Figure S1

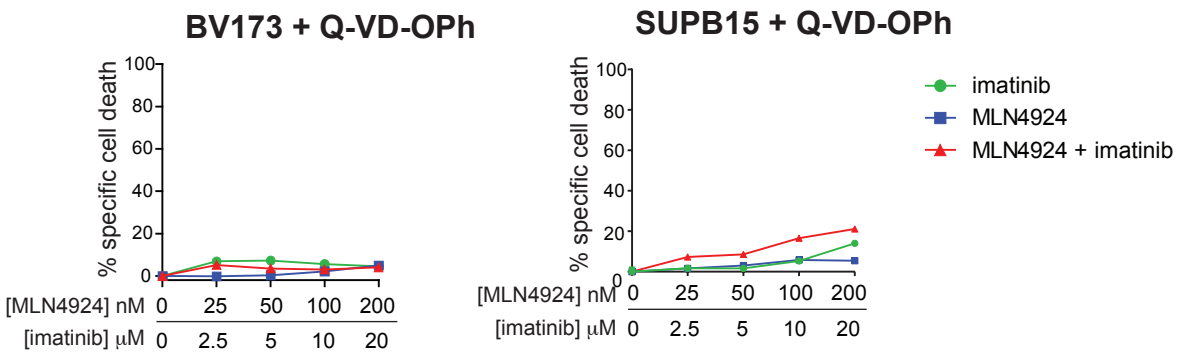

Figure S1. MLN4924 and imatinib induce caspase-dependent cell death in BV173 and SUPB15 cells. BV173 and SUPB15 were exposed for 3 days to various concentrations of MLN4924 and imatinib, with the pan-caspase inhibitor Q-VD-OPh. Three independent experiments were performed, cultures were done in triplicate. Means ± SEM of percent specific cell death are depicted in the graphs.

Figure S2

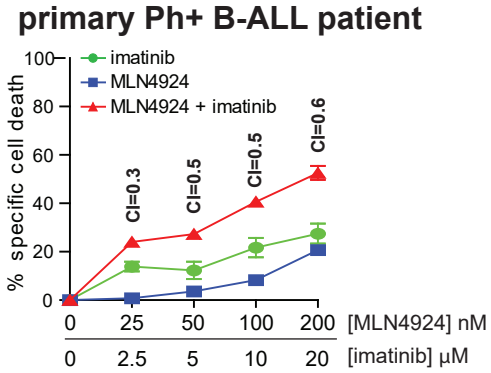

Figure S2. MLN4924 synergizes with imatinib for the induction of cell death in primary Ph+ leukemia cells. Primary CD34+ leukemic blasts from a previously untreated adult Ph+ B-ALL patient were cultured overnight with IL3, IL6, IL7 and SCF, and subsequently treated for 3 days with various concentrations of MLN4924 and imatinib in the presence of these cytokines. Cultures were performed in triplicate (technical replicates), means ± SEM are shown.

