SUPPLEMENTAL METHODS

Cell lines, xenografts and patient samples

REH, RS4;11 and Nalm-6 cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA). Nalm-20, Nalm-21, Tom-1, RCH-ACV, Kasumi-2, 697, KOPN-8 cells were obtained from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). SMS-SB cells were a kind gift from Dr. Ralph B. Arlinghaus (The University of Texas MD Anderson Cancer Center, USA). BALL1 and Tanoue were purchased from the RIKEN BioResource Center (RIKEN, Japan). Cell lines were maintained in RPMI 1640 (Hyclone, Logan, UT, USA) supplemented with 2 mmol/l L-glutamine, 100 μl/ml penicillin, 100 μg/ml streptomycin (CellGro, Manassas, VA, USA) and 10% (REH, RS4;11, Nalm-6, SMS-SB, BALL-1, Tanoue and HPB-NULL) or 20% (Nalm-20, Nalm-21, Tom-1, RCH-ACV, Kasumi-2, 697) fetal bovine serum (FBS, Hyclone). Cell line authenticity was verified via STR fingerprinting.

Xenograft leukemia samples derived from untreated de novo B cell precursor ALL (B-ALL) patients were established using the NOD/SCID/huALL xenograft model as previously described[1]. The immunophenotype of primary patient samples was assessed according to the standards of the European Group for the Immunological Characterization of Leukemias.[2] Patient samples were obtained after informed consent and in accordance with the institution's ethical review board at the University of Ulm, Germany. For ex vivo experiments, xenograft-expanded BCP-ALL cells were isolated from leukemia bearing recipients and cultured in RPMI 1640 medium supplemented with 10% fetal calf serum and 1% L-glutamine (Gibco Life Technologies, Darmstadt, Germany) at 37°C in humidified air/5% CO_2 as described earlier [3,4]. All xenograft

samples contained more than 90% of BCP-ALL cells as estimated by flow cytometry staining for human CD19 and/or CD45.

ICN12 xenograft-expanded cells were kindly provided by Dr. Markus Müschen (University of California, San Francisco). Cells were propagated in Alpha MEM supplemented with 20% fetal bovine serum, 100 μl/ml penicillin, 100 μg/ml streptomycin, GlutaMAX (Life Technologies) and Sodium-Pyruvate.

B-ALL patient samples for in vitro analysis of PRT318 efficacy were obtained, after informed consent from patients fulfilling diagnostic and immunophenotypic criteria for B-ALL at the Leukemia Department at MD Anderson Cancer Center. Patient consent for samples used in this study was obtained in accordance with the Declaration of Helsinki on protocols that were reviewed and approved by the Institutional Review Board at MD Anderson Cancer Center.

PRT318 (PRT060318) was kindly provided by Portola Pharmaceuticals (San Francisco, CA). LY294002, R406 and PRT062607 were purchased from Selleck Chemicals (Houston, TX).

CRISPR/Cas9 and FOXO1-AAA expression

The CRISPR design tool (http://crispr.mit.edu/) was used to identify target specific sgRNA sequences. To ensure specificity only high-scoring (>50) sgRNA sequences were selected. To further reduce chances of off-target effects at least two distinct sgRNAs were used per target. Depending on the nature of the target proteins sgRNAs were then cloned into either one of two hSpCas9 containing backbone vectors. pX330 backbone vector was used for targets where knockout could be verified via flow cytometry (SYK). sgRNA sequences targeting Igμ and MYC were cloned into the PX458 backbone vector. Both vectors were a kind gift from Feng Zhang (Addgene plasmid

#42230 and #48138).[5] Cells were transfected with the NEON™ Transfection System (Life Technologies) using predetermined conditions according to manufacturers instructions. Knockout of SYK was confirmed via flow cytometry. To verify knockout of MYC, GFP^+ cells were sorted (FACSJazz, BD) 48 h after electroporation and subjected to immunoblot analysis of MYC protein. Low Igμ surface expression levels forced us to verify successful Igμ-KO through a combination of GFP and surface Igμ staining. Briefly, cells were stained for surface Igμ 48 h after electroporation and assessed via flow cytometry. Gating on GFP^+ and $Ig\mu^-$ cells allowed the selection of Igμ-KO cells (Supplementary Figure 5). For all knockout experiments cells transfected with the respective backbone vector lacking target gRNA served as control.

Western Blotting

The following antibodies were used for immunoblot analysis: anti-Igα, anti-SYK, anti-pZAP-70 (T319)/pSYK (Y352), anti-SHP1, anti-BLNK, anti-BTK, anti-PLCγ2, anti-VAV1, anti-GAPDH, anti-AKT, anti-pAKT (S473), anti-ERK1/2, anti-pERK1/2 (T202/Y204), anti-CD19, anti-pCD19 (Y531), anti-FOXO1, anti-pFOXO1(T24)/pFOXO3a (T32)/pFOXO4 (T28), anti-p27, anti-MYC (Cell Signaling Technologies, Danvers, MA); anti-LYN, anti-pLYN (Y396), anti-human IgM, anti-pBTK (Y223), anti-pVAV1 (Y174) (Abcam, Cambridge, MA). Cells were lysed in RIPA Buffer (Sigma Aldrich) containing PhosSTOP Phosphatase Inhibitor Cocktail and cOmplete Protease Inhibitor Cocktail (Roche, Indianapolis, IN) and loaded onto NuPAGE® Novex® 4%-12% gradient gels (Life Technologies). After electrophoresis protein was transferred onto PVDF membranes (EMD Millipore, Darmstadt, Germany). Membranes were blocked for 1 h at room temperature with PBS containing 0.1% Tween (PBS-T) and either 5% BSA (phoshoproteins) or 5% Milk (total protein). Primary antibodies were diluted in blocking

solution at the recommended concentrations. Membranes were incubated overnight at 4 °C. The following day membranes were washed three times for 10 min with PBS-T and incubated with species-specific HRP-linked secondary antibody (GE Healthcare) (diluted 1:10000) for 1 h at room temperature. Protein was visualized via ECL detection (Pierce, Rockford, IL) according to supplier's instructions. Densitometric analysis was performed with ImageJ 1.48v (NIH).

B-ALL xenograft mouse models

In vivo xenograft experiments were conducted according to the national animal welfare law and approved by the appropriate authority (Regierungspräsidium Tübingen, Germany). Xenograft leukemia cells were transplanted onto female NOD/SCID mice (Jackson Laboratory/Charles River, MA) with a median age of 12 weeks. Following successful engraftment as indicated by more than 5% human $CD19^+$ cells in the peripheral blood, mice were randomized (5 per group) to receive either 30 mg/kg PRT318 in 5% Captisol or 5% Captisol twice daily via intraperitoneal injection.[6,7] According to pharmacokinetic analysis this dosing schedule results in an average steady state plasma concentration of 2.5 μM PRT318. Mice were killed 10 days after beginning of therapy and the amount of leukemia was assessed in bone marrow of left and right femur and tibia, peripheral blood, spleen, and central nervous system via flow cytometry. For survival analysis sublethally irradiated NOD/SCID mice were inoculated with 10^6 patient-derived leukemia cells (ICN12). After engraftment mice were randomized into groups of seven receiving either 100 mg/kg PRT062607 dissolved in H_20 or H_20 alone daily for 5 weeks. Mice exhibiting signs of overt leukemia were killed and the presence of leukemia was confirmed via flow cytometric analysis of $hCD45^+$ and $hCD19^+$ B-ALL blast

cells in the spleen and bone marrow. Experiments were performed in agreement with the appropriate authority at the University of California, San Francisco.

