



# Lymph-Node Targeted TLR9 Agonists Enable Potent Cellular Immune Responses Against Cancer & Infectious Disease

STING & TLR-Targeting Therapies Summit 2022

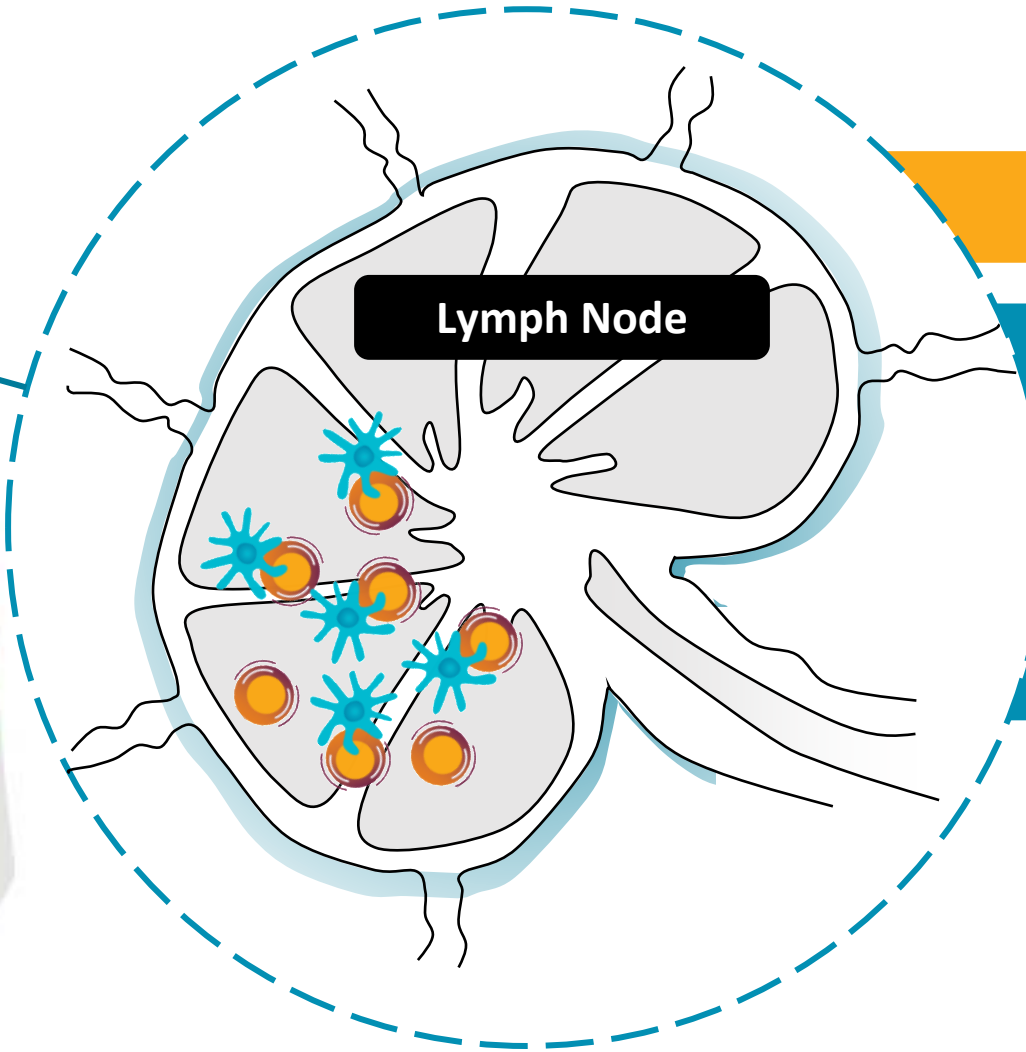
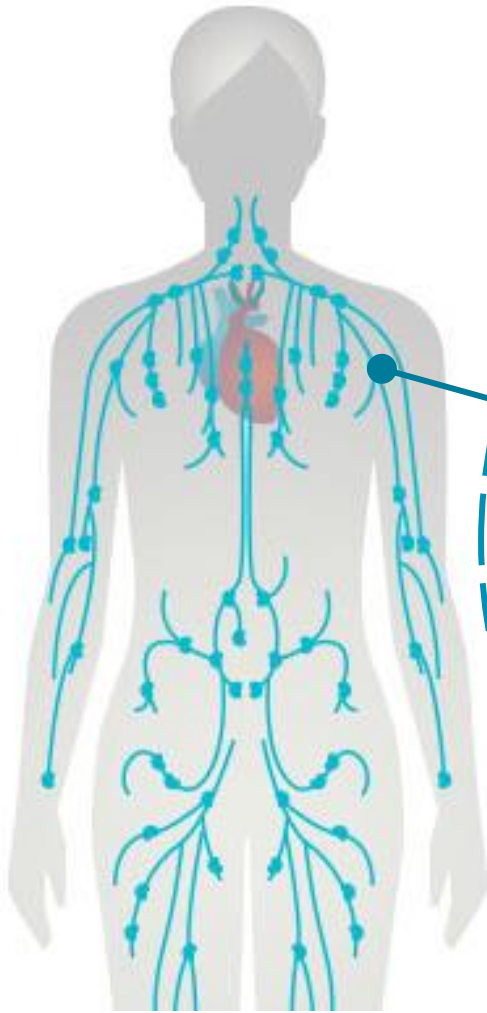
Pete DeMuth, PhD  
Chief Scientific Officer  
Boston, Massachusetts

# How does **Lymph Node Targeting** of TLR-9 Agonists Impact Immunological Activity?

- 1) Designing a system to target immune agents to **lymph nodes**
- 2) Boosting prophylactic activity for vaccines against **infectious disease**
- 3) Boosting therapeutic activity for immunotherapies in **oncology**



# Lymph Nodes are Where the Immune Response is Orchestrated



**The Immune “School House”**

**Numerous Immune Cells**

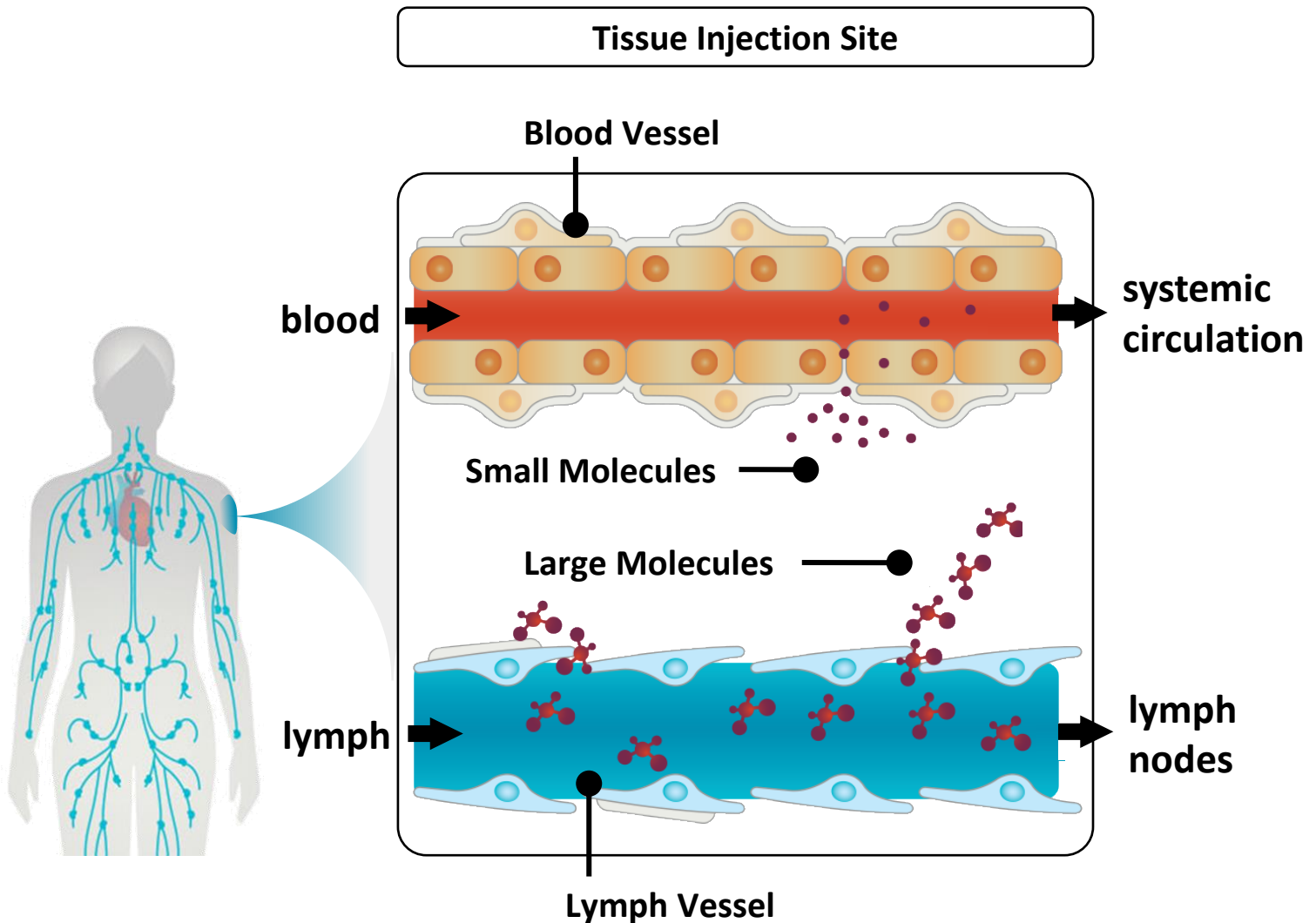
**Response Coordination**

**APC : T and B Cell Interaction**

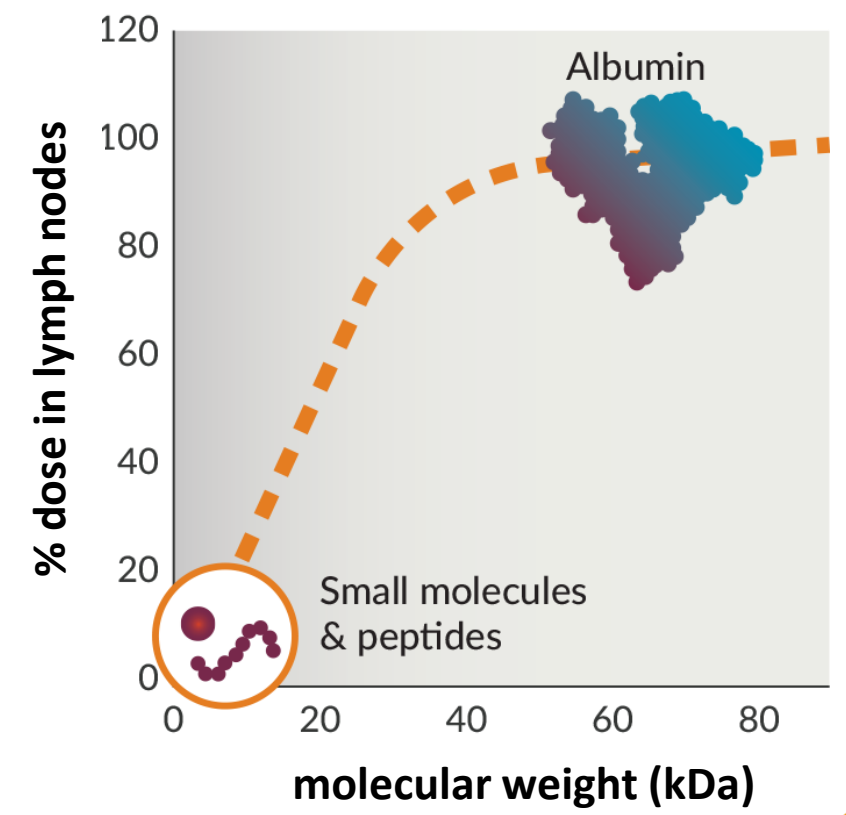
- **Expansion**
- **Persistence**
- **Anti-tumor Activity**
- **Pathogen-specific Immunity**



