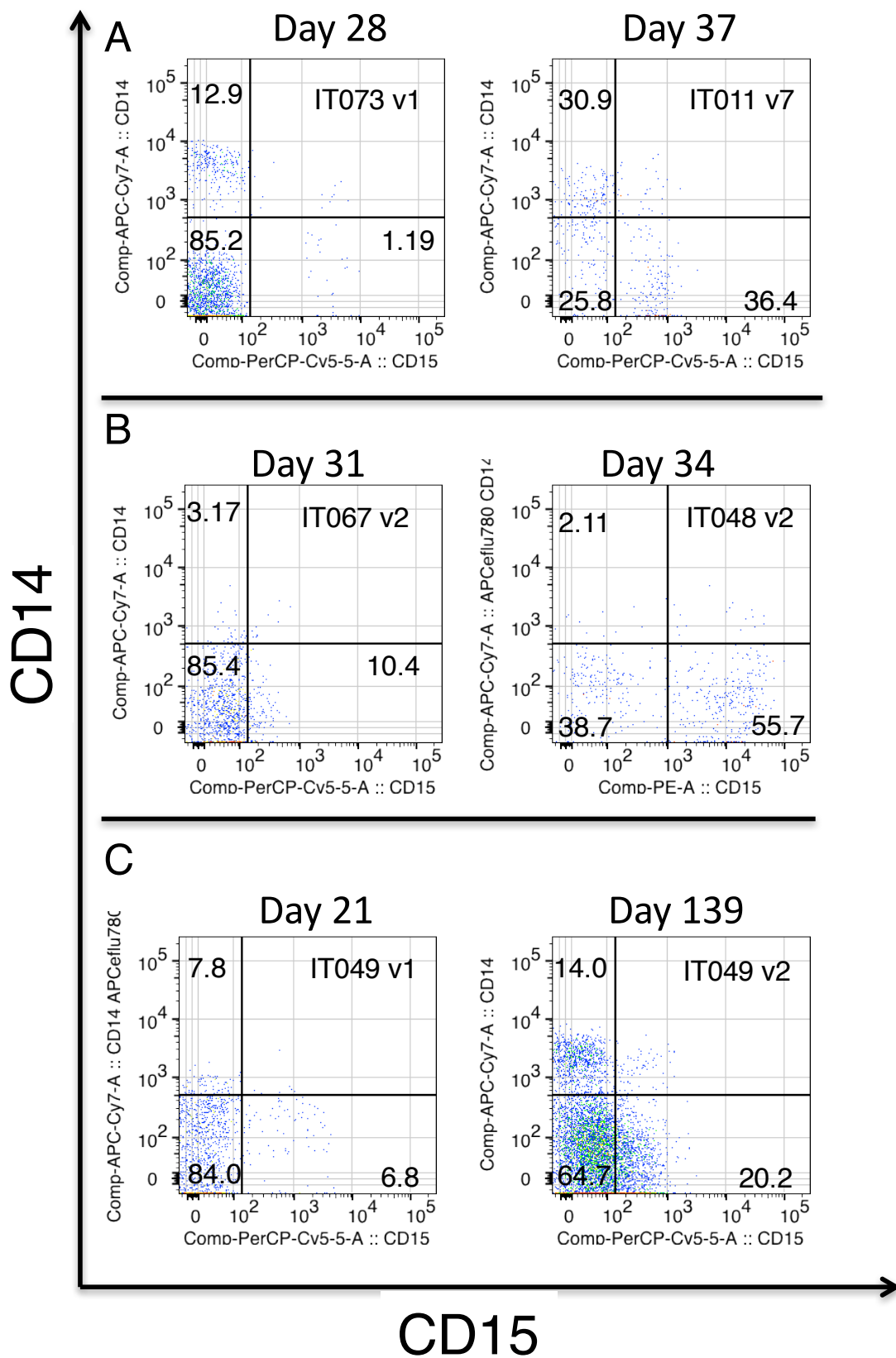


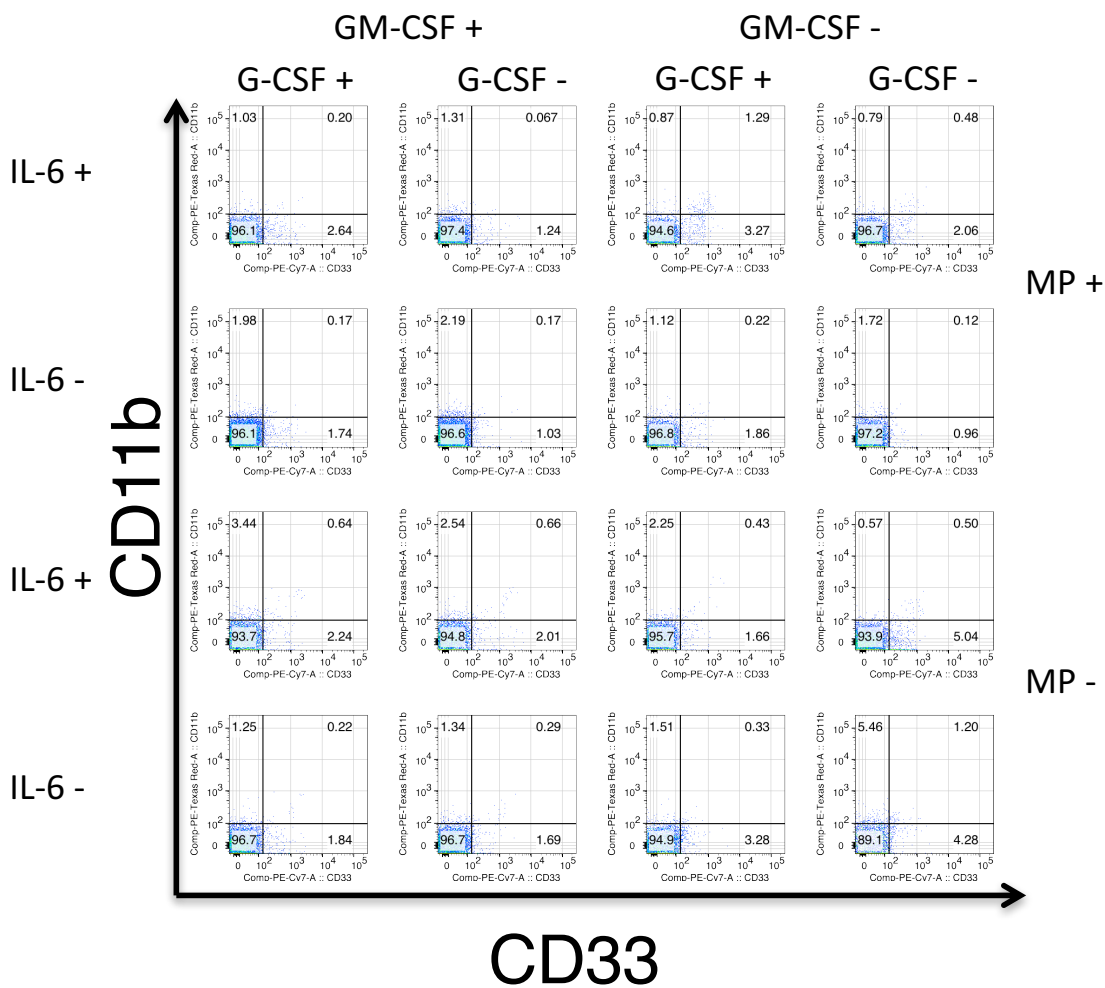
Supplementary Figure 1. CD52 expression on lineage⁺ cells and MDSCs

The histogram shows CD52 expressions on PMN-MDSCs (red), e-MDSCs (orange), M-MDSCs (blue), MDSCs and lineage (CD3, CD56, and CD19)⁺ cells (dark green). The light green histogram shows the isotype control. M-MDSCs are CD52 positive, but PMN-MDSCs are CD52 low or negative. e-MDSCs show heterogeneous expression of CD52. Similar data were obtained from at least 5 individual patients.



