

Chromosomal rearrangement-enhanced mRNA stability drives the oncogenic potential of fusion genes in pediatric leukemia

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Supplementary information

Supplementary materials and methods

Cells and cell culture

Human embryonic kidney HEK293T cell lines were provided by Invitrogen (Grand Island, NY). The mouse pro-B cell line BaF3 was purchased from the Shanghai Institute of JIHE SHENG WU KEJI Biology (Shanghai, China). Human NALM-6 cell lines was purchased were purchased from the American Type Culture Collection (Rockville, USA). Human REH cell lines was purchased from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). Human HEK293T cells were cultured in DMEM medium. BaF3 cells were cultured in RPMI 1640 medium supplemented with 2 ng/mL IL-3. NALM-6 and REH cells were cultured in RPMI 1640 medium. All medium were supplemented with 10% fetal bovine serum (Gibco BRL) and 1% penicillin/streptomycin. Cells were cultured in an incubator at 37°C with 5% CO₂. All cell lines were routinely tested for mycoplasma using Mycoplasma Detection Kit (Bimake, Houston, TX, USA) and passaged for a maximum of two months. All cell lines were authenticated utilizing short tandem repeat (STR) profiling every 6 months.

Plasmids and reagents

The coding sequences of PAX5-ETV6 and STK38-PXT1 were subcloned into the pCDH-MSCV-GFP (PMSCV) plasmid. shRNA sequences for human IGF2BP3 and mouse Igf2bp3 were cloned to pLKO.1 vector. The targeting sequences are as follows: shIGF2BP3, TCTGCGGCTTGTAAGTCTATT; shIgf2bp3#1, CGCGGAGAAGTCCATTACTAT; shIgf2bp3#2, CCTACCCACAATTTGAGCAAT. The primers used for PCR identification in animal studies are as follows: STK38-PXT1-F: 5'-ATGGCAATGACAGGCTCAAC-3', STK38-PXT1-R: 5'-TTATTTCCCTCCTGGTGATGC-3'. The packaging plasmid pRΔ8.9 and envelope plasmid pMD.G were kindly provided by Dr. D.B. Kohn (University of Southern California). 102 FDA-approved antitumor drugs including venetoclax, as well as actinomycin D, were purchased from Selleck Chemicals (Houston, Texas).

The fusion and gene expression analysis of ALL samples

First, the patient-derived ALL cells from bone marrow were obtained, and the RNA-seq detection was performed by Shanghai Tissue bank Biotechnology Co., Ltd. Then the STAR-

Fusion v1.7.0 was used to identify fusions from RNA-seq data across ALL samples in our CNpALL cohort.

Cellular proliferation, colony formation and cell apoptosis analysis

The total cell number was determined by trypan blue exclusion with manual counting in Burker chambers. The cell colony formation ability was detected by soft agar (Sigma, Shanghai) colony formation. We used Nitroterazolium Blue chloride (Aladdin, Shanghai) to color the cell clones. The apoptosis quantification was detected by PI/Annexin V-staining, and Annexin V-stained cells were analyzed to measure the cell apoptosis rate.

The mRNA half-life detection

The target gene was transfected into HEK293T or BaF3 cells, then actinomycin D (5 $\mu\text{g}/\text{mL}$, Apexbio) was used to block de novo RNA synthesis. Samples are collected at different time points after the addition of actinomycin D for mRNA extraction. Specific PCR primers were used to detect the expression levels of the corresponding mRNA (**Table S1**). Half-life ($t_{1/2}$): the time required for the relative expression level of mRNA to decrease to half. Simple linear regression was used to calculate the half-life of mRNA.

RNA sequencing assay of BaF3 cells

The sequencing of samples based on BaF3 cells was entrusted to Shanghai Biochip Co., Ltd. The first step of transcriptome sequencing (RNA-seq) involved extracting RNA from cells and enriching for target RNA, then converting the RNA into complementary DNA (cDNA), fragmenting it, and ligating adapters. Finally, these adapters were sequenced on the Illumina HiSeq 2000 platform for analysis.

Quantitative real-time PCR

Total RNA was isolated from cells with Trizol reagent (Invitrogen), and cDNA was transcribed using a transcript kit (TransGen Biotech). qRT-PCR analysis was performed using the SYBR Green (Bio-Rad) method on the QuantStudio 6 (Thermo). The gene expression was normalized to the expression of the gene encoding GAPDH. Sequences of the primers for qRT-PCR are shown in **Table S1**. Expression levels were normalized to the GAPDH mRNA level using the $2^{-\Delta\Delta\text{Ct}}$ method.

MERIP-qPCR experimental procedure

The target gene was transfected into HEK293T or BaF3 cells. Extract the mRNA required for the experiment, using non-fragmented mRNA for detection. Retain 10% of the mRNA sample as input, and the remaining sample was subjected to IP with m6A-specific antibody. Mix 15 μL of Magnetic Beads A/G Blend, RNasin Plus RNase inhibitor (40 U/ μL), 300 μg of mRNA sample, 200 μL of IP buffer, and 25 μL of m6A-specific antibody. Incubate at 4°C with end-over-end rotation for 4 hours, followed by washing 4 times. Add 100 μL of elution buffer to the magnetic beads, and incubate the mixture continuously at 4°C with shaking for 4 hours. Finally, the washed mRNA was detected for content using qPCR. Detail primers are shown in **Table S1**.

Supplementary figures

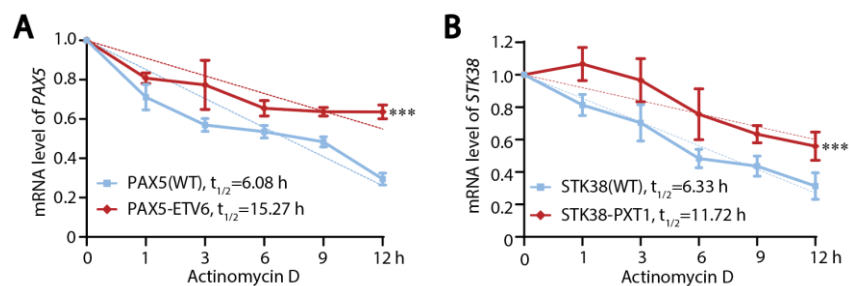


Figure S1. mRNA half-lives for *PAX5-ETV6* and *STK38-PXT1* versus wild type. (A) The mRNA half-life of *PAX5(WT)* and *PAX5-ETV6*. Fusions were overexpressed in HEK293T cells by plasmid transfection. After 24h, actinomycin D inhibited mRNA synthesis by 5 μ g/mL. Samples were collected to extract mRNA at the indicated times after being treated with actinomycin. $t_{1/2}$ was calculated by Simple Linear Regression. (B) The mRNA half-life of *STK38(WT)* and *STK38-PXT1*. (A, B) Data are represented as mean \pm SD (n=3). The significance analysis was conducted by two-way ANOVA analysis. ***, $P < 0.001$ vs. indicated.

