SUPPLEMENTARY APPENDIX

Targeting intermediary metabolism enhances the efficacy of BH3 mimetic therapy in hematologic malignancies

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Supplemental information

Supplemental Figure S1. First *in vivo* dose of navitoclax resulted in marked ultrastructural changes in CLL cells. CLL cells isolated from two different patients prior to receiving navitoclax and 4 h after the first *in vivo* dosing during day 1 of the lead-in-period (L1D1) were incubated *ex vivo* for a further 8 h, before fixation in 2% glutaraldehyde and transmission electron microscopy. CLL cells from patients exposed to navitoclax exhibited chromatin condensation and rupture of the outer mitochondrial membrane. Scale bar – 500 nm.

Supplemental Figure S2. Rapid development of resistance to BH3 mimetic-mediated apoptosis in haematological cell lines. (**A**) Exposure of MAVER-1, K562 and H929 cells to ABT-199 (10 nM), A-1331852 (10 nM) and A-1210477 (10 μM), respectively resulted in a time dependent induction of apoptosis as assessed by western blotting for the indicated proteins. (**B-D**) Schemes used for developing the other three models of drug resistance as described in the Methods. Assessment of apoptosis by PS externalisation showed that resistance developed in the different cellular models following exposure for 4 h to ABT-199 (10 nM), A-1331852 (10 nM) and A-1210477 (5 μM), respectively. ***P $\leqslant$0.001; Error bars = Mean ± SEM (n=3).

Supplemental Figure S3. Resistance to BH3 mimetic-mediated apoptosis could not be attributed to marked changes in the expression levels of BCL2 family members. Immunoblots of BCL-2 family members showed no major changes in the indicated cell lines, during the development of resistance, depicted as A, C and E. A is the sensitive parent line, whereas C and E are relatively more resistant to the BH3 mimetics compared to A. * in the PUMA immunoblot depicts a non-specific band.

Supplemental Figure S4. Protein-protein interactions among anti- and pro-apoptotic BCL-2 family proteins are similar in both sensitive and resistant cells. Immunoprecipitates of BIM and PUMA in MAVER-1, K562 and H929 (A and E) cells showed no major differences in the binding of the indicated proteins. BC represents the beads control.

Supplemental Figure S5. Resistance to BH3 mimetics in the different resistance models can be overcome by simultaneous inhibition of multiple BCL-2 members. (**A-C**) Sensitive and resistant lines of MAVER-1, K562 and H929 from the different resistance models, as explained in methods and depicted in Supplemental figure 2, were exposed for 4 h to ABT-199 (10 nM), A-1331852 (10 nM) or A-1210477 (5 μM) and the extent of apoptosis assessed by PS externalisation. ***P ⩽0.001, **P⩽0.01; Error bars = Mean ± SEM (n=3).

Supplemental Figure S6. Glutamine deprivation sensitises the different cell lines to BH3 mimetic-mediated apoptosis. (**A-C**) Sensitive and resistant lines of MAVER-1, K562 and H929 from the different resistance models, as explained in methods and depicted in Supplemental figure 2, were deprived of glutamine for 16 h and the extent of apoptosis assessed following a 4 h exposure to ABT-199 (10 nM), A-1331852 (10 nM) or A-1210477 (5 μM). ***P ⩽0.001; Error bars = Mean ± SEM (n=3).

Supplemental Figure S7. Modulation of intermediary metabolism enhances sensitivity to BH3 mimetics in distinct haematological cell lines Exposure to GPNA (5 mM for 48 h), CB-839 (10 μM for 72 h), SB204990 (1 μM for 72 h), GSK2194069 (100 nM for 48 h), simvastatin (250 nM for 72 h) or torin-1 (10 nM for 24 h) enhances the sensitivity of the chemoresistant MAVER-1 and H929 cells to their respective BH3 mimetics. Apoptosis was assessed by PS externalisation following exposure for 4 h to ABT-199 (10 nM) or A-1210477 (5 μM). ***P ⩽0.001; *P⩽0.1; Error bars = Mean ± SEM (n=3).

