

Engineered Regulatory T cells ameliorate lung injury in the LPS model of acute lung injury

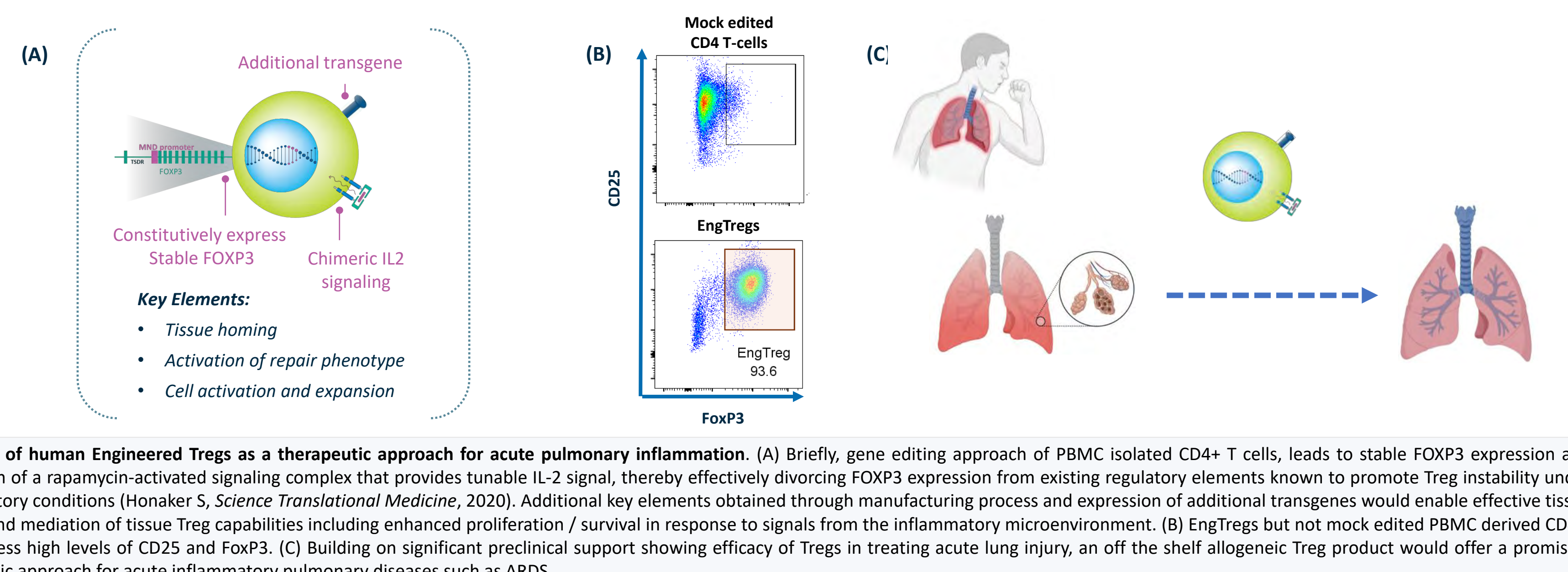
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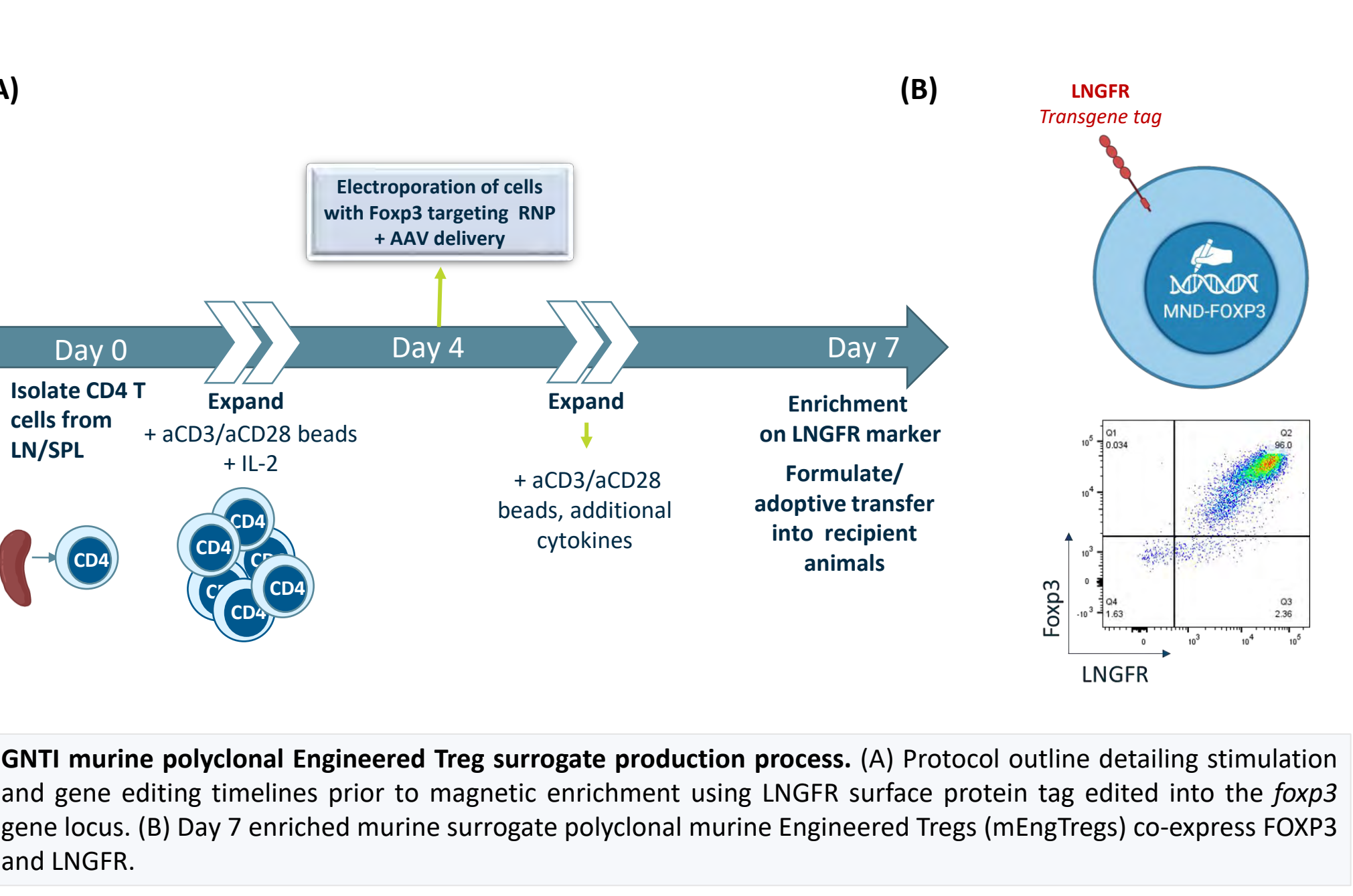
ABSTRACT

