Supplementary Table S1. Kinase selectivity of acalabrutinib as assessed by DiscoveRX KinomeScan screening.

Ambit Gene Symbol	Percent of Control Binding	Ambit Gene Symbol	Percent of Control Binding
AAK1	86	BMPR2	100
ABL1	100	**BMX**	**34**
ABL1(E255K)	89	BRAF	64
ABL1(F317I)	100	BRAF(V600E)	44
ABL1(F317L)	100	BRK	79
ABL1(H396P)	100	BRSK1	97
ABL1(M351T)	100	BRSK2	100
ABL1(Q252H)	100	**BTK**	**0.05**
ABL1(T315I)	100	CAMK1	87
ABL1(Y253F)	100	CAMK1D	98
ABL2	97	CAMK1G	95
ACVR1	100	CAMK2A	90
ACVR1B	96	CAMK2B	92
ACVR2A	99	CAMK2D	89
ACVR2B	96	CAMK2G	84
ACVRL1	76	CAMK4	91
ADCK3	95	CAMKK1	89
ADCK4	88	CAMKK2	93
AKT1	93	CDC2L1	100
AKT2	100	CDC2L2	100
AKT3	92	CDK11	92
ALK	100	CDK2	82
AMPK-α1	86	CDK3	97
AMPK-α2	84	CDK5	100
ANKK1	96	CDK7	74
ARK5	99	CDK8	99
ASK1	73	CDK9	87
ASK2	99	CDKL2	100
AURKA	100	CDKL3	100
AURKB	100	CDKL5	100
AURKC	100	CHEK1	100
AXL	100	CHEK2	94
BIKE	100	CIT	100
BLK	**61**	CLK1	94

BMPR1A	100	CLK2	94
BMPR1B	100	CLK3	98
CLK4	100	EPHA1	76
CSF1R	84	EPHA2	91
CSK	86	EPHA3	100
CSNK1A1L	92	EPHA4	100
CSNK1D	92	EPHA5	100
CSNK1E	90	EPHA6	100
CSNK1G1	78	EPHA7	94
CSNK1G2	82	EPHA8	91
CSNK1G3	100	EPHB1	100
CSNK2A1	54	EPHB2	69
CSNK2A2	100	EPHB3	98
CTK	100	EPHB4	100
DAPK1	100	EPHB6	96
DAPK2	80	**ERBB2**	**2.1**
DAPK3	99	ERBB3	95
DCAMKL1	100	**ERBB4**	**4.3**
DCAMKL2	100	ERK1	97
DCAMKL3	99	ERK2	97
DDR1	100	ERK3	94
DDR2	100	ERK4	89
DLK	95	ERK5	100
DMPK	99	ERK8	99
DMPK2	95	ERN1	85
DRAK1	87	FAK	96
DRAK2	88	FER	100
DYRK1A	100	FES	100
DYRK1B	93	FGFR1	100
DYRK2	98	FGFR2	99
EGFR	**75**	FGFR3	87
EGFR(E746-A750DEL)	85	FGFR3(G697C)	77
EGFR(G719C)	97	FGFR4	100
EGFR(G719S)	100	FGR	100
EGFR(L747-E749DEL, A750P)	87	FLT1	100
EGFR(L747-S752DEL, P753S)	100	FLT3	100
EGFR(L747-T751DEL, SINS)	89	FLT3(D835H)	86
EGFR(L858R)	94	FLT3(D835Y)	100
EGFR(L858R, T790M)	94	FLT3(ITD)	100

