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Supplemental Data

LXR Signaling Couples

Sterol Metabolism to Proliferation

in the Acquired Immune Response

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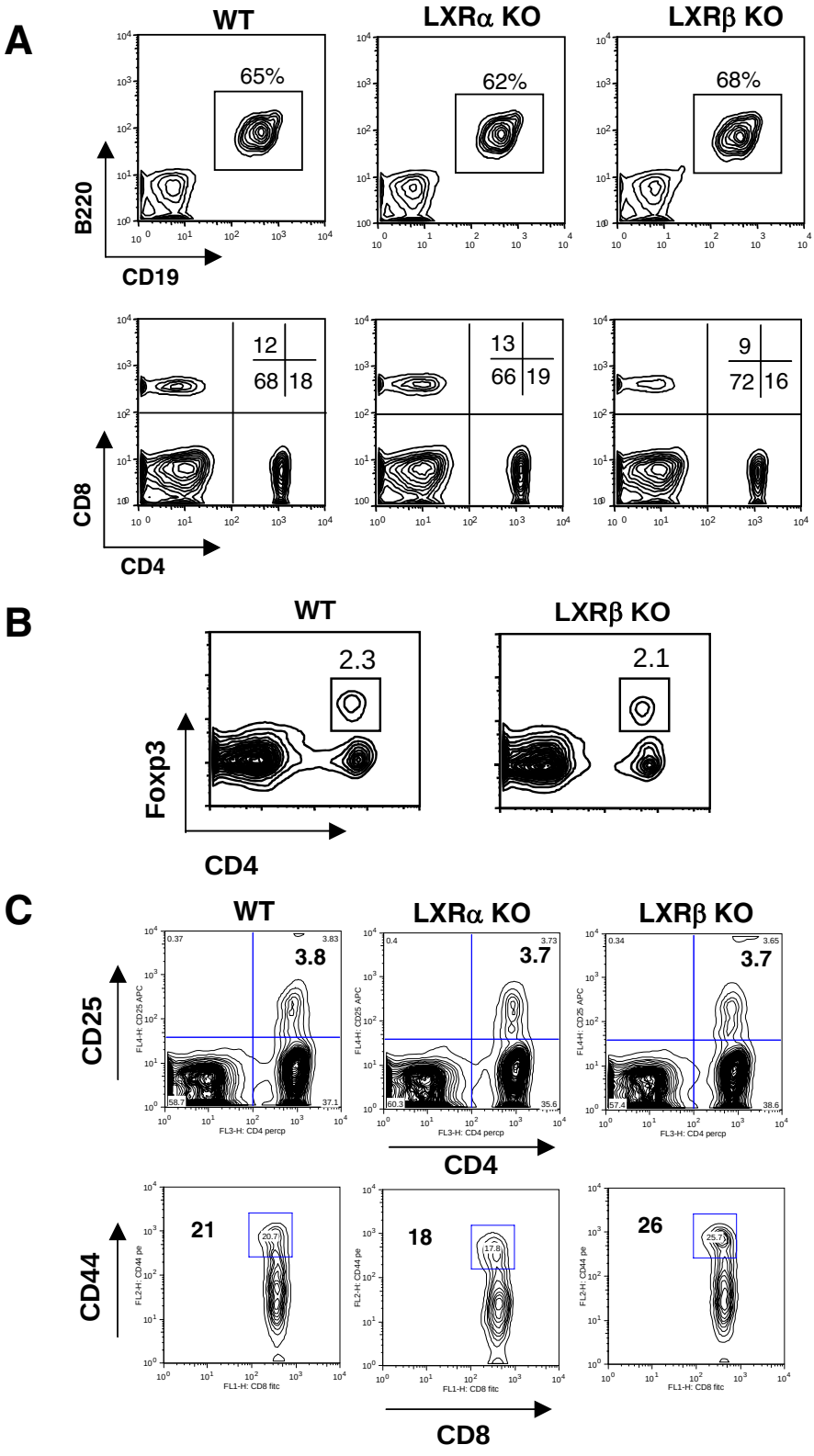

Figure S1. Normal distribution of T and B lymphocytes in LXR null mice. (A) Splenocytes from male, 6-8 week old WT, LXRα KO and LXRβ KO mice were stained with anti-CD4, CD8, CD19 and B220. The frequency of B cells is shown in the upper

panel and the frequency of CD4 and CD8 T cells are marked in the upper right quadrant of lower panels. (B) Spleen cells from WT and LXRβ KO mice were stained for CD4 and intracellular Foxp3. (C) Splenocytes from WT, LXRα KO and LXRβ KO mice were stained with anti-CD4, CD8, CD44 and CD25. FACS plots are representative of one mouse per genotype repeated greater than 5 times.

