Figure S1. Tissue transglutaminase (TG2) forms a complex with the NF-κB component p65 and affects the affinity between IκBα and p65 in MCL cells.

Jeko-1 cells were treated with A23187 (2 μM for 24 hrs) for TG2 activation, the specific TG2 inhibitors monodansylcadaverine (MDC; 50 μM for 24 hrs), or a L-type calcium channel blocker perillyl alcohol (POH; 1 mM for 24 hrs). Cell lysates were prepared from untreated and treated Jeko-1 samples that were immunoprecipitated using an anti-TG2 antibody and probed with anti-TG2 and anti-p65 antibodies. Immunoprecipitation of MCL cell lysates with an anti-TG2 antibody pulled down p65 component of NF-κB. The changes in TG2 activity altered the intensity of TG2-conjugated p65 bands.

Figure S2. Calcium blockers suppressed NF-κB activity in MCL regardless of treatment duration.

(A) Nuclear extracts from SP-53 and Jeko-1 cells with or without TG2-specific inhibitors, MDC (50 μM for 4 hrs) and BPA (1 mM for 4 hrs), were subjected to ELISA assays to evaluate p50 and p65 DNA-binding activities. Changes in p50 and p65 DNA-binding activity levels before and after treatment with MDC or BPA were measured. The colorimetric values in untreated samples were subtracted from those measured in treated samples. The results are shown as the mean ± standard deviation. MDC and BPA suppressed NF-κB DNA-binding activity in MCL cells not only with long incubation time, 24 hrs but also with short incubation time, 4 hrs. MDC, monodansylcadaverine; BPA, 5-biotinamidopentylamine.

(B) TG2-specific inhibitors do not affect the cell viability and proliferation of MCL cells. The cytotoxicity of TG2-specific inhibitors, MDC and BPA, on a patient sample and MCL cell lines, Jeko-1, SP53, Mino, and REC-1 were measured using CellTiter-Blue® fluorometric cell viability assay (Promega). Cells were cultured for 24 hrs with MDC or BPA. The drugs were serially diluted as indicated using maximal drug doses (50 μM MDC and 1 mM BPA). Cytotoxicity was indicated as a ratio compared to cell viability without treatment. The results are shown as the mean ± standard deviation of triplicate. MDC, monodansylcadaverine; BPA, 5-biotinamidopentylamine.

Figure S2.

(C) Nuclear extracts from SP-53 and Jeko-1 cells that were untreated or treated with BAPTA/AM (60 μM for 4 hrs) or POH (1 mM for 4 hrs) were analyzed for p50 and p65 DNA-binding activities using ELISA assays. Changes in p50 and p65 DNA-binding activity levels before and after treatment with BAPTA/AM or POH were measured. The colorimetric values in untreated samples were subtracted from the values measured in treated samples. The results are shown as the mean ± standard deviation. POH, perillyl alcohol. Calcium blockers also suppressed NF-κB activity of MCL cells even in short-timed treatment.

Figure S3. NF-κB is constitutively expressed in CD45+CD19- MCL-ICs.

(A) Nuclear extracts of CD45+CD19- MCL-ICs from five different patients were analyzed for p50 and p65 expression by immunoblots. TBP (TATA-binding protein) was used as a control. The Jeko-1 cell line was used as a positive control, and normal B cells were used as negative controls. MCL-ICs have constitutively upregulated NF-κB p50 and p65 expression.

(B) ELISA assays to measure p50 and p65 DNA-binding activities were performed using nuclear lysates of CD45+CD19- MCL-ICs from five different patients. Raji cells with no drug treatments were used as a positive control, and normal blood cells were used as a negative control. The results are shown as the mean ± standard deviation. Elevated p50 and p65 DNA-binding activities were noted in MCL-ICs compared to normal blood cells with statistical significance (*P* values by unpaired *t*-test with negative control and patient samples are 0.04 in p50 and 0.009 in p65).

Figure S4. Calcium blockers affect NF-κB activity in CD45+CD19- MCL-ICs.

(A) Stem-like MCL cells constitutively expressed the polymeric forms of IκBα. Cell lysates prepared from stem-like cells (CD45+CD19-) from five different patients were immunoprecipitated and immunoblotted with an anti-IκBα antibody. Analysis indicated the presence of dimeric forms (66 kDa) of IκBα. IP, immunoprecipitation; WB, western blot.

(B) Calcium blockers inhibited NF-κB DNA-binding activities in primary CD45+CD19- MCL-ICs with short incubation period. Nuclear extracts from CD45+CD19- MCL-ICs that were untreated or treated with BAPTA/AM (60 μM for 4 hrs) or POH (1 mM for 4 hrs) were analyzed using ELISA assays to evaluate p50 and p65 DNA-binding activities. The relative ratio values of NF-κB DNA-binding activities after drug treatment are shown as the mean ± standard deviation. *, $P<0.05$ by unpaired *t*-test. POH, perillyl alcohol.

Figure S5. CD45+CD19- MCL-ICs isolated from patient samples are bortezomib-resistant.

(A) CD45+CD19- MCL-ICs and CD45+CD19+ cells isolated from five different MCL patients were tested for the chemotherapy sensitivity to bortezomib. Cells were cultured for 16 hr after the addition of bortezomib. Cell viability was determined by CellTiter-Blue® fluorometric assay (Promega) and was indicated as a ratio compared to cell viability without treatment. The mean IC_{50} value of bortezomib for CD45+CD19- MCL-ICs was higher than those of the CD45+CD19+ MCL cells, Jeko-1, or even REC-1, which was reported as a bortezomib-resistant cell line. Bars represent averages; *, $P<0.05$ by unpaired *t*-test.

(B) Proteasome activities of BAPTA/AM-treated and POH-treated cells were maintained at comparable levels to bortezomib-treated cells. Five different CD45+CD19- MCL-ICs were treated with bortezomib (100 nM), BAPTA/AM (60 μM), or POH (1 mM) for 16 hrs and compared based on proteasome activities. Proteasome activity was assessed using a colorimetric assay based on substrate cleavage (CHEMICON® Proteasome Activity Assay Kit, Chemicon International). *, $P<0.05$ by unpaired *t*-test. BTZ, bortezomib; POH, perillyl alcohol.

