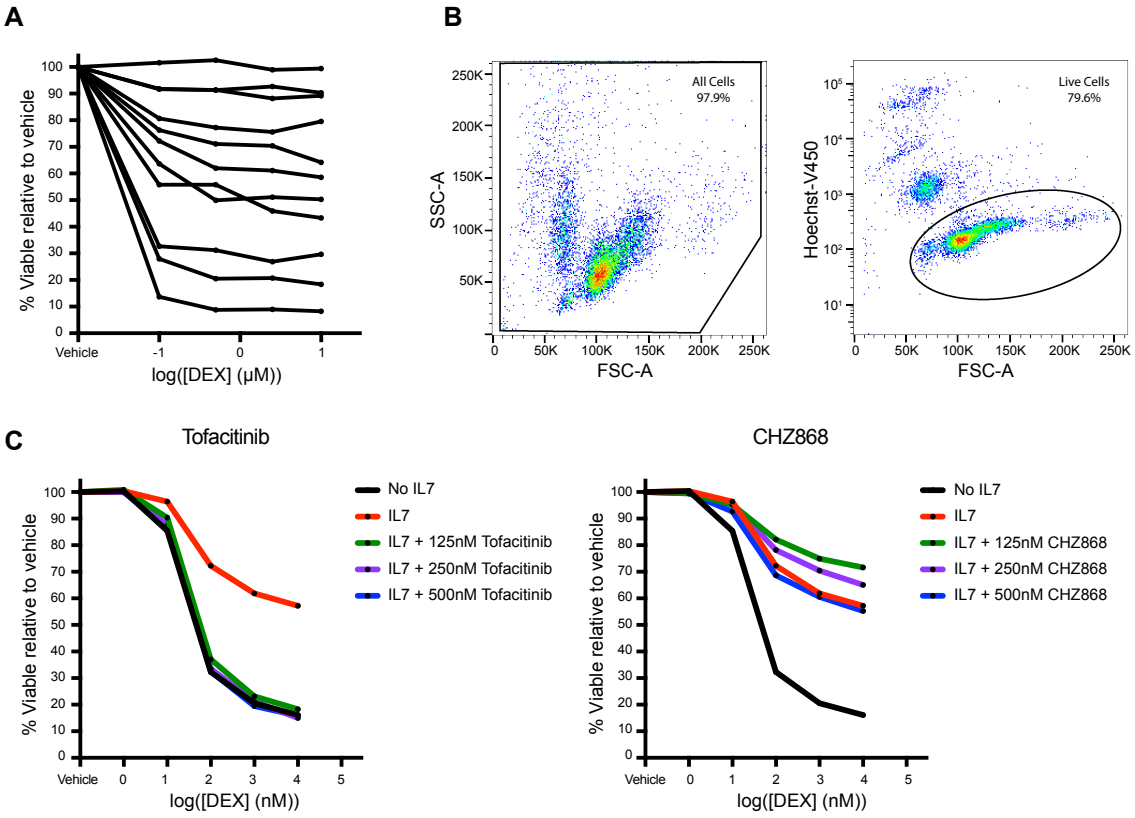

Figure S1: (A) Viability of 11 representative primary diagnostic T-ALL samples exposed to increasing concentrations of DEX for 48 hours in the presence of 25ng/mL IL7. **(B)** Gating strategy used to assess viability by Hoechst stain and flow cytometry in CCRF-CEM cells. **(C)** Viability of CCRF-CEM cells exposed to DEX in the presence of 25ng/mL IL7 in the absence or presence of increasing concentrations of tofacitinib (left) or CHZ868 (right) for 72 hours in technical triplicate. Viability data in (C) are representative of two independent experiments.

