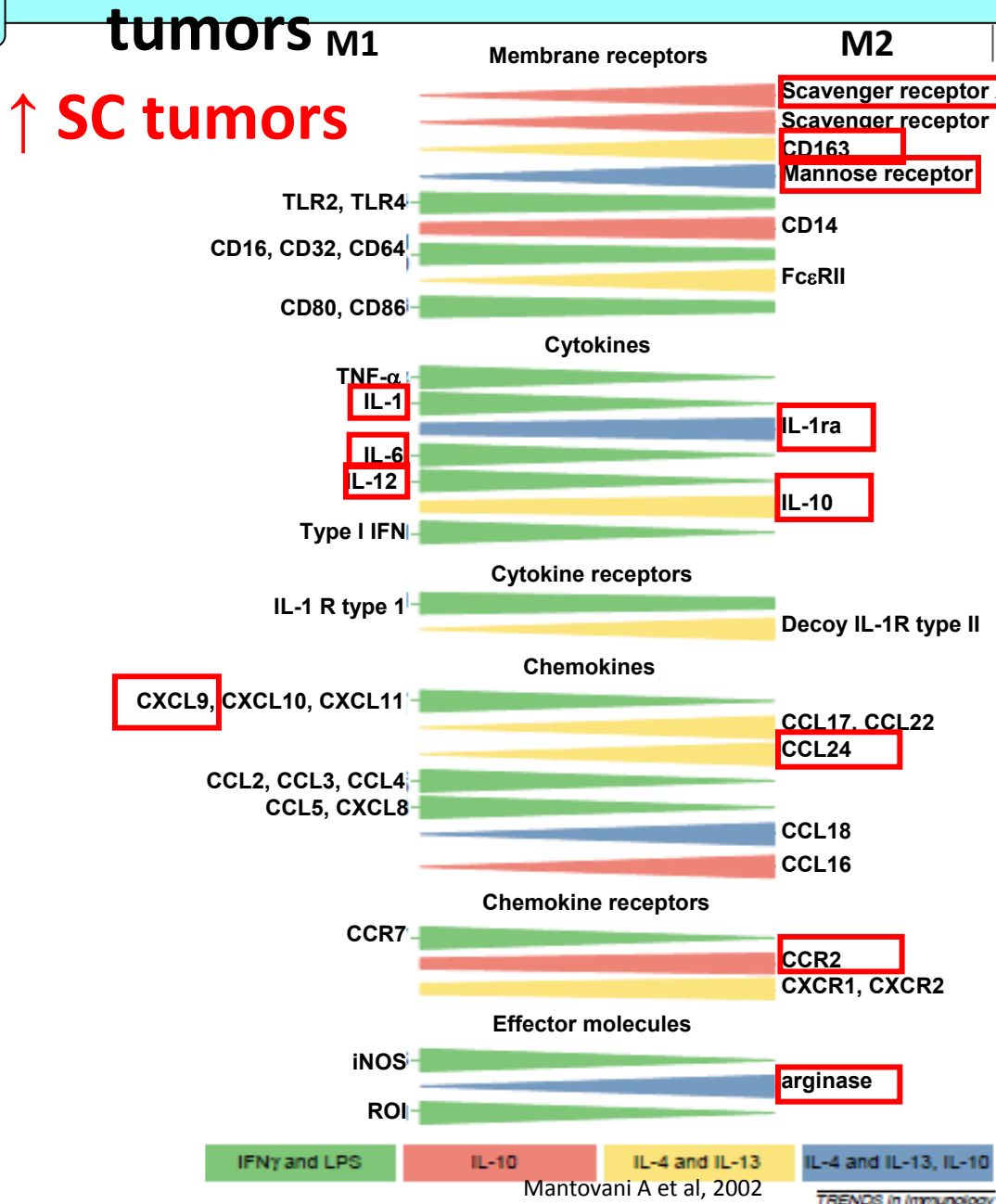
FoxP3

CD206





# M2 macrophage markers predominate in kidney tumors



**↑ IK