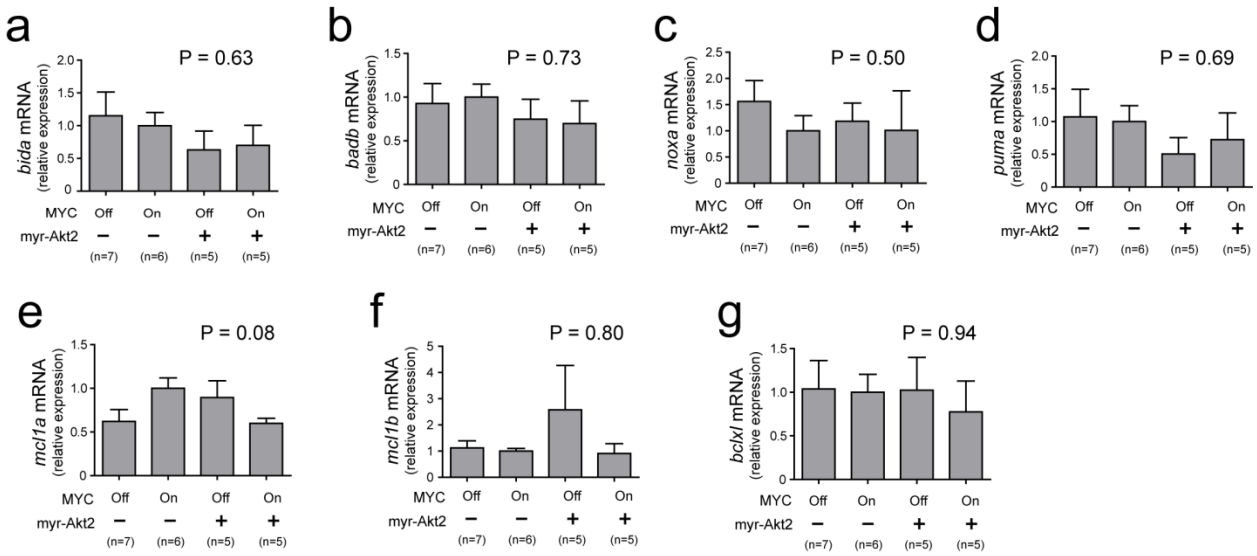
SUPPLEMENTARY FIGURES AND LEGENDS

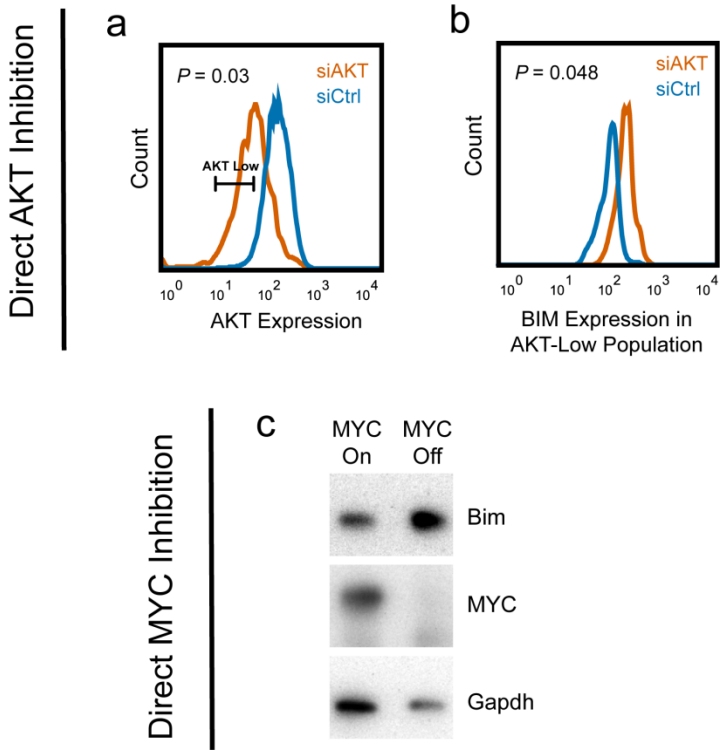
Reynolds et al.

