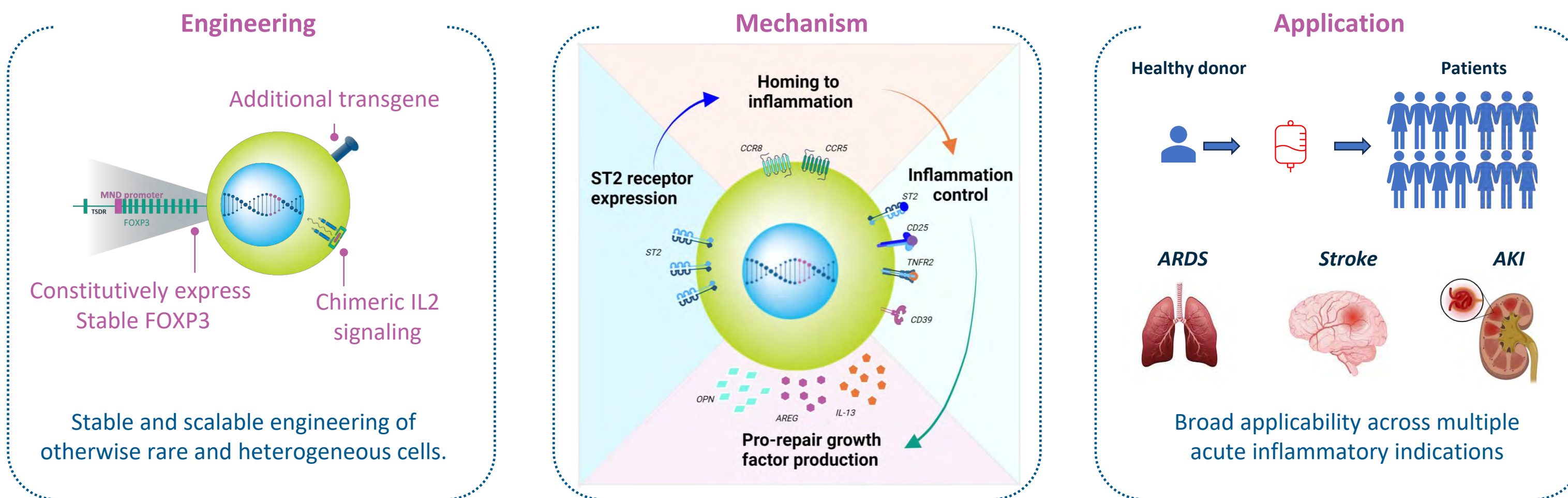


# Allogeneic Engineered T Regulatory Cells Improve Disease Outcome in Preclinical Models of Acute Lung Injury

Maegan Hoover, Priya Saikumar, Yash Agarwal, Madison Milaszewski, Kassandra J Baron, Nicole R Reed, Carlos E Frias, Abigail Doherty, Heth R Turnquist, Tiffany F Chen, Thomas Wickham, Payam Zarin

