Electronic Supplementary Material (ESI) for Metallomics.

Supplementary Data

Integrity of Zinc Finger Motifs in PML protein is Necessary for Inducing its Protein Degradation by Antimony

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Materials and Methods

Reagents

All reagents were of analytical grade. Milli-Q water (Millipore) was used throughout the experiment. Trizma® HCl and Trizma® Base, phenazene methosulfate, decylubiquinone, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were purchased from Sigma (St. Louis, MO, USA). Phenylstibine oxide (PSO), phenanthroline monohydrate (PTMH), sodium arsenite (iAs^{III}), were purchased from Wako Pure Chemical Industries, Ltd.(Osaka, Japan). Trivalent antimony was obtained from Tri Chemicals (Yamanashi, Japan).

Antibodies

Primary antibodies RARα, PRAM−1, rabbit anti-Poly (ADP-ribose) polymerase (PARP) polyclonal antibody, rabbit anti-human PML, anti-human SUMO-1, Ub antibody were purchased from Santa Cruz Biotechnology (CA, USA). Primary antibodies; β-actin, anti-cleaved caspase-3 antibody, caspase-3, GAPDH were purchased from Cell Signaling Technology (Danvers, MA). Anti-FLAG mouse monoclonal antibody was purchased from Sigma.

Ultra-sonication for Increasing the Cell Permeability of EDTA

PMLV transfected HEK293T cells were seeded on T25 cell culture flask and exposure to 500μM EDTA and then sonicated at different voltage on ice for different time (2-3min), and then exposed to 8μM of iAs^{III} at 37°Cfor 3h. Proteins were extracted with RIPA buffer. PML V protein solubility changes and degradation were determined by western-blot.

UV absorption spectroscopy

Phenanthroline dissolved in the spectroscopic grade methanol was mixed with trivalent antimony water solution at different ratio. The UV spectra of the mixture were recorded in the wavelength range 230–500 nm with a UV–vis spectrophotometer (Shimadzu, Japan).

Figure Legends

Fig.S1. Proposed Metabolism Pathway of Arsenic (iAs^{III}) and Antimony (Sb^{III}) *in vivo*

Proposed methylation reaction and its intermediate metabolites of arsenic (**A**) and antimony (**B**) *in vivo.* In particular, Sb^{III} was found to be unable to methylate to more toxic mono- and dimethylated antimony compounds in animal, which is different from arsenic.

Fig.S2. Establishment of EDTA in preventing of PML protein solubility changes induced by arsenic trioxide

PML over-expressed HEK293T cells were sonicated by ultrasonicator for 5mins in presence of EDTA (500μM), and then exposed to iAs^{III} at 8μM for 3h **(A)**. PML over-expressed HEK293T cells were sonicated by ultrasonicator at different conditions (voltage, power1.5%; power 2.15%) in presence of EDTA, and then treated with iAs^{III} for 3h **(B)**. PML protein solubility changes as well as degradations in soluble and insoluble pellet fractions were determined by western blot. Asterisks indicate unspecific bands.

Fig.S3. UV spectra of Phenanthroline mixed with Sb^{III} at different ratio.

Phenanthroline(100μM) was mixed with Sb^{III} at different ratio (1:1, 1:10, 1:50). The UV absorption spectrum of the mixture was detected by UV–vis spectrophotometer in the wavelength range 230–500 nm.

Fig.S4. Effect of Phenylstibine Oxide (PSO) on Mutant PML Proteins Solubility Change and Modifications

Flag-PMLV (C212A) and (L218P) overexpressed HEK293T cells were exposed to PSO at indicated concentrations for 6h. Then, PMLV (C212A) (**A**) and (L218P) (**B**) protein solubility changes as well as degradations in soluble and insoluble pellet fractions were determined by western blot. Asterisks indicate unspecific bands.

