

Supplementary Figures

S1: AML blasts share the immunophenotype of MDSC and alter arginine metabolism. **a)** Blood samples from newly-diagnosed AML patients were stained for a panel of surface markers. The whole blood profile (FSC and SSC) and expression of surface markers is shown (histograms) for one representative patient. Red line = Fluorochrome conjugated antibody; Black line = Isotype control. **b)** Arginase II and iNOS expression was determined by RT-PCR. Representative data from nine patients are shown. GAPDH was used as the housekeeping gene to ensure equal loading **c)** Whole cell lysates of AML blasts from 10 patients, or monocytes and neutrophils from healthy donors were tested for the ability to convert arginine into urea using a colorimetric assay. Arginase activity is found for AML blasts but not myeloid cells from healthy donors. **d)** No correlation between arginase activity of AML blast lysate as measured by colorimetric assay, and intracellular arginase II concentration measured by ELISA ($p=0.29$).

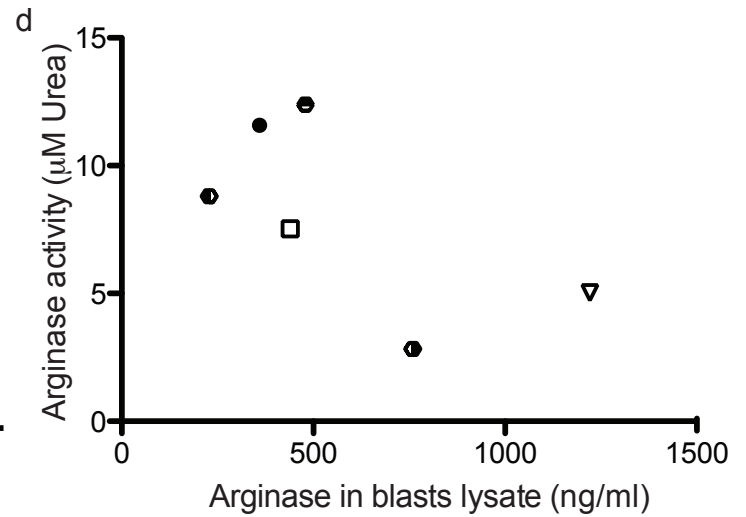
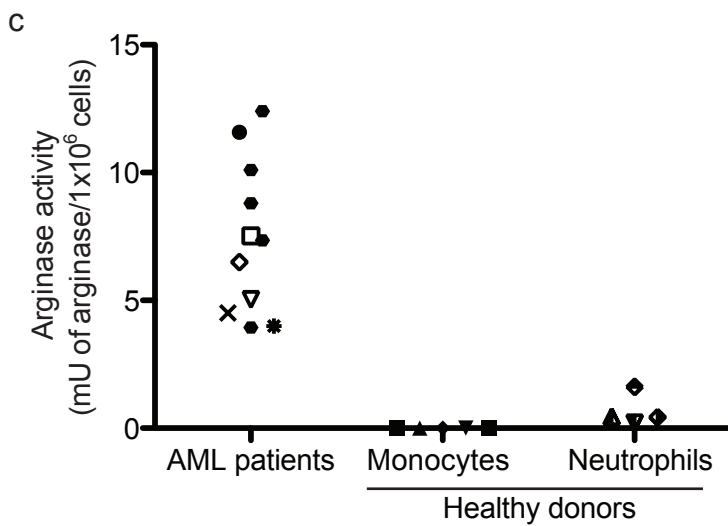
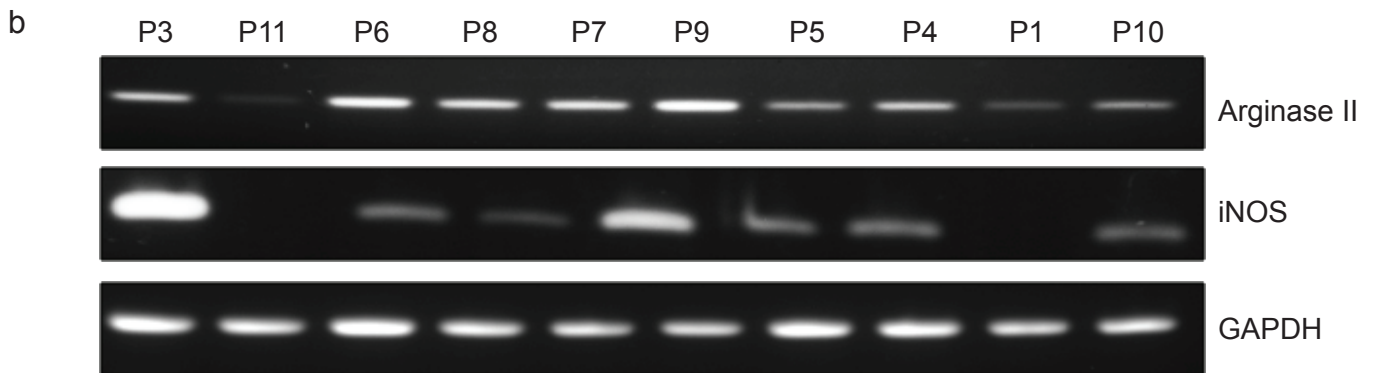
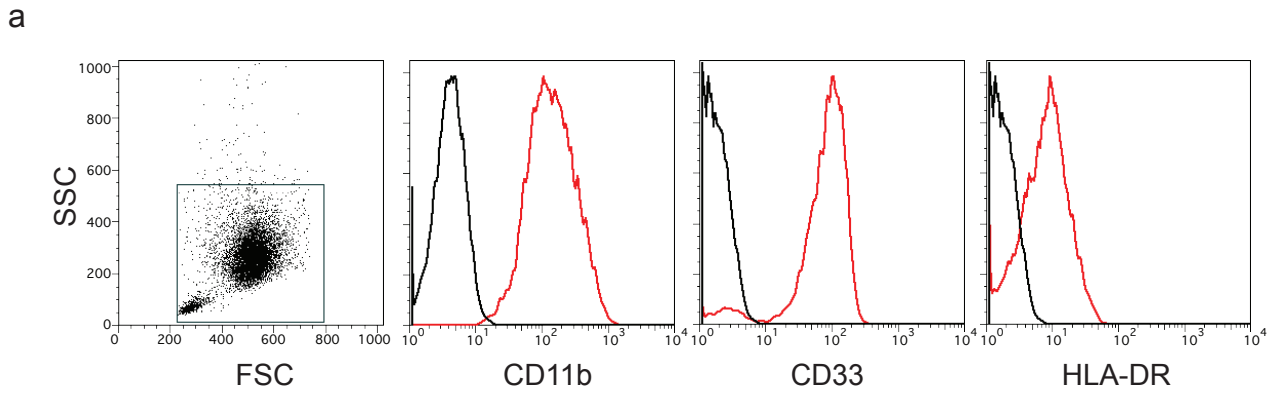
S2: Arginase I is not raised in the plasma of AML patients compared to healthy controls. **a)** Plasma from 15 AML patients and 15 healthy controls was analysed by ELISA for arginase I ($p=0.03$). **b)** The arginase activity in plasma from 2 patients decreases towards normal levels after 1 cycle of chemotherapy ($p=0.04$).

