SUPPLEMENTAL INFORMATION

Preclinical evaluation of a novel SHIP1 phosphatase activator for inhibition of PI3K signaling in malignant B cells

Elizabeth Lemm*, Beatriz Valle-Argos*, Lindsay D. Smith, Johanna Richter, Yohannes Gebreselassie, Matthew Carter, Jana Karolova, Michael Svaton, Karel Helman, Nicola Weston-Bell, Laura I Karydis, Chris T Williamson, Georg Lenz, Jeremy D Pettigrew, Curtis Harwig, Freda K Stevenson, Mark S Cragg, Francesco Forconi, Andrew J Steele, Jennifer L Cross, Lloyd F Mackenzie, Pavel Klener, Graham Packham

*These authors contributed equally to this work

Supplementary Methods

Microscopy

CLL samples were treated with soluble goat $F(ab')_2$ anti-IgM for 30 minutes or left untreated as a control. $1x10^6$ cells were fixed and permeabilized with acetone and embedded in 2% (w/v) low melting temperature agarose (Merck). The agarose blocks containing fixed cells were incubated with 4% (v/v) fetal bovine serum, rabbit anti-phosphorylated SHIP1 (Y^{1022}) (Cell Signaling Technology), then donkey anti-rabbit Alexa488 and/or anti-goat Alexa568-conjugated secondary antibodies (Life Technologies). Cells were additionally stained with 4',6-diamidino-2-phenylindole (DAPI; Sigma). Agarose gels were mounted with Mowiol (Sigma) and images collected using either a Leica CTR 5000 microscope coupled to a Leica DFC300FX microscope camera or a Leica CTR6500 microscope coupled to a Leica TCS-SP5 digital confocal system.

Annexin V/propidium iodide staining

Cells ($1x10^6$) were washed in phosphate buffered saline and resuspended in 300 µl of annexin V staining buffer (10 mM HEPES HCl (pH 7.4), 140 mM NaCl, 2.5 mM $CaCl_2$) supplemented with 2.5 µg/ml fluorescein isothiocyanate-labelled annexin V (Protein Core Facility, University of Southampton) and 12.5 µM propidium iodide (Invitrogen). Cells were incubated at room temperature in the dark for 15 minutes before analysis by flow cytometry (Canto II system, BD Biosciences). Unstained cells were used to set gates for identification of annexin V/propidium iodide negative and positive cells.

Characterization of PDX models - whole exome sequencing

PDXs were derived as previously described (1). Primary lymphoma cells were obtained from patients with DLBCL according to the Declaration of Helsinki. The experiment was approved by the Ethics Committee of the General University Hospital Prague under number 63/16.

Germline, parental tumor and PDX-derived DNA were sequenced using a NextSeq 500 (Illumina) instrument according to manufacturer's protocols. Sequencing libraries were prepared using

SureSelectXT Human All Exon V6+UTR kit (Agilent Technologies) or by Atlas Biolabs with libraries prepared using Nimblegen SeqCap ERZ Human Exome Library v 2.0 (Roche Nimblegen) on the HiSeq 2000 (Illumina) instrument.

Sequence reads from PDX samples were first aligned against the mouse reference genome mm10 combined with the human reference genome hg19 and murine reads were filtered out from further analysis by a custom script to reduce risk of contamination. Remaining reads were then aligned against the human reference genome hg19. All alignments were performed by BWA (2). Genomic variants were called with samtools and VarScan 2 (3,4). Variant annotation was performed using SnpEff (5). Only nonsynonymous variants in gene coding regions with coverage of at least 10 reads with mapping quality and base quality higher than 20 in all related samples were compared together based on their frequency. Variants present in the relevant patient's germline DNA at a frequency >0.05 were excluded from analysis in all cases. We compared variants with an allele fraction ≥0.2 in at least one of the compared samples that were present in at least 3 reads in both patient's sample and derived PDX samples. All variant filtering was done in RStudio (http://www.R-project.org; http://www.rstudio.com) and frequencies and counts of variants were plotted using the ggplot2 library (http://ggplot2.org). These variants were then manually reviewed in Integrative Genomics Viewer (http://www.broadinstitute.org/igv) and clear sequencing artefacts or variants present but not called in the germline sample were also excluded. A list of 573 genes of special interest was created based on recent publications of frequently mutated genes in DLBCL samples (6-13) and variants present in these genes were highlighted.

Copy number variants were predicted using CNVkit (14) with normalization to pooled normal samples sequenced on the same instrument using the same library preparation kits. Inferred segmental changes were calculated using the fused lasso method (15) and plotted in diagrams for parental tumor and PDX samples.

Downloadable copies of Supplementary Tables 4-6 and the full list of 573 DLBCL-related genes are available at supplemental-tables and genelist.

Statistics

To evaluate *in vivo* drug responses, we plotted graphs showing mean tumour volumes against time for each model (TMD8 cells and three DLBCL PDX). For the purpose of assessing the statistical significance of treatment effectiveness, we calculated the difference between mean tumor volumes in the control and treatment groups, and between each treatment group, for each time point. For the daily time series of these differences in tumor volumes, statistical hypothesis tests of linear trend slopes equality to zero were performed. The Bonferroni correction was used to smooth the significance level for multiple simultaneous hypothesis tests. We assumed that the calculated differences in mean tumor volumes were generated by a process which includes a deterministic linear trend in the form of

$$y_t = \beta_0 + \beta_1 t + \varepsilon_t$$

where y_t denotes the data-generating stochastic process of the analyzed differences, t = 1, 2, …T is a time variable, T signifies the length of the experiment in days, and ε_t is the Gaussian IID white noise. We performed a total of 21 statistical hypothesis tests about β_1 = 0 across the four experiments and the Bonferroni correction of 10% and 1% simultaneous significance was used, resulting in individual significance levels of 0.4762% and 0.0476%, respectively.

All other statistical comparisons were performed using Prism 6 software (GraphPad Software, La Jolla, CA, USA).

Supplementary Table 1. Details of CLL samples used for *in vitro* studies.

Sample[a]	*IGHV* status[b]	CLL (% cells)[c]	ZAP70 expression[d]	CD38 expression[d]	CD49d expression[d]	sIgM expression[e]	sIgM signal capacity[f]
110F*	U	85	56	91	86	527	49
164B*	M	77	2	18	10	28	1
276D	M	95	0	2	84	784	78
279A/279A*	M	89	1	2	4	28	74
281D/281D*	M	88	1	8	3	7	16
348D/348D*	M	98	1	0	21	53	4
351A	M	94	nd	56	nd	10	6
351B	M	93	nd	90	nd	29	71
368E	M	97	19	85	1	31	36
409C	U	80	21	47	79	112	60
409D	U	81	23	24	75	99	64
409E*	U	84	16	17	75	95	59
452	M	61	nd	8	nd	30	43
452B*	M	89	6	4	40	17	54
470D*	M	88	4	1	6	24	2
475C*	M	91	5	9	3	12	10
482D/482D*	M	94	4	99	52	nd	2
483A	M	98	1	1	18	65	2
489D*	M	93	2	0	10	37	32
495A	M	90	nd	9	nd	58	75
498B	M	79	4	1	18	15	12
500F/500F*	U	89	36	81	2	45	39
501B	U	96	19	90	24	137	52
501C	U	96	35	81	nd	77	52
511A	U	96	36	22	3	32	39
519C*	M	95	0	4	nd	15	61
523C*	M	83	0	0	1	nd	56
523H	M	92	3	2	2	45	39
525B*	M	90	5	0	17	24	19
528A	U	95	60	14	nd	132	69
528B	U	91	56	3	25	151	59
531B*	U	97	6	0	17	50	5
541	M	86	0	0	nd	179	79
549	M	76	1	6	nd	33	22
550B	U	89	2	1	15	130	87
551A*	U	95	21	100	8	129	69
560B	M	84	0	1	14	20	3
567	M	86	73	85	nd	97	46
575B	M	97	2	1	11	87	64
575E	M	95	0	1	65	99	69
580C*	M	83	0	5	5	16	5
585	U	90	39	86	nd	133	39
585A	U	95	28	87	nd	175	8
588A*	M	94	5	7	nd	30	5
588B	M	90	3	11	17	34	10

595A	U	95	3	70	nd	26	10
595D/595D*	U	95	4	77	12	36	20
600	M	94	0	0	nd	13	22
602	U	93	0	36	nd	19	24
604A	M	92	2	0	nd	94	44
604B	M	88	0	1	14	59	73
604E/604E*	M	87	2	0	13	95	84
604G	M	89	0	3	4	81	40
607	U	91	23	90	nd	26	60
609	M	88	0	18	nd	25	57
609C	M	94	0	0	15	53	79
615	M	93	5	8	nd	32	69
628A	U	97	9	24	10	32	14
629B*	U	94	0	14	11	20	19
629C	U	95	2	17	1	12	6
635	U	87	12	1	nd	67	82
635D/635D*	U	95	2	3	63	50	78
636	M	94	0	0	nd	24	2
637	M	83	2	0	nd	25	17
640	M	87	1	0	nd	53	13
643A	M	91	0	0	nd	15	25
643C*	M	88	1	0	13	31	32
644	U	90	46	26	nd	48	31
654	M	85	0	0	ND	21	36
657B*	M	83	0	0	11	18	2
657D	M	90	2	4	4	6	2
661	M	81	7	1	nd	98	73
665	M	85	0	72	nd	468	68
668	U	92	35	0	nd	71	54
668/668B*	U	93	9	1	15	88	42
669	U	91	39	3	nd	36	52
674B/674B*	U	85	38	22	27	295	89
681	M	96	0	6	46	212	60
681A	M	97	2	6	34	185	31
684D	M	94	5	11	10	33	21
686A	M	88	23	18	18	70	78
687C*	U	79	62	5	81	94	59
704	U	99	35	41	22	31	70
706*	M	82	nd	5	60	706	86
707	M	82	0	1	13	32	6
711*	U	80	2	8	17	123	51
715	U	92	4	77	13	145	73
716A	M	86	1	0	25	35	83
716B*	M	88	1	4	4	34	65
727/727*	M	96	0	0	24	52	58
731	U	94	4	5	28	42	19
732	U	91	13	47	34	85	47
735A*	M	83	0	5	3	16	21
739	U	98	1	81	10	99	76
739A*	U	97	1	77	4	83	75

740A	M	81	nd	5	2	11	26
746A	M	83	0	8	10	23	5
747*	U	79	1	7	10	247	43
754A	M	82	16	1	12	20	10
754B/754B*	M	86	9	4	5	14	2
771	U	96	46	38	39	94	73
774A*	U	97	15	5	5	40	25
778*	U	86	32	28	84	27	41
780	U	99	6	86	30	65	81
780A	U	99	2	62	18	81	81
780B	U	98	30	98	49	47	81
791A*	M	87	5	33	4	47	28
794*	M	86	4	4	4	15	2
794A	M	86	8	5	3	23	37
803	U	95	45	25	5	33	30
803B	U	93	55	30	5	38	20
803D	U	nd	nd	nd	nd	nd	nd
805*	U	86	6	3	3	41	49
807	M	93	0	19	4	45	60
816A*	U	80	0	4	93	43	73
818*	U	90	2	91	6	59	69
822*	U	90	57	91	2	64	28
834	M	92	11	97	11	155	70
882*	U	84	56	84	17	96	34
929	U	90	25	75	2	134	58

Diagnosis of CLL was according to the IWCLL-NCI 2008 criteria and the monoclonal B-cell population in the peripheral blood had a typical $IgM^{+}IgD^{+}$ CLL phenotype in all cases. None of the patients had received any (immuno)chemotherapy, steroids, idelalisib or ibrutinib for the 6 months prior to collection. [a]Where suffix is not shown, this is the first sample obtained from that patient, typically obtained shortly after diagnosis. A, B, C etc. indicate subsequent samples from the same patient. The presence or absence of an asterisk indicates whether the sample was used fresh (Figure 1A, Supplementary Figure 1) or following cryopreservation (all other experiments), respectively, or both. [b]*IGHV* mutation status (M, mutated; U, unmutated). [c]Percentage of CLL cells. [d]Percentage of ZAP70, CD49d or CD38 positive cells. [e]Mean fluorescence intensity of IgM positive cells. [f]Maximal percentage of cells with increased intracellular calcium following treatment with soluble anti-IgM (16). nd, not done.

Supplementary Table 2. AQX-435-induced growth inhibition in DLBCL cell lines.

Cell line	Malignancy[a]	IC_{50} (µM)[b]
WSU-DLCL2	GCB-DLBCL	1.66
SU-DHL-4	GCB-DLBCL	2.01
SU-DHL-6	GCB-DLBCL	4.46
SU-DHL-5	GCB-DLBCL	2.16
Toledo	GCB-DLBCL	4.59
OCI-LY19	GCB-DLBCL	2.40
KARPAS-422	GCB-DLBCL	2.57
TMD8	ABC-DLBCL	2.90
U2932	ABC-DLBCL	2.53
HBL1	ABC-DLBCL	2.06
OCI-LY10	ABC-DLBCL	19.1

[a]ABC-DLBCL, activated B cell-like diffuse large B-cell lymphoma; GCB, germinal center B cell-like diffuse large B-cell lymphoma. [b]IC_{50} values were determined using CellTitre Glo (Promega) in 96-well plates after 72 hours of drug exposure and using DMSO-treated cells as controls. Data are means derived from up to 6 individual determinations.

Supplementary Table 3. Features of DLBCL PDX models.

Model	Subtype[a]	Tissue	Features	Patient outcome
VFN-D1	Non-GCB	Lymph node	Derived at first relapse after failure of obinotuzumab-CHOP-based therapy	Patient died of refractory lymphoma
VFN-D2	GCB	Malignant ascites	Derived at second relapse after failure of rituximab-CHOP and RDHAP-based therapies	Patient died of refractory lymphoma
VFN-D3	Non-GCB	Lymph node	Derived from diagnostic sample	Patient achieved complete response with R-CHOP and is currently alive

[a]Assigned as germinal centre B cell (GCB)-like or non-GCB-like according to the Hans algorithm (17).

Supplementary Table 4. A complete list of protein coding variants which passed the filtering described in supplementary methods, that were found in both the parental tumor samples and the derived PDXs. Variants in genes of special interest based on a list of frequently mutated genes are highlighted in red.

