

# Supramolecular Metal Displacement allows On-Fluorescence Analysis of Manganese(II) in Living Cells

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## Supporting information

### Materials.

The cell culture medium Dulbecco's modified Eagle's medium (DMEM-F12) with L glutamine, FBS (fetal bovine serum), F12 and F12K medium, FluoZin-1 acetoxymethyl ester (Fz1AM), and Pluronic acid F127 were obtained from Invitrogen. Calcein blue acetoxymethyl ester (CBAM) was obtained from Sigma, and TransIT-LT1 transfection reagent was obtained from Mirus. Intracellular fluorescence was visualized using a Leica DMIRE2 fluorescence microscope equipped with DAPI LP (excitation filter 340-380 nm) and FITC BP (excitation filter 480-540 nm) filters whose parameters were optimal for CB and Fz1, respectively. High resolution images were acquired on a TE2000-U inverted microscope (Nikon Instruments Inc.) using a Sony Cool Snap ES 1300 × 1030 camera controlled by MetaMorph software.

### Association Constants.

Association constants were determined by direct titration or by competition in 50 mM HEPES buffer at pH 7.2 and 100 mM KNO<sub>3</sub>. Data in Figures S1-3 were used to determine affinity constants by nonlinear least squares fitting of the 1:1 binding isotherm. (Connors, K. A. *Binding constants: the measurement of molecular complex stability*, Wiley New York; 1987.) The binding affinity was obtained by fitting fluorescence titration data with the following equation in which  $F_0$  and  $F_{\max}$  are the minimum and maximum fluorescence, respectively, and  $[L]_t$  is total concentration of the dye,  $[M^{2+}]_t$  is the total concentrations of the metal ion, and  $K_d$  is the dissociation constant.

$$F = F_0 + (F_{\max} - F_0) \times \frac{([L]_t + [M^{2+}]_t + K_d) - \sqrt{([L]_t + [M^{2+}]_t + K_d)^2 - 4[L]_t[M^{2+}]_t}}{2 \times [L]_t}$$

### Cell culture.

Cells were kept at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>. For imaging experiments, the cells were grown in 96-well plates for 2 days, reaching 60-70% confluence. HEK 293 cells were cultured in DMEM F12 medium supplemented with 10% of FBS. A549 cells were cultured in F12K medium supplemented with 10% of FBS.

### A549 transient transfection.

For transient transfection A549 was grown to a confluence of 40-50%. A solution was prepared as follows: 9 mL of free FBS F12K medium, 0.28 mL TransIT-LT1, 0.1 mL of DNA (DMT1, IRES, GFP, 1 mg/mL). The solution was left for 40 minutes at room temp and then added to 92 mL of regular medium. The cells were incubated for 36/48 hours and then washed with regular medium. The yield of transfection was around 25%.

### **A549 Mn<sup>2+</sup> measurements.**

Transiently transfected cells were washed with F12 medium supplemented with 10% of FBS and a solution of MnCl<sub>2</sub> (0.0001 M) was added to reach a concentration of 5 mM. The cells were incubated for three hours at 37 °C. A solution of dyes was prepared in HBSS with 4.4 mM of Fz1AM (from a 0.005 M stock solution in DMSO), 22.2 mM of CBAM (from a 0.005 M stock in DMSO), and 4.4 mM of Pluronic acid F127. The cells were carefully washed and a solution of the two dyes was added. The cells were incubated for one hour, washed carefully and a solution of CdCl<sub>2</sub> (0.0001 M) was added to reach a concentration of 2.5 mM. The cells were incubated for one additional hour and washed.

### **HEK 293 Mn<sup>2+</sup> measurements.**

The experiment was run in a 96-well plate with a confluence of 60-70%. The cells were washed and a solution of MnCl<sub>2</sub> (0.0001 M) was added. The cells were incubated for three hours at 37°C and 5% CO<sub>2</sub>. The cells were washed carefully and an aliquot of the solution of the two dyes was added. A solution of dyes was prepared in HBSS with 3.6 mM for Fz1AM, 26.6 mM of CBAM, 3.6 mM of Pluronic acid F127. The cells were incubated for one hour, washed carefully and a solution of CdCl<sub>2</sub> (0.0001 M) was added to reach a concentration of 10 mM. The cells were incubated for one additional hour and washed. For all the experiments pictures were taken with bright field, green field and blue field.

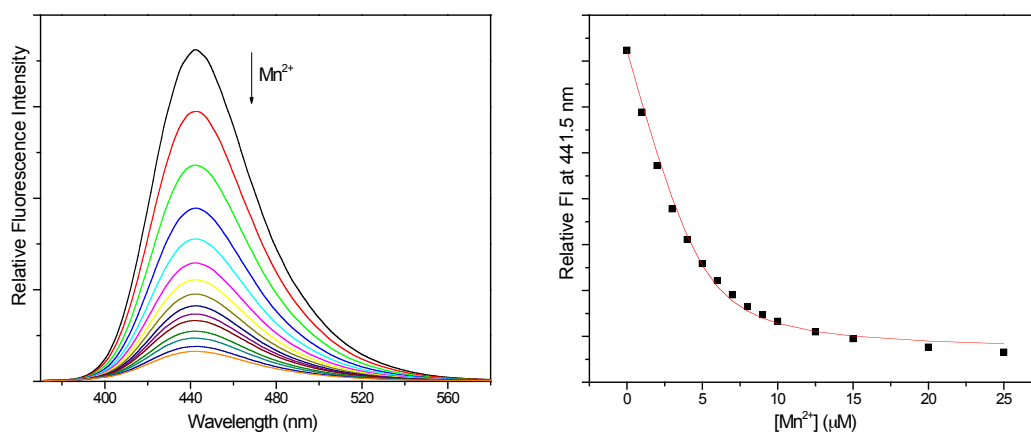
### **Quantitation.**

The experiment was run in a 96-well plate using eight wells for each concentration of Mn<sup>2+</sup> with a confluence of 60-70%. The cells were washed and a solution of MnCl<sub>2</sub> (0.0001 M) was added to reach the desired concentration (1 mM, 5 mM, 10 mM, 50 mM). The cells were incubated for three hours at 37°C