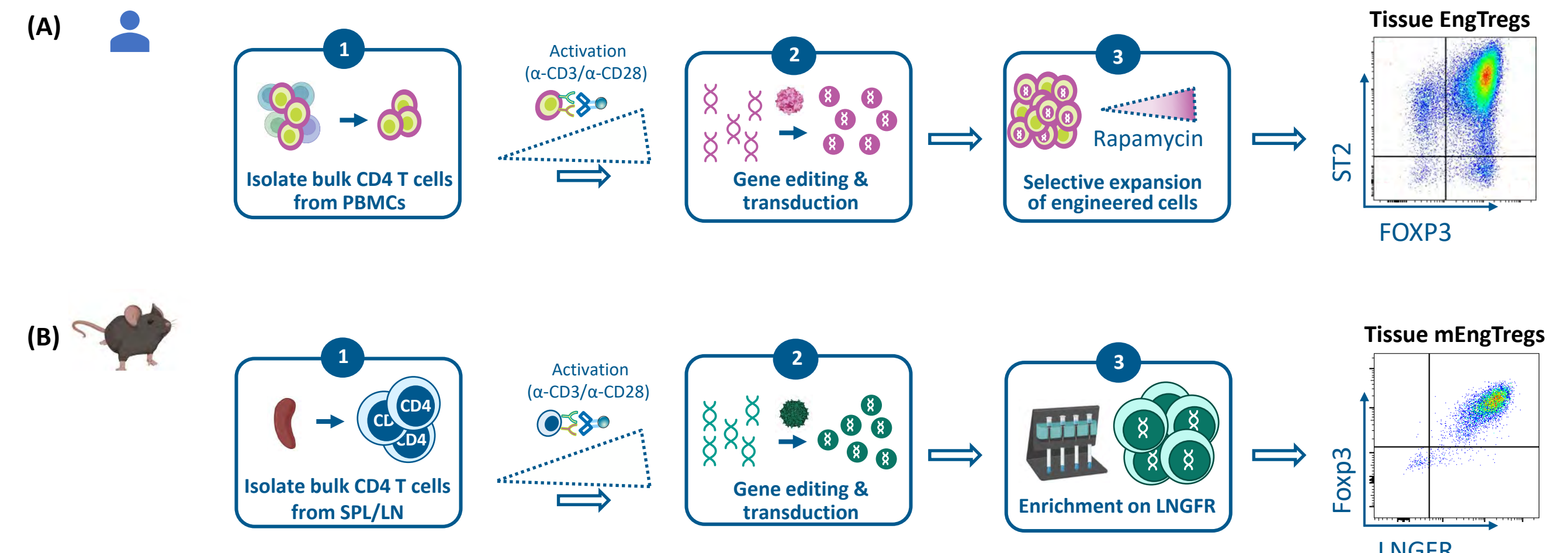
GentiBio, Inc., Cambridge, MA, USA

## Premise



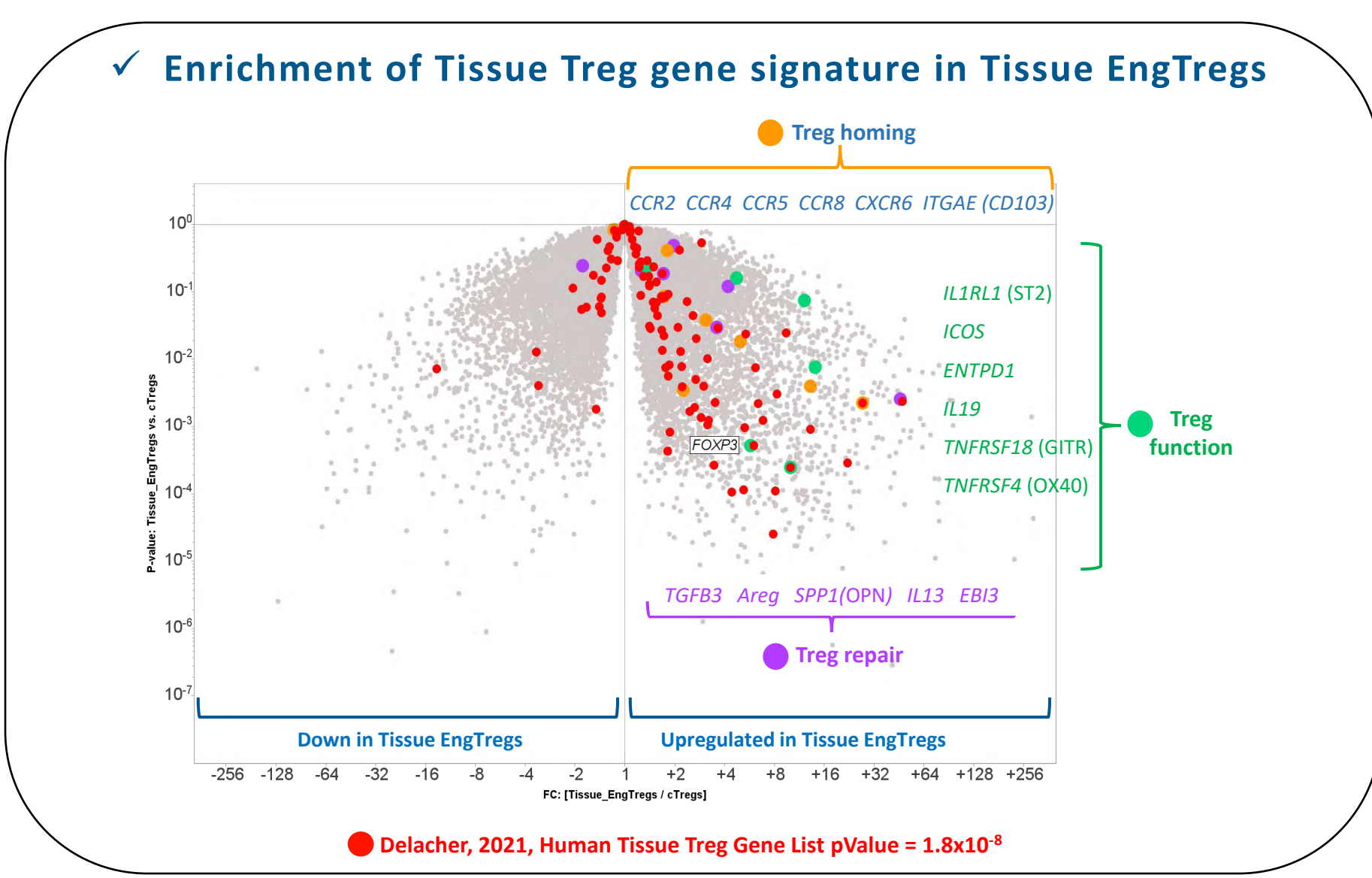
**Human Engineered Tissue Tregs as a therapeutic approach for acute inflammatory and ischemic diseases.** Panel 1: Gene edited CD4<sup>+</sup> T cells express stable FOXP3 and a rapamycin-activated signaling complex (CISC) that provides tunable IL-2 signal, divorcing FOXP3 expression from existing regulatory elements known to promote Treg instability under inflammatory conditions (Honaker S, *Science Translational Medicine*, 2020, Cook P, *Molecular Therapy*, 2023). Panel 2: Expression of further transgenes, such as the IL-33 receptor ST2, enables effective tissue homing and enhanced expression of programs associated with activation, function, and repair in response to inflammatory signals. Panel 3: Scalable manufacturing of an allogeneic cell product from healthy donors would allow for an off-the-shelf treatment applicable across a broad range of acute inflammatory and ischemic diseases often associated with poor prognosis.

