SUPPLEMENTARY APPENDIX

Non-genotoxic MDM2 inhibition selectively induces a pro-apoptotic p53 gene signature in chronic lymphocytic leukemia cells

Carmela Ciardullo,[1] Erhan Aptullahoglu,[1] Laura Woodhouse,[2] Wei-Yu Lin,[1] Jonathan P Wallis,[3] Helen Marr,[3] Scott Marshall,[4] Nick Bown,[5] Elaine Willmore[1] and John Lunec[1]

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Supplementary Methods

Supplementary Figure Legends

SUPPLEMENTARY METHODS

Patients and cell isolation

CLL cells, normal peripheral blood mononuclear cells (PBMCs, n=6) and normal bone marrow mononuclear cells (BMMCs, n=5) were isolated by density gradient centrifugation (Lymphoprep, Axis-Shield) following the manufacturer's protocol. CLL cells and normal PBMCs were re-suspended at a density of 5.5 x 10^6 cells/ml in RPMI-1640 supplemented with 10% FCS, 2 mM glutamine, 1 mM sodium pyruvate, 1% penicillin/streptomycin and incubated at 37°C, 5% CO_2. BMMCs were re-suspended in Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 20% FCS, 2 mM glutamine, 1 mM sodium pyruvate and 1% penicillin/streptomycin. CD34+ haematopoietic stem cells were isolated from BMMCs using a MACS immunomagnetic selection system (CD34 MicroBead Kit Ultra Pure, Miltenyi Biotec) and cultured for less than 7 days in StemMACS HSC Expansion Media XF supplemented with StemMACS HSC Expansion Cocktail (Miltenyi Biotec).

The purity of the isolated CD34+ hematopoietic progenitor cells was evaluated by flow cytometry. CD34+ cells and total BMMCs were stained using a fluorescent antibody (CD34-PE, clone: AC136, # 130-081-002, Miltenyi Biotec, UK) recognizing an epitope different from that recognized by the bead-bound CD34 monoclonal antibody QBEND/10. For optimal discrimination of CD34+ cells, cells were also counterstained with an antibody against CD45 (CD45‑FITC, # 130-080-202, Miltenyi Biotec, UK). Staining was carried out for 5 minutes at 4°C, cells were washed, resuspended in buffer and analysed at the Newcastle University Flow Cytometry Core Facility (FCCF) using a FACSCanto II (BD Biosciences).

Freshly isolated leukemic and normal cells were used immediately in all experiments and harvested at the indicated time points to asses viability, apoptosis, gene expression and for western blot analyses.

***Ex vivo* cytotoxicity assay**

5 x 10^5 CLL cells/well were seeded into a flat-bottom 96-well microtiter plate in triplicate and exposed to increasing concentrations of RG7388 for 48 hours. The final DMSO concentration (0.5%) was kept constant in each experiment.

Activated-XTT solution was prepared immediately before use by mixing XTT Reagent and the Activation Reagent in a 50:1 ratio. Cytotoxicity was evaluated by adding 50 µl of activated-XTT solution to 100 µl of drug-treated cells. Cells were then incubated for 8 hours at 37°C, 5% CO_2 and the microtiter plate was periodically assessed for the visual appearance of an orange color. After incubation, absorbance at 450nm was measured using a FluoStar Omega plate reader (BMG Labtech).

Western blot analysis

Thirty microgram aliquots of protein were electrophoresed on 4-20% denaturing SDS-polyacrylamide gradient gels (Bio-Rad, UK) and transferred onto nitrocellulose membrane (Amersham, UK). The samples were probed with antibodies against p53 (clone DO-7, Vector Laboratories), MDM2 (Calbiochem, OP46), p21 (Calbiochem, OP64), PARP (Trevigen, 4338-MC-50), PUMA (Calbiochem, PC686) and actin (Cell Signaling, 9284S). Peroxidase-conjugated anti-mouse (Dako, P0447) or anti-rabbit (Dako, P0448) secondary antibodies, ECL reagent (Amersham, UK) and blue light sensitive X-ray film (Fujifilm) were used for detection.

Apoptosis assay

5 x 10^5 CLL cells/well were seeded into a flat-bottom 96-well microtiter plate in triplicate and exposed to increasing concentrations of RG7388 for 24 hours. The final DMSO concentration (0.5%) was kept constant in each experiment.

Reconstituted Caspase-Glo 3/7 assay reagent (Promega, UK) was used for caspase detection in treated cells. The reagent provides a proluminescent caspase-3/7 substrate, which contains the tetrapeptide sequence DEVD, in combination with luciferase and a cell-lysing agent. The addition of the reconstituted Caspase-Glo 3/7 reagent directly to the assay well results in cell lysis, followed by caspase cleavage of the DEVD substrate, and the generation of luminescence. The amount of luminescence detected is proportional to the amount of caspase activity in the sample. Briefly, drug treated cells were mixed in a 1:1 ratio with reconstituted Caspase-Glo® 3/7 reagent and incubated for 30 minutes in the dark. Luminescence was measured with a FLUOstar Omega plate reader (BMG Labtech).

Co-culture and stimulation of CLL cells with CD40L-expressing cells

CD40L NIH-3T3 mouse fibroblasts were seeded into 24-well plates (3×10^5 cells/ml) in RPMI medium containing 10% heat-inactivated FCS, 2 mM L-glutamine and 1% penicillin/streptomycin and allowed to adhere. The cells were irradiated (35 Gy) to prevent proliferation and incubated at 37°C, 5% CO_2 overnight. CLL cells were thawed and resuspended at a density of 2×10^7 cells/mL in RPMI supplemented with 10% FCS, glutamine, 1% penicillin/streptomycin and 10 ng/mL human IL4. Cells were then seeded onto the CD40L monolayer at a density of 1×10^7 per well and incubated at 37°C, 5% CO_2. Cells were monitored daily and began to proliferate after approximately 96 hours.

After 96 hours, CLL cells were counted as a pre-treatment control and exposed to increasing concentrations of RG7388. Cells were counted at 24 hour intervals thereafter. At 6 and 24 hour intervals after drug exposure, cells were harvested for western immunoblot and qRT-PCR mRNA expression profiling. Growth inhibitory and cytotoxic response to drug was measured after 48 hours drug exposure. Cell counts were performed using a Coulter Counter (Beckman Coulter, High Wycombe, UK) with the threshold set at 5-12 μm. Counts were plotted against

time and as a concentration-dependent response curve generated from the cell counts at the terminal 48hr exposure period expressed as a % of the vehicle control. Throughout the experiment, CLL cells were re-seeded onto a fresh CD40L feeder layer every 72 hours and drug-containing medium was replenished with fresh medium containing the same concentration of RG7388 every 48 hours.

Cell cycle analysis of CD34+ haematopoietic stem cells

Positively-selected CD34+ cells were incubated with RG7388 for 24 hours. Cells were washed with PBS and fixed with cold 70% ethanol for 30 minutes on ice. Cells were then resuspended in 500 μL PBS with 100 μg/mL propidium iodide (Sigma) and 200 μg/mL RNAse A (Sigma). Samples were analyzed on a FACSCaliburTM flow cytometer using CellQuest Pro software (Becton Dickinson, Oxford, UK). Cell cycle distribution was determined using Cyflogic software (CyFlo Ltd, Turku, Finland).

SUPPLEMENTARY FIGURE LEGENDS

Figure S1

PUMA protein expression following treatment with RG7388 and time dependent loss of viability

(A) Western blot analysis showing representative examples of dose dependent PUMA protein induction by *ex vivo* treatment of CLL samples with RG7388 for 24hours (CLL265 and CLL268). Two examples of non-functional p53 stabilisation with minimal downstream PUMA expression are included (CLL281 and CLL297) together with one example of high basal PUMA expression which is further increased by RG7388 (CLL 302). (B) RG7388 dose and exposure time dependent loss of viability measured by XTT assay for two representative wild-type p53 CLL patient samples exposed to a concentration range of 0.1-10μM RG7388 for 12, 24 and 48hrs. Results are expressed as % relative to DMSO vehicle control, presented as mean ± standard error of mean (SEM) for three intra-experimental repeats.