# Albumin is the Ideal Carrier to Transport Immunotherapies and Vaccines into Lymph Nodes

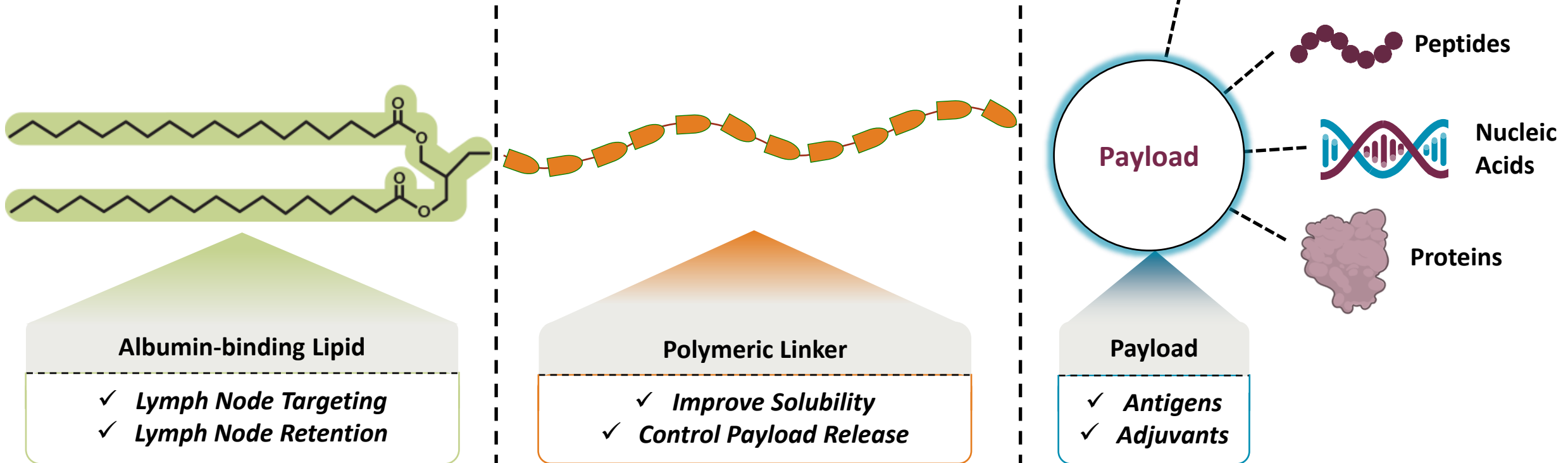


**Molecular Size Drives Lymphatic Targeting**



# Amphiphile (AMP) Platform Enables Lymph Node Delivery of Validated Therapeutics with Modular Application

## AMP: A Modular Conjugation Approach for Delivery of Immune Therapeutics to the Lymph Nodes



# Targeting the Lymph Nodes with AMP to Orchestrate Immunity

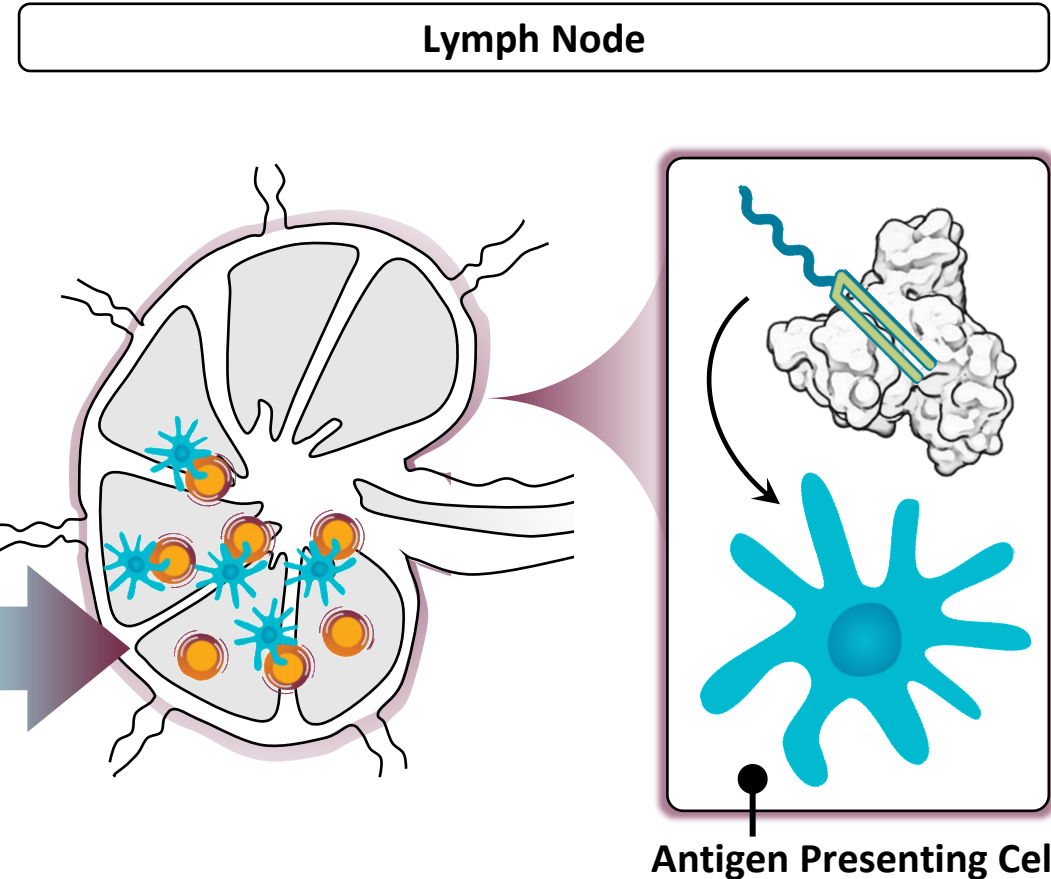
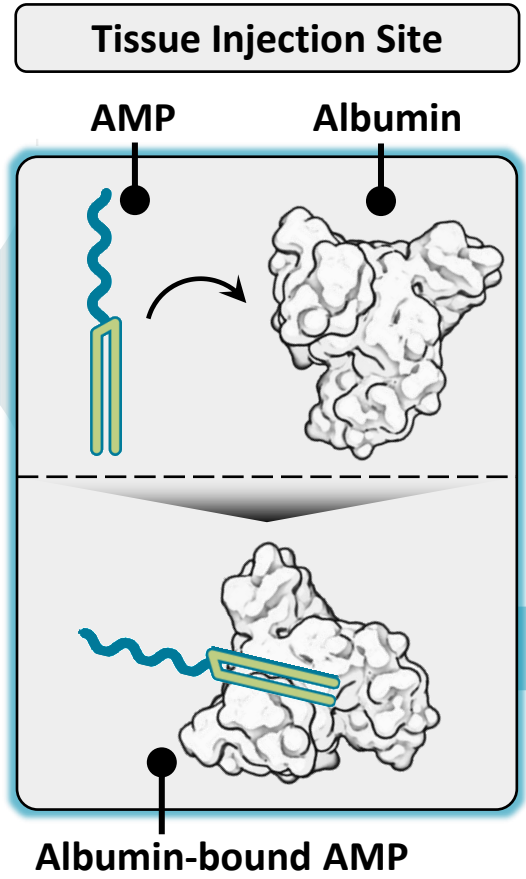
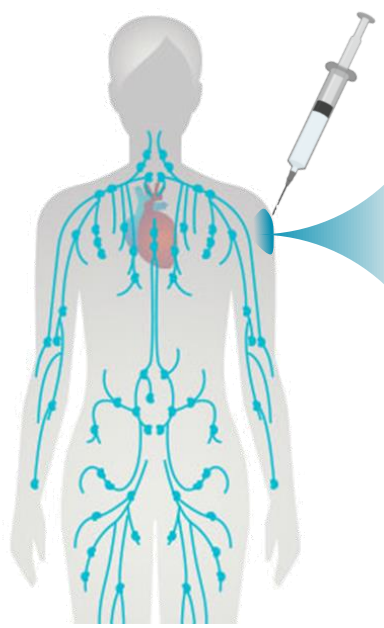
1 Subcutaneous Injection

2 Albumin Binding

3 Lymph Node Targeting

4 Delivery to Immune Cells

5 Immune Activation



- APC Activation
- Cytokines
- T Cell Priming
- Ab Generation

# Designing a Lymph Node Targeting CpG DNA



**AMP Modification: Albumin Binding Lipid for Lymph Node Targeting**

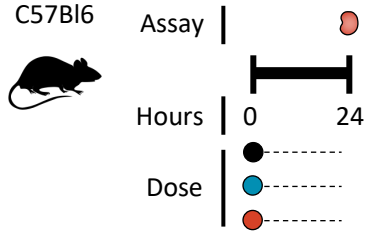
**CpG DNA: TLR-9 Agonist**

- Potent TLR-9 immuno-activator
- AMP modification gives >10-fold improved lymph node targeting

