STAT5 does not drive steroid resistance in T-cell acute lymphoblastic leukemia despite the activation of *BCL2* and *BCLXL* following glucocorticoid treatment

Jordy C.G. van der Zwet, Valentina Cordo', Jessica G.C.A.M. Buijs-Gladdines, Rico Hagelaar, Willem K. Smits, Eric Vroegindeweij, Laura T.M. Graus, Vera M. Poort, Marloes Nulle, Rob Pieters and Jules P.P. Meijerink°

Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands

°*Current affiliation: Acerta Pharma, Oss, the Netherlands.*

Correspondence: J.PP. Meijerink
j.meijerink@prinsesmaximacentrum.nl

Received: November 23, 2021.
Accepted: May 30, 2022.
Early view: June 23, 2022.

https://doi.org/10.3324/haematol.2021.280405

Abstract

Physiological and pathogenic interleukin-7-receptor (IL7R)-induced signaling provokes glucocorticoid resistance in a subset of patients with pediatric T-cell acute lymphoblastic leukemia (T-ALL). Activation of downstream STAT5 has been suggested to cause steroid resistance through upregulation of anti-apoptotic *BCL2*, one of its downstream target genes. Here we demonstrate that isolated STAT5 signaling in various T-ALL cell models is insufficient to raise cellular steroid resistance despite upregulation of BCL2 and BCL-XL. Upregulation of anti-apoptotic *BCL2* and *BCLXL* in STAT5-activated T-ALL cells requires steroid-induced activation of NR3C1. For the *BCLXL* locus, this is facilitated by a concerted action of NR3C1 and activated STAT5 molecules at two STAT5 regulatory sites, whereas for the *BCL2* locus this is facilitated by binding of NR3C1 at a STAT5 binding motif. In contrast, STAT5 occupancy at glucocorticoid response elements does not affect the expression of NR3C1 target genes. Strong upregulation of BIM, a NR3C1 pro-apoptotic target gene, upon prednisolone treatment can counterbalance NR3C1/STAT5-induced BCL2 and BCL-XL expression downstream of IL7-induced or pathogenic IL7R signaling. This explains why isolated STAT5 activation does not directly impair the steroid response. Our study suggests that STAT5 activation only contributes to steroid resistance in combination with cellular defects or alternative signaling routes that disable the pro-apoptotic and steroid-induced BIM response.

Introduction

Synthetic steroids remain a vital cornerstone drug in the treatment of pediatric T-cell acute lymphoblastic leukemia (T-ALL). Upon binding with steroids, the glucocorticoid receptor (NR3C1) dimerizes and migrates to the nucleus where it functions as a transcription factor and regulates the expression of multiple steroid-response genes, including the pro-apoptotic gene *BIM*.[1-3] Resistance to steroid treatment predicts for inferior outcome and therefore remains a major clinical problem in T-ALL.[4,5] An important signaling pathway that interferes with steroid sensitivity is the interleukin-7-receptor (IL7R) pathway. Both physiological IL7 signaling or the presence of activating mutations in various IL7R signaling molecules have been linked to steroid resistance in T-ALL patients.[6,7]

Recently, the mechanisms underlying IL7R-dependent survival and/or steroid resistance have been studied. IL7R signaling results in downstream activation of the PI3K-AKT and STAT5 pathways, but also activates MAPK-ERK signaling.[6,8-12] For these individual downstream signaling pathways, different mechanisms have been identified which may contribute to steroid resistance (Figure 1). Activation of the PI3K-AKT pathway is reported to drive phosphorylation of NR3C1, which blocks its migration to the nucleus.[13] Additionally, PI3K-AKT signaling can upregulate various anti-apoptotic proteins including BCLXL and MCL1.[6] AKT can also inhibit transcription of the important glucocorticoid receptor target gene *BIM* via an inhibitory phosphorylation of the FOXO3A transcription factor.[14] Epigenetic silencing of the *BIM* locus, as found in some ALL patient-derived xenograft models, has also been proposed as an important mechanism of resistance to steroids.[15,16] IL7R signaling mutations, found in approximately 35% of pediatric T-ALL patients, or physiological IL7 signaling activate downstream MAPK-ERK signaling.[6,7,12] Activated ERK phosphorylates BIM-L and BIM-EL proteins, which therefore lose their potential to bind and neutralize anti-apoptotic BCL2 protein family members including BCL2, BCL-XL and MCL1, hence resulting in steroid resistance.[12] MEK inhibitors, and to a limited extent the JAK inhibitor ruxolitinib, showed

synergy with steroid treatment by restoring functional BIM levels, thereby representing promising targeted compounds to overcome steroid resistance in T-ALL.[6,12]

Activation of the glucocorticoid receptor NR3C1 facilitates transcriptional upregulation of glucocorticoid target genes, including *BIM* and *IL7R*.[17] Recently, it was shown that by upregulating IL7Rα, steroid treatment could paradoxically induce steroid resistance,[18] since enhanced IL7-induced signaling activates STAT5 and consequentially enhances the expression of the pro-survival gene *BCL2*[18] and the *PIM1* kinase gene.[19] Thus far, upregulation of BCL2 is regarded as the driving mechanism for IL7-induced survival and steroid resistance.[7,17,18,20,21]

Here we explore the significance of STAT5 signaling downstream of physiological or mutant IL7R signaling in relation to steroid resistance in pediatric T-ALL. We studied whether induced expression of the constitutively active N642H STAT5 mutant can activate BCL2 and drive steroid resistance.

Methods

Cell preparation and cytotoxicity assays

An extended description of the generation of SUPT-1 and P12-ICHIKAWA cell lines can be found in the *Online Supplementary Methods*. For quantitative real-time reverse-transcription polymerase chain reaction (RTQ-PCR), immunoblot analysis and cytotoxicity assays, SUPT-1 and CCRF-CEM cells were plated in RPMI-1640 medium as described in the *Online Supplementary Methods*. For activation of IL7R signaling, the medium of the CCRF-CEM cells was supplemented with 10 ng/mL IL7 (R&D systems). For RTQ-PCR and immunoblot analysis experiments, cells were plated at a concentration of $1x10^6$ cells/mL overnight. For cytotoxicity assays, cells were plated at a concentration of $0.2x10^6$ cells/mL, and cell viability was determined after 4 days by a methylthiazolyldiphenyl-tetrazolium bromide (MTT, Sigma Aldrich) assay.

Immunoblot analysis and immunoprecipitation

Cell pellets of treated cell suspensions were lysed using kinase lysis buffer, and protein eluates were loaded on BioRad Mini-Protean® TGX™ any-kd precast gels.[12] Proteins were transferred to 0.2 μm nitrocellulose membranes using the Trans-Blot® Turbo™ Transfer System (BioRad). Primary antibodies used for immunoblot analysis are listed in the *Online Supplementary Methods*. Loading controls were equivalent in experiments in which multiple membranes were used. Immunoprecipitation was performed as previously described,[12] and as

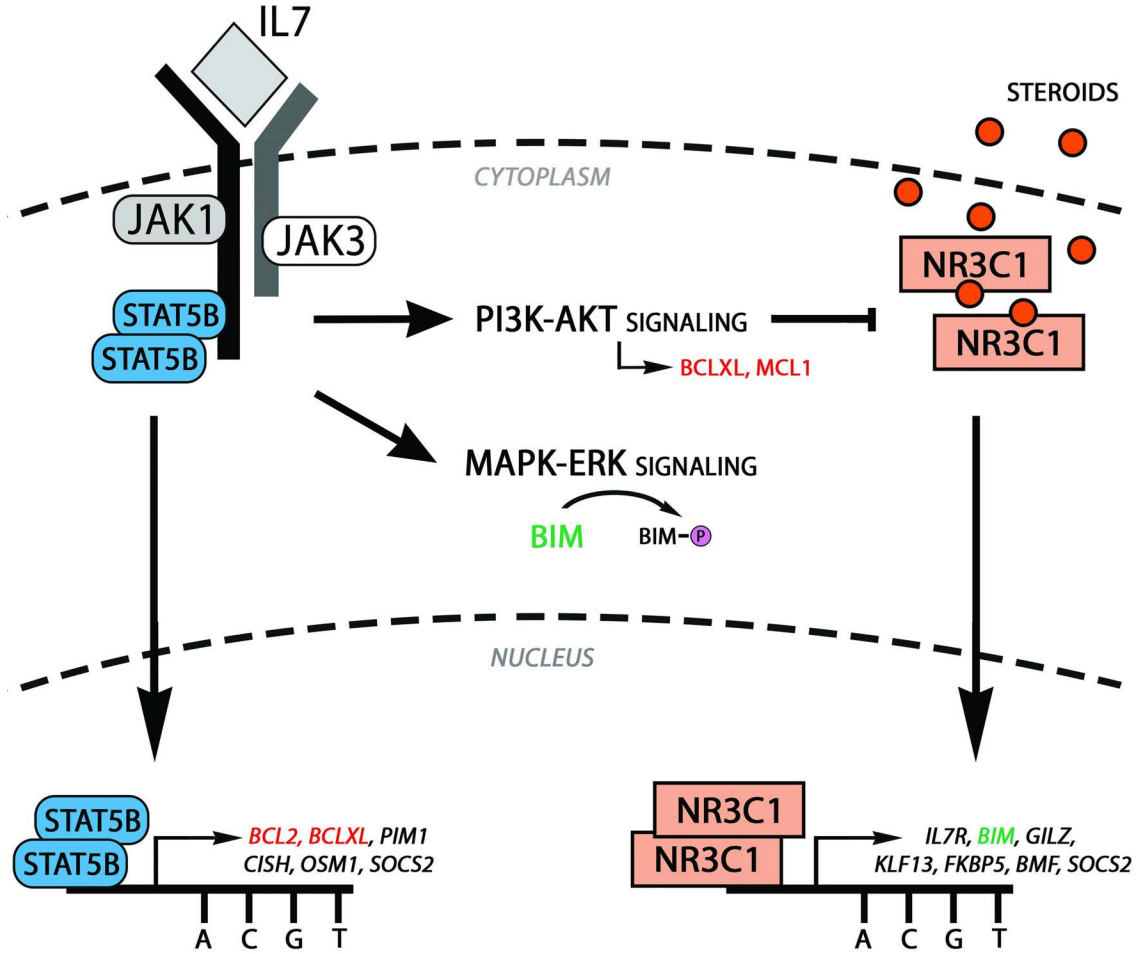


Figure 1. Schematic overview of IL7-receptor- and steroid-induced signaling. In the presence of IL-7, IL7Rα (left) and IL7Rγ (right) subunits heterodimerize, allowing transphosphorylation of JAK1 and JAK3 kinases and phosphorylation of IL7Rα as a docking site for STAT5. As a result, downstream PI3K-AKT, MAPK-ERK and STAT5 signaling pathways are activated. Activated STAT5 migrates to the nucleus as a homodimer to regulate the transcription of canonical STAT5 target genes, including anti-apoptotic *BCL2* and *BCLXL*. Activated PI3K-AKT signaling upregulates anti-apoptotic BCLXL and MCL1 and can regulate the nuclear translocation of the activated glucocorticoid receptor (NR3C1). Activated MAPK-ERK signaling phosphorylates and therefore inactivates pro-apoptotic BIM. Upon exposure to steroids, NR3C1 migrates to the nucleus as a homodimer and induces the expression of NR3C1 target genes (including pro-apoptotic BIM) at glucocorticoid response element sites.

described in more detail in the *Online Supplementary Methods*.

