SUPPLEMENTAL MATERIALS and METHODS

Cell culture

The number of passages between thawing and use in the described experiments ranged between two and five. Cell line authenticity was confirmed by STR-based analysis (Laragen Inc). Mycoplasma testing was performed regularly (every 2 months) using Mycoplasma PCR detection kit (ABM, Canada).

For chase experiments MCL cell lines were seeded at $1x10^6$/ml in the presence of 100 μg/ml of Cycloheximide and cultured up to 12h. Following treatment cells were lysed and used for immunoblotting.

For stimulation with soluble factors, MCL cell lines were seeded at $1.5x10^6$/ml in the presence of 5 μg/ml of soluble goat F(ab')2 anti-human IgM antibody (sol-IgM; Sothern Biotech, Birmingham, AL, USA) or 25 ng/mL soluble human FABB (sol-BAFF; Cell signaling Technology, Danvers, MA, USA). Following stimulation cells were lysed and used for immunoblotting.

MCL primary samples were cultured on BAFF-expressing or CD40-L expressing mouse fibroblasts cells as previously described.[1-3] Briefly, stroma cells were seeded to achieve 80-90% confluence, after 24 hours MCL cells were plated at a 10:1 ratio and incubated for another 24 hours.

Gene expression manipulation and cell line generation

Gene knockdown plasmids were delivered using lentiviruses. HEK293T cells (ATCC) were co-transfected with Lipofectamine 2000, psPAX2 and pMD2.G packaging plasmids (Addgene) and pLKO.1-based MISSION® Lentiviral shRNA (Sigma Aldrich) to produce lentiviral particles. Target cells were transduced with lentivirus and control knockdown cells were transduced with pLKO.1 empty vector-containing lentivirus. Cells were selected at 48 hours post-transduction for 7 days with up to 10 uM puromycin.

Gene overexpression utilized CRISPR activation in a two-step protocol. Following the above description, CRISPRa dCas9-VPR (Horizon Discovery) lentiviral particles were produced using

HEK293T cells co-transfection and Mino and JeKo-1 cell lines were transdcued. Cells were selected at 48 hours post-transduction for 14 days with blasticidin to establish Mino dCas9-VPR and JeKo-1 dCas9-VPR CRISPRa ready cell lines. sgRNA lentiviral particles were produced using sgRNA lentiviral plasmids (Horizon Discovery) HEK293T cells co-transfection as described above. sgRNA lentivirus was transduced to Mino dCas9-VPR and JeKo-1 dCas9-VPR cells. Target cells were selected at 48 hours post-transduction for 7 days with up to 10 uM puromycin.

Cell viability testing

To measure cell proliferation, cells were plated in 96-wellplates (10,000 cells/well, 3 wells/sample) with drugs and incubated for 72 h at 37 °C in 5% CO2. After incubation, relative numbers of viable cells were measured using a tetrazolium-based colorimetric assay (CellTiter Aqueous One Solution Cell Proliferation Assay, Promega) on the SpectraMax iD3 (Molecualr Devices).

Apoptosis was measured using the ApoScreen Annexin V (Southern Biotech). Briefly, cells were resuspended in 250 µl µL Annexin V binding buffer containing 2.5 µL Annexin V-APC and 2.5 µL 7-aminoactinomycin D (7-AAD). Cells were then analyzed by flow cytometry.

Quantitative PCR

Total RNA was extracted using Homogenizer Mini Columns and the total RNA Kit I (Omega Bio-Tek). Complementary DNA (cDNA) was synthesized using qScript cDNA Supermix (QuantaBio), then was prepared according to the manufacturer's recommendations using PerfeCTaFastMix II (Quantabio). *CDK5* TaqMan probe (Hs00358991_g1) and reference probe (*GAPDH* Hs02758991_g1) from ThermoFisher Scientific were used. *XBP1s* mRNA levels were quantified using spliced-variant specific primers described in van Schadewijk, *et al* [4]: XBP1-F (5'- TGCTGAGTCCGCAGCAGGTG-3') and XBP1-R (5'- GCTGGCAGGCTCTGGGGAAG-3') and normalized against *GAPDH:* GAPDH-F (5' – AATCCCATCACCATCTTCCA – 3') and GAPDH-R (5' – TGGACTCCACGACGAACTCA – 3'). PowerUp SYBR Green Master Mix for

qPCR (ThermoFisher Scientific, USA). Experiments were carried out in triplicates. The comparative Ct method was used for analysis ($2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = \Delta CtP - \Delta CtK$; P = probe and K = reference sample).

Transcriptomics

For bulk RNA sequencing, JeKo-1, JeKo-1-IR, JeKo-1 CDK5-OE and JeKo-1-IR CDK5-KD were collected and RNA was isolated as described above. RNA integrity and quality were evaluated using the Agilent 2100 Bioanayzer (#5067-1511, Agilent Technologies). Library prep and sequencing was performed on an Illumina Hiseq 2500 through City of Hope's Integrative Genomics Core.

Data was exported and analyzed using Partek flow (https://www.partek.com/). Briefly, Fastq files were trimmed, then aligned to the human reference genome (h38) using STAR (v.2.7.8.) and finally counts were normalized. Up/downregulated genes were defined by statistical significance (Padj<0.05). Gene set enrichment analysis was performed using GSEA (v.4.3.3.) under Hallmark.

Somatic mutations in the drug-resistant cell line was performed following strategy of the Integrated Mutation Analysis Pipeline for RNA-seq data [5] with the matched control before drug treatment. In brief, the reads were first trimmed by Trimmomatic with "ILLUMINACLIP:TruSeq3-PE.fa:2:30:10 SLIDINGWINDOW:4:15 MINLEN:36" option and aligned with STAR which was followed by coordinate sorting with samtool sort. Duplicates were marked and removed by MarkDuplicates in GATK, which was followed by reads splitting with SplitNCigarReads and base recalibration with BaseRecalibrator and ApplyBQSR. Mutations were then called by Mutect2 with germline source from gnomAD and panel of normal from 1000 Genomes and artifacts were filtered with FilterMutectCalls using the learned orientation priors and Mutect2 call statistics. The alignment was further filtered to keep only reads overlapping candidate variant sites using Samtools and GATK FilterSamReads. And the reads were extracted by Samtools and remapped with HISAT2. The mutations were called again with Mutect2 following reads splitting by SplitNCigarReads and filtered by a custom script to remove mutations within a suite of RNA-editing and artifact blacklists, including TCGA PON,

RADAR, DARNED, RNA-EDI databases, and HLA, immunoglobulin, and pseudogene regions. The final mutations were then annotated with Funcotator and VEP and mutations with less than 4 supporting reads or 10 total reads or lower than 4% allele frequency were removed. Potential germline mutations with over 1% frequency in dbSNPs and gnomAD were removed and only functionally relevant variants: missense, nonsense, frameshift, splice-site, and in-frame indels were kept. Finally, cross-reference filtered variants against COSMIC was performed to flag known cancer-associated mutations and functional impact was predicted by SIFT and PolyPhen.