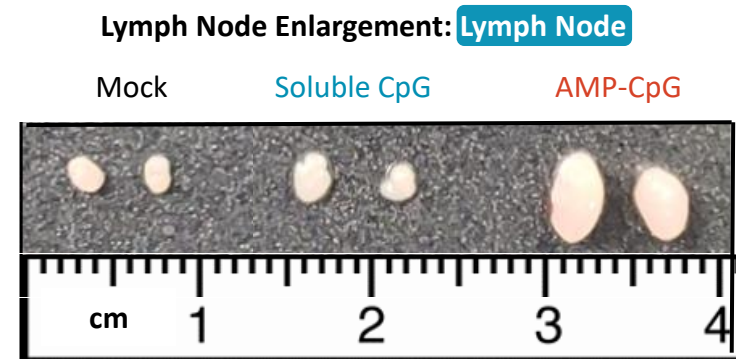
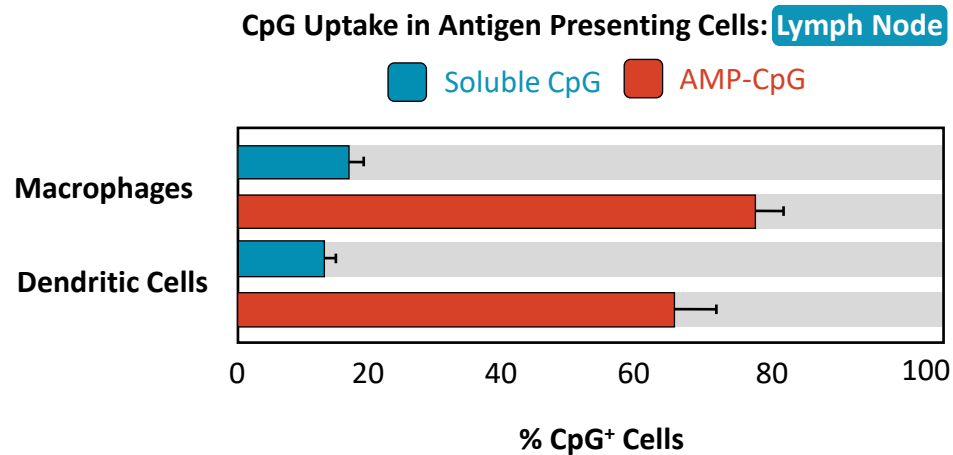
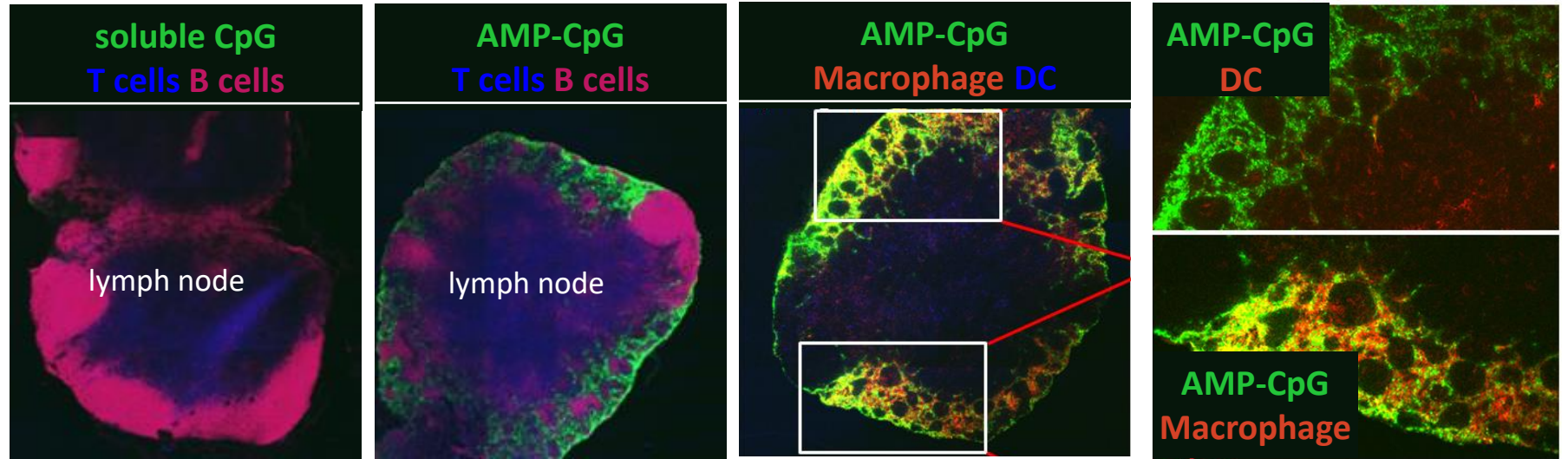


# AMP-CpG Targets the Lymph Nodes for Efficient Uptake into Resident APCs

## Experimental Schema:



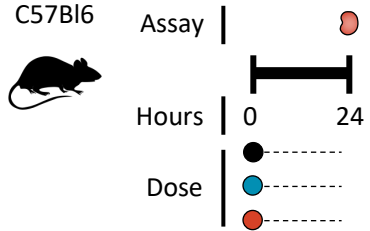
- Mock Dose
- Soluble CpG
- AMP-CpG
- LN Collection



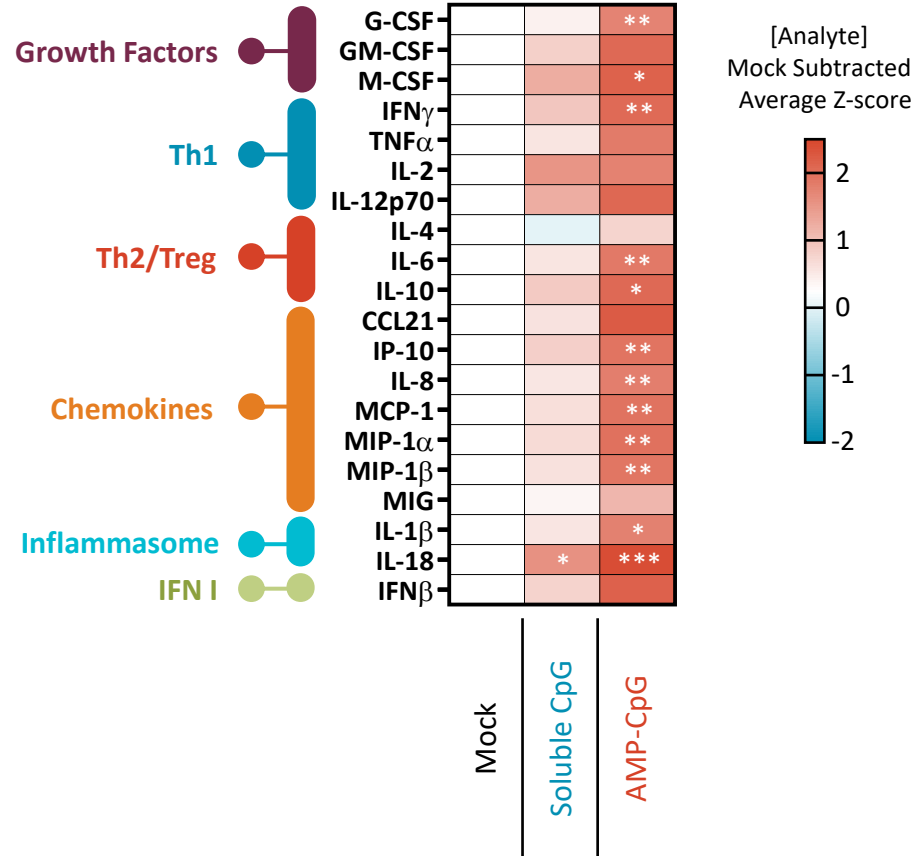


# AMP-CpG Induces Coordinated Inflammation, Innate Immune Cell Activation in Draining Lymph Nodes

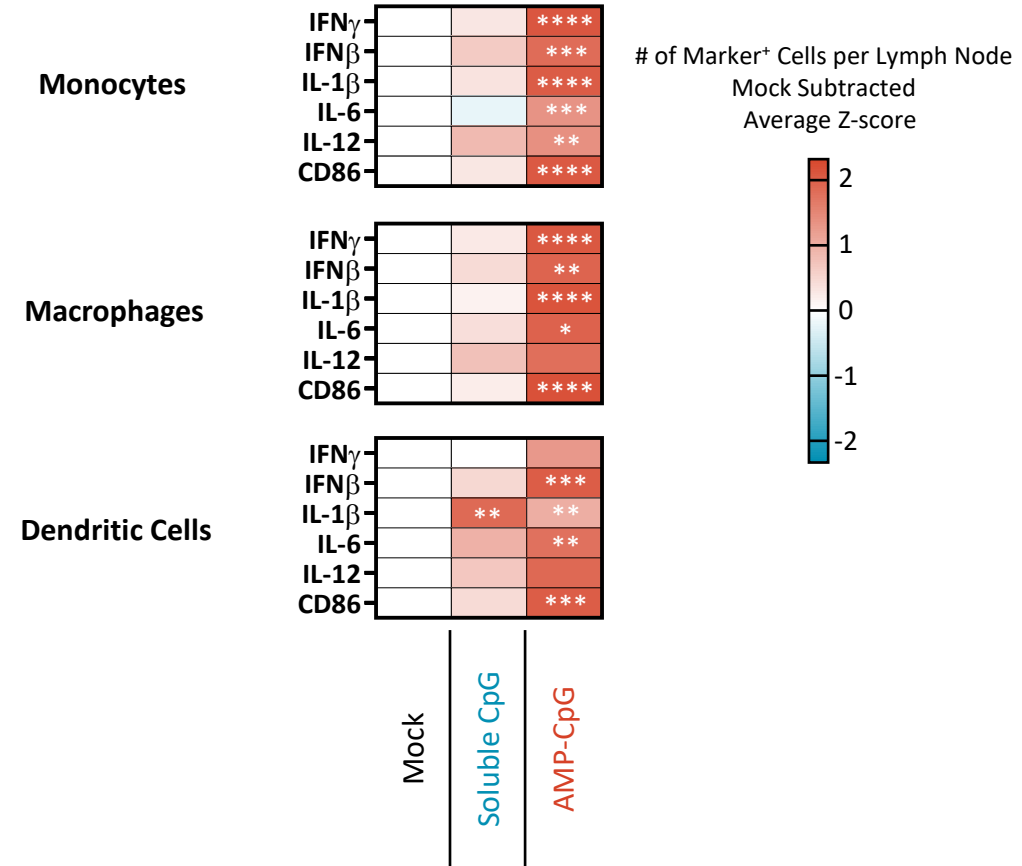
## Experimental Schema:



## Lymph Node Proteomics: 24 hours

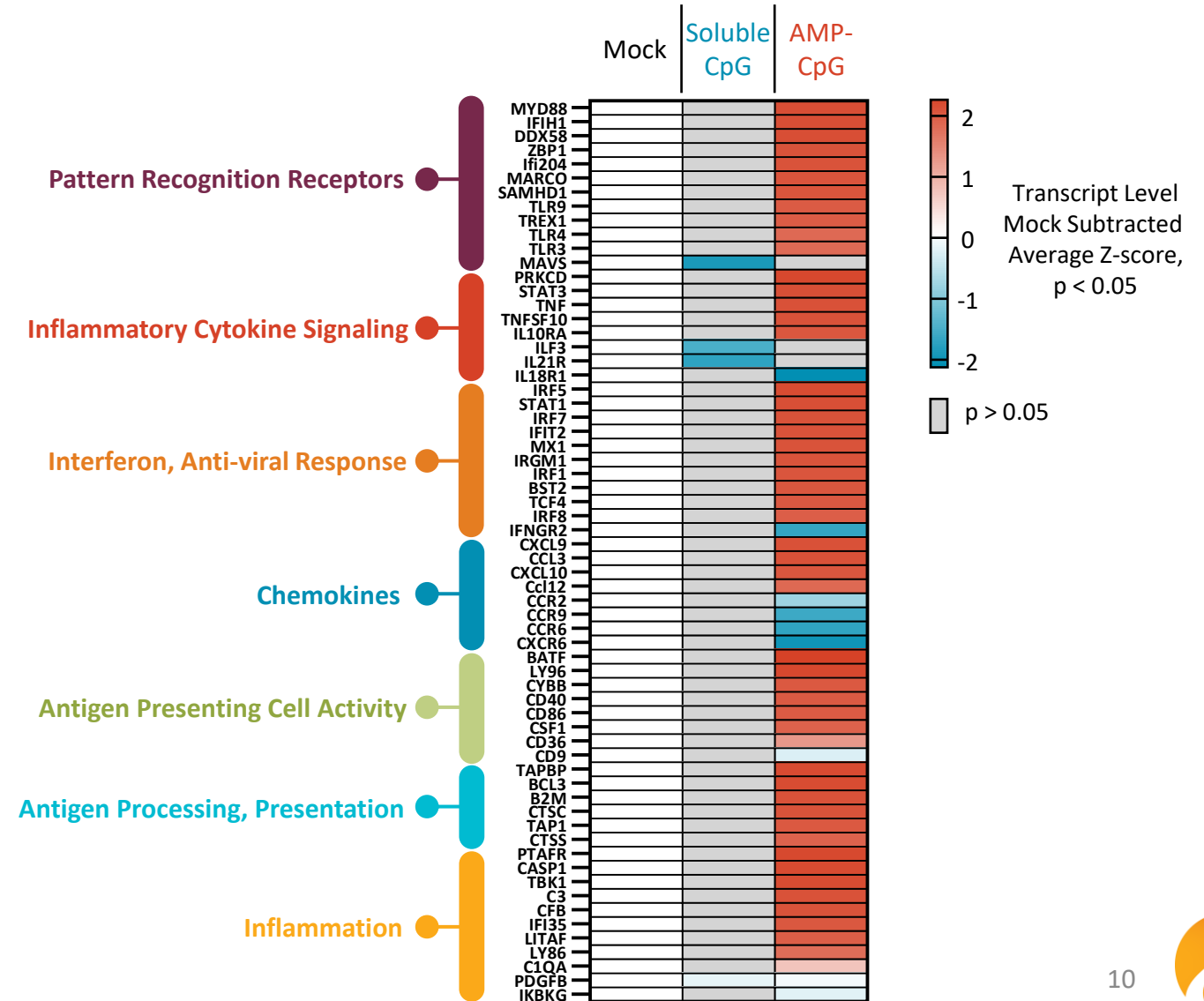
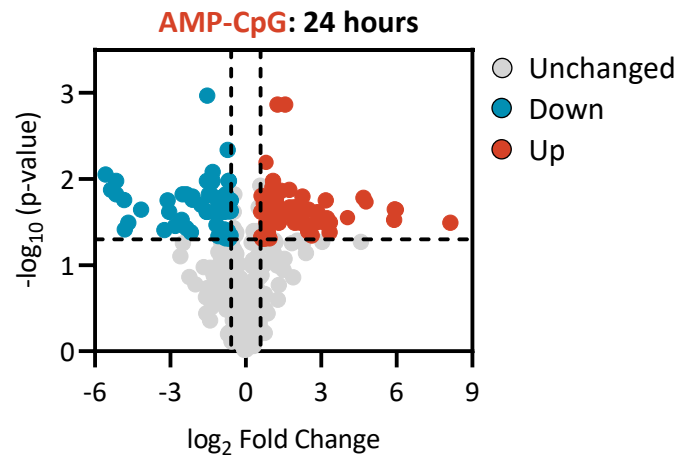
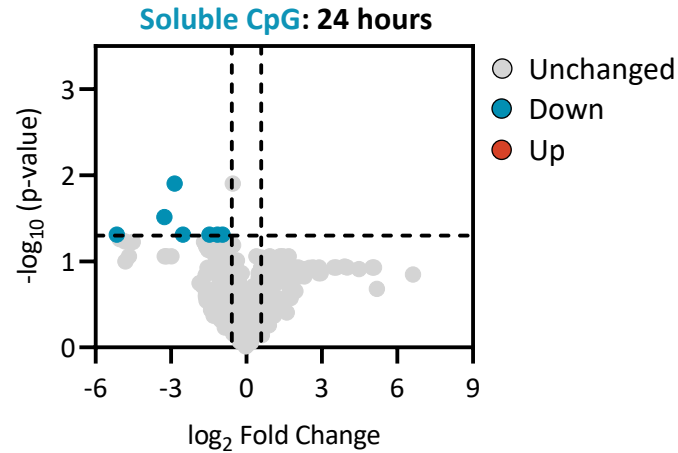


## Lymph Node Innate Cell Recruitment and Activation: 24 hours



# AMP-CpG Induces Potent Transcriptional Reprogramming of the Lymph Node Immune Response

## Lymph Node Transcriptomics: 24 hours



## The AMP Platform Efficiently Targets the Lymph Nodes



- Enhanced Lymph Node Delivery and Retention
  - Increased Uptake into APCs
  - Potent APC Activation
- Inflammatory Transcriptional Programming
  - Robust Cytokine/Chemokine Milieu



A background image showing a close-up of a handwritten musical score on a page. A fountain pen is positioned diagonally across the page, pointing towards the right. The score consists of several staves of music with notes, rests, and some handwritten text. The word "Romance" is visible in the upper right portion of the score. The overall image has a soft, slightly blurred appearance.

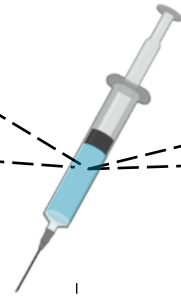
# AMP-CpG Vaccine Adjuvant for Infectious Disease



**How can **Lymph Node Targeting** Improve TLR-9 Adjuvant Activity to Generate Potent T and B Cell Responses Against Infectious Disease?**

# ELI-005: Designing a Lymph Node Targeted Protein Subunit Vaccine for SARS-CoV-2

**(1) SARS-CoV-2 Spike RBD Protein Antigen**



**(2) AMP-CpG Adjuvant**



**AMP Modification: Albumin Binding Lipid for Lymph Node Targeting**

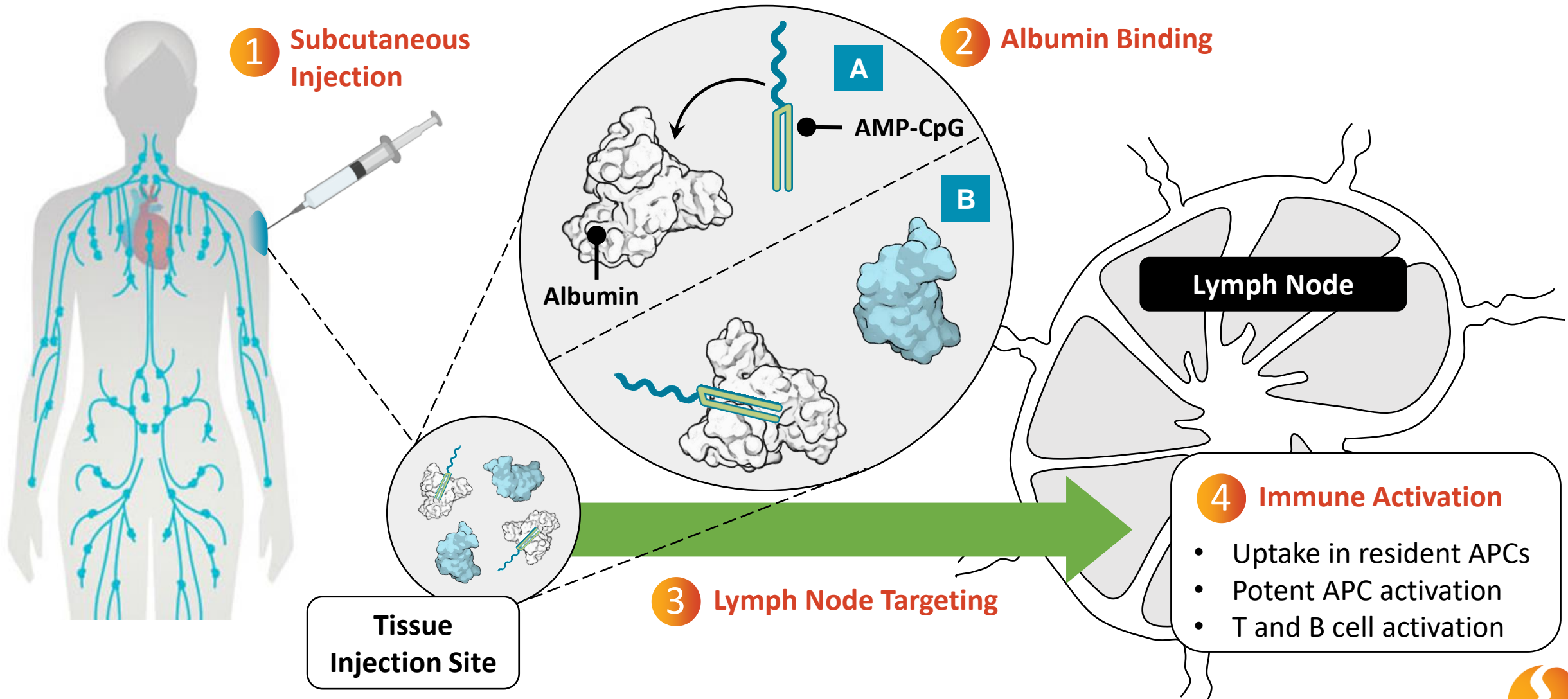
**CpG DNA: TLR-9 Agonist**

- Target of nAb responses
- Known CD4 and CD8 T cell target
- MW~34 kDa predicts suitable lymph node targeting

- Potent TLR-9 immuno-activator
- AMP modification gives >10-fold improved lymph node targeting
- CpG has proven safety and activity in humans

