Figure S1. Expression and activity of SK-1 was enhanced in drug-resistant K562/IMA-3 compared to parental K562 cells. (A-B) The expression levels of SK-1 mRNA (A) and protein (B) were measured using Q-PCR and Western blotting, respectively, in drug resistant K562/IMA-3 compared to sensitive parental K562 cells (lanes 2 and 1, respectively). Levels of rRNA or beta-actin were used as controls in Q-PCR or Western blotting, respectively. In B, relative expression levels of SK-1 (normalized to actin levels) are shown below each lane. (C) To examine whether overexpression of SK-1 also results in increased enzyme activity, we labeled K562 and K562/IMA-3 cells with 5 μM exogenous C_{17}-sphingosine (C_{17}-Sph), used as a substrate, and measured the generation of C_{17}-S1P at 3 hr using LC/MS. The data shown represent at least two independent trials performed in duplicates, and error bars represent standard deviation. P <0.05 was considered significant (*).

Figure S2. Ectopic expression of SK-1-V5, and knock-down of SK-1 using siRNA in K562 versus K562/IMA-3 cells. (A) The expression of SK-1-V5 in K562 cells was confirmed using an antibody that recognizes the V5 tag on Western blots (lane 2). Cell extracts obtained from vector-transfected K562 cells were used as negative controls (lane 1). Beta-actin was used as a loading control. (B) The effectiveness of siRNA knock-down of SK-1 was determined using real-time PCR, compared to non-targeting scrambled siRNA. (C) Effects of knock-down of SK-1 using siRNA on the generation of S1P was measured by the conversion of C_{17}-Sph to C_{17}-S1P using LC/MS/MS in K562/IMA-3 cells. The data shown represent at least two independent trials performed in duplicates, and error bars represent standard deviation. P <0.05 was considered significant (*).

Figure S3. Detection of S1P1-4 mRNA levels in response to siRNA treatments. Effects of siRNA targeted against S1P1, S1P2, S1P3 and S1P4 on their target mRNA levels in K562/IMA-3 cells were measured using Q-PCR. The data shown represent at least two independent trials performed in duplicates, and error bars represent standard deviation. $P < 0.05$ was considered significant (*).

Figure S4. Examining the role of SK-2 knock-down in the regulation of Bcr-Abl1 in K562/IMA-3 cells. Effects of Scr and SK-2 siRNA on the mRNA levels of SK-2 were examined using semi-quantitative RT-PCR (left panel, lanes 1and 2, respectively). Effects of Scr and SK-2 siRNA on Bcr-Abl1 protein levels were measured using Western blotting (right panel, lanes 1 and 2, respectively). Actin mRNA or protein levels were used as controls in left and right panels, respectively. The data shown represent at least two independent trials performed in duplicates. Relative expression levels of SK-2 and Bcr-Abl1 (normalized to actin levels) are shown below each lane.

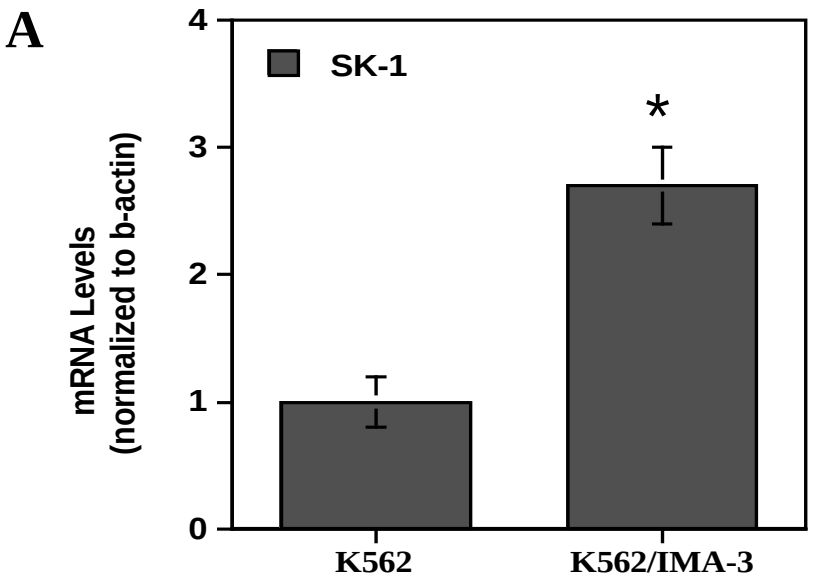
Figure S5. Effects of the inhibition of SK-1 and S1P2 on imatinib-induced cell death. (A-B) Effects of SKI-II and JTE-013, alone or in combination on imatinib-induced death in K562 (A) and K562/IMA-1 (B) cells (at 0.1 and 0.2, or 0.5 and 1.0 μM for 48 hr) were determined using the caspase 3 activity assay kit. The data shown represent at least two independent trials performed in duplicates, and error bars represent standard deviation. $P < 0.05$ was considered significant (*).

