Supporting Information

Revealing CD38 Cellular Localization Using a Cell Permeable, Mechanism-Based Fluorescent Small Molecule Probe

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**General experimental methods.** Reagents, unless specified otherwise, were purchased from commercial suppliers in the highest purity available and used as supplied. $^1$H NMR was performed on INOVA 400/500/600 spectrometers, $^{13}$C NMR was performed on INOVA 400 spectrometer, and 2D NMR was performed on INOVA 500/600 spectrometers. NMR data was analyzed by MestReNova (version 8.1.1). $^1$H NMR chemical shifts are reported in units of ppm relative to tetramethylsilane. $^1$H NMR data are reported in the following manner: chemical shift (multiplicity, integration). Both sulforhodamine and tetramethylrhodamine fluorescence signals from protein gels were recorded by Typhoon 9400 Variable Mode Imager (GE Healthcare Life Sciences) with settings of Green laser at 532 nm and emission collection filter of 580BP30 (for tetramethylrhodamine) and 610BP30 (sulforhodamine). Detection using PMT650V (normal or high sensitivity), and analyzed by ImageQuant TL v2005. LC-MS experiments were carried out on a Shimadzu HPLC LC20-AD and Thermo Scientific LCQ Fleet with a Sprite TARGA C18 column (40 × 2.1 mm, 5 μm, Higgins Analytical, Inc.) monitoring at 215 and 260 nm with positive mode for mass detection. Solvents for LC-MS were water with 0.1% acetic acid (solvent A) and acetonitrile with 0.1% acetic acid (solvent B). Compounds were eluted at a flow rate of 0.3 mL/min with 0% solvent B for 2 min, followed by a linear gradient of 0% to 10% solvent B over 2 min, followed by a linear gradient of 10% to 100% solvent B over 5 min, and finally 100% solvent B for 1 min before equilibrating the column back to 0% solvent B over 1 min. Preparative HPLC experiments were done on Beckman Coulter System Gold 125p Solvent Module & 168 Detector with a TARGA C18 column (250 × 20 mm, 10 μm, Higgins Analytical, Inc.) monitoring at 215 and 260 nm. Solvents for HPLC were water with 0.1% trifluoroacetic acid or TFA (solvent A) and acetonitrile with 0.1% TFA (solvent B). Compounds were eluted at
a flow rate of 8.0 mL/min with 0% solvent B for 10 min, followed by first a linear gradient of 0% to X% (X depends on different compounds) solvent B over 60 min, then a linear gradient of X% to 100% solvent B over 5 min, and finally 100% solvent B for 5 min before equilibrating the column back to 0% solvent B over 5 min.

**Scheme S1:** Entire Synthetic Scheme for CD38 probe, SR101-F-araNMN

1-((2R,3S,4R,5R)-4-(benzoyloxy)-5-((benzoyloxy)methyl)-3-fluorotetrahydrofuran-2-yl)-3-carbamoylpyridin-1-ium (2)

