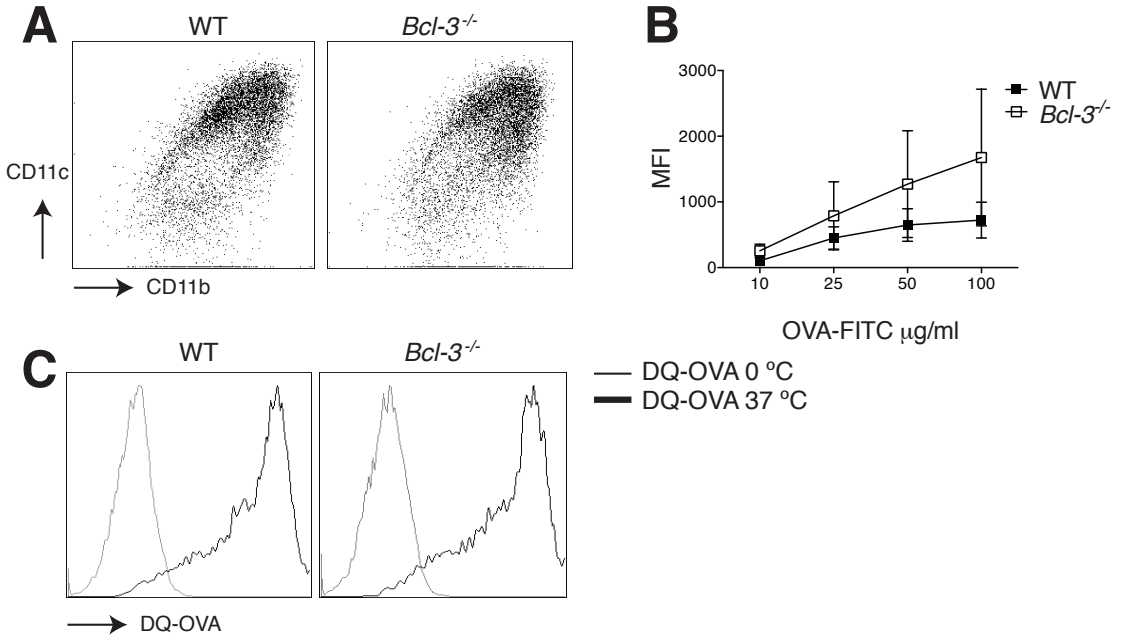
