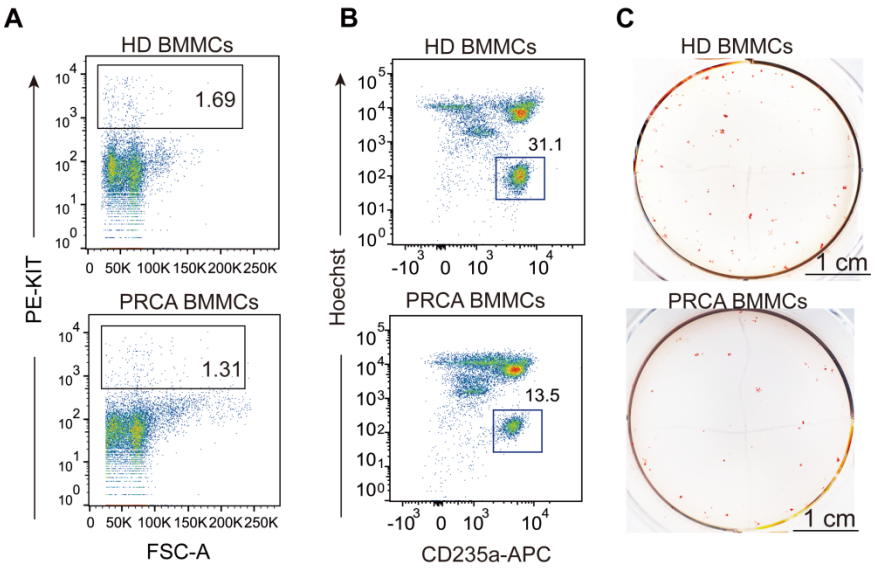
