LILRB4 signalling in leukaemia cells mediates T cell suppression and tumour infiltration

Mi Deng1,20, Xun Gui2,20, Jaehyup Kim3,20, Li Xie4, Weina Chen1, Zunling Li1,5, Lical He1,6, Yuanzhi Chen7,8, Heyu Chen1, Weiguang Luo1,9, Zhigang Lu1,9, Jingjing Xie1,9, Hywyn Churchill1, Xiyang Xu2, Zhan Zhou1, Guojin Wu3, Chenyi Yu2,10, Samuel John4, Kouyuki Hirayasu1, Nam Nguyen1, Xiaoye Liu1, Fangfang Huang1,13, Leike Li2, Hui Deng2, Haidong Tang2, Ali H. Sadek1, Lingbo Zhang10, Tao Huang4, Yizhou Zou8, Benjamin Chen15, Hong Zhu16,17, Hisashi Arase1, Ningshao Xia1, Youxing Jiang1, Robert Collins18, M. James Yo19, Jade Homsi18, Nisha Unni18, Cheryl Lewis17, Guo-Qiang Chen4, Yang-Xin Fu3, X. Charlene Liao14, Zhiqiang An14, Junke Zheng14, Ningyan Zhang14 & Cheng Cheng Zhang14

Immune checkpoint blockade therapy has been successful in treating some types of cancer but has not shown clinical benefits for treating leukaemia1. This result suggests that leukaemia uses unique mechanisms to evade this therapy. Certain immune inhibitory receptors that are expressed by normal immune cells are also present on leukaemia cells. Whether these receptors can initiate immune-related primary signalling in tumour cells remains unknown. Here we use mouse models and human cells to show that LILRB4, an immunoreceptor tyrosine-based inhibition motif-containing receptor and a marker of monocytic leukaemia, supports tumour cell infiltration into tissues and suppresses T cell activity via a signalling pathway that involves APOE, LILRB4, SHP-2, uPAR and ARG1 in acute myeloid leukaemia (AML) cells. Deletion of LILRB4 or the use of antibodies to block LILRB4 signalling impeded AML development. Thus, LILRB4 orchestrates tumour invasion pathways in monocytic leukaemia cells by creating an immunosuppressive microenvironment. LILRB4 represents a compelling target for the treatment of monocytic AML.

To identify novel mechanisms for AML development and immune regulation, we analysed the relationship between gene expression of known co-stimulating and co-inhibitory receptors and the overall survival of patients with AML as documented in The Cancer Genome Atlas (TCGA) database. Expression of the mRNA that encodes leukocyte immunoglobulin-like receptor B4 (LILRB4), an immune inhibitory receptor that is restrictively expressed on monocytic cells2–4 and monocytic AML cells (FAB M4 and M5 AML subtypes)3 ranked at the top of the list for negative correlation with patient survival (Fig. 1a, Extended Data Fig. 1a–d, Supplementary Table 1). Notably, LILRB4 levels were higher on monocytic AML cells than on normal monocytes (Fig. 1b).

The extracellular domain of LILRB4 inhibits T cell activity5. To test whether LILRB4 expressed on AML cells suppresses T cells, we cultured LILRB4-positive leukaemia cells, LILRB4-negative leukaemia cells, or normal haematopoietic cells together with either autologous T cells or T cells from healthy donors. Only LILRB4-positive monocyctic AML cells substantially suppressed T cell proliferation (Fig. 1c, Extended Data Fig. 1e, f). We then deleted LILRB4 from human monocytic AML THP-1 and MV4-11 cells and found that the ability of AML cells to suppress T cells was reduced upon LILRB4 knockout (LILRB4−/−) and was restored by forced expression of wild-type LILRB4 (LILRB4+/−, WT), but not by forced expression of a mutant LILRB4 with deleted intracellular domain (as LILRB4−/−, intΔ) (Fig. 1d, Extended Data Fig. 2a–g). Moreover, when wild-type THP-1 cells and human T cells were cultured in separate transwells, LILRB4-mediated T cell inhibition was also observed and could be reversed by anti-LILRB4 blocking antibodies (Extended Data Fig. 2h–p). Blocking LILRB4 resulted in an increase in T cell cytotoxicity and cytokine release (Extended Data Fig. 2q, u). These in vitro data suggest that, instead of the extracellular domain5, the intracellular signalling of LILRB4 in AML cells is required for suppression of T cell activity.

Next, we used humanized mouse xenograft models and an immunocompetent mouse model to investigate LILRB4 function in immune checkpoint blockade. Subcutaneous implantation of THP-1 cells—but not of LILRB4−/− THP-1 cells—resulted in the development of AML in human T cell-reconstituted mice, and this was blocked by anti-LILRB4 treatment (Extended Data Fig. 3a–i). Doxycycline-induced deletion of LILRB4 in an established disseminated leukaemia model in humanized mice also impaired leukaemia development and restored T cells (Fig. 1e, f, Extended Data Fig. 3j–l). In addition, we subcutaneously implanted human LILRB4-expressing mouse C1498 AML cells (LILRB4−/− C1498 into C57BL/6 mice to establish a syngeneic immunocompetent mouse model. To exclude the anti-tumour effects of Fc effector functions, we treated tumour-bearing mice with anti-LILRB4 with the Fc glycosylation site N297A mutation8. Blockade of LILRB4 effectively lowered tumour burden and prolonged survival; depletion of CD8+ T cells eliminated the anti-tumour effects of the anti-LILRB4 antibody (Extended Data Fig. 3m–r). These results suggest that the tumour-supportive effect of LILRB4 depends on inhibition of host T cells. The anti-LILRB4 antibody treatment generated tumour-specific memory T cells (Extended Data Fig. 3s). Similar results were obtained in the disseminated hLILRB4−/−C1498 syngeneic mouse model (Extended Data Fig. 3x–z). Finally, blockade of LILRB4 reduced leukaemia development in xenografts derived from primary human monocyctic AML cells (Fig. 1g–i, Extended Data Fig. 4a) and increased the number of engraftable autologous human T cells (Extended Data Fig. 4b). Together, our in vitro and in vivo results suggest that LILRB4 is a promising target for the treatment of AML.

1Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX, USA. 2Texas Therapeutics Institute, Brown Foundation Institute of Molecular Medicine, McGovern Medical School, University of Texas Health Science Center, Houston, TX, USA. 3Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX, USA. 4Department of Pathophysiology, Key Laboratory of Cell Differentiation and Apoptosis of Chinese Ministry of Education, Shanghai Jiao Tong University School of Medicine, Shanghai, China. 5Taishan Immunology Program, Basic Medicine School, Binzhou Medical University, Yantai, China. 6Key Laboratory of Laboratory Medicine, Ministry of Education, School of Laboratory Medical and Life Science, Wenzhou Medical University, Wenzhou, China. 7School of Public Health, Xiamen University, Xiamen, China. 8Department of Immunology, Xiangya Medical School, Central South University, Changsha, China. 9Institute of Biomedical Sciences and the Fifth People’s Hospital of Shanghai, Fudan University, Shanghai, China. 10Xiangya Medical School, Central South University, Changsha, China. 11Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX, USA. 12Department of Immunoochemistry, Research Institute for Microbial Diseases and Laboratory of Immunoochemistry, World Premier International Immunology Frontier Research Center, Osaka University, Osaka, Japan. 13Department of Hematology, Zhongshan Hospital, Xiamen University, Xiamen, China. 14Immune-Onc Therapeutics, Inc., Palo Alto, CA, USA. 15Department of Radiation Oncology, University of Texas Southwestern Medical Center, Dallas, TX, USA. 16Department of Clinical Sciences, University of Texas Southwestern Medical Center, Dallas, TX, USA. 17Harold C. Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, Dallas, TX, USA. 18Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA. 19Department of Hematopathology, Division of Pathology and Laboratory Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA. 20These authors contributed equally: Mi Deng, Xun Gui, Jaehyup Kim. *e-mail: zhiqiang.an@uth.tmc.edu; zhengjunke@sjtu.edu.cn; ningyan.zhang@uth.tmc.edu; alec.zhang@utsouthwestern.edu

© 2018 Springer Nature Limited. All rights reserved.
LILRB4 expressed on leukaemia cells suppresses T cell proliferation. a, Surface expression of LILRB4 quantified by flow cytometry analysis of samples from 105 patients with AML (n = 1–34 for each classification (see Methods), mean ± s.e.m.). b, Comparison of surface expression of LILRB4 on normal monocytes and neoplastic monocytes from the same patients (n = 6 independent patients), MFI, mean fluorescence intensity. c, Autologous T cells (pT, patient T cells) isolated from a patient with monocytic AML (AML#1) or allogeneic THP-1 cells in NSG mice (n = 5 mice) were labeled with GFP and CD45+LILRB4+ (%), and overall survival (d) and body weight (e) over time. f–j, Comparison of transendothelial migration (f, n = 3 biologically independent samples, mean ± s.e.m.), short-term (g, 20 h, n = 5 mice) and long-term (h, 16 days, n = 5 mice) infiltration of hLILRB4+CD149 or control CD149 cells (GFP+), and overall survival (i) and body weight (j) over time. k, Comparison of short-term (20 h) infiltration of indicated wild-type or modified THP-1 cells in NSG mice (n = 5 mice). l–n, Comparison of short-term (20 h) infiltration of human primary monocytic AML cells (AML#21) in NSG mice (n = 4 mice) after treatment with anti-LILRB4 antibody or control IgG (n = 8 biologically independent samples). Experiment repeated for 16 independent patient samples with similar results (Extended Data Fig. 4a). See Methods for definition of box plot elements in b–n. All P values from two-tailed Student’s t-test.

indicate that LILRB4 signalling in monocytic AML cells suppresses T cell-mediated anti-tumour immunity.

