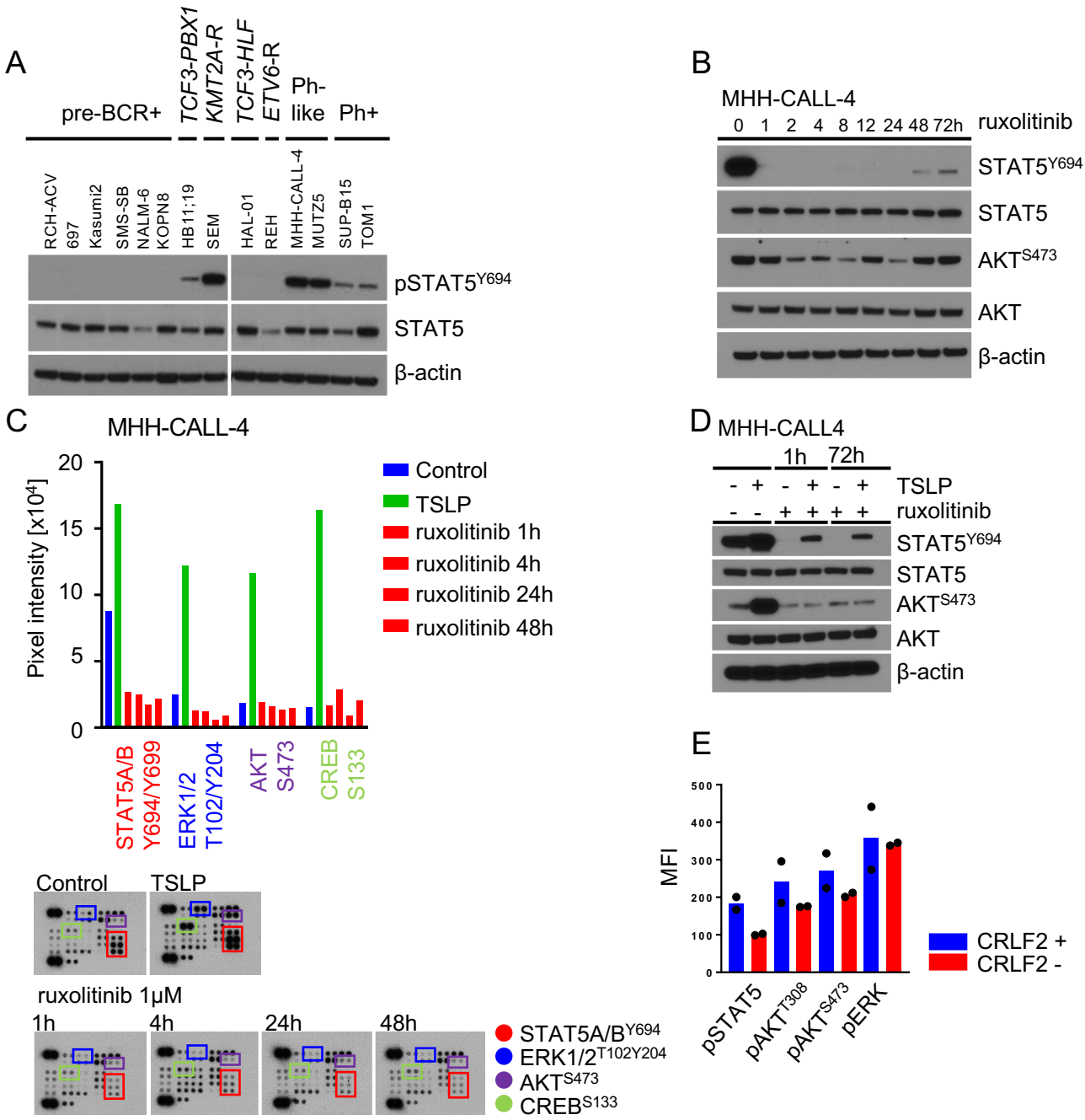
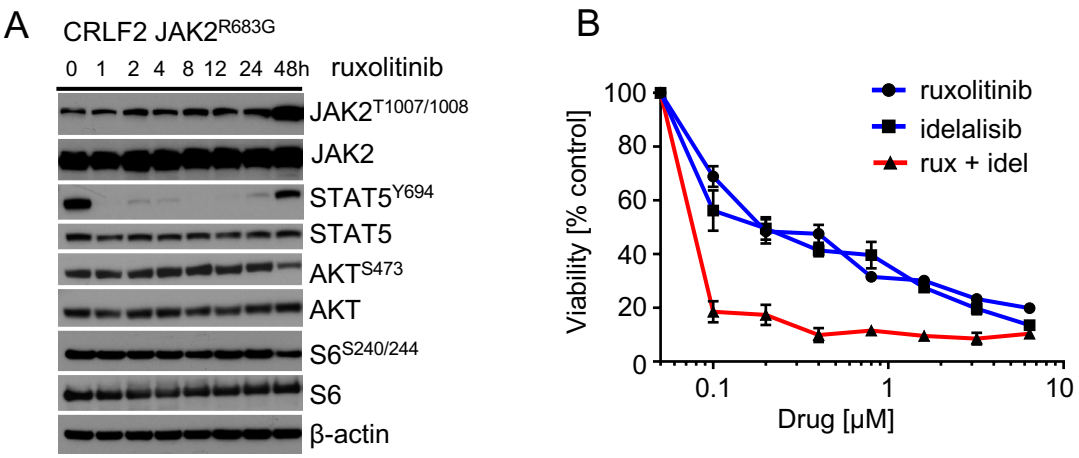
Supplemental Figure 1