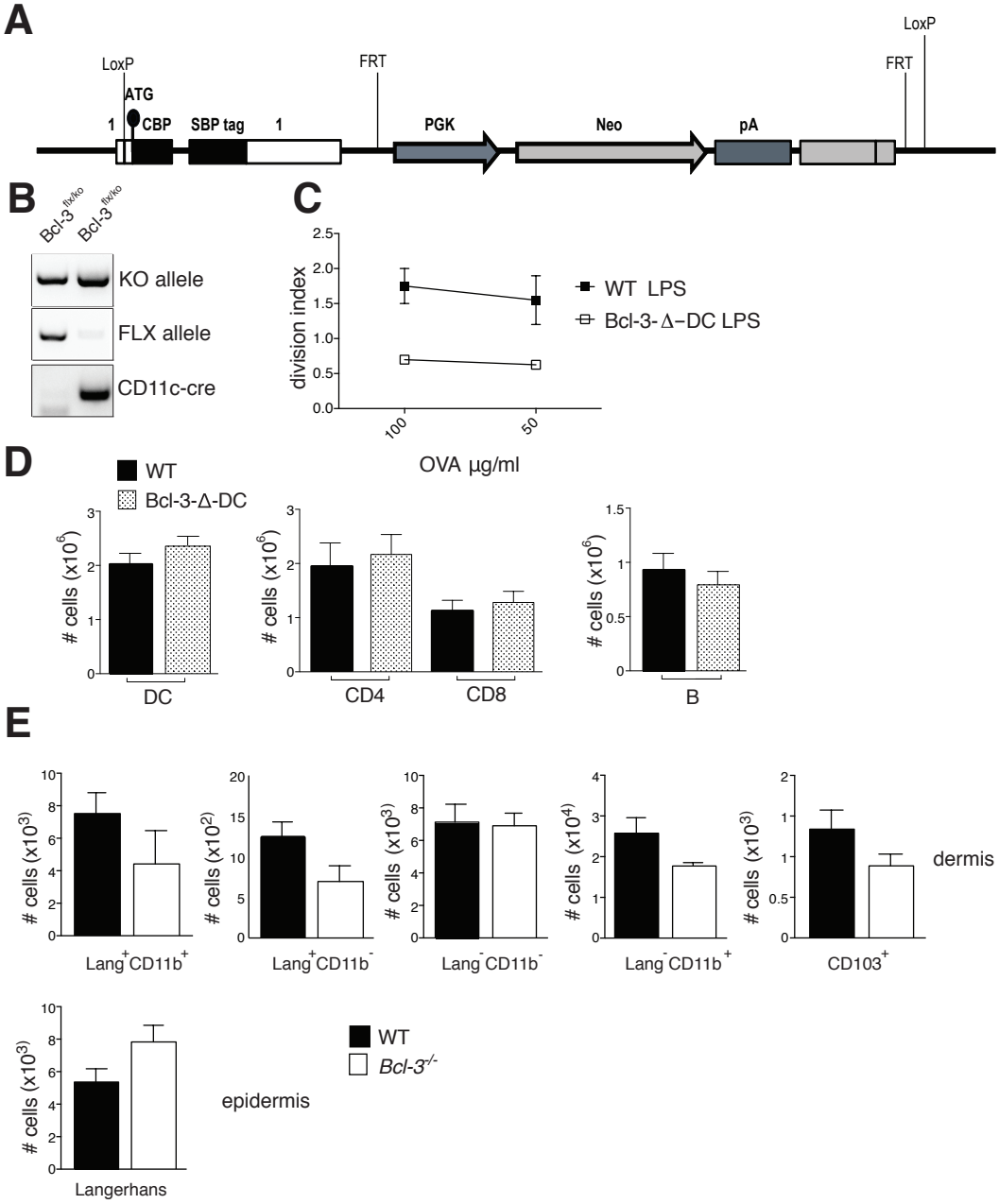
# Supplemental Fig. 1



