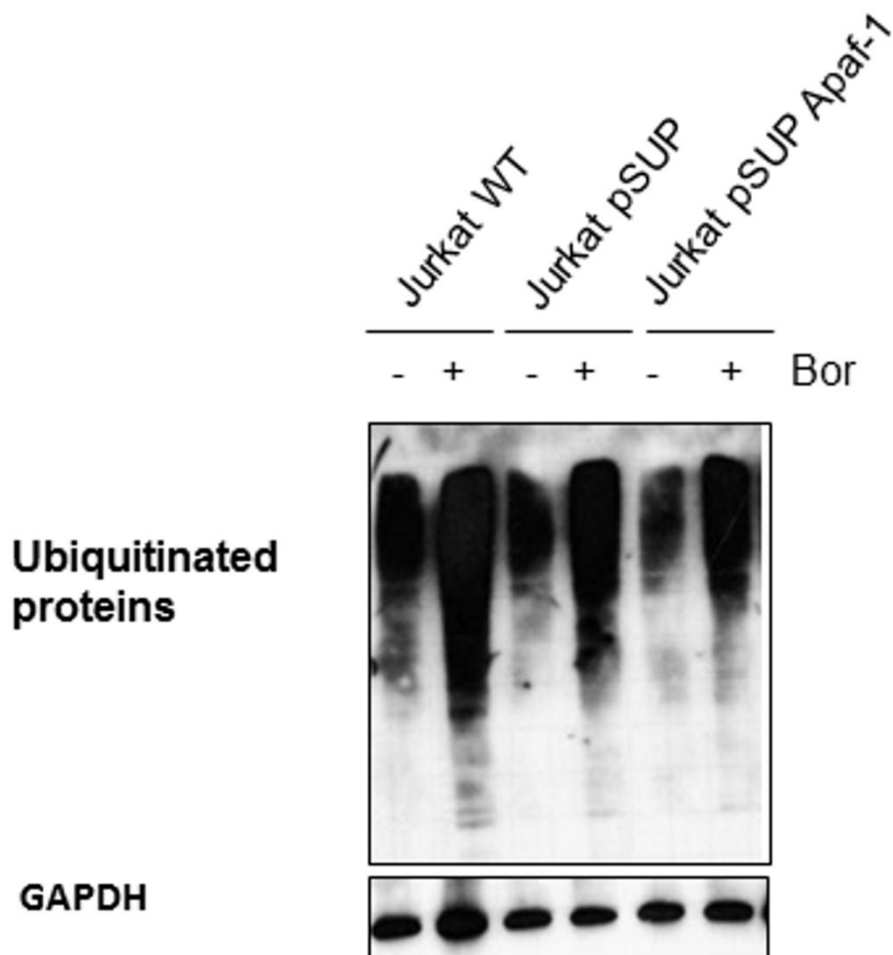
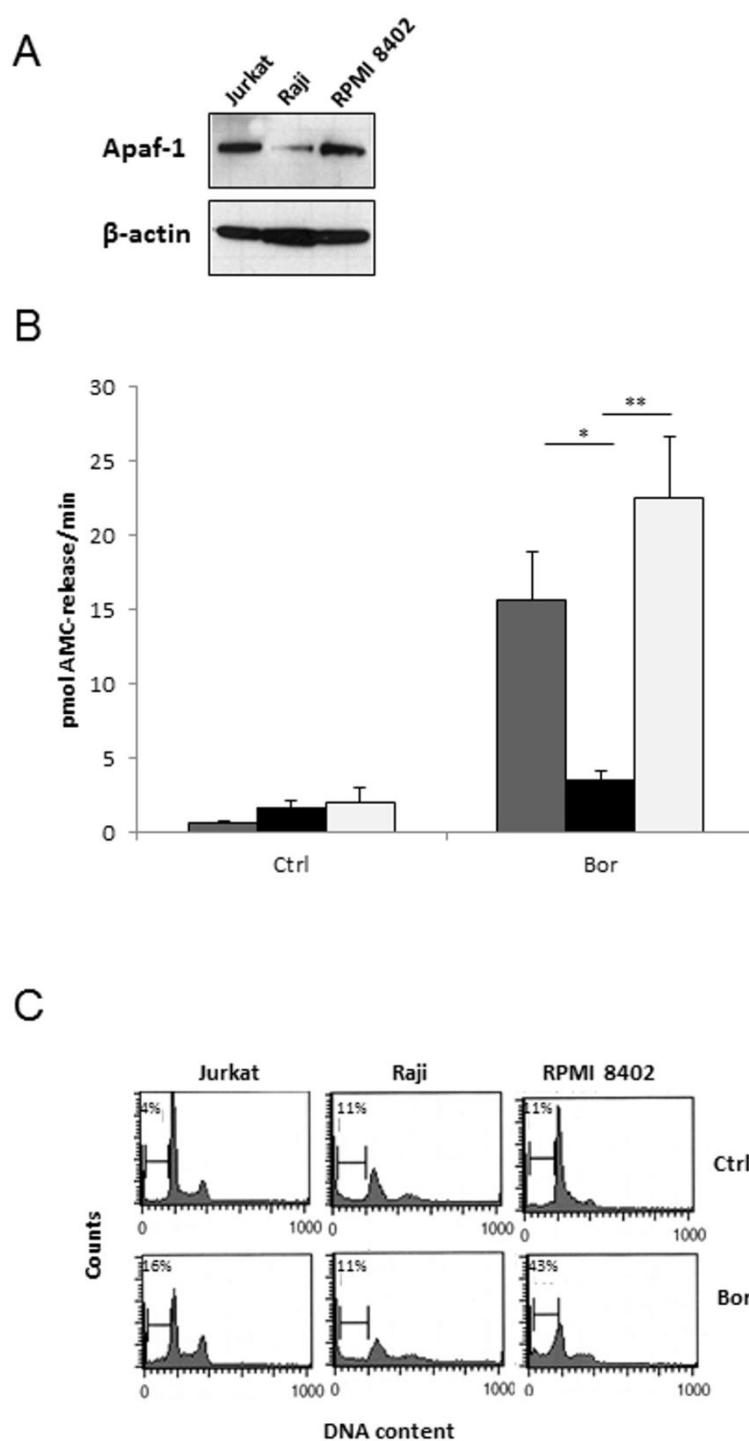


