# Supplementary Information

## FAK mediates a compensatory survival signal parallel to PI3K-AKT in *Pten*-null T-ALL cells

Dewen You,[1*] Junping Xin,[1] Andrew Volk,[1,4] Wei Wei,[1] Rachel Schmidt,[1] Gina Scurti[1,3], Sucha Nand,[5] Eun-Kyoung Breuer,[1,7] Paul C Kuo,[1,3] Peter Breslin,[1,8,9] Ameet Kini,[1,2] Michael I Nishimura[1,3], Nancy J Zeleznik-Le[1,4,6], and Jiwang Zhang[1,2,4*]

# Experimental Procedures

**Mice and drug treatments:** *Fak*$^{fx/fx}$ mice (B6.129-*Ptk2*$^{tm1Lfr}$/Mmcd, Stock Number: 009967-UCD) were purchased from Mutant Mice Regional Resource Center (MMRRC). *Pten*$^{fx/fx}$ mice were kindly provided by Dr. Tak W. Mak of the Campbell Family Institute for Breast Cancer Research (University of Toronto, Toronto, Canada), and were maintained in our laboratory as *Mx-1-Cre*$^{+}$*Pten*$^{fx/+}$ (Zhang et al., 2011). All mice were maintained according to the standards set forth in the National Institutes of Health Guidelines for the Care and Use of Animals in the animal facility at Loyola University Chicago in a C57BL/6 background. All experiments performed on these animals were approved by Loyola University's Institutional Animal Care and Use Committee. *Fak*$^{fx/fx}$ mice were crossed with *Mx-1-cre*$^{+}$*Pten*$^{fx/+}$ mice to obtain *Mx-1-Cre*$^{+}$*Pten*$^{fx/+}$*Fak*$^{fx/+}$ mice. We backcrossed *Mx1-Cre*$^{+}$*Pten*$^{fx/+}$*Fak*$^{fx/+}$ mice to generate *Mx-1-Cre*$^{+}$*Pten*$^{fx/fx}$*Fak*$^{fx/fx}$ mice and their corresponding littermate controls. Mouse genotypes were confirmed by PCR. Primers used for mouse genotyping are listed in Table S1. At 4~5 weeks of age, mice were injected with pI:C (polyinosine-policytidine) at a dose of 25 μg/g to induce target gene(s) deletion using either a single injection or injection every other day for a total of five

injections as indicated in the text. After a single pI:C injection to *Pten*$^{-/-}$ and *Pten*$^{-/-}$*Fak*$^{-/-}$ mice, we repeatedly checked the PB of these mice for T-ALL development. Most mice showed obvious diseased phenotypes when there were ≥ 20,000 μl$^{-1}$ WBC and above 20% blasts in their PB. We initiated injections to these mice with inhibitors until they reached 35,000-60,000 μl$^{-1}$ WBC (**Fig.5 H**), and above 30% blasts (**Fig. S2F, G**) in their PB. We injected these T-ALL mice with vehicle, or a pan-PI3K inhibitor LY294002 (LY, Sigma) by intraperitoneal (i.p.) injection at 40 mg/kg every other day (Bondar et al., 2002; Hu et al., 2002; Nagata et al., 2004), or GDC-0941 (GDC, APExBIO) orally at 75 mg/kg daily (Folkes et al., 2008), or an mTOR inhibitor KU0063794 (KU, Sigma) at 30 mg/kg by i.p. injection every other day until analysis, or amaximum of 15 injections. GDC was dissolved in 10% DMSO, 5% Tween-20 (Sigma), 85% water. LY and KU were dissolved in 5% Tween-80 (Sigma) and 5% PEG-400 (Hampton Research).

**Table S1. Primers used for mouse genotype analysis**

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|---|
| *Fak*-P1: 5'-GAGAATCCAGCTTTGGCTGTTG-3' |
| *Fak*-P2: 5'-GAATGCTACAGGAACCAAATAAC-3' |
| *Fak*-P3: 5'-GACCTTCAACTTCTCATTTCTCC-3' |
| *Fak*-P1 + *Fak*-P2 produce a 290bp *WT* band and 400bp *fx* band |
| *Fak*-P1 + *Fak*-P3 produces a 327bp deleted (*Δ*) *Fak* band |
| *Pten*-P1: 5'-CTCCTCTACTCCATTCTTCCC-3' |
| *Pten*-P2: 5'-ACTCCCACCAATGAACAA AC-3' |
| *Pten*-P3: 5'-GTCACCAGGATGCTTCTGAC-3' |
| *Pten*-P4: 5'-ACTATTGAACAGAATCAACCC-3' |
| *Pten*-P1 + *Pten*-P2 produce a 228bp *WT* band and 335bp *fx* band |
| *Pten*-P3 + *Pten*-P4 produces a 500bp deleted (*Δ*) *Pten* band |

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|---|
| *Cre*-P1: 5'-TACCTGGAAAATGCTTCTGT-3' |
| *Cre*-P2: 5'-TGATCTCCGGTATTGAAACT-3' |
| *Cre*-P1+ *Cre*-P2 produces an 810bp band. |

**Cell culture and inhibitor treatment:** Thymocytes that were recovered from all genotypes of mice were adapted in RPMI-1640 (SH30027. 01, Hyclone Laboratories Inc., Thermo Scientific) supplemented with 10% FBS (16000, GIBCO, Invitrogen), penicillin/streptomycin (100 units/ 100 μg/ml), 2 mM glutamine, 50 μM β-mercaptoethanol, 10 mM Heps, 1 mM MEM sodium pyruvate (11360-070, GIBCO, Invitrogen), 1:100 MEM nonessential amino acids (11140-050, GIBCO, Invitrogen), and 100 ng/ml IL-2 (BD Bioscience). Cells were cultured under suspension condition (without feeder layers or ECM culture), or on OP9 feeder layers (OP9), or on MatriGel (MG, BD Bioscience)-, collagen (CLG, GIBCO)-, or fibronectin (FN, sigma)-coated plates at 37°C in a 5% $CO_2$ humidified incubator. Primary murine and human T-ALL cell lines, including JURKAT (Shan et al., 2000), CCRF-CEM, HPB-ALL, LOUCY, KOPT-K1, and MOLT-4 were cultured in RPMI-1640 medium supplemented with 10% FBS, and penicillin/streptomycin (100 units/100 μg/ml) under suspension condition (without feeder layers or ECM culture), or on OP9 feeder layers (OP9), or on MatriGel (MG, BD Bioscience)-, collagen (CLG, GIBCO)-, or fibronectin (FN, sigma)-coated plates at 37°C in a 5% $CO_2$ humidified incubator. Only CUTLL1 cells were cultured with20% FBS (Palomero et al., 2006). As previously reported (Ragni et al., 1984), IL-2 was added to primary cell culture media at a final concentration of 100 ng/ml. We titrated the optimized concentration of LY, KU, Rapamycin (RA, mTOR inhibitor, Sigma), MCL-1 inhibitor (MI, EMD Millipore), inhibiting PI3K/mTOR/MCL-1 signaling, and PF, SRC inhibitor (SI, SelleckChem), suppressing the activation of FAK/SRC on primary thymocytes or T-ALL cells. Human T-ALL cell lines were cultured on OP9 feeder layers, or on MG-, CLG-,

FN-coated plates. We tested the effects on these cells using combined drug treatment at optimized concentrations. After 36 hours drug treatment, cells were collected for cell viability analysis by measuring the percentage of AnnexinV$^+$ cells.

**Colony-forming assay and treatment with inhibitors:** As described previously, mononuclear cells (MNC) from BM were seeded into methylcellulose together with cytokines (Stemcell Technologies, Cat. 03434), and thymocytes or primary T-ALL cells were grown in methylcellulose with IL-2 (100 ng/ml) and Phytohemagglutinin (PHA, 2μg/ml, Roche, Cat. 11249738001) (Ragni et al., 1984). In colony forming assays, human leukemia cells were cultured in methylcellulose without cytokines (Stemcell Technology, Cat. H4100) in the presence of different treatments as described in the main text. All cells were cultured at 37°C in a 5% $CO_2$ humidified incubator, and the number of colonies was counted at day 7 after seeding. To test the efficiency of induced *Pten/Fak* deletion by colony PCR, colonies for genotyping were picked on day 5 after growth in methylcellulose. Three experiments were performed; colony numbers were 150, 120, and 100, respectively. The primers for *Pten* and *Fak* genotyping are listed in Table S1.

**Flow cytometry and antibodies:** Bone marrow (BM), spleens (SP), thymuses (TH) and peripheral blood (PB) were collected after the mice had been sacrificed. MNC were isolated from these tissues after red blood cell lysis. Cells were suspended in FACS buffer (1×PBS supplemented with 2% FBS) at a concentration of $1\times10^7$ cells/ml and aliquotted into flow cytometry tubes (100 μl per tube) for antibody staining. Antibodies used in this study were purchased from eBioscience Inc. They include: FITC-Lin (CD3, CD8, Ter119, B220, Gr1), APC-c-Kit, PE-Sca1, PE-Cy5-FLK2, Biotin-CD150, Biotin-CD34, PE-Cy5.5-IL7R, PE-Cy7-CD16/32, SA-APC-Cy7, PE-Mac1, APC-Gr1, PerCP-Ter119, FITC-CD41, APC-B220, PE-Cy5-

B220, APC-CD8, PE-CD4, FITC-CD4, and Biotin-F4/80. Data were collected using a BD LSR *Fortessa* cell analyzer and analyzed by *FlowJo10.0* software. For hematopoietic stem/progenitor sorting, c-Kit$^+$ hematopoietic cells were enriched from BM MNC using *EasySep*™ Mouse CD117 (c-Kit) Positive Selection Kit (eBioscience Inc.). C-Kit$^+$-enriched cells were stained with Lin, IL-7R, c-Kit, Sca1, Flk2, CD16/32, and CD34 surface markers. As previously reported (Zhang et al., 2006), LSK, LSKF$^-$, LSKF$^+$, CMP, GMP, EMP and CLP were purified by FACS using BD Aria III. T-ALL cells were purified by staining MNC from spleens or thymuses of T-ALL mice with FITC-CD3, APC-CD8, PE-CD4, and PE-Cy5-c-Kit, followed by FACS sorting.

