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CD36 drives metastasis and relapse in acute myeloid leukemia

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40 **SIGNIFICANCE**

41 CD36 promotes blast migration and extramedullary disease in acute myeloid leukemia and represents a
42 critical target that can be exploited for clinical prognosis and patient treatment.

43

44 **ABSTRACT**

45 Identifying mechanisms underlying relapse is a major clinical issue for effective cancer treatment. The
46 emerging understanding of the importance of metastasis in hematological malignancies suggests that it
47 could also play a role in drug resistance and relapse in acute myeloid leukemia (AML). In a cohort of
48 1,273 AML patients, we uncovered that the multifunctional scavenger receptor CD36 was positively
49 associated with extramedullary dissemination of leukemic blasts, increased risk of relapse after intensive
50 chemotherapy, and reduced event-free and overall survival. CD36 was dispensable for lipid uptake but
51 fostered blast migration through its binding with thrombospondin-1. CD36-expressing blasts, which were
52 largely enriched after chemotherapy, exhibited a senescent-like phenotype while maintaining their
53 migratory ability. In xenograft mouse models, CD36 inhibition reduced metastasis of blasts and prolonged
54 survival of chemotherapy-treated mice. These results pave the way for the development of CD36 as an
55 independent marker of poor prognosis in AML patients and a promising actionable target to improve the
56 outcome of patients.

57 INTRODUCTION

58 Acute myeloid leukemia (AML) is a heterogeneous group of diseases characterized by uncontrolled
59 proliferation of clonal neoplastic hematopoietic precursor cells and impaired function of normal
60 hematopoiesis, leading to neutropenia, anemia, and thrombocytopenia (1). If untreated, patients die of
61 infection, bleeding, or organ failure, usually in a matter of weeks. Induction therapy, which combines 7
62 days of cytarabine (AraC) and 3 of anthracycline, is highly effective in killing leukemic cells, but despite a
63 high rate of complete remission after these cytotoxic agents, most patients relapse (2). In AML patient-
64 derived xenografts (PDX) in immunodeficient mice (non-obese diabetic scid gamma mouse, i.e. NSG)
65 treated by AraC, we found CD36, a plasma membrane receptor and fatty acid transporter, among the most
66 strongly upregulated genes in chemoresistant cells (3). In this study, we investigated the clinical relevance
67 of this observation, and the mechanisms by which CD36 expression may contribute to chemoresistance
68 and relapse.

69 We and others have shown that AML blasts are highly dependent on fatty acid metabolism and oxidative
70 phosphorylation (OXPHOS) to bypass chemotherapy (3-5). In a number of tumors (6-8), an increased
71 CD36 expression has been found to support cancer cell metabolism through the transport of fatty acids.
72 However, CD36 is a multi-liganded receptor also binding molecules such as oxidized low-density
73 lipoprotein (oxLDL) (9) or Thrombospondins 1 and 2 (TSP1 and TSP2) (10), and CD36 is involved in a
74 number of functions besides metabolism, including angiogenesis, tumor immunity and metastasis (11-13).
75 Metastatic cancers are largely resistant to treatments as compared to primary tumor cells, and represent the
76 most frequent cause of death in cancer patients. In solid cancers as in leukemia (8,14), metastatic niches
77 provide a microenvironment which fuels cell metabolism, protecting tumor cells from stresses and
78 chemotherapies. Among the different mechanisms of relapse, entry of leukemic blasts into a senescent-
79 like state is an important driver of chemoresistance and AML relapse (15). Senescent cells can stimulate
80 tumor progression through their senescence-associated secretory phenotype (SASP) which comprises pro-
81 inflammatory and pro-invasive molecules that can stimulate motility, invasion, and metastasis (16).

82 Until recently (17), because leukemic cells circulate in lymph and blood as their normal counterparts do,
83 leukemia were generally not referred to as metastatic diseases. However, the presence at diagnosis of an
84 extramedullary disease (EMD) in AML patients has been described for decades, with an incidence ranging
85 from 2.5% (18) to 31% (19). The prognostic significance of EMD in AML was found as of no value in
86 some studies (20), whereas others found EMD to convey a poor prognosis, independent of other
87 established risk factors (18,19,21,22). These discrepancies between studies partly result from the difficulty
88 to accurately score EMD. As recently shown in a multi-center cohort of AML patients, EMD was
89 significantly associated with a decreased median overall survival (OS) and was predictive of a reduced
90 even-free survival (EFS) only when histology was added to the clinical examination (22). This highlights
91 the need to better understand the molecular mechanisms driving EMD and to identify reliable biomarkers.
92 The impact of EMD in AML relapse after chemotherapy also needs to be deciphered.
93 Here, to uncover the molecular mechanisms behind dissemination of leukemic blasts in extramedullary
94 organs, we combined *in vitro* and *in vivo* experiments using AML cell lines and patient cells, including
95 single-cell analysis, alongside with a clinical study from a large cohort of 1,273 AML patients, some of
96 whom have received an intensive chemotherapy regimen and have been followed for at least three years.
97 Our findings highlight CD36 as an actionable driver of leukemic blasts metastasis and unravel new
98 mechanisms contributing to AML relapse.

99

100 **MATERIALS AND METHODS**

101 **Patients**

102 Inclusion criteria were: newly diagnosed AML or relapse according to the WHO 2008 classification and
103 age 15 years or older. This study included 1,273 patients admitted at the Hematology department of
104 Toulouse University Hospital-IUCT-O and/or registered in the regional oncology network from January
105 1st 2016 to December 31st 2020 (TUH cohort). Data are gathered in an electronic clinical research form. A
106 written informed consent was obtained from all patients in accordance with the Declaration of Helsinki,
107 allowing the collection of clinical and biological data in an anonymized database. Samples from AML

108 patients were stored in the HIMIP collection (BB-0033-00060). In accordance with French law, the
109 HIMIP collection was declared to the Ministry of Higher Education and Research (DC 2008-307
110 collection 1) and obtained a transfer agreement (AC 2008-129) after approval by the Comité de Protection
111 des Personnes Sud-Ouest et Outremer II (ethics committee).

112 Cytogenetic and molecular risk classifications were in accordance with the Medical Research Council and
113 ELN 2010 classifications, respectively (23,24). Details on treatment with first-line chemotherapy regimen
114 used over time have been reported elsewhere (25). The decision-making process with regard to intensive
115 or non-intensive treatment was based on initial characteristics such as white blood cell count,
116 cytogenetics, age, secondary AML, performance status, and comorbidities. EMD status was determined at
117 AML diagnosis by clinical examination.

118 Endpoints, including response, EFS, LFS, CIR and OS, were assessed according to standard criteria (1).
119 Bone marrow assessment in patients treated with intensive chemotherapy was performed after blood
120 recovery or in case of delayed recovery between day 35 and 45.

121

122 ***In vivo* animal studies**

123 NSG (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) mice were used for transplantation of AML cell lines or
124 primary AML samples. Mice were housed in sterile conditions using HEPA-filtered micro-isolators and
125 fed with irradiated food and sterile water in the Animal core facility of the Cancer Research Center of
126 Toulouse (France). All animals were used in accordance with a protocol reviewed and approved by the
127 Institutional Animal Care and Use Committee of Région Midi-Pyrénées (France). Six to 9 weeks-old male
128 or female mice were randomly assigned to experimental groups before cell injection or drug treatments.

129

130 **Primary patients sample phenotyping**

131 Multi-parameter flow cytometry (MFC) was performed on whole bone marrow (BM) or blood specimens
132 using a standard stain-lyse-wash procedure with ammonium chloride lysis. 1×10^5 cells were stained per
133 analysis tube (see antibody list in **Table S1**), and data were acquired on at least 1×10^4 blasts when

134 specimen quality permitted. Data on standardized 8- to 10-color staining combinations were acquired on
135 Navios instruments analyzed using Kaluza (Beckman-Coulter). A blasts gate including CD45 dim
136 mononuclear cells was analyzed according to cytomorphologic data.

137

138 **Xenograft mouse models**

139 NSG mice were produced at the Genotoul Anexplor platform in Toulouse (France), using breeders
140 obtained from Charles River Laboratories. To assess response to chemotherapy in PDX models, mice (6–
141 9-week-old) were sublethally treated with busulfan (30 mg/kg) 24 h before injection of leukemic cells.
142 Leukemia samples were thawed at 37 °C, washed in IMDM 20% FBS, and suspended in Hank's balanced
143 salt solution at a final concentration of $2\text{--}10 \times 10^6$ cells in 200 μL for tail vein injection; 8–18 weeks after
144 AML cell transplantation, once cell engraftment was confirmed by flow cytometry on peripheral blood or
145 bone marrow aspirates, NSG mice were treated by daily intraperitoneal injection of 60 mg/kg AraC or
146 vehicle (PBS) for 5 days. AraC was kindly provided by the pharmacy of the TUH. Mice were sacrificed at
147 day 8 post-AraC treatment to harvest human leukemic cells from murine bone marrow.

148 To establish CLDX, NSG mice were treated with busulfan (20 mg/kg) 24 h before injection of AML cell
149 lines. Cells were thawed and washed as previously described, suspended in HBSS at a final concentration
150 of $0.2\text{--}2 \times 10^6$ in 200 μL before intravenous injection. Mice were treated by daily intraperitoneal injection
151 of 30 mg/kg AraC for 5 days (starting day 10 for U937 and day 17 for OCIAML3 post-injection) and
152 sacrificed at day 8 or later as indicated. When indicated, mice received concomitantly with AraC until
153 sacrifice thrice weekly an intravenous injection of PBS (100 μL) containing either 5 μg of monoclonal
154 CD36Ab FA6.152 (Stem Cell Technologies 60084) or 5 μg of mouse control IgG (Stem Cell
155 Technologies 60070). Mice survival time was also determined. All animals were used in accordance with
156 a protocol reviewed and approved by the Institutional Animal Care and Use Committee of Région Midi-
157 Pyrénées (France).

158

159 **¹⁴C palmitate uptake and oxidation assay**

160 Cells were preincubated for 3-hour with [1-¹⁴C] palmitate (1 μCi/ml; Perkinelmer, Boston, MA) and non-
161 labeled (cold) palmitate. Palmitate was coupled to a fatty acid-free BSA in a molar ratio of 5:1. Following
162 incubation, ¹⁴CO₂ and ¹⁴C-ASM were measured as previously described (26). Briefly, assayed medium is
163 transferred into a custom-made Teflon 48-well trapping plate. The plate is clamped and sealed, and
164 perchloric acid is injected through the perforations in the lid into the medium, which drives CO₂ through
165 the tunnel into an adjacent well, where it is trapped in 1N NaOH. Following trapping, the media is spun
166 twice and ¹⁴C-ASM measured by scintillation counting. Aliquots of NaOH and medium are transferred
167 into scintillation vials, and radioactivity is measured on a multipurpose scintillation counter (LS 6500;
168 Beckman Coulter). All assays are performed in triplicates, and data are normalized to viable cell number.

169

170 **RNA Extraction and Real-Time RT-qPCR.**

171 Total cell RNA was isolated and extracted using the ZYMO RNA kit (Zymo). For mouse tissues, total
172 RNA was isolated by Qiazol extraction and purified using RNeasy minicolumns (Qiagen, Hilden,
173 Germany). 300-1000 ng total RNA was reverse transcribed using the High Capacity cDNA Reverse
174 Transcription kit (Applied Biosystem 4368814), SYBR Green PCR Master Mix (Applied Biosystem
175 4309155), and 300 nmol/L primers on an Applied Biosystem (Massachusetts, United States) StepOne
176 instrument. Relative gene expression was determined using the 2^{-ΔCT} or 2^{-ΔΔCT} method and
177 normalized to m36B4 or hRPLP0 expression as described in Figure legends. Primer sequences are
178 provided in Supplementary Material and methods.

179

180 **Migration assay**

181 For migration assays, 24 mm diameter, 8 μm pore transwells (Corning 353097) were used. Cell
182 suspension in 0%FBS αMEM medium was added in the upper chamber. The lower chamber was filled
183 with 500 μL of medium with or without 10% FBS. As indicated, anti-CD36 blocking antibodies (1

184 $\mu\text{g/mL}$) (FA6-152, Stem Cell Technologies 60084 or Cayman chemical JC63.1 10009893) and/or
185 recombinant TSP1 (R&D Systems 3074-TH) were added in the upper chamber. Leukemic cells were
186 allowed to migrate at 37°C for 24 hr. Inserts were removed and cell concentration was measured by cell
187 counting with trypan blue coloration.

188

189 **Immunohistochemistry**

190 Subcutaneous adipose tissue samples were immediately immersed in 10% neutral buffered formalin
191 fixative after sampling for 24 hours before storage in PBS at 4°C . After paraffin embedding, $3\mu\text{m}$
192 thickness paraffin sections were dewaxed (successive toluene and descending alcohols bathes) and stained
193 with Haematoxilin and Eosin. Labeling of $3\text{-}\mu\text{m}$ sections of paraffin-embedded adipose tissues samples
194 was performed out after antigen retrieval (Ptlink low pH, reference K8005, Dako) for 30 min, using anti-
195 human specific Ku-80 antibody (2753S Cell Signaling, rabbit monoclonal antibody, dilution 1:150) and
196 incubated for 50 min at room temperature. Staining was carried out with Dakostainer automated system
197 using a biotinylated anti-rabbit antibody (reference ABK125 Microm) for 25 min at room temperature,
198 followed by HRP (1:150, 25 min room temperature) and DAB as a chromogen. The stained slides were
199 imaged by light microscopy on a Nikon Eclipse Ci-L microscope with a DS138 Fi3 Camera and NIS
200 Elements D software.

201

202 **Publicly accessible transcriptomic databases of AML patients used in this study**

203 The following AML cohorts were used as indicated: GEO accession no. GSE14468 (Verhaak cohort (27)),
204 BeatAML cohort (28), TCGA cohort (29). FAB M3 patients, and Patients who did not receive intensive
205 chemotherapy were removed for analysis. Patients with overall survival value inferior to 1 year but still
206 alive were removed in Kaplan-Meier curves. Other publicly accessible transcriptomic databases of AML
207 patients used in this study were GSE40871, Klco et al. (30); GSE97631, Farge et al. (3); GSE146544, Duy
208 et al. (15).