VFN-D1

Chr[a]	Position (hg19)	REF[b]	ALT[c]	Genesymbol	AA change[d]	DNA change[e]	Transcript	Patient AF[f]	Patient Depth[g]	PDX AF[f]	PDX Depth[g]
chr1	156518447	C	G	IQGAP3	p.Arg640Pro	c.1919G>C	NM_178229.4	0.355556	90	0.350877	57
chr1	203274876	G	A	BTG2	p.Glu48Lys	c.142G>A	NM_006763.2	0.4	20	0.478261	23
chr1	231339708	G	A	TRIM67	p.Val544Met	c.1630G>A	NM_001004342.3	0.586667	75	0.554455	101
chr1	37948805	C	T	ZC3H12A	p.Arg465*	c.1393C>T	NM_025079.2	0.333333	18	0.444444	18
chr10	45869806	G	A	ALOX5	p.Val27Met	c.79G>A	NM_000698.3	0.415094	53	0.467742	62
chr10	99160983	AC	A	RRP12	p.Gly2fs	c.5delG	NM_001145114.1	0.5	34	0.439024	41
chr11	30033718	G	A	KCNA4	p.Arg170Cys	c.508C>T	NM_002233.3	0.37931	58	0.4	80
chr11	58979015	G	A	MPEG1	p.Leu442Phe	c.1324C>T	NM_001039396.1	0.322581	93	0.421053	95
chr11	67926195	G	A	SUV420H1	p.Arg368Trp	c.1102C>T	NM_001300907.1	0.385965	57	0.478873	71
chr11	71293674	A	T	KRTAP5-11	p.Cys70*	c.210T>A	NM_001005405.2	0.428571	91	0.516854	89
chr12	49422914	TGAGCCCAGATGAGGGAAAC	T	KMT2D	p.Arg4721fs	c.14162_14180delGTTTCCCTCATCTGGGCTC	NM_003482.3	0.333333	75	0.216495	97
chr12	49426919	G	A	KMT2D	p.Gln3857*	c.11569C>T	NM_003482.3	0.376623	77	0.425	80
chr14	91883151	C	T	CCDC88C	p.Ser31Asn	c.92G>A	NM_001080414.3	0.75	48	0.842105	38
chr14	94517703	T	A	DDX24	p.Gln805Leu	c.2414A>T	NM_020414.3	0.411765	119	0.523077	130
chr15	45007641	T	A	B2M	p.Tyr30Asn	c.88T>A	NM_004048.2	0.821429	56	0.927273	55
chr15	51839591	C	A	DMXL2	p.Leu194Phe	c.582G>T	NM_001174116.1	0.724138	29	0.85	20
chr15	93486192	T	TA	CHD2	p.Tyr316_Ile317fs	c.947_948insA	NM_001042572.2	0.397959	98	0.449541	109
chr16	2813048	C	A	SRRM2	p.Pro840His	c.2519C>A	NM_016333.3	0.404255	47	0.648148	54
chr16	77325368	G	T	ADAMTS18	p.Ala1066Glu	c.3197C>A	NM_199355.2	0.306452	62	0.352113	71
chr16	81819656	C	T	PLCG2	p.Ala21Val	c.62C>T	NM_002661.4	0.379747	79	0.557143	70
chr17	40475329	T	A	STAT3	p.Asp566Val	c.1697A>T	NM_003150.3	0.348148	135	0.402516	159

chr17	42285113	C	T	UBTF	p.Arg623Gln	c.1868G>A	NM_001076683.1	0.393939	66	0.436364	110
chr17	80046084	G	A	FASN	p.Thr898Met	c.2693C>T	NM_004104.4	0.372093	86	0.494845	97
chr2	125547527	T	A	CNTNAP5	p.Leu933*	c.2798T>A	NM_130773.3	0.433333	30	0.395349	43
chr2	182358063	G	A	ITGA4	p.Gly389Arg	c.1165G>A	NM_000885.4	0.333333	72	0.462687	67
chr2	242707170	C	T	D2HGDH	p.Pro317Leu	c.950C>T	NM_001287249.1	0.333333	60	0.361702	47
chr2	26703760	C	T	OTOF	p.Arg566Gln	c.1697G>A	NM_001287489.1	0.523077	65	0.416667	48
chr2	37283727	GC	G	HEATR5B	p.Ala752fs	c.2254delG	NM_019024.1	0.956522	23	0.8125	16
chr2	46770261	C	T	RHOQ	p.Leu26Phe	c.76C>T	NM_012249.3	0.404762	42	0.407407	27
chr20	35060849	G	A	DLGAP4	p.Met243Ile	c.729G>A	NM_014902.5	0.368421	19	0.210526	19
chr21	35147383	G	A	ITSN1	p.Glu523Lys	c.1567G>A	NM_001001132.1	0.307692	52	0.42029	69
chr22	26688908	G	A	SEZ6L	p.Val211Met	c.631G>A	NM_001184773.1	0.363636	22	0.466667	15
chr3	49412923	A	C	RHOA	p.Tyr34Asp	c.100T>G	NM_001664.2	0.354545	110	0.459677	124
chr4	126373626	C	T	FAT4	p.Arg3821*	c.11461C>T	NM_001291285.1	0.362069	58	0.397059	68
chr4	134084339	C	G	PCDH10	p.Thr1002Ser	c.3005C>G	NM_032961.1	0.313725	51	0.5	56
chr4	159091444	C	A	FAM198B	p.Trp322Cys	c.966G>T	NM_001031700.2	0.470588	68	0.44186	86
chr5	16877734	C	T	MYO10	p.Arg35Gln	c.104G>A	NM_012334.2	0.366972	109	0.432836	134
chr6	161160209	C	T	PLG	p.Arg663*	c.1987C>T	NM_000301.3	0.375635	197	0.365854	205
chr6	26093137	A	G	HFE	p.Thr281Ala	c.841A>G	NM_000410.3	0.836066	61	0.913043	69
chr6	37138928	G	A	PIM1	p.Val181Met	c.541G>A	NM_001243186.1	0.37931	29	0.625	32
chr6	37139203	G	C	PIM1	p.Glu272Asp	c.816G>C	NM_001243186.1	0.37931	29	0.5	46
chr6	37140818	C	T	PIM1	p.Tyr309Tyr	c.927C>T	NM_001243186.1	0.45	120	0.452991	117
chr6_mcf_hap5	2703745	G	A	HLA-B	p.Gln204*	c.610C>T	NM_005514.6.5	0.909091	11	0.944444	18
chr6_qbl_hap6	2618175	C	T	HLA-B	p.Gly50Asp	c.149G>A	NM_005514.6.2	0.8	25	0.947368	38
chr7	142565462	C	T	EPHB6	p.Ala324Val	c.971C>T	NM_001280794.2	0.428571	49	0.5	46
chr8	140715058	G	A	KCNK9	p.Arg60Trp	c.178C>T	NM_001282534.1	0.235849	106	0.269231	104
chr8	143569788	G	A	BAI1	p.Trp791*	c.2372G>A	NM_001702.2	0.558442	77	0.602273	88
chr9	113194299	G	A	SVEP1	p.Ala1750Val	c.5249C>T	NM_153366.3	0.463636	110	0.473684	114
chr9	113194865	C	T	SVEP1	p.Asp1704Asn	c.5110G>A	NM_153366.3	0.222222	135	0.426829	164

chr9	138379846	G	C	PPP1R26	p.Glu1164Gln	c.3490G>C	NM_014811.3	0.522388	67	0.430769	65
chr9	139793328	C	G	TRAF2	p.Gln46Glu	c.136C>G	NM_021138.3	0.36	50	0.522388	67
chr9	35310565	G	A	UNC13B	p.Arg288Gln	c.863G>A	NM_006377.3	0.464286	56	0.409091	66
chr9	39099925	C	A	CNTNAP3	p.Gly993Val	c.2978G>T	NM_033655.3	0.462264	106	0.503546	141
chrX	24383078	C	T	SUPT20HL1	p.Thr734Met	c.2201C>T	NM_001136234.1	0.783784	37	0.857143	42
chrX	86087129	G	A	DACH2	p.Glu424Lys	c.1270G>A	NM_001139515.1	0.854545	55	0.966102	59
chr11	58979177	G	A	MPEG1	p.Leu388Phe	c.1162C>T	NM_001039396.1	0.353535	99	0.45045	111
chr20	62571739	C	T	UCKL1	p.Val453Met	c.1357G>A	NM_001193379.1	0.451613	155	0.425287	174
chr6	37139025	G	A	PIM1	p.Arg213Lys	c.638G>A	NM_001243186.1	0.5	36	0.363636	22
chr6	37139033	C	T	PIM1	p.Pro216Ser	c.646C>T	NM_001243186.1	0.483871	31	0.5	28
chr6	37139063	G	A	PIM1	p.Glu226Lys	c.676G>A	NM_001243186.1	0.486486	37	0.324324	37
chr7	112724299	C	T	GPR85	p.Val160Met	c.478G>A	NM_001146265.1	0.339623	53	0.58	50
chr1	206858812	C	T	MAPKAPK2	p.Gln80*	c.238C>T	NM_004759.4	0.136364	22	0.357143	14
chr12	11803087	G	A	ETV6	p.Ser9Asn	c.26G>A	NM_001987.4	0.438596	57	0.543478	46
chr12	11803094	G	C	ETV6	p.Lys11Asn	c.33G>C	NM_001987.4	0.482143	56	0.533333	45
chr12	92539304	G	A	BTG1	p.Pro3Leu	c.8C>T	NM_001731.2	0.290323	31	0.125	32
chr2	96810609	C	T	DUSP2	p.Gly134Asp	c.401G>A	NM_004418.3	0.34375	32	0.212121	33
chr4	115997291	T	C	NDST4	p.Asp301Gly	c.902A>G	NM_022569.1	0.0559441	143	0.284848	165
chr5	141059554	C	T	ARAP3	p.Gly167Asp	c.500G>A	NM_022481.5	0.371429	35	0.380952	42
chr6	37138549	G	A	PIM1	p.Gly119Asp	c.356G>A	NM_001243186.1	0.241379	29	0.444444	27
chr6	37138775	G	A	PIM1	p.Glu161Lys	c.481G>A	NM_001243186.1	0.425	40	0.441176	34
chr9	130507352	C	T	SH2D3C	p.Ala77Thr	c.229G>A	NM_001142531.1	0.6	15	0.571429	21

17
18

VFN-D2

Chr[a]	Position (hg19)	REF[b]	ALT[c]	Genesymbol	AA change[d]	DNA change[e]	Transcript	Patient AF[f]	Patient Depth[g]	PDX AF[f]	PDX Depth[g]

chr1	10725422	G	A	CASZ1	p.Arg75Trp	c.223C>T	NM_001079843.2	0.525	80	0.485714	35
chr10	100152196	G	A	PYROXD2	p.Thr352Met	c.1055C>T	NM_032709.2	0.459459	185	0.185185	108
chr12	21327603	G	A	SLCO1B1	p.Gly107Arg	c.319G>A	NM_006446.4	0.441718	163	0.335878	131
chr14	23898487	C	A	MYH7	p.Arg403Leu	c.1208G>T	NM_000257.3	0.436047	172	0.411765	85
chr14	35231301	G	GTGCTC	BAZ1A	p.Thr1302_Pro1303fs	c.3904_3905insGAGCA	NM_013448.2	0.308989	178	0.327044	159
chr5	16681558	T	C	MYO10	p.Lys1415Arg	c.4244A>G	NM_012334.2	0.518248	137	0.516129	124
chr5	67591097	A	G	PIK3R1	p.Asn201Asp	c.601A>G	NM_001242466.1	0.385185	135	0.45283	159
chr8	11687883	C	T	FDFT1	p.Ser278Leu	c.833C>T	NM_001287742.1	0.418033	122	0.413333	75
chr8	3224671	T	C	CSMD1	p.Thr1000Ala	c.2998A>G	NM_033225.5	0.384615	117	0.39726	73
chr1	196963350	C	T	CFHR5	p.Gln191*	c.571C>T	NM_030787.3	0.354839	62	0.674419	43
chr1	23885724	C	CTA	ID3	p.Ser65_Gln66fs	c.193_194insTA	NM_002167.4	0.421053	76	0.419355	31
chr1	94654488	A	G	ARHGAP29	p.Phe529Ser	c.1586T>C	NM_004815.3	0.459459	37	0.489796	49
chr10	5415963	C	T	UCN3	p.Arg94Trp	c.280C>T	NM_053049.2	0.478723	94	0.347826	23
chr10	89725228	G	T	PTEN	p.Ter404Leuext*?	c.1211G>T	NM_000314.4	0.448276	29	0.636364	33
chr11	100814584	T	C	ARHGAP42	p.Ile343Thr	c.1028T>C	NM_152432.2	0.385542	83	0.579545	88
chr12	40153970	A	G	SLC2A13	p.Leu602Ser	c.1805T>C	NM_052885.3	0.573034	89	0.54878	82
chr12	40672017	G	A	LRRK2	p.Gly732Glu	c.2195G>A	NM_198578.3	0.489583	96	0.385827	127
chr12	86373447	C	T	MGAT4C	p.Glu353Lys	c.1057G>A	NM_013244.3	0.551724	87	0.460674	89
chr13	38160321	G	A	POSTN	p.Arg284*	c.850C>T	NM_001135934.1	0.398305	118	0.288889	90
chr15	75684864	A	ACACCATT	SIN3A	p.Val857_Gly858fs	c.2569_2570insAATGGTG	NM_001145357.1	0.31677	161	0.291667	144
chr17	27002001	G	A	SUPT6H	p.Arg120His	c.359G>A	NM_003170.3	0.419847	131	0.454545	77
chr17	74942491	G	A	MGAT5B	p.Glu628Lys	c.1882G>A	NM_001199172.1	0.478992	119	0.466667	75
chr17	7578393	A	C	TP53	p.His179Gln	c.537T>G	NM_000546.5	0.461538	182	0.488636	88
chr19	15131469	C	T	CCDC105	p.Pro291Leu	c.872C>T	NM_173482.2	0.528302	53	0.428571	21
chr2	170048414	T	G	LRP2	p.Asn2987Thr	c.8960A>C	NM_004525.2	0.514706	68	0.325	80
chr2	202131210	A	G	CASP8	p.Met1?	c.1A>G	NM_001080124.1	0.85	40	0.928571	28
chr2	223917774	G	A	KCNE4	p.Glu127Lys	c.379G>A	NM_080671.3	0.377358	53	0.371429	35
chr2	74328555	A	G	TET3	p.Lys1547Arg	c.4640A>G	NM_001287491.1	0.484375	64	0.466667	30

