A PI3K p110β–Rac signalling loop mediates Pten-loss-induced perturbation of haematopoiesis and leukaemogenesis

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The tumour suppressor PTEN, which antagonizes PI3K signalling, is frequently inactivated in haematologic malignancies. In mice, deletion of PTEN in haematopoietic stem cells (HSCs) causes perturbed haematopoiesis, myeloproliferative neoplasia (MPN) and leukaemia. Although the roles of the PI3K isoforms have been studied in PTEN-deficient tumours, their individual roles in PTEN-deficient HSCs are unknown. Here we show that when we delete PTEN in HSCs using the Mx1–Cre system, p110β ablation prevents MPN, improves HSC function and suppresses leukaemia initiation. Pharmacologic inhibition of p110β in PTEN-deficient mice recapitulates these genetic findings, but suggests involvement of both Akt-dependent and -independent pathways. Further investigation reveals that a p110β–Rac signalling loop plays a critical role in PTEN-deficient HSCs. Together, these data suggest that myeloid neoplasia driven by PTEN loss is dependent on p110β via p110β–Rac-positive-feedback loop, and that disruption of this loop may offer a new and effective therapeutic strategy for PTEN-deficient leukaemia.

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Dysregulation of the molecular pathways involved in the self-renewal, differentiation and proliferation of haematopoietic stem cells (HSCs) can cause leukaemia. Notably, the serine/threonine kinase Akt, which acts downstream of PI3 kinase (PI3K), is hyper-phosphorylated in up to 80% of acute myeloid leukaemia (AML) cases. This is unlikely to be due to mutations in upstream receptor tyrosine kinases alone. In chronic myelogenous leukaemia, PI3K/Akt signalling can also be activated through downregulation of the phosphatase and tensin homologue (PTEN) by BCR-ABL.1-3 PTEN is a lipid phosphatase that counteracts PI3K signalling by dephosphorylating phosphatidylinositol-3,4,5-trisphosphate (PIP3). PTEN is frequently inactivated in haematological malignancies,4–9, including in AML and T cell acute lymphoblastic leukaemia (T-ALL).5,6 Notably, PTEN expression is often reduced in the disease through several other modes of PTEN regulation, for example, microRNAs, epigenetic modifications and ubiquitination,7–9, which likely contribute to the high frequency of Akt phosphorylation in myeloid leukaemia.

In mice, genetic ablation of PTEN in the haematopoietic system leads to HSCLP, in the bone marrow (BM), myeloproliferative neoplasia (MPN) and transplantable acute leukaemia (myeloid or T-cell leukaemia).10–12 In patients, MPNs such as chronic myelogenous leukaemia or myelofibrosis can progress to AMI.13 Class I PI3Ks are heterodimeric lipid kinases that produce the lipid second messenger PIP3 on stimulation of cells by many growth factors. Class I PI3Ks are divided into class IA (p110α, p110β and p110δ) and class IB (p110γ) enzymes; of these, the p110α and p110β isoforms are ubiquitously expressed, while p110δ and p110γ are enriched in leukocytes. Work in several different murine models has documented distinct requirements for different PI3K isoforms in particular tumour types.14,15 For example, p110δ is essential in a model of mutant Kras-induced lung adenocarcinoma.16 Recently, we showed that Ras-mutated myeloid leukaemia is also dependent on the p110α isoform, and combined pharmacologic inhibition of p110α and mitogen-activated protein kinase kinase (MEK) could be an effective therapeutic strategy for Ras-mutated myeloid malignancies.17 Although p110β plays a less prominent role in receptor tyrosine kinase (RTK) signalling, it mediates G protein-coupled receptor (GPCR) and integrin signalling,18–20 and has been shown to interact specifically with Rho family GTPases Rac1 and CDC42 (ref. 21). Several recent studies demonstrated that p110β is required in many, but not all, PTEN-deficient solid tumours.20,22,23 However, it is not known which PI3K isoforms are most important for myeloid neoplastic transformation driven by PTEN loss.

A number of pan-class I PI3K and dual class I/mTOR inhibitors are now in clinical trials for cancer, including leukaemia. However, targeting PI3K with these inhibitors could potentially lead to severe toxicity, which could be prevented by targeting single PI3K isoforms. To this end, numerous isoform-selective compounds are available and toxicity, which could be prevented by targeting single PI3K isoforms. To this end, numerous isoform-selective compounds are available and targeting the PI3K pathway with these inhibitors could potentially lead to severe toxicity, which could be prevented by targeting single PI3K isoforms. To this end, numerous isoform-selective compounds are available and targeting the PI3K pathway with these inhibitors could potentially lead to severe toxicity, which could be prevented by targeting single PI3K isoforms.

In the present study, we used genetically engineered mouse models to determine which of the class IA PI3K isoforms are most important in mediating the effects of Pten loss in HSCs. We show that, in the setting of Pten loss, p110β is the main PI3K isoform responsible for MPN development and HSC depletion in the BM. Furthermore, we show that isoform-selective PI3K inhibitors recapitulate our genetic findings. We also found that a signalling loop featuring p110β and Rac plays an important role in the absence of Pten. Our results suggest that targeting p110β and/or Rac may lead to an effective therapeutic strategy for PTEN-deficient myeloid leukaemia.

Results

p110β ablation prolongs survival of Mx-1-Cre;Ptenfl/fl mice. To explore the roles of the class IA PI3K isoforms in HSCs and leukaemic transformation in the absence of Pten, we crossed Mx-1-Cre; Ptenfl/fl mice15 with strains that carry homozygous floxed alleles of p110α (ref. 25) or p110β (ref. 20), or with a p110δ germline knockout strain26 to allow for simultaneous deletion of Pten and PI3K isoforms in HSCs. As Mx1 is an interferon-responsive promoter expressed in HSCs and all other haematopoietic cells, the expression of Cre recombinase can be induced by administration of double-stranded RNA poly(I)-poly(C) (plpC), which induces an interferon response.27 Mx-1-Cre; Ptenfl/fl; p110α/α, Mx-1-Cre; Ptenfl/fl; p110β/β (PtenA/dPtenA/d), Mx-1-Cre; Ptenfl/fl; p110δ/δ (PtenA/dPtenA/d) and Mx-1-Cre; Ptenfl/fl; p110δ/δKO/KO (PtenA/dPtenA/d; p110δ/δ−/−) mice were treated with plpC to induce Cre-mediated excision of floxed alleles at 4 weeks of age (Supplementary Fig. 1a). This leads to the deletion of Pten and/or PI3K isoforms in haematopoietic cells, including HSCs or progenitor cells in the BM, spleen, thymus and liver.11,28 Consistent with previous studies10,11, all PtenA/d mice developed MPN and reached the survival end point 20–40 days post injection (DPI; Fig. 1a). PtenA/d; p110δ/δ and PtenA/d; p110δ−/− mice also developed MPN with slightly extended survival (Fig. 1a). Notably, PtenA/d; p110β/β mice lived the longest, with median survival significantly longer than that of any other group (Fig. 1a). Further observation revealed that, whereas control, PtenA/d, PtenA/d; p110α/α and PtenA/d; p110δ/δ animals developed MPN, six out of nine PtenA/d; p110β/β mice succumbed to T-ALL (Fig. 1b). BM from the three PtenA/d, p110β/β mice that did develop MPN was analysed for excision of the p110β allele. Notably, we found that BM of these mice had incomplete deletion of Pik3cb alleles suggesting that p110β is critical for the development of MPN in this model (Supplementary Fig. 1b). Deletion of p110β in HSCs using Mx1–Cre in animals that are wild-type (WT) for Pten does not significantly affect blood counts (Supplementary Fig. 1c).

