Figure S1. AVA4746 increases the mean fluorescence intensity of PE-HUTS-21 antibodies in a dose-dependent manner. Corresponding representative flow cytometry histograms of the PE-HUTS-21 antibody MFI for Fig. 1. LAX7R or TXL3 cells were treated with progressively increasing dosages of (A) AVA4746 or (B) VCAM-1 without Mn^{2+}. LAX7R or TXL3 cells were treated with progressively increasing dosages of (C) AVA4746 or (D) VCAM-1 with Mn^{2+}. MFI, mean fluorescence intensity; VCAM-1, vascular cell adhesion molecule 1.

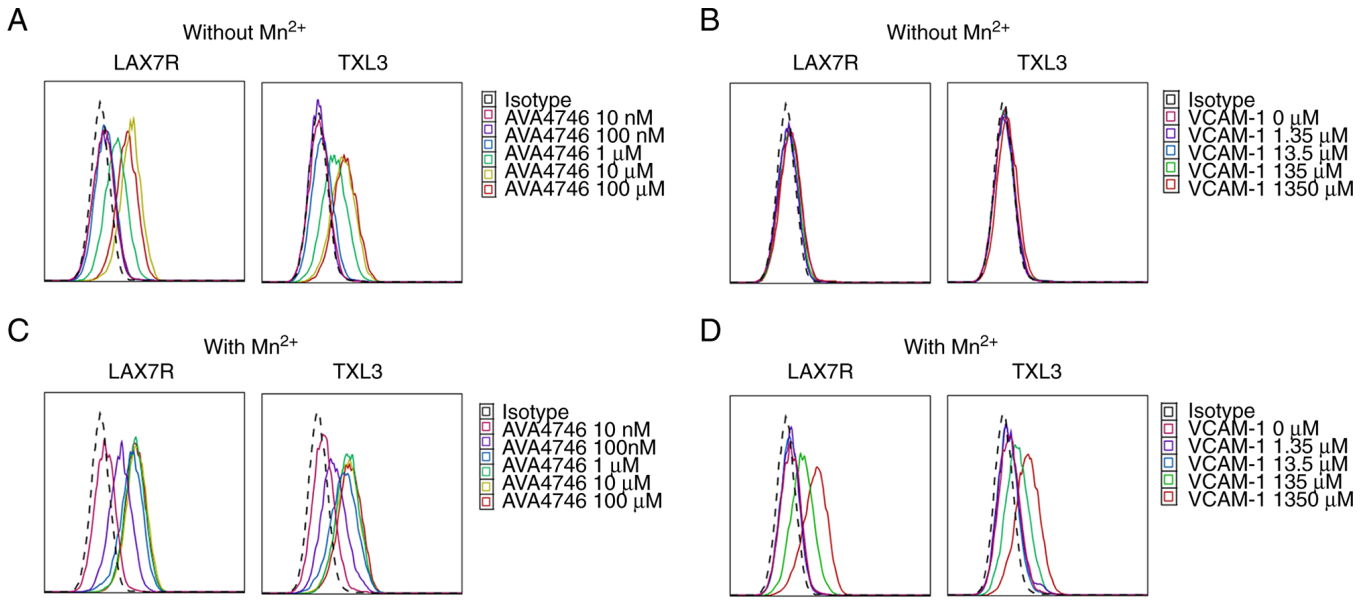

Figure S2. AVA4746 decreases integrin α4 expression but does not affect α5 and α6 in B-lineage acute lymphoblastic leukemia cells. (A) LAX7R, (B) LAX7, (C) LAX53, (D) LAX56 and (E) REH cells were treated with AVA4746 at 1 (red curve), 5 (blue curve) and 25 μM (orange curve) for either 24 or 96 h, where 0 was the DMSO control (green curve). Histograms representing the cell surface expression of integrins α4, α5 and α6 using flow cytometry are shown. ISO, isotype control.

