PTEN encodes a lipid phosphatase that is underexpressed in many cancers owing to deletions, mutations or gene silencing3,9. PTEN dephosphorylates phosphatidylinositol (3,4,5)-triphosphate, thereby opposing the activity of class I phosphatidylinositol 3-kinases that mediate growth- and survival-factor signalling through phosphati-

dylinositol 3-kinase effectors such as AKT and mTOR2. To determine whether continued PTEN inactivation is required to maintain malignancy, here we generate an RNA interference-based transgenic mouse model that allows tetracycline-dependent regulation of PTEN in a time- and tissue-specific manner. Postnatal Pten knockdown in the haematopoietic compartment produced highly disseminated T-cell acute lymphoblastic leukaemia. Notably, reactivation of PTEN mainly reduced T-cell leukaemia dissemination but had little effect on tumour load in haematopoietic organs. Leukaemia infiltration into the intestine was dependent on CCR9 G-protein-coupled receptor signalling, which was amplified by PTEN loss. Our results suggest that in the absence of PTEN, G-protein-coupled receptors may have an unan-
ticipated role in driving tumour growth and invasion in an unsupportive environment. They further reveal that the role of PTEN loss in tumour maintenance is not invariant and can be influenced by the tissue microenvironment, thereby producing a form of intratu-



**Figure 1** | Pten shRNA transgenic mice develop disseminated CD4+ CD8+ double-positive T-cell leukaemia. a, Outline of the targeting construct and the embryonic stem (ES) cell-targeting strategy. FRT, FLP recognition target; pA, polyadenylation site; PGK, phosphoglycerate kinase promoter; SA, splice acceptor site; TRE, tetracycline-responsive element promoter. ATG* denotes truncated ATG sequence; *Hygromycin denotes ATG-less hygromycin resistance gene. b, Immunoblot (western blot) analysis of MEFs from shPten;R26-rtTA2 transgenic mice and the frequent inactivation of PTEN in human T-cell acute lymphoblastic leukaemia (T-ALL),4 we focused on the effects of PTEN suppression and reactivation in the lymphoid compartment. We crossed mice transgenic for an shRNA against luciferase (shLuc) and shPten mice to a Vav-
tTA transgenic line, which expresses a ‘Tet-off’ Tet transactivator in early B and T cells39 and drives shRNA expression in a manner that is silenced upon Dox addition (Extended Data Fig. 2 and data not shown). The Vav-tTAshPten mice displayed thymic hyperplasia (Extended Data Fig. 2a–d) and, by 16 weeks, a subset deteriorated and had to be eutha-
nized (Fig. 1c), whereas control animals remained healthy (P < 0.001). Diseased mice showed massive enhanced green fluorescent protein (eGFP)-positive tumours that consisted of Thy1.2+ CD4+ CD8+ double-



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Human T-ALLs with PTEN loss often overexpress MYC and harbour NOTCH1 and CDKN2A mutations\(^1\). Analysis of murine shPten-expressing tumours by spectral karyotyping, comparative genomic hybridization (CGH) and sequencing of the gene encoding the T-cell receptor β-chain showed that most primary tumours were clonal and harboured the same recurrent translocations between the Tcre locus and Myc observed in a Pten knockout model and a small subset of human T-ALL (Extended Data Figs 3b, c and 4a, and data not shown)\(^1\)\(^,\)\(^2\). One shPten T-ALL showed a Cdkn2a deletion by CGH and six out of eight tumours analysed showed activating mutations in the Notch1 PEST domain (Fig. 1e and Extended Data Figs 3c, d and 4b). Gene set enrichment analysis (GSEA) of gene expression profiles obtained from shPten leukemias demonstrated enrichment for a human PTEN-mutated T-ALL signature, and profiles from human PTEN-mutated T-ALLs were enriched for a murine shPten signature (Extended Data Fig. 5a, b). Thus, although all the T-cell leukemias were initiated by a Pten shRNA, they acquire molecular features reminiscent of the human disease\(^1\)\(^,\)\(^2\)\(^,\)\(^3\)\(^,\)\(^4\).

The leukaemia arising in shPten mice was highly malignant, and rapidly produced disease when transplanted into recipient mice (Extended Data Fig. 6a). Of note, because the Vav-tTA;shPten transgenic mice were of a mixed genetic background, Rag1\(^-/-\) recipients were used to avoid graft rejection. These recipients succumbed to a highly disseminated form of T-ALL consisting of CD4\(^+\) CD8\(^+\) double-positive cells that rapidly took over the haematopoietic organs, accumulated to high levels in the peripheral blood, and spread to the liver, kidney and intestine (Fig. 2d and Extended Data Fig. 6b). Notably, decreased PTEN levels were associated with disease dissemination and lower survival in T-ALL patients (Fig. 1f and Extended Data Fig. 6c), and were also linked with intestinal infiltration in patients with peripheral T-cell lymphoma (Extended Data Fig. 6d, e). The association between PTEN loss and disease dissemination in murine and human T-cell malignancies underscores the relevance of the model to human disease.

We reasoned that the transplanted leukemias described above would be ideal for our experiments as they are highly malignant such that individual primary isolates can be studied for their response to different perturbations in multiple secondary recipients. Recipients were monitored for disease development by weekly analysis of peripheral blood for the presence of eGFP\(^+\) (shPten-expressing) cells. Upon disease manifestation, a cohort of mice was given Dox to silence the shRNA and reactivate PTEN. Notably, Dox treatment almost tripled the survival time of mice harbouring Vav-tTA;shPten leukaemia (Fig. 2a; \(P < 0.0001\)) but had no effect on mice harbouring Pten\(^-/-\) leukaemia (Extended Data Fig. 6f). Immunoblotting of leukemic cells collected from mice indicated that the system worked as expected: hence, Dox addition led to upregulation of Pten messenger RNA (Extended Data Fig. 7a–c and data not shown), silenced eGFP and re-established PTEN to endogenous levels (Fig. 2b and Extended Data Fig. 7d). Therefore, PTEN reactivation had a marked anticaner effect but was by no means curative.

Leukaemia-bearing mice showed magnetic resonance imaging (MRI) signals in multiple haematopoietic compartments, the liver and intestine (Extended Data Fig. 6h, i and data not shown). Although PTEN reactivation had no overt effect on tumour growth in the lymph nodes or spleen, it visibly decreased tumour infiltration into intestine and liver (Fig. 2c and Extended Data Fig. 6g–i). These findings were corroborated by immunohistochemistry (IHC) and flow cytometric quantification of CD4\(^+\) leukemic cells (Fig. 2e, f). Notably, Dox treatment had a minimal impact on the proliferation or apoptosis of leukemic cells residing in the lymph nodes and spleen, but triggered apoptosis in leukemic cells that had disseminated into the intestine (Fig. 2g and Extended Data Fig. 7e–g). Thus, the impact of PTEN expression on disease progression is dictated by the anatomical location of the leukemic cell.

