SUPPLEMENTAL DATA

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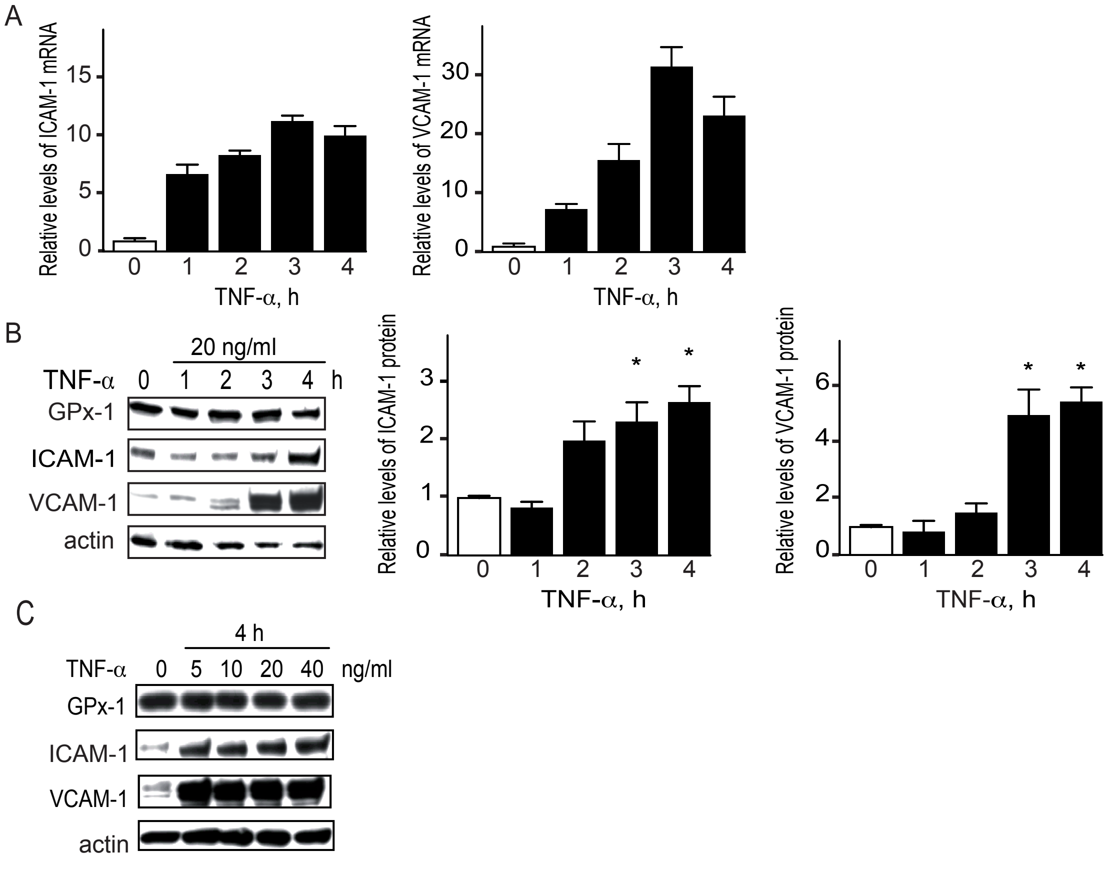
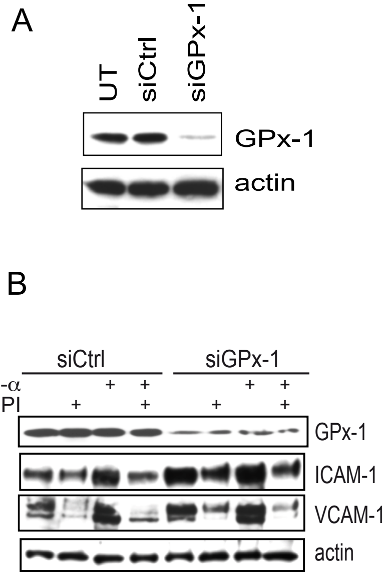
Suppl. Fig. 1. Tumor necrosis factor-α and ICAM-1 and VCAM-1 expression. (A) HMVEC were exposed to tumor necrosis factor-alpha (TNF-α) (20 ng/ml) for 1- 4 h, and ICAM-1 and VCAM-1 mRNA were measured by qRT-PCR (n=3-4) ($p<0.0001$ by ANOVA). (B) The effect of TNF-α on GPx-1, ICAM-1, and VCAM-1 protein expression was determined by Western immunoblotting in a time-dependent (n=4-5), and (C) dose-dependent (n=5-6) fashion. Representative blots are shown. Data are presented as mean ± SEM. Means were significantly different by ANOVA $p<0.05$. Additionally, pairwise comparison shows *$p<0.05$ vs. no treatment.