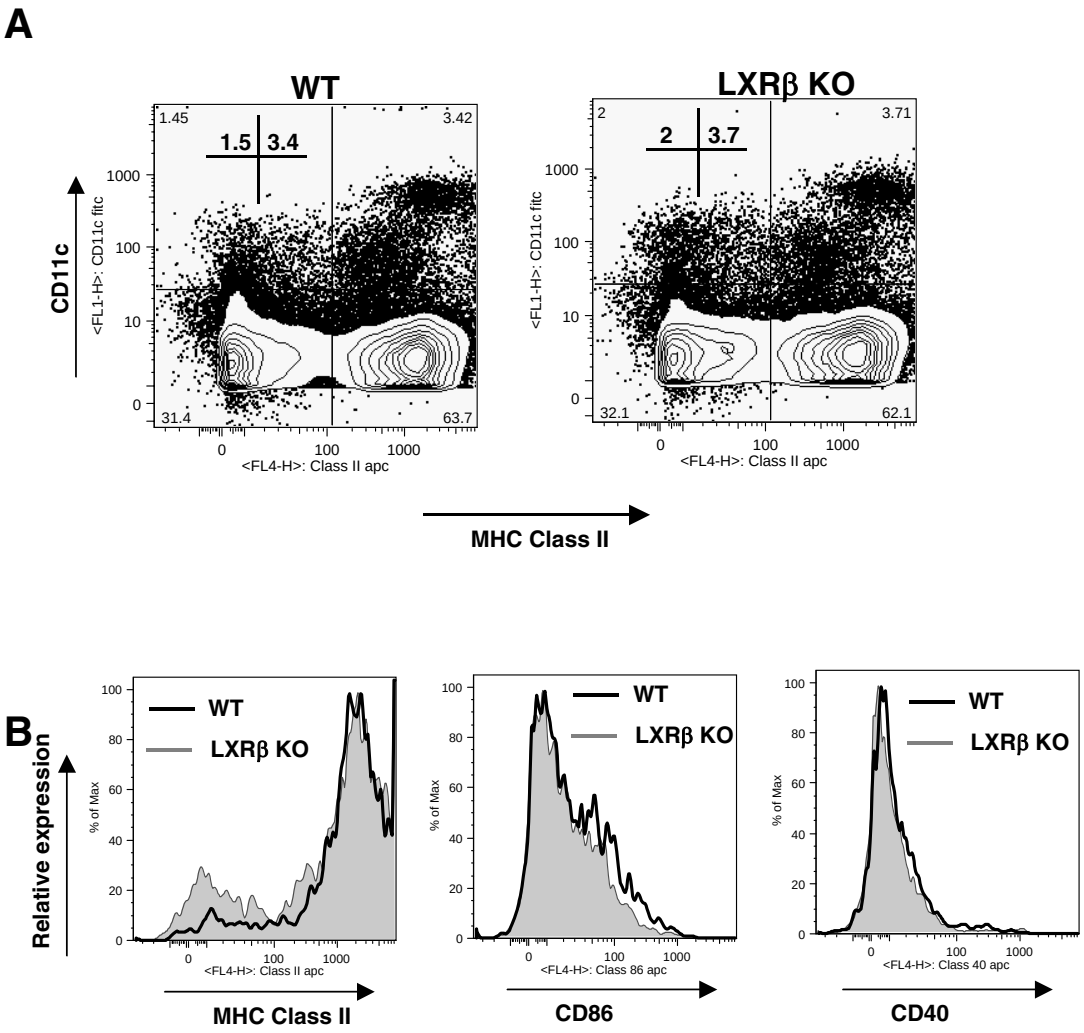

Figure S2. Normal APC phenotype in aged LXRβ null mice. (A,B) Spleen cells from 5 month old WT and LXRβ KO mice were stained for CD11c, MHC class II, CD40, and CD86 expression. FACS plots are representative of one mouse per genotype repeated greater than 3 times.

