



# IL2-LNPs As A Targeted mRNA-LNP Treg Immunotherapy

**Erin Maule**

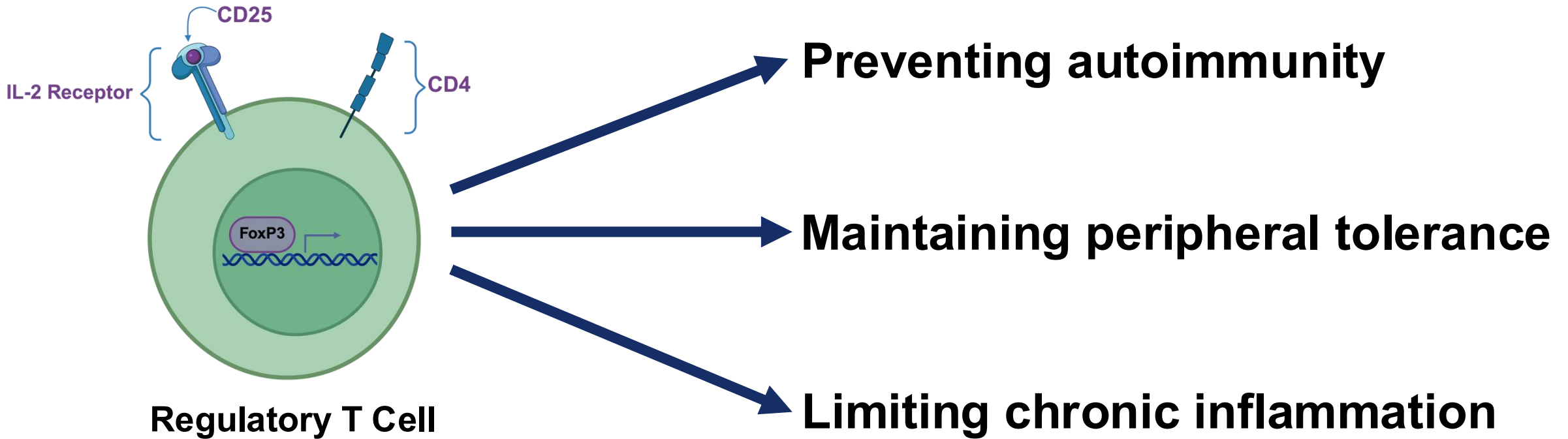
Immunology PhD Candidate

Betts Lab

University of Pennsylvania

April 16<sup>th</sup>, 2026

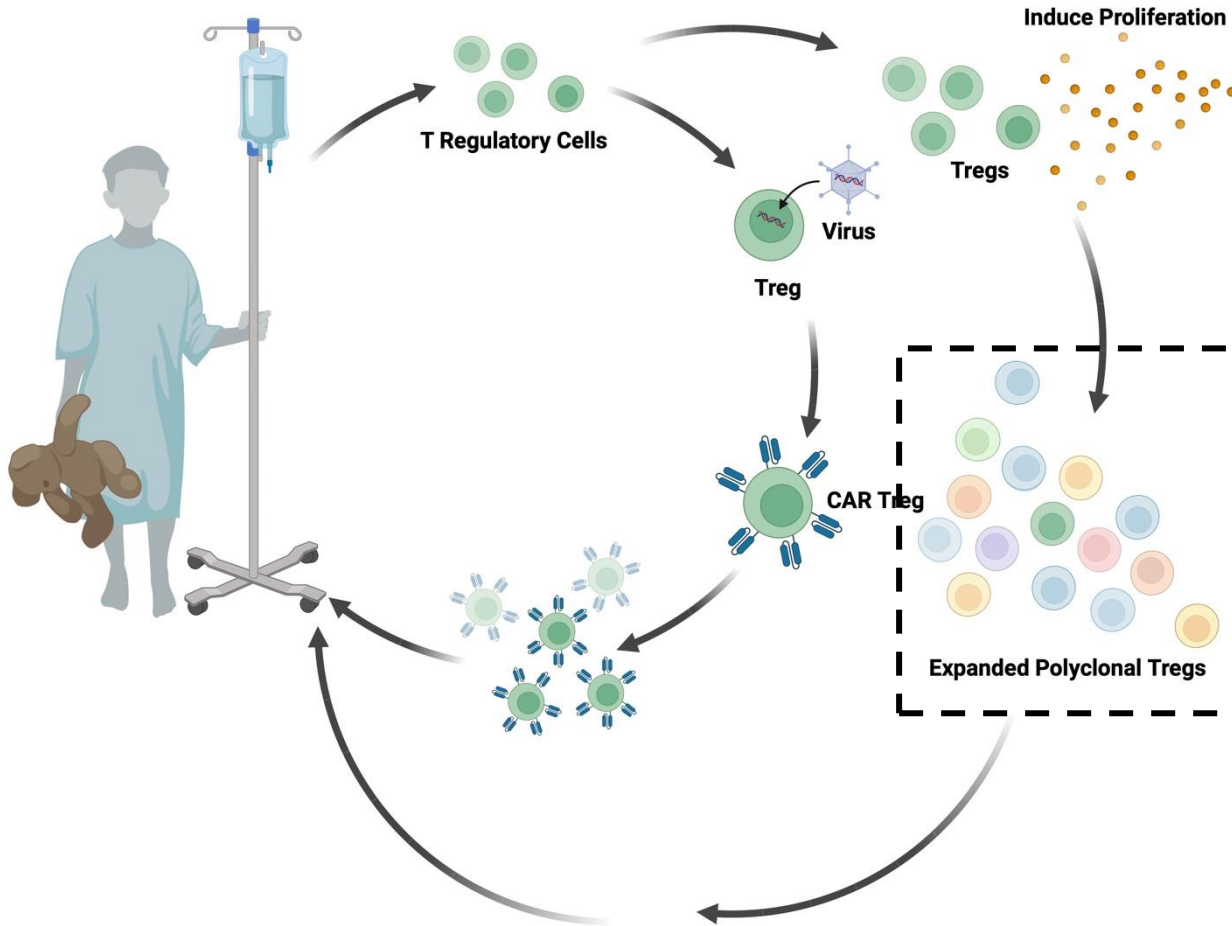
# Regulatory T Cells Are Important for Maintaining Immunological Homeostasis



