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Liver X receptor β is required for the survival of single-positive thymocytes by regulating IL-7R α expression

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Liver X receptors (LXRs) are known as key transcription factors in lipid metabolism and have been reported to play an important role in T-cell proliferation. However, whether LXRs play a role in thymocyte development remains largely unknown. Here, we demonstrated that LXR β deficiency caused a reduction in single-positive (SP) thymocytes, whereas the transitions from the double-negative to SP stage were normal. Meanwhile, LXR β -null SP thymocytes exhibited increased apoptosis and impairment of the IL-7R α -Bcl2 axis. In addition, the LXR agonist T0901317 promoted the survival of SP thymocytes with enhanced IL-7R α expression in wild-type mice but not in LXR β -deficient mice. Mechanistically, LXR β positively regulated the expression of IL-7R α via direct binding to the *Il7r* allele in SP thymocytes, and forced expression of IL-7R α or Bcl2 restored the survival of LXR β -defective SP thymocytes. Thus, our results indicate that LXR β functions as an important transcription factor upstream of IL-7R α to promote the survival of SP thymocytes.

Keywords: LXR β ; IL-7R α ; Single-positive thymocytes; Cell survival; Transcriptional regulation

Cellular & Molecular Immunology (2021) 18:1969–1980; <https://doi.org/10.1038/s41423-020-00546-y>

INTRODUCTION

T lymphocytes, including CD8⁺ and CD4⁺ T cells, which are essential mediators for cellular and humoral immune responses, are produced following sequential developmental stages in the thymus.¹ T-cell precursors migrate from the bone marrow (BM) to the thymic cortex, where they differentiate into CD4⁺CD8⁻ double-negative (DN) thymocytes, which are conventionally divided into four subsets (DN1–DN4) based on CD44 and CD25 expression.^{1,2} After the rearrangement of the T-cell receptor (TCR), DN4 thymocytes expressing pre-TCR sequentially develop into CD8⁺ (for mice) or CD4⁺ (for humans) TCR β ⁻ immature single-positive (ISP) and CD4⁺CD8⁺ double-positive (DP) thymocytes, in which DP thymocytes undergo positive and negative selection and then differentiate into immature CD4⁺TCR β ⁺ or CD8⁺TCR β ⁺ single-positive (SP) thymocytes.^{2,3} Positive selection allows the proliferation of cells with TCRs that recognize self-peptide-MHC (major histocompatibility complex) with appropriate affinity or causes cell death by neglecting cells with TCRs that fail to interact with self-MHC.^{2,4} Negative selection permits the survival of cells with TCRs that recognize self-MHC or self-peptide-MHC with weak affinity.^{2,4} Finally, the cells mature into MHC-II-restricted CD4⁺ or MHC-I-restricted CD8⁺ SP thymocytes in the thymic medulla and migrate to the peripheral lymphoid organs, where they become naive CD4⁺ or CD8⁺ T cells.^{1,2}

In addition to TCR signaling, interleukin-7 (IL-7) signaling is crucial for thymocyte development and survival during the progression

from the DN to DP stage.^{5,6} IL-7 signaling is transduced by the IL-7 receptor (IL-7R), which is a heterodimeric complex consisting of an IL-7R α -chain (IL-7R α , encoded by *Il7r*) and a common γ -chain.^{5,7} Upon IL-7 binding, the receptor complex activates the tyrosine kinases JAK1 and JAK3, which then activate Stat5 and eventually promote cell survival by inducing the expression of anti-apoptotic factors such as Bcl2 in thymocytes.^{7,8} Beyond the DP stage, IL-7 signaling still plays an essential role in the final maturation and survival of CD4⁺ and CD8⁺ SP thymocytes,^{9,10} consistent with the “on-off-on” pattern of IL-7R α expression at the DN, DP, and SP stages.^{5,11} Given the central role of IL-7R α in IL-7 signaling, the regulation of IL-7R α expression in thymocytes is being elucidated step by step.¹² In the absence of IL-7, ephrinb1 and ephrinb2, the ligands for erythropoietin-producing hepatocellular kinases, interact directly with IL-7R α and then stabilize the expression of IL-7R α in thymocytes by retarding its internalization.¹³ As a receptor, the surface IL-7R α protein is downregulated by clathrin-mediated endocytosis upon IL-7 binding, where the IL-7R α protein prefers to degrade in a JAK3-dependent manner¹⁴ rather than recycle in a Vps34-dependent manner.¹⁵ In addition to the above posttranslational regulation processes, several transcription factors are reportedly involved in the regulation of *Il7r* transcription. The transcription factor Foxo1 promotes the survival of naive T cells by upregulating *Il7r* transcription via direct binding to its 5'-regulatory region, although Foxo1 is dispensable for T-cell development due to its low expression in the thymus.¹⁶ In contrast, the transcriptional repressor

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Received: 26 March 2020 Accepted: 23 August 2020

Published online: 22 September 2020

Gfi1 controls developmental maturation during the DP-to-SP transition partially by suppressing the transcription of the *Foxo1-Il7r* axis.¹⁷ Moreover, the transcription factor Foxp1 inhibits *Il7r* transcription by antagonizing Foxo1 at the same locus and then controls the quiescence of naive T cells.¹⁸ In addition, GA binding protein α , a member of the Ets family, maintains *Il7r* transcription in DN thymocytes by occupying the promoter of the *Il7r* gene.¹⁹ Despite our increasing knowledge of the regulation of IL-7Ra expression in DN thymocytes and peripheral naive T cells, the regulatory factors of IL-7Ra expression and the underlying mechanism in SP thymocytes remain to be further elucidated.

Liver X receptors (LXRs), including two isoforms, namely, LXRA (encoded by *Nr1h3*) and LXR β (encoded by *Nr1h2*), are ligand-activated nuclear receptors and play pivotal roles in lipid metabolism. Transcriptional activity of LXRs is induced in response to increased cellular levels of oxysterols, cholesterol, and several intermediates in the cholesterol biosynthetic pathway, as well as synthetic agonists, such as T0901317 and GW3965.²⁰ LXRA and LXR β exhibit appreciable protein sequence homology but have different tissue distributions: the former is highly expressed in the intestine, liver, adipocytes, and macrophages, and the latter is ubiquitously expressed.^{20,21} Upon heterodimer formation with retinoid X receptors (RXRs), the LXR-RXR complex binds to an LXR response element (LXRE) containing repeated canonical "RGGTCA" consensus sequences separated by four nucleotides and then controls lipid homeostasis by regulating the expression of a series of genes involved in cholesterol, fatty acid, and phospholipid metabolism.^{20–22} In addition to their roles in lipid metabolism, LXRs have several important functions in the regulation of immune responses. For example, LXR agonists exert potent anti-inflammatory effects via transrepression of the expression of several inflammatory genes in different mouse models of inflammatory disease.²³ Moreover, upon bacterial infection, LXRA promotes macrophage survival by upregulating the expression of SP α , an anti-apoptosis factor.²⁴ In addition, LXR agonists exert anti-proliferative effects by promoting cholesterol efflux via the induction of ABCG1 expression in T lymphocytes.²⁵ Despite this progress, little is known regarding the roles of LXRs in thymocyte development. In this study, we determined that LXR β was necessary for the survival of SP thymocytes. LXR β directly bound to the *Il7r* 5'-regulatory region and then promoted *Il7r* transcription in SP thymocytes. Notably, overexpression of IL-7Ra or Bcl2 rescued the defects caused by LXR β deficiency in SP thymocytes. Thus, our findings suggest that LXR β positively regulates the survival of SP thymocytes by promoting *Il7r* transcription via direct binding to its 5'-regulatory region.

MATERIALS AND METHODS

Animals and T0901317 treatment

LXRA- and LXR β -deficient mice²⁶ were provided by Dr. J.Å. Gustafsson (Karolinska Institutet) and were in the C57BL/6J background. C57BL/6J (CD45.1 and CD45.2) mice were obtained from the Jackson Laboratory. WT, α KO, and β KO mice were analyzed at 12–14 weeks of age unless noted otherwise, and both sexes were included without randomization or "blinding". BM chimeras were generated in irradiated 3- to 5-month-old recipients and then analyzed after 12–16 weeks of reconstitution. All mouse experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committees of the Third Military Medical University. WT and β KO mice were intraperitoneally (i.p.) injected with T0901317 (S7076, Selleck, 0.4 mg/ml in olive oil, 0.2 mg/d per mouse) or 0.5 ml of olive oil for 1 day or 7 continuous days.

