Supplemental Materials for

FBXW7β isoform drives transcriptional activation of a proinflammatory TNF cluster in human pro-B cells

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Contains Supplemental Methods, Supplemental Figures, and Supplemental Tables

SUPPLEMENTAL METHODS

Dataset usage
We accessed RNA-seq data from the St. Jude Cloud, an initiative of St. Jude Children's Research Hospital (https://www.stjude.cloud) and from the Cancer Cell Line Encyclopedia (CCLE) (https://sites.broadinstitute.org/ccle). St. Jude Cloud data used for this analysis (EGAD00001002704 and EGAD00001002692) were accessed by permission from the Computational Biology Committee through the European Bioinformatics Institute (EMBL-EBI).

Spearman correlation
Correlations and their significance were computed using the nonparametric Spearman's rank-order correlation implemented in R function cor.test().

Cell culture
REH cells were maintained in RPMI-1640 medium supplemented with 10% FBS, 2 mmol/L l-glutamine, 25 mM HEPES, and antibiotic-antimycotic at 37°C and 5% CO_2. 293T cells were cultured in DMEM with the same supplements.

Cell lysis for immunoprecipitation (IP) and immunoblotting (IB)
Cells were centrifuged at 250 × g for 5 minutes. The cell pellet was lysed on ice in buffer containing 150mM NaCl, 50mM Tris pH 8.0, 1% Triton X-100, and 2 × Halt protease and phosphatase inhibitors (Thermo Fisher 78446, 100×). Protein concentration of lysates was maintained at 2 – 4 µg/µL. Cell debris was pelleted at 5000 × g for 5 minutes. Supernatant was collected for direct IB or IP before IB.

In-solution digestion prior to mass spectrometry
Cell pellets were lysed solubilized and digested with the iST kit (PreOmics GmbH, Martinsried, Germany) per manufacturers protocol. Briefly, the resulting pellet was solubilized, reduced and alkylated by addition of SDC buffer containing TCEP and 2-chloroacetamide then heated to 95°C for 10 minutes. Proteins were enzymatically hydrolyzed for 1.5 hours at 37°C by addition of LysC and trypsin. The resulting peptides were de-salted, dried by vacuum centrifugation and reconstituted in 0.1% TFA containing iRT peptides (Biognosys Schlieren, Switzerland).

Mass Spectrometry (MS) Analysis
Samples were analyzed on a QExactive HF mass spectrometer (Thermo Fisher Scientific San Jose, CA) coupled with an Ultimate 3000 nano UPLC system and EasySpray source. Peptides were separated by reverse phase (RP)-HPLC on Easy-Spray RSLC C18 2um 75 µm id × 50 cm column at 50°C. Mobile phase A consisted of 0.1% formic acid and mobile phase B of 0.1% formic acid/acetonitrile. Peptides were eluted into the mass spectrometer at 300 nL/min with each RP-LC run comprising a 95-minute gradient from 1 to 3% B in 5 min, 3-45%B in 90 min. The mass spectrometer was set to repetitively scan m/z from 300 to 1400 (R = 120,000) followed by data-dependent MS/MS scans on the twenty most abundant ions, dynamic exclusion with a repeat count of 1, repeat duration of 30s, (R=15000) and a nce of 27. FTMS full scan AGC target value was 3e6, while MSn AGC was 2e5, respectively. MSn injection time was 32 ms; microscans were set at one. Rejection of unassigned, 1, 6-8 and >8 charge states was set.

MS raw data processing
Peak lists obtained from MS/MS spectra were identified using a combination of three search engines (MSGF+, Comet, and X!Tandem).The search was conducted using SearchGUI. Protein

identification was conducted against a concatenated target/decoy version of the Homo sapiens complement of the UniProtKB. The decoy sequences were created by reversing the target sequences in SearchGUI. The identification settings were as follows: Trypsin, Specific, with a maximum of 2 missed cleavages, 10.0 ppm as MS1 and 0.02 Da as MS2 tolerances; fixed modifications: Carbamidomethylation of Cys, variable modifications: Oxidation of Met and Acetylation of protein N-term. Peptides and proteins were inferred from the spectrum identification results using PeptideShaker version 1.16.45. Peptide Spectrum Matches (PSMs), peptides and proteins were validated at a 1.0% False Discovery Rate (FDR) estimated using the decoy hit distribution.

Plasmids, transfections, viral vectors, and transductions

FBXW7 expression plasmids for transient overexpression:

Each FBXW7 isoform ORF (without the stop codon) was cloned into a pcDNA3.1+ plasmid backbone (pcDNA3.1+/C-(K)-DYK vector) that contained the Kozak sequence N-terminal to the gene insert and a FLAG tag C-terminal to the gene insert by Genscript. The empty pcDNA3.1+ vector control used in the experiments did not contain a FLAG tag. These plasmids were used for transient overexpression in 293T cells via the Lipofectamine 3000 reagent (Thermo Fisher).

Plasmids for CRISPR-Cas9 genome editing:

LentiV_Cas9_puro (cc60) viral vector (gift from Junwei Shi) was used to transduce the REH cell line, yielding the polyclonal REHCas9 cells after puromycin selection.

Each of the CRISPR guide sequences was cloned into the Lenti_gRNA-GFP(LRG)_2.1T plasmid individually. The empty LRG_2.1T plasmid was a gift from Junwei Shi. After cloning, the two CRISPR guide plasmids (in their DNA form) were electroporated into REHCas9 cells simultaneously with the Lonza AMAXA Nucleofector 2b device (device program O-013 and Nucleofector Solution V were used for REH). GFP+ electroporated cells were FACS-sorted into single cell clones at 48h post-transfection. Because the two guides targeted introns that flank the exon of interest, the transient expression of the two guides allowed removal of the specific exon in some of the single cell clones selected through GFP expression. Single cell clones were expanded and subject to PCR-based genotyping to screen for successful editing by the two guides (“2-cut”).

Viral FBXW7 expression plasmids for stable reconstitution or overexpression:

The following sequences were gene synthesized and subcloned into the MIGR1 retroviral vector with the BglII and EcoRI restriction enzymes – the Kozak sequence, start codon, N-terminal VSVG tag, respective FBXW7 isoform ORF, C-terminal FLAG tag, and two stop codons (TGATAA).

CRISPR-Cas9 genome editing and reconstitution

REH cells were stably transduced with LentiV_Cas9_puro (cc60) viral construct, yielding the REHCas9 cells. To generate a pan-FBXW7 KO single-cell clone, REHCas9 cells were transiently transfected with two CRISPR Lenti_gRNA-GFP(LRG)_2.1T DNA constructs simultaneously to remove the coding exon 2 of FBXW7. Finally, the pan-FBXW7 KO cells were stably reconstituted with individual FBXW7 isoform MIGR1 retroviral constructs that carried EGFP for Fluorescence-Activated Cell Sorting (FACS) selection.