chr20	55840962	G	A	BMP7	p.Gln73*	c.217C>T	NM_001719.2	0.517241	145	0.488372	43
chr20	55841018	A	G	BMP7	p.Met54Thr	c.161T>C	NM_001719.2	0.55102	98	0.590909	22
chr3	123010175	G	A	ADCY5	p.Arg688Trp	c.2062C>T	NM_001199642.1	0.493506	77	0.431034	58
chr3	74411062	A	G	CNTN3	p.Val448Ala	c.1343T>C	NM_020872.1	0.416667	72	0.438356	73
chr4	5830235	C	T	CRMP1	p.Arg595His	c.1784G>A	NM_001014809.2	0.354167	192	0.147727	88
chr4	94547585	AG	A	GRID2	p.Arg692fs	c.2075delG	NM_001286838.1	0.544776	134	0.142857	98
chr5	140432524	C	T	PCDHB1	p.Ser490Phe	c.1469C>T	NM_013340.3	0.36	75	0.522727	88
chr5	61688763	CAG	C	DIMT1	p.Ser239fs	c.716_717delCT	NM_014473.2	0.434483	145	0.373684	190
chr5	94878980	C	T	TTC37	p.Val48Ile	c.142G>A	NM_014639.3	0.428571	56	0.378378	74
chr6	31324143	G	T	HLA-B	p.Tyr140*	c.420C>A	NM_005514.6.3	0.944444	36	1	25
chr7	99359680	T	A	CYP3A4	p.Lys412*	c.1234A>T	NM_001202855.2	0.43554	287	0.463878	263
chr9	117552800	C	T	TNFSF15	p.Val171Ile	c.511G>A	NM_001204344.1	0.459016	183	0.287958	191
chr9	123852647	C	A	CNTRL	p.Asn104Lys	c.312C>A	NM_007018.4	0.375	56	0.506667	75
chr9	126139119	G	T	CRB2	p.Lys1212Asn	c.3636G>T	NM_173689.6	0.509091	55	0.625	24
chr9	126139126	C	G	CRB2	p.Pro1215Ala	c.3643C>G	NM_173689.6	0.480769	52	0.714286	28
chr9	137982149	C	T	OLFM1	p.Arg87Trp	c.259C>T	NM_001282611.1	0.382857	175	0.333333	105
chrX	70783295	GATTCAA	G	OGT	p.Ile795_Gln796del	c.2383_2388delATTCAA	NM_181672.2	0.782609	46	0.769231	52

19
20

VFN-D3

Chr[a]	Position (hg19)	REF[b]	ALT[c]	Genesymbol	AA change[d]	DNA change[e]	Transcript	Patient AF[f]	Patient Depth[g]	PDX AF[f]	PDX Depth[g]
chr14	96180289	T	G	TCL1A	p.Ile39Leu	c.115A>C	NM_001098725.1	0.446154	65	0.538462	143
chr1	203274817	G	C	BTG2	p.Gly28Ala	c.83G>C	NM_006763.2	0.125	48	0.365385	104
chr1	223568661	G	A	CCDC185	p.Arg615His	c.1844G>A	NM_152610.2	0.847826	46	0.495146	103
chr1	41945111	C	T	EDN2	p.Arg169Gln	c.506G>A	NM_001956.3	0.25	48	0.423077	130
chr1	43637288	C	T	EBNA1BP2	p.Arg117Gln	c.350G>A	NM_001159936.1	0.32	25	0.442308	52

chr10	135044253	G	A	UTF1	p.Arg154Gln	c.461G>A	NM_003577.2	0.425	40	0.398058	103
chr10	6521018	C	G	PRKCQ	p.Arg430Thr	c.1289G>C	NM_001242413.2	0.555556	27	0.372549	51
chr10	95326613	G	A	FFAR4	p.Val46Met	c.136G>A	NM_001195755.1	0.586207	58	0.56962	79
chr10	99342401	G	A	ANKRD2	p.Arg259His	c.776G>A	NM_001129981.2	0.392857	56	0.375	152
chr11	102272772	G	A	TMEM123	p.Thr108Ile	c.323C>T	NM_052932.2	0.347826	46	0.435484	62
chr11	61547372	T	A	MYRF	p.Ile769Asn	c.2306T>A	NM_001127392.1	0.392857	56	0.578947	76
chr11	62569088	G	A	NXF1	p.Arg219*	c.655C>T	NM_001081491.1	0.276596	47	0.522936	109
chr11	70331881	G	A	SHANK2	p.Thr918Met	c.2753C>T	NM_133266.4	0.458333	72	0.564103	117
chr11	76831837	G	A	CAPN5	p.Val457Ile	c.1369G>A	NM_004055.4	0.241379	58	0.357724	123
chr12	108006584	G	A	BTBD11	p.Val150Ile	c.448G>A	NM_001017523.1	0.377049	61	0.464286	112
chr12	32729320	C	T	FGD4	p.Ser10Phe	c.29C>T	NM_139241.2	0.25	20	0.510638	47
chr12	65260552	G	A	TBC1D30	p.Ser387Asn	c.1160G>A	NM_015279.1	0.294118	17	0.431818	44
chr13	113173350	GGA	G	TUBGCP3	p.Ser619fs	c.1857_1858delTC	NM_001286277.1	0.25	16	0.590909	22
chr13	38143462	T	G	POSTN	p.Glu745Asp	c.2235A>C	NM_001135934.1	0.157895	19	0.375	32
chr13	43798262	G	A	ENOX1	p.Thr576Met	c.1727C>T	NM_001127615.1	0.52381	21	0.442308	52
chr14	20841852	T	A	TEP1	p.Met2197Leu	c.6589A>T	NM_007110.4	0.484848	33	0.477612	67
chr14	20841854	G	C	TEP1	p.Ala2196Gly	c.6587C>G	NM_007110.4	0.516129	31	0.469697	66
chr14	35482640	T	G	SRP54	p.Val193Gly	c.578T>G	NM_001146282.1	0.568966	58	0.544444	90
chr14	55511044	A	AG	SOCS4	p.Arg429_Val430fs	c.1286_1287insG	NM_080867.2	0.333333	18	0.538462	39
chr14	86089014	C	T	FLRT2	p.Leu386Phe	c.1156C>T	NM_013231.4	0.416667	36	0.361702	47
chr15	24921172	G	T	NPAP1	p.Arg53Leu	c.158G>T	NM_018958.2	0.536585	41	0.422222	90
chr15	24921658	A	C	NPAP1	p.Lys215Thr	c.644A>C	NM_018958.2	0.372093	86	0.489796	196
chr15	34137113	G	A	RYR3	p.Trp4449*	c.13347G>A	NM_001036.3	0.314815	54	0.418367	98
chr15	42278110	G	A	PLA2G4E	p.Pro710Leu	c.2129C>T	NM_001206670.1	0.454545	44	0.467532	77
chr15	75015147	G	A	CYP1A1	p.Arg98Trp	c.292C>T	NM_000499.3	0.320755	53	0.396552	116
chr16	55735796	C	A	SLC6A2	p.Pro594Thr	c.1780C>A	NM_001043.3	0.263158	19	0.367647	68
chr16	71007800	T	C	HYDIN	p.Ile1721Val	c.5161A>G	NM_001270974.1	0.184211	76	0.207547	159
chr17	10402365	G	A	MYH1	p.Gln1304*	c.3910C>T	NM_005963.3	0.238095	21	0.588235	51

chr17	11865224	A	G	DNAH9	p.Asn4295Ser	c.12884A>G	NM_001372.3	0.384615	78	0.401235	162
chr17	4578596	A	G	PELP1	p.Leu230Ser	c.689T>C	NM_001278241.1	0.416667	24	0.542373	59
chr17	72477892	G	A	CD300A	p.Ala119Thr	c.355G>A	NM_001256841.1	0.393939	33	0.333333	60
chr17	8702423	G	A	MFSD6L	p.Arg6Trp	c.16C>T	NM_152599.3	0.354839	31	0.396226	106
chr18	44030275	G	A	RNF165	p.Arg19Gln	c.56G>A	NM_001256758.1	0.232558	43	0.378947	95
chr18	59157866	A	G	CDH20	p.Asp27Gly	c.80A>G	NM_031891.2	0.27551	98	0.386243	189
chr18	72344362	C	T	ZNF407	p.His463Tyr	c.1387C>T	NM_001146189.1	0.297872	47	0.328571	70
chr19	13248324	T	G	NACC1	p.Ile420Ser	c.1259T>G	NM_052876.3	0.176471	17	0.414634	41
chr19	16942313	C	T	SIN3B	p.Thr79Ile	c.236C>T	NM_001297595.1	0.3125	16	0.551724	29
chr19	18960969	T	A	UPF1	p.Cys183Ser	c.547T>A	NM_001297549.1	0.4375	32	0.537313	67
chr19	2129444	G	C	AP3D1	p.Ala202Gly	c.605C>G	NM_001261826.1	0.365079	63	0.3125	112
chr19	21606258	A	T	ZNF493	p.Lys266Ile	c.797A>T	NM_001076678.2	0.555556	18	0.5	24
chr19	36352753	G	A	KIRREL2	p.Arg446Gln	c.1337G>A	NM_032123.6	0.666667	18	0.54386	57
chr19	47282088	C	T	SLC1A5	p.Arg73His	c.218G>A	NM_001145144.1	0.421053	38	0.367647	68
chr19	5653425	G	A	SAFB	p.Ser507Asn	c.1520G>A	NM_001201338.1	0.409091	22	0.314815	54
chr2	198285841	G	A	SF3B1	p.Ser71Leu	c.212C>T	NM_001005526.1	0.392857	28	0.367347	49
chr2	209036721	G	A	C2orf80	p.Arg149Cys	c.445C>T	NM_001099334.2	0.458333	24	0.387097	62
chr2	27249157	G	A	MAPRE3	p.Ala263Thr	c.787G>A	NM_012326.2	0.162791	43	0.282828	99
chr2	84955003	C	T	DNAH6	p.Arg3395*	c.10183C>T	NM_001370.1	0.466667	30	0.22807	57
chr2	98737829	G	T	VWA3B	p.Ala204Ser	c.610G>T	NM_144992.4	0.380952	42	0.463768	69
chr20	44236765	G	A	WFDC9	p.Ser85Leu	c.254C>T	NM_147198.3	0.473684	57	0.5	66
chr20	62737878	C	T	NPBWR2	p.Ala103Thr	c.307G>A	NM_005286.2	0.368421	76	0.474747	198
chr21	31768574	A	G	KRTAP13-1	p.Gln57Arg	c.170A>G	NM_181599.2	0.404255	47	0.527778	108
chr22	20103993	T	C	TRMT2A	p.Gln56Arg	c.167A>G	NM_001257994.1	0.38	50	0.515789	95
chr22	23230404	A	C	IGLL5	p.Glu22Ala	c.65A>C	NM_001256296.1	0.9	10	0.933333	45
chr3	15126539	T	C	ZFYVE20	p.Asp135Gly	c.404A>G	NM_022340.2	0.65625	32	0.411765	34
chr3	164908039	T	G	SLITRK3	p.Lys194Gln	c.580A>C	NM_014926.2	0.179487	39	0.292308	65
chr3	173997143	C	T	NLGN1	p.Thr451Ile	c.1352C>T	NM_014932.3	0.304348	69	0.255556	90

chr3	35729256	GT	G	ARPP21	p.Ser96fs	c.288delT	NM_001267617.1	0.538462	13	0.388889	18
chr3	37163140	G	T	LRRFIP2	p.His74Asn	c.220C>A	NM_001282691.1	0.133333	30	0.477273	44
chr3	38182641	T	C	MYD88	p.Ter160Argext*?	c.478T>C	NM_001172566.1	0.37037	27	0.55	60
chr4	126370555	C	T	FAT4	p.Thr2797Ile	c.8390C>T	NM_001291285.1	0.25	24	0.425	40
chr4	20717844	C	A	PACRGL	p.Gln189Lys	c.565C>A	NM_001258346.1	0.631579	19	0.4	30
chr4	44176914	C	A	KCTD8	p.Glu439*	c.1315G>T	NM_198353.2	0.468085	47	0.584906	53
chr4	85742333	C	T	WDFY3	p.Val499Met	c.1495G>A	NM_014991.4	0.285714	28	0.428571	56
chr5	176016578	C	T	CDHR2	p.Pro1056Leu	c.3167C>T	NM_001171976.1	0.367647	68	0.411392	158
chr5	57913591	G	A	RAB3C	p.Arg49His	c.146G>A	NM_138453.2	0.416667	24	0.5	34
chr6	110056434	G	T	FIG4	p.Met193Ile	c.579G>T	NM_014845.5	0.785714	14	1	18
chr6_cox_hap2	2836811	A	T	HLA-B	p.Tyr147Asn	c.439T>A	NM_005514.6.4	0.763158	38	0.963303	109
chr6_cox_hap2	2836836	G	C	HLA-B	p.Asn138Lys	c.414C>G	NM_005514.6.4	0.790698	43	0.978417	139
chr6_cox_hap2	2836882	T	C	HLA-B	p.Tyr123Cys	c.368A>G	NM_005514.6.4	0.911111	45	0.956897	116
chr6_cox_hap2	2836897	G	T	HLA-B	p.Thr118Asn	c.353C>A	NM_005514.6.4	0.741935	31	0.884058	69
chr7	142881493	C	T	TAS2R39	p.Arg328*	c.982C>T	NM_176881.2	0.428571	56	0.45045	111
chr7	146829418	C	T	CNTNAP2	p.Arg389Trp	c.1165C>T	NM_014141.5	0.416667	36	0.407895	76
chr7	15725797	ATGG	A	MEOX2	p.His76_His77del	c.228_230delCCA	NM_005924.4	0.466667	15	0.190476	42
chr7	6193773	C	G	USP42	p.Ser863Trp	c.2588C>G	NM_032172.2	0.54386	57	0.467742	124
chr7	95750622	C	A	SLC25A13	p.Val638Phe	c.1912G>T	NM_001160210.1	0.268293	41	0.405063	79
chr8	144993545	G	A	PLEC	p.Arg3509Cys	c.10525C>T	NM_000445.4	0.454545	44	0.47619	126
chr8	25708195	T	G	EBF2	p.Lys537Asn	c.1611A>C	NM_022659.3	0.269231	52	0.382022	89
chr8	97318780	G	A	PTDSS1	p.Val189Met	c.565G>A	NM_001290225.1	0.454545	44	0.451613	62
chr9	13107023	C	T	MPDZ	p.Glu2019Lys	c.6055G>A	NM_001261406.1	0.473684	38	0.480769	52
chrX	133551199	A	G	PHF6	p.Lys279Glu	c.835A>G	NM_001015877.1	0.4375	16	0.384615	26
chrX	135432145	G	A	GPR112	p.Val2094Ile	c.6280G>A	NM_153834.3	0.285714	21	0.253968	63
chrX	35820689	C	T	MAGEB16	p.His126Tyr	c.376C>T	NM_001099921.1	0.306122	49	0.537736	106
chrX	41007811	G	C	USP9X	p.Asp537His	c.1609G>C	NM_001039590.2	0.351852	54	0.444444	99
chrX	67942430	G	A	STARD8	p.Arg914His	c.2741G>A	NM_001142503.2	0.340659	91	0.4	135

chrX	90691016	A	AT	PABPC5	p.Tyr147_Ala148fs	c.441_442insT	NM_080832.2	0.5	44	0.48	75

[a]Chromosome; [b]Reference allele; [c]Alternative allele; [d]Amino-acid change; [e]DNA sequence change; [f]Allele frequency in patient's sample/PDX; [g]Read depth in patient's sample/PDX.