Figure S3

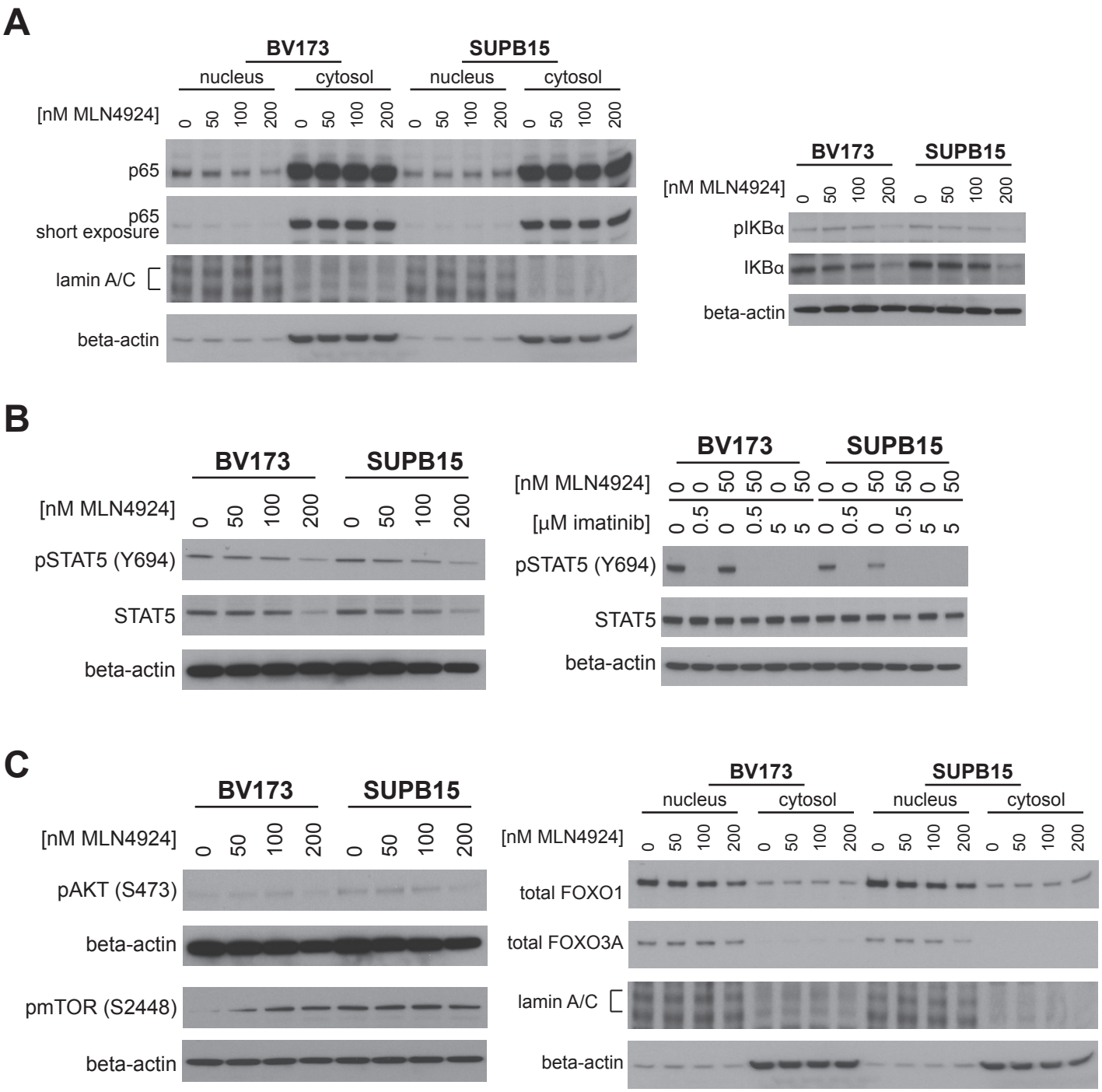

Figure S3. MLN4924 treatment has no appreciable effect on NF-κB, STAT5, and AKT signaling in BV173 and SUPB15 cells. (A) Immunoblotting analysis of nuclear and cytosolic NF-κB p65 protein levels (right panels) and phospho-IκBα and total IκBα levels in whole cell extracts (right panels), in BV173 and SUPB15 cells exposed overnight to various concentrations of MLN4924, as indicated. **(B)** Immunoblotting analysis of phospho-STAT5 and total STAT5 levels in BV173 and SUPB15 cells treated overnight with MLN4924 and imatinib, as indicated. **(C)** Immunoblotting analysis of phospho-AKT and phospho-mTOR in whole cell extracts, and nuclear and cytosolic FOXO1 and FOXO3a protein level in BV173 and SUPB15 cells treated overnight with various concentrations of MLN4924, as indicated. Beta-actin and lamin A/C blotting was used to control for loading.

