# Clinicopathological features and prognostic value of SOX11 in childhood acute lymphoblastic leukemia

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**Supplementary Table 1. Dataset sample sizes**

| Group | Hemap | GSE47051 | PanALL |
|---|---|---|---|
| T-ALL | 385 | 30 | 0 |
| HeH | 151 | 18 | 279 |
| Low hyperdiploid | 0 | 0 | 51 |
| KMT2A | 301 | 5 | 136 |
| KMT2A-like | 0 | 0 | 5 |
| TCF3-PBX1 | 81 | 6 | 78 |
| ETV6-RUNX1 | 171 | 16 | 187 |
| ETV6-RUNX1-like | 0 | 0 | 42 |
| Ph | 137 | 5 | 123 |
| Ph-like | 0 | 0 | 359 |
| B-cell | 75 | 0 | 0 |
| HSC | 21 | 0 | 0 |
| T-cell | 247 | 0 | 0 |
| BCP-ALL | 1304 | 0 | 0 |
| AML | 1713 | 0 | 0 |
| MCL | 100 | 0 | 0 |
| BCL2/MYC | 0 | 0 | 18 |
| CRLF2 | 0 | 0 | 16 |
| DUX4 | 0 | 0 | 106 |
| HLF | 0 | 0 | 9 |
| iAMP21 | 0 | 0 | 40 |
| IKZF1 N159Y | 0 | 0 | 8 |
| Low hypodiploid | 0 | 0 | 78 |
| MEF2D | 0 | 0 | 43 |
| Near haploid | 0 | 0 | 29 |
| NUTM1 | 0 | 0 | 11 |
| Other | 0 | 0 | 125 |
| PAX5 P80R | 0 | 0 | 44 |
| PAX5alt | 0 | 0 | 148 |
| ZNF384 | 0 | 0 | 49 |
| ZNF384-like | 0 | 0 | 4 |

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BCL2/MYC, BCL2/MYC rearranged; BCP-ALL, B-cell precursor ALL; CRLF2, CRLF2 (non-Ph-like); DUX4, DUX4 rearranged; HeH, high hyperdiploid; HLF, TCF3/TCF4-HLF; HSC, hematopoietic stem cell; iAMP21, intrachromosomal amplification of chromosome 21; IKZF1 N159Y, IKZF1 missense alteration encoding p.Asn159Tyr; KMT2A, KMT2A rearranged; MCL, mantle cell lymphoma; MEF2D, MEF2D rearranged; NUTM1, NUTM1 rearranged; PAX5alt, PAX5 alterations; PAX5 P80R, PAX5 p.Pro80Arg (P80R) alteration; Ph, Philadelphia chromosome; T-ALL, T-cell ALL; ZNF384, ZNF384 rearranged.

**Supplementary Table 2. RT-qPCR primer sequences**

| Primer | Sequence 5’→ 3’ |
|---|---|
| *SOX11* forward | CGGTCAAGTGCGTGTTTCTG |
| *SOX11* reverse | CACTTTGGCGACGTTGTAGC |
| *PBGD* forward | CGCATCTGGAGTTCAGGAGTA |
| *PBGD* reverse | CCAGGATGATGGCACTGA |
| *SOX4* forward | GGCACATCAAGCGACCCATG |
| *SOX4* reverse | CGTTTGCCCAGCCGCTTGG |
| *SOX12* forward | CGAGGACGACGATGAAGACG |
| *SOX12* reverse | GGACCATCCTCCACAGCTC |
| *ETV6-RUNX1* forward | TGCACCCTCTGATCCTGAAC |
| *ETV6-RUNX1* reverse | AACGCCTCGCTCATCTTGC |