## Engineered Treg Generation

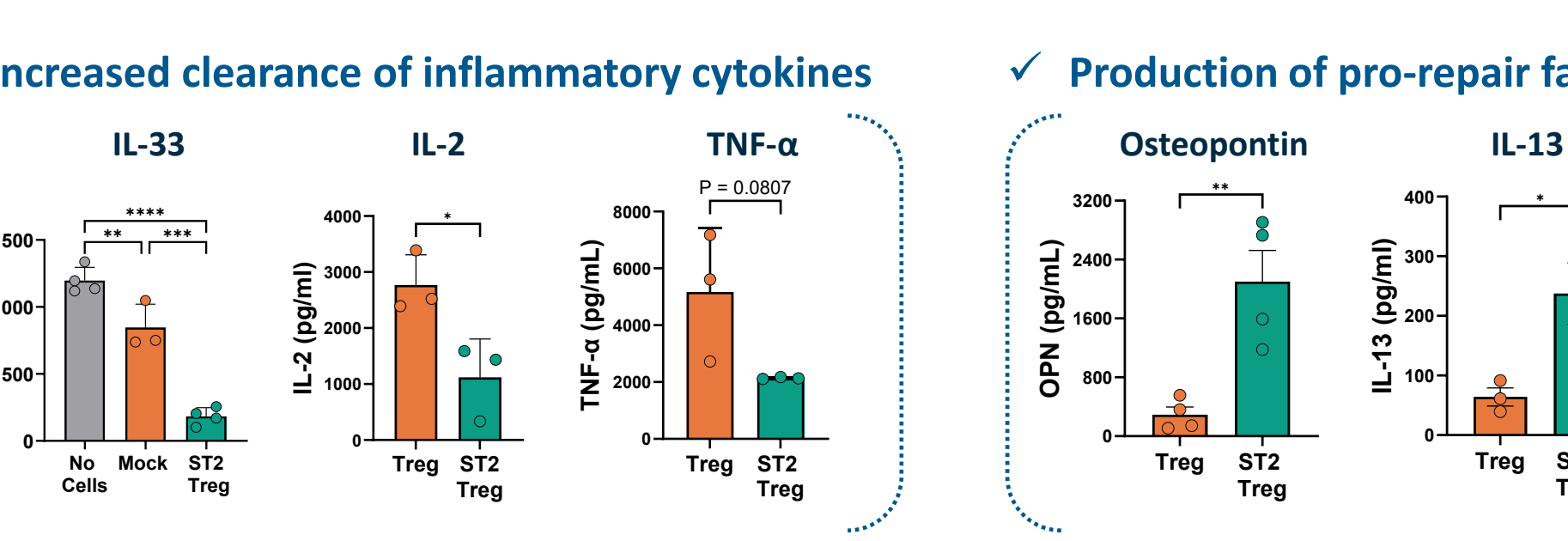
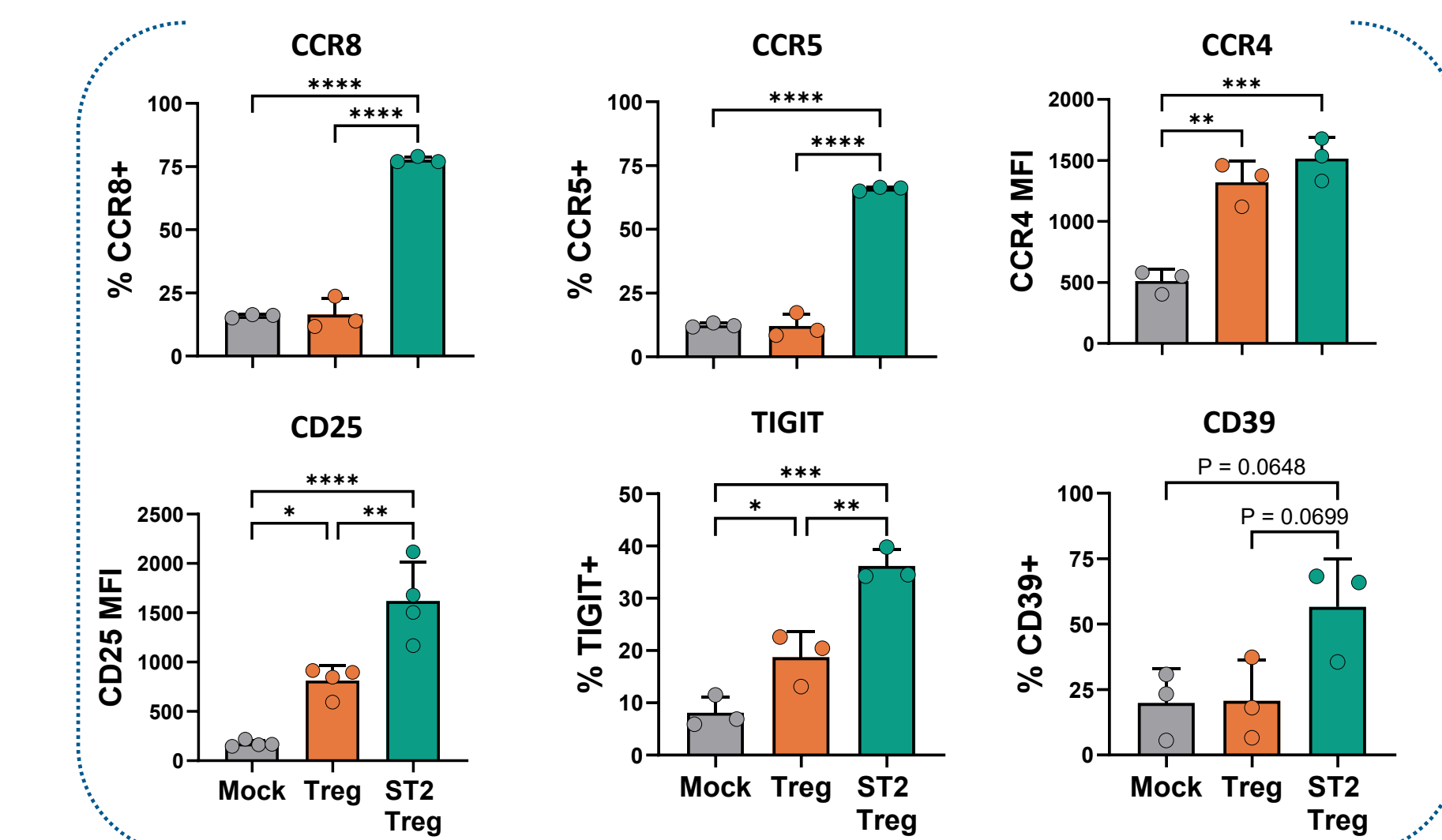
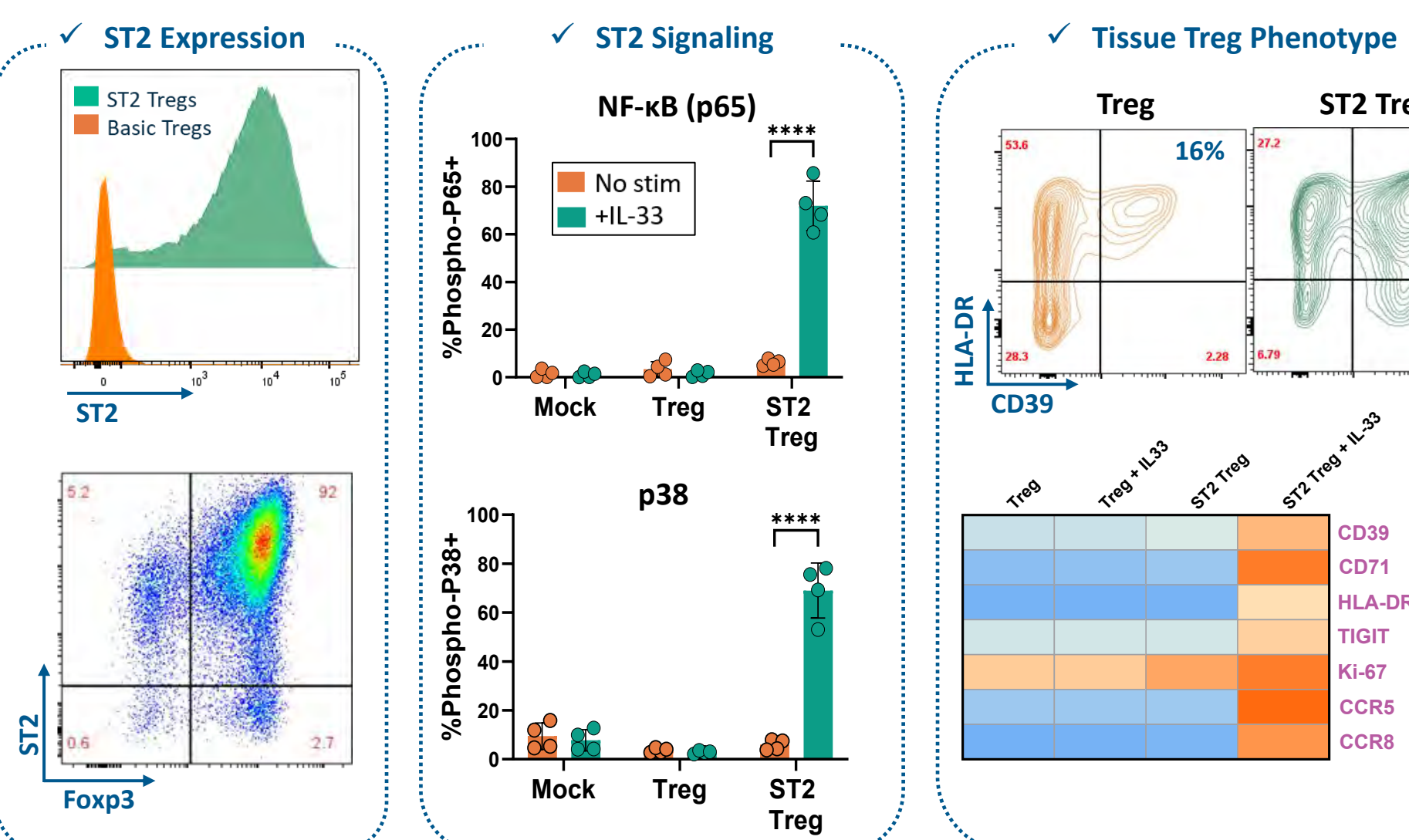