EGFR(L861Q)	84	FLT3(K663Q)	100
EGFR(S752-I759DEL)	95	FLT3(N841I)	95
FLT4	98	KIT(V559D,V654A)	100
FRK	59	LATS1	93
FYN	100	LATS2	96
GAK	100	LCK	90
GCN2(S808G)	90	LIMK1	13
GRK1	100	LIMK2	98
GRK4	100	LKB1	100
GRK7	100	LOK	73
GSK3A	100	LTK	86
GSK3B	100	LYN	82
HCK	62	LZK	83
HIPK1	90	MAK	90
HIPK2	99	MAP3K1	100
HIPK3	100	MAP3K15	100
HIPK4	88	MAP3K2	100
HPK1	89	MAP3K3	100
HUNK	100	MAP3K4	86
ICK	100	MAP4K2	100
IGF1R	98	MAP4K3	99
IKK-α	100	MAP4K4	97
IKK-β	100	MAP4K5	99
IKK-ε	100	MAPKAPK2	100
INSR	100	MAPKAPK5	100
INSRR	99	MARK1	100
IRAK1	100	MARK2	59
IRAK3	100	MARK3	82
ITK	**96**	MARK4	100
JAK1(JH1domain)	100	MAST1	100
JAK1(JH2domain)	71	MEK1	100
JAK2(JH1domain)	81	MEK2	82
JAK3(JH1domain)	**97**	MEK3	97
JNK1	98	MEK4	100
JNK2	98	MEK6	98
JNK3	100	MELK	76
KIT	84	MERTK	77
KIT(D816V)	83	MET	91
KIT(L576P)	63	MET(M1250T)	90

KIT(V559D)	53	MET(Y1235D)	100
KIT(V559D,T670I)	55	MINK	100
MKNK1	99	PAK7	100
MKNK2	100	PCTK1	100
MLCK	99	PCTK2	100
MLK1	89	PCTK3	100
MLK2	100	PDGFRA	100
MLK3	94	PDGFRB	95
MRCKA	98	PDPK1	67
MRCKB	98	PFTAIRE2	76
MST1	87	PFTK1	100
MST1R	89	PHKG1	100
MST2	65	PHKG2	92
MST3	100	PI3KCA	100
MST4	91	PI3KCB	100
MTOR	100	PI3KCD	100
MYLK	100	PI3KCG	100
MYLK2	89	PIK4CB	100
MYO3A	100	PIM1	84
MYO3B	99	PIM2	79
NDR1	98	PIM3	84
NDR2	100	PIP5K1A	92
NEK1	97	PIP5K2B	100
NEK2	100	PKAC-α	77
NEK5	95	PKAC-β	88
NEK6	100	PKMYT1	100
NEK7	80	PKN1	94
NEK9	73	PKN2	86
NIM1	100	PLK1	100
NLK	100	PLK2	95
OSR1	100	PLK3	100
p38-α	84	PLK4	80
p38-β	98	PRKCD	100
p38-δ	57	PRKCE	100
p38-γ	100	PRKCH	87
PAK1	87	PRKCQ	100
PAK2	78	PRKD1	97
PAK3	73	PRKD2	100
PAK4	88	PRKD3	100

PAK5	100	PRKG1	70
PAK6	94	PRKG2	95
PRKR	92	STK35	100
PRKX	100	STK36	100
PRP4	86	STK39	100
PYK2	95	SYK	100
QSK	100	TAK1	100
RAF1	77	TAO1	100
RET	100	TAOK1	100
RET(M918T)	71	TAOK3	100
RET(V804L)	93	TBK1	100
RET(V804M)	100	**TEC**	**6.4**
RIOK1	92	TESK1	83
RIOK2	80	TGFBR1	92
RIOK3	100	TGFBR2	96
RIPK1	100	TIE1	89
RIPK2	60	TIE2	98
RIPK4	100	TLK1	100
ROCK1	100	TLK2	98
ROCK2	100	TNIK	82
ROS1	100	TNK1	90
RPS6KA1	100	TNK2	93
RPS6KA2	100	TNNI3K	38
RPS6KA3	100	TRKA	100
RPS6KA4	90	TRKB	100
RPS6KA5	84	TRKC	99
S6K1	100	TSSK1B	100
SBK1	100	TTK	91
SGK	100	**TXK**	**24**
SgK110	90	TYK2(JH1domain)	97
SIK	88	TYK2(JH2domain)	100
SIK2	100	TYRO3	100
SLK	100	ULK1	100
SNARK	100	ULK2	100
SRC	78	ULK3	99
SRMS	67	VEGFR2	100
SRPK1	67	WEE1	100
SRPK2	85	WEE2	95
SRPK3	93	YANK2	59

