Targeting the endoplasmic reticulum-mitochondria interface sensitizes leukemia cells to cytostatics

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 doi:10.3324/haematol.2018.197368

Received: May 9, 2018.

Accepted: October 4, 2018.

Pre-published: October 11, 2018.

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Targeting the ER-Mitochondria Interface Sensitizes Leukemia Cells Towards Cytostatics

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SUPPLEMENTARY METHODS

Compounds
PS89 was synthesized as described previously.(1) The PS89 photo probe was labeled with a rhodamine reporter dye by click chemistry (5-TAMRA-Azide; Jena Bioscience, Jena, Germany). Etoposide, daunorubicin, 6-mercaptopurine, dexamethasone and vincristine were purchased from Sigma Aldrich (St Louis, MO, USA). ABT-199 was obtained from LKT Laboratories (St Paul, MN, USA), Z-IETD-FMK from R&D Systems (Minneapolis, MN, USA) and QVD-OPh from Merck Millipore (Darmstadt, Germany).

Apoptosis assay
Apoptosis was determined according to Nicoletti *et al*.(2) In brief, stimulated cells were stained with 50 µg/ml propidium iodide (PI, Sigma Aldrich) in 0.1% Triton X-100 permeabilization buffer and percentage of apoptotic cells at subG1 was determined using a FACSCanto II flow cytometer (BD, Franklin Lakes, NJ, USA) and FlowJo software v7.6.5 (Tree Star, Ashland, OR, USA). Apoptosis of daunorubicin treated HL-60 cells was determined using YO-PRO-1 nucleic acid stain.
PDX cells and PBMCs were analyzed with identical equipment and the percentage of viable or apoptotic cells, respectively, was determined by forward/side scatter (FSC/SSC) gating as previously described (3). For cell death analysis of CD34+ cells, PBMCs were isolated and stained with FITC conjugated-anti-CD34 antibody. Propidium iodide was used to determine the cell death of at least 25000 CD34 positive cells by flow cytometry. Specific apoptosis was calculated as follows: [(experimental apoptosis (%) - spontaneous apoptosis (%)) / (100% - spontaneous apoptosis (%))] x 100

Proliferation assay
Cells were allowed to proliferate for 72 h in presence or absence of stimulants and stained with CellTiter-Blue reagent (Promega, Fitchburg, WI, USA) for 4 h. Fluorescence was measured on a SpectraFluor Plus microplate reader (Tecan, Männedorf, Switzerland) and normalized towards DMSO control.

Western Blot
Chemiluminescent western blotting was performed according to standard procedures. Protein amount was quantified by BCA assay (Uptima BC Assay Kit, Interchim, Montlucon, France) and equal protein load was determined by actin staining or stainfree detection (4) using a ChemiDoc Touch Imaging System (Bio-Rad, Hercules, CA, USA), as indicated. Proteins were transferred to Amersham PVDF membranes (GE Healthcare) by tank blotting. Densitometric quantification of the band intensity of 3 independent western blot experiments was performed by using Image J software.

Antibodies for Western blot

Primary antibody	Origin	Supplier
Actin Clone C4	Mouse	Millipore, Darmstadt, Germany
BAP31 B-10	Mouse	Santa Cruz, Dallas, TX, USA
BAP31 C-15	Goat	Santa Cruz, Dallas, TX, USA
Bcl-2 2872	Rabbit	Cell Signaling, Danvers, MA, USA
Bcl-xL 2762	Rabbit	Cell Signaling, Danvers, MA, USA
Caspase-3, Active C8487	Rabbit	Sigma Aldrich, St Louis, MO, USA
Caspase-8 1C12	Mouse	Cell Signaling, Danvers, MA, USA
Cytochrome c 4272	Rabbit	Cell Signaling, Danvers, MA, USA
PARP 9542	Rabbit	Cell Signaling, Danvers, MA, USA
PDI C81H6	Rabbit	Cell Signaling, Danvers, MA, USA
VDAC 4866	Rabbit	Cell Signaling, Danvers, MA, USA

Secondary antibody	Origin	Supplier
Anti-goat IgG, HRP 705-035-147	Donkey	Dianova, Hamburg, Germany
Anti-mouse IgG, HRP 7076	Goat	Cell Signaling, Danvers, MA, USA
Anti-rabbit IgG, HRP 172-1019	Goat	Bio-Rad, Hercules, CA, USA

Transfection
Gene silencing was performed using GenaxxoFect reagents (Genaxxon, Ulm, Germany) according to manufacturer's instructions and ON-TARGET*plus* SMARTpool siRNA (GE Dharmacon, Lafayette, CO, USA) against human PDIA1 and BCAP31. Overexpression of PDIA1 was performed with FuGene HD reagent (Promega, Fitchburg, WI, USA). PDI vector was kindly provided by W. Ou (Bethesda, MD, USA).(5)

Target network analysis
Protein-protein interaction network analysis was performed using STRING v10 (6) with subsequent refinement of functional enrichment by Gene Ontology (GO) classification.(7)

Confocal microscopy
Hela cells were incubated with the PS89 photo probe followed by UV crosslinking to cellular targets at 365 nm and coupling of a rhodamine reporter dye by click chemistry (5-TAMRA-Azide; Jena Bioscience, Jena, Germany). BAP31 was subsequently stained using anti-BAP31 HPA003906 (Sigma Aldrich) and goat anti-rabbit Alexa 488 (Thermo Fisher, Waltham, MA, USA) according to Prestige Antibody IF procedure. Confocal microscopy was performed on a Leica SP8 LSM system (Leica, Wetzlar, Germany) and co-localization was evaluated using Leica LAS X software.

Fluorescence Correlation Spectroscopy (FCS)
FCS measurements were performed on a Leica TCS SP8 SMD microscope combined with a Picoquant LSM Upgrade Kit. For all measurements, 63x Zeiss water immersion lens and ibidi 8 well µ-slides with glass bottoms were used. The effective volume (V_{eff}) and structure parameter (κ) were measured at the start of each experiment using 1nM ATTO488 dye solution (ATTO-TEC GmbH, Siegen, Germany). Ten or more different points were measured in every well for 45 s per point. The samples included two different concentrations (50 nM and 250 nM) of the freely diffusing PS89 probe in buffer and two different concentration ratios [S1: 50nM/50nM, (1:1), S2: 50 nM/200 nM, (1:4)] of the PS89 plus the recombinant human BAP31 protein (Abcam plc, Cambridge, UK), respectively. All solutions were diluted using 50 mM Tris-HCL, pH = 8 buffer with a 5% DMSO. All concentrations were also verified with nanodrop spectrophotometer. FCS curves were analyzed using the Picoquant SymPhoTime V 5.2.4.0 software. Control measurements to determine the diffusion time and concentration of PS89 were fitted with a single diffusing species and a triplet state (eq. 1). Subsequent measurements to determine the diffusion time and concentration of PS89+BAP31, were fitted for two diffusing species and a triplet state (eq. 1). In this case, one of the two species diffusion times was confined to the diffusion time of PS89 obtained in the control experiments.