Suppl. Fig. 2. Role for ROS in TNF-α-mediated activation of endothelial cells. (A) Effect of siRNAs on GPx-1 protein was determined by immunoblot. (B) 48 hrs after transfection, cells were pretreated 1 hr with diphenylene iodonium (DPI), an NADPH oxidase inhibitor, before TNF-α exposure (20 ng/ml). ICAM-1 and VCAM-1 expression was determined by immunoblotting 4 hrs following TNF-α treatment (n=4). Representative blots are shown.

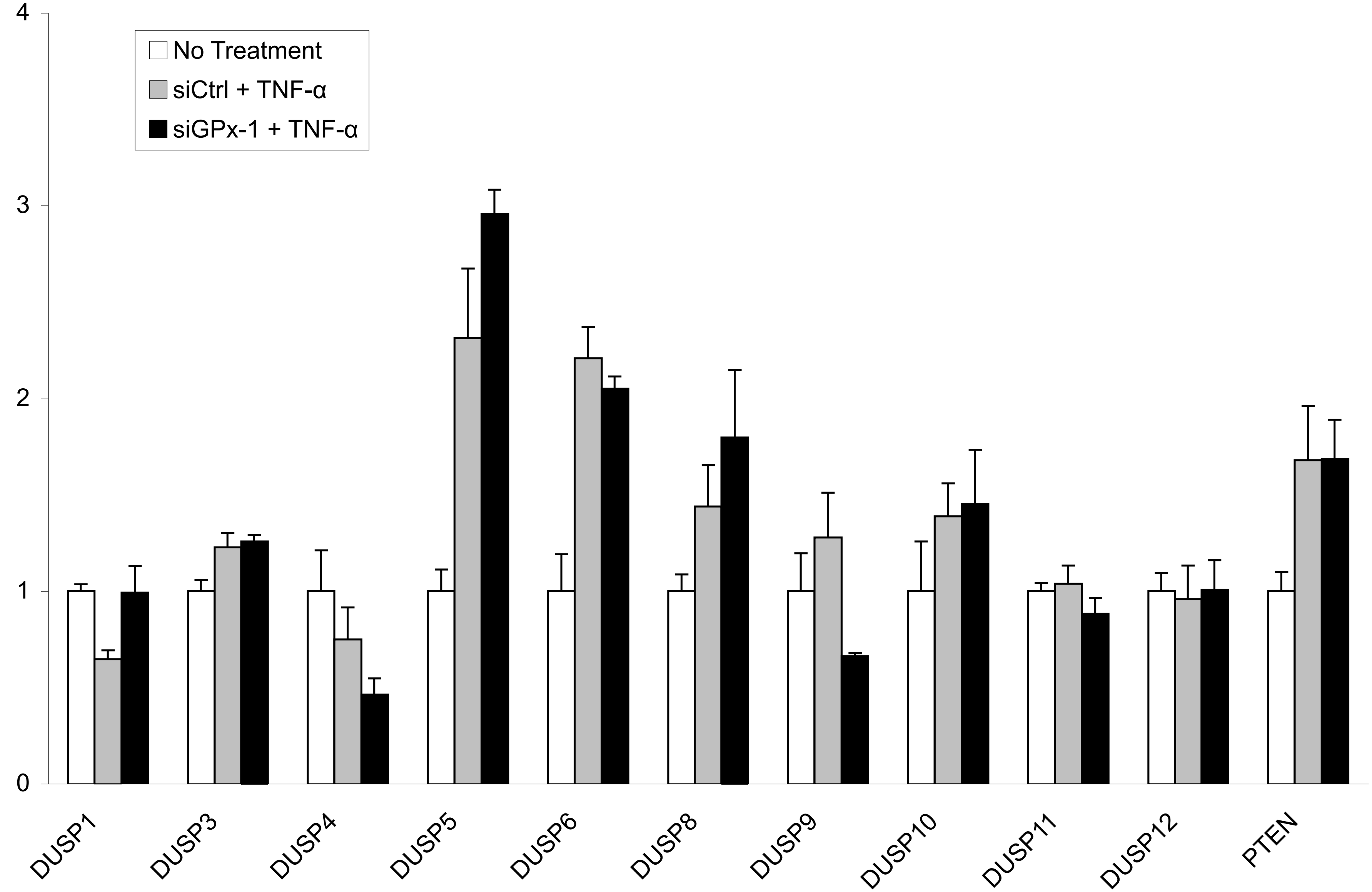
Suppl. Fig. 3. Microarray data analysis. Overview of gene changes in the DUSP family and PTEN using the Affymetrix Human Genome U133A 2.0 gene array in siControl with TNF-α versus non-treated and siGPx-1 with TNF-α versus non-treated cells.



