

## **Materials and methods**

### **Isolation of CD34<sup>+</sup> cells from patients with MDS-EB**

Three MDS-EB patients and three healthy donors (HDs) were enrolled. The study was performed in accordance with the Declaration of Helsinki and was approved by the local institutional review board. All patients and HDs provided written informed consent. For flow cytometry sorting of CD34<sup>+</sup> cells, bone marrow mononuclear cells obtained from three MDS-EB patients and three HDs were incubated (15 minutes) with the monoclonal antibodies CD45 FITC/CD34 PE (341071; BD Biosciences, San Jose, CA). Sorting of the CD34<sup>+</sup> cells was then conducted on a BD FACS Aria II cell sorter (BD Biosciences), aiming for 15,000 to 20,000 CD34<sup>+</sup> cells and a purity of >90%.

### **ScAb-seq surface labeling, single-cell capture and library preparation.**

ScAb-seq surface antibody libraries were pipetted 24 h before the experiments. For most antibodies, 1  $\mu$ l was used for surface library preparation. Antibodies recognizing epitopes with well-known high surface expression were further diluted in PBS, and 1  $\mu$ l was added to the surface library (for example, CD7, CD11b, CD22, CD33, CD34, CD44, CD45, CD45RA, CD56, CD123, CD366 or CD371). (1, 2) Sorted cells (approximately  $1.2 \times 10^5$ – $1.4 \times 10^5$ ; described in the “Cell sorting for scAb-seq” section) were centrifuged for 5 min at  $350 \times g$  and resuspended in the surface library mixture. In cases where different biological samples or sorted populations were combined in the same run, the sorted cells were labeled individually with oligonucleotide-coupled cell hashing antibodies (BD single-cell multiplexing kit) for 25 min on ice, washed three times, each followed by 5 min of centrifugation at  $350 \times g$ , pooled and then subjected to scAb-seq cell surface labeling. The cells were then labeled for 30 min at 4 °C and washed three times, each followed by 5 min of centrifugation at  $350 \times g$ . The cells were resuspended in sample buffer (BD Rhapsody Cartridge reagent kit), and between  $1 \times 10^4$  and  $2 \times 10^4$  cells were captured with a BD Rhapsody single-cell system following the manufacturer’s instructions. Antibody tag libraries, multiplex libraries and targeted mRNA gene expression libraries were generated following the manufacturer’s instructions. For the mRNA libraries, the targeted panel or the whole-transcriptome analysis library preparation protocol was used according to the manufacturer’s instructions (BD). The resulting libraries were quality checked by a Qubit and Bioanalyzer, pooled and sequenced using NextSeq500 or Illumina NovaSeq S2 (Illumina; high-output mode).

### **ScRNA-seq and scAb-seq data analysis**

The raw FASTQ files were processed using the BD Rhapsody Sequence Analysis Pipeline for mapping.(3, 4) The processed data were converted into a matrix format. We used Seurat (V4.3.0.1) in R (V4.2.3) for quality control of postalignment data to remove low-quality cells and genes.(5) The quality control metrics included the total UMI count (nCount\_RNA), total number of detected genes (nFeature\_RNA),

mitochondrial gene percentage (percent\_mt), and cell complexity. For both the experimental and validation cohorts, we removed cells whose nCount\_RNA was less than 500 and whose nFeature\_RNA was less than 200. The cells whose percent\_mt was greater than 25% were also removed. Additionally, we filtered out genes with zero counts in the data matrix and genes expressed in fewer than five cells. Subsequently, downstream analysis was performed using the Seurat package. To analyze the scAb-seq data, we extracted the antibody-specific labels. The data underwent standard normalization and scaling. On the basis of the flow cytometry results, we selected specific subsets of cells. The scRNA-seq and scAb-seq data were then integrated using cell labels. The integrated data were analyzed using the Seurat package. The key steps included data normalization, identification of highly variable features, scaling, and dimensionality reduction via PCA. Neighbors were identified, and cells were clustered on the basis of transcriptomic similarities. UMAP plots were generated to visualize cell clusters and groupings, highlighting specific markers (e.g., CD38, CD33, CD45RA, and CD123) to assess their expression patterns across different cell populations.(6)

### **Differential Gene Analysis and Visualization**

We used the "FindAllMarkers" function in the Seurat package to identify signature genes in each cell subpopulation by comparing gene expression patterns. To further analyze differentially expressed genes between groups, we employed the "FindMarkers" function. To control the false-positive rate, we applied the Bonferroni correction method to adjust p values, considering an adjusted p value ( $p\_val\_adj < 0.05$ ) as statistically significant.

For visualization, we generated bubble plots using R and the ggplot2 package. These plots display gene expression differences between cell subpopulations or groups, with each point representing a gene. The position of the point indicates the gene's identity, its size reflects the percentage of gene expression (pct.exp), and its color represents the average expression value (avg.exp.scaled).

### **Gene Set Variation Analysis**

In this study, we performed gene set enrichment analysis (GSEA) via the "gsva()" function of the GSVA R package. To obtain high-quality reference gene sets, we utilized the R package msigdb, which provides an interface to the Molecular Signatures Database (MSigDB) website [www.gsea-msigdb.org](http://www.gsea-msigdb.org). This allowed us to conveniently download and import gene sets from the database. MsigDB is a widely used public database that contains a large number of gene sets related to different biological processes and disease states.

### **GO and Pathway Enrichment Analysis and Visualization**

For GO enrichment analysis, we used the clusterProfiler package in R. During the analysis, we employed a statistical test based on the hypergeometric distribution and

compared p values to ensure the accuracy of the results. To further explore the roles of signature genes in specific biological pathways, we conducted pathway enrichment analysis using the pathview package. Like in the GO enrichment analysis, we also compared the p values of the pathway enrichment analysis to ensure the accuracy of the results.

### **Transplant procedures**

Allografts were generated according to previous studies.(7-9) Positive MRD intervention and relapse treatment were performed as previously described.(7, 10-12)

### **Definitions and evaluation**

Engraftment, infection, nonrelapse mortality (NRM), relapse, disease-free survival (DFS), and overall survival (OS) were defined according to previous studies.(7, 8) The definition and grade of acute graft-versus-host disease (GVHD) are based on the pattern and severity of organ involvement.(8, 13) The definition and grades of chronic GVHD are in accordance with the National Institute of Health criteria.(14)

### **End-points**

The primary study endpoint was the CIR.(7) The secondary endpoints were the engraftment rate; acute GVHD grades II-IV and chronic GVHD; and NRM, OS, and DFS.(8, 13) To determine whether there was any difference in relapse between the posttransplant MRD-positive and MRD-negative groups, the cumulative incidence approach was used with a one-sided confidence interval (CI) for the difference in the Kaplan–Meier estimate of the 1-year CIR. With a planned sample size of 143 MDS-EB patients, 80% power can be achieved against the hypothesis of CIRs of 23% and 1.6% for patients in the posttransplant MRD-positive and MRD-negative groups, respectively, at a significance level of  $P=0.05$  in the one-tailed Student's t test.

### **References**

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## Figure legends.

### Figure S1. Enriched differentially expressed genes and EMP1 regulatory network in MDS-EB\_CD34<sup>+</sup>CD38<sup>-</sup> cells.

**a-b.** Gene set enrichment analysis (GSEA) comparing HD\_CD34<sup>+</sup>CD38<sup>-</sup> cells and MDS-EB\_CD34<sup>+</sup>CD38<sup>-</sup> cells, highlighting pathways that are highly enriched in MDS-EB\_CD34<sup>+</sup>CD38<sup>-</sup> cells.

**Abbreviations:** MDS-EB=myelodysplastic syndrome with excess blasts; HD=healthy donor

### Figure S2. Enriched differentially expressed genes and EMP1 regulatory network in MDS-EB CD34<sup>+</sup>CD38<sup>-</sup> cells.

**a.** Enrichment of differentially expressed genes between MDS-EB\_CD34<sup>+</sup>CD38<sup>-</sup> cells and HD\_CD34<sup>+</sup>CD38<sup>-</sup> cells, with the red section showing genes upregulated in MDS-EB\_CD34<sup>+</sup>CD38<sup>-</sup> cells and the blue section showing genes downregulated in MDS-EB\_CD34<sup>+</sup>CD38<sup>-</sup> cells.

**b.** The EMP1 gene regulatory network is present in MDS-EB\_CD34<sup>+</sup>CD38<sup>-</sup> cells but absent in HD\_CD34<sup>+</sup>CD38<sup>-</sup> cells.

**Abbreviations:** MDS-EB=myelodysplastic syndrome with excess blasts; HD=healthy donor

### Figure S3. Greater stemness of CD34<sup>+</sup>CD38<sup>-</sup>CD33<sup>+</sup> cells in patients with MDS-EB than that of CD34<sup>+</sup>CD38<sup>-</sup> cells in HDs.

**a.** Differential expression of genes between CD34<sup>+</sup>CD38<sup>-</sup>CD33<sup>+</sup> cells from patients with MDS-EB and CD34<sup>+</sup>CD38<sup>-</sup> cells from HDs.

**b.** Enrichment of differentially expressed genes between CD34<sup>+</sup>CD38<sup>-</sup>CD33<sup>+</sup> cells from patients with MDS-EB and CD34<sup>+</sup>CD38<sup>-</sup> cells from HDs, with the red section showing genes upregulated in CD34<sup>+</sup>CD38<sup>-</sup>CD33<sup>+</sup> cells and the blue section showing genes downregulated in CD34<sup>+</sup>CD38<sup>-</sup>CD33<sup>+</sup> cells.

**c-d.** Gene set enrichment analysis (GSEA) comparing CD34<sup>+</sup>CD38<sup>-</sup>CD33<sup>+</sup> cells of patients with MDS-EB with CD34<sup>+</sup>CD38<sup>-</sup> cells of HDs, highlighting pathways that are highly enriched in CD34<sup>+</sup>CD38<sup>-</sup>CD33<sup>+</sup> cells.

**Abbreviations:** MDS-EB=myelodysplastic syndrome with excess blasts; HD=healthy donor

### Figure S4. Stronger stemness of CD34<sup>+</sup>CD38<sup>-</sup>CD45RA<sup>+</sup> cells in patients with MDS-EB than that of CD34<sup>+</sup>CD38<sup>-</sup> cells in HDs.

**a.** Differential expression of genes between CD34<sup>+</sup>CD38<sup>-</sup>CD45RA<sup>+</sup> cells from patients with MDS-EB and CD34<sup>+</sup>CD38<sup>-</sup> cells from HDs.

**b.** Enrichment of DEGs between CD34<sup>+</sup>CD38<sup>-</sup>CD45RA<sup>+</sup> cells from patients with MDS-EB and CD34<sup>+</sup>CD38<sup>-</sup> cells from HDs, with the red section showing genes upregulated in CD34<sup>+</sup>CD38<sup>-</sup>CD45RA<sup>+</sup> cells and the blue section showing genes downregulated in CD34<sup>+</sup>CD38<sup>-</sup>CD45RA<sup>+</sup>

cells.

**c-d.** Gene set enrichment analysis (GSEA) comparing CD34<sup>+</sup>CD38<sup>-</sup>CD45RA<sup>+</sup> cells of patients with MDS-EB with CD34<sup>+</sup>CD38<sup>-</sup> cells of HDs, highlighting pathways that are highly enriched in CD34<sup>+</sup>CD38<sup>-</sup>CD45RA<sup>+</sup> cells.

**Abbreviations:** MDS-EB=myelodysplastic syndrome with excess blasts; HD=healthy donor

**Figure S5. Greater stemness of CD34<sup>+</sup>CD38<sup>-</sup>CD123<sup>+</sup> cells in patients with MDS-EB than that of CD34<sup>+</sup>CD38<sup>-</sup> cells in HDs.**

**a.** Differential expression of genes between CD34<sup>+</sup>CD38<sup>-</sup>CD123<sup>+</sup> cells from patients with MDS-EB and CD34<sup>+</sup>CD38<sup>-</sup> cells from HDs.

**b.** Enrichment of differentially expressed genes between CD34<sup>+</sup>CD38<sup>-</sup>CD123<sup>+</sup> cells from patients with MDS-EB and CD34<sup>+</sup>CD38<sup>-</sup> cells from HDs, with the red section showing genes upregulated in CD34<sup>+</sup>CD38<sup>-</sup>CD123<sup>+</sup> cells and the blue section showing genes downregulated in CD34<sup>+</sup>CD38<sup>-</sup>CD123<sup>+</sup> cells.

**c-d.** Gene set enrichment analysis (GSEA) comparing CD34<sup>+</sup>CD38<sup>-</sup>CD123<sup>+</sup> cells of patients with MDS-EB with CD34<sup>+</sup>CD38<sup>-</sup> cells of HDs, highlighting pathways that are highly enriched in CD34<sup>+</sup>CD38<sup>-</sup>CD123<sup>+</sup> cells.

**Abbreviations:** MDS-EB=myelodysplastic syndrome with excess blasts; HD=healthy donor

**Figure S6. Stronger stemness of CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells in patients with MDS-EB than that of CD34<sup>+</sup>CD38<sup>-</sup> cells in HDs.**

**a.** Differential expression of genes between CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells from patients with MDS-EB and CD34<sup>+</sup>CD38<sup>-</sup> cells from HDs.

**b.** Enrichment of differentially expressed genes between CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells from patients with MDS-EB and CD34<sup>+</sup>CD38<sup>-</sup> cells from HDs, with the red section showing genes upregulated in CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells and the blue section showing genes downregulated in CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells.

**c-d.** Gene set enrichment analysis (GSEA) comparing CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells from patients with MDS-EB with CD34<sup>+</sup>CD38<sup>-</sup> cells from HDs, highlighting pathways that are highly enriched in CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells.

**Abbreviations:** MDS-EB=myelodysplastic syndrome with excess blasts; HD=healthy donor

**Figure S7. The stemness of CD34<sup>+</sup>CD38<sup>-</sup> cell subsets in patients with MDS-EB.**

**a.** Venn diagram illustrating the intersection of genes expressed by CD34<sup>+</sup>CD38<sup>-</sup>Cocktail<sup>+</sup> cells, CD34<sup>+</sup>CD38<sup>-</sup>CD45RA<sup>+</sup> cells, CD34<sup>+</sup>CD38<sup>-</sup>CD33<sup>+</sup> cells, and CD34<sup>+</sup>CD38<sup>-</sup>CD123<sup>+</sup> cells.

**b.** Pathways commonly enriched across the four types of CD34<sup>+</sup>CD38<sup>-</sup> cells, including CD34<sup>+</sup>CD38<sup>-</sup>Cocktail<sup>+</sup> cells, CD34<sup>+</sup>CD38<sup>-</sup>CD45RA<sup>+</sup> cells, CD34<sup>+</sup>CD38<sup>-</sup>CD33<sup>+</sup> cells, and CD34<sup>+</sup>CD38<sup>-</sup>CD123<sup>+</sup> cells.

**Abbreviations:** MDS-EB=myelodysplastic syndrome with excess blasts

**Figure S8. Greater stemness of CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells than that of CD34<sup>+</sup>CD38<sup>-</sup>CD45RA<sup>+</sup> cells in patients with MDS-EB.**

- a. Differential expression of key genes between CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells and CD34<sup>+</sup>CD38<sup>-</sup>CD45RA<sup>+</sup> cells.
- b. Enrichment of differentially expressed genes between CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells and CD34<sup>+</sup>CD38<sup>-</sup>CD45RA<sup>+</sup> cells, with the plot showing genes upregulated in CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells.
- c. Gene set enrichment analysis (GSEA) comparing CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells with CD34<sup>+</sup>CD38<sup>-</sup>CD45RA<sup>+</sup> cells, highlighting pathways that are highly enriched in CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells.

**Abbreviations:** MDS-EB=myelodysplastic syndrome with excess blasts

**Figure S9. Greater stemness of CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells than that of CD34<sup>+</sup>CD38<sup>-</sup>CD123<sup>+</sup> cells in patients with MDS-EB.**

- a. Differential expression of key genes between CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells and CD34<sup>+</sup>CD38<sup>-</sup>CD123<sup>+</sup> cells.
- b. Enrichment of differentially expressed genes between CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells and CD34<sup>+</sup>CD38<sup>-</sup>CD123<sup>+</sup> cells, with the red section showing genes upregulated in CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells and the blue section showing genes downregulated in CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells.
- c-d. Gene set enrichment analysis (GSEA) comparing CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells with CD34<sup>+</sup>CD38<sup>-</sup>CD123<sup>+</sup> cells, highlighting pathways that are highly enriched in CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells.

**Abbreviations:** MDS-EB=myelodysplastic syndrome with excess blasts

**Figure S10. Representative flow cytometry plots showing gating strategy of MDS-SC. a.** Healthy control, **b.** newly diagnosed MDS-EB patient, **c.** MDS-EB patient with positive posttransplantation MRD, **d.** relapsed MDS-EB patient.

