

Online Supporting Material

Supplemental TABLE 1 List of primers used for Q-PCR analysis

Gene	Forward (5' to 3')	Reverse (5' to 3')
Cell cycle arrest proteins		
<i>CDC2¹</i>	CAGAGCTTTGGGCACTCCCAATAA	ATGGGATGCTAGGCTTCCTGGTTT
<i>Cdc25A</i>	TGAGCAACCACTGGAGGTGAAGAA	AGCTGGACTACATCCCAACAGCTT
<i>CDK2</i>	AGATGGACGGAGCTTGTTATCGCA	TGGCTTGGTCACATCCTGGAAGAA
<i>CDK4</i>	TTTGTGGCCCTCAAGAGTGTGAGA	AAAGCCTCCAGTCGCCTCAGTAAA
<i>Cyclin A</i>	ATGAGCATGTCACCGTTCCTCCTT	TCAGCTGGCTTCTTCTGAGCTTCT
<i>Cyclin D1</i>	AGAAGCTGTGCATCTACACCGACA	TGATCTGTTTGTTCCTCCGCCT
<i>DPI</i>	AGCGCAACAGGAAAGGAGAGAAGA	TCGTCTGCCACTTCGTTGTAGGAA
<i>GADD153</i>	AGGGAGAACCAGGAAACGGAAACA	TCCTGCTTGAGCCGTTTCATTCTCT
Apoptosis		
<i>AKT2</i>	AGCACAGGTTCTTCCTCAGCATCA	TGATTGTGATGGACTGGGCGGTAA
<i>Bcl-2</i>	GCATGCGGCCTCTGTTTGATTCT	AGGCATGTTGACTTCACTTGTGGC
<i>Caspase-3</i>	TCATTATTCAGGCCTGCCGTGGTA	TGGATGAACCAGGAGCCATCCTTT
<i>c-jun</i>	GGAACAGGTGGCACAGCTTAAACA	TTGCAACTGCTGCGTTAGCATGAG
<i>FoxO1</i>	GCTGCATCCATGGACAACAACAGT	TGAGCTAGTTCGAGGGCGAAATGT
<i>p19</i>	TCGTGGTTCACATCTCGTGGTTCA	CCCATCATCATGACCTGGTCTTCT
<i>p21</i>	TCAAAGGCCCGCTCTACATCTTCT	TAGGAACCTCTCATTCAACCGCCT
<i>P38</i>	TGTCGACTTGCTGGAGAAGATGCT	TCATAAGGATCGGCCACTGGTTCA
<i>P53</i>	GCCGTCCCAAGCAATGGATGATTT	TCTGGCATTCTGGGAGCTTCATCT
Selenoproteins		
<i>Dio1</i>	TGGTCGTGGGTAAAGTGCTTCTGA	

<i>Gpx1</i>	TGGTATCCAGTTGTCGTGGCTGAA TCAGGAGAACGCCAAGAACGAAGA TCTCGAAGAGCATGAAGTTGGGCT
<i>Gpx2</i>	AGCTCTGGGCCTTCACAGAATGAT TCCACACCTGCCCTTTATTGGTCT
<i>Gpx3</i>	AGTGGCACCATTTACGAGTACGGA AAGCCCAGAATGACCAGACCGAAT
<i>Gpx4</i>	ATACGCTGAGTGTGGTTTGCGGAT ATCGAATTTGACGTTGTAGCCCGC
<i>Selh</i>	ACGCAAACCTCAAATTCCTGAGCC TCAGCATGAGGGCTTCTACCCAAA
<i>Selk</i>	ATCAATCATCTGCGTGGCCCTAGT TGAGGACACAGAGCAGTCCTTGTT
<i>Selm</i>	ATGACAGCTGAACCGCCTAAAGGA AGGGAGGTGTTTCATCACCAGGTT
<i>Selo</i>	TTCTATGCAGCGAAGCCATGTTCC TACCATCATAGAACACGTCGCGCA
<i>Sels</i>	AATGTGGGACAGCATGCAAGAAGG CCGCAAAGGCTTTCTGTCCGATTT
<i>Selt</i>	TTCTTTGGCATGCAAGCTCCTAGC TGGAAGGTGACCAGATTCCAGCTT
<i>Selv</i>	AAAGAGCCTGGAGCAGCAATTTCC ATGGACCAGTCTCCCGTTCACAAA
<i>Sep15</i>	TTCATCGGAGGCATGCAGAGAGTT TGACAGCATCCTCTGCAATCAGGA
<i>Sepn1</i>	TCAACGAGAGCTTCATCAGCACCT TTGGCATTGATGTGATGGACCACG
<i>Sepp1</i>	AGCCAGGACCAAAGCTCCTTATGT TAGATGCCTGCAGTATGCACAGGT
<i>Sepw1</i>	ATGGCTACGTGGACACAGAAAGCA AGCCACGAGAACATCAGGGAAAGA
<i>Txnrd1</i>	TTGCAATCCAGGCAGGAAGATTGC AGCTTTCTCCTCAGAAAGGCCACA
<i>Txnrd2</i>	TGGACGAGATGCATCCCAGTGTTA ATCCTTGAGTAACTTCGCCTGCGT
	Control protein
<i>HPRT</i>	GGCCAGACTTTGTTGGATTTG TGCGCTCATCTTAGGCTTTGT