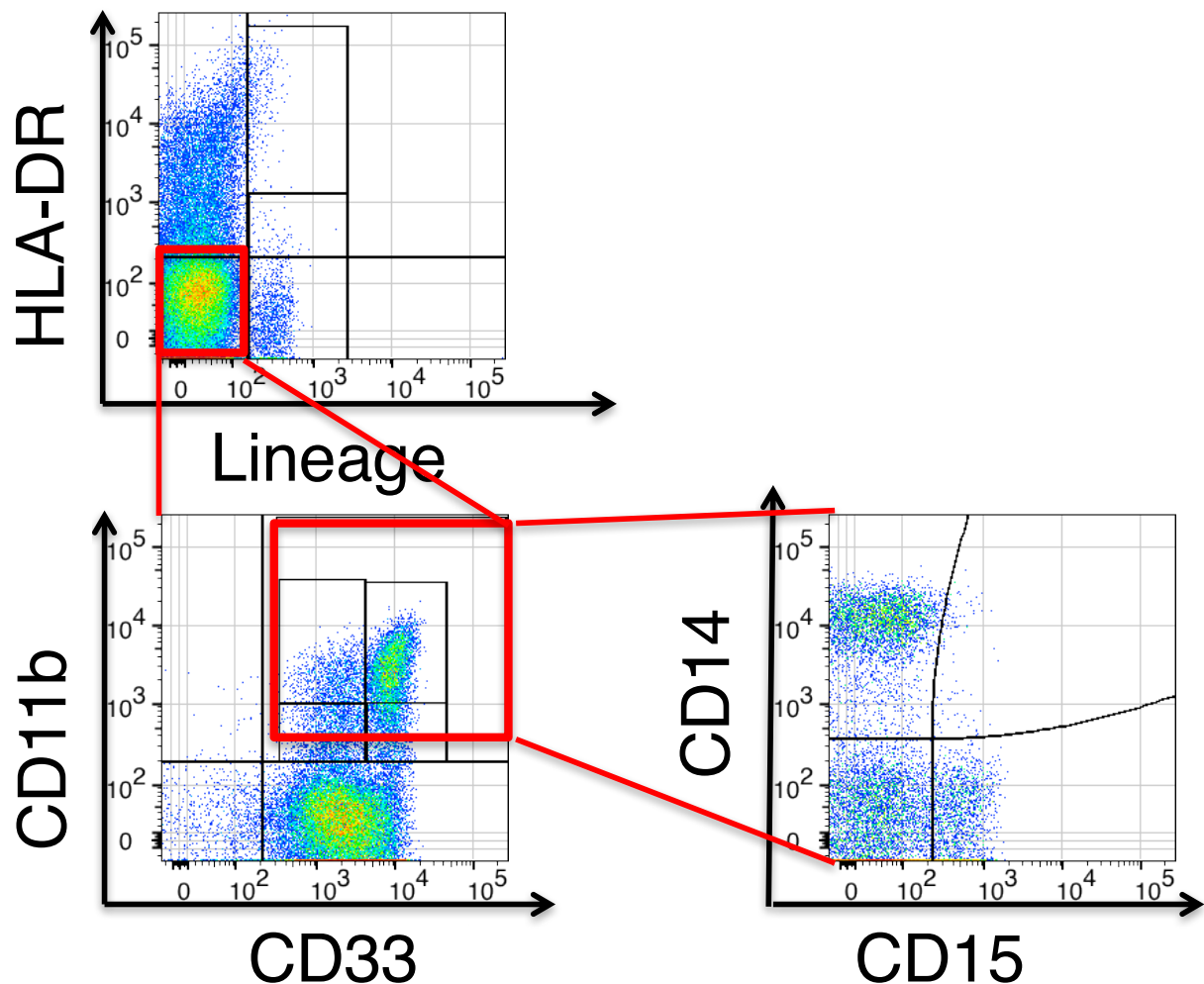
Supplementary Figure 2. No distinct population of M-MDSC phenotype is detected early after ITx with induction therapy including alemtuzumab

Dot plots show expression of CD14 and CD15 on MDSCs from PBMCs during week 3 to 5 after full multivisceral transplantation without induction therapy (A) or Itx with induction therapy with alemtuzumab (B). Dot plots in C show expression of CD14 and CD15 on MDSCs in PBMCs on day 21 and week 20 after Itx from patient no. IT049, who received alemtuzumab. The sample numbers (the upper quadrant of each panel) and percentages of each MDSC subset (upper-left, lower-left, or lower-right quadrant) are indicated in the dot plot figures. The indicated data shows representative data, and similar trends were observed in other samples from different patients.