209

210 **Establishment of the CD36 gene signature**

211 Global analysis of frequency vs genes on normalized Affymetrix data showed that CD36 expression
212 followed a Pareto distribution in TCGA and BEATAML databases whereas it followed a normal
213 distribution in the Verhaak database. Only patients with non-zero CD36 expression were considered. In
214 order to standardize the cutoff of differential expression among the three databases and establish
215 experimental groups, based on a 95% confidence interval (i.e., p-value < 0.05) of CD36 expression,
216 patients with a z-score > 1.64 were categorized as “High CD36” and < 1.64 as the baseline group. Welch’s
217 t-test of means for difference in variances was performed between the two groups to determine the
218 differentially expressed genes. Then, data points were filtered to preserve overexpressed genes with a p-
219 value < 0.05 in the High CD36 (positive “statistic”) group. Corresponding test statistic values were then
220 listed in decreasing order. Gene symbols were assigned unique Ensembl IDs to mitigate redundancies
221 from gene synonyms and awkward formatting. The top 1000 genes in each database were used to select
222 the common genes among the different databases.

223

224 **Statistical analysis**

225 For univariate survival analyses of OS and LFS in patients, Kaplan-Meier survival curves were drawn and
226 differences in survival functions were tested using the Log-Rank test. Univariate survival analyses used
227 Cumulative Incidence Functions and Gray’s test for relapse (CIR), since non-relapse mortality was treated
228 as competing events. Hazard Ratios (HR) and 95% confidence intervals (CI) were assessed using a
229 standard Cox model, for OS and EFS and a proportional subdistribution hazard model which is an
230 extension of the Cox model for the situation of competing risks, for CIR. All reported p-values were two-
231 sided and the significance threshold was < 0.05. Statistical analyses were performed on STATA® version
232 13 (STATA Corp., College Station, TX).

233 We assessed the statistical analysis of the difference between two sets of data using two-tailed (non-
234 directional) non-parametric Mann-Whitney test or unpaired test according to samples distribution, with or
235 without Welch’s correction depending on samples variance. For graphs with control condition normalized

236 to 1, we used a one sample t test with a hypothetical value set to 1. For multiple comparisons, one-way
237 Anova or two-way Anova were performed with multiple comparison tests. For paired tests, Wilcoxon test
238 was used. For contingency analysis of clinical data, we addressed Fisher's Exact test on raw data before
239 normalization in percentages. For survival analyses, we used Log-rank (Mantel-Cox) test. Analyses were
240 performed using Graphpad Prism (v9). A p value of less than 0.05 indicates significance. *p < 0.05;
241 **p < 0.01; ***p < 0.001; ****p < 0.0001; ns, not significant.

242

243 **Data Availability Statement**

244 All raw data generated in this study are available upon request from the corresponding author. The data
245 analyzed in this study were obtained from Gene Expression Omnibus (GEO) at [GSE14468](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE14468), [GSE40871](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE40871),
246 [GSE97631](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE97631), [GSE146544](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE146544). Data from the BeatAML study was obtained from <http://www.vizome.org>, and
247 TCGA AML dataset (29) was obtained from <https://cbioportal.org>.

248

249 **RESULTS**

250 **CD36 is an independent prognostic marker of AML progression**

251 To study if CD36 has a clinical prognostic value, we measured CD36 protein expression by flow
252 cytometry in AML blasts of 1,273 AML patients from the Toulouse University Hospital (TUH). Based on
253 the percentage of blasts expressing CD36 at diagnosis, AML patients were stratified as CD36-high
254 (CD36-expressing blasts \geq 20%, 407/1273, 32%) vs CD36-low (**Fig. 1a**). In this cohort, 435 AML
255 patients were treated with an intensive chemotherapy regimen combining 3 days of daunorubicin or 5 days
256 of idarubicin and 7 days of cytarabine. Although several features impact patients' prognosis, as already
257 well described (1), a multivariate analysis revealed that CD36 was significantly associated with a worse
258 event free survival (EFS; HR: 1.55; 95%CI: 1.17–2.05, p=0.002), and a worse overall survival (OS; HR:
259 1.69; 95%CI: 1.18–2.41, p=0.005; **Table 1**). Median EFS of CD36-high patients was half that of CD36-
260 low patients (252 days vs 538 days respectively (HR:1.65, p<0.0001; **Fig. 1b**)), whereas median OS,

261 which was 462 days in CD36-high patients, was not reached after 3 years in CD36-low patients (HR: 1.88,
262 $p < 0.0001$; **Fig. 1c**). Furthermore, a high CD36 protein expression at diagnosis was associated with an
263 increased cumulative incidence of relapse (CIR) after intensive chemotherapy (**Fig. 1d**), and a multivariate
264 analysis showed that a high expression of CD36 was associated with a shorter CIR (SHR: 1.53; 95%CI:
265 1.07–2.19, $p = 0.02$; **Table 1**). We next characterized the molecular and cytogenetic profile of patients
266 exhibiting high percentage of CD36 expressing blasts. Consistent with our findings, intermediate and
267 unfavorable karyotypes had higher percentage of CD36 expressing blasts (**Fig. 1e**). To provide an
268 overview of the genomic landscape of CD36 AML clusters, we plotted targeted NGS data from 224 AML
269 patients to examine the co-occurrence of mutations. We also calculated odds ratios for recurrent
270 cytogenetic abnormalities. Comparison of the genomic landscape in 224 AML did not reveal a pattern
271 related to CD36 expression, except for FLT3 abnormalities (**Fig. 1f-g**). We next examined the set of
272 recurrent karyotype abnormalities ($n = 1202$) showing an enrichment of AML with KMT2A (11q23) or
273 t(9;22) abnormalities in CD36 high group and an increase in t(15;17) and t(8;21) AML in CD36 low group
274 (**Fig. 1h**).

275 Altogether, these clinical findings obtained on a large patient cohort reveal that a high percentage of blasts
276 expressing CD36 at diagnosis is an independent prognostic marker of disease progression, thus warranting
277 the study of CD36 contribution to AML progression and relapse.

278

279 **CD36 inhibition delays AML relapse after chemotherapy**

280 Using additional PDX, we confirmed our previous observation (3) that AraC treatment *in vivo* induced a
281 sustained increase in CD36 expression at the plasma membrane of residual AML blasts (**Fig. S1a-b**). To
282 decipher whether CD36 is a promoter or a consequence of AML progression and relapse, we performed
283 experiments using two human AML cell lines strongly expressing CD36 protein i.e. U937 and OCIAML3,
284 U937 exhibiting two distinct subpopulations expressing low and high levels of CD36 at baseline, while
285 OCIAML3 cells do not exhibit such phenotypical heterogeneity (**Fig. S1c**). In both models, treating cells
286 with AraC *in vitro* increased CD36 protein expression (**Fig. S1d**). Using shRNA, we knocked-down CD36

287 and then engrafted these cells and their control counterparts into NSG mice. After engraftment, mice were
288 treated with vehicle or AraC for 5 days, and survival experiments were performed. In both models,
289 knocking down CD36 did not affect the OS of untreated mice (data not shown) but significantly increased
290 the survival of AraC-treated mice (**Fig. 2a-b**). We then asked if CD36 inhibition might have a therapeutic
291 potential once the disease was established. To this aim, we engrafted mice with U937 cells, and 10 days
292 later randomized mice into four groups receiving vehicle only, AraC only, FA6-152 anti-CD36 blocking
293 antibody only, or a combination of AraC and CD36 antibody. Whereas inhibition of CD36 did not
294 increase the OS in untreated mice, AraC increased mice survival, and importantly, adding a CD36
295 antibody to AraC further significantly prolonged mice survival (**Fig. 2c**). Of note, neither CD36
296 knockdown with shRNA nor its inhibition with the FA6-152 blocking antibody affected the number of
297 viable blasts (**Fig. S1e-h**). Together, these data unveil a new therapeutic modality to potentialize
298 chemotherapeutic effects, delaying time to relapse.

299 We then questioned the mechanisms behind CD36-dependent relapse after chemotherapy. We first
300 hypothesized that CD36 mediates lipid transfer in leukemic blasts. Surprisingly, CD36 silencing by
301 shRNA, which reduced CD36 protein expression in basal as well as in AraC-treated cells (**Fig. S1i-k**), did
302 not affect cell neutral lipid content, measured by Bodipy staining (**Fig. S1l-m**). Treatment with JC63.1
303 CD36 antibody which blocks fatty acid and oxLDL uptake (31,32) or with FA6-152 CD36 antibody which
304 in addition inhibits CD36 binding to TSP1/2 (33-36) both failed to affect lipid content (**Fig. S1n-o**).
305 Invalidation of CD36 using siRNA or FA6-152 CD36 blocking antibody did not affect ¹⁴C-palmitate
306 uptake and oxidation (**Fig. S1p-aa**), suggesting that CD36 is dispensable for lipid uptake in AML blasts.

307 To further investigate the role of CD36 in AML blast biology, we generated a CD36 gene signature
308 comprising 249 genes that were all highly expressed along with CD36 in transcriptomic data from three
309 AML patient databases (**Fig. 2d, Table S2**). Interestingly, this signature was enriched in primary AML
310 cells treated with AraC compared to control cells in two different databases (GSE40871 (30), GSE146544
311 (15)), consistent with our previous observations (GSE97631) (3)) (**Fig. 2e**).

312

313 **CD36 triggers blast migration**

314 A Gene Ontology (GO) analysis of the above defined CD36 signature highlighted an enrichment in
315 migration and adhesion processes (**Fig. 3a**), including the GO term “positive regulation of mononuclear
316 cell migration” (**Fig. S2a**), while no enrichment for GO terms related to lipid transport and metabolism
317 was found (data not shown). Considering that CD36 involvement in metastasis is described in several
318 tumors (6,12,37), we hypothesized that CD36 could drive leukemic blast migration. Knocking-down
319 CD36 with shRNA or siRNA decreased AML cell migration (**Fig. 3b and S2b-c**). FA6-152 CD36
320 blocking antibody, but not JC63.1 antibody, significantly decreased U937 and OCIAML3 migration (**Fig.**
321 **3c; Fig. S2d**). In fresh patient’ samples, FA6-152 antibody decreased migration (**Fig. 3d**). FA6-152
322 antibody blocks the binding of CD36 to its ligand TSP1, which is well known to promote cell migration in
323 various tumor models (38-40). Invalidation of TSP1 using shRNA (**Fig. S2e-f**) decreased migration of
324 U937 (**Fig. 3e**) as well as of OCIAML3 cells (**Fig. S2g**). Adding A6.1 anti-TSP1 antibody also decreased
325 U937 and OCIAML3 cell migration, and noteworthy, CD36 knock-down with shRNA abolished this
326 effect (**Fig. 3f and S2h**). Conversely, recombinant TSP1 increased migration of both U937 and
327 OCIAML3 cells. This effect was hampered by the addition of CD36 blocking antibody (**Fig. 3g, Fig. S2i**),
328 indicating that TSP1 promotes cell migration through its binding to CD36. As observed with FA6-152
329 anti-CD36 antibody, A6.1 TSP1 blocking antibody inhibited migration of fresh patient cells (**Fig. 3h**).
330 Together, these data highlight that CD36 fosters AML blast migration, at least in part through its binding
331 to TSP1.

332 Because migration is usually associated with metastasis in solid tumors, we asked whether blasts could
333 migrate and localize into extramedullary organs. To visualize AML cell distribution *in vivo*, we injected a
334 large panel of bioluminescent AML cell lines into NSG mice. In all models, AML cells localized
335 throughout the body as shown by the acquisition of bioluminescence in mice during the time course of
336 AML progression, and on isolated organs at sacrifice (**Fig.3i and Fig.S2j, k**). Analyses of human-specific
337 CD45 expression by RT-qPCR in NSG mice grafted with U937 cells confirmed the presence of human
338 blasts in the bone marrow, but also in different organs including spleen, perigonadal adipose tissues and

339 liver (**Fig. S2l**). Immunohistochemistry using a human-specific anti-Ku-80 antibody showed that AML
340 blasts present in subcutaneous adipose tissues were scattered within the stroma, closely surrounding
341 adipocytes (**Fig.3j**), ruling out that the PCR results corresponded to blasts present in blood vessels. To
342 further rule out that these extramedullary localizations might result from an experimental bias linked to the
343 intravenous modality of blast injection, we injected OCIAML3 cells orthotopically into the right femur of
344 NSG mice. Flow cytometry analyses revealed the presence of human CD45+ blasts in the contralateral
345 femur, but also in spleen, subcutaneous adipose tissue, perigonadal adipose tissue and lung (**Fig. 3k**). This
346 tissue distribution was comparable to that observed after intravenous injection of OCIAML3 cells (**Fig.**
347 **S2m**), establishing that leukemic cells are able to migrate out of the bone marrow and engraft into distant
348 locations. Interestingly, CD36 expression was increased in cells that had migrated from the site of
349 injection, as shown comparing blasts from the bone marrow of injected femurs to contralateral femurs and
350 extramedullary organs excepting lungs (**Fig. 3l**). In addition, we pretreated OCIAML3 cells with IGG or
351 FA6-152 anti-CD36 antibody, injected these cells intra-venously into NSG mice which were then treated
352 with either IGG or FA6-152 antibody. Ten days later, while untreated control cells started engrafting in
353 the bone marrow (BM), perigonadal adipose tissue (PGAT), liver and lungs, minimal to no engraftment
354 was detected in mice injected with FA6-152 pretreated cells (**Fig. S2n**), suggesting that even in the
355 absence of chemotherapy, CD36 is required for AML cell extravasation and migration. Altogether, these
356 results establish that AML blasts can migrate from the bone marrow into different organs in xenograft
357 models.

358

359 **Residual blasts maintained their migratory properties after chemotherapy**

360 We observed that treating cells with AraC increased CD11b, CD14, CD15 and CD44 protein expression
361 levels (**Fig. 4a, Fig. S3a**), consistent with a AraC-induced partial differentiation of blasts towards a
362 monocytic phenotype. Monocytes are particularly endowed with migratory capacities, and interestingly, a
363 “Bone marrow monocyte” gene signature was enriched in primary AML cells treated with AraC as
364 compared to control cells in three databases (GSE40871 (30), GSE97631 (3), GSE146544 (15)) (**Fig. 4b**).