(A) Human or (B) murine CD4<sup>+</sup> T cells are isolated and activated via  $\alpha$ -CD3/ $\alpha$ -CD28 microbeads (Panel 1). Cells are genetically modified using lentiviral transduction and/or CRISPR-Cas9 mediated knock-in of transgenes delivered by AAV vectors (Panel 2). For human EngTregs, CISC receptor expression enables selective expansion of edited cells, while mouse surrogate EngTregs rely on enrichment by the extracellular LNGFR tag (Panel 3).

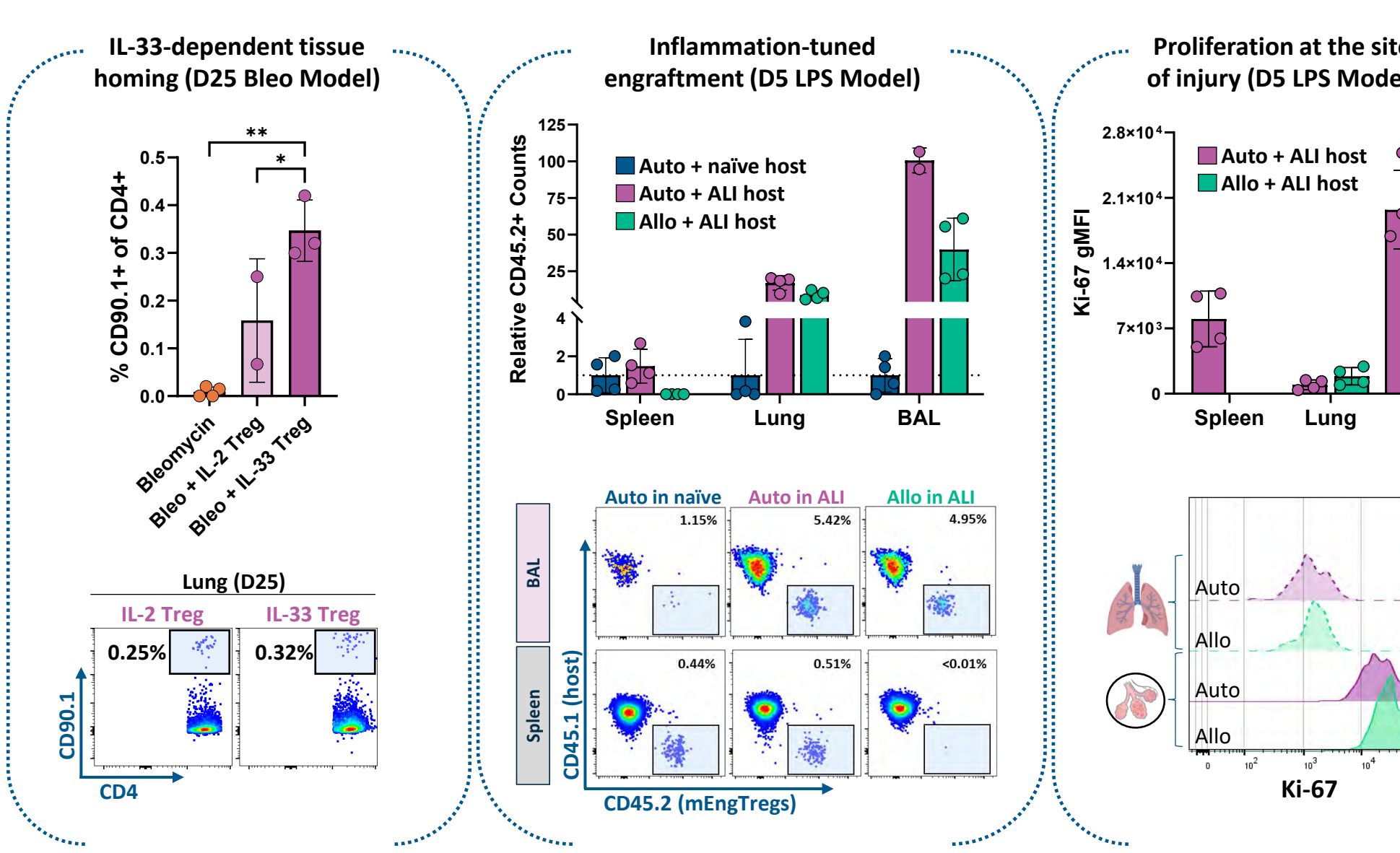
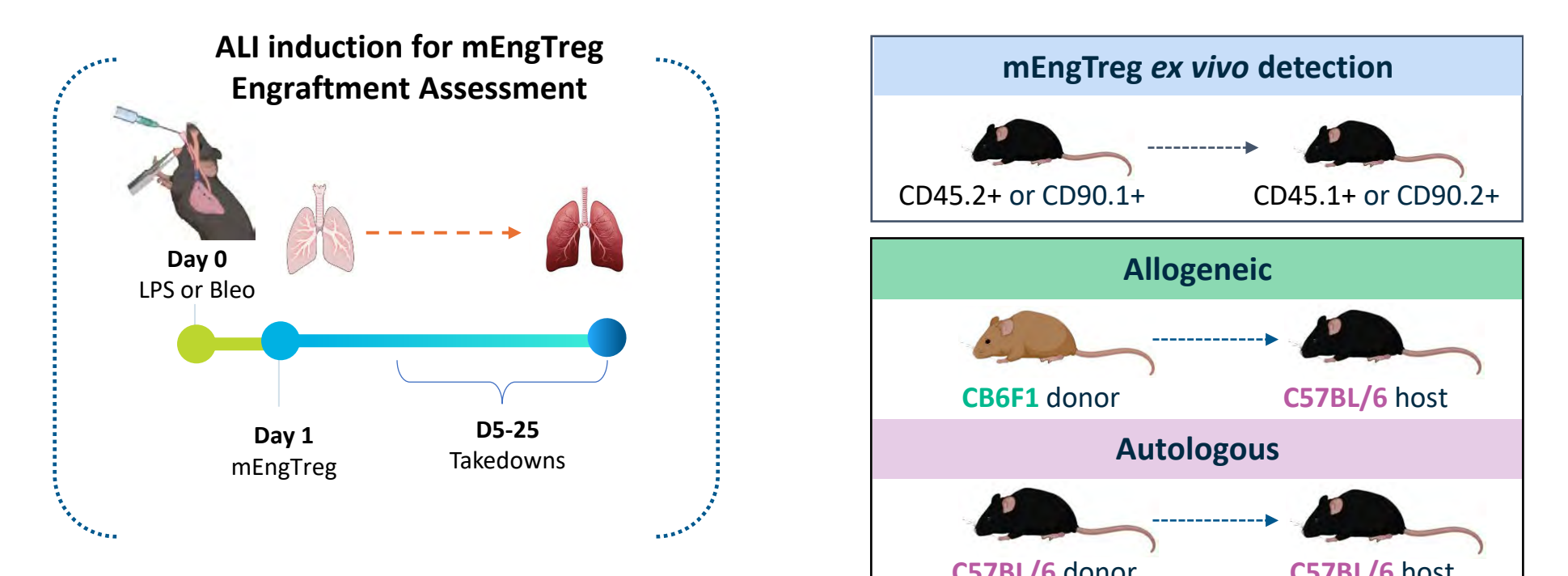
## 1. Tissue EngTregs signal through ST2 to drive a repair ready phenotype