Supplementary Figure 1



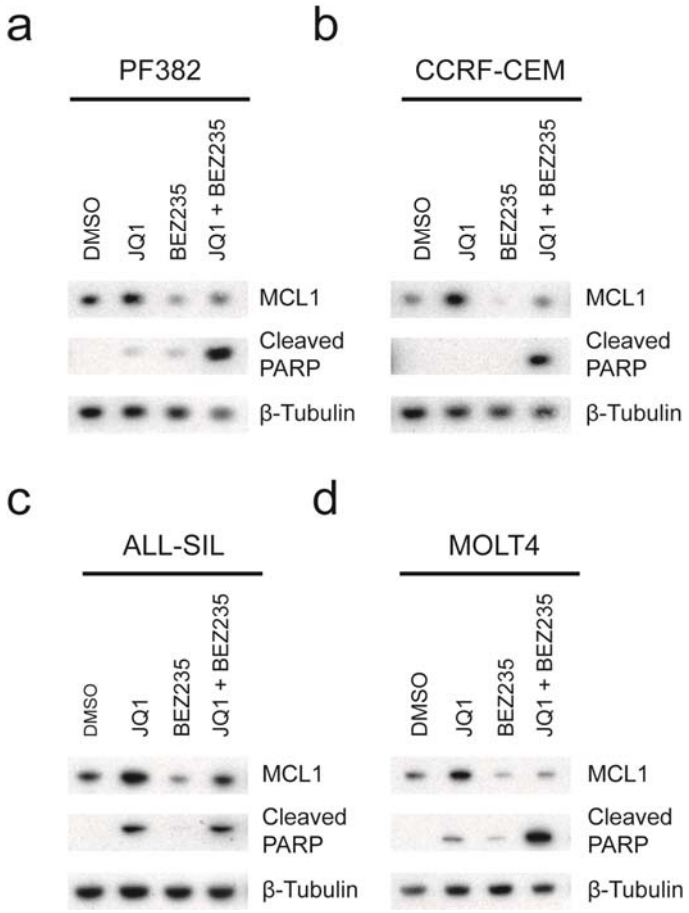
Supplementary Figure 1. mRNA expression of zebrafish BCL2 family members in zebrafish T-ALLs expressing activated MYC and/or AKT transgenes. (**a** to **g**) Quantitative RT-PCR analysis of mRNA expression of the BH3-only pro-apoptotic and anti-apoptotic BCL2 family members known to be functionally conserved in zebrafish, using RNA from T-ALL cells isolated from *rag2:MYC-ER; rag2-EGFP-bcl2* zebrafish that also expressed either *rag2:myr-Akt2* or *rag2:mCherry* control. T-ALL cells were sorted from animals in 4-hydroxytamoxifen ("MYC On"), or 4 days after tamoxifen removal ("MYC Off"). Note that endogenous expression of zebrafish *bcl2* could not accurately be assessed, because expression of a *rag2:EGFP-bcl2* transgene was used in all conditions to avoid comparing live vs. dying cells. Significance was assessed using the Kruskal-Wallis test, a statistical method designed to assess whether at least one of the conditions is significantly different from the others. Error bars represent mean +/- standard error of the mean for experiments performed in triplicate.

Supplementary Figure 2



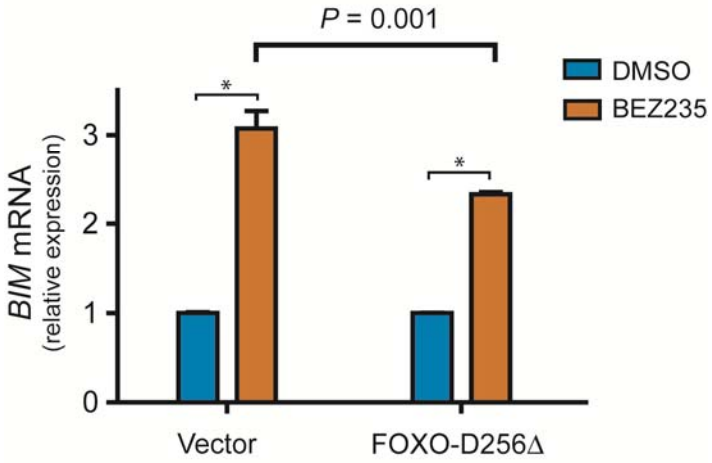
Supplementary Figure 2. Direct downregulation of AKT or MYC induces BIM upregulation. (**a**) Transfection of the human T-ALL cell line CCRF-CEM using siRNA targeting AKT or control was performed, and total AKT protein levels were analyzed using flow cytometry. Bracket indicates the gate used to define the AKT-low population for analysis of BIM protein expression. (**b**) BIM protein expression was measured by flow cytometry by gating on the AKT-low population in siAKT-transfected cells. An identical gating strategy was used to assess BIM expression in control siRNA-transfected cells. *P* values were calculated using a one-sided Welch t test, using the difference in mean fluorescence intensity of experiments performed in duplicate. (**c**) Murine 4188 cells, which are induced by a doxycycline-repressible human MYC transgene, were treated with vehicle ("MYC On") or doxycycline ("MYC Off") for 48 hours, and Western blot analysis was performed for the indicated proteins.