Figure S2

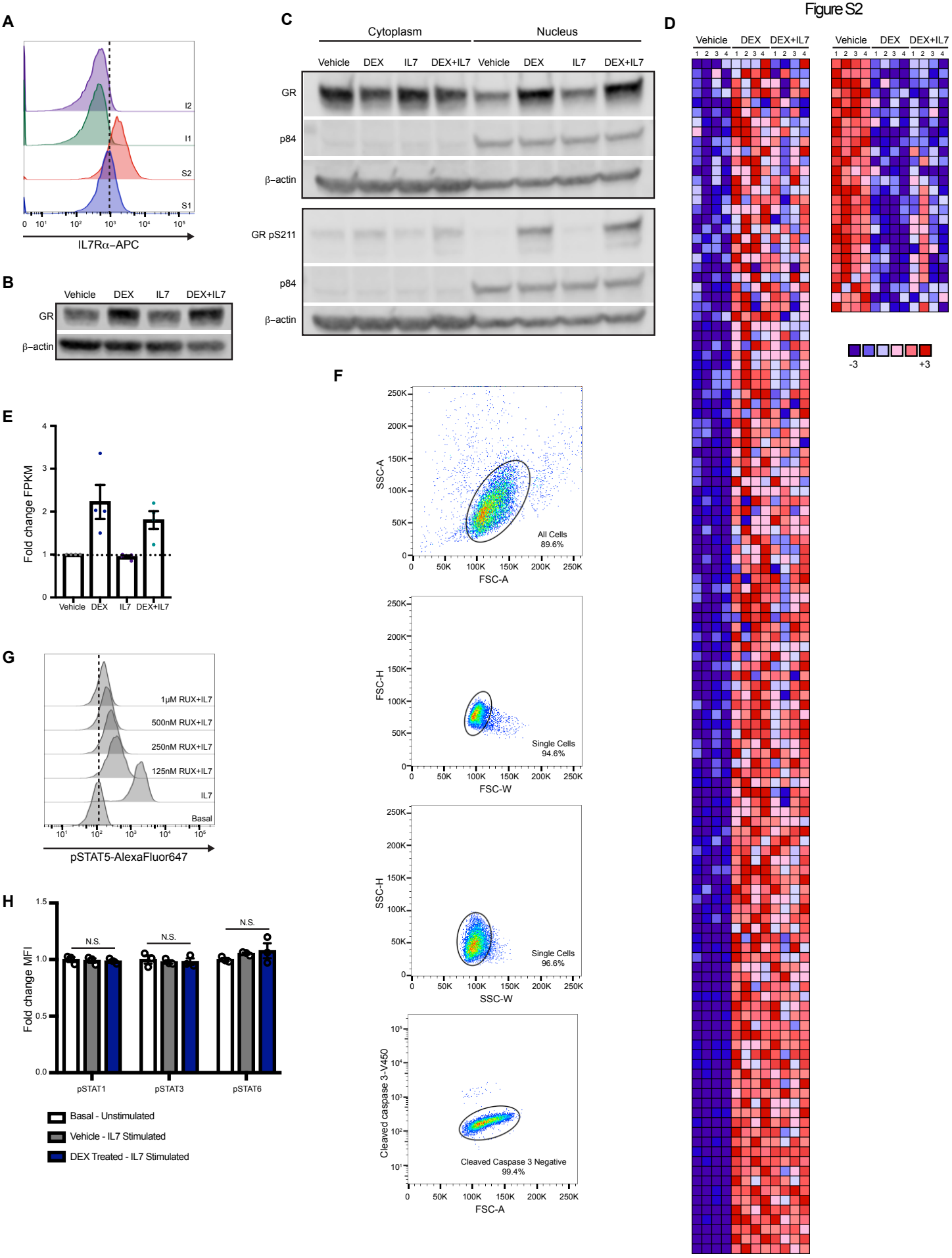
A
I2
I1
S2
S1
IL7Rα–APC
B
Vehicle DEX IL7 DEX+IL7
GR
β–actin
C
Cytoplasm
Nucleus
Vehicle DEX IL7 DEX+IL7 Vehicle DEX IL7 DEX+IL7
GR
p84
β–actin
GR pS211
p84
β–actin
D
Vehicle DEX DEX+IL7
Vehicle DEX DEX+IL7
-3
+3
E
Fold change FPKM
Vehicle DEX IL7 DEX+IL7
F
SSC-A
FSC-A
All Cells
89.6%
FSC-H
FSC-W
Single Cells
94.6%
SSC-H
SSC-W
Single Cells
96.6%
Cleaved caspase 3-V450
FSC-A
Cleaved Caspase 3 Negative
99.4%
G
1μM RUX+IL7
500nM RUX+IL7
250nM RUX+IL7
125nM RUX+IL7
IL7
Basal
pSTAT5-AlexaFluor647
H
Fold change MFI
N.S.
N.S.
N.S.
pSTAT1
pSTAT3
pSTAT6
Basal - Unstimulated
Vehicle - IL7 Stimulated
DEX Treated - IL7 Stimulated