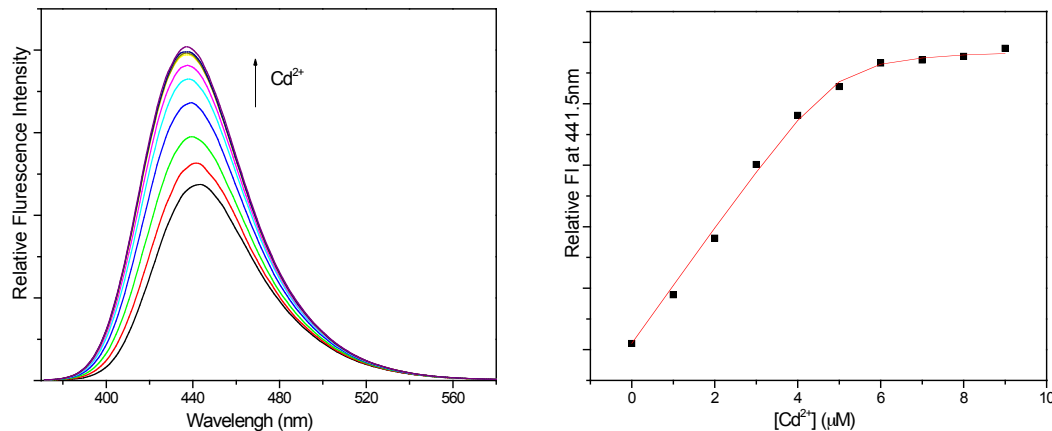
and 5% CO<sub>2</sub>. The cells were washed carefully and an aliquot of the solution of the two dyes was added. A solution of dyes was prepared in HBSS with 3.6 mM for Fz1AM, 26.6 mM of CBAM, 3.6 mM of Pluronic acid F127. The cells were incubated for one hour, washed carefully and a solution of CdCl<sub>2</sub> (0.0001 M) was added to reach a concentration of 10 mM. The cells were incubated for one additional hour and washed.

### **Statistical Analysis:**

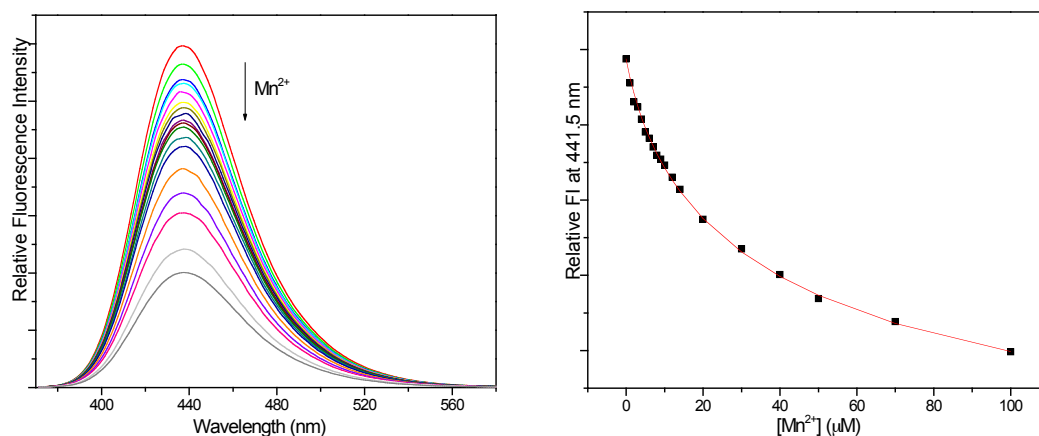
Significance was determined by a Student's two tailed T test with a threshold p-value of  $p \leq 0.05$  (less than 5% chance that the null hypothesis is true). Error bars are expressed as standard deviation from the mean.



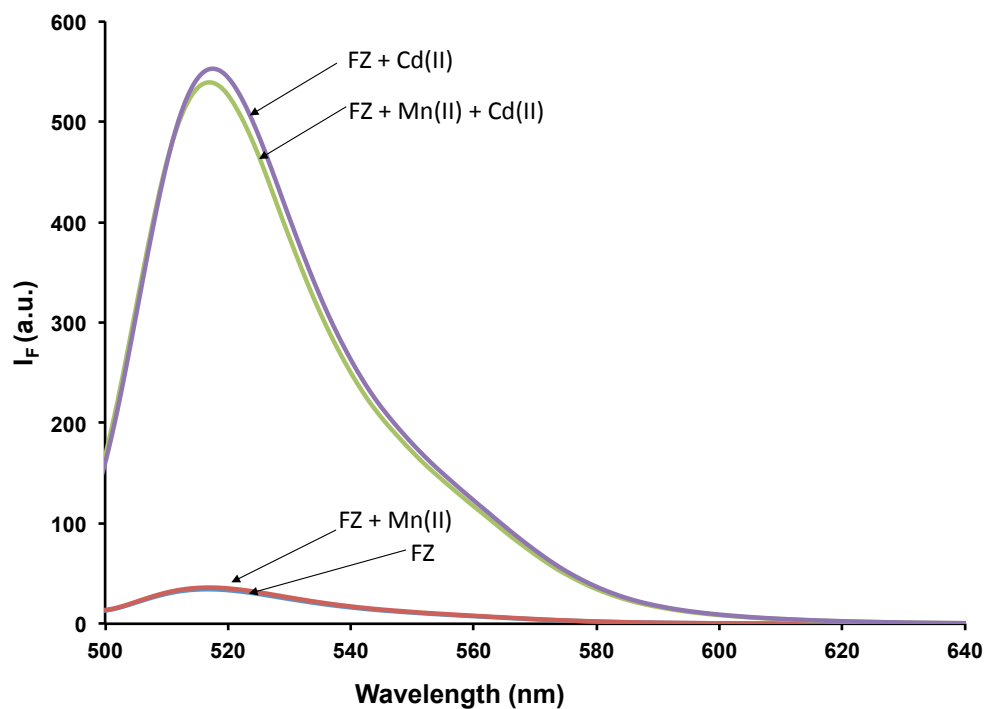
**Figure S1.** Fluorescent response for the direct titration of an aqueous solution (pH=7.2) containing 5  $\mu\text{M}$  Calcein Blue, 50 mM HEPES and 100 mM  $\text{KNO}_3$  with  $\text{MnCl}_2$ . (left) Spectra contain 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12.5, 15, 20 and 25  $\mu\text{M}$   $\text{MnCl}_2$ , excitation was at 350 nm. (right) Plot of relative fluorescent intensity of Calcein Blue (5  $\mu\text{M}$ ) at 441.5 nm as a function of manganese concentration.