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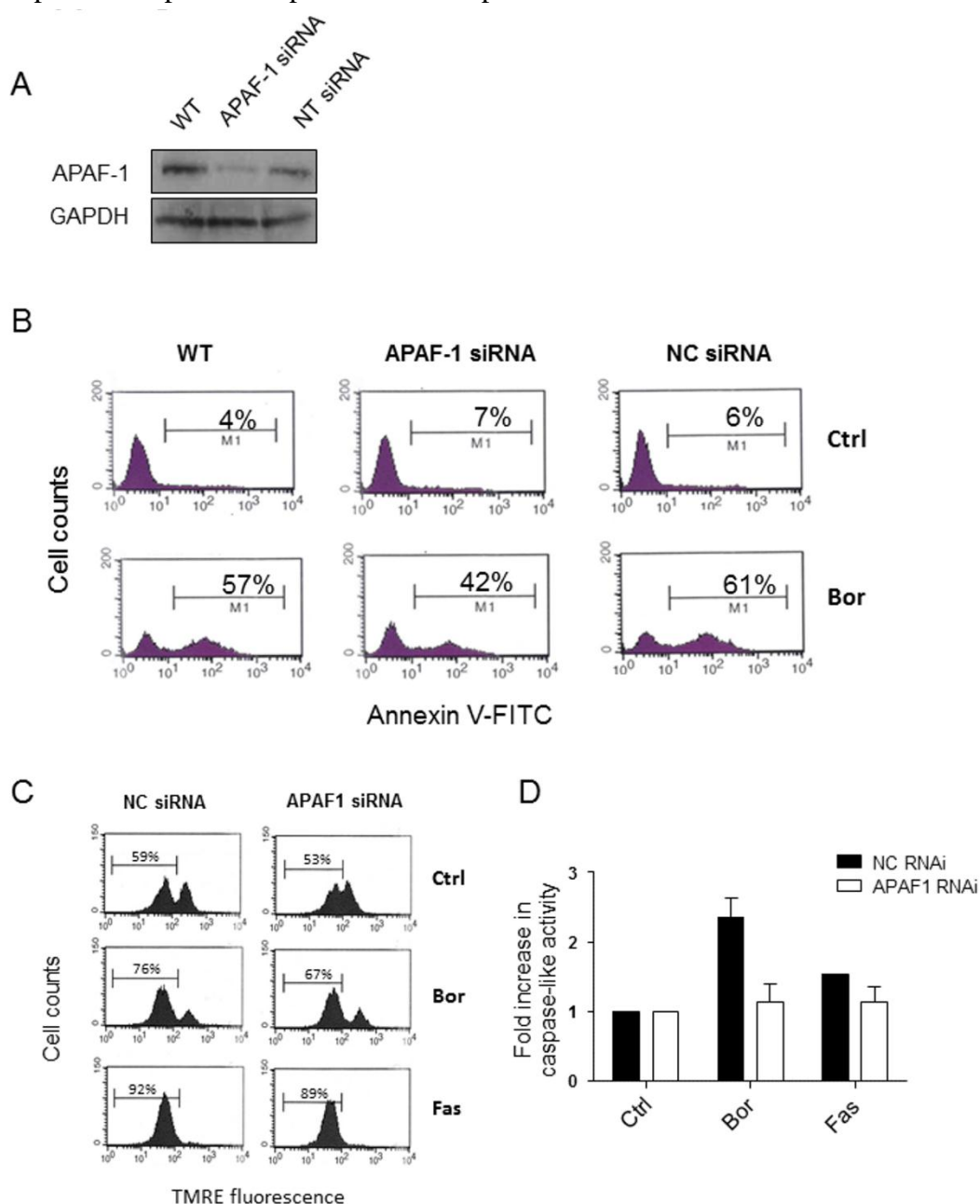
Supplemental Figure 1. The proteasome inhibitor bortezomib induces an increase in ubiquitinated proteins. Jurkat WT, pSUP and pSUP Apaf-1 cells were treated with bortezomib for 14 h and ubiquitinated proteins were detected using anti-ubiquitin antibodies. GAPDH expression was monitored to control for equal loading of protein.



