

Supplementary Materials for

Human skin-resident host T cells can persist long term after allogeneic stem cell transplantation and maintain recirculation potential

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The PDF file includes:

Figs. S1 to S10

Other Supplementary Material for this manuscript includes the following:

Data file S1
MDAR Reproducibility Checklist
List of Reagents

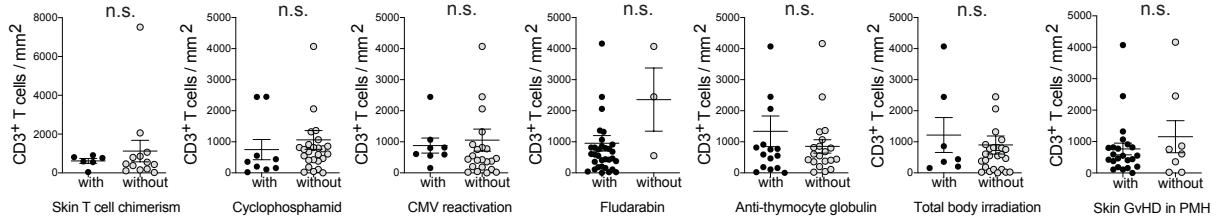


Figure S1. The impact of allo-HSCT associated treatments and complications on T cell density in the human skin. CD3^+ T cells from skin punch biopsies of allo-HSCT patients were enumerated by flow cytometry. Patients were stratified according to the presence (with) or absence (without) of the indicated treatment associated parameters.

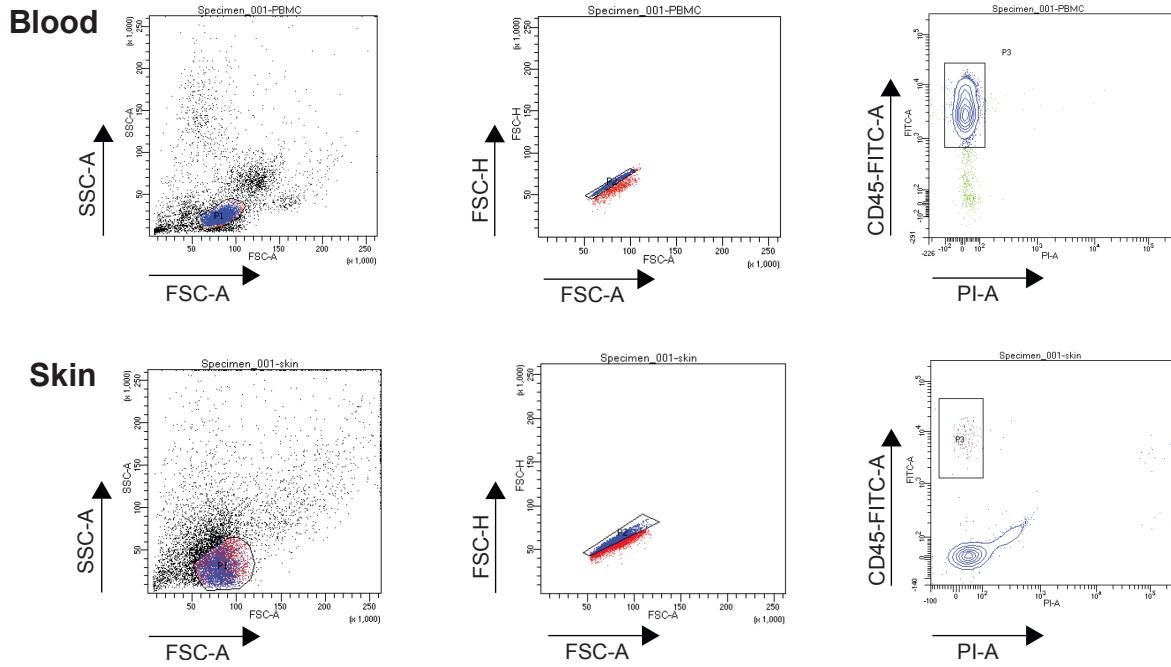


Figure S2. Sorting strategy for blood and skin lymphocytes after allo-HSCT. Shown is the sorting strategy that served lymphocyte isolation for further downstream analyses by scRNAseq and high-dimensional flow cytometry.