Figure S1

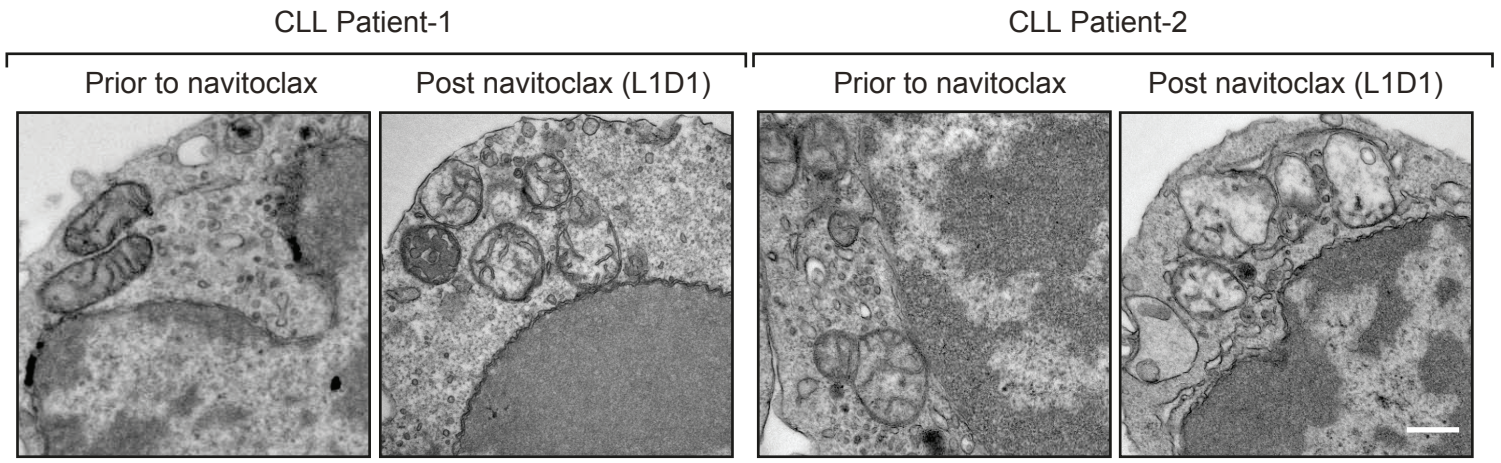

CLL Patient-1
CLL Patient-2
Prior to navitoclax
Post navitoclax (L1D1)
Prior to navitoclax
Post navitoclax (L1D1)