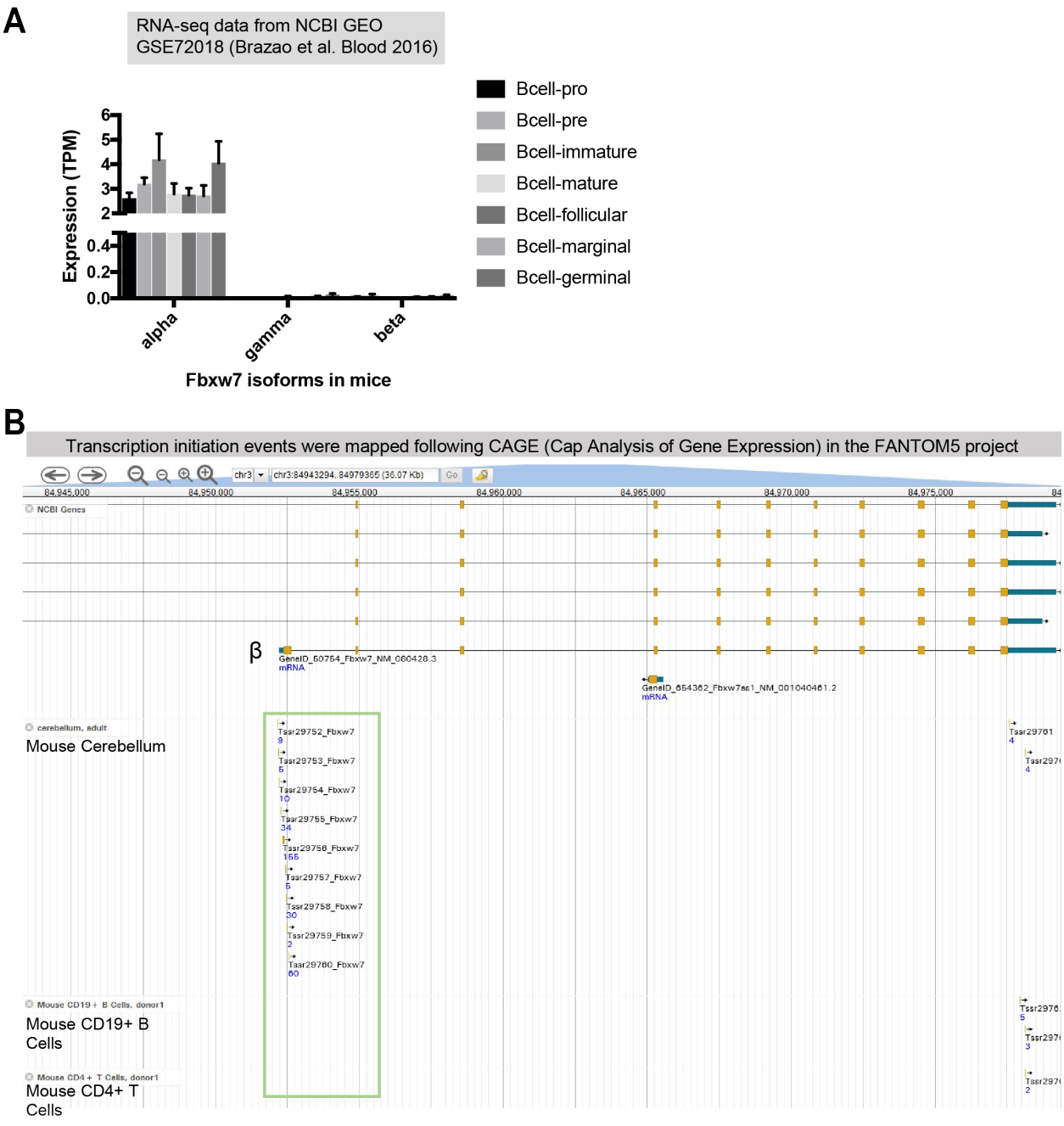
Custom antibody generation

FBXW7β peptide-KLH conjugate antigen preparation, immunization of BALB/c and C57BL/6 mice, and antiserum preparation were performed by GenScript. Antigen peptide sequences are indicated in Supplemental Figure 4.

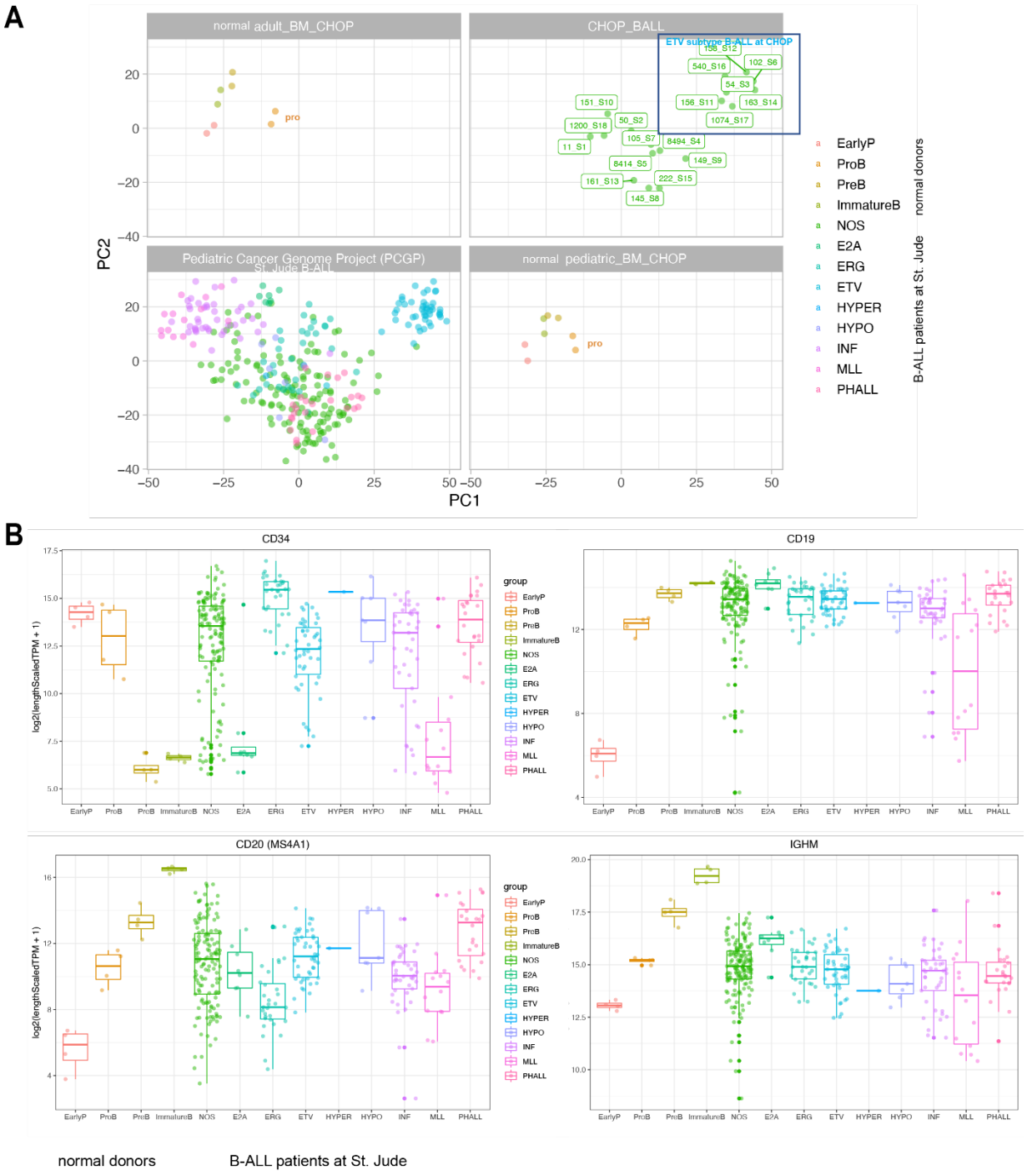
MG132 treatment
REH cells were left untreated or treated with 1:1000 DMSO vehicle or 10 μM MG132 (Peptide Institute 3175-v) for 4 hours in complete RPMI-1640.