Confocal microscopy

Cells were synchronized through serum starvation for 12 h followed by treatment with 1 μM PRT318 or 20 μM LY294002 for 6 h. 10^6 cells were then stained with CellTracker™ Green CMFDA (Molecular Probes®, Life Technologies) according to manufacturers instructions and seeded onto coverslips (Ted Pella, Redding, CA) precoated with Poly-L-Ornithine (Sigma Aldrich). After 2 h Cells were fixed with 4% paraformaldehyde (Electron Microscopy Science, Hatfield, PA) for 10 min, washed once with PBS and then permeabilized for 30 min at room temperature with blocking/permeabilization solution (in PBS: 0.1% BSA w/v, 10% FBS, 0.3% v/v TRITON® X-100 (BIORAD, Hercules, CA)). Cells were incubated with FOXO1 antibody (Cell Signaling Technologies) diluted 1:100 in blocking buffer (in PBS: 0.1% BSA w/v, 10% FBS) overnight at 4 °C. Following two washing steps cells were incubated with Alexa Fluor® 647-conjugated species specific secondary antibody (Molecular Probes®, Life Technologies) for 1.5 hours, washed again with PBS and mounted on coverslips with ProLong® Gold Antifade Mountant containing DAPI (Life Technologies). Cells were visualized on an Olympus FV1000 Laser Confocal Microscope. To determine the cellular distribution of FOXO1 captured images were analyzed with Slidebook 5.5v (Intelligent Imaging Innovations, Denver, CO).

Supplemental Table 1. Depicted are the genes most differentially expressed between pro-B and pre-B cells. GEP data was obtained from the NCBI GEO GEP database (GSE45460, Lee ST et al.). Expression data for pro-B and pre-B cells was averaged, followed by selection of the most differentially expressed genes (Cutoff: ±1.5 log2).

Up in pre-B	Pre-B – Pro-B (Ave.)	Down in pre-B	Pre-B – Pro-B (Ave.)
TCL1A	3.409484875	DNTT	-3.970801625
ADAM23	3.39671975	CD34	-3.36188325
CAMK2D	2.814519375	ERG	-3.2757345
IRF4	2.776612625	MYO5C	-2.669143875
TNFRSF17	2.75213025	TCF7L2	-2.322865125
KLRK1	2.642934625	GBP4	-2.247708875
KMO	2.631260875	GIMAP4	-2.246645
FCRLA	2.63115675	TSPAN7	-2.158570875
IKZF3	2.55654725	MPO	-2.05548725
MYO3A	2.53473575	ELK3	-1.972691
C13orf18	2.516602625	CCND2	-1.94018325
CAMK4	2.373186875	NPY	-1.92724375
ADARB1	2.345757875	HPGDS	-1.883255125
IGKC	2.32370975	SOCS2	-1.8701565
LYN	2.31681725	IFITM3	-1.842949375
IGF2	2.28297525	MS4A3	-1.8368695
KIAA1407	2.227569125	PECAM1	-1.82905075
PLEKHA2	2.1536035	CLC	-1.823461375
PLEKHG1	2.148306625	SPINK2	-1.81431
ACSM3	2.145306625	CD99	-1.807950375
C20orf103	2.143419	LST1	-1.79281475
FCRL1	2.039112125	AIF1	-1.791898375
PARP15	2.008830375	SEMA6A	-1.791159
P2RY10	2.007818	SPG20	-1.7818535
MPEG1	2.006921375	GNG11	-1.748639375
BEST3	2.00088925	IRAK3	-1.74363775
IGKC	1.997911625	LRRC70	-1.73812075
SNX25	1.9600115	NPCDR1	-1.663513875
IGKV3D-11	1.91861575	PLEK	-1.657183125
FBXO25	1.917923125	ATP2B4	-1.63642575
ATXN7L1	1.87686725	C1orf54	-1.62824175
ABCB4	1.86574025	TFPI	-1.624913
VNN2	1.86509025	CD109	-1.597598875
CCDC112	1.85018125	PRG2	-1.591376625
IGK@	1.834047375	TNFRSF1A	-1.589034375
ROR1	1.83379075	MYC	-1.58497575

WASF1	1.833325625	CPA3	-1.57857225
KLHL14	1.825049125	RNASE2	-1.57695775
GCET2	1.802085875	PRKCH	-1.57691225
MTSS1	1.79681825	P2RY14	-1.571509625
GPR160	1.784055	SRGN	-1.56066175
LOC100133207	1.777806375	LCP2	-1.5517245
CD180	1.774211	NIPSNAP3B	-1.54061325
LOC652493	1.765745375	IGF2BP2	-1.53788275
HCK	1.75581725	MEF2C	-1.517344375
SLAMF6	1.753883125	FAM134B	-1.500649625
TCL6	1.7506425		
KCNA3	1.7410005		
C6orf192	1.691661875		
TMSB15A	1.685059875		
IKZF2	1.66435725		
BMP3	1.656715875		
MAP3K1	1.639192625		
CHP	1.637050625		
S1PR1	1.620007625		
VLDLR	1.607713875		
RNF144B	1.596045125		
ANXA2	1.59383625		
TFDP2	1.5924425		
IGHM	1.5602		
PCDH9	1.54592575		
MTMR1	1.540679		
VSIG6	1.533327		
KLRC4	1.526390125		
BIRC3	1.519319375		
AFF3	1.505205625		

Supplemental Table 2. Genetics and immunophenotype of B-ALL cell lines and xenografts used in the study. Pro-B = cytoplasmic-igμ^-/surface IgM$^-$; pre-B = cytoplasmic-igμ^+/surface IgM$^-$. n.a.: not available

Name	Fusiongenes	Additional genetic abnormalities	Cytoplasmic Igμ	Surface IgM	pre-BCR	Developmental Stage
Cell lines						
REH	TEL/AML1	n.a.	-	-	-	pro-B
RS4;11	MLL/AF4	hyperdiploid, +8, +18	-	-	-	pro-B
SFO3	-	n.a.	-	-	-	pro-B
Nalm-20	BCR/ABL	hyperdiploid, +8, +8	-	-	-	pro-B
Nalm-21	BCR/ABL	near diploid, -Y, +8	-	+	-	pro-B
Tom-1	BCR/ABL	hyperdiploid, +8,+16	-	-	-	pro-B
RCH-ACV	TCF3/PBX1	hyperdiploid, +8,+16	+	-	+	pre-B
ICN12	TCF3/PBX1	n.a.	+	-	+	pre-B
SMS-SB	none	n.a.	+	-	+	pre-B
Nalm-6	t(5;12)	diploid	+	-	+	pre-B
Tanoue	unknown	n.a.	+	+	-	mature-B
Xenografts						
X002	none	n.a.	-	-	-	pro-B
X089	none	n.a.	-	-	-	pro-B
X112	none	n.a.	-	-	-	pro-B
X068	none	n.a.	-	-	-	pro-B
X018	none	n.a.	+	-	+	pre-B
X116	none	n.a.	+	-	+	pre-B
X006	none	*IKZF1 del ex2*	+	-	+	pre-B
X120	*E2A/PBX1*	n.a.	+	-	+	pre-B
X134	none	*P2RY8/CRLF2*	+	-	+	pre-B
X135	BCR/ABL	n.a.	+	-	+	pre-B

Supplemental Table 3. Characteristics of B-ALL primary patient samples.

Sample	Diagnosis	Age at Diagnosis	Immunophenotype	Cytogenetics	Fusiongenes	Previous Therapies
pre-B ALL 1	relapsed B-ALL	18y	CD10+, CD19+, CD22+, CD34+, CD38+, CD52+, cytoCD79a+, CD123+, **cytoIgμ+**	Hypodiploid, 45,XY,-7	-	Augmented-BFM, Blinatumomab, Coltuximab, hyper-CVAD+everolimus
pre-B ALL 2	relapsed B-ALL	66y	CD10+, CD19+, CD20+(dim), CD22+(dim), **cyIgμ+**,CD38+, CD52+, CD81+, CD38+	Hyperdiploid, 49~51,XX, add(3)(q29), del(4)(q21q35), +6, del(6)(q21q25)x2, +2~5mar[cp4	-	Hyper-CVAD+rituximab, Coltuximab
pro-B ALL 1	relapsed B-ALL	20y	CD10+, CD13+(partial), CD20+(partial), CD22+, CD33+, CD34+, CD38+, CD45+, CD52+, CD58+, CD81+	Hyperdiploid, 49,XY, +X, add(3)(p25), add(7)(p11.2), add(9)(p24), dup(11)(q13q23), +14, del(17)(q23), +21	-	Augmented hyper-CVAD, FLAM, Blinatumumab, Coltuximab
pro-B ALL 2	relapsed B-ALL	60y	CD19+, CD20+, CD10+, HLA-DR+, CD34+, CD38+, TdT+	Hyperdiploid, 53,XX, +X, del(4)(q33), +8, t(9;22)(q34;q11.2), +10, +15, +21, +der(22)t(9;22)	BCR-ABL	Hyper-CVAD+ dasatinib

Supplemental Table 4. CRISPR/Cas9 guide RNA (gRNA) sequences.