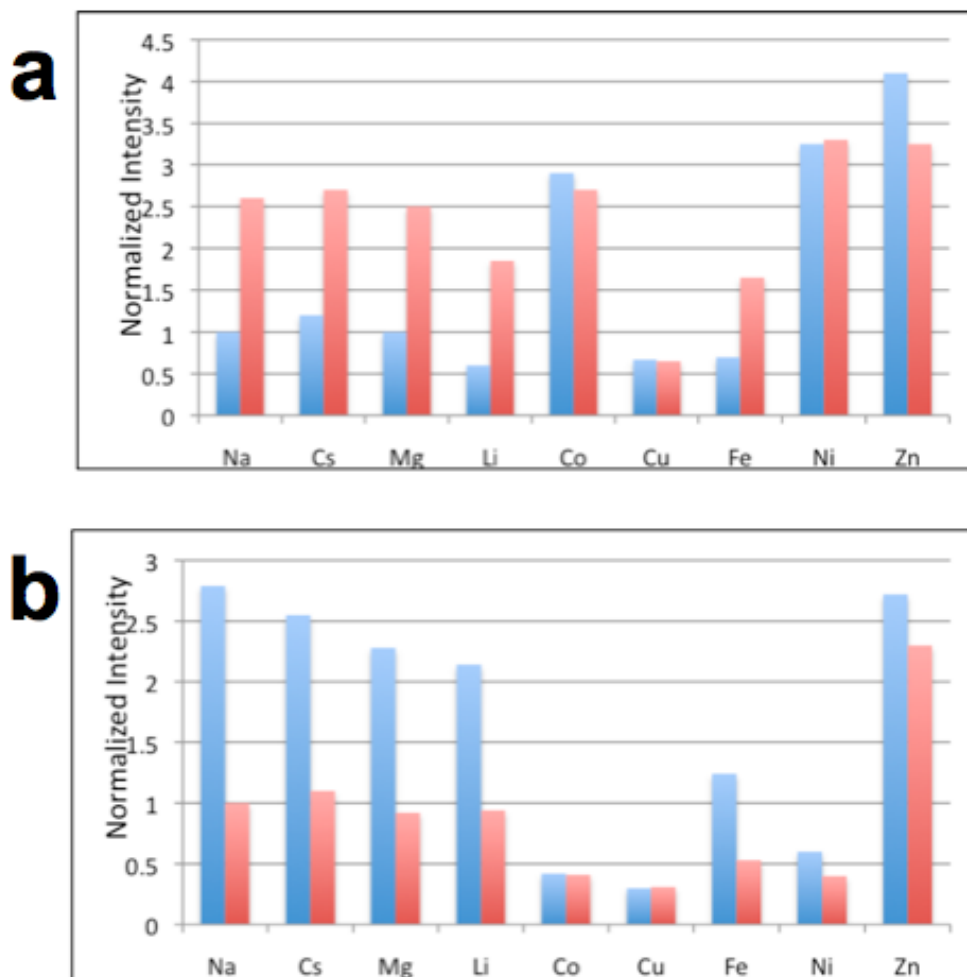
**Figure S2** Fluorescent response for the direct titration of aqueous solution (pH=7.2) containing 5  $\mu\text{M}$  Calcein Blue, 50 mM HEPES and 100 mM  $\text{KNO}_3$  with  $\text{Cd}(\text{ClO}_4)_2$ . (left) Spectra contain 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9  $\mu\text{M}$   $\text{Cd}(\text{ClO}_4)_2$ , excitation was at 350 nm. (right) Plot of relative fluorescent intensity of Calcein Blue (5  $\mu\text{M}$ ) at 441.5 nm as a function of  $\text{Cd}^{2+}$  concentration.



**Figure S3.** Fluorescent response for the direct competition titration of aqueous solution (pH=7.2) containing 5  $\mu M$  Calcein Blue, 7  $\mu M$   $Cd(ClO_4)_2$ , 50 mM HEPES and 100 mM  $KNO_3$  with  $MnCl_2$ . (left) Spectra contain 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 20, 30, 40, 50, 70 and 100  $\mu M$   $MnCl_2$ , excitation was at 350 nm. (right) Plot of relative fluorescent intensity at 441.5 nm as a function of manganese concentration.