(3S,4R,5R)-5-((benzoyloxy)methyl)-3-fluorotetrahydrofuran-2,4-diyl dibenzoate (1.396 g, 3.0 mmol, 1.0 eq, 1) was dissolved in anhydrous dichloromethane (DCM, 10 mL), followed by addition of 33% HBr in acetic acid (2.22 mL, 37.1 mmol, 12.4 eq), yielding a clear, yellow reaction mixture. Reaction proceeded overnight at room temperature and confirmed to be complete by TLC (20% ethyl acetate in hexanes). The reaction mixture was diluted with DCM
(100 mL) and washed using ice cold NaHCO₃ (2 x, 100 mL each), and ice cold water (2 x, 100 mL each). The organic layers were dried over anhydrous Na₂SO₄, and then concentrated on a rotary evaporator and further dried under high vacuum. ((2R,3R,4S)-3-(benzoyloxy)-5-bromo-4-fluorotetrahydrofuran-2-yl)methyl benzoate (referred to as the glycosyl bromide compound) was the product obtained and was used without further purification. To a separate round bottom flask, nicotinamide (724 mg, 5.93 mmol, 2.1 eq) was dissolved in anhydrous acetonitrile (19.48 mL, 0.30 M with respect to nicotinamide) under N₂. The mixture was refluxed under N₂ for five minutes, after which the mixture was allowed to cool to room temperature. Once nicotinamide begun to precipitate, the mixture was added to the glycosyl bromide compound obtained (1.17 g, 2.76 mmol). Reaction continued at room temperature. Initially, the reaction mixture was cloudy because the nicotinamide was not completely dissolved. However, as the reaction proceeded, the mixture became clear and colorless. The reaction was allowed to go overnight at room temperature. The reaction mixture was concentrated on a rotary evaporator and purified on a silica gel column (1 L ethyl acetate, followed by 20:1 DCM:MeOH until product begun eluting, at which point 10:1 DCM:MeOH was used). Compound 2 was obtained as a white solid (1.256 g, 90% yield). Rₜ was 0.3 in 10:1 CH₂Cl₂:CH₃OH. ¹H NMR (400 MHz, Methanol-d₄) δ 9.71 (s, 1H), 9.40 (dd, J = 6.4, 1.1 Hz, 1H), 9.12 (dt, J = 8.1, 1.5 Hz, 1H), 8.34 (dd, J = 8.1, 6.3 Hz, 1H), 8.21 – 8.13 (m, 2H), 8.13 – 8.03 (m, 2H), 7.72 – 7.66 (m, 1H), 7.65 – 7.57 (m, 1H), 7.58 – 7.51 (m, 2H), 7.47 (tt, J = 7.6, 1.5 Hz, 2H), 7.09 (dd, J = 18.0, 3.2 Hz, 1H), 6.00 (dd, J = 3.2, 1.3 Hz, 1H), 5.91 – 5.81 (m, 1H), 5.06 – 4.97 (m, 2H), 4.92 – 4.88 (m, 1H). LC-MS (ESI) calcd. for C₂₅H₂₂FN₂O₆⁺ [M⁺] 465.15, obsd. 465.08.
H NMR

LC-MS

Total Ion Chromatogram

Mass/charge: 464-466

Exact Mass: 465.15
3-carbamoyl-1-((2R,3S,4R,5R)-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyridin-1-ium (3)

Compound 2 (415 mg, 0.89 mmol, 1.0 eq) was dissolved in anhydrous methanol (9 mL), while keeping the flask under N₂ and on ice. K₂CO₃ (497 mg, 3.56 mmol, 4.0 eq) was added to the reaction mixture and the reaction was allowed to proceed for 5 hours at 0 °C. Reaction became yellow as it proceeded. Reaction was confirmed to be complete by detection of product mass on LC-MS (260 nm), after which methanol (10 mL) was added to the reaction mixture. The mixture was centrifuged to remove the K₂CO₃, and the solution was concentrated on a rotary evaporator to afford an orange product. Product was dissolved in methanol and purified via HPLC purification. The fractions were checked by LC-MS and those that contained the desired product were lyophilized to give the product as a yellow solid (327 mg, 85% yield). Preparative HPLC: retention time tᵣ = 11 min with 0% solvent B until 18 min followed by a linear gradient of 0% - 10% solvent B over 11 min, then from 10% - 100% solvent B over 10 min. 

¹H NMR (400 MHz, Methanol-d₄) δ 9.78 (s, 1H), 9.44 (dd, J = 6.3, 1.2 Hz, 1H), 9.04 (dt, J = 8.0, 1.5 Hz, 1H), 8.28 (dd, J = 8.0, 6.3 Hz, 1H), 6.70 (dd, J = 8.1, 5.0 Hz, 1H), 5.48 (dt, J = 52.0, 5.0 Hz, 1H), 4.51 (ddd, J = 18.2, 6.2, 5.2 Hz, 1H), 4.21 (dt, J = 7.1, 3.5 Hz, 1H), 4.01 (ddd, J = 12.5, 3.3, 2.2 Hz, 1H), 3.88 (dd, J = 12.6, 3.7 Hz, 1H). LC-MS (ESI) calcd. for C₁₁H₁₄FN₄O₄⁺ [M⁺] 257.09, obsd. 257.00.
F-araNMN alkyne