Figure S6. Calcium blockers have synergic cytotoxic effects with bortezomib in CD45+CD19- MCL-ICs.

(A) Representative non-fixed 7-AAD-staining flow cytometric profiles show CD45+CD19- MCL-ICs isolated from 5 different patients after 16 hr-incubation with or without bortezomib (100 nM) and the combination of bortezomib (100 nM) and POH (1mM). Dead cell fractions are indicated as red spots in boxes, and live cells are indicated as blue spots. The proportions of dead cells were increased after treatment with bortezomib. Furthermore, The combination of bortezomib and POH readily caused more cell death than bortezomib alone. POH sensitized the sensitivity of CD45+CD19- MCL-ICs to bortezomib.

(B) POH improved the bortezomib sensitivity in CD45+CD19- MCL-ICs. The chemosensitivities of CD45+CD19- MCL-ICs to bortezomib were compared with or without POH using CellTiter-Blue® fluorometric cell viability assay (Promega). Cells were cultured for 16 hrs with bortezomib or POH alone or with combination of bortezomib and POH. The drugs were serially diluted as indicated using maximal drug doses (100 nM bortezomib and 2 mM POH). Cytotoxicity was indicated as a ratio compared to cell viability without treatment. The results are shown as the mean ± standard deviation of triplicate. BTZ, bortezomib; POH, perillyl alcohol.

Figure S1.

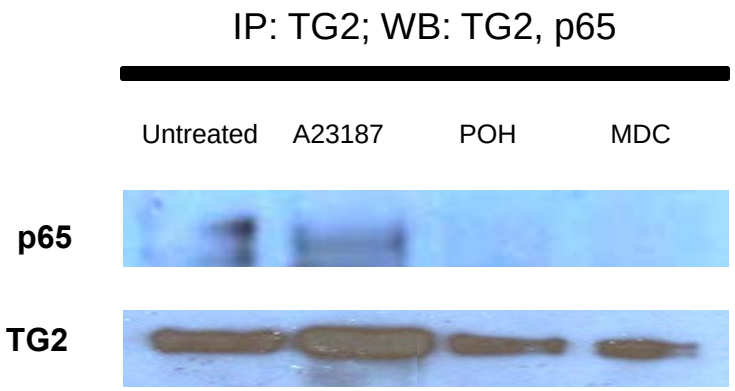
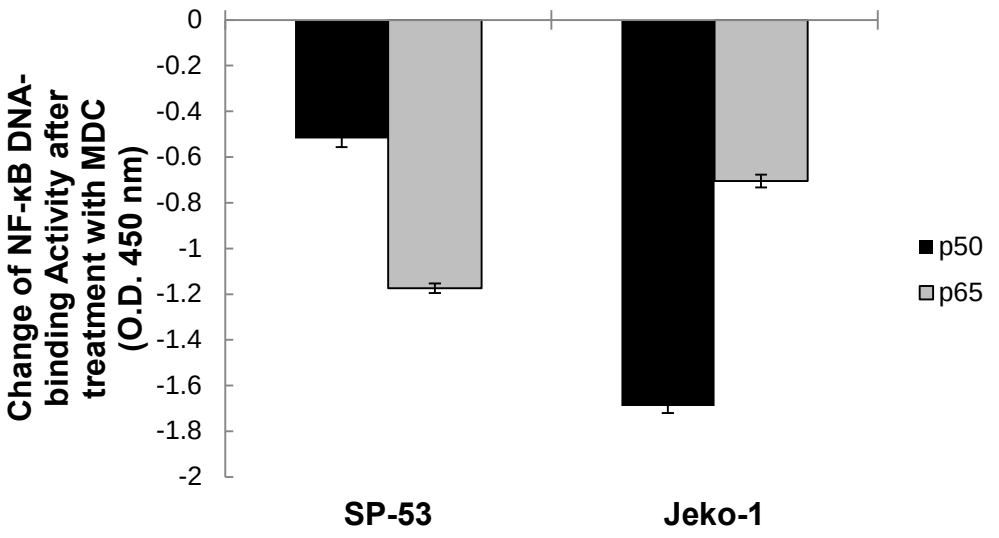


Figure S2.

(A)


Change of NF-κB DNA-binding Activity after treatment with MDC (O.D. 450 nm)
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
-1.4
-1.6
-1.8
-2
SP-53
Jeko-1
p50
p65

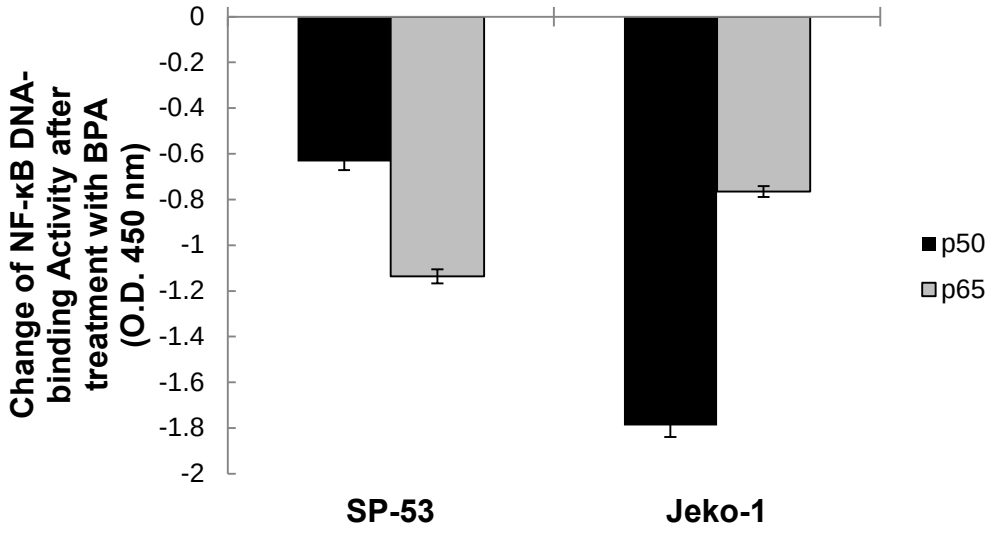

Change of NF-κB DNA-binding Activity after treatment with BPA (O.D. 450 nm)
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
-1.4
-1.6
-1.8
-2
SP-53
Jeko-1
p50
p65

Figure S2.