Quantitative real-time reverse-transcription polymerase chain reactions

Isolation of RNA, cDNA synthesis and RTQ-PCR were performed as previously described.[22,23] The expression of NR3C1 or STAT5 target genes was calculated using the delta CT (dCT) method as percentage of GADPH expression. Fold expression change was calculated relative to the non-doxycycline-induced (-dox), non-prednisolone treated (-pred) condition. The primers used are described in the *Online Supplementary Methods*.

Processing and visualization of chromatin immunoprecipitation sequencing data

An extended description of chromatin immunoprecipitation (ChIP)-sequencing can be found in the *Online Supplementary Methods*. DNA eluates were sequenced using the Illumina NextSeq500 platform of the Utrecht Sequence facility. Raw reads were aligned to the CRCh38 human genome, using the Burrows-Wheeler Aligner tool[24] with default settings. Narrowpeak calling with default settings from MACS2[25] was used for peak calling. BamCoverage from deeptools[26] was used to normalize input signal for figures, using the reads per genomic content method. Peaks were visualized using Integrative Genomics Viewer.[27] Bedtools[28] was used to select unique and overlapping peaks for STAT5/NR3C1. These are visualized using the plotheatmap function from deeptools. MEME-ChIP was used to detect motifs within regions of 50 bp up or down of the summit of NR3C1 or STAT5 peak summits (101 bp window), using default settings.[29]

Data availability

Affymetrix U133 Plus2 microarray data for the 117 patients as previously published[30] were normalized using the robust multi-array average, computed by the affy package.[31] Microarray data are available at *http://www.ncbi.nlm.nih.gov/geo/* under accession number GSE26713. Data analysis is described in the *Online Supplementary Methods*. A selection of STAT5 target genes ChIP-sequencing files are available at Gene Expression Omnibus under GEO series accession number: GSE171976, *https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE171976*.

Results

Activation of STAT5 target genes does not predict steroid resistance in T-cell acute lymphoblastic leukemia

To study the significance of STAT5 activation in relation to steroid resistance in pediatric T-ALL, we investigated the expression of STAT5 target genes (e.g., *BCL2*, *BCL2L1* (*BCLXL*), *PIM1*, *CISH*, *OSM1* and *SOCS2*) in our historic microarray dataset of 117 samples from treatment-naïve pediatric T-ALL patients.[30] The leukemia subtype and the IL7R signaling pathway mutational status of these patients, as previously determined,[6,30] are displayed in Figure 2A. Cluster analysis based on the expression of STAT5 target genes separated T-ALL patients into two major clusters, with cluster B representing patients with high expression of STAT5 target genes. In relation to leukemic subtype, cluster B was significantly enriched for *TLX3*-rearranged leukemias (P<0.0001), whereas *TLX1-/NKX2.1*- rearranged patients or *TAL1/2*- and *LMO1/2*-rearranged T-ALL patients were strongly associated with cluster A (P=0.0126 and P<0.0001, respectively) (Figure 2B). Moreover, activating IL7R signaling mutations in *IL7R*, *JAK1*, *JAK3* and/or *STAT5B* genes were also strongly associated with STAT5 transcriptional activity (P<0.0001) (Figure 2C), in line with previous observations that these mutations are also associated with *TLX3*-rearranged patients.[6,30,32] Enrichment of *TLX3*-rearranged leukemia and IL7R signaling mutations in samples that display high expression of these STAT5 target genes was verified by performing similar analysis on RNA-sequencing data available from the St. Jude database (*Online Supplementary Figure S1*).[32] These results highlight the contribution of *IL7R*, *JAK1*, *JAK3* and/or *STAT5B* mutations to the activation of STAT5 in T-ALL. In relation to *ex-vivo* prednisolone cytotoxic response levels, as determined for 84 out of these 117 T-ALL patients' samples, we observed that the prednisolone concentrations lethal to 50% of the T-ALL cells (LC_{50}) were comparable for patients with high or low STAT5 transcriptional activity (Figure 2D). Moreover, no difference in event-free survival was observed between the two groups (log-rank test P=0.24) (Figure 2E). This suggests that the predicted STAT5 activity does not alone predict for sensitivity to steroid treatment at diagnosis, either by not having an immediate impact on steroid sensitivity or due to the presence of alternative resistance mechanisms.

STAT5 activation does not drive steroid resistance in IL7R-mutant cell lines

To further explore the relation between the STAT5-regulated transcriptional program and steroid resistance, we generated SUPT-1 or P12-Ichikawa derivative lines that can be induced to express wild-type IL7Rα ($IL7R^{WT}$) or cysteine-mutant IL7Rα molecules ($IL7R^{PILT240\text{-}244RFCPH}$ or $IL7R^{PIL240\text{-}242QSPSC}$) following exposure to doxycycline. We previously demonstrated that induced expression of cysteine-mutant IL7Rα molecules, in contrast to wild-type IL7Rα, provoked steroid resistance in otherwise steroid-sensitive T-ALL SUPT-1 cells.[12] Overexpression of mutant IL7Rα resulted in STAT5 phosphorylation and thus STAT5

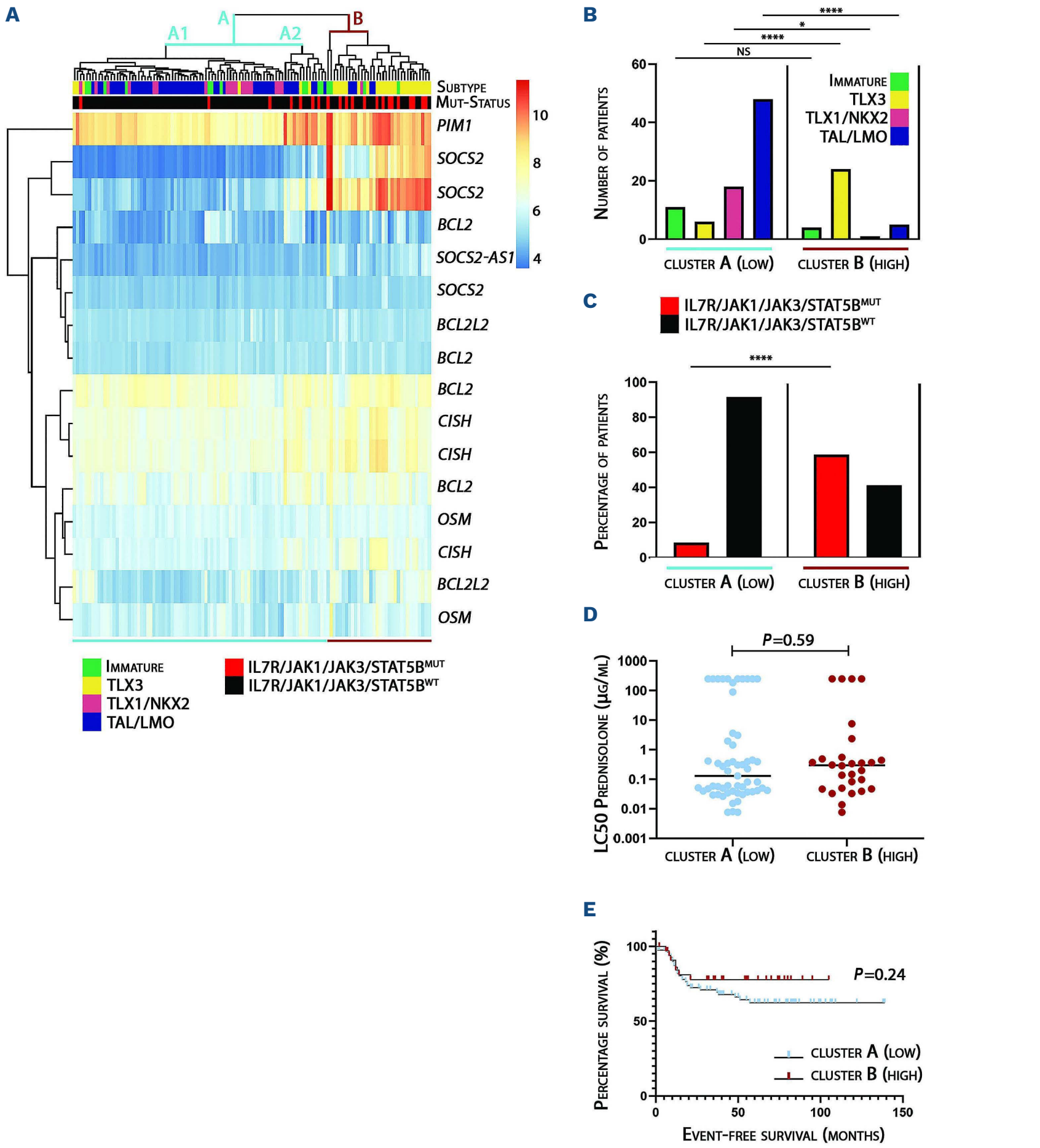


Figure 2. STAT5 transcriptional activity in pediatric patients with T-cell acute lymphoblastic leukemia does not predict steroid resistance. (A) Unsupervised clustering of STAT5 target gene expression of 117 treatment-naïve pediatric patients with T-cell acute lymphoblastic leukemia (T-ALL) using Affymetrix U133 Plus2 microarrays. Genes used for clustering were already described STAT5 target genes *BCL2*, *BCL2L1* (*BCLXL*), *PIM1*, *CISH*, *OSM1* and *SOCS2*. For most of these genes, multiple probes were used. Euclidean distances were used to determine clusters. The leukemic subtype and IL7R-pathway mutational status for these patients had been previously determined. Cluster B represents patients with the highest STAT5 transcriptional activity. The four major T-ALL subtypes (characterized by specific oncogenic rearrangements, clustered as described by Homminga *et al.*[30] are represented in green, yellow, pink and blue. Patients who harbor a mutation in the *IL7R*, *JAK1*, *JAK3* or *STAT5* gene are indicated in red (*versus* no mutations in these genes in black). (B) Representation of the four major leukemic T-ALL subtypes in both STAT5 transcriptional clusters. Statistical differences were calculated by the χ^2 test; *P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001. (C) IL7R/JAK1/JAK3/STAT5B mutation status of primary patients' blasts related to STAT5 transcriptional clusters. Statistical differences were calculated by the χ^2 test; *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. (D) Statistical analysis of *in vitro* prednisolone sensitivity of patients in low *versus* high STAT5 transcriptional clusters (Mann-Whitney-test). (E) Kaplan-Meier analysis of event-free survival between patients in low *versus* high STAT5 transcriptional clusters (long-rank test). LC50: concentrations lethal to 50% of cells.

activation, while no effect was seen upon wild-type IL7Rα overexpression (Figure 3A). Examining the activation of STAT5 target genes, both mutant IL7Rα molecules strongly induced BCL2 and BCL-XL protein expression, but only in the presence of prednisolone. To further validate this concept, we studied the expression of *BCL2* and *BCLXL* and various other STAT5 target genes such as *PIM1* and *CISH* in SUPT-1 cells expressing IL7R^{WT} or a cysteine-mutant IL7R variant. Expression of these STAT5 target genes was also studied in the presence of prednisolone with or without targeted inhibitors of JAK1/2 (ruxolitinib), MEK (selumetinib) and/or AKT (MK2206) (Figure 3B).[6,7,12] Again, steroid treatment of IL7R-mutant overexpressing cells led to a nearly two-fold increase in *BCL2* and *BCLXL* expression (Figure 3C). As expected, the JAK inhibitor ruxolitinib effectively blocked downstream STAT5B, MAPK-ERK and AKT pathways (*Online Supplementary Figure S2*), and also blocked steroid-dependent upregulation of *BCL2*, *BCLXL*, *CISH* and *PIM1* (Figure 3C). Treatment with the MEK inhibitor selumetinib, the AKT inhibitor MK2206, or their combination could not block transcriptional upregulation of these genes. Similar results were obtained in the context of IL7-induced signaling in the T-ALL cell line CCRF-CEM, indicating that this reflects a general mechanism in T-ALL cells (*Online Supplementary Figure S3A, B*).

