

Supplementary Information

Bridging the Marrow: A Co-Culture-Platform of Leukemia Cells and MS5-derived Stromal Cells or Adipocytes

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Supplementary Figures

Figure S1

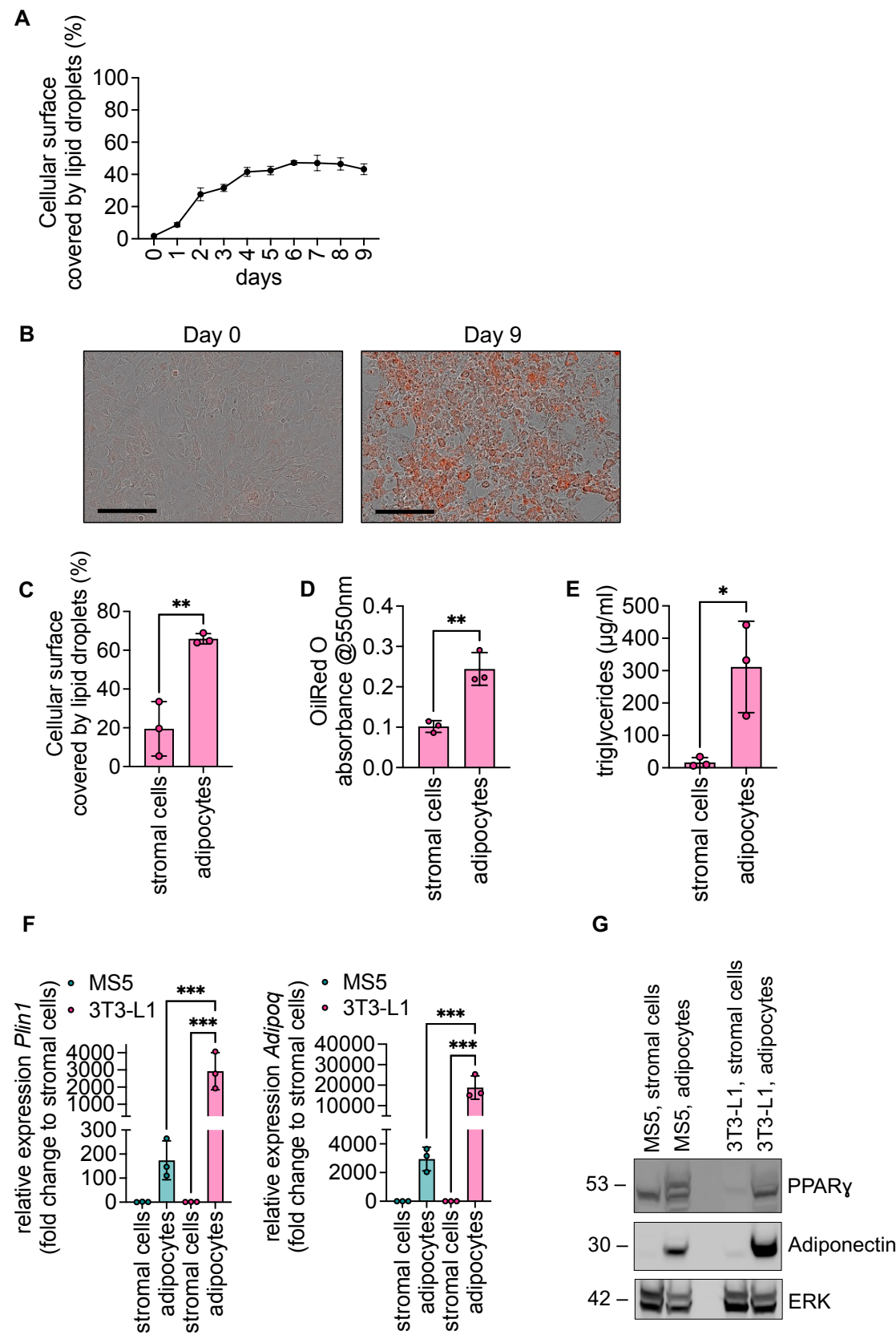


Fig S1. Adipogenic differentiation of MS5 and 3T3-L1 cells.

(A) Cellular surface covered by lipid droplets during the course of adipogenic differentiation of MS5 cells is shown. (B – D) 3T3-L1 stromal cells (day 0 of adipogenic differentiation) and adipocytes (day 9 of adipogenic differentiation) were stained with OilRed O and imaged in the Incucyte S3 (B). Cellular surface covered by lipid droplets (C) and quantification of OilRed O staining are shown (D). (E) Triglycerides were determined in stromal cells (day 0) and adipocytes (day 9). (F – G) mRNA (F) and protein (G) expression of MS5 and 3T3-L1 stromal cells (day 0) and adipocytes (day 9) is depicted. Data are presented as mean $\pm$ SD from three independent experiments performed in at least triplicates. Unpaired t-test (C, D, E) or ordinary one-way ANOVA with Bonferroni's multiple comparisons test (F), *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Images (B, G) are representative of three independent experiments. Scale bar equates to 200 μm .

