Table S1. *Primary ALL samples characteristics*

Patient	Sample ID	Sample Type	Karyotype
1	ICN13	Diagnosis	***MLL-AF4***
2	LAX2	Relapse	***BCR-ABL1*** **p210, T315I** Mutant
3	TXL2	Diagnosis	***BCR-ABL1*** **p210**
4	TXL3	Diagnosis	***BCR-ABL1*** **p210**
5	SFO2	Relapse	***BCR-ABL1*** **p210**
6	ICN6	Diagnosis	***TEL-AML1***
7	ICN12	Diagnosis	***E2A-PBX1***
8	SFO1	Relapse	Unknown
9	SFO3	Relapse	Unknown
10	LAX6	Diagnosis	***Unknown***
11	LAX7	Diagnosis	Normal
12	LAX7R	Relapse of LAX7	Normal
13	LAX3	Diagnosis	Normal
14	LAX12	Diagnosis	Unknown
15	LAX20	Diagnosis	Unknown
16	LAX1	Diagnosis	T-ALL
17	LAX1R	Relapse of LAX1	T-ALL
18	LAX27	Relapse	T-ALL
	ICN1	Diagnosis	***BCR-ABL1*** **p210**

Sample type and karyotype of primary ALL samples.

Table S2. *Viability of primary ALL Cells 15 days post-treatment with EZN-3042 and chemotherapy*

	Mock	STD	VDL or Nilotinib	STD	VDL or Nilotinib + EZN-3088	STD	VDL or Nilotinib + EZN-3042	STD
SFO2	86.7%	± 1.9	88.4%	± 5.8	73.4%	± 1.8	3.6%	± 0.5
SFO3*	77.2%	± 6.2	34.5%	± 5.1	31.6%	± 4.1	2.7%	± 0.9
LAX7R	76.8%	± 6.5	78.3%	± 1.4	84.6%	± 1.5	12.8%	± 3.0

**Day 21 Post-Treatment*

Table S3. *Percent Viability* (*Annexin V-/ 7AAD-*) of *SFO2 After Treatment with EZN-3042 and Chemotherapy*

SFO2	Mock	STD	VDL or Nilotinib	STD	Nilotinib + EZN-3088	STD	Nilotinib + EZN-3042	STD
Day 9	57.0%	± 3.2	30.2%	± 8.8	23.2%	± 7.9	28.6%	± 3.9
Day 15	48.6%	± 8.7	31.2%	± 6.4	39.7%	± 8.3	1.4%	± 0.8

Table S4. *Percent Viability* (*Annexin V-/ 7AAD-*) *TXL3 After Treatment with EZN-3042 and Chemotherapy*

TXL3	Mock	STD	VDL or Nilotinib	STD	Nilotinib + EZN-3088	STD	Nilotinib + EZN-3042	STD
Day 13	34.1%	± 9.1	18.2%	± 3.1	28.3%	± 2.0	4.8%	± 0.7

Legend: (**S2**) Mean viability percent of triplicates of various treatment groups are shown for Day 15 of SFO2 and LAX7R of Figure 5C and 5H, respectively. Day 21 mean percent viabilities of SFO3 shown for Figure 5F. P-Values ≤ 0.01,for survivin-targeting EZN-3042 co-treatment compared to EZN-3088 co-treatment for SFO2, SFO3, and US7R or p-values equaling 0.0007, 0.01, and 0.0001, respectively, on indicated post-treatment timepoints. (**S3**) SFO2 annexin V staining of respective treatment conditions for treatment days 9, 13, and 15 are shown resulted in a mean reduction in the annexin V-/7-AAD- population by EZN-3042 + nilotinib to 1.4% ± 0.8%, compared EZN-3088 + nilotinib of 39.7% ± 8.3% of respective triplicates, on day 15 post-treatment (p-value>0.0007). (**S4**) Annexin V staining of respective treatment conditions for treatment days 9 of TXL3 resulted in a mean reduction in the annexin V-/7-AAD- population by EZN-3042 + nilotinib to 4.8% ± 0.7%, compared EZN-3088 + nilotinib of 28.3% ± 2.0% of respective triplicates, on day 13 post-treatment (p-value>0.00003). Statistical analyses performed using a student paired T-test. .

Table S5. *Sequences of relevant oligonucleotide primers*

PCR primers

hBIRC5 F	5'-CATCTCTACATTCAAGAACTGG-3' (Bhojwani et al, 2006)
hBIRC5 R	5'-GGTTAATTCTTCAAACTGCTTC -3' (Bhojwani et al, 2006)
hGAPDH F	5'-GTTGCCATCAATGACCCCTTCATTG-3'
hGAPDH R	5'-GCTTCACCACCTTCTTGATGTCATC-3'
mHPRT F	5'-GGGGGCTATAAGTTCTTTGC-3'
mHPRT R	5'-TCCAACACTTCGAGAGGTCC-3'

Survivin overexpression cloning primers

hBIRC5 F	5'-GTCATACTCGAGGCCACCATGGGTGCCCCGACGTTGCC–3'
hBIRC5 R	5'-ACGCCGAATTCTCAATCCATGGCAGCCAGCTGCT–3'