Flow cytometry and antibodies

Flow cytometry data were acquired with a FACSCanto II (BD Biosciences) and analyzed with FlowJo software (Tree Star). Biotinylated Lin markers, including CD4 (clone GK1.5), CD8 α

(clone 53-6.7), B220 (clone RA3-6B2), CD11b (clone M1/70), CD11c (clone N418), NK1.1 (clone PK136), Gr-1 (clone RB6-8C5), and TER-119 (clone TER-119), were used for Lin depletion in BM cells or thymocytes excluding CD4 and CD8 markers. Surface staining for CD4 (clone RM4-5), CD8 α (clone 53-6.7), CD44 (clone IM7), CD62L (clone MEL-14), CD24 (clone M1/69), Qa2 (clone 69H1-9-9), CD25 (clone PC61), CD69 (clone H1.2F3), TCR β (clone H57-597), CD5 (clone 53-7.3), Sca-1 (clone D7), c-Kit (clone 2B8), CD45.1 (clone A20), CD45.2 (clone 104), IL-7Ra (clone A7R34), and Thy1.1 (clone HIS51) was performed in PBS containing 2% fetal bovine serum (FBS). Intracellular staining for Bcl2 (clone BCL/10C4) was performed with the Cytofix/Cytoperm Fixation/Permeabilization Kit (554722, BD Biosciences). Intranuclear staining for Ki-67 (556027, BD Biosciences) was performed with the Transcription Factor Staining Buffer Set (562574, BD Biosciences). For detection of Caspase activation, thymocytes or splenocytes were analyzed with the FAM Caspase-3 and -7 Assay Kit (V35118, Invitrogen) or the Violet Live Cell Caspase Probe (565521, BD Biosciences) according to the manufacturer's instructions. Annexin V/7AAD staining was performed with the PE Annexin V Apoptosis Detection Kit I (559763, BD Biosciences), and mitochondrial membrane potential was detected by the Mitochondria Staining Kit (JC-1) (MJ101, MultiSciences) according to the manufacturer's instructions. For in vivo incorporation of BrdU, an analog of thymidine, mice were administered BrdU (2 mg of BrdU in 0.2 ml of PBS, i.p.) 12 h (for thymocytes) or 24 h (for lymphocytes) before sacrifice. BrdU incorporation was detected by staining with the APC BrdU Flow Kit (552598, BD Biosciences) according to the manufacturer's instructions. For p-Stat5 detection, surface-stained thymocytes were stimulated with IL-7 (1 ng/ml; 217-17, Pepro-Tech) at 37 °C for 1 h. The stimulated thymocytes were immediately fixed with Phosflow Lyse/Fix Buffer (558049, BD Biosciences), followed by permeabilization with Phosflow Perm Buffer I (557885, BD Biosciences), and successively stained with an anti-p-Stat5 antibody (9314S, Cell Signaling Technology) and an A647-conjugated anti-rabbit IgG antibody (4414S, Cell Signaling Technology).

Generation of BM chimeras

For generation of competitive BM chimeras, donor LSK cells from β KO (CD45.2) and WT (CD45.1) mice were mixed (1:1), and a total of 6000 LSK cells were then transferred into lethally irradiated (6 Gy) recipient (CD45.1 and CD45.2) mice. Recipient mice were analyzed after 16 weeks of reconstitution. For rescuing BM chimeras, a total of 2000 LXR β -null (CD45.2) LSK cells transduced separately with a retroviral construct containing LXR β , IL-7Ra, Bcl2, or empty coding sequence were transferred into lethally irradiated recipients (CD45.1). Recipients were analyzed after 12 weeks of reconstitution.

RT-qPCR

To compare gene expression between LXR β -defective and WT CD4SP and CD8SP thymocytes, the cells were sorted directly into TRIzol LS reagent (10296, Invitrogen). The extracted RNA samples were reverse-transcribed with HiScript II Q RT SuperMix (R223-01, Vazyme). The corresponding cDNA samples were analyzed with AceQ qPCR SYBR Green Master Mix (Q111, Vazyme) on a CFX96 Touch Real-Time System (Bio-Rad). The housekeeping gene *Actb* was used as a reference control. The primer sets used were as follows: *Actb*: 5'-AATCGTGCCTGACATCAAAG and 5'-GGATCCATACCCAAGAAGG; *Srebf1c*: 5'-GAGCCATGGATTGCACATT and 5'-GGCCAGAGAAGCAGAAGAGA; *Il7r*: 5'-AAAGCATGATGTGGCCTACC and 5'-GACTCCAC TCGCTCCAGAAG; *Nr1h2*: 5'-AAACGATCTTCTCCGACCA and 5'-ATGG CTAGTCCGGTGAAGTG (identical to the cDNA sequence transcribed from exon 5–6,²⁶ which is excised in LXR β -deficient cells).

ChIP

The sorted WT CD4SP thymocytes were treated with T0901317 (5 μ M/ml) or DMSO for 4 h, and the ChIP procedure was performed

with the anti-LXR β antibody (PA1-333, Invitrogen) or rabbit isotype IgG (A7016, Beyotime) coupled with Dynabeads Protein G (10004D, Invitrogen) as previously described.²⁷ The qPCR primer sets used were as follows: *Actb* + 0.1 K: 5'-CATGGTGTCCGTTCTGAGTG and 5'-TTCTTTGCAGCTCCTTCGTT; *Il7r* -0.8 K: 5'-TGGCATGAGATCACCAAT and 5'-CGCCTGGTATTTGTCAACCT; *Il7r* -3.9 K: 5'-TTGTGGGTGAGCAATGGTAA and 5'-TCTGACAAAGCAGTGCCATC; *Srebfl* + 9.8 K: 5'-CAGAAGCTTGCCTGGACCATT and 5'-AGGCAACTATTGGCCTTCT.

Retroviral constructs and transduction

The bicistronic retroviral vector MigR1 and the vector expressing Bcl2 were obtained from H.H. Xue (University of Iowa). The *Il7r* and *Nr1h2* coding sequences were amplified and cloned into the MigR1 vector. We cloned the *Il7r* promoter (-272 to +134) and the WT and mutant 5'-regulatory regions (-4097 to -3428) and then fused the promoter to the WT or mutant 5'-regulatory region before insertion into the modified self-inactivated retroviral reporter construct as previously described.²⁸ All sequences were verified by DNA sequencing. Retrovirus production was described previously.²⁸ For rescuing BM chimeras, Lin⁻ BM cells enriched by the depletion of Lin⁺ cells coupled with BeaverBeads Streptavidin (22305, Beaver) were cultured for 24 h with IMDM with 50 μ M 2-mercaptoethanol, 100 μ g/ml Amp/Strep, 50 ng/ml SCF (250-03, PeproTech), 20 ng/ml TPO (315-14, PeproTech), and 15% FBS. The cells were "spin-infected" at 1000 \times g for 90 min at 37 °C with the corresponding retrovirus in the presence of 8 μ g/ml Polybrene (H9268, Sigma-Aldrich). After another 24 h of culture, a total of 2000 transduced LSK cells were transferred into irradiated recipients. For the *Il7r* retroviral reporter, the sorted WT or LXR β -deficient CD4SP thymocytes were activated by plate-bound anti-CD3 antibody (0.5 μ g/ml, clone 145-2C11) with 0.5 μ g/ml anti-CD28 antibody (clone 37.51) for 48 h at 37 °C and subsequently "spin-infected" at 1000 \times g for 90 min at 37 °C with the corresponding retrovirus in the presence of 8 μ g/ml polybrene, 10 ng/ml IL-7 (217-17, PeproTech), and 20 ng/ml IL-2 (130-098-221, Miltenyi Biotec).

Statistical analysis

Statistical analysis was conducted with Prism 6.0 software (GraphPad). Error bars indicate the mean \pm SEM. The statistical significance of differences was calculated by unpaired two-tailed Student's *t*-test (two groups), paired two-tailed Student's *t*-test (for BM chimera competition and rescue experiments), or one-way ANOVA with Tukey's post hoc test (more than two groups). *P* values < 0.05 were considered statistically significant. Significance was defined as follows: **P* < 0.05; ***P* < 0.01; ****P* < 0.001; ns, not significant.

RESULTS

LXR β deficiency causes a reduction in peripheral naive T cells
Recently, several reports have revealed that cholesterol metabolism plays an important role in T-cell activation and proliferation by modulating TCR signaling by adjusting the components of the plasma membrane.^{29,30} As key transcription factors in cholesterol metabolism, LXRs also play a role in the regulation of T-cell proliferation by promoting cholesterol efflux.²⁵ Based on these findings and the essential role of TCR signaling in thymocyte development, it can be reasonably hypothesized that LXRs play a role in thymocyte development. To verify this possibility, we first examined the frequencies and numbers of naive (CD44^{lo}CD62L^{hi}) T cells in LXR α -deficient (hereafter designated α KO), LXR β -deficient (hereafter designated β KO), and wild-type (WT) mice, and only β KO mice showed obvious reductions in naive CD4⁺ and CD8⁺ T splenocytes (Fig. 1a). Similarly, the frequencies of naive CD4⁺ and CD8⁺ T cells in peripheral blood lymphocytes (PBLs) of β KO mice were diminished compared with those of WT and α KO

mice (Supplementary Fig. S1). The reduction of LXR β -null naive T cells was unlikely to be associated with increased apoptosis or decreased proliferation, as evidenced by the negligible differences in Caspase-3/7 activation, Bcl2 and IL-7R α expression, BrdU incorporation and Ki-67 expression between WT and LXR β -deficient T splenocytes (Supplementary Fig. S2). We next investigated the cell numbers in spleens and the frequencies in inguinal lymph nodes (LNs) of naive CD4⁺ and CD8⁺ T cells from WT and β KO mice at different ages and observed that the difference in naive T cells between WT and β KO mice at 12–14 weeks of age was the most significant (Fig. 1b, c), suggesting that the reduction in naive T cells in β KO mice might result from decreased thymic output. To confirm this hypothesis, recent thymic emigrant (RTE) cells, which are precursors of peripheral naive T cells and directly migrate from mature SP thymocytes with high CD24 and low Qa2 expression,³¹ were analyzed in WT, α KO, and β KO mice. As expected, both the frequencies and numbers of RTE cells in naive CD4⁺ and CD8⁺ T splenocytes from β KO mice were markedly diminished (Fig. 1d). Taken together, our results demonstrate that a lack of LXR β results in a reduction in peripheral naive T cells and that the reduction can be attributed to decreased RTE cells in β KO mice.

