Metabolism of selenite in human lung cancer cells: X-ray absorption and fluorescence studies

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SUPPORTING INFORMATION

REFERENCE 25

Carter, E. A.; Rayner, B. S.; McLeod, A. I.; Wu, L. E.; Marshall, C. P.; Levina, A.; Aitken, J. B.; Witting, P. K.; Lai, B.; Cai, Z.; Vogt, S.; Lee, Y.; Chen, C.; Tobin, M. J.; Harris, H. H.; Lay, P. A. *Mol. BioSyst*. **2010**, *6*, 1316–1322.

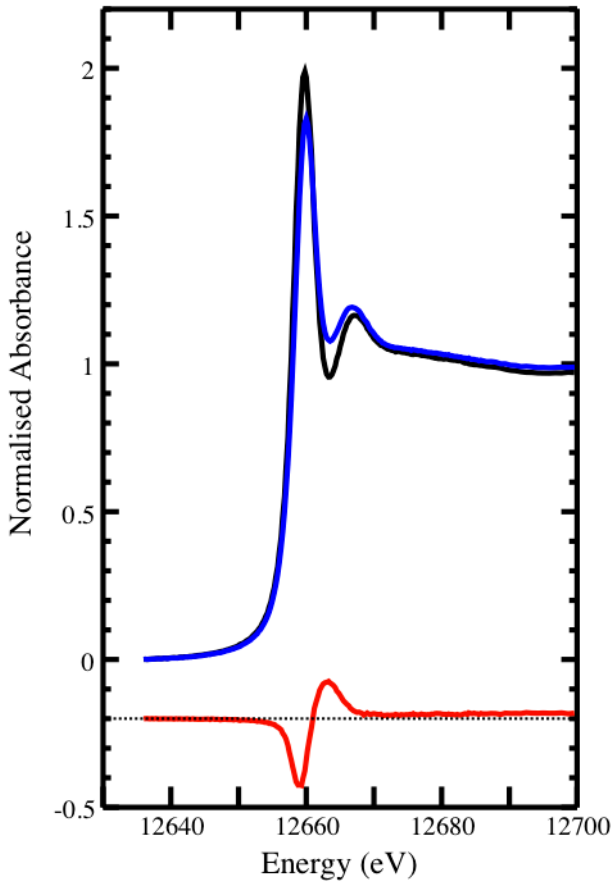


Figure S1. Difference plot of Se K-edge X-ray absorption near-edge spectra of A549 cells treated with 5 μM selenite for 24 h (blue solid line) or 48 h (black solid line), with the residual (red line shown offset) showing the difference between them.