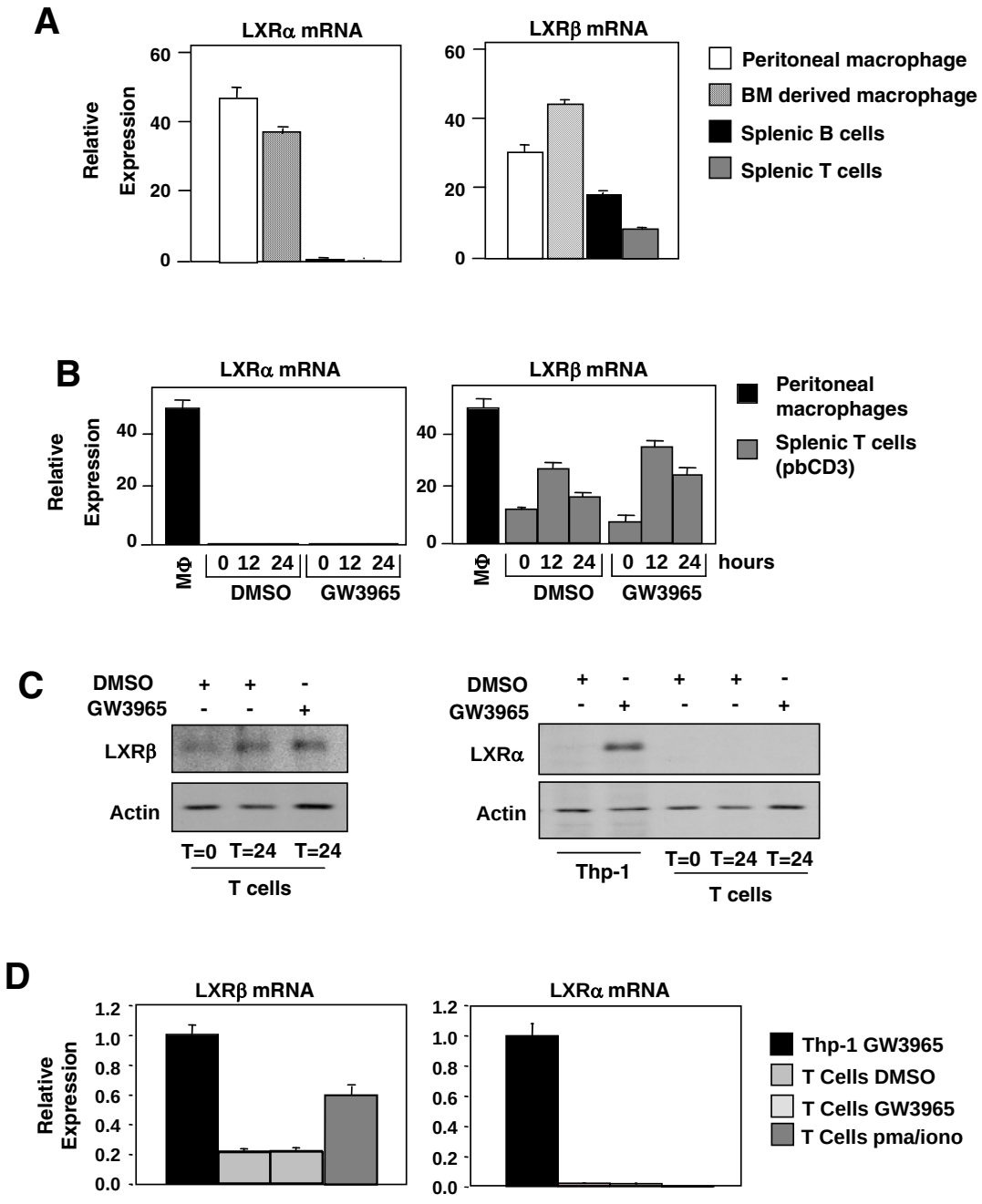

Figure S3. LXRβ is expressed in quiescent and activated lymphocytes. (A) Real-time PCR analysis of mRNA from C57BL/6 thioglcollate- elicited peritoneal macrophages, bone marrow derived macrophages, *ex vivo* purified splenic B and T cells. (B) Purified splenic T cells were activated for 12- 24 h with pbCD3 and LXR ligand as indicated and Real-time PCR analysis was performed. (C,D) Purified human T cells were cultured with LXR ligand and PMA/ionomycin for 24 h as indicated. (C) Whole cell lysates were collected at the indicated time and probed for LXRα and LXRβ expression by Western. (D) mRNA was collected at the indicated time and analyzed for LXRα and LXRβ gene expression by real-time PCR.

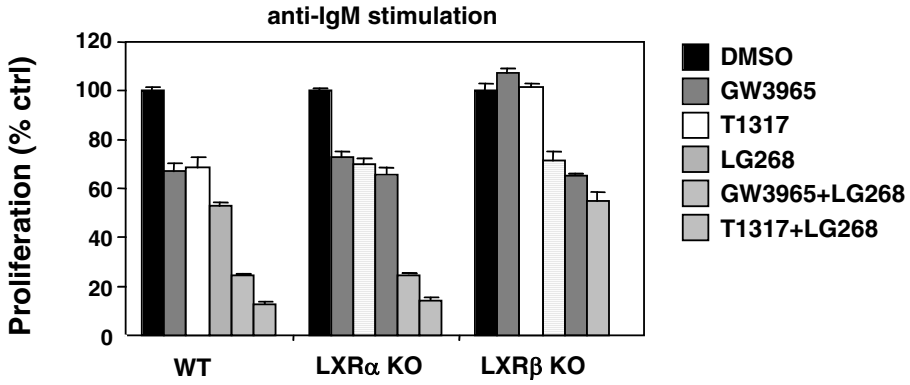

Figure S4. Gain of LXR function inhibits B lymphocyte proliferation. Spleen cells from 6-8 week old WT, LXRα KO and LXRβ KO mice were stimulated with anti-IgM(Fab')$_2$. Cultures were treated with LXR ligands GW3965 (2μM), T1317 (1μM) and RXR ligand LG268 (100nM) as indicated as indicated. ^{3}H-thymidine was added to cultures after 72 h for the final 16 h.