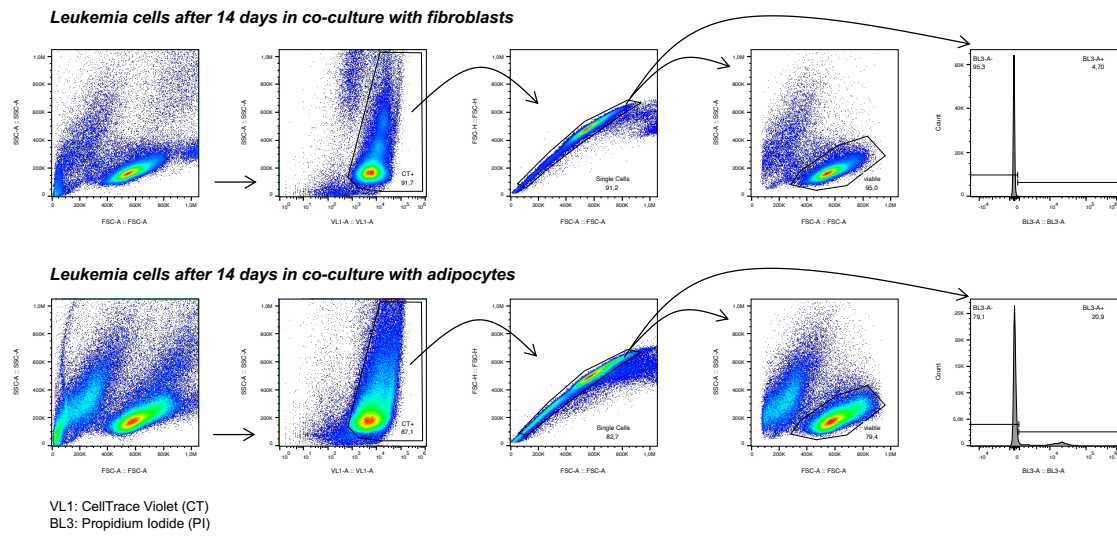
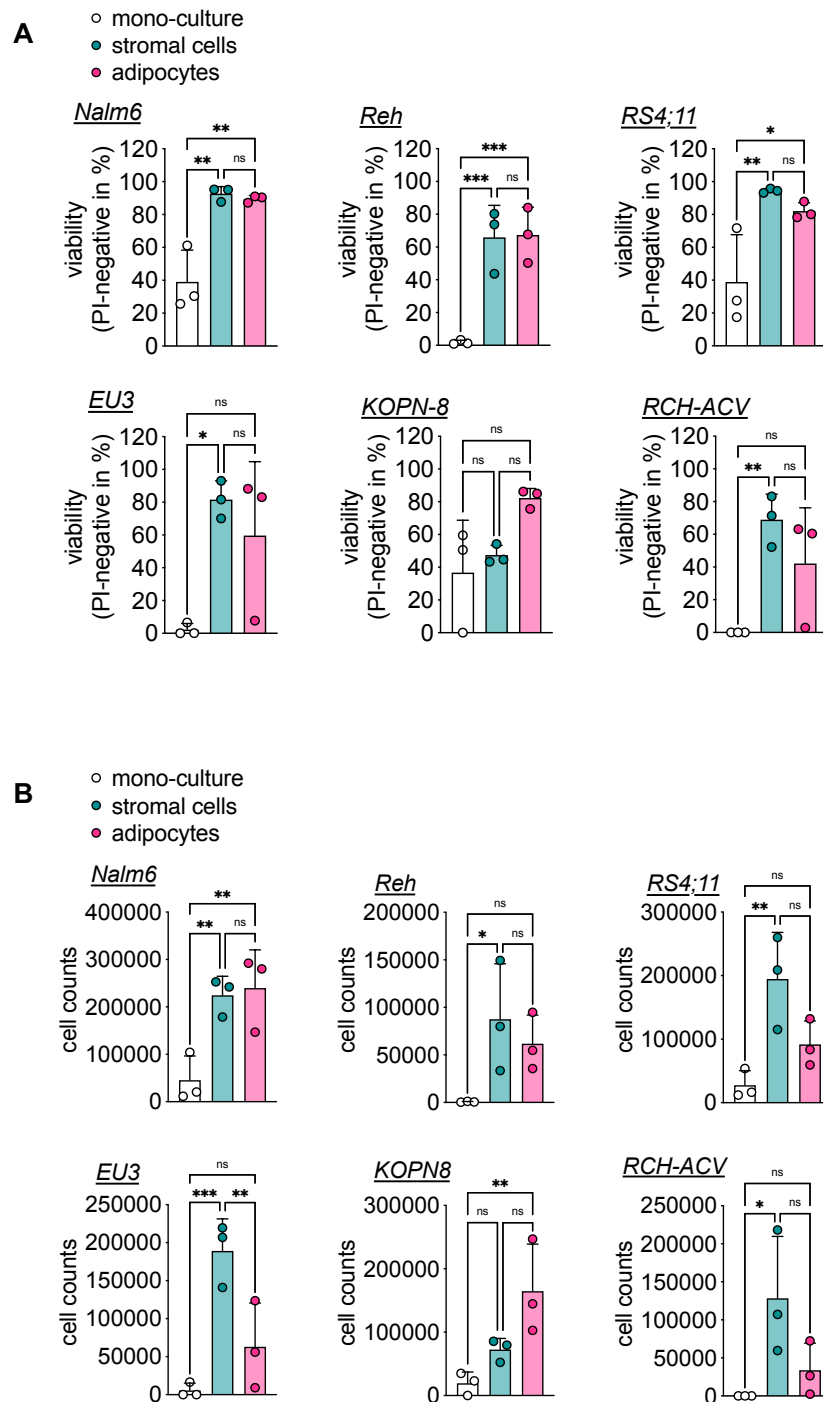
Figure S2