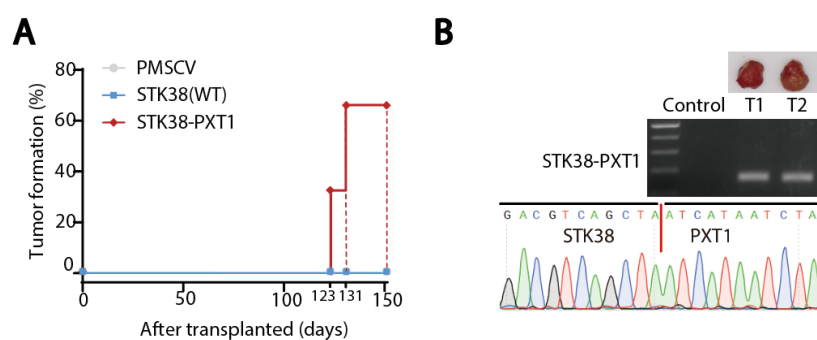


Figure S2. The effect of *STK38-PXT1* fusion on BaF3 xenograft tumorigenicity. (A) The oncogenic transformation activity of *STK38-PXT1* fusion. BaF3 cells transduced with vector-PMSCV virus, *STK38(WT)* and *STK38-PXT1* were subcutaneously injected into nude mice. (B) Identification of *STK38-PXT1* in transplanted tumors. After 150 days post-transplantation, all mice were euthanized, and tumors were excised for photography; mice without tumor formation were represented by a segment of their tail. Then the tumors were extracted for RNA isolation and the presence of *STK38-PXT1* was detected by RT-PCR.

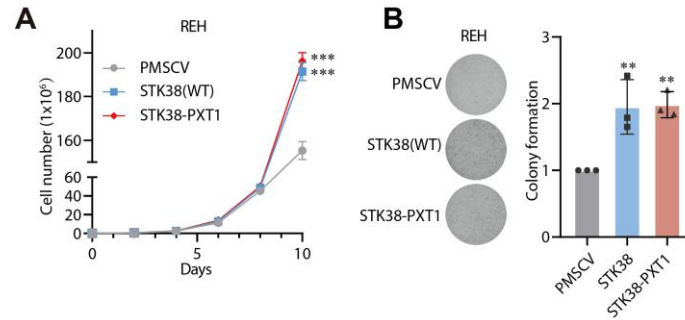


Figure S3. The effect of STK38-PXT1 on REH cell proliferation and colony formation. (A) Proliferation curve of REH cells overexpressing STK38 (WT), STK38-PXT1. REH cells were transfected with lentivirus PMSCV, STK38 (WT), STK38-PXT1. (B) The clonality of REH cells overexpressing STK38 (WT), STK38-PXT1. (A, B) Data are represented as mean \pm SD (n=3). (A) The significance analysis was conducted by two-way ANOVA analysis. ***, $P < 0.001$ vs. PMSCV. (B) The significance analysis was conducted by one-way ANOVA analysis. **, $P < 0.01$ vs. PMSCV.

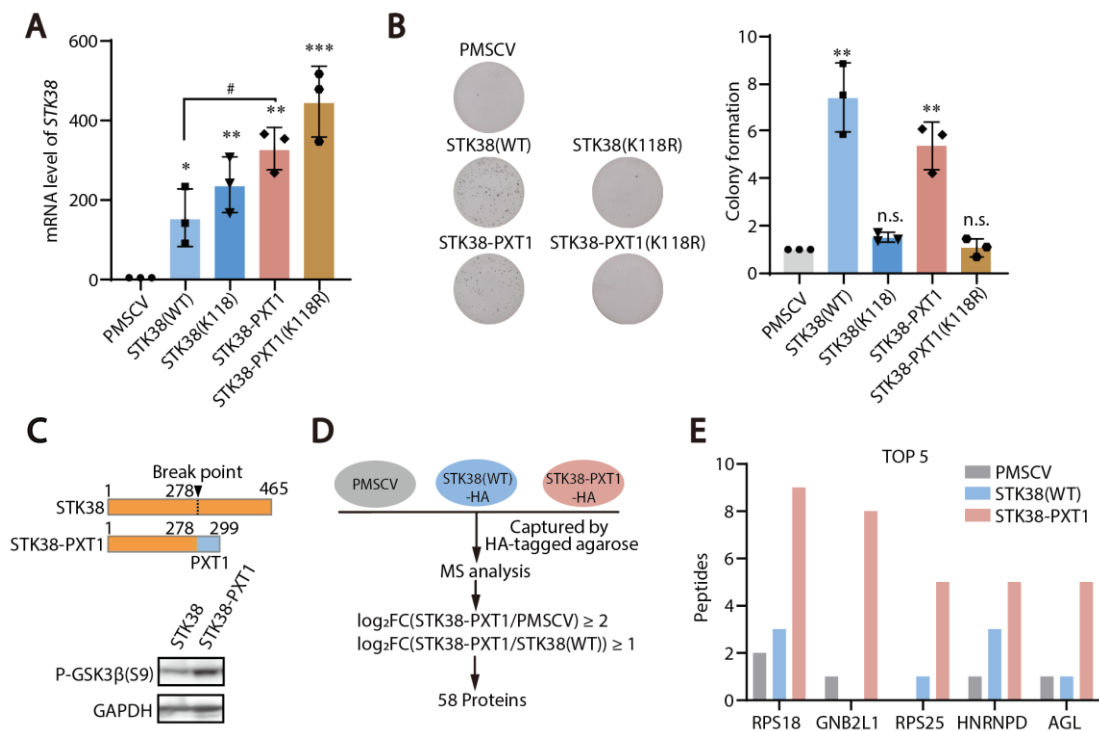


Figure S4. The effect of STK38-PXT1 overexpression on leukemogenesis and underlying potential mechanisms (A) The mRNA level of *STK38* in the BaF3 cells overexpressing STK38-PXT1. (B) The clonality of BaF3 cells overexpressing STK38-PXT1. (A, B) BaF3 cells were transfected with lentivirus PMSCV, STK38 (WT), STK38(K118R), STK38-PXT1, and

STK38-PXT1(K118R). (C) p-GSK3 β (Ser9) of BaF3 cells expressing STK38(WT) or STK38-PXT1. (D) Workflow for the identification of STK38(WT) and STK38-PXT1 interaction proteins. HEK293T cells were transfected with PMSCV, STK38(WT)-HA, or STK38-PXT1-HA. Lysates were captured with HA-tagged agarose and analyzed by LC-MS/MS. Candidate STK38-PXT1-preferential interactors were defined by $\log_2(\text{STK38-PXT1/PMSCV}) \geq 2$ and $\log_2(\text{STK38-PXT1/STK38 (WT)}) \geq 1$. (E) Peptide counts for the top five STK38-PXT1-preferential interactors identified by MS across PMSCV, STK38(WT), and STK38-PXT1 samples. (A, B) Data are represented as mean \pm SD (n=3). The significance analysis was conducted by one-way ANOVA analysis. n.s., $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ vs. PMSCV. #, $P < 0.05$ vs. indicated.