For primary MCL tumors RNA sequencing, 5 normal samples (B cells enriched from PBMCs from healthy donors) and 58 MCL samples were used. Descriptive methods can be found at Yi et al.[6]. For the survival curve MCL samples were stratified in two groups based on the mean CDK5 expression (above-mean vs below-mean expression) and the difference in survival was evaluated using a log-rank test.

Proteomics

For proteomics assays 300 µg from JeKo-1, JeKo-1-IR, JeKo-1 CDK5-OE and JeKo-1-IR CDK5-KD were processed using urea denaturation and Lys-C/trypsin dual digestion. 40 µg of peptides from each sample was labeled with distinct TMTpro tags. Peptides from different samples were pooled after checking labeling efficiencies and processed using SMOAC phosphopeptide enrichment protocol. The TiO2 and IMAC fraction from SMOAC were kept separate and half of each fraction was loaded on a 50 cm C18 column. Flow-through from the SMOAC protocol was subjected to offline high pH RP-fractionation. Peptides were fractionated into 96 fractions and then condensed to final 12 fractions for MS analysis. An estimated 2 µg peptides from each of the twelve global fractions were cleaned using Thermo C18 Spin Tips and 500 ng peptides per fraction was loaded on column for global proteomics. Mass spectrometry data was acquired on the Thermo Orbitrap Eclipse mass spectrometer equipped with a FAIMS Pro interface. Each peptide fraction was separated using 120-minute LC gradient on a 50 cm C18 EasySpray column. Phosphoproteomics and global proteomics data was acquired with FAIMS TMT-MS2 workflow.

Raw spectra were analyzed through Proteome Discoverer v.s.2.4 and normalized using Internal Reference Scaling-Sample Loading (IRS-SL) method in R (v.3.6.). Deep expression proteins were identified using the Mascot v2.7. search engine and phosphopetides were identified using Byonic (v4.0.12.). Kinase activity profiling was determined using IKAP or KSEA. Statistical significance was defined as Padj<0.05.

Data obtained by Dr. Patrick Pirrote's group (Integrative Mass spectrometry at Tgen and COH)

Immunoblotting

Cells were lysed in lysis buffer (20 mM Tris, 150 mM NaCl, 1 % NP-40, 1 mM NaF, 1 mM sodium phosphate, 1 mM $NaVO_3$, 1 mM EDTA, 1 mM EGTA), supplemented with protease inhibitor cocktail (Roche), phosphatase inhibitor cocktail 2 (Sigma-Aldrich) and 1 mM phenylmethylsulfonyl fluoride. Protein quantification in cell lysates was measured using Pierce BCA protein Assay kit (ThermoFisher). Primary antibodies used in Western blot analysis and secondary horseradish peroxidase-conjugated anti-mouse and anti-rabbit were purchased from Cell Signaling Technology and Novus Biologicals (**Supplemental Table 1**). Protein bands were developed using SuperSignal West Femto Maximum Sensitivity Substrate (ThermoFisher Scientific) according to kit instructions. In each case, a representative of 3 independent experiments is shown. Densitometry was performed using ImageJ/Fiji software to quantify band intensities and results were normalized to the loading control. In the case of pIRE1α, after normalization to the loading control, the ratio of the phosphorylated protein to its corresponding total protein was calculated.

Co-Immunoprecipitation

Whole cell lysates were pre-cleared with beads for 45min at room temperature and then incubated at 4 °C overnight with 2 μg of pull-down antibody (CDK5, BTK or Ire1α) or rabbit IgG isotype control. Pierce Protein A Magnetic Beads (25 μL, ThermoFisher Scientific) were added to lysates and samples were incubated for 1h at room temperature. Beads were washed 3

times in cell lysis buffer (see Immunoblotting) and subjected to immunoblotting. 10% source protein was used as input control.

In vivo MCL model

All mouse care and experiment were conducted in accordance with established institutional guidance and approved protocols of the Institutional Animal Care and Use Committee at City of Hope. 5-week old NOD.Cg-Prkdcscid Il2rgtm1Wjl/Sz (NSG; The Jaxon Laboratories) mice were pre-conditioned with 25 mg/kg of busulfan via intraperitoneal injection (I.P.). The following day, $1x10^6$ JeKo-1 or JeKo-1 CDK5-OE cells were inoculated via tail vain injection (I.V.). Circulating MCL cells were detected using CD19-BV395 and CD5-PECy7 antibodies by flow cytometry. Mice were monitored for survival and euthanized when the human endpoints were met.

Statistical analysis

At least 3 biological replicates were used in all experiments shown throughout the manuscript, unless noted otherwise. Error bars represent standard error of the mean (SEM) unless otherwise indicated. Statistical analysis was performed with Student t test or one-way ANOVA with Tukey's multiple comparisons test, when indicated. For survival, Kaplan-Meier analysis with Logrank (Mantel-Cox) was used. All analysis was performed in GraphPad Prism software. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ throughout the manuscript.

1. Godbersen JC, Humphries LA, Danilova OV, Kebbekus PE, Brown JR, Eastman A*, et al.* The Nedd8-activating enzyme inhibitor MLN4924 thwarts microenvironment-driven NF-kappaB activation and induces apoptosis in chronic lymphocytic leukemia B cells. *Clin Cancer Res* 2014 Mar 15; **20**(6)**:** 1576-1589.

2. Puente XS, Jares P, Campo E. Chronic lymphocytic leukemia and mantle cell lymphoma: crossroads of genetic and microenvironment interactions. *Blood* 2018 May 24; **131**(21)**:** 2283-2296.

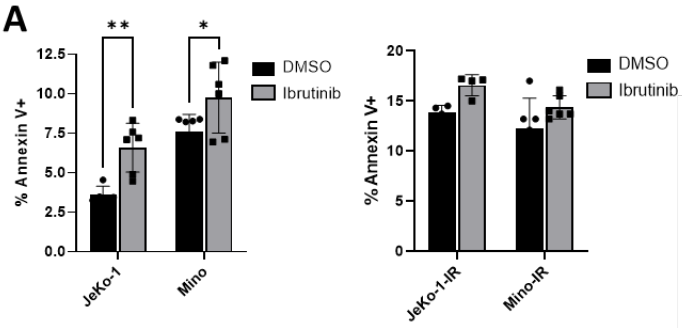
3. Paiva C, Rowland TA, Sreekantham B, Godbersen C, Best SR, Kaur P*, et al.* SYK inhibition thwarts the BAFF - B-cell receptor crosstalk and thereby antagonizes Mcl-1 in chronic lymphocytic leukemia. *Haematologica* 2017 Nov; **102**(11)**:** 1890-1900.

