Supplementary Materials

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Antibodies

For immunoblotting and immunofluorescence staining we used: rabbit antisera specific for p110β (Santa Cruz, H-198), p110α, and p110δ (gift of Bart Vanhaesebroeck), a mAb specific for p110α (Transduction Laboratories), a rabbit antiserum that recognizes all class IA PI3K regulatory isoforms (anti-pan p85, Upstate, 06-195), a mouse mAb specific for p55γ (Abcam, V2), a mouse mAb specific for p85α (Upstate, UB93-3), a purified mouse IgM mAb antibody specific for PtdIns (3,4,5)P_3 (Echelon Biosciences, Z-P345). Rabbit and mouse antibodies specific for total and phosphorylated forms of AKT (11E7 #4685, 193H12 #4058 and 587F11 #4051), FOXO1/3a (#9464), FOXO1/4 (#9461), ERK1/2 (D13.14.4E #4370 and 197G2 #4377), rS6 (61H9 #4838, 2F9 #4856, and #2211), and 4eBP-1 (53H11 #9644 and #9451) were from Cell Signaling Technology. For flow cytometry we used: anti-human CD8 APC, anti-human CD4 AF647 (BD biosciences), anti-human CD19 FITC, anti-human CD20 FITC, anti-human CD14 PE or APC, anti-human CD16 PE or APC, anti-human CD33 FITC or APC, anti-human CD34 PE or APC, anti-human CD38 FITC, anti-human CD11b APC, anti-human CD11c APC, and mIgG1 isotype controls (Caltag), and anti-CD19 PE, anti-B220 APC or APC-AF750, anti-CD43 PE, anti-CD25 PC7, anti-CD24 PC5, anti-BP-1 FITC or PE, anti-IgM PC5.5 or PC7, anti-IgD PE, anti-CD11b APC, anti-GR1 PC7, anti-CD3 PC5.5,

anti-CD34 APC, anti-Sca1 PC7, anti-CD117 PE, anti-Thy1.1 PE, anti human CD4 AF488 or Pacific blue (eBioscience).

Retroviral vectors

pMIG (MSCV-IRES-GFP) was provided by R. Hawley (American Red Cross), pMIT (MSCV-IRES-Thy1.1) by P. Marrack (National Jewish Hospital), pMIC (MSCV-IRES-human CD4) by Jason Cyster (UCSF). pMSCV-IRES-FOXO3a/ FOXO3a.A3-Thy1.1, were constructed as previously described (1). pMSCV-Cre-IRES-hCD8 and empty vector was a generous gift provided by Patrick Stern Hynes (Harvard). High-titer helper-free retrovirus stocks were prepared by transient co-transfection of 293T cells with indicated vectors and ψ^- ecotropic packaging vector and used as previously described (1, 2).

Expansion and infection of leukemic cell lines

After 7 days of culture, single colonies were transferred to 48 well plates containing RPMI20. Leukemic-colony forming cells (L-CFC) were expanded to 24 well plates on day 2 of liquid culture, 12 well plates on day 4-6 of liquid culture, 6 well plates on day 6-10 of liquid culture. Established cultures of WT, β null, and α null L-CFC were later switched to 10% FCS (RPMI10) for long-term passaging. α/β null L-CFC cultures were consistently maintained in RPMI20. All L-CFC were cultured in fresh RPMI20 ≥12 hr prior to and during all in vitro studies unless specifically stated. For studies involving retroviral transduction of established p190 L-CFC, 2 x 10^6 cells in 1.5 ml of culture

medium were mixed with 1.5 ml viral supernatant with 8 μg/ml polybrene and spinoculated in 6 well plates at 37°C for 45 min. at 450 x *g*. After incubation overnight at 37°C, 5% CO_2, cells were analyzed by flow cytometry as indicated and/or expanded into fresh culture medium.

Culture Media

RPMI20 media contained: RPMI1640, 20% heat-inactivated FCS, with 1 mM Na Pyruvate, 2 mM glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml), gentamicin (50 μg/ml), 50 μg/ml β-mercaptoethanol, and 5 mM HEPES additives (Hyclone).

Amino Acid Starvation

p190 L-CFC maintained in RPMI20 were washed twice with ice-cold HBSS (no Ca^{2+}/ Mg^{2+}; Hyclone) and were starved of leucine by resuspension in pre-heated (37°C) leucine-free RPMI 1640 media (MP Biomedicals) containing 10% dialyzed FCS (Hyclone) and supplemented with 1 mM Na Pyruvate, 2 mM glutamine, 50 *μ*g/ml β-mercaptoethanol, and 5 mM HEPES additives (Hyclone) for the indicated amount of time. Control conditions consisted of RPMI1640 with matched 10% dialyzed FCS containing the same supplemental additives as above.

Immunoblotting

Cells were washed in 1x PBS, and whole cell lysates were created by lysing 2- to 4 x 10^6 cells for all signaling studies and 1 x 10^6 cells for deletion assessment in ice cold

RIPA lysis buffer (120 mM NaCl, 1% Triton X-100, 40 mM HEPES (pH 7.4), 1 mM Na_2EDTA, 10mM Na Pyrophosphate, 10 mM Na glycerophosphate, 50mM NaF, 0.5mM Na_3VO_4, supplemented with phosphatase inhibitor cocktails I and II (Sigma), and protease inhibitor cocktail (Sigma)) for 20 min (vortex every 5 min) on ice. Lysates were subsequently spun at 16K x *g* for 10 min. Supernatant was resuspended in 2X sample buffer and boiled for 15 min. Lysates corresponding to equal cell numbers were electrophoresed and transferred to nitrocellulose at 4°C.

