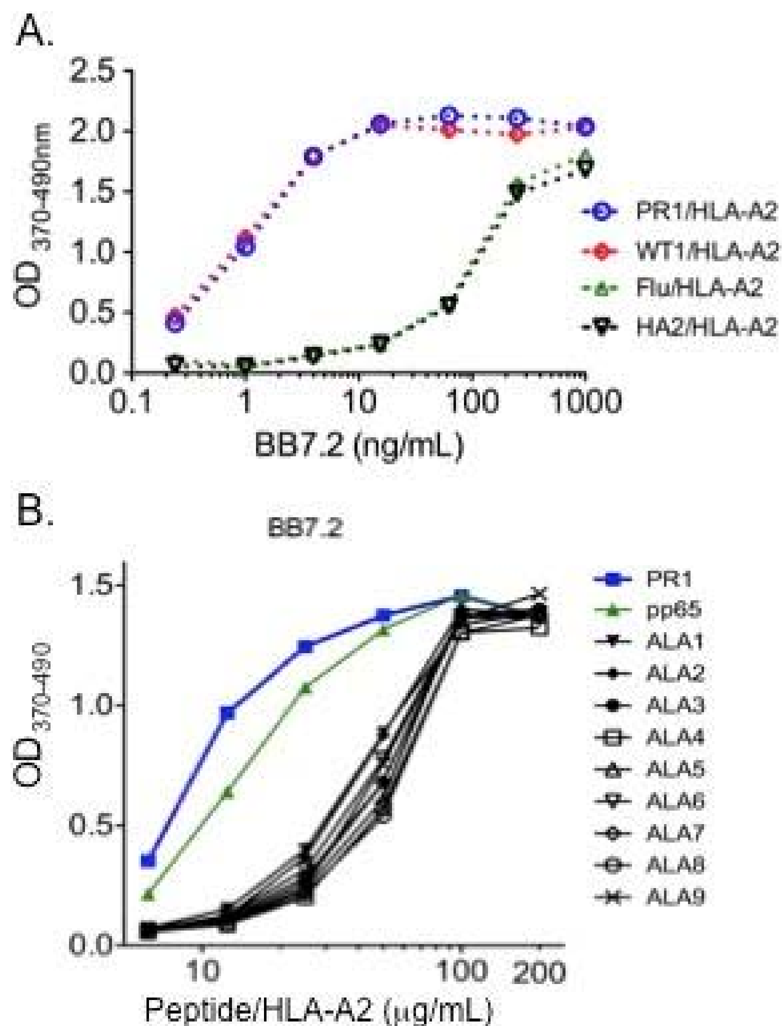
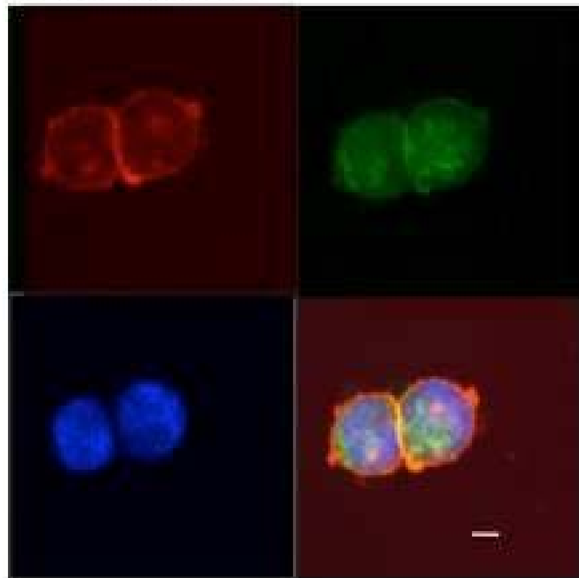


