Supplementary Information

Proteomic screen reveals Fbw7 as a modulator of the NF-κB pathway

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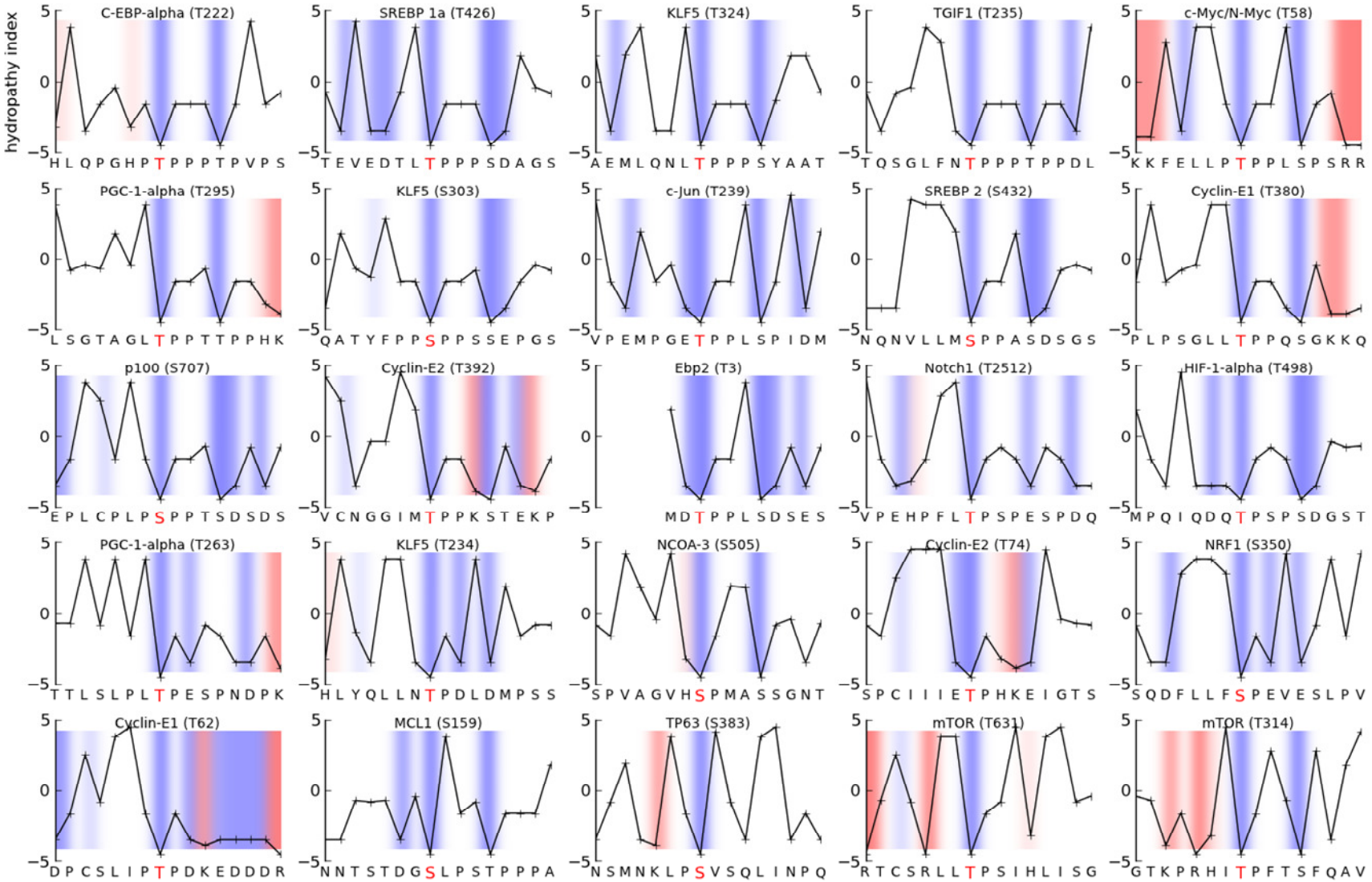
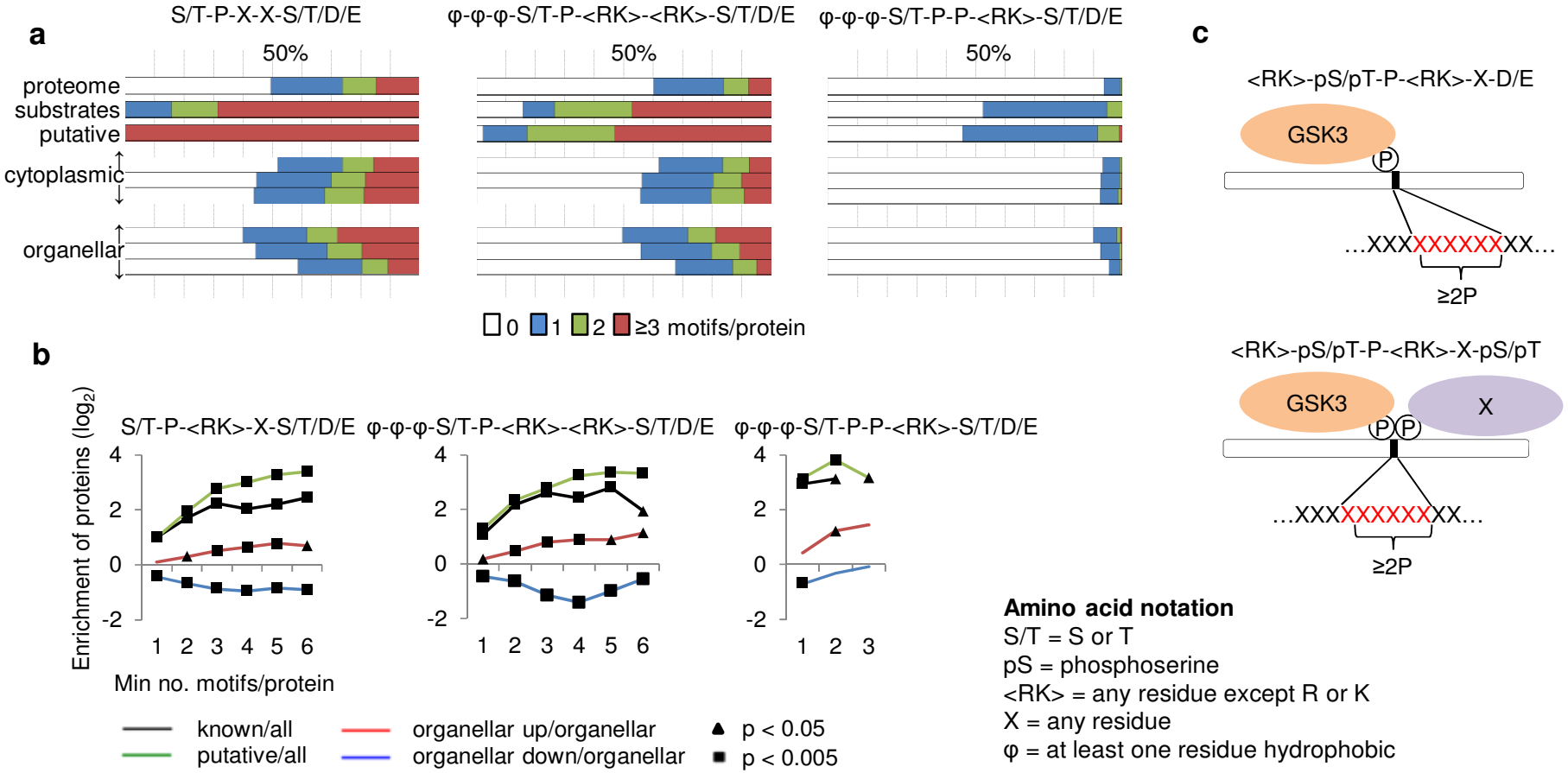
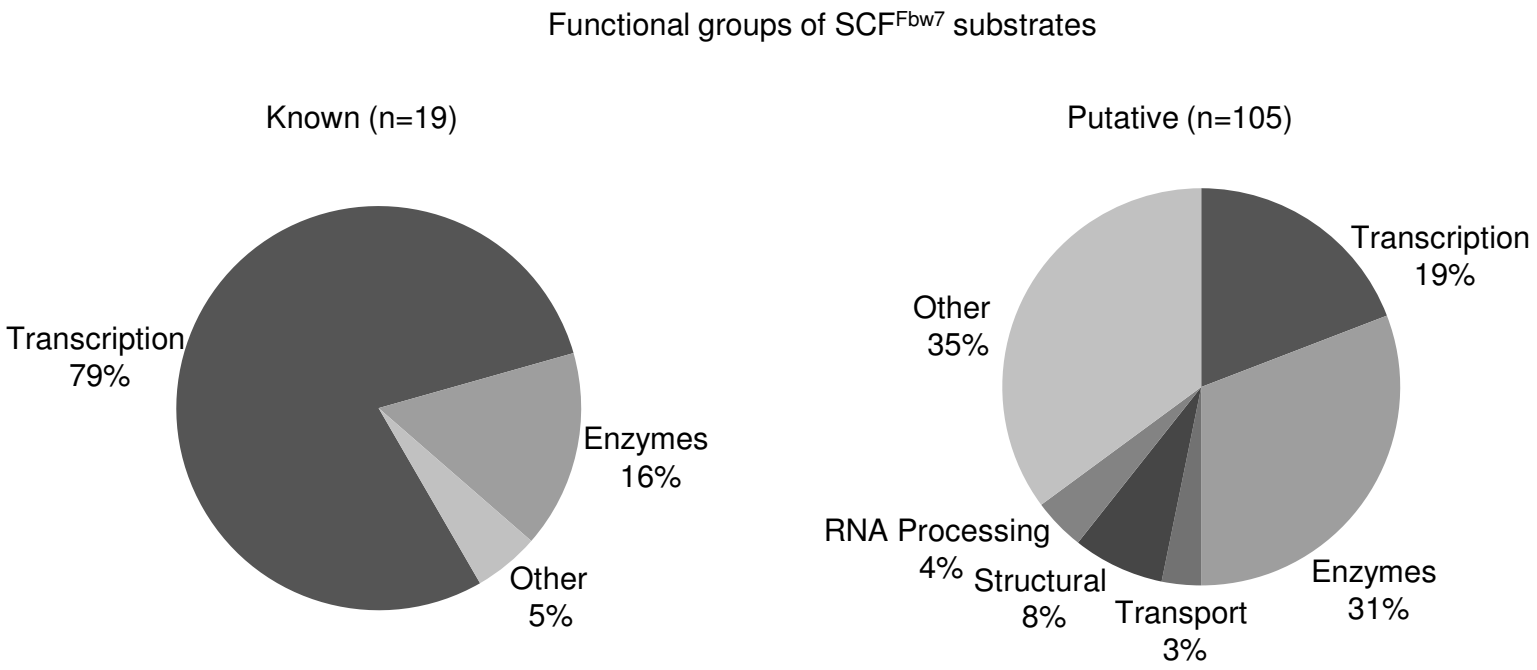