Figure S4

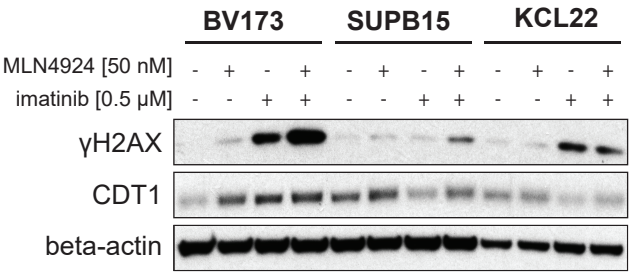

Figure S4. MLN4924 treatment induces accumulation of replication licensing factor CDT1 and induces phosphorylation of H2AX, which is exacerbated by imatinib co-treatment. Immunoblot analysis of BV173, SUPB15 and KCL22 Ph+ leukemia cells cells treated overnight with 50 nM MLN4924 + 0.5 μM imatinib, as indicated. Beta-actin was used to control for loading.

Figure S5

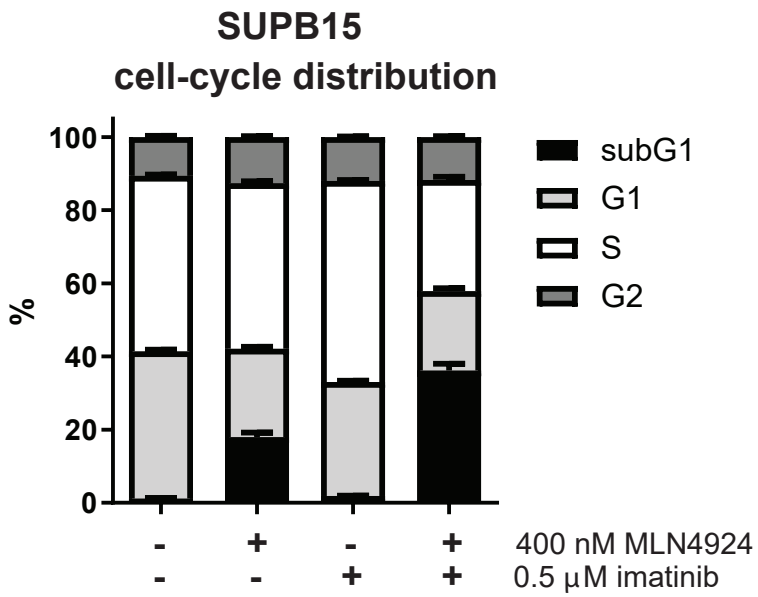

Figure S5. Cell-cycle distribution of SUPB15 cells upon MLN4924 and imatinib treatment.
Bargraphs depicting BrdU-incorporation and cell cycle analysis of SUPB15 cells treated overnight with 400 nM MLN4924, 0.5 µM imatinib or the combination of both, as indicated. Graph depicts percentage of cells in the different cell-cycle stages.