A round bottom flask containing compound 3 (39 mg, 0.15 mmol, 1.0 eq) was purged with N\textsubscript{2} and put on ice. \textit{m}-Cresol (1.5 mL) and diphosphoryl chloride (0.093 mL, 0.675 mmol, 4.5 eq) was added to the flask while on ice. The reaction was allowed to proceed at 0 °C, and product formation was monitored by LC-MS (260 nm). Additional diphosphoryl chloride was added after 3 hr (21 μL, 1.0 eq) and 5 hr (21 μL, 1.0 eq). However, even after 5 hr, based on LC-MS, the starting material compound 3 was not completely consumed. After 5 hr, the reaction mixture was immediately used for the next step in the synthesis. To a separate reaction flask that was purged with N\textsubscript{2}, 3-butyne-1-ol (2 mL) was added to the flask while under N\textsubscript{2}. The flask was put on a mixture of dry ice, ice and acetone to keep below 0 °C. The reaction mixture for preparing F-araNMN alkyne was transferred via syringe and slowly added to the flask containing 3-butyne-1-ol while kept below 0 °C. The product, F-araNMN alkyne, was formed based on LC-MS (260 nm) monitoring. After 4 hr, the reaction was quenched with cold water (10 mL), washed with ether (3 x 20 mL), and then purified by HPLC. The fractions were checked by LC-MS and those that contained the desired product were lyophilized to give the product as a white solid (3.77 mg, 8.0% yield). Preparative HPLC: t\textsubscript{R} = 13.8 min with 0% solvent B until 20 min. Followed by a linear gradient of 0% - 100% solvent B over 10 min. \textsuperscript{1}H NMR (599 MHz, Deuterium Oxide) δ 9.28 (s, 1H), 9.12 (d, \textit{J} = 6.3 Hz, 1H), 8.83 (dt, \textit{J} = 8.1, 1.4 Hz, 1H), 8.13 (dd, \textit{J} = 8.0, 6.4 Hz, 1H), 6.58 (dd, \textit{J} = 10.6, 4.5 Hz, 1H), 5.37 (dt, \textit{J} = 51.2, 4.4 Hz, 1H), 4.47 (dt, \textit{J} = 17.1, 4.9 Hz, 1H), 4.31 – 4.26 (m, 1H), 4.20 – 4.14 (m, 1H), 4.08 – 4.01 (m, 1H), 3.85 – 3.74 (m, 2H), 2.40 – 2.31 (m, 2H), 2.12 (t, 1H). \textsuperscript{13}C NMR (151 MHz, Deuterium Oxide)
Oxide) $\delta$ 146.31, 143.50, 141.30, 128.18, 95.19, 94.00, 93.87, 84.00, 71.34, 63.88, 63.18, 63.17, 20.00. LC-MS (ESI) calcd. for C$_{15}$H$_{19}$FN$_2$O$_7$P$^+$ [M$^+$] 389.09, obsd. 389.08.

$^1$H NMR
2-azidoacetic acid

To a round-bottom flask containing water (40 mL) was added sodium azide (4.677 g, 72 mmol, 2.0 eq). The flask was kept on ice during the addition of 2-bromoacetic acid (5.0 g, 36 mmol, 1.0 eq) to yield a clear, colorless reaction mixture. The reaction was let go overnight at room temperature. The reaction mixture was acidified to pH 2 with 1 M HCl and then extracted with ether (3 x 75 mL). Ether extracts were combined and dried using Na$_2$SO$_4$. Ether was evaporated and product was obtained in quantitative yield and used with no further purification. $^1$H NMR (300 MHz, Chloroform-$_d$) $\delta$ 9.61 (s, 1H), 3.97 (s, 2H).
** tert-butyl (2-(2-(2-azidoacetamido)ethoxy)ethoxy)ethyl)carbamate**

To a reaction mixture of 15 mL DCM with azidoacetic acid 4 (202.12 mg, 2.0 mmol, 2.0 eq), HBTU (758.5 mg, 2.0 mmol, 2.0 eq), DIEA (348 µL, 2.0 mmol, 2.0 eq) was added the amine (**tert**-butyl (2-(2-(2-azidoacetamido)ethoxy)ethoxy)ethyl)carbamate (248.32 mg, 1.0 mmol, 1.0 eq, synthesized according to reported method¹). The reaction was done overnight at room temperature and monitored by TLC (DCM:MeOH 10:1, visualized using iodine stain). The reaction was quenched by adding water and the product was extracted with DCM (2 x 30 mL). The product was concentrated on a rotary evaporator and purified on a silica gel column (DCM:MeOH 20:1). Compound 5 (298 mg) was obtained in 90% yield. Rf was 0.4 in 10:1 DCM:MeOH.