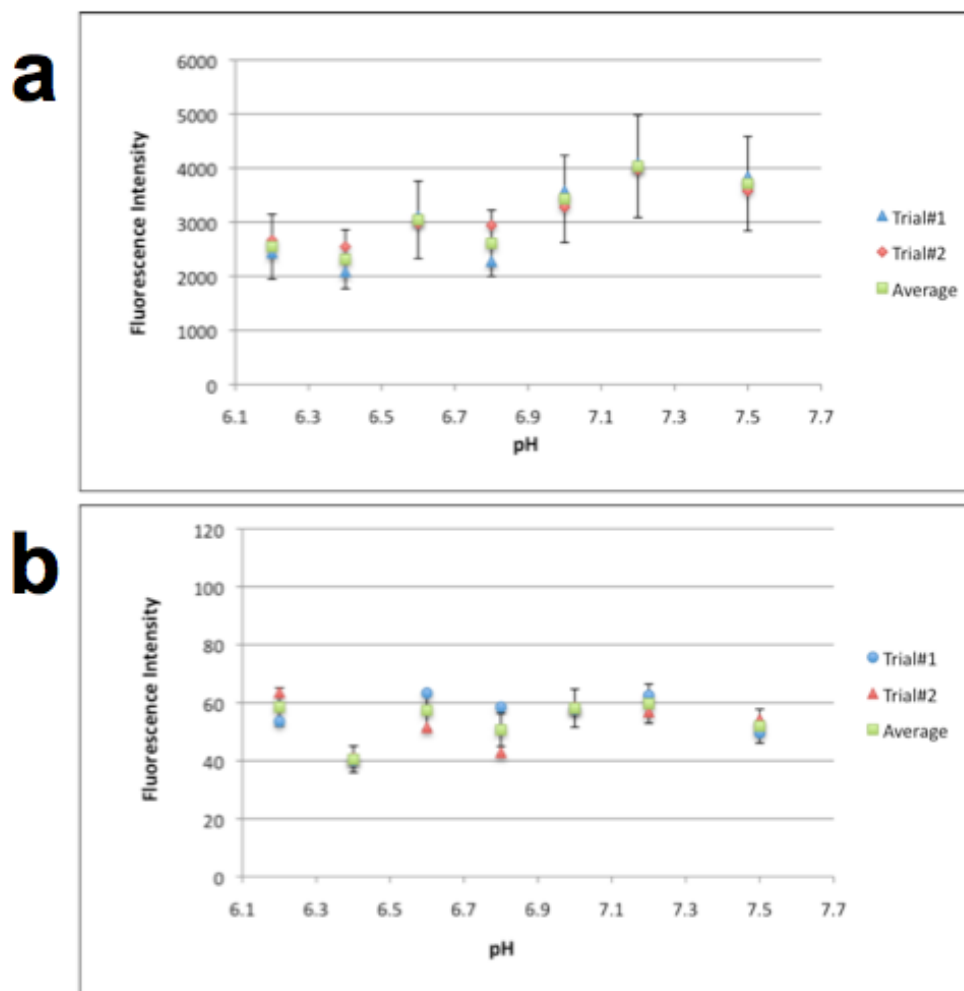


**Figure S4.** Fluorescence spectra of FluoZin-1 (FZ) in the presence or absence of metal ions. Solutions contained 0.1 M  $\text{KNO}_3$ , 50 mM HEPES, pH=7.0, and 10  $\mu\text{M}$  FZ. Where indicated,  $\text{Cd}(\text{ClO}_4)_2$  and  $\text{MnCl}_2$  were present in 10  $\mu\text{M}$  concentration. The affinity of FZ for Mn(II) cannot be accurately determined from these data, but apparently it is 1-2 orders of magnitude weaker than that for Cd(II).

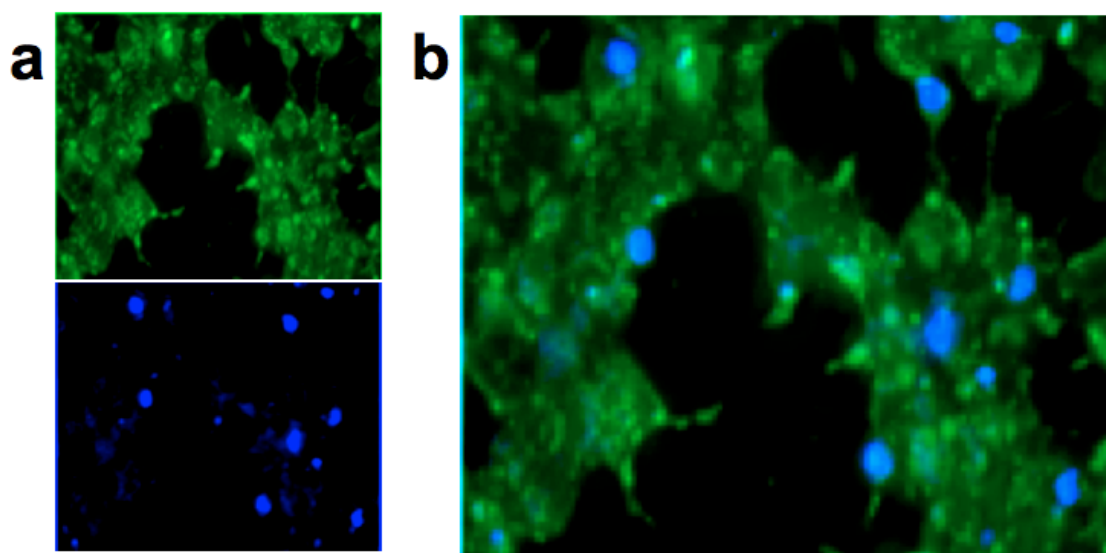


**Figure S5.** Metal-ion response screening for Calcein-Blue/Fluozin-1 system. The relative fluorescent intensity was measured for a solution containing 10 $\mu$ M Fluozin-1, 10 $\mu$ M CalceinBlue, 10 $\mu$ M Cd<sup>2+</sup>, 10 $\mu$ M ion (blue bars) and subsequent addition of 50 $\mu$ M Mn<sup>2+</sup> (red bars). The solutions were prepared in 50mM HEPES, 100mM KNO<sub>3</sub> at pH=7.2. **(a)** Response for excitation at 493 nm and **(b)** for excitation at 350 nm.

