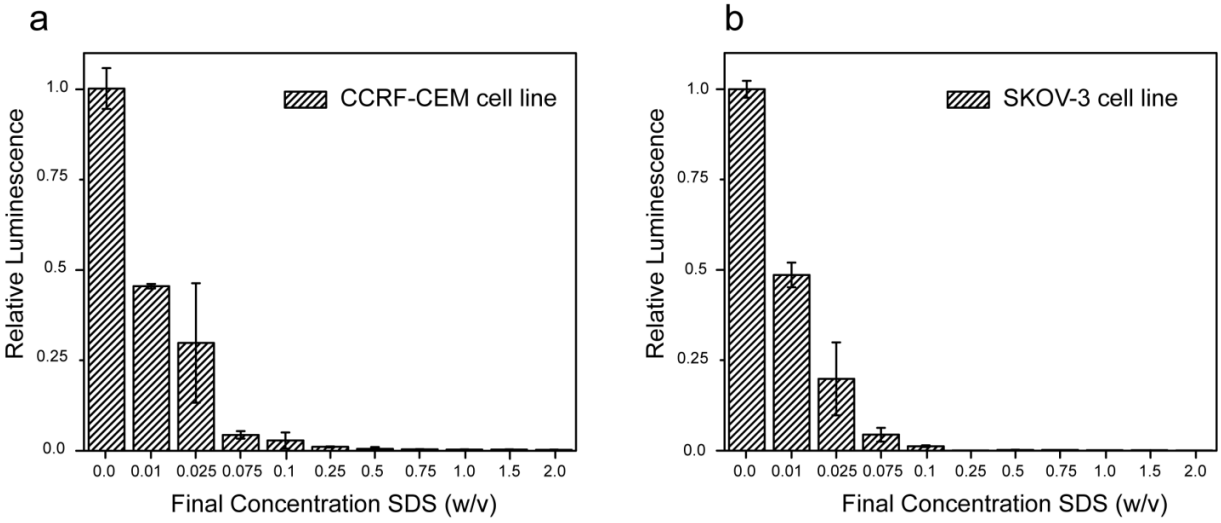
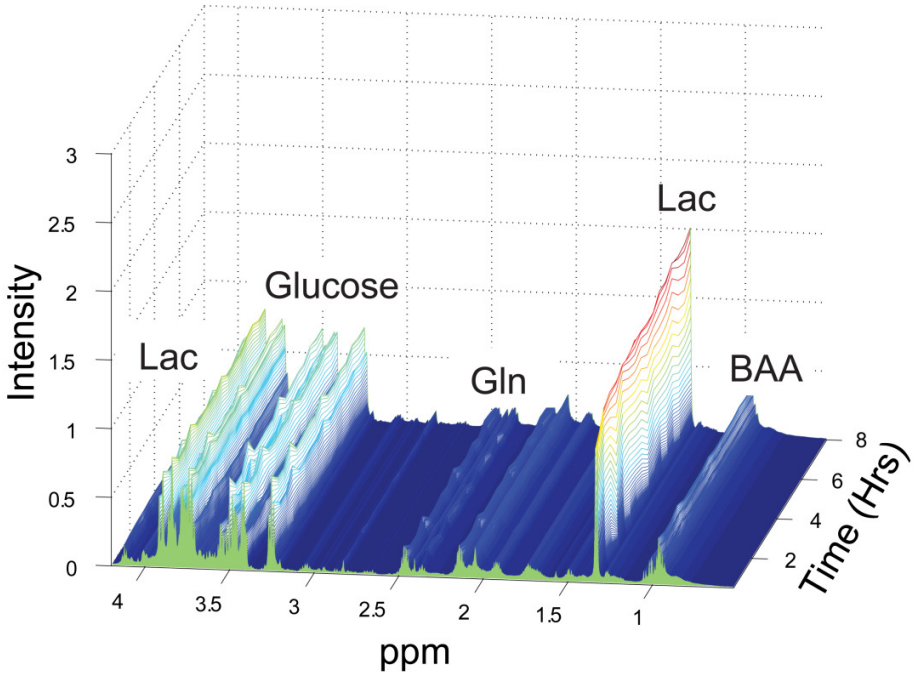
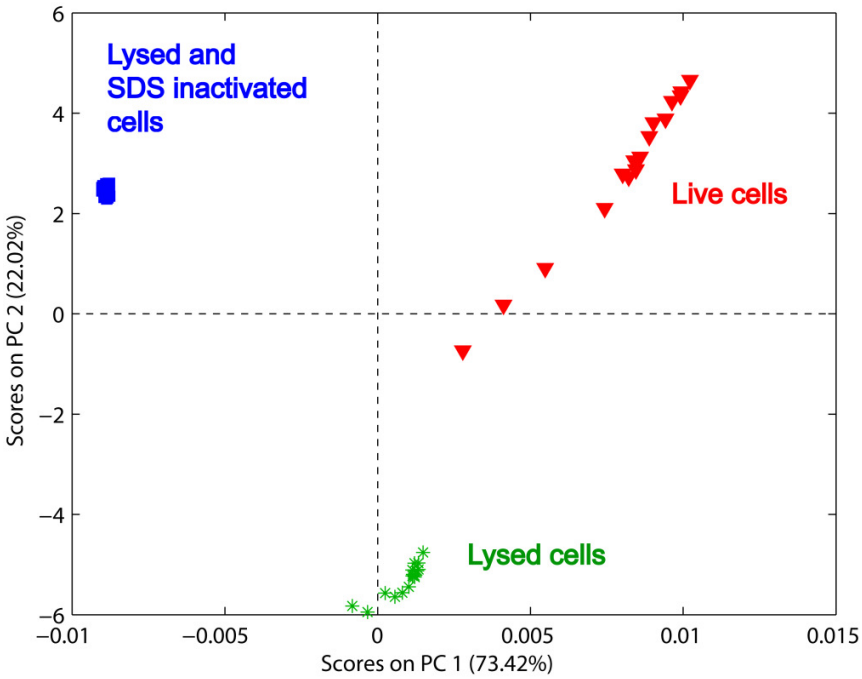


