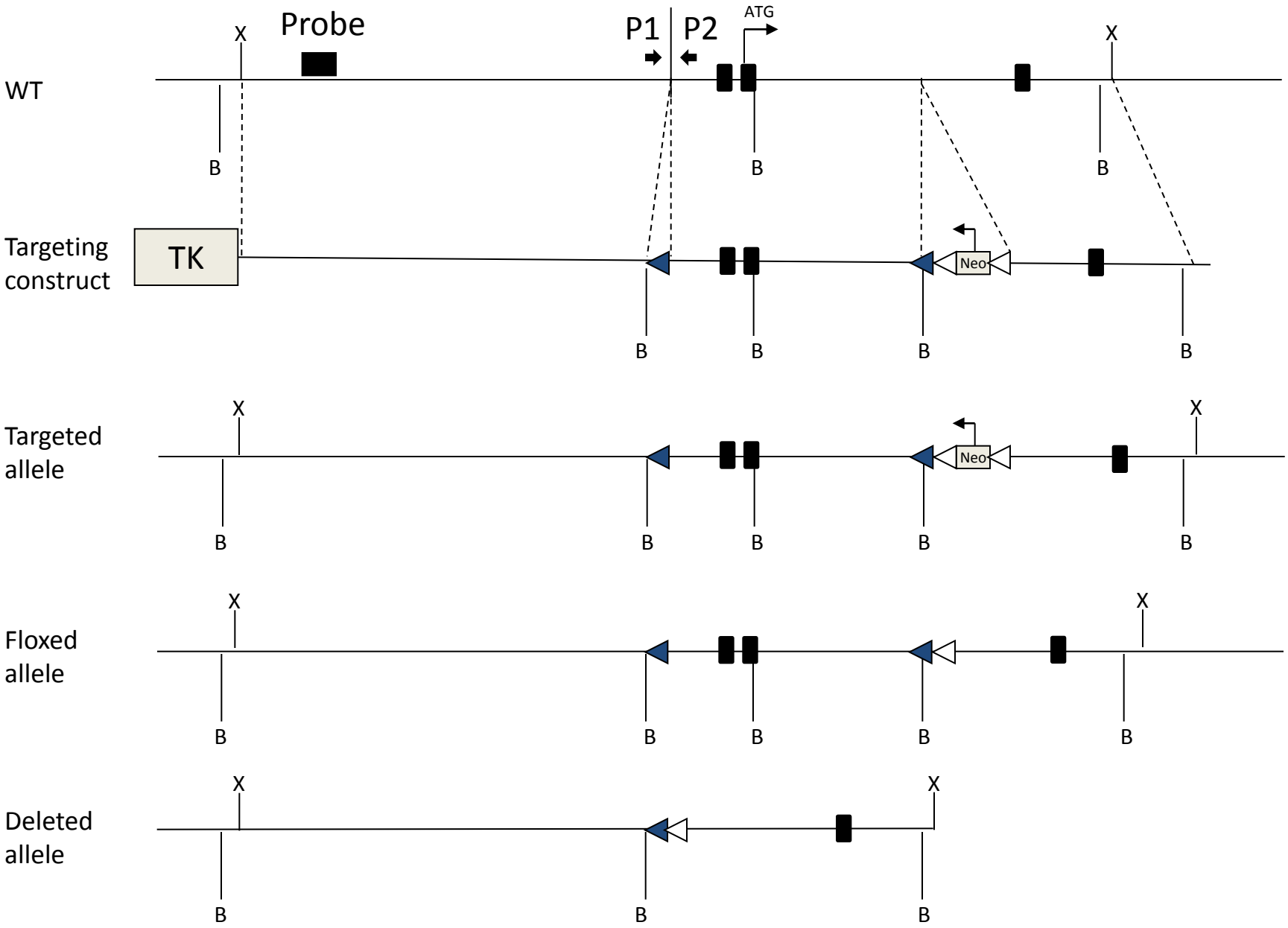
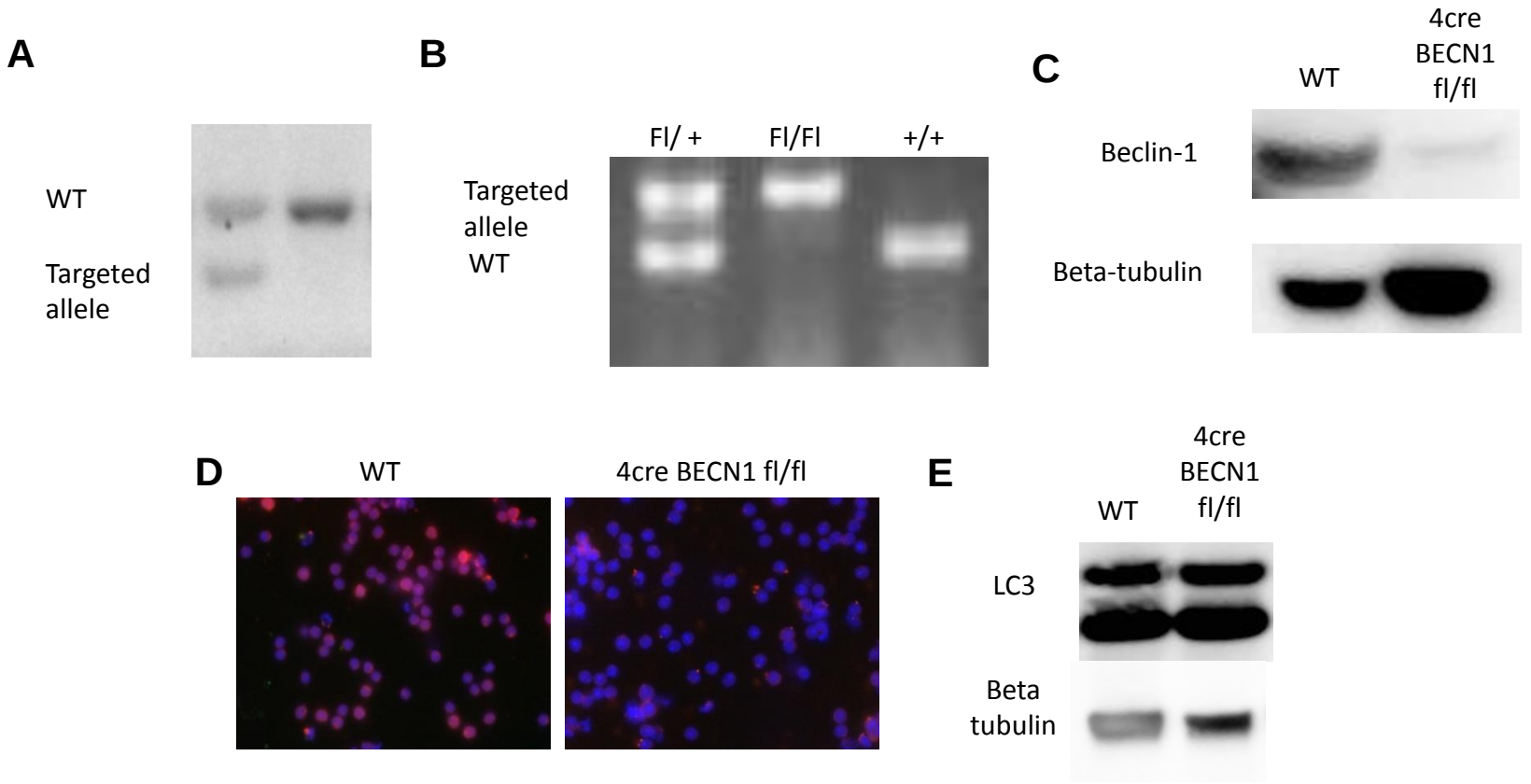
