Supporting Information

for

# Heavy Metals Induce Decline of Derivatives of 5-Methycytosine in Both DNA and RNA of Stem Cells

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**Assay of Caspase 3 Activity**

The Caspase 3 activity assay was performed using Caspase 3 Activity Assay Kit (Beyotime) according to the manufacture's recommended procedure. Briefly, heavy metals treated and control cells (~ $10^6$ cells) were lysed in 100 μL of the lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate) (Beyotime) in ice for 15 min, and centrifuged at 13,000 for 10 min at 4ºC. The supernatant was collected for determination of Caspase 3 activity. In addition, the protein concentration was measured by BCA Protein Assay Kit (Beyotime) according to the manufacture's recommended procedure.

**Quantitative Real-time PCR**

One μg of isolated total RNA was used to generate cDNA with PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (TaKaRa). qPCR was performed using a CFX96™ Real-Time PCR Detection System (Bio-Rad Laboratories) and SYBR® Premix Ex Taq II (Tli RNaseH Plus) (TaKaRa) according to the manufacturer's instructions. PCR primers were according to previous study.[1] And the sequences were listed in Supplementary Table 6.

**Method Validation**

The calibration curves of 5-mdC, 5-hmdC, 5-fodC, 5-cadC, 5-mrC, 5-hmrC, 5-forC, and 5-carC were constructed by plotting the mean peak area ratios of 5-mdC/$10^2$ dG, 5-hmdC/$10^6$ dG, 5-fodC/$10^6$ dG, 5-cadC/$10^6$ dG, 5-mrC/$10^2$ rG, 5-hmrC/$10^6$ rG, 5-forC/$10^6$ rG, 5-carC/$10^6$ rG versus the mean molar ratios of 5-mdC/$10^2$ dG, 5-hmdC/$10^6$ dG, 5-fodC/$10^6$ dG, 5-cadC/$10^6$ dG, 5-mrC/$10^2$ rG, 5-hmrC/$10^6$ rG, 5-forC/$10^6$ rG, 5-carC/$10^6$ rG based on data obtained from triplicate measurements using the BDEPE labeling strategy combined with LC-ESI-MS/MS analysis. The results showed that good linearities were obtained with a correlation coefficient ($R^2$) being greater than 0.99 (Supplementary Table 7). The accuracy and reproducibility of the developed method were evaluated with the REs and RSDs being less than 12.0% and 12.6% (Supplementary Table 8-15), respectively, demonstrating that good accuracy and reproducibility were achieved.

**Reference**

1. Hu, X., Zhang, L., Mao, S. Q., Li, Z., Chen, J., Zhang, R. R., Wu, H. P., Gao, J., Guo, F., Liu, W., Xu, G. F., Dai, H. Q., Shi, Y. G., Li, X., Hu, B., Tang, F., Pei, D., and Xu, G. L. (2014) Tet and TDG mediate DNA demethylation essential for mesenchymal-to-epithelial transition in somatic cell reprogramming, *Cell Stem Cell 14*, 512-522.

Supplementary Table 1. The MRM parameters for the analysis of nucleosides by LC-ESI-MS/MS.