Table S6. *Sequences of shRNA and EZN-3042*

shRNA target sequences

Non-Silencing	5'-CGGACTTGAATGGAATGATAAT- 3'
hBIRC5	5'- CCTTAGCAATGTCTTAGGAAA -3' (targeting survivin sequence 519–539)

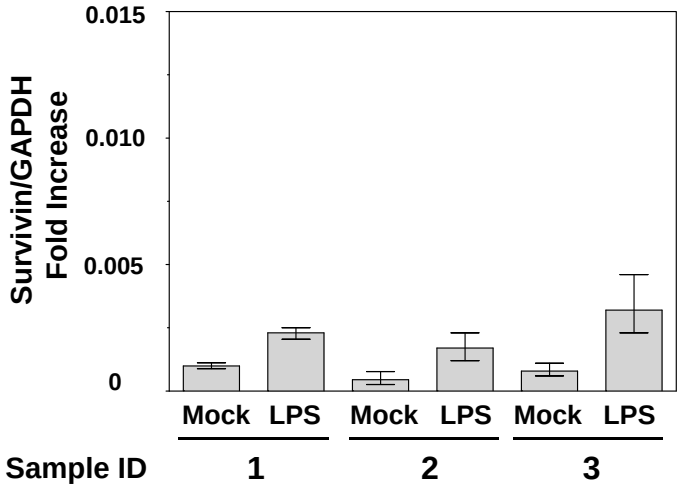
LNA Sequences

EZN-3088	5'-**CGT**cagtatgcg**AAT**c–3'
EZN-3042	5'-**CTCA**atccatgg**CAG**c–3' (targeting position 418–433)

***Bold capital letters denote LNA modifications**

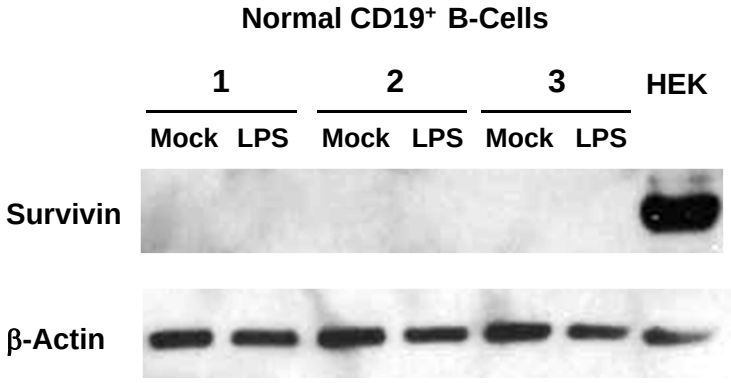
Figure S1. *CD19⁺ survivin expression in healthy volunteer samples*

A



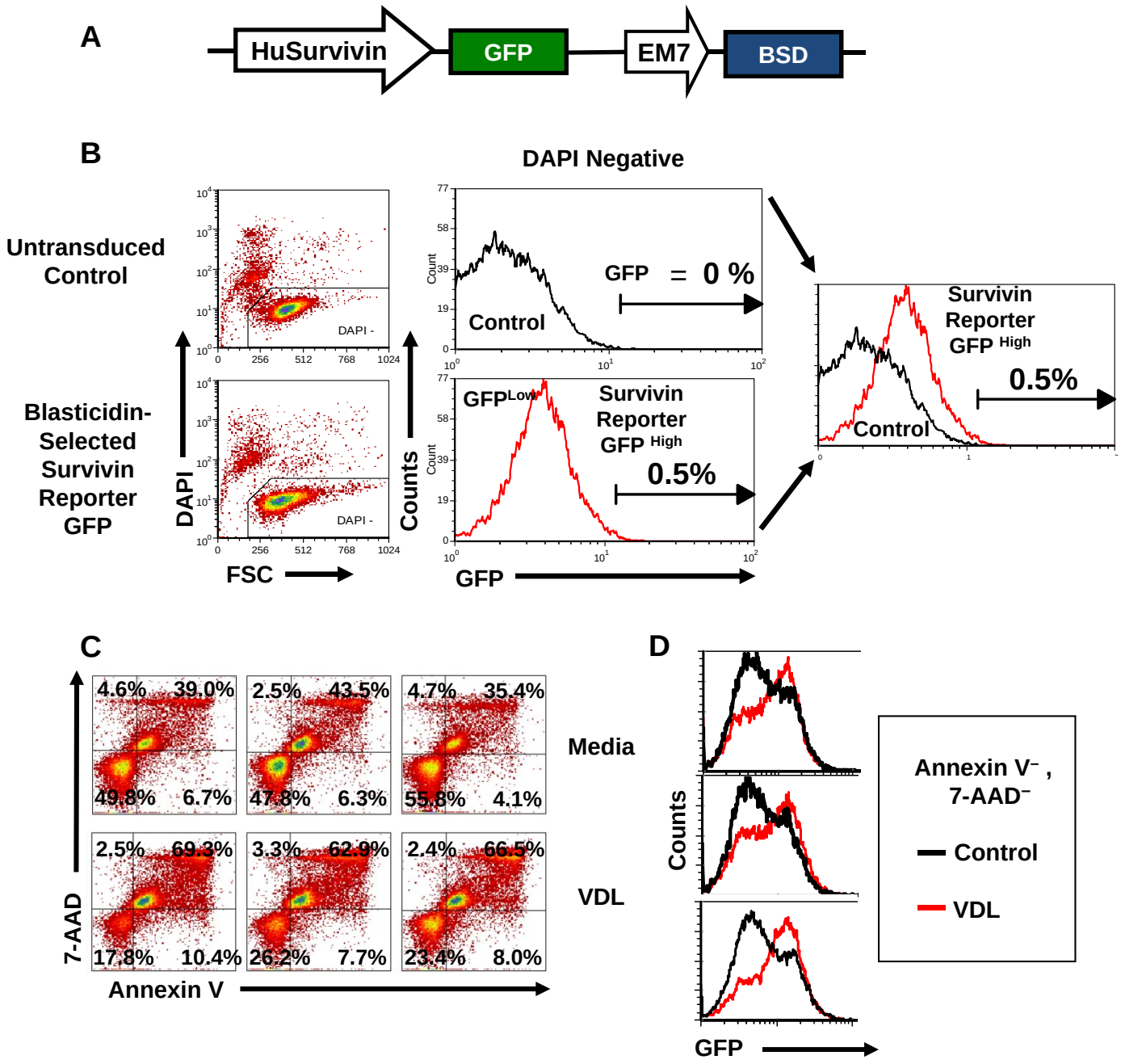
Case	LPS	Sample ID	Tissue Type	Phenotype
1	-	Normal B-Cells	Peripheral Blood	$CD19^+$
	LPS	Normal B-Cells	Peripheral Blood	$CD19^+$
2	-	Normal B-Cells	Peripheral Blood	$CD19^+$
	LPS	Normal B-Cells	Peripheral Blood	$CD19^+$
3	-	Normal B-Cells	Peripheral Blood	$CD19^+$
	LPS	Normal B-Cells	Peripheral Blood	$CD19^+$