Kovacs et al supplementary figures



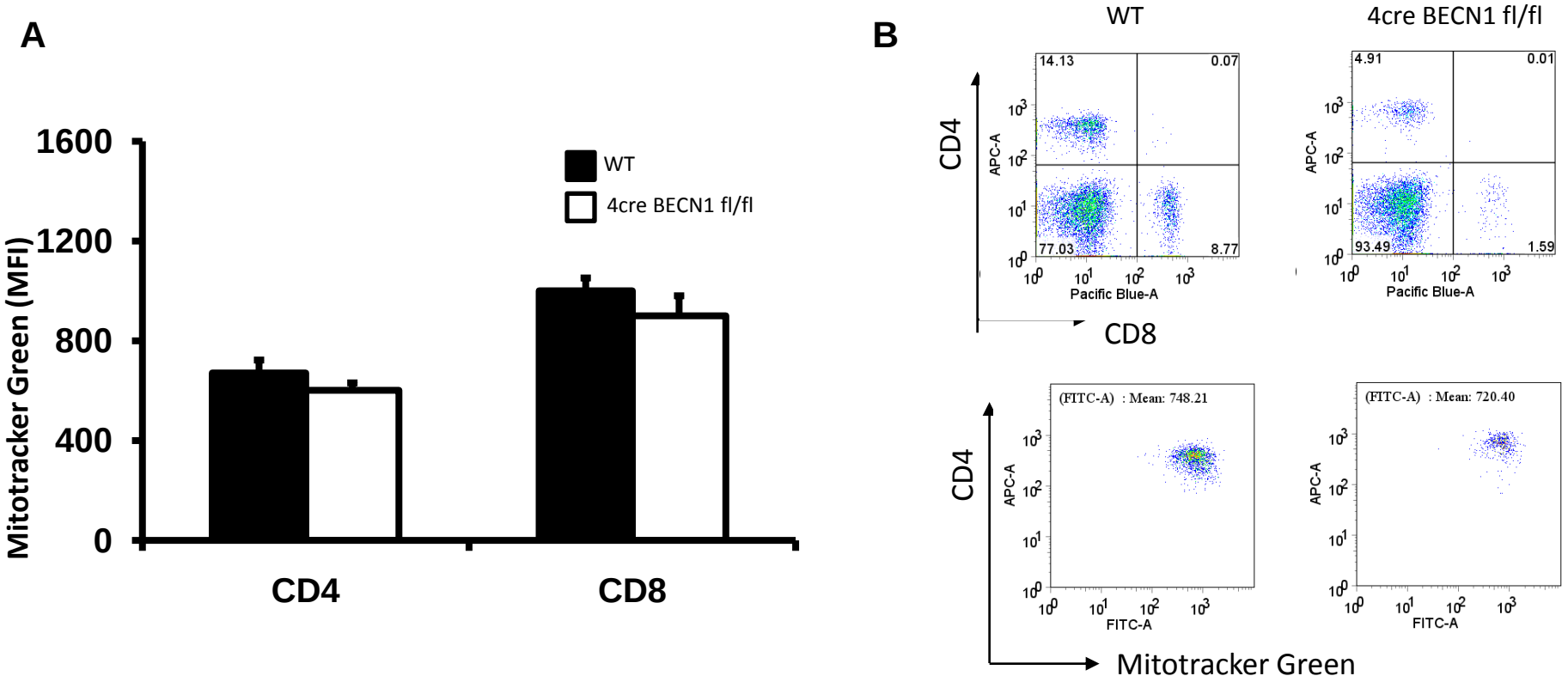
Supplementary Figure 1

Supplementary Figure 1. The targeting strategy for generating BECN1 fl/fl mice.