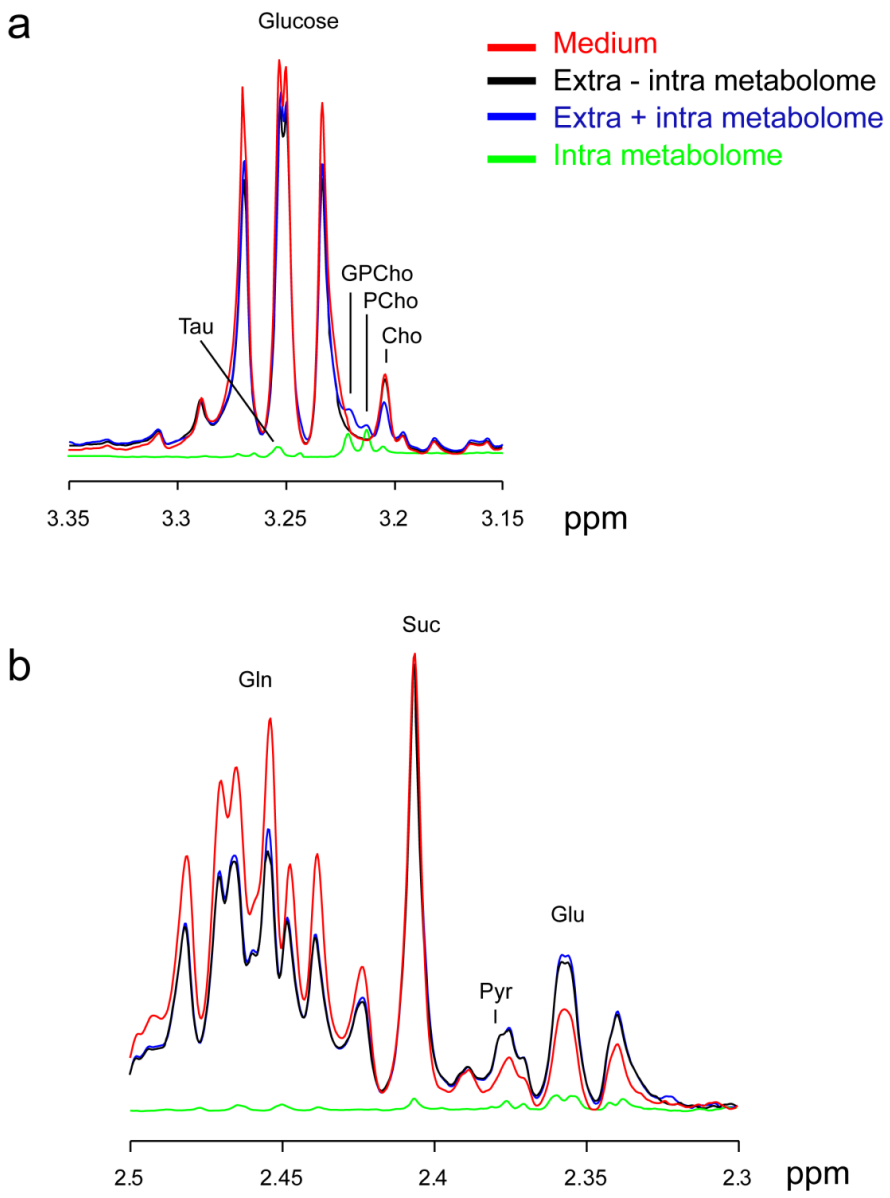
Supplementary Figure S1| Assessment of residual enzymatic activity following addition of different amounts of SDS to CCRF-CEM and SKOV-3 cell lines. Relative luminescence intensity after addition of different amounts of SDS (final concentration ranging from 0.01 to 2%) to (a) CCRF-CEM and (b) SKOV-3 cells. The luminescence intensities are normalized to the luminescence value of the sample without SDS. Each bar and the error bars represent the mean-normalized luminescence and the SEM of 6 independent replicates, respectively.



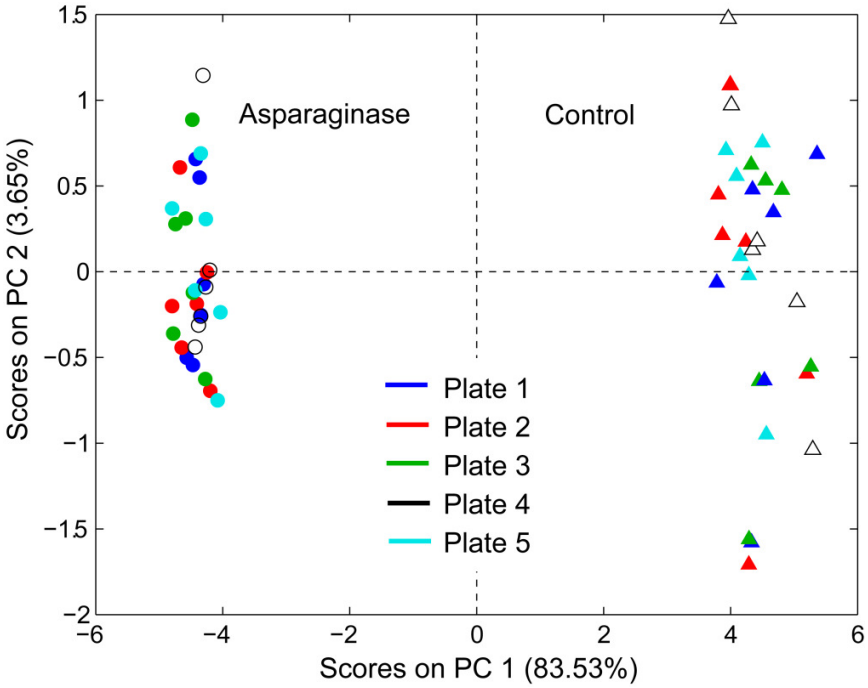
Supplementary Figure S2 | Assessment of residual metabolism in lysed CCRF-CEM cells. Series of 1D NMR spectra acquired every 30 minutes for a total of eight hours on cells lysed by ultrasonication without quenching the enzymatic activity. NMR signal intensities were normalized, as for the spectra in **Figure 1**(**b-c**), using the first spectrum of the time series (BAA, branched amino acids lactate; Gln, glutamine).