tumors**

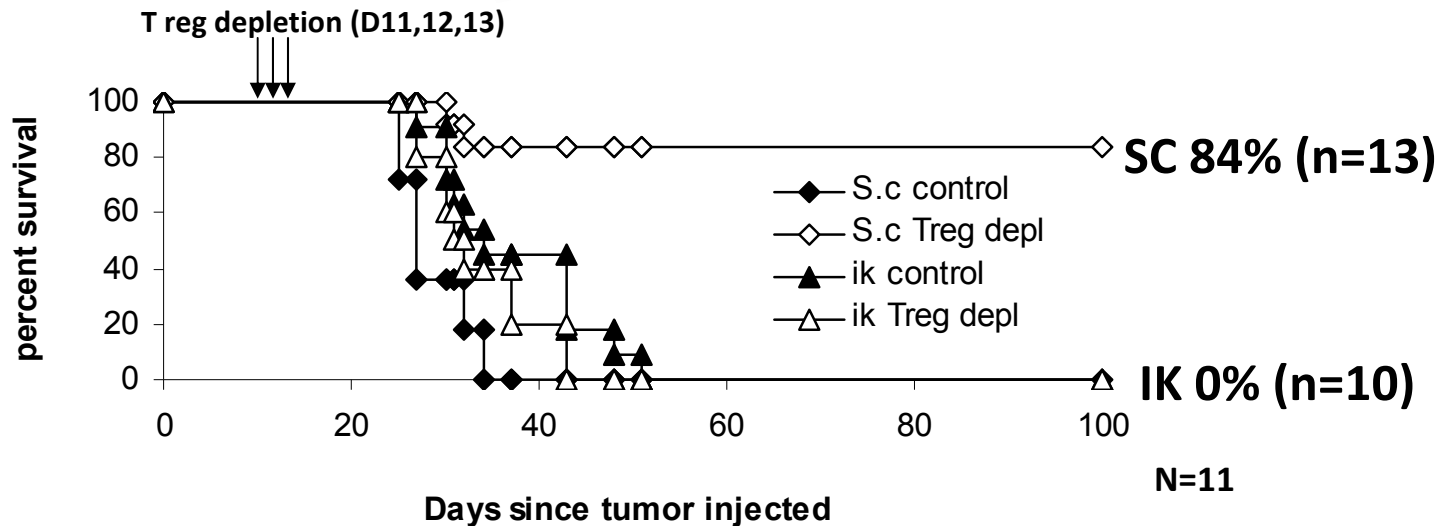
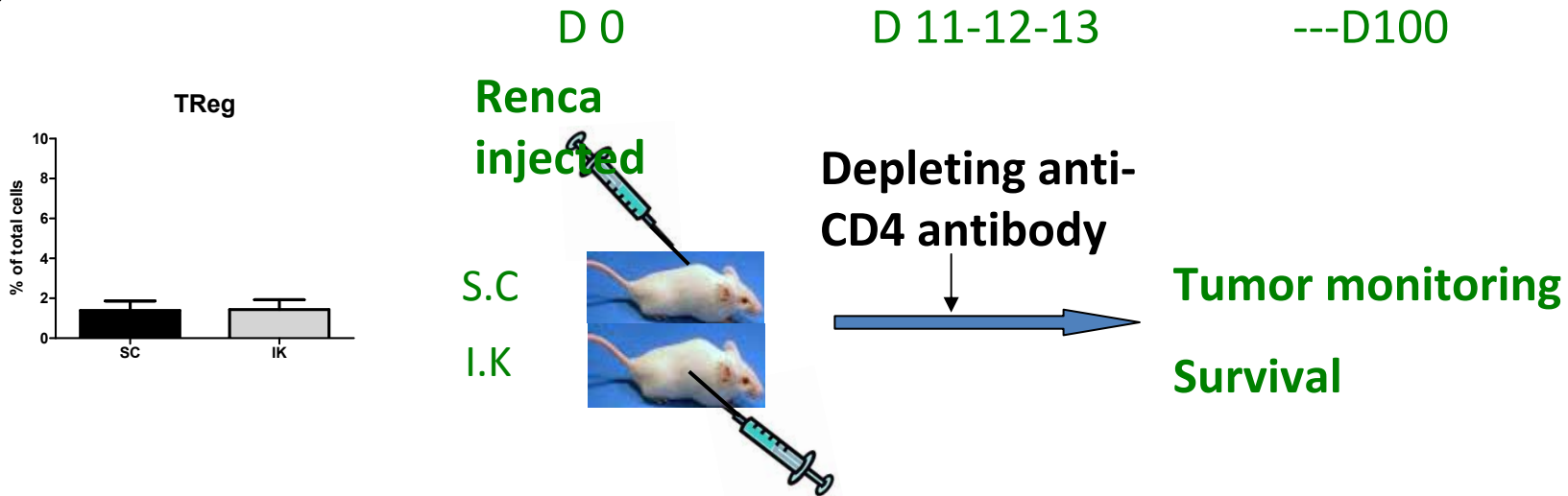
➤ Factors for the M2 switch  
➔ LIF

