Supporting Information to:

ATM-mediated up-regulation of NKG2D ligands on leukemia cells by resveratrol results in enhanced NK cells susceptibility.

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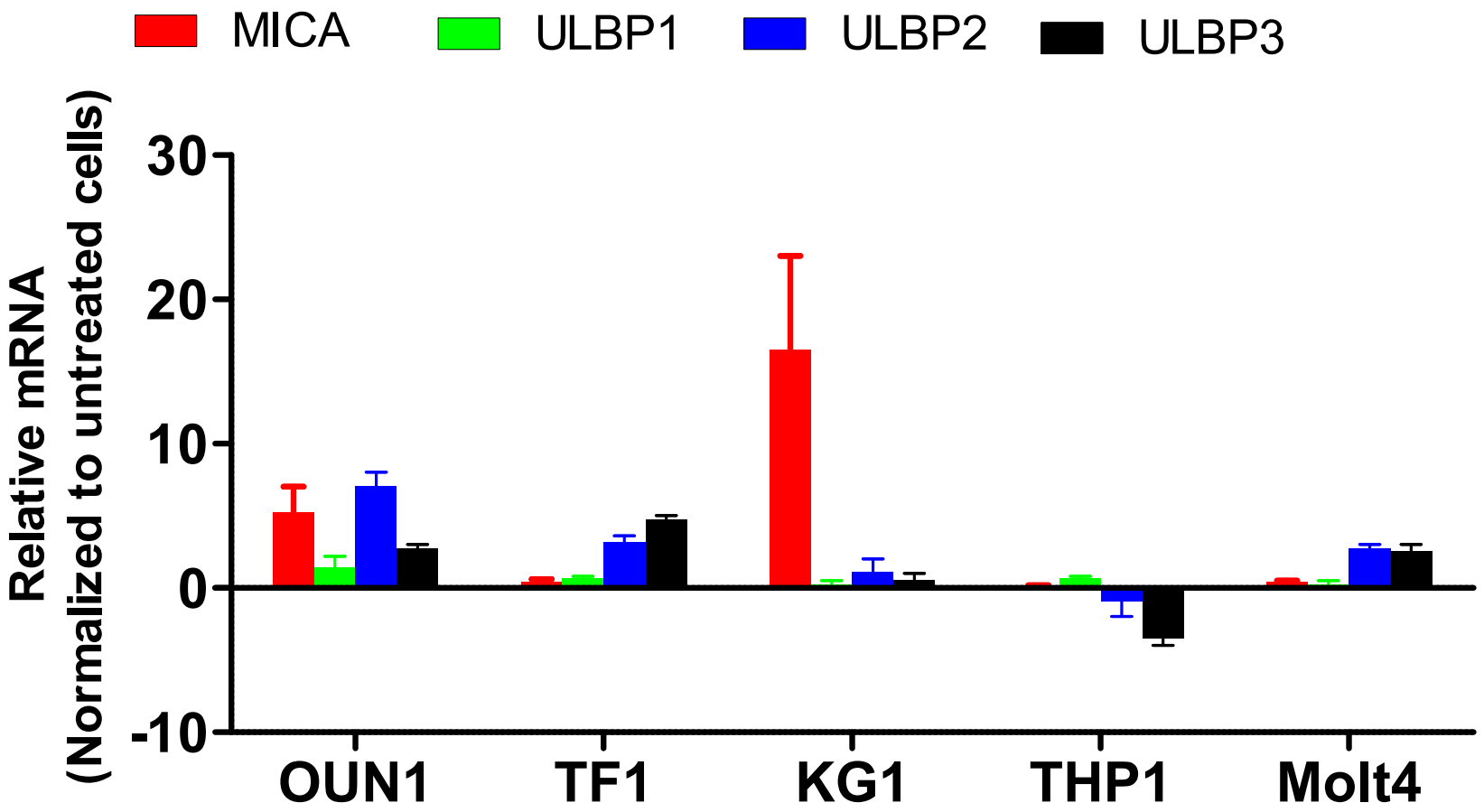
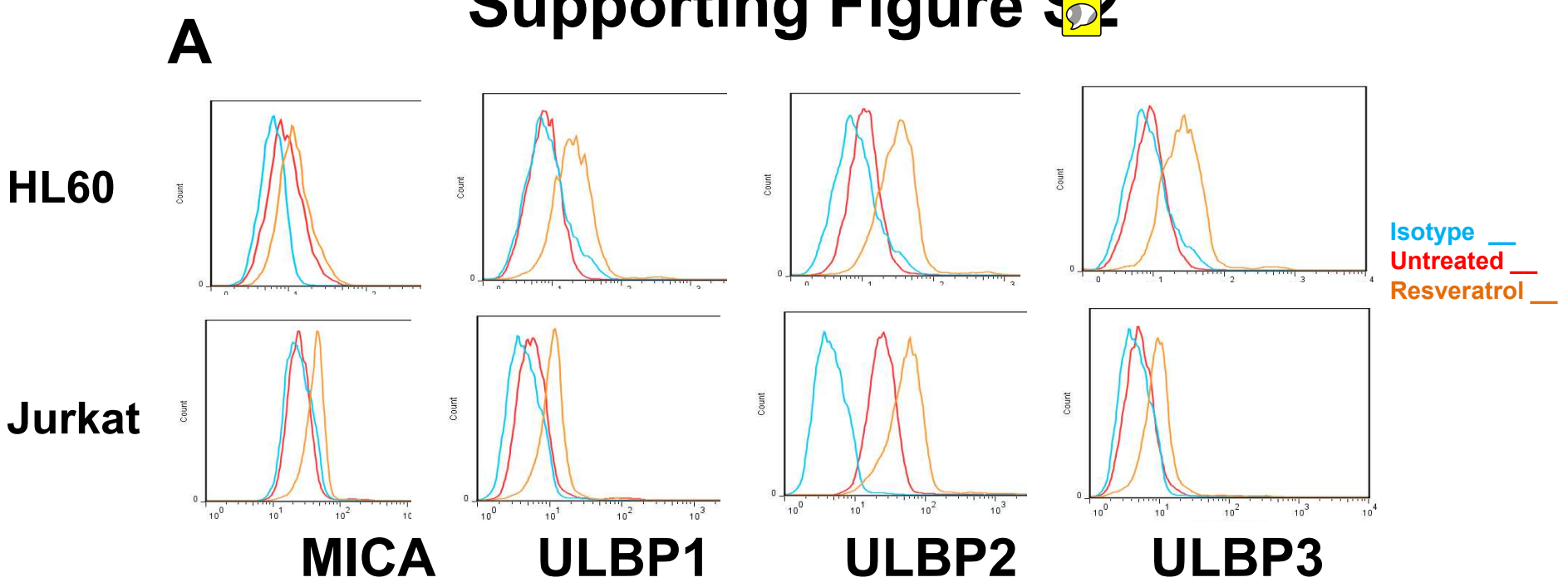
Supporting Figures 1 – 3