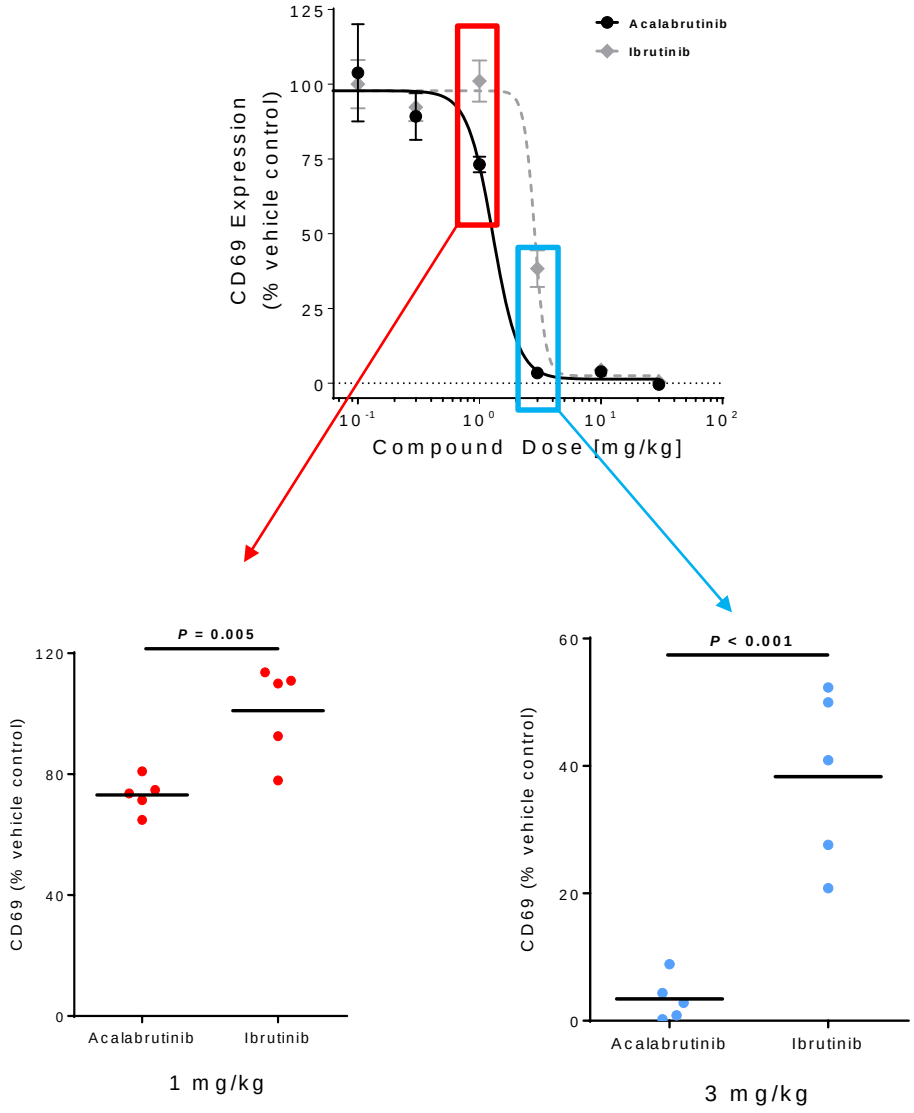
STK16	89	YANK3	100
STK33	73	YES	83
YSK1	92	ZAK	84
YSK4	90	ZAP70	98

Acalabrutinib was screened at 1 μM in ATP site-dependent competition binding assays by contract with DiscoverRX (Ambit Biosciences; San Diego, CA).[1] The results for the primary screen binding interactions are reported as '% of control binding', where lower numbers indicate stronger hits in the matrix. Values of >35% are considered 'no hits'. BTK had the lowest percentage of control binding at 0.05% indicating a high probability of a potent interaction. 353 kinases were assessed in the screen. Kinases in bold contain a conserved cysteine residue that aligns with the Cys481 in BTK.

