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High-dimensional immune profiling of atopic dermatitis reveals a dysfunctional OX40⁺ regulatory T cell subset

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- Conflict of Interest: none

- ✓ **Learning Objective:** To understand how dysfunctional OX40⁺ Tregs contribute to immune dysregulation and disease severity in AD
- ✓ **Takeaway Message:** OX40⁺ Tregs are expanded, exhibit impaired function and Th2-skewing in AD, providing mechanistic rationale for OX40-targeted therapies

Introduction

- **Treg Paradox in Atopic Dermatitis**
  - Tregs play a central role in immune tolerance and suppression of Th2 responses
  - **Conflicting reports:** ↑ frequency vs. ↓ function?
  - Expression of dysfunction markers (OX40, CRTH2) linked to impaired suppressive capacity

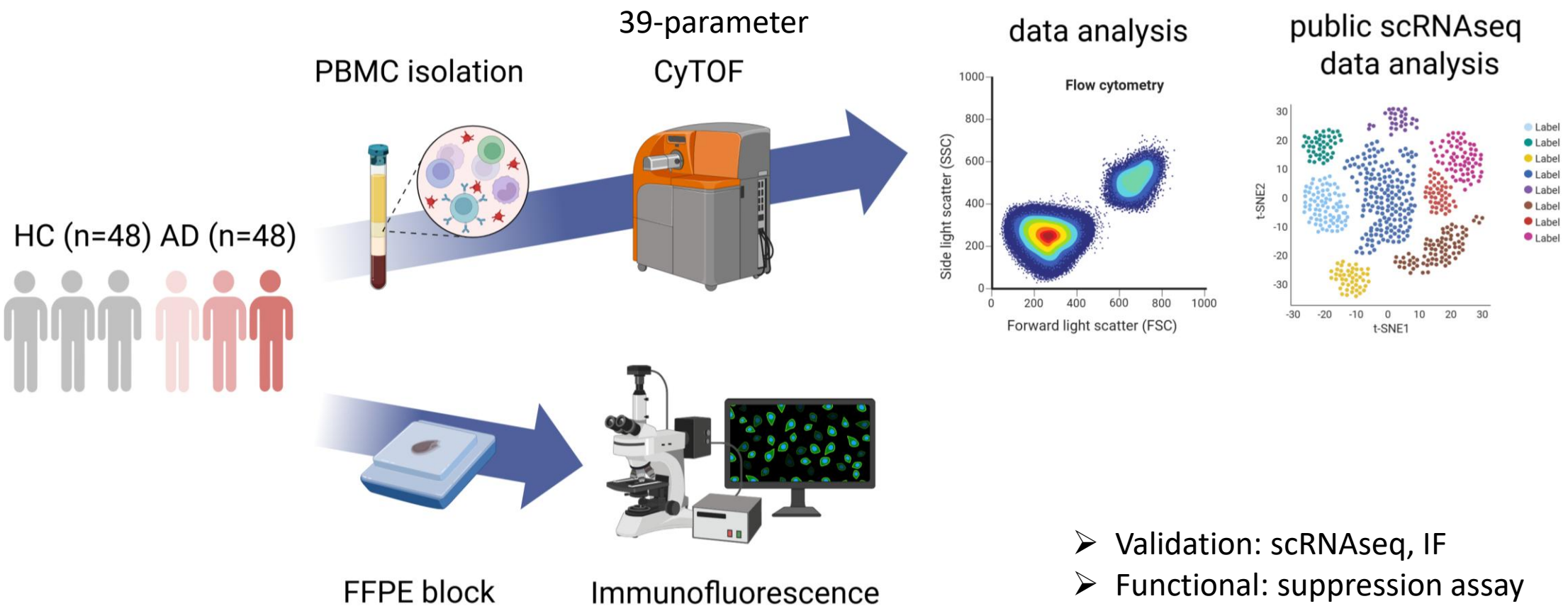
- **Knowledge Gap**
  - Limited high-dimensional **protein-level** characterization of Tregs in AD
  - **Heterogeneity** and **functional states of Treg subsets** remain **poorly understood**

Martin-Cruz L et al. Int Arch Allergy Immunol 2024;185:503-18; Reefer AJ et al. J Allergy Clin Immunol 2008;121:415-22; Roesner LM et al. Allergy 2015;70:1656-60; Garcia EM et al. Int Arch Allergy Immunol 2020;181:353-6; Vu MD et al. Blood 2007;110:2501-10; Zhang X et al. Cell Rep 2018;24:607-18; Chantveerawong T et al. Allergy 2022;77:681-5; Boonpiyathad T et al. Allergy 2020;75:84-94.

Objectives

- What is the **true functional state** of Tregs in AD?
- Are there **distinct Treg subsets** that drive immune dysregulation in AD?
- How does Treg heterogeneity relate to **disease severity**?
- Can high-dimensional immune profiling reveal **novel therapeutic targets** in AD pathogenesis?

