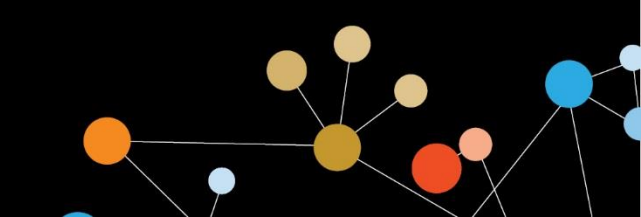
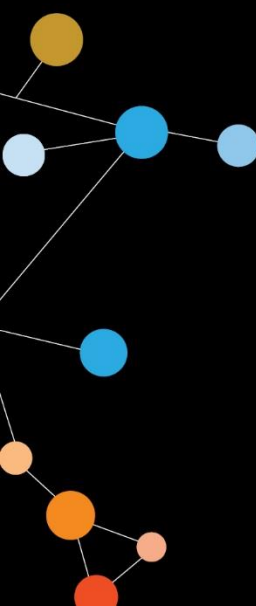


SITC 2016

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NOVEMBER 9-13, 2016



Society for Immunotherapy of Cancer





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Mechanisms of Chitosan/IL-12 Immunotherapy for the Treatment of Bladder Cancer

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University of North Carolina and North Carolina State University



Society for Immunotherapy of Cancer

#SITC2016



Presenter Disclosure Information

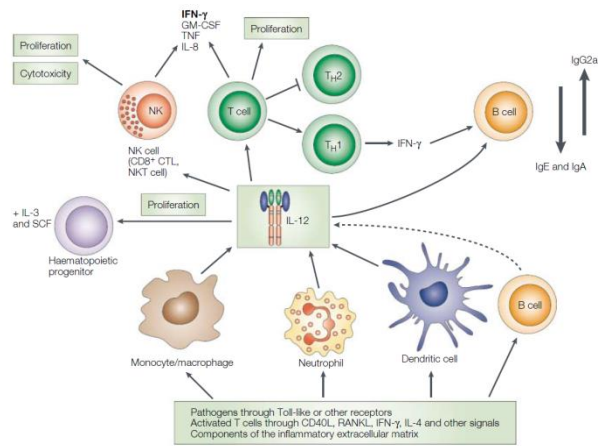
Sean Smith

The following relationships exist related to this presentation:

VivImmune LLC, Ownership, Cofounder

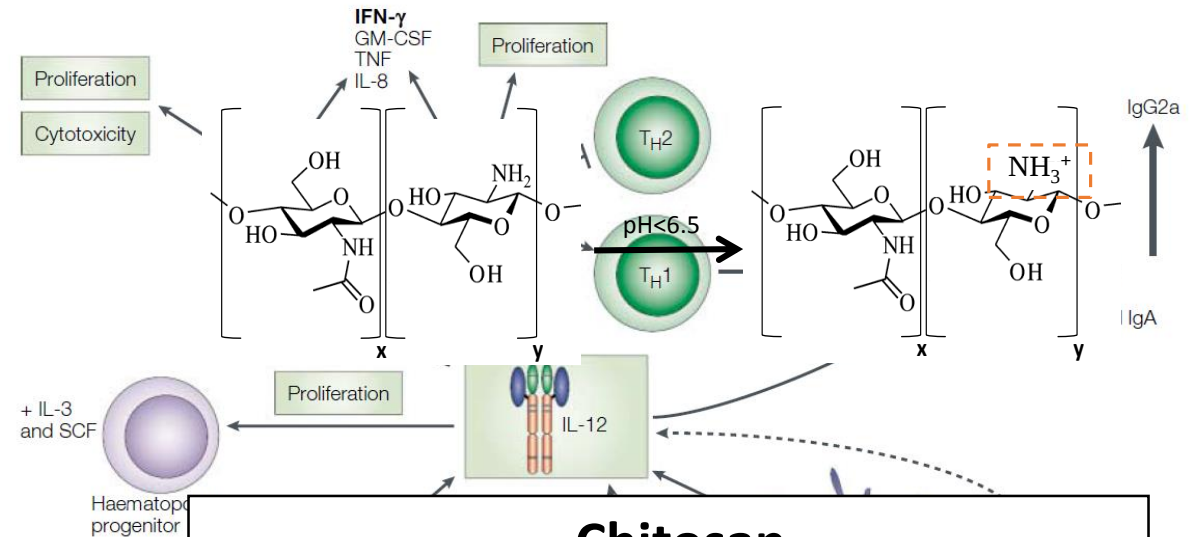
#SITC2016

Chitosan/IL-12 Combines Potency with Delivery



Interleukin-12

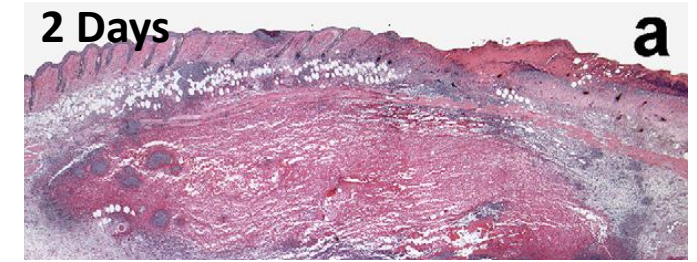
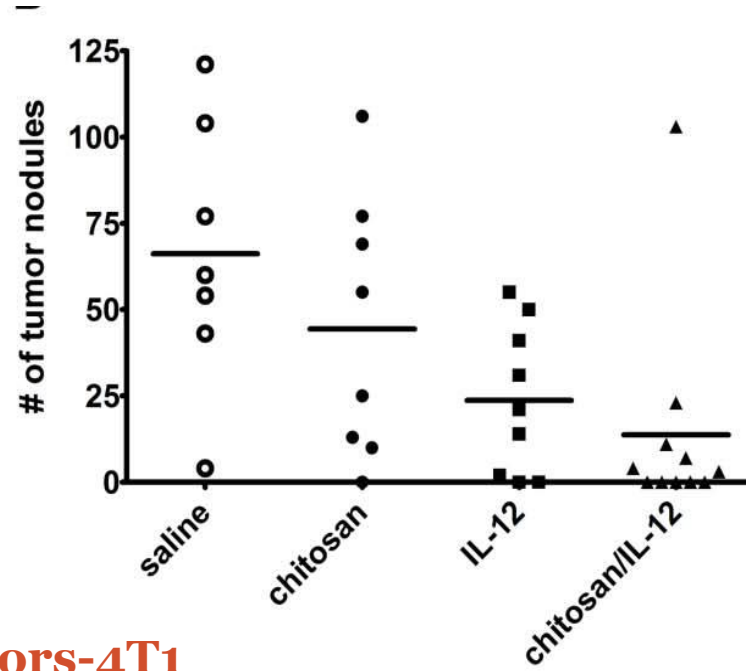
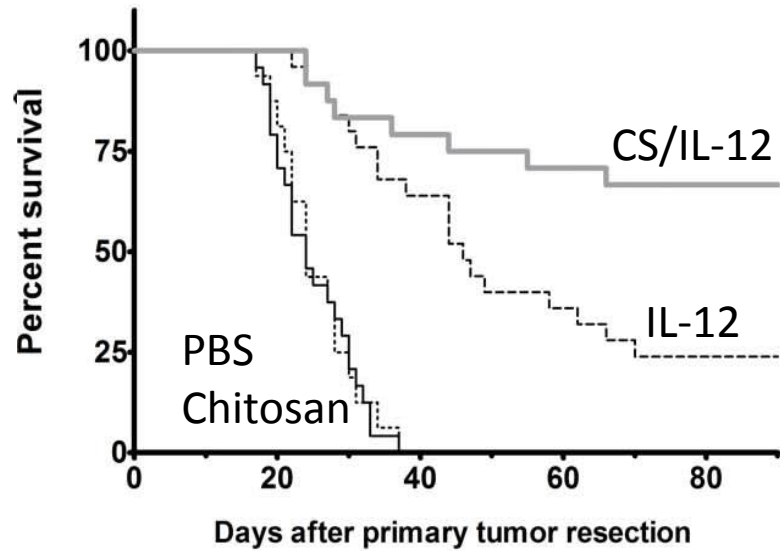
1. Pro-inflammatory cytokine
2. Enhances T_H1 response
3. Toxic when given systemically



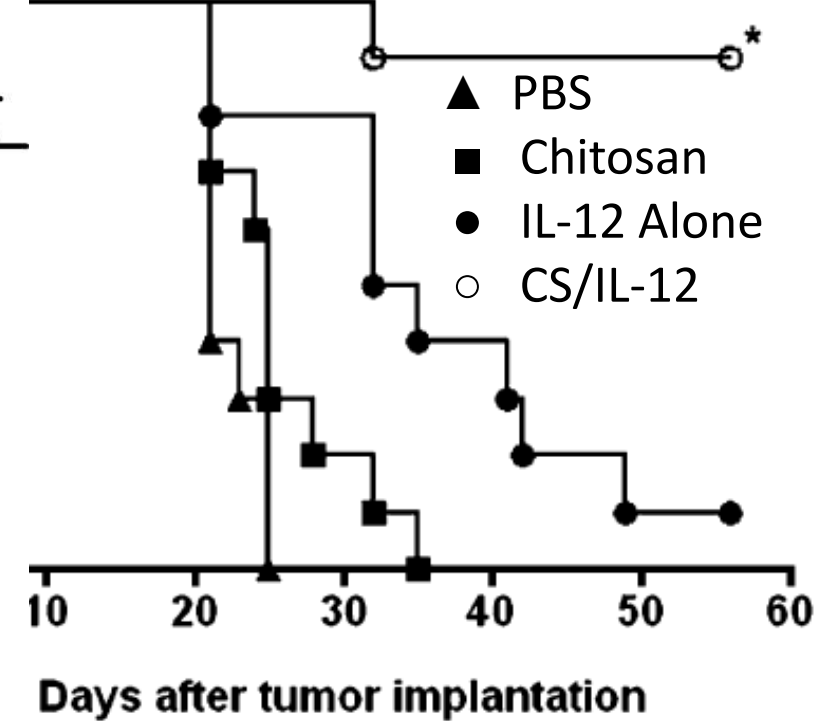
Chitosan

1. Biopolymer
2. Soluble in mild acids
3. Gels *in vivo*

Intratumoral Chitosan/IL-12



on Tumors-MC38



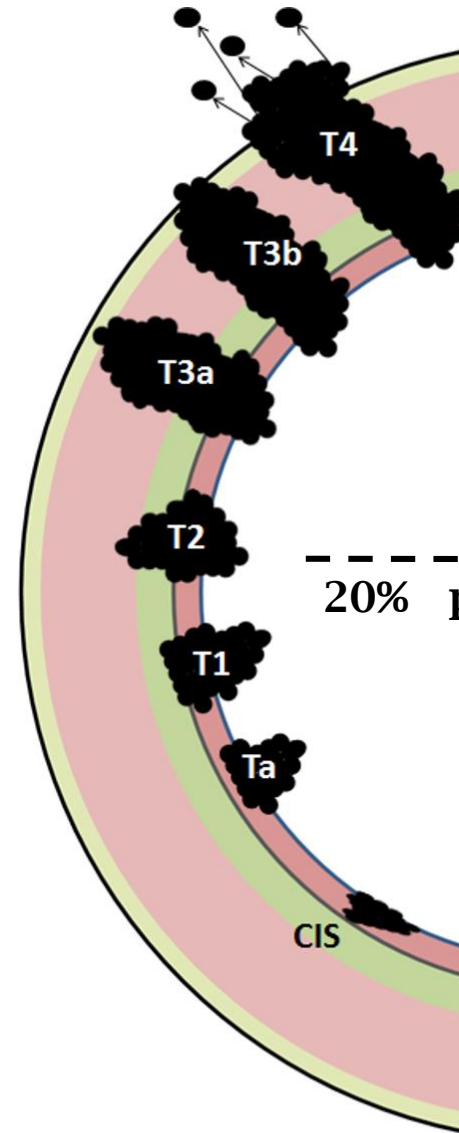
Breast Tumors-4T1



Bladder Cancer: Recurrence is the Issue

- 5th most common cancer in USA
 - Highest rate of recurrence
- Intravesical BCG
 - Standard of care for 35 years
 - Does not promote tumor specific memory

Can we use CS/IL-12 to initiate an adaptive immune response in the bladder?