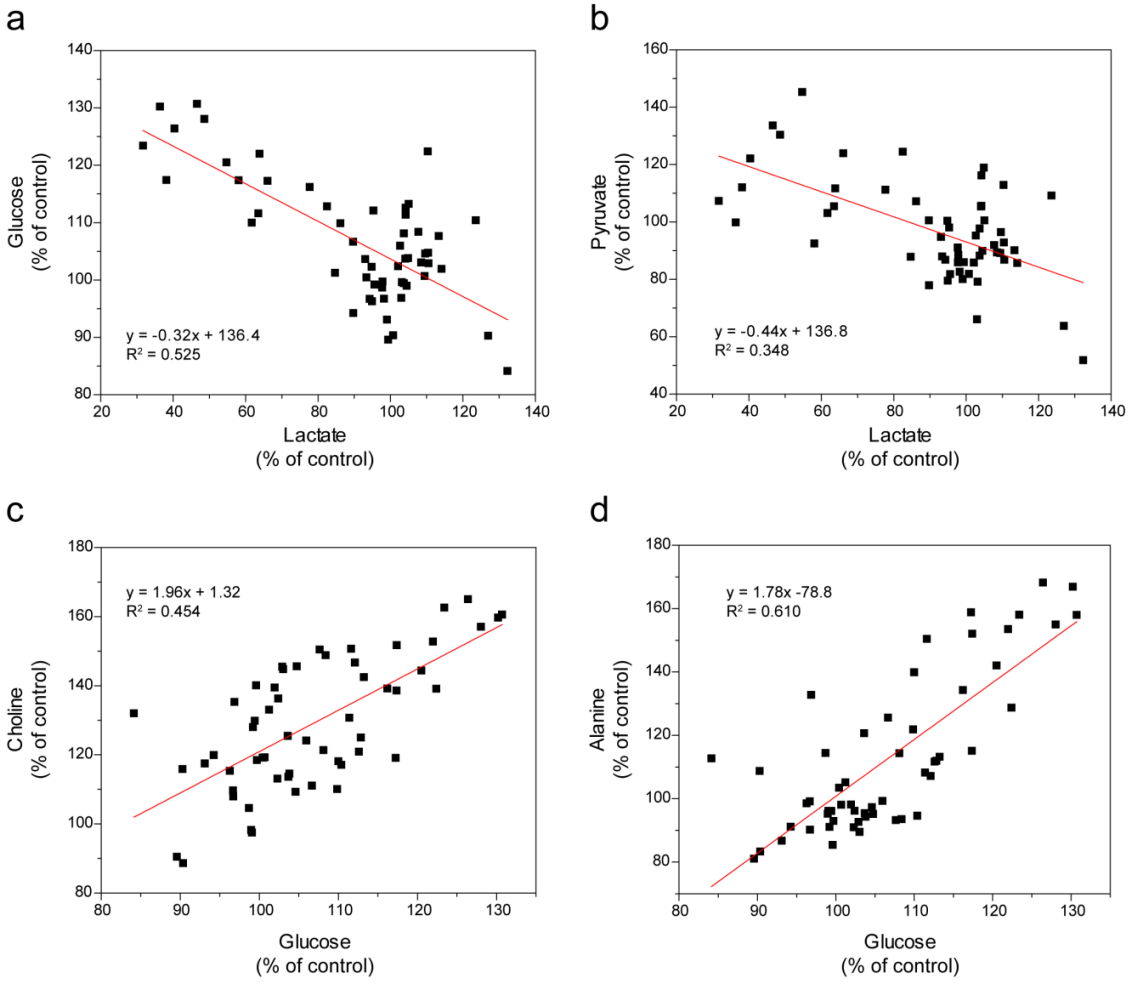
Supplementary Figure S3 | Assessment of residual metabolism in CCRF-CEM cells using multivariate analysis. Principal component analysis was performed on ^{1}H NMR spectra of CCRF-CEM cells acquired every 30 minutes over a period of 8 hours for live cells, cell lysed using only ultrasonication and cells simultaneously lysed and SDS inactivated. 16 spectra per condition were included in the multivariate analysis.



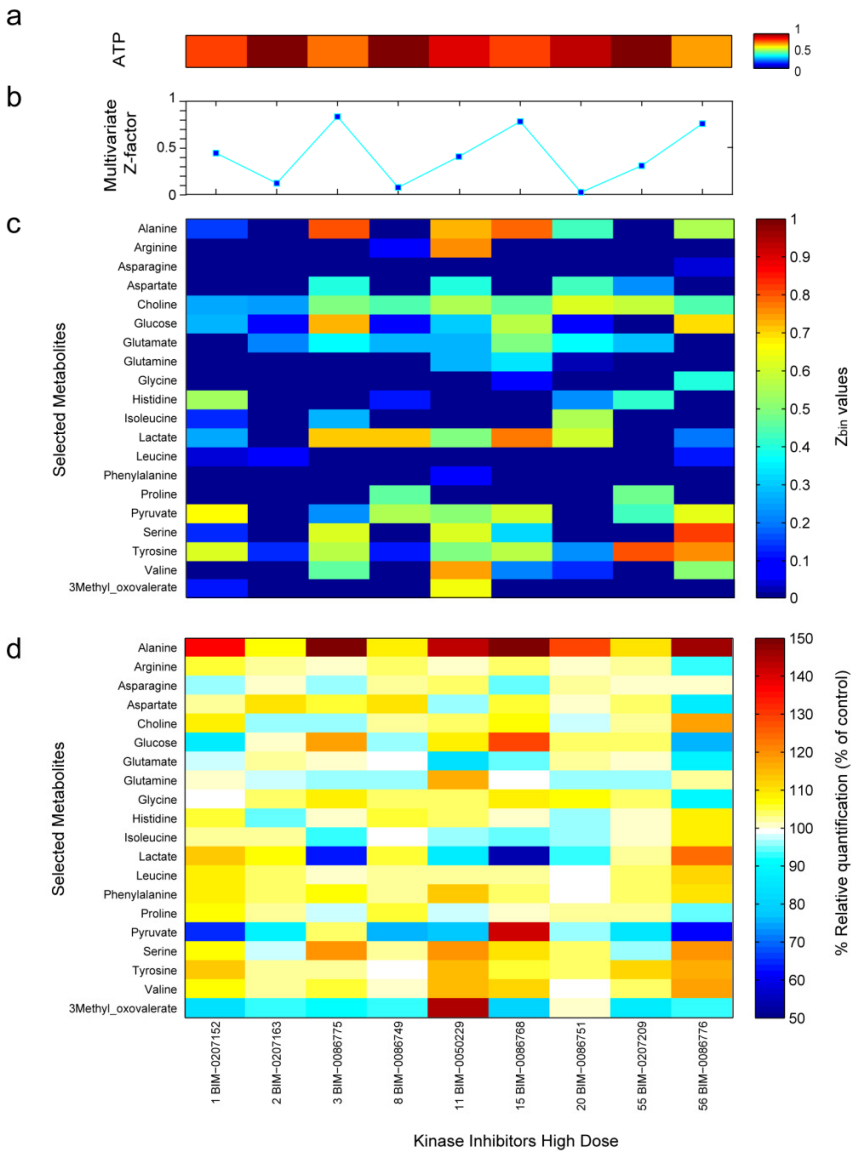
Supplementary Figure S4 | Contribution of intracellular metabolome to the NMR spectra acquired on the entire well content. NMR spectra were acquired on the entire well content (including both endo- and exo-metabolomes), on the media incubated in the same 96-well plate where cells were seeded (in an empty well, not seeded with cells) and on the exo-metabolome and endo-metabolome previously separated. Representative expanded sections of ^{1}H NMR spectrum show the individual contributes of exo- and/or endo-metabolome to the NMR spectrum acquired for the entire well content (Tau, taurine; Gluc, glucose; GPCho, glycerophosphocholine; PCho, phosphocholine; Cho, choline; Pyr, pyruvate; Gln, glutamine; Glu, glutamate; Suc, succinate).