LXR β is necessary for the maintenance of SP thymocytes

To investigate the cause of the reduction in RTE cells due to LXR β knockout, we examined T-cell development in the thymus fractionated by CD4 and CD8 expression. The DN thymocytes, including the DN1–DN4 stages, were comparable (Fig. 2a, b and Supplementary Fig. S3), but the frequencies and numbers of both CD4⁺ and CD8⁺ SP thymocytes (hereafter designated CD4SP and CD8SP, respectively) in β KO mice were obviously decreased compared with those in WT and α KO mice (Fig. 2a, b). Although the frequency of DP thymocytes in β KO mice was slightly increased, the cell number was similar to those in WT and α KO mice (Fig. 2a, b), suggesting that the reduction in SP thymocytes caused by LXR β deficiency is probably not the result of the developmental block at the DP stage. To test this hypothesis, positive and negative selection, which are important processes during the transition from the DP to SP stage, were investigated. We analyzed the surface expression of CD69, TCR β , CD24, and CD5, upregulation of which is considered to be one of the characteristics of positive selection,² in DP thymocytes and found that these surface markers all displayed similar expression in WT and LXR β -null DP thymocytes (Supplementary Fig. S4a). Moreover, DP thymocyte death *in vivo* can be attributable to either negative selection (in response to strong TCR signals) or death by neglect (in response to weak or no signal).⁴ Therefore, the response of DP thymocytes to a gradient of TCR stimulation was used to imitate strong TCR signals *in vitro*, and WT and LXR β -deficient DP thymocytes exhibited similar sensitivities to death induced by anti-CD3/CD28 stimulation (Supplementary Fig. S4b). Under *in vitro* conditions mimicking death by neglect, WT and LXR β -null DP thymocytes exhibited comparable levels of cell death (Supplementary Fig. S4c). These data indicate that LXR β plays a redundant role during the transitions from the DN to SP stage.

To reveal the reason for the reduction in SP thymocytes due to LXR β deficiency and avoid possible ISP interference in CD8SP thymocytes, we assessed the transition from the immature to mature SP stage. Although the frequency of immature (CD24⁺CD69⁺) CD4SP thymocytes in β KO mice was increased and the mature (CD24⁻CD69⁻) CD4SP frequency was decreased relative to the frequencies in WT and α KO mice, the numbers of both immature and mature CD4SP thymocytes were obviously decreased in β KO mice (Fig. 2c). Meanwhile, the immature and mature CD8SP thymocyte numbers in β KO mice were also dramatically diminished compared with those in WT and α KO mice (Fig. 2d). As expected, the numbers of ISP (CD24⁺CD69⁻) thymocytes in these mice were similar, although the frequency in

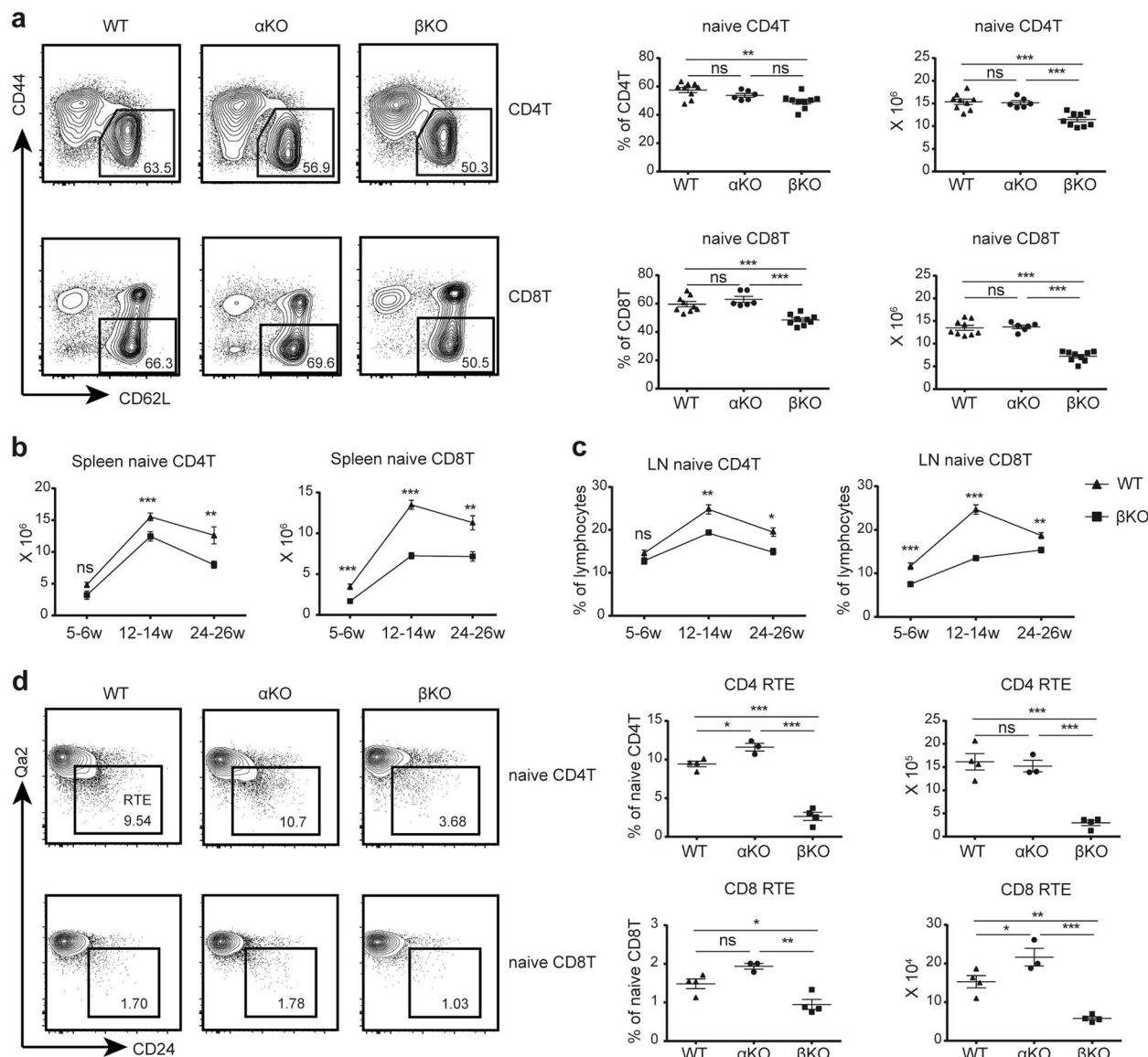


Fig. 1 Lack of LXR β results in a decrease in peripheral naive T cells. **a** Representative contour plots (left), percentages (middle), and numbers (right) of naive CD4⁺ (top) and CD8⁺ (bottom) T splenocytes of the indicated cells from WT, α KO, and β KO mice. **b, c** Cell numbers in spleens (**b**) and frequencies in inguinal LNs (**c**) of naive CD4⁺ (left) and CD8⁺ (right) T cells from WT and β KO mice at the indicated ages ($n \geq 3$). **d** Representative contour plots (left), percentages (middle), and numbers (right) of CD4⁺ (top) and CD8⁺ (bottom) RTE splenocytes of the indicated cells from WT, α KO, and β KO mice. The numbers in the outlined areas (**a, d**) indicate percent cells in each subset. The data are representative of at least three independent experiments. The data are the means \pm SEMs. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant by one-way ANOVA with Tukey's post hoc test (**a, d**) or unpaired two-tailed Student's *t*-test (**b, c**)

β KO mice was ~ 2.5 -fold higher (Fig. 2d), suggesting that the existence of the ISP subset has little influence on CD8SP thymocytes. Our results demonstrate that the reduction in SP thymocytes is unlikely to be attributable to maturation arrest at the SP stage in β KO mice.

To avoid the potential effects of an altered microenvironment in the thymus caused by LXR β knockout, we generated BM chimeric mice by reconstituting irradiated recipients (CD45.1 and CD45.2) using a mixture of LSK (Lin⁻Sca-1⁺c-Kit⁺) cells, which phenotypically express Sca-1 and c-Kit but lack lineage (Lin) markers in BM cells,³² at a ratio of 1:1 from β KO (CD45.2) and WT (CD45.1) donor mice (Supplementary Fig. S5a). This competitive BM chimeric experiment enabled us to compare the abilities of WT and LXR β -deficient LSK cells to differentiate into mature SP thymocytes and peripheral naive T cells in identical environments. As expected, the thymocytes of LXR β -defective origin showed significantly

decreased contributions among both CD4SP and TCR β ⁺CD8SP thymocytes and similar contributions among DN, ISP, and DP thymocytes compared with those of WT origin in the same mice (Supplementary Fig. S5b, d). Moreover, the CD4⁺ and CD8⁺ T splenocytes of LXR β -null origin were dramatically diminished relative to those of WT origin in the same mice (Supplementary Fig. S5c, d). These data indicate that LXR β is required for the maintenance of SP thymocytes in a cell-intrinsic manner.

