Supplementary Material

Dual Targeting of Acute Leukemia and Supporting Niche by CXCR4-Directed Theranostics

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Running title: CXCR4 theranostics for targeting acute leukemia

Keywords: acute leukemia / microenvironment / C-X-C chemokine receptor 4 / in vivo molecular imaging / theranostics

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Conflict of interest: H.J.W. is shareholder of Scintomics (Germany). All other authors have no relevant conflicts of interest to declare.

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Table S1

Clinical characteristics of patients who provided primary material for PDX.

	ALL230	**ALL0**	**AML356**	**AML346**
Subtype	T-ALL	T-ALL	AML M5	AML M7
ID / RR	ID	ID	RR	RR
Age	4	17	5	1
Gender	m	m	m	w
Cytogenetics	46XY, t(11;14); (p32;q11)	N/A	N/A	del5q, del13q

Table S2

Characteristics of patients treated with Pentixather.

	Patient 1	**Patient 2**	**Patient 3**
Subtype	AML M1	tAML	AML M0
Age at diagnosis	46	65	38
Gender	M	F	M
Molecular genetics	NPM1mut, FLT3-ITD	FLT3-ITD, MLL-PTD, RUNX1mut	no mutation
Cytogenetics	46, XY	46, XY	45, XY, -7
Previous therapy	7+3 → MRD+ FLAG-IDA Flu-Bu and alloSCT (matched unrelated donor)	S-HAM → MRD+ FLAMSA-Bu-Cy-ATG and alloSCT (matched related donor)→ CR, MLL-MLL+ relapse → Azacytidine DLI	ICE → refractory HAM → refractory Azacytidine (bridging) TBI (12Gy)-Flu-Cy and alloSCT (haploidentical related donor) → CR
Comorbidities	none	Breast cancer	none
Organ dosimetry (pre-therapeutic estimates for ^{90}Y-Pentixather)	Kidney: 4.7 Gy/GBq Liver: 2.4 Gy/GBq Spleen: 2.4 Gy/GBq BM: 2.3 Gy/Gbq	Kidney: 5.8 Gy/GBq Liver: 2.4 Gy/GBq Spleen: 3.0 Gy/GBq BM: 4.9 Gy/Gbq	Kidney: 7.9 Gy/GBq Liver: 3.0 Gy/GBq Spleen: 4.4 Gy/GBq BM: 1.7 Gy/Gbq

Fig. S1.

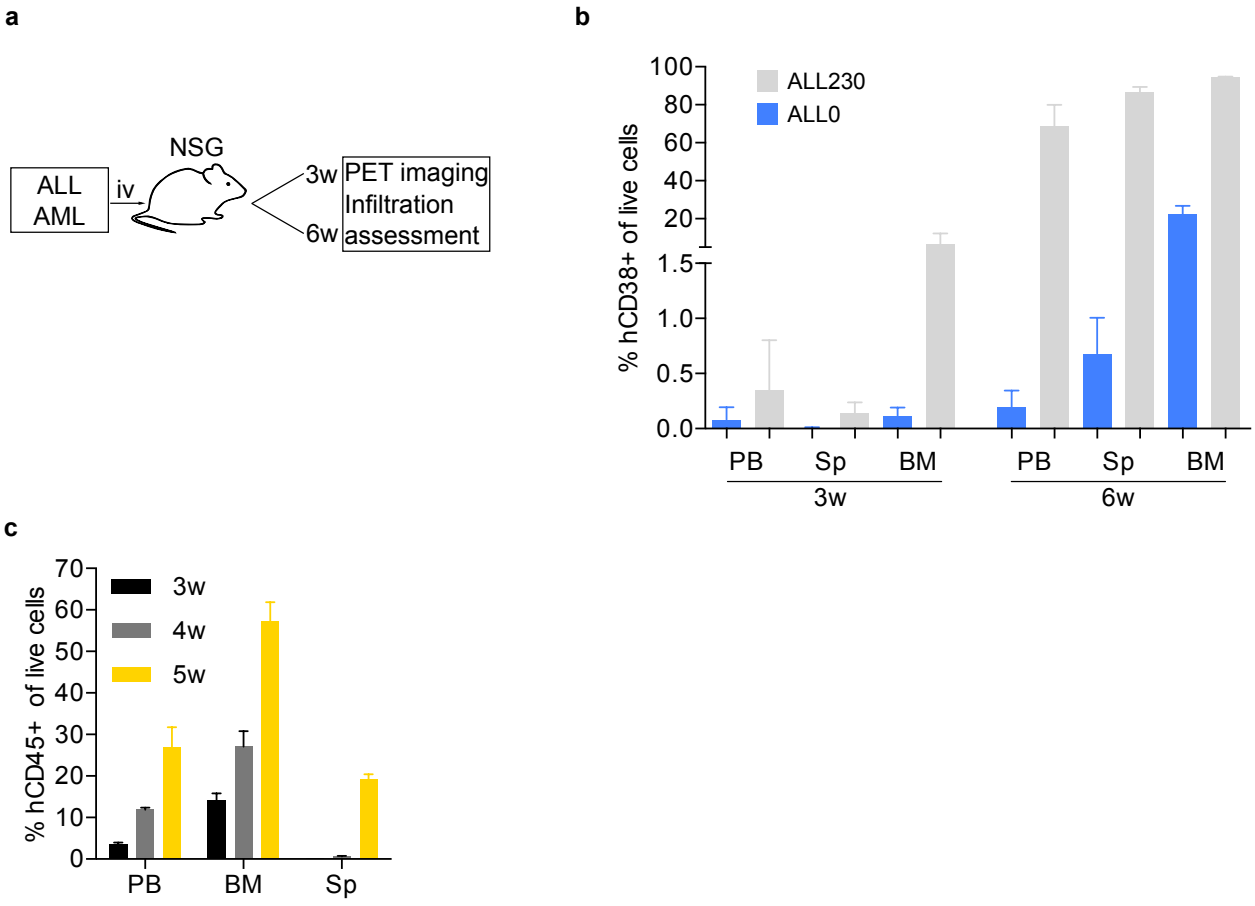


Fig. S1. PDX establishment. **a)** Schematic of PDX establishment. **b,c)** Progressive infiltration of Sp, BM and PB by ALL (B) and AML356 (C) PDX.

Fig. S2.

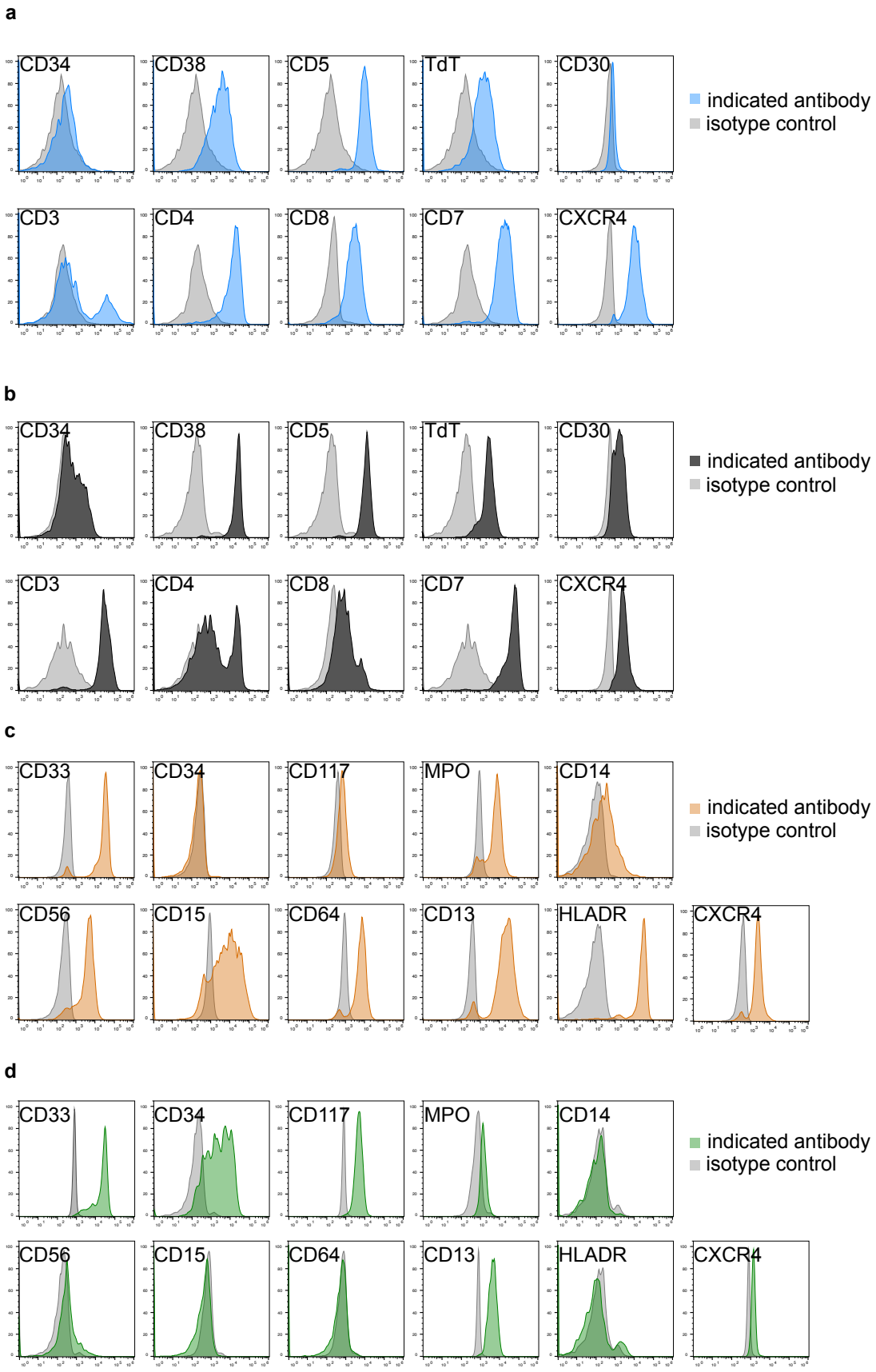


Fig. S2. PDX surface markers determined by flow cytometry. **a)** ALL230, **b)** ALL0 and **c)** AML356 **d)** AML346.

Fig. S3.

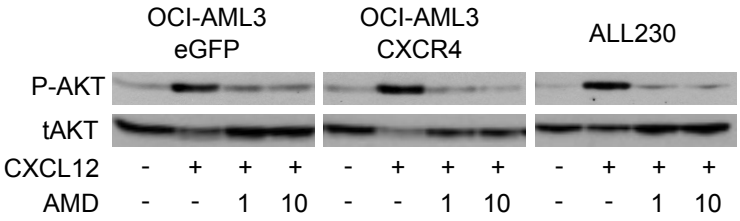


Fig. S3. P-AKT immunoblotting. OCI-AML3 and ALL230 showing AKT phosphorylation upon 100ng/µl CXCL12 pretreatment that can be blocked with 1 and 10µM AMD3100.

Fig. S4.

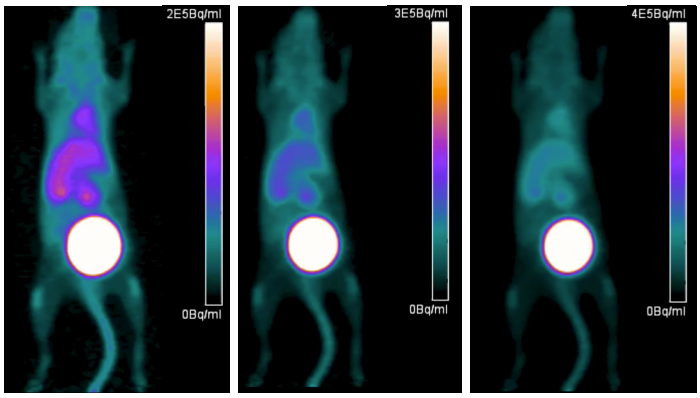


Fig. S4. Representative images of a healthy NSG control mouse imaged with Ga-Pentixafor (n=3 mice, different intensities for 1 mouse are shown).

Fig. S5.

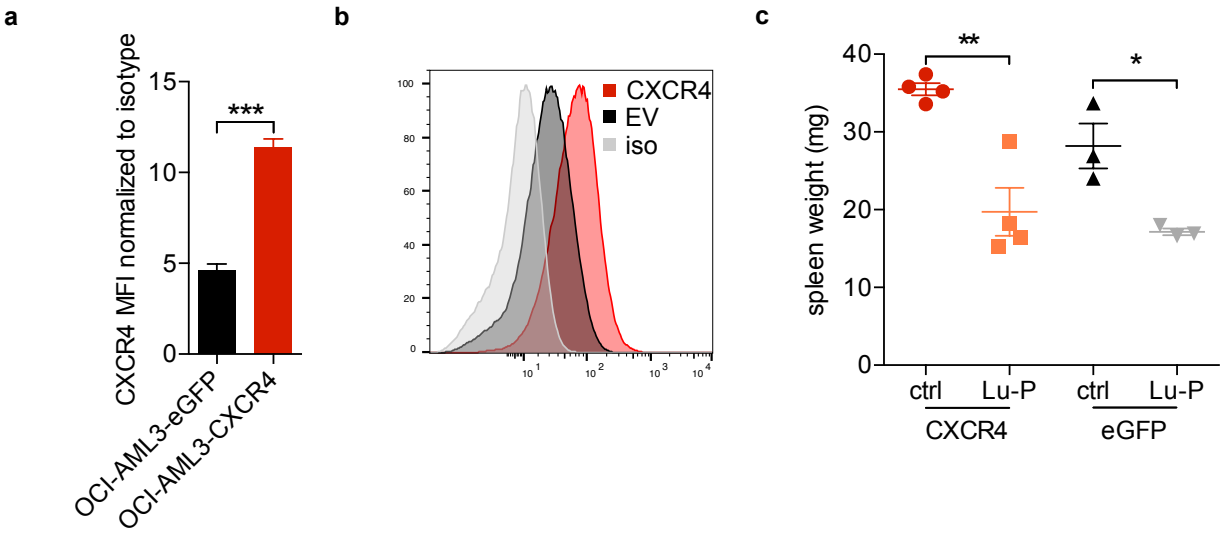


Fig. S5. Pentixather therapy in AML xenografts with enforced CXCR4 expression**. a)** CXCR4 surface expression of OCI-AML3-eGFP and OCI-AML3-CXCR4 depicted as mean fluorescence intensity (MFI) relative to isotype antibody (n=3 replicates per cell line) . **b)** Representative FACS plots of A). **c)** Spleen weight of OCI-AML3-eGFP/CXCR4 xenografts after 3d treatment with ctrl or Lu-P (n=4 for CXCR4, n=3 for eGFP)

Fig. S6

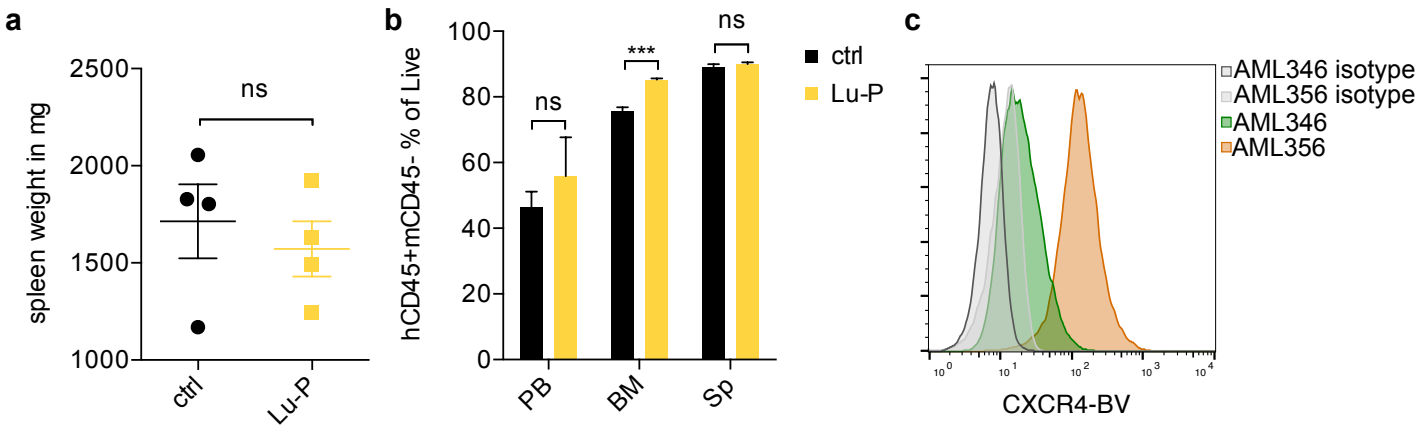
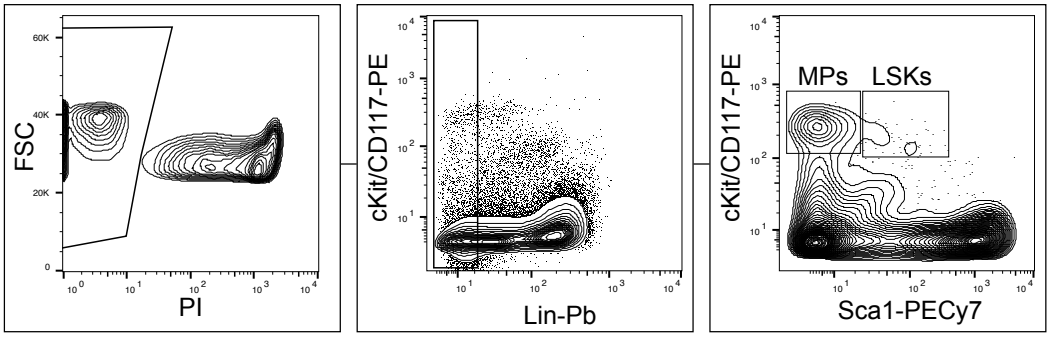


Fig. S6. Lu-P therapy in AML346. **a)** spleen weight **b)** infiltration assessment by flow cytometry of AML346 mice treated with Lu-P for 7d. (n=4 mice per group). **c)** CXCR4 surface expression of AML346 compared to AML356.

Fig. S7.

a



b

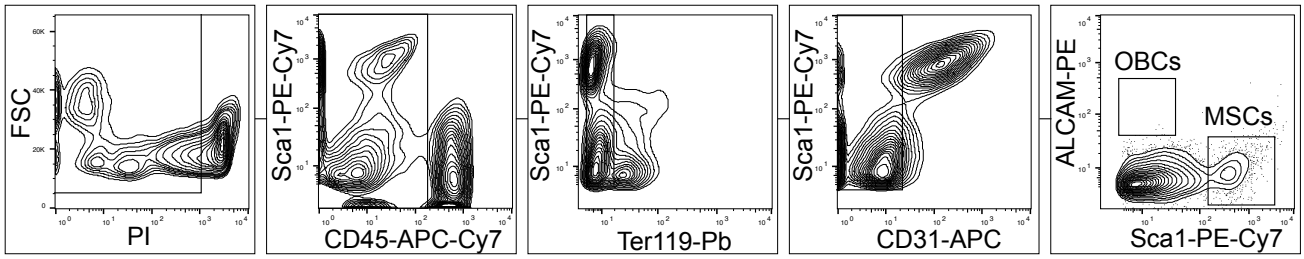


Fig. S7. Flow cytometry gating strategy. **a)** LSKs and MPs; **b)** osteoblastic cells (OBCs) and MSCs.

Fig. S8.

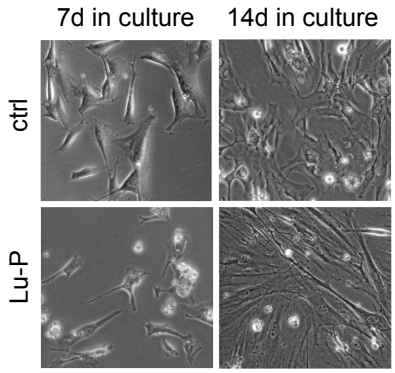


Fig. S8. Representative images of cultured stromal cells.

Fig. S9.

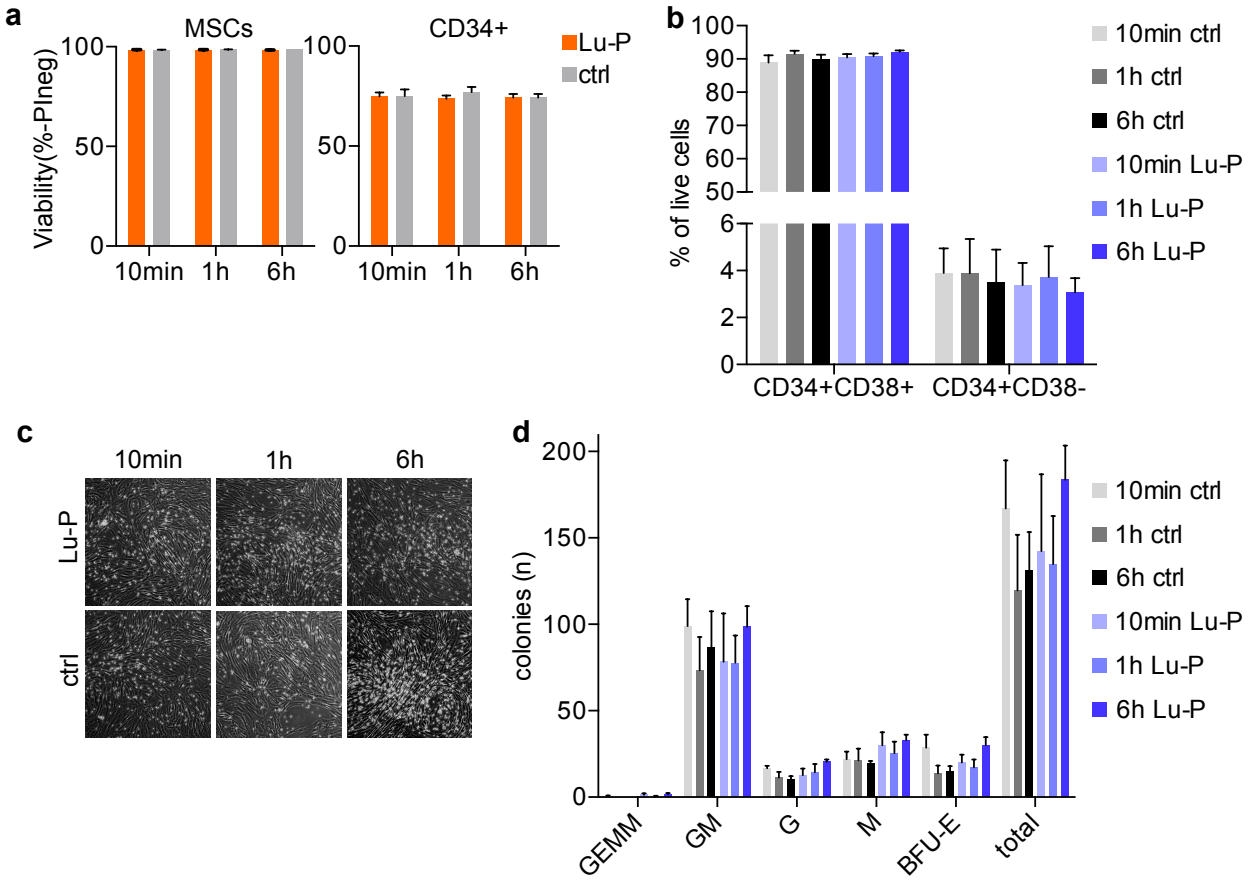


Fig. S9. Lu-P treatment of primary human MSCs (for 10min, 1h, 6h). **a)** Viability of MSCs and CD34+ cells after co-culture. **b)** CD34 and CD38 expression of CD34+ cells after co-culture. **c)** Representative images of MSCs and CD34+ cells in co-culture. **d)** Colony forming unit assay with CD34+ cells after co-culture with MSCs from 3 healthy individuals. PI: propidium iodide, GEMM: granulocyte, erythrocyte, monocyte, megakaryocyte; GM: granulocyte, monocyte; G: granulocyte; M: monocyte; BFU-E: burst forming unit, erythrocyte.

Fig. S10.

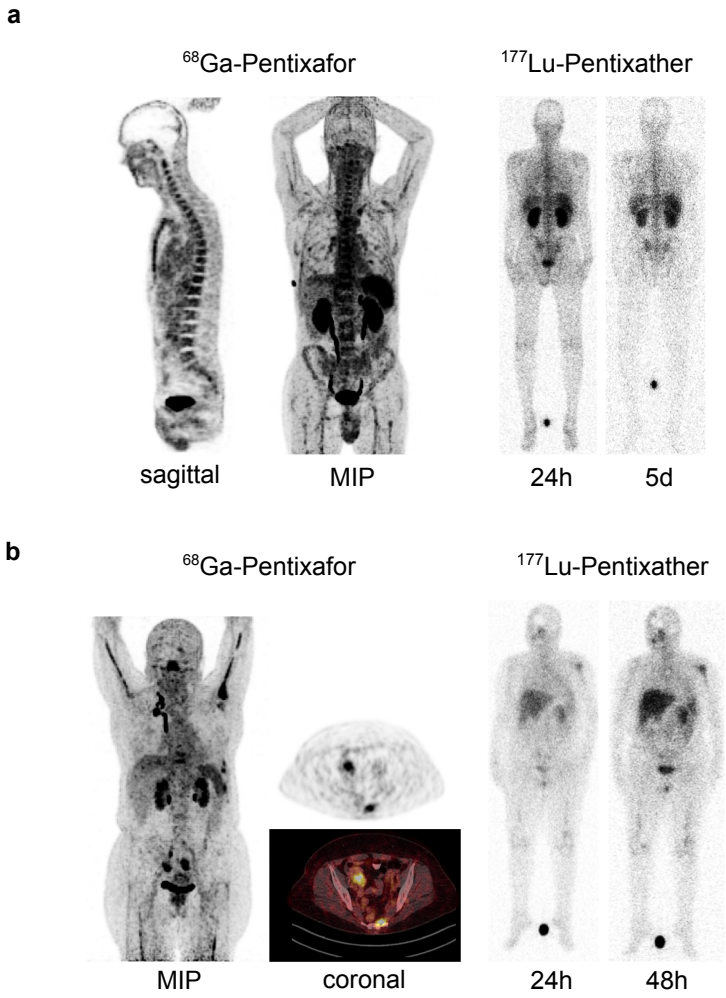


Fig. S10. ^{68}Ga-Pentixafor PET imaging and planar whole-body scintigraphic images after injection of 200MBq Lu-P in **a)** patient 1 and **b)** patient 2 (activity injected for pre-therapeutic dosimetry)**.**