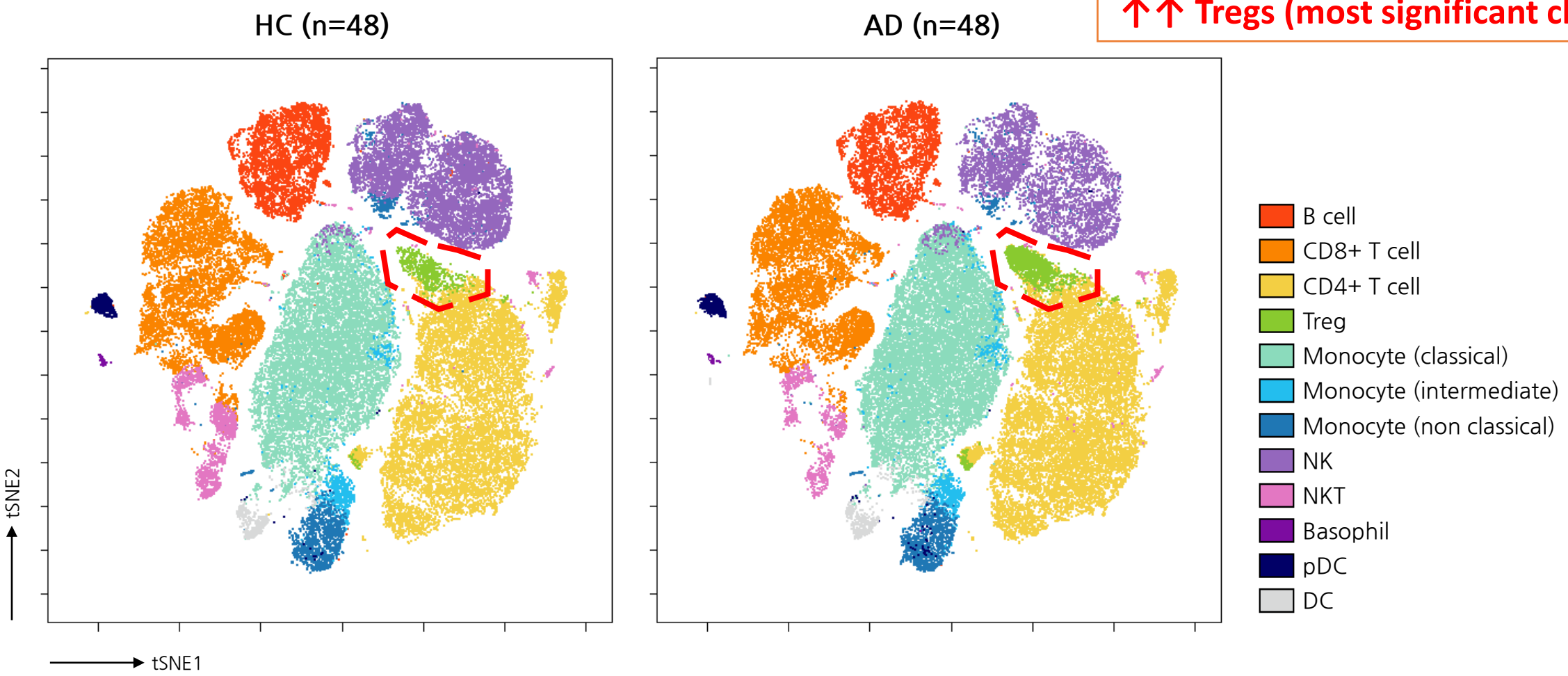
Study design



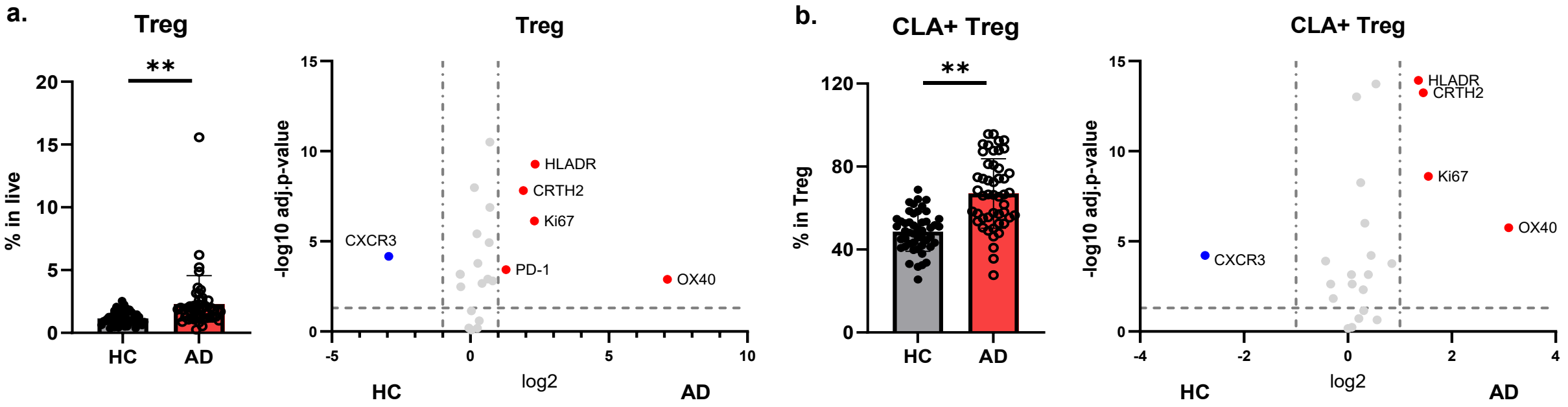
- Validation: scRNAseq, IF
- Functional: suppression assay