(B)

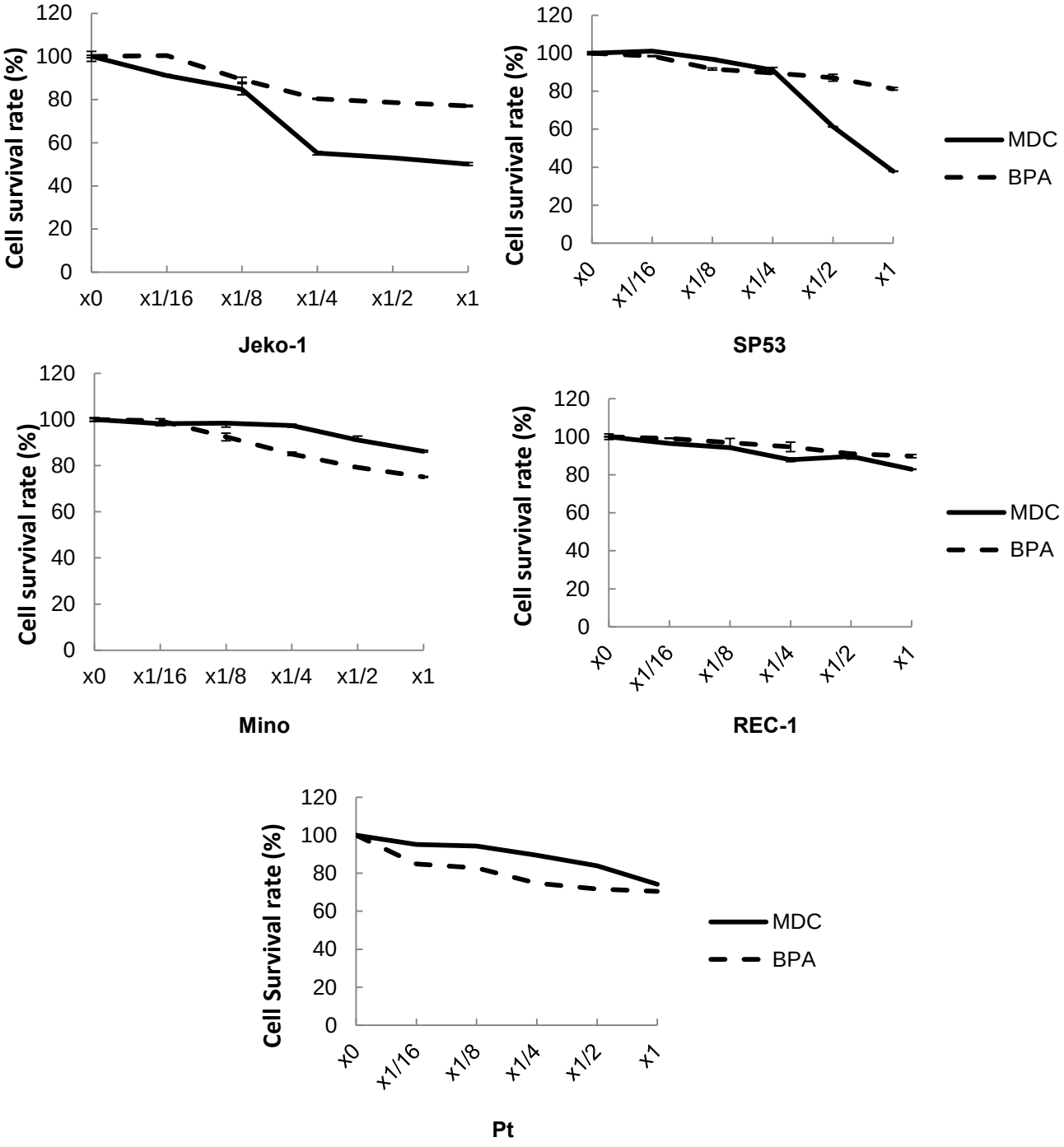
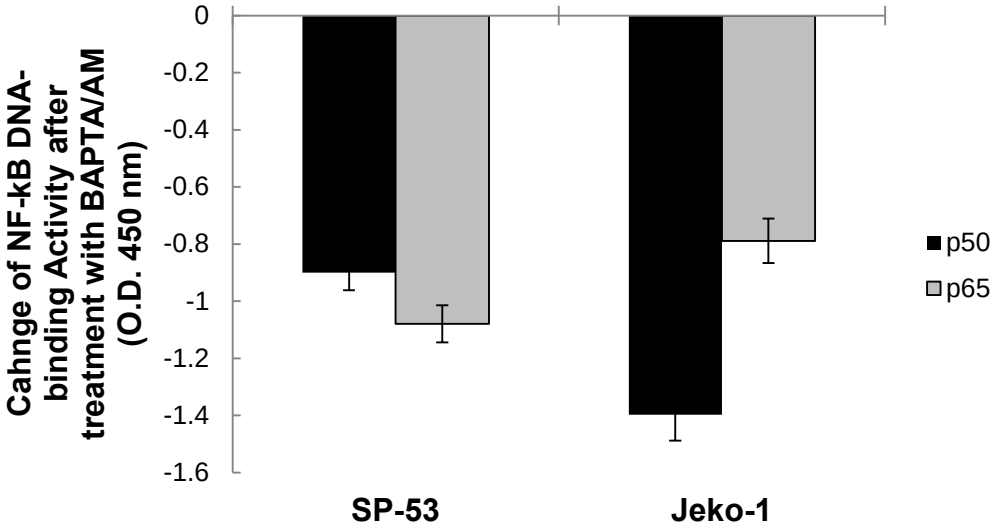

Cell survival rate (%)
120
100
80
60
40
20
0
x0
x1/16
x1/8
x1/4
x1/2
x1
Jeko-1
SP53
Mino
REC-1
Pt
Cell Survival rate (%)
MDC
BPA

Figure S2.