¹*AKT2*, RAC-beta serine/threonine-protein kinase; *Bcl-2*, B-cell lymphoma 2; *CDC2*, cyclin-dependent kinase 1; *Cdc25A*, cell division cycle 25 homolog A; *CDK2*, cyclin-dependent kinase 2; *CDK4*, cyclin-dependent kinase 4; *Dio1*, deiodinase, iodothyronine, type I; *DPI*, transcription

factor Dp-1; *FoxO1*, forkhead box O1; *GDAI153*, growth arrest and DNA damage-inducible gene 153; *Gpx1*, cytosolic glutathione peroxidase; *Gpx2*, gastro-intestinal glutathione peroxidase; *Gpx3*, plasma glutathione peroxidase; *Gpx4*, phospholipid- hydroperoxide glutathione peroxidase; *HPRT*, hypoxanthine-guanine phosphoribosyltransferase; *p19*, cyclin-dependent kinase inhibitor 2A; *p21*, cyclin-dependent kinase inhibitor 1; *p38*, mitogen-activated protein kinase p38; *p53*, tumor suppressor protein 53; *Selh*, selenoprotein H; *Selk*, selenoprotein K; *Selm*, selenoprotein M; *Selo*, selenoprotein O; *Sels*, selenoprotein S; *Selt*, selenoprotein T; *Selv*, selenoprotein V; *Sep15*, 15 kDa selenoprotein; *Sepn1*, selenoprotein N; *Sepp1*, selenoprotein P; *Sepw1*, selenoprotein W; *Txnrd1*, thioredoxin reductase-1; *Txnrd2*, thioredoxin reductase-2;

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Supplemental TABLE 2 Name, type, dilution, and source of primary antibodies

Antibody	Isotype	Dilution	Source
p53	Rabbit	1,000	Cell Signaling Technology (Beverly, MA)
Bcl-2	Rabbit	1,000	Cell Signaling Technology (Beverly, MA)
c-jun	Rabbit	1,000	Santa Cruz Biotechnology (Santa Cruz, CA)
Sepp1	Rabbit	1,000	Santa Cruz Biotechnology (Santa Cruz, CA)
Sep15	Rabbit	1,000	Abcam (Cambridge, MA)
β -Actin	Rabbit	1,000	Cell Signaling Technology (Beverly, MA)

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Supplemental TABLE 3 Stepwise regression analysis between mRNA levels of selenoprotein genes (independent variables) and cell death genes (dependent variables) in Expt. 2¹