PBMCs from HC and AD patients

↓ NK, NKT cells
↑ CD4+ naive T cells
↑↑ **Tregs (most significant change)**

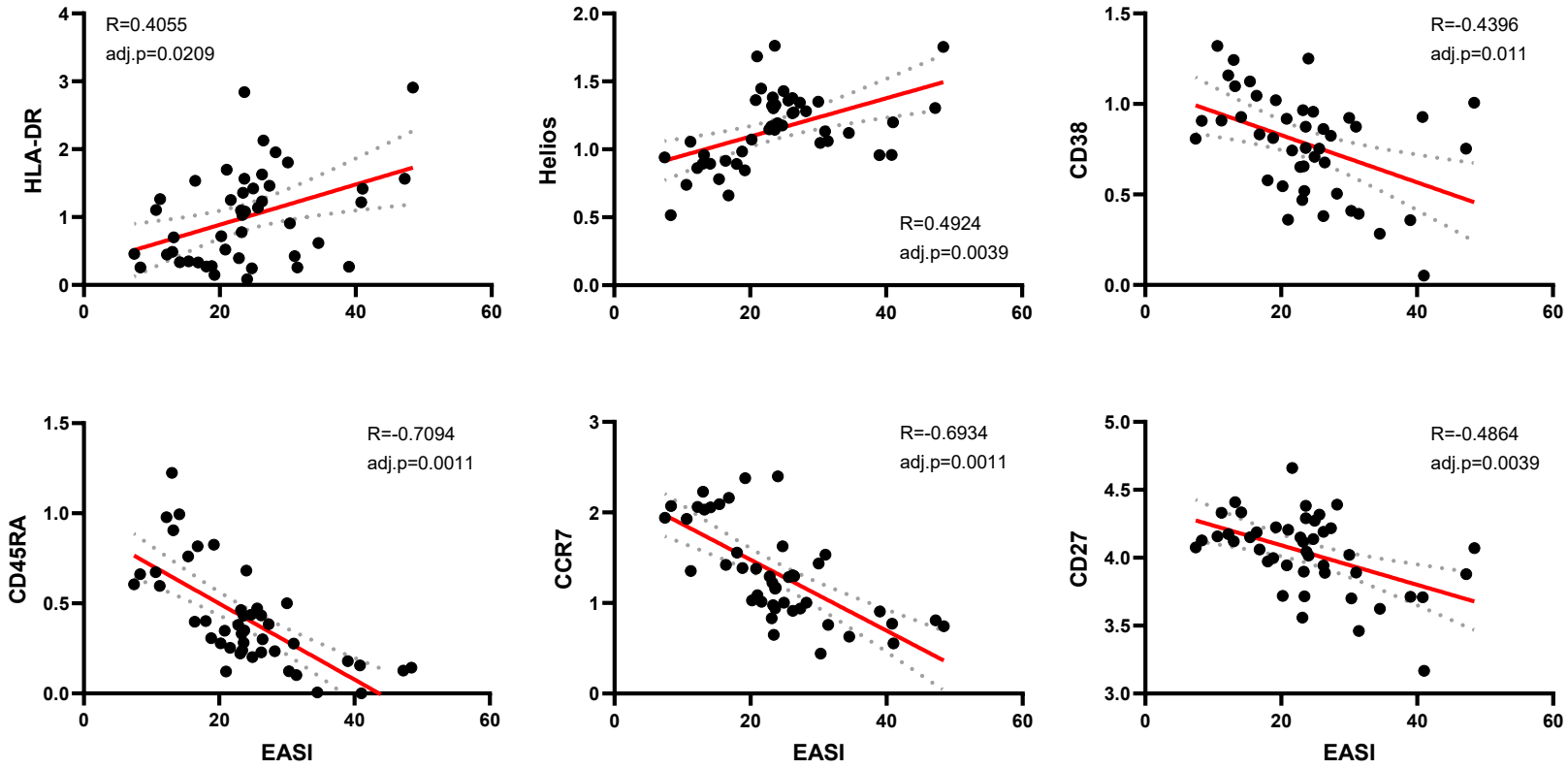


Treg/CLA+ Treg frequency and functional markers



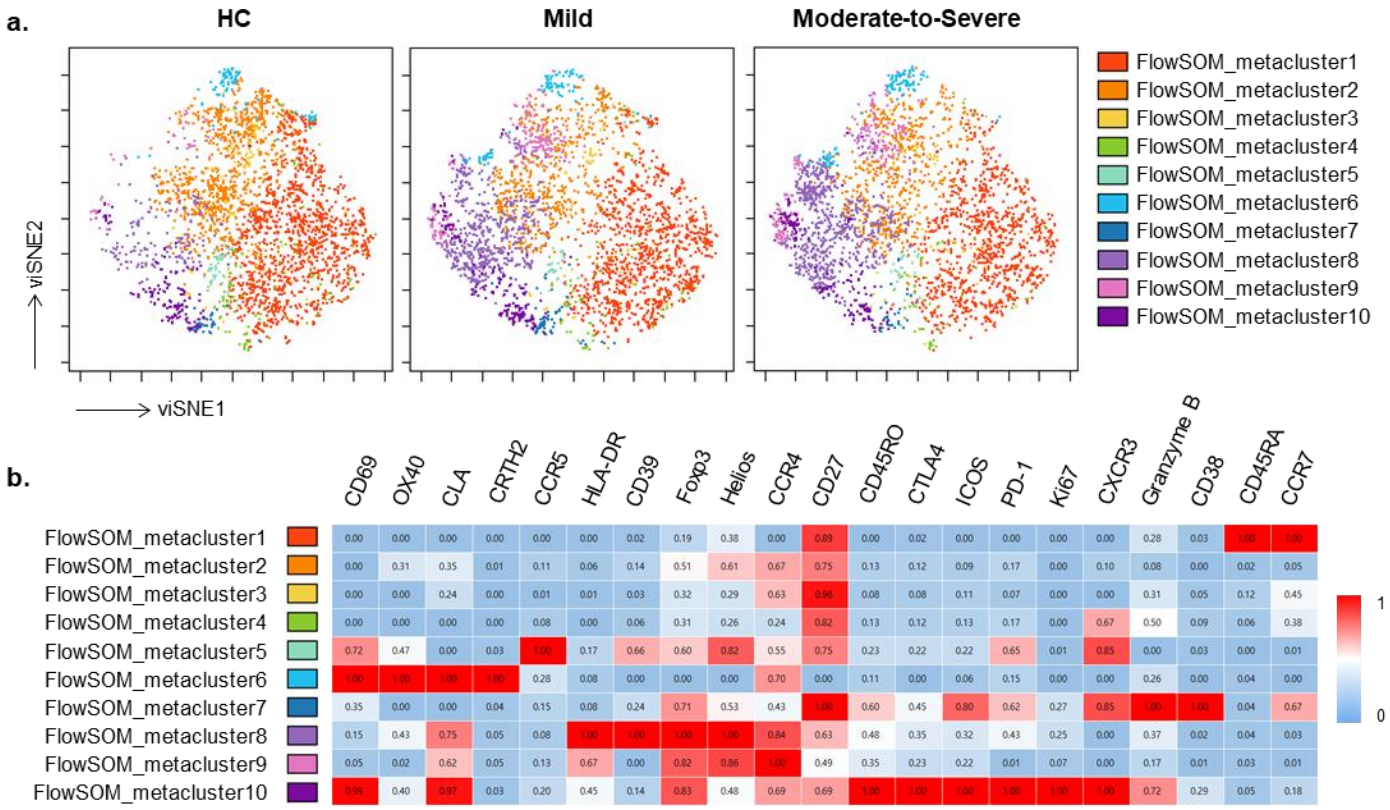
- Key findings: ↑ Treg frequency
  ↑ Activation markers (HLA-DR, Ki67, OX40)
  ↓ CXCR3

Correlation between EASI score and marker expression