One of the characteristic features of monocytic AML is enhanced extramedullary infiltration of tumour cells. In addition to tumour shrinkage (Extended Data Fig. 3m), antibody blockade of LILRB4 resulted in a decrease in leukaemic infiltration into internal organs, including bone marrow, liver and brain (Extended Data Fig. 3n–q). Although anti-LILRB4 antibody treatment did not reduce the size
of subcutaneous C1498 tumours in C57BL/6 mice depleted of CD8+ T cells (Extended Data Fig. 3m), treatment with anti-LILRB4 antibody did lead to a decrease in leukaemia cell infiltration into the liver (Extended Data Fig. 3w). We hypothesized that, as well as inhibiting T cells, LILRB4 promotes leukaemia infiltration. To test this hypothesis, we performed transendothelial migration and homing assays and monitored leukaemia infiltration relative to LILRB4 expression on leukaemia cells. Human AML THP-1 cells depleted of LILRB4 had lower transendothelial migration in vitro than cells that expressed LILRB4 (Fig. 2a). Deletion of LILRB4 reduced homing and engraftment of AML cells to haematopoietic organs (Fig. 2b, c), and resulted in prolonged survival of xenografted mice (Fig. 2d) and delayed body weight loss (Fig. 2e). By contrast, forced expression of human LILRB4 in mouse AML C1498 or WEHI-3 cells had the opposite effects (Fig. 2f–j, Extended Data Fig. 5a–e). Antibody-mediated LILRB4 blockade in LILRB4-expressing AML cells had the same effects as LILRB4 knockout (Extended Data Fig. 5f–t). Leukaemia infiltration depended on LILRB4 expression and its intracellular signalling in leukaemia cells (Fig. 2k) but not the Fc effector functions of the antibody (Extended Data Fig. 5u, v). Furthermore, LILRB4 blockade reduced the infiltration ability of primary monocytic AML cells (Fig. 2l–n, Extended Data Fig. 4c–e). Our results are concordant with previous studies showing that the frequency of circulating LILRB4+ AML blasts is lower than that of LILRB4− AML blasts5 and that LILRB4+ chronic lymphocytic leukaemia cells are associated with lymphoid tissue involvement15. The bone marrow, liver, and brain—to which LILRB4+ AML cells tend to migrate—are known to have certain immune privileges11–13. Thus, LILRB4-mediated migration, which supports enhanced extramedullary infiltration of monocytic AML cells, may also contribute to immune evasion. The blockade of immune inhibitory and migration functions of AML cells by anti-LILRB4 antibodies suggests that these functions of LILRB4 are regulated by extracellular mechanisms. Integrin-α5β3 is the ligand for gp49B1, the mouse LILRB4 orthologue14. However, a variety of integrin-α5β3 complexes did not activate human LILRB4 reporter cells (Extended Data Fig. 6a, b). Unexpectedly, human serum and mouse serum were capable of activating the LILRB4 reporter but not reporters for other LILRBs (Fig. 3a). Through protein liquid chromatography fractionation followed by reporter assays and mass spectrometry we identified APOE, which specifically activated LILRB4 and mouse PirB reporters (Fig. 3b, Extended Data Fig. 6c–j). Serum from wild-type mice, but not Apoe-null mice, activated the LILRB4 reporter (Fig. 3c). In addition, both liposome-reconstituted APOE protein (APOE–POPC) and lipid-free APOE activated LILRB4 reporter cells (Fig. 3d). Binding of APOE to THP-1 cells was significantly decreased by deletion of LILRB4 (Fig. 3e). We confirmed specific binding of recombinant APOE to LILRB4 using microscale thermophoresis (MST), surface plasmon resonance (SPR) and bio-layer interferometry (Octet). The dissociation constant was 210 nM as determined by MST (Fig. 3f, Extended Data Fig. 6k, l). Mutagenesis studies showed that the N-terminal domain of APOE, and P35 and W106 in the first immunoglobulin domain and Y121 in the linker region between two immunoglobulin domains of LILRB4, are critical for APOE-mediated activation of LILRB4 (Fig. 3g, h, Supplementary Table 2, Extended Data Fig. 6m).

The finding that APOE activates the immune inhibitory receptor LILRB4 is consistent with the immunosuppressive function of APOE15,16. To determine whether suppression of T cells by LILRB4 depends on APOE, we examined proliferation of T cells co-cultured with LILRB4 reporter cells activated by APOE proteins. Data on LILRB4 mutants that interfere with APOE activation are highlighted in red (g, h, low-to-high outline and line at mean in box plots), i, T cells from healthy donors were incubated with indicated irradiated wild-type, LILRB4KO or APOEKO THP-1 cells. T cells were analysed by flow cytometry after 7 days. j–l, C57BL/6 mouse spleen cells (E) were incubated with irradiated human LILRB4-expressing (GFP LILRB4) or control (GFP) C1498 cells (T) at indicated E:T ratios. Cells were supplemented with 5% serum from wild-type or ApoeKO mice, cultured with anti-CD3 and anti-CD28-coated beads for 60 h, and then stained with anti-CD3 antibody. j, Representative flow plots from samples at E:T of 20:1. k, Percentages of CD3+ T cells (mean ± s.e.m.). l, Effects of APOE–POPC rescue of ApoeKO serum. m, Expression of human LILRB4 in mouse leukaemia C1498 cells increases leukaemia cell infiltration in wild-type recipient mice but not in ApoeKO recipient mice. e, j, Experiments were repeated independently three times with similar results. See Methods for definition of box plot elements in i, l, m. All P values from two-tailed Student’s t-test, NS, not significant. a–d, n = 3 biologically independent samples; g–i, k, l, n = 4 biologically independent samples; m, n = 5 mice.
with control or APOEKO human AML cells. AML cells deficient in APOE restored proliferation of T cells and suppressed migration of leukaemia cells (Fig. 3i, Extended Data Fig. 6n–i). Moreover, the percentage of T cells in co-culture was lower when C1498 cells ectopically expressing LILRB4 were treated with wild-type mouse serum compared to those treated with APOEKO mouse serum (Fig. 3j, k). Addition of liposome-reconstituted APOE to a co-culture of mouse spleen cells and LILRB4-expressing AML cells decreased the T cell percentage (Fig. 3i). Furthermore, expression of LILRB4 increased infiltration of C1498 cells to bone marrow and liver in wild-type mice but not in APOEKO recipients (Fig. 3m). These data indicate that APOE activates LILRB4 on human mononcytic AML cells to suppress T cell proliferation and support AML cell migration.

We sought to identify the signalling downstream of LILRB4 that is required for T cell suppression and leukaemia infiltration. The phosphatases SHP-1 (also known as PTPN6), SHP-2 (also known as PTEN11) and SHIP (also known as INPP5D) can be recruited to the intracellular domain of LILRB4. The level of phosphorylation of SHP-2, but not of SHP-1 or SHIP, was lower in LILRB4KO AML cells than in wild-type cells (Fig. 4a, Extended Data Fig. 7a). Lack of SHP-2, but not of SHP-1 or SHIP, reversed T cell suppression by THP-1 cells (Fig. 4b, Extended Data Fig. 7b–d), and decreased short-term (20 h) and long-term (21 days) infiltration of THP-1 cells (Fig. 4c, d). Our results suggest that SHP-2 is a mediator of LILRB4 signalling.

Our ingenuity pathway analysis showed that the activity of the key transcription factors NF-κB and RELA in the NF-κB pathway17, which is positively regulated by SHP-228, was strongly inhibited by loss of LILRB4 (Fig. 4e, Supplementary Tables 3, 4). Consistently, phosphorylation of IKKα/β and levels of nuclear NF-κB were decreased in LILRB4KO AML cells (Fig. 4f, g, Extended Data Fig. 7a). Inhibition of NF-κB signalling reversed T cell suppression and reduced AML cell infiltration in a LILRB4-dependent manner (Fig. 4h, i, Extended Data Fig. 7e, f). Therefore the effects of LILRB4 are mediated through the NF-κB pathway, which is particularly robust in monocytic AML among AML subtypes.

Consistent with our result that AML cells inhibit T cell proliferation in transwells (Extended Data Fig. 2o, p), conditioned medium from wild-type THP-1 cells suppressed T cell activity but conditioned medium from LILRB4KO cells did not (Fig. 4j). Among proteins that were present at higher levels in the conditioned medium of wild-type THP-1 cells than in that of LILRB4KO cells (Extended Data Fig. 7g–i), the urokinase receptor uPAR is highly expressed by monocytic AML cells20. uPAR, an NF-κB target, is known to promote cancer invasion, metastasis, survival and angiogenesis.15,22 The addition of recombinant uPAR decreased proliferation of T cells co-cultured with LILRB4KO THP-1 cells in a dose-dependent manner (Fig. 4k). Overexpression of uPAR (PLAUR) or ARG1 rescued the infiltration defect in LILRB4KO MV4-11 cells (n = 5 mice). A, f, g, j, k, i, m. Experiments repeated independently three times with similar results. See Methods for definition of box plot elements in b–d, h–m. All P values from two-tailed Student’s t-test.
METHODS

Mice. C57 BL/6 and NOD-SCID IL2γ-null (NSG) mice were purchased from and maintained at the animal core facility of University of Texas Southwestern Medical Center (UTSW). Apoe KO (Apoe<sup>−/−</sup>) mice were purchased from the Jackson Laboratory. Animal work described in this manuscript has been approved and conducted under the oversight of the UT Southwestern Institutional Animal Care and Use Committee (IACUC). For each experiment, the same sex- and age-matched (4–8 weeks) mice were used and randomly allocated to each group; and for tumour size measurement and in vivo lumina imaging experiments, experimenters were blinded to the treatment conditions of the mice. The minimum number of mice in each group was calculated based on results from our prior relevant studies.<sup>12,16</sup> For the subcutaneous tumour model, the tumour size was calculated as (width x width x length) cm<sup>3</sup>. The maximal tumour measurement permitted by UTSW IACUC is 2 cm in diameter. In none of the experiments were these limits exceeded (see Source Data). We complied with all relevant ethical regulations and used approved animal study protocols.

Cell culture. 293T cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C in 5% CO<sub>2</sub> and normal O<sub>2</sub>. Human umbilical vein/vascular endothelial cells (HUVECs) (ATCC, CRL-1730) were cultured in endothelial cell growth medium plus growth factor, cytokines and supplements (EGM-BulletKit, Lonza) at 37 °C in 5% CO<sub>2</sub> and normal O<sub>2</sub>. Human monocytic AML cells (THP-1 (ATCC, TIB-202), MV4-11 (ATCC, CRL-2991), and U937 (ATCC, CRL-1593.2)) and mouse AML cells (WEHI-3 (ATCC, TIB-68)) were cultured in Roswell Park Memorial Institute (RPMI) 1640 supplemented with 10% FBS at 37 °C in 5% CO<sub>2</sub> and normal O<sub>2</sub>. Mouse primary bone marrow monocytes were cultured in DMEM supplemented with 10% FBS at 37 °C in 5% CO<sub>2</sub> and normal O<sub>2</sub>. All cell lines were routinely tested for mycoplasma contamination (R&D Systems).

Human primary leukaemia cells. Primary human AML and B-cell acute lymphoblastic leukaemia (B-ALL) samples were obtained from the tissue banks at UTSW and University of Texas MD Anderson Cancer Center (MDACC). Informed consent was obtained under protocols reviewed and approved by the Institutional Review Board at UTSW and MDACC (IRB STU 122013-023 by UTSW and LAB10-0682 by MDACC). The UTSW cohort included 105 patients with AML subtypes by the French-American-British (FAB) classification, acute myeloblastic leukaemia (AUL; n = 39), acute megakaryoblastic leukaemia (B-ALL; n = 42), acute myelomonocytic leukaemia (M4, n = 16), acute monocytic leukaemia (M5, n = 2), acute megakaryoblastic leukaemia (M7, n = 1) and patients with undifferentiated leukaemia (AUL; n = 1) and transient myeloproliferative disorder (TAM; n = 2) (Supplementary Table 1). Samples were frozen in FBS with 10% DMSO and stored in liquid nitrogen. Primary leukaemia samples used in patient-derived xenografts (PDXs), co-culture, short-term infiltration assay and western blotting were summarized in Supplementary Table 5. We have complied with all relevant ethical regulations with approved study protocols.