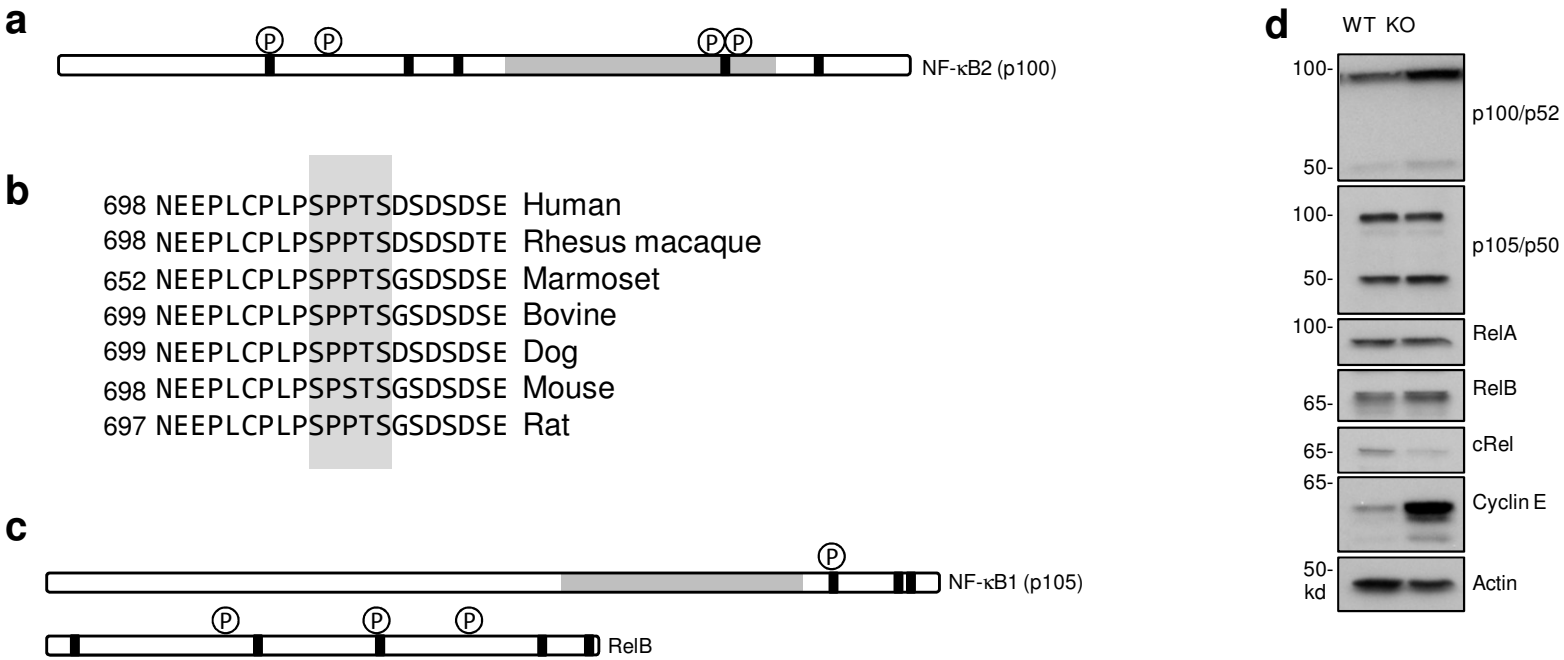
Supplementary Figure S1 Hydrophobicity and acid association constant (pKa) analysis of known Fbw7 degrons. The solid line shows the hydropathy index according to the Kyte-Doolittle[47] scale of amino acids in a surrounding degrons of 24 previously reported substratesand the p100 degron described here. The scale ranges from -4.5 (hydrophilic) to 4.5 (hydrophobic). Enlarged red letters indicate the central GSK3b phosphorylation sites. Most degrons contain at least one leading hydrophobic residue. The electrical characteristics of amino acids are also indicated with acidic residues in blue and basic in red. Degrons are arranged according to the number of prolines from high (upper left) to low (lower right).