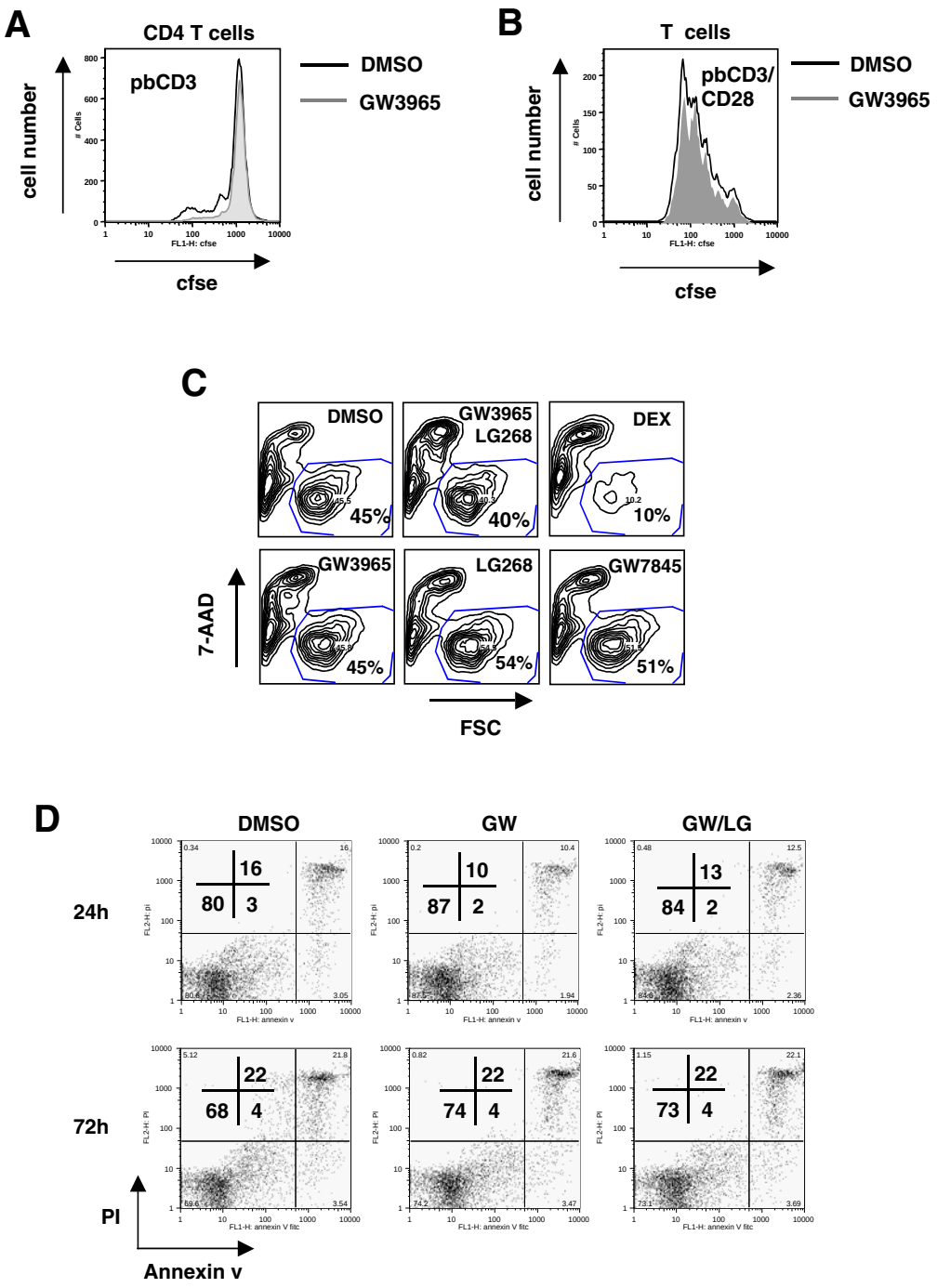

Figure S5. Gain of LXR function negatively regulates human CD4 T cell expansion, but can be rescued by costimulation and does not induce apoptosis. (A,B) Purified CFSE labeled human T cells were and stimulated with pbCD3, soluble anti-CD28 and GW3965 for 96 h. Cultures were stained with anti- CD4 and 7-AAD at the indicated times. $5x10^4$ counting beads were added to samples to serve as an internal counting control. (C) Purified WT mouse T cells were activated with pbCD3 and cultured with LXR (GW3965) , RXR (LG268), GR (dexamethasone), and PPARγ (GW7845) ligands or DMSO control. Cultures were stained with 7-AAD at 24 h. (D) Purified human T cells

were stimulated with pbCD3 and GW3965 for 24- 72 h. Cultures were stained for annexin V and PI at the indicated times.