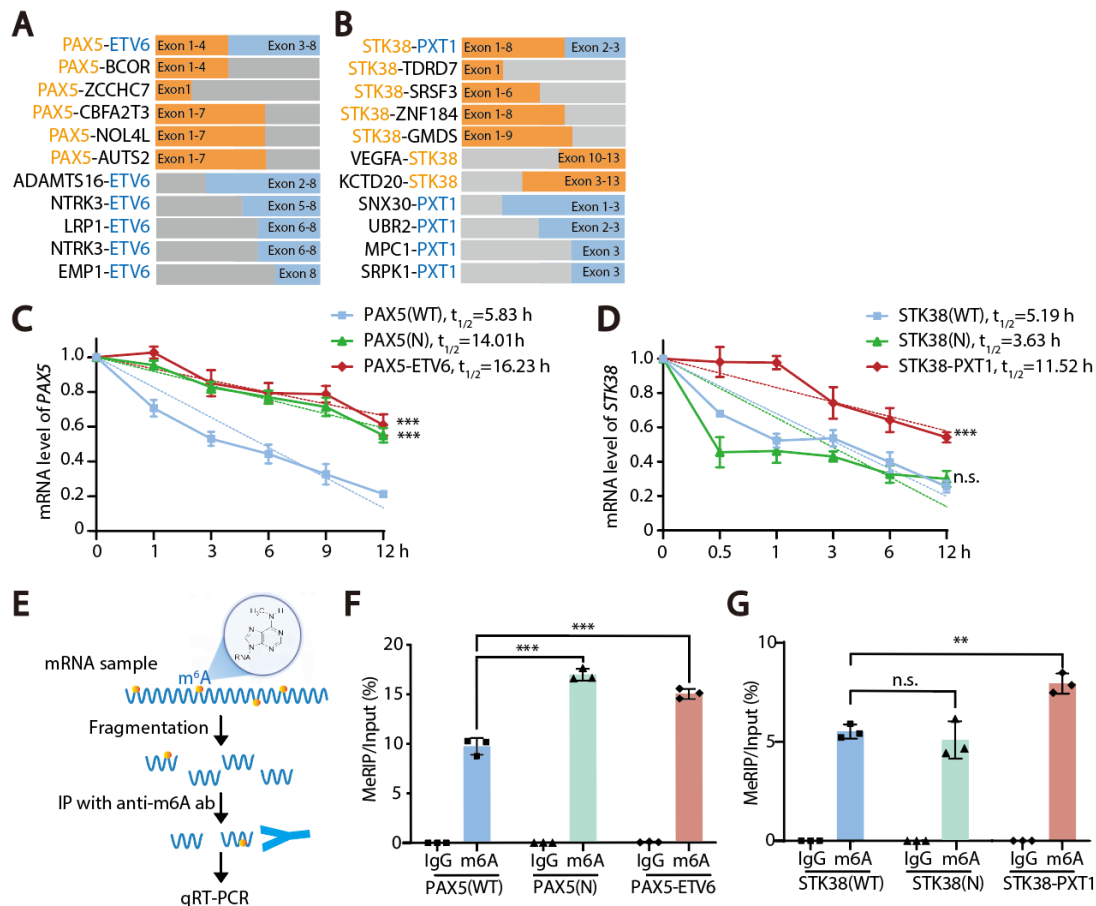


Figure S5. The mRNA half-life and m6A modification of PAX5 fusion and STK38-PXT1. (A) Structural schematic diagram of PAX5 fusions from pan-cancer database. (B) Structural schematic diagram of STK38 fusions from pan-cancer database. (C) mRNA half-life of

PAX5(WT), *PAX5(N)* and *PAX5-ETV6* in HEK293T cell. **(D)** mRNA half-life of *STK38(WT)*, *STK38(N)* and *STK38-PXT1* in HEK293T cell. **(E)** Schematic diagram of MeRIP-qPCR process. **(F)** The m6A modification level of *PAX5(WT)*, *PAX5(N)* and *PAX5-ETV6* in HEK293T cell. **(G)** The m6A modification level of *STK38(WT)*, *STK38(N)* and *STK38-PXT1* in HEK293T cell. (C, D) The significance analysis was conducted by two-way ANOVA analysis. n.s., $P>0.05$; ***, $P<0.001$ vs. *PAX5(WT)* (C) and *STK38(WT)* (D). (F, G) The significance analysis was conducted by one-way ANOVA analysis. n.s., $P>0.05$; **, $P<0.01$; ***, $P<0.001$ vs. indicated.

