

**Supporting Information**

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**Transgenerational Reproductive Effects of Arsenite Are Associated  
with H3K4 Dimethylation and SPR-5 Downregulation in *Caenorhabditis*  
*elegans***

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**Summary of Supporting Information:**

1. Total pages: 7
2. Total tables: 4

## ■ MATERIALS AND METHODS

### **Estimation of Total Worms Numbers for Measuring Arsenic Species Accumulation Assay.**

Nematodes from the control, F0, F1, and F2 generations were obtained in the same manner as described in the transgenerational assays. Total worms of F0, F1, and F2 generations were counted by the “dilution method” (Supporting Information). Briefly, worms from each generation were washed off from the NGM plate. Nematodes were then collected by centrifugation at 2000 rpm for 2 min at 4 °C. The collected nematodes were re-suspended in 10 ml of K-medium (0.032 M KCl, 0.051 M NaCl) and then performed serial dilutions until appropriated concentration of worm number were observed. Subsequently, worm numbers were counted under the microscope by pipetting 10 µl from the well-suspension mixture. At least 10 repeats were performed. Total worm numbers can be calculated from the following formula: Total worm numbers = worms counted x 1000 x dilution factor. Around 50,000 worms of each condition were used.

**STable 1. Primers used for real-time PCR.**

Gene	Sequence
<i>spr-5</i>	forward: 5'-GTTGTTGCATCAGGTAAACTTCGA-3' reverse: 5'-GCTCACATTGATGGGCTTGATAG-3'
$\beta$ -actin	reverse: 5'- GTGTGACGACGAGGTTGCCGCTTGTAGAC-3' reverse: 5'- GGTAAAGGATCTTCATGAGGTAATCAGTAAGATCAC-3'
<i>mlc-2</i>	forward: 5' - TTGACAGGAACTGACCCAGAGG -3' reverse: 5' - ATAGCCTTGACCTCATCCTCG-3'

## ■ RESULTS AND DISCUSSION

**S**Table 2. ANOVA analysis for growth and reproduction assays.

Arsenite (mM)		Body length	Brood size
		<i>p</i> -value (Tukey HSD)	
0	0.1	0.989	0.912
	0.25	0.996	0.014
	0.5	0.029	0.003
	1	0.000	0.000
0.1	0	0.989	0.912
	0.25	1.000	0.105
	0.5	0.422	0.026
	1	0.001	0.000
0.25	0	0.996	0.014
	0.1	1.000	0.105
	0.5	0.044	0.937
	1	0.000	0.035
0.5	0	0.029	0.003
	0.1	0.422	0.026
	0.25	0.044	0.937
	1	0.023	0.360
1	0	0.000	0.000
	0.1	0.001	0.000
	0.25	0.000	0.035
	0.5	0.023	0.360

**STable 3.** ANOVA analysis for transgeneration assays.

Generation		Brood size	<i>spr-5</i> mRNA level	Dimethylated histone H3K4 level
		<i>p</i> -value (Tukey HSD)		
Control	<b>F0</b>	0.000	0.045	0.002
	<b>F1</b>	0.000	0.021	0.002
	<b>F2</b>	0.000	0.014	0.000
	<b>F3</b>	0.000	0.022	0.018
	<b>F4</b>	0.000	0.992	0.719
	<b>F5</b>	0.000	0.985	0.995
<b>F0</b>	<b>Control</b>	0.000	0.045	0.002
	<b>F1</b>	0.000	0.997	1.000
	<b>F2</b>	0.007	0.999	0.976
	<b>F3</b>	0.031	1.000	1.000
	<b>F4</b>	0.000	0.247	0.039
	<b>F5</b>	0.000	0.485	0.034
<b>F1</b>	<b>Control</b>	0.000	0.021	0.002
	<b>F0</b>	0.000	0.997	1.000
	<b>F2</b>	1.000	1.000	0.983
	<b>F3</b>	1.000	1.000	1.000
	<b>F4</b>	0.696	0.116	0.033
	<b>F5</b>	0.803	0.263	0.030
<b>F2</b>	<b>Control</b>	0.000	0.014	0.000
	<b>F0</b>	0.007	0.999	0.976
	<b>F1</b>	1.000	1.000	0.983
	<b>F3</b>	1.000	1.000	0.972
	<b>F4</b>	0.891	0.105	0.003

	<b>F5</b>	0.936	0.268	0.004
<b>F3</b>	<b>Control</b>	0.000	0.022	0.018
	<b>F0</b>	0.031	1.000	1.000
	<b>F1</b>	1.000	1.000	1.000
	<b>F2</b>	1.000	1.000	0.972
	<b>F4</b>	0.675	0.148	0.168
	<b>F5</b>	0.764	0.343	0.111
<b>F4</b>	<b>Control</b>	0.000	0.992	0.719
	<b>F0</b>	0.000	0.247	0.039
	<b>F1</b>	0.696	0.116	0.033
	<b>F2</b>	0.891	0.105	0.003
	<b>F3</b>	0.675	0.148	0.168
	<b>F5</b>	1.000	1.000	0.993
<b>F5</b>	<b>Control</b>	0.000	0.985	0.995
	<b>F0</b>	0.000	0.485	0.034
	<b>F1</b>	0.803	0.263	0.030
	<b>F2</b>	0.936	0.268	0.004
	<b>F3</b>	0.764	0.343	0.111
	<b>F4</b>	1.000	1.000	0.993

**STable 4. Analysis of significance between factors in transgenerational data (Figures 3 - 5) by two-way ANOVA.**

Main effects	Interaction (arsenite vs. generation)
Brood size	0.085
<i>spr-5</i> mRNA level	0.055
Dimethylated histone H3K4 level	0.074