Immunoblot densitometry
ImageJ bundled with Java 1.8.0_172 for Mac OS X (NIH) was used for quantification of overexpressed or endogenous FBXW7 protein isoforms. Peak area measurements were normalized to those of GAPDH control, and the values were further divided by those of lane 1.

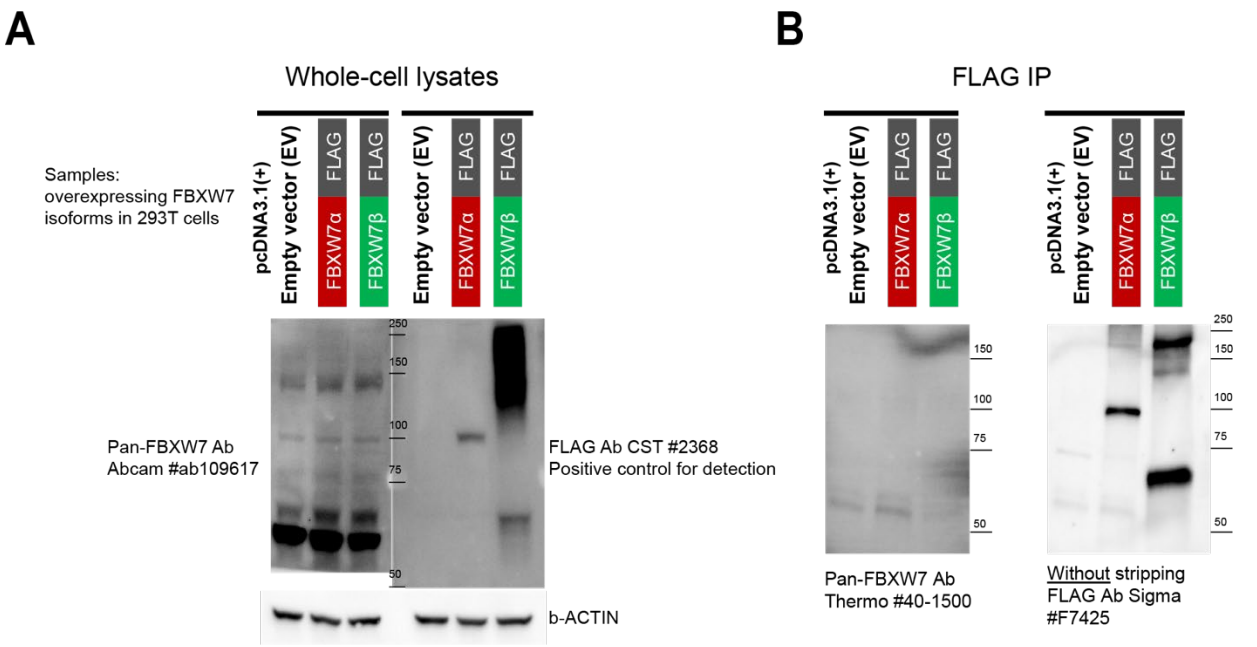
SUPPLEMENTAL FIGURES



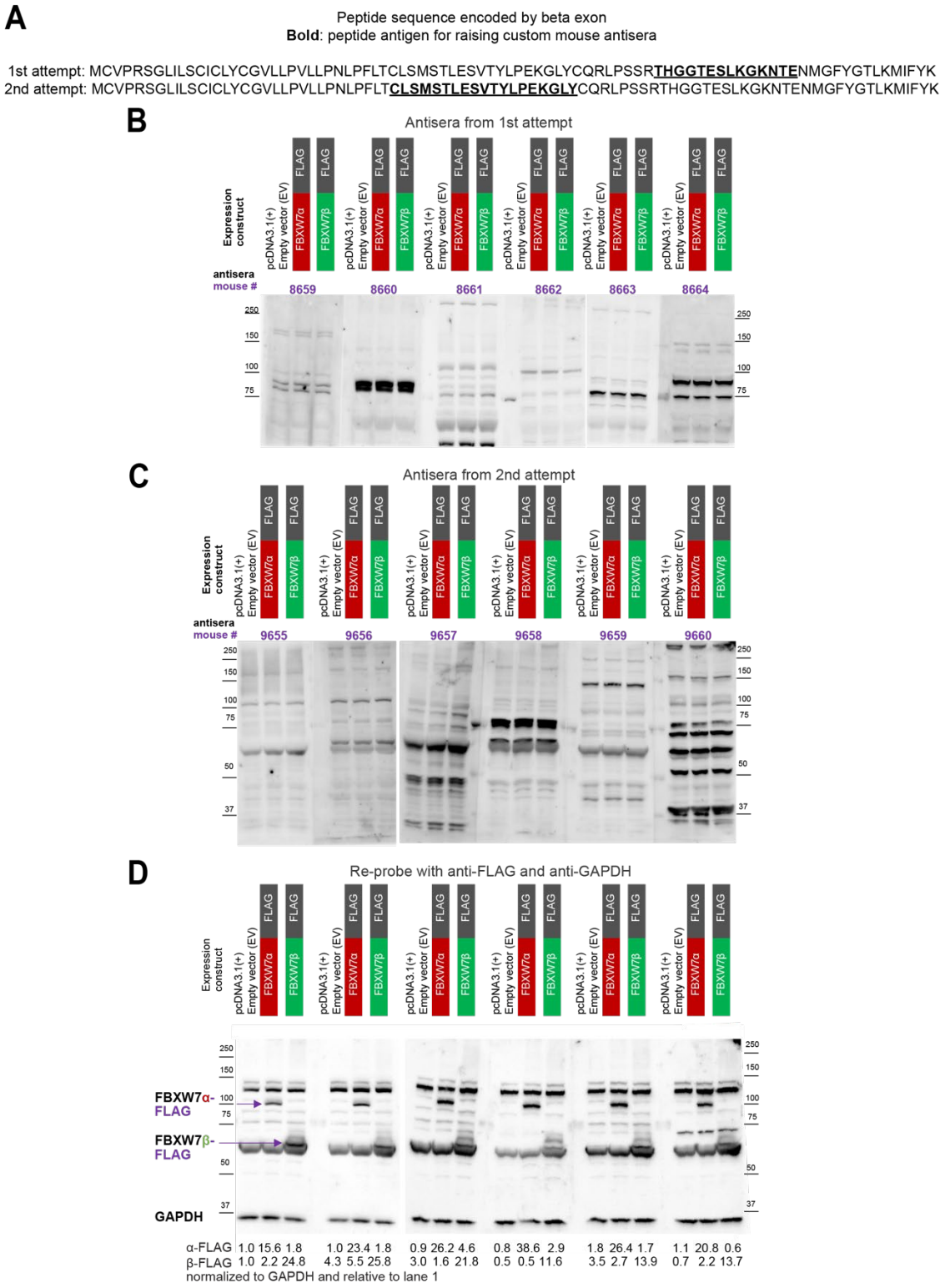
Supplemental Figure 1. Existing RNA-seq and 5' Cap Analysis of Gene Expression (CAGE) datasets provide no evidence of *Fbxw7β* expression in mouse hematopoietic tissues. (A) RNA-seq of various mouse B-cell subsets from NCBI GEO GSE72018 (Brazao T et al. Blood 2016) was reanalyzed to quantify *Fbxw7* isoform expression. HTSeq v0.8.0 was used in intersection-strict mode against the Ensembl mouse gene annotation (GRCm38/mm10 v87). **(B)** FANTOM5 CAGE transcriptional start site mapping dataset. Mouse *Fbxw7β* RNA expression is detected in nervous tissues but not in lymphocytes. Viewed on Mouse Genome Informatics (MGI) at Jackson Labs: https://tinyurl.com/Mm-FBXW7b