**BrdU staining and proliferation analysis:** Mice were injected with BrdU at 100 μg/g 24 hours prior to being sacrificed. MNC were collected from BM, spleens and thymuses of mice and initially stained with indicated surface markers followed by BrdU antibody staining using BrdU Flow Kits (BD Pharmingen$^{TM}$) according to the protocol provided by vendor. BrdU$^+$ cell percentage (representing the proliferation rate of the cells) was examined using a BD LSR *Fortessa* cell analyzer.

**Annexin-V staining and apoptosis analysis:** MNC from BM, spleens or thymuses of mice and cultured cells were stained with Annexin-V utilizing Annexin-V Flow kits (BD Pharmingen) according to the protocol provided by the vendor. Percentages of Annexin-V$^+$ cells (representing early and late apoptotic cells) were examined using a BD LSR *Fortessa* cell analyzer.

**Intracellular antibody staining:** To analyze the expression and activities of Fak and Akt in thymocytes, MNC from thymuses were recovered and fixed immediately in 4% Paraformaldehyde (PFA) for 5 minutes to protect the epitopes. The cells were permeabilized with Cytofix/ Cytoperm$^{TM}$ (BD Bioscience) and stained with antibodies to specifically recognize pan-Fak, p-Fak$^{Y397}$, and then washed with FACS buffer, followed by surface staining for APC-

CD8, and PE-CD4. The expression and activation of Fak were measured on a BD LSR *Fortessa* cell analyzer by examining the intensity of fluorescent antibody staining.

**Quantitative RT-PCR (qRT-PCR) for gene expression:** RNA was isolated from cells using *TRIzol* (Invitrogen). The cDNA was generated from RNA by using SuperScript® III Reverse Transcriptase (Life Technologies). Levels of mRNA of genes of interest were examined by qRT-PCR using QuantiFast Probe Assay DP Kit (Qiagen) following the instructions provided by the vendors. *Gapdh* was used as control. The primers for qRT-PCR used in this study are listed in Table S2.

**Table S2. Primers for qRT-PCR and human *FAK* shRNAs**

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| *Bcl-2* forward: 5'-GATGACTGAGTACCTGAACCG-3' |
| *Bcl-2* reverse: 5'-CAGAGACAGCCAGGAGAAATC-3' |
| *Bcl-xL* forward: 5'-GGAAAGCGTAGACAAGGAGATG-3' |
| *Bcl-xL* reverse: 5'-GCATTGTTCCCGTAGAGATCC-3' |
| *Mcl-1* forward: 5'-TTGTAAGGACGAAACGGGAC-3' |
| *Mcl-1* reverse: 5'-TCTAGGTCCTGTACGTGGAAG-3' |
| *c-Myc* forward: 5'-GCTGTTTGAAGGCTGGATTTC-3' |
| *c-Myc* reverse: 5'-GATGAAATAGGGCTGTACGGAG-3' |
| *CyclinD1* forward: 5'-GCCCTCCGTATCTTACTTCAAG-3' |
| *CyclinD1* reverse: 5'-GCGGTCCAGGTAGTTCATG-3' |
| *CyclinD2* forward: 5'-GTGTTCCTATTTCAAGTGCGTG-3' |
| *CyclinD2* reverse: 5'-AGCCAAGAAACGGTCCAG-3' |
| *cIAP1* forward: 5'-TGGAGATGACCCCTGGATAG-3' |
| *cIAP1* reverse: 5'-ACAAACTCCTGACCCTTCATC-3' |

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|---|
| *cIAP2* forward: 5'-ATCTACGTCAGACTCCCCAG-3' |
| *cIAP2* reverse: 5'-CTCCTACTGAAGCCCATTTCC-3' |
| *p21* forward: 5'-GTGGCCTTGTCGCTGTCTTG-3' |

**(Continued Table S2 )**

| |
|---|
| *p21* reverse: 5'-AGAAATCTGTCAGGCTGGTCTGC-3' |
| *p27* forward: 5'-AGGAGAGCCAGGATGTCAGC-3' |
| *p27* reverse: 5'-CAGAGTTTGCCTGAGACCCAA-3' |
| *p57* forward: 5'-GGCCAATGCGAACGACTTCT-3' |
| *p57* reverse: 5'-AGGAGCCACGTTTGGAGAGG-3' |
| *Gapdh* forward: 5'-GTGAGGCCGGTGCTGAGTAT-3' |
| *Gapdh* reverse: 5'-TCATGAGCCCTTCCACAATG-3' |
| Human *FAK* shRNA1 V2LHS_248262, antisense sequence:<br>5'-TTGAATCTCATAATCCCTG-3' |
| Human *FAK* shRNA2 V2LHS_57326, antisense sequence:<br>5'-TAAGTTCAGTAAACCTGGG-3' |
| Human *FAK* shRNA3 V3LHS_325803, antisense sequence:<br>5'-ATCTCTAACATATAATCGC-3' |
| Human *FAK* shRNA4 V3LHS_325804, antisense sequence:<br>5'-AGACTTCTTTTCTAGTGCA-3' |
| Human *FAK* shRNA5 V3LHS_325806, antisense sequence:<br>5'-ACACCTTATCATTCGACCG-3' |
| Human *FAK* shRNA6 V3LHS_325807, antisense sequence:<br>5'-AAACCAACATCTTTTTCTA-3' |

**Analysis of the effects of over-expression of *Fak$^{WT}$* or *Fak$^{Y397F}$* in *Pten$^{-/-}$Fak$^{-/-}$* thymocytes and T-ALL cells:** We cloned *Fak$^{wt}$* and *Fak$^{Y397F}$* (from Drs. Filippo G. Giancotti and Dr. Wei Qiu) into MSCV-IRES-EGFP retroviral construct (Addgene). We transduced these retroviral constructs into *Pten$^{-/-}$Fak$^{-/-}$* thymocytes and T-ALL cells, and compared to empty vector MSCV-IRES-EGFP-transduced *Pten$^{-/-}$Fak$^{-/-}$* and *Pten$^{-/-}$* thymocytes and T-ALL cells (controls). The sorted GFP$^{+}$ cells were cultured on MG-coated plate and treated with DMSO, LY (50 μM), KU (30 μM), RA (0.2 μM), MI (20 μM), and LY+PF (20 μM). After 36 hours of treatment, the percentage of AnnexinV$^{+}$ cells was measured. The transduction efficiency was measured by FACS and confirmed by Western blotting.

**Isolation and treatment of primary human T-ALL cells and normal CD3$^{+}$ lymphocytes:** An IRB protocol for the use of human primary samples was approved in advance by Loyola University's Institutional Review Board. Efficiency of the inhibitors, LY (30 μM), KU (15 μM), and PF (10 μM), was measured by western blotting with a treated sample from patient-2. Normal healthy donor blood obtained by apheresis was acquired from Key Biologics, Inc. (Memphis, TN). PBMCs were isolated by density gradient centrifugation using *Ficoll Hyopaque Plus* (GE Life sciences, Cat. #17-1440-03) and washed a minimum of three times with DPBS (Corning Cellgro, Cat. #21-031-CV). One hundred million PBMCs were enriched for CD3$^{+}$ cells using the *MagCellect* Human CD3$^{+}$ T Cell Isolation Kit (R&D Systems, Cat. #MAGH101). Briefly, PBMCs were resuspended in *MagCellect* buffer and incubated with *MagCellect* Human CD3$^{+}$ T Cell biotinylated antibody cocktail at 2-8°C for 15 minutes. *MagCellect* streptavidin ferrofluid was added to the cell suspension, mixed and incubated again at 2-8°C for 15 minutes. Approximately 3ml of *MagCellect* buffer was added to the cell suspension and the tube was placed on a cell separator magnet for six minutes. The non-binding CD3$^{+}$-enriched cell

suspension was transferred to a new tube and placed on the magnet for another 6 minutes. The final $CD3^+$-enriched cell suspension was collected and transferred to a 15ml conical for further processing. With proper informed authorization, we obtained fourteen T-ALL patient samples from the tissue bank of the Department of Pathology of Loyola University Chicago, to test the effects of inhibition of AKT and FAK signaling pathways on the survival of these cancer cells. We succeeded in growing eight of these primary patient samples on MG-coated plates, and used two healthy $CD3^+$ lymphocyte samples as controls. The former samples had been freshly isolated from BM or PB of T-ALL patients and frozen under liquid nitrogen. Cells were thawed and immediately cultured on MG-coated plates for 36 hours. These cells were then: 1) collected for PTEN, FAK, SRC, GSK3β, S6K, and $pFAK^{Y397}$, $pFAK^{Y925}$, pSRC, pAKT, $pAKT^{T308}$, $pAKT^{S473}$, pGSK3β, pS6K measurement by western blotting; 2) treated with LY, KU, or PF individually, or in combinations. After 36 hours of treatment, cells were collected for cell viability analysis by measuring the $AnnexinV^+$ cell percentage. Normal $CD3^+$ lymphocyte controls were treated in the same manner.