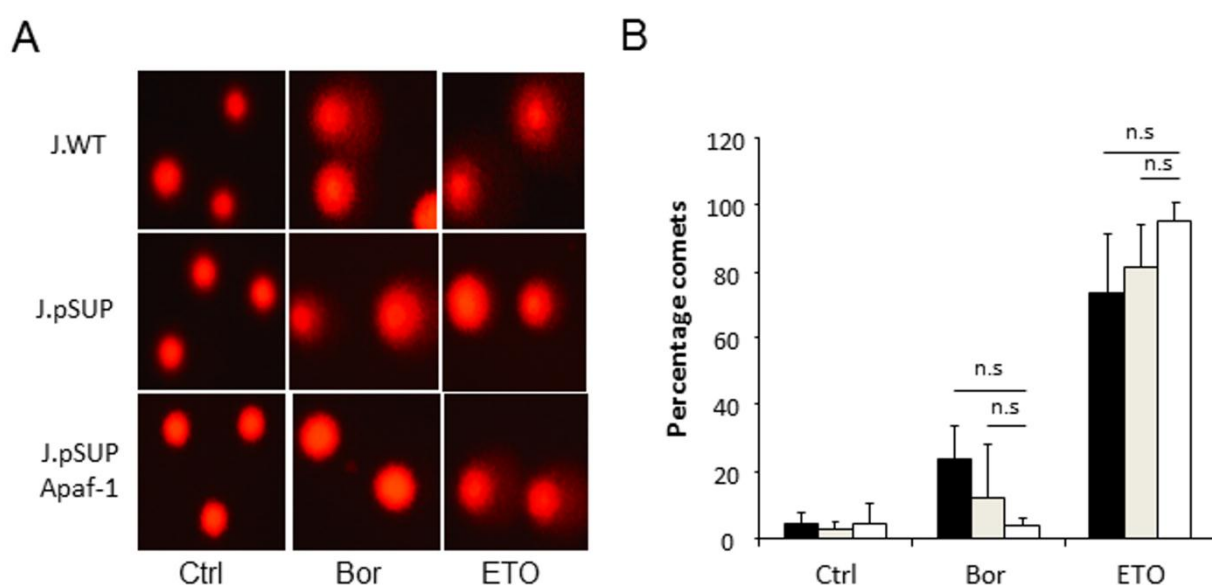
Supplemental Figure 2. Bortezomib induced caspase-3 activation is Apaf-1-dependent. (A) Apaf-1 protein expression in cytosolic fractions of Jurkat, Raji and RPMI 8402 cells. (B) Caspase-3-like activity in Jurkat (dark grey bars), Raji (black bars) and RPMI 8402 (light grey bars) cells after treatment with 50 nM bortezomib for 14 h. (C) Representative histograms showing the percentages of hypodiploid (apoptotic) cells upon treatment of Jurkat, Raji and RPMI 8402 cells with 50 nM bortezomib for 14 h. One asterisk (*) corresponding to a P value <0,05, two asterisks (**) corresponding to a P value <0,01.



Supplemental Figure 3. Transient silencing of Apaf-1 expression reduces apoptosis in bortezomib-treated RPMI 8402 cells. (A) Western blot of RPMI 8402 leukemia cells transfected with 200 nM siRNA against APAF1 or 200 nM non-targeting siRNA (NC) versus non-treated (NT) cells. GAPDH was used as a loading control. (B-D) RPMI 8402 cells transfected for 36 h or non-transfected with siRNA against Apaf-1 were treated with 50 nM bortezomib for 12 h and apoptosis was assessed by annexin V-FITC labeling, drop in mitochondrial membrane potential (MMP), and caspase-3 like activity. Results are presented as percentage of PS-positive cells or percentage of cells with drop in MMP. Data from one representative experiment are shown. The caspase-3 results are presented as fold increase of caspase-3-like activity to control and are shown as mean \pm SEM from two independent experiments performed in duplicates.