$$G_{3D}(\tau) = \frac{1}{N} \cdot \left(1 + \frac{T}{1-T} e \frac{\tau}{\tau_t}\right) . \left(1 + \frac{4D \cdot \tau}{{\omega_r}^2}\right)^{-1} \cdot \left(1 + \frac{4D \cdot \tau}{{\omega_z}^2}\right)^{-\frac{1}{2}}$$

$$V_{Eff,3D} = \pi^{\frac{3}{2}} . {w_0}^2 . z_0 \quad ; \quad D_{3D} = \frac{{w_0}^2}{4\tau} \quad ; \quad < C > = \frac{<N>}{V_{Eff} \cdot N_A} \quad (1)$$

Co-immunoprecipitation
Preparation of cell lysates (Triton-X lysis buffer, 500 µg protein per sample determined by BCA assay) and co-IP using the µMACS Protein G MicroBeads kit (Miltenyi Biotech, Bergisch Gladbach, Germany) was performed according to manufacturer's instructions. Precipitation: Goat anti-BAP31 C-15 (Santa Cruz). Detection: Mouse anti-BAP31 B-10 (Santa Cruz) and Mouse anti-CASP8 1C12 (Cell Signaling).

Flow cytometric analysis of calcium, MMP and ROS
The following dyes were used for fluorescence staining. Calcium: Cal-520 (AAT Bioquest, Sunnyvale, CA, USA); Mitochondrial membrane potential (MMP): JC-1 (Enzo, Farmingdale, NY; USA); Reactive oxygen species (ROS): Carboxy-H_2DCFDA (Thermo Fisher). PI counterstaining was used to exclude

dead cells. Sample preparation was performed according to manufacturers' instructions and cells were analyzed on a FACSCanto II flow cytometer (BD).

Cell cycle analysis
Cellular DNA content was examined by propidium iodide staining and flow cytometry.(2) Cell cycle analysis was performed using FlowJo software v7.6.5 (Tree Star, Ashland, OR, USA).

Colony formation assay
Jurkat and VCR-R CEM were stimulated for 4 h, washed with PBS and reseeded at a density of 5.000 cells/ml in 0.4% methylcellulose and 40% FCS supplemented medium to grow into colonies. After 7 or 5 days of proliferation, respectively, colonies were stained with MTT (0.25 mg/ml) for 3 h. Images of each well were analyzed with ImageJ software (open source) and the number of colony forming units (CFU) per well was normalized towards DMSO control. HL-60 cells were treated for 24h with PS89 and 6-MP, washed and reseeded (2000 cells/ml) in methylcellulose medium (Human Methylcellulose complete media, R&D Systems, Minneapolis, MN, USA). Clonogenic growth was monitored by counting the colonies after 7 days, respectively.

Data collection and statistics
Data from at least three independent experiments are expressed as mean ± SEM and statistical analysis was performed with GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA). For Western blot and confocal microscopy, representative images of at least three independent data sets are shown. Synergism was calculated according to the Bliss independence model (8) as described in the following equitation: $Y_P = Y_{ab}/(Y_a + Y_b - Y_aY_b)$, where Ya is the cytotoxic effect of drug a and Yb the effect of drug b. $Y_P > 1$: synergy, $Y_P < 1$: antagonism, $Y_P = 1$: additivity.

SUPPLEMENTARY REFERENCES

1. Eirich J, Braig S, Schyschka L, et al. A small molecule inhibits protein disulfide isomerase and triggers the chemosensitization of cancer cells. Angewandte Chemie International Edition. 2014;53(47): 12960-12965.
2. Nicoletti I, Migliorati G, Pagliacci M, et al. A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. Journal of immunological methods. 1991;139(2): 271-279.
3. Ehrhardt H, Schrembs D, Moritz C, et al. Optimized anti–tumor effects of anthracyclines plus Vinca alkaloids using a novel, mechanism-based application schedule. Blood. 2011;118(23): 6123-6131.
4. Ladner CL, Yang J, Turner RJ, et al. Visible fluorescent detection of proteins in polyacrylamide gels without staining. Analytical biochemistry. 2004;326(1): 13-20.
5. Ou W, Silver J. Role of protein disulfide isomerase and other thiol-reactive proteins in HIV-1 envelope protein-mediated fusion. Virology. 2006;350(2): 406-417.
6. Szklarczyk D, Franceschini A, Wyder S, et al. STRING v10: protein–protein interaction networks, integrated over the tree of life. Nucleic acids research. 2014: gku1003.
7. Consortium GO. Gene ontology consortium: going forward. Nucleic acids research. 2015;43(D1): D1049-D1056.
8. Bliss CI. The calculation of microbial assays. Bacteriol Rev. 1956 Dec;20(4): 243-258.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. (**a**) Jurkat cells were treated with PS89 and etoposide (ETO) for 48 h, permeabilized and stained with propidium iodide. Cell cycle was analyzed by flow cytometry. (**b**) Jurkat cells were incubated with ETO and allowed to proliferate for 72 h in presence or absence of PS89. Viable cells were determined by CellTiter-Blue staining and normalized towards DMSO control. (**c**) Jurkat cells stimulated for 4 h with PS89 and ETO were washed and reseeded at low density (5.000 cells/ml) in medium with increased viscosity. The number of colonies was quantified after 7 days of proliferation and normalized towards DMSO control. (**d**) Jurkat or (**e**) HL-60 cells were treated with increasing concentrations of 6-mercaptopurine in combination with 25µM PS89. After 48h apoptosis rate was determined by FACS measurements. (**f)** Combined treatment of HL-60 and Jurkat cells with indicated concentrations of dexamethasone (DEX) and PS89 for 48h and analysis of apoptosis rate. (**g**) Vincristine resistant CEM cells stimulated for 4 h with PS89 and vincristine (VCR) were washed and reseeded in medium with increased viscosity. After 5 days, the number of colonies was quantified and normalized towards DMSO control. (**h**) HL-60 cells were treated for 24h with PS89 and 6-mercaptopurine and reseeded in low density in methylcellululose medium. Clonogenic growth was monitored by counting the colonies after 7 days.

Supplementary Figure S2. CCRF-CEM and HL-60 cells were cultured for 24h in drug supplemented medium as indicated and cleavage of PARP and caspase 3 activation was analyzed by Western Blotting.

Supplementary Figure S3. Vincristine resistant (VCR-R) CEM cells were treated with PS89 and VCR in presence or absence of the pan-caspase inhibitor Q-VD. Percentage of apoptotic cells was determined by FACS analysis after 48 h.

Supplementary Figure S4. PDI silenced Jurkat cells (siRNA transfection, 24 h) were incubated with etoposide (ETO) and allowed to proliferate for 72 h. Viable cells were determined by CellTiter-Blue staining and normalized towards DMSO control. PDI expression was analyzed by immunoblotting as shown in Figure 2a.

Supplementary Figure S5. Control of secondary antibody background staining, immuno-fluorescence of BAP31 primary and goat anti-rabbit Alexa 488 secondary antibodies and rhodamine reporter dye background staining (5-TAMRA-Azide) with and without PS89 photo probe after UV crosslinking and coupling to the rhodamine reporter using equal settings as in Fig 3a. Nuclei were stained with Hoechst 33342.