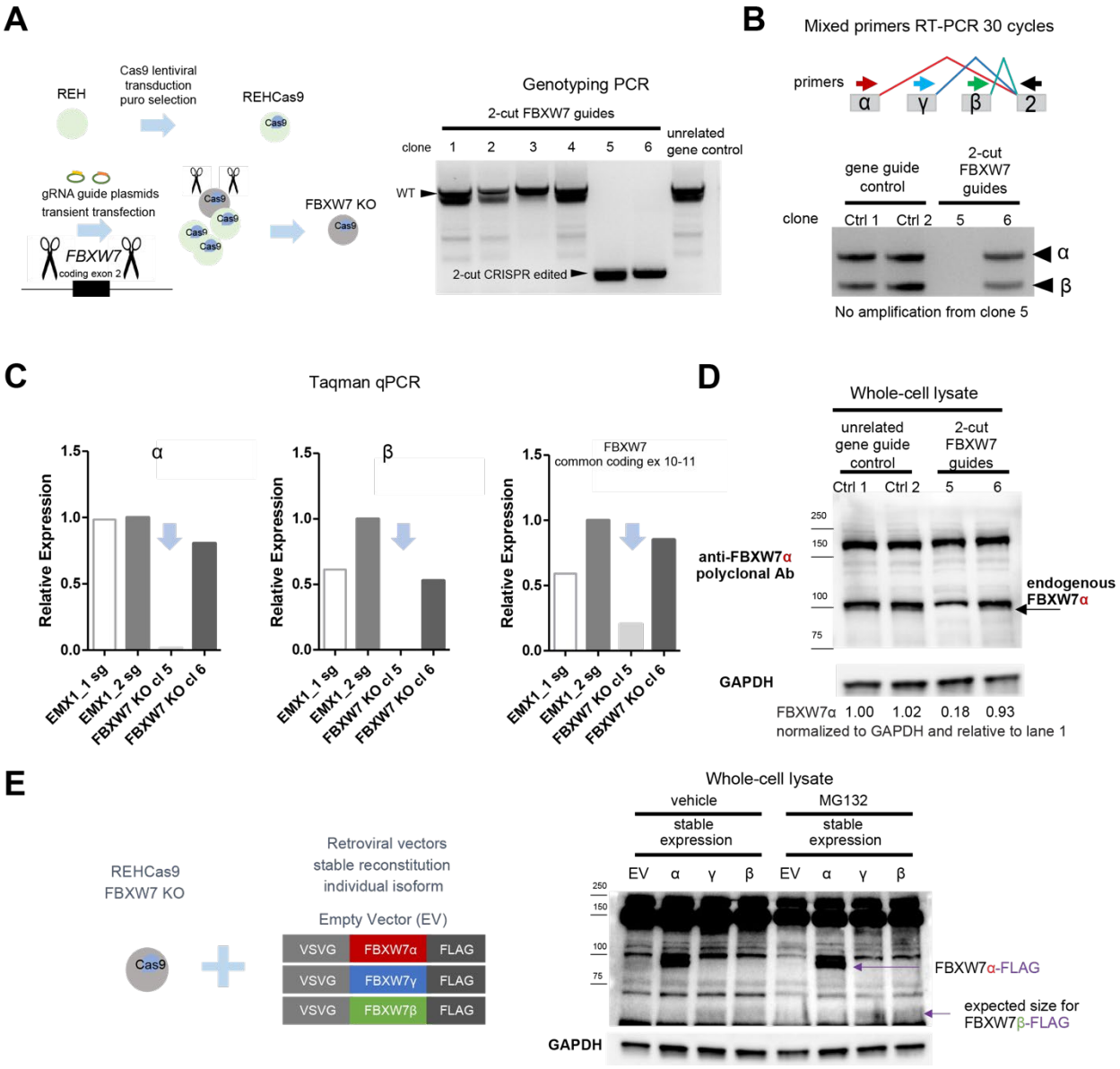
Supplemental Figure 2. Gene expression of primary B-ALL patient samples compared to normal counterparts. (A) Principal component analysis of transcriptome profiles of B-ALL patients at Children's Hospital of Philadelphia (CHOP) (n=18), St. Jude Children's Hospital (n=313), and normal BM cell subsets (pediatric n=2, adult n=2; total n=4). **(B)** Transcript expression of B-cell and hematopoietic markers in B-ALL patients at St. Jude Children's Hospital (n=313) and in normal BM cell subsets (early progenitors, pro-, pre-, immature B cells; n=4). Salmon algorithm (https://combine-lab.github.io/salmon), tximport, DESeq2, and ggplot2 R packages were used for analysis.



