Immunology:

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Davinna L. Ligons[‡1], Ceren Tuncer[§1], Brett A. Linowes[‡], Izzet Mehmet Akcay[§2], Sema Kurtulus[§2,3], Emre Deniz[§2], Belkis Atasever Arslan[§4,5], Safak Isil Cevik[§2], Hilary R. Keller[‡], Megan A. Luckey[‡], Lionel Feigenbaum[¶], Tarik Möröy[‖], Tulin Ersahin[**], Rengul Atalay[**], Batu Erman[§‡‡1,6], and Jung-Hyun Park[‡1,7]

From the [‡]Experimental Immunology Branch, National Cancer Institute, Bethesda, Maryland 20892, the [§]Biological Sciences and Bioengineering Program, Faculty of Engineering and Natural Sciences, Sabanci University, Orta Mahalle, Üniversite Caddesi No. 27, Tuzla, Istanbul 34956, Turkey, the [¶]Science Applications International Corporation-Frederick, National Cancer Institute–Frederick Cancer Research and Development Center, Frederick, Maryland 21702, the [‖]Institut de Recherches cliniques de Montréal, Département de microbiologie et immunologie, Université de Montréal, Montréal (Québec) H2W 1R7, Canada, the [**]Department of Molecular Biology and Genetics, Bilkent University, Bilkent, Ankara 06800 Turkey, and the [‡‡]Sabanci University, Nanotechnology Research and Application Center, SUNUM, Istanbul 34956, Turkey

Background: Expression of the IL-7Rα gene is up-/down-regulated during T/B-lymphocyte development.
Results: IL-7Rα gene transcription is repressed by the transcription factor Gfi1, specifically in $CD8^+$ T-lymphocytes.
Conclusion: Treatment by dexamethasone down-regulates Gfi1, which contributes to glucocorticoid receptor mediated up-regulation of IL-7R expression.
Significance: The mechanism by which the IL-7R gene gets turned on and off during development is a critical issue in biology.

Interleukin-7 receptor α (IL-7Rα) is essential for T cell survival and differentiation. Glucocorticoids are potent enhancers of IL-7Rα expression with diverse roles in T cell biology. Here we identify the transcriptional repressor, growth factor independent-1 (Gfi1), as a novel intermediary in glucocorticoid-induced IL-7Rα up-regulation. We found Gfi1 to be a major inhibitory target of dexamethasone by microarray expression profiling of 3B4.15 T-hybridoma cells. Concordantly, retroviral transduction of Gfi1 significantly blunted IL-7Rα up-regulation by dexamethasone. To further assess the role of Gfi1 *in vivo*, we generated bacterial artificial chromosome (BAC) transgenic mice, in which a modified *Il7r* locus expresses GFP to report *Il7r* gene transcription. By introducing this BAC reporter transgene into either Gfi1-deficient or Gfi1-transgenic mice, we document *in vivo* that IL-7Rα transcription is up-regulated in the absence of Gfi1 and down-regulated when Gfi1 is overexpressed. Strikingly, the *in vivo* regulatory role of Gfi1 was specific for $CD8^+$, and not $CD4^+$ T cells or immature thymocytes. These results identify Gfi1 as a specific transcriptional repressor of the *Il7r* gene in CD8 T lymphocytes *in vivo*.

A critical issue in biology is the mechanism by which genes get turned on and off during development and differentiation. Because IL-7 receptor (IL-7R) proteins provide critical survival signals to developing lymphocytes, the expression of the *Il7r* gene that encodes the IL-7Rα receptor protein is tightly regulated at different stages of T and B lymphocyte development and precisely timed to stages when selection and programmed cell death occur in the immune system (1–3). The expression of IL-7Rα follows an on-off-on pattern in the thymus at the $CD4^-CD8^-$ double negative (DN),[8] $CD4^+CD8^+$ double positive (DP), and $CD4^+CD8^-$ or $CD4^-CD8^+$ single positive (SP) stages, respectively (4). Thus, developmental cues during thymocyte differentiation control IL-7Rα expression. During $CD8^+$ memory cell generation in the peripheral immune system, *Il7r* gene expression again correlates with developmental outcome, in that long-lived memory cell precursors up-regulate IL-7Rα expression and short-lived $CD8^+$ cells lose IL-7Rα expression (5). Notably, up-regulated IL-7Rα expression is not sufficient to drive long-lived memory $CD8^+$ T cell generation, even though IL-7Rα up-regulation clearly marks progenitors of this T cell subset (6, 7). Importantly, the differentiation signals that match IL-7Rα expression to CD8 T cell fate remain unknown.

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[1] These authors contributed equally to this work.
[2] Supported by the Scientific and Technological Research Council of Turkey scholarships.
[3] Present address: Div. of Immunobiology, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH 45229.
[4] Supported by the Turkish National Academy of Science post-doctoral fellowship.
[5] Present address: Department of Electroneurophysiology, Uskudar University, Istanbul, Turkey.
[6] Supported by TUBITAK 1001 Grant 109T315, National Institutes of Health-FIRCA Grant 1R03TW008208, and National Institutes of Health-GRIP Grant 1R01TW007270. To whom correspondence may be addressed: FENS, Sabanci University, Istanbul, Turkey. Tel.: 90-216-483-9530; Fax: 90-216-483-9550; E-mail: batu@sabanciuniv.edu.
[7] To whom correspondence may be addressed: Exp. Immunol. Branch, NCI, National Institutes of Health, Bldg. 10, Rm. 5B17, Bethesda, MD 20892. Tel.: 301-451-7641; Fax: 301-496-0887; E-mail: parkhy@mail.nih.gov.

[8] The abbreviations used are: DN, double negative; DP, double positive; SP, single positive; GR, glucocorticoid receptor; Dex, dexamethasone; BAC, bacterial artificial chromosome; IRES, internal ribosome entry site; ZF, zinc finger; LIP, lymphopenia-induced homeostatic proliferation; TCR, T cell receptor; GABP, GA-binding protein; Gfi1, growth factor independent-1.

In T cells, IL-7Rα expression is thought to be primarily regulated at the transcriptional level through an array of nuclear factors whose expression is also tightly controlled during development and activation. Several transcription factors that control *Il7r* gene expression have been identified. The promoter of *Il7r* contains binding sites for the PU.1 transcription factor, which is necessary for the IL-7Rα expression in developing B cells (8, 9). The same site is occupied in T cells by another ETS family transcription factor, GABP (10). Promoter occupancy by these factors likely prevents CpG methylation of promoter sequences and subsequent down-regulation of expression in mature T cells (11). Additionally, in human thymopoiesis, Notch may be complementing these ETS family proteins by acting through a conserved RBP-Jk/CSL binding site close by in the promoter of the *Il7r* gene (12). Therefore, down-regulation of Notch expression at the DP stage may be causative of the complete loss of *Il7r* gene transcription in murine DP thymocytes. Also, the potential role of microRNAs acting on the *Il7r* gene locus, specifically at the DP stage has not been addressed and needs to be tested. Furthermore, the zinc finger protein Gfi1, for which a regulatory role was originally proposed in T cells and more recently confirmed in pro-B cells, was shown to bind to a putative intronic silencer (13–15). Additionally, glucocorticoid receptor (GR), Runx1/3, FoxOA1/3, and FoxP1 were all shown to bind to a putative enhancer in an evolutionarily conserved region 3.5 kb upstream of the gene (16–21). Finally FoxP3 was found to bind near the promoter in T_{reg} cells to suppress IL-7Rα transcription (22). Importantly, however, how these factors interact with each other and what controls the mechanism of developmental stage-specific differences in *Il7r* gene transcription remains ill defined.

In the present study, we addressed this issue first by profiling gene expression in 3B4.15 T hybridoma cells that respond to dexamethasone (Dex) treatment by up-regulating IL-7Rα expression (23). We identified Gfi1 as a novel target of Dex and we further documented that either Gfi1 overexpression or treatment with the glucocorticoid receptor (GR) inhibitor RU486 (Mifepristone) in 3B4.15 cells prevented IL-7Rα up-regulation by Dex. These results indicate that Gfi1 is either controlled by GR or cooperates with it to down-regulate IL-7Rα expression. To further assess the role of Gfi1 *in vivo*, we then generated a novel bacterial artificial chromosome (BAC) transgenic mouse that reports transcriptional activity of the *Il7r* gene locus. We show that Gfi1 is a transcriptional repressor of the *Il7r* gene locus, but only in CD8 lineage cells, by assessing *Il7r* reporter activity in Gfi1-deficient and Gfi1-transgenic thymocytes and T cells. Our observations place Gfi1 as a lineage-specific and developmental stage-dependent transcriptional repressor of IL-7Rα *in vivo*.

EXPERIMENTAL PROCEDURES

Animals—C57BL/6 and RAG2-deficient mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Gfi1-deficient ($Gfi1^{KO}$) and Gfi1-transgenic ($Gfi1^{Tg}$) mice have been previously described (24, 25). Animal experiments were approved by the National Cancer Institute Animal Care and Use Committee, and all mice were cared for in accordance with National Institutes of Health (NIH) guidelines.

Generation of 7RIG-BAC^{Tg} Transgenic Mice—A BAC clone (RP23-365P6) containing the *Il7r* gene locus was modified by recombineering an IRES-EGFP cassette into the 3′ UTR region of the gene in *Escherichia coli* (26). Briefly, a targeting vector was generated containing (1) an HincII fragment of the pIRES2EGFP plasmid (Clontech) (2), an SV40 late poly(A) signal sequence PCR amplified from the pGL3Basic plasmid (Promega), (3) a KpnI fragment of the pLTM260 plasmid containing an Frt and a loxP-flanked Neomycin resistance gene with a PGK promoter and a bGH poly(A) signal and (4) two flanking regions (210 and 300 bp long) homologous to the *Il7r* 3′ UTR PCR amplified from BAC DNA. This reporter BAC DNA was purified by sucrose gradient centrifugation and was injected into fertilized B6 oocytes to generate transgenic mice as described (27). Founder mice were identified by flow cytometric detection of GFP expression on peripheral blood lymphocytes. One transgenic line out of 4 founders that recapitulated IL-7Rα expression patterns on peripheral T and B lymphocytes was selected for further study and named for this study "7RIG-BAC^{Tg}." Note that this transgene is unique compared with a recently reported BAC transgene, as we utilized an internal ribosome entry site (IRES) element in the 3′ UTR to ensure GFP reporter expression coincident with transgenic IL-7Rα expression (28). The transgene was introduced into a $Gfi1^{Tg}$ or $Gfi1^{KO}$ background by breeding.