¹Reported method: [Source](https://example.com)
N-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-2-azidoacetamide (5)

Compound 5 (200 mg, 0.60 mmol, 1.0 eq) was dissolved in trifluoroacetic acid (5 mL). The reaction mixture was stirred at room temperature for 1 hr and was concentrated to yield the product without further purification. LC-MS (ESI) calcd. for C_{8}H_{18}N_{5}O_{3} [(M+H)^{+}] 232.13, obsd. 232.08

LC-MS

Sulforhodamine 101 chloride (4)

To a flame dried round bottom flask was added anhydrous DCM (2 mL) followed by dissolving of SR101 (50 mg, 0.082 mmol, 1.0eq). The flask was then placed on ice before addition of oxalyl chloride (31.1 µL, 0.412 mmol, 5 eq) and dimethylformamide (a few drops). The reaction was let go overnight at 4 °C in order to minimize double substitution. Product formation was monitored using LC-MS
(260 nm). Reaction was concentrated on a rotary evaporator and dried further using high vacuum to ensure all oxalyl chloride was gone. Product obtained was violet and used without any further purification.

**SR101 azide**

Compound 6 (20 mg, 0.08 mmol, 1.0 eq) was dissolved in anhydrous DCM (1 mL) followed by addition of diisopropylethylamine (28 µL, 0.16 mmol, 2.0 eq). This mixture was then added to compound 7 (50 mg, 0.08 mmol, 1.0 eq) followed by addition of 4-dimethylaminopyridine or DMAP (2 mg, 0.016 mmol). The reaction proceeded overnight at room temperature. Product formation was monitored using LC-MS (260 nm). Reaction was concentrated on a rotary evaporator and purified by silica gel column (DCM:MeOH 20:1). R_f was 0.45 in DCM:MeOH 20:1. The product SR101 azid (45 mg) was obtained in 69% yield as a violet solid. ^1H NMR (400 MHz, Chloroform-d) δ 8.83 (d, J = 1.9 Hz, 1H), 8.01 (dd, J = 7.9, 1.9 Hz, 1H), 7.88 – 7.74 (m, 1H), 7.19 (d, J = 7.9 Hz, 1H), 6.75 (s, 2H), 6.19 (t, J = 5.6 Hz, 1H), 3.75 (s, 2H), 3.62 (ddt, J = 13.8, 10.2, 5.2 Hz, 8H), 3.54 – 3.37 (m, 10H), 3.32 (q, J = 5.3 Hz, 2H), 3.08 – 2.93 (m, 4H), 2.84 – 2.56 (m, 4H), 2.16 – 2.02 (m, 4H), 2.02 – 1.86 (m, 4H). ^13C NMR (101 MHz, Chloroform-d) δ 168.01, 161.75, 161.41, 154.70, 152.07, 151.06, 146.85, 141.88, 134.51, 130.68, 127.65, 127.45, 126.89, 123.03, 113.43, 104.68, 77.36, 70.12, 70.08, 69.52, 69.21, 53.82, 51.83, 50.84, 50.35, 50.09, 43.05, 42.14, 39.14, 27.38, 20.56, 19.86, 19.72, 18.49, 18.46, 17.28, 17.21, 12.05, 12.02. LC-MS (ESI) calcd. for C_{39}H_{46}N_{7}O_{6}S_{2} [(M+H)^+] 820.28, obsd. 820.64.
SR101-F-araNMN

F-araNMN alkyne (101.6 μL of a 74.3 mM aqueous solution, 7.6 μmol), SR101 azide (2.0 mL of a 3.35 mM DMF solution, 6.7 μmol), Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (as ligand, 215.9 μL of an 100 mM DMF solution, 21.6 μmol), copper sulfate (1.080 mL of a 20 mM aqueous solution, 21.6 μmol), sodium ascorbate (1.727 mL of a 20 mM aqueous solution, 34.5 μmol) were added to DMF (500 μL) and water (500 μL) in that order. The resulting reaction mixture was stirred at room temperature overnight. Quantitative yield was determined from HPLC (260 nm) followed by purification by HPLC. Preparative HPLC: t_R = 43 min with a linear gradient of 0% - 35% solvent B from 10 – 25 min, then 35% - 50% solvent B from 25 – 50 min and 50% - 95% solvent B from 50 – 55 min. 1H NMR (500 MHz, Methanol-d_4) δ 9.64 (s, 1H), 9.37 (s, 1H), 9.06 (d, J = 7.4 Hz, 1H), 8.66 (s, 1H), 8.40 – 8.16 (m, 2H), 8.16 – 8.01 (m, 1H), 7.44 (d, J = 7.9 Hz, 1H), 6.69 (dd, J = 10.2, 4.2 Hz, 1H), 6.63 (s, 2H), 5.42 (d, J = 51.7 Hz, 1H), 5.07 (s, 2H), 4.58 – 3.98 (m, 7H), 3.77 – 3.43 (m, 16H), 3.39 (t, J = 5.4 Hz, 2H), 3.26 (t, J = 5.2 Hz, 2H), 3.13 – 2.94 (m, 5H), 2.77 – 2.46 (m, 4H), 2.19 – 2.00 (m, 4H), 1.98 – 1.77 (m, 4H). LC-MS (ESI) calcd. for C_{54}H_{64}FN_{9}O_{16}PS_{2}^{+} (M^{+}) 1208.36 and [(M^{+})/2] 604.68, obsd. 604.92, 1208.42
**Figure S1.** Time course of labeling WT CD38 with Rh-(6-F-araNAD) and SR101-F-araNMN. All time points are in minutes. (A) Labeling reaction with SR101-F-araNMN. (B) Labeling reaction with SR101-F-araNMN and 1 mM NAD. (C) Labeling reaction with SR101-F-araNMN and 100 μM nicotinamide. (D) Labeling reaction with Rh-F-araNAD. Protein ladder is on the left, listed first.

**Experimental procedure associate with Figure S1:** Fluorescent labeling of purified CD38 in vitro. CD38 (1 μM) and fluorescent probe (either SR101-F-araNMN or Rh-F-araNAD, at 10 μM) in 10 μL reaction buffer (25 mM HEPES, 50 mM NaCl, pH 7.4) were incubated at room temperature. To determine whether NAD or nicotinamide affected fluorescent labeling efficiency, NAD (1 mM) or nicotinamide (100 μM) was added simultaneously with SR101-F-araNMN to the reaction mixture. Then, samples were mixed with 2 μL 6x protein loading buffer to quench the reaction at specific time points. The samples were heated at 100°C for 10 min and then resolved by SDS-PAGE. Before staining with Coomassie blue, the fluorescence image of the gel was recorded using a Typhoon 9400 Variable Mode Imager with settings of Green laser at 532 nm and emission collection filter of 610BP30. Detection was done using an approximate PMT650V (normal sensitivity), and data was analyzed by ImageQuant TL v2005.
**Figure S2.** In-gel fluorescence analysis for labeling CD38 with SR101-F-araNMN in either live cells or whole cell lysate. Lysis 1 contained less protease inhibitors (only protease inhibitor cocktail or PIC used at 100x) than Lysis 2 (contained PIC at 20x, phosphatase inhibitor at 100x, PMSF at 1.0 mM, EDTA at 10 mM). Therefore, lysates from Lysis 2 showed a weaker fluorescent band around 20 kD than in Lysis 1, indicating that this 20 kD band was from CD38 cleavage. Lanes 1: live cell labeling then in-gel fluorescence analysis of the whole cell lysates; Lanes 2: whole cell lysate was obtained first followed by probe labeling; Lanes 3: whole cell lysate without CD38 probe. Protein ladder is on the left.
Figure S3. In-gel fluorescence analysis of CD38 in HL-60 (RA and untreated) and Raji cells to compare amount of intracellular versus cell surface CD38. Lanes 1, cell surface CD38 was blocked with 6-alkyne-F-araNAD then labeling intracellular CD38 with SR101-F-araNMN (fluorescence indicating intracellular CD38); Lanes 2, cells were only labeled with SR101-F-araNMN (fluorescence indicating all CD38); Lanes 3, No labeling molecules were added to the cells. Protein ladder is on the left.