Supplemental Figure 3. Commercial antibodies against pan-FBXW7 epitopes fail to detect FBXW7β. (A) Western Blot of 293T cells transiently transfected with *FBXW7* isoform overexpression constructs using various antibodies. **(B)** 293T cells were transiently transfected with *FBXW7* isoform overexpression constructs, lysed, immunoprecipitated with anti-FLAG agarose beads, and immunoblotted with Thermo #40-1500 FBXW7 antibody first. Without stripping, the same membrane was blotted with FLAG antibody Sigma #F7425. Overexpression of FBXW7 protein isoforms was confirmed with the FLAG antibody.



Supplemental Figure 4. Attempts to generate FBXW7β-specific antibodies did not lead to successful protein detection. (A) Amino acid sequence encoded by the FBXW7 β exon. Highlighted sequences were used as two different immunogens for two antisera generation attempts. **(B)(C)** Antisera Western Blot compared to **(D)** anti-FLAG Western Blot in 293T cells transiently transfected with *FBXW7* isoform overexpression constructs. Presence of overexpressed isoforms was confirmed after stripping anti-FBXW7β sera and re-probing with anti-FLAG (Sigma #F7425).



Supplemental Figure 5. Generation of pan-*FBXW7* KO REHCas9 single cell clone with 2-cut CRISPR-Cas9 genome editing followed by reconstitution. (A) Genotyping KO single cell clones with genomic DNA PCR: short amplicon reflects removal of *FBXW7* coding exon 2 shared by all isoforms. The control sample was transfected with one CRISPR guide targeting an unrelated gene *EMX1*. **(B)** Mixed primers RT-PCR (30 cycles) to examine RNA expression of KO single cell clone after 2-cut CRISPR-Cas9 genome editing. **(C)** Taqman qPCR to examine RNA expression of KO single cell clone after 2-cut CRISPR-Cas9 genome editing. Transcript quantity was relative to control sample EMX1_2 sg, where one CRISPR guide was used to target the unrelated gene *EMX1*. **(D)** Lack of FBXW7α protein expression in KO single cell clone, Western Blot with anti-FBXW7α (Bethyl rabbit polyclonal #A301-720A). **(E)** Stable reconstitution of REHCas9 FBXW7 KO cells followed by Western Blot with anti-FLAG (CST #2368). FBXW7α-FLAG protein is readily detected. FBXW7β-FLAG is detected at ~64 kDa. MG132 treatment does not affect FBXW7 protein levels. Results representative of 3 independent experiments.

SUPPLEMENTAL TABLES

Supplemental Table 1. Taqman real-time qPCR primers and probes

Target	Type	Sequence 5'->3'
FBXW7al	Primer 1	CGA ACT CCA GTA GTA TTG TGG ACC T
	Primer 2	TTC TTT TCA TTT TTG TTG TTT TTG TAT AGA
	PrimeTime Probe	/56-FAM/CC CGT TCA C/ZEN/C AAC TCT CCT CCC CA/3IABkFQ/
FBXW7ga	Primer 1	GAG CCT CTA CCA CAT CAA ACT
	Primer 2	AAA GAG CGG ACC TCA GAA CC
	PrimeTime Probe	/56-FAM/AG CAT TAG C/ZEN/A TCA TTG CCC AAG GC/3IABkFQ/
FBXW7be	Primer 1	CAG CCG GAC ACA CGG G
	Primer 2	GGT CCA ACT TTC TTT TCA TTT TGT AAA
	PrimeTime Probe	/56-FAM/AA ATA CAG A/ZEN/A AAT ATG GGT TTC TAC GGC ACA TTA AAA ATG /3IABkFQ/
FBXW7common(ex10-11)	Primer 1	TGG GAT ATC AAA ACA GGA CAG TGT
	Primer 2	TAA ACA GGT CAC AGC ACT CTG ATG
	PrimeTime Probe	/56-FAM/ACAAACATT/ZEN/GCAAGGTCCCAACAAG/3IABkFQ/
GAPDH	Primer 1	TGT AGT TGA GGT CAA TGA AGG G
	Primer 2	ACA TCG CTC AGA CAC CAT G
	PrimeTime Probe	/56-FAM/AA GGT CGG A/Zen/G TCA ACG GAT TTG GTC /3IABkFQ/
CD52	Primer 1	GTT TGG CTG GTG TCG TTT
	Primer 2	GCC ACG AAG ATC CTA CCA AA
	PrimeTime Probe	/56-FAM/TGA GAG TCC /ZEN/AGT TTG TAT CTG TAC CAT AAC CA/3IABkFQ/
LST1	Primer 1	GTC CCT GAT CCC TGA CCT AA
	Primer 2	GAA GGA CCA CTG CCA GAA G
	PrimeTime Probe	/56-FAM/TCG CGG AAT /ZEN/GAT GAT ATA TGT ATC TAC GGG /3IABkFQ/
LTB	Primer 1	CAG CTG CCC ACC TCA TAG
	Primer 2	ACA GTA GAG GTA ATA GAG GCC G
	PrimeTime Probe	/56-FAM/AAA CGC CTG /ZEN/TTC CTT CGT CGT CT/3IABkFQ/
MME	Primer 1	AAG AAA CAG CGA TGG ACT CC
	Primer 2	GCA GCT GAT TTT ATG CAG TCT G
	PrimeTime Probe	/56-FAM/AGG AGC AGG /ZEN/ACA AGG ACC GAG A/3IABkFQ/
FRG2C	Primer 1	TTC CAA GGA TAT CTG CCA AGA C
	Primer 2	CAG CAG TGG AGG ATC TTG ATT
	PrimeTime Probe	/56-FAM/CCA GAA GAG /ZEN/GAG TGC AAC TTG ACG T/3IABkFQ/
PCDH10	Primer 1	CTC AGT GCA ATT GGA GAA GAG A
	Primer 2	CCA GGA AGC CGA CAT AGT AAG
	PrimeTime Probe	/56-FAM/AGT GGT CAT /ZEN/GGA GAC AGT GAA CAG G/3IABkFQ/

Supplemental Table 2. Regular PCR primers	
RT-PCR mixed primers	Sequence 5'->3'
FBXW7_syy_al_F	CAGCAAAAGACGACGAACTGG
FBXW7_syy_ga_F	CAGGACATTTGGTAGGGGAAGG
FBXW7_syy_be_F	TGACAGGGCATAGTCTCCTCC
FBXW7_syy_2_R	AAAGAGCGGACCTCAGAACC
Genotyping PCR primers flanking coding exon 2	Sequence 5'->3'
FBXW7_syy_gD2_F	AGCCTAATAACTGTGAGAGTGGG
FBXW7_syy_gD2_R	AAGGGAAGAAACCAGCCAGATC

Supplemental Table 3. CRISPR guide and Morpholino sequences		
CRISPR guide combination for coding exon 2 removal		
CRISPR guide	Sequence 5'->3'	
all FBXW7 sg#1	CTTACGACATTAGGGGCTAG	
all FBXW7 sg#2	CTAGGGTAGACATTTATGTA	
Morpholinos		
Target	Name	Sequence 5'->3'
FBXW7al	MOalpha	ATTGAATATACTCACTTTTGTTGTT
FBXW7ga	MOgamma	TTCAAATGTGTGAGACTTACCCGTC
FBXW7be	MObeta	GAAGAAAACAGCTTACTTACTTTGT
Random control oligo	MOctrl	25N, mixture of up to 4^25 different sequences

