CD44 engagement enhances acute myeloid leukemia cell adhesion to the bone marrow microenvironment by increasing VLA-4 avidity

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Supplemental Methods

Cell lines

Murine BM stromal cells (M2-10B4, ATCC-CRL-1972) were obtained from the ATCC (Manassas, Virginia, USA). AML cell lines were purchased from DSMZ (Braunschweig, Germany): OCI-AML3 (ACC-582), KG-1a (ACC-421), HL-60 (ACC-3), MOLM-13 (ACC-554), MOLM-14 (ACC-777), MV4-11 (ACC-102). Cell lines were cultured for less than 15 passages or authenticated by DNA fingerprinting and STR-technology (1).

Antibodies

Antibodies are listed in Supplemental Table 2.

Flow cytometry

Whole BM aspirates and AML cells were stained with monoclonal antibodies (Supplemental Table 2) or corresponding isotype controls. Classical blast gating was performed, based on side scatter properties and (low) CD45 expression (2). αCD13, αCD14, αCD33, αCD34, and αCD117 were used to further discriminate various AML subtypes and other cell types (3), in combination with CD44 and CD49d determination. Measurements were performed using the Gallios system (Beckman Coulter, Brea, California, USA). For cell line experiments, CD44 and CD49d expression were determined using a FC-500 system (Beckman Coulter). Viability was assessed using Annexin V-FITC and 7AAD.

RNA interference and lentiviral transduction

RNA interference and lentiviral transduction experiments were performed as described (4). The following short hairpin RNA (shRNA) constructs selected from the Mission TRC shRNA

library (Sigma-Aldrich, St. Louis, Missouri, USA) were used: shRNA CD44 (CCGGCCGTTGGAAACATAACCATTACTCGAGTAATGGTTATGTTTCCAACGGTTTTTG), shRNA CD49d (CCGGGCTCCGTGTTATCAAGATTATCTCGAGATAATCTTGATAACACGGAGCTTTTT) and scrambled control shRNA (CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTGTTTTT). Transduced cells were selected for puromycin resistance prior to further analysis.

Shear flow assay

Shear flow assays were conducted as described (5). µ-slides (Ibidi, Martinsried, Germany) were coated with protein A/VCAM-1/Fc (R&D Systems, Minneapolis, Minnesota, USA) or HA or HA followed by protein A/VCAM-1/Fc. Mononuclear cells from BM aspirates of AML patients (blast content: 75-95%, determined by flow cytometry) were preincubated with 10 µg/mL HA (Sigma-Aldrich), with 2 µg/mL αCD49d (clone HP2/1), with 5 µg/mL αCD44 Fab fragments (clone 515) for 10 min, where indicated. Src family kinase inhibition was performed using 10 µM PP2 (Merck, Darmstadt, Germany) for 30 min. Midostaurin was used at 1 µM (Selleckchem, Houston, USA) for 60 min. The cells were allowed to accumulate at subphysiological shear stress (0.5 dyn/cm^2), and then subjected to physiological shear stress (2 dyn/cm^2). Frequencies of adhesive categories were determined as percentages of cells flowing immediately over the substrate using customized image analysis software (Wimasis, Córdoba, Spain) (6-8).

Western blotting

AML cells were analyzed as described (9) using antibodies against phosphorylated and non-phosphorylated IkB, Akt, ERK, FAK, paxillin and PPIA. For VCAM-1/Fc treatment protein A Dynabeads^{TM} (Invitrogen, Carlsbad, California, USA) were incubated for 1 h at room

temperature with VCAM-1/Fc (3.5 µg/mL) (R&D Systems). 3 x 10^6 cells were either untreated or pretreated with high molecular weight HA (10 µg/mL) (Sigma-Aldrich) for 2 min and then incubated with VCAM-1/Fc coated Dynabeads™ (2 x 10^6 cells per 15 µl beads) for 20 min at 37°C. Cells were lysed and used for western blotting.

Quantitative real time PCR (qPCR)

RNA isolation and cDNA synthesis were performed as described (9). qPCR was performed on the ViiA 7 System (Applied Biosystems, Foster City, California, USA) using TaqMan Gene Expression Assays. Results were quantified by normalization to 18S rRNA or GAPDH expression using the ΔCT method.

Viability assay

OCI-AML3 cells were seeded into 24-well plates at 2 x 10^5 cells/well and cultured on wells coated with or without VCAM-1 (7.5 µg/ml). Cells were previously stimulated with 10 µg/ml HA for 10 minutes at 37°C and then treated with 0.1 and 0.5 µM doxorubicin final concentration for 24 hours. Viable cell count was performed with TC10™ Trypan Blue dye (0.4 %) and evaluated with TC20™ automated cell counter according to the manufacturer`s instructions (Bio-Rad, USA).

Flow cytometric HA-binding assay

To detect HA binding, OCI-AML3 cells were incubated with HA-FITC (AbLab, Vancouver, Canada) for 15 min at room temperature with/without pretreatment with αCD44 (clone 515) or soluble HA (Sigma) for 10 min.

In vivo engraftment and mobilization

10 NSGS mice were engrafted with secondary (previously expanded in NSG mice) human AML cells originally derived from Patient 46. One week after *i.v.* injection of the cells, 5 mice were treated with 100 μg αCD44 antibody (clone 515) and the other 5 mice with the same volume of PBS *i.v.* One day after treatment with the αCD44 antibody mice were sacrificed and blood, BM and spleen were analyzed for human cell content via anti-human CD45 (clone J33), CD34 (clone 581) and CD38 (clone LS198-4-3) antibody staining.

Xenotransplant treatment study

5 million MOLM-13 cells were injected i.v. into the tail vein of NOD/SCID mice. Mice were randomized into 4 groups (a 7 mice): control, cytarabine (AraC), anti-CD49d or AraC + anti-CD49d combination. 3 days after injection treatment was started. Control group 1 received inVivoPure Dilution Buffer 5 ml/kg/day ip (Days 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48, 51, 54, 57). Group 2 was given with 5 mg/kg/day iv Day(s) 0-42 AraC. Group 3 was given 10 mg/kg/day ip (Days: 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48, 51, 54, 57) anti-CD49d (clone PS2) antibody. Group 4 received AraC and anti-CD49d (clone PS2) in combination. The treatment study was conducted at Charles River Laboratory. The experiment was terminated at day 74 after cell injection. At this time, all mice of the control group had died, 1 mouse was still alive in AraC group, 2 mice were alive in PS2 group and 1 mouse was still alive in combination group. Blood, spleen, lymph node and bone marrow were harvested to measure number of human CD45 positive cells by flow cytometry. Counting beads were used to determine absolute numbers. Significant outliers were removed.

Supplemental shear flow assay

Shear flow assays were conducted as described in the main manuscript. µ-slides (Ibidi, Martinsried, Germany) were coated with HA, protein A/VCAM-1/Fc or HA followed by protein A/VCAM-1/Fc. OCI-AML3 cells were treated with/without low molecular weight HA (Cat# 40583, Sigma) and high molecular weight HA (Cat# 41897, Sigma) or with/without 15 mM methyl-beta-cylcodextrin (MβCD) (Sigma-Aldrich) and HA for 10 min at 37 °C where indicated. For avidity assay 6-channel µ-slides (Ibidi, Martinsried, Germany) were coated with αCD49d (clone HP2/1) overnight at 4 °C.

Determination of VLA-4 conformation

VLA-4 conformation assay has been described in (10). Shortly, AML cells were resuspended in HBSS (10 mM HEPES, 0.2% BSA, 1 mM $CaCl_2$, 1 mM $MgCl_2$). Viability, CD44, CD44v6, CD49d, and CD29 expression were cytometrically determined prior to each experiment. Cells were incubated with LDV and with/without HA in the presence of an excess of αCD29 mAb (clone HUTS-21, detecting the ligand occupied conformation of VLA-4) for 30 min at 37 °C. Subsequently, the mean fluorescence intensity (MFI) of labeled HUTS-21 mAb was cytometrically determined. EC_{50} values for HUTS-21 binding report LDV ligand binding affinity, as described (11).