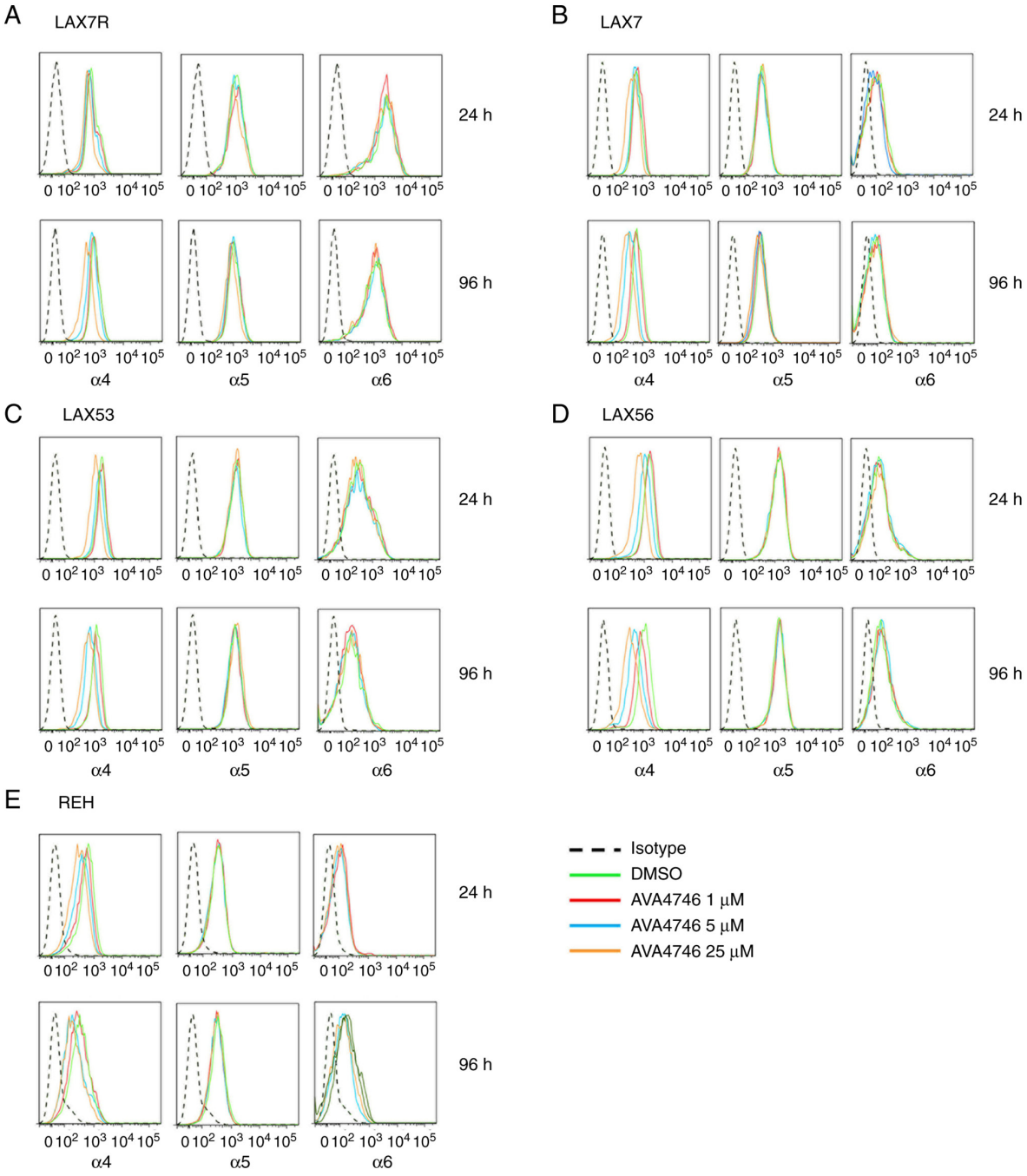

Figure S3. Reductions in the expression of integrin α4 induced by AVA4746 is partially reversed by the proteasome inhibitor MG132. (A) Schematic of the experimental design. (B) LAX7R, LAX53, LAX56 and REH B-lineage acute lymphoblastic leukemia cells were pre-treated with the proteasome inhibitor MG132 (1 μM) for 2 h, followed by treatment with either DMSO (0.1%) or AVA4746 25 μM for 96 h. Cell lysates were analyzed for integrin α4 protein expression by western blotting. β-actin was used as loading control. α4, α4-integrin.