Supplemental Table 4. Key flow cytometry and immunoblotting reagents			
Flow cytometry reagents			
Specificity	Fluorochrome	Manufacturer	Catalog Number
Human TruStain FcX		Biolegend	422302
CD34	PE	Beckman Coulter	IM1459U
IgM	FITC	Beckman Coulter	B30655
CD19	APC	Beckman Coulter	IM2470U
IgD	PE	Thermo Fisher	12-9868
Immunoblotting antibodies			
Specificity	Manufacturer	Catalog Number	
FLAG	Cell Signaling Technology	2368S	
FLAG	Sigma	F7425	
FBXW7	Abcam	ab109617	
FBXW7	Thermo Fisher	40-1500	
FBXW7α	Bethyl	A301-720A	
β-ACTIN HRP	Cell Signaling Technology	12262	
GAPDH HRP	Cell Signaling Technology	3683S	
anti-mouse IgG HRP	Cytiva	NA931-1ML	
anti-rabbit IgG HRP	Cytiva	NA934-1ML	

Supplemental Table 5. List of 150 genes harboring Local Splicing Variants denoted in Venn Diagram intersection in Fig 1B.

ABHD15-AS1	HOMEZ	RANBP1
AC093642.3	IDS	RBSN
AKAP9	IFT122	RCOR3
ALKBH4	IGHG1	RHOT1
ANKRD36	IGHG2	RNFT1
ANKRD36B	IL3RA	RP11-1023L17.1
ARL17B	INTS4	RP11-1212A22.1
ASPH	INVS	RP11-156P1.3
ATG9A	IP6K2	RP11-164J13.1
BCOR	ITFG2	RP11-178C3.1
BCORL1	JPX	RP11-33N16.3
BMS1P20	KB-1572G7.2	RP11-43F13.1
C14ORF159	KIAA0125	RP11-514P8.7
C1QTNF3-AMACR	KLC1	RP11-798G7.5
C8ORF58	L3MBTL1	RP11-864I4.1
C9ORF156	LHPP	RP11-875O11.1
CA5BP1	LINC00893	RP3-477O4.14
CAPN15	LINC01237	RP4-717I23.3
CAPN3	LRRC27	RPP14
CASP8	LRRC37A	RRP7BP
CCDC39	LRRC37A4P	RSRC2
CENPO	LUC7L3	RSRP1
CEP250	MGME1	S100A13
CTBP2	MLLT10	S100PBP
CTD-3092A11.1	MRS2	SCMH1
DHX34	MSTO2P	SCYL3
EDRF1	MTERF4	SELO
ELMOD3	NAT1	SFXN2
ENSG00000163386	NDUFAF6	SGK3
ENSG00000225241	NPIPB3	SLC16A1-AS1
ENSG00000231486	NPIPB5	SMG1P5
ENSG00000232637	OGG1	SRP14-AS1
ENSG00000255168	OVOL2	STAG3L1
FAM111A	PACRGL	STRN3
FAM122C	PARG	TCAIM
FAM173B	PAX8	TCEB3-AS1
FAM228B	PAX8-AS1	TCTN1
FANCA	PCBP1-AS1	TMEM128
FANCD2	PCNX	TMEM161B-AS1
FBXO41	PFKM	TNFSF12
FBXW7	PIGL	TNFSF12-TNFSF13
GALK2	PKD1P1	UBC
GKAP1	PLGLB1	WDR27
GSN	PMS2P4	WIBG
GTPBP10	POLR2J3	ZNF131
HERC2P3	PPIP5K1	ZNF250
HLA-DRB5	PRDM2	ZNF254
HLA-G	PREPL	ZNF273
HLA-K	PSMC6	ZNF551
HMGCR	PTAR1	ZNF83

Supplemental Table 6. List of 188 genes differentially expressed in FBXW7 alpha vs beta knockdown cells (MOα vs MOβ). Per Gene Set Enrichment Analysis (collection C5 at www.gsea-msigdb.org), they are enriched in the following top 10 biological processes: dry skin, calcium ion binding, regulation of cell death, tissue development, growth, biological adhesion, Golgi apparatus, regulation of growth, cell-cell signaling, and structural molecule activity. List ranked by log2FoldChange of genes expressed in MOα relative to MOβ.

1. ENSG00000263244	56. CTPS2	111.HR	166.CCDC198
2. OLR1	57. CDKN2A	112.ZHX3	167.IGFBP1
3. ENSG00000280571	58. H2BC20P	113.NQO1	168.PPARGC1A
4. C19orf33	59. SMN1	114.KCNJ2	169.CPA4
5. KRT6A	60. ENSG00000279149	115.CDH24	170.ACAN
6. PINX1	61. NIT2	116.PURPL	171.LINC01139
7. KRT18	62. PLCH1	117.PCDH10	172.BMP4
8. GALNT6	63. DPEP1	118.ENSG00000259660	173.ALDH3A1
9. ACOX2	64. FRG2B	119.TMC8	174.FLG
10. MSLN	65. FRG2C	120.ZNF467	175.FBXO10
11. SH3RF2	66. FAXC	121.MC1R	176.ENSG00000224066
12. KRT8	67. SMAD9	122.XKR4	177.ENSG00000232725
13. ENSG00000225339	68. PTPN22	123.DENND2B	178.SMIM11B
14. PLPP2	69. ENSG00000263731	124.IGFBP4	179.HDX
15. SMOC1	70. ENSG00000268205	125.ENSG00000124224	180.PRDM6
16. FAM83A	71. TMSB4XP4	126.BMP3	181.ENSG00000267459
17. ALPP	72. SELL	127.ARSJ	182.ENSG00000284969
18. DAW1	73. LTB	128.EGR1	183.GAPDHP62
19. ENSG00000281379	74. CD52	129.ZCCHC18	184.ENSG00000261641
20. PDE1C	75. MPO	130.PMEPA1	185.ENSG00000224837
21. FOLR1	76. MAP2	131.NRXN1	186.RPL21P16
22. NPTX1	77. ENSG00000210082	132.SDC2	187.RMRP
23. NKD1	78. SYNGR1	133.WWTR1	188.RPL36AP37
24. SCHIP1	79. CPZ	134.PTPN4	
25. KRT80	80. LST1	135.SCARA3	
26. ANXA3	81. NNMT	136.ALPK2	
27. MTARC1	82. HYPK	137.KCNJ2-AS1	
28. FAM153A	83. ARL17B	138.MBNL1-AS1	
29. ARAP3	84. GBP4	139.KRT19	
30. EPPK1	85. DHCR7	140.WFDC1	
31. CELSR1	86. LMO2	141.C11orf87	
32. WWC1	87. MRPL10	142.RNF144B	
33. MISP	88. MME	143.OLFML3	
34. TINAGL1	89. NRN1	144.NEB	
35. PCDHB2	90. FBXW7	145.SLC4A4	
36. LINC00114	91. PCDH9	146.TXK	
37. PCAT2	92. DNAJB2	147.ENSG00000261434	
38. ZDHHC21	93. UVSSA	148.LPCAT2	
39. TBX3	94. CHD7	149.ENSG00000241489	
40. FBXL16	95. LANCL2	150.FGF5	
41. WDR72	96. FGD6	151.ENSG00000257386	
42. POSTN	97. NBPF19	152.NECTIN4	
43. MAGED4	98. NID2	153.C13orf46	
44. CASP1	99. KCNJ16	154.GDF6	
45. ENSG00000262172	100.POLH	155.POU6F1	
46. CDKN2B	101.ENSG00000269837	156.OGN	
47. CPLX2	102.ABHD4	157.LOC105369760	
48. AGR2	103.PHLDA3	158.KCNMA1	
49. NPAS2	104.TENM4	159.EYA2	
50. ENSG00000226239	105.SAMHD1	160.LINC00648	
51. CCDC182	106.KDM7A	161.P3H2	
52. ARRDC3-AS1	107.SLIT2	162.BAZ2B	
53. FBLN2	108.ATXN7	163.ENSG00000243696	
54. NR2F2	109.INKA2	164.ENSG00000285625	
55. GASK1B	110.SLC7A7	165.PDE4C	