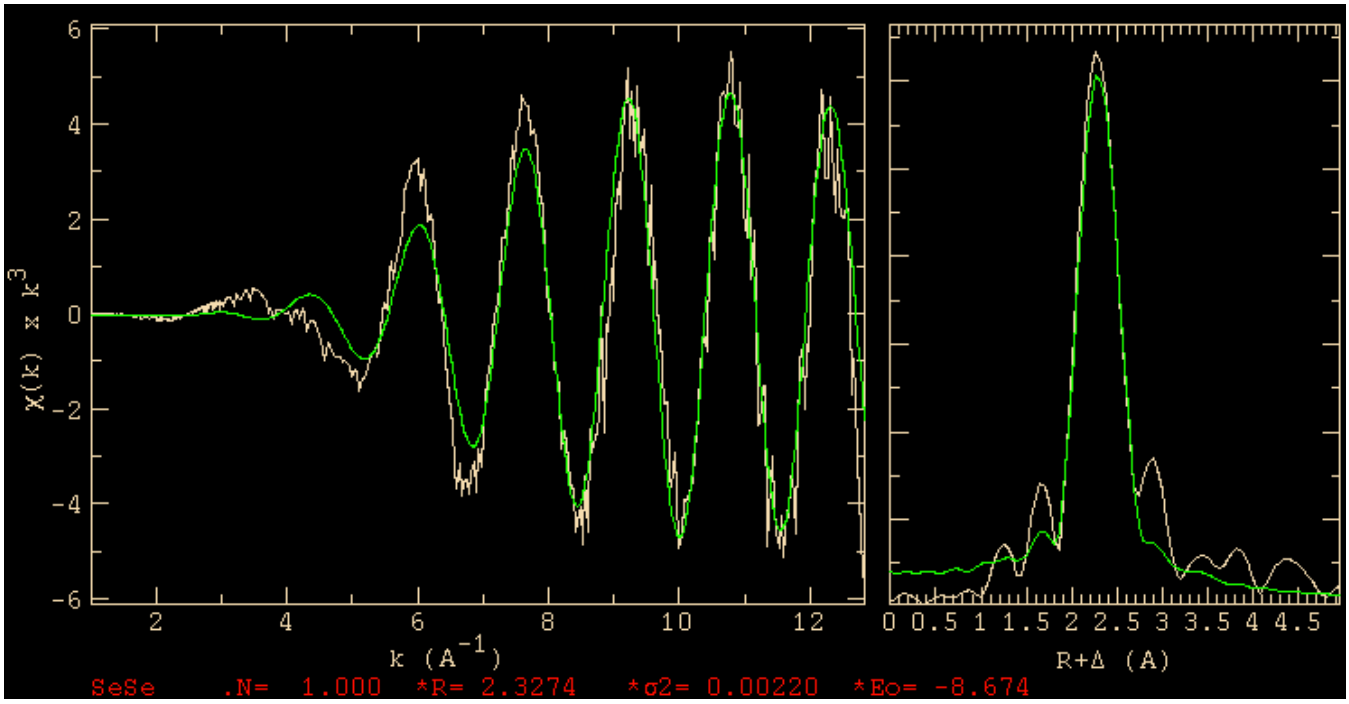


Figure S2. EXAFS spectrum (left) and corresponding Fourier Transform (right) of CysSeSeCys (white). A single Se scatterer is fit to the spectrum (a C scatterer was not included in the fit) with a Se-Se bond length of 2.33 Å and the calculated fit is shown in green. Fit parameters are shown in the red text. The fit error is 0.35.