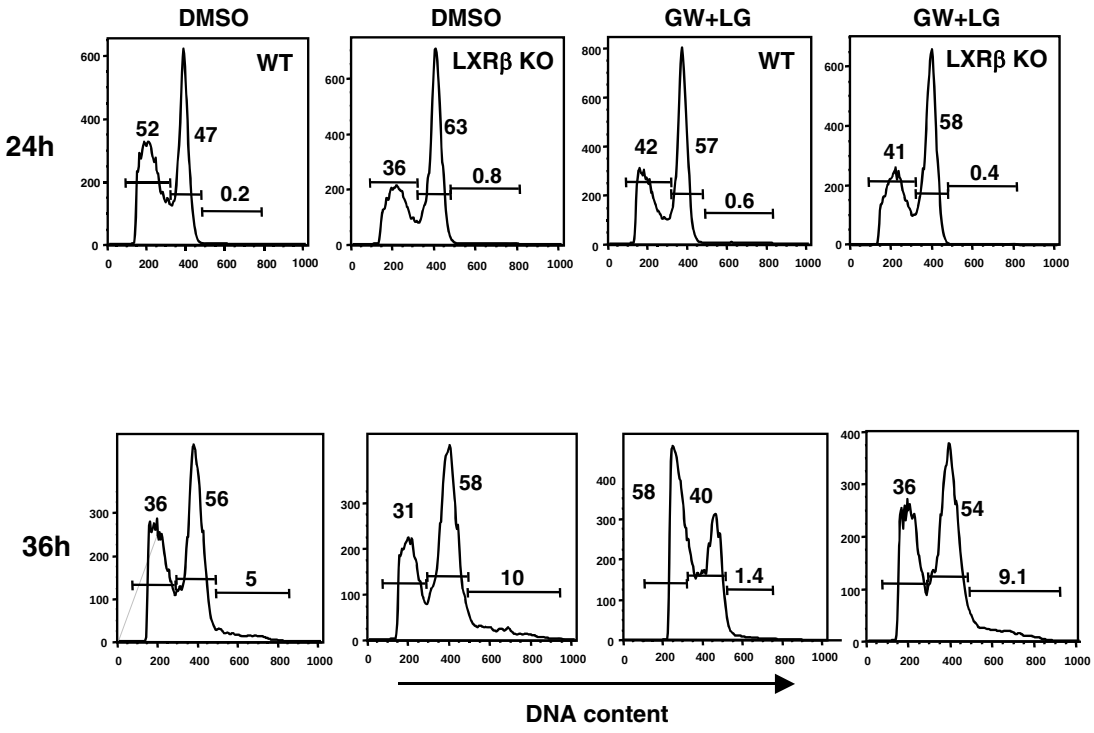

Figure S6. LXRβ signaling regulates cell cycle progression. WT and LXRβ KO T cells were stimulated with pbCD3 and LXR/RXR ligands (GW3965/LG268) as indicated. Cells were stained for DNA content with propidium iodide at the indicated times and analyzed by flow cytometry.