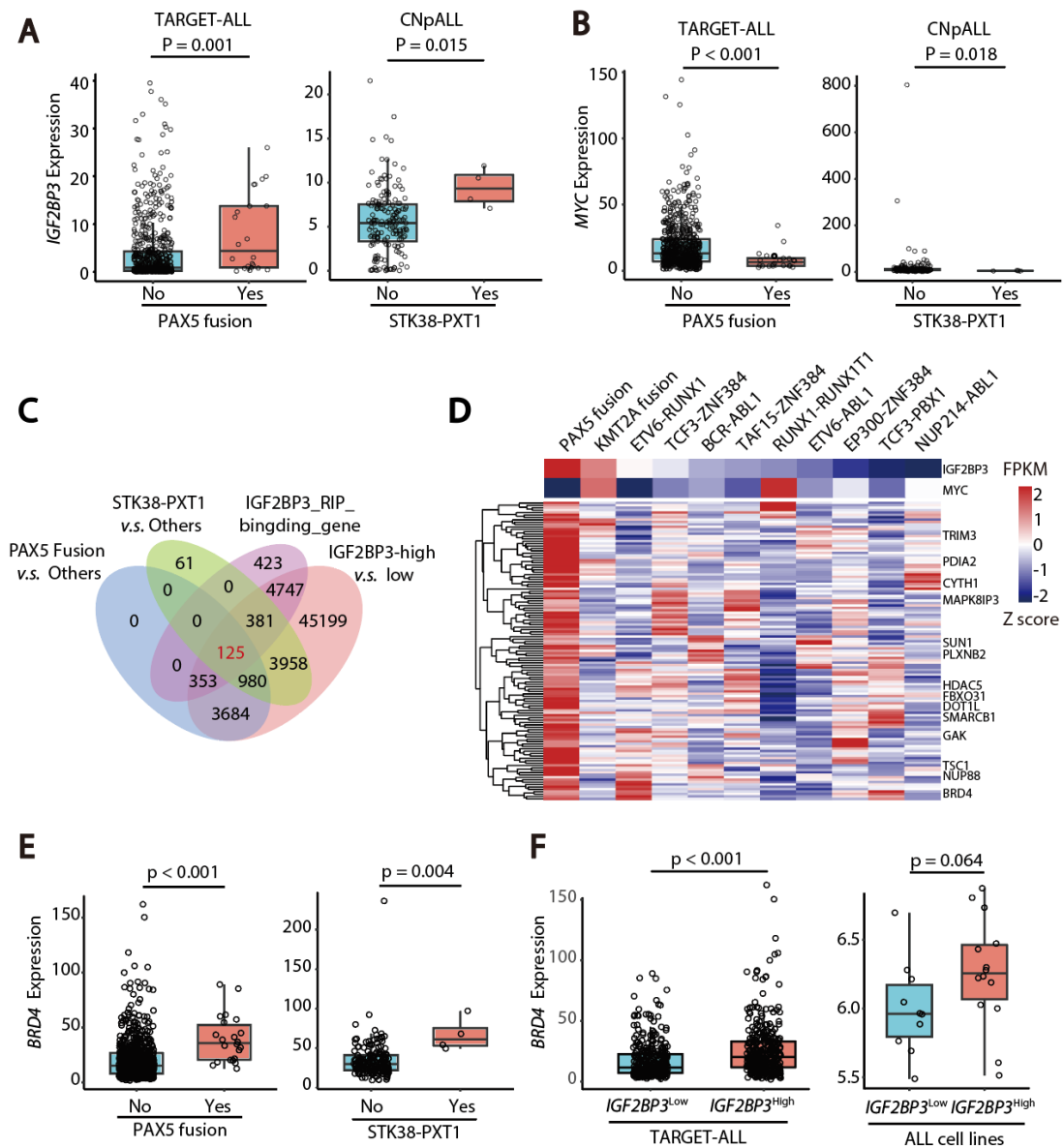


Figure S6. The potential downstream of IGF2BP3 as well as the mechanism of *IGF2BP3* upregulation in the *PAX5* fusion or *STK38-PXT1* positive ALL. (A) *IGF2BP3* mRNA

expression in cases with or without PAX5 fusions (TARGET-ALL, left) and STK38-PXT1 (CNpALL, right). **(B)** *MYC* mRNA expression in the same comparisons as in (A) for TARGET-ALL (left) and CNpALL (right). **(C)** Venn diagram showing overlap among DEGs in PAX5-fusion versus other ALL, STK38-PXT1 versus other ALL, *IGF2BP3* RIP-seq binding targets, and genes higher in *IGF2BP3*-high versus *IGF2BP3*-low samples. **(D)** Heat map of *IGF2BP3*, *MYC*, and 125 overlaid genes in (C). **(E)** *BRD4* expression in PAX5-fusion (TARGET-ALL, left) and STK38-PXT1 samples (CNpALL, right). **(F)** *BRD4* expression stratified by *IGF2BP3* level in TARGET-ALL (left) and in ALL cell lines (right). (A, B, E, F) The significance analysis was conducted by two-tailed unpaired Student's t-test.

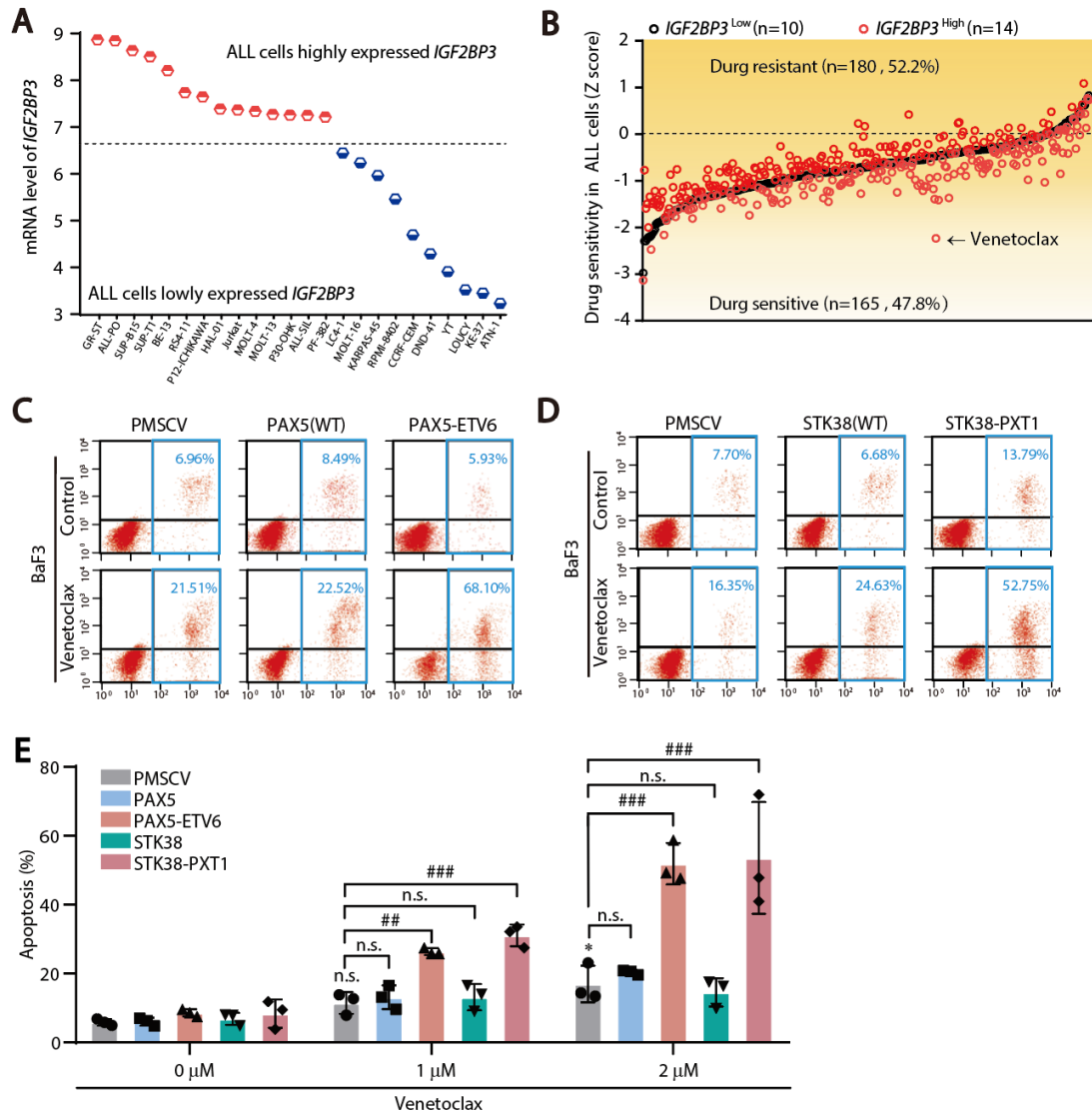


Figure S7. The effect of venetoclax on the cells with PAX5-ETV6 or STK38-PXT1. (A)