Supplementary Figure S6. (a) Example of FCS autocorrelation curves and residual plots for all measured samples. (**b**) Two different concentrations (50 nM, 250 nM) of the freely diffusing PS89 in buffer solution were analyzed by single-point FCS. Diffusion coefficients were measured after 1 species fitting of the autocorrelation curves (N>15). Bars represent mean + SEM. (**c**) Diffusion values of the two different species of PS89 in combination with 200nM BAP31. (**d**) Concentration values for all measured samples acquired by single-point FCS were measured and verified after 1 or 2 species fitting of the autocorrelation curves (N>15, N>10). Bars represent mean + SEM.

Supplementary Figure S7. (**a**) CEM cells or (**b**) ALL patient derived xenograft (PDX) cells were treated with PS89 and vincristine for indicated time points and expression and cleavage of caspase 8 and BAP31 was analyzed by western blotting.

Supplementary Figure S8. Normalization of protein amounts of cleaved caspase 8 to immunoprecipitated BAP31 in Jurkat cells treated with PS89 and etoposide.

Supplementary Figure S9. Caspase-8 deficient (CASP8 -/-) or wildtype Jurkat cells were treated with PS89 25 µM and etoposide (ETO 250 nM or 500 nM, respectively). Percentage of apoptotic cells was determined by FACS analysis after 48 h. Knockout of CASP8 was verified by immunoblotting.

Supplementary Figure S10. Cytosolic calcium levels in PS89, vincristine or daunorubicin treated (**a**) CCRF- CEM, (**b**) HL-60 and (**c**) ALL PDX cells were analyzed by FACS measurements after 24h or 48h, as indicated.

Supplementary Figure S11. Cytochrome release into the cytosol after treatment of CCRF-CEM cells with PS89 and vincristine was determined after 48h by cytosol-mitochondrial fractionation and western blotting.

Supplementary Figure S12. (**a)** CCRF-CEM and (**b**) HL-60 cells were treated with PS89 and cytostatics. Intracellular ROS levels were evaluated by FACS measurements after 24h and 48h.

SUPPLEMENTARY TABLES

Supplementary Table S1. (a,b) Synergistic interaction of PS89 and cytostatics in cells treated according to Figure 1a and 1b was evaluated by using the Bliss independence model.

Supplementary Table S2. Classification and cytogenetic characteristics of PDX samples.

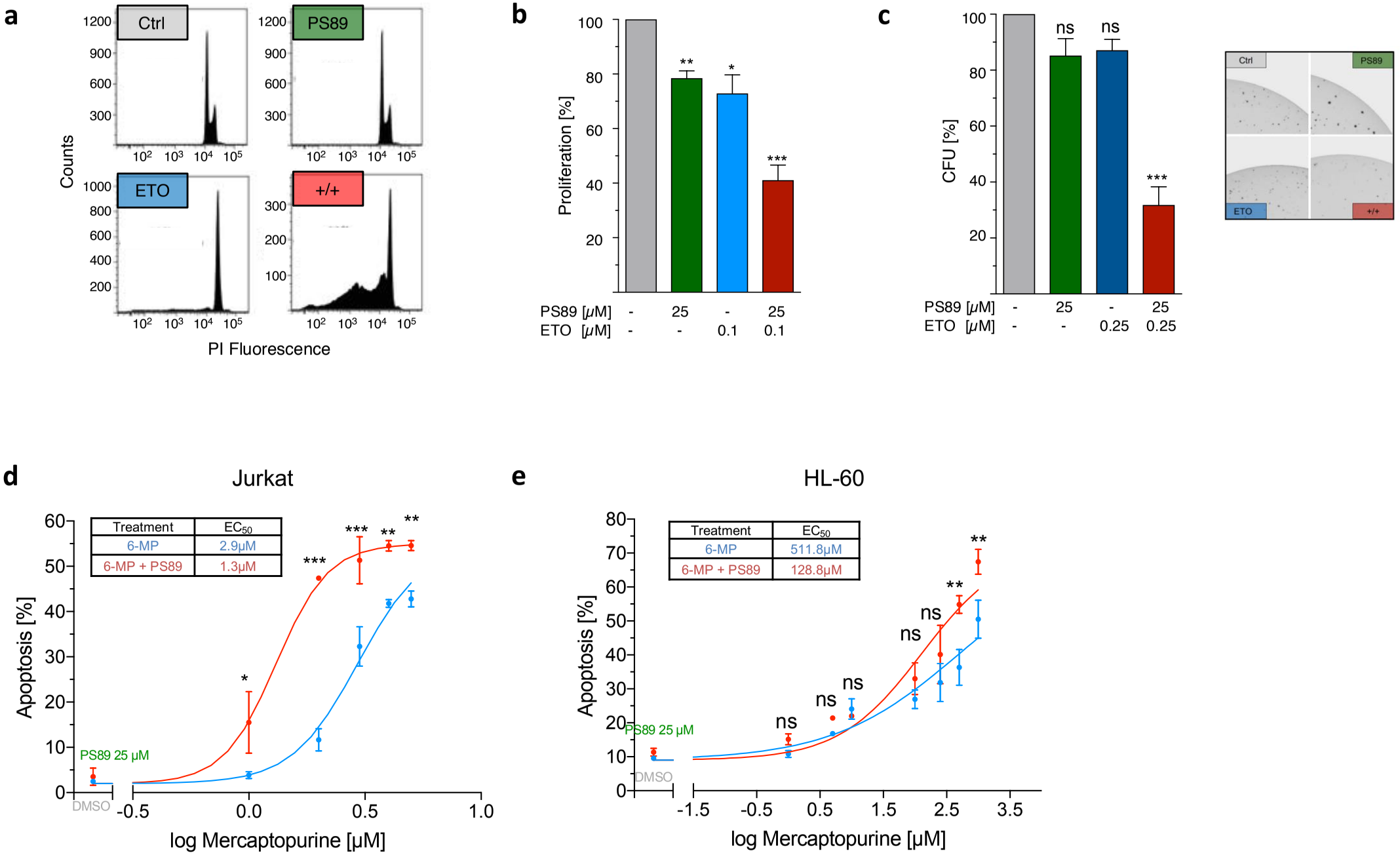
Supplementary Table S3. (a,b) Respective p-values of PBMCs, CD34+, ALL and AML patient samples treated with PS89 and vincristine or daunorubicin were calculated by ordinary one-way ANOVA test. Green fields indicate statistically significant effects (p-values <0,05). **(c)** The Brown-Forsythe statistical test demonstrates that group variances of CD34postive treated cells as shown in Fig 1f are statistically equal.

Supplementary Table S4. List of PS89 target proteins identified by ABPP (n=42) matching defined criteria: (1) Probe / DMSO: >3-fold enrichment (log_2 Probe / DMSO >1.6) and -log_{10} p-value >2. (2) Probe / PS89 log_2 enrichment >0. Ranks were assigned according to the degree of enrichment and their reproducibility. The overall score was calculated as the average of all ranks with double weighting the Probe/PS89 competition values.