Supplemental Figure 1



Supplemental figure 2.



Supplemental Figure 3

Supplementary TABLE 1 Primers for qRT-PCR

	Applied Biosystems (Human)	Ref.Sequence
GAPDH	4333764T	NM_002046.3
GPx-1	Hs00829989_gH	NM_201397.1, NM_000581.2
ICAM-1	Hs00164932_m1	NM_000201.1
VCAM-1	Hs00365485_m1	NM_080682.1, NM_001078.2
CD97	Hs00173542_m1	NM_078481.2, NM_001025160.1, NM_001784.3
TNFRSF1B	Hs00153550_m1	NM_001066
TAK1	Hs00177373_m1	NM_145333.1, NM_145331.1, NM_145332.1, NM_003188.2
NFkB1	Hs00231653_m1	NM_003998.2
NFkB2	Hs00174517_m1	NM_001077493.1, NM_001077494.1 NM_002502.3
MAPK1	Hs00177066_m1	NM_138957.2, NM_002745.4
CCND3	Hs00236949_m1	NM_001760.2
DUSP4	Hs00154826_m1	NM_001394.5
	Applied Biosystems (Mouse)	
GAPDH	Mm99999915_g1	NM_008084.2
GPx-1	Mm00656767_g1	NM_008160.5

Supplemental TABLE 2. Validation of microarray data by using qRT-PCR

	Microarray				**qRT-PCR**			
Gene Symbol	No Treatment	siCtrl + TNF-α	siGPx-1 + TNF-α	ANOVA p-value	No Treatment	siCtrl + TNF-α	siGPx-1 + TNF-α	ANOVA p-value
GPx-1	1.00 ± 0.03	1.13 ± 0.01	0.02 ± 0.00	<0.0001	1.00 ± 0.00	1.13 ± 0.07	0.03 ± 0.00	<0.0001
ICAM-1	1.00 ± 0.04	3.59 ± 0.09	4.37 ± 0.17	<0.0001	1.00 ± 0.00	5.62 ± 0.23	11.44 ± 0.69	<0.0001
VCAM-1	1.00 ± 0.01	4.31 ± 0.08	3.84 ± 0.21	<0.0001	1.01 ± 0.04	18.16 ± 0.61	21.79 ± 0.41	<0.0001
CD97	1.00 ± 0.11	1.44 ± 0.53	2.71 ± 0.36	0.04	1.11 ± 0.01	0.85 ± 0.29	2.72 ± 0.33	0.004
TNFRSF1B	1.00 ± 0.14	2.05 ± 0.10	1.25 ± 0.06	0.001	1.00 ± 0.00	2.03 ± 0.29	1.39 ± 0.15	0.02
TAK1	1.00 ± 0.08	1.15 ± 0.08	1.10 ± 0.04	0.38	1.00 ± 0.00	1.27 ± 0.08	1.26 ± 0.16	0.20
NFKB2	1.00 ± 0.08	3.98 ± 0.30	3.72 ± 0.09	<0.0001	1.00 ± 0.00	2.93 ± 0.79	2.98 ± 0.30	0.046
MAPK1	1.00 ± 0.04	0.93 ± 0.02	0.86 ± 0.04	0.06	1.00 ± 0.00	1.09 ± 0.18	1.01 ± 0.07	0.84
CCND3	1.00 ± 0.03	1.05 ± 0.05	0.33 ± 0.02	<0.0001	1.03 ± 0.04	0.99 ± 0.18	0.22 ± 0.02	0.002
DUSP4	1.00 ± 0.12	0.75 ± 0.10	0.45 ± 0.04	0.02	1.03 ± 0.06	0.85 ± 0.16	0.40 ± 0.06	0.01

	qRT-PCR			
Gene Symbol	No Treatment	Ad5Bgl II + TNF-α	AdGPx-1 + TNF-α	ANOVA p-value
GPx-1	1.00 ± 0.00	0.58 ± 0.06	95.47 ± 11.86	<0.0001
ICAM-1	1.00 ± 0.00	14.80 ± 1.32	7.59 ± 2.14	0.002
VCAM-1	1.00 ± 0.00	12.37 ± 0.87	3.57 ± 0.32	<0.0001
CD97	1.00 ± 0.00	0.63 ± 0.07	0.20 ± 0.07	0.0002
TNFRSF1B	1.00 ± 0.00	0.57 ± 0.02	0.49 ± 0.17	0.02
TAK1	1.00 ± 0.00	0.81 ± 0.13	0.69 ± 0.23	0.40
NFKB2	1.00 ± 0.00	2.30 ± 0.32	1.39 ± 0.50	0.09
MAPK1	1.00 ± 0.00	0.65 ± 0.10	0.47 ± 0.14	0.02
CCND3	1.00 ± 0.00	0.83 ± 0.13	0.73 ± 0.29	0.60

Supplementary TABLE 3. GSEA-leading edge genes upregulated in siGPX-1 plus TNF-α compared to siControl plus TNF-α

Gene Symbol	Description	GPx-1 >Control[a] Fold Change	GPx-1>NT[b] Fold Change	GPx-1=NT[c] Fold Change	GPx-1<NT[d] Fold Change
CD14	CD 14 molecule	6.83	4.91		
DTNA	Dystrobrevin, alpha	6.06	1.80		
PTPN22	Protein tyrosine phosphatase, non-receptor type 22	4.10			5.21
DYNLT1	Dynein, light chain, Tctex-type 1	3.82		1.13	
AP3S1	Adaptor-related protein complex 3, sigma 1 subunit	2.83			1.94
API5	Apoptosis inhibitor 5	2.73	1.46		
IGFBP5	Insulin-like growth factor binding protein 5	2.61		1.13	
ETNK1	Ethanolamine kinase 1	2.06	1.39		
MLLT11	Myeloid/lymphoid or mixed-lineage leukemia, translocated to 11	2.01		1.19	
IL1A	Interleukin-1, alpha	1.93	4.74		
CD97	CD 97 molecule	1.88	2.71		
GZMA	Granzyme A	1.88		1.02	

[a] Fold change of genes upregulated in siGPx-1 plus TNF compared to siControl plus TNF-α.

[b] Fold change of genes that are upregulatead in siGPx-1 plus TNF compared to no treatment (NT).

[c] Fold change of genes thatare not different between siGPx plus TNF compared to no treatment.

[d] Fold change of genes that are more highly expressed in no treatment conditions compared to siGPx-1 plus TNF-α.

