SUPPLEMENTARY MATERIAL

Supplementary Figure 1: Gating strategy for analysis of co-IR expression in immune cell subsets from patient PBMCs

PBMCs were isolated from 28 SSc patients and 19 age-matched controls. The cells were stained with a 16-color antibody panel for flow cytometric analysis of immune cell subsets and co-IR expression. Flow cytometry data were manually gated as follows: (A) Cells were first gated on forward and side scatter to select for lymphocytes, then gated on height versus area followed by width versus height to gate out doublets. Cells were then gated on Red A-A negative to gate out dead cells, CD14+ monocytes and CD19+ B cells. (B) Live non-B cell lymphocytes were gated into CD3+ and CD3- fractions. The CD3+ fraction was gated multiple ways: CD4+ and CD8+ T cells, Vα24+ and CD1d tetramer+ (iNKT cells) and γδ TCR+ (γδ T cells). Within the CD4+ gate, Tregs were gated as $CD25^{hi}CD127^{lo}$ and the $CD25^{lo}$ fraction was designated conventional T cells (Tconv). (C) CD4+ and CD8+ T cell fractions were further separated in CD45RO+ (effector/memory) and CD45RO- (naïve) subsets.

Supplementary Figure 2: Frequencies of T cell subsets in SSc patients compared to healthy controls

Frequency of lymphocyte subsets identified by manual gating as described in Suppl. Fig. 1 was compared between SSc patients and healthy controls. Graphs show (A) percentages of CD4+, CD8+, γδ TCR+, and iNKT cells within the CD3+ gate +/- SEM, (B) percentage of $CD25^{hi}$ $CD127^{lo}$ Tregs within the CD4+ gate. +/- SEM, (C) percentages of CD45RO+ memory cells

within each T cell type +/- SEM. Each symbol represents an individual subject. Statistics was done with t test * $p \leq 0.05$.

Supplementary Figure 3: Gating strategy to determine co-IR-positive cells

PD-1, TIGIT, TIM-3 and LAG-3 were gated based on Fluorescence Minus One (FMO) samples in which antibodies to individual co-IRs were left out. Dot plots show PD-1 (A), TIGIT (B), TIM-3 (C) and LAG-3 (D) gating compared to the corresponding FMO sample.

Supplementary Figure 4: Co-IR expression on CD45RO- T cell subsets

Co-IR expression was determined on CD45RO- naive T cell subsets. (A-D) Percentages of PD-1+ (A), TIGIT+ (B), TIM-3+ (C) and LAG-3+ (D) cells within the CD45RO- gate of Tconv, Tregs, and CD8+ T cells +/- SEM. Statistics was done with t test * $p \leq 0.05$.

Supplementary Figure 5: Co-IR expression in SSc patients with high percentages of Tregs

SSc patients were divided into those with high levels of Tregs and those with normal levels and co-IR expression on multiple T cell populations from these patients was compared. (A) Graph shows percentages of $CD4+CD25^{hi}CD127^{lo}$ Tregs within CD4+ T cells. The blue box designates patients with normal levels of Tregs and the red box designates patients with high levels of Tregs. (B-E) The percentage of PD-1+ (B), TIGIT+ (C), TIM-3 (D) and LAG-3+ (E) cells within the indicated CD45RO+ T and NK cells populations from $Treg^{hi}$ vs $Treg^{lo}$ patients +/- SEM. Each symbol represents an individual subject. Co-IR expression on iNKTs only graphed if >20 events were recorded. Statistics was done with t test * $p \leq 0.05$.