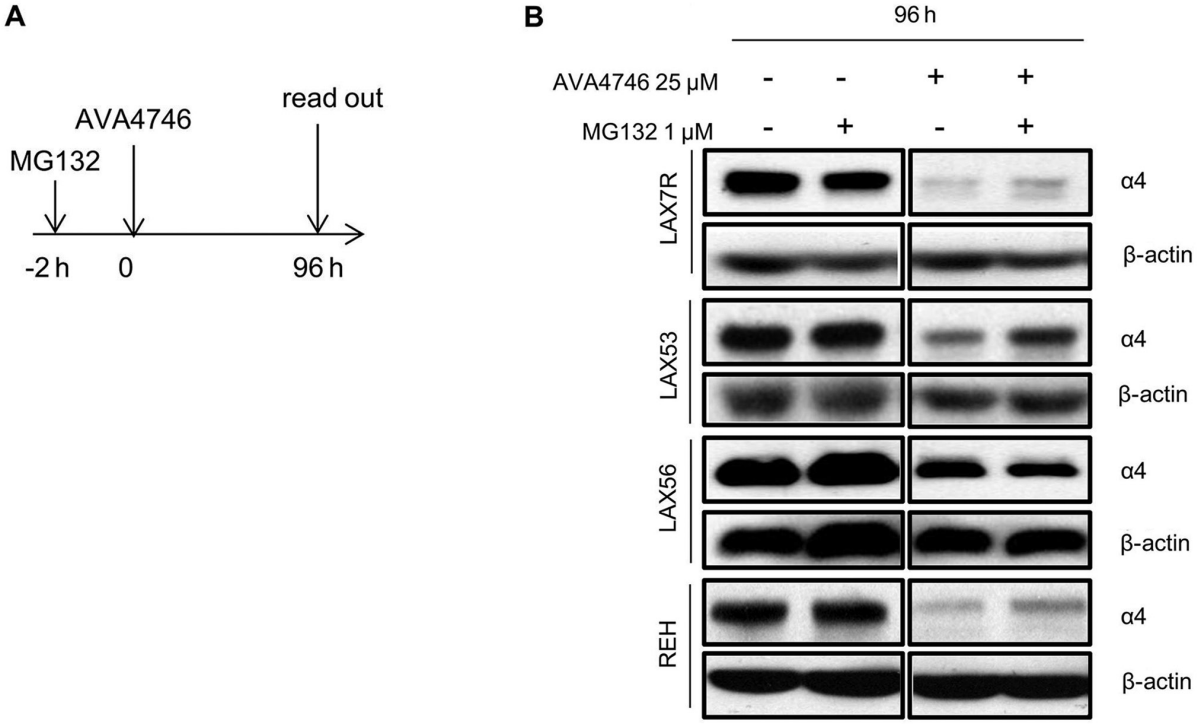

Figure S4. AVA4746 does not affect AKT phosphorylation but may regulate other cell signaling molecules in B-lineage acute lymphoblastic leukemia cells. (A) LAX56 and LAX7R cells were serum starved overnight and stimulated with either 20% FBS or 10 μg/ml human VCAM-1, with or without AVA4746 treatment (25 μM), for 1 h. Western blot analysis of p-AKT, total AKT protein levels with β-actin as the loading control are shown. (B) Following overnight serum starvation, LAX53 cells were stimulated with stroma OP9 cells for 1 or 24 h with AVA4746 at doses of 0, 5 and 25 μM. Western blot analysis of phosphotyrosine with β-actin as the loading control is shown. Red box shows potential cell signaling changes by AVA4746. p-, phosphorylated; hVCAM1, human vascular cell adhesion molecule 1.