Figure S2

CLL sample with subclonal 17p deletion treated with RG7388 shows functional activation of p53, upregulation of pro-apoptotic p53-targets and cytotoxicity

(A) Western immunoblot of a CLL sample (CLL 0269) harbouring a subclonal (22%) 17p deletion treated with RG7388 for 6 and 24 hours. Sample shows stabilisation of p53 and induction of MDM2, p21 and cleaved PARP. (B) qRT-PCR results for CLL 0269 treated with RG7388 for 6 and 24 hours showing fold-change of mRNA for p53-target genes and in particular preferential induction of *PUMA.* Data are presented as mean ± standard error of mean (SEM) of three repeats (C) Increase in Caspase 3/7 activity in CLL 0269 exposed to RG7388 for 24 hours. Data are presented as mean ± standard error of mean (SEM) for three repeats (D) Response of CLL 0269 exposed to increasing concentrations of RG7388 for 48

hours. Concentration-dependent decrease in cell viability assessed by XTT assay indicates sensitivity to RG7388. Data are presented as mean ± standard error of mean (SEM) for three repeats.

Figure S3

RG7388 induces cytotoxicity in cells isolated from CLL patients for the *TP53* wild-type subset, regardless of disease stage, *IGHV* status and other cytogenetics abnormalities.

(A) RG7388 LC_{50} values for CLL samples from patients with Binet A, B or C stage of disease. (B) RG7388 LC_{50} values for CLL samples from patients with mutated or unmutated *IGHV* genes.

(C) RG7388 LC_{50} values for CLL samples from patients with trisomy of chromosome 12 (+12), deletion of chromosome 13q, deletion of chromosome 11q and no cytogenetics abnormalities. Horizontal bars represent the median. The p-values were calculated by Mann–Whitney test.

Figure S4

RG7388 inhibits CLL proliferation induced by microenvironment stimuli

(A) CLL cells from patient 0260 were cultured on CD40L-expressing feeder cells for 96 hours followed by treatment with increasing concentrations of RG7388 at the start of proliferation. Cells were counted at 24 hour intervals for a further 96 hours. (B) (C) Concentration-dependent reduction in CLL cell numbers after 48 hours of RG7388 exposure compared with untreated controls (CLL sample 0260, CLL sample 0278). (D) (E) Western immunoblots blot of a p53-functional CLL sample (CLL 0278) unstimulated or stimulated with CD40L/IL4 and then treated with RG7388 for 6 and 24 hours. Stimulated sample shows a greater stabilisation of p53 and induction of MDM2 and p21 but no cleaved PARP compared to the unstimulated counterpart. (F) qRT-PCR results for fold-change of *PUMA*, *MDM2* and *CDKN1A* mRNA

measured following 6 and 24 hours exposure to RG7388 in CLL 0278 cells which were left unstimulated or stimulated with CD40L/IL4.

Figure S5

Caspase 3/7 activation is triggered by RG7388 selectively in CLL cells but not in normal PBMC or BMMC cells.

Caspase 3/7 activity for n=9 p53-functional CLL samples, n=1 p53-non-functional CLL sample, n=3 normal BMMC samples and n=6 normal PBMC samples exposed to 1 μM RG7388 for 24 hours. Caspase 3/7 activity is represented as % change relative to DMSO solvent control. Data are presented as mean ± standard error of mean (SEM). p-value is calculated by Mann–Whitney test.

Figure S6

MDM2 inhibition by RG7388 induces cell cycle distribution changes in positively-selected CD34+ haematopoietic stem cells

(A) FACS plots showing CD34+ cells (left) sorted to high purity by antibody-based magnetic bead cell separation (MACS) from mononuclear cells isolated from human bone marrow (right). Flow cytometry of enriched CD34+ cells (left) shows that of the live cells, >80% express CD34, of which 39% co-express CD45. (B) FACS histograms showing CD34+ expression in mononuclear cells isolated from human bone marrow (left) and haematopoietic CD34+ cells purified by MACS selection (right). (C) Cell cycle distribution analysis of CD34+ cells incubated with RG7388 for 24 hours showing an increased proportion of cells in G0/G1 phase and a reduction of cells in S phase. (D) Bar-chart showing a slight increase of Sub-G1 events in CD34+ cells exposed to RG7388 for 24 hours.

Figure S7

***MDM2* and *PUMA* basal expression levels vary across CLL samples but do not predict response to RG7388 in the *TP53* wild-type subset**

(A) Basal *MDM2* expression levels expressed as Ct values measured by qRT-PCR in CLL samples, normal BMMCs, normal PBMCs and normal CD34+ cells. The median *MDM2* basal levels in CLL samples was statistically different to that of PBMCs. Ct values are expressed as mean of at least three repeats. P-values are calculated by Mann-Whitney test. (B) Basal *PUMA* expression levels expressed as Ct values and measured by qRT-PCR in CLL samples, normal BMMCs, normal PBMCs and normal CD34+ cells. The median *PUMA* basal levels in CLL samples was not different to that of normal cells. Ct values are expressed as mean of at least three repeat. p-values are calculated by Mann-Whitney test. (C) *MDM2* mRNA expression levels expressed as Ct values and measured by qRT-PCR in CLL samples grouped according to different cytotoxic responses to RG7388. The distribution of *MDM2* basal levels in CLL samples showing sensitivity to RG7388 (LC_{50}<1μM) was not statistically significantly different to that of samples showing intermediate response/resistance (LC_{50}>1μM) to RG7388. Ct values are expressed as mean of at least three repeats. P-values were calculated by Mann-Whitney test. (D) *PUMA* mRNA levels expressed as Ct values and measured by qRT-PCR in CLL samples showing different cytotoxic responses to RG7388. The distribution of *PUMA* basal levels in CLL samples showing sensitivity to RG7388 (LC_{50}<1μM) was not statistically significantly different to that of samples showing intermediate response/resistance (LC_{50}>1μM) to RG7388. Ct values are expressed as mean of at least three repeats. P-values were calculated by Mann-Whitney test. (E) (F) Comparison between (E) *MDM2* and (F) *PUMA* basal Ct values and RG7388 LC_{50} concentrations for CLL samples. The r-value indicates Pearson's correlation coefficient with associated p-value indicating no significant correlation.

Figure S8

***MDM2* and *PUMA* fold inductions by RG7388 are alone not biomarkers of drug-induced cytotoxicity for the subset of wild-type p53 CLL cases**

(A) (B) Correlation of (A) *MDM2* and (B) *PUMA* mRNA fold induction (exposure 1 µM RG7388 for 6 hours) and RG7388 LC_{50} concentrations of CLL samples. The r-value is Pearson's correlation coefficient and associated p-values indicate there is no significant correlation.

Figure S9

Combination treatments of CLL cells *ex vivo* with RG7388 and either venetoclax (ABT199) or Ibrutinib

(A) (B) Combination treatment with RG7388 and ABT199 compared with either agent alone showing a significant additive effect on cell killing. Drug exposure was 48hours and viability measured by XTT assay. (C) (D) Combination treatment with RG7388 and Ibrutinib showing no additional effect of Ibrutinib over that seen with RG7388 alone.