Target	Cell Line	gRNA1 (forward)	gRNA2 (forward)	gRNA3 (forward)	Cas9 Backbone Plasmid	target region
Ig*μ*	RCH-ACV	GTGGATGGGATGGATCAACGC	GTGCTATGCATTGGGTGCGCC	-	hSpCas9(BB)-2A-GFP (PX458)	VDJ-Region
Ig*μ*	SMS-SB	GCTCTGTGACCGCCGCAGACA	GTTGGGAGTATCTATCATAGT	-	hSpCas9(BB)-2A-GFP (PX458)	VDJ-Region
SYK	-	GTTTCGGCAACATCACCCGGG	GACCATCGAGCGGGAGCTGAA	GAAGATTACCTGGTCCAGGG	hSpCas9(BB)-2A-GFP (PX458)	Exon 1
MYC	-	GTATTTCTACTGCGACGAGG	GCCGTATTTCTACTGCGACG	GCTGCACCGAGTCGTAGTCG	hSpCas9(BB)-2A-GFP (PX458)	Exon 2

Supplemental Table 5. FOXO1-3A specific gene set used to identify active pre-BCR signaling in pre-B ALL cases of the St. Jude GEP dataset.

FOXO1-3A_UP gene set (Cutoff: 2)		
CD247	DUSP10	CDH15
TXNIP	TM6SF1	PLA2G4C
LOC730101	KIAA1683	GNG11
IFIT2	MERTK	TP63
ARPP-21	FNDC5	
ACTA2	LRRC3B	
ATP9A	KLHL2	
CSMD1	IL1B	
ANGPT2	DDIT3	
OSCAR	CCPG1	
LOC285501	CYTL1	
PLS3	ANG	
CASP1	LOC100131707	
MEI1	TMEM71	
LITAF	LY6H	
MUC6	NRG3	
TGFB1I1	LDHD	
BTG2	PADI4	
NR3C1	XAGE1	
LOC728253	FAIM3	
GBP4	EFNA1	
FOXP1	DYRK2	
SORBS1	TRPS1	
AFF3	BCL6B	
PPP1R15A	ISG20	
COL6A3	ICA1	
SPTA1	HS.400256	
TNFAIP3	GPR160	
HIST2H4A	SHISA2	
DNAJB9	CD69	
LDB2	TACC2	
TSC22D3	ARRDC5	
LOC90925	GIMAP4	
GAB1	CSAG1	
PP14571	DPEP1	
RGS1	RASD1	
LOC100128888	C10ORF10	

Supplemental Table 6. Genes most differentially expressed in pre-B and non-pre B ALL (> 2 fold change). Log2 transformed gene expression values were averaged for each group and then subtracted (pre-B – pro-B).

High in pre-B ALL				Low in pre-B ALL			
Gene ID	Fold change (log2)	Gene ID	Fold change (log2)	Gene ID	Fold change (log2)	Gene ID	Fold change (log2)
NME3	1.00	RAG2	1.17	GNG11	-3.48	TMEM156	-1.66
ARNTL2	1.00	TGFBR2	1.17	CYTL1	-3.15	P2RY14	-1.66
COCH	1.01	LRRC15	1.18	STK32B	-2.95	PIEZO1	-1.64
ZAP70	1.01	C11orf49	1.18	SOCS2	-2.92	GPR56	-1.63
DACT1	1.01	BCAT1	1.18	POU4F1	-2.82	IFIT1	-1.62
LIPC	1.02	MERTK	1.18	IFI44L	-2.72	ECM1	-1.61
ADAM23	1.02	HIP1R	1.18	TSPAN7	-2.71	H2BFS	-1.60
GALNT14	1.02	IGLL3P	1.18	ITGA6	-2.44	FSCN1	-1.60
PRL	1.02	MT1X	1.18	MRC1	-2.43	TNFRSF21	-1.59
TSSC1	1.02	LGALS1	1.19	NPR1	-2.42	DNTT	-1.59
TCL6	1.03	KIAA0922	1.19	CYB5R2	-2.37	RAG1	-1.59
FAIM	1.03	BLK	1.19	WDFY3	-2.36	SLC2A5	-1.59
ADK	1.03	MTX2	1.20	EGFL7	-2.26	EFNB1	-1.56
ITM2C	1.03	EPHA3	1.20	EFNA1	-2.23	CD69	-1.55
DPY19L2P2	1.04	TACC2	1.22	H1F0	-2.22	NPDC1	-1.53
DYNC2LI1	1.04	GP5	1.24	EPHA7	-2.13	CTGF	-1.52
DOCK10	1.04	ODZ4	1.24	AIF1	-2.09	MAN1A1	-1.52
ROBO1	1.05	CTNNBL1	1.24	CD99	-1.99	ITPR1	-1.51
PARP1	1.05	PLS3	1.25	ST3GAL6	-1.98	MX1	-1.50
ACCN1	1.06	MAPKBP1	1.26	ELK3	-1.97	SCN3A	-1.49
TUBB2A	1.08	LINC00094	1.26	BMP2	-1.92	PLXND1	-1.49
DSTN	1.09	PSEN2	1.26	LGMN	-1.90	TERF2	-1.48
PSAT1	1.10	PBK	1.26	CD34	-1.89	GIMAP4	-1.47
QRSL1	1.10	EPB41L2	1.26	PDE4B	-1.86	KIAA0226L	-1.46
KIF21B	1.10	SIT1	1.27	ALOX5	-1.81	BANK1	-1.46
IGHM	1.10	SEMA4C	1.27	PLAG1	-1.80	FKBP5	-1.45
ATP1A3	1.11	PHACTR1	1.28	SERINC5	-1.79	SEPP1	-1.45
PKIG	1.11	UGT8	1.29	SH3BP5	-1.78	CSF3R	-1.45
CTSC	1.11	BACH2	1.29	USP18	-1.78	MME	-1.42
CD38	1.11	VASH2	1.29	LTB	-1.77	NR3C2	-1.42
PPM1E	1.12	NAV2	1.29	SMAD1	-1.76	C1orf38	-1.40
WASF1	1.13	SORBS1	1.31	LST1	-1.72	LPAR6	-1.39
VIPR2	1.13	FAM3C	1.31	KCNK12	-1.70	CSGALNACT1	-1.39
ALDH1A1	1.14	CRIP1	1.32	TIE1	-1.70	SLC35E3	-1.39
ZNF167	1.14	RGS2	1.32	IRAK3	-1.69	BAALC	-1.38

