Table S1. Characterization and source of antibodies

CD	Antigen	clone	conjugate	Source/company*	catalog number
CD34	HPCA-1	581	PE	BD Biosciences	555822
CD38	T10	HIT2	APC	BD Biosciences	555462
CD44	HERMES	515	PE	BD Biosciences	550989
CD44	HERMES	BJ18	FITC	BioLegend	338804
CD45	LCA	2D1	APC-H7	BD Biosciences	560178
CD45	LCA	HI30	APC-Cy7	BioLegend	304014
CD45	LCA	HI30	V500	BD Biosciences	560777
n.c.	pAKT	M89-61	PE	BD Biosciences	560378
n.c.	pS6	N7-548	AlexaFluor647	BD Biosciences	560435
n.c.	Actin	2Q1055	none	Santa Cruz Biotechnology	Sc-58673
n.c.	AKT	polyclonal	none	Cell Signaling	9272S
n.c.	pAKT	D9E	none	Cell Signaling	4060S
n.c.	S6	5G10	none	Cell Signaling	2217S
n.c.	pS6	D57.2.2E	none	Cell Signaling	4858S

*Antibodies were purchased from BioLegend (San Diego, CA), BD Biosiences (San Jose, CA), Cell Signaling (Danvers, MA) and Santa Cruz Biochtechnology (Santa Cruz, CA). Abbrevations: CD, cluster of differentiation; HPCA-1, human precursor cell antigen-1; LCA, leukocyte common antigens; n.c., not (yet) clustered; APC, allophycocyanin; FITC, fluorescein; PE, phycoerythrin.

Table S2. Patients' characteristics

Patient No.	Gender (f/m)	Age (years)	Source	Diagnosis, Phase of CML	*BCR-ABL1* (% by IS) in PB	*BCR-ABL1* mutations	White Blood Cell Count (G/l)	Platelet Count (G/l)	Hemoglobin (g/dl)	Blasts (%) PB	Blasts (%) BM	Treatment before sampling
1	m	27	PB	CML-CP	56.436	n.d.	160.60	177	13.2	1	2	none
2	m	43	PB	CML-CP	55.677	n.d.	73.21	153	13.9	2	4	none
3	m	43	PB	CML-BP*	47.839	n.d.	44.56	769	9.0	20	25	hydroxyurea
4	f	92	PB	CML-CP	44.031	n.d.	280.70	821	9.8	6	n.a.	none
5	f	57	BM	CML-CP	35.033	n.d.	35.24	1378	11.5	1	5	none
6	m	72	PB	CML-CP	39.693	n.d.	332.48	309	8.4	1	1	none
7	m	43	BM	CML-BP	18.348	n.d.	15.32	20	10.8	18	18	dasatinib
8	m	37	PB	CML-CP	44.945	n.d.	339.56	683	10.4	4	1	none
9	f	61	BM	CML-CP	35.736	n.d.	19.06	342	13.1	1	1	none
10	f	53	PB	CML-CP	34.946	n.d.	47.20	1789	6.9	5	3-4	none
11	f	57	BM	CML-CP	53.465	n.d.	39.14	150	12.1	1	1	none
12	m	59	BM	CML-CP	51.975	n.d.	222.12	789	10.5	1	1-2	none

Abbreviations: No., number; f, female; m, male; PB, peripheral blood; BM, bone marrow; CML, chronic myeloid leukemia; CP, chronic phase; BP, blast phase; n.d., not detected (BP) or not determined (CP); n.a., not available; IS, International Scale. *In this patient, a primary BP of CML was diagnosed.

Table S3. Primer sequences used for quantitative RT-PCR

Gene	Sequence
NOXA	5'-CGCGCAAGAACGCTCAACC-3' (forward)
	5'-CACACTCGACTTCCAGCTCTGCT-3' (reverse)
PUMA	5'-GGATGGCGGACGACCTCAAC-3' (forward)
	5'-CCGCTGCTGCTCTTCTTGTC-3' (reverse)
BIM	5'-TGTCTGACTCTGACTCTCTGACTGA-3' (forward)
	5'-GAAGGTTGCTTTGCCATTTGGTC-3' (reverse)
ABL1	5'-TGTATGATTTTGTGGCCAGTGGAG-3' (forward)
	5'-GCCTAAGACCCGGAGCTTTTCA-3' (reverse)

Abbreviations: RT-PCR, real time polymerase chain reaction; NOXA, phorbol-12-myristate-13-acetate-induced protein; PUMA, p53 upregulated modulator of apoptosis; BIM, BCL-2-like protein 11; ABL1, Abelson murine leukemia viral oncogene homolog.