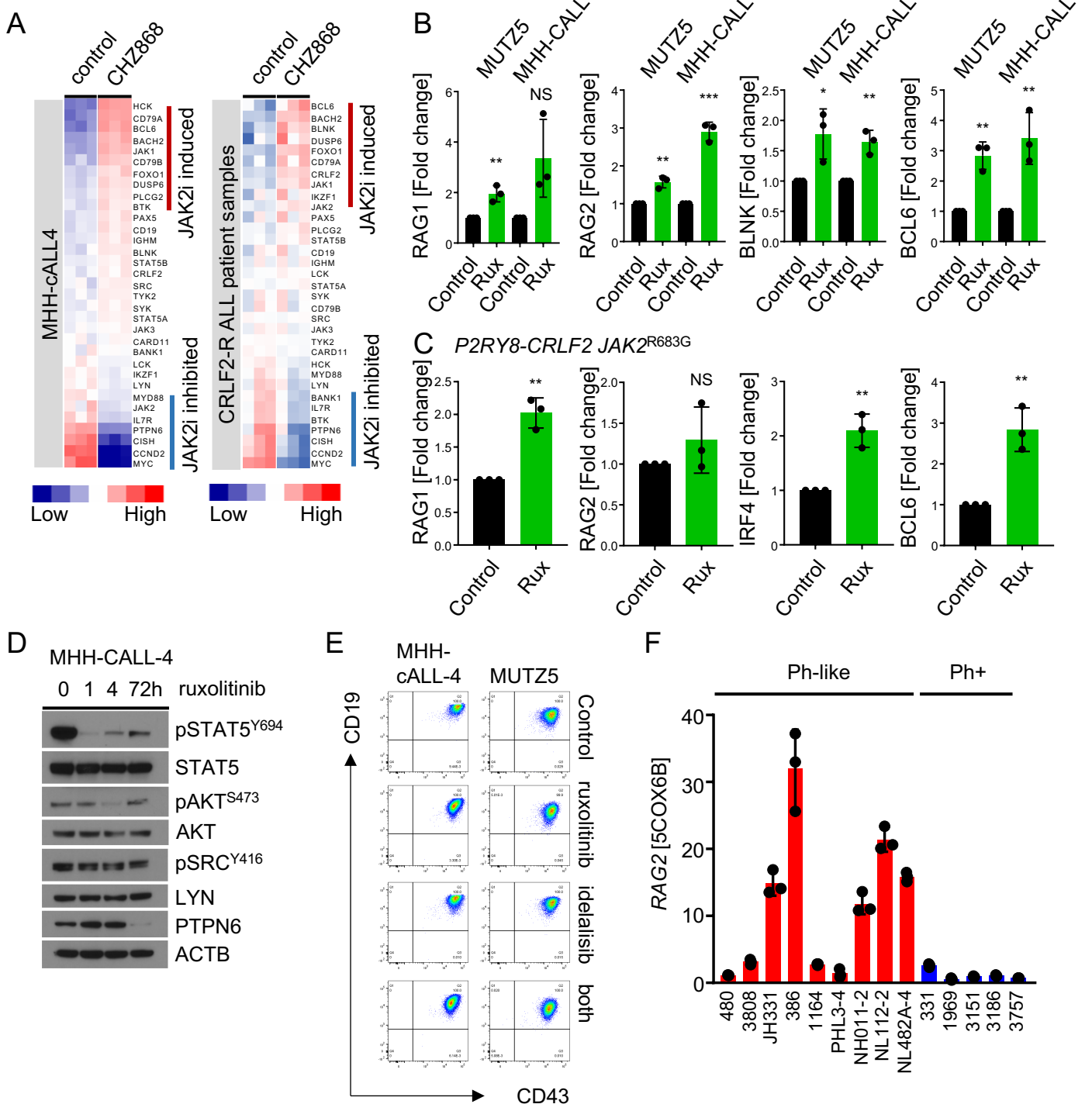
(A) Western blot analysis of pSTAT5 in multiple B-ALL cell lines using beta-actin as loading control. **(B)** Protein analysis of indicated targets in MHH-CALL-4 cells treated with 1 μM ruxolitinib for the indicated timepoints. **(C)** Phosphoprotein analysis using the human phospho-kinase array from R&D Systems in MHH-CALL-4 cells treated with TSLP (25 nM x 30 min) or ruxolitinib (1 μM x 1h). A scan of each blot for each condition is included on the bottom. The colored boxes indicate changes that were observed with TSLP stimulation (n=1). **(D)** MHH-CALL-4 cells were treated either with TSLP (25 nM x 30min) or ruxolitinib (1μM x 1h or 72h) alone or TSLP was added to the ruxolitinib treaded samples (25nM; 30 min). Protein expression of pSTAT5^{Y694}, pAKT^{S473}, and the corresponding total protein controls were tested via Western blot using beta-actin as control. **(E)** MUTZ5 cells isolated from the spleen of terminally sick NSG mice **(shown in Figure 2E)** were tested for signaling activation via phosphoflow in CRLF2-null and CRLF2-expressing cells by gating on the corresponding cells (see Figure 2E). Only two of the three injected mice were available for this analysis.

Supplemental Figure 2



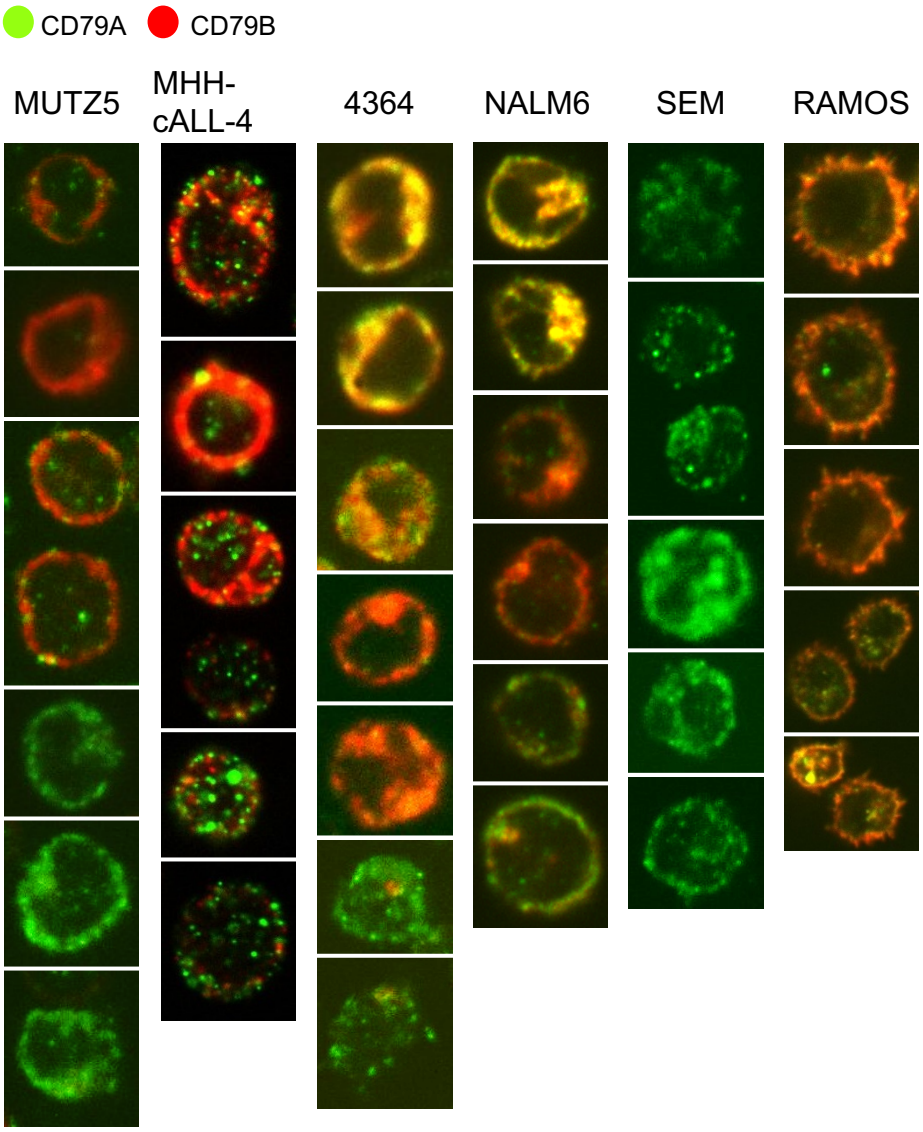
(A) Protein analysis of indicated targets in murine CRLF2/JAK2^{R683G} Ph-like ALL cells treated with 500 nM ruxolitinib for the indicated time points. β-actin was used as loading control. **(B)** Viability of mCRLF2-JAK2 cells treated with increasing concentrations of ruxolitinib and idelalisib (blue) or with 1 μM idelalisib and increasing concentrations of ruxolitinib (red) was assessed (n=3). Data are represented as individual values with mean ± SEM bars.