TUBB6	1.14	CLEC11A	1.32	STAG3	-1.68	MN1	-1.38
PPPDE1	1.15	TMSB15A	1.32	TBC1D9	-1.67	KCNA5	-1.37
CD96	1.16	SYT1	1.33	SMAGP	-1.66	FHIT	-1.36
GATM	1.16	CLEC2D	1.35	NPY	-1.66	SLC27A3	-1.35
PCDH9	1.36	FGF9	2.08	MYO1B	-1.31	ALOX5AP	-1.10
RORB	1.36	IGFBP7	2.13	PROM1	-1.31	HIST1H2BH	-1.09
SPAG6	1.38	FAT1	2.17	XBP1	-1.31	RNASET2	-1.09
CD72	1.39	RHOBTB1	2.18	PECAM1	-1.30	HHEX	-1.08
SPIB	1.39	TRIB2	2.20	IPCEF1	-1.29	HIST1H2AG	-1.07
CCDC69	1.39	SLC27A2	2.34	IFI44	-1.28	ARMCX2	-1.07
KCNJ12	1.40	BIK	2.57	FAM49A	-1.27	FOS	-1.07
MYBPH	1.42	PRKCZ	2.61	GIMAP6	-1.26	PTPLA	-1.06
ODC1	1.43	ADARB1	2.72	CD1C	-1.26	SH3TC1	-1.06
ARL4C	1.43	NYNRIN	2.91	B4GALT6	-1.25	ETS2	-1.06
RNF144A	1.46	PBX1	3.30	ISG20	-1.24	DSE	-1.06
MAGEF1	1.46	C20orf103	3.65	TNFRSF1A	-1.24	KCTD12	-1.05
ELOVL2	1.47	FHOD3	1.96	CCND2	-1.24	GSN	-1.05
RASAL1	1.47	WNT16	1.97	C15orf5	-1.23	SERPINB6	-1.04
AEBP1	1.49	IL12RB2	2.01	SEMA6A	-1.23	YES1	-1.04
POLM	1.49	LRMP	2.05	CXCR7	-1.22	DAB2	-1.03
ITGA8	1.50	CCDC165	2.05	TCEAL4	-1.22	PLSCR1	-1.03
KCNMB3	1.52	ROR1	2.07	PRKCH	-1.21	SYNGR1	-1.03
TMEM121	1.58			CDKN1A	-1.21	SESN1	-1.02
QPRT	1.58			DRAM1	-1.20	CLEC2B	-1.02
SLAMF1	1.61			ENOSF1	-1.20	NEDD9	-1.01
KCNA3	1.62			PCTP	-1.20	GPR171	-1.01
NFATC4	1.62			RECK	-1.19	HIST1H2BK	-1.11
UCK2	1.72			DHRS3	-1.18	AKR1C3	-1.11
SYNPO	1.73			IL3RA	-1.16	CD97	-1.11
GLDC	1.76			RRAS2	-1.16	PEG10	-1.10
SLC2A6	1.76			C13orf15	-1.16	PSD3	-1.10
IGLL1	1.76			FHL1	-1.15	STAP1	-1.10
CCDC81	1.76			FUCA1	-1.15		
NRGN	1.77			MYRIP	-1.14		
AOX1	1.80			FCGRT	-1.14		
MAP3K1	1.85			FLT3	-1.13		
LAMA5	1.92			NFE2	-1.12		
SH3BP4	1.94			GNA15	-1.12		
NID2	1.96			IFI27	-1.12		

Supplemental Table 7. Genes most differentially expressed (>2 absolute fold change) in RCH-ACV and SMS-SB after Ig*μ* KO.

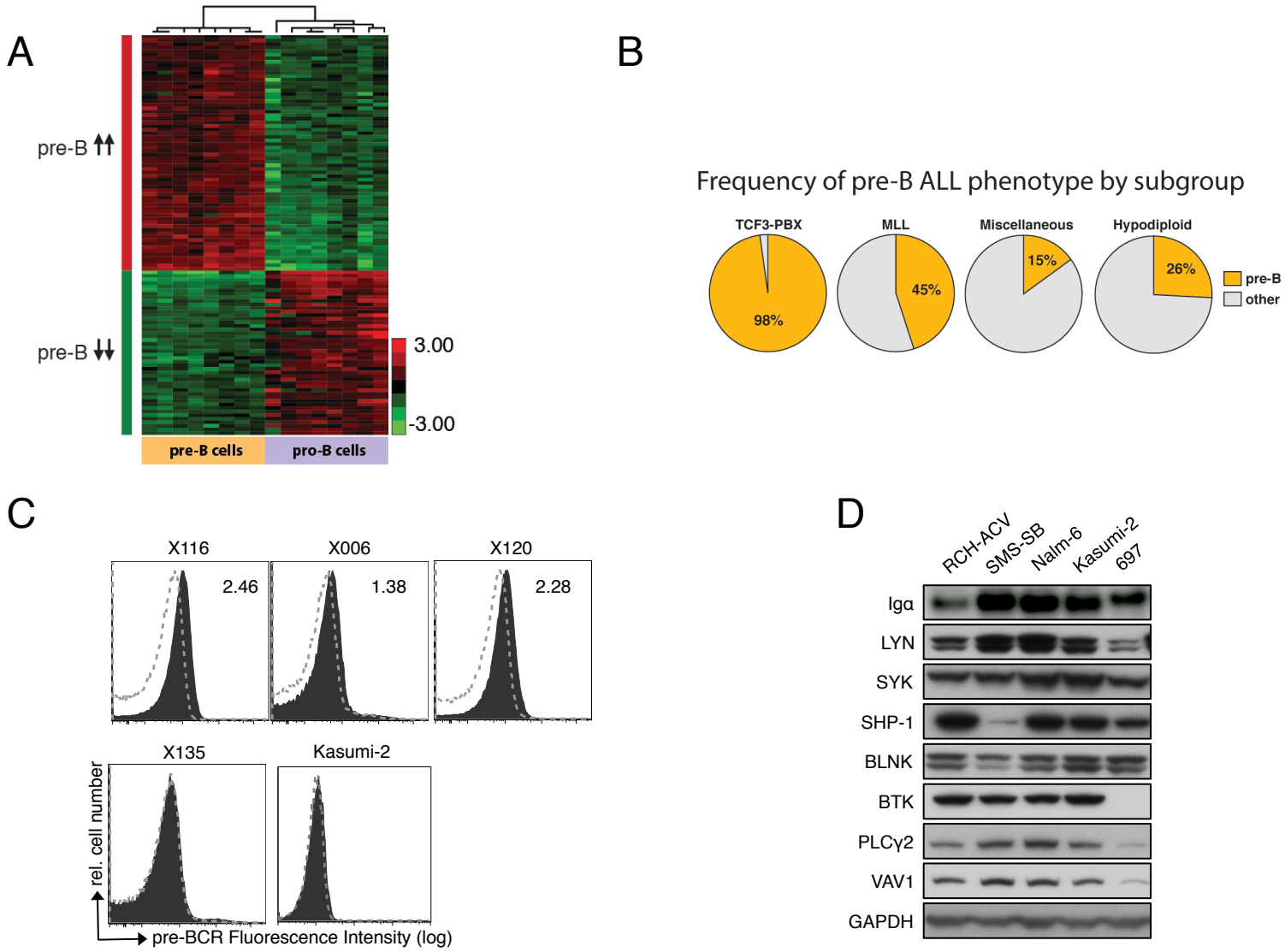
Downregulated	**RCH-ACV**		**SMS-SB**		
ILMN_Gene	**Ig*μ* KO1**	**Ig*μ*KO2**	**Ig*μ*KO1**	**Ig*μ*KO2**	**Average**
PRICKLE1	-2.4682	-2.4682	-1.3646	-2.1662	-2.1168
ALOX5AP	-1.3968	-1.1217	-1.9901	-2.5903	-1.7747
SMAGP	-1.4951	-0.9479	-1.8569	-2.7940	-1.7735
ITGB7	-1.6519	-2.2220	-1.4468	-1.6591	-1.7449
IL4I1	-0.8086	-1.8951	-1.7411	-1.7411	-1.5465
RASAL3	-1.2238	-1.8041	-1.4139	-1.6062	-1.5120
ELOVL6	-1.2493	-2.0233	-0.9935	-1.3509	-1.4043
CSPG4	-0.7698	-0.7698	-1.5534	-2.4805	-1.3934
PTPN6	-1.0967	-1.1581	-1.2496	-2.0237	-1.3820
TNF	-1.2640	-1.4302	-1.2595	-1.5729	-1.3817
TLR10	-1.0476	-1.4359	-1.3949	-1.6438	-1.3806
PPP1R16B	-1.0634	-1.3642	-1.3249	-1.7220	-1.3686
ITGB2	-1.3442	-1.4571	-1.0296	-1.6161	-1.3618
VGF	-0.6741	-1.1776	-1.4916	-2.0603	-1.3509
CSF1R	-0.8774	-1.2049	-1.6533	-1.6533	-1.3473
C19ORF51	-2.1405	-1.0472	-0.7541	-1.3866	-1.3321
TMC6	-1.2948	-1.4101	-0.9049	-1.6617	-1.3179
PLEK	-1.6054	-1.5277	-0.9854	-1.0650	-1.2959
EYA4	-1.3918	-1.8898	-0.9366	-0.9366	-1.2887
TIMP1	-1.2227	-0.9938	-1.4437	-1.4777	-1.2845
KCNJ16	-1.0740	-1.0290	-1.3597	-1.6563	-1.2798
RASAL3	-0.7041	-1.9176	-1.0542	-1.4309	-1.2767
SLC39A14	-1.0532	-1.7159	-0.8814	-1.3625	-1.2533
LIMS2	-1.4033	-1.1976	-0.9415	-1.2957	-1.2095
EHD4	-0.8046	-2.0628	-0.9233	-1.0343	-1.2062
SLC17A9	-1.1642	-1.1371	-0.9180	-1.4298	-1.1623
NPW	-1.5808	-1.3462	-0.6691	-0.9895	-1.1464
ELOVL6	-1.1441	-1.4841	-1.0100	-0.9357	-1.1435
POLR3G	-0.9626	-1.5587	-0.8686	-1.1653	-1.1388
RINL	-0.8970	-0.7945	-1.3339	-1.5001	-1.1314
STEAP3	-1.4163	-1.2707	-0.7765	-1.0552	-1.1297
SLC7A2	-1.0939	-1.5471	-0.8642	-0.9942	-1.1249
TRIB1	-0.8931	-1.6978	-0.8961	-0.8961	-1.0958
HBEGF	-0.6716	-0.8192	-1.3500	-1.5420	-1.0957
COTL1	-1.2271	-1.2348	-1.2081	-0.6986	-1.0922
SMAGP	-0.9926	-0.9888	-0.8123	-1.5110	-1.0762
CST7	-1.4671	-1.0464	-0.8858	-0.8858	-1.0713
SLAMF1	-0.8998	-0.9857	-1.1254	-1.2657	-1.0691
LOC644590	-0.9093	-0.9919	-1.3325	-0.9913	-1.0562
SLC29A1	-0.9383	-0.6162	-1.2662	-1.3966	-1.0543