(C)


Cahnge of NF-kB DNA-binding Activity after treatment with BAPTA/AM (O.D. 450 nm)
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
-1.4
-1.6
SP-53
Jeko-1
p50
p65

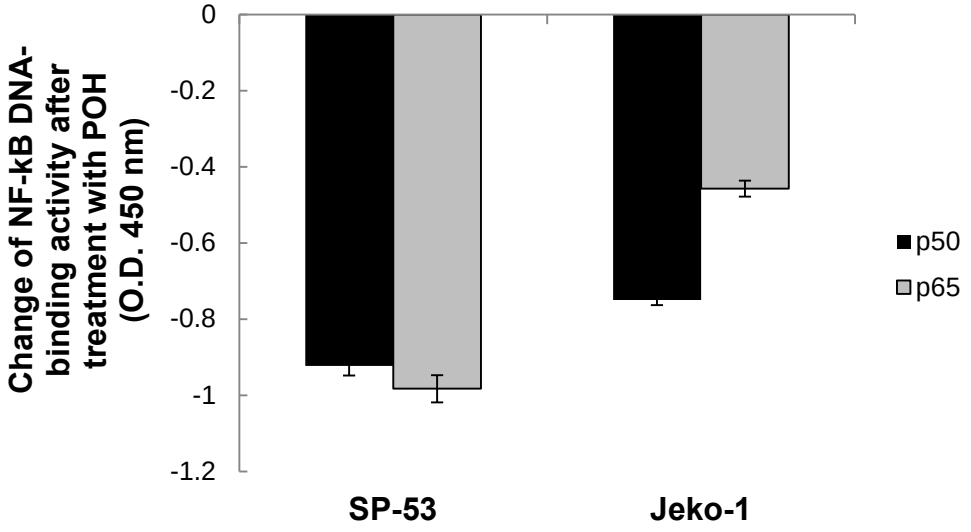

Change of NF-kB DNA-binding activity after treatment with POH (O.D. 450 nm)
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
SP-53
Jeko-1
p50
p65

Figure S3.

(A)

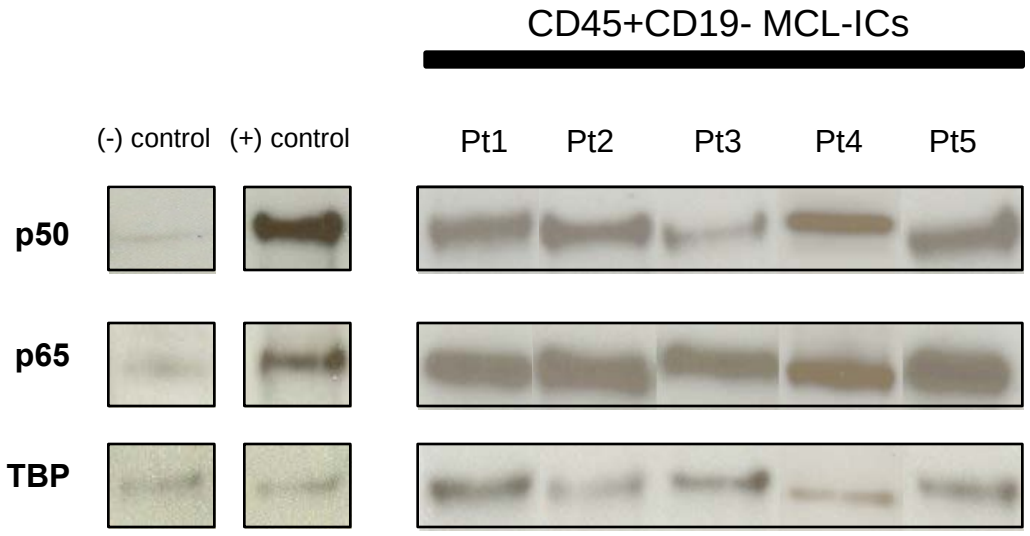

CD45+CD19- MCL-ICs
(-) control
(+) control
Pt1
Pt2
Pt3
Pt4
Pt5
p50
p65
TBP

Figure S3.

(B)