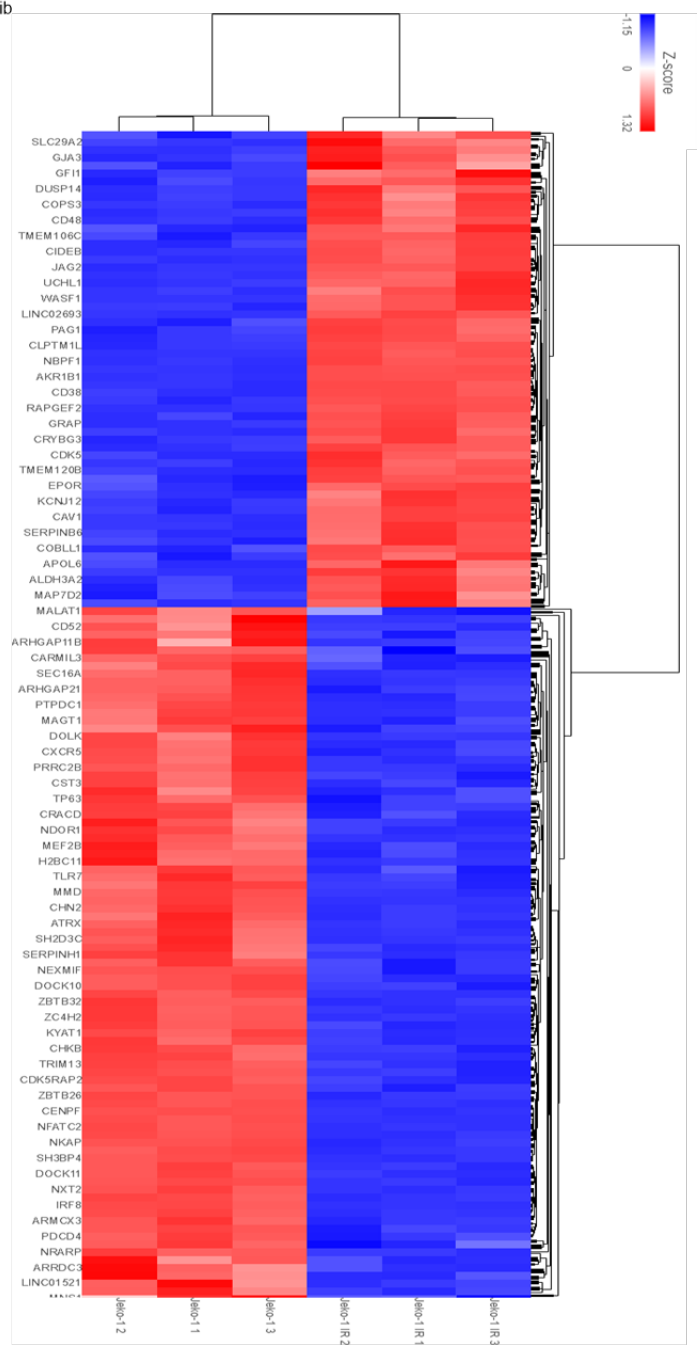
4. van Schadewijk A, van't Wout EF, Stolk J, Hiemstra PS. A quantitative method for detection of spliced X-box binding protein-1 (XBP1) mRNA as a measure of endoplasmic reticulum (ER) stress. *Cell Stress Chaperones* 2012 Mar; **17**(2)**:** 275-279.

5. Tang G, Liu X, Cho M, Li Y, Tran DH, Wang X. Pan-cancer discovery of somatic mutations from RNA sequencing data. *Commun Biol* 2024 May 23; **7**(1)**:** 619.

6. Yi S, Yan Y, Jin M, Bhattacharya S, Wang Y, Wu Y*, et al.* Genomic and transcriptomic profiling reveals distinct molecular subsets associated with outcomes in mantle cell lymphoma. *J Clin Invest* 2022 Feb 1; **132**(3).

Sup. Fig. 1





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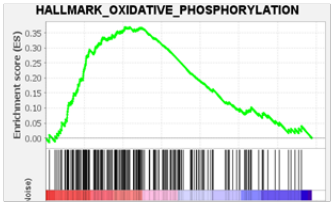
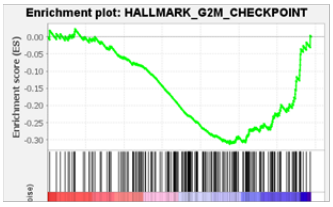


NES: 1.65 p: <0.005



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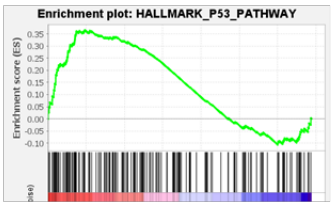
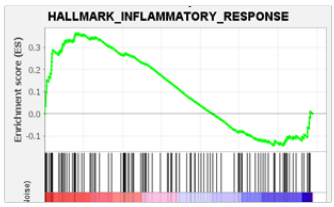
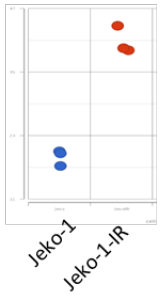


NES: 1.6 p: <0.001



NES: 1.5 p: <0.05

D



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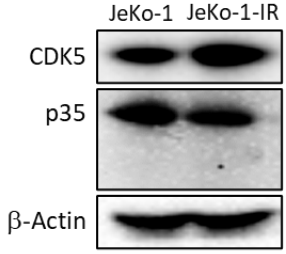
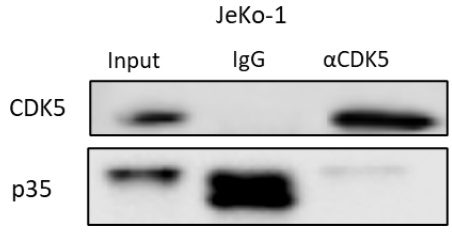




Supplemental Figure 1. RNA-Seq results of Acquired Ibrutinib Resistance models

(A) Ibrutinib-resistant vs. parental cell lines treated with ibrutinib (5 μM for JeKo-1, 1 μM for Mino) or vehicle control for 48 hours. Apoptosis was measured by Annexin V (n=4-6).

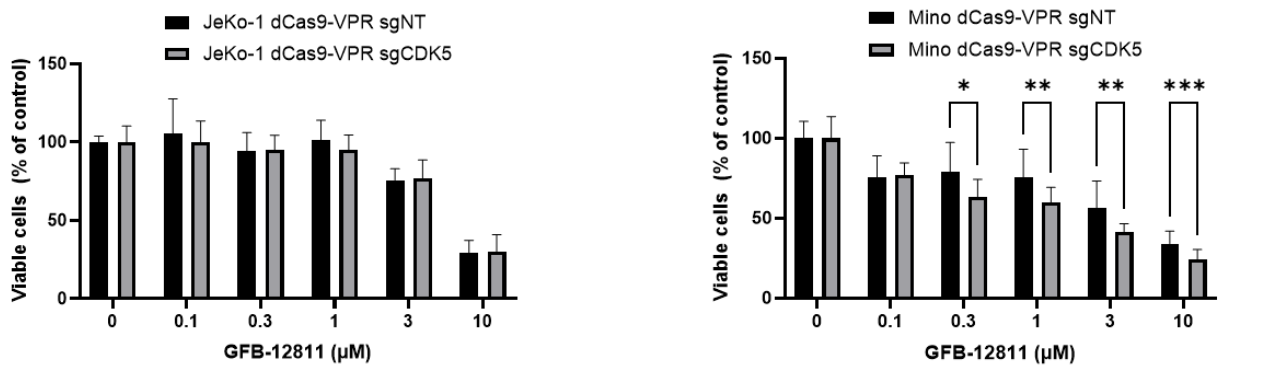
(B) Heat map for JeKo-1 and JeKo-1-IR.