Experimental procedure associate with Figure S3: In-gel fluorescence analysis of intracellular CD38 using 6-alkyne-F-araNAD and SR101-F-araNMN. Lysis buffer recipe: Tris-HCl pH 7.9 (25 mM), NaCl (150 mM), Glycerol (10%), Igepal (1%), 25 uL protease inhibitor cocktail (PIC, Sigma-Aldrich, #P8340)/500 uL, PMSF (1.0 mM), EDTA pH 8.0 (5 mM). HL-60 cells were treated with 1 μM RA in cell culture media for 24 h in a 5% CO2 incubator at 37 °C. Raji cells were not treated with RA. Untreated HL-60 and Raji cells were cultured using the same media except that no RA was used. Cells were harvested by centrifugation at 25 °C, 1500 rpm for 5 min. The cell pellet was washed with 1 mL of PBS. The cells were resuspended in 100 μL PBS and 6-alkyne-F-araNAD was added to a final concentration of 10 μM. Following incubation, the cells were collected at 25 °C, 2000 rpm for 2 min. Then, the reaction mixture was removed. The cell pellet was washed once with PBS to remove any residual 6-alkyne-F-araNAD. The 6-alkyne-F-araNAD incubation was not done for those samples that only received SR101-F-araNMN labeling. Cells were resuspended in 100 μL of fresh PBS (reaction volume), and SR101-F-araNMN was added to a final concentration of 10 μM and allowed to incubate at RT for 8 min. Following incubation, cells were collected by centrifugation at 25 °C, 2000 rpm for 2 min. The cell pellet was washed twice using 1 mL of PBS. Then, about 30 μL lysis buffer followed by freeze/thaw lysis (the samples were frozen at -80 °C and then removed from -80 °C and thawed on ice for ~30 min while vortexing briefly every 5 min). Once samples were fully thawed on ice, centrifuged at 4 °C, 14,000 rpm for 10 min to collect the supernatant (proteins solubilized by detergent in lysis buffer). Protein concentration in the cell lysate was determined using the Bradford assay, and protein lysate was mixed with 2 μL of 6x SDS-containing protein loading buffer. The samples were heated at 100 °C for 10 min and then resolved by SDS-PAGE. Before staining with Coomassie blue, the fluorescence image
of the gel was recorded using a Typhoon 9400 Variable Mode Imager with settings of Green laser at 532 nm and emission collection filter of 610BP30 (sulforhodamine). Data was analyzed using ImageQuant TL v2005.

Figure S4. Confocal images of Raji cells fixed and permeabilized with paraformaldehyde (PFA) followed by methanol after labeling of CD38 with SR101-F-araNMN in live cells. (A) Only SR101-F-araNMN was used for labeling of CD38. Confocal microscope settings: laser power: 6.0%, pinhole: 1.1 airy units, master gain for PMT: 860. (B) 6-Alkyne-F-araNAD was first used to label plasma membrane CD38 and then SR101-F-araNMN was used to label intracellular CD38. Confocal microscope settings: laser power: 24%, pinhole: 1.1 airy units, master gain for PMT: 1060. An increase in laser power and master gain was necessary in order to have enough fluorescence emission to see the signal. This indicates a low amount of intracellular active CD38.