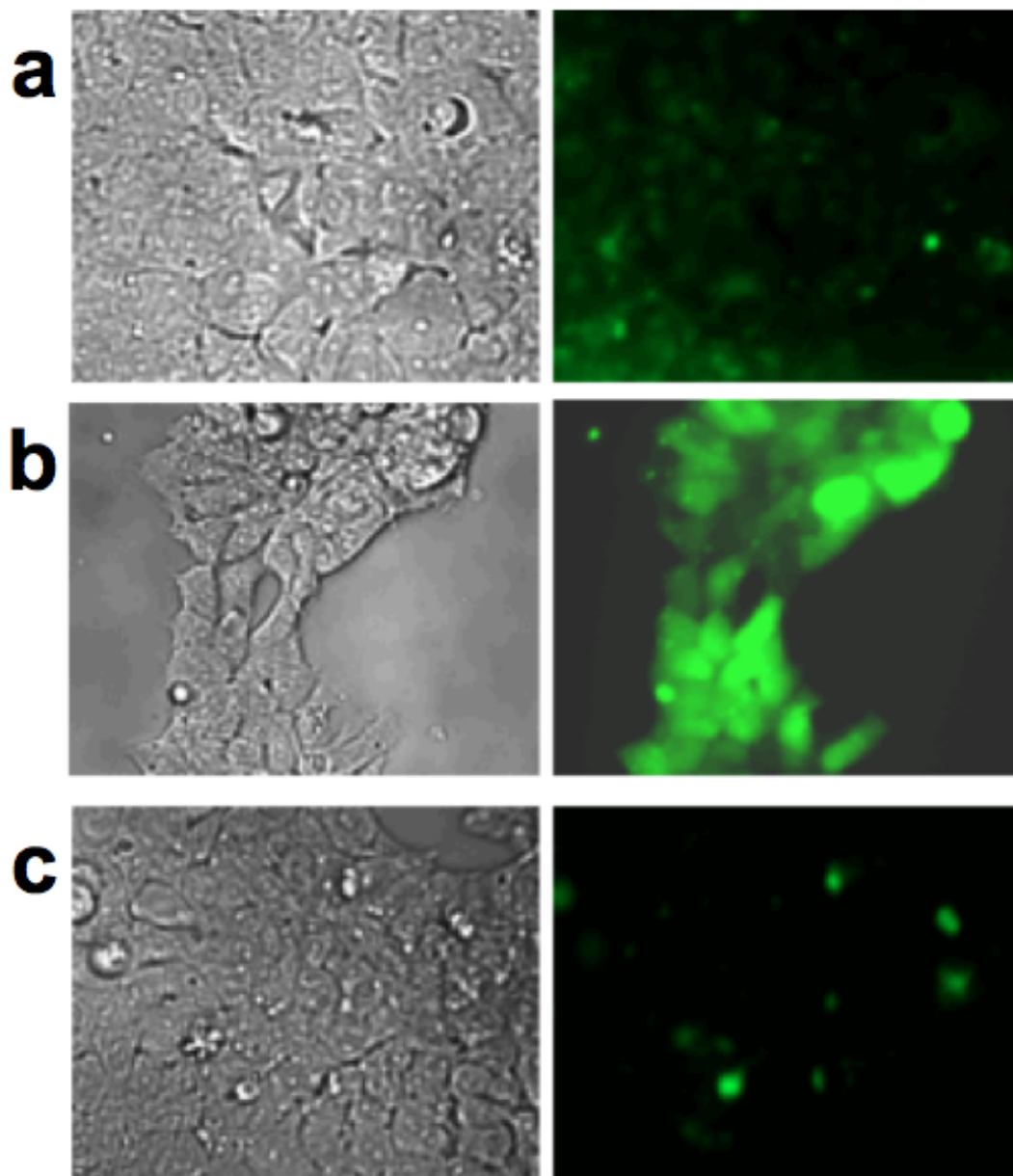




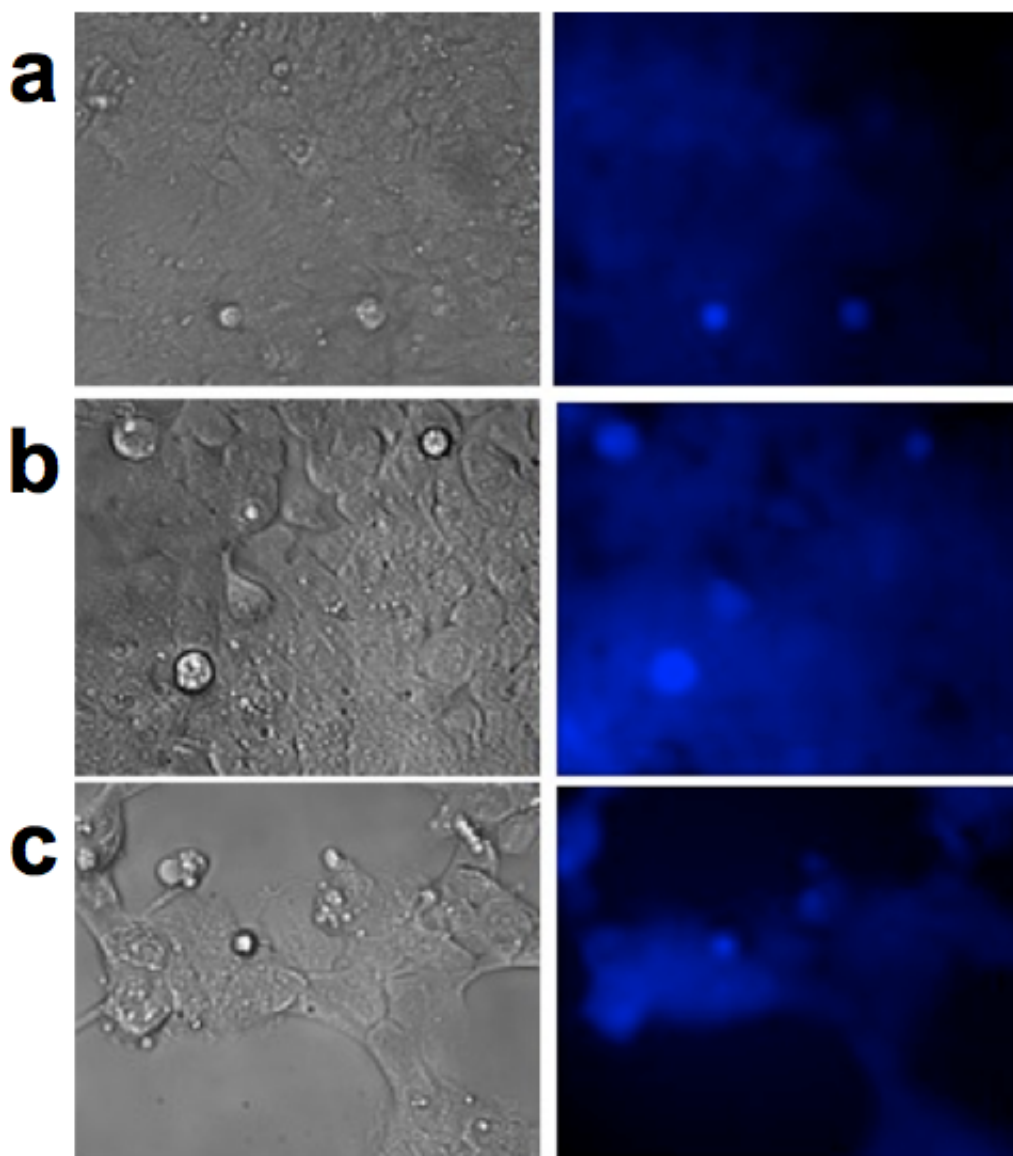
**Figure S6.** Fluorescence Intensity vs. pH for the system containing 10uM FluoZin-1, 10uM Calcein Blue, 10 uM  $\text{Cd}^{2+}$ , 50 uM  $\text{Mn}^{2+}$ . The solutions were made in HEPES buffer (50mM),  $\text{KNO}_3$  (100mM), and the pH was adjusted by adding NaOH. **(a)** Excitation at 350 nm, **(b)** Excitation at 493 nm. Error bars from the pool of sets are shown.