Static cell adhesion assay

Flat 96-well plates were coated with 1µg/mL recombinant human VCAM-1-Fc (R&D systems) over night at 4°C. OCI-AML3 cells were treated with either αCD49d (5 µg/mL, clone HP2/1) antibody or αCD44 antibody (5 µg/mL, clone 515). Afterwards the cells were treated with/without HA (AMO Germany) for 30 min and aliquoted into the coated plates at a final concentration of $1x10^6$/mL. Plates were incubated for 1 h or 10 min at 37 °C followed by

fixation and crystal violet staining. Absorbance was determined at 595 nm (MolecularDevices SpectraMax iD3 microplate reader). Results were shown as mean OD values of triplicate wells ± SD.

E-Selectin adhesion assay

The wells of a 96 well plate were coated over night with 0.5 µg/ml E-selectin-Fc on 2 µg/ml ProteinA at 4°C. OCI-AML3 cells (1 million/ml) were pre-incubated or not for 10 minutes at 37°C with anti-CD44 (clone 515, 5 µg/ml), and then allowed to adhere to the E-selectin coating at 37°C for 30 min (100 µl cell suspension/well). Adhesion was quantified using CyQuant Cell Proliferation Kit (Thermo Fisher). Each condition was assessed in technical triplicates.

Supplemental VLA-4 cluster assay

Mononuclear cells from BM aspirates of AML patients (blast content 75-95%) were pretreated for 10 min with 10 µg/mL HA and/or for 24 h with 10 µM cobimetinib (APExBIO, Houston, USA), and/or for 90 min with 5 µM idelalisib (APExBIO) and allowed to adhere for 30 min at 37 °C before fixation with 4% paraformaldehyde (PFA). Slides were stained with primary αCD49d antibody (clone AHP1225) and Cy3-conjugated secondary anti–rabbit antibody.

OCI-AML3 cells were pretreated for 10 min with 10 µg/mL HA and/or for 30 min with 7.5 µM MßcD and allowed to adhere for 30 min at 37°C before fixation with 4% PFA. Slides were stained with primary αCD49d antibody (clone AHP1225) and Cy3-conjugated secondary anti-rabbit antibody.

Supplemental Stroma binding

Falcon culture slides were coated with 20 µg/mL fibronectin for 1 h at 37 °C. 70 000 M2 stroma cells were seeded and cultured overnight. M2 stroma cells were pretreated with 20 µg/mL αVCAM-1 antibody (clone 429) for 20 min before OCI-AML3 cells (0.5-1.0 x 10^6 cells) were seeded on M2 stromal cells and co-cultured for 30 min at 37°C. Cells were washed, fixed with 4% PFA, and stained using DAPI Antifade Reagent. Images were taken with an Olympus IX81 microscope (UPLSAPO 20xO/0.85 objective). Numbers of cells were determined in 10-12 pictures for each treatment with ImageJ software.

Immunofluorescence microscopy

Falcon culture slides were coated with 7.5 µg/mL VCAM-1 for 3 hours at room temperature and overnight at 4 °C. OCI-AML3 were treated with/without 10 µg/mL HA for 10 min, and allowed to adhere for 30 min at 37°C before fixation with 4% PFA. Slides were stained with primary αCD49d antibody (clone AHP1225) and αCD29 antibody (clone 12G10) and Cy3-conjugated secondary anti-rabbit and AF488-conjugated anti-mouse antibody. Images were taken with an Olympus IX81 microscope.

Reverse transcription PCR

For detection of CD44v transcription by reverse transcription PCR (RT-PCR), cDNA from AML samples was amplified by panCD44 or CD44 variant exon specific primers and visualized by agarose gels, as described in (12).

Co-Immunoprecipitation

OCI-AML3 cells were stimulated with HA where indicated. For immunoprecipitation (IP) of CD49d, cells were lysed in 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Triton-X-100, 0.1% SDS, 1% sodium deoxycholate, 1 mM sodium orthovanadate, 1 mM aprotinin and

1mM leupeptin. The cleared lysates were incubated with antibody-coated protein A/G agarose beads (Merck). The beads were washed in lysis buffer, then subjected to western blot analysis.

Statistical analysis

Statistics were performed using GraphPad Prism 8. Normal distribution was determined using the Kolmogorov–Smirnov test. Two normally distributed groups were compared using t-test. Nonparametric data sets were analyzed by Wilcoxon signed-rank tests for paired analysis or Mann-Whitney test for unpaired analysis. Three or more groups of data were analyzed with the one-way ANOVA with post-hoc tests. Results were considered significantly different when $P < 0.05$, with values at $P < 0.05$ marked as *, $P < 0.01$ as **, $P < 0.001$ as *** and $P < 0.0001$ as ****.

Supplemental Figure Legends

Supplemental Figure 1.

(A) Representative histograms of CD44 and CD49d surface expression of the cell lines HL-60, MV4-11, MOLM-13, MOLM-14 and KG-1a are shown. (B) Mononuclear cells from BM aspirates of AML patients pretreated with/without αCD44 antibody clone 515 (αCD44) and/or αCD49d clone HP2/1 (αCD49d) were injected into the tail veins of NSG mice. After 3 h the number of AML cells that had homed to BM and spleen of the recipients was determined by flow cytometry using human-specific αCD44 (clone J.173) and αCD49d (clone 9F10) antibodies. The homing rate was defined as the number of measured leukemic cells per 1 million measured cells per 1 million injected cells (data for double treatment from 4 of the 5 primary samples shown in Figure 1 C i).

Supplemental Figure 2.

(A) In 4 of 5 homing experiments shown in Figure 1C i, the viability of patient cells treated with/without αCD44 (clone 515) for 3 h *in vitro* was determined in parallel via AnnexinV/7AAD staining. (B) In 4 of 5 experiments shown in Figure 1C i, the percentage of CD44 and CD49d positive patient cells with and without corresponding blocking antibody (αCD44 (clone 515) and αCD49d (clone HP2/1)) treatment was determined before injection into recipient mice. (C) OCI-AML3 cells pretreated with/without αCD44 (clone 515) were allowed to adhere to E-Selectin. (D i) OCI-AML3 cells transduced with shCD44 or control shRNA (shCont) were perfused over HA for 1 min at 0.5 dyn/cm^2. Three runs were averaged per experiment and 3 independent experiments were performed. (ii) CD44 mRNA expression was determined via qPCR from OCI-AML3 cells transduced with shCD44 or control shRNA (shCont) (n = 3). (E i) OCI-AML3 cells transduced with shCD49d or control shRNA (shCont)

were perfused over VCAM-1 for 1 min at 0.5 dyn/cm2. Five runs were performed. (ii) CD49d (ITGA4) mRNA expression was determined via qPCR from OCI-AML3 cells transduced with shCD49d or control shRNA (shCont) (n = 3). Unpaired t-tests were used in C+D. (F i) In the experiment shown in Figure 1 E proliferation of primary AML cells after 3 h and 3 d was determined using CellTrace^{TM} dye dilution (n = 4). One-way ANOVA with multiple comparisons was used. (ii) Representative histograms of CellTrace^{TM} dye intensity are shown in AML cells found in BM and spleen after 3 h or 3 d. (G) Human AML cells in murine organs (bone marrow: BM, blood and spleen: SPL) were determined as CD45^{+}/CD34^{+}cells one day after αCD44 antibody or PBS treatment of the mice (n = 5 per group).

Supplemental Figure 3.

(A) HA binding capacity of the OCI-AML3 cell line was determined by flow cytometry using fluorescein-labeled HA (HA-FITC). OCI-AML3 were pretreated with a blocking αCD44 antibody (clone 515) or soluble HA (n = 5). One-way ANOVA with multiple comparisons was used. Representative histograms are shown in ii. (B) Primary AML cells from 1 patient were perfused over VCAM-1. Where indicated, cells were pretreated with soluble HA or/and αCD49d antibody (clone HP2/1, abrogating VLA-4-mediated interactions). Runs were performed in triplicates. (C) OCI-AML3 (n = 14) (i) and AML patient samples (n = 7) (ii) were pretreated with/without HA (10 µg/mL) for 10 min. CD49d and CD44 surface expression was determined via flow cytometry. Relative mean fluorescence intensity ratio = MFIR. Paired t-tests were used.