We next assessed the phosphorylation state of key phosphatidylinositol 3-kinase (PI3K) effectors in tissue sections by IHC and pathway functionality by positron emission tomography (PET) of \(^{18}\)F-fluorodeoxyglucose (FDG) uptake into leukaemia cells\(^6\). The heterogeneous responses correlated with the ability of PTEN to effectively suppress aberrant PI3K signalling; whereas S6 and AKT phosphorylation were reduced in disseminated leukemic cells obtained from the intestine, it persisted in the leukemic cells collected from the spleen of the same animal (Fig. 3a and Extended Data Fig. 8). Similarly, mice displayed a marked reduction in FDG signal stemming from the liver and intestine within 2 days of PTEN reactivation, an effect that could not simply be accounted for by loss of leukaemia burden (Fig. 3b, c). Conversely, the FDG signal emanating from the spleen and bone marrow remained strong (Fig. 3b, d and Supplementary Videos 1 and 2). The divergent responses to PTEN
activation in a clonal leukaemia suggest that the control of the PI3K pathway can be markedly affected by microenvironmental factors.

Surprisingly, untreated NCr nude mice transplanted with the same number of shPten tumour cells survived as long as Rag1−/− recipient mice treated with Dox, and did not show a survival advantage following Dox addition (Fig. 4a). The untreated NCr recipients displayed vastly reduced intestinal dissemination of leukaemic cells compared to normal and thymectomized Rag1−/− recipients (Fig. 4b and Extended Data Fig. 9a, b, g, h), whereas spleen and lymph nodes were strongly affected (Fig. 4c and Extended Data Fig. 9c, d). Apparently, genetic differences between Rag1−/− and NCr mice contribute to variation in disease aggressiveness and the response to PTEN reactivation.

Whereas Rag1−/− mice are defective in immunoglobulin and T-cell receptor gene rearrangement, NCr mice have mutations in Foxn1, a gene that controls terminal differentiation of epithelial cells in the thymus and other organs17. Among other changes, NCr mice show decreased...
expression of Ccl25 (refs 18, 19), which encodes a chemokine that is mainly expressed by epithelial cells in the thymus and small intestine and acts as an important chemoattractant for T cells in the gut20,21. CCL25 acts through CCR9, a G-protein-coupled receptor that can signal through the PI3K pathway and is expressed on a subset of developing thymocytes22,23. Signalling through a related receptor, CCR7, is important for leukaemia dissemination into the central nervous system24; moreover, the CCL25/CCR9 network is required for T-cell dissemination during inflammatory bowel disease, which can be countered by CCR9 antagonists currently in clinical trials25–27. CCL25 levels were decreased in the intestine of NCr mice (Extended Data Fig. 9e, f), whereas CCR9 was highly expressed on the shPten leukaemia cells (Fig. 4d). Notably, CCR9 expression was not affected by PTEN reactivation as determined by fluorescence-activated cell sorting and RNA-seq analysis (Extended Data Fig. 9i and data not shown).

To test whether PTEN influences T-ALL homing and survival in the intestine by modulating CCL25 signalling, shPten T-ALL leukaemia isolates were treated with CCL25 (± Dox to modulate PTEN), and cell signalling and motility was assessed in short-term culture. Whereas CCL25 stimulation had little impact on PI3K signalling in the presence of PTEN, Pten knockout sensitized cells to CCL25-induced AKT phosphorylation and, to a lesser extent, S6 phosphorylation (Fig. 4e). Similar results were obtained with two human T-ALL lines transduced with either shPten or a control shRNA (Fig. 4f and Extended Data Fig. 9j). CCL25 addition also increased migration of murine shPten T-ALL cells in a transwell assay, and the effect was largely abrogated by PTEN reactivation (Extended Data Fig. 9k).

Dual-colour in vivo competition experiments were performed to assess the contribution of CCR9 signalling to T-ALL dissemination (Fig. 4g). After identifying shRNAs efficient at knocking down Ccr9 (Extended Data Fig. 10a, b), eGFP+ shPten leukaemia cells were transduced with either shCcr9 or shRenilla control shRNAs co-expressing the mCherry red fluorescent protein (Fig. 4g and Extended Data Fig. 10d). Upon transplantation and subsequent disease development, mice were euthanized and the fraction of eGFP/mCherry+ cells versus all eGFP+ cells was determined in various organs (Fig. 4g, h). shCcr9-expressing T-ALL cells showed significantly decreased abundance in the intestine but not the spleen or liver (Fig. 4h and Extended Data Fig. 10c, d). Mice transplanted with shPten leukaemia cells were also treated with a small molecule inhibitor for CCR9 that is in clinical trials for the treatment of inflammatory bowel disease28. Although the effects on survival were modest, leukaemia dissemination was reduced in the intestine, whereas cells in the spleen and liver were unaffected (Extended Data Fig. 10e–h and data not shown). Hence, in the intestine, PTEN suppression promotes leukaemic cell dissemination and maintenance by modulating CCL25–CCR9 signalling.

In human cancers, PTEN deletions often coincide with tumour expansion, metastasis and a generally worse prognosis29,30. Results confirmed and extended for T-cell disease in this report. Using a powerful new mouse model enabling reversible suppression of endogenous PTEN expression, we show that PTEN loss can promote tumour cell survival at distant sites by amplifying weak environmental cues that enable tumour cells to survive in an otherwise non-supportive microenvironment. Accordingly, the promiscuous yet passive ability of PTEN to attenuate PI3K signalling may be influenced by the nature and intensity of phosphatidylinositol (3,4,5)-triphosphate-generating signals in different microenvironments, and targeting such tissue-specific signals might present a valid strategy to treat cancer spread. Still, the requirement for PTEN loss in tumour maintenance is not absolute and can be influenced by genetic context29,30 and, as shown here, the tumour microenvironment. These observations paint a more complex picture of how PTEN inactivation drives tumour maintenance, and reveal an interplay between tumour and microenvironment that would not be predicted from studies on cultured cells. Nonetheless, this interplay produces a form of intratumoral heterogeneity that is independent of genotype but can affect disease progression and perhaps the clinical response to molecularly targeted therapies.

**METHODS SUMMARY**

**ES cell targeting and generation of Pten shRNA transgenic mice.** KH2 ES cells expressing the reverse transactivator (rtTA2) from the Rosa26 promoter were electroporated with Pten shRNAs cloned into a recombination-mediated cassette exchange vector (ctGMG) targeting the Collal locus, and correctly targeted and functional ES cell clones were identified and used to generate live mice by tetraploid embryo complementation. The number of each shRNA is used as index and refers to the position of the first nucleotide of the shRNA guide strand relative to the refseq cDNA sequence. MEFs were generated from 13.5-day-old embryos according to standard protocols. Mice were bred to CMV-rtTA, CAGGS-rtTA and Var+rtTA trans-activator lines to generate heterozygous (for example, shPten+/-CMV-rtTA) double-transgenic mice using standard breeding techniques. To induce shRNA expression, Pten and firefly luciferase (Luc) or Renilla luciferase (Renilla) shRNA mice bred to CMV-rtTA or CAGGS-rtTA mice were put on food containing 625 mg kg−1 Dox (Harlan Teklad) immediately after weaning. Dox food was also used to shut off shRNA expression in Var+rtTA transgenic animals at different time points. All mouse experiments were performed in accordance with institutional and national guidelines and regulations and were approved by the Institutional Animal Care and Use Committee (IACUC no. 06-02-97-17 (Cold Spring Harbor Laboratory) and no. 11-06-017 (Memorial Sloan Kettering Cancer Center)).