Supplementary TABLE 4. GSEA leading edge genes downregulated in siGPx-1 plus TNF-α compared to siControl plus TNF-α[a]

Gene Symbol	Description	Control >GPx-1[b] Fold Change	Control>NT[c] Fold Change	Control=NT[d] Fold Change	Control<NT[d] Fold Change
GPx-1	Glutathione peroxidase 1	59.48		1.13	
CRIP1	Cysteine-rich protein 1	8.28		1.18	
NR4A3	Nuclear receptor subfamily 4, group A, member 3	6.92	16.67		
SF3A2	Splicing factor 3a, subunit 22, 66 kDa	5.93		1.12	
SLC11A1	Solute carrier family 11, member 1	5.03	4.49		
TGM2	Transglutaminase 2	4.81		1.14	
GPR65	G protein-coupled receptor 65	4.31	1.27		
METTL7A	Methyltransferase like 7A	4.26	1.65		
ABAT	4-aminobutyrate aminotransferase	3.92	2.07		
AHSG	Alpha-2-Hs-glycoprotein	3.91			1.33
STARD7	StAR-related lipid transfer (START) domain containing 7	3.85		1.13	
GPx-3	Glutathione peroxidase 3	3.82	1.51		
PDE1A	Phosphodiesterase 1A, calmodulin-dependent	3.42	2.88		
DUSP4	Dual specificity phosphatase 4	3.32			1.34
UST	Uronyl-2-sulfotransferase	3.31		1.19	
SELL	Selectin L	3.18	2.80		
CCND3	Cyclin D3	3.13		1.19	
POLR3K	Polymerase (RNA) III (DNA direct) polypeptide K, 12.3 kDa	2.93			1.65
MKI67	Antigen identified by monoclonal antibody Ki-67	2.72			1.92
CEBPD	CCAAT/enhancer binding protein (C/EBP), delta	2.75	36.03		
RGS4	Regulator of G-protein signaling 4	2.49			3.45
CKS1B	CDC28 protein kinase regulatory subunit 1B	2.48			1.63
NR4A2	Nuclear receptor subfamily 4, group A, member 2	2.37	6.72		
RRM2	Ribonucleotide reductase M2 polypeptide	2.28			3.18
SERPINA3	Serpin peptidase inhibitor, clade A, member 3	2.23	12.68		
CENPE	Centromere protein E	2.20			1.82

[a] An additional 45 genes downregulated between 1.8 and 2.2-fold are not listed.

[b] Fold change of genes that are upregulated in siControl plus TNF-α compared to siGPx-1 plus TNF-α.[c] Fold change of genes that are upregulated in siControll plus TNF-α compared to no treatment (NT).

[c] Fold change of genes that are not statistically different and between siControl plus TNF-α compared to no treatment.

[e] Fold change of genes that are more highly expressed.