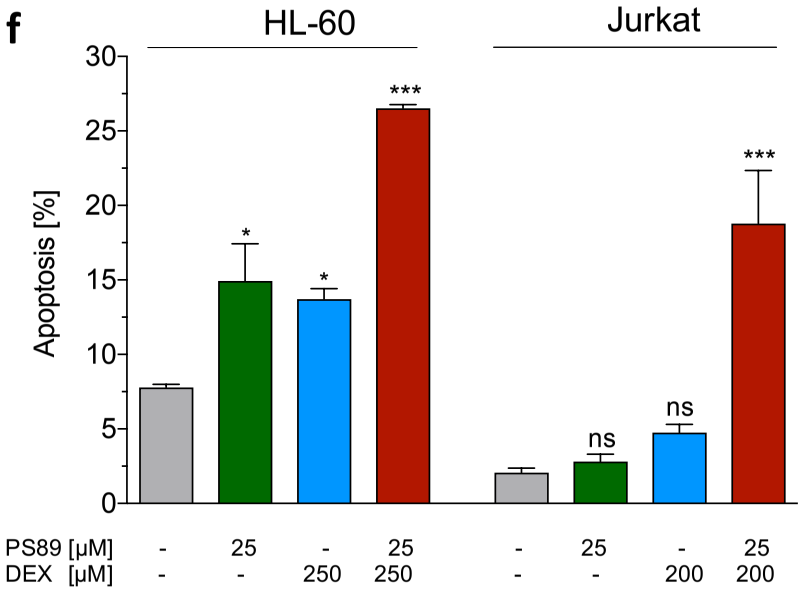
Supplementary Table S5. Synergistic interaction of PS89 and ABT-199 in Jurkat cells was evaluated by using the Bliss independence model.

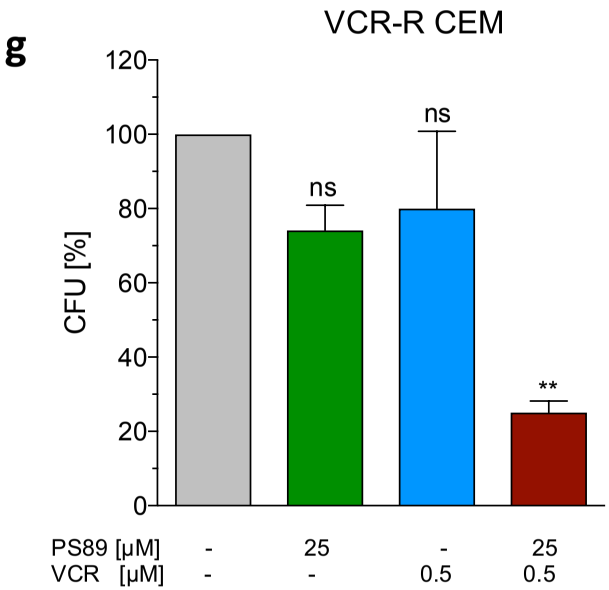
Supplement

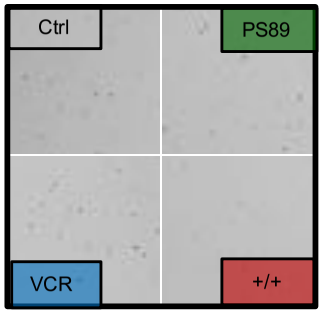
S1



S1


f
HL-60
Jurkat
Apoptosis [%]
30
25
20
15
10
5
0
*
*
***
ns
ns
***
PS89 [µM] - 25 - 25 - 25 - 25
DEX [µM] - - 250 250 - - 200 200


g
VCR-R CEM
CFU [%]
120
100
80
60
40
20
0
ns
ns
**
PS89 [µM] - 25 - 25
VCR [µM] - - 0.5 0.5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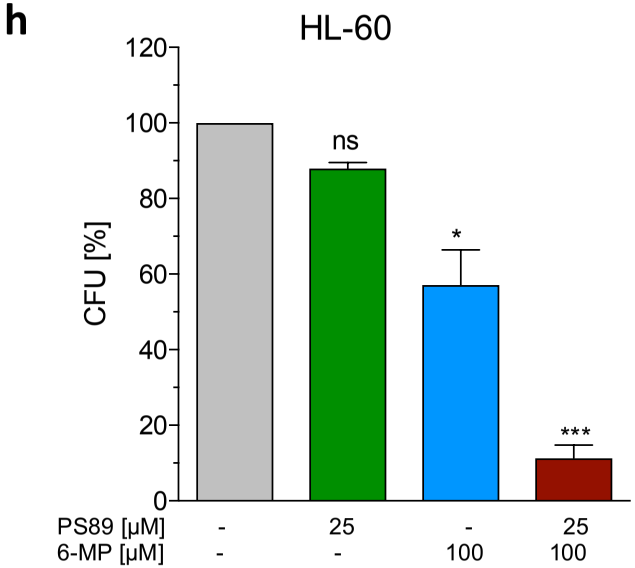
PS89
VCR
+/+


h
HL-60
CFU [%]
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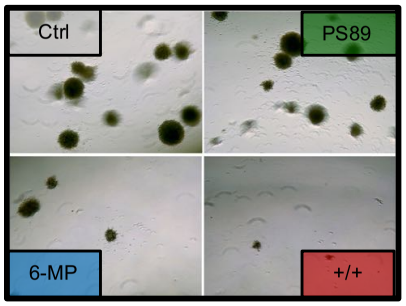
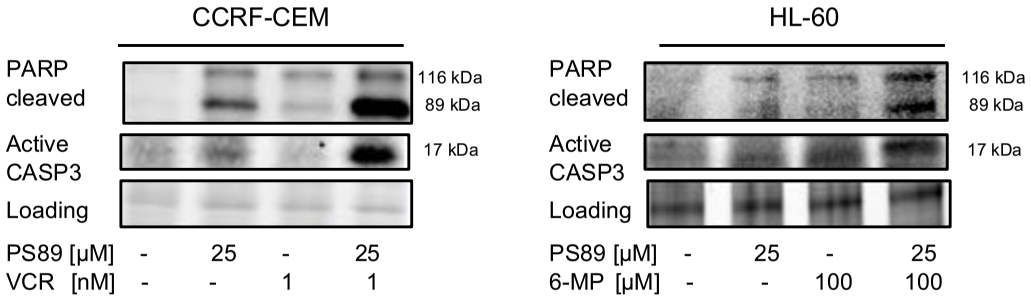
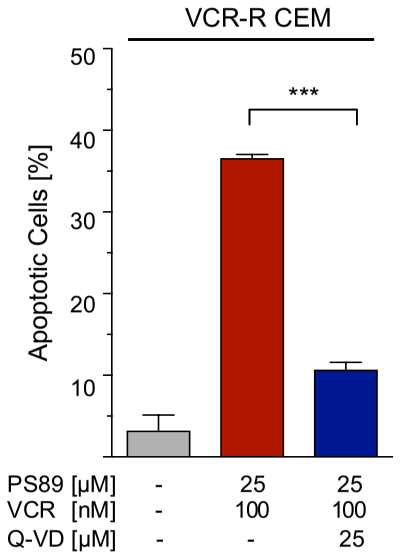
100
80
60
40
20
0
ns
*
***
PS89 [µM] - 25 - 25
6-MP [µM] - - 100 100