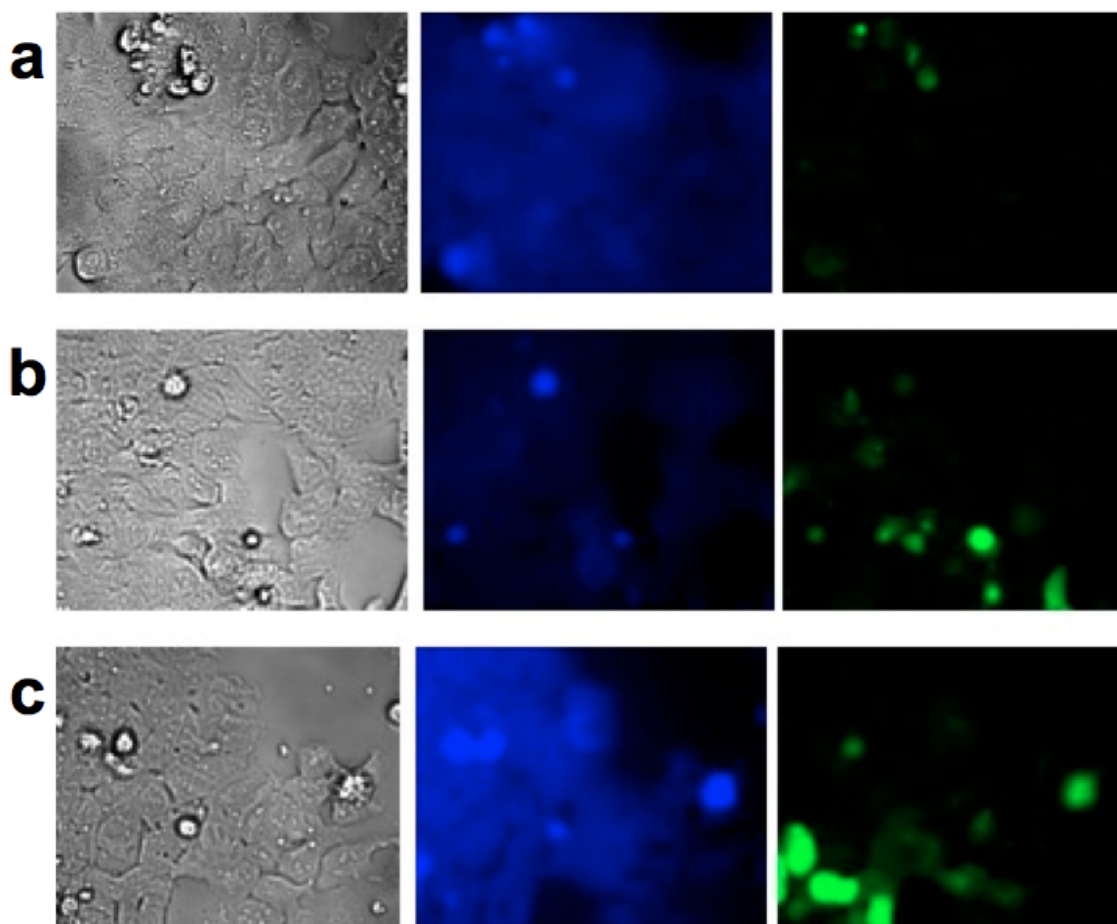
**Figure S7.** Fluorescence microscopy (40X magnification) of *DMT-1* cells treated with 5  $\mu\text{M}$   $\text{Mn}^{2+}$ , 3.6  $\mu\text{M}$  Fz1AM, 26.6  $\mu\text{M}$  CB-AM and 10  $\mu\text{M}$   $\text{Cd}^{2+}$ . **(a)** The assay provides heterogeneous, green on, blue off, fluorescence in the field of view. **(b)** Anticorrelation of blue and green intensity is consistent with differential quenching depending on local  $\text{Mn}^{2+}$  concentration. However, differential distribution of dyes and metals cannot be excluded.



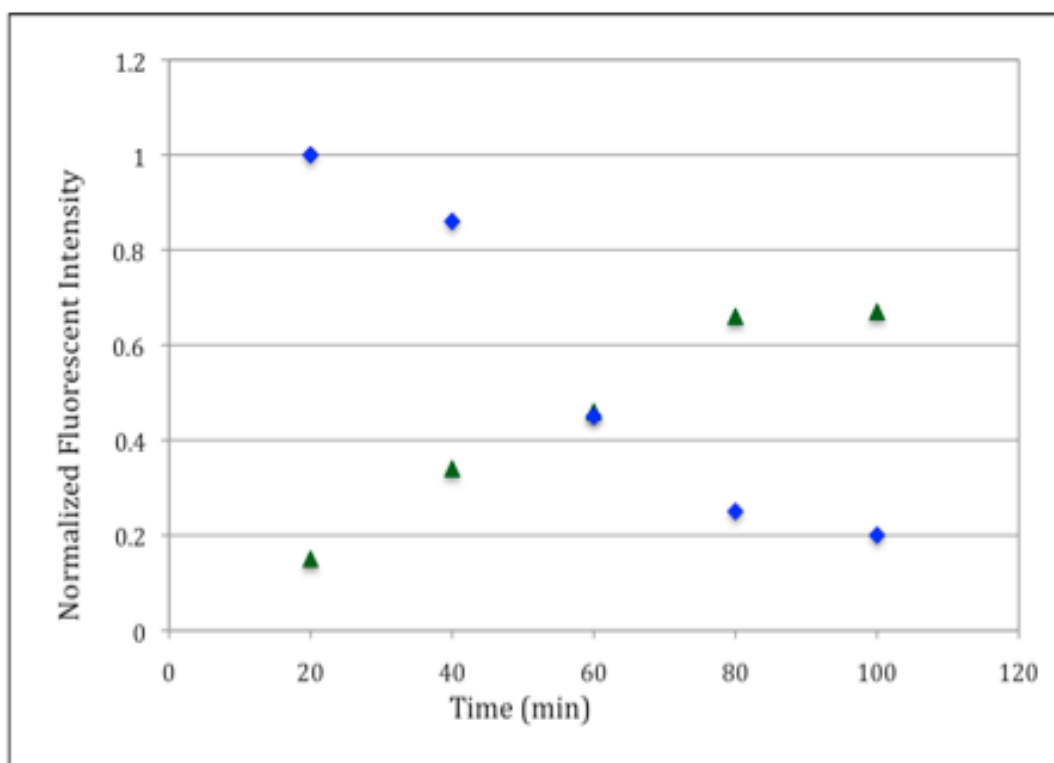
**Figure S8.** Control experiments carried out on *DMT-1* cells with: (a) 3.6  $\mu\text{M}$  Fz1AM ; (b) 3.6  $\mu\text{M}$  Fz1AM and 10  $\mu\text{M}$  of  $\text{Cd}^{2+}$  ; (c) 3.6  $\mu\text{M}$  Fz1AM and 5  $\mu\text{M}$  of  $\text{Mn}^{2+}$  .