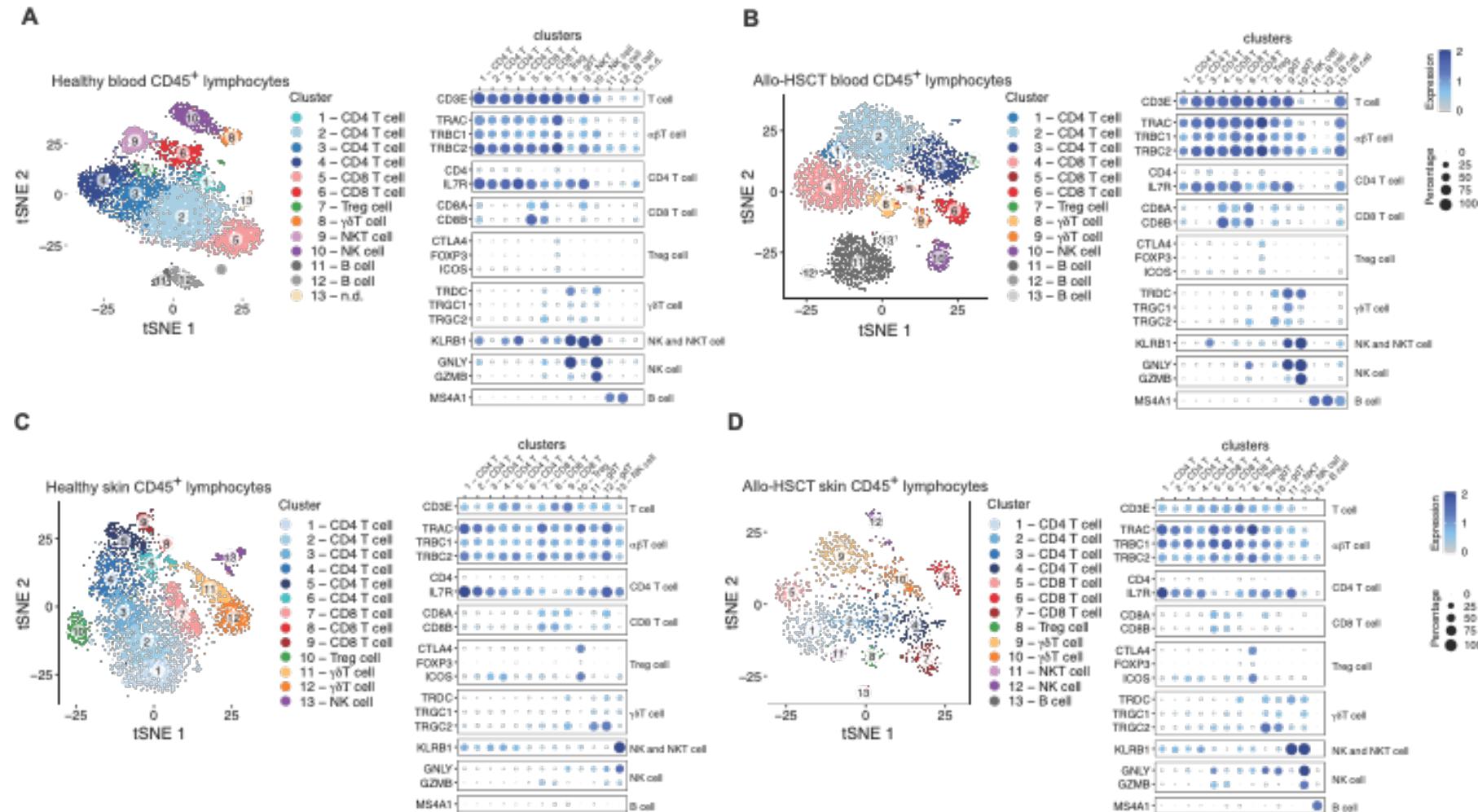


Figure S3. Cell type annotation and clustering of single-cell RNA sequencing data. (A-D, left panels) Louvain clusters depicted in the reduced space calculated by t-SNE. (A-D, right panels) The cluster annotation in the legend was performed based on the average cluster expression of cell type-specific marker genes. The circle size reflects the percentage of cells expressing the marker genes within the respective cluster.