Ctrl
PS89
6-MP
+/+

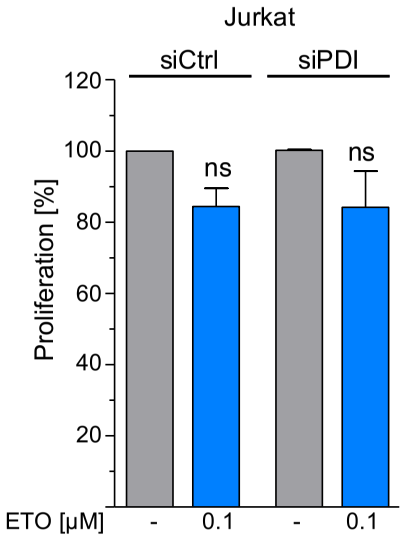
S2


CCRF-CEM
PARP cleaved
116 kDa
89 kDa
Active CASP3
17 kDa
Loading
PS89 [µM] - 25 - 25
VCR [nM] - - 1 1
HL-60
PARP cleaved
116 kDa
89 kDa
Active CASP3
17 kDa
Loading
PS89 [µM] - 25 - 25
6-MP [µM] - - 100 100

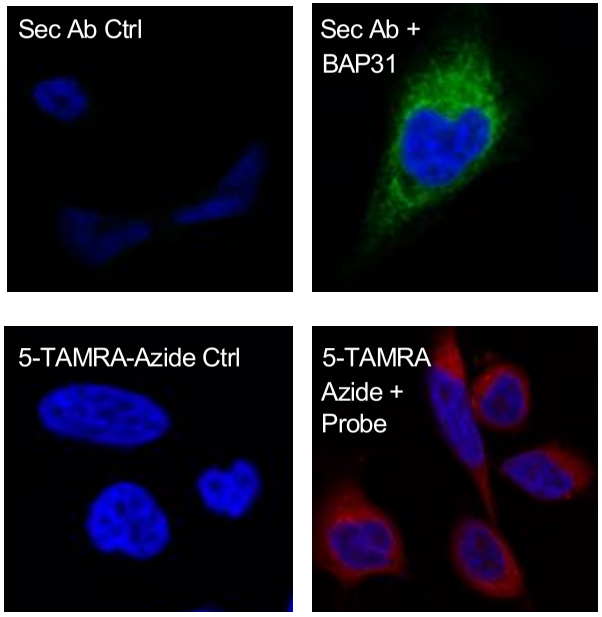
S3


VCR-R CEM
Apoptotic Cells [%]
50
40
30
20
10
***
PS89 [µM] - 25 25
VCR [nM] - 100 100
Q-VD [µM] - - 25

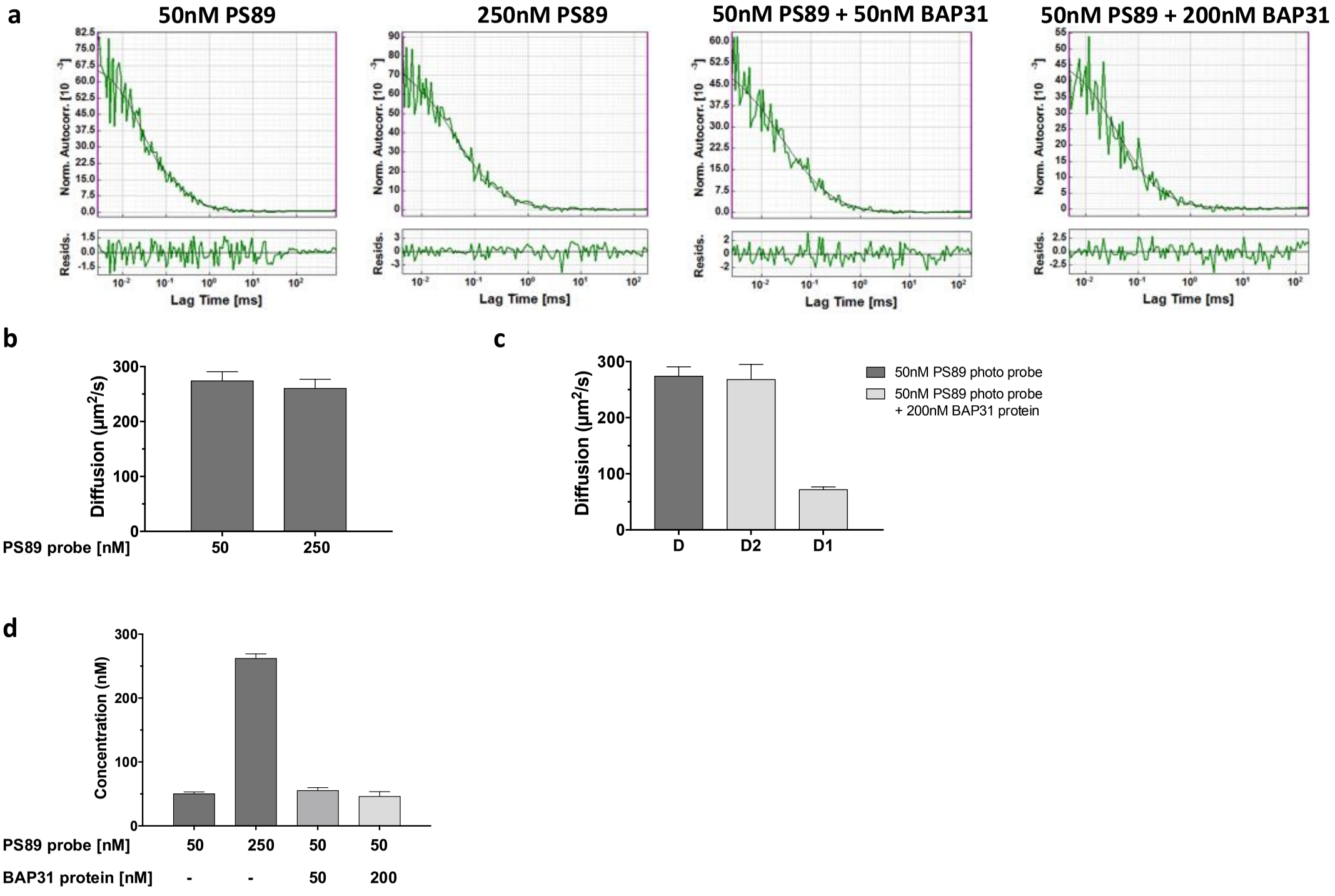
S4


Jurkat
siCtrl
siPDI
Proliferation [%]
120
100
80
60
40
20
ns
ns
ETO [µM] - 0.1 - 0.1