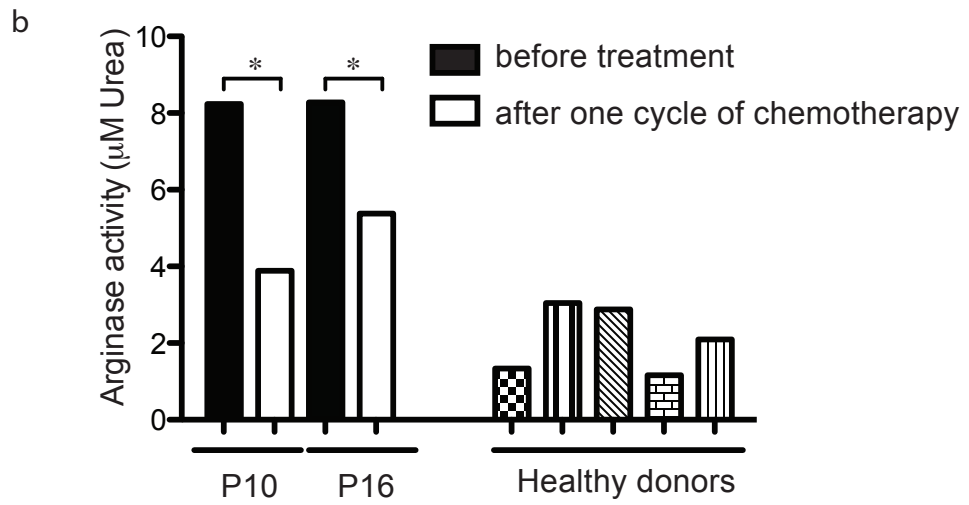
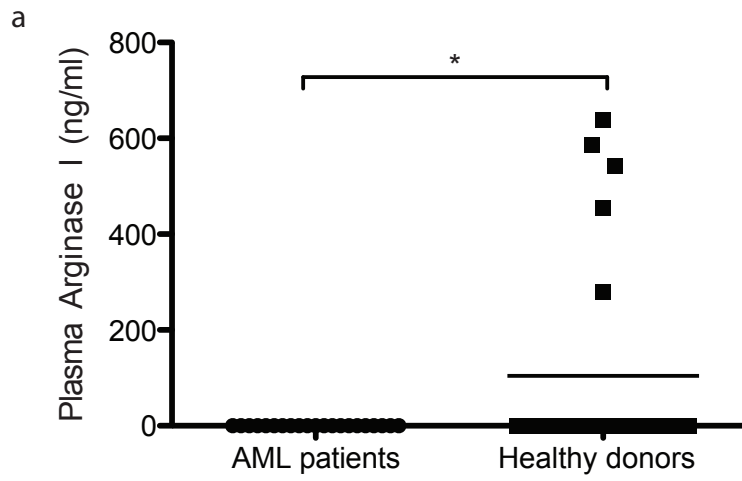
S3. CD206⁺ monocytes suppress T cell proliferation. CD206⁺ monocytes generated from 48 hour culture in AML patient plasma, significantly suppress T cell proliferation in MLR compared to monocytes cultured in the plasma of healthy patients. (***) $p=0.0001$