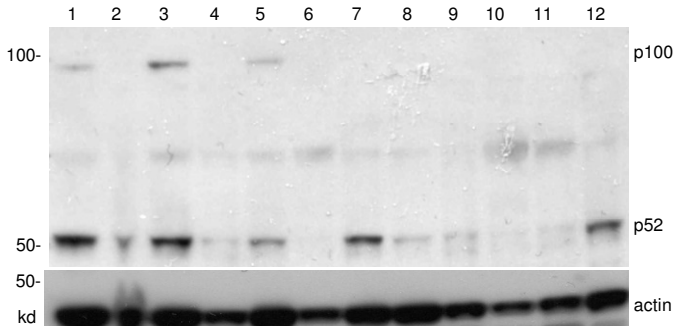
Supplementary Figure S2 Fbw7 degron motif distribution. (**a**) The proportion of the proteins with the indicated number of motifs in the human proteome, known Fbw7 substrates and in the pools identified in the TMT/MS analysis is shown. Arrows indicate up (↑) or down-regulated (↓) subpopulations of Fbw7 KO cells. Proteins with degron motifs are enriched in the nuclear/organellar (NO) pool and known substrates for the more stringent motifs and multiple less stringent motifs (two-tailed binomial test, $p < 0.05$), while proteins with motifs are depleted in the down-regulated NO pool (two-tailed binomial test, $p < 0.005$). (**b**) Enrichment of proteins grows among known substrates and up-regulated proteins in the NO pool of *FBW7* KO cells for more restrictive motifs or higher motif counts. Down-regulated proteins in the NO pool are eliminated by increased selectivity more rapidly than proteins in the reference pool. Statistically significant changes (two-tailed binomial test) are indicated for $p < 0.05$ (▲) and $p < 0.005$ (■). (**c**) Construction of predicted Fbw7 phosphodegrons used as criterion iii) in the substrate identification (Fig. 1b). The central serine or threonine is a predicted GSK3b phosphorylation site (Netphos, score ≥ 0.49) and the +4 position holds either an acidic residue (upper panel) or a serine or threonine predicted to be phosphorylated by any kinase (lower panel, score ≥ 0.50). Since known Fbw7 substrates contain proline-rich degrons (Supplementary Table S2), at least 2 prolines are required in the motif.



