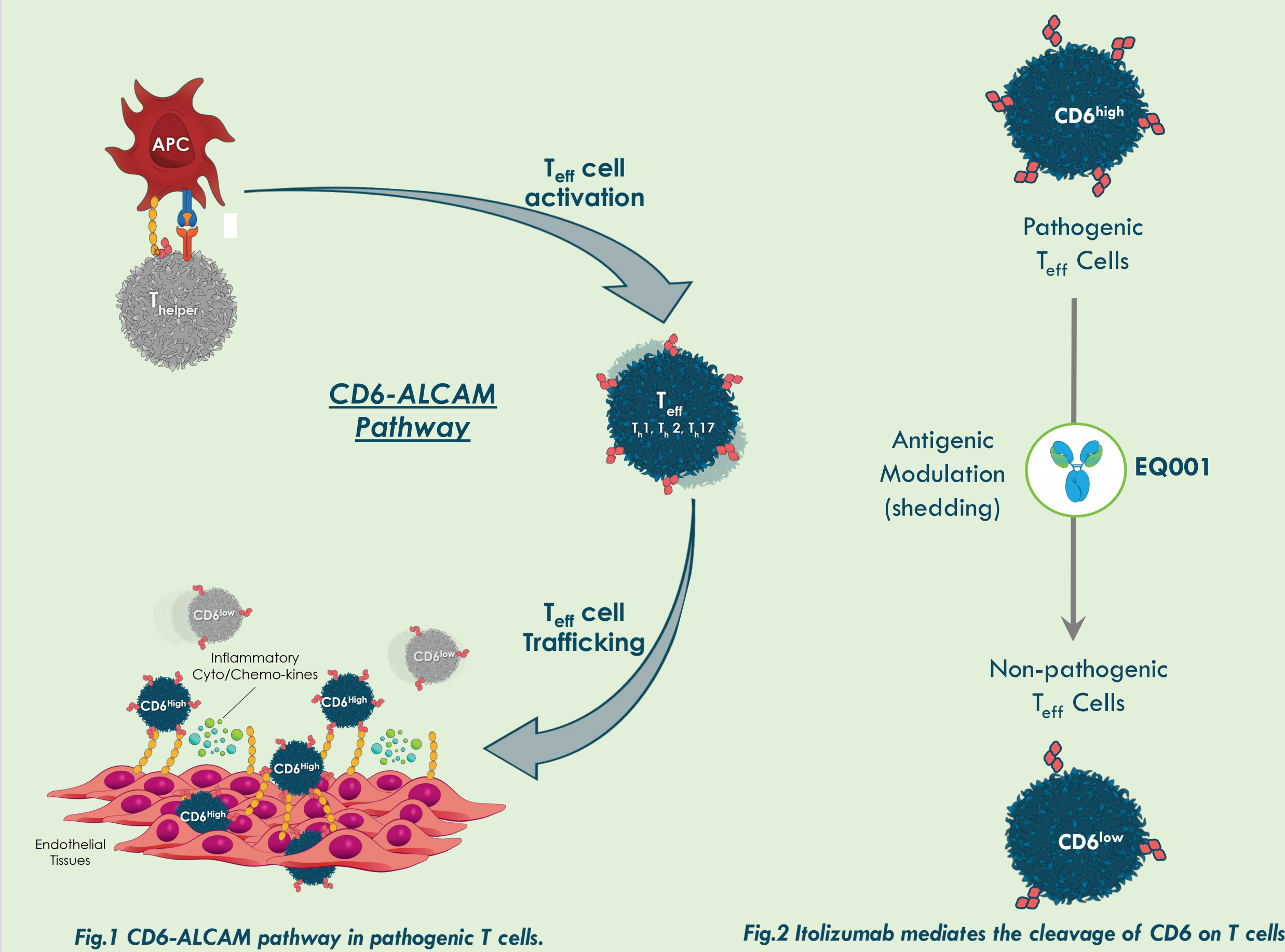


Surface CD6 regulates T cell adhesion and migration through direct ligand interaction and stabilization of VLA4