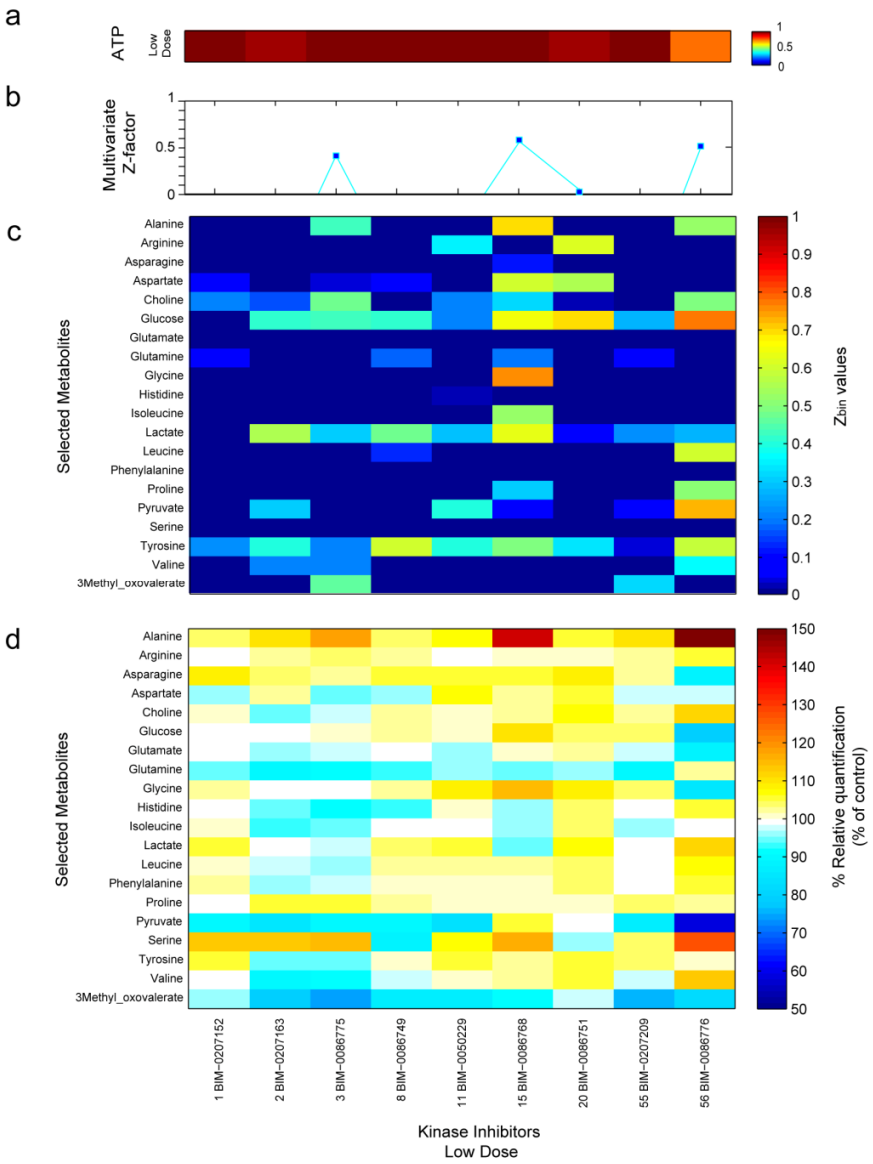
Supplementary Figure S5 | Assessment of plate-to-plate variability. PCA score plot (PC1 *versus* PC2) obtained from the analysis of the ^{1}H-NMR spectra acquired on simultaneously lysed and SDS-inactivated CCRF-CEM cell samples with and without asparaginase (1 u/ml) treatment. The cells were seeded and treated in randomized well positions, 6 wells per plate, over 5 different plates (in total 60 ^{1}H-NMR spectra, 30 replicates per treatment condition). We observed excellent grouping within each treatment condition and separation between the two groups along the first principal component (PC1 85.29%) without any relation to the plate of origin of the specific sample analyzed. Similarly also the higher principal components indicated no separation between samples prepared from different plates. In addition, the similar multivariate Z-factor values (calculated as detailed in Methods, **Eq. 2)** for each plate (**Supplementary Table 1)** support the robustness and the reproducibility of the method.



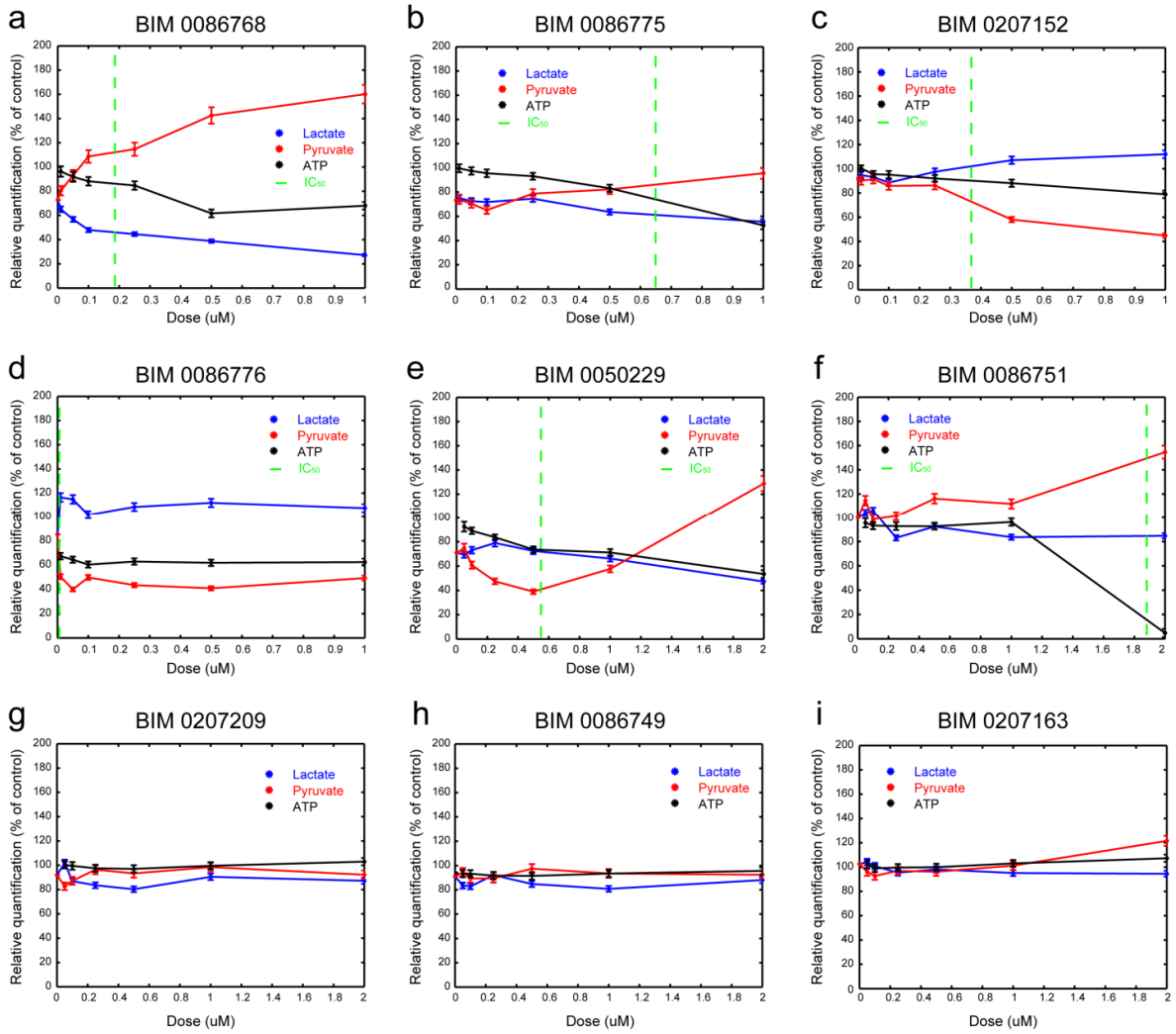
Supplementary Figure S6 | Pair-wise correlation of metabolites obtained screening a library of kinase inhibitors. Pair-wise correlation of relative concentrations (as percent of control) of selected metabolites obtained by screening CCRF-CEM cells following 24 hours of treatment with 56 KIs. **(a)** Correlation between lactate and glucose, **(b)** lactate and pyruvate, **(c)** glucose and choline and **(d)** glucose and alanine are shown.