Stepwise Regression		R ²	P value
DU145 cells			
² Y ₁	Y ₁ =-0.086X ₃ +1.03	0.29	0.11
Y ₃	Y ₃ =0.0022X ₁ -0.0031X ₂ -0.006X ₄ +0.015	0.77	0.02
Y ₄	Y ₄ =0.10X ₁ -0.17X ₃ -0.89X ₅ +1.0	0.73	0.04
Y ₅	Y ₅ =-0.22X ₂ +2.27	0.56	0.01
Y ₆	Y ₆ =-0.23X ₂ +2.78	0.42	0.04
Y ₇	Y ₇ =0.42X ₂ -0.39X ₃ +1.04	0.88	0.00
Y ₈	Y ₈ =0.04X ₂ -0.086	0.65	0.01
Y ₁₀	Y ₁₀ =0.30X ₃ +2.14X ₅ -2.7X ₈ -0.80	0.78	0.02
Y ₁₁	Y ₁₁ =0.021X ₁ +0.028X ₂ +0.05X ₃ -0.11X ₄ -0.15X ₇ -0.12	0.96	0.01
Y ₁₂	Y ₁₂ =0.053X ₂ +0.044	0.57	0.01
HTC116 cells			
Y ₄	Y ₄ =0.014X ₁ -0.07X ₆ -0.19X ₉ +1.25	1.00	0.02
Y ₇	Y ₇ =6.84X ₁₀ -325.90X ₁₁ -10.53	1.00	0.01
Y ₉	Y ₉ =0.087X ₉ -0.16	0.93	0.01
Y ₁₁	Y ₁₁ =-0.028X ₉ +0.28	0.70	0.08
Y ₁₂	Y ₁₂ =4.91X ₇ -0.12X ₁₀ +0.27	1.00	0.01

¹Data were from cells treated with the control porcine serum, the Se-biofortified serum, and the MSA-balanced serum.

²Y₁: DP1; Y₂: Cdc2; Y₃: Cdc25A; Y₄: CyclinA; Y₅: CDK2; Y₆: CDK4; Y₇: GADD153; Y₈: p53;

Y₉: p38; Y₁₀: c-jun; Y₁₁: Caspase3; Y₁₂: FoxO1; X₁: Gpx4; X₂: Sep15; X₃: Selt; X₄: Gpx1; X₅:

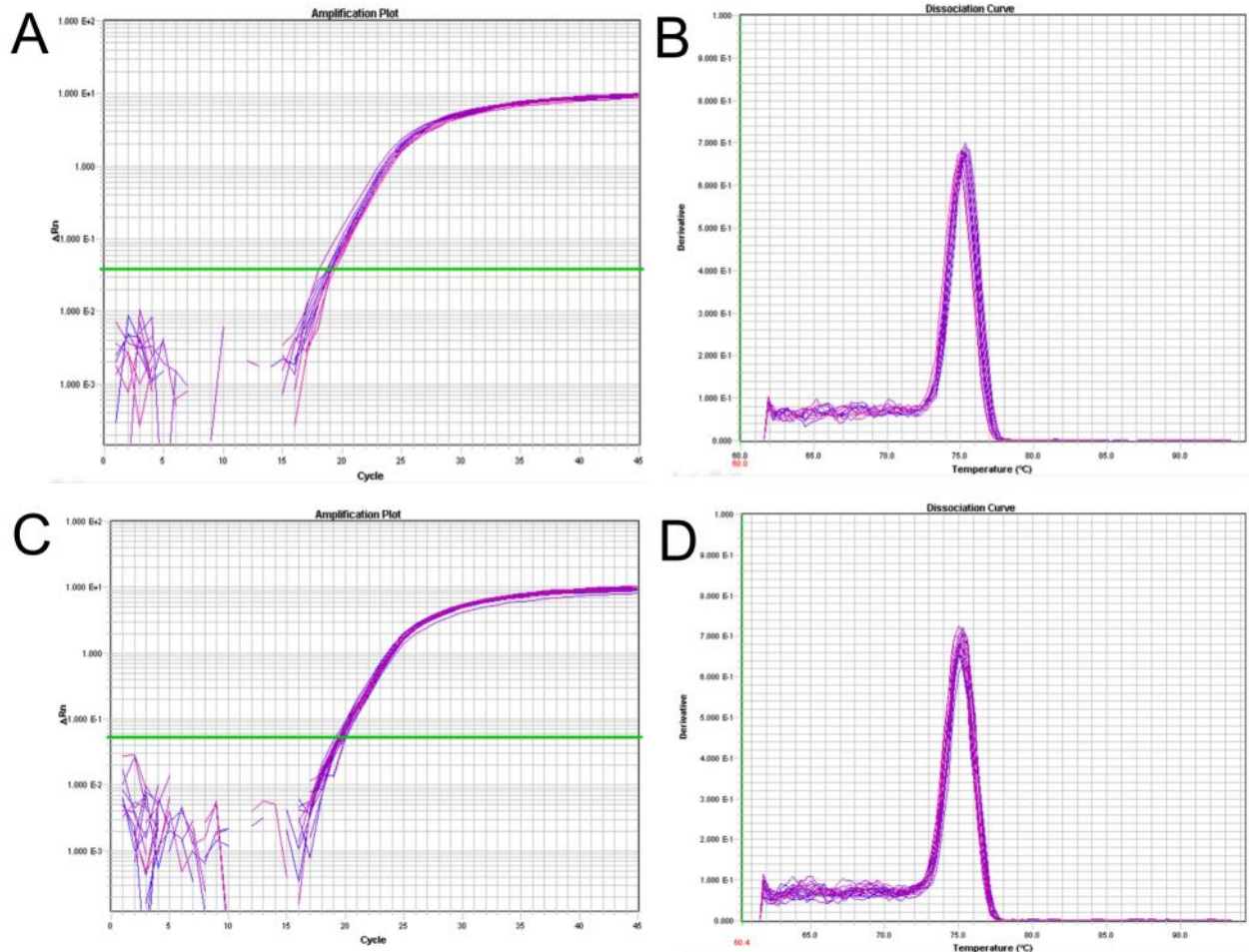
Sepp1; X₆: Sels; X₇: Txnrd2; X₈: Selm; X₉: Selh; X₁₀: Selk; X₁₁: Sepn1.