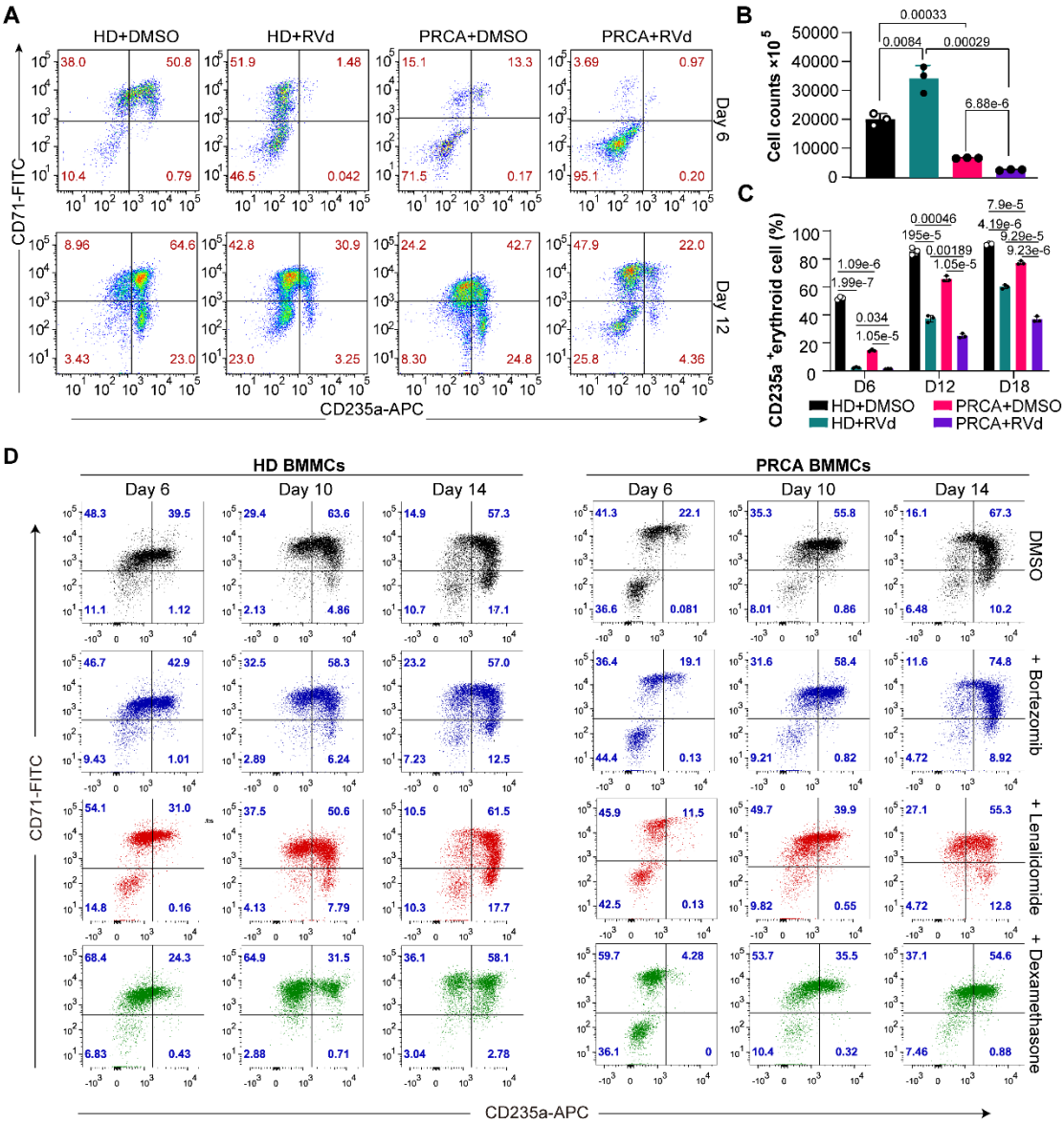
Supplemental material