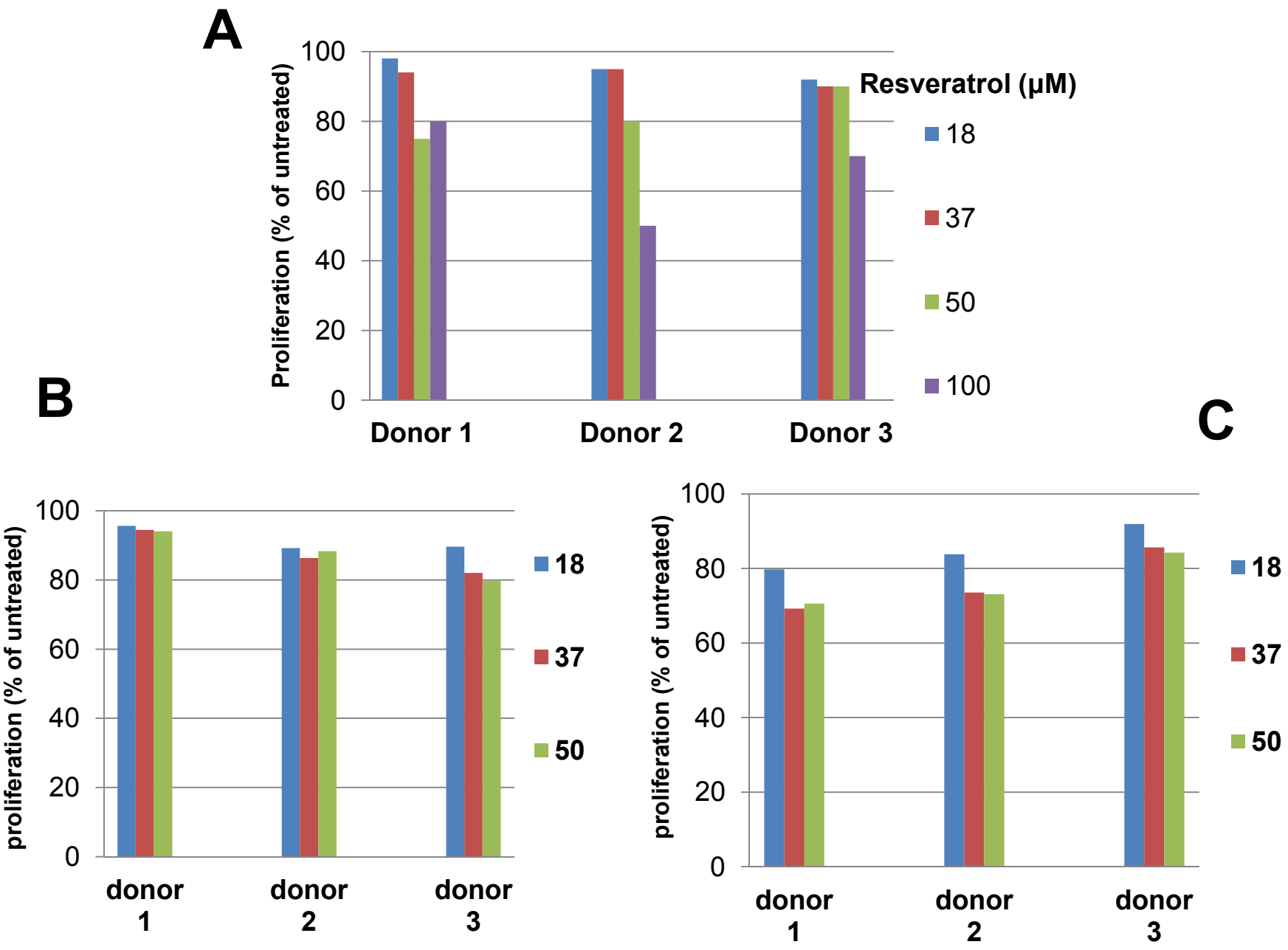
Supporting Figure S1



Supporting Figure S2



Supporting Figure S3



Supporting Figure Legends

Supporting figure S1:

Effect of resveratrol on the expression of NKG2D-Ls transcript in leukemia cells

Leukemia cells were cultured for 24 hours with resveratrol (37 μM) or with vehicle. Total RNA was extracted and the expression of NKG2D-Ls transcripts including MICA, ULBP1, ULBP2, and ULBP3 were assessed by RT-PCR. The transcripts levels relative to those of β actin and normalized to those of untreated cells measured in three independent experiments are shown.

Supporting Figure S2.

Role of p53 signaling in NKG2D-L expression induced by resveratrol

(A) The p53 null HL60 cells and Jurkat were treated with 37 μM of resveratrol or vehicle for 48 hours and stained with antibodies specific for MICA, ULBP1, ULBP2, and ULBP3 and analyzed with flow cytometry. Representative results of three independent experiments are shown.

(B) Leukemia cells, including OUN1, TF1 and Molt4 cells, were treated with Pifithrin-α (10 μM) for two hours and then cultured in the presence of resveratrol for another 48 hours and their expression of NKG2D-Ls was assessed with flow cytometry. A representative result from three independent experiments using Molt4 cells is shown.

(C) HL60 and Jurkat cells were treated with resveratrol (37 μM) for 12 hours and a cell cycle analysis was performed using flow cytometry. Representative results of three independent experiments are shown.

Supporting Figure 3.

Effect of resveratrol on activated NK cells, T cells and PHA-activated PBMC:

A) Purified NK cells from three different donors were resuspended in NK culture medium supplemented with IL-2 (100 U/ml) and IL-15 (10 ng/ml) and cultured for 48 hours and thereafter cultured for other 48 hours in the presence or absence of resveratrol and assessed for viability and proliferation using an MTT colorimetric assay (Roche, Indianapolis, IN, USA).

B) T cells were activated by culturing PBMC from three different donors in RPMI medium supplemented with 10% FBS and anti CD3 anti CD28 beads (invitrogen) for 48 hours and cultured for other 48 hours in the presence or absence of resveratrol and assessed for viability and proliferation using an MTT colorimetric assay .

C) PBMC were treated with 5 µg/ml of PHA for 48 hours and thereafter cultured for other 48 hours with or without resveratrol and assessed with an MTT assay. The figure shows results from three different donors.