Supplemental Figure 3



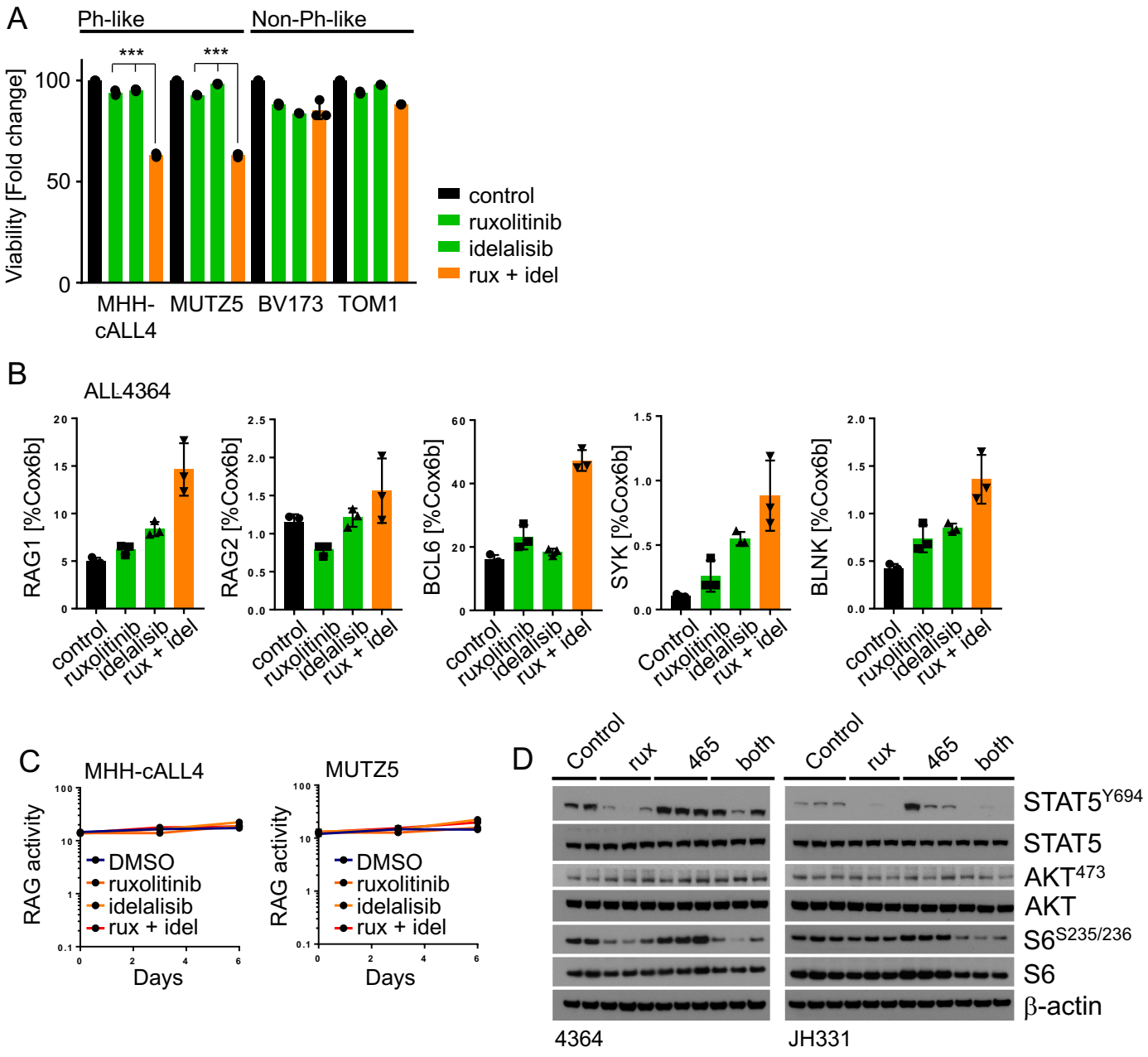
(A) Supervised meta-analysis of gene expression data (GSE61696). RT-PCR validation of gene expression data for the indicated targets in **(B)** MUTZ5 and MHH-CALL-4 and **(C)** Ph-like ALL4364 PDX model treated in vitro with 1 μM ruxolitinib for 12h (n=3 independent experiments/biologic replicates each with 3 technical replicates). **(D)** MHH-CALL4 cells were treated with 1 μM ruxolitinib for indicated timepoints. Protein levels for the indicated targets were determent via Western blotting. (E) Flow cytometric analysis of CD19 and CD43 expression in MHH-CALL-4 cells treated with ruxolitinib (1 μM), idelalisib (1 μM) or both (n=3). (F) *RAG2* expression levels were tested in the indicated PDX samples (Ph-like red; Ph+ blue) via RT-PCR using *COX6B* as control (n=3). Data are represented as individual values with mean ± SEM bars.

Supplemental Figure 4



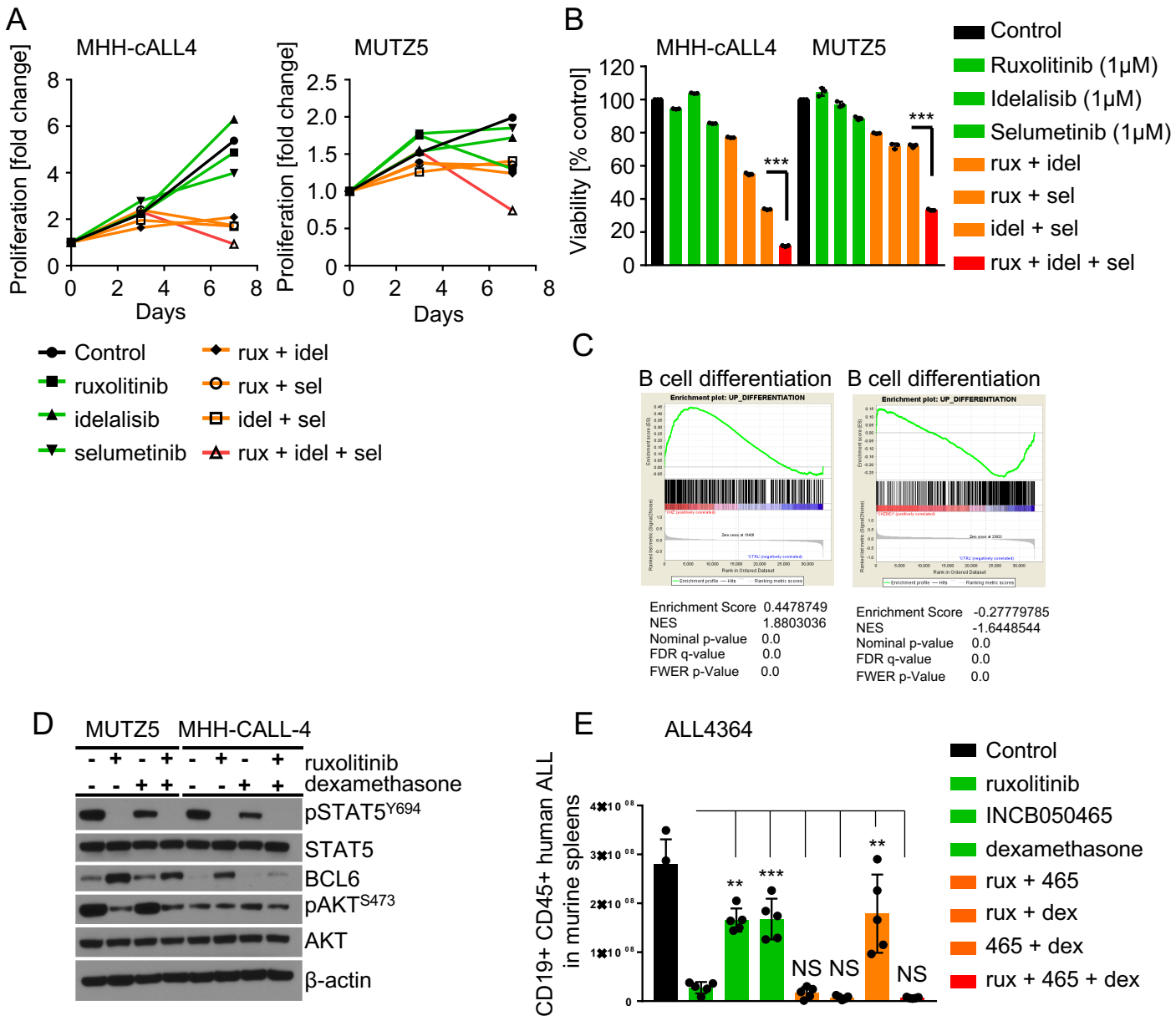
Immunofluorescence microscopy for CD79A (green) and CD79B (red) in Ph-like ALL cell lines (MUTZ5, MHH-CALL-4) and one Ph-like ALL PDX sample (ALL4364). The CD79B-negative *KMT2A-R* B-ALL cell line SEM was used as negative control, and the CD79A+/CD79B+ lymphoma cell line Ramos was used as positive control.