**Treg dysfunction in pancreatic microenvironment is highly implicated in T1D pathogenesis.**

**Bolstering Treg function is an attractive strategy for T1D focused immunotherapies.**

# Need for Scalable, *In Vivo* Treg Immunotherapy Strategies



## Clinical trials of autologous polyclonal Tregs in T1D:

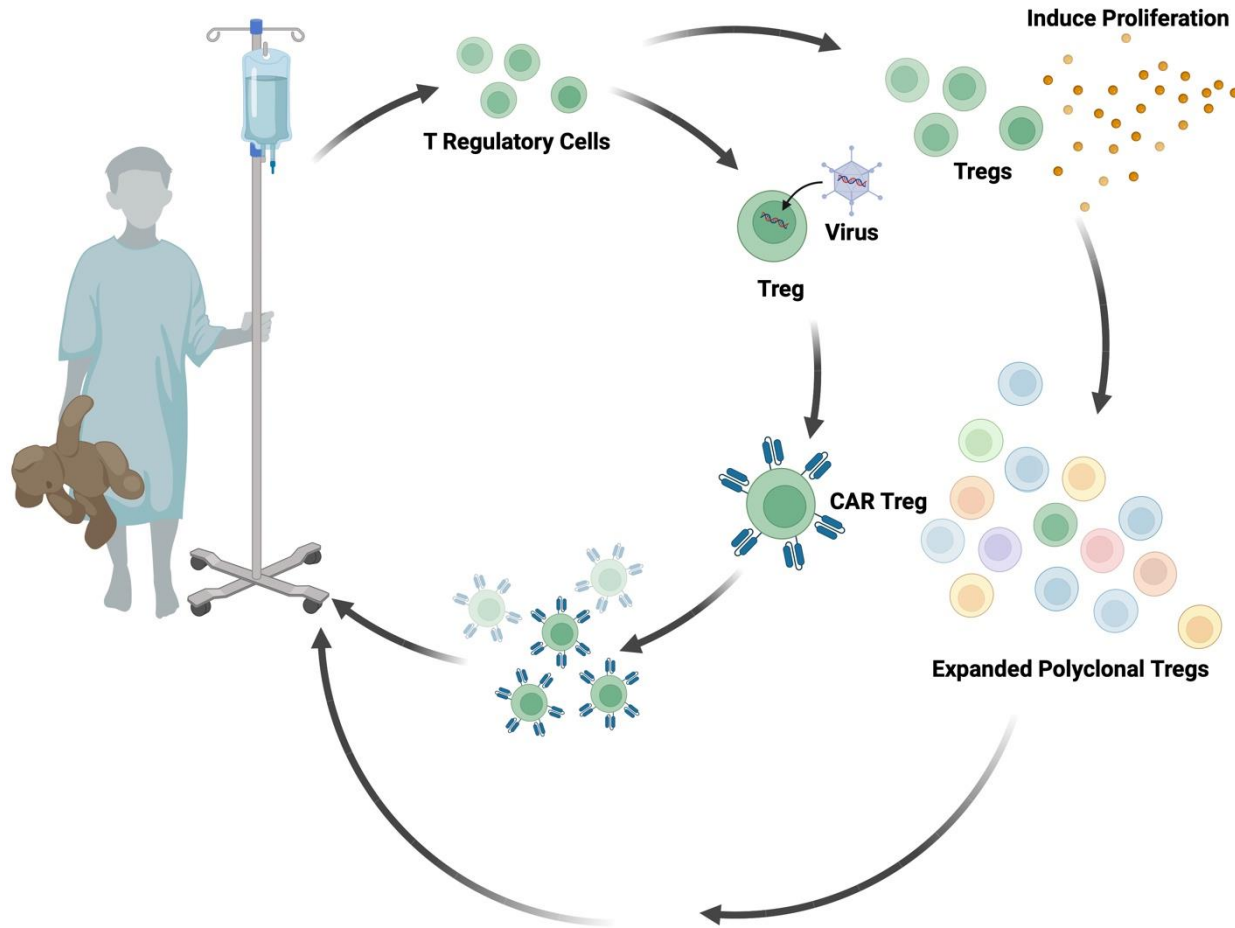
- ‘**T1DM immunotherapy using CD4+CD127lo/-CD25+ polyclonal Tregs**’<sup>1</sup>
  - University of California San Francisco
  - NCT01210664
- ‘**T1DM immunotherapy using polyclonal Tregs+IL-2 (TILT)**’<sup>2</sup>
  - University of California San Francisco
  - NCT02772679
- ‘**Safety and Efficacy of CLBS03 in adolescents with recent onset type 1 diabetes**’<sup>3</sup>
  - The Sanford Project T-Rex Study – Lisata Therapeutics, Inc.
  - NCT02691247

(1) Bluestone, et al. *Science Translational Medicine*. (2015) (3) Caladrius Biosciences. *Caladrius biosciences reports top-line data for the phase 2a*

(2) Dong, et al. *JCI Insight*. (2021)

*Sanford project: T-Rex trial of CLBS03 for recent onset type 1 diabetes*. (2019)