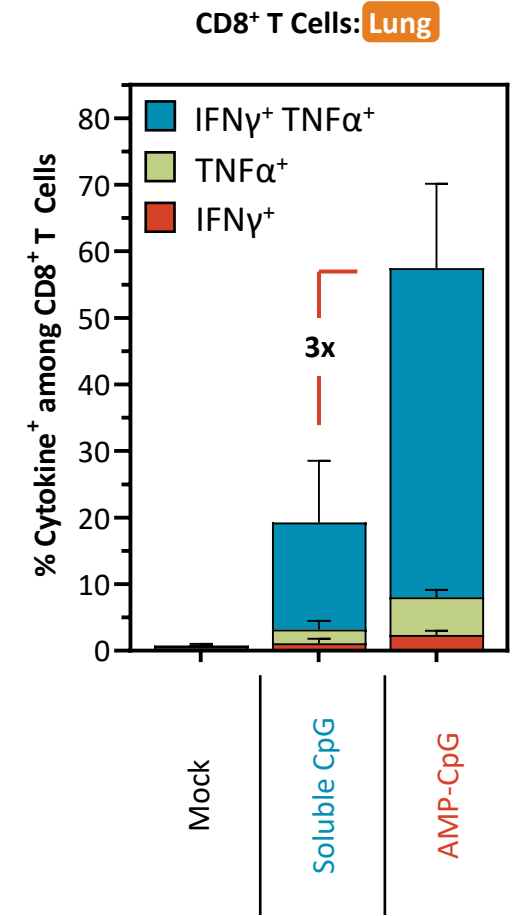
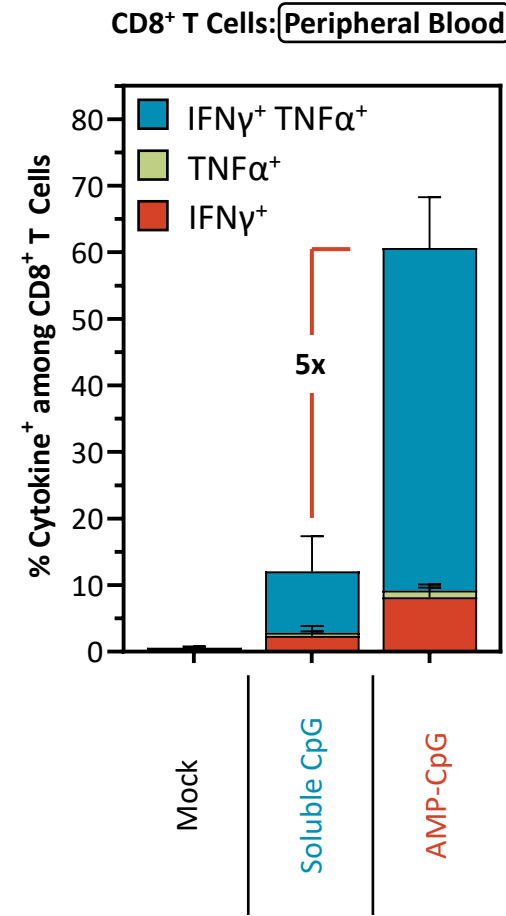
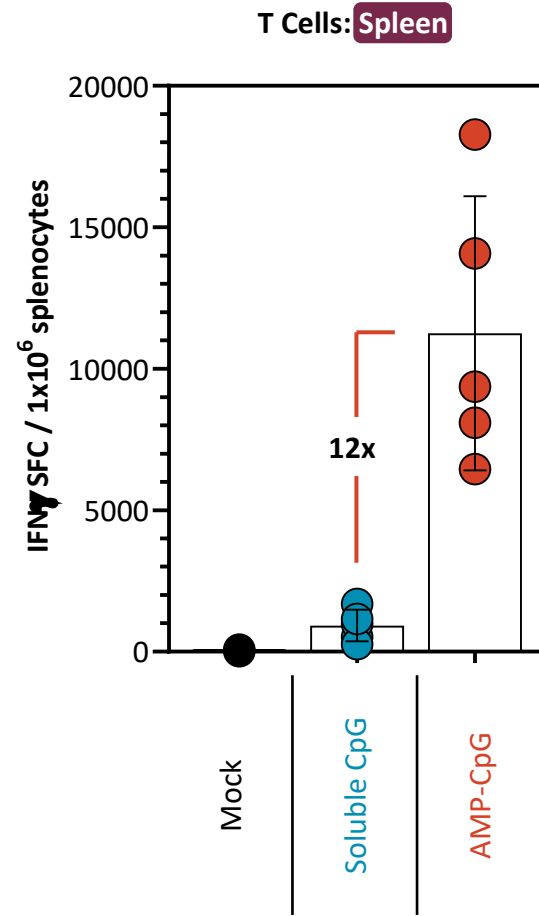
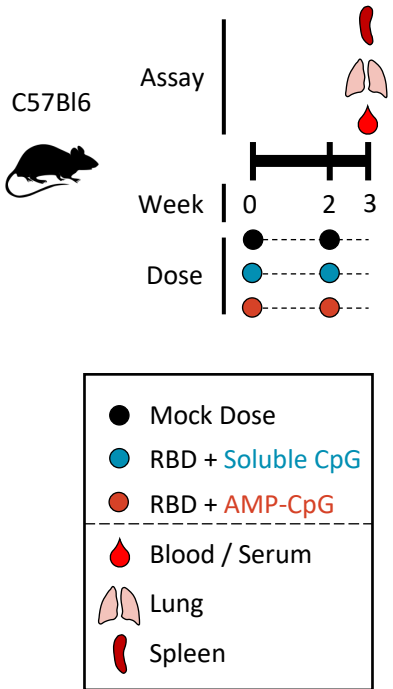


# ELI-005: Designing a Lymph Node Targeted Protein Subunit Vaccine for SARS-CoV-2



# AMP-CpG Vaccination Induces Potent Polyfunctional T Cell Responses Targeting SARS CoV-2

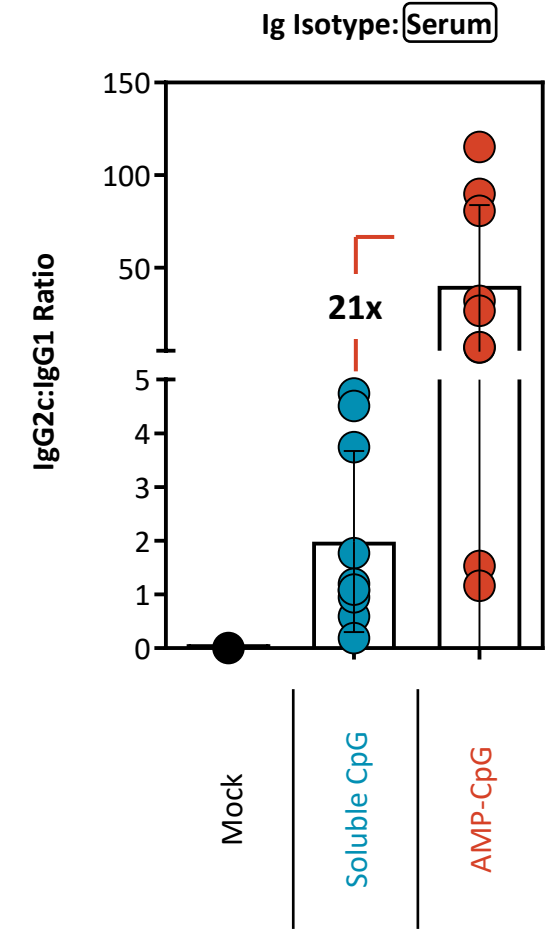
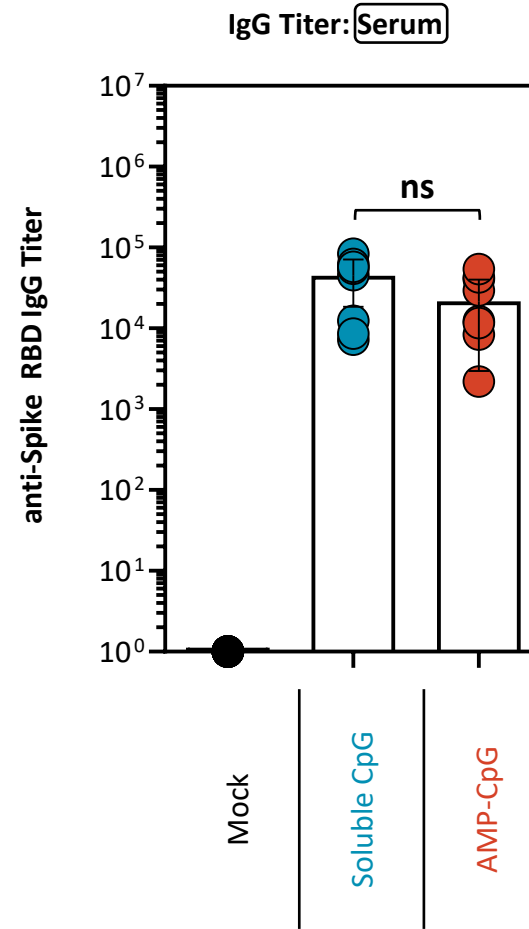
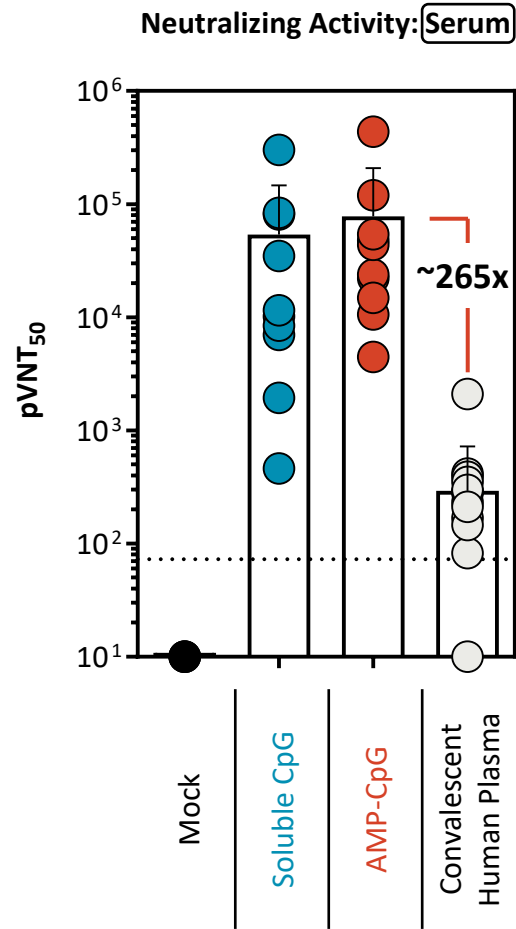
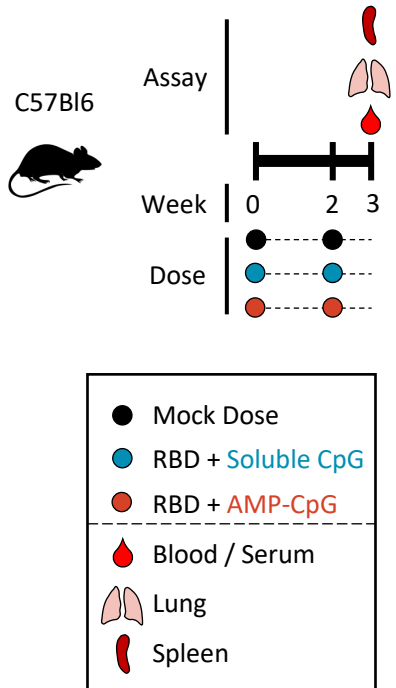
## Experimental Schema:





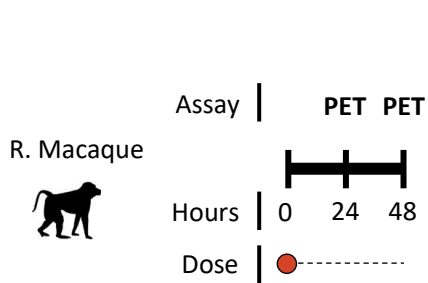
# AMP-CpG Vaccination Induces Potent Neutralizing Antibodies with Optimal Th1 Dominant Isotype Profile