RRP12	-1.2584	-1.4194	-0.7135	-0.8161	-1.0519
STOM	-1.1731	-1.2855	-0.6963	-1.0091	-1.0410
HS.10862	-1.1196	-1.4995	-0.7061	-0.8325	-1.0394
IFI30	-1.2246	-0.7233	-1.1039	-1.0944	-1.0365
MYBBP1A	-1.1876	-1.2488	-1.0343	-0.6683	-1.0348
DHCR7	-0.7411	-1.1430	-1.1139	-1.1176	-1.0289
LY9	-0.8167	-0.8167	-0.9188	-1.5057	-1.0145
DYNC1I1	-0.7656	-0.7656	-1.2669	-1.2394	-1.0094

Upregulated	**RCH-ACV**		**SMS-SB**		
ILMN_Gene	**Ig*μ* KO1**	**Ig*μ*KO2**	**Ig*μ*KO1**	**Ig*μ*KO2**	**Average**
FNDC5	2.2593	2.1283	2.8788	2.8818	2.5371
LOC100129878	1.5796	2.0268	2.4302	2.5279	2.1411
LOC648868	1.2585	1.7784	2.7918	2.5315	2.0901
LOC100132810	1.5332	1.7873	2.2954	2.4221	2.0095
C10ORF10	2.3614	2.1426	1.6997	1.7668	1.9926
MGC24125	1.5327	1.6009	1.9174	2.5291	1.8950
HPS4	1.4577	1.9099	1.7760	2.1046	1.8120
CCM2	1.6189	2.3456	1.3698	1.6741	1.7521
LOC728175	1.2248	1.5160	1.7613	2.2877	1.6974
NAV2	0.8713	1.1514	2.3397	2.3993	1.6904
GZMK	1.1728	1.3650	2.0699	2.0610	1.6672
TNFRSF17	0.8987	1.5593	2.0726	2.0427	1.6433
SELL	1.1759	1.8934	1.5754	1.8812	1.6315
LOC100128888	1.8935	1.5313	1.4021	1.6428	1.6175
BACH2	1.1386	1.4068	1.7984	2.1126	1.6141
BACH2	1.7277	1.1783	1.7757	1.7720	1.6134
GAB1	2.2639	1.6955	1.0751	1.2335	1.5670
DTX1	1.5038	1.3609	1.6820	1.6977	1.5611
ARPP-21	0.8353	0.9694	2.1523	2.1593	1.5291
CTGF	1.6035	1.3915	1.5649	1.5392	1.5248
YPEL3	1.2547	1.9619	1.5088	1.3507	1.5190
SPTA1	1.3026	1.1083	1.6575	1.8664	1.4837
ARPP-21	1.2536	0.8265	1.8098	2.0331	1.4808
BACH2	1.2948	1.1759	1.6165	1.7140	1.4503
SH2D4B	1.4844	1.0232	1.5360	1.7226	1.4416
MPP1	1.1293	1.2130	1.5143	1.7414	1.3995
SOCS2	1.1617	1.3497	1.4170	1.5970	1.3814
DPEP1	1.9022	2.0094	0.7531	0.8462	1.3777
LOC645993	1.2254	1.7737	1.2709	1.2263	1.3741
BTNL3	1.2815	1.6512	1.2827	1.2447	1.3650
MXD3	1.1608	1.6635	1.3024	1.2955	1.3556
CLEC2D	1.0370	1.0688	1.6579	1.6322	1.3490
GBP4	1.6559	1.3792	1.1601	1.1589	1.3386