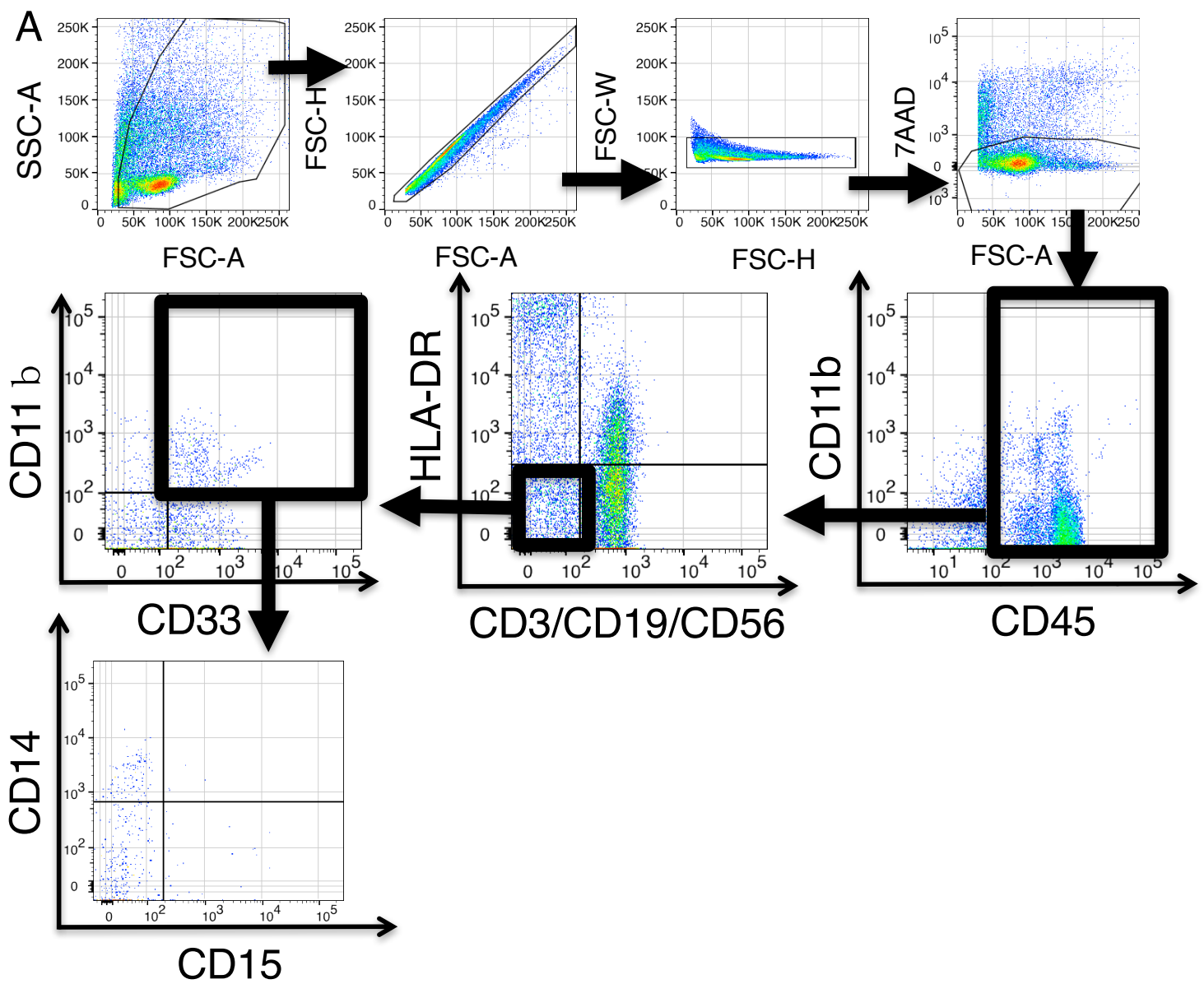
Supplementary Figure 3. No expansion of MDSCs is detected in PBMC culture in medium supplemented with G-CSF, GM-CSF, IL-6, and/or MP.