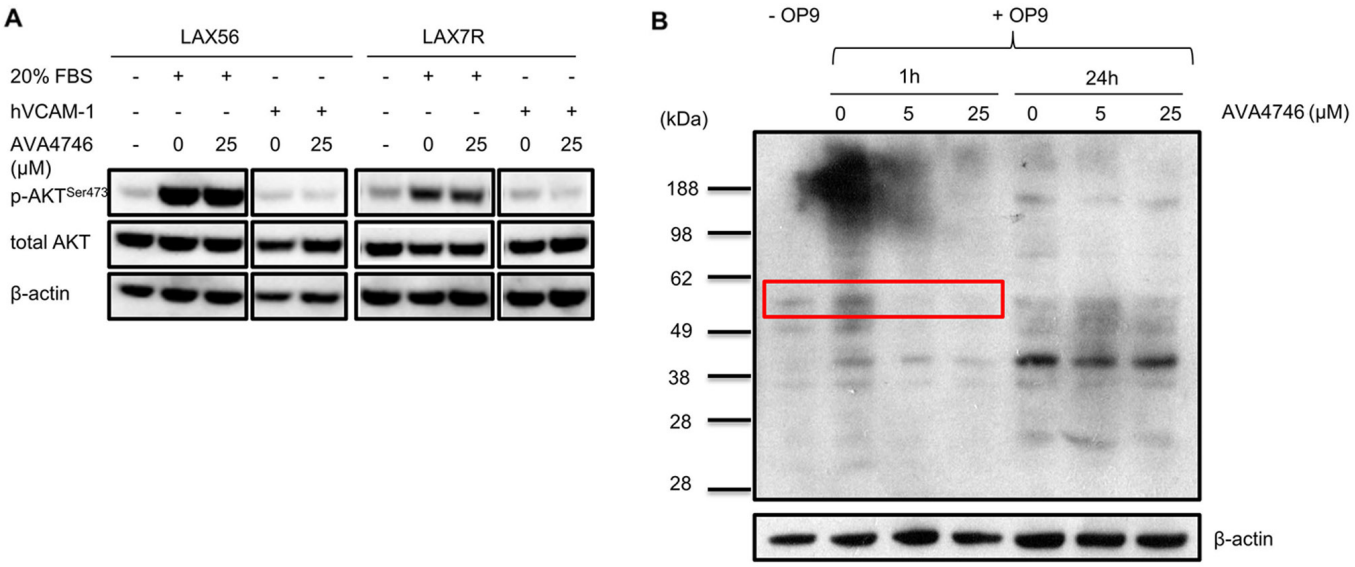

Figure S5. AVA4746 detaches pre-B-ALL cells from the stromal cell line OP9. (A) RS4;11, (B) SupB15, (C) KASUMI-2, (D) 697, (E) BEL-1, (F) BV173, (G) RCH and (H) TOM-1 B-ALL cells were seeded into plates with or without OP9 cells for 4 h and then treated with either DMSO control or the AVA4746 (25 μM) overnight. Percentage of adherent cells are shown, which was determined by cell counting using trypan blue exclusion. Each experiment was performed in triplicate. *P<0.05 and **P<0.01, B-ALL, B-lineage acute lymphoblastic leukemia.

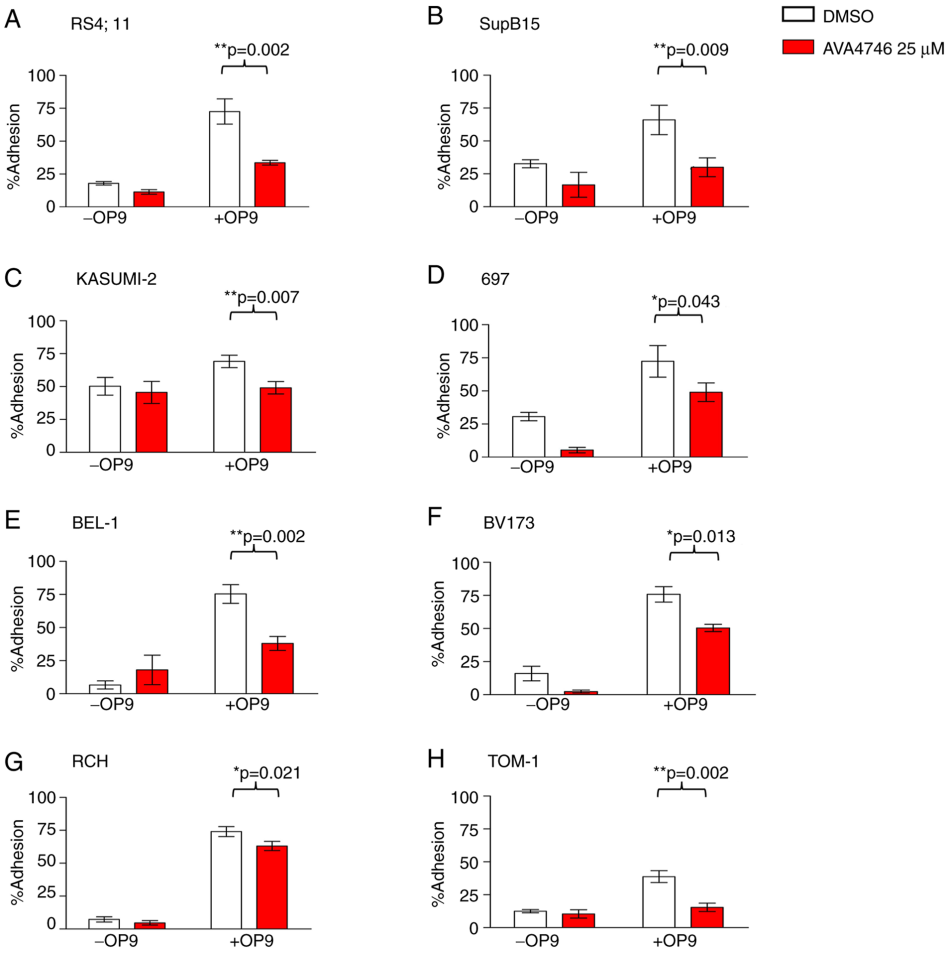

Figure S6. Stromal cells support the viability in B-ALL cells. (A) TXL3, (B) LAX56 and (C) LAX7R B-ALL cells were seeded into uncoated wells or wells pre-coated the stromal cell line OP9 and with treated for 48 h with either DMSO or AVA4746 25 μM, with or without VDL chemotherapy (vincristine 5 nM, dexamethasone 0.05 nM, L-asparaginase 2.5x10-3 IU/ml). Percentage of cell apoptosis (% Annexin-V-positive population) was determined using flow cytometry. Experiments were performed in triplicate. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. NS, not significant; B-ALL, B-lineage acute lymphoblastic leukemia; VDL, vincristine, dexamethasone and L-asparaginase.

