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Supplemental Table 1 Primers used for cloning novel porcine selenoprotein gene fragments

Gene	Length (bp)	Primer pairs (5' to 3' direction)
Gpx2 [1]	924	CTTACATTGCCAAGTCCTTCTACG
		3'-site adapter primer [3]
Sepp1 [1]	1705	ATCAACAAGAAGAAAACCAAACAGA
		3'-site adapter primer
Sepn1 [1]	595	CTCTGAATGTGGACATGGAGTGG
		TGGAC(T/C)AC(A/G)GTGCC(A/G)TTGGGCA
Sephs2 [2]		
5′-fragment	571	GTCCGCTCCGTATTAACTGG
		TGTGCCAACGTGCTGAGTG
3′-fragment	1661	TCCCTACATGATGGGGCGCA
		3'-site adapter primer
Sep15 [2]		
5′-fragment	538	AGACGGCGGCGATGGCG
		CAAGGTAACAAAAGGACAGGACGAAAT
3′-fragment	821	CGACAACGGGAACATTGCTGAA
		3'-site adapter primer

[1]Incomplete CDS.

[2]After assembling the respective 5′- and 3′-fragments, we obtained the complete CDS of 2203 bp for *Sephs2* and 1230 bp for *Sep15*.

[3]The sequence of 3'-site adapter primer was 5′- CTGATCTAGAGGTACCGGATCC -3′, similar to the adapter sequence of oligo $d(T)_{18}$-adapter primer. The later was initially used to amplify the poly A+ RNA in the reverse transcription with total RNA as template, and its sequence was 5′-CTGATCTAGAGGTACCGGATCCTTTTTTTTTTTTTTTTTVN-3′. V = A or C or G; N = A or C or G or T.

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Supplemental Table 2 Primers used for the Q-PCR of the target and reference genes

Protein [1]	Gene	Accession number	Primer pairs (5′ to 3′ direction)
GPX1	*Gpx1*	AF532927	GATGCCACTGCCCTCATGA
			TCGAAGTTCCATGCGATGTC
GPX4	*Gpx4*	NM_214407	TGAGGCAAGACGGAGGTAAACT
			TCCGTAAACCACACTCAGCATATC
TrxR1	*Txnrd1*	AF537300	GATTTAACAAGCGGGTCATGGT
			CAACCTACATTCACACACGTTCCT
D1	*Dio1*	AY533206	CATGGCCAAGAACCCTCACT
			CCAGAAATACTGGGCACTGAAGA
D3	*Dio3*	AY533208	TGAAGTGGAGCTCAACAGTGATG
			TGTCGTCAGACACGCAGATAGG
SelK	*Selk*	DQ372075	CAGGAAACCCCCCTAGAAGAA
			CTCATCCACCGGCCATTG
SelW	*Sepw1*	NM_213977	CACCCCTGTCTCCCTGCAT
			GAGCAGGATCACCCCAAACA
GPX2	*Gpx2* [2]	DQ898282	AGAATGTGGCCTCGCTCTGA
			GGCATTGCAGCTCGTTGAG
SPS2	*Sephs2* [2]	EF033624	TGGCTTGATGCACACGTTTAA
			TGCGAGTGTCCCAGAATGC
Sep15	*Sep15* [2]	EF178474	ACAGCCCTGCCAAGCAGAT
			AACAGGGAGGCTGGGTAACAC
SelN	*Sepn1* [2]	EF113595	ACCTGGTCCCTGGTGAAAGAG
			AGGCCAGCCAGCTTCTTGT
SelP	*Sepp1* [2]	EF113596	AACCAGAAGCGCCAGACACT
			TGCTGGCATATCTCAGTTCTCAGA
β-actin	*Actb*	AY550069	CCCAAAGCCAACCGTGAGAA
			CCACGTACATGGCTGGGGTG
GAPDH	*Gapdh*	AF017079	CAGCAATGCCTCCTGTACCA
			CCACGATGCCGAAGTTGTC

[1]D1, iodothyronine deiodinase 1; D3, iodothyronine deiodinase 3; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GPX1, glutathione peroxidase 1; GPX2, glutathione peroxidase 2; GPX4, glutathione peroxidase 4; SelK, selenoprotein K; SelN, selenoprotein N; SelP, selenoprotein P; SelW, selenoprotein W; Sep15, selenoprotein 15kDa; SPS2, selenophosphate synthetase 2; and TrxR1, thioredoxin reductase 1.