Table S1. Characteristics of the CLL cohort

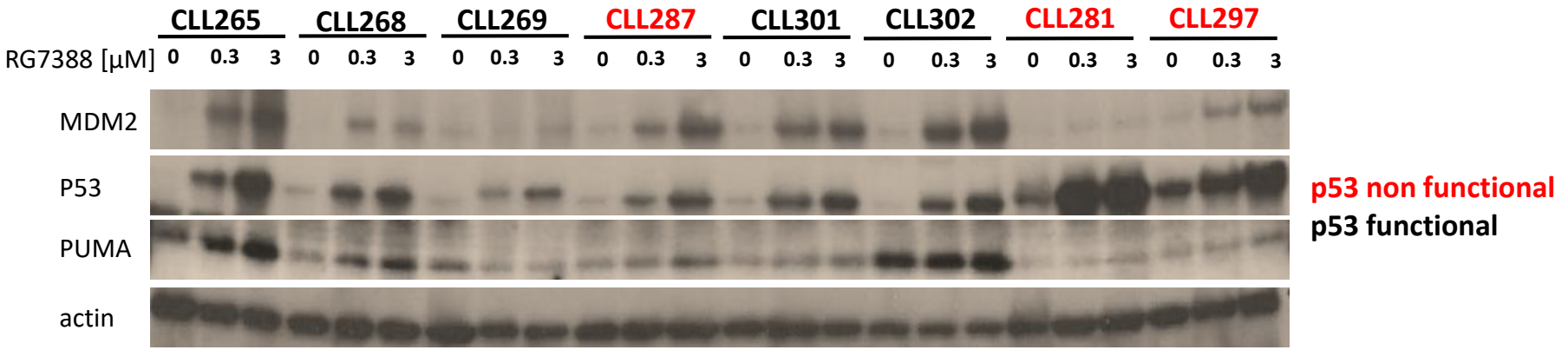
Tumour ID	Age	Binet Stage	IgVH	Cytogenetics	*TP53* status (NGS)	*TP53* mutation	RG7388 LC_{50} (µM)
212	57	C	U	12+	WT		0.7
216	81	A	M	del13q	WT		0.36
217	66	C	U	del11q	MUT	c.524G>A, p.R175H	1.96
218	84	A	M	none	WT		0.22
219	86		U	del13q	WT		0.26
220	57	A	M	del13q	WT		0.26
221	86	C	M	none	WT		0.17
222	76	C	M	del11q, del13q, 12+	WT		0.82
224	69	C	M	bi13q	MUT	c.578A>T, p.H193L	5.28
225	66		M	none	WT		0.2
226	58	A	M	none	WT		0.32
227	71	A	M	bi13q	WT		0.89
228	79	B	U	12+	WT		0.18
229	67	B	M	del13q	WT		6.5
230	79	B	U	12+	WT		0.16
231	60	A	M	none	WT		0.87
233	66	C	U	del11q, del13q	WT		>10
234	81	B	U	del11q, del13q	WT		0.27
235	77	A	M	bi13q	WT		3.71
237	77	B	U	del17p	MUT	c.701A>G, p.Y234C	6.06
239	56		M	del13q	WT		0.1
240	72		U	none	WT		>10
241	80	A	M	none	WT		>10
242	78	C	M	none	WT		0.2

243	76	A	M	none	WT		0.72
244	72		M	del13q	MUT	c.832C>T, p.P278S	0.62
245	79	A	U	del13q	WT		2.19
246	76	C		none	WT		0.24
247	83	A		none	WT		0.2
248	67	A	M	del13q	MUT	c.850A>T, p.T284S	>1
249	66	C		del13q	WT		2.98
250	85	B	M	del13q	WT		0.71
251	64	B	M	del17p, del13q	MUT	c.742C>T, p.R248W; c.673-2A>C	>10
252	68	C		del13q	WT		0.13
253	61		M	del13q	WT		0.65
254	72	Apro		del11q, del13q	WT		0.53
255	80			NA	NA		8.37
257	72	A		del13q, del 11q	WT		0.12
258	72			del13q, del11q	MUT	c.626_627delGA, p.R209fs*6	0.98
259	88		U	del13q	WT		0.58
260	89			del13q	WT		0.39
261	79	C	M	del13q	MUT	c.626_627delGA, p.R209fs*6	>3
262	72	A		none	WT		0.24
263	80	A		none	WT		0.64
264	71			del11q	WT		0.42
265	86			del13q, del11q	WT		0.28
266	59			del13q	WT		0.37
267	60	A	M	del13q	WT		0.24
268	83	A	M	del13q	WT		0.15

269	63			del17p	WT		0.34
270	75			del13q	WT		0.35
272	69		M	none	WT		0.33
273	61		U	none	MUT	c.1067G>C, p.G356A; c.1069A>C, p.K357Q	>10
275	88		M	none	WT		0.88
276	67	C		none	WT		1.7

Table S2. Primers used for qRT-PCR			
Gene Symbol	**Gene name**	Primer Forward (5'-3')	Primer Reverse (5'-3')
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase	CGACCACTTTGTCAAGCTCA	GGGTCTTACTCCTTGGAGGC
CDKN1A	Cyclin Dependent Kinase Inhibitor 1A	TGTCCGTCAGAACCCATGC	AAAGTCGAAGTTCCATCGCTC
MDM2	Mouse double minute 2 homolog	AGTAGCAGTGAATCTACAGGGA	CTGATCCAACCAATCACCTGAAT
PUMA (BBC3)	p53 upregulated modulator of apoptosis	ACCTCAACGCACAGTACGA	CTGGGTAAGGGCAGGAGTC
BAX	BCL2 Associated X	CCCGAGAGGTCTTTTTCCGAG	CCAGCCCATGATGGTTCTGAT
FAS	Fas cell surface death receptor	AGATTGTGTGATGAAGGACATGG	TGTTGCTGGTGAGTGTGCATT
FDXR	Ferredoxin reductase	CAGCATTGGGTATAAGAGCCG	GGCCTGGCACATCCATAACC
TNFRSF10B	Tumor necrosis factor receptor superfamily, member 10b	ATGGAACAACGGGGACAGAAC	CTGCTGGGGAGCTAGGTCT
TP53INP1	Tumor protein p53 inducible nuclear protein 1	TCTTGAGTGCTTGGCTGATACA	GGTGGGGTGATAAACCAGCTC
ZMAT3	Zinc finger matrin-type 3	CCTTACTTCAATCCCCGCTCT	CTTCGCCAGCTCCAACATTAC
GADD45A	Growth arrest and DNA damage inducible alpha	GAGAGCAGAAGACCGAAAGGA	CAGTGATCGTGCGCTGACT
WIP1 (PPM1D)	Protein phosphatase, Mg2+/Mn2+ dependent 1D	TTTCTCGCTTGTCACCTTGC	TTCCAAGAACCACCCCTGAG
BCL2	Protein phosphatase 1, regulatory subunit 50	GGTGGGGTCATGTGTGTGG	CGGTTCAGGTACTCAGTCATCC
MCL1	MCL1 apoptosis regulator, BCL2 family member	GTGCCTTTGTGGCTAAACACT	AGTCCCGTTTTGTCCTTACGA
BCL2L1 (BCL-XL)	BCL2 Like 1	QIAGEN QUANTITECT PRIMER ASSAY QT00236712	
PMAIP1 (NOXA)	Phorbol-12-myristate-13-acetate-induced protein 1	TGCTACACAATGTGGCGTC	ACTTGGACATGGCCTCCCTTA
BCL2L11 (BIM)	BCL2-Like 11	TAAGTTCTGAGTGTGACCGAGA	GCTCTGTCTGTAGGGAGGTAGG

A


CLL265
CLL268
CLL269
CLL287
CLL301
CLL302
CLL281
CLL297
RG7388 [µM] 0 0.3 3 0 0.3 3 0 0.3 3 0 0.3 3 0 0.3 3 0 0.3 3 0 0.3 3 0 0.3 3
MDM2
P53
PUMA
actin
p53 non functional
p53 functional