## Experimental Schema:

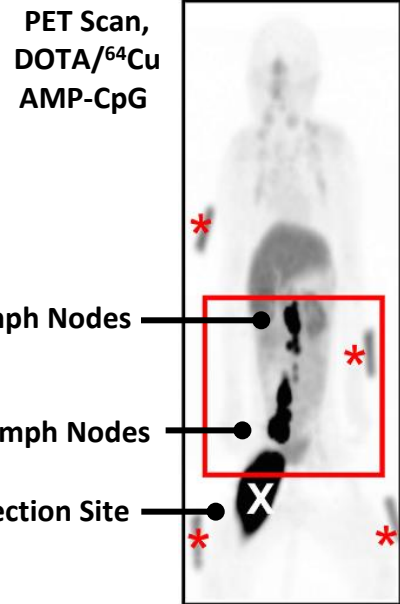


# AMP-CpG Accumulates in NHP Lymph Nodes and Induces Potent T cell Responses

## Experimental Schema:



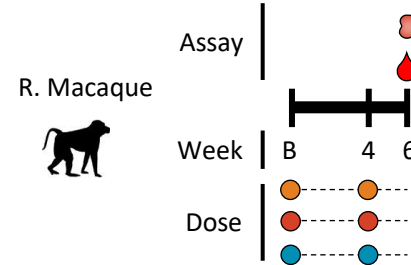
## Lymph Node



Lymph Nodes	% Dose 48h
Inguinal	~12%
Iliac	~30%

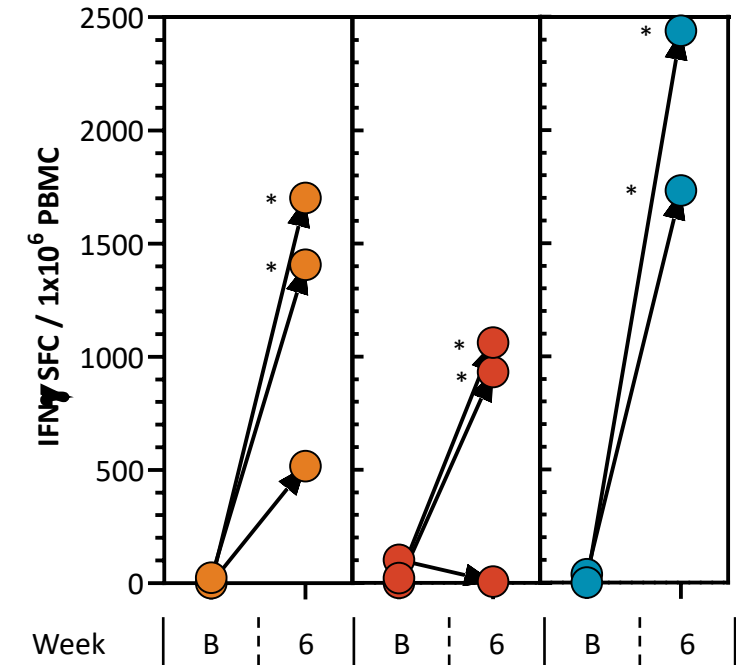
● AMP-CpG
PET   PET Scan

## Experimental Schema:



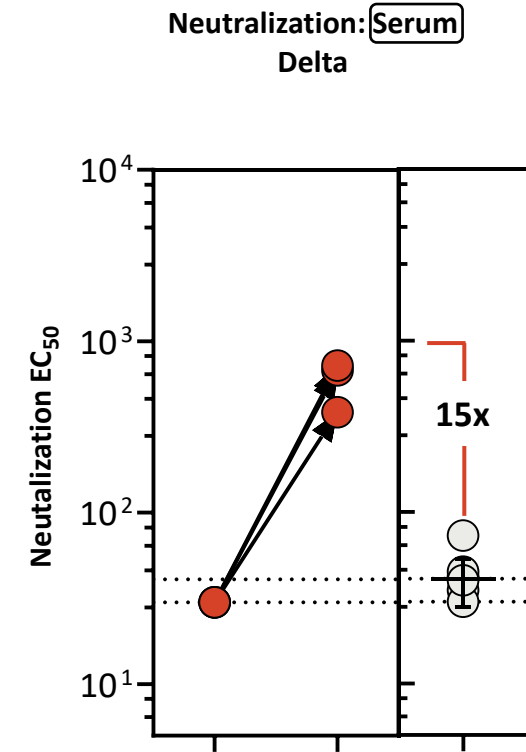
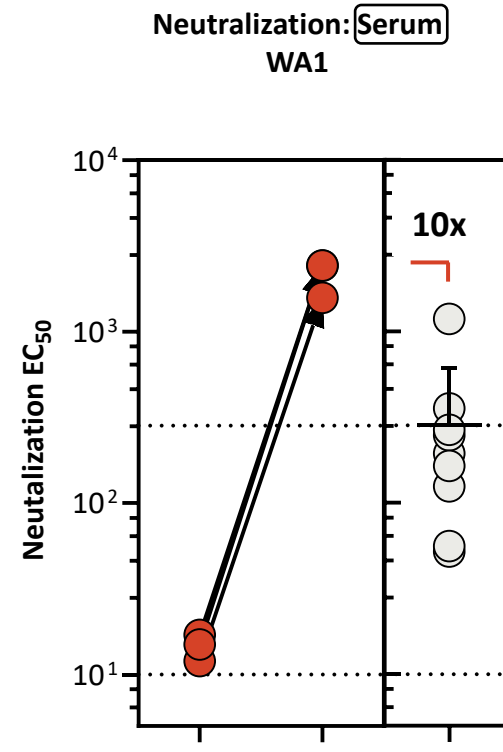
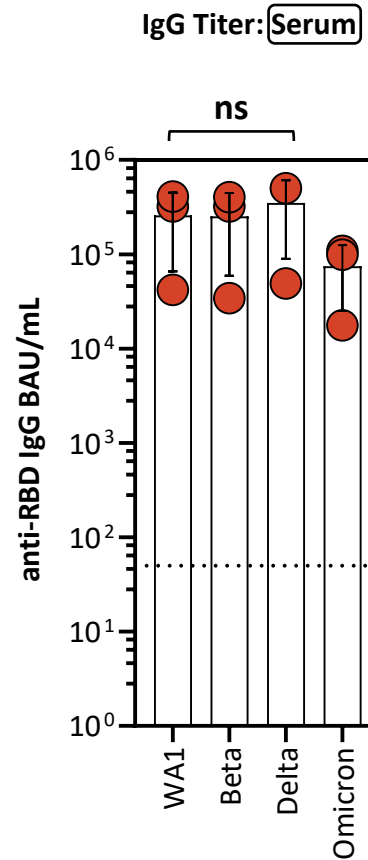
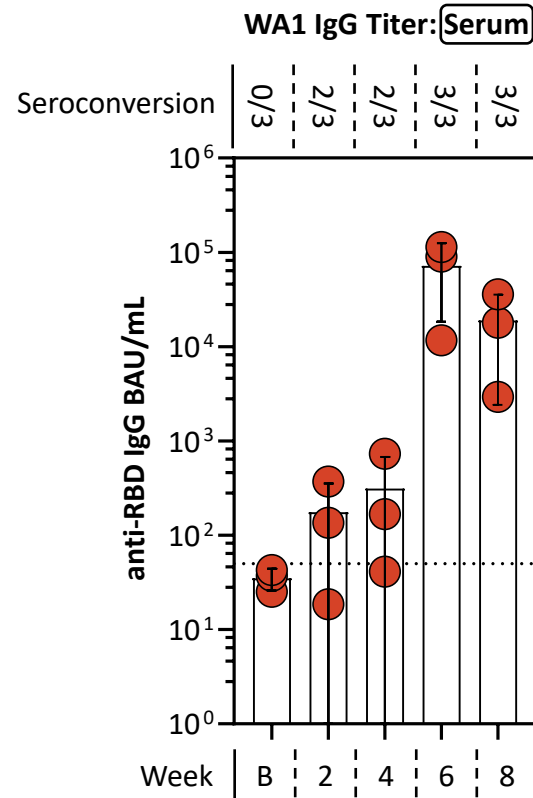
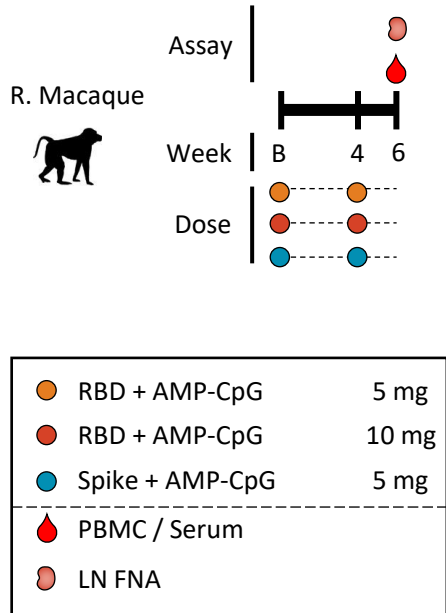
● RBD + AMP-CpG	5 mg
● RBD + AMP-CpG	10 mg
● Spike + AMP-CpG	5 mg
● PBMC / Serum	
● LN FNA	

## T Cells: Peripheral Blood



# AMP-CpG Induces Potent Cross-reactive Neutralizing Antibody Responses

## Experimental Schema:



## ELI-005: A Lymph Node Targeted Vaccine Against COVID-19



- Potent T Cell Responses in Blood, Lung, Spleen
- Neutralizing Th1-biased Antibody Responses
  - Dose Sparing
  - Responses in Aged Animals
  - Cross-reactive Responses to VOC



- Lymph Node Accumulation
  - Potent T Cell Responses
- Cross-reactive Neutralizing Antibody Responses



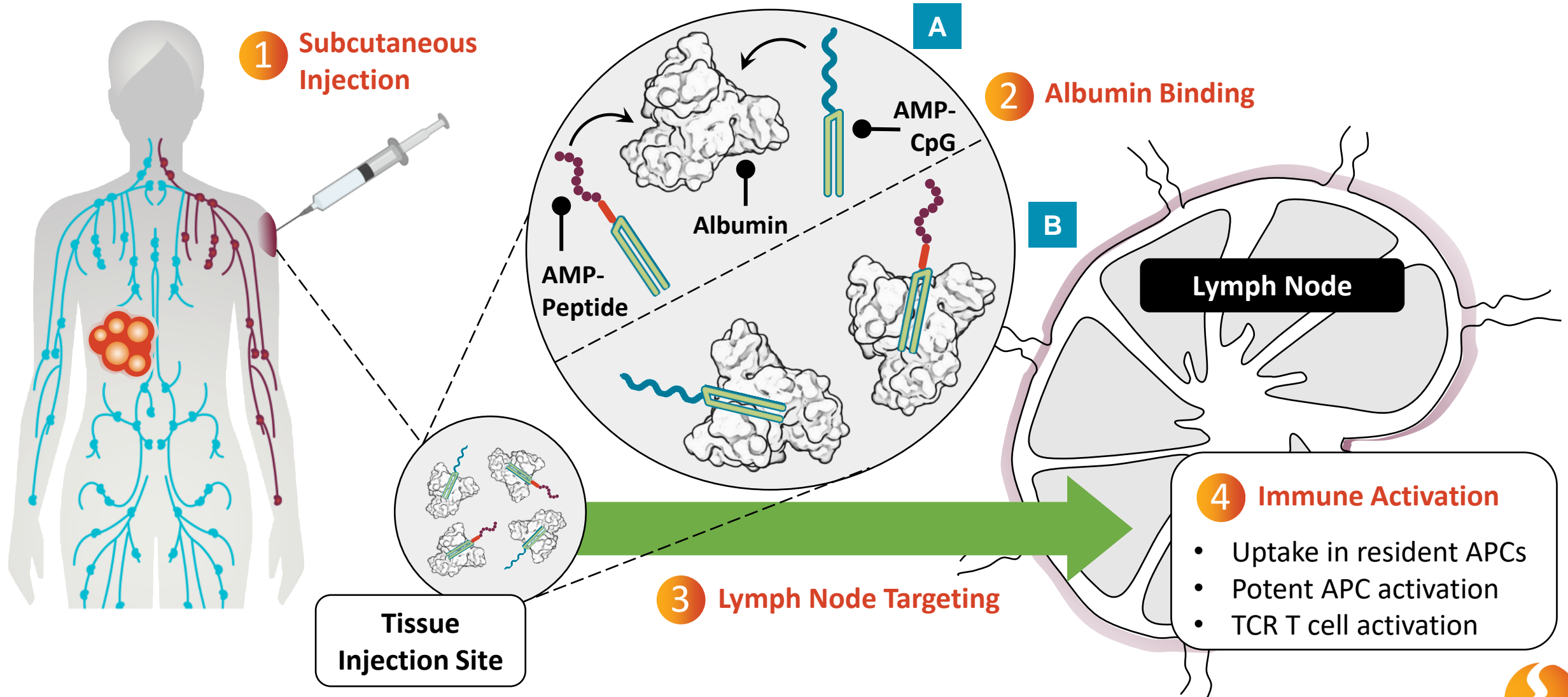
A background image showing a close-up of a fountain pen writing on a sheet of music paper. The paper is filled with handwritten musical notation, including staves with notes and rests. The word "Romance" is written in cursive at the top of the page. A red banner with white text is overlaid across the middle of the image.