➤ Growth factors  
➔ GM-CSF  
➔ M-CSF

➤ Trafficking  
➔ CCL2, CCL1  
➔ CX3CL1, CCL6

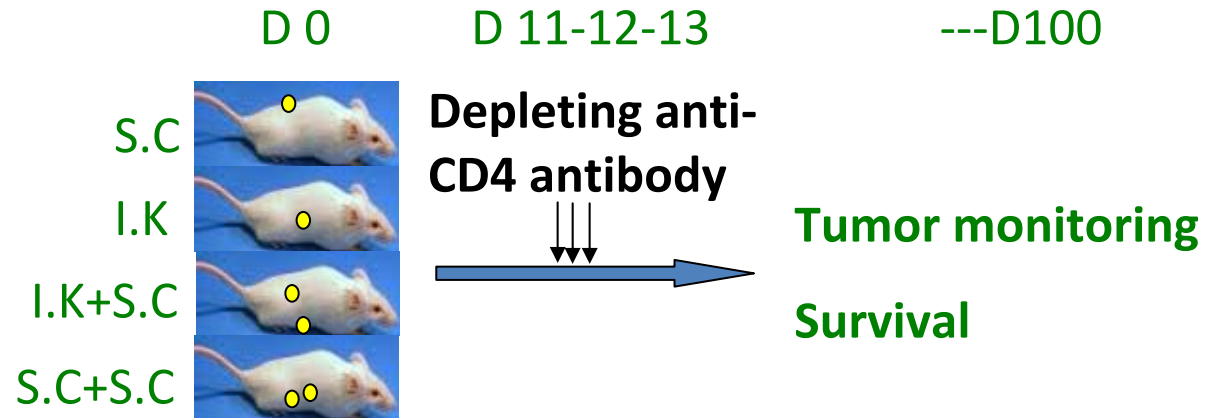
➤ Activity  
➔ Arginase  
➔ IL-10

# CD4<sup>+</sup> T cell depletion triggers regression of SC tumors but not IK tumors

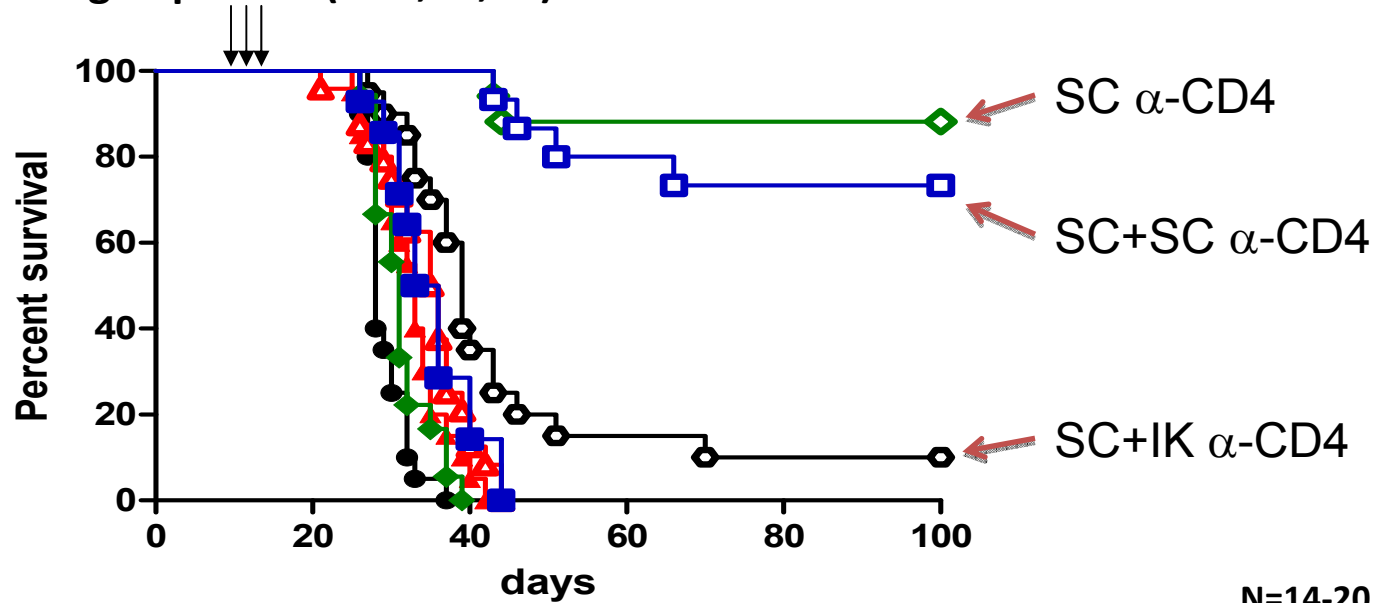


1 experiment representative of 3

# Immune response after CD4+ depletion may be systemic



T reg depletion (D11,12,13)