To more precisely assess the role of LXR β in regulating the maintenance of SP thymocytes, T0901317, an efficient agonist of LXR β with an EC₅₀ of 50 nM,³³ was used. Compared with the vehicle treatment group, T0901317-treated WT mice exhibited obvious increases in CD4SP and CD8SP thymocyte frequencies and a corresponding decrease in DP thymocyte frequency (Fig. 2e), although the increases in SP thymocyte numbers were not significant (Supplementary Fig. S6a), possibly as a result of the

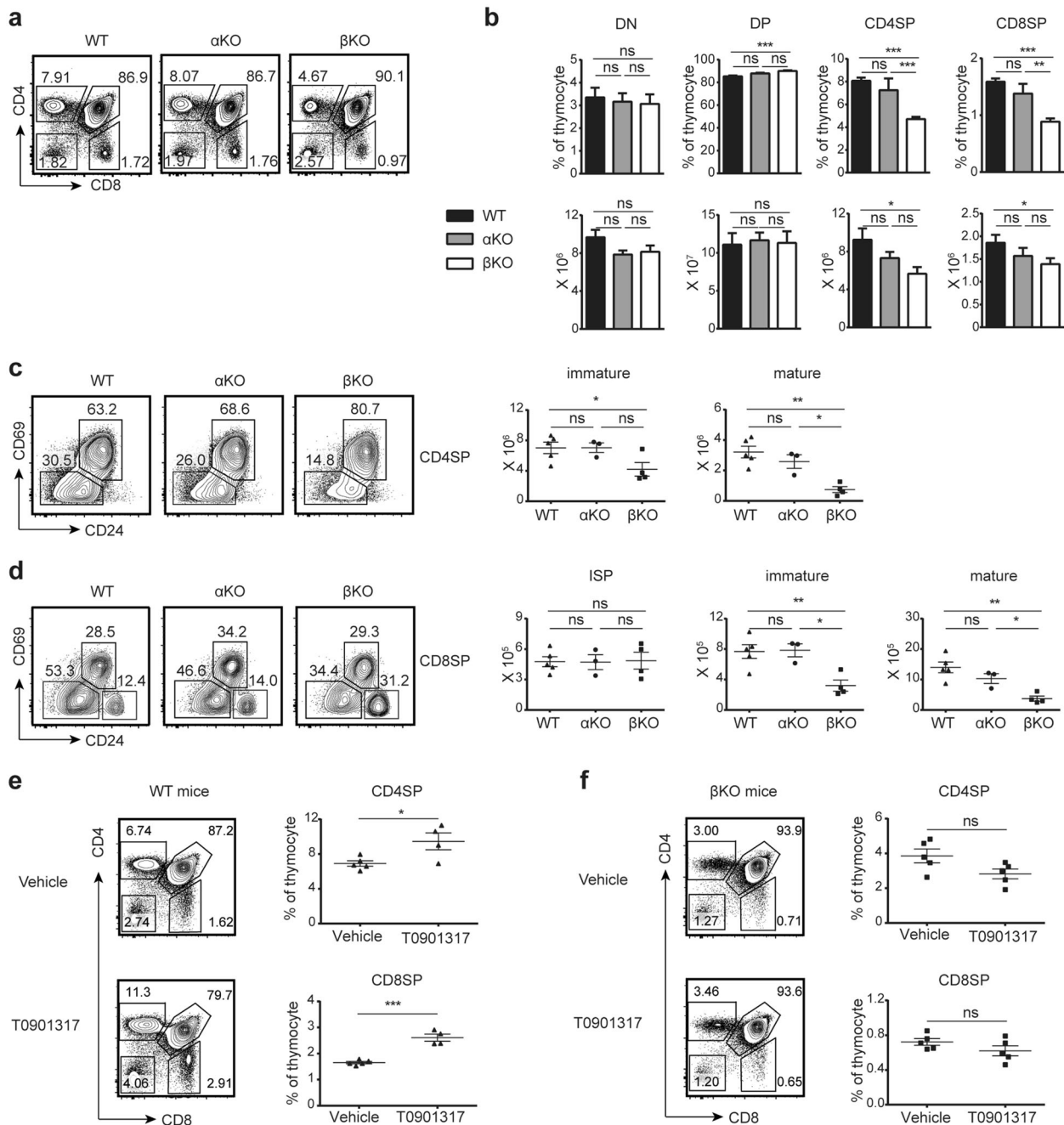


Fig. 2 LXR β is required for the maintenance of SP thymocytes. **a** Flow cytometry fractionating whole thymocytes from WT, α KO, and β KO mice into DN (bottom left), DP (top right), CD4SP (top left), and CD8SP (bottom right) subsets. **b** The percentages (top) and cell numbers (bottom) of the indicated subsets as described in **(a)** are summarized ($n \geq 6$). **c, d** Flow cytometry fractionating CD4SP (**c**) and CD8SP (**d**) thymocytes as described in **(a)** into immature and mature without/with ISP subsets (left). The cell numbers of the indicated subsets are summarized on the right. **e, f** Representative contour plots (left) and percentages (right) of CD4SP (top) and CD8SP (bottom) thymocytes in WT (**e**) and β KO (**f**) mice at day 7 after the indicated treatment. The numbers adjacent to outlined areas (**a, c–f**) indicate percent cells in each subset. The data are representative of at least two independent experiments. The data are the means \pm SEMs. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant by one-way ANOVA with Tukey's post hoc test (**b–d**) or unpaired two-tailed Student's t -test (**e, f**)

variation in total thymocyte number. The increases in the frequency of SP thymocytes were unlikely to be associated with DP thymocytes, as evidenced by the similar cell numbers of DP thymocytes between T0901317- and vehicle-treated mice (Supplementary Fig. S6a). Meanwhile, we did not observe any appreciable differences in immature and mature CD4SP and CD8SP thymocytes between T0901317- and vehicle-treated mice (Supplementary Fig. S7), confirming the above hypothesis that LXR β is dispensable for the maturation of SP thymocytes.

T0901317 is also an agonist of other LXRs, such as LXR α and farnesoid X receptor with EC₅₀ values of 20 nM and 5 μ M, respectively.³⁴ We thus examined the effect of T0901317 treatment in the absence of LXR β and did not observe any obvious differences in DP, CD4SP, and CD8SP thymocytes between T0901317- and vehicle-treated β KO mice (Fig. 2f and Supplementary Fig. S6b). Collectively, these findings demonstrate that LXR β has a cell-intrinsic effect on the maintenance of SP thymocytes, and the reduction in SP thymocytes due to LXR β

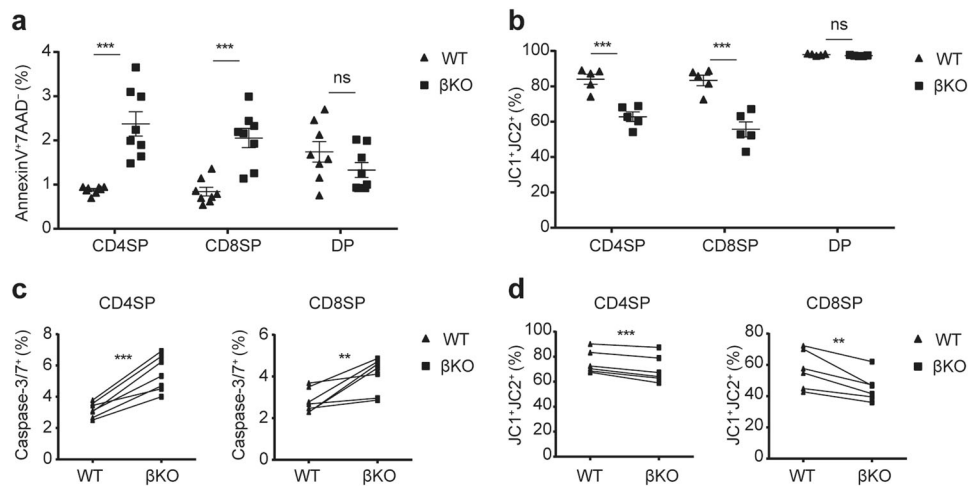


Fig. 3 LXR β is necessary for the survival of SP thymocytes. **a, b** The percentages of AnnexinV⁺7AAD⁻ (**a**) and JC1⁺JC2⁺ (**b**) subsets in the indicated thymocytes from WT and β KO mice. **c, d** Percentages of Caspase-3/7⁺ (**c**) and JC1⁺JC2⁺ (**d**) subsets in the indicated donors from competitive BM chimeras after 16 weeks of reconstitution as described in Supplementary Fig. S5a. The data are representative of at least two independent experiments. The data are the means \pm SEMs. ** $P < 0.01$; *** $P < 0.001$; ns, not significant by unpaired (**a, b**) or paired (**c, d**) two-tailed Student's *t*-test

deficiency is not attributable to either the transition blocks from the DN to SP stage or the maturation arrest at the SP stage.