Acute respiratory distress syndrome (ARDS), is marked by acute pulmonary inflammation and life-threatening hypoxia, resulting from different etiologies including viral infections such as SARS-CoV2. Although patient outcomes have been improved by implementation of prone positioning, mechanical ventilation and corticosteroid administration, ARDS continues to have few therapeutic options and a high rate of morbidity and mortality. A key driver of tissue damage in ARDS remains the widespread dysregulation of the pulmonary immune response, ultimately resulting in tissue damage and fluid buildup in the lung parenchyma. Regulatory T cells (Tregs) are known to play a key role in tissue repair and establishing peripheral immune tolerance and their accumulation in the lung is associated with improved clinical outcomes in the context of SARS-CoV2 associated ARDS. We evaluate the efficacy of polyclonal murine Engineered Tregs (mEngTregs), generated by gene editing from splenic CD4 T cells, in an LPS induced model of acute lung injury (ALI) selected due to similarities with viral ARDS kinetics and inflammatory manifestation. We find that mEngTreg treated animals show significantly improved disease outcome as assessed by measurements of body weight, pulse oximetry and lung volume. mEngTregs were found to effectively home to the site of pulmonary inflammation and resemble endogenous Tregs in expression of key markers such as FOXP3, CD25, ST2 and CCR4. Importantly, the presence of the mEngTreg in the lung interstitium correlated with significantly reduced immune infiltrates such as neutrophils as well as lower levels of inflammatory cytokines such as IFN-g and TNF- α in the bronchoalveolar lavage (BAL). Finally, histopathology analysis of lung tissue from LPS ALI mice treated with mEngTregs appeared to show lower tissue damage or potential repair when compared to LPS ALI mice. Overall, our work lends supports to CD4 derived Engineered Tregs as a therapeutic approach for acute pulmonary diseases.