**The regulation of FAK activity by PTEN and analysis of the interaction between PTEN and FAK in human T-ALL cells:** $PTEN^{WT}$, $PTEN^{C124S}$, $PTEN^{G129E}$ (Addgene), and $PTEN^{Y138L}$ (from Dr. Nick Leslie in Heriot-Watt University, Edinburgh) were subcloned into MSCV-IRES-EGFP retroviral construct (Addgene). These retroviral constructs were transduced into JURKAT cells, using empty vector (EV) MSCV-IRES-EGFP as a control. Sorted $GFP^+$ cells were cultured on MG-coated plates, followed by western blotting to measure $pFAK^{Y397}$, $pAKT^{T308}$, $pAKT^{S473}$, and FAK, PTEN, AKT levels. To test the interaction between FAK and PTEN, FAK and PTEN antibodies were used for immunoprecipitation (IP) of the transduced JURKAT cells, followed by western blotting detection.

Similar to transduced JURKAT cells, HPB-ALL cells were cultured on MG-coated plates, and collected for immunoprecipitation (IP) with PTEN and FAK antibodies, followed by detection by western blotting.

**The connection between FAK and NF-κB:** JURKAT and HBP-ALL cells were treated with the c-IAP inhibitor birinapant (BI, Selleckchem, S7015) and the specific Rac GTPase inhibitor EHop-016 (EH, Selleckchem, S7319) on MG-coated plate, respectively. After 6 hours of treatment, the cells were collected, lysed and western blotting was performed to test for pPAK1$^{T423}$, PAK1, RAC1/CDC42, c-IAP1, p-P65, and P65 levels.

**PB analysis and smear staining:** PB was collected from mice into 5% EDTA buffer. WBC counts, platelet numbers, and hemoglobin concentrations were determined using a *Hemavet 950FS* (Drew Scientific Inc.). Blood smears were stained with Wright's Giemsa stain (Exaxol Chemical Corporation) for hematopoietic cell morphologic analysis.

**T-ALL transplantation and *in vivo* treatments:** CD3$^{+}$c-Kit$^{Low}$ cells were isolated from *Pten*$^{-/-}$ or *Pten*$^{-/-}$*Fak*$^{-/-}$ T-ALL, or *WT* mice (CD45.1$^{+}$) and transplanted into lethally-irradiated recipient mice (CD45.2$^{+}$) together with support BM cells (CD45.2$^{+}$). Each mouse received $1\times10^{4}$ T-ALL cells and $2\times10^{5}$ support BM cells. From day 14 to Day 40 post-transplantation, the recipient mice were treated with vehicle, or a pan-PI3K inhibitor LY by intraperitoneal (i.p.) injection at 40 mg/kg every other day (Bondar et al., 2002; Hu et al., 2002; Nagata et al., 2004), or GDC by oral at 75 mg/kg daily (Folkes et al., 2008), or a mTOR inhibitor KU at 30 mg/kg by i.p. injection every other day until analysis, or a maximum of 15 injections. Recipient mice that had received *Pten*$^{-/-}$ T-ALL cells were also treated with a FAK inhibitor PF at 50mg/kg by i.p. injection every other day from day 14 to day 40 after transplantation. GDC was dissolved in 10% DMSO, 5%

Tween-20 (Sigma), 85% water. LY, KU, and PF were dissolved in 5% Tween-80 (Sigma) and 5% PEG-400 (Hampton Research).

**Tissue histologic analysis:** Livers, spleens and kidneys were collected from mice and fixed in 10% zinc formalin buffer for three days. Paraffin sections were cut and stained with hematoxylin and eosin kit (H & E, EXAXOL chemical corporation).

**Targeting human *FAK* shRNAs with lentiviral and retroviral constructs:** The shRNAs sequenced to target human *FAK* were subcloned from the pGIPZ lentiviral construct (Thermo Scientific) into MSCV-IRES-EGFP retroviral construct (Addgene). After transduction of the shRNAs (listed in Table S2) into JURKAT cells, $GFP^+$ cells were isolated by FACS sorting, followed by *FAK* knockdown efficiency assessment using western blotting.

**Analysis of NF-κB activation:** Nuclear and cytoplasmic fractions were isolated from thymocytes, T-ALL cells and JURKAT cells using NE-PER® Nuclear and Cytoplasmic Extraction Reagents (Thermo Scientific). The levels of P65 in these two fractions were subsequently measured by western blotting. Imaging flow cytometry (Amnis), ImageStream$^X$MaxII (EMD Millipore), was also used to test the activity of NF-κB after *FAK* knockdown in JURKAT cells by measuring nuclear: cytoplasmic ratio similarity of P65 to DAPI.

**Table S3. Antibodies used for western blotting**

| Target | Source | Company | Cat. |
|---|---|---|---|
| Pten | Rabbit | Cell Signaling | 9559S |
| Pten | Rabbit | Santa Cruz | sc-6817 |
| Pten | Rabbit | Invitrogen | 51-2400 |
| phospho-Fak$^{Y397}$ | Rabbit | Cell Signaling | 3283S |

| | | | |
|---|---|---|---|
| phospho-Fak$^{Y397}$ | Rabbit | Invitrogen | 44-624G |
| Fak | Rabbit | Cell Signaling | 3285S |

**(Continued Table S3)**

| | | | |
|---|---|---|---|
| Fak | Mouse | Invitrogen | Aho1272 |
| Fak | Mouse | Abcam | Ab98961 |
| Phospho-Pyk2$^{Y402}$ | Rabbit | Cell Signaling | 3291S |
| Pyk2 | Rabbit | Cell Signaling | 3292S |
| phospho-Src$^{Y416}$ | Rabbit | Cell Signaling | 2101S |
| Src | Rabbit | Cell Signaling | 2123S |
| phospho-Erk$^{T202/Y204}$ | Rabbit | Cell Signaling | 9101S |
| Erk | Rabbit | Cell Signaling | 4695S |
| phospho-Akt$^{T308}$ | Rabbit | Cell Signaling | 9275 |
| phospho-Akt$^{S473}$ | Rabbit | Cell Signaling | 4058L |
| Akt | Rabbit | Cell Signaling | 4685 |
| phospho-Jnk$^{T183/S185}$ | Rabbit | Cell Signaling | 4668S |
| Jnk | Rabbit | Cell Signaling | 9258S |
| P53 | Rabbit | Cell Signaling | 2527 |
| p-P65$^{S536}$(p-NF-κB$^{S536}$) | Rabbit | Cell Signaling | 3033L |
| P65(NF-κB) | Rabbit | Cell Signaling | 4764 |
| P65(NF-κB) | Mouse | Cell Signaling | 6956 |
| Bad | Rabbit | Cell Signaling | 9268S |
| Mcl-1 | Rabbit | Cell Signaling | 4572S |
| p-Gsk-3β$^{S9}$ | Rabbit | Millipore | 07-835 |

| | | | |
|---|---|---|---|
| Gsk3β | Rabbit | Cell Signaling | 9315S |
| Mcl-1 | Mouse | Santa Cruz | sc-56152 |

**(Continued Table S3)**

| | | | |
|---|---|---|---|
| Mcl-1 | Rabbit | Santa Cruz | sc-819 |
| Bcl-2 | Rabbit | Santa Cruz | sc-492 |
| Bcl-xL | Rabbit | Santa Cruz | sc-7195 |
| Gapdh | Goat | Santa Cruz | sc-20357 |
| Bim | Goat | Santa Cruz | sc-31687 |
| Bim | Rabbit | Santa Cruz | sc-11425 |
| c-Myc | Rabbit | Santa Cruz | sc-788 |
| c-Myc | Rabbit | Cell Signaling | 5605 |
| phospho-p70-S6K$^{T421/S424}$ | Rabbit | Cell Signaling | 9204 |
| p70-S6K | Rabbit | Cell Signaling | 2708 |
| p57$^{kip2}$ | Rabbit | Cell Signaling | 2557 |
| Paxillin (D9G12) | Rabbit | Cell Signaling | 12065 |
| phospho-Paxillin$^{Tyr118}$ | Rabbit | Cell Signaling | 2541 |
| p130 Cas (E1L9G) | Rabbit | Cell Signaling | 13383 |
| phosphor-P130 Cas$^{Y165}$ | Rabbit | Cell Signaling | 4015 |
| phospho-FAK$^{Y407}$ | Rabbit | Santa Cruz | sc-16664 |
| phospho-FAK$^{Y925}$ | Rabbit | Cell Signaling | 3284 |
| H3 | Rabbit | Cell Signaling | 9715 |
| phospho-H3$^{S10}$ | Rabbit | Cell Signaling | 9701 |
| p27$^{kip1}$ (F-8) | Mouse | Santa Cruz | sc-1641 |

| | | | |
|---|---|---|---|
| $p27^{kip1}$ (F-5) | Mouse | Santa Cruz | sc-6246 |
| Rb | Rabbit | Cell Signaling | 9309 |

**(Continued Table S3)**

| | | | |
|---|---|---|---|
| phospho-$Rb^{S780}$ | Rabbit | Cell Signaling | 9307 |
| Rac1/Cdc42 | Rabbit | Cell Signaling | 4651 |
| phospho-PAK1 (Thr423)/PAK2 (Thr402) | Rabbit | Cell Signaling | 2601 |
| PAK1 | Rabbit | Cell Signaling | 2602 |
| c-IAP1 | Rabbit | Abcam | ab2399 |
| β-actin | Rabbit | Santa Cruz | sc-7210 |
| anti-goat IgG-HRP | Rabbit | Santa Cruz | sc-2768 |
| anti-goat IgG-HRP | Donkey | Santa Cruz | sc-2304 |
| anti-mouse IgG-HRP | Horse | Cell Signaling | 7076 |
| anti-rabbit IgG-HRP | Goat | Cell Signaling | 7074 |

**Western blotting:** Cell lysates were extracted using Cell Lysis Buffer (Cell Signaling) followed by a brief sonication. The supernatants were collected after centrifugation at 4°C, 14,000×g for 20 minutes. Protein samples were separated by SDS-PAGE on 10 or 12% acrylamide gels. Proteins were transferred onto nitrocellulose or PVDF membranes to examine the target proteins by antibody blotting. Antibodies used in this study are listed in Table S3. The band quantifications using *Multi Gauge 3.0* were normalized to corresponding controls, which were set as 1.00, after being normalized to corresponding loading standards in western blot.

