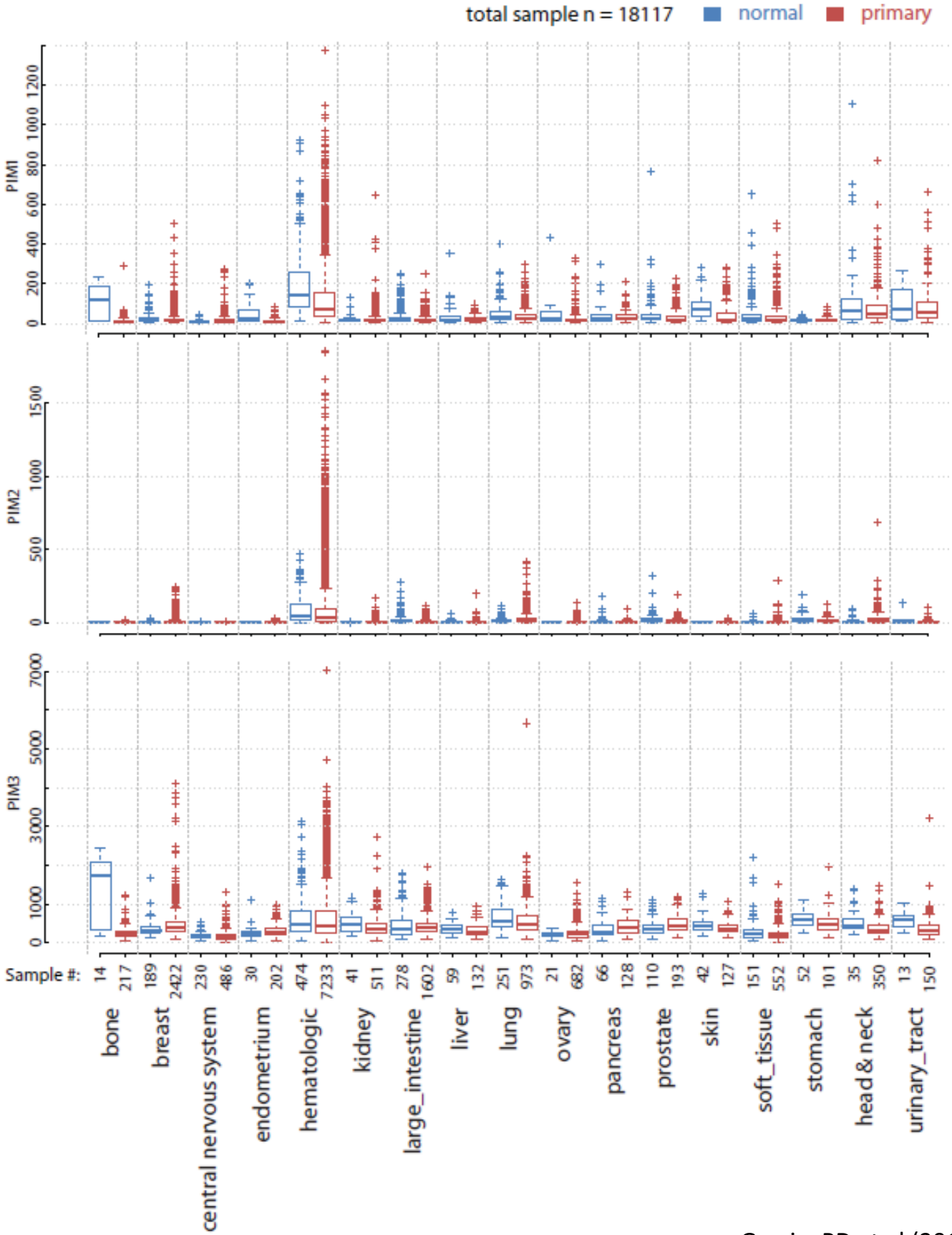
Supplementary Figure 1: Normal vs. Tumor expression of PIM kinases mRNA across 17 tissue types



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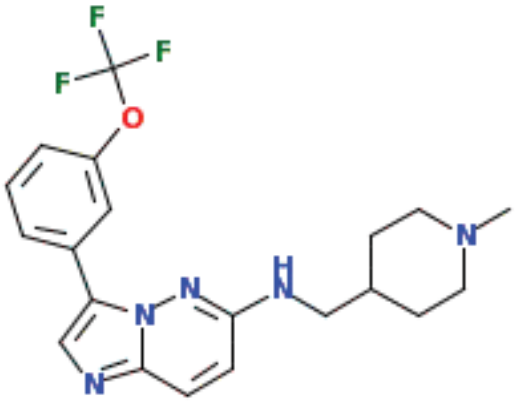
<u>Supplementary Figure 1 Legend</u>: PIM1, PIM2 and PIM3 mRNA expression