LXR β is required for survival of SP thymocytes

Since the transitions from the DN to SP stage and the maturation of SP thymocytes in β KO mice are normal, the reduction in LXR β -null SP thymocytes might result from defective homeostasis itself. To confirm this possibility, the proliferation and apoptosis of SP thymocytes in WT and β KO mice were tested. Consistent with the previous results in T splenocytes (Supplementary Fig. S2d, e), we observed comparable levels of BrdU incorporation and Ki-67 expression in both CD4SP and CD8SP thymocytes in WT and β KO mice (Supplementary Fig. S8), indicating that LXR β has a redundant role in the proliferation of SP thymocytes. In contrast, the frequencies of apoptotic cells as measured by Annexin V/7AAD staining were approximately 2.5- to 3-fold higher in LXR β -deficient CD4SP and CD8SP thymocytes than in WT cells, although the frequencies of apoptotic DP thymocytes were similar (Fig. 3a). We also examined the mitochondrial membrane potentials, disruption of which is an early intracellular event of apoptosis,³⁵ and noted that the frequencies of JC1 aggregates (JC1⁺JC2⁺) in LXR β -null CD4SP and CD8SP thymocytes decreased by 25% and 33%, respectively, compared with those in WT cells, while DP thymocytes displayed comparable levels (Fig. 3b), consistent with our aforementioned result (Fig. 3a) that SP thymocytes lacking LXR β were more prone to apoptosis. Moreover, using the competitive BM chimeras described in Supplementary Fig. S5a, we observed that both CD4SP and CD8SP thymocytes of LXR β -deficient origin also showed clearly increased frequencies of apoptotic cells, as detected by Caspase-3/7 activation (Fig. 3c) and decreased levels of JC1 aggregates (Fig. 3d) compared with those of WT origin in the same mice, consistent with the data obtained from WT and β KO mice (Fig. 3a, b). Taken together, our results demonstrate that LXR β is necessary for the survival but not proliferation of SP thymocytes in a cell-intrinsic manner.

LXR β regulates the IL-7R α -Bcl2 axis in SP thymocytes

To investigate the cause of increased apoptosis in LXR β -defective SP thymocytes, we further examined IL-7 signaling, which plays vital roles in the survival of thymocytes.⁶ The expression of IL-7R α was approximately 1.5- to 2-fold lower in both LXR β -deficient CD4SP and CD8SP thymocytes than in WT cells, whereas it exhibited comparable levels in WT and LXR β -null DP thymocytes

(Fig. 4a). Notably, the JAK3/Stat5/Bcl2 axis is a major signaling pathway in thymocytes that is activated upon IL-7 binding to the receptor complex and plays a key role in thymocyte survival.⁶ Therefore, we assessed Stat5 phosphorylation (p-Stat5) and Bcl2 expression and observed that both were obviously decreased in CD4SP and CD8SP thymocytes lacking LXR β , although the levels were similar in WT and LXR β -deficient DP thymocytes (Fig. 4b, c). Moreover, in the competitive BM chimeras described in Supplementary Fig. S5a, both CD4SP and CD8SP thymocytes of LXR β -defective origin displayed clear reductions in IL-7R α and Bcl2 expression compared with those of WT origin in the same mice (Fig. 4d, e). In addition, compared with the vehicle treatment group, LXR agonist T0901317-treated WT mice showed obvious increases in IL-7R α expression in CD4SP and CD8SP thymocytes (Fig. 4f), while the differences between T0901317- and vehicle-treated β KO mice were not perceptible (Fig. 4g). Collectively, these findings suggest that LXR β plays an essential role in the survival of SP thymocytes by regulating IL-7R α -Bcl2 signaling.

LXR β promotes *Il7r* transcription via direct binding to its locus. It has been reported that the surface expression of IL-7R α can be regulated by several processes, including transcription,^{16,18,19} degradation,¹⁴ and protein trafficking by internalization/recycling.¹³⁻¹⁵ On the other hand, as a key transcription factor in cholesterol metabolism, LXR β might influence IL-7R α clustering and endosome formation by modulating the membrane composition in the presence of IL-7. To determine the reason for the reduction in surface IL-7R α caused by LXR β knockout, we examined the total (surface plus intracellular) IL-7R α expression in permeabilized cells. Although we did not observe any appreciable difference between WT and LXR β -null DP thymocytes, the total IL-7R α expression in both LXR β -deficient CD4SP and CD8SP thymocytes was obviously decreased (Fig. 5a). Importantly, compared with the vehicle treatment group, LXR agonist T0901317-treated WT CD4SP and TCR β ⁺CD8SP thymocytes showed significant increases in *Il7r* transcripts by the reverse-transcription quantitative PCR (RT-qPCR) assay (Fig. 5b), implying that LXR β might be involved in the transcriptional regulation of IL-7R α expression in SP thymocytes. To elucidate the molecular mechanism by which LXR β regulates the mRNA expression of IL-7R α in SP thymocytes, we performed an RT-qPCR assay in sorted WT and LXR β -defective CD4SP and CD8SP thymocytes. The *Nr1h2* transcripts were barely detectable in the knockout samples

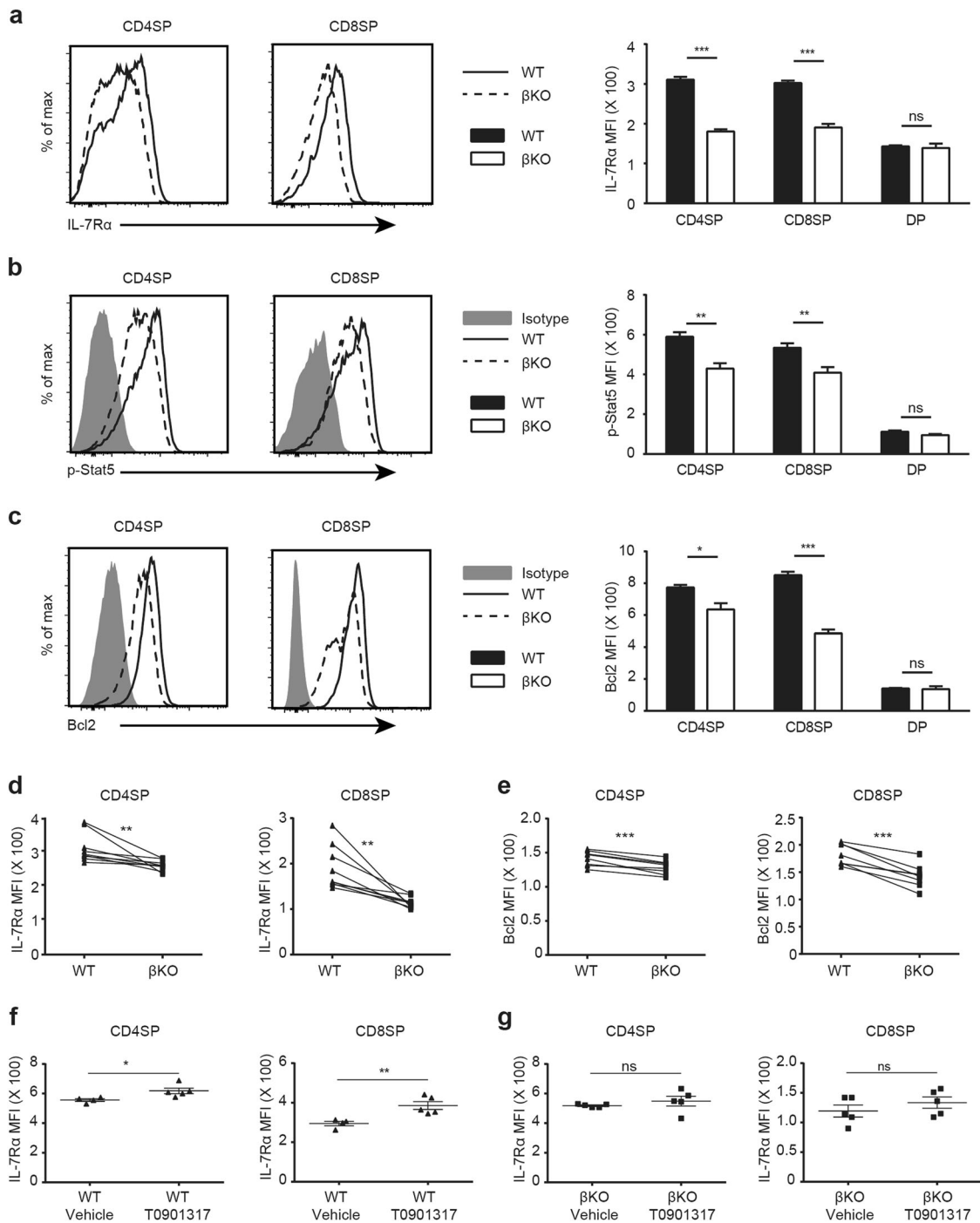


Fig. 4 LXR β is necessary for the expression of the IL-7R α -Bcl2 axis in SP thymocytes. **a–c** Representative histograms (left) and the mean fluorescence intensities (MFIs) (right) of IL-7R α (**a**), p-Stat5 (**b**), and Bcl2 (**c**) expression in the indicated thymocytes from WT and β KO mice ($n = 5$). **d**, **e** MFIs of IL-7R α (**d**) and Bcl2 (**e**) expression in the indicated donors from competitive BM chimeras after 16 weeks of reconstitution as described in Supplementary Fig. S5a. **f**, **g** MFIs of IL-7R α expression in CD4SP (left) and CD8SP (right) thymocytes from WT (**f**) and β KO (**g**) mice at day 7 after the indicated treatment. The data are representative of at least two independent experiments. The data are the means \pm SEMs. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant by unpaired (**a–c**, **f**, **g**) or paired (**d**, **e**) two-tailed Student's t -test

