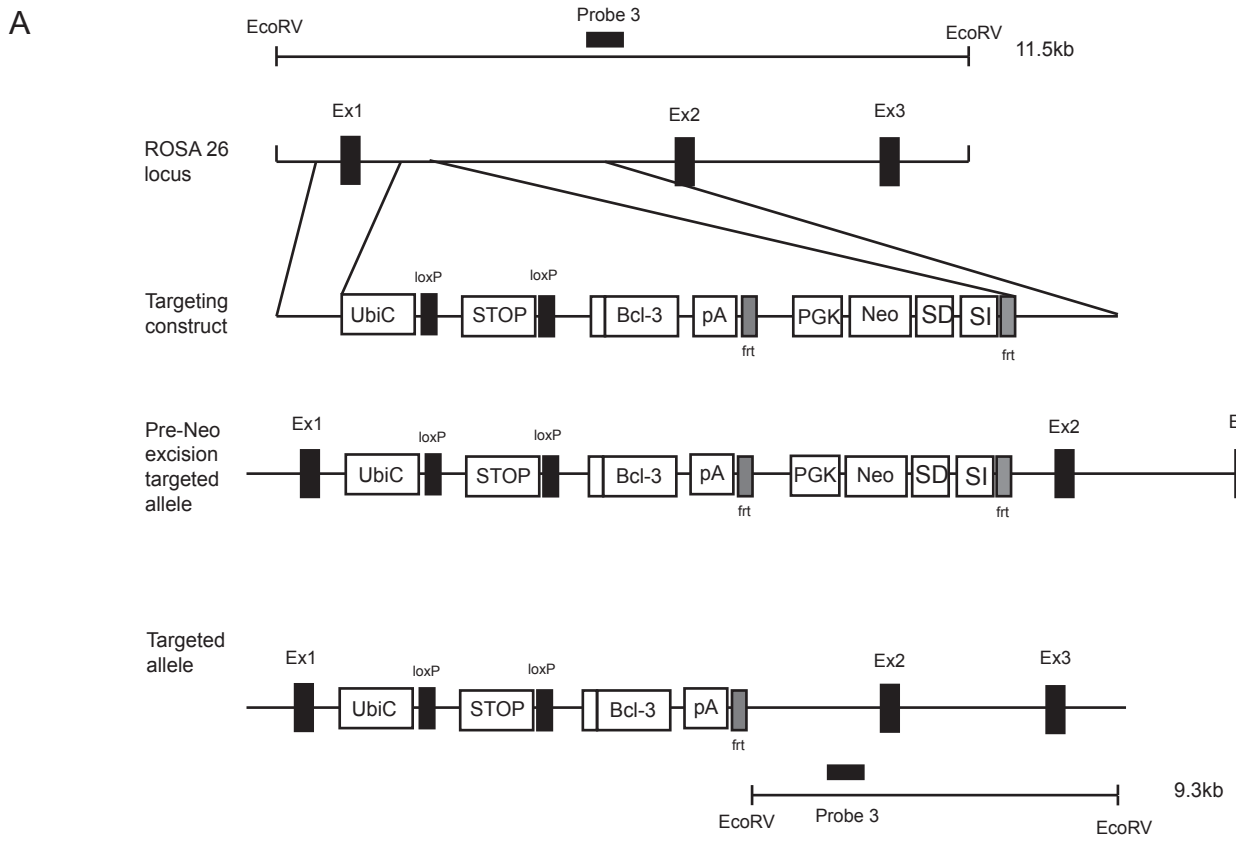
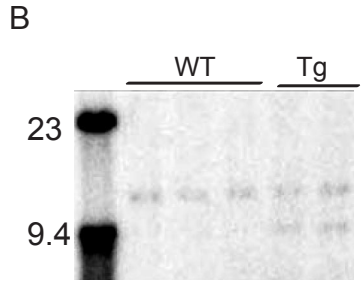
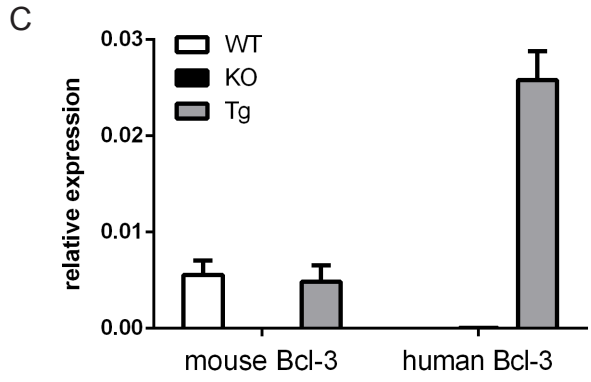
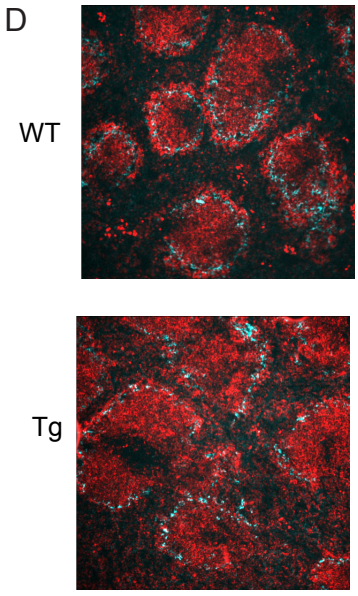
Supplemental Figure 3



Generation of mice that conditionally express a Bcl-3 transgene.