Figure S6

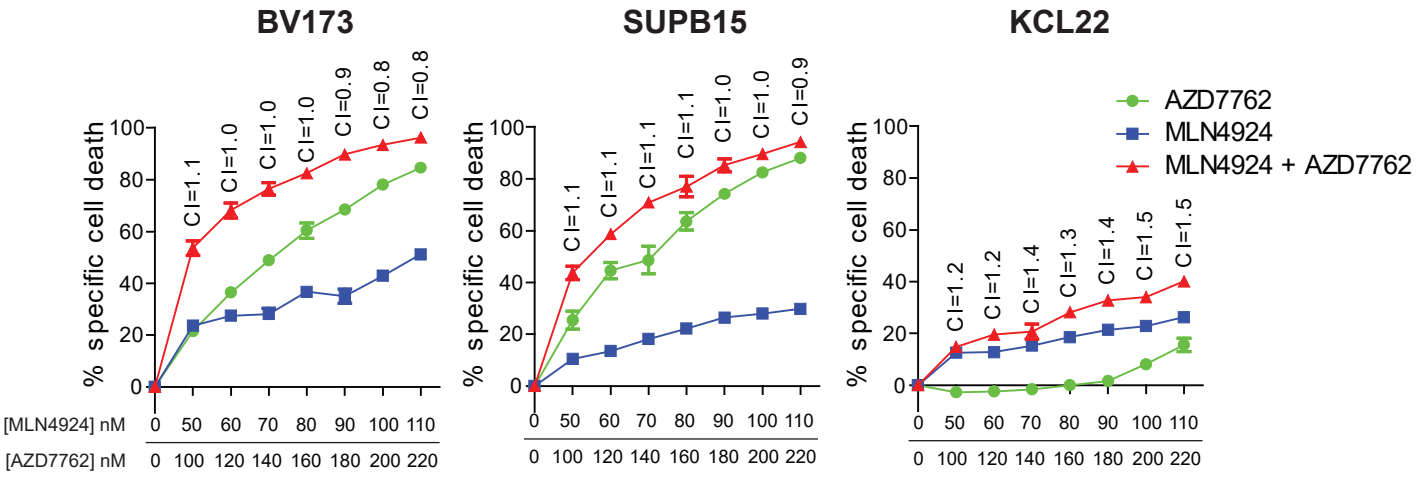

Figure S6. Additive effects of MLN4924 and the CHK1-inhibitor AZD7762 in Ph+ leukemia cells. BV173, SUPB15 and KCL22 cells were exposed for three days to various concentrations of MLN4924 and AZD7762, as indicated. Means ±SEM of percent specific cell death are depicted, experiments were performed in triplicate. CI values of relevant data point are shown.

