SUPPLEMENTARY APPENDIX

Tocilizumab, tacrolimus and methotrexate for the prevention of acute graft-*versus*-host disease: low incidence of lower gastrointestinal tract disease

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SUPPLEMENTAL METHODS

Serum Cytokine Analysis. Serum was isolated from patients prior to onset of conditioning, and on days 7, 14 and 28 post-transplantation. IL-2, IL-4, IL-6, IL-10, IFN-γ, TNF-α, and IL-17A levels were measured using the BD Cytometric Bead Array Human Th1/Th2/Th17 Cytokine Kit (BD Biosciences), according to the manufacturer's instructions. Samples were acquired on a BD FACSCanto II flow cytometer (BD Biosciences) and analyzed using the FCAP Array v3.0.1 software (BD Biosciences). Soluble IL-6R levels were measured using the Human IL-6R alpha Quantikine ELISA Kit (R and D Systems, Minneapolis, MN). This assay has been reported to detect three forms of the sIL-6R; free sIL-6R, sIL-6R in complex with IL-6, and sIL-6R in an immune complex with Tocilizumab.[44]

Immune Reconstitution. Multi-parameter flow cytometry was utilized for the testing of T cells and NK after mononuclear cell enrichment by density gradient separation. The antibodies used included: CD3-HPC-H7, CD4-PE-Cy, CD8-APC, and CD56-PE. Regulatory T cells were detected using the BD Pharmingen (San Diego, CA) FoxP3 staining kit according to instructions, with the addition of CD127-PE-Cy7. Tregs were defined as $CD3^+$ $CD4^+$ $CD25^+$ FoxP3 $^+$ and $CD127^-$. Th17 cells where tested after activation with PMA and Ionomycin in the presence of brefeldin A, then fixed and permeablized before intracellular antigen staining with IL-17-PE. B cell subsets were tested on whole blood samples (300 µL) that were lysed and washed twice prior to the addition of antibodies so as to remove serum Ig that can inhibit surface Ig staining. The antibodies used included: IgD-FITC, IgM-APC, CD27-APC-H7, CD38-PE-Cy7, CD21-FITC, and CD69-APC, with initial gating on $CD19^+$ B cells. Minimally 300,000 debris-free events were collected. The following $CD19^+$ subsets were defined: Antigen inexperienced: $CD27^-$ IgD^+, Pre-Switch Memory: $CD27^+$ IgD^+, Post switch Memory: $CD27^+$ IgD^-, Plasma cells: IgM^- IgD^- $CD27^+$ $CD38^{hi}$; Immature/Transitional: $CD21^-$, Activated: $CD69^+$. All antibodies were obtained from BD Pharmingen and testing was performed on a BD Canto II cytometer within 24 hours of cell collection. Patients in hematological relapse at the time of sampling were not assessed.

SUPPLEMENTAL TABLE 1. DEFINITION OF EVENTS AND COMPETING RISKS FOR SPECIFIC OUTCOMES

Outcome	Event(s)	Competing risks
Event-free survival		
Acute GVHD-free survival	Grade II-IV aGVHD or death	None
Disease-free survival	Relapse or death	None
Overall survival	Death	None
Cumulative incidence		
Treatment related mortality	Death	Relapse
Relapse	Relapse	Death w/o relapse
Acute GVHD	Grade II-IV aGVHD	Relapse or death
Chronic GVHD	Chronic GVHD	Relapse or death
Neutrophil engraftment	1st of 3 consecutive days with an ANC>500	Death w/o engraftment
Platelet engraftment	1st day of a sustained platelet count above 20,000 w/o any platelet transfusions for the preceding 7 days	Death w/o engraftment

All outcomes used loss to follow up / end of study as censoring

SUPPLEMENTAL TABLE 2. PATIENT CHARACTERISTICS OF CYTOKINE CONTROL POPULATION

Variable	Value
N	11
Age, median (range)	61 (27-67)
Sex (M/F)	7/4
Disease (n, %)	
AML	2 (18)
MDS	3 (27)
Myelofibrosis	2 (18)
Myeloproliferative Disorder	1 (9)
Hodgkin Lymphoma	2 (18)
Non-Hodgkin Lymphoma	1 (9)
Donor Type (n, %)	
MRD	9 (82)
MUD	2 (18)
Preparative Regimen (n, %)	
Myeloablative	6 (55)
Recued Intensity	5 (45)
Graft Source (n, %)	
Bone Marrow	0 (0)
Peripheral Blood	11 (100)

AML, acute myelogenous leukemia; MDS, myelodysplasia; MRD, matched related donor; MUD, matched unrelated donor

SUPPLEMENTAL FIGURES

Supplemental Figure 1: **Acute GVHD outcomes by tissue site.** Cumulative incidence of grades II-IV acute GVHD in skin (panel A), liver (panel B), upper GI tract (panel C) and lower GI tract (panel D) of patients that received Tocilizumab for GVHD prophylaxis.