B



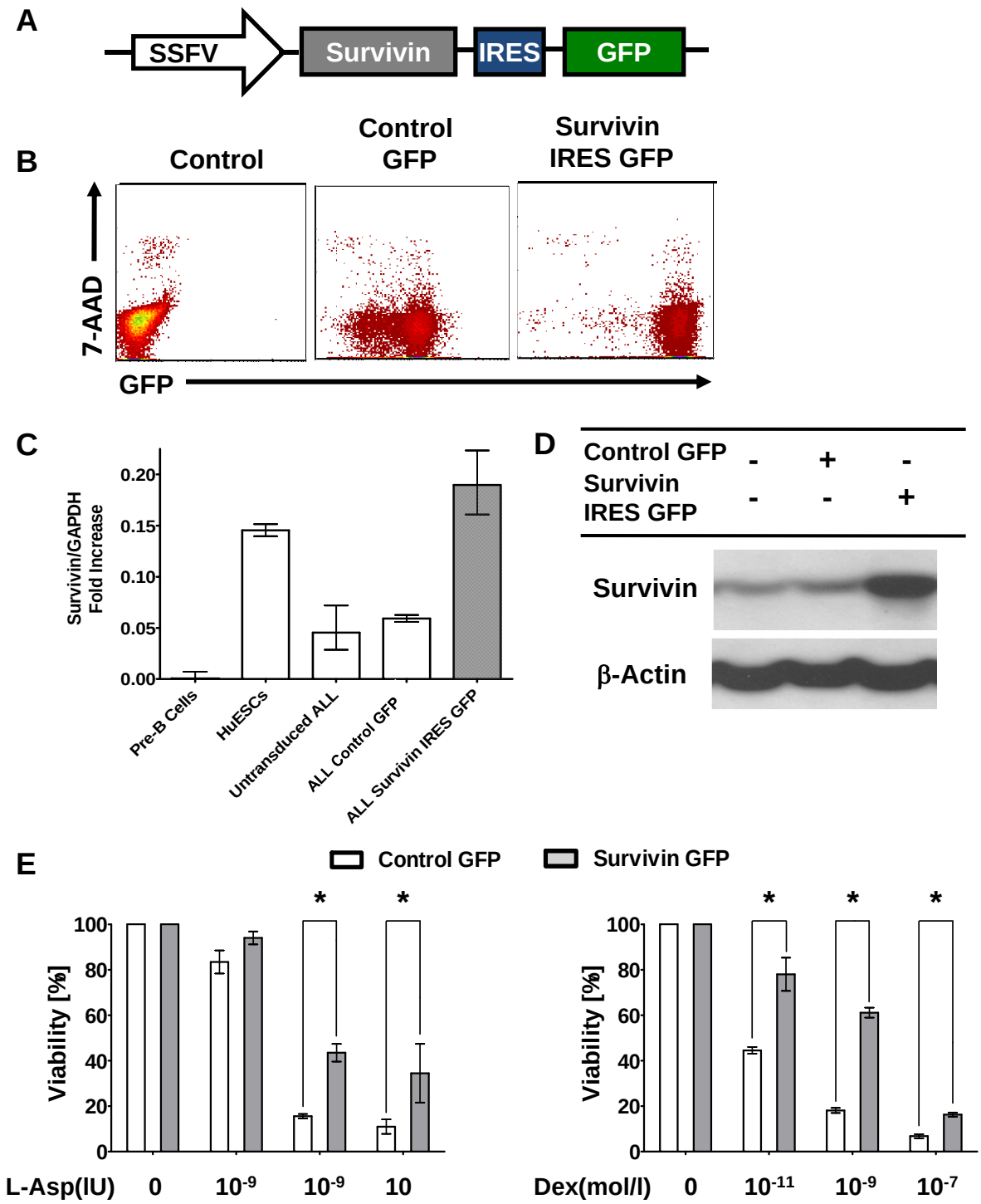
Legend: Peripheral blood from healthy volunteers were sorted for $CD19^+$ cells by FACS, following ficoll separation of mononuclear cells (MNCs). (**A**) Subsequently, respective samples were stimulated with 2.5 μg/ml of lipopolysaccharide (LPS) or mock treatment with vehicle control for 48 hours. Real-time PCR results for healthy volunteer samples analyzed in triplicate., (**B**) Western blot and real-time PCR (data not shown) for survivin expression in both LPS unstimulated and stimulated conditions, was determined.