Fig.S1

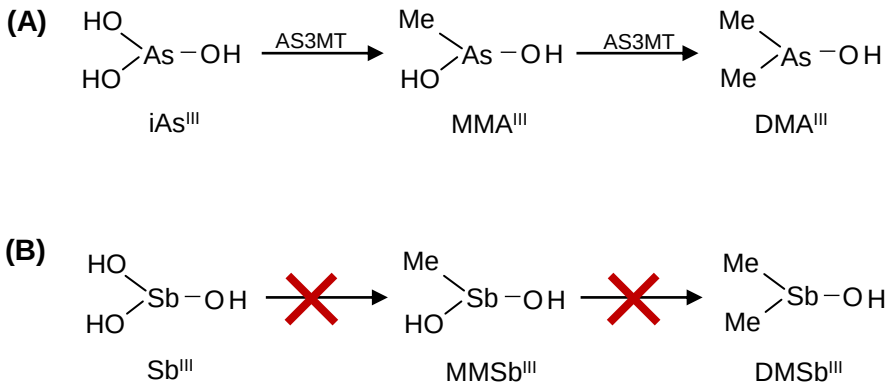

(A)
HO
HO
As
OH
AS3MT
Me
HO
As
OH
AS3MT
Me
Me
As
OH
iAsIII
MMAIII
DMAIII
(B)
HO
HO
Sb
OH
Me
HO
Sb
OH
Me
Me
Sb
OH
SbIII
MMSbIII
DMSbIII

Fig.S2

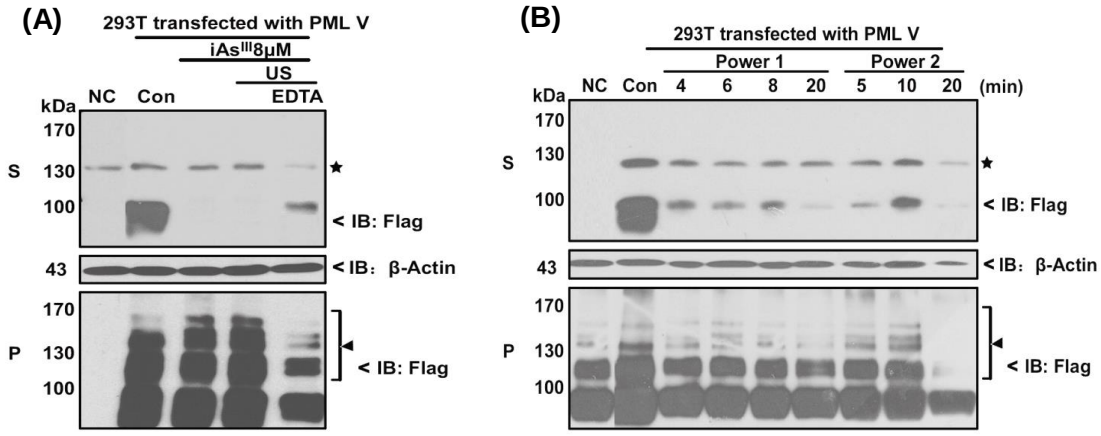

(A)
293T transfected with PML V
iAs^{III}8μM
US
NC
Con
EDTA
kDa
170
130
100
S
< IB: Flag
43
< IB：β-Actin
170
130
100
P
< IB: Flag
(B)
293T transfected with PML V
Power 1
Power 2
NC
Con
4
6
8
20
5
10
20
(min)
kDa
170
130
100
S
< IB: Flag
43
< IB：β-Actin
170
130
100
P
< IB: Flag