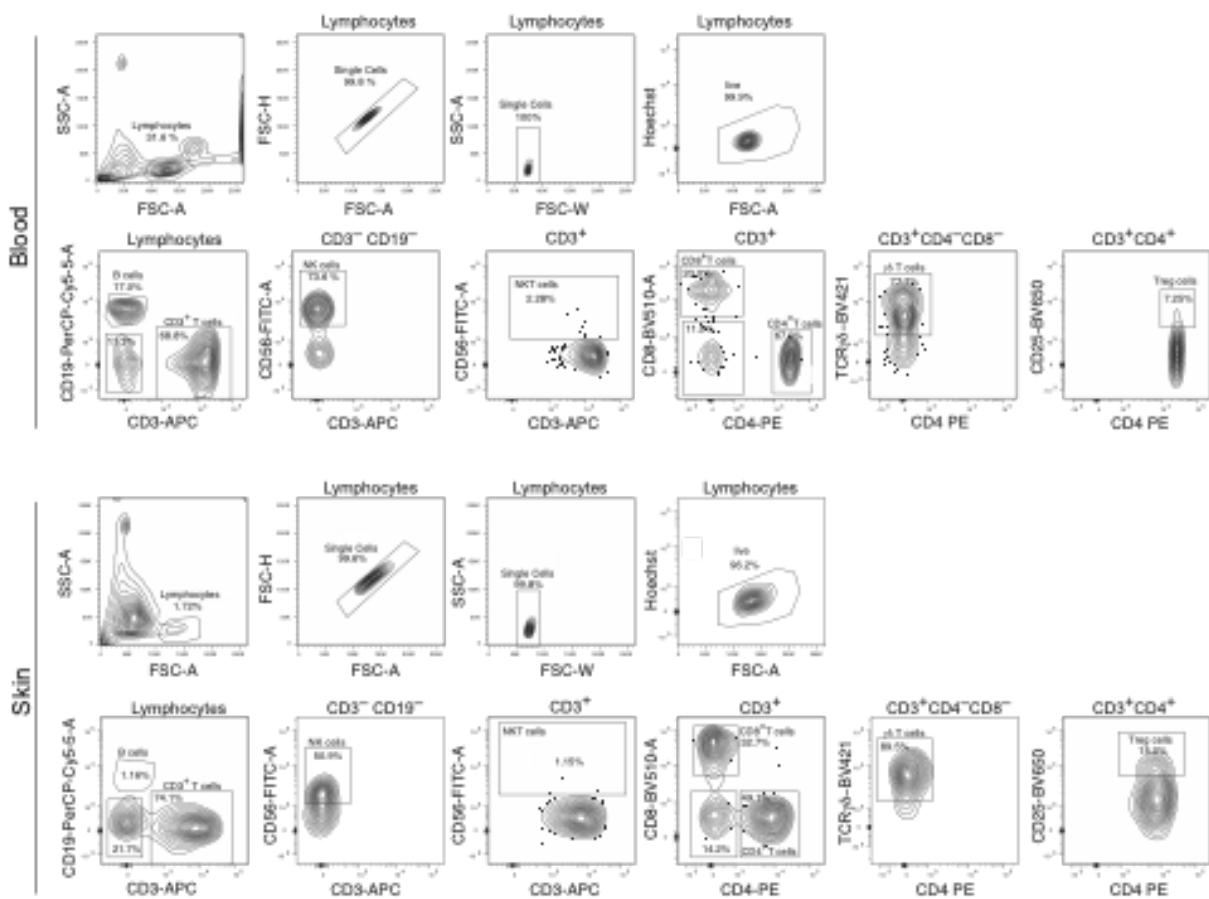


Figure S4. Gating strategy for the identification of immune cell subsets in skin and blood by flow cytometry. PBMC and cells from skin punch biopsies were stained with antibodies directed against lineage specific surface markers. Shown is the gating strategy for immune subset identification and quantification as summarized in Figure 1H.