# Need for Scalable, *In Vivo* Treg Immunotherapy Strategies

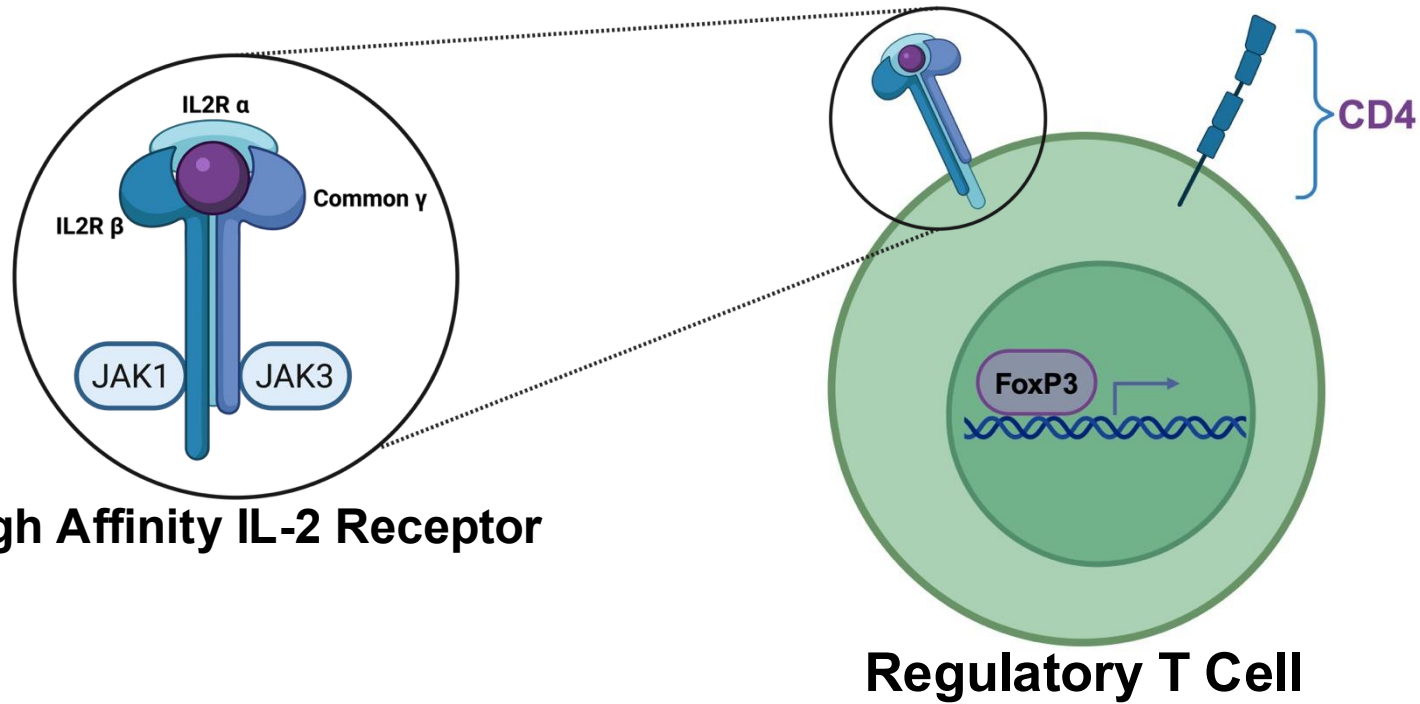


- *Ex vivo* approaches have had difficulties in maintaining Treg phenotype after re-infusion<sup>1-4</sup>.
- *Ex vivo* approaches are labor intensive, expensive, and patient specific.
- Transient *in vivo* Treg modulation by mRNA-LNPs may be more broadly applicable.

(1) Bluestone, et al. *Frontiers Immunology*. (2023) (3) Hasan, et al. *International Immunopharmacology*. (2025)

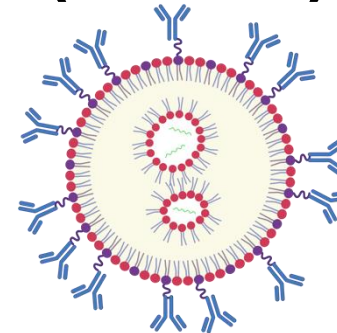
(2) Raffin, et al. *Nature Reviews Immunology*. (2020) (4) Marek-Trzonkowska, et al. *Pediatric Diabetes*. (2013)

# Targeting IL-2 Receptor to Target Tregs

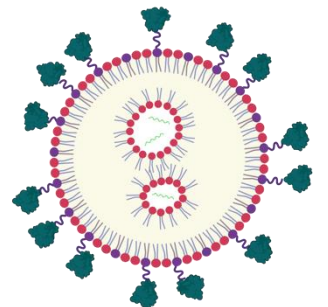


## Two Strategies to Target IL2R

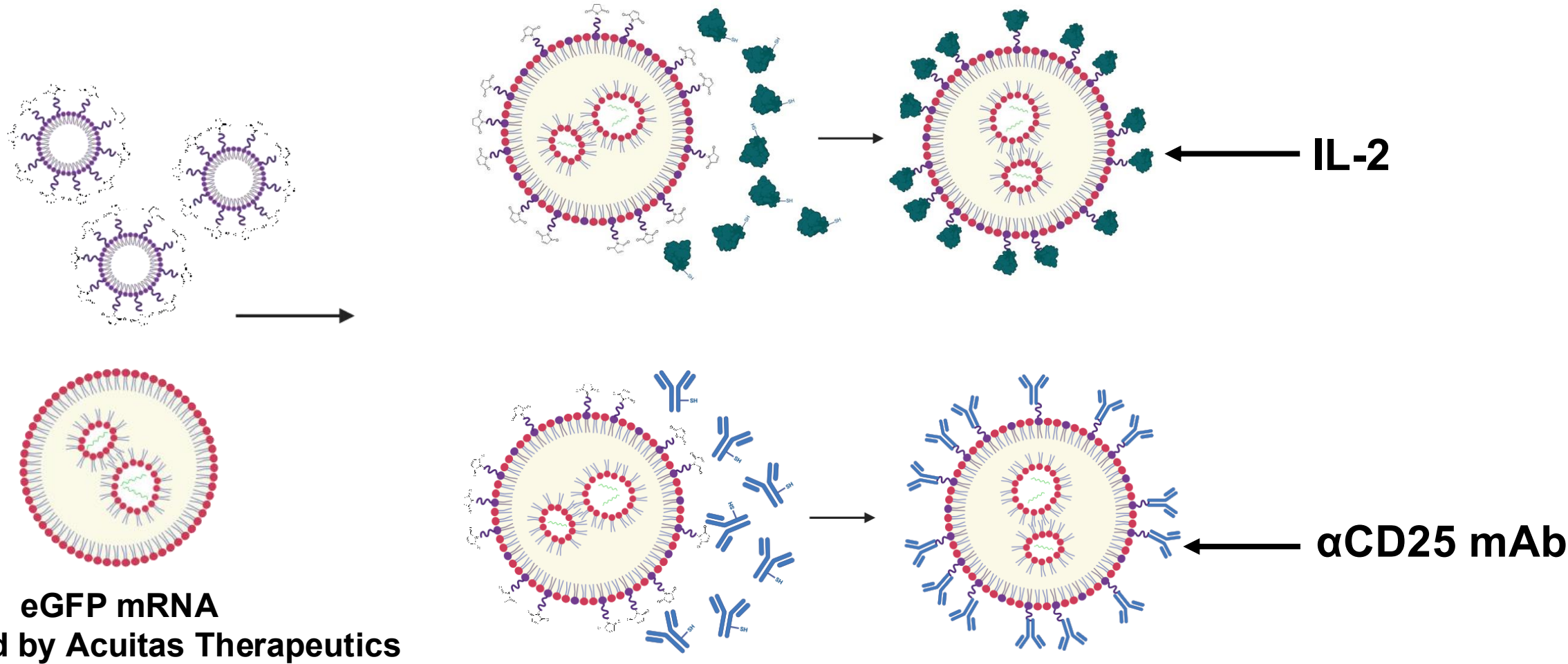
$\alpha$ CD25 mAb  
(IL2R $\alpha$ )