Expression levels of *IGF2BP3* in 24 ALL cell lines in GDSC database. According to the expression level of *IGF2BP3*, the 24 ALL cell lines were divided into 14 cell lines with high expression (red) and 10 cell lines with low expression (blue). **(B)** Z score, the mean sensitivity score, of 24 ALL cell lines mentioned above to 345 anti-tumor compounds. *IGF2BP3* Low: Z score of 10 cell lines with low levels of *IGF2BP3* expression, marked in black; *IGF2BP3* High: Z score of the 14 cell lines with high levels of *IGF2BP3* expression, marked in red. **(C)** Representative flow-cytometry plots showing venetoclax-induced apoptosis in Ba/F3 cells overexpressing vector-PMSCV, PAX5 (WT), or PAX5-ETV6. **(D)** Representative flow-cytometry plots showing venetoclax-induced apoptosis in Ba/F3 cells overexpressing vector-PMSCV, STK38 (WT), or STK38-PXT1. **(C-D)** Apoptosis of cells induced by 2 μ M venetoclax was investigated by flow cytometry with Annexin V/PI double staining. **(E)** Quantification of venetoclax-induced apoptosis in BaF3 cells overexpressing vector-PMSCV, PAX5(WT), PAX5-ETV6, STK38(WT) and STK38-PXT1. **(E)** Data are represented as mean \pm SD (n=3). The significance analysis was conducted by one-way ANOVA analysis. n.s., P>0.05; *, P<0.05 vs. PMSCV. n.s., P>0.05; ##, P<0.01; ###, P<0.001 vs. indicated.

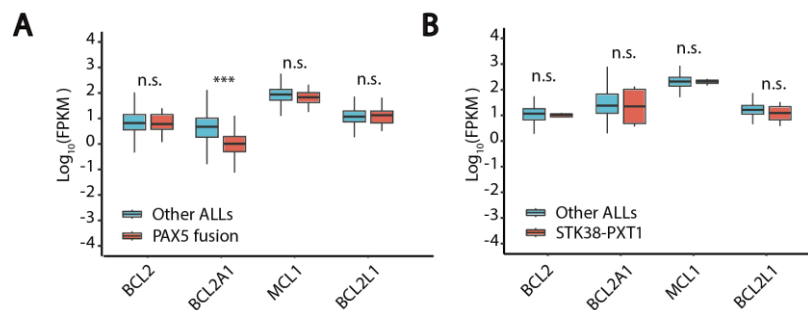


Figure S8. The expression level of anti-apoptotic family proteins in ALL with PAX5 fusion or STK38-PXT1. (A) Log₁₀(FPKM) of *BCL2*, *BCL2A1*, *MCL1*, and *BCL2L1* in PAX5 fusion versus other ALLs (TARGET-ALL). **(B)** Log₁₀(FPKM) of the same genes in STK38-PXT1 versus other ALLs (CNpALL). **(A, B)** The significance analysis was conducted by two-tailed unpaired Student's t-test. n.s., P>0.05; ***, P<0.001.

Supplementary tables

Table S1. Specific PCR primers

Name	Specific PCR primers
GAPDH-F	GTCATCCATGACAACCTTTGG
GAPDH-R	GAGCTTGACAAAGTGGTCGT
PAX5-F	GAAAAATTATCCGACTCCTC
PAX5-R	TGTGACTGGAAGCTGGGACTG
STK38-F	AGCACATGCTCGGAAGGAAA
STK38-R	GAACAAGCCGTACCTCACCA
Igf2bp3-F	CAAAGGGGTTGGGGAGGAAG
Igf2bp3-R	ATGGAGCCGGTGGATTTTGT

Table S2. The fusions with mRNA upregulation of partner gene.

Fusion.name	Left partner gene		Right partner gene	
	log ₂ FC	log ₁₀ (P_value)	log ₂ FC	log ₁₀ (P_value)
AL713998.3--AL713998.1	6.222048	2.134122243	5.791141	1.841809223
PDCD6IPP1--AC138649.5	4.86912	1.764061415	5.166115	3.634786228
PHACTR3--RBM38	4.701417	6.272734997	1.971395	5.018697322
AC139491.6--AC139491.8	4.599559	14.22716379	5.764199	9.670982801
SIK1--SERINC2	3.840083	1.71404285	2.458446	2.192216512
AC006427.2--TAPT1-AS1	3.643966	1.761458682	1.826769	1.438534951
AC110023.1--AC091078.1	3.058589	3.282287334	3.008313	2.900328077
SLC7A5--ZFPM1	2.614066	1.766959358	2.759086	1.060941258
PIM3--PLXNB2	2.518564	1.775492059	2.187941	1.686920832
RNU6-3P--SNORD13	2.454678	2.076627979	1.809349	1.411251677
GLT1D1--SLC15A4	2.397478	1.643675477	1.851806	1.725990649
LINC00621--SLC25A13	2.356188	1.348566296	2.304524	1.060942188
RNU6-9--SNORA20	2.315168	11.08128863	1.094784	4.302725212
AC008740.1--CRYM-AS1	2.214831	5.458771323	2.137733	5.581563853
CTSD--LSP1	2.167376	1.659978638	1.287203	1.363803275
RNU6-2--SNORD13	2.113436	13.69015362	1.801866	13.98454563
PAX5--ETV6	2.105401	3.298874131	1.604696	3.3960304
PAX5--NOL4L	2.082179	2.113837942	2.401667	2.462575011
SLC7A5--CBFA2T3	2.081732	2.777109361	1.117829	0.68931774
MKNK2--MOB3A	1.968826	6.092565238	1.992339	6.8416633
SIK1--KLF2	1.946382	1.503318294	1.263268	1.427811573