| Analytes | Precursor ion | Product ion | DP/V | EP / V | CE / V | CXP / V |
|---|---|---|---|---|---|---|
| dA | 252.2 | 136.1 | 34.0 | 5.0 | 21.0 | 4.0 |
| dC | 228.2 | 112.1 | 22.0 | 4.0 | 15.0 | 4.0 |
| dG | 268.2 | 152.2 | 32.0 | 4.0 | 16.0 | 5.0 |
| T | 243.2 | 127.0 | 30.0 | 4.5 | 16.0 | 3.0 |
| rA | 268.2 | 136.2 | 32.0 | 5.0 | 24.0 | 3.0 |
| rC | 244.2 | 112.1 | 25.0 | 4.0 | 20.0 | 3.0 |
| rG | 284.2 | 152.2 | 32.0 | 5.0 | 24.0 | 3.0 |
| rU | 245.2 | 113.3 | 34.0 | 5.5 | 17.0 | 4.0 |
| 5-mdC | 242.2 | 126.2 | 22.0 | 4.5 | 21.0 | 3.0 |
| 5-hmdC | 258.2 | 142.2 | 20.0 | 4.0 | 15.0 | 3.0 |
| 5-fodC | 256.2 | 140.2 | 38.0 | 4.0 | 18.0 | 4.0 |
| 5-cadC | 272.2 | 156.2 | 42.0 | 5.0 | 14.0 | 5.0 |
| 5-mrC | 258.2 | 126.2 | 23.0 | 5.0 | 20.0 | 3.0 |
| 5-hmrC | 274.2 | 142.1 | 31.0 | 5.5 | 16.0 | 5.0 |
| 5-forC | 272.2 | 140.1 | 30.0 | 4.0 | 19.0 | 4.0 |
| 5-carC | 288.2 | 156.2 | 36.0 | 4.0 | 16.0 | 4.0 |
| 5-mdC-BDEPE | 413.2 | 297.1 | 20.0 | 7.0 | 27.0 | 4.0 |
| 5-hmdC-BDEPE | 429.2 | 313.3 | 32.0 | 7.0 | 22.0 | 4.0 |
| 5-fodC-BDEPE | 427.2 | 311.2 | 15.0 | 5.0 | 18.0 | 3.0 |
| 5-cadC-BDEPE | 632.3 | 516.3 | 37.0 | 8.0 | 24.0 | 4.0 |
| 5-mrC-BDEPE | 429.2 | 297.1 | 58.0 | 6.0 | 30.0 | 5.0 |
| 5-hmrC-BDEPE | 445.2 | 313.3 | 52.0 | 8.0 | 25.0 | 3.0 |
| 5-forC-BDEPE | 443.3 | 311.2 | 50.0 | 8.0 | 26.5 | 4.0 |
| 5-carC-BDEPE | 648.4 | 516.3 | 56.0 | 6.0 | 30.0 | 5.0 |

Supplementary Table 2. Limits of detections (LODs) of 5-mdC, 5-hmdC, 5-fodC, 5-cadC, 5-mrC, 5-hmrC, 5-forC, and 5-carC with and without BDEPE labeling.

| | LODs (fmol) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5-mdC | 5-hmdC | 5-fodC | 5-cadC | 5-mrC | 5-hmrC | 5-forC | 5-carC |
| Without labeling | 5.10 | 10.30 | 15.06 | 31.02 | 5.60 | 11.15 | 16.07 | 30.20 |
| BDEPE labeling | 0.04 | 0.06 | 0.05 | 0.07 | 0.04 | 0.06 | 0.05 | 0.08 |
| Detection sensitivities increased folds | 128 | 172 | 305 | 443 | 140 | 186 | 321 | 377 |

Supplementary Table 3. Contents change of eight cytosine modifications and TCA metabolites in mouse ES cells by heavy metals treatments. Value > 0, increase; value < 0, decrease. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

| | As | Cd | Cr | Sb |
|---|---|---|---|---|
| 5-mdC | 10.3% | 10.4% | 11.5% | 12.9% |
| 5-hmdC | -41.7%** | -44.7%** | -47.9%** | -29.8%* |
| 5-fodC | -49.1%** | -53.1%** | -43.4%** | -49.0%** |
| 5-cadC | -76.8%*** | -75.3%** | -54.9%** | -69.3%** |
| 5-mrC | -4.2% | 5.6% | 12.5% | 1.4% |
| 5-hmrC | -54.6%* | -44.9%* | -66.6%** | -46.1%* |
| 5-forC | -42.1%* | -71.0%** | -70.8%*** | -44.6%** |
| 5-carC | -41.3%* | -34.8%* | -43.3%* | -57.9%** |
| 2-HG | 56.7% | 177.6%** | 488.0%*** | 118.5%** |
| 2-KG | -23.3% | -52.6%** | 3.8% | -45.2%** |
| Isocitrate | -38.6%** | -53.9%** | -47.7%** | -39.7%** |
| Citrate | -40.7%* | -66.5%** | -7.4% | -52.2%** |
| Oxaloacetate | -33.9% | -59.4%* | -56.8%** | -71.4%** |
| Malate | -52.8%** | -72.9%*** | -42.5%** | -70.5%*** |
| Fumarate | -41.8%** | -68.0%*** | -25.8%* | -65.4%*** |
| Succinate | -27.8% | -60.2%* | -41.4%* | -57.0%** |