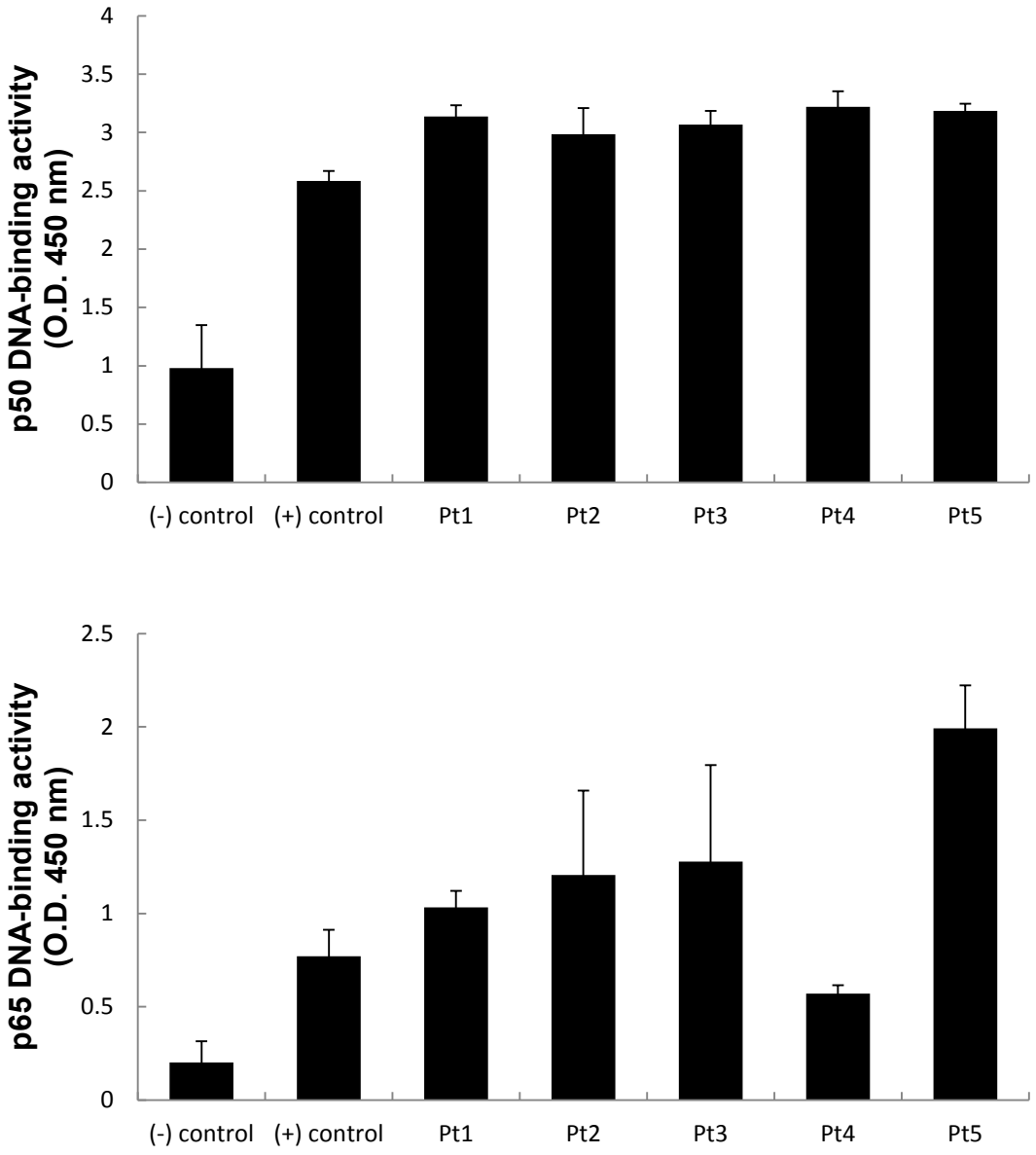

p50 DNA-binding activity (O.D. 450 nm)
4
3.5
3
2.5
2
1.5
1
0.5
0
(-) control
(+) control
Pt1
Pt2
Pt3
Pt4
Pt5
p65 DNA-binding activity (O.D. 450 nm)
2.5
2
1.5
1
0.5
0
(-) control
(+) control
Pt1
Pt2
Pt3
Pt4
Pt5

Figure S4.

(A)

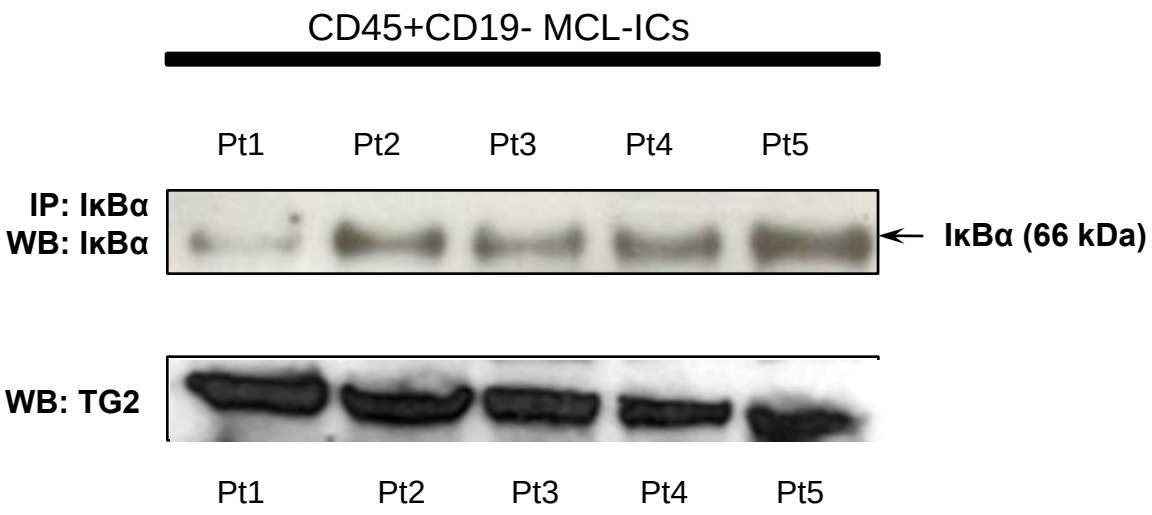

CD45+CD19- MCL-ICs
Pt1
Pt2
Pt3
Pt4
Pt5
IP: IκBα
WB: IκBα
IκBα (66 kDa)
WB: TG2
Pt1
Pt2
Pt3
Pt4
Pt5

Figure S4.

(B)