(C) GSEA enrichment plots of RNA-Seq results for JeKo-1-IR vs JeKo-1.

(D) Principal component analysis for CDK5 in JeKo-1 and JeKo-1-IR model (n=3).

(E) Immunoblot of CDK5, p35 and β-actin protein levels in a representative experiment in JeKo-1 and JeKo-1-IR models (left) and pull down of CDK5 and Co-IP with p35 in JeKo-1 (right panel). Representative experiment.

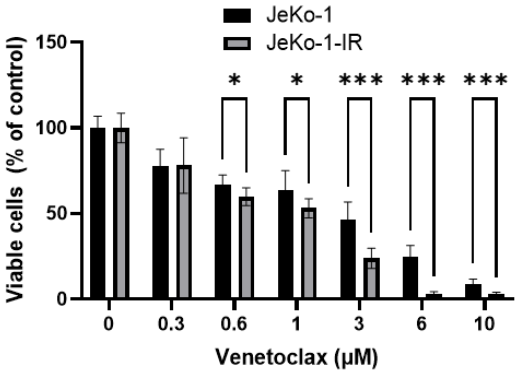
Data are mean ± SEM, *$p<0.05$ and **$p<0.01$.

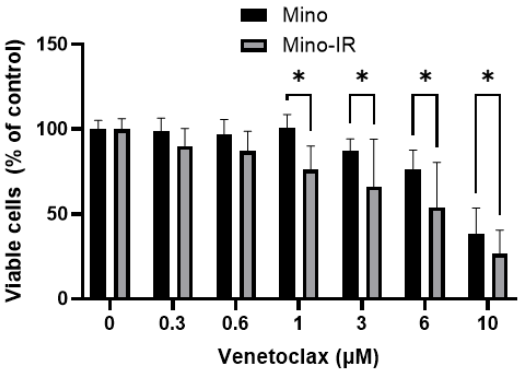


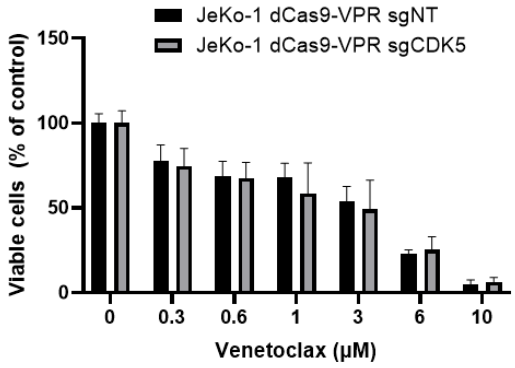
Supplemental Figure 2. CDK5 inhibitor effects in CDK5-OE cells

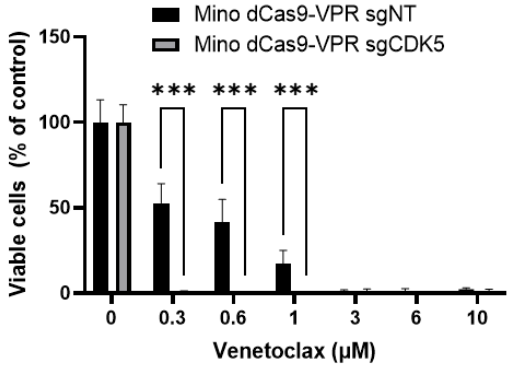
CDK5 overexpressing vs control cells were treated with GFB-12811 or vehicle control for 72h; proliferation was measured by colorimetric MTS assay (n=6-9).

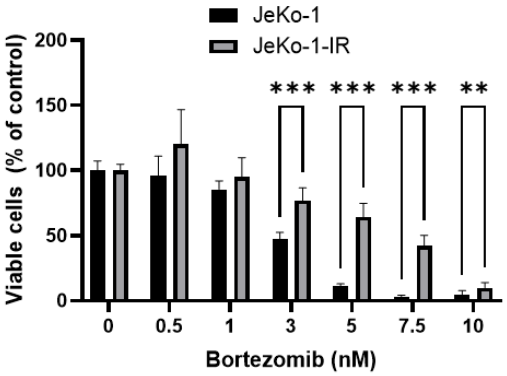
Data are mean ± SEM. **p<0.05, **p<0.01 and ***p<0.001.

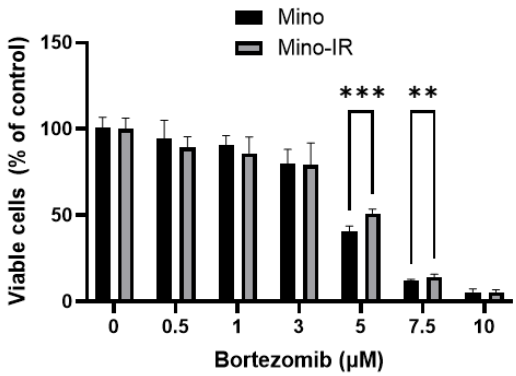

JeKo-1
JeKo-1-IR
Viable cells (% of control)
Venetoclax (μM)
0 0.3 0.6 1 3 6 10
* * *** *** ***

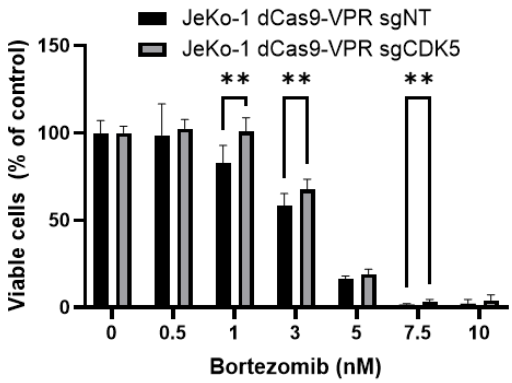

Mino
Mino-IR
Viable cells (% of control)
Venetoclax (μM)
0 0.3 0.6 1 3 6 10
* * * *