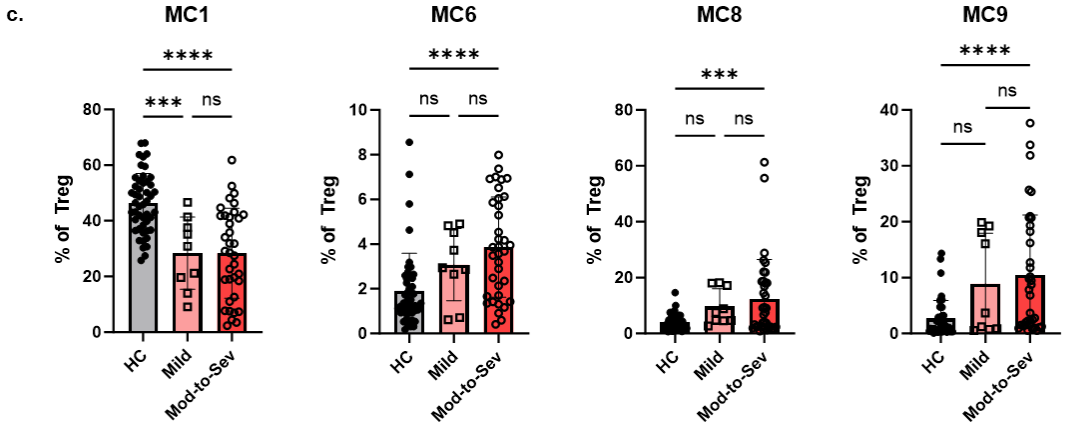
- Treg phenotype correlates with disease severity:
  - ✓ Positive correlation: HLA-DR, Helios (activation)
  - ✓ Negative correlation: CD38, CD45RA, CCR7, CD27 (naïve/resting)

Identification of a distinct CRTH2+ OX40+ Treg meta-cluster



- MC6: CRTH2^{hi} OX40^{hi}
  - ✓ Lowest: FoxP3, CTLA-4, CD27
  - ✓ Ki-67, PD-1, CD39, CD38: relatively low levels.