Supplementary Figure 6: Cell viability and subset composition in MLR cultures

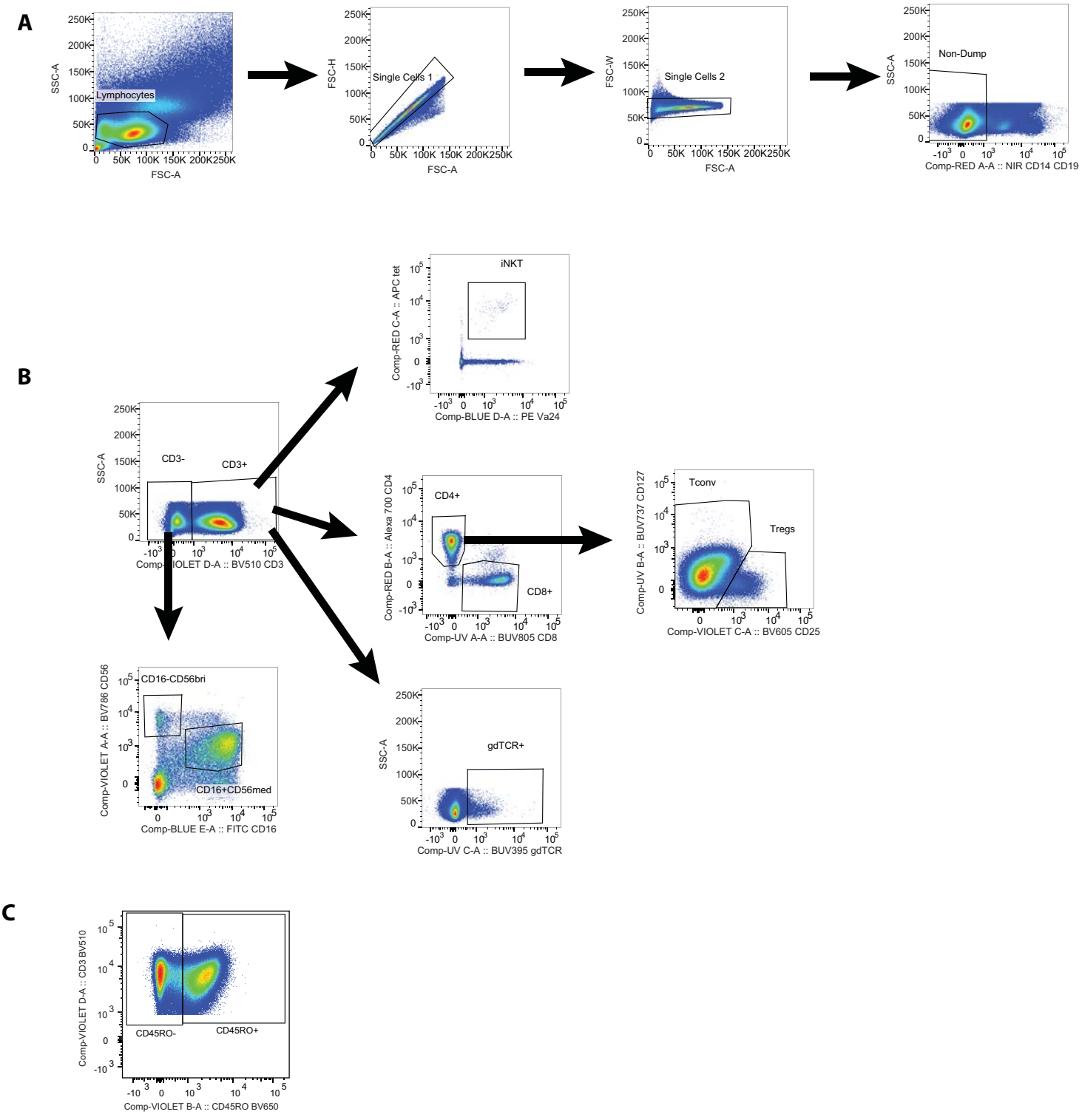
(A) Percentage of live cells in MLR cultures treated with isotype, anti-PD-1, anti-TIGIT, and anti-TIM-3 antibodies, as determined by 123 eCount beads (eBioscience) and flow cytometry. (B) Percentage of CD4+ T cells within the live cell gate in MLR cultures treated with isotype, anti-PD-1, anti-TIGIT, and anti-TIM-3 antibodies. (C) Percentage of CD8+ cells within the live cell gate in MLR cultures treated with isotype, anti-PD-1, anti-TIGIT, and anti-TIM-3 antibodies. (D) Total numbers of CD4+ cells within the live cell gate in MLR cultures treated with isotype, anti-PD-1, anti-TIGIT, and anti-TIM-3 antibodies. (E) Total numbers of CD8+ cells within the live cell gate in MLR cultures treated with isotype, anti-PD-1, anti-TIGIT, and anti-TIM-3 antibodies.