Supplemental Figure 5



(A) Viability analysis via flow cytometry in 2 Ph-like and 2 Ph+ ALL cell lines after 3 days of treatment with ruxolitinib, idelalisib, or a combination of both (each 1 µM, n=3 independent experiments). **(B)** Cells from the Ph-like *CRLF2-R* PDX ALL4364 model were treated for 4h with 1µM ruxolitinib and 2µM idelalisib or both drugs. RT-PCR was performed, and expression levels of the indicated targets was tested in comparison to *COX6B* as control (n=3 technical repeats). **(C)** MUTZ5 and MHH-CALL-4 cells were lentivirally transduced with a RAG enzyme activity reporter. Both cell lines were treated with ruxolitinib and idelalisib for 6 days with a concentration of 1 µM for each drug. **(D)** Protein analysis of pSTAT5 and pS6 of isolated ALL PDX cells from the in vivo experiment described in Figure 4 D and E. Three mice were used for each treatment group. Data for (A) are represented as individual values with mean ± SEM bars, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by unpaired t-test.

Supplemental Figure 6



(A) MHH-CALL-4 and MUTZ5 cells were treated with the indicated kinase inhibitors, and cell proliferation was monitored over time using an automated cell counter (7 days, n=3). **(B)** 7AAD/ANNEXIN V flow cytometry analysis of MHH-CALL-4 and MUTZ5 cells treated with indicated kinase inhibitors (1 μM each; 3 days, n=3). **(C)** Gene set enrichment analysis (GSEA) plots demonstrate i) enrichment of B cell differentiation gene sets in CRLF2-rearranged B-ALL PDXs cells treated with CHZ868 compared to vehicle-treated controls (left panel) or ii) cotreatment with dexamethasone (right panel) for treatment details see[44]. Statistics are shown. **(D)** MHH-CALL-4 and MUTZ5 cells were treated with ruxolitinib (1μM) or dexamethasone (10 nM) for 4h and subjected to a protein analysis of the indicated target genes. **(E)** Mice were injected via tail vein with Ph-like ALL PDX ALL4364 cells. Once 5% blast were detected in the blood, therapy of the indicated drug combination was initiated (see Material and Methods for dosing). Mice were treated for 4 weeks and sacrificed 12h after the treatment has stopped and spleen and blood cells were analysis via flow cytometry for CD19+/CD45+ ALL cells. Data are represented as individual values with mean ± SEM bars, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by unpaired t-test (B) or ANOVA with Dunnett post-test for multiple comparisons (E).

Supplemental Table 1

Patient	USI	Age (years)	WBC Count (10e3 cells/uL)	Sex	Race/ Ethnicity	Genomic Lesion(s)	Cytogenetics	Notes	Induction Chemotherapy Regimen or Trial	TKI	HSCT
Ph-like B-ALL (n=14)											
1	**UP_386**	64	28.6	F	Caucasian	*P2RY8-CRLF2, JAK2* R683G	46,XX		hyperCVAD	no	unknown
2	**UP_480**	49	2.9	M	Caucasian	*IGH-CRLF2*	46,XY		E2993	no	yes
3	**UP_810**	62	9.7	M	Caucasian	*CRLF2+	46,XY		hyperCVAD	no	no
4	**UP_1164**	42	19.3	M	Caucasian	*P2RY8-CRLF2, JAK2* K682delinsGGE	n/a		hyperCVAD	no	yes
5	**UP_2142**	29	136.2	M	Caucasian	*IGH-CRLF2*	46,XY,del(9)(p21p21)[6]/46,XY[24]	Positive FISH for deletion of 9p21 (*CDKN2A* and CDKN2B): nuc ish(*9p11-q11.1x2),(*CDKN2Ax1)[130/200]	C10403	no	yes
6	**UP_2549**	28	2.2	M	Caucasian	*IGH-CRLF2, JAK2* R938Q	46,XY		C10403	no	no
7	**UP_3211**	63	14.5	M	Caucasian	*IGH-CRLF2, JAK2* F694L	n/a		hyperCVAD	no	no
8	**UP_3808**	43	42.1	M	Caucasian	*IGH-CRLF2, JAK2* R683G	46,XY		hyperCVAD	no	unknown
9	**UP_4986**	37	193.5	M	Hispanic/ Latino	*IGH-CRLF2, JAK2* L977L *and* C616_C618delinsGV	46,XY		E1910/arm A	no	no
10	**UP_4988**	60	60.5	F	Hispanic/ Latino	*IGH-CRLF2, JAK2 F694S*	46,XX, del(7)(p11.2)[7]/46,XX[13]	*KRAS* G12R & *NOTCH1* VUS	E1910/arm A	no	yes
11	**UP_5049**	41	46	F	Caucasian	Ph-like kinase fusions not detected	45,XX, add(3)(q?24), -7[6]/ 46,XX[4]	*FLT3* internal tandem duplication	yes	no	yes
12	**UP_5318**	29	23.2	M	Caucasian	truncated *EPOR*, *TBL1XR1-PIK3CA*	46,XY,t(2;19)(p21;p13.1)[2]/46,XY[18]	Negative for *ABL1* and *JAK2* fusion genes on panel.	C10403	no	no
13	**UP_6005**	75	24.2	M	Hispanic/ Latino	*IGH-CRLF2, KRAS* G12D	48,XY,+8,+21[12]/46,XY[2]	*BCORL1* P562Rfs*43 and *FLT3* N676K pathogenic mutations on CPD	ECOG 1910	no	no
14	**UP_6096**	76	48.2	F	Caucasian	*TP53* R280S	35~38,XX,-3,-7,-9,dic(10;18)(q26;p11.2),der(12)t(9;12)(q13;p13),-13,-15,-16,-17,add(17)(p11.2),-19,-20,+1~2mar[cp11]/46,XX[1]	*BCOR* D1712N (VUS) and *TPMT* A154T and Y240C	weekly vincristine, IT cytarabine	no	no