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Introduction

- CD6 is a costimulatory receptor on T cells that, through binding to its ligand "Activated Leukocyte Cell Adhesion Molecule" (ALCAM), modulates both the activity and trafficking of pathogenic CD6^{high} CD4⁺ T cells.¹
- ALCAM expression is reported in several cell types including hematopoietic stem cells, cancer stem cells, intestinal epithelial crypts, the central nervous system and the microvascular endothelium.^{2,3}
- During inflammation, expression levels of ALCAM are increased at the inflamed tissues as well the infiltration of T cells expressing high levels of CD6. Those observations suggest that the CD6-ALCAM pathway is important for T cells migration, however their exact role in this process is not completely understood (Fig.1).^{4,5}
- Immune cells migration from the blood stream to the inflamed tissues requires 3 main steps: rolling, firm adhesion and trans-endothelial migration, all processes involving integrins and adhesion molecules such as VLA4 and LFA1.
- We studied the involvement of CD6 and ALCAM in the static adhesion and in the endothelial transmigration using an in vitro adhesion assay and a Boyden chamber assay. To identify the exact role of each component, we treated T cells expressing CD6 with a monoclonal antibody that is currently tested in phase 3 for GVHD (NCT05263999), itolizumab. Itolizumab not only blocks the binding of CD6 to ALCAM, but it also induces the stripping of the receptor on the T cells membrane in the presence of APCs, generating CD6^{low} T cells (Fig.2).⁶



OBJECTIVES:

- To investigate changes in CD6 levels in the adhesion of CD4⁺ T cells to the endothelial monolayer
- To study changes in expression of proteins involved in adhesion and migration induced by itolizumab

Methods

Adhesion assay

HUVECs were plated at 20,000 cells per well in black with clear flat bottom Corning 96 well half area plate for 72 hours. Total PBMCs were thawed and activated with Anti-Human CD3 at 5µg/ml and anti-Human CD28 at 1µg/ml and incubated O/N. Activated PBMCs are treated with isotype or itolizumab at 20µg/ml overnight to generate CD6^{high} and CD6^{low} cells. The next day, the CD6^{high} and CD6^{low} cells are enriched for T cells. Isolated CD6^{high} and CD6^{low} cells are treated with Cell Biolabs, Inc. 500X Leukotracker™ Solution for 60 minutes at 2uL/ml. Then, the labeled T cells are plated at 50,000 cells per well and incubated for the designated time points. The cells were then washed twice to remove non-binding cells, lysed and the plate is read at 480/520 nm.

Migration assay

T-cell chemotaxis was assayed using 24-well Transwell chambers with 3 µm pores. HUVEC cells were plated in growth media for 48 hours. After 48 hours, total PBMCs were placed in the upper chambers in 200 µL of serum free medium. The chemokine CXCL12 was placed in the lower well and the chambers were incubated for 24 hours. Migrated cells in the bottom chamber and non-migrated cells in the upper chamber were collected and analyzed by FACS. All experiments were conducted in triplicate. For the treated PBMCs, cells were rested overnight (O/N) and pre-incubated 1h with the different antibodies. For activated PBMCs, cells were rested for 24h and pre-activated for 2h with anti-CD3 5µg/ml and anti-CD28 1µg/ml.