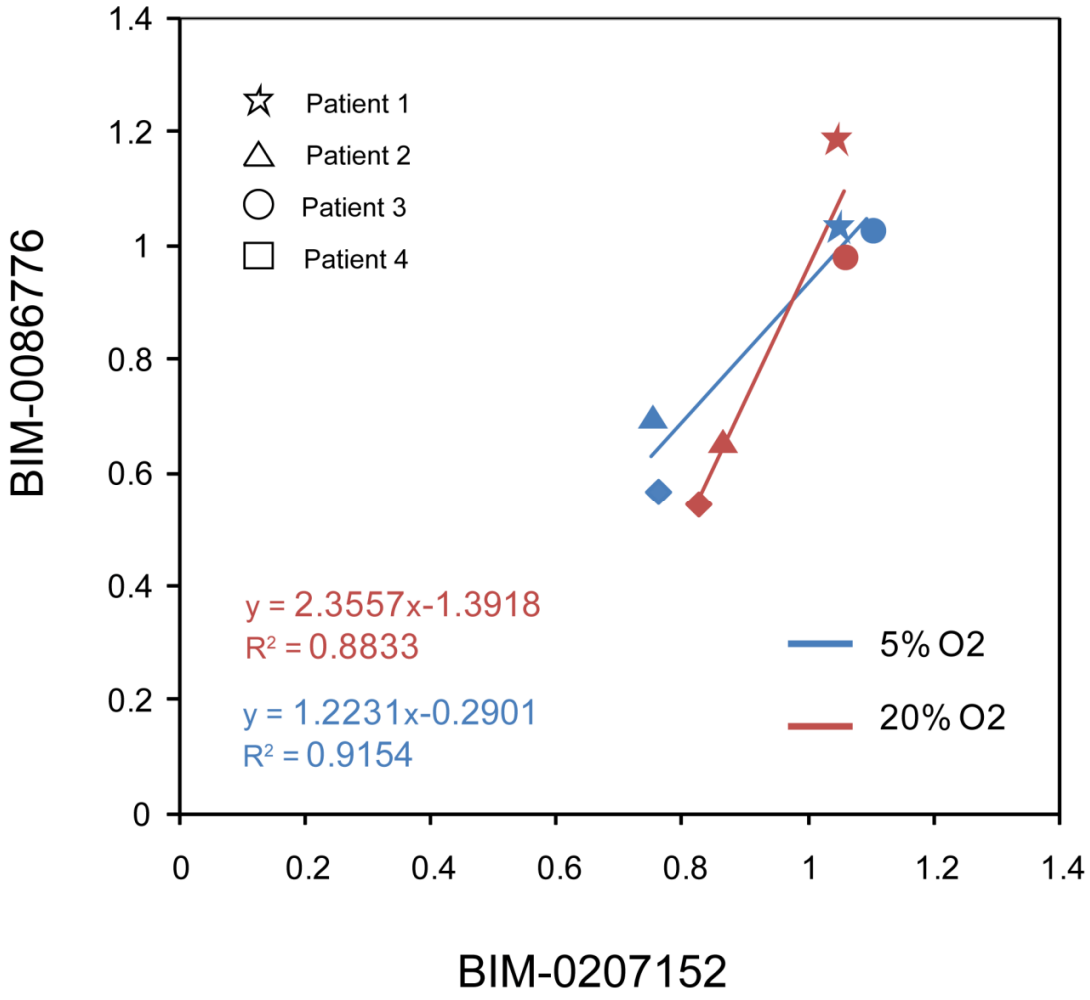
Supplementary Figure S7 | High-content NMR-based metabolomic screening of nine selected KIs at 1 µM as final concentration (high dose). (**a**) ATP assay was used to measure the cell viability of CCRF-CEM cells following 24 hours of treatment with 9 selected KIs. The drugs were eEF-2 kinase inhibitor (BIM-0207152), *5-Iodo-indirubin-3'*-monoxime (BIM-0207163), MK2a inhibitor (BIM-0086775), ERK Inhibitor II, FR180204 (BIM-0086749), PKC inhibitor (BIM-0050229), K-252a, Nocardiopsis sp. (BIM-0086768), *Fascaplysin, Synthetic* (BIM-0086751), Wee1 Inhibitor II (BIM-0207209), and NF-KB activation inhibitor (BIM-0086776) all administered at 1 µM (high dose). (**b**) Principal component analysis was performed on the ^{1}H NMR spectra acquired in triplicates and a multivariate Z-factor value was calculated for each KI. For a subset of all the observed metabolites we determined the Z_{bin} values (**c**) and their relative concentration (**d**, as percent of control).



Supplementary Figure S8 | High-content NMR-based metabolomic screening of nine selected KIs at 0.1 µM as final concentration (low dose). (**a**) ATP assay was used to measure the cell viability of CCRF-CEM cells following 24 hours of treatment with 9 selected KIs. The drugs were eEF-2 kinase inhibitor (BIM-0207152), *5-Iodo-indirubin-3'*-monoxime (BIM-0207163), MK2a inhibitor (BIM-0086775), ERK Inhibitor II, FR180204 (BIM-0086749), PKC inhibitor (BIM-0050229), K-252a, Nocardiopsis sp. (BIM-0086768), *Fascaplysin, Synthetic* (BIM-0086751), Wee1 Inhibitor II (BIM-0207209), and NF-KB activation inhibitor (BIM-0086776) all administered at 0.1 µM (low dose). (**b**) Principal component analysis was performed on the ^{1}H NMR spectra acquired in triplicates and a multivariate Z-factor value was calculated for each KI. For a subset of all the observed metabolites we determined the Z_{bin} values (**c**) and their relative concentration (**d**, as percent of control).