365 We thus questioned the migration ability of blasts bypassing chemotherapy. Although to a lesser extent
366 than in control conditions, U937 cells that survived 4 days of AraC treatment were still able to migrate,
367 and, noteworthy, in both control and AraC-treated cells, CD36 blocking antibody FA6-152 decreased
368 migration (**Fig. 4c**). We then studied the ability to migrate of viable CD36-high cells sorted after 4 days of
369 treatment with AraC. While about 83% of U937 cells died, the percentage of living CD36-positive cells
370 raised from 70% in untreated cells up to 90% in resistant ones and very few CD36-low cells survived,
371 precluding their analysis (**Fig. 4d**). CD36 expression was strongly increased in migrating compared to
372 non-migrating cells in both control and AraC-treated CD36-positive U937 cells (**Fig. 4e, f**). CD36-
373 expressing blasts migrated more efficiently than CD36-negative cells in control condition, and, even
374 though AraC partially decreased their migration, CD36-high cell were still efficiently migrating, similarly
375 to untreated CD36-low cells (**Fig. 4g**).

376 We next engrafted mice with control or CD36 knocked-down U937 cells, treated mice with AraC for 5
377 days, and once disease relapse had translated into clinical signs, the presence of blasts was evaluated in
378 different organs. At relapse, CD36 knock-down had increased the tumor burden in the bone marrow (**Fig.**
379 **4h**), while it had reduced it in the spleen (**Fig. 4i**) and in several extramedullary tissues (**Fig. 4j-m**), albeit
380 not in the liver (data not shown). Thus, the increased time to relapse in mice co-treated with chemotherapy
381 and CD36 inhibition (**Fig. 2a-c**) likely does not result from a blast reduction in the bone marrow but from
382 a decreased blast dissemination into extramedullary organs.

383

384 **Senescent-like state induced by cytarabine chemotherapy occurs specifically in CD36-high AML**

385 **blasts**

386 We observed that AraC treatment increased both cell size and granularity, two common features of
387 senescent cells, and noteworthy, this increase almost exclusively occurred within the CD36-high viable
388 cell population (**Fig. 5a-b**). In addition, senescence in AraC-treated cells translated into an increased β -
389 galactosidase activity, which also occurred selectively in CD36-high cells, as measured by a 2.5-fold
390 increase in C12FDG fluorescence (**Fig. 5c-d**). To confirm these data, we treated U937 cells for 4 days

391 with PBS or AraC, sorted viable CD36-high and -low cells and analyzed the expressions of several cyclin-
392 dependent kinase inhibitors (*P21*, *P16*, *P15*), as well as that of cytokines of the SASP (*CCL2*, *IL1b*,
393 *TNFa*). As previously (**Fig. 4d**), the very small number of CD36-low cells surviving after AraC treatment
394 hindered these analyses. Expressions of all these well-established senescence markers were markedly
395 increased by AraC treatment in CD36-high cells (**Fig. 5e**). Similarly, size, granularity, β -galactosidase
396 activity and expressions of senescent markers, were all increased in chemoresistant OCIAML3 cells after
397 4 days of treatment with AraC (**Fig. S3b-d**), specifically in CD36-high cells (**Fig. S3b-c**). Interestingly,
398 we found that *TSP1* mRNA, which was found to be part of the SASP (41), was strongly increased by
399 AraC (**Fig. 5e, Fig.S3d**), especially within the CD36-high population (**Fig. 5e**). Of note, blocking CD36
400 with FA6-152 antibody did not alter AraC-induced increase in β -galactosidase activity of U937 or
401 OCIAML3 cells (**Fig. 5f, Fig. S3e**). However, a significant decrease in SASP-related genes *TNF α* and
402 *TSP1* mRNA level was observed in U937 cells, although no significant effect was observed regarding the
403 expressions of other senescent markers (**Fig. 5g-m**).

404 To decipher if senescence-like state and migration are functionally related, we compared the senescent
405 profile of AraC-treated migrating and non-migrating cells. After 4 days of AraC or vehicle treatment,
406 viable U937 cells were FACS-sorted and submitted to migration assay. In AraC-treated condition, β -
407 galactosidase activity was increased in cells that had migrated compared to non-migrating cells (**Fig. S3f-**
408 **g**), while no difference was found regarding expressions of CDKi and SASP related genes (data not
409 shown). These data suggest that chemotherapy resistant cells that migrate preserve their senescent-like
410 profile, and can even increase senescence-associated β -galactosidase activity. Together, these results show
411 that chemotherapy increases the expressions of CD36 and its ligand TSP1 in blasts which retain their
412 migration ability, and triggers a senescent-like phenotype specifically in CD36-high blasts, suggesting that
413 senescent-like state and migration properties co-exist within CD36-high blasts bypassing chemotherapy.

414
415 **Single-cell transcriptomic analysis reveals enrichment of CD36-high, migration and senescence-like**
416 **gene signatures in a cluster emerging after standard chemotherapy**

417 We next asked whether primary AML patients cells persisting after chemotherapy also exhibit a
418 senescent-like state associated with migration *in vivo*. To this aim, we analyzed the transcriptomes of more
419 than 30,000 single viable AML cells from a PDX model (TUH07 (42)) (**Fig. 6a**). The Seurat-based
420 unsupervised hierarchical clustering analysis uncovered 8 distinct clusters before (PBS) and after AraC
421 treatment (**Fig. 6b**). While the cell count within each cluster varied before and after AraC treatment,
422 cluster 8 exhibited the highest increase in cell count after AraC treatment (**Table S3**). A detailed analysis
423 of cluster 8 revealed heterogeneity within this cluster, which consisted of two sub-clusters exhibiting
424 either high or low expression of the CD36 gene signature (**Fig. 6c**). Strikingly, the sub-cluster highly
425 expressing the CD36 gene signature was significantly enriched in gene sets related to both senescence and
426 migration processes, with a large proportion of cells exhibiting a strong overlay between the two
427 signatures (**Fig. 6c**). Correlation plots emphasized the global co-linearity among CD36, migration, and
428 senescence across all cells, while the cluster 8 displayed the highest expression across all of the compared
429 phenotypes (**Fig. S3h-j**). These findings further strengthened that migration and senescence-like states
430 coexist within high CD36-expressing blasts enriched after chemotherapy *in vivo*.

431

432 **CD36 is positively associated with extramedullary disease in AML patients**

433 We assessed the clinical relevance of these findings in patients from the TUH cohort. Interestingly,
434 patients with hyperleukocytosis had a higher percentage of CD36+ blasts (**Fig. 7a**). The presence of EMD
435 at diagnosis was documented in 631 patients and among them, 166 (26.3%) exhibited EMD with 1, 2, or
436 more than 2 organs involved, in agreement with other reports (19,20). In the TUH cohort, both EMD and a
437 high CD36-positive blast percentage were independent prognostic factors of relapse and reduced OS
438 (**Table S4**). We found that the presence of an EMD was significantly correlated with a higher percentage
439 of CD36+ blasts at diagnosis (**Fig. 7b**), regardless of the number of organs involved (**Fig. 7c**). Analysis of
440 CD36 expression in the bone marrow of patients from the TUH cohort showed a higher percentage of
441 CD36 positive blasts in patients exhibiting minimal residual disease (MRD) after chemotherapy compared
442 to patients without MRD (**Fig.7d**). Using a CD36-gating strategy in diagnosis/MRD paired patient

443 samples, we found a significant increase in cell size at MRD compared to diagnosis, a feature of senescent
444 cells, specifically in CD36-high patients (**Fig. 7e**). Very interestingly, among patients classified as EMD-
445 negative, a high CD36 expression identified a group at higher risk for both CIR and reduced OS (**Fig. 7f-**
446 **g**). Together, these clinical data highlight that CD36 expression in blasts is positively associated with
447 EMD in patients.

448

449 **DISCUSSION**

450 Despite the availability in the recent years of new treatments targeting key survival and metabolic
451 vulnerabilities in leukemic blasts, AML remains an aggressive cancer with a poor prognosis, due to
452 frequent relapses after chemotherapy. Here, our study highlights CD36 as a prognostic marker and
453 therapeutic target of metastasis to thwart relapse in AML.

454 In patient- and -cell line-derived xenografts in NSG mice, we have previously found and here confirmed
455 that CD36 is overexpressed in chemoresistant blasts (3). While CD36 has been shown to support fatty acid
456 oxidation in chronic myeloid leukemia (8), our data suggest that, unlike FABP4 (43), CD36 is dispensable
457 for lipid metabolism in AML blasts. Although inhibiting lipid transport function of CD36 might require to
458 knock-out instead of knock-down its expression, as recently shown in AML (44), the absence of
459 significant enrichment in GO terms related to lipid metabolism in the CD36 gene signature constitutes an
460 additional evidence of the uncoupling between CD36 and lipid metabolism in leukemic blasts.

461 Strikingly, our work demonstrated that CD36 fosters AML blast migration. In xenografted mice, a wide
462 range of AML cell lines developed EMD in a variety of organs. This phenotype was also observed in the
463 immuno-competent syngeneic AML model MLL/AF9 (45), showing that EMD in NSG mice does not
464 result from an impaired immune response. Interestingly, AraC treatment, which increases CD36
465 expression, also triggers the differentiation of leukemic cells towards a monocytic phenotype. Monocytic
466 cells migrate out of the marrow to perform their functions in tissues. While chemotherapies were used in
467 lymphomas to enhance CD34+ hematopoietic stem cells mobilization in order to collect them before
468 autologous transplantation (46), their impact on leukemic blasts mobilization has not been investigated.

469 Importantly, while having no effect in untreated cells in contrast to findings obtained with MOLM13 cells
470 (47), we demonstrated that CD36 inhibition delayed time to relapse after chemotherapy, as observed in a
471 AML mouse model (44), this being associated with a decreased EMD.

472 Using a set of senescence markers, we demonstrated that chemotherapy-induced senescence occurs
473 mostly, if not only, in CD36-high cells. While CD36 inhibition did not impair β -galactosidase activity, it
474 decreased the expression of some senescence-related secreted factors including TNF α and TSP1. These
475 results are in accordance with a recently published paper (48) showing that CD36 inhibition in muscle
476 senescent cells, although not impacting β -galactosidase activity nor the number of senescent cells,
477 decreased the expression of some SASP-related genes. This confers to CD36 a role in the secretory
478 phenotype of senescent cells, as previously described (49). Together, these data suggest that CD36, rather
479 than contributing to leukemic blasts entry into senescence, could play a role in the secretory phenotype of
480 senescent blasts treated with chemotherapy AraC. Importantly, we demonstrated that AraC-surviving
481 blasts were still able to migrate. The preservation of migratory abilities of CD36-high cells bypassing
482 chemotherapy and exhibiting a senescence-like state was further strengthened by single-cell analysis of a
483 PDX treated with AraC (42). A cell cluster emerging after chemotherapy was not only overexpressing the
484 CD36 gene signature but also enriched in both senescence and migration gene sets. Among the
485 components of the SASP that could support leukemic blasts migration, TSP1 was strongly increased by
486 chemotherapeutic treatment, especially in the CD36-high cell population. The sustained induction of both
487 CD36 and TSP1 by AraC treatment likely contributes to maintain the capacity of CD36-high cells to
488 migrate, even after a chemotherapeutic stress.

489 The clinical relevance of these results was analyzed in a large cohort of 1,273 AML patients from the
490 TUH. CD36 was found by multivariate analysis to be an independent marker of AML progression, with a
491 higher hazard ratio than major parameters that are characteristic of aggressive AML (FLT3-ITD, adverse
492 cytogenetics, etc.). A previous study performed on a smaller cohort (266 patients) also reported the poor
493 outcome of AML patients with a high CD36 expression (50). In the TUH cohort, the pejorative role of
494 CD36 is further emphasized by the fact that patients with MRD after induction chemotherapy had a higher

495 percentage of CD36-positive blasts at diagnosis than patients without MRD. Interestingly, both
496 leukocytosis and EMD were significantly associated with a high percentage of CD36-positive blasts at
497 diagnosis and EMD was found to convey a poor prognosis, as recently shown (22). Importantly, among
498 patients classified as EMD-negative, CD36 expression, whose evaluation is easily performed in routine
499 practice, discriminated a group of patients with a higher CIR and a reduced OS. The impact of CD36 on
500 patients classification is reinforced by the fact that its expression is not clearly associated with mutational
501 profiles commonly utilized in clinical practice for AML.

502 The present study has some limitations. Inhibition of CD36 by shRNA or blocking antibodies did not
503 impact lipid uptake in leukemic blasts. These methods however do not result in complete loss of CD36
504 activity and it is thus possible that the amount of protein remaining active is sufficient to allow normal
505 lipid metabolism in blasts. CD36 knockout by genetic approaches, although not clinically easy to
506 duplicate, could reveal a role of CD36 in lipid metabolism in leukemic blasts. We showed that CD36
507 fosters leukemic blasts migration, at least in part through its binding with TSP1 which was strongly
508 released by blasts surviving chemotherapy. However, one cannot rule out the possibility that TSP1 may
509 also be secreted by the microenvironment of different organs in response to chemotherapy which could
510 impact blasts metastasis.

511
512 In conclusion, whereas most AML studies are focused on leukemic cells present in the bone marrow, our
513 study unravels the importance of blast dissemination and EMD triggered by AraC. It highlights the non-
514 canonical role of CD36 in this process, opening a new therapeutic strategy through CD36 inhibition in
515 combination to chemotherapy to impair this metastatic process.