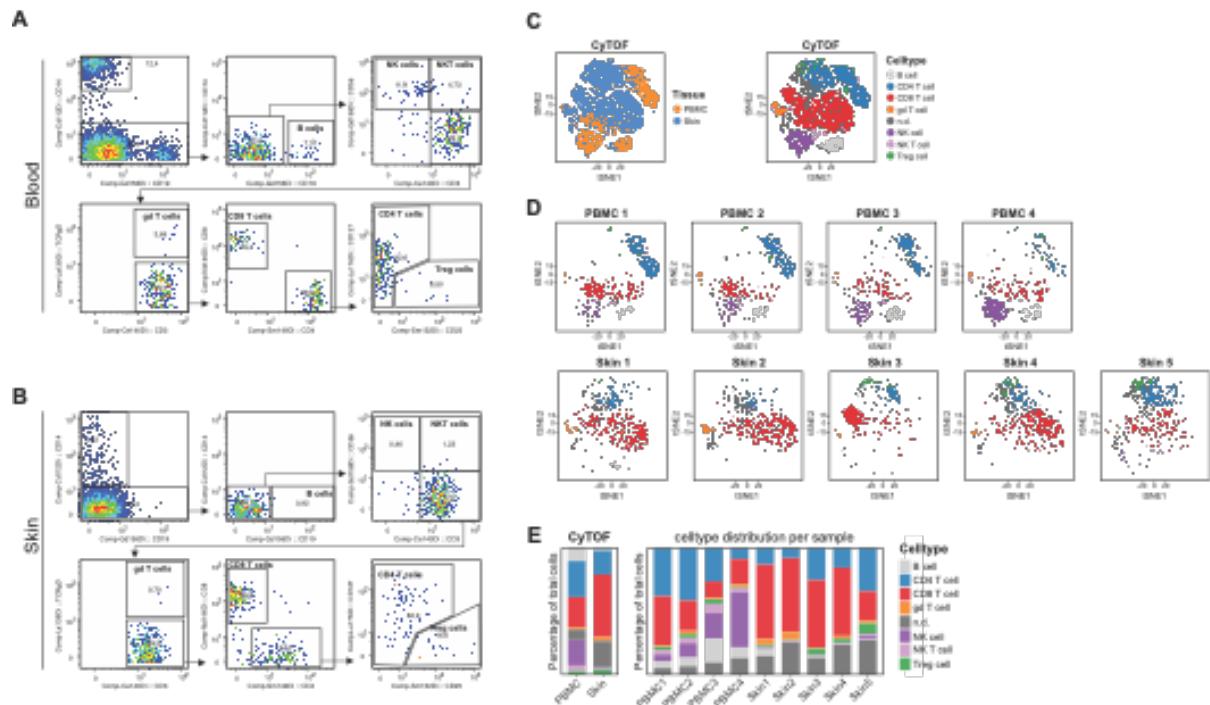


Figure S5. Cell type annotation by CyTOF analysis in healthy skin and blood lymphocytes. (A-B) CyTOF gating strategy for the blood (A) and skin (B). (C) t-SNE dimensionality reduction of normalized protein expression values of blood and skin samples from healthy donors measured by CyTOF (PBMC: n = 4, skin: n = 5). Each donor was downsampled to 11930 total cells. The different colors depict the tissue of origin (left) and the annotated cell type (right) as determined by gating in FlowJo. (D) t-SNE dimensionality reduction of normalized protein expression values measured from the blood and skin samples for each donor, as measured by CyTOF. The different colors depict the annotated cell type as determined by gating in FlowJo. (E) Distribution of cell types in the skin and blood of healthy donors annotated by manual gating in FlowJo on protein expression values measured by CyTOF. The left plot shows the average distribution of all donors while the right plot depicts the distribution of each donor. Raw data files were obtained from a public repository (Wong et al. *Immunity* 45, 442-456 (2016)).