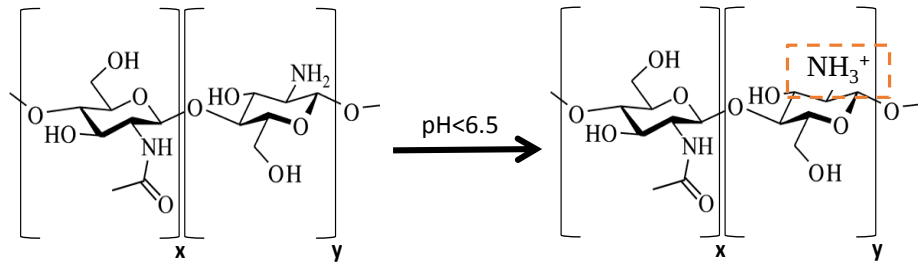
Muscle Invasive

- 30% of cases
- Treated with chemotherapy, radiation, and cystectomy
- High mortality

Non-Muscle Invasive

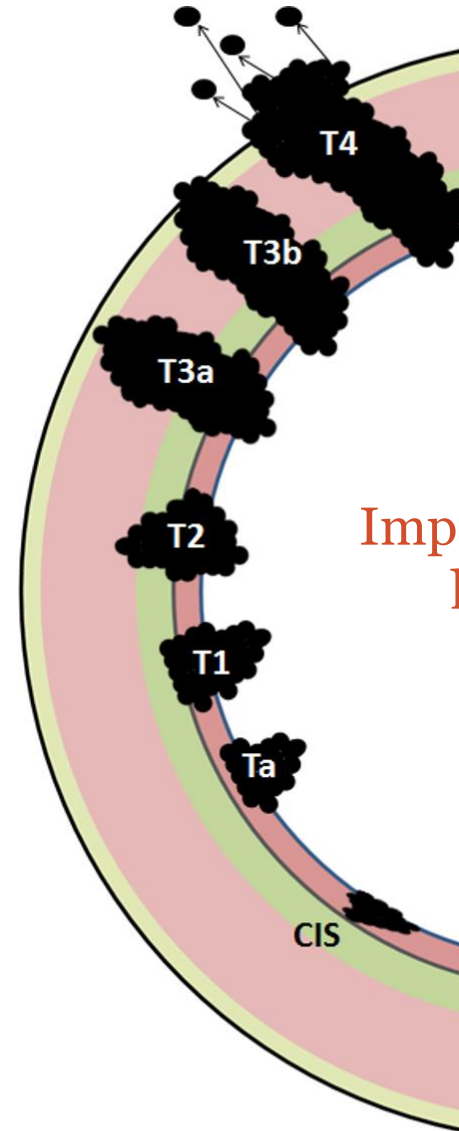
- 70% of cases
- Treated by transurethral resection + intravesical BCG
- High recurrence rate
- Low Mortality

Chitosan Provides Enhanced Intravesical Delivery



Chitosan

1. Gels *in vivo*
2. **Viscous**
3. **Mucoadhesive**
4. **Absorption Enhancing**



Impermeable nature of the bladder
limits therapeutic exposure

MB49 Implantation and Treatment

Our murine models imitate orthotopic, superficial bladder cancer.

Day

0

Implant 75k MB49

7

1x Treat

Gross hematuria develops

10

2x Treat

14

3x Treat

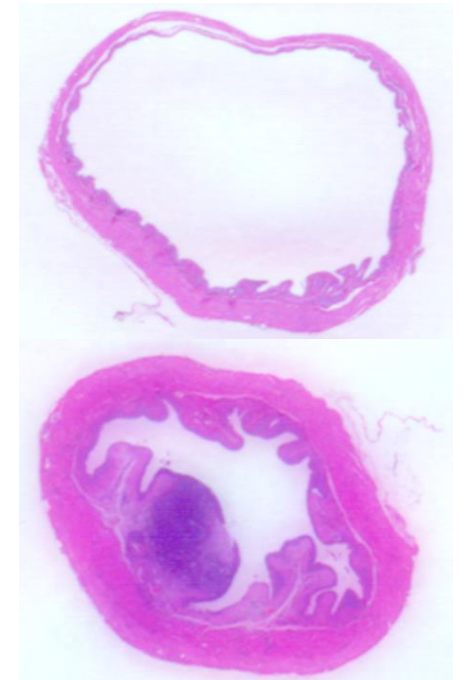
17

4x Treat

21

28

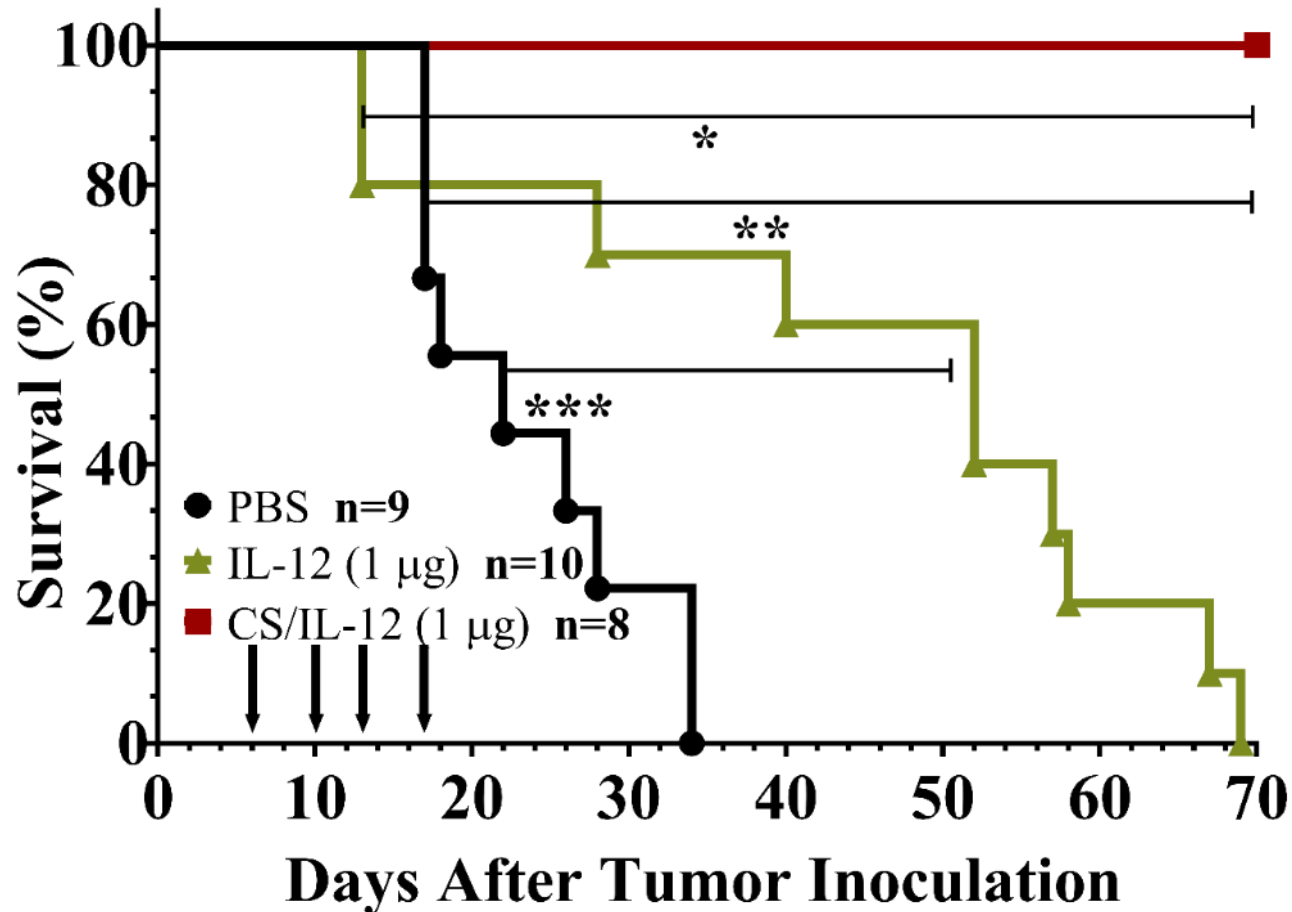
Typical death of control mice



Tumors are instilled and treated intravesically.

Intravesical Chitosan/IL-12 Against MB49 Bladder Cancer

Orthotopic Tumor



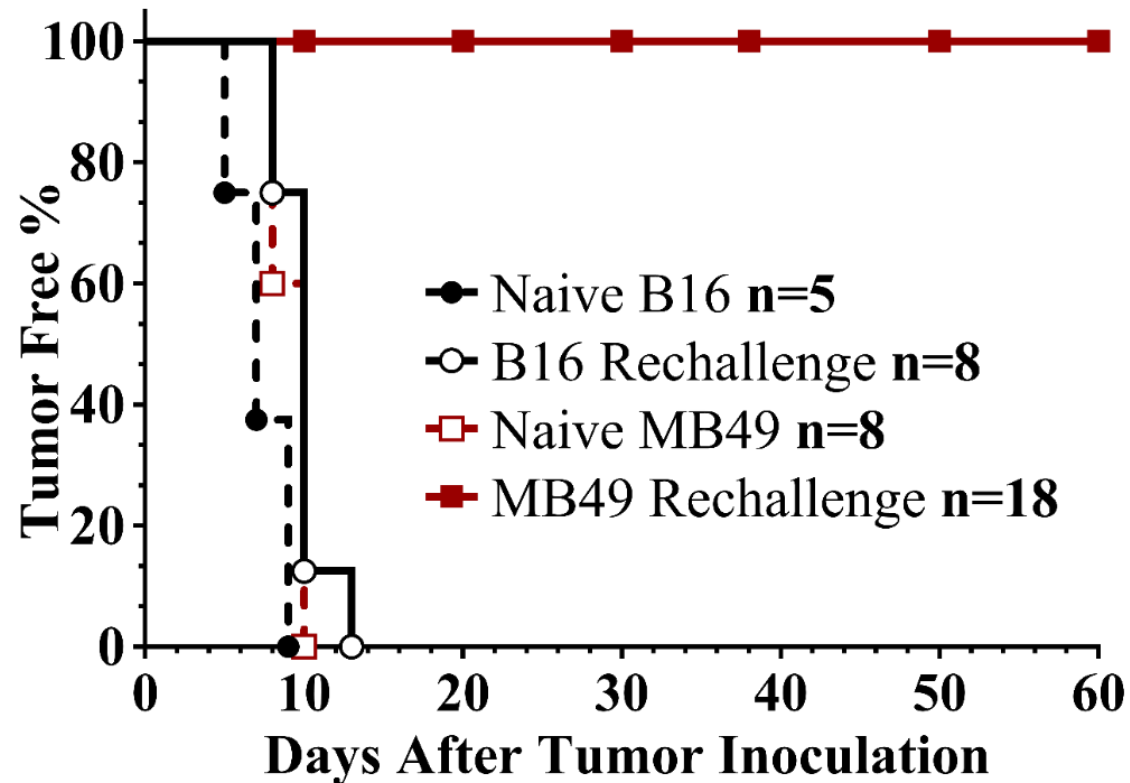
Can CS/IL-12 eliminate orthotopic bladder tumors?

Intravesical CS/IL-12 eliminates orthotopic tumors and is more effective than IL-12 alone.

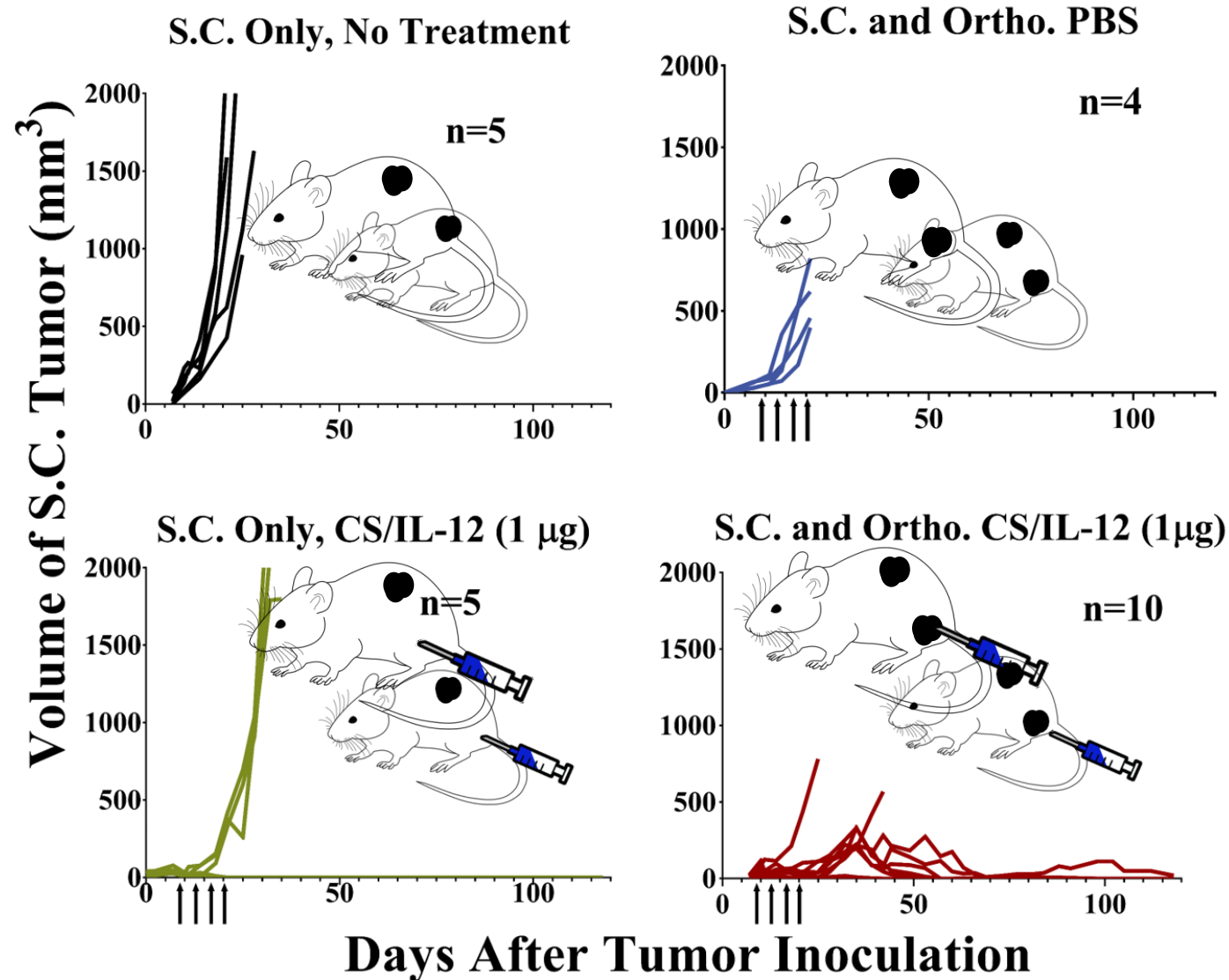
Intravesical Chitosan/IL-12 Generates Systemic Immunity

Distant Rechallenge

Can a local treatment generate a systemic response?