Genotyping of IL-7Rα KO Allele by Quantitative Real Time-PCR—Originally, the IL-7Rα-deficient ($IL\text{-}7R\alpha^{KO}$) mouse was generated by inserting a 1-kb $MC1^{neo}$ cassette into a HindIII site within the third exon of the *Il7r* gene, around position 90 of the 180-amino acid long extracellular domain (1). The originally inserted neo^r gene is a modified neo^r gene from pMC1Neo as described in Thomas and Capecchi (29). In this altered neomycin resistance gene, a synthetic translation initiation sequence "5′-gccaatatgggatcggcc-3′" is introduced. We used the reverse sequence of this synthetic translation initiation sequence in combination with an *Il7r* exon3-specific primer to amplify a short PCR fragment. The exon 3-specific primer corresponds to the amino acid sequence "GSSNICV" of the IL-7Rα extracellular domain. Copy numbers of the IL-7Rα KO allele was determined by real time-PCR using primers IL7Rex3GSSNICV and MC1neo-R.

Cell Culture and Flow Cytometric Analysis—Thymocytes or LN cells were prepared by processing thymus and LN into single cell suspensions and filtering through a 0.70-μm cell strainer (BD Biosciences). For cell culture or stimulation, processed cells were incubated at 5×10^6 cells/ml in 7.5% CO_2 at 37 °C in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 units/ml of penicillin/streptomycin, 2 mM L-glutamine, 1× minimal essential medium vitamins/nonessential amino acids, and 50 μM β-mercaptoethanol. For dexamethasone treatment, cells were incubated with 10 μM water-soluble dexamethasone (catalog number D4902, Sigma) for 18 h in the presence or absence of 10 μM mifepristone (RU486; catalog number M8046, Sigma). For flow cytometry, one million 3B4.15 hybridoma, thymocytes, or lymph node cells were used per staining with the corresponding antibodies and incubated for 45 min on ice. After washing with FACS buffer (1× HBSS, 0.5% sodium azide, 0.5% BSA), cells were analyzed on LSRII,

ARIAII, FACSCanto, or FACSCalibur flow cytometers (BD Biosciences). Dead cells were excluded by forward light scatter gating and propidium iodide or 7-Aminoactinomycin D staining. Antibodies with the following specificities were used for staining: Qa-2 (clone 69H1-9-9), CD44 (clone IM7), HSA (clone M1/69), IL-7Rα (clone A7R34), IL-2Rα (clone PC61.5), IL-21R (clone ebio4A9), CD8α (clone 53-6.7); all from eBioscience); γc-chain (clone 4G3), IL-4Rα (clone mIL4R-M1), CD4 (clone GK1.5), TCRβ (clone H57–957), and B220 (RA3–6B2) (all from BD Biosciences); and IL-2Rβ (clone 5H4) from Biolegend. Data were analyzed with software designed by the Division of Computer Research and Technology at the NCI or with FlowJo 9.4.3 software (Treestar).

Adoptive Transfer—Purified LNT cells from 7RIG-BAC^{Tg} and Gfi^{Tg}7RIG-BAC^{Tg} mice were labeled with CellTrace Violet (Invitrogen) and adoptively transferred into RAG2-deficient host mice. 4×10^6-Labeled cells were intravenously injected, and spleen and lymph node cells from host mice were harvested 5 days later. Single cell suspensions were stained for surface IL-7Rα and TCRβ expression and analyzed by flow cytometry.

RNA Isolation and Northern Blot Analysis—Total RNA was isolated using TRIzol (Invitrogen). Equal amounts of RNA were resolved in a 1.5% agarose gel under denaturing conditions and blotted onto Hybond-N$^+$ nylon membranes (Amersham Biosciences). Radioactive probes for detecting specific gene expression were generated using the EZ-strip DNA kit (Ambion) and used to hybridize with RNA-blotted membrane in UltraHyb hybridization solution (Ambion) at 42 °C. The next day, membranes were washed two times with 2× SSC, 0.1% SDS for 30 min and two more times with 0.1× SSC, 0.1% SDS at 55 °C. Membranes were exposed to a PhosphorImager screen (Amersham Biosciences) and analyzed.

Expression Plasmids and Gene Transfer—Full-length and truncation mutants of murine Gfi1 cDNAs were C-terminal FLAG epitope-tagged and cloned into the pBluescript II plasmid using oligonucleotides incorporating 5′ XhoI and 3′ NotI restriction sites. All cDNAs were transferred from pBluescript II to a retroviral expression plasmid, LZRSpBMN-linker-IRES-EGFP, using XhoI and NotI restriction enzymes. This resulted in bicistronic expression of FLAG epitope-tagged Gfi1 cDNA variants with an EGFP reporter gene. The following oligonucleotide pairs were used to amplify Gfi1 truncations: D-ZF, M13+dZFsrev; mGfi1-ZF, ZFsfor+T7; and D-SNAG, dSNAGfor+T7. LZRSpBMN-linker-IRES-EGFP with full-length or truncated Gfi1 cDNAs were transfected into Phoenix-Eco retroviral packaging cell lines with a plasmid encoding ecotropic retrovirus envelope proteins (pCL-Eco Addgene plasmid 12371) (30) and supernatants were collected for 2 days, pooled, and filtered through 45-μm filters. 3B4.15 cells were infected by spin infection in the presence of 6 μg/ml of Polybrene (Sigma).

Oligonucleotides Used in this Study—The following oligonucleotides were used to PCR amplify Gfi1 cDNA constructs. Restriction enzyme sites (XhoI and NotI) used for cloning are shown in bold lettering, FLAG epitope tag sequence is shown in italics, and the start and stop codons are underlined; M13, 5′-CGC CAG GGT TTT CCC AGT CAC GAC-3′; T7, 5′-TAA TAC GAC TCA CTA TAG GG-3′; dSNAGfor, 5′-**ATC TCG AGG** CCA CCA TGC CAG GGC CGG ACT ACT CC-3′; ZFsfor, 5′-AT**C TCG AG**G CCA CCA TGT CCT ACA AAT GCA TCA AAT G-3′; dZFsrev, 5′-AT**G CGG CCG** CTA *TTT ATC GTC ATC GTC TTT GTA GTC CAT GGA TCC* TTT GTA GGA GCC GCC G-3′; dSNAGrev, 5′-AT**G CGG CCG** CTA *TTT ATC GTC ATC GTC TTT GTA GTC CAT GGA TCC* AGA ACG CGG CTG GTG ATA G-3′; IL7Rex3GSSNICV, 5′-GGT AGC AGC AAT ATA TGT GTG-3′; MC1neo-R, 5′-GGC CGA TCC CAT ATT GGC-3′.

Microarray Analysis—Expression analysis was performed on 3B4.15 T hybridoma cells, either untreated or treated with 1 μM dexamethasone (Sigma) for 16 h. Total RNA was extracted using TriReagent (Sigma), RNA quality was confirmed on an RNA 6000 Nano chip (AGT-5067-1511) in an Agilent Bioanalyzer. Double-stranded cDNA was generated using a SuperScript cDNA Synthesis Kit (Invitrogen), and the cDNA was then labeled with Cy-3, cleaned, quantified, and hybridized according to the manufacturer's protocols (Roche-Nimblegen). Nimblegen full genome Mouse Expression arrays (12X135K RO5543797) were washed and scanned at the Sabanci University Nanotechnology Research and Application Center-SUNUM. Results were processed using the ANAIS software (31). Array quality was assessed at the probe level. Values for 3 probes for each gene in each array were combined to summarize gene expression from probe sets. Robust Multi-Array Analysis background normalization and quantile normalization were performed for intra- and inter-array normalization, respectively. Genes with signal intensities above a 95% random threshold were chosen for further studies. Differentially expressed genes were obtained based on the following criteria: fold-change ≥2.5 and analysis of variance p value ≤ 0.01. Hierarchical clustering was applied to the top 500 differentially expressed genes with Genesis software (32). The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (33) and are accessible through GEO Series accession number GSE39296.

RESULTS

Glucocorticoids Induce IL-7Rα Expression by Down-regulating Expression of Gfi1—Treatment of primary T lymphocytes and T cell lines with glucocorticoids, such as Dex, results in the up-regulation of surface IL-7Rα expression (17, 34). T cells normally express high levels of surface IL-7Rα but the I-E^k-restricted, PCC-specific T cell hybridoma 3B4.15 expresses only low levels of IL-7Rα (23). Nevertheless, when treated with glucocorticoids such as Dex, 3B4.15 cells dramatically up-regulate both IL-7Rα mRNA and cell surface protein expression (Fig. 1, *A* and *B*). These results parallel those previously obtained using the transformed murine T cell line KKF, which responds to Dex by up-regulating IL-7Rα protein expression (16). Dex treatment induces nuclear localization and binding of the glucocorticoid receptor transcription factor to an evolutionarily conserved region 3.5 kb upstream of the transcriptional start site of the *Il7r* gene (16). Thus, Dex-induced IL-7Rα up-regulation has been considered to be a direct transcriptional effect of activated GRs. To understand other gene regulators that control this phenotypic change, we compared the gene expression profiles of mock treated *versus* Dex-treated 3B4.15 cells by high coverage Nimblegen expression arrays with 135,000 features.