(Fig. 5c), indicating that these samples could be further used. A target gene of LXR β , encoding the transcription factor SREBP1c (encoded by *Sreb1c*, a specific transcript of the *Sreb1* gene), which plays an important role in cholesterol biosynthetic process,^{21,36} was used as a positive control. As expected, compared with WT cells, LXR β -null CD4SP and CD8SP thymocytes exhibited significant reductions in *Sreb1c* transcripts (Fig. 5c). Meanwhile, the *Il7r* transcripts in LXR β -defective CD4SP and

CD8SP thymocytes were obviously decreased relative to those in WT cells, although the reduction in CD8SP thymocytes was less than that in CD4SP thymocytes (Fig. 5c), possibly as a result of ISP interference in CD8SP thymocytes. These results indicate that LXR β is involved in the transcriptional regulation of IL-7R α expression in SP thymocytes.

We therefore investigated whether LXR β regulates the *Il7r* gene by directly binding to its genomic locus. Using the UCSC browser

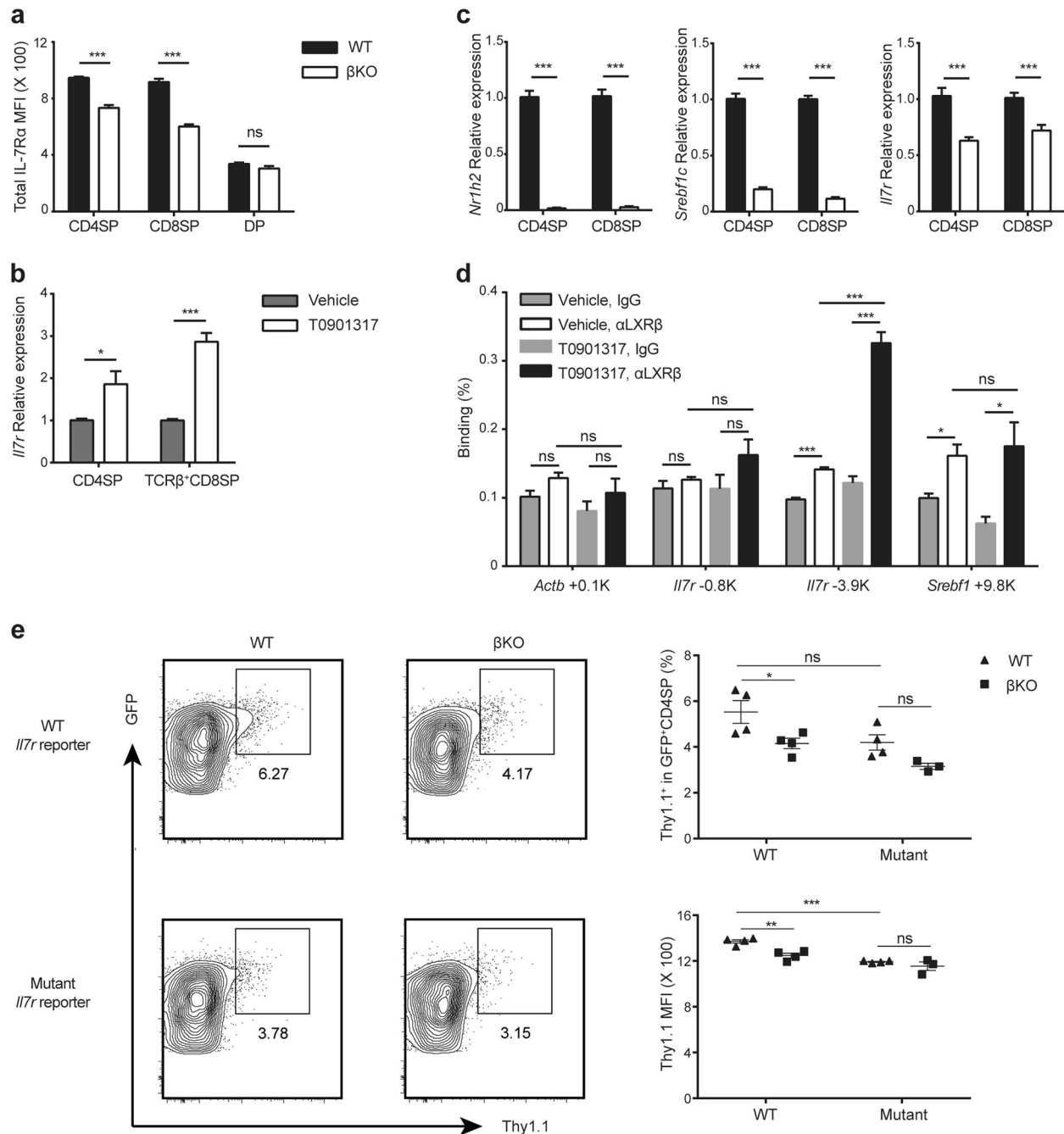


Fig. 5 LXR β regulates IL-7R α expression via direct binding to its allele. **a** MFIs of total IL-7R α expression as measured by surface and intracellular staining in the indicated subsets from WT and β KO mice ($n = 4$). **b** RT-qPCR of the *Il7r* transcript in sorted CD4SP and TCR β ⁺CD8SP thymocytes from WT mice at day 1 after the indicated treatment, which were normalized to the corresponding vehicle-treated transcripts ($n = 3$). **c** RT-qPCR of *Nr1h2* (left), *Sreb1c* (middle), and *Il7r* (right) transcripts in sorted WT and β KO CD4SP and CD8SP thymocytes, which were normalized to the corresponding WT transcripts ($n = 3$). **d** Direct binding of LXR β to the *Il7r* 5'-regulatory region. ChIP was performed on sorted WT CD4SP thymocytes treated with vehicle or T0901317 for 4 h using an anti-LXR β or isotype IgG antibody. The *Actb* + 0.1 K, *Il7r* -0.8 K, *Il7r* -3.9 K, and *Sreb1c* + 9.8 K regions were measured by qPCR in enriched DNA segments and then normalized to the corresponding input segments. **e** LXR β enhanced the activity of the *Il7r* 5'-regulatory region. The sorted WT and β KO CD4SP thymocytes were activated by anti-CD3/CD28 stimulation and subsequently transduced with retroviruses containing the *Il7r* reporters as described in Supplementary Fig. S9c. After 24 h of incubation, representative contour plots of the Thy1.1⁺ subset in transduced (GFP⁺) CD4SP thymocytes are shown on the left. The Thy1.1 frequencies (top) in GFP⁺ cells and Thy1.1 MFIs (bottom) in Thy1.1⁺GFP⁺ subsets are summarized on the right. The numbers adjacent to outlined areas (**e**) indicate percent cells in each subset. The data are representative of at least two independent experiments. The data are the means \pm SEMs. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant by unpaired two-tailed Student's *t*-test

(<http://genome.ucsc.edu/>) and published data³⁷ for chromatin immunoprecipitation (ChIP) coupled to next-generation sequencing (ChIP-seq) assays of stable LXR expression in LXR double-knockout immortalized murine BM-derived macrophages,³⁸ we

identified a conserved peak (-3.9 K, upstream of the transcriptional starting site, TSS) enriched at the *Il7r* allele only in 3xFlag-LXR β -expressing cells (Supplementary Fig. S9a). To examine whether LXR β occupancy on the *Il7r* locus exists in SP thymocytes,

sorted CD4SP thymocytes were treated with vehicle or T0901317, and a ChIP-quantitative PCR (ChIP-qPCR) assay was performed. The +0.1 K region of *Actb*, a housekeeping gene, and the *Il7r* -0.8 K region, both of which have no LXRE consensus sequence, were used as negative controls. In comparison with each isotype IgG control sample, we did not observe any obviously enriched binding to the *Actb* +0.1 K and *Il7r* -0.8 K regions in all samples (Fig. 5d). The +9.8 K region of the *Srebf1* locus, occupied by LXR β in murine hepatocytes²¹ and macrophages,³⁹ was used as a positive control. As expected, we observed clearly enriched binding of LXR β to the *Srebf1* +9.8 K region in T0901317- and vehicle-treated cells (Fig. 5d). Importantly, we observed obvious enriched binding of LXR β to the *Il7r* -3.9 K region, and the binding in T0901317-treated cells was significantly higher than that in vehicle-treated cells (Fig. 5d), which might be attributable to the increased activity of LXR β in T0901317-treated cells.