PBMCs were cultured for 6 days in medium supplemented with G-CSF (the first and third columns from the left), GM-CSF (the left two columns on the left), IL-6 (the first and third rows from the top), and/or MP (the top two rows). Dot plots show expression of CD33 and CD11b in single and live lineage-HLA-DR⁻ cells. Few typical CD33⁺CD11b⁺ MDSCs were detected in this culture condition.



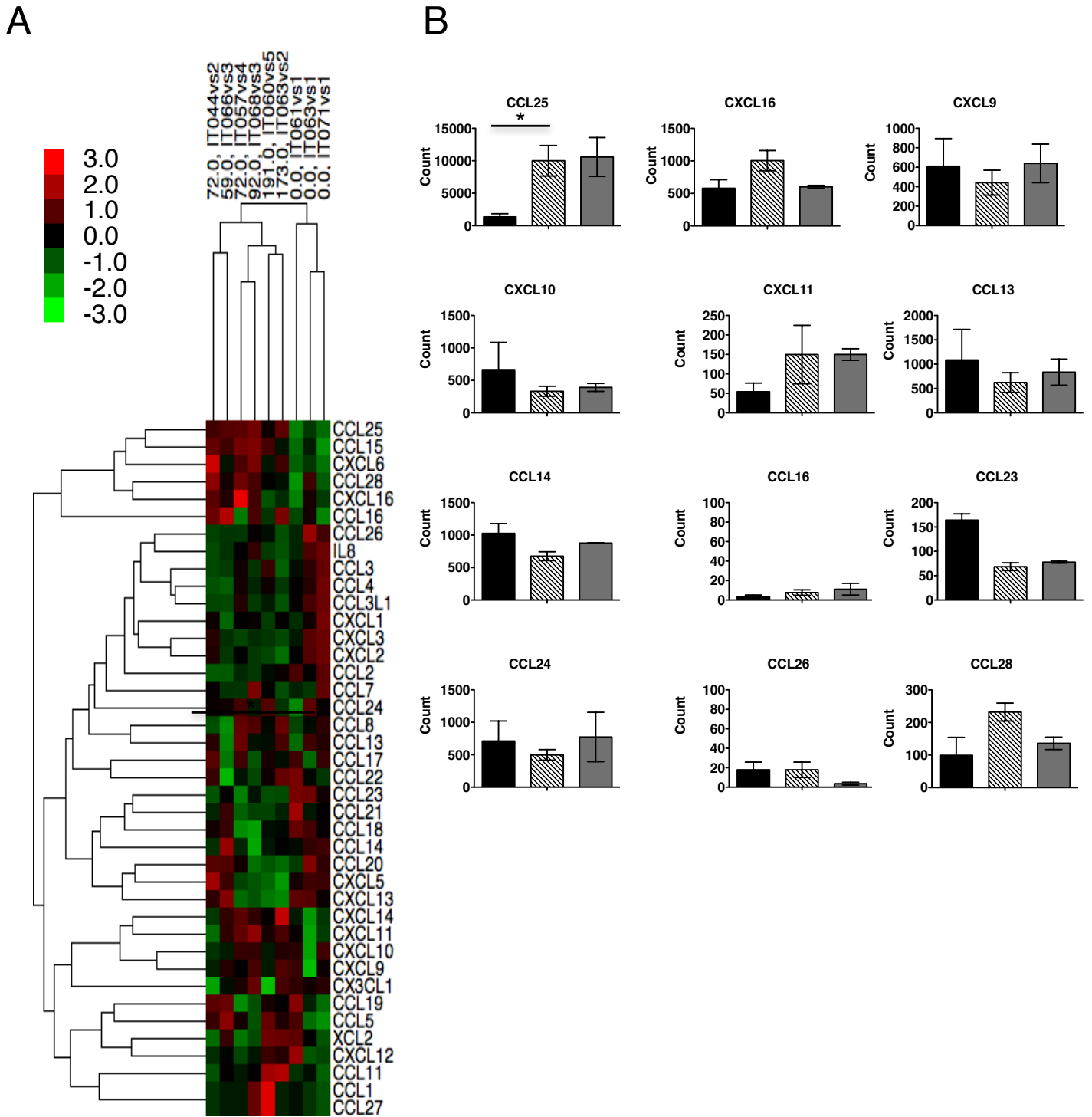
Supplementary Figure 4. Cells with MDSC phenotype differentiate from BMCs in culture medium supplemented with G-CSF, GM-CSF, IL-6, and MP.