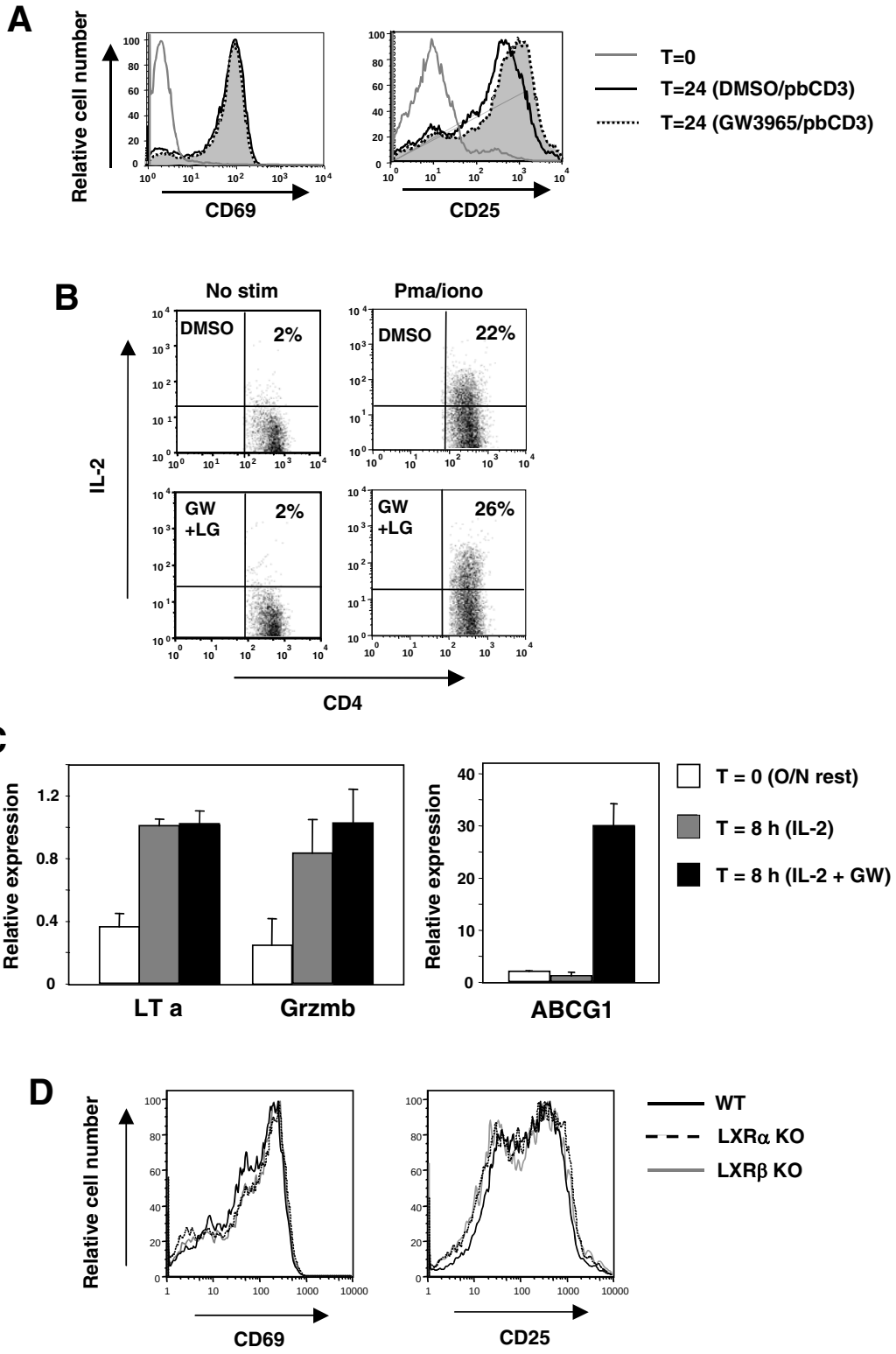

Figure S7. Gain- and loss- of LXR function does not perturb activation or IL-2 signaling. (A) CD69 and CD25 expression on WT T cells *ex vivo* or after 24h activation with pbCD3 in the presence LXR/RXR ligand (GW3965/LG268) as indicated. (B) IL-2 production from purified WT T cells activated with pbCD3 for 24 h and then cultured

with Brefeldin A and restimulated with PMA/ionomycin. After 5h stimulation, cells were stained with anti-CD4 and CD8, then fixed, permeabilized, stained for intracellular IL-2 and analyzed by flow cytometry. (C) Real-time PCR analysis of LTa and GRZMb mRNA from Purified WT T cells stimulated with pbCD3 for 5 days, rested for 18 h and