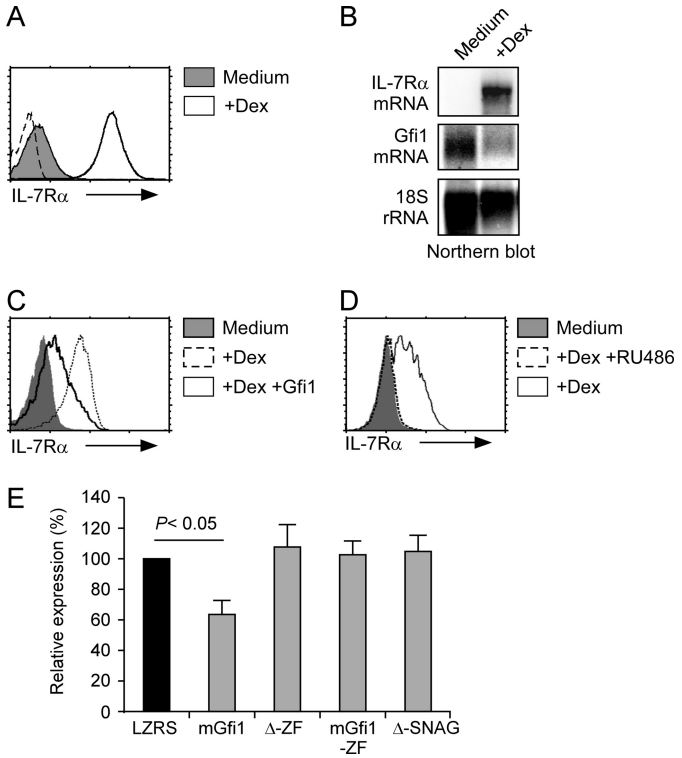


FIGURE 1. **Glucocorticoids up-regulate IL-7Rα expression while down-regulating Gfi1.** *A*, Dex induces surface IL-7Rα expression on 3B4.15 hybridoma. Single parameter histograms of IL-7Rα expression on 3B4.15 hybridoma cells incubated overnight in medium or with Dex. Isotype staining controls are shown in *dotted line*. *B*, Dex-induced IL-7Rα expression inversely correlates with Gfi1 expression. Northern blot analysis of total RNA from overnight medium or Dex-treated 3B4.15 cells with probes indicated on the *left*. *C*, Gfi1 overexpression inhibits Dex-induced IL-7Rα expression. Histograms show surface IL-7Rα expression on control retrovirus-infected 3B4.15 cells incubated for 16 h, either in medium (*filled histogram*) or with Dex (*dotted line*). *Solid histogram* shows IL-7Rα expression on Dex-treated, Gfi1-expressing retrovirus-infected 3B4.15 cells. *D*, Dex-induced IL-7Rα expression is a glucocorticoid receptor dependent event. Histograms show IL-7Rα expression on 3B4.15 cells incubated for 16 h in medium (*filled histogram*), with dexamethasone (*solid histogram*), or with Dex in the presence of Mifepristone (RU486) (*dotted line*). *E*, zinc finger and SNAG domains of Gfi1 are required for inhibiting Dex-induced IL-7Rα expression. IL-7Rα surface expression on 3B4.15 cells was quantified into linear fluorescence units, with IL-7Rα expression on empty LZRS retrovirus-infected cells set equal to 100. Inhibition of IL-7Rα expression by retroviruses expressing either full-length (*mGfi1*), or domain deletions of Gfi1 were compared. Zinc finger domain deleted (Δ-*ZF*), N-terminal truncation containing only the ZF domain (*mGfi1-ZF*), and SNAG domain deleted (Δ-*SNAG*).

Microarray results confirmed that Dex-treated 3B4.15 T hybridoma cells indeed up-regulated IL-7Rα gene expression (supplemental Fig. S1).

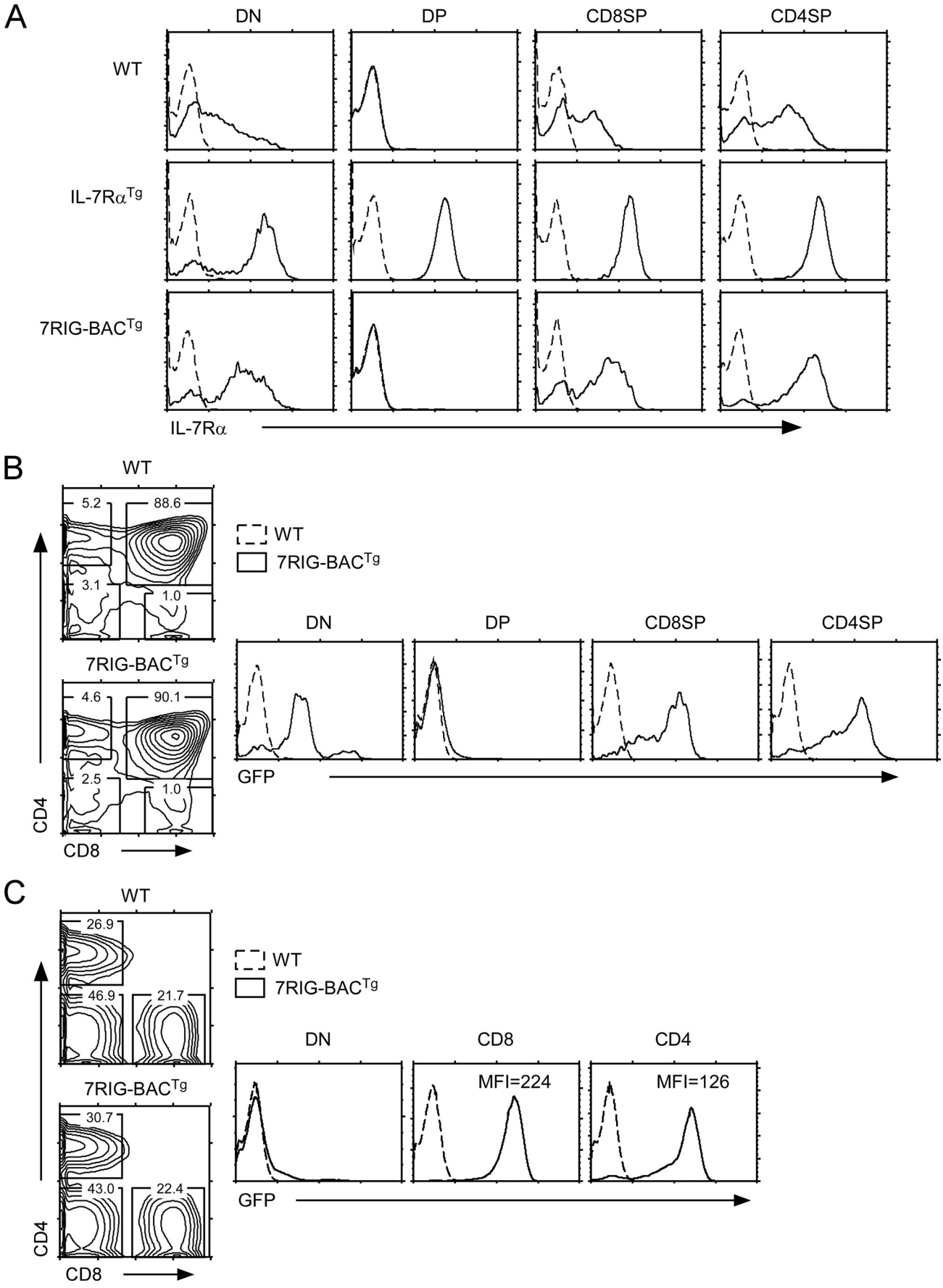
To confirm the specificity of Dex signaling in 3B4.15 hybridomas, first, we examined the expression profiles of the known Dex-regulated genes, *GILZ* (Tsc22d3) and *GITR* (Tnfrsf18) and found that these genes were positioned in the top 520 differentially expressed genes (supplemental Fig. S1) (35–37). Next, we further analyzed the expression profiles of all transcription factors that have previously been reported to regulate *Il7r* transcription. Among these were: glucocorticoid receptor, Gfi1, its close homolog Gfi1b, GABPα and its partners GABPβ1 and GABPβ2, PU.1 (Sfpi1), Runx1/3, NF-κB, FoxOA1/3, FoxP1, and FoxP3. Notably, we found that among these transcription factors, Gfi1 was the only one that passed our differentially expressed gene criteria of fold-change ≥ 2.5 and analysis of variance p value ≤ 0.01. Thus, Dex treatment results in an up-regulation of *Il7r* transcription and a dramatic down-regulation of the zinc finger repressor protein Gfi1 mRNA expression (Fig. 1*B* and supplemental Fig. S1).

Gfi1 was previously proposed to repress IL-7Rα transcription during lymphocyte development (14). Consequently, we wished to assess whether Gfi1 down-regulation would contribute to IL-7Rα up-regulation in 3B4.15 cells. To test this idea, we retrovirally overexpressed Gfi1 in 3B4.15 cells. Strikingly, Gfi1 overexpression inhibited IL-7Rα up-regulation by dexamethasone, and Gfi1 overexpressing 3B4.15 cells remained IL-7Rα low (Fig. 1*C*). Thus, Dex induces the down-regulation of endogenous Gfi1 expression, but retroviral overexpression of Gfi1 is maintained even in the presence of Dex, and results in these cells remaining IL-7Rα low. This effect was indeed directly dependent on GR, because Dex treatment in the presence of RU-486, which is a competitive inhibitor of Dex for GR binding, completely inhibited IL-7Rα up-regulation (Fig. 1*D*).

Next, to understand the mechanism of Gfi1-mediated repression of IL-7Rα expression, we retrovirally overexpressed a series of truncated Gfi1 cDNAs and assessed their effects on the Dex response of 3B4.15 cells (supplemental Fig. S2, *A* and *B*). Although expression of full-length Gfi1 significantly suppressed IL-7Rα re-expression, truncated Gfi1 lacking the demethylase-recruiting Snail-Gfi (SNAG) domain or the DNA binding zinc finger (ZF) domain failed to do so (Fig. 1*E*). Thus, repression of IL-7Rα expression by Gfi1 requires both its transcriptional repressor and the DNA binding domains and uncover Gfi1 to be a potent transcriptional inhibitor of IL-7Rα expression that acts downstream of Dex signaling, which de-represses *Il7r* transcription.