Supplementary Table 4. High resolution mass spectrometry parameters for the analysis of metabolites in TCA cycle.

| Compounds | Detection ion form | Theoretical *m/z* | Detected *m/z* | Error (ppm) | Detection mode |
|---|---|---|---|---|---|
| 2-HG | $[M-H]^-$ | 147.0299 | 147.0299 | 0 | negative |
| 2-KG | $[M-H]^-$ | 145.0142 | 145.0144 | 1.4 | negative |
| Isocitrate | $[M-H]^-$ | 191.0197 | 191.0194 | -1.6 | negative |
| Citrate | $[M-H]^-$ | 191.0197 | 191.0194 | -1.6 | negative |
| Oxaloacetate | $[M-H-CO_2]^-$ | 87.0088 | 87.0088 | 0 | negative |
| Malate | $[M-H]^-$ | 133.0142 | 133.0143 | 0.8 | negative |
| Fumarate | $[M-H]^-$ | 115.0037 | 115.0038 | 0.9 | negative |
| Succinate | $[M-H]^-$ | 117.0193 | 117.0194 | 0.9 | negative |

Supplementary Table 5. Calibration curves for the analysis of metabolites in TCA cycle.

| Compounds | Linear range/pmol | Calibration curve | | |
|---|---|---|---|---|
| | | Slope | Intercept | $R^2$ |
| 2-HG | 5-500 | 0.0052 | 0.0093 | 0.9991 |
| 2-KG | 5-500 | 0.0006 | -0.0013 | 0.9971 |
| Isocitrate | 5-500 | 0.0018 | -0.0117 | 0.9990 |
| Citrate | 5-500 | 0.0019 | -0.0104 | 0.9998 |
| Oxaloacetate | 5-500 | 0.0048 | -0.0061 | 0.9967 |
| Malate | 5-500 | 0.0006 | 0.0044 | 0.9970 |
| Fumarate | 5-500 | 0.0010 | -0.0063 | 0.9999 |
| Succinate | 5-500 | 0.0064 | -0.0130 | 0.9959 |

Supplementary Table 6. Sequences of the PCR primers.

| Sequence name | Sequences (5’ → 3’) |
|---|---|
| GAPDH forward | GCCAGCCTCGTCCCGTAGACA |
| GAPDH reverse | CAACAATCTCCACTTTGCCACTGC |
| TET1 forward | CATTCTCACAAGGACATTCACAACA |
| TET1 reverse | AGTAAAACGTAGTCGCCTCTTCCTG |
| TET2 forward | GCTCCAATATACAAGAAGCTTGCAC |
| TET2 reverse | TATTGAGGGTGACCACCACTGTACT |
| TET3 forward | CTATCAGAACCAGGTGACCAATGAG |
| TET3 reverse | ACAGTGCACCCATTGTAGAGGTTAT |

Supplementary Table 7. Calibration curves for the analysis of 5-mdC, 5-hmdC, 5-fodC, 5-cadC, 5-mrC, 5-hmrC, 5-forC, and 5-carC by BDEPE labeling coupled with LC-ESI-MS/MS analysis.