516
517

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546 **AUTHOR CONTRIBUTIONS**

547 Conceptualization: TF, JN, IA, JES, CR, FV, FC, AC

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555

556 **COMPETING INTERESTS STATEMENT**

557 The authors declare no conflict of interest.

558 **SUPPLEMENTARY MATERIALS**

559 MATERIAL AND METHODS

560 FIG. S1. Characterization of CD36 role in lipid metabolism in blasts

561 FIG. S2. CD36 triggers OCIAML3 migration and analysis of blasts dissemination in vivo

562 FIG. S3. Effect of AraC on AML blasts and single cell correlation plots from the PDX TUH07

563 TABLE S1 Human antibodies for flow cytometry (TUH cohort)

564 TABLE S2 CD36 gene signature

565 TABLE S3 Single-cell analysis (TUH07)

566 TABLE S4 Multivariate analysis of the TUH cohort

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711

712 **TABLE 1 Multivariate analysis of the TUH cohort**

713

		Haz. Ratio/ Robust SHR (CIR)	[95% Conf. Interval]	Std. Err.	z	P> z	Statistics
Event Free Survival (EFS)	CD36	1.55	[1.174 - 2.051]	0.22	3.09	0.002	**
	Age > 60 years	1.64	[1.222 - 2.192]	0.24	3.3	0.001	**
	WBC > 50G/L	1.85	[1.314 - 2.591]	0.32	3.54	0.000	***
	Allo-SCT	0.52	[0.375 - 0.709]	0.08	-4.09	0.000	***
	Favorable Cytogenetics	0.61	[0.361 - 1.03]	0.16	-1.85	0.064	ns
	Adverse Cytogenetics	1.34	[0.955 - 1.892]	0.23	1.7	0.090	ns
	FLT3-ITD mutation	1.31	[0.938 - 1.832]	0.22	1.59	0.113	ns
	NPM1 mutation	0.55	[0.394 - 0.775]	0.10	-3.43	0.001	**
	AML secondary status	1.53	[1.134 - 2.06]	0.23	2.78	0.005	**
Overall Survival (OS)	CD36	1.69	[1.176 - 2.418]	0.31	2.84	0.005	**
	Age > 60 years	1.39	[0.947 - 2.036]	0.27	1.68	0.093	ns
	WBC > 50G/L	1.84	[1.192 - 2.833]	0.41	2.76	0.006	**
	Allo-SCT	0.30	[0.187 - 0.47]	0.07	-5.17	0.000	***
	Favorable Cytogenetics	0.45	[0.203 - 1.003]	0.18	-1.95	0.051	ns
	Adverse Cytogenetics	1.41	[0.903 - 2.204]	0.32	1.51	0.131	ns
	FLT3-ITD mutation	1.46	[0.964 - 2.216]	0.31	1.79	0.074	ns
	NPM1 mutation	0.61	[0.394 - 0.941]	0.14	-2.23	0.026	*
	AML secondary status	1.85	[1.272 - 2.697]	0.36	3.22	0.001	***
Cumulative Incidence of Relapse (CIR)	CD36	1.53	[1.07 - 2.193]	0.28	2.33	0.020	*
	Age > 60 years	1.54	[1.088 - 2.192]	0.28	2.43	0.015	*
	WBC > 50G/L	1.57	[1.008 - 2.458]	0.36	1.99	0.046	*
	Allo-SCT	0.77	[0.519 - 1.128]	0.15	-1.35	0.177	ns
	Favorable Cytogenetics	0.76	[0.451 - 1.29]	0.20	-1.01	0.312	ns
	Adverse Cytogenetics	0.86	[0.524 - 1.424]	0.22	-0.57	0.566	ns
	FLT3-ITD mutation	1.19	[0.763 - 1.869]	0.27	0.78	0.437	ns
	NPM1 mutation	0.67	[0.437 - 1.014]	0.14	-1.9	0.058	ns
	AML secondary status	1.37	[0.921 - 2.05]	0.28	1.56	0.120	ns

714

715 Allo-SCT: Allogeneic stem-cell transplantation

716 WBC: White blood cells

717

718

719

720 **FIGURE LEGENDS**

721 **Fig.1. CD36 expression in blasts at diagnosis is associated with human AML progression and**
722 **relapse. a.** AML patients from the TUH cohort (1273 patients) were classified as low and high CD36
723 expressers (CD36-expressing blasts <20%, Low, n=866 and CD36-expressing blasts ≥20%, High, n=407).
724 **b.** Kaplan-Meier curve for even-free survival according to CD36 expression (CD36 Low n=298 and CD36
725 High n=136). **c.** Kaplan-Meier curve for overall survival according to CD36 expression (CD36 Low
726 n=298 and CD36 High, n=136). **d.** Cumulative incidence of relapse according to CD36 expression (CD36
727 Low n=254, CD36 High, n=105). **b, c, d.** Log-Rank test. **e.** Box plot of CD36 expression on blasts
728 according to cytogenetic risk (n=1191). Box plot shows the 10th percentile, first quartile, median, third
729 quartile, and 90th percentile. Mann Whitney test was performed. **f.** Landscape of somatic mutations
730 detected in diagnostic samples (n=224) by sequencing with a panel of 52 genes. The number of mutations
731 for each patient is shown at the top, while the frequencies of each mutation are located at the right. **g.**
732 Forrest plot showing mutation enrichment at AML diagnosis based on blast CD36 level by logarithmic
733 odds ratio. Fisher's exact test was performed (p = 0.029 for FLT3). **h.** Forrest plot showing enrichment of
734 recurrent cytogenetic anomalies at AML diagnosis according to CD36 expression by logarithmic odds
735 ratio. Fisher's exact test was performed (p < 0.0001 for t(15;17) ; p = 0.0008 for t(9;22) ; p = 0.0002 for
736 t(8;21) ; p = 0.044 for t(11q23;x)). The circles (in the center of the error bars) represent the odds ratios.
737 The error bars represent 95% confidence interval of odds ratio.

738

739

740 **Fig.2. CD36 inhibition delays AML relapse after chemotherapy.** **a.** Percentage of mice engrafted with
741 U937 cells transduced with shCtrl (n=11) or shCD36 (n=11) and treated with vehicle or AraC, surviving
742 over time after treatment. **b.** Same as **a.** with OCIAML3 (shCtrl (n=18), shCD36 (n=22)). **c.** Percentage of
743 mice engrafted with U937 cells surviving over time after treatment with the indicated combinations of
744 PBS or AraC administered for the first 5 days, with either control IgG or FA6-152 anti-CD36 antibody
745 injected 3 times/week (n=7 PBS-IGG, n=7 PBS-anti-CD36, n=7 AraC-IGG, n=6 AraC-anti-CD36). **d.**
746 Venn diagram of significantly co-overexpressed genes along with CD36 in TCGA, Verhaak and
747 BEATAML cohorts. **e.** GSEA of CD36 gene signature in AraC versus untreated AML blasts from 3
748 different transcriptomic analyses. a-c, log-rank (Mantel-Cox) test. e, one sample t test. *P<0.05, **P<0.01.

749

750 **Fig.3. CD36 triggers blast migration. a.** GO analysis of migration-related biological processes
751 significantly enriched in the CD36 gene signature. **b.** Number of migrating shCtrl or shCD36 U937 cells
752 in the lower chamber of the transwell assay (normalized to control) (n=5). **c.** As in **b.** but with CD36-
753 blocking antibodies (FA6-152 or JC53.1) (n=4). **d.** Number of migrating cells in primary AML samples
754 treated or not with CD36 blocking antibody (FA6-152) (n=7). **e.** Number of migrating shCtrl or shTSP1
755 U937 cells (normalized to control) (n=3). **f.** Number of migrating shCtrl or shCD36 U937 cells treated or
756 not with TSP1-blocking antibody (A6.1) (normalized to shCtrl) (n=5). **g.** Number of migrating U937 cells
757 treated or not with recombinant TSP1, in the presence or not of CD36-blocking antibody (FA6-152)
758 (normalized to control) (n=3). **h.** As in **d.** but with TSP1-blocking antibody (A6.1) (n=5). **i.**
759 Bioluminescence imaging of mice injected with the indicated AML cell line stably expressing a luciferase
760 reporter from day 1 to day 64 after injection. **j.** Representative histological section showing anti-human
761 Ku-80 labeling of U937 AML blasts in the subcutaneous adipose tissue 17 days post-xenograft. Positive
762 brown nuclei highlight the infiltration of human blasts in murine adipose tissue either as dense infiltrate
763 (left) or scattered isolated cells (right). Scale bar left image: 50 μ M, right image: 10 μ M. **k.** Percentage of
764 viable OCIAML3 cells in the indicated tissues 14 days after orthotopic injection in the femur. **l.**
765 Expression of CD36 (MFI) in viable OCIAML3 cells measured in the injected and contralateral femur and
766 different organs of mice 14 days after orthotopic injection. Values are represented as mean \pm SEM. **b, c, e,**
767 one sample t test. **d, h,** paired t test. **f, g** ordinary one-way ANOVA with Tukey's multiple comparisons
768 test. **l,** matched one-way ANOVA. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

769

770 **Fig.4. Residual blasts maintained their migratory properties after chemotherapy.** **a.** CD11b, CD14,
771 CD15 and CD44 cell surface expression in viable control or AraC-treated (2 μ M) U937 cells for 96h
772 (n=4). **b.** GSEA of “HAY_BONE_MARROW_MONOCYTE” gene signature in AraC versus untreated
773 AML samples from 3 different transcriptomic analyses. **c.** Number of migrating cells in the presence or
774 absence of CD36-blocking antibody (FA6-152) sorted from control or 2 μ M AraC treated cells for 4 days
775 (normalized to control) (n=3). **d.** Percentage of viable CD36-low and CD36-high cells in PBS or 2 μ M
776 AraC treated U937 cells for 4 days (n=4). **e.** Representative histograms of CD36 expression in non-
777 migrating vs migrating CD36-low and CD36-high subpopulations sorted from control or AraC-treated
778 U937 cells (2 μ M) for 4 days. Plain histogram, non-migrating cells; striped histogram, migrating cells. **f.**
779 Quantification of CD36 expression in non-migrating vs migrating cells in the same conditions as in **e.** (n =
780 3). **g.** Number of migrating cells from CD36-low and CD36-high subpopulations sorted from control or
781 2 μ M AraC treated U937 cells for 4 days (normalized to Ctrl CD36-low cells) (n=3). **h, i.** Quantification in
782 the bone marrow (**h**) and spleen (**i**) of viable U937 cells transduced with shCtrl (n=9) or shCD36 (n=9) in
783 mice relapsing after AraC treatment. **j-m.** Quantification of U937 cells by RT-qPCR (hCD45 mRNA
784 normalized to m36B4) in SCAT (**j**), PGAT (**k**), lung (**l**) and kidneys (**m**) in mice relapsing after AraC
785 treatment. Data expressed as $2^{-\Delta\text{CT}}$ (values multiplied by 1000) (n=4-5 shCtrl, n=5-7 shCD36). Values
786 are represented as mean \pm SEM. **a,** Mann-Whitney or unpaired t test depending on sample distribution. **b,**
787 unpaired t test. **c, g,** ordinary one-way ANOVA with Tukey’s multiple comparisons test. **f,** Ordinary two-
788 way ANOVA with multiple comparisons correction. **h-m,** unpaired t test with or without Welch’s
789 correction depending on sample variance. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

790

791 **Fig.5. Senescent-like state induced by cytarabine chemotherapy occurs specifically in CD36-high**
792 **AML blasts. a, b.** FSC/SCC analysis on gated CD36-low and CD36-high cells from U937 treated or not
793 with 2 μ M AraC for 4 days (n=4). **c.** Representative histogram of C12FDG staining on gated CD36-low
794 and CD36-high cells from U937 treated or not with 2 μ M AraC for 4 days. **d.** Quantification of C12FDG
795 staining performed in **c.** (normalized to control CD36-low cells) (n=4). **e.** Expression of senescence-
796 associated genes measured by RT-qPCR in CD36-low and CD36-high subpopulations sorted from control
797 or 2 μ M AraC treated U937 cells for 4 days (normalized to Ctrl CD36-low cells). mRNA of the gene of
798 interest normalized to the housekeeping gene, data expressed as $2^{\Delta\Delta Ct}$ (n=4). **f.** C12FDG staining on
799 U937 cells treated or not with 2 μ M AraC and CD36 blocking antibody (FA6-152) for 4 days. **g-m.**
800 Expression of senescence-associated genes measured by RT-qPCR in 2 μ M AraC treated U937 cells with
801 or without CD36 blocking antibody (FA6-152) for 4 days (normalized to AraC-treated cells). mRNA of
802 the gene of interest normalized to the housekeeping gene, data expressed as $2^{\Delta\Delta Ct}$ (n=5). Values are
803 expressed as mean \pm SEM. **b,** unpaired t test. **d, e, f,** ordinary one-way ANOVA with Tukey's multiple
804 comparisons test. **g-m,** one sample Wilcoxon or t test depending on sample distribution. *P<0.05,
805 **P<0.01, ***P<0.001.

806

807 **Fig.6. Single-cell transcriptomic analysis reveals enrichment of CD36-high, migration and**
808 **senescence-like gene signatures in a cluster emerging after standard chemotherapy. a.** Schematic
809 representation of the single-cell RNA-seq experiment performed on the TUH07 PDX previously published
810 in Bosc et al. 2021 (GEO accession GSE178910). **b.** UMAP plot of 31,604 single cells from PDX TUH07
811 using Seurat. Colors indicate k-means clusters (k=9). **c.** Cluster 8 was isolated on the Seurat object and
812 bifurcated into two groups “CD36-High” and “CD36-Low” according to the enrichment level for the
813 CD36 gene signature. Expression level of gene sets related to senescence
814 (FRIDMAN_SENESCENCE_UP) and migration (WU_CELL_MIGRATION) are shown.
815

816 **Fig.7. CD36 is positively associated with extramedullary disease in AML patients.** **a.** Percentage of
817 CD36+ blasts in the BM of patients with (LS+) or without (LS-) leukocytosis at diagnosis (n=596 LS-,
818 n=46 LS+). **b.** Percentage of CD36+ blasts in the BM of patients presenting (EMD+, n=166) or not
819 (EMD-, n=465) clinical signs of EMD at diagnosis. **c.** Percentage of CD36+ blasts in the BM of patients
820 depending on the number of organs involved in EMD (n=465 for 0 organs, n=102 for 1 organ, n=45 for 2
821 organs, n=19 for more than 2 organs). **d.** Percentage of CD36+ blasts in the BM of patients at diagnosis,
822 exhibiting MRD or not after chemotherapy (n=89 MRD-, n=67 MRD+). **e.** FSC/SCC analysis on blasts
823 from CD36 low (n=12) and CD36 high (n=13) patients followed up from diagnosis to MRD. **f.**
824 Cumulative incidence of relapse in TUH patients according to CD36 expression and presence of EMD at
825 diagnosis (n=267). **g.** Overall survival in TUH patients according to CD36 expression and presence of
826 EMD at diagnosis (n=319). Values are represented as mean \pm SEM. a, b, d, Mann-Whitney test. C,
827 ordinary one-way ANOVA with Tukey's multiple comparisons test. e, Wilcoxon matched-pairs signed
828 rank test. f, g, Log-Rank test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