FLT1--CDX2	1.910141	1.909698103	9.928784	2.518161477
RNU6-9--SNORD13	1.850025	1.83783521	1.683141	1.483806425
RNU6-33P--Z93241.1	1.840877	9.636695131	1.391302	4.951582748
PAX5--AUTS2	1.801358	1.427010118	2.676011	1.796913581
RNU6-4P--TMEM107	1.798287	2.995319533	1.090378	1.397981433
YPEL5--GNAS	1.793707	4.039126132	1.092312	3.593744883
RNU6-33P--SNORD13	1.758092	2.660786228	1.916942	1.854911133
MKNK2--METRNL	1.661278	2.485593428	1.269334	2.428944366
PAX5--CBFA2T3	1.617686	1.647705178	1.240506	1.806007696
ZEB1--GNAS	1.610407	1.643675477	1.694615	1.06067047
NUP214--ABL1	1.550979	1.778422723	1.551588	1.787516336
SLC66A2--CTDP1	1.524007	2.790264913	1.430831	1.369098227
ZCCHC7--PAX5	1.416107	1.480614788	1.397932	1.571258862
MEF2D--HNRNPUL1	1.404458	1.512580764	1.072729	1.560288161
AL365295.1--AL356020.1	1.383408	1.995268624	1.403986	5.394051996
ETV6--ARNT	1.337475	1.556284643	3.509374	1.835795692
PLEKHO2--SLC51B	1.205654	1.349005212	3.656317	2.010477727
PABPC1--EIF1B	1.200483	2.43930395	1.960306	2.861206281
PAX5--ZCCHC7	1.191649	1.894716082	1.148316	1.951070679
STK38--PXT1	1.189507	3.041253327	4.363556	3.152875952
NOL4L--RBM38	1.172027	1.44238654	1.638697	1.438534823
LPCAT1--CLPTM1L	1.132157	1.796601183	1.286005	2.461716995
CTDP1--NFATC1	1.129225	2.216063689	2.823167	1.074809362
TPM4--PTMA	1.098715	1.362558488	1.292914	1.662029018
MOB3A--GNA15	1.087105	1.958851932	1.331997	2.711614965
BSG--RNF126	1.055961	1.340157582	1.369012	1.754198856
MKNK2--REXO1	1.02069	1.319644545	1.009528	1.659978638
DUX4L4--IGH@-ext	10.26654	60.38100704	-0.691958	0.132456367
DUX4L4--IGH@-ext	6.810196	15.18101248	-1.507835	0.391179905
TLX1NB--TRDC	6.153203	1.331675517	-0.730636	0.399603991
AC093642.5--RPL23AP60	5.726451	1.735665329	-13.09306	0.019201268
TLX1NB--RPP30	5.575933	2.293823619	0.741371	1.450105111
NKX2-1--TRAJ42	5.129953	1.335199331	0.042343	0.487678455
SIK1--GNAS	4.863286	1.793284543	-0.10745	0.145274497
DUX4--IGH@-ext	4.649656	9.02165989	-1.507835	0.391179905
SFTA3--TRDC	4.04183	1.490716004	0.075894	0.490071166
SIK1--PTMA	3.278034	3.276562988	0.197299	0.319857808
TRDV2--TRAC	3.221798	15.6876546	0.751805	3.583063541
NKD2--SLC12A7	3.198069	2.866334479	0.500602	1.185859146
SEMA6A--FEM1C	3.002357	1.670306271	0.812881	4.714339513
AC007952.4--SNORD3H	2.884153	1.041955939	8.674227	3.530850856
SIK1--ACTB	2.668294	2.180045659	-0.053547	0.117140961
BCL9--MEF2D	2.641028	1.818463484	0.175216	0.300370928
DUX4--IGH@-ext	2.550988	8.754919041	-1.183849	0.13403449

SYTL3--EZR	2.378346	1.485022989	0.410461	0.531947913
PIM3--CRELD2	2.284602	1.653772609	0.716197	0.785135412
RNU6-3P--RPL23	2.275656	1.700006448	0.977521	1.295896892
RNU6-3P--EIF4A2	2.224215	3.825448441	-0.132799	0.192578829
RNU6-9--CSKMT	2.216114	26.93004357	0.728535	8.13863336
RNU6-4P--SNORA20	2.205429	21.03201052	0.601532	7.609866472
BCL2L11--PTMA	2.197166	2.900815977	0.235907	0.512538447
RNU6-3P--SNHG12	2.169115	4.297595314	0.61965	1.853470271
ZNF384--TCF3	2.164294	1.725990649	-0.831292	0.423190576
RNU6-9--SCARNA11	2.125562	4.950229205	0.34445	1.978111169
MKNK2--DAZAP1	2.105134	2.155955332	-0.501932	0.437985664
MAFF--CSNK1E	2.07668	10.81198971	0.518081	4.205758205
RNU6-33P--RPSA	2.075424	8.353135154	0.683799	2.409292758
RNU6-36P--EIF4A2	2.071509	4.349438344	-0.065109	0.00600977
PIK3R5--UBB	2.070434	1.627452128	-0.694601	0.966242707
PAX5--BCOR	2.066366	1.52840224	0.534798	0.817279853
RNU6-3P--NCL	2.060368	13.44153846	-0.046821	0.283150874
GNA11--SGTA	2.040955	1.742693797	0.90639	1.082670945
FBRSL1--NOC4L	2.024921	3.715905095	0.289741	0.822837255
GNA11--CSNK1G2	1.995971	1.692824648	0.136743	0.32723613
RNU6-4P--SNORA14B	1.957311	7.911963037	0.643799	3.174087354
RNU6-1--RPL23	1.954321	1.596551321	0.810292	0.90480322
PIM3--BRD1	1.925519	7.011071538	0.772686	1.493731766
MAFK--PTMA	1.907675	1.516118085	0.043648	0.044135327
CXCR4--RBM38	1.898152	1.069427035	1.411131	1.321628025
MKNK2--DOT1L	1.89701	2.59074734	0.775478	1.521938439
PFKFB3--RBM17	1.88239	1.438973848	0.372886	0.916480242
NKD2--CLPTM1L	1.836044	1.537549677	0.531266	0.785859446
YPEL5--PTMA	1.810482	8.131180234	0.282185	1.314909724
FGFRL1--CTBP1	1.796572	3.376841466	-0.236854	0.029363192
GNA12--RNPC3	1.796084	1.611308529	0.051339	0.072067097
RORA--B2M	1.787104	1.516118085	0.098801	0.171665964
MAP2K3--TMEM11	1.768672	1.643675477	0.026234	0.060716321
NADK--SSU72	1.750879	1.838607474	0.11106	0.246761111
SEMA6A--NR3C1	1.748182	1.363803275	0.35229	0.343584093
AC078983.1--CAND1	1.723392	2.060855526	0.242447	0.400079092
TAL1--TRDC	1.701255	1.306894737	0.663506	1.038796387
CDKN1A--EEF1A1P5	1.676123	2.115963778	0.420951	0.962348828
SENP3-EIF4A1--GNAS	1.653588	1.725995497	0.35975	0.867929142
MKNK2--CSNK1G2	1.632167	1.490601217	0.01594	0.139265334
FOCAD--LINC01239	1.589218	1.068601532	6.106922	2.185785454
SLC16A3--RNF166	1.574675	1.122860951	2.080496	1.759477123
TXLNG--AP1S2	1.535681	1.759477123	0.884202	1.219346502
AC010332.1--ZNF880	1.513516	0.859356959	1.554774	1.576353064