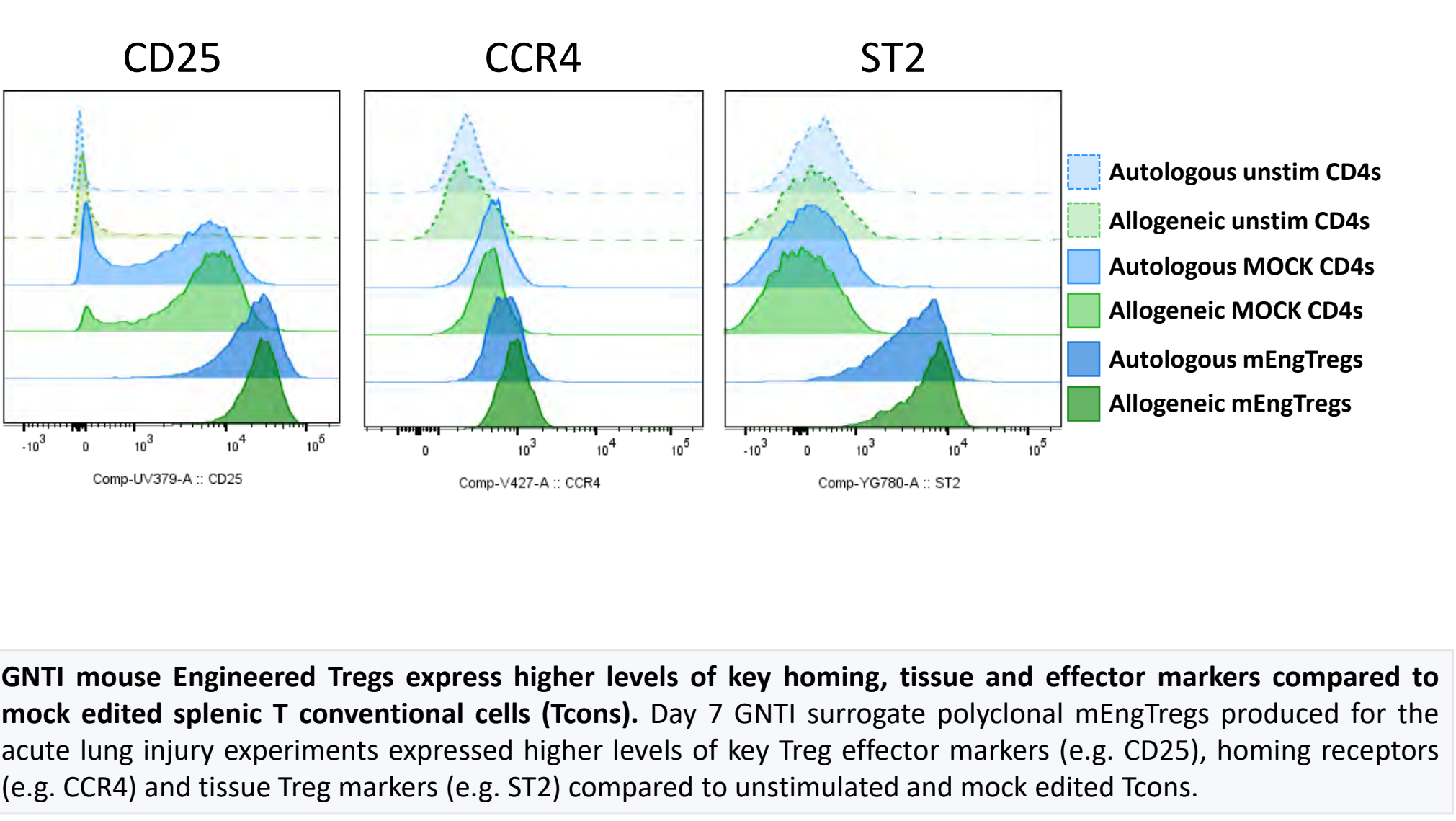
PREMISE



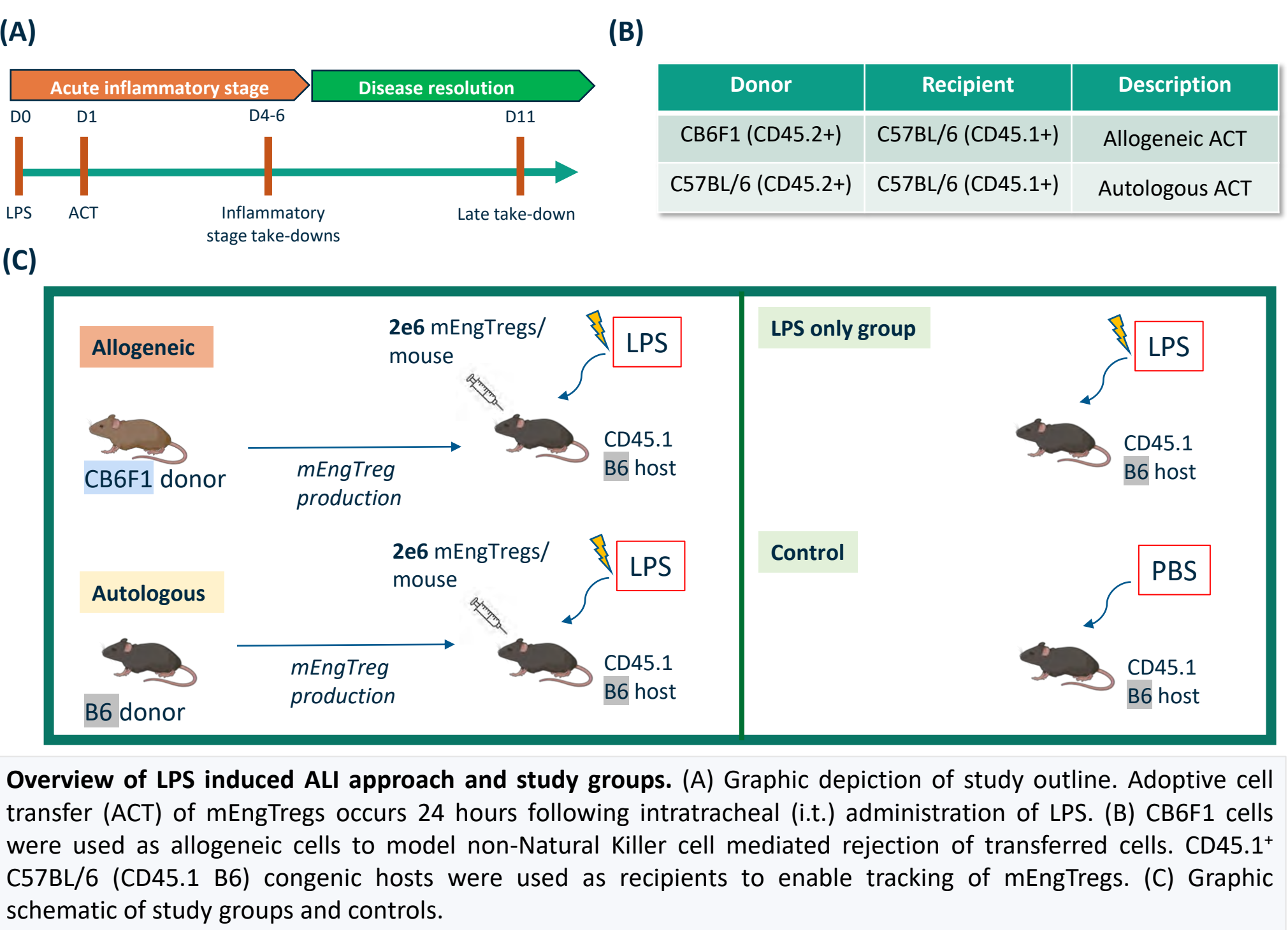
1. Methods: GNTI murine polyclonal Engineered Treg surrogate process



