Supportive Information Figure 1



Supporting Information Figure 1: (A) Genomic PCRs to detect the floxed (Flx) and Cre-deleted (KO) Bcl-3 alleles, as well as the CD11c-Cre transgene (Cre) in $CD11c^{+}$ cells (DCs) and in $CD3^{+}$ (T) cells that were not treated or were activated with α CD3 and α CD28 (2µg/ml 145-2C11 and 4µg/ml BE0015-1, respectively; BioXCell) for 24h. Cells were isolated from spleens of [Bcl-3^{flx/-}: CD11c-Cre] and [Bcl-3^{flx/-}] mice with MACS micro beads (Miltenvi Biotec). The remaining low level of the FIx allele in CD11c⁺ cells likely reflects minor cellular contamination, as this allele is nearly absent in more highly purified CD11c⁺ DCs cells [13]. (B) WT, Bcl- $3^{-/-}$ and Bcl-3- Δ -DC mice were injected with STAg (10µg/ml) i.v (or not injected) and IL12-p40 was measured in serum 6h later. Mean ± SEM; n=3 mice/group. (C) CD11c⁺ splenic DCs were isolated from WT and $Bcl-3^{-/-}$ and 10^5 cells were stimulated with STAg (5µg/ml), LPS (100ng/ml) and polyI:C (100µg/ml) o.n. Levels of IL-12-p40 in supernatant were assessed with ELISA. Mean ± SEM; n=3 mice/group. (D) WT, $Bc/-3^{-/-}$ and Bcl-3- Δ -DC mice were infected with 20 cysts of T. gondii (ME49 strain). Cells from peritoneal cavity (PEC) were isolated one week later and 3x10⁵ cells were plated o.n in the presence of LPS (100ng/ml), CpG (1µg/ml), Pam3CSK (1µg/ml) and poly I:C (100µg/ml). Levels of IL-12-p40 in supernatant were assessed with ELISA. Mean ± SEM; n=3-4 mice/group. (E) WT, $Bcl-3^{-/-}$ and Bcl-3- Δ -DC mice were infected as above and cells from PECs were isolated one week later and stained for CD11c and MHC-II. Absolute numbers of these cells (inflammatory monocytes derived) shown as mean ±SEM; n=4 mice/group. (F) WT, Bcl- $3^{-/-}$ and Bcl- 3^{-} DC mice were infected as above and levels of IFNy and IL-12p40 in the PEC were measured by CBA and ELISA, respectively. Mean \pm SEM; n=4 mice/group. (G) WT. *Bcl-3^{-/-}* and Bcl-3- Δ -DC mice were infected as above. Cells from spleen and mesenteric lymph nodes (LN) were isolated 3 and/or 7 days later and stained for CD11c and MHC-II. Absolute numbers of DCs shown as mean ±SEM; n=4 mice/group. *p<0.05 and **p<0.01