Supplementary Figure S3 Functional classification of the known and putative Fbw7 substrates. To get an overview of the biological functions of potential SCF^{Fbw7} substrates Gene ontology (GO)-analysis was performed. Proteins were subdivided into functional groups based on their molecular function or activity. Other indicates the 40 identified Fbw7 candidate substrates that are uncharacterized or have unknown molecular functions.



Supplementary Figure S4 Fbw7 degron motifs in NF-κB proteins. (**a**) The position of Fbw7 degron-motifs (black boxes) and predicted GSK3b sites (circled P) at S222, T291, S707 and S711 in NF-κB2. (**b**) The stringent Fbw7 degron-motif is conserved across species. (**c**) In addition to p100, only NF-κB1 and RelB of the NF-κB proteins contain Fbw7 degron-motifs (**d**) Levels of NF-κB proteins in FBW7 WT versus KO cells show p100 and RelB levels are elevated in KO cells.



Supplementary Figure S5 NF-κB2 is elevated in several primary paediatric B-cell Acute lymphoblastic leukaemia. Whole cell extracts from B-cells from 12 patients were analysed by WB. Endogenous p100 and p52 levels were probed.

	Total	Cyt	Nuc	Unique Cyt	Unique Nuc
Proteins	7816	6620	6036	1780	1196
Up	1074	968	212	862	106
Down	892	335	614	278	557
Peptides	41238	32019	28905	12333	9219
Quant. proteins	7544	6407	5822	1722	1137
Quant. peptides	37621	29252	25927	11694	8369

Supplementary Table S1. Quantitative proteomics of HCTFbw7KO versus HCTFbw7WT cells. Cytoplasmic (Cyt) and nuclear (Nuc) fractions were analysed. The number of identified proteins and peptides are indicated. Proteins were identified by at least one peptide, at false discovery rate (FDR) 1%. Quant. indicates the number of peptides with associated HCD spectra, containing valid reporter ion peak intensities. Significantly up- and down-regulated proteins in Fbw7 KO cells were determined by SAM analysis at q-value cut off 2%.

	Ingenuity Canonical Pathways	p-value
Nucleus	Oxidative Phosphorylation	3.16E-41
	Mitochondrial Dysfunction	3.16E-36
	Ubiquinone Biosynthesis	7.94E-25
Cytoplasm	Mitochondrial Dysfunction	5.01E-22
	Oxidative Phosphorylation	3.16E-21
	Ubiquinone Biosynthesis	4.37E-09
	N-Glycan Biosynthesis	2.19E-04
	Regulation of eIF4 and p70S6K Signaling	8.71E-04
	RhoA Signaling	1.07E-03
	Rac Signaling	1.82E-03
	Pyruvate Metabolism	1.82E-03
	Aldosterone Signaling in Epithelial Cells	2.45E-03
	Caveolar-mediated Endocytosis Signaling	2.69E-03
	Integrin Signaling	3.16E-03
	EIF2 Signaling	3.24E-03
	Myc Mediated Apoptosis Signaling	3.80E-03
	Granzyme B Signaling	5.37E-03
	Germ Cell-Sertoli Cell Junction Signaling	7.24E-03
	Tumoricidal Function of Hepatic NK Cells	7.76E-03
	mTOR Signaling	9.33E-03

Supplementary Table S2. Most significantly affected pathways identified by ingenuity pathway analysis of proteins with different levels in FBW7KO cells.