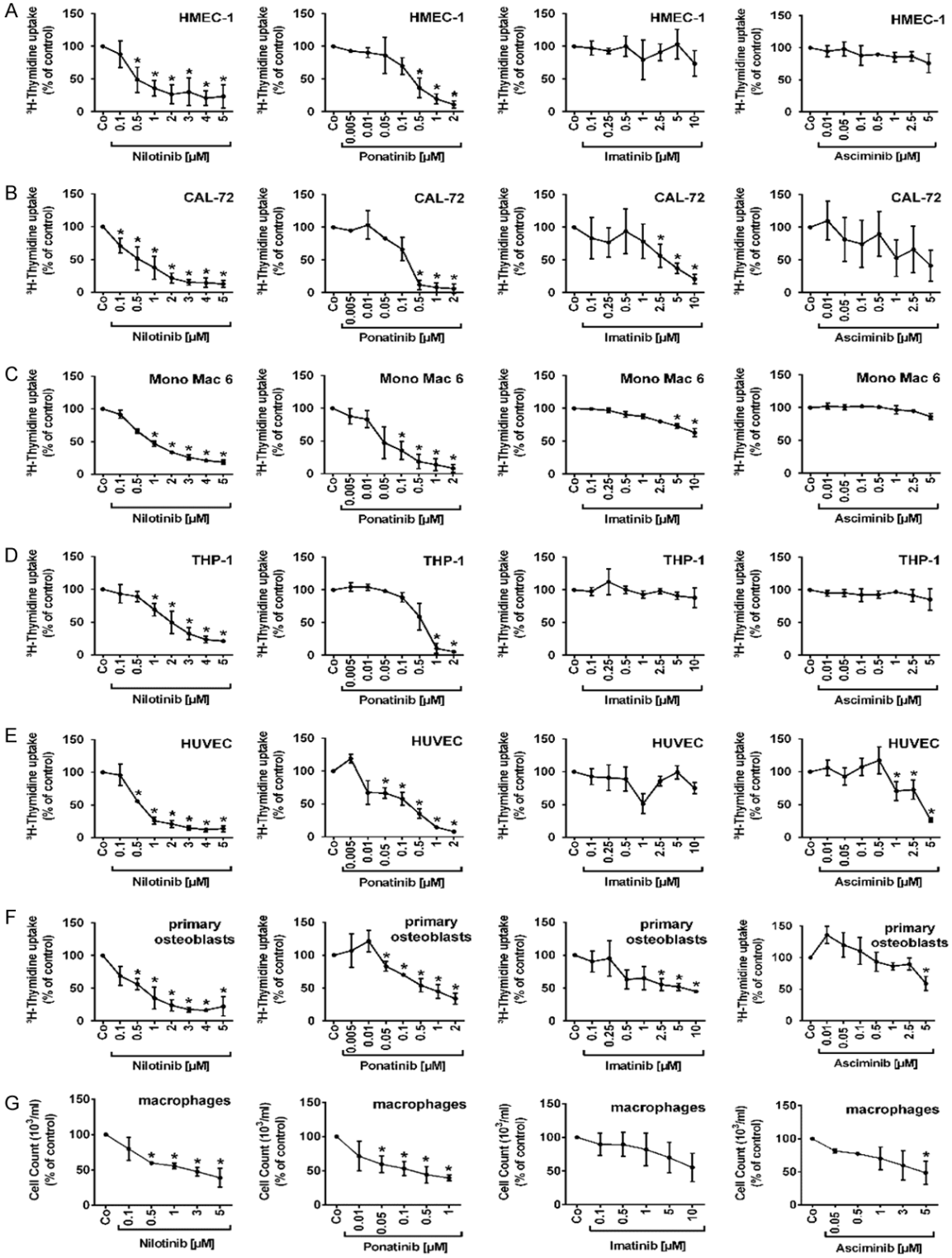

Figure S1. Effects of various tyrosine kinase inhibitors on proliferation of niche cells. HMEC-1 (A), CAL-72 (B), Mono Mac 6 (C), THP-1 (D), HUVEC (E), primary osteoblasts (F), and primary macrophages isolated from healthy donors (G), were incubated in control medium (Co) or in various concentrations of nilotinib (0.1-5 µM), ponatinib (0.005-2 µM), imatinib (0.1-10 µM) or asciminib (0.01-5 µM) at 37 °C for 48 hours. Then, cell counts (primary macrophages) or ^{3}H-thymidine uptake (all other cell types) were measured. Results show cell counts or ^{3}H-thymidine uptake as percentage of control and represent mean ± SD of 3 independent experiments. Asterisk (*): P<0.05 compared to control.