| Compound molar ratio | Linear range | Calibration curve | | |
|---|---|---|---|---|
| | | Slope | Intercept | $R^2$ |
| 5-mdC/$10^2$ dG | 0.1-10 | 0.8463 | 0.0463 | 0.9999 |
| 5-hmdC/$10^6$ dG | 50-5000 | 6.5E-5 | 9.0E-9 | 0.9988 |
| 5-fodC/$10^6$ dG | 0.5-50 | 7.9E-5 | -1.1E-9 | 0.9997 |
| 5-cadC/$10^6$ dG | 0.1-10 | 1.1E-4 | 4.1E-9 | 0.9959 |
| 5-mrC/$10^2$ rG | 0.02-2 | 0.7490 | 0.0017 | 0.9968 |
| 5-hmrC/$10^6$ rG | 0.1-10 | 6.3E-5 | 7.2E-9 | 0.9993 |
| 5-forC/$10^6$ rG | 0.1-10 | 7.2E-5 | -5.4E-8 | 0.9980 |
| 5-carC/$10^6$ rG | 0.1-10 | 4.8E-5 | -5.6E-9 | 0.9955 |

Supplementary Table 8. Accuracy and precision for the determination of 5-mdC by BDEPE labeling coupled with LC-ESI-MS/MS analysis.

| | Nominal 5-mdC/$10^2$ dG | 0.10 | 0.20 | 0.50 | 1.00 | 2.00 | 5.00 | 10.00 |
|---|---|---|---|---|---|---|---|---|
| Day 1 *n*=3 | Measured mean 5-mdC/$10^2$ dG | 0.11 | 0.19 | 0.52 | 0.98 | 2.16 | 4.65 | 10.25 |
| | RSD (%) | 5.1 | 4.2 | 2.1 | 4.6 | 9.8 | 3.6 | 8.5 |
| | RE (%) | 11.2 | -4.0 | 4.7 | -2.4 | 8.1 | -7.0 | 2.5 |
| Day 2 *n*=3 | Measured mean 5-mdC/$10^2$ dG | 0.09 | 0.19 | 0.54 | 1.06 | 1.91 | 5.17 | 9.06 |
| | RSD (%) | 2.3 | 6.7 | 5.7 | 8.5 | 8.7 | 8.7 | 4.2 |
| | RE (%) | -7.7 | -5.3 | 8.4 | 6.2 | -4.4 | 3.3 | -9.4 |
| Day 3 *n*=3 | Measured mean 5-mdC/$10^2$ dG | 0.11 | 0.22 | 0.50 | 0.97 | 2.15 | 4.79 | 8.80 |
| | RSD (%) | 6.2 | 10.2 | 5.7 | 10.6 | 12.2 | 6.5 | 6.2 |
| | RE (%) | 5.1 | 8.0 | -0.8 | -3.5 | 7.6 | -4.3 | 12.0 |

Supplementary Table 9. Accuracy and precision for the determination of 5-hmdC by BDEPE labeling coupled with LC-ESI-MS/MS analysis.

| | Nominal 5-hmdC/$10^6$ dG | 50 | 100 | 200 | 500 | 1000 | 2000 | 5000 |
|---|---|---|---|---|---|---|---|---|
| Day 1 *n*=3 | Measured mean 5-hmdC/$10^6$ dG | 51 | 96 | 196 | 485 | 962 | 1847 | 4752 |
| | RSD (%) | 6.5 | 2.4 | 3.6 | 9.5 | 7.2 | 3.8 | 1.5 |
| | RE (%) | 2.7 | -3.5 | -1.9 | -2.8 | -3.8 | -7.7 | -5.0 |
| Day 2 *n*=3 | Measured mean 5-hmdC/$10^6$ dG | 48 | 106 | 222 | 462 | 1062 | 2066 | 4892 |
| | RSD (%) | 7.9 | 8.4 | 5.6 | 6.5 | 9.5 | 11.5 | 8.4 |
| | RE (%) | -2.7 | 6.5 | 11.3 | -7.5 | 6.3 | 3.3 | -2.2 |
| Day 3 *n*=3 | Measured mean 5-hmdC/$10^6$ dG | 45 | 94 | 214 | 455 | 962 | 1962 | 5242 |
| | RSD (%) | 5.7 | 4.5 | 1.5 | 8.9 | 4.5 | 6.6 | 3.8 |
| | RE (%) | -9.4 | -5.9 | 7.3 | -9.0 | -1.9 | -1.9 | 4.9 |