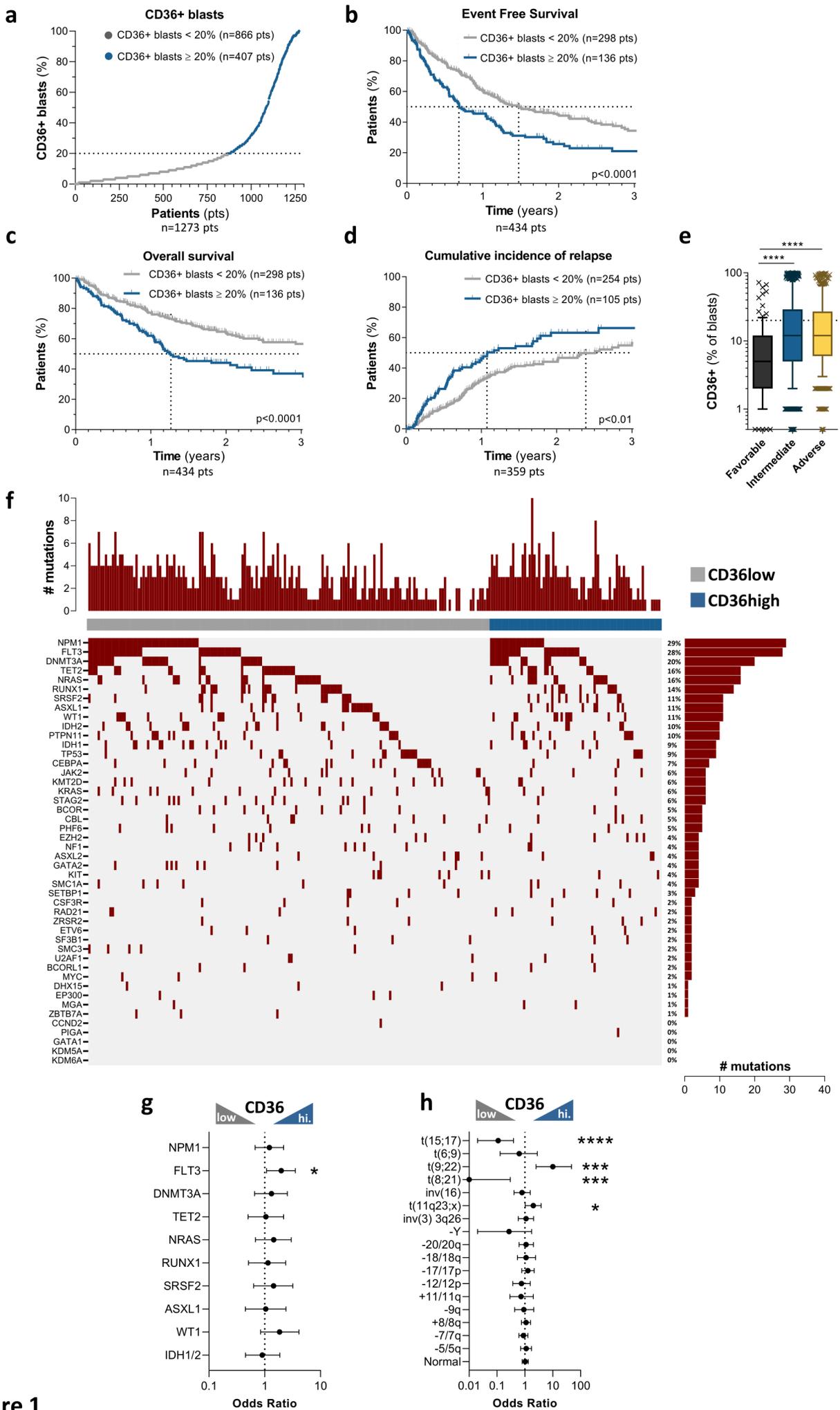


Figure 1

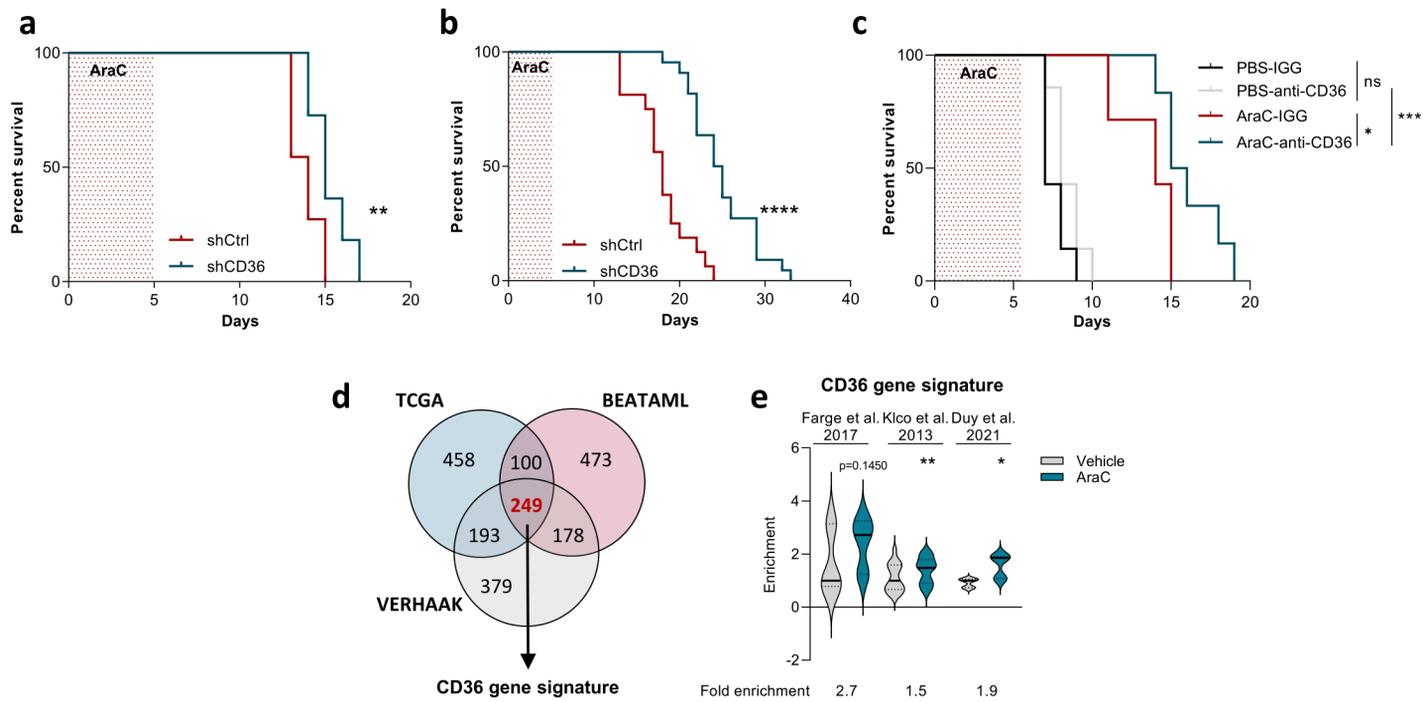


Figure 2

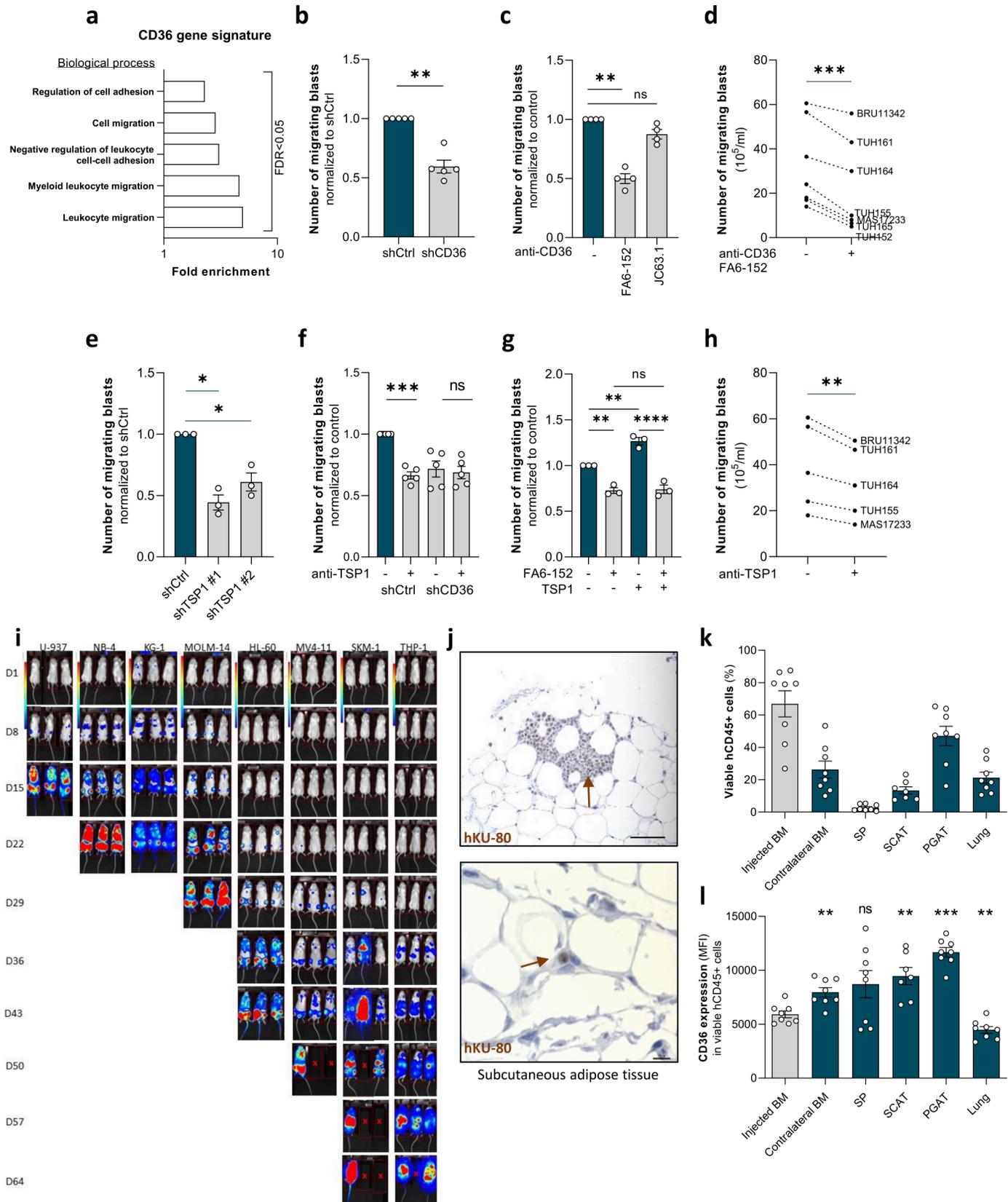


Figure 3

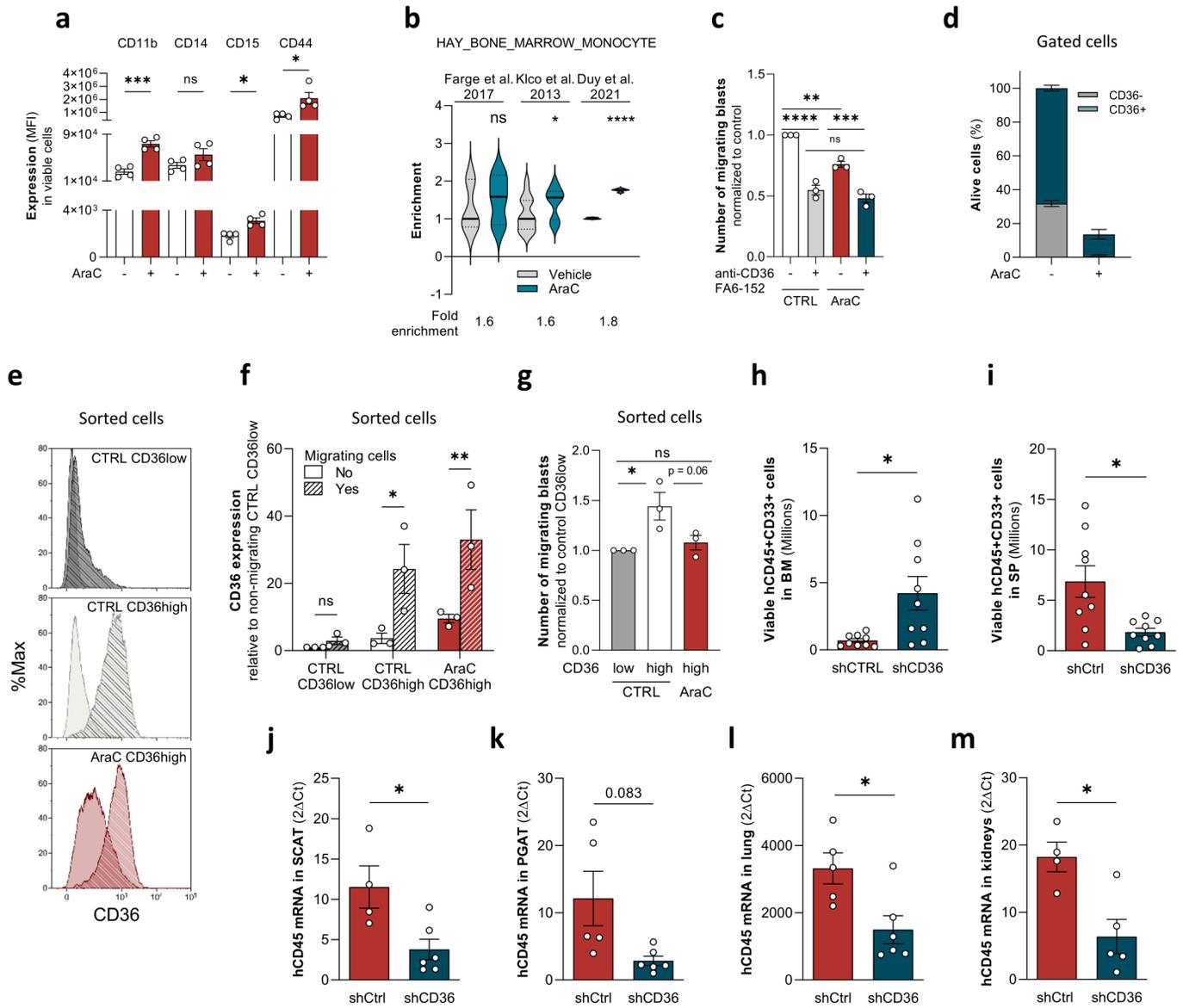


Figure 4

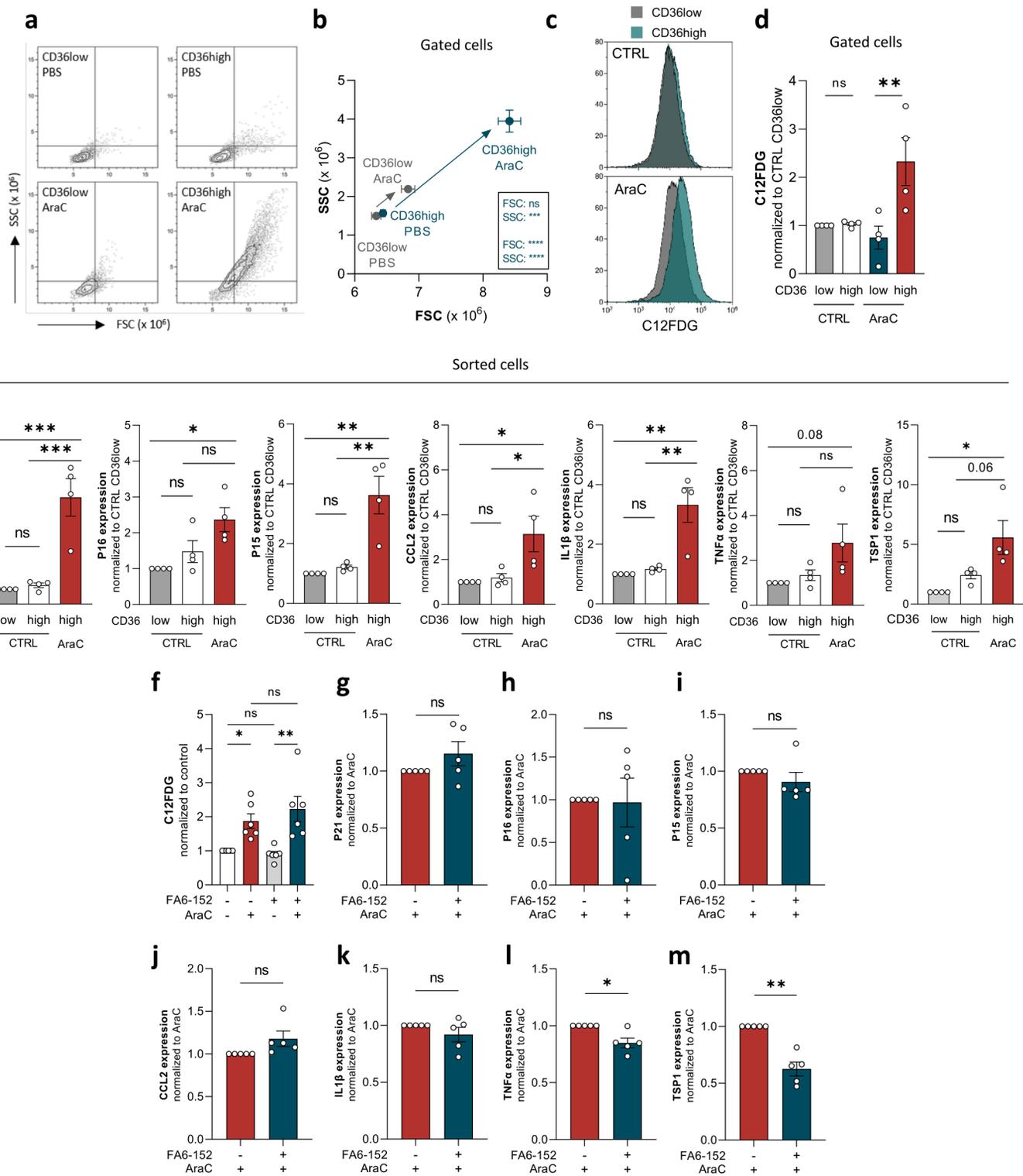
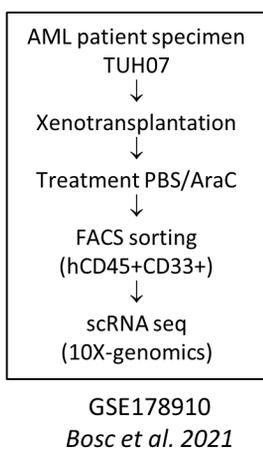
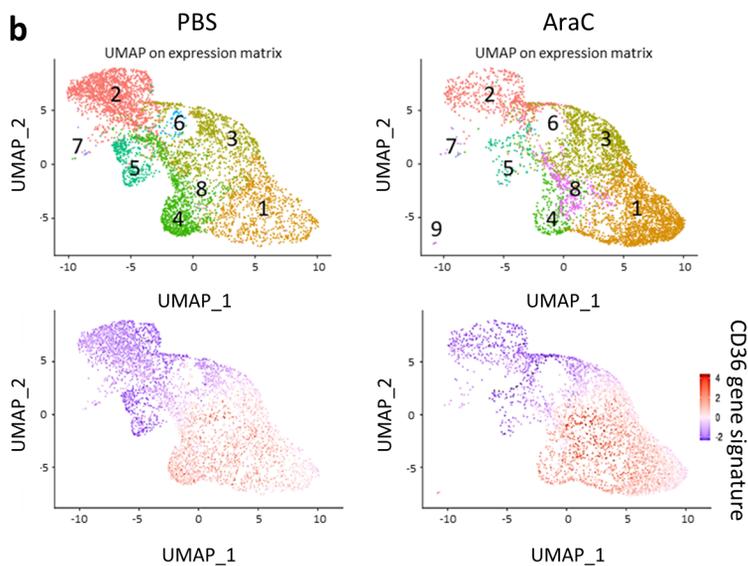
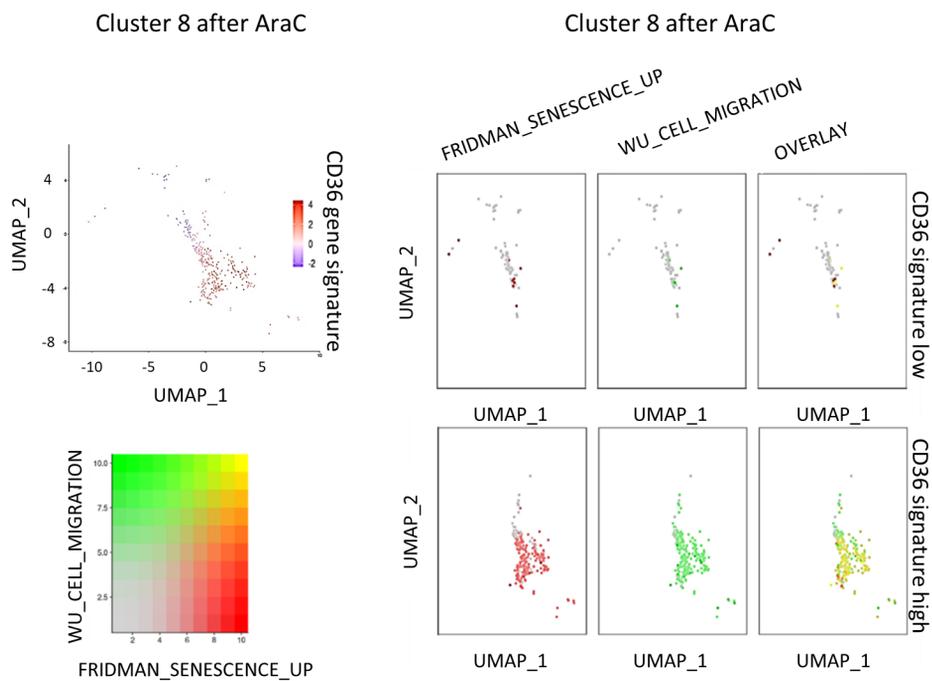


Figure 5

a**b****c****Figure 6**

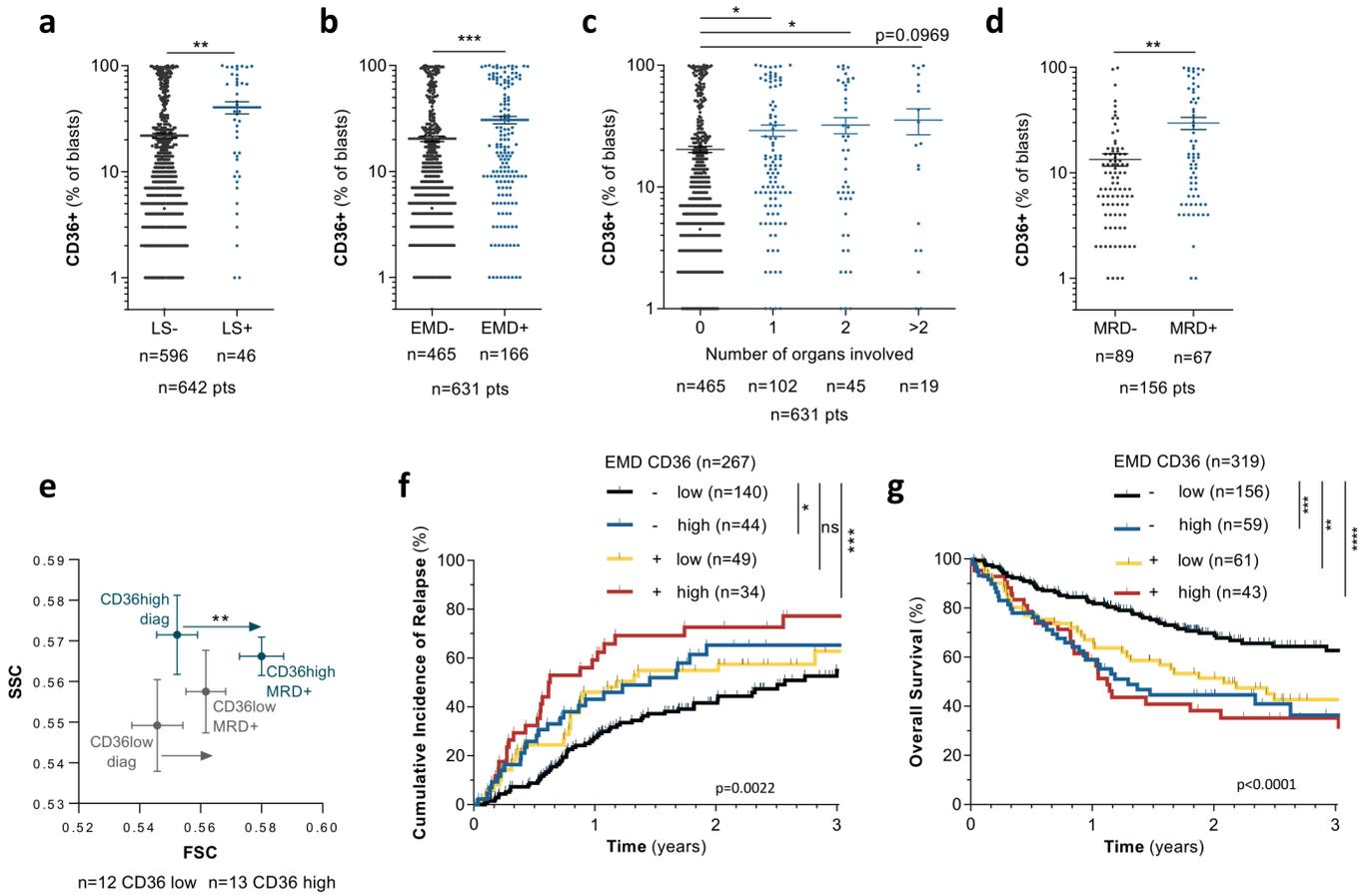


Figure 7

SUPPLEMENTAL MATERIALS

CD36 drives metastasis and relapse in acute myeloid leukemia

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Short running title: CD36-dependent metastasis in acute myeloid leukemia

Conflict of interest: The authors declare no conflict of interest.

Keywords: acute myeloid leukemia, metastasis, CD36

MATERIAL AND METHODS

Cell lines, primary cultures, and culture conditions

Primary AML cells, peripheral blood or bone marrow patients' samples were frozen in FCS with 10% DMSO and stored in liquid nitrogen. The percentage of blasts was determined by flow cytometry and morphologic characteristics before purification. Cells were thawed at 37 °C, washed in thawing media composed of IMDM, 20% FBS. Cells were maintained in IMDM, 20% FBS, and 1% Pen/Strep (GIBCO) for all experiments.

Human AML cell lines were maintained in MEMa-media (Gibco) supplemented with 10% FBS (Invitrogen) in the presence of 100 U/ml of penicillin and 100 µg/ml of streptomycin, and were incubated at 37 °C with 5% CO₂. Cells were split every 2–3 days and maintained in an exponential growth phase. All AML cell lines were purchased at DSMZ or ATCC. These cell lines have been routinely tested for Mycoplasma contamination in the laboratory. The U937 cells were obtained from the DSMZ in February 2012 and from the ATCC in January 2014.

Bioluminescent xenograft mouse models