Next, we attempted to assess whether direct binding of LXR β to the *Il7r* allele influences the transcription of IL-7Ra. We screened two nonconserved and one conserved LXR-binding sequence in the 5'-regulatory region of the *Il7r* locus (Supplementary Fig. S9b, c) using variations of the LXRE consensus sequence, "RGKKCR", because only 6.3%³⁹ or up to 7.3%⁴⁰ of the peaks reportedly contain the canonical LXRE consensus motif, as determined by de novo motif analysis. To evaluate the accuracy of this putative conserved binding site, a reporter assay using a self-inactivating retroviral system was executed in CD4SP thymocytes. We cloned the 5'-regulatory region of *Il7r* (-4097 to -3428) harboring the WT or mutant conserved LXR β -binding motif and *Il7r* promoter (-272 to +134) and then fused the WT or mutant 5'-regulatory region and promoter to a sequence including the coding sequence of Thy1.1, as a reporter of transcriptional activity, and the PGK-GFP cassette, which acts as an indicator of retroviral transduction (Supplementary Fig. S9c). The sorted WT and LXR β -deficient CD4SP thymocytes were activated by anti-CD3/CD28 stimulation and were subsequently transduced with the corresponding retroviruses (Supplementary Fig. S9d). After another 24 h of incubation, we observed that the GFP⁺ WT CD4SP thymocytes transduced with the retrovirus containing the WT LXR β -binding sequence of the *Il7r* 5'-regulatory region exhibited a higher frequency and MFI of the Thy1.1⁺ subset than the transduced LXR β -defective CD4SP thymocytes, while both WT and LXR β -null GFP⁺ cells transduced with the mutant retrovirus exhibited similar low frequencies and MFIs of Thy1.1⁺ subsets (Fig. 5e), indicating that LXR β positively regulates *Il7r* transcription via direct binding to the *Il7r* allele. Taken together, these results demonstrate that LXR β promotes IL-7Ra expression in SP thymocytes via direct DNA binding.

Forced IL-7Ra expression restores the survival of LXR β -deficient SP thymocytes

To demonstrate that the IL-7Ra-Bcl2 axis plays a functionally important role in mediating the regulation of SP thymocyte survival by LXR β , we separately introduced the coding sequences of LXR β , IL-7Ra, and Bcl2 into LXR β -defective LSK cells using a bicistronic retroviral vector, MigR1 (MSCV-IRES-GFP), in which GFP serves as an expression indicator. This system enabled us to directly compare the rescue efficacy of LXR β -null SP thymocytes derived from retrovirus-transduced (marked as GFP⁺) and nontransduced (marked as GFP⁻) LSK cells in the same BM chimeric mouse. After 12 weeks of reconstruction, we observed that the GFP⁺ CD4SP and CD8SP thymocytes derived from the LSK cells transduced with empty vector (EV) were similar to the corresponding GFP⁻ subsets, including cell frequencies, apoptosis levels, and IL-7Ra expression (Fig. 6a, b). In contrast, the LXR β -overexpressing CD4SP and CD8SP subsets displayed significantly increased frequencies, decreased apoptosis levels, and elevated IL-7Ra expression compared with the corresponding GFP⁻ cells (Fig. 6c, d), indicating that recovering LXR β expression

completely rescues the defects of SP thymocytes caused by LXR β knockout. Importantly, IL-7Ra overexpression clearly caused not only reductions in apoptotic cells but also increases in CD4SP and CD8SP thymocytes (Fig. 6e, f), although the difference in apoptosis in CD4SP thymocytes was not statistically significant, as a result of merely a slight increase in IL-7Ra expression (Fig. 6f), confirming that forced IL-7Ra expression is sufficient for restoring the survival of LXR β -deficient SP thymocytes. In addition, ectopic expression of Bcl2, which is a downstream anti-apoptosis factor in IL-7 signaling, also led to several predictable results, namely, more CD4SP and CD8SP thymocytes, fewer apoptotic cells, and unaltered IL-7Ra expression (Fig. 6g, h). Collectively, our findings demonstrate that overexpression of either the LXR β or IL-7Ra-Bcl2 axis rescues the defects of SP thymocytes caused by LXR β deficiency.

DISCUSSION

It is well known that LXRs have general roles in the regulation of lipid metabolism and exert an anti-inflammatory or anti-proliferative effect in a number of cell types, such as macrophages and T lymphocytes;²³ however, whether LXRs play a role in thymocytes remains unknown. In this study, we demonstrate that LXR β is required for the survival of SP thymocytes. On the one hand, LXR β deficiency induced SP thymocyte apoptosis (Fig. 3a, b) and then caused reductions in both immature and mature SP thymocytes (Fig. 2c, d). Similar phenomena were observed with competitive BM chimeras, namely, elevated apoptosis (Fig. 3c, d) and decreased SP thymocytes (Supplementary Fig. S5b, d) of LXR β -defective origin. On the other hand, the LXR agonist T0901317 could increase the frequencies of both CD4SP and CD8SP thymocytes in the presence of LXR β (Fig. 2e) but not in the absence of LXR β (Fig. 2f). Meanwhile, the LXR β -null SP thymocytes overexpressing LXR β exhibited lower levels of apoptosis (Fig. 6d) and, finally, higher cell levels were obtained (Fig. 6c). These "gain-and loss-of-function" data provide solid evidence that LXR β plays both necessary and sufficient roles in the survival of SP thymocytes.

Although many genes involved in lipid metabolism and inflammation are reportedly regulated by LXRs,²⁰⁻²² little is known regarding the LXR target genes involved in other functions. Here, we identified IL-7Ra, which plays an essential role in thymocyte survival, as a target gene of LXR β in SP thymocytes, and the series of events is listed as follows. First, both the protein (Figs. 4a and 5a) and mRNA (Fig. 5c) expression levels of IL-7Ra in LXR β -null SP thymocytes were obviously decreased. Meanwhile, IL-7Ra expression in SP thymocytes of LXR β -deficient origin from competitive BM chimeras was also diminished (Fig. 4d). Second, the LXR agonist T0901317 efficiently promoted the expression of IL-7Ra in WT SP thymocytes (Figs. 4f and 5b) but not in LXR β -defective cells (Fig. 4g). Moreover, forced expression of LXR β significantly enhanced the expression of IL-7Ra in LXR β -null SP thymocytes (Fig. 6d). These data demonstrate that the transcriptional activity or expression level of LXR β is positively correlated with the expression of IL-7Ra in SP thymocytes. Third, the ChIP-qPCR assay showed that LXR β could directly bind to the 5'-regulatory region of the *Il7r* allele and that the LXR agonist T0901317 obviously enhanced the DNA-binding capacity of LXR β (Fig. 5d). Finally, although the induction of reporter Thy1.1 driven by the 5'-regulatory region and promoter of the *Il7r* gene was not strong, the retroviral reporter experiment still clearly displayed that only WT CD4SP thymocytes transduced with the retrovirus containing the WT LXR β -binding sequence exhibited higher expression of Thy1.1 than either the transduced LXR β -deficient cells or the cells transduced with the mutant retrovirus (Fig. 5e), indicating that the LXR β -binding motif is necessary for the binding of LXR β to the *Il7r* locus. Collectively, these findings strongly support our hypothesis that LXR β positively regulates the transcription of IL-7Ra in SP