Supplemental Figure 1:



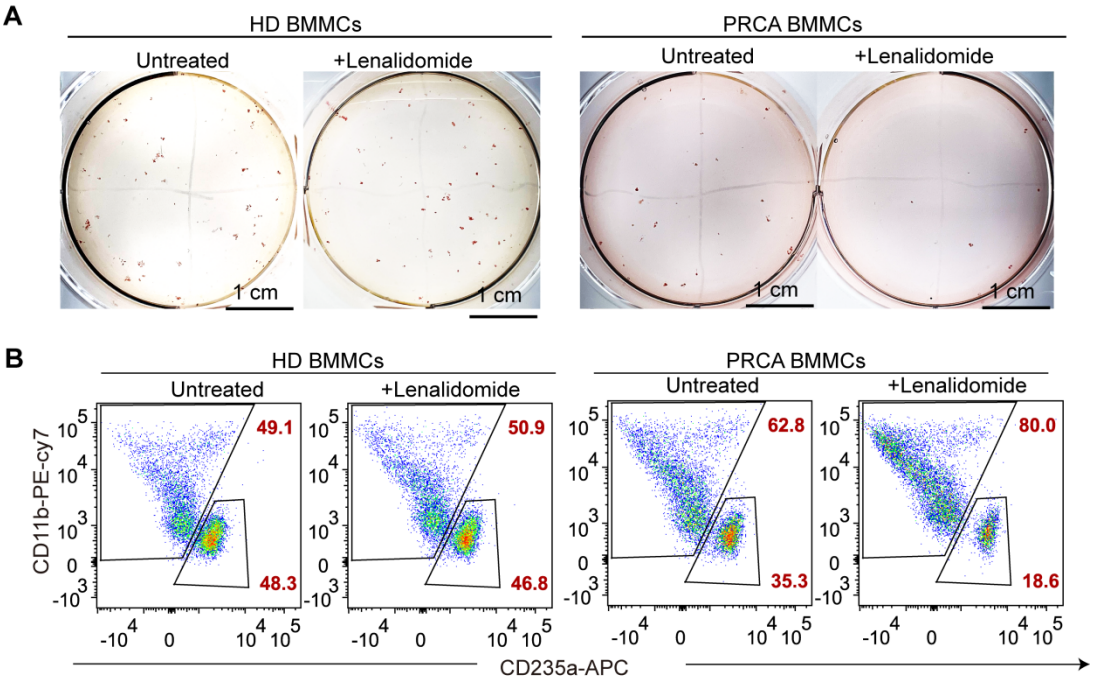
A, Representative flow cytometry results showing the percentages of HSPCs in bone marrow from healthy donors (HD, upper) and the PRCA patient (lower). **B,** Representative flow cytometry results showing the percentages of enucleated cells of BMMCs from HD (upper) and the PRCA patient (lower) after undergoing ex vivo erythroid culture. **C,** Representative results of colony-forming assays performed with BMMCs from the PRCA patient and HD. A total of 10,000 cells were seeded in each 3.5 cm diameter plate, and the colonies were counted after 14 days. The experiment was repeated three times with similar results.