Supplementary Figure 7: Hierarchical clustering of cytokine production and fibroblast gene expression

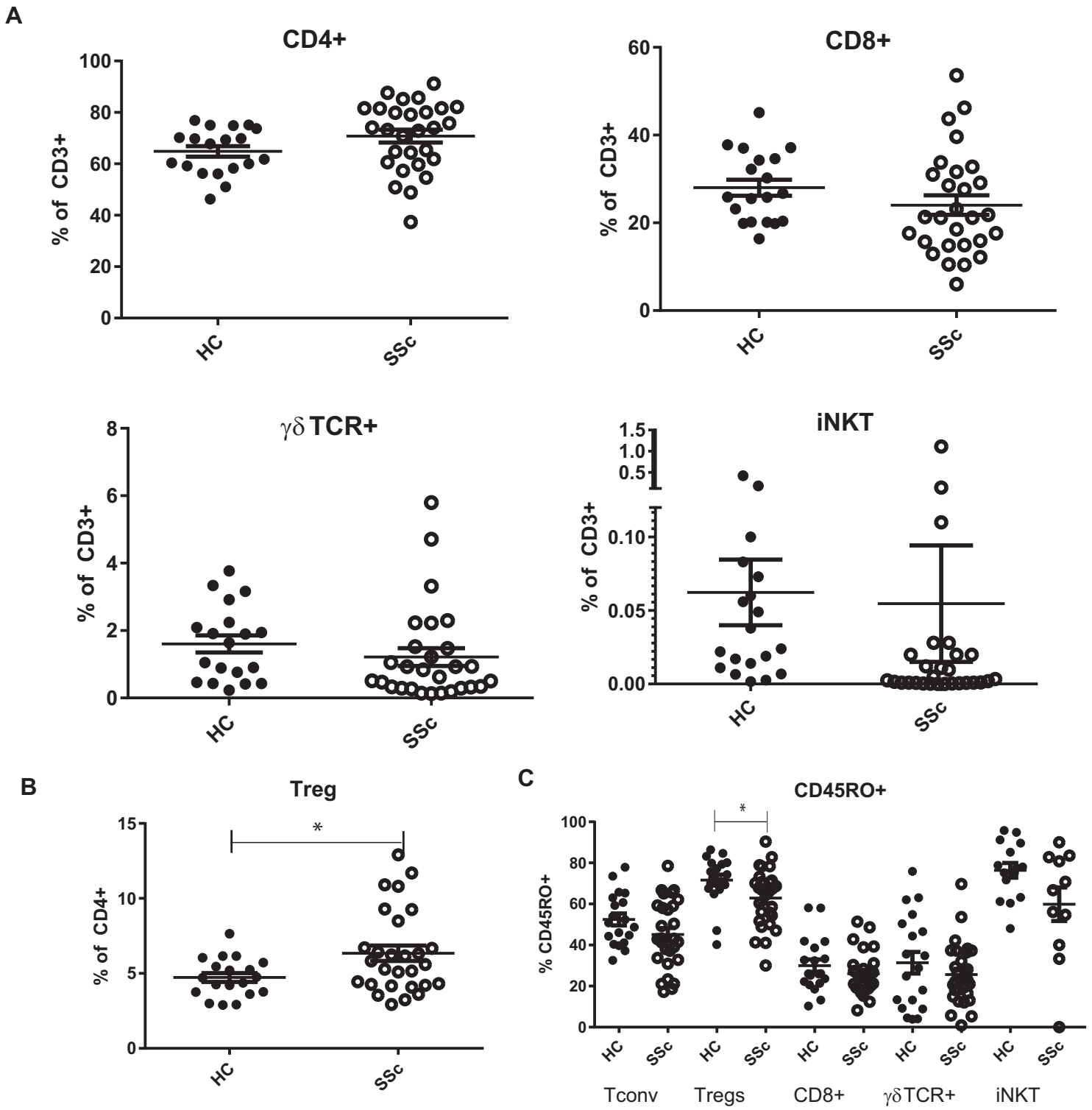
(A) Heatmap of the quantification of cytokines in the supernatants from MLR cultures treated with blocking antibodies to co-IRs. (B) SSc fibroblasts were cultured for 24 hrs with supernatants from MLR cultures in Fig. 3. Fibroblast RNA was prepared and expression of selected genes measured with Nanostring. Heatmap shows hierarchical clustering of gene expression vs anti-PD-1-, anti-TIGIT-, anti-TIM-3- and isotype-treated cultures of each individual healthy and SSc donor.

