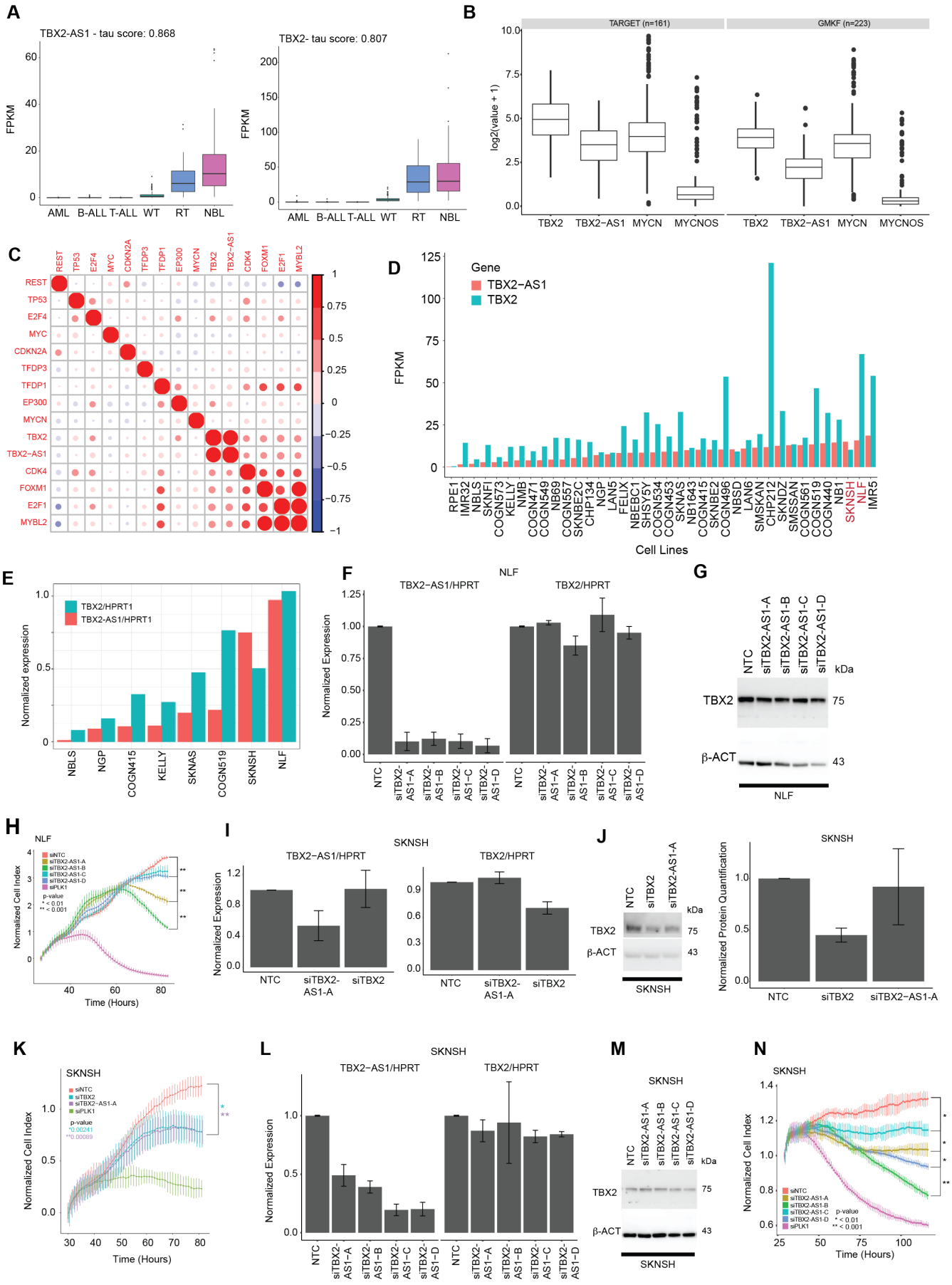
A
TBX2-AS1 - tau score: 0.868
TBX2- tau score: 0.807
FPKM
AML B-ALL T-ALL WT RT NBL
B
TARGET (n=161)
GMKF (n=223)
log2(value + 1)
TBX2 TBX2−AS1 MYCN MYCNOS
C
REST TP53 E2F4 MYC CDKN2A TFDP3 TFDP1 EP300 MYCN TBX2 TBX2−AS1 CDK4 FOXM1 E2F1 MYBL2
D
Gene
TBX2−AS1
TBX2
FPKM
Cell Lines
E
TBX2/HPRT1
TBX2-AS1/HPRT1
Normalized expression
NBLS NGP COGN415 KELLY SKNAS COGN519 SKNSH NLF
F
NLF
TBX2−AS1/HPRT
TBX2/HPRT
Normalized Expression
NTC siTBX2-AS1−A siTBX2-AS1−B siTBX2-AS1−C siTBX2-AS1−D
G
NTC siTBX2-AS1-A siTBX2-AS1-B siTBX2-AS1-C siTBX2-AS1-D
kDa
TBX2 75
β-ACT 43
NLF
H
NLF
siNTC
siTBX2-AS1-A
siTBX2-AS1-B
siTBX2-AS1-C
siTBX2-AS1-D
siPLK1
p-value
* < 0.01
** < 0.001
Normalized Cell Index
Time (Hours)
I
SKNSH
TBX2−AS1/HPRT
TBX2/HPRT
Normalized Expression
NTC siTBX2-AS1-A siTBX2
J
NTC siTBX2 siTBX2-AS1-A
kDa
TBX2 75
β-ACT 43
SKNSH
SKNSH
Normalized Protein Quantification
NTC siTBX2 siTBX2−AS1-A
K
SKNSH
siNTC
siTBX2
siTBX2−AS1-A
siPLK1
p-value
*0.00241
**0.00089
Normalized Cell Index
Time (Hours)
L
SKNSH
TBX2−AS1/HPRT
TBX2/HPRT
Normalized Expression
NTC siTBX2-AS1−A siTBX2-AS1−B siTBX2-AS1−C siTBX2-AS1−D
M
SKNSH
NTC siTBX2-AS1-A siTBX2-AS1-B siTBX2-AS1-C siTBX2-AS1-D
kDa
TBX2 75
β-ACT 43
SKNSH
N
SKNSH
Normalized Cell Index
Time (Hours)
siNTC
siTBX2-AS1-A
siTBX2-AS1-B
siTBX2-AS1-C
siTBX2-AS1-D
siPLK1
p-value
* < 0.01
** < 0.001

Supplementary Fig. S7: Validation of *TBX2-AS1* expression in NBL cell lines and impact on NBL cell growth. (A) Expression of *TBX2-AS1* and *TBX2* across pediatric cancers. **(B)** Comparison of expression levels of *TBX2, TBX2-AS1, MYCN,* and *MYCNOS* in the NBL TARGET (un-stranded) and NBL GMKF (stranded) RNA-sequencing cohorts show high concordance. Pearson's correlation between the 14 common samples for these genes was r=0.979, 0.954, 0.983, and 0.877, respectively. **(C)** Correlation between *TBX2-AS1* and other genes previously shown to be regulated by TBX2. **(D)** Expression of *TBX2-AS1* and *TBX2* in 38 NBL cell lines. **(E)** RT-qPCR validation of *TBX2-AS1* and *TBX2* expression in 8 NBL cell lines. **(F)** RT-qPCR expression of *TBX2-AS1* and *TBX2* for NLF cell line treated with non-targeting control (siNTC) and four different siRNAs targeting *TBX2-AS1.