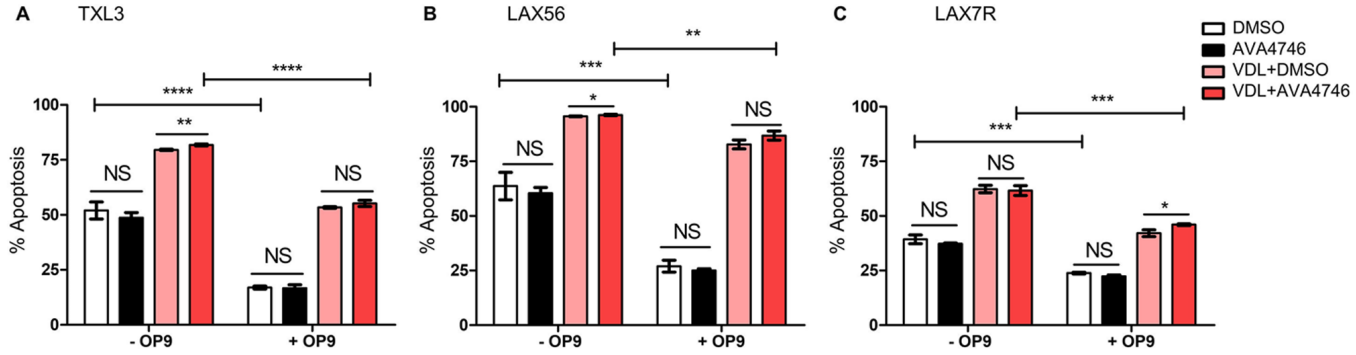

Figure S7. AVA4746 does not change leukemia distribution at 8 h post-treatment. (A) A schematic of the leukemic cell distribution experimental design. Following 8 h of treatment with AVA4746 (60 mg/kg) or PBS control, with or without VDL (vincristine 0.5 mg/kg, dexamethasone 10.5 mg/kg, and L-asparaginase 1500 IU/kg; n=6 per group), mice were sacrificed before (B) PB, (C) BM and (D) SPC were collected and analyzed using flow cytometry. The total MNC number, percentage (%) of human $CD45^+CD19^+$ population, leukemic cell number, MFI of human α4, % of mouse $CD45^+$ and mouse cell number are also shown. NS, not significant. $^{**}P<0.01$ and $^{***}P<0.001$. MNC, mononuclear cell; PB, peripheral blood; BM, bone marrow; SPC, spleen; VDL, vincristine, dexamethasone and L-asparaginase; Nb, number.