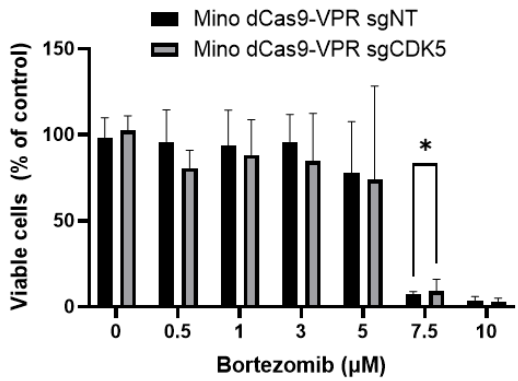

JeKo-1 dCas9-VPR sgNT
JeKo-1 dCas9-VPR sgCDK5
Viable cells (% of control)
Venetoclax (μM)
0 0.3 0.6 1 3 6 10


Mino dCas9-VPR sgNT
Mino dCas9-VPR sgCDK5
Viable cells (% of control)
Venetoclax (μM)
0 0.3 0.6 1 3 6 10
*** *** ***


JeKo-1
JeKo-1-IR
Viable cells (% of control)
Bortezomib (nM)
0 0.5 1 3 5 7.5 10
*** *** *** **

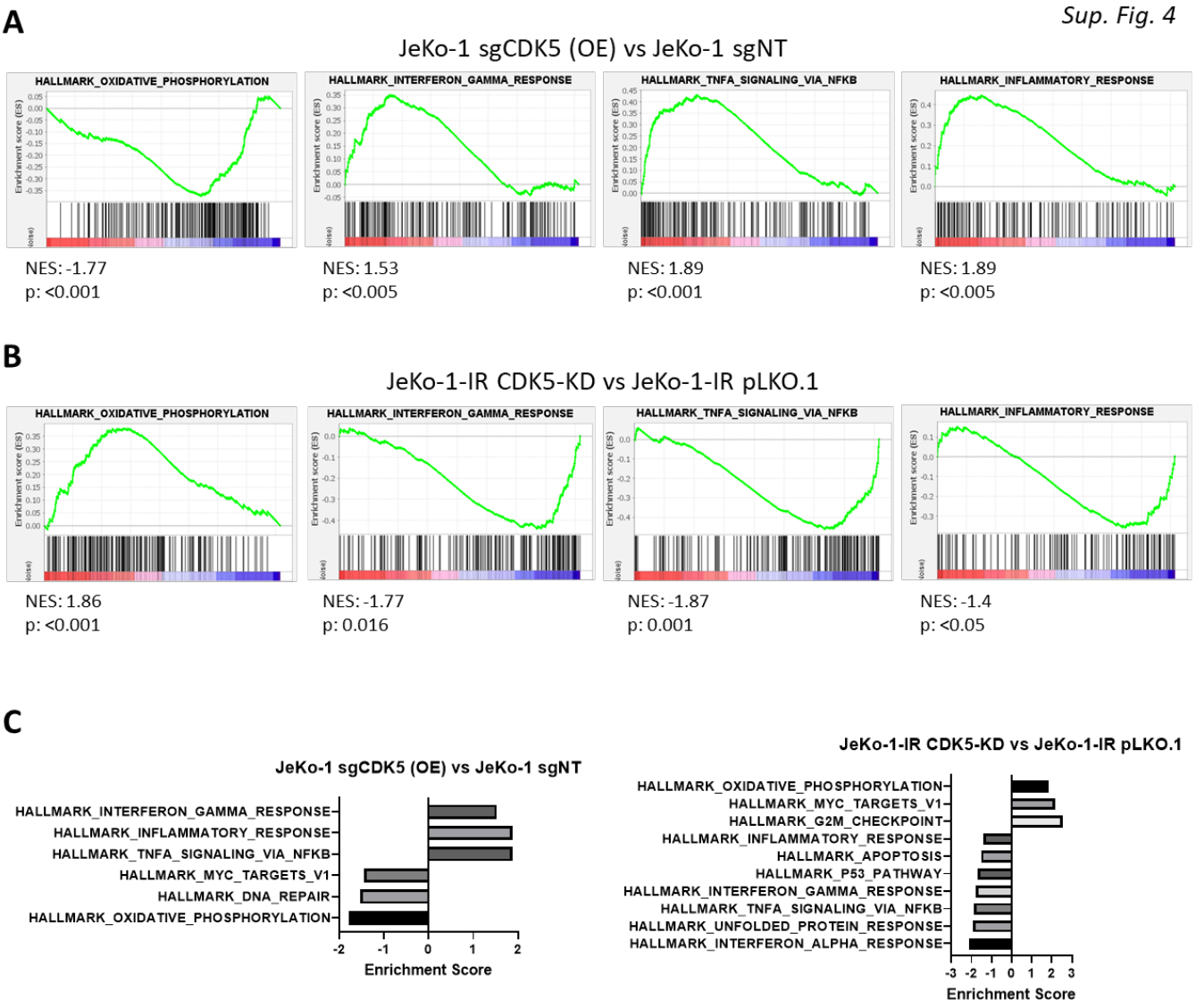

Mino
Mino-IR
Viable cells (% of control)
Bortezomib (μM)
0 0.5 1 3 5 7.5 10
*** **


JeKo-1 dCas9-VPR sgNT
JeKo-1 dCas9-VPR sgCDK5
Viable cells (% of control)
Bortezomib (nM)
0 0.5 1 3 5 7.5 10
** ** **


Mino dCas9-VPR sgNT
Mino dCas9-VPR sgCDK5
Viable cells (% of control)
Bortezomib (μM)
0 0.5 1 3 5 7.5 10
*