Supplemental Figure 2:



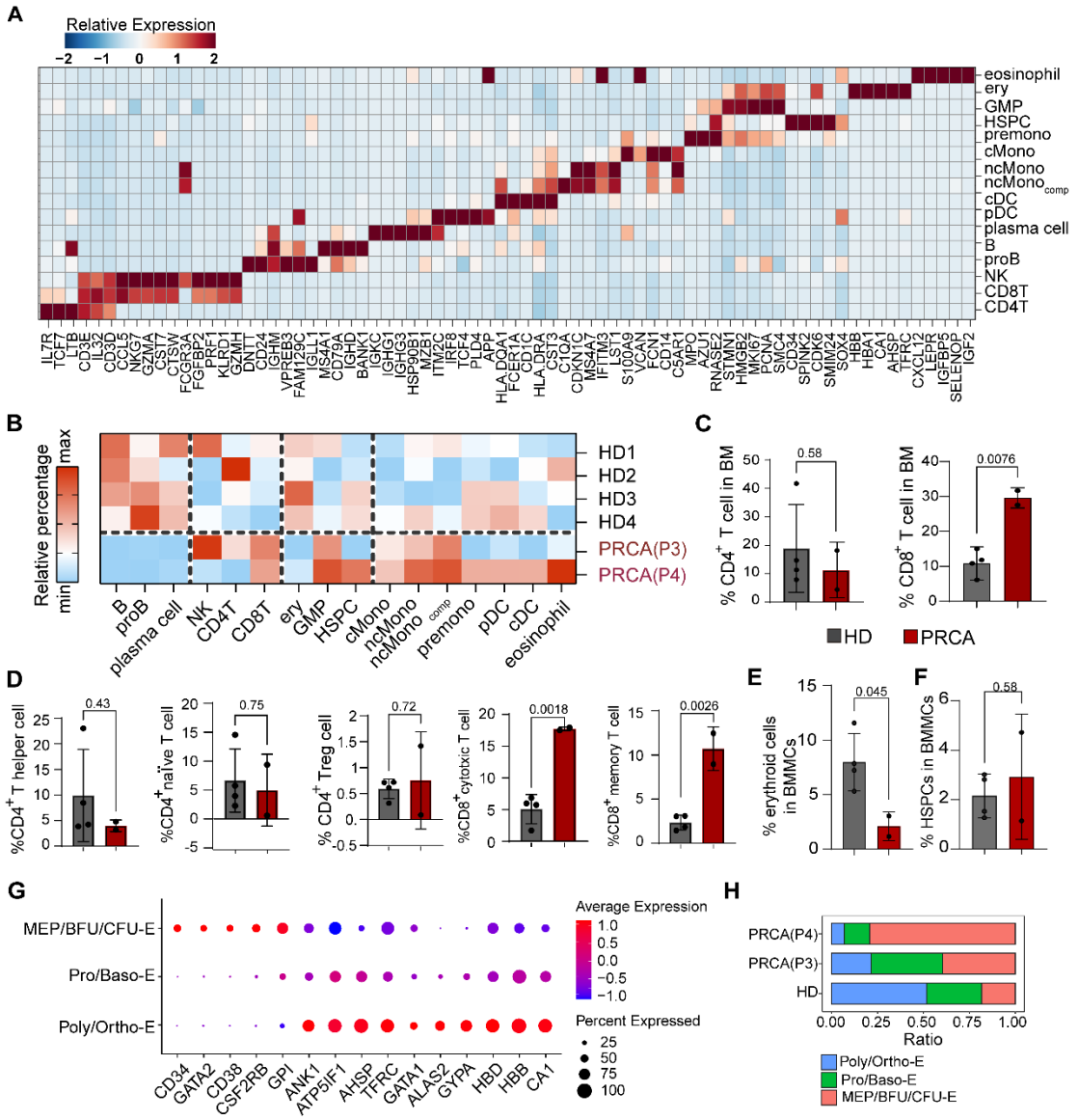
A, BMMCs from HD (left) and PRCA (right) were cultured in serum-free erythroid medium, with or without RVd. Dexamethasone was administered at 250 nM, lenalidomide at 250 nM, and bortezomib at 10 nM. Flow cytometry analyses were performed on days 6 and 12. **B,** Quantification of the impact of drugs on erythroid proliferation. Cell numbers were counted on day 18 from three independent biological replicates. **C,** Quantification of CD235a+ erythroid cells on day 6, 12, and 18 (n=3, biological replicates). Data are presented as mean values +/- SEM. Two-tailed paired Student's t-test. **D,** BMMCs from HD (left) and the PRCA patient (right) were cultured in serum-free erythroid medium with indicated treatments. Flow cytometry analyses were performed on days 6, 10, and 14. The experiment was repeated three times with similar results.