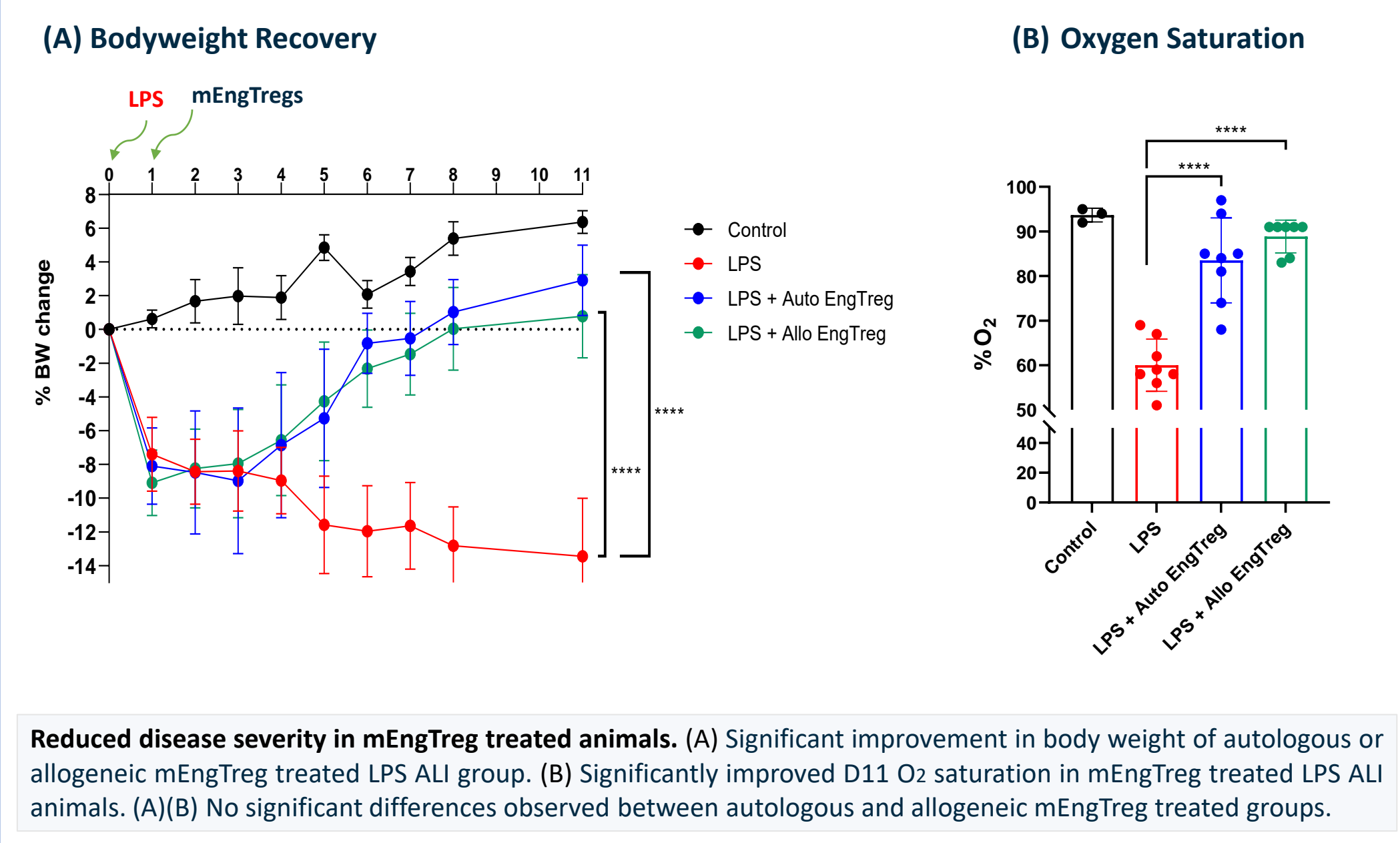
2. Results: Expression of key Treg markers by GNTI mouse Engineered Tregs