Supplemental Figure 4.

(A) VLA-4 activation by inside-out signaling. VLA-4 binding strength to its ligand VCAM-1 can be modulated by either increased avidity mediated by receptor clustering or increased

affinity by distinct conformational states. (B) OCI-AML3 cells with/without HA or Mn^{2+} (positive control) were incubated with the indicated LDV concentrations in presence of an excess of HUTS-21 mAb. The HUTS-21 antibody detects the epitope on the hybrid domain of the CD29 subunit only exposed in the ligand-occupied conformation. Fitting of the data was done using the sigmoidal dose-response equation with variable slope using GraphPad Prism 8 software. Each point represents the mean of triplicates. The EC_{50} values calculated for control and HA treatment indicate a resting state of the VLA-4 integrin. (C) Confocal images and CD29 cluster analysis of OCI-AML3 and of primary AML cells from BM aspirates were performed. Cells were pretreated with/without HA and then settled on immobilized VCAM-1, followed by fixation and staining with αCD29 (green) monoclonal antibody (clone 12G10) where indicated (1 representative of 2 different patients is shown). CD29 clusters for each treatment were quantified using ImageJ software (n = 50 cells, unpaired t-test). (D) OCI-AML3 cells with/without αCD44 or αCD49d were cultured on VCAM-1 for 1 h. Absorbance was determined at 595 nm (n = 3, one-way ANOVA with multiple comparisons). (E) OCI-AML3 cells were perfused over an anti-CD49d (clone HP2/1) substrate or an isotype control substrate for 1 min at 0.5 dyn/cm^2 with/without HA pretreatment. Categories of interaction (tethers) are expressed as frequencies of cells in direct contact with the substrate. (F i) Confocal images of OCI-AML3 cells that were pretreated with HA and with/without methyl-beta-cyclodextrin (MßcD). Cells were stained with αCD49d (red) monoclonal antibody (clone AHP1225). (ii) CD49d clusters were quantified for each treatment using ImageJ software (n = 50 cells). (iii) OCI-AML3 cells were perfused over VCAM-1, HA or VCAM-1 + HA substrate for 1 min at 0.5 dyn/cm^2. Where indicated, cells were pretreated with/without HA and/or with/without MßcD. Arrests are expressed as frequencies of cells in direct contact with the substrate. One-way ANOVAs with multiple comparisons were used.

Supplemental Figure 5.

(A) RT-PCR analysis of CD44 variant transcripts. 5 forward primer (5 fw) and v2, v3, v4, v5, v6, v7, v8, v9, v10 reverse (rv) primer combination were used in cDNA from HL60, MV4-11, MOLM13, MOLM14, OCI-AML3 cell line as well as from 6 different primary AML samples and 2 normal $CD34^+$ samples. (B) Co-Immunoprecipitation (IP) of CD49d or isotype control following western blot (WB) determination of CD44v6 and CD49d in the pull-down of OCI-AML3 cell lysates. HA treatment where indicated.

Supplemental Figure 6.

(A) Protein lysates from native, control shRNA (shCont) and shCD44-transduced OCI-AML3 cells were treated with/without 10 µg/ml HA for 10 min and tested for their Src, phospho-Src, Akt, phospho-Akt and PPIA content by western blot. (B) Confocal images of OCI-AML3 that were pretreated with/without HA and/or (i) with/without the MEK inhibitor cobimetinib (cobi) or (ii) with/without the PI3K inhibitor idelalisib (idela). Cells were stained with αCD49d (red) monoclonal antibody (clone AHP1225). CD49d clusters were quantified for each treatment using ImageJ software (n = 50 cells). (C) OCI-AML3 cells were perfused over VCAM-1 substrate for 1 min at 0.5 dyn/cm^2. Where indicated, cells were pretreated with/without HA and/or with/without the Src kinase inhibitor PP2 and/or with/without the multikinase inhibitor midostaurin (mido) and/or with/without the PI3K inhibitor idelalisib (idela). Arrests are expressed as frequencies of cells in direct contact with the substrate. One-way ANOVAs with multiple comparisons were used.

Supplemental Figure 7.

(A) OCI-AML3 cells transduced with shCD44 or control shRNA (shCont) were pretreated with/without HA and allowed to adhere to M2 stromal cells for 30 min. (B) Native OCI-AML3

were pretreated with/without HA and allowed to adhere to αVCAM-1 antibody (clone 429) pretreated M2 stromal cells for 30 min where indicated. The number of AML cells that had bound to stromal cells was counted on 10-12 bright field images with additional DAPI staining by fluorescence microscopy. One-way ANOVAs with multiple comparisons were used. Images were taken at 20x magnification. Bars, 20 µm.

Supplemental Figure 8.

(A) For VCAM-1 treatment, protein A Dynabeads^{TM} (Invitrogen, Carlsbad, California, USA) were incubated for 1 h at room temperature with VCAM-1/Fc (3.5 µg/mL) (R&D Systems). 3 x 10^6 OCI-AML3 cells were either untreated or pretreated with high molecular weight HA (10 µg/mL) (Sigma-Aldrich) for 2 min and then incubated with VCAM-1 coated dynabeads (2 x 10^6 per 15 µL beads) for 20 min at 37 °C. Cells were analyzed via flow cytometry. (B) Protein lysates from OCI-AML3 cells treated with/without HA- and with/without VCAM-1-coated beads were tested for their PPIA, IkB alpha, phospho-IkB alpha, ERK, phospho-ERK, focal adhesion kinase (FAK), phospho-FAK, paxillin (Pax) and phospho-Pax content by western blot. Expression intensities were quantified with ImageJ software and phosphorylation was normalized to total protein content. Bottom: 3 independent experiments were quantified, using one-way ANOVAs with multiple comparisons. Top: individual experiment.

Supplemental Figure 9

Total number of $CD45^+$ cells in untreated, cytarabine, anti-CD49d (clone PS2) antibody treated or cytarabine + anti-CD49d treated MOLM-13 xenotransplants were determined in bone marrow (A), spleen (B), lymph-node-like structures (C) and blood (D) by flow cytometry (n = 5-7; mean ± sem.). Data of mice that were euthanized due to experiment end at day 74 are

shown in red. (E) Overall survival was determined (7 mice per group). Mice that were still alive at experiment end were censored.