Figure S7

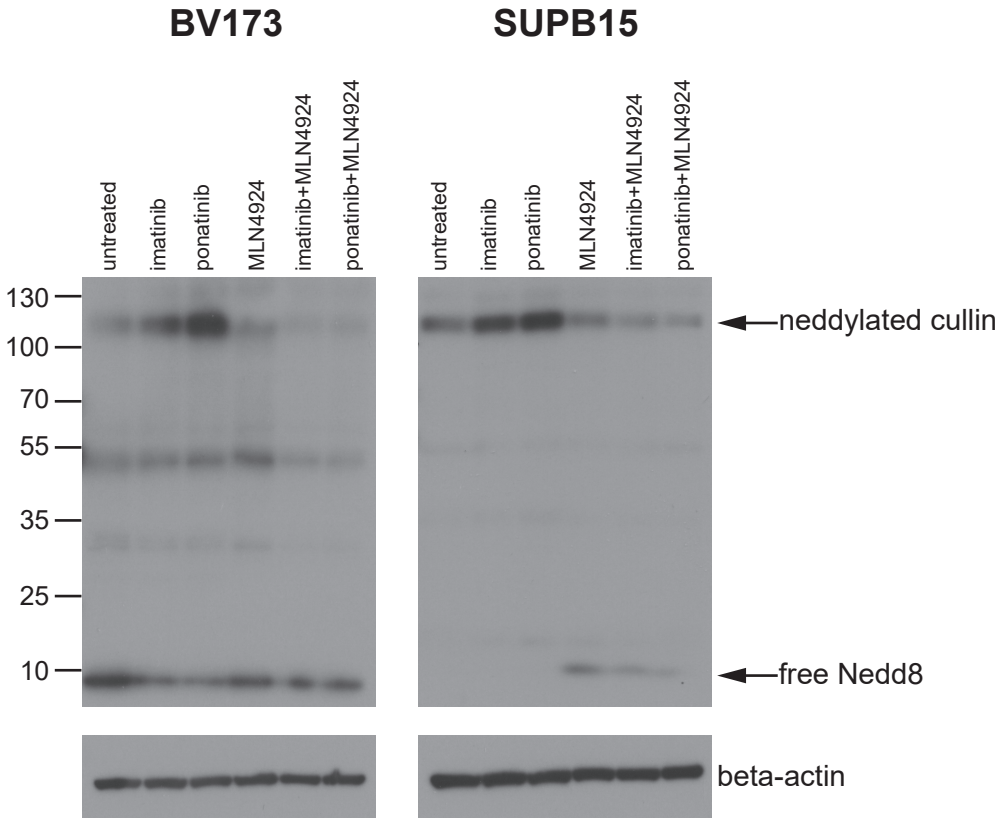

Figure S7. ABL TKIs imatinib and ponatinib alter global protein neddylation and potentiate NAE1 inhibition by MLN4924 in Ph+ leukemia cells.
Immunoblot analysis of free Nedd8 and protein-conjugated Nedd8 in BV173 and SUPB15 Ph+ leukemia cells treated overnight with 2.5 uM imatinib, 20 nM ponatinib, 100 nM MLN4924, the combination of imatinib + MLN4924, or ponatinib + MLN4924, as indicated. Numbers reflect molecular size in kDa. Bands respresenting neddylated cullin and free Nedd8 are indicated. Beta-actin blotting was used to control for loading.

Figure S8

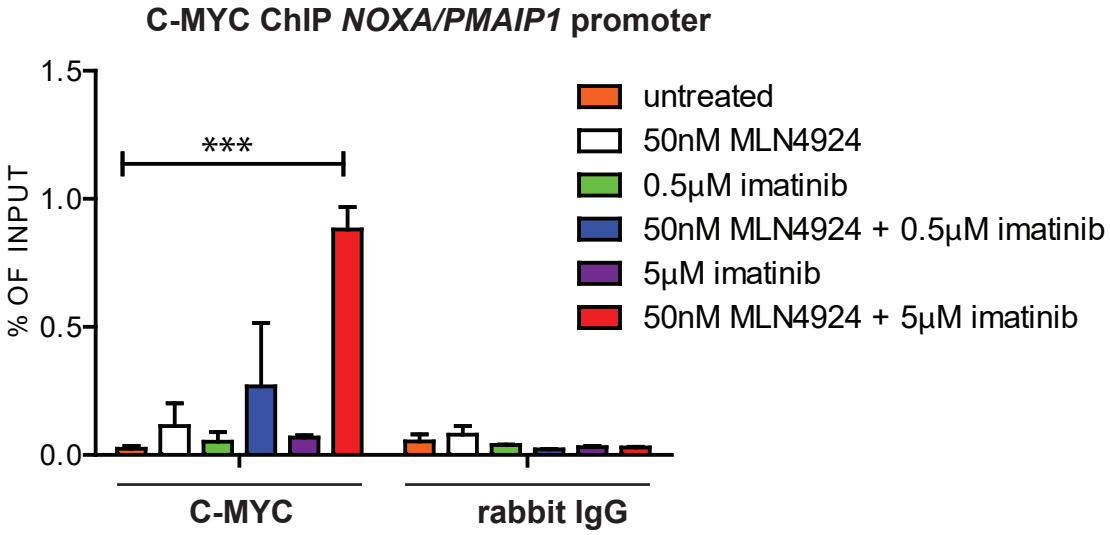

Figure S8. MLN4924 and imatinib treatment results in binding of C-MYC to the *NOXA* promoter. Chromatin immunoprecipitation (ChIP) analysis of BV173 treated overnight with MLN4924 and imatinib as indicated. Binding of C-MYC and control rabbit IgG to the *NOXA/PMAIP1* promoter as determined by ChIP, shown as percent of input. A representative example of 2 independent experiments is shown, PCR quantification was performed in triplicate, means ± SEM are shown (*** $p<0.001$, One-way ANOVA).