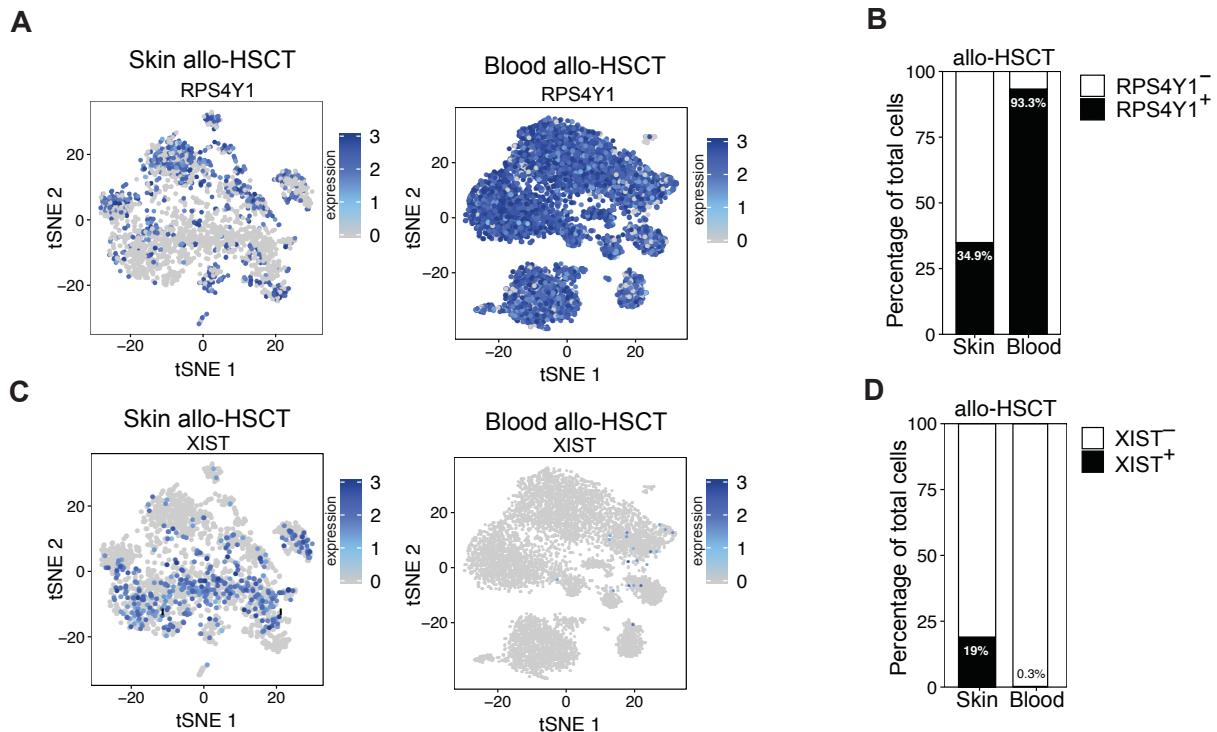


Figure S6. Evaluation of sex gene-associated transcripts in resident skin lymphocytes in the context of a sex-mismatched transplantation. (A) Normalized single-cell expression of the Y-linked gene *RPS4Y1* depicted in the reduced space calculated by t-SNE. (B) Distribution of cells according to the expression status of the Y-linked gene *RPS4Y1* in the scRNA-seq data. The number inside the bar indicates the percentage of *RPS4Y1*-expressing cells in the total analyzed lymphocyte population. (C, D) Lymphocytes were analyzed for gene expression of *XIST* as shown in A and B.

