



3-Mercaptopyruvate sulfur transferase is a protein persulfidase

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Supplementary Table 1: Reagents

| Reagent | Source | Identifier |
|--|--------------------------|-------------------|
| NEBuilder HiFi DNA Assembly Master Mix | New England Biolabs | E2621 |
| Quikchange Site-Directed Mutagenesis Kit | Agilent | 200519 |
| Dithiothreitol | Panreac AppliChem | A2948 |
| Zeba™ Spin Desalting Columns | Thermo Scientific | 89882, 87766 |
| Sodium mercaptopyruvate dihydrate | Sigma-Aldrich | 90374 |
| 3-mercaptoplactic acid | Santa Cruz Biotechnology | sc-482537 |
| L-cysteine hydrochloride monohydrate | Sigