Targeting hyperactive platelet-derived growth factor receptor-β signaling in T-cell acute lymphoblastic leukemia and lymphoma

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Supplementary Data

Targeted Locus Amplification (TLA)

Preparation of the samples for TLA was performed as described (1). In brief, cells were crosslinked using formaldehyde and DNA was digested with NlaIII. The samples were ligated, crosslinks reversed, and the DNA purified. To obtain circular chimeric DNA molecules for PCR amplification, the DNA molecules were trimmed with NspI and ligated at a DNA concentration of 5 ng/µl to promote intramolecular ligation. Importantly, NspI was chosen for its RCATGY recognition sequence that encompasses the CATG recognition sequence of NlaIII. As a consequence, only a subset of NlaIII (CATG) sites were (re-)digested, generating DNA fragments of approximately 2 kb and allowing the amplification of entire restriction fragments. Sequences of the PDGFRB primers are (5 to 3): PDGFRB_F TGGTGGGCATATAACTCATC and PDGFRB_R CCCAGCCAATAAAGACATCA. After ligation, the DNA was purified, PCR products were purified and library prepped using the Illumina NexteraXT protocol and sequenced on an Illumina Miseq sequencer. Reads were mapped using BWA-SW, which is a Smith-Waterman alignment tool. This allows partial mapping, which is optimally suited for identifying break-spanning reads. The human genome version hg19 was used for mapping.

Array Comparative Genomic Hybridization

Patient DNA was profiled on a custom 180K oligonucleotide array platform (Agilent SurePrint G3 Human CGH microarrays, G4449A, design ID: 022060). In brief, genomic DNA from patients and controls were labelled using random prime labelled using random prime labelling with Cy3 and Cy5 dyes (Perkin Elmer, Waltham, MA, USA). Next, hybridization was performed according to the manufacturer's instructions (Agilent Technologies, Santa Clara, CA) and data was analysed using our in-house developed arrayCGHbase tool(2).

Complete cDNA sequence of MYH9::PDGFRB fusion

ATGGCACAGCAAGCTGCCGATAAGTATCTCTATGTGGATAAAAACTTCATCAACAATCCGCTGGCCCAG
GCCGACTGGGCTGCCAAGAAGCTGGTATGGGTGCCTTCCGACAAGAGTGGCTTTGAGCCAGCCAGCC
TCAAGGAGGAGGTGGGCGAAGAGGCCATCGTGGAGCTGGTGGAGAATGGGAAGAAGGTGAAGGT
GAACAAGGATGACATCCAGAAGATGAACCCGCCCAAGTTCTCCAAGGTGGAGGACATGGCAGAGCTC
ACGTGCCTCAACGAAGCCTCGGTGCTGCACAACCTCAAGGAGCGTTACTACTCAGGGCTCATCTACAC
CTATTCAGGCCTGTTCTGTGTGGTCATCAATCCTTACAAGAACCTGCCCATCTACTCTGAAGAGATTGTG
GAAATGTACAAGGGCAAGAAGAGGCACGAGATGCCCCCTCACATCTATGCCATCACAGACACCGCCTA
CAGGAGTATGATGCAAGACCGAGAAGATCAATCCATCTTGTGCACTGGTGAATCTGGAGCTGGCAAGA
CGGAGAACACCAAGAAGGTCATCCAGTATCTGGCGTACGTGGCGTCCTCGCACAAGAGCAAGAAGGA

CCAGGGCGAGCTGGAGCGGCAGCTGCTGCAGGCCAACCCCATCCTGGAGGCCTTCGGGAACGCCAA
GACCGTGAAGAATGACAACTCCTCCCGCTTCGGCAAATTCATTCGCATCAACTTTGATGTCAATGGCTA
CATTGTTGGAGCCAACATTGAGACTTATCTTTTGGAGAAATCTCGTGCTATCCGCCAAGCCAAGGAAG
AACGGACCTTCCACATCTTCTATTATCTCCTGTCTGGGGCTGGAGAGCACCTGAAGACCGATCTCCTGT
TGGAGCCGTACAACAAATACCGCTTCCTGTCCAATGGACACGTCACCATCCCCGGGCAGCAGGACAAG
GACATGTTCCAGGAGACCATGGAGGCCATGAGGATTATGGGCATCCCAGAAGAGGAGCAAATGGGCC
TGCTGCGGGTCATCTCAGGGGTTCTTCAGCTCGGCAACATCGTCTTCAAGAAGGAGCGGAACACTGA
CCAGGCGTCCATGCCCGACAACACAGCTGCCCAAAAGGTGTCCCATCTCTTGGGTATCAATGTGACCG
ATTTCACCAGAGGAATCCTCACCCCGCGCATCAAGGTGGGACGGGATTACGTCCAGAAGGCGCAGAC
TAAAGAGCAGGCTGACTTTGCCATCGAGGCCTTGGCCAAGGCGACCTATGAGCGGATGTTCCGCTGG
CTGGTGCTGCGCATCAACAAGGCTCTGGACAAGACCAAGAGGCAGGGCGCCTCCTTCATCGGGATCC
TGGACATTGCCGGCTTCGAGATCTTTGATCTGAACTCGTTTGAGCAGCTGTGCATCAATTACACCAATG
AGAAGCTGCAGCAGCTCTTCAACCACACCATGTTCATCCTGGAGCAGGAGGAGTACCAGCGCGAGGG
CATCGAGTGGAACTTCATCGACTTTGGCCTCGACCTGCAGCCCTGCATCGACCTCATTGAGAAGCCAG
CAGGCCCCCCGGGCATTCTGGCCCTGCTGGACGAGGAGTGCTGGTTCCCCAAAGCCACCGACAAGA
GCTTCGTGGAGAAGGTGATGCAGGAGCAGGGCACCCACCCCAAGTTCCAGAAGCCCAAGCAGCTGA
AGGACAAAGCTGATTTCTGCATTATCCACTATGCCGGCAAGGTGGATTACAAAGCTGACGAGTGGCTG
ATGAAGAACATGGATCCCCTGAATGACAACATCGCCACACTGCTCCACCAGTCCTCTGACAAGTTTGTC
TCGGAGCTGTGGAAGGATGTGGACCGCATCATCGGCCTGGACCAGGTGGCCGGCATGTCGGAGACC
GCACTGCCCGGGGCCTTCAAGACGCGGAAGGGCATGTTCCGCACTGTGGGGCAGCTTTACAAGGAG
CAGCTGGCCAAGCTGATGGCTACGCTGAGGAACACGAACCCCAACTTTGTCCGCTGCATCATCCCCAA
CCACGAGAAGAAGGCCGGCAAGCTGGACCCGCATCTCGTGCTGGACCAGCTGCGCTGCAACGGTGT
TCTCGAGGGCATCCGTATCTGCCGCCAGGGCTTCCCCAACAGGGTGGTCTTCCAGGAGTTTCGGCAG
AGATATGAGATCCTGACTCCAAACTCCATTCCCAAGGGTTTCATGGACGGGAAGCAGGCGTGCGTGCT
CATGATAAAAGCCCTGGAGCTCGACAGCAATCTGTACCGCATTGGCCAGAGCAAAGTCTTCTTCCGTG
CCGGTGTGCTGGCCCACCTGGAGGAGGAGCGAGACCTGAAGATCACCGACGTCATCATAGGGTTCCA
GGCCTGCTGCAGGGGCTACCTGGCCAGGAAAGCATTTGCCAAGCGGCAGCAGCAGCTTACCGCCATG
AAGGTCCTCCAGCGGAACTGCGCTGCCTACCTGAAGCTGCGGAACTGGCAGTGGTGGCGGCTCTTCA
CCAAGGTCAAGCCGCTGCTGCAGGTGAGCCGGCAGGAGGAGGAGATGATGGCCAAGGAGGAGGAG
CTGGTGAAGGTCAGAGAGAAGCAGCTGGCTGCGGAGAACAGGCTCACGGAGATGGAGACGCTGCA
GTCTCAGCTCATGGCAGAGAAATTGCAGCTGCAGGAGCAGCTCCAGGCAGAAACCGAGCTGTGTGCC
GAGGCTGAGGAGCTCCGGGCCCGCCTGACCGCCAAGAAGCAGGAATTAGAAGAGATCTGCCATGAC
CTAGAGGCCAGGGTGGAGGAGGAGGAGGAGCGCTGCCAGCACCTGCAGGCGGAGAAGAAGAAGA
TGCAGCAGAACATCCAGGAGCTTGAGGAGCAGCTGGAGGAGGAGGAGAGCGCCCGGCAGAAGCTG
CAGCTGGAGAAGGTGACCACCGAGGCGAAGCTGAAAAAGCTGGAGGAGGAGCAGATCATCCTGGA
GGACCAGAACTGCAAGCTGGCCAAGGAAAAGAAACTGCTGGAAGACAGAATAGCTGAGTTCACCAC
CAACCTCACAGAAGAGGAGGAGAAATCTAAGAGCCTCGCCAAGCTCAAGAACAAGCATGAGGCAAT
GATCACTGACTTGGAAGAGCGCCTCCGCAGGGAGGAGAAGCAGCGACAGGAGCTGGAGAAGACCC
GCCGGAAGCTGGAGGGAGACTCCACAGACCTCAGCGACCAGATCGCCGAGCTCCAGGCCCAGATCG
CGGAGCTCAAGATGCAGCTGGCCAAGAAAGAGGAGGAGCTCCAGGCCGCCCTGGCCAGGTGTCCAC
GTGAGCTGCCGCCCACGCTGCTGGGGAACAGTTCCGAAGAGGAGAGCCAGCTGGAGACTAACGTGA
CGTACTGGGAGGAGGAGCAGGAGTTTGAGGTGGTGAGCACACTGCGTCTGCAGCACGTGGATCGGC
CACTGTCGGTGCGCTGCACGCTGCGCAACGCTGTGGGCCAGGACACGCAGGAGGTCATCGTGGTGC
CACACTCCTTGCCCTTTAAGGTGGTGGTGATCTCAGCCATCCTGGCCCTGGTGGTGCTCACCATCATCT
CCCTTATCATCCTCATCATGCTTTGGCAGAAGAAGCCACGTTACGAGATCCGATGGAAGGTGATTGAGT
CTGTGAGCTCTGACGGCCATGAGTACATCTACGTGGACCCCATGCAGCTGCCCTATGACTCCACGTGGG
AGCTGCCGCGGGACCAGCTTGTGCTGGGACGCACCCTCGGCTCTGGGGCCTTTGGGCAGGTGGTGG

AGGCCACGGCTCATGGCCTGAGCCATTCTCAGGCCACGATGAAAGTGGCCGTCAAGATGCTTAAATCC
ACAGCCCGCAGCAGTGAGAAGCAAGCCCTTATGTCGGAGCTGAAGATCATGAGTCACCTTGGGCCCC
ACCTGAACGTGGTCAACCTGTTGGGGGCCTGCACCAAAGGAGGACCCATCTATATCATCACTGAGTAC
TGCCGCTACGGAGACCTGGTGGACTACCTGCACCGCAACAAACACACCTTCCTGCAGCACCACTCCGA
CAAGCGCCGCCCGCCCAGCGCGGAGCTCTACAGCAATGCTCTGCCCGTTGGGCTCCCCCTGCCCAGC
CATGTGTCCTTGACCGGGGAGAGCGACGGTGGCTACATGGACATGAGCAAGGACGAGTCGGTGGAC
TATGTGCCCATGCTGGACATGAAAGGAGACGTCAAATATGCAGACATCGAGTCCTCCAACTACATGGCC
CCTTACGATAACTACGTTCCCTCTGCCCCTGAGAGGACCTGCCGAGCAACTTTGATCAACGAGTCTCCA
GTGCTAAGCTACATGGACCTCGTGGGCTTCAGCTACCAGGTGGCCAATGGCATGGAGTTTCTGGCCTC
CAAGAACTGCGTCCACAGAGACCTGGCGGCTAGGAACGTGCTCATCTGTGAAGGCAAGCTGGTCAAG
ATCTGTGACTTTGGCCTGGCTCGAGACATCATGCGGGACTCGAATTACATCTCCAAAGGCAGCACCTTT
TTGCCTTTAAAGTGGATGGCTCCGGAGAGCATCTTCAACAGCCTCTACACCACCCTGAGCGACGTGTG
GTCCTTCGGGATCCTGCTCTGGGAGATCTTCACCTTGGGTGGCACCCCTTACCCAGAGCTGCCCATGA
ACGAGCAGTTCTACAATGCCATCAAACGGGGTTACCGCATGGCCCAGCCTGCCCATGCCTCCGACGAG
ATCTATGAGATCATGCAGAAGTGCTGGGAAGAGAAGTTTGAGATTCGGCCCCCCTTCTCCCAGCTGGT
GCTGCTTCTCGAGAGACTGTTGGGCGAAGGTTACAAAAAGAAGTACCAGCAGGTGGATGAGGAGTT
TCTGAGGAGTGACCACCCAGCCATCCTTCGGTCCCAGGCCCGCTTGCCTGGGTTCCATGGCCTCCGAT
CTCCCCTGGACACCAGCTCCGTCCTCTATACTGCCGTGCAGCCCAATGAGGGTGACAACGACTATATCA
TCCCCCTGCCTGACCCCAAACCCGAGGTTGCTGACGAGGGCCCACTGGAGGGTTCCCCCAGCCTAGC
CAGCTCCACCCTGAATGAAGTCAACACCTCCTCAACCATCTCCTGTGACAGCCCCCTGGAGCCCCAGG
ACGAACCAGAGCCAGAGCCCCAGCTTGAGCTCCAGGTGGAGCCGGAGCCAGAGCTGGAACAGTTGC
CGGATTCGGGGTGCCCTGCGCCTCGGGCGGAAGCAGAGGATAGCTTCCTGTAG

***MYH9-PDGFRB* cloning**

A custom made hPGK-backbone containing the full length MYH9-PDGFRB fusion was made by VectorBuilder (Chicago, IL, USA). The fusion was re-amplified with Q5 High Fidelity 2x Master Mix (NEB, Ipswich, MA, USA) and primers 1 and 2 using a touchdown PCR method for cloning into pMIGII-IRES-GFP (MSCV) backbone. The PCR product was purified using Isolate II PCR & Gel Kit (Bioline, London, UK) and digested with EcoRI (NEB, Ipswich, MA, USA). MSCV backbone was also digested with EcoRI and dephosphorylated with quick CIP (NEB, Ipswich, MA, USA). After the final round of purification using Isolate II PCR & Gel Kit, the eluates were ligated using T4 DNA Ligase (NEB, Ipswich, MA, USA) at 4°C overnight. The ligation product was transformed into NEB® Stable Competent *E. coli* (NEB, Ipswich, MA, USA). Colonies were assessed by colony PCR using MyTaq Red Mix (Bioline, London, UK). Plasmid DNA was obtained from positive colonies using ZymoPUREII Plasmid Midiprep Kit (Zymo Research, Irvine, USA). DNA was Sanger Sequenced to confirm the sequence of the *MYH9-PDGFRB* fusion.

Cloning primers	
Primer 1	CCCGGAATTCGCCACCATGGCACAGCAAG
Primer 2	CCCGGAATTCGCTGGGTCTACAGGAAGCTA
Colony PCR primers	
Primer 3	CCTCGATCCTCCCTTTATCC
Primer 4	GAGCCCTGAGTAGTAACGCT

Clinical information MYH9::PDGFRB T-LBL patient

A 7 year old boy was referred to the department of pediatric hematology-oncology because of palpable supraclavicular lymph nodes and mild tiredness. Chest X-ray and CT-scan showed a mediastinal mass. A supraclavicular lymph node biopsy was performed and T-cell lymphoblastic lymphoma diagnosed. Cerebrospinal fluid was initially negative and 12% lymphoblasts were found in bone marrow, resulting in a diagnosis of stage 4 T-cell lymphoblastic lymphoma with CD2+, CD45+, CD8+, CD4+, sCD3 weak+ and cyCD3+ immunophenotype. Karyotyping (46,XY,add(22)(q12)[2]/46,XY[20]) and FISH (Kreatech) diagnostics were performed on the bone marrow sample which showed a TCR beta (7q35) rearrangement with absence of t(9;22), 11q23 and TAL1 rearrangements. Treatment according to EORTC-58951-CLG (3) was initiated. In the first weeks of treatment a clinical deterioration and disease progression was seen as the patient developed skin rash, pleural effusion and ascites. Lymphoblasts were found in the pleural effusion and in the cerebrospinal fluid on day 13, and the patient developed hyperleukocytosis. The patient was assigned to the very high risk group due to poor corticosteroid response at the end of prephase and absence of remission at the end of induction treatment (M2 marrow). Treatment continued with an intensified Berlin-Frankfurt-Münster based consolidation 1 (3), and continued consolidation with high dose chemotherapy (VANDA course and HR-1 block) until hematopoietic stem cell transplantation (HSCT) with his HLA-matched sibling brother. Four months after transplantation a relapse was detected in the left orbit and local irradiation, 20 Gy, was administered with positive effect. Seven months after HSCT the patient developed a testicular relapse and palliative care was started. The patient died one month later.

Immunohistochemistry

Tissue was cut (3 µm) and mounted on TOMO® slides (Matsunami, Bellingham, WA, USA). Slides were stained with primary antibodies directed against human PDGFRB. Slides were stained with the Benchmark Ultra platform, Roche Diagnostics (Ventana Medical Systems, Inc, Tucson, AZ, USA) according to manufacturer`s protocols. Staining conditions were as follows: PDGFRB (clone 28E1, rabbit/monoclonal, Cell Signaling Technology, Danvers, Massachusetts, USA) 1:50, 32 min incubation, OptiView kit, pretreatment CC1 (32min). Specimens were analyzed by light microscopy (Olympus BX50, Tokyo, Japan) and scanned images (Nano Zoomer 2.0 -RS Virtual Slide Light microscope scanner and NDP View Software, version NDP.view2, both Hamamatsu Photonics K.K., Hamamatsu City, Japan). Staining intensities were compared to positive controls.

Retroviral transduction

Viral supernatant was produced by transfecting 4 x 10^6 HEK293T cells plated overnight in T75 flasks with lipofectamine™ 2000 Transfection Reagent (ThermoFisher), containing 10 µg purified plasmid DNA and 10 µg EcoPac. Viral supernatant was collected and filtered through a 0.45 µM filter. 1 x 10^6 Ba/F3 cells were loaded onto Nunc™ Non-Treated Multidishes (150239, ThermoScientific). Viral supernatant was loaded onto Ba/F3 cells with 8 µg/mL polybrene (28728-5-4, Sigma Aldrich) and spun for 90 min at 2500 rpm 30°C.

Western blotting

Cells were lysed with radioimmunoprecipitation assay (RIPA) buffer supplemented with phosphatase inhibitors (PhosSTOP™, Roche) and proteinase inhibitor (AA Blocks). Protein concentration was measured with the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific).

Denatured protein was loaded on a 4-12% Bolt Bis-Tris Plus gel (ThermoFisher), proteins were separated by electrophoresis for 1hr then transferred onto PVDF membrane using iBlot 2 (Invitrogen). Antibodies used are: pPDGFRB (#3161, Cell Signaling Technology), PDGFRB (#3169, Cell Signaling Technology), β-actin (A1978, Sigma-Aldrich), pSTAT3 (#9131S, Bioké BV), pSTAT5 (#9351S, Bioké BV), pGSK3B (#5558S, Bioké BV), pAKT (4060S, Bioké BV).

Phospho-STAT5 flow cytometry
Cells were treated for 1h with 1μM CP-673451 in 10% RPMI, washed and fixed with PFA (Life Technologies). Cells were permeabilised with ice cold 90% methanol (Sigma-Aldrich) and stored overnight. Samples were stained for 1h with pSTAT5 (612599, BD Biosciences) and isotype control (557783, BD Biosciences). After washing away excess antibody, cells were analyzed by flow cytometry (LSRFortessa, BD Biosciences) and data analysis was performed using FlowJo software.

PDGFRB stimulation
HEK293T-PDGFRB^{wt} were a kind gift from Prof. Paul Ekert. Cells were serum starved for 6 hours prior to 1 hour 1 μM CP-673451 treatment or DMSO followed by 8 minute stimulation with 20 ng/mL PDGF-BB (100-14B-10UG, PreproTech).

***Ex* vivo culture transduced HSPCs (BMT)**
Transduced HSPCs were cultured in RPMI 1640 medium supplemented with 20% fetal bovine serum (FBS) and 100 μg/ml Primocin (Jomar Life Research, Australia) at 37°C with 5% CO2.

Immunophenotyping organs (BMT)
Organs were made into single cell suspension. Cells were stained with CD4-V450 (75-0042-U100 , Cytek Biosciences), CD8-APC-Cy7 (25-0081-U100 , Cytek Biosciences), CD11b-PE (50-0112-U100 , Cytek Biosciences), Gr1-APC (20-5931-U100 , Cytek Biosciences).

Patient information
In Figure 3D, samples from two different patients cohorts were used. Samples labelled T-ALL1, 2, 3, 4, 5, and T-LBL1 were sourced from patients recruited at Ghent University Hospital. The samples T-ALL6, 7, 8, 9, 10, 11 are part of the Pediatric Preclinical Testing Consortium (including patients from St Jude Children's Research Hospital) with IDs T-ALL6 = ETP1, T-ALL7 = ETP3, T-ALL8= SJTALL056732_D1, T-ALL9 = ETP6, T-ALL10 = SJTALL187_D1, T-ALL11 = SJTALL009_D1.

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Supplementary Table 1: Immunophenotypes of mice that received MYH9::PDGFRB in the bone marrow transplant model.

		Organ			
	EFS	**Peripheral Blood**	**Spleen**	**Bone Marrow**	**Thymus**
Mouse 1	119 days	22.3 % GFP Major GFP+ Clone (22.3%): CD8+	27.7% GFP Major GFP+ Clone (27.7%): CD8+	3.41% GFP Major GFP+ Clone (3.41%): CD8+	80.2% GFP Major GFP+ Clone (80.2%): CD8+/CD4+CD8+
Mouse 2	86 days (non leukemic event)	10.4% GFP Major GFP+ Clone (10.4%): CD8+	17.7% GFP Major GFP+ Clone (17.7%): CD8+	7.11% GFP Major GFP+ Clone (7.11%): Gr1+ CD11b+	23.7% GFP Major GFP+ Clone (23.7%): CD8+
Mouse 3	126 days	64.5% GFP Major GFP+ Clone (64.5%): CD8+	41.6% GFP Major GFP+ Clone (27%): CD8+ Minor GFP+ Clone (14.6%): Gr1+ CD11b+	54.1% GFP Major GFP+ Clone (44.96%): Gr1+ CD11b+ Minor GFP+ Clone (9.14%): CD8+	91.2% GFP Major GFP+ Clone (91.2%): CD8+/CD4+CD8+
Mouse 4	72 days	65.3% GFP Major GFP+ Clone (45%): Gr1+ CD11b+ Minor GFP+ Clone (20.3%): CD8+	42% GFP Major GFP+ Clone (28%): Gr1+ CD11b+ Minor GFP+ Clone (14%): CD8+	55.8% GFP Major GFP+ Clone (35%): Gr1+ CD11b+ Minor GFP+ Clone (20.8%): CD8+	54.4% GFP Major GFP+ Clone (54.4%): CD8+/CD4+CD8+
Mouse 5	101 days	33.8% GFP Major GFP+ Clone (33.8%): Gr1+ CD11b+	33.6% GFP Major GFP+ Clone (33.6 %): Gr1+ CD11b+	17.6% GFP Major GFP+ Clone (10.72%): Gr1+ CD11b+ Minor GFP+ Clone (6.88%): CD8+	12.5% GFP Major GFP+ Clone (12.5%): CD8+
Mouse 6	56 days	73.3% GFP Major GFP+ Clone (73.3%): Gr1+ CD11b+	98.7% GFP Major GFP+ Clone (80.1%): Gr1+ CD11b+ Minor GFP+ Clone (18.6%): CD8+	99.8% GFP Major GFP+ Clone (99.8%):	N/A

Supplementary Table 2: Immunophenotypes of mice that received EBF1::PDGFRB in the bone marrow transplant model.

		Organ			
	EFS	**Peripheral Blood**	**Spleen**	**Bone Marrow**	**Thymus**
Mouse 1	45 days (non leukemic event)	8.96% GFP (no staining performed)	9.2% GFP Major GFP+ Clone (9.2%): Gr1+CD11b+	0.88% GFP	N/A
Mouse 2	112 days (non leukemic event)	13.4% GFP Major GFP+ Clone (13.4%): CD8+	15.8% GFP Major GFP+ Clone (15.8%): CD8+	16.7% GFP Major GFP+ Clone (16.7%): CD8+	N/A
Mouse 3	Still alive at >150 days post transplant	N/A	N/A	N/A	N/A
Mouse 4	62 days (non leukemic event)	11.1% GFP Major GFP+ Clone (11.1%): CD8+	25.2% GFP Major GFP+ Clone (25.2%): CD8+	27.2% GFP Major GFP+ Clone (27.2%): Gr1+ CD11b+	69.2% GFP Major GFP+ Clone (69.2%): CD8+/CD4+CD8+
Mouse 5	83 days (non leukemic event)	27% GFP Major GFP+ Clone (27%): Gr1+CD11b+	58.5% GFP Major GFP+ Clone (58.5%): Gr1+ CD11b+	36.5% GFP Major GFP+ Clone (36.5%): CD8+	N/A
Mouse 6	100 days (non leukemic event)	9.66% GFP Major GFP+ Clone (%):	20.6% GFP Major GFP+ Clone (20.6%): Gr1+ CD11b+	20.5% GFP Major GFP+ Clone (20.5%): Gr1+ CD11b+	0.63% GFP

Supplementary Figure Legends

Figure S1

(A) Array CHG of the t(5;22) MYH9::PDGFRB T-LBL patient. Red bars depict losses whereas blue bars are gains.
(B) TLA of a T-LBL case with PDGFRB (chromosome 5) as viewpoint. Additional reads were detected at chromosome 22 suggesting the presence of a (5;22) translocation.

Figure S2

(C) (p)PDGFRB (Tyr751) Western blot analysis of Ba/F3 transduced cells with MYH::PDGFRB, EBF1::PDGFRB (positive control[1]), NRAS(G12D) (negative control, no PDGFRB) and JAK3(M511I) (negative control, no PDGFRB)
(D) (p)PDGFRB (Tyr751) Western blot analysis on $PDGFRB^{wt}$ stably transduced HEK293T cells. Cells were pre-treated for 1 hour with 1µM CP-673451 or DMSO followed by 8 minutes of stimulation with 20 ng/mL PDGF-BB.

Figure S3

(A) Flow cytometric analysis of murine stem cells harvested from C57BL/6JAusB mice transduced with *MYH9::PDGFRB-GFP* construct. Cells were cultured without cytokines (IL-3, IL-6 and SCF) and were analyzed at days 0, 5, 7 and 14.
(B) White Blood Cell count and % GFP of peripheral blood from MYH9::PDGFRB mice (n=6). Mice were bled weekly.
(C) Gating strategy for immunophenotyping of peripheral blood, spleen, BM and thymus from MYH9::PDGFRB/EBF1::PDGFRB mice.

Figure S4

(A) (p)PDGFRB (Tyr751) Western blot analysis of PDX samples treated *ex vivo* for 1 hour with 0 or 1 µM CP-673451.
(B) pSTAT5 (Y694) flow cytometry analysis of CTV-1 and T-ALL1 treated with DMSO vehicle control or 1 µM CP-673451 for 1 hour.

Figure S5

(A) Body weight changes in both control (90% PEG300, 10% N-methyl pyrrolidone) and treated (CP-673451) NSG-SGM3 mice during the course of the experiment

Figure S6

(A) Schematic overview of a conditional PDGFRB gain-of-function mouse model generated as previously described [2]. After CD2-iCre induced recombination of loxP sites, *Pdgfrb* is being overexpressed from the *ROSA26* promotor.
(B) *Pdgfrb* mRNA expression in thymi from wild type (WT) versus *CD2-iCre*$^{tg/+}$ *R26-Pdgfrb*$^{tg/tg}$ mice (PDGFRB) ($p<0.0001$)
(C) PDGFRB Western blot analysis of thymi from wild type (WT) versus *CD2-iCre*$^{tg/+}$ *R26-Pdgfrb*$^{tg/tg}$ (PDGFRB) mice

(D) Aging cohort of *Lck-Cre*$^{tg/+}$ *Pten*$^{fl/fl}$ (n=12, median survival 94 days) and *Lck-Cre*$^{tg/+}$ *Pten*$^{fl/fl}$ R26-*Pdgfrb*$^{tg/tg}$ (n=13, median survival 91 days) mice

(E) (p)PDGFRB (Tyr751) Western blot analysis of thymi from *Lck-Cre*$^{tg/+}$ *Pten*$^{fl/fl}$ R26-*Pdgfrb*$^{tg/tg}$ mice

Supplementary References

1. S J Welsh, M.L.C., M Togni, C G Mullighan & J Hagman *Deregulation of kinase signaling and lymphoid development in EBF1-PDGFRB ALL leukemogenesis.* Leukemia, 2017. **34**: p. 38-48.
2. Tim Pieters, S.T.S., Lisa Demoen, André Almeida, Lieven Haenebalcke, Filip Matthijssens, Kelly Lemeire, Jinke D'Hont, Frederique Van Rockeghem, Tino Hochepied, Beatrice Lintermans, Lindy Reunes, Tim Lammens, Geert Berx, Jody J. Haigh, Steven Goossens & Pieter Van Vlierberghe, *Novel strategy for rapid functional in vivo validation of oncogenic drivers in haematological malignancies.* Scientific Reports, 2019. **9**: p. 10577.

Figure S1

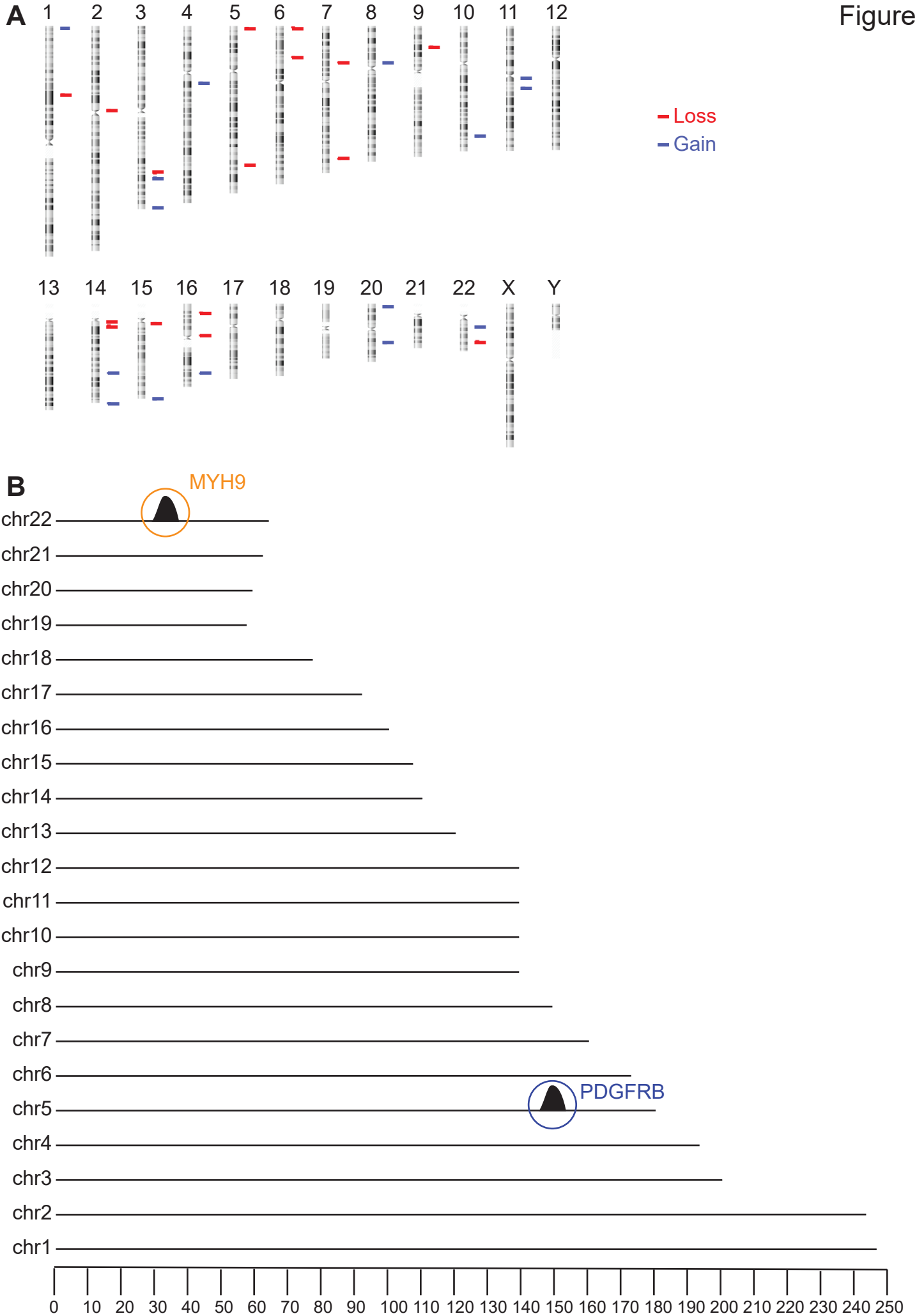

A
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
Loss
Gain
B
MYH9
chr22
chr21
chr20
chr19
chr18
chr17
chr16
chr15
chr14
chr13
chr12
chr11
chr10
chr9
chr8
chr7
chr6
chr5
chr4
chr3
chr2
chr1
PDGFRB
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
250
Chromosomal position (Mb)

Figure S2

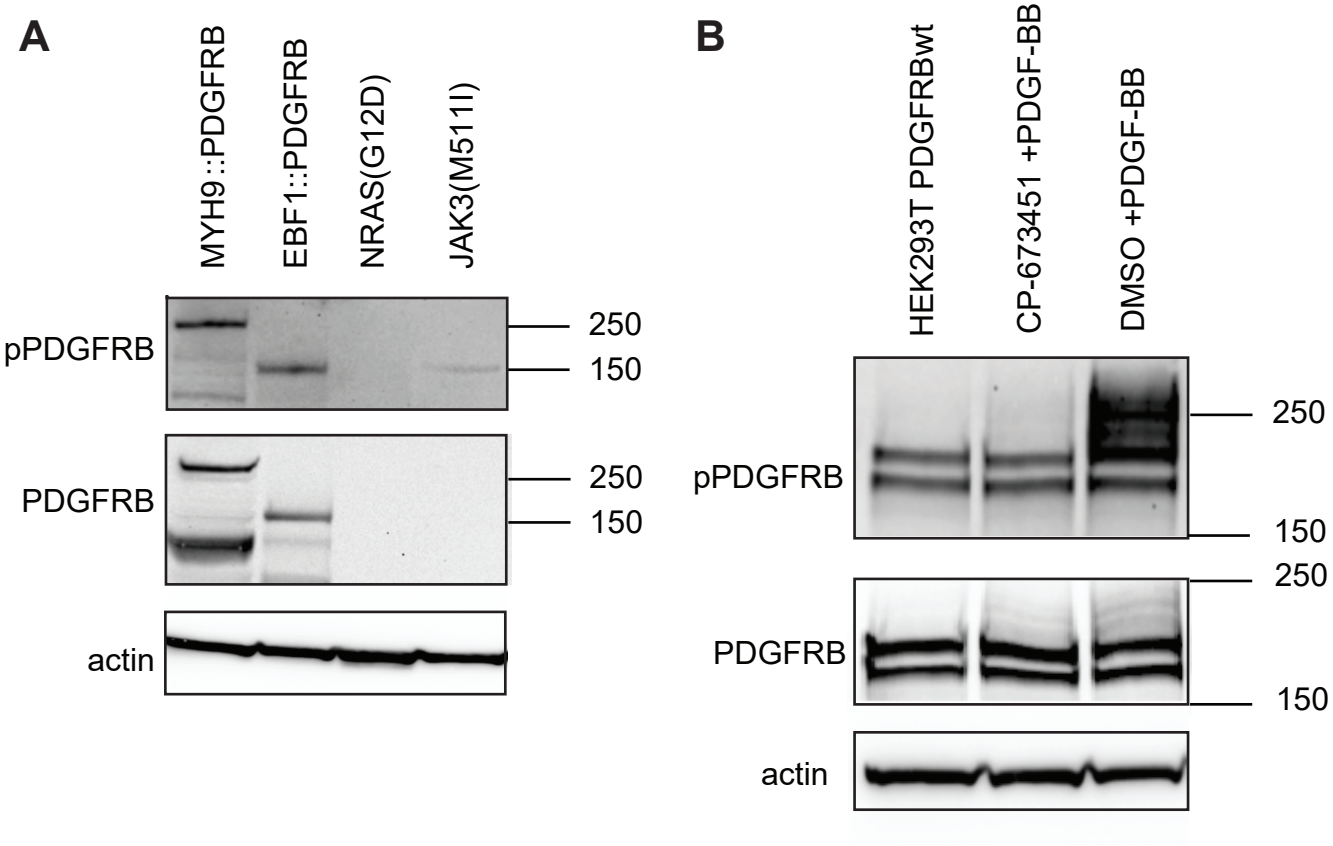

A
MYH9::PDGFRB
EBF1::PDGFRB
NRAS(G12D)
JAK3(M511I)
pPDGFRB
250
150
PDGFRB
250
150
actin
B
HEK293T PDGFRBwt
CP-673451 +PDGF-BB
DMSO +PDGF-BB
pPDGFRB
250
150
PDGFRB
250
150
actin