allo-HSCT skin

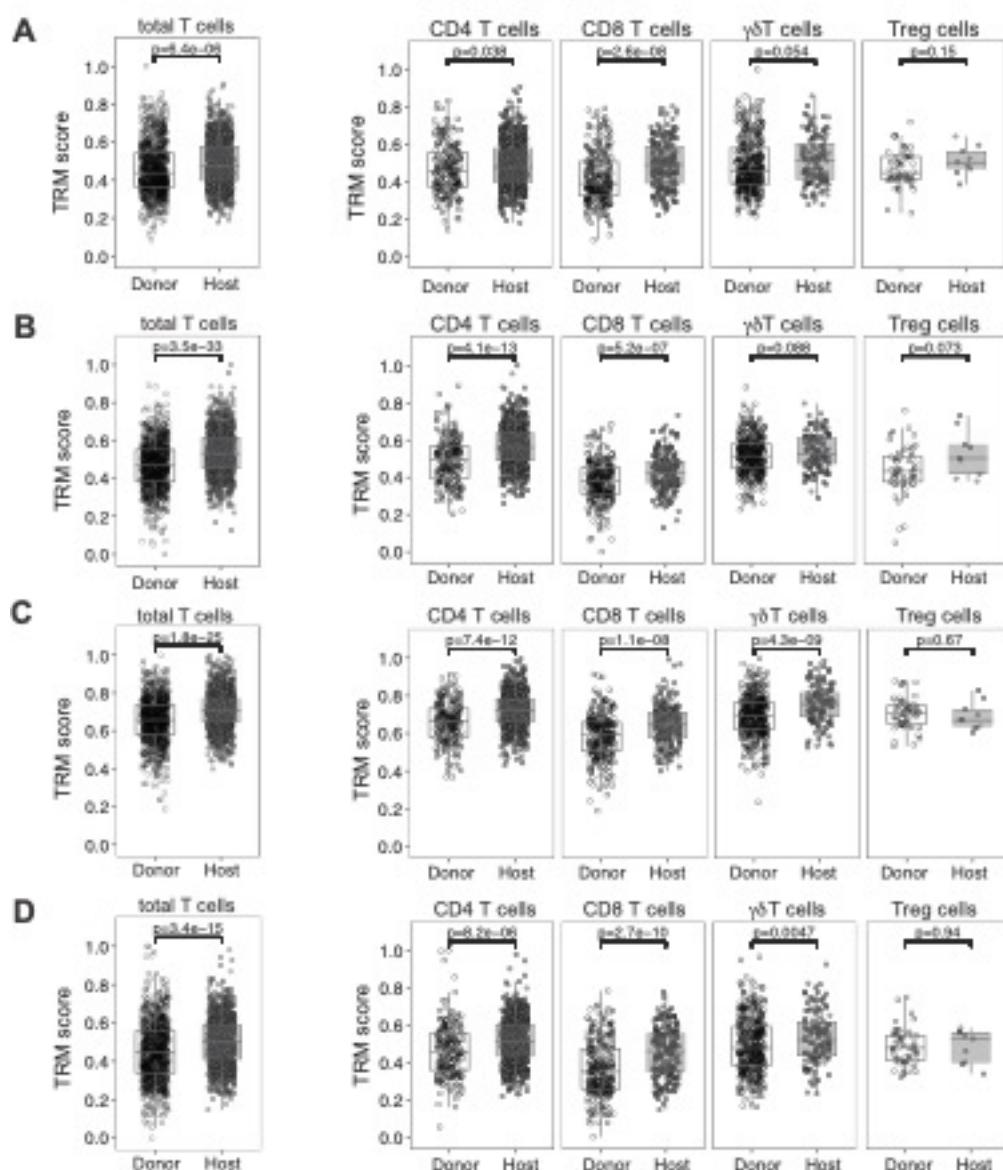


Figure S7. Differential enrichment of TRM signatures in distinct host versus donor skin lymphocyte subsets after allo-HSCT. (A-D). Total skin T cells (left) and their respective memory T cells subsets (right) from the skin were tested for enrichment of a T_{RM} signature that was derived from multiple previously published data sets (A, (31); B, (20); C, (18); D, (53)). Gene set expression scores for residency signatures were calculated for each single cell using the AddModuleScore function of the R package Seurat v3.0.0. To obtain the T_{RM} signature, scores were calculated for the downregulated genes (defined previously as the circulatory signature) and were subtracted from the scores calculated for the upregulated genes (defined previously as the residency signature) as described before (31). Two-tailed, unpaired Student's t-test.

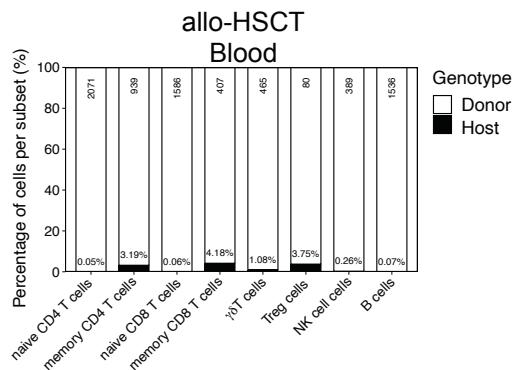


Figure S8. Differential distribution of host versus donor cells within blood immune cell subsets after allo-HSCT. Cell identity annotation by average cluster expression of cell-type specific markers and genotype distribution within each of the annotated cell subsets in the blood.