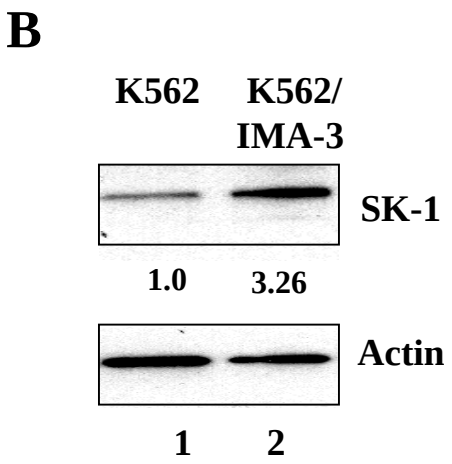
Figure S6. Increased stability of Bcr-Abl1 in drug-resistant K562 cells was associated with its altered ubiquitination, and inhibition of SK-1 and S1P2 signaling enhanced ubiquitination of Bcr-Abl1. (A) The ubiquitination of Bcr-Abl1 in K562 and K562/IMA-3 cells were examined by immunoprecipitation (IP) studies in the absence/presence of lactacystin (LACT) at 5 μM using the anti-Bcr-Abl1 antibody in IP, followed by Western blotting using an anti-ubiquitin antibody (lanes 1,3, and 2,4, respectively), as described in Materials and Methods. The IP samples obtained using the anti-Abl1 antibody from K562 and K562/IMA-3 cell extracts (20 μl and 10 μl, respectively) were used in Western blotting as controls (lower panel). (B) The ubiquitination of Bcr-Abl1 in sensitive and resistant cells (K562 and K562/IMA-1, respectively) was also determined using co-IP followed by Western blotting after transfection of cells for the expression of an HA-tagged ubiquitin peptide, in the absence/presence of LACT (lanes 1,3, and 2,4, respectively). The anti-HA antibody was used in IP, followed by the detection of Bcr-Abl1 on Western blots using anti-Abl antibody. Total immunoprecipitated HA-ubiquitin levels were used as controls for equal loading (lower panel). (C) Effects of the inhibition of SK and S1P2 using SKI-II and JTE-013, respectively, (alone or in combination) compared to untreated controls (lanes 2-4, and 1, respectively) on the ubiquitination of Bcr-Abl1 was assessed in drug-resistant K562/IMA-1 cells using co-IP followed by Western blotting after transfection of cells for the expression of an HA-tagged ubiquitin peptide as described in (B). The data shown represent at least two independent trials. In B and C, relative expression levels of Bcr-Abl1-Ub (normalized to HA levels) are shown below each lane.

Figure S7. Effects of the inhibition of SK-1 and S1P2 on cell survival/growth in response to imatinib treatment in MNCs isolated from CML patients. (A-C) Effects of the SK and S1P2 inhibitors, SKI-II and JTE-013, alone or in combination, on imatinib-induced death in MNCs obtained from CML patients at CP, patient 1 (A), at BC, patients 2 and 3 (B and C, respectively) at 0.5 and 1.0 μM for 48 hr) were determined using MTT assay. (D) The mRNA levels of SK-1 and S1P2 were measured using Q-PCR in MNCs isolated from the BC patient, who expressed the T315I-Bcr-Abl1 compared to MNCs isolated from a healthy volunteer. The data shown represent at least two independent trials performed in duplicates, and error bars represent standard deviation. $P < 0.05$ was considered significant (*).

Figure S8. Regulation of nilotinib-mediated growth inhibition in 32D/wt- or 32/Y253H-Bcr-Abl1 cells in the presence of SK and S1P2 inhibitors. Effects of SKI-II and JTE-013, alone or in combination, on growth of 32D/wt- (A) or 32D/Y253H-Bcr-Abl1 (B) cells in response to nilotinib treatment (at 0.05, 0.1, or 0.5 μM for 48 hr) were determined using MTT assay. (C) The mRNA levels of SK-1 and S1PR2 were examined in 32D/wt-, 32D/Y253H-or 32D/T315I-Bcr-Abl1 cells using RT-PCR. 32D/vector cells were used a controls. The data shown represent at least two independent trials performed in duplicates, and error bars represent standard deviation. $P < 0.05$ was considered significant (*).