Supplementary Figure 3



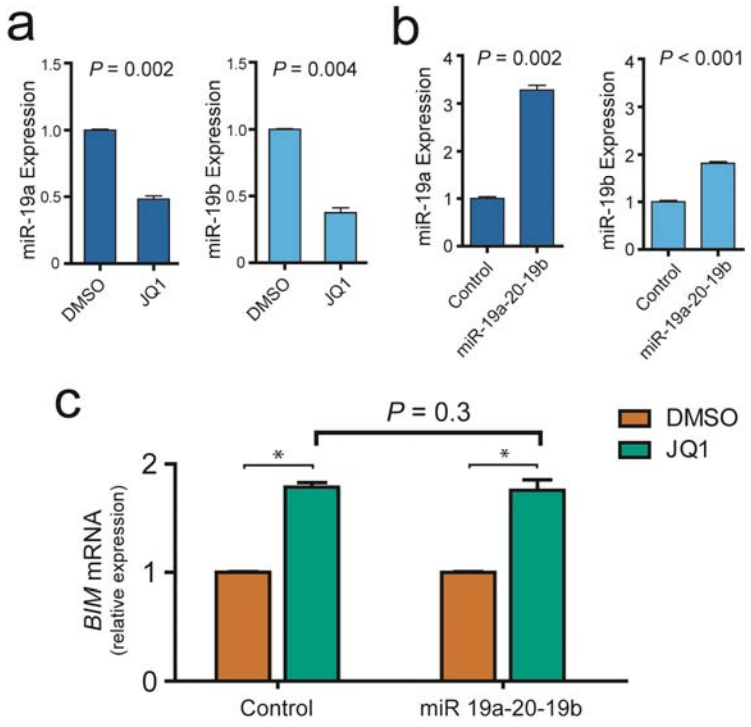
Supplementary Figure 3. Effect of BEZ235 and JQ1 treatment on MCL1 protein levels in human T-ALL cell lines. (**a** to **d**) The indicated human T-ALL cell lines were treated using DMSO, JQ1 (1 μM), BEZ235 (500 nM), or both drugs in combination for 24 hours, and Western blot analysis was performed using the indicated antibodies. Note that MCL1 levels are similar between control cells and those treated with the combination of JQ1 and BEZ235, despite effective induction of the apoptotic marker cleaved PARP.

Supplementary Figure 4



Supplementary Figure 4. Expression of dominant-negative FOXO impairs BEZ235-induced upregulation of *BIM.* The human T-ALL cell line PF382 was transfected with a FOXO-D256Δ expression vector or pCS2 control vector, and cells were treated with 500 nM BEZ235. After 24 hours, *BIM* mRNA expression was assessed using Q-RT-PCR analysis. β-actin was used as the Q-RT-PCR control. Error bars represent mean +/- standard error of the mean for experiments performed in triplicate. *P* values were calculated using a one-sided Welch t test. *, P < 0.001. Note that the plasmids used for these transfection experiments did not contain a selection marker, thus these results may underestimate the effect of dominant-negative FOXO on BIM expression in these cells.

Supplementary Figure 5



Supplementary Figure 5. Expression of the MYC targets miR-19a, miR-20 and miR-19b does not detectably rescue JQ1-induced *BIM* mRNA upregulation. (**a**) Quantitative RT-PCR analysis for expression of the miR-19a and mir-19b microRNAs in the human T-ALL cell line CCRF-CEM treated with DMSO or JQ1 (1 μM) for 24 hours. (**b**) CCRF-CEM cells were infected with an MSCV retrovirus driving expression of miR-19a, miR-20 and miR-19b, and Q-RT-PCR analysis was performed to confirm effective microRNA upregulation. MSCV-puro retrovirus was used as the control. (**c**) Effect of 1 μM JQ1 treatment for 24 hours on *BIM* mRNA expression was assessed using Q-RT-PCR analysis of CCRF-CEM cells expressing control MSCV, or an MSCV retroviral construct that drives expression of miR-19a, miR-20 and miR-19b. *P* values were calculated using a one-sided Welch t test. *, P < 0.001.