N=14-20

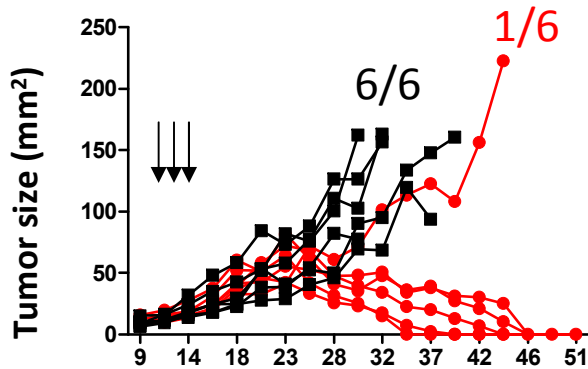
3 independent experiments pooled

# Kidney tumor inhibits rejection of subcutaneous tumor

## SC alone

N=6

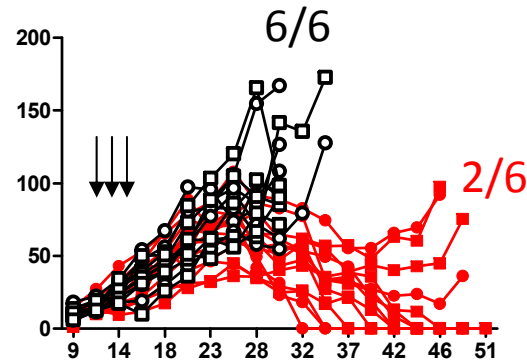
- SC T Reg D
- SC Control



## SC + SC

N=6

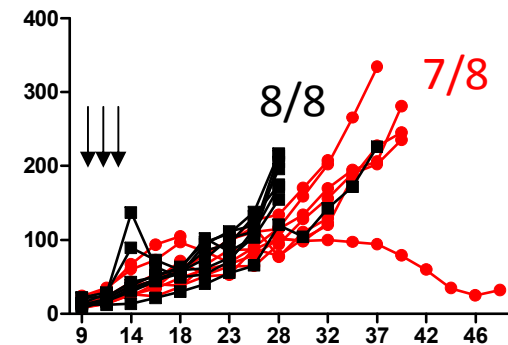
- T Reg D right
- T Reg left
- Control right
- Control left



## SC + IK

N=8

- SC+IK TReg D
- SC+IK Control

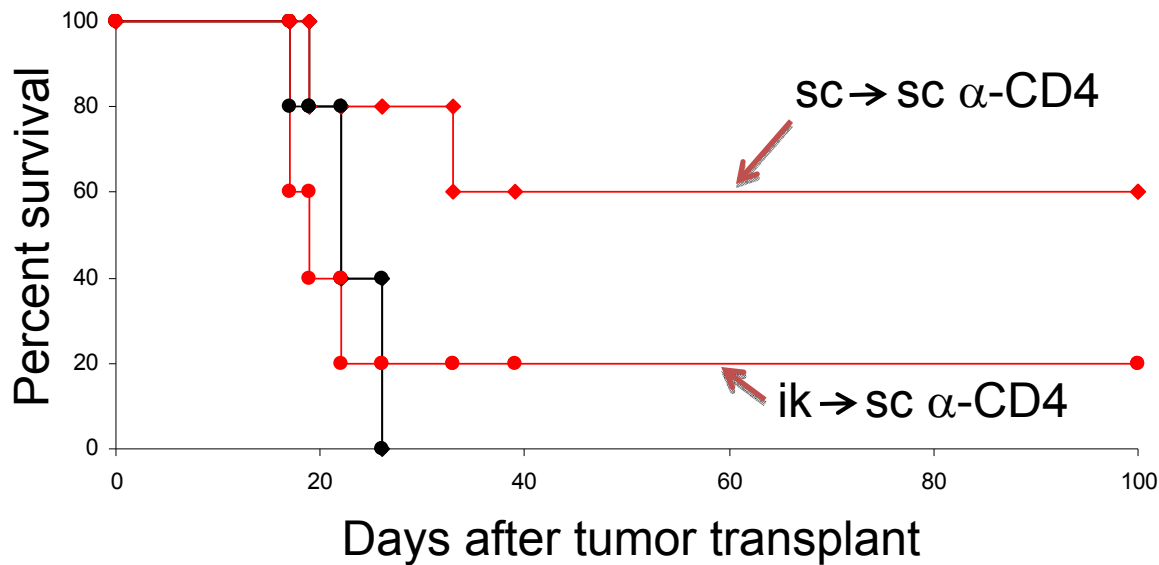
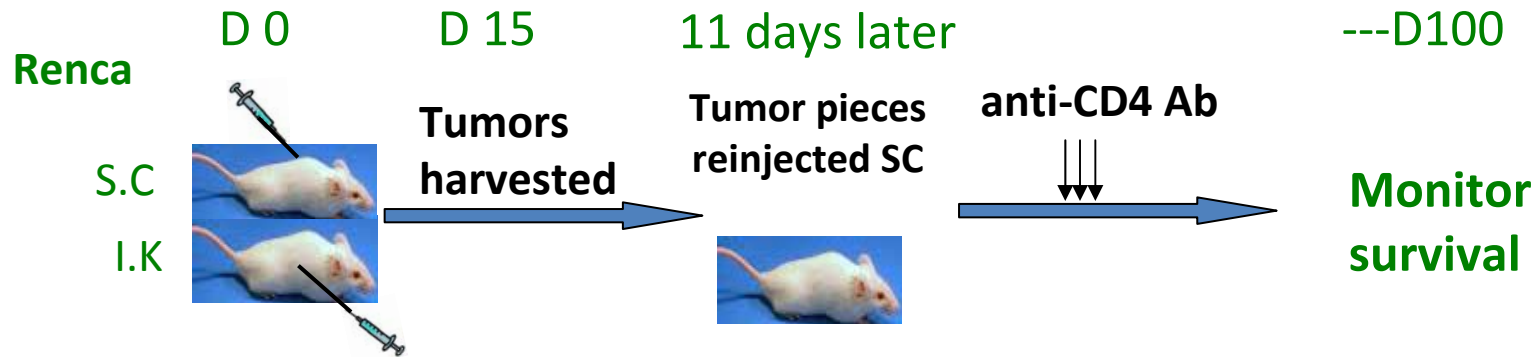