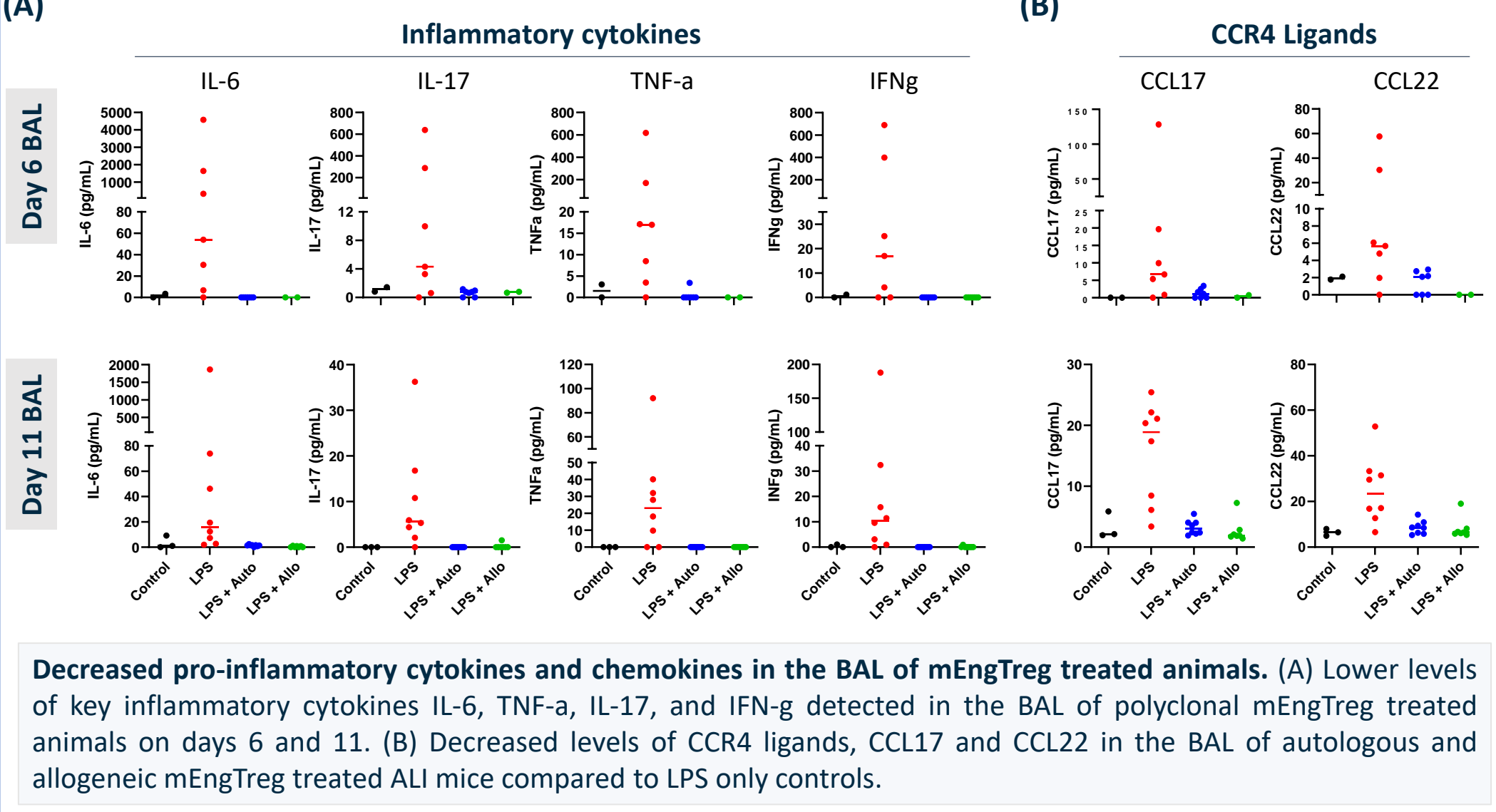
3. Methods: Overview of LPS induced ALI approach and study groups



