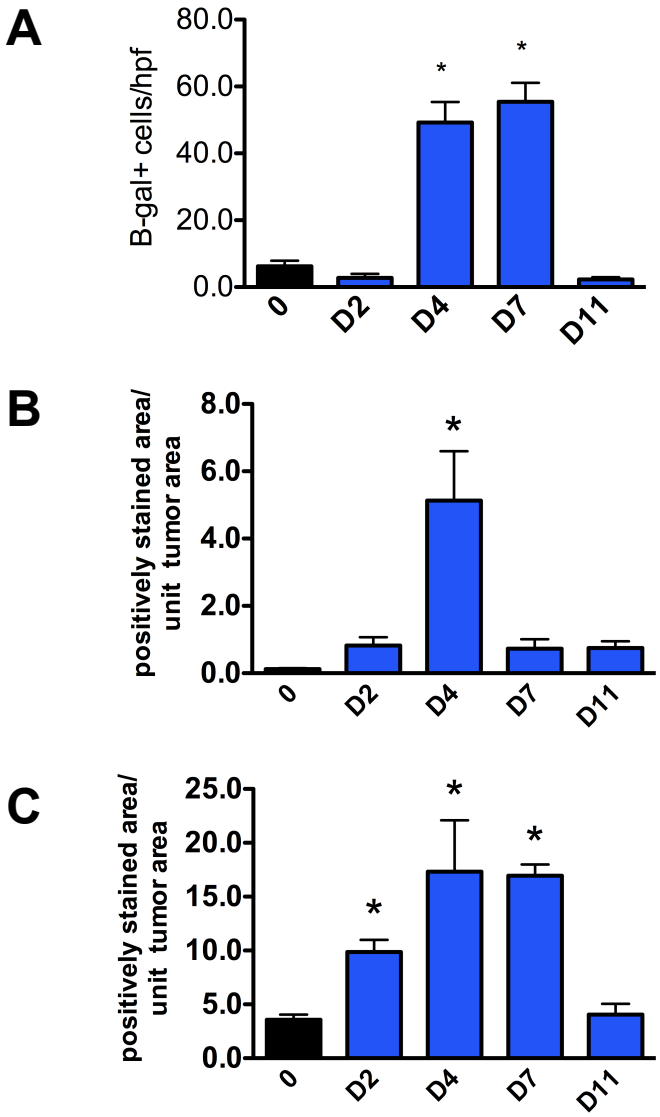
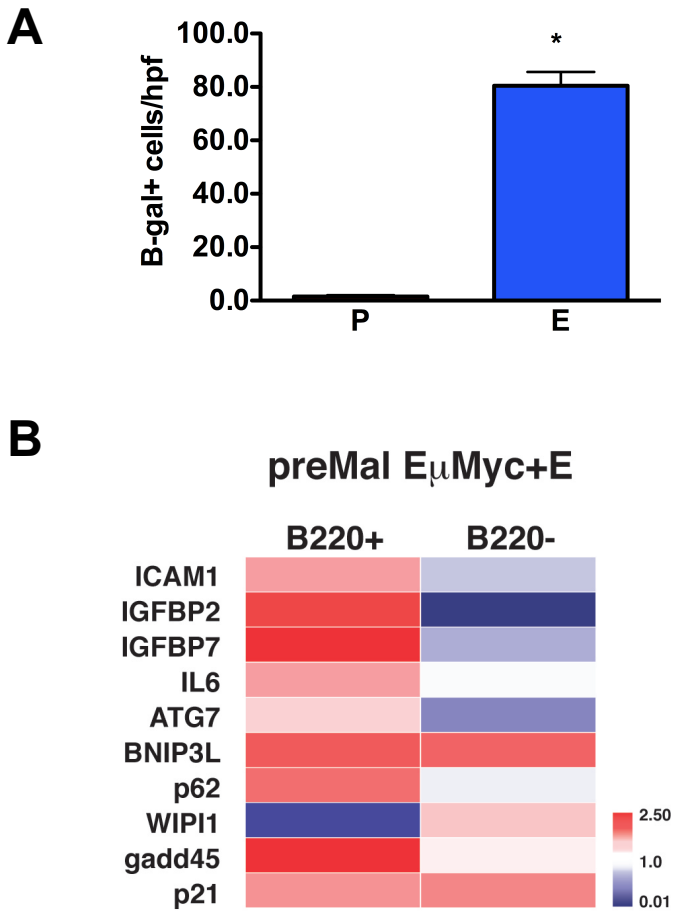
Supplementary Figure S9