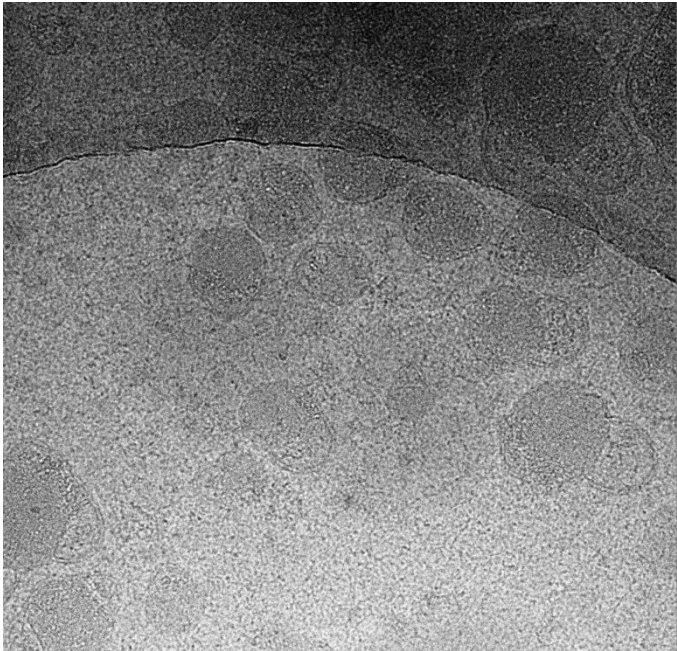
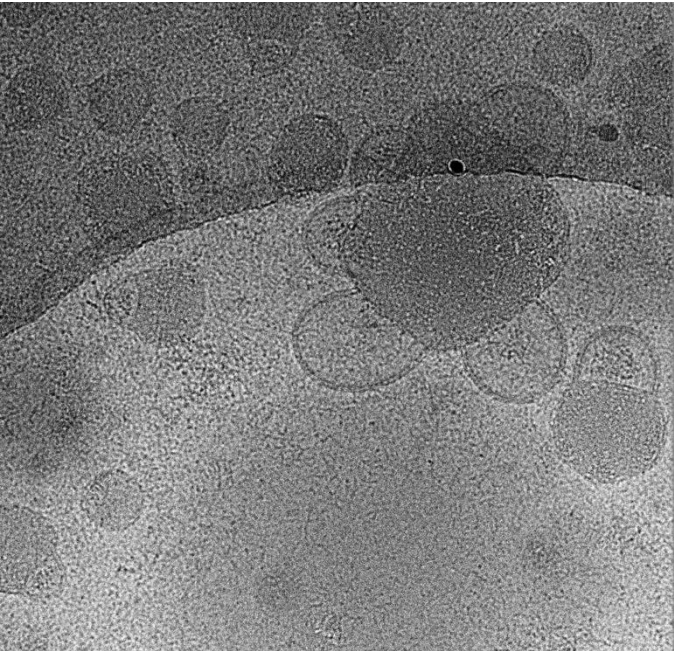
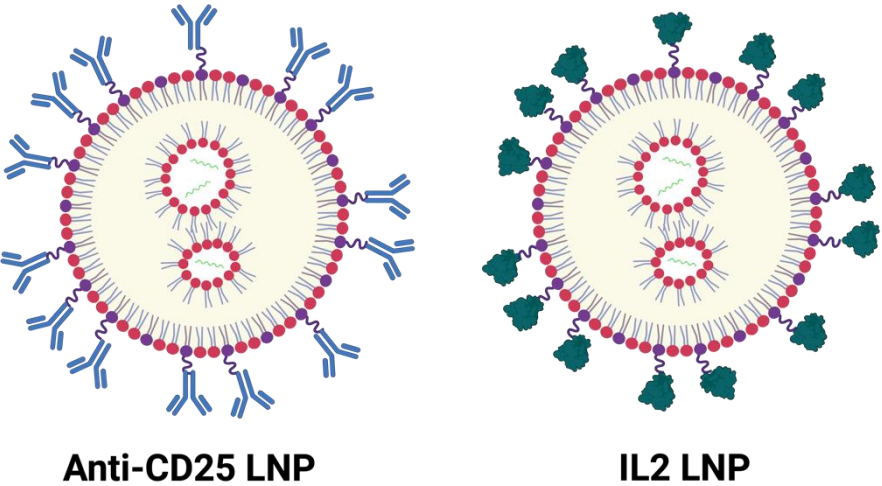
IL2