**Online Content** Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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**Author Contributions** C.M. and S.W.L. designed the study. C.M., C.S., P.K.P. and L.L. performed shRNA design and testing, targeting vector construction and E.S. cell targeting. C.M., B.B., I.A., C.S. and L.L. performed mouse breeding, transplantation experiments and analysed data. C.M. and I.A. performed *in vitro* migration assays and analysed data. C.M. and H.B. ran the mouse MRI experiments and analysed data. C.M. and B.B. performed the 18F-FDG-PET experiments and analysed data. J.H. performed CGH analysis, and J.H. and C.M. analysed data. J.T.-F. performed the histopathological analysis of mouse and human tumours, and J.T.-F. and C.M. analysed data. B.B. and C.M. performed paraffin embedding, sectioning and IHC staining of mouse tissues and analysed data. C.M. performed flow cytometry, immunoblotting and analysed data. J.N. performed the RNA-seq sample processing and J.N., J.M., J.R.D., C.S. and C.M. analysed data. C.S. ran the GSEA analysis and comparison with human expression data. M.A. and M.R.S. performed the SKY analysis of mouse tumours, and M.A., M.R.S. and C.M. analysed data. S.W.L. supervised the project. C.M., C.S., B.B. and S.W.L. wrote the paper. All authors reviewed the manuscript.

**Author Information** Data sets from RNA-seq analysis were deposited at the Sequence Read Archive (SRA) at the European Nucleotide Archive under the accession number PRJEB5498. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to S.W.L. (lowes@mskcc.org).
METHODS

Constructs and shRNAs. To identify potent shRNAs targeting murine and human Pten, various 97-bp oligonucleotides predicted from sensor-based and other shRNA design algorithms (Extended Data Fig. 4c and data not shown) were cloned into the miR-30 cassette of the MLP vector and tested as described previously (Extended Data Fig. 1a). The two most efficient murine Pten shRNAs (Pten.1522 and Pten.2049, numbers refer to the position of the first nucleotide of the shRNA guide strand relative to the refseq cDNA sequence) were cloned into a recombination-mediated cassette exchange (RCME) vector (CTGM) targeting the Collal1 locus (see Fig. 1a)3–5. For knockdown of human Pten, the Pten.1522 shRNA, which showed complete overlap with the human Pten sequence, was used. For knockdown of murine Cr9, multiple shRNAs were designed, cloned and tested as described above. The two most efficient shRNAs, shCr9.904 (97-mer: 5′-TGGCTGTGACAGGCAGGAAAGATGGAAGCTGATAGGACCA CAGATGTATAAAGGTTGTAAACTGTTGG-3′) and shCr9.2357 (97-mer: 5′-TGGCTGTGACAGGCAGGAAAGATGGAAGCTGATAGGACCA CAGATGTATAAAGGTTGTAAACTGTTGG-3′) were cloned into a LMN-cherry vector (MSCV-miR-30b-peek-NRES-mCherry) for dual-colour competition assays (see below).

ES cell targeting and generation of transgenic mice. Two potent shRNAs against murine Pten were cloned into a cassette that links eGFP and shRNA expression downstream of the CMV promoter and targeted into a defined locus downstream of the collagen, type I, alpha 1 (Collal1) gene in KH2 ES cells expressing the reverse transactivator (rtTA2) from the Rosa26 promoter36 by RMCE (Fig. 1a and Extended Data Fig. 1a)3–4. Southern blotting showed correct transgene insertion, and Dox-inducible knockdown of endogenous Pten was confirmed by western blot analysis (Extended Data Fig. 1b).

Germine transgenic mice were generated by tetraploid embryo complementation. MEFs were generated from 13.5-day-old embryos according to standard protocols. Since both shRNAs caused a similar degree of Pten knockdown and P2K pathway activation and equally promoted tumorigenesis in vivo in transgenic mice experiments (Extended Data Fig. 1c, d and data not shown), we focused subsequent analysis on a single (shPten.1522) transgenic line.

Mice were bred to CMV-rtTA, CAGGS-rtTA and Vav-rtTA transactivator lines4,5,35 to generate compound heterozygous or homozygous (for example, shPten1/−:CMV-rtTA3/− or shPten1/−:Vav-rtTA3/−) double-transgenic mice using standard breeding techniques. To induce shRNA expression, Pten and firefly luciferase (Luc) or Renilla luciferase (Renilla) shRNA mouse lines were bred to CMV-rtTA or CAGGS-rtTA mice were put on food containing 625 mg/kg Dox (Harlan Teklad) immediately after weaning. As predicted from knockout mice33, most double-transgenic mice harboring the inducible shPten allele together with the broadly expressing CMV-rtTA or the CAGGS-rtTA transactivator strains36 developed tumours within 12 months of Dox addition (Extended Data Fig. 2; data not shown). Dox food was also used to shut off shRNA expression in Vav-rtTA transgenic animals at different time points.

For the Vav-rtTA/shPten mice survival studies, a number of Vav-rtTA−/−shPten−/− mice (n = 49) and controls (Vav-rtTA−/−shLuc−/− (n = 20), Vav-rtTA−/−shPten−/− (n = 68), Vav-rtTA−/−shPten−/−, +Dox (n = 10)) were generated and analysed. No difference in phenotype was observed between heterozygous (shPten1/−:Vav-rtTA3/−) and homozygous (shPten1/−:shPten1/−:Vav-rtTA3/−) mice. All mice experiments were performed in accordance with institutional and national regulations and were approved by the Institution Animal Care and Use Committee (IACUC no. 06-02-97-17 (Cold Spring Harbour Laboratory) and no. 11-06-017 (Memorial Sloan Kettering Cancer Center)).

Statistics and reagents. For all murine survival studies, a group size of at least five animals per condition was chosen, which allowed the detection of twofold differences in survival with a power of 0.89, assuming a two-sided test with a significance threshold of 0.05 and a standard deviation of less than 50% of the mean. For the primary animals, all mice with the correct genotype were included in the analysis. For the transfection experiments, all mice receiving similar amounts of transfected cells as determined by flow cytometric and/or whole-body immunoluminescent evaluation 5–10 days after transplant were included in the analysis. For the reactivation and treatment experiments, animals were assigned into different groups by random picking from the non-selected transplanted group of mice 5–10 days after transplant. Blinding of animals in the reactivation/inhibitor treatment studies was not feasible, because of requirements by the local animal housing facility to mark cages if containing special food/treatment.