Supplemental Table 1

Patient	USI	Age (years)	WBC count (10e3 cells/uL)	Sex	Race/ Ethnicity	Genomic lesion(s)	Cytogenetics	Notes	Induction Chemotherapy Regimen or Trial	TKI	HSCT
Ph+ B-ALL (n=33)											
15	**UP_408**	21.9	31.3	M	Caucasian	BCR-ABL1 p190	46,XY[11]	BCR-ABL1+ by RT-PCR	E2993	no	unknown
16	**UP_1120**	n/a	161	M	n/a	BCR-ABL1 p190	n/a		unknown	unknown	unknown
17	**UP_1182**	61	35.1	F	Caucasian	BCR-ABL1 p190	46,XX,t(9;22)(q34;q11.2)[9]		hyperCVAD	yes - imatinib	unknown
18	**UP_1441**	37	35.1	F	Hispanic/Latino	BCR-ABL1 p190	n/a		hyperCVAD	yes - imatinib	yes
19	**UP_1866**	64	19.4	M	n/a	BCR-ABL1 p190	53~55,XY,+X,+der(1)t(1;7)(p13;q11.2),+2,+6,-7,t(9;22)(q34;q11.2),+10,+11,+14,+18,+21,+der(22)t(9;22),+mar[cp16]/46,XY[4]		hyperCVAD	yes - imatinib	no
20	**UP_1969**	52	17.7	F	Caucasian	BCR-ABL1 p210	46,XX,t(9;22)(q34;q11.2)[10]/45,XX,-7,t(9;22)(q34;q11.2)[3]/46,XX[12]		hyperCVAD	yes - imatinib	no
21	**UP_2270**	54	12.6	M	Caucasian	BCR-ABL1 p210	46,XY,t(9;22)(q34;q11.2)[10]/46,XY[15]		hyperCVAD	yes - dasatinib	no
22	**UP_2802**	78	6.8	F	Caucasian	BCR-ABL1 p190	46,XX,t(9;22)(q34;q11.2)[6]/46,XX[2]		methotrexate & cytarabine	yes - dasatinib	no
23	**UP_3151**	65	58.3	F	Black/African American	BCR-ABL1 p190	n/a		hyperCVAD	yes - dasatinib	no
24	**UP_3186**	53	75.4	F	Caucasian	BCR-ABL1 p210	46,XX		hyperCVAD	yes - dasatinib	yes
25	**UP_3285**	88	56	M	Caucasian	BCR-ABL1 p190	46,XY,der(9)del(9)(p22)t(9;22)(q34;q11.2),der(22)t(9;22)[10]/46,XY,-8,der(9)t(8;9)(q12;p22)t(9;22)(q34;q11.2)x2,der(22)t(9;22)[8]		prednisone	yes - dasatinib	no
26	**UP_3304**	42	18.6	F	Caucasian	BCR-ABL1 p210	46,XX		hyperCVAD	yes - dasatinib	no
27	**UP_3360**	40	22.7	F	Caucasian	BCR-ABL1 p190	46,XX		S0805	yes - dasatinib	yes
28	**UP_3434**	59	30.9	M	Caucasian	BCR-ABL1 p190	46,XY		S0805	yes - dasatinib	yes
29	**UP_3529**	79	126.1	F	Caucasian	BCR-ABL1 p210	46,XX,t(9;22)(q34;q11.2)[9]		prednisone, cytarabine, methotrexate	yes - dasatinib	unknown
30	**UP_3757**	45	45.3	M	Caucasian	BCR-ABL1 p210	46,XY,der(2)t(1;2)(q12;q37),t(7;9;22)(q11.2;q34;q11.2)[7]		S0805	yes - dasatinib	yes
31	**UP_4260**	53	38.6	M	Black/African American	BCR-ABL1 p210	46,XY,t(9;22)(q34;q11.2)[2]/44,XY,der(4)t(9;22)(q34;q11.2)t(4;9)(p15.2;q21),-7,-9,der(22)t(9;22)[14]/46,XY[4]		hyperCVAD	yes - dasatinib	no
32	**UP_5204**	74.3	73.5	M	Caucasian	BCR-ABL1 p210	46,XY,t(9;22)(q34;q11.2)[5]/50,idem,+Y,t(7;15)(p13;q15),+8,+10,+21[6]		prednisone	yes - dasatinib	no

* HSCT = hematopoietic stem cell transplantation, TKI = tyrosine kinase inhibitor, USI = unique specimen identifier, WBC = white blood cell

Supplemental Table 2

Cell line	Genetic rearrangement	*JAK2* mutation	Malignancy type	LDA positive	Source
MUTZ5	*IGH-CRLF2*	R683G	B-ALL	yes	DSMZ
MHH-CALL-4	*IGH-CRLF2*	I682F	B-ALL	yes	DSMZ
BV173	*BCR-ABL1*		B-ALL		DSMZ
TOM-1	*BCR-ABL1*		B-ALL		DSMZ
SUP-B15	*BCR-ABL1*		B-ALL		DSMZ
HAL-01	*TCF3-HLF*		B-ALL		DSMZ
RCH-ACV	*TCF3-PBX1*		B-ALL		DSMZ
697	*TCF3-PBX1*		B-ALL		DSMZ
Kasumi-2	*TCF3-PBX1*		B-ALL		DSMZ
HAL-01	*TCF3-HLF*		B-ALL		DSMZ
NALM-6	*ETV6-PDGFRB*		B-ALL		DSMZ
KOPN-8	*KMT2A-MLLT1*		B-ALL		DSMZ
HB11;19	*KMT2A-MLLT1*		B-ALL		Gift from Dr Patrick Brown (Johns Hopkins University; Baltimore, Maryland)
SEM	*KMT2A-AFF1*		B-ALL		DSMZ
REH	*ETV6-RUNX1*		B-ALL		DSMZ
SMS-SB	PDGFRB+		B-ALL		Gift from Dr Markus Muschen (City of Hope; Duarte, California)
Ramos	*MYC-IGH*		Burkitt lymphoma		DSMZ

ALL PDX USI	Genetic rearrangement	*JAK2* mutation	Other genetic alterations	LDA positive	Diagnosis/ Relapse	Source
UP_ALL2142	*IGH-CRLF2*			yes	diagnosis	Penn SCXC
ALL121	*IGH-CRLF2*	R683G		yes	relapse	CHOP CCCR
JH331	*IGH-CRLF2*	R683G		yes	diagnosis	COG
ALL2128	*IGH-CRLF2*			no	diagnosis	CHOP CCCR
UP_ALL588	*P2YR8-CRLF2*			yes	relapse	Penn SCXC
ALL4364	*P2YR8-CRLF2*	R683G		yes	relapse	CHOP CCCR
ALL185GD	*P2RY8-CRLF2*		*PAX5-AUTS2*, *CDKN2A*/B del	yes	diagnosis	COG
NL482A	*BCR-JAK2*			yes	diagnosis	COG
NL112	*STRN3-JAK2*			yes	diagnosis	COG
PAXDBJ	*GOLAG5-JAK2*			yes	diagnosis	CHOP CCCR
UP_ALL240	*IGH-EPOR*			yes	diagnosis	Penn SCXC
NL432	*EBF1-PDGFRB*			yes	diagnosis	COG
NH011	*NUP214-ABL1*		*IKZF1*, *CDKN2A*, *PAX5* dels	yes	diagnosis	COG
ALL-NT	*RCSD1-ABL1*			yes	relapse	CHOP CCCR
PHL3	*ETV6-ABL1*			yes	diagnosis	COG
TVA1	*ETV6-ABL1*			yes	diagnosis	Gift from Drs Vo and Fruman (University of California, Irvine)
UP_ALL3529	*BCR-ABL1*			(yes; Ph+)	diagnosis	Penn SCXC
UP_ALL5204	*BCR-ABL1*			(yes; Ph+)	diagnosis	Penn SCXC
ALL1807 (STL-CHPB1719)	*TCF3-HLF*		*IKZF1* del	no	relapse	CHOP CCCR