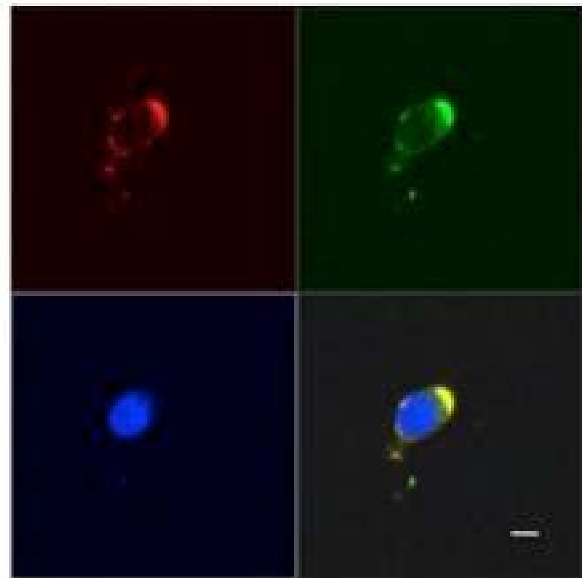
Figure S1

(A) ELISA assay showing binding of BB7.2, a control HLA-A2 specific antibody, to monomers consisting of HLA-A2 and PR1 (blue circles) or control peptides WT1 (red diamonds), Flu (green triangles) and HA-2 (black triangles), which increases with increasing BB7.2 concentration. **(B)** Control antibody BB7.2, which binds HLA-A2 independent of the bound peptide, shows similar binding to PR1/HLA-A2 and pp65/HLA-A2 monomer in ELISA. BB7.2 binding to monomers consisting of HLA-A2, β2m and modified PR1 peptide (VLQELNVTV) analogues constructed with alanine substitutions at position 1 to 9 (ALA1-ALA9). BB7.2 shows similar binding to all modified peptide/HLA-A2 monomers.

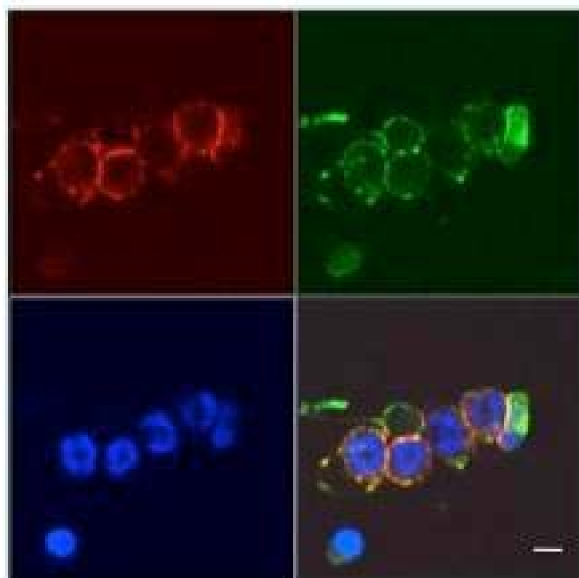
A. AML1



B. AML2



C. AML3



D. AML5

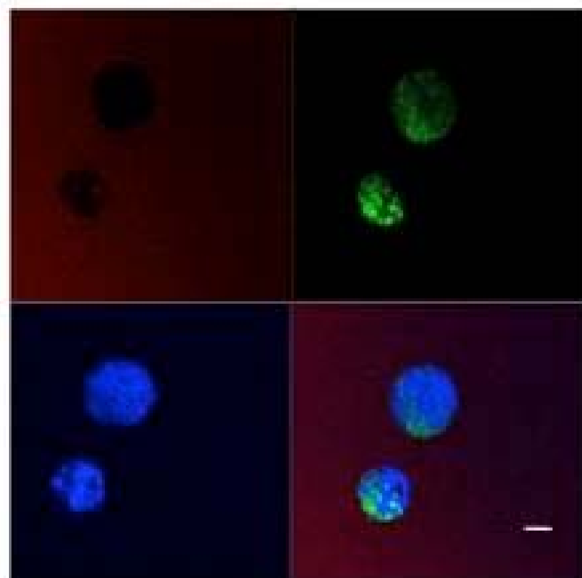


Figure S2

Heterogeneous PR1 expression on AML cells. Primary leukemia cells (from patients in Table 1) were co-stained with Alexa Fluor (AF) 488-conjugated anti-HLA-A2 (green) (left panels) and AF647-conjugated 8F4 (red) and Dapi (blue, middle panels). **(A)** AML-M1; **(B)** AML-M2; **(C)** AML-M5; **(D)** AML-M1. Images were viewed using Leica Microsystems SP2 SE confocal microscope (Illinois, USA) with 10x/25 air, 63x/1.4 oil objectives and Leica Type F immersion liquid. Leica LCS software (version 2.61) was used for image analysis. Scale bar on merged images, 10µm.