Human normal monocytes and macrophages. Human normal monocytes (CD14<sup>+</sup>) cells were isolated by the AutoMACS Pro Separation System (Miltenyi Biotech) from the mononuclear cells fraction of normal peripheral blood. In brief,buffy coat was purchased from Interstate Blood Bank and the mononuclear cell layer was separated by Ficoll Hypaque (17144003, GE Lifesciences) density gradient separation. Mononuclear cells were treated with red blood cell lysis buffer to remove red blood cells and then incubated with CD14 microbead-conjugated antibody (130-050-201, Miltenyi Biotech) for 15 min at 4 °C. CD14-positive cells were then isolated using the positive selection program according to the manufacturer’s protocol. One million CD14<sup>+</sup> cells were plated in macrophage culture medium, Isco’s modified Dulbecco’s medium (IMDM) (12440053, Thermo Fisher) supplemented with 10% human AB serum (MT35080C1, Fisher Scientific), 1% NEAA (11-140-050, Fisher), 2 μL/mL-α-tinutum-α-glutamine (SH3003402, Fisher), per each well of a 6-well plate and cultured for 7 days. After incubation, most of the cells were adherent to the plastic surface and stained positive for CD14 and other markers specific for macrophages. TCGA analyses. Data were obtained from the TCGA acute myeloid leukaemia database (version: 16 August 2016). The patients were classified into AML subtypes by the French-American-British (FAB) classification, acute myeloblastic leukaemia with maturation (M1, n = 9), acute myeloblastic leukaemia with maturation (M2, n = 34), acute promyelocytic leukaemia (M3, n = 10), acute myelomonocytic leukaemia (M4, n = 34), acute monocytic leukaemia (M5, n = 25), acute erythroid leukaemia (M6, n = 2), and acute megakaryoblastic leukaemia (M7, n = 1) and patients with undifferentiated leukaemia (AUL; n = 1) and transient myeloproliferative disorder (TAM; n = 2) (Supplementary Table 1). Samples were frozen in FBS with 10% DMSO and stored in liquid nitrogen. Primary leukaemia samples used in patient-derived xenografts (PDXs), co-culture, short-term infiltration assay and western blotting were summarized in Supplementary Table 5. We have complied with all relevant ethical regulations with approved study protocols.

Primary human leukaemia cells. Primary human AML and B-cell acute lymphoblastic leukaemia (B-ALL) samples were obtained from the tissue banks at UTSW and University of Texas MD Anderson Cancer Center (MDACC). Informed consent was obtained under protocols reviewed and approved by the Institutional Review Board at UTSW and MDACC (IRB STU 122013-023 by UTSW and LAB10-0682 by MDACC). The UTSW cohort included 105 patients with AML subtypes by the French-American-British (FAB) classification, acute myeloblastic leukaemia (AUL; n = 39), acute megakaryoblastic leukaemia (B-ALL; n = 42), acute myelomonocytic leukaemia (M4, n = 16), acute monocytic leukaemia (M5, n = 2), acute megakaryoblastic leukaemia (M7, n = 1) and patients with undifferentiated leukaemia (AUL; n = 1) and transient myeloproliferative disorder (TAM; n = 2) (Supplementary Table 1). Samples were frozen in FBS with 10% DMSO and stored in liquid nitrogen. Primary leukaemia samples used in patient-derived xenografts (PDXs), co-culture, short-term infiltration assay and western blotting were summarized in Supplementary Table 5. We have complied with all relevant ethical regulations with approved study protocols.

Human normal monocytes and macrophages. Human normal monocytes (CD14<sup>+</sup>) cells were isolated by the AutoMACS Pro Separation System (Miltenyi Biotech) from the mononuclear cells fraction of normal peripheral blood. In brief, buffy coat was purchased from Interstate Blood Bank and the mononuclear cell layer was separated by Ficoll Hypaque (17144003, GE Lifesciences) density gradient separation. Mononuclear cells were treated with red blood cell lysis buffer to remove red blood cells and then incubated with CD14 microbead-conjugated antibody (130-050-201, Miltenyi Biotech) for 15 min at 4 °C. CD14-positive cells were then isolated using the positive selection program according to the manufacturer’s protocol. One million CD14<sup>+</sup> cells were plated in macrophage culture medium, Isco’s modified Dulbecco’s medium (IMDM) (12440053, Thermo Fisher) supplemented with 10% human AB serum (MT35080C1, Fisher Scientific), 1% NEAA (11-140-050, Fisher), 2 μL/mL-α-tinutum-α-glutamine (SH3003402, Fisher), per each well of a 6-well plate and cultured for 7 days. After incubation, most of the cells were adherent to the plastic surface and stained positive for CD14 and other markers specific for macrophages. TCGA analyses. Data were obtained from the TCGA acute myeloid leukaemia database (version: 16 August 2016). The patients were classified into AML subtypes (FAB classification) M0 (undifferentiated acute myeloblastic leukaemia), M1 (acute myeloblastic leukaemia with maturation), M2 (acute myeloblastic leukaemia with minimal maturation), M3 (acute lymphoblastic leukaemia), M4 (acute myelomonocytic leukaemia), M5 (acute monocytic leukaemia), M6 (acute erythroid leukaemia), M7 (acute megakaryoblastic leukaemia) and TAM (transient myeloproliferative disorder) (n = 1) and patients with undifferentiated leukaemia (AUL; n = 1) and transient myeloproliferative disorder (TAM; n = 2) (Supplementary Table 1). Samples were frozen in FBS with 10% DMSO and stored in liquid nitrogen. Primary leukaemia samples used in patient-derived xenografts (PDXs), co-culture, short-term infiltration assay and western blotting were summarized in Supplementary Table 5. We have complied with all relevant ethical regulations with approved study protocols.
Cas9 expression in engrafted leukaemia cells. The knockout was validated by flow cytometry.

Leukaemia cell and T cell co-culture assay. In the co-culture assay, human T cells (5 × 10^6 per well) isolated from peripheral blood (PBMCs) from a healthy donor were stimulated with anti-CD3/CD28-coated beads (11163D, Thermofisher) and 50 U/ml rhIL-2 for 4–6 h in triplicate wells. Anti-CD3 and anti-CD28 antibodies were used to detect normal haematopoietic stem progenitor cells and autologous T cells, via tail-vein injection. Cells were cryopreserved in liquid nitrogen until use. To measure the ability of AML cells to migrate transendothelially, 5 × 10^6 mouse primary peripheral blood or bone marrow mononuclear cells were injected intravenously into each NSG mouse. Three weeks after implantation, mice had 30 to 50% engraftment of human T cells. At 3 weeks post implantation, 1 × 10^6 human AML THP-1 cells, including wild-type or LILRB4KO THP-1 cells or THP-1 cells stably expressing luciferase (THP-1–Luc–GFP) were subcutaneously implanted. Mice were immediately given 10 mg/kg of anti-LILRB4 antibodies or control IgG intravenously 7 days after leukaemia cell implantation and were treated twice a week until euthanization. Tumour growth was monitored over time by luminescence imaging (maximum, 1 × 10^8 p/sec/cm^2/sr; min, 5 × 10^8 p/sec/cm^2/sr). For survival curve experiments, death was recorded when moribund animals were euthanized. For primary PDXs, each NSG mouse was given 5–10 × 10^6 human primary peripheral blood or bone marrow mononuclear cells, which contain leukaemia cells and other normal compartments such as normal haematopoietic stem progenitor cells and autologous T cells, via tail-vein injection. Cells were cryopreserved in liquid nitrogen until use. To test the infiltration ability of mouse leukaemia cells, 5 × 10^6 C1498 GFP+ cells were implanted. Mice were immediately given 10 mg/kg of anti-LILRB4 antibodies or control IgG intravenously. Three to four weeks after transplantation, the peripheral blood, bone marrow, spleen and liver were assessed for engraftment. Leukaemia growth was monitored over time by flow cytometry of human cells in peripheral blood. The presence of more than 1% of human leukaemia cells in mouse tissue was considered successful engraftment of primary AML cells. One to four months after transplantation, the peripheral blood, bone marrow, spleen, and liver were assessed for engraftment.

For the human PBMC (hPBMC)-humanized model, 1 × 10^6 hPBMCs were injected intravenously into each NSG mouse. Three weeks after implantation, mice had 30 to 50% engraftment of human T cells. At 3 weeks post implantation, 1 × 10^6 human AML THP-1 cells, including wild-type or LILRB4KO THP-1 cells or THP-1 cells stably expressing luciferase (THP-1–Luc–GFP) were subcutaneously implanted. Mice were immediately given 10 mg/kg of anti-LILRB4 antibodies or control IgG intravenously 7 days after leukaemia cell implantation and were treated twice a week until euthanization. Tumour growth was monitored over time by luminescence imaging (maximum, 1 × 10^8 p/sec/cm^2/sr; min, 5 × 10^8 p/sec/cm^2/sr). For survival curve experiments, death was recorded when moribund animals were euthanized. For primary PDXs, each NSG mouse was given 5–10 × 10^6 human primary peripheral blood or bone marrow mononuclear cells, which contain leukaemia cells and other normal compartments such as normal haematopoietic stem progenitor cells and autologous T cells, via tail-vein injection. Cells were cryopreserved in liquid nitrogen until use.

To test the infiltration ability of mouse leukaemia cells, 5 × 10^6 C1498 GFP+hILRB4 cells or C1498 GFP cells were injected intravenously into wild-type C57BL/6 mice. Mice were euthanized after 20 h. GFP was used to detect leukaemia cells by flow cytometry. The number of leukaemia cells in recipient liver, spleen and bone marrow were normalized to numbers in peripheral blood cells, and were reported as a ratio.

To test the homing ability of haematopoietic stem/progenitor cells (HSPCs), 1 × 10^6 human cord blood mononuclear cells were injected intravenously into an NSG mouse. Mice were treated with 10 mg/kg of anti-LILRB4 antibodies or control IgG immediately after injection of monocytes and were euthanized after 20 h. CFSE-positive cells were analysed by flow cytometry.

To measure the ability of AML cells to migrate transendothelially, 3 × 10^6 HUVECs were cultured on a transwell membrane (pore size 8 µm). After 3 days, 1 × 10^5 indicated leukaemia cells were seeded in the upper chamber. In indicated experiments, leukaemia cells were treated with antibodies or proteins in the upper chamber. After 18 h, cells in the lower chamber were counted.