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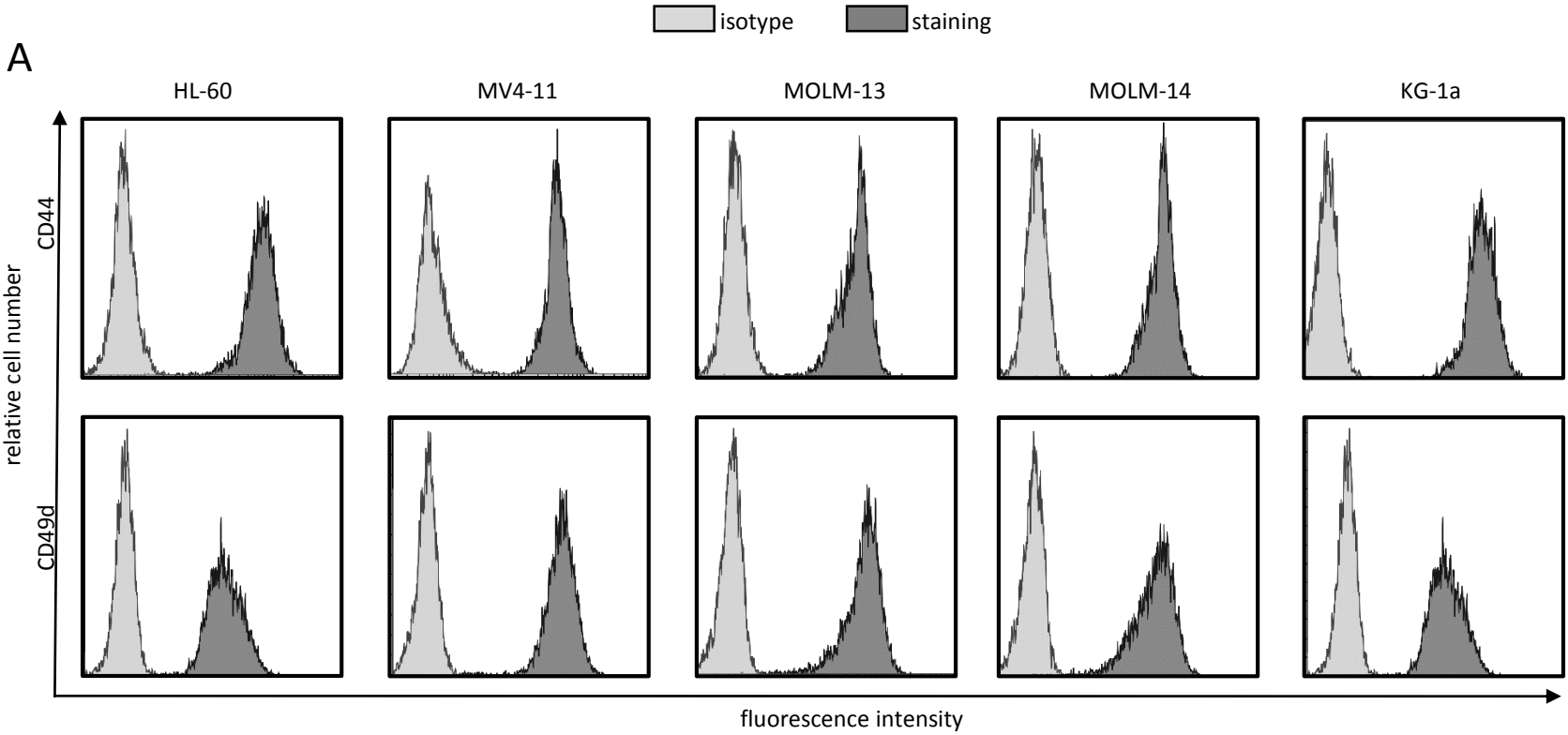
Supplemental Table 1: AML patient characteristics.

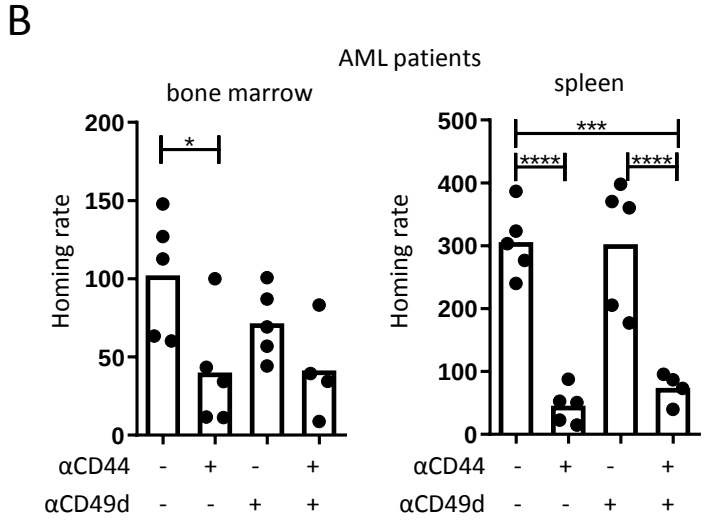
ID	gender	age	FAB type	WBC count (10^9/l)	PB blast count (%)	BM blast count (%)	karyotype	molecular genetics	Used for
9	M	85	2	344.38	100	96	normal	normal	*Fig 1A, 1Ci,, 2Bii, 3Aii, 3Aiii, 3Biii, 3Dii, 4Bii, 4C, 4D, 6B, Supp. Fig.1B,2A, 2B, 3Cii, 4C, 5A*
16	M	75	0	3.74	52	90	normal	normal	*Fig 1Ci, 3Aiii, 4Aii, 4C, Supp. Fig. 1B, 2A,2B, 3Cii, 5A*
20	F	69	0	24.68	52	100	normal	normal	*Fig 2Bii, 3Aiii, 3Biii, 4C, 4D, 5B, 6B, Supp. Fig.3Cii*
36	F	55	2	6.12	59	91	normal	*NPM1, FLT3-ITD*	*Fig 1Ci, 3Biii, 6B, Supp. Fig. 1B, 2A, 2B 3Cii, 5A*
42	F	30	2/4	186.26	84	96	N/A	*FLT3-ITD*	*Fig 1A, 1Ci, 3Aiii, 3Biii, 4C, 6B, Supp. Fig.1B,2A,2B 3Cii, 5A,*
46	F	52	0	240.10	95	86	normal	*NPM1, FLT3-ITD*	*Fig 1A, 1Ci, 1E, 2Bii, 3Aiii, 3Bii, 3Biii, 4C, 4D, 6B,, Supp.Fig 1B,.2F, 2G, 3Cii, 5A*
47	F	55	2	95.37	60	81	normal	*NPM1, FLT3-ITD*	*Fig 1A, 3Aiii, 4C, 5B, Supp. Fig. 3Cii, 5A*
54	M	64	1	129.12	81	90	normal	*CEBPA* (monoallelic)	*Fig 1A, 2Bii, 4D, 5B,*
56	M	75	2/4	169.25	99	96	-Y, add4q, add15q	*NPM1, FLT3-ITD*	*Fig 2Bii, 3Biii, 4D, 5B,, Supp. Fig. 3B,*

Supplemental Table 2: List of used antibodies. FACS: flow cytometry; WB: Western blotting; IF: immunofluorescence microscopy; Blocking: Blocking experiment