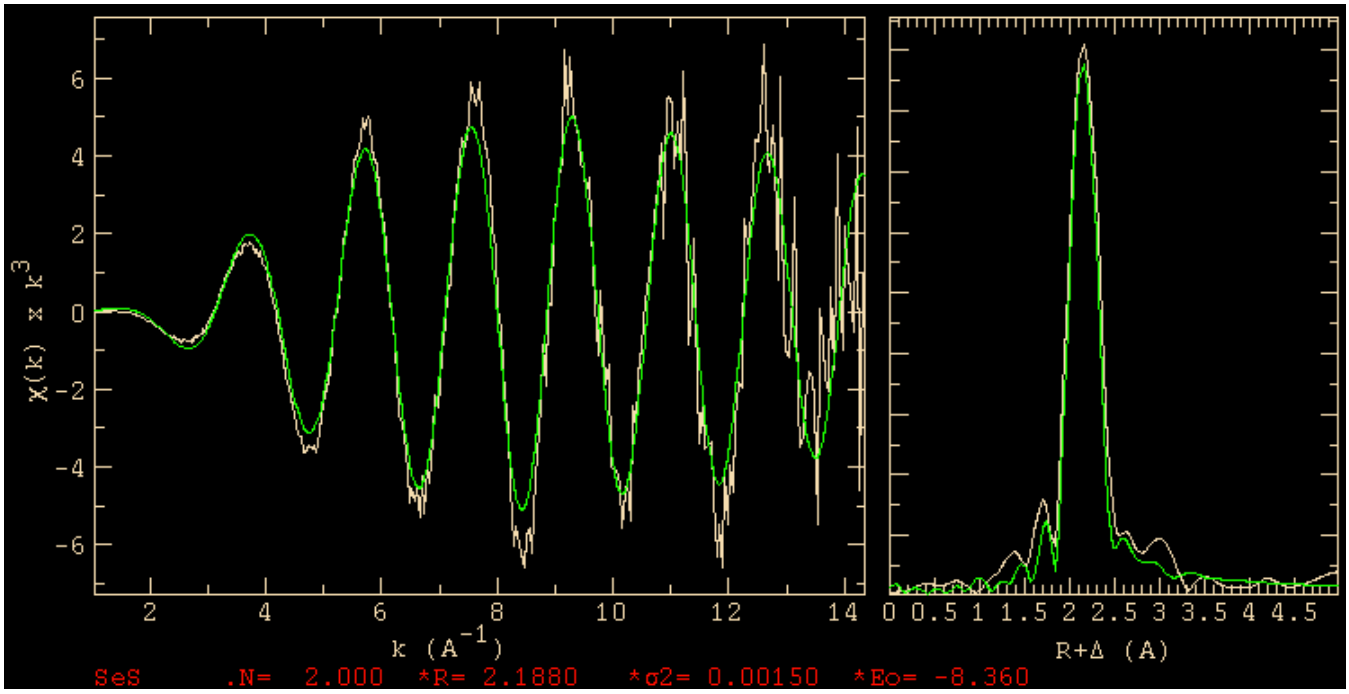


Figure S3. EXAFS spectrum (left) and corresponding Fourier Transform (right) of GSSeSG (white). Two S scatterers are fit to the spectrum with a Se-S bond length of 2.19 Å and the calculated fit is shown in green. Fit parameters are shown in the red text. The fit error is 0.36.

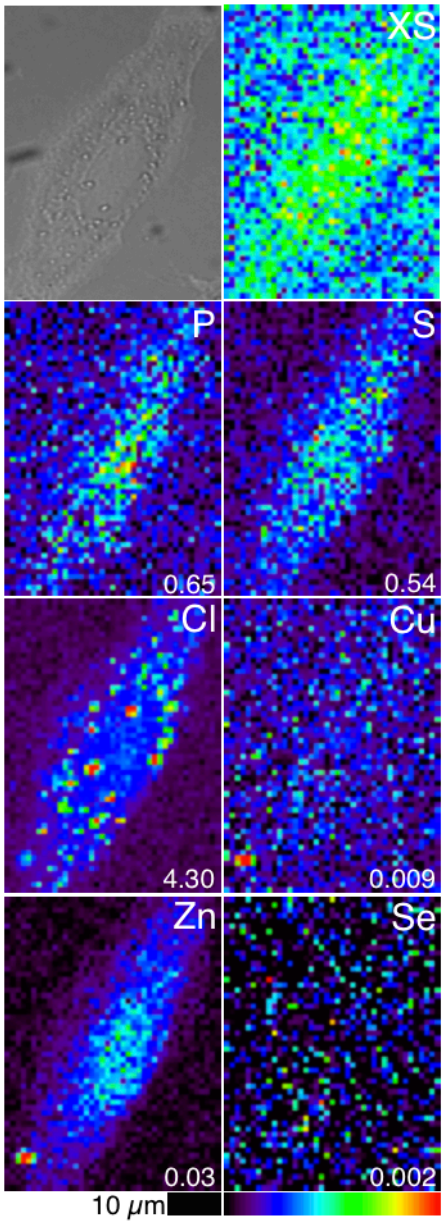
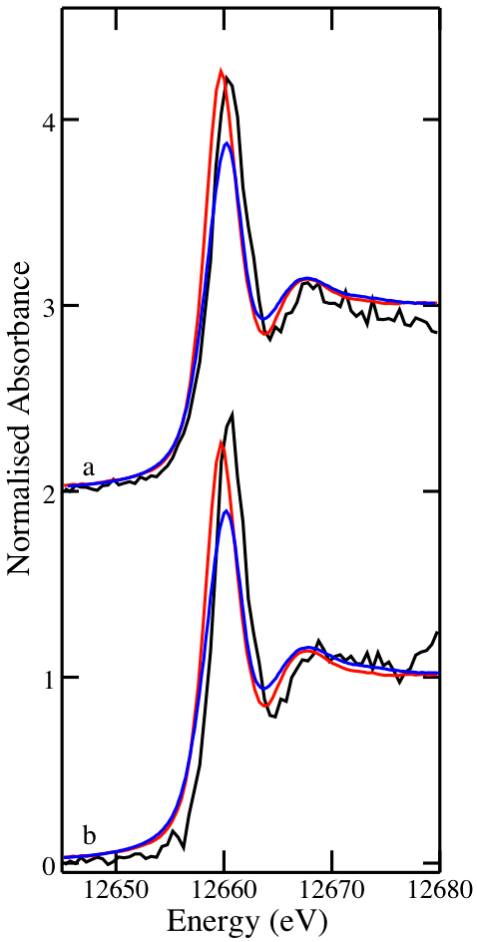


Figure S4. Optical micrograph (top left), scattered X-ray (XS) and XRF elemental distribution maps of P, S, Cl, Cu, Zn and Se of an A549 cell treated with PBS as a vehicle control for 24 h. Maximum elemental area density (μg cm^{-2}) is given at the bottom right of each map. High concentration spots in the Cl map are due to the formation of PBS crystals on the outside of the cell after washing.