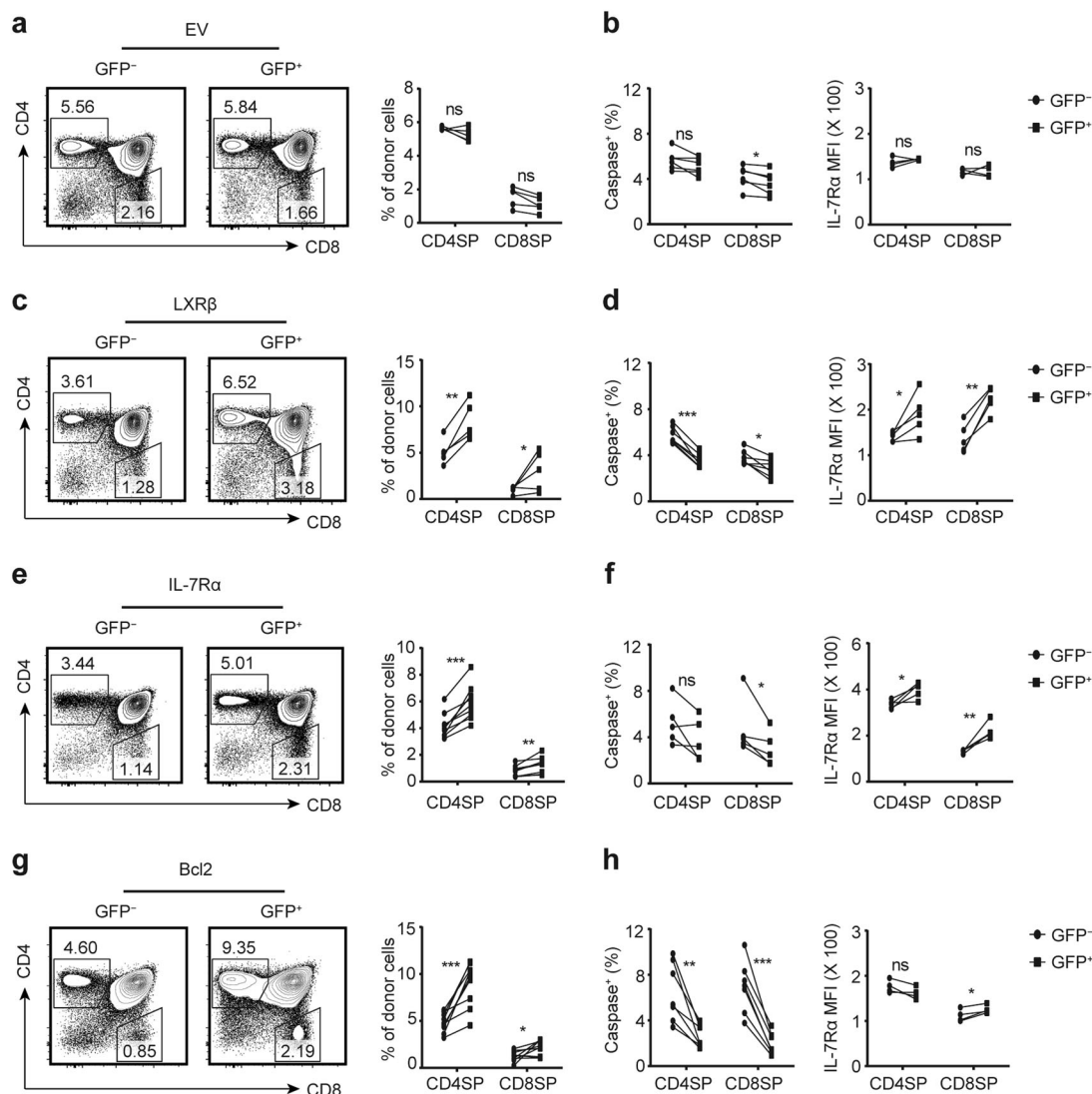


Fig. 6 Overexpression of LXR β /IL-7R α /Bcl2 rescues the defects of SP thymocytes caused by LXR β knockout. **a, c, e, g** Representative contour plots (left) and frequencies (right) of the indicated SP thymocytes derived from LXR β -defective LSK cells transduced (GFP⁺) separately with EV (**a**), LXR β (**c**), IL-7R α (**e**), and Bcl2 (**g**) retroviruses, or not transduced (GFP⁻), after 12 weeks of reconstitution. **b, d, f, h** The frequencies (left) of Caspase⁺ subsets and the MFIs (right) of IL-7R α expression in the indicated SP thymocytes as described in (**a**), (**c**), (**e**), and (**g**), respectively. The data are representative of at least two independent experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant by paired two-tailed Student's *t*-test

thymocytes via direct binding to the *Il7r* allele. Notably, although there is no canonical LXRE consensus motif in the 5'-regulatory region of the *Il7r* genomic locus, the retroviral *Il7r* reporter assay has proven, at least partially, the important role of the noncanonical LXR β -binding sequence in the regulation of IL-7R α expression. In addition, the spaces between two motifs in the *Il7r* 5'-regulatory region are more than four nucleotides in length, although there are three noncanonical LXRE motifs. Thus, further studies will be required to better define LXR-binding sequences, investigate whether LXR β functions independently or identify the cooperating factors, and better elucidate the mechanisms underlying IL-7R α regulation in SP thymocytes.

Interestingly, LXR β seems to exert its transcriptional effect on IL-7R α expression only in SP thymocytes, as evidenced by the negligible differences in IL-7R α expression in DP thymocytes (Figs. 4a and 5a) and T splenocytes (Supplementary Fig. S2c) between WT and β KO mice but an obvious decrease in LXR β -defective SP thymocytes (Figs. 4a and 5a, c), and there are several possible mechanisms by which this cell type-dependent

regulation could be achieved. First, a transcriptional regulatory protein, possibly a transcription factor, the expression of which begins in SP thymocytes, such as Foxo1,¹⁷ may cooperate with LXR β to regulate *Il7r* transcription. In fact, the *Il7r* 5'-regulatory region, as described in Supplementary Fig. S9a, also contains a Foxo-responsive element,¹⁶ indicating that the interaction between LXR β and Foxo1 may occur via binding to the same *Il7r* 5'-regulatory region and may thereby orchestrate *Il7r* transcription. Next, because of the "on-off-on" pattern of IL-7R α expression at the DN, DP, and SP stages,^{5,11} a transcriptional repressor that is highly expressed in DP thymocytes but expressed at low levels in SP thymocytes, such as Gfi1,¹⁷ allows LXR β to perform its transcriptional activity specifically in SP thymocytes. Finally, there are many transcription factors that regulate IL-7R α expression in peripheral T cells.^{8,12} For example, compared with the expression in SP thymocytes, Foxo1 is significantly upregulated in peripheral T cells,¹⁶ suggesting that Foxo1 plays a more important role than LXR β in the regulation of IL-7R α expression, leading to LXR β becoming redundant in peripheral T cells.

CD4SP and CD8SP thymocytes have different sensitivities to the activity of LXR β , although LXR β plays an important role in the survival of both CD4SP and CD8SP thymocytes by upregulating IL-7Ra expression. That is, the decrease in CD8SP thymocytes was more than that in CD4SP thymocytes in the “loss-of-function” experiments, and the increase in CD8SP thymocytes was still more than that in CD4SP thymocytes in the “gain-of-function” experiments. In addition to the survival role of IL-7 signaling in SP thymocytes, a previous study revealed that intrathymic IL-7 signaling plays an additional role in CD8 lineage choice.⁴¹ Since IL-7 signaling plays a dual role in CD8SP thymocytes and LXR β positively regulates the expression of IL-7Ra, a receptor and transducer in IL-7 signaling, CD8SP thymocytes should have a high sensitivity to LXR β activity. In addition, several important studies have shown that IL-7 signaling represses the expression of IL-7Ra by inducing Gfi1, specifically in CD8⁺ T cells,^{42,43} and Gfi1 overexpression suppresses IL-7Ra expression only in CD8SP thymocytes,⁴³ indicating that this negative regulation of IL-7Ra expression by IL-7 itself also exists in CD8SP thymocytes and results in increased sensitivity to LXR β activity. Moreover, it has been reported that the ablation of Acat1, a key gene that converts free cholesterol to cholesteryl esters for storage, elevates the level of plasma membrane cholesterol and then leads to faster TCR clustering and stronger TCR signaling in CD8⁺ T cells, whereas Acat1 fails in CD4⁺ T cells.²⁹ Similarly, ablation of LXR β , another key gene that promotes cholesterol efflux by upregulating ABCG1 expression,²⁵ may enhance the plasma membrane cholesterol level in CD8SP but not CD4SP thymocytes, resulting in faster IL-7Ra trafficking, which eventually elevates the sensitivity of CD8SP thymocytes to IL-7. Indeed, the surface IL-7Ra level in LXR β -defective CD4SP and CD8SP thymocytes was decreased by 42% and 37%, respectively (Fig. 4a); however, the total IL-7Ra level (surface plus intracellular) was reduced by 22% and 34%, respectively (Fig. 5a), at least partially confirming our hypothesis that the IL-7Ra trafficking in LXR β -null CD8SP thymocytes is faster than that in CD4SP thymocytes. Nevertheless, the detailed mechanisms underlying the difference in LXR β function between CD4SP and CD8SP thymocytes await further elucidation.

In conclusion, we have demonstrated that LXR β plays an essential role in the survival of SP thymocytes by controlling the IL-7Ra-Bcl2 axis. Importantly, LXR β directly regulates IL-7Ra expression in SP thymocytes, and ectopic IL-7Ra or Bcl2 restores the survival of LXR β -null SP thymocytes. Our findings identify IL-7Ra as a novel LXR β target gene in SP thymocytes and provide new insights into the cell type-dependent and metabolism-independent functions of LXR β in the regulation of IL-7Ra expression.

ACKNOWLEDGEMENTS

We thank Dr J.Å. Gustafsson (Karolinska Institutet) for providing LXR α - and LXR β -deficient mice. We thank Dr H.H. Xue (University of Iowa) for providing retroviral vectors. We thank the core facility center of Third Military Medical University for cell sorting. This work was supported by grants from the National Key Research and Development Program of China (No. 2016YFA0502203 to X.Z. and No. 2016YFA0502204 to Y.W.), the National Natural Science Foundation of China (No. 81571537 to T.Z., No. 31770949 to X.Z., No. 31770972 to Z.X., and No. 81571604 to J.Z.), and the Chongqing Basic and Frontier Research Project (No. cstc2015jcyjBX0086 to H.He.).

AUTHOR CONTRIBUTIONS

H.Hu., X.W., and D.M. performed the experiments and analyzed the data with assistance from Y.F., L.Z., Z.L., S.T., X.L., and Y.Ca. X.Z., Y.W., T.Z., H.He., Z.X., and J.Z. procured several grants. Y.Ch. provided LXR α - and LXR β -deficient mice. X.Z., H.Hu., and T.Z. designed the study. X.Z. and H.Hu. wrote the paper. X.Z., Y.W., and T.Z. supervised the study.

ADDITIONAL INFORMATION

The online version of this article (<https://doi.org/10.1038/s41423-020-00546-y>) contains supplementary material.

Competing interests: The authors declare no competing interests.

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