# AMP-CpG for Cancer Immunotherapy: TCR-T ACT

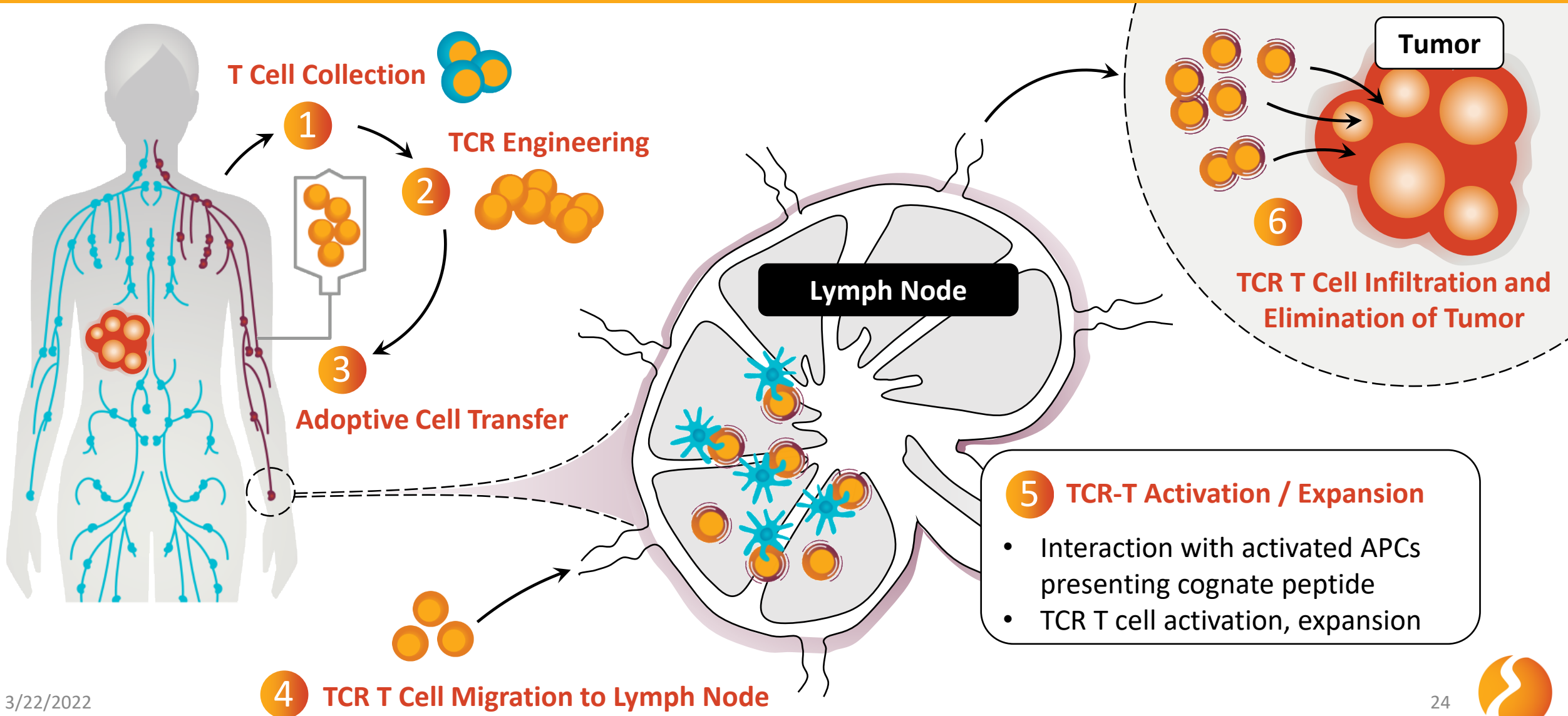


**How can **Lymph Node Targeting** Improve TLR-9 Adjuvant Activity to Enhance TCR-T Cell Therapy Against Solid Tumors?**

# Designing a Lymph Node Targeted Booster for TCR-T Cell Therapy

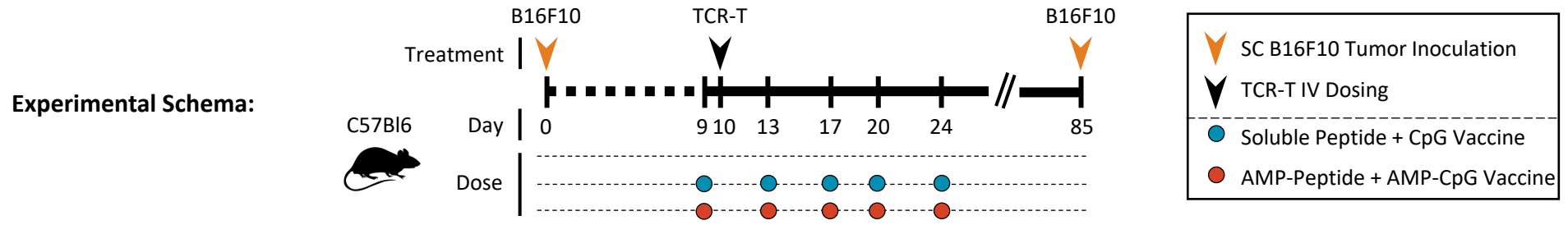


# Designing a Lymph Node Targeted Booster for TCR-T Cell Therapy

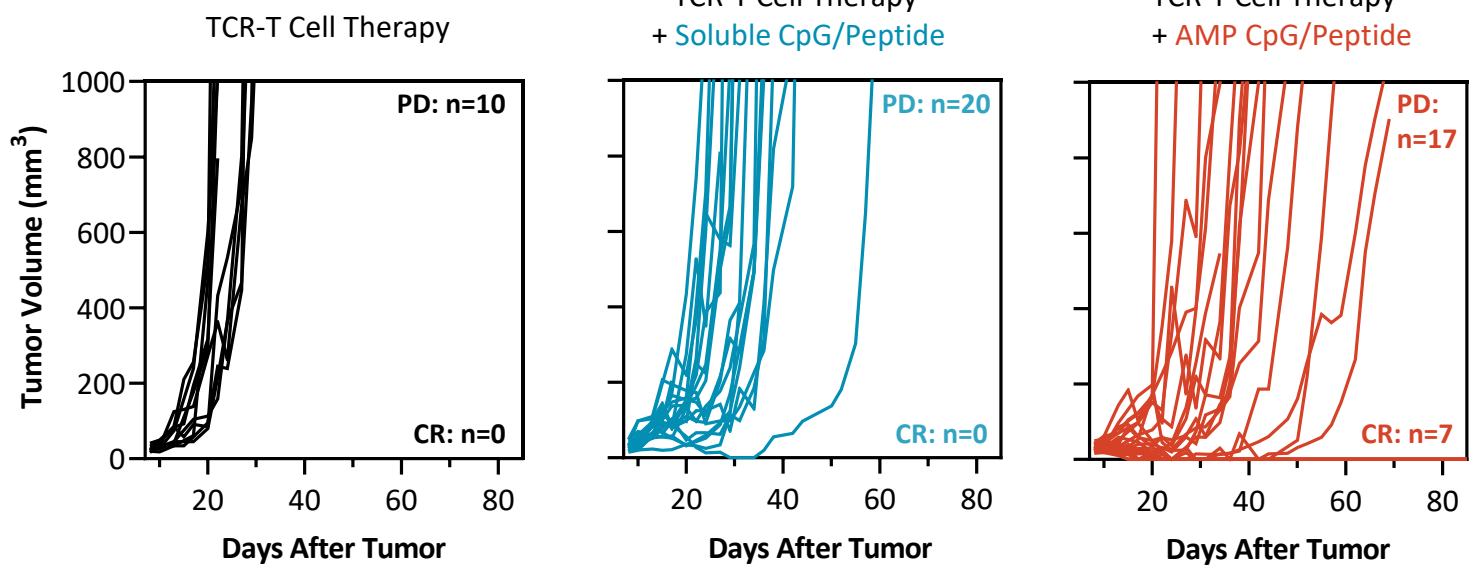




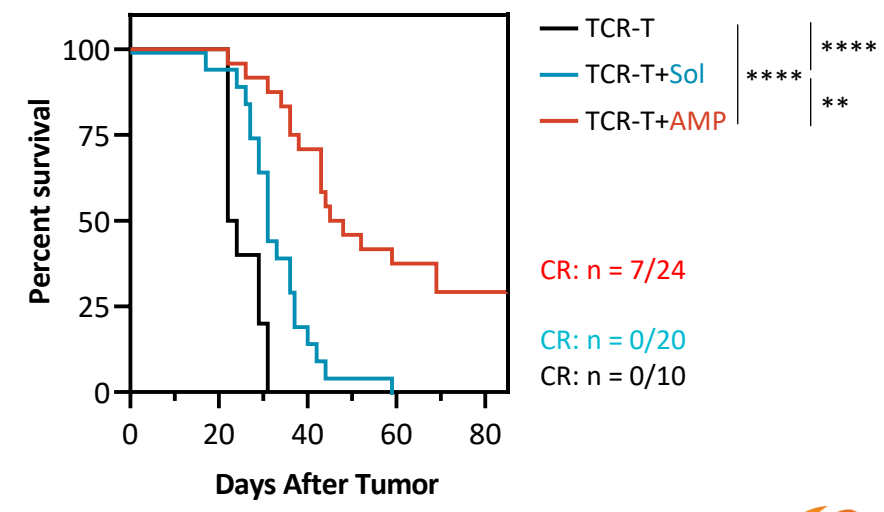
# AMP-Boosting Potently Enhances TCR-T Therapy to Eliminate Established Solid Tumors