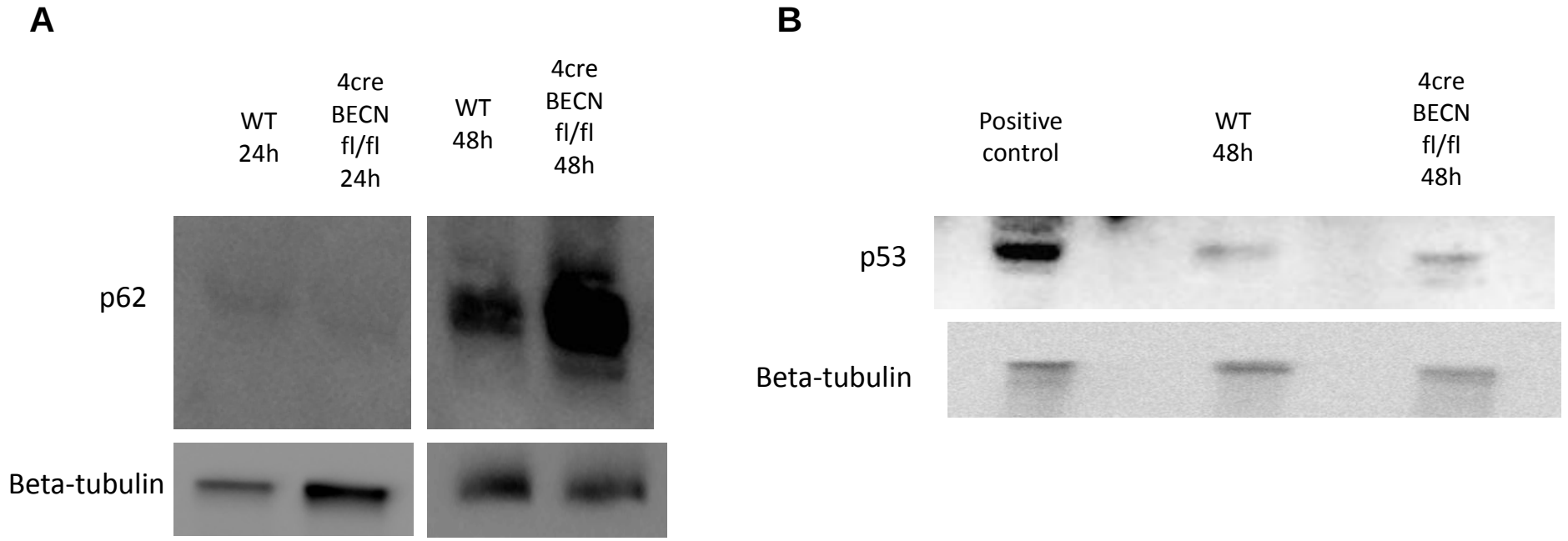
The 10.4 kb genomic fragment containing beclin-1 exons 1 to 3 (filled black boxes) was subcloned from the bacterial artificial chromosome (BAC) clone. Using homologous recombination techniques in bacteria, we inserted a loxp site (filled arrow) in front of exon 1 and another loxp site downstream of exon 2. The Neo resistant cassette is flanked by two FRT sites (open arrows) that allow deletion of Neo by Flipase. B, BamH1; X, Xba I; E1. Genomic DNA purified from ES cells transfected with a beclin-1 targeting vector was digested by BamH1 and subjected to southern analysis using the probe.