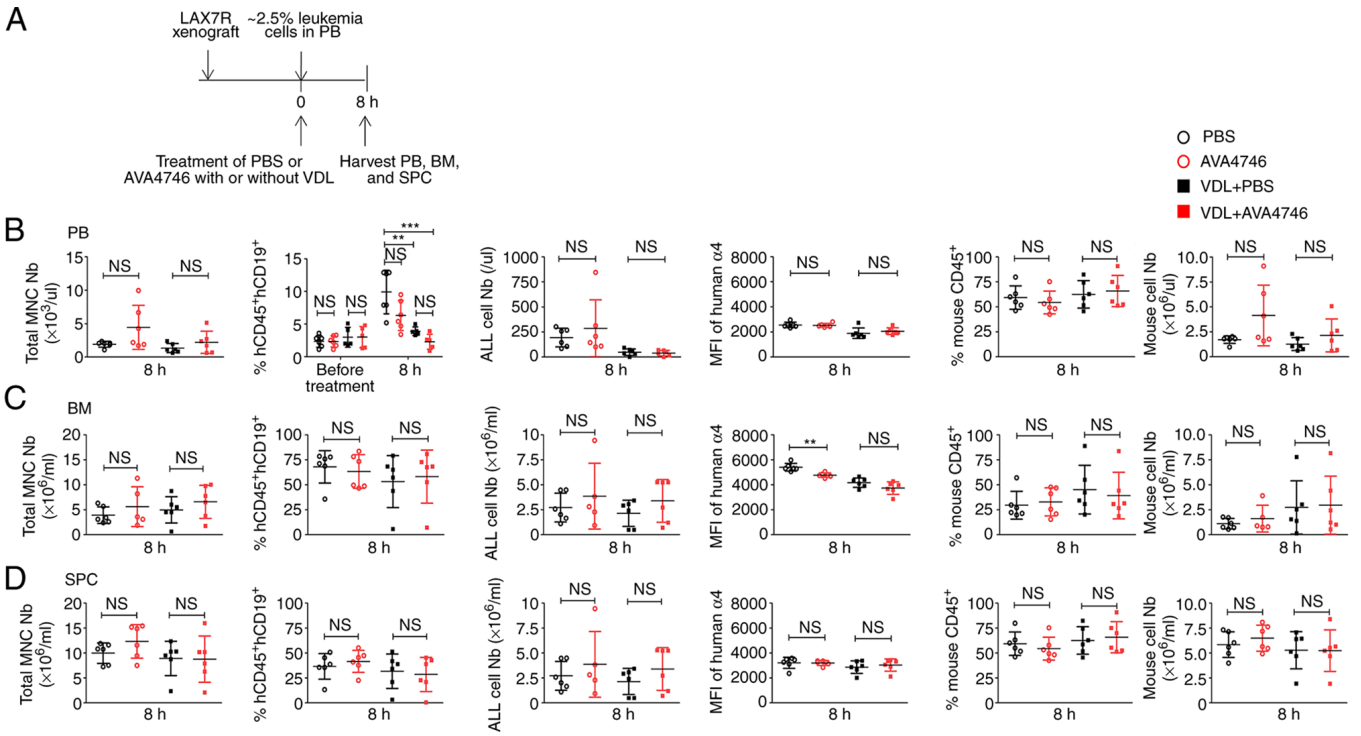

Figure S8. Corresponding representative hCD45^{+}hCD19^{+} flow cytometry dot plots for Fig. 4. Representative hCD45^{+} (y axis) and hCD19^{+} (x axis) dot plots of whole mononuclear cells isolated from (A) PB, (B) BM or (C) SPC were shown. PB, peripheral blood; BM, bone marrow; SPC, spleen.

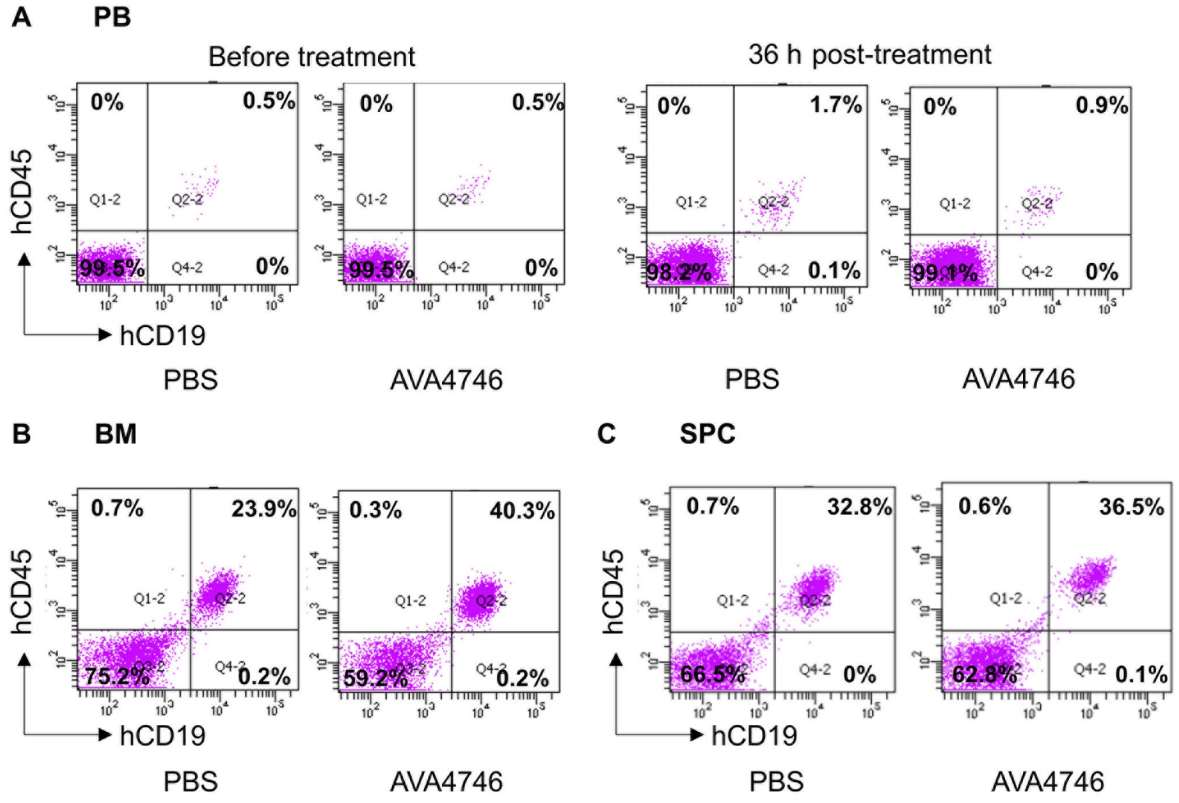

Figure S9. TBC3486 inhibits tube formation by HUVEC *in vitro*. HUVECs treated with either 0 or 25 μM TBC3486 for 30 min were seeded onto Matrigel-coated plates for 4-6 h. Tube formation was assessed by analyzing the number of (A) nodes, (B) segments, (C) meshes and (D) the total area. Images were analyzed with the ImageJ software coupled with the angiogenesis analyzer plug-in. **P<0.01 and ***P<0.001. Nb, number.