Histopathological analysis of moribund animals of PtenA/d, PtenA/d; p110β/β and PtenA/d; p110β−/− mice at 20–40 days post plpC showed that they developed massive splenomegaly with a marked increase in cells expressing myeloperoxidase, a marker used to detect leukaemic cells of the myeloid lineage, in both the spleen and liver (Supplementary Fig. 2a), confirming MPN development in these mice. Notably, thymuses in these moribund mice appeared normal (Supplementary Fig. 2b). Different murine models has documented distinct requirements for different PI3K isoforms in particular tumour types.14,15 For example, p110δ is essential in a model of mutant Kras-induced lung adenocarcinoma.16 Recently, we showed that Ras-mutated myeloid leukaemia is also dependent on the p110α isoform, and combined pharmacologic inhibition of p110α and mitogen-activated protein kinase kinase (MEK) could be an effective therapeutic strategy for Ras-mutated myeloid malignancies.17 Although p110β plays a less prominent role in receptor tyrosine kinase (RTK) signalling, it mediates G protein-coupled receptor (GPCR) and integrin signalling,18–20 and has been shown to interact specifically with Rho family GTPases Rac1 and CDC42 (ref. 21). Several recent studies demonstrated that p110β is required in many, but not all, PTEN-deficient solid tumours.20,22,23 However, it is not known which PI3K isoforms are most important for myeloid neoplastic transformation driven by PTEN loss.

A number of pan-class I PI3K and dual class I/mTOR inhibitors are now in clinical trials for cancer, including leukaemia. However, targeting PI3K with these inhibitors could potentially lead to severe toxicity, which could be prevented by targeting single PI3K isoforms. To this end, numerous isoform-selective compounds are currently under development with some already in clinical trials.4,5 The p110β-selective inhibitor idelalisib (referred to here as GS1101) has been remarkably effective in treating indolent B-cell lymphoma,6,7 and is now approved by the FDA for the treatment of chronic lymphocytic leukaemia.4,5 In the case of solid tumours, p110α-selective inhibitors have shown great promise in early-phase trials for patients with tumours bearing PI3KCA mutations.4,5 Notably, selective inhibitors of p110β are in clinical trials as anticancer reagents for advanced solid tumours with PTEN deficiency (NCT01458067). Thus, unravelling the role of the p110β isoform, and its contribution to leukaemic transformation driven by PTEN loss, would inform rational approaches in targeting the PI3K pathway with a better therapeutic window.

In the present study, we used genetically engineered mouse models to determine which of the class IA PI3K isoforms are most important in mediating the effects of Pten loss in HSCs. We show that, in the setting of Pten loss, p110β is the main PI3K isoform responsible for MPN development and HSC depletion in the BM. Furthermore, we show that isoform-selective PI3K inhibitors recapitulate our genetic findings. We also found that a signalling loop featuring p110β and Rac plays an important role in the absence of Pten. Our results suggest that targeting p110β and/or Rac may lead to an effective therapeutic strategy for PTEN-deficient myeloid leukaemia.

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Previous studies have shown that Pten deletion in T-cell progenitors causes malignant transformation in the thymus and leads to T-cell lymphoma/T-ALL within 50–150 days. Mice with Mx1-Cre-mediated deletion of Pten\textsuperscript{+/−}, Pten\textsuperscript{+/−},p110Δ/Δ or Pten\textsuperscript{+/−},p110Δ/Δ showed infiltrating MPN disease and became moribund within 20–40 days after plpC, earlier than the disease latency for T-cell disease development. Since Pten\textsuperscript{+/−},p110Δ/Δ mice did not develop MPN, they survived longer and developed T-cell lymphoma/T-ALL ~50–70 days post plpC injection, a timeline consistent with previous reports on T-cell lymphoma/T-ALL formation in models of Pten loss in T-cell progenitors\textsuperscript{26,29}. This suggests that p110β ablation does not prevent T-ALL formation driven by Pten loss.

To examine further the PI3K isoform dependence in T-ALL induced by Pten loss, we investigated the roles of p110α and p110β in a different T-ALL model driven by Pten ablation in T-cell progenitors using Lck–Cre\textsuperscript{30}. Interestingly, deletion of either p110α or p110β had no effect on T-ALL in this model (Supplementary Fig. 2f). These results underscore the distinct roles of p110β in myeloid and lymphoid neoplasia induced by Pten deletion in haematopoietic cells.