B-ALL patient specimen (adult)	Genetic rearrangement	*JAK2* mutation	LDA positive	Diagnosis/ Relapse	Source
240	*IGH-EPOR*		yes	diagnosis	Penn SCXC
480	*IGH-CRLF2*		yes	diagnosis	Penn SCXC
1003	*IGH-CRLF2*		yes	relapse	Penn SCXC
2142	*IGH-CRLF2*		yes	diagnosis	Penn SCXC
3808	*IGH-CRLF2*	R938Q	yes	diagnosis	Penn SCXC
4988	*IGH-CRLF2*	F694S	yes	relapse	Penn SCXC
386	*P2RY8-CRLF2*	R683G	yes	diagnosis	Penn SCXC
331	*BCR-ABL1*			relapse	Penn SCXC
1969	*BCR-ABL1*			diagnosis	Penn SCXC
3186	*BCR-ABL1*			diagnosis	Penn SCXC
3151	*BCR-ABL1*			diagnosis	Penn SCXC
3757	*BCR-ABL1*			diagnosis	Penn SCXC
4835	*BCR-ABL1*			diagnosis	Penn SCXC
3958	trisomy 13, del(17p)			diagnosis	Penn SCXC

* CHOP CCCR = Children's Hospital of Philadelphia Center for Childhood Cancer Research (Philadelphia, Pennsylvania), COG = Children's Oncology Group (Monrovia, California), DMSZ = Deutsche Sammlung von Mikroorganismen und Zellkulturen (https://www.dsmz.de/; Leibniz Institutite; Braunschweig, Germany), Penn SCXC = University of Pennsylvania Stem Cell and Xenograft Core (Philadelphia, Pennsylvania)

Supplemental Table 3

Drug Name	Target	Vendor	Product ID#
ruxolitinib (formerly INCB18424)	JAK1/2	Incyte Corporation	
ruxolitinib	JAK1/2	SelleckChem	S1378
idelalisib (formerly CAL-101)	PI3Kδ	SelleckChem	S2226
parsaclisib (INCB050465)	PI3Kδ	Incyte Corporation	
dasatinib	SRC/ABL/BTK	LC Laboratories	D-3307
selumetinib	MEK	LC Laboratories	S-4490
dexamethasone	glucocorticoid receptor	Mylan	67457-422-54

Supplemental Table 4

Antibodies used for Western blotting			
Antigen	**Clone**	**Manufacturer**	**ID #**
pJAK2-Tyr1007/1008		Cell Signaling	3771S
JAK2	D2E12	Cell Signaling	3230S
pSTAT5-Tyr694	D47E7	Cell Signaling	4322S
STAT5	D2O6Y	Cell Signaling	94205S
STAT5	C-17	Santa Cruz	SC-835-G
pAKT-Ser473	D9E	Cell Signaling	4060L
AKT	C67E7	Cell Signaling	4691L
pERK-Thr202/Tyr204	D13.14.4E	Cell Signaling	4370S
ERK	3A7	Cell Signaling	9107S
pS6-Ser240/244	D68F8	Cell Signaling	5364L
S6	5G10	Cell Signaling	2217L
pSRC-Tyr416		Cell Signaling	2101S
LYN	C13F9	Cell Signaling	2796S
PTPN6	C14H6	Cell Signaling	3759S
CD79A		Cell Signaling	3351S
CD79B	D7V2F	Cell Signaling	96024S
CD19	D4V4B	Cell Signaling	90176S
pBTK-Tyr223	D9T6H	Cell Signaling	87141S
BTK	D6T2C	Cell Signaling	56044S
BCL6	D65C10	Cell Signaling	5650S
beta-actin	AC-15	Sigma-Aldrich	A5441
pFOXO1-T24/FOXO3-T32		Cell Signaling	9464S
FOXO1	c29h4	Cell Signaling	2880S

Immunohistochemistry

Antigen	**Clone**	**Manufacturer**	**ID #**
CD79A	HM47/A9	ThermoFisher	MA-513212
CD79B	EPR6861	Abcam	ab134147

Flow cytometry

Antigen (mouse)	**Clone**	**Manufacturer**	**ID #**
CD19-APC-Cy7	1D3	BD	557655
TSLPR (CRLF2)-PE	eBio1A6	ThermoFisher	12-5499-42

Antigen (human)	**Clone**	**Manufacturer**	**ID #**
CD10-PE-Cy7	eBioCB-CALLA	ThermoFisher	25-0106-42
CD19-APC-Cy7	SJ25-C1	ThermoFisher	A15429
CD19-BV786	SJ25C1	BD	563325
CD19-PE	H1B19	ThermoFisher	12-0199-42
CD43-PE	CD43-10G7	Biolegend	343204
CD45-APC	HI30	ThermoFisher	MHCD4505
CD45-APC	2D1	ThermoFisher	17-9459-42
CD79A-Alexa488	ZL7-4	BioRad	MCA1298A488
CD79B-PE-Cy5	CB3-1	BD	551063
TSLPR (CRLF2)-PE	eBio1A6	ThermoFisher	12-5499-42
TSLPR (CRLF2)-PerCP	1A6	ThermoFisher	46-5499-41
$pAKT^{T308}$-Alexa 488	D25E6	Cell Signaling	43506S
$pAKT^{S473}$-Alexa 647	D9E	Cell Signaling	4075S
$pERK^{T202/Y204}$- Alexa 647	D13.14.4E	Cell Signaling	4284S

Supplemental Table 5

MUTZ5
Sequence variant(s)

Gene	Genomic Position (hg19)	Reference	Nucleotide	Amino Acid	VAF	Comment
IKZF1	chr7:50444470-50444471	NM_006060.5	c.400_401insGGGCTCCG	p.Val134Glyfs*62	0.58	
JAK2	chr9:5078360-5078360	NM_004972.3	c.2047A>G	p.Arg683Gly	0.41	COSM29300
CDC25C	chr5:137621491-137621491	NM_001790.4	c.1312C>G	p.Gln438Glu	0.41	
NSD1	chr5:176637415-176637415	NM_022455.4	c.2015C>T	p.Thr672Ile	0.49	
SF1	chr11:64534522-64534522	NM_004630.3	c.1432A>T	p.Met478Leu	0.53	
SH2B3	chr12:111885984-111885984	NM_005475.2	c.1606G>A	p.Ala536Thr	0.42	rs140649197

Copy Number Variant(s)