Supplementary Table 5. A complete list of protein coding variants which passed the filtering described in supplementary methods and were found only in the derived PDX models and not the parental tumor samples. Variants in genes of special interest based on a list of frequently mutated genes are highlighted in red.

VFN-D1

Chr[a]	Position (hg19)	REF[b]	ALT[c]	Genesymbol	AA change[d]	DNA change[e]	Transcript	Patient AF[f]	Patient Depth[g]	PDX AF[f]	PDX Depth[g]
chr15	23889151	G	T	MAGEL2	p.Pro1247Thr	c.3739C>A	NM_019066.4	0	0	0.2	30
chr11	125893317	G	T	CDON	p.Leu19Met	c.55C>A	NM_001243597.1	0.0263158	76	0.20635	63
chr13	110831303	G	T	COL4A1	p.Pro809Thr	c.2425C>A	NM_001845.4	0	0	0.23529	17
chr16	58545362	G	T	NDRG4	p.Arg353Leu	c.1058G>T	NM_001130487.1	0	0	0.2	25
chr20	4202658	G	T	ADRA1D	p.Arg411Ser	c.1231C>A	NM_000678.3	0	0	0.2	15
chr6	26020763	G	T	HIST1H3A	p.Ala16Ser	c.46G>T	NM_003529.2	0	0	0.25	40
chr1	41094490	C	A	RIMS3	p.Val236Leu	c.706G>T	NM_014747.2	0.0491803	122	0.29268	123
chr16	47488041	C	A	ITFG1	p.Val104Phe	c.310G>T	NM_030790.3	0.0487805	82	0.32099	81
chr17	57915712	C	T	VMP1	p.Ala344Val	c.1031C>T	NM_030938.3	0.015873	63	0.20588	68
chr19	12902605	AG	A	JUNB	p.Gln7fs	c.21delG	NM_002229.2	0.0454545	44	0.23684	38
chr2	163057091	C	T	FAP	p.Arg396Lys	c.1187G>A	NM_001291807.1	0.0178571	56	0.375	56
chr3	49711998	G	T	APEH	p.Cys30Phe	c.89G>T	NM_001640.3	0.0416667	24	0.21053	19
chr3	52518558	G	T	NISCH	p.Ala520Ser	c.1558G>T	NM_007184.3	0.0416667	24	0.2	20
chr6	37140782	G	A	PIM1	p.Val297Val	c.891G>A	NM_001243186.1	0.0210526	95	0.2	100
chr6	44143859	C	G	CAPN11	p.His296Asp	c.886C>G	NM_007058.3	0.0104167	96	0.26582	79
chrX	41203018	G	T	DDX3X	p.Leu236Phe	c.708G>T	NM_001193416.1	0.047619	42	0.23529	34
chr1	156697310	G	T	ISG20L2	p.Asn45Lys	c.135C>A	NM_030980.1	0	0	0.21429	28
chr1	167691425	G	T	MPZL1	p.Ala13Ser	c.37G>T	NM_001146191.1	0	0	0.33333	18
chr1	167691455	G	T	MPZL1	p.Val23Leu	c.67G>T	NM_001146191.1	0	0	0.2	20
chr1	171553578	G	T	PRRC2C	p.Gln2552His	c.7656G>T	NM_015172.3	0	0	0.21429	14

chr1	200842802	C	A	GPR25	p.Leu213Met	c.637C>A	NM_005298.3	0	0	0.23529	17
chr10	17271471	G	T	VIM	p.Gly17Val	c.50G>T	NM_003380.3	0	0	0.2	20
chr11	116661258	C	A	APOA5	p.Gln229His	c.687G>T	NM_001166598.1	0	0	0.3	10
chr11	13031458	G	T	RASSF10	p.Arg112Leu	c.335G>T	NM_001080521.2	0	0	0.30769	13
chr12	107713780	G	T	BTBD11	p.Val355Phe	c.1063G>T	NM_001018072.1	0	0	0.25	16
chr12	133084936	G	T	FBRSL1	p.Gln163His	c.489G>T	NM_001142641.1	0	0	0.2	15
chr14	103592962	G	T	TNFAIP2	p.Gln56His	c.168G>T	NM_006291.2	0	0	0.22222	18
chr15	41611938	C	A	OIP5	p.Val144Phe	c.430G>T	NM_007280.1	0	0	0.2	20
chr15	41793745	G	T	ITPKA	p.Ala192Ser	c.574G>T	NM_002220.2	0	0	0.25	12
chr15	43501575	G	T	EPB42	p.Asn273Lys	c.819C>A	NM_000119.2	0	0	0.26667	15
chr15	54306082	G	T	UNC13C	p.Glu328*	c.982G>T	NM_001080534.1	0	0	0.21429	14
chr16	30751032	G	T	SRCAP	p.Arg3224Ile	c.9671G>T	NM_006662.2	0	0	0.22222	18
chr17	46620582	G	T	HOXB2	p.Leu307Ile	c.919C>A	NM_002145.3	0	0	0.2	20
chr17	74236295	G	T	RNF157	p.His9Gln	c.27C>A	NM_052916.2	0	0	0.23077	13
chr17	7751798	C	A	KDM6B	p.Ser731Tyr	c.2192C>A	NM_001080424.1	0	0	0.35714	14
chr19	49000772	G	T	LMTK3	p.Ala1214Asp	c.3641C>A	NM_001080434.1	0	0	0.26316	19
chr19	55944541	C	A	SHISA7	p.Lys533Asn	c.1599G>T	NM_001145176.1	0	0	0.2	15
chr2	239147819	G	T	HES6	p.His106Gln	c.318C>A	NM_001142853.2	0	0	0.23529	17
chr2	47596684	G	T	EPCAM	p.Ala14Ser	c.40G>T	NM_002354.2	0	0	0.22222	18
chr3	128720805	G	T	EFCC1	p.Asp112Tyr	c.334G>T	NM_024768.2	0	0	0.28571	14
chr3	49724399	G	T	MST1	p.His237Asn	c.709C>A	NM_020998.3	0	0	0.38462	13
chr3	49724403	G	T	MST1	p.His235Gln	c.705C>A	NM_020998.3	0	0	0.30769	13
chr5	132159114	G	T	SHROOM1	p.Ala685Asp	c.2054C>A	NM_001172700.1	0	0	0.21053	19
chr5	148753957	G	T	IL17B	p.Ala173Asp	c.518C>A	NM_014443.2	0	0	0.25	20
chr5	176759209	G	T	LMAN2	p.Pro317Thr	c.949C>A	NM_006816.2	0	0	0.23077	13
chr5	176830879	C	T	F12	p.Ala411Thr	c.1231G>A	NM_000505.3	0	0	0.21429	14
chr6	106960878	G	T	AIM1	p.Gly221Val	c.662G>T	NM_001624.2	0	0	0.27273	11
chr6	36098407	G	T	MAPK13	p.Lys16Asn	c.48G>T	NM_002754.4	0	0	0.2	15

chr6	37138563	C	T	PIM1	p.Pro124Ser	c.370C>T	NM_001243186.1	0	0	0.23529	34
chr7	150761849	G	T	SLC4A2	p.Val152Leu	c.454G>T	NM_001199692.1	0	0	0.25714	35
chr8	55370994	G	T	SOX17	p.Ser99Ile	c.296G>T	NM_022454.3	0	0	0.21429	14
chrX	153994672	G	T	DKC1	p.Ala149Ser	c.445G>T	NM_001142463.2	0	0	0.25	32
chrX	34149603	G	T	FAM47A	p.Leu265Met	c.793C>A	NM_203408.3	0	0	0.2	25
chrX	46434324	G	T	CHST7	p.Val320Leu	c.958G>T	NM_019886.3	0	0	0.29412	17
chrX	53457415	G	T	RIBC1	p.Gln245His	c.735G>T	NM_001031745.4	0	0	0.2	20

30
31

VFN-D2

Chr[a]	Position (hg19)	REF[b]	ALT[c]	Genesymbol	AA change[d]	DNA change[e]	Transcript	Patient AF[f]	Patient Depth[g]	PDX AF[f]	PDX Depth[g]
chr1	116931321	G	A	ATP1A1	p.Gly188Glu	c.563G>A	NM_000701.7	0	116	0.32	100
chr1	176838140	G	C	ASTN1	p.Leu1163Val	c.3487C>G	NM_207108.2	0	118	0.34589	292
chr1	179399737	T	G	AXDND1	p.Phe495Val	c.1483T>G	NM_144696.5	0	41	0.22973	74
chr1	223179209	G	T	DISP1	p.Lys1490Asn	c.4470G>T	NM_032890.3	0	28	0.2	20
chr1	63300476	T	A	ATG4C	p.Tyr348Asn	c.1042T>A	NM_032852.3	0.0114286	175	0.23423	222
chr11	66297402	G	T	BBS1	p.Glu484Asp	c.1452G>T	NM_024649.4	0	81	0.2	30
chr15	40954323	C	A	CASC5	p.Tyr2296*	c.6888C>A	NM_144508.4	0	195	0.25444	169
chr17	33316507	A	C	LIG3	p.Glu238Asp	c.714A>C	NM_002311.4	0	70	0.2	35
chr17	41561472	G	A	DHX8	p.Ala23Thr	c.67G>A	NM_004941.1	0	242	0.22222	99
chr17	63010434	A	T	GNA13	p.Phe264Ile	c.790T>A	NM_001282425.1	0	130	0.22137	131
chr18	28737398	A	G	DSC1	p.Leu96Pro	c.287T>C	NM_004948.3	0	96	0.22581	93
chr19	15375367	G	A	BRD4	p.Leu354Phe	c.1060C>T	NM_014299.2	0	275	0.28022	182
chr20	36962889	G	T	BPI	p.Val448Phe	c.1342G>T	NM_001725.2	0.0033898	295	0.28061	196
chr22	20077574	A	G	DGCR8	p.Ser367Gly	c.1099A>G	NM_001190326.1	0	135	0.26136	88
chr4	84509364	C	A	AGPAT9	p.Leu186Ile	c.556C>A	NM_001256421.1	0.006993	143	0.34524	84

chr4	96035902	G	T	BMPR1B	p.Glu59*	c.175G>T	NM_001203.2	0.01	300	0.22105	190
chr7	44149689	G	A	AEBP1	p.Gly409Asp	c.1226G>A	NM_001129.4	0	191	0.25676	74
chr8	125384159	G	T	TMEM65	p.His80Gln	c.240C>A	NM_194291.2	0	38	0.21429	14
chrX	41205854	A	G	DDX3X	p.Thr532Ala	c.1594A>G	NM_001193416.1	0.0089286	112	0.25325	154
chr1	150482355	C	A	ECM1	p.Leu88Ile	c.262C>A	NM_001202858.1	0.0212766	47	0.55405	148
chr1	248790005	G	T	OR2T11	p.Ala142Asp	c.425C>A	NM_001001964.1	0.016129	62	0.27273	11
chr10	126480314	G	T	METTL10	p.Ser30*	c.89C>A	NM_212554.2	0.0465116	43	0.21053	19
chr10	21805577	G	T	SKIDA1	p.Ala392Glu	c.1175C>A	NM_207371.3	0.0140845	71	0.22222	18
chr11	60233452	T	A	MS4A1	p.Ile132Asn	c.395T>A	NM_021950.3	0.015873	63	0.25373	67
chr11	60233473	T	G	MS4A1	p.Leu139Arg	c.416T>G	NM_021950.3	0.0169492	59	0.31035	58
chr11	62455481	G	T	LRRN4CL	p.Ala167Asp	c.500C>A	NM_203422.3	0.030303	33	0.33333	15
chr12	1969341	A	G	CACNA2D4	p.Phe637Ser	c.1910T>C	NM_172364.4	0.0092593	108	0.40845	71
chr13	99536071	G	C	DOCK9	p.Thr822Ser	c.2465C>G	NM_001130048.1	0.0089286	112	0.24272	103
chr16	3016759	G	T	KREMEN2	splice_acceptor_variant	c.487G>T	NM_001253725.1	0.0178571	56	0.22222	27
chr16	50667345	G	T	NKD1	p.Val356Leu	c.1066G>T	NM_033119.4	0.015625	64	0.31579	19
chr2	226447631	G	T	NYAP2	p.Gly500Cys	c.1498G>T	NM_020864.1	0.0294118	34	0.23529	17
chr20	43530195	G	T	YWHAB	p.Glu7Asp	c.21G>T	NM_003404.4	0.0212766	47	0.25	24
chr20	61050106	G	T	GATA5	p.Pro158Thr	c.472C>A	NM_080473.4	0.0227273	44	0.2	15
chr22	50988318	G	T	KLHDC7B	p.Gly575Trp	c.1723G>T	NM_138433.3	0.0322581	31	0.22222	18
chr3	133119341	G	T	BFSP2	p.Met138Ile	c.414G>T	NM_003571.3	0.0212766	47	0.2	20
chr5	110074872	G	T	SLC25A46	p.Ala18Ser	c.52G>T	NM_138773.1	0.025641	39	0.21053	19
chr6	126070938	G	T	HEY2	p.Glu6*	c.16G>T	NM_012259.2	0.0166667	60	0.22222	18
chrX	57313446	G	T	FAAH2	p.Arg63Ile	c.188G>T	NM_174912.3	0.0222222	45	0.23529	17
chr1	52824735	G	T	CC2D1B	p.Asp407Glu	c.1221C>A	NM_032449.2	0	14	0.46154	13
chr10	114903688	C	T	TCF7L2	p.Pro208Leu	c.623C>T	NM_001146284.1	0	71	0.22222	36
chr16	30581744	G	T	ZNF688	p.Asn94Lys	c.282C>A	NM_001024683.1	0	33	0.2	15
chr19	18121516	G	T	ARRDC2	p.Arg378Leu	c.1133G>T	NM_001025604.2	0	40	0.21739	23
chr4	143130063	A	C	INPP4B	p.Leu318Arg	c.953T>G	NM_001101669.1	0	107	0.23596	89