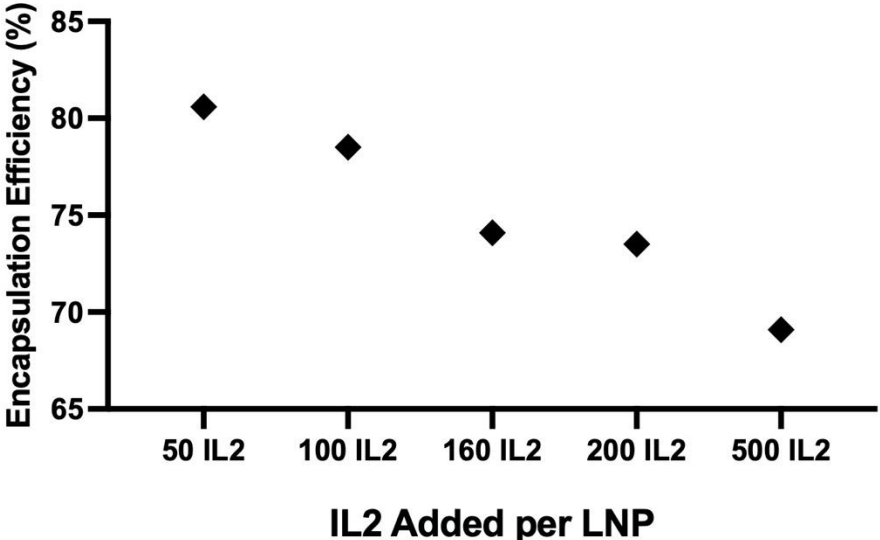
# mRNA-LNP Targeting Strategy for Tregs



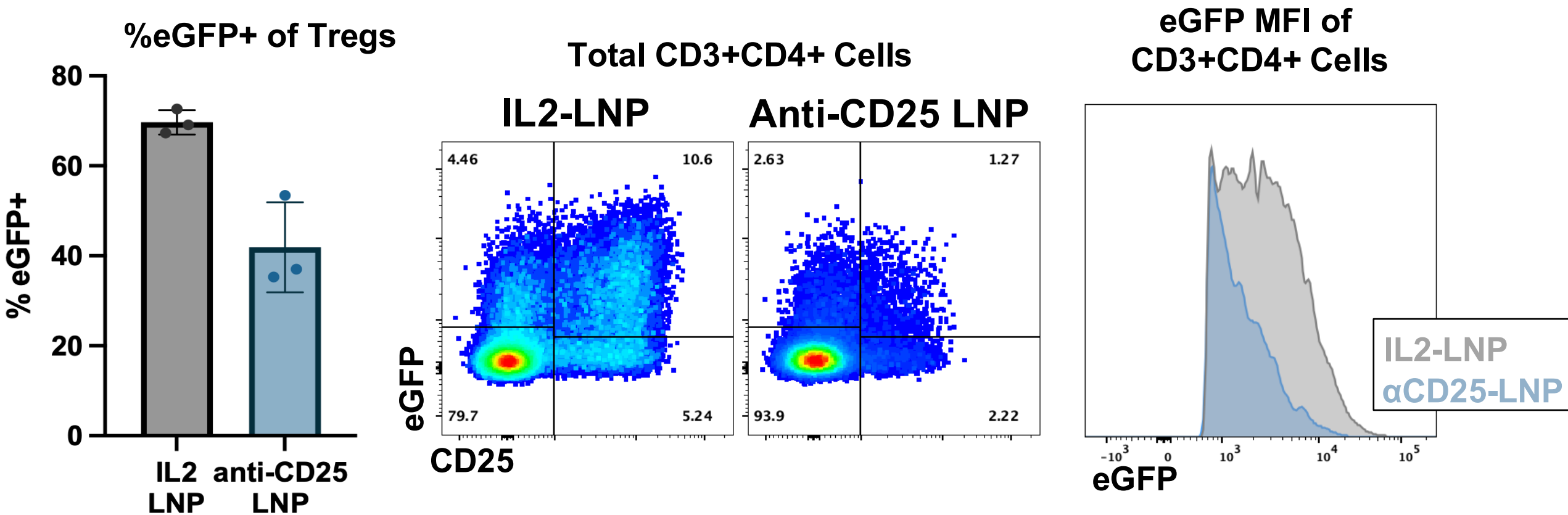
# mRNA-LNP Targeting Strategy for Tregs



Cryo-EM performed by M. Hajialyani

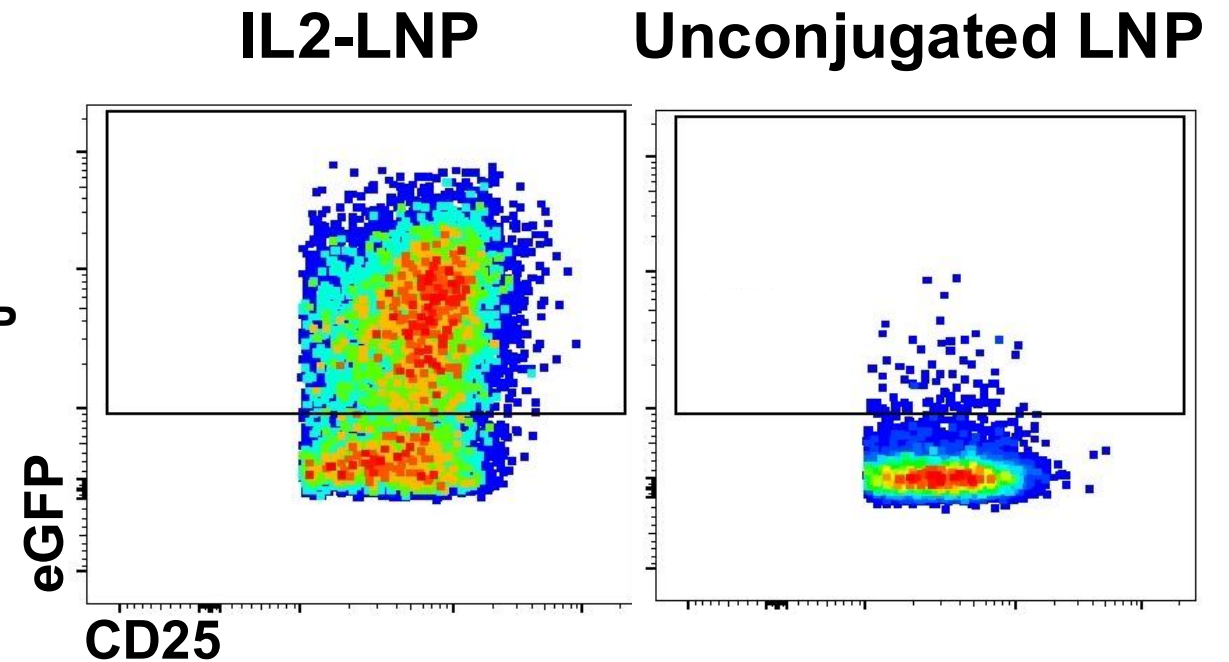
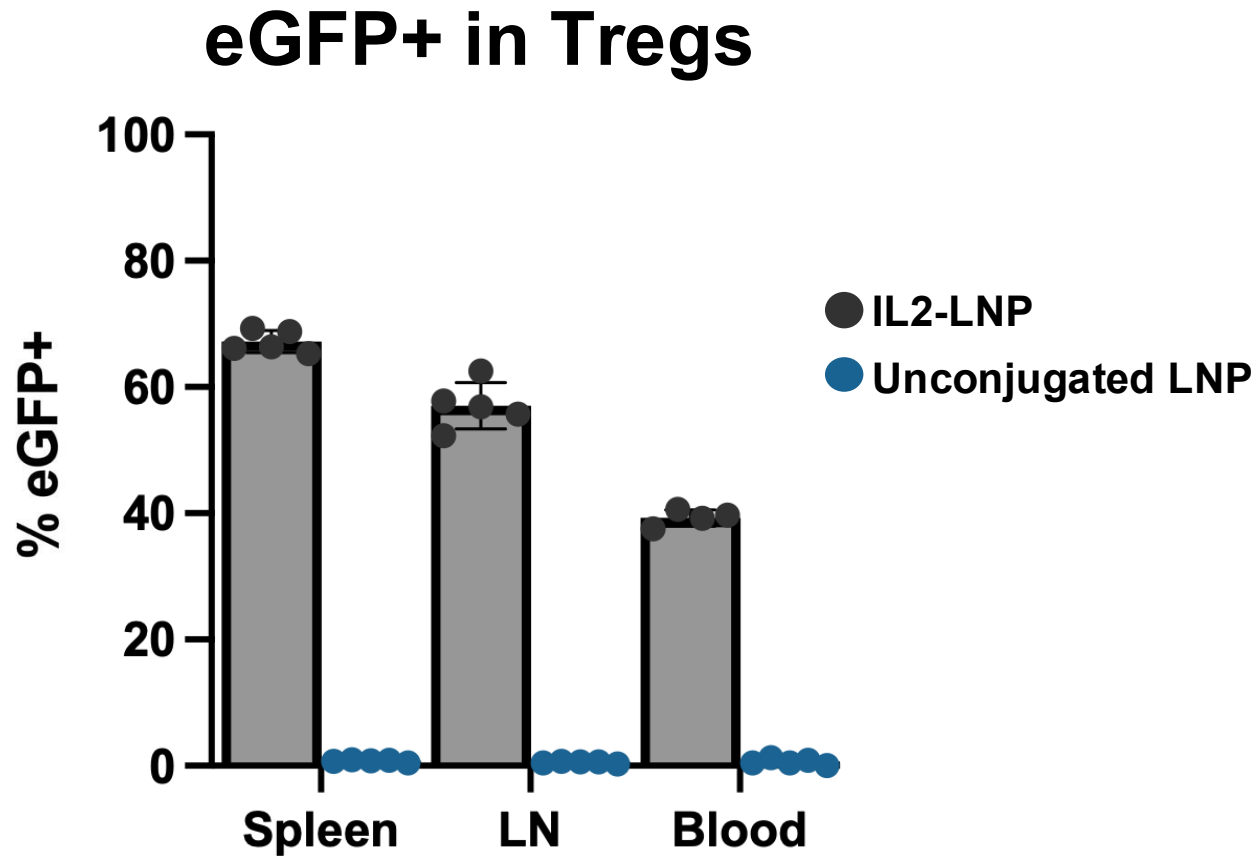