Supplementary TABLE 5. Microarray data of antioxidant genes

		Microarray		
Gene Symbol	No Treatment	siCtrl + TNF-α	siGPx-1 + TNF-α	ANOVA p-value
Heme oxygenase (HMOX)				
HMOX-1	1.00 ± 0.01	0.95 ± 0.02	0.44 ± 0.02	<0.0001
HMOX-2	1.00 ± 0.12	0.84 ± 0.14	0.86 ± 0.09	0.54
Superoxide dismutase (SOD)				
SOD-1	1.00 ± 0.02	1.19 ± 0.04	1.13 ± 0.05	0.03
SOD-2	1.00 ± 0.03	6.52 ± 0.25	6.56 ± 0.25	<0.0001
Glutathione peroxidase (GPx)				
Non-selenium GPx	1.00 ± 0.06	0.78 ± 0.02	0.65 ± 0.03	0.002
GPx-3	1.00 ± 0.03	1.18 ± 0.06	0.55 ± 0.05	0.0002
GPx-4	1.00 ± 0.01	1.42 ± 0.01	1.33 ± 0.01	<0.0001
GPx-5	1.00 ± 0.42	0.29 ± 0.08	1.68 ± 0.10	0.02
Glutathione S-transferase (GST)				
GST	1.00 ± 0.14	1.04 ± 0.03	1.08 ± 0.17	0.09
GST subunit-4	1.00 ± 0.12	1.44 ± 0.16	1.10 ± 0.08	0.10
GST subunit-13	1.00 ± 0.05	1.05 ± 0.03	1.04 ± 0.04	0.61
GSTA4	1.00 ± 0.01	0.73 ± 0.05	0.88 ± 0.04	0.009
GSTM1	1.00 ± 0.04	1.29 ± 0.04	1.24 ± 0.05	0.006
GSTM2	1.00 ± 0.05	1.25 ± 0.05	1.01 ± 0.11	0.09
GSTM3	1.00 ± 0.07	1.05 ± 0.02	0.79 ± 0.01	0.01
GSTM4	1.00 ± 0.07	0.87 ± 0.05	1.17 ± 0.08	0.06
GSTM5	1.00 ± 0.13	0.99 ± 0.17	0.76 ± 0.12	0.46
GST like	1.00 ± 0.04	0.98 ± 0.03	1.14 ± 0.05	0.06
GST theta-1	1.00 ± 0.06	1.30 ± 0.03	0.95 ± 0.03	0.002
GST theta-2	1.00 ± 0.19	1.12 ± 0.07	1.02 ± 0.03	0.78
GST pi	1.00 ± 0.07	0.90 ± 0.03	1.11 ± 0.05	0.07
GST zeta-1	1.00 ± 0.05	0.83 ± 0.01	0.64 ± 0.02	0.0007
Microsomal GST-2	1.00 ± 0.05	1.22 ± 0.04	1.26 ± 0.05	0.016
Microsomal GST-3	1.00 ± 0.04	1.18 ± 0.05	1.27 ± 0.02	0.006
Glutathione synthetase (GSS)	1.00 ± 0.01	0.80 ± 0.05	0.73 ± 0.07	0.016
Glutathione reductase (GSH-R)	1.00 ± 0.12	1.08 ± 0.05	0.93 ± 0.04	0.45
Thioredoxin				
Thioredoxin	1.00 ± 0.01	0.98 ± 0.03	0.94 ± 0.02	0.23
Thioredoxin-2	1.00 ± 0.09	1.15 ± 0.09	1.02 ± 0.07	0.43
Thioredoxin-like	1.00 ± 0.03	1.20 ± 0.04	1.13 ± 0.02	0.009
Thioredoxin delta-3	1.00 ± 0.33	0.93 ± 0.14	1.18 ± 0.25	0.77
Thioredoxin reductase-1	1.00 ± 0.10	0.68 ± 0.04	0.78 ± 0.03	0.03
Thioredoxin reductase, mitochondrial	1.00 ± 0.01	1.10 ± 0.23	0.90 ± 0.23	0.77
Thioredoxin reductase II beta	1.00 ± 0.07	0.96 ± 0.12	0.84 ± 0.03	0.39
Thioredoxin-related transmembrane protein	1.00 ± 0.21	0.62 ± 0.04	0.97 ± 0.05	0.14
Thioredoxin peroxidase	1.00 ± 0.01	0.93 ± 0.03	0.94 ± 0.01	0.09
Catalase	1.00 ± 0.02	0.93 ± 0.04	0.78 ± 0.02	0.005
UDP-glucose dehydrogenase	1.00 ± 0.01	0.64 ± 0.05	0.67 ± 0.03	0.0006
Glucose-6-phosphate dehydrogenase	1.00 ± 0.04	0.78 ± 0.04	0.68 ± 0.05	0.006
NADPH oxidase-4	1.00 ± 0.06	0.73 ± 0.02	0.94 ±0.05	0.017
Uncoupling protein-2	1.00 ± 0.10	0.97 ±0.12	0.55 ± 0.02	0.02

Uncoupling protein homolog	1.00 ± 0.03	0.97 ± 0.04	0.47 ± 0.05	0.0002
Mitochondrial uncoupling protein-5	1.00 ± 0.08	0.91 ± 0.04	1.15 ± 0.07	0.097
Peroxiredoxin-2	1.00 ± 0.07	0.85 ± 0.09	0.90 ± 0.12	0.56
Peroxiredoxin-3	1.00 ± 0.05	0.79 ± 0.05	0.90 ± 0.04	0.05