Expression levels in cancer tissues derived from 17 major organs and their normal counterpart (hematologic tissues are group together) were determined as described in Figure 1 of the main paper text. The number of samples for each tissue type is indicated at the bottom of the figure. The median expression of each gene is represented by the center line within each box, and the first and third quartiles are depicted by the edges of the box. The whiskers extending from each box indicate expression values that are within 1.5 times the inter-quartile range (IQR) from the upper or lower quartile. Outliers that are at a distance of great than 1.5*IQR from the box are plotted individually as plus signs.

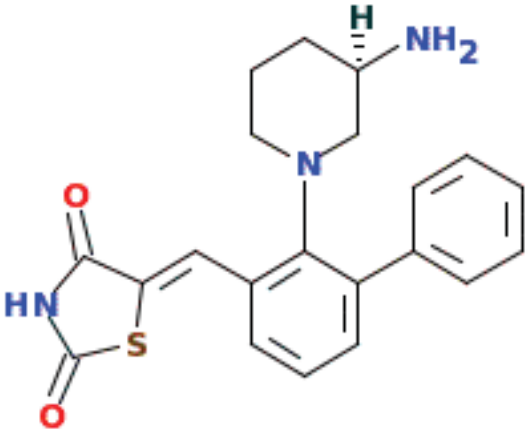
Supplementary Figure 2:
Comparison of LGB321 on-target activity vs. Previously described pan-PIM inhibitors

Compound	PIM1 K_i (nM)	PIM2 K_i (nM)	PIM3 K_i (nM)	KMS11-luc GI_{50} (N) (μM)
LGB321	0.001	0.002	0.001	0.017 (26)
SGI-1776	16	610	24	4.5 (6)
AZD1208	0.017	0.16	0.23	0.67 (2)

SGI-1776



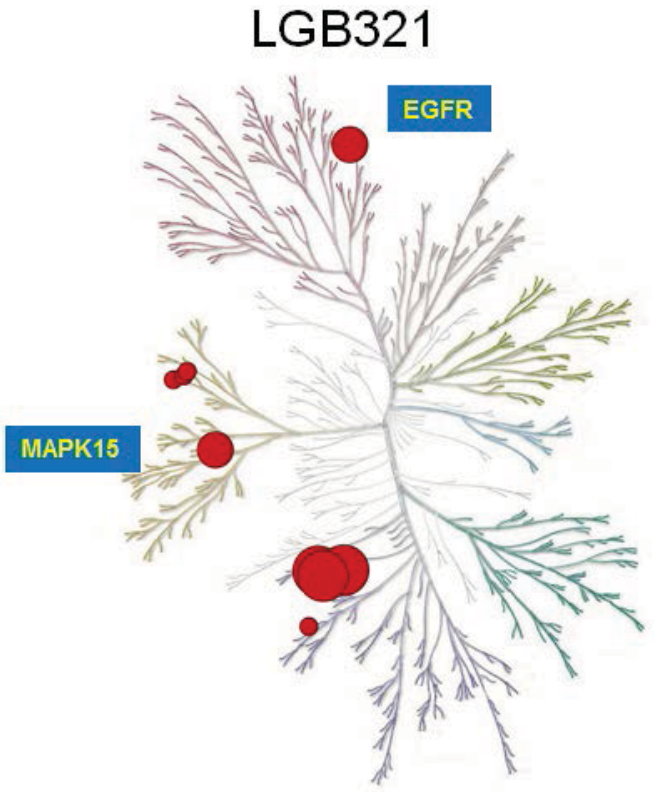
AZD1208



<u>Supplementary Figure 2 Legend</u>: The biochemical potency for SGI-1776 and AZD1208 was measured by the same methodology reported for LGB321 in Figure 2. The potency in KMS11-luc cell was determined as in the mail Figure 3.