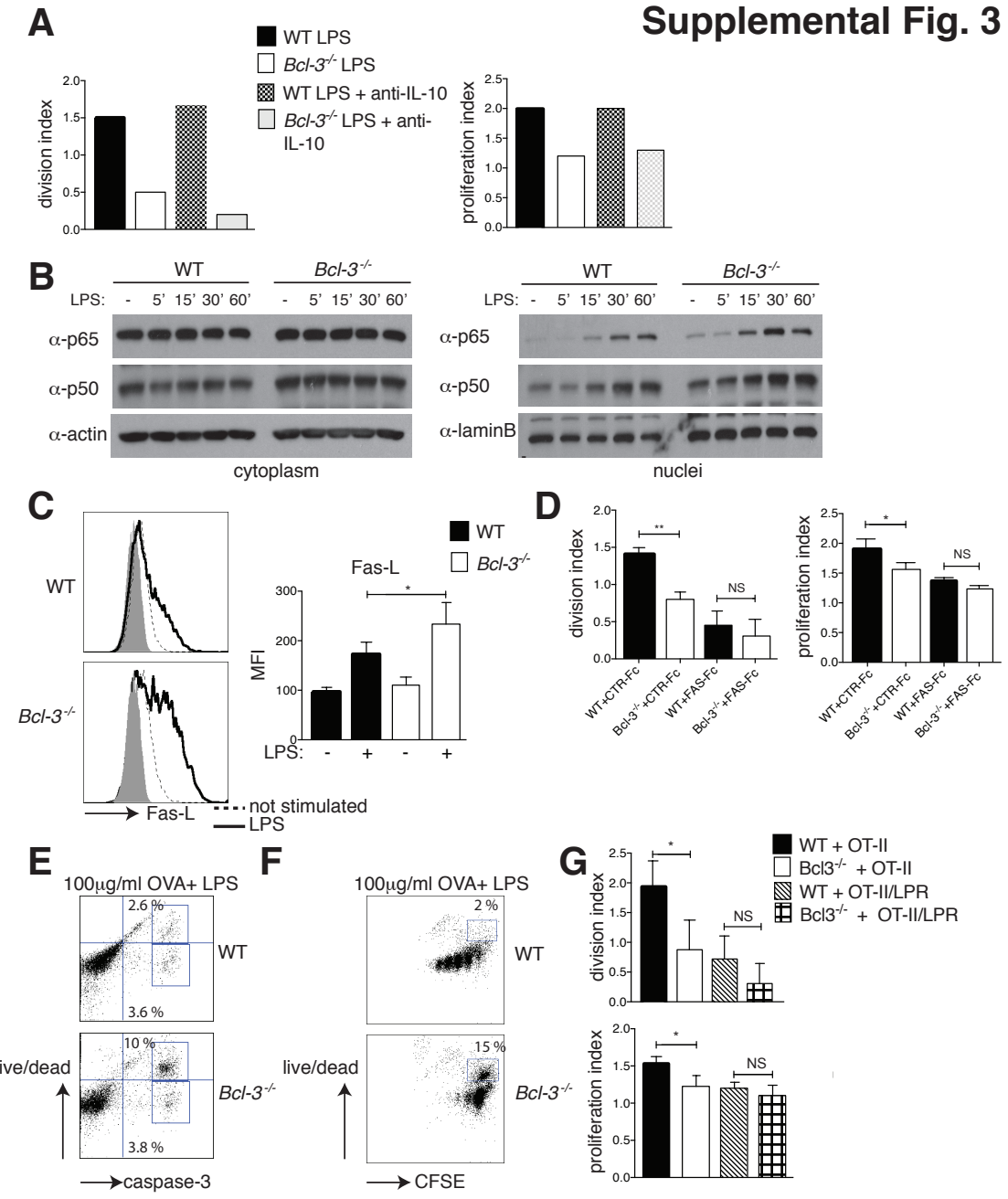

**Supplemental Figure 1. (A)** WT and *Bcl-3*$^{-/-}$ BMDCs were cultured with GM-CSF for 7-8 days, stained for CD11c and CD11b and analyzed by flow cytometry. FACS data are representative of 10 experiments. Similar numbers of WT and *Bcl-3*$^{-/-}$ BMDCs were obtained in these cell culture experiments. **(B)** WT and *Bcl-3*$^{-/-}$ BMDCs were incubated with increasing amounts of OVA-FITC (Invitrogen) for 30 min, stained and gated for CD11c and CD11b and uptake analyzed by flow cytometry. Mean ±SEM; n=3 mice/group. **(C)** WT and *Bcl-3*$^{-/-}$ BMDCs were pulsed with DQ-OVA (Invitrogen) for 15 min, extensively washed and incubated for 30 min at either 4°C or 37°C. Ag processing unquenched DQ-OVA fluorescence, which was recorded as mean fluorescent intensity (MFI). Similar data were obtained in 2 additional experiments.

## Supplemental Fig. 2




**Supplemental Figure 2. (A)** Schematic diagram of the targeting vector used to generate *Bcl-3*$^{flx/flx}$ conditional knockout mice. The Neo cassette is flanked by FRT sites, and exon 1 is flanked by LoxP sites. A tandem affinity purification (TAP) tag was introduced at the translation start site of Bcl-3 (the TAP tag was derived from the pNTAP vector (Agilent Technologies). *Bcl-3*$^{flxNeo/WT}$ mice were generated by Ozgene (Australia) via homologous recombination of the targeting

