SUPPLEMENTARY FIGURE 1

HEK 293T cells were transiently transfected with pMIG-R1 or pMIG-cMyb by transient calcium phosphate transfection. Cells were lysed 24 hours later in 20 mM Tris pH 7.4, 100 mM NaCl, 10 mM EDTA, 1 mM EGTA, 1% Triton X-100 containing EDTA-free protease inhibitor cocktail (Roche, Indianapolis, IN). Fifty micrograms of protein was fractionated on 10% SDS-polyacrylamide gel and transferred to Protran nitrocellulose transfer membranes (Whatman, Dassel, Germany). Membranes were blocked in PBS + 0.05% Tween-20 (PBS-T) with 5% non-fat dry milk for 1 hour and then incubated overnight at 4°C with either anti-c-Myb (clone 1-1, Millipore, Bedford, MA) or anti-HA (clone Y11, Santa Cruz Biotechnology, Santa Cruz, CA). Membranes were washed three times in PBS-T and probed with either anti-rabbit-HRP or anti-mouse-HRP conjugated antibody in PBS-T for 1 hour at room temperature. After washing the membrane three times in PBS-T, the proteins were detected by enhanced chemiluminescence (Amersham, Piscataway, NJ). Asterisk (*) represents a nonspecific interaction that serves as a loading control.
SUPPLEMENTARY FIGURE 2. Overexpression of Bcl-xL fails to rescue B-lineage development from Myb<sup>−/−</sup> Mb1-cre LMPPs. LMPPs were isolated from Myb<sup>−/−</sup> Mb1-cre bone marrow by fluorescence activated cell sorting, seeded at 5000 cells per well on OP-9 stromal cells and transduced with MIG-R1, MIG-BclxL or MIG-Ebf1. LMPPs were cultured in the presence of SCF, Flt3L, and IL-7 and analyzed 10 days later by flow cytometry for the expression of B220 and CD19 and the number of GFP+ B220+ CD19+ cells were determined.