B

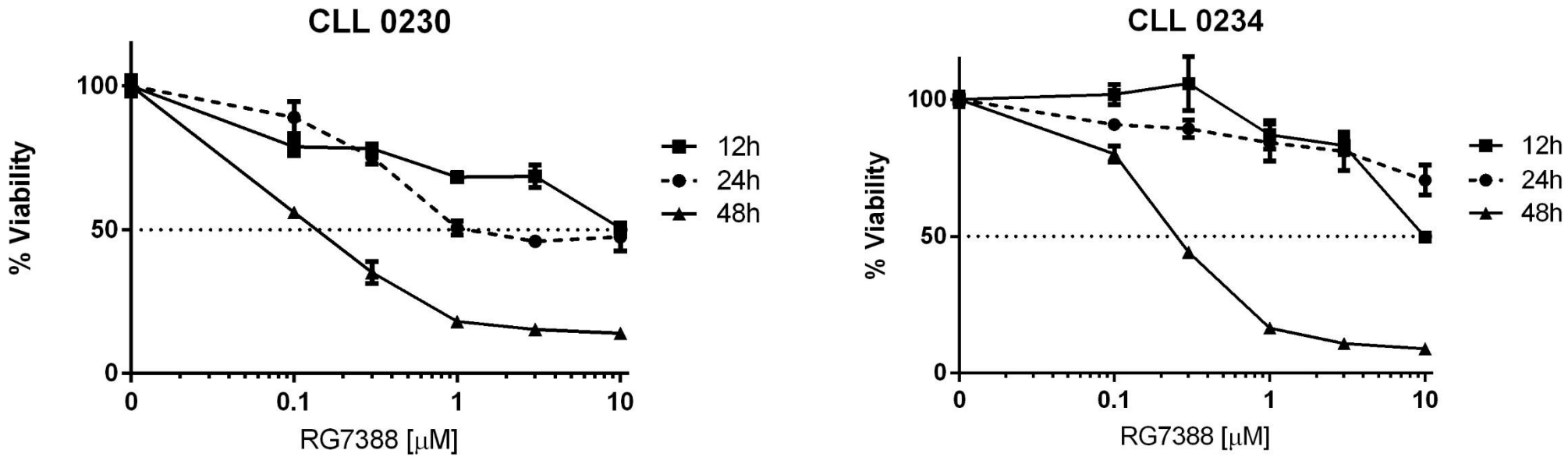

CLL 0230
% Viability
100
50
0
0
0.1
1
10
RG7388 [µM]
12h
24h
48h
CLL 0234
% Viability
100
50
0
0
0.1
1
10
RG7388 [µM]
12h
24h
48h

Figure S1

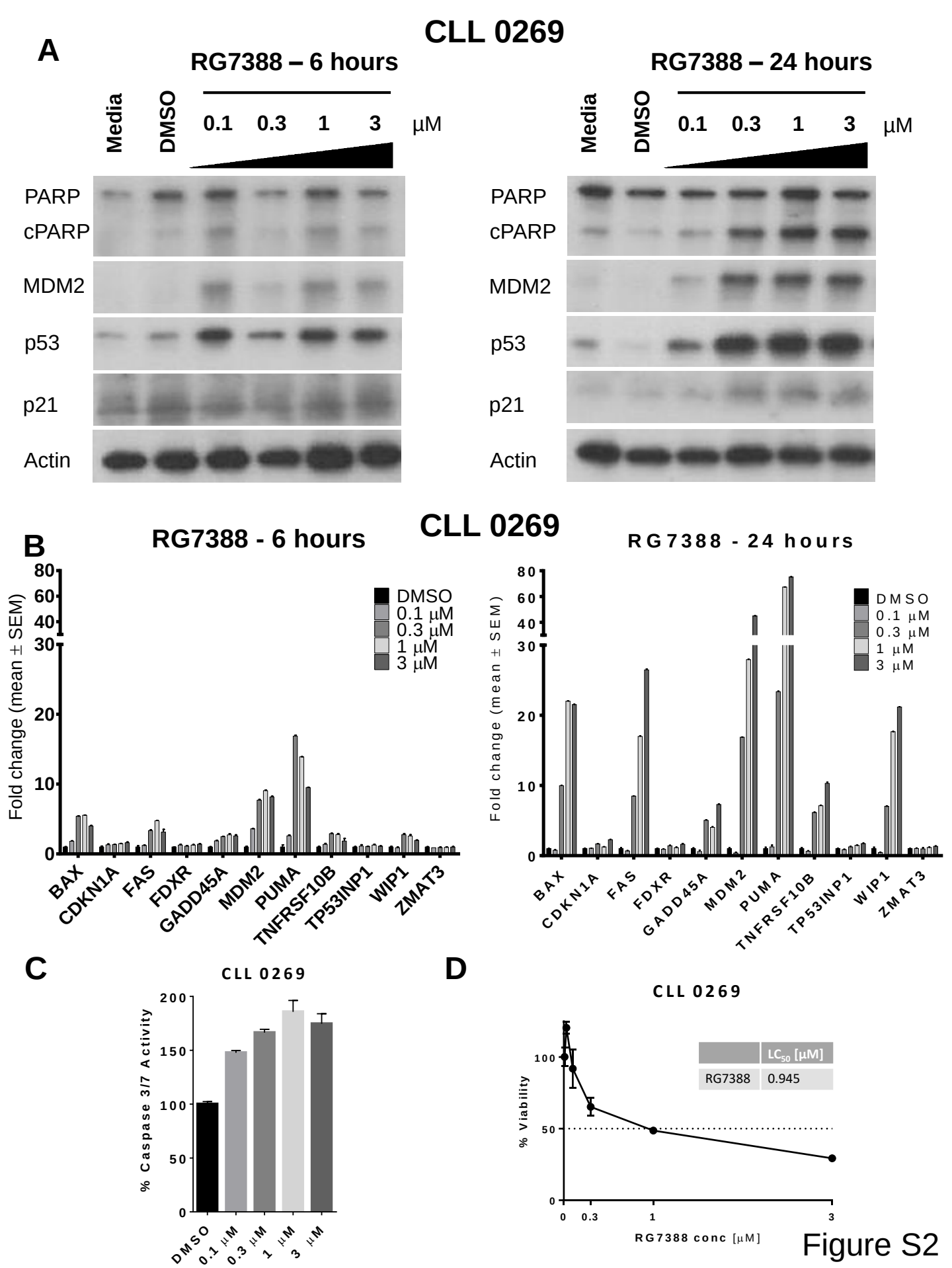
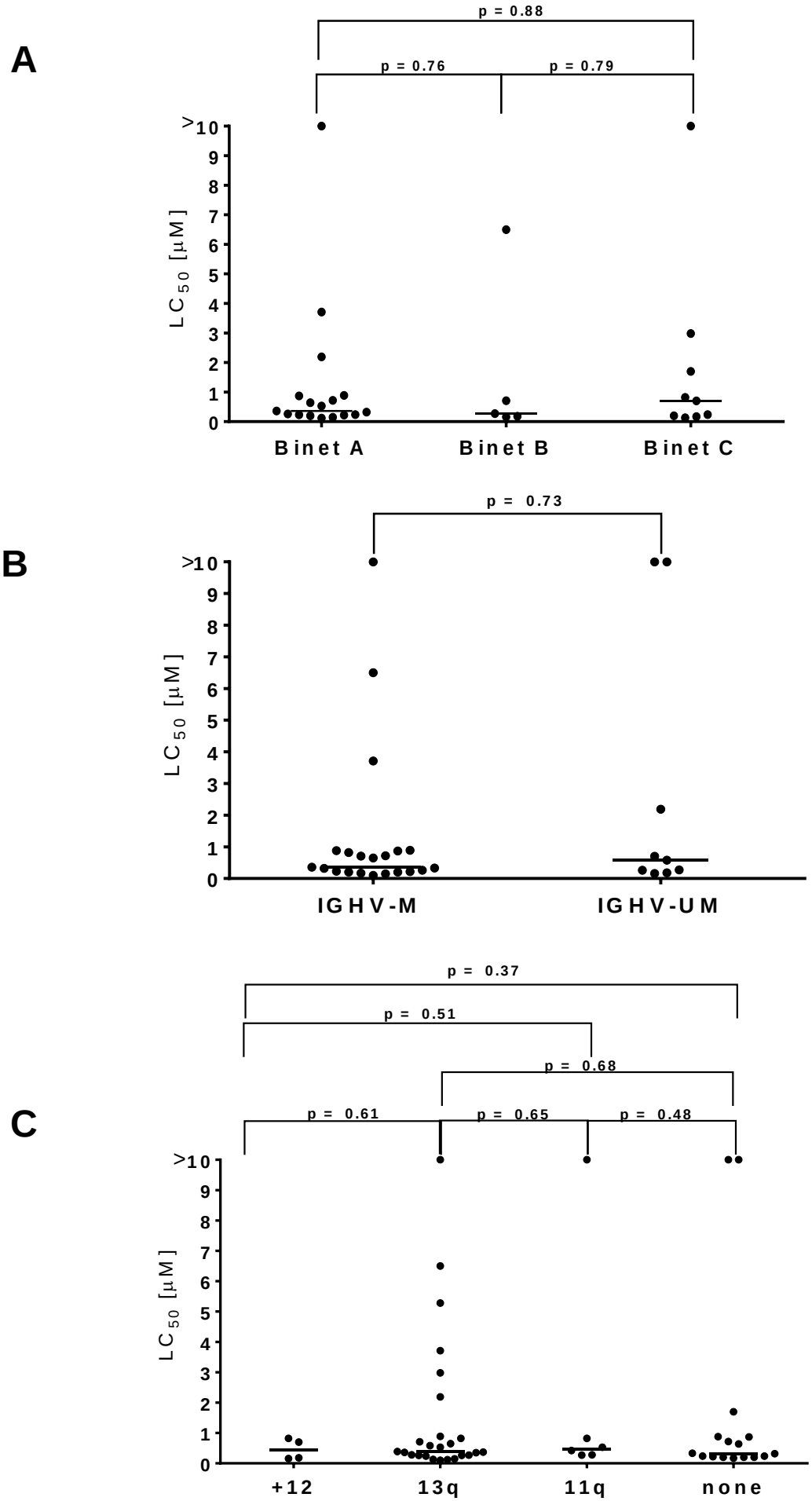