Intravesical Chitosan/IL-12 Generates Systemic Immunity

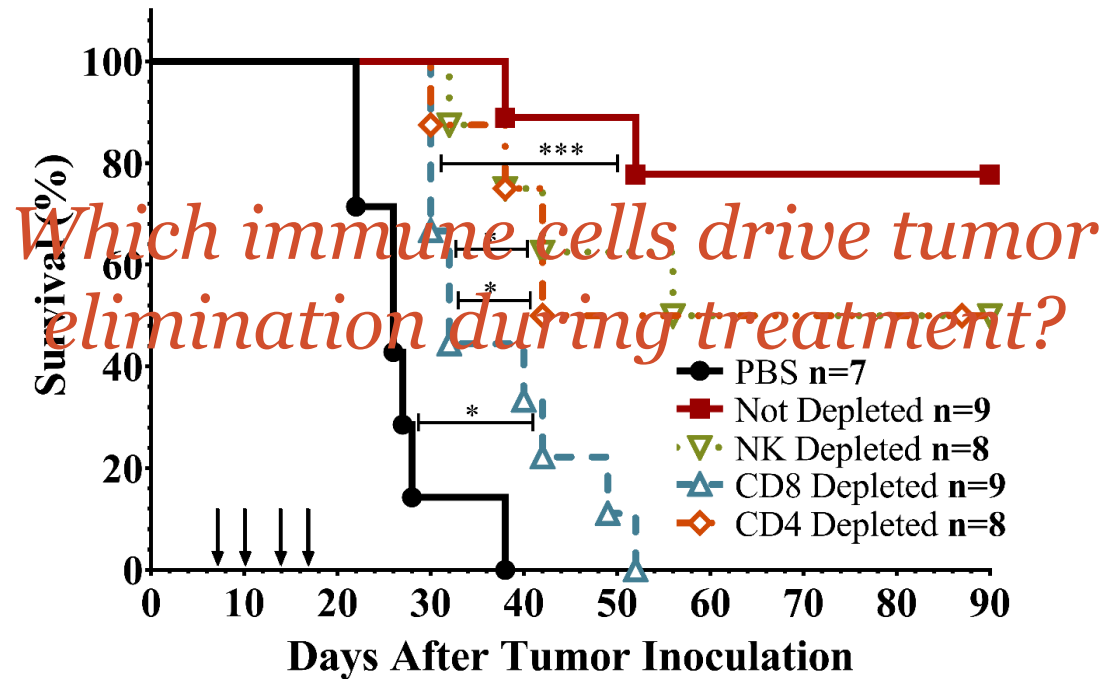


Intravesical treatment generates a systemic tumor specific immunity as well as an abscopal response.

Can a local treatment generate a systemic response?

Depletion Studies Reveal Key Roles for CD8+ and CD4+ T-cells

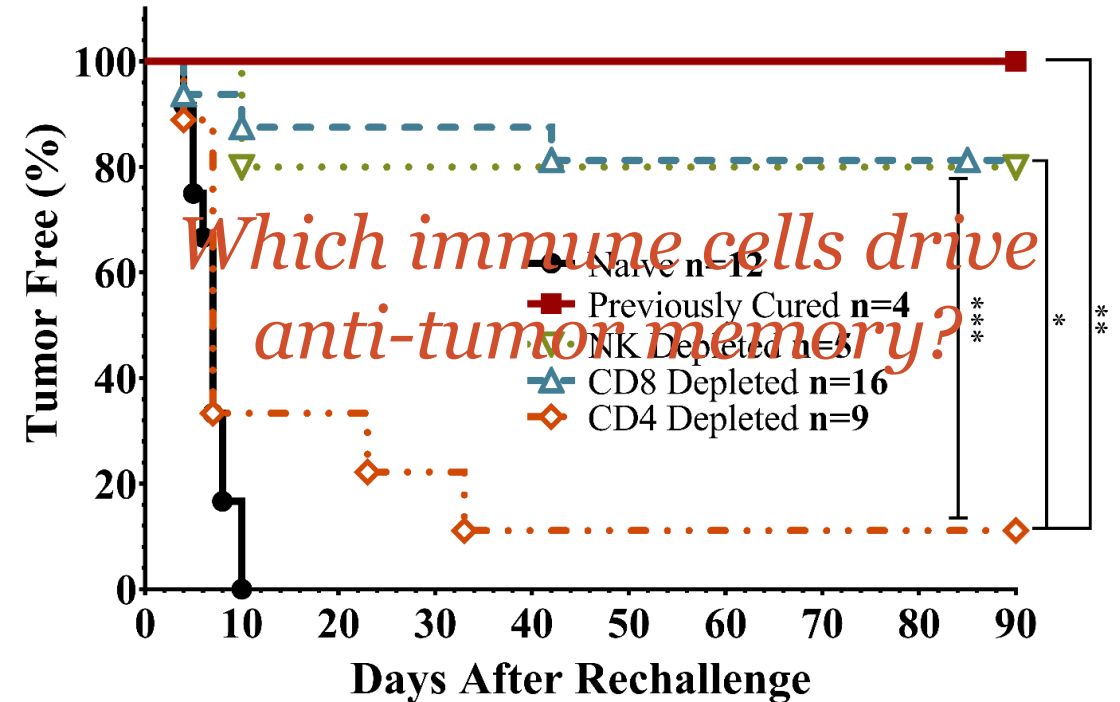
Orthotopic Tumor



CD8+ T-cells are primary drivers of tumor elimination during treatment.

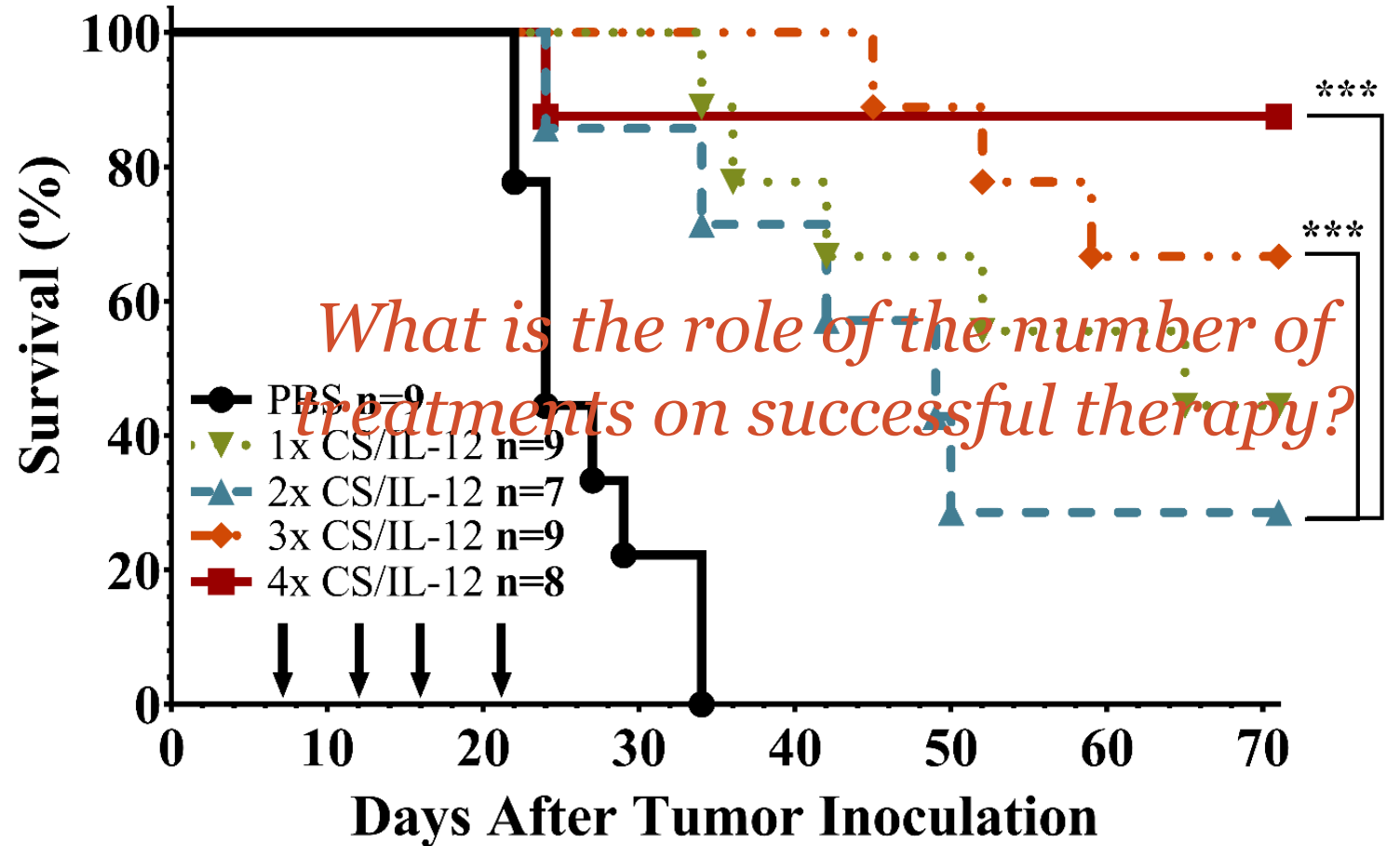
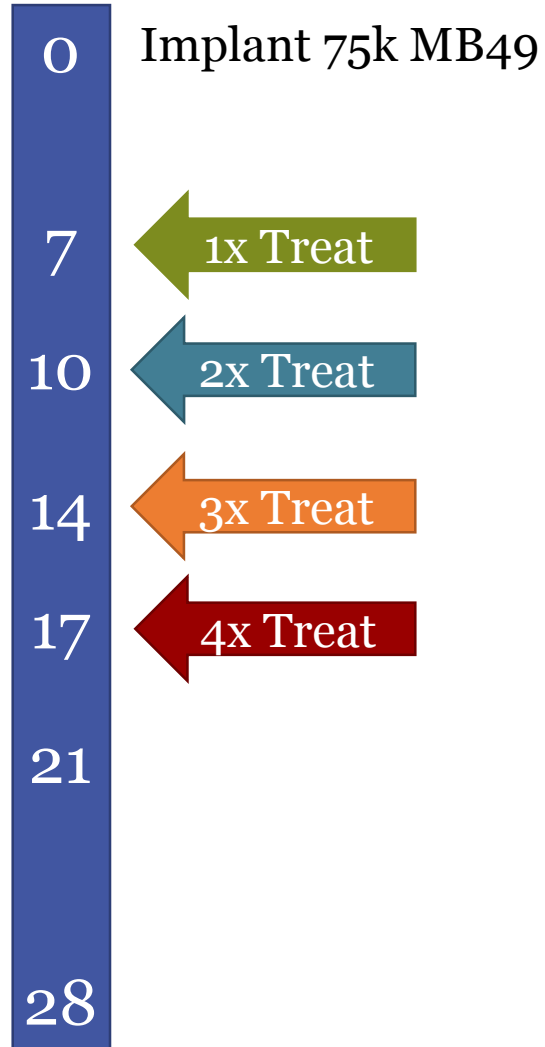
Smith et al. *Oncolimmunology* (In Press)

Distant Rechallenge



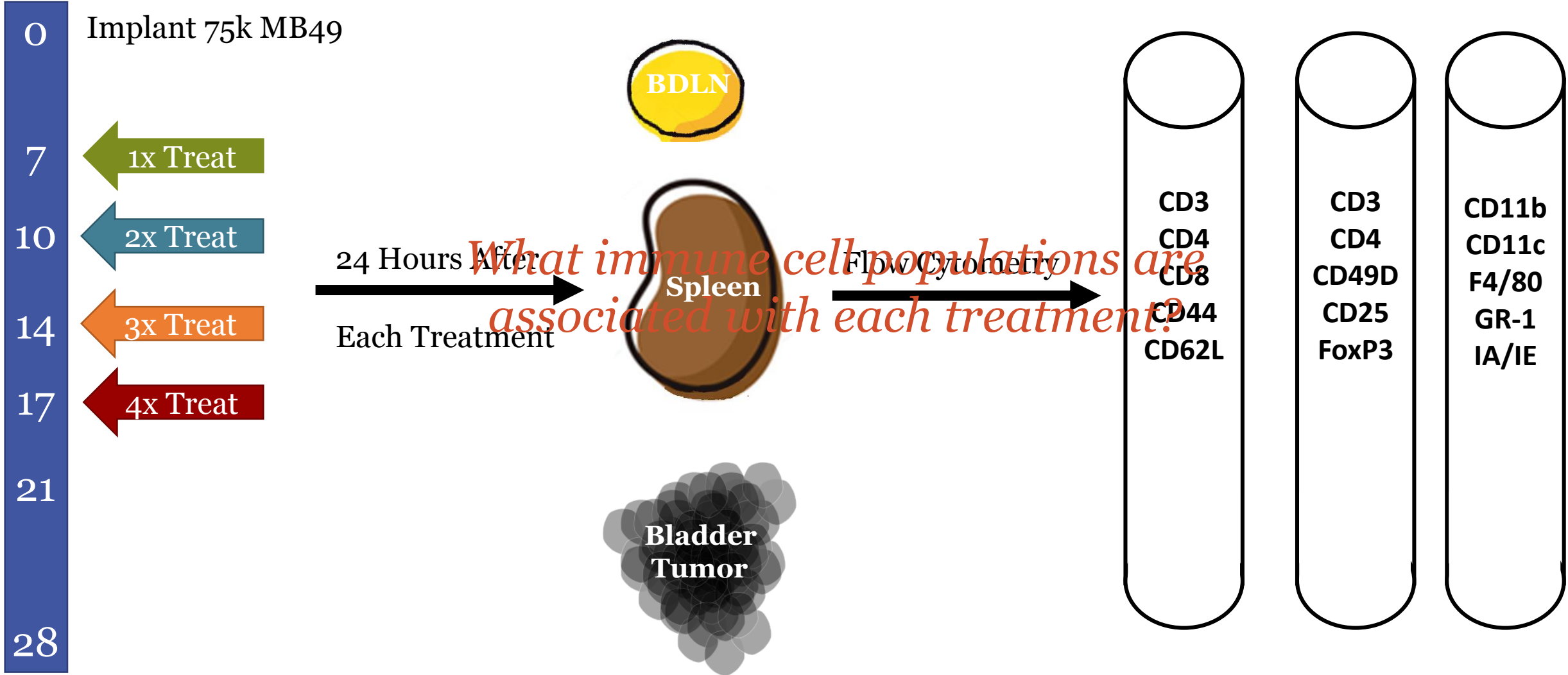
CD4+ T-cells drive tumor rejection upon rechallenge.

Importance of Treatment Number



Four treatments provide the greatest protection, but even a single treatment eliminates 50% of tumors.

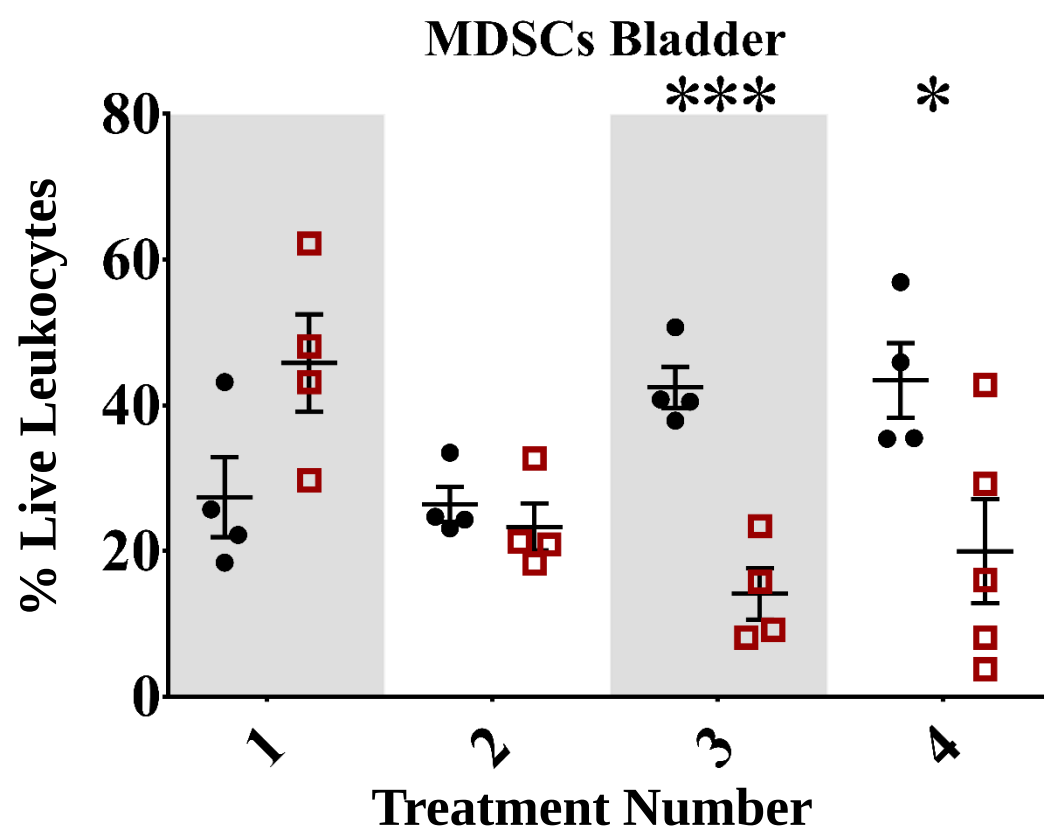
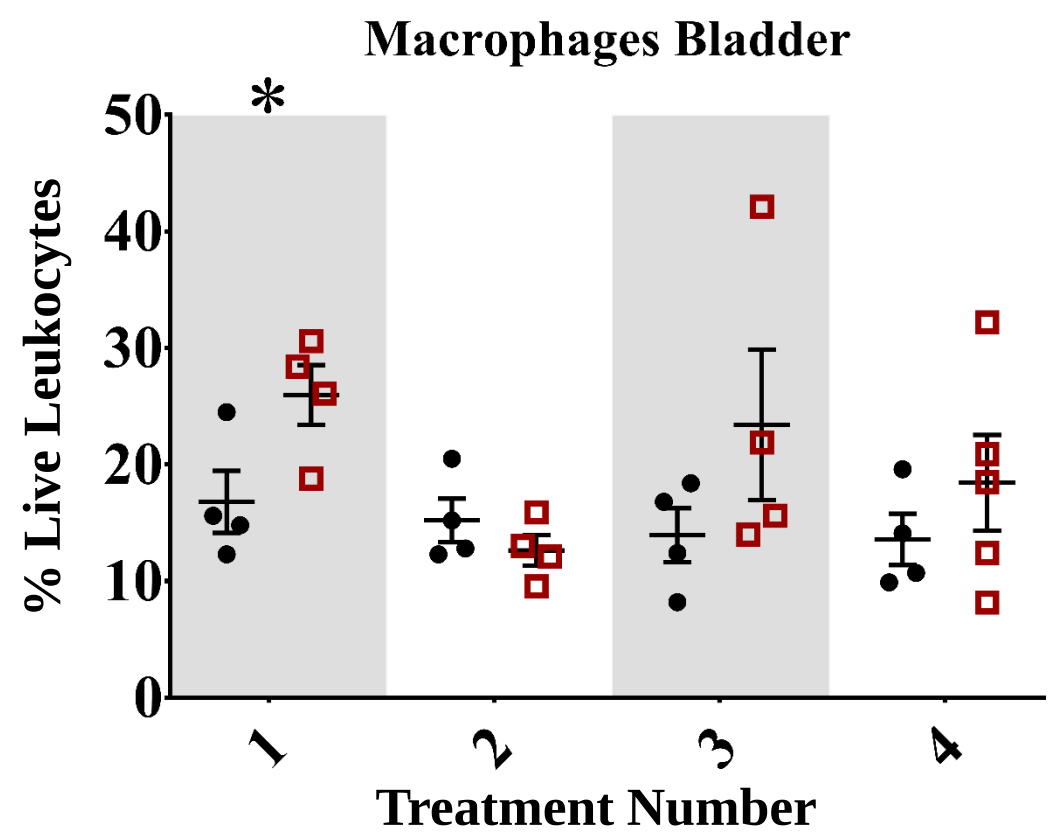
Shifting Immune Cell Populations



Initial Influx of Macrophages and Granulocytes in Bladder

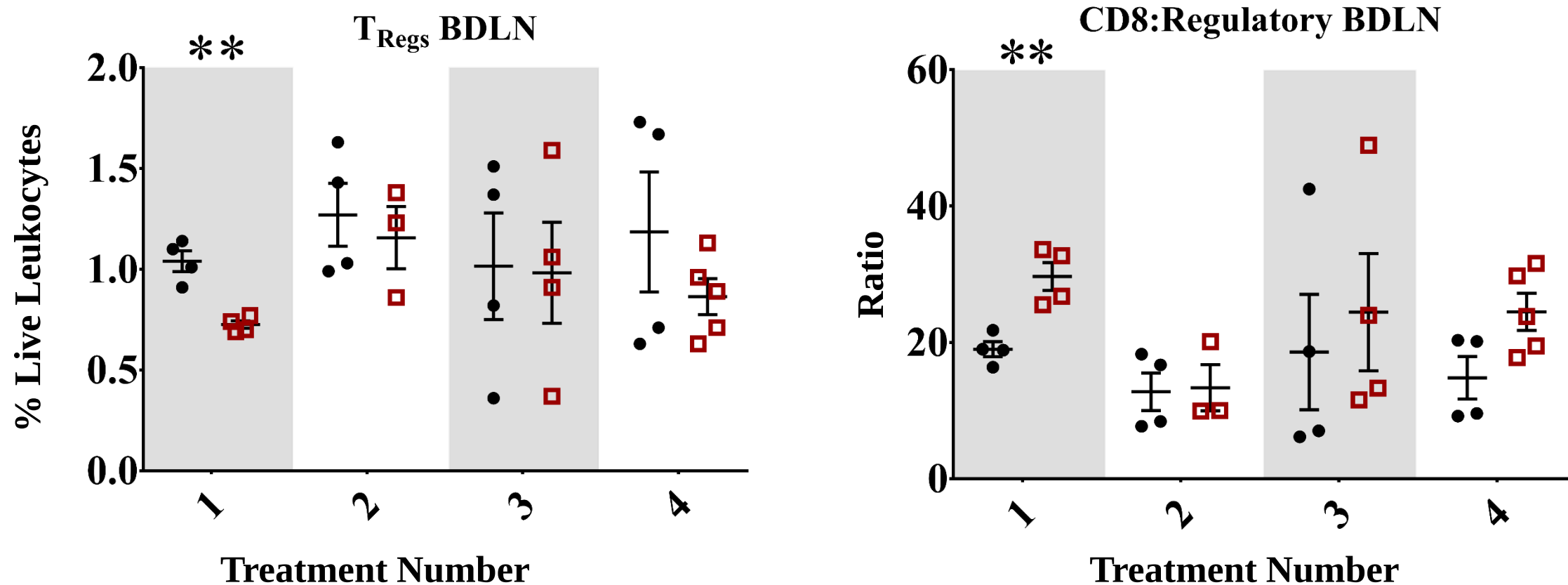
CS/IL-12 leads to enhanced macrophage infiltration in the bladder.

CS/IL-12 causes an initial influx of granulocytes followed by a decrease in MDSCs in the bladder.



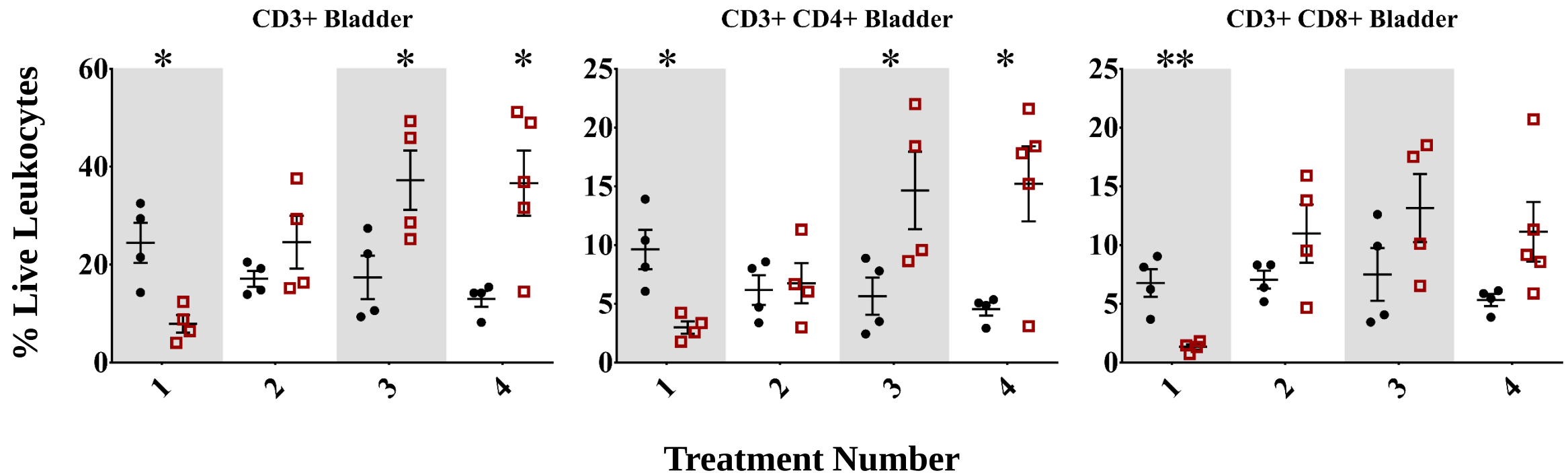
Initial Reduction of Regulatory T-cell in Draining Lymph Node

CS/IL-12 reduces T_{Reg} infiltration in the BDLNs after the first treatment.



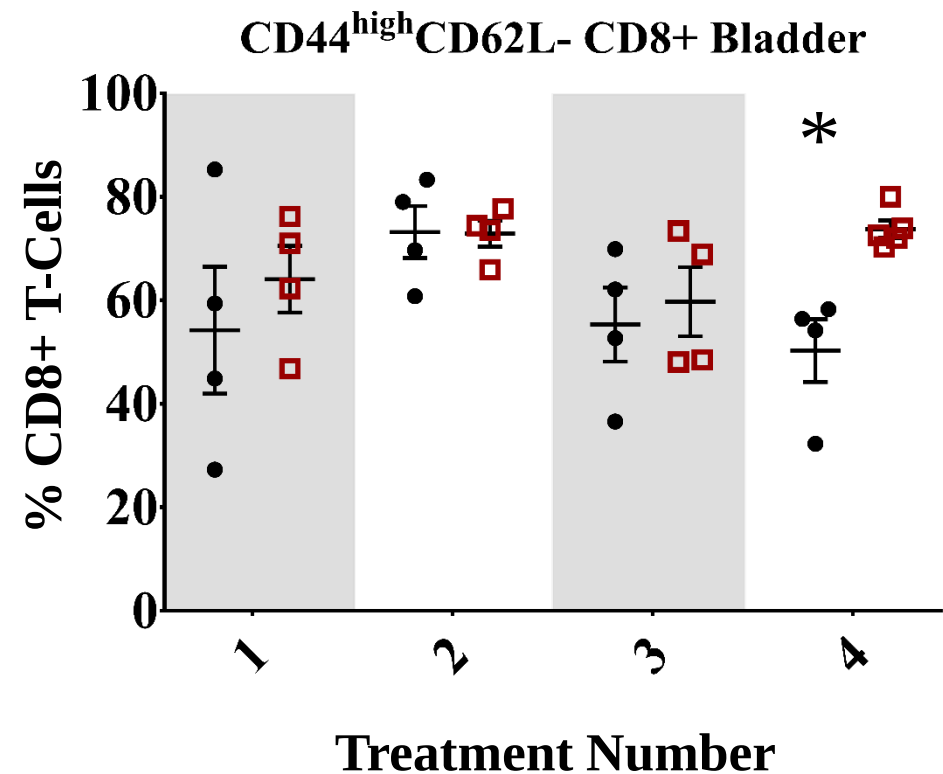
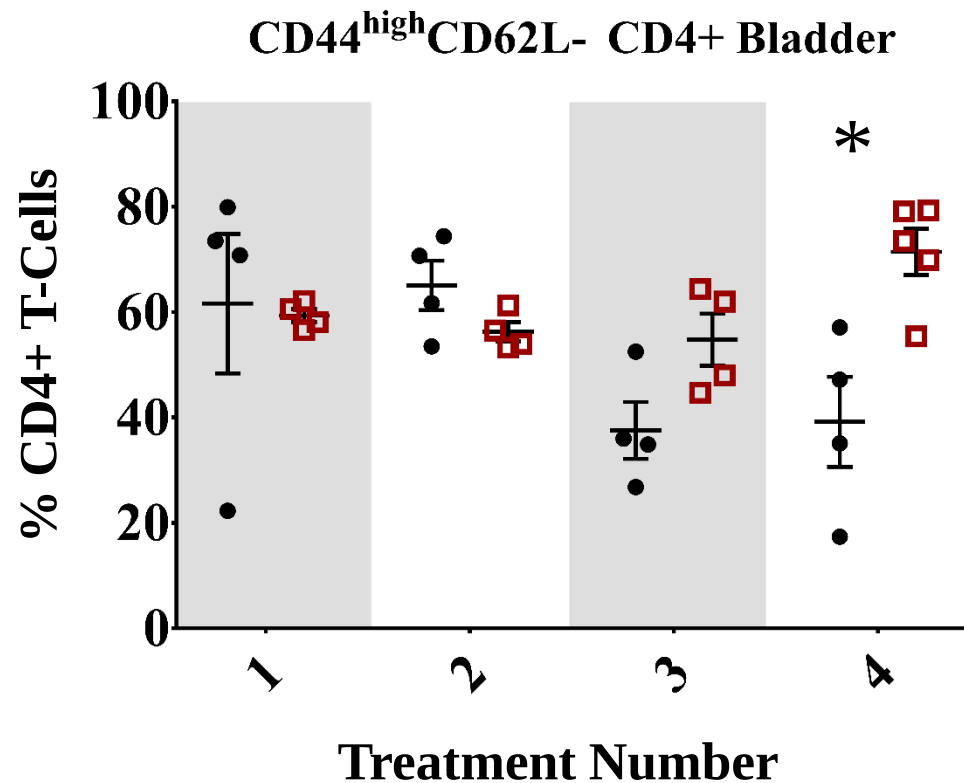
Delayed Infiltration of CD4+ T-cells into the Bladder

CS/IL-12 leads to enhanced CD3+ and CD4+ T-cell infiltration in the bladder after the third treatment in the bladder.



Effector-Memory T-Cells Become Dominant in the Bladder

Effector memory T-cells are the dominant phenotype in the bladder for both CD4+ and CD8+ cells by the 4th treatment.



Take Home Messages

- Intratumoral CS/IL-12 is effective in a range of tumor models
- Intravesical CS/IL-12 eliminates the majority of bladder tumors
 - Generates long-lasting memory
 - Effectors are different for initial and memory responses
 - Systemic and dynamic
 - Early treatments effects can have delayed outcomes

Strategic delivery of cytokines maximizes their therapeutic potential.

Acknowledgements



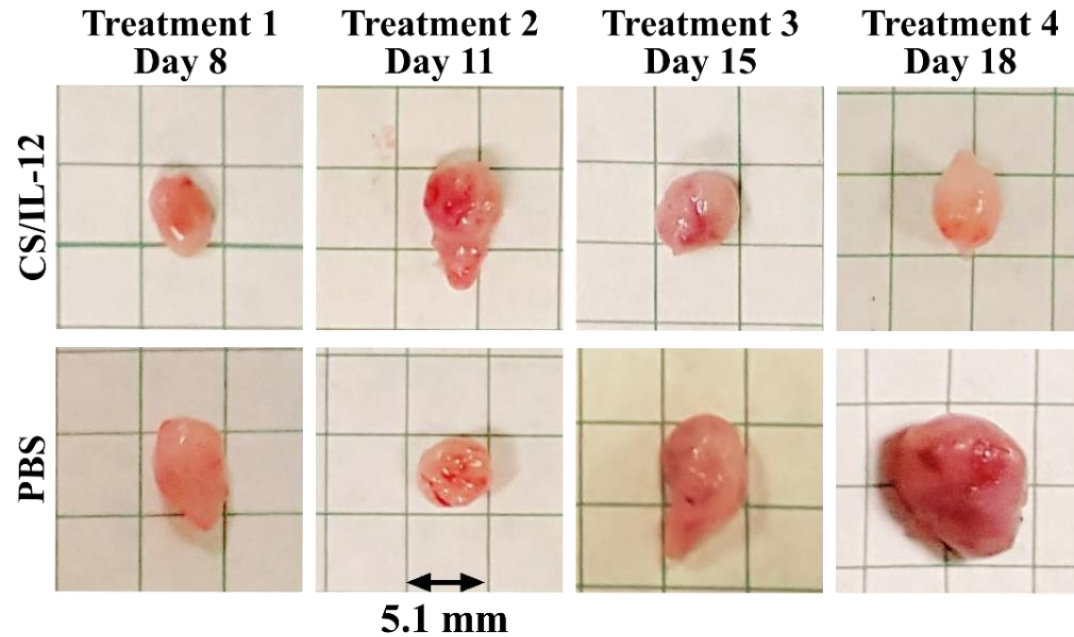
A decorative network graph with colored nodes (blue, orange, yellow, red, white) and lines connecting them, located in the corners of the slide.

Questions?

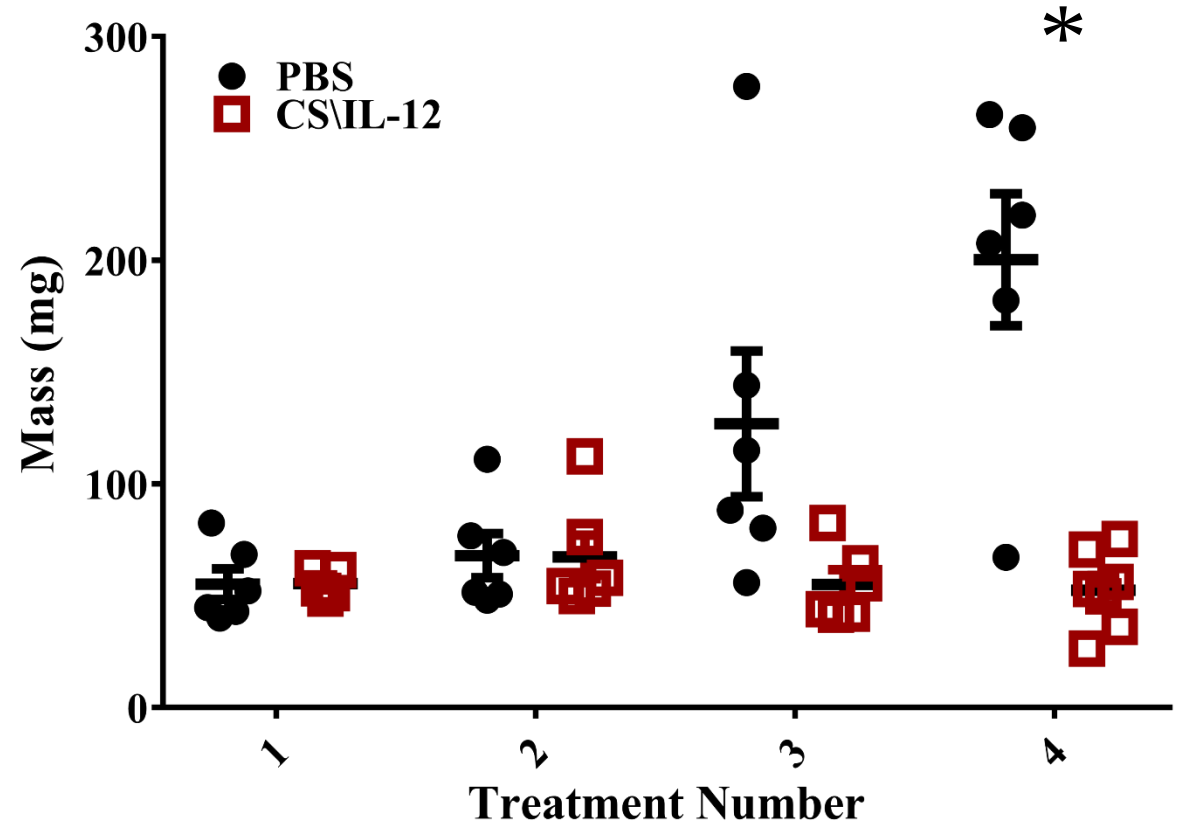
Come see the rest of the data at
poster #349.