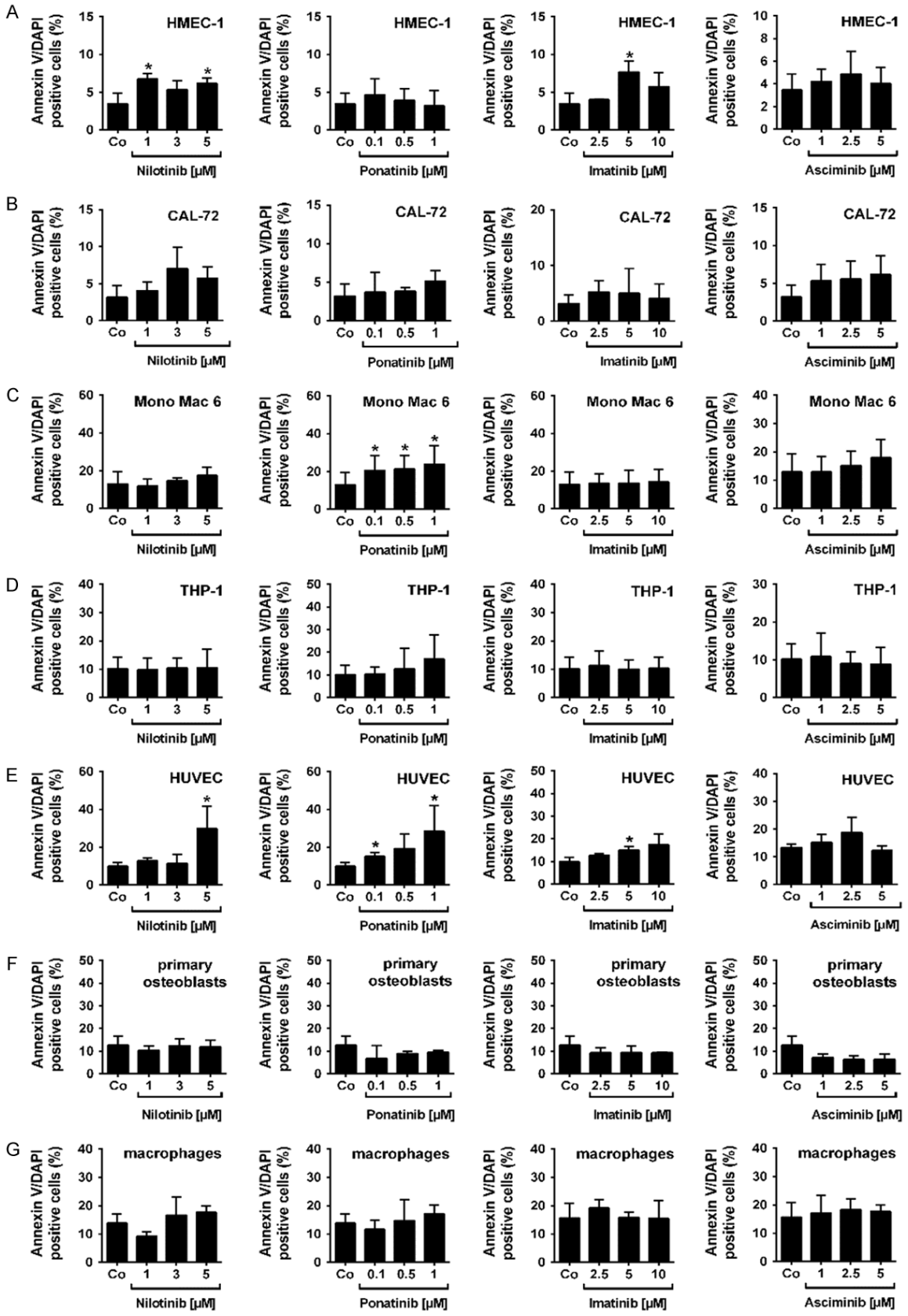

Figure S2. Effects of various tyrosine kinase inhibitors on survival of niche cells. HMEC-1 cells (A), CAL-72 cells (B), Mono Mac 6 cells (C), THP-1 cells (D), HUVEC cells (E), primary osteoblasts (F) and primary macrophages isolated from healthy donors (G) were incubated in control medium (Co) or in medium containing various concentrations of

nilotinib (1-5 μM), ponatinib (0.1-1 μM), imatinib (2.5-10 μM) or asciminib (1-5 μM) at 37 °C for 48 hours. Then, cells were examined by flow cytometry to determine the percentage of apoptotic (AnnexinV/DAPI-positive) cells. Results represent the mean ± SD of 3 independent experiments. Asterisk (*): P<0.05 compared to control.