* siTBX2-AS1-A is referred to as siTBX2-AS1 in the main figures. Three independent knockdown experiments are represented. **(G)** Western blot for TBX2 expression after independent treatment of multiple siRNAs targeting *TBX2-AS1* in NLF. **(H)** Representative image of NLF cell growth as measured by RT-ces assay following siRNA treatments. siPLK1 is a positive control. Cell index is normalized to time point when siRNA reagent is added at 24 hours post cell plating. All siTBX2-AS1 treatments resulted in significant growth inhibition. **(I)** RT-qPCR expression of *TBX2-AS1* and *TBX2* for SKNSH cell line treated with siNTC, siTBX2-AS1, and siTBX2. Three independent knockdown experiments are plotted, each plated in triplicate. **(J)** Representative Western blot for TBX2 expression after siTBX2 or siTBX2-AS1 treatment compared to NTC in SKNSH. Right panel: Protein quantification derived from ImageJ analysis of Western blots for three independent knockdown experiments. **(K)** Representative image of SKNSH cell growth as measured by RT-ces assay following siRNA treatments. Cell index is normalized to time point when siRNA reagent is added at 24 hours post cell plating. Both siTBX2-AS1 and siTBX2 show significant growth inhibition, consistent with results observed for the NLF cell line. **(L)** RT-qPCR expression of *TBX2-AS1* and *TBX2* for SKNSH cell line treated with siNTC and four different siRNAs targeting *TBX2-AS1.* Two independent knockdown experiments are represented. **(M)** Western blot for TBX2 expression after independent treatment of multiple siRNAs targeting *TBX2-AS1* in SKNSH. **(N)** Image of SKNSH cell growth as measured by RT-ces assay following independent treatments of four siRNAs targeting siTBX2-AS1. Cell index is normalized to time point when siRNA reagent is added at 24 hours post cell plating with index set to start at one.