Unexpectedly, whereas expression of both mutant IL7R isoforms strongly raised steroid resistance, this effect seemed independent of upregulation of pro-survival proteins BCL2 and BCL-XL via STAT5; while the JAK inhibitor ruxolitinib inhibits upregulation of BCL2 and BCL-XL and reverts steroid resistance in this model, both AKT-inhibitor treatment and combined MEK- and AKT- inhibitor treatment also sensitized SUP-T1 cells to prednisolone treatment (Figure 3D) regardless of the STAT5-driven and steroid-induced BCL2 and BCL-XL induction (Figure 3C). Similar findings were observed for T-ALL CCRF-CEM cells, in which these inhibitors reverted IL7-induced steroid resistance independently of the expression levels of BCL2 and BCL-XL via STAT5 (*Online Supplementary Figure S3B,C*). Therefore, we conclude that STAT5 activation and consequent upregulation of BCL2 and BCL-XL do not have a direct negative impact on steroid sensitivity as studied in SUPT-1 cell line models (Figure 2D).

STAT5^{N642H}-induced SUPT1 cells remain steroid sensitive despite enhanced *BCL2* and *BCLXL* levels

To further exclude a direct role of active STAT5 signaling in relation to steroid resistance, we generated doxycycline-inducible SUPT-1 and P12-Ichikawa derivative lines that express wild-type STAT5B (STAT5^{WT}) or the constitutively active mutant isoform STAT5^{N642H} (Figure 4A). This activating and transforming *STAT5B* mutation, found in 4/117 patients of our patient cohort (i.e., 3.4%), solely activates the STAT5-signaling pathway and therefore serves as a STAT5-focused model.[6,33-37] Expression of STAT5B^{N642H,} but not STAT5^{WT}, led to activated STAT5B signaling without affecting the MAPK-ERK or PI3K-AKT signaling pathways (Figure 4A, *Online Supplementary Figure S4A*).[6,33-37] In line with these results, expression of STAT5B^{WT} was ineffective at activating the expression of downstream target genes, while STAT5B^{N642H} induced the expression of canonical STAT5 target genes (Figure 4B, *Online Supplementary Figure S4B*). Moreover, the expression of STAT5 target genes was further boosted in STAT5^{N642H} cells upon steroid treatment (Figure 4B). In addition to enhanced expression of *BCLXL* and other classical STAT5 target genes such as *PIM1*, *CISH* and *OSM1*, *BCL2* expression was greatly dependent on the steroid treatment. Despite upregulation of BCL2 and BCL-XL anti-apoptotic molecules in the presence of active STAT5 signaling (STAT5^{N642H}) and steroid treatment, the cytotoxic steroid response did not change for SUPT-1 or P12-Ichikawa cells (Figure 4C, D). This again demonstrates that STAT5 signaling alone does not provoke steroid resistance despite the upregulation of anti-apoptotic BCL2 and BCL-XL.

NR3C1-STAT5B co-binding enhances the expression of canonical STAT5 target genes

The enhanced STAT5 transcriptional activity during steroid treatment has previously been attributed to steroid-induced transcriptional upregulation of *IL7RA* by NR3C1, which can further enhance STAT5 activation and subsequently the upregulation of *BCL2* in the presence of IL7.[18] As the induction of *BCL2* and *IL7RA* also occurs in parallel in IL7-exposed and inhibitor-treated CCRF-CEM cells (Figure 3B, *Online Supplementary Figure S5A*), an alternative hypothesis could be that NR3C1 and STAT5B act in a single transcriptional complex and co-regulate an identical set of target genes. To explore this possibility, we performed NR3C1 co-immunoprecipitation experiments using SUPT-1 STAT5B^{WT} and STAT5B^{N642H} cells and identified that NR3C1 and STAT5B could indeed physically interact (Figure 5A). This interaction seemed to be independent of the phosphorylation status of STAT5B, as both (unphosphorylated) wild-type STAT5 and (phosphorylated) mutant STAT5B bound NR3C1 to equal extents following exposure to steroids. Moreover, NR3C1 also bound to STAT5 in the non-prednisolone treated condition (*Online Supplementary Figure S5B*). To explore whether (mutant) STAT5 and NR3C1 could bind to the same transcriptional regulatory regions, we performed ChIP-sequencing for NR3C1 and STAT5. While wild-type STAT5 was expressed in a non-phosphorylated, transcriptionally inactive form, we observed that it could still bind to many regulatory sites that were also bound by the phosphorylated and constitutively active STAT5B^{N642H} isoform (Figure 5B). While binding of STAT5B^{WT} or STAT5B^{N642H} to regulatory sites was independent of steroid exposure, NR3C1 only bound to DNA upon

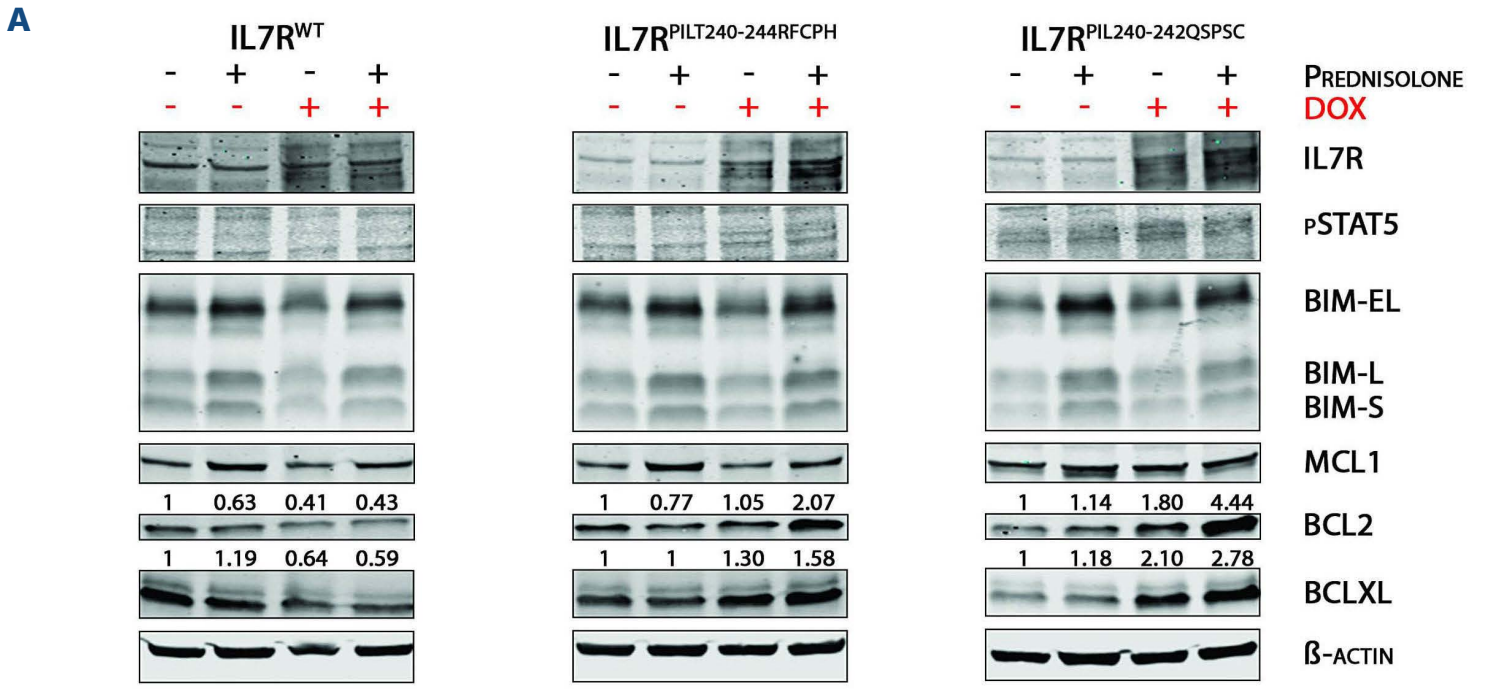


Figure 3. Steroid treatment and inhibition of MAPK-ERK and PI3K-AKT signaling enhance the expression of STAT5 target genes in mutant IL7R cell lines. (A) Ligand-independent STAT5B pathway activation in wild-type and cysteine-mutant IL7R overexpressing SUPT-1 cell lines. Protein band intensity for BCL2 and BCL-XL are represented relative to the condition without doxycycline induction and without prednisolone treatment (-dox-pred). Loading controls were equivalent between different membranes used. (B) Graphic representation of *Online Supplementary Figure S1*: selective inhibition of IL7R^{MUT} activated pathways by ruxolitinib (1 µM, JAK1/2 inhibitor), selumetinib (1 µM, MEK inhibitor) and MK2206 (0.5 µM, AKT inhibitor). (C) Expression of STAT5B target genes in wild-type and mutant IL7R overexpressing SUPT-1 cells. Cells were treated with targeted inhibitors for 30 min before doxycycline induction. Steroid-exposed cells were treated with prednisolone (250 µg/mL) for 16 h. Representative data from two independent experiments with identical conditions. (D) Steroid sensitivity of wild-type and mutant IL7R overexpressing SUPT-1 cells in the absence or presence of targeted inhibitors. Steroid sensitivity was determined by a 4-day MTT read-out. Representative data from a biological duplicate.

prednisolone treatment, which was expected based on its nuclear translocation upon interaction with steroids.[1] We identified genomic regions that were predominantly bound by either STAT5B or NR3C1, denoted as STAT5 or NR3C1 unique binding sites. Interestingly, many genomic regions were bound by both STAT5B and NR3C1 upon steroid exposure, suggesting that STAT5B and NR3C1 can co-regulate a common set of target genes. Motif analysis for NR3C1 and NR3C1/STAT5 binding sites revealed significant enrichment of classical glucocorticoid response element motifs[38] and STAT5 motifs, but not at binding sites that were uniquely bound by STAT5 (Figure 5C, *Online Supplementary Table S1*).