**Figure 1 | Ablation of p110β prolonged survival of animals on Pten loss.** (a) Survival of Pten\textsuperscript{+/−}(blue; n = 9; median survival = 26 days), Pten\textsuperscript{+/−},p110α\textsuperscript{+/−} (green; n = 9; median survival = 31 days), Pten\textsuperscript{+/−},p110β\textsuperscript{+/−} (red; n = 9; median survival = 55 days), Pten\textsuperscript{+/−},p110α−/− (orange; n = 7; median survival = 37 days) and control animals (black) represented as DPI. The log-rank test was used to compare survival between groups. (b) Survival time and the development of MPN and T-ALL in each group of mice as indicated. Survival of Pten\textsuperscript{+/−} (n = 9; median survival = 26 days), Pten\textsuperscript{+/−},p110α\textsuperscript{+/−} (n = 9; median survival = 31 days), Pten\textsuperscript{+/−},p110β\textsuperscript{+/−} (n = 9; median survival = 55 days), Pten\textsuperscript{+/−},p110α−/− (n = 7; median survival = 37 days) and control animals (black) represented as DPI. Black dots represent MPN disease cases and red dots represent T-ALL cases in each group. The log-rank test was used to compare survival between groups. (c) Histopathology of the spleen (×1 and ×40) and liver (×100) taken at 26 DPI. Increased splenomegaly and disruption of spleen architecture as well as increased myeloid cell infiltration after Pten deletion. Scale bar, 5 μm. (d) Total cellularity of the spleen is represented as mean ± s.d. of n = 5 except Pten\textsuperscript{+/−},p110α−/− (n = 10). Two-way ANOVA test was applied to compare the spleen cellularity. (e) Representative flow cytometry plots of Mac1\textsuperscript{+} Gr1\textsuperscript{+} cells in the spleen. Samples were analysed at 26 DPI. Bar graphs represent the mean ± s.d. of n = 9 (Pten\textsuperscript{+/−},p110α−/−), 10 (Pten\textsuperscript{+/−},p110β−/−) or 11 (ctrl) or 13 (Pten\textsuperscript{+/−} and Pten\textsuperscript{+/−},p110β−/−). Two-way ANOVA test was applied to compare the Mac1-Gr1 cells in the spleen. *p < 0.05, **p < 0.01, ***p < 0.001
p110β mediates myeloid expansion induced by Pten loss. To further characterize disease in the Pten<sup>+/−</sup>:p110<sup>−/−</sup> mice, we killed mice of each genotype at 26 DPI, the time point at which the Pten<sup>+/−</sup> mice become moribund. Similar to previous reports, all animals in the Pten<sup>+/−</sup> group displayed massive splenomegaly, increased spleen cellularity and loss of spleen architecture at this time point (Fig. 1c,d). Both Pten<sup>+/−</sup>:p110<sup>−/−</sup> and Pten<sup>+/−</sup>: p110<sup>−/−</sup> mice showed evidence of MPN similar to that of Pten<sup>+/−</sup> mice, suggesting that ablation of p110β or p110δ failed to rescue this disease phenotype. Notably, Pten<sup>+/−</sup>:p110<sup>−/−</sup> mice had significantly reduced spleen cellularity and size, compared with Pten<sup>+/−</sup> mice (Fig. 1c,d). Consistently, pathological analysis of the spleen and liver revealed infiltration of myeloid cells in Pten<sup>+/−</sup>, Pten<sup>+/−</sup>:p110<sup>−/−</sup> and Pten<sup>+/−</sup>:p110<sup>−/−</sup> animals, but not in Pten<sup>+/−</sup>:p110<sup>−/−</sup> mice (Fig. 1c). Flow cytometric analysis confirmed an increased population of myeloid cells (Mac1<sup>+</sup>) in the BM, spleen and peripheral blood of Pten<sup>+/−</sup> animals (Fig. 1e; Supplementary Fig. 3). Again, the numbers of Mac1<sup>+</sup> Gr1<sup>+</sup> cells in these organs in Pten<sup>+/−</sup>:p110<sup>−/−</sup> mice but not in Pten<sup>+/−</sup>:p110<sup>−/−</sup> and Pten<sup>+/−</sup>:p110<sup>−/−</sup> animals were consistently reduced compared with those of Pten<sup>+/−</sup> mice (Fig. 1e; Supplementary Fig. 3), suggesting that ablation of p110β suppressed myeloid cell expansion on Pten loss.

To further validate the role of p110β in the myeloid expansion caused by Pten loss, we performed colony assays in methylcellulose supplemented with myeloid growth factors. Compared with wild-type controls, both BM and spleen cells from Pten<sup>+/−</sup> animals generated an increased number of colonies, which was significantly reduced in Pten<sup>+/−</sup>:p110<sup>−/−</sup> mice (Fig. 2a). Together, these results demonstrate that p110β is required for MPN development in the absence of Pten.

To determine whether the contribution of p110β to myeloid neoplasia in the absence of Pten is a cell-autonomous or indirect effect, we transplanted whole BM cells from Pten<sup>+/−</sup>, Pten<sup>+/−</sup>;p110<sup>−/−</sup>, or control mice into recipient mice (Fig. 2b). Four weeks after transplantation, all groups were treated with pIpC, and the relative frequency of CD45.2<sup>+</sup> donor-derived Mac1<sup>+</sup> Gr1<sup>+</sup> cells was monitored over 16 weeks. The proportion of Pten<sup>+/−</sup> donor-derived myeloid cells expanded significantly 4 weeks after pIpC and remained elevated during the course of the experiment. In contrast, the Pten<sup>+/−</sup>:p110<sup>−/−</sup> donor-derived myeloid population remained stable during the entire experiment, with levels much comparable to that of wild-type control mice (Fig. 2c). There were no significant differences in the percentage of donor-derived CD3-positive T cells among any of the groups tested (Supplementary Fig. 4a). The percentage of donor-derived B220-positive B cells was reduced after Pten depletion, and deletion of p110β did not alter B-cell chimaerism (Supplementary Fig. 4b). These data suggest that p110β mediates the expansion of myeloid cells in a cell-autonomous manner.

**p110β perturbs HSC homeostasis on loss of Pten.** Earlier studies showed that HSC-specific deletion of Pten leads to the exhaustion of HSCs in the BM, their accumulation in the periphery and extramedullary haematopoiesis<sup>10,11</sup>. Hence, we wanted to test whether p110β ablation could rescue HSCs. In Pten<sup>+/−</sup> mice, the numbers of both the Lin<sup>−</sup> Sca-1<sup>+</sup> c-kit<sup>−</sup> (LSK) cells, containing HSCs and the CD150<sup>+</sup> CD48<sup>−</sup> Lin<sup>−</sup> Sca-1<sup>+</sup> c-kit<sup>−</sup> population, which is enriched for long-term HSCs (LT-HSCs) were significantly reduced at 26 DPI, consistent with previous findings<sup>11</sup> (Fig. 3a; Supplementary Fig. 5a). Ablation of p110β was able to partially rescue LSK cells and restore LT-HSCs in Pten-null BM (Fig. 3a). Loss of Pten did not change the total number of myeloid progenitors, or the frequencies of the common myeloid progenitors and granulocyte macrophage progenitors or megakaryocyte-erythroid progenitors, but led to a significant decrease in the number of common lymphoid progenitors consistent with original reports<sup>11</sup> (Supplementary Fig. 5b).

