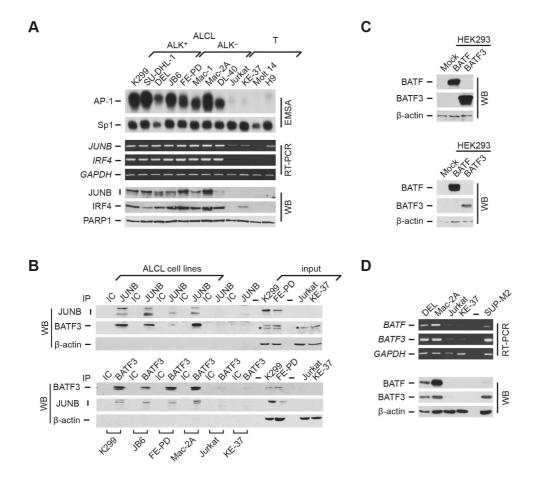
Supplementary Figure 1



Supplementary Figure 1. Characterization of the AP-1 complex in ALCL. (A) Analysis of AP-1 DNA-binding activity and JUNB and IRF4 expression in human lymphoma cell lines. Upper panel, nuclear extracts of the indicated lymphoma cell lines were analyzed by EMSA for DNA-binding activity at the AP-1 TRE site. DNA-binding of Sp1 was analyzed as a control. Center panel, RT-PCR analyses of JUNB and IRF4 mRNA expression in the various cell lines. The GAPDH control is the same as in Figure 4 A. Lower panel, analyses of JUNB and IRF4 protein expression in the various cell lines by immunoblotting. The expression of PARP1 is shown as a control. (B) JUNB and BATF3 co-immunoprecipitations. Whole cell extracts of various cell lines were immunoprecipitated (IP) with anti-JUNB (upper panel) or anti-BATF3 as well as respective isotype controls (IC). Detection of (co-)immunoprecipitated proteins was performed by immunoblotting (WB). Note, that JUNB - BATF3 and BATF3 - JUNB co-immunoprecipitations are specifically detected in ALCL, but not in control cell lines. β-actin and input protein extracts were analyzed as controls. *, non-specific band. (C) Specificity of BATF and BATF3 antibodies. Upper panel, whole cell extracts of HEK293 cells transfected with empty plasmid (Mock) or plasmids encoding BATF or BATF3 were analyzed by immunoblotting for expression of BATF (antibody #8638; Cell Signaling) or BATF3 (antibody AF7437; R&D Systems). Lower panel, analysis of the same extracts as in the upper panel by use of antibody to BATF (sc-100974) and BATF3 (sc-162246; both Santa Cruz). Positions of BATF or BATF3 are indicated. Expression of β-actin was analyzed as a control. (D) Characterization of BATF and BATF3 in SUP-M2. Upper panel, RT-PCR analyses of BATF and BATF3 mRNA expression in the various cell lines. The expression of GAPDH was analyzed as a control. Lower panel, analyses of BATF and BATF3 protein expression in the various cell lines by immunoblotting. The expression of β-actin is shown as a control.