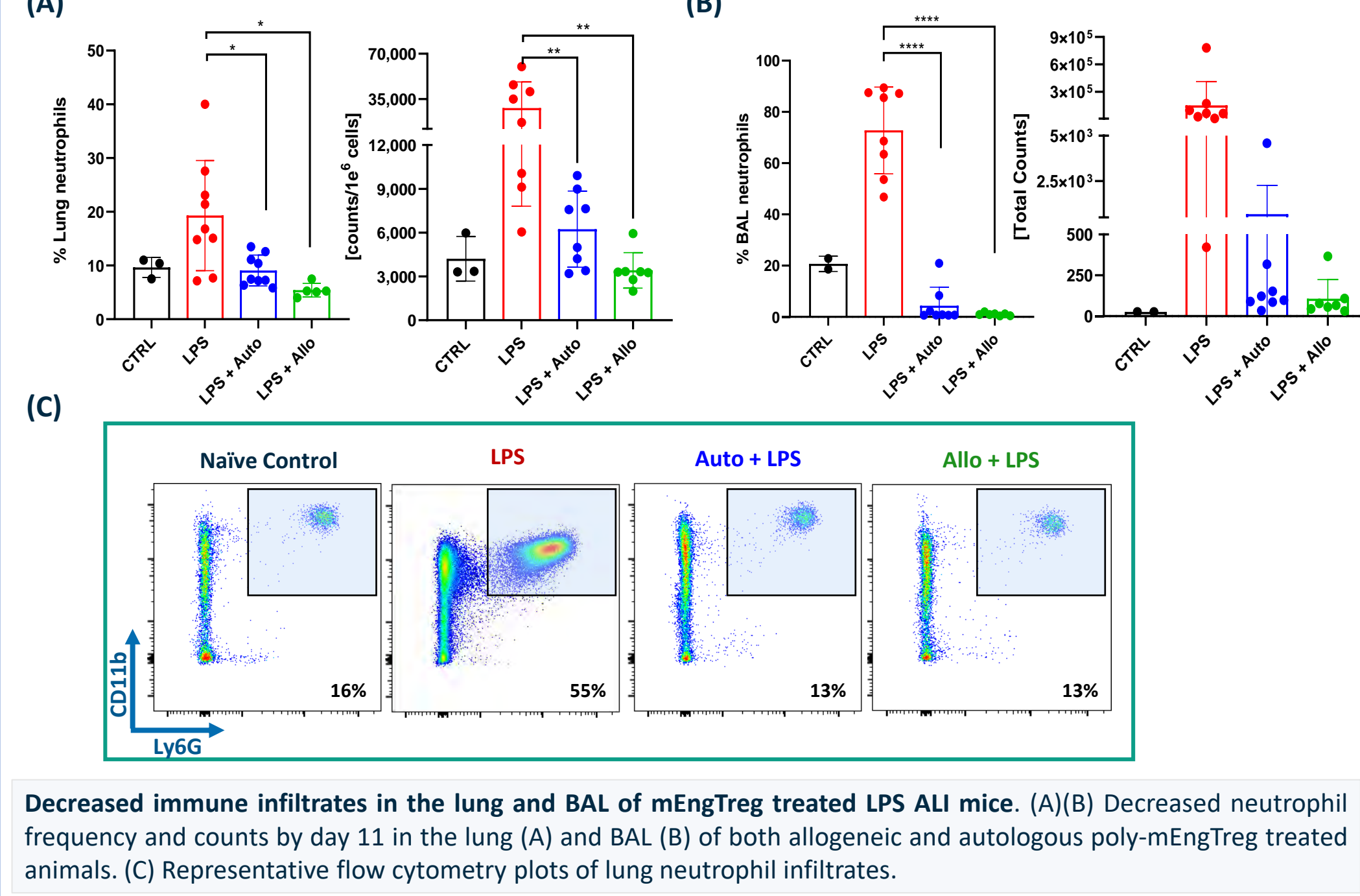
4. Results: Reduced ALI disease severity in autologous and allogeneic mEngTreg treated mice



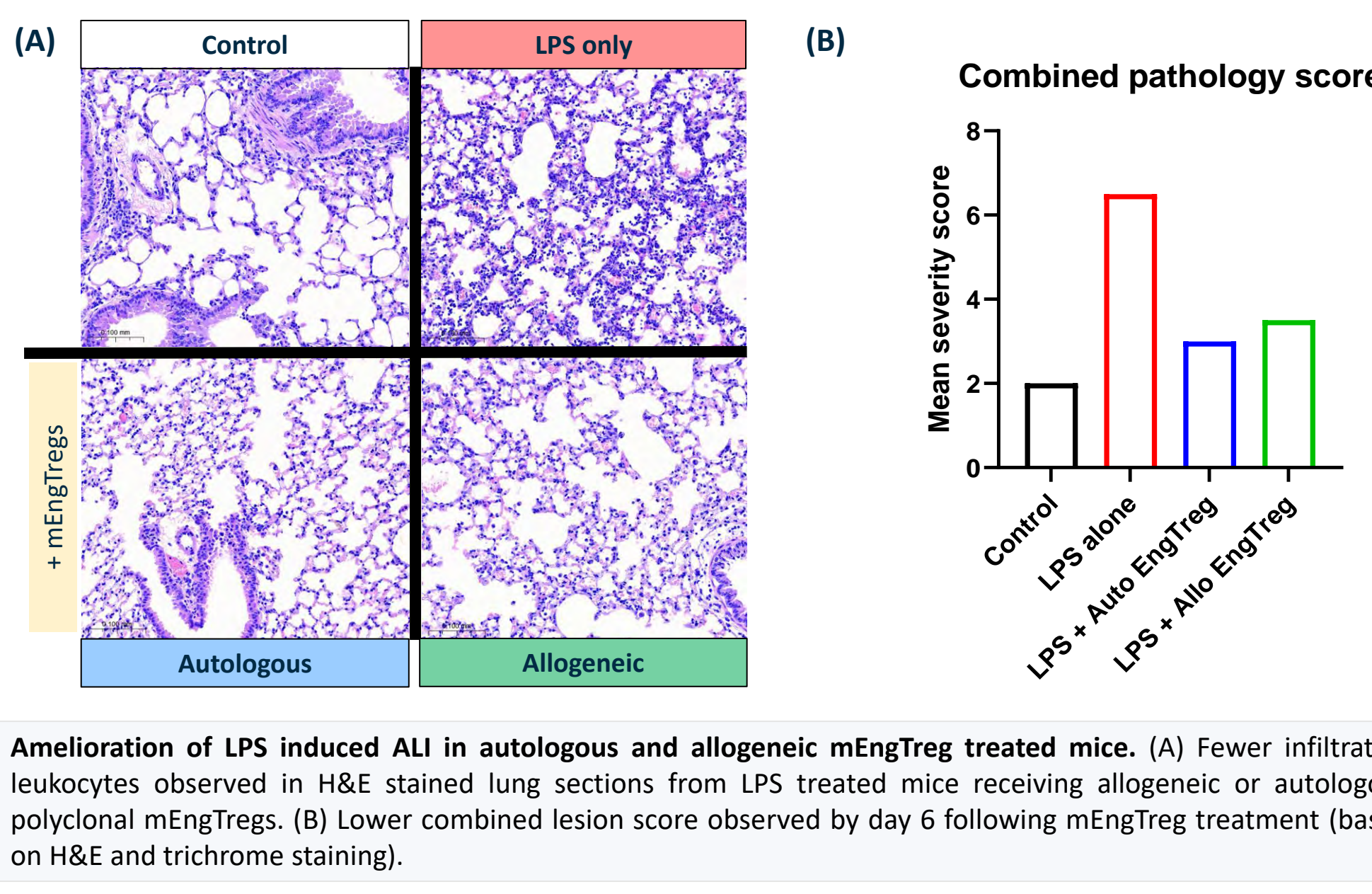
5. Results: Lower concentration of pro-inflammatory cytokines and chemokines detected in the BAL of mEngTreg treated mice