Figure S2. *Endogenous survivin expression in ALL cells*



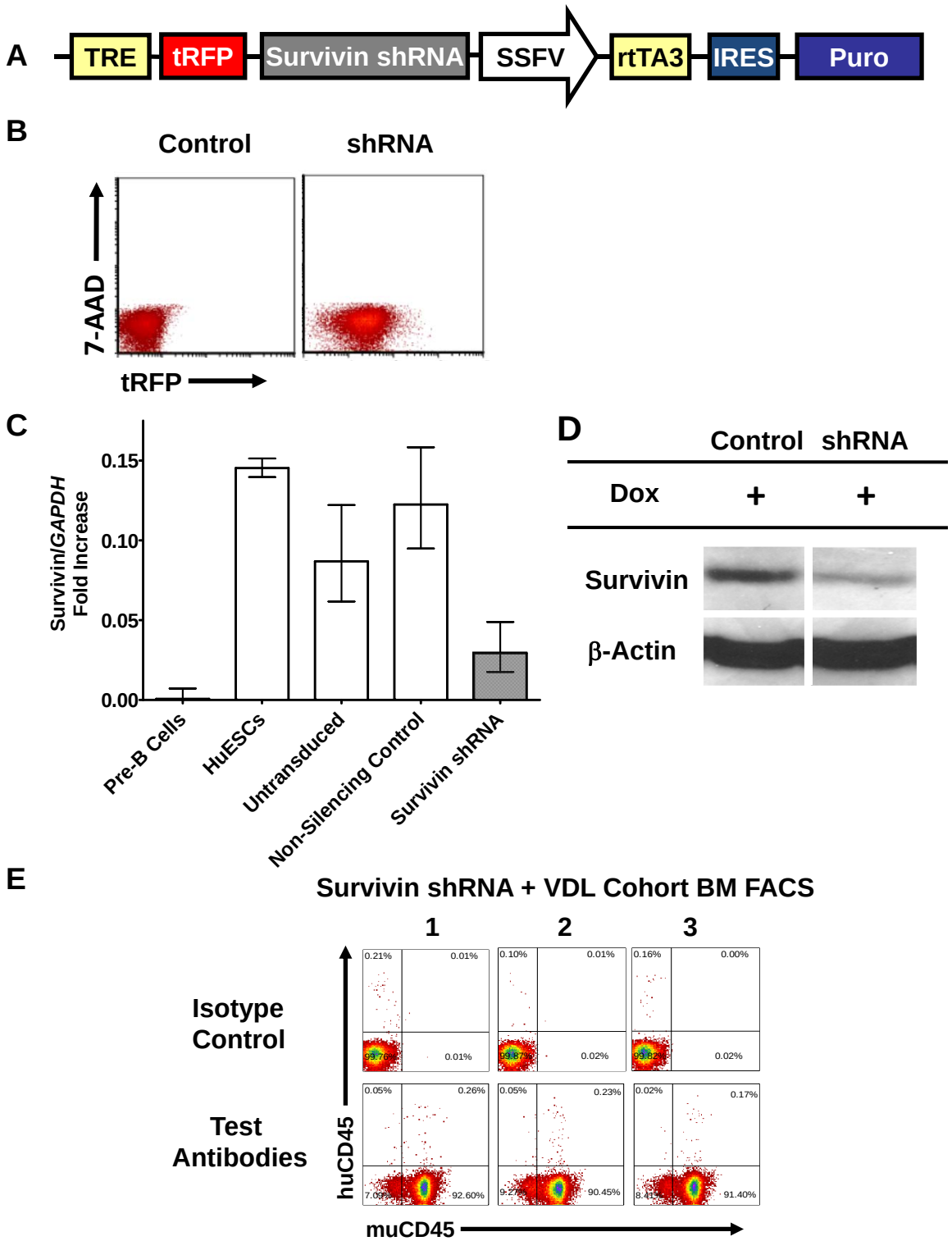
Legend: (**A**) Schematic of the human survivin promoter-driven GFP reporter (Survivin reporter GFP). (**B**) FAC Sorting of survivin expressing ALL cells, depicting transduction control under blasticidin selection (8 µg/mL), untransduced control, and survivin reporter GFP transduced ALL. Cells characterized to be highly expressing GFP compared to untransduced controls, were 0.5% of the total DAPI negative population. (**C**) Primary ALL transduced with the survivin reporter GFP were treated in triplicate with either media control or a combination of Vincristine (1 nM), Dexamethasone (0.1 nM), and L-Asparaginase (0.01 IU) (VDL) for 48 hours and stained for annexin V/7-AAD. (**D**) Histograms for show enriched survivin promoter activity in VDL treated annexin V^-/7-AAD^- versus control.