Supplementary Figure S9 | Dose response experiments at different concentrations of selected KIs. ATP assay and high-content NMR-based metabolomic screening were used to measure the cell viability and the metabolomic response of CCRF-CEM cells following 24 hours of treatment using six different concentrations of KI in the range of 0.05-2 µM. These doses are within the clinical relevant dose range for other KIs. ATP levels and relative concentrations of lactate and pyruvate calculated (as % of control) are reported as mean values +/- SEM. IC_{50} values were calculated based on a 50% reduction of ATP levels induced by treatment (**Supplementary Table S4**). The drugs were **a**) K-252a, Nocardiopsis sp. (BIM-0086768), **b**) MK2a inhibitor (BIM-0086775), **c**) EF-2 kinase inhibitor (BIM-0207152), **d**) NF-KB activation inhibitor (BIM-0086776), **e**) PKC inhibitor (BIM-0050229), **f**) *Fascaplysin, Synthetic* (BIM-0086751), **g**) Wee1 Inhibitor II (BIM-0207209), h) ERK Inhibitor II, FR180204 (BIM-0086749), and **i**) *5-Iodo-indirubin-3'*-monoxime (BIM-0207163). For BIM-0086749, BIM-0207163, BIM-0207209 the IC50 values were not determined as these KIs failed to cause changes not only in ATP levels but also in lactate and pyruvate at the considered treatment doses in CCRF-CEM cells.



Supplementary Figure S10 | Cell viability of acute lymphoblastic leukemia primary cells from four patients following 24 hours of NF-kB activation and eEF-2 kinase inhibitor treatments. ATP measurements were performed on acute lymphoblastic leukemia primary cells from four patients. The cells were seeded for 24 hours in a 384-well plate at two different oxygen levels (20% and 5%) following individual treatment using a library of 246 kinase inhibitors (final concentration 1µM). Relative luminescence values were calculated by dividing the luminescence value of each sample by that of primary cells within the same plate receiving solvent control. The two kinase inhibitors BIM-0086776 (NF-kB activation inhibitor) and BIM-0207152 (eEF-2 kinase inhibitor) depicted similar response in the cellular survival regardless of the oxygen level in the environment. The Pearson correlation coefficient of the drug responses was 0.93983 and 0.95677 at 20% and 5% oxygen culture, respectively.

Supplementary Table S1. List of metabolites detected in CCRF-CEM cells using the NMR-based high content screening method. Metabolites are divided based on whether they are detectable either only in the extracellular or in both extra- and intracellular metabolome of CCRF-CEM cells contained in one well of a 96-well plate.

Extracellular	Intracellular	Extracellular + intracellular
2-Oxoisocaproate	-	2-Oxoisocaproate
3-Methyl-2-oxovalerate	-	3-Methyl-2-oxovalerate
-	AMP	-
-	ATP	-
Acetate	Acetate	Acetate
Acetone	Acetone	Acetone
Alanine	Alanine	Alanine
Arginine	-	
Asparagine	Asparagine	Asparagine
Aspartate	Aspartate	Aspartate
Choline	Choline	Choline
Creatine	-	Creatine
Cystine	-	Cystine
Ethanol	Ethanol	Ethanol
Formate	Formate	Formate
Fructose	-	Fructose
Fumarate	-	Fumarate
Glucose	Glucose	Glucose
Glutamate	Glutamate	Glutamate
Glutamine	Glutamine	Glutamine
-	Glycero-3-phosphocholine	Glycero-3-phosphocholine
Glycine	Glycine	Glycine
Histidine	-	Histidine
Isoleucine	-	Isoleucine
Lactate	Lactate	Lactate
Leucine	-	Leucine
Lysine	-	Lysine
Myo-Inositol	Myo-Inositol	Myo-Inositol
Methanol	Methanol	Methanol
Methionine	-	Methionine
Niacinamide	-	Niacinamide
Ornithine	-	Ornithine
Panthotenate	-	Panthotenate
Phenylalanine	-	Phenylalanine
-	Phosphocholine	Phosphocholine
Proline	-	Proline
Propionate	-	Propionate
Pyridoxine	-	Pyridoxine
Pyruvate	-	Pyruvate
Serine	-	Serine
Succinate	Succinate	Succinate
-	Taurine	-*
Threonine	-	Threonine
Tryptophan	-	Tryptophan
Tyrosine	-	Tyrosine
Valine	-	Valine
trans-4-Hydroxy-L-proline	-	trans-4-Hydroxy-L-proline
*Taurine covered by glucose signals of the medium		

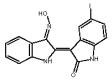
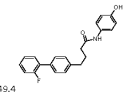
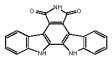
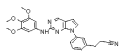
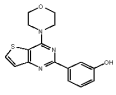
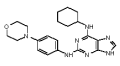
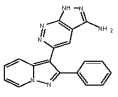
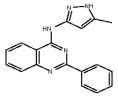
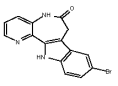
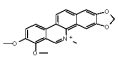
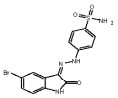
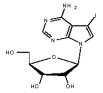
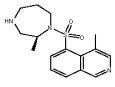
Supplementary Table S2. Comparison of percent variability explained by the first two components (PC1 % and PC2 %) of the principal component analysis and multivariate Z-factor values (Z, calculated using equation 2) of one-dimensional (1D) ^{1}H NMR spectra acquired on CCRF-CEM leukemia cells as solvent control and treated asparaginase at A1, 1 u/ml for 5 different plates.

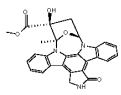
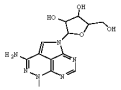
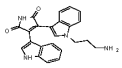
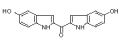
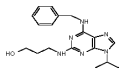
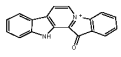
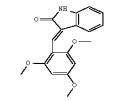
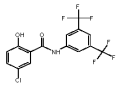
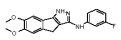
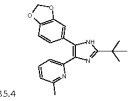
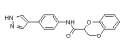
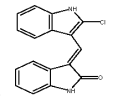
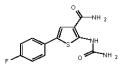
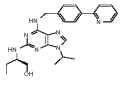
	PC1 %	PC2 %	Z
Plate1	87.77	3.97	0.90
Plate2	88.04	3.06	0.82
Plate3	88.14	3.93	0.77
Plate4	85.97	4.023	0.83
Plate5	87.55	4.86	0.84
All Plates	83.53	3.65	0.79

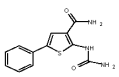
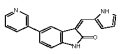
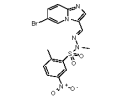
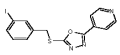
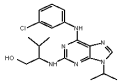
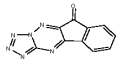
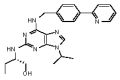
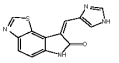
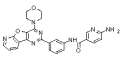
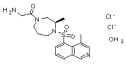
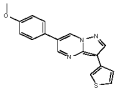
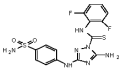
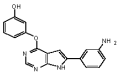
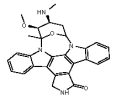
Supplementary Table S3. Comparison of percent variability explained by the first two components (PC1 % and PC2 %) of the principal component analysis and multivariate Z factor values (Z, calculated using equation 2) of ^{1}H NMR spectra acquired using one-dimensional (1D), CPMG and 1D projections of 2D JRES spectra (pJRES) for CCRF-CEM leukemia cells treated with dexamethasone (Dex), dichloroacetate (DCA), rapamycin, (Rap) vincristine (Vin) and asparaginase at three different doses (A1, 1 u/ml; A01, 0.1 u/ml; A001, 0.01 u/ml).