Supplementary Table 1: Clinical features of Systemic Sclerosis patients and healthy controls used for in vitro culture experiments

Characteristic	HC	SSc
	n=5	n=6
Sex, number males/females	1/4	1/5
Age	53 (37-69)	62 (48-67)
Age of Onset, median	NA	53 (33-67)
Disease Duration	NA	9 years (2-27)
Race, Hispanic-White/Black/White	0/0/5	0/0/6
Number dSSc/lSSc	NA	1/5
Modified Rodnan Skin Score	NA	4 (0-17)
Lungs		
ILD	NA	1
PAH	NA	3



Suppl. Figure 1



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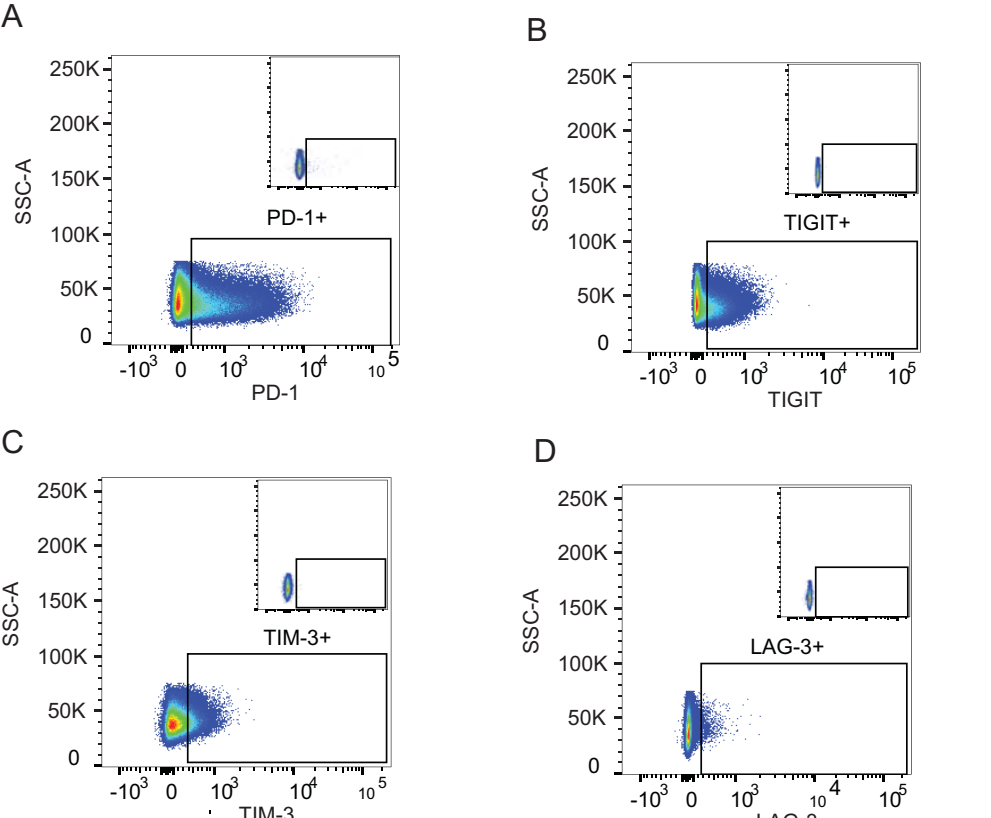