Apoptosis and cell cycle flow cytometry

For apoptosis or nuclear visualization, DAPI and 7-AAD (Invitrogen) was used, and annexin V PE or APC (Caltag). For cell cycle analysis, single cell suspensions were fixed in ice-cold 70% ethanol dropwise and kept at -20°C overnight to be subsequently stained with propidium iodide using standard methods. All data analysis was calculated using FlowJo software (Treestar) and for quantitation of DNA content the Watson pragmatic model was used. For PKH26 labeling, single cell suspensions were washed twice with 1X PBS and labeled with 2 μM PKH26 (Sigma) for 10 min at RT. Cells were subsequently washed three times with RPMI10 and analyzed by flow cytometry. EdU (5-ethynyl-2'-deoxyuridine) incorporation was determined in human CD4+ p190 cells with Click-iT EdU flow cytometry assay kit (Invitrogen) using the AF647 azide and propidium iodide components as per manufacturer's instructions.

EdU detection

BM was collected and lysed of RBCs, and incubated with Fc block (BD Biosciences) 5 min prior to staining with anti-human CD4 AF488. Cells were subsequently fixed with Click-iT fixative and permeabilized with Click-iT saponin-based reagent. To detect EdU incorporation cells were stained with Click-iT reaction cocktail, washed, and subsequently prepared for DNA content analysis with ribonuclease A and propidium iodide.

Detection of PtdIns(3,4,5)P_3 formation by immunofluorescence microscopy

p190 L-CFC maintained in RPMI20 were plated onto poly-L-lysine-coated 8 well chamber slides and fixed with 4% paraformaldehyde (PFA) in TBS (150 mM NaCl, 10 mM Tris-HCl, pH 7.5). Treated cells were incubated with 50 nM wortmannin for the indicated times prior to fixation. Cells were washed and permeabilized with saponin solution (0.2% saponin, 2% FBS, and 1% BSA, in TBS, pH 7.5) at RT for 30 min. Cells were blocked with 10% BSA and 5% goat serum for 1 hr and subsequently stained with anti-PIP3 (PtdIns(3,4,5)P_3) antibody (1:100, Z-P345) in TBS and incubated at 4°C overnight. Cells were washed and subsequently stained with rhodamine (TRITC)-labeled goat anti-mouse IgM antibody (1.5 μg/ml, Jackson ImmunoResearch Labs) at RT for 30 min. Slides were mounted with VECTASHIELD (Vector Laboratories) containing 0.3 μg/ml DAPI and placed on coverslips. Immunofluorescence images were generated using a Zeiss Axioskop (NA= 0.55) using the AxioVision camera and software (Carl Zeiss) and images were exported and manipulated in Photoshop CS or CS2 (Adobe Systems).

In vivo transplant studies

Animals were monitored daily for signs of illness. Recipient mice were euthanized based on at least one of the following criteria: markedly elevated eGFP+ or human CD4+ white blood cell counts, weight loss, lack of mobility, prominent splenomegaly, or palpable peri-ocular lymphomas encompassing the skull. To determine disease burden, the BM (femur and tibias) was flushed and spleens disaggregated, and following RBC lysis, the numbers of eGFP+ or human CD4+ cells in the marrow and spleen were assessed for immunophenotype by flow cytometry. Cells from leukemic animals were expanded in RPMI20 and further characterized by flow cytometry and immunoblotting as described (Figure 4 and Supplementary Figure 3). Drug trials contained six treatment arms (5 mice each): imatinib (70 mg/kg b.i.d.), rapamycin (7 mg/kg per day), imatinib (70 mg/kg b.i.d.) plus rapamycin (7 mg/kg per day), PI-103 (40 mg/kg b.i.d.), imatinib (70 mg/kg b.i.d.) plus PI-103 (40 mg/kg b.i.d.), and double placebo. A 2 day treatment regimen began when animals established disease (11 days post transplant). Mice received 1.125 mg of EdU (resuspended in sterile 0.9% saline) i.p. 2 hr prior to sacrifice. Imatinib (100 mg tablet; Novartis), was crushed, suspended in diH_2O, and was administered i.p. every 12 hr. Rapamycin (LC laboratories), resuspended in 75% DMSO/ 25% saline, was prepared fresh prior to injection and administered i.p. every 24 hr. PI-103, resuspended in 75% DMSO/ 25% saline, was prepared fresh prior to injection and administered i.p. every 12 hours. Apoptosis, EdU incorporation, and

numbers of hCD4+ cells in the BM (femurs and tibias), spleens, and peripheral blood, were assessed by flow cytometry following the 2 day treatment regimen.