**Abbreviations:** MDS-SC=myelodysplastic syndrome stem cell; MDS-EB=myelodysplastic syndrome with excess blasts; MRD=measurable residual disease

**Figure S11. Association of CD34<sup>+</sup>CD38<sup>-</sup>Cocktail<sup>+</sup> cells with relapse in a representative patient.**

- a. UMAP distribution plot of three HDs and three MDS-EB cell lines.
- b. UMAP distribution plot of CD34<sup>+</sup>CD38<sup>-</sup> cell subsets from three MDS-EB patients.
- c. UMAP distribution plot of the four subsets of CD34<sup>+</sup>CD38<sup>-</sup> cells, including CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells, CD34<sup>+</sup>CD38<sup>-</sup>CD45RA<sup>+</sup> cells, CD34<sup>+</sup>CD38<sup>-</sup>CD33<sup>+</sup> cells, and CD34<sup>+</sup>CD38<sup>-</sup>CD123<sup>+</sup> cells.
- d. Kinetics of the percentages of CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> cells in the CD34<sup>+</sup> cell population of patient three from pretransplantation to relapse.
- e. Morphological characteristics of the bone marrow at the time of relapse in patient three.

**Abbreviations:** MDS-EB=myelodysplastic syndrome with excess blasts; HD=healthy donor

## **Figure S12. Screening and enrollment**

**Figure S13. Relationship between pretransplantation tumor burden determined by the traditional MFC MRD method and the MDS-SC-based assay and transplant outcomes for patients with MDS-EB who underwent allo-SCT (n=143).** Kaplan–Meier estimates of relapse and DFS according to pretransplantation tumor burden detected by the traditional MFC MRD method (a-b), pretransplantation CD34<sup>+</sup>CD38<sup>-</sup>CD123<sup>+</sup> MDS-SCs (c-d), pretransplantation CD34<sup>+</sup>CD38<sup>-</sup>CD33<sup>+</sup> MDS-SCs (e-f) and pretransplantation CD34<sup>+</sup>CD38<sup>-</sup>CD45RA<sup>+</sup> MDS-SCs (g-h).

**Abbreviations:** MFC=multiparameter flow cytometry; MRD=measurable residual disease; MDS-SC=myelodysplastic syndrome stem cell; MDS-EB=myelodysplastic syndrome with excess blasts; allo-SCT=allogeneic stem cell transplantation; DFS=disease-free survival.

**Figure S14. Relationships between posttransplantation residual disease determined by the traditional MFC MRD method or the MDS-SC-based MRD assay and transplant outcomes for patients with MDS-EB who underwent allo-SCT (n=143).** Kaplan–Meier estimates of relapse and DFS, respectively, according to the pretransplantation residual detected by the traditional MFC MRD method (a-b) as well as the pretransplantation CD34<sup>+</sup>CD38<sup>-</sup>CD123<sup>+</sup> MDS-SCs (c-d), pretransplantation CD34<sup>+</sup>CD38<sup>-</sup>CD33<sup>+</sup> MDS-SCs (e-f) and pretransplantation CD34<sup>+</sup>CD38<sup>-</sup>CD45RA<sup>+</sup> MDS-SCs (g-h) the peri-transplantation kinetics of CD34<sup>+</sup>CD38<sup>-</sup> cocktail<sup>+</sup> MDS-SCs (i-l) detected by the MDS-SC-based MRD assay. Groups E, F1, F2, and G indicate patients with low MDS-SC burdens before SCT and negative MDS-SC-based MRD after SCT, high MDS-SC burdens before SCT and negative MDS-SC-based MRD after SCT, low MDS-SC burdens before SCT and positive MDS-SC-based MRD after SCT as well as high MDS-SC burdens before SCT and positive MDS-SC-based MRD after SCT, respectively, according to CD34<sup>+</sup>CD38<sup>-</sup> cocktail<sup>+</sup> MDS-SCs determined by MFC. Groups E, F and G indicate patients with low MDS-SC burdens before SCT and negative MDS-SC-based MRD after SCT, high MDS-SC burdens before SCT or positive MDS-SC-based MRD after SCT, as well as high MDS-SC burdens before SCT and positive MDS-SC-based MRD after SCT, respectively, according to CD34<sup>+</sup>CD38<sup>-</sup> cocktail<sup>+</sup> MDS-SCs determined by MFC.

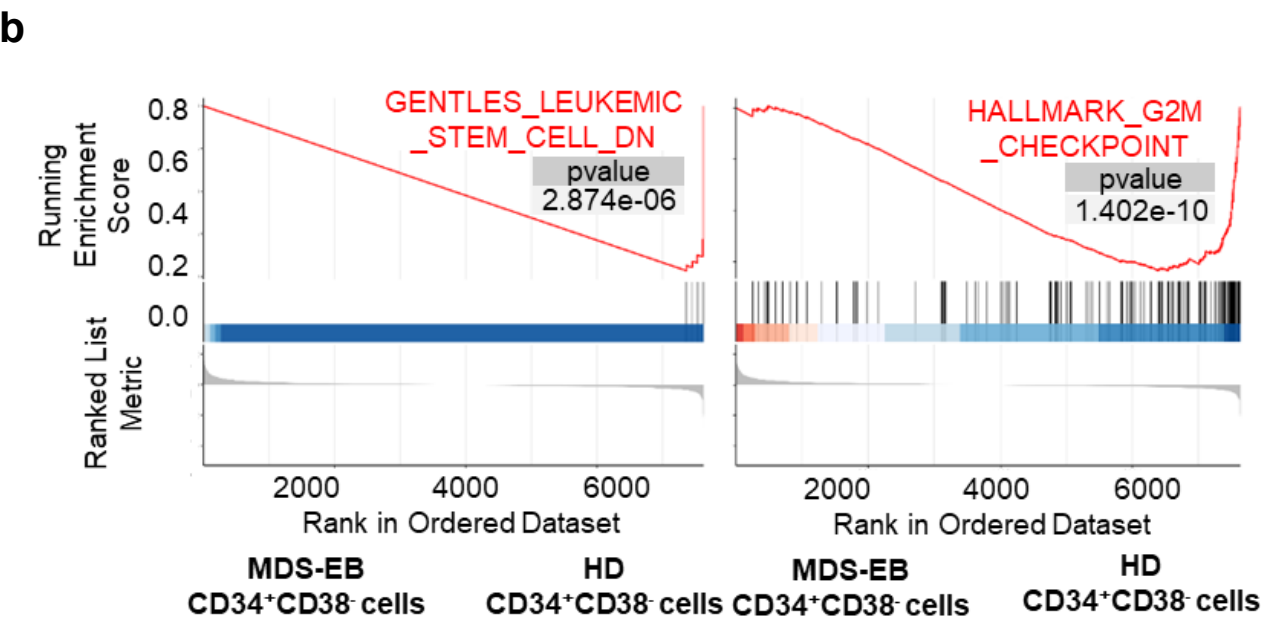
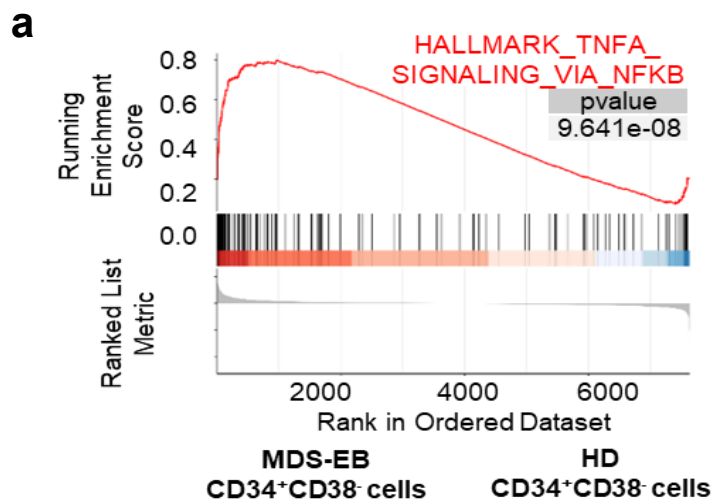
**Abbreviations:** MFC=multiparameter flow cytometry; MRD=measurable residual disease; MDS-SC=myelodysplastic syndrome stem cell; MDS-EB=myelodysplastic syndrome with excess blasts; allo-SCT=allogeneic stem cell transplantation; DFS=disease-free survival.

**Figure S15. Model performance evaluation of pre-MDS-SC, pre-transplantation tumor burden, post-MRD and post-MDS-SC detected by MFC in relapse prediction.**

- a. Decision curves comparing net benefit of pre-transplantation tumor burden and pre-transplantation MDS-SCs.
- b. Decision curves comparing net benefit of post-transplantation traditional MRD and post-transplantation MDS-SCs.
- c. Calibration plots of pre-transplantation tumor burden and pre-transplantation MDS-SCs.
- d. Calibration plots of post-transplantation traditional MRD and post-transplantation MDS-SCs.

**Abbreviations:** MDS-EB=myelodysplastic syndrome with excess blasts; allo-SCT=allogeneic stem cell transplantation; MDS-SC=myelodysplastic syndrome stem cell; MFC=multiparameter flow cytometry.

Figure S1



**Figure S2**

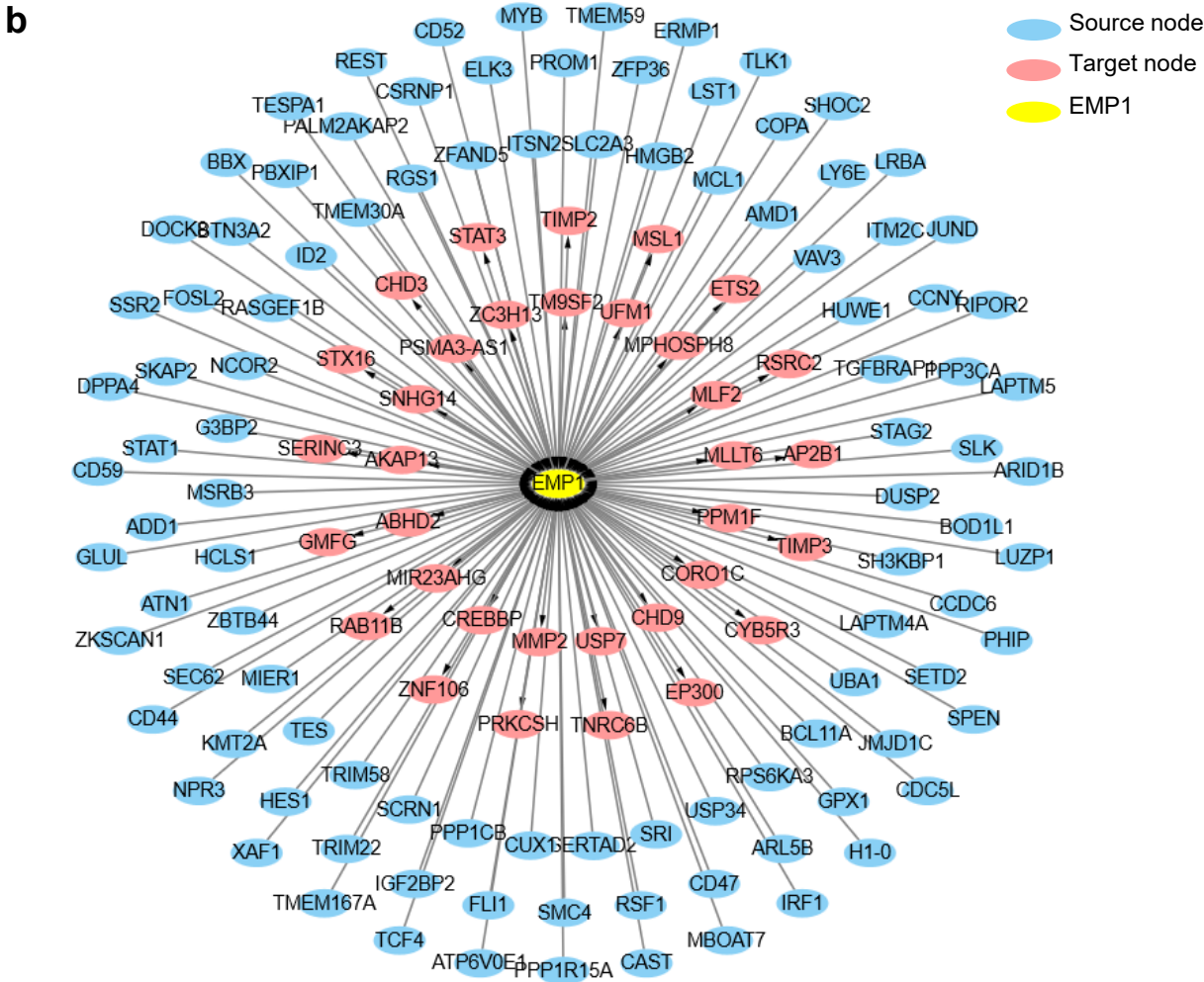
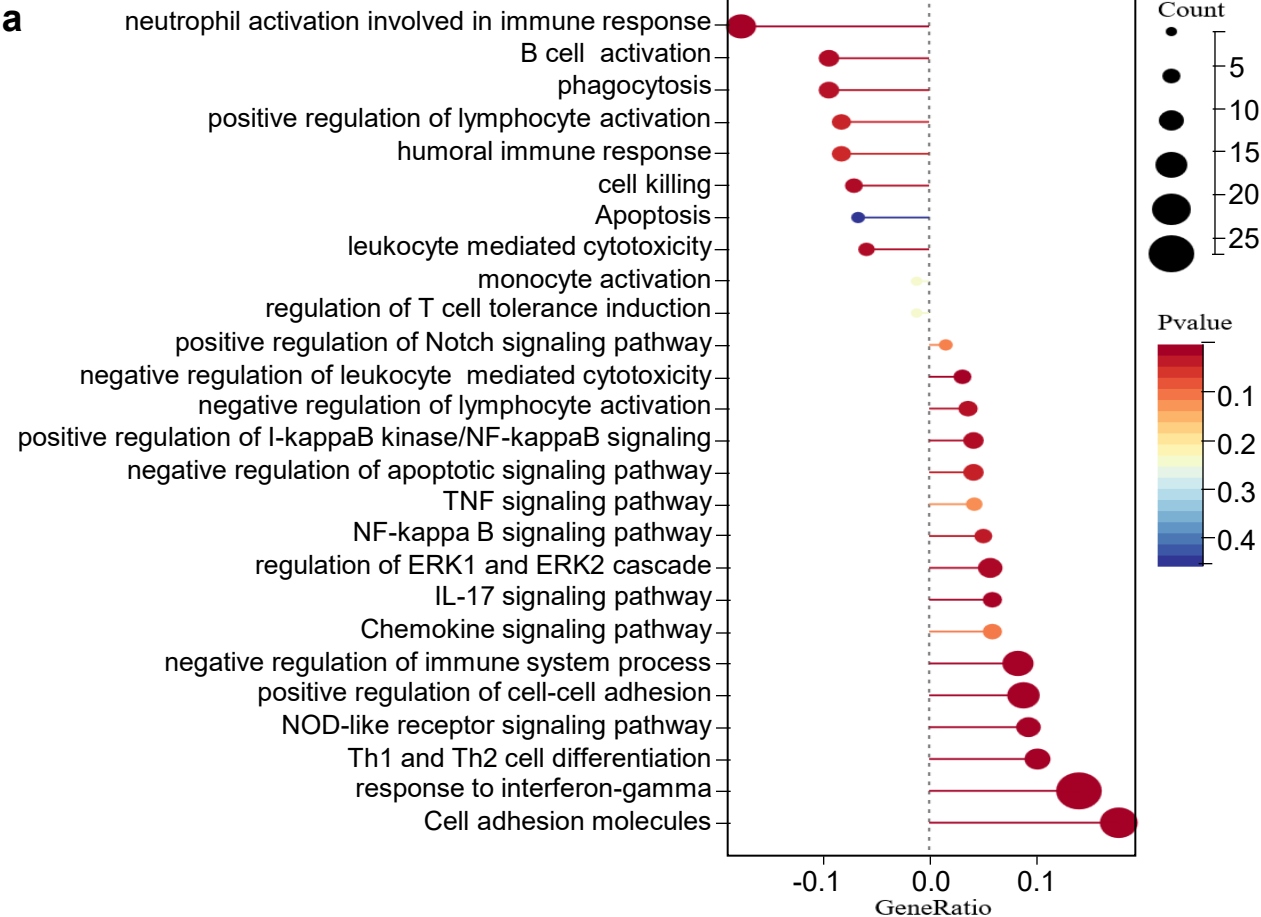