Garcia, PD et al (2013)

Supplementary Figure 3: KINOMESCAN™ of 1 μM LGB321



Ambit Gene Symbol	Entrez Gene Symbol	Percent Control
PIM1	PIM1	0.5
PIM3	PIM3	0.5
PIM2	PIM2	0.75
EGFR(L858R)	EGFR	2.1
ERK8	MAPK15	2.4
EGFR(L747-E749del, A750P)	EGFR	2.5
EGFR	EGFR	2.6
CLK2	CLK2	14
CLK4	CLK4	28
PIK3CB	PIK3CB	32
CLK1	CLK1	33
MYLK	MYLK	34
NLK	NLK	36
PRKD3	PRKD3	40

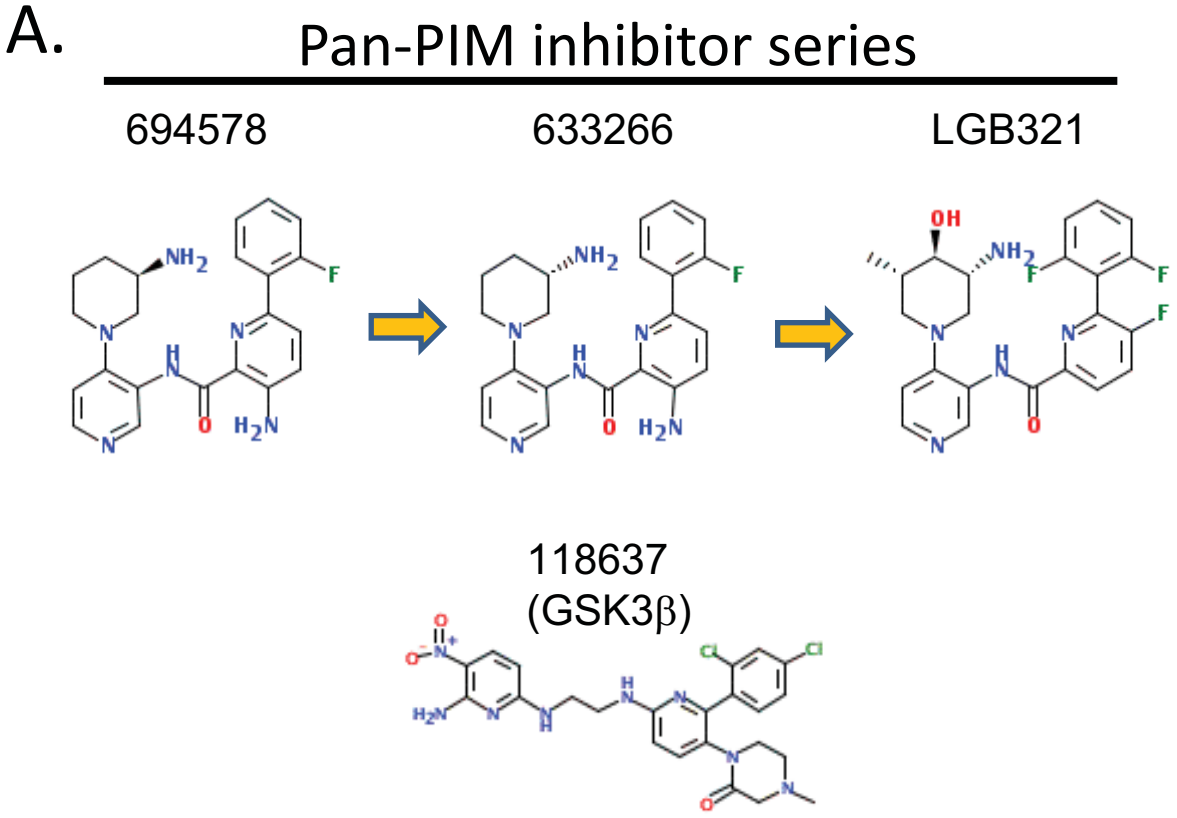
Selectivity score type	# of Hits	# of non-mutant kinases	Selectivity Score
< 35 % of control	10	386	0.026
< 10 % of control	5	386	0.013
< 1 % of control	3	386	0.008