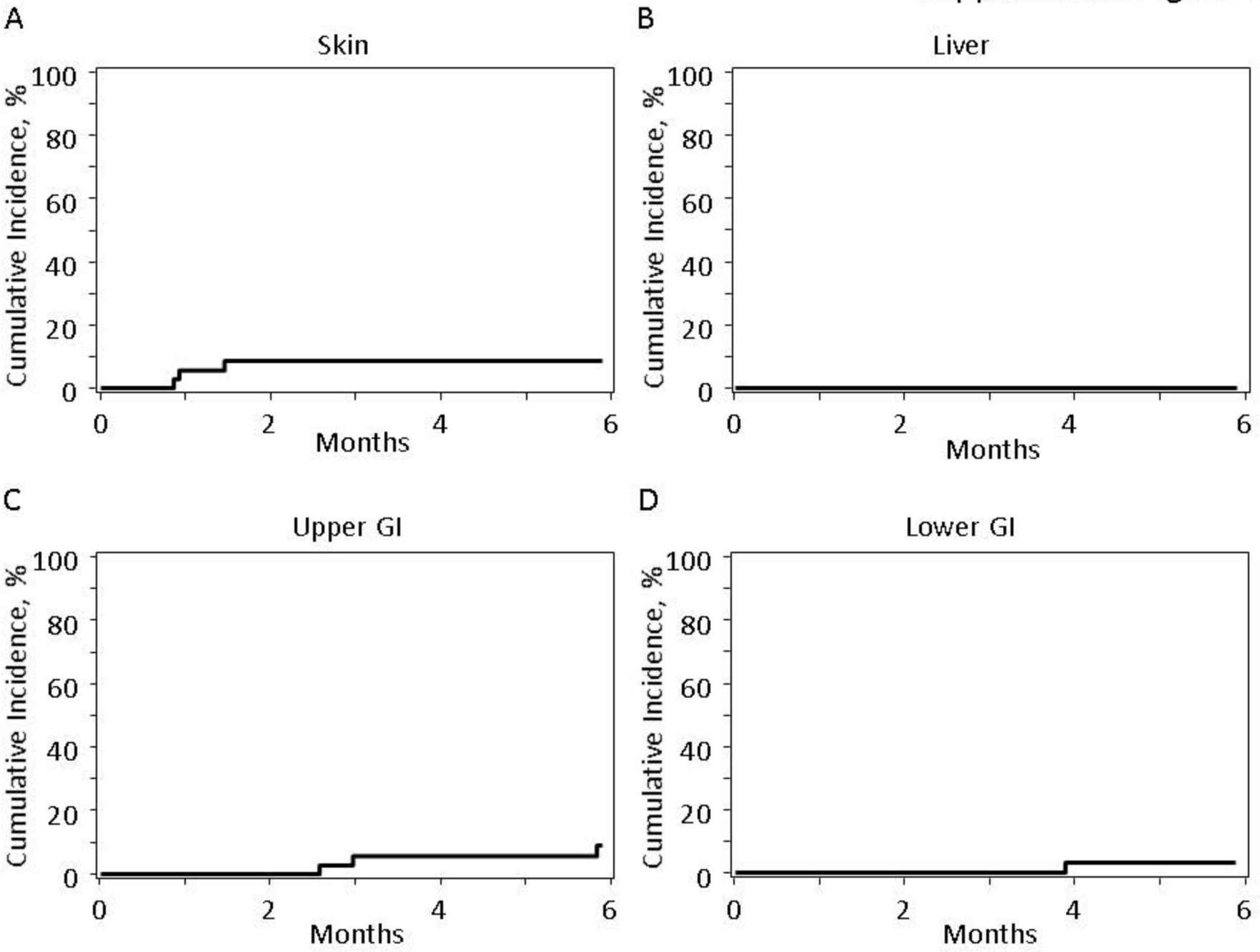
Supplemental Figure 2: **Effect of Tocilizumab administration on interleukin 6 and soluble interleukin 6 receptor levels based on conditioning regimen.** (A). Concentration of IL-6 in the serum on days 7, 14 and 28 from patients that were treated with Tocilizumab and received reduced intensity versus myeloablative conditioning. (B). Concentration of soluble IL-6 receptor in the serum on days 7, 14, and 28 from patients that were treated with Tocilizumab and received reduced intensity versus myeloablative conditioning. Statistics: *$p<0.05$, **$p<0.01$, ***$p<0.001$