CLL 0269
A
RG7388 – 6 hours
RG7388 – 24 hours
Media
DMSO
0.1
0.3
1
3
µM
PARP
cPARP
MDM2
p53
p21
Actin
CLL 0269
B
RG7388 - 6 hours
RG7388 - 24 hours
Fold change (mean ± SEM)
DMSO
0.1 µM
0.3 µM
1 µM
3 µM
BAX
CDKN1A
FAS
FDXR
GADD45A
MDM2
PUMA
TNFRSF10B
TP53INP1
WIP1
ZMAT3
C
CLL 0269
% Caspase 3/7 Activity
DMSO
0.1 µM
0.3 µM
1 µM
3 µM
D
CLL 0269
% Viability
LC50 [µM]
RG7388
0.945
RG7388 conc [µM]

Figure S2


A
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p = 0.76
p = 0.79
LC50 [µM]
>10
9
8
7
6
5
4
3
2
1
0
Binet A
Binet B
Binet C
B
p = 0.73
LC50 [µM]
>10
9
8
7
6
5
4
3
2
1
0
IGHV-M
IGHV-UM
C
p = 0.37
p = 0.51
p = 0.68
p = 0.61
p = 0.65
p = 0.48
LC50 [µM]
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9
8
7
6
5
4
3
2
1
0
+12
13q
11q
none

Figure S3

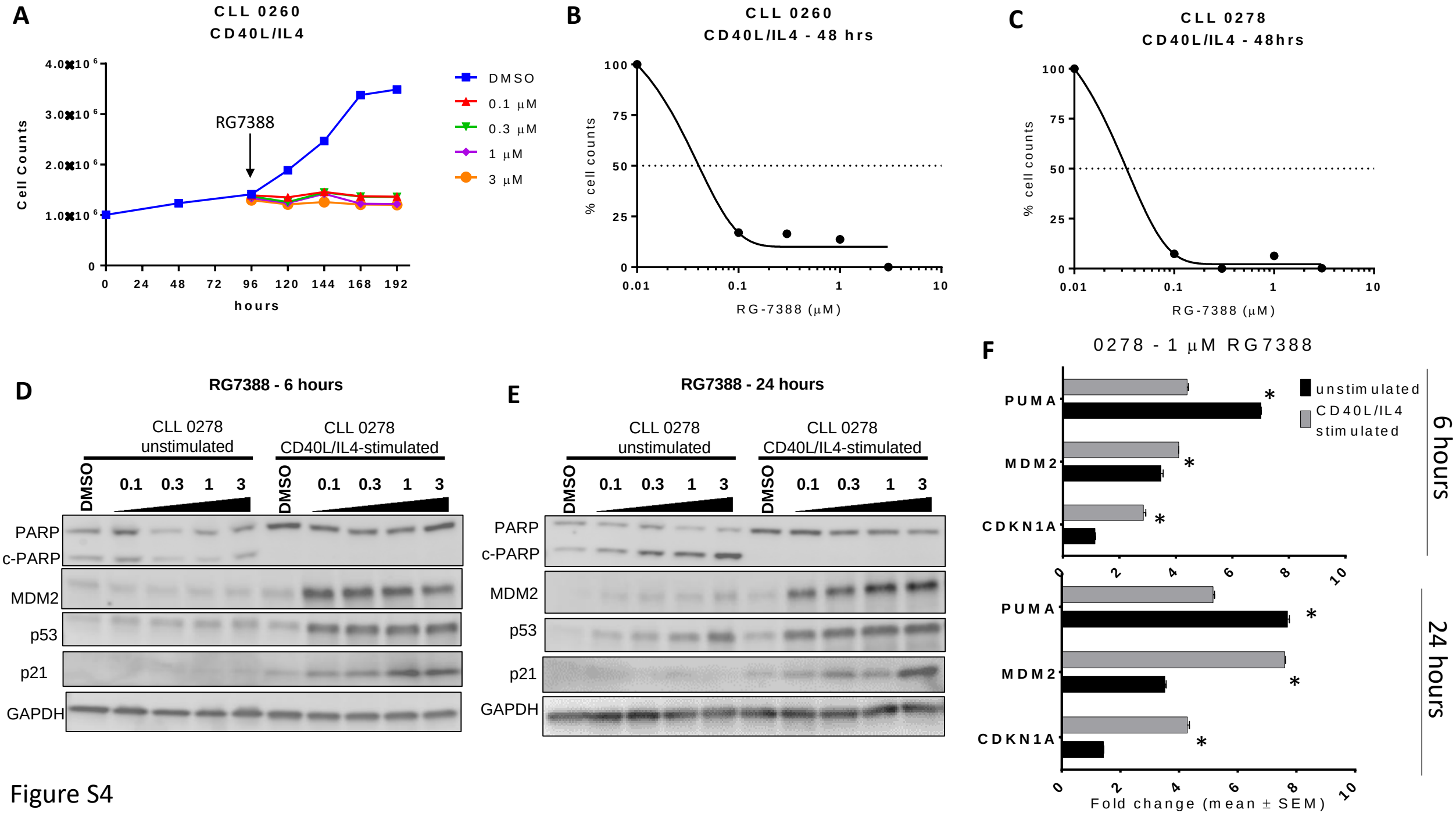

A
CLL 0260
CD40L/IL4
Cell Counts
hours
RG7388
DMSO
0.1 µM
0.3 µM
1 µM
3 µM
B
CLL 0260
CD40L/IL4 - 48 hrs
% cell counts
RG-7388 (µM)
C
CLL 0278
CD40L/IL4 - 48hrs
% cell counts
RG-7388 (µM)
D
RG7388 - 6 hours
CLL 0278 unstimulated
CLL 0278 CD40L/IL4-stimulated
DMSO
0.1
0.3
1
3
PARP
c-PARP
MDM2
p53
p21
GAPDH
E
RG7388 - 24 hours
CLL 0278 unstimulated
CLL 0278 CD40L/IL4-stimulated
F
0278 - 1 µM RG7388
unstimulated
CD40L/IL4 stimulated
PUMA
MDM2
CDKN1A
6 hours
24 hours
Fold change (mean ± SEM)