Figure S1


A
SK-1
mRNA Levels
(normalized to b-actin)
4
3
2
1
0
*
K562
K562/IMA-3


B
K562
K562/
IMA-3
SK-1
1.0
3.26
Actin
1
2

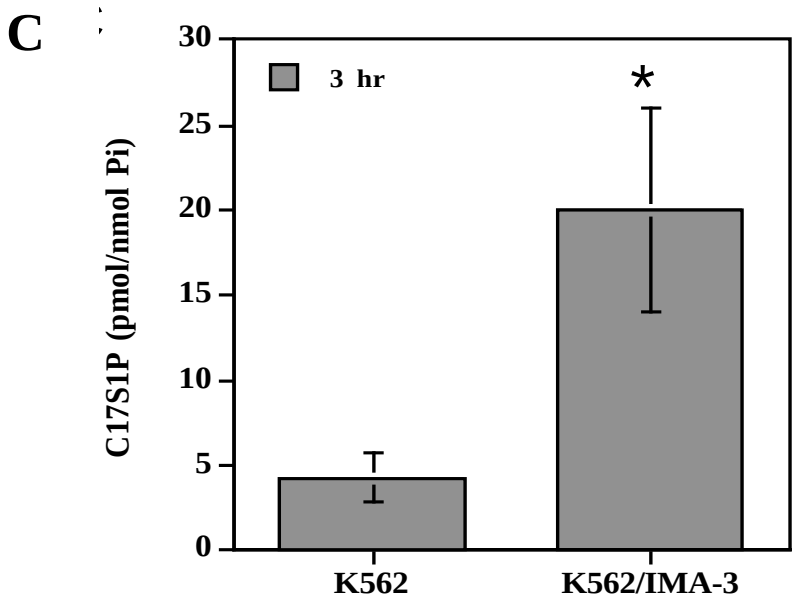

C
3 hr
C17S1P (pmol/nmol Pi)
30
25
20
15
10
5
0
*
K562
K562/IMA-3

Figure S2

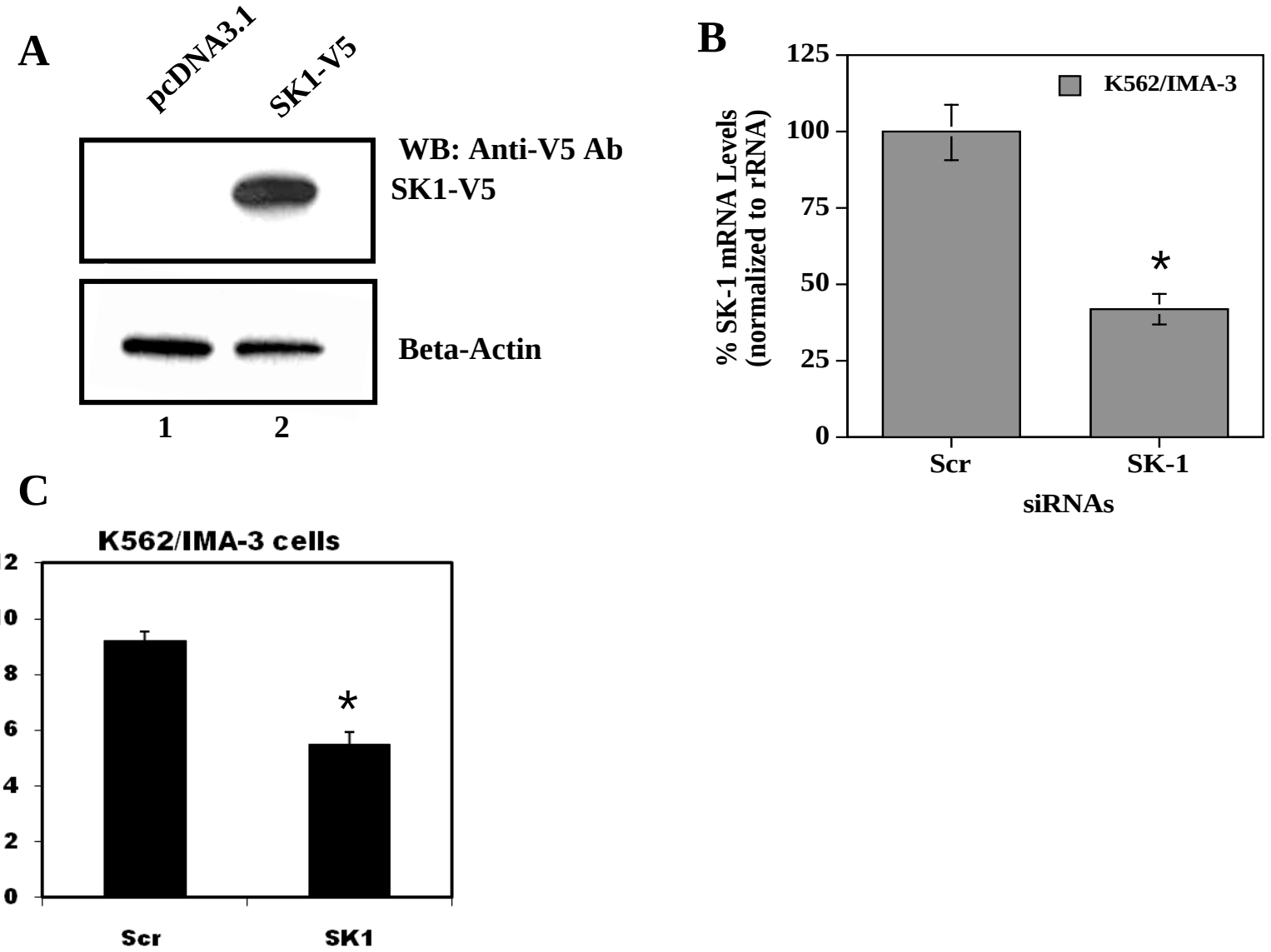

A
pcDNA3.1
SK1-V5
WB: Anti-V5 Ab
SK1-V5
Beta-Actin
1
2
B
K562/IMA-3
% SK-1 mRNA Levels
(normalized to rRNA)
125
100
75
50
25
0
*
Scr