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Supplemental TABLE 4 Doses of selenium in various forms used in previous studies

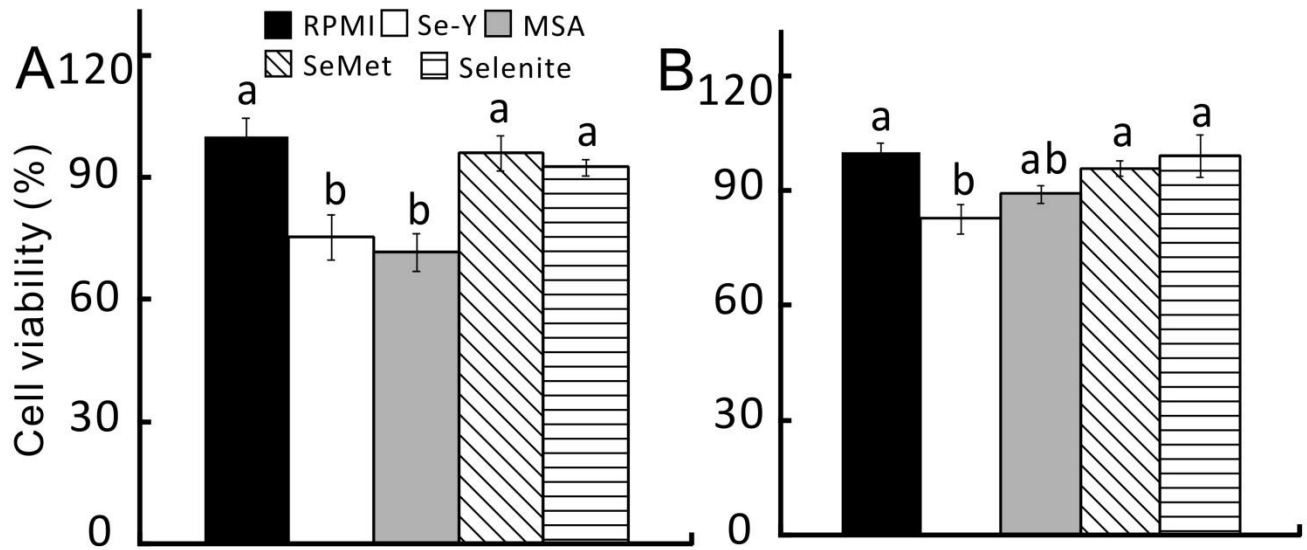
Forms	Se, μ M	Cell Lines	Reference
MSA	5-10	PC-3	33
	3-30	LNCaP	34
	3-5	DU145	35
SeMet	45-130	MCF7S, DU145 and UACC375	31
	32	HT-29, HCA-7, Caco-60 and Caco-pcDNA	32
	25-400	PC-3	33
Sodium	5	DU145	35
selenite	10-100	B16 and pB16	36
	20	NB4	37

