

# Engineering Precise Production of Lipopolysaccharides for Intratumoral Immune Activation By *Salmonella* Typhimurium

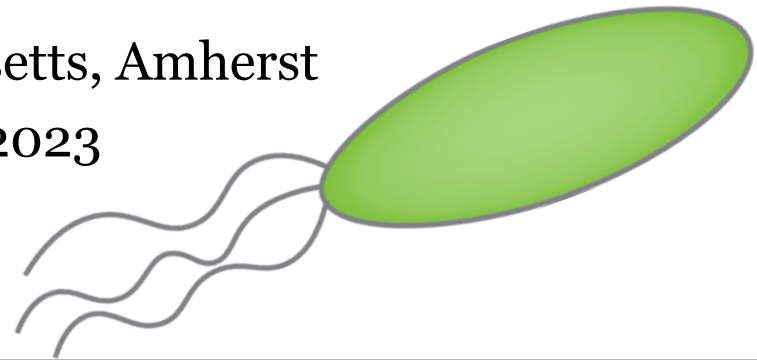
Lars Howell

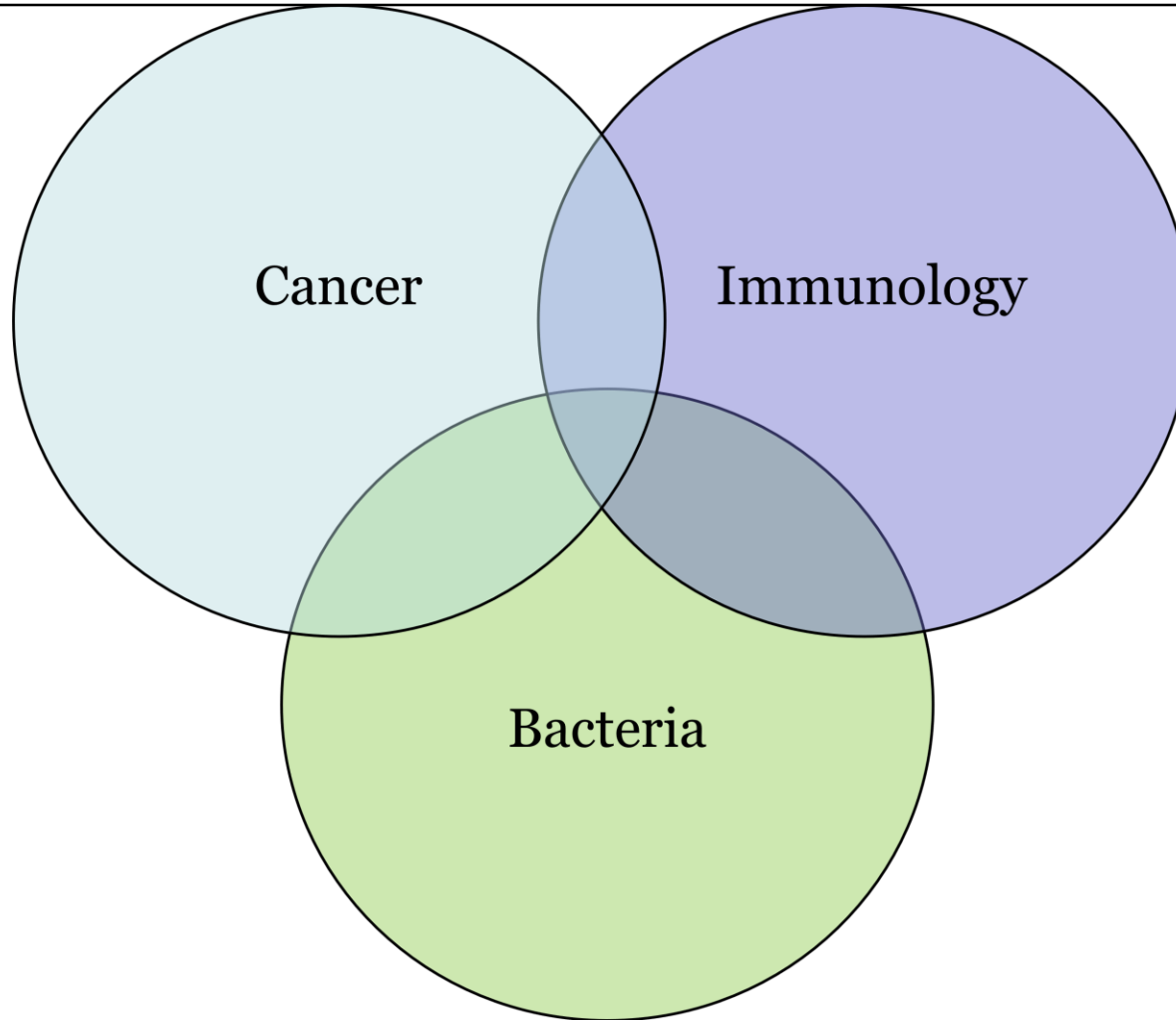
Forbes Lab

University of Massachusetts, Amherst

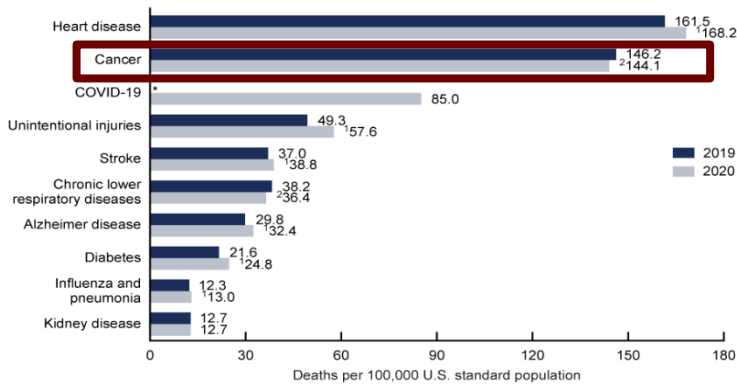
December 10, 2023

ICME

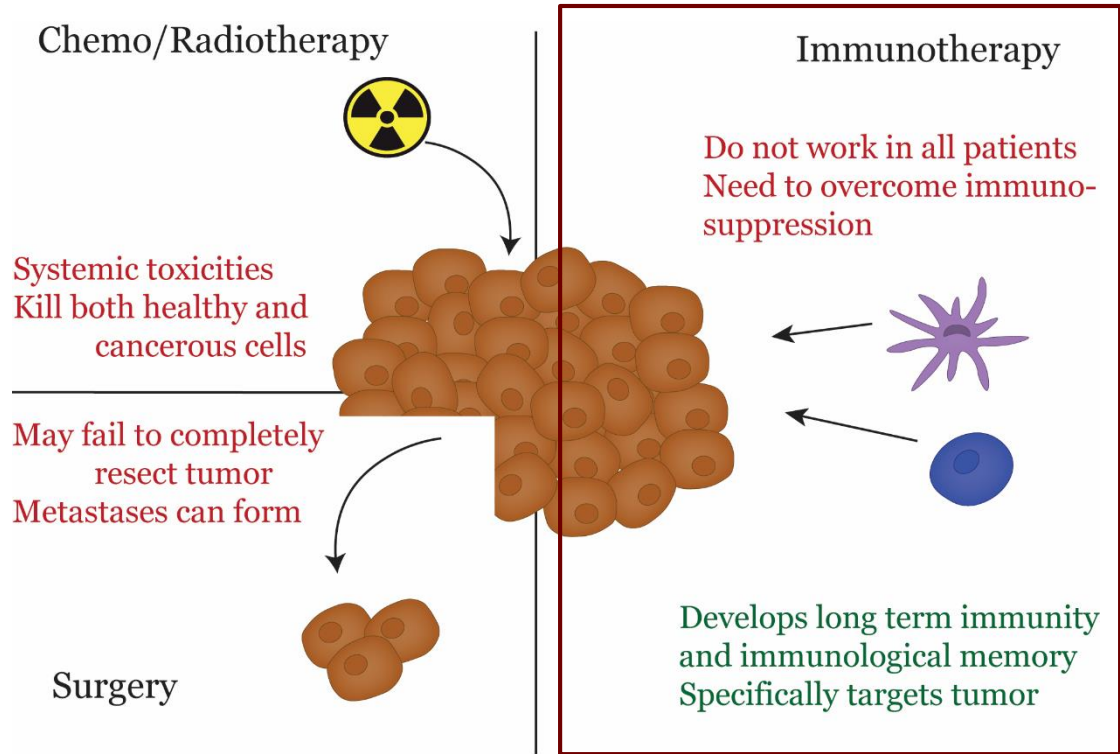




# Cancer is the second leading cause of death in the US



Total patient out of pocket costs:  
**\$21.09 billion per year**

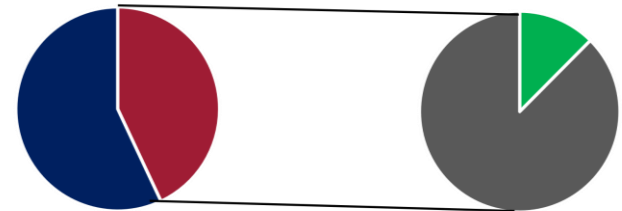


# Immunotherapies are class of promising treatments

**Developing therapies to specific tumor markers is expensive:**

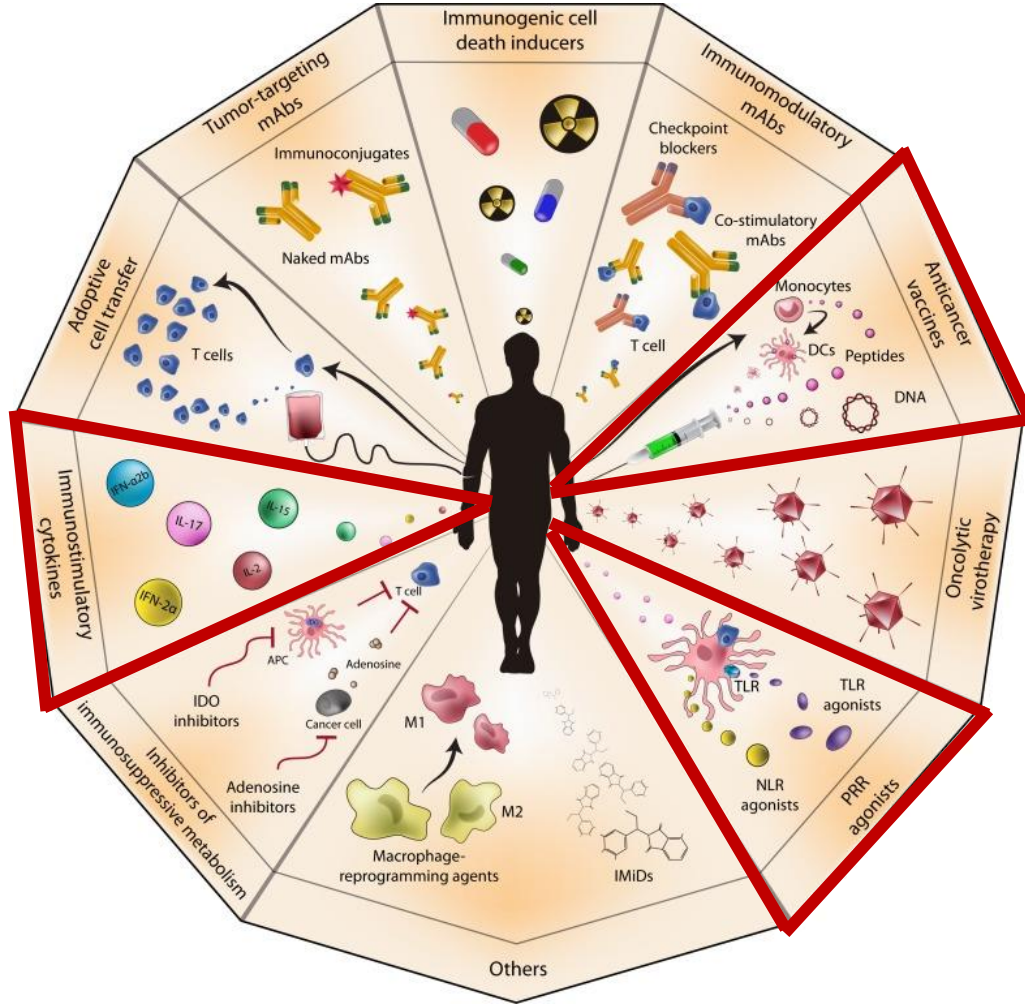
- Monoclonal antibodies: \$149,622/year
- CAR T cells: \$373,000–\$475,000/dose

Patients eligible for checkpoint blockade      Patient response to checkpoint blockade



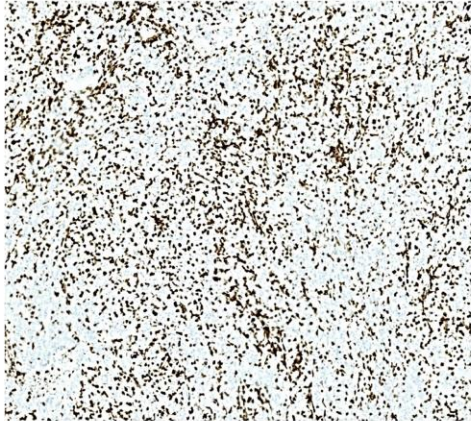
■ Eligible ■ Ineligible ■ Responsive ■ Nonresponsive

**How can we improve immunotherapies?**

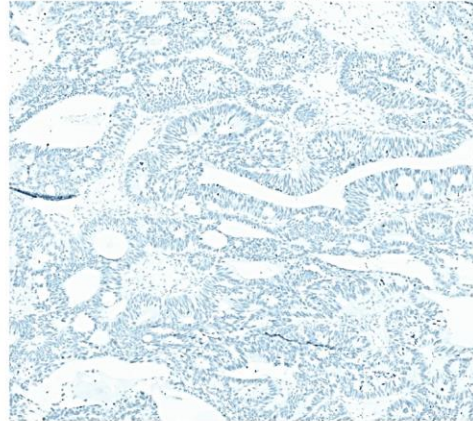


# Immunologically cold tumors are less responsive

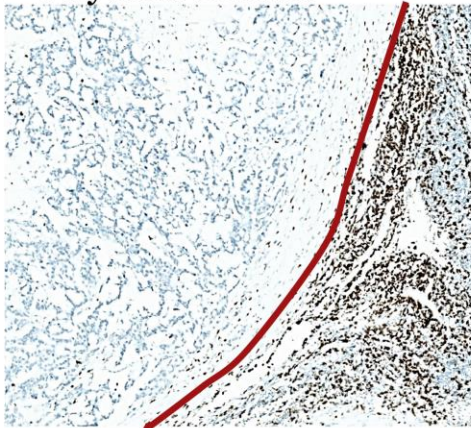
Hot – Lots of T cells



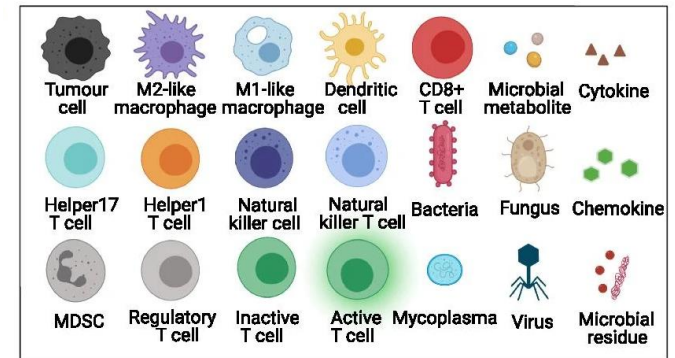
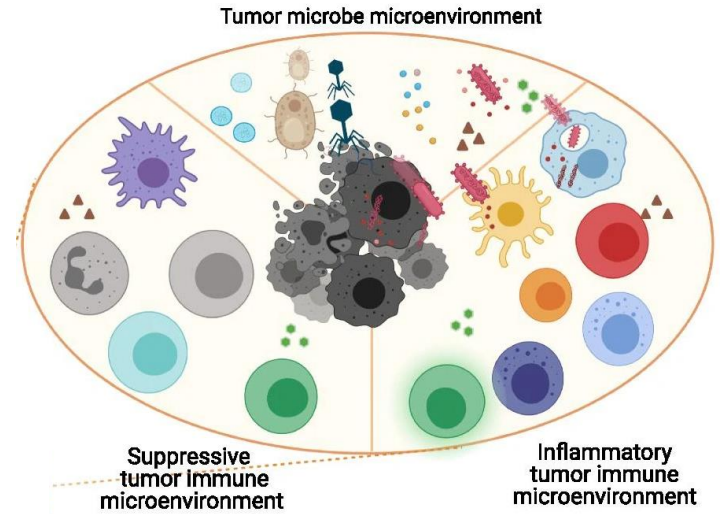
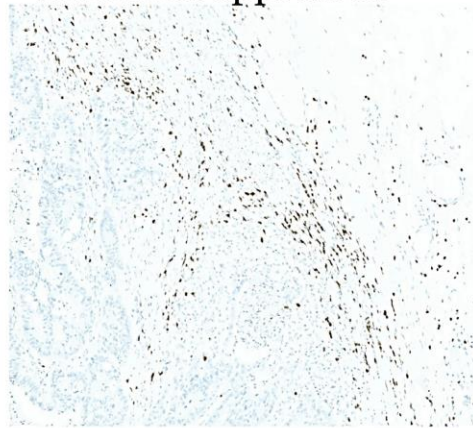
Cold – No T cells



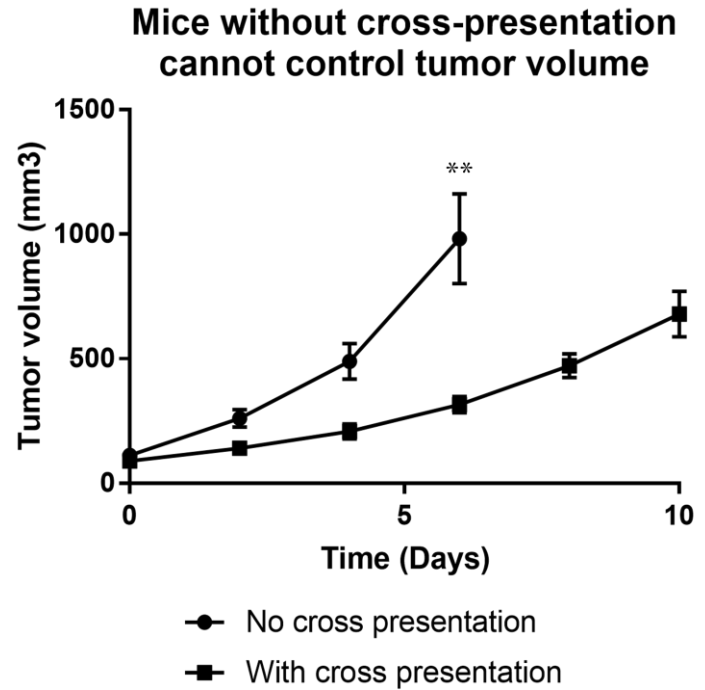
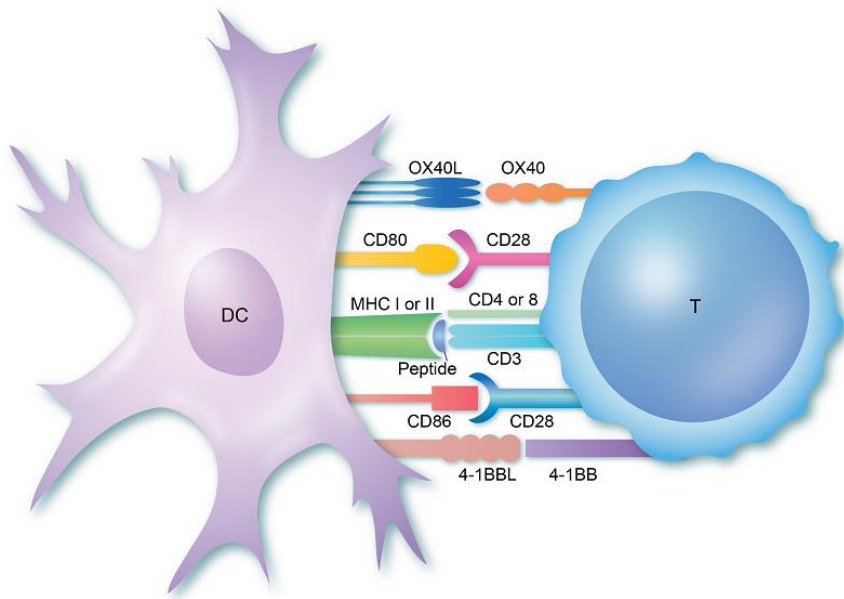
Physical Barrier



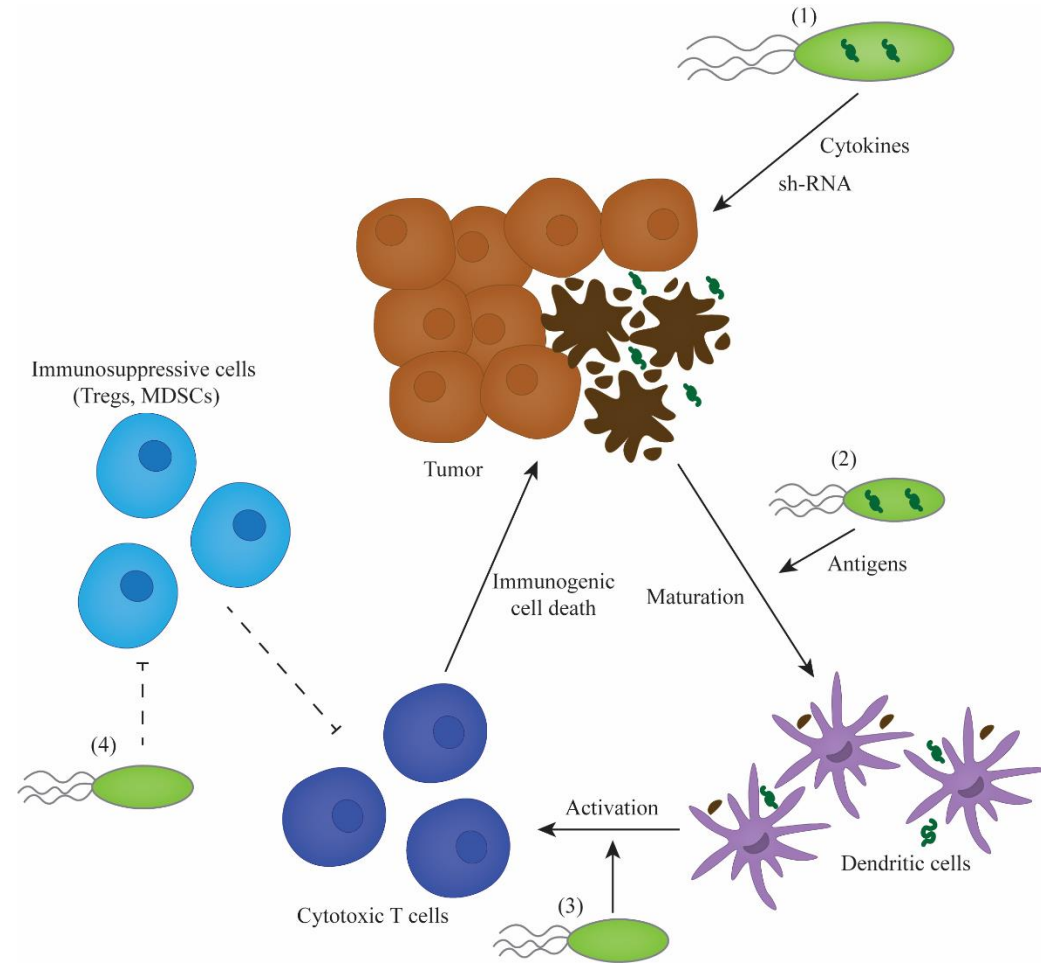
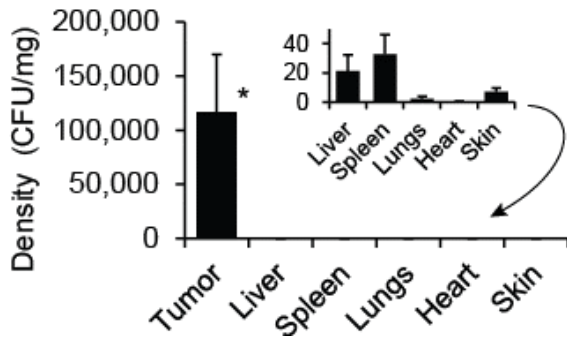
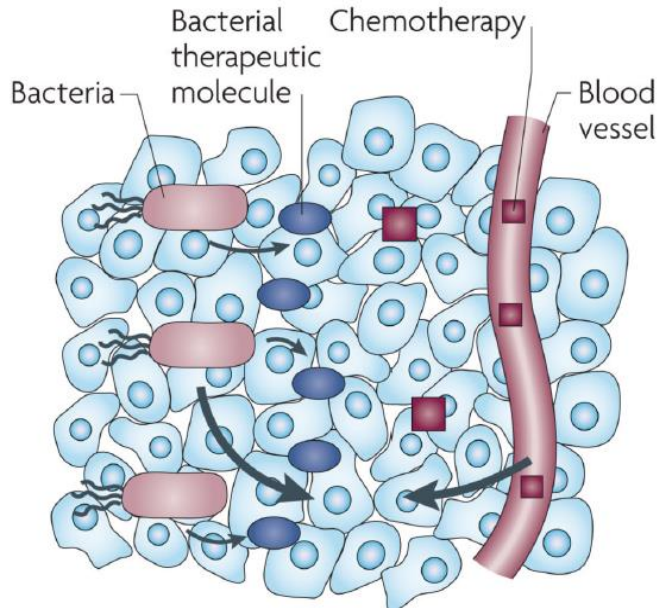
Immunosuppressed



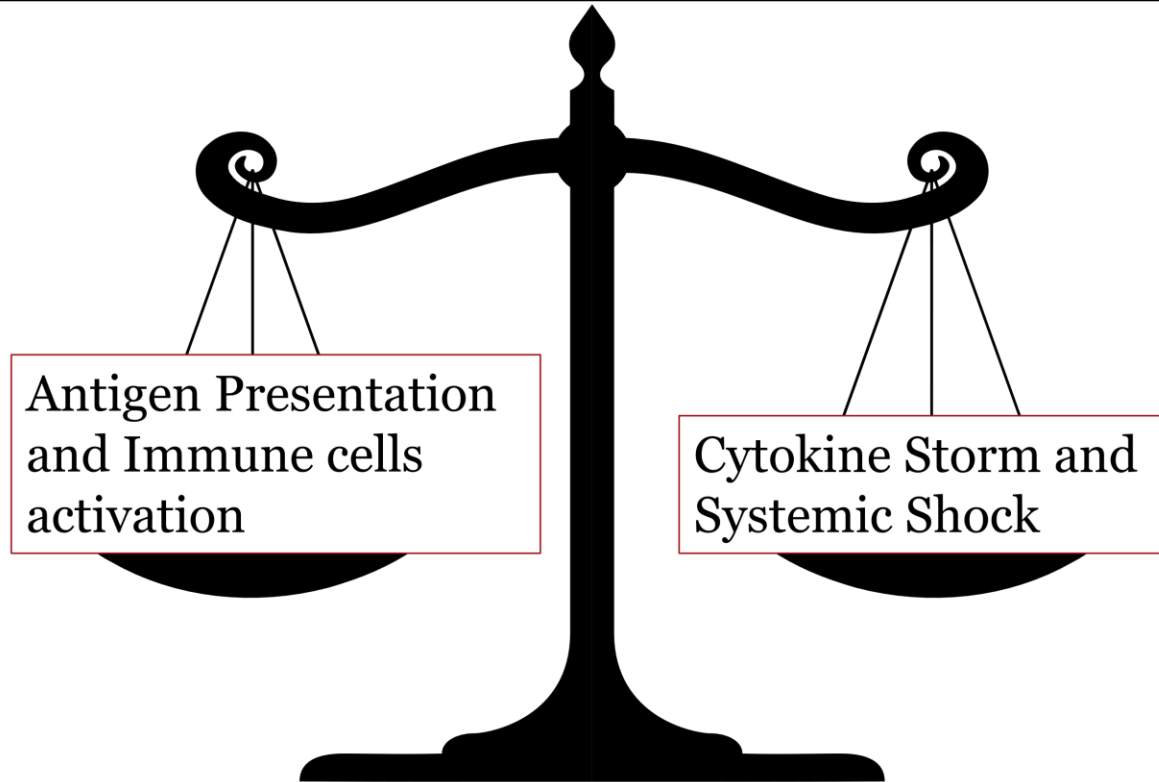
# Antigen cross-presentation is key to control tumors



# Bacteria overcome transport barriers to treat tumors



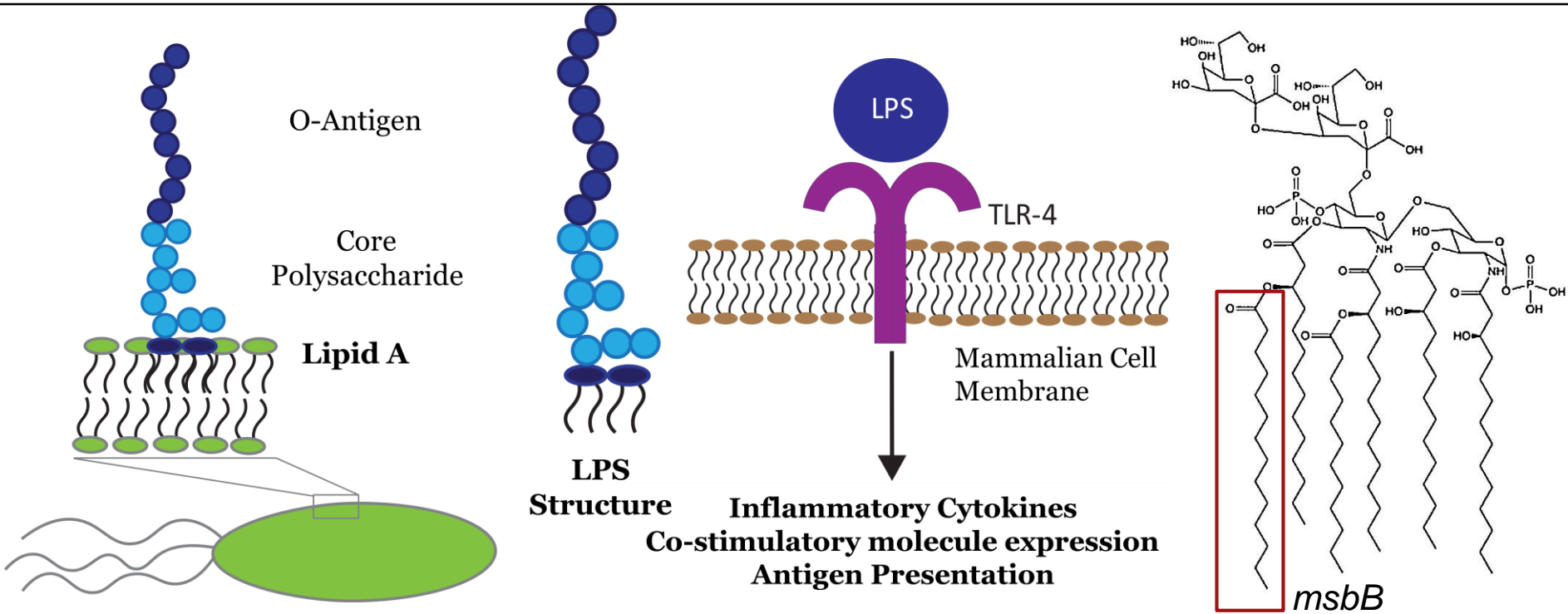
# Bacterial immunotherapies need to strike a balance



Many therapeutic bacterial strains have removed msbB and LPS production entirely



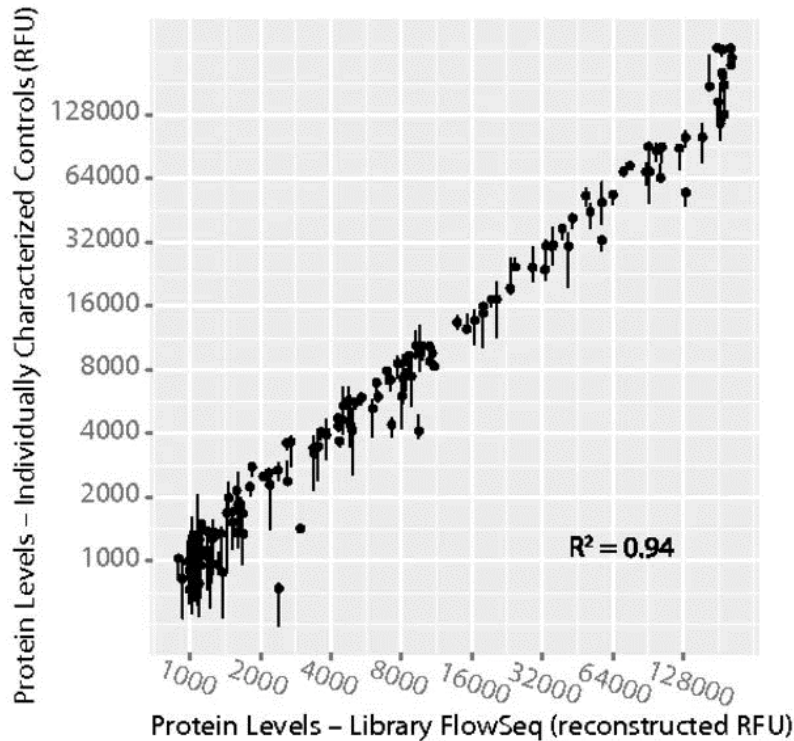
# Hypothesis



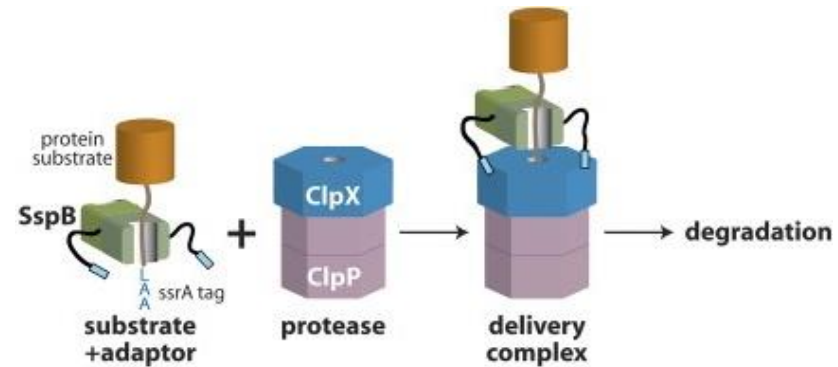
Production of LPS within the tumor can increase anti-tumor immune effects and Salmonella can deliver LPS controllable allowing for specific delivery

# Protein levels can be controlled using genetic tools

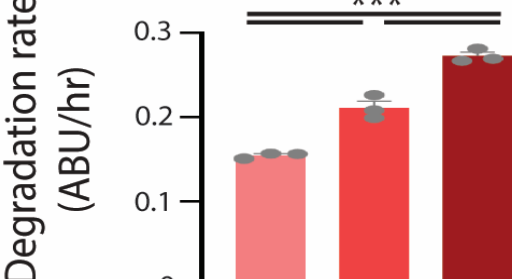
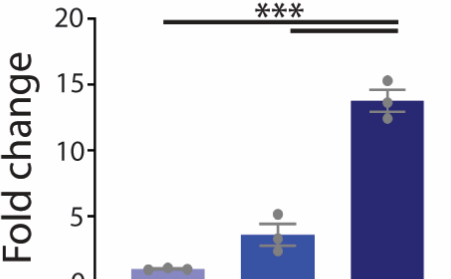
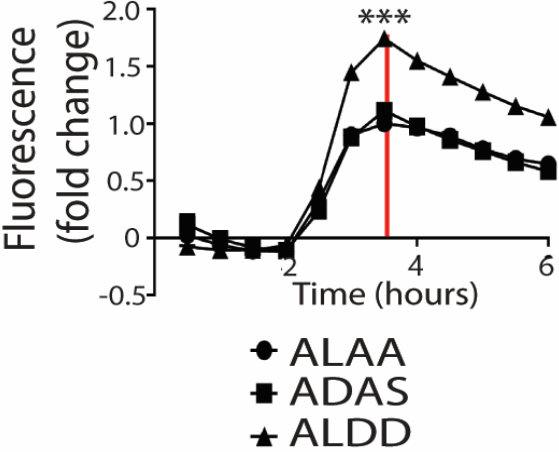
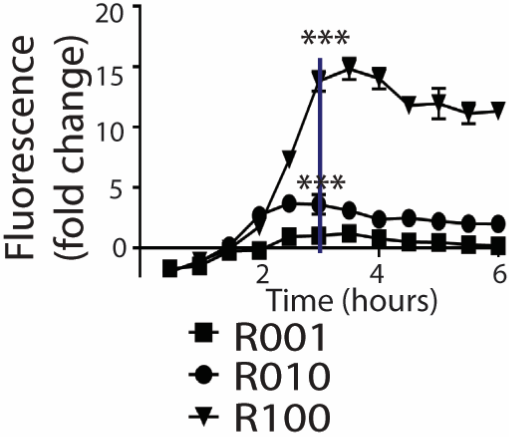
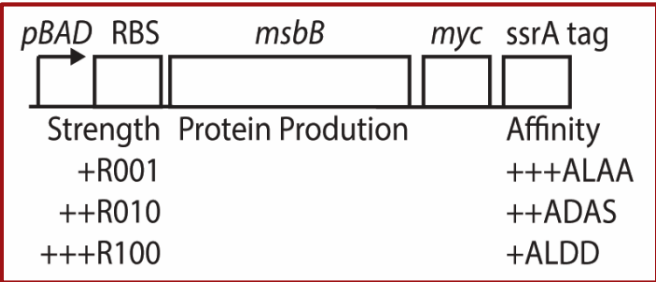
Ribosomal binding sites control the rate of protein translation



Degradation tags localize proteins to proteases for destruction

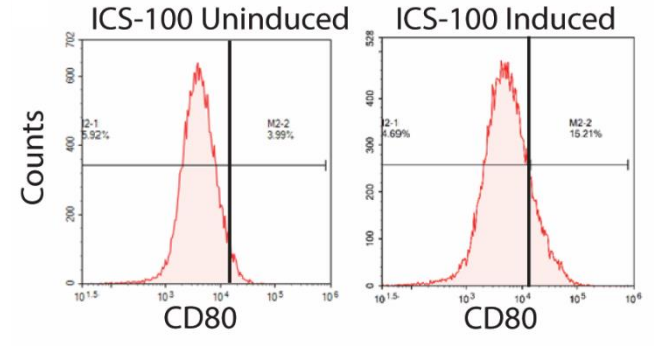
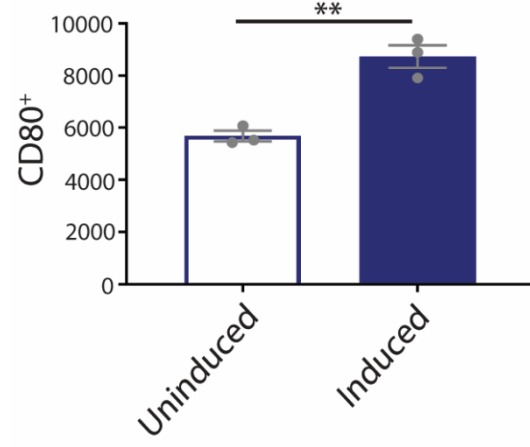
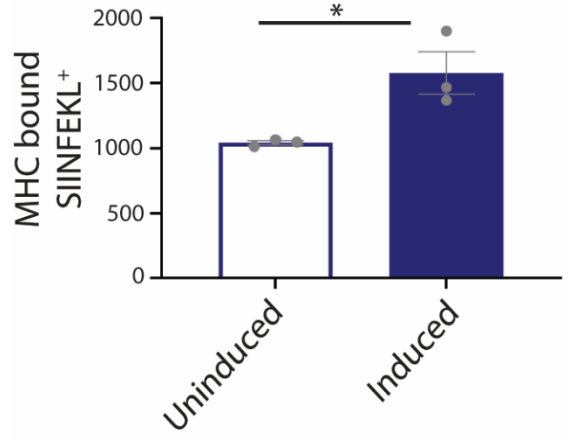
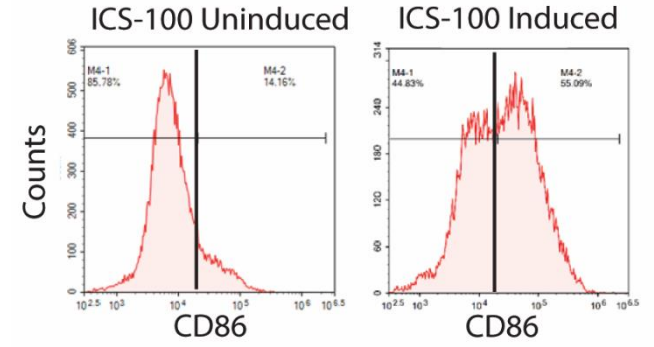
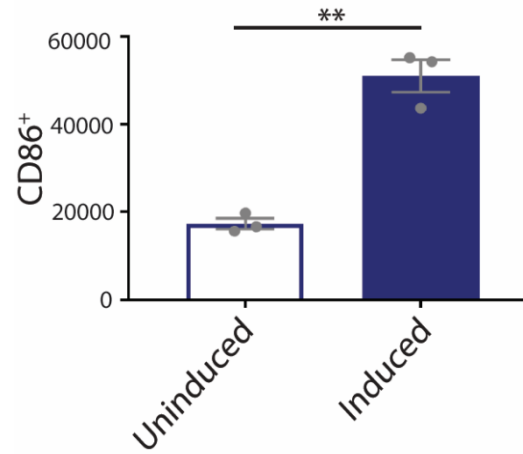
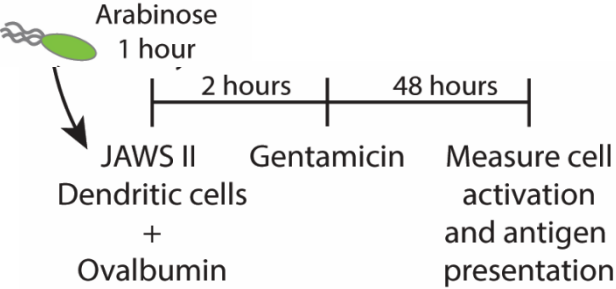


# RBSs and degradation tags control *msbB* production

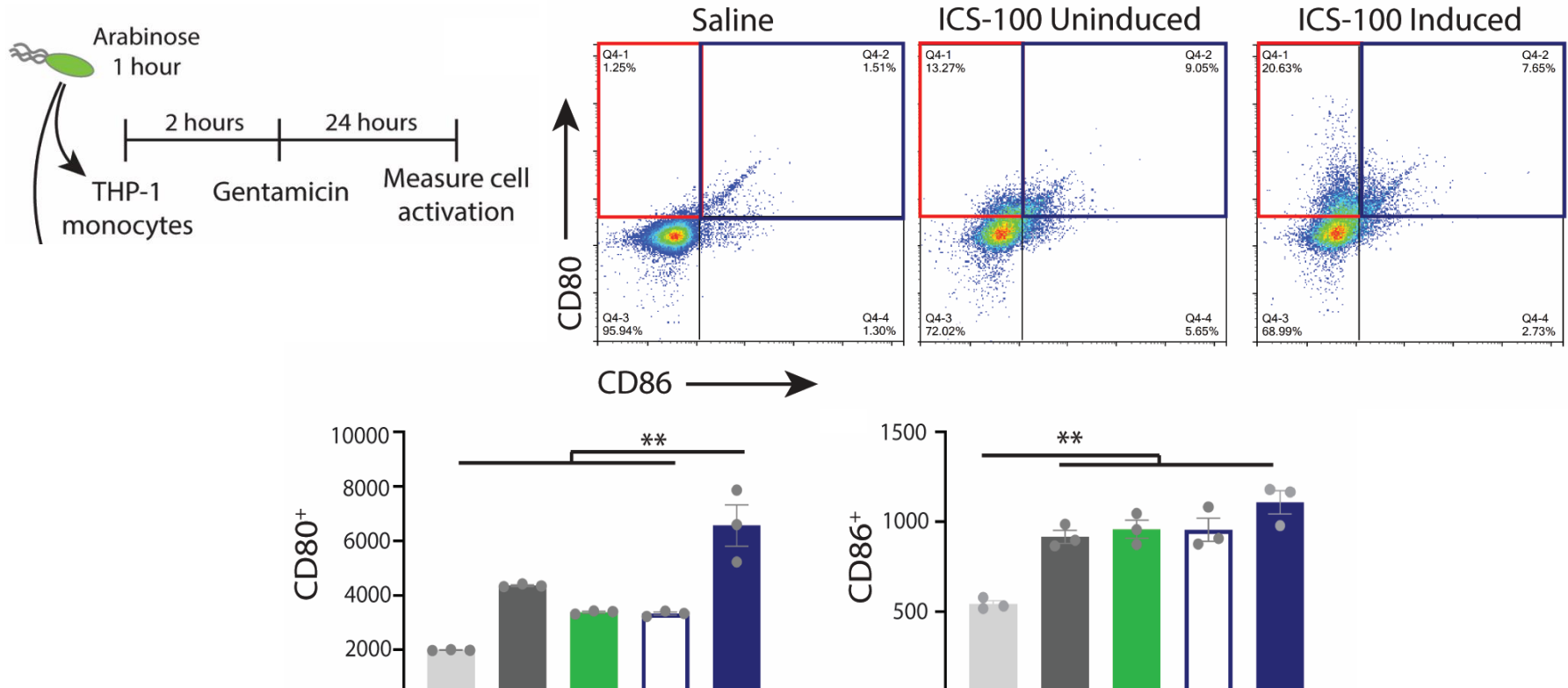


**Moving forward we selected 3 RBSs with ALDD  
Renamed as ICS-1, ICS-10, and ICS-100**

# Co-culture with ICS activates dendritic cells *in vitro*

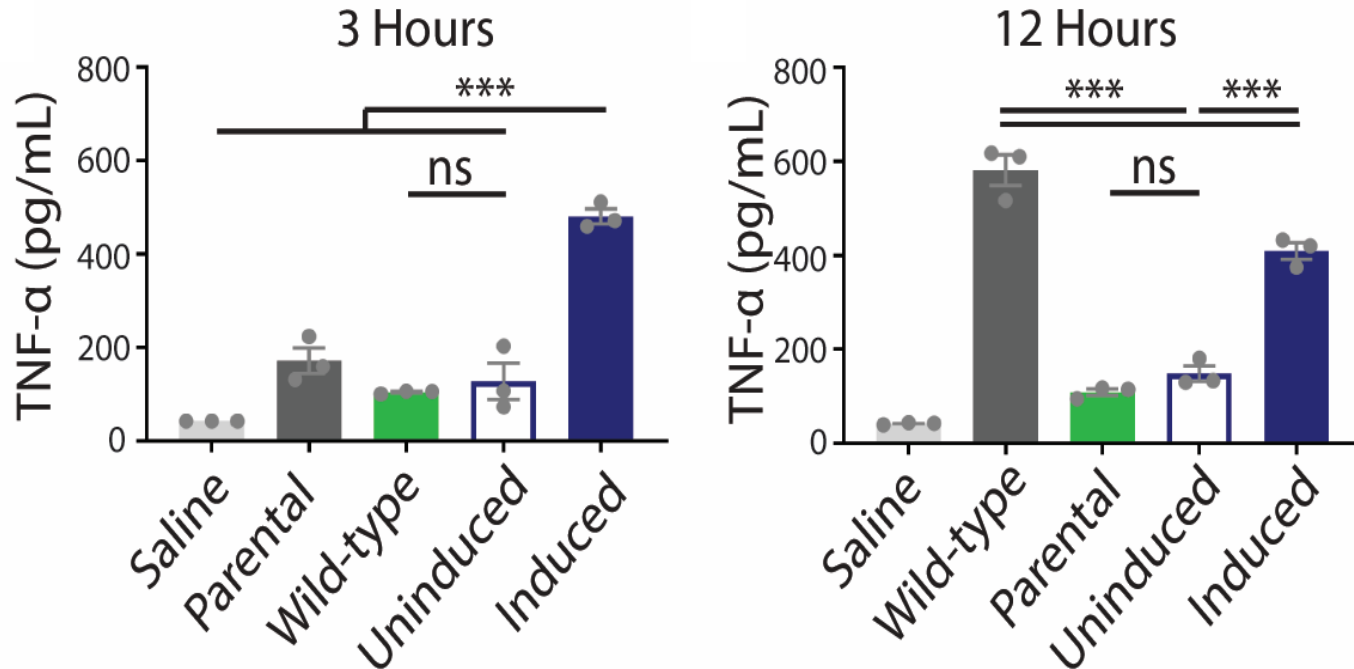


# Co-culture with ICS activates monocytes *in vitro*



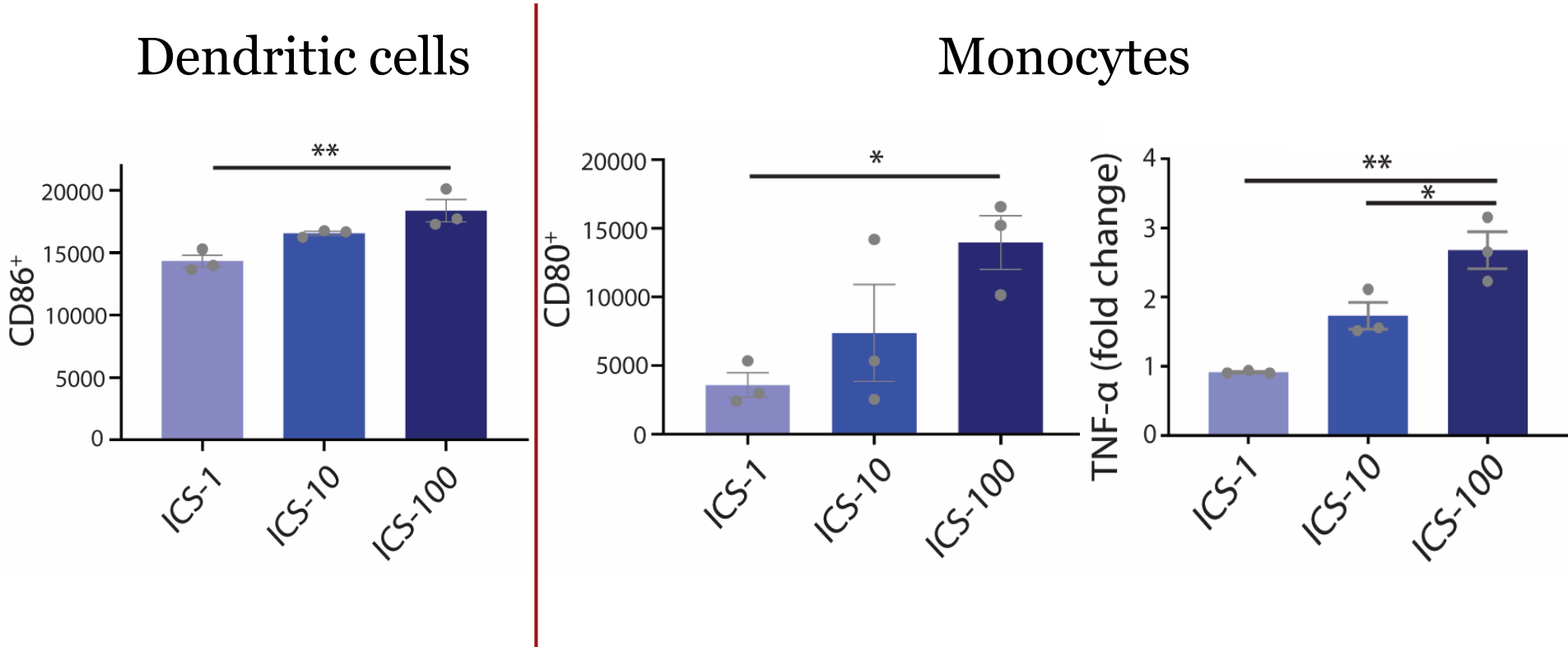
**This *in vitro* data demonstrates increases in innate immune activation and suggests therapeutic efficacy**

# Uninduced ICS does not stimulate inflammation



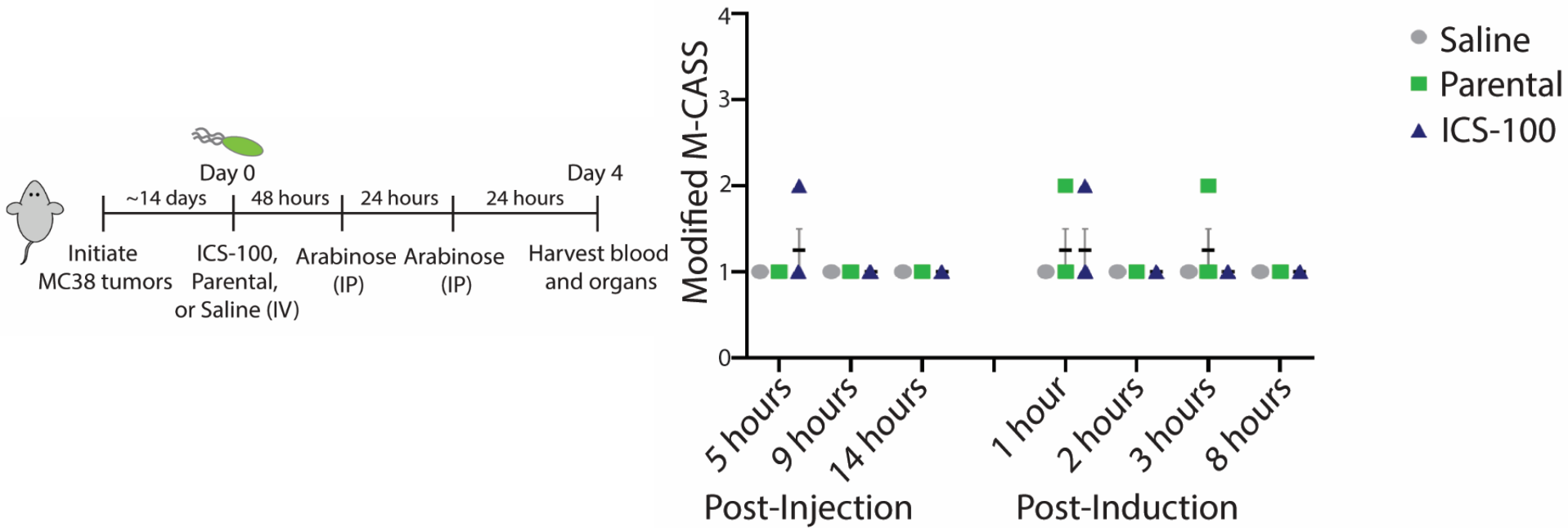
**ICS-100 induces inflammation less than wild-type Salmonella when induced, and the same as control while uninduced**

# Changing RBSs proportionally impacts cell activation



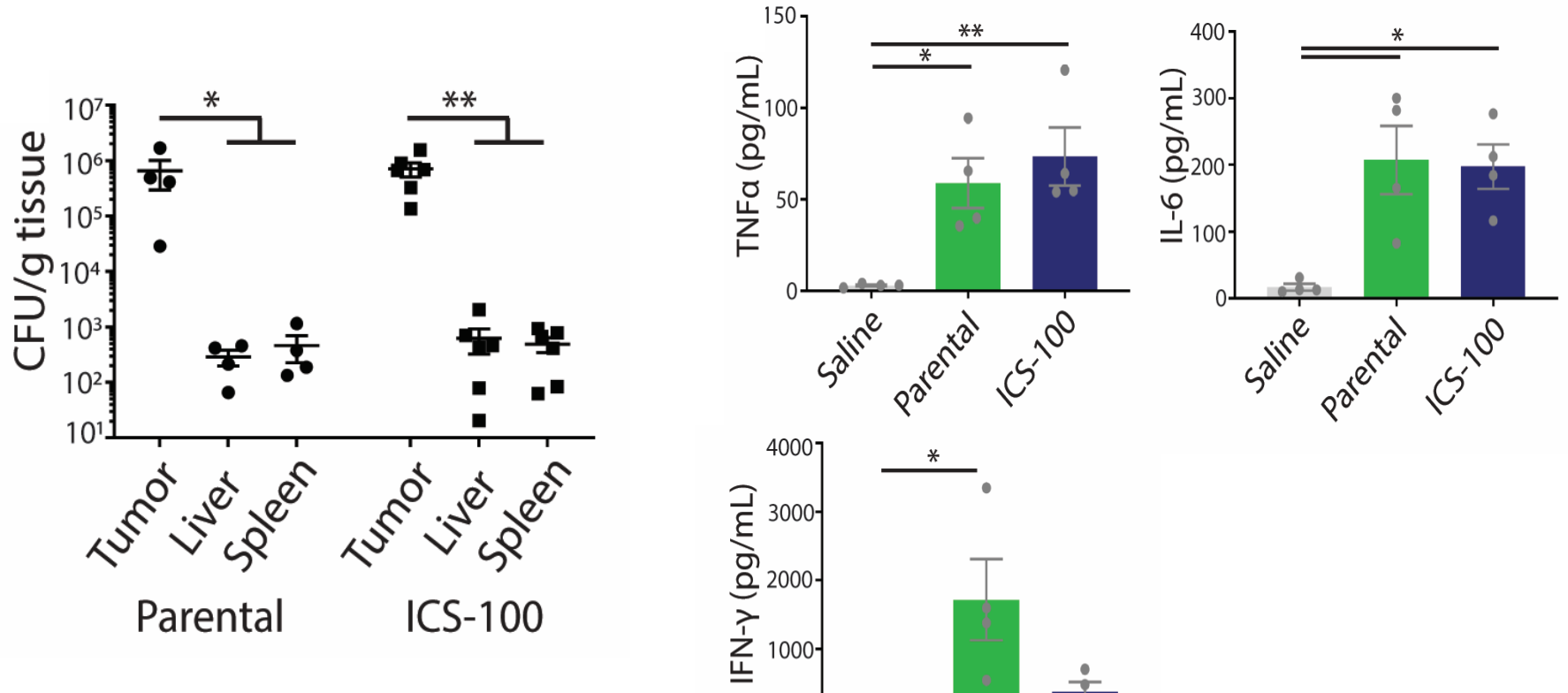
**Induced ICS-100 leads to the strongest activation effect**

# ICS demonstrates a safe injection profile *in vivo*



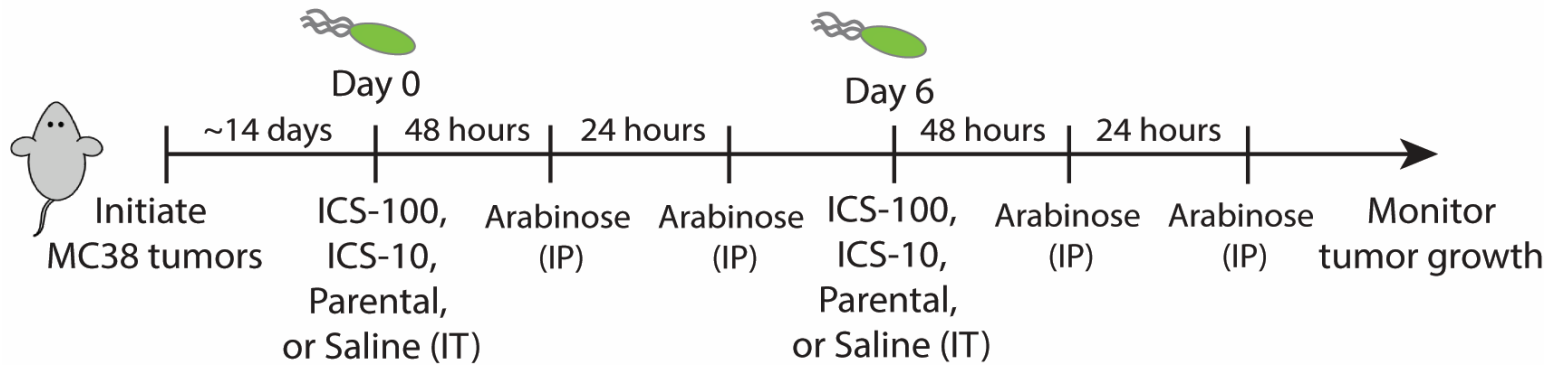


# ICS maintains tumor specificity and cytokine profile

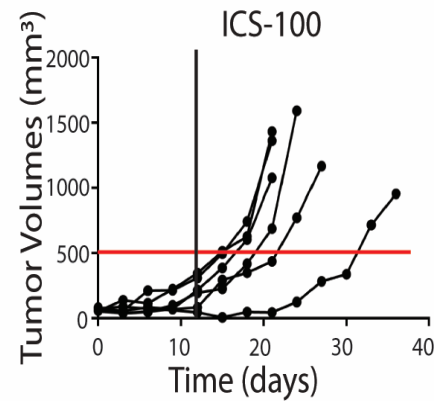
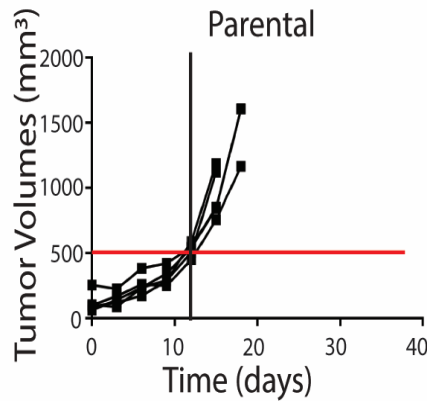
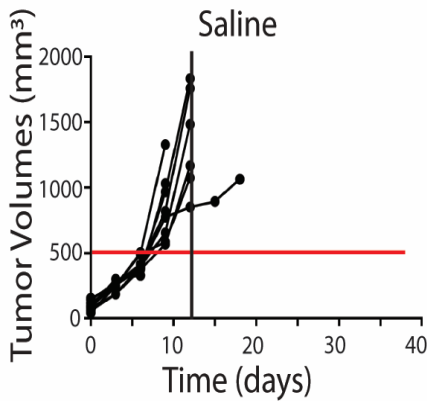
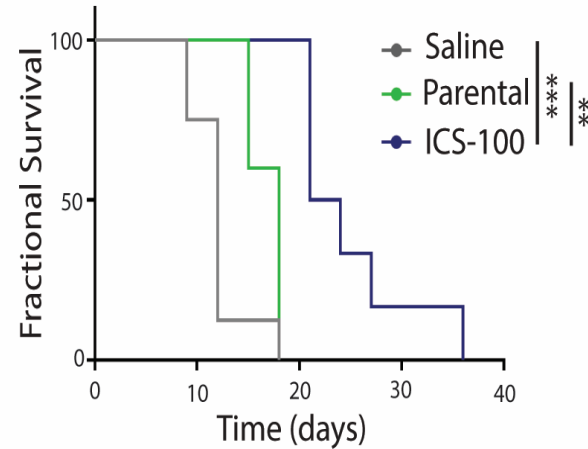
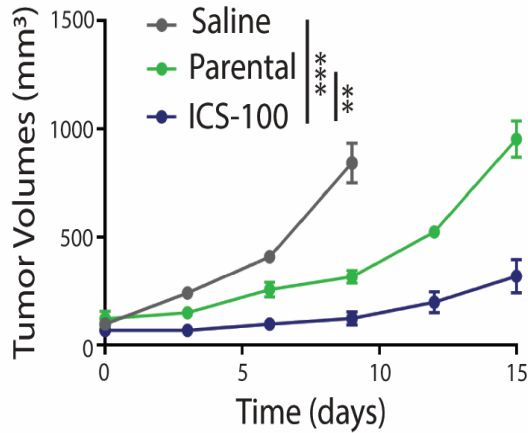


**Intravenous injection and systemic induction of ICS-100 supports a safe delivery profile**

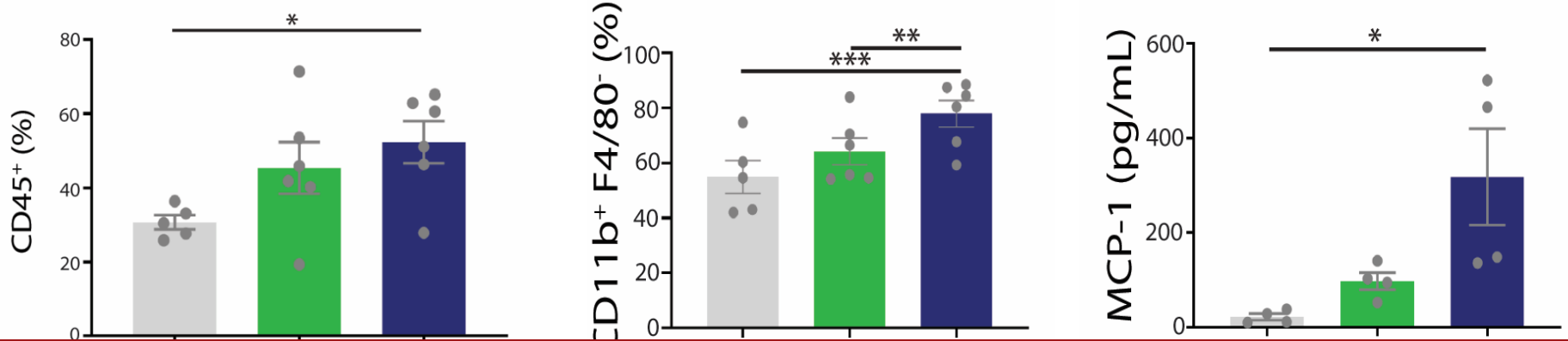
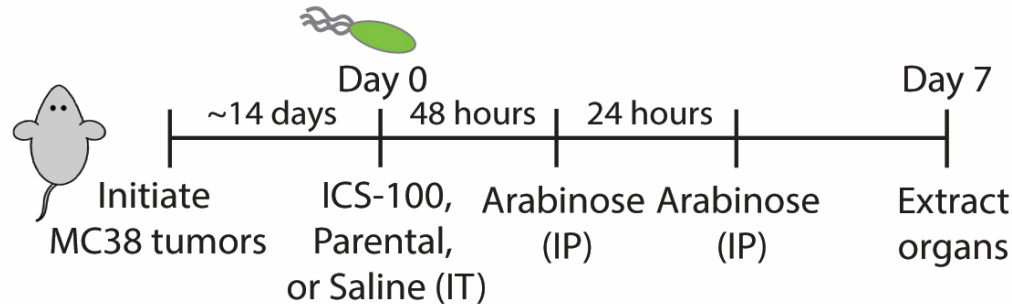
# So, what happens in tumors?



# ICS reduces tumor volume and increases survival

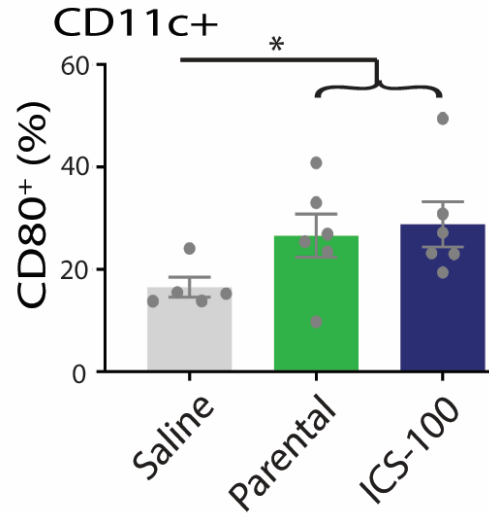
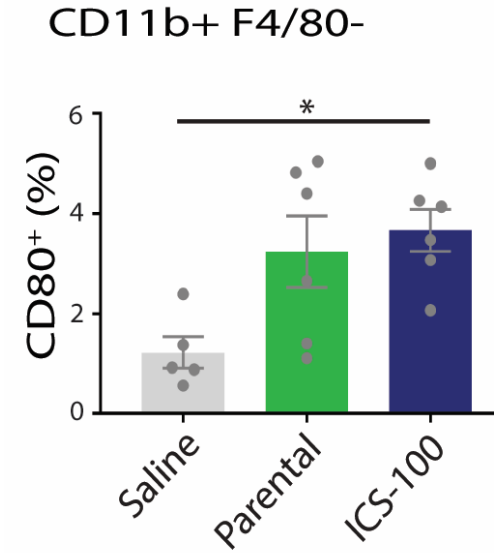
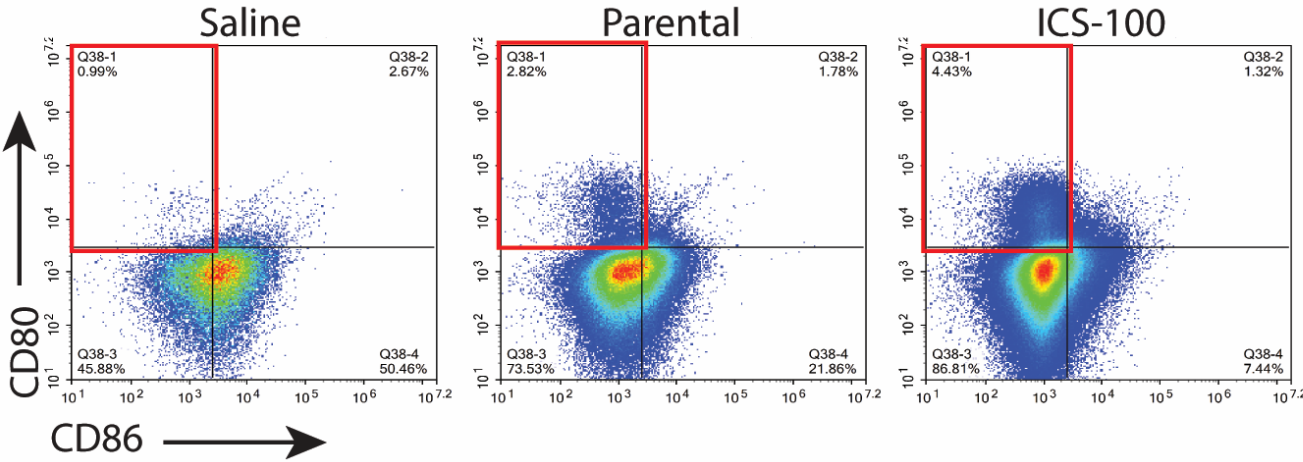


# ICS influences immune infiltration

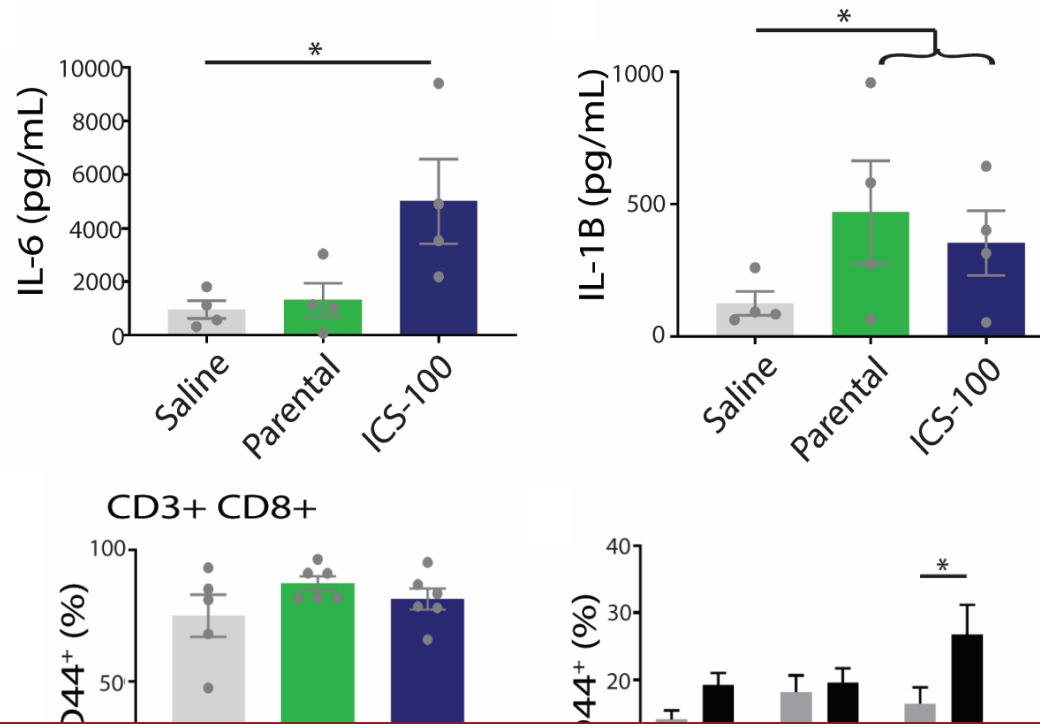


**ICS-100 increases the overall immune population within tumors, driven primarily by monocytes and neutrophils**

# Antigen presenting cells are activated in the tumor

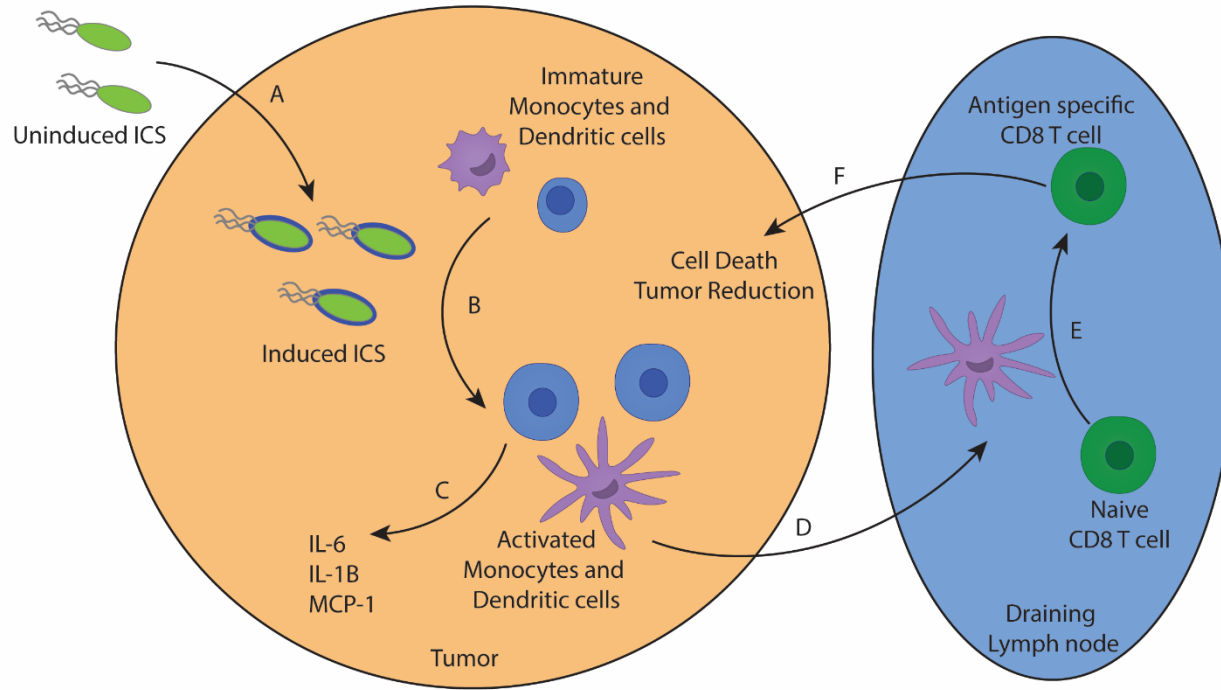
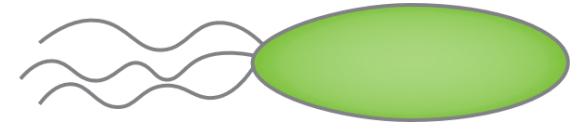


# ICS induces an inflammatory tumor environment



**ICS increases intratumoral innate immune activation as well as antigen aware T cells in the draining lymph nodes**

# Conclusions



Salmonella are a targeted delivery system for immunogenic protein therapeutics

# Acknowledgements and Questions

Dr. Neil Forbes, Dr. Ashish Kulkarni, Dr. Lisa Minter

## Forbes Lab

- Dr. Vishnu Raman
- Dr. Shane Taylor
- Dr. Shoshana Bloom
- Dr. Chris Hall
- Simin Manole
- Shradha Khanduja
- Chinmay Deshpande
- Victoria Wetherby
- Alec Reitter
- Abie Meyerson

## ICME Travel Grant



U.S. DEPARTMENT OF  
**ENERGY**

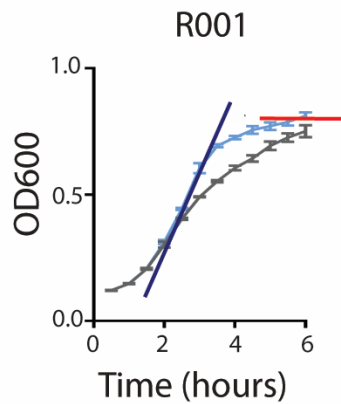
Office of Science



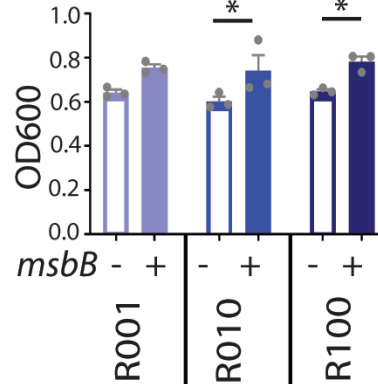


# *msbB* induction improves bacterial growth

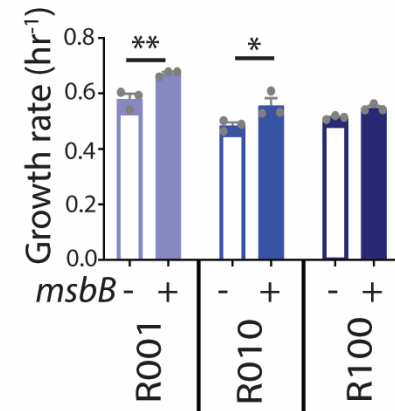
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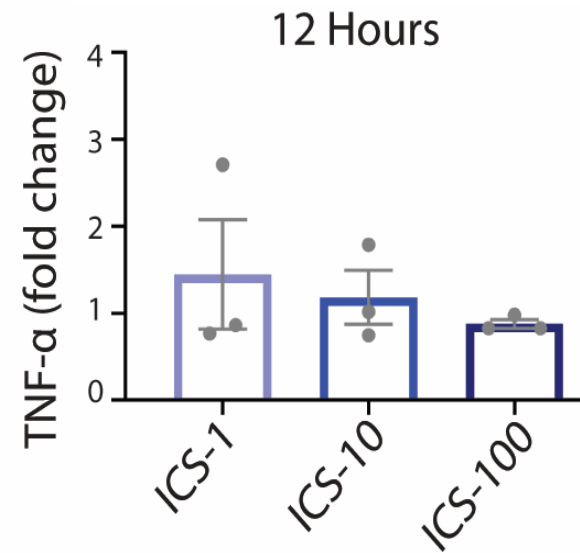
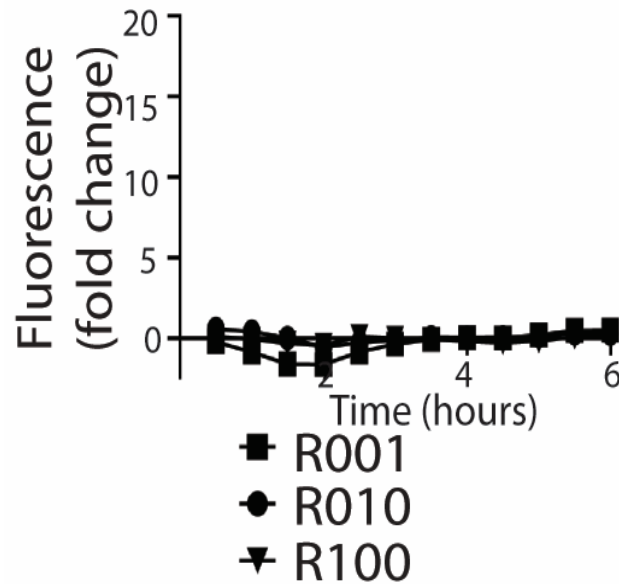
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# Uninduced *msbB* does not promote inflammation



# Changes in the overall immune populations