Western Blotting

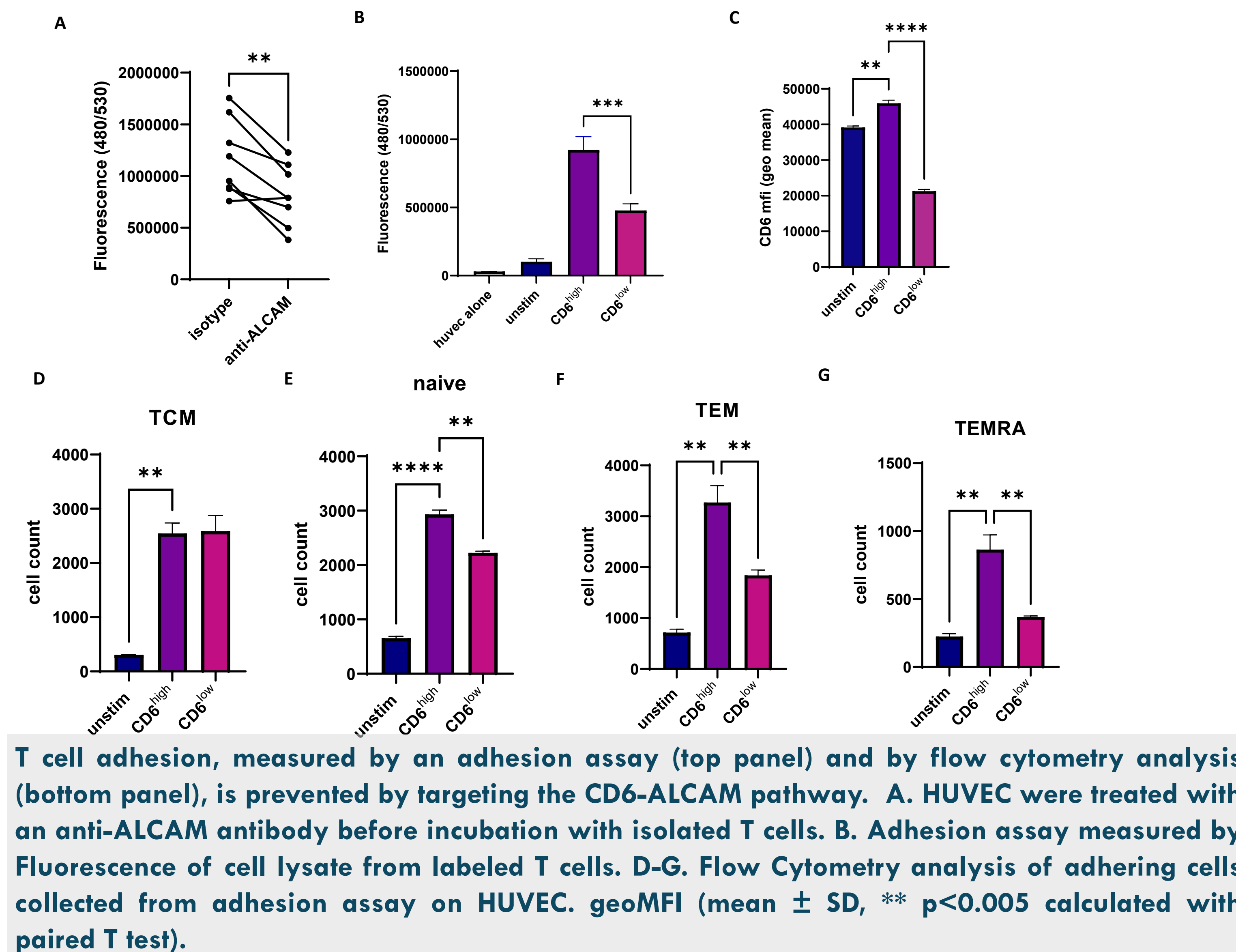
Total cell lysate was generated using RIPA buffer from total PBMCs activated for 24h with anti-CD3 and anti-CD28 at, then treated with isotype or itolizumab for 24h to generate CD6^{high} and CD6^{low} T cells.