Appropriate statistical tests were applied as indicated, including non-parametric tests for experiments where sample size was too small to assess normal distribution. All t-tests and Chi-squared test were two-sided. For all tests, variation was calculated as standard deviation and included in the graphs as error bars. To investigate whether Pten expression has an impact on tumour dissemination in human T-ALL, we performed IHC staining for Pten on bone marrow sections from 31 patients with newly diagnosed T-ALL, for which clinical data on disease dissemination was available.

Owing to the relatively low number of patient specimens and because the variables were nonlinear, we analysed the data in a contingency table using Fisher’s exact test. We also re-analysed the contingency table using Berger’s test, with similar results37. For probing an association between Pten expression status and intestinal infiltration in human T-cell lymphoma patients, the same statistical tests were applied. All antibodies used for western blot analysis were purchased from Cell Signaling Technology unless otherwise specified, including the antibodies against Pten (cat. no. 9188), PAK1 (T308, cat. no. 2962), AKT (cat. no. 6061), GAPDH (4939, cleaved, cat. no. 4951), and for intracellular flow cytometric analysis of P56(V523/236) directly. Pacific Blue fluorescence-coupled antibodies were purchased from Cell Signaling (cat. no. 8520).

For antibodies for flow cytometry were purchased from BioLegend unless otherwise specified. Mouse antibodies included CD3 (clone 145-2C11), CD4 (clone GK1.5), CD8 (clone 53-6.7), Th1.2 (clone 30-H12), CD45 (clone 30-F11), CCR9 (clone 9B1), CD11b (clone M1-70), Gr-1 (clone RB6-8C5), CD45 (clone IM7), CD25 (clone PC61). All cell lines were tested for absence of mycoplasma and authenticated by flow cytometry and western blotting.

Transplantation experiments. For transplantation, single-cell suspensions were generated from primary tumours and 1 × 105 cells were injected into sublethally (450 rad) irradiated recipient female Rag1−/− mice (on a C57/B6 background, cat. no. 2216) or NCr nude mice (on an inbred albino background, cat. no. 2019) via tail vein injection. All mice used as transplant recipients were purchased from Jackson Laboratory. Mice were monitored by serial flow cytometric analysis of the peripheral blood. Once eGFP+ cells reached >5% of total leukocytes, cohorts of mice were started on Dox containing food as indicated.

Analysis of human T-ALL and PTCL patient samples. For the analysis of survival of Pten normal versus Pten altered patients with T-ALL, published genomic and miRNA expression data on patients with T-ALL was used (accession number GSE4073). Pten altered (n = 20) included patients with Pten deletion, mutation, underexpression (<0.8 sigma after z scoring) and any combination of such alterations, and Pten normal (n = 62) included all other patients with available data. For IHC analysis, samples from patients with T-ALL were analysed as individual bone marrow biopsies. For the T-cell lymphoma samples, tissue microarrays were constructed as previously published using a fully automated Beckman Instruments, ATA-27. The study cohort comprised 84 patients with T-cell lymphomas and 31 patients with T-ALL. The T-cell lymphomas could be subdivided into enteropathy-associated T-cell lymphoma (4) and peripheral T-cell lymphoma involving bowel or gastrointestinal tract (7), T/NK cell lymphoma (4), angioimmunoblastic T-cell lymphoma (9), anaplastic large cell lymphoma (14), and peripheral T-cell lymphoma with non bowel or gastrointestinal tract involvement (46). All samples were consecutively ascertained at the Memorial Sloan Kettering Cancer Center (MSKCC) between 2001 and 2012. Use of tissue samples was approved with an Institutional Review Board Waiver and the Human Bio-specimen Utilization Committee. All biopsies were evaluated at MSKCC, and the histological diagnosis was based on H&E staining. The Pten antibody (rabbit monoclonal antibody from Cell Signalling, 138G6, no. 9559) was used at a 1:30 dilution. IHC analysis was performed on the Ventana Discovery XT automated platform according to the manufacturer's instructions. Results were scored as 0, 1, 2 for Pten with 0 = no staining of tumour cells, with endothelial cell- and macrophage-positive; 1 = weak staining of tumour cells, compared to endothelial cell- and macrophage-positive; 2 = strong staining of tumour cells, compared to endothelial cell- and macrophage-positive. Representative images were taken using the Olympus BX41 model, DP20 camera, at ×60 objective.

MRI. The mice were anesthetized with 2–3% isoflurane delivered in O2 and allowed to breathe spontaneously during the imaging study. The mice were positioned supine in a custom-made acrylic cradle fitted with a snout mask for continuous delivery of anaesthesia. Non-invasive, MRI-compatible monitors (pulse-oximetry, respiration, body temperature, and core temperature probe, SA Instruments) were used for continuous monitoring of vital signs while the animal underwent MRI imaging. During imaging, body temperature was kept strictly within 36.5–37.5 °C using a computer-assisted air heating system (SA Instruments). A volume radiofrequency coil (diameter 11.2 mm) used in transmit and receive mode were used for all imaging acquisitions. Following localizer anatomical scout scans, a 2D multi-slice T2-weighted RARE sequence along the coronal slice direction with fat suppression was obtained with the following parameters: TR = 12,500 ms, TE = 40 ms, RARE factor = 8, field of view (FOV) = 80 × 80 × 80 mm3, FA = 90°, and matrix = 512 × 256 × 256. Yielding an in-plane resolution of 0.234 × 0.234 mm2, slice thickness = 0.9 mm total scanning time = 10 min 40 s.

18F-FDG-PET analysis. For PET analysis, mice were fasted for 6 h before intravenous injection of 0.5 mCi 18F-FDG. Mice were kept for 1 h under isoflurane anesthesia and subsequently imaged on a Focus 120 microPET (Siemens) or in
some cases an Inveon MicroPET/CT (Siemens). Image normalization and analysis was performed using the ASI Pro MicroPET analysis software and the Inveon Workplace software package (Siemens Medical Solutions).

CGH. CGH experiments were performed using standard Agilent 244K mouse whole genome arrays, and hybridizations were carried out according to the manufacturer’s recommendations. Data processing, normalization and segmentation were carried out as described19.

Multiplex-FISH (M-FISH)/spectral karyotyping analysis. Cells were cultured in RPMI-1640 with 1 g-lactalbumin (PA) supplemented with 10% FBS, 1% penicillin-streptomycin and 50 U ml−1 penicillin.interferon 2 and 5 μg ml−1 concanavalin A for 48–72 h at 37 °C and 5% CO2. To prepare metaphases, colcemid at a final concentration of 0.1 μg ml−1 was added to the cells for 120 min. Spinning at 300 g for 8 min was followed by hypotonic treatment in pre-warmed 0.075 M KCl for 20 min at 37 °C. Cells were fixed in cold ethanol/acetic acid (3:1) and air-dried slides were prepared.