S5

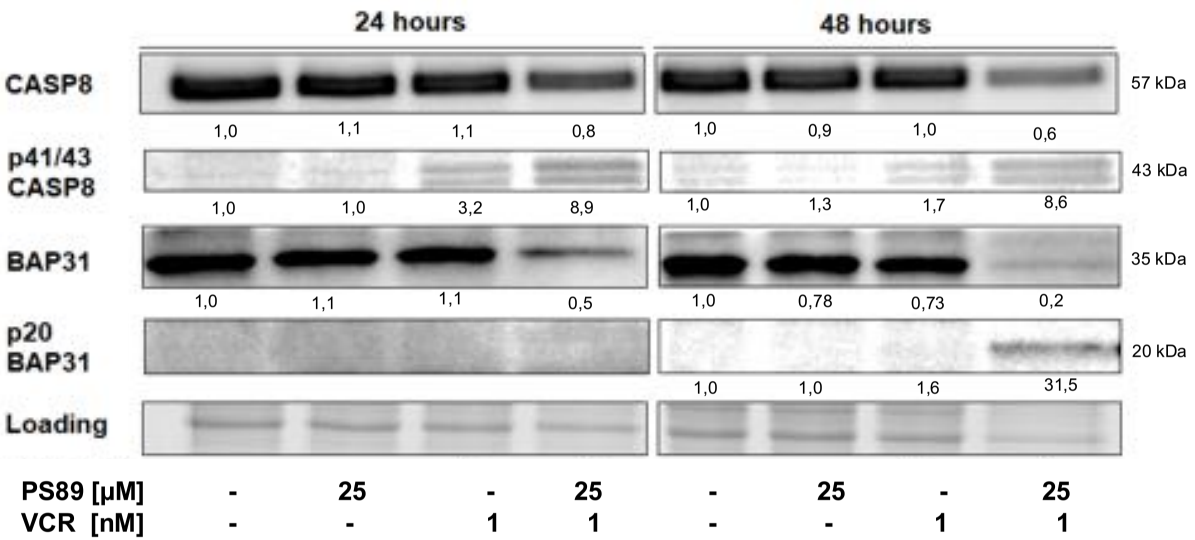

Sec Ab Ctrl
Sec Ab + BAP31
5-TAMRA-Azide Ctrl
5-TAMRA Azide + Probe

S6

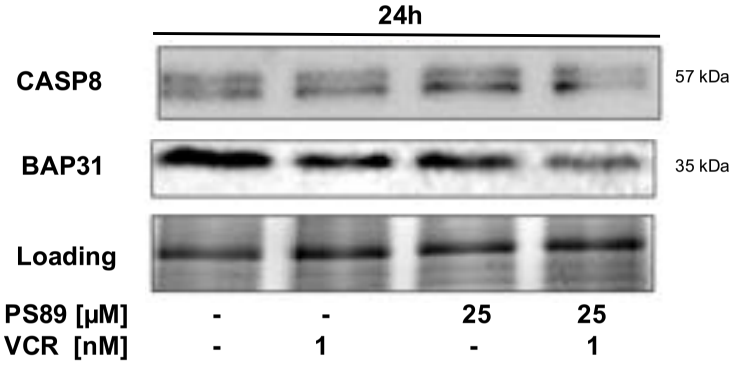

a
50nM PS89
250nM PS89
50nM PS89 + 50nM BAP31
50nM PS89 + 200nM BAP31
Norm. Autocorr. [10 -3]
Resids.
Lag Time [ms]
b
Diffusion (μm²/s)
PS89 probe [nM]
50
250
c
Diffusion (μm²/s)
50nM PS89 photo probe
50nM PS89 photo probe
+ 200nM BAP31 protein
D
D2
D1
d
Concentration (nM)
PS89 probe [nM]
50
250
50
50
BAP31 protein [nM]
-
-
50
200

S7

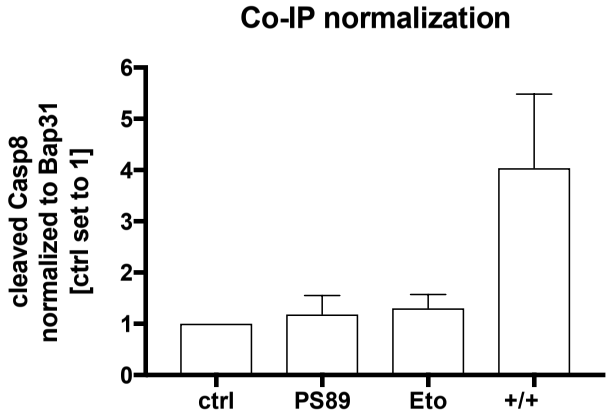
a


24 hours
48 hours
CASP8
57 kDa
1,0 1,1 1,1 0,8
1,0 0,9 1,0 0,6
p41/43 CASP8
43 kDa
1,0 1,0 3,2 8,9
1,0 1,3 1,7 8,6
BAP31
35 kDa
1,0 1,1 1,1 0,5
1,0 0,78 0,73 0,2
p20 BAP31
20 kDa
1,0 1,0 1,6 31,5
Loading
PS89 [µM] - 25 - 25 - 25 - 25
VCR [nM] - - 1 1 - - 1 1

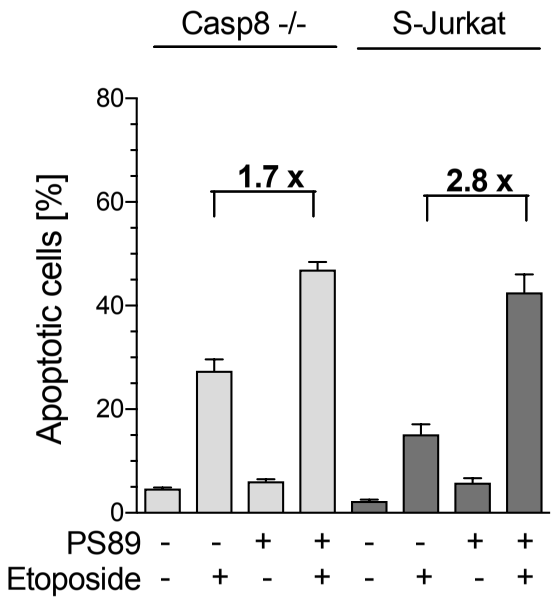
b


24h
CASP8
57 kDa
BAP31
35 kDa
Loading
PS89 [µM] - - 25 25
VCR [nM] - 1 - 1

S8

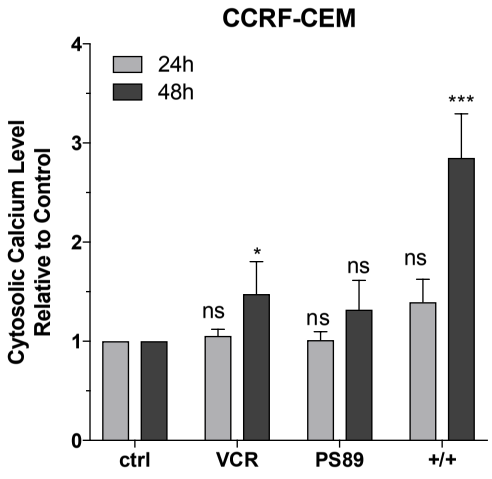

Co-IP normalization
cleaved Casp8
normalized to Bap31
[ctrl set to 1]
0
1
2
3
4
5
6
ctrl
PS89
Eto
+/+