## Anti-tumor Therapeutic Efficacy

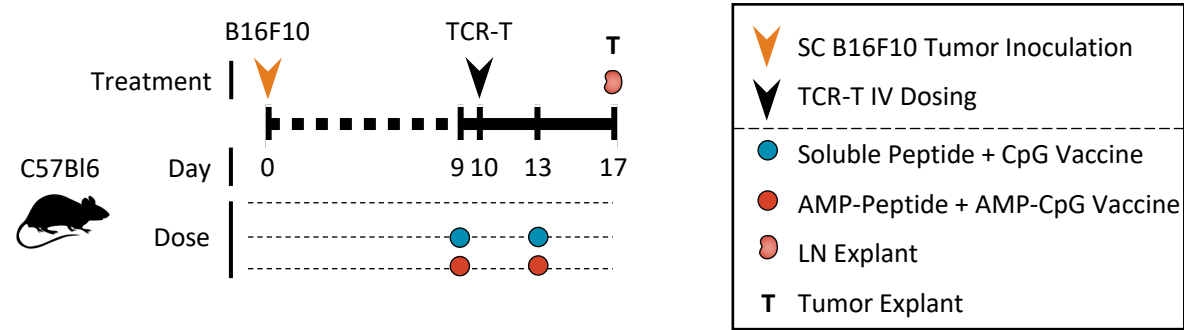


## Overall Survival

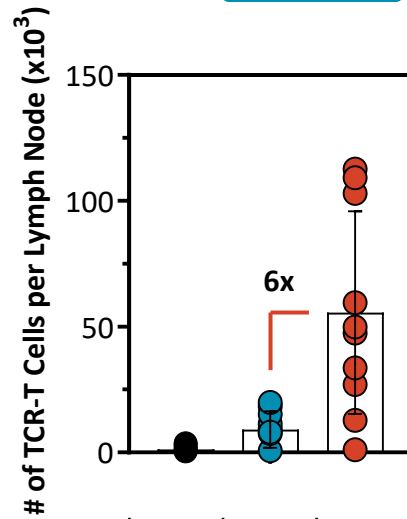


# AMP-Boosting Enhances TCR-T Expansion and Function in Lymph Nodes and Solid Tumors

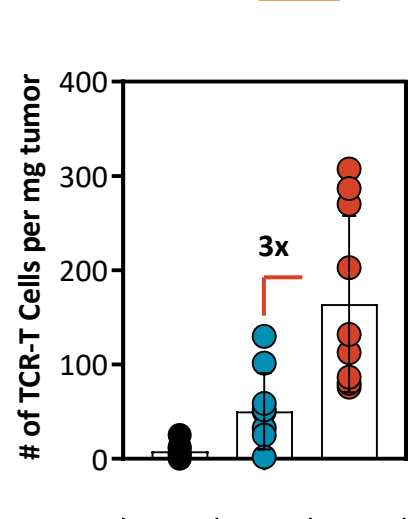
## Experimental Schema:



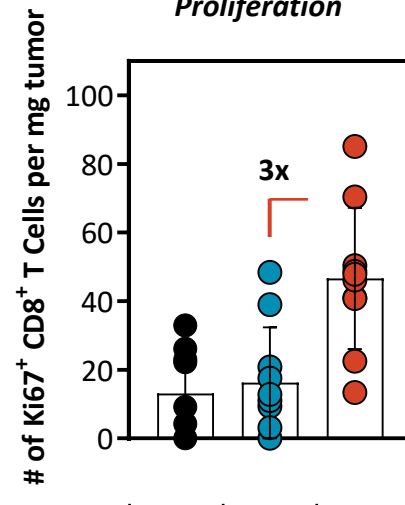
**T Cells: Lymph Node**



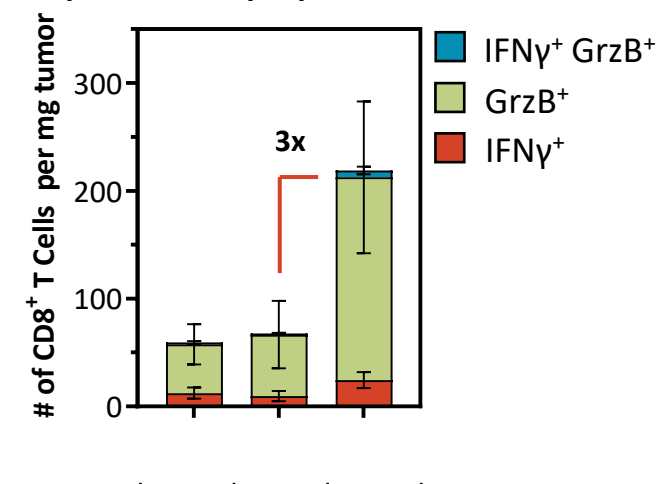
**T Cells: Tumor**



**T Cells: Tumor Proliferation**

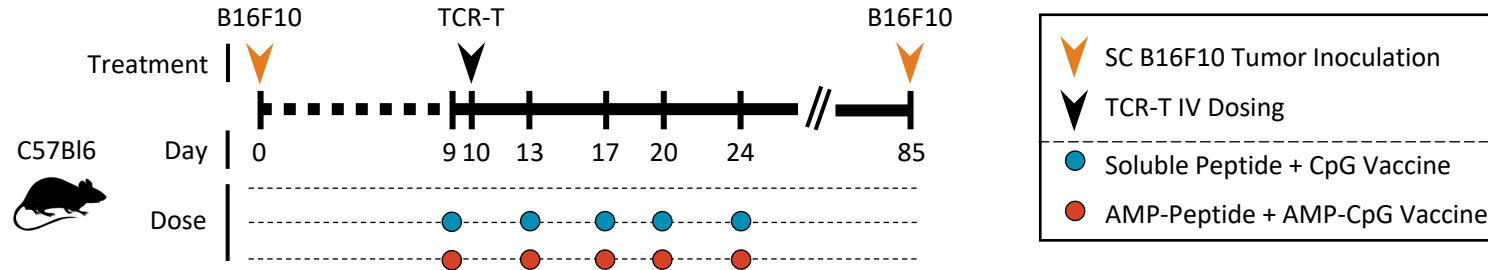


**T Cells: Tumor Cytokine and Cytolytic Function**

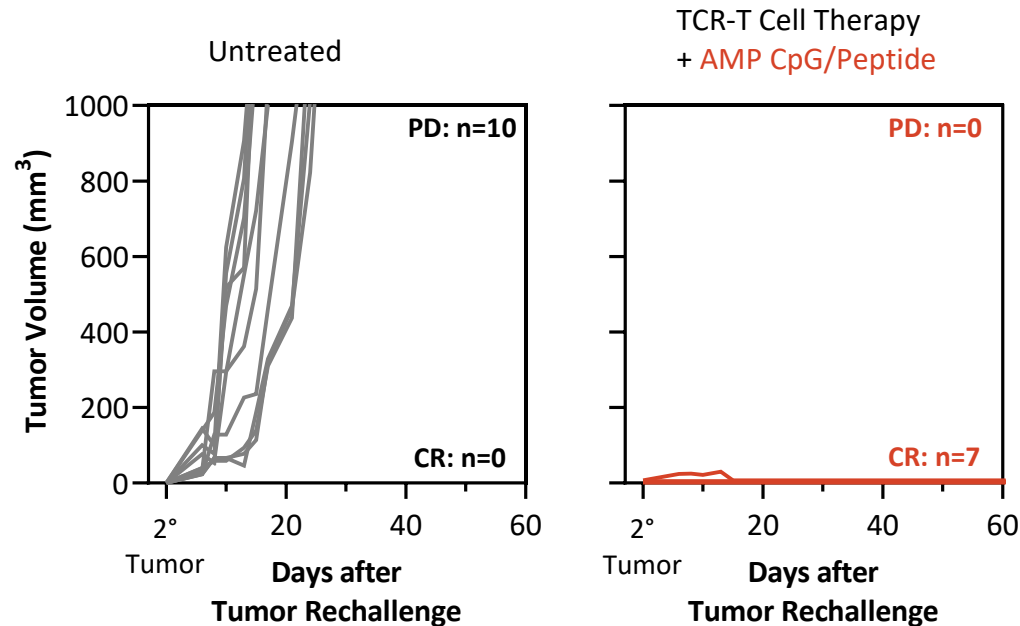


# AMP-Boosting Promotes Complete Durable Protection Against Solid Tumor Recurrence

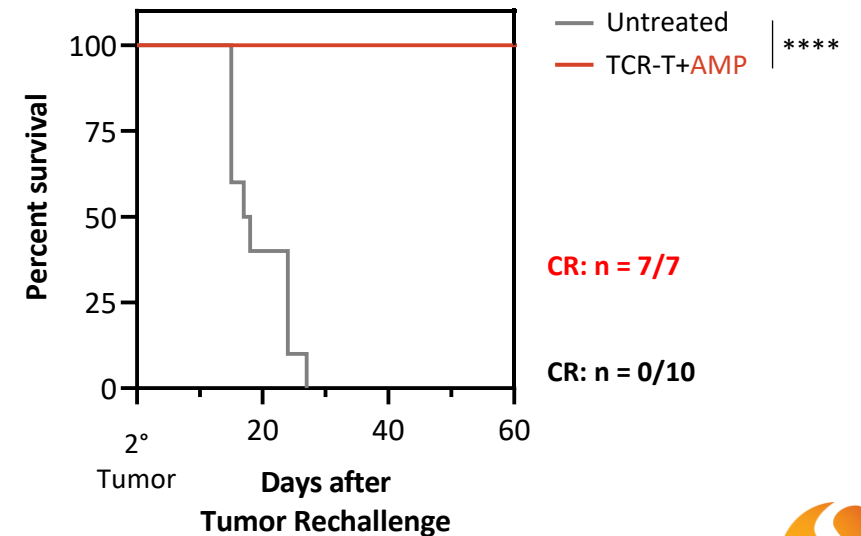
## Experimental Schema:



## Protection Against Tumor Recurrence



## Overall Survival



## AMP-Boosting of TCR-T Cell Therapy for Solid Tumors



- Increased TCR-T Cell Expansion
- Enhanced TCR-T Cell Lymph Node Activation
  - Improved Solid Tumor Infiltration
    - Solid Tumor Elimination
- Durable Protection Against Recurrence



## The AMP Platform Efficiently Targets the Lymph Nodes



- Enhanced Lymph Node Delivery and Retention
  - Potent APC Activation
- Inflammatory Transcriptional Programming
  - Robust Cytokine/Chemokine Milieu

## ELI-005: A Lymph Node Targeted Vaccine Against COVID-19



- Potent T Cell Responses in Blood, Lung, Spleen
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- Potent T Cell Responses
- Cross-reactive Neutralizing Antibody Responses

## AMP-Boosting of TCR-T Cell Therapy for Solid Tumors



- Increased TCR-T Cell Expansion
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- Durable Protection Against Recurrence





**Martin Steinbuck PhD, Aniela Jakubowski MS, Lochana Seenappa MS,  
Erica Palmer, Lisa McNeil PhD, Dylan Drakes PhD, Abdul Abbas MS,  
Jackie Shields MS, Chris Haqq MD PhD**

Liu, Irvine, et al. **Nature** 2014  
Steinbuck, DeMuth, et al. **Science Advances** 2021  
Martin, Irvine, et al. **Biomaterials** 2021