Figure S3

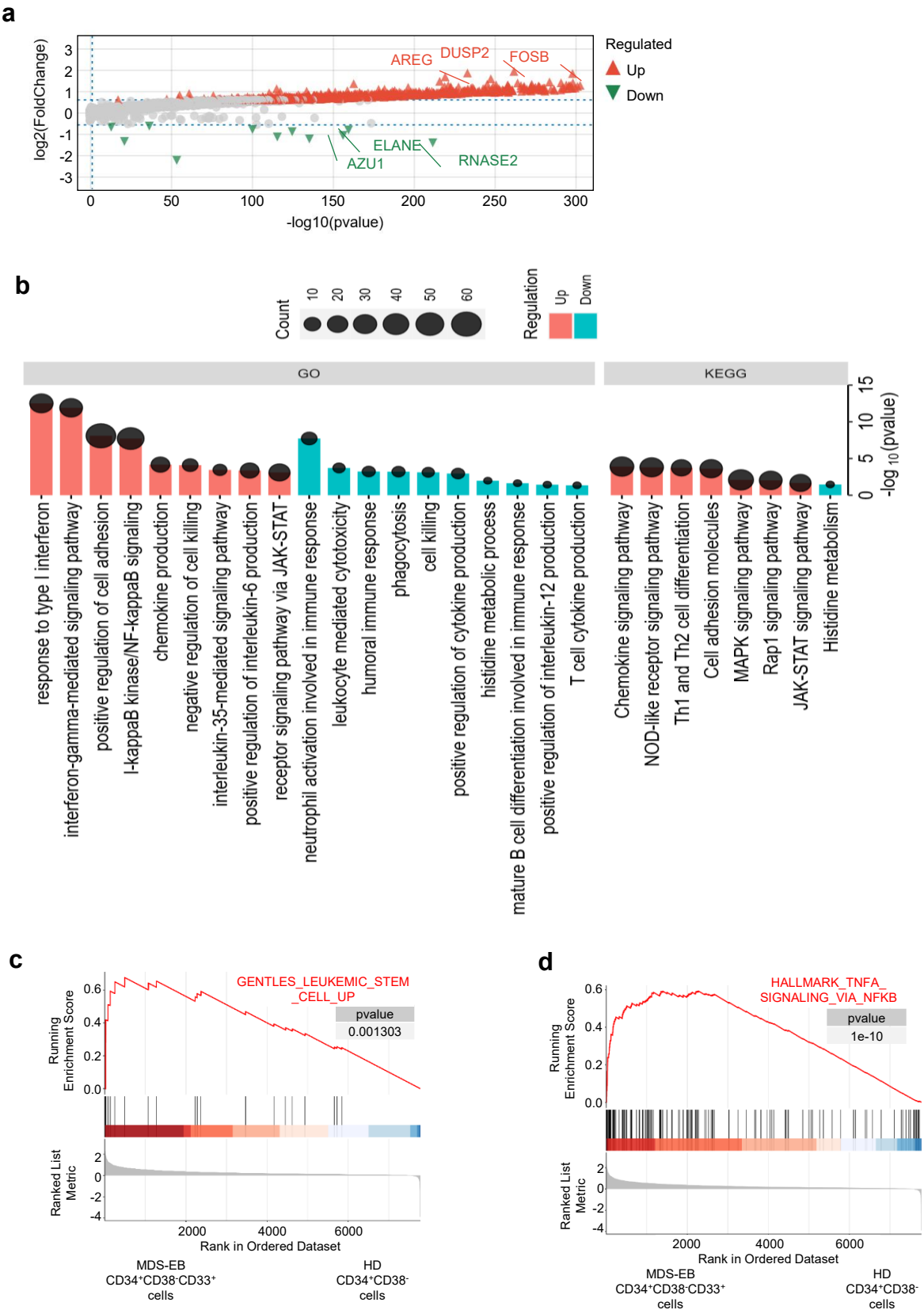


Figure S4

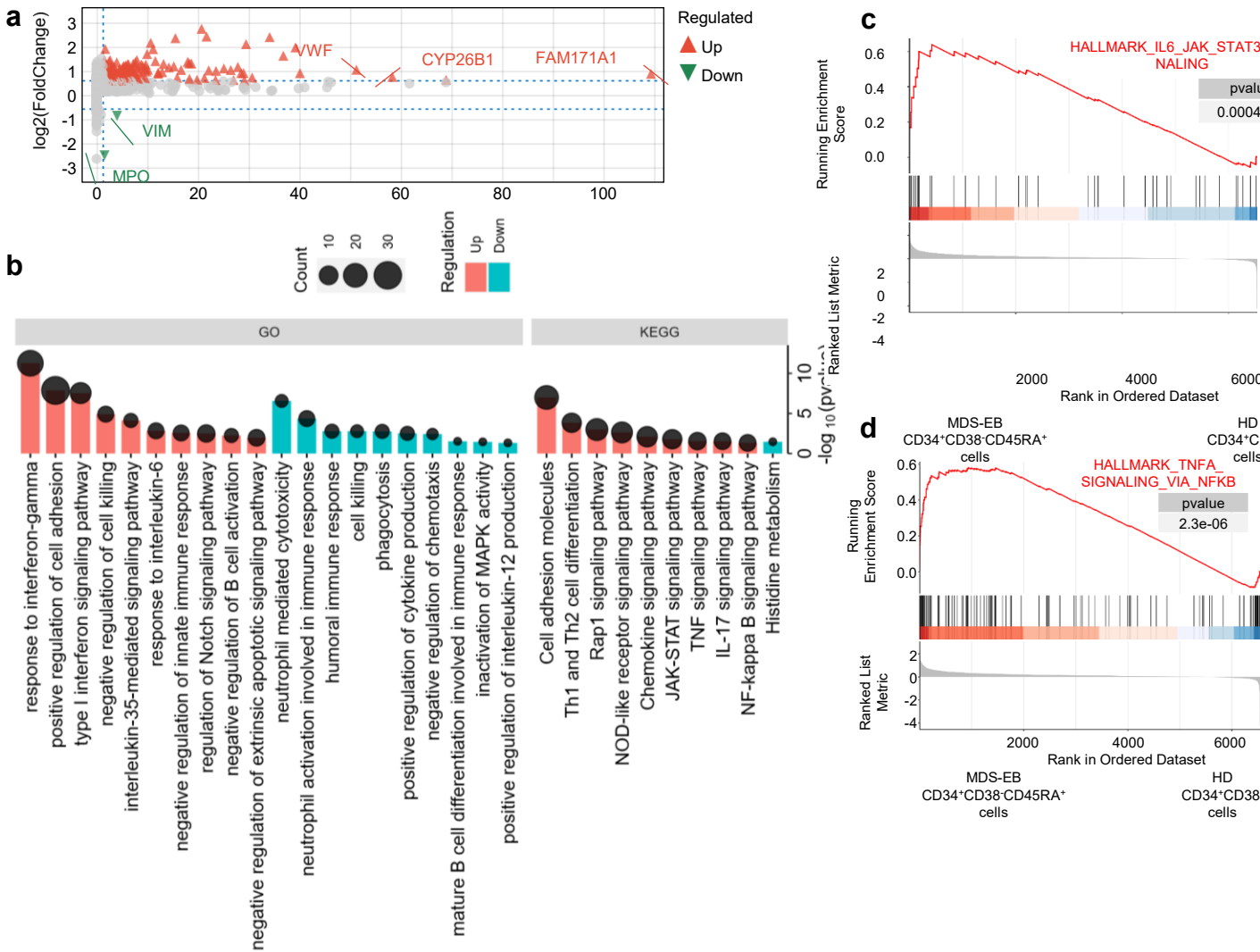


Figure S5

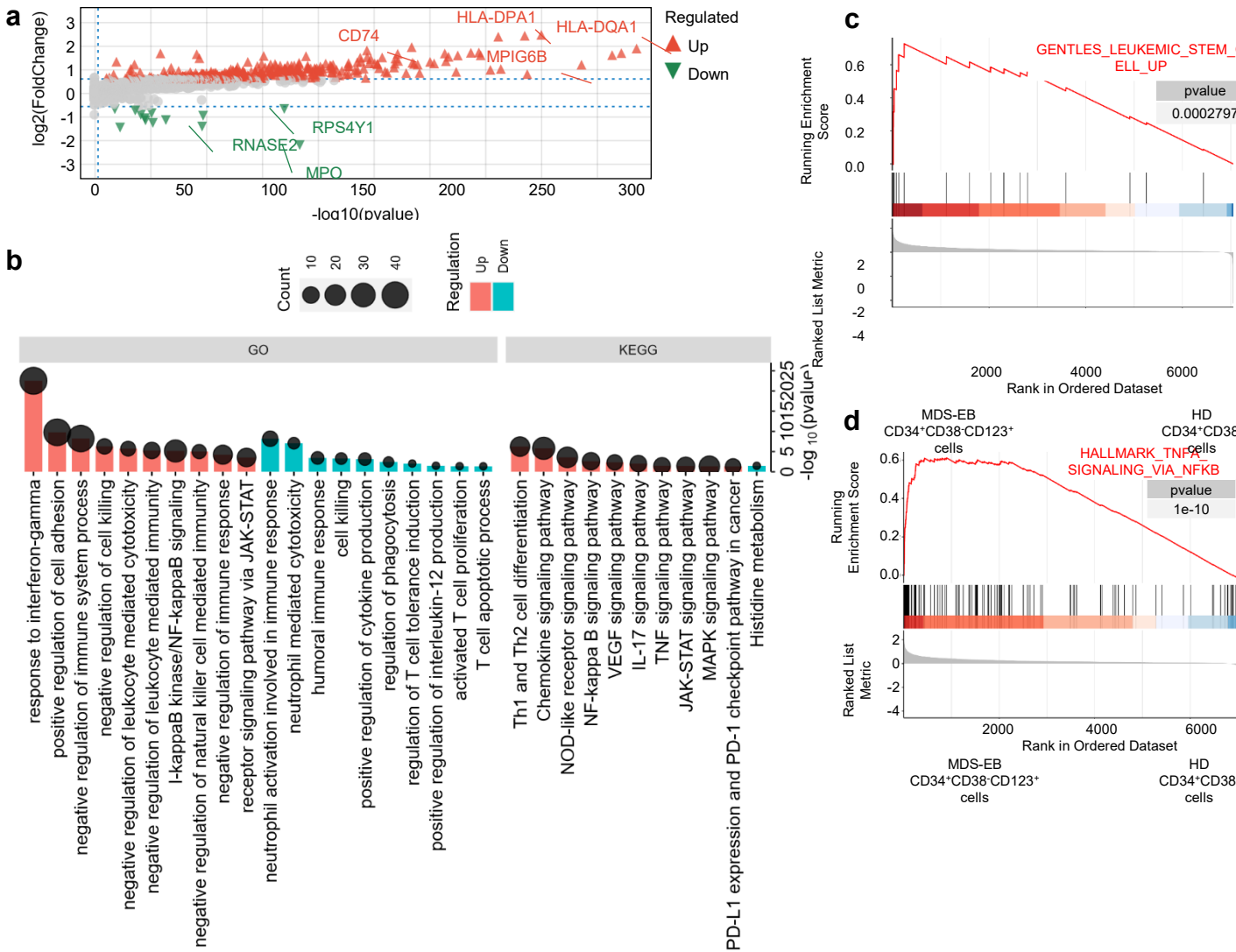


Figure S6

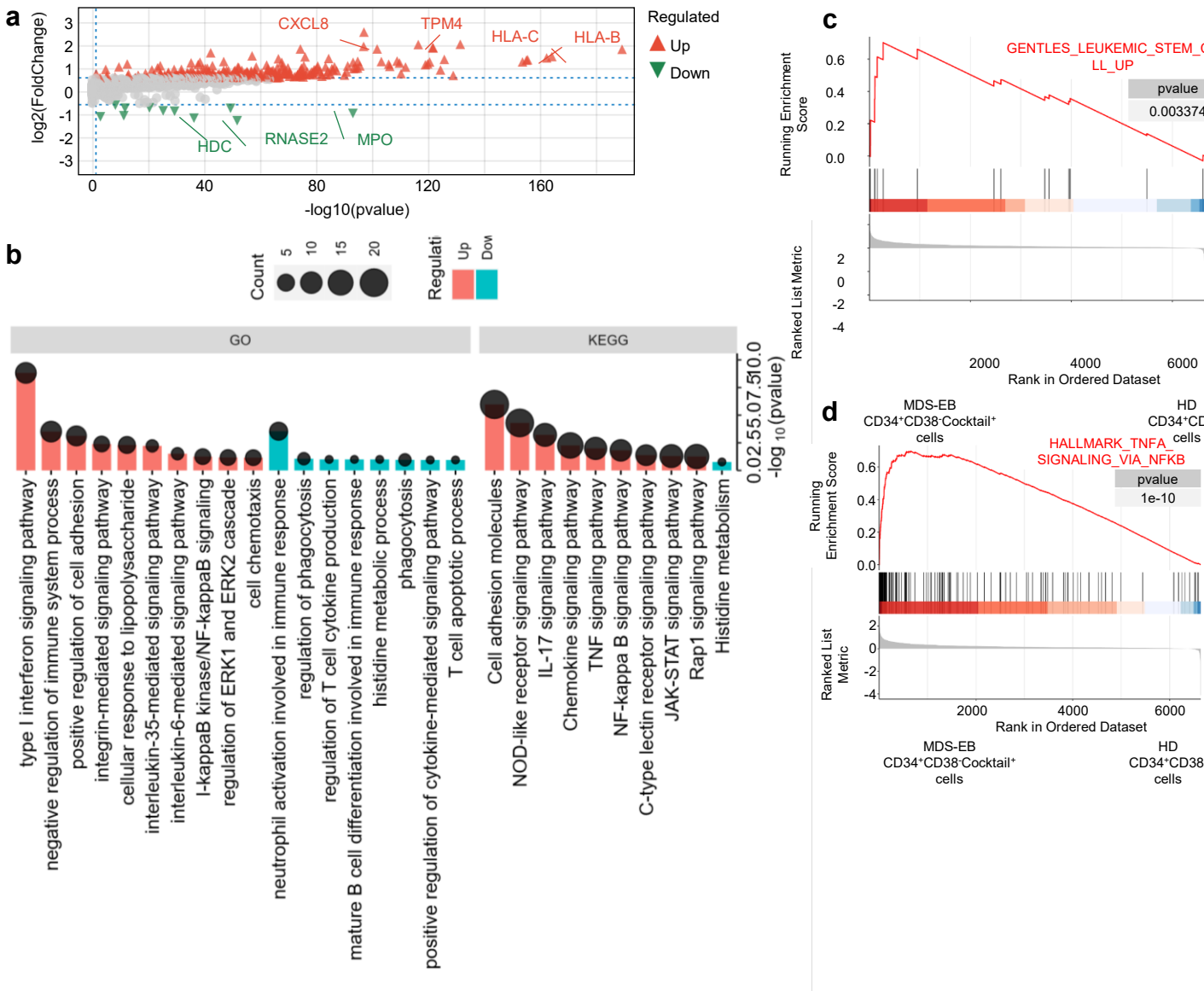
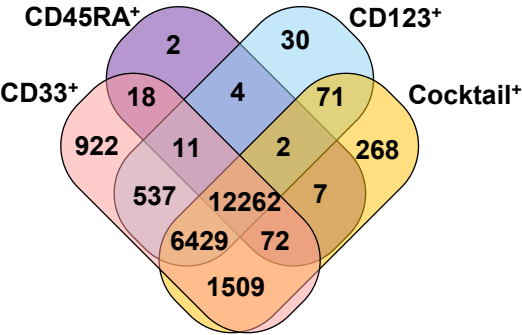