Supplemental Figure 4. Bortezomib induces limited DNA damage in Jurkat cells. Jurkat WT, pSUP, and pSUP-Apaf-1 were treated with 50 nM bortezomib or 10 μ M etoposide for 14 h and DNA damage was measured using the comet assay, as described in Materials and Methods. (A) Typical fluorescence microscope images depicting comet tails. (B) Quantification of comet tails in control, bortezomib-, and etoposide-treated cells, respectively. Jurkat WT (black bars), pSUP (grey bars) and pSUP-Apaf-1 (white bars).



SUPPLEMENTARY TABLE 1. Meta-analysis of APAF1 expression in the Human Gene Expression Map^a

^aAssessment of APAF1 expression in the Human Gene Expression Map (27). Direction of change and p-values, respectively, are indicated for the respective “groups” for the three probes targeting the APAF1 gene. Here, the groups “anaplastic large cell lymphoma”, “B-cell lymphoma” and “CD138+ plasma cell lymphoma” are assessed relative to the 96 biological groups. Significant increased expression is indicated by plus (+), significant decreased expression indicated by minus (-) and non-differentially expressed is indicated by \pm . A p-value of 0.05 was considered significant.

| Group | APAF1 | | | | | |
|---------------------------------------|-------------------|----------------------|-------------------|-----------------------|-------------------|----------------------|
| | 204859_s_at | | 211553_s_at | | 211554_s_at | |
| | Expression change | p-value | Expression change | p-value | Expression change | p-value |
| Anaplastic large cell lymphoma (n=27) | \pm | 0.417 | - | 7.08×10^{-4} | \pm | 0.059 |
| B-cell lymphoma (n=213) | + | 6.7×10^{-5} | + | 1.5×10^{-4} | + | $<1 \times 10^{-10}$ |
| CD138+ plasma cell lymphoma (n=120) | - | $<1 \times 10^{-10}$ | \pm | 0.145 | \pm | 0.438 |

SUPPLEMENTARY TABLE 2.

APAF1 expression in blood neoplasm cell lines from the Human Gene Expression map^a

^aAssessment of APAF1 expression in the Human Gene Expression Map (27). The database encompasses 5372 samples hybridized to the Affymetrix HG-U133A platform for comparison of the expression levels relative to cell lines. Direction of change and p-values, respectively, are indicated for the respective cell lines for probes targeting the APAF1 gene. Significant increased expression is indicated by plus (+), significant decreased expression indicated by minus (-) and non-differentially expressed is indicated by \pm . A p-value of 0.05 was considered significant.

| Cell line | APAF1 | | | | | |
|--------------|-------------------|----------------------|-------------------|----------------------|-------------------|----------------------|
| | 204859_s_at | | 211553_s_at | | 211554_s_at | |
| | Expression change | p-value | Expression change | p-value | Expression change | p-value |
| AMO-1 | \pm | 0.878 | \pm | 1 | \pm | 1 |
| ccrf-cem | \pm | 1 | \pm | 1 | \pm | 1 |
| CMK | \pm | 1 | \pm | 0.289 | \pm | 0.812 |
| FR4 | \pm | 1 | \pm | 1 | \pm | 0.993 |
| HL60 | + | $<1 \times 10^{-10}$ | + | $<1 \times 10^{-10}$ | + | $<1 \times 10^{-10}$ |
| Jurkat | \pm | 1 | \pm | 0.864 | \pm | 0.875 |
| K562 | \pm | 0.69 | \pm | 0.488 | + | 9.1×10^{-5} |
| KM4 | \pm | 1 | \pm | 1 | \pm | 0.896 |
| MOLT4 | \pm | 1 | \pm | 1 | \pm | 0.06 |
| NCI-H929 | \pm | 1 | \pm | 1 | \pm | 1 |
| NCU-MM1 | \pm | 1 | \pm | 1 | \pm | 0.984 |
| RJ2.2.5 | \pm | 1 | \pm | 0.968 | \pm | 0.704 |
| RPMI-8226 | \pm | 0.651 | \pm | 0.991 | \pm | 1 |
| SKMM1 | \pm | 1 | \pm | 1 | \pm | 1 |
| SR | \pm | 1 | \pm | 1 | \pm | 1 |
| Su-dhl1 | \pm | 0.998 | \pm | 0.883 | \pm | 0.264 |
| ts cell line | \pm | 0.722 | \pm | 0.217 | \pm | 0.722 |
| U937 | \pm | 0.944 | \pm | 0.164 | \pm | 1 |