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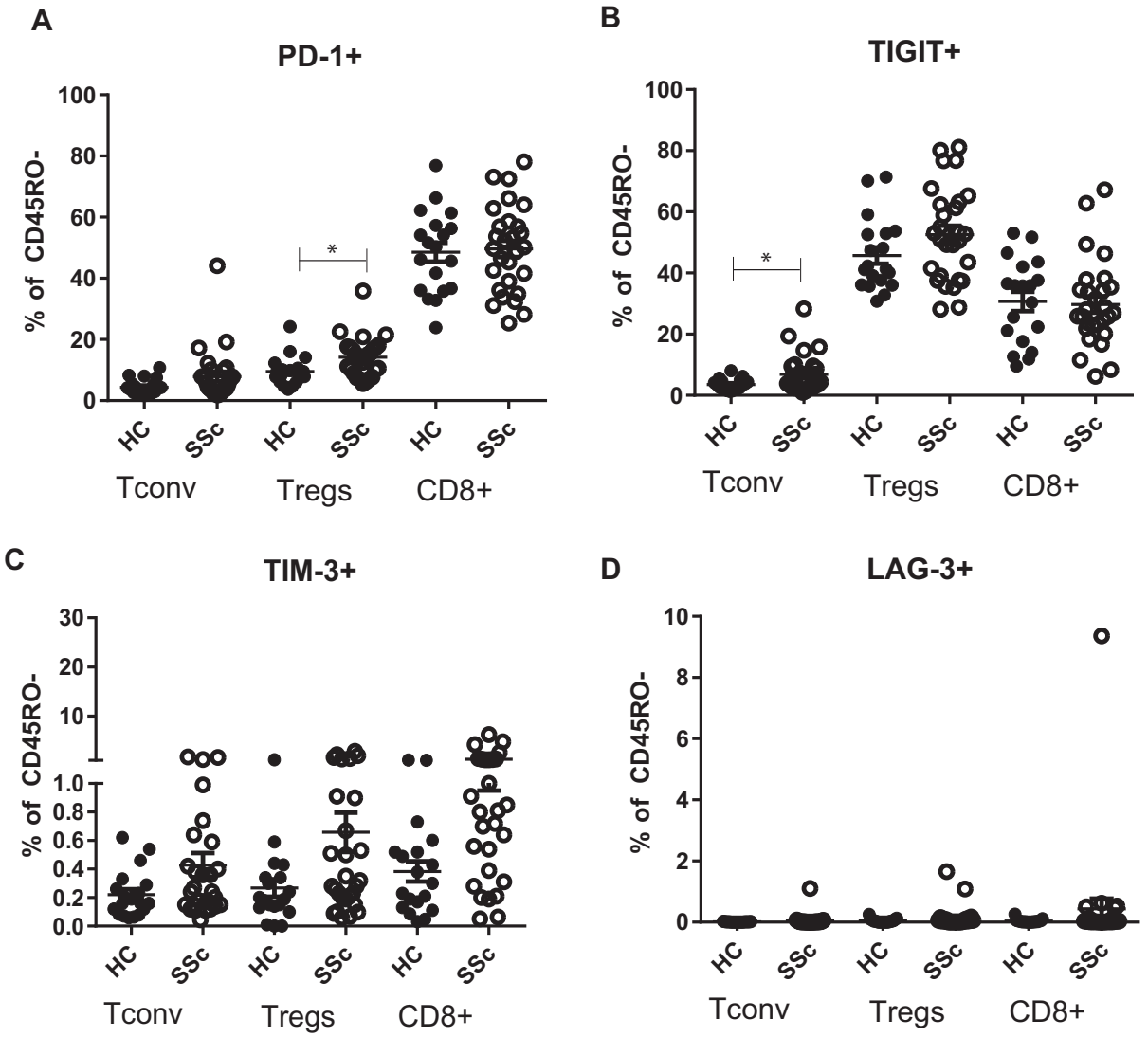