Figure S4

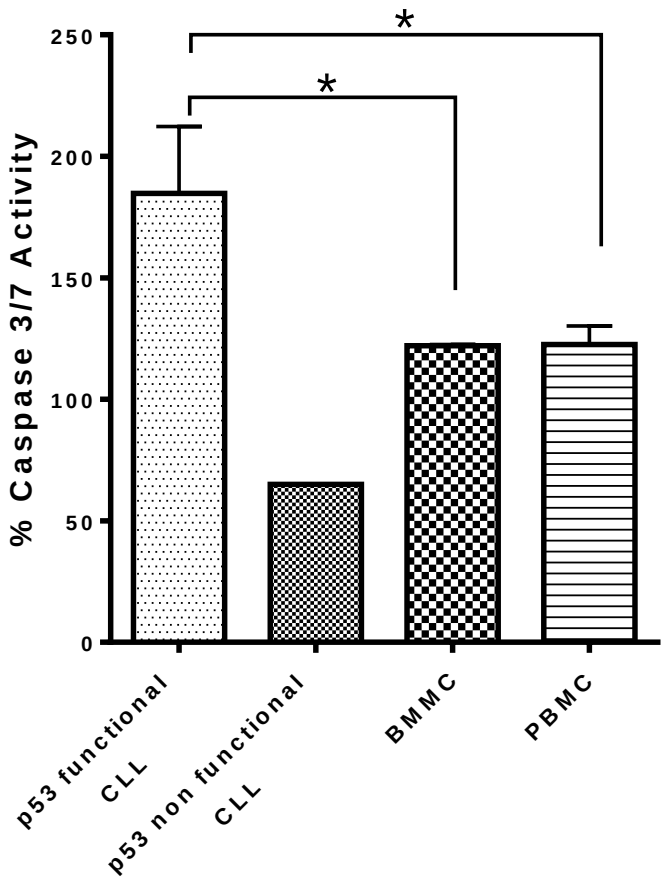

RG7388 - 1 μM - 24 hours
% Caspase 3/7 Activity
250
200
150
100
50
0
*
*
p53 functional CLL
p53 non functional CLL
BMMC
PBMC

Figure S5

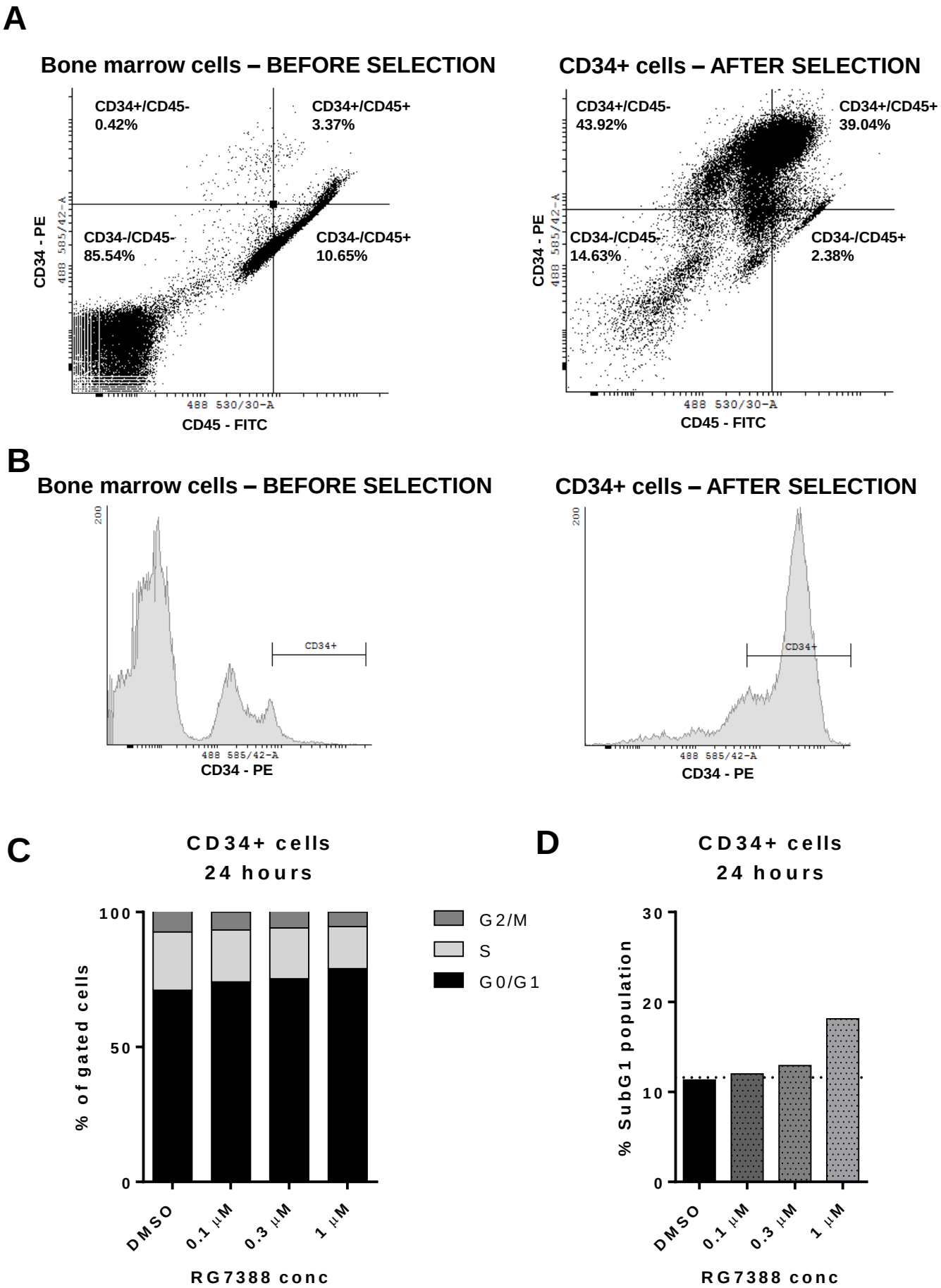

A
Bone marrow cells – BEFORE SELECTION
CD34+/CD45-
0.42%
CD34+/CD45+
3.37%
CD34-/CD45-
85.54%
CD34-/CD45+
10.65%
CD34 - PE
488 585/42-A
488 530/30-A
CD45 - FITC
CD34+ cells – AFTER SELECTION
CD34+/CD45-
43.92%
CD34+/CD45+
39.04%
CD34-/CD45-
14.63%
CD34-/CD45+
2.38%
CD34 - PE
488 585/42-A
488 530/30-A
CD45 - FITC
B
Bone marrow cells – BEFORE SELECTION
200
CD34+
488 585/42-A
CD34 - PE
CD34+ cells – AFTER SELECTION
200
CD34+
488 585/42-A
CD34 - PE
C
CD34+ cells
24 hours
100
50
0
% of gated cells
G2/M
S
G0/G1
DMSO
0.1 µM
0.3 µM
1 µM
RG7388 conc
D
CD34+ cells
24 hours
30
20
10
0
% SubG1 population
DMSO
0.1 µM
0.3 µM
1 µM
RG7388 conc