chr5	163483	G	T	PLEKHG4B	p.Cys743Phe	c.2228G>T	NM_052909.3	0	32	0.2	20
chr9	35712111	G	T	TLN1	p.Ala1191Glu	c.3572C>A	NM_006289.3	0	40	0.26316	38
chrX	3030539	G	T	ARSF	p.Cys572Phe	c.1715G>T	NM_001201538.1	0	45	0.21429	28
chr1	109792835	G	T	CELSR2	p.Arg45Leu	c.134G>T	NM_001408.2	0	44	0.21053	19
chr1	176863891	C	A	ASTN1	p.Ser916Ile	c.2747G>T	NM_001286164.1	0	119	0.31293	147
chr1	176863966	C	G	ASTN1	p.Gly891Ala	c.2672G>C	NM_001286164.1	0	48	0.26563	64
chr1	182829306	T	C	DHX9	p.Ile440Thr	c.1319T>C	NM_001357.4	0	46	0.43836	73
chr1	44056951	G	A	PTPRF	p.Val420Met	c.1258G>A	NM_002840.3	0	42	0.2	20
chr1	45671790	G	T	ZSWIM5	p.Ala78Glu	c.233C>A	NM_020883.1	0	49	0.23077	13
chr1	77334265	G	T	ST6GALNAC5	p.Lys33Asn	c.99G>T	NM_030965.1	0	38	0.27273	22
chr1	78959193	G	T	PTGFR	p.Met255Ile	c.765G>T	NM_000959.3	0	18	0.24	25
chr10	116075491	G	T	AFAP1L2	p.Ser147Tyr	c.440C>A	NM_001001936.2	0	37	0.23529	17
chr10	133946947	G	T	JAKMIP3	p.Glu255Asp	c.765G>T	NM_001105521.2	0	52	0.21739	23
chr10	71266587	C	A	TSPAN15	p.Phe246Leu	c.738C>A	NM_012339.3	0	47	0.23529	17
chr11	13031251	G	T	RASSF10	p.Arg43Leu	c.128G>T	NM_001080521.2	0	21	0.22222	18
chr11	2424444	C	A	TSSC4	p.Pro194His	c.581C>A	NM_001297658.1	0	61	0.24	25
chr11	64600431	G	T	CDC42BPG	p.Ala911Asp	c.2732C>A	NM_017525.2	0	66	0.26316	19
chr11	65413941	G	T	SIPA1	p.Arg479Leu	c.1436G>T	NM_006747.3	0	43	0.25	12
chr11	72437693	G	T	ARAP1	p.Arg161Ser	c.481C>A	NM_001040118.2	0	50	0.33333	18
chr12	111748080	G	T	CUX2	p.Lys498Asn	c.1494G>T	NM_015267.3	0	46	0.28571	21
chr12	115112133	G	T	TBX3	p.Ala516Asp	c.1547C>A	NM_005996.3	0	25	0.36364	11
chr12	40499268	G	T	SLC2A13	p.Leu115Met	c.343C>A	NM_052885.3	0	25	0.28571	14
chr12	49933258	C	A	KCNH3	p.Thr20Lys	c.59C>A	NM_012284.1	0	42	0.25	16
chr12	57919442	G	T	MBD6	p.Ala231Ser	c.691G>T	NM_052897.3	0	49	0.20588	34
chr12	88462350	T	G	CEP290	p.Lys2028Asn	c.6084A>C	NM_025114.3	0	32	0.32	50
chr13	21563279	A	G	LATS2	p.Tyr214His	c.640T>C	NM_014572.2	0	59	0.28125	32
chr13	21751298	G	T	MRPL57	p.Lys81Asn	c.243G>T	NM_024026.4	0	43	0.27273	11
chr13	23912662	G	C	SACS	p.Pro1638Ala	c.4912C>G	NM_001278055.1	0	76	0.27941	68

chr14	100759349	G	T	SLC25A29	p.Gly61Gly	c.183C>A	NM_001039355.2	0	32	0.26667	15
chr15	40648299	G	T	PHGR1	p.Gly15Val	c.44G>T	NM_001145643.1	0	16	0.28571	14
chr16	31087999	G	T	ZNF646	p.Gln118His	c.354G>T	NM_014699.3	0	47	0.23529	17
chr16	88502434	G	T	ZNF469	p.Leu2824Phe	c.8472G>T	NM_001127464.1	0	16	0.22222	18
chr17	1373558	G	T	MYO1C	p.Arg813Ser	c.2437C>A	NM_001080779.1	0	33	0.21429	14
chr17	47583878	G	T	NGFR	p.Gln142His	c.426G>T	NM_002507.3	0	53	0.2	25
chr17	62496442	G	T	DDX5	p.Arg482Ser	c.1444C>A	NM_004396.3	0	37	0.25807	31
chr17	79518087	G	T	C17orf70	p.Gln145Lys	c.433C>A	NM_025161.5	0	45	0.22727	22
chr19	11526796	G	T	RGL3	p.Leu152Met	c.454C>A	NM_001035223.3	0	23	0.22222	18
chr19	17323056	G	T	MYO9B	p.Lys2137Asn	c.6411G>T	NM_004145.3	0	41	0.2	15
chr19	17417132	G	T	MRPL34	p.Gly75Cys	c.223G>T	NM_023937.3	0	21	0.36364	11
chr19	36223009	G	T	KMT2B	p.Val1880Phe	c.5638G>T	NM_014727.2	0	40	0.25	16
chr19	3942260	G	T	NMRK2	p.Asp233Tyr	c.697G>T	NM_001289117.1	0	17	0.2	15
chr19	39804885	G	T	LRFN1	p.Asp364Glu	c.1092C>A	NM_020862.1	0	35	0.23529	17
chr19	4446672	G	T	UBXN6	p.Gln196Lys	c.586C>A	NM_001171091.1	0	43	0.2	30
chr19	5783461	G	T	PRR22	p.Ala266Glu	c.797C>A	NM_001134316.1	0	30	0.2	20
chr19	6714015	G	T	C3	p.Thr254Asn	c.761C>A	NM_000064.2	0	73	0.21053	19
chr2	101971729	T	G	CREG2	p.Gln237His	c.711A>C	NM_153836.3	0	120	0.2973	111
chr2	110372773	G	T	SOWAHC	p.Gly236Val	c.707G>T	NM_023016.3	0	25	0.23077	13
chr2	54856248	G	T	SPTBN1	p.Lys659Asn	c.1977G>T	NM_003128.2	0	36	0.2381	21
chr22	25010759	G	T	GGT1	p.Gly61Cys	c.181G>T	NM_001288833.1	0	81	0.20408	49
chr22	43539122	C	T	MCAT	p.Gly78Asp	c.233G>A	NM_014507.3	0	58	0.275	40
chr3	127379851	G	T	PODXL2	p.Gly327Val	c.980G>T	NM_015720.3	0	57	0.25	28
chr3	130714891	G	T	ATP2C1	p.Ala688Ser	c.2062G>T	NM_001001485.2	0	14	0.2	20
chr3	187443366	G	T	BCL6	p.Ala587Asp	c.1760C>A	NM_001130845.1	0	152	0.38843	121
chr4	114276768	A	T	ANK2	p.Ser2332Cys	c.6994A>T	NM_001148.4	0	70	0.34043	47
chr4	23816205	G	T	PPARGC1A	p.Pro301Thr	c.901C>A	NM_013261.3	0	55	0.2	20
chr4	3444801	G	T	HGFAC	p.Cys108Phe	c.323G>T	NM_001297439.1	0	79	0.2	15

chr4	99950049	A	T	METAP1	p.Lys49Met	c.146A>T	NM_015143.2	0	82	0.23188	69
chr5	171638865	G	T	UBTD2	p.Pro225His	c.674C>A	NM_152277.2	0	12	0.25	12
chr5	180622225	G	T	TRIM7	p.Arg493Ser	c.1477C>A	NM_203293.2	0	69	0.21739	23
chr5	72744159	G	T	FOXD1	p.Ala10Asp	c.29C>A	NM_004472.2	0	55	0.2	20
chr5	74696100	A	T	COL4A3BP	p.Leu475*	c.1424T>A	NM_001130105.1	0	67	0.26531	98
chr6	17804631	T	A	KIF13A	p.Lys805Asn	c.2415A>T	NM_001105566.2	0	125	0.27007	137
chr6_qbl_hap6	2985262	G	T	C6orf25	p.Arg93Leu	c.278G>T	NM_025260.3.4	0	41	0.2	20
chr7	103191602	C	T	RELN	p.Glu2072Lys	c.6214G>A	NM_005045.3	0	66	0.33333	48
chr7	45122120	G	T	NACAD	p.Pro1220His	c.3659C>A	NM_001146334.1	0	27	0.23529	17
chr7	48311783	T	G	ABCA13	p.Tyr840*	c.2520T>G	NM_152701.4	0	20	0.41936	31
chr8	144464621	G	T	RHPN1	p.Val605Phe	c.1813G>T	NM_052924.2	0	44	0.36364	11
chr8	144512441	G	T	MAFA	p.Pro46Thr	c.136C>A	NM_201589.3	0	28	0.3	10
chrX	152686670	C	A	ZFP92	p.Leu279Ile	c.835C>A	NM_001136273.1	0	50	0.21053	19
chrX	152721728	T	C	HAUS7	p.Thr244Ala	c.730A>G	NM_017518.7	0	39	0.2	20
chrX	49104732	G	T	CCDC22	p.Glu391Asp	c.1173G>T	NM_014008.4	0	40	0.28571	14

32
33

VFN-D3

Chr[a]	Position (hg19)	REF[b]	ALT[c]	Genesymbol	AA change[d]	DNA change[e]	Transcript	Patient AF[f]	Patient Depth[g]	PDX AF[f]	PDX Depth[g]
chr2	112615888	C	G	ANAPC1	p.Gln451His	c.1353G>C	NM_022662.3	0.0416667	24	0.2	55
chr4	77675883	C	T	SHROOM3	p.Thr1416Met	c.4247C>T	NM_020859.3	0.0054945	182	0.36	400
chr10	97392772	G	T	ALDH18A1	p.Ala249Asp	c.746C>A	NM_001017423.1	0	0	0.37931	29
chr12	115118737	C	T	TBX3	p.Val202Ile	c.604G>A	NM_005996.3	0	0	0.46	50
chr14	59834210	G	A	DAAM1	p.Gly964Ser	c.2890G>A	NM_001270520.1	0	0	0.29032	31
chr17	59161917	C	G	BCAS3	p.Ala821Gly	c.2462C>G	NM_001099432.1	0	0	0.53571	28
chr22	23230373	G	A	IGLL5	p.Ser47Asn	c.140G>A	NM_001178126.1	0	0	0.96078	51

chr4	55129873	A	G	PDGFRA	p.Tyr136Cys	c.407A>G	NM_006206.4	0	0	0.46316	95

[a]Chromosome; [b]Reference allele; [c]Alternative allele; [d]Amino-acid change; [e]DNA sequence change; [f]Allele frequency in patient's sample/PDX; [g]Read depth in patient's sample/PDX.

Supplementary Table 6. A complete list of protein coding variants which passed the filtering described in supplementary methods, that were found only in the parental tumor samples and not the derived PDXs. Variants in genes of special interest based on a list of frequently mutated genes are highlighted in red. Note, no protein coding variants were found only in the parental tumor for PDX model VFN-D3.