Figure S3. *Ectopic survivin expression in primary pre-B ALL cells*



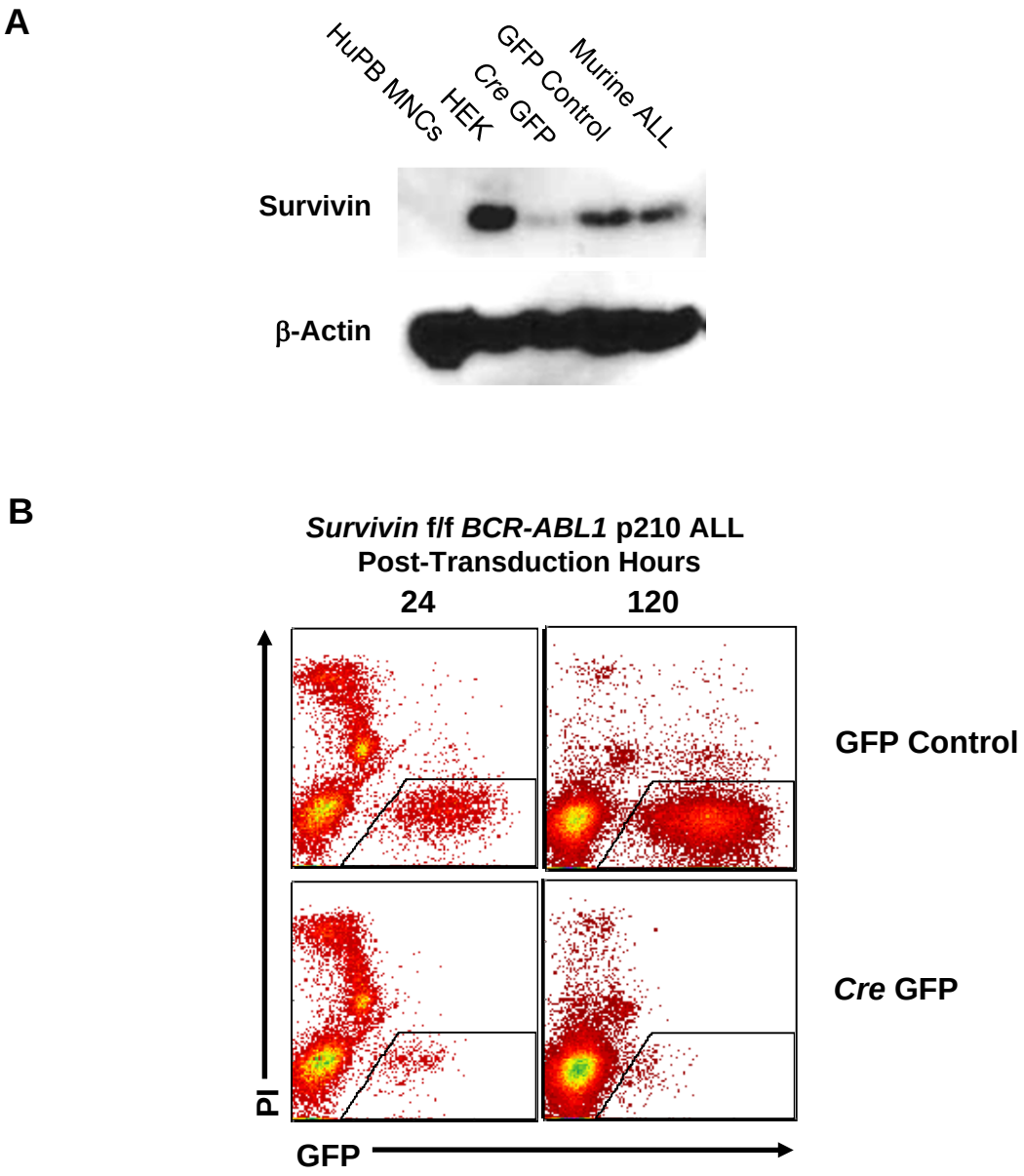
Legend: (**A**) A lentiviral Survivin IRES GFP was used to sort ALL cells ectopically expressing survivin by flow cytometry (**B**). (**C**) Survivin expression was detected by qPCR to be a mean 3.2 fold higher than in control GFP cells and by Western blot more than 3-fold (**D**). (**E**) Diminished sensitivity of survivin IRES GFP ALL cells versus control GFP cells to single agents L-Asparaginase and Dexamethasone was observed in respective MTT assays.