Identification of a distinct CRTH2+ OX40+ Treg meta-cluster

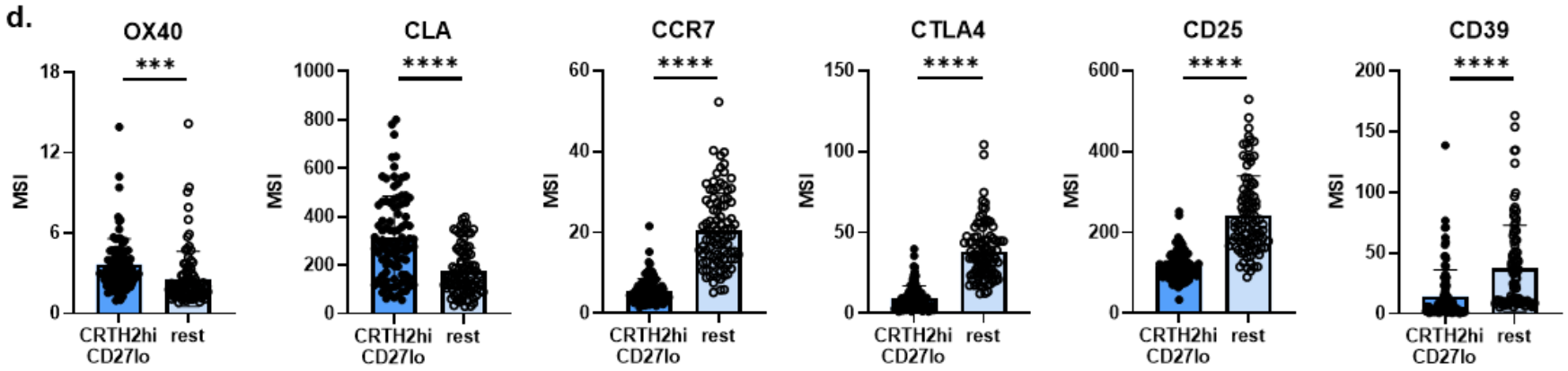


- MC6: expanded in patients with M2S AD

- Functional impairment

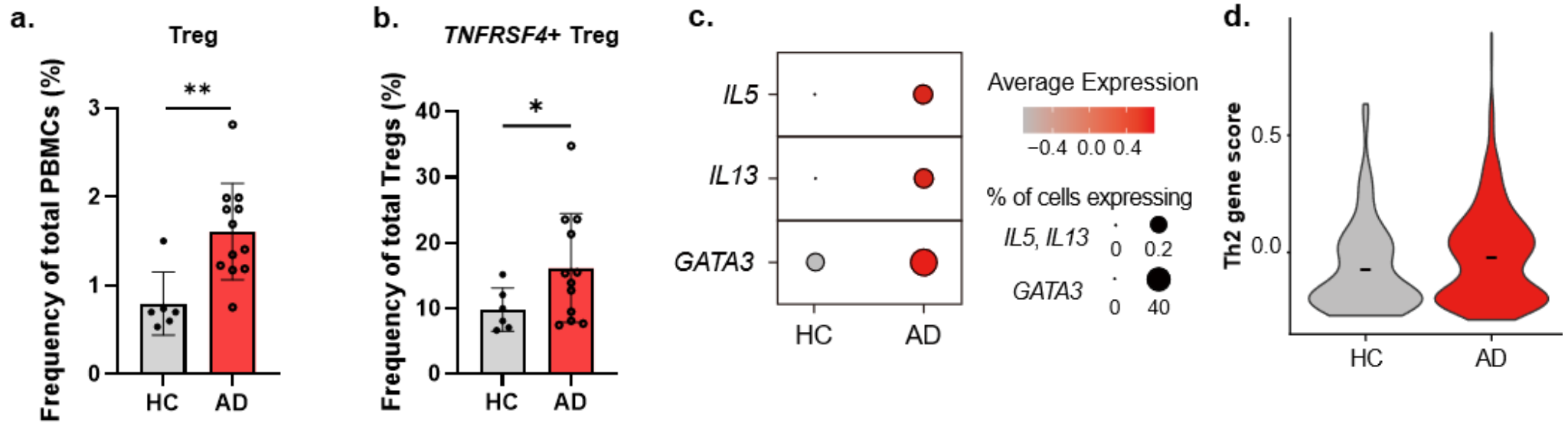
  ↑ OX40, CLA ↓ CCR7, CTLA-4, CD25, CD39 (key functional and homing markers)