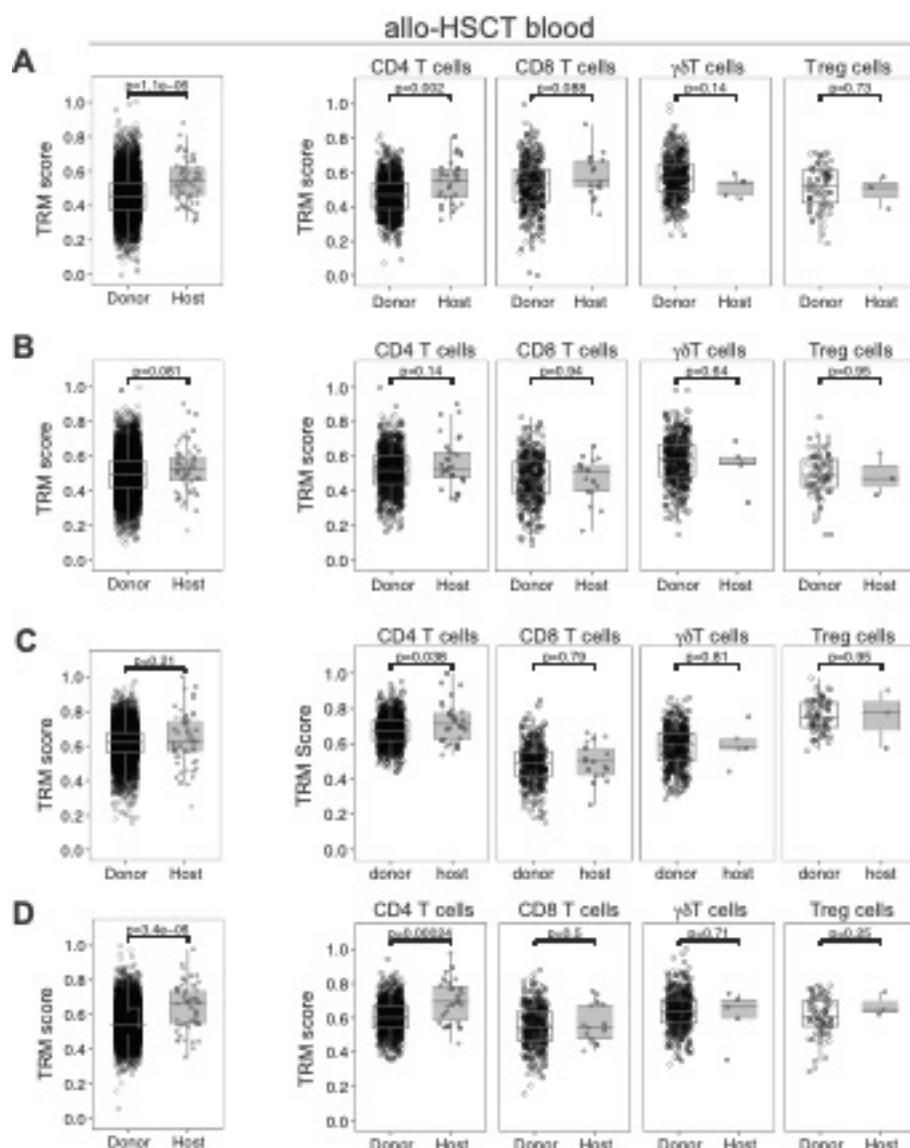


Figure S9. Differential enrichment of the TRM signature in distinct host versus donor blood lymphocyte subsets after allo-HSCT. (A-D) Total memory blood T cells (left) and their respective blood memory T cells subsets (right) were tested for enrichment of a T_{RM} signature that was derived from multiple previously published data sets (A, (31); B, (20); C, (18); D, (53)). The analysis was performed as in Fig.S7.