SK-1
siRNAs
C
K562/IMA-3 cells
17C-S1P
(pmol/mg protein)
12
10
8
6
4
2
0
*
Scr
SK1

Figure S3

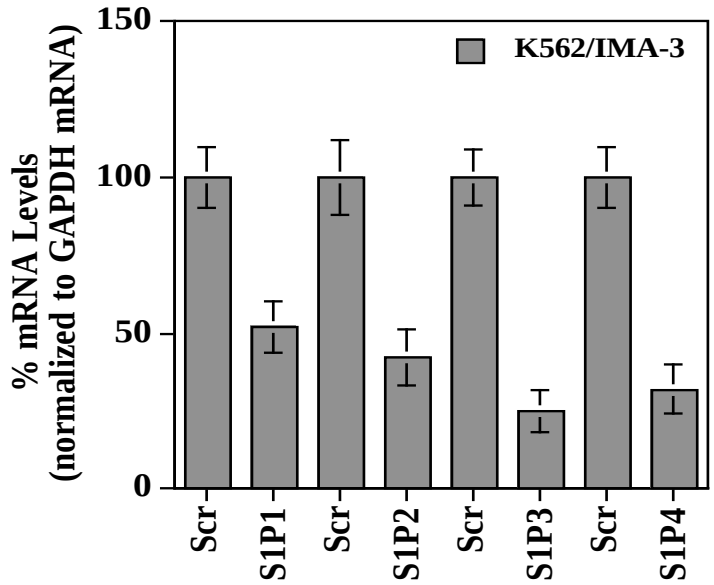

% mRNA Levels
(normalized to GAPDH mRNA)
150
100
50
0
K562/IMA-3
Scr
S1P1
Scr
S1P2
Scr
S1P3
Scr
S1P4

Figure S4

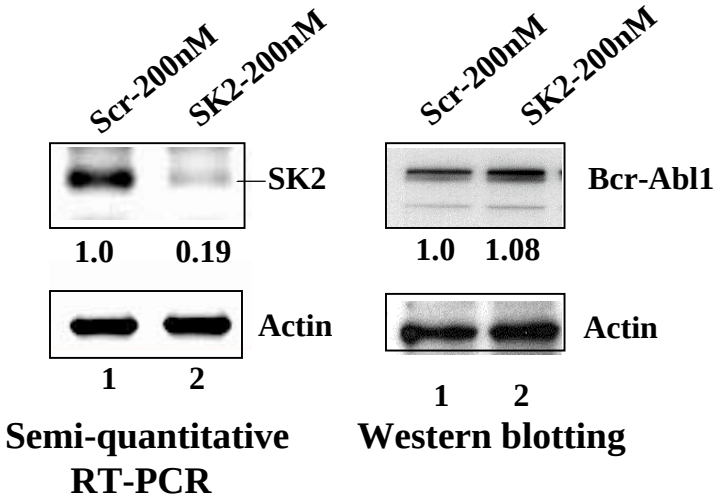

Scr-200nM
SK2-200nM
SK2
1.0
0.19
Actin
1
2
Semi-quantitative RT-PCR
Scr-200nM
SK2-200nM
Bcr-Abl1
1.0
1.08
Actin
1
2
Western blotting