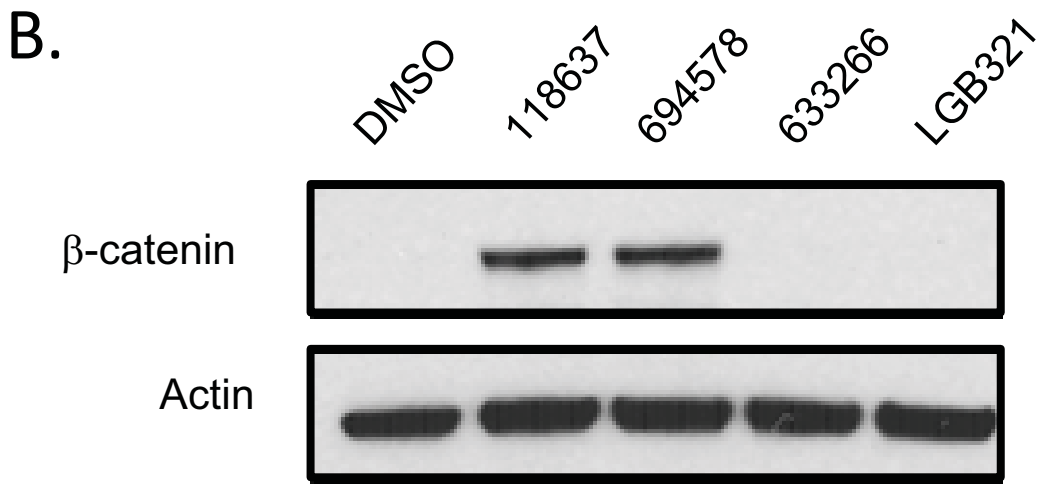
Supplementary Figure 3 Legend: The complete data set, including all kinases tested in this assay, was published in Burger MT, Han W, Lan J, et al. Structure Guided Optimization, in Vitro Activity, and in Vivo Activity of Pan-PIM Kinase Inhibitors. ACS Med Chem Lett 2013.

For a more detailed description of KINOMESCAN's assay technology, see Fabian et al. A small molecule-kinase interaction map for clinical kinase inhibitors. Nat. Biotechnol. 23, 329-336 (2005).

Garcia, PD et al (2013)

Supplementary Figure 4: Biochemical and cellular selectivity of the LGB321 PIM inhibitor series towards GSK3β





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C.

	118637	694578	633266	LGB321
PIM2 (IC_{50})	N.D.	0.031	<0.002	<0.002
GSK3β (IC_{50})	<0.003	<0.003	0.013	4.4
GSK3β (EC_{50}) / MV 4-11	0.33	N.D	N.D.	>20

Supplementary Figure 4 Legend – Biochemical and cellular selectivity of the LGB321 PIM inhibitor series towards GSK3β

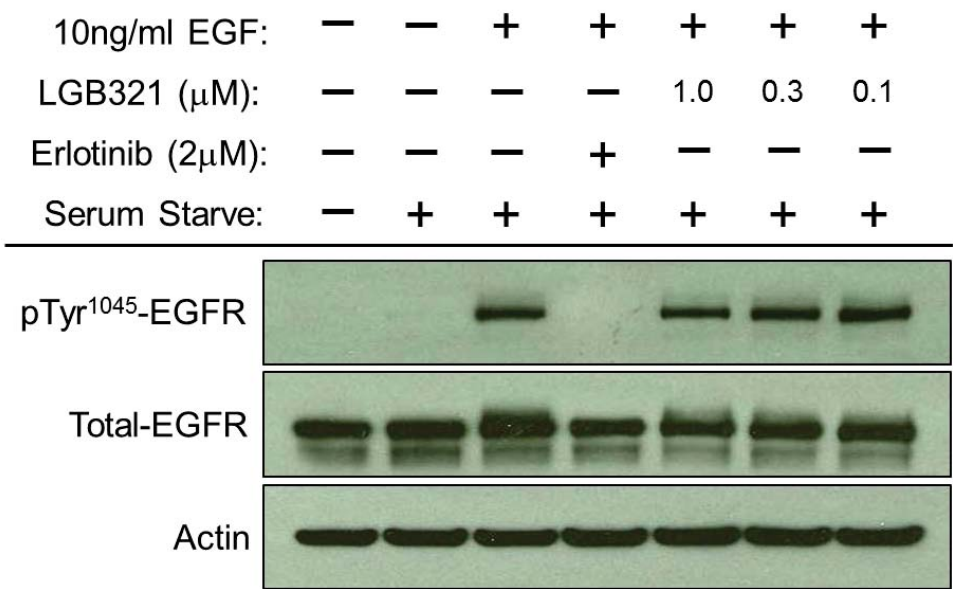
Cellular inhibition of GSK3β results in stabilization of β-catenin[1]. Compounds from the PIM inhibitor series (694578, 633266 & LGB321) were tested along with a control GSK3β inhibitor (118637)[2] on MV-411 cells for β-catenin stabilization. A) Representative compounds from the PIM series leading to LGB321. B) Western blot analysis of β-catenin levels in MV-411 cells after treatment with the control GSK3β inhibitor or PIM compounds. C) Activity table of the control GSK3β inhibitor and PIM compounds in PIM2 & GSK3β biochemical assays as well as a quantitative (MSD assay similar to the ones described for BAD and S6RP in main paper text) cellular assay for GSK3β inhibition.

