

Supplementary information

Study on the properties and synergistic antioxidant effects of novel bifunctional fusion proteins expressed using the UTuT6 system

Qi Yan¹; Jingyan Wei^{1,2,3, *}; Junxia Song¹; Mengna Li¹; Xin Guan^{1, *};

Jian Song^{4, *}

¹ College of Pharmaceutical Science, Jilin University, Changchun, 130021, P. R. China.

² Key Laboratory for Molecular Enzymology and Engineering of the Ministry of Education, Jilin University, Changchun, 130000, P. R. China.

³ Institute of Theoretical Chemistry, Jilin University, 130023 Changchun, PR China.

⁴ School of microelectronics, Shanghai University, Shanghai, 201800, China

***The corresponding authors:**

E-mail address: jsong@shu.edu.cn (J. S); weijy@jlu.edu.cn (J. W); guan@jlu.edu.cn(X. G).

Supplementary Results

1. Supplementary tables

Table S1: Primer sequences used for Se-hGPx_{UAG} fusion proteins cloning.

<i>Primers</i>	<i>Sequences 5'→3'</i>
hGPx1-BamHI-F	CGCGGATCCGATGTGTGCTGCTC
hGPx1 _{UAG} -linker-R	GAGCCACCTCCGCCTGAACCGCCTCCACCGGCACAGCT GGGCCCTTG
hGPx1 _{UAG} -long linker-R	CCGCCTGAACCGCCTCCACCAGATCCACCGCCAC CGGAGGCACAGCTGGGCCCTTG
linker-SOD3-72P-F	CGGAGGTGGCTCAGGCGGTGGCGGCTCACACGTTCA CCAGTTCG
SOD3-72P-HindIII-R	CCCAAGCTTTTAGTCTTCACCAGC
SOD3-72P-BamHI-F	CGGGATCCCACGTTACACGTTTCG
hGPx4-EcoRI-F	CCGGAATTCGATGTGTGCTGCTC
hGPx4 _{UAG} -linker-R	GAGCCACCTCCGCCTGAACCGCCTCCACCGAAATAGTGG GGCA
hGPx4 _{UAG} -long linker-R	CCGCCTGAACCGCCTCCACCAGATCCACCGCCAC CGGAGAAATAGTGGGGCA

Gene accession number used in this study shown below: GPx1: NM_000581; GPx4: NM_001367832; SOD3: NM_003102.

Table S2: Primer sequences used for Se-hGPx4_{UAG} fusion protein mutation.

<i>Primers</i>	<i>Sequences 5'→3'</i>
GPx4-C2S-NdeI-F	GGGAATTCCATATGTGCGCGTCC
GPx4-C10S-NdeI-F	GGGAATTCCATATGTGCGCGTCCCGGGACGACTGGCGCTCGG CGCGCTCC
GPx4-C37S-F	GGCTTCGTGTCGATCGTCACC
GPx4-C37S-R	GGTGACGATCGACACGAAGCC
GPx4-C66S-F	ACGCTGAGTCGGGTTTGCGG
GPx4-C66S-R	CCGCAAACCCGACTCAGCGT

GPx4-C75S-F	CCTTCCCGTCGAACCAGTT
GPx4-C75S-R	AACTGGTTCGACGGGAAGG
GPx4-C107S-F	CAGCAAGATCTCGGTGAACGGGG
GPx4-C107S-R	CCCCGTTACCGAGATCTTGCTG
GPx4-C148S-F	CGACAAGAACGGCTCGGTGGTGAAGCGCT
GPx4-C148S-R	AGCGCTTCACCACCGAGCCGTTCTTGTCG

Table S3: Thiol content of Se-hGPx4_{UAG} / Se-hGPx4_{UAG}-L₃-SOD3-72P and mutants

Cys residues reacting with DTNB	Se- hGPx4 _{UAG}	Se-hGPx4 _{UAG} - L ₃ -SOD3-72P	C10S	C37S
Theoretical	7	8	7	7
Detected	7.4±0.2	2.78±0.3	1.8±0.32	2.9±0.29