VFN-D1

Chr[a]	Position (hg19)	REF[b]	ALT[c]	Genesymbol	AA change[d]	DNA change[e]	Transcript	Patient AF[f]	Patient Depth[g]	PDX AF[f]	PDX Depth[g]
chr13	23929290	G	T	SACS	p.Asp340Glu	c.1020C>A	NM_001278055.1	0.214286	14	0	0
chr2	240111695	G	T	HDAC4	p.Ser58*	c.173C>A	NM_006037.3	0.266667	15	8	0
chr2	241810063	G	T	AGXT	p.Ala121Ser	c.361G>T	NM_000030.2	0.272727	11	0	0
chr20	3641279	G	T	GFRA4	p.Asp177Glu	c.531C>A	NM_022139.3	0.3	10	0	0
chr6	40360256	G	T	LRFN2	p.Pro599Gln	c.1796C>A	NM_020737.1	0.214286	14	0	0
chr8	38067946	G	T	BAG4	p.Ala401Ser	c.1201G>T	NM_001204878.1	0.230769	13	0	0
chrX	153041855	G	T	PLXNB3	p.Asp1615Tyr	c.4843G>T	NM_001163257.1	0.214286	14	0	0
chrX	71425705	G	T	ERCC6L	p.Ser971Tyr	c.2912C>A	NM_017669.2	0.230769	13	0	0

VFN-D2

Chr[a]	Position (hg19)	REF[b]	ALT[c]	Genesymbol	AA change[d]	DNA change[e]	Transcript	Patient AF[f]	Patient Depth[g]	PDX AF[f]	PDX Depth[g]
chr2	233709134	G	T	GIGYF2	p.Gly1052Val	c.3155G>T	NM_001103146.1	0.300752	133	0	0
chr10	27688070	G	T	PTCHD3	p.Ala486Glu	c.1457C>A	NM_001034842.3	0.2	25	0	0
chr10	68687667	A	T	LRRTM3	p.Lys331Asn	c.993A>T	NM_178011.4	0.285714	63	0	0
chr10	73572290	A	T	CDH23	p.Gln905Leu	c.2714A>T	NM_001171933.1	0.287671	73	0	0
chr11	60233410	TAATGA	T	MS4A1	p.Ile118fs	c.354_358delAATGA	NM_021950.3	0.339286	56	0	0

chr11	94326718	C	A	PIWIL4	p.Pro354His	c.1061C>A	NM_152431.2	0.461538	117	0	0
chr13	113508670	A	G	ATP11A	p.Lys690Arg	c.2069A>G	NM_015205.2	0.343434	99	0	0
chr13	33017810	A	T	N4BP2L2	p.Asp273Glu	c.819T>A	NM_001278432.1	0.422764	123	0	0
chr14	24724652	C	G	TGM1	p.Lys521Asn	c.1563G>C	NM_000359.2	0.348404	376	0	0
chr15	42434825	G	A	PLA2G4F	p.Arg744Cys	c.2230C>T	NM_213600.3	0.360294	136	0	0
chr15	86225395	G	T	AKAP13	p.Arg325Leu	c.974G>T	NM_001270546.1	0.345679	81	0	0
chr18	7038814	C	A	LAMA1	p.Gly520Cys	c.1558G>T	NM_005559.3	0.238806	67	0	0
chr19	39665657	G	A	PAK4	p.Met395Ile	c.1185G>A	NM_001014831.2	0.327869	244	0	0
chr20	**30309616**	**T**	**G**	**BCL2L1**	**p.Asn136His**	**c.406A>C**	**NM_138578.1**	**0.344**	**125**	**0**	**0**
chr3	42676723	C	A	NKTR	p.Thr343Lys	c.1028C>A	NM_005385.3	0.358491	53	0	0
chr4	90743452	C	A	SNCA	p.Gly84Val	c.251G>T	NM_000345.3	0.311111	270	0	0
chr5	137426581	G	A	WNT8A	p.Arg310His	c.929G>A	NM_001300938.1	0.328063	253	0	0
chr5	178417671	T	C	GRM6	p.Lys312Glu	c.934A>G	NM_000843.3	0.285714	77	0	0
chr5	26881552	A	C	CDH9	p.Leu688Arg	c.2063T>G	NM_016279.3	0.327869	122	0	0
chr5	74450146	C	T	ANKRD31	p.Gly578Arg	c.1732G>A	NM_001164443.1	0.369863	73	0	0
chr7	95024002	T	A	PON3	p.Glu33Asp	c.99A>T	NM_000940.2	0.266187	139	0	0
chr8	18729679	A	G	PSD3	p.Ile232Thr	c.695T>C	NM_015310.3	0.225806	62	0	0
chr9	37327819	GTATCACC	G	ZCCHC7	p.Tyr326fs	c.976_982delTATCACC	NM_001289119.1	0.304348	115	0	0
chrX	102931875	G	T	MORF4L2	p.Ser27Arg	c.81C>A	NM_001142418.1	0.2	15	0	0
chrX	21755733	T	C	SMPX	p.Glu72Gly	c.215A>G	NM_014332.2	0.813559	59	0	0

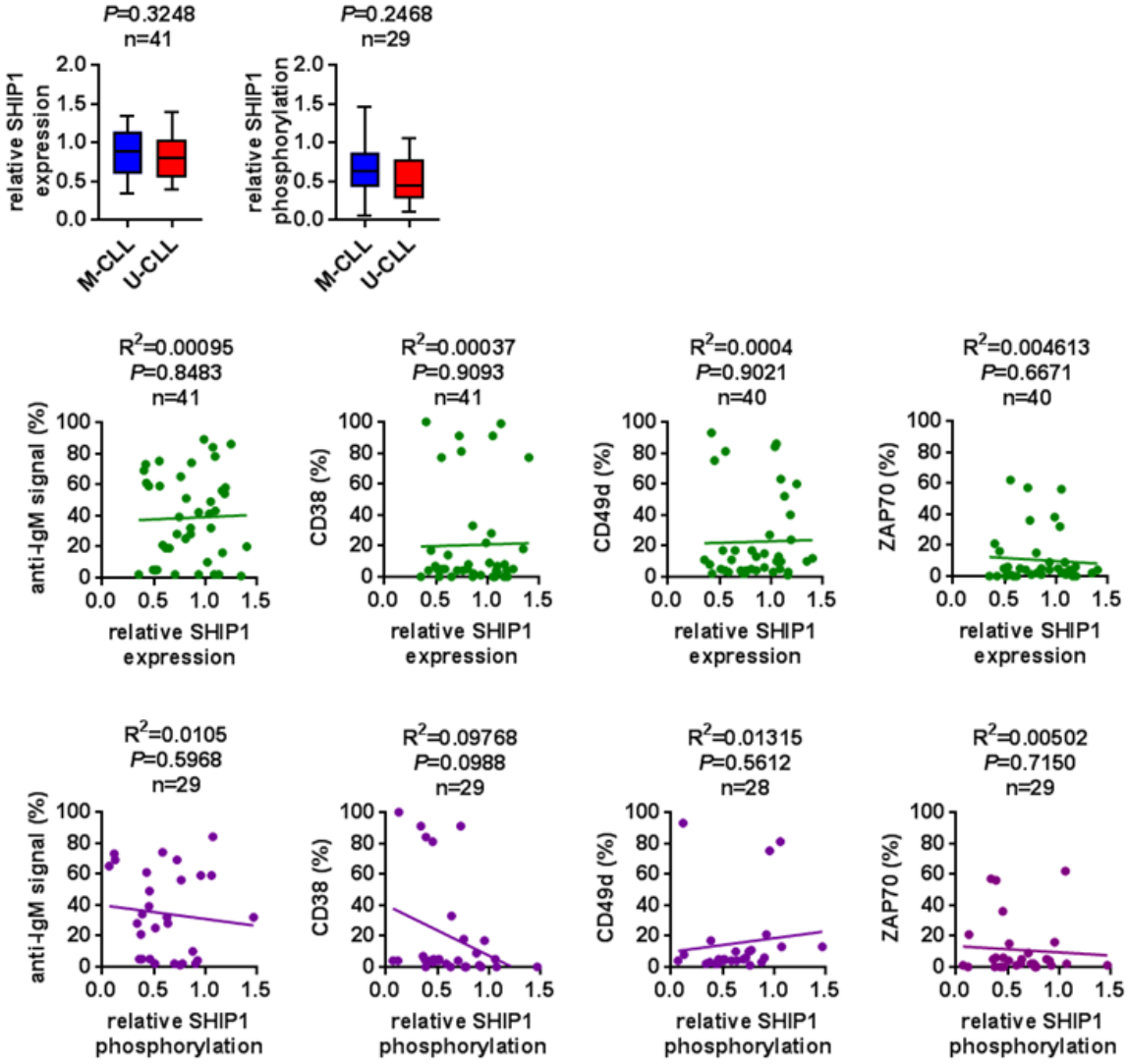
[a]Chromosome; [b]Reference allele; [c]Alternative allele; [d]Amino-acid change; [e]DNA sequence change; [f]Allele frequency in patient's sample/PDX; [g]Read depth in patient's sample/PDX.

Supplementary Table 7. Statistical significance of effects of compounds on tumor volume in *in vivo* models. Table shows P-values of partial t-tests about zero slope of mean tumor volume differences.

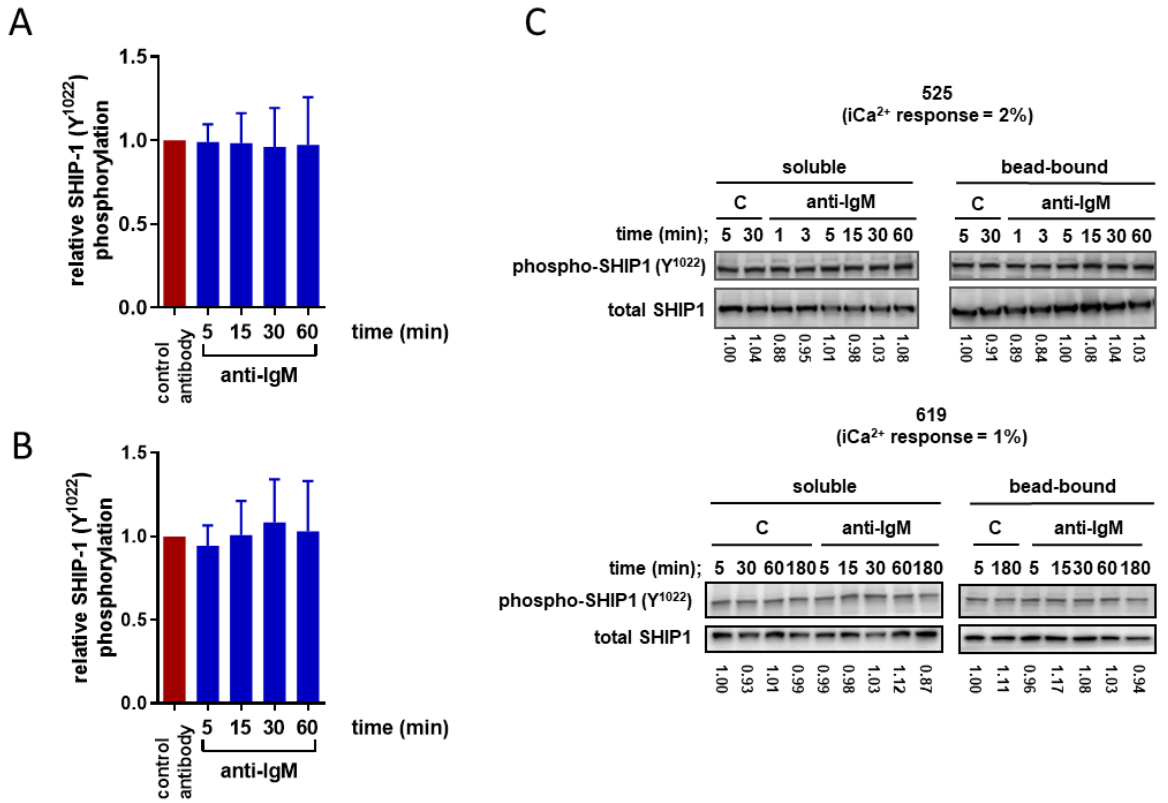
Differences	TMD 8	VFN-D1	VFN-D2	VFN-D3
vehicle vs. AQX-435	0.0000**	0.0040*	0.0001**	0.0000**
vehicle vs. ibrutinib	0.0000**	0.0064	0.4465	0.0573
vehicle vs. ibrutinib and AQX-435	N/A	0.0000**	0.0000**	0.0000**
ibrutinib vs. AQX-435	0.5538	0.4629^{+}	0.0004**	0.0017*
AQX-435 vs. ibrutinib and AQX-435	N/A	0.0000**	0.7029	0.0016*
ibrutinib vs. ibrutinib and AQX-435	N/A	0.0001**	0.0001**	0.0000**

*Statistically significant difference at the 10% simultaneous significance level. **Statistically significant difference at the 1% simultaneous significance level. $^{+}$Non-significant negative slope. N/A, not applicable.

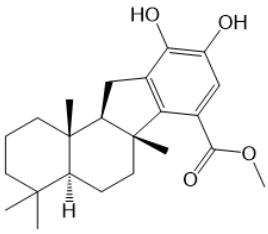
Supplementary Fig. 1. SHIP1 expression and phosphorylation. Immunoblot analysis was performed to quantify SHIP1 expression and phosphorylation (Y^{1022}) in snap-frozen pellets from fresh CLL samples. Total SHIP1 and phospho-SHIP1 was normalized to HSC70 expression with values for RL cells (positive control) set to 1.0. Graphs show SHIP1 expression or phosphorylation relative to *IGHV* mutation status, sIgM signaling capacity and expression of CD38, CD49d or ZAP70. Results of statistical comparisons are shown (Mann-Whitney test for U- versus M-CLL, otherwise Pearson's correlation analysis).



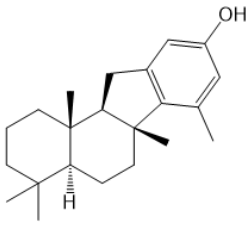
Supplementary Fig. 2. Effect of anti-IgM on SHIP1 phosphorylation. Anti-IgM signaling responsive CLL samples were treated with control antibodies, or anti-IgM for the indicated times and expression of total and phospho-SHIP1 was analyzed by immunoblotting. Graphs show mean (±SD) SHIP1 phosphorylation with values for control cells set to 1.0 for **A,** soluble (n=7) and **B,** immobilized (n=8) antibodies. **C**, Analysis of effects of anti-IgM or control (C) antibodies on SHIP1 phosphorylation in two anti-IgM signaling non-responsive samples. Numbers under the blots show quantitation of SHIP1 phosphorylation relative to control antibody (5 minutes)-treated cells.