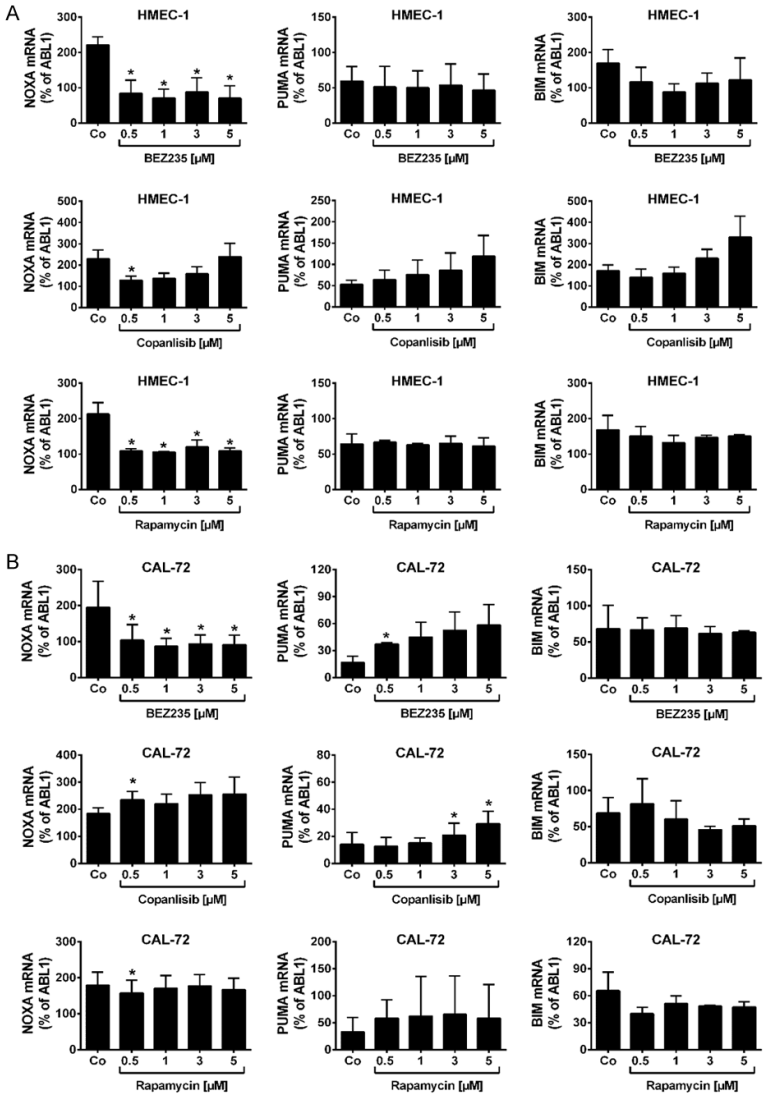

Figure S3. Effects of PI3K inhibitors on mRNA expression of pro-apoptotic BCL2 members. HMEC-1 cells (A) and CAL-72 cells (B) were incubated in control medium (Co) or in medium containing BEZ235 (0.5-5 μM), copanlisib (0.5-5 μM) or rapamycin (0.5-5 μM) at 37 °C for 48 hours. Then, cells were harvested and expression of NOXA, PUMA and BIM mRNA was determined by qPCR analysis. Relative expression levels of NOXA, PUMA and BIM mRNA were calculated by employing ABL1 as a reference gene. NOXA, PUMA and BIM mRNA levels are expressed as percent of ABL1 mRNA levels and represent the mean ± SD from three independent experiments. Asterisk (*): P<0.05 compared to control.

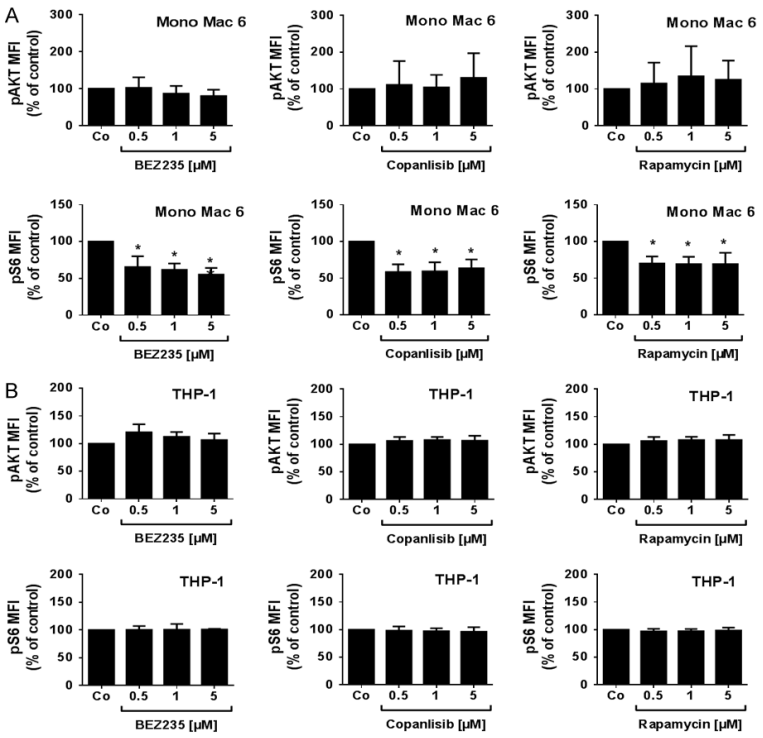

Figure S4. Effects of BEZ235, copanlisib and rapamycin on expression of phosphorylated (p) AKT and pS6 in monocytic cell lines. Mono Mac 6 cells (A) and THP-1 cells (B) were incubated in control medium (Co) or in medium containing various concentrations of BEZ235 (0.5-5 μM), copanlisib (0.5-5 μM) or rapamycin (0.5-5 μM) at 37 °C for 4 hours. Then, cells were permeabilized and stained with fluorochrome-labeled monoclonal antibody against pAKT (S473) and pS6 (S235/236). Expression of pAKT and pS6 was determined by flow cytometry. Results show median fluorescence intensity (MFI) values expressed as percent of control and represent the mean ± SD from three independent experiments. Asterisk (*): $P<0.05$ compared to control.

