Electronic Supplementary Material (ESI) for Metallomics.

The Effect of Metalloprotein Inhibitors on Cellular Metal Ion Content and Distribution

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SUPPORTING INFORMATION

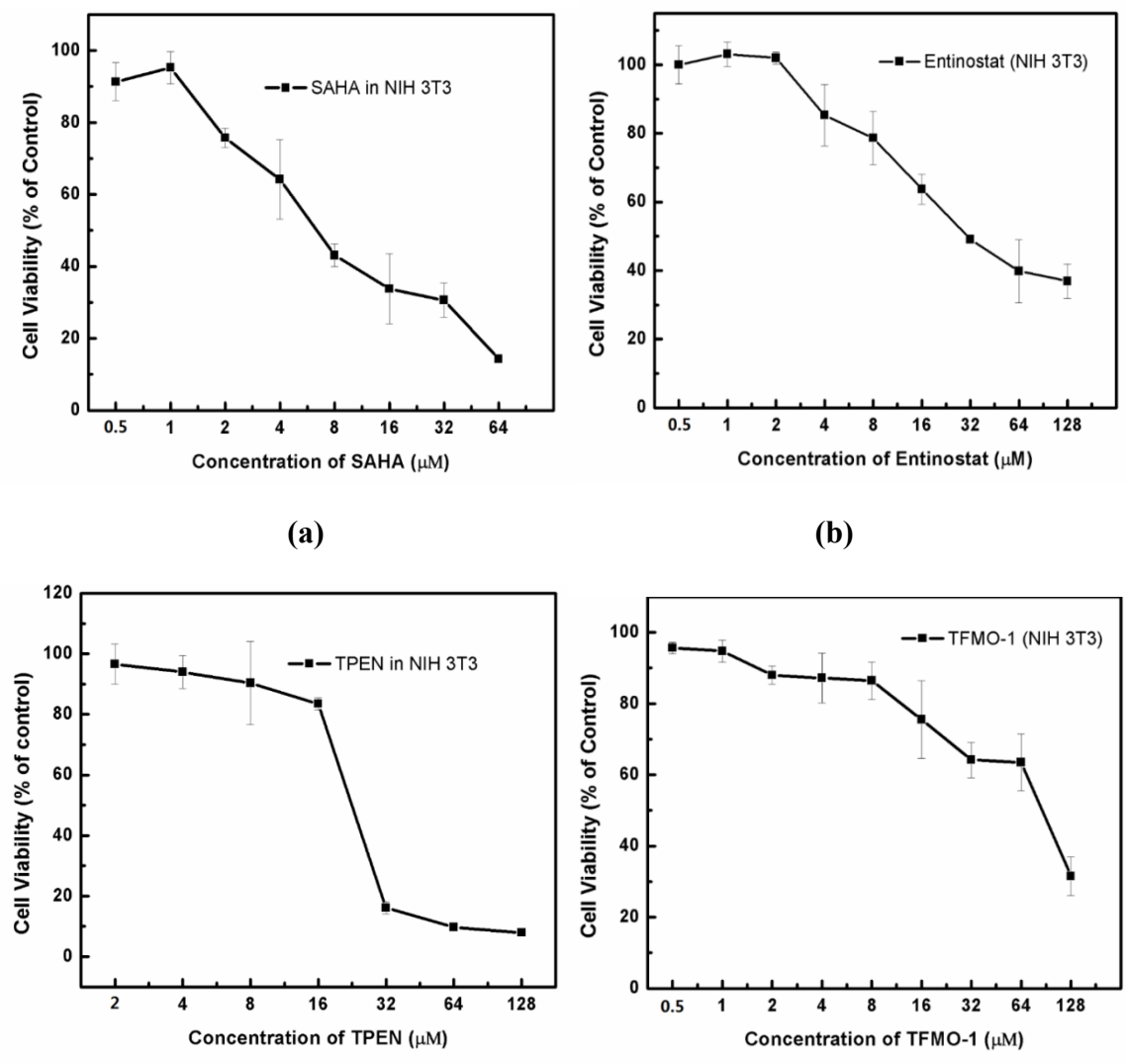

Fig.S1. Dose response curve for NIH3T3 cell viability with: (a) SAHA; (b) Entinostat; (c) TFMO-1; and (d) TPEN. Cells were aliquoted into a 96-well culture plates and cell viability was determined by comparing untreated controls to treated groups using an MTS assay performed in triplicate.