**Figure S1. The Expression of *Fak* in Murine T-lymphocytes (Related to Figure 1).**

The expression of *Fak* in $CD4^-CD8^-$, $CD4^+CD8^+$, $CD4^+CD8^-$, and $CD4^-CD8^+$ murine T-lymphocytes was examined by intracellular staining and fluorescence-activated cell sorting (FACS) analysis.

**Figure S2. *Fak* Deletion does not Change the Development of Myeloproliferative Disorder (MPD) but Delays T-ALL Development in *Pten*$^{-/-}$ Mice (Related to Figure 2).**

**(A-C)** Representative images of FACS analysis of premature myeloid cells ($Gr1^+Mac1^+$) in BM, SP, and PB (**A**), percentages of $Gr1^+Mac1^+$, $CD3^+$, $B220^+$ cells in BM, SP, and PB (**B**), Data shown are Mean ± S.E.M., * compared to *WT*, P<0.001, determined by *Student's t-test*. Absolute number of WBC, Plt, and RBC (**C**) of *WT*, *Pten*$^{-/-}$, *Pten*$^{-/-}$*Fak*$^{-/-}$, and *Fak*$^{-/-}$ mice on day10 following five-pI:pC injections. Data shown are Mean ± s.d. ***, **, * compared to *WT*, P<0.001, <0.01, <0.05; $$, $ compared to *Pten*$^{-/-}$, P<0.01, <0.05, determined by *Two-way ANOVA tests*. (**D)** Kaplan-Meier survival curve of *WT*, *Pten*$^{-/-}$, *Pten*$^{-/-}$*Fak*$^{+/-}$, and *Pten*$^{-/-}$*Fak*$^{-/-}$ mice after 5×pI:pC treatments. The genotypes of the mice were confirmed by PCR (middle panel) and Western blot (bottom panel) of splenocytes recovered from moribund or *WT* animals. (**E)** Representative data showing FACS images of BM and SP of *Pten*$^{-/-}$, and *Pten*$^{-/-}$*Fak*$^{-/-}$ mice with obvious T-ALL phenotypes, using *WT* as control. $CD4^+CD8^{low/+}CD45^{high}$ cells are defined as T-ALL cells (Borowitz et al., 1993; Guo et al., 2008). (**F&G)** Giemsa staining of PB smears of *Pten*$^{-/-}$ and *Pten*$^{-/-}$*Fak*$^{-/-}$ mice with T-ALL and MPD; *WT* and *Fak*$^{-/-}$ mice were used as controls. Insets are ×1000 images of individual cells (**F**), average number of nucleated cells/field of PB (**G**). Data shown are Mean ± s.d. * compared to *WT*, P<0.001, determined by *Two-way ANOVA*

*tests*. (**H)** H&E staining for splenic structure from *WT*, *Pten*$^{-/-}$, and *Pten*$^{-/-}$*Fak*$^{-/-}$ mice on day45 after a single pI:pC injection and T-ALL *Pten*$^{-/-}$*Fak*$^{-/-}$ mice at their death.

**Figure S3. *Fak* Deletion does not significantly Affect the Cell-cycle of Thymocytes nor does it completely Block the Development of T-ALL in *Pten*$^{-/-}$ Mice (Related to Figure 3).**

**(A-C)** Representative FACS images of PE-CD4 and APC-CD8 staining (**A**), AnnexinV analysis (**B**), and cell-cycle analysis (**C**) of thymocytes from *WT*, *Pten*$^{-/-}$, *Pten*$^{-/-}$*Fak*$^{-/-}$, and *Fak*$^{-/-}$ mice before T-ALL development with a single pI:pC treatment. Data shown are Mean ± S.E.M. * compared to *WT*, P<0.05; $ compared to *Pten*$^{-/-}$, P<0.05, determined by *Student's t-test.*

**Figure S4. Not all Downstream Targets of NF-κB, or Cell-cycle Related Genes Expressions are Affected by *Fak* Deletion in *Pten*-null thymocytes or T-ALL cells (Related to Figure 4).**

**(A)** Relative mRNA levels of *c-Myc*, *CyclinD1*, *CyclinD2*, *cIAP1*, *cIAP2*, *p21*, p27, and *p57* in *Pten*$^{-/-}$, *Pten*$^{-/-}$*Fak*$^{-/-}$ thymocytes, and *Pten*$^{-/-}$, *Pten*$^{-/-}$*Fak*$^{-/-}$ T-ALL cells were examined by qRT-PCR. Data shown are Mean ± s.d. * compared to *WT*, P<0.001, determined by *Two-way ANOVA test*. **(B)** The levels of pPyk2, pPak1, pJnk1/2, pRb, pH3, and the protein levels of Rac1/Cdc42, Cyclin D1, Cyclin A2, p57, p27, p21, c-Myc in *Pten*$^{-/-}$, *Pten*$^{-/-}$*Fak*$^{-/-}$ cells were evaluated by western blot. The images shown are representative of three independent experiments.

**Figure S5. Fak is Activated when cultured on OP9 Feeder Layer Cells, or on MG/CLG/ FN-coated Plates *in vitro*. Inhibition of both mTor and Fak Enhanced the Survival of Recipient Mice Receiving *Pten*$^{-/-}$ T-ALL Cells (Related to Figure 5).**

**(A-B)** The levels of pFak, pAkt, and pS6k in *Pten*$^{-/-}$ thymocytes and T-ALL cells cultured in suspension condition, or on OP9, MG-coated plates (**A**) and also on CLG/FN-plates (**B**) were

examined by western blot. Images shown are representative of three independent experiments. (**C)** Optimizing the concentrations of LY, KU, RA, and MI on thymocytes of *WT*, *Pten*$^{-/-}$, and *Pten*$^{-/-}$*Fak*$^{-/-}$ mice. (**D&E**) Sensitivity of thymocytes **(D)** and T-ALL cells **(E**) from *Pten*$^{-/-}$, and *Pten*$^{-/-}$*Fak*$^{-/-}$ mice to DMSO (DO), LY, KU, RA, MI, and LY+PF on CLG/FN-coated plates. Using *WT* as control. Data shown are Mean ± S.D. * compared to DMSO treatment, $ compared to *WT*, # compared to *Pten*$^{-/-}$, £ compared to LY treatment, P<0.001, determined by *2-way ANOVA tests*. (**F**) Sorting of GFP$^{+}$ cells from *Migr1*-transduced *Pten*$^{-/-}$ and *Pten*$^{-/-}$*Fak*$^{-/-}$ thymocytes or T-ALL cells, as well as *Fak*$^{wt}$ *and Fak*$^{Y397F}$-transduced *Pten*$^{-/-}$*Fak*$^{-/-}$ thymocytes and T-ALL cells. (**G**) Kaplan-Meier survival curve of recipient mice (CD45.2$^{+}$) treated with vehicle, KU, or PF alone, or KU+ PF together since day 14 after transplantion with CD3$^{+}$c-Kit$^{low}$ cells isolated from the BM of *Pten*$^{-/-}$ T-ALL mice (CD45.1$^{+}$). * compared to vehicle treatment, $ compared to PF or KU treatment, P<0.05. (**H&I**) Kaplan-Meier survival curve for *Pten*$^{-/-}$ and *Pten*$^{-/-}$*Fak*$^{-/-}$ T-ALL mice treated with vehicle, LY, or GDC (**H**), and recipient mice (CD45.2$^{+}$) treated with vehicle, LY, or GDC since day 14 after transplantion with CD3$^{+}$c-Kit$^{low}$ cells isolated from BM of *Pten*$^{-/-}$, or *Pten*$^{-/-}$*Fak*$^{-/-}$ T-ALL mice (CD45.1$^{+}$) (**I**). * compared to corresponding vehicle treatment; $compared to *Pten*$^{-/-}$ mice with corresponding vehicle or LY/GDC treatment.

**Figure S6. *PTEN*-mutant Human T-ALL Cell Lines have Two Parallel Survival Signaling Pathways, PI3K/AKT/mTOR/MCL-1 and FAK/NF-κB/BCL-xL/BCL-2 (Related to Figure 6).**

**(A)** The levels of pFAK, pSRC, pAKT, pS6K, and the expression of PTEN in JURKAT and HPB-ALL cells when cultured in suspension, or on OP9, or on MG/CLG/FN-coated plates. (**B&C)** Optimization of the concentration of LY, KU, RA, MI, PF, or SI in treatments to

JURKAT cells when cultured on MG-coated plates (**B**), or growth in methylcellulose (**C**). (**D)** Viability of human T-ALL cell lines, CCRF-CEM, HBP-ALL, MOLT-4, LOUCY, and KOPT-K1, when cultured in suspension, or on OP9, or on MG/CLG/FN-coated plates, treated with DMSO (DO), LY, KU, RA, MI, PF, LY+PF, KU+PF, RA+PF, MI+PF, or SI, LY+SI, KU+SI, RA+SI, MI+SI. Data shown are Mean ± s.d. ***, * compared to DO treatment, P<0.001, <0.05; $$$, $ compared to suspension culture, P<0.001, <0.05; ### compared to each single inhibitor treatment, P<0.001. P values were determined by *Two-way ANOVA tests*. (**E&F)** The nuclear localization of P65, measured by Amnis imaging flow cytometry, in JURKAT cells with knockdown of *FAK* by shRNA1 and shRNA6. Data shown are Mean ± S.D. * compared to Scrambled cells, P<0.001, determined by *Student's t-test* (**E**). The viability of JURKAT cells with *FAK* inhibition by shRNA1 or shRAN6 and treated with DO, LY, KU, RA, or MI when cultured on OP9 feed layer cells (**F**). Data shown are Mean ± s.d. * compared to DO treatment, $ compared scrambled cells, P<0.001, determined by *Two-way ANOVA tests*.