# IL2-LNPs Demonstrate Higher eGFP MFI after Delivery

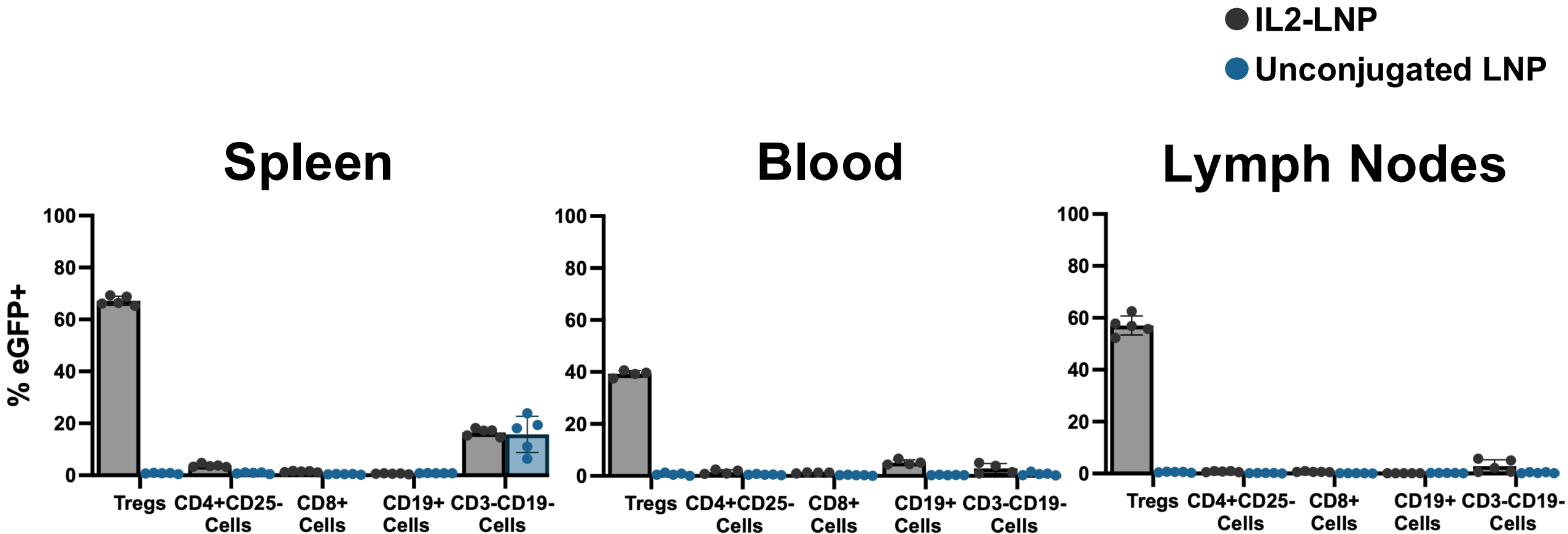


Tregs: CD3+CD4+CD127-CD25+  
5µg LNPs delivered IV. Spleen collected at 24 hours post-LNP treatment.