Figure S6

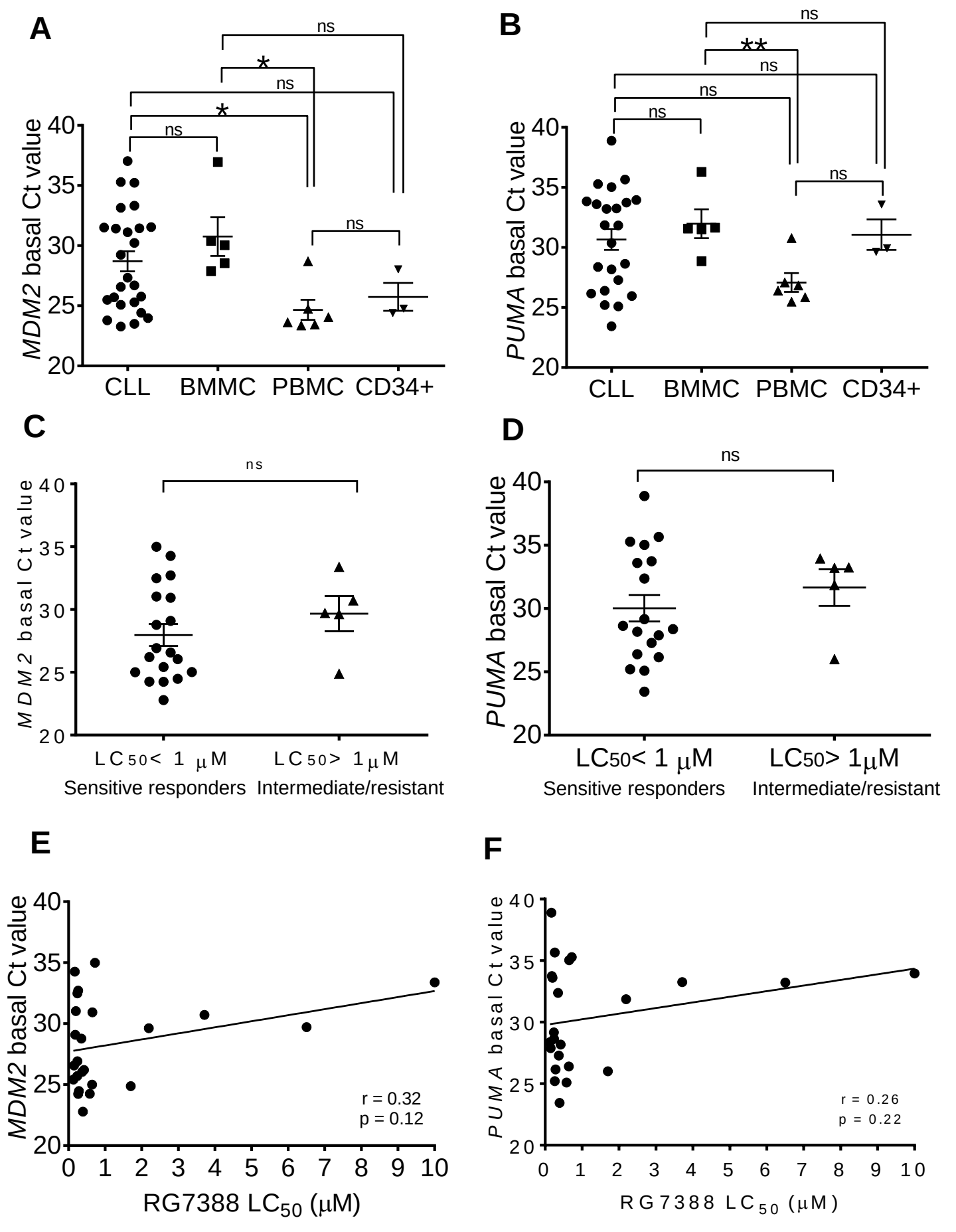
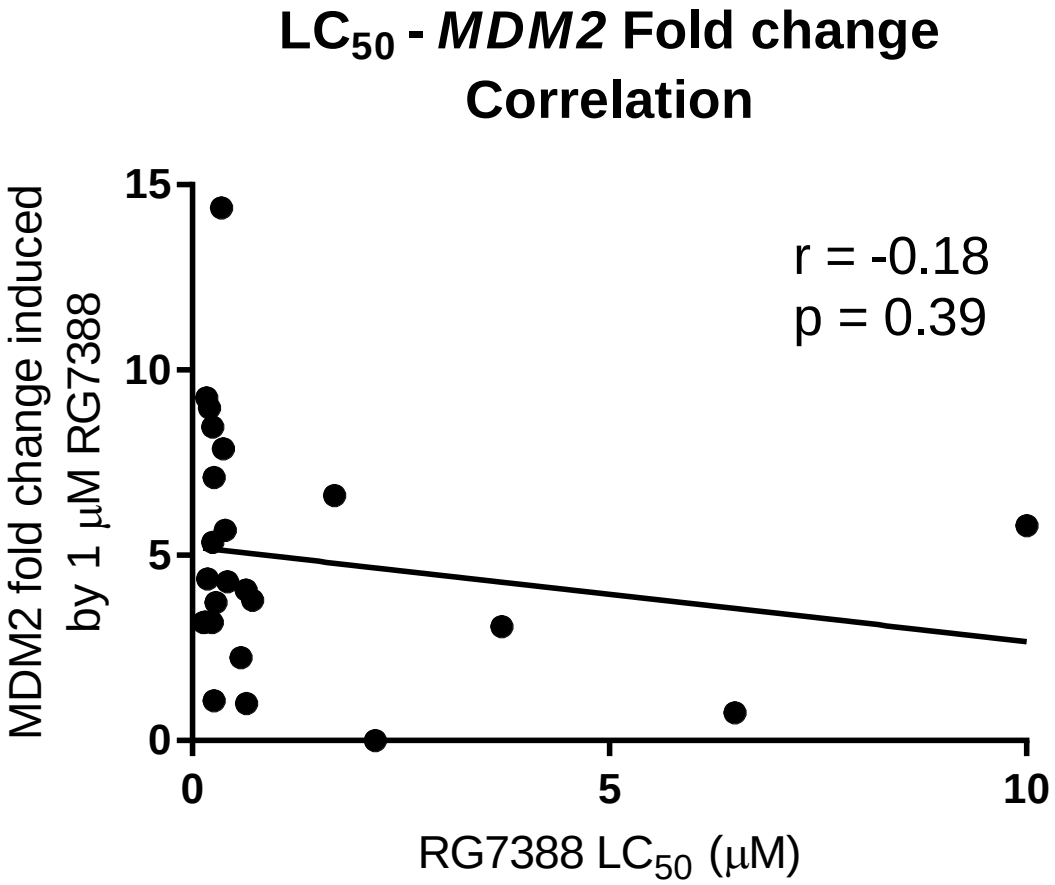

A
ns
*
ns
*
ns
ns
MDM2 basal Ct value
40
35
30
25
20
CLL
BMMC
PBMC
CD34+
B
ns
**
ns
ns
ns
ns
PUMA basal Ct value
40
35
30
25
20
CLL
BMMC
PBMC
CD34+
C
ns
MDM2 basal Ct value
40
35
30
25
20
LC50< 1 μM
LC50> 1μM
Sensitive responders
Intermediate/resistant
D
ns
PUMA basal Ct value
40
35
30
25
20
LC50< 1 μM
LC50> 1μM
Sensitive responders
Intermediate/resistant
E
MDM2 basal Ct value
40
35
30
25
20
r = 0.32
p = 0.12
0 1 2 3 4 5 6 7 8 9 10
RG7388 LC50 (μM)
F
PUMA basal Ct value
40
35
30
25
20
r = 0.26
p = 0.22
0 1 2 3 4 5 6 7 8 9 10
RG7388 LC50 (μM)

Figure S7

A


LC50 - MDM2 Fold change Correlation
MDM2 fold change induced by 1 μM RG7388
r = -0.18
p = 0.39
15
10
5
0
0
5
10
RG7388 LC50 (μM)