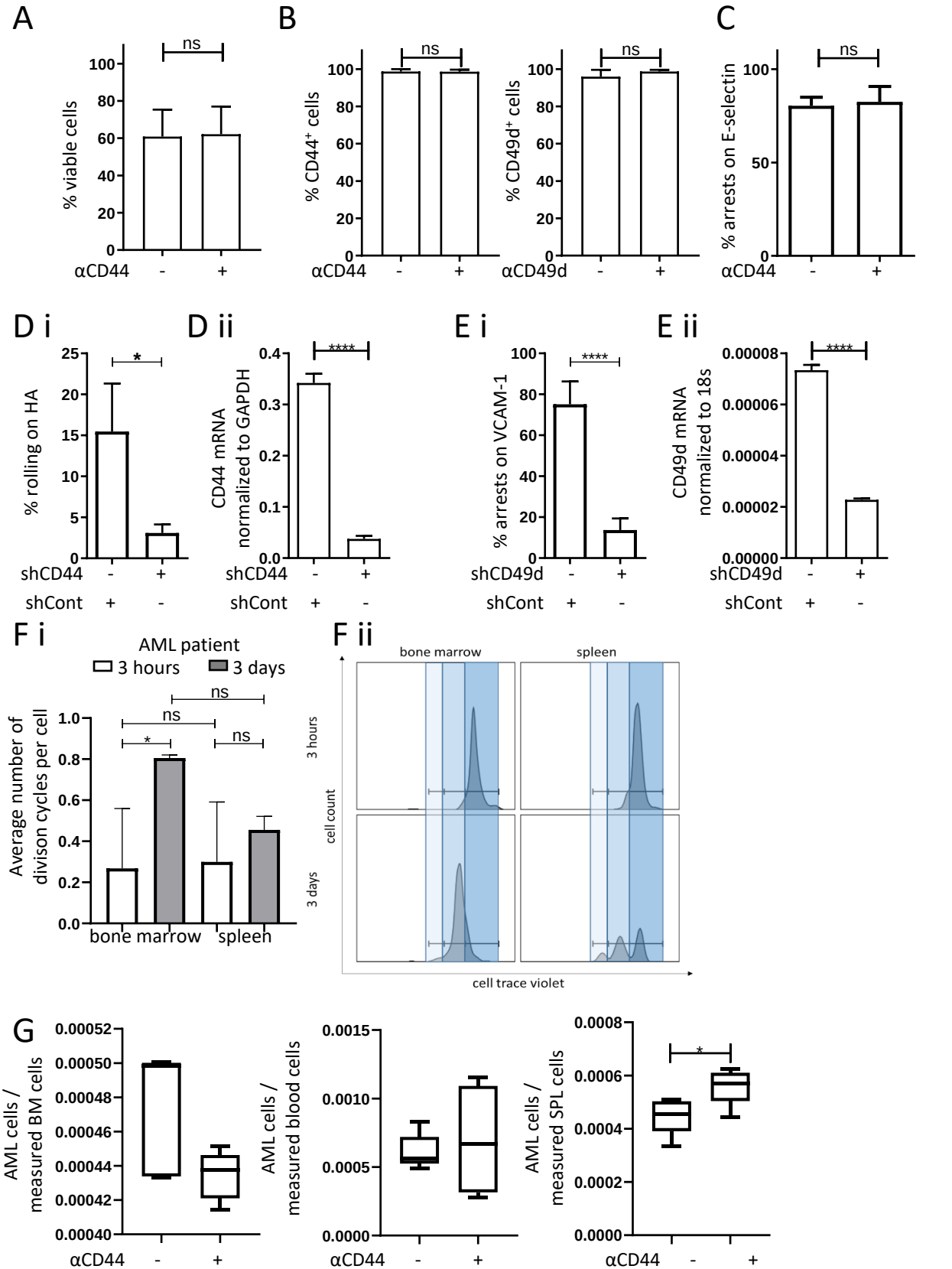
Target	Company	Cat#	Usage
CD44-FITC	BioLegend	103006	FACS
CD44-FITC	IO Test	IM1219U	FACS
CD44v6-FITC	eBioscience	BMS125FI	FACS
CD49d-PE	BD Biosciences	555503	FACS
CD44 Fab (515)	BD Pharmingen	550990	Blocking
CD49d (HP2/1)	Merck MIllipore	MAB1383	Blocking
CD49d (PS2)	Merck Millipore	CBL1304	Blocking
CD49d (AHP1225)	Bio Rad	AHP1225	IF
aMouse-HRP	Cell Signaling	7076	WB
aRabbit-HRP	Cell Signaling	7074	WB
Phospho-IκBα (Ser32) (14D4)	Cell Signaling	2859S	WB
IκBα (44D4)	Cell Signaling	4812S	WB
Akt (pan) (11E7)	Cell Signaling	4685S	WB
Phospho-Akt (Thr308)	Cell Signaling	9275S	WB
Cyclophilin A	Cell Signaling	2175S	WB
Phospho-p44/42 MAPK Rabbit (Erk1/2) (Thr202/Tyr204)	Cell Signaling	9101	WB
p44/42 MAPK (Erk1/2)	Cell Signaling	9102	WB
mTOR	Cell Signaling	2983S	WB
Phospho-mTOR	Cell Signaling	5536S	WB
aRabbit Cy3	Jackson ImmunoResearch	711-165-152	IF
aMouse AF488	Jackson ImmunoResearch	715-545-151	IF
CD44-FITC	Beckman Coulter	A32537	FACS
CD13-ECD	Beckman Coulter	B36286	FACS
CD14-PC7	Beckman Coulter	A22331	FACS
CD33-PC5.5	Beckman Coulter	B36289	FACS
CD117-APC	Beckman Coulter	B36300	FACS
CD34-APC-AF750	Beckman Coulter	B92463	FACS
CD38-PB	Beckman Coulter	B92396	FACS
CD45-KrO	Beckman Coulter	B36294	FACS
Annexin V-FITC	eBioscience	BMS306FI-20	FACS
7AAD	eBioscience (ThermoFisher)	00-6993-50	FACS
CD29	Abcam	ab30394	IF
CD34	Abcam	ab8536	IF
Phospho-Src Family (Tyr416)	Cell Signaling	2101	WB
Src Antibody	Cell Signaling	2108	WB
VCAM-1	Fisher Scientific	BDB555645	Blocking
VCAM-1	Thermo Fisher	16-1061-82	Blocking
CD15	Biolegend	323039	FACS
FAK	Cell Signaling	3285	WB
Phospho-FAK	Cell Signaling	3283	WB
Paxillin	Cell Signaling	2542	WB
Phospho-Paxillin	Cell Signaling	2541	WB