Assessing IL-7Rα Transcription in Vivo Using a Novel IL-7Rα Reporter Mouse—Untreated 3B4.15 cells express high levels of Gfi1 and low levels of IL-7Rα. Mature resting T cells, on the other hand, express only low levels of Gfi1 and high levels of IL-7Rα (38). Therefore, Gfi1 levels in T cells *in vivo* do not correspond to those in 3B4.15 T hybridomas. To test whether Gfi1 can also control IL-7Rα expression *in vivo*, we generated a novel IL-7Rα transcriptional reporter transgenic mouse. We used a 210-kb BAC fragment containing the *Il7r* gene locus and inserted an IRES-EGFP cassette in the 3′ untranslated region (UTR) of the *Il7r* gene. We used this construct in pronuclear injections to generate transgenic mice (7RIG-BAC^{Tg}) (supplemental Fig. S3).

Because the BAC construct contained the full *Il7r* gene locus, including putative transcriptional control regions, we expected the transgenic *Il7r* gene to faithfully reproduce expression of endogenous *Il7r*. To test this, we assessed IL-7Rα surface levels on freshly isolated 7RIG-BAC^{Tg} thymocytes. Endogenous IL-7Rα displays a characteristic on-off-on pattern during the progression through the DN-DP-SP stages of thymocyte development (3, 39). Indeed, 7RIG-BAC^{Tg} thymocytes showed the expected on-off-on pattern for IL-7Rα expression in the thymus. These data suggest that transgenic IL-7Rα transcription is appropriately controlled in 7RIG-BAC^{Tg} thymocytes (Fig. 2*A*). Notably, IL-7Rα expression by 7RIG-BAC^{Tg} differed from that of a human CD2 promoter/enhancer-driven IL-7Rα transgene (IL-7Rα^{Tg}), in its ability to down-regulate IL-7Rα expression on



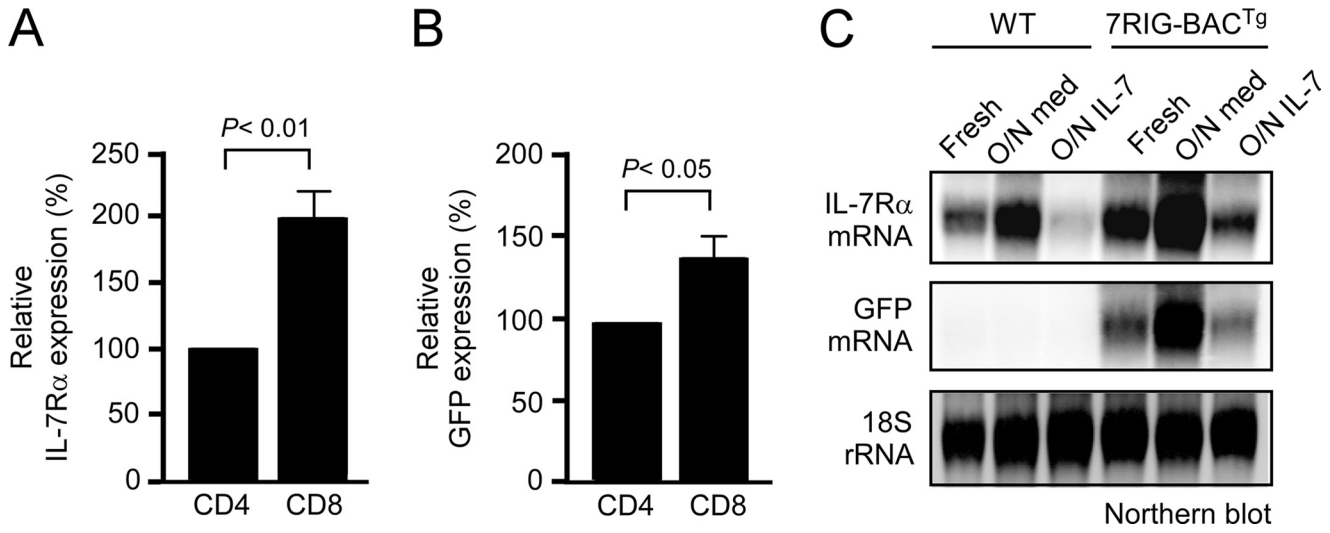


FIGURE 3. **Transcriptional regulation of GFP reporter expression.** *A,* relative surface IL-7Rα expression on CD4 and CD8 LNT cells. Surface IL-7Rα levels on 7RIG-BAC^{Tg} T cells were quantified in mean fluorescence intensity and normalized to IL-7Rα levels on CD4 cells. *Bar graph* shows mean ± S.E. from three independent experiments. *B,* relative GFP expression in CD4 and CD8 LN T cells. Intracellular GFP levels in 7RIG-BAC^{Tg} T cells were quantified in the mean fluorescence intensity and normalized to GFP levels in CD4 cells. *Bar graph* shows mean ± S.E. from three independent experiments. *C,* purified LN T cells from WT or 7RIG-BAC^{Tg} mice were assessed for IL-7Rα and GFP mRNA expression by Northern blot analysis with probes indicated on the *left*. Total RNA was isolated from fresh, overnight medium incubated, or overnight IL-7-treated LNT cells.

DP thymocytes (Fig. 2*A*). These results support the expectation that the BAC transgene retained all endogenous regulatory elements for correct IL-7Rα expression. Notably, IL-7Rα surface levels on 7RIG-BAC^{Tg} transgenic cells were significantly higher compared with WT controls, presumably because *Il7r* gene transcription was active both from the endogenous and the transgenic *Il7r* locus. To demonstrate that the 7RIG-BAC^{Tg} transgene faithfully reports *Il7r* gene transcription, we assessed GFP expression in thymocytes. GFP expression also followed the on-off-on pattern of IL-7Rα expression in DN, DP, and SP thymocytes, respectively, indicating that transcription from the transgenic locus was correctly inhibited in DP thymocytes (Fig. 2*B*). We also assessed IL-7Rα reporter expression in peripheral LN cells. Mature B cells do not express IL-7Rα. Accordingly, we found that 7RIG-BAC^{Tg} DN lymph node cells, which include all mature B cells, were negative for GFP expression (Fig. 2*C*). CD4 and CD8 lymph node T cells, on the other hand, correctly expressed high levels of GFP. Interestingly, CD8 T cells reported higher levels of *Il7r* transcription than CD4 T cells based on their GFP levels (Fig. 2*C*). These data confirm that 7RIG-BAC^{Tg} reporter mice faithfully represent endogenous IL-7Rα expression *in vivo* and reveal a hitherto unappreciated difference in IL-7Rα transcription levels in CD4 and CD8 T cells.

Cytokine-induced Regulation of 7RIG-BAC^{Tg} Expression—To further document distinct IL-7Rα expression in CD4 and CD8 T cells, we quantified surface IL-7Rα and intracellular GFP levels in freshly isolated 7RIG-BAC^{Tg} LN T cells. We confirmed statistically significant higher levels of both IL-7Rα expression and transcription in CD8 T cells in multiple experiments (Fig. 3, *A* and *B*). Moreover, such lineage-specific IL-7Rα expression was developmentally set as CD4 and CD8 T cells incubated overnight in medium in the absence of *in vivo* signals still displayed distinct levels of IL-7Rα expression (data not shown).

The *Il7r* gene locus is exquisitely sensitive to cytokine signaling. For instance, *in vivo* IL-7 signaling down-regulates *Il7r* transcription and steady-state levels of IL-7Rα mRNA in T cells (13). To determine whether the transgenic IL-7Rα gene locus also responds to cytokine treatment, we incubated LN T cells from either WT or 7RIG-BAC^{Tg} mice overnight in medium or in the presence of IL-7. The next day, total RNA was extracted and IL-7Rα mRNA signals were assessed and compared with those from freshly isolated T cells. In both WT and 7RIG-BAC^{Tg} T cells, overnight release from the cytokine-rich *in vivo* environment highly up-regulated IL-7Rα mRNA expression (Fig. 3*C*). Furthermore, overnight IL-7 signaling potently suppressed IL-7Rα mRNA expression in both WT and transgenic T cells. These results suggest that both the endogenous and the transgenic *Il7r* loci are regulated in a cytokine-dependent manner. More importantly, GFP mRNA levels also faithfully replicated IL-7Rα expression, which confirms the validity of this transgenic model as an *in vivo* IL-7Rα reporter (Fig. 3).

Restoring T Cell Development in IL-7Rα-deficient Mice by 7RIG-BAC^{Tg}—IL-7Rα deficiency results in severely impaired T cell development and peripheral T cell homeostasis. Because 7RIG-BAC^{Tg} replicates expression of endogenous *Il7r*, we wished to know if the BAC transgene could restore T cell

FIGURE 2. **7RIG-BAC^{Tg} faithfully reports IL-7Rα expression *in vivo*.** *A,* cell surface IL-7Rα expression during thymocyte development. IL-7Rα expression on gated thymocyte subpopulations from WT mice (*upper panel*), 7RIG-BAC^{Tg} (*lower panel*), and a human CD2 mini-cassette driven IL-7Rα^{Tg} mice (*middle panel*) are shown (*solid line*) over isotype control staining (*dotted line*). *B,* assessing IL-7Rα transcription using GFP reporter activity in thymocyte subpopulations. Total thymocytes from WT and 7RIG-BAC^{Tg} mice were stained for CD4 and CD8 surface markers, and GFP expression was determined in individual subpopulations. Data are representative of four independent experiments. *C,* lineage specific IL-7Rα transcription in LNT cells. Total LN cells from WT and 7RIG-BAC^{Tg} mice were stained for CD4 and CD8 surface markers, and GFP expression was determined in CD8 and CD4 LN T cells. Mean fluorescence intensity of surface IL-7Rα are shown for CD8 and CD4 T cells, respectively. Data are representative of four independent experiments.