Supplementary Table 10. Accuracy and precision for the determination of 5-fodC by BDEPE labeling coupled with LC-ESI-MS/MS analysis.

| | Nominal 5-fodC/$10^6$ dG | 0.5 | 1.0 | 2.0 | 5.0 | 10.0 | 20.0 | 50.0 |
|---|---|---|---|---|---|---|---|---|
| Day 1 | Measured mean 5-fodC/$10^6$ dG | 0.52 | 0.95 | 2.0 | 4.5 | 9.1 | 19.4 | 46.2 |
| *n*=3 | RSD (%) | 9.5 | 7.5 | 4.3 | 3.8 | 4.5 | 10.2 | 11.2 |
| | RE (%) | 5.0 | -2.4 | 1.8 | -9.4 | -8.8 | -2.9 | -7.5 |
| Day 2 | Measured mean 5-fodC/$10^6$ dG | 0.47 | 1.07 | 2.2 | 5.5 | 10.6 | 19.9 | 51.1 |
| *n*=3 | RSD (%) | 5.2 | 6.5 | 4.8 | 8..4 | 2.8 | 6.6 | 7.5 |
| | RE (%) | -4.4 | 7.6 | 10.8 | 9.3 | 6.3 | -2.4 | 2.3 |
| Day 3 | Measured mean 5-fodC/$10^6$ dG | 0.48 | 0.94 | 1.9 | 4.7 | 9.0 | 21.8 | 49.8 |
| *n*=3 | RSD (%) | 5.4 | 6.8 | 8.9 | 2.5 | 6.4 | 8.4 | 5.8 |
| | RE (%) | -3.8 | -5.4 | -6.1 | -7.0 | -10.4 | 9.1 | -0.5 |

Supplementary Table 11. Accuracy and precision for the determination of 5-cadC by BDEPE labeling coupled with LC-ESI-MS/MS analysis.

| | Nominal 5-cadC/$10^6$ dG | 0.10 | 0.20 | 0.5 | 1.0 | 2.0 | 5.0 | 10.0 |
|---|---|---|---|---|---|---|---|---|
| Day 1 *n*=3 | Measured mean 5-cadC/$10^6$ dG | 0.09 | 0.18 | 0.49 | 0.96 | 1.9 | 5.0 | 9.5 |
| | RSD (%) | 3.8 | 5.2 | 6.8 | 9.5 | 10.4 | 11.2 | 5.5 |
| | RE (%) | -2.9 | -2.4 | -0.8 | -3.8 | -4.0 | 0. | -5.5 |
| Day 2 *n*=3 | Measured mean 5-cadC/$10^6$ dG | 0.09 | 0.21 | 0.46 | 1.02 | 1.9 | 4.7 | 9.6 |
| | RSD (%) | 6.5 | 8.8 | 6.5 | 4.5 | 6.6 | 2.5 | 3.9 |
| | RE (%) | -7.7 | 2.3 | -7.0 | 2.8 | -2.6 | -6.7 | -3.8 |
| Day 3 *n*=3 | Measured mean 5-cadC/$10^6$ dG | 0.11 | 0.21 | 0.51 | 1.12 | 1.9 | 5.2 | 9.5 |
| | RSD (%) | 11.2 | 8.1 | 6.5 | 4.2 | 6.2 | 5.4 | 6.5 |
| | RE (%) | 8.1 | 5.7 | 3.0 | 12.0 | -2.2 | 4.5 | -5.4 |

Supplementary Table 12. Accuracy and precision for the determination of 5-mrC by BDEPE labeling coupled with LC-ESI-MS/MS analysis.