Figure S7

a



b

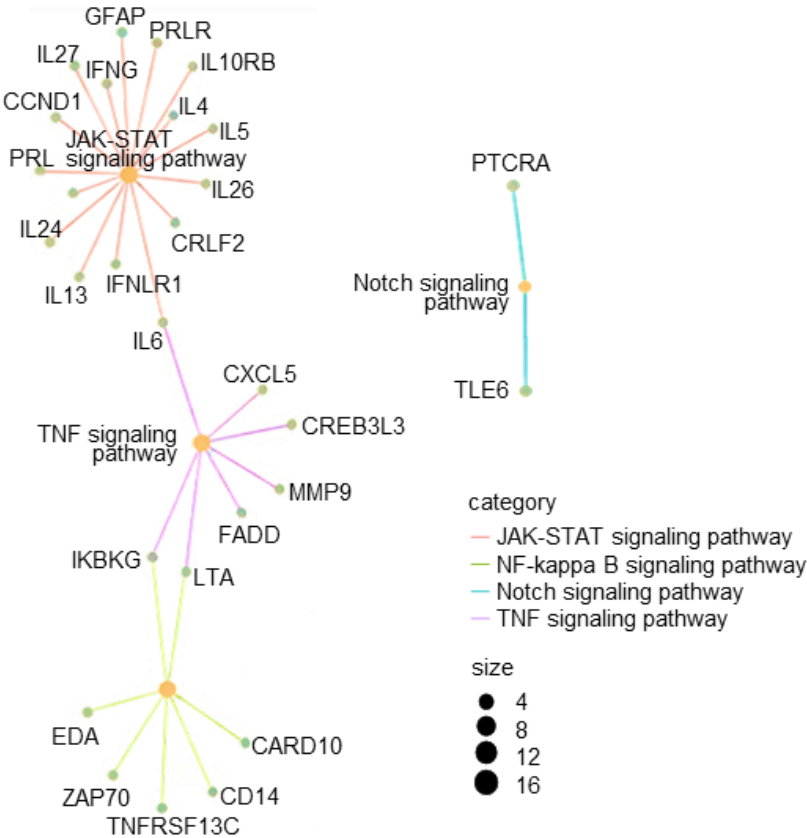


Figure S8

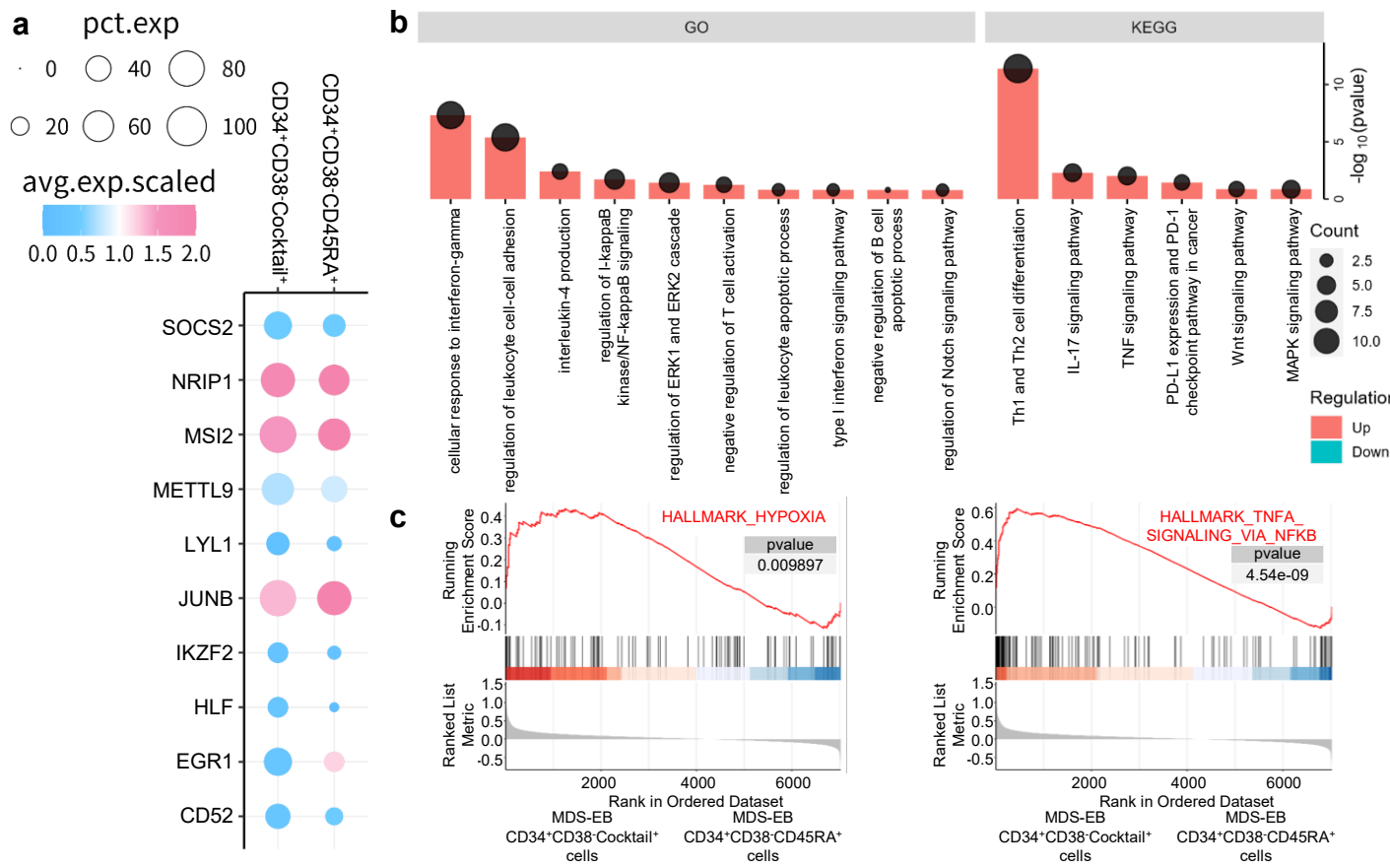


Figure S9

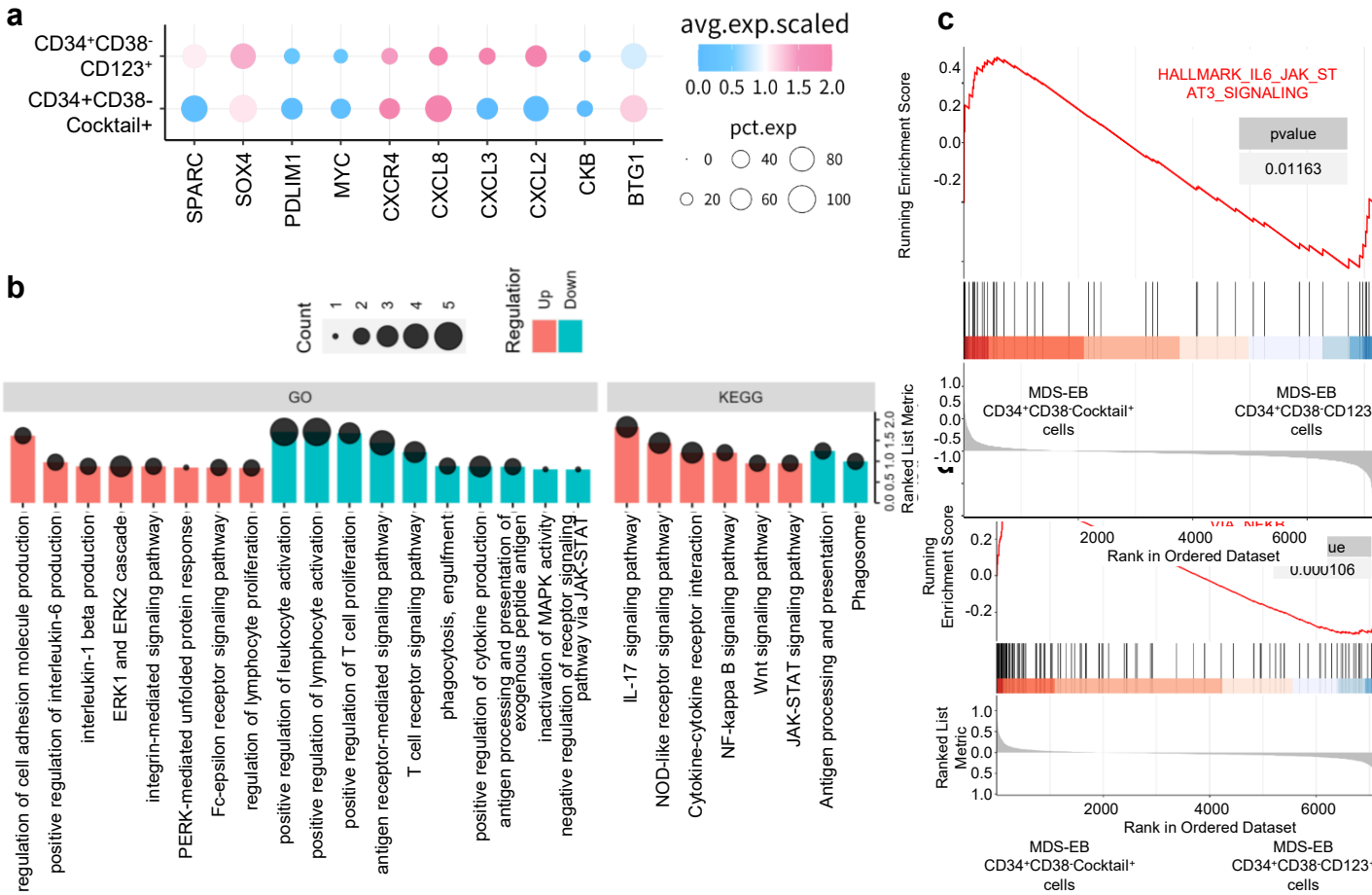
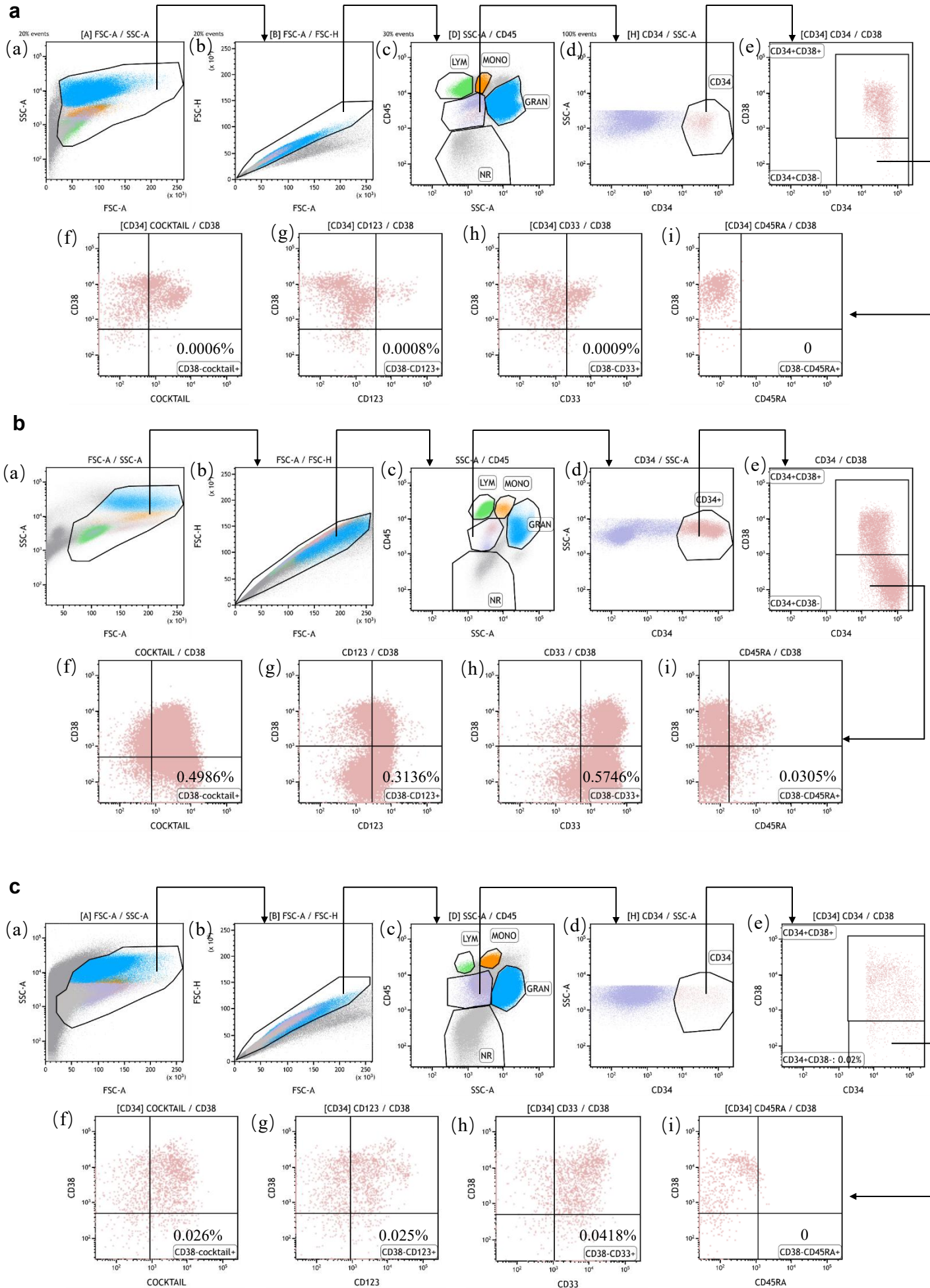


Figure S10



**d**

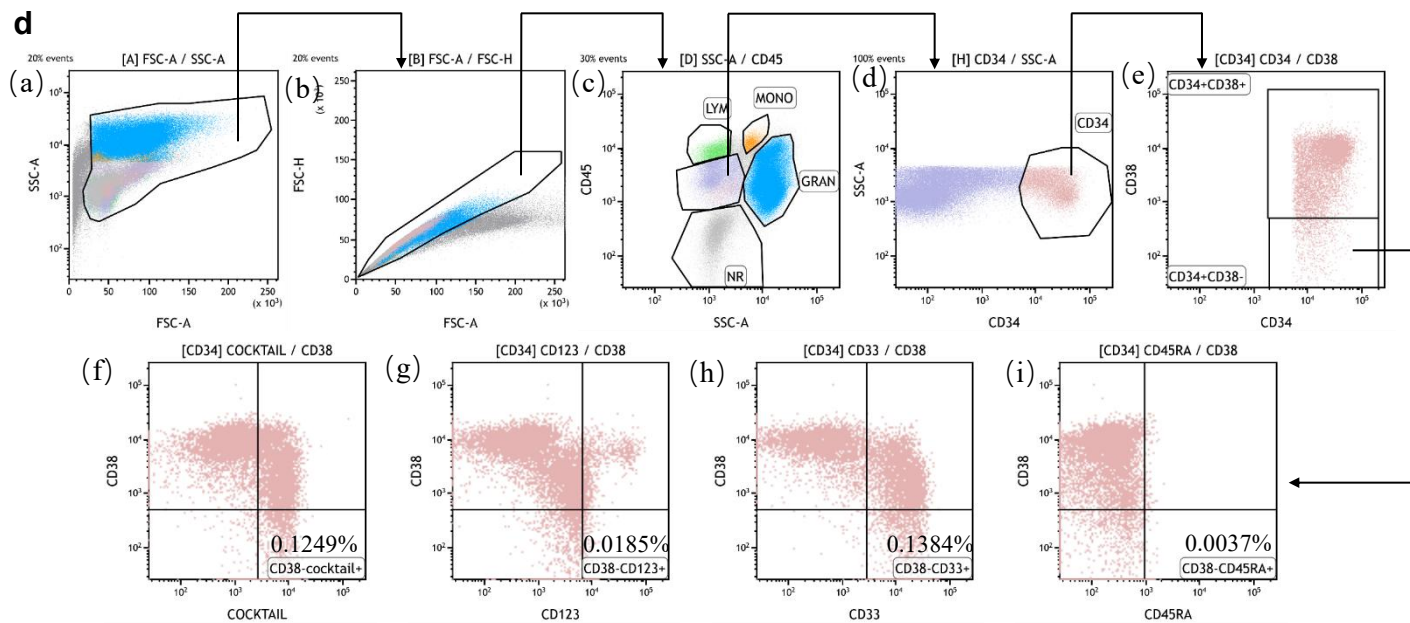
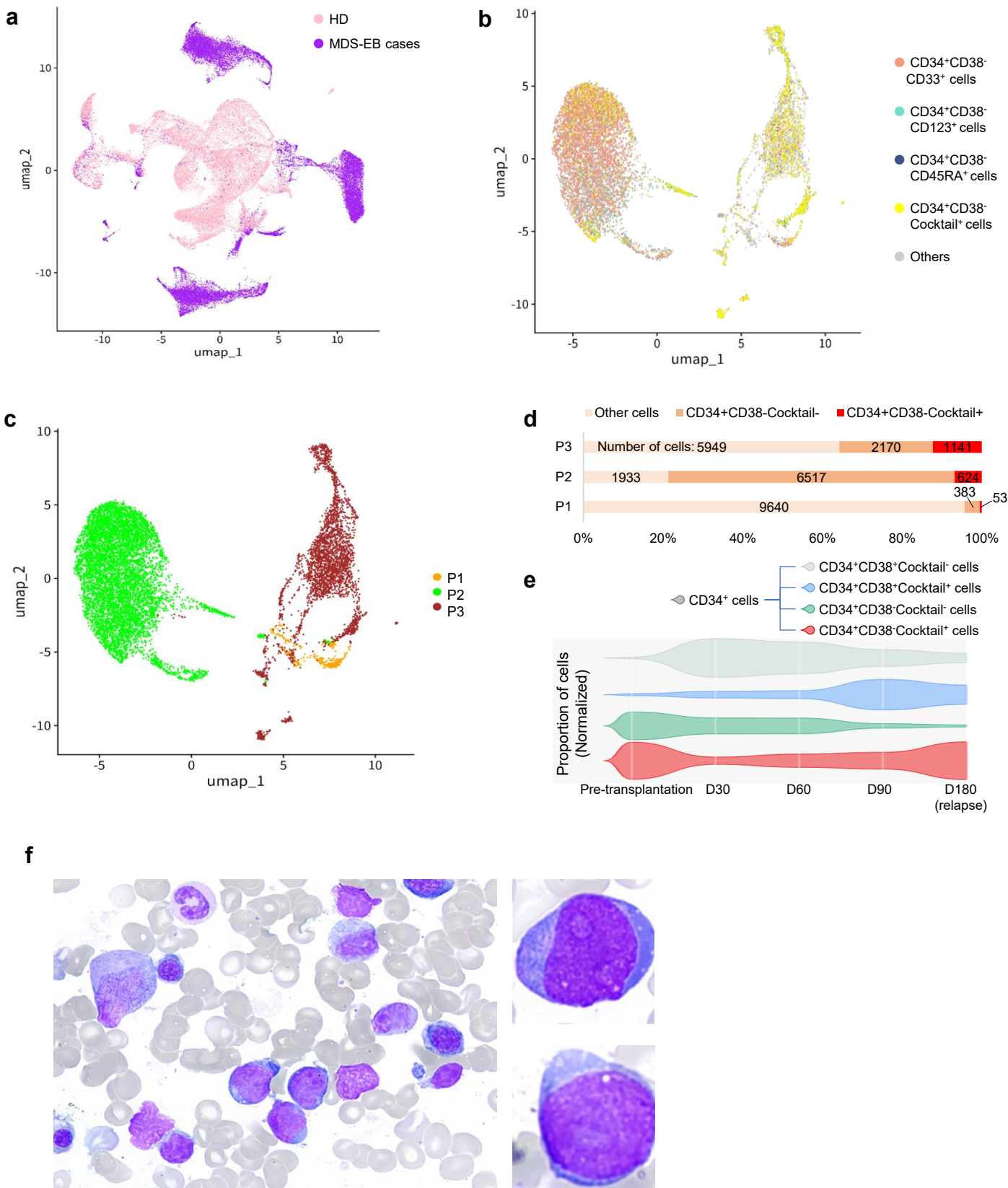