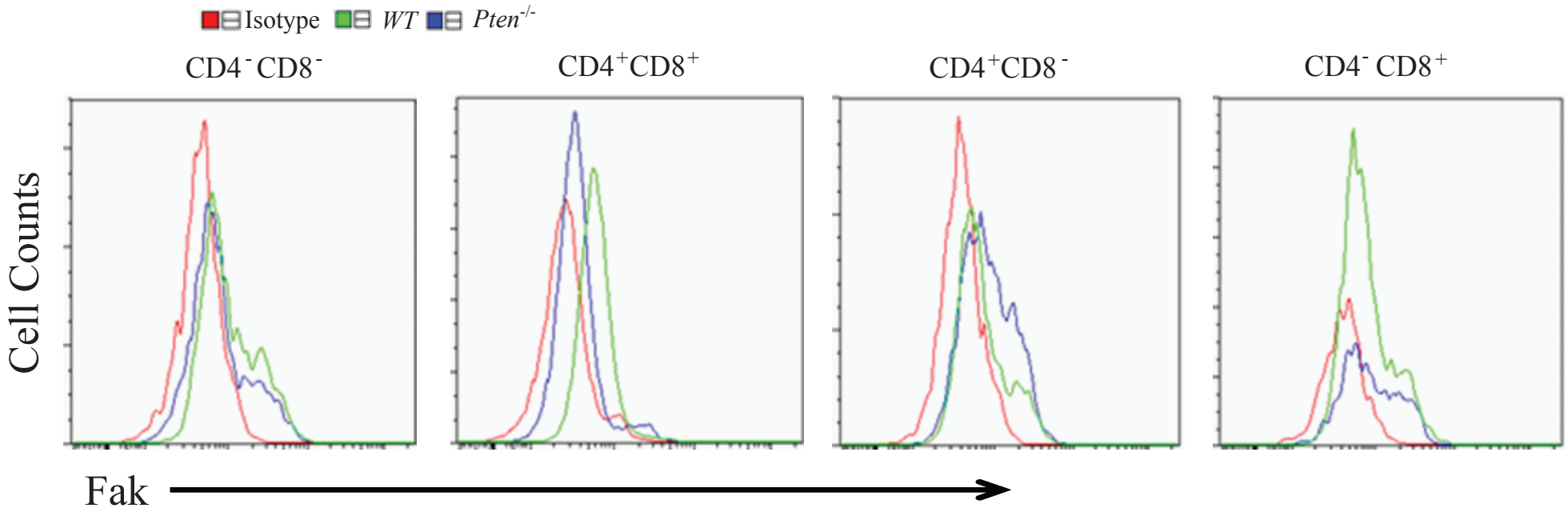
**Figure S7. BCL-xL/BCL-2 are Regulated by NF-κB in *PTEN*-mutant Human T-ALL Cells (Related to Figure 6).**

(**A&B)** The levels of pP65, and the expression of PTEN, BCL-2, and BCL-xL in JURKAT, KOPT-K1, and CCRF-CEM cells (**A**), and the levels of cytoplasmic (C) and nuclear (N) P65 in JURKAT cells (**B**) when cultured on MG-coated plates with DMSO (DO), BA, or ABT-737 treatment. Images shown are representative of three independent experiments. Data shown are Mean ± s.e.m. * compared to DO treatment, P<0.001, determined by *Student's t-test*.

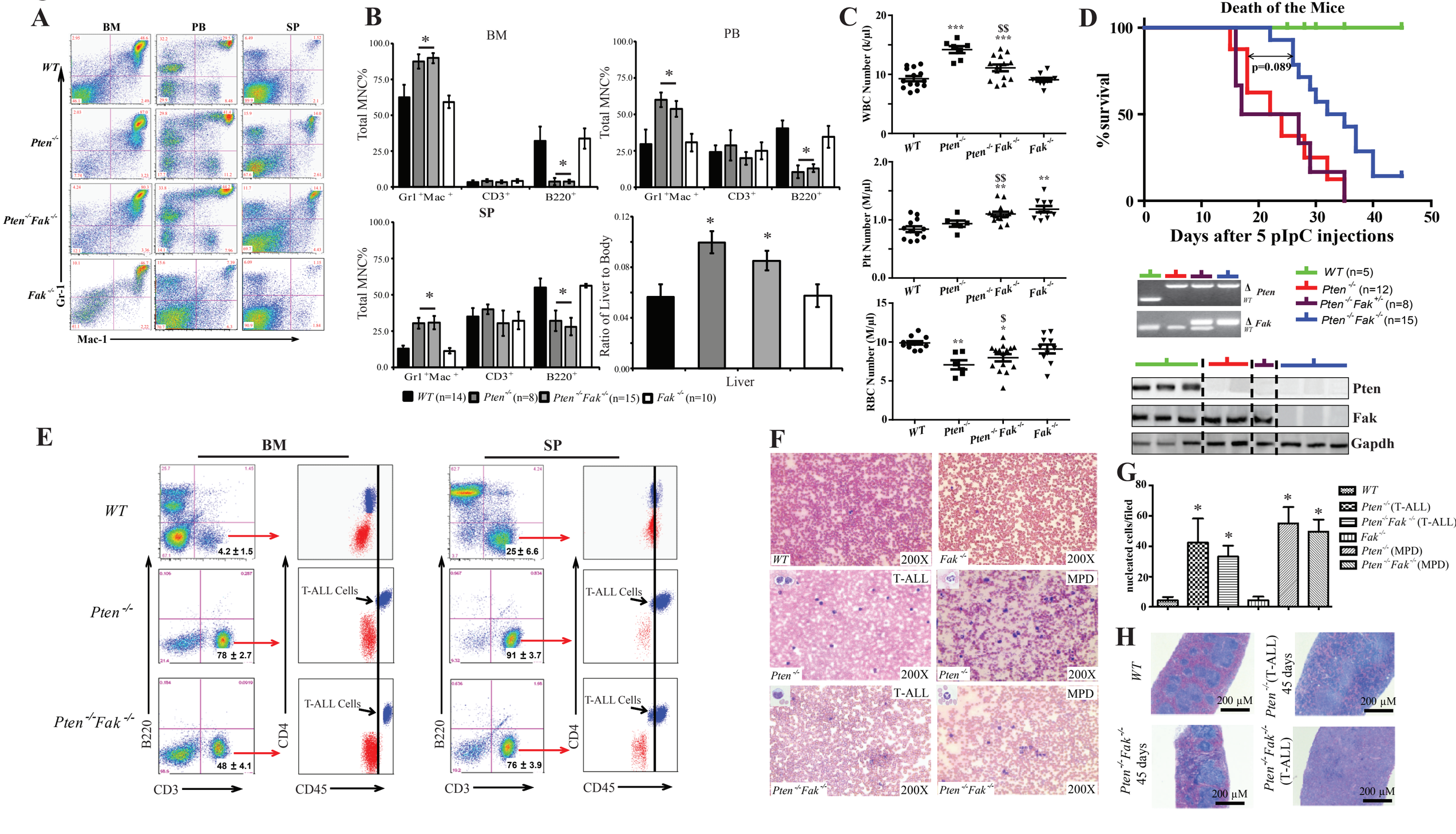
# References

Bondar, V. M., Sweeney-Gotsch, B., Andreeff, M., Mills, G. B., and McConkey, D. J. (2002). Inhibition of the phosphatidylinositol 3'-kinase-AKT pathway induces apoptosis in pancreatic carcinoma cells in vitro and in vivo. Molecular cancer therapeutics *1*, 989-997.
Borowitz, M. J., Guenther, K. L., Shults, K. E., and Stelzer, G. T. (1993). Immunophenotyping of acute leukemia by flow cytometric analysis. Use of CD45 and right-angle light scatter to gate on leukemic blasts in three-color analysis. American journal of clinical pathology *100*, 534-540.
Folkes, A. J., Ahmadi, K., Alderton, W. K., Alix, S., Baker, S. J., Box, G., Chuckowree, I. S., Clarke, P. A., Depledge, P., Eccles, S. A., *et al.* (2008). The identification of 2-(1H-indazol-4-yl)-6-(4-methanesulfonyl-piperazin-1-ylmethyl)-4-morpholin-4-yl-t hieno[3,2-d]pyrimidine (GDC-0941) as a potent, selective, orally bioavailable inhibitor of class I PI3 kinase for the treatment of cancer. J Med Chem *51*, 5522-5532.
Guo, W., Lasky, J. L., Chang, C. J., Mosessian, S., Lewis, X., Xiao, Y., Yeh, J. E., Chen, J. Y., Iruela-Arispe, M. L., Varella-Garcia, M., and Wu, H. (2008). Multi-genetic events collaboratively contribute to Pten-null leukaemia stem-cell formation. Nature *453*, 529-533.
Hu, L., Hofmann, J., Lu, Y., Mills, G. B., and Jaffe, R. B. (2002). Inhibition of phosphatidylinositol 3'-kinase increases efficacy of paclitaxel in in vitro and in vivo ovarian cancer models. Cancer Res *62*, 1087-1092.
Nagata, Y., Lan, K. H., Zhou, X., Tan, M., Esteva, F. J., Sahin, A. A., Klos, K. S., Li, P., Monia, B. P., Nguyen, N. T., *et al.* (2004). PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. Cancer Cell *6*, 117-127.
Palomero, T., Barnes, K. C., Real, P. J., Glade Bender, J. L., Sulis, M. L., Murty, V. V., Colovai, A. I., Balbin, M., and Ferrando, A. A. (2006). CUTLL1, a novel human T-cell lymphoma cell line with t(7;9) rearrangement, aberrant NOTCH1 activation and high sensitivity to gamma-secretase inhibitors. Leukemia *20*, 1279-1287.
Ragni, M. V., Winkelstein, A., Evans, T. L., Lewis, J. H., Bontempo, F. A., Spero, J. A., and Rabin, B. S. (1984). T lymphocyte colony assay in hemophiliacs. Blood *64*, 105-109.
Shan, X., Czar, M. J., Bunnell, S. C., Liu, P., Liu, Y., Schwartzberg, P. L., and Wange, R. L. (2000). Deficiency of PTEN in Jurkat T cells causes constitutive localization of Itk to the plasma membrane and hyperresponsiveness to CD3 stimulation. Mol Cell Biol *20*, 6945-6957.
Zhang, J., Grindley, J. C., Yin, T., Jayasinghe, S., He, X. C., Ross, J. T., Haug, J. S., Rupp, D., Porter-Westpfahl, K. S., Wiedemann, L. M., *et al.* (2006). PTEN maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention. Nature *441*, 518-522.
Zhang, J., Xiao, Y., Guo, Y., Breslin, P., Zhang, S., Wei, W., Zhang, Z., and Zhang, J. (2011). Differential requirements for c-Myc in chronic hematopoietic hyperplasia and acute hematopoietic malignancies in Pten-null mice. Leukemia *25*, 1857-1868.