We then specifically studied co-occupancy of STAT5 and

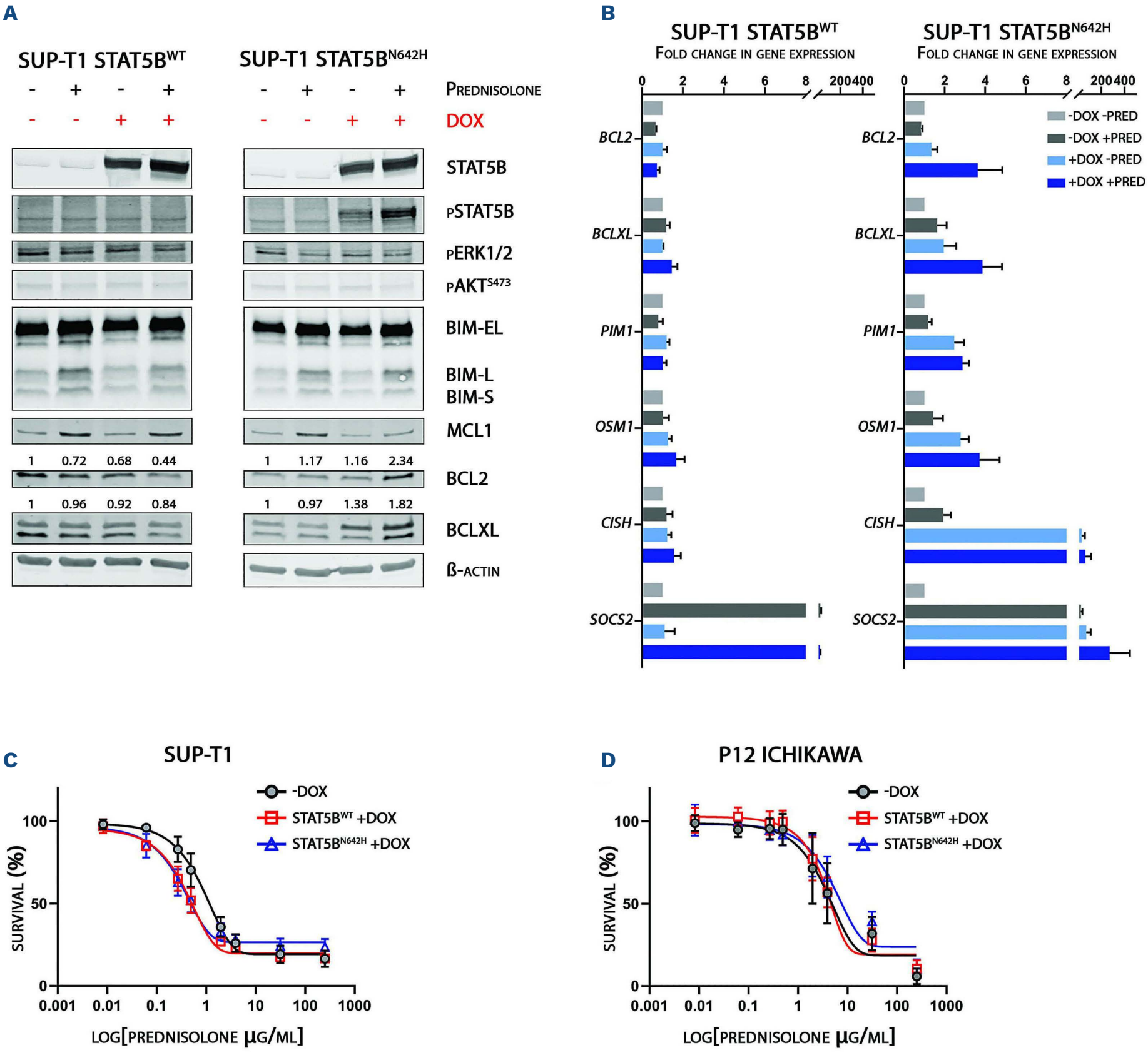
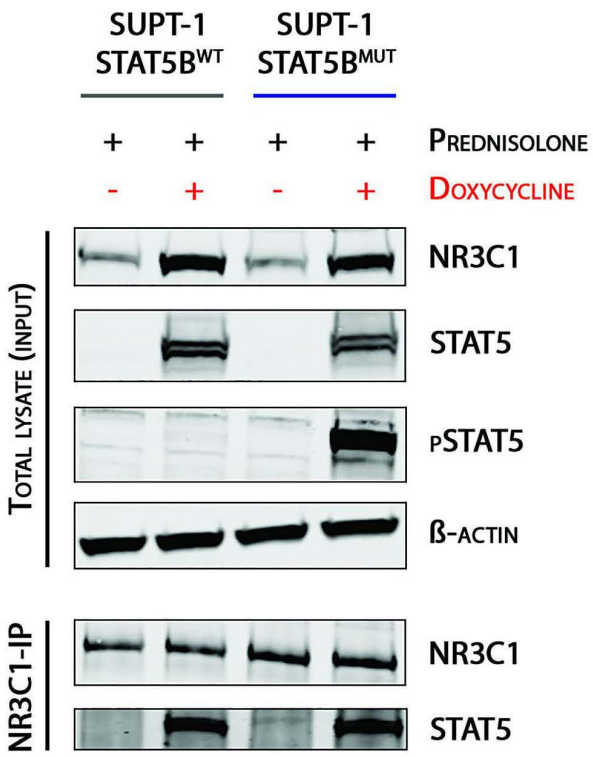
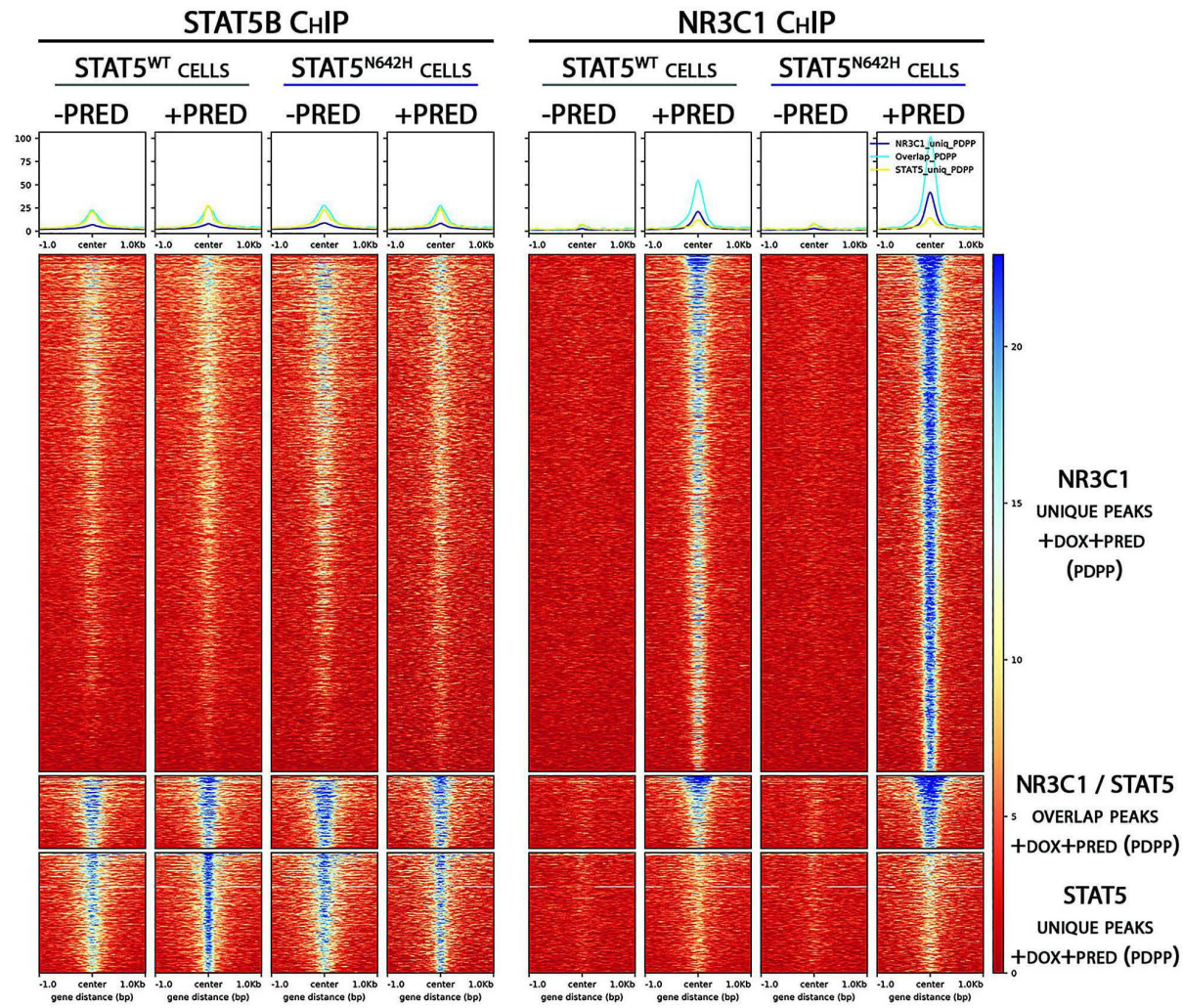


Figure 4. Overexpression of STAT5B^{N642H} does not provoke steroid resistance despite enhanced expression of anti-apoptotic molecules upon steroid treatment. (A) Activation of STAT5B signaling and expression of anti-apoptotic Bcl2 family proteins in STAT5B wild-type and mutant overexpressing SUPT-1 cells. Protein band intensity for BCL2 and BCL-XL represented relative to the condition without doxycycline induction and without prednisolone treatment (-dox-pred). Loading controls were equivalent between different membranes used. (B) Expression of STAT5 target genes in STAT5B^{WT} and STAT5B^{N642H} overexpressing SUPT-1 cells in the absence and presence of prednisolone treatment (16 h, 250 µg/mL). Data from biological triplicates with standard deviation indicated. (C) Steroid sensitivity of wild-type and mutant STAT5B overexpressing SUPT-1 and P12 ICHIKAWA cell lines. Steroid sensitivity was determined by a 4-day MTT read-out. Data from biological triplicates with standard deviation indicated.

A

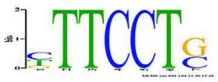


B



C

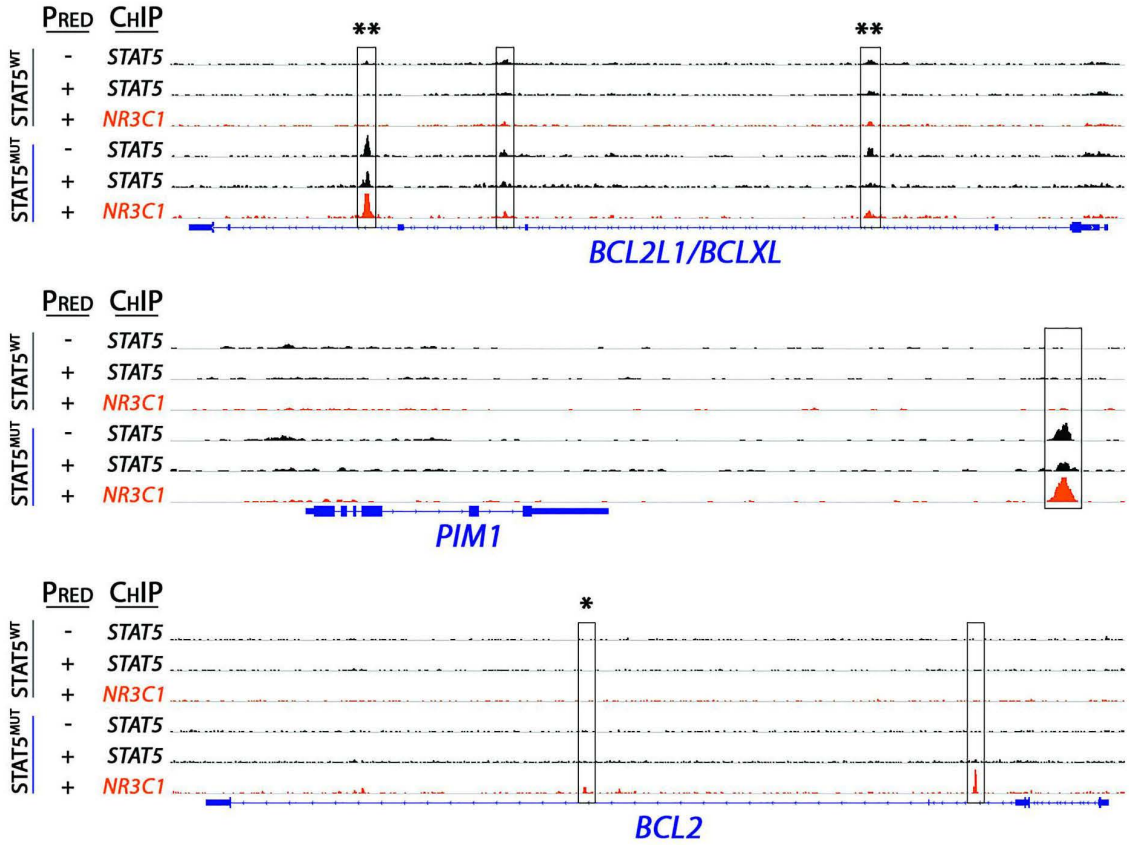
NR3C1 unique peaks (n=2593):

Motif	Sites	E-value	Gene
	283	$6.1E^{-30}$	*STAT5*
	581	$9.1E^{-27}$	*STAT5*
	927	$2.7E^{-401}$	*NR3C1*

NR3C1 / STAT5 overlap peaks (n=365):

Motif	Sites	E-value	Gene
	57	$3.6E^{-27}$	*STAT5*
	29	$7.0E^{-03}$	*STAT5*
	20	$3.8E^{-02}$	*STAT5*
	42	$7.4E^{-35}$	*NR3C1*

D



* = STAT5 motif in peak called in NR3C1 unique set
* = NR3C1 motif in peak called in NR3C1 unique set
** = STAT5 motif in peak called in NR3C1/STAT5 overlap set
** = NR3C1 motif in peak called in NR3C1/STAT5 overlap set

Continued on following page.