| | Nominal 5-mrC/$10^2$ rG | 0.02 | 0.05 | 0.10 | 0.20 | 0.50 | 1.00 | 2.00 |
|---|---|---|---|---|---|---|---|---|
| Day 1 | Measured mean 5-mrC/$10^2$ rG | 0.022 | 0.048 | 0.11 | 0.22 | 0.49 | 0.94 | 2.16 |
| *n*=3 | RSD (%) | 10.6 | 9.5 | 2.5 | 6.5 | 4.7 | 8.2 | 6.9 |
| | RE (%) | 9.3 | -4.7 | 9.9 | 7.8 | -2.1 | -6.4 | 8.1 |
| Day 2 | Measured mean 5-mrC/$10^2$ rG | 0.020 | 0.051 | 0.10 | 0.19 | 0.55 | 1.04 | 2.08 |
| *n*=3 | RSD (%) | 2.8 | 12.6 | 10.3 | 6.9 | 5.8 | 9.5 | 6.5 |
| | RE (%) | -2.2 | 2.6 | -3.5 | -5.3 | 9.1 | 3.5 | 4.2 |
| Day 3 | Measured mean 5-mrC/$10^2$ rG | 0.018 | 0.048 | 0.09 | 0.21 | 0.49 | 0.92 | 1.87 |
| *n*=3 | RSD (%) | 4.7 | 8.7 | 9.2 | 12.4 | 6.3 | 5.6 | 8.2 |
| | RE (%) | -7.8 | -3.5 | -11.3 | 4.3 | -3.0 | -7.7 | -6.3 |

Supplementary Table 13. Accuracy and precision for the determination of 5-hmrC by BDEPE labeling coupled with LC-ESI-MS/MS analysis.

| | Nominal 5-hmrC/$10^6$ rG | 0.1 | 0.2 | 0.5 | 1.0 | 2.0 | 5.0 | 10.0 |
|---|---|---|---|---|---|---|---|---|
| Day 1 *n*=3 | Measured mean 5-hmrC/$10^6$ rG | 0.11 | 0.19 | 0.47 | 0.95 | 1.9 | 4.6 | 11.0 |
| | RSD (%) | 4.9 | 11.5 | 7.6 | 5.6 | 5.9 | 7.3 | 6.5 |
| | RE (%) | 10.5 | -2.8 | -4.3 | -4.4 | -2.2 | -7.6 | 10.1 |
| Day 2 *n*=3 | Measured mean 5-hmrC/$10^6$ rG | 0.11 | 0.19 | 0.51 | 0.96 | 1.9 | 4.6 | 9.6 |
| | RSD (%) | 11.2 | 7.5 | 9.3 | 3.8 | 5.4 | 3.7 | 9.2 |
| | RE (%) | 9.6 | -2.3 | 3.2 | -3.6 | -4.3 | -7.5 | -3.7 |
| Day 3 *n*=3 | Measured mean 5-hmrC/$10^6$ rG | 0.09 | 0.20 | 0.52 | 1.01 | 2.2 | 5.2 | 9.5 |
| | RSD (%) | 5.1 | 6.5 | 6.5 | 9.6 | 6.4 | 4.9 | 4.8 |
| | RE (%) | -0.2 | 2.2 | 5.2 | 1.6 | 11.3 | 4.5 | -5.4 |

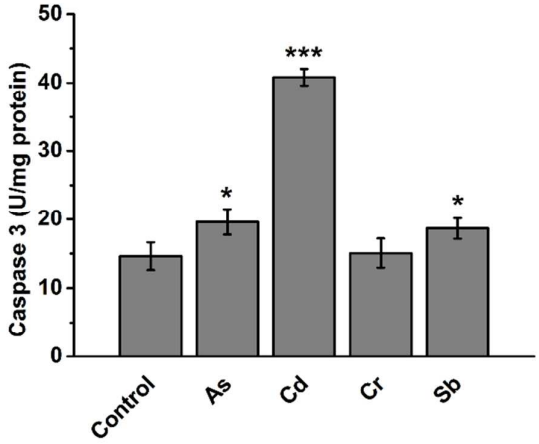
Supplementary Table 14. Accuracy and precision for the determination of 5-forC by BDEPE labeling coupled with LC-ESI-MS/MS analysis.