Supplemental Figure 1





Supplemental Figure 2



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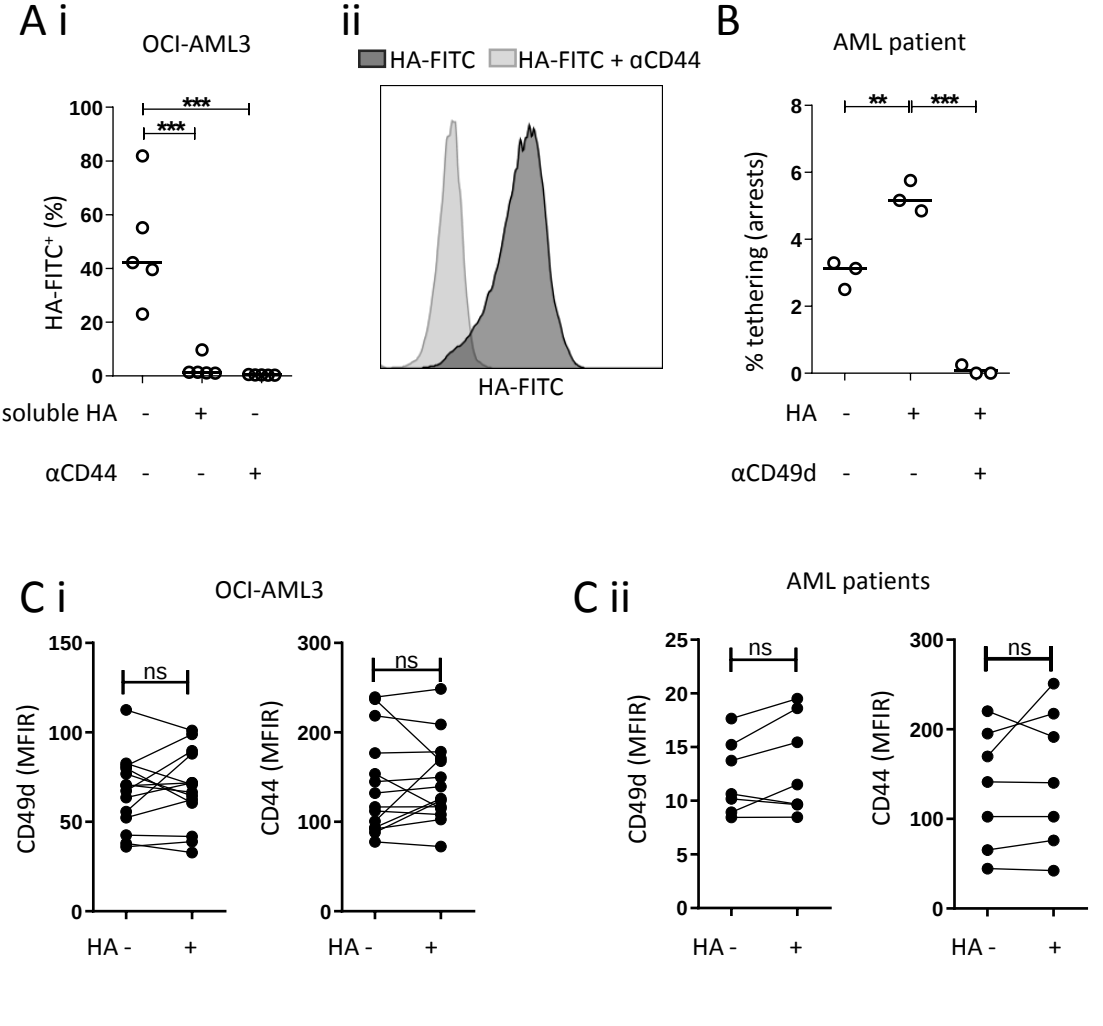
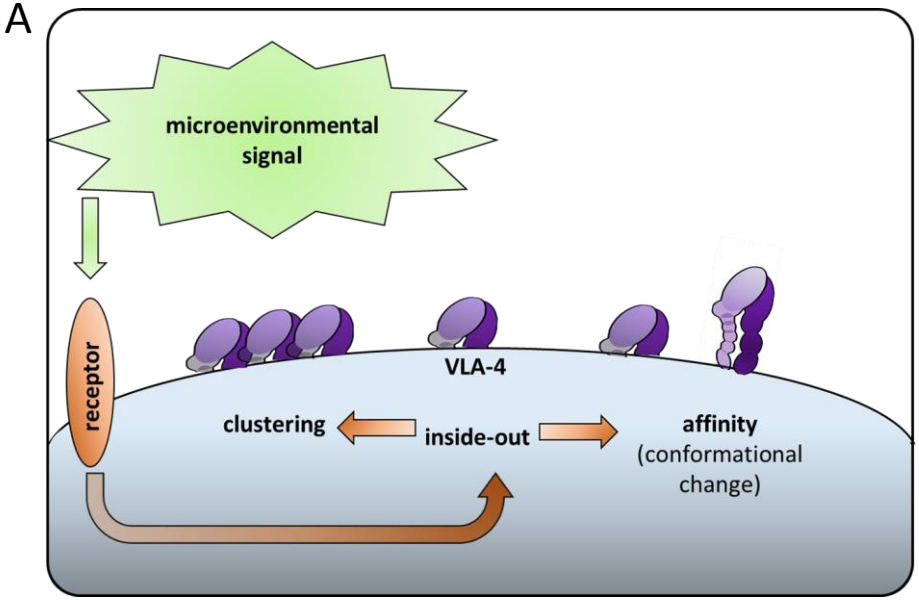
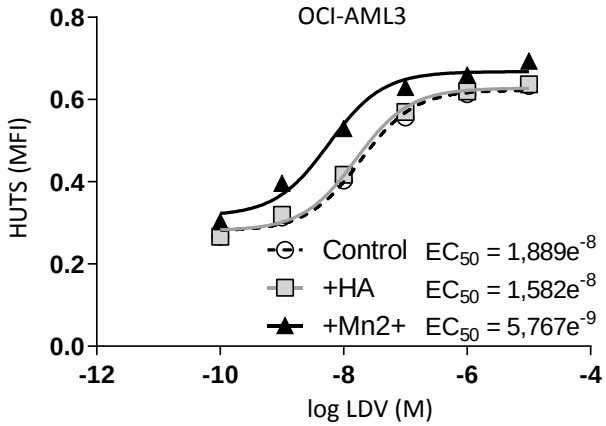
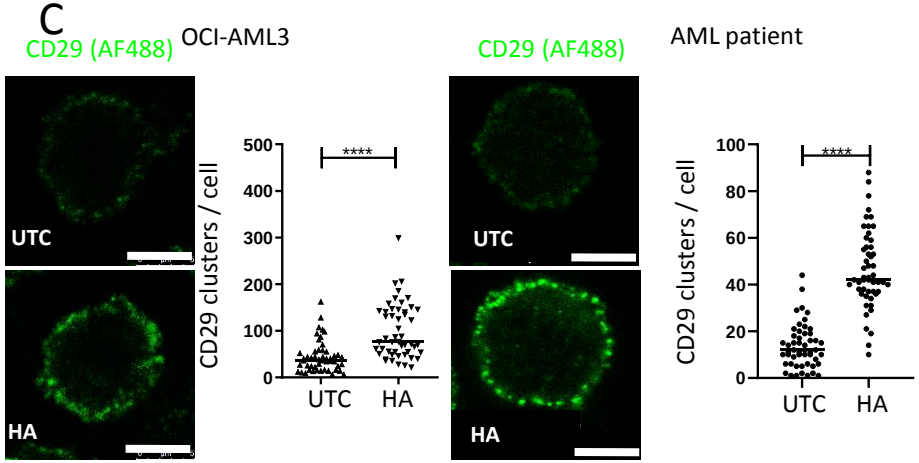
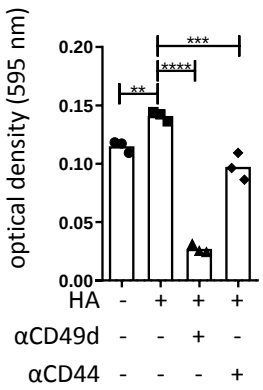