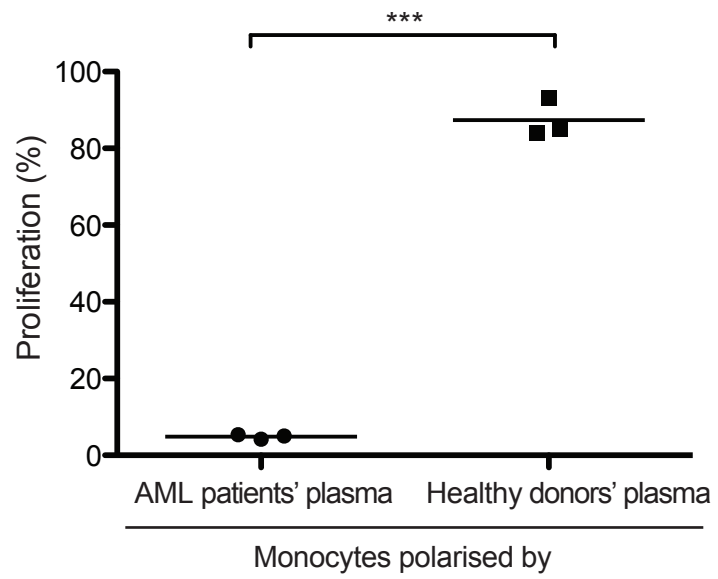
S4. Human CD34⁺ HSCs cultured with AML plasma retain colony-forming potential. **a)** Human CD34⁺ HSCs previously cultured for 3 days in either AML plasma or with AML plasma containing L-NMMA or NOHA, or healthy plasma, were transferred to a methylcellulose gel. Colony-formation was assessed after 7 days using an inverted microscope. (colony-forming units: AML plasma vs. AML plasma with inhibitors, ** p=0.029; AML plasma vs. healthy donor plasma, ***p=0.0007) **b)** Human CD34⁺ HSCs maintain CD34 expression in the presence of AML patient plasma. HSCs were cultured in the presence of AML plasma, AML plasma with L-NMMA or NOHA, or healthy plasma. CD34⁺ expression was assessed on Day 3 of culture by flow cytometry. Data from three separate experiments are shown. (AML plasma vs. AML plasma with inhibitors, * p=0.027; AML plasma vs. healthy donor plasma, ***p=0.0002)