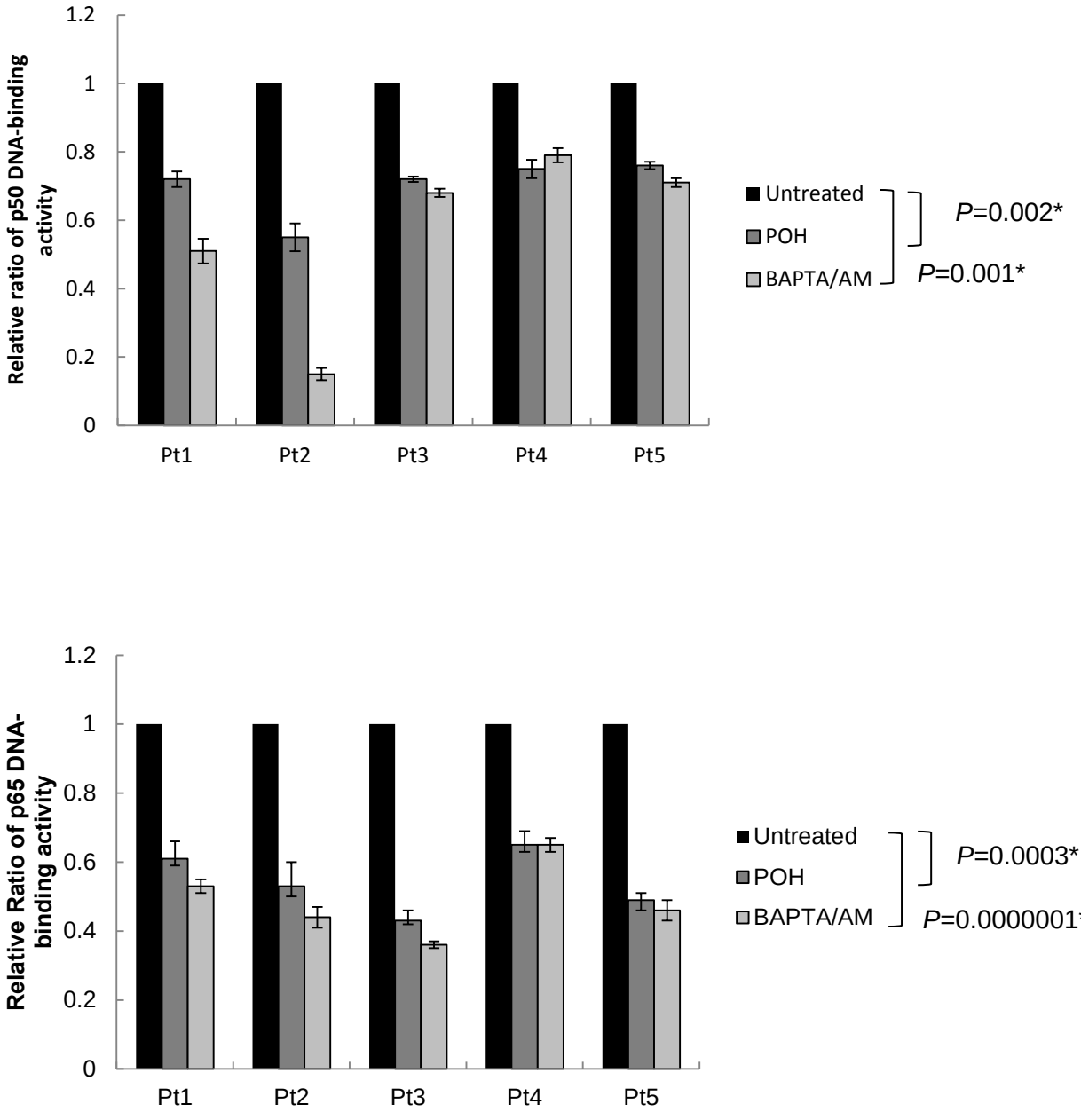

Relative ratio of p50 DNA-binding activity
1.2
1
0.8
0.6
0.4
0.2
0
Pt1
Pt2
Pt3
Pt4
Pt5
Untreated
POH
BAPTA/AM
P=0.002*
P=0.001*
Relative Ratio of p65 DNA-binding activity
1.2
1
0.8
0.6
0.4
0.2
0
Pt1
Pt2
Pt3
Pt4
Pt5
Untreated
POH
BAPTA/AM
P=0.0003*
P=0.0000001*

Figure S5.

(A)

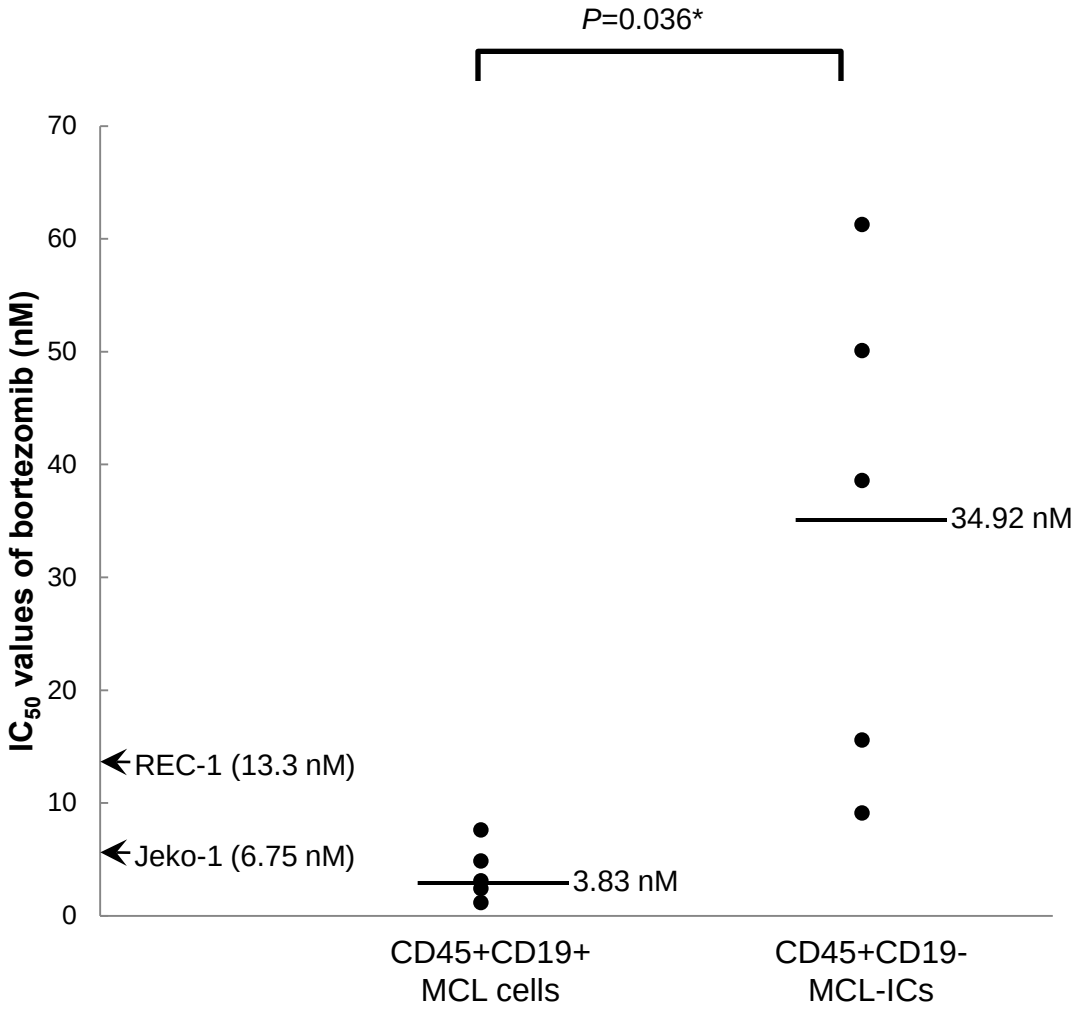

P=0.036*
70
60
50
40
30
20
10
0
IC50 values of bortezomib (nM)
34.92 nM
REC-1 (13.3 nM)
Jeko-1 (6.75 nM)
3.83 nM
CD45+CD19+
MCL cells
CD45+CD19-
MCL-ICs

Figure S5.