Figure S2

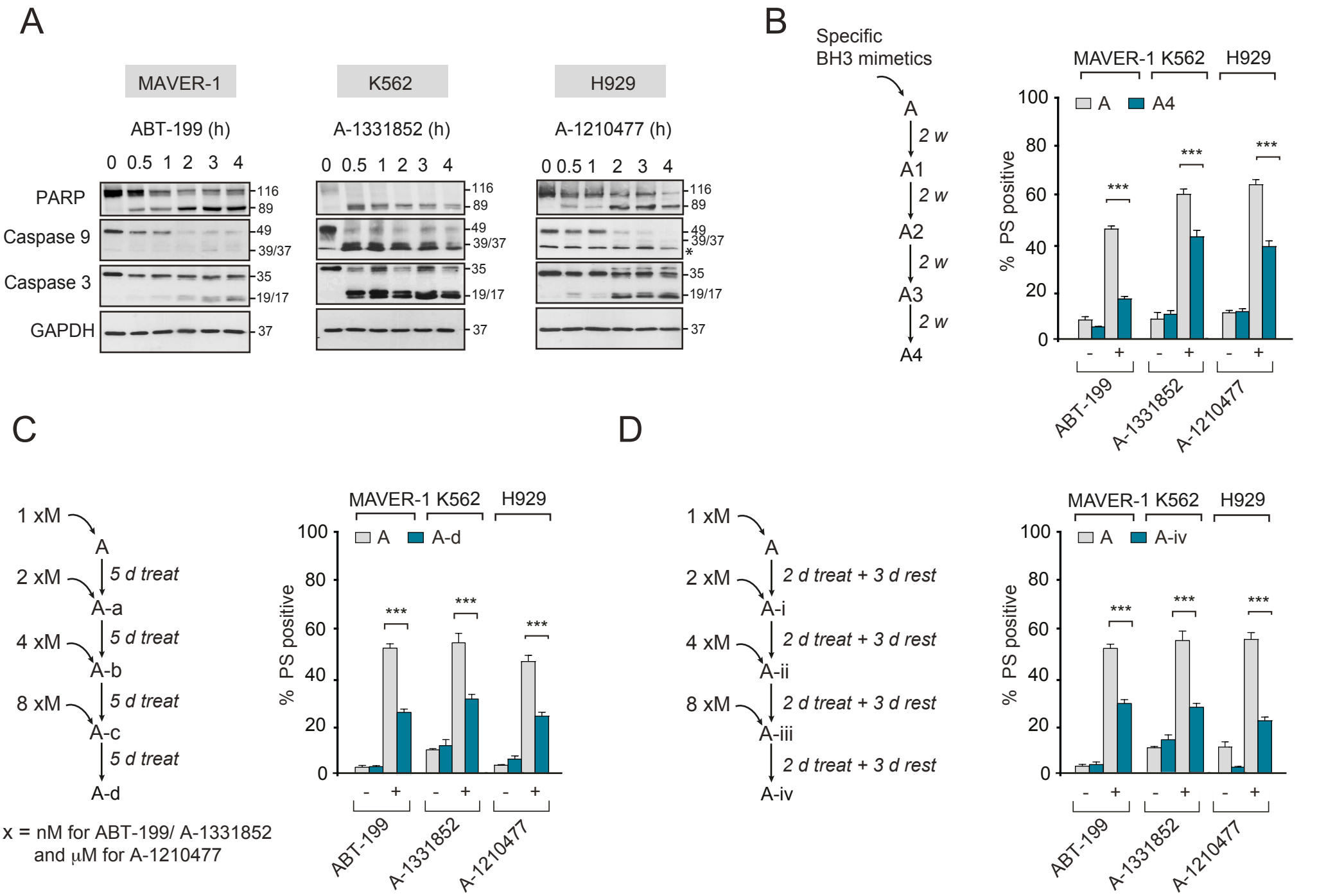

A
MAVER-1
K562
H929
ABT-199 (h)
A-1331852 (h)
A-1210477 (h)
0 0.5 1 2 3 4
PARP
Caspase 9
Caspase 3
GAPDH
116
89
49
39/37
35
19/17
37
*
B
Specific
BH3 mimetics
A
2 w
A1
A2
A3
A4
MAVER-1 K562 H929
A A4
% PS positive
ABT-199
A-1331852
A-1210477
C
1 xM
2 xM
4 xM
8 xM
5 d treat
A-a
A-b
A-c
A-d
x = nM for ABT-199/ A-1331852
and μM for A-1210477
A A-d
D
2 d treat + 3 d rest
A-i
A-ii
A-iii
A-iv
A A-iv