S9

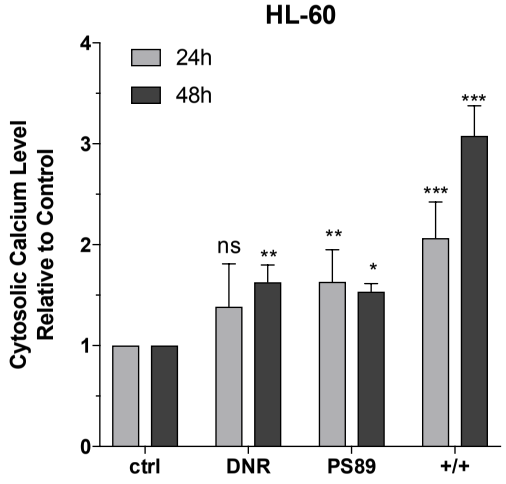

Casp8 -/-
S-Jurkat
Apoptotic cells [%]
0
20
40
60
80
1.7 x
2.8 x
PS89 - - + + - - + +
Etoposide - + - + - + - +

S10

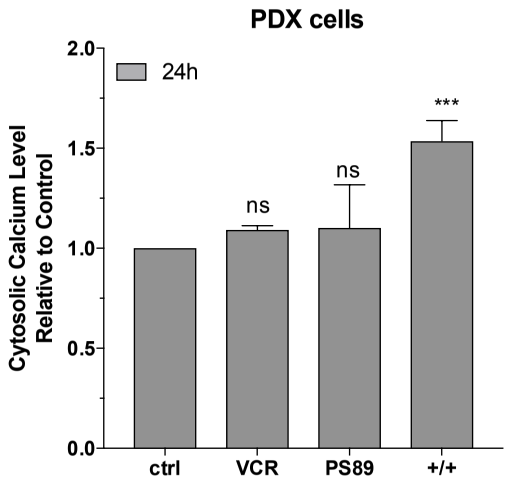
a


CCRF-CEM
24h
48h
Cytosolic Calcium Level
Relative to Control
0
1
2
3
4
ns
*
ns
ns
ns
***
ctrl
VCR
PS89
+/+

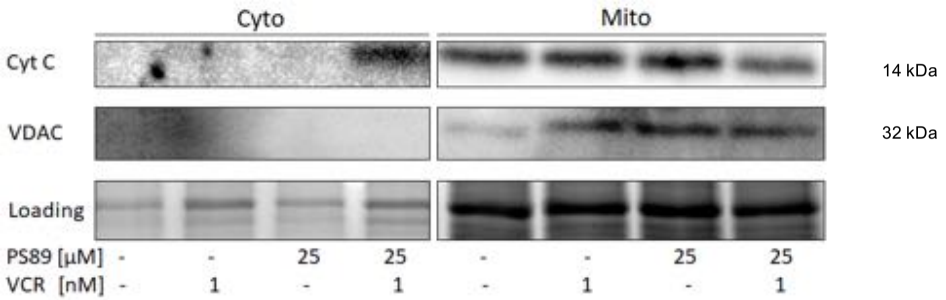
b


HL-60
24h
48h
Cytosolic Calcium Level
Relative to Control
0
1
2
3
4
ns
**
**
*
***
***
ctrl
DNR
PS89
+/+

c

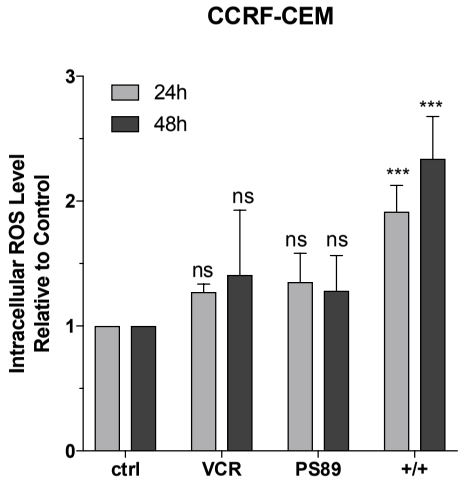

PDX cells
24h
Cytosolic Calcium Level
Relative to Control
0.0
0.5
1.0
1.5
2.0
ns
ns
***
ctrl
VCR
PS89
+/+

S11

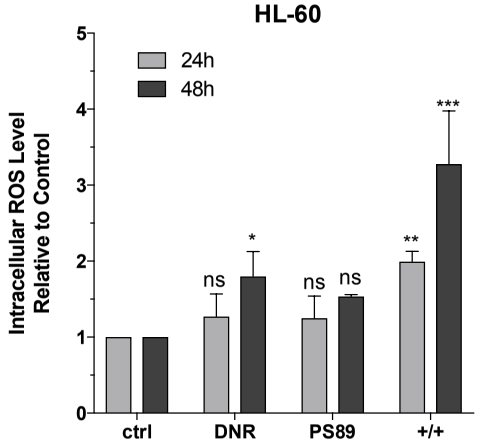

Cyto
Mito
Cyt C
VDAC
Loading
14 kDa
32 kDa
PS89 [µM] - - 25 25 - - 25 25
VCR [nM] - 1 - 1 - 1 - 1

S12

a


CCRF-CEM
24h
48h
Intracellular ROS Level
Relative to Control
ns
ns
ns
ns
***
***
ctrl
VCR
PS89
+/+

b


HL-60
24h
48h
Intracellular ROS Level
Relative to Control
ns
*
ns
ns
**
***
ctrl
DNR
PS89
+/+

Supplementary tables

Table S1

a

Bliss values (> 1 indicates synergism)	
0,1μM ETO + PS89	1,10
0,25μM ETO + PS89	1,22
0,5μM ETO + PS89	1,65
1μM ETO + PS89	1,72
1,5μM ETO + PS89	1,38
2μM ETO + PS89	1,18
5μM ETO + PS89	1,00
10 μM ETO + PS89	1,03

b

Bliss values (> 1 indicates synergism)			
	HL60	CCRF-CEM	VCR-CEM
DNR/VCR+PS89	1,98	2,73	5,96

c

p-values (Ordinary one-way ANOVA)	PBMC	ALL-168	ALL-230
25μM PS89	0,07	0,08	0,11
5nM VCR	0,79	0,06	0,05
25μM PS89+5nM VCR	0,07	0,002	0,002

d

p-values (Ordinary one-way ANOVA)	PBMC	AML-372	AML-393	AML-491	CD34+
25μM PS89	0,81	0,73	>0,99	>0,99	0,97
5nM DNR	0,67	0,82	>0,99	0,95	-
25μM PS89+5nM DNR	0,39	0,18	0,95	0,39	-
20nM DNR	0,11	0,02	0,08	<0,001	0,97
25μM PS89+20nM DNR	0,003	<0,001	0,02	<0,001	0,37

e

Brown-Forsythe test	CD34+ cells
F (DFn, DFd)	0,7336 (2,6)
p-value	0,5188
p-value summary	ns
Are SDs significantly different (p< 0,05)?	No

Table S2

Sample	Type	Subtype	Disease Stage	Sex	Age	Cytogenetics	NPM1	FLT3	BCR-ABL	MLL
ALL-168	ALL	preB ALL	initial diagnosis	female	5	46, XX, der(19)t(1;19)(q23;q13),inc			neg.	neg.
ALL-230	ALL	T ALL	initial diagnosis	male	4	46, XY, t(11;14);(p32;q11)			neg.	neg.
AML-372	AML	M0	relapse	male	42	complex, including -17	wt	wt		neg.
AML-393	AML	M4	relapse	female	47	46,XX,ins(10;11)(p12;q23q23)	wt	wt		MLL-AF10
AML-491	AML		relapse	female	53	46,XX,del(7)(q2?1)	wt	wt		neg.