(B)

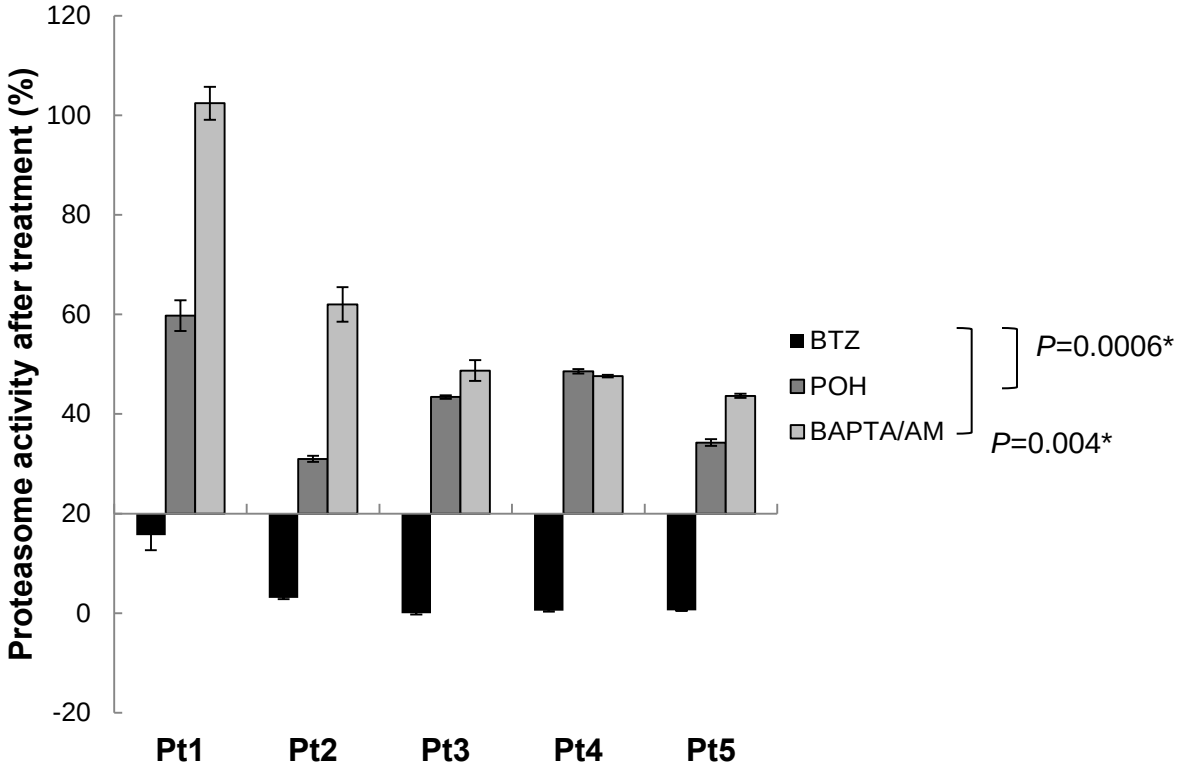

120
100
80
60
40
20
0
-20
Proteasome activity after treatment (%)
Pt1
Pt2
Pt3
Pt4
Pt5
BTZ
POH
BAPTA/AM
P=0.0006*
P=0.004*

Figure S6.

(A)