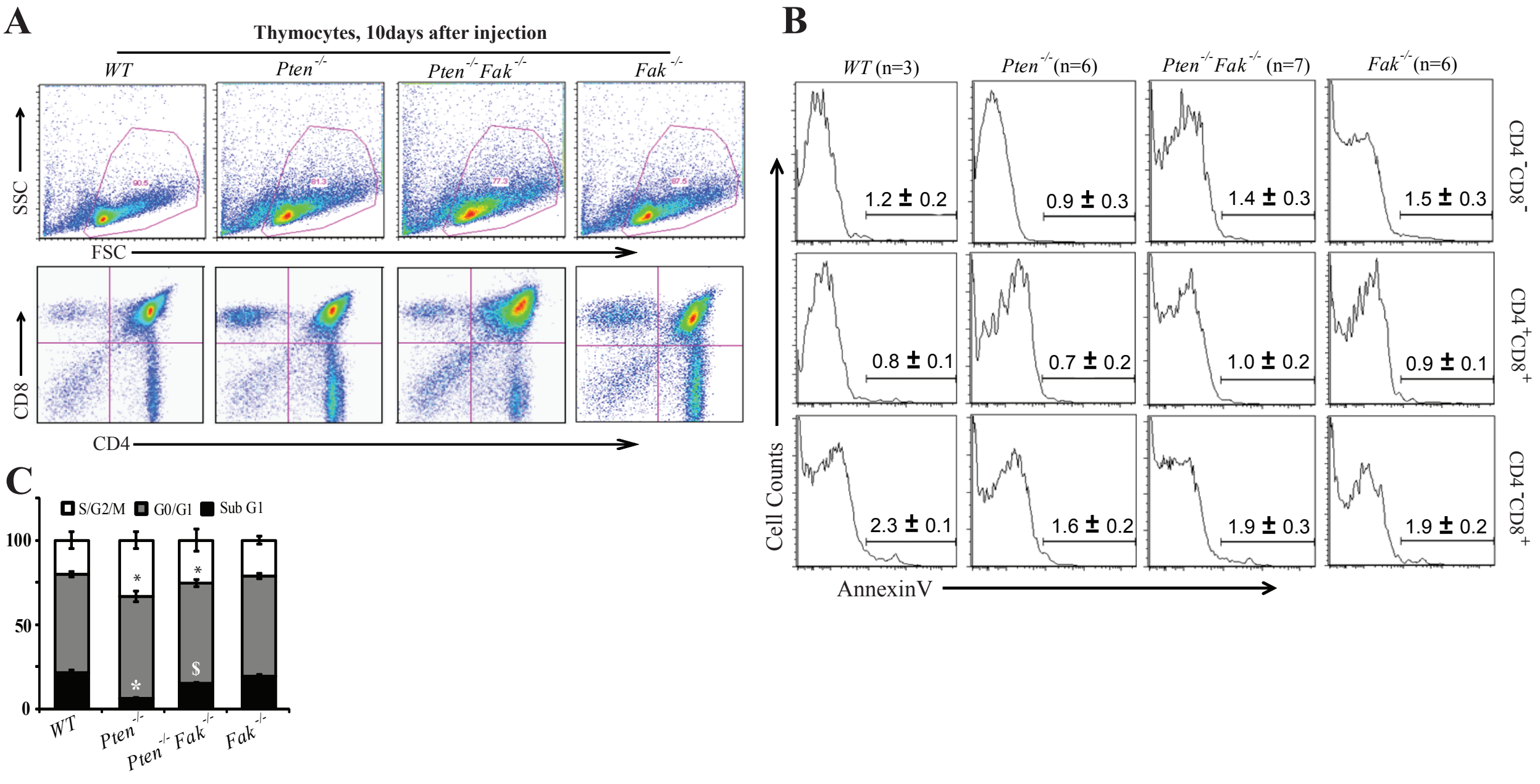
# Fig. S1


Isotype
WT
Pten-/-
CD4-CD8-
CD4+CD8+
CD4+CD8-
CD4-CD8+
Cell Counts
Fak

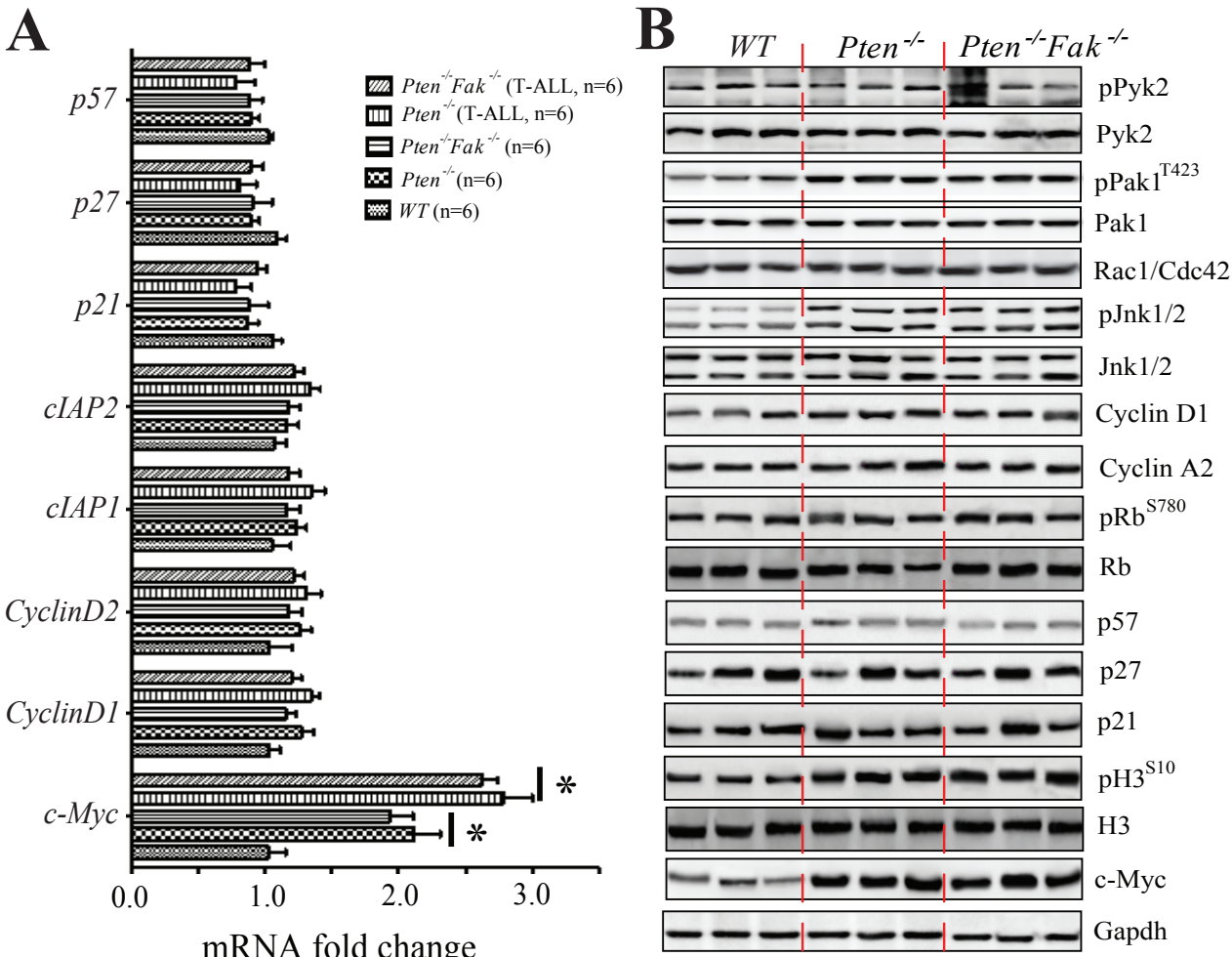
# Fig. S2


A
BM
PB
SP
WT
Pten-/-
Pten-/-Fak-/-
Fak-/-
Gr-1
Mac-1
B
BM
PB
SP
Total MNC%
Gr1+Mac+
CD3+
B220+
Ratio of Liver to Body
Liver
WT (n=14) Pten-/- (n=8) Pten-/-Fak-/- (n=15) Fak-/- (n=10)
C
WBC Number (k/μl)
Plt Number (M/μl)
RBC Number (M/μl)
WT
Pten-/-
Pten-/-Fak-/-
Fak-/-
D
Death of the Mice
% survival
p=0.089
Days after 5 pIpC injections
WT (n=5)
Pten-/- (n=12)
Pten-/-Fak+/- (n=8)
Pten-/-Fak-/- (n=15)
Δ Pten
Δ Fak
Pten
Fak
Gapdh
E
BM
SP
WT
Pten-/-
Pten-/-Fak-/-
4.2 ± 1.5
78 ± 2.7
48 ± 4.1
25± 6.6
91 ± 3.7
76 ± 3.9
T-ALL Cells
B220
CD4
CD3
CD45
F
WT
Fak-/-
Pten-/-
Pten-/-Fak-/-
200X
T-ALL
MPD
G
nucleated cells/filed
WT
Pten-/-(T-ALL)
Pten-/-Fak-/- (T-ALL)
Fak-/-
Pten-/- (MPD)
Pten-/-Fak-/- (MPD)
H
WT
Pten-/-(T-ALL) 45 days
Pten-/-Fak-/- 45 days
Pten-/-Fak-/- (T-ALL)
200 μM