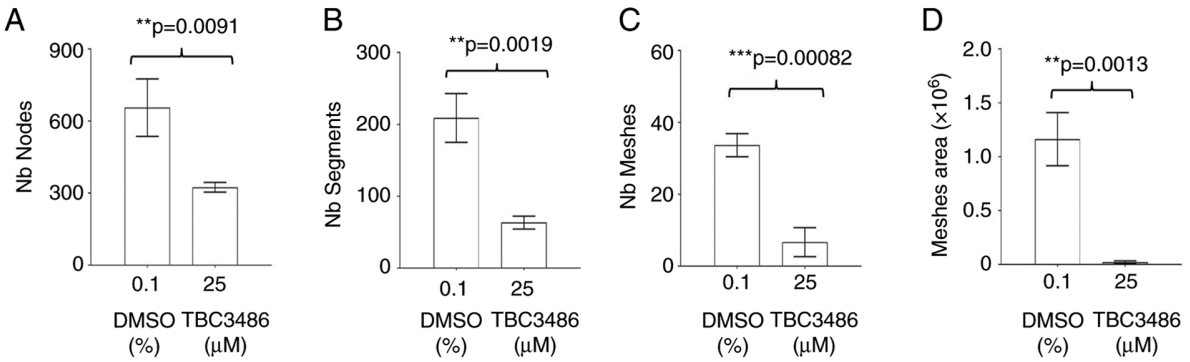

Figure S10. AVA4746 in combination of chemotherapy does not prolong the survival of NSG mice injected with RS4;11 and TXL3 cells. (A) Treatment regimen of 21 days with AVA4746 and 4 weeks with VDL after RS4;11 cell injection. (B) Kaplan-Meier survival curve of RS4;11-engrafted mice. P-values for PBS vs. AVA4746 and PBS + VDL vs. AVA4746 +VDL were 0.1842 and 0.0515, respectively. (C) Treatment regimen of 14 days with AVA4746 and 14 days with VD after TXL3 cell injection. (D) P-value of PBS vs. AVA4746 and PBS + VD vs. AVA4746 + VD were 0.003 and 0.080, respectively. *P<0.05. VDL, vincristine 0.5 mg/kg once a week, dexamethasone 10.5 mg/kg five times a week and L-asparaginase 1500 IU/kg five times a week; i.p., intraperitoneal; Bid, twice a day. P.o., oral gavage.