**Supplementary Table 3. Used corticosteroid and chemotherapy concentrations**

| Cell line | Prednisolone (µM) | Dexamethasone (µM) | Asparaginase (U/ml) | Vincristine (nM) |
|---|---|---|---|---|
| 697 | 0, 0.01, 0.1, 1 | 0, 0.01, 0.1, 1 | 0, 0.001, 0.01, 0,1 | 0, 0.1, 0.25, 0.5, 1 |
| RCH-ACV | 0, 0.01, 0.1, 1 | 0, 0.01, 0.1, 1 | 0, 0.001, 0.01, 0.1, 0.5 | 0, 0.1, 0.25, 0.5, 1, 2 |
| REH | 0, 100, 500, 1000 | 0, 10, 50, 100 | 0, 0.001, 0.01, 0.1 | 0, 0.1, 0.25, 0.5, 1 |

**Supplementary Table 4. siRNA sequences**

| siRNA | Primers 5' → 3' | Reference |
|---|---|---|
| siSCR sense | GUUGCGUAGCGUACGUCGCAA | |
| siSCR antisense | UUGCGACGUACGCUACGCAAC | |
| siSOX11 sense | GAUAAGAUGUCGUGACGCA | [1] |
| siSOX11 antisense | UGCGUCACGACAUCUUAUC | [1] |

**Supplementary Table 5. Transfection protocols**

| Cell line | Solution | Reaction |
|---|---|---|
| 697 | SG | CA-137 |
| RCH-ACV | SG | CM-137 |
| REH | SF | CA-137 |

**Supplementary Table 6. Kruskal-Wallis H test and Mann-Whitney U test was used to evaluate differential expression of *SOX11* in distinct leukemia subgroups**

| Mann-Whitney U test (p-value) | | | |
|---|---|---|---|
| **Groups** | **Hemap** | **GSE47051** | **PanALL** |
| TCF3-PBX1 vs rest | $9.09 \times 10^{-20}$ | $8.63 \times 10^{-2}$ | $1.69 \times 10^{-27}$ |
| ETV6-RUNX1 vs rest | $3.04 \times 10^{-52}$ | $1.81 \times 10^{-9}$ | $2.09 \times 10^{-78}$ |
| | **Kruskal-Wallis H test (p-value)** | | |
| | $3.12 \times 10^{-104}$ | $3.16 \times 10^{-7}$ | $5.57 \times 10^{-142}$ |

**Library preparation and RNA sequencing**

The quality of the total RNA samples was ensured with Advanced Analytical Fragment Analyzer. Library preparation was done according to Illumina TruSeq® Stranded mRNA Sample Preparation Guide (part # 15031047) (Illumina, San Diego, CA, USA). The high quality of the libraries was confirmed with Advanced Analytical Fragment Analyzer and the concentrations of the libraries were quantified with Qubit® Fluorometric Quantitation (Life Technologies). Good quality libraries were exclusively selected and sequenced.

The samples were sequenced with Illumina HiSeq 2500 instrument using Truseq v2 Rapid sequencing chemistry. The samples were normalized and pooled for the automated onboard cluster preparation in HiSeq 2500. The 18 libraries were pooled into one pool and run in 2 lanes. Single-read sequencing with 1 x 50 bp read length was used, followed by 8 + 8 bp dual index run. The base calling was performed using Illumina's standard bcl2fastq2 software, automatic adapter trimming was used.

**RNA sequencing and methylation analysis of patient samples**

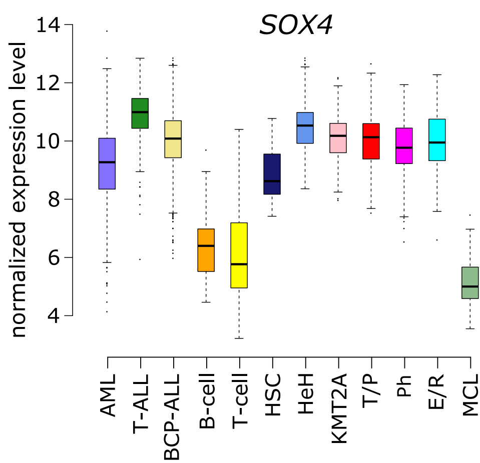
Briefly, strand-specific RNA-seq libraries were prepared from rRNA-depleted RNA using the ScriptSeq V2 Kit and paired-end sequenced to a minimum of 50 bp using a Hiseq2000/2500 or MiSeq instrument (Illumina), producing on average, 46 million read pairs per sample. Sequence reads were aligned to the human 1000 Genomes build 37 (GRCh37) using Tophat 2 (2.0.4) and gene expression levels were normalized to fragments per kilobase per million mapped reads (FPKM) using Cufflinks version 2.2.0.