Figure S11



**Figure S12**

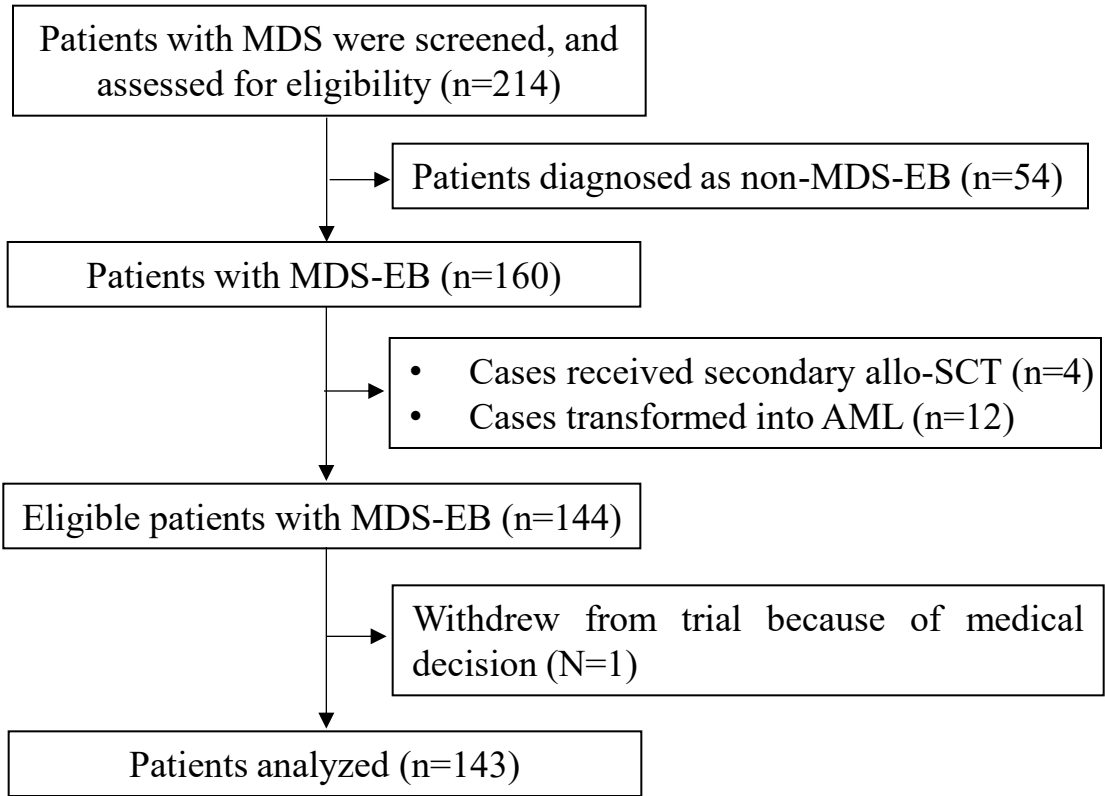


Figure S13

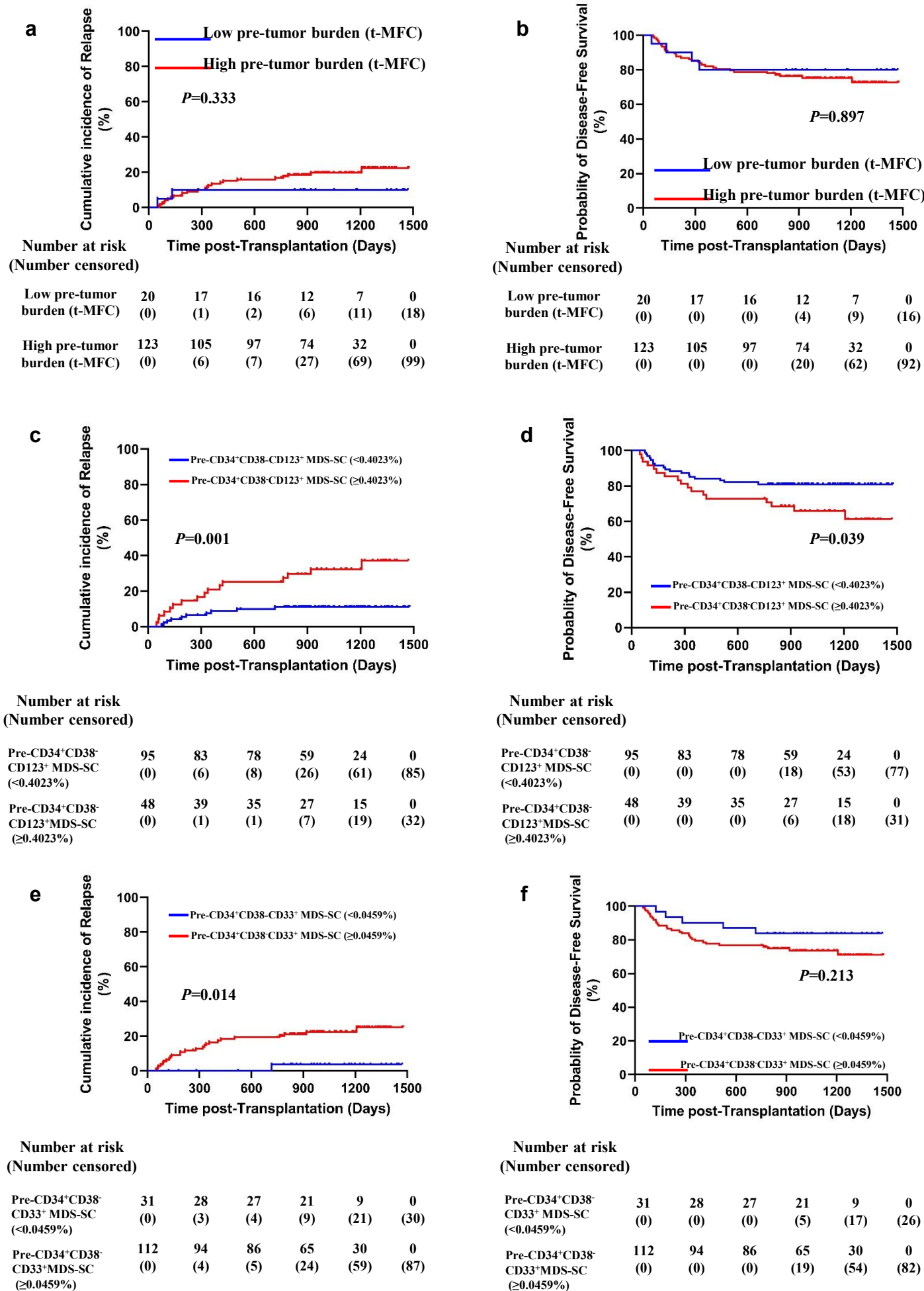
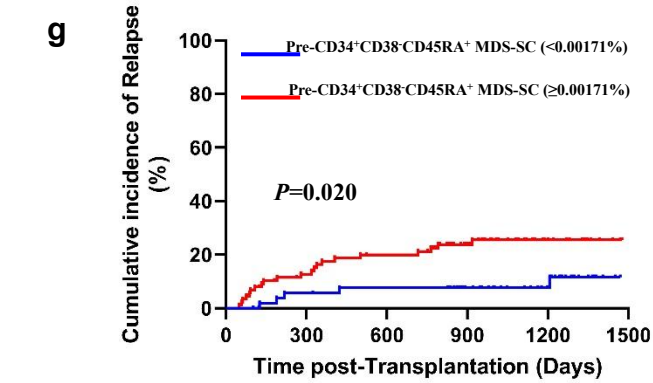
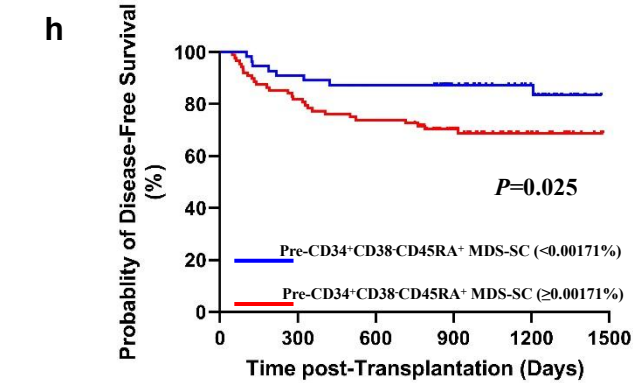


Figure S13 Continued

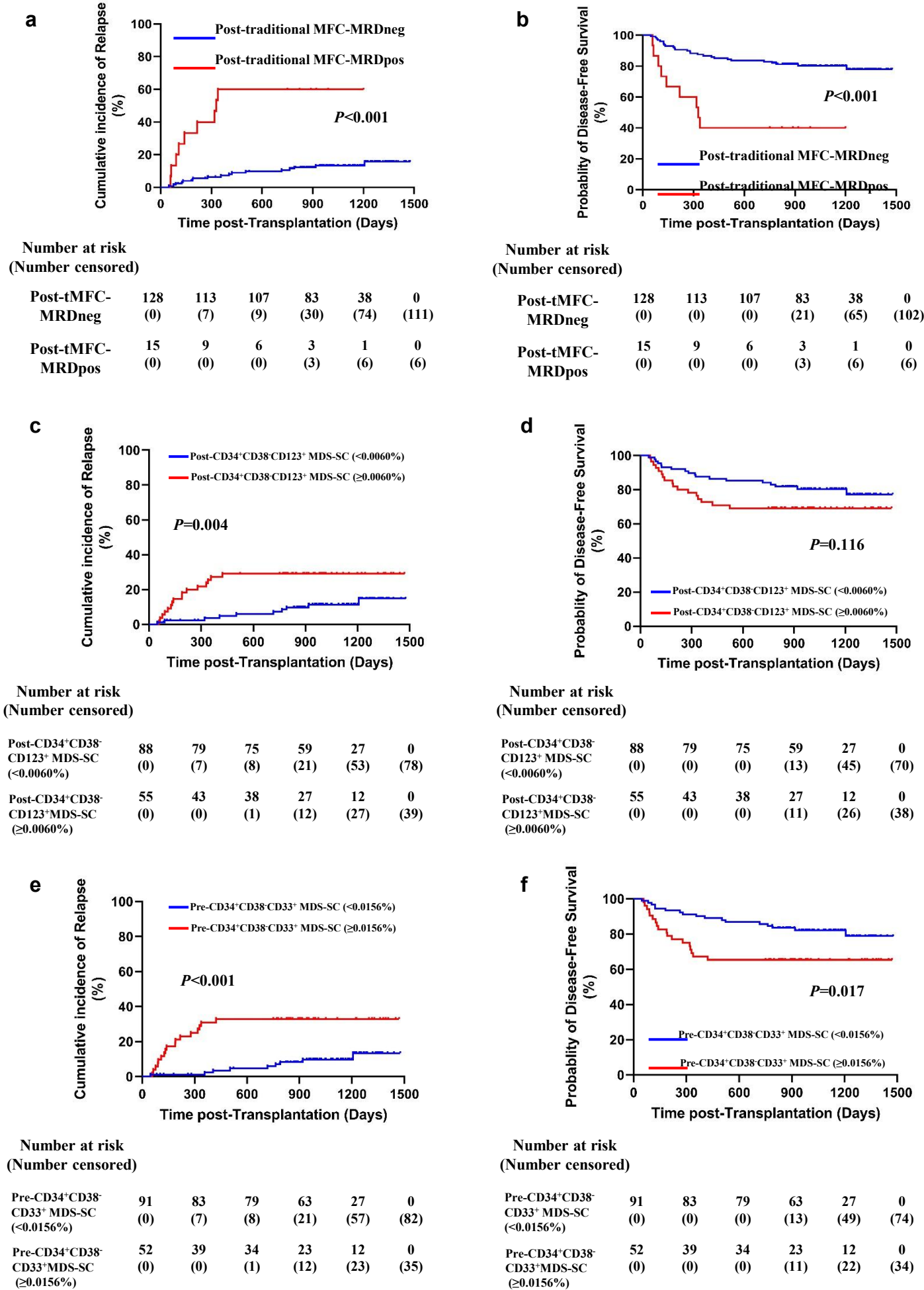


|                                                                                | Number at risk<br>(Number censored) |           |           |            |            |           |
|--------------------------------------------------------------------------------|-------------------------------------|-----------|-----------|------------|------------|-----------|
| Pre-CD34 <sup>+</sup> CD38 <sup>-</sup> CD45RA <sup>+</sup> MDS-SC (<0.00171%) | 55<br>(0)                           | 50<br>(2) | 48<br>(3) | 39<br>(12) | 23<br>(28) | 0<br>(50) |
| Pre-CD34 <sup>+</sup> CD38 <sup>-</sup> CD45RA <sup>+</sup> MDS-SC (≥0.00171%) | 88<br>(0)                           | 72<br>(5) | 65<br>(6) | 47<br>(21) | 16<br>(52) | 0<br>(67) |

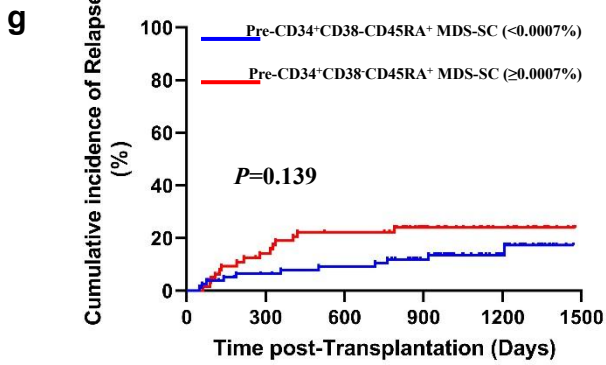


|                                                                                | Number at risk<br>(Number censored) |           |           |            |            |           |
|--------------------------------------------------------------------------------|-------------------------------------|-----------|-----------|------------|------------|-----------|
| Pre-CD34 <sup>+</sup> CD38 <sup>-</sup> CD45RA <sup>+</sup> MDS-SC (<0.00171%) | 55<br>(0)                           | 50<br>(0) | 48<br>(0) | 39<br>(9)  | 23<br>(25) | 0<br>(47) |
| Pre-CD34 <sup>+</sup> CD38 <sup>-</sup> CD45RA <sup>+</sup> MDS-SC (≥0.00171%) | 88<br>(0)                           | 72<br>(0) | 65<br>(0) | 47<br>(15) | 16<br>(46) | 0<br>(61) |