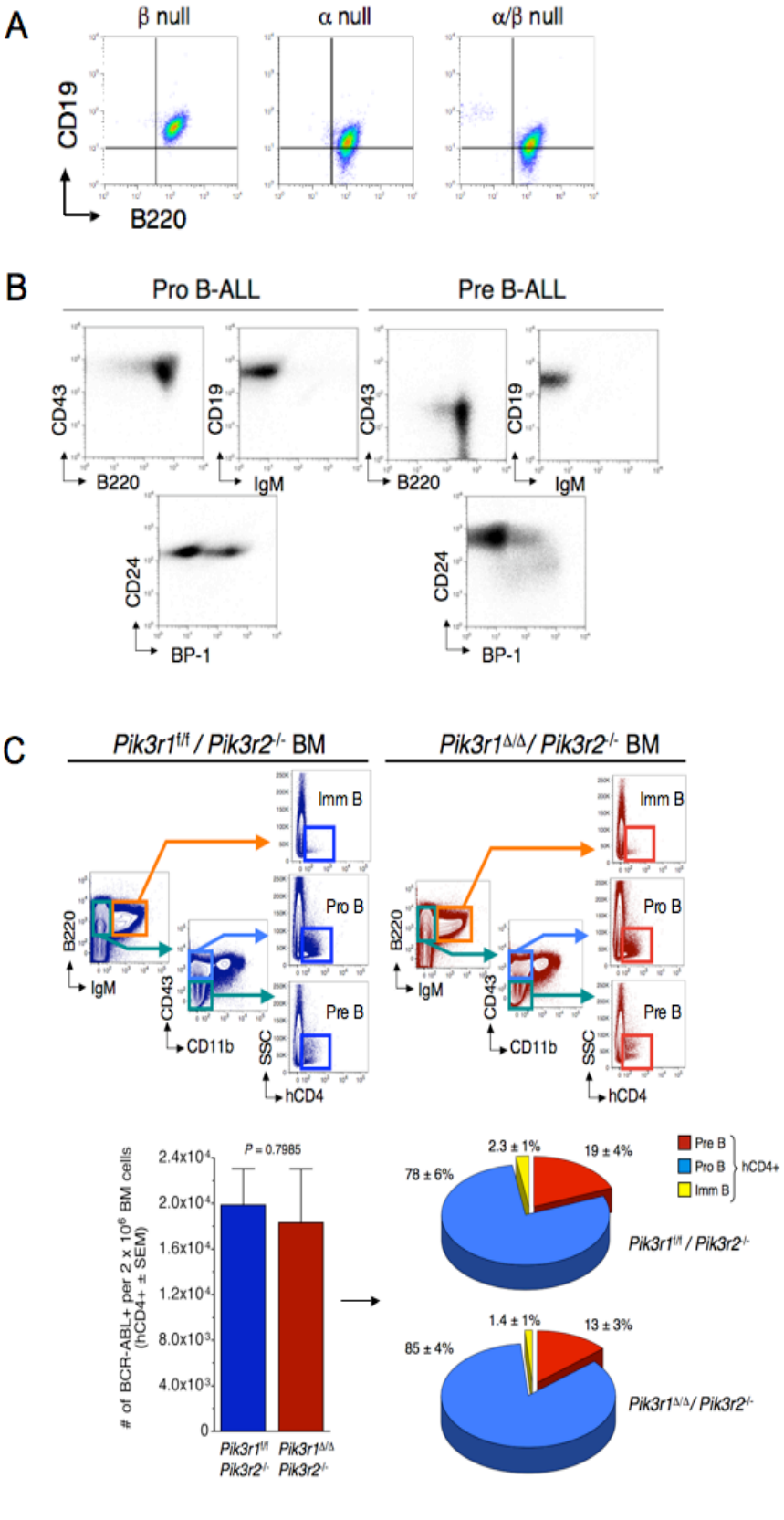
In vitro drug combination studies

p190 L-CFC (generated from M3630 clonal cultures of 3-wk-old Balb/c BM; 2 x 10^4 cells) were incubated in triplicate wells of 96-well culture plates for 48 hr in the presence of increasing concentrations of imatinib, rapamycin, and PI-103. Cell viability and median-effect dose (IC_{50}) was determined using the MTS assay (Cell Titer 96 AQueous One solution cell proliferation assay kit; Promega) where cells were reacted with 20 _l MTS/PMS solution at 37°C, 5% CO_2 for ~1 hr after which absorbance was read at 490 nm. Absorbance values were normalized to vehicle control (0.2% DMSO) treated cells and expressed as % cell viability. A range of fixed ratios of drug combinations was employed to assess synergy using the combination index (CI) with CalcuSyn software (Biosoft) using the median-effect method. The ranges of CI are the following: <0.1 very strong synergism, 0.1-0.3 strong synergism, 0.3-0.7 synergism, 0.7-0.85 moderate synergism, 0.85-9.0 slight synergism, 0.90-1.10 nearly additive, 1.10-1.20 slight antagonism, 1.20-1.45 moderate antagonism, 1.45-3.3 antagonism.