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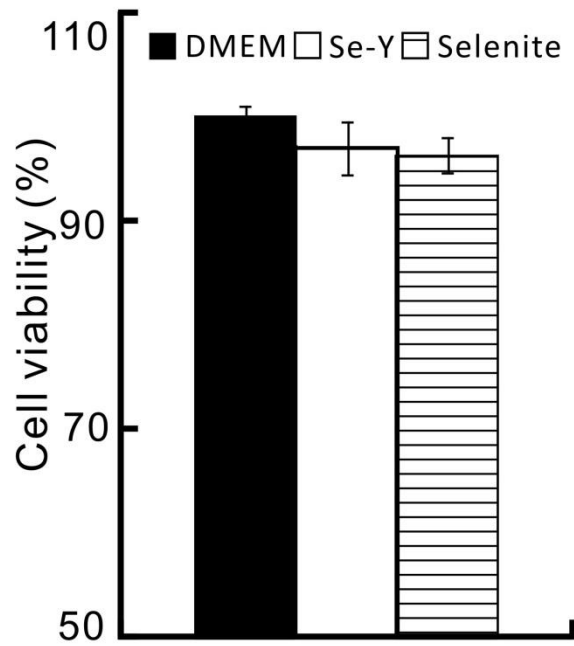
Supplemental FIGURE 1 Amplification plots (A and C) and dissociation curves (B and D) based on Q-PCR data used to detect HPRT mRNA abundances in DU145 cells (A and B) and HCT116 cells (C and D) in Expt. 2. The assay for HPRT mRNA abundances in the five treatments (Control, Se-Y, MSA, SeMet and Selenite) was conducted simultaneously on one 384-well plate. (A and C) The cycle threshold (Ct) value was the projection of crossing point on X-axis of ΔRn threshold line ($Y = 0.04-0.05$). The amplification plot in the increased logarithmic phase and the smaller Ct value represented the higher abundance of initial mRNA template that can be detected after less PCR cycles. (B and D) The single sharp dissociation curve cluster of HPRT represented the gene's specific amplification.

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Supplemental FIGURE 2 Effects of the Se-biofortified porcine serum (Se-Y) treatment on cell viability of the human prostate cancer cells (DU145, A) and the human colon cancer cells (HTC116, B), in comparison with the RPMI 1640 medium (supplemented with 100 IU of penicillin, 100 μ g of streptomycin/mL and 10% fetal bovine serum) or that medium was added with different Se compounds to match the Se concentration in Se-Y in Expt. 2. The cells were seeded at 1×10^4 cells/well in 24-well plates and incubated with the designated Se sources for 144 h. Cell viability was determined using MTT assay. Data are means \pm SE, n = 5. Bars not sharing a common letter differ ($P < 0.05$).

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Supplemental FIGURE 3 Effects of the Se-biofortified porcine serum (Se-Y) treatment on cell viability of the human hepatocellular carcinoma cells (HepG2), in comparison with the DMEM medium (supplemented with 100 IU of penicillin, 100 µg of streptomycin/mL and 10% fetal bovine serum) or that medium added with sodium selenite to match the Se concentration in Se-Y in Expt. 2. The cells were seeded at 1×10^4 cells/well in 24-well plates and incubated with the designated Se sources for 36 h. Cell viability was determined using MTT assay. Data are means \pm SE, n = 6.