

Toxicology

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Arsenite sensitizes to ferroptosis by disrupting selenium metabolism and reducing GPx4 expression

7 **Supplemental Method**

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9 **Biotin-PEAC₅ Maleimide Labeling assay (BPML)**

10 Purified protein (20 µg) was mixed with the metals in Tris-HCl (pH 7.5, 100 mM) and incubated
11 (37°C, 30 min) to bind the metal to the protein. Then, 0.25% SDS was added and incubated (37°C,
12 60 min), followed by the addition of biotin-PEAC₅-maleimide (BPM) (30 µM) (37°C, 30 min).
13 Methanol and chloroform precipitation was performed and the precipitate was washed with methanol
14 to remove unreacted BPM. The resulting residue was dissolved by adding SDS-sample buffer and
15 heating at 95°C for 5 min. 20 µL of the eluate was subjected to SDS-PAGE and detected by Avidin-
16 HRP.

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18 **Patient data analysis**

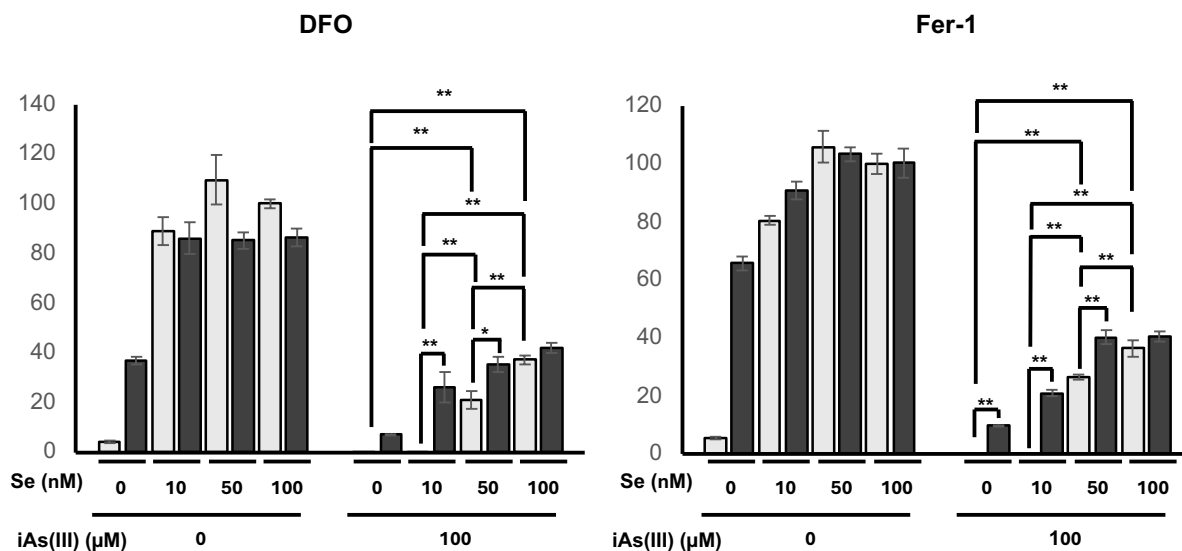
19 Gene expression and survival analyses were performed using GEPIA2 (Gene Expression Profiling
20 Interactive Analysis 2) an online tool for analyzing RNA sequencing data from The Cancer Genome
21 Atlas (TCGA) and Genotype-Tissue Expression (GTEx) projects. The gene expression profile of
22 PRDX6 (Ensembl ID: ENSG00000117592.8) across all tumor samples and paired normal tissues was
23 performed.

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PRDX6 KO

■ control ■ inhibitor



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29 Supplemental Figure 1. Effect of the selenite on the arsenite induced ferroptosis.

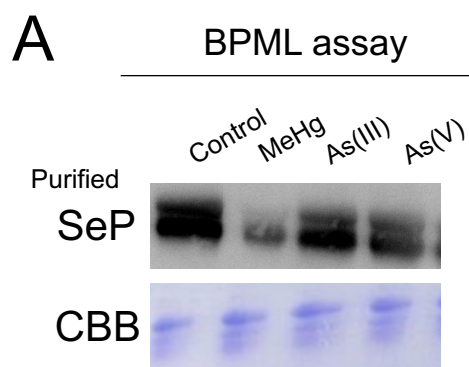
30 PRDX6 KO HT-1080 cells were pretreated with DFO and Fer-1 for 1 hr, with or without the
 31 indicated concentration of selenite and exposed to As(III). After 24 hr, cell viability was measured.
 32 Black column indicates with ferroptosis inhibitor (DFO or Fer-1). Mean±S.D., n=3. *P<0.05,
 33 **P<0.01 vs indicated group. Statistical analysis was performed by ANOVA followed by Tukey's
 34 test.