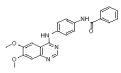
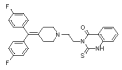
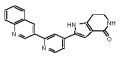
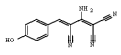
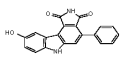
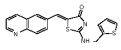
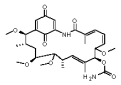
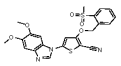
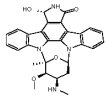
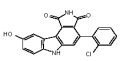
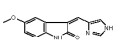
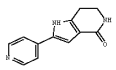
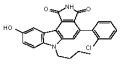
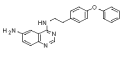
Drug Tr.	**1D**			**CPMG**			**pJRES**		
	PC1 %	PC2 %	Z	PC1 %	PC2 %	Z	PC1 %	PC2 %	Z
Dex	51.98	31.93	-1.86	58.80	26.40	0.15	64.01	10.15	0.41
DCA	90.57	6.42	0.86	94.60	3.16	0.91	93.14	2.13	0.90
Rap	70.00	19.49	0.60	79.63	10.87	0.74	79.91	5.88	0.72
Vin	58.40	30.92	-2.62	59.73	16.17	-1.97	55.81	12.38	0.47
A1	87.77	3.97	0.90	89.67	5.32	0.91	91.27	2.28	0.90
A01	88.12	7.30	0.75	92.04	3.69	0.86	90.86	2.96	0.88
A001	87.94	7.33	0.68	91.17	3.64	0.84	91.02	2.83	0.79

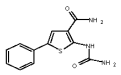
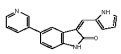
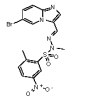
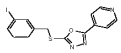
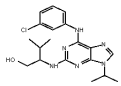
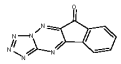
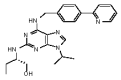
Supplementary Table S4. List of 56 kinase inhibitors screened.

Compound ID	Formula	Mol Wt	Structure	Compound Vendor	Vendor Compound ID	Salt Form	Real Mol Wt	Comments
BIM-0207152	C27H45N2	397.66		Calbiochem	324515	HYDROIODIDE SALT	524.6	eEF-2 Kinase Inhibitor, NH125
BIM-0207163	C16H10IN3	403.17		Calbiochem	402086		403.2	Indirubin-3'-monoxime, 5-Iodo-
BIM-0086775	C22H20FN3	349.4		EMD Biosciences	475863			MK2a Inhibitor
BIM-0207133	C20H11N3O	325.32		Calbiochem	181305		325.3	Acriflavin A, Synthetic
BIM-0207143	C24H23N5O	429.47		Calbiochem	218713		429.5	Keratinocyte Differentiation Inducer
BIM-0207183	C16H15N3O	313.37		Calbiochem	528111	Chloride SALT	349.8	PI 3-K? Inhibitor IV
BIM-0207192	C21H27N7	393.49		Calbiochem	554717		393.5	Reversine
BIM-0086749	C18H13N7	327.34		EMD Biosciences	328007			ERK Inhibitor II, FR180204
BIM-0086759	C18H15N5	301.35		EMD Biosciences	361555			GSK-3 Inhibitor XIII
BIM-0207134	C15H10BrN	328.16		Calbiochem	191500		328.2	1-Azakenpaullone
BIM-0050229	C21H18NO	348.37		LOPAC	C 2932	CHLORIDE SALT		PKC inhibitor; affects translocation of PKC from cytosol to plasma membrane
BIM-0207144	C14H11BrN	395.23		Calbiochem	219445		395.2	Cdk2 Inhibitor II
BIM-0207165	C11H13IN4	392.15		Calbiochem	407900		392.1	5-Iodotubercidin
BIM-0207193	C16H21N3	319.42		Calbiochem	555550	Chloride SALT	355.9	Rho Kinase Inhibitor

BIM-0086768	C27H21N3O	467.47		EMD Biosciences		420298		K-252a, Nocardiopsis sp.
BIM-0053458	C13H16N6O	320.3				154020		
BIM-0207135	C23H20N4O	384.43		Calbiochem		203294 Chloride SALT	420.9	Bisindolylmaleimide III, Hydrochloride
BIM-0086675	C17H12N2O	292.29		EMD Biosciences		343021		Flt-3 Inhibitor II
BIM-0086735	C18H24N6O	340.42		EMD Biosciences		203600		Bohemine
BIM-0086751	C18H11N2O	271.29		EMD Biosciences		341251 Chloride SALT		Fascaplysin, synthetic
BIM-0050990	C18H17NO5	311.33		LOPAC	I0658			Casein kinase-1 (CK-1 delta/epsilon) inhibitor.
BIM-0207156	C15H8ClF6NO	383.67		Calbiochem		401482	383.7	IKK-2 Inhibitor V
BIM-0086693	C18H16FN3O2	325.34		EMD Biosciences		521233		PDGF Receptor Tyrosine Kinase Inhibitor IV
BIM-0086716	C20H21N3O2	335.4		EMD Biosciences		616453 Chloride SALT		TGF-b RI Inhibitor III
BIM-0207195	C18H15N3O3	321.33		Calbiochem		555555	321.3	Rho Kinase Inhibitor V
BIM-0086736	C17H11ClN2O	294.74		EMD Biosciences		217695		Cdk1 Inhibitor
BIM-0086762	C12H10FN3O2S	279.29		EMD Biosciences		401481		IKK-2 Inhibitor IV
BIM-0207137	C24H29N7O	431.53		Calbiochem		203881	431.5	CR8, (R)-Isomer

BIM-0207157	C12H11N3O2	261.3		Calbiochem	401483	261.3	IKK-2 Inhibitor VI
BIM-0086686	C18H13N3O	287.32		EMD Biosciences	420126	METHANESULFONATE (MESYLATE) SA	JAK3 Inhibitor VI
BIM-0207186	C16H14BrN5O4S	452.28		Calbiochem	528116	H2O	470.3 PI 3-K? Inhibitor VIII
BIM-0086753	C14H10IN3OS	395.22		EMD Biosciences	361541		GSK-3b Inhibitor II
BIM-0086780	C19H25ClN6O	388.89		EMD Biosciences	540500		Purvalanol A
BIM-0086695	C10H4N6O	224.18		EMD Biosciences	521275		PDK1/Akt/Flt Dual Pathway Inhibitor
BIM-0207138	C24H29N7O	431.53		Calbiochem	203882	431.5	CR8, (S)-Isomer
BIM-0086696	C13H8N4O	268.29		EMD Biosciences	527450		PKR Inhibitor
BIM-0207178	C25H21N7O4	467.48		Calbiochem	524611	467.5	PIKfyve Inhibitor
BIM-0086708	C18H26Cl2N6O4S	465.39		EMD Biosciences	555554		Rho Kinase Inhibitor IV
BIM-0086723	C17H13N3OS	307.37		EMD Biosciences	676489		VEGF Receptor 2 Kinase Inhibitor IV
BIM-0086737	C15H13F2N7O2S	425.44		EMD Biosciences	217714		Cdk1/2 Inhibitor III
BIM-0086758	C18H14N4O	318.33		EMD Biosciences	361554		GSK-3b Inhibitor XII, TWS119
BIM-0086710	C28H26N4O3	466.53		EMD Biosciences	569397		Staurosporine, Streptomyces sp.