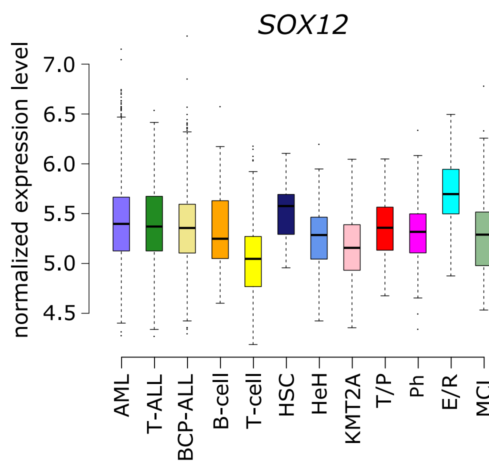
Briefly, DNA methylation levels were measured using the Infinium HumanMethylation 450k BeadChip assay (Illumina). Intensity signals from the 450k array were converted to methylation beta values (ranging from 0-1) followed by probe filtering and normalization using Peak Based Correction as previously described [2].

## References

1. Kuo PY, et al. High-resolution chromatin immunoprecipitation (ChIP) sequencing reveals novel binding targets and prognostic role for SOX11 in mantle cell lymphoma. *Oncogene* 2015; **34**: 1231-1240.

2. Nordlund J, et al. Genome-wide signatures of differential DNA methylation in pediatric acute lymphoblastic leukemia. *Genome Biol* 2013; **14**: r105.



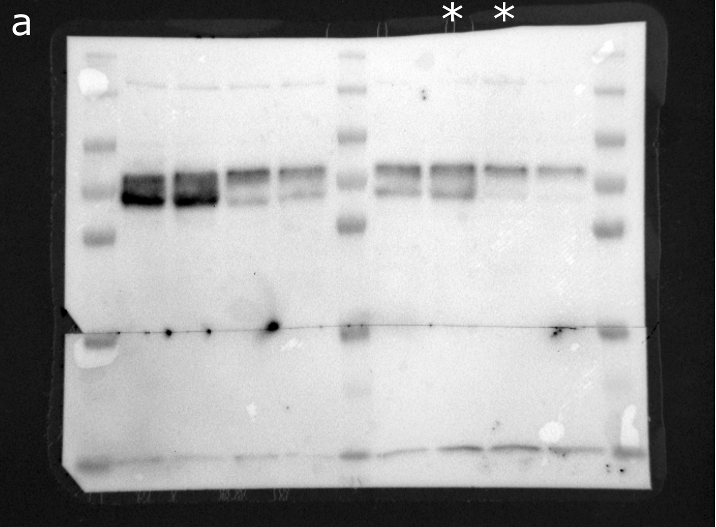

**Supplementary Figure 1. *SOX4* and *SOX12* expressions.** Expression boxplots of *SOX4* and *SOX12* in healthy cells, leukemias and mantle cell lymphomas. Data source: Combined microarray dataset [1,2]. ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; Ph, Philadelphia chromosome; BCP -ALL, B-cell precursor ALL; E/R, ETV6-RUNX1 subtype; HeH, high hyperdiploid subtype; HSC, hematopoietic stem cell; KMT2A, KMT2A rearrangement subtype; MCL, mantle cell lymphoma; T/P TCF3-PBX1 subtype.