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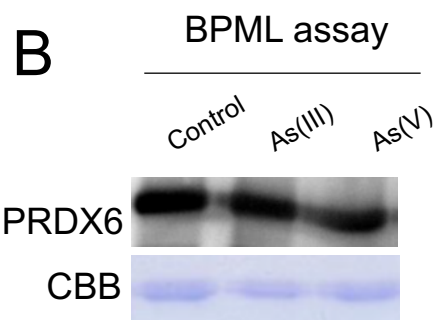
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52 **Supplemental Figure 2. Covalent binding between Cys residues of the proteins by arsenic.** Each
53 protein and metal were reacted in a 1:100 molar ratio and subjected to the BPML assay.

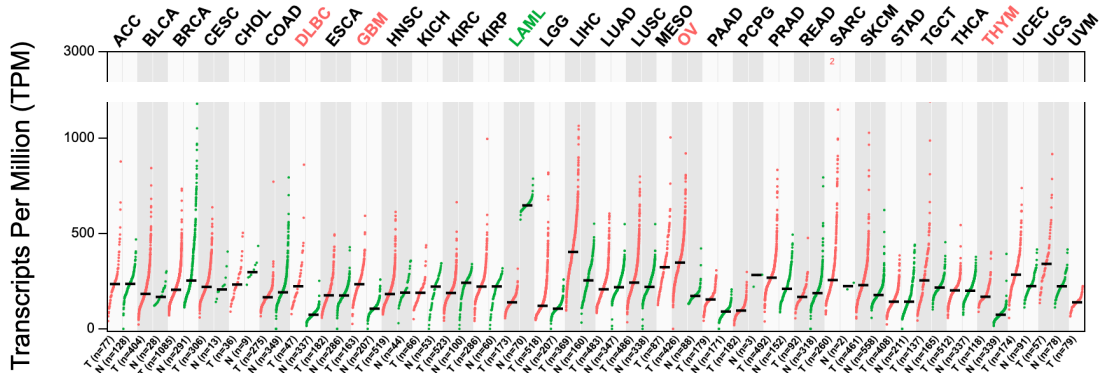
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66 **Supplemental Figure 3. Expression of PRDX6 in various cancer tissues.**

67 Comparison of tumor and normal tissue mRNA level obtained from the TCGA database by GEPIA2.

68 Red plots indicates cancer patients, and Green plots indicates healthy subjects. If the title of the cancer

69 are colored, it means there is significant change. ACCA (drenocortical carcinoma), BLCA (Bladder

70 Urothelial Carcinoma), BRCA (Breast invasive carcinoma), CESC (Cervical squamous cell

71 carcinoma and endocervical adenocarcinoma), CHOL (Cholangio carcinoma), COAD (Colon

72 adenocarcinoma), DLBC (Lymphoid Neoplasm Diffuse Large B-cell Lymphoma), ESCA

73 (Esophageal carcinoma), GBM (Glioblastoma multiforme), HNSC (Head and Neck squamous cell

74 carcinoma), KICH (Kidney Chromophobe), KIRC (Kidney renal clear cell carcinoma), KIRP

75 (Kidney renal papillary cell carcinoma), LAML (Acute Myeloid Leukemia), LGG (Brain Lower

76 Grade Glioma), LIHC (Liver hepatocellular carcinoma), LUAD (Lung adenocarcinoma), LUSC

77 (Lung squamous cell carcinoma), MESO (Mesothelioma), OV (Ovarian serous cystadenocarcinoma),

78 PAAD (Pancreatic adenocarcinoma), PCPG (Pheochromocytoma and Paraganglioma), PRAD

79 (Prostate adenocarcinoma), READ (Rectum adenocarcinoma), SARC (Sarcoma), SKCM (Skin

80 Cutaneous Melanoma), STAD (Stomach adenocarcinoma), TGCT (Testicular Germ Cell Tumors),

81 THCA (Thyroid carcinoma), THYM (Thymoma), UCEC (Uterine Corpus Endometrial Carcinoma),

82 UCS (Uterine Carcinosarcoma), UVM (Uveal Melanoma).

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