Figure S5

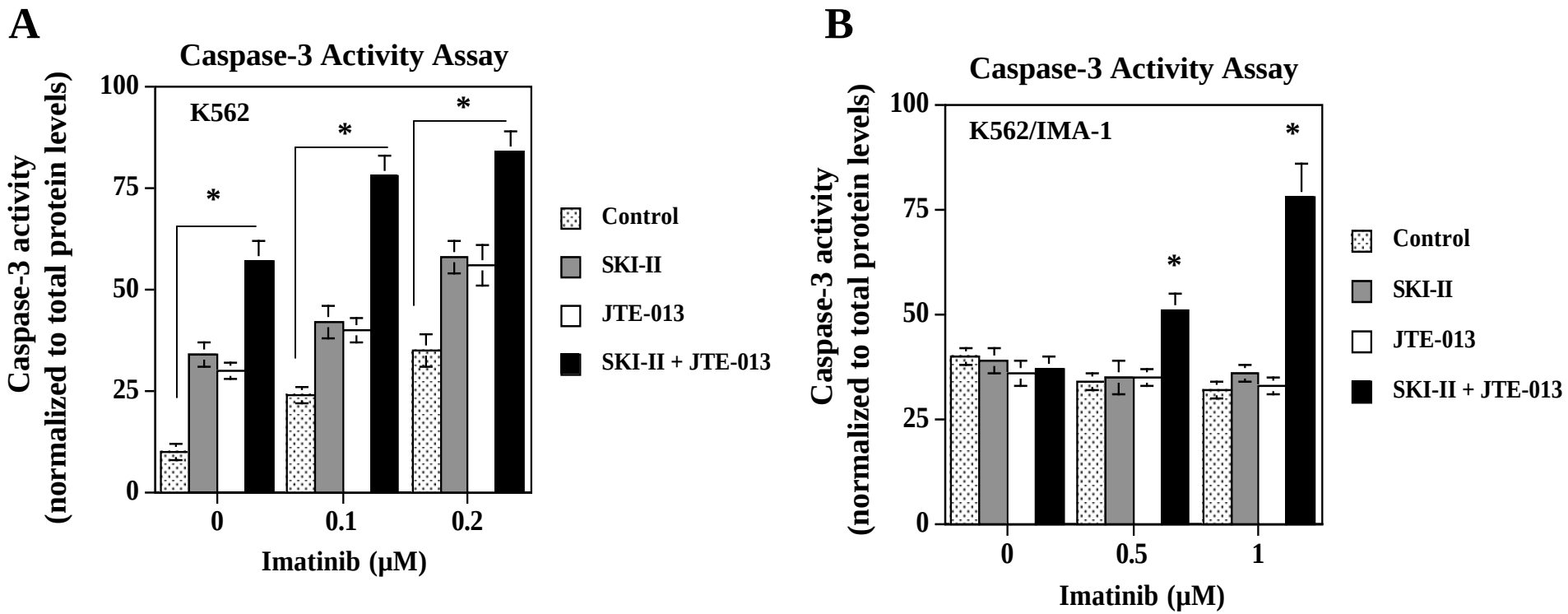

A
Caspase-3 Activity Assay
K562
Caspase-3 activity
(normalized to total protein levels)
100
75
50
25
0
0
0.1
0.2
Imatinib (μM)
*
*
*
Control
SKI-II
JTE-013
SKI-II + JTE-013
B
Caspase-3 Activity Assay
K562/IMA-1
Caspase-3 activity
(normalized to total protein levels)
100
75
50
25
0
0
0.5
1
Imatinib (μM)
*
*
Control
SKI-II
JTE-013
SKI-II + JTE-013