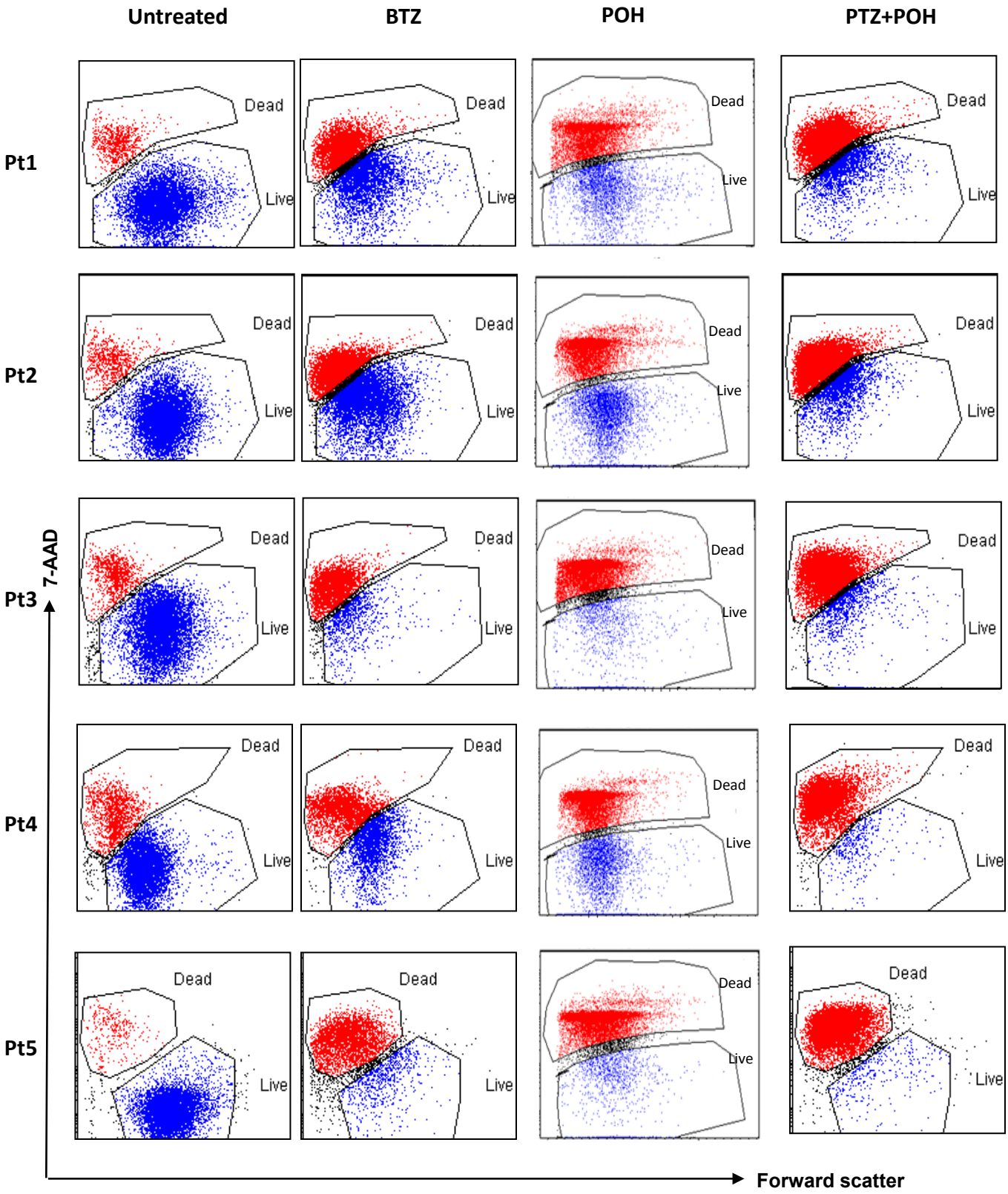
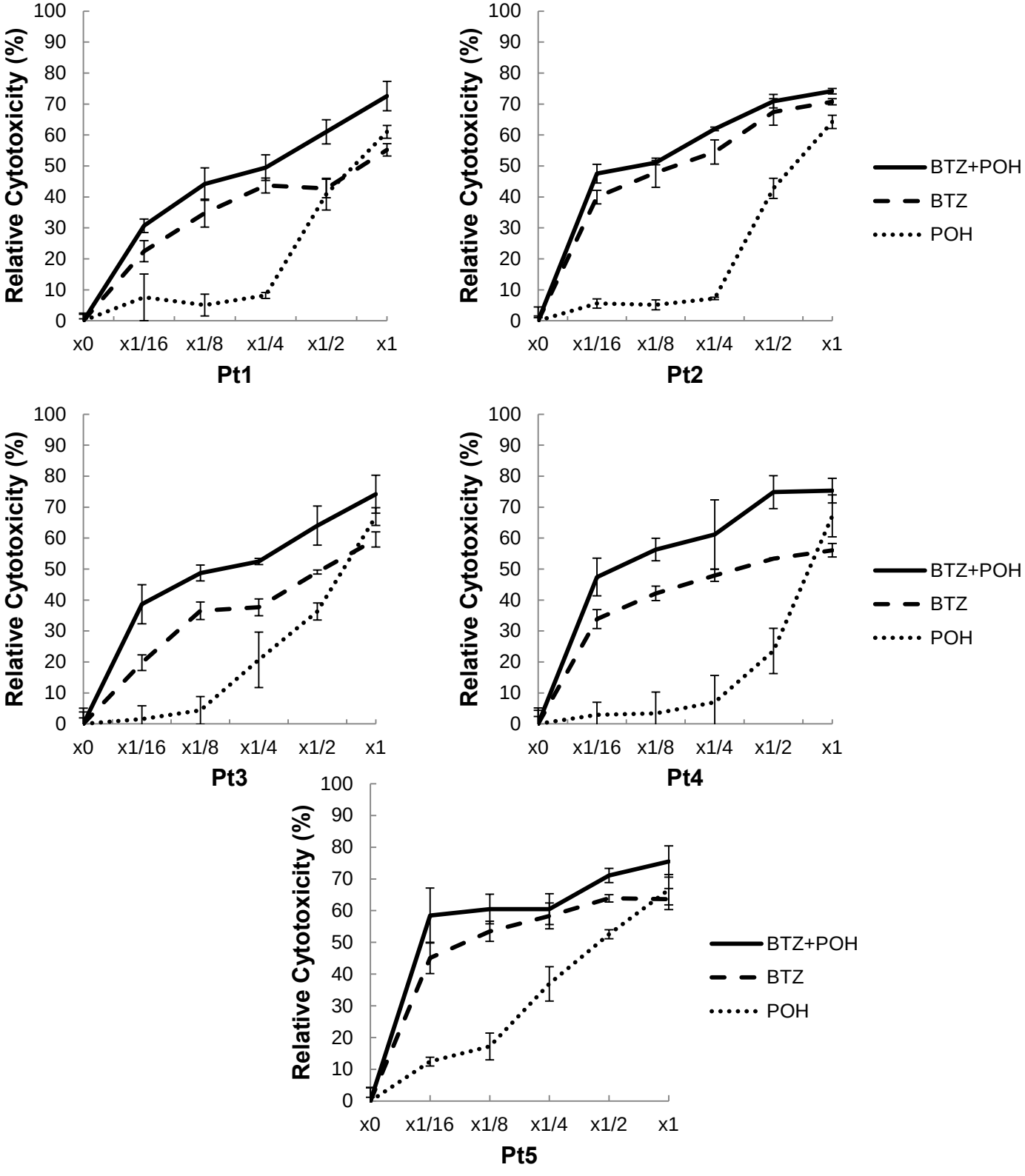

Untreated
BTZ
POH
PTZ+POH
Pt1
Pt2
Pt3
Pt4
Pt5
Dead
Live
7-AAD
Forward scatter

Figure S6.

(B)


Relative Cytotoxicity (%)
100
90
80
70
60
50
40
30
20
10
0
x0
x1/16
x1/8
x1/4
x1/2
x1
Pt1
Pt2
Pt3
Pt4
Pt5
BTZ+POH
BTZ
POH