**Figure 2 | Ablation of p110β blocked MPN development in a cell-autonomous manner.** (a) Colony-forming assays were performed with 1 × 10<sup>4</sup> (BM) or 5 × 10<sup>5</sup> (spleen) cells obtained from corresponding animals. Cells were cultured in M3434 medium. Colonies were counted 7 days after incubation. Data are represented as mean ± s.e.m. (n = 3, in duplicates). *P < 0.05, **P < 0.01, ***P < 0.001. (b) BM cells were isolated from CD45.2<sup>+</sup> WT, Pten<sup>+/−</sup> and Pten<sup>+/−</sup>:p110<sup>−/−</sup> animals and transplanted into CD45.1<sup>+</sup> mice as shown in the figure (n = 10 for each group). (c) The myeloid cell reconstitution potential of the CD45.2<sup>+</sup> cells was measured at regular intervals for 16 weeks. WT and Pten<sup>+/−</sup>:p110<sup>−/−</sup>-derived myeloid cells have a stable amount of CD45.2<sup>+</sup> myeloid cells; however, Pten<sup>+/−</sup>-derived myeloid cells expanded significantly and reached to 60% at week 16 (n = 10 for all groups, **P < 0.001). Two-way ANOVA test was applied to compare the myeloid cell potential.
between the BM and extramedullary tissues in stem cell numbers. (HSC (bottom right) cells in the spleen measured at 26 DPI. Numbers represent total cell counts per spleen (Pten we measured the frequency and absolute numbers of HSCs and extramedullary haematopoiesis in the spleen seen after Pten loss, p110 Supplementary Fig. 5c,d). These results suggest that deletion of NATURE COMMUNICATIONS | DOI: 10.1038/ncomms9501 ARTICLE

Fig. 3 | p110β deletion restores HSCs to both the bone marrow (BM) and the spleen. (a) Representative flow cytometry plots of ctrl, PtenΔ/Δ and PtenΔ/Δ;p110βΔ/Δ BM showing LSK cells (Lin−Sca-1−c-kit+) , ST-HSC (CD150+CD48−Lin− Sca-1−c-kit+) and LT-HSC (CD150−CD48−Lin− Sca-1−c-kit+) cells (left panels) gated on total living CD45.2+Lin+ cells. Total counts LSK (top) and LT-HSC (bottom right) of lineage-negative cells in the BM measured at 26 DPI in ctrl (n = 13), PtenΔ/Δ (n = 12) and PtenΔ/Δ;p110βΔ/Δ (n = 18) animals. Two-way ANOVA test was applied to compare the stem cell numbers. (b) Representative flow cytometry plots of control, PtenΔ/Δ and PtenΔ/Δ;p110βΔ/Δ spleen cells showing LSK cells (Lin−Sca-1−c-kit+), ST-HSC (CD150+CD48−Lin− Sca-1−c-kit+) and LT-HSC (CD150−CD48−Lin− Sca-1−c-kit+) cells (left panels). Total LSK (top) and ST-HSC and LT-HSC (bottom right) cells in the spleen measured at 26 DPI. Numbers represent total cell counts per spleen (n = 7 (ctrl, PtenΔ/Δ) and 10 (PtenΔ/Δ;p110βΔ/Δ)). Two-way ANOVA test was applied to compare the stem cell numbers. *P<0.05, **P<0.01, ***P<0.001.

Fig. 5b). This decrease in the number of common lymphoid progenitors in the Pten-deficient BM was not rescued by the deletion of p110β (Supplementary Fig. 5b). To determine whether p110β is responsible for the increased extramedullary haematopoiesis in the spleen seen after Pten loss, we measured the frequency and absolute numbers of HSCs and progenitors in the spleens of PtenΔ/Δ and PtenΔ/Δ;p110βΔ/Δ animals. Pten deficiency led to a significant increase in the number of LSK cells, as well as LT-HSCs, short-term HSCs (ST-HSCs) and progenitors in the spleens of PtenΔ/Δ animals (Fig. 3b; Supplementary Fig. 5c,d),1,3,1 which was partially suppressed in the spleens of PtenΔ/Δ;p110βΔ/Δ animals (Fig. 3b; Supplementary Fig. 5c,d). These results suggest that deletion of p110β normalizes the distribution of phenotypic LSK cells between the BM and extramedullary tissues in PtenΔ/Δ;p110βΔ/Δ animals compared with PtenΔ/Δ controls. Thus, our findings are consistent with the idea that p110β contributes to the perturbed HSC homeostasis observed in the absence of Pten leading to extramedullary haematopoiesis and the development of MPN.

p110β mediates leukaemia initiation in the absence of Pten. It has been reported that Pten loss in the BM leads to the depletion of HSCs and the generation of leukaemia-initiating cells10,11. To determine whether the p110β isoform also uniquely plays critical roles in these processes in the Pten-loss setting, we performed competitive multi-lineage repopulation assays to compare the contribution of marked (CD45.2) PtenΔ/Δ, PtenΔ/Δ;p110βΔ/Δ, PtenΔ/Δ;p110βΔ/Δ donor BM cells to that of wild-type competitor cells (CD45.1) following transplantation into lethally irradiated mice. Consistent with earlier reports, the contribution of donor-derived cells to the peripheral blood was progressively decreased and eventually depleted for PtenΔ/Δ donors, but not for control donors10,11 (Fig. 4a). HSCs derived from PtenΔ/Δ;p110βΔ/Δ mice also failed to sustain long-term reconstitution. In striking contrast,
HSCs derived from \( Pten^{+/A}\times p110^\beta^{+/A} \) mice were able to reconstitute recipient animals for more than 20 weeks (Fig. 4a). In addition, the majority of \( Pten^{+/A} \) and \( Pten^{+/A}\times p110x^{+/A} \) recipient mice developed T-ALL as evidenced by the abundance of donor-derived CD45.2\(^+\)CD3\(^+\),CD4\(^+\) or CD3\(^+\)CD4\(^-\)T lymphoblasts at the experimental end point of 20 weeks in these mice (Fig. 4b; Supplementary Fig. 6). In contrast, recipients of BM from wild-type control mice and from \( Pten^{+/A}\times p110x^{+/A} \) animals remained leukaemia free with few CD45.2\(^+\)CD3\(^+\) cells at the experimental end point (Fig. 4b; Supplementary Fig. 6). Furthermore, analysis of the BM at week 20 showed that the donor chimaerism in the LSK, ST-HSC and LT-HSC compartments was significantly improved in the \( Pten^{+/A} \) and \( Pten^{+/A}\times p110x^{+/A} \) groups (Fig. 4c). These data suggest that, in the absence of \( Pten \), \( p110^\beta \) is the major PI3K isomter critical for the loss of HSCs and for leukaemia initiation.