Short-term infiltration assay of leukaemia cells and homing assay of haematopoietic stem/progenitor cells. Cells (5 × 10^5 cells per mouse) were injected intravenously into NSG mice. Animals were treated with 10 mg/kg of anti-LILRB4 antibodies or control IgG immediately after injection of leukaemia cells. Mice were euthanized by CO2 asphyxiation and death was assured by cervical dislocation after 20 h. Peripheral blood, bone marrow, spleen, and liver were collected, and single-cell suspensions were examined by flow cytometry. CFSE, GFP, or indicated markers such as anti-human CD45 and anti-human CD33 were used to detect target leukaemia cells in indicated experiments. Numbers of leukaemia cells in recipient liver, spleen and bone marrow are reported as a ratio relative to cell numbers in peripheral blood.

To test the infiltration ability of mouse leukaemia cells, 5 × 10^3 C1498 GFP hILRB4 cells or C1498 GFP cells were injected intravenously into wild-type C57BL/6 or APOe-null mice. Mice were euthanized after 20 h. GFP was used to detect leukaemia cells by flow cytometry. The number of leukaemia cells in recipient liver, spleen and bone marrow were normalized to numbers in peripheral blood cells, and were reported as a ratio.

To test the homing ability of haematopoietic stem/progenitor cells (HSPCs), 1 × 10^6 human cord blood mononuclear cells were injected intravenously into an NSG mouse. Mice were treated with 10 mg/kg of anti-LILRB4 antibodies or control IgG immediately after injection of monocytes and were euthanized after 20 h. Anti-human CD45 and anti-human CD34 were used to detect human HSPCs by flow cytometry. Similarly, to test the infiltration ability of monocytes, 5 × 10^5 CD14+positive selected monocyte from health donor PBMC were labelled by CFSE and injected intravenously into an NSG mouse. Mice were treated with 10 mg/kg of anti-LILRB4 antibodies or control IgG immediately after injection of human leukaemia cells in the circulation of NSG mice. Macrophages were depleted by treating NSG mice with clodronate (dichloromethylene bisphosphonate) liposomes (50 µg per mouse) (200 µl of stock solution 3 days before leukaemia cell implantation), resulting in >70% depletion of CD45+CD11b+ F4/80+ macrophages in the circulation of NSG mice. NSG mice were rendered neutropenic by intra-peritoneal injection of 200 µg anti-ly-6G mAb (BP0075–1, Bioxcell) on days –3, –2, –1, and 0 after leukaemia cell implantation, resulting in >80% depletion of CD45+CD11b+CD11c+ neutrophils in the circulation of NSG mice.

Mouse AML xenografts. Xenografts were performed essentially as described. In brief, 6–8-week-old NSG mice were used for transplantation. Human leukaemia cells (1 × 10^6) were resuspended in 200 µl PBS for each mouse intravenously inject. Mice were immediately given 10 mg/kg of anti-LILRB4 antibodies or control IgG intravenously. Three to four weeks after transplantation, the peripheral blood, bone marrow, spleen and liver were assessed for engraftment. Leukaemia growth was monitored over time by flow cytometry of human cells in peripheral blood. The presence of more than 1% of human leukaemia cells in mouse tissue was considered successful engraftment of primary AML cells. One to four months after transplantation, the peripheral blood, bone marrow, spleen, and liver were assessed for engraftment.

For the human PBMC (hPBMC)-humanized model, 1 × 10^6 hPBMCs were injected intravenously into each NSG mouse. Six weeks after implantation, mice had 10 to 50% engraftment of human cells. THP-1 cells (1 × 10^7) that stably express luciferase (THP-1–Luc–GFP) were subcutaneously implanted. Mice were immediately given 10 mg/kg of anti-LILRB4 antibodies or control IgG intravenously 7 days after leukaemia cell implantation and were treated twice a week until euthanization. Tumour growth was monitored over time by luminescence imaging (maximum, 1 × 10^8 p/sec/cm^2/sr; min, 5 × 10^8 p/sec/cm^2/sr). For survival curve experiments, death was recorded when moribund animals were euthanized. For primary PDXs, each NSG mouse was given 5–10 × 10^6 human primary peripheral blood or bone marrow mononuclear cells, which contain leukaemia cells and other normal compartments such as normal haematopoietic stem progenitor cells and autologous T cells, via tail-vein injection. Cells were cryopreserved in liquid nitrogen until use.
we conducted adoptive transfer of spleen cells (5 × 10^6 cells per mouse) from anti-LILRB4-treated mice into normal recipient C57BL/6 mice. Four out of five transplanted mice rejected the control C1498-GFP mouse leukemia cells, and these mice were not susceptible to rechallenge with threefold higher numbers (3 × 10^6 cells per mouse) of C1498-GFP leukemia cells. Of 5 mice that received adoptive transfer of spleen cells from naive mice, none rejected the control C1498-GFP mouse leukemia cells.

Chimeric receptor LILRB4-Fc assay. We constructed a stable chimeric receptor reporter cell system as described^4, 5 to test the ability of a ligand to bind to the ECD of individual LILRBs, PirB, gp49B1 and LILRB4 site mutants and to trigger the activation of the chimerically fused intracellular domain of paired immunoglobulin-like receptor β3, which signals through the adapter DAP-12 to activate the NFAT promoter. If an agonist or antagonist binds the ECD and activates or suppresses the chimeric signaling domain, an increase or decrease, respectively, in GFP expression is observed. A competition assay was used to screen LILRB4 blocking antibodies. In brief, APOE proteins (C102, Novoprotein; 10 µg/ml) or human AB serum (10%, diluted in PBS) were pre-coated onto 96-well plates at 37°C for 3 h. After two washes with PBS, 2 × 10⁴ LILRB4 reporter cells were seeded in each well; meanwhile, indicated anti-LILRB4 antibodies were added into the culture medium. After 16 h, the percentage of GFP⁺ reporter cells was analysed by flow cytometry. The threshold of activation is 2 times that of negative control treatment.

Fast protein liquid chromatography and mass spectrometry. Ten per cent human AB serum in PBS was loaded onto a 16/60 Superdex 200 gel filtration column and eluted with PBS and 2 mM EDTA. Eighty fractions (40 ml) were collected and analyzed for APOEs using the amine-coupling kit from GE. LILRB4-hFc was injected into one reference sensors that were loaded only with LILRB4-Fc. Background wavelength shifts were measured from reference sensors that were loaded only with LILRB4-Fc. association (300 s) and dissociation (600 s) of APOEs. Background wavelength shifts were measured from reference sensors that were loaded only with LILRB4-Fc.

Western blotting and co-immunoprecipitation. Whole cells were lysed in Laemmli sample buffer (Sigma-Aldrich) supplemented with protease inhibitor cocktail (Roche Diagnostics). Samples were separated on SDS–PAGE gels (Bio-Rad) and transferred onto nitrocellulose membranes (Bio-Rad) for protein detection. Primary antibodies including anti-SHP-1 (Cell Signaling, 3759, 1:1,000), anti-phospho-SHP-1 Tyr564 (Cell Signaling, 8499, 1:500), anti-phospho-SHP-1 Tyr564 (Invitrogen, PA53708, 1:500), anti-SHP-2 (Cell Signaling, 3997, 1:1,000), anti-phospho-SHP-2 Tyr580 (Cell Signaling, 3703, 1:500), anti-SHIP1 (Cell Signaling, 2758, 1:1,000), anti-NFκB p65 (Cell Signaling, 8242, 1:1,000), anti-IκKαβ (Cell Signaling, 11930, 1:1,000), anti-IκKβ (Cell Signaling, 8943, 1:1,000), anti-phospho-IκKα/β Ser176/180 (Cell Signaling, 2697, 1:1,000), anti-IκB-α (Cell Signaling, 4814, 1:1,000), anti-phospho-IκB-α Ser32 (Cell Signaling, 2859, 1:500), anti-Lamin-B2 (Cell Signaling, 12255, 1:1,000), anti-arginase-1 (Cell Signaling, 9819, 1:1,000), anti-μPAR (Invitrogen, MON R-4, 1:200), anti-LILRB4 (Santa Cruz, sc-366213, 1:200), anti-APOE (Creative Diagnostics, DCAB-2367, 1:250), anti-IκB-α (Sigma-Aldrich, AB2666, 1:1,000), and anti-α-tubulin (Sigma-Aldrich, MAB2705, 1:1,000), as well as heronerdish peroxidase conjugated secondary antibodies (Cell Signaling, 7074, 1:1,000, and 7076, 1:1,000) and chemi-luminescent substrate (Invitrogen), were used. Specific cellular compartment fractions were carried out using the NE-Per nuclear/cyttoplasmic extraction kit (Thermo Fisher, 78833) or the plasma membrane protein extraction kit (Abcam, ab65400). Proteins from plasma membrane fraction were further incubated with anti-LILRB4 antibodies and dye bands protein a (Thermo Fisher, 10001D) for further immunoprecipitation and western blotting.

Immunohistochemistry. Haematoxylin staining and immunostaining were performed at the UTSW Pathology Core Facility. Antibodies used were against LILRB4 (laboratory produced, 1:100), CD3 (Abcam, ab16669, 1:100), PD-1 (Thermo Fisher, J116, 14-9989-82, 1:100) and arginase-1 (Cell Signaling, 98195, 1:100). The images were visualized using the Hamamatsu NanoZoomer 2.0-HT (Meyer instruments) and viewed in NDPview2 software (Hamamatsu).

Cytokine antibody array and arginase activity assay. To examine the secreted protein from leukemia cells, conditioned media were applied to a human cytokine antibody array (AAH-CYT-100, RayBioc) for the quantitative detection of 120 human proteins. Image J (NIH) was used for quantification. Arginase activity was determined in condition medium of indicated leukemia cells by a QuantiChrom Arginase assay kit (DARG100, BioAssay system).

RNA-seq analysis. RNA was purified from sorted cells with Qiagen RNeasy Mini Kit and then reverse-transcribed with SuperScript III Reverse Transcriptase (Invitrogen) according to the manufacturer’s instructions. RNA-seq was performed at the UTSW Genomics and Microarray Core Facility. The cDNA was sonicated using a Covaris S2 ultrasonicator, and libraries were prepared with the KAPA High Throughput Library Preparation Kit. Samples were end-repaired, and the 3’ ends were adenylated and barcoded with multiplex adapters. PCR-amplified libraries were purified with AmpureXP beads and validated on the Agilent 2100 Bioanalyzer. Before being normalized and pooled, samples were quantified by Qubit (Invitrogen) and then run on an Illumina HiSeq 2500 instrument using PE100 SBS v3 reagents to generate 51-bp single-end reads. Before mapping, reads were trimmed to remove low-quality regions in the ends. Trimmed reads were mapped to the human genome (HM19) using TopHat v2.0.1227 with the UCSC iGenomes GTf file from Illumina.