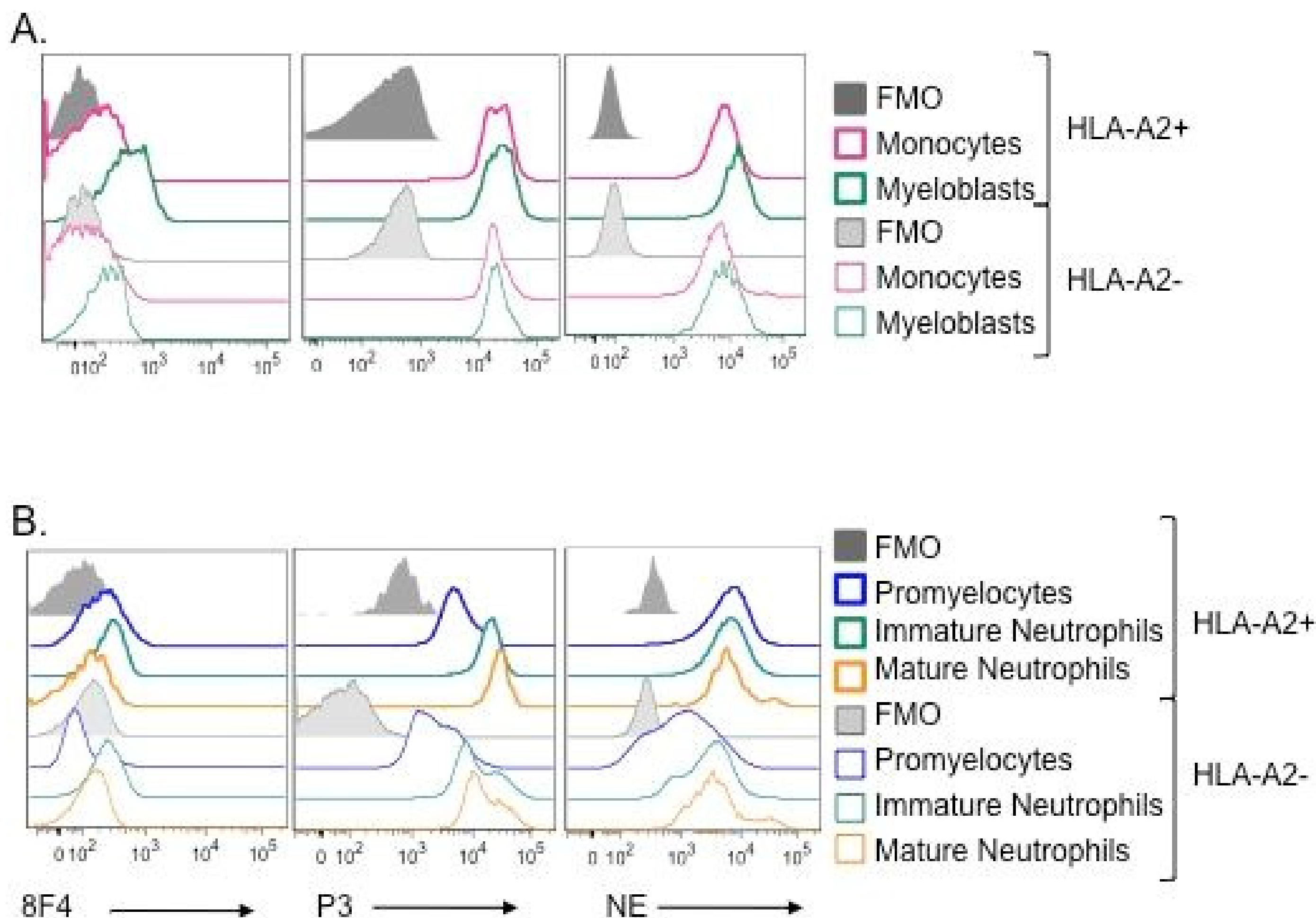


Figure S3

Expression of HLA-A2/PR1, NE and P3 during normal granulopoiesis in healthy bone marrow. Healthy donor ND10 fresh bone marrow cells were stained with fluorochrome-conjugated antibodies directed to CD45, CD33, CD11b, CD16, and HLA-A2 and 8F4, or NE and P3. Granulocytes were identified based on scatter characteristics and then examined for expression of CD11b and CD16. Promyelocytes were identified as $CD11b^{hi}/CD16^{lo}$; Immature Granulocytes were $CD11b^{low}/CD16^{lo}$; Mature Granulocytes stained brightly for both markers CD11b and CD16. Cells were fixed and permeabilized prior to NE and P3 staining to measure intracellular proteases. **(A)** P3 and NE are expressed in myeloblasts and monocytes, and PR1/HLA-A2 is weakly expressed on HLA-A2+ myeloblasts. **(B)** PR1/HLA-A2 is weakly expressed on promyelocytes and immature granulocytes, but not on mature granulocytes, while P3 and NE are expressed in all myeloid cells.