Figure 5. NR3C1 co-regulates the expression of transcriptional STAT5 target genes by co-binding at STAT5-regulated genes. (A) Immunoblot analysis of NR3C1-immunoprecipitation in STAT5B^{WT} and STAT5B^{N642H} SUPT-1 cells. Steroid-exposed cells were treated with prednisolone (250 μg/mL) for 16 h. Loading controls were equivalent between different membranes used. (B) Reads per genomic content-normalized centered heatmap of unique and overlapping NR3C1 and STAT5 chromatin-immunoprecipitation (ChIP) sequencing binding peaks of doxycycline-induced STAT5B^{WT} and STAT5B^{N642H} SUPT-1 cells with (+dox+pred) or without (+dox-pred) prednisolone treatment (16 h, 250 μg/mL). Blue represents more reads, indicating confirmed binding of protein at the genome. (C) MEME-ChIP motif analysis of NR3C1 and STAT5 motifs significantly enriched in NR3C1/STAT5 unique or overlapping peak sets. (D) ChIP sequencing identified binding of NR3C1 and STAT5 transcription factors at the STAT5 target genes *BCL2*, *BCLXL* and *PIM1*.

NR3C1 at the regulatory sites of selected STAT5 target genes (Figure 5D, *Online Supplementary Figure S5C*). For *BCLXL* and *PIM1*, we identified STAT5 regulatory sites that were bound by STAT5B^{N642H} but not by wild-type STAT5B, indicating that binding of STAT5 at these regulatory sites is dependent on STAT5 phosphorylation. Interestingly, we found that NR3C1 could also bind to these sites upon steroid exposure in mutant STAT5 cells. For the *BCL2* locus, two NR3C1 peaks were identified and bound by NR3C1 only in STAT5^{N642H} cells, with one site harboring a STAT5 binding motif. Interestingly, no clear STAT5 binding was observed at the *BCL2* locus, suggesting that STAT5 might be unable to independently regulate *BCL2* expression in the absence of steroid treatment or nuclear NR3C1. This is also reflected by the lack of *BCL2* expression in STAT5^{N642H}-overexpressing cells in the absence of steroid treatment (Figure 4B, *Online Supplementary Figure S3B*). We did not identify glucocorticoid response elements at any of the NR3C1-bound sites in *BCLXL*, *PIM* or *BCL2*, suggesting that NR3C1 may be recruited to these binding sites by binding to STAT5B rather than physical binding to DNA. Combined, these data revealed that NR3C1 can interact with STAT5B and bind in or near STAT5-bound genomic sites, even in the absence of a conserved glucocorticoid response element binding motif. Binding of STAT5 to various 'canonical STAT5 target genes' seems dependent on its active (phosphorylated) form, which enables recruitment of NR3C1 to these sites following steroid exposure to further boost the expression of these target genes.

In a reciprocal manner, ChIP-sequencing analysis identified STAT5 binding near NR3C1-binding sites that lack conserved STAT5 binding motifs. Examination of various NR3C1 target genes including *BIM*, *KLF13*, *FKBP5* and *GILZ* and the newly proposed NR3C1 target genes *BMF* and *MCL1* revealed various NR3C1 binding sites that harbor conserved glucocorticoid response element sequences in *BIM*, *KLF13* and *FKBP5* (Figure 6A, B, *Online Supplementary Figure S6A, B*). Remarkably, binding of STAT5^{WT} and STAT5^{N624H} was observed at glucocorticoid response element sites of *KLF13* and *FKBP5* that were not flanked by conserved STAT5 binding sequences. This suggests that STAT5 molecules can be recruited to these sites by (direct) interaction with NR3C1, independently of their activation (phosphorylation) state. The significance of STAT5 binding at these sites remains unknown, as the expression of NR3C1 target genes, including *BIM*, following steroid exposure remained unaffected upon co-incubation with inhibitors of JAK1/2, MEK, or AKT or of both MEK and AKT (Figure 6C).

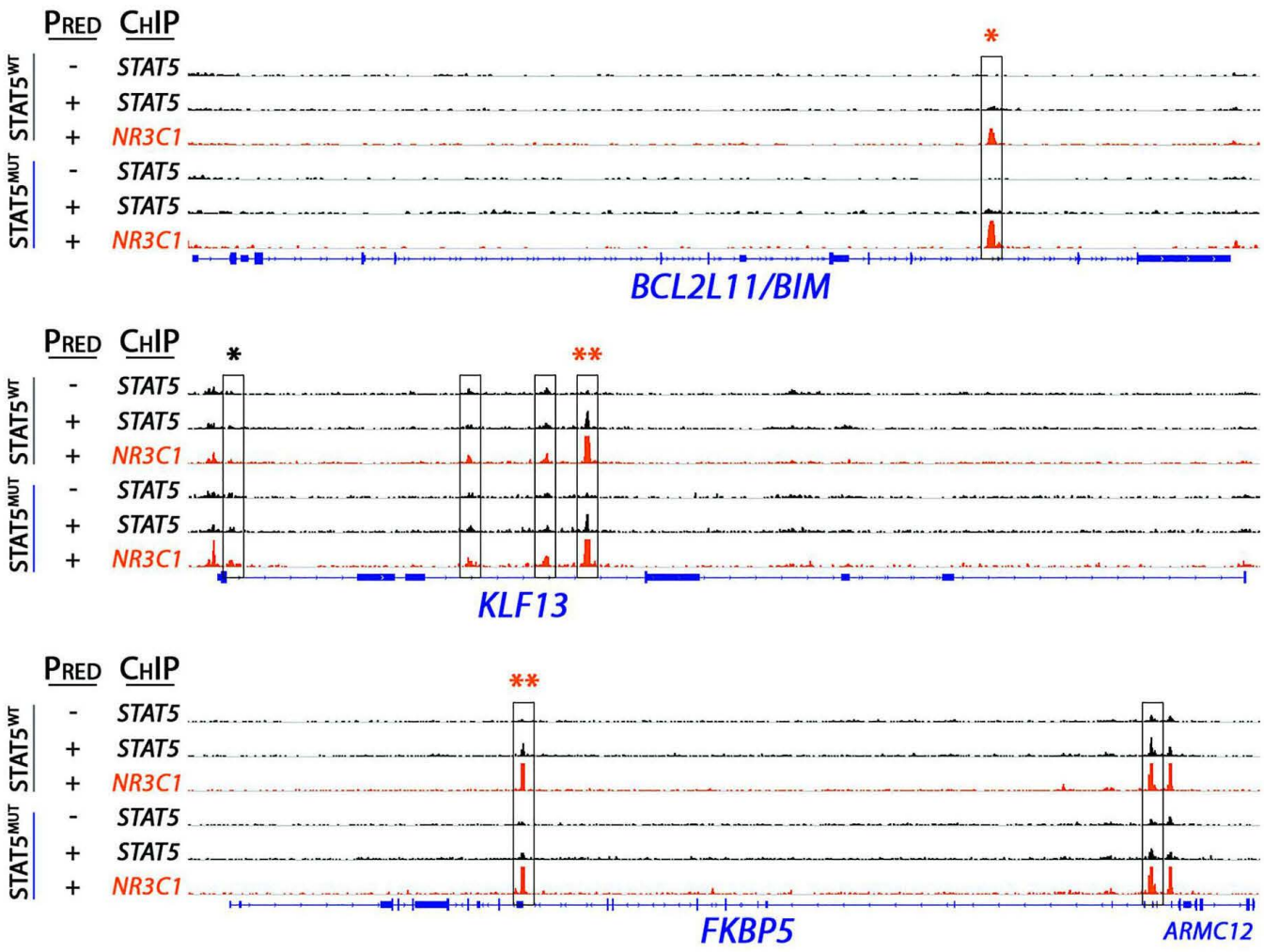
NR3C1-induced BIM binds to enhanced BCL2 and BCL-XL protein in STAT5B^{N642H} cells

We then explored whether the pro-apoptotic steroid response (upregulation of pro-apoptotic BIM via NR3C1) could effectively counter-balance the strong upregulation of anti-apoptotic BCL2 and BCL-XL molecules downstream of activated STAT5 during steroid treatment. To do this, we performed BIM-immunoprecipitation experiments in non-induced or doxycycline-induced STAT5B^{N642H} SUPT-1 cells that were exposed to prednisolone treatment. As shown in Figure 7, steroid treatment led to increased expression of BIM in its active and unphosphorylated form (total lysate, lanes 2 and 4). In line with previous results,[12] active BIM strongly bound to BCL2, BCL-XL and MCL1 (immunoprecipitation, lane 2). Steroid treatment again enhanced the expression of BCL2 and BCL-XL in doxycycline-induced STAT5B^{N642H} cells (total lysate, lane 4). However, immunoprecipitated BIM could effectively bind the upregulated BCL2 and BCL-XL, despite their increased expression (immunoprecipitation, lane 4). Since STAT5^{N642H} overexpression in SUPT-1 cells does not confer steroid resistance (Figure 4C, D), BIM seems to counter the enhanced STAT5/NR3C1 driven anti-apoptotic induction of BCL2 and BCL-XL and therefore preserves a sensitive steroid response. Thus, our study highlights that steroid sensitivity is not solely defined by the upregulation of anti-apoptotic proteins, but is regulated by a tight balance between anti- and pro-apoptotic molecules.