Briefly, 6 to 10-week-old NOD.Cg-Prkdc scid/J (NSG) mice were obtained from Charles River France or bred in-house and maintained on a 12-h light and 12-h dark cycle, under specific pathogen-free conditions with sterilized food and water provided ad libitum. Animals were injected via tail vein at day 0 with 0.2x10⁶ viable luciferase-expressing AML cell lines in 0.1mL sterile PBS. A bioluminescence measurement was performed at day 1, then once a week, following injection of endotoxin-free luciferin (Promega #E6552, 30 mg/kg) using an Optima PhotonIMAGER imaging system and analyzed using M3vision software (Biospace Lab, Nesles-la-Vallée, France). Daily monitoring of mice for symptoms of disease (weight loss >20%, ruffled coat, hunched back, weakness, reduced motility) determined the time of killing for injected animals with signs of distress. After the final time point, for ex vivo organ imaging, 10 min after receiving luciferin, mice were killed and organs were collected and washed with PBS. Images were then immediately acquired in indicated organs. Results were expressed as percentage of total signal (100*(organ signal/total bioluminescence)).

Assessment of leukemic engraftment

At the end of the experiment, NSG mice were humanely killed in accordance with European ethics protocol. Bone marrow (mixed from tibias and femurs), spleen and liver were dissected and flushed in HBSS with 1% FBS. Mononuclear cells from bone marrow and spleen were labeled with anti-hCD33, anti-mCD45.1, anti-hCD45, anti-hCD3, and/or anti-hCD44 (all from BD) antibodies to determine the fraction of viable human blasts (hCD3⁻hCD45⁺mCD45.1⁻hCD33⁺/hCD44⁺AnnV⁻ cells) by flow cytometry. Adipose tissues and lungs were dissociated with collagenase NB4 (Standart Grade from Coger) 0.4 U/mL for 30 minutes. Acquisitions were performed on a CytoFLEX flow cytometer with CytoExpert software v2.0 (Beckman Coulter), and analyses done with Flowjo v10.4.2. The number of AML cells/ μ L of peripheral blood and number of AML cells in total leukemia burden (in bone marrow, spleen, subcutaneous and perigonadic adipose tissues, liver and lungs) were determined using CountBright beads (Invitrogen) as described in the provided protocol.