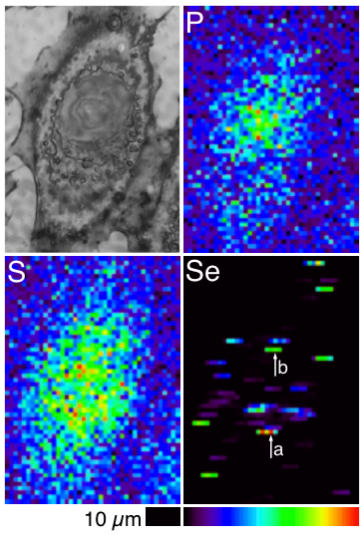


Figure S5. Se K-edge μ-XANES spectra of Se hotspots in an A549 cell treated with 5 μm selenite. The experimental spectra (a and b, black) are overlaid with the spectra of elemental Se (red) and GSSeSG (blue). The optical micrograph (top left) and scattered X-ray (XS) and elemental distribution maps of S and Se of the cell are shown with arrows indicating the locations from which spectra (a) and (b) were collected.

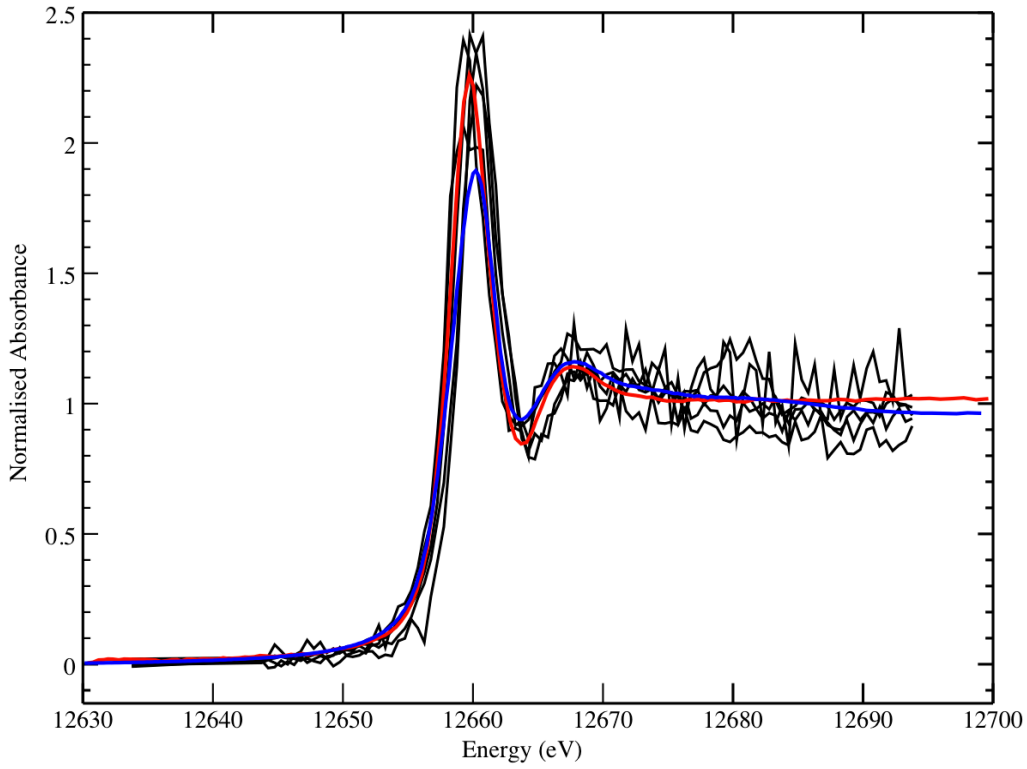


Figure S6. Se K-edge μ-XANES of regions of high Se content in A549 cells treated with 5 μm selenite for 24 h. The experimental spectra (black) are overlaid with the spectra of elemental Se (red) and GSSeSG (blue).