vector in C57BL/6J-derived ES cells. The Neo cassette was removed by crossing the *Bcl-3*$^{WT/floxNeo}$ mice with mice carrying an FLPe recombinase transgene. The FLPe transgene was subsequently removed from *Bcl-3*$^{flx/WT}$ mice in crosses. *Bcl-3*$^{-/WT}$ mice were generated by germline Cre-mediated deletion (EIIa-cre, Ozgene) of the loxP-flanked sequences and this Cre transgene was also subsequently removed in crosses. *Bcl-3*$^{flx/flx}$, *Bcl-3*$^{-/flx}$ and *Bcl-3*$^{-/-}$ mice were generated by appropriate intercrosses of the latter lines, and Bcl-3-Δ-DC mice (Bcl-3 knockout in CD11c+ cells) were generated by crosses of *Bcl-3*$^{flx/flx}$ and *Bcl-3*$^{-/flx}$ with mice carrying the CD11c-driven Cre recombinase transgene. **(B)** Detection of the Bcl-3 loxP-flanked (floxed; flx) allele and the Cre-mediated loxP-deleted allele (KO) in CD11c$^{+}$ sorted BMDCs generated from mice with genotypes as indicated (with or without CD11c-Cre transgene). **(C)** WT and Bcl-3-Δ-DC BMDCs were loaded with different doses of OVA, stimulated with LPS (100ng/ml) o.n., and cultured with CFSE-labeled OT-II T cells for 72h. After staining and gating for CD4, T cells were analyzed by flow cytometry (division index). Mean ± SEM; n=3 mice/group. **(D)** Splenocytes from WT and Bcl-3-Δ-DC mice were stained for CD4, CD8α, B220, MHC-II and CD11c and CD4, CD8, B and DC cell populations were assessed with countbright beads. Total numbers shown as mean ±SEM; n=3 mice/group. **(E)** Flow cytometric analysis of dermal and epidermal cell suspensions from ears of WT and *Bcl-3*$^{-/-}$ mice. After gating out dead cells, live cells were gated on CD11c$^{+}$MHC-II$^{+}$ and analyzed for expression of Langerin (Lang), CD11b and CD103, as indicated. Lang$^{+}$ cells in the epidermis are Langerhans cells. The cell numbers for each population were assessed with countbright beads. Total numbers shown as mean ±SEM; n=5 mice/group.




**Supplemental Figure 3. (A)** WT and *Bcl3*$^{-/-}$ BMDCs were co-cultured with CFSE-labeled OT-II cells and T cells analyzed as in Figure 4C (standard conditions and analysis), except that indicated co-cultures also contained anti-IL-10 blocking antibodies (10μg/ml) (JES5-2A5, BioXcell). **(B)** WT and *Bcl3*$^{-/-}$ BMDCs were stimulated for the indicated times with LPS (100 ng/ml). Cytoplasmic (left panels) and nuclear (right panels) fractions were analyzed by immunoblot with anti-p65

and anti-p50 antibodies. As a control for protein loading, the membranes were immunoblotted with an anti-actin antibody for the cytoplasmic fraction, and with anti-laminB antibody for the nuclear fraction. **(C)** WT and *Bcl-3*$^{-/-}$ BMDCs were left unstimulated or stimulated with LPS (100ng/ml) for 24h, then stained for CD11c, CD11b and FasL and analyzed by flow cytometry for FasL (MFI) after gating on CD11c and CD11b. Isotype control staining is shown by shaded histograms. Representative FACS data in left panels and summary in right panel, shown as mean ± SEM; n=4 mice/group. **(D)** WT and *Bcl3*$^{-/-}$ BMDCs were OVA loaded, LPS stimulated and co-cultured with CFSE-labeled OT-II cells and T cells analyzed as in (A) (standard conditions and analysis; Figure 4C), except for the presence of FAS-Fc or control CTR-Fc in co-cultures. T cell proliferation data shown as mean ± SEM; n=3 mice/group. **(E)** WT and *Bcl3*$^{-/-}$ BMDCs were OVA loaded, LPS stimulated, then co-cultured with OT-II cells and T cells analyzed after staining and gating for CD4 (standard analysis). Here T cells were analyzed for live/dead (live/dead fixable kit) and caspase-3 activity. FACS plots shown are representative of 4 experiments. **(F)** WT and *Bcl3*$^{-/-}$ BMDCs were treated and co-cultured with CFSE labeled OT-II and CD4+ T cells gated on as in (C), but now analyzed for live/dead and CFSE. FACS plots shown are representative of 4 experiments. **(G)** WT and *Bcl3*$^{-/-}$ BMDCs were treated, co-cultured with CFSE-labeled OT-II or OT-II/LPR T cells and T cells analyzed as in (A). Data shown as mean ± SEM; n=4/group.