Supplemental Figure 3:



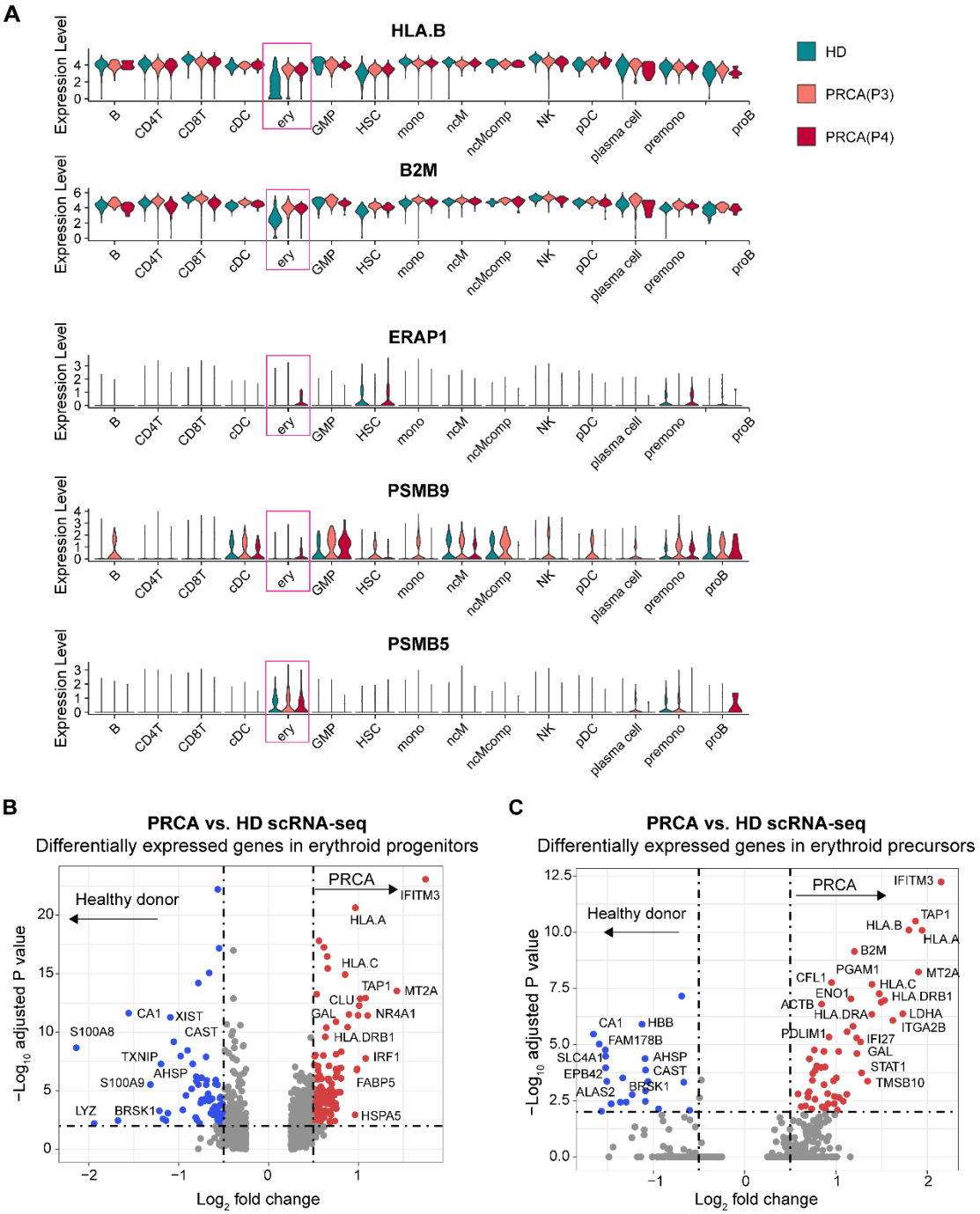
A, Colony-forming assays of BMMCs from the PRCA patient or HD, with or without lenalidomide. A total of 10,000 cells were seeded in each 3.5 cm diameter plate, and the colonies were counted after 14 days. The experiment was repeated three times with similar results. **B,** Representative flow cytometry results showing the percentages of myeloid and erythroid lineages in total cells washed from each colony plate in Extended Data Fig. 2B. Cells were distinguished by the myeloid marker CD11b and the erythroid marker CD235a.

Supplemental Figure 4:



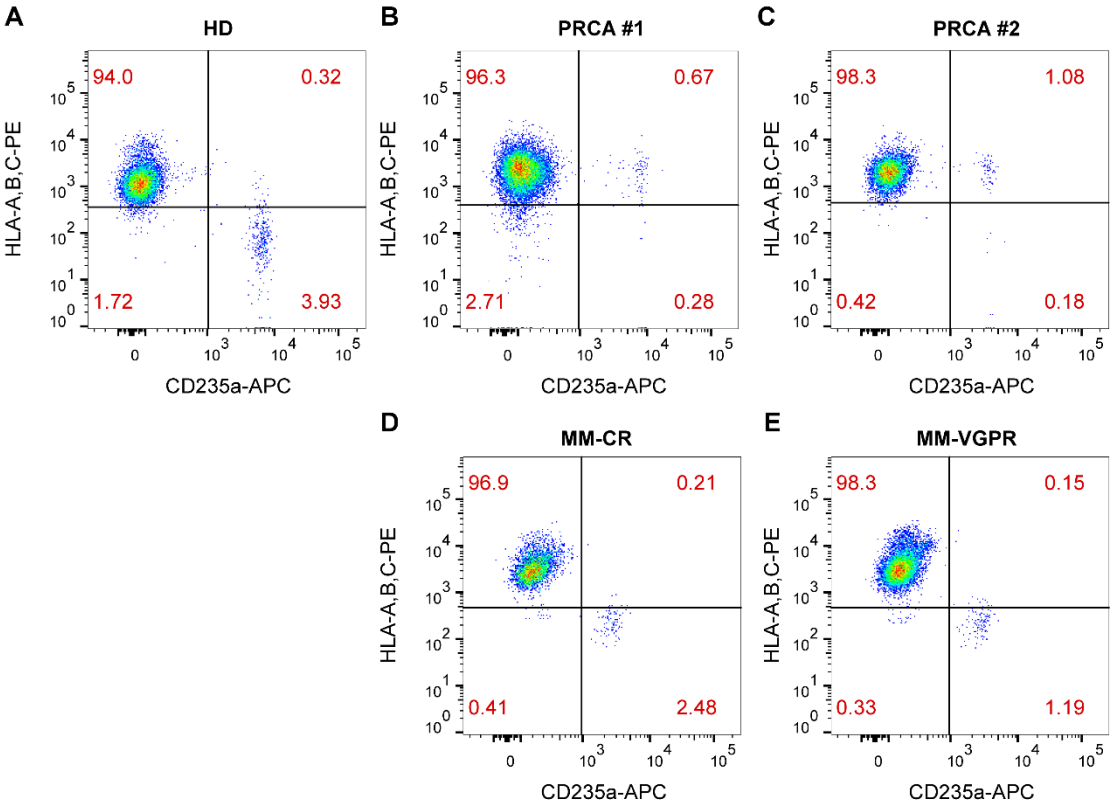
A, Heatmap showing the normalized mean expression levels of discriminative gene sets for each cell type in the bone marrow. **B,** Distributions of cell types in BMMCs from the PRCA patient (n = 2; samples from two PRCA timepoints of the patient as biological replicates) and HD (n = 4; samples from four individuals as biological replicates). Cell-type fractions are standardized by Z scores across different samples. **C,** Percentages of CD4+ (left) and CD8+ T cells (right) in the bone marrow. Two-tailed paired Student's t-test. **D,** Percentages of CD4+ helper, CD4+ naïve, regulatory T cells, CD8+ cytotoxic, and CD8+ memory T cells in the bone marrow. Two-tailed paired Student's t-test. **E-F,** Percentages of erythroid cells (**E**) and HSPCs (**F**) in bone marrow obtained from single-cell RNA sequencing. Data are presented as mean values +/- SEM. Two-tailed paired Student's t-test. **G,** Dot plot showing the normalized mean expression levels of discriminative gene sets for each erythroid cell type. **H,** Proportional distribution of erythroid cell types in the bone marrow across different samples.

Supplemental Figure 5:



A, Expression levels of antigen processing and proteasome genes in BMMCs based on scRNA-Seq. **B,** Volcano plot of genes differentially expressed in erythroid progenitors (CD71+CD235-) from PRCA patient and healthy donors(HD), 200 erythroid progenitor cells were used in each sample for DEG analysis. Genes with log2(fold change) > 0.5 or < -0.5 are shown in red or blue. C, Volcano plot of genes differentially expressed in erythroid precursors (CD71+CD235+) from PRCA patient and HD, 60 erythroid precursor cells were used in each sample for DEG analysis. Genes with log2(fold change) > 0.5 or < -0.5 are shown in red or blue.

Supplemental Figure 6:

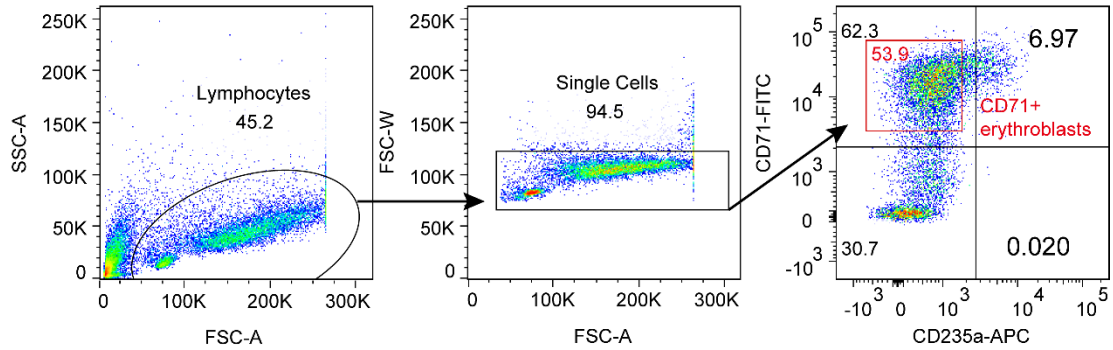


A-E, Representative flow cytometry results showing MHC-I levels on erythroid cells in the PBMCs from a healthy donor (HD) (A), PRCA patient #1 (B), PRCA patient #2 (C), and two multiple myeloma patients treated with three courses of RVd, achieving complete response (CR) (D) and very good partial responses (VGPR) (E), respectively.

The sample from PRCA patient #1 was collected from Peking University People's Hospital and is the primary focus of this study. The sample from PRCA patient #2 was collected from Peking Union Medical College Hospital[1] to validate of the expression of MHC-I on erythroid cells.

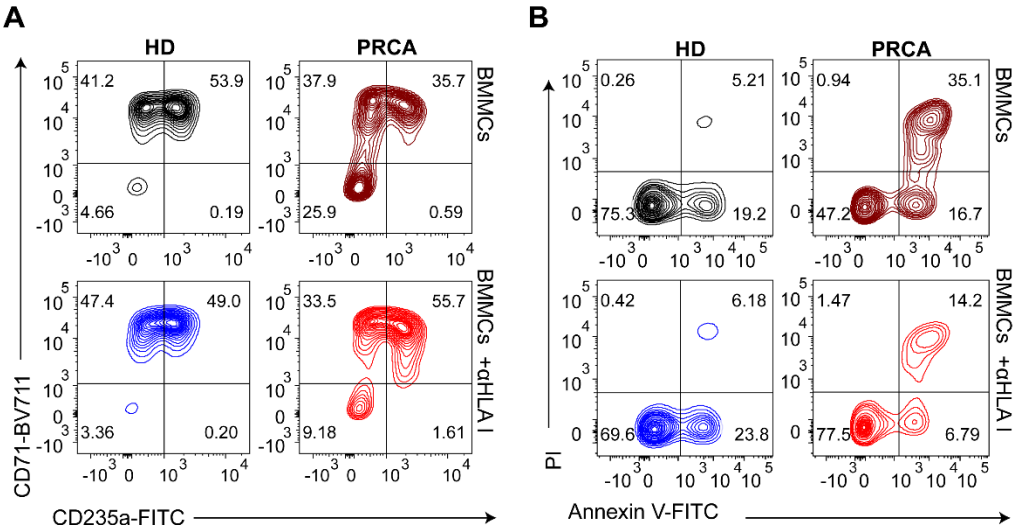
Supplemental Figure 7:

A



A, Flow cytometric gating strategy used for sorting CD71+ erythroblasts.

Supplemental Figure 8 (Fig 6):



A, BMMCs from HD (left) and PRCA (right) were cultured in the coculture medium, with or without anti-HLA class I antibody. Flow cytometry analyses were performed on day 9. **B,** Apoptosis assay on erythroblasts from (A). Cells were cultured in coculture medium, with or without anti-HLA class I antibody. Apoptosis ratio was examined by propidium Iodide (PI) and Annexin-V staining.

References

1. Jin X*, et al.* Pure red cell aplasia and minimal residual disease conversion associated with immune reconstitution in a patient with high-risk multiple myeloma. *Chronic Diseases and Translational Medicine* **9**, 341-344 (2023).