Figure S3

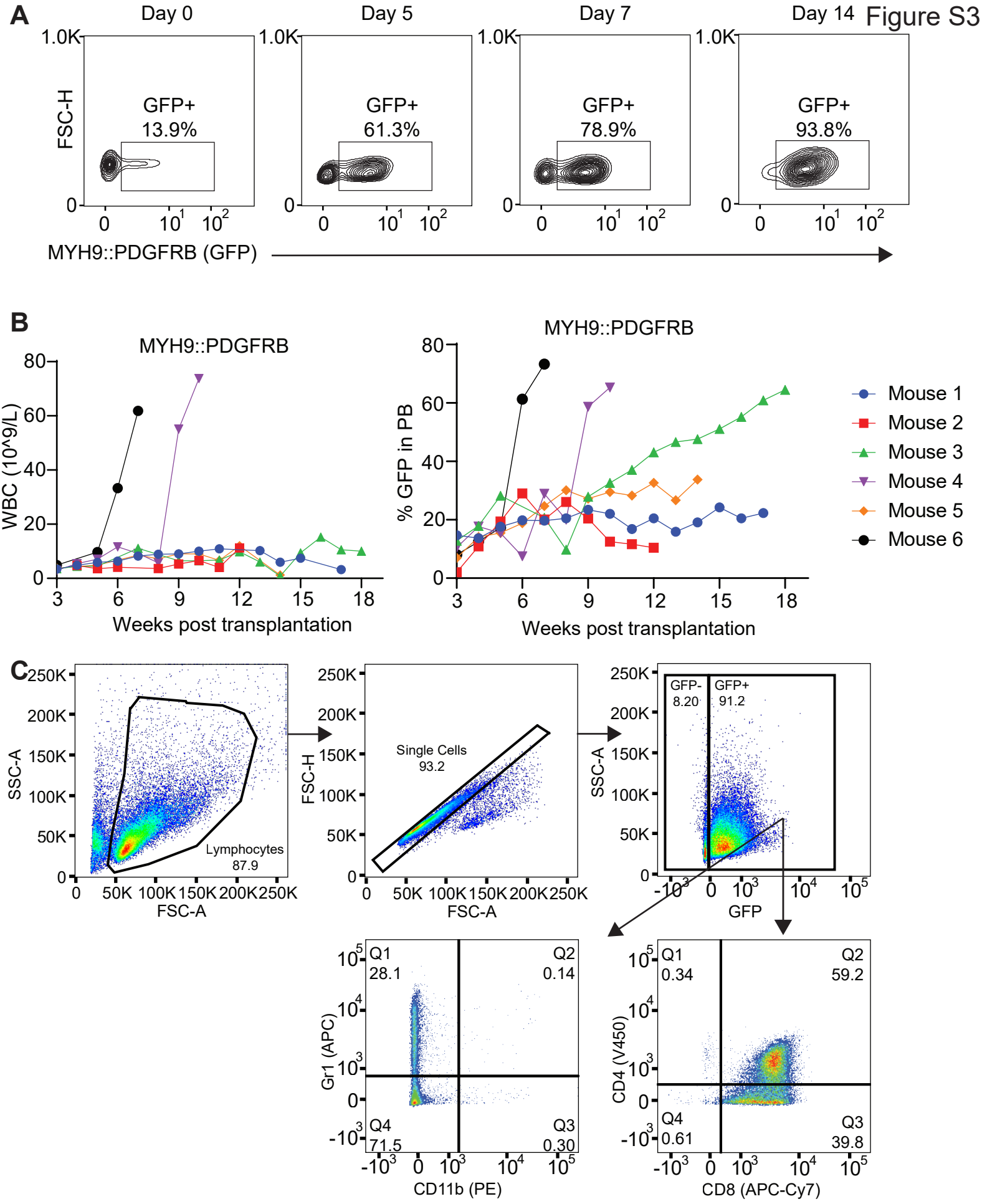

A
Day 0
Day 5
Day 7
Day 14
1.0K
FSC-H
GFP+
13.9%
GFP+
61.3%
GFP+
78.9%
GFP+
93.8%
0
10^1
10^2
MYH9::PDGFRB (GFP)
B
MYH9::PDGFRB
WBC (10^9/L)
Weeks post transplantation
% GFP in PB
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
C
SSC-A
FSC-A
Lymphocytes
87.9
FSC-H
Single Cells
93.2
GFP-
8.20
GFP+
91.2
GFP
Gr1 (APC)
CD11b (PE)
Q1
28.1
Q2
0.14
Q4
71.5
Q3
0.30
CD4 (V450)
CD8 (APC-Cy7)
Q1
0.34
Q2
59.2
Q4
0.61
Q3
39.8

Figure S4

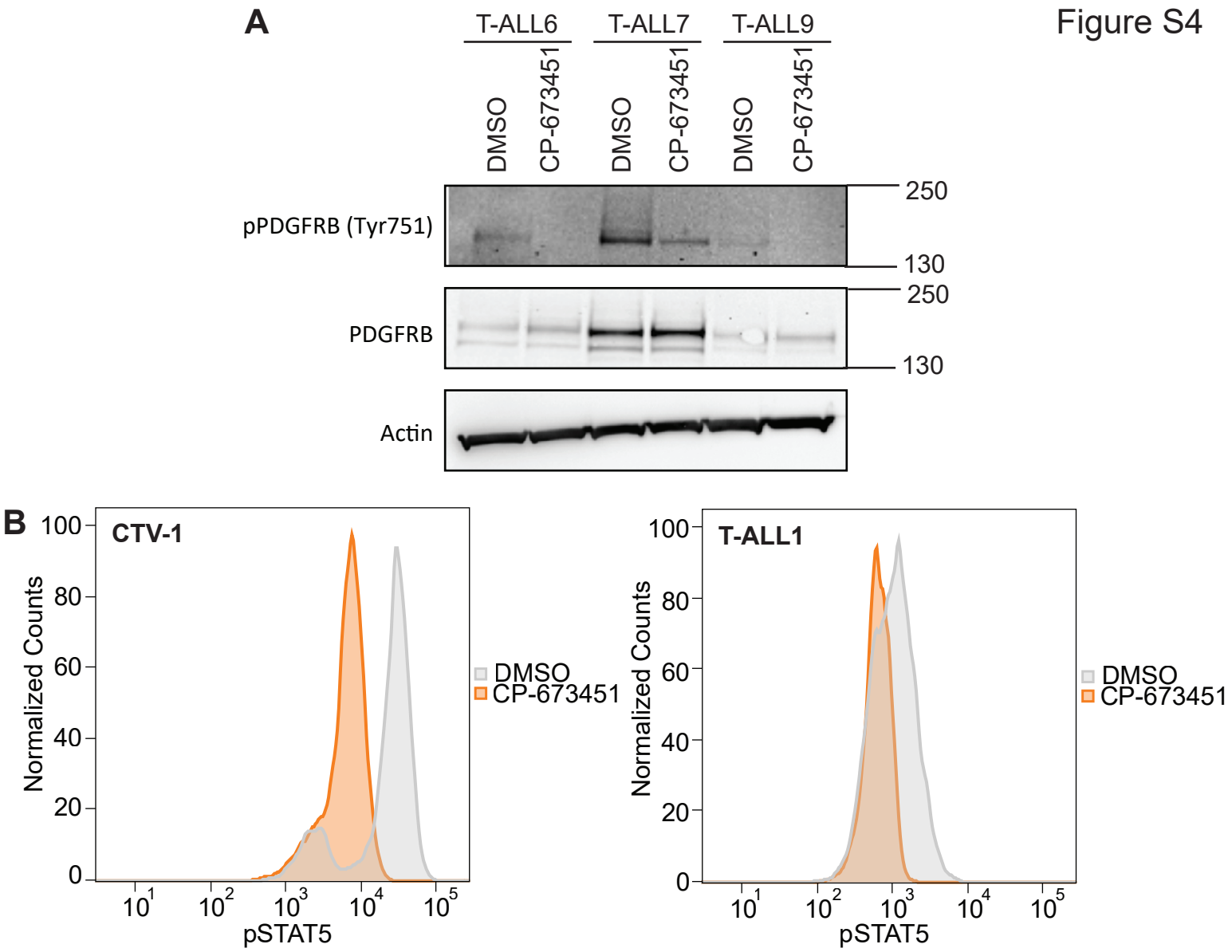

A
T-ALL6
T-ALL7
T-ALL9
DMSO
CP-673451
DMSO
CP-673451
DMSO
CP-673451
pPDGFRB (Tyr751)
250
130
PDGFRB
250
130
Actin
B
CTV-1
Normalized Counts
100
80
60
40
20
0
10^1
10^2
10^3
10^4
10^5
pSTAT5
DMSO
CP-673451
T-ALL1
Normalized Counts
100
80
60
40
20
0
10^1
10^2
10^3
10^4
10^5
pSTAT5
DMSO
CP-673451