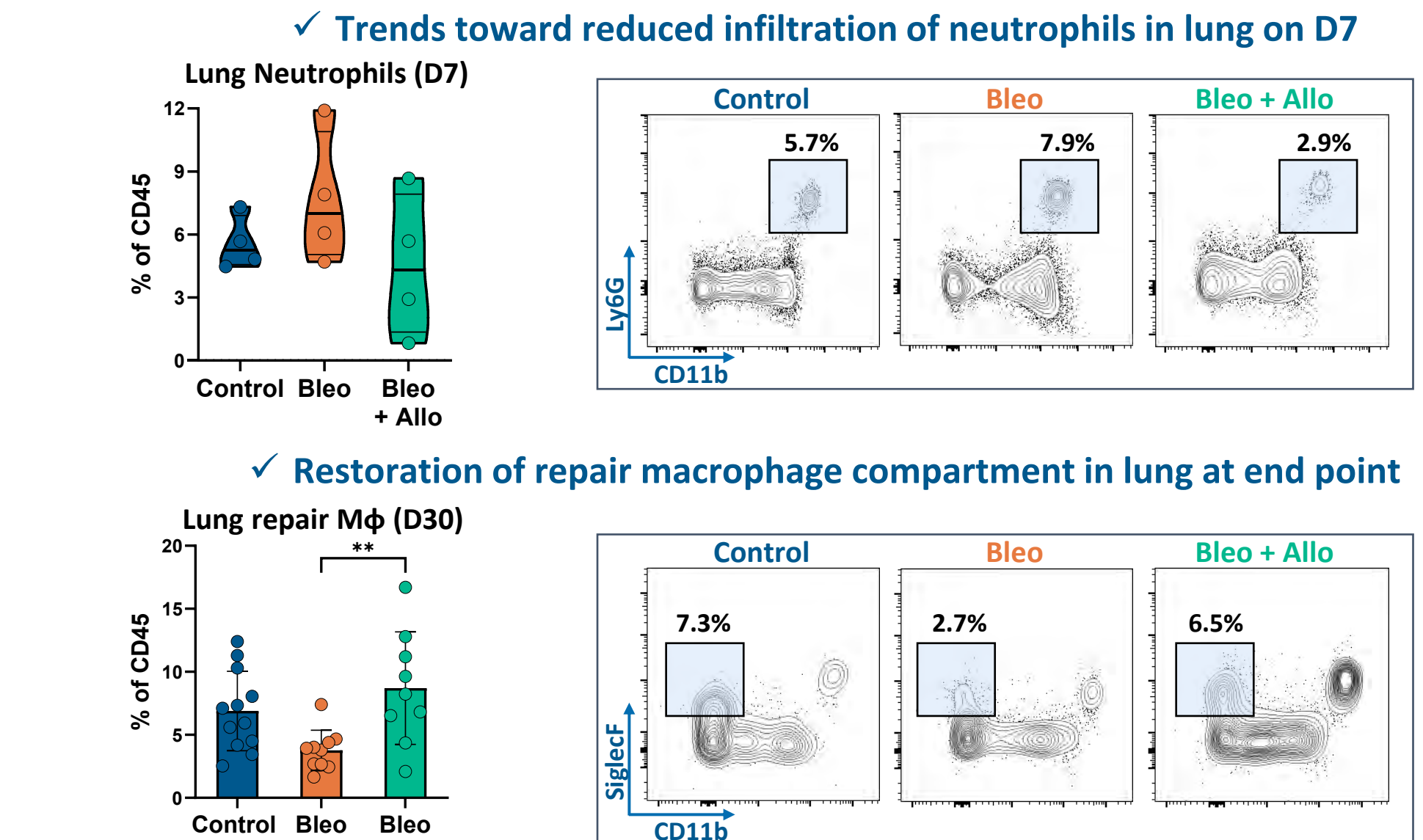
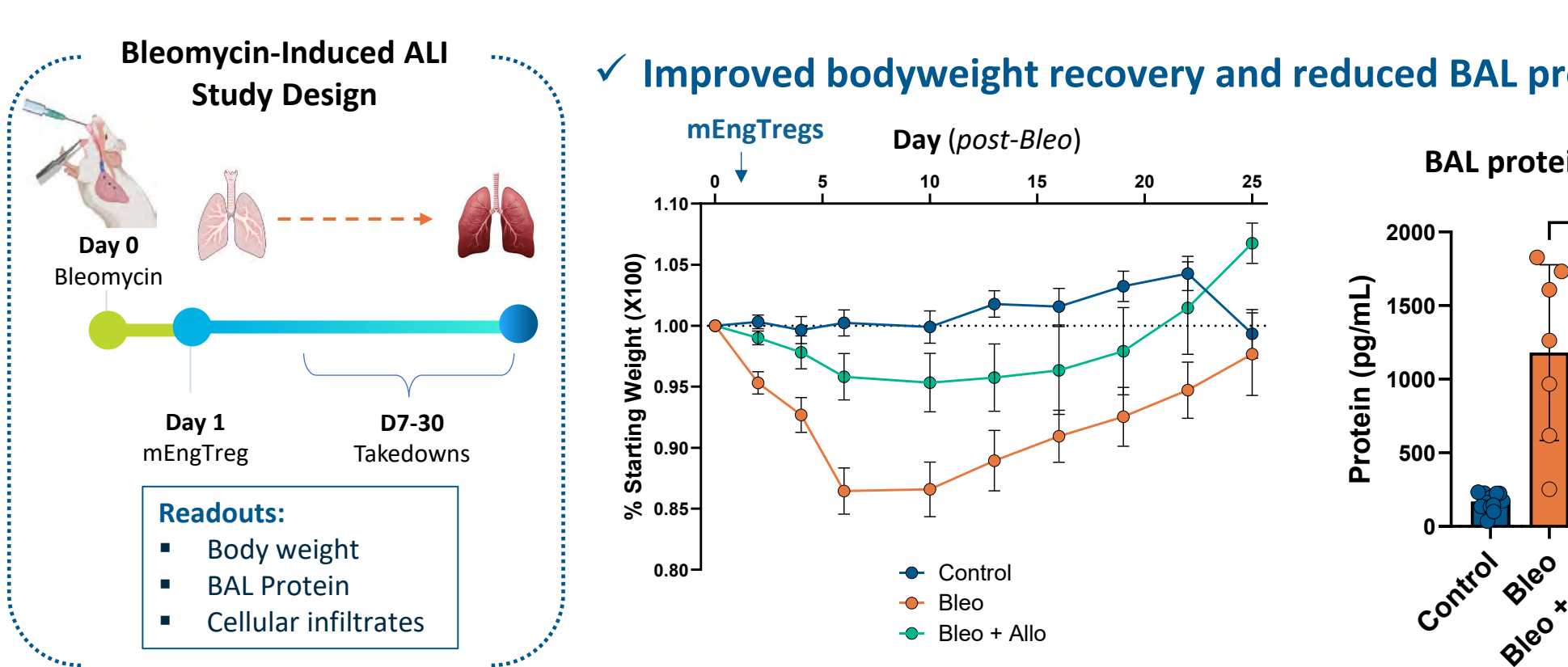
Tissue EngTregs signal through ST2 and express high levels of Treg homing, activation, and tolerogenic markers