Methods for data normalization and analysis were based on the use of ‘internal standards’ that characterize some aspects of the system’s behaviour, such as technical variability, as presented elsewhere. Genes with fold change > 2, P < 0.01 and RPMK >.0.1 were deemed to be significantly differentially expressed between the two conditions and were used for pathway analysis and upstream transcription factor analysis. Pathway analysis was conducted using the DAVID
Upstream transcription-factor analysis was conducted using QIAGEN’s Ingenuity tool (http://www.ingenuity.com/).

**Molecular docking of LILRB4 with APOE.** Docking of LILRB4 with APOE was performed on ZDOCKpro module of the Insight II package. The general protocol for running ZDOCK includes two consecutive steps of calculation described as geometry search and energy search, running in program ZDOCK and RDOCK, respectively. LILRB4 crystal structure (3P2T) and APOE3 structure (2L7B) were obtained from the Protein Data Bank database. The top 50 ZDOCK poses were submitted to RDOCK refinement. Poses with high scores in both ZDOCK and RDOCK were selected as candidate complex for LILRB4–APOE interaction analysis (Supplementary Table 2).

**Statistical analyses.** Representative data from four independent experiments or indicated independent samples are presented as dot plots (means ± s.e.m.) or as box-and-whisker plots (median values (line), 25th–75th percentiles (box outline) and minimum and maximum values (whiskers)). Statistical significance for two-sample comparisons was calculated by two-tailed Student’s t-test, and significance for survival was calculated by the log–rank test. The multivariate analysis (TCGA data was analysed by Cox regression. The difference was considered statistically significant if $P < 0.05$. NS, not significant; exact $P$ values are shown. Pearson’s correlation analyses were performed with RStudio software (the R Foundation).

**Code availability.** The custom code for Pearson’s correlation analysis in RStudio is shown below.

```r
# setwd("~/file paths")
draw.graph = function(sam) {
    file = paste(sam,".tsv", sep = ")
    df1 = read.table(file, header = T)
    df2 = read.table(file, sep = ")
    df = merge(df1, df2, by.x = "sample", by.y = "sample")
    df = df[!is.na(df[,2]),]
    r = cor.test(df[,2], df[,3])
    P = cor.test(df[,2], df[,3])$p.value
    jpeg(filename = paste(sam,".jpeg", sep = "
    width = 280, height = 280, units = "px", pointsize = 12,
    quality = 75
    plot(df[,2], df[,3], xlab = "Title", ylab = sam,
    pch = 16, cex = 1, col = "red",
    main = paste("r = ",round(\text{r}estimate,2), ",\"t\", P = ",round(p,8), sep = "
    reg = lm(df[,2]-df[,3])
    abline(reg)
    dev.off()
} for (s in sample) {
    draw.graph(s)
}
```

**Reporting summary.** Further information on experimental design is available in the Nature Research Reporting Summary linked to this paper.

**Data availability**

The TCGA datasets analysed are available in the UCSC Xena Browser (https://xena.ucsc.edu). The RNA-seq datasets generated in the current study have been deposited in NCBI SRA database with the SRA accession number SRP155049.

31. Piedrahita, J. A., Zhang, S. H., Hagaman, J. R., Oliver, P. M. & Maeda, N. Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. *Proc. Natl Acad. Sci. USA* **89**, 4471–4475 (1992).

32. Zheng, J. et al. Inhibitory receptors bind ANGPTLs and support blood stem cells and leukaemia development. *Nature* **485**, 656–660 (2012).

33. Kang, X. et al. The ITIM-containing receptor LAIR1 is essential for acute myeloid leukaemia development. *Nat. Cell Biol.* **17**, 665–677 (2015).

34. Deng, M. et al. A motif in LILRB2 critical for Angptl2 binding and activation. *Blood* **124**, 924–935 (2014).

35. Zheng, J. et al. Ex vivo expanded hematopoietic stem cells overcome the MHC barrier in allogeneic transplantation. *Cell Stem Cell* **9**, 119–130 (2011).

36. Lu, Z. et al. Fasting selectively blocks development of acute lymphoblastic leukemia via leptin-receptor upregulation. *Nat. Med.* **23**, 79–90 (2017).

37. Zhang, C. C., Kaba, M., Iizuka, S., Huynh, H. & Lodish, H. F. Angiopoietin-like 5 and IGFBP2 stimulate ex vivo expansion of human cord blood hematopoietic stem cells as assayed by NOD/SCID transplantation. *Blood* **111**, 3415–3423 (2008).

38. Zheng, J., Huynh, H., Umitkawa, M., Silvany, R. & Zhang, C. C. Angiopoietin-like protein 3 supports the activity of hematopoietic stem cells in the bone marrow niche. *Blood* **117**, 470–479 (2011).

39. Cawthorne, C., Swindell, R., Stratford, I. J., Dive, C. & Welman, A. Comparison of doxycycline delivery methods for Tet-inducible gene expression in a subcutaneous xenograft model. *J. Biomol. Tech.* **18**, 120–123 (2007).

40. Denisov, I. G., Grinkova, Y. V., Lazarides, A. A. & Sigal, S. G. Directed self-assembly of monodisperse phospholipid bilayer Nanodiscs with controlled size. *J. Am. Chem. Soc.* **126**, 3477–3487 (2004).

© 2018 Springer Nature Limited. All rights reserved.
Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | LILRB4 expression in patients with AML negatively correlated with overall survival and T cell proliferation.