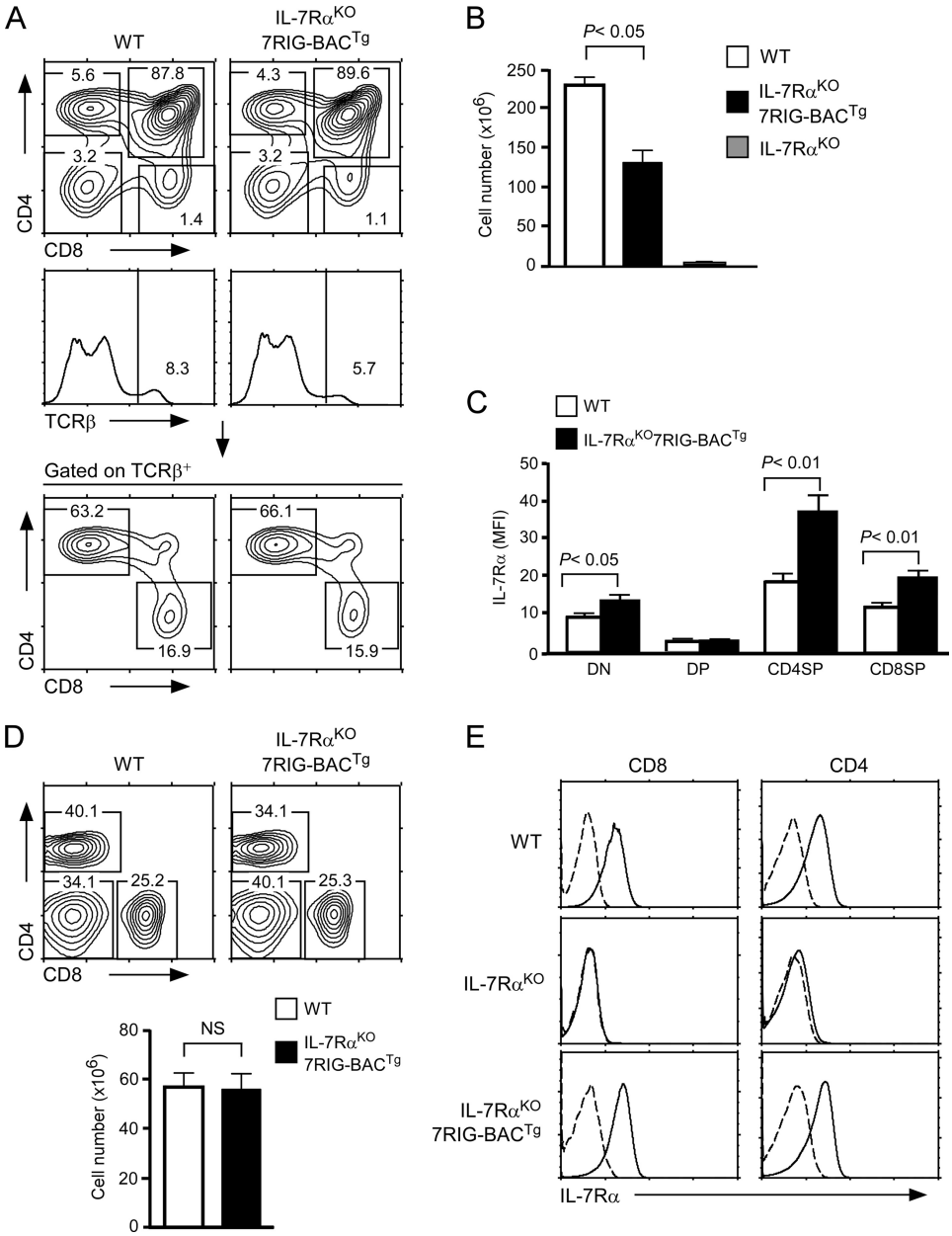


FIGURE 4. **7RIG-BAC^{Tg} restores thymocyte development and T cell homeostasis in IL-7Rα^{KO} mice.** *A*, thymocyte development in IL-7Rα^{KO}7RIG-BAC^{Tg}. Total thymocytes from WT and IL-7Rα^{KO}7RIG-BAC^{Tg} mice were assessed for CD4, CD8, and TCRβ surface marker expression (*top* and *middle*). Mature TCRβ^{hi} thymocytes were analyzed for CD4 and CD8 profiles (*bottom*). *B*, total thymocyte numbers in IL-7Rα^{KO}7RIG-BAC^{Tg} mice. Thymocyte numbers from WT, IL-7Rα^{KO}7RIG-BAC^{Tg}, and IL-7Rα^{KO} mice were determined. *Bar graph* shows mean ± S.E. from three independent experiments. *C*, surface IL-7Rα on WT and 7RIG-BAC^{Tg} thymocytes. IL-7Rα expression was determined on WT and IL-7Rα^{KO}7RIG-BAC^{Tg} thymocyte subpopulations. *Bar graph* shows mean ± S.E. from three independent experiments. *D*, peripheral T cell homeostasis in IL-7Rα^{KO}7RIG-BAC^{Tg} mice. LN cells were isolated, counted, and phenotyped for CD4 and CD8 expression (*top*). Bar graph shows total LN numbers (*bottom*). Data show mean ± S.E. from four independent experiments. *E*, surface IL-7Rα on WT and 7RIG-BAC^{Tg} LNT cells. IL-7Rα expression was assessed and quantified on WT and IL-7Rα^{KO}7RIG-BAC^{Tg} LN cell subpopulations. Data show representative histograms from three independent experiments.

defects in IL-7Rα^{KO} mice. To test this, we crossed 7RIG-BAC^{Tg} into IL-7Rα^{KO} mice to generate IL-7Rα^{KO}7RIG-BAC^{Tg} mice (supplemental Fig. S4*A*). In these mice, thymic αβ T cell development was largely restored as demonstrated by thymocyte CD4 *versus* CD8 profiles and TCRβ surface expression (Fig. 4*A*). Also, thymic NKT cell development and γδ T cell generation was dramatically improved compared with IL-7Rα^{KO} mice (supplemental Fig. S4, *B* and *C*). Notably, total thymocyte numbers were also restored compared with IL-7Rα^{KO} mice, but they did not fully recover to WT levels (Fig. 4*B*). To further understand this, we analyzed surface IL-7Rα levels on thymocytes, and we found that IL-7Rα^{KO}7RIG-BAC^{Tg} mice expressed significantly higher levels of IL-7Rα than WT mice (Fig. 4*C*). This is presumably caused by insertion of multiple copies of the BAC transgene into the genome as usually observed in BAC transgenesis. Elevated levels of IL-7Rα, however, have been shown to increase IL-7 consumption and competition for IL-7, which results in an overall decrease in thymocyte numbers (40, 41). Thus, decreased total thymocyte numbers might reflect quantitative differences in surface IL-7Rα expression between WT

and IL-7Rα^{KO}7RIG-BAC^{Tg} thymocytes. Interestingly, however, no major differences were observed in peripheral LN T cell numbers and CD4/CD8 profiles (Fig. 4*D*), despite surface IL-7Rα levels on transgenic LN T cells being still higher than WT counterparts (Fig. 4*E*). Thus, thymocytes and LN T cells are differently affected by IL-7Rα levels, likely because of the different mechanisms of proliferation and homeostasis in these two organs. Collectively, we find that BAC transgenic IL-7Rα is expressed and regulated in a developmentally correct fashion, and consequently restores T cell development and maintenance in IL-7Rα-deficient mice.

Gfi1 Overexpression Suppresses Il7r Gene Transcription in CD8 T Cells—Using these reporter mice, next we asked whether Gfi1 can suppress IL-7Rα transcription and expression *in vivo*. To this end, we introduced the 7RIG-BAC^{Tg} onto mice expressing a T lineage-specific, human CD2 promoter driven Gfi1 transgene (25) to generate Gfi1^{Tg}7RIG-BAC^{Tg} mice and determined their IL-7Rα transcription by analyzing GFP expression. Strikingly, in Gfi1^{Tg}7RIG-BAC^{Tg} thymocytes, we found that GFP levels were significantly down-regulated by Gfi1, but that this was specific and restricted to CD8SP thymocytes (Fig. 5*A*). These results suggest that transgenic Gfi1 expression fails to suppress IL-7Rα transcription in any other thymocyte subpopulation than in CD8SP cells. Such a specific effect of Gfi1 on CD8 lineage cells was further confirmed in peripheral CD8 T cells. We found both surface IL-7Rα and intracellular GFP levels selectively down-regulated in CD8, but not in CD4 T cells (Fig. 5*B*). Thus, these data document that Gfi1 only down-regulates IL-7Rα gene transcription and surface protein expression in CD8 T lymphocytes, and that all other thymocyte subpopulations and CD4 T cells are not susceptible to repression of this gene by Gfi1.

To further assess the specificity of Gfi1 transcriptional repression, we examined expression of other members of the γc cytokine receptor family. The γc cytokine family is composed of IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 (42), and antibodies are available for surface staining of each member of the γc receptor family with the exception of IL-9. Consequently, we examined expression of γc cytokine receptors for IL-2Rα, -2Rβ, γc, IL-4Rα, IL-7Rα, and IL-21R on WT and Gfi1^{Tg} CD8 T cells (Fig. 5*C*). Strikingly, IL-7Rα was the only cytokine receptor that was significantly affected by Gfi1 overexpression (Fig. 5*D*), which revealed a highly selective effect of Gfi1 on IL-7Rα and reaffirmed its specificity for CD8 T cells.