Supplemental Figure 3. CDK5 and resistance to venetoclax and bortezomib

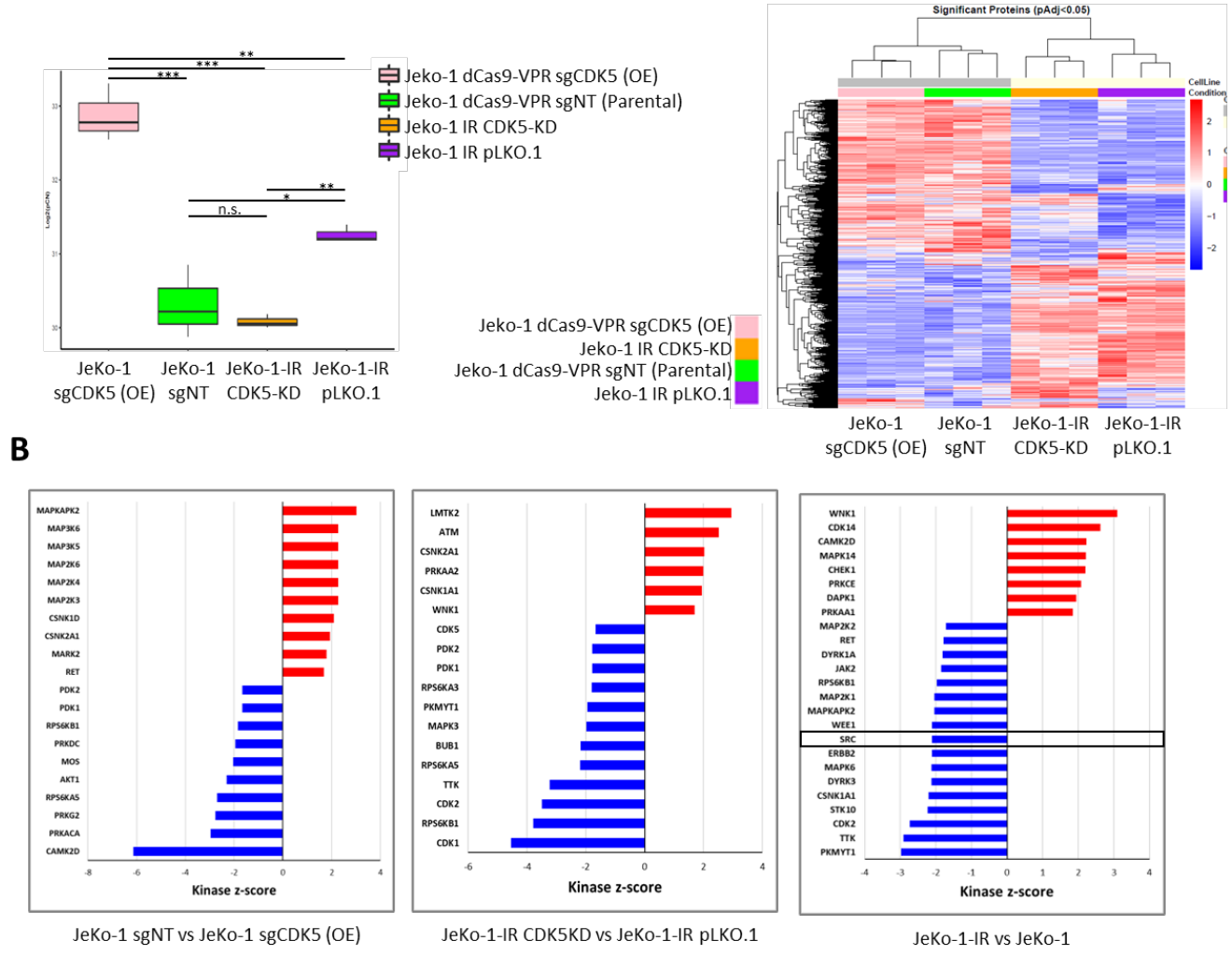
(A) Ibrutinib resistant and parental cell lines (top panels) and CDK5-overexpressing and control cells (bottom panels) were treated with venetoclax or vehicle control for 72h; proliferation was measured by colorimetric MTS assay (n=6-9).
(B) Ibrutinib resistant and parental cell lines (top panels) and CDK5-overexpressing and control cells (bottom panels) were treated with bortezomib or vehicle control for 72h; proliferation was measured by colorimetric MTS assay (n=6-9).
Data are mean ± SEM. **$p<0.05$, **$p<0.01$ and ***$p<0.001$.



Supplemental Figure 4. RNA-seq in MCL cell lines after CDK5 manipulation

(A-B) GSEA enrichment plots of RNA-Seq results for: (top) JeKo-1 CDK5-overexpressing vs control cells gCDK5 (OE) and (bottom) Jeko-1-IR CDK5-KD vs control cells.

(C) GSEA Hallmark Pathway analysis JeKo-1-IR vs parental and Jeko-1-IR CDK5-KD vs control cells. Showing top significantly enriched pathways ranked by NES (normalized enrichment score) (n=3).

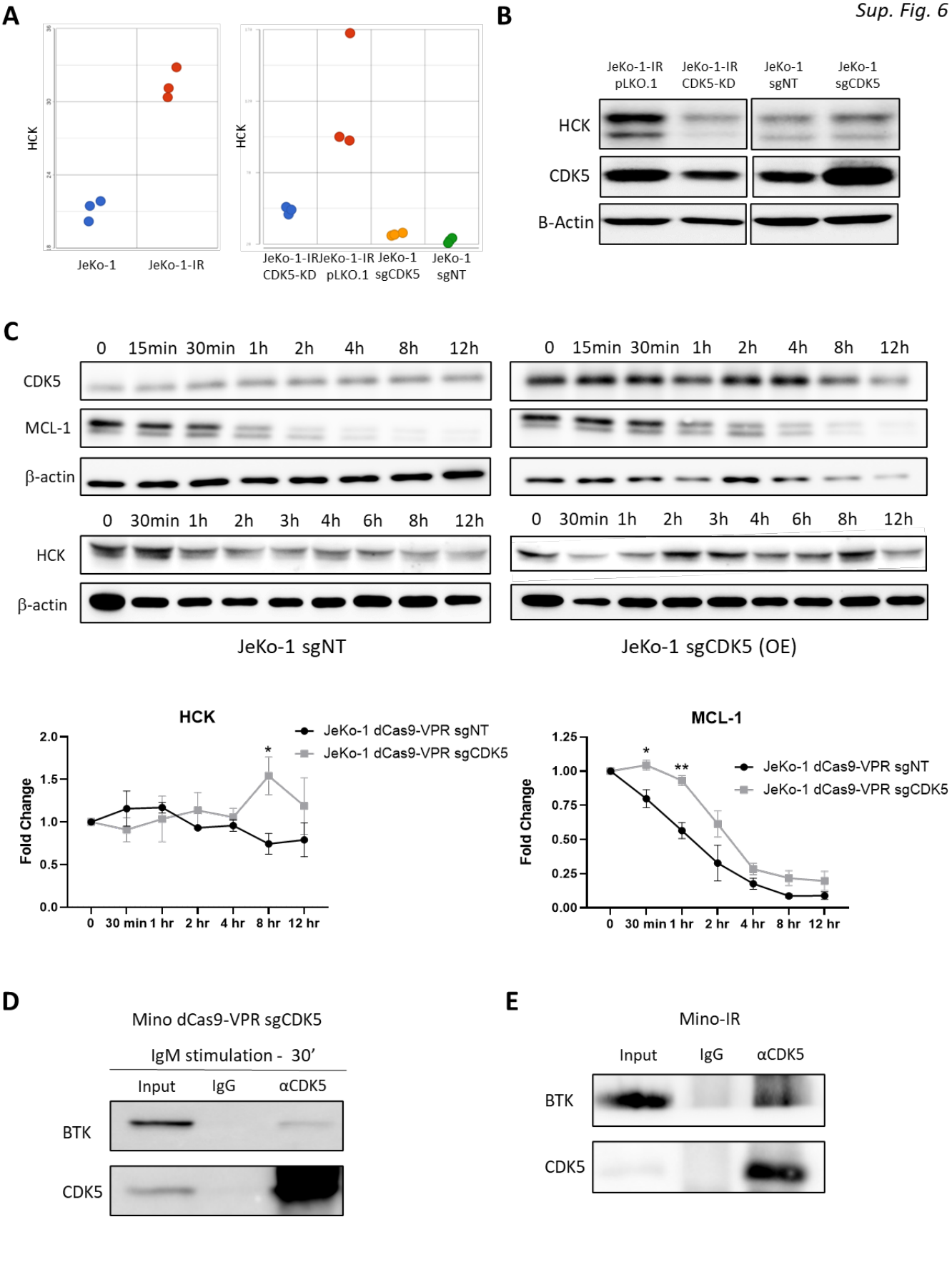


Supplemental Figure 5. Proteomics analysis in JeKo-1 models

(A) CDK5 protein abundance in global proteomics analysis and heat map of differential protein expression in JeKo-1 parental, IR and with CDK5 manipulation.