Importance of Treatment Number

Bladder Morphology

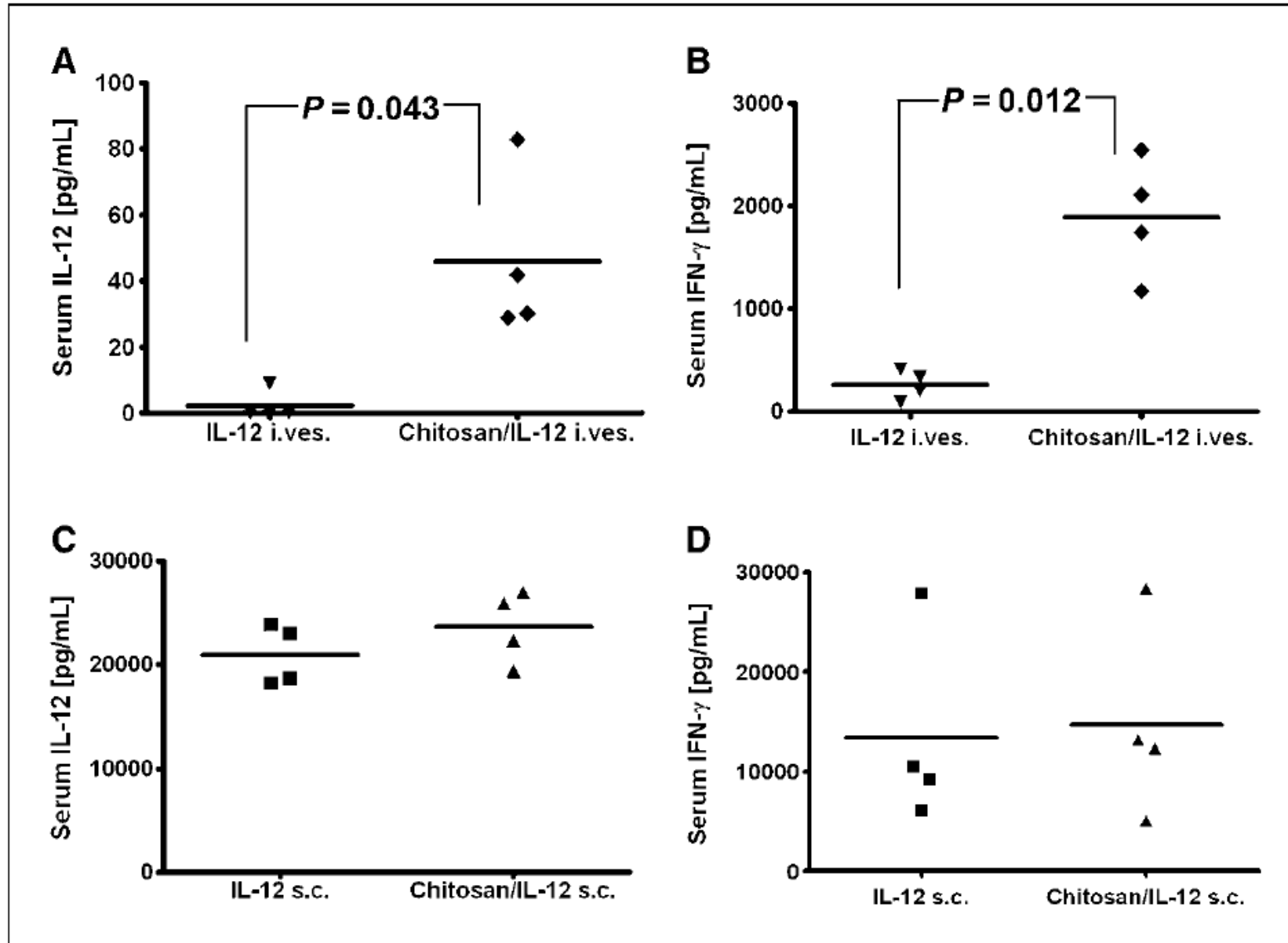


Bladder Mass

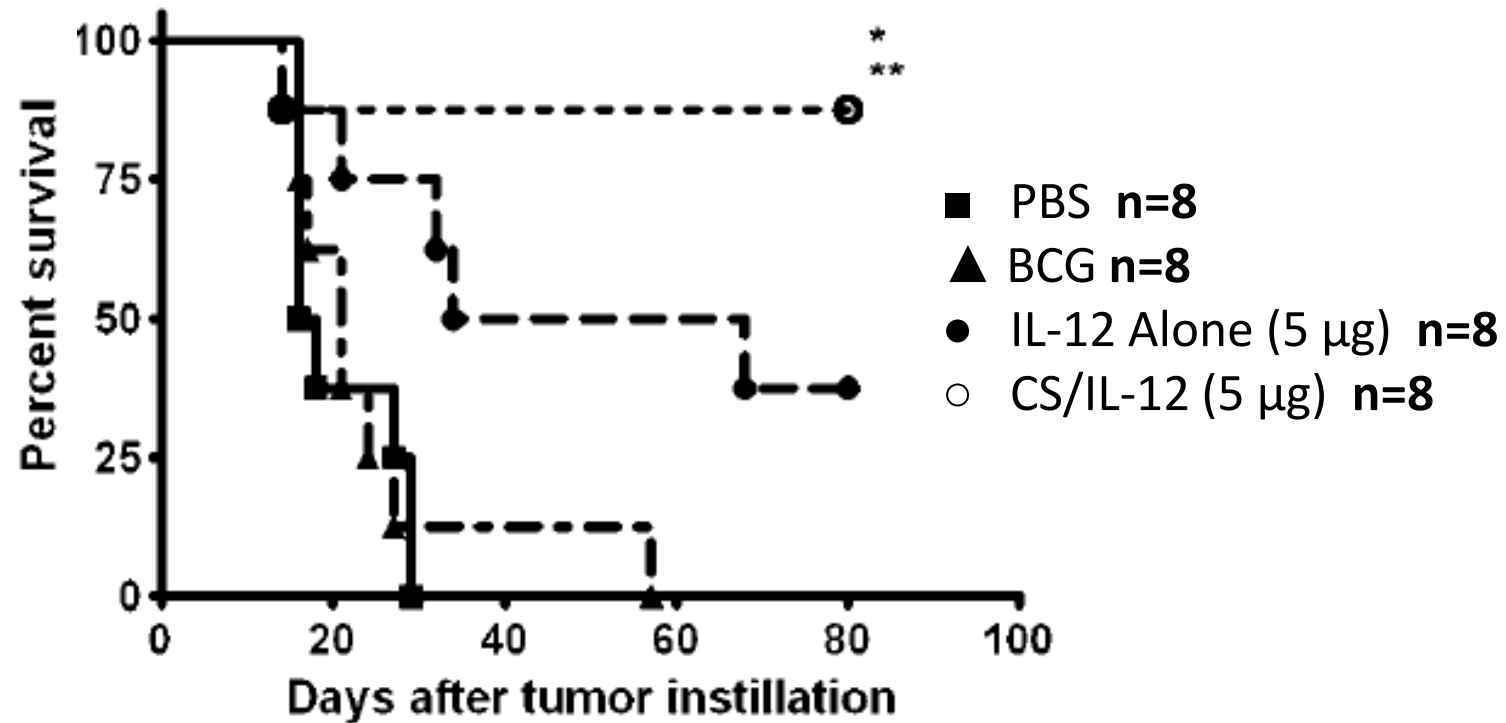
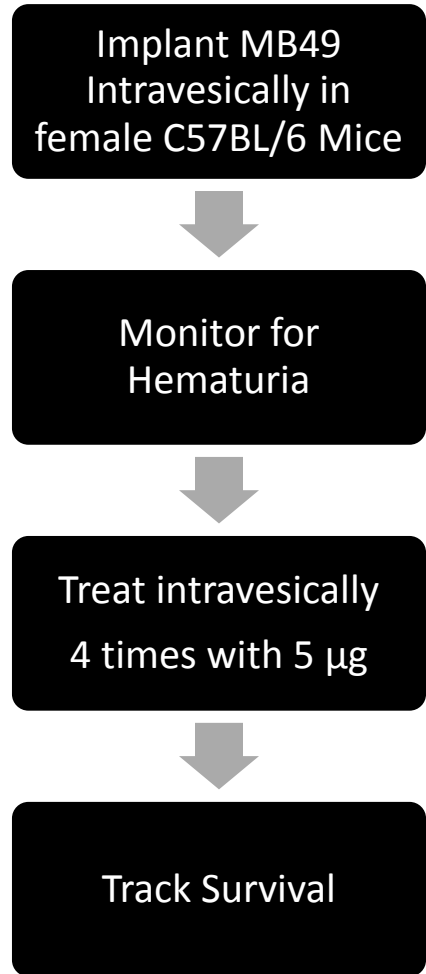


Differences in tumor sizes begin to appear by the third treatment.

Chitosan's Effect on Serum Levels of IL-12 and IFN γ

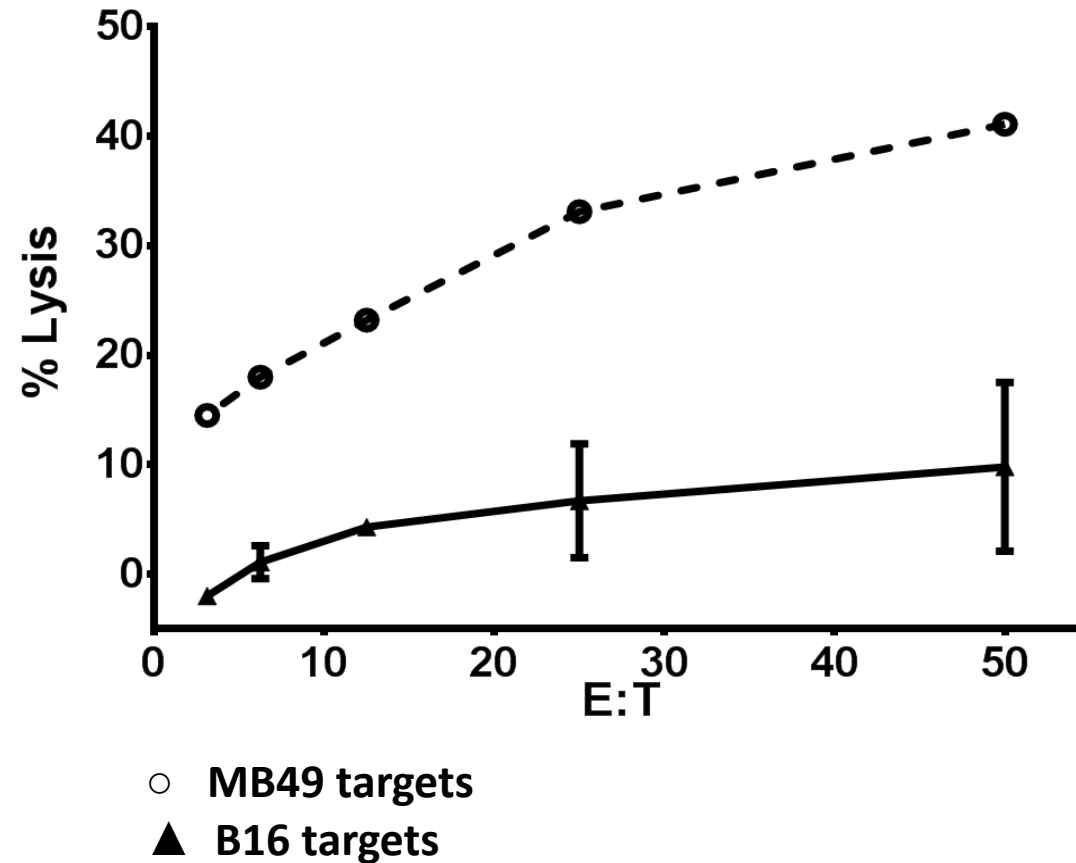
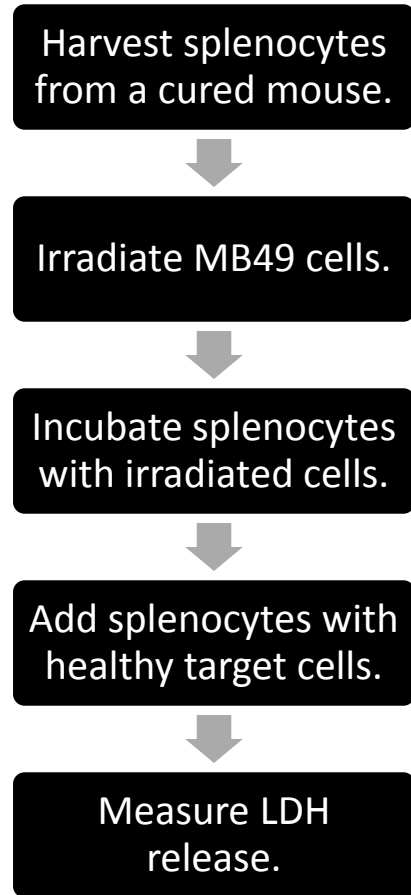


Chitosan/IL-12 Immunotherapy is superior to BCG



CS/IL-12 is superior to BCG and IL-12 alone at eradicating intravesical MB49 tumors.

Splenocytes from cured mice kill targets in a tumor specific manner.



Efficacy of BCG?

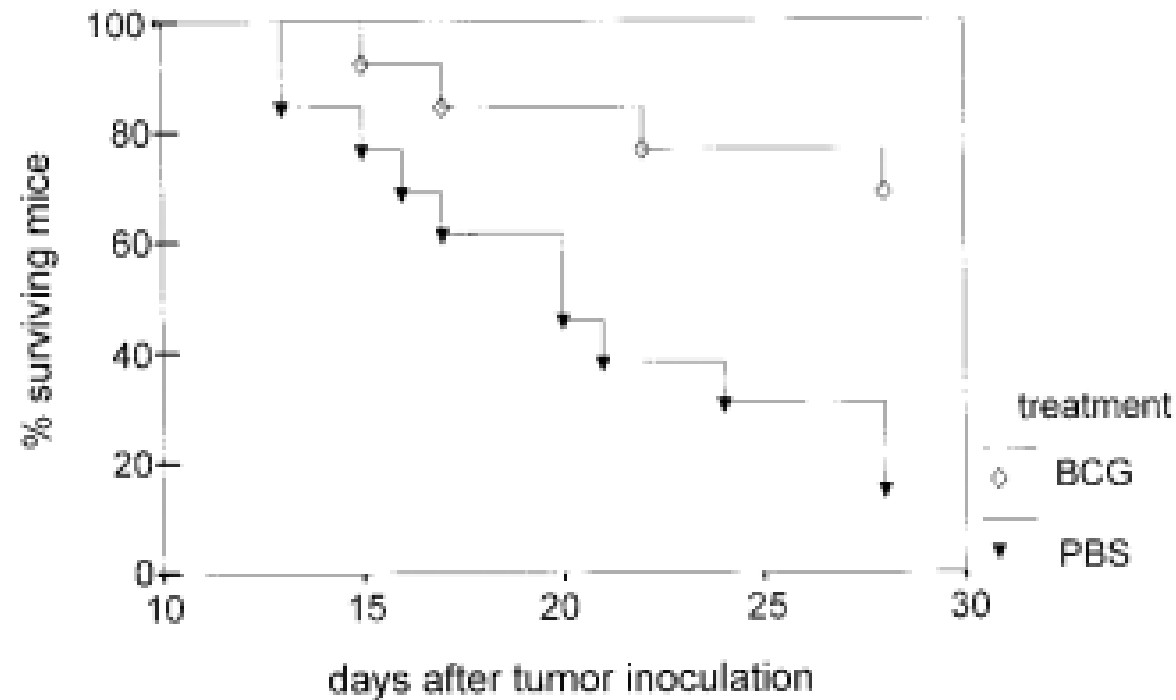
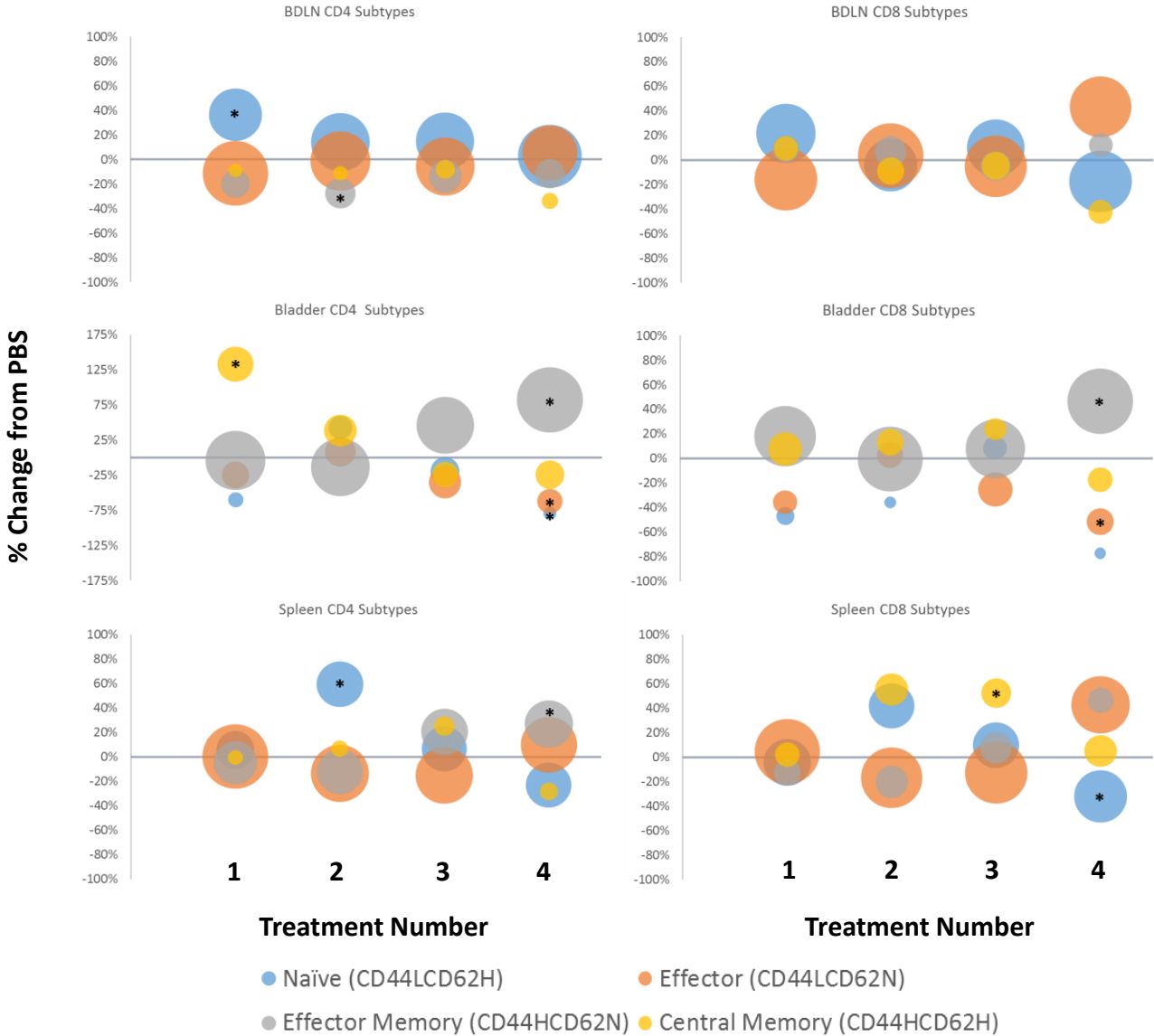