The M-FISH hybridization was performed with a panel of mouse M-FISH probes (21 × Mouse mFISH probe kit, MetaSystems) according to the manufacturer’s instructions. In brief, the probes were denatured at 75 °C for 5 min and pre-annealed at 37 °C for 30 min. Slides were incubated in 0.1 × SSC for 1 min, denatured in 0.07N NaOH at room temperature for 1 min, quenched in 0.1 × SSC at 4 °C and 2 × SSC at 4 °C for 1 min each, dehydrated in an ethanol series and air dried. M-FISH probe was applied onto the slides and hybridization was performed for 48 h in a humidified chamber at 37 °C. Following hybridization, the slides were washed in 0.4 × SSC at 72 °C for 2 min, followed by a wash in 2 × SSC, 0.05% Tween20 at room temperature for 2 min. Counterstaining was performed using DAPI (4,6-diamidino-2-phenylindole) and mounted with phenylenediamine.

Slides were visualized using a Leica DMRXA-TRF epifluorescence microscope equipped with special filter blocks (Chroma Technology). For image acquisition, a Sensys CCD camera (Photometrics) with a Kodak KAF 1400 chip was used. Both the camera and microscope were controlled with Leica Q-FISH software (Leica Microsystems Imaging Solutions). Images were analysed using the Leica MCK-Software package (Leica Microsystems Imaging Systems).

Western blotting, flow cytometry and antibodies. Western blotting was performed according to standard protocols. In brief, tissues were either snap frozen in liquid nitrogen and homogenized, or dissociated into single cells using 100 μm nylon mesh (Cellstrainer, BD Falcon). Protein was extracted using standard protein lysis buffer (20 mM Tris (pH7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na3VO4) supplemented with a protease inhibitor cocktail (Complete Roche Diagnostics) and quantified using a Bradford Protein Assay (Bio-Rad). Proteins were separated on a polyacrylamide gel (ProteinIII, Bio-Rad) and transferred to a PVDF membrane (Immobilon-F, Millipore). Protein bands were resolved using fluorochrome-conjugated secondary antibodies on an Odyssey scanner (Licor). All western blot experiments were replicated at least twice.

For the analysis of pAkt and pS6 induction after CCL25 stimulation, shPten T-ALL cells derived from spleen tissues of tumour-bearing shPten mice and adapted to cell culture were either left untreated or treated with Dox for 4 days to reactivate PTEN, starved over night at 0.5% FBS and then treated with CCL25 for the indicated time points. For the CCL25 stimulation assay the human T-ALL cell lines HPA-ALL and TALL1, the cells were infected with a retroviral vector coexpressing a mCherry-positive cell fractions. Relative mCherry fractions from different organs were determined by normalizing to the spleen fraction as 100%.

CCFR inhibitor treatment. The CCR9 inhibitor fedl in the presence of 10 ng ml−1 IL-23 in Opti-Gen Glutamax medium (Gibco/Life Technologies) and infected with either shCcr9 or shRenilla control shRNAs. Approximately 0.75 × 106 infected shPten cells (20–30 μm) were implanted into recipient Rag−/− mice irradiated with 450 rad, and monitored for disease development. Diseased mice were euthanized, and spleen, bone marrow, liver, lung, small intestine and kidney tissues were collected and minced to generate single-cell suspensions for flow cytometric measurement of GFP- and mCherry-positive cell fractions. Relative mCherry fractions from different organs were determined by normalizing to the spleen fraction as 100%.

Clonality analysis of murine shPten tumours. Clonality analysis by PCR amplification and sequencing of murine TCRβ gene segments was performed as previously described11. In brief, clonality was assayed at V-D and J rearrangements in a murine T-ALL cell line on a PTC-200. PCR products were analyzed using agarose gel electrophoresis and the consensus primers located 5’ of DJ1 and DJ2 gene segments and consensus downstream primers located within Vβ gene segments, were amplified. TCRβ gene segments of interest were further analyzed for point mutations in the mouse mm9 genome and its corresponding transcript sequences using an in-house pipeline and quality assessment pipeline. Transcription expression levels were estimated as fragments per kilobase of transcript per million mapped reads (FPKM) and gene FPKMs were computed by summing the transcript FPKMs for each gene using the Cuffdiff2 program14.
We called a gene ‘expressed’ in a given sample if it had a FPKM value $\geq 0.5$ based on the distribution of FPKM gene expression levels and excluded genes that were not expressed in any sample from the final gene expression data matrix for downstream analysis. Differentially expressed genes were identified using LIMMA and false discovery rate was estimated by Benjamini–Hochberg method. GSEA and the Database for Annotation, Visualization and Integrated Discovery (DAVID) were used to assess pathway enrichment. All mouse RNA-seq data sets are submitted to the European Nucleotide Archive (ENA) and can be accessed under the accession number PRJEB5498 at http://www.ebi.ac.uk/ena/data/view/PRJEB5498.

GSEA. To test for mouse shPten signature enrichment in PTEN-disrupted human T-ALL, we established a shPten-dependent signature using the 100 most upregulated genes in shPten T-ALL samples (untreated, $n = 3$) against PTEN-restored samples (Dox-treated, $n = 4$) samples. Publicly available human T-ALL gene expression profiles (GSE28703, $n = 47$) were processed using RMA (quantile normalization) and supervised for PTEN status (PTEN disrupted including PTEN deletion, mutation or both, $n = 10$; PTEN wild-type, $n = 37$) according to the published sample genetic features annotation. Statistical significance of GSEA results was assessed using 1,000 sample permutations. For enrichment of human PTEN-T-ALL signature in mouse shPten T-ALL (Dox-off) profiles against PTEN-restored (Dox-on) profiles, a human PTEN-disrupted signature was generated by including the 100 most upregulated genes in PTEN-disrupted versus PTEN-wild type T-ALL samples. Mouse genes were ranked by supervising untreated to Dox-treated shPten T-ALL. Statistical significance of human PTEN-disrupted signature enrichment was assessed using 1,000 gene set permutations.

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Extended Data Figure 1 | Pten shRNA-transgenic mice enable conditional expression of PTEN and develop tumours after prolonged Pten knockdown.