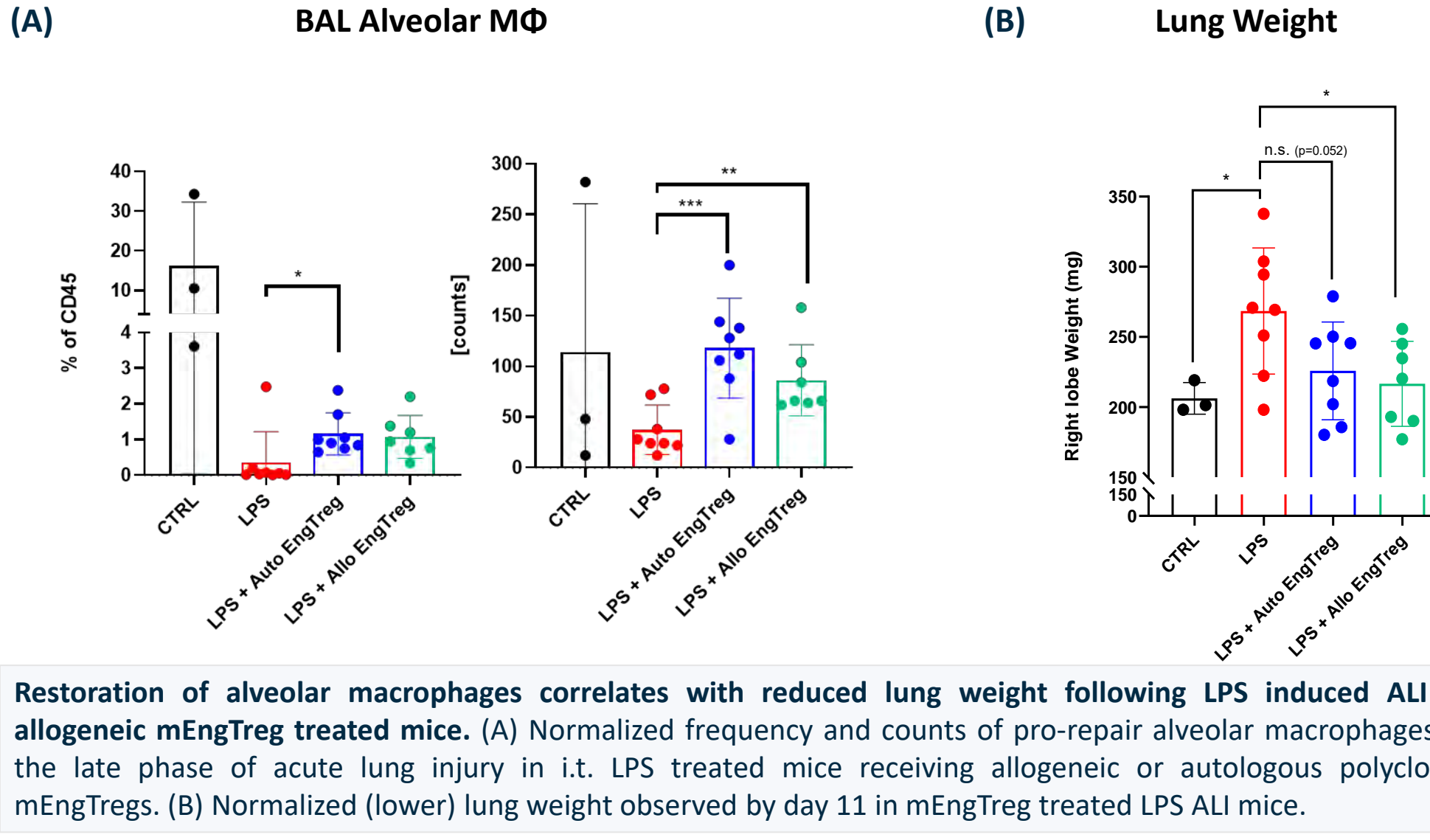
6. Results: Reduced neutrophil infiltrates detected in the lungs and BAL of LPS ALI mice treated with mEngTregs