then restimulated with IL-2 for 8h. (D) CD69 and CD25 expression on WT, *Lxr*a-/- and *Lxr*β-/- T cells after 24h activation with pbCD3.

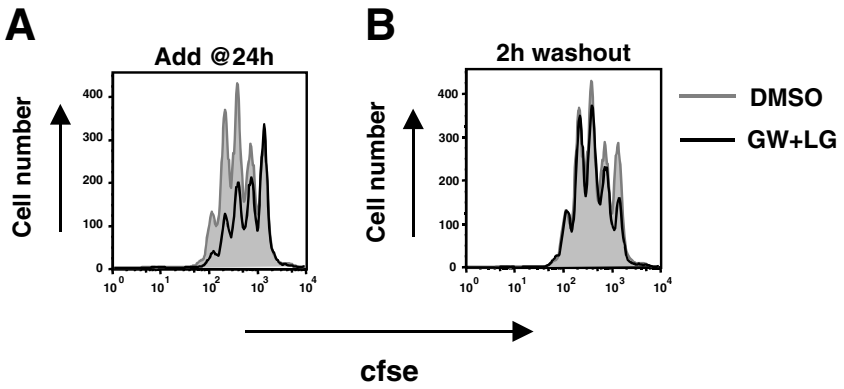

Figure S8. Sustained LXR signaling is required to inhibit proliferation. (A) CFSE dilution of purified WT T cells stimulated with pbCD3 for 72h. In addition, indicated cultures received LXR/RXR ligands (GW3965/LG268) at 24h. (B) Cultures were pre-treated with LXR/RXR agonist for 2 h, then washed, placed in fresh media and stimulated with pbCD3. Cells were harvested at 72 h and analyzed by flow cytometry. $5x10^4$ counting beads were added to serve as an internal counting control and samples were analyzed via flow cytometry.