Single cell analysis

Next generation sequencing of single cell gene expression (NextSeq 550) was performed on each of the two experimental groups (vehicle or AraC) of the PDX patient TUH07 (GEO accession GSE178910) published in (1), to produce raw FASTQ reads. Gene expression data had been rendered from FASTQ reads using CellRanger software by 10x Genomics. The quality control, principle component analysis (PCA), and t-SNE/UMAP dimensionality reduction procedures to integrate and visualize the scRNAseq expression data of the patients was performed using R package Seurat 3.0 by Satija Lab. The corresponding input parameters and functional R scripts to execute the aforementioned procedures are publicly available on the Zenodo repository (accession 5137701). Cells were classified into their corresponding clusters on a UMAP projection based upon their phenotypic characteristic at diagnosis and response to therapies. Clusters significantly increasing in cell count after Arac treatment in proportion to the entire cell population were considered “enriched”, while clusters that significantly decreased in cell count were considered “sensitive”. A standard Z-test for difference in proportions had

been utilized to facilitate this classification and determine the statistical significance of each change in cluster size according to its calculated z-value (**Table S2**).

In order to assign enrichment values to the metadata of the single-cell samples, the Seurat function “AddModuleScore” had been implemented for the gene expression signatures: CD36, FRIDMAN_SENESCENCE_UP, and WU_MIGRATION (**Table S2**) with the “search” parameter set as “TRUE”. The enrichment values pertaining to each cell were z-standardized using the base-R “scale” function.

To visualize the enrichment regions of the aforementioned gene expression signatures, the method “FeaturePlot” was used on the PDX category subset by patient number and treatment type. To maintain visual continuity across each FeaturePlot, the maximum and minimum z-scores for the color gradient were standardized to the 0.999 and 0.001 percentile expression values respectively in order to mitigate the effect of outliers. For the correlation analyses performed among the gene expression signatures at the cellular level, the function “FeatureScatter” had been implemented to both derive linear Pearson correlation coefficients (r) and to visualize the effect of each individual cluster on the overall relationship between the variables examined. Differential CD36 signature expression was derived for each cluster using the “col_t_welch” function from the matrixTests R library, which produced the relevant test statistics for a two-sample t-test of unpaired means for different variances (**Table S2**). The same process was then performed for senescence and migratory signatures (**Table S2**).

The cluster 8 was isolated using base-R command “subset” (with parameter ‘subset = “seurat_clusters” == 8’) on the Seurat object belonging to patient TUH07-AraC. To better assess the overlapping effects of CD36 with senescence and migration within this cluster, it had been bifurcated into two groups “CD36 High” and “CD36 Low” by assigning a new “CD36” field to the cluster’s Seurat metadata. Using the base-R “ifelse” function, all cells with z-scores greater than zero in CD36 signature expression were assigned “CD36 High”, and cells having less than $z = 0$ were assigned “CD36 Low” and recorded on the “CD36” metadata field. To facilitate the rendering of the blended plot visualizing the overlap of senescence and migratory activity exclusive to the CD36 enrichment within cluster 8, the “FeaturePlot” Seurat function was utilized with its functions: “blend = TRUE”, “features = c(“FRIDMAN_SENESCENCE_UP”, “WU_CELL_MIGRATION”)", and “split.by = “CD36””.

GSEA analysis

GSEA analysis was performed using GSEA version 4.0 (Broad Institute). Gene signatures used in this study were from Broad Institute database, literature, or in-house built. Following parameters were used: Number of permutations = 1000, permutation type = gene_set. Other parameters were left at default values.

Plasmid cloning, shRNA, lentiviral production, and leukemic cell transduction

Control shRNA (MFCD07785395), CD36 shRNA (TRCN0000056998) and TSP1 shRNA plasmids (TRCN0000226405 and TRCN0000219072) on a pLKO.1 backbone were purchased from Sigma. shRNA, lentiviral production and cell transduction was performed as previously described (2).

SA- β -gal activity measurement by C12-FDG staining

C12-FDG staining was performed as previously (3). Briefly, cells were treated with 100 nM Bafilomycin A1 (Invivogen tirl-bafa1) for 2 hours then incubated with 20 μ M C12-FDG (Abcam ab273642) in the dark for 1 hour at 37 °C. Cells were then washed with PBS and stained for flow cytometry as per usual.

Number of viable cells

U937 and OCIAML3 cells (300,000/mL) were seeded for 72-96h in MEMa-media (Gibco) supplemented with 10% FBS (Invitrogen) in the presence of 100 U/ml of penicillin and 100 μ g/ml of streptomycin, and diluted in half with fresh media at 48h. Two technical replicated were seeded in a 24-well plate for each condition. Alive cells were counted every 24h on a Malassez chamber using Trypan Blue staining.

Primers

Primers sequences are : **CCL2 (human)** Forward - CATGAAAGTCTCTGCCGCC and Reverse - GGGGCATTGATTGCATCTGGC), **P21 (human)** Forward - TGCCGAAGTCAGTTCCTTGT and Reverse - GTTCTGACATGGCGCCTCC; **P16 (human)** Forward - GGGGTCCGGTAGAGGAGG and Reverse - GCCCATCATCATGACCTGGA; **P15 (human)** Forward -

GGGACTAGTGGAGAAGGTGC and Reverse - CCATCATCATGACCTGGATCG; **IL1- β (human)**
- Forward - AGCCATGGCAGAAGTACCTG and Reverse - CCTGGAAGGAGCACTTCATCT;
TNF- α (human) - Forward - GCCCATGTTGTAGCAAACCC and Reverse -
TATCTCTCAGCTCCACGCCA; **TSP1 (human)** - Forward -
CTCAGGACCCATCTATGATAAAACC and Reverse - AAGAAGGAAGCCAAGGAGAAGTG;
CD45 (human) Forward - CACTGCAGGGATGGATCTCA and Reverse -
TGCGTAGAGCTTTTACCACTTGAA; **RPLP0 (human)** Forward -
TAGTTGGACTTCCAGGTCGC and Reverse - CGTCCTCGTGGAAGTGACAT and **36b4 (mouse)**
Forward - AGTCGGAGGAATCAGATGAGGAT and Reverse - GGCTGACTTGGTTGCTTTGG.

Molecular analysis

The presence of *FLT3-ITD* was tested as described (4). Electrophoregram peaks were quantified using GeneMarker 2.2 (SoftGenetics, State College, PA). Extended DNA resequencing was performed using Illumina NextSeq500 and Haloplex HS (Agilent, Santa Clara, CA) targeted on the complete coding regions of 52 genes: *ASXL1*, *ASXL2*, *ATM*, *BCOR*, *BCORL1*, *CBL*, *CCND2*, *CEBPA*, *CSF3R*, *CUX1*, *DDX41*, *DHX15*, *DNMT3A*, *EP300*, *ETV6*, *EZH2*, *FLT3*, *GATA1*, *GATA2*, *IDH1*, *IDH2*, *JAK2*, *KDM5A*, *KDM6A*, *KIT*, *KMT2D*, *KRAS*, *MGA*, *MPL*, *MYC*, *NF1*, *NPM1*, *NRAS*, *PHF6*, *PIGA*, *PPM1D*, *PRPF8*, *PTPN11*, *RAD21*, *RUNX1*, *SETBP1*, *SF3B1*, *SMC1A*, *SMC3*, *SRSF2*, *STAG2*, *TET2*, *TP53*, *U2AF1*, *WT1*, *ZBTB7A*, and *ZRSR2*. Data were processed through two algorithms from GATK (<https://software.broadinstitute.org/gatk>), HaplotypeCaller (scaling accurate genetic variant discovery) to tens of thousands of samples (5). The mean depth was 2,190 reads. Identified variants were curated manually and named according to the rules of the Human Genome Variation Society (hgvs.org). Molecular data are stored in the European Nucleotide Archive (<https://www.ebi.ac.uk/ena/>).

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SUPPLEMENTAL FIGURES

Fig. S1. Characterization of CD36 role in lipid metabolism in blasts. a-d. Cell surface CD36 expression (**a, b**) of viable human AML blasts in the BM of vehicle- or AraC-treated AML PDX. **c.** Representative flow cytometry histograms of basal CD36 expression in U937 and OCIAML3 cells. **d.** CD36 protein expression in AML cells treated with AraC for 24h, relative to that of PBS-treated cells. AraC concentrations used were 2 μ M for U937 and 10 μ M for OCIAML3 cells (n=5). **e-f.** Viable cell number of shCtrl and shCD36 U937 (**e**) and OCIAML3 (**f**) cells proliferating over 72h (n=4). **g-h.** Viable cell number of U937 (**g**) and OCIAML3 (**h**) cells proliferating over 96h in the presence of FA6-152 antiCD36 antibody (0-10 μ g/ml) (n=3). **i.** Representative flow cytometry histograms of CD36 expression in shCtrl and shCD36 U937 cells treated with vehicle or 2 μ M AraC for 24h. **j-k.** CD36 expression assessed by flow cytometry in shCtrl or shCD36 cells treated with vehicle or AraC (2 μ M for U937 (**j**), 10 μ M for OCIAML3 (**k**)) for 24h (n=4-6). Values are normalized to control. **l-m.** Neutral lipid staining measured by flow cytometry using the Bodipy probe, in viable shCtrl or shCD36 U937 (**l**) and OCIAML3 (**m**) cells treated with vehicle or AraC (2 μ M for U937, 10 μ M for OCIAML3) for 24h (n=3-4). Values are normalized to control. **n-o.** Neutral lipid content measured by flow cytometry using the Bodipy probe, in viable U937 treated with vehicle or AraC (2 μ M) and/or CD36 blocking antibody (JC63.1, (**n**) or FA6-152 (**o**) for 24h (n=3-4). Values are normalized to control. **p.** Representative flow cytometry histograms of CD36 expression in siCtrl and siCD36 U937 cells treated with vehicle or 2 μ M AraC for 24h. **q.** CD36 expression in siCtrl and siCD36 U937 cells treated with vehicle or 2 μ M AraC for 24h (n=4). Values are normalized to control. **r, s.** 14C palmitate uptake (**r**) and oxidation (**s**) in siCtrl and siCD36 U937 cells treated with vehicle or AraC (2 μ M) for 24h (n=4). Values are normalized to the number of viable cells per condition and reported as a fold change to control. **t-u.** 14C palmitate uptake (**t**) and oxidation (**u**) in U937 cells treated with vehicle or AraC (2 μ M) and/or CD36 blocking antibody (FA6-152) for 24h (n=4). Values are normalized to the number of viable cells per condition and reported as a fold change to control. **v.** Representative flow cytometry histograms of CD36 expression in siCtrl and siCD36 OCIAML3 cells treated with vehicle or 2 μ M AraC for 24h. **w.** CD36 expression in siCtrl and siCD36 OCIAML3 cells treated with vehicle or 10 μ M AraC for 24h (n=4). Values are normalized to control. **x-y.** 14C palmitate uptake (**x**) and oxidation (**y**) in siCtrl and siCD36 OCIAML3 cells treated

with vehicle or AraC (10 μ M) for 24h (n=4). Values are normalized to the number of viable cells per condition and reported as a fold change to control. **z-aa**. ¹⁴C palmitate uptake (**z**) and oxidation (**aa**) in OCIAML3 cells treated with vehicle or AraC (10 μ M) and/or CD36 blocking antibody (FA6-152) for 24h (n=4). Values are normalized to the number of viable cells per condition and reported as a fold change to control. Values are expressed as mean \pm SEM. a, Mann-Whitney or unpaired t test depending on sample distribution, with or without Welch's correction depending on sample variance. b, Wilcoxon matched-pairs signed rank test. d, one sample t test. e-h, ordinary two-way ANOVA. j-aa, ordinary one-way ANOVA with Tukey's multiple comparisons test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Fig. S2. CD36 triggers OCIAML3 migration and analysis of blasts dissemination in vivo. **a.** GSEA gene signature of “GO_POSITIVE_REGULATION_OF_MONONUCLEAR_CELL_MIGRATION” was performed in CD36 overexpressing patients from BEATAML, Verhaak and TCGA cohorts compared to the baseline CD36 expressers. **b-c.** Number of migrating cells in shCtrl or shCD36 OCIAML3 cells (**b**) and in siCtrl or siCD36 OCIAML3 cells (**c**) in the lower chamber of the transwell assay. Data are normalized to shCtrl/siCtrl for each experiment (n=8 and n=3). **d.** Number of migrating OCIAML3 cells treated or not with CD36 blocking antibodies (FA6-152 or JC63.1 as indicated) in the lower chamber of the transwell assay. Data are normalized to control (n=3). **e.** TSP1 mRNA expression in shCtrl or shTSP1 U937 cells. Data are normalized to shCtrl (n=3). **f.** TSP1 mRNA expression in shCtrl or shTSP1 OCIAML3 cells. Data are normalized to shCtrl (n=3). **g.** Number of migrating shCtrl or shTSP1 OCIAML3 cells in the lower chamber of the transwell assay. Data are normalized to control (n=3). **h.** Number of migrating shCtrl or shCD36 OCIAML3 cells in the lower chamber of the transwell assay, in the presence or absence of TSP1 blocking antibody (A6.1). Data are normalized to shCtrl (n=5). **i.** Number of migrating OCIAML3 cells treated or not with recombinant TSP1, in the presence or not of CD36-blocking antibody (FA6-152). Data are normalized to control (n=3). **j.** Acquisition of bioluminescent signals from organs harvested from mice injected with the indicated AML cell line stably expressing a luciferase reporter, at final time point. **k.** Quantification of bioluminescent signals in each organ (percentage compared to total bioluminescent signal). **l.** Quantification of human cells in the bone marrow (BM), spleen (SP), perigonadal adipose tissue (PGAT), and liver after intra-venous injection of U937 cells in NSG mice by RT-qPCR (n=2-3). hCD45 mRNA normalized to m36B4, data expressed as $2\Delta Ct$ (values multiplied by 1000). **m.** Flow cytometry analysis of human viable CD45+ AML blasts in the bone marrow (BM), spleen (SP), subcutaneous adipose tissue (SCAT), perigonadal adipose tissue (PGAT), liver, lung and blood 24 days after intra-venous injection of OCIAML3 (n=6). Values are expressed as mean \pm SEM. **n.** OCIAML3 cells incubated with IGG or FA6-152 anti-CD36 antibody (1 μ g/ml) for 30 minutes were injected intra-venously in NSG mice. Mice were treated with either control IgG or FA6-152 anti-CD36 antibody injected 3 times/week. At day 10 after injection, tumor burden was analyzed by flow cytometry in different organs including bone marrow (BM), perigonadal adipose tissue (PGAT), liver and lung. **a.** unpaired t test with or without Welch’s correction depending on sample