MC6: CRTH2^{hi}OX40^{hi} with lowest suppressive markers



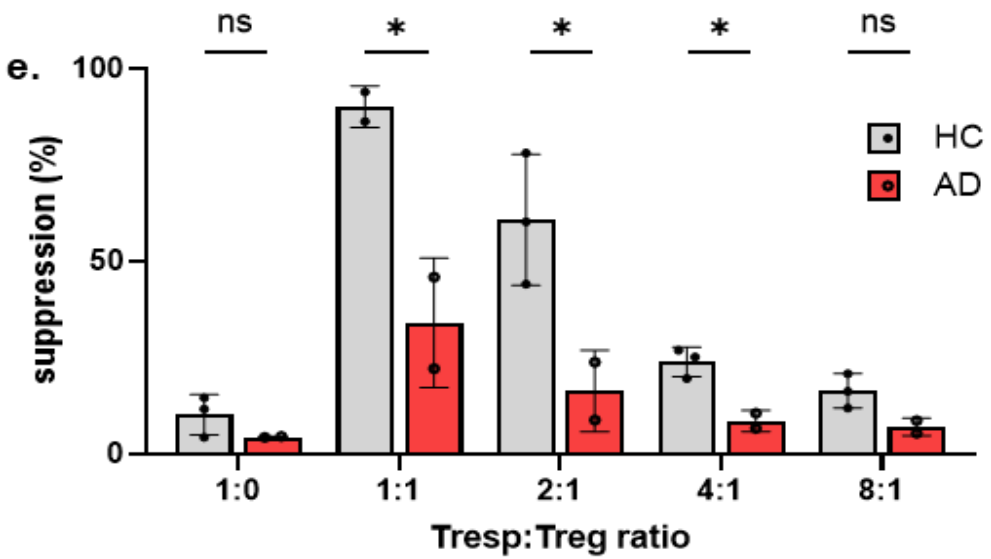
OX40+ Treg in PBMC

scRNA-Seq



- Increased frequency: Total Tregs & TNFRSF4+ Tregs
- TNFRSF4+ Tregs exhibit Th2-skewed signature
  - ↑ IL5, IL13, GATA3 expression
  - Higher Th2 gene scores

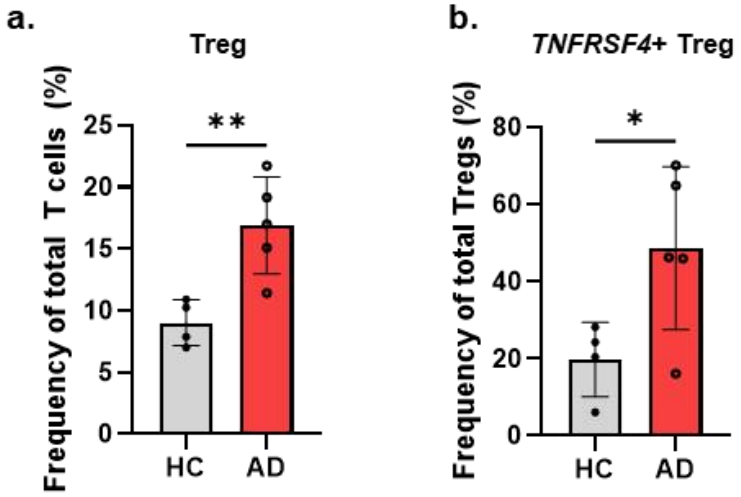
OX40+ Treg in PBMC



Data: mean ± SD. *p<0.05, unpaired t-test. HC n=3, AD n=2

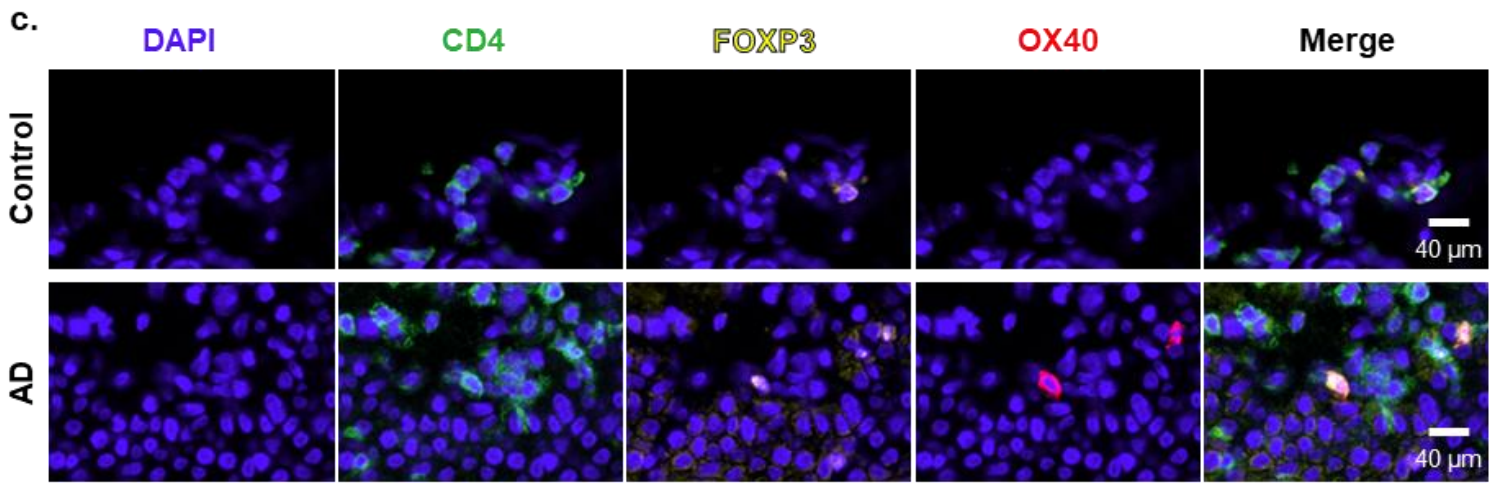
- OX40+ Tregs from AD patients exhibited reduced suppressive capacity

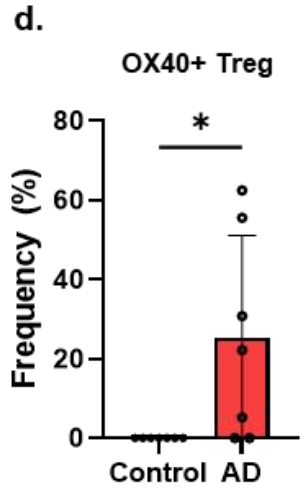
OX40+ Treg in skin



▶ Treg and *TNFRSF4*+ Treg is increased in AD lesional skin.