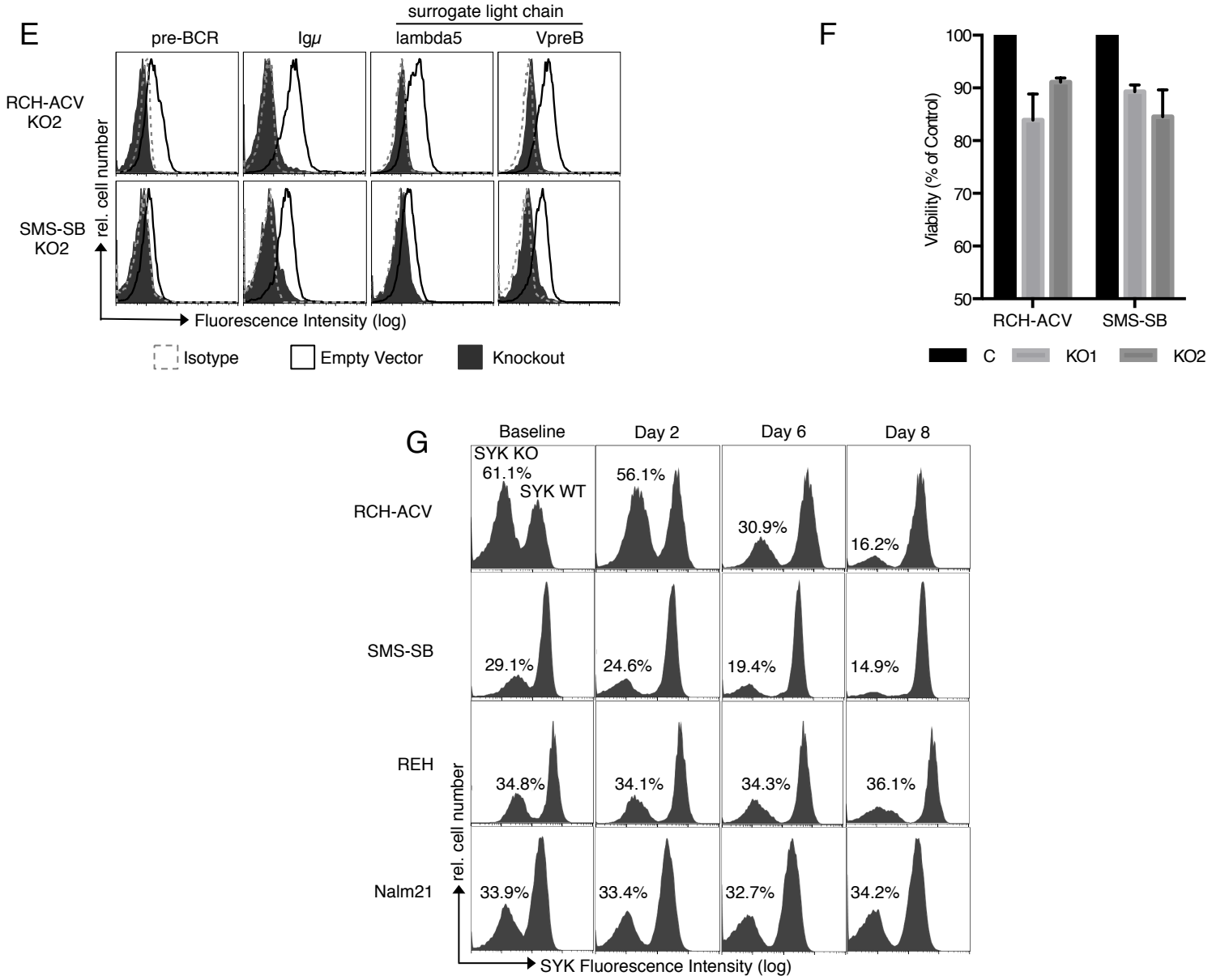
ARPP-21	1.2087	0.6234	1.6283	1.8807	1.3353
LOC100129878	1.5281	2.0248	0.7177	1.0470	1.3294
RCAN1	1.4686	1.9379	0.7175	1.1809	1.3262
LOC399804	0.7415	0.9880	1.7927	1.7343	1.3141
ARPP-21	0.7990	0.8684	1.8735	1.7054	1.3116
NYNRIN	0.9031	1.0356	1.5551	1.7193	1.3033
HIP1R	1.1500	1.7627	1.0630	1.2102	1.2965
S1PR4	0.9341	1.1220	1.4721	1.5792	1.2768
HS.434957	1.4684	0.9833	1.1566	1.3502	1.2396
MYLK	1.2675	1.0441	1.1053	1.5371	1.2385
RCAN1	1.3266	1.2087	1.0881	1.3130	1.2341
GAB1	1.4392	1.7006	0.6152	1.1628	1.2295
C20ORF103	1.2471	1.6403	0.9310	1.0388	1.2143
LRRC26	1.0806	1.4062	1.1859	1.1795	1.2130
RASD1	0.9218	1.1061	1.3005	1.4842	1.2032
CMTM8	0.9765	1.2378	1.2015	1.3321	1.1870
RASAL1	1.1037	1.0351	1.2502	1.3162	1.1763
YPEL5	1.4437	1.2492	0.8362	1.1190	1.1620
CXCR4	0.9661	1.3879	0.8356	1.3936	1.1458
LOC90925	1.2295	1.5326	0.8226	0.9699	1.1387
LOC389816	0.9506	1.3491	1.1107	1.1020	1.1281
SHROOM3	0.9526	0.7612	1.3478	1.4462	1.1270
RAG1	1.2766	1.2470	1.0561	0.9267	1.1266
LOC100130503	1.1988	0.6834	1.2245	1.3940	1.1252
MME	1.5357	1.2703	0.7432	0.9207	1.1175
KLHL24	1.1397	1.1342	1.1570	1.0190	1.1125
TMEM71	0.6785	1.0861	1.3109	1.3562	1.1079
LOC100130123	1.2800	0.6672	1.0794	1.3391	1.0914
ZFP36L2	0.7961	1.0503	1.0072	1.4731	1.0817
AKAP12	1.1064	0.6663	0.9463	1.5907	1.0774
GPR137C	1.4334	0.9614	0.6372	1.2535	1.0714
YPEL1	0.9821	0.8289	1.1424	1.3110	1.0661
TXNIP	1.4691	1.0400	0.8289	0.9222	1.0651
RAB37	0.8603	1.3944	0.6852	1.3159	1.0639
CXCR4	0.9756	1.1435	0.9389	1.1976	1.0639
SOCS2	0.9893	0.9816	1.0863	1.1501	1.0518
PIM1	1.2959	0.9938	1.0427	0.8622	1.0486
LOC100130476	0.6085	1.3336	1.0580	1.1258	1.0315
LOC100130503	0.7344	0.6212	1.0273	1.7402	1.0308
AXUD1	1.3704	0.6926	0.9538	1.0922	1.0272
LOC730101	1.1103	0.8570	1.0736	1.0222	1.0157
CLEC2D	0.8102	1.0686	0.8777	1.2784	1.0087
ADCY9	0.6181	0.6417	1.3321	1.4296	1.0054

Supplemental Figure 1



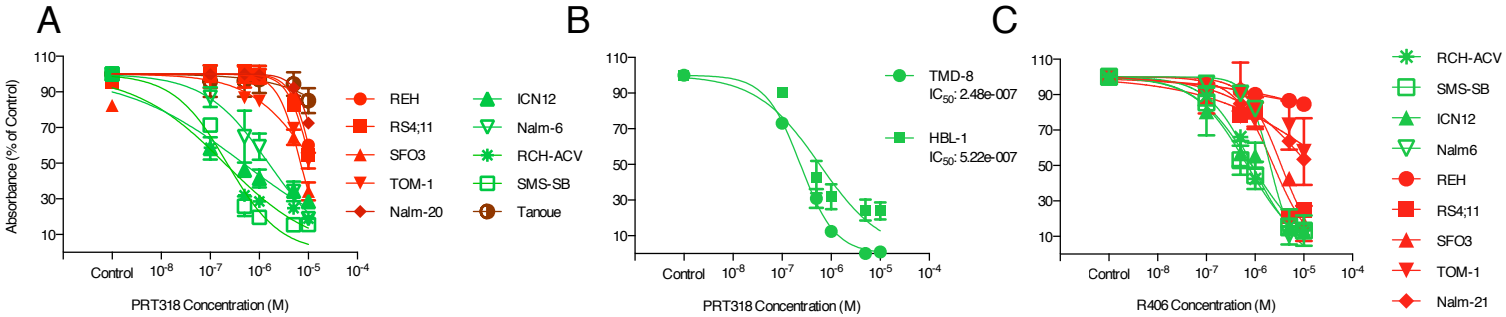
Supplemental Figure 1. (A) Genes distinguishing normal pro-B from pre-B cells. Gene expression data derives from the NCBI GEO database (GSE45460, Lee ST et al. 2012). Heatmap depicts log2-transformed, mean-centered expression values. (B) Frequency of the pre-B ALL phenotype by cytogenetic subgroup (C) Pre-BCR expression in xenograft cells and cell lines. (D) Expression of pre-BCR signaling molecules in pre-B ALL cells.