Figure S14

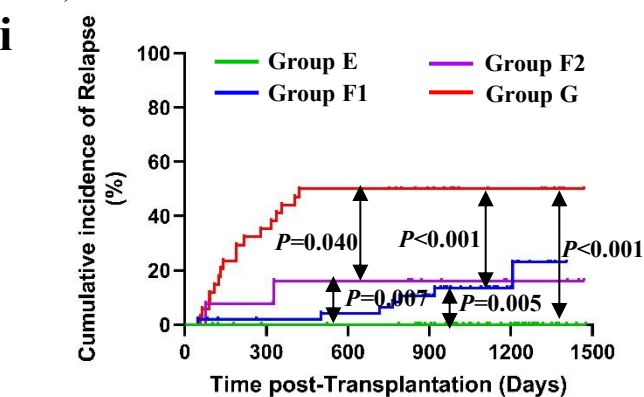


**Figure S14 Continued**



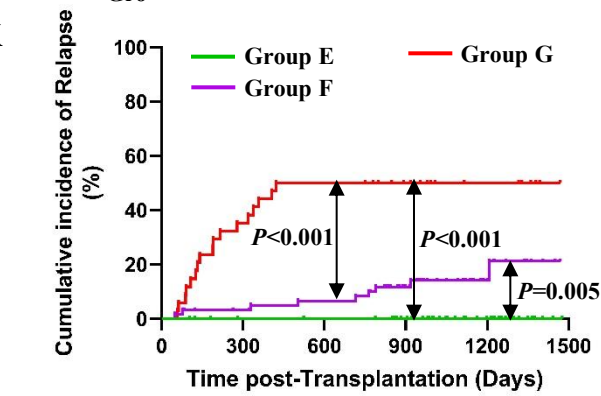
Number at risk  
(Number censored)

|                                                                               |           |           |           |            |            |           |
|-------------------------------------------------------------------------------|-----------|-----------|-----------|------------|------------|-----------|
| Pre-CD34 <sup>+</sup> CD38 <sup>-</sup> CD45RA <sup>+</sup> MDS-SC (<0.0007%) | 78<br>(0) | 69<br>(4) | 66<br>(5) | 54<br>(15) | 25<br>(44) | 0<br>(67) |
| Pre-CD34 <sup>+</sup> CD38 <sup>-</sup> CD45RA <sup>+</sup> MDS-SC (≥0.0007%) | 65<br>(0) | 53<br>(3) | 47<br>(4) | 32<br>(18) | 14<br>(36) | 0<br>(50) |



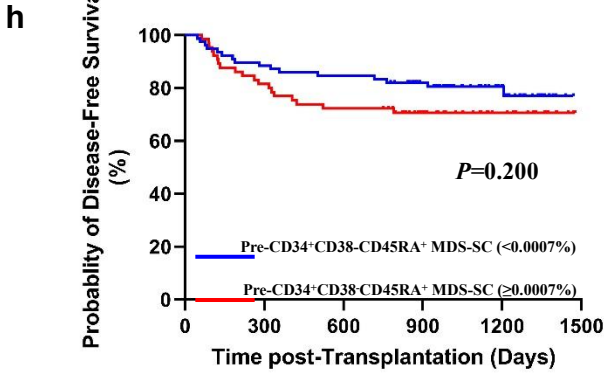
Number at risk  
(Number censored)

|          |           |           |           |            |            |           |
|----------|-----------|-----------|-----------|------------|------------|-----------|
| Group E  | 46<br>(0) | 42<br>(4) | 41<br>(5) | 36<br>(10) | 19<br>(27) | 0<br>(46) |
| Group F1 | 50<br>(0) | 46<br>(3) | 45<br>(3) | 32<br>(13) | 11<br>(34) | 0<br>(43) |
| Group F2 | 13<br>(0) | 12<br>(0) | 10<br>(1) | 6<br>(5)   | 3<br>(8)   | 0<br>(11) |
| Group G  | 34<br>(0) | 22<br>(0) | 17<br>(0) | 12<br>(5)  | 6<br>(11)  | 0<br>(17) |



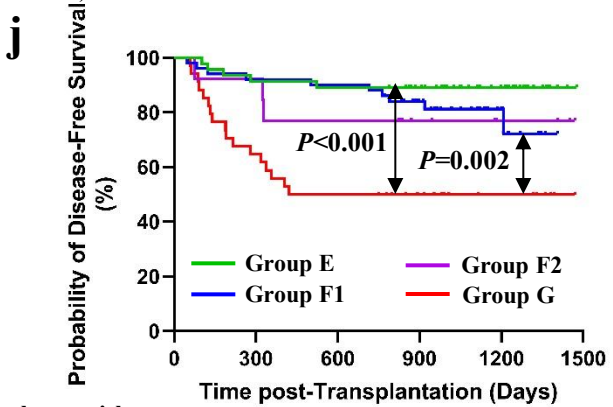
Number at risk  
(Number censored)

|         |           |           |           |            |            |           |
|---------|-----------|-----------|-----------|------------|------------|-----------|
| Group E | 46<br>(0) | 42<br>(4) | 41<br>(5) | 36<br>(10) | 19<br>(27) | 0<br>(46) |
| Group F | 63<br>(0) | 58<br>(3) | 55<br>(4) | 38<br>(18) | 14<br>(42) | 0<br>(54) |
| Group G | 34<br>(0) | 22<br>(0) | 17<br>(0) | 12<br>(5)  | 6<br>(11)  | 0<br>(17) |



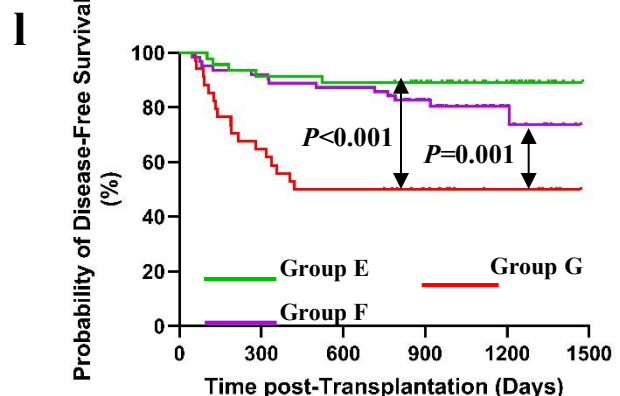
Number at risk  
(Number censored)

|                                                                               |           |           |           |            |            |           |
|-------------------------------------------------------------------------------|-----------|-----------|-----------|------------|------------|-----------|
| Pre-CD34 <sup>+</sup> CD38 <sup>-</sup> CD45RA <sup>+</sup> MDS-SC (<0.0007%) | 78<br>(0) | 69<br>(0) | 66<br>(0) | 54<br>(10) | 25<br>(39) | 0<br>(62) |
| Pre-CD34 <sup>+</sup> CD38 <sup>-</sup> CD45RA <sup>+</sup> MDS-SC (≥0.0007%) | 65<br>(0) | 53<br>(0) | 47<br>(0) | 32<br>(14) | 14<br>(32) | 0<br>(46) |



Number at risk  
(Number censored)

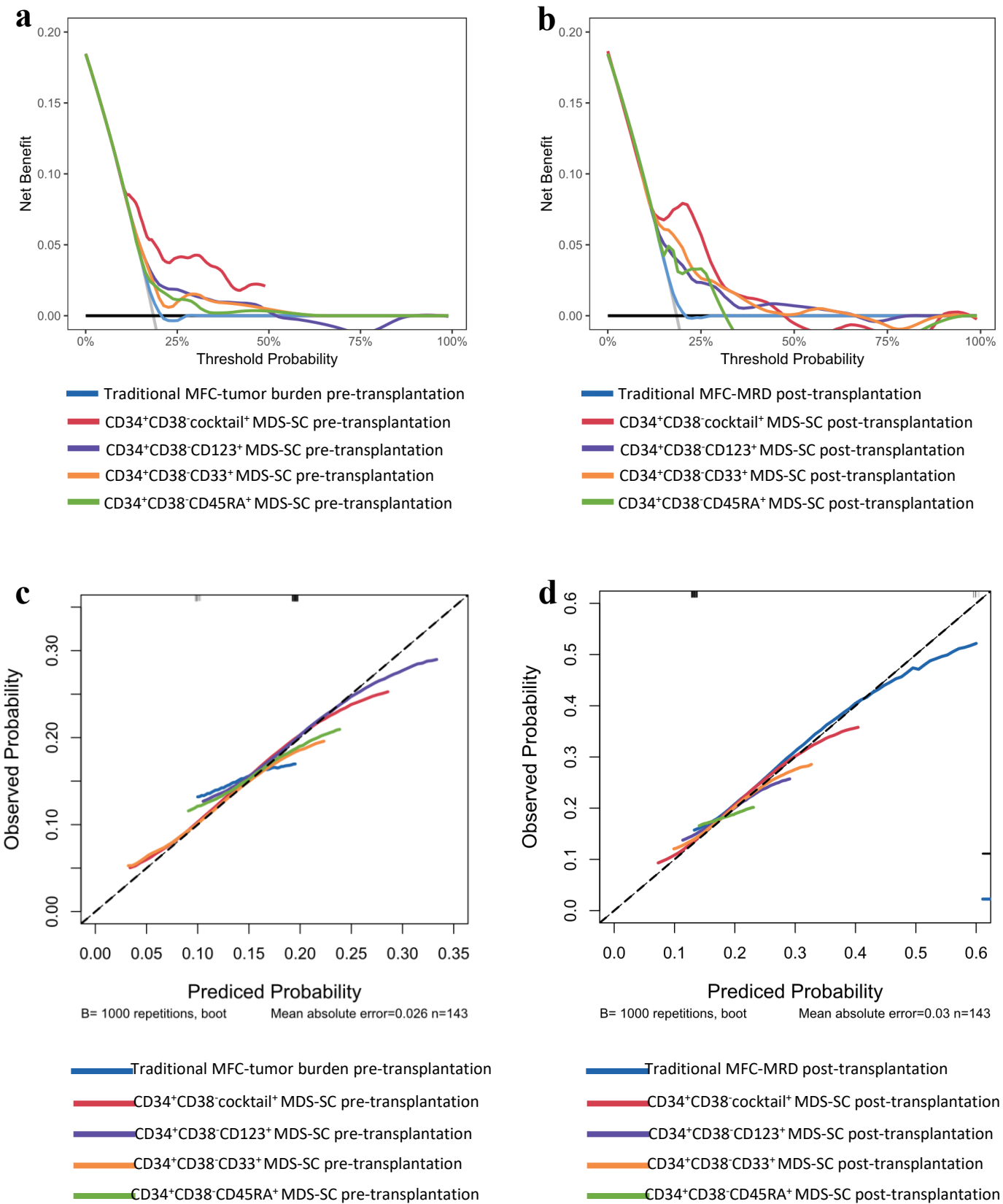
|          |           |           |           |            |            |           |
|----------|-----------|-----------|-----------|------------|------------|-----------|
| Group E  | 46<br>(0) | 42<br>(0) | 41<br>(0) | 36<br>(5)  | 19<br>(22) | 0<br>(41) |
| Group F1 | 50<br>(0) | 46<br>(0) | 45<br>(0) | 32<br>(10) | 11<br>(31) | 0<br>(40) |
| Group F2 | 13<br>(0) | 12<br>(0) | 10<br>(0) | 6<br>(4)   | 3<br>(7)   | 0<br>(10) |
| Group G  | 34<br>(0) | 22<br>(0) | 17<br>(0) | 12<br>(5)  | 6<br>(11)  | 0<br>(17) |



Number at risk  
(Number censored)

|         |           |           |           |            |            |           |
|---------|-----------|-----------|-----------|------------|------------|-----------|
| Group E | 46<br>(0) | 42<br>(0) | 41<br>(0) | 36<br>(5)  | 19<br>(22) | 0<br>(41) |
| Group F | 50<br>(0) | 46<br>(0) | 45<br>(0) | 32<br>(14) | 11<br>(38) | 0<br>(40) |
| Group G | 34<br>(0) | 22<br>(0) | 17<br>(0) | 12<br>(5)  | 6<br>(11)  | 0<br>(17) |

Figure S15



**Table S1 Characteristics of sample for Ab-seq.****Patient characteristics**

| Patient | Sex    | Age(years) | Bone marrow blasts before transplantation | Relapse after transplantation |
|---------|--------|------------|-------------------------------------------|-------------------------------|
| P1      | Female | 60         | 6.5%                                      | No                            |
| P2      | Female | 37         | 7.5%                                      | No                            |
| P3      | Male   | 72         | 4.5%                                      | Yes                           |

**Healthy donor characteristics**

| Healthy donor | Sex  | Age(years) | Bone marrow blasts before transplantation |
|---------------|------|------------|-------------------------------------------|
| H1            | Male | 33         | 2%                                        |
| H2            | Male | 51         | 0.5%                                      |
| H3            | Male | 50         | 0.5%                                      |

**Table S2. Patient characteristics**

| Characteristics                                             | Total       | Low pre-SCT MDS-<br>SC burden group | High pre-SCT MDS-<br>SC burden group | <i>P</i><br>value | Negative post-SCT<br>MDS-SC group | Positive post-SCT<br>MDS-SC group | <i>P</i><br>value |
|-------------------------------------------------------------|-------------|-------------------------------------|--------------------------------------|-------------------|-----------------------------------|-----------------------------------|-------------------|
| Number of patients                                          | 143         | 59                                  | 84                                   |                   | 98                                | 45                                |                   |
| Male sex, n (%)                                             | 87 (60.8%)  | 33 (55.9%)                          | 54 (64.3%)                           | 0.314             | 61 (62.2%)                        | 26 (57.8%)                        | 0.611             |
| Median age (range, years)                                   | 48 (16-72)  | 49 (28-69)                          | 48 (16-72)                           | 0.780             | 50 (8-69)                         | 48 (16-72)                        | 0.869             |
| Diagnosis, n (%)                                            |             |                                     |                                      | 0.707             |                                   |                                   | 0.955             |
| MDS-EB1                                                     | 13 (9.1%)   | 6 (10.2%)                           | 7 (8.3%)                             |                   | 9 (9.2%)                          | 4 (8.9%)                          |                   |
| MDS-EB2                                                     | 130 (90.9%) | 53 (89.8%)                          | 77 (90.9%)                           |                   | 89 (90.8%)                        | 41 (91.1%)                        |                   |
| Median time from diagnosis to transplant<br>(range, months) | 4 (0.5-67)  | 4 (0.5-12)                          | 4.8 (0.5-67)                         | 0.058             | 4 (0.5-67)                        | 4 (1-23)                          | 0.625             |
| IPSS-M                                                      |             |                                     |                                      | 0.621             |                                   |                                   | 0.656             |
| Very high                                                   | 60 (42.0%)  | 21 (35.6%)                          | 39 (46.4%)                           |                   | 40 (40.8%)                        | 20 (44.4%)                        |                   |
| High                                                        | 56 (39.2%)  | 25 (42.4%)                          | 31 (36.9%)                           |                   | 39 (39.8%)                        | 17 (37.8%)                        |                   |
| Moderate high                                               | 21 (14.7%)  | 10 (16.9%)                          | 11 (13.1%)                           |                   | 16 (16.3%)                        | 5 (11.1%)                         |                   |
| Low                                                         | 6 (4.2%)    | 3 (5.1%)                            | 3 (3.6%)                             |                   | 3 (3.1%)                          | 3 (6.7%)                          |                   |
| Therapy before transplantation, n (%) *                     |             |                                     |                                      | 0.895             |                                   |                                   | <0.001            |
| Yes                                                         | 50 (35.0%)  | 21 (35.6%)                          | 29 (34.5%)                           |                   | 21 (21.4%)                        | 29 (64.4%)                        |                   |
| No                                                          | 93 (65.0%)  | 38 (64.4%)                          | 55 (65.5%)                           |                   | 77 (78.6%)                        | 16 (35.6%)                        |                   |
| Transplantation modalities, n (%)                           |             |                                     |                                      | 0.933             |                                   |                                   | 0.058             |
| MSDT                                                        | 33 (23.1%)  | 13 (22.0%)                          | 20 (23.8%)                           |                   | 17 (17.3%)                        | 16 (35.6%)                        |                   |
| Haplo-SCT                                                   | 92 (64.3%)  | 39 (66.1%)                          | 53 (63.1%)                           |                   | 67 (68.4%)                        | 25 (55.6%)                        |                   |
| MUDT                                                        | 18 (12.6%)  | 7 (11.9%)                           | 11 (13.1%)                           |                   | 14 (14.3%)                        | 4 (8.8%)                          |                   |
| Donor-recipient relationship, n (%)                         |             |                                     |                                      | 0.995             |                                   |                                   | 0.513             |
| Parent-child                                                | 14 (9.8%)   | 6 (10.2%)                           | 8 (9.5%)                             |                   | 10 (10.2%)                        | 4 (8.9%)                          |                   |
| Sibling-sibling                                             | 50 (35.0%)  | 20 (33.9%)                          | 30 (35.7%)                           |                   | 34 (34.7%)                        | 16 (35.6%)                        |                   |
| Child-parent                                                | 58 (40.6%)  | 24 (40.7%)                          | 34 (40.5%)                           |                   | 37 (37.8%)                        | 21 (46.7%)                        |                   |
| Other                                                       | 21 (14.7%)  | 9 (15.3%)                           | 12 (14.3%)                           |                   | 17 (17.3%)                        | 4 (8.9%)                          |                   |
| ABO match grafts, n (%)                                     |             |                                     |                                      | 0.992             |                                   |                                   | 0.219             |
| Matched                                                     | 85 (59.4%)  | 35 (59.3%)                          | 50 (59.5%)                           |                   | 54 (55.1%)                        | 31 (68.9%)                        |                   |