- 25% of Tregs in AD lesions are OX40+ (vs. ~0% in controls)

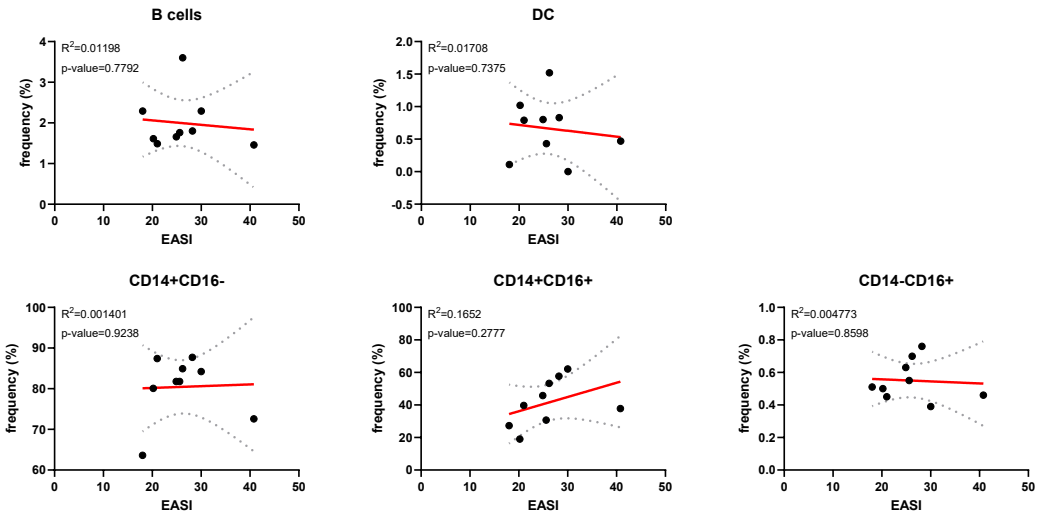




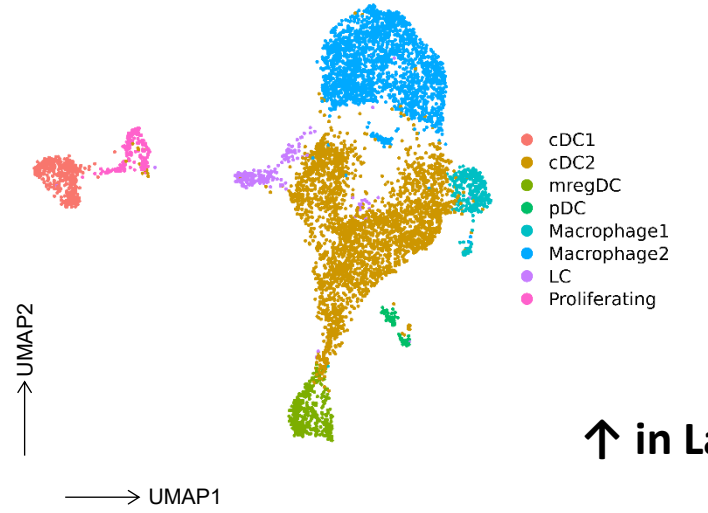
OX40-OX40L axis in AD

Flow cytometry - OX40L in blood APCs

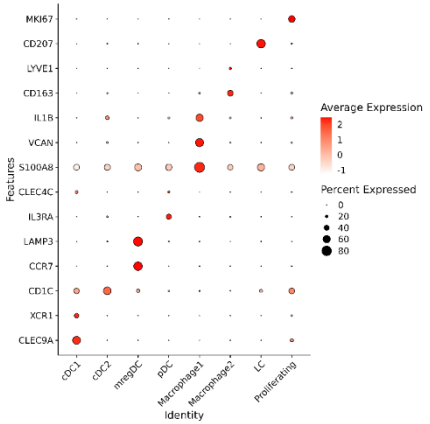
No correlation with severity in blood

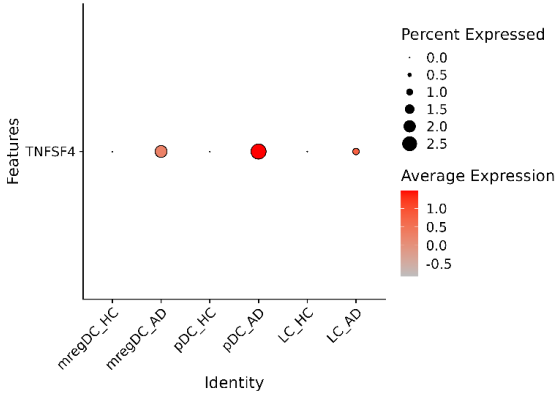


scRNAseq - OX40L (TNFSF4) in skin APCs



↑ in Langerhans cells, mregDCs, pDCs



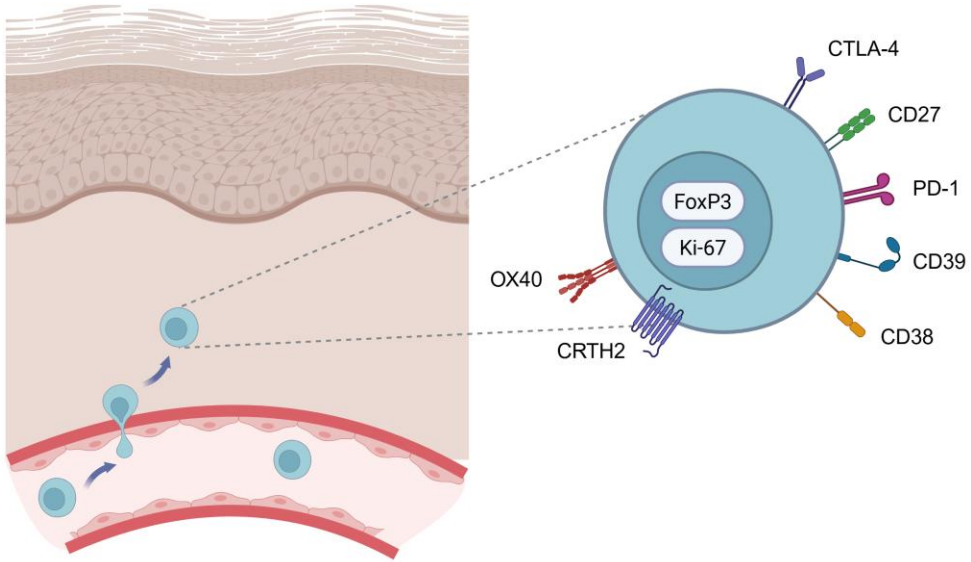


- Tissue-specific OX40L upregulation may drive local Treg dysfunction

Graphical Summary

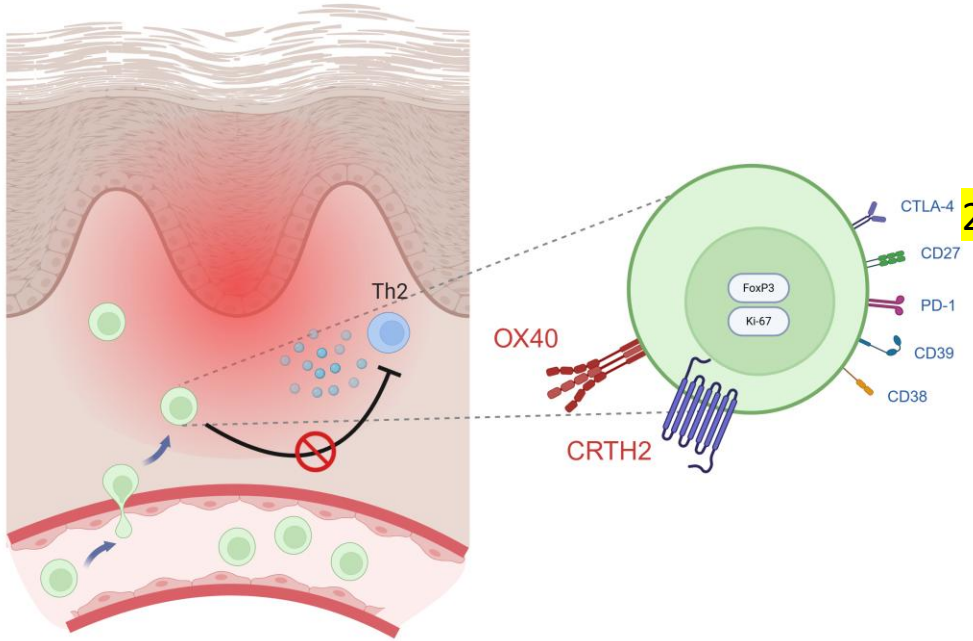
Healthy control (n=48)

Effective regulation of inflammation



Atopic dermatitis (n=48)

Improper regulation of inflammation



Increased OX40⁺ Tregs in blood and lesional skin

25% of Tregs in AD lesions

Impaired suppression

Decreased Treg functional markers in OX40⁺ Tregs

CTLA-4, CD27, CD39, FoxP3

Conclusion

1. **OX40+ Tregs are expanded and dysfunctional in AD**
   - ✓ Correlate with disease severity
   - ✓ Exhibit Th2-skewed transcriptional signature
   - ✓ Show impaired suppressive capacity *in vitro*

2. **Tissue-specific OX40-OX40L axis activation**
   - ✓ Blood APCs: No change
   - ✓ Skin APCs: ↑ OX40L in Langerhans cells, DCs

3. **Clinical implications**
   - ✓ Strong mechanistic rationale for OX40/OX40L blockade
   - ✓ Potential biomarker for patient stratification
   - ✓ Supports precision medicine approaches (rocatinlimab, telazorlimab, amlitelimab trials)

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