a, Analysis of correlation between mRNA levels of immune-modulating molecules and the overall survival of patients with AML (n = 160, divided into three groups based on gene expression) in TCGA database (https://xena.ucsc.edu) by Kaplan–Meier log-rank test. b, Kaplan–Meier analysis of correlations between LILRB4 mRNA level and the overall survival of patients (n = 160) from the TCGA database, performed in Xena browser (https://xena.ucsc.edu). Low, n = 57; medium, n = 48; high, n = 55. The P value was from Kaplan–Meier log-rank test. c, mRNA expression data from the TCGA database were analysed as a function of the AML subtype of the patient. M0, n = 16; M1, n = 42; M2, n = 39; M3, n = 16; M4, n = 35; M5, n = 18; M6, n = 2; M7, n = 3; and two not-classified AML samples. Pairwise comparisons between M4 and each one of the other subtypes (all P < 0.0001), as well as between M5 and each one of the other subtypes (all P < 0.0001), using two-sample t-test. Mean and s.e.m. values are shown. d, A multivariable Cox regression analysis to assess the association, with adjustment for confounders that include age, cytogenetics and PML-RAR mutation in TCGA database. The total sample size was 79. *P < 0.05 is considered significant. e, f, Autologous T cells isolated from individual patients with monocytic AML or B-ALL were incubated with irradiated LILRB4-positive or LILRB4-negative primary leukaemia cells from the same patients. pT, patient T cells. Allogeneic T cells isolated from healthy donors were incubated with irradiated LILRB4-positive or LILRB4-negative primary leukaemia cells from indicated patients with AML or B-ALL at an E:T of 10:1. nT, normal T cells. After culture with anti-CD3/CD28/CD137-coated beads and rhIL-2 for 14 days, T cells were stained with anti-CD3, anti-CD4, and anti-CD8 antibodies and analysed by flow cytometry. e, f, P values from two-tailed Student's t-test. P values in black indicate significance of CD3^+CD8^+ cells; P values in red indicate significance of CD3^+CD4^+ cells. n = 2 or 3 biologically independent samples with mean and s.e.m. See raw data for e and f in Source Data.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | LILRB4 suppresses T cell proliferation in vitro. a, Schematic of preparation of LILRB4-modulated THP-1 cells and examination of LILRB4 expression on the cell surfaces by flow cytometry. WT, THP-1 cells treated with scrambled control; LILRB4-KO, LILRB4-knockout THP-1 cells; LILRB4-WT, forced expression of wild-type LILRB4 in LILRB4-KO THP-1 cells; LILRB4-KO-intΔ, forced expression of intracellular domain-deleted mutant LILRB4 in LILRB4-KO THP-1 cells. b, Loss of LILRB4 on THP-1 cells reduces T cell suppression. Representative photograph of Fig. 1c (scale bar, 100 μm). c, Loss of LILRB4 on THP-1 cells does not affect cell proliferation (n = 3 biologically independent samples with mean and s.e.m.). d, Examination of LILRB4 expression on cell surface of LILRB4-KO MV4-11 cells by flow cytometry. e, Loss of LILRB4 on MV4-11 cells reduces T cell suppression. T cells isolated from healthy donors incubated in the lower chambers of a 96-well transwell plate with irradiated MV4-11 cells (E:T ratio of 2:1) in the upper chamber separated by a membrane with 3-μm pores. After culture with anti-CD3/CD28-coated beads and rhIL-2 for 7 days, representative cells were photographed using an inverted microscope (scale bar, 100 μm) (e) and T cells were stained with anti-CD3 and analysed by flow cytometry (f). n = 4 biologically independent samples. g, Loss of LILRB4 on MV4-11 cells does not affect cell proliferation (n = 3 biologically independent samples with mean and s.e.m.). h, i, T cells (E, effector cells) isolated from healthy donors were incubated with indicated irradiated THP-1 cells (T, target cells) without direct contact in transwells for 2 days. E:T = 2:1. T cells were treated with BrdU for 30 min followed by BrdU and 7-AAD staining for flow cytometry analysis. Representative flow cytometry plots are shown in h and the cell cycle status is summarized in i. Control, T cells cultured without THP-1 cells. n = 3 biologically independent samples with mean and s.e.m. j, k, T cells (E, effector cells) isolated from healthy donors were stained with CFSE and incubated with indicated irradiated THP-1 cells (T, target cells) without direct contact in transwells for 2 days. A representative flow cytometry plot is shown in j and the percentages of proliferating T cells indicated by CFSE-low staining is shown in k. n = 3 biologically independent samples with mean and s.e.m. LILRB4 increases PD-1 expression on T cells in coculture of leukaemia cells and T cells. T cells (E, effector cells) isolated from healthy donors were incubated with indicated irradiated THP-1 cells (T, target cells) in a non-contact manner for 5 days. E:T = 2:1. T cells were stained with anti-LAG-3, anti-TIM-3, anti-TIGIT, anti-PD-1 and anti-Fasl antibodies for flow cytometry analysis. Representative flow cytometry plots and the mean of fluorescence intensities, at the right-upper corner (black, WT; red, KO), are shown. Experiments were performed three times with similar results. m, n, Anti-LILRB4 antibody had no effect on proliferation of THP-1 cells (m) or T cells (n). m, The growth of THP-1 cells during 7 days treatment with IgG or anti-LILRB4 antibody (n = 3 biologically independent samples with mean and s.e.m.). n, The numbers of human primary T cells after 5 days treatment with IgG or anti-LILRB4 antibody in vitro (n = 3 biologically independent samples with mean and s.e.m.). o, p, Primary T cells and irradiated THP-1 cells (E:T ratio, 2:1) were placed in the lower and upper chamber, respectively, and treated with 10 μg ml−1 control IgG or anti-LILRB4 antibodies. o, Representative photographs of T cells (scale bar, 100 μm). p, T cells stained with anti-CD3 and analysed by flow cytometry. n = 4 biologically independent samples. q, Primary T cells stimulated with anti-CD3/CD28/CD137-coated beads were co-cultured with wild-type or LILRB4-KO THP-1 cells with indicated E:T ratios for 4 h (n = 3 biologically independent samples with mean and s.e.m.). Cytotoxicity of leukaemia cells was determined by PI staining in flow cytometry analysis. r–u, CD8+ T cells (5 × 104 cells) stimulated with anti-CD3/CD28/CD137-coated beads were co-cultured with 5 × 103 THP-1 cells that stably express GFP and treated with 100 μg ml−1 anti-LILRB4 antibodies or control IgG for 5 days. s, t, n = 4 biologically independent samples; u, n = 3 biologically independent samples with mean and s.e.m. Representative flow plots (r) of the percentages of T cells (GFP+) and surviving leukaemia cells (GFP−), and quantification of T cells (s), GFP+ leukaemia cells (t), and secretion of IFNγ (u), are shown. b, d, e, h, j, o, r. Experiments repeated independently three times with similar results. See Methods for definition of box plot elements in f, p, s, t. All P values were from two-tailed Student’s t-test.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Inhibition of LILRB4 reduces leukaemia development in humanized immunocompromised mice and syngeneic mice. a–c, Wild-type or LILRB4KO THP-1 cells (3 × 10⁶ cells per mouse) were subcutaneously implanted into hPBMC-repopulated NSG mice (WT, n = 14 mice with mean and s.e.m.; LILRB4KO, n = 10 mice, mean and s.e.m.; also see Source Data). Tumour size (a), quantification of CD3+ cells at day 31 in peripheral blood of recipient mice (b) and representative flow plots showing CD4+ and CD8+ T cells (c) are shown. d, e, LILRB4 increases PD-1 expression on tumour-infiltrated T cells. Wild-type or LILRB4KO THP-1 cells were subcutaneously implanted into hPBMC-repopulated NSG mice. Three weeks after implantation, 7 out of 10 WT group mice had large tumours and 3 out of 10 knockout-group mice had tiny tumours. These tumours were dissected for immunohistochemistry and flow cytometry staining with anti-LILRB4, anti-CD3, anti-CD8 and anti-ARG1 antibodies. Left corner images are magnified from yellow highlighted regions. In CD3 and PD-1 staining images, orange dashed lines indicate the tumour boundary. Black arrowheads indicate PD-1 positive cells. Scale bar, 100 μm. e, Tumours were dissected and cells in tumour region were stained with anti-CD3 and anti-PD-1 antibodies for flow cytometry analysis. The percentages of PD-1+ T cells (ratio of PD-1+CD3+ to CD3+ cells) were calculated. f–l, THP-1 cells were transplanted into hPBMC-repopulated NSG mice, and mice were treated with control IgG or anti-LILRB4 antibodies after 6 days (10 mg kg⁻¹; n = 5). Leukaemia development was monitored by luminescence imaging (f); luminescence flux (radiance) at day 26 (g; n = 5) and T cell numbers at day 26 in representative mice (h, i). j, k, Engraftment of human T cells and intravenously transplanted Dox-inducible LILRB4-knockout THP-1 cells (GFP+) in NSG mice at day 7 before Dox administration (n = 5). l, Representative flow plot shows that LILRB4 was successfully deleted in engrafted leukaemia cells in bone marrow of Dox-fed mouse at the endpoint. n.s., not significant. m–w, Mouse AML C1498 cells (3 × 10⁶ cells per mouse) that stably express LILRB4–IRES–GFP were subcutaneously implanted into C57BL/6 mice. Anti-LILRB4-N297A antibodies or control IgG were intravenously injected at 6, 9, 12, 15, 18 and 21 days after implantation of tumour cells. Two groups of mice were treated with anti-CD8 antibodies at 3, 6, 9 and 12 days after implantation of tumour cells to achieve CD8+ T cell depletion. m, Tumour growth of subcutaneously implanted human LILRB4-expressing mouse AML C1498 cells (hLILRB4 C1498) in C57BL/6 mice with anti-LILRB4-N297A antibodies or control antibody treatment (n = 5 mice). Also see Source Data. n, Survival curve of subcutaneous hLILRB4 C1498 tumour-bearing mice (n = 12 mice). As for tumour size, anti-LILRB4 antibodies decreased the tumour weight (o, n = 5 mice) but did not do so in the absence of CD8+ T cells (p, n = 5 mice). The percentage of CD8+ T cells in spleen was significantly negatively correlated with tumour size (q, n = 5 mice) but not in the absence of CD8+ T cells (r, n = 5 mice). s, Adoptive transplantation of spleen cells from control mice or tumour-bearing mice that were cured by anti-LILRB4-N297A treatment (n = 5 mice). Tumour size was monitored as a function of time. Arrow indicates day of rechallenge in mice that had eliminated leukaemia with three times the number of AML cells (n = 4 mice). Also see Source Data. Anti-LILRB4 antibodies reduced the infiltration of leukaemia cells into host tissues (t–v, n = 5 mice) and even CD8+ cells were depleted (w, n = 5 mice). x–z, C57BL/6 mice were intravenously injected with human LILRB4-expressing mouse AML C1498 cells (3 × 10⁶ cells per mouse) that expressed GFP. Anti-LILRB4-N297A antibodies (n = 9 mice) or control IgG (n = 9 mice) were intravenously injected at 6, 9, 12, 15 and 18 days after implantation of tumour cells. Anti-LILRB4 antibodies decreased the percentage of leukaemia cells in bone marrow (x). Anti-LILRB4 antibodies increased CD8+ T cells (y). The percentage of CD8+ T cells in bone marrow was significantly negatively correlated with the percentage of leukaemia cells (z). c, i, I, Experiments repeated independently three times with similar results. See Methods for definition of box plot elements in b, e, g, h, j, k, o, p, t–y. All P values (except n, long-rank test; and q, r, z, Pearson’s correlation) from two-tailed Student’s t-test.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Anti-LILRB4 antibodies reduce leukaemia development by restoring autologous T cells in PDX mice and inhibiting primary AML cell infiltration. **a**, Primary peripheral blood or bone marrow mononuclear AML cells (5 × 10⁶ to 1 × 10⁷ cells per mouse) from each of sixteen human patients (three shown in Fig. 1g–i, also see Supplementary Table 5) were injected into NSG mice followed by treatment with IgG or anti-LILRB4 antibodies (10 mg kg⁻¹ twice a week by intravenous injection). Percentages of human CD45⁺ LILRB4⁺ AML cells collected from haematopoietic tissues including bone marrow, spleen, liver and peripheral blood 2–4 months after transplantation, as determined by flow cytometry, are shown. **b**, Percentages of autologous human T cells collected from haematopoietic tissues including bone marrow, spleen, liver and peripheral blood 2–4 months after transplantation, as determined by flow cytometry; and representative flow plots of CD3⁺ CD8⁺ T cells in bone marrow of mice in three PDXs. **c–e**, Comparison of infiltration of human primary monocytic AML cells in NSG mice (n = 5 mice) after treatment with anti-LILRB4 antibody or IgG control. **c, d**, Primary human peripheral blood mononuclear cells from patients with monocytic AML were injected. The quantifications in c are also shown in Fig. 2l–n. **e**, Mouse liver cells with xenografted primary human monocytic AML cells (human CD45⁺ LILRB4⁺ cells) were injected. See Methods for definition of box plot elements in a–e. All P values from two-tailed Student’s t-test.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | LILRB4 promotes infiltration of AML cells. a, c, LILRB4 expression on mouse C1498 (a) or WEHI-3 (c) AML cells that stably express Lilrb4 (also known as Lilrb4a). b, d, Forced expression of LILRB4 did not affect proliferation of mouse C1498 (b, n = 3 biologically independent samples with mean and s.e.m.) or WEHI-3 (d, n = 3 biologically independent samples with mean and s.e.m.) AML cells. e, Forced expression of human LILRB4 promoted transendothelial migration of mouse AML WEHI-3 cells (n = 3 biologically independent samples with mean and s.e.m.). f, NSG mice (n = 6 mice) were injected with 1 × 10^6 THP-1 cells followed immediately by IgG or anti-LILRB4 antibody treatment and were monitored by bioluminescence imaging. g, h, Anti-LILRB4 antibodies decreased AML cell infiltration into internal organs. Mice were killed at 21 days for ex vivo bioluminescence imaging of internal organs after transplantation of 1 × 10^6 luciferase-expressing THP-1 cells. Images of luminescence flux (radiance) from representative mice are shown (g). 1, GI tract; 2, legs; 3, lung; 4, spleen; 5, liver; 6, kidneys; 7, brain; 8, heart. Infiltrated leukaemia cells formed tumour nodules in liver (h). i, j, Anti-LILRB4 antibodies did not affect LILRB4-negative cancer cells. LILRB4 is expressed on mouse C1498 (a) or WEHI-3 (c) AML cells but not on U937 cells as analysed by flow cytometry (i). Isotype IgG was used as control. NSG mice were injected with U937 human AML cells, which do not express LILRB4, and then treated with anti-LILRB4 antibodies (j). IgG served as control antibodies. Mice were killed at day 25 post-transplant for analysis of LV, BM, SP and PB by flow cytometry. The presence of human AML cells was detected by anti-human CD45 antibody staining (n = 4 mice with mean and s.e.m.). k–t, Anti-LILRB4 antibodies decreased infiltration of THP-1 (k–o) or MV4-11 (p–t) human AML cells. Comparison of transendothelial migration abilities of GFP-expressing THP-1 (k) or CFSE-labelled MV4-11 (p) cells after treatment with anti-LILRB4 antibody or IgG control in a transwell assay (n = 3 biologically independent samples with mean and s.e.m.). Comparison of the homing abilities of GFP-expressing THP-1 or CFSE-labelled MV4-11 cells (5 × 10^6 per mouse) that were injected into NSG mice followed immediately by IgG or anti-LILRB4 antibody treatment. Numbers of leukaemia cells (GFP+ in l, CFSE+ in q) in LV, SP and BM normalized to that in PB as determined by flow cytometry 20 h after injection (n = 5 mice). NSG mice were injected with 1 × 10^6 THP-1 or MV4-11 cells followed immediately by IgG or anti-LILRB4 antibody treatment (n = 6 mice for THP-1 or 5 mice for MV4-11 xenografts). Percentages of MV4-11 cells (stained with anti-human CD45) as determined by flow cytometry in indicated organs at day 21 post-xenografts. Percentages of MV4-11 cells (stained with anti-human CD45) as determined by flow cytometry in indicated organs at day 21 post-xenografts. Percentages of MV4-11 cells (stained with anti-human CD45) as determined by flow cytometry in indicated organs at day 21 post-xenografts. Percentages of MV4-11 cells (stained with anti-human CD45) as determined by flow cytometry in indicated organs at day 21 post-xenografts. Percentages of MV4-11 cells (stained with anti-human CD45) as determined by flow cytometry in indicated organs at day 21 post-xenografts. Percentages of MV4-11 cells (stained with anti-human CD45) as determined by flow cytometry in indicated organs at day 21 post-xenografts.

