

Supplementary Material

Lead (Pb) exposure reduces global DNA methylation level by non-competitive inhibition and alteration of *dnmt* expression

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1. Supplementary Tables

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2. Supplementary Figures

Figure S1. Polyacrylamide gels showing the effect of Pb²⁺ and Ca²⁺ on the endonuclease activity of Hpa II at DNA concentrations of (A) 0.15 μM and (B) 0.75 μM.

Figure S2. Agarose gel of genomic DNA before and after sonication. Genomic DNA was sheared into fragments of ~ 100-300 bp in length. Lane 1: 1 kbp DNA ladder (NEB). Lane 2 and 3: Genomic DNA before and after sonication. Lane 4: 100 bp DNA ladder (NEB).

Figure S3. A typical polyacrylamide gel of DNA fragments digested by *HpaII* after different durations of methyltransferase reactions (0 – 10 mins) catalyzed by *M.SssI* under different Ca concentrations. Presence of Ca does not affect the activity of *M.SssI*. [DNA] = 0.15 μM.

Figure S4. Effect of Pb²⁺ (100 and 500 ppb) on zebrafish gDNA methylation assessed using a FCS-based assay. *: p<0.05 (ANOVA, Duncan's multiple-range test); n≥5, n: number of samples. Data is presented as mean ± standard deviation.

1. Supplementary Tables

Table S1. Sequence of DNA fragment used for the evaluation of the methyltransferase activity of M. Sss I.

Name	Sequence	No. CG sites.
MMTV (147bp)	ACTTGCAACAGTCCTAACATTCACCTCTTGTGTGTTTGTGTCTGTT CGCCAT CCCGTCT CCGCTCGT CACTTATCCTTCACTTCCAGAGGGTCCCC CGCAC ACCC CGGCG ACCCTCAGGT CGGCCG ACT GCGGC ACAGTTTTTTG	10

2. Supplementary Figures

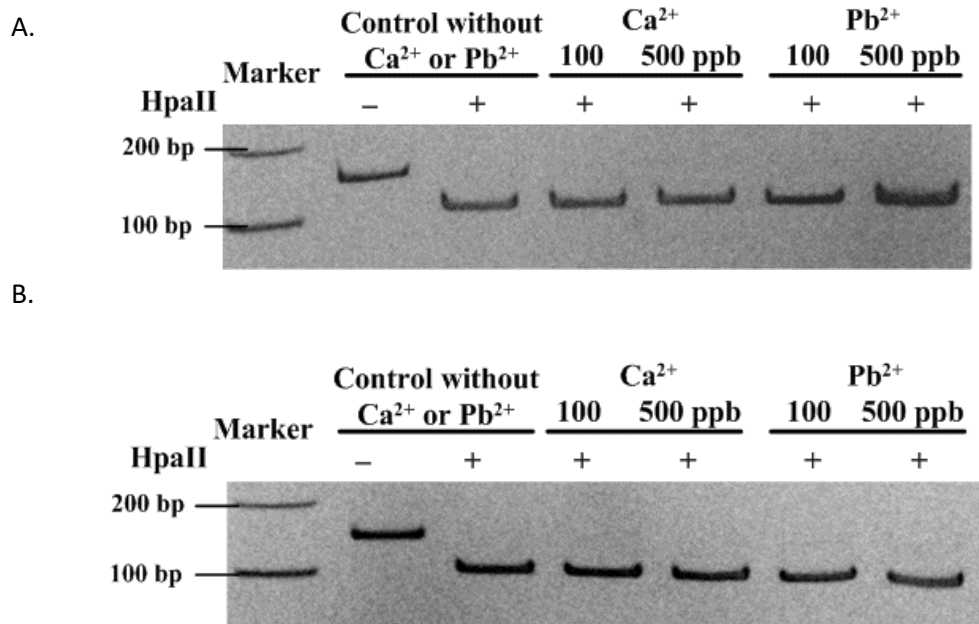


Figure S1. Polyacrylamide gels showing the effect of Pb²⁺ and Ca²⁺ on the endonuclease activity of Hpa II at DNA concentrations of (A) 0.15 μM and (B) 0.75 μM.