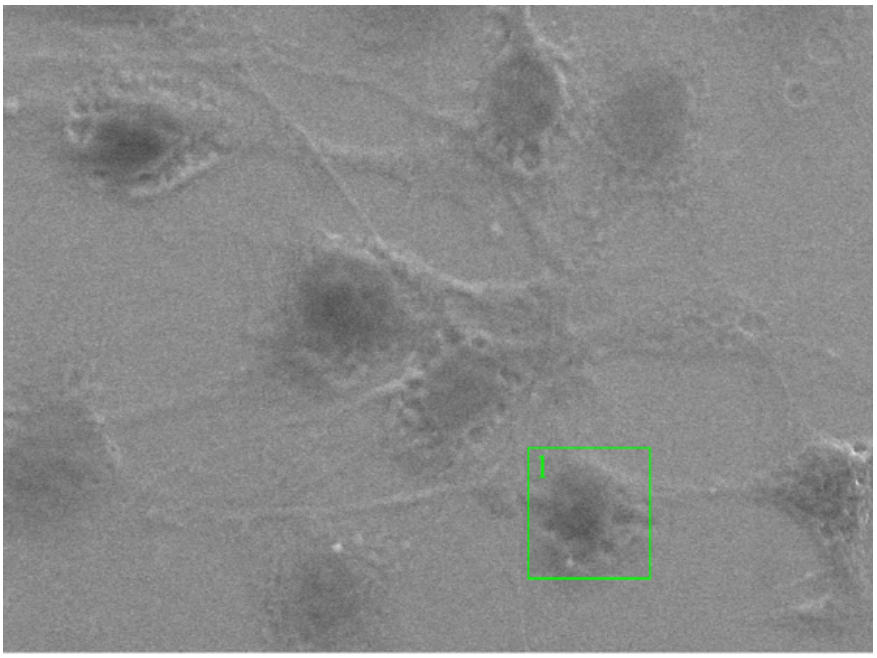

Fig. S2. SEM image of NIH3T3 growth on a glass cover slip. The green square contains one NIH3T3 cell.

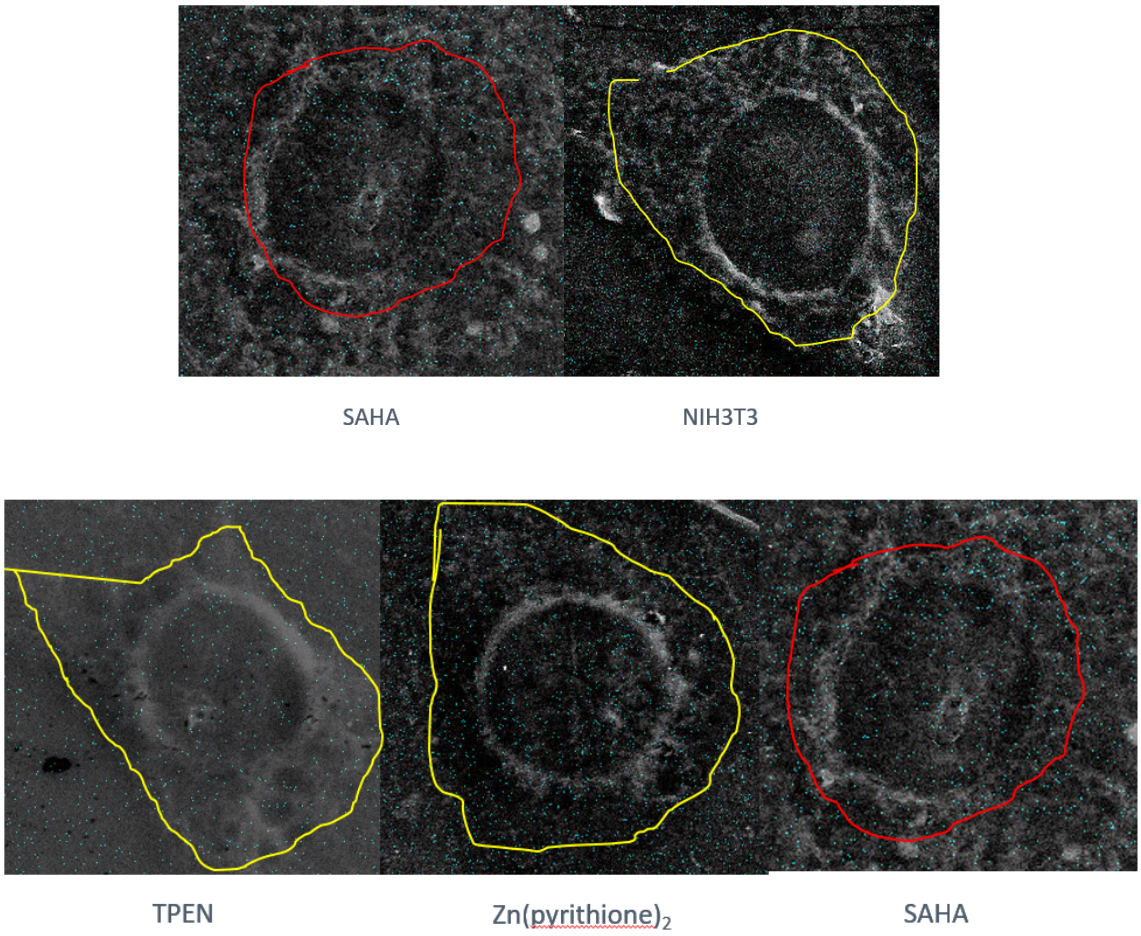

Fig. S3. SEM-EDX mapping image of NIH3T3 cells (green dots represent Zn) with different treatments. *Top*: Comparison of the EDX mapping of cells treated with 1.7 μM SAHA and untreated NIH 3T3 cells. *Bottom*: Comparison of the EDX mapping of cells treated with 1.7 μM TPEN, 1.7 μM $ZnCl_2$/0.28 μM pyrithione, and 1.7 μM SAHA (same data as top image). The colored lines represent the cell outlines.