Gfi1 Deficiency Up-regulates Il7r Transcription in CD8 T Cells—Such a CD8 lineage-specific effect of Gfi1 was intriguing. One potential explanation could be that Gfi1 is expressed in all thymocytes and T cells, but that its repressor activity is only limited to CD8 T cells. Consequently, we wished to test whether removal of Gfi1 would up-regulate IL-7Rα expression in T cells other than in CD8 lineage cells. To this end, we generated Gfi1^{KO}7RIG-BAC^{Tg} mice and analyzed the GFP levels in thymocytes and mature LN T cells (supplemental Fig. S5*A* and Fig. 6*A*) (24). Notably, Gfi1 deficiency failed to de-repress IL-7Rα transcription in most thymocytes, with the exception of CD8SP thymocytes (supplemental Fig. S5*A*). DP thymocytes, which express high levels of Gfi1 and are completely silent for IL-7Rα transcription, were still negative for GFP expression, when Gfi1 expression was ablated. These results indicate that Gfi1 is not required to suppress IL-7Rα expression in this particular subset. Rather, the effect of Gfi1 was highly restricted to post-selection CD8 lineage cells as GFP expression was quantitatively increased in both CD8SP thymocytes and LN CD8 T cells only (supplemental Fig. S5*A* and Fig. 6*B*).

Because absent Gfi1 was sufficient to up-regulate IL-7Rα expression on CD8 T cells, next, we wished to know whether this would correlate with the Dex effect that we observed in 3B4.15 cells (Fig. 1, *A* and *B*). To this end, we stimulated WT and Gfi1^{Tg} T cells with Dex and assessed their surface IL-7Rα expression after overnight culture (Fig. 6*C*). Although WT CD8$^+$ T cells significantly up-regulated IL-7Rα as previously observed (17), IL-7Rα levels on Gfi1^{KO} CD8$^+$ T cells were unaffected by stimulation with Dex (Fig. 6*C*). Notably, IL-7Rα expression on Gfi1^{KO} CD4$^+$ T cells was still up-regulated by Dex indicating that Gfi1 effect is CD8 lineage specific (supplemental Fig. S5*B*). These data strongly suggest that a major role for Dex is to suppress Gfi1, and that Gfi1 directly controls IL-7Rα expression in CD8 T cells.

Gfi1 deficiency has been proposed to promote CD8 memory phenotype cell generation (43). Because memory CD8 T cells express high levels of IL-7R (42), we wished to test whether increased IL-7Rα is a consequence of memory cell differentiation or a direct effect of Gfi1 deficiency. GFP levels in CD8 T cell subsets demonstrated that IL-7Rα transcription was significantly increased in both naive (CD44^{low}) and activated/memory (CD44^{high}) phenotype CD8 T cells (Fig. 6*D*). Thus, we conclude that absent Gfi1 expression de-represses IL-7Rα transcription and expression in all CD8 T cells independently of their differentiation status.

In Vivo Analysis of IL-7Rα Transcription Using 7RIG-BACTg on a Single Cell Basis—We wished to know if using the 7RIG-BAC^{Tg} could provide us with new insights on IL-7Rα expression that so far has not been experimentally feasible to assess. Adoptive transfer of CD8 T cells into chronic lymphopenic mice results in lymphopenia-induced homeostatic proliferation (LIP). Slowly dividing homeostatic proliferation is dependent on IL-7, whereas rapid proliferation is IL-7 independent and driven by commensal antigens (44). To further understand the role of IL-7 in this process, it would be important to assess how IL-7Rα expression is regulated during LIP. To do so, we assessed surface IL-7Rα and intracellular GFP expression on day 5 adoptively transferred 7RIG-BAC^{Tg} CD8 T cells. Analyzing IL-7Rα transcription in adoptively transferred cells on a single cell basis has not been possible so far. IL-7 signaling suppresses IL-7Rα expression under steady-state conditions (13). Surprisingly, however, surface IL-7Rα levels remained largely unchanged during IL-7-driven LIP as assessed on slowly dividing cells (Fig. 7, *A*, *left*, and *B*). Strikingly, in the same cells, *Il7r* transcription was dramatically down-regulated upon cell division, as demonstrated by reduced GFP expression in cell trace-diluted cells (Fig. 7, *A*, *right*, and *B*). These data indicate that surface IL-7Rα expression is not a reliable marker for *Il7r* transcription, at least during homeostatic proliferation. They further suggest the operation of a transcription-independent mechanism of surface IL-7R expression, which could be either increased recycling of endocytosed IL-7Rα, stabilization of pre-

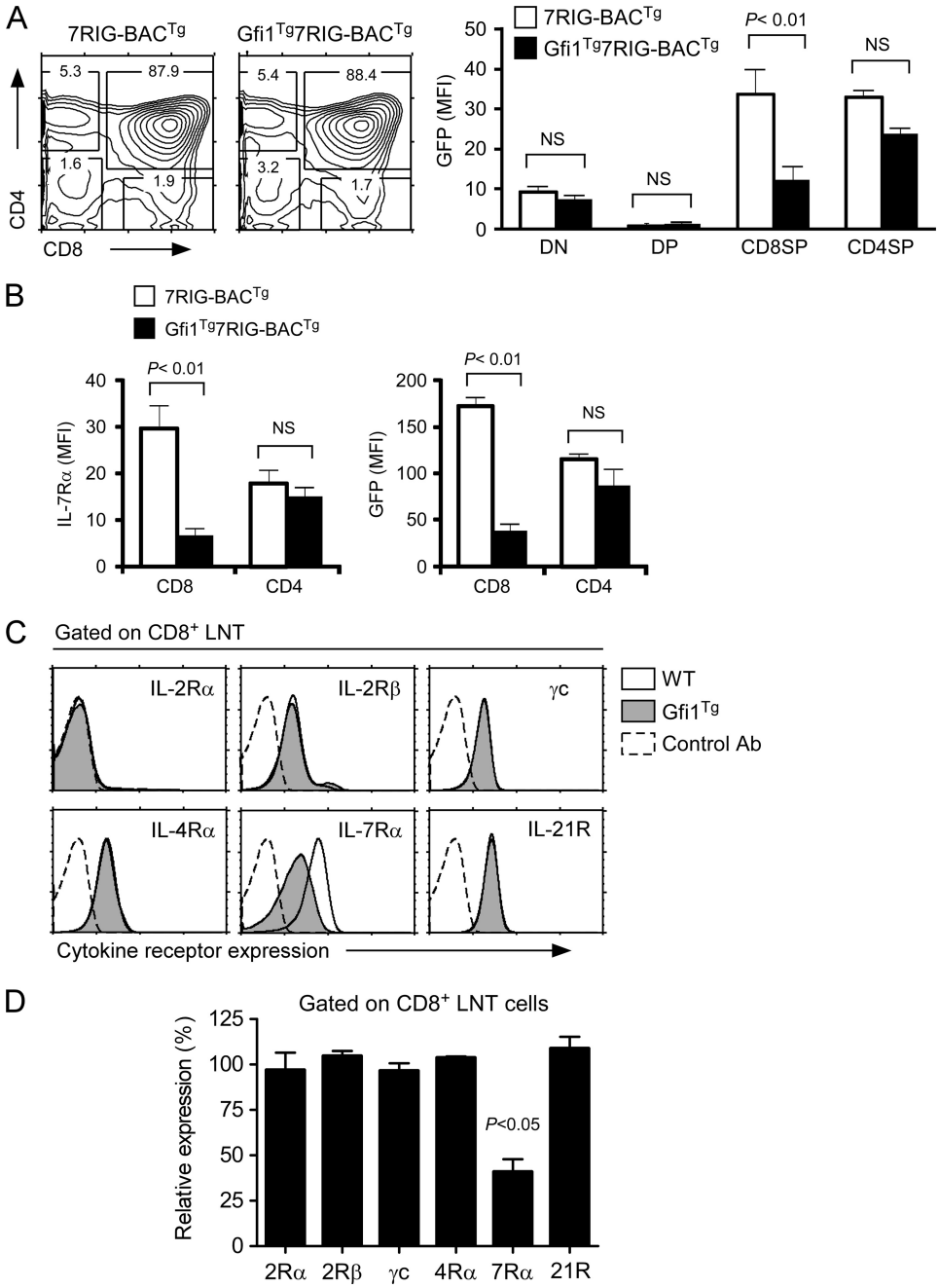


FIGURE 5. **Gfi1 suppresses IL-7Rα expression and transcription *in vivo*.** *A,* suppression of GFP reporter activity by overexpression of Gfi1. IL-7Rα transcriptional activities in individual thymocyte subpopulations were determined using the 7RIG-BAC^{Tg} on WT or Gfi1^{Tg} backgrounds. Contour plots are representative of four independent experiments with 4 WT and 5 Gfi1^{Tg} mice transgenic for 7RIG-BAC^{Tg}. *Bar graph* shows mean ± S.E. four independent experiments. *B*, IL-7Rα surface expression and transcription in Gfi1^{Tg} transgenic CD4 and CD8 LNT cells. The effect of Gfi1 was assessed in WT or Gfi1^{Tg} mice transgenic for 7RIG-BAC^{Tg}. Cell surface IL-7Rα and GFP expression were determined by flow cytometry. *Bar graph* shows mean ± S.E. from three independent experiments with 3 WT and 5 Gfi1^{Tg}7RIG-BAC^{Tg} mice. *C,* expression of γc cytokine receptor families on Gfi1^{Tg} CD8^{+} LNT cells. Surface expression of the indicated γc cytokine receptors were determined on gated CD8 T cells from WT and Gfi1^{Tg} mice. Data are representative of three independent experiments. *D*, Gfi1^{Tg} specifically suppresses IL-7Rα expression on CD8^{+} T cells. Surface cytokine receptor levels on Gfi1^{Tg} CD8^{+} T cells were quantified and normalized to levels on WT CD8^{+} T cells. *Bar graph* shows mean ± S.E. of three independent experiments.

existing IL-7Rα proteins or a yet unknown post-transcriptional mechanism. We are currently in the process of addressing these possibilities.