Name	#degrons	#motifs	Degron sequences
SREBP[48]	2	9	TEVEDTL**TP**PP**S**DAGS (T426;1a)
			NQNVLLM**SP**PA**S**DSGS (S432;2)
mTOR[49]	2	7	RTCSRLL**TP**SI**H**LISG (T631)
			GTKPRHI**TP**FT**S**FQAV (T314)
Notch1[50]	1	6	VPEHPFL**TP**SP**E**SPDQ (T2512)
HIF1α[51]	1	6	MPQIQDQ**TP**SP**S**DGST (T498)
PGC-1α[52]	2	5	LSGTAGL**TP**PT**T**PPHK (T295)
			TTLSLPL**TP**ES**P**NDPK (T263)
c-Myc[53]	1	5	KKFELLP**TP**PL**S**PSRR (T58)
N-Myc[54]			
TGIF1[55]	1	5	TQSGLFN**TP**PP**T**PPDL (T235)
Cyclin-E1[56]	2	3	DPCSLIP**TP**DK**E**DDDR (T62)
			PLPSGLL**TP**PQ**S**GKKQ (T380)
Cyclin-E2[57]	2	3	SPCIIIE**TP**HK**E**IGTS (T74)
			VCNGGIM**TP**PK**S**TEKP (T392)
KLF5[58]	3	3	QATYFPP**SP**PS**S**EPGS (S303)
			AEMLQNL**TP**PP**S**YAAT (T324)
			HLYQLLN**TP**DL**D**MPSS (T234)
c-Jun[59]	1	3	VPEMPGE**TP**PL**S**PIDM (T239)
SRC-3[60]	1	3	SPVAGVH**SP**MA**S**SGNT (S505)
TP63[61]	1	2	NSMNKLP**S**V**L**SQ**L**INPQ (S383)
MCL1[62]	1	2	NNTSTDG**SL**PS**T**PPPA (S159)
NRF1[63]	1	2	SQDFLLF**SP**EV**E**SLPV (S350)
Presenilin 1[64]	0	1	
Ebp2*[65]	1	1	MD**TP**PL**S**DSES (T3)
c-Myb[66, 67]	0	1	
C/EBPα[68]	1	1	HLQPGHP**TP**PP**T**PVPS (T222)
LT40*[69]	1	1	TCFKKPP**TP**PP**E**PET (T701)

Supplementary Table S3. Known Fbw7 substrates. The characterized degrons and number of motifs (S/T-P-X-X-S/T/D/E) for known targets are indicated. Residues constrained by the motif are marked in bold. Mismatching residues are marked in red. The offset of the motif in the protein is given in parentheses as specified by the referenced article. Asterisks mark pseudosubstrates that bind to Fbw7 but the interaction does not lead to degradation. 15 of the 19 bona fide substrates have more than one motif. None of the 25 reported degrons have a basic residue (R or K) in the +2 or -1 positions counting from the first amino acid of the motif. A total of 17 degrons from 14 substrates have more than 2 prolines in the -2 to +5 positions, with 13 degrons having a proline in both the +1 and +2 positions.

Cohort 1 n=178

Gene name	Rho	P-value
BCL2	-.261**	0.00042
BCL2L1	-.291**	8.0E-05
MCL1	-.180*	0.016
BCL2L11	-.183*	0.014
MYC	-.329**	7.48E-06
CCND1	-.138	0.067
IL2RG	-.165*	0.028
WNT10A	-.335**	4.80E-06

Cohort 2 n=297

Gene name	Rho	P-value
BCL2	-.221**	1.214E-04
BCL2L1	-.375**	2.323E-11
BCL2L11	-.181**	1.733E-03
IL2RG	-.189**	1.062E-03
WNT10A	-.369**	5.029E-11

Supplementary Table S4. mRNA expression correlation analysis of Fbw7 and NF-kB2 target genes in primary pediatric B-cell acute lymphoblastic leukemia. Gene specific mRNA expression levels were determined by Affymetrix U133 plus 2.0 GeneChips. Rho = Spearman's coefficient correlation, (-) denotes inverse correlation, Significant correlations; P <0.05*, P <0.005** (all P values are 2-sided).

Target	Direction	Sequence
bactin	forward	5-GTGGGAGTGGGTGGAGGC-3
	reverse	5-TCAACTGGTCTCAAGTCAGTG-3
P100	forward	5-AGCCTGGTAGACACGTACCG-3
	reverse	5-CCGTACGCACTGTCTTCCTT-3
SKP2	forward	5-TTGTCCGCAGGCCTAAGCTA-3
	reverse	5-TGCCATAGAGACTCATCAGACGC-3
CYCD1	forward	5-GTGCTGCGAAGTGGAAACC-3
	reverse	5-ATCCAGGTGGCGACGATCT-3

Supplementary Table S5. Primer sequences used in qPCR experiments to assess gene specific mRNA levels.

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