Human Leukemia assays

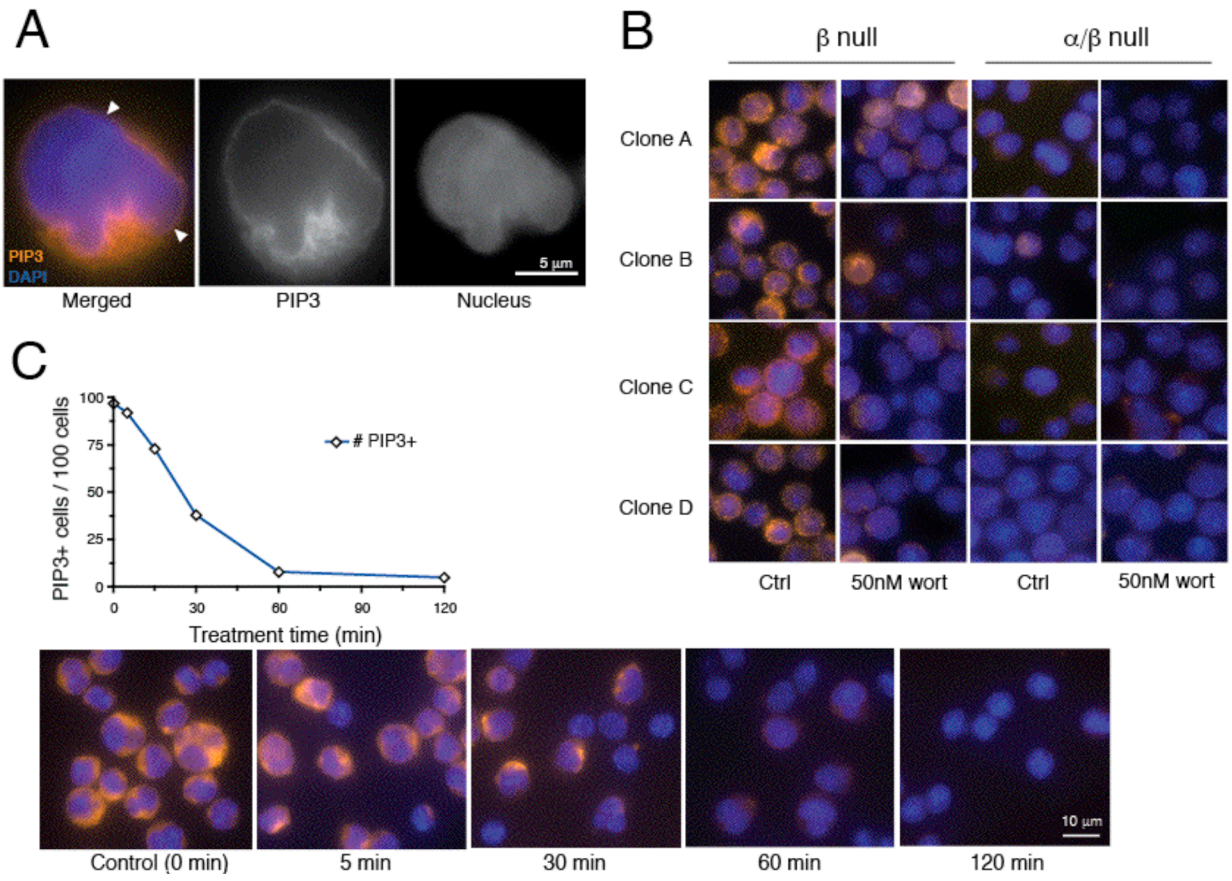
Human leukemia cells were thawed and washed with IMDM containing 10% FCS and Ficoll-Paque Plus (GE Healthcare) separated and/or followed by isolation of the CD34+CD19+ fraction by using the CD19 MultiSort kit and/or CD34 microbeads

(Miltenyi Biotec) and grown and plated before being used in assays. Magnetically sorted leukemic progenitors were kept overnight in IMDM supplemented with 30% FCS, 2mM glutamine, 5mM HEPES, penicillin (100 U/ml), streptomycin (100 _g/ml), and human recombinant cytokines (Peprotech): IL-3 (30 ng/ml), IL-7 (100 ng/ml), Flt-3 ligand (100 ng/ml), SCF (30 ng/ml) at 3.3 x10^6 cells/ml. Following the resting period, leukemic progenitors (3 x 10^4 to 7 x 10^4 cells) were plated in complete 0.9% MethoCult GF+ H4435 (Stem Cell Technologies) supplemented with recombinant human cytokines for lymphoid promotion and myeloid support as follows: IL-3 (100 ng/ml), IL-6 (20 ng/ml), GM-CSF (20 ng/ml), G-CSF (20 ng/ml), IL-7 (100 ng/ml), Flt-3 ligand (30 ng/ml), SCF (100 ng/ml), and EPO (3 U/ml). Methycellulose colony formation was assayed jn 48-well plates, each containing 350 μl of complete methylcellulose with or without indicated concentrations of inhibitors. Colonies (>100 μm) from primary cells were scored 12 to 14 days later where indicated. For cytologic analysis, unsorted and post-sorted cells were centrifuged onto slides using a Shandon Cytocentrifuge 4 (Thermo Scientific), fixed in methanol and stained with May-Grunwald/Giemsa (Sigma). Light microscopy pictures were captured on an Olympus BX60 using a 40x objective.