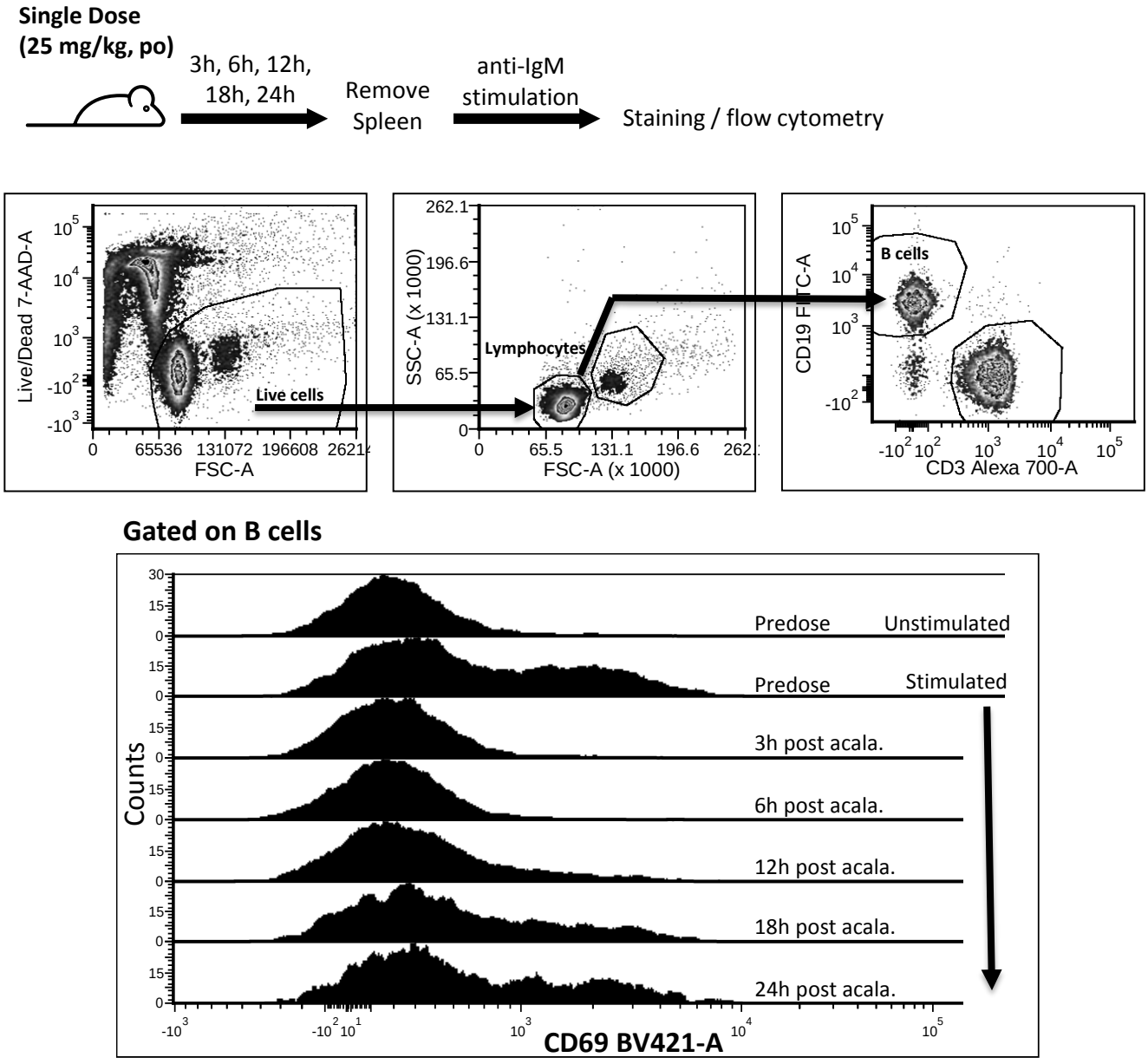
Supplemental reference:

1) Fabian M. A., et al. (2005) A small molecule-kinase interaction map for clinical kinase inhibitors. Nat. Biotechnol. 23, 329–336

Supplementary Figure S1

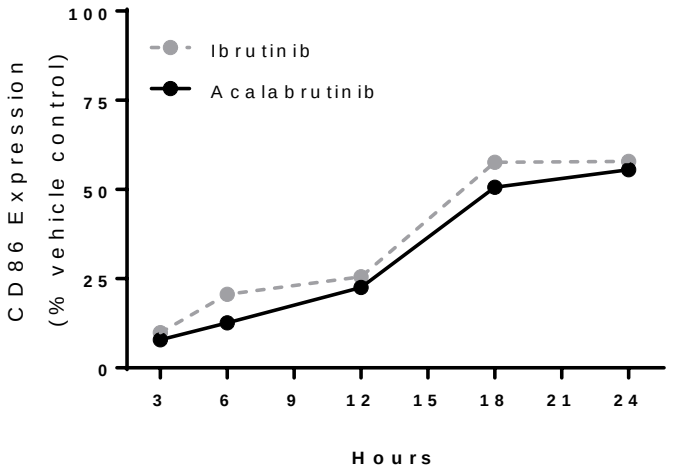


Supplementary Figure S2



Supplementary Figure S3

A



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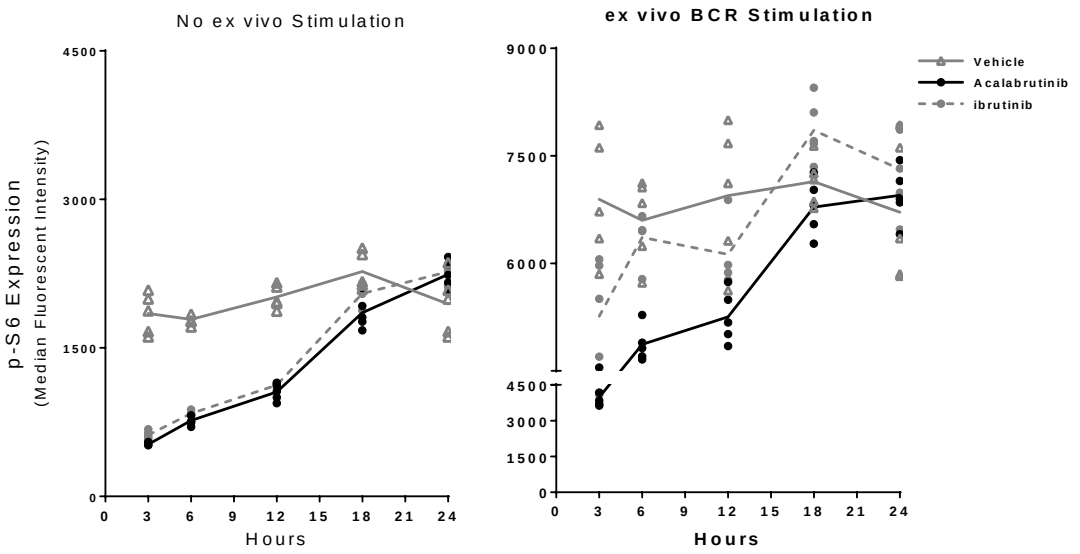
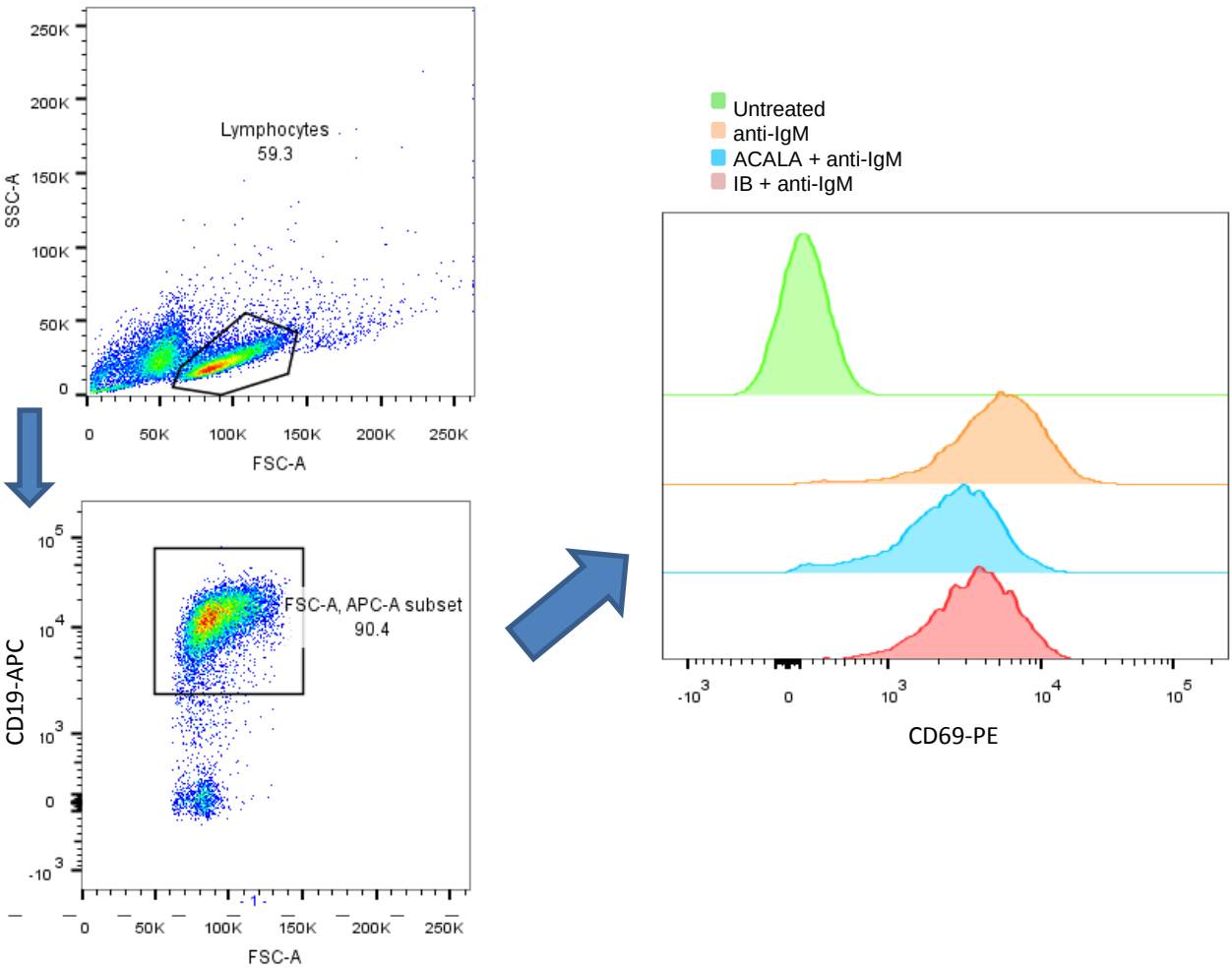
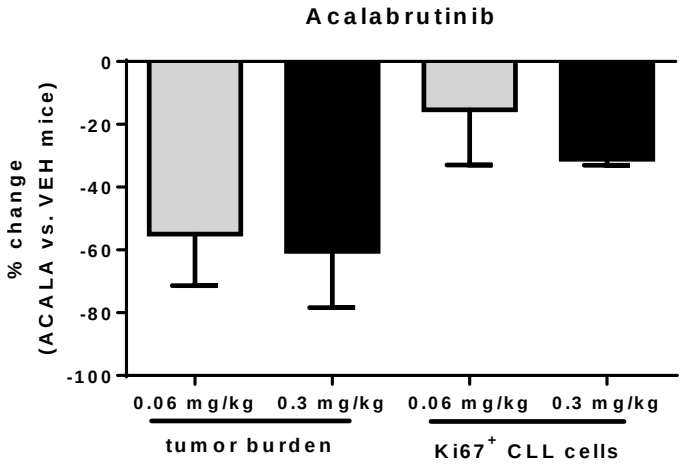