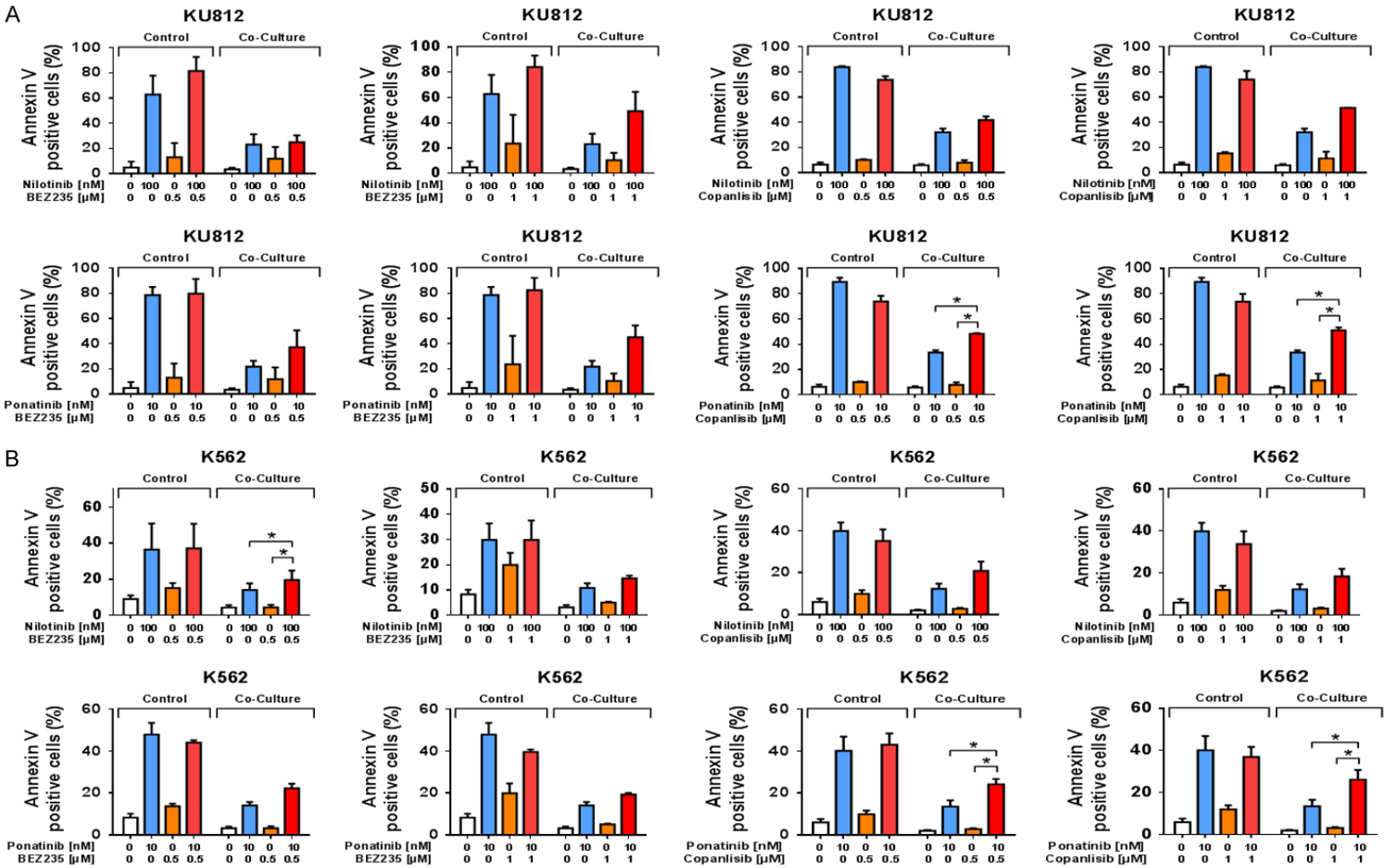

Figure S5. Effects of BEZ235 and copanlisib on niche-induced TKI resistance of CML cells. KU812 cells (A) and K562 cells (B) were incubated in control medium (Co) or in medium containing nilotinib (100-250 nM), ponatinib (10-25 nM), BEZ235 (0.5-1 µM) or copanlisib (0.5-1 µM) or in various drug combinations (BEZ235+nilotinib, BEZ235+ponatinib, copanlisib+nilotinib, copanlisib+ponatinib) in the absence (Control) or presence (Co-culture) of CAL-72 cells at 37°C for 48 hours. Then, cells were examined by flow cytometry to determine the percentage of apoptotic (AnnexinV/DAPI-positive) cells. Results represent the mean ± SD of three independent experiments. Asterisk (*): P<0.05.