# IL2-LNPs Effectively Target Tregs *In Vivo* at 24 Hours

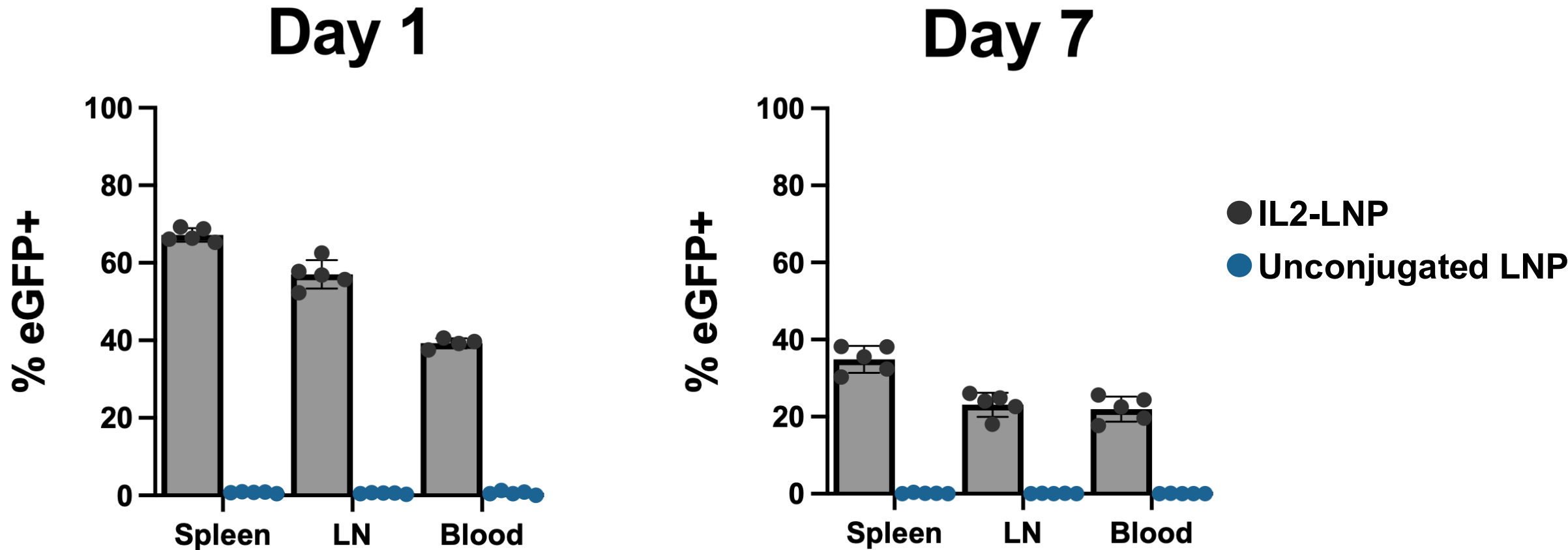


# Minimal Background Uptake of IL2-LNPs By Other Immune Subsets



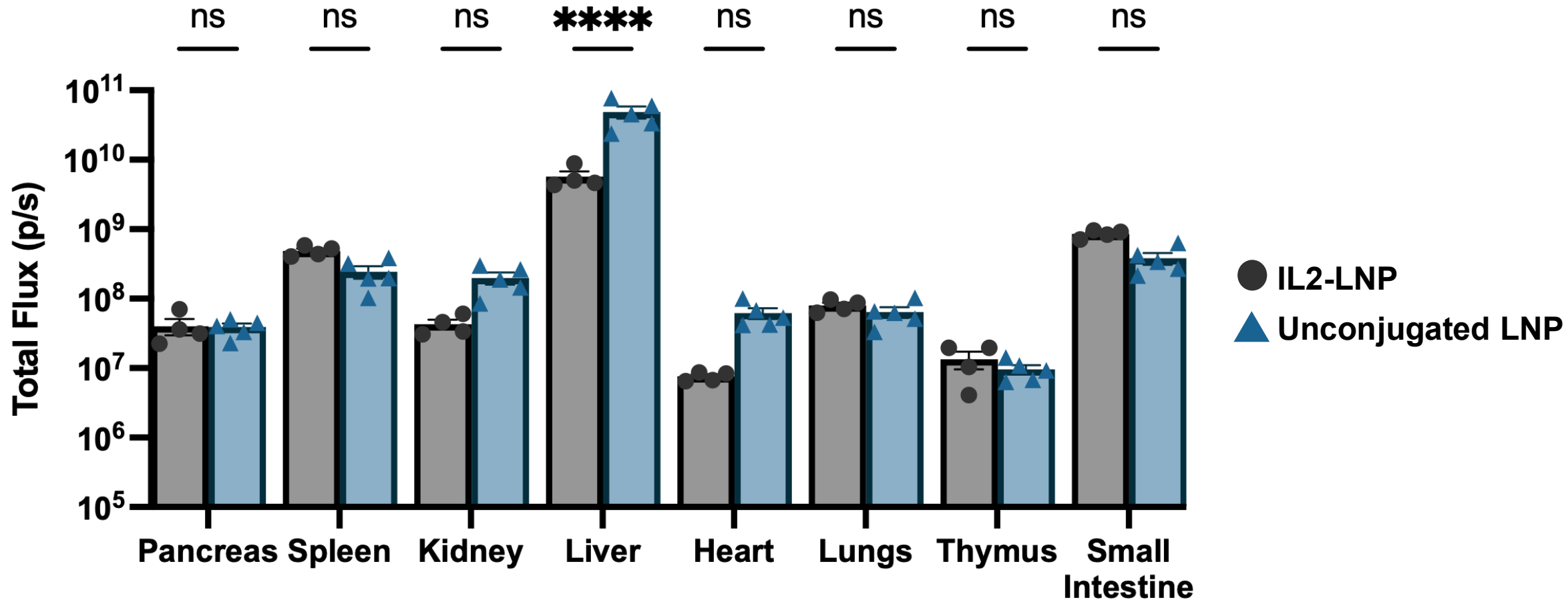
Tregs: CD3+CD4+CD127-CD25+  
5 $\mu$ g LNPs delivered IV. Spleen, blood, and lymph nodes collected at 24 hours post-LNP treatment.

# Sustained eGFP Expression in Circulating Tregs

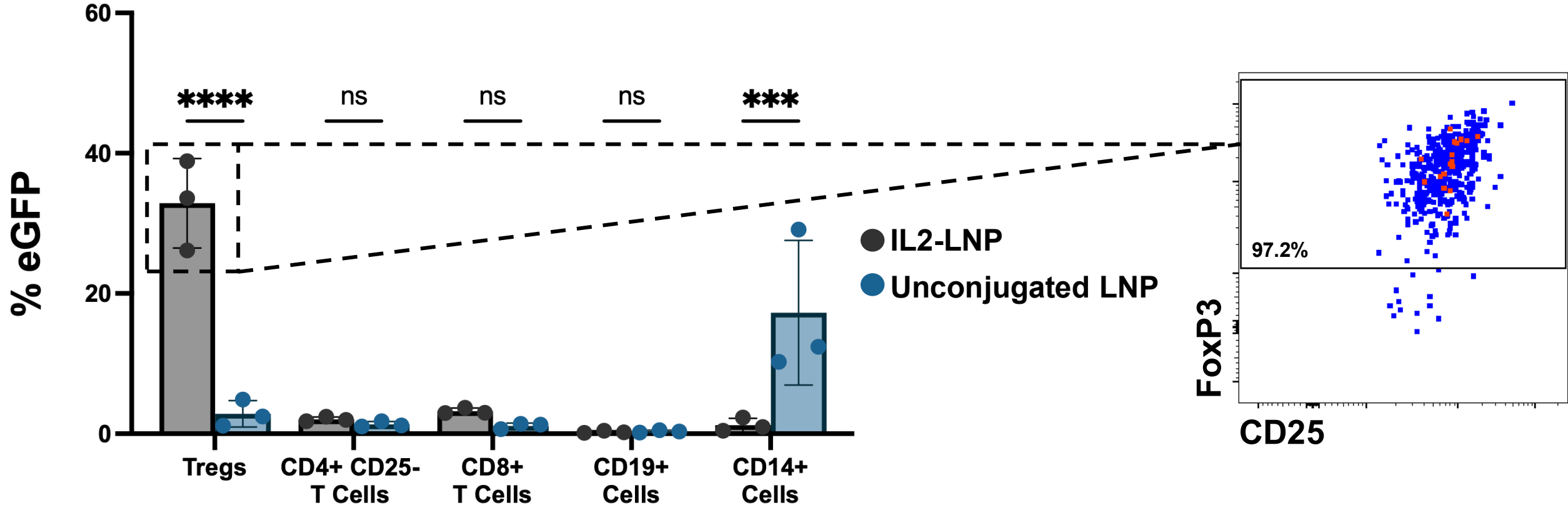


Tregs: CD3+CD4+CD127-CD25+  
5µg LNPs delivered IV. Spleen collected 1 day and 7 days post-LNP treatment.