MAFK--SUN1	1.509876	1.354209525	0.89497	1.014452101
ABL1--BCR	1.493564	2.372857499	0.148172	0.29106765
CDKN1A--GNAS	1.438914	1.423782123	-0.004348	0.101497292
KLF10--YWHAZ	1.391784	13.47151315	0.261255	2.482086545
KLF10--AZIN1	1.383321	10.38847331	0.427835	2.134252348
PIM3--PTMA	1.342965	1.692824648	-0.252799	0.220309735
GNA15--MOB3A	1.322221	1.298261364	1.923496	1.736291832
AC131097.3--THAP4	1.320508	3.321934242	0.583736	1.902714941
ITGB2--SUMO3	1.316731	4.744958218	0.200213	0.326256051
AC244502.1--TLX1NB	1.30984	0.751531255	6.242535	1.337754601
ARHGEF7--ING1	1.29711	2.290225974	-0.111987	0.001763831
LSP1--SF1	1.287203	1.363803275	0.132153	0.272143969
SH3TC1--IGH@-ext	1.282154	1.378679924	-0.82903	0.107565907
ARHGAP12--AC012494.1	1.271837	0.959673438	8.168267	2.135541207
MOB3A--IGH@-ext	1.268871	2.457185795	-0.96385	0.0234908
SCAF4--MYH9	1.189933	1.595244616	0.586191	1.261862923
B3GNT5--KLHL24	1.186879	8.1670402	0.050257	0.190959229
CXCR4--PTMA	1.167698	7.705823183	0.795451	9.376443917
SEN3P3--EIF4A1--METRNL	1.158849	0.978875896	1.156319	1.45424452
FLT1--HMGB1	1.158563	2.175162409	0.185988	0.331303519
BACH2--SYNCRIP	1.146014	2.812439616	0.385664	2.061691199
SLC7A5--BANP	1.136214	5.158198541	0.839304	4.936070986
SCAF4--SON	1.105797	1.516118085	0.67122	1.1913914
BACH2--PNRC1	1.09307	1.455203223	0.500557	1.490439282
YPEL5--BCL11A	1.080265	2.557340429	0.855468	1.58134776
YPEL5--YWHAZ	1.05862	1.393635234	0.301544	0.571357393
PRDM10--APLP2	1.047056	1.393635234	-0.026054	0.234790927
ZNF384--EP300	1.044715	3.02929014	0.093761	0.516636176
LATS2--ZMYM2	1.023834	6.812329484	0.246897	2.4052188
YWHAZ--ZNF706	1.018671	1.775492059	0.315192	1.772815414
YPEL5--FOSL2	1.014619	1.26678974	1.166204	3.328234925
SH3TC1--TBC1D14	1.004686	2.038956335	0.601059	0.839350711
RUNX1--LHFPL3-AS1	-0.854597	0.201465225	10.39868	2.498071182
MLLT10--AC097462.3	0.465618	0.551948923	10.1463	1.514435222
ITPR1--RIT2	0.505513	0.506957152	9.824007	1.984285788
PGS1--GIP	0.384528	0.68931774	9.670081	1.859531426
CCDC26--AC103718.1	0.790115	0.986725065	8.88815	1.838040324
TCF3--HLF	0.812444	1.374308526	8.198867	3.235485537
TCF3--PBX1	0.895484	4.197595268	7.969384	8.093606845
PRKCB--CACNG3	-0.521821	0.172416285	7.903027	1.635523522
RUNX1--RUNX1T1	-0.047595	0.130771164	7.633872	2.575342775
ZCCHC7--AC114757.1	0.830649	0.587609992	7.318608	1.39515141
CRBN--GABRB2	-0.61754	0.628745335	7.244197	2.596598089

EWSR1--FEV	-1.744699	1.032657383	7.067241	1.391735204
RUNX1--BCL2L14	0.006252	0.450743807	6.957345	1.933064772
FDFT1--DEFB135	0.216829	0.284619685	6.517531	10.03804189
OAZ1--PQLC1	0.903537	1.042468011	6.425012	13.06550717
MAD1L1--ELFN1	0.892929	0.334849272	6.394255	1.835740032
RUNX1--MMP16	0.335835	0.705906845	6.367398	1.88692166
TPGS2--CCDC178	-0.414049	0.241900789	6.324548	1.46081299
USP9Y--XGY1	-5.705099	0.166676487	6.150875	1.572571222
B2M--AC107398.3	0.185868	0.392192913	5.699795	1.810781928
DIAPH1--JAKMIP2-AS1	-0.042938	0.052269846	5.456419	2.027119725
ETV6--BCL2L14	-1.43195	1.043171618	5.260303	3.372552419
PSMD2--ECE2	0.043883	0.100477399	5.178203	1.413019686
KMT2A--CT45A1	-1.262666	0.657910572	5.121956	2.372128999
AC131097.3--AC093642.5	0.422351	0.237317085	5.079359	6.810836714
CPSF6--SPATA48	-0.552948	0.627738925	5.05181	1.839911491
CD74--CAMK2A	-0.904951	3.042408181	4.672377	8.953540282
IGH@-ext--CRLF2	0.242889	0.153471236	4.632812	3.061106047
GPSM2--SPATA42	-0.182091	0.028821434	4.397726	1.388255956
SLC12A7--IRX2	0.940984	0.688516243	4.271735	5.872147541
CD81--DUSP8	-0.805118	1.025776127	4.008108	4.314581011
IGH@-ext--DUX4L4	-1.487923	0.036312868	3.760177	5.221065813
P2RY8--CRLF2	-0.792397	0.955813139	3.687682	3.10480948
B2M--SKI	0.737025	0.901717781	3.544577	1.771223212
EIF4A1--KLF2	-0.364888	0.259420026	3.528952	2.479069303
TTYH3--PDGFA	-0.226552	0.405446555	3.372324	2.483201805
EBF1--PDGFRB	0.655933	0.514164573	3.353728	1.703618297
OAZ1--KLF2	0.200338	0.283470093	3.337182	6.060896601
CD74--ARSI	-0.484012	1.76234915	3.268155	11.07016078
SLC12A7--NKD2	0.36261	0.434416194	3.238995	2.390267549
MAFK--PDGFA	0.638339	2.298743939	3.160024	4.845746714
IGH@-ext--DUX4	-1.487923	0.036312868	3.108713	3.373665264
IGH@-ext--DUX4	-2.405588	0.2214723	3.053589	3.304396159
ARID4B--GNG4	-0.38465	0.204317157	2.979813	1.703621533
MEF2D--BCL9	0.325981	0.561573664	2.95063	2.541422904
PAX5--VPS9D1-AS1	-1.601833	0.052269849	2.873202	1.796913581
OAZ1--TMPRSS9	0.033241	0.138834114	2.827957	1.81031046
LAPTM5--SERINC2	0.394437	0.855979871	2.774279	1.776340686
MAD1L1--PDGFA	0.822349	0.928807039	2.75312	1.643677006
PIM3--SIK1	0.630783	1.122511277	2.656698	2.176856738
CBFA2T3--ZFPM1	0.836132	1.038773643	2.616418	1.775492168
ANKRD11--SLC7A5	0.989449	1.38136395	2.614066	1.766959358
B2M--RBM38	0.317001	0.614658265	2.608318	1.775492032
OAZ1--SERINC2	-0.451243	0.611345802	2.466227	2.689110402
SH3TC1--WFS1	0.17695	0.503412567	2.165493	2.083520727