Table S1: Alternative parameters fit to EXAFS spectra of A549 cells treated with 5 μM selenite for 24 h, which give poorer fits than the fit shown in Table 1.[a]

scatterer	coordination Number (N)	interatomic distance (R, Å)	Debye-Waller factor (σ^2, Å^2)	$-\Delta E_0$ (eV)	fit error
S	2	2.270(2)	0.0023(2)	-4.2(5)	0.43
Se	2	2.316(3)	0.0053(1)	21(1)	0.35
Se	3	2.317(3)	0.0071(1)	20.7(9)	0.32
S	1.4	2.416(4)	0.0007(2)	12(1)	0.31
Cu	0.6	2.198(5)	0.0023(2)		

[a]The k-range was 1 – 14.2 Å^{-1} and a scale factor (S_0^2) of 0.9 was used for all fits. $\Delta E_0 = E_0 - 12658$ (eV) where E_0 is the threshold energy. Values in parentheses are the estimated standard deviation derived from the diagonal elements of the covariance matrix and are a measure of precision. The fit-error is defined as $[\Sigma k^6(\chi_{exp}-\chi_{calc})^2/\Sigma k^6\chi_{exp}{}^2]^{½}$.

Table S2: Elemental area densities (μg cm^{-2}) of A549 control cells and cells treated with 5 μM selenite for 20 min.[a]

Treatment	P	S	Cl	K	Ca × 10^{-3}	Fe × 10^{-3}	Cu × 10^{-3}	Zn × 10^{-3}	Se × 10^{-3}	Area (μm^2)
Control (1 h) (n =8)	0.4(1)	0.3(1)	1.2(4)	0.01(1)	14(4)	2.6(5)	1.4(1)	18(4)	0.23(2)	900(200)
5 μM selenite (n = 6)	0.6(1)*	0.5(2)*	1.4(2)	0.07(2)*	30(10)*	12(3)*	1.7(1)*	9(2)*	0.28(3)*	900(600)

[a]Data represent the mean and standard deviation (in brackets) for n cells. A statistically significant difference between treatment and controls was accepted at the 95% confidence interval with P-values determined by Student t-test assuming unequal variance. *$P < 0.05$ versus control.

Table S3: Elemental area densities (μg cm^{-2}) of the nuclei of A549 control cells and cells treated with 5 μM selenite for 20 min.[a]

Treatment	P	S	Cl	K	Ca × 10^{-3}	Fe × 10^{-3}	Cu × 10^{-3}	Zn × 10^{-3}	Se × 10^{-3}	Area (μm^2)
Control (1 h) (n = 8)	0.8(2)	0.4(1)	2.0(5)	0.02(1)	29(4)	3.3(7)	1.6(2)	30(6)	0.25(4)	160(80)
5 μM selenite (n = 6)	1.5(3)*	1.2(4)*	3.1(8)*	0.16(4)*	80(30) *	20(8)*	2.2(3)*	22(5)*	0.4(1)*	130(50)

[a]Data represent the mean and standard deviation (in brackets) for n cells. A statistically significant difference between treatment and controls was accepted at the 95% confidence interval with P-values determined by Student t-test assuming unequal variance. *$P < 0.05$ versus control.

Table S4: Elemental area densities (μg cm^{-2}) of the non-nuclear region of A549 control cells and cells treated with 5 μM selenite for 20 min.[a]

Treatment	P	S	Cl	K	Ca × 10^{-3}	Fe × 10^{-3}	Cu × 10^{-3}	Zn × 10^{-3}	Se × 10^{-3}	Area (μm^2)
Control (1 h) (n = 8)	0.3(1)	0.21(7)	1.0(3)	0.011(5)	11(3)	2.4(4)	1.4(1)	16(3)	0.22(2)	800(200)
5 μM selenite (n = 6)	0.37(9)	0.4(1)*	1.0(1)	0.05(1)*	23(7)*	11(3)*	1.6(1)*	7(1)*	0.25(2)*	800(600)

[a]Data represent the mean and standard deviation (in brackets) for n cells. A statistically significant difference between treatment and controls was accepted at the 95% confidence interval with P-values determined by Student t-test assuming unequal variance. *$P < 0.05$ versus control.