© 2018 Springer Nature Limited. All rights reserved.
Extended Data Fig. 6 | APOE induces LILRB4 activation to suppress T cells and support AML cell migration in vitro. 

a, Schematic of the LILRB4 reporter system. b, Human and mouse integrin heterodimer proteins cannot activate the LILRB4 reporter (n = 3 biologically independent samples with mean and s.e.m.). Human and mouse sera were used as positive controls. The threshold of activation is twice that of negative control treatment. c, Flow cytometry demonstrating that anti-LILRB4 antibody binds to human LILRB4 reporter cells. d, LILRB4 activation as indicated by percentage of GFP+ cells in the presence and absence of 10% human serum (HS) with or without anti-LILRB4 antibody or control IgG (n = 3 biologically independent samples with mean and s.e.m.). e, Flow chart of ligand identification of potential ligands of LILRB4 in human serum. f, Fractionation of LILRB4-stimulating activities from human serum by fast protein liquid chromatography. The positive control was 10% human serum. g, Proteins identified from the LILRB4 stimulating fractions by mass spectrometry. PSMs, peptide spectrum matches. h, Both human and mouse APOE proteins can activate LILRB4 reporter (n = 3 biologically independent samples with mean and s.e.m.). Human and mouse sera were used as positive controls. The threshold of activation is twice that of negative control treatment. i, APOE proteins from different sources all activate LILRB4. APOE (20 µg ml−1) purified from human plasma, His-tagged or tag-free recombinant human APOE (rhAPOE) (20 µg ml−1) expressed by 293T mammalian cells, or rhAPOE (20 µg ml−1) expressed by bacteria all activate the LILRB4 reporter. These APOE all represent human APOE3 (n = 3 biologically independent samples with mean and s.e.m.). j, APOE2, APOE3 and APOE4 all activate the LILRB4 reporter (n = 3 biologically independent samples with mean and s.e.m.). Forty micrograms per millilitre APOEs were coated on plates or directly added in cell culture medium (soluble). k, l, Three APOE isoforms bind to human LILRB4. k, Binding kinetics of APOE2, 3 and 4 to LILRB4-Fc were measured using SPR. LILRB4-Fc was immobilized on protein A biosensor tips and incubated with APOE concentrations ranging from 1.5625 nM to 100 nM. l, Binding kinetics of APOE2, 3, and 4 to LILRB4-Fc were measured using bio-layer interferometry (Octet). LILRB4-Fc was immobilized on protein A biosensor tips and incubated with APOE concentrations ranging from 44 nM to 1,176 nM. m, As shown in Fig. 3h, mutation of W106 and Y121, located in the first immunoglobulin domain and in the linker between two immunoglobulin domains, respectively, significantly reduced activation of LILRB4 by APOE. n, p, Examination of APOE expression in APOE-knockout THP-1 and MV4-11 cells by immunoblots. Primary T cells and irradiated THP-1 or MV4-11 cells (E:T = 2:1) were incubated in the lower and upper chambers, respectively. T cells were photographed (o, q, scale bar, 100 µm) and quantified by flow cytometry (Fig. 3i and r, n = 4 biologically independent samples) after 7 days. s, t, Loss of APOE suppresses transendothelial migration of human AML THP-1 and MV4-11 cells (n = 4 biologically independent samples with mean and s.e.m.). c, k, l, n–q, These experiments were repeated independently three times with similar results. See Methods for definition of box plot elements in r. All P values are from two-tailed Student’s t-test.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | LILRB4 upregulates phosphorylation of SHP-2, NFκB signalling and expression of uPAR and ARG1 to suppress T cell activity and support leukaemia migration. a, Phosphorylated SHP-2 and IκBα were downregulated upon LILRB4 knockout in MV4-11 cells. b, Co-immunoprecipitation demonstrated that LILRB4 interacts with SHP-2 in THP-1 cells. c, SHP1, SHP2 and SHIP were individually knocked out by CRISP–Cas9 in THP-1 cells as detected by western blotting. d, Primary T cells and irradiated THP-1 cells (E:T = 2:1) were cultured in the lower and upper chambers, respectively. T cells were photographed (scale bar, 100 µm) after 7 days. e, f, Two different NFκB inhibitors restored T cell proliferation from the suppression by THP-1 cells in an LILRB4-dependent manner (n = 4 biologically independent samples). THP-1 cells were pretreated with various doses of NFκB inhibitors for 1 h. Primary T cells and irradiated pretreated THP-1 cells (E:T = 2:1) were cultured in the lower and upper chambers, respectively. T cells were photographed (e, scale bar, 100 µm) and analysed by flow cytometry (f) after 7 days. g, h, Loss of LILRB4 decreased secreted protein production in THP-1 cells as determined by a human cytokine antibody array (g) and the blot intensities were quantified by ImageJ software (h, n = 3 biologically independent samples with mean and s.e.m.). Red boxes indicate proteins that were changed upon LILRB4 knockout; blue boxes indicate positive controls. i, Surface uPAR was downregulated in LILRB4KO THP-1 and MV4-11 AML cells. j, T cells were incubated with irradiated indicated THP-1 cells supplemented with indicated concentrations of recombinant uPAR proteins for 7 days. T cells were photographed. k, T cells isolated from healthy donors were cultured with anti-CD3/CD28-coated beads and rhIL-2 and supplemented with indicated concentrations of uPAR proteins for 3 days (n = 4 biologically independent samples). Representative cells were photographed using an inverted microscope and T cells were analysed by flow cytometry. p, Autologous T cells isolated from individual patients with monocytic AML were incubated with irradiated LILRB4-positive or LILRB4-negative primary leukaemia cells from the same patients at an E:T of 10:1, supplemented with recombinant anti-LILRB4 antibodies, APOE-VLDL, uPAR or ARG1. pT, patient T cells. After culture with anti-CD3/CD28/CD137-coated beads and rhIL-2 for 14 days, T cells were stained with anti-CD3, anti-CD4, and anti-CD8 antibodies and analysed by flow cytometry. n = 3 biologically independent samples with mean and s.e.m. q, Supplementation of recombinant uPAR or ARG1 to the medium rescued the decrease in transmigration ability of LILRB4KO THP-1 or LILRB4KO MV4-11 cells across endothelium (n = 3 biologically independent samples with mean and s.e.m.). Scale bar, 100 µm. a–e, g, i, j, l, n, Experiments repeated independently three times with similar results. See Methods for definition of box plot elements in f, k, o. All P values from two-tailed Student’s t-test. See raw data for p in Source Data.
Extended Data Fig. 8 | Detection of SHP-2–NFκB signalling and uPAR and ARG1 expression in primary human monocytic AML cells. a, LILRB4-positive or -high CD33⁺ AML cells (red box) and LILRB4-negative or -low CD33⁺ AML cells (blue box) were gated for further intracellular staining of SHP-2 phosphorylated at Y580, IKKα/β phosphorylated at S176/S180, NFκB phosphorylated at S529, uPAR and ARG1. Isotype IgG was used as negative control. Red numbers indicate MFI of LILRB4-positive or -high CD33⁺ AML cells; blue numbers indicate MFIs of LILRB4-negative or -low CD33⁺ AML cells. This experiment was repeated with eight individual patient samples with similar results.

b, Quantification of individual staining in LILRB4-positive or -high CD33⁺ AML cells versus in LILRB4-negative or -low CD33⁺ AML cells. n = 8 independent patients; see Methods for definition of box plot elements. P values from two-tailed Student’s t-test. c, Schematic for the mechanisms by which LILRB4 suppresses T cells and promotes leukaemia infiltration.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Comparison of LILRB4-mediated intracellular signalling in leukaemia cells and in normal haematopoietic cells.

a, Comparison of LILRB4 surface expression on normal monocytes from healthy donors (n = 25 individual donors with mean and s.e.m.) and neoplastic monocytic cells from patients with AML (n = 53 individual patients with mean and s.e.m.).
b, Comparison of LILRB4 surface expression on normal monocytes from two healthy donors and on wild-type and LILRB4KO THP-1 cells. This experiment was repeated independently three times with similar results.
c, Anti-LILRB4 antibody did not affect homing ability of normal monocytes. Human normal monocytes (b) selected through CD14-positive selection. These isolated monocytes were pooled and stained by CFSE. After staining, monocytes (5 × 10⁶ for each mouse) were injected into NSG mice followed immediately by antibody treatment, and then the mice (n = 4 mice, see Methods for definition of box plot elements) were killed 20 h after transplant. The number of CFSE⁺ cells in liver, spleen and bone marrow were normalized to that in peripheral blood as determined by flow cytometry.
d, e, APOE activates LILRB4 intracellular signalling in leukaemia cells. Indicated THP-1 cells and primary AML (M5) cells were serum-starved overnight and then treated with the indicated concentration of human recombinant APOE protein for the indicated time. Phospho-SHP-2, phospho-NFκB and ARG1 were examined by western blotting.
f, The effect of APOE on normal monocytes or in vitro differentiated macrophages. Normal monocytes were isolated from healthy donors and macrophages were derived from these monocytes after one week of differentiation in vitro. Cells were serum-starved overnight and then treated with the indicated concentrations of human recombinant APOE protein for the indicated times. Phospho-SHP-2, phospho-NFκB and ARG1 were examined by western blotting.
g, APOE induces uPAR upregulation on AML cells. Normal monocytes were isolated from healthy donors. Indicated primary AML cells and normal monocytes were serum-starved overnight and then treated with 20 µg ml⁻¹ human recombinant APOE protein for eight hours. Surface uPAR was examined by flow cytometry. Representative flow plots are shown and MFIs are shown in top right corner (black, PBS control; red, APOE treatment). Experiments were performed three times with similar results. P values from two-tailed Student’s t-test.
Extended Data Fig. 10 | See next page for caption.
Extended Data Fig. 10 | Anti-LILRB4 does not affect engraftment of normal haematopoietic cells. **a**, LILRB4 and CD34 co-staining patterns for representative samples of human cord blood mononuclear cells (hCB MNCs). N/G, neutrophils and granulocytes; M/D, monocytes, macrophages and dendritic cells; L/P, lymphocytes, haematopoietic stem and progenitor cells. This experiment was repeated independently three times with similar results. **b**, Anti-LILRB4 antibody did not affect homing ability of normal haematopoietic progenitor cells. hCB MNCs ($1 \times 10^7$) were injected into NSG mice followed immediately by antibody treatment, and then the mice ($n = 3$ mice with mean and s.e.m.) were killed 20 h after transplant. The number of CD45$^+$CD34$^+$ HSCs in liver, spleen, and bone marrow were normalized to that in peripheral blood as determined by flow cytometry. **c–e**, Anti-LILRB4 antibodies inhibited leukaemia development in hCB-humanized NSG mice. **c**, Schematic of the experiment to test whether anti-LILRB4 antibody inhibits leukaemia development in hCB-humanized NSG mice. **d**, Leukaemia development was monitored over time by luminescence imaging. This experiment was repeated independently twice with similar results. **e**, Frequency of engrafted leukaemia, normal human cells, including human B cells, human myeloid cells and human T cells in peripheral blood over time and haematopoietic tissues of hCB-humanized mice at 24 days after leukaemia transplantation. $n = 3$ mice with mean and s.e.m. All $P$ values from two-tailed Student's $t$-test.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- **n/a**
- Confirmed

☐ The exact sample size \((n)\) for each experimental group/condition, given as a discrete number and unit of measurement

☐ An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

☐ The statistical test(s) used AND whether they are one- or two-sided

☐ Only common tests should be described solely by name; describe more complex techniques in the Methods section.