Figure S5

A

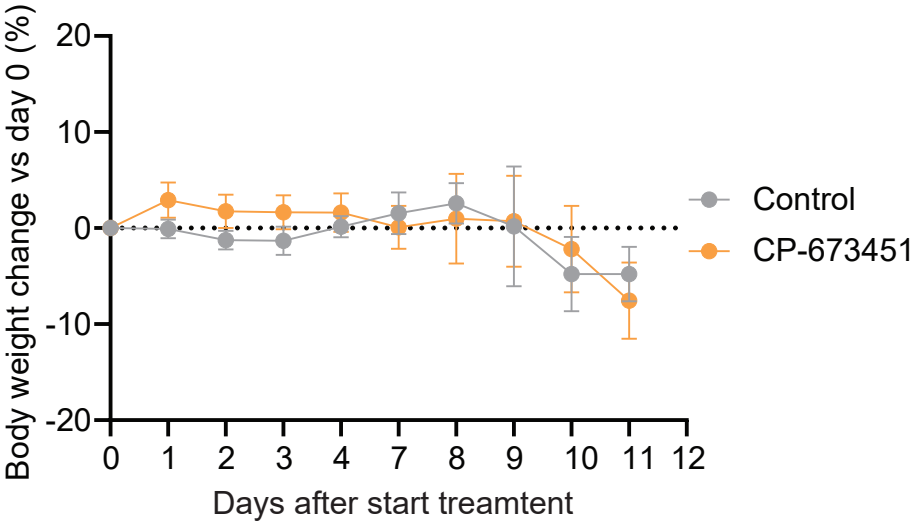
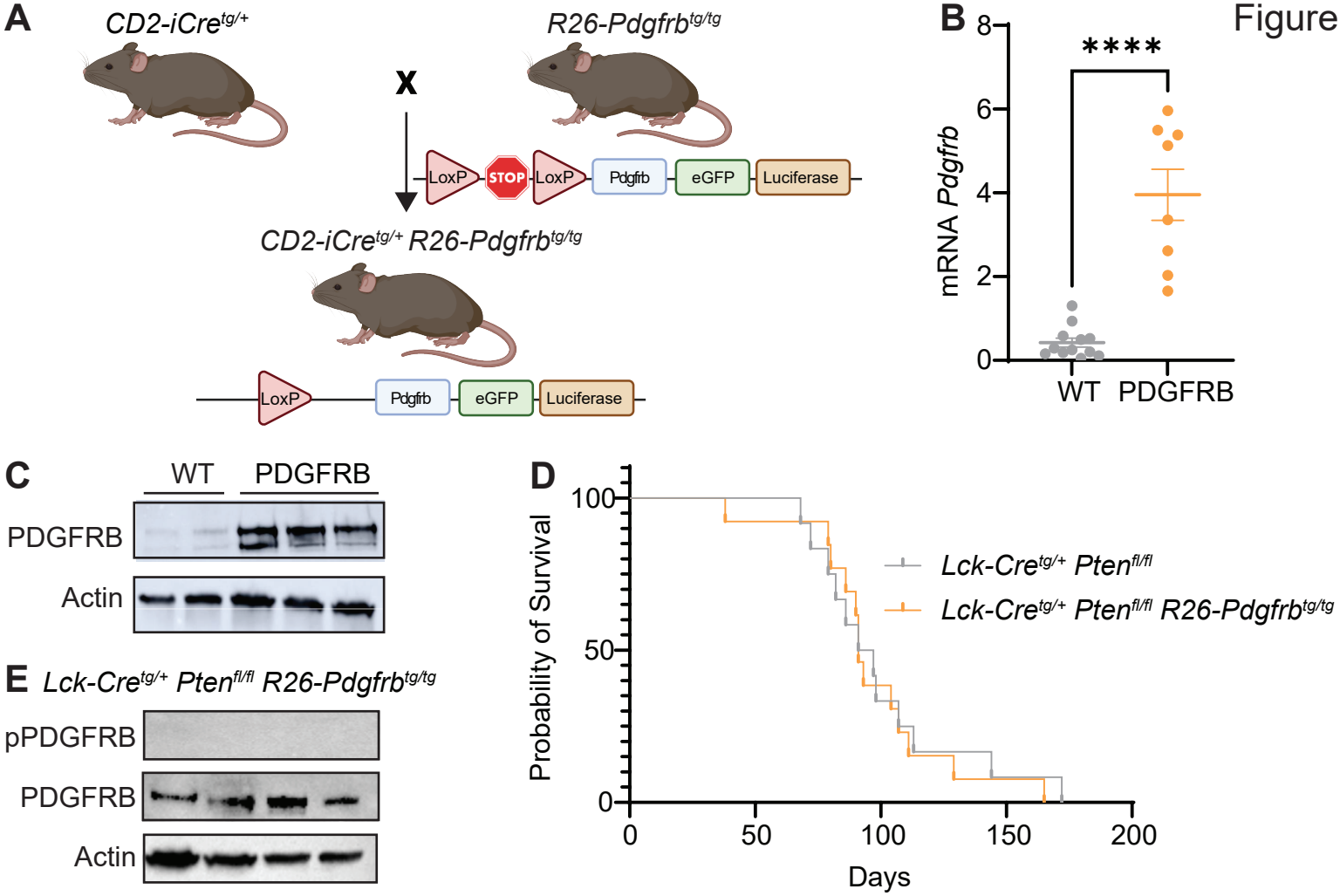

Body weight change vs day 0 (%)
20
10
0
-10
-20
0
1
2
3
4
7
8
9
10
11
12
Days after start treamtent
Control
CP-673451

Figure S6


A
CD2-iCre tg/+
R26-Pdgfrb tg/tg
x
LoxP
STOP
LoxP
Pdgfrb
eGFP
Luciferase
CD2-iCre tg/+ R26-Pdgfrb tg/tg
LoxP
Pdgfrb
eGFP
Luciferase
B
mRNA Pdgfrb
8
6
4
2
0
****
WT
PDGFRB
C
WT
PDGFRB
PDGFRB
Actin
D
Probability of Survival
100
50
0
0
50
100
150
200
Days
Lck-Cre tg/+ Pten fl/fl
Lck-Cre tg/+ Pten fl/fl R26-Pdgfrb tg/tg
E Lck-Cre tg/+ Pten fl/fl R26-Pdgfrb tg/tg
pPDGFRB
PDGFRB
Actin