Supplemental Table 7. List of 228 genes differentially expressed in FBXW7 alpha vs beta reconstituted cells (KO + α vs KO + β). Per Gene Set Enrichment Analysis (collection C5 at www.gsea-msigdb.org), they are enriched in the following top 10 biological processes: intrinsic component of plasma membrane, locomotion, neurogenesis, cell morphogenesis, cell projection organization, cell-cell adhesion, biological adhesion, taxis, cell migration, and ion transport. List ranked by log2FoldChange of genes expressed in KO + α relative to KO + β.

1. ENSG00000261093	58. TBC1D30	115.FOXO6	172.ANO2
2. FAM86B2	59. ENSG00000237356	116.SYT1	173.TRDV2
3. USP41	60. OTOGL	117.MLLT11	174.CACNA1E
4. CBSL	61. ITGAL	118.MYO10	175.LTA
5. ENSG00000283228	62. SOHLH2	119.IRX3	176.ELN
6. BDNF-AS	63. CEACAM21	120.PCDHB13	177.TRPM2
7. ENSG00000261459	64. ENSG00000270112	121.LFNG	178.RCAN3
8. ENSG00000285000	65. FAM53A	122.ARHGAP24	179.IL6ST
9. LINC01159	66. VWA8	123.BBS9	180.LOC102723996
10. FAAH2	67. BCDIN3D-AS1	124.SBK1	181.LTB
11. LOC100653049	68. SNORC	125.PDGFRB	182.CASKIN1
12. EPHA1-AS1	69. PCDH1	126.USP18	183.UMODL1
13. ZNF287	70. SRGAP1	127.IFI44	184.FGF18
14. LGR6	71. WTIP	128.PTPN6	185.DNAH10
15. TDRP	72. MYCN	129.PABPC4L	186.LINC00467
16. ENSG00000239922	73. PDZRN4	130.CDKN2C	187.EIF3CL
17. PECAM1	74. NIPSNAP3B	131.PALM	188.GPX3
18. MIR646HG	75. PCDH10	132.SP4	189.TMC8
19. PKHD1L1	76. KLHDC7A	133.SNHG5	190.PPL
20. ENSG00000244558	77. WNK3	134.FBXW7	191.PAWR
21. RAPSN	78. LUCAT1	135.SNHG3	192.MME
22. PIP4K2C	79. DSC3	136.SH3KBP1	193.IL16
23. SLCO2B1	80. NRP1	137.LIMD2	194.GRIN2B
24. SGMS2	81. DAPK1	138.ENSG00000281398	195.ENSG00000271046
25. RHPN2	82. HDAC9	139.RFTN1	196.MEG9
26. SLC5A2	83. GNG11	140.FRG2C	197.C3
27. IMPACT	84. ARSG	141.PAG1	198.FANCB
28. ENSG00000258590	85. SLITRK1	142.RASD1	199.MYOM1
29. RTBDN	86. GPER1	143.PPM1F	200.ENSG00000225096
30. DOCK1	87. MBP	144.DTX1	201.MPDZ
31. CHRM2	88. MAP1A	145.SNHG26	202.PRODH
32. CFAP299	89. BIN1	146.NETO1	203.DIO2
33. TTC38	90. LINC00158	147.MT2A	204.LINC00670
34. ENSG00000264058	91. HBEGF	148.LINGO3	205.EVA1A
35. ZNF521	92. SLCO5A1	149.CD19	206.DIO2-AS1
36. CDH12	93. ENSG00000272933	150.LCK	207.DNAAF8
37. CHURC1-FNTB	94. LPAR4	151.ZNF423	208.PLA2G10
38. IDI2-AS1	95. TFPI	152.ERMAP	209.MYO16
39. KRT18P27	96. DSC2	153.DOCK9	210.OR2A42
40. DNAH14	97. ENSG00000272070	154.RIPOR2	211.ENSG00000271893
41. PCDH10-DT	98. SPTBN2	155.LOC105375924	212.ENSG00000279593
42. ATP1A2	99. IFI27L2	156.KCNK12	213.PRRG1
43. TRPS1	100.FAT4	157.TNF	214.TBR1
44. ENSG00000271930	101.RGMA	158.COL5A1	215.ANO9
45. TLR7	102.AGAP1	159.BEST3	216.STAB2
46. SSPN	103.ZNF469	160.SEMA6A	217.ENSG00000233974
47. PPP1R14A	104.VPS13B	161.TRAC	218.MUC5B
48. KIF26B	105.NTRK1	162.SLC35E3	219.LPAL2
49. ENSG00000258592	106.HIC1	163.SPAG1	220.RYR1
50. ZNF385D	107.RGL1	164.CD52	221.LHX1
51. SALL1	108.CAMK2D	165.FLT1	222.CCR6
52. LHX9	109.OPN3	166.ENSG00000204282	223.ZSWIM8-AS1
53. CAP2	110.PKIG	167.LST1	224.TMEM265
54. LIPI	111.ABCA9	168.ICOSLG	225.SCARNA2
55. ENSG00000227579	112.ZNRF1	169.GBGT1	226.ENSG00000196302
56. XYLT1	113.CD248	170.MT1X	227.ENSG00000280064
57. SRSF12	114.SEMA3D	171.TMEM52	228.ENSG00000259948

Supplemental Table 8. Top 300 genes whose expression highly correlates with FBXW7 beta exon usage in the St. Jude B-ALL patient cohort. The genes identified in both knockdown and reconstitution experiments (MME/CD10, LTB, CD52, and LST1) were ranked between 22nd and 256th out of the 41,275 genes whose RNA expression were analyzed, or in the top 0.62%. Some other genes that highly correlated with FBXW7 beta-exon usage (ADGRG1, ECM1, PTP4A3, and CD200) mapped to the locomotion and cell migration biological processes annotated by collection C5 on the Gene Set Enrichment Analysis website (www.gsea-msigdb.org).