☐ A description of all covariates tested

☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons

☐ A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)

☐ For null hypothesis testing, the test statistic (e.g. \(F, t, r\)) with confidence intervals, effect sizes, degrees of freedom and \(P\) value noted

☒ Give \(P\) values as exact values whenever suitable.

☒ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings

☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

☐ Estimates of effect sizes (e.g. Cohen's \(d\), Pearson's \(r\)), indicating how they were calculated

☐ Clearly defined error bars

☐ State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

BD FACSDiVa™ and BD CellQuest Pro softwares were used to collect data from flow cytometry; Living Image® 4.2 was used to collect data from in vivo imaging IVIS Lumina system; NPDview2 software was used to collect immunohistochemistry data.

Data analysis

Flow cytometric analyses were performed with the FlowJo analysis software (FlowJo 9.3.2 and FlowJo 10); RNA-seq data were analyzed with TopHat v2.0.1227, DAVID 6.8, and QIAGEN Ingenuity tool Ingenuity® Pathway Analysis (IPA). Statistical analyses, such as t-test and long-rank test, were performed with the GraphPad Prism 6 software (GraphPad). The Pearson’s correlation analyses were performed with the RStudio software (the R Foundation) and custom code below:

```r
setwd("~/file paths")

draw.graph = function(sam) {
  file = paste(sam, ".tsv", sep = "")
  df1 = read.table(file, sep = "\t", header = T)
  df2 = read.table(file, sep = "\t", header = T)
  df = merge(df1, df2, by.x = "sample", by.y = "sample")
  df = df[!is.na(df[,2]),]
  r = cor.test(df[,2], df[,3])
  p = cor.test(df[,2], df[,3])$p.value
}
```
Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The RNA-seq datasets generated in the current study have been deposited in NCBI SRA database with the SRA accession number SRP155049. TCGA AML datasets are available in UCSC Xena Browser (https://xena.ucsc.edu).
Figure 1c, Extended Data Figures 1e, 1f, 3a, 3m, 3s and 7p have associated raw data in Source Data.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☑ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size: The minimum number of mice in each group was determined based on our prior animal studies. Sample size was determined on the basis of control samples and the experimental samples. We have used the data from at least two or three independent experiments when necessary. The P values results from statistical analyses suggested that the samples sizes in our study are sufficient.

Data exclusions: No data were excluded from the analyses.

Replication: For all editing experiments findings were replicated in at least two biologically independent samples.

Randomization: All samples were assigned to groups randomly.

Blinding: For tumor size measurement and in vivo lumina imaging experiments, the technicians were not aware of treatment condition of the mice.

Reporting for specific materials, systems and methods
## Antibodies

Antibodies were obtained from the following vendors: anti-human CD45-PE (BD Pharmingen, HI30), CD45-FITC (BD Pharmingen, HI30), CD45-APC (BD Pharmingen, HI30), anti-human CD34-FITC (BD Pharmingen, 55582), anti-human CD19-PE (eBioscience, HIB19), anti-human CD20-PE (BD Pharmingen, SS5623), anti-human CD11b-APC (eBioscience, ICRF44), anti-human LILRB4-APC (eBioscience, ZM4.1), anti-human LILRB4-PE (Biolegend, ZM4.1), anti-human CD14-APC (eBioscience, RPA-T4), anti-human CD3-FITC (BioLegend, HIT3a), anti-human CD3-Pacific blue (BD Pharmingen, SP34-2), anti-human CD8-PE (BD Pharmingen, 555367), anti-human CD28-APC (eBioscience, CD28.2), anti-human CD40L-APC (eBioscience, 24-31), anti-human PD1-APC (Biolegend, EH12.2H7), anti-human TIM3-APC (eBioscience, F38-2E2), anti-human TIGIT-APC (eBioscience, MBSA43), anti-human LAG3-APC (eBioscience, 3DS23H), anti-human FasL-PE (Biolegend, 24-31), anti-uPAR-APC (Biolegend, VIM5), anti-mouse CD3-APC (BioLegend, 17A2), anti-mouse CD8a-PE (BioLegend, 555623), anti-mouse CD49b-APC (eBioscience, DX5), anti-mouse CD49F-PE (eBioscience, GoH3), anti-mouse CD11b-APC (BioLegend, M1/71), anti-mouse CD11c-APC (eBioscience, N418), anti-mouse F4/80-APC (BioLegend, BM8), anti- His-tag-APC (R&D systems, AD1.1.10), IgG isotype-control-APC (eBioscience, P3.6.2.8.1), anti-LILRB4-Alexa Fluor 647 (Biolegend, ZM4.1), anti-human CD3-FITC (Biolegend, HIM3-4), fixable cell viability dye eFluor 450 (Bioscience, Cat#65-0863-14), anti-p-SHP-2 (Y580)-PE (Cell signaling, Cat#33285), anti-p-PI3Kα/β (S176/180) (16A6) (Cell signaling, Cat#2697), anti-NFkB (S529)-PE (eBioscience, B33B4WP), anti-uPAR-PE (Biolegend, VIM5), anti-Arginase-1 (D4E3M) (Cell signaling, Cat#93668), rabbit IgG Isotype control-PE (Cell signaling, Cat#5742), mouse IgG Isotype control-PE (eBioscience, m2a-15F8), anti-rabbit IgG-PE (Jackson Immunoresearch Lab, Cat#111-116-144), anti-asialo GM1 (Cedarlane, CL8955), anti-Ly-6G (Bioxcell, BP0075-1), anti-C08 (Bioxcell, YTS 169.4.2), anti-SHP-1 (Cell signaling, 3759), anti-phospho-SHP-1 Tyr564 (Cell signaling, 8849), anti-phospho-SHP-1 Tyr564 (Invitrogen, PA537708), anti-SHP-2 (Cell signaling, 3397), anti-phospho-SHP-2 Tyr580 (Cell signaling, 3703), anti-SHPI (Cell signaling, 2727), anti-phospho-SHPI 1 Tyr1020 (Cell signaling, 3941), anti-NFkB p65 (Cell signaling, 8242), anti-IKKα (Cell signaling, 11930), anti-IKKβ (Cell signaling, 8943), anti-IκBα (Cell signaling, 4814), anti-IκBα Ser32 (Cell signaling, 2859), anti-Lamin-B2 (Cell signaling, 12255), anti-uPAR (Invitrogen, MON R-4-02), anti-LILRB4 (Santa cruz, sc-366213), anti-APOE (Creative diagnostics, DCABH-2367), anti-β-actin (Sigma-Aldrich, A2066), anti-α-tubulin (Sigma-Aldrich, MABT205), anti-CD3 (Abcam, ab16669), anti-PD-1 (Thermo Fisher, 1116, 14-9988-82), anti-Arginase-1 (Cell signaling, 9819S), CD14 microbead-conjugated antibody (130-050-201, Miltenyi Biotech, Auburn, CA), anti-CD3/CD28-coated beads (111610, Thermo Fisher), anti-CD3/CD28/CD137-coated beads (111630, Thermo Fisher), horseradish peroxidase (HRP) conjugated secondary antibodies (Cell signaling, 7074 and 7076), Anti-LILRB4 blocking antibodies were developed in the labs of Drs. Cheng Cheng Zhang and Zhiqiang An.

**Validation**

Antibody validations were performed by antibody suppliers per quality assurance literature provided by each supplier. Validations of antibody made in house were performed by investigators in the labs of Drs. Cheng Cheng Zhang and Zhiqiang An.

## Eukaryotic cell lines

**Policy information about cell lines**

**Cell line source(s)**

THP-1, MV4-11, U937, C1498, WEHI-3, HEK293, HUVEC cells were purchased from ATCC; Reporter cells were described in Blood. 2014; 124(6):924-935

**Authentication**

Cell lines (THP-1, MV4-11, U937, C1498, WEHI-3, HEK293, HUVEC) and reporter cells were routinely authenticated using GenePrint 10 system (Promega) and matched with earliest passages of cell lines.

**Mycoplasma contamination**

Cell lines (THP-1, MV4-11, U937, C1498, WEHI-3, HEK293, HUVEC) and reporter cells were routinely tested using a mycoplasma-contamination kit (R&D Systems) and tested negative.

**Commonly misidentified lines**

No commonly misidentified cell lines were used.

(See ICLAC register)

## Animals and other organisms

**Policy information about studies involving animals**

ARRIVE guidelines recommended for reporting animal research

**Laboratory animals**

4-8 week old C57Bl/6j either male or female and NOD/SCID/IL-2R-null (NSG) mice either male or female were used in the study.

**Wild animals**

This study did not involve wild animals
Field-collected samples
This study did not involve samples collected from field

Flow Cytometry

Plots

Confirm that:

☑️ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
☑️ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
☑️ All plots are contour plots with outliers or pseudocolor plots.
☑️ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation
Human peripheral blood mono-nucleated cells (PBMC) of healthy donors were isolated from fresh blood samples by Ficoll-Paque, or isolated from transplanted mice for standard flow cytometry analyses.

Instrument
BD Biosciences FACS Aria sorting, BD FACSCalibur and FACS Aria for analyzing

Software
Cellquests for data collection; FlowJo 9 and 10 for data analysis.

Cell population abundance
Flow cytometry sorted cells were confirmed >90% purity.

Gating strategy
Cells were gated on FSC/ SSC- area, live(PI negative; cell line, PBMC, primary leukemia cells), transfected cells (GFP or tdTomato positive)

☑️ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.