variance. b-h, one sample or Wilcoxon t test depending on sample distribution. i, ordinary one-way ANOVA with Tukey's multiple comparisons test. n. Mann-Whitney tests. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Fig. S3. Effect of AraC on monocytes markers expression and senescence in OCIAML3 cells, and PDX TUH07 single-cell RNAseq correlation plots. **a.** CD11b, CD14, CD15 and CD44 cell surface expression in viable OCIAML3 cells in control conditions or in the presence of 10 μ M AraC for 96h (n=4). **b.** FSC/SCC analysis on gated CD36^{low} and CD36^{high} cells from OCIAML3 treated or not with 10 μ M AraC for 4 days (n=3). **c.** C12FDG staining (MFI) in gated CD36^{low} and CD36^{high} OCIAML3 cells treated or not with 10 μ M AraC for 4 days. Data are normalized to control CD36^{low} cells (n=3). **d.** Expression of senescence-associated genes in AraC-treated OCIAML3 cells expressed as a Log₂ fold change in comparison to untreated cells. mRNA of the gene of interest was normalized to the human housekeeping gene, data expressed as $2^{\Delta\Delta Ct}$. Data are normalized to control (n=3). **e.** C12FDG staining on OCIAML3 cells treated or not with 10 μ M AraC and CD36 blocking antibody (FA6-152) for 4 days. Data are normalized to control (n=4). **f-g.** U937 cells were treated with 2 μ M AraC for 4 days and viable cells were FACS sorted and submitted to migration assay. C12FDG staining was then performed in migrating and non-migrating cells. **f.** Representative histogram of C12FDG staining in non-migrating and migrating AraC-treated cells. **g.** Quantification of C12FDG staining performed in **f.** Data are normalized to non-migrating AraC-treated cells (n=4). **h.** Correlation between expression levels of gene sets related to migration (WU_CELL_MIGRATION) and the CD36 gene signature. **i.** Correlation between expression levels of gene sets related to senescence (FRIDMAN_SENESCENCE_UP) and the CD36 gene signature. **j.** Correlation between expression levels of gene sets related to senescence (FRIDMAN_SENESCENCE_UP) and migration (WU_CELL_MIGRATION). Values are represented as mean \pm SEM. a and b, Mann-Whitney or unpaired t test depending on sample distribution, with or without Welch's correction depending on sample variance. c and e, ordinary one-way ANOVA with Tukey's multiple comparisons test. d and g, one sample t test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

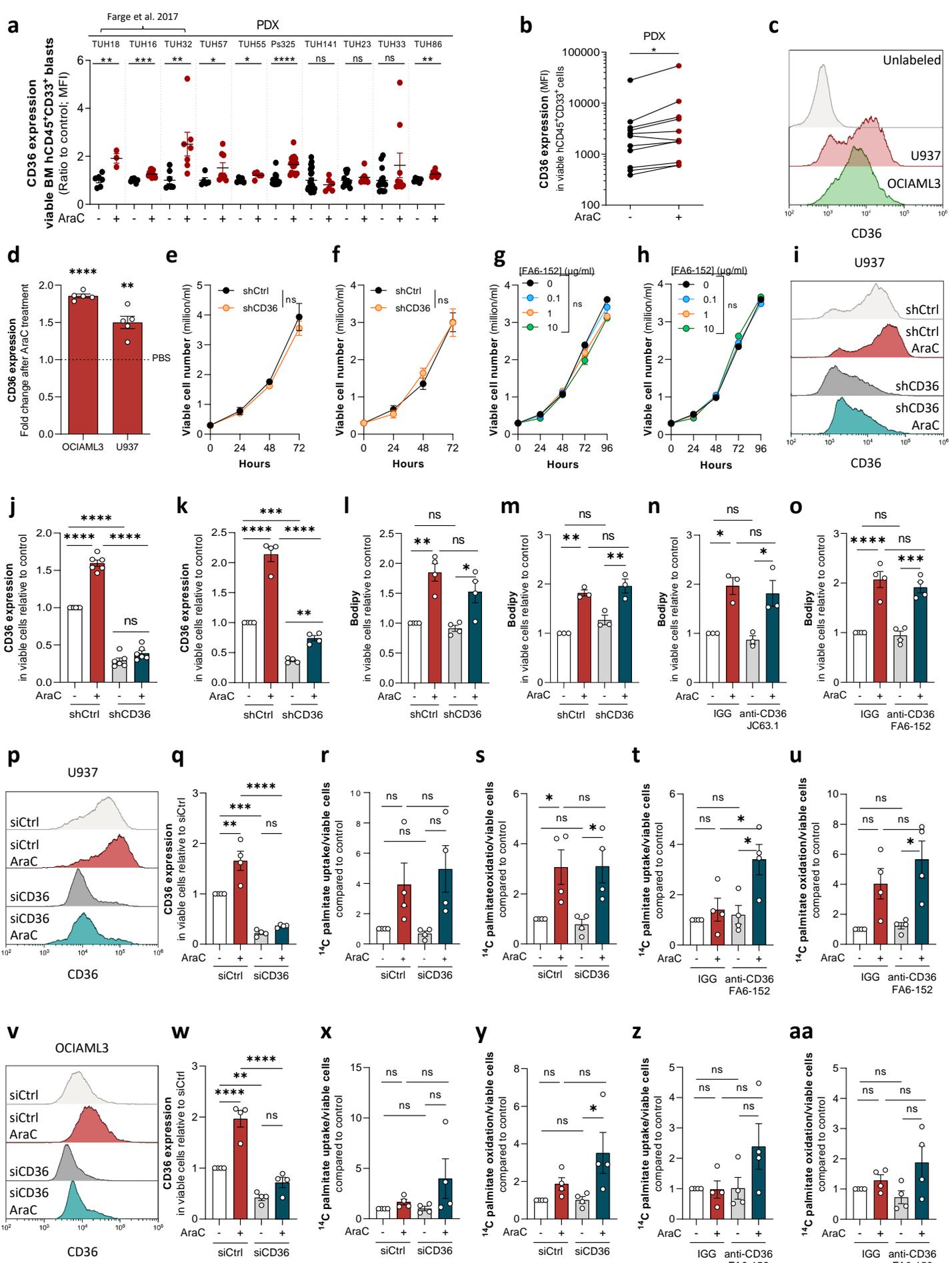


Figure S1

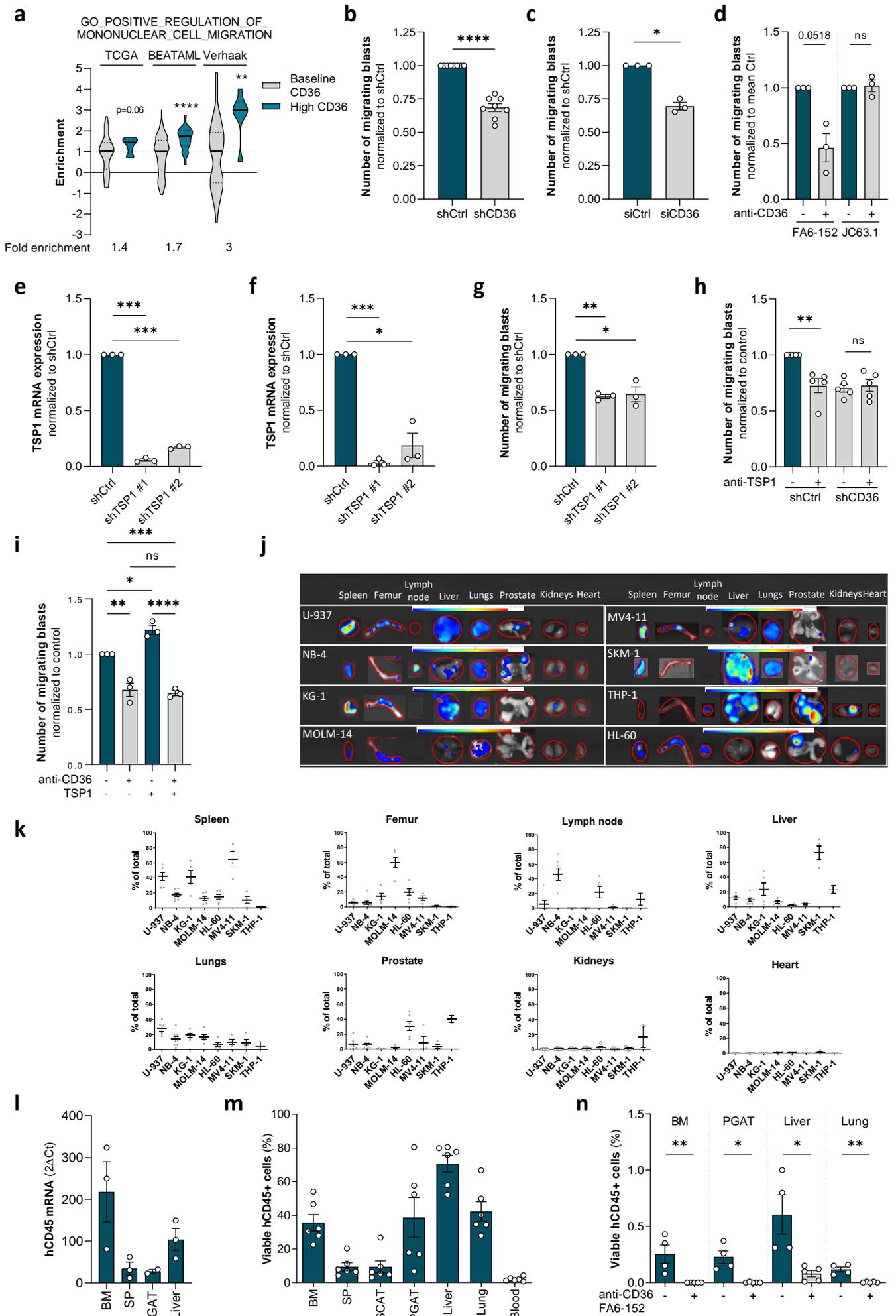


Figure S2

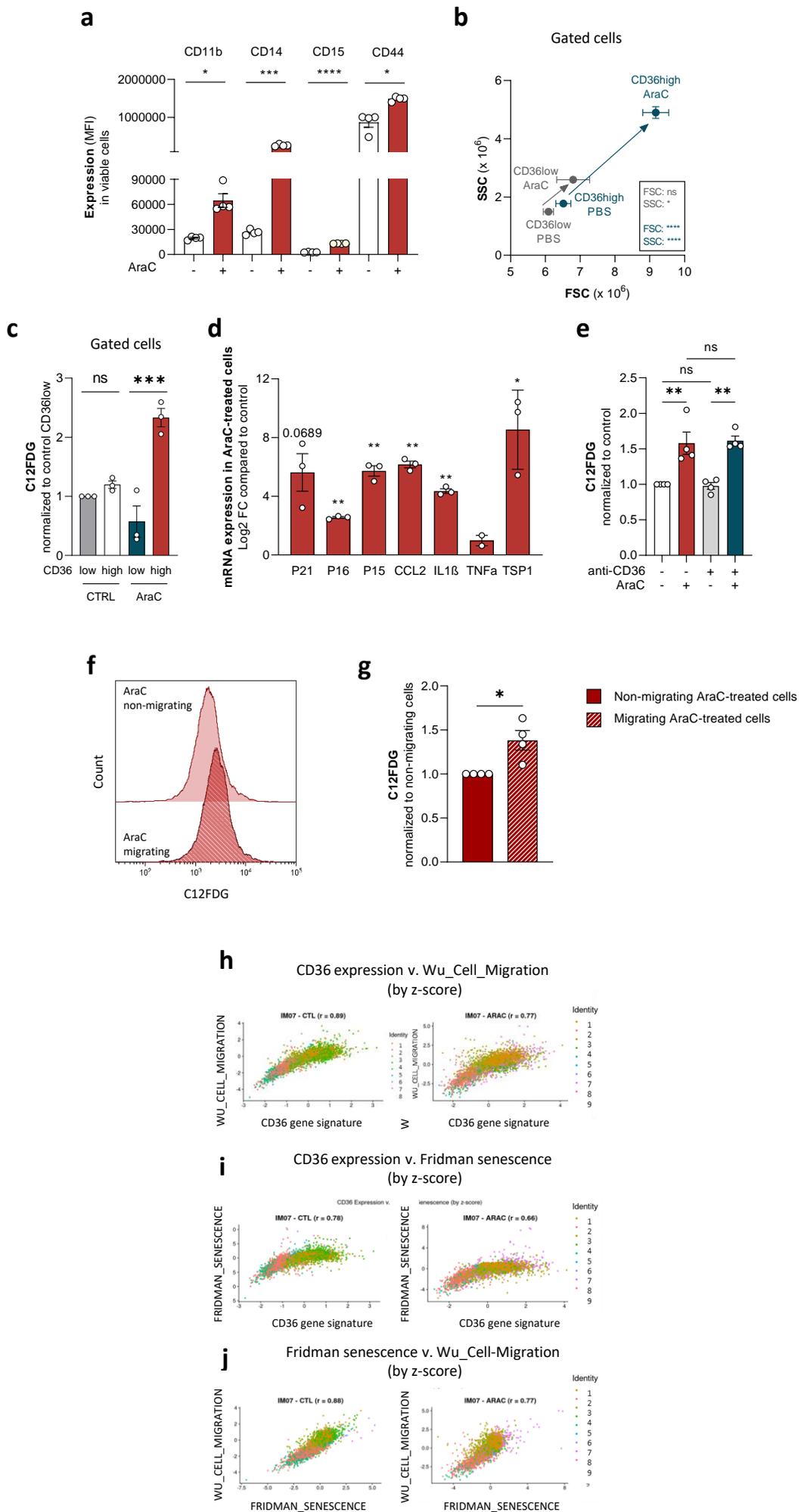


Figure S3