Figure S6

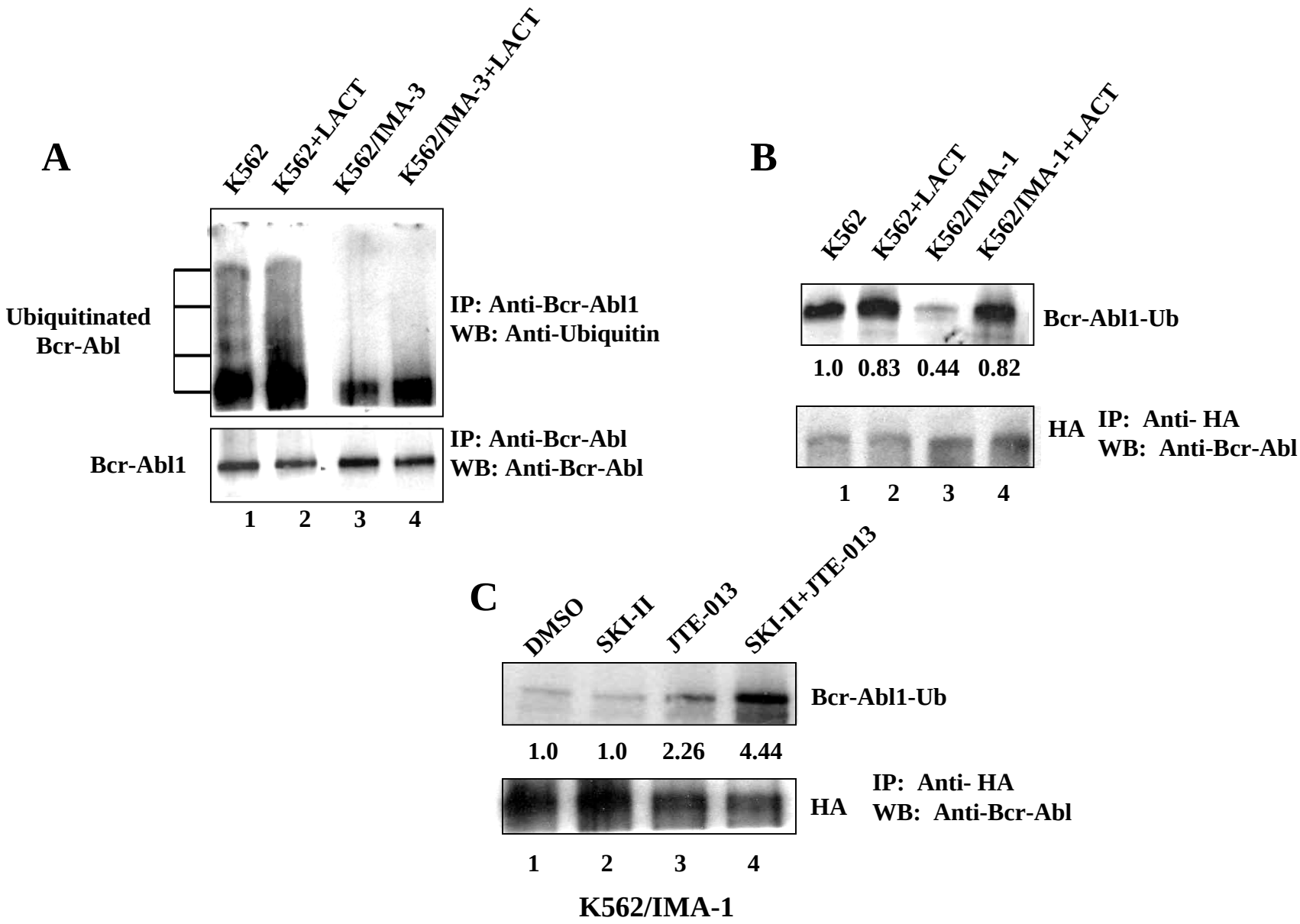

A
K562
K562+LACT
K562/IMA-3
K562/IMA-3+LACT
Ubiquitinated Bcr-Abl
IP: Anti-Bcr-Abl1
WB: Anti-Ubiquitin
Bcr-Abl1
IP: Anti-Bcr-Abl
WB: Anti-Bcr-Abl
1 2 3 4
B
K562
K562+LACT
K562/IMA-1
K562/IMA-1+LACT
Bcr-Abl1-Ub
1.0 0.83 0.44 0.82
HA
IP: Anti- HA
WB: Anti-Bcr-Abl
1 2 3 4
C
DMSO
SKI-II
JTE-013
SKI-II+JTE-013
Bcr-Abl1-Ub
1.0 1.0 2.26 4.44
HA
IP: Anti- HA
WB: Anti-Bcr-Abl
1 2 3 4
K562/IMA-1