Experimental procedure associate with Figure S4: SR101-F-araNMN labeling of CD38 in live Raji cells followed by fixation with PFA and methanol. Raji cells were kept in cell culture media (GIBCO RPMI Medium 1640 with 10% GIBCO Heat-inactivated Fetal Bovine Serum) in a 5% CO2 incubator at 37 °C. The cells were harvested from 4 mL cell culture (1 × 10^6 cells/mL) by centrifugation at 25 °C, 1500 rpm for 5 min. The cell pellet was washed using 1 ml of PBS. Cells were suspended in 100 μL PBS (reaction volume). In one experimental procedure, these cells were directly incubated with 10 μM SR101-F-araNMN at RT for 8 min to label all the CD38 molecules. In another experimental procedure, the cells were first treated with the non-fluorescent and cell impermeable probe, 6-alkyne-F-araNAD at 10 μM at RT for 8 min, to block the cell surface CD38. Following incubation with 6-alkyne-F-araNAD, cells were collected by centrifugation at 25 °C, 2000 rpm for 2 min. The cell pellet was washed using 1 mL of PBS. Then, cells were suspended in 100 μL fresh PBS (reaction volume), and SR101-F-araNMN was added to a final concentration of 10 μM and allowed to incubate at RT for 8 min. For both experiments, after incubation with SR101-F-araNMN, the cells were washed twice using 1 mL cold PBS (PBS at 4 °C) and then suspended in 1 mL of 2% paraformaldehyde (PFA). Cells suspended in PFA were left on ice for 5 min followed by 10 min at RT. Following PFA fixation, cells were collected by centrifugation at 4 °C, 2000 rpm for 4 min. Cells were washed three times with 1 mL cold PBS (PBS at 4 °C) to wash away PFA. Finally, cells were suspended in 1 mL of cold methanol (methanol stored in -20 °C for at least 1 h prior to use) and held at -20 °C for 2 min. Methanol was removed by centrifugation at 4 °C, 2500 rpm for 5 min. Cells were then suspended in fresh 1 mL of cold methanol (stored in -20 °C for at least 1 h prior to use) and
incubated on ice for 40 min. Methanol was then removed by centrifugation at 4 °C, 2500 rpm for 5 min. Cells were washed once with 1 mL cold PBS (PBS at 4 °C). Finally, cells were suspended in 100 µL of PBS. Then, 10 µL of the cell suspension was applied onto a microscope slide and covered with a micro cover glass. Confocal images (8 line average) of cells were acquired with a Zeiss LSM 710 Confocal Microscope with a 63x/1.4 oil immersion objective. Green 561 nm (DPSS laser) was used for sulforhodamine (SR) fluorescence. Emission signals in the range of 566-717 nm (SR emission) were detected.

Figure S5. Western blot analysis following subcellular fractionation of Raji and RA treated HL-60 cells. Subcellular fractions were Nucleus (N), Membrane (M) and Cytosolic (C). Whole cell lysate (W) was run as a positive control. (A) Blotting for CD38. (B) Blotting for Histone 3 as a nucleus marker protein. (C) Blotting for Na⁺/K⁺ ATPase α1 as a plasma membrane marker protein. Image J quantification was done to analyze band intensity for CD38 and Na⁺/K⁺ ATPase α1 between nucleus and membrane fractions. Using ImageJ quantification of protein band intensity and relating CD38 band intensity to CD38 amount, showed the nucleus fraction for RA-treated HL-60 cells contained 22% of that present in the membrane fraction. CD38 amount in the nucleus fraction for Raji cells contained 23% of that present in the membrane fraction. However, the nucleus fraction for RA-treated HL-60 cells contained about 23% of the plasma membrane marker protein compared to that contained in the membrane fraction. Also, the nucleus fraction for Raji cells contained 21% of the plasma membrane marker protein compared to that in the membrane fraction. Therefore, the presence of CD38 in the nucleus fraction is mostly from contamination of the nucleus fraction from proteins that should have been collected in the membrane fraction alone.