A
β null
α null
α/β null
CD19
B220
B
Pro B-ALL
Pre B-ALL
CD43
CD19
B220
IgM
CD24
BP-1
C
Pik3r1f/f / Pik3r2-/- BM
Pik3r1Δ/Δ/ Pik3r2-/- BM
Imm B
Pro B
Pre B
B220
IgM
CD43
CD11b
SSC
hCD4
# of BCR-ABL+ per 2 x 10^6 BM cells (hCD4+ ± SEM)
P = 0.7985
2.4x10^4
2.0x10^4
1.6x10^4
1.2x10^4
8.0x10^3
4.0x10^3
0
Pik3r1f/f Pik3r2-/-
Pik3r1Δ/Δ Pik3r2-/-
2.3 ± 1%
19 ± 4%
78 ± 6%
Pre B
Pro B
Imm B
hCD4+
Pik3r1f/f / Pik3r2-/-
1.4 ± 1%
13 ± 3%
85 ± 4%
Pik3r1Δ/Δ/ Pik3r2-/-

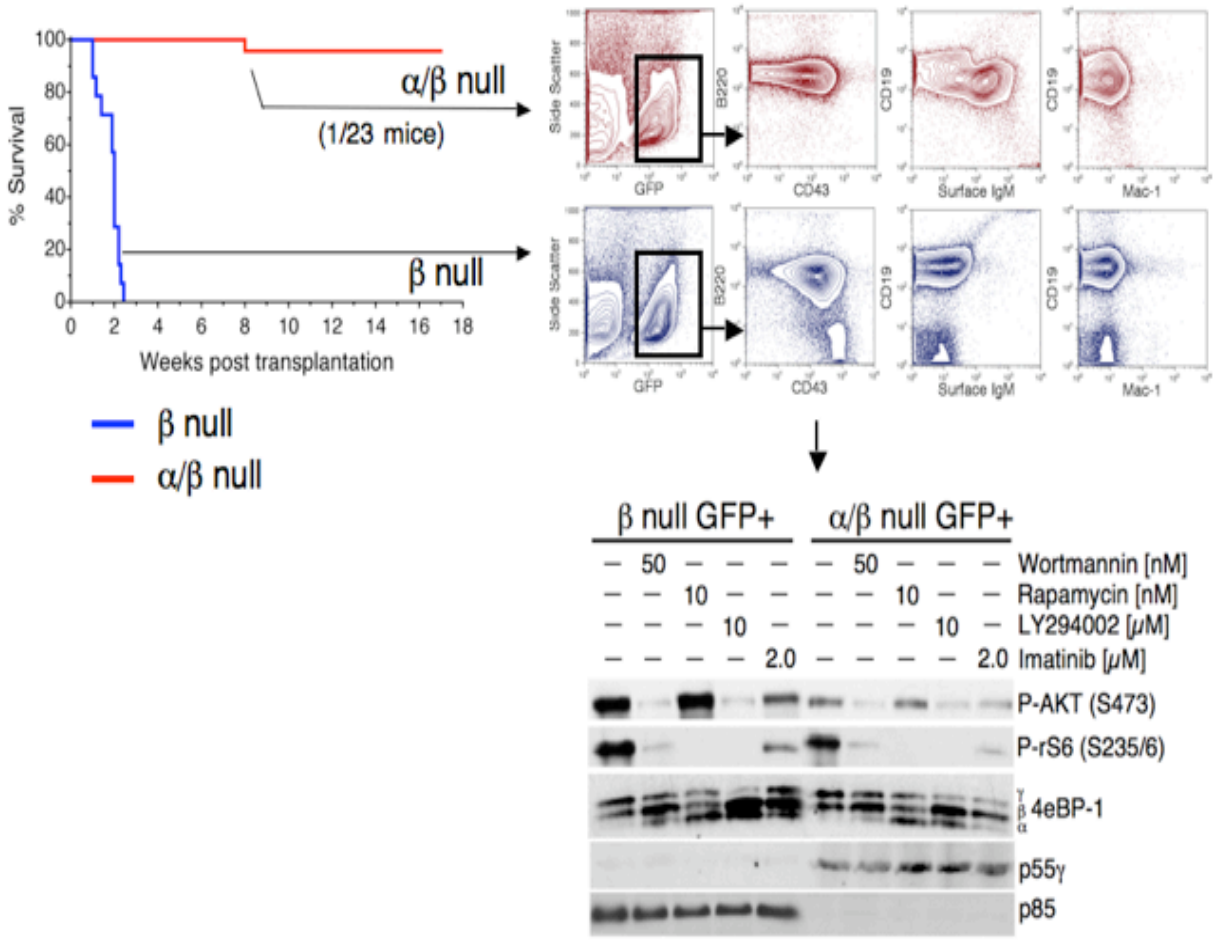
Supplemental Figure 1

Immunophenotype of p190 L-CFC. (**A**) p190 L-CFC in expansion phase were stained for CD19 and B220 expression. Cells were analyzed by flow cytometry. n= 5. The CD19-Cre mice have Cre knocked into the CD19 locus, such that the mice express one intact copy of the *CD19* gene and a lower level of CD19 protein on B lineage cells. This results in intermediate expression on α null and α/β null L-CFC relative to β null pro/pre-B cells. (**B**) Representative immunophenotypes of two groups of pro-B and pre-B-ALL observed in L-CFC from M3630 cultures, with no apparent distinctions in growth or signaling (data not shown). (**C**) B lymphoid subsets and target of leukemic transformation in infected cultures. The BM from 3-wk-old mice of indicated genotypes was spinoculated with p190 (MSCV-BCR-ABL-IRES-hCD4) and plated overnight in RPMI20 containing IL-7 (10 ng/ml). The BM was subsequently analyzed by flow cytometry 14 hr post infection. Total numbers and immunophenotype of p190 infected cells (hCD4+) are enumerated as indicated. Immunophenotype of hCD4+ cells are represented (mean ± s.d.): pro-B (B220+CD43+IgM-), pre-B (B220+CD43-IgM-), immature-B (B220+IgM+). Target of leukemic transformation arises predominantly from the pro-B fraction, with no significant differences in total hCD4+ events or immunophenotype across genotypes. n= 3 mice.