Fig S2. Staining with CellTrace Violet allows robust discrimination of leukemia cells from both, stromal cells or adipocytes.

Gating strategy of leukemia cells in co-culture with fibroblasts (upper panel) or adipocytes (lower panel) is depicted.

Figure S3**Fig S3. MS5-derived stromal cells and adipocytes can maintain survival and proliferation of B-precursor ALL cell lines.**

(A – B) B-precursor ALL cell lines Nalm6, Reh, RS4;11, EU3, KOPN-8, and RCH-ACV were cultured alone or together with stromal cells or adipocytes derived from MS5 cells. Cell

viability (A) or cell counts of the number of viable cells per well (B) were determined after 7 days. Data are presented as mean + SD from three independent experiments performed in triplicates; dots represent individual experiments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; one-way ANOVA with Tukey's multiple comparisons test.

Figure S4

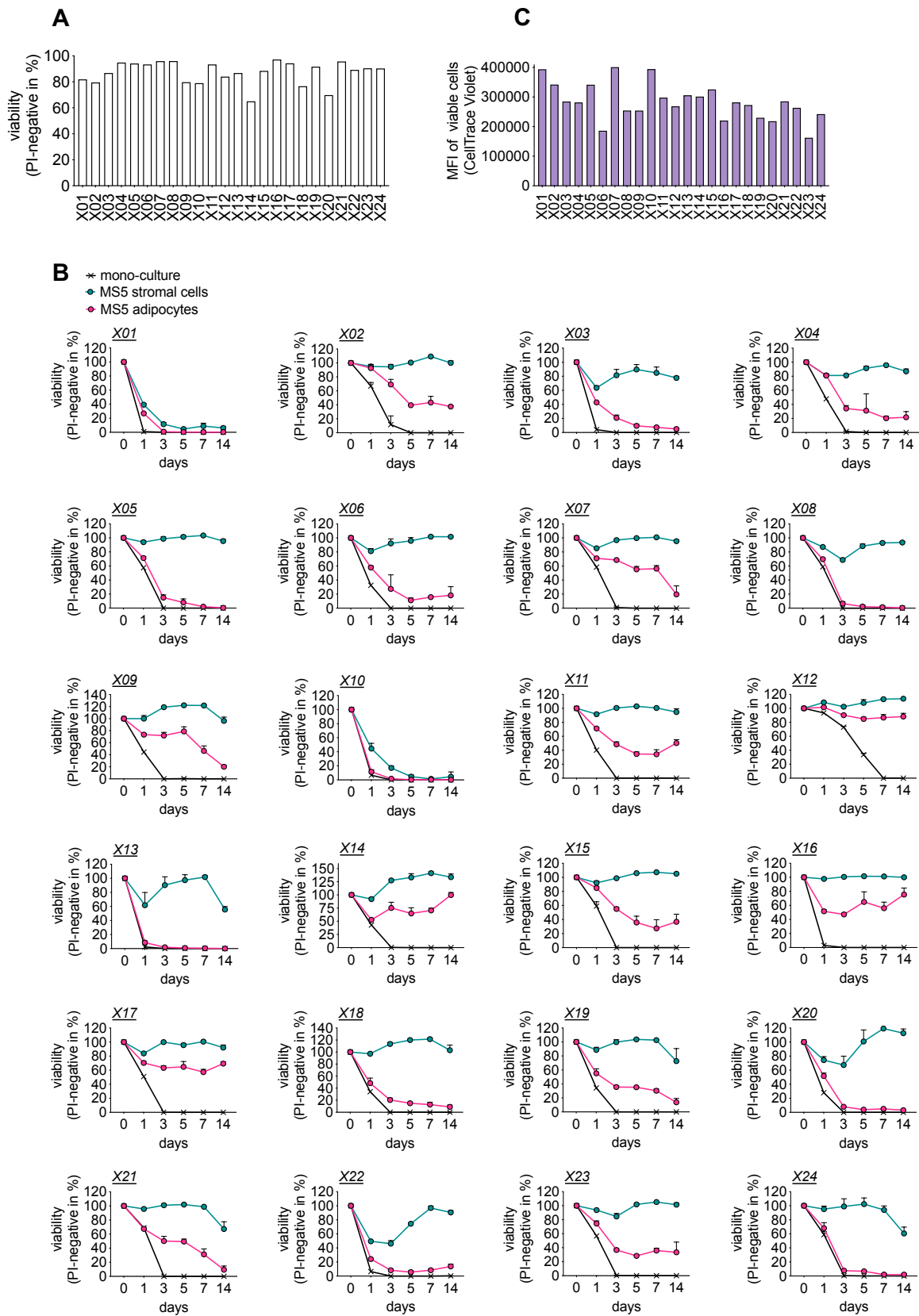


Fig S4. Co-culture of MS5-derived stromal cells or adipocytes with PDX B-precursor ALL cells.

(A) Viability of PDX B-precursor ALL samples (n=24) on day 0. (B) Individual PDX B-precursor ALL samples (n=24) were cultured alone or together with stromal cells or adipocytes derived from MS5 cells. Viability as determined by propidium-iodide (PI)-negative cells on different days after seeding is shown. Data are presented as mean + SD of samples measured in triplicates. (C) Mean fluorescence intensity (MFI) of CellTrace-labelled PDX samples on day 0 is depicted.