Supplemental Figure 1



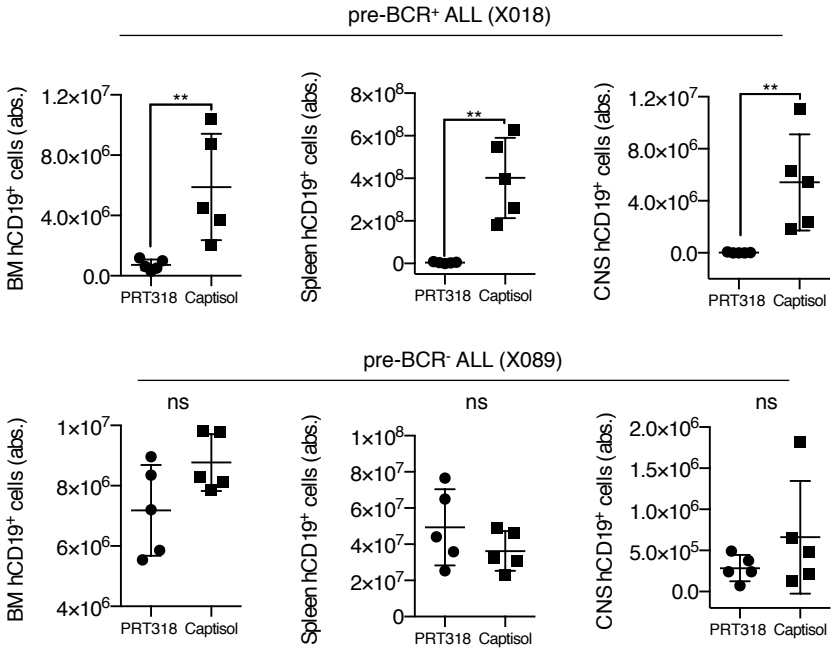
Supplemental Figure 1. (E) Successful abrogation of pre-BCR expression in RCH-ACV and SMS-SB cells through alternative gRNAs (KO2). (F) Viability of Igμ-KO cells in comparison to control (G) Transfection of B-ALL cells with SYK-specific CRISPR/Cas9 vectors results in stable knockout of SYK in a fraction of cells (SYK-KO). In SYK-dependent cells (RCH-ACV, SMS-SB) SYK KO cells disappear over time, in SYK-independent cells (REH, Nalm21) their numbers remain stable.

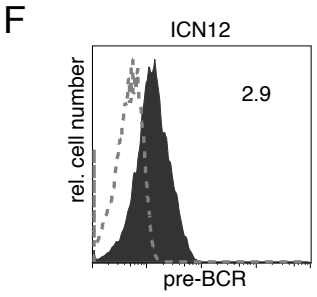
Supplemental Figure 2



D

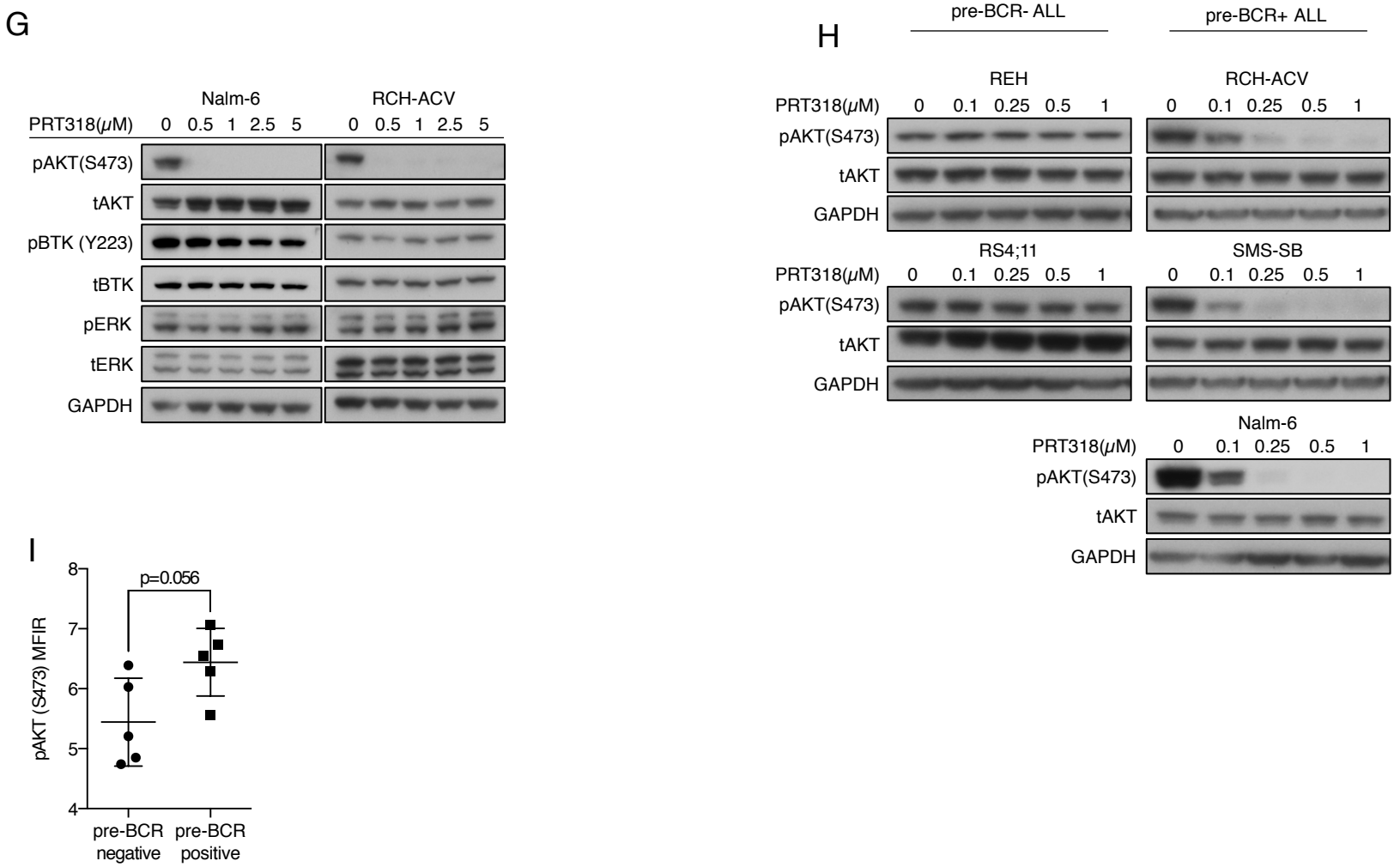
Stage	Cellline	pre-BCR	IC_{50} R406 [95% CI]
pro-B ALL	REH	-	> 20 μM
	RS4;11	-	2.4 μM [1.3 - 4.3]
	SFO3	-	3.5 μM [2.3-5.3]
	Nalm-20	-	13 μM [8.1 - 20.7]
	TOM-1	-	> 20 μM
pre-B ALL	RCH-ACV	+	0.9 μM [0.7-1.1]
	SMS-SB	+	0.7 μM [0.47-1.03]
	ICN12	+	0.9 μM [0.54-1.36]
	Nalm-6	+	2 μM [1.46-2.87]





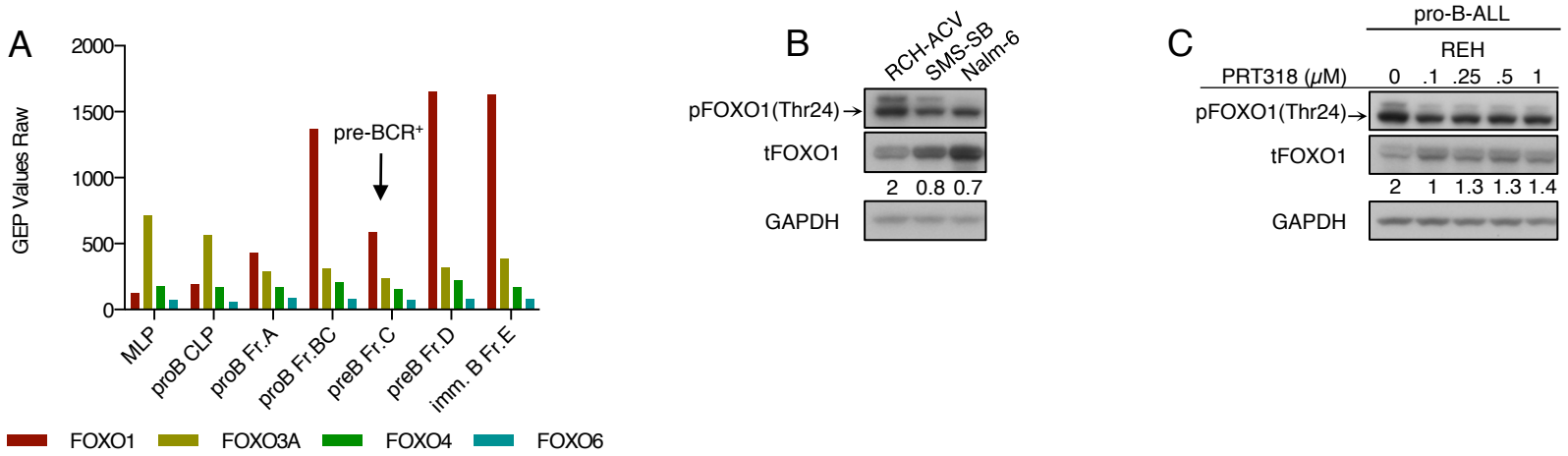
Supplemental Figure 2. (A) Dose response curves of PRT318 in pre-BCR$^+$ (green) and pre-BCR$^-$ (red) ALL cells and in (B) SYK-dependent DLBCL cells. (C) Dose response curves and (D) IC_{50} values of R406 in pre-BCR$^+$ and pre-BCR$^-$ B-ALL cells. (E) Extent of leukemia cell infiltration in the bone marrow (BM), spleen and central nervous system (CNS) after 10 days of PRT318 or 5% Captisol (control). **p<0.01 Mann-Whitney test. (F) Pre-BCR expression in the t(1;19)$^+$ xenograft cell line ICN12.