Supplementary Table

S Table 1. Table of patients' characteristics

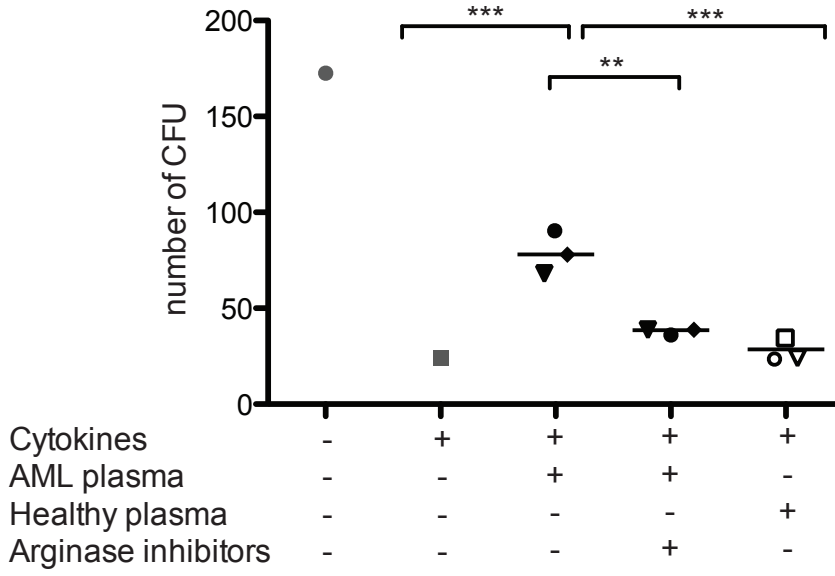




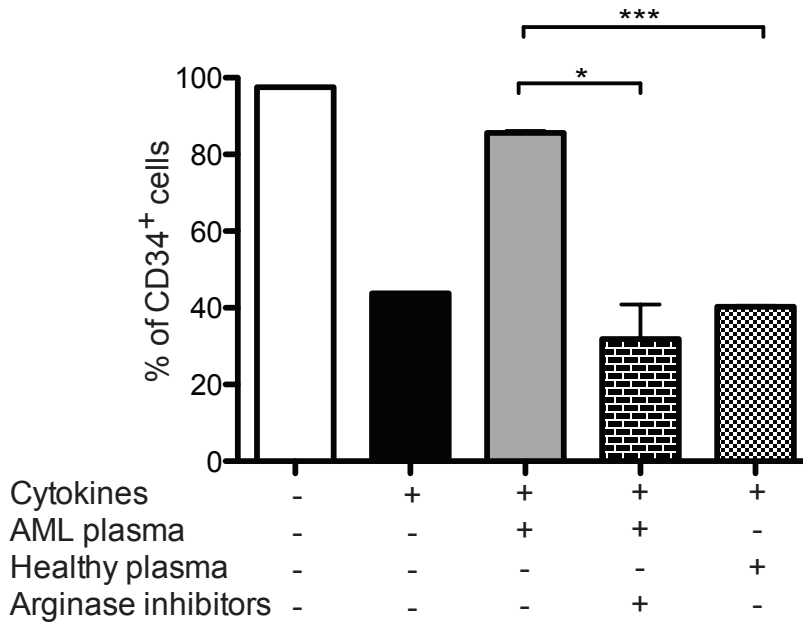


Supplementary Figure 3

a



b



Patient	Sample origin	Timing	Sex	Age	Diagnostic white cell count (x10 ³ /uL)	Cytogenetics	FAB	Immunophenotype
P1	Blood	Diagnosis	F	3	338	49,6,+8,+8,t(10;13;11)(p13;q14;q23)[9]/46,XX[1]	M5	CD11b+/CD33+/CD14(12%)/CD34-
P2	Blood	Diagnosis	M	44	246	Normal	M4	CD11b+/CD33+/CD34-/CD15+(15%)
P3	Blood	Diagnosis	F	77	2.6	Normal	M5	CD11b+/CD33+/CD14+/CD34-/ CD117+/ CD64+
P4	Blood	Diagnosis	F	64	3.1	Normal	M5	CD11b+/CD34+/CD33+/CD38+/CD15+(30%)/CD7+(40%)/CD11b+
P5	Blood	Diagnosis	F	89	5.7	Normal	M1	CD11b+/CD34+/CD33+(30%)
P6	Blood	Diagnosis	F	88	1.3	Normal	M0-1	CD11b+/CD34+/CD33-
P7	Blood	Diagnosis	F	13	37	MLL, t(9;11)	M4	CD11b+/CD33+/CD15+(65%)/CD19+/CD117+/ NG2+
P8	Blood	Diagnosis	F	26	5.0	46XX, t(5;17)	M3	CD11b+/CD33+/CD34-
P9	Blood	Diagnosis	F	58	76	Normal	M5	CD11b+/CD34+/CD15+/CD33+/CD38+/CD71+/CD117+
P10	Blood	Diagnosis	F	3	30	Constitutional +21, GATA-1+	M7	CD11b+/CD33+/MPO+/ CD41-/CD117+/CD34+/GLYA-/CD10+/CD19+
P11	Blood	Diagnosis	M	39	185	Normal	M4Eo	CD11b+/CD33+/CD15+(22%)/CD34-
P12	Blood	Diagnosis	M	80	7	Normal	CMML	CD11b+/CD34+/CD33+
P13	Blood	Diagnosis	F	68	47.2	Normal	M5	CD34+/CD117+/CD14+/CD64+/CD300e+/CD11b+/CD15+/CD16+
P14	Blood	Diagnosis	F	56	166	Normal	M4	CD11b+/CD33+/CD34+
P15	Blood	Diagnosis	F	27	10.4	Normal	Mixed Lineage	CD34+ (10%) of which 50% MPO; CD13+/CD33+(60%); CD7+/CD2+/TdT+ (50%)

Supplementary Table 1