qPCR array

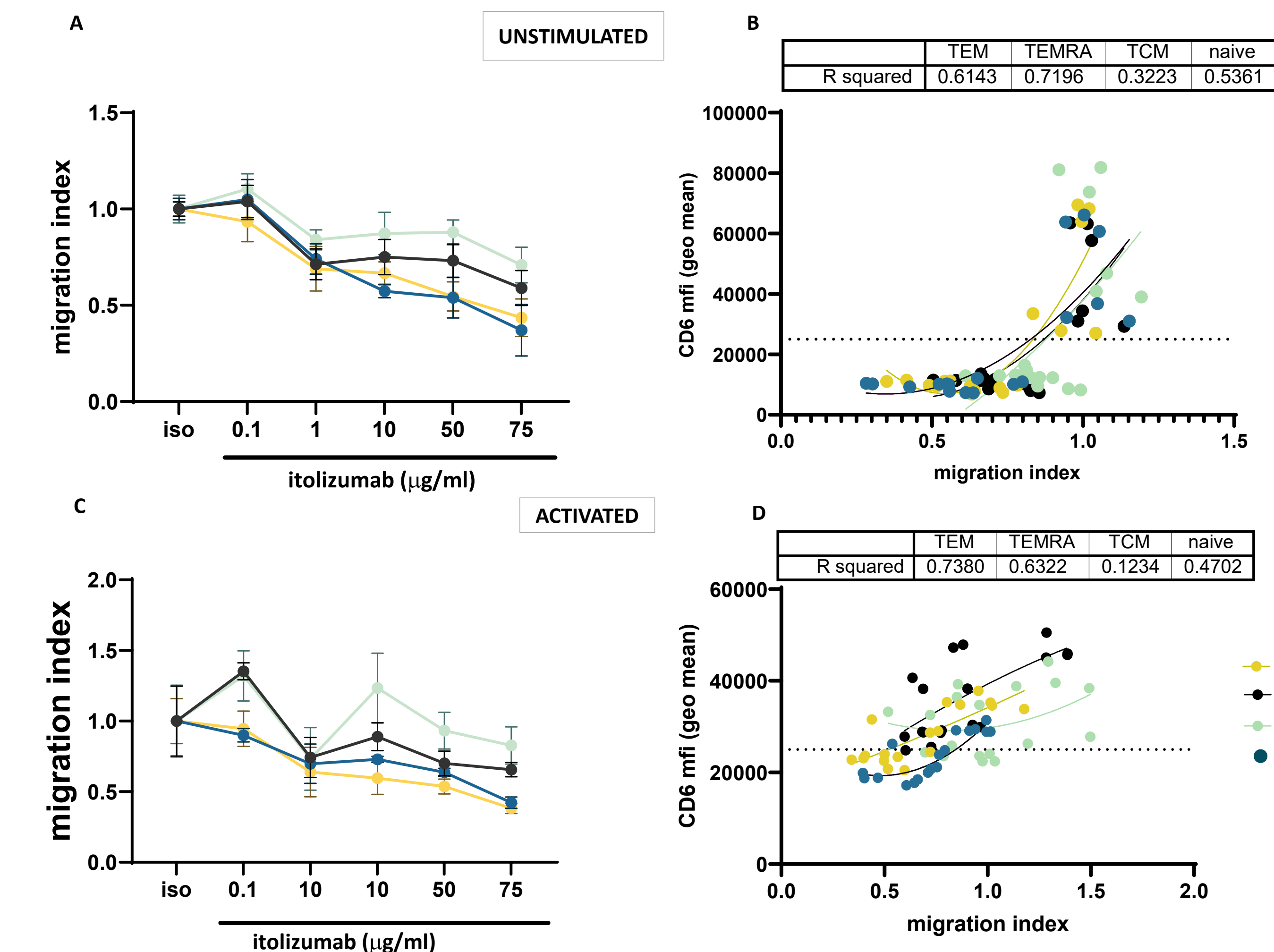
Total RNA was isolated from enriched CD3⁺ T cells using the RNAqueous isolation kit. Gene expression was analyzed using the RT2 Profiler PCR Array for cell motility from Qiagen and the qScript One-Step SYBR Green mix from Quantabio.