Figure S4. *Knockdown of survivin expression in ALL cells*



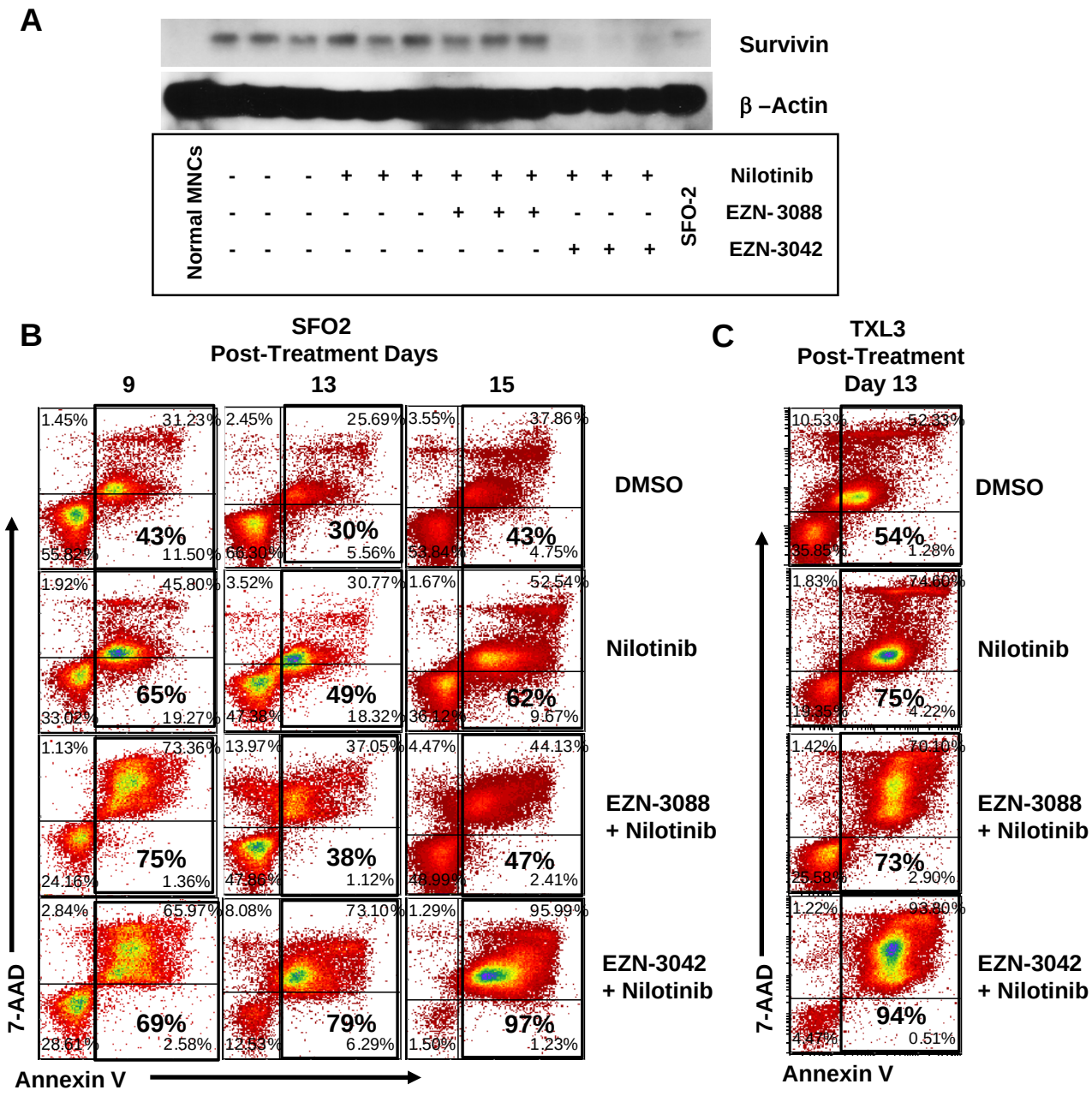
Legend: (**A**) Knockdown of survivin was performed using an inducible lentiviral shRNA vector. (**B**) Inducible knockdown by doxycycline induction was determined via RFP expression confirmation by FACS analysis. (**C**) Knockdown was quantified by qPCR and confirmed to achieve a mean 76% knockdown of survivin mRNA transcript. (**D**) Western Blot determined that survivin protein was knocked down. (**E**) CD45 BM FACS of Survivin shRNA cohort (n=3).