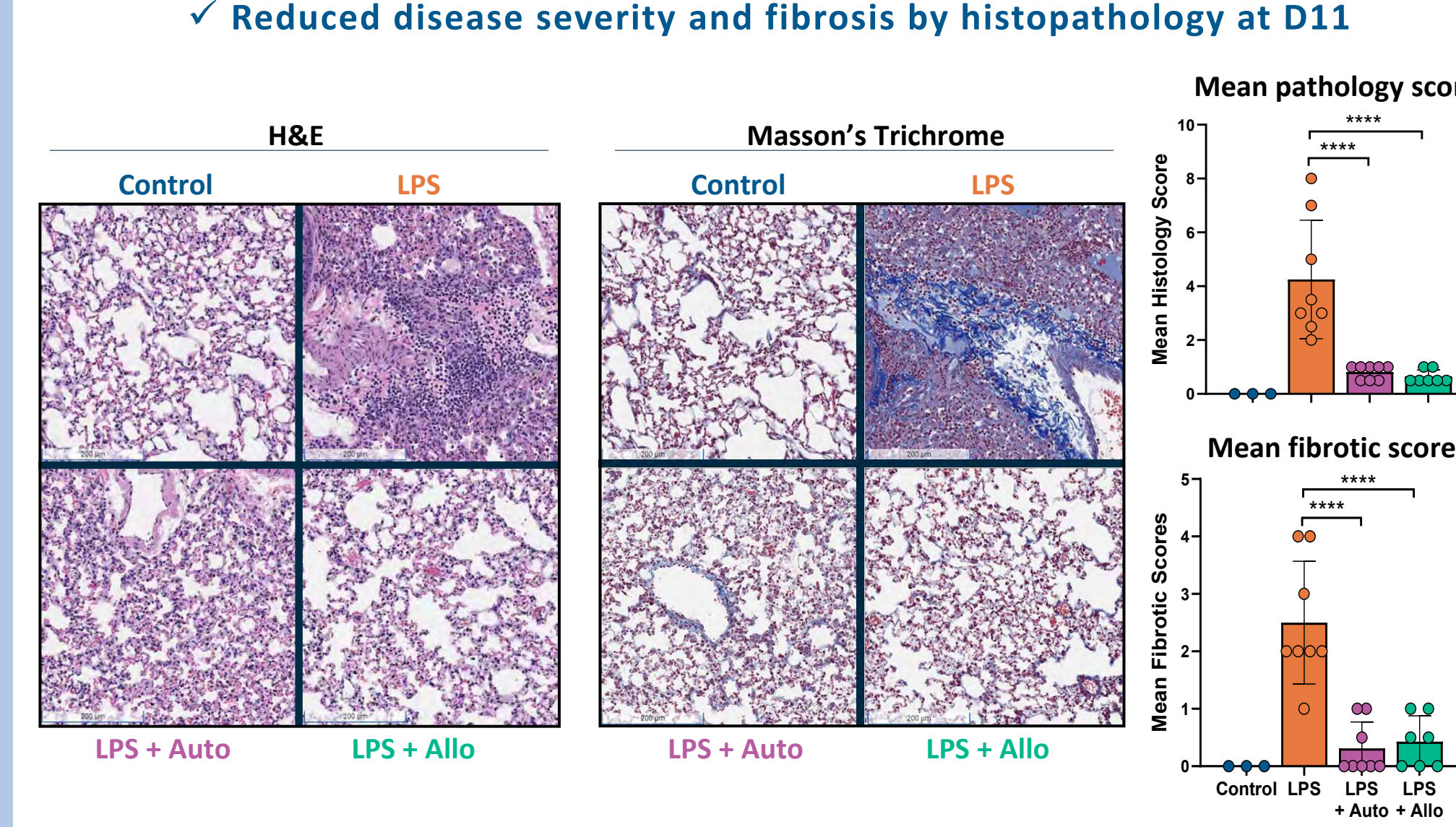
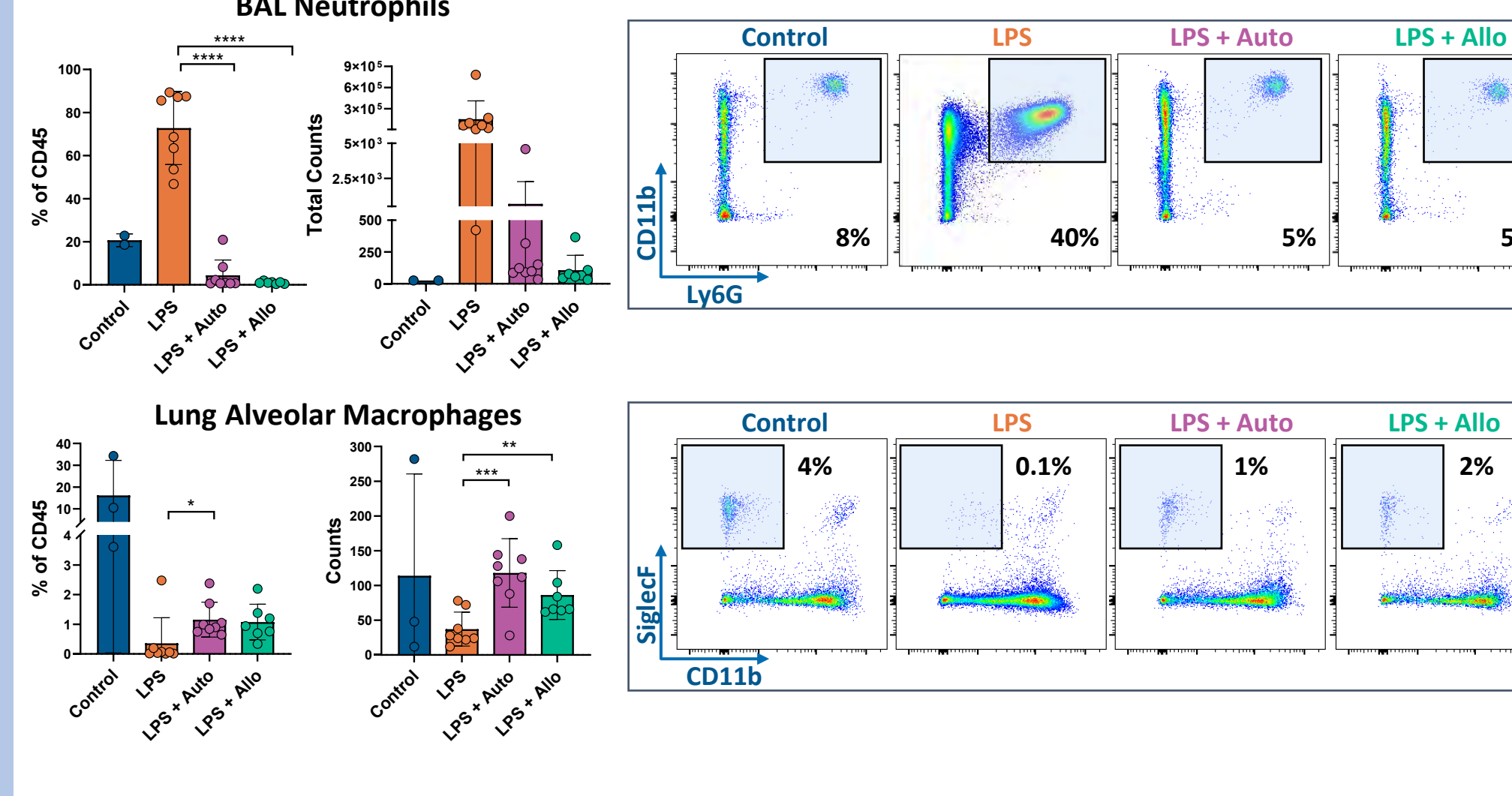
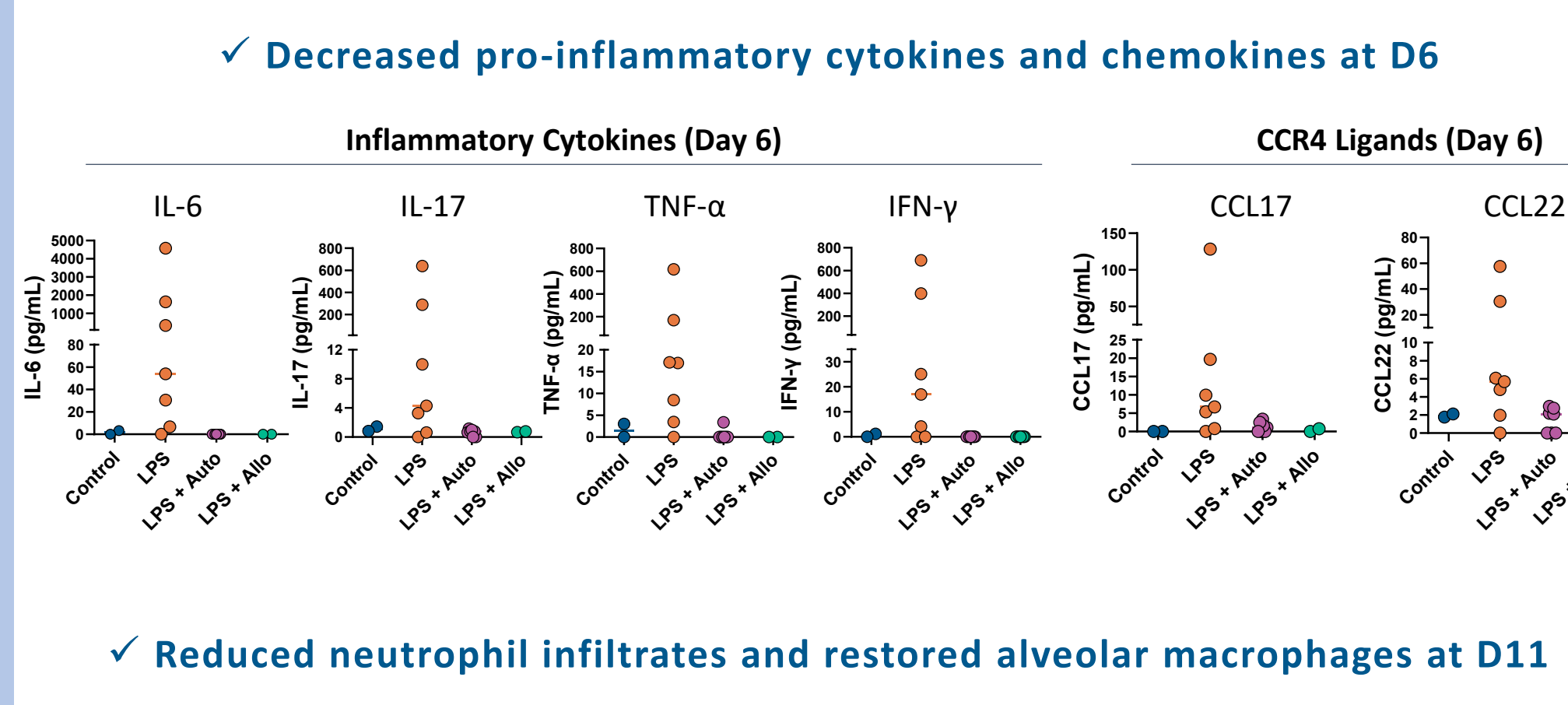
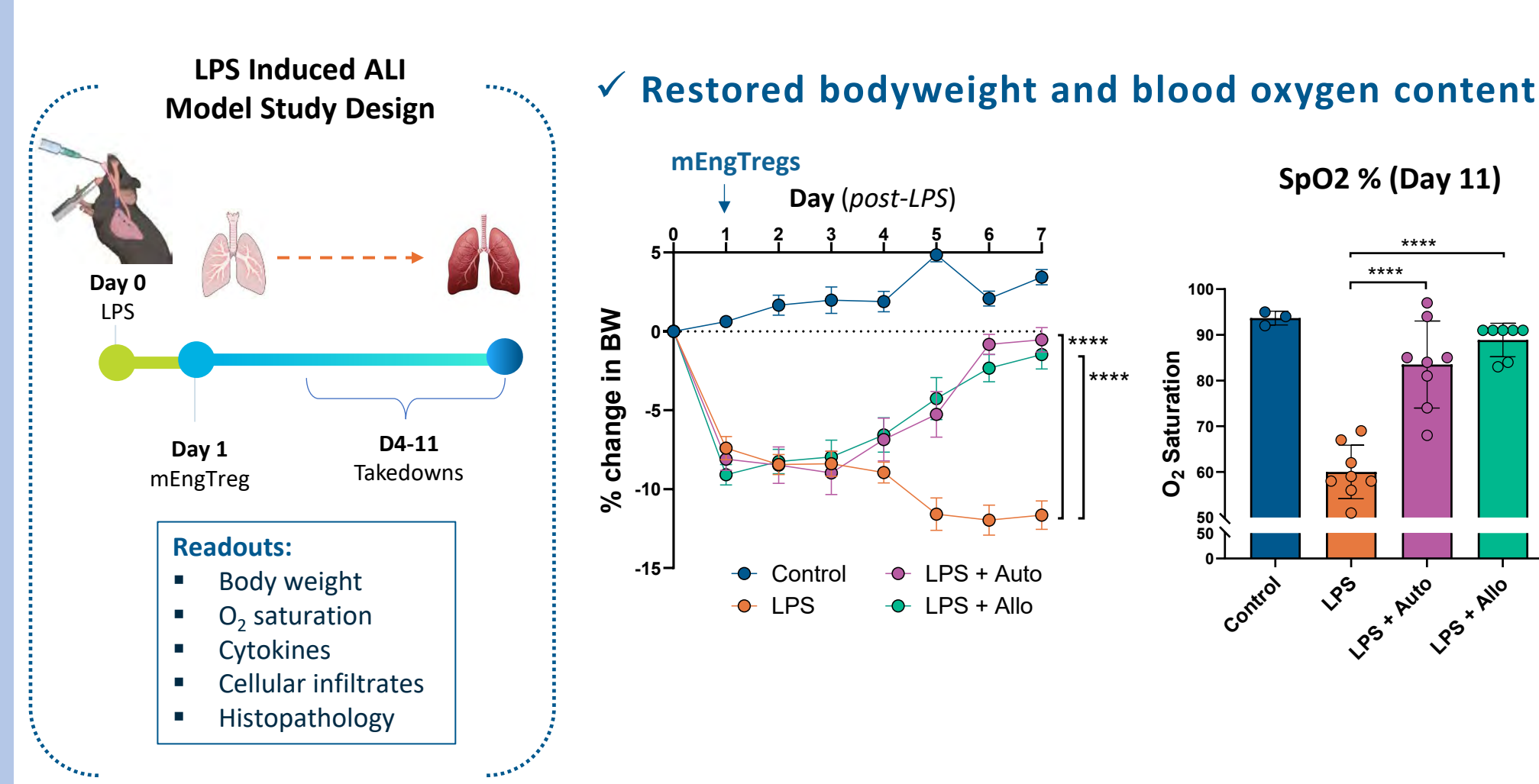
## 2. Mouse Tissue EngTregs exhibit inflammation tuned homing and persistence in preclinical models of acute lung injury