Because Gfi1 suppresses *Il7r* transcription, next we wished to assess the effect of Gfi1 on IL-7Rα expression during LIP. Day 5 adoptively transferred Gfi1^{Tg}7RIG-BAC^{Tg} cells displayed a comparable pattern of surface IL-7Rα and intracellular GFP expression to WT cells, in that GFP levels steadily decreased upon proliferation (Fig. 7*C*, *right*) but surface IL-7Rα levels remain largely unaffected (Fig. 7*C, left*). Thus, the dichotomoy of IL-7Rα transcription and surface protein expression during LIP still remained distinct in Gfi1^{Tg}7RIG-BAC^{Tg} cells. Moreover, LIP further down-regulated IL-7Rα transcription in Gfi1^{Tg} cells, which is presumably mediated by a mechanism independent of Gfi1. Importantly, Gfi1 overexpression significantly impaired CD8 T cell LIP, which correlated with lower

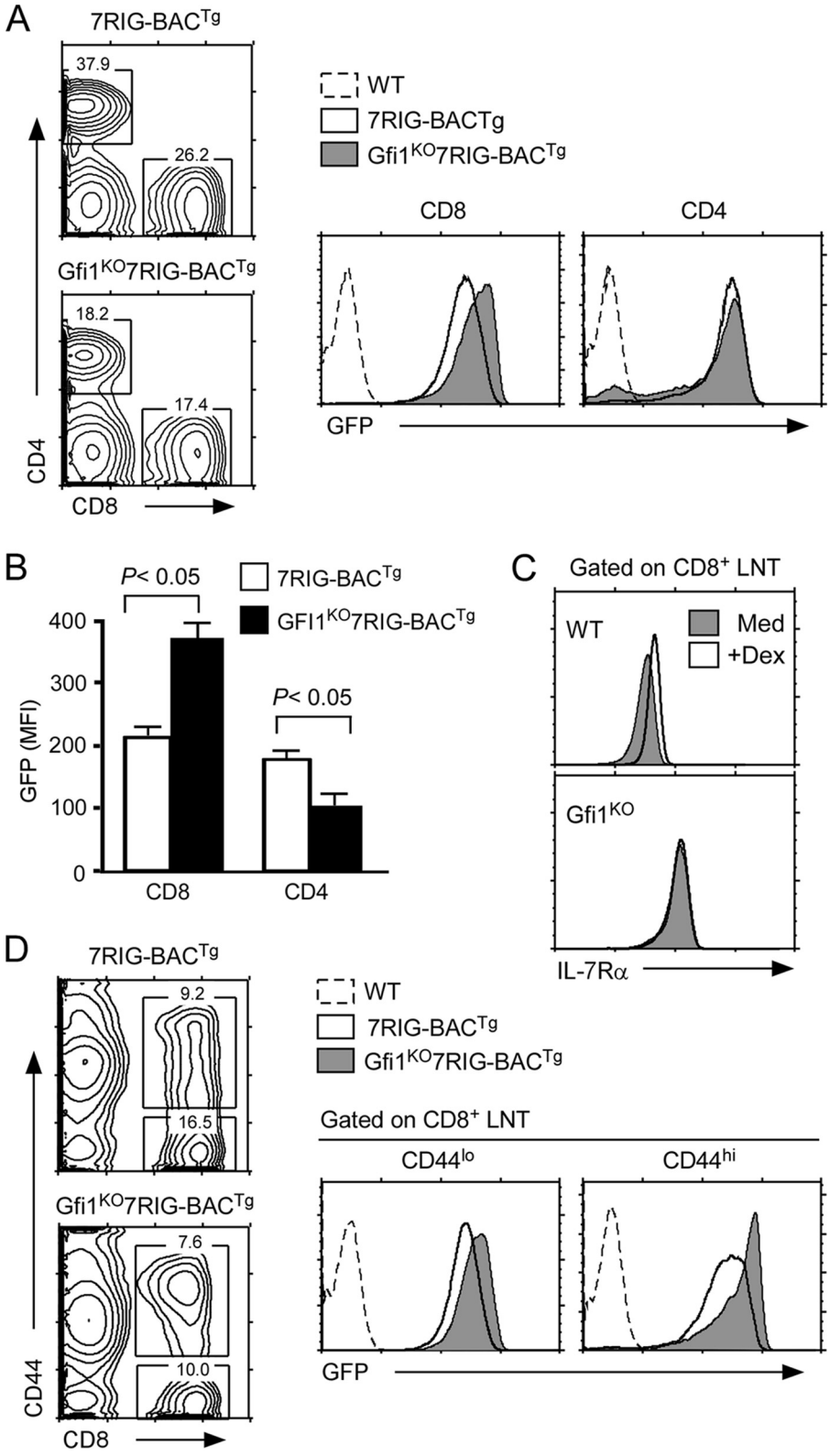


FIGURE 6. **Gfi1 deficiency de-represses IL-7Rα transcription and expression in CD8 lineage cells.** *A*, increased GFP reporter activity in Gfi1^{KO}7RIG-BAC^{Tg} CD8 T cells. GFP expression was assessed in CD8 and CD4 T cells from WT or Gfi1^{KO} mice expressing 7RIG-BAC^{Tg}. Data are representative of four independent experiments. *B*, quantification of GFP expression in Gfi1^{KO}7RIG-BAC^{Tg} T cells. Mean fluorescence intensity of intracellular GFP levels were determined from 7RIG-BAC^{Tg} and Gfi1^{KO}7RIG-BAC^{Tg} LNT cells. *Bar graph* shows the mean ± S.E. of two independent experiments. *C*, Dex effect on CD8^{+} LNT IL-7Rα expression. Surface IL-7Rα expression was determined on WT and Gfi1^{KO} CD8 T cells incubated overnight in medium or Dex. *D*, the effect of Gfi1 on IL-7Rα transcription is independent of activation/differentiation status in CD8 T cells. GFP reporter expression was assessed in freshly isolated CD44^{lo} and CD44^{hi} CD8 T cells from 7RIG-BAC^{Tg} and Gfi1^{KO}7RIG-BAC^{Tg} mice. Data are representative of four independent experiments.

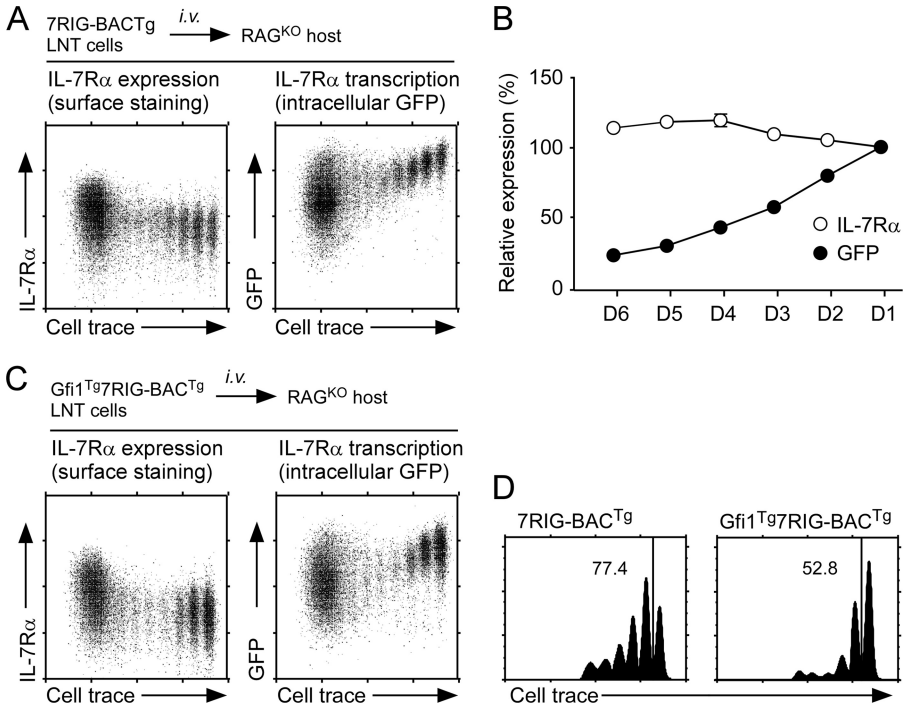


FIGURE 7. ***In vivo* effects of Gfi1 on IL-7Rα expression.** *A*, IL-7Rα expression during lymphopenia-induced homeostatic proliferation. Surface IL-7Rα expression and intracellular GFP expression were assessed on day 5 adoptively transferred 7RIG-BAC^{Tg} CD8^{+} T cells in RAG-2-deficient host mice. Cell division was monitored by CellTrace Violet dye dilution. Dot plots are representative of three independent experiments. *B*, IL-7Rα expression and transcription in proliferating donor cells. Mean fluorescence intensity of surface IL-7Rα (*open circle*) and intracellular GFP (*closed circle*) were assessed for each cell division (*D1* to *D6*), and normalized to nondividing cells (*D1*), which was set to 100 (%). Graph shows the mean ± S.E. from three independent experiments. *C*, IL-7Rα expression on proliferating Gfi1^{Tg}7RIG-BAC^{Tg} CD8^{+} T cells. Surface IL-7Rα expression and intracellular GFP expression was assessed on day 5 adoptively transferred Gfi1^{Tg}7RIG-BAC^{Tg} CD8^{+} T cells in RAG-2-deficient host mice. Cell division was monitored by CellTrace Violet dye dilution. Dot plots are representative of three independent experiments. *D*, Gfi1 impairs lymphopenia-induced homeostatic proliferation. CellTrace Violet dilutions on slow dividing donor 7RIG-BAC^{Tg} or Gfi1^{Tg}7RIG-BAC^{Tg} were quantitated. *Numbers* indicate the percentage of cells that have undergone one or more divisions during homeostatic proliferation. Data are representative of three independent experiments.