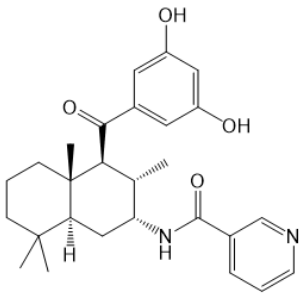
Supplementary Fig. 3. Structure of SHIP1 activator. The figure shows the structure of the natural product SHIP1 activator pelorol, its closely related analogue MN100 and AQX-435. Shown below are the calculated logP (clogP) values which provide a comparative measure of the compounds' lipophilicities. clogP values were calculated using ChemDraw Professional (Perkin Elmer).



pelorol
clogP 7.50

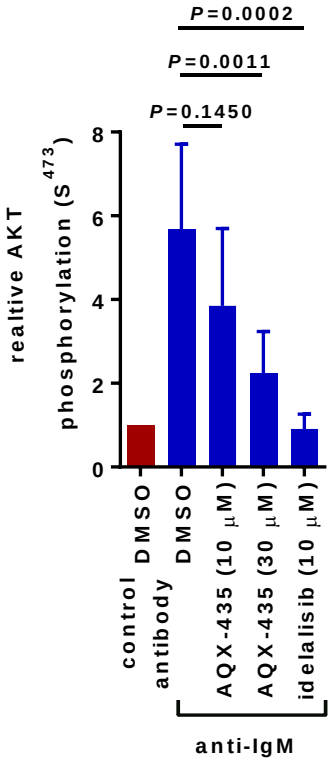


AQX-MN100
clogP 7.93

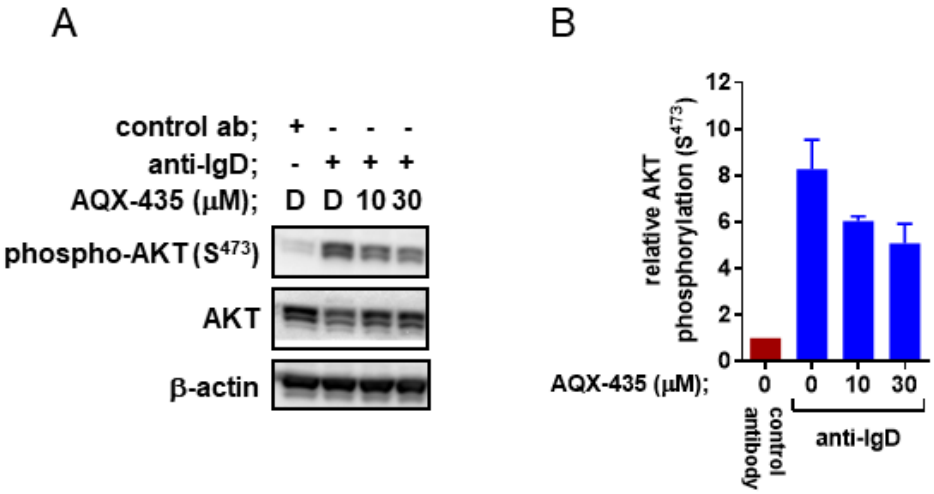


AQX-435
clogP 5.85

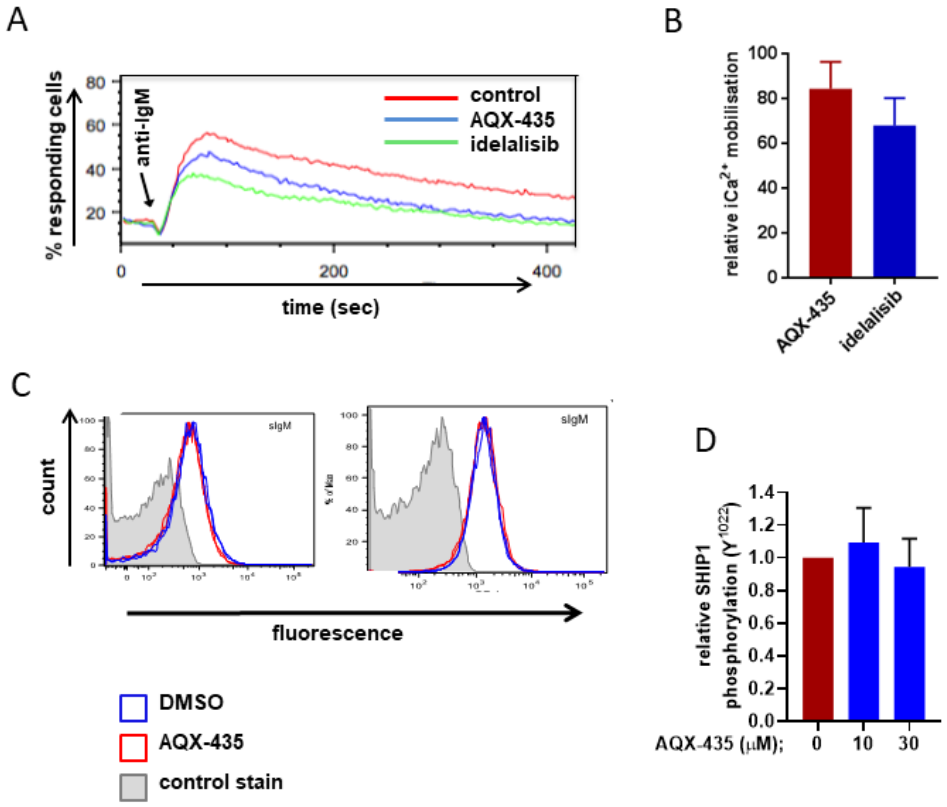
Supplementary Fig. 4. Comparison between AQX-435 and idelalisib for effects on AKT S^{473} phosphorylation. CLL samples (n=9) were pre-treated with the indicated concentrations of AQX-435 or idelalisib, or DMSO as a control, for 30 minutes and then stimulated with bead-bound anti-IgM or control antibody. Graph shows mean (±SD) S^{473} AKT phosphorylation with values for control antibody/DMSO-treated cells set to 1.0 and the statistical significance of the indicated differences (Student's t-test).



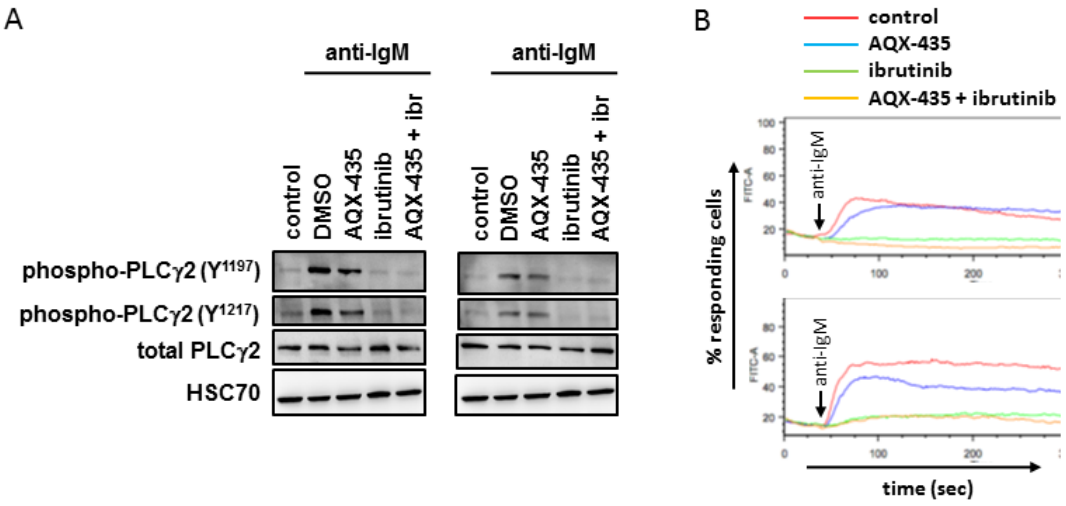
Supplementary Fig. 5. Effect of AQX-435 on anti-IgD-induced signalling. CLL samples were pre-treated with the indicated concentrations of AQX-435 or DMSO (D) as a control for 30 minutes and then stimulated with bead-bound anti-IgD or control antibody (ab) for 30 minutes. Figure shows **A,** representative immunoblot analysis for phosphorylation of AKT S^{473}, total AKT and β-actin as an additional loading control. **B,** Quantitation of AKT S^{473} phosphorylation for all samples analysed (n=3). Graph shows mean (±SD) AKT S^{473} phosphorylation with values for control antibody/DMSO-treated cells (C) set to 1.0.



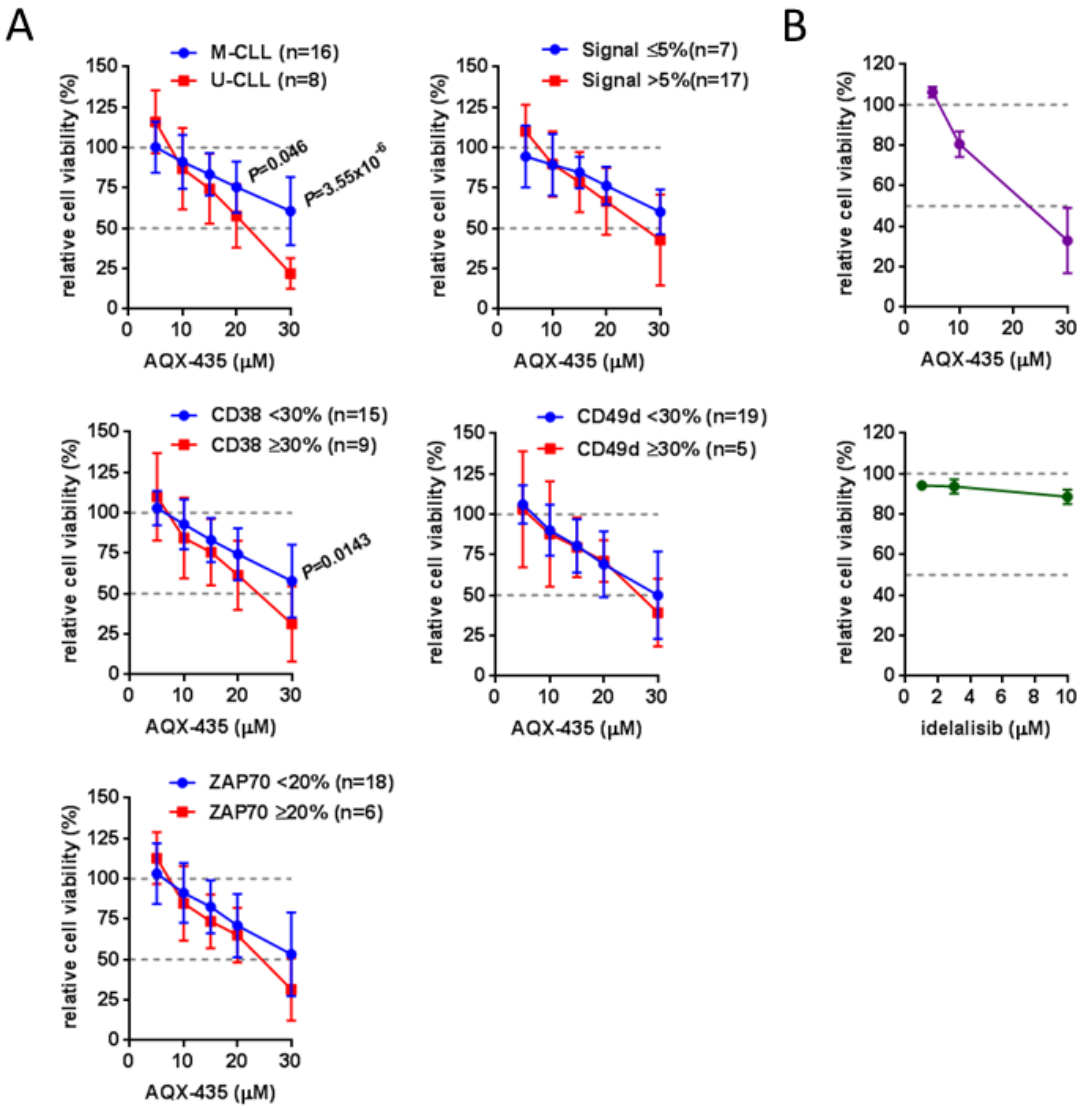
Supplementary Fig. 6. Effect of AQX-435 on anti-IgM-induced intracellular Ca^{2+} mobilization, sIgM expression and SHIP1 phosphorylation. **A** and **B,** CLL samples were pre-treated with AQX-435 (30 µM) or idelalisib (10 µM), or left untreated as a control for 30 minutes. Cells were then incubated with the Ca^{2+}-sensitive probe Fluo3-AM (FluoroPure Grade, Life Technologies), in the continued presence of AQX-435/idelalisb for an additional 30 minutes before analysis using flow cytometry. "Baseline" data was collected for ~30 seconds before addition of anti-IgM. **A,** Representative results (percentage of responding cells). **B,** Quantitation for all samples analyzed (n=3 for both). Graph shows mean (±SD) iCa^{2+} mobilization in AQX-435 or idelalisib-treated cells relative to control anti-IgM-treated cells (set to 100%). **C,** CLL samples were treated with AQX-435 (30 µM) or DMSO for 6 hours. Samples were stained with pacific blue-conjugated anti-CD19, PerCP-Cy5.5-conjugated anti-CD5 and PE-conjugated anti-surface IgM (Biolegend) in duplicate and fluorescence quantified by flow cytometry. Graphs show sIgM expression in $CD5^{+}CD19^{+}$ cells for AQX-435 (red) or DMSO (blue) treated cells. The solid grey histogram shows staining of cells with a PE-conjugated mIgG2a isotype control antibody (Biolegend). Figure shows results for 2 samples and is representative of a total of 5 samples analyzed. **D**, CLL samples (n=4) were treated with AQX-435 (10 or 30 µM) for 2 hours and then expression of total/phospho-SHIP1 was analyzed by immunoblotting. Graph shows mean (±SD) SHIP1 phosphorylation in AQX-435-treated cells relative to DMSO-treated cells (set to 1.0).



Supplementary Fig. 7. AQX-435 does not alter ibrutinib-mediated inhibition of calcium signaling. CLL samples (n=2) were pre-treated with AQX-435 (30 μM) and/or ibrutinib (100 nM), or DMSO as a control, for 30 minutes. **A,** Cells were then stimulated with bead-bound anti-IgM or control antibody for 15 minutes and expression of total/phospho-PLCγ2 was analyzed by immunoblotting. HSC70 was analyzed as an additional loading control. **B,** Drug-treated cells were incubated with the Ca^{2+}-sensitive probe Fluo3-AM 30 minutes before analysis using flow cytometry. "Baseline" data was collected for ~30 seconds before addition of anti-IgM. Graphs show percentage of responding cells.