Supplemental Figure 2

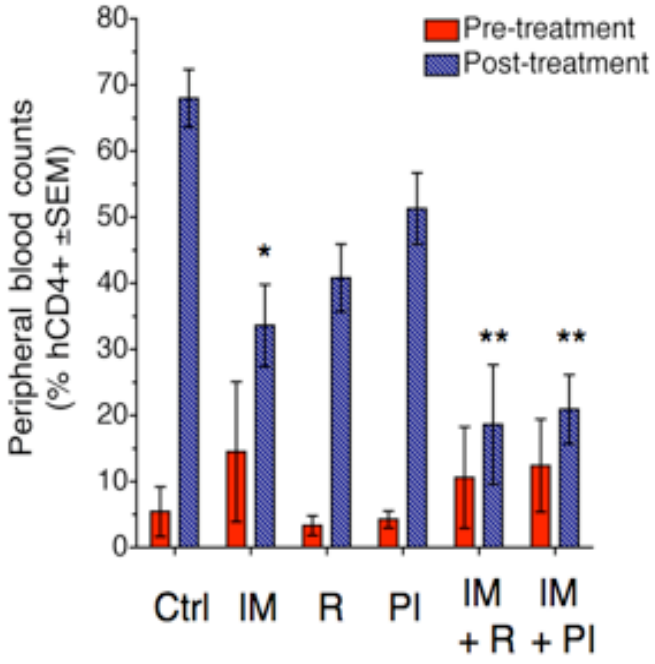
α/β-null L-CFC have severe defects in PtdIns(3,4,5) production. (A) Control β-null cells are distinguished by PIP3+ immunostaining in the inner edge of the plasma membrane, inside cytoplasmic puncta, and in areas circling the nuclear membrane (arrowheads). (B) Multiple clones (distinguished as A-D) were assessed for PIP3 production. Cells were treated with or without wortmannin (50 nM) 30 min prior to fixation. After fixation, β-null and α/β-null L-CFC were immunolabeled with anti-PIP3 antibodies (orange). Nuclei were stained by DAPI (blue). (C) Control p190 cells were treated with wortmannin (50 nM) over a time course of 5 min to 2 hr. Cells were visualized with 40X (for quantitation of % positive cells), 63X (shown, B), and 100X (shown, A) objectives.



Supplemental Figure 3

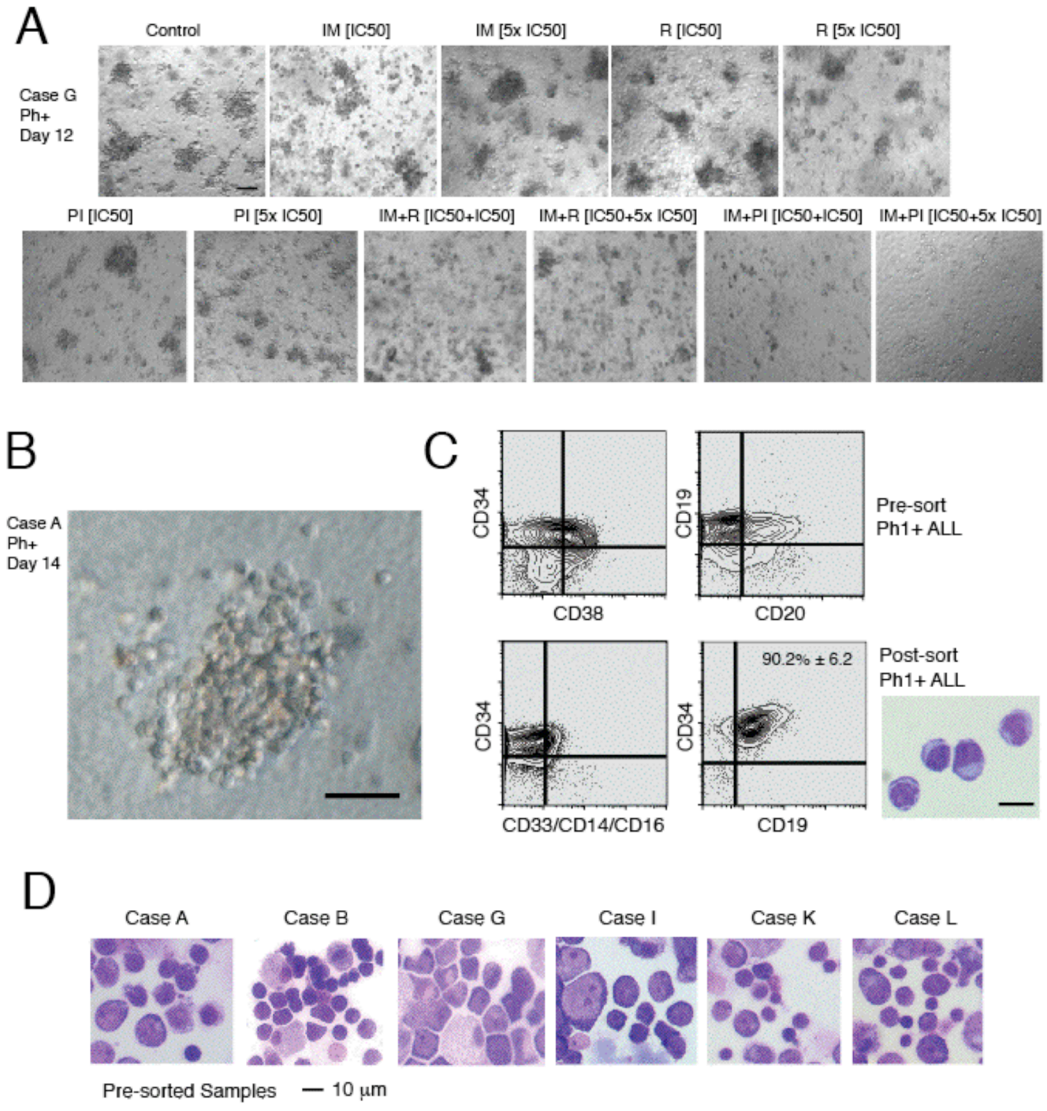
Emergent α/β-null leukemic blasts displayed detectable p-AKT. (**A**) A single mouse (1/23) injected with α/β null L-CFC developed B-ALL that retained complete deletion of *Pik3r1* gene products. 5/23 mice emerged with mixtures of pre/pro-B and pro-B lymphoblasts that were non-deleted (p85α+) by immunoblot analysis of pure p190 cells recovered from the BM and spleen. 17 mice were disease free (≤ 2% eGFP+ or human CD4+ cells) upon inspection of the BM at the cessation of the Kaplan-Meier survival