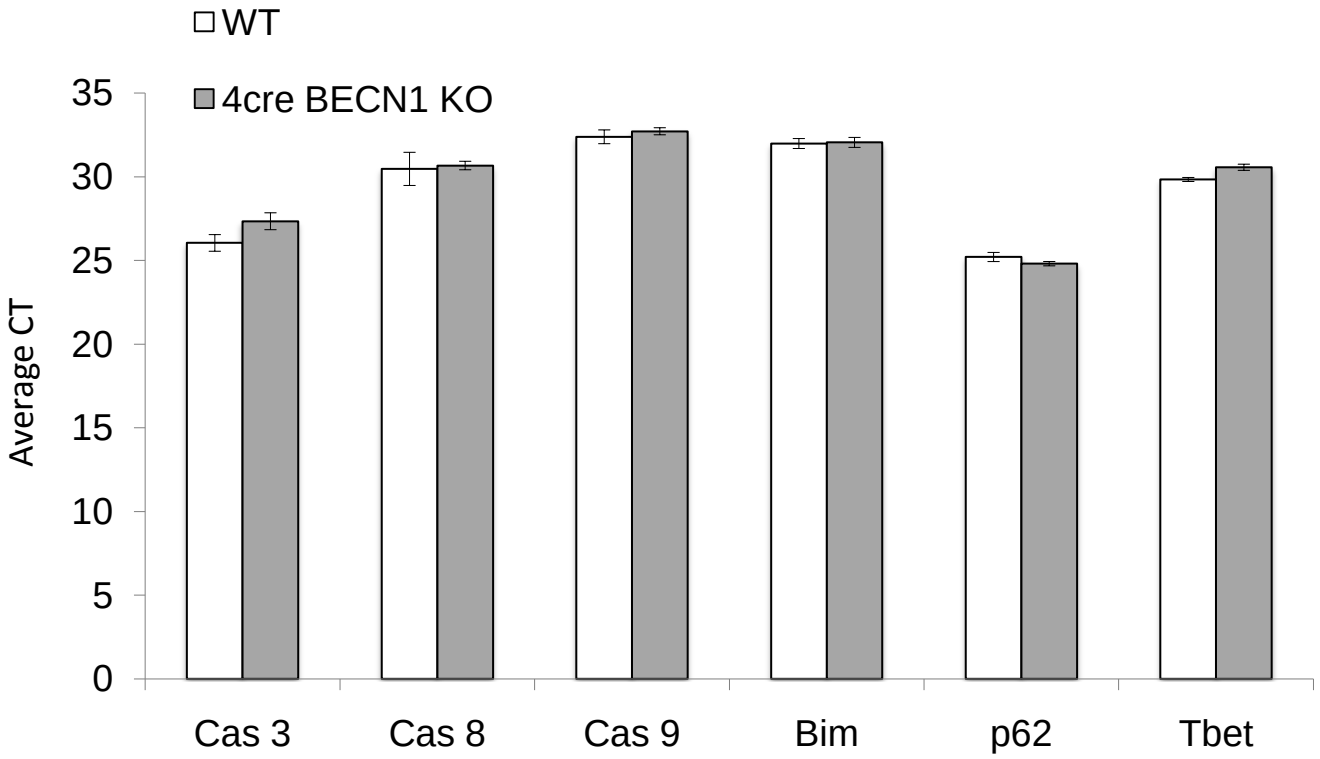
Supplementary Figure 2. Absence of Beclin 1 protein and autophagy in 4cre BECN1 fl/fl T cells. A, detection of Beclin 1 targeting by Southern blot. Genomic DNA purified from ES cells transfected with a Beclin-1 targeting vector was digested by BamH1 and subjected to southern analysis. The wild type band migrates at around 7.5 kb and the conditional "knock-out" band migrates at around 5.9 kb. B, PCR analysis of tail DNA isolated from mouse tails using P1 and P2 primers which flanks 5' loxp site. C, Western blot analysis on extracts made from thymocytes from control (WT) and 4cre BECN1 fl/fl (KO) mice was completed to assess the levels of BECN1 and beta-tubulin (control) D, $CD4^+$ T cells from control (WT) and 4cre BECN1 fl/fl (KO) mice were analyzed for Beclin 1 expression by immunofluorescence (red) and nuclei were stained with Hoechst 33342 dye (blue). E, murine $CD4^+$ T cells were cultured in Th1 conditions for 48h. Western blot analysis on extracts made from $CD4^+$ re-purified T cells was performed to determine the cellular levels of LC3 I and LC3 II and beta-tubulin (control). All results are representative of $n \geq 3$ independent experiments.