|                                                               |                   |                   |                   |       |                   |                   |       |
|---------------------------------------------------------------|-------------------|-------------------|-------------------|-------|-------------------|-------------------|-------|
| Major mismatch                                                | 18 (12.6%)        | 7 (11.9%)         | 11 (13.1%)        |       | 12 (12.2%)        | 6 (13.3%)         |       |
| Minor mismatch                                                | 30 (21.0%)        | 13 (22.0%)        | 17 (20.2%)        |       | 25 (25.5%)        | 5 (11.1%)         |       |
| Bidirectional mismatch                                        | 10 (7.0%)         | 4 (6.8%)          | 6 (7.1%)          |       | 7 (7.1%)          | 3 (6.7%)          |       |
| Donor-recipient sex matched grafts, n(%)                      |                   |                   |                   | 0.462 |                   |                   | 0.719 |
| Male-male                                                     | 55 (38.5%)        | 23 (39.0%)        | 32 (38.1%)        |       | 39 (39.8%)        | 16 (35.6%)        |       |
| Male-female                                                   | 33 (23.1%)        | 15 (25.4%)        | 18 (21.4%)        |       | 24 (24.5%)        | 9 (20.0%)         |       |
| Female-male                                                   | 33 (23.1%)        | 10 (16.9%)        | 23 (27.4%)        |       | 22 (22.4%)        | 11 (24.4%)        |       |
| Female-female                                                 | 22 (15.4%)        | 11 (18.6%)        | 11 (13.1%)        |       | 13 (13.3%)        | 9 (20.0%)         |       |
| Cell compositions in allografts                               |                   |                   |                   |       |                   |                   |       |
| Infused nuclear cells, (range, 10 <sup>8</sup> /kg)           | 9.64 (3.46-19.90) | 9.60 (4.47-18.60) | 9.93 (3.46-19.90) | 0.245 | 9.58 (3.46-18.28) | 9.94 (6.19-19.90) | 0.523 |
| Infused CD34 <sup>+</sup> cells, (range, 10 <sup>8</sup> /kg) | 3.63 (0.56-13.06) | 3.35 (0.56-10.71) | 3.75 (0.72-13.06) | 0.350 | 3.67 (0.56-12.41) | 3.63 (0.96-13.06) | 0.608 |

**Abbreviations:** SCT=stem cell transplantation; MDS-SC=myelodysplastic syndrome stem cell; MDS-EB=myelodysplastic syndrome with excess blasts; IPSS-M=molecular international prognostic scoring system; MSDT=human leukocyte antigen-matched sibling donor transplantation; Haplo-SCT=Haploidentical stem cell transplantation; MUDT=HLA-matched unrelated donor transplantation

\* Therapy for patients with MDS before transplantation was performed according to a previous publication (Lancet Haematol, 2021;8(8):e552-e561) and recommendations (Zhonghua Xue Ye Xue Za Zhi, 2019;40(2):89-97).

**Table S3. Outcomes of total patients with myelodysplastic syndromes (n=143)**

| Outcomes                                         |                            |
|--------------------------------------------------|----------------------------|
| Cumulative 100-day incidence of II-IV acute GVHD | 25.9% (95%CI, 18.6%-33.2%) |
| Cumulative 1-year incidence of chronic GVHD      | 46.4% (95%CI, 37.8%-55.0%) |
| 3-year CIR                                       | 17.7% (95%CI, 11.4%-24.1%) |
| 3-year NRM                                       | 6.3% (95%CI, 2.3%-10.3%)   |
| 3-year DFS                                       | 76.0% (95%CI, 68.9%-83.1%) |
| 3-year OS                                        | 81.7% (95%CI, 75.2%-88.2%) |

Abbreviations: GVHD=graft-versus-host disease; CIR=cumulative incidence of relapse; NRM=nonrelapse mortality; DFS=Disease-free survival; OS=overall survival

**Table S4. Transplant outcomes of MDS patients classified into different subgroups according to traditional MFC method and MDS-SC-based tumor burden before transplantation (n=143).**

|                                                                                      | 3-year CIR                     | 3-year NRM                    | 3-year DFS                     | 3-year OS                      |
|--------------------------------------------------------------------------------------|--------------------------------|-------------------------------|--------------------------------|--------------------------------|
| Traditional MFC-tumor burden pre-transplantation                                     |                                |                               |                                |                                |
| Not-Detected (n=20)                                                                  | 10.0%<br>(95% CI, 0.0%-23.5%)  | 10.0%<br>(95% CI, 0.0%-23.5%) | 80.0%<br>(95% CI, 62.6%-97.4%) | 80.0%<br>(95% CI, 62.6%-97.4%) |
| Detected (n=123)                                                                     | 19.0%<br>(95% CI, 11.9%-26.1%) | 5.7%<br>(95% CI, 1.6%-9.8%)   | 75.3%<br>(95% CI, 67.7%-82.9%) | 81.9%<br>(95% CI, 75.0%-88.8%) |
| P value                                                                              | 0.333                          | 0.485                         | 0.643                          | 0.790                          |
| CD34 <sup>+</sup> CD38 <sup>-</sup> cocktail <sup>+</sup> MDS-SC pre-transplantation |                                |                               |                                |                                |
| Negative (n=59)                                                                      | 3.4%<br>(95% CI, 0.0%-8.0%)    | 10.2%<br>(95% CI, 2.4%-18.0%) | 86.4%<br>(95% CI, 77.6%-95.2%) | 88.1%<br>(95% CI, 79.9%-96.3%) |
| Positive (n=84)                                                                      | 27.9%<br>(95% CI, 15.1%-40.8%) | 3.6%<br>(95% CI, 0.0%-7.6%)   | 68.5%<br>(95% CI, 58.3%-78.7%) | 78.6%<br>(95% CI, 69.8%-87.4%) |
| P value                                                                              | <0.001                         | 0.116                         | 0.011                          | 0.240                          |
| CD34 <sup>+</sup> CD38 <sup>-</sup> CD123 <sup>+</sup> MDS-SC pre-transplantation    |                                |                               |                                |                                |
| Negative (n=95)                                                                      | 10.5%<br>(95% CI, 4.3%-16.7%)  | 8.4%<br>(95% CI, 2.8%-14.0%)  | 81.1%<br>(95% CI, 73.3%-88.9%) | 82.9%<br>(95% CI, 75.3%-90.5%) |
| Positive (n=48)                                                                      | 31.9%<br>(95% CI, 18.3%-45.5%) | 2.1%<br>(95% CI, 0.0%-6.2%)   | 66.0%<br>(95% CI, 52.3%-79.7%) | 79.2%<br>(95% CI, 67.6%-90.8%) |
| P value                                                                              | 0.001                          | 0.141                         | 0.039                          | 0.614                          |
| CD34 <sup>+</sup> CD38 <sup>-</sup> CD33 <sup>+</sup> MDS-SC pre-transplantation     |                                |                               |                                |                                |
| Negative (n=31)                                                                      | 3.2%<br>(95% CI, 0.0%-9.5%)    | 12.9%<br>(95% CI, 0.9%-24.9%) | 83.9%<br>(95% CI, 71.0%-96.8%) | 87.1%<br>(95% CI, 75.3%-98.9%) |
| Positive (n=112)                                                                     | 21.8%<br>(95% CI, 14.0%-29.6%) | 4.5%<br>(95% CI, 0.6%-8.3%)   | 73.7%<br>(95% CI, 65.5%-81.9%) | 80.2%<br>(95% CI, 72.8%-87.6%) |
| P value                                                                              | 0.014                          | 0.092                         | 0.213                          | 0.403                          |
| CD34 <sup>+</sup> CD38 <sup>-</sup> CD45RA <sup>+</sup> MDS-SC pre-transplantation   |                                |                               |                                |                                |

|                 |                                |                              |                                |                                |
|-----------------|--------------------------------|------------------------------|--------------------------------|--------------------------------|
| Negative (n=55) | 7.3%<br>(95% CI, 0.3%-8.0%)    | 5.5%<br>(95% CI, 0.0%-11.5%) | 87.3%<br>(95% CI, 78.5%-96.1%) | 87.3%<br>(95% CI, 78.5%-96.1%) |
| Positive (n=88) | 24.4%<br>(95% CI, 15.2%-33.6%) | 6.8%<br>(95% CI, 1.5%-12.1%) | 68.8%<br>(95% CI, 59.0%-78.6%) | 78.1%<br>(95% CI, 69.3%-86.9%) |
| P value         | 0.020                          | 0.752                        | 0.025                          | 0.185                          |

**Abbreviations:** MDS= myelodysplastic syndromes; MDS-SC= myelodysplastic syndromes stem cell; CIR=cumulative incidence of relapse; NRM=non-relapse mortality; DFS=Disease-free survival; OS=overall survival; CI=confidence interval

**Table S5. Comparison of diagnostic accuracy of traditional MFC method and MDS-SC-based tumor burden before transplantation (n=143).**

|                           | Traditional MFC | CD34 <sup>+</sup> CD38 <sup>-</sup> cocktail <sup>+</sup> | CD34 <sup>+</sup> CD38 <sup>-</sup> CD123 <sup>+</sup> | CD34 <sup>+</sup> CD38 <sup>-</sup> CD33 <sup>+</sup> | CD34 <sup>+</sup> CD38 <sup>-</sup> CD45RA <sup>+</sup> |
|---------------------------|-----------------|-----------------------------------------------------------|--------------------------------------------------------|-------------------------------------------------------|---------------------------------------------------------|
|                           | Tumor burden    | MDS-SC                                                    | MDS-SC                                                 | MDS-SC                                                | MDS-SC                                                  |
| Sensitivity*              | 92.3%           | 92.3%                                                     | 61.5%                                                  | 96.2%                                                 | 80.8%                                                   |
| Specificity               | 15.4%           | 48.7%                                                     | 72.7%                                                  | 25.6%                                                 | 42.7%                                                   |
| Negative predictive value | 90.0%           | 96.6%                                                     | 89.5%                                                  | 96.8%                                                 | 90.9%                                                   |
| Positive predictive value | 19.5%           | 28.6%                                                     | 33.3%                                                  | 22.3%                                                 | 23.9%                                                   |
| Youden index              | 0.077           | 0.410                                                     | 0.342                                                  | 0.218                                                 | 0.235                                                   |
| C-index                   | 0.527           | 0.679                                                     | 0.640                                                  | 0.598                                                 | 0.621                                                   |

Abbreviations: MDS-SC= myelodysplastic syndromes stem cell, MFC=multiparameter flow cytometry

\* Sensitivity=True positive/(True positive + False negative); Specificity=True negative/(True negative + False positive); Negative predictive value=True negative/(True negative + False negative); Positive predictive value=True positive/(True positive + False positive); Youden's index=Sensitivity+Specificity-1.

True-negative refers tumor burden test negative patients who did not experience relapse. True-positive refers to tumor burden test positive patients who experienced relapse according to the literature (Bone Marrow Transplant, 2020;55(5):843-850).

**Table S6. Multivariate analysis of factors associated with outcomes of patients with myelodysplastic syndrome (n=143).**

| Covariate                                                | Univariate analysis |              |         | Multivariate analysis |              |         |
|----------------------------------------------------------|---------------------|--------------|---------|-----------------------|--------------|---------|
|                                                          | HR                  | 95% CI       | P-value | HR                    | 95% CI       | P-value |
| 3-year Relapse                                           |                     |              |         |                       |              |         |
| Pre-SCT MDS-SC* (high tumor burden vs. low tumor burden) | 9.651               | 2.278-40.897 | 0.002   | 8.587                 | 2.022-36.465 | 0.004   |
| IPSS-M (very high vs. others)                            | 3.717               | 1.615-8.556  | 0.002   | 3.219                 | 1.396-7.421  | 0.006   |
| 3-year NRM                                               |                     |              |         |                       |              |         |
| Grades III-IV acute GVHD (yes vs. no)                    | 5.654               | 1.412-22.633 | 0.014   | 5.654                 | 1.412-22.633 | 0.014   |
| 3-year DFS                                               |                     |              |         |                       |              |         |
| Pre-SCT MDS-SC* (high tumor burden vs. low tumor burden) | 2.676               | 1.214-5.898  | 0.015   | 2.374                 | 1.074-5.248  | 0.033   |
| IPSS-M (very high vs. others)                            | 3.609               | 1.766-7.373  | <0.001  | 3.324                 | 1.631-6.845  | 0.001   |
| 3-year OS                                                |                     |              |         |                       |              |         |
| IPSS-M (very high vs. others)                            | 3.701               | 1.608-8.521  | 0.002   | 3.701                 | 1.608-8.521  | 0.002   |

**Abbreviations:** HR=hazard ratio; CI=confidence interval; Pre-SCT=pre-stem cell transplantation; MDS-SC=myelodysplastic syndrome; IPSS-M = molecular international prognostic scoring system; NRM=nonrelapse mortality; GVHD=graft-versus-host disease; DFS=Disease-free survival; OS=overall survival

\* MDS-SC were determined by multiparameter flow cytometry according to CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> phenotype.

**Table S7. Transplant outcomes of MDS patients underwent chemotherapy classified into different subgroups according to CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> MDS-SC-based tumor burden before transplantation (n=50).**

|                                                                                      | 2-year CIR                     | 2-year NRM                   | 2-year DFS                      | 2-year OS                       |
|--------------------------------------------------------------------------------------|--------------------------------|------------------------------|---------------------------------|---------------------------------|
| CD34 <sup>+</sup> CD38 <sup>-</sup> cocktail <sup>+</sup> MDS-SC pre-transplantation |                                |                              |                                 |                                 |
| Low burden (n=23)                                                                    | 4.3%<br>(95% CI, 0.0%-12.9%)   | 4.3%<br>(95% CI, 0.0%-12.9%) | 91.3%<br>(95% CI, 79.7%-100.0%) | 91.3%<br>(95% CI, 79.7%-100.0%) |
| High burden (n=27)                                                                   | 44.4%<br>(95% CI, 25.2%-63.7%) | 0.0%<br>(95% CI, 0.0%-0.0%)  | 55.6%<br>(95% CI, 36.8%-74.4%)  | 63.0%<br>(95% CI, 44.8%-81.2%)  |
| P value                                                                              | 0.002                          | 0.279                        | 0.008                           | 0.028                           |

**Abbreviations:** MDS= myelodysplastic syndromes; MRD=minimal/measurable residual disease; MDS-SC= myelodysplastic syndromes stem cell; CIR=cumulative incidence of relapse; NRM=non-relapse mortality; DFS=Disease-free survival; OS=overall survival; CI=confidential interval

**Table S8. Transplant outcomes of MDS patients who didn't received chemotherapy classified into different subgroups according to CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> MDS-SC-based tumor burden before transplantation (n=93).**

|                                                                                      | 2-year CIR                     | 2-year NRM                   | 2-year DFS                     | 2-year OS                      |
|--------------------------------------------------------------------------------------|--------------------------------|------------------------------|--------------------------------|--------------------------------|
| CD34 <sup>+</sup> CD38 <sup>-</sup> cocktail <sup>+</sup> MDS-SC pre-transplantation |                                |                              |                                |                                |
| Low burden (n=72)                                                                    | 6.9%<br>(95% CI, 1.1%-12.9%)   | 9.7%<br>(95% CI, 2.8%-16.6%) | 83.3%<br>(95% CI, 74.7%-91.9%) | 87.2%<br>(95% CI, 79.4%-95.0%) |
| High burden (n=21)                                                                   | 35.2%<br>(95% CI, 12.9%-57.5%) | 4.8%<br>(95% CI, 0.0%-14.1%) | 60.0%<br>(95% CI, 38.0%-82.0%) | 76.2%<br>(95% CI, 58.0%-94.4%) |
| P value                                                                              | 0.003                          | 0.476                        | 0.057                          | 0.229                          |

**Abbreviations:** MDS= myelodysplastic syndromes; MRD=minimal/measurable residual disease; MDS-SC= myelodysplastic syndromes stem cell; CIR=cumulative incidence of relapse; NRM=non-relapse mortality; DFS=Disease-free survival; OS=overall survival; CI=confidential interval