Fig.S3

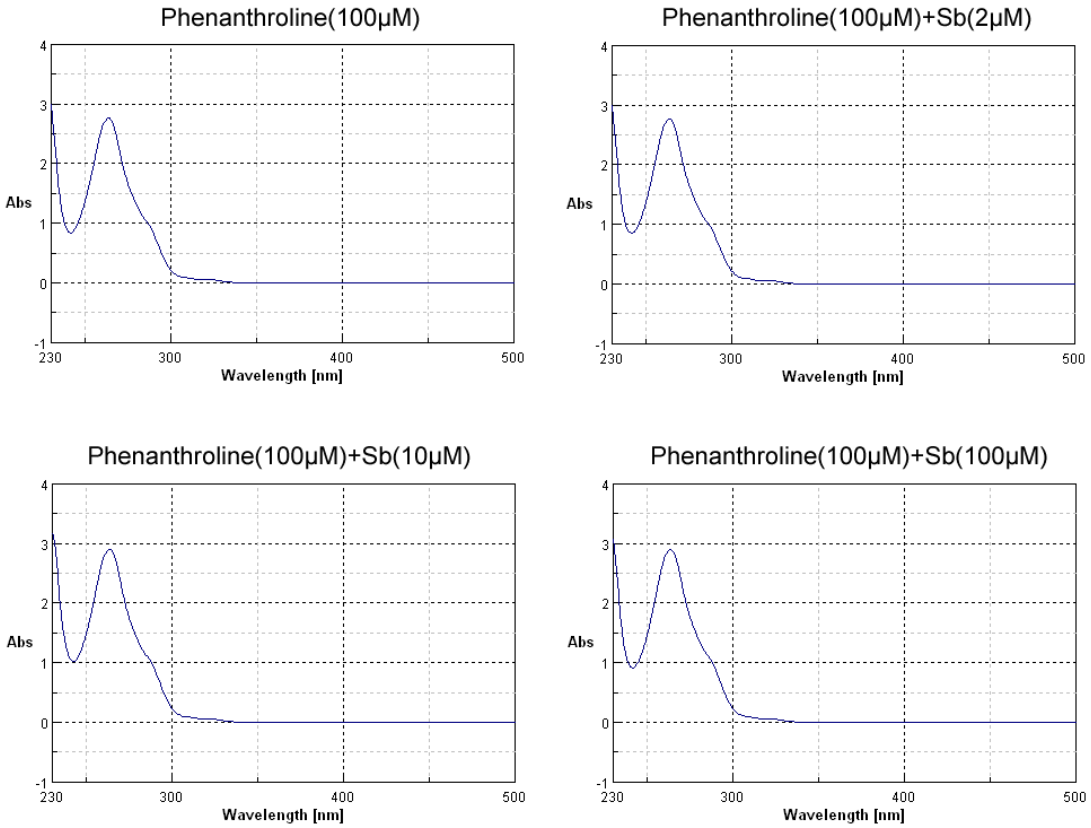
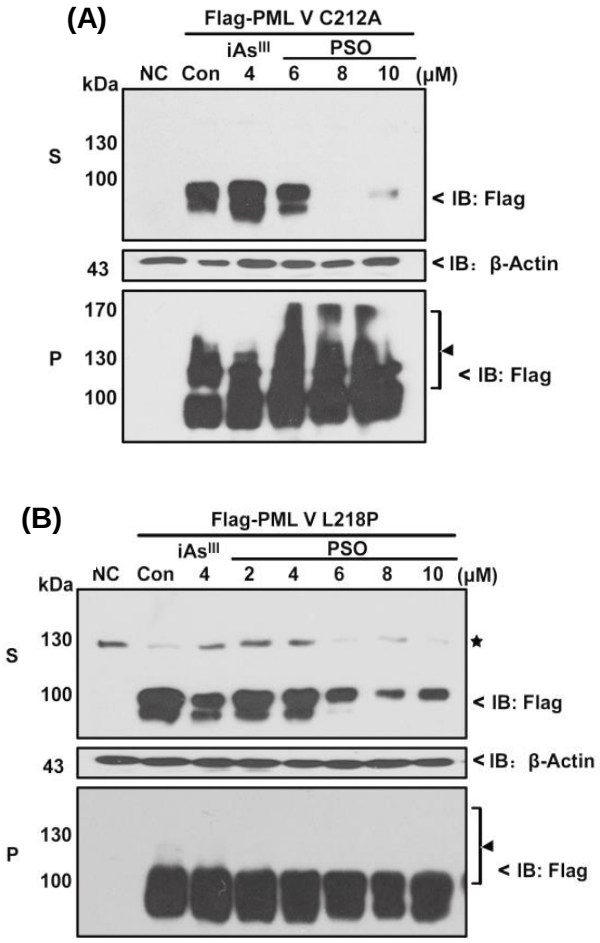

Phenanthroline(100μM)
Phenanthroline(100μM)+Sb(2μM)
Phenanthroline(100μM)+Sb(10μM)
Phenanthroline(100μM)+Sb(100μM)
Abs
Wavelength [nm]
4
3
2
1
0
-1
230
300
400
500

Fig.S4


(A)
Flag-PML V C212A
iAs III
PSO
NC Con 4 6 8 10 (μM)
kDa
S
130
100
< IB: Flag
43
< IB: β-Actin
170
P
130
100
< IB: Flag
(B)
Flag-PML V L218P
iAs III
PSO
NC Con 4 2 4 6 8 10 (μM)
kDa
S
130
100
< IB: Flag
43
< IB: β-Actin
P
130
100
< IB: Flag