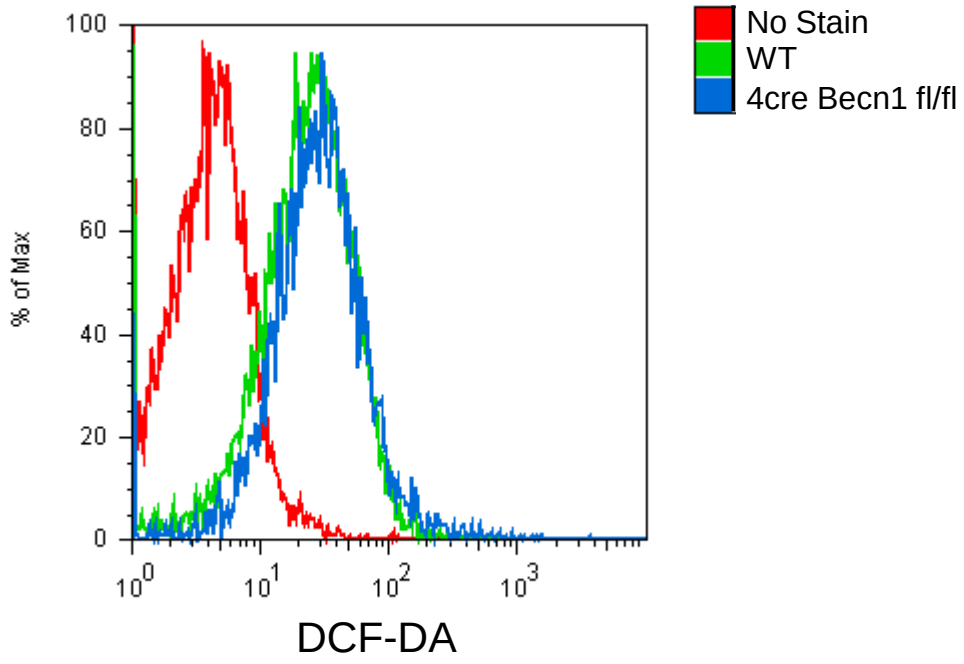
Supplementary Figure 3. Beclin 1 deficient CD4⁺ T cells do not accumulate mitochondria. A, Flow cytometry analysis of lymphocytes in blood samples from both WT (filled bar) and 4cre BECN1 fl/fl (open bar) mice was completed to assess mitochondrial accumulation. Summary of results from one representative experiment (A) and sample dotplot analysis (B) is presented upon staining with MitoTracker Green according to Materials and Methods. The mean fluorescent intensity of MitoTracker Green was analyzed for both CD4⁺ and CD8⁺ populations and data was summarized for more than 15 mice each (WT and 4cre BECN1 fl/fl (means ± SEM). This data is representative of at least 5 independent experiments.



Supplementary Figure 4. p62 accumulates over a 48h period upon activation while p53 was not noticeably accumulated. WT and 4cre BECN1 fl/fl CD4+ T cells were stimulated for 24h and 48h by anti-CD3 and anti-CD28. (A) At 24 and 48h, cells CD4+ cells were re-isolated by positive selection and extract was made for western. The results shown are western blot analysis of the chaperone protein p62 for both WT and 4cre BECN1 fl/fl. (B) CD4+ cells were re-isolated at 48h by positive selection and extract was made for western. The results shown are western blot analysis of p53 protein for both WT and 4cre BECN1 fl/fl and a positive control for p53.



Supplementary Figure 5. mRNA levels of p62 and cell death machinery were not altered in 4cre BECN1 fl/fl. Real-time PCR was performed on RNA isolated from WT and 4cre BECN1 fl/fl $CD4^+$ T cells cultured in Th0 condition for 24h. Error bars represent SEM.



Supplementary Figure 6. Similar levels of ROS were observed in WT and 4cre BECN1 fl/fl CD4+ T cells cultured in the Th1 condition. WT and 4cre BECN1 fl/fl $CD4^+$ T cells cultured in the Th1 condition for 48h. The cells were stained for CD4 and DCF-DA and analyzed by flow cytometry.