Supplementary Figure S4



Supplementary Figure S5

A



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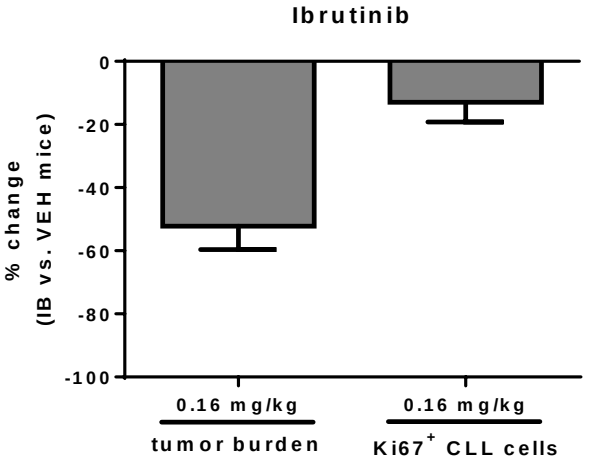

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1: Acalabrutinib is a more potent BCR-inhibitor than ibrutinib *in vivo* at 1mg/kg and 3mg/kg. Mice (5/group/dose) were orally given vehicle, acalabrutinib or ibrutinib. After 3 hours, spleens were extracted and splenocytes stimulated with anti-IgM for 18 h, followed by CD69 expression analysis by flow cytometry. Red dots represent 1mg/kg dose and blue dots represent 3mg/kg dose. Statistics were determined by unpaired student t-test.

Supplementary Figure S2: *In vivo* gating strategy for murine cells. Mice (5/group) received 25 mg/kg of vehicle, acalabrutinib or ibrutinib at time zero. Spleens were extracted at various time points and splenocytes stimulated with anti-IgM for 18 h. Live cells were gated out, followed by a lymphocytes gated on FSC and SSC. B-cells were identified as CD19+/CD3-. CD69 expression analysis was then preformed on 7-AAD-/CD19+/CD3- cells. Representative histograms are shown.

Supplementary Figure S3: In vivo acalabrutinib treatment inhibits BCR signal transduction similarly to ibrutinib. Mice (5/group) received 25 mg/kg of vehicle, acalabrutinib or ibrutinib at time zero. Spleens were extracted at various time points and splenocytes stimulated with anti-IgM for 18 h, followed by CD86 expression analysis (A) and phospho-S6 expression analysis (B) by flow cytometry. Grey dots represent ibrutinib and black dots represent acalabrutinib.

Supplementary Figure S4: *In vitro* gating strategy for patient MNCs. CLL patient MNCs were pretreated with BCR-inhibitors for 3 hours and then stimulated with or without anti-IgM for 18 h, followed by CD69 expression analysis by flow cytometry. Lymphocytes were gated by FSC and SSC. CLL cells were identified as CD19+. CD69 expression analysis was then preformed. Representative histograms are shown for untreated cells (green), anti-IgM stimulated cells (orange), acalabrutinib (ACAL) + anti-IgM (blue) and ibrutinib (IB) + anti-IgM (red).

Supplementary Figure S5: Acalabrutinib demonstrates similar changes in proliferation and tumor burden in the CLL xenograft mouse model as ibrutinib. Matched CLL MNCs (n=3) were used in separate but identical experiments comparing vehicle to 0.06mg/kg (light grey bars) and 0.3mg/kg (black bars) acalabrutinib (A) and vehicle to 0.16mg/kg (dark grey

bars) ibrutinib (B). MNCs were harvested from NSG mouse spleens (n=2-5 per patient/experiment) after 3 weeks of treatment. Shown is the % change in tumor burden (percentage of CLL cells among human CD45+ cells in the spleen) and proliferation (Ki67+ CLL cells) compared to experimental vehicle controls.