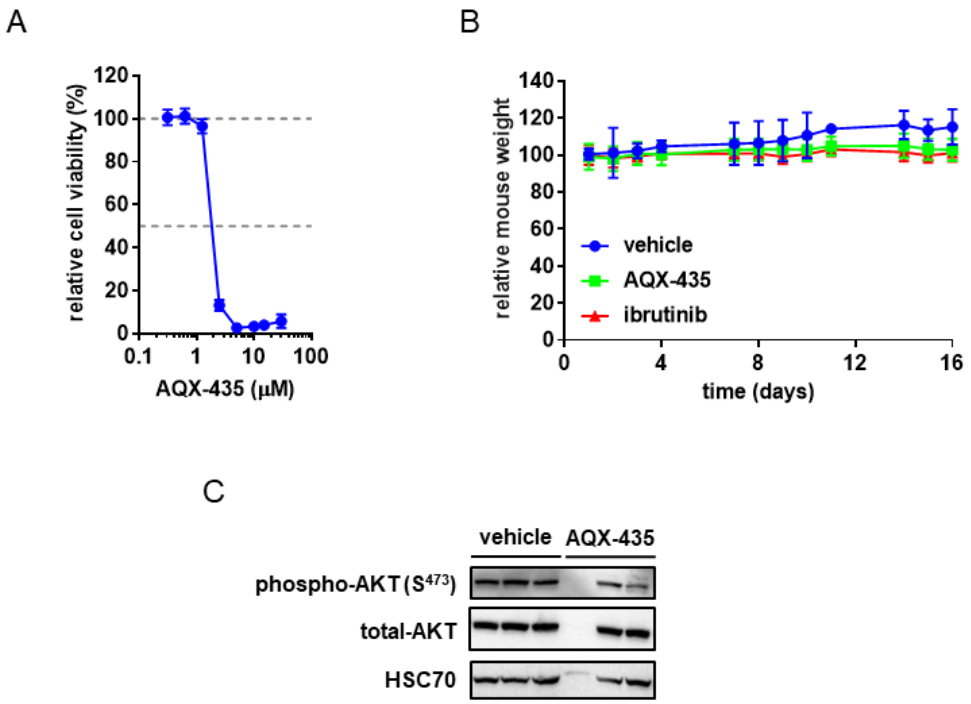


Supplementary Fig. 8. AQX-435-induced apoptosis in CLL samples. CLL samples were treated with various concentrations of AQX-435 (5–30 μM), idelalisib (1-10 μM) or DMSO as a control. After 24 hours, the proportion of viable (annexin V^-/PI^-) cells was determined using annexin V/PI staining. Results were normalized to values for DMSO-treated cells (set to 100% for each sample). **A,** Effect of AQX-435 in 24 samples was compared across disease subsets defined by *IGHV* mutation status, sIgM signaling capacity and expression of CD38, CD49d or ZAP70 (using cut-offs of 30%, 30% and 20%, respectively) (18-21). Graphs show means (±SD) and the statistical significance of differences between groups at each concentration (Student's t-test). Where values are not shown, $P>0.05$. **B,** Comparison of effects of AQX-435 and idelalisib in 3 samples (mean (±SD) percentage of viable cells).

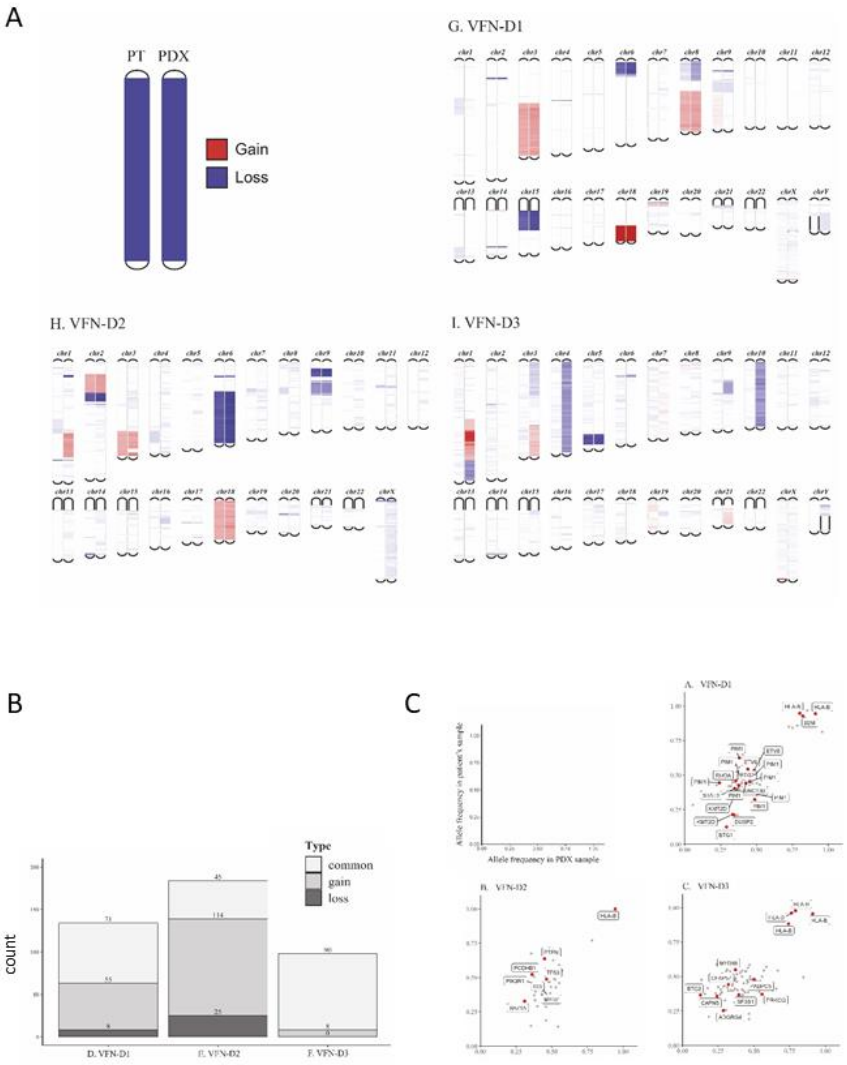


Supplementary Fig. 9. Inhibition of anti-IgM-induced AKT phosphorylation in WSU-FSCCL cells**.** WSU-FSCCL cells were pre-treated with AQX-435 or DMSO as a control for 1 hour and then incubated with soluble anti-IgM or control antibody for 15 minutes at 37 °C. Expression of total and phospho-AKT (S^{473}) was analyzed by immunoblotting. Experiment is representative of two separate experiments.

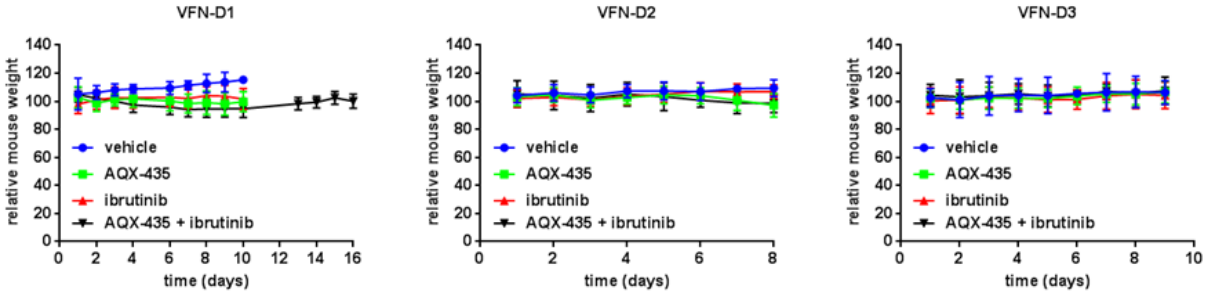
Supplementary Fig. 10. Effect of AQX-435 on TMD8 cell viability *in vitro* and on mouse weight and AKT phosphorylation *in vivo*. **A,** TMD8 cells were incubated with the indicated concentrations of AQX-435 for 72 hours and cell viability was determined by annexin V/PI staining and flow cytometry. Graph shows mean (±SD) percentage of viable (annexin V^-/PI^-) cells derived from three independent experiments relative to DMSO control (set to 100%). **B,** Relative mouse weight during treatment with vehicle control, AQX-435 (10 mg/kg, ip) or ibrutinib (25 mg/kg po). Graph shows mean (±SD) with day 0 values set to 100. **C,** Immunoblot analysis of phospho-AKT (S^{473}), total AKT and HSC70 in TMD8 tumors resected from mice treated with vehicle or AQX-435 for 5 days. Note we were unable to obtain intact protein from one tumor from the AQX-435-treated mice.



Supplementary Fig. 11. Characterization of DLBCL PDX models. **A,** Karyotype diagram charts showing the copy number variation as calculated by CNVkit in the parental sample (left chromosomes) and the derived PDX model (right chromosomes). Inferred segmental changes are marked in shades of blue (loss) and red (gain). **B,** Stacked bar charts showing numbers of common protein coding variants in both the parental tumor sample and the PDX, and those gained or lost in the PDX model. **C,** Scatter plots showing the allele frequency of common protein coding variants in each PDX model sample compared to its matched parental tumor. Variants in genes of special interest based on a list of frequently mutated genes described in the supplemental methods are highlighted in red



Supplementary Fig. 12. Effects of combined treated with AQX-435 and ibrutinib in PDX models. Mice bearing DLBCL PDX tumors were treated with AQX-435, ibrutinib or the combination of AQX-435 and ibrutinib. Graphs show relative mouse weight (mean ±SD) during treatment with day 0 values set to 100.



1. Klanova M, Soukup T, Jaksa R, Molinsky J, Lateckova L, Maswabi BC*, et al.* Mouse models of mantle cell lymphoma, complex changes in gene expression and phenotype of engrafted MCL cells: implications for preclinical research. Lab Invest **2014**;94(7):806-17 doi 10.1038/labinvest.2014.61.
2. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics **2009**;25(14):1754-60 doi 10.1093/bioinformatics/btp324.
3. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N*, et al.* The Sequence Alignment/Map format and SAMtools. Bioinformatics **2009**;25(16):2078-9 doi 10.1093/bioinformatics/btp352.
4. Koboldt DC, Zhang Q, Larson DE, Shen D, McLellan MD, Lin L*, et al.* VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome research **2012**;22(3):568-76 doi 10.1101/gr.129684.111.
5. Cingolani P, Patel VM, Coon M, Nguyen T, Land SJ, Ruden DM*, et al.* Using Drosophila melanogaster as a Model for Genotoxic Chemical Mutational Studies with a New Program, SnpSift. Frontiers in genetics **2012**;3:35 doi 10.3389/fgene.2012.00035.
6. Pasqualucci L, Dalla-Favera R. Genetics of diffuse large B-cell lymphoma. Blood **2018**;131(21):2307-19 doi 10.1182/blood-2017-11-764332.
7. Reddy A, Zhang J, Davis NS, Moffitt AB, Love CL, Waldrop A*, et al.* Genetic and Functional Drivers of Diffuse Large B Cell Lymphoma. Cell **2017**;171(2):481-94 e15 doi 10.1016/j.cell.2017.09.027.
8. Dubois S, Viailly PJ, Mareschal S, Bohers E, Bertrand P, Ruminy P*, et al.* Next-Generation Sequencing in Diffuse Large B-Cell Lymphoma Highlights Molecular Divergence and Therapeutic Opportunities: a LYSA Study. Clin Cancer Res **2016**;22(12):2919-28 doi 10.1158/1078-0432.CCR-15-2305.
9. Karube K, Enjuanes A, Dlouhy I, Jares P, Martin-Garcia D, Nadeu F*, et al.* Integrating genomic alterations in diffuse large B-cell lymphoma identifies new relevant pathways and potential therapeutic targets. Leukemia **2018**;32(3):675-84 doi 10.1038/leu.2017.251.
10. Pasqualucci L, Trifonov V, Fabbri G, Ma J, Rossi D, Chiarenza A*, et al.* Analysis of the coding genome of diffuse large B-cell lymphoma. Nat Genet **2011**;43(9):830-7 doi 10.1038/ng.892.
11. Zhang J, Grubor V, Love CL, Banerjee A, Richards KL, Mieczkowski PA*, et al.* Genetic heterogeneity of diffuse large B-cell lymphoma. Proc Natl Acad Sci U S A **2013**;110(4):1398-403 doi 10.1073/pnas.1205299110.
12. Lohr JG, Stojanov P, Lawrence MS, Auclair D, Chapuy B, Sougnez C*, et al.* Discovery and prioritization of somatic mutations in diffuse large B-cell lymphoma (DLBCL) by whole-exome sequencing. Proc Natl Acad Sci U S A **2012**;109(10):3879-84 doi 10.1073/pnas.1121343109.
13. Morin RD, Mendez-Lago M, Mungall AJ, Goya R, Mungall KL, Corbett RD*, et al.* Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. Nature **2011**;476(7360):298-303 doi 10.1038/nature10351.
14. Talevich E, Shain AH, Botton T, Bastian BC. CNVkit: Genome-Wide Copy Number Detection and Visualization from Targeted DNA Sequencing. PLoS computational biology **2016**;12(4):e1004873 doi 10.1371/journal.pcbi.1004873.
15. Tibshirani R, Wang P. Spatial smoothing and hot spot detection for CGH data using the fused lasso. Biostatistics **2008**;9(1):18-29 doi 10.1093/biostatistics/kxm013.
16. Mockridge CI, Potter KN, Wheatley I, Neville LA, Packham G, Stevenson FK. Reversible anergy of sIgM-mediated signaling in the two subsets of CLL defined by VH-gene mutational status. Blood **2007**;109(10):4424-31 doi blood-2006-11-056648 [pii] 10.1182/blood-2006-11-056648.
17. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G*, et al.* Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood **2004**;103(1):275-82 doi 10.1182/blood-2003-05-1545.

18. Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL*, et al.* Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood **1999**;94(6):1840-7.
19. Zucchetto A, Bomben R, Dal Bo M, Sonego P, Nanni P, Rupolo M*, et al.* A scoring system based on the expression of six surface molecules allows the identification of three prognostic risk groups in B-cell chronic lymphocytic leukemia. J Cell Physiol **2006**;207(2):354-63 doi 10.1002/jcp.20570.
20. Orchard JA, Ibbotson RE, Davis Z, Wiestner A, Rosenwald A, Thomas PW*, et al.* ZAP-70 expression and prognosis in chronic lymphocytic leukaemia. Lancet **2004**;363(9403):105-11 doi 10.1016/S0140-6736(03)15260-9.
21. Rassenti LZ, Huynh L, Toy TL, Chen L, Keating MJ, Gribben JG*, et al.* ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease progression in chronic lymphocytic leukemia. N Engl J Med **2004**;351(9):893-901 doi 10.1056/NEJMoa040857.