**Table S9. Transplant outcomes of MDS patients classified into different subgroups according to traditional MFC method and MDS-SC-based MRD after transplantation (n=143).**

|                                                                                       | 3-year CIR                     | 3-year NRM                   | 3-year DFS                      | 3-year OS                      |
|---------------------------------------------------------------------------------------|--------------------------------|------------------------------|---------------------------------|--------------------------------|
| Traditional MFC-MRD post-transplantation                                              |                                |                              |                                 |                                |
| Negative (n=128)                                                                      | 12.8%<br>(95% CI, 6.9%-18.6%)  | 7.0%<br>(95% CI, 2.6%-11.5%) | 80.2%<br>(95% CI, 73.1%-987.3%) | 85.2%<br>(95% CI, 79.1%-91.3%) |
| Positive (n=15)                                                                       | 60.0%<br>(95% CI, 33.7%-86.3%) | 0.0%<br>(95% CI, 0.0%-0.0%)  | 40.0%<br>(95% CI, 15.3%-64.7%)  | 51.4%<br>(95% CI, 25.1%-77.7%) |
| P value                                                                               | <0.001                         | 0.292                        | <0.001                          | 0.002                          |
| CD34 <sup>+</sup> CD38 <sup>-</sup> cocktail <sup>+</sup> MDS-SC post-transplantation |                                |                              |                                 |                                |
| Negative (n=96)                                                                       | 6.5%<br>(95% CI, 1.4%-11.7%)   | 8.3%<br>(95% CI, 2.8%-13.9%) | 85.1%<br>(95% CI, 77.8%-92.4%)  | 90.6%<br>(95% CI, 84.7%-96.5%) |
| Positive (n=47)                                                                       | 40.4%<br>(95% CI, 26.2%-54.7%) | 2.1%<br>(95% CI, 0.0%-6.3%)  | 57.4%<br>(95% CI, 43.3%-71.5%)  | 63.1%<br>(95% CI, 49.0%-77.2%) |
| P value                                                                               | <0.001                         | 0.148                        | <0.001                          | <0.001                         |
| CD34 <sup>+</sup> CD38 <sup>-</sup> CD123 <sup>+</sup> MDS-SC post-transplantation    |                                |                              |                                 |                                |
| Negative (n=88)                                                                       | 10.5%<br>(95% CI, 3.9%-17.1%)  | 9.1%<br>(95% CI, 3.0%-15.1%) | 80.4%<br>(95% CI, 72.0%-88.8%)  | 86.4%<br>(95% CI, 79.1%-93.7%) |
| Positive (n=55)                                                                       | 29.1%<br>(95% CI, 17.0%-41.2%) | 1.8%<br>(95% CI, 0.0%-5.4%)  | 69.1%<br>(95% CI, 56.9%-81.3%)  | 74.0%<br>(95% CI, 62.2%-85.8%) |
| P value                                                                               | 0.004                          | 0.079                        | 0.116                           | 0.087                          |
| CD34 <sup>+</sup> CD38 <sup>-</sup> CD33 <sup>+</sup> MDS-SC post-transplantation     |                                |                              |                                 |                                |
| Negative (n=91)                                                                       | 9.1%<br>(95% CI, 3.0%-15.1%)   | 8.8%<br>(95% CI, 2.9%-14.6%) | 82.2%<br>(95% CI, 74.2%-90.2%)  | 87.9%<br>(95% CI, 81.2%-94.6%) |
| Positive (n=52)                                                                       | 32.7%<br>(95% CI, 19.8%-45.6%) | 1.9%<br>(95% CI, 0.0%-5.7%)  | 65.4%<br>(95% CI, 52.5%-78.3%)  | 70.6%<br>(95% CI, 58.1%-83.1%) |
| P value                                                                               | <0.001                         | 0.102                        | 0.017                           | 0.015                          |
| CD34 <sup>+</sup> CD38 <sup>-</sup> CD45RA <sup>+</sup> MDS-SC post-transplantation   |                                |                              |                                 |                                |

|                 |                                |                              |                                |                                |
|-----------------|--------------------------------|------------------------------|--------------------------------|--------------------------------|
| Negative (n=78) | 13.1%<br>(95% CI, 5.4%-20.7%)  | 6.4%<br>(95% CI, 0.9%-11.9%) | 80.5%<br>(95% CI, 71.7%-89.3%) | 87.2%<br>(95% CI, 79.8%-94.6%) |
| Positive (n=65) | 23.2%<br>(95% CI, 12.8%-33.6%) | 6.2%<br>(95% CI, 0.3%-12.0%) | 70.7%<br>(95% CI, 59.5%-81.9%) | 75.0%<br>(95% CI, 64.4%-85.6%) |
| P value         | 0.139                          | 0.946                        | 0.200                          | 0.084                          |

**Abbreviations:** MDS= myelodysplastic syndromes; MRD=minimal/measurable residual disease; MDS-SC= myelodysplastic syndromes stem cell; CIR=cumulative incidence of relapse; NRM=non-relapse mortality; DFS=Disease-free survival; OS=overall survival; CI=confidential interval

**Table S10. Comparison of diagnostic accuracy of traditional MFC method and MDS-SC-based MRD assay after transplantation (n=143).**

|                           | Traditional<br>MFC-MRD | CD34 <sup>+</sup> CD38 <sup>-</sup> cocktail <sup>+</sup><br>MDS-SC | CD34 <sup>+</sup> CD38 <sup>-</sup> CD123 <sup>+</sup><br>MDS-SC | CD34 <sup>+</sup> CD38 <sup>-</sup> CD33 <sup>+</sup><br>MDS-SC | CD34 <sup>+</sup> CD38 <sup>-</sup> CD45RA <sup>+</sup><br>MDS-SC |
|---------------------------|------------------------|---------------------------------------------------------------------|------------------------------------------------------------------|-----------------------------------------------------------------|-------------------------------------------------------------------|
| Sensitivity*              | 34.6%                  | 73.1%                                                               | 61.5%                                                            | 65.4%                                                           | 57.7%                                                             |
| Specificity               | 94.9%                  | 76.1%                                                               | 66.7%                                                            | 70.1%                                                           | 57.3%                                                             |
| Negative predictive value | 86.7%                  | 92.7%                                                               | 88.6%                                                            | 90.1%                                                           | 85.9%                                                             |
| Positive predictive value | 60.0%                  | 40.4%                                                               | 29.1%                                                            | 32.7%                                                           | 23.1%                                                             |
| Youden index              | 0.295                  | 0.491                                                               | 0.282                                                            | 0.355                                                           | 0.150                                                             |
| C-index                   | 0.650                  | 0.748                                                               | 0.649                                                            | 0.688                                                           | 0.582                                                             |

Abbreviations: MDS-SC= myelodysplastic syndromes stem cell, MRD=minimal/measurable residual disease; MFC=multiparameter flow cytometry

\* Sensitivity=True positive/(True positive + False negative); Specificity=True negative/(True negative + False positive); Negative predictive value=True negative/(True negative + False negative); Positive predictive value=True positive/(True positive + False positive); Youden's index=Sensitivity+Specificity-1.

True-negative refers MRD-test negative patients who did not experience relapse. True-positive refers to MRD-test positive patients who experienced relapse according to the literature (Bone Marrow Transplant, 2020;55(5):843-850).

**Table S11. Multivariate analysis of factors associated with outcomes of patients with myelodysplastic syndrome (n=143).**

| Covariate                                        | Univariate analysis |              |         | Multivariate analysis |              |         |
|--------------------------------------------------|---------------------|--------------|---------|-----------------------|--------------|---------|
|                                                  | HR                  | 95% CI       | P-value | HR                    | 95% CI       | P-value |
| 3-year Relapse                                   |                     |              |         |                       |              |         |
| Post-SCT MDS-SC* (positive vs. negative)         | 7.108               | 2.980-16.958 | <0.001  | 6.286                 | 2.533-15.601 | <0.001  |
| IPSS-M (very high vs. others)                    | 3.717               | 1.615-8.556  | 0.002   | 5.613                 | 2.331-13.516 | <0.001  |
| Post-Traditional MFC MRD (positive vs. negative) | 7.324               | 3.207-16.723 | <0.001  | 5.806                 | 2.348-14.357 | <0.001  |
| 3-year NRM                                       |                     |              |         |                       |              |         |
| Grades III-IV acute GVHD (yes vs. no)            | 5.654               | 1.412-22.633 | 0.014   | 5.654                 | 1.412-22.633 | 0.014   |
| 3-year DFS                                       |                     |              |         |                       |              |         |
| Post-SCT MDS-SC* (positive vs. negative)         | 3.426               | 1.750-6.708  | <0.001  | 3.088                 | 1.520-6.274  | 0.002   |
| IPSS-M (very high vs. others)                    | 3.609               | 1.766-7.373  | <0.001  | 4.636                 | 2.220-9.682  | <0.001  |
| Post-Traditional MFC MRD (positive vs. negative) | 4.570               | 2.121-9.845  | <0.001  | 4.033                 | 1.767-9.204  | 0.001   |
| 3-year OS                                        |                     |              |         |                       |              |         |
| Post-SCT MDS-SC* (positive vs. negative)         | 4.267               | 1.900-9.585  | <0.001  | 3.827                 | 1.627-9.002  | 0.001   |
| IPSS-M (very high vs. others)                    | 3.701               | 1.608-8.521  | 0.002   | 4.551                 | 1.937-10.695 | 0.002   |
| Post-Traditional MFC MRD (positive vs. negative) | 3.578               | 1.502-8.524  | 0.004   | 2.632                 | 1.034-6.701  | 0.042   |

**Abbreviations:** HR=hazard ratio; CI=confidence interval; Pre-SCT=pre-stem cell transplantation; MDS-SC=myelodysplastic syndrome; IPSS-M = molecular international prognostic scoring system; NRM=nonrelapse mortality; GVHD=graft-versus-host disease; DFS=Disease-free survival; OS=overall survival

\* MDS-SC were determined by multiparameter flow cytometry according to CD34<sup>+</sup>CD38<sup>-</sup>cocktail<sup>+</sup> phenotype.

**Table S12. Transplant outcomes of MDS patients classified into different subgroups according to pre-transplantation MDS-SC-based tumor burden and post-transplantation MDS-SC-based MRD (n=143).**

|                                               | 2-year CIR                        | 2-year NRM                    | 2-year DFS                        | 2-year OS                         |
|-----------------------------------------------|-----------------------------------|-------------------------------|-----------------------------------|-----------------------------------|
| Traditional MFC-MRD post-transplantation      |                                   |                               |                                   |                                   |
| Group1: Pre-MDS-SCneg & post-MDS-SCneg (n=46) | 0.0% * † ‡<br>(95% CI, 0.0%-0.0%) | 10.9%<br>(95% CI, 1.8%-20.0%) | 89.1% †<br>(95% CI, 80.1%-98.1%)  | 89.1% **<br>(95% CI, 80.1%-98.1%) |
| Group2: Pre-MDS-SCpos & post-MDS-SCneg (n=50) | 12.8% #<br>(95% CI, 3.0%-22.5%)   | 6.0%<br>(95% CI, 0.0%-12.7%)  | 81.2% ##<br>(95% CI, 71.0%-91.4%) | 92.0% #<br>(95% CI, 84.6%-99.4%)  |
| Group3: Pre-MDS-SCneg & post-MDS-SCpos (n=13) | 15.4% \$<br>(95% CI, 0.0%-35.9%)  | 7.7%<br>(95% CI, 0.0%-22.8%)  | 76.9%<br>(95% CI, 54.0%-99.8%)    | 84.6%<br>(95% CI, 57.0%-100.0%)   |
| Group4: Pre-MDS-SCpos & post-MDS-SCpos (n=34) | 50.0%<br>(95% CI, 32.8%-67.2%)    | 0.0%<br>(95% CI, 0.0%-0.0%)   | 50.0%<br>(95% CI, 33.1%-66.9%)    | 58.8%<br>(95% CI, 42.3%-75.3%)    |

**Abbreviations:** MDS= myelodysplastic syndromes; MRD=minimal/measurable residual disease; MDS-SC= myelodysplastic syndromes stem cell; CIR=cumulative incidence of relapse; NRM=non-relapse mortality; DFS=Disease-free survival; OS=overall survival; CI=confidential interval

\* indicates  $P=0.005$  comparison between group 1 and group 2

† indicates  $P=0.007$  comparison between group 1 and group 3

‡ indicates  $P<0.001$  comparison between group 1 and group 4

£ indicates  $P=0.004$  comparison between group 1 and group 4

\*\* indicates  $P=0.002$  comparison between group 1 and group 4

# indicates  $P<0.001$  comparison between group 2 and group 4

## indicates  $P=0.002$  comparison between group 2 and group 4

\$ indicates  $P=0.040$  comparison between group 3 and group 4

**Table S13. Transplant outcomes of MDS patients classified into different subgroups according to pre-transplantation MDS-SC-based tumor burden and post-transplantation MDS-SC-based MRD (n=143).**

|                                                | 2-year CIR                      | 2-year NRM                    | 2-year DFS                       | 2-year OS                        |
|------------------------------------------------|---------------------------------|-------------------------------|----------------------------------|----------------------------------|
| Traditional MFC-MRD post-transplantation       |                                 |                               |                                  |                                  |
| Group1: Pre-MDS-SCneg & post-MDS-SCneg (n=46)  | 0.0% * †<br>(95% CI, 0.0%-0.0%) | 10.9%<br>(95% CI, 1.8%-20.0%) | 89.1% †<br>(95% CI, 80.1%-98.1%) | 89.1% £<br>(95% CI, 80.1%-98.1%) |
| Group2: Pre-MDS-SCpos or post-MDS-SCpos (n=63) | 13.4% ‡<br>(95% CI, 4.6%-22.2%) | 6.3%<br>(95% CI, 0.3%-12.4%)  | 80.3% #<br>(95% CI, 70.1%-90.5%) | 88.4% #<br>(95% CI, 80.4%-96.4%) |
| Group3: Pre-MDS-SCpos & post-MDS-SCpos (n=34)  | 50.0%<br>(95% CI, 32.8%-67.2%)  | 0.0%<br>(95% CI, 0.0%-0.0%)   | 50.0%<br>(95% CI, 33.1%-66.9%)   | 58.8%<br>(95% CI, 42.3%-75.3%)   |

**Abbreviations:** MDS= myelodysplastic syndromes; MRD=minimal/measurable residual disease; MDS-SC= myelodysplastic syndromes stem cell; CIR=cumulative incidence of relapse; NRM=non-relapse mortality; DFS=Disease-free survival; OS=overall survival; CI=confidential interval

\* indicates  $P=0.005$  comparison between group 1 and group 2

† indicates  $P<0.001$  comparison between group 1 and group 3

£ indicates  $P=0.002$  comparison between group 1 and group 3

‡ indicates  $P<0.001$  comparison between group 2 and group 3

# indicates  $P=0.001$  comparison between group 2 and group 3

## Key resources table

| REAGENT or RESOURCE                   | SOURCE                  | IDENTIFIER              |
|---------------------------------------|-------------------------|-------------------------|
| Antibodies                            |                         |                         |
| PE anti-human CD7                     | Biolegend               | #Cat 343106             |
| PE anti-human CD11b                   | Biolegend               | #Cat 301306             |
| PE anti-human CD22                    | Biolegend               | #Cat 363504             |
| PE anti-human CD366 (Tim-3)           | Biolegend               | #Cat 345006             |
| PerCP/Cyanine5.5 anti-human CD123     | Biolegend               | #Cat 306016             |
| FITC anti-human CD45RA                | Biolegend               | #Cat 304106             |
| Brilliant Violet 421™ anti-human CD34 | Biolegend               | #Cat 343610             |
| APC anti-human CD38                   | Biolegend               | #Cat 303510             |
| Brilliant Stain Buffer                | BD Pharmingen           | #Cat 563794             |
| Hu/NHP CD56 PE                        | BD Pharmingen           | #Cat 556647             |
| Hu CD371 PE                           | BD Pharmingen           | #Cat 562566             |
| Hu CD45 Horizon V500                  | BD Pharmingen           | #Cat 560777             |
| PE/Cyanine7 anti-human CD33           | Biolegend               | #Cat 303434             |
| Software and algorithms               |                         |                         |
| R                                     | R                       | R                       |
| Seurat                                | Seurat                  | Seurat                  |
| ggplot2 package                       | ggplot2 package         | ggplot2 package         |
| GSVA R package                        | GSVA R package          | GSVA R package          |
| msigdb R package                      | msigdb R package        | msigdb R package        |
| clusterProfiler package               | clusterProfiler package | clusterProfiler package |
| pathview package                      | pathview package        | pathview package        |
| SPSS software                         | SPSS software           | SPSS software           |