Supplemental Figure 3: **Effect of Tocilizumab administration on cytokine production.** Concentration of IL-2, IL-4, IL-10, TNF-α, IFN-γ, and IL-17 in the serum of patients that were treated with Tocilizumab for the prevention of acute GVHD prior to the start of conditioning and at days 7, 14 and 28. Statistics: **$p<0.01$, ***$p<0.001$.

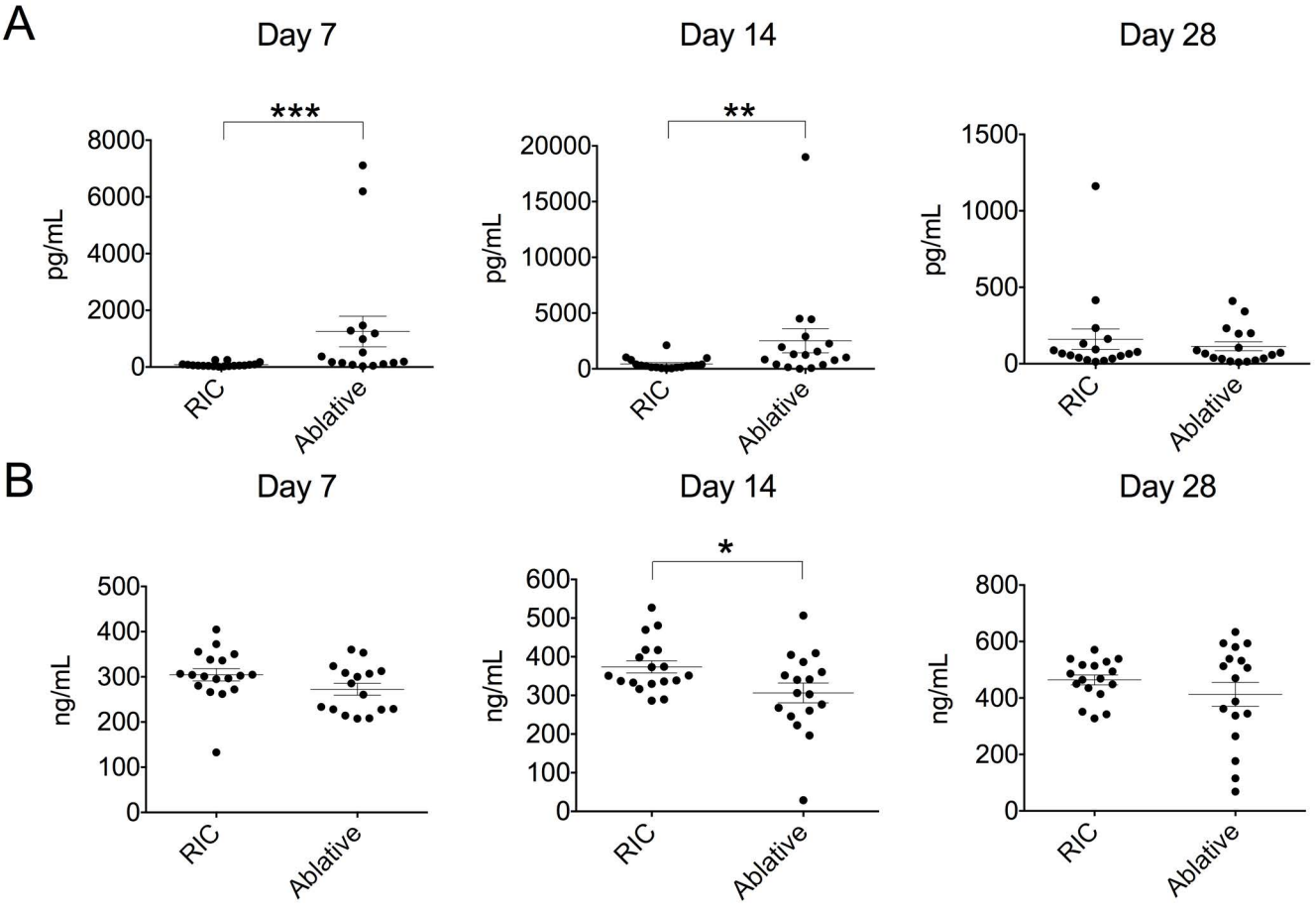
Supplemental Figure 4: **Reconstitution of Treg and Th17 subsets.** The percentage of the indicated subset within $CD4^+$ T cells (upper panels) and the number of cells per mm3 (microliter) of blood for regulatory T cells (Tregs) and T_H17 cells. Data are shown for individual patients together with the median and 25^{th} and 75^{th} quartiles. The shaded area indicates the range of values for healthy controls. Samples were obtained at 1 month (n=33). 3 months (n=29), 6 months (n=22), and 12 months (n=13). The indicated subsets were defined as described in methods.