[2]Cloned in this study.

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Supplemental Table 3 Growth performance of pigs fed diets containing 0, 0.3, or 3.0 mg Se/kg for 8 wk [1]

Measures	BD	BD + 0.3 mg Se/kg	BD + 3.0 mg Se/kg
Initial body weight, *kg*	10.3 ± 0.3	10.3 ± 0.2	10.3 ± 0.2
Final body weight, *kg*	47.3 ± 0.7	48.2 ± 0.4	45.7 ± 0.3
Feed intake, *g/d*	1,258.2 ± 28.3	1,226.4 ± 30.8	1,163.8 ± 21.5
Body weight gain, *g/d*	665.4 ± 16.4	665.9 ± 21.3	627.7 ± 11.1
Gain /Feed	0.53 ± 0.03	0.54 ± 0.03	0.54 ± 0.02

[1] Values are mean ± SE (n = 10).

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Supplemental Table 4 Tissue enzyme activities of pigs fed diets containing 0, 0.3, or 3.0 mg Se/kg for 8 wk[1]

Measures[2]	Liver	Testis	Thyroid	Pituitary
Glutathione reductase, *EU/mg protein*				
BD	49.3 ± 1.3	35.6 ± 2.4	12.8 ± 0.8	5.6 ± 0.3
BD + 0.3 mg Se/kg	50.2 ± 1.6	26.3 ± 1.1	15.1 ± 1.1	5.6 ± 0.0
BD + 3.0 mg Se/kg	42.4 ± 3.1	34.1 ± 4.7	13.6 ± 0.5	5.7 ± 0.4
Glutathione S-transferase, *EU/mg protein*				
BD	92.9 ± 8.3	51.2 ± 2.1	12.3 ± 2.8	29.4 ± 3.5
BD + 0.3 mg Se/kg	94.0 ± 10.6	53.1 ± 2.3	9.0 ± 2.6	24.9 ± 1.9
BD + 3.0 mg Se/kg	86.0 ± 6.0	48.1 ± 4.6	9.2 ± 2.7	27.9 ± 4.3
Superoxide dismutase, *EU/mg protein*				
BD	459.6 ± 34.8	206.2 ± 8.2	78.3 ± 7.7	124.8 ± 6.5
BD + 0.3 mg Se/kg	470.2 ± 29.2	211.9 ± 14.5	82.4 ± 6.4	126.9 ± 3.6
BD + 3.0 mg Se/kg	466.3 ± 28.4	199.0 ± 9.6	68.5 ± 12.4	134.5 ± 6.2

[1]Values are mean ± SE (n = 6).
[2]The units for the enzyme activities were described in the "Materials and methods" section.