Table S5: Ratio of nuclear to non-nuclear region elemental area densities (μg cm^{-2}) of A549 control cells and cells treated with 5 μM selenite for 20 min.[a]

Treatment	P	S	Cl	K	Ca	Fe	Cu	Zn	Se	Area (μm^2)
Control (1 h) (n = 8)	2.8(7)	2.1(4)	2.0(3)	2.4(6)	2.6(5)	1.3(3)	1.1(2)	1.9(3)	1.1(1)	0.3(2)
5 μM selenite (n = 6)	4(1)*	2.8(3)*	3.1(8)*	3.0(4)	3.6(5)*	1.9(7)	1.4(1)*	3.3(4)*	1.5(4)*	0.20(8)

[a]Data represent the mean and standard deviation (in brackets) for n cells. A statistically significant difference between treatment and controls was accepted at the 95% confidence interval with P-values determined by Student t-test assuming unequal variance. *$P < 0.05$ versus control.

Table S6: Elemental area densities (μg cm^{-2}) of A549 control cells and cells treated with 5 μM selenite for 24 h.[a]

Treatment	P	S	Cl	K	Ca × 10^{-3}	Fe × 10^{-3}	Cu × 10^{-3}	Zn × 10^{-3}	Se × 10^{-3}	Area (μm^2)
Control (n = 4)	0.23(7)	0.15(4)	1.5(3)	0.13(4)	5(2)	2.2(3)	1.5(1)	8(3)	0.22(4)	1100(400)
5 μM selenite (n = 7)	0.39(6)*	0.33(6)*	0.51(3)*	0.003(2)*	2(1)	8.2(4)*	3.8(4)*	5(1)	50(30)*	1800(300)

[a]Data represent the mean and standard deviation (in brackets) for n cells. A statistically significant difference between treatment and controls was accepted at the 95% confidence interval with P-values determined by Student t-test assuming unequal variance. *$P < 0.05$ versus control.

Table S7: Elemental area densities (μg cm^{-2}) of the nuclei of A549 control cells and cells treated with 5 μM selenite for 24 h.[a]

Treatment	P	S	Cl	K	Ca × 10^{-3}	Fe × 10^{-3}	Cu × 10^{-3}	Zn × 10^{-3}	Se × 10^{-3}	Area (μm^2)
Control (n = 4)	0.42(8)	0.26(5)	2.7(8)	0.24(3)	8(4)	2.8(5)	1.7(2)	16(4)	0.23(9)	160(20)
5 μM selenite (n = 7)	1.0(1)*	0.6(1)*	0.60(7)*	0.007(6)*	5(3)	9(1)*	4.6(9)	9(3)	40(30)*	280(80)

[a]Data represent the mean and standard deviation (in brackets) for n cells. A statistically significant difference between treatment and controls was accepted at the 95% confidence interval with P-values determined by Student t-test assuming unequal variance. *$P < 0.05$ versus control.

Table S8: Elemental area densities (μg cm^{-2}) of the non-nuclear region of A549 control cells and cells treated with 5 μM selenite for 24 h.[a]

Treatment	P	S	Cl	K	Ca × 10^{-3}	Fe × 10^{-3}	Cu × 10^{-3}	Zn × 10^{-3}	Se × 10^{-3}	Area (μm^2)
Control (n = 4)	0.19(5)	0.12(3)	1.2(2)	0.11(2)	4(1)	2.0(2)	1.4(1)	6(2)	0.22(4)	900(400)
5 μM selenite (n = 7)	0.29(4)*	0.27(5)*	0.50(2)*	0.002(2)*	2(1)*	8.0(4)*	3.6(3)*	5(1)	40(30)*	1500(300)*

[a]Data represent the mean and standard deviation (in brackets) for n cells. A statistically significant difference between treatment and controls was accepted at the 95% confidence interval with P-values determined by Student t-test assuming unequal variance. *$P < 0.05$ versus control.

Table S9: Ratio of nuclear to non-nuclear region elemental area densities (μg cm^{-2}) of A549 control cells and cells treated with 5 μM selenite for 24 h.[a]

Treatment	P	S	Cl	K	Ca	Fe	Cu	Zn	Se	Area (μm^2)
Control (n = 4)	2.3(4)	2.2(3)	2.2(8)	2.2(2)	2.3(9)	1.4(2)	1.19(8)	2.6(3)	1.1(4)	0.2(1)
5 μM selenite (n = 7)	3.5(6)*	2.3(2)*	1.2(1)	4(2)*	3(1)	1.1(1)	1.3(2)*	2.0(3)*	1.0(5)	0.19(6)

[a]Data represent the mean and standard deviation (in brackets) for n cells. A statistically significant difference between treatment and controls was accepted at the 95% confidence interval with P-values determined by Student t-test assuming unequal variance. *$P < 0.05$ versus control.