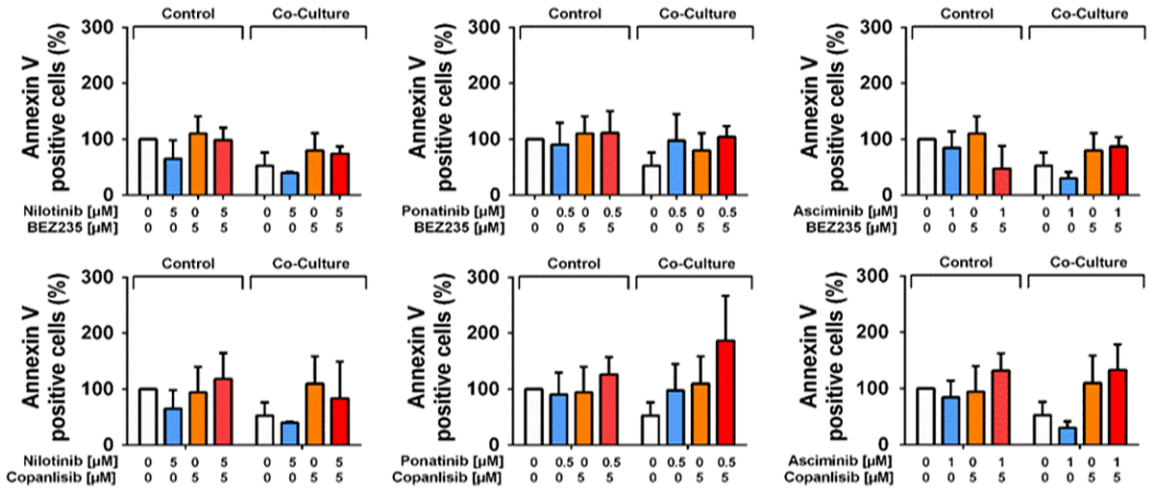

Figure S6. Effects of BCR-ABL1 TKI and PI3K inhibitors on normal CD34$^+$ BM cells. Normal CD34$^+$ stem/progenitor cells (healthy bone marrow, BM) were incubated in control medium (0) or in medium containing nilotinib (5 μM), ponatinib (0.5 μM), asciminib (1 μM), BEZ235 (5 μM), or copanlisib (5 μM), or in medium containing combinations of these drugs (as indicated) in the absence or in presence (Co-culture) of CAL-72 cells at 37 °C for 48 hours. Then, cells were examined by flow cytometry to determine the percentage of CD34$^+$/CD38$^-$/Annexin-V-positive cells among DAPI-negative cells. Results represent the mean ± SD of three independent experiments.