| | Nominal 5-forC/$10^6$ rG | 0.1 | 0.2 | 0.5 | 1.0 | 2.0 | 5.0 | 10.0 |
|---|---|---|---|---|---|---|---|---|
| Day 1 *n*=3 | Measured mean 5-forC/$10^6$ rG | 0.09 | 0.20 | 0.51 | 0.95 | 1.9 | 5.0 | 9.3 |
| | RSD (%) | 9.8 | 4.8 | 2.8 | 1.9 | 5.4 | 0.6 | 5.8 |
| | RE (%) | -5.9 | -0.9 | 2.5 | -4.4 | -2.2 | -0.9 | -7.4 |
| Day 2 *n*=3 | Measured mean 5-forC/$10^6$ rG | 0.09 | 0.21 | 0.46 | 1.03 | 1.9 | 4.9 | 10.4 |
| | RSD (%) | 8.9 | 11.5 | 3.7 | 2.9 | 0.2 | 6.5 | 5.4 |
| | RE (%) | -8.9 | 2.5 | -7.6 | 3.6 | -3.6 | -2.9 | 3.6 |
| Day 3 *n*=3 | Measured mean 5-forC/$10^6$ rG | 0.10 | 0.21 | 0.45 | 1.04 | 2.1 | 4.5 | 10.8 |
| | RSD (%) | 2.4 | 3.1 | 2.3 | 5.5 | 8.8 | 9.7 | 4.8 |
| | RE (%) | 3.4 | 4.5 | -8.8 | 4.6 | 6.6 | -10.7 | 8.4 |

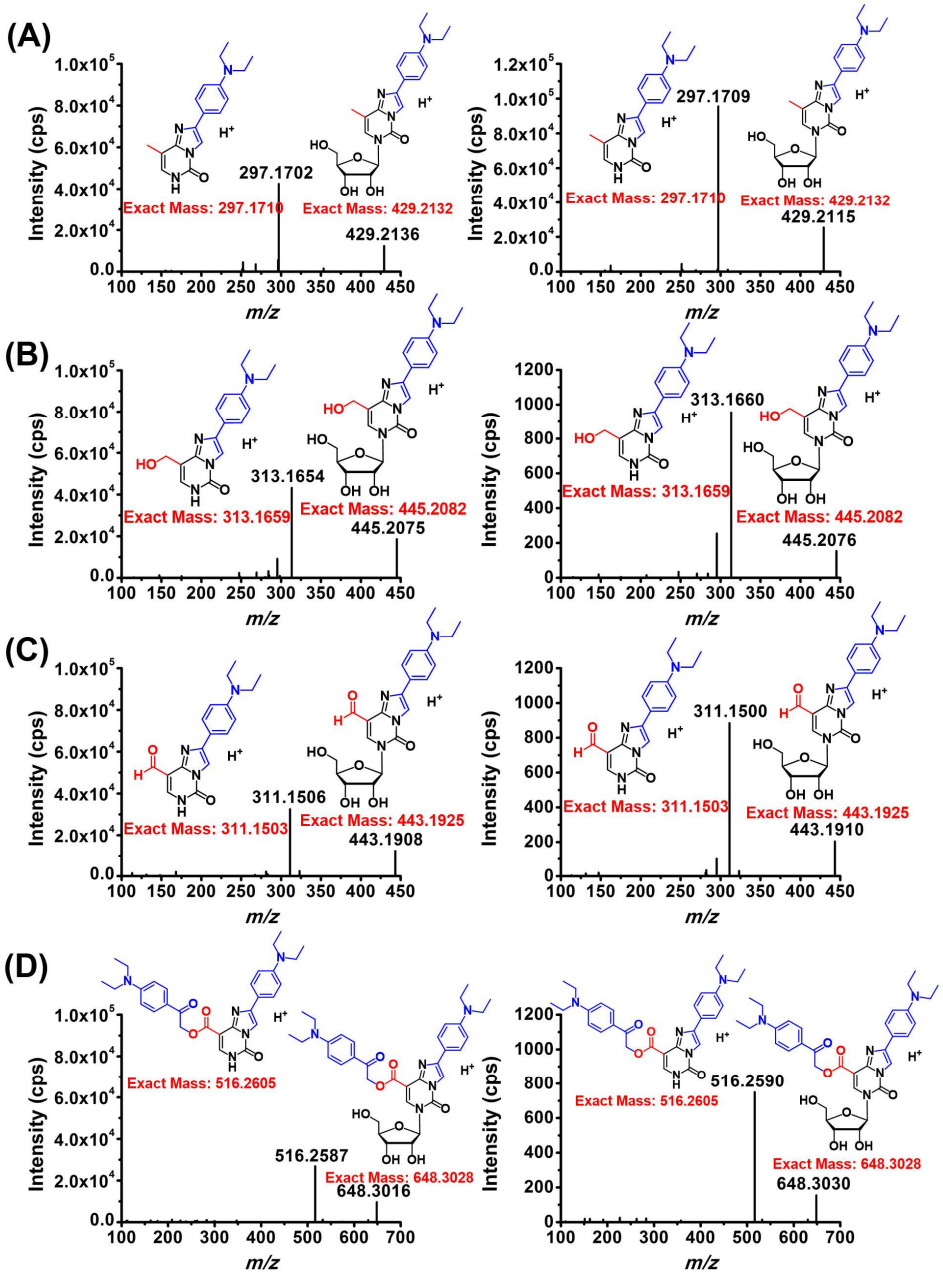
Supplementary Table 15. Accuracy and precision for the determination of 5-carC by BDEPE labeling coupled with LC-ESI-MS/MS analysis.