Figure S5. *Conditional deletion of survivin abrogates leukemia in vitro*



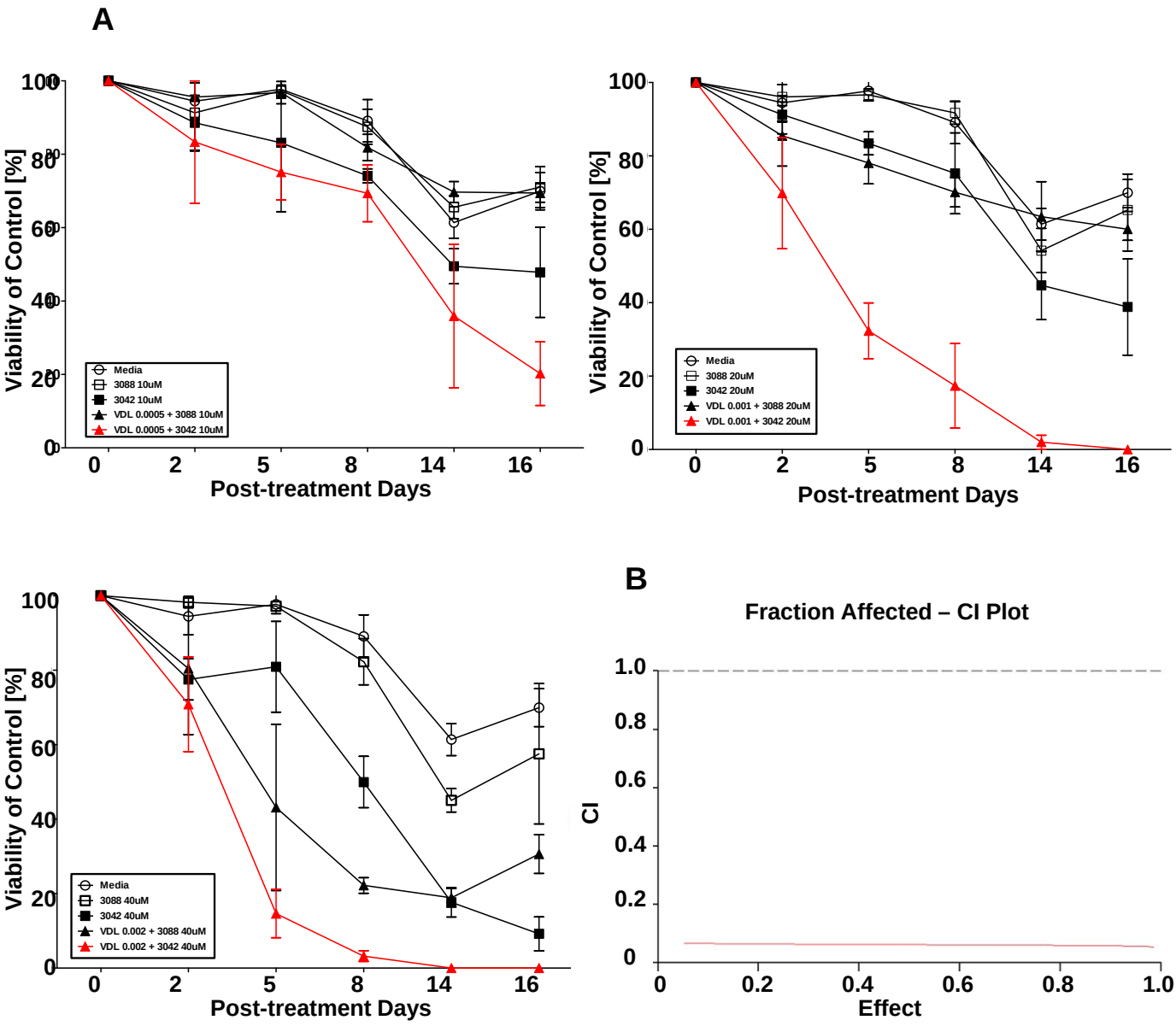
Legend: (A) Western blot confirmation of survivin-deletion using *Cre*-GFP and GFP control vectors. (**B**) *Cre*-GFP and GFP control transduced *BCR-ABL* p210 murine leukemia were monitored for GFP expression by FACS. Mean 24 hour to 120 hour growth in percent GFP positive cells was 243% and 105%, for *BCR-ABL1* controls. Mean 24 hour to 120 hour growth in percent GFP positive cells for *BCR-ABL1 Cre*-GFP transduced cells was -18%, with representative FACS plot shown for hours 24 and 120 shown, respectively. Statistical analyses at 120 hours performed using a paired student T-test.

Figure S6. *Annexin V staining of in vitro relapse model*



Legend: (**A**) Western blot analysis of survivin protein levels for treatment groups, confirmed knockdown with EZN-3042 treatment, in triplicate for each experimental condition of Ph^+ ALL sample SFO2. (**B**) SFO3 annexin V staining of respective treatment conditions for treatment days 9, 13, and 15 are shown resulted in a mean reduction in the annexin $V^-/7\text{-}AAD^-$ population by EZN-3042 + nilotinib to 1.43% ± 0.8%, compared EZN-3088 + nilotinib of 39.7% ± 8.3% of respective triplicates, on day 15 post-treatment (p-value>0.0007). (**C**) Annexin V staining of respective treatment conditions for treatment days 9 of TXL3 resulted in a mean reduction in the annexin $V^-/7\text{-}AAD^-$ population by EZN-3042 + nilotinib to 4.8% ± 0.7%, compared EZN-3088 + nilotinib of 28.3% ± 2.0% of respective triplicates, on day 13 post-treatment (p-value>0.00003). Statistical analyses performed using a student paired T-test. .