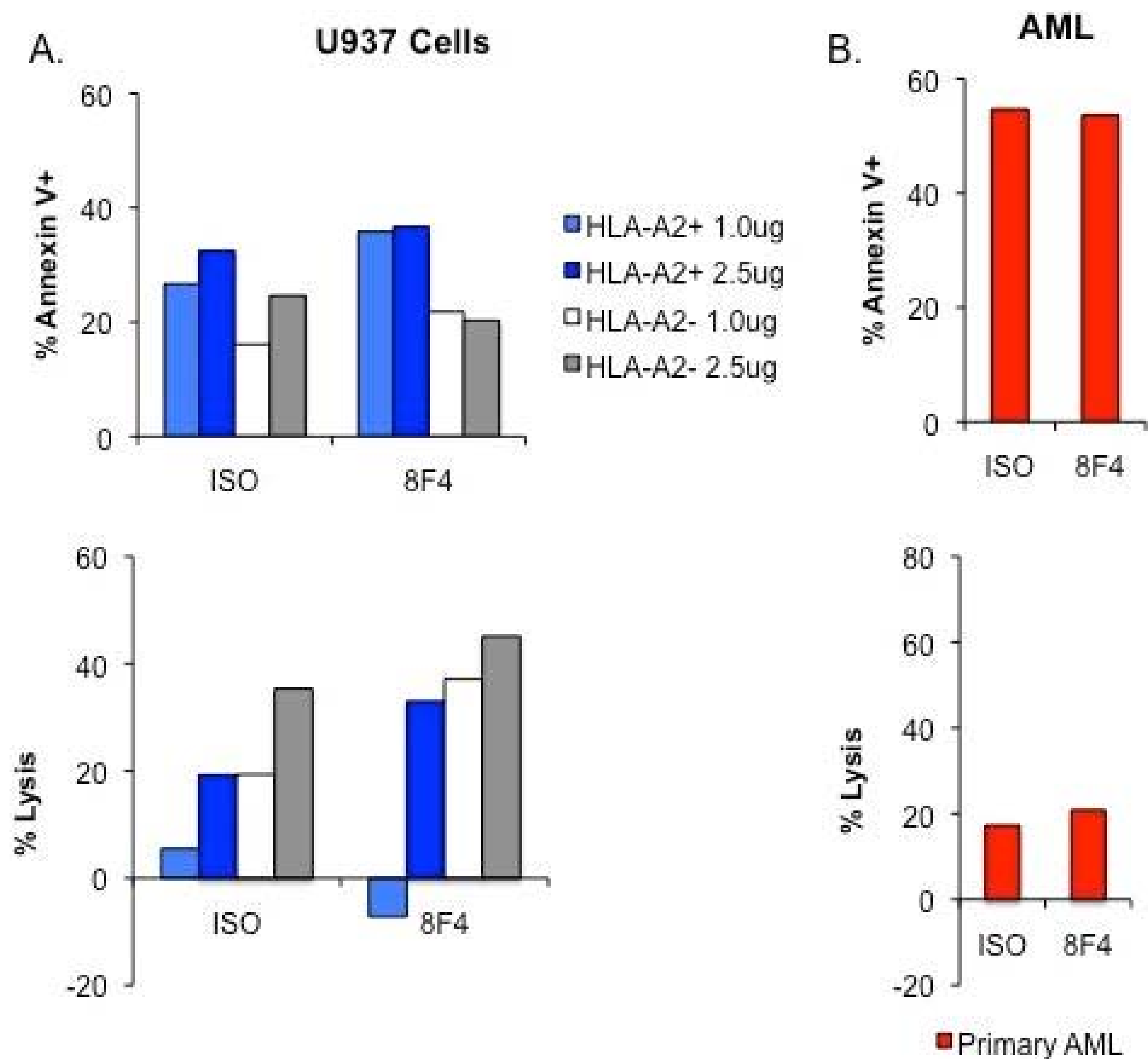


Figure S4

8F4 does not directly mediate apoptosis or cytolysis of PR1/HLA-A2+ cells. HLA-A2 transfected or non-transfected U937 cells or AML9 were plated in replicate with 8F4 or isotype control mAb for 12 hours-5 days. Cells were stained with Annexin V and Sytox Red, counted with counting beads and examined by flow cytometry. **(A)** Left panels show U937 at 60hrs and **(B)** right panels show HLA-A2+ blasts from patient AML9 after 36hrs. Upper panels in A and B show %Annexin V+ cells; whereas the lower panels show %Lysis relative to untreated viable cell counts.