2. Supplementary Figures

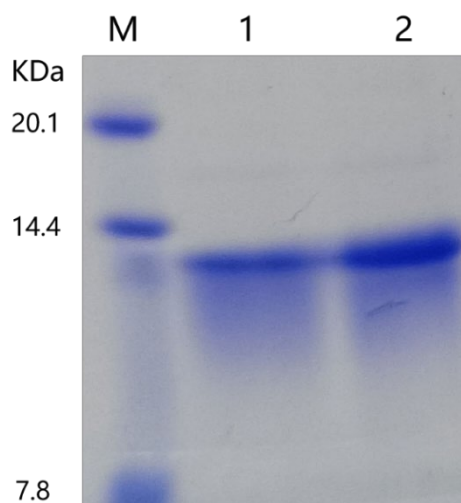


Figure. S1. SDS-PAGE analysis of SOD3-72P protein.

M: Marker; 1: reduced SOD3-72P; 2: non-reduced SOD3-72P.

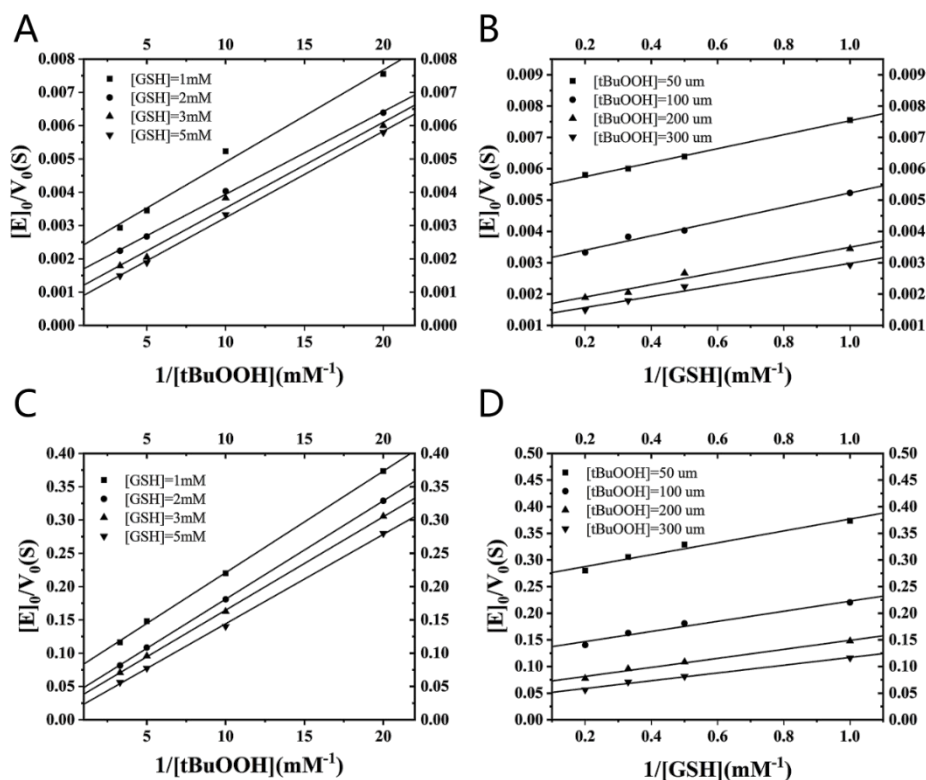


Figure. S2. (A)(C) $[E]_0/V_0$ versus $1/[tBuOOH]$ (mM^{-1}) at $[GSH]=1$ mM (square), 2 mM (circle), 3 mM (triangle), 5 mM (down triangle). (B)(D) $[E]_0/V_0$ versus $1/[GSH]$ (mM^{-1}) at $[tBuOOH]=50\mu\text{M}$ (square), $100\mu\text{M}$ (circle), $200\mu\text{M}$ (triangle), $300\mu\text{M}$ (down triangle).