(B) Kinase-substrate enrichment analysis (KSEA) in JeKo-1 models: Left panel: parental vs IR model, middle panel: control vs CDK5 overexpression, right panel: IR vs IR CDK5-KD (n=3). Padj<0.05.

Sup. Fig. 6


A
HCK
JeKo-1
JeKo-1-IR
JeKo-1-IR CDK5-KD
JeKo-1-IR pLKO.1
JeKo-1 sgCDK5
JeKo-1 sgNT
B
JeKo-1-IR pLKO.1
JeKo-1-IR CDK5-KD
JeKo-1 sgNT
JeKo-1 sgCDK5
HCK
CDK5
B-Actin
C
0 15min 30min 1h 2h 4h 8h 12h
CDK5
MCL-1
β-actin
0 30min 1h 2h 3h 4h 6h 8h 12h
HCK
β-actin
JeKo-1 sgNT
JeKo-1 sgCDK5 (OE)
HCK
JeKo-1 dCas9-VPR sgNT
JeKo-1 dCas9-VPR sgCDK5
Fold Change
0 30 min 1 hr 2 hr 4 hr 8 hr 12 hr
MCL-1
JeKo-1 dCas9-VPR sgNT
JeKo-1 dCas9-VPR sgCDK5
Fold Change
0 30 min 1 hr 2 hr 4 hr 8 hr 12 hr
D
Mino dCas9-VPR sgCDK5
IgM stimulation - 30'
Input
IgG
αCDK5
BTK
CDK5
E
Mino-IR
Input
IgG
αCDK5
BTK
CDK5

Supplemental Figure 6. Src kinases in Ibrutinib resistance

(A) Principal component analysis for HCK in JeKo-1 models: parental vs IR (left panel), control vs CDK5 overexpression, IR vs IR-CDK5-KD (right panel) (n=3).

(B) Protein lysates from JeKo-1 cells with manipulated CDK5 were subjected for immunoblotting. A representative blot of 3 independent experiments is shown.

(C) Protein lysates from JeKo-1 CDK5 overexpressing and control cells from ciclohexamide experiments were subjected for immunoblotting (top panels; representative blot of 3 independent experiments is shown) and summary quantification (bottom panels).

(D) Protein lysates grom Mino CDK5 overexpressing cells were subjected to CDK5 immunoprecipitation experiment after 30' of IgM stimulation. A representative blot of 3 independent experiments is shown.

(E) Protein lysates grom Mino-IR cells were subjected to CDK5 immunoprecipitation experiment. A representative blot of 3 independent experiments is shown.

Data are mean ± SEM. **p<0.05 and **p<0.01.

Supplemental Table 1. List of Antibodies

Antibody	Clone	Cat. #	Company
Immunoblotting			
AKT		9272	Cell signaling
B-actin	13E5	4970	Cell signaling
BTK	D3H5	8547	Cell signaling
BTK	D6T2C	56044	Cell signaling
CDK5	D1F7M	14145	Cell signaling
CDK5	1H3	12134	Cell signaling
ERK		9102	Cell signaling
GAPDH	14C10	2118	Cell signaling
HCK	E117F	14643	Cell signaling
Ire1α	14C10	3294	Cell signaling
MCL-1	D2W9E	94296	Cell signaling
p35/P25	C64B10	2680	Cell signaling
PCLγ2		3872	Cell signaling
p-AKT	S473	4058	Cell signaling
p-BTK	Y223	5082	Cell signaling
p-ERK	T202/Y204	9101	Cell signaling
p-Ire1α	Ser724	NB100-2323	Novus Biologicals
p-PCLγ2	Y1217	3871	Cell signaling
p-Src family	Tyr-416 / E6G4R	59458	Cell signaling
Vinculin	E1E9V	13901	Cell signaling
Xbp1s	E9V3E	40435	Cell signaling
Flow cytometry			
CD45-PERCPCy5.5	564105	HI30	BD biosciences
CD19-BV395	302298	HIB19	Biolegend
CD5-PECy7	571696	UCHT2	BD Biosciences
Live/Dead Fixable aqua		L34965	ThermoFisher