Figure S2: (A) Histograms of IL7Rα expression in scramble control (S1 and S2) and IL7Rα KO (I1 and I2) CCRF-CEM cell clones. **(B)** Evaluation of GR expression by Western blot in CCRF-CEM cells treated with or without 1μM DEX and/or 100ng/mL IL7 for 24 hours. **(C)** Evaluation of total and phosphorylated GR (pS211) expression by Western blot in cytoplasmic and nuclear protein fractions from CCRF-CEM cells treated with or without 100ng/mL IL7 for 15 minutes followed by vehicle control or 1μM DEX for one hour. An anti-β-actin antibody was used as a cytoplasmic loading control and an anti-p84 antibody was used as a nuclear loading control. **(D)** Heatmap of GR target genes as determined by RNA-seq in scramble control clones (n=4) treated with or without 1μM DEX and 100ng/mL IL7 for four hours. **(E)** Fold change in the fragments per kilobase million (FPKM) values of *IL7RA* transcript as determined by RNA-seq in four independent scramble control clones treated with or without 1μM DEX in the presence or absence of 100ng/mL IL7 for four hours. **(F)** Gating strategy used for measurement of intracellular proteins by flow cytometry in CCRF-CEM cells. **(G)** Representative histograms of pSTAT5 in CCRF-CEM cells treated with 1μM DEX in the absence of IL7 for 24 hours followed by a one-hour exposure to vehicle control or increasing concentrations of RUX prior to a 15-minute stimulation with 100ng/mL IL7. **(H)** Fold change in the MFI of pSTAT1, pSTAT3, or pSTAT6 following a 15 minute stimulation with 100ng/mL IL7 in CCRF-CEM cells cultured in vehicle control or 1μM DEX in technical triplicate for 24 hours. Statistical significance was assessed using one-way ANOVA with Tukey's method for multiple comparisons adjustment. With the exception of the RNA-seq experiment, all data are representative of three independent experiments.