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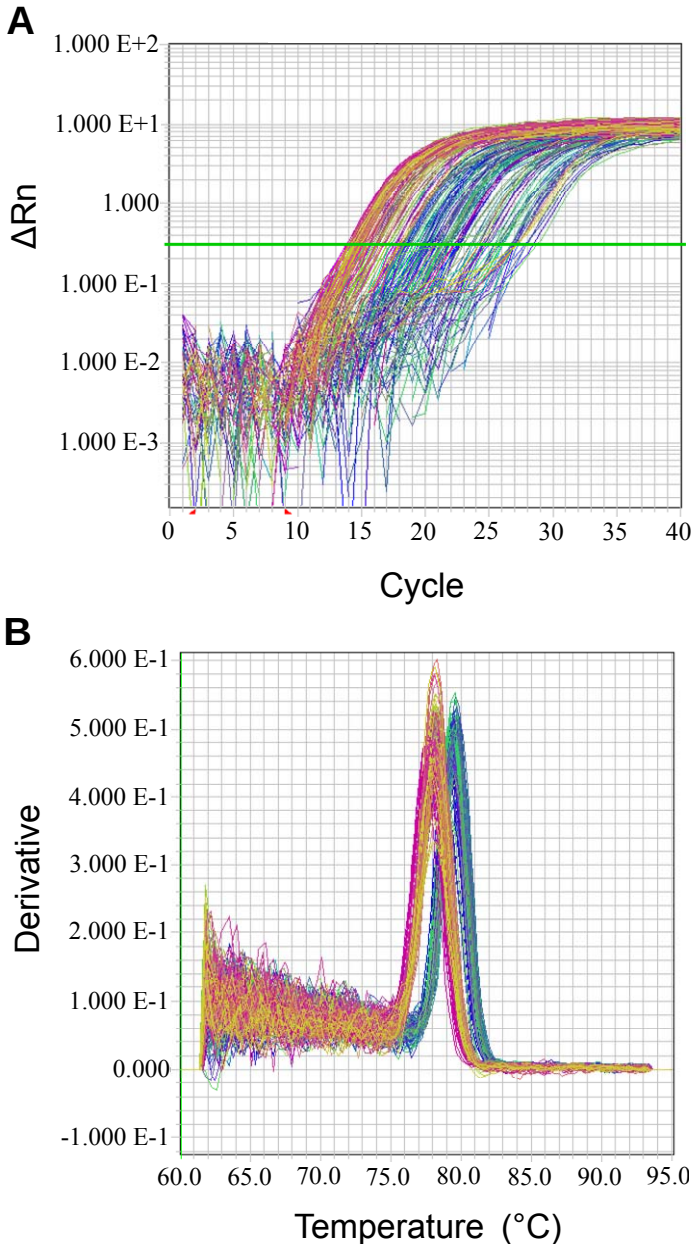

Supplemental Figure 1. Amplification plots (*A*) and dissociation curves (*B*) in the Q-PCR detecting mRNA abundances. Taking *Gpx1* (in cold color) and *Actb* (in warm color) as an example, the assay for mRNA abundances of every two genes in the four tissues (liver, testis, thyroid, and pituitary) were conducted on the same 384-well plate at one time. (*A*) The cycle threshold (Ct) value was the projection of crossing point on X-axis of ΔRn threshold line (Y = 0.3) and the amplification plot in the increased logarithmic phase. The smaller Ct value represented the higher abundance of initial mRNA template that could be detected after less PCR cycles. (*B*) The single sharp dissociation curve cluster of *Actb* (and *Gpx1*) represented specific amplification of the genes.

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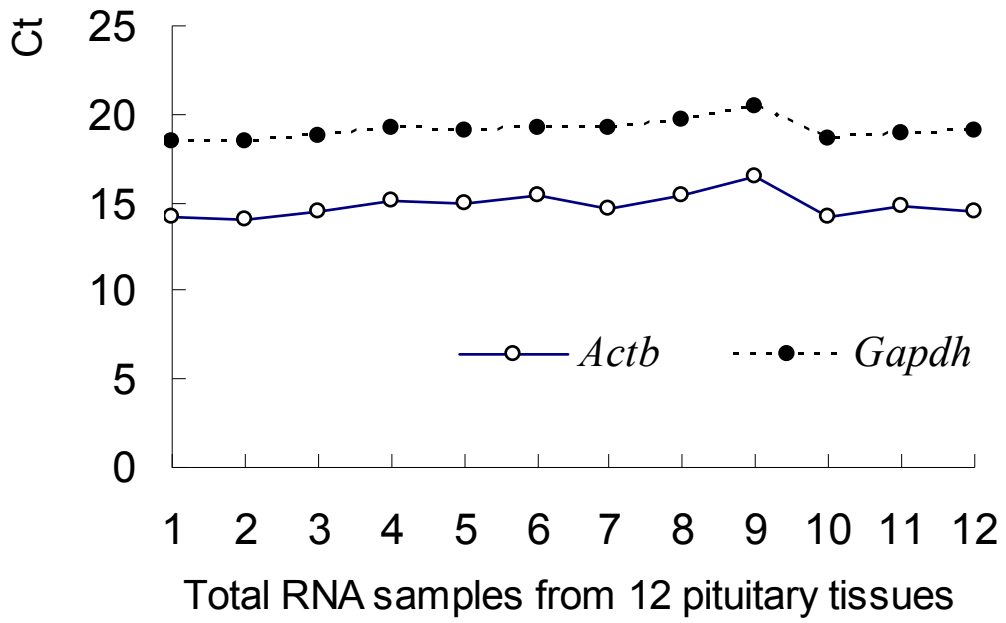