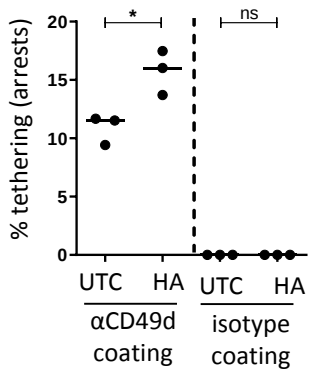
Supplemental Figure 4

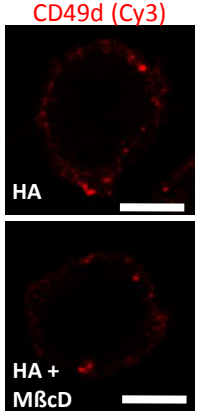


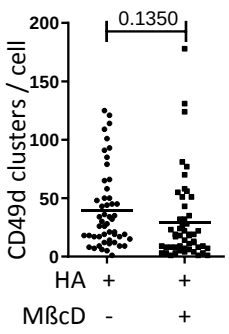


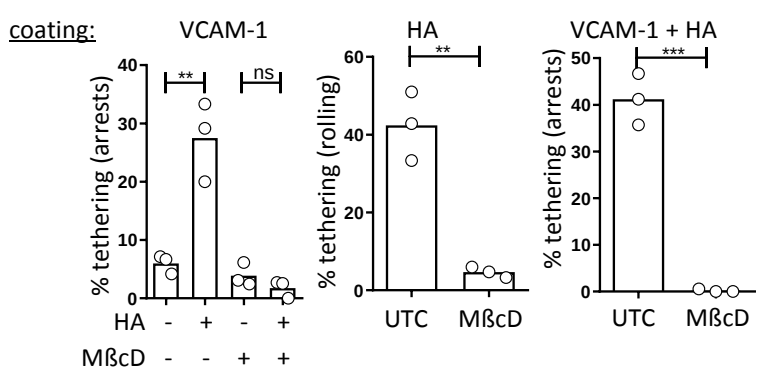






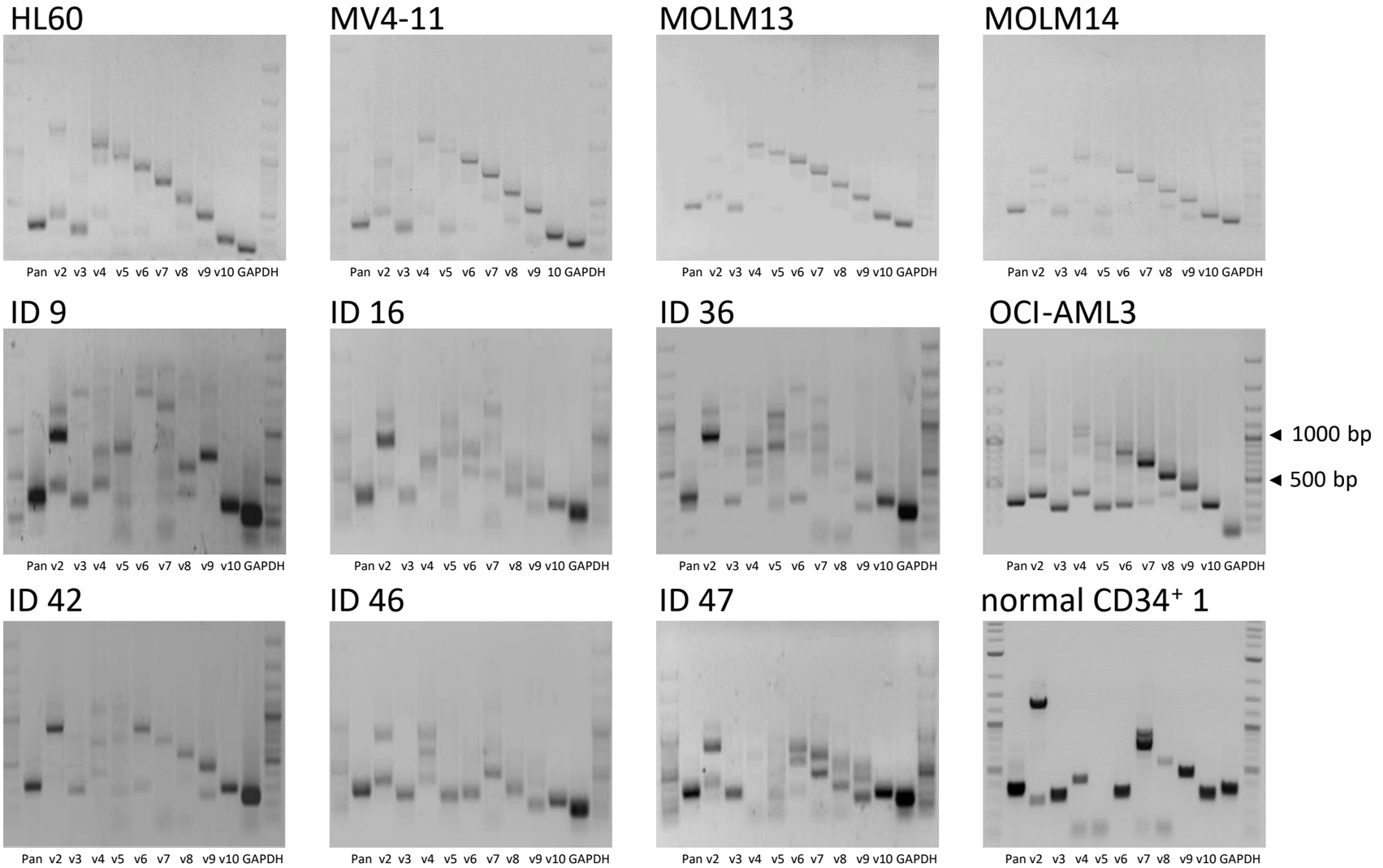




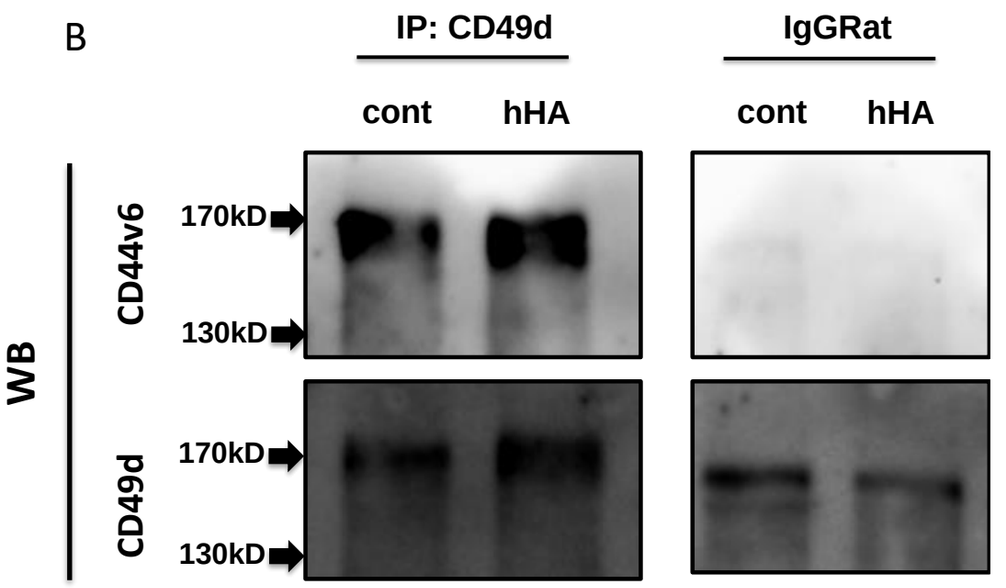


Supplemental Figure 5

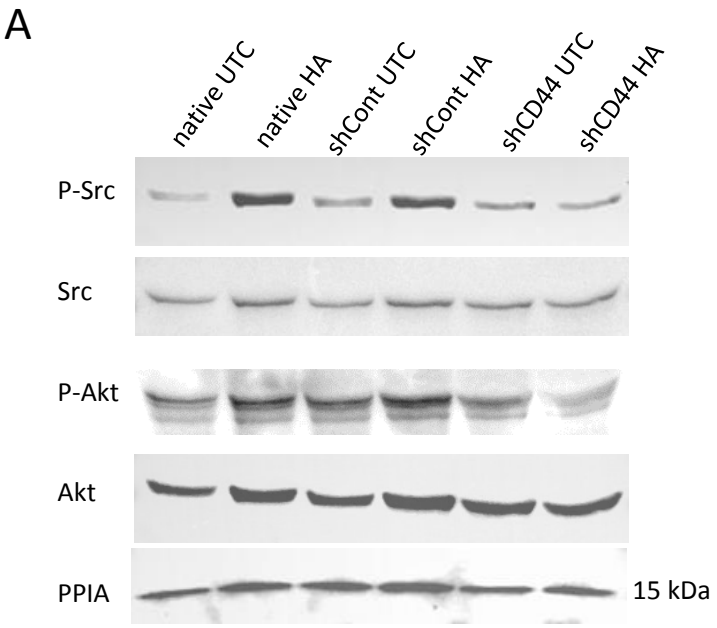
A

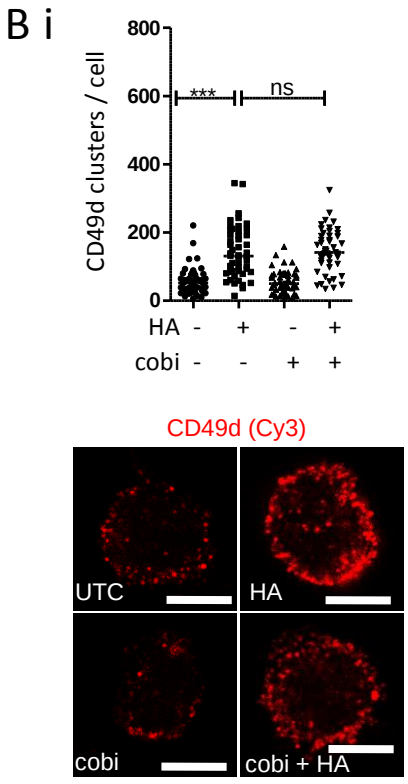


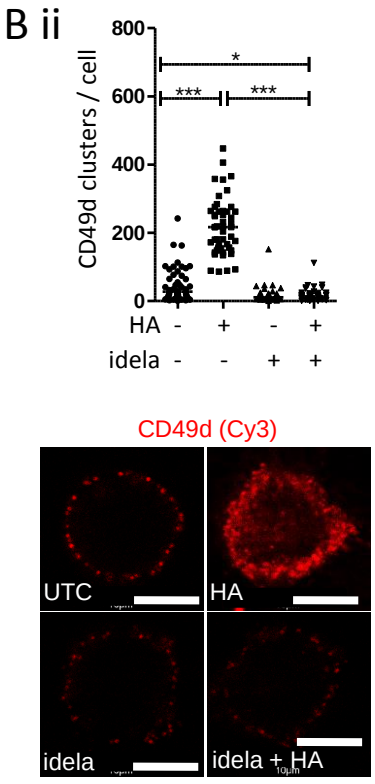