Chromosome	Band	Abnormality	Gene	Comments
X	partial Xp	loss	*KDM6A*	Homozygous deletion
2	partial 2p	loss	*MSH6*	
4	partial 4p	loss	*LEF1*	
5	partial 5p	loss	*EBF1*	
7	partial 7p	loss	*IKZF1*	
9	partial 9p	loss	*CDKN2A*/B, *PAX5*	Homozygous deletion of *CDKN2A*/*2B*
10	partial 10p	loss	*PTEN*	
10	partial 12p	loss	*ETV6*	Homozygous deletion

MHH-CALL-4
Sequence variant(s)

Gene	Genomic Position (hg19)	Reference	Nucleotide	Amino Acid	VAF	Comment
JAK2	chr9:5078357-5078357	NM_004972.3	c.2044A>T	p.Ile682Phe	0.48	COSM303887
ETV6	chr12:12038883-12038896	NM_001987.4	c.1177_1189del	p.Lys393Profs*8	0.83	
KRAS	chr12:25398251-25398251	NM_033360.3	c.68T>G	p.Leu23Arg	0.44	rs730880472;COSM303853
KMT2D	chr12:49426916-49426917	NM_003482.3	c.11571_11572insT	p.Gln3858Serfs*154	0.1	
USH2A	chr1:216052344-216052344	NM_206933.2	c.8320G>A	p.Ala2774Thr	0.46	rs111033533;COSM4783112
ABL1	chr9:133760372-133760372	NM_005157.5	c.2695C>T	p.Pro899Ser	0.45	rs767171554

Copy Number Variant(s)

Chromosome	Band	Abnormality	Gene	Comments
7	partial 7p	loss	*IKZF1*	
9	partial 9p	loss	*CDKN2A*/*2B*, *PAX5*	Homozygous loss of *CDKN2A*/*2B*
12	partial 12p	loss	*ETV6*	

Supplemental Table 6

Viral construct	Insert	Notes	Vector	Ligation method
CRLF2	PCR product, Genescript OHu23392D		lentiCRISPRv2 blast Addgene 398239[1]	Gibson (NEB HiFi)
retro myc IRES mcherry	myc tag (NotI, AgeI cloning sites, for C-terminal myc epitope tag)		IRES mCherry Addgene 80139[2]	oligo ligation/T4 ligase (invitrogen)
PAX-JAK2	PCR of N-term PAX5 , Genescript OHu14569D PCR of C-term JAK2 (Addgene 23915)	Junction in PMID: 22897847	retro myc IRES mCherry	Gibson (NEB HiFi)
EBF-PDGFRB	PCR of N-term EBF1, Genescript OHu20245D PCR of C-term PDGFRB, Genescript OHu25829D	Junction in PMID: 22897847	retro myc IRES mCherry	Gibson (NEB HiFi)
JAK2 R683G	PCR product, Addgene JAK2 (23915)[3]		retro myc IRES mCherry	Gibson (NEB HiFi)

Viral construct	Notes	Source
MSCV-RFP	Control vector for GFPi	Kind gift from Jocelyne Demengeot[4]
MSCV-GFPi Luciferase-T2A-GFP	RAG activity reporter	Kind gift from Jocelyne Demengeot[4]

References for constructs:

1. Stringer, B.W., et al. A reference collection of patient-derived cell line and xenograft models of proneural, classical and mesenchymal glioblastoma. Scientific reports 9, 4902 (2019).
2. Dick, H.M., Wright, P., Chapman, C.M., Zacharias, F.J. & Nicholls, J.T. Adverse reactions to practolol: some observations on the possible relevence to immune mechanisms. Allergy 33, 71-75 (1978).
3. Wang, S., et al. JAK2-binding long noncoding RNA promotes breast cancer brain metastasis. The Journal of clinical investigation 127, 4498-4515 (2017).
4. Trancoso I, Bonnet M, Gardner R, Carneiro J, Barreto VM, Demengeot J, and Sarmento LM. A Novel Quantitative Fluorescent Reporter Assay for RAG Targets and RAG Activity. *Frontiers in immunology.* 2013;4(110).

Supplemental Table 7

Quantitative RT-PCR primers	Sequence
BCL6	F_TGAGAAGCCCTATCCCTGTG
	R_TGTGACGGAAATGCAGGTTA
BLNK	F_CCAGCTGGCCTTCAGAGAAA
	R_ATGAAAAGCTGGGTAGGGGC
RAG1	F_ATAGAAGAAAGCAACACAAAAGC
	R_ATACTGAGTTCAATCCCTGAAGA
RAG2	F_ATAGCAAGAGCTCTACACACTCC
	R_AAAAATCAGATCAGAAATCCTCA
SYK	F_CACAAAGCAAAGGCAGTCCC
	R_AGGGGGAAAGTAAGCCAAGC
COX6B	F_AACTACAAGACCGCCCCTTT
	R_GCAGCCAGTTCAGATCTTCC

sgRNAs purchased from IDT	Sequence
CD79B	CD79B.1.AA_AACACCTCGGAGGTCTACCA
CRLF2	CRLF2.1.AC_ACCTGCAACGTCACCATAGA