Figure S3

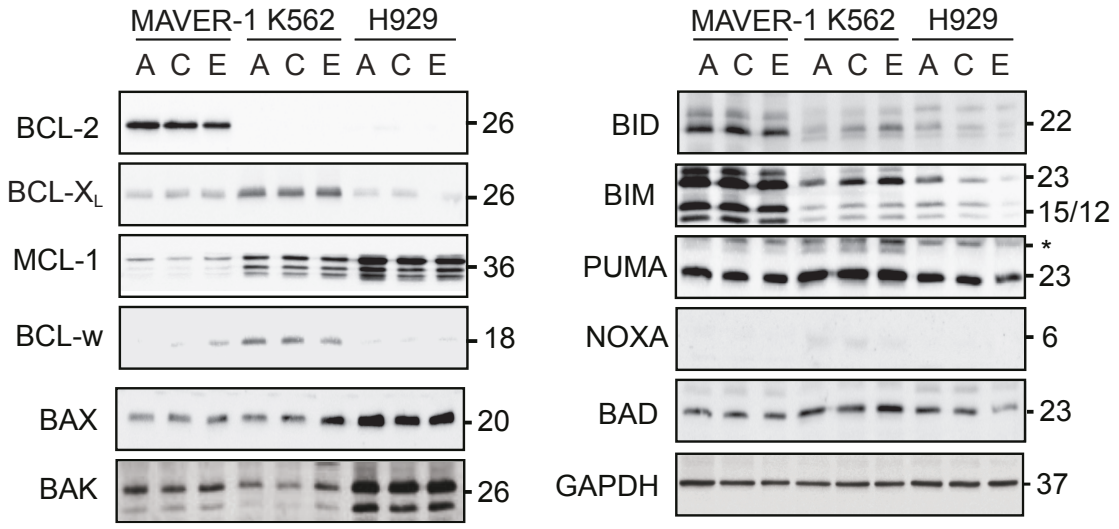

MAVER-1 K562 H929
A C E A C E A C E
BCL-2
BCL-X$_L$
MCL-1
BCL-w
BAX
BAK
26
26
36
18
20
26
MAVER-1 K562 H929
A C E A C E A C E
BID
BIM
PUMA
NOXA
BAD
GAPDH
22
23
15/12
*
23
6
23
37