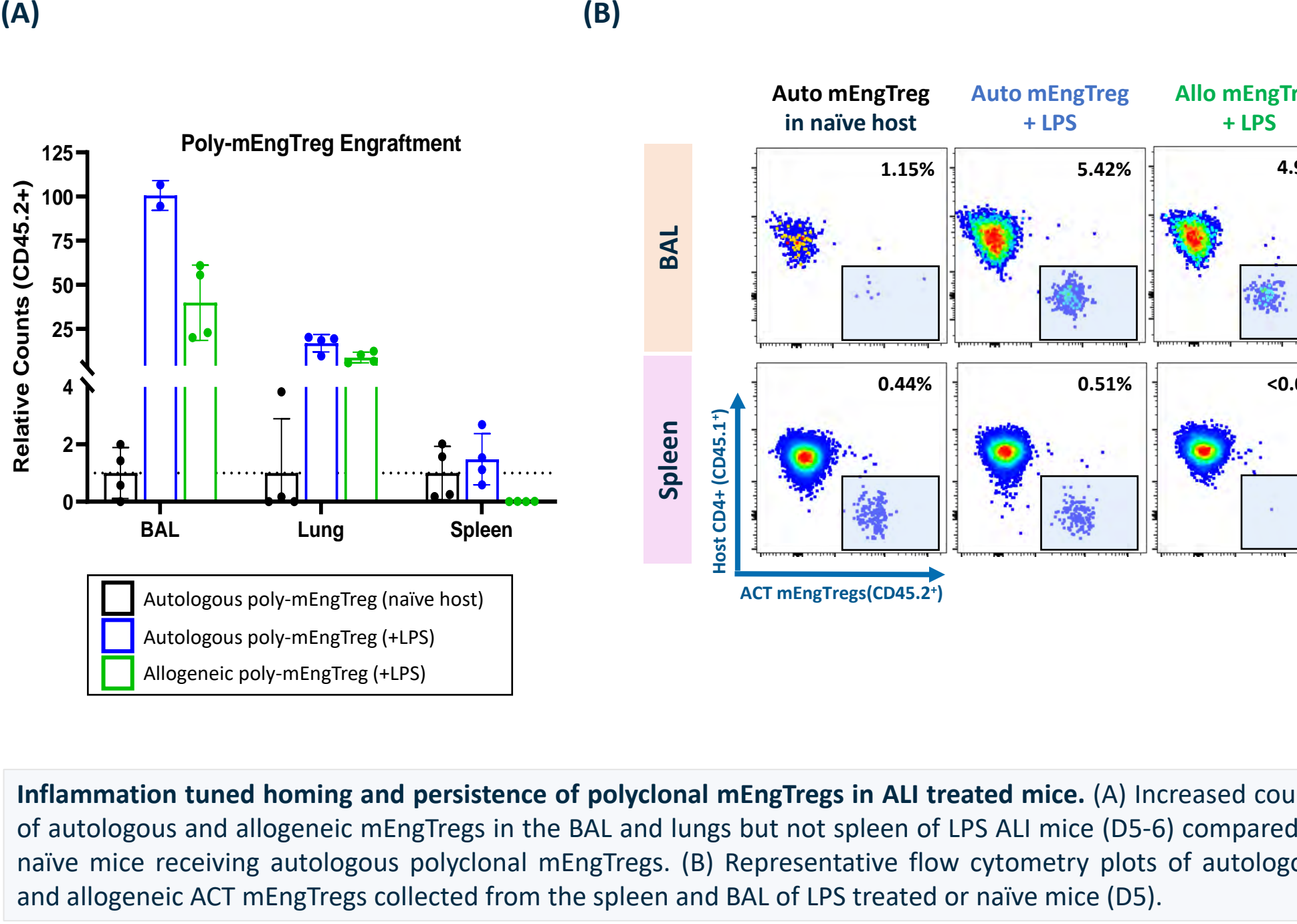
7. Results: Reduced disease severity based on histopathology in polyclonal mEngTreg treated ALI mice



8. Results: Restoration of alveolar macrophages in mEngTreg treated ALI mice correlates with lower lung weight



9. Results: Specific homing and persistence of allogeneic mEngTregs to site of tissue damage in LPS induced ALI model



CONCLUSIONS

- GentiBio's Engineered Treg platform overcomes the scaling limitations of sorted donor Treg cells by starting with more abundant T cell sources and specifically enriching edited cells with an engineered IL-2 signaling receptor.
- Genti surrogate murine engineered Treg cells express key markers of pulmonary thymic Tregs including FOXP3, CD25, CCR4 and ST2.
- Equivalent efficacy and improved disease outcome observed in LPS induced acute lung injury mice treated with allogeneic or autologous mEngTregs based on body weight and pulse oximetry measurements.
- Fewer day 11 inflammatory infiltrates observed in the lung and BAL of autologous and allogeneic mEngTreg treated mice, with normalized counts of alveolar macrophages, reduced BAL inflammatory cytokines and normalized lung weight suggesting return towards pulmonary immune homeostasis.
- High frequency of allogeneic mEngTregs detected during the inflammatory phase of disease and at the site of inflammation while lower persistence is observed in distal sites with lower inflammation during ALI.
- This data lends support to the use of allogeneic CD4 derived Engineered Tregs as a powerful off-the-shelf therapeutic approach for acute onset inflammatory diseases including vARDS.

ACKNOWLEDGEMENT: The laboratory of Dr. David Rawlings at Seattle Children's Hospital pioneered the gene editing approach to produce engineered Tregs. The research groups of Dr. Jason Mock and Dr. Heth Turnquist contributed to the models of acute lung injury used to generate this data.

We make Tregs. Better.