analysis (see also *Supplementary Table 1*). All emergent β null p190 blasts from the spleen, BM, and LNs lacked maturation markers and had a pro/pre-B lymphoblastic phenotype (CD19+, B220+, CD43+/-, IgM-, and IgD-) whereas the α/β-null cells from the single mouse displayed surface Ig+ B-ALL like disease (B220+, IgM+, IgD-, and CD43+/-). We did not observe any significant variation of cell morphology from spleen and BM cytospins of the α/β-null injected mice compared to the β−null injected mice. (**B**) Emerging α/β-null leukemic blasts were purified from the BM after ex vivo passages until proper purity was established (≥ 98% eGFP+, 5-7 days ex vivo passaging in culture media (20% FCS), see *Methods*). The pure eGFP+ cells were assessed for p85 expression and AKT/mTOR signaling by immunoblot analysis. Note the detectable p-AKT with concomitant upregulation of p55γ.



Supplemental Figure 4

In vivo investigation of combining PI-103 or rapamycin with imatinib. Peripheral blood was collected from mice at 10 days post transplantation (d.p.t.) with p190 L-CFC and at 13-14 d.p.t. (after 48 hr period of treatment, see *Methods*) at the time of sacrifice. Peripheral blood was lysed of RBCs and stained for human CD4 and B220. **P* <0.01, ***P* <0.001 (comparing post treatment Ctrl vs drug); 1-way ANOVA, mean % B220+ human CD4+ events ± SEM is reported as shown, n= 5 mice per treatment group.



Supplemental Figure 5

Morphology of Ph1+ leukemic progenitors before and after treatment. (A) Colonies from case sample G were analyzed by light microscopy for all indicated treatment conditions 14 days post plating in methylcellulose (100 μm scale bar). Similar results were observed in other Ph1+ samples. (B) Leukemic CFU-pre B scored on days 12-14 from

all cases exhibited lymphoid morphology (case sample A is depicted; 50 μm scale bar). (C) CD34+CD19+ leukemic progenitors were magnetically sorted from ficoll preparations of collected peripheral blood from case samples. All samples used for clonogenic potential were CD34+, CD38-/+, CD19+ and with some cases displaying low myeloid marker expression in tandem with CD19. Representative immunophenotype from pre-sorted and post-sorted samples is depicted. All post-sorted samples exhibited lymphoid morphology as depicted (10 μm scale bar). (D) May-Grunwald/Giemsa stained cytospin preparations from unsorted peripheral blood of indicated patient samples are shown.

Supplementary References

1. Kharas, M.G., Deane, J.A., Wong, S., O'Bosky, K.R., Rosenberg, N., Witte, O.N., and Fruman, D.A. 2004. Phosphoinositide 3-kinase signaling is essential for ABL oncogene-mediated transformation of B-lineage cells. *Blood* **103**:4268-4275.
2. Wong, S., McLaughlin, J., Cheng, D., and Witte, O.N. 2003. Cell context-specific effects of the BCR-ABL oncogene monitored in hematopoietic progenitors. *Blood* **101**:4088-4097.

Supplementary Table 1.