Supplemental Figure 2. Cycle threshold (Ct) of mRNA abundances of *Actb* and *Gapdh* in the 12 porcine pituitary samples. Each point represented the average value of duplicated assays for one pig. Pearson correlation analysis was performed to evaluate the consistency of the two reference (housekeeping) genes with a coefficient of 0.94 ($P < 0.01$, $n = 12$).

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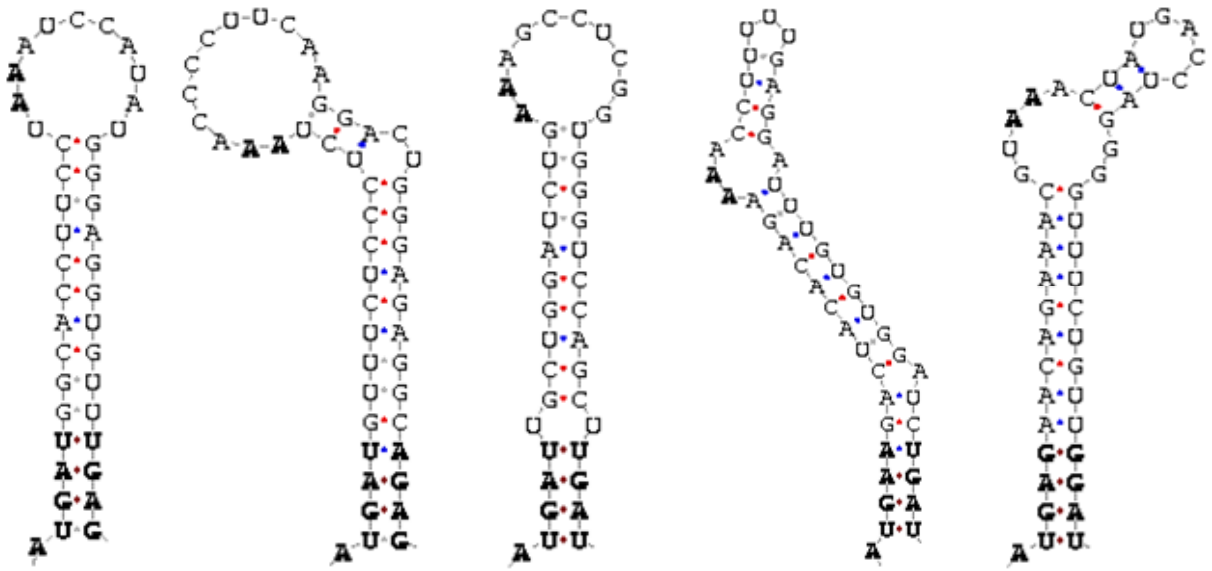

Gpx2 *Sephs2* *Sepn1* *Sep15* *Sepp1*

Supplemental Figure 3. The secondary structures of SECIS elements of the five novel porcine selenoprotein genes. Form 1: *Gpx2*, *Sephs2*, and *Sepn1*; and Form 2: *Sep15* and *Sepp*. Conserved nucleotides were shown in bold.