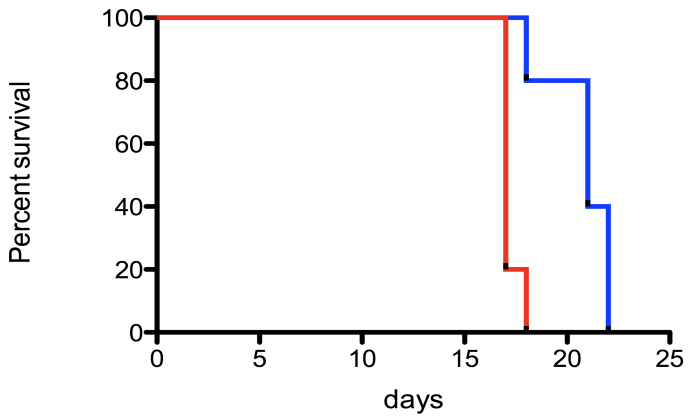
Supplementary Figure S9. Quantitation of SA-β−gal staining and Gr1 and F4/80 immunostaining. Sections from lymph nodes of untreated mice and mice sacrificed 2, 4, 7 and 11 days after chronic daily dosing with everolimus were stained with SA-β-gal, anti-Gr1 and anti-F4/80. **(A)** Quantitation of SA-β-gal staining. Positively stained cells per high power field for 10 consecutive fields were counted for untreated (n=4) and everolimus-treated mice (n=2 mice per time point). **(B and C)** Percentage area stained with anti-Gr1 (B) and anti-F4/80 (C) was analyzed using Metamorph imaging software (Molecular Devices, CA). Red immunofluorescent staining was quantitated by determining a fluorescent threshold and subsequently an integrated morphometric analysis. Nuclei were also electronically counted in order to determine that each image had a similar density and overall number of cells to be analyzed. Two images were quantitated for each untreated (n=4) and everolimus-treated mice (n=2 mice per time point). Error bars represent the SEM. Groups were compared to untreated control using a one way ANOVA with Dunnet's multiple comparison post test correction. *$p<0.05$.

Supplementary Figure S10



Supplementary Figure S10. Markers of senescence in premalignant Eμ−*Myc* mice. (A) 4 week old Eμ-*Myc* mice were treated with daily placebo (n=4) or everolimus (n=4) and sacrificed after 4 days. Splenic sections were stained SA-β-gal. Positively stained cells per high power field for 10 consecutive fields were counted. Error bars represent the SEM. p values were generated using a Student's unpaired 2-tailed t-test, *p<0.05. **(B)** Bone marrow was harvested from mice treated as in (A) and live primary B-cells (B220+) were isolated following incubation with APC conjugated anti-mouse/human B220 (CD45R) antibody (eBioscience), subsequent washing and sorting using the BD FACSAria™ II cell sorter (BD Biosciences). The non-B220 stained fraction of bone marrow was also collected (B220-). Dead cells were detected by Fluoro-gold staining (Molecular Probe) and excluded from analysis. Cells were then harvested for quantitave real time PCR analysis as described in the Materials and Methods section of the manuscript. Heatmap representation of qPCR expression of a panel of senescence markers in both the B220+ and B220- fractions of mice treated with E as compared to P (fold change over P=1) where the mean relative expression levels are represented by a color scale where red=increased expression; white=no change; and blue=reduced expression.

Supplementary Figure S11



Supplementary Figure S11. Overall survival of mice transplanted with a p53 mutant Eμ-*Myc* lymphoma. Syngeneic mice were injected with lymphoma cells (tumor#229). Dosing with placebo or everolimus was started 72 hours after injection (n= 5 mice per group). Median survival was 17 days for placebo and 21 days for everolimus (p=0.005).