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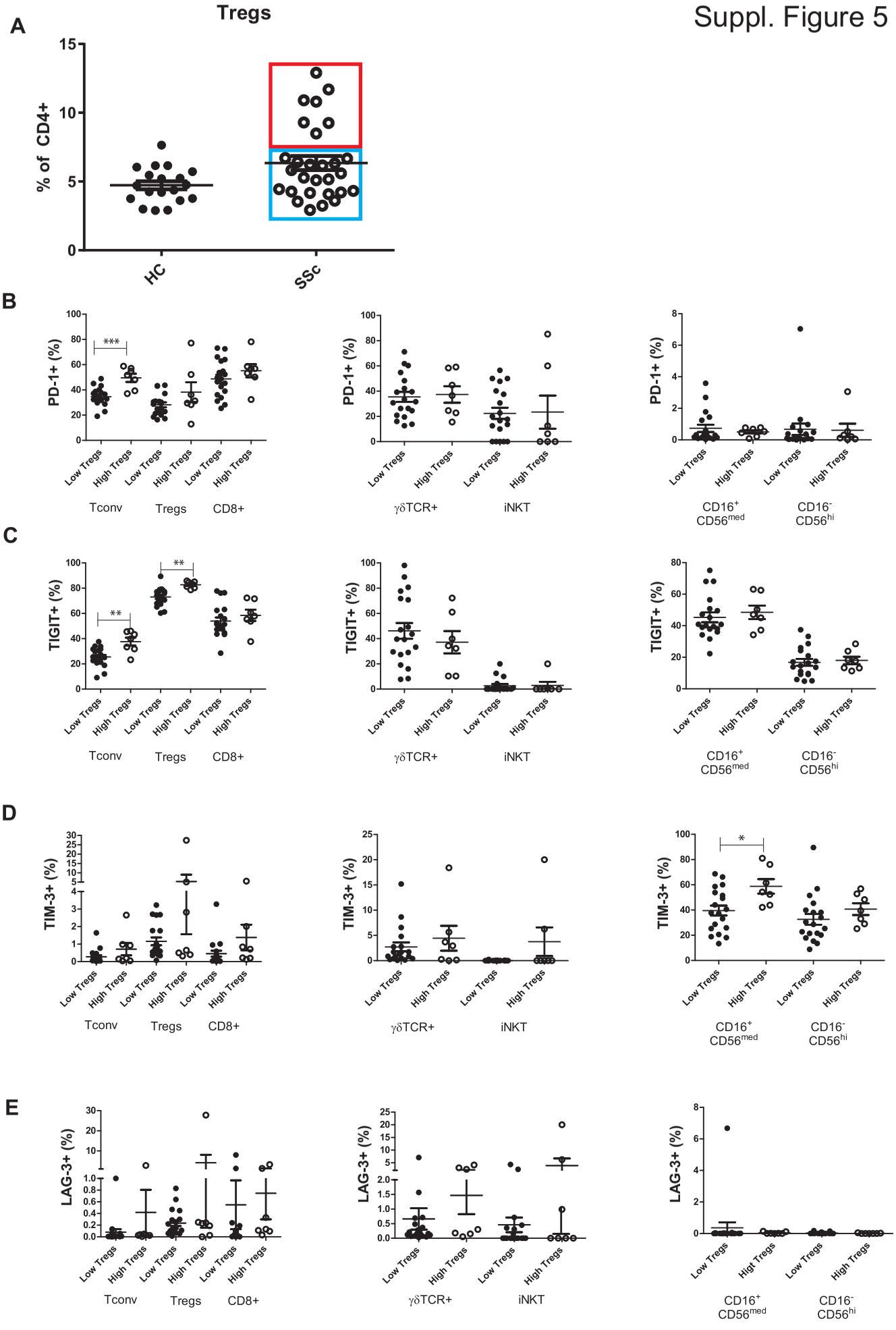
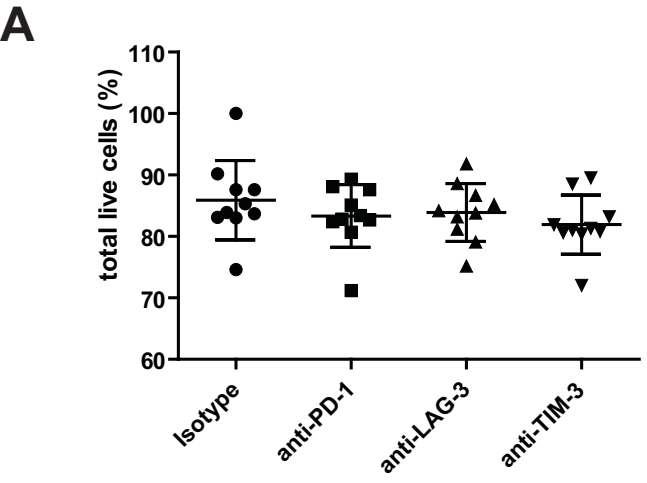