TCF3--ZNF384	-0.202669	0.178297246	2.164294	1.725990649
OAZ1--MKNK2	-0.428086	1.093063016	2.162514	4.991195543
U3--KCTD1	-0.085534	0.265598498	2.154447	7.164936042
GSE1--SLC7A5	0.438568	0.633048726	2.150025	1.982616486
PPP6R2--SCO2	-0.269515	0.420542564	2.08562	1.725990649
BCOR--PAX5	0.534798	0.817279853	2.066366	1.52840224
JARID2--PTP4A1	0.337347	0.751806892	2.025401	1.62745969
PLXNB2--SCO2	-0.068101	0.234708434	2.016351	2.192216512
OAZ1--REXO1	-0.249692	0.271209703	1.869666	1.827119238
ST3GAL1--NDRG1	0.86738	8.629284776	1.852136	18.82898612
TRABD--SCO2	-0.962742	0.797359207	1.820837	1.563355593
YWHAZ--KLF10	0.292077	1.358612977	1.778159	9.860938405
CYTH1--USP36	0.7792	10.69859267	1.769663	19.18328324
SBF1--SCO2	-0.230157	0.098582041	1.744357	1.924078524
ETV6--NCOA2	0.823411	0.681226398	1.714163	1.637320947
LMNB2--MKNK2	0.57558	0.718422002	1.669642	1.676361675
KLHDC4--SLC7A5	-1.14745	1.108990897	1.663555	1.649652019
GPSM1--RALGDS	0.080206	0.392192918	1.647572	1.6046623
OAZ1--KLF16	-0.320881	1.271592476	1.611146	4.480461585
LAPTM5--TSC22D3	0.746147	1.643675477	1.583448	1.45424452
HNRNPK--DAPK1	0.255355	0.864407446	1.534868	2.303911415
EGFL7--AGPAT2	0.205877	0.42406424	1.534234	2.711617643
CBFA2T3--SLC7A5	0.493413	1.511061806	1.528605	4.426929751
GPSM1--NOTCH1	0.978447	0.964346262	1.469931	1.595244616
TTYH3--LFNG	0.912733	1.35672781	1.412477	2.833217006
PFKP--KLF6	-0.078042	0.234311399	1.40846	3.902035016
SLC12A7--CLPTM1L	0.044563	0.145312828	1.392098	2.023400813
RGPD5--BCL2L11	0.804401	1.466626905	1.35564	1.82496488
MBP--ZNF516	0.237228	0.785696582	1.34967	4.084063992
OAZ1--TCF3	0.694768	0.017489021	1.346664	2.132930029
ZC3H7A--SNN	0.826674	1.500530924	1.344521	1.393635234
YWHAE--CRK	0.861654	1.588455014	1.34292	1.60872672
BCR--ABL1	-0.201064	0.12961818	1.338937	4.923060986
HMG2--STMN1	0.859339	1.508820818	1.31679	1.814217768
DDX3X--MLLT10	-0.946216	0.861774044	1.311994	2.332636373
OR51S1--TP53I11	-2.63103	0.689008836	1.311833	11.76059439
MTAP--CDKN2B-AS1	-0.447408	0.281106494	1.301153	2.363139981
BANP--SLC7A5	0.794949	2.262069411	1.293492	4.458361579
FBRSL1--RBM38	0.020104	0.420542564	1.239836	1.627452128
AGPAT3--SUMO3	0.129924	0.242129704	1.238352	1.70936762
YAF2--RYBP	0.359041	3.637435302	1.225131	8.531883765
G3BP2--CNOT6L	0.708022	1.563355593	1.21743	1.45424452
MAD1L1--MAFK	0.471469	1.620410637	1.209478	2.208348153
OAZ1--SGTA	0.627124	0.020014493	1.179691	2.234393284

CXCR4--GNAS	0.893958	1.510749519	1.169315	2.616440091
GALNS--RNF166	0.850203	0.448030957	1.164768	2.154901604
GRAMD4--CERK	0.446789	0.732561518	1.157083	3.101243823
HNRNPA1P49-- HNRNPA1	0.538255	0.633003909	1.141056	1.766959358
TTYH3--METRNL	0.163888	0.045120532	1.135452	3.477334011
CXCR4--KLF11	0.159951	0.356424241	1.130711	1.306195395
B2M--GNAS	0.386147	1.811379658	1.127635	7.983650443
CPSF6--CSNK1D	0.141351	0.303222124	1.120189	1.363803275
NUP98--RAP1GDS1	-0.349163	0.310382214	1.111837	1.385140115
OAZ1--METRNL	0.048558	0.249315588	1.107374	3.661834016
WDR6--RBM38	-0.438711	0.676263821	1.061754	1.866881551
PPP6R2--SBF1	-0.245096	0.7227021	1.044262	2.901953674
SBNO2--TMEM259	0.9267	1.095989907	1.022378	1.692824648
TTYH3--RBM38	0.092062	0.099316976	1.021337	2.290225974
TET3--AC073046.1	0.354851	0.639527095	1.006484	1.719076732
TPM4--PTMAP5	0.557796	1.060920921	1.006114	1.455705994

Table S3. The prediction of m6A modification sites in *PAX5-ETV6* fusion

Position	Partner	Sequence	Score (combined)
43	<i>PAX5</i>	CAGCA GG <u>A</u> CA GGACA	0.583
48	<i>PAX5</i>	GGACA GG <u>A</u> CA UGGAG	0.568
614	<i>ETV6</i>	AAAGA GG <u>A</u> CU UUCGC	0.590
843	<i>ETV6</i>	CCAUU GA <u>A</u> CU GUUGC	0.589
1322	<i>ETV6</i>	AUAGC AG <u>A</u> CU GUAGA	0.561
1437	<i>ETV6</i>	CCAAC GG <u>A</u> CU GGCUC	0.599

Table S4. The prediction of m6A modification sites in *STK38-PXT1* fusion

Position	Partner	Sequence	Score (combined)
545	<i>STK38</i>	AAAAA AG <u>A</u> CA CUCUG	0.564
565	<i>STK38</i>	AGAGG AG <u>A</u> CU CAGUU	0.567
602	<i>STK38</i>	GCCAU AG <u>A</u> CU CUAUU	0.651
662	<i>STK38</i>	CUUUU GG <u>A</u> CA GCAAG	0.607
681	<i>STK38</i>	AUGUG AA <u>A</u> CU UUCUG	0.582
689	<i>STK38</i>	CUUUC UG <u>A</u> CU UUGGU	0.621

708	<i>STK38</i>	GCACA GG <u>A</u> CU GAAAA	0.766
727	<i>STK38</i>	ACAUA GG <u>A</u> CA GAAUU	0.667