# Biodistribution of Luciferase Expression



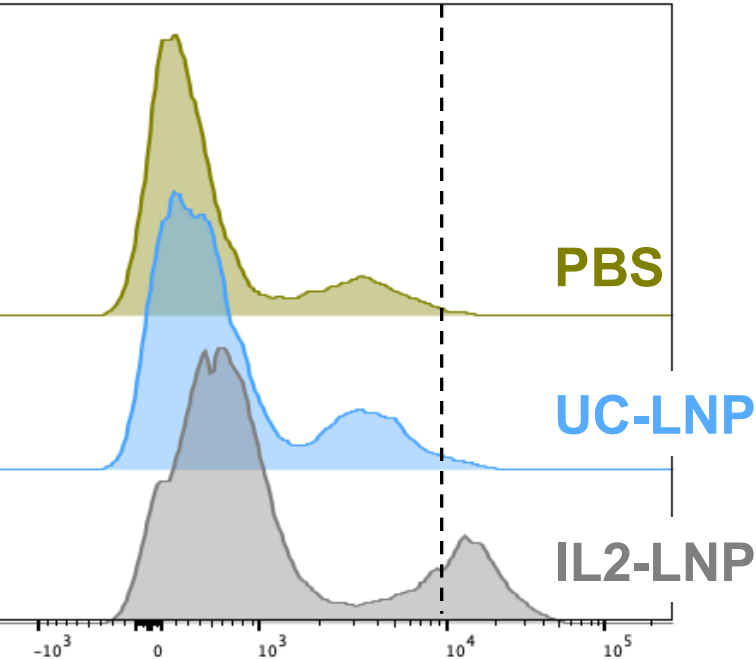
# IL2-LNPs Target Primary Human Tregs *In Vitro*



n = 3 Human Organ Donor Splenocytes  
 Splenocytes treated with 2µg per 1E6 cells of LNP; eGFP signal assessed after 48hours of culturing  
 Statistics done with Two-Way ANOVA

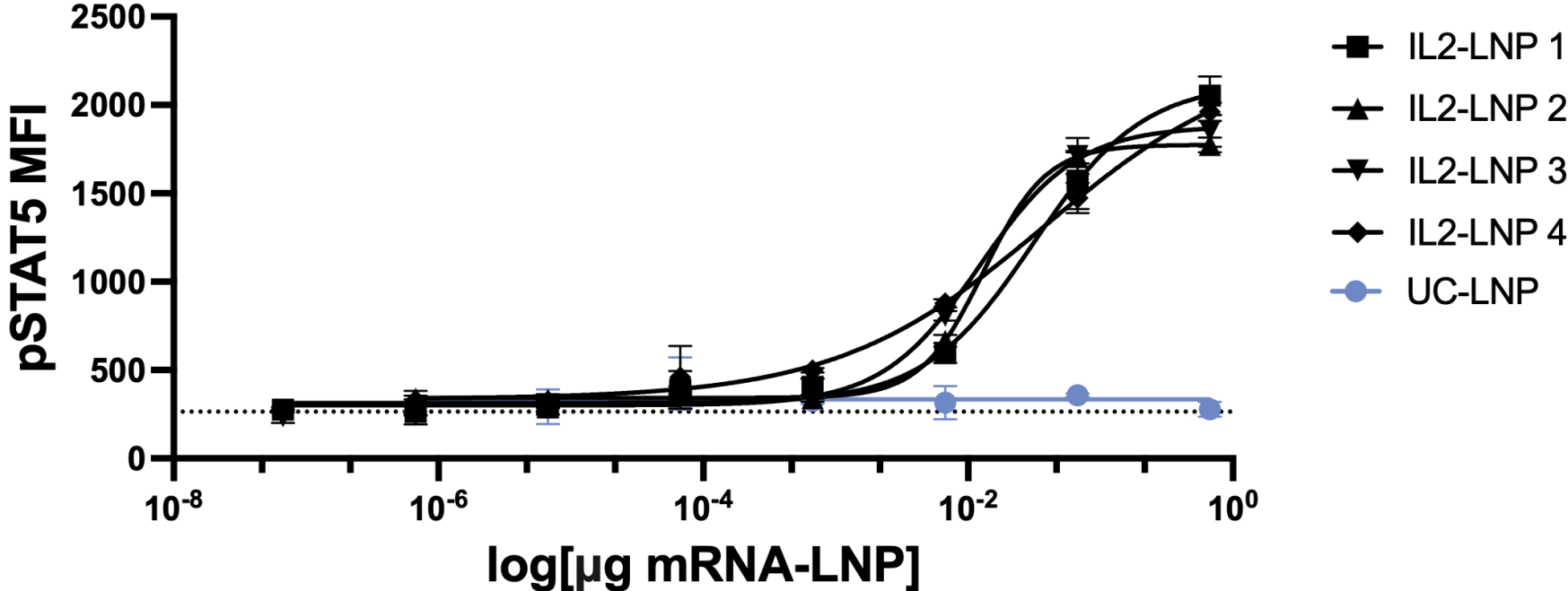
# Conjugated IL-2 Retains Biological Activity and Signals Through IL-2R

CD3+CD4+ Cells



FoxP3

CD3+CD4+IL2R $\alpha$ + Cells



# Conclusions

- IL-2 conjugated mRNA-LNPs enable direct *in vivo* targeting of regulatory T cells
  - Avoids the need for *ex vivo* cell product production
  - Highly specific LNP delivery to human Tregs *in vitro* and mouse Tregs *in vivo*
  - Direct IL-2 conjugation is superior to anti-CD25 antibody mediated delivery
  - IL-2 maintains biologic activity when conjugated to mRNA-LNPs
- Current studies will apply this strategy to modulate pancreas homing properties of Tregs for T1D amelioration
  - Delivery of CXCR2 mRNA to allow Tregs to respond to elevated chemokine ligands expressed in inflamed islets (CXCL1, 2, 6, 8)

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