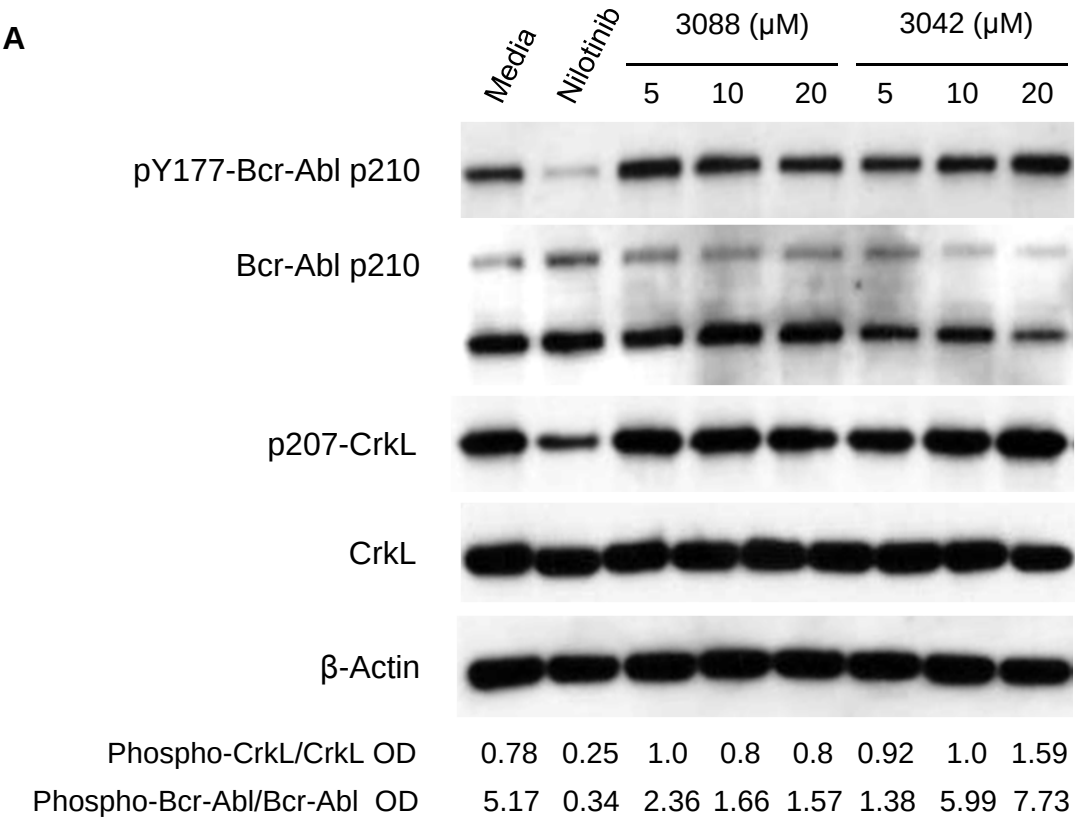
Figure S7. *Combination index (CI) for EZN-3042 + VDL*



Legend: (**A**) SFO3 was treated with media only, VDL alone. or in combination with EZN-3088 or EZN-3042. Viability was determined on days noted by trypan blue exclusion, for the various drug concentrations. (**B**) Synergistic effects of EZN-3042 plus VDL on primary human ALL cells. CI plot calculated on the basis of viability assessed by trypan blue exclusion indicating synergistic effects (C<1) of the combination of EZN-3042 plus VDL above an affected fraction F_a <0.0, for day 16 post-treatment. The CI Index for F_a = 0.5 was calculated as 0.06 ± 0.05. CI Index was calculated using CalcuSyn software application of the Chou-Talalay Method (Cambridge, United Kingdom).

Figure S8. *EZN-3042 does not interfere with BCR-ABL1 and downstream target CrkL*

A



Phospho-CrkL/CrkL OD	0.78	0.25	1.0	0.8	0.8	0.92	1.0	1.59
Phospho-Bcr-Abl/Bcr-Abl OD	5.17	0.34	2.36	1.66	1.57	1.38	5.99	7.73

Legend: SFO2 (ALL cells) were co-cultured with OP9 cells and treated with media only, single agent scrambled antisense EZN-3088 (3088) (5 – 20 μM or survivin antisense EZN-3042 (3042) (5 - 20 μM) for 6 days. Pre-treated ALL were subsequently treated with either vehicle or Nilotinib (500 nM) before cell harvesting 4 hours post TKI-treatment. Cell lysates then analyzed by (**A**) Western blot analysis for phospho-CrkL, total CrkL, phospho-*BCR-ABL1*, total *BCR-ABL1,* survivin, and β-Actin was determined. The optical density (OD) ratio of phospho-CrkL to total CrkL or phospho-*BCR-ABL1* OD to total *BCR-ABL1* OD, was calculated using Image J software (U.S. National Institutes of Health; Bethesda, MD).