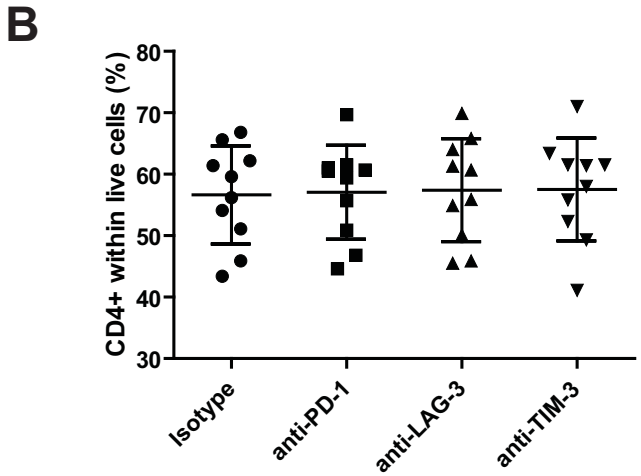
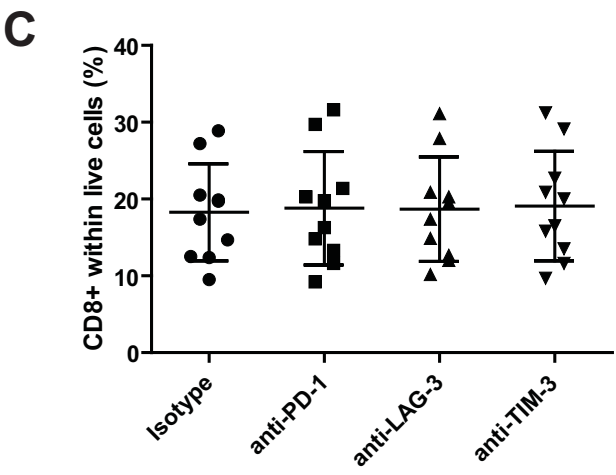
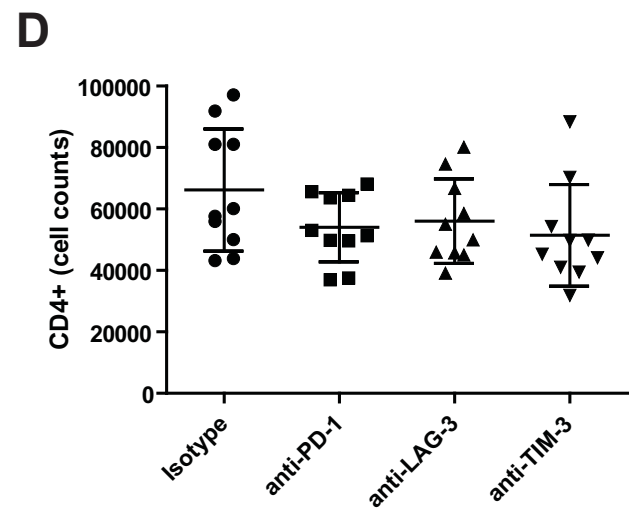
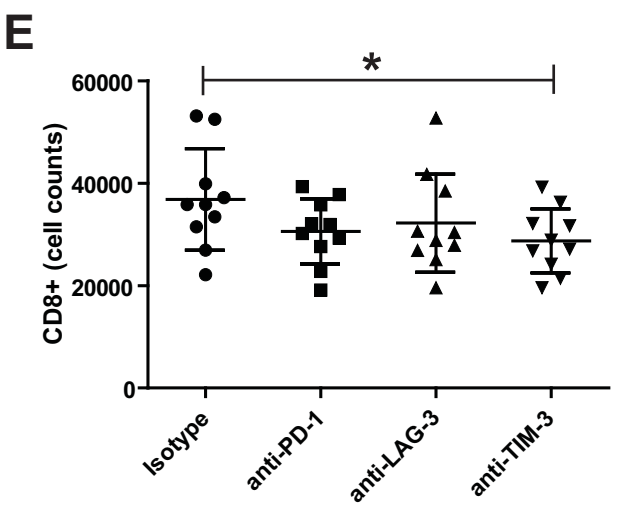