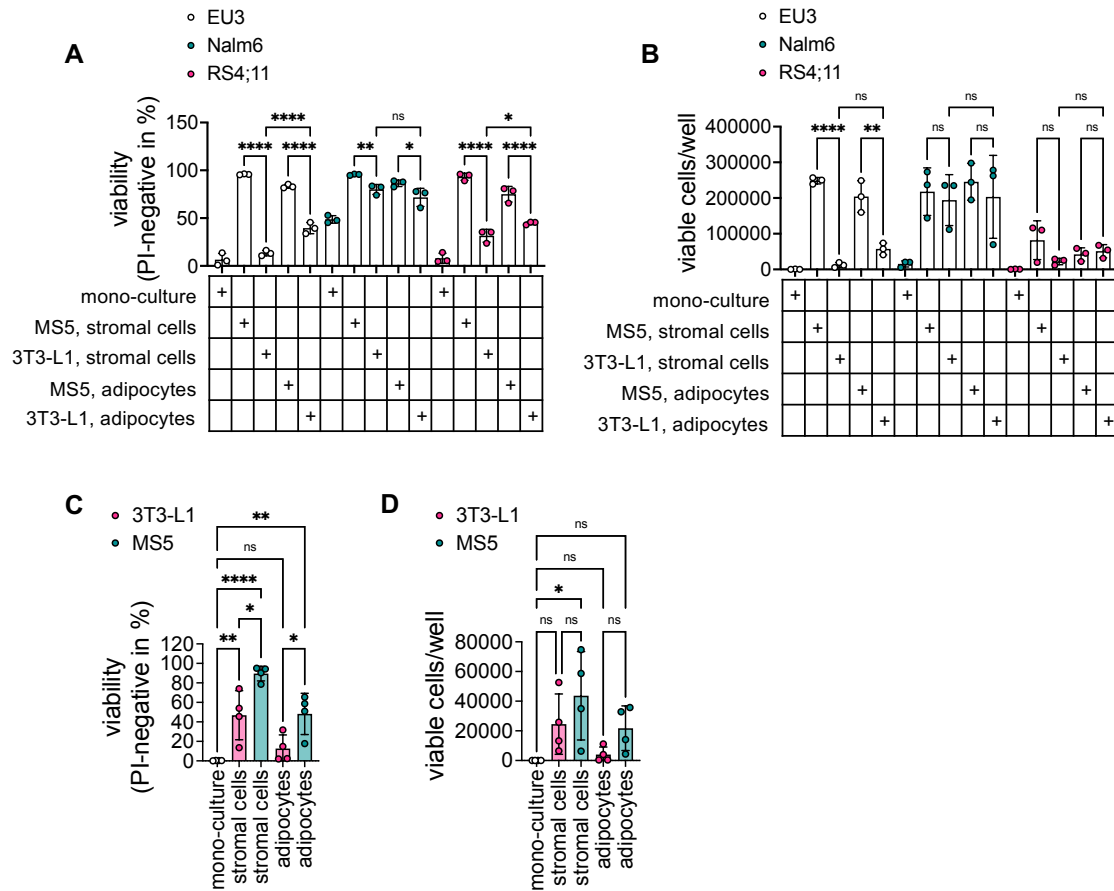
Figure S5

Fig S5. Co-culture of leukemia cells with MS5-derived or 3T3-L1-derived stromal cells or adipocytes. (A – B) B-precursor ALL cell lines EU3, Nalm6 and RS4;11 were cultured alone or together with MS5-derived or 3T3-L1-derived stromal cells or adipocytes. Cell viability (A) or viable cells/well (B) were determined after 7 days. Data are presented as mean $\pm$ SD from three independent experiments performed in five technical replicates; dots represent individual experiments. (C – D) PDX B-precursor ALL samples X07, X12, X14, and X17 were cultured alone or together with 3T3-L1- or MS5-derived stromal cells or adipocytes. Viability (C) and the number of viable cells/well (D) were determined after 7 days in culture. Data are presented as mean $\pm$ SD from four individual PDX samples performed in three technical replicates; dots represent individual PDX samples. Ordinary one-way ANOVA with Šidák's multiple comparisons test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$.

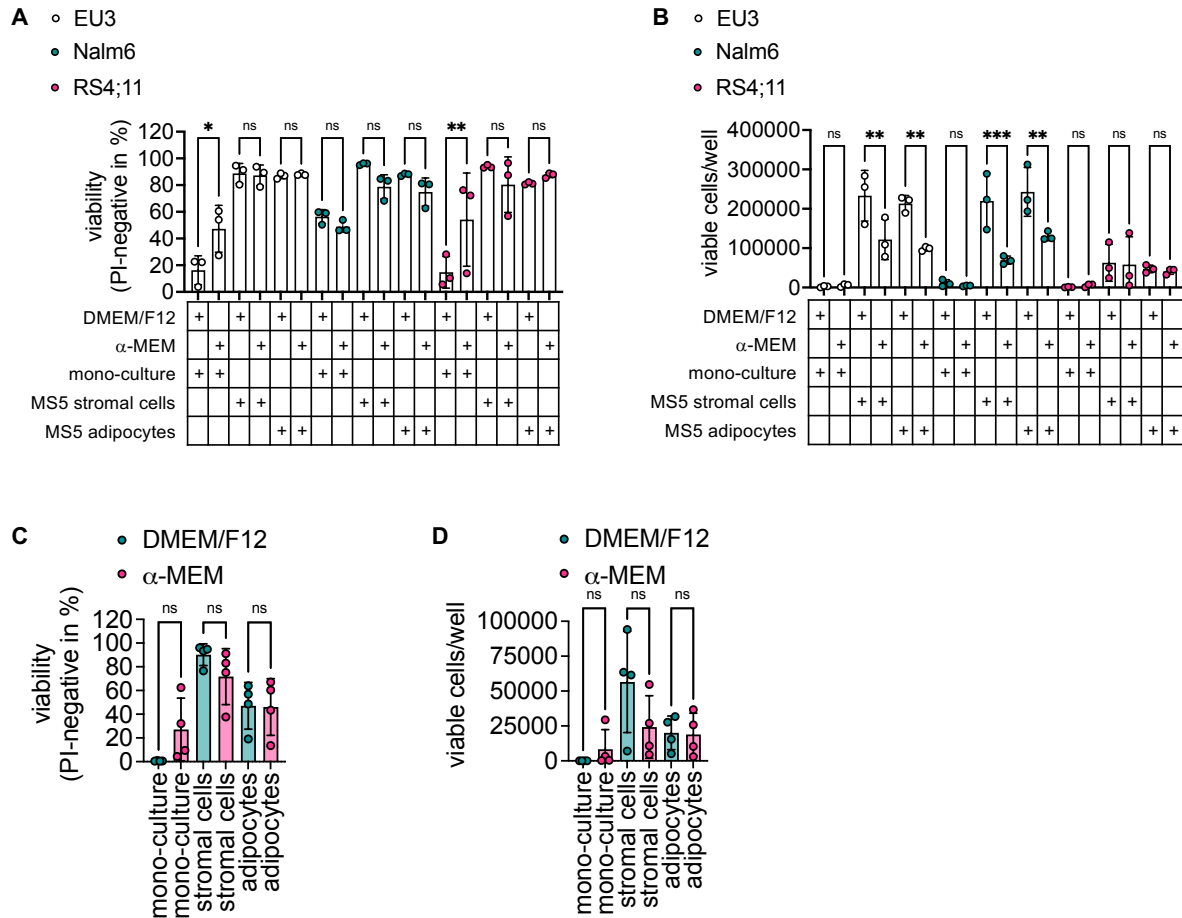
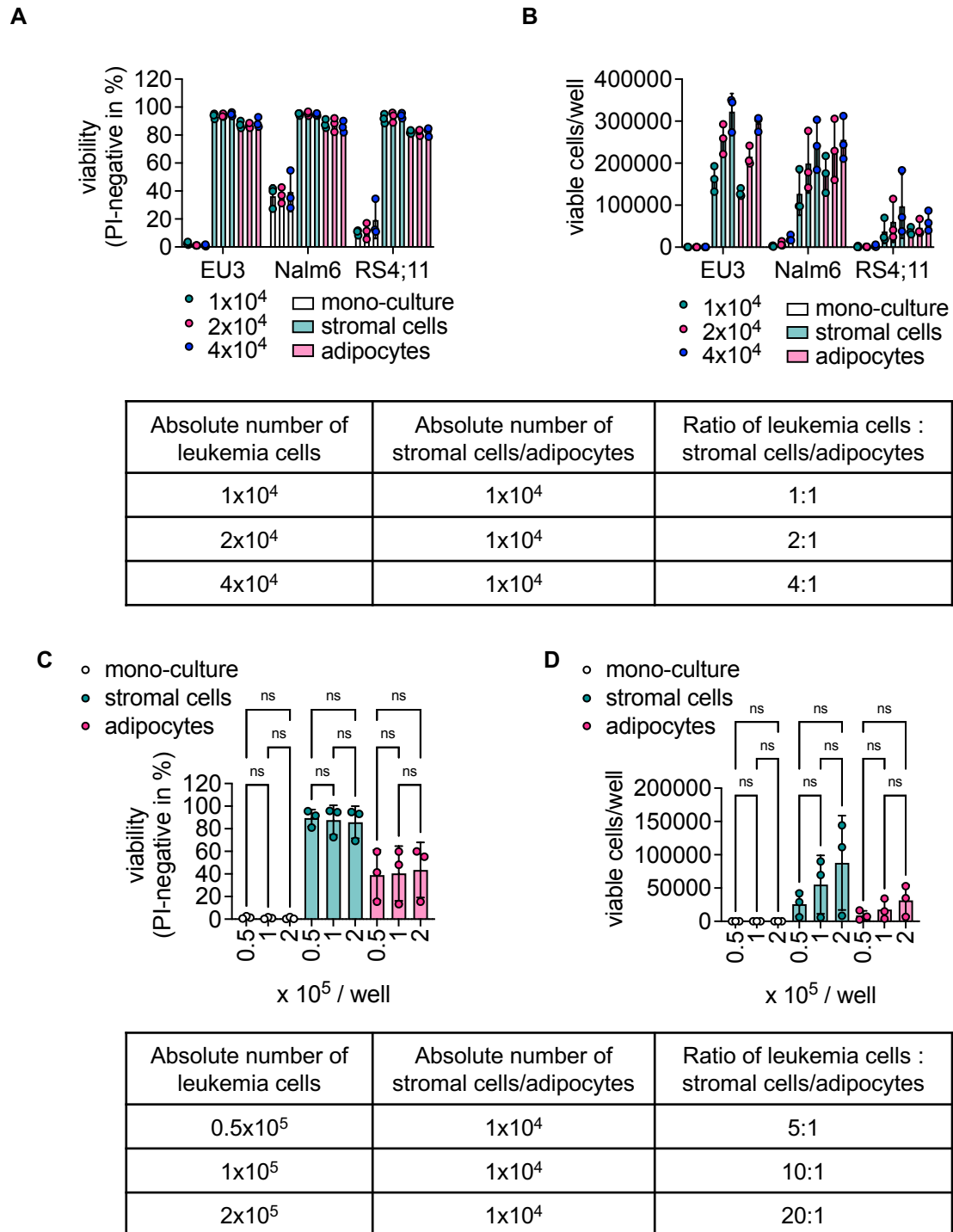
Figure S6

Fig S6. Co-culture of leukemia cells with MS5-derived stromal cells or adipocytes cultured in DMEM/F12 or alpha-MEM. (A – B) B-precursor ALL cell lines EU3, Nalm6 and RS4;11 were cultured alone or together with MS5-derived stromal cells or adipocytes in either DMEM/F12 or alpha-MEM. Cell viability (A) or viable cells/well (B) were determined after 7 days. Data are presented as mean $\pm$ SD from three independent experiments performed in five technical replicates; dots represent individual experiments. (C – D) PDX B-precursor ALL samples X07, X12, X14, and X17 were cultured alone or together with MS5-derived stromal cells or adipocytes in either DMEM/F12 or alpha-MEM. Viability (C) and the number of viable cells/well (D) were determined after 7 days in culture. Data are presented as mean $\pm$ SD from four individual PDX samples performed in three technical replicates; dots represent individual