Figure S4

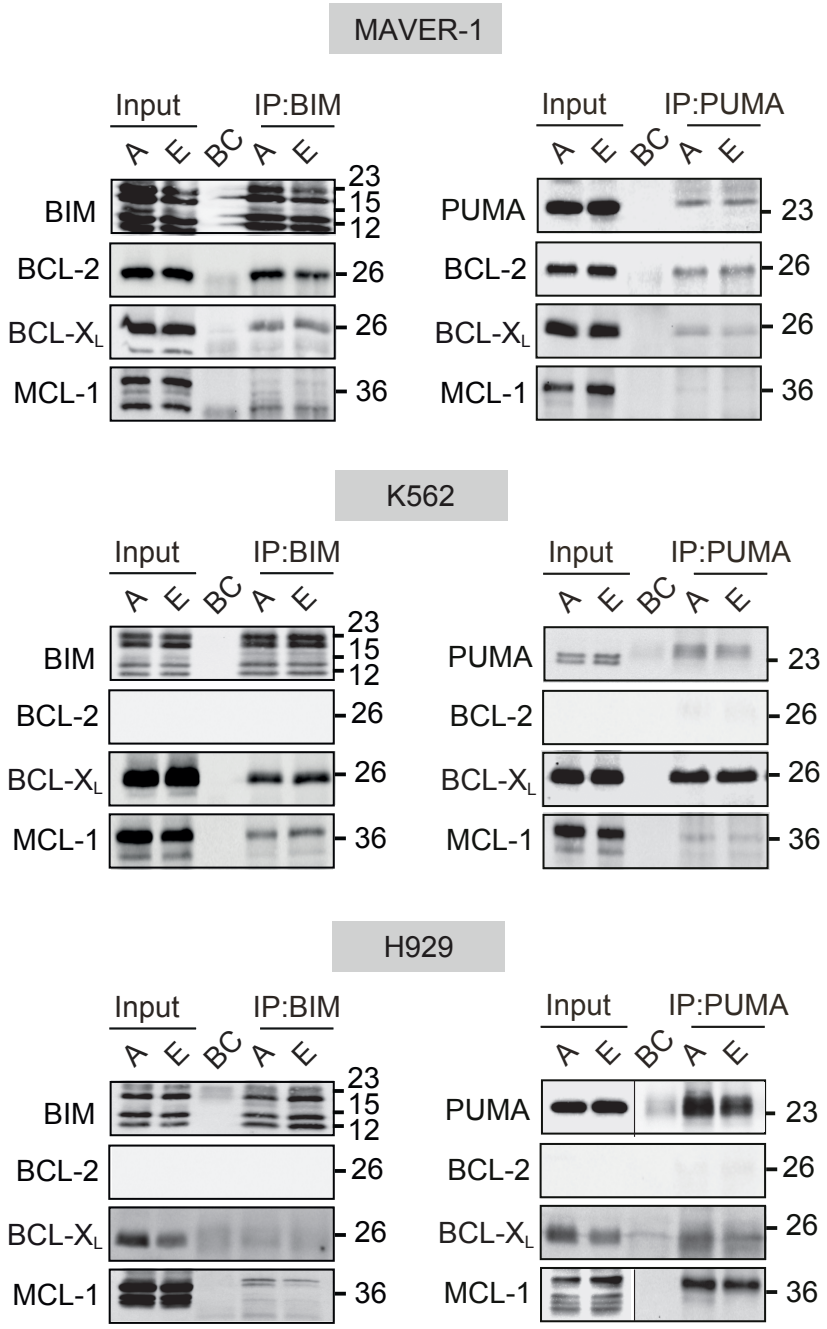

MAVER-1
Input
IP:BIM
IP:PUMA
A
E
BC
BIM
PUMA
BCL-2
BCL-X$_L$
MCL-1
23
15
12
26
36
K562
H929

Figure S5

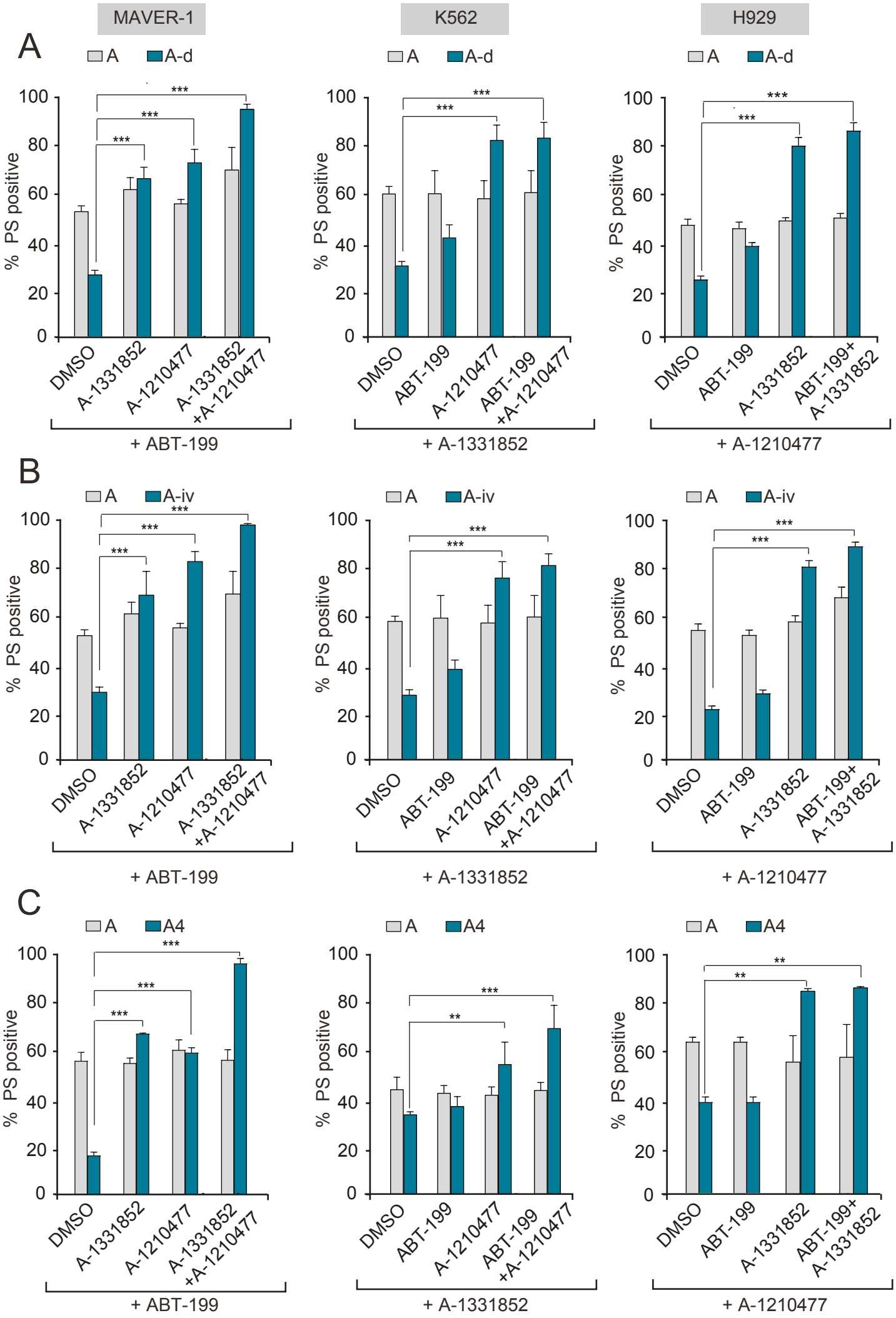

MAVER-1
K562
H929
A
A
A-d
% PS positive
DMSO
A-1331852
A-1210477
A-1331852 +A-1210477
ABT-199
ABT-199 +A-1210477
ABT-199+ A-1331852
+ ABT-199
+ A-1331852
+ A-1210477
B
A
A-iv
C
A
A4