Figure S3

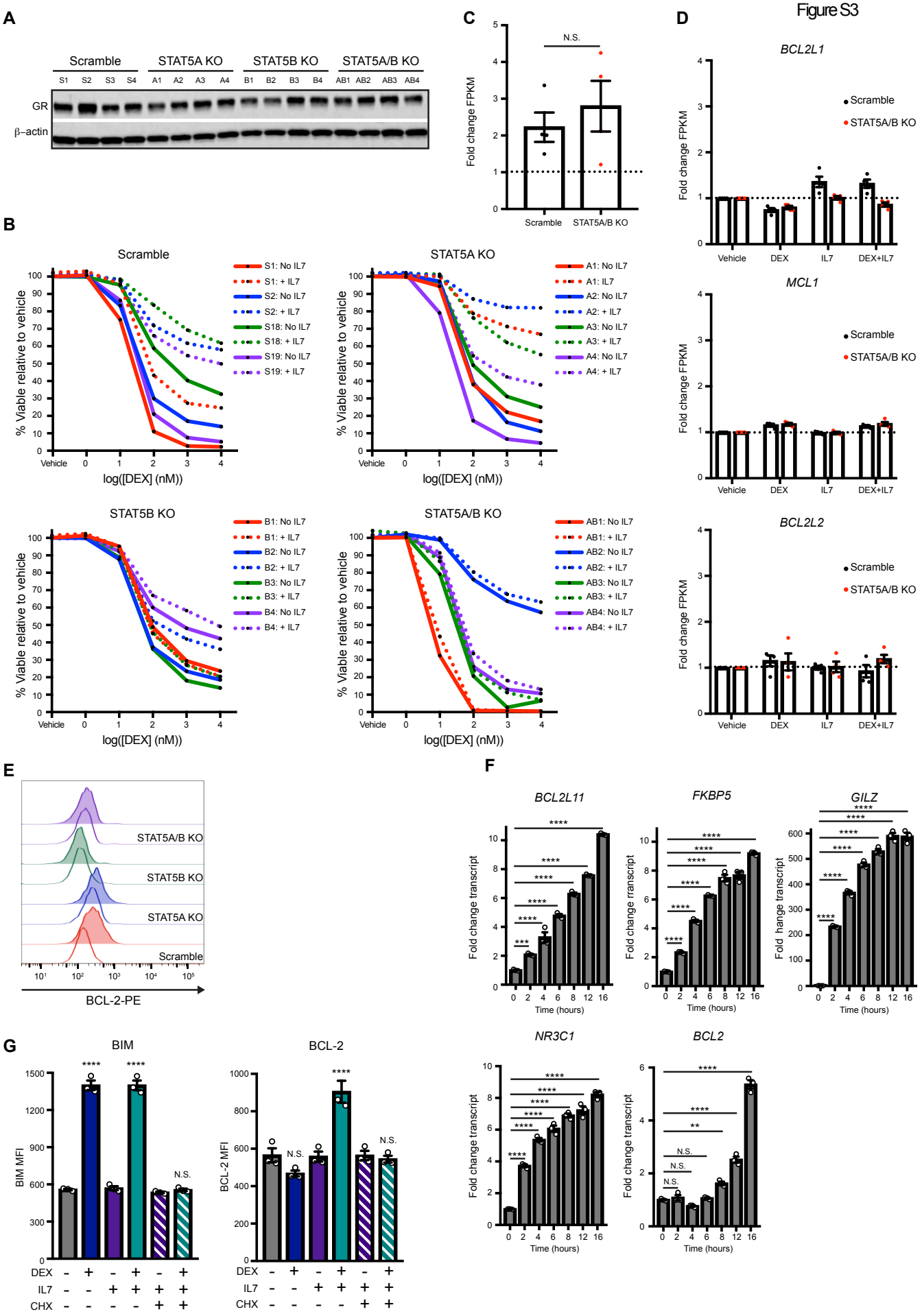
A
Scramble
STAT5A KO
STAT5B KO
STAT5A/B KO
S1 S2 S3 S4 A1 A2 A3 A4 B1 B2 B3 B4 AB1 AB2 AB3 AB4
GR
β–actin
B
Scramble
STAT5A KO
STAT5B KO
STAT5A/B KO
% Viable relative to vehicle
log([DEX] (nM))
Vehicle
S1: No IL7
S1: + IL7
S2: No IL7
S2: + IL7
S18: No IL7
S18: + IL7
S19: No IL7
S19: + IL7
A1: No IL7
A1: IL7
A2: No IL7
A2: + IL7
A3: No IL7
A3: + IL7
A4: No IL7
A4: + IL7
B1: No IL7
B1: + IL7
B2: No IL7
B2: + IL7
B3: No IL7
B3: + IL7
B4: No IL7
B4: + IL7
AB1: No IL7
AB1: + IL7
AB2: No IL7
AB2: + IL7
AB3: No IL7
AB3: + IL7
AB4: No IL7
AB4: + IL7
C
Fold change FPKM
N.S.
Scramble
STAT5A/B KO
D
BCL2L1
MCL1
BCL2L2
Fold change FPKM
Scramble
STAT5A/B KO
Vehicle
DEX
IL7
DEX+IL7
E
STAT5A/B KO
STAT5B KO
STAT5A KO
Scramble
BCL-2-PE
F
BCL2L11
FKBP5
GILZ
NR3C1
BCL2
Fold change transcript
Fold change rranscript
Fold hange transcript
Time (hours)
G
BIM
BCL-2
BIM MFI
BCL-2 MFI
N.S.
DEX
IL7
CHX

Figure S3: (A) Evaluation of GR expression by Western blot in scramble control and STAT5 KO CCRF-CEM cell clones (n=4 per genotype). **(B)** Viability of scramble control and STAT5 KO CCRF-CEM cell clones (n=4 per genotype) treated with DEX without (solid lines) or with (dotted lines) 25ng/mL IL7 for 72 hours. **(C)** Fold change in the FPKM for *IL7RA* in scramble control (n=4) and STAT5A/B KO (n=4) CCRF-CEM cell clones following exposure to 1μM DEX for four hours relative to vehicle control. **(D)** Fold change in the FPKM for *BCL2L1, MCL1,* and *BCL2L2* transcripts as determined by RNA-seq analysis of scramble control (n=4) and STAT5A/B double KO (n=4) CCRF-CEM cell clones treated in the absence or presence of 100ng/mL IL7 and/or 1μM DEX for 16 hours. **(E)** Histograms of BCL-2 protein expression in a representative clone from each group treated with 100ng/mL IL7 in the absence (unfilled histograms) versus presence (filled histograms) of 1μM DEX for 48 hours. **(F)** Fold change in transcript expression of four known primary GR target genes and *BCL2* following exposure to 1μM DEX and 100ng/mL IL7 for various time points relative to cells exposed to vehicle control as determined by qPCR performed in technical triplicate. **(G)** MFI of BIM and BCL-2 protein expression performed in technical triplicate in CCRF-CEM cells exposed to vehicle control or 1μM DEX with or without 100ng/mL IL7 in the absence or presence of 10μg/mL CHX for 24 hours. Significance is relative to the vehicle control condition. Statistical significance was assessed using a two-sample t-test (C) or one-way ANOVA with Tukey's method for multiple comparisons adjustment (F and G). With the exception of the RNA-seq experiment, all data are representative of three independent experiments. ****$p<0.0001$, ***$p<0.001$, **$p<0.01$, *$p<0.05$, N.S. – not significant.

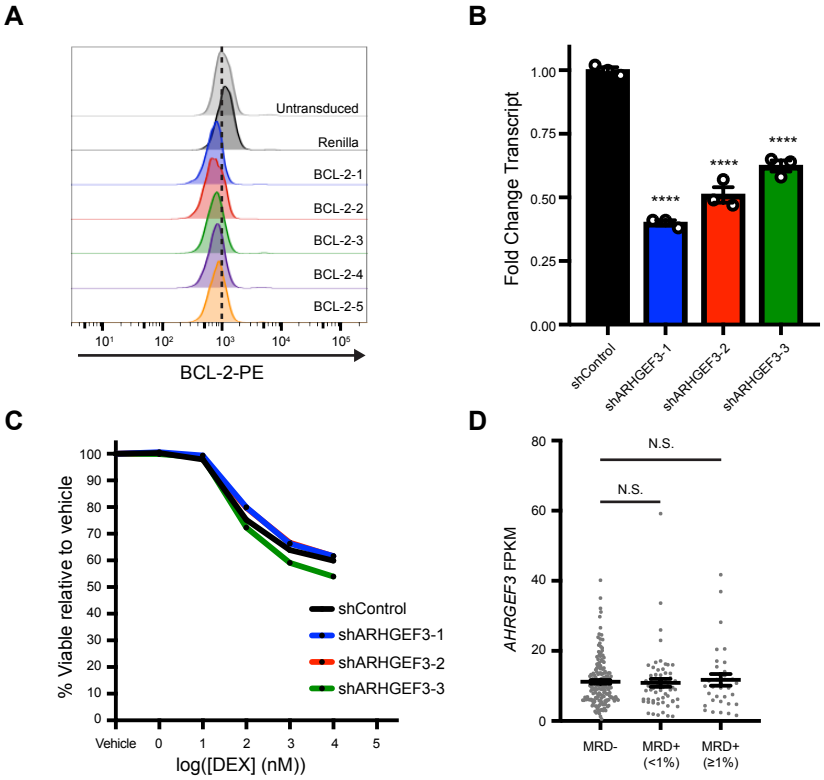

Figure S4: (A) Representative histograms of BCL-2 protein expression in untransduced CCRF-CEM cells and CCRF-CEM cells transduced with a non-targeting shRNA control (shControl) or a *BCL2*-targeting shRNA (shBCL2-1-5). **(B)** Fold change in the transcript expression of *ARHGEF3* in CCRF-CEM cells transduced with shControl or an *ARHGEF3*-targeting shRNA (shARHGEF3-1-3). **(C)** Viability of shRNA-transduced CCRF-CEM cells treated with DEX in the presence of 25ng/mL IL7 in technical triplicate for 72 hours. **(D)** FPKM values for *ARHGEF3* transcript obtained from published RNA-seq data from diagnostic samples from patients enrolled on COG AALL0434, stratified based on day 29 bone marrow MRD. Statistical significance was assessed using one-way ANOVA with Tukey's method for multiple comparisons adjustment (B and D). All CCRF-CEM cell data are representative of three independent experiments. ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05, N.S. – not significant.

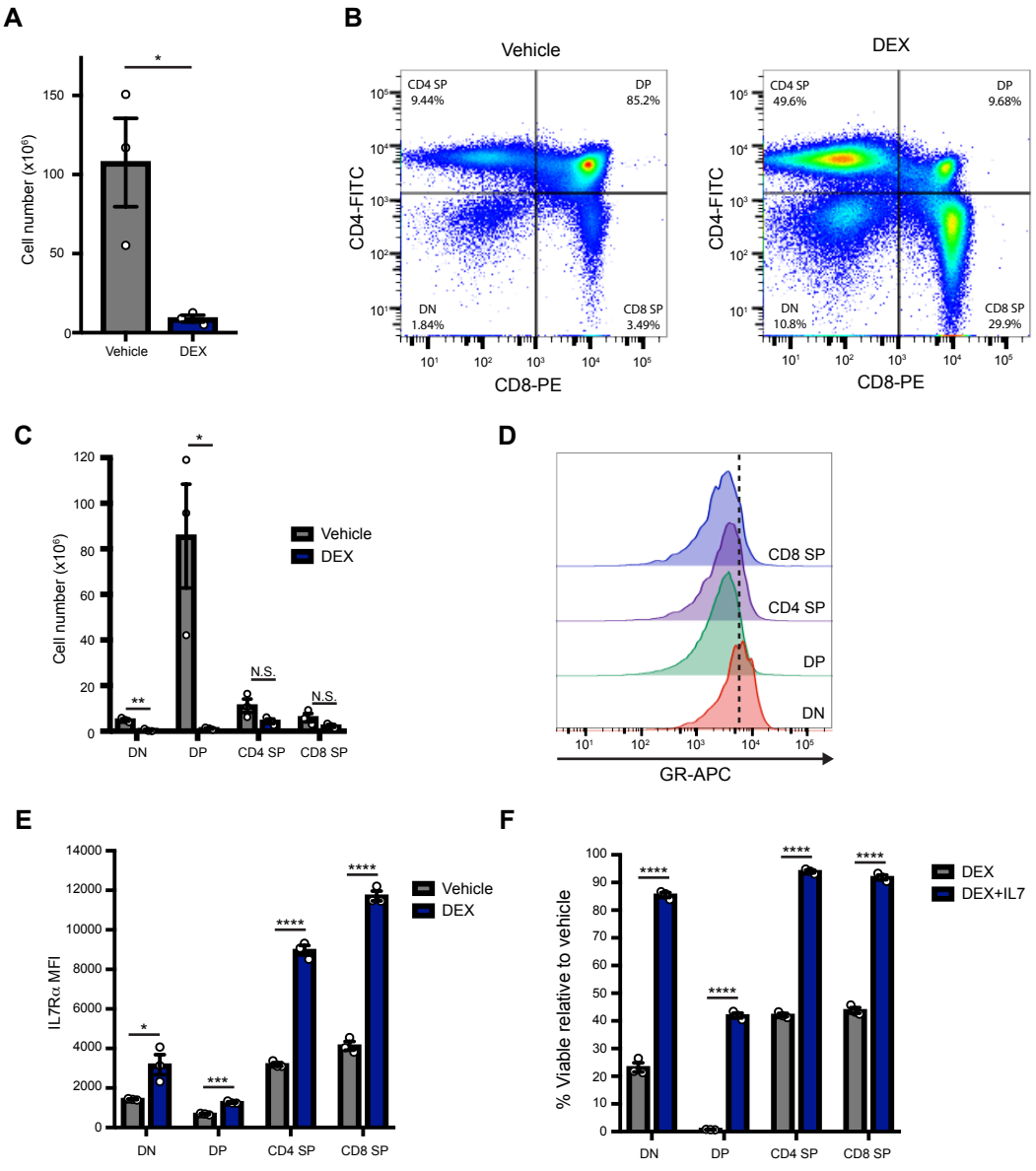

Figure S5: (A) Absolute number of thymocytes in thymi isolated from mice treated with vehicle control (n=3) or DEX (n=3) at 2mg/kg/day for three days. **(B)** Representative FACS plots demonstrating the distribution of the major thymocyte subpopulations in thymi isolated from mice treated with vehicle control or DEX at 2mg/kg/day for three days. **(C)** Absolute number of thymocytes in each of the major thymocyte subpopulations in thymi isolated from mice treated with vehicle control (n=3) or DEX (n=3) at 2mg/kg/day for three days. **(D)** Histograms of the basal expression of GR in the major murine thymocyte subpopulations. **(E)** MFI of IL7Rα in human thymocytes treated ex vivo in the presence or absence of 1μM DEX in technical triplicate for 24 hours. **(F)** Viability of human thymocytes treated ex vivo with 1μM DEX with or without 25ng/mL IL7 in technical triplicate for 24 hours. Statistical significance was assessed using two-sample t-tests (A, C, E, and F). All

murine thymocyte data are representative of three independent experiments. All human thymocyte data are representative of two independent experiments. ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05.

Figure S6

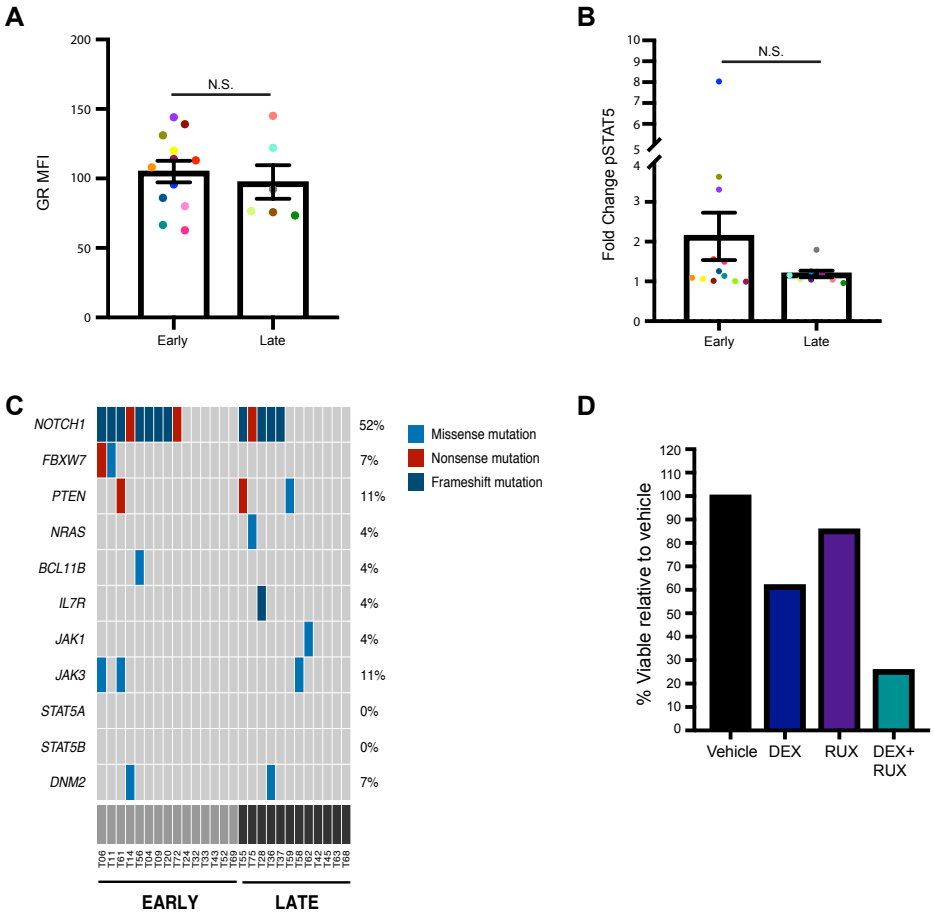

Figure S6: (A) MFI of GR protein in 12 early and 6 late T-ALLs. Some samples were not analyzed due to limitations in cell number. Statistical significance was analyzed using a two-sample t-test. **B)** MFI of pSTAT5 in 12 early and 9 late T-ALL PDX samples following stimulation with 100ng/mL IL7 for 15 minutes in technical triplicate. Some samples were not analyzed due to limitations in cell number. **(C)** Oncoplot depicting the occurrence of mutations in genes previously implicated in T-ALL in 15 early and 12 late T-ALL PDX samples. **(D)** Viability relative to vehicle control of cells from T-ALL T24 exposed to 25ng/mL IL7 with or without 1μM DEX and/or 500nM RUX in vitro for 48 hours. N.S. – not significant.