**Figure S9.** Control experiments carried out on *DMT-1* cells with: (a) 26.6 μM CBAM ; (b) 26.6 μM CBAM and 10 μM of Cd<sup>2+</sup> ; (c) 26.6 μM CBAM and 5 μM of Mn<sup>2+</sup>

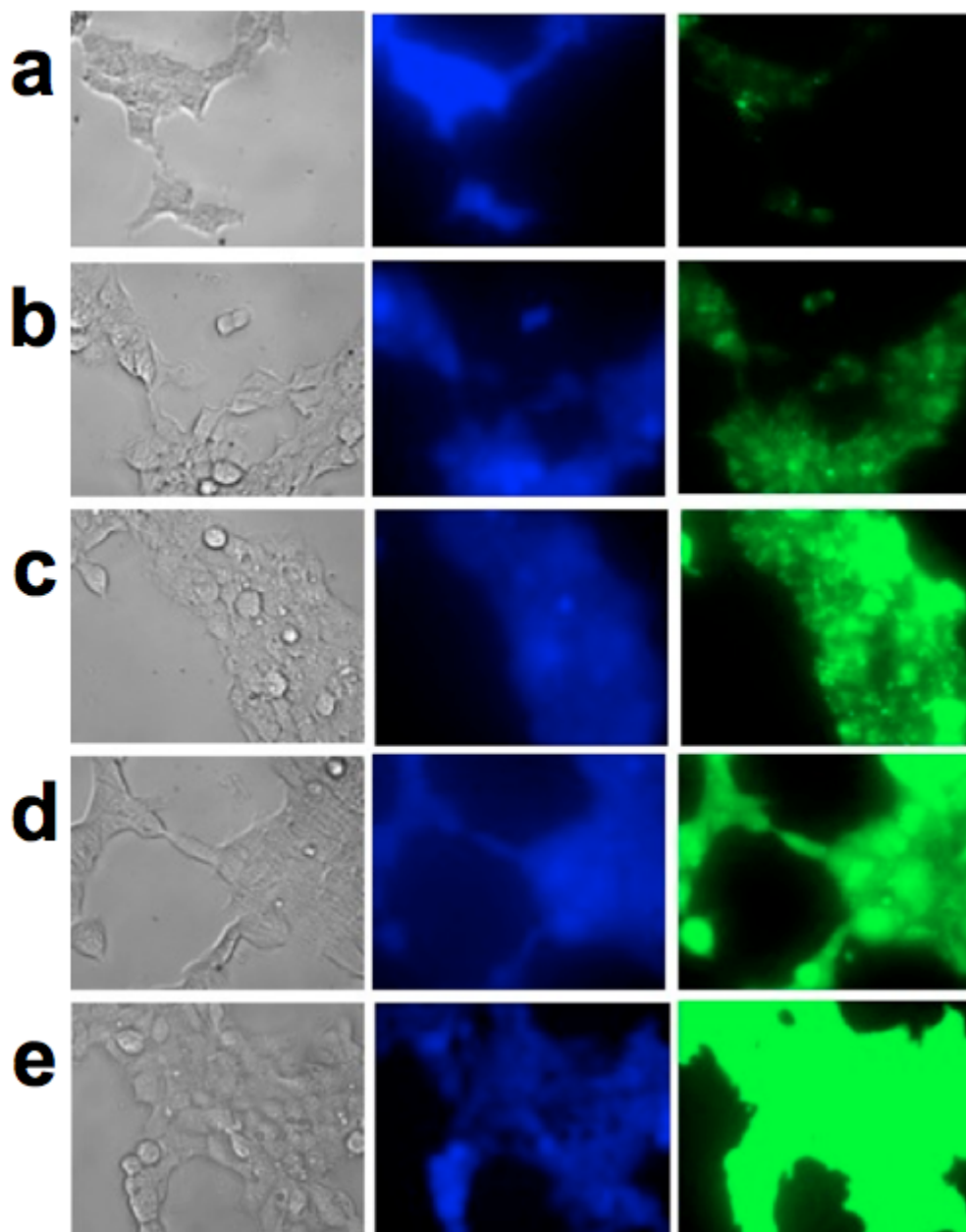


**Figure S10.** Control experiments carried out on *DMT-1* cells with: (a) 26.6  $\mu\text{M}$  CBAM and 3.6  $\mu\text{M}$  Fz1AM; (b) 26.6  $\mu\text{M}$  CBAM, 3.6  $\mu\text{M}$  of Fz1AM and 5  $\mu\text{M}$  of  $\text{Mn}^{2+}$  ; (c) 26.6  $\mu\text{M}$  CBAM, 3.6  $\mu\text{M}$  of Fz1AM and 10  $\mu\text{M}$  of  $\text{Cd}^{2+}$

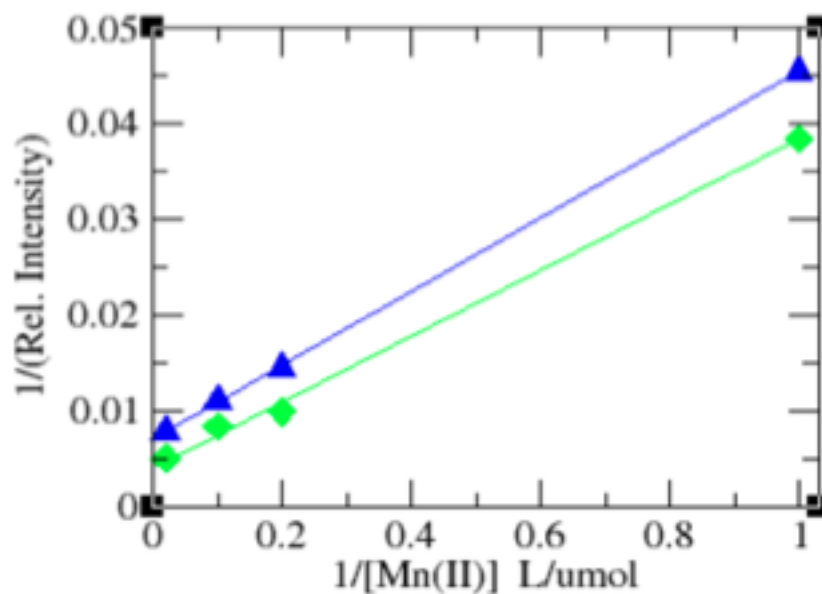


**Figure S11.** Plot showing fluorescent intensity over time for excitation at 493 nm

(  ) and at 350 nm (  )