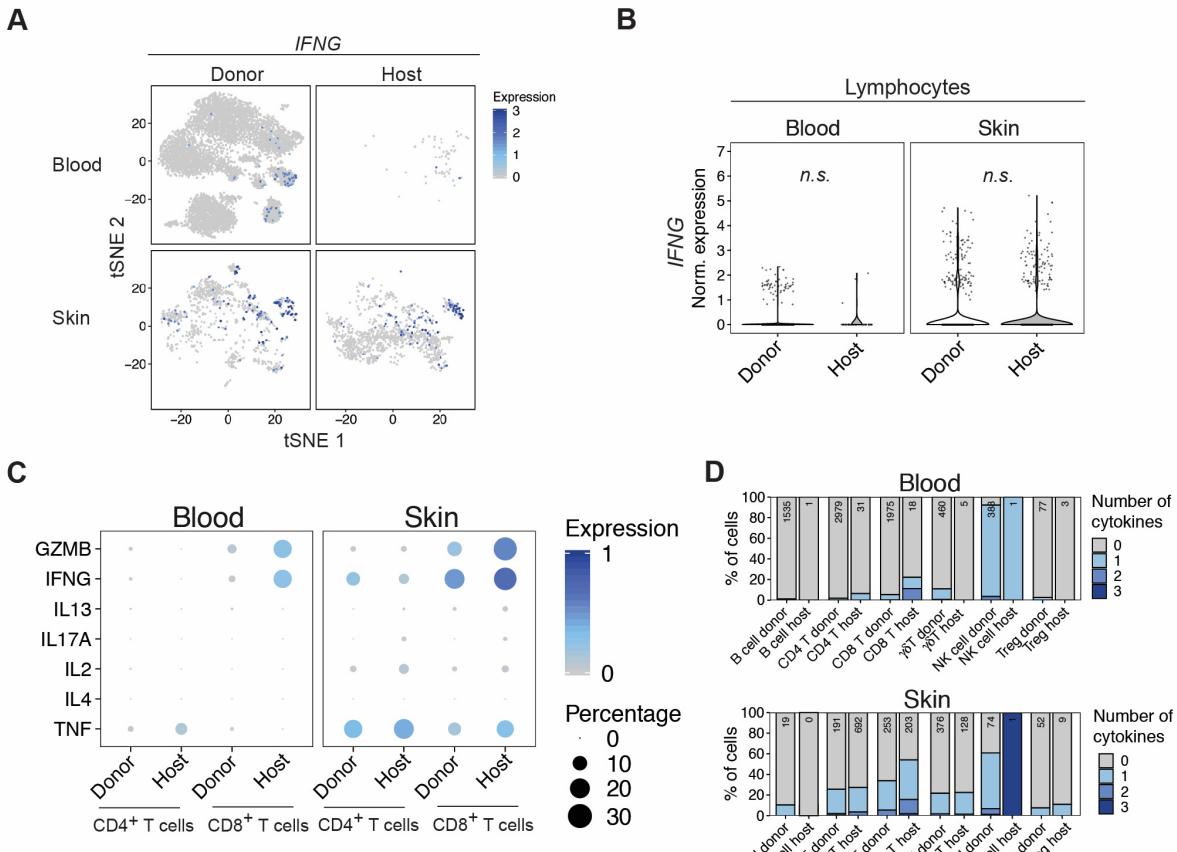


Figure S10. Differential expression of pro-inflammatory cytokines in host versus donor cells in the skin and blood after allo-HSCT. (A) Normalized single-cell gene expression of *IFNG* in all lymphocytes from the blood (top) and the skin (bottom) of the allo-HSCT patient (Patient ID 8), depicted for donor (left) and host (right) cells separately in the reduced space calculated by t-SNE. (B), normalized single-cell gene expression of *IFNG* shown as violin plots for host and donor lymphocytes from the blood (left) and the skin (right) of the allo-HSCT patient. Expression was compared between genotypes using the Wilcoxon Rank Sum test with Bonferroni correction of p-values for multiple testing. n.s., not significant. (C), Average gene expression and percentage of cells per genotype expressing different proinflammatory cytokines in CD4⁺ and CD8⁺ T cells from the blood (left) and the skin (right) after allo-HSCT. Comparison between genotypes with the Wilcoxon Rank Sum test with Bonferroni correction of p-values for multiple testing. not significant. (D), Distribution of cells expressing 1, 2, 3 or none of the cytokines shown in (C) in each of the cell types identified in blood (top) and skin (bottom) of the allo-HSCT patient. Numbers indicate number of cells per subset. A cytokine was considered to be expressed in a cell if there was at least one read count detected for the respective gene in that cell.