Supplemental Table 8

Gene expression signature Up-differentiation									
BCL6	*PAFAH1B3*	*PHKA2*	*CMTM7*	*PGLS*	*WDR90*	*CLK1*	*TRAPPC1*	*ECH1*	*ABI1*
FCRLA	*BLNK*	*HDAC5*	*PSRC1*	*PNRC2*	*SFSWAP*	*GABARAP*	*DESI1*	*CENPN*	*WDR81*
CD72	*DDIT4*	*B3GNT8*	*DYNLT1*	*APOBEC1*	*PCYT1A*	*RNF167*	*UTRN*	*CD24*	*ZFP36L1*
TMPRSS3	*CCNG2*	*DNTT*	*RHOQ*	*ZBTB20*	*ZCCHC6*	*RMND5B*	*FOXP1*	*RAB37*	*PPP2R5A*
LAPTM5	*TIFA*	*TMEM71*	*KLHL24*	*CSRP2*	*CARHSP1*	*INPPL1*	*SYAP1*	*REEP5*	*PRR14*
UBLCP1	*AP003419.11*	*CXCR4*	*IRF8*	*GPR155*	*CCNB2*	*KIF23*	*RDH11*	*ERO1B*	*FAM107B*
NATD1	*ARNTL*	*SORD*	*PLEKHO1*	*B3GNT2*	*MANBA*	*GIT2*	*CD53*	*LYRM2*	*PRKCG*
SPP1	*WBP1*	*LGR5*	*CDC42EP3*	*SRGN*	*RFX5*	*TGFBR2*	*SKAP2*	*HIVEP1*	*PLEKHO2*
YPEL5	*IL2RA*	*PIK3AP1*	*ITM2B*	*MTURN*	*PIGH*	*ZFP36L2*	*DDIT3*	*LAMP2*	*CARNS1*
ADSSL1	*ZFPM1*	*IGHV1-69*	*TESK2*	*WDFY1*	*CHKB*	*TNFRSF13C*	*RSPH9*	*AMDHD1*	*KDM3B*
RGS2	*RPL3L*	*EQTN*	*EBF1*	*RDH12*	*RAMP1*	*PTPRC*	*AC027682.1*	*TPP1*	*ZC3H7A*
IL12A	*SMARCA4*	*MTSS1*	*ATP6AP1*	*STX5*	*CENPF*	*TCTA*	*EHD2*	*KIF22*	*NR3C1*
PECAM1	*CTD-2545M3.6*	*NUSAP1*	*ARHGAP17*	*RELB*	*CENPE*	*ASPM*	*FBXO11*	*UCP2*	*TTC28*
NTMT1	*HERPUD1*	*MYLIP*	*SLC15A2*	*POLM*	*PCMTD2*	*ATP6V1D*	*IQSEC1*	*STK17B*	*TRIM6-TRIM34*
PIPOX	*HIST3H2A*	*MXD3*	*TSPYL4*	*SYK*	*CIC*	*SLA*	*PXK*	*BIRC3*	*TRIP4*
RAB43	*CDKN1A*	*IL7R*	*SERP1*	*OGT*	*MPP1*	*NT5C2*	*HEXB*	*GTPBP2*	*STK11IP*
MYL4	*BLK*	*SPATA6*	*TSPAN17*	*MYB*	*PIK3CG*	*CDCA3*	*ATXN7L1*	*RELL1*	*ZDHHC14*
SBK1	*TXNIP*	*RSPH1*	*COX7A2L*	*TCIRG1*	*DNASE1*	*FCHO1*	*TMA16*	*MKI67*	*GPSM3*
PFN2	*CPEB4*	*KLF13*	*ELK3*	*PLGRKT*	*CCPG1*	*IFFO1*	*MDM1*	*CRLF2*	*ZBTB18*
BACH2	*UNC93B1*	*IRF4*	*LGALS9*	*SKIL*	*NDUFAF3*	*ZEB2*	*TRIM59*	*IFT22*	*CCNI*
MXD4	*PHKA1*	*ATP2A3*	*PNRC1*	*TRIOBP*	*VAMP8*	*UNC119B*	*LMO2*	*NCAPD2*	*ATG12*
EGFL6	*PLEKHG2*	*CDC25B*	*ZFAND3*	*PRKCB*	*MYO18A*	*TOB1*	*NRM*	*ITGAM*	*IRF2*
MBD4	*PIAS3*	*TBX6*	*PINK1*	*LITAF*	*TUBA1A*	*GFI1B*	*TRAFD1*	*CTNNA1*	*IFNA7*
SELL	*SIT1*	*NFKBIE*	*DDX54*	*RDM1*	*HINT3*	*TRAM1*	*CCDC88A*	*PCBD1*	*IFNA7*
YPEL3	*GFRA1*	*EMP1*	*PITPNM1*	*ARHGAP45*	*DUSP2*	*FOXO1*	*GRINA*	*CDC37L1*	*PHF1*
CXXC5	*CPT1A*	*ABCB4*	*ITPR2*	*PQLC3*	*INPP5K*	*AC013264.1*	*IFT122*	*PIAS1*	*PNPLA8*
SLC23A1	*H2AFV*	*PLEKHA2*	*TCF19*	*CRLF1*	*FRYL*	*SPEF1*	*SIRT7*	*AGFG2*	*ZRSR1*
PREP	*BTG2*	*IFT80*	*TSPO*	*PARP4*	*SAT1*	*HSPA2*	*CEP89*	*ADCY7*	*REEP2*
FCGR2C	*HBP1*	*ARHGAP18*	*MGST1*	*KMT2A*	*DUSP6*	*SDC4*	*HIPK3*	*TNFRSF13B*	*PPP1R18*
EPS8	*MYADM*	*ETS2*	*LIMD2*	*FMNL3*	*COTL1*	*CD37*	*PLAT*	*SLC7A7*	*STK10*
CHST15	*FAM63A*	*METTL7A*	*TCN2*	*ARPC5L*	*DUSP10*	*ZNF821*	*RHOH*	*RSRP1*	*SLC27A4*
AP1S2	*GFRA2*	*AKAP12*	*NEDD9*	*BAZ2B*	*TRIM16*	*RAB33B*	*FAM13B*	*BIRC2*	*TMEM229B*
TP53INP1	*ELOF1*	*BLVRB*	*IGKV1-6*	*TCF4*	*NNT*	*CEACAM1*	*XRCC6*	*CD79A*	*ARNT*
ZAP70	*SMIM14*	*CNP*	*NFAT5*	*P2RX3*	*CBFA2T3*	*CDK5RAP2*	*WSB2*	*CORO1B*	*EPC1*
BTG1	*GDI1*	*DNAH8*	*AKR1B10*	*PSAP*	*FAM64A*	*ATL2*	*MKRN1*	*RACGAP1*	*SRPK2*
SEC63	*ATP8A1*	*OTUB2*	*TAX1BP3*	*ABCD1*	*RCHY1*	*RB1*	*SERPINI1*	*SLAMF1*	*SLC30A4*
RABGAP1L	*TRIM11*	*GAS7*	*WDPCP*	*KANSL1L*	*MARCKS*	*NDC80*	*LASP1*	*RP11-286N22.8*	*CST5*
PTGR1	*IGKV4-1*	*SLC12A6*	*HIST1H1C*	*GADD45A*	*BTRC*	*TK2*	*OSBPL9*	*IQGAP1*	*STAT2*
GLCCI1	*PSEN2*	*CAST*	*CEACAM3*	*NEIL3*	*IP6K1*	*ATP1B3*	*CHFR*	*DGKZ*	*RBM4B*
EMP3	*TLE6*	*CTSE*	*MAP1LC3B2*	*LUC7L2*	*RCSD1*	*CD38*	*GPM6A*	*SH3YL1*	*CENPA*
MSH5	*THBD*	*CD22*	*MYO7A*	*GSN*	*POC1B-GALNT4*	*STX7*	*SMIM3*	*SOX4*	*TM6SF1*
DOK3	*ID2*	*MTOR*	*KMT2E*	*PLD4*	*SNAP29*	*TGFB1*	*GP2*	*RRM2*	*CHCHD5*
NFKBIA	*RAG2*	*GPAM*	*CDKN2C*	*PDE4B*	*LGALS1*	*CCM2*	*GLRX*	*FGD6*	*CCDC28A*
PHLDA1	*TP53INP2*	*ABCA1*	*MAP7*	*ICAM2*	*NIPBL*	*MAST2*	*STK3*	*UBE2H*	*RP11-192H23.4*
CERK	*CDKN1B*	*CTDSP2*	*TRIB2*	*TMEM9B*	*ST8SIA4*	*SNAPC3*	*RNF103*	*RRM2B*	*SYVN1*
CD93	*CD79B*	*ACP5*	*CKLF*	*SLAMF6*	*AAK1*	*IL16*	*CNOT6L*	*CD19*	*ANKRD13A*
RAG1	*SLAMF7*	*CIT*	*HES6*	*LHPP*	*CNN3*	*VRK3*	*ZNF207*	*CMC2*	*C12orf57*
CDKN2D	*PBXIP1*	*EHBP1L1*	*RP11-307N16.6*	*MAP3K1*	*SESN3*	*TSPAN13*	*MEF2D*	*SPC25*	*CAT*
HSPA1A	*GRN*	*EIF2AK3*	*EDIL3*	*CASP6*	*SELP*	*HIVEP2*	*NSMF*	*ELK4*	*LTK*
SLC12A3	*TNFRSF19*	*PLXDC1*	*VPREB3*	*SH3KBP1*	*RB1CC1*	*SP4*	*BCL7A*	*CYBA*	*INPP5D*