Fig. 4. Kaplan-Meier analysis of murine survival after intravesical BCG immunotherapy. Mice received four intravesical instillation treatments with BCG. The control group received four PBS instillations. The experiment was terminated at day 28 due to rapid tumor growth. $P = 0.005$, log-rank test.

Changes in T-Cell Memory Subsets

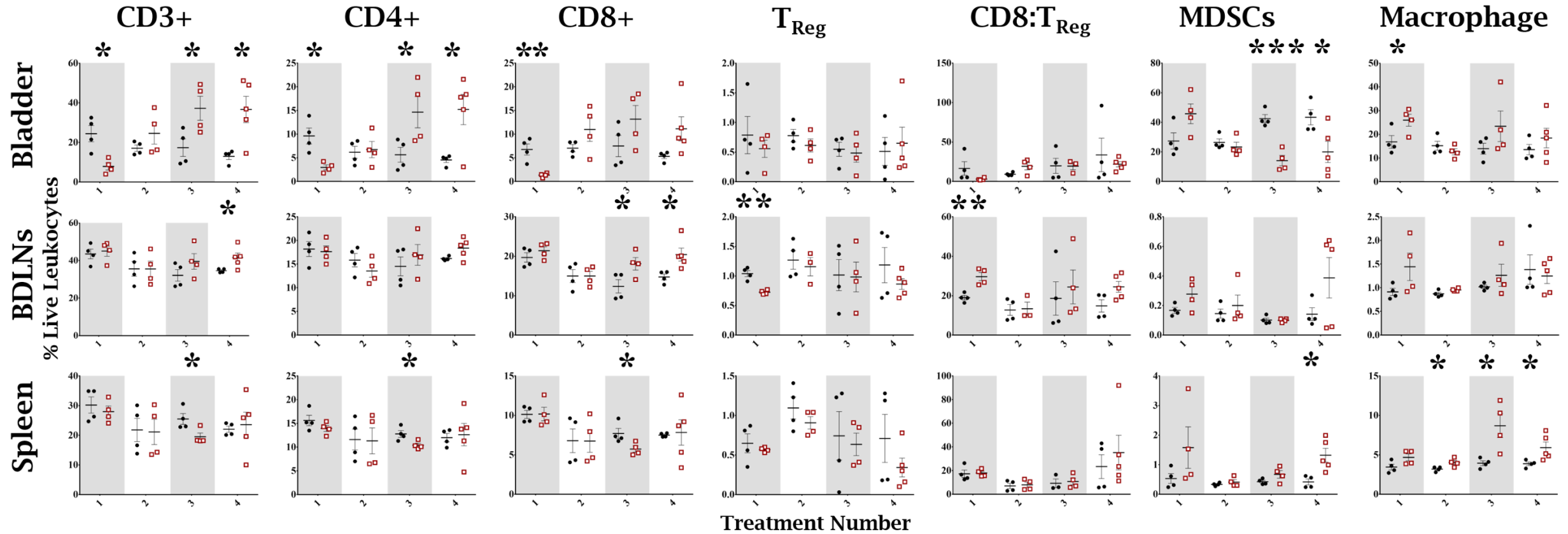


Infiltration Overview

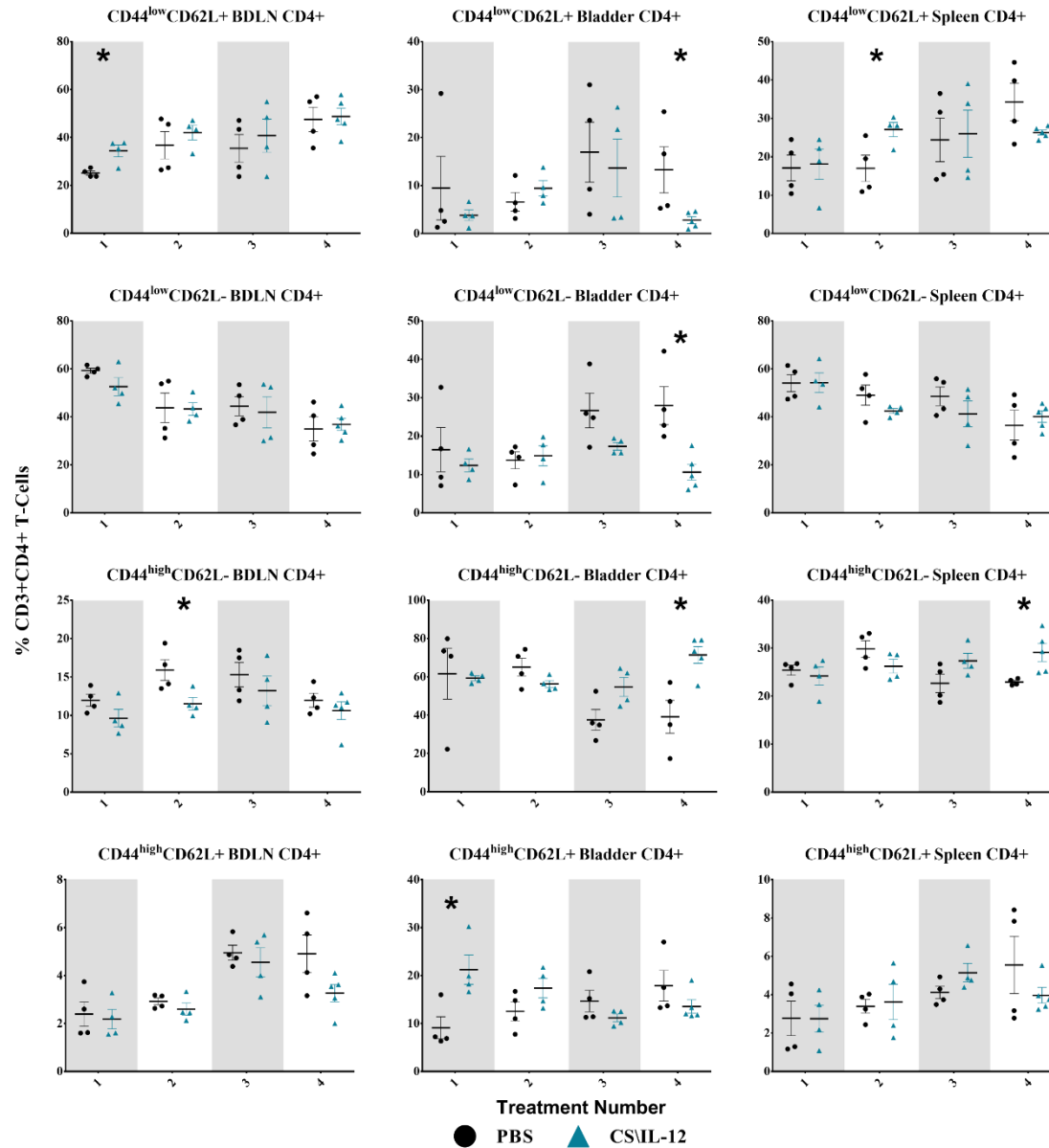


Bladder	MΦ, Gran, CD4-T _{CM} CD4, CD8	CD4, CD8, T _{EM} MDCS	CD4, T _{EM} CD4-T _{Naive} , T _{EFF} MDCS
BDLN	CD8:T _{Reg} , CD4-T _{Naive} T _{Reg}	CD8 CD4-T _{EFF}	CD8-T _{EFF}
Spleen	MΦ, CD4-T _{Naive}	MΦ, CD8-T _{CM} CD3, CD4, CD8, NK	MΦ, DC, MDCS, CD4-T _{EM} CD8-T _{Naive}

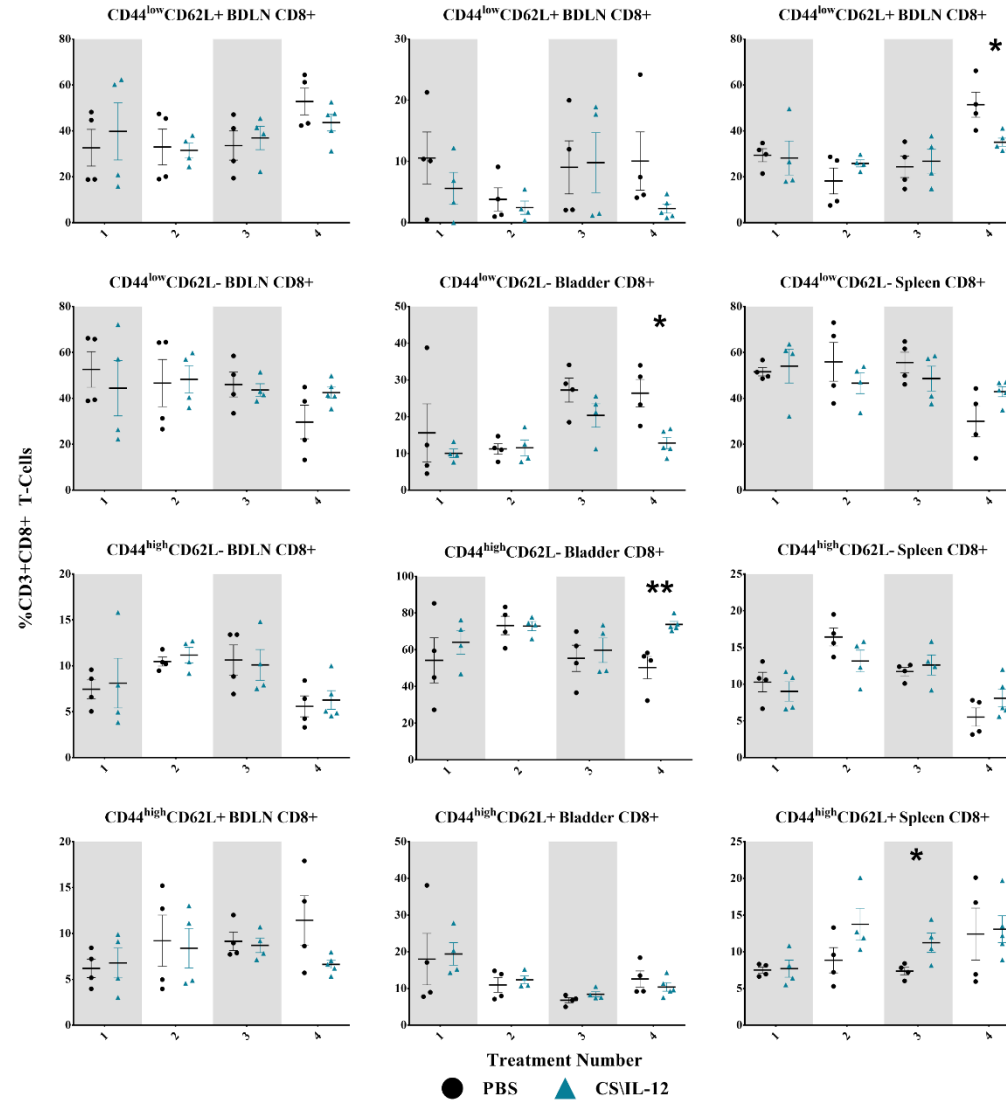
Mechanisms: Importance of Treatment Number