To determine the cellular mechanism underlying the improved reconstitution of \( Pten \)-null BM cells on loss of \( p110^\beta \), we examined the cell cycle status, senescence, apoptosis and homing properties of HSCs. As reported earlier, we also found that loss of \( Pten \) led to increased cycling of HSCs and reduced the number of quiescent HSCs (Supplementary Fig. 7a).\(^{11}\) Although ablation of \( p110^\beta \) resulted in a tendency towards rescuing these effects, the results did not reach statistical significance (Supplementary Fig. 7a). Moreover, we did not observe any change in the proportion of whole BM or LSK cells expressing senescence-associated \( \beta \)-gal activity or undergoing apoptosis in any of the groups tested (Supplementary Fig. 7b,c). We then performed homing assays, in which fluorescently labelled BM cells were transplanted into irradiated wild-type hosts, and donor-derived cells were quantified after 24 h. We found that \( Pten \) deficiency significantly reduced the homing capacity of transplanted cells to
the BM, and p110β ablation could partially rescue the homing potential (Fig. 4d). We also found that p110β ablation does not affect homing in Pten-null-type BM cells (Fig. 4d). Thus, we conclude that p110β is responsible, at least in part, for the reduced homing activity of Pten-deficient HSCs.

**Inhibition of p110β suppresses myeloid leukaemogenesis.** To determine whether our findings using a genetic method could be recapitulated by pharmacologic approaches utilizing PI3K isoform-selective inhibitors at effective doses as published in earlier studies, we first examined the effects of PI3K isoform inhibition on myeloid progenitor function. We cultured BM cells and splenocytes from Pten+/− animals in methylcellulose supplemented with myeloid growth factors in the presence of PI3K inhibitors. As reported in previous studies10, the pan-PI3K inhibitor GD0941 and the mTOR inhibitor RAD001 significantly suppressed the increased colony formation arising from Pten deletion in a dose-dependent manner (Fig. 5a). Consistent with our genetic findings, inhibition of p110b by BYL719 (a p110b-selective inhibitor)21, and p110β with GS1101 (ref. 33) had a modest effect on the expansion of myeloid cells in the context of Pten loss (Fig. 5a). Since p110γ is also expressed in leukocytes, we tested the p110γ inhibitor NVSP315 (ref. 34). Interestingly, inhibition of p110γ with NVSP315 showed some effect on BM cells, but not on splenocytes (Fig. 5a). Notably, inhibition of p110β with KIN193 (a p110β-selective inhibitor, also known as AZD6482)14,22 significantly reduced formation of both BM- and spleen-derived myeloid colonies in methylcellulose in a dose-dependent manner (Fig. 5a) suggesting that pharmacologic inhibition of p110β is highly effective in suppressing myeloid cell expansion driven by Pten loss.

To further determine the effects of pharmacologic inhibition of PI3K isoforms in vivo, we tested a group of plpC-induced Pten+/− animals with BYL719, KIN193, IC87114 (a p110β-selective inhibitor in the same class as GS1101, but with better bioavailability in mice)35, AS605240 (p110γ-selective inhibitor suitable for in vivo studies)36 or a vehicle control 10 days after induction (Fig. 5b). All the inhibitors were used at the effective doses in vivo as published in earlier studies36–38. Treatment with BYL719 (ref. 37), IC87114 (ref. 38) or AS605240 (ref. 36) resulted in a minimal survival benefit compared with vehicle-treated animals (Fig. 5c). Notably, mice treated with KIN193 had a significantly longer survival as compared with mice in any other group (Fig. 5c). Moreover, KIN193-treated animals appeared healthy and had significantly reduced spleen weights and normal-looking spleen architecture compared with vehicle-treated mice (Fig. 5d; Supplementary Fig. 8), consistent with our genetic data that ablation of p110β largely prevented myeloid leukaemia in Pten-deficient mice.

Next, we examined PI3K/Akt signalling in Pten-null BM cells in response to isoform-selective inhibition. As expected, vehicle-treated Pten-null BM cells from Pten+/− animals showed markedly increased Akt phosphorylation compared with wild-type BM cells (Fig. 5e). Interestingly, inhibition of either p110α, p110β or p110γ led to a significant reduction of p-Akt, compared with the controls (Fig. 5e). We also examined Akt activation by measuring basal p-Akt levels in LSK cells by intracellular phosopho-flow cytometry after Pten deletion and short-term isoform-selective inhibitor treatment of lineage-negative cells from Pten+/− BM. We detected significantly higher basal levels of p-Akt in Pten+/− LSK cells compared with WT LSKs, and again inhibition of p110α, p110β, p110γ or p110γ led to a significant reduction of p-Akt compared with the vehicle group (Fig. 5f). Since p110β is not the only isoform responsible for mediating Akt signalling in Pten-deficient BM and HSCs, Akt signalling alone is not sufficient to explain the specific biological effects of p110β ablation or inhibition observed in our study.

**Pten-deficient HSCs depend on the p110β–Rac axis.** Recent data suggested that p110α, p110β and p110γ bind to and are activated by the Ras subfamily of GTPases, while p110β instead binds to and is activated by the Rho subfamily GTPases, Rac1 and CDC42 via its ‘Ras-binding domain’ (RBD)17,21. Previous studies also reported that an intact RBD was required for signalling and oncogenic transformation by wild-type p110β, suggesting a potential role for the interaction of Rho GTPase with p110β in transformation19,40. Notably, Rac1 and CDC42 can also be activated downstream of PI3K by PI3P-dependent guanine-nucleotide exchange factors41,42. It has been previously reported that Rac plays important roles in the homing and survival of HSCs43,44. Given the significant rescue of HSCs in Pten+/−, p110β+/−/A mice, we hypothesized that a unique positive-feedback signalling loop might exist between p110β and Rac, in which p110β is activated by Rac and Rac could in turn be activated by the phosphatidylinositol products of p110β in the setting of Pten-null haematopoietic cells.