	BIM-0086659	C23H20N4O	400.43	EMD Biosciences	189404		Aurora Kinase Inhibitor II
	BIM-0086668	C28H25F2N	489.58	EMD Biosciences	266788		Diacylglycerol Kinase Inhibitor II
	BIM-0207170	C21H16N4O	340.38	Calbiochem	475864 H2O	358.4	MK-2 Inhibitor III
	BIM-0051107	C13H8N4O	236.23	LOPAC	T 6943		Protein tyrosine kinase inhibitor
	BIM-0207207	C20H12N2O	328.32	Calbiochem	681637 H2O	346.3	Wee1/Chk1 Inhibitor
	BIM-0207140	C18H13N3O	351.45	Calbiochem	217699	351.4	Cdk1 Inhibitor IV, RO-3306
	BIM-0086681	C30H42N2O	574.66	EMD Biosciences	375670		Herbimycin A, Streptomyces sp.
	BIM-0207160	C22H19N3O	469.53	Calbiochem	401488	469.5	IKK-3 Inhibitor IX
	BIM-0207189	C28H26N4O	482.53	Calbiochem	539644	482.5	UCN-01
	BIM-0207208	C20H11ClN	362.77	Calbiochem	681640	362.8	Wee1 Inhibitor
	BIM-0086789	C13H11N3O	241.25	EMD Biosciences	572650		SU9516
	BIM-0206950	C12H11N3O	213.24	Calbiochem	217707	213.2	
	BIM-0207209	C24H19ClN	418.87	Calbiochem	681641	418.9	Wee1 Inhibitor II
	BIM-0086776	C22H20N4O	356.42	EMD Biosciences	481406		NF-KB Activation Inhibitor

BIM-0207157	C12H11N3O2	261.3		Calbiochem	401483	261.3	IKK-2 Inhibitor VI
BIM-0086686	C18H13N3O	287.32		EMD Biosciences	420126	METHANESULFONATE (MESYLATE) SA	JAK3 Inhibitor VI
BIM-0207186	C16H14BrN5O3S	452.28		Calbiochem	528116	H2O	470.3 PI 3-K? Inhibitor VIII
BIM-0086753	C14H10IN3S	395.22		EMD Biosciences	361541		GSK-3b Inhibitor II
BIM-0086780	C19H25ClN6O	388.89		EMD Biosciences	540500		Purvalanol A
BIM-0086695	C10H4N6O	224.18		EMD Biosciences	521275		PDK1/Akt/Flt Dual Pathway Inhibitor
BIM-0207138	C24H29N7O	431.53		Calbiochem	203882	431.5	CR8, (S)-Isomer

Supplementary Table S5. IC_{50} values obtained from the dose response experiments performed using 6 different concentrations of 9 KIs in a clinically relevant range (0.05-2 μM). IC_{50} values were determined as the treatment dose that induced a 50% reduction of ATP levels. No ATP level-dependent inhibition was observed following treatment of CCRF-CEM cells with BIM-0086749, BIM-0207163, BIM-0207209 in the range of doses used in this study.

Kinase Inhibitors	IC_{50} (μM) ± SEM
BIM 0086768	0.190 ± 0.0078 μM
BIM 0086775	0.646 ± 0.0180 μM
BIM 0207152	0.374 ± 0.0281 μM
BIM 0086776	0.006 ± 1.275 e-^{04} μM
BIM 0050229	0.545 ± 0.0087 μM
BIM 0086751	1.879 ± 0.055 μM
BIM 0207209	No Effect
BIM 0086749	No Effect
BIM 0207163	No Effect

Supplementary Methods

miRNA transfections

For transfection of HeLa cells, miR16 and miR122 precursors and pre-miR (as negative control; Applied Biosystems, TX) were used. The cells were transfected with TransIT-TKO (Mirus Bio Corp., WI) according to the manufacturer's instruction. Fifty μl of transfection mixture containing 50 nM of miRNAs and TransIT-TKO transfection reagent in Opti-mem (Invitrogen, CA) was incubated for 20-25 minutes at room temperature and added into each well. The cells were cultured with the transfection mixture for 24 hours before proceeding to the metabolomic assays. All the experiments were performed in 6 replicates per condition.

ATPlite luminescence measurements

ATP Detection Assay (Perkin Elmer, MA) was used according to manufacturer's instruction to quantify the viability of cells in each well. Briefly, 50 μL of the reagent were added to each well, and thoroughly mixed for 2 minutes at 600 rpm using a plate shaker. Luminescence was measured using Biosystems Analyst HT microplate reader (LJL Biosystems, Inc., Sunnyvale, CA). Relative luminescence values were calculated by dividing the luminescence value of each treated sample by that of cells within the same plate receiving solvent control (negative controls).

Multivariate Z factor and Z_{bin} factor

The projected value $P_i^{C/T}$ for the *i*-th sample was calculated as a weighted sum of the solvent control (C) or drug treatment (T) spectral data values $x_{ij}^{C/T}$ for all the j data points (np) of the

NMR spectra. The weights are obtained from the loadings values of the first principal component of a principal component analysis (PCA).

$$P_i^{C/T} = \sum_{j=1}^{np} L_j^1 \cdot x_{ij}^{C/T} \qquad \textbf{(S1)}$$

The multivariate Z factor is then calculated as:

$$Z = 1 - 3 \cdot \frac{\sigma^C + \sigma^T}{\left|\mu^C - \mu^T\right|} \qquad \textbf{(S2)}$$

Where μ^C and μ^T, and σ^C and σ^T are the means and standard deviations of P^C (solvent control) and P^T (drug treatment), respectively. The Z-factor can have values within the range of $-\infty \leq Z \leq 1$; Z-factor values between 0.5 and 1 indicate an excellent separation of groups; $0 \leq Z \leq 0.5$ indicates moderate separation while $Z = 0$ points to poor separation. For $Z < 0$, the method is not suitable for screening.

Alternatively, a calculation of Z_{bin} factor values for every point/bin (*i*) of the spectrum can be calculated as follows:

$$Z_{bin} = 1 - 3 \cdot \frac{\sigma_i^C + \sigma_i^T}{\left|\mu_i^C - \mu_i^T\right|} \qquad \textbf{(S3)}$$

where μ_i^C and μ_i^T, and σ_i^C and σ_i^T are the means and standard deviations at each point/bin (*i*) of the NMR spectra for solvent control (C) and drug treated (T) samples, respectively.