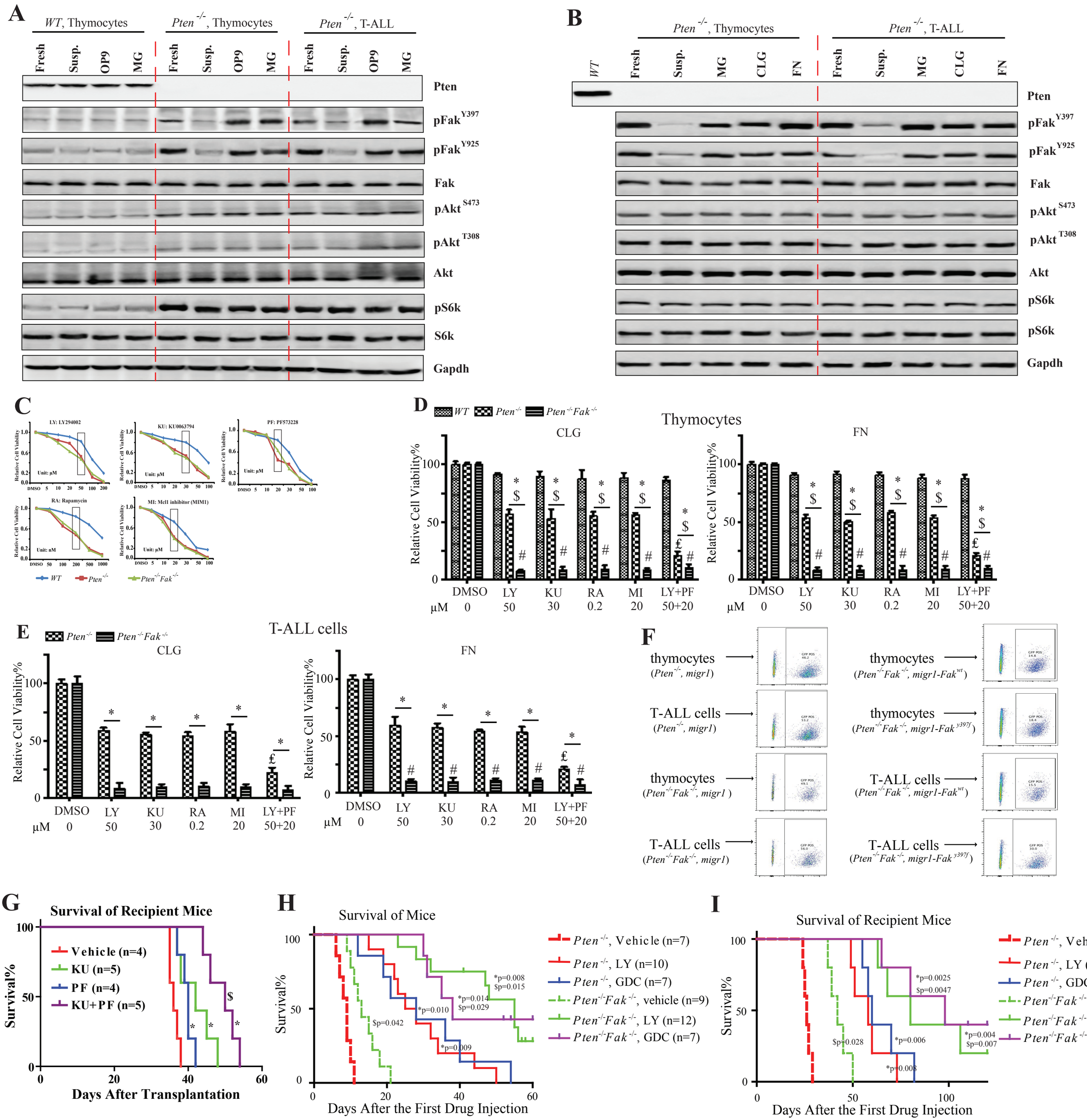
# Fig. S3


A
Thymocytes, 10days after injection
WT
Pten-/-
Pten-/-Fak-/-
Fak-/-
SSC
FSC
CD8
CD4
90.5
81.3
77.3
87.5
B
WT (n=3)
Pten-/-(n=6)
Pten-/-Fak-/- (n=7)
Fak-/-(n=6)
CD4-CD8-
CD4+CD8+
CD4-CD8+
1.2 ± 0.2
0.9 ± 0.3
1.4 ± 0.3
1.5 ± 0.3
0.8 ± 0.1
0.7 ± 0.2
1.0 ± 0.2
0.9 ± 0.1
2.3 ± 0.1
1.6 ± 0.2
1.9 ± 0.3
1.9 ± 0.2
Cell Counts
AnnexinV
C
S/G2/M
G0/G1
Sub G1
100
50
0
*
*
*
$
WT
Pten-/-
Pten-/-Fak-/-
Fak-/-

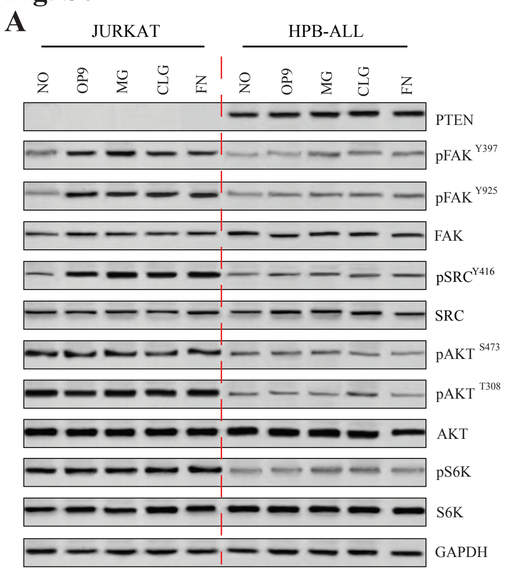
# Fig. S4

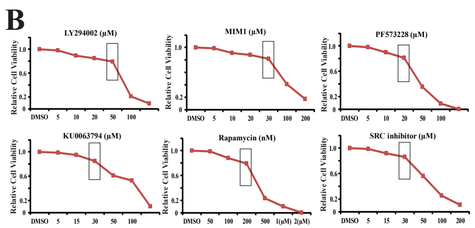

A
p57
p27
p21
cIAP2
cIAP1
CyclinD2
CyclinD1
c-Myc
Pten-/-Fak-/- (T-ALL, n=6)
Pten-/- (T-ALL, n=6)
Pten-/-Fak-/- (n=6)
Pten-/- (n=6)
WT (n=6)
*
*
0.0
1.0
2.0
3.0
mRNA fold change
B
WT
Pten-/-
Pten-/-Fak-/-
pPyk2
Pyk2
pPak1T423
Pak1
Rac1/Cdc42
pJnk1/2
Jnk1/2
Cyclin D1
Cyclin A2
pRbS780
Rb
p57
p27
p21
pH3S10
H3
c-Myc
Gapdh