IL-7Rα levels on Gfi1^{Tg} CD8 T cells (Fig. 5*B*). Thus, Gfi1 suppresses IL-7Rα expression and also IL-7-dependent proliferation. Taken together, these results uncover Gfi1 as a critical regulator of IL-7Rα transcription and expression *in vivo*, but exclusively in CD8 lineage thymocytes and T cells.

DISCUSSION

To understand the molecular mechanisms of IL-7Rα expression during T cell development and activation, here we generated a novel BAC GFP-reporter transgene and utilized this tool to assess IL-7Rα transcription *in vivo*. The BAC transgene was constructed by inserting a GFP-reporter and an IRES element into the 3′ UTR of the murine *Il7r* gene. Consequently, BAC reporter mice overexpressed full-length IL-7Rα proteins in addition to GFP. We affirmed the developmentally correct expression of reporter transgenes by assessing lymphocyte differentiation in IL-7Rα-deficient mice reconstituted with the BAC construct (IL-7Rα^{KO}7RIG-BAC^{Tg}). In these mice, development of all IL-7Rα-dependent lymphoid cell populations, including αβ-, γδ-T cells, B-cells, and NKT cells, were restored. Thus, the transgenic *Il7r* gene locus in 7RIG-BAC^{Tg} mice is correctly and lineage specifically regulated, and it equipped us with a new tool to assess IL-7Rα transcription and expression *in vivo*.

IL-7Rα is the ligand-specific subunit of the functional IL-7 receptor, which is composed of the IL-7Rα chain and the γc-chain (4). In contrast to the γc-chain, IL-7Rα expression is dynamic and is actively regulated during T cell development and differentiation. All developing thymocytes and all mature T cells express IL-7Rα, albeit at varying degrees. However, immature DP thymocytes are unique in that they have completely terminated IL-7Rα expression. Such peculiar absence of IL-7Rα on pre-selection DP cells was proposed to reflect a critical thymic selection mechanism that ensures a random but self-MHC-specific TCR repertoire (39, 45, 46). Accordingly, absent IL-7Rα expression renders DP thymocytes dependent on selecting TCR signals and not on nondiscriminatory IL-7 signaling for survival. However, the molecular mechanisms that terminate IL-7Rα expression in DP cells remain unclear. Along this line, the molecular circuitry that re-induces IL-7Rα expression upon positive selection also remains unmapped. This is even more intriguing as TCR signaling in mature T cells down-regulates IL-7Rα expression, but in immature DP thymocytes, TCR signaling induces IL-7Rα expression. Recent studies have suggested that re-expression of IL-7Rα on DP cells is dependent on positive selecting TCR signals in a NFAT- and MAPK-dependent fashion and to a lesser degree on Akt (47). However, the detailed mechanism and molecular events remain unknown. Using 7RIG-BAC^{Tg} mice, here we establish that absent IL-7Rα expression in DP cells is a transcriptionally regulated event. Pre-selected DP cells failed to express GFP, while positive selection and maturation resulted in re-expression of GFP. With the 7RIG-BAC^{Tg} reporter mouse, it is now feasible

to test nuclear factors that regulate IL-7Rα transcription *in vivo*. We anticipate further applications of these reporter mice in dissecting the thymic signals that lead to termination as well as re-induction of *Il7r* transcription under various *in vitro* and *in vivo* settings.

In this regard, we were able to assess the effect of a transcriptional repressor on *Il7r* gene expression *in vivo*. Gfi1 has been considered as a potential *Il7r* regulator in immature thymocytes because of its high level of expression in pre-selection DP thymocytes and its potent suppression of IL-7Rα in pre-B cells. As such, Gfi1^{KO}7RIG-BAC^{Tg} mice offered a unique opportunity to assess the effect of Gfi1 on IL-7Rα transcription in DP thymocytes *in vivo*. The complete absence of GFP signals in Gfi1^{KO}7RIG-BAC^{Tg} DP thymocytes strongly suggested that transcriptional silencing of *Il7r* gene expression does not require Gfi1, at least in pre-selection DP thymocytes. In mature T cells, Gfi1 clearly plays a more critical role. In fact, Gfi1 was first reported to suppress IL-7Rα expression by analyzing peripheral mature T cells (13). Nevertheless, a definitive and direct role of Gfi1 in IL-7Rα transcription has remained highly controversial. In some cases, surface IL-7Rα expression on Gfi1^{KO} T cells was found to be not, or insignificantly different (38), and also increased IL-7Rα levels in Gfi1^{KO} mice had been suggested to be indirect, *i.e.* due to high proportions of memory cells under Gfi1 deficiency (43). Moreover, increased Gfi1 mRNA levels in human IL-7Rα^{high} effector memory CD8 T cells contradicted a repressor role for Gfi1 in IL-7Rα expression (48). Our current data showing significantly increased GFP expression in Gfi^{KO} CD8 T cells, however, clearly documents Gfi1 as an *in vivo* repressor for IL-7Rα transcription.

Nevertheless, contradictory observations still keep open the possibility of a more complex regulatory network, with several Gfi1 co-factors being involved. As such, the *cis*-elements participating in the Gfi1 control of *Il7r* gene transcription are still not well defined. In B lymphocytes, Gfi1 can bind to the second intron of the *Il7r* gene to suppress transcription (14). In myeloid cells, Gfi1 can also associate with PU.1, an ETS family transcription factor that binds to the promoter of IL-7Rα and activates its transcription (49). However, PU.1 is not expressed in T lymphocytes. Rather another ETS family transcription factor, GABP, is thought to occupy the ETS site in the promoter of the *Il7r* gene (50). Whether Gfi1 can interact with the GABP protein is not known. But in T cells, GABP and Gfi1 were shown to have opposing roles so that a direct interaction and mutual suppression cannot be excluded (51). Thus, in addition to its role in binding the *Il7r* gene promoter, GABP may control Gfi1 expression as GABP-deficient splenocytes lack Gfi1 expression, presumably due to the positively acting GABP binding sites in the Gfi1 promoter (52).

With Gfi1 emerging as a key control factor in IL-7Rα expression and also in many aspects of T and B cell immunology, it is critical to know what controls Gfi1 expression. So far, an autoregulatory role for Gfi1 as well as a transregulatory role for Gfi1b has been documented (53). Also, downstream signaling of the GTPase Cdc42 has been proposed to suppress Gfi1 expression in resting T cells (54). The current study now proposes that glucocorticoids are a new family of molecules that can control Gfi1 expression. Glucocorticoids have been known to take part in thymocyte development and selection (55–58). Recently, conditional deletion of GR in pre-selection thymocytes documented significantly reduced thymus cellularity with an altered TCR repertoire and increased negative selection (59). Mechanistically, glucocorticoids have been shown to intersect with TCR signaling and increase TCR signaling thresholds to promote thymic selection (60). Our current data now also proposes a role for Gfi1 downstream of GR signaling, and it would be informative to assess the contribution of Gfi1 to the GR-deficient phenotype.

In peripheral T cells, glucocorticoids are immunosuppressive, but they also up-regulate IL-7Rα transcription and expression. The identification of Gfi1 as a novel target of Dex sheds new light onto pre-existing observations on the effects of glucocorticoids and in this regard, the discovery of Gfi1 as an intermediary of Dex in IL-7Rα expression suggests that other Dex-induced events might also employ such a mechanism. Although the up-regulation of IL-7Rα expression in Dex-treated B cells, which normally do not express IL-7Rα, could be another case of an Gfi1-mediated Dex effect (34), inhibition of cytokine production in Dex-treated cells also could be explained along this line. Further studies are planned to address these possibilities and to delineate a direct Dex effect *versus* Gfi1-mediated Dex effects.

Although Gfi1 expression is down-regulated by Dex signaling, and overexpression of Gfi1 can overcome Dex-mediated up-regulation of IL-7Rα, we found, as expected, that GR is critical for Dex-mediated IL-7Rα up-regulation. GR binds to a putative *Il7r* enhancer in an evolutionarily conserved sequence 3.5 kb upstream of the transcriptional start site (16). The GR site in this evolutionarily conserved sequence is 50 base pairs away from a FoxO1 transcription factor binding site, which positively regulates gene expression in both naive CD4$^+$ and CD8$^+$ T cells (20, 21). Whether this enhancer, the PU.1/GABP binding promoter, and the putative Gfi1-binding intronic silencer are the only functional control elements in the *Il7r* gene locus is not known. In fact, a series of nuclear factors are known to regulate IL-7Rα expression. In addition to Gfi1, two other transcriptional repressors have been identified that down-regulate *Il7r* gene expression in T cells. The forkhead family transcription factor FoxP1 suppresses IL-7Rα expression by competing with the *Il7r* transactivator FoxO1 for enhancer occupancy (19). On the other hand, FoxP3, which is specifically expressed in regulatory T cells, directly suppress IL-7Rα expression (22). Overexpression of a Gfi1 family member, Gfi1b, can also repress IL-7Rα expression in T lymphocytes, but presumably this effect is through the same intronic Gfi1 binding site, because the DNA binding domains of these two factors are very similar (61). Notably, in our expression profiling experiments, Gfi1 was the only nuclear factor that showed a significant difference in expression upon Dex treatment.

Collectively, the present study identified and tested a novel *Il7r* transcriptional control mechanism *in vivo* using a newly established IL-7Rα reporter mouse. We validated these reporter mice by complementing IL-7Rα deficiency, and we utilized this tool to assess *Il7r* transcription downstream of Gfi1. Using a lymphopenia-induced homeostatic proliferation model, we further documented the superiority of these reporter

mice in assessing IL-7Rα gene transcription in T cells to traditional methods. Finally, the current study not only resolves the controversies surrounding the role of Gfi1 as a transcriptional repressor of IL-7Rα in CD8 T cells but also demonstrates that IL-7Rα expression in any other T cell population is independent of Gfi1. The molecular basis for such lineage-specific regulation of cytokine receptor expression is intriguing and important, and we think that our findings will provide a new venue to identify critical players for controlling IL-7Rα expression.

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