**a**, Western blot analysis of PTEN protein knockdown in NIH 3T3 cells infected with different Pten shRNAs at low multiplicity of infection. l.e., long exposure; s.e., short exposure. **b**, PTEN protein knockdown assessed by western blot in ES cell clones targeted with two different Pten shRNAs, either treated with Dox or left untreated. **c**, MEFs from Rosa26-rtTA;shPten.1522 transgenic mice, wild-type control mice, or Pten1/2 mice were treated with Dox for the indicated times and analysed for PTEN, GFP and ACTB expression by western blot. **d**, Overall survival of mice receiving bone marrow cells from tTA-transgenic mice infected with an inducible TRE-GFP-miR-30 (TGM) retroviral vector expressing shPten.1522, shPten.2049 or control after irradiation with 600 rad. **e**, Fluorescence image of a CAGGS-rtTA;shPten.1522 mouse on Dox for 5 days and a CAGGS-rtTA-only control mouse. **f**, Flow cytometric analysis of the peripheral blood of a CAGGS-rtTA;shPten.1522 mouse on Dox and an off Dox control mouse for myeloid (CD11b) and GFP marker expression 10 days after initiating Dox food. **g**, Overall survival curve of CMV-rtTA;shPten.1522 double-transgenic and control mice (single-transgenic shPten.1522 or CMV-rtTA). Dox treatment for shRNA induction was started after weaning (at ~4 weeks of age). **h**, Situs of a tumour-bearing CAGGS-rtTA;shPten.1522 double-transgenic mouse. A large thymic tumour (full arrow), as well as enlarged lymph nodes (dashed arrows) and spleen (arrowhead), are visible. **i**, Immunohistochemical staining of kidney sections from a CAGGS-rtTA;shPten.1522 mouse for the indicated antigens. Arrows highlight a tumour infiltrate around a kidney venule. Scale bars, 100 μm.
Extended Data Figure 2 | Vav-tTA;shPten transgenic mice with targeted shPten expression in the haematopoietic lineage display thymic hyperplasia by 6 weeks, and a subset develops thymic tumours infiltrating multiple peripheral organs. a, Brightfield (left) and fluorescence (right) images of spleen and thymus from Vav-tTA;shLuc (control) and Vav-tTA;shPten double-transgenic mice at 5 weeks. b, Fluorescence-activated cell sorting analysis of spleen and thymus single-cell suspensions from Vav-tTA;shLuc/shPten mice for CD4/CD8 expression. c, IHC analysis of thymic tissue from 6-week-old Vav-tTA;shLuc and Vav-tTA;shPten mice. Sections were stained with haematoxylin and eosin (H&E), anti-GFP or anti-PTEN antibodies, showing heterogeneous GFP staining and correspondingly variable PTEN knockdown. Scale bars, 200 µm for H&E and GFP, 100 µm for PTEN. The insets are ×2 magnifications. d, Thymus weight of 6-week-old Vav-tTA;shLuc and Vav-tTA;shPten mice (n = 5 for both groups, P < 0.006 by t-test). e, Brightfield and GFP-fluorescence images of a Vav-tTA;shPten mouse with tumours. f, IHC staining of spleen, liver and kidney tissues from a Vav-tTA;shPten mouse with primary T-cell disease. Sections were stained for H&E, GFP, PTEN and phospho-AKT(S473) as indicated, showing heterogeneous staining owing to variable tumour infiltration. Scale bars, 100 µm, insets 25 µm.
Extended Data Figure 3 | Immunophenotype, chromosomal aberrations and Notch1 mutations observed in murine shPten tumours. a, Flow cytometric analysis of organ infiltration by primary tumours in Vav-tTA;shPten.1522 transgenic mice. Single-cell suspensions of indicated tissues were analysed for eGFP, Thy1.2, CD4 and CD8 expression. BM, bone marrow; LN, lymph node; PB, peripheral blood. b, Spectral karyotyping analysis of a T-cell tumour arising in Vav-tTA;shPten.1522 mice, showing a t(14;15) translocation. c, CGH analysis of a Vav-tTA;shPten.1522 leukaemia. Genomic tumour DNA was analysed on Affymetrix CGH SNP arrays and compared to normal skin tissue. x axis indicates genomic coordinate and y axis represents log2(tumour/germline). d, Schematic of the murine NOTCH1 protein generated using protein paint (http://explore.pediatriccancer genomeproject.org/proteinPainter), highlighting the different NOTCH1 protein domains and the mutations detected in the murine shPten and Pten−/− T-ALL tumours.
### Extended Data Figure 4 | Summary of karyotyping and Notch1 sequencing of shPten T-ALL tumours.

**a** Results from a multiplex FISH analysis of three different primary shPten-induced T-ALL tumours. At least ten cells were analysed for each sample, and chromosomal gains, deletions or translocations are highlighted.

| Mouse No. | Karyotype |
|-----------|-----------|
| CM90      | 40~42, XY, +Y[1], -X[1], +2[8], -8[1], -18[1] [cp10] |
| CM92      | 41, XX, +15 |
| CM122     | 37~41, XX, t(14;15) [6], -X[1], +10[1], -3[1], -8[1], -9[1], -16[1], +4[1], +10[1] [cp11] |

**b** Summary of Notch1 mutations identified in shPten and Pten knockout tumours.

| GeneName | Chr | mm9_Pos | Class | AAChange | ProteinGI | mRNA_acc | Sample Name |
|----------|-----|---------|-------|----------|-----------|----------|-------------|
| Notch1   | 2   | 26315225 | frameshift | S2475fs | 224967065 | NM_008714 | shPten tumor 1 primary |
| Notch1   | 2   | 26315225 | frameshift | S2475fs | 224967065 | NM_008714 | shPten tumor 1 secondary |
| Notch1   | 2   | 26335472 | missense | N454D   | 224967065 | NM_008714 | shPten tumor 2 |
| Notch1   | 2   | 26315567 | frameshift | R2361fs | 224967065 | NM_008714 | shPten tumor 2 |
| Notch1   | 2   | 26315564 | frameshift | F1692S | 224967065 | NM_008714 | shPten tumor 3 |
| Notch1   | 2   | 26322049 | missense | F1692S | 224967065 | NM_008714 | shPten tumor 4 |
| Notch1   | 2   | 26315567 | frameshift | R2361fs | 224967065 | NM_008714 | shPten tumor 5 |
| Notch1   | 2   | 26316068 | frameshift | L2195fs | 224967065 | NM_008714 | shPten tumor 6 |
| Notch1   | 2   | 26315566 | frameshift | R2361fs | 224967065 | NM_008714 | shPten tumor 6 |

**c** Sequence of all shRNAs targeting murine Pten that were tested in the study. Sense and guide strand are highlighted in red.