Table S3

Gene names	Protein names	Mol. weight [kDa]	Probe / DMSO				Probe / PSB				Overall Score
			log2 enrichment		-log10 p-value		log2 enrichment		-log10 p-value		
			enrichment	Rank	enrichment	Rank	enrichment	Rank	enrichment	Rank	
C21orf33	ES1 protein homolog, mitochondrial	25.84	4.767	3	4.662	14	4.642	1	4.318	1	3.5
TMX1	Thioredoxin-related transmembrane protein 1	31.791	3.417	9	6.568	2	1.758	6	3.141	3	4.8
BCAP31	B-cell receptor-associated protein 31	27.991	3.725	6	5.819	4	1.543	7	2.345	6	6.0
SLC29A1	Equilibrative nucleoside transporter 1	50.219	4.584	4	4.770	13	2.130	3	2.049	7	6.2
P4HB	Protein disulfide-isomerase	57.116	5.131	1	6.927	1	1.289	10	1.956	10	7.0
EPHX2	Bifunctional epoxide hydrolase 2	62.915	3.419	8	3.416	30	3.893	2	3.315	2	7.7
PDIA6	Protein disulfide-isomerase A6	47.837	3.409	10	6.669	15	1.096	15	2.731	4	10.5
HSD17B12	Very-long-chain 3-oxoacyl-CoA reductase	34.324	2.893	16	4.242	19	1.516	8	1.999	9	11.5
SOAT1	Sterol O-acyltransferase 1	64.734	2.832	15	3.930	23	1.982	5	1.868	12	12.0
HM13	Minor histocompatibility antigen H13	36.813	4.037	5	4.637	12	1.012	17	1.637	17	14.2
CYP51A1	Lanosterol 14-alpha demethylase	58.805	2.502	18	3.158	35	2.018	4	1.668	15	15.2
PDIA4	Protein disulfide-isomerase A4	72.932	3.247	12	5.485	6	0.946	23	1.668	14	15.2
PDIA3	Protein disulfide-isomerase A3	56.782	3.464	7	6.366	3	0.836	26	1.660	16	15.7
TMEM33	Thioredoxin domain-containing protein 8	40.369	2.628	17	4.840	11	1.217	14	1.553	20	16.0
TMEM214	Transmembrane protein 214	77.15	2.315	21	4.007	22	0.916	24	2.683	5	16.8
TMEM212	Thioredoxin domain-containing protein 12	19.206	2.982	14	4.372	16	1.217	13	1.096	25	17.7
CYB5B	Cytochrome b5 type B	14.846	2.357	20	3.202	33	1.308	9	1.113	24	19.8
HMOX2	Heme oxygenase 2	36.032	3.322	11	4.212	20	0.966	21	1.093	26	20.8
KTL3	Kitaelin-3	60.541	1.757	38	5.427	8	0.803	33	1.891	8	21.0
ATP2A2	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	109.73	2.994	29	4.398	16	0.900	25	1.630	16	22.2
SPCS2	Signal peptidase complex subunit 2	17.027	2.060	30	2.835	40	1.277	11	1.469	21	22.3
VAT1	Synaptic vesicle membrane protein VAT-1 homolog	41.92	4.635	2	5.195	8	0.799	28	0.674	36	23.0
HCCS	Cytochrome c-type heme lyase	36.601	2.256	24	5.015	10	0.959	22	0.888	31	23.3
HADHA	Trifunctional enzyme subunit alpha, mitochondrial	82.999	1.803	36	4.103	21	0.760	29	1.755	13	23.5
SQLE	Squalene monooxygenase	53.067	2.996	28	3.425	29	0.976	20	1.447	22	23.5
TMPO	Lamina-associated polypeptide 2, isoforms beta/gamma	50.67	1.819	35	3.183	34	0.882	18	1.671	18	23.8
DHCR7	7-dehydrocholesterol reductase	54.469	1.801	42	4.356	17	0.960	19	1.136	23	23.8
PARK7	Protein deglycase DJ-1	19.891	2.125	26	2.823	41	1.282	12	0.982	28	24.5
ENDOD1	Endonuclease domain-containing 1 protein	55.019	1.998	32	3.019	37	0.728	30	1.908	11	25.2
CLPTM1L	Cleft lip and palate transmembrane protein 1-like protein	58.355	2.466	19	3.692	25	0.713	31	1.026	27	26.7
VDAC2	Voltage-dependent anion-selective channel protein 2	31.566	2.261	23	5.264	7	0.415	37	0.892	30	27.3
PRKCSH	Glucosidase 2 subunit beta	60.192	1.815	41	3.063	36	1.060	16	0.685	32	28.8
DNPEP1	Thioredoxin reductase 1, cytoplasmic	60.078	2.092	27	3.288	31	0.592	34	0.901	29	30.7
VDAC3	Voltage-dependent anion-selective channel protein 3	30.656	2.300	22	5.173	9	0.266	40	0.557	37	30.8
ALDH3A2	Fatty aldehyde dehydrogenase	44.648	2.986	13	3.772	24	0.435	36	0.164	41	31.8
SACM1L	Phosphatidylinositide phosphatase SAC1	86.966	1.850	40	3.446	27	0.800	32	0.852	34	33.2
LRRC59	Leucine-rich repeat-containing protein 59	34.93	2.049	31	3.437	28	0.531	35	0.727	35	33.2
COMT	Catechol O-methyltransferase	24.449	1.987	33	2.588	42	0.803	27	0.455	38	34.2
FKBP8	Peptidyl-prolyl cis-trans isomerase FKBP8	44.591	1.764	39	3.279	32	0.330	38	0.671	33	35.5
CANX	Calnexin	67.567	1.789	37	3.097	26	0.278	41	0.308	40	37.5
DCXR1	DCIA domain-containing protein 1	27.626	1.871	34	2.993	39	0.327	39	0.357	39	38.2
NT5DC1	5-nucleotidase domain-containing protein 1	51.844	2.129	25	2.998	38	0.120	42	0.086	42	38.5

Table S4

Bliss values (> 1 indicates synergism)	
0,5μM ABT-199 + PS89	1,21
1μM ABT-199 + PS89	1,53
2μM ABT-199 + PS89	1,54
3μM ABT-199 + PS89	1,92
5μM ABT-199 + PS89	1,53
10μM ABT-199 + PS89	1,48
25μM ABT-199 + PS89	1,06
50 μM ABT-199 + PS89	1,00
100 μM ABT-199 + PS89	0,94