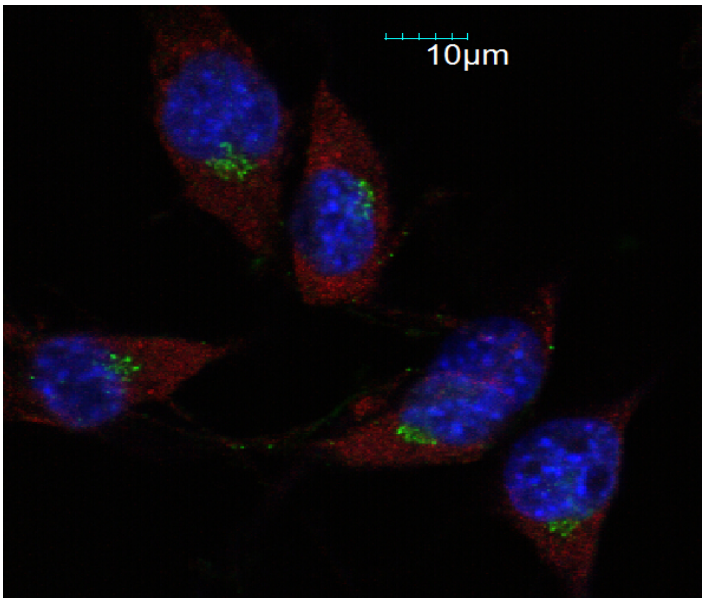

Fig. S4. Confocal image of NIH3T3 cells. The Golgi body (green) was stained using CellLight GFP BacMam 2.0; the mitochondria (red) was stained by MitoTracker® Red CMXRos; and the nuclei (blue) was stained by Hoechst 33342.

Table S1. Cellular metal (Fe, Cu, Zn) content of treated and untreated samples obtained from ICP-OES with Bradford assay normalization (normalized for protein content).

Cells	Cell Mass (mg/ml)	Zn in Cell(ng/g)	Zn /protein (ng/mg)	Cu in cell(ng/g)	Cu /protein (ng/mg)	Fe in cell (ng/g)	Fe /protein (ng/mg)
Untreated	0.49	86.2±2.2	175.9	14.5±2.6	29.6	26.2±1.1	53.5
1.7 µM SAHA	0.51	94.1±2.1	184.5	19.9±2.4	39.0	35.1±1.9	68.6
1.7 µM TPEN	0.56	80.9±1.5	144.4	17.2±3.1	31.7	39.2±3.9	70.0
1.7 µM $ZnCl_2$ +0.28 µM pyrithione	0.76	178.6±3.2	235.0	29.0±3.7	38.1	47.3±2.4	61.8

Table S2. Overall Zn content in the treated and untreated cells.

NIH3T3 Cells	Cell Mass (mg/ml)	Zn in cell (ng/g)	Zn/protein (ng/mg)
Untreated	3.59	594.12±32.12	165.49±8.94
1.7 µM SAHA	1.50	269.53±43.23	179.69±28.80
5.1 µM Entinostat	3.29	503.32±89.14	152.98±27.08
5.1 µM TFMO-1	4.71	734.78±98.41	156.00±20.89
1.7 µM TPEN	2.90	509.22±65.51	175.59±22.59
3.4 µM TPEN	2.17	309.75±68.25	142.73±31.43
5.1 µM TPEN	1.67	228.61±61.37	136.89±36.71
1.7 µM $ZnCl_2$ + 0.28 µM pyrithione	1.97	571.41±72.41	290.06±36.75

Table S3. Relative cellular Zn and P content from SEM-EDX in treated and untreated cells.

NIH3T3 Cells	Mole Concentration of Zn (%)	Mole Concentration of P (%)	Molar ratio of Zn/P
Untreated	56.46±3.93	43.54±3.93	1.23/1
1.7 µM SAHA	56.53±0.61	43.94±0.61	1.29/1
5.1 µM Entinostat	57.64±3.96	42.36±3.96	1.36/1
5.1 µM TFMO-1	55.14±4.17	44.86±4.17	1.20/1
1.7 µM TPEN	56.39±0.87	43.61±0.87	1.29/1
3.4 µM TPEN	49.48±1.60	50.52±1.60	0.98/1
5.1 µM TPEN	47.13±1.60	52.87±1.60	0.89/1
1.7 µM $ZnCl_2$ +0.28 µM pyrithione	61.97±0.63	38.03±0.63	1.63/1

Table S4. Comparison of the cellular Zn concentration of 1.7 µM entinostat treated samples and untreated samples from SXRF.

Cells	Zn in cell (µg/cm^2)
Untreated	0.098±0.011
1.7 µM entinostat treated	0.103±0.008