Notably, we detected higher levels of Rac–GTP in the BM of Pten+/− mice, which could be suppressed by deletion of p110β but not p110α (Fig. 6a). To investigate whether the binding of p110β to Rac is important in mediating p110β activity in Pten-deleted BM cells, we mutated the two highly conserved key residues within the p110β RBD to generate a p110β–S205D/K224A double mutant lacking the binding activity to Rac1 (ref. 21), and performed an add-back experiment with either wild-type or RBD-mutant p110β in BM cells derived from Pten+/−,p110β+/−/A mice (Fig. 6b; Supplementary Fig. 10). Colony-forming assays revealed that, while adding back a wild-type p110β in Pten+/−,p110β+/−/A BM cells restored myeloid colony numbers comparable to those of Pten+/−/A BM cells, the RBD-mutant p110β failed to rescue colony formation (Fig. 6c). To determine whether p110β affects Rac–GTP levels in HSCs/progenitor cells (HSPCs), we performed the Rac–GTP assay either on Lin-negative Pten+/− BM cells or Pten+/−,p110β+/−/A BM cells expressing wild-type p110β and RBD-mutant p110β. We detected higher levels of Rac–GTP in the Pten-deficient cells compared with WT control cells, and these levels were significantly reduced in Pten+/−,p110β+/−/A cells. Adding back wild-type p110β to Pten+/−,p110β+/−/A HSC/P cells, partially rescued Rac–GTP levels but adding back RBD-mutant p110β failed to rescue Rac activity (Fig. 6d; Supplementary Fig. 10). Together, these data suggest that the interaction of p110β with Rac plays an important role in mediating the myeloid clonogenic activity driven by Pten loss.

To further investigate the functional dependency of Pten-deleted leukaemic cells on the p110β–Rac axis, we utilized NSC23766, a potent Rac inhibitor45 in our Pten-null model (Fig. 7a). We found that treatment of these mice for 10 days led to a reduced disease burden, as demonstrated by reduced spleen size and cellularity (Fig. 7a; Supplementary Fig. 9). Similarly, treatment of Pten+/− mice with NSC23766 resulted in a significant reduction of HSC and myeloid progenitor numbers in the spleen compared with vehicle controls (Fig. 7b; Supplementary Fig. 9). Treatment with NSC23766 also led to a significantly prolonged survival of Pten+/−/A mice (Fig. 7c), recapitulating the findings for genetic ablation or pharmacological inhibition of p110β in Pten-null animals.

Since Rac is required for p110β activation downstream of GPCRs21, we assessed the functional importance of the Rac–p110β signalling axis in HSC function in response to activation of CXCR4, a GPCR important in the regulation of HSPCs. We used Transwell migration assays with CXCL12, a...
potent chemo-attractant of stem cells that signals through CXCR4. Lineage-negative BM cells from PtenΔ/Δ mice showed increased migration towards CXCL12 compared with WT control cells (Fig. 7d). This migration was abolished by the GPCR inhibitor pertussis toxin (PTX; Fig. 7d). Interestingly, we observed significantly reduced migration of PtenΔ/Δ;p110βΔ/Δ cells, and of PtenΔ/Δ cells treated with either KIN193 or NSC23766, compared with PtenΔ/Δ and PtenΔ/Δ;p110βΔ/Δ cells (Fig. 7d). This suggests that deletion of p110β, or pharmacologic inhibition of either p110β or Rac, partially interferes with the migration of PtenΔ/Δ.
cells towards a CXCL12 gradient, likely through perturbed GPCR signalling.

Because murine models of haematopoietic-specific Rac1 and Rac2 deficiency have revealed differential roles of Rac proteins in terms of HSPC function, we wanted to understand which Rac isoform is more important in the absence of Pten. To this end, we used siRNA to knockdown either Rac1 or Rac2, or both, and performed colony assays on Pten<sup>Δ/Δ</sup> and Pten<sup>Δ/Δ</sup>;p110β<sup>Δ/Δ</sup> BM cells (Fig. 7c; Supplementary Fig. 10). We also tested the Rac inhibitor NSC23766, which targets both Rac1 and Rac2 (ref. 46). Knockdown of either Rac1 or Rac2, or their combined knockdown or pharmacological inhibition significantly reduced colony formation by Pten<sup>Δ/Δ</sup> cells to levels obtained with Pten<sup>Δ/Δ</sup>; p110β<sup>Δ/Δ</sup> BM cells (Fig. 7f). However, knockdown of Rac1 or Rac2, the combination, or NSC23766 treatment did not further suppress colony formation beyond the effects of p110β deletion (Fig. 7f), suggesting that there is no additive or synergistic effect of Rac inhibition with p110β deficiency in Pten<sup>Δ/Δ</sup> cells. Together, these results suggest that p110β–Rac1/2 work in concert to mediate the effects of Pten loss in promoting myeloid neoplasia.

**Discussion**

We and others have reported that Pten-deficient solid tumours frequently rely on p110β (refs 20,23,47). In this study, we report for the first time an essential role for p110β in promoting haematologic neoplasia driven by Pten deletion in HSCs despite the expression of four different PI3K isoforms in haematopoietic cells. We have also found that p110β contributes to HSC depletion in the BM after Pten deletion. Interestingly, we found that Mx1–Cre-mediated deletion of p110β in HSPCs of animals that are WT for Pten does not significantly affect blood counts. In fact, these animals appear healthy for many months after excision, suggesting that targeting p110β may lead to an effective therapy for myeloid leukaemia with little toxicity to normal HSCs.

Despite the marked impact of genetic deletion or pharmacologic inhibition of p110β on MPN, and the significantly delayed