References List

1. Yeow K, Novo-Perez L, Gaillard P et al. A cellular assay for measuring the inhibition of glycogen synthase kinase-3 via the accumulation of beta-catenin in Chinese hamster ovary clone K1 cells. Assay. Drug Dev.T echnol. (2006) 4:451-460.

2. Wagman AS, Johnson KW, Bussiere DE. Discovery and development of GSK3 inhibitors for the treatment of type 2 diabetes. Curr. Pharm. Des (2004)10:1105-1137.

Supplementary Figure 5: LGB321 does not inhibit EGFR signaling in HCC1954 cells



Supplementary Figure Legend 5

HCC1954 breast carcinoma cells were serum starved overnight and then treated for 3 hours with either the EGFR inhibitor Erlotinib (2 μM) as a positive control, or a the pan PIM inhibitor LGB321 (1, 0.3 or 0.1 μM). Cells were then stimulated for 15 minutes with 10 ng/mL EGF followed by 30 minutes of lysis at 4°C. Cleared lysates (20 ug/lane) were then resolved by SDS-PAGE and transferred to a nitrocellulose membrane for Western blotting using commercial antibodies.

Supplementary Table 1:

Sensitivity to LGB321 and Erlotinib of Lung Cells in the CCLE

Cell Line	Erlotinib [GI_{50} (μM)]	LGB321 [GI_{50} (μM)]
HCC827	0.07	>8
HCC4006	0.07	N.D.
NCI-H3255	0.08	>8
PC-14	0.08	>8
NCI-H1666	0.20	>8
CAL-12T	0.30	>8
NCI-H1734	0.31	>8
NCI-H2073	0.44	>8
SK-MES-1	0.66	>8
NCI-H322	0.67	>8
NCI-H1573	0.85	>8
NCI-H1944	0.87	>8
LUDLU-1	0.88	>8
HARA	0.99	>8
Calu-3	1.1	>8
DMS 53	N.D.	0.05
VMRC-LCD	>8	0.05
NCI-H1155	>8	1.8
MOR/CPR	>8	3.2
NCI-H810	>8	4.3
NCI-H889	>8	4.9
COR-L279	N.D.	6.3
EBC-1	>8	6.7

Supplementary Table 2

Sensitivity to LGB321 of hematological malignacies cell lines

	Cell line	LGB321 [GI_{50} (μM)]	Malignancy type	Other Annotations
ALL	CCRF-HSB-2	0.014	T-ALL	
	HH	0.059	CTLC	
	697	0.092	pre-B-ALL	
	RCH-ACV	0.26	pre-B-ALL	
	SEM	1.3	B-ALL	
	JURKAT	2.8	T-ALL	
	RPMI-8402	3.2	B-ALL	
	MOLT-16	3.5	T-ALL	
	PEER	4.3	T-ALL	
	RS4;11	5.4	B-ALL	
	KE-37	5.9	T-ALL	
	MOLT-4	7.4	T-ALL	
	MOLT3	9.7	T-ALL	
	CCRF-CEM	10	T-ALL	
	CEMC1	10	T-ALL	
	HPB-ALL	10	T-ALL	
	Loucy	10	T-ALL	
	P12-ICHIKAWA	10	T-ALL	
	PF-382	10	T-ALL	
	Reh	10	B-ALL	
	SUP-T1	10	T-ALL	
AML	M-07e	0.014	AML	
	NOMO-1	0.014	AML	
	MOLM-16	0.014	AML	
	KG-1	0.014	AML	
	UKE1	0.014	AML	JAK2-V617F
	EOL-1	0.014	AML	
	MV-4-11	0.021	AML	Flt3-ITD
	Set-2	0.031	AML	JAK2-V617F
	CMK	0.041	AML	
	CMK-11-5	0.12	AML	
	HEL 92.1.7	0.16	AML	JAK2-V617F
	OCI-M1	0.20	AML	
	MUTZ-8	0.20	AML	
	TF-1	0.37	AML	
	OCI-AML-3	0.58	AML	
	MOLM-13	0.69	AML	Flt3-ITD
	MONO-MAC-1	1.1	AML	
	THP-1	1.3	AML	
	OCI-AML2	3.1	AML	
	SKM-1	3.7	AML	
	PL-21	5.1	AML	

Supplementary Table 2 (cont.)