Experimental procedure associate with Figure S5: Subcellular fractionation of RA HL-60 and Raji cell lysates and detection of CD38 in different subcellular fractions. All buffers were supplemented with 1x protease inhibitor cocktail (PIC, Sigma-Aldrich, #P8340), 1 mM
PMSF and 1 mM DTT before use. Recipes for the buffers used within the protocol include: **buffer 1** – Sucrose (250 mM), Tris-HCl pH 7.9 (50 mM), MgCl₂ (5 mM); **buffer 2a** – Sucrose (1 M), Tris-HCl pH 7.9 (50 mM), MgCl₂ (5 mM); **buffer 2b** – Sucrose (2 M), Tris-HCl pH 7.9 (50 mM), MgCl₂ (5 mM); **buffer 4** – HEPES (20 mM), MgCl₂ (1.5 mM), NaCl (0.5 M), EDTA (0.2 mM), Glycerol (20%), Triton X-100 (1%); **buffer 3** – Tris-HCl pH 7.9 (20 mM), NaCl (0.4 M), Glycerol (15%), Triton X-100 (1.5%). HL-60 cells were treated with 1 μM RA in cell culture media for 24 h in a 5% CO₂ incubator at 37 °C. Raji cells were not treated with RA. Both Raji and RA treated HL-60 cells (~3.0 x 10⁷) were washed twice with PBS and pelleted for 4 min at 500 g. The cell pellet was suspended in 1 ml buffer 1 and cell lysis was performed by slowly pushing lysate through a 25 gauge needle. To ensure cell lysis has occurred, 10 μL of the lysate was loaded onto a hemocytometer and examined under an optical microscope. The suspension was centrifuged at 800 g for 15 min to obtain supernatant 1 and pellet 1. Supernatant 1 was centrifuged again at 1,000 x g for 15 min to obtain supernatant 2 and pellet 2. Supernatant 2 was saved to isolate the cytosolic and membrane proteins, whereas pellet 2 was discarded. Pellet 1 was dissolved in 1 ml buffer 1 and centrifuged at 1,000 x g for 15 min to obtain supernatant 3 and pellet 3. Supernatant 3 was added to supernatant 2 for isolating cytosolic and membrane proteins and stored on ice until later use. Pellet 3 (used to isolate nuclei) was suspended in 1 ml buffer 2a and layered onto a 3 ml cushion of buffer 2b in a 5.0 mL ultracentrifuge tube (thinwall polyallomer tube, Beckman Coulter cat. #: 326819). Afterwards, swing-bucket ultracentrifugation (Beckman Coulter SW 55 Ti rotor) at 80,000 x g for 35 min was carried out to obtain supernatant 4 and pellet 4. Pellet 4 (the nuclear pellet) was taken up in buffer 4 and incubated 1 h while on a rotating mixer at 4 °C. Then, the suspension was slowly passaged 20 times through a 25 gauge needle followed by centrifugation at 9,000 x g for 30 min to obtain supernatant 5 and pellet 5. Supernatant 5 contained the nuclear proteins. Pellet 5 was discarded. The pooled supernatants 2 and 3 were centrifuged for 1 h at 100,000 x g in a swing-bucket (SW 55 Ti rotor) ultracentrifuge to obtain supernatant 6 and pellet 6. Supernatant 6 contained the cytosolic proteins. Pellet 6 was dissolved in buffer 3 and incubated 1 h while on a rotating mixer at 4 °C. The suspension was centrifuged at 9,000 x g for 30 min to obtain supernatant 7 and pellet 7. Supernatant 7 contained the membrane proteins. Pellet 7 was discarded. Then, 2.0% of each fraction was resolved by SDS-PAGE analysis and transferred to a PVDF membrane. Membranes were then blocked for 1 h in a solution of 5% dry non-fat milk in TBS-Tween before probing with primary antibody in 5% dry non-fat milk in TBS-Tween for 1 h at RT. Membranes were probed with secondary antibodies in TBS-Tween for 1 h at RT before visualizing with ECL. Antibodies used for the Western blotting include: mouse anti-human CD38 from BD Biosciences (cat. #: 611114) used at 1/2000 dilution (v/v), Histone H3 (nuclear protein marker) antibody from Cell Signaling (cat. #: 9715) used at 1/5000 dilution (v/v), Na+/K+ ATPase (membrane protein marker) antibody from Cell Signaling (cat. #: 3010) used at 1/1000 dilution (v/v), as well as horseradish peroxidase-linked anti-mouse and anti-rabbit antibodies from Santa Cruz (cat. #: sc-2005 and sc-2004, respectively).
**Figure S6.** In-gel fluorescence analysis for SR101-F-araNMN specificity for CD38. Lanes 1, no SR101-F-araNMN added to the cells; Lanes 2, SR101-F-araNMN labeling live cells before collecting whole cell lysate. Protein ladder is on the left, listed first.

**Figure S7.** In-gel fluorescence showing molecular weight range from 150 to 20 kD

**References cited in Supporting Information:**

(1) Marom, H.; Miller, K.; Bechor-Bar, Y.; Tsarfaty, G.; Satchi-Fainaro, R.; Gozin, M. J Med Chem 2010, 53, 6316.