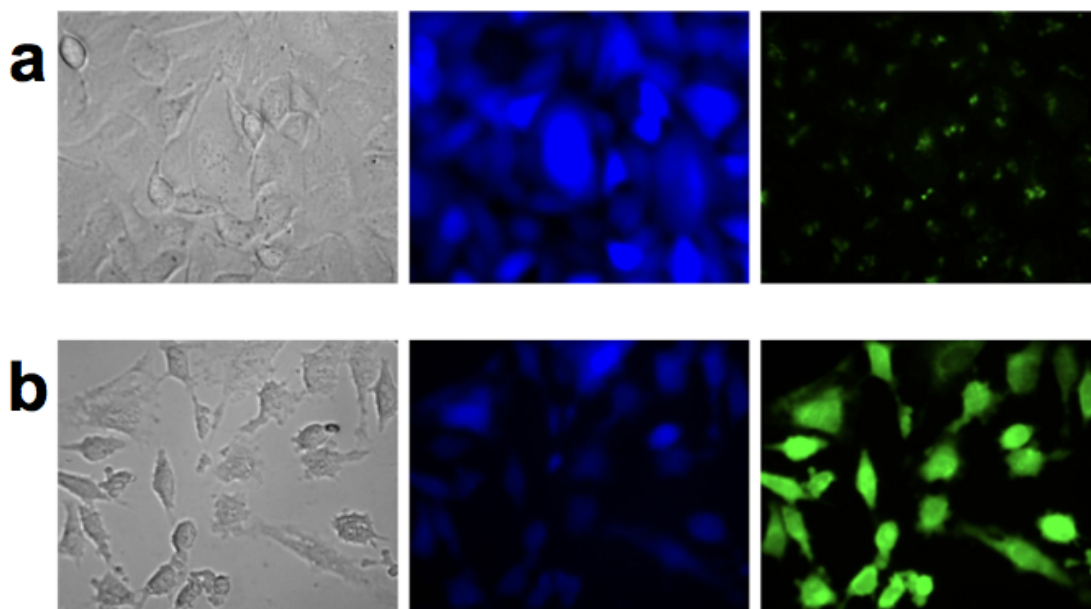


**Figure S12.** Pictures showing green and blue fluorescence in *DMT-1* cells in increasing levels of supplementation: (a) 0  $\mu\text{M}$ , (b) 1  $\mu\text{M}$ , (c) 5  $\mu\text{M}$ , (d) 10  $\mu\text{M}$  and (e) 50  $\mu\text{M}$  of  $\text{Mn}^{2+}$  with 26.6  $\mu\text{M}$  CBAM, 3.6  $\mu\text{M}$  of Fz1AM and 10  $\mu\text{M}$  of  $\text{Cd}^{2+}$ .

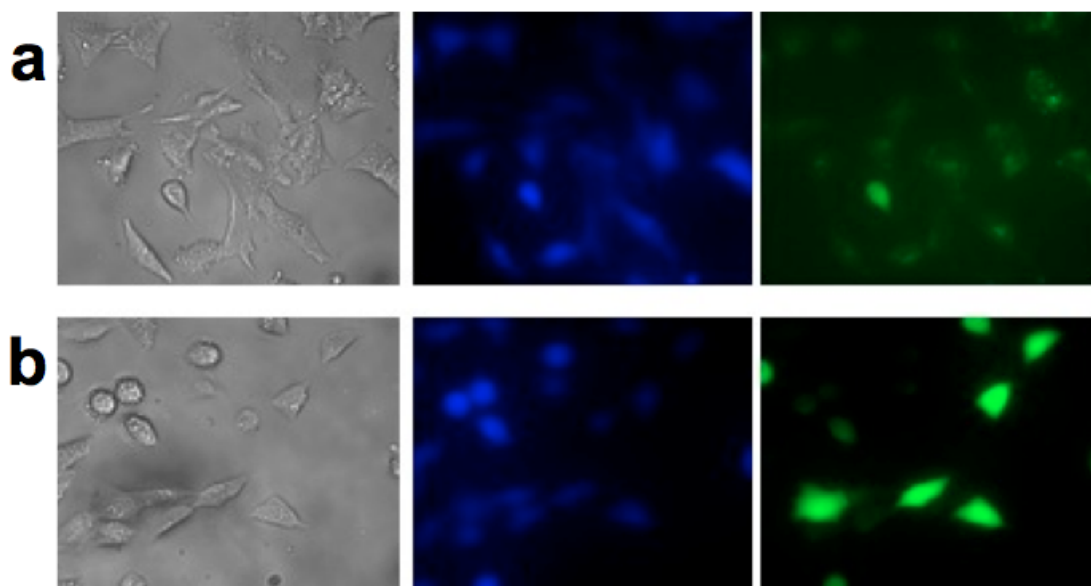


**Figure S13.** Double reciprocal plot of intensity as a function of  $Mn^{2+}$  concentration. Both intensities were corrected for intensity of cells with zero  $Mn^{2+}$ . Lines are simple regression  $R^2$  (green) = 0.9974,  $R^2$  (blue) = 0.999799.





**Figure S14.** Assay of  $\text{Mn}^{2+}$  in A549 cells. Images were obtained upon treating A549 cells with (a) CBAM, Fz1AM and 1 mM  $\text{CdCl}_2$ . (b) CBAM, Fz1AM, 1mM  $\text{CdCl}_2$  and 10 mM of  $\text{Mn}^{2+}$ .



**Figure S15.** A549 cells transfected with DMT-1/GFP: **(a)** 22.2  $\mu\text{M}$  CBAM, 4.4  $\mu\text{M}$  Fz1AM, 2.5  $\mu\text{M}$   $\text{Cd}^{2+}$ ; **(b)** 22.2  $\mu\text{M}$  CBAM, 4.4  $\mu\text{M}$  Fz1AM, 2.5  $\mu\text{M}$   $\text{Cd}^{2+}$  and 5  $\mu\text{M}$   $\text{Mn}^{2+}$ .