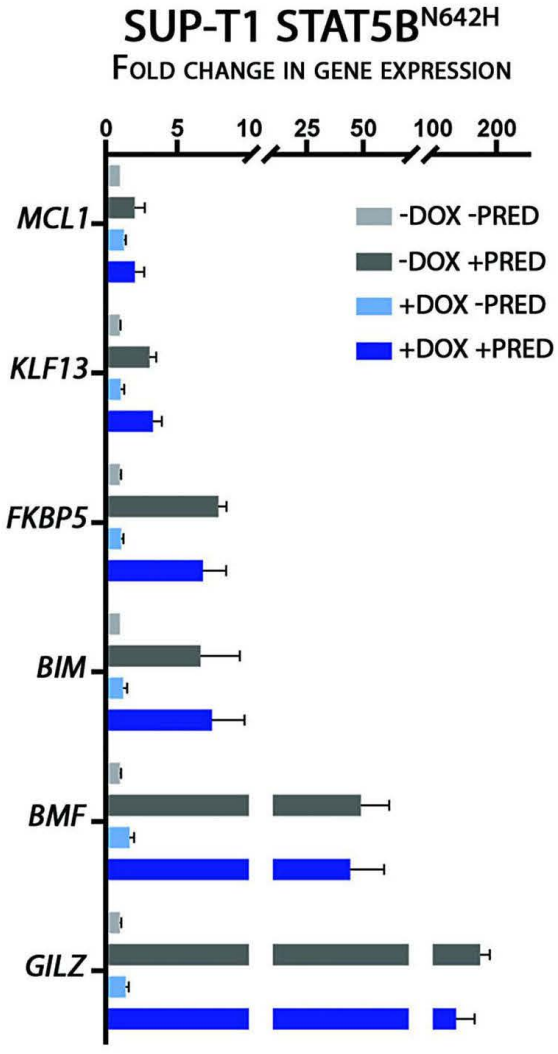
Discussion

Resistance to synthetic steroids remains a problem in the treatment of pediatric ALL. Aberrant activation of the IL7R signaling cascade is frequently observed in T-ALL and is due either to production of IL7 by stromal cells in the leukemia microenvironment[7] or to activating mutations in the IL7R signaling pathway.[6,12] Activation of the IL7R pathway has been strongly related to steroid resistance via various pro-survival mechanisms downstream of the PI3K-

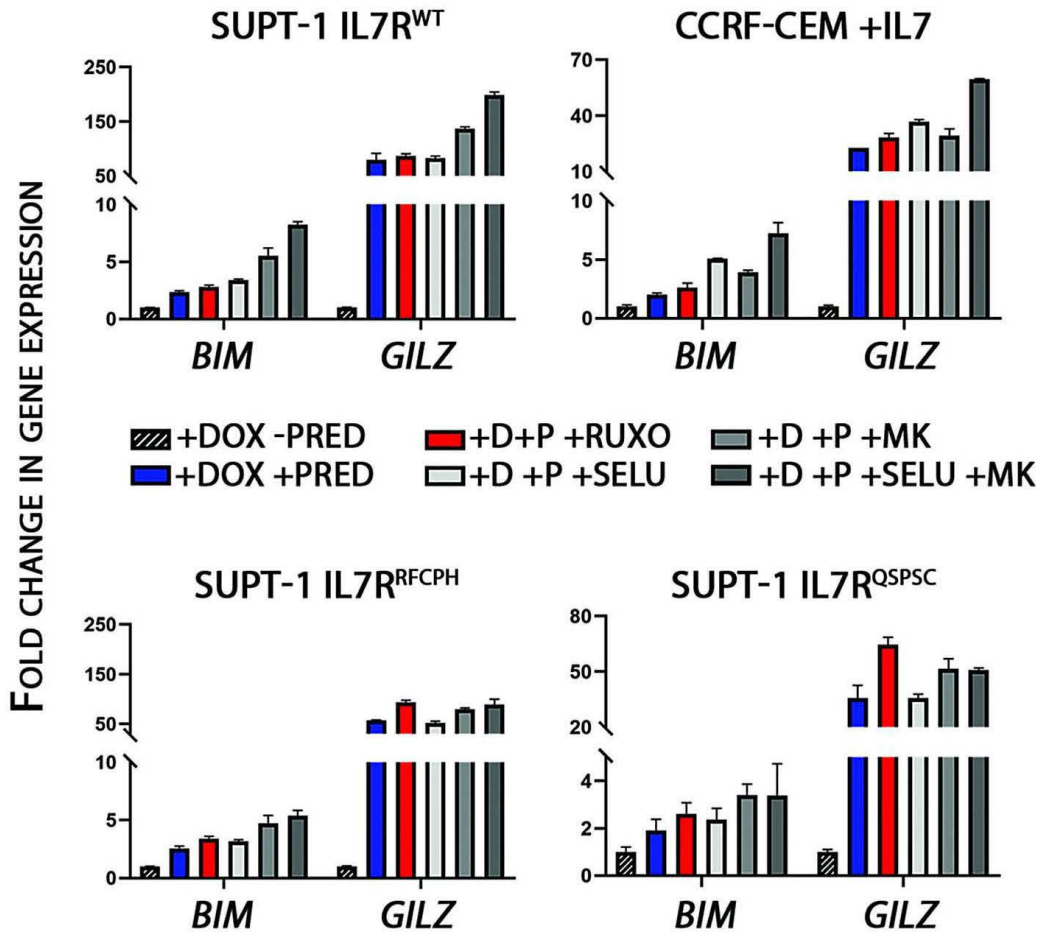
A



B



C



Continued on following page.

Figure 6. Intact NR3C1 transcriptional activity outbalances steroid-dependent enhanced expression of anti-apoptotic molecules. (A) Chromatin immunoprecipitation (ChIP) sequencing identified binding of NR3C1 and STAT5 transcription factors at glucocorticoid response elements of the NR3C1 target genes *BIM*, *FKBP5* and *KLF13*. (B) Gene expression of NR3C1 target genes (*MCL1*, *KLF13*, *FKBP5*, *BIM*, *BMF* and *GILZ*) in mutant STAT5 overexpressing SUPT-1 cells in the absence or presence of overnight steroid treatment (250 μg/mL). Data from biological triplicate with standard deviations indicated. (C) Gene expression of NR3C1 target genes (*BIM* and *GILZ*) in wild-type and mutant IL7R overexpressing SUPT-1 cells in the absence and presence of targeted inhibitors. Cells were treated with targeted inhibitors 30 min before doxycycline-induction. Steroid-exposed cells were treated with prednisolone (250 μg/mL) for 16 h. Representative data from biological duplicates.

AKT, JAK-STAT and MAPK-ERK signaling pathways. Understanding these mechanisms of resistance is of utmost importance, as it may reveal new therapeutic strategies to revert steroid resistance using targeted agents.

In the last decade, various steroid resistance mechanisms that are linked to the IL7R pathway have been uncovered. Interestingly, many of these mechanisms involve the inactivation or activation of pro-apoptotic (e.g., BIM, BMF) and anti-apoptotic Bcl-2 family members (e.g., BCL2, BCL-XL, MCL1), respectively. In healthy lymphocytes, steroid-induced upregulation of BIM is sufficient to neutralize anti-apoptotic Bcl-2 family members, to antagonize their function and to effectuate cellular apoptosis during early T-cell selection processes.[39,40] Overexpression of BCL2 in healthy thymocytes has been demonstrated to outbalance pro-apoptotic BIM, resulting in the abnormal survival and resistance of cells to steroid-induced cell death.[41-44] Similarly, upregulation of *BCL2* downstream of STAT5 has been proposed to drive IL7-induced steroid resistance in T-ALL patients' samples.[7,18] Here we demonstrate that upregulation of *BCL2* and *BCLXL* by STAT5 is not sufficient to induce steroid resistance in various T-ALL cellular models. Previously, we demonstrated that aberrant activation of the MEK-ERK signaling pathway is one of the major drivers of steroid resistance in IL7R-, JAK1- and RAS-mutant cells, since activated ERK phosphorylates and inactivates pro-apoptotic BIM.[12] MEK inhibitors (and to a lesser extent the JAK inhibitor ruxolitinib) can re-sensitize IL7-induced or IL7R signaling mutant T-ALL patients' cells to steroid treatment. Other studies also revealed the importance of the pro-apoptotic BIM, since epigenetic silencing of *BIM* also results in steroid resistance in T-ALL.[15,16]

Our current study suggests that the anti-apoptotic response of activated STAT5 signaling by itself is insufficient to drive steroid resistance. In fact, combined MEK and AKT inhibition in mutant-IL7R T-ALL cell models resensitized these cells to steroid treatment, despite elevated *BCL2* and *BCLXL* expression levels by activated STAT5 in steroid-treated conditions. The NR3C1-induced expression of BIM seems sufficient to counteract the increased expression of BCL2 and BCL-XL that is caused by steroid/STAT5, through direct binding. Activation of STAT5 may contribute to steroid resistance when the steroid-induced pro-apoptotic BIM response is disabled by (epi-)genetic changes or by MAPK-ERK and PI3K-AKT signaling events.[12-16] This is also supported by our observation that active STAT5 signaling, as measured by the expression of various downstream target genes in primary T-ALL patients' cells, is not associated with steroid resistance in T-ALL patients.

Interestingly, we did not observe clear STAT5 binding at the *BCL2* locus, despite this harboring a STAT5-binding motif. We demonstrate that this site is bound by NR3C1 upon steroid treatment, only in STAT5-activated cells, resulting in high BCL2 expression. Our results are in line with those of a previous study,[20] suggesting that STAT5 itself is unable to regulate the expression of *BCL2* despite the presence of a STAT5-binding site. Combined with our observation that *BCL2* is induced in steroid-treated $STAT5^{N642H}$ cells, we suggest that nuclear NR3C1 is vital for *BCL2* expression in STAT5-activated cells. Our study reveals that expression of *BCL2* and the enhanced expression other STAT5-regulated genes following exposure to steroids reflect a common mechanism in different STAT5-activated T-ALL models. Whereas regulatory sites of many genes can be bound by both inactive (non-phosphorylated) $STAT5^{WT}$ and constitutively active (phosphorylated) $STAT5^{N642H}$, various canonical STAT5 target genes were only bound by active STAT5. Remarkably, NR3C1 is also recruited to these sites upon steroid treatment, which points to important direct co-regulation of NR3C1 in the induction of STAT5 target genes. Although we only analyzed a limited set of genes in more detail, we observed that NR3C1 can be recruited to these sites in the absence of glucocorticoid response element sequences, confirming that NR3C1 can act as a transcriptional co-factor. In combination with our immunoprecipitation results, we suggest that this co-occupancy is caused by direct binding between NR3C1 and STAT5, rather than genomic binding of NR3C1 and STAT5 at overlapping sites. In line with this, we also observed that STAT5 can be recruited to NR3C1-bound target genes irrespective of its activation state. Various of these sites did not contain STAT5-binding motifs, again implying that STAT5 binds directly to NR3C1 rather than binding directly to specific DNA sequences. In contrast to the expression of STAT5 target genes, binding of STAT5 at NR3C1-bound target genes did not further enhance the relative expression of NR3C1 downstream target genes, and the expression of these genes also remained unaffected by ruxolitinib treatment. Matched transcriptome studies are required to determine whether the interaction between NR3C1 and STAT5 can regulate the expression of specific genes, or whether it causes more general aberrant transcriptional activity. Additional research is also required to

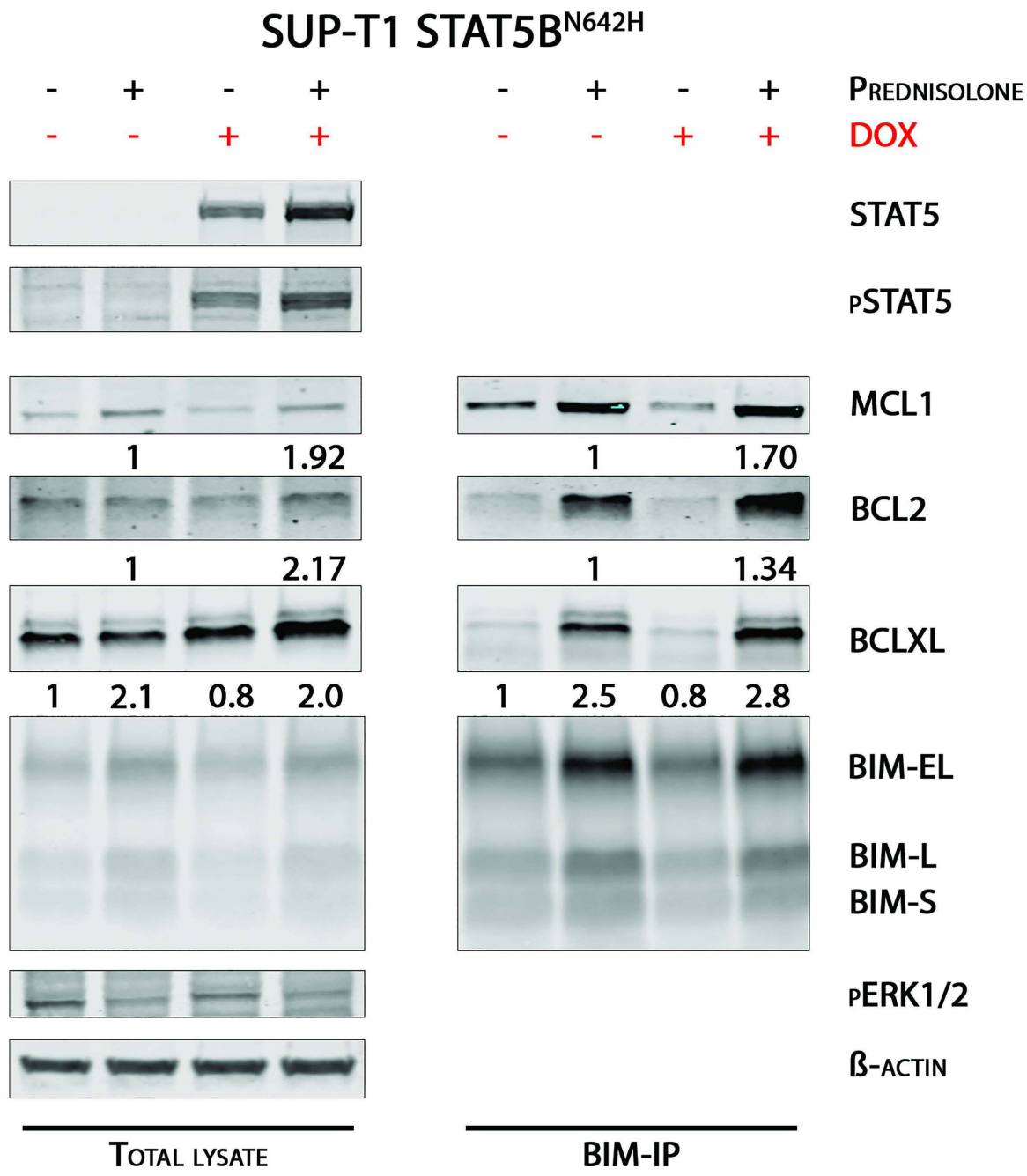


Figure 7. Steroid-induced BIM in STAT5-activated cells binds the enhanced expression of BCL2 and BCL-XL. Immunoblot analysis of BIM-immunoprecipitation in STAT5B^{N642H} SUPT-1 cells (left: total lysate, right: BIM-immunoprecipitation). Steroid-exposed cells were treated with prednisolone (250 μg/mL) for 16 h. The protein abundancy of BCL2, BCL-XL and BIM was quantified, and the expression in cells without doxycycline induction that were treated with prednisolone was taken as the reference. Loading controls were equivalent between different membranes used.

study the exact mechanism by which NR3C1 and STAT5 interact at these genomic regions, and in what ways this physical interaction depends on Tyr694 phosphorylation or a non-dimer form of STAT5.

Co-binding of STAT5 with NR3C1 has previously been reported to occur during glucocorticoid-induced signaling in T cells, mammary and hepatocyte epithelial cells.[45-48] Apart from acting as a transcription factor, STAT5 is known to regulate chromatin accessibility of the TCRγ-locus or immunoglobulin heavy-chain,[49,50] and induces epigenetic changes at EZH2- and SUZ12-binding sites in STAT5B^{N642H} mutated cells.[37] Therefore, STAT5-dependent chromatin remodeling might render (certain) gene sites accessible for NR3C1. This would give an alternative explanation why certain STAT5-target gene sites are only bound by NR3C1 in SUPT-1 cells that overexpress the constitutively active mutant STAT5 molecule.

Our results warrant more detailed research into the mechanisms of STAT5 and NR3C1 cooperation in STAT5-induced and NR3C1/steroid-induced signaling. The complexity of STAT5-regulated transcription is exemplified by the interaction of STAT5 with the transcription factor TLX1 in T-ALL cases that harbor a NUP214-ABL1 fusion. Co-binding of these transcription factors drives the expression of *BCL2* and *MYC* in these leukemias.[51] STAT5 therefore seems to act as a versatile transcription factor that can interact with various other transcription factors to promote the transcription of STAT5-regulated genes. In fact, TLX1 and STAT5 are predominantly found at the same enhancer sites in *NUP214-ABL1*-positive leukemias, and BET protein inhibitors diminish the expression of *BCL2* and *MYC*. Moreover, deacetylase inhibitors can also inhibit STAT5-mediated transcription by relocating BET proteins.[52] These findings and our data highlight the

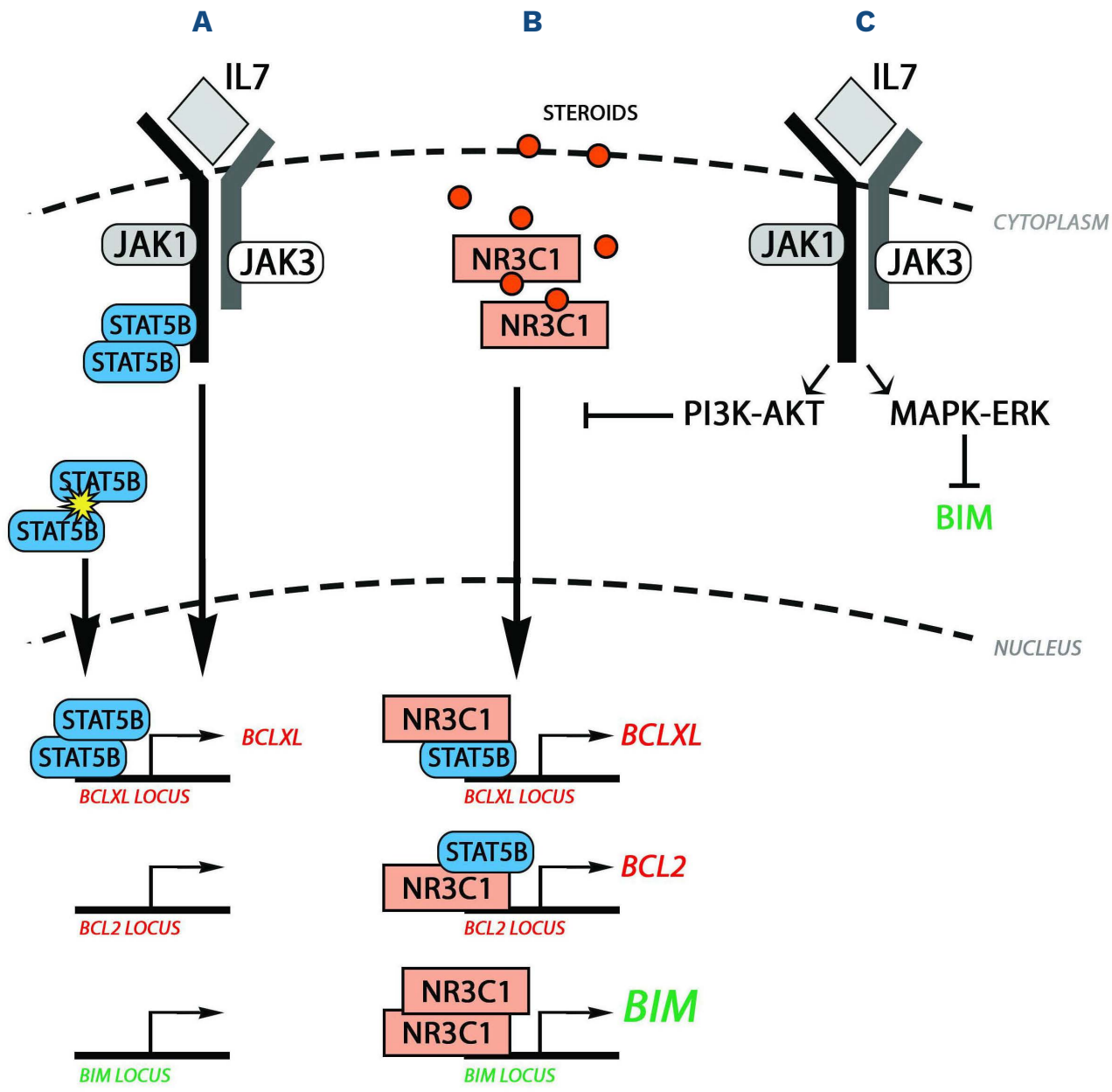


Figure 8. Model of STAT5/NR3C1-regulated transcription and steroid responsiveness. (A) Sole STAT5 activation leads to the transcription of STAT5-target genes (such as *BCLXL*, but also *PIM1*, *OSM1* and *CISH*). (B) In the presence of steroid treatment, NR3C1 functions as a transcriptional factor to induce the expression of NR3C1-target genes (such as *BIM*) at glucocorticoid response elements. In the presence of active STAT5 signaling, NR3C1 also functions as a co-factor at STAT5 transcriptional binding sites (such as *BCLXL*). Moreover, NR3C1 facilitates the expression of *BCL2* by binding at the *BCL2* locus in the presence of active STAT5 signaling. Our work suggests that NR3C1-induced BIM expression is sufficient to neutralize enhanced (STAT5/NR3C1-regulated) BCL-XL and BCL2 expression, therefore securing a steroid-sensitive phenotype. (C) In the presence of BIM-inactivating events by active PI3K-AKT and/or MAPK-ERK signaling (in the context of active IL7R-signaling), BIM abundancy or function is diminished. As a result, BIM is unable to neutralize enhanced BCL-XL and BCL2 expression, thus resulting in steroid resistance.

plasticity of STAT5 as a transcription factor, and its ability to induce local or broad epigenetic changes in leukemia.

In conclusion, we identified that NR3C1 can directly co-regulate the expression of STAT5 target genes including *BCL2* and *BCLXL*, without influencing the steroid response. We demonstrated that NR3C1/STAT5-regulated expression of *BCL2* and *BCLXL* can be counterbalanced by the upregulation of the pro-apoptotic and steroid response gene *BIM* (Figure 8). Therefore, in the absence of other BIM-inactivating mechanisms by aberrant IL7 signaling, STAT5 activation itself seems insufficient to provoke steroid resistance in T-ALL. Multi-omic screening of primary patient material, before and after induction therapy, is required to extrapolate our findings to patients.

Disclosures

No conflicts of interest to disclose.

Contributions

JvdZ designed the study, performed research, and wrote the manuscript. JBG, RH, WKS, EV, LG, MN, and VP performed research. RP and VC provided critical input and wrote the manuscript. JM designed and supervised the study and wrote the manuscript.

Acknowledgments

This study was sponsored by grants from the "Kinderen Kankervrij" foundation; KiKa-219 (to JvdZ), KiKa-92 and KiKa-295 (to WKS), KiKa-335 (to VP), KiKa-244 (to EV) and KWF-2016-10335 (to VC).

Data-sharing statement

The data that support the findings of this study are openly available at http://www.ncbi.nlm.nih.gov/geo/ under accession number GSE26713 (microarray data) and at Gene Expression Omnibus under GEO series accession number: GSE171976, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE171976 (ChIPseq files).

References

1. Weikum ER, Knuesel MT, Ortlund EA, Yamamoto KR. Glucocorticoid receptor control of transcription: precision and plasticity via allostery. Nat Rev Mol Cell Biol. 2017;18(3):159-174.
2. Wang Z, Malone MH, He H, McColl KS, Distelhorst CW. Microarray analysis uncovers the induction of the proapoptotic BH3-only protein Bim in multiple models of glucocorticoid-induced apoptosis. J Biol Chem. 2003;278(26):23861-23867.
3. Jing D, Bhadri VA, Beck D, et al. Opposing regulation of BIM and BCL2 controls glucocorticoid-induced apoptosis of pediatric acute lymphoblastic leukemia cells. Blood. 2015;125(2):273-283.
4. Lauten M, Moricke A, Beier R, et al. Prediction of outcome by early bone marrow response in childhood acute lymphoblastic leukemia treated in the ALL-BFM 95 trial: differential effects in precursor B-cell and T-cell leukemia. Haematologica. 2012;97(7):1048-1056.
5. Kaspers GJ, Pieters R, Van Zantwijk CH, Van Wering ER, Van Der Does-Van Den Berg A, Veerman AJ. Prednisolone resistance in childhood acute lymphoblastic leukemia: vitro-vivo correlations and cross-resistance to other drugs. Blood. 1998;92(1):259-266.
6. Li Y, Buijs-Gladdines JG, Cante-Barrett K, et al. IL-7 receptor mutations and steroid resistance in pediatric T cell acute lymphoblastic leukemia: a genome sequencing study. PLoS Med. 2016;13(12):e1002200.
7. Delgado-Martin C, Meyer LK, Huang BJ, et al. JAK/STAT pathway inhibition overcomes IL7-induced glucocorticoid resistance in a subset of human T-cell acute lymphoblastic leukemias. Leukemia. 2017;31(12):2568-2576.
8. Oliveira ML, Akkapeddi P, Ribeiro D, Melao A, Barata JT. IL-7R-mediated signaling in T-cell acute lymphoblastic leukemia: an update. Adv Biol Regul. 2019;71:88-96.
9. Barata JT, Silva A, Brandao JG, Nadler LM, Cardoso AA, Boussiotis VA. Activation of PI3K is indispensable for interleukin 7-mediated viability, proliferation, glucose use, and growth of T cell acute lymphoblastic leukemia cells. J Exp Med. 2004;200(5):659-669.
10. Zenatti PP, Ribeiro D, Li W, et al. Oncogenic IL7R gain-of-function mutations in childhood T-cell acute lymphoblastic leukemia. Nat Genet. 2011;43(10):932-939.
11. Cante-Barrett K, Spijkers-Hagelstein JA, Buijs-Gladdines JG, et al. MEK and PI3K-AKT inhibitors synergistically block activated IL7 receptor signaling in T-cell acute lymphoblastic leukemia. Leukemia. 2016;30(9):1832-1843.
12. van der Zwet JCG, Buijs-Gladdines J, Cordo V, et al. MAPK-ERK is a central pathway in T-cell acute lymphoblastic leukemia that drives steroid resistance. Leukemia. 2021;35(12):3394-3405.
13. Piovan E, Yu J, Tosello V, et al. Direct reversal of glucocorticoid resistance by AKT inhibition in acute lymphoblastic leukemia. Cancer Cell. 2013;24(6):766-776.
14. Xie M, Yang A, Ma J, et al. Akt2 mediates glucocorticoid resistance in lymphoid malignancies through FoxO3a/Bim axis and serves as a direct target for resistance reversal. Cell Death Dis. 2019;9(10):1013.
15. Bachmann PS, Gorman R, Papa RA, et al. Divergent mechanisms of glucocorticoid resistance in experimental models of pediatric acute lymphoblastic leukemia. Cancer Res. 2007;67(9):4482-4490.
16. Bachmann PS, Piazza RG, Janes ME, et al. Epigenetic silencing of BIM in glucocorticoid poor-responsive pediatric acute lymphoblastic leukemia, and its reversal by histone deacetylase inhibition. Blood. 2010;116(16):3013-3022.
17. Franchimont D, Galon J, Vacchio MS, et al. Positive effects of glucocorticoids on T cell function by up-regulation of IL-7 receptor alpha. J Immunol. 2002;168(5):2212-2218.
18. Meyer LK, Huang BJ, Delgado-Martin C, et al. Glucocorticoids paradoxically facilitate steroid resistance in T cell acute lymphoblastic leukemias and thymocytes. J Clin Invest. 2020;130(2):863-876.
19. De Smedt R, Morscio J, Reunes L, et al. Targeting cytokine- and therapy-induced PIM1 activation in preclinical models of T-cell acute lymphoblastic leukemia and lymphoma. Blood. 2020;135(19):1685-1695.
20. Ribeiro D, Melao A, van Boxtel R, et al. STAT5 is essential for IL-7-mediated viability, growth, and proliferation of T-cell acute lymphoblastic leukemia cells. Blood Adv. 2018;2(17):2199-2213.
21. Maude SL, Dolai S, Delgado-Martin C, et al. Efficacy of JAK/STAT pathway inhibition in murine xenograft models of early T-cell precursor (ETP) acute lymphoblastic leukemia. Blood. 2015;125(11):1759-1767.
22. Meijerink J, Mandigers C, van de Locht L, Tonnissen E, Goodsaid F, Raemaekers J. A novel method to compensate for different amplification efficiencies between patient DNA samples in quantitative real-time PCR. J Mol Diagn. 2001;3(2):55-61.
23. Cante-Barrett K, Mendes RD, Smits WK, van Helsdingen-van Wijk YM, Pieters R, Meijerink JP. Lentiviral gene transfer into human and murine hematopoietic stem cells: size matters. BMC Res Notes. 2016;9:312.
24. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25(14):1754-1760.
25. Zhang Y, Liu T, Meyer CA, et al. Model-based analysis of ChIP-Seq (MACS). Genome Biol. 2008;9(9):R137.
26. Ramirez F, Ryan DP, Gruning B, et al. deepTools2: a next generation web server for deep-sequencing data analysis. Nucleic Acids Res. 2016;44(W1):W160-165.
27. Robinson JT, Thorvaldsdottir H, Winckler W, et al. Integrative genomics viewer. Nat Biotechnol. 2011;29(1):24-26.
28. Quinlan AR, Hall IM. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics. 2010;26(6):841-842.
29. Machanick P, Bailey TL. MEME-ChIP: motif analysis of large DNA datasets. Bioinformatics. 2011;27(12):1696-1697.
30. Homminga I, Pieters R, Langerak AW, et al. Integrated transcript and genome analyses reveal NKX2-1 and MEF2C as potential oncogenes in T cell acute lymphoblastic leukemia. Cancer Cell. 2011;19(4):484-497.
31. Gautier L, Cope L, Bolstad BM, Irizarry RA. affy--analysis of Affymetrix GeneChip data at the probe level. Bioinformatics. 2004;20(3):307-315.

32. Liu Y, Easton J, Shao Y, et al. The genomic landscape of pediatric and young adult T-lineage acute lymphoblastic leukemia. Nat Genet. 2017;49(8):1211-1218.
33. de Araujo ED, Erdogan F, Neubauer HA, et al. Structural and functional consequences of the STAT5B(N642H) driver mutation. Nat Commun. 2019;10(1):2517.
34. Govaerts I, Jacobs K, Vandepoel R, Cools J. JAK/STAT pathway mutations in T-ALL, including the STAT5B N642H mutation, are sensitive to JAK1/JAK3 inhibitors. Hemasphere. 2019;3(6):e313.
35. Kontro M, Kuusanmaki H, Eldfors S, et al. Novel activating STAT5B mutations as putative drivers of T-cell acute lymphoblastic leukemia. Leukemia. 2014;28(8):1738-1742.
36. Bandapalli OR, Schuessele S, Kunz JB, et al. The activating STAT5B N642H mutation is a common abnormality in pediatric T-cell acute lymphoblastic leukemia and confers a higher risk of relapse. Haematologica. 2014;99(10):e188-192.
37. Pham HTT, Maurer B, Prchal-Murphy M, et al. STAT5BN642H is a driver mutation for T cell neoplasia. J Clin Invest. 2018;128(1):387-401.
38. Strahle U, Klock G, Schutz G. A DNA sequence of 15 base pairs is sufficient to mediate both glucocorticoid and progesterone induction of gene expression. Proc Natl Acad Sci U S A. 1987;84(22):7871-7875.
39. Willis SN, Fletcher JI, Kaufmann T, et al. Apoptosis initiated when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak. Science. 2007;315(5813):856-859.
40. Strasser A. The role of BH3-only proteins in the immune system. Nat Rev Immunol. 2005;5(3):189-200.
41. Gratiot-Deans J, Merino R, Nunez G, Turka LA. Bcl-2 expression during T-cell development: early loss and late return occur at specific stages of commitment to differentiation and survival. Proc Natl Acad Sci U S A. 1994;91(22):10685-10689.
42. Siegel RM, Katsumata M, Miyashita T, Louie DC, Greene MI, Reed JC. Inhibition of thymocyte apoptosis and negative antigenic selection in bcl-2 transgenic mice. Proc Natl Acad Sci U S A. 1992;89(15):7003-7007.
43. Sentman CL, Shutter JR, Hockenbery D, Kanagawa O, Korsmeyer SJ. bcl-2 inhibits multiple forms of apoptosis but not negative selection in thymocytes. Cell. 1991;67(5):879-888.
44. Wojciechowski S, Tripathi P, Bourdeau T, et al. Bim/Bcl-2 balance is critical for maintaining naive and memory T cell homeostasis. J Exp Med. 2007;204(7):1665-1675.
45. Stocklin E, Wissler M, Gouilleux F, Groner B. Functional interactions between Stat5 and the glucocorticoid receptor. Nature. 1996;383(6602):726-728.
46. Stoecklin E, Wissler M, Moriggl R, Groner B. Specific DNA binding of Stat5, but not of glucocorticoid receptor, is required for their functional cooperation in the regulation of gene transcription. Mol Cell Biol. 1997;17(11):6708-6716.
47. Mueller KM, Themanns M, Friedbichler K, et al. Hepatic growth hormone and glucocorticoid receptor signaling in body growth, steatosis and metabolic liver cancer development. Mol Cell Endocrinol. 2012;361(1-2):1-11.
48. Petta I, Dejager L, Ballegeer M, et al. The interactome of the glucocorticoid receptor and its influence on the actions of glucocorticoids in combatting inflammatory and infectious diseases. Microbiol Mol Biol Rev. 2016;80(2):495-522.
49. Ye SK, Agata Y, Lee HC, et al. The IL-7 receptor controls the accessibility of the TCRgamma locus by Stat5 and histone acetylation. Immunity. 2001;15(5):813-823.
50. Bertolino E, Reddy K, Medina KL, Parganas E, Ihle J, Singh H. Regulation of interleukin 7-dependent immunoglobulin heavy-chain variable gene rearrangements by transcription factor STAT5. Nat Immunol. 2005;6(8):836-843.
51. Vanden Bempt M, Demeyer S, Broux M, et al. Cooperative enhancer activation by TLX1 and STAT5 drives development of NUP214-ABL1/TLX1-positive T cell acute lymphoblastic leukemia. Cancer Cell. 2018;34(2):271-285.e7.
52. Pinz S, Unser S, Buob D, Fischer P, Jobst B, Rascle A. Deacetylase inhibitors repress STAT5-mediated transcription by interfering with bromodomain and extra-terminal (BET) protein function. Nucleic Acids Res. 2015;43(7):3524-3545.