B

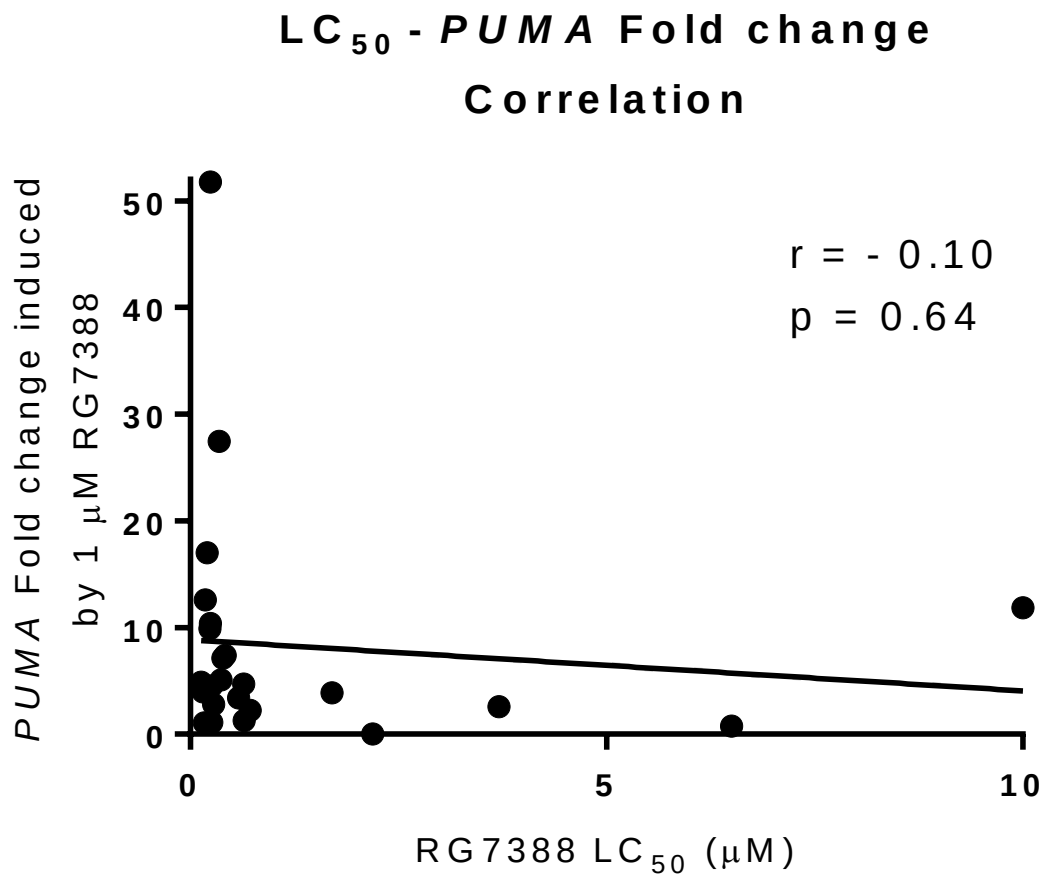
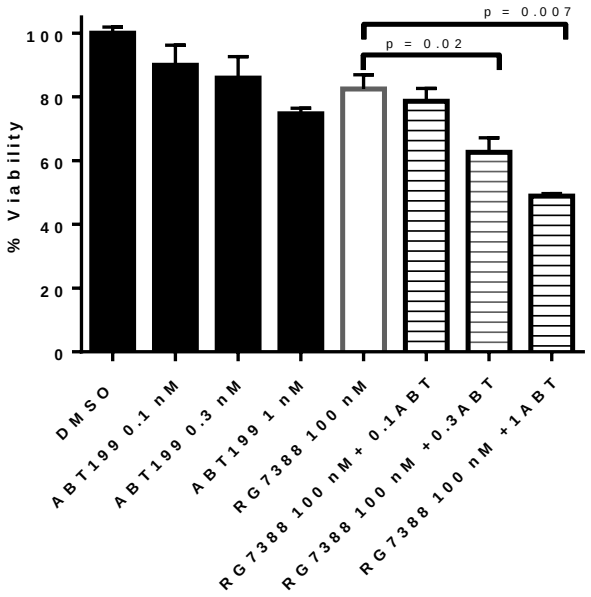
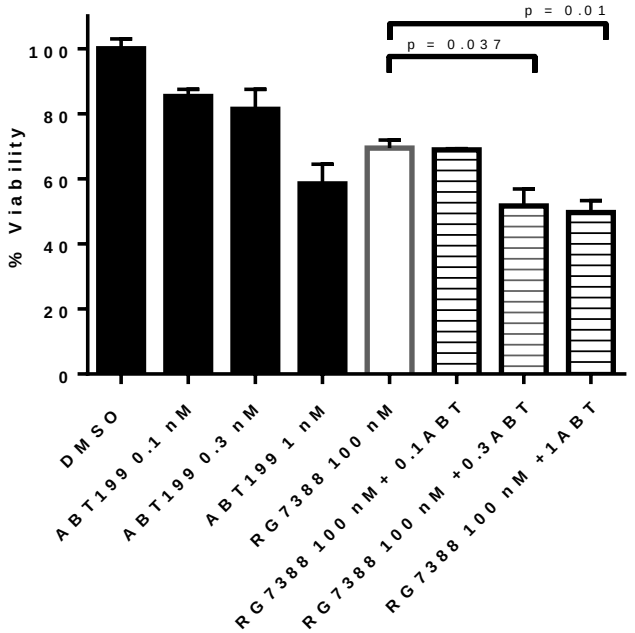

LC50 - PUMA Fold change Correlation
PUMA Fold change induced by 1 μM RG7388
r = - 0.10
p = 0.64
50
40
30
20
10
0
0
5
10
RG7388 LC50 (μM)

Figure S8

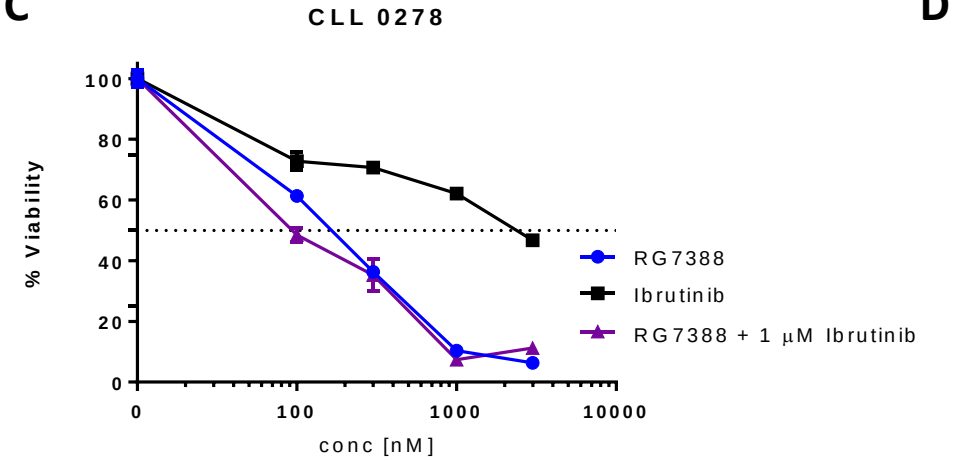
A


CLL0307 (TP53 WT)
% Viability
100
80
60
40
20
0
p = 0.007
p = 0.02
DMSO
ABT199 0.1 nM
ABT199 0.3 nM
ABT199 1 nM
RG7388 100 nM
RG7388 100 nM + 0.1ABT
RG7388 100 nM +0.3ABT
RG7388 100 nM +1ABT

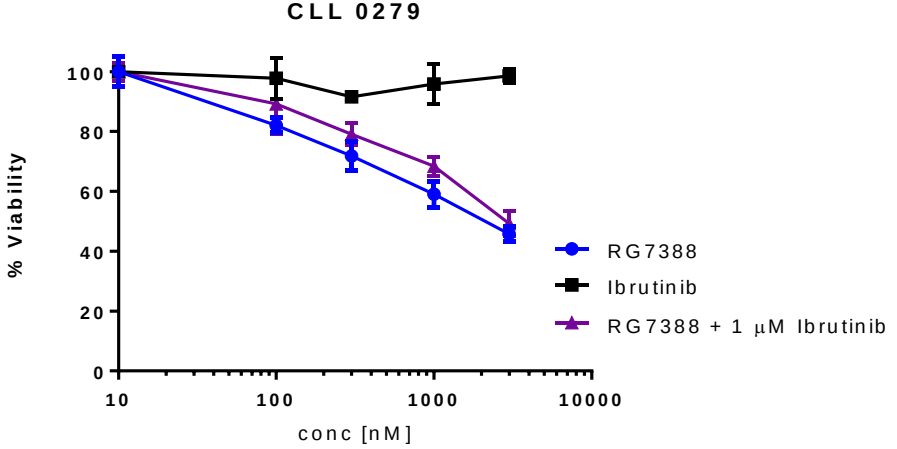
B


CLL0308 (TP53 WT)
% Viability
100
80
60
40
20
0
p = 0.01
p = 0.037
DMSO
ABT199 0.1 nM
ABT199 0.3 nM
ABT199 1 nM
RG7388 100 nM
RG7388 100 nM + 0.1ABT
RG7388 100 nM +0.3ABT
RG7388 100 nM +1ABT

C


CLL 0278
% Viability
100
80
60
40
20
0
0
100
1000
10000
conc [nM]
RG7388
Ibrutinib
RG7388 + 1 μM Ibrutinib

D


CLL 0279
% Viability
100
80
60
40
20
0
10
100
1000
10000
conc [nM]
RG7388
Ibrutinib
RG7388 + 1 μM Ibrutinib