	Cell line	LGB321 [GI_{50} (μM)]	Malignancy type	Other Annotations
AML (cont.)	F-36P	6.0	AML	
	SIG-M5	7.6	AML	
	P31/FUJ	8.8	AML	
	NB-4	9.1	AML	
	OCI-AML5	10	AML	
CML	MOLM-6	0.014	CML	BCR-ABL positive
	JURL-MK1	0.014	CML	BCR-ABL positive
	BV-173	0.055	CML	BCR-ABL positive
	KYO-1	0.26	CML	BCR-ABL positive
	LAMA-84	0.27	CML	BCR-ABL positive
	EM-2	1.2	CML	BCR-ABL positive
	K-562	3.1	CML	BCR-ABL positive
	MEG-01	3.6	CML	BCR-ABL positive
	KCL-22	4.8	CML	BCR-ABL positive
	CML-T1	5.9	CML	BCR-ABL positive
HL	L-428	0.037	Hodgkin Lymphoma	
	L-540	0.082	Hodgkin Lymphoma	
	L-1236	3.6	Hodgkin Lymphoma	
	HD-MY-Z	10	Hodgkin Lymphoma	
MM	KMS-26	0.020	MM	
	LP-1	0.032	MM	
	MOLP-8	0.058	MM	
	RPMI 8226	0.068	MM	
	KMS-20	0.073	MM	
	KMS-12-BM	0.12	MM	
	KMS-28BM	0.15	MM	
	KMS-34	0.16	MM	
	MM1-S	0.20	MM	
	KMS-21BM	0.24	MM	
	AMO-1	0.27	MM	
	NCI-H929	0.27	MM	
	KMS-11	0.41	MM	
	EJM	0.65	MM	
	L-363	1.00	MM	
	KMM-1	1.8	MM	
	KMS-27	2.5	MM	
	KHM-1B	10	MM	
	OPM-2	10	MM	
B-cell NHL	JM1	0.014	DLBCL-GC	
	CA46	0.014	Burkitt Lymphoma	
	U-2932	0.036	DLBCL-ABC	TAK
	Toledo	0.071	DLBCL-GC	BCL2
	HBL-1	0.23	DLBCL-ABC	CD79+MyD88

Supplementary Table 2 (cont.)

	Cell line	LGB321 [GI_{50} (μM)]	Malignancy type	Other Annotations
B-cell NHL (cont.)	JeKo-1	0.25	MCL	
	DOHH-2	0.26	DLBCL-GC	
	RIVA	0.32	DLBCL-ABC	
	SU-DHL-8	0.40	DLBCL-ABC	
	SU-DHL-2	0.47	DLBCL-ABC	A20
	Z-138	1.2	MCL	
	SSK41	1.6	B-Cell NHL undefined	
	BL-70	3.0	Burkitt Lymphoma	
	Mino	3.4	MCL	
	SU-DHL-4	4.0	DLBCL-GC	Tonic BCR
	RL	4.1	DLBCL-GC	
	BL-41	4.1	Burkitt Lymphoma	
	NU-DHL-1	4.2	DLBCL-GC	
	WSU-DLCL2	4.3	DLBCL-GC	
	HT	5.3	DLBCL-GC	
	OCI-LY3	5.4	DLBCL-ABC	CARD11
	U-937	6.0	DLBCL-GC	
	SU-DHL-5	6.6	DLBCL-ABC	
	BJAB	7.7	DLBCL-GC	
	DB	8.2	DLBCL-GC	BCL2
	MC116	10	B-Cell NHL undefined	
	SU-DHL-10	10	DLBCL-GC	
T-cell NHL	SR-786	0.44	T-Cell ALCL	
	KARPAS -299	2.7	T-Cell ALCL	
	SUP-T11	4.9	T-Cell ALCL	
	SU-DHL-1	6.0	T-Cell ALCL	
	DEL	6.1	T-Cell ALCL	