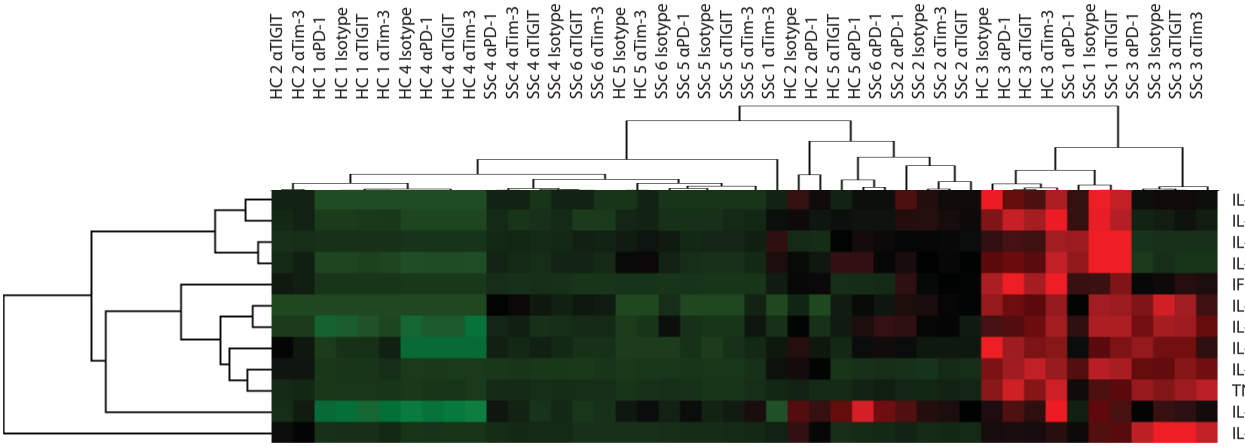




Suppl. Figure 6

Suppl. Figure 7

A



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