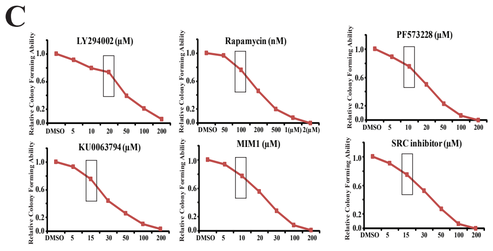
# Fig. S5

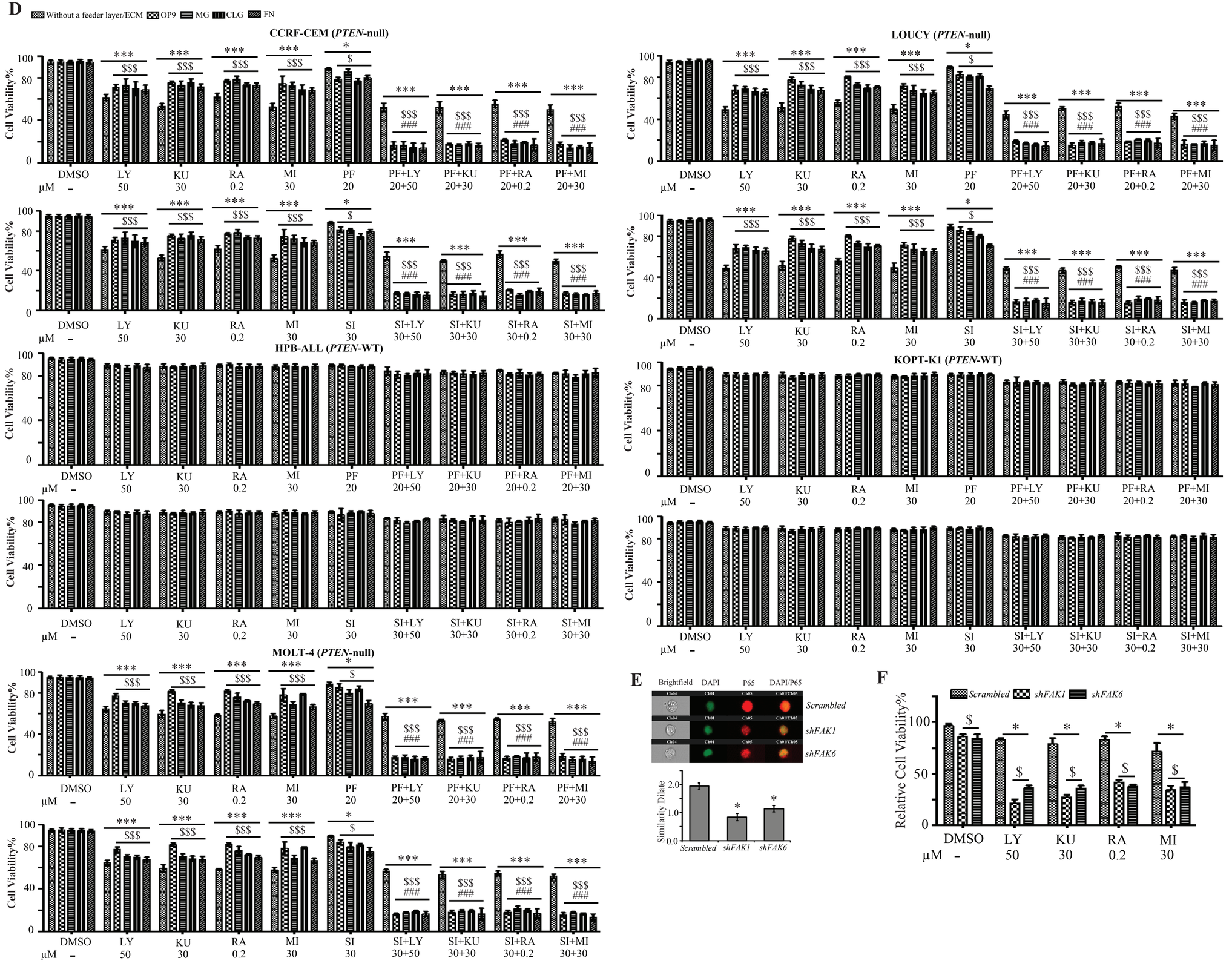

A
WT, Thymocytes
Pten -/-, Thymocytes
Pten -/-, T-ALL
Fresh
Susp.
OP9
MG
Pten
pFak Y397
pFak Y925
Fak
pAkt S473
pAkt T308
Akt
pS6k
S6k
Gapdh
B
Pten -/-, Thymocytes
Pten -/-, T-ALL
WT
Fresh
Susp.
MG
CLG
FN
Pten
pFak Y397
pFak Y925
Fak
pAkt S473
pAkt T308
Akt
pS6k
pS6k
Gapdh
C
LY: LY294002
KU: KU0063794
PF: PF573228
RA: Rapamycin
MI: Mcl1 inhibitor (MIM1)
Relative Cell Viability
Unit: μM
Unit: nM
DMSO
WT
Pten -/-
Pten -/-Fak -/-
D
WT
Pten -/-
Pten -/-Fak -/-
Thymocytes
CLG
FN
Relative Cell Viability%
DMSO
LY
KU
RA
MI
LY+PF
μM
0
50
30
0.2
20
50+20
E
Pten -/-
Pten -/-Fak -/-
T-ALL cells
CLG
FN
Relative Cell Viability%
DMSO
LY
KU
RA
MI
LY+PF
μM
0
50
30
0.2
20
50+20
F
thymocytes (Pten -/-, migr1)
T-ALL cells (Pten -/-, migr1)
thymocytes (Pten -/-Fak -/-, migr1 )
T-ALL cells (Pten -/-Fak -/-, migr1)
thymocytes (Pten -/-Fak -/-, migr1-Fak wt)
thymocytes (Pten -/-Fak -/-, migr1-Fak y397f)
T-ALL cells (Pten -/-Fak -/-, migr1-Fak wt)
T-ALL cells (Pten -/-Fak -/-, migr1-Fak y397f)
G
Survival of Recipient Mice
Survival%
Days After Transplantation
Vehicle (n=4)
KU (n=5)
PF (n=4)
KU+PF (n=5)
H
Survival of Mice
Survival%
Days After the First Drug Injection
Pten -/-, Vehicle (n=7)
Pten -/-, LY (n=10)
Pten -/-, GDC (n=7)
Pten -/-Fak -/-, vehicle (n=9)
Pten -/-Fak -/-, LY (n=12)
Pten -/-Fak -/-, GDC (n=7)
*p=0.008
$p=0.015
*p=0.014
$p=0.029
*p=0.010
$p=0.042
*p=0.009
I
Survival of Recipient Mice
Survival%
Days After the First Drug Injection
Pten -/-, Vehicle (n=5)
Pten -/-, LY (n=5)
Pten -/-, GDC (n=5)
Pten -/-Fak -/-, vehicle (n=5)
Pten -/-Fak -/-, LY (n=5)
Pten -/-Fak -/-, GDC (n=5)
*p=0.0025
$p=0.0047
$p=0.028
*p=0.006
*p=0.004
$p=0.007
*p=0.008

# Fig. S6

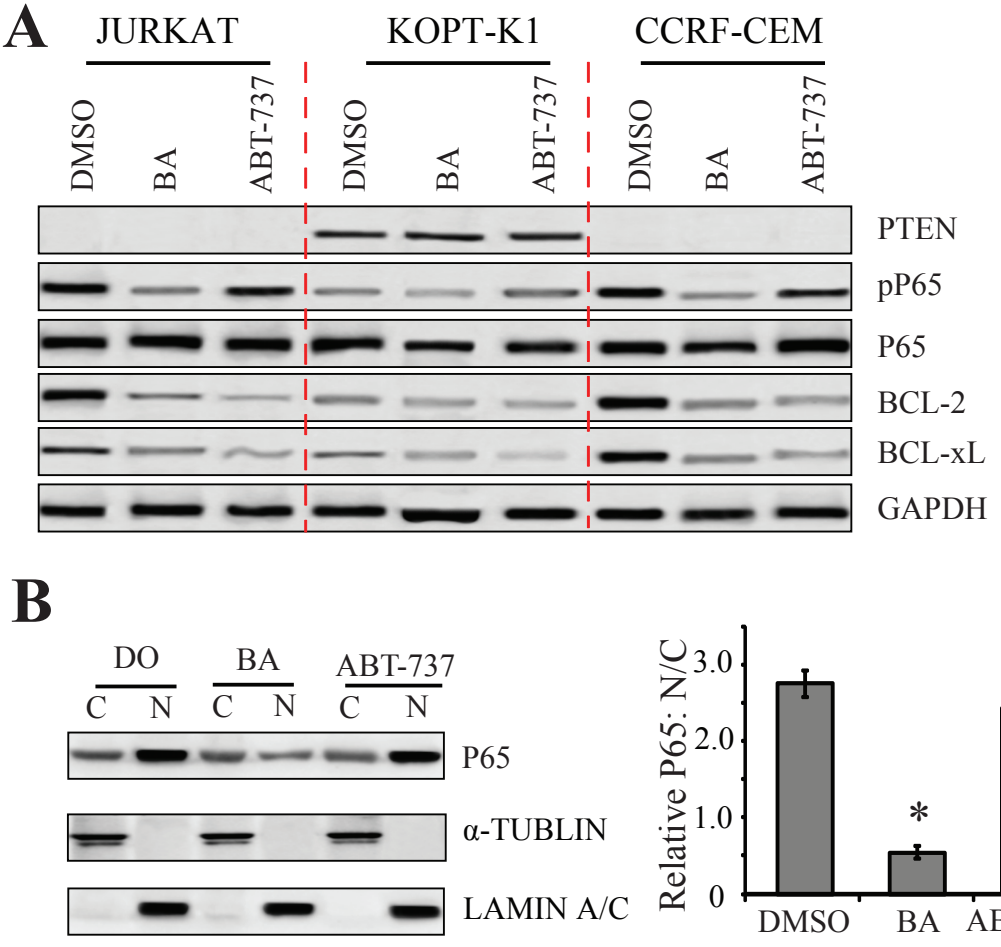

A
JURKAT
HPB-ALL
NO
OP9
MG
CLG
FN
PTEN
pFAK Y397
pFAK Y925
FAK
pSRC Y416
SRC
pAKT S473
pAKT T308
AKT
pS6K
S6K
GAPDH


B
LY294002 (μM)
MIM1 (μM)
PF573228 (μM)
KU0063794 (μM)
Rapamycin (nM)
SRC inhibitor (μM)
Relative Cell Viability


C
LY294002 (μM)
Rapamycin (nM)
PF573228 (μM)
KU0063794 (μM)
MIM1 (μM)
SRC inhibitor (μM)
Relative Colony Forming Ability


D
Without a feeder layer/ECM
OP9
MG
CLG
FN
CCRF-CEM (PTEN-null)
LOUCY (PTEN-null)
HPB-ALL (PTEN-WT)
KOPT-K1 (PTEN-WT)
MOLT-4 (PTEN-null)
Cell Viability%
DMSO
LY
KU
RA
MI
PF
SI
PF+LY
PF+KU
PF+RA
PF+MI
SI+LY
SI+KU
SI+RA
SI+MI
μM
E
Brightfield
DAPI
P65
DAPI/P65
Ch04
Ch01
Ch05
Ch01/Ch05
Scrambled
shFAK1
shFAK6
Similarity Dilate
F
Relative Cell Viability%

# Fig. S7


A
JURKAT
KOPT-K1
CCRF-CEM
DMSO
BA
ABT-737
PTEN
pP65
P65
BCL-2
BCL-xL
GAPDH
B
DO
BA
ABT-737
C
N
P65
α-TUBLIN
LAMIN A/C
Relative P65: N/C
3.0
2.0
1.0
0
*
DMSO
BA
ABT-737