Days since tumor injected

Anti-CD4 days 11, 12 and 13

1 experiment representative of 3

# Kidney tumors do not respond as well as subcutaneous tumors when pieces are transplanted under the skin



one experiment (n=5)

**AIM: Determine the reasons for the differential responses to immunotherapy of tumors in different locations**

Immune related differences:

cells and molecules of the tumor microenvironment

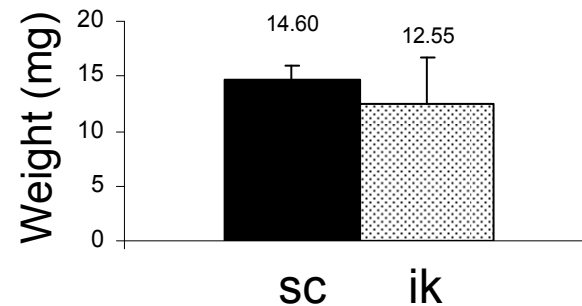
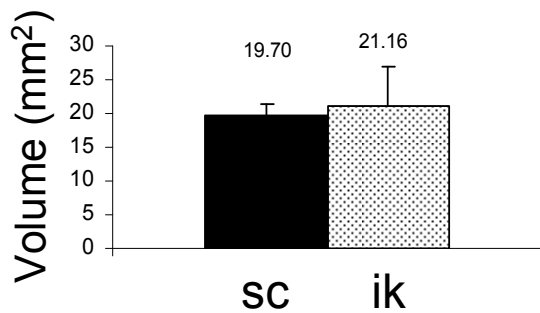
Differences in intrinsic tumor qualities:

resistance to apoptosis, MHC expression,  
morphological/structural differences

**-What do the tumors look like before treatment?**

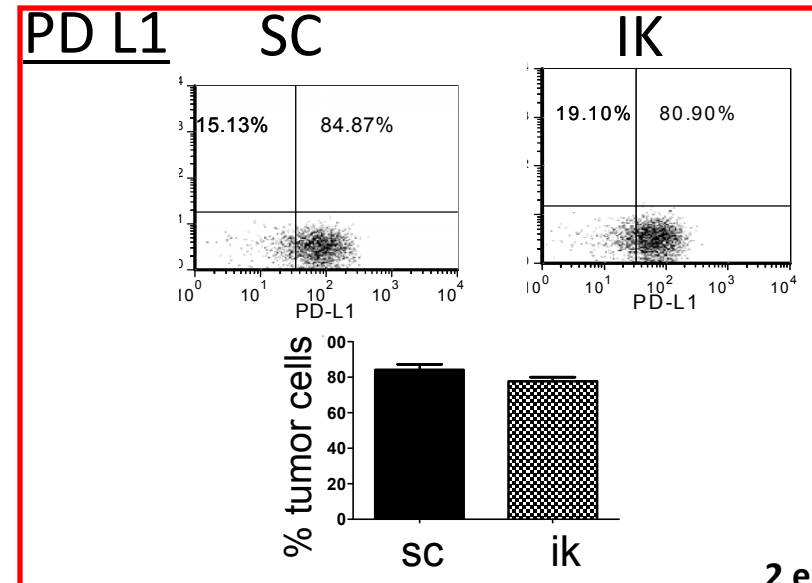
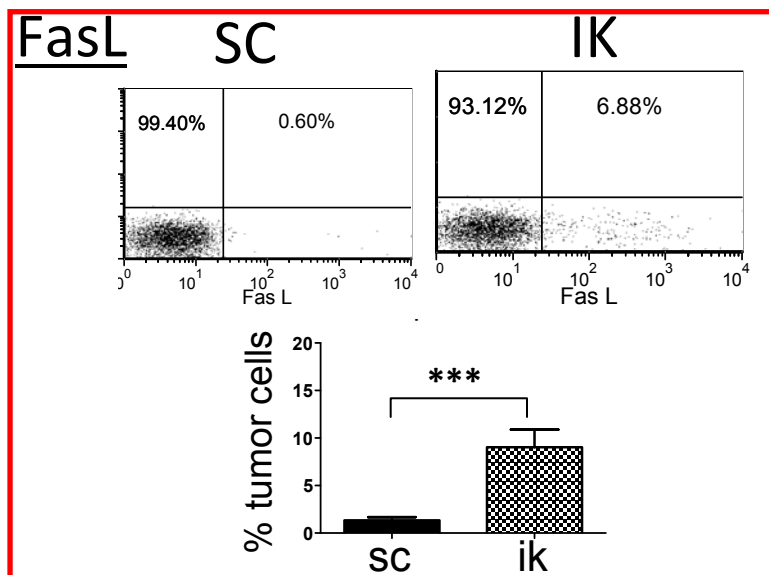
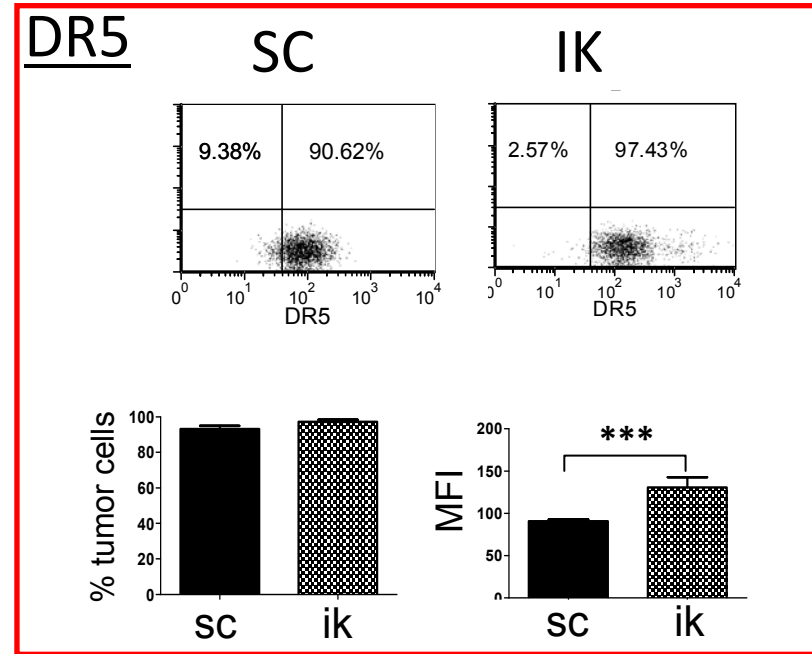
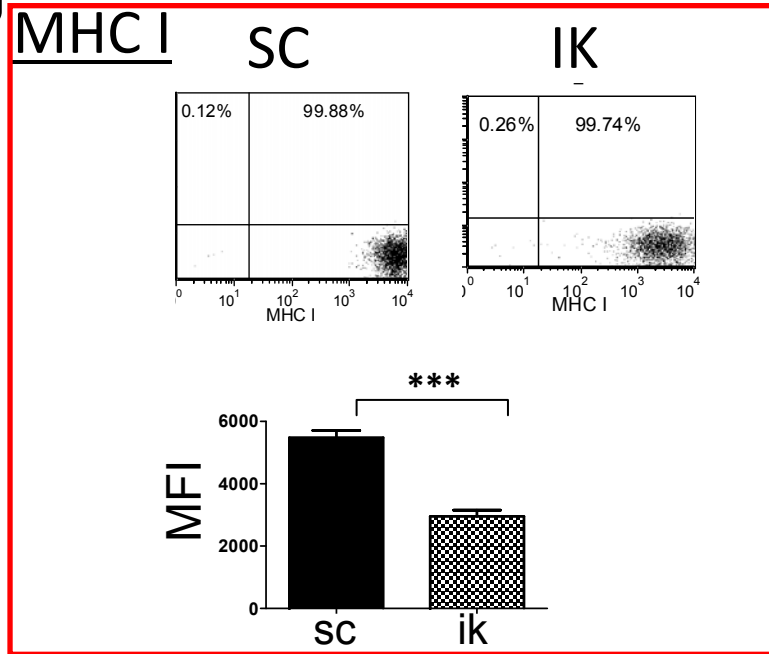
# SC and IK tumors are same size / weight before treatment