| | Nominal 5-carC/$10^6$ rG | 0.1 | 0.2 | 0.5 | 1.0 | 2.0 | 5.0 | 10.0 |
|---|---|---|---|---|---|---|---|---|
| Day 1 | Measured mean 5-carC/$10^6$ rG | 0.09 | 0.19 | 0.44 | 0.99 | 1.9 | 5.1 | 9.6 |
| *n*=3 | RSD (%) | 3.9 | 5.5 | 6.5 | 4.5 | 9.9 | 7.8 | 5.6 |
| | RE (%) | -1.3 | -2.4 | -10.8 | -0.4 | -2.4 | 2.5 | -3.7 |
| Day 2 | Measured mean 5-carC/$10^6$ rG | 0.11 | 0.20 | 0.50 | 1.03 | 1.9 | 5.3 | 9.4 |
| *n*=3 | RSD (%) | 9.3 | 10.8 | 6.4 | 7.7 | 6.2 | 3.5 | 6.4 |
| | RE (%) | 4.0 | 2.4 | 1.3 | 4.3 | -4.0 | 6.3 | -5.8 |
| Day 3 | Measured mean 5-carC/$10^6$ rG | 0.11 | 0.20 | 0.47 | 1.09 | 1.9 | 4.8 | 10.4 |
| *n*=3 | RSD (%) | 6.5 | 3.5 | 9.3 | 7.8 | 7.5 | 9.8 | 5.2 |
| | RE (%) | 8.2 | -1.3 | -4.4 | 9.1 | -2.4 | -3.3 | -4.3 |

Supplementary Figure 1. Quantification of the activity of Caspase 3 in mouse ES cells treated by heavy metals. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Data are represented as mean ± SEM in triplicate experiments.



Supplementary Figure 2. Confirmation of the cytosine modifications in RNA of mouse ES cells by high-resolution mass spectrometry. (A) Product-ion spectra of BDEPE labeled 5-mrC standard (left) and 5-mrC in RNA of mouse ES cells (right). (B) Product-ion spectra of BDEPE labeled 5-hmrC standard (left) and 5-hmrC in RNA of mouse ES cells (right). (C) Product-ion spectra of BDEPE labeled 5-forC standard (left) and 5-forC in RNA of mouse ES cells (right). (D) Product-ion spectra of BDEPE labeled 5-carC standard (left) and 5-carC in RNA of mouse ES cells (right).



Supplementary Figure 3. mRNA expressions of (A) TET1, (B) TET2, and (C) TET3.