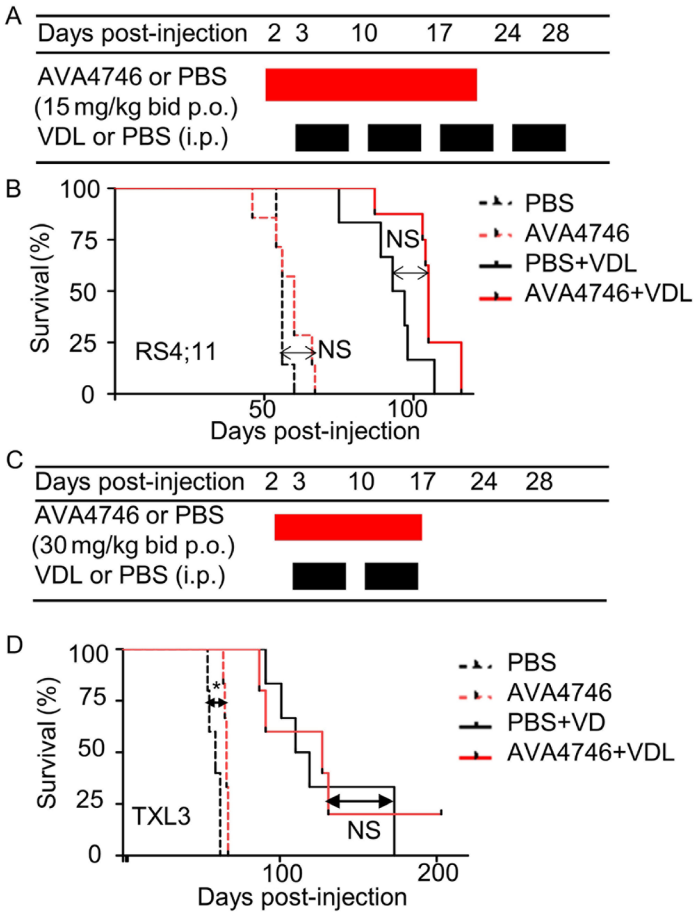

Table SI. Cytogenetic information and integrin expression of B-ALL cells

ALL	Status	Karyotypes/ cytogenetics	% human α4
LAX7R	Relapse of LAX7	IZKF1 KRAS G12V	99.8
LAX7	Diagnosis	IZKF1	99.1
LAX53	Diagnosis	unknown	99.8
LAX56	Relapse	46X,Y t(Y;7)	99.0
TXL3	Diagnosis	BCR-ABL1	99.8
ICN24	Diagnosis	unknown	99.2
REH	Cell line	TEL-AML1	98.0
RS4;11	Cell line	MLL-AF4	98.0
BEL-1	Cell line	MLL-AF4	100.0
697	Cell line	E2A-PBX1	98.2
KASUMI-2	Cell line	E2A-PBX1	99.7
RCH	Cell line	E2A-PBX1	99.8
SUP B15	Cell line	BCR-ABL1	98.5
BV173	Cell line	BCR-ABL1	99.9

Cytogenetic information and integrin expression percentages of B-ALL cells are shown in Table SI (24,25).

Table SII. MFI of HUST-21 of AVA4746 or VCAM-1 treatment with or without Mn^{2+} in LAX7R and TXL3. Combined values indicate average of experiments #1 and #2.

LAX7R

Without Mn^{2+}	Vehicle control	AVA4746 (nM)					VCAM-1 (μM)			
		10	100	1000	10000	100000	1.35	13.5	135	1350
Exp#1	374±14	373±14	423±19	789±19	1457±20	1288±25	323±9	325±19	348±20	389±35
Exp#2	299±8	309±10	381±5	742±3	1188±12	1330±4	288±7	298±3	312±8	391±11
Combined[a]	337±43	341±37	402±26	766±28	1323±148	1309±28	306±21	312±19	330±24	390±23

With Mn^{2+}	Vehicle control	AVA4746 (nM)					VCAM-1 (μM)			
		10	100	1000	10000	100000	1.35	13.5	135	1350
Exp#1	411±13	966±14	1509±28	1752±30	1852±16	1828±33	390±13	398±14	763±48	1330±117
Exp#2	402±14	813±2	1351±11	1608±4	1579±4	1778±19	381±9	401±10	750±33	1162±17
Combined	406±13	890±85	1430±89	1680±81	1716±150	1803±36	385±11	399±11	757±37	1246±118

TXL3

Without Mn^{2+}	Vehicle control	AVA4746 (nM)					VCAM-1 (μM)			
		10	100	1000	10000	100000	1.35	13.5	135	1350
Exp#1	388±23	326±7	405±4	747±16	1210±5	1282±11	312±5	315±15	335±2	373±2
Exp#2	314±2	299±4	410±9	824±1	1209±9	1327±15	300±4	302±10	302±6	423±49
Combined	351±43	312±16	408±7	786±43	1209±7	1305±27	306±7	309±13	318±18	398±41

With Mn^{2+}	Vehicle control	AVA4746 (nM)					VCAM-1 (μM)			
		10	100	1000	10000	100000	1.35	13.5	135	1350
Exp#1	413±16	815±14	1269±18	1474±18	1509±18	1543±30	380±12	399±14	585±31	1120±63
Exp#2	440±16	792±35	1229±36	1448±13	1445±28	1554±31	415±31	407±18	723±41	1123±47
Combined	427±20	804±27	1249±34	1461±20	1477±41	1549±28	397±28	403±15	654±82	1122±50

[a]Combined means average of experiments 1 and 2

Table SIII. Percentages of adhered cells following AVA4746 treatment in LAX7R and TXL3. Combined values indicate average of experiments #1 and #2.

LAX7R

		AVA4746 (nM)				
Experiment	Vehicle control	10	100	1000	10000	100000
Exp#1	91.3±1.1	90.6±0.9	88.1±0.5	85.0±1.9	51.9±2.9	20.0±2.9
Exp#2	83.1±1.9	76.9±3.9	75.6±1.9	65.6±2.9	55.0±1.9	16.3±2.2
Combined[a]	87.2±4.7	83.8±7.9	81.9±7.0	75.3±10.8	53.4±2.8	18.1±3.1

TXL3

		AVA4746 (nM)				
Experiment	Vehicle control	10	100	1000	10000	100000
Exp#1	92.3±1.1	91.3±1.1	88.8±0.8	85.5±1.9	66.0±3.8	16.0±3.0
Exp#2	80.5±5.2	77.0±3.1	71.5±1.5	66.5±2.3	59.5±2.6	21.0±1.7
Combined	86.4±7.3	84.1±8.1	80.1±9.5	76.0±10.6	62.8±4.6	18.5±3.5

[a]Combined means average of experiments 1 and 2