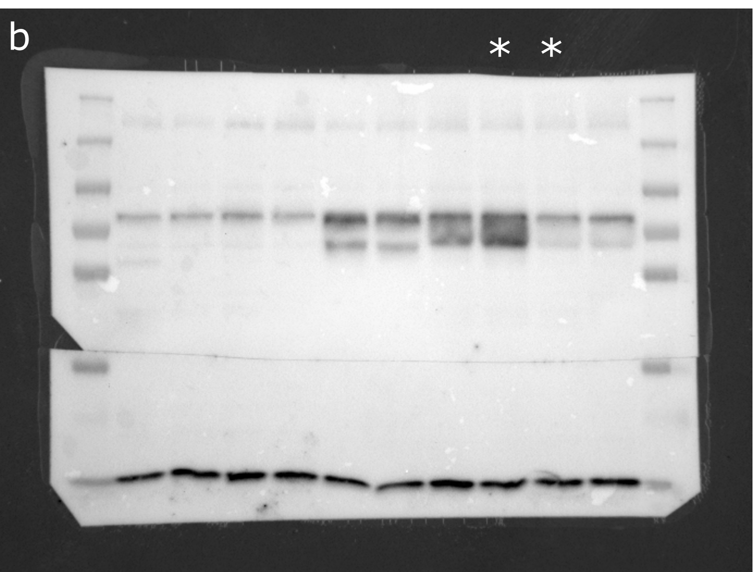
**References**

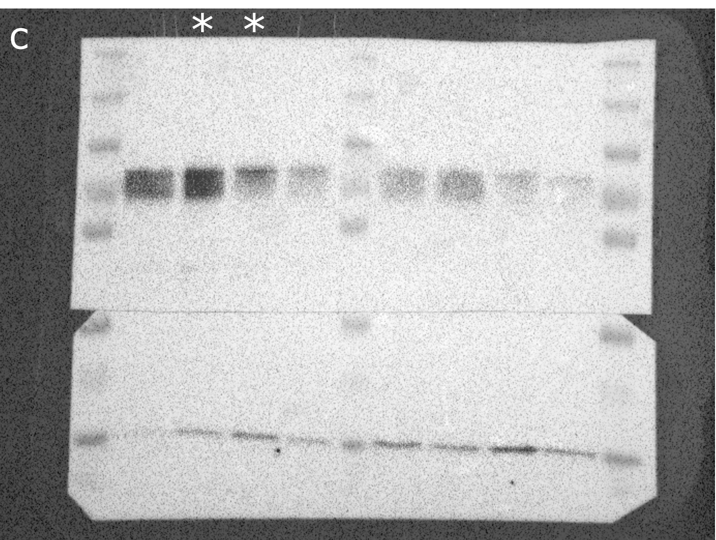

1. Heinäniemi, M., et al. Transcription-coupled genetic instability marks acute lymphoblastic leukemia structural variation hotspots. *Elife*. 5, e13087 (2016).
2. Pölönen, P., et al. Hemap: An Interactive Online Resource for Characterizing Molecular Phenotypes across Hematologic Malignancies. *Cancer Res*. 79, 2466-2479 (2019).