L-CFC injected (clone)	Leukemic occurrence	B-ALL immunophe-notype	p85 status of p190 cells	Observed time post-transplantation (Days)	% p190+ in BM / spleen
WT (A)	Yes	Pro > Pre	WT	8	45 / 60
WT (A)	Yes	Pro > Pre	WT	8	28 / 35
WT (A)	Yes	Pro = Pre	WT	21	52 / 65
WT (A)	Yes	Pro > Pre	WT	23	35 / 36
α null (A)	Yes	Pro = Pre	NA	15	46 / 35
α null (A)	Yes	Pro = Pre	NA	37	50 / 51
α null (A)	Yes	Pro > Pre	NA	37	53 / 55
α null (A)	Yes	NA	NA	32	NA
β null (A)	Yes *	Pro	β null	7	10 / 15
β null (A)	Yes *	Pro > Pre	β null	11	80 / 88
β null (A)	Yes *	Pro < Pre	β null	12	78 / 56
β null (A)	Yes *	Pro > Pre	β null	12	72 / 75
β null (A)	Yes *	Pro < Pre	β null	14	79 / 70
β null (A)	Yes *	Pro < Pre	β null	14	67 / 54
β null (A)	Yes *	Pro < Pre	β null	14	75 / 62
β null (A)	Yes *	Pro < Pre	β null	17	75 / 72
β null (B)	Yes *	Pro	β null	7	38 / 46
β null (B)	Yes	Pro	β null	10	42 / 68
β null (C)	Yes *	Pro > Pre	β null	13	53 / 78
β null (C)	Yes *	Pro	β null	13	60 / 73
β null (D)	Yes	Pro > Pre	β null	15	48 / 61
β null (D)	Yes *	Pro > Pre	β null	16	75 / 84
β null (E)	Yes	Pro > Pre	β null	16	63 / 61

β null (E)	Yes	Pro > Pre	β null	16	74 / 82
α/β null (A)	Yes	Pro > Pre	β null	56	82 / 56
α/β null (A)	Yes *	Pro	β null	56	63 / 60
α/β null (A)	Yes	sIgM >> Pre	α/β null	56	62 / 67
α/β null (A)	Yes	Pro > Pre	β null	87	59 / 60
α/β null (A)	No	Pro	NA	87	0.3 / 0
α/β null (A)	No	Pro	NA	87	0.6 / 0
α/β null (A)	No	Pro	NA	92	0.5 / 0
α/β null (A)	No	Pro	NA	93	0.1 / 0
α/β null (A)	Yes	Pro	β null	93	22 / 20
α/β null (A)	Yes	Pro	β null	93	56 / 44
α/β null (B)	No	NA	NA	119	0.1 / 0
α/β null (B)	No	NA	NA	119	0.1 / 0.1
α/β null (B)	No	NA	NA	119	0.2 / 0.1
α/β null (B)	No	NA	NA	119	0.1 / 0
α/β null (C)	No	NA	NA	122	0.5 / 0
α/β null (C)	No	NA	NA	122	0.4 / 0.1
α/β null (C)	No	NA	NA	122	0.3 / 0
α/β null (C)	No	NA	NA	122	0.8 / 0.2
α/β null (D)	No	NA	NA	126	0 / 0
α/β null (D)	No	NA	NA	139	0 / 0
α/β null (D)	No	NA	NA	139	0 / 0
α/β null (E)	No	NA	NA	139	0.1 / 0
α/β null (E)	No	NA	NA	139	0.1 / 0

Detailed Kaplan-Meier survival analysis.

Characteristics of NOD-SCID mice injected with indicated genotypes of p190 L-CFC are described above with individual clones designated (A, B, C, etc.). Mice that were

euthanized with excessive leukemic disease burden (as described in Methods section) that was confirmed by flow cytometry (eGFP+ or human CD4+) were reported as 'yes'. Mice that lacked any disease burden or showed minimal residual disease (< 2% eGFP+) at the end of observation were reported as 'no' in the leukemic occurrence column (* indicates mice that had severe ocular lymphomas near cell injection site). Immunophenotype of the eGFP+ or human CD4+ events are reported in the B-ALL immunophenotype column (pro-B-ALL= CD43+ B220+ IgM-; pre-B-ALL= CD43- B220+ IgM-; sIgM+ B-ALL= CD43- B220+ IgM+). The p85 expression status of p190 cells from the BM was determined by immunoblotting as described in *Supplemental Figure 3*.

Supplementary Table 2

Sample #	Age	Prior Rx	Disease Status**	Cytogenetics	Diagnosis	Immunophenotype
A	15	Chemo, imatinib	R2	t(9;22); trisomy 8; t(3;10)	Ph+ ALL	CD34+ CD19+ CD20+/- $CD11b^{low}$ $CD33^{low}$
B	63	Chemo, imatinib	R2	t(9;22); der(7;12)	Ph1+ ALL	$CD34^{hi}$ CD19+ CD20+/- $CD14^{low}$ CD11c+/-
C	28	Chemo	R3	NA	Pre-B ALL	CD34+/- CD19+ CD20+/-
G	30	None	N	t(9;22)	CML, lymphoid BC*	CD34+/- CD19+/- CD20+/- CD11b+/- CD14+/- CD16+/- CD33+/- CD11c+/-
H	NA	NA	NA	t(9;22)	Ph+ ALL	$CD34^{hi}$ CD19+ $CD11b^{low}$
I	42	None	N	t(9;22)	Ph+ ALL	$CD34^{hi}$ $CD19^{low}$
K	23	Chemo	R4	Hyperdiploid with structural anomalies	Pre-B ALL	$CD34^{low}$ CD19+ CD20+ CD11b+ $CD13^{low}$ CD33+ CD11c+
L	22	Chemo	R3	Normal	Pre-B ALL	CD34+ CD19+ CD20+/- $CD11b^{low}$ $CD13^{low}$ CD33+

**N = new diagnosis, R1 = 1^{st} relapse, R2 = 2^{nd} relapse, etc.
*BC = blast crises
NA = not available