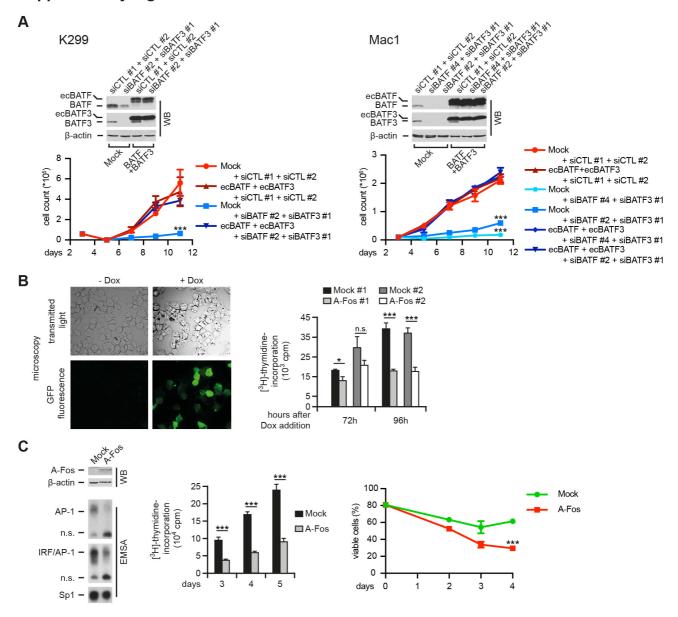
Supplementary Figure 3



Supplementary Figure 3. (A) Ectopic expression of BATF and BATF3 rescues ALCL cells from BATF and BATF3 siRNA-induced cellular toxicity. K299 (left) and Mac-1 (right) cells transduced with control virus (Mock) or cDNAs encoding BATF and BATF3 were treated with control siRNAs (siCTL #1 and siCTL #2) or a combination of siRNAs targeting the untranslated regions of BATF or BATF3 (siBATF #2 or #4 and siBATF3 #1). Knock-down and ectopic expression of the respective proteins was confirmed by immunoblotting (WB, top panels). Note, that ectopically expressed BATF and BATF3 are larger in size compared to the wt variants due to remaining P2A fusion protein residues. Bottom, cell numbers over time. Note, that the toxic effect of BATF and BATF3 knock-down is rescued by ectopic expression of BATF and BATF3. (B) Left, inducible A-Fos expression in FE-PD cells. Following Dox addition for 48 h, > 50% of cells were GFP-positive. Cells were analyzed by transmitted light (top) and GFP-fluorescence microscopy (bottom). Right, reduced [3H]-thymidine incorporation following A-Fos induction and enrichment of induced cells. Cells were enriched following 72 and 96 h, and [3H]-thymidine was added after an additional 48 h. Data of triplicates from two independent experiments (#1 and #2) are represented as means ± SD. (C) K299 cells were transiently transfected with empty plasmid (Mock) or A-Fos expression plasmid along with pEGFP. 48 hours after electroporation, transfected GFP+ cells were enriched and analyzed by immunoblotting (WB) for expression of A-Fos by use of FLAG antibody (upper left panel) and by EMSA for AP-1 and IRF/AP-1 DNA-binding activity (lower left panel). β-actin and of Sp1 DNA-binding activity were controls. Center, [3H]-thymidine incorporation of enriched Mock and A-Fos transfected cells, as determined 3, 4, and 5 days after enrichment. Data of triplicates are represented as means ± SD. One of three independent experiments is shown. Right panel, enriched Mock and A-Fos cells were cultured for the indicated times, and the percentage of viable, PI-negative cells was determined by propidium-iodide (PI) staining and flow cytometry. One of three independent experiments is shown. ***, P < 0.001.