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**Figure 6 | Pten-deficient haematopoietic cells depend on the p110β–Rac1 signalling axis.** (a) Rac–GTP activity assay was performed on BM after 7 DPI in the corresponding genotypes. Rac–GTP and total Rac were measured and normalized (n = 3; mean with s.e.m.). Two-way ANOVA test was applied to compare the Rac–GTPase/RAC levels. **b** Pten<sup>Δ/Δ</sup>;p110β<sup>Δ/Δ</sup> BM cells were transduced with retrovirus expressing HA-tagged wild-type P110β or RBD-mutant P110β, and briefly selected in the presence of puromycin. HA immunoblotting was performed to show expression of RBD-mutant and wild-type P110β (n = 3 for each group). **c** Cells from WT control, Pten knockout or Pten<sup>Δ/Δ</sup>;p110β<sup>Δ/Δ</sup> animals were cultured in methylcellulose with myeloid growth factors in the presence of puromycin for 7 days, and colonies were counted at the end of day 7 (n = 3 for each group). Two-way ANOVA test was applied to compare the colony numbers. (d) Lineage-negative cells were isolated from WT control, Pten<sup>Δ/Δ</sup> or Pten<sup>Δ/Δ</sup>;p110β<sup>Δ/Δ</sup> mice, and the Rac–GTP activity assay was performed as in (a). Rac–GTP and total Rac were measured and normalized to WT (n = 6; mean with s.e.m.). Two-way ANOVA test was applied to compare the Rac–GTPase/RAC levels. **a** P < 0.01, ***P < 0.001.
**Figure 7 | Pten-deficient haematopoietic cells depend on the p110α-Rac1 signalling axis.** (a) NSC23766 was given at 2.5 μg g⁻¹ daily intraperitoneally (IP). Spleen size and cellularity of vehicle-treated (n = 3) and NSC23766-treated Pten<sup>Δ/Δ</sup> animals (n = 3) at 17 DPI. *P < 0.05; **P < 0.001; ***P < 0.0001. Two-way ANOVA test was applied to compare spleen size. (b) Quantification of the numbers of LSK (Lin-Sca-1⁺ c-kit⁻), ST-HSC (CD150⁺CD48⁻ Lin⁻ Sca-1⁺ c-kit⁺) or LT-HSC (CD150⁺CD48⁻ Lin⁻ Sca-1⁺ c-kit⁻) cells in the spleens of control, Pten<sup>Δ/Δ</sup> animals treated with vehicle, or Pten<sup>Δ/Δ</sup> animals treated with NSC23766 at 17 DPI, determined by flow cytometry. The numbers represent total cell counts per spleen (n = 3; ctrl, Pten<sup>Δ/Δ</sup> vehicle and NSC23766 treated). (c) Survival of Pten<sup>Δ/Δ</sup> animals treated with vehicle (blue; n = 7; median survival = 22 days) or NSC23766 (red; n = 7; median survival = 42 days). **P < 0.005. The log-rank test was applied to compare survival. (d) Lineage-negative BM cells were isolated from control, Pten<sup>Δ/Δ</sup> or Pten<sup>Δ/Δ</sup>:p110β<sup>Δ/Δ</sup> Pten<sup>Δ/Δ</sup>:p110β<sup>Δ/Δ</sup> on 7 DPI, and lineage-negative cells were pretreated for 3 h with KIN193, NSC23766 (50 μM) or pertussis toxin (PTX; 100 ng ml⁻¹). Migration towards the lower chamber (supplemented with CXCL12, 50 nM) was measured as the fraction of migrated cells relative to untreated Pten-null bone marrow-derived cells. Numbers of replicates are n = 4 (basal, Pten<sup>Δ/Δ</sup> no Cxcl12; Pten<sup>Δ/Δ</sup> + PTX), 10 (Pten<sup>Δ/Δ</sup>), 6 (Pten<sup>Δ/Δ</sup>:P110β<sup>Δ/Δ</sup> and 6 (Pten<sup>Δ/Δ</sup> + KIN193). Statistical comparisons were all performed relative to the Pten<sup>Δ/Δ</sup> group. (e) Pten<sup>Δ/Δ</sup> and Pten<sup>Δ/Δ</sup>:p110β<sup>Δ/Δ</sup> BM cells were transfected with two different siRNAs targeting scrambled, mouse Rac1, mouse Rac2 alone, or both Rac1 and Rac2 and subjected to immunoblotting 48 h later. (f) Pten<sup>Δ/Δ</sup> and Pten<sup>Δ/Δ</sup>:p110β<sup>Δ/Δ</sup> BM cells were transfected with different siRNAs targeting Rac1 and Rac2 and subjected to immunoblotting 48 h later. (g) A working model of signalling cascades downstream of p110β and the signalling loop of p110β and Rac1.
onset of leukaemia, a fraction of Pten+/a;p110β/a/a animals succumbed to T-ALL at a later time. Berenjeno et al.48 showed that in Pten+/−; mice, inactivation of p110β led to reduced PIP3 generation in lymphoma tissues, but had little impact on lymphoma formation. It is possible that these tumours become p110β independent through the acquisition of secondary alterations. In fact, Yilmaz et al.15 have documented the presence of cytogenetic alterations in leukaemic blasts from Pten+/a/a animals. Alternatively, isoform dependency may shift with cell differentiation. For example, the isoform dependency in the skin hamartoma driven by Pten loss changed from a p110β dependency in the basal layer of the epidermis to a p110α dependency in the suprabasal cells as the basal cells underwent stepwise differentiation to become suprabasal cells37. In this study, we also provide evidence that neither p110x nor p110β has any effect on T-ALL driven by T lymphocyte-specific deletion of Pten using Lck−Cre. In this system, it has been shown that p110β and p110γ contribute to T-ALL induced by Pten loss in T cells28. These studies provide additional data that accentuate the distinct roles of p110β in the HSCs and in myeloid and lymphoid tumour initiation in the absence of Pten.

Interestingly, we found that inhibition of p110β, p110x or p110δ could similarly reduce p-Akt in Pten-deficient BM and HSCs, suggesting an Akt-independent pathway specific to p110β is important in Pten-deficient HSCs in promoting myeloid neoplasia. We report a new mechanistic insight that may explain the unique role of p110β in this setting. Since p110β binds to Rac rather than to Ras via its RBD, unlike the other class I PI3K isoforms21, we investigated the role of the p110β–Rac axis in the setting of myeloid neoplasia induced by Pten loss in HSCs. Notably, Rac signalling is not only important for the activation of p110β but it itself is also activated by PIP3 via PIP3-activated guanine-nucleotide exchange factors, forming a potential signalling loop (Fig. 7g)14,49. We found strong evidence that this loop is indeed active in our Pten-deficient model. We show that Rac was activated in Pten-null BM cells, and this activation was suppressed in Pten+/p110β double KOs, but not in Pten+/p110x double KOs. This hypothesis was further supported by our finding that only wild-type p110β, but not the RBD mutant of p110β, rescued colony formation in Pten+/p110β-deficient BM cells. Notably, the effect of the RBD-mutant p110β on inhibiting colony formation in Pten-null BM cells is comparable to that of p110β deletion. An intact RBD was required to be reported for membrane localization of p110β for both signalling and oncogenic transformation by wild-type p110β in cultured cells40. Our data suggest that the interaction of p110β–Rac may play an important role in mediating p110β activity downstream of GPCRs and tyrosine kinases in the context of Pten deficiency. Moreover, pharmacologic inhibition of p110β or Rac in Pten-deficient mice resulted in a strikingly similar functional rescue in vivo, with a reduction in extramedullary haematopoiesis in the spleen and improved survival.

Of the three isoforms of Rac family GTPases, Rac2 is expressed specifically in haematopoietic cells, while Rac1 and Rac3 are ubiquitously expressed44,45,50. The Rac inhibitor NSC23766 targets all three Rac members: Rac1, 2 and 3 (ref. 46). Both Rac1 and CDC42 have been shown to bind p110β in a recent study21. Binding to Rac2/3 was not tested, but might also be expected, based on the homology of their effector domains with that of Rac1. Previous studies have suggested that Rac1 and Rac2 play both distinct and overlapping roles in HSCs and progenitor cells, while the role of Rac3 in haematopoiesis has not been defined44,51. It also has been shown that targeting both Rac1 and Rac2 was effective in a mouse model of BCR–ABL-induced MPN, as well as in a mouse model of MLL–AF9 AML52,53. Interestingly, our data show that the effect of knockdown of Rac2 is comparable to that of combined knockdown of both Rac1 and Rac2, or a pan-Rac inhibitor NSC23766. Since Rac2 is primarily expressed in haematopoietic cells, our data suggest that Rac2 could potentially be a better pharmacologic target with reduced toxicity.