BMCs were cultivated for 6 or 7 days in medium supplemented with G-CSF, GM-CSF, IL-6, and MP. Dot plots show representative MDSC phenotypes differentiated from BMCs. Dot plots show expression of HLA-DR and lineage in single and live mononuclear cells (the top dot plot) and CD33 and CD11b in lineage⁻HLA-DR⁻ cells (the lower left dot plot). The lower right dot plot shows expression of CD14 and CD15 in lineage⁻HLA-DR⁻CD33⁺CD11b⁺ cells. All three subsets of CD33⁺CD11b⁺ MDSC were detected (the lower right dot plot). Similar results were obtained in 5 independent experiments.



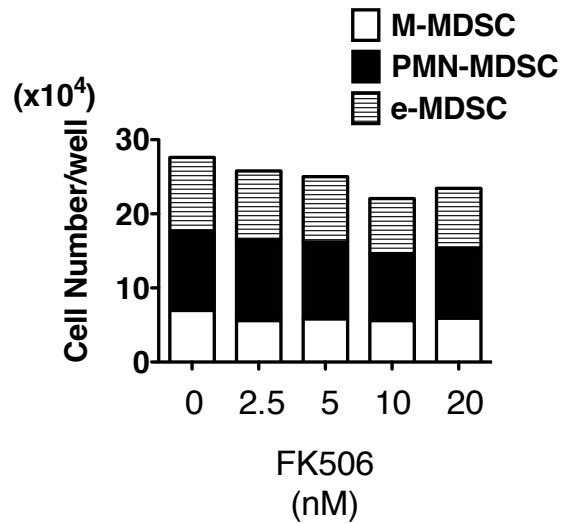
Supplementary Figure 5. Phenotype of MDSCs in LPC after ITx.

Mononuclear cells obtained in LPC were labeled with fluorescent-labelled antibodies and analyzed by flow cytometry. The cells were gated on an expanded lymphocyte and monocyte population based on FSC vs SSC (the left of top row dot plot), and doublet cells [FSC-A vs FSC-H (the second from the left of the top row), and FSC-H vs FSC-W (the third from the left of the top row)] and dead cells (FSC-A vs 7-aad; the right of the top row) were excluded. CD45⁺ cells were gated (the right of the second row), and then, lineage-HLA-DR⁻ (the middle of the second row) CD33⁺CD11b⁺ cells (the left of the second row) were defined as MDSCs. MDSCs were further classified as CD14⁻CD15⁻ (e-MDSCs), CD14⁺CD15⁻ (M-MDSCs), and CD14⁻CD15⁺ (PMN-MDSCs). Representative data from the LPC sample of no. IT052 v2 are shown.



Supplementary Figure 6. Heat-Map data for mRNA expression of chemokines in intestinal transplant grafts.

(A) The heat map shows color-coded expression levels of differentially expressed mRNA for indicated chemokine ligands using the NanoStrings® platform. The dendrogram for each sample shows similarity of the expression profiles, resulting in categorization as pre-transplant grafts and grafts 2-3 months and approximately 6 months after ITx. The dendrogram for each chemokine ligand shows similarity of profile for mRNA expression of chemokine ligands in the mucosa of intestinal grafts. (B) Bar graphs show the mean normalized counts of mRNA \pm SEM for the indicated chemokine ligands; mRNAs were extracted from pre-transplant grafts (black bar, $n = 3$), intestinal grafts at 3 months (striped bar, $n = 3$), and intestinal grafts at 6 months (gray bar, $n = 2$) after ITx. Statistical p values were calculated using one-way ANOVA with Bonferroni post hoc tests and are indicated in the graphs (* $p < 0.05$).



Supplementary Figure 7. FK506 does not affect MDSC differentiation from BMCs.

The bar graph shows numbers of M-MDSCs (white bars), PMN-MDSCs (black bars), and e-MDSCs (striped bars). BMCs were cultured for 8 days in culture medium supplemented with G-CSF and GM-CSF, IL-6, and/or various concentrations of FK506 as indicated under the X-axis of the bar graphs.