Results

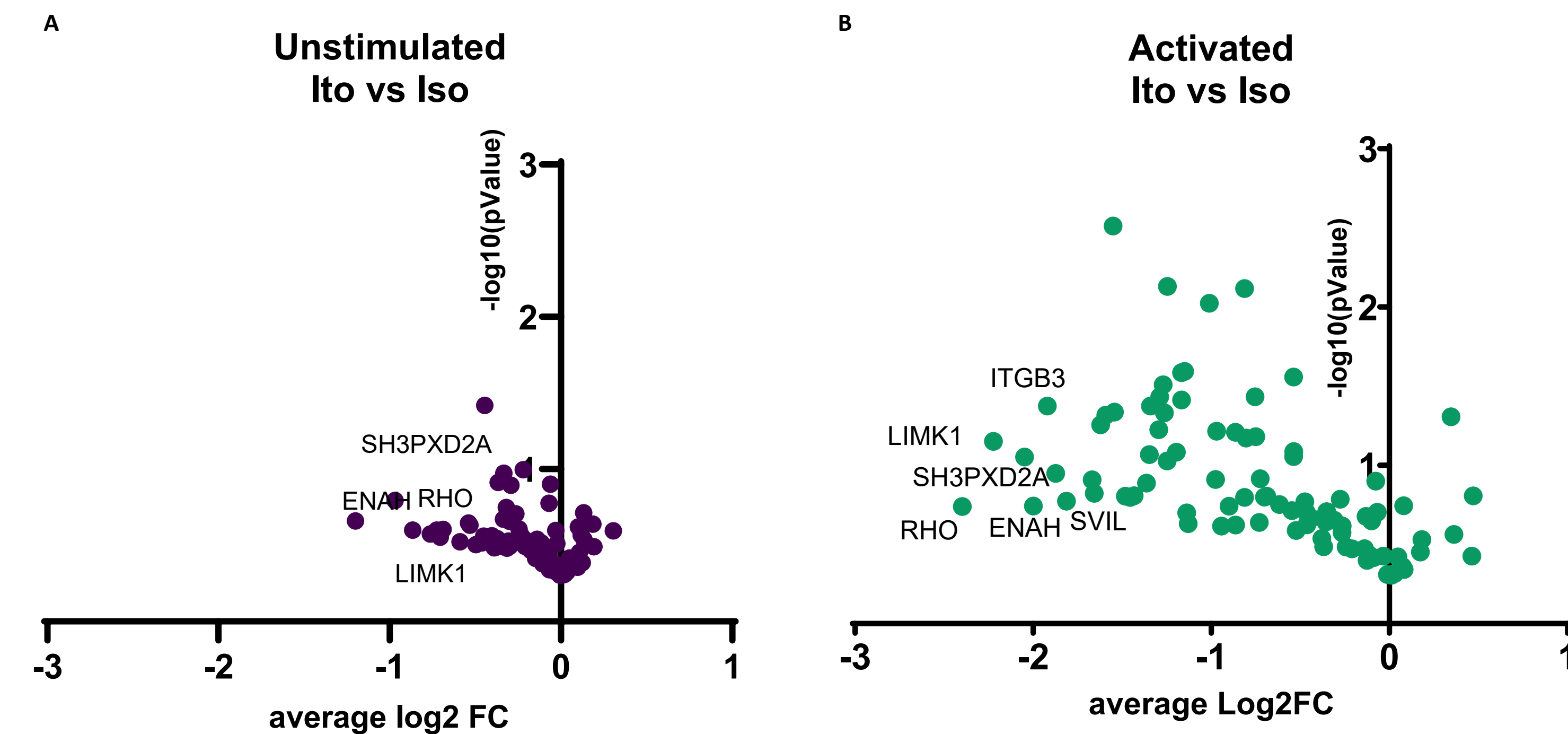
Adhesion to endothelial cells is reduced in CD6 low T cells