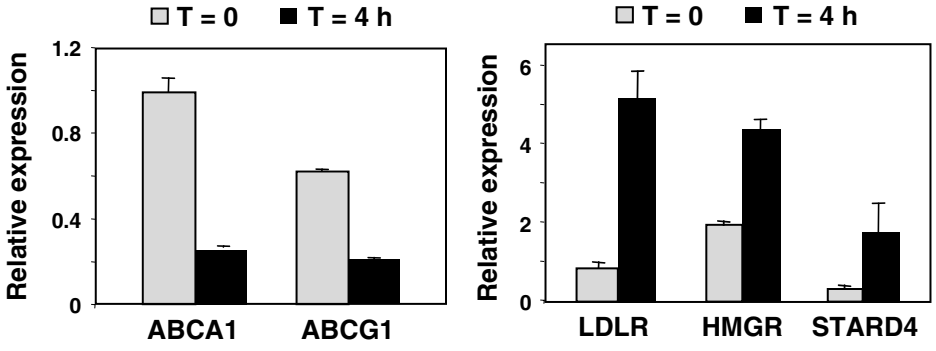

Figure S9. Reciprocal regulation of SREBP-2 and LXR transcriptional programs during human lymphocyte activation. Real-time PCR analysis of ABCA1, ABCG1, LDLR, HMGCR and STARD4 mRNA from purified human peripheral blood lymphocyes *ex vivo* or stimulated with pma/ionomycin for 4h.

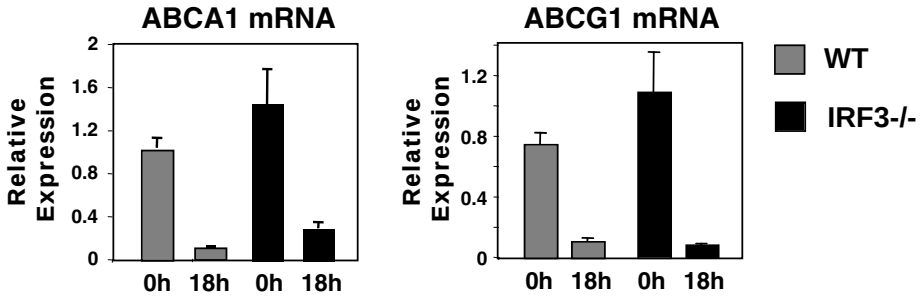

Figure S10. IRF-3 does not influence TCR mediated downregulation of LXR target genes in lymphocytes. Realtime PCR analysis of ABCA1 and ABCG1 from purified WT and IRF3-/- T cells ex vivo or after stimulation with pbCD3 for 18 h**.**

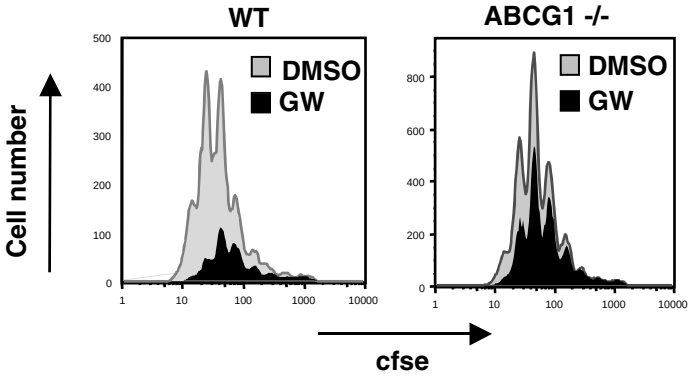

Figure S11. LXR inhibits proliferation through ABCG1-dependent alteration of cholesterol homeostasis. CFSE dilution of purified ABCG1-/- and WT T cells stimulated with pma/ionomycin and GW3965 for 96 h. $5x10^4$ counting beads were added to serve as an internal counting control and samples were analyzed via flow cytometry.