## 3. Allogeneic Tissue mEngTregs reduce disease and improve pro-repair microenvironment in the Bleomycin induced lung injury model



## 4. Reduced disease severity in autologous and allogeneic Tissue mEngTreg treated mice in the LPS induced acute lung injury model



## CONCLUSIONS

- GentiBio's Engineered Treg platform overcomes scaling and stability limitations of Treg therapeutics by starting with more abundant T cell sources and enriching FOXP3<sup>+</sup> edited cells with an engineered IL-2 signaling receptor.
- Additional ST2 engineering bestows Tissue EngTregs with a pro-repair phenotype, expressing greater levels of Treg activation markers, tissue homing receptors, and tolerogenic factors.
- Murine surrogate EngTregs specifically accumulate and proliferate at sites of injury, particularly when primed with IL-33, supporting an inflammation-tuned, ST2-driven, homing mechanism.
- Allogeneic Tissue mEngTregs drive efficacy in the bleomycin induced lung injury model, reducing disease severity and restoring key pro-repair populations at the site of inflammation.
- Allogeneic and autologous Tissue EngTregs show equivalent efficacy in the LPS induced ALI model, reducing inflammation and improving disease outcome by translatable biomarkers.
- Improved lung fibrotic scores in the later stage of disease in Tissue mEngTreg treated LPS ALI mice suggests potential mEngTreg driven protection against downstream fibrosis following acute tissue injury.
- These data support the use of allogeneic CD4 derived Engineered Tregs as a powerful off-the-shelf therapeutic approach for acute onset inflammatory diseases such as ARDS.

ACKNOWLEDGEMENTS: The laboratory of Dr. David Rawlings at Seattle Children's Hospital pioneered the gene editing approach to produce engineered Tregs. The research group of Dr. Jason Mock contributed scientific discussions regarding models of acute lung injury.

\*, \*\*, \*\*\*, \*\*\*\* = p-value < 0.01, 0.005, 0.001 and 0.0001 respectively.

We make Tregs. Better.