(A) Cloning strategy; shown are the genomic Rosa26 locus, the targeting construct, and the targeted allele, before and after excision of a Neo cassette as well as the EcoRV restriction fragments that are labeled with the probe indicated. Bcl-3 Tg mice on a C57BL/6 background were generated by Ozgene, Australia. Briefly, human Bcl-3 cDNA was cloned into the pNTAP vector (Stratagene) carrying a Tap-tag and then subcloned into the UBiC_N targeting vector (Ozgene; Koentgen F et al (2010). *Methods Mol Biol* 602:55-77). This vector contains a UbiC promoter and a loxP-flanked STOP cassette to control expression of the Bcl-3 Tg. The end of the second loxP site is located 15 base pairs upstream of the translation initiation site of the tagged Bcl-3 transgene. Genomic fragments of the Rosa26 gene were amplified by PCR from C57BL/6 genomic DNA to generate 5′ and 3′ homology arms that were used to flank a FRT-PGK-NeoR-FRT selection cassette. Forward and reverse DNA sequencing and restriction enzyme digests confirmed the integrity of the final targeting construct. Following homologous recombination of the targeting vector in C57BL/6 ES cells, correctly targeted clones were identified by Southern blot analysis. Targeted ES cell clones were microinjected into C57BL/6 blastocysts to generate chimeric animals. After establishment of germline transmission, the PGK-NeoR cassette was deleted by breeding to a transgenic line containing Flpe recombinase. Selective breeding was used to eliminate the Flpe gene and Bcl-3 Tg mice were maintained on a pure C57BL/6 genetic background. (B) Representative Southern blot from founder mice showing integration of the transgene in mice containing the targeted-allele specific EcoRV fragment labeled with the probe shown in (A). (C) Real time quantitation of mouse and human Bcl-3 transcripts in WT, Bcl-3$^{-/-}$ and mb1-cre mediated expression of the human Bcl-3 transgene in B cells; data are shown for n=2/group as expression relative to beta actin. RNA was extracted from bead-sorted CD43- B cells from two mice of each genotype with Qiagen RNEasy. cDNA was generated using Qiagen Quantitect Reverse Transcription Kit and Bcl-3 transcripts were quantitated using primers specific for mouse and human Bcl-3 with SyBRGreen on an Applied Biosystems StepOnePlus (fw primer [shared human and mouse]: 5'-CCCATGGAACACCCCCTTTC; human rev primer: 5'-TTGTTGTAGATGTCGAGCTCCC; mouse rev: 5'-TCCCAAGCTTGAAAAGGCTGA). Data are shown as expression relative to beta actin. (D) Bcl-3 transgenic mice display reduced numbers of MZ B cells relative to FO B cells. MZ B cells are located outside the ring of MOMA1$^{+}$ macrophages. Spleen sections from 6 week old WT and Tg mice were stained with anti-IgM Alexa Fluor-488 (Invitrogen) and MOMA1 Alexa Fluor-647 (AbD Serotec) shown in colors as indicated. Magnification 20x.