| shPten | 97-mer oligo |
|--------|--------------|
| shPten.1967 | TGCTGTTCGACGTGAGCG CCCAGATGTAGTGACAATGAA TAGTGAGCCACAGAT GTAATCGTCGATCACTAAGATCGGATAGTGCCTCGGA |
| shPten.2049 | TGCTGTTCGACGTGAGCG AAAGATCGCAGTTACCAAATTA TAGTGAGCCACAGAT GTAAATTTGTGAATGTCTGATCCTTC TGCGCTACTGCCTCGGA |
| shPten.1766 | TGCTGTTCGACGTGAGCG CATCGATAGCATTTGCAGTAATA TAGTGAGCCACAGAT GTAATATCTGCACGCTCTATACTGC TGCGCTACTGCCTCGGA |
| shPten.1522 | TGCTGTTCGACGTGAGCG ACCAGCTAAAGGTGAAGATATA TAGTGAGCCACAGAT GTAATATCTGCACGCTCTATACTGC TGCGCTACTGCCTCGGA |
| shPten.1687 | TGCTGTTCGACGTGAGCG CTGTGGAATATACGTGGTTTCAATAGTGAGCCACAGAT GTAATCGGAAAGCTAAGTTACCCAAA TGCGCTACTGCCTCGGA |
| shPten.1178 | TGCTGTTCGACGTGAGCG ATTCTGGAAGATCTGGACAA TAGTGAGCCACAGAT GTAATCGGAAAGCTAAGTTACCCAAA TGCGCTACTGCCTCGGA |
| shPten.1202 | TGCTGTTCGACGTGAGCG ACTAAGTGAAGATGACAATCAT TAGTGAGCCACAGAT GTAATCGGAAAGCTAAGTTACCCAAA TGCGCTACTGCCTCGGA |
| shPten.2052 | TGCTGTTCGACGTGAGCG AATAGCAGCTACAAAATTTGAA TAGTGAGCCACAGAT GTAATCGGAAAGCTAAGTTACCCAAA TGCGCTACTGCCTCGGA |
| shPten.1780 | TGCTGTTCGACGTGAGCG ACAGTATAGGAGTGGACAGATTA TAGTGAGCCACAGAT GTAATCGGAAAGCTAAGTTACCCAAA TGCGCTACTGCCTCGGA |
| shPten.1198 | TGCTGTTCGACGTGAGCG CGACGGAATAGTACAGGCTGACACTGAGTAGGATATAGTAGTAAGGCCACAGAT GTAATCGGAAAGCTAAGTTACCCAAA TGCGCTACTGCCTCGGA |
| shPten.1523 | TGCTGTTCGACGTGAGCG ACGCTAAGGTTGAAAGATATAGTAGTAAGGCCACAGAT GTAATCGGAAAGCTAAGTTACCCAAA TGCGCTACTGCCTCGGA |
| shPten.1794 | TGCTGTTCGACGTGAGCG CCAAGTTGCTTTGAAATGGACACAGAT TAGTGAGCCACAGAT GTAATCGGAAAGCTAAGTTACCCAAA TGCGCTACTGCCTCGGA |
| shPten.2196 | TGCTGTTCGACGTGAGCG CCAAGTTGCTTTGAAATGGACACAGAT TAGTGAGCCACAGAT GTAATCGGAAAGCTAAGTTACCCAAA TGCGCTACTGCCTCGGA |
Extended Data Figure 5 | GSEA shows similar gene expression patterns in human and mouse T-ALL lacking Pten. a, GSEA of a mouse shPten signature in PTEN-altered human T-ALL was tested after establishing the shPten-dependent signature using the 100 most upregulated genes in shPten T-ALL samples (untreated, n = 3) against PTEN-restored samples (Dox-treated, n = 4) as determined by RNA-seq analysis (data not shown). Publicly available human T-ALL gene expression profiles (GSE28703, n = 47) were processed using RMA (quantile normalization) and supervised for PTEN status (PTEN altered including PTEN deletion, mutation or both, n = 10; PTEN wild-type (WT), n = 37) according to the published sample annotation18. Statistical significance of GSEA results was assessed using 1,000 samples permutations. b, For enrichment of human PTEN T-ALL signature in mouse shPten knockdown (kd) T-ALL (Dox-off) profiles against Pten-restored (Dox-on) profiles, a human PTEN-disrupted signature was generated by including the 100 most upregulated genes in PTEN-disrupted versus PTEN-wild type T-ALL samples. Mouse genes were ranked by supervising untreated to Dox-treated shPten T-ALLs. Statistical significance of human PTEN-disrupted signature enrichment was assessed using 1,000 gene set permutations.
Extended Data Figure 6 | Secondary recipients of shPten T-ALL cells display extensive intestinal tumour infiltration similar to a subset of human patients characterized by peripheral T-cell lymphoma and low PTEN expression. a, Overall survival of sublethally irradiated Rag1<sup>−/−</sup> mice transplanted with 1 x 10<sup>5</sup> T-ALL cells from primary Vav-tTA;shPten.L52 mice compared to untransplanted mice (n = 5 for both groups, P < 0.003). b, IHC staining for eGFP expression in the indicated tissues from secondary T-ALL transplant recipients. Scale bars, 400 μm, 100 μm for insets. c, Overall survival of PTEN normal (WT) versus PTEN-altered patients with T-ALL analysed from published data on patients with T-ALL<sup>15</sup>, P = 0.02). PTEN-altered (n = 20) includes patients with PTEN deletion, mutation, underexpression (<0.8 sigma after z scoring) and any combination of such alterations; PTEN normal (n = 62) include all other patients with available data. d, PTEN IHC staining of tissue microarrays of tumour sections from Memorial Sloan Kettering Cancer Center patients with peripheral T-cell lymphomas. Examples of low (top panel) and high (bottom panel) PTEN expression samples are shown. e, Contingency table showing a significant association (P < 0.003; Fisher’s exact test) between low expression of PTEN and intestinal infiltration in PTCL patients. f, Overall survival of Rag1<sup>−/−</sup> mice transplanted with T-ALL cells from Pten<sup>fl/fl</sup>; Lck-cre mice<sup>6</sup> Dox (n = 5 for each group). g, Weight of spleen (n = 4) and lymph nodes (n = 8) in Rag1<sup>−/−</sup> mice transplanted with Vav-tTA;shPten leukaemic cells untreated or treated with Dox for 5 days (∆ s.d.). h, MRI of Rag1<sup>−/−</sup> mice transplanted with Vav-tTA;shPten leukaemic cells untreated or treated with Dox for 5 days, 14 days after transplant. Arrows highlight lymph nodes (LN) and increased signals in the liver. Representative images for one out of three analysed mice per condition are shown. i, MRI imaging of the intestine and liver of the same mice as in h are shown. Dashed arrows highlight the liver, full arrows the intestine.
Extended Data Figure 7 | *Pten* reactivation affects multiple pathways and increases apoptosis in tumour cells infiltrating the intestine, but not in the spleen. a, b, Heatmap of top 30 upregulated (a) and downregulated (b) genes after *Pten* reactivation as determined by RNA-seq on CD4-sorted leukaemic samples isolated from the spleen. Three mice with *Pten* knocked down and four mice with reactivated *Pten* were analysed. *Pten* is one of the top 50 upregulated genes after reactivation, but is not included on the list. c, Bubblegraph visualization of the most significantly affected pathways as determined by DAVID pathway analysis. *y* axis represents relative pathway enrichment in *Pten* reactivated versus *Pten* knockdown leukaemic cells, and size of the bubble graph is inversely proportional to *P* value. d, IHC analysis for expression of GFP and PTEN in spleen, lymph node (LN) and liver from *shPten*-tumour-transplanted mice ≥Dox treatment (5 days after start of Dox treatment; *n* = 3 per group). Representative sections are shown. Scale bars, 100 μm for full images, 20 μm for insets. e. *In vivo* 5-bromodeoxyuridine (BrdU) uptake in leukaemic cells isolated from the lymph nodes of mice transplanted with Vav-TA*shPten* primary T-ALL tumours ≥Dox. *n* = 3 for each group (± s.d.). f, TUNEL staining of spleen and intestinal sections of *Rag1*^−/−^ mice serially transplanted with Vav-TA*shPten* leukaemia cells and either left untreated or treated with Dox 24 h before sectioning. Scale bars, 200 μm (×2.5) and 50 μm (×10). g, Quantification of TUNEL-stained sections from the intestinal sections in f. TUNEL-positive cells from three representative areas of 1 mm² from two different intestine sections were counted for each condition (*P* < 0.01) (± s.d.).
**Extended Data Figure 8 | AKT and S6 protein phosphorylation is affected by PTEN reactivation in the intestine.**