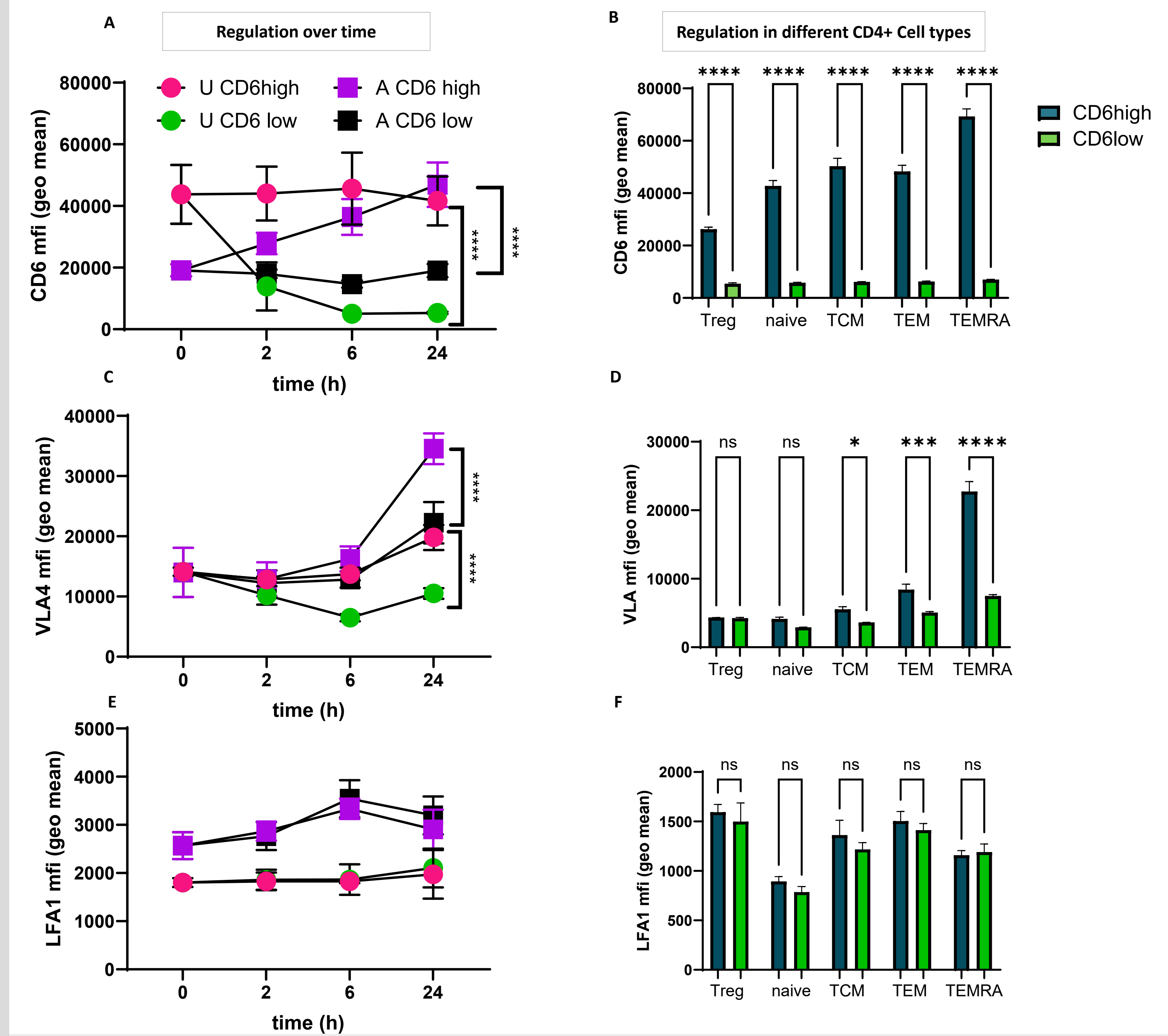
Migration of Effector CD4+ T cells correlates with CD6 levels



CD6 modulation prevents the upregulation of genes involved in cell motility during T cells activation



VLA4 integrin, but not LFA-1, is highly expressed in Effector T cells, and is regulated by CD6 levels



Western blotting analysis (A) of VLA4 levels in activated total PBMCs treated with isotype or itolizumab for 24h and quantification (B) from 3 different donors. (mean $\pm$ SD, * p<0.05 calculated with paired T test).

Conclusions

- Reduction of CD6 on T cells or blocking of ALCAM on the HUVEC, prevents the adhesion of Effector T cells to the endothelial monolayer.
- Itolizumab treatment significantly reduced the Trans-endothelial migration of pathogenic T cells, with high correlation of Effector T cells migration index with the CD6 levels on the cell surface.
- Itolizumab treatments reduced the expression of gene important for cell motility and downregulated VLA4 integrin levels on the cells surface and the total protein levels.

Altogether, this data identified a new role of the CD6-ALCAM pathway in adhesion and trans-endothelial migration of Effector T cells and showed that CD6 expression at the membrane is required for the expression and stabilization of the integrin VLA4, suggesting a direct role of CD6-ALCAM pathway and indirect T cell mobility regulation through downregulation of VLA4.

Acknowledgments

We would like to acknowledge the whole Equillum research team for constant feedbacks and suggestions on the project.

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Disclosures

This study was funded by Equillum, Inc., with the support of ONO Pharmaceutical Co. Valeria Marrocco, Jeanette Ampudia, and Cherie Ng are currently employees and stockholders of Equillum. Stephen Connelly is currently an employee, stockholder, and officer of Equillum.

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