Figure S6

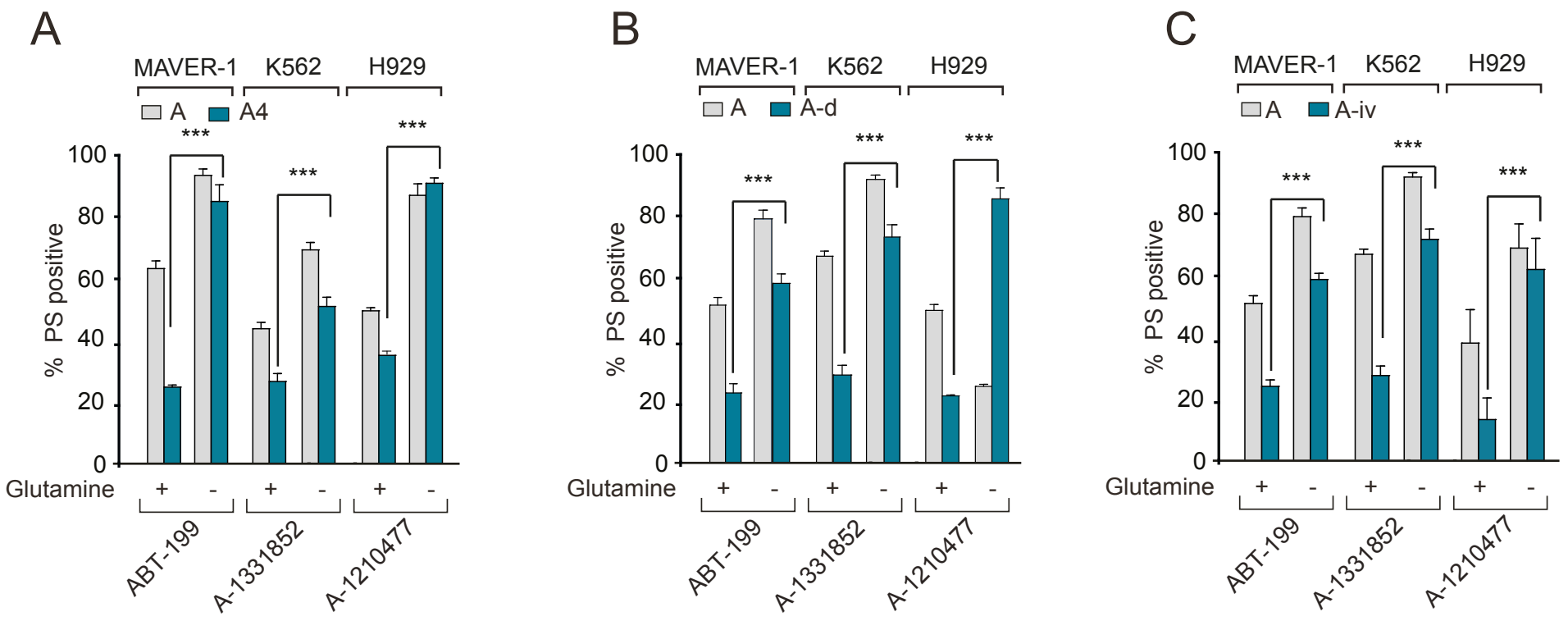
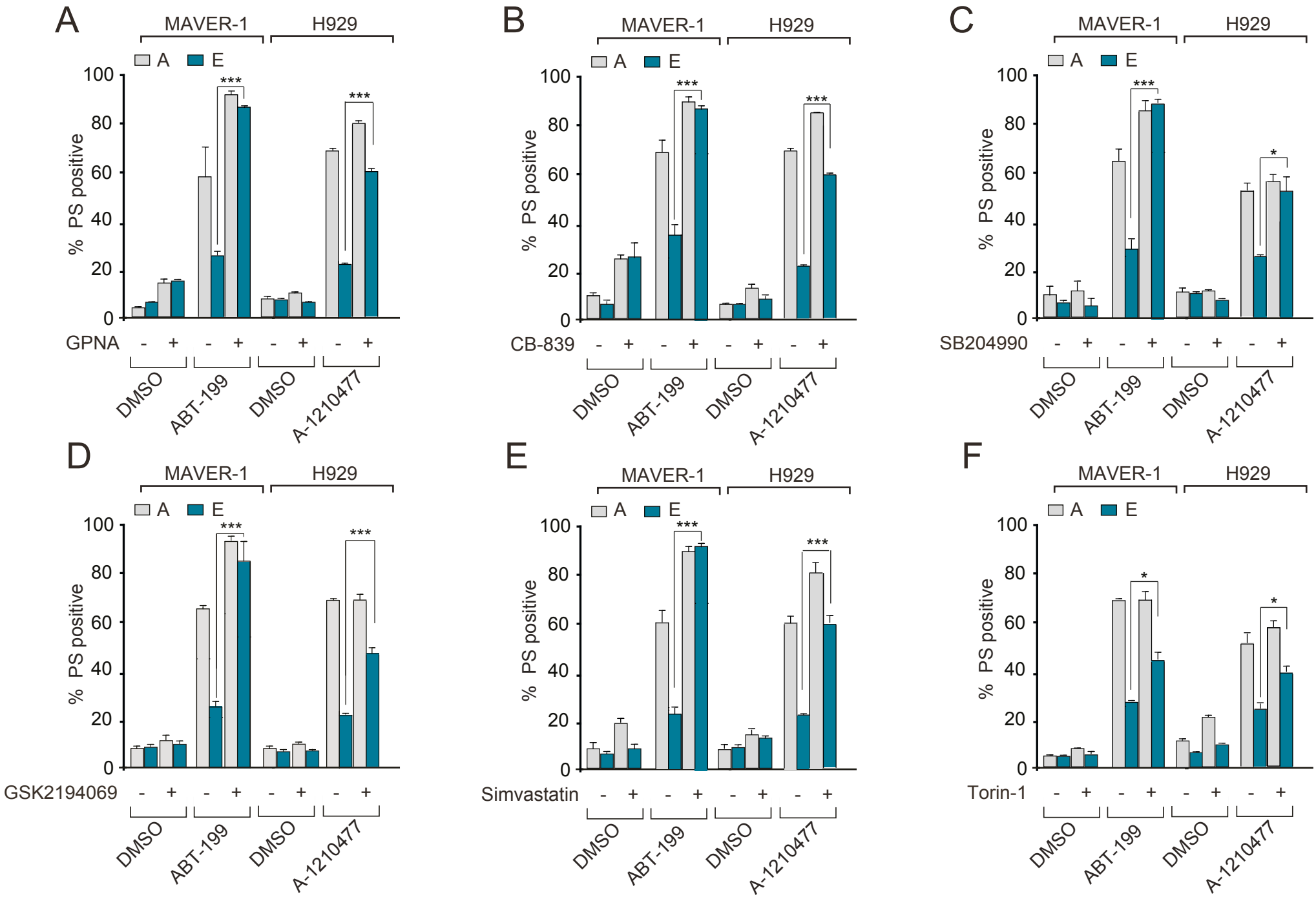

A
MAVER-1
K562
H929
A
A4
***
***
***
100
80
60
40
20
0
% PS positive
Glutamine
+
-
+
-
+
-
ABT-199
A-1331852
A-1210477
B
MAVER-1
K562
H929
A
A-d
***
***
***
100
80
60
40
20
0
% PS positive
Glutamine
+
-
+
-
+
-
ABT-199
A-1331852
A-1210477
C
MAVER-1
K562
H929
A
A-iv
***
***
***
100
80
60
40
20
0
% PS positive
Glutamine
+
-
+
-
+
-
ABT-199
A-1331852
A-1210477

Figure S7


A
B
C
D
E
F
MAVER-1
H929
A
E
% PS positive
100
80
60
40
20
0
***
*
GPNA
CB-839
SB204990
GSK2194069
Simvastatin
Torin-1
- + - + - + - +
DMSO
ABT-199
DMSO
A-1210477