Supplemental Figure 5: **B cell reconstitution in patients that received Tocilizumab for GVHD prophylaxis.** (A,B). The number of cells per mm^3 (i.e., microliter) is shown in panel A, and the percentage of gated $CD19^+$ B cells is shown in panel B. The B cell subsets were defined as shown in Methods. Data are shown for individual patients together with the median and 25^{th} and 75^{th} quartiles. The shaded area indicates the range of values for healthy controls. Samples were obtained at 1 month (n=33), 3 months (n=29), 6 months (n=22), and 12 months (n=13).

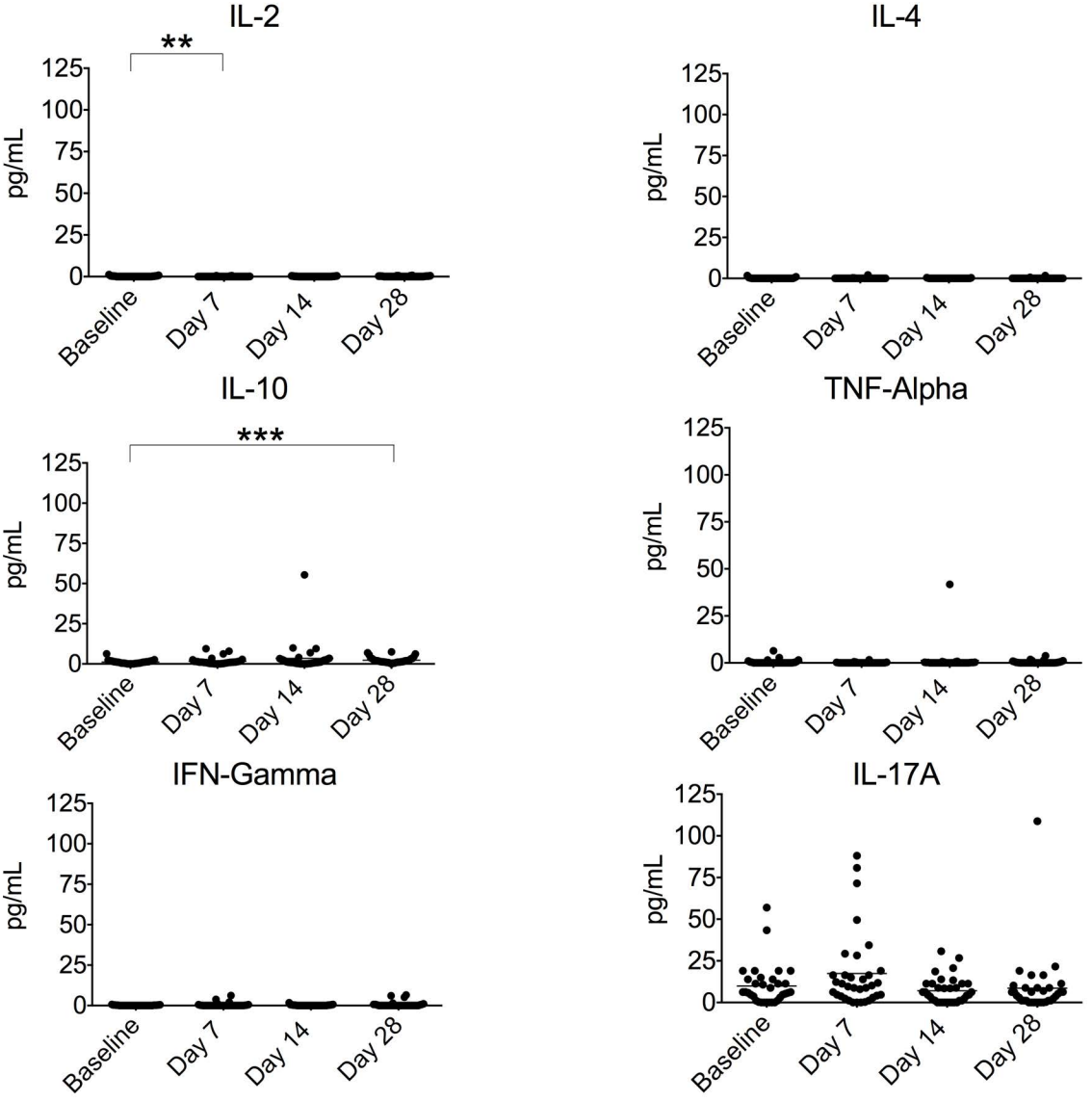
Supplemental Figure 1



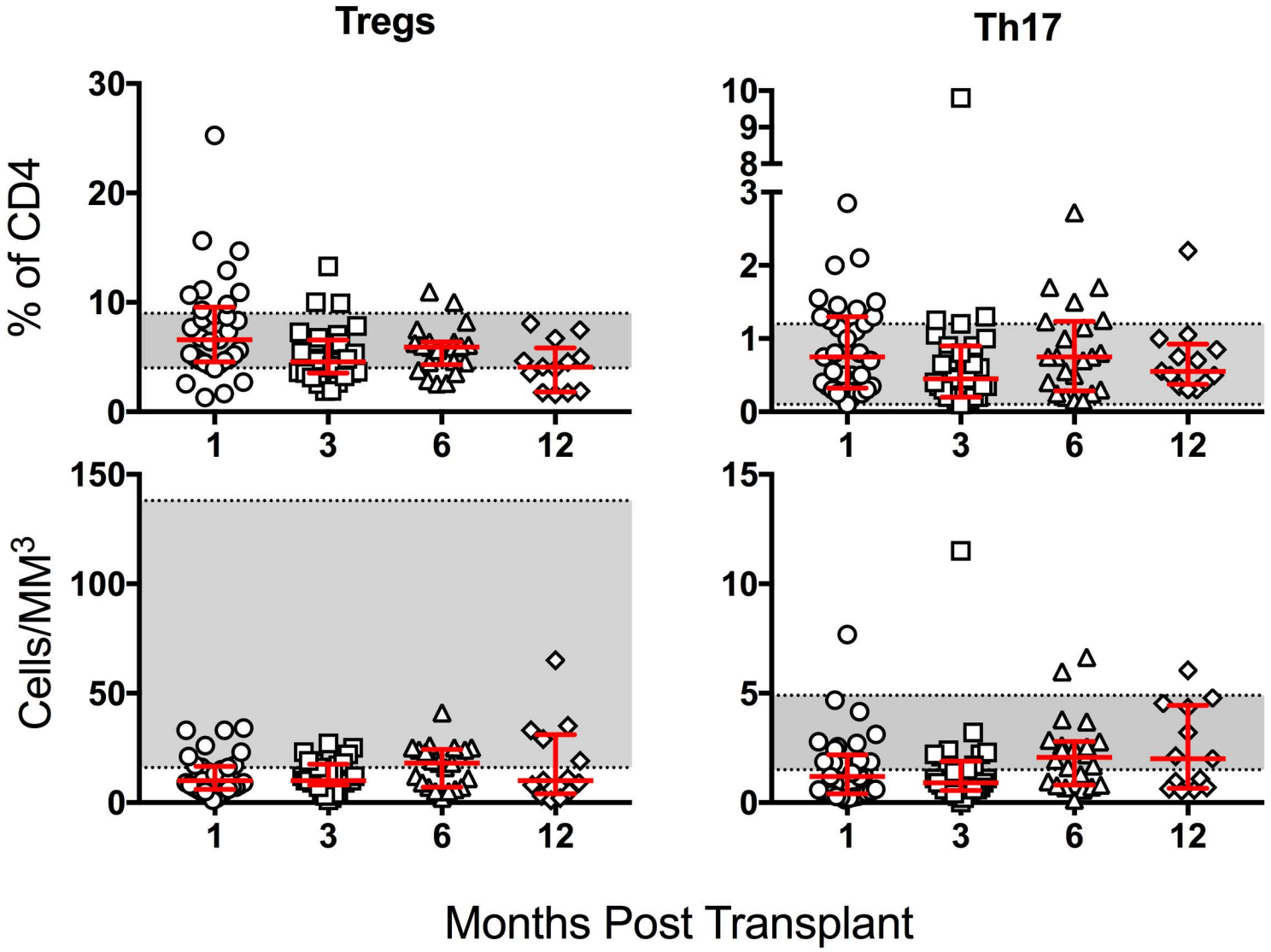
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5