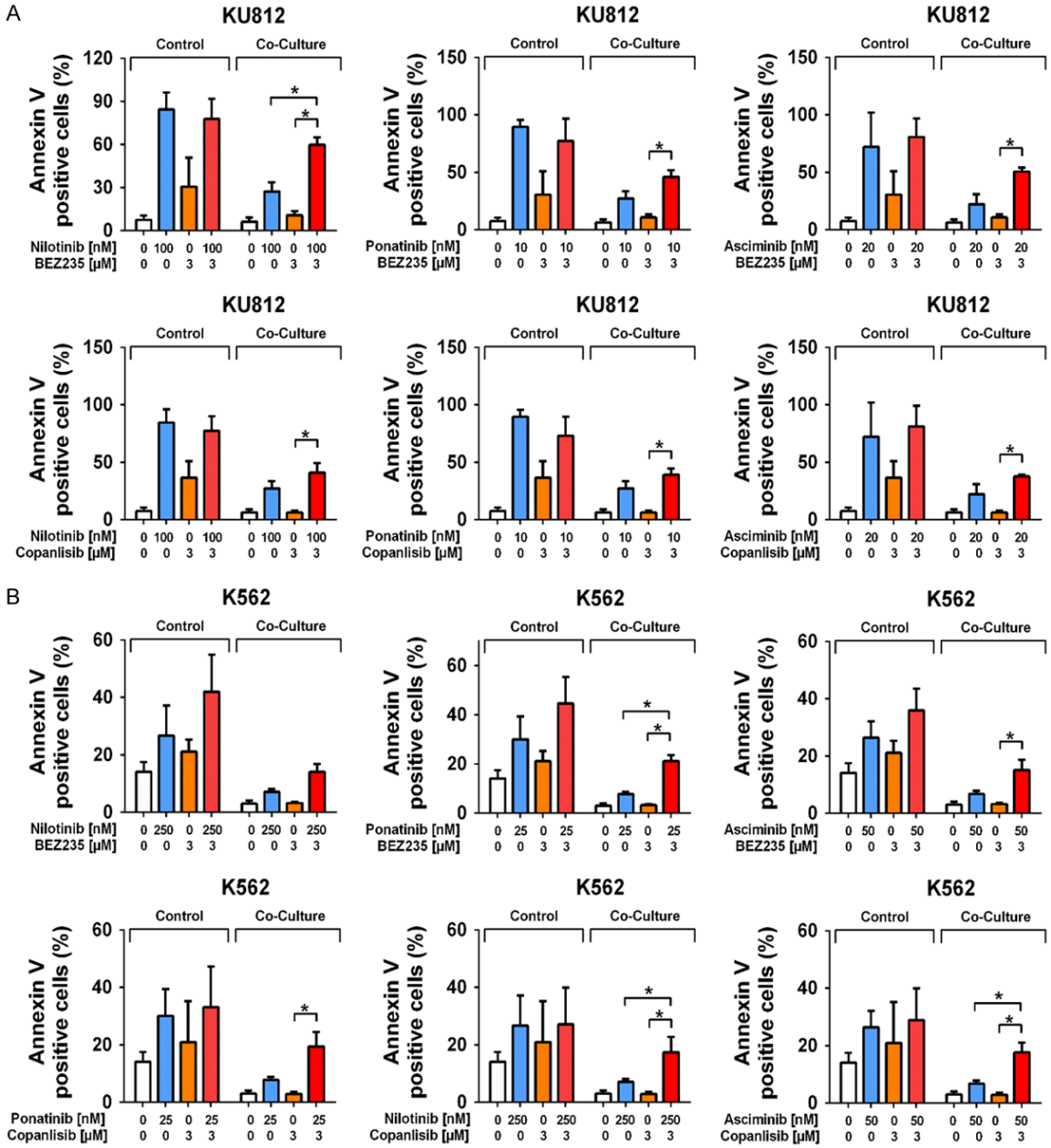

Figure S7. Effects of BEZ235 and copanlisib on osteoblast-induced resistance of CML cells. CAL-72 cells were pre-treated with BEZ235 (3 μM) or copanlisib (3 μM) at 37°C for 24 hours. Afterwards, cells were washed and co-cultured with KU812 cells (A) or K562 cells (B). These cells were incubated in control medium (0) or in medium containing nilotinib (100-250 nM), ponatinib (10-25 nM), asciminib (20-50 nM) or in various drug combinations (as indicated) in the absence (Control) or presence of drug-pre-treated CAL-72 cells (Co-culture). Then, $CD45^+$ KU812 cells and $CD44^-$ K562 cells were examined by multi-color flow cytometry to determine the percentage of apoptotic (AnnexinV/DAPI-positive) cells. Results represent the mean ± SD of three independent experiments. Asterisk (*): $P<0.05$.

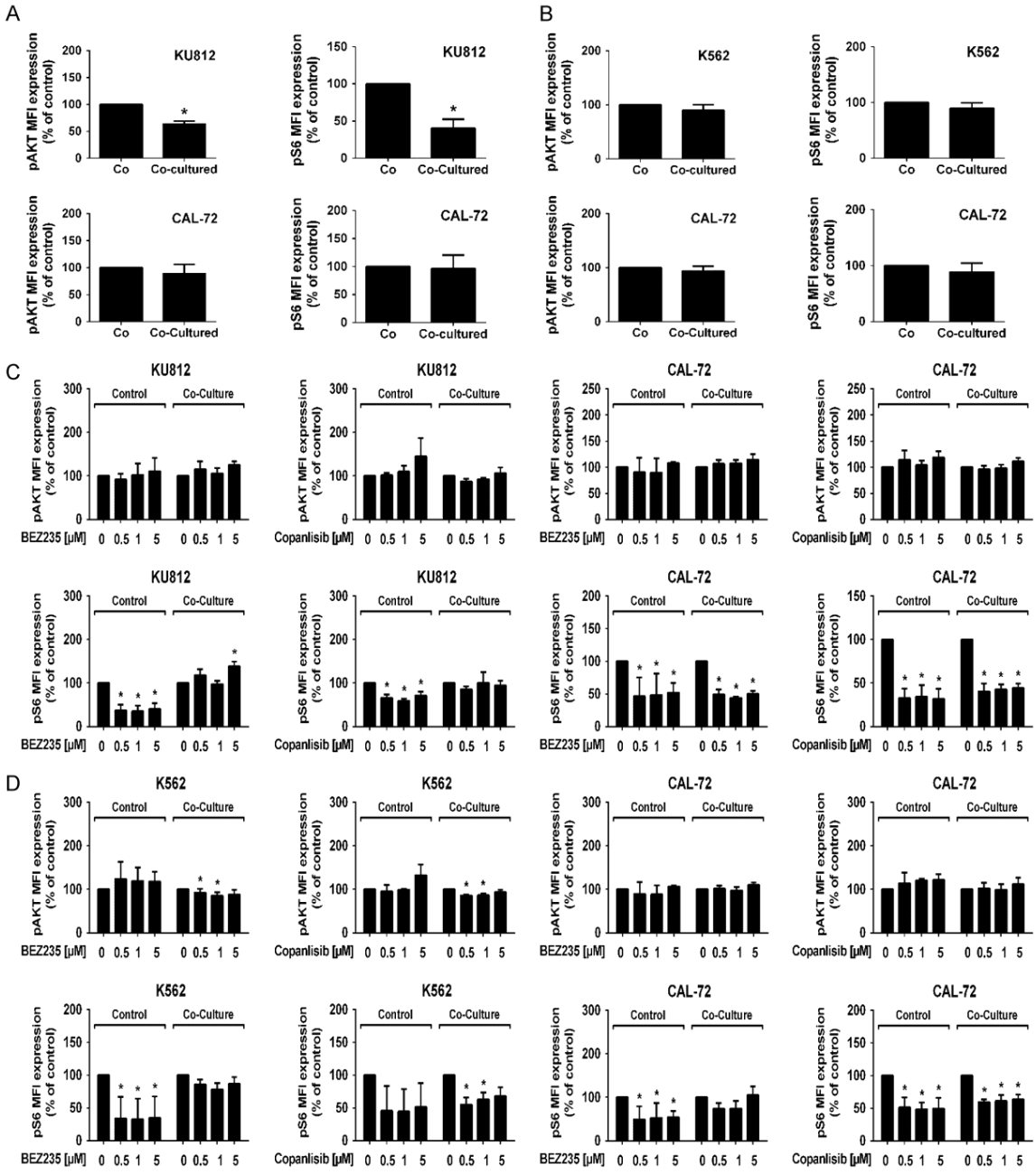

Figure S8. Effects of BEZ235 and copanlisib on expression of phosphorylated (p) AKT and S6 in CML cells and CAL-72 cells. KU812 (A) and K562 cells (B) were incubated in medium in the absence (Co) or presence (Co-culture) of CAL-72 cells at 37 °C for 48 hours. KU812 cells (C) and K562 cells (D) were incubated in control medium (0) or in medium containing BEZ235 (0.5-5 μM) or copanlisib (0.5-5 μM) in the absence (Control) or presence (Co-culture) of CAL-72 cells at 37 °C for 48 hours. Then, cells were permeabilized and stained with antibodies against pAKT (S473) and pS6 (S235/236). Expression of pAKT and pS6 was determined by flow cytometry. Results show median fluorescence intensity (MFI) values expressed as percent of control and represent the mean ± SD from 3 independent experiments. Asterisk (*): P<0.05 compared to control.