1. LINC02273	76. CORO6	151. OR4Q3	226. MRPL49P2
2. VWA2	77. ASB13	152. CD27-AS1	227. AC027309.1
3. FBXW7-AS1	78. CD27	153. SCNN1A	228. LRRC70
4. TMEM236	79. AP002761.3	154. AL139246.2	229. LINC01507
5. AC097375.4	80. LINC02538	155. AC108724.2	230. LIMK2
6. SERPINB9	81. AC010260.1	156. AC025423.4	231. MAPRE1P1
7. ZC3H12D	82. LINC02452	157. MDS2	232. ARHGAP42P4
8. TSPAN7	83. RGL1	158. ABCA4	233. AL512310.2
9. BLACE	84. DUSP27	159. MSR1	234. PLCH1
10. SPAAR	85. AC025423.3	160. KHDRBS3	235. AC139769.3
11. ADGRG1	86. AC004466.1	161. GOLGA8B	236. PFN1P6
12. ECM1	87. AC139769.2	162. LINC02571	237. ZNF90
13. ELFN2	88. NBPF15	163. AC087752.3	238. MYLK
14. FP325335.1	89. LINC02487	164. NPAP1P6	239. AL161782.1
15. MME-AS1	90. ARHGEF17	165. EMP2	240. NRP1
16. PTP4A3	91. HDAC7	166. AC025423.2	241. AL713998.1
17. CD200	92. RPL23AP49	167. AC129915.3	242. AL021408.2
18. CTDSPL	93. SLC2A7	168. AL355916.3	243. MRVI1-AS1
19. Z94160.2	94. CRELD2	169. AL669970.1	244. TRERNA1
20. SLC35E3	95. AC007207.1	170. SYNPO2	245. AC006487.1
21. AC008149.1	96. CNKSR3	171. MZB1	246. CA6
22. MME	97. DUX4L26	172. TNRC6C-AS1	247. ABLIM1
23. TMPRSS11E	98. AC009271.1	173. MDM2	248. AC025754.1
24. PRX	99. LGMN	174. AC018552.3	249. MRGPRG-AS1
25. AC004704.1	100. LINC01416	175. VAMP5	250. SLC37A3
26. AC093916.1	101. TP53INP1	176. AL161912.4	251. SSX2
27. S100Z	102. MIR4520-1	177. TDRD1	252. TGM7
28. ZNF793	103. SOAT2	178. SFXN1	253. CYTL1
29. LARGE2	104. LINC01224	179. RIPOR2	254. RPL10P19
30. C2orf73	105. AL513523.2	180. AL354993.2	255. DPEP1
31. SCHIP1	106. AC069272.1	181. AL356234.2	256. LST1
32. IQCJ-SCHIP1	107. NDUFAF6	182. AC027277.1	257. AGGF1P3
33. AC069023.1	108. B4GALT6	183. CEP68	258. NLRP12
34. AC079340.2	109. AF067845.5	184. GPR17	259. SIDT1
35. AC139718.2	110. RAI14	185. AL645939.1	260. ACVR1C
36. LINC01922	111. RPS6KA2-IT1	186. AC079776.4	261. NEURL1B
37. MRC1	112. ELK3	187. PEAK1	262. BMPR1B
38. MAMLD1	113. AC006305.1	188. OR2B6	263. KCNA2
39. AFAP1L2	114. FAR2P1	189. CSMD2	264. DENND3
40. RIPPLY3	115. ZEB1-AS1	190. SERINC5	265. AC011095.1
41. TSPAN15	116. LTB	191. CLIC5	266. HACD1
42. ZNF793-AS1	117. AC139769.1	192. GPA33	267. TMEM263
43. NT5E	118. SHISA9	193. AL161912.1	268. NPR1
44. AC092894.1	119. LINC01539	194. AL669970.3	269. AF067845.2
45. AC100797.5	120. XG	195. TMC6	270. ORMDL3
46. RNU6ATAC36P	121. SDK2	196. HNRNPA1P70	271. AL136309.3
47. IFNG-AS1	122. NR3C2	197. MRVI1	272. IL26
48. RAB11FIP5	123. LINC01013	198. PCLO	273. KLF2P1
49. MMP28	124. CREG2	199. GFY	274. DGKA
50. ADGRF1	125. PIM1	200. LINC01905	275. AL139415.2
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51. FBLN5	126. LINC01825	201. CMTM8	276. AC127029.2
52. AL355974.2	127. MAP3K19	202. LINC01290	277. AC243967.1
53. AC131211.1	128. EGLN1	203. MYO1B	278. LAMB4
54. JPH1	129. SEMA6A-AS2	204. AL355916.2	279. AC093278.2
55. AC022148.1	130. AC092839.1	205. AF067845.4	280. C1R
56. AC139718.1	131. CLUHP10	206. GRB7	281. AL139415.1
57. AC008060.4	132. AC104564.5	207. FAM129A	282. KCNK15
58. SLC16A14	133. DLGAP2-AS1	208. ITPR3	283. PPP2R2C
59. CRHBP	134. ST6GALNAC3	209. AP003555.2	284. AF067845.1
60. PIK3IP1	135. ALOX5	210. AL161912.2	285. TCN2
61. DLGAP2	136. NFASC	211. AC017100.1	286. LINC00670
62. ARHGAP29	137. BNIP3P39	212. RN7SL88P	287. NRN1
63. FRG2C	138. CCL17	213. AC040970.1	288. LINC00544
64. FBXW7	139. AC079209.1	214. AC100803.3	289. GRIK5
65. AL356234.3	140. AC080078.2	215. LTA	290. AC092614.1
66. AC008060.1	141. AL138881.1	216. AC006305.3	291. GPRIN3
67. AC010542.1	142. CD52	217. SV2A	292. AC004706.1
68. AL359220.1	143. AC011447.1	218. LINC01841	293. LINC02074
69. AC087627.1	144. LINC01471	219. BNIP3P15	294. AC100803.1
70. CMTM2	145. SEMA6A	220. LGALS7	295. GSDMA
71. ZNF366	146. LINC02577	221. GAB1	296. LINC02564
72. AC005383.1	147. UNC93B3	222. AL669970.2	297. CERNA1
73. FGF16	148. SEMA6A-AS1	223. SLFN14	298. AL512428.1
74. C14orf132	149. AC246785.3	224. LYVE1	299. AC084816.1
75. AC018781.1	150. AL731567.1	225. CHST15	300. NPR2