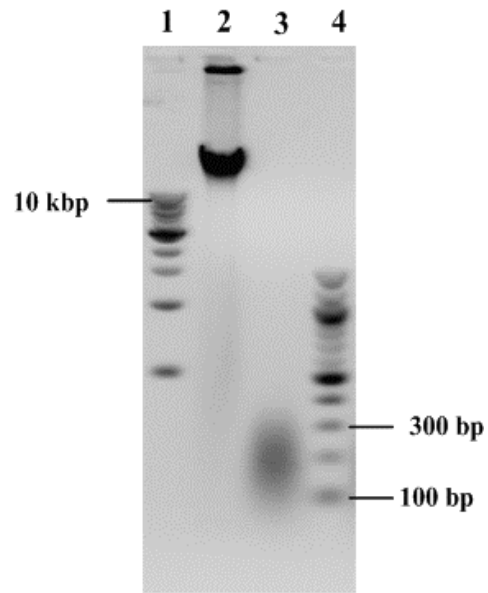


Figure S2. Agarose gel of genomic DNA before and after sonication. Genomic DNA was sheared into fragments of ~ 100-300 bp in length. Lane 1: 1 kbp DNA ladder (NEB). Lane 2 and 3: Genomic DNA before and after sonication. Lane 4: 100 bp DNA ladder (NEB).

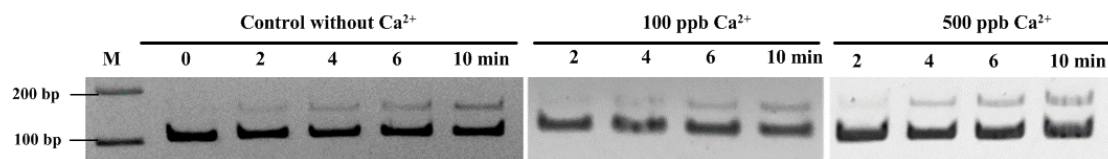


Figure S3. Polyacrylamide gel of DNA fragments digested by *HpaII* after different durations of methyltransferase reactions (0 – 10 mins) catalyzed by M.SssI under different Ca concentrations. Presence of Ca does not affect the activity of *M.SssI*.

[DNA] = 0.15 μ M.

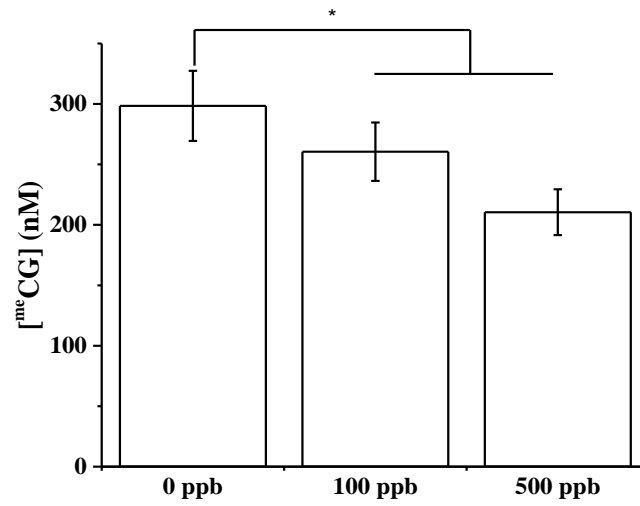


Figure S4. Effect of Pb²⁺ (100 and 500 ppb) on zebrafish gDNA methylation assessed using a FCS-based assay. *: p<0.05 (ANOVA, Duncan's multiple-range test); n≥5, n: number of samples. Data is presented as mean ± standard deviation.