B

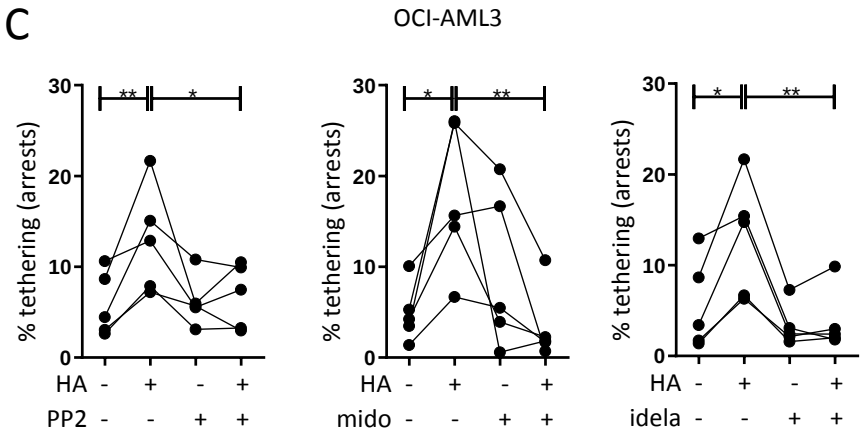


Supplemental Figure 6



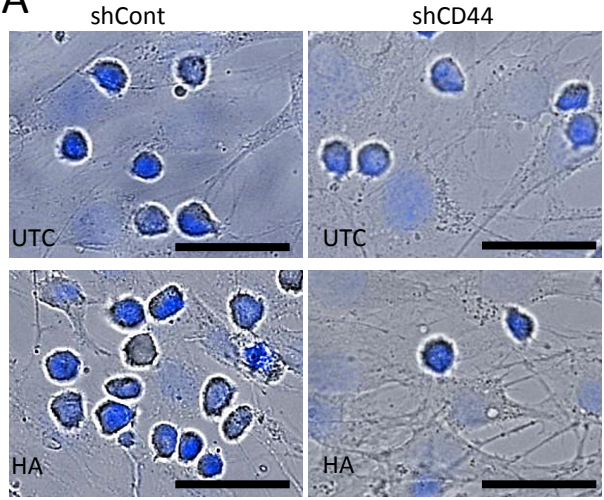


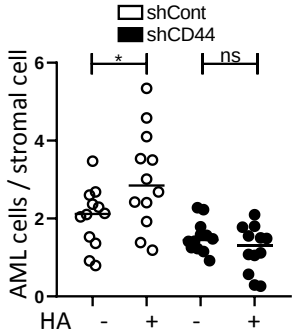




Supplemental Figure 7

A





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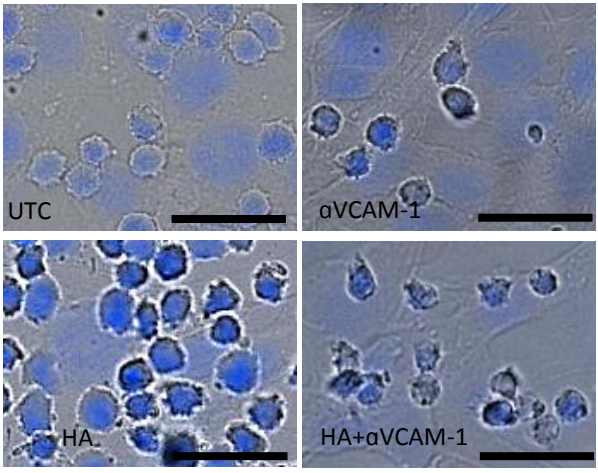
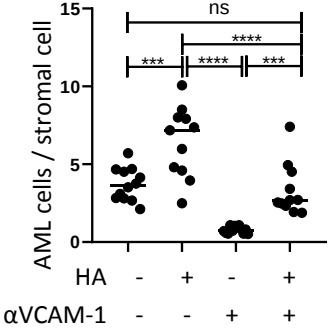
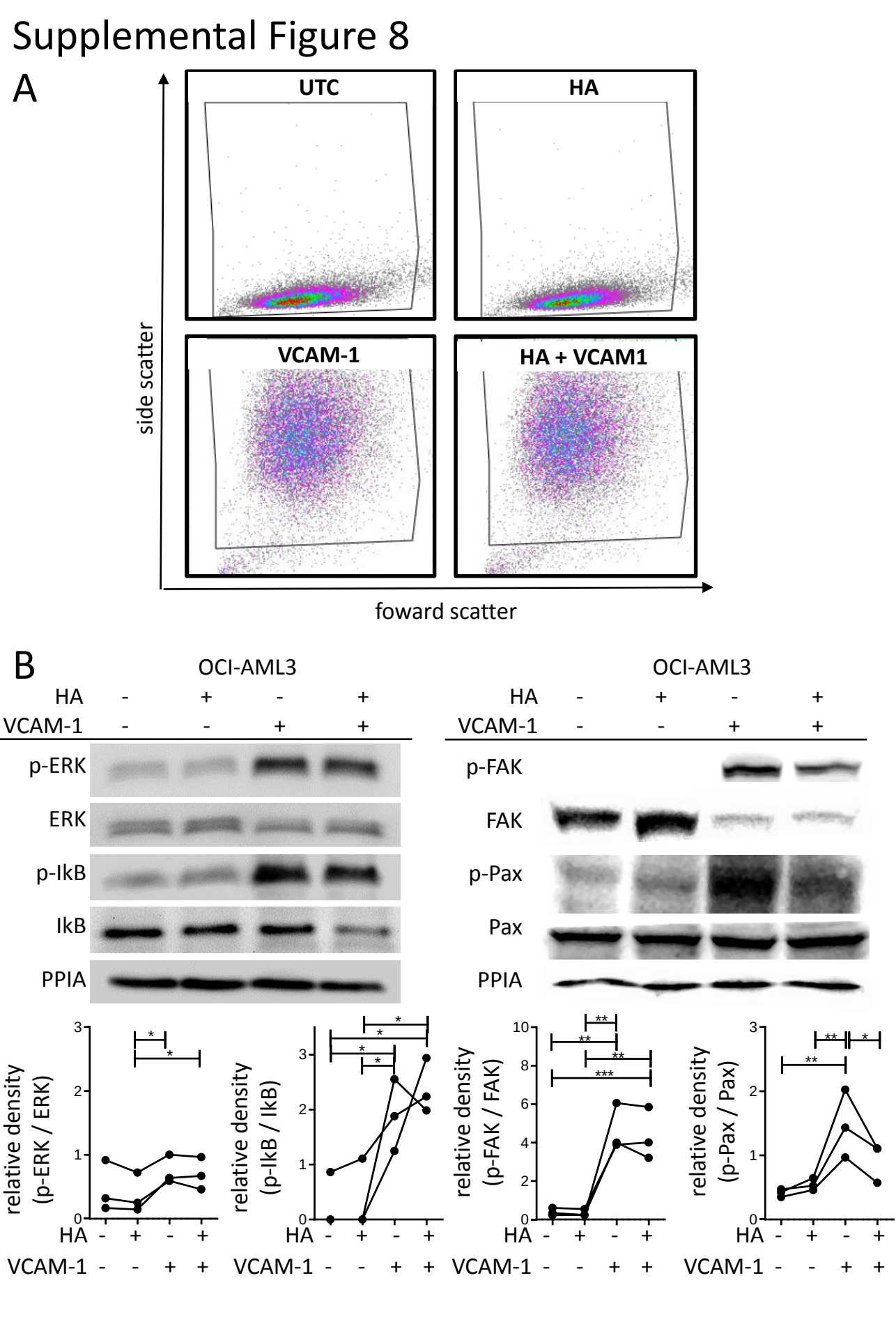




Supplemental Figure 8



Supplemental Figure 9