PDX samples. Ordinary one-way ANOVA with Šidák's multiple comparisons test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Figure S7



independent experiments performed in three technical replicates; dots represent individual experiments. (C – D) 0.5×10^5 , 1×10^5 or 2×10^5 PDX B-precursor ALL cells ($n=3$; X12, X14, and X17) were cultured alone or together with MS5-derived stromal cells or adipocytes. Viability (C) and the number of viable cells/well (D) were determined after 7 days in culture. Data are presented as mean $\pm$ SD from three individual PDX samples performed in three technical replicates; dots represent individual PDX samples. Ordinary one-way ANOVA with Šidák's multiple comparisons test.

Figure S8

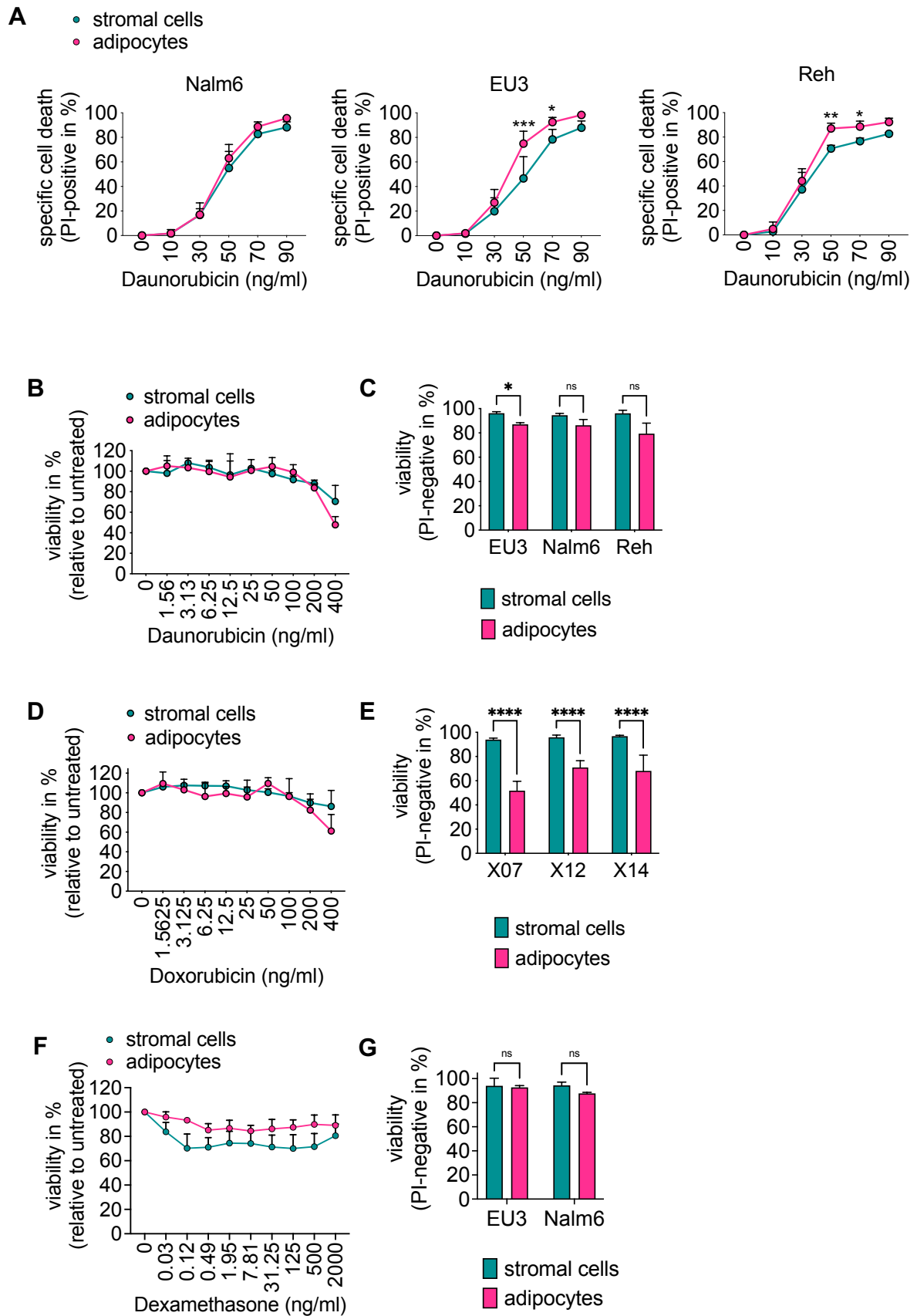


Fig S8. MS5-derived fibroblasts and adipocytes are resistant to anthracycline- or dexamethasone-induced cell death.