**a**, IHC staining for phospho-S6 (pS235/236-S6) and phospho-AKT (pS473AKT) of spleen sections from Rag1^-/-^ mice transplanted with Vav-tTA;shPten tumour cells from primary mice and either treated with Dox or left untreated 2 days after treatment begin (n = 3 per group). Scale bars, 100 μm, 20 μm for insets. Representative images are shown.

**b**, IHC staining for pS473-AKT (bottom) in the intestine, showing very low pAKT signal in the intestinal epithelial cells independent of Dox treatment status (arrows; bottom left and right panels), conversely strong staining for pAKT was detected in some of the infiltrating tumour cells (arrow heads). The signal was reduced concomitantly with the overall reduction of the Pten-shRNA-linked GFP signal (top) after 36 h of Dox treatment (+ Dox; right panels).

**c**, Representative histogram of flow cytometric analysis for intracellular pS6 signal in CD4^+^ cells isolated from spleen and intestine of Rag1^-/-^ mice transplanted with shPten tumour cells and either treated with Dox for 5 days or left untreated.

**d**, Flow cytometric quantification of pS6 signal in CD4^+^ cells isolated from the intestine (d) and spleens (e) of Rag1^-/-^ mice transplanted with primary shPten tumours and treated ± Dox for 5 days (n = 4 for each condition, P < 0.04 for the intestine and not significant for the spleen by paired t-test). MFI, mean fluorescent intensity, error bars represent s.d.
Extended Data Figure 9 | NCr mice display a reduced intestinal tumour infiltration, which is not dependent on the absence of the thymus.

a, Brightfield pictures of the intestinal sites of Rag1-/- and NCr nude mice serially transplanted with shPten tumours (top four panels) and fluorescence images (FI) of cells infiltrating the small intestine in these mice (bottom four panels). Scale bars, 800 μm (top FI panels) and 100 μm (bottom FI panels). Pictures were taken on a Nikon SMZ 1000 stereomicroscope. b, Quantification of the intestinal infiltration in transplanted Rag1-/- or NCr mice by flow cytometry (P < 0.03). c, d, Weight of lymph nodes (P < 0.01) (c) and spleens (P = n.s.) (d) in transplanted Rag1-/- and NCr mice. e, CCL25 expression in the small intestine of Rag1-/- and NCr mice measured by ELISA. f, Western blot analysis of CCL25 expression in the small intestine of Rag1-/- and NCr mice. g, Overall survival of Rag1-/- and thymectomized Rag1-/- mice after transplant with shPten T-ALL cells (n = 5 per group). h, H&E and immunohistochemical analysis of CD3 expression of spleen, liver and intestine from Rag1-/- and thymectomized Rag1-/- mice transplanted with shPten T-ALL cells. Scale bars, 200 μm for spleen and liver and 100 μm for intestinal samples. i, Flow cytometric measurement of CCR9 expression on shPten leukaemia cells either in the absence of Dox (Pten knocked down) or Dox-treated (Pten reactivated). One representative analysis out of four analysed on/off Dox pairs is shown. A CCR9 negative B-cell line was used as control. j, Immunoblot analysis of PTEN, p-AKT(S473) and ACTB expression in human HBP-ALL T-ALL cells infected with either a control shRNA (shRenilla) or a shRNA targeting PTEN, and either starved or stimulated for 15 min with 500 ng ml⁻¹ CCL25. k, shPten tumour cell migration across a Boyden chamber in the presence or absence of 1 μg ml⁻¹ Dox and 500 ng ml⁻¹ CCL25. One representative experiment of two is shown, samples were run in triplicate; **P < 0.01, ***P < 0.001 by t-test (± s.d.).
Extended Data Figure 10 | CCR9 inactivation by shRNA knockdown or by pharmacologic inhibition attenuates intestinal tumour infiltration.

a, CCR9 expression on the surface of shPten tumour cells either infected with a control shRNA (shRenilla) (left) or with a shRNA targeting Ccr9 (right) as measured by flow cytometry, compared to uninfected cells, respectively.

b, Flow cytometry-based quantification of CCR9 suppression in shCcr9-infected shPten T-ALL cells compared to shRenilla-infected cells, n = 5 for each cohort (± s.d.).

c, Raw percentage of shRenilla/shCcr9-expressing shPten T-ALL cells ± s.d. in different tissue compartments of mice 12 days after transplantation, determined by flow cytometry, n = 5 for each cohort. P < 0.0005 (intestine).

d, IHC analysis for mCherry (left, shRenilla-mCherry; right, shCcr9-mCherry)-expressing cells in tissue sections of mice from c. Spleen, liver and intestinal sections of mice transplanted with shRenilla- or shCcr9-infected T-ALL cells were analysed for mCherry expression. Representative stains from one mouse out of three analysed mice are shown. Scale bars, 100 μm, insets 20 μm.

e, IHC staining for eGFP expression in representative sections of small intestine, liver and spleen of Vav-tTA;shPten-tumour-bearing mice treated with vehicle or the CCR9 inhibitor CCX8037 (n = 3). Scale bars, 400 μm (×2.5) and 100 μm (×10).

f, Flow cytometric quantification of intestinal tumour infiltration in Rag1<sup>−/−</sup> mice transplanted with Vav-tTA;shPten leukaemia cells and treated with vehicle (n = 4) or a small molecule inhibitor of CCR9 (n = 5). *P < 0.05 by t-test (± s.d.).

g, Immunoblot analysis of p-AKT expression 15 min after stimulation of shPten leukaemia cells with CCL25 in the absence or presence of indicated concentrations of CCX8037.

h, IHC analysis of eGFP and p-AKT signal in representative sections of small intestine from Vav-tTA;shPten-tumour-bearing mice treated with vehicle or the CCR9 inhibitor CCX8037. Scale bars, 100 μm, 25 μm for insets.