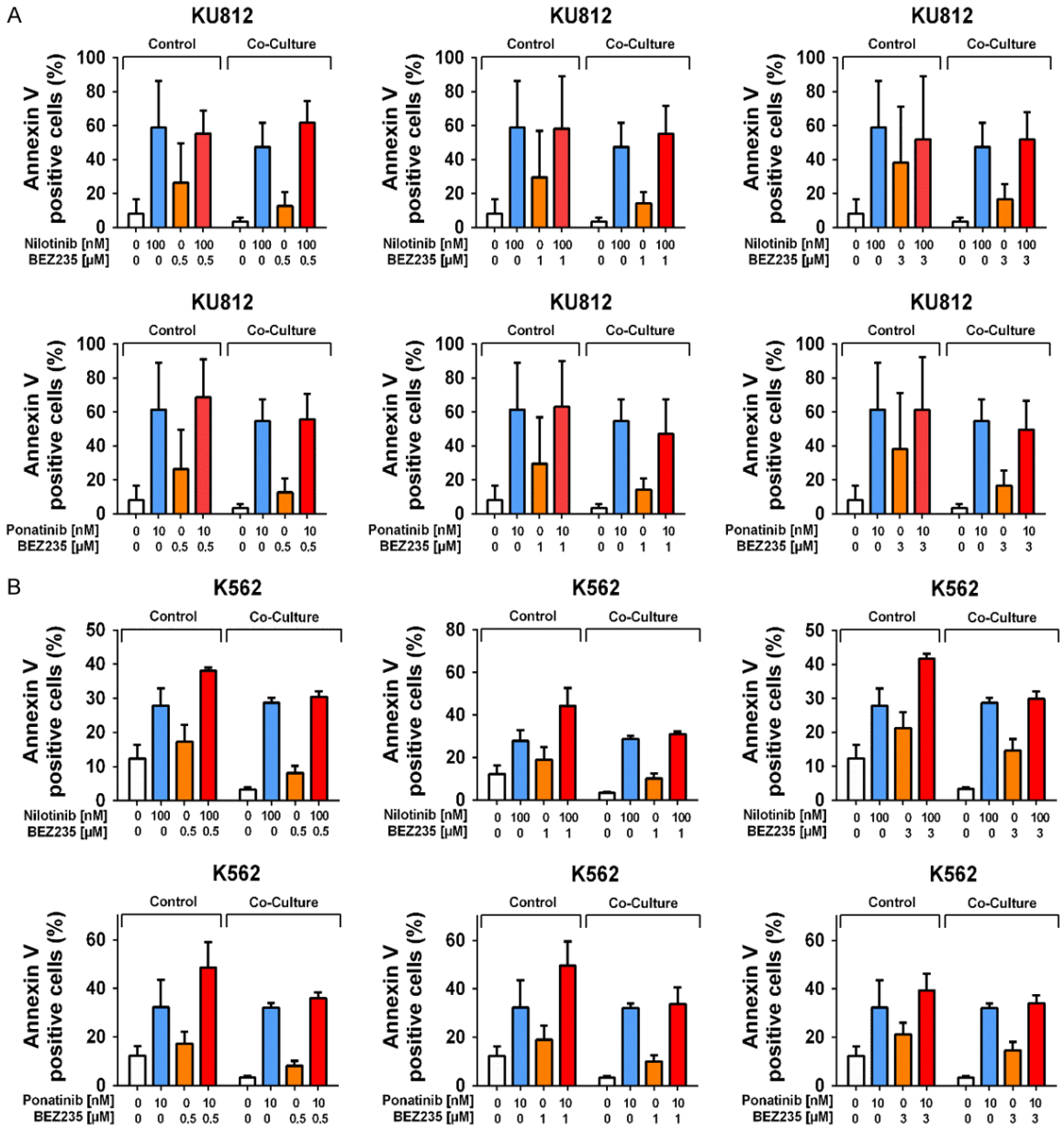

Figure S9. Effects of BEZ235 on vascular niche-induced TKI resistance of CML cells. KU812 cells (A) and K562 cells (B) were incubated in control medium (Co) or in medium containing nilotinib (100-250 nM), ponatinib (10-25 nM), BEZ235 (0.5-3 µM) or in drug combinations (BEZ235+nilotinib and BEZ235+ponatinib) in the absence (Control) or in the presence (Co-culture) of HMEC-1 cells at 37°C for 48 hours. Then, cells were examined by multi-color flow cytometry to determine the percentage of apoptotic (AnnexinV/DAPI-positive) $CD45^+$ KU812 and $CD44^-$ K562 cells. Results represent the mean ± SD of three independent experiments.