Supplemental Table 2. List of acquired mutations in JeKo1-IR

JeKo-1 vs JeKo-1-IR											
Sample	Gene	chr	start	end	strand	mutClass	mutType	refBase	varBase	AAC	MAF
Jeko1_D	VPS13D	chr1	12244275	12244275	+	MISSENSE	SNP	C	A	p.P69T	0.231
Jeko1_D	ALDH4A1	chr1	18883304	18883304	-	MISSENSE	SNP	T	C	p.N193S	0.291
Jeko1_D	SNIP1	chr1	37552706	37552706	-	MISSENSE	SNP	C	G	p.S89T	0.073
Jeko1_D	SETDB1	chr1	150961094	150961094	+	MISSENSE	SNP	A	T	p.E1011V	0.202
Jeko1_D	RAB3GAP2	chr1	220190402	220190402	-	MISSENSE	SNP	T	C	p.N536D	0.386
Jeko1_D	HLX	chr1	220880359	220880359	+	MISSENSE	SNP	G	A	p.A168T	0.056
Jeko1_D	KLHL29	chr2	23642756	23642756	+	MISSENSE	SNP	T	G	p.N282K	0.251
Jeko1_D	TTC27	chr2	32630679	32630679	+	MISSENSE	SNP	C	T	p.T82I	0.095
Jeko1_D	BCL11A	chr2	60461284	60461284	-	MISSENSE	SNP	A	T	p.L543Q	0.06
Jeko1_D	DQX1	chr2	74522605	74522605	-	MISSENSE	SNP	C	A	p.M490I	0.345
Jeko1_D	TMEM237	chr2	201626057	201626057	-	MISSENSE	SNP	C	G	p.L376F	0.114
Jeko1_D	TLR10	chr4	38774177	38774177	-	MISSENSE	SNP	T	A	p.N472Y	0.067
Jeko1_D	GEMIN5	chr5	154891346	154891346	-	MISSENSE	SNP	C	T	p.R1386Q	0.328
Jeko1_D	FAM193B	chr5	177536604	177536604	-	MISSENSE	SNP	A	T	p.L277Q	0.066
Jeko1_D	TBP	chr6	170562157	170562157	+	MISSENSE	SNP	T	A	p.S141T	0.19
Jeko1_D	AKAP9	chr7	92096783	92096783	+	MISSENSE	SNP	A	T	p.Q3275L	0.619
Jeko1_D	MNX1	chr7	157010047	157010047	-	MISSENSE	SNP	C	T	p.G102S	0.06
Jeko1_D	IMPAD1	chr8	56964040	56964040	-	MISSENSE	SNP	T	A	p.D278V	0.437
Jeko1_D	PDP1	chr8	93923359	93923359	+	MISSENSE	SNP	G	A	p.G434S	0.313
Jeko1_D	AGTPBP1	chr9	85578939	85578939	-	MISSENSE	SNP	C	A	p.C1068F	0.106
Jeko1_D	MTPAP	chr10	30322404	30322404	-	MISSENSE	SNP	T	G	p.L402F	0.224
Jeko1_D	BTAF1	chr10	92027197	92027197	+	MISSENSE	SNP	A	G	p.Q1768R	0.219
Jeko1_D	TAF5	chr10	103368429	103368429	+	MISSENSE	SNP	T	G	p.L147R	0.102
Jeko1_D	RASGRP2	chr11	64742110	64742110	-	MISSENSE	SNP	C	A	p.D26Y	0.298
Jeko1_D	SORL1	chr11	121496922	121496922	+	MISSENSE	SNP	C	A	p.P271H	0.296
Jeko1_D	LRMP	chr12	25004487	25004487	+	MISSENSE	SNP	C	T	p.P49L	0.333
Jeko1_D	LETMD1	chr12	51059366	51059366	+	NONSENSE	SNP	G	T	p.E353*	0.149
Jeko1_D	ZBTB39	chr12	57004533	57004533	-	MISSENSE	SNP	C	A	p.A129S	0.089
Jeko1_D	SYNE2	chr14	64126773	64126773	+	MISSENSE	SNP	T	A	p.L4579Q	0.103
Jeko1_D	SYNE3	chr14	95417852	95417852	-	MISSENSE	SNP	G	A	p.R968C	0.094
Jeko1_D	TSC2	chr16	2065607	2065607	+	MISSENSE	SNP	C	G	p.A563G	0.21
Jeko1_D	NFATC2IP	chr16	28954607	28954607	+	MISSENSE	SNP	C	T	p.S168F	0.199
Jeko1_D	LMNB2	chr19	2431598	2431598	-	MISSENSE	SNP	C	T	p.E591K	0.27
Jeko1_D	MYO9B	chr19	17195126	17195126	+	MISSENSE	SNP	A	T	p.E1233D	0.064
Jeko1_D	RIMBP3	chr22	18607144	18607144	-	MISSENSE	SNP	G	C	p.P1431A	0.102
Jeko1_D	CHCHD10	chr22	23767547	23767547	-	MISSENSE	SNP	A	T	p.S30T	0.129
Jeko1_D	RAB9A	chrX	13709188	13709188	+	MISSENSE	SNP	T	C	p.Y148H	0.052

Supplemental Table3. List of acquired mutations in Mino-IR

Mino vs Mino-IR											
Sample	**Gene**	**chr**	**start**	**end**	**strand**	**mutClass**	**mutType**	**refBase**	**varBase**	**AAC**	**MAF**
Mino_D	HECTD3	chr1	45007567	45007567	-	MISSENSE	SNP	C	T	p.R450H	0.078
Mino_D	UTP25	chr1	2.1E+08	2.1E+08	+	MISSENSE	SNP	G	C	p.E689Q	0.086
Mino_D	ZNF672	chr1	2.49E+08	2.49E+08	+	MISSENSE	SNP	G	T	p.D39Y	0.11
Mino_D	TMEM214	chr2	27034111	27034111	+	MISSENSE	SNP	A	G	p.N66D	0.068
Mino_D	ASNSD1	chr2	1.9E+08	1.9E+08	+	MISSENSE	SNP	G	C	p.V480L	0.131
Mino_D	ASNSD1	chr2	1.9E+08	1.9E+08	+	MISSENSE	SNP	T	C	p.S482P	0.112
Mino_D	GOLGA4	chr3	37326948	37326948	+	MISSENSE	SNP	G	C	p.V1688L	0.4
Mino_D	CENPU	chr4	1.85E+08	1.85E+08	-	MISSENSE	SNP	A	G	p.L263P	0.149
Mino_D	RASA1	chr5	87268820	87268820	+	MISSENSE	SNP	G	C	p.L123F	0.125
Mino_D	CTNNA1	chr5	1.39E+08	1.39E+08	+	MISSENSE	SNP	G	C	p.G53A	0.159
Mino_D	TCOF1	chr5	1.5E+08	1.5E+08	+	MISSENSE	SNP	C	T	p.A143V	0.058
Mino_D	CYFIP2	chr5	1.57E+08	1.57E+08	+	MISSENSE	SNP	G	C	p.A437P	0.057
Mino_D	OARD1	chr6	41070096	41070096	-	MISSENSE	SNP	C	T	p.G36R	0.079
Mino_D	ZNF292	chr6	87257117	87257117	+	MISSENSE	SNP	G	A	p.S1163N	0.067
Mino_D	CAPZA2	chr7	1.17E+08	1.17E+08	+	MISSENSE	SNP	G	C	p.A105P	0.153
Mino_D	ZNF212	chr7	1.49E+08	1.49E+08	+	MISSENSE	SNP	C	T	p.H334Y	0.143
Mino_D	RASSF4	chr10	44993322	44993322	+	MISSENSE	SNP	C	T	p.A320V	0.081
Mino_D	TAPBPL	chr12	6458803	6458803	+	MISSENSE	SNP	C	T	p.L355F	0.2
Mino_D	EEF1AKMT1	chr13	20757571	20757571	-	MISSENSE	SNP	G	T	p.P10T	0.545
Mino_D	MTHFS	chr15	79889222	79889222	-	MISSENSE	SNP	G	A	p.R113W	0.086
Mino_D	FANCI	chr15	89306145	89306145	+	NONSENSE	SNP	T	G	p.L1103*	0.101
Mino_D	IGF1R	chr15	98913247	98913247	+	MISSENSE	SNP	A	T	p.K598M	0.097
Mino_D	ROGDI	chr16	4797748	4797748	-	MISSENSE	SNP	A	G	p.V263A	0.13
Mino_D	THRA	chr17	40083961	40083961	+	MISSENSE	SNP	G	A	p.A117T	0.081
Mino_D	MEX3C	chr18	51177112	51177112	-	MISSENSE	SNP	A	G	p.F237L	0.106
Mino_D	SHKBP1	chr19	40590285	40590285	+	MISSENSE	SNP	C	T	p.A544V	0.1
Mino_D	CEP250	chr20	35502836	35502836	+	MISSENSE	SNP	A	C	p.L1489F	0.067
Mino_D	PRAF2	chrX	49073882	49073882	-	MISSENSE	SNP	G	A	p.R36C	0.105