Figure S7

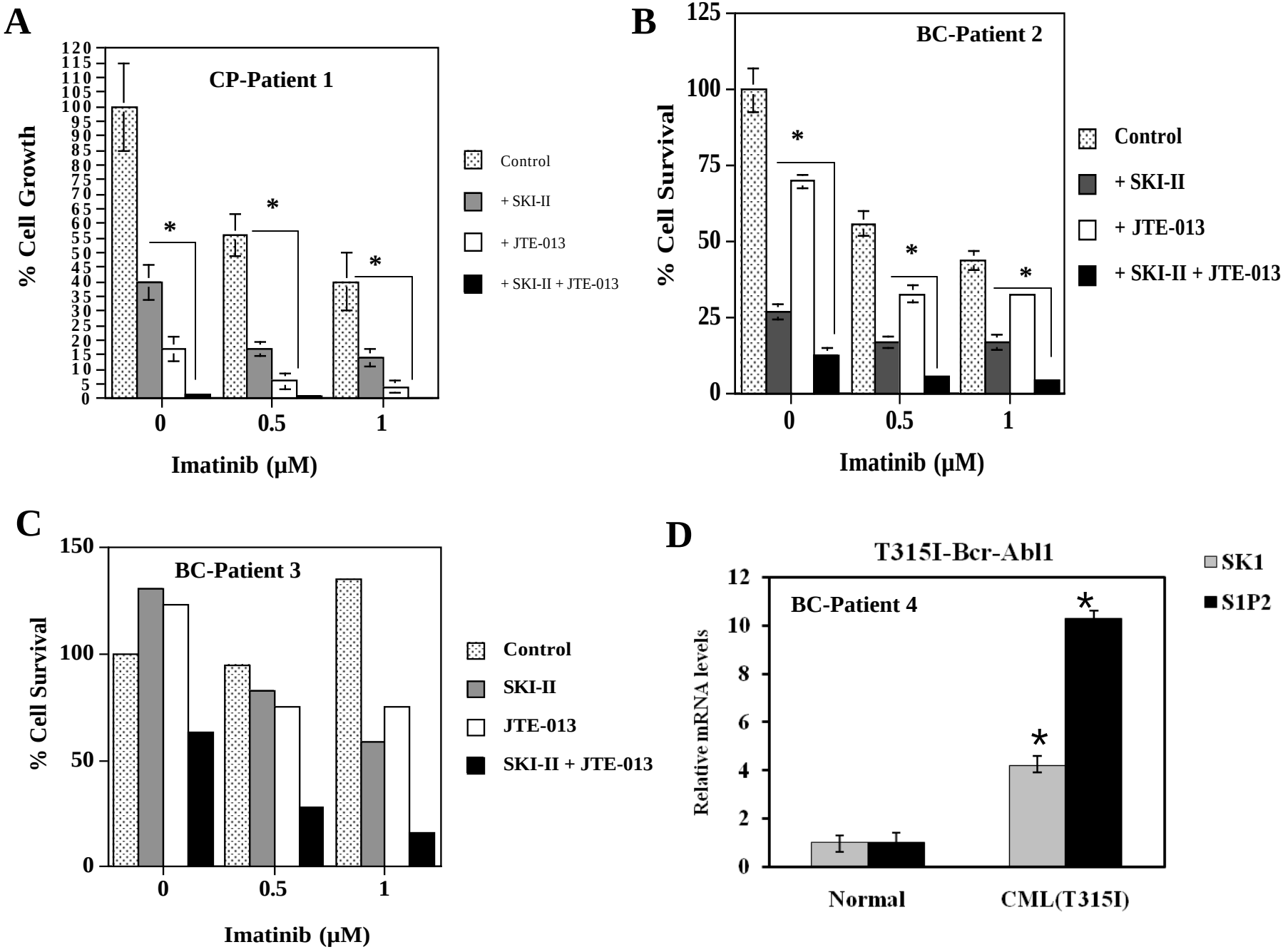
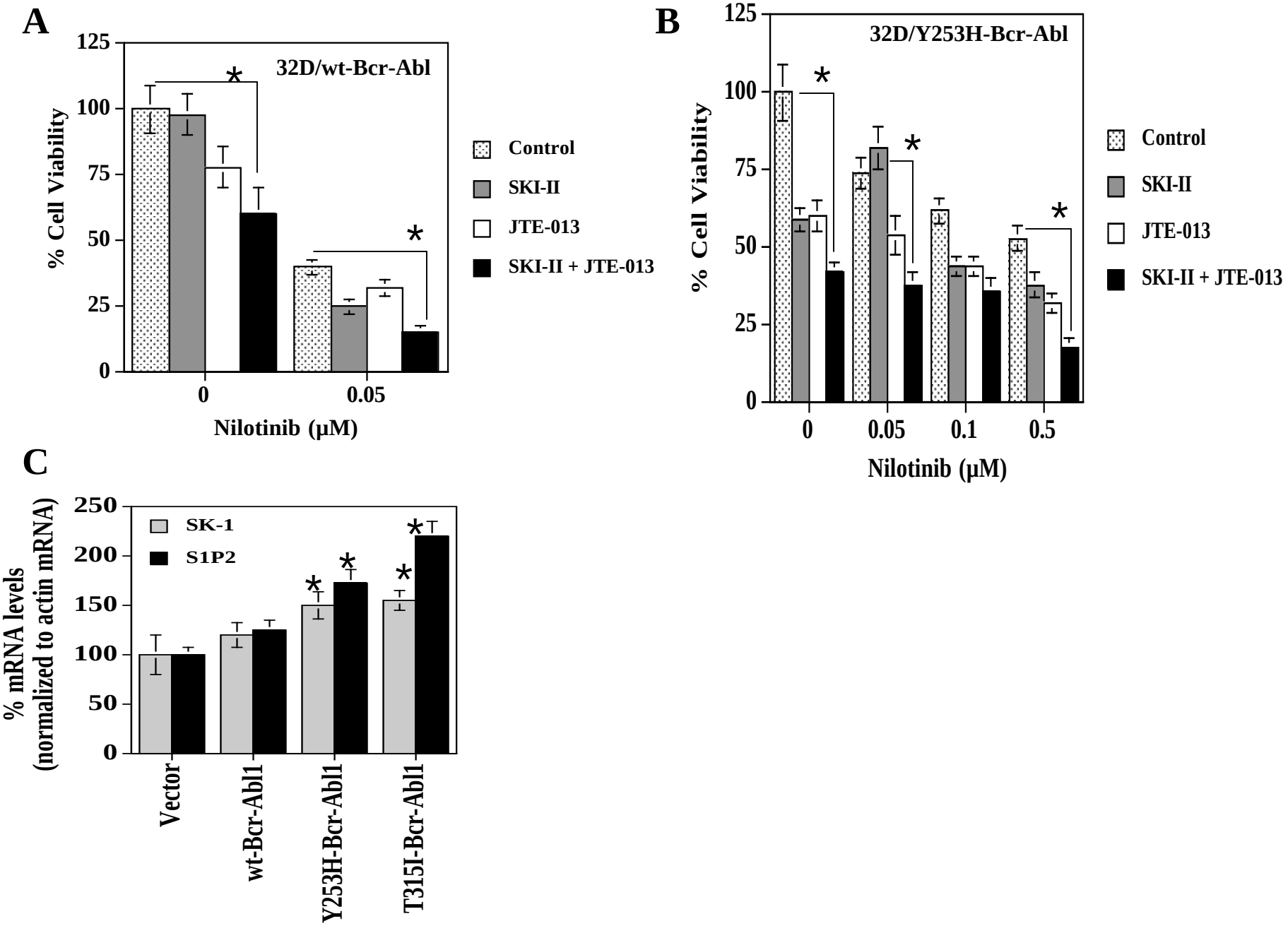

A
CP-Patient 1
% Cell Growth
Imatinib (μM)
Control
+ SKI-II
+ JTE-013
+ SKI-II + JTE-013
B
BC-Patient 2
% Cell Survival
Imatinib (μM)
Control
+ SKI-II
+ JTE-013
+ SKI-II + JTE-013
C
BC-Patient 3
% Cell Survival
Imatinib (μM)
Control
SKI-II
JTE-013
SKI-II + JTE-013
D
T315I-Bcr-Abl1
BC-Patient 4
Relative mRNA levels
SK1
S1P2
Normal
CML(T315I)

Figure S8


A
32D/wt-Bcr-Abl
% Cell Viability
125
100
75
50
25
0
0
0.05
Nilotinib (μM)
Control
SKI-II
JTE-013
SKI-II + JTE-013
B
32D/Y253H-Bcr-Abl
% Cell Viability
125
100
75
50
25
0
0
0.05
0.1
0.5
Nilotinib (μM)
Control
SKI-II
JTE-013
SKI-II + JTE-013
C
% mRNA levels
(normalized to actin mRNA)
250
200
150
100
50
0
SK-1
S1P2
Vector
wt-Bcr-Abl1
Y253H-Bcr-Abl1
T315I-Bcr-Abl1