(A) Leukemia cell lines Nalm6, EU3 and Reh were stimulated with increasing concentrations of daunorubicin as indicated in co-culture with either MS5-derived stromal cells or adipocytes. Cell death was assessed by PI positivity at the FACS after 96 hours. (B, D, F) MS5-derived stromal cells or adipocytes were treated with increasing concentrations of daunorubicin (B), doxorubicin (D) or dexamethasone (F). Viability was determined after seven days using CellTiter-Glo®. (C, E, G) Corresponding viability of untreated leukemia cells from Fig S8A (C), Fig 5D and E (E) or Fig 5F (G). Data are presented as mean + SEM (A) or + SD (B – G) from at least three independent experiments performed in triplicates (A – D, F and G) or three technical replicates of the individual PDX B-precursor ALL samples (E). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; two-way ANOVA with Šídák's multiple comparisons test (A, C, E, G).

Supplementary Methods

Determination of cellular surface covered by lipid droplets

The cellular surface covered by lipid droplets was determined in an automated manner by using the live-cell analysis system Incucyte S3 (Sartorius) followed by analysis with the Incucyte 2019B Rev2 software and the basic analyzer software module. Analysis was performed on at least 3 different wells of a 12-well plate with MS5 or 3T3-L1 cells undergoing adipogenic differentiation. At least 4 different images were taken per well with a 10x or 20x magnification. Analysis was performed on phase contrast images to determine (i) the area of the well covered by lipid droplets, and (ii) the area of the well covered by cells. From this the percentage of the cellular surface covered by lipid droplets was calculated ((i) divided by (ii) multiplied by 100).

Adipogenic differentiation of 3T3-L1 cells

3T3-L1 cells were differentiated based on a previously published differentiation protocol (1). In brief, 3.5×10^3 3T3-L1 cells were seeded into each well of a 96-well plate or 4×10^4 into each well of a 12-well plate on day minus 3 of adipogenic differentiation in their usual culture medium, i.e. DMEM (Gibco) supplemented with 10% FCS and 2% L-Glutamine. On day 0 of adipogenic differentiation, the culture medium was changed to differentiation medium (DMEM plus 10% FCS and 2% L-Glutamine supplemented with 500 μ M IBMX, 5 μ g/ml insulin, 5 μ M dexamethasone, 125 μ M indomethacin, 1 nM T3 and 1 μ M rosiglitazone). On day 4 of adipogenic differentiation, the differentiation medium was exchanged by maturation medium (DMEM plus 10% FCS and 2% L-Glutamine supplemented with 5 μ g/ml insulin and 1 μ M rosiglitazone). The maturation medium was replaced every three to four days by fresh maturation medium. 3T3-L1 cells are considered to be mature and fully differentiated adipocytes after 8 - 10 days of adipogenic differentiation (2, 3).

Supplementary Table 1. Primer sequences

<i>Adipoq</i>	gttcctcttaatcctgccagtcacgcc (f), ggaccaagaagacctgcatctcctttctc (r)
<i>Fabp4</i>	cgatgattacatgaaagaagtgaggagtgagg (f), tatgatgctcttcaccttcctgtcgtctg (r)
<i>Glut4</i>	tgagctgaaggatgagaaacggaagttgga (f), ctaagagcaccgagaccaacgtgaagacc (r)
<i>Hprt</i>	gctggtgaaaaggacctc (f), cacaggactagaacacct (r)
<i>Il6</i>	gatggatgctaccaaactgga (f), tctgaaggactctggctttg (r)
<i>Mcp1</i>	aggtccctgtcatgcttctg (f), ggatcatcttgctggtgaa (r)
<i>Plin</i>	caatgcctatgagaagggtgtacagggtg (f), caactcattggcagctgtgaactgggtg (r)
<i>Pparg</i>	gaccactcgcattcctttgacatcaagcc (f), tgatcgcactttggtattcttgagcttcag (r)

References

1. Caratti G, Stifel U, Caratti B, Jamil AJM, Chung KJ, Kiehntopf M, et al. Glucocorticoid activation of anti-inflammatory macrophages protects against insulin resistance. *Nat Commun.* 2023;14(1):2271.
2. Kaczmarek I, Suchy T, Strnadova M, Thor D. Qualitative and quantitative analysis of lipid droplets in mature 3T3-L1 adipocytes using oil red O. *STAR Protoc.* 2024;5(2):102977.
3. Sun W, Yu Z, Yang S, Jiang C, Kou Y, Xiao L, et al. A Transcriptomic Analysis Reveals Novel Patterns of Gene Expression During 3T3-L1 Adipocyte Differentiation. *Front Mol Biosci.* 2020;7:564339.