Supplemental Figure 2



Supplemental Figure 2. (G) Effects of PRT318 on AKT phosphorylation in Nalm-6 and RCH-ACV cells (pre-BCR$^+$). (H) Representative immunoblots depicting the effect of PRT318 on pAKT in pre-BCR$^+$ and pre-BCR$^-$ cells. 0.5 μM PRT318 completely blocks AKT phosphorylation in pre-BCR-dependent cells. (I) pAKT levels in pre-BCR$^+$ and pre-BCR$^-$ xenograft cells.

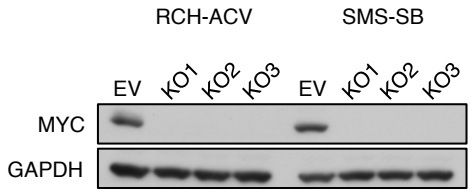
Supplemental Figure 3



Supplemental Figure 3. (A) FOXO1 gene expression predominates in hematopoietic progenitors committed to the B-cell lineage (Fr. A to Fr. E). Data was kindly provided by the Immunological Genome Project. Y-axis represents raw gene expression values. Displayed are mean values from three independent experiments. (B) Expression and phosphorylation of FOXO1 in pre-BCR+ ALL. Numbers indicate phospho-densitometry analysis of pFOXO1/tFOXO1. (C) Effects of PRT318 on pFOXO1 in REH cells. Numbers indicate phospho-densitometry analysis of pFOXO1/tFOXO1.

Supplemental Figure 4

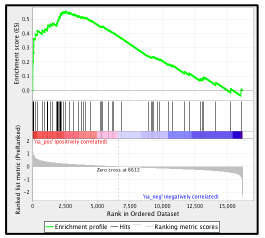
A



B

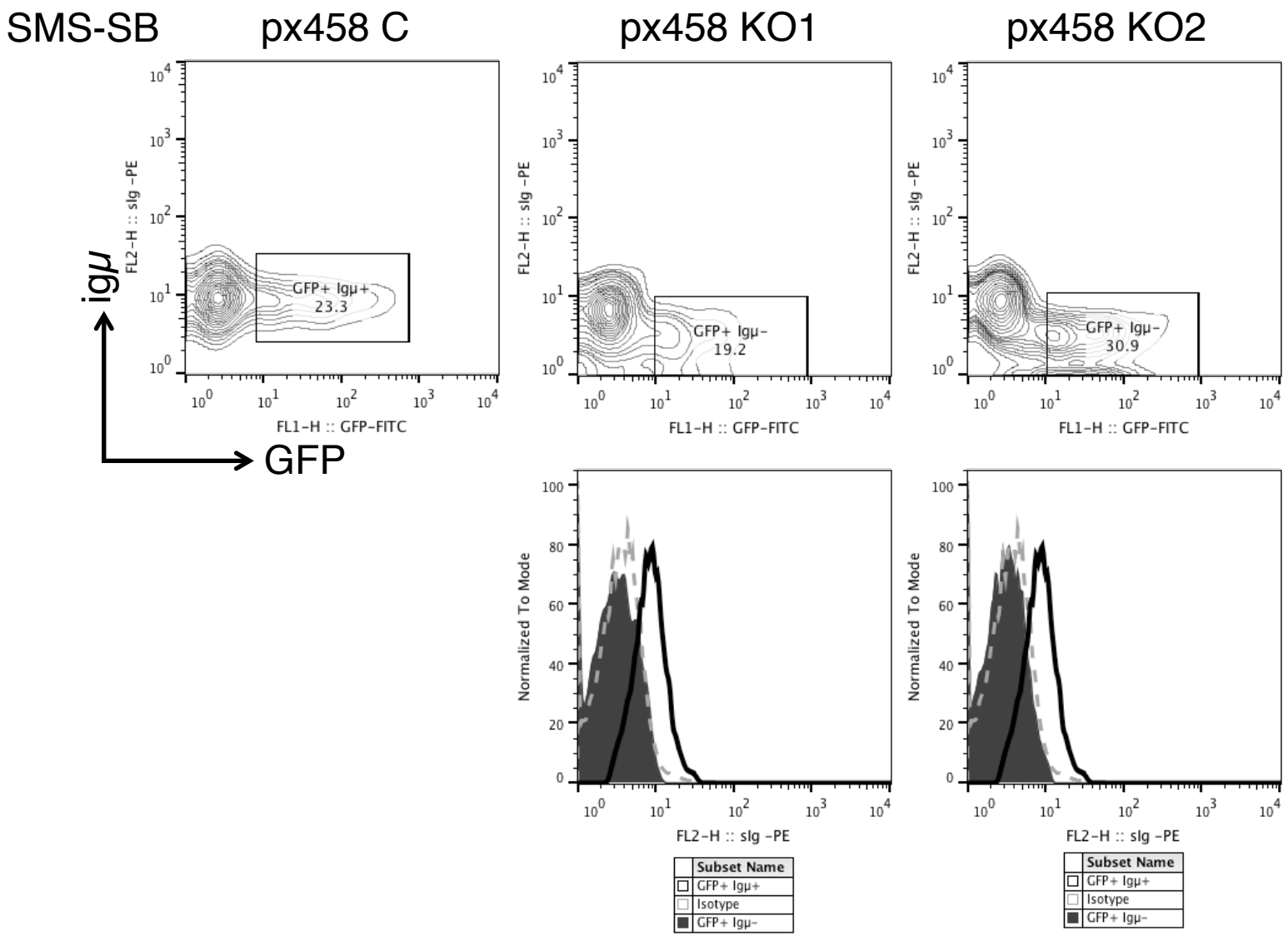
Gene Set Name	FDR (q-Value)	NOM p-Value
ODONNELL_TARGETS_OF_MYC_AND _TFRC_UP	0.007	<0.001
SCHLOSSER_MYC_AND_SERUM_RES PONSE_SYNERGY	0.087	<0.001
PID_MYC_ACTIVPATHWAY	0.147	0.002
SCHUHMACHER_MYC_TARGETS_UP	0.182	0.006

ODONNELL_TARGETS_OF_ MYC_AND_TFRC_UP



Supplemental Figure 4. (a) Successful knockout of MYC with three distinct gRNAs (b) Gene sets associated with MYC downmodulation, which were found to be significantly enriched in RCH-ACV cells expressing FOXO1-AAA.

Supplementary Figure 5



Supplementary Figure 5. 48h after transfection of SMS-SB cells with control vector (px458 C) or igμ-specific CRISPR/Cas9 knockout vector (px458 KO1 and px458 KO2). The cells were stained for surface Igμ expression. GFP$^+$ cells express px458. GFP$^+$ Cells transfected with px458 control are Igμ^+; GFP$^+$ cells transfected with px458 KO1 or KO2 loose Igμ expression. Histograms depict the gates from the blots in the top row. The black line represents the GFP$^+$/Igμ^+ gate in px458 EV; Dark filled histograms represent GFP$^+$/Igμ^- gates in the px458 gRNA1/2 blots. Grey dotted line represents Isotype control staining.