Yilmaz et al. have shown that the mTOR inhibitor rapamycin can rescue HSC depletion and can suppress the development of leukaemia in vivo. More recently, the Armstrong and Morrison groups reported that mTORC1 and mTORC2 play critical roles in haematopoiesis and Pten-loss-driven leukaemogenesis, respectively33,34. By ablation or inhibition of p110β, we obtained similar results suggesting that the activation of mTOR in HSCs by Pten loss may be mediated largely by p110β. A recent study demonstrated that Rac1 regulates the activity of both mTORC1 and mTORC2 (ref. 55), providing a potential Akt-independent link between p110β–Rac and mTOR. Together, these data suggest that p110β–Rac acts upstream of Akt and mTOR. We feel that our data are most consistent with the working model shown in Fig. 7g, in which p110β works in a signalling loop with Rac to generate the key signals arising from Pten loss. Notably, these signals include both Akt-dependent and Akt-independent pathways leading to cell proliferation and migration.

In summary, our results provide the first evidence that P3K–p110β plays an essential role in controlling HSC function in the setting of Pten loss56. We have also uncovered a specific role for p110β in myeloid leukaemia induced by Pten deficiency. In contrast, we found that p110β is dispensable for T-ALL induced by Pten loss. Most importantly, our data show that a p110β–Rac signalling loop is important for the induction of myeloid neoplasia in the absence of Pten. Thus, second-generation p110β- or Rac-selective inhibitors may interrupt this loop, thereby providing a promising new therapeutic strategy for Pten-deficient myeloid leukaemias while preserving normal haematopoiesis.

Methods
Animal experiments. Mice were housed in a pathogen-free animal facility at Dana-Farber Cancer Institute (DFCI). All animal experiments were performed in accordance with the relevant regulatory standards, guidelines and regulations, and were approved by the DFCI-Institutional Animal Care and Use Committee (IACUC). Dead and moribund animals were included in survival curves. Pten+/− mice (obtained from Hong Wu’s Laboratory) were crossed with Pten+/−; animals carrying an Mx-1-Cre transgene (obtained from Dr Gary Gilliland’s laboratory) to obtain Mx-1−Cre−/−; Pten+/− mice (Pten+/−); p110x and p110β mutants were obtained by crossing Mx-1−Cre−/−; Pten−/−; mice with p110x+/−, p110x−/− and Mx-1−Cre−/−; Pten−/−; p110β−/−, and Mx-1−Cre−/−; Pten−/−; p110β−/− mice (Pten−/−; p110β−/−). P110β−/− germline knockout animals (James Ihle’s laboratory) were backcrossed to the C57BL/6 background for more than nine generations and then crossed with Mx-1−Cre−/−; Pten−/−; mice to obtain Mx-1−Cre−/−; Pten−/−; p110β−/− (Pten−/−; p110β−/−). Thirty-day-old male and female animals were induced by intraperitoneal injection with pIpC (25 mg g−1; GE Healthsciences) every other day for 5 days. WT3x-M1−Cre− mice were used as controls for all experiments. Peripheral blood was obtained by retro-orbital venous blood sampling. Periperal blood counts were analysed on a Hemavet 950FS blood analyzer (Drew Scientific).

Flow cytometry. All data acquisition was performed on a LSRII (BD) flow cytometer, and results were analysed using FlowJo v.8.8.7 (Tree Star). Antibodies used for flow cytometry were directly coupled and directed against B220 (APC, BD Pharmingen), Kit (PE-Cy7, Biologic), CD3 (PE-Cy7, BD Bioscience), CD4 (APC-H7, BD Pharmingen; CD4, ECD, Beckman Coulter), CD11b (PE, BD Bioscience), CD16/32 (PE, eBioscience), CD34 (FITC, BD Pharmingen), CD45.2 (PerCP-Cy5.5, BD Pharmingen), CD48 (APC-Cy7, BD Pharmingen), CD127 (ECD, BD Pharmingen), CD130 (PerCP-Cy5.5, Biologic), Gr1 (APC-Alexa 700, BD Bioscience), lineage cocktail (APC, BD Pharmingen), Sca1 (Brilliant Violet 421, BioLegend), K67 (Alexa 488, BD Bioscience). Dead cells were excluded using either DAPI or Vivid-Aqua.

Chemotaxis assays. Fresh BM cells were isolated from mutant mice and from control animals, and lineage-negative cells were depleted using magnetic separation
(lineage depletion kit, Miltenyi Biotec) and were resuspended in PBS containing 0.5% BSA. Lineage-negative cells (0.5–1 million) were pretreated for 3 h at 37°C with the lineage depletion kit (Clontech) and were then placed in wells containing CXCL12 (50 nM, PeproTech), and incubated for 3 h at 37°C. Cells migrating from the top to the bottom chamber were collected and quantified using a Countess cell counting machine (Invitrogen).

Compounds. TGXX21 was obtained from Chemedia; NSC23766 and IC87114 from Selleck Chemicals; GDC0941 from MedChemExpress; KIN193 from Haoyuan Chemexpress; NVP-BYL719, NVSPi3, AS605240 and IC87114 were synthesized by Chemietik (Indianapolis, USA). Mice were dosed once by intraperitoneal injection of KIN193 (20 mg kg

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Long-term competitive repopulation assays. Recipient mice (4–6 weeks old female mice; B6-SJL strain) received two doses of 540 rads each, delivered 3 h apart. Nucleated BM cells from control and Pten

Plasmid constructs. Mutations were generated in the pBabe-HA wild-type P110β by site-directed mutagenesis to obtain p110β-S250D/R224A double mutant to obtain Ras-binding mutant P110β. This plasmid was used to transduce murine BM cells and for colony-forming experiments.

Statistical methods. Two-tailed T-tests (two groups) and analysis of variance (ANOVA) tests (multiple groups) and log-rank statistics (Mantel-Cox test; survival studies) were used, and calculated with the use of Prism 6.0 (GraphPad). Data are represented as mean ± s.d. unless otherwise noted. *P < 0.05; **P < 0.01; ***P < 0.001.

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