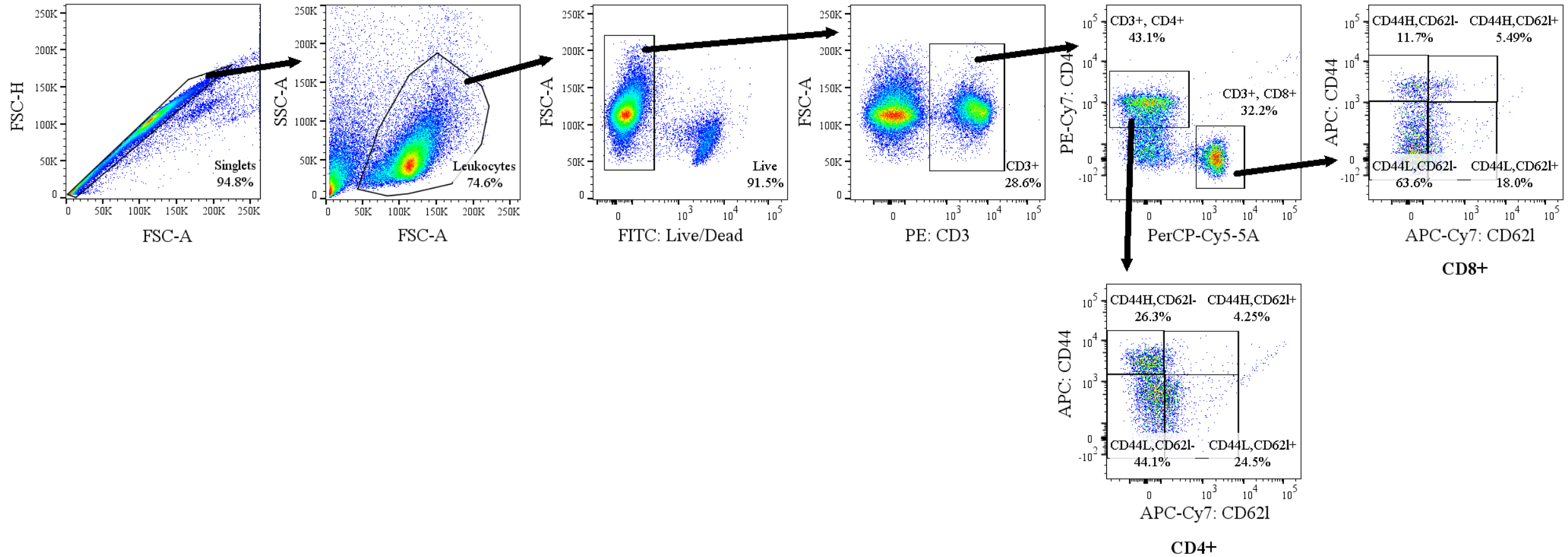
Memory CD4



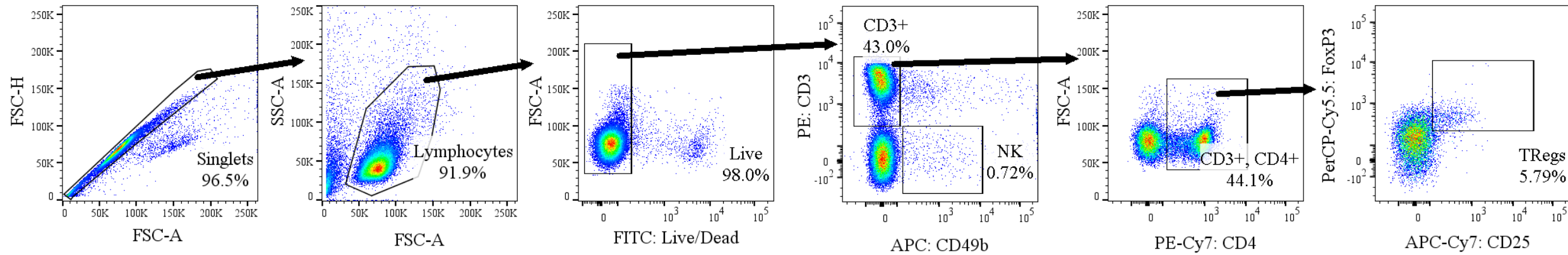
Memory CD8



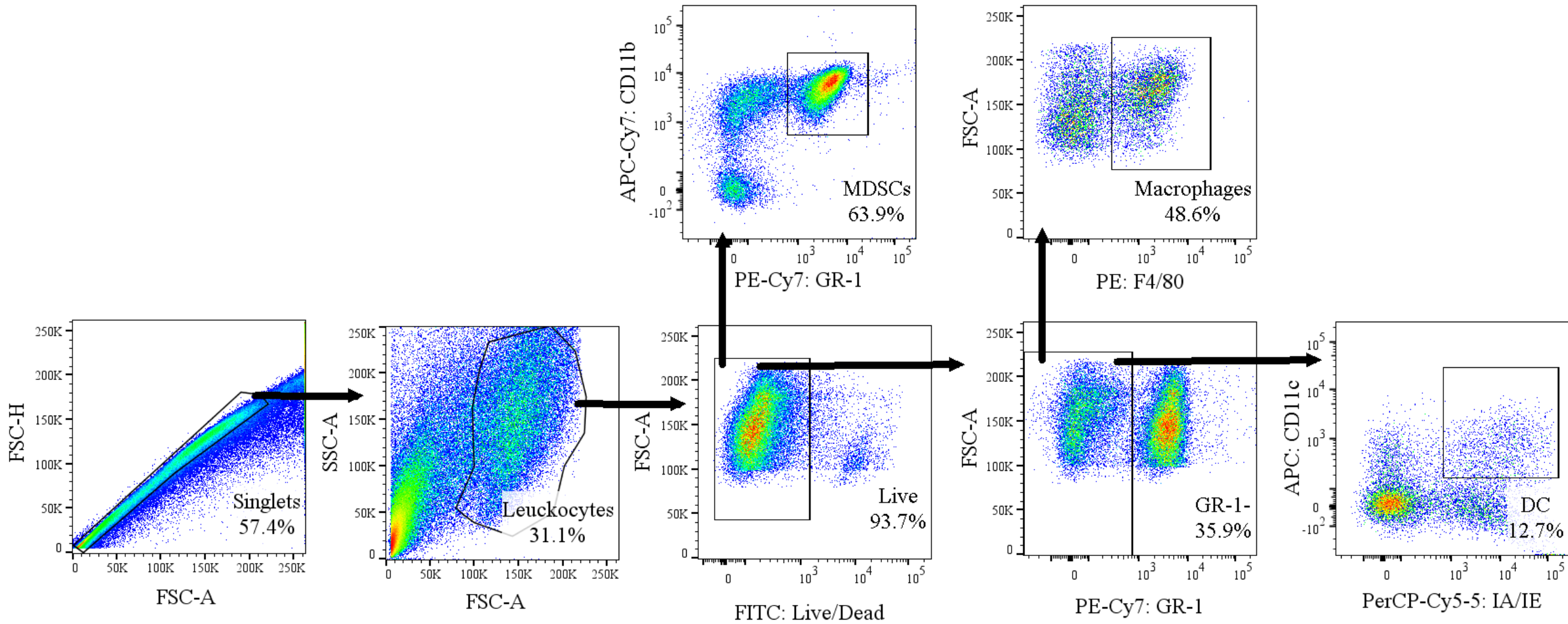
Memory Panel



Regulatory Panel

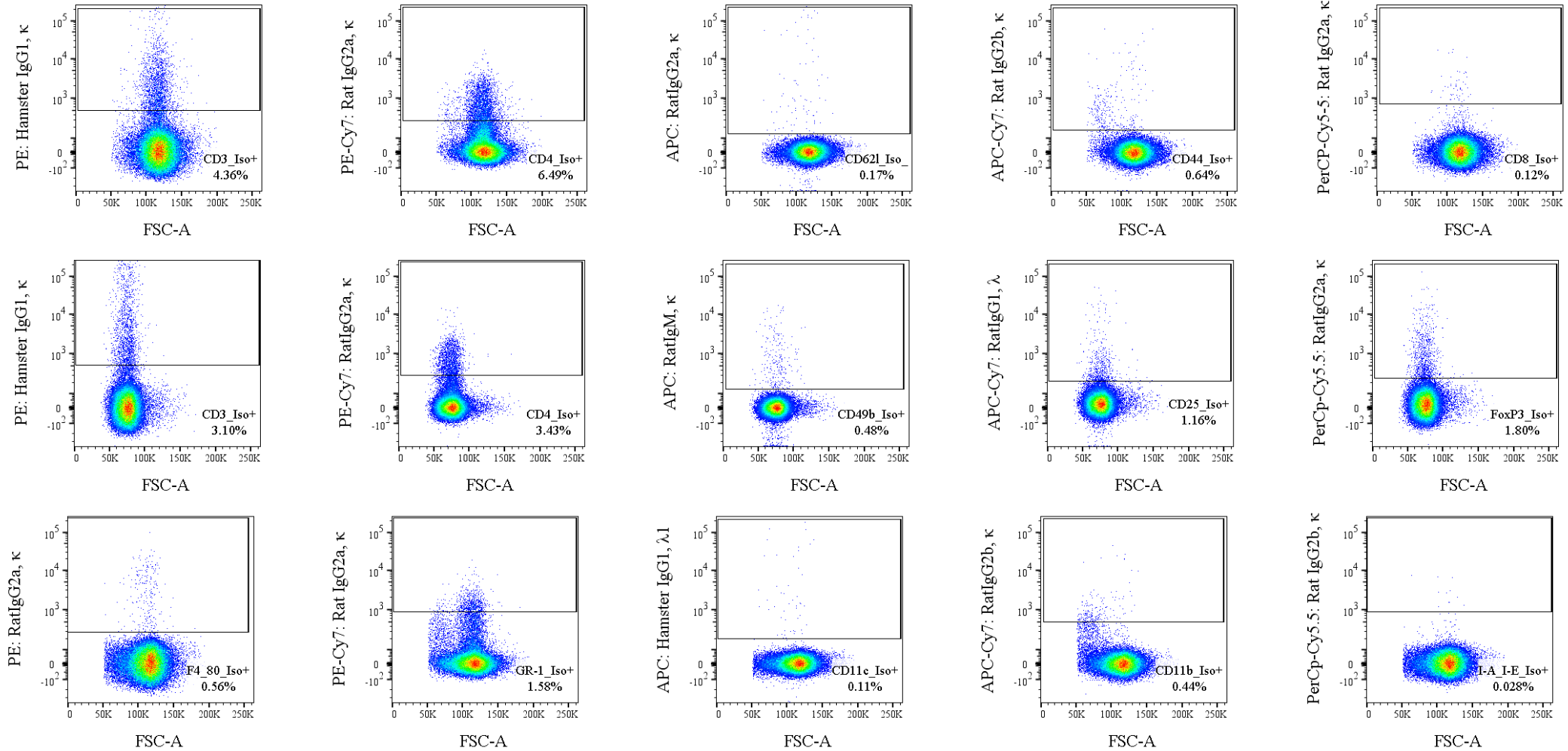


Myeloid Panel

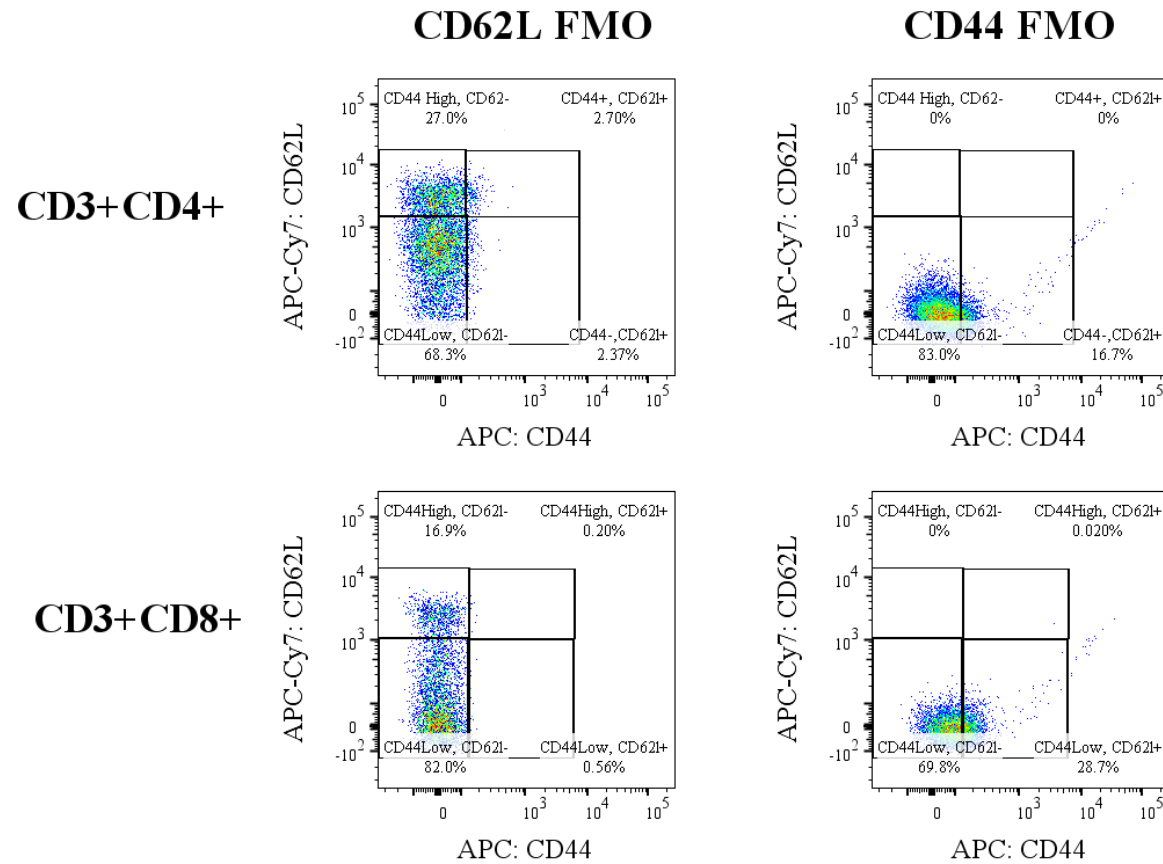


Isotypes

A



B



Depletion