D10 before treatment



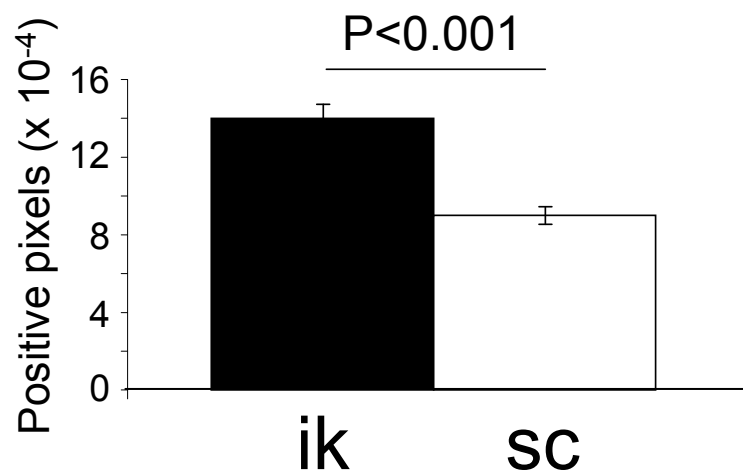
1 experiment representative of 3

# Phenotype of tumors: Higher level of MHC I in SC tumor and higher level of DR5 and expression of Fas L in IK tumors

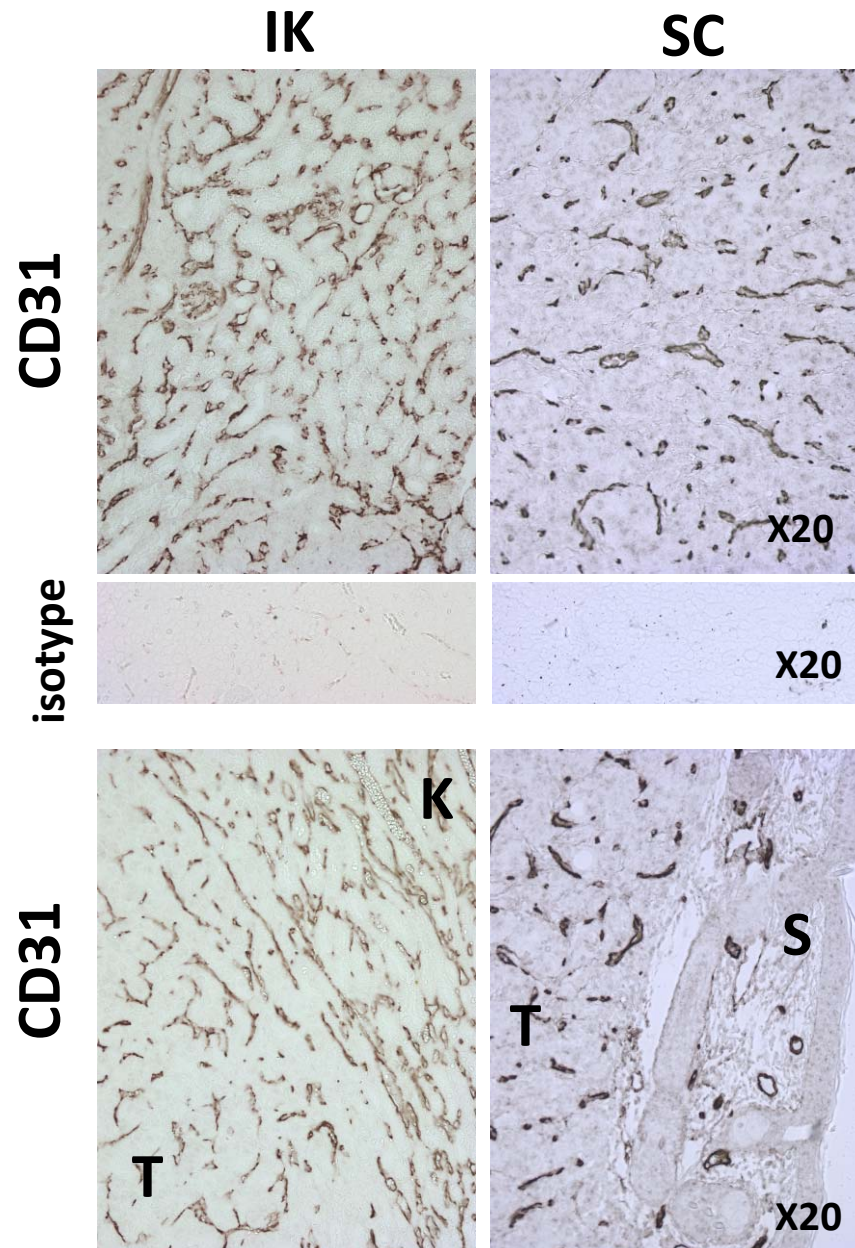




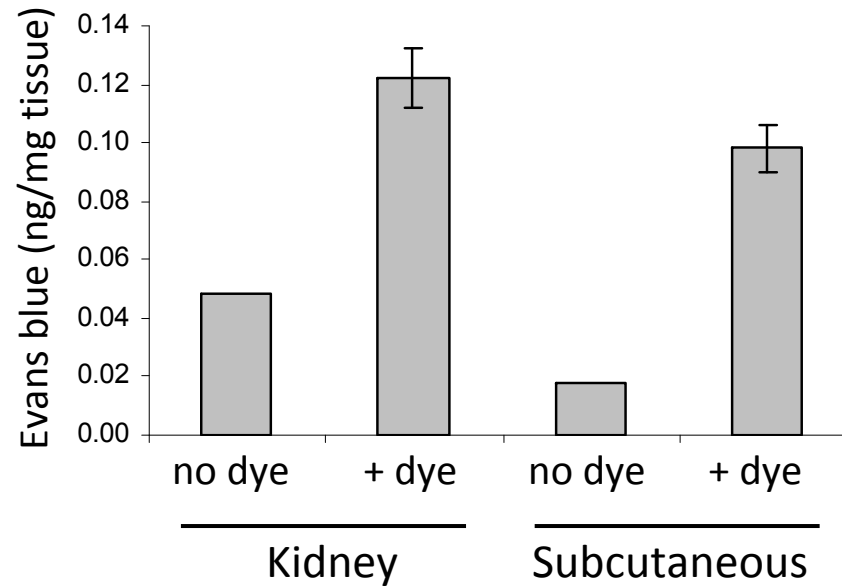
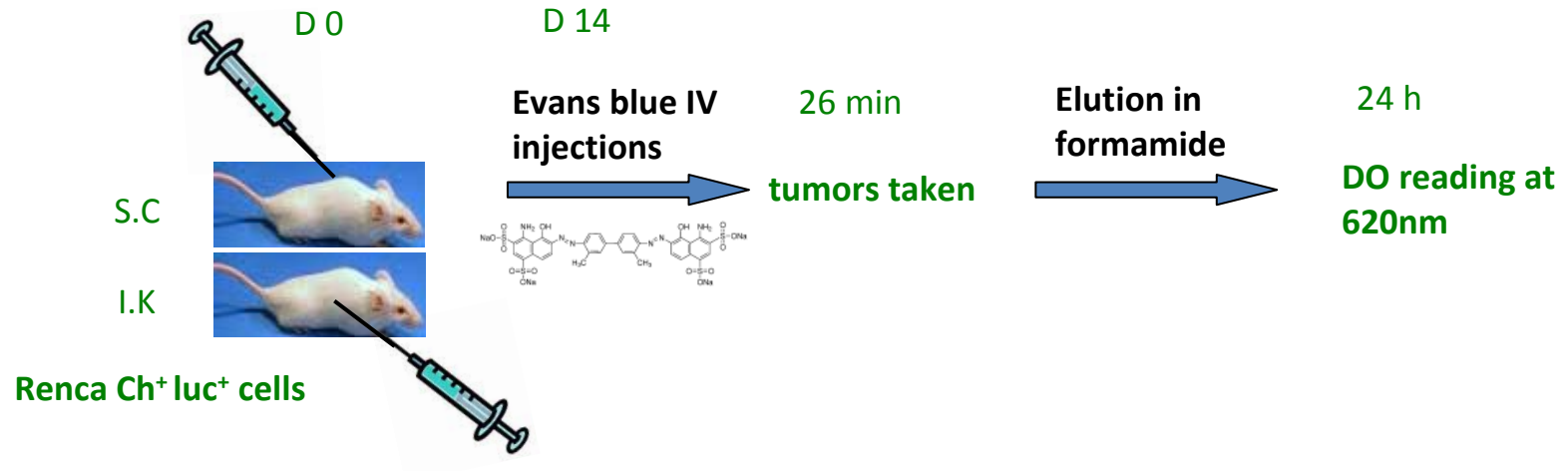
# Kidney tumors are more highly vascularized



(5 tumors, 10 fields/tumor)



# No difference in tumor vessel permeability



N = 5 tumors, representative of 3 experiments

# Summary

- Subcutaneous tumors eradicated by Trimab or  $T_{reg}$  depletion but kidney tumors are not
- M2 macrophage microenvironment in kidney tumors
- Higher frequency of  $F4/80^{hi}CD11b^{int}FoxP3^{+}$  macrophages in kidney tumors
- Immunosuppression may be systemic
- More blood vessels and higher MHCII in subcutaneous tumors

# Thanks

Peter MacCallum Cancer Center

➤ Immunology program

**Christel Devaud**  
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➤ **Peter Mac molecular genomics facility**

➤ **Histology lab**

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