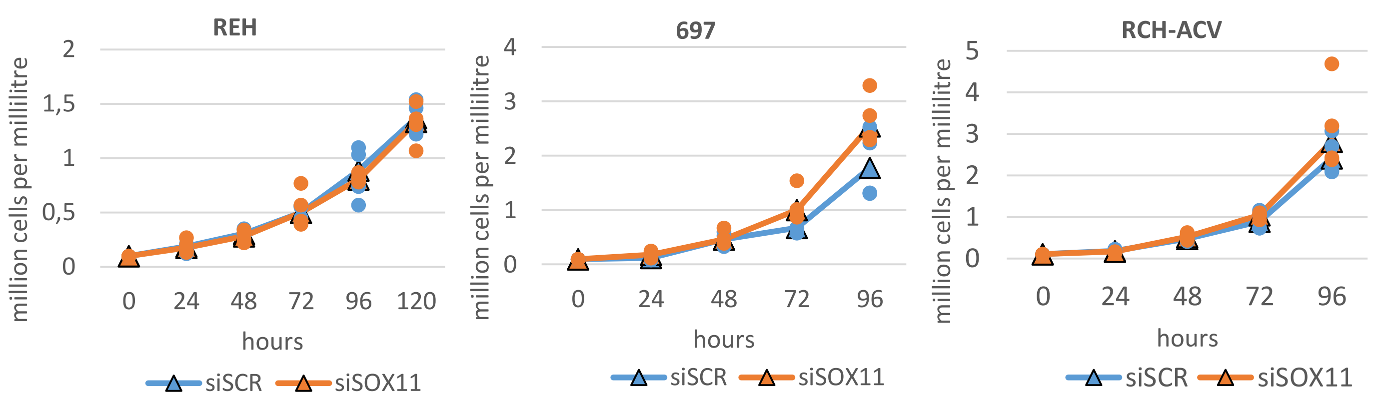

**Supplementary Figure 2. *SOX11* expression. a.** Changes in *E/R* expression has no marked change on *SOX11* expression (N=2). **b.** Effects of decitabine treatment on *SOX11* expression in REH cell line (N=2).




**Supplementary Figure 3. Original Western Blots for Figure 4b. a.** 697 **b.** RCH-ACV **c.** REH.
*) marks cropped lines for the figure 4 B western blots.




**Supplementary Figure 4. Proliferation assay in REH, 697 and RCH-ACV cell lines after *SOX11* knockdown.** Proliferation was assayed by counting the cells every 24 hours after the siRNA (siSOX11 = *SOX11* siRNA, siSCR = control siRNA) transfection. *SOX11* knockdown was confirmed with RT-qPCR and N=4.

697: Vincristine
Cell viability
Concentration nM
siSCR siSOX11

697: Asparaginase
Cell viability
Concentration U/ml
siSCR siSOX11

697: Dexamethasone
Cell viability
Concentration µM
siSCR siSOX11

697: Prednisolone
Cell viability
Concentration µM
siSCR siSOX11

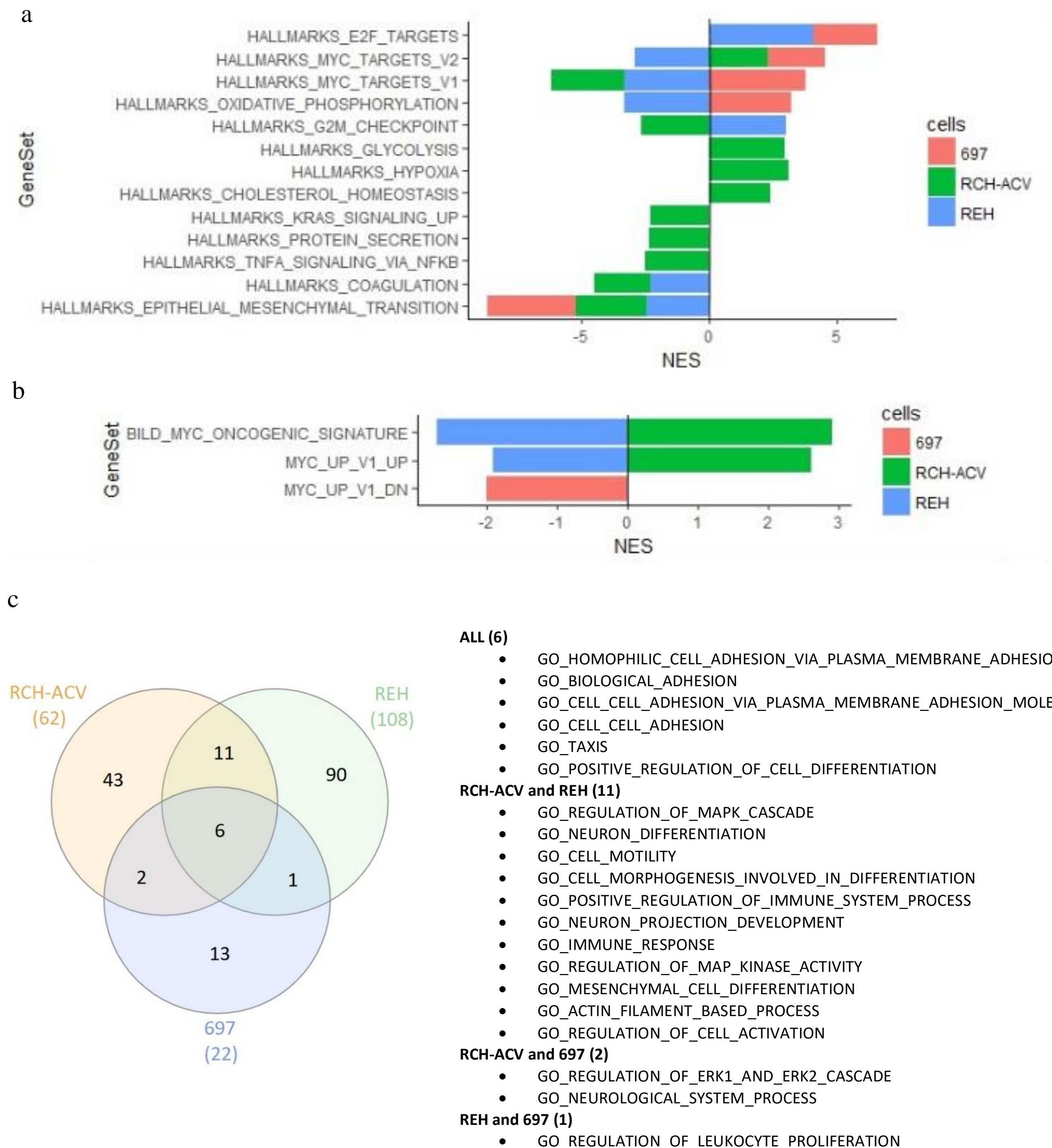
REH: Vincristine
Cell viability
Concentration nM
siSCR siSOX11

REH: Asparaginase
Cell viability
Concentration U/ml
siSCR siSOX11

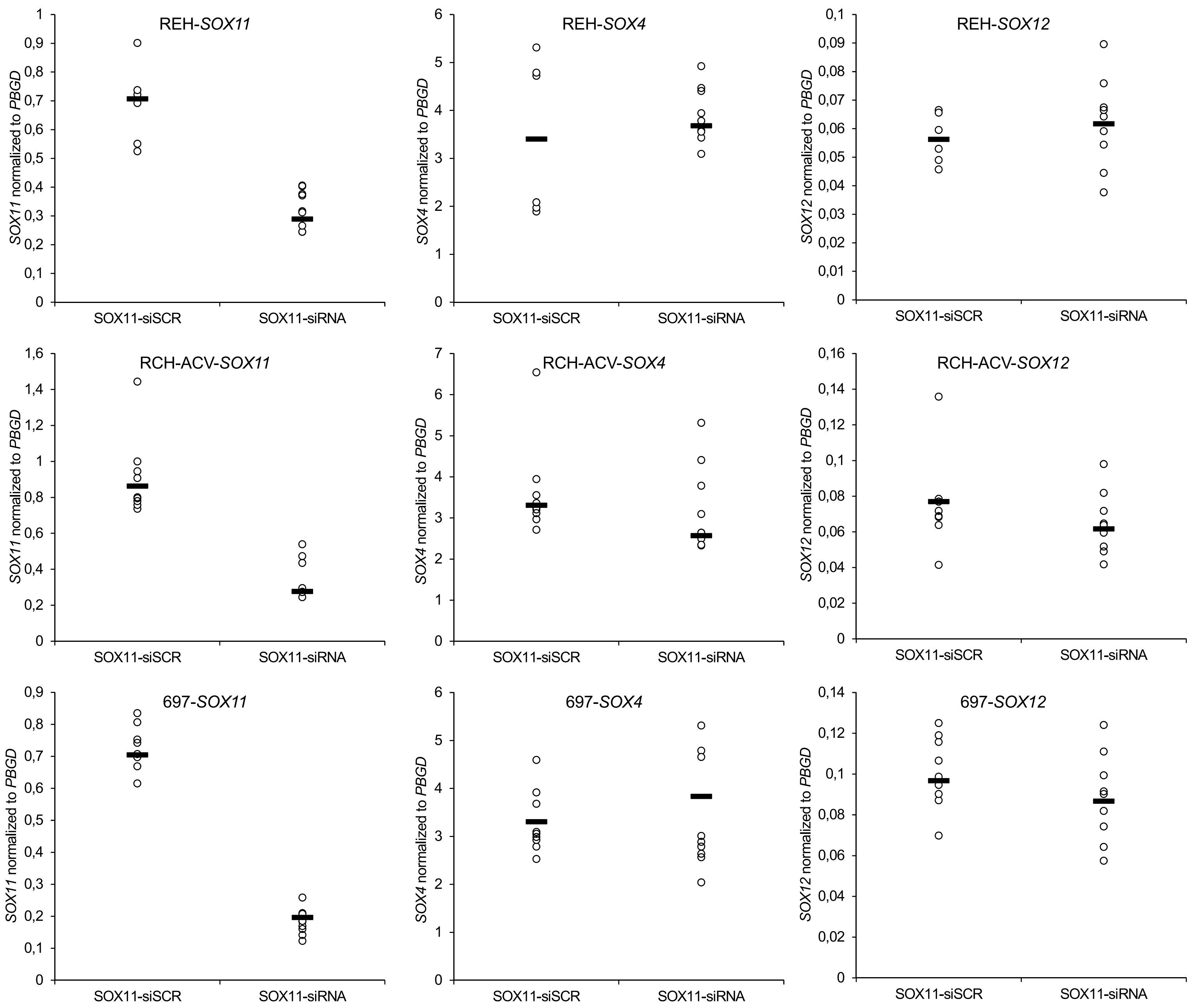
REH: Dexamethasone
Cell viability
Concentration µM
siSCR siSOX11

REH: Prednisolone
Cell viability
Concentration µM
siSCR siSOX11

**Supplementary Figure 5. Chemotherapy agent and corticosteroid tretments on REH and 697 cell lines.** Cell viabilities after siRNA transfection, *SOX11* siRNA (siSOX11) and control siRNA (siSCR) followed by drug treatments (697: 72 hours, REH: 96 hours; N=2). *SOX11* knockdown was confirmed with RT-qPCR and cell viability assessments were carried out with Alamar Blue assay.




**Supplementary Figure 6. Enrichment of gene sets in 697, RCH-ACV and REH. a.** Enrichment of hallmark gene sets retrieved from molecular signature database (MSigDB) in REH, RCH-ACV and 697 cell lines. Adjusted p-value of enrichment for all gene sets < 0.01. **b.** Enrichment of founder gene sets of "MYC targets V2" gene set in REH, RCH-ACV and 697 cell lines. Adjusted p-value of enrichment for all gene sets < 0.02. **c.** Venn diagram of significantly enriched GO terms in *SOX11* siRNA treated REH, RCH-ACV and 697 cell lines and list of GO terms overlapping between cell lines.




***S*upplementary Figure 7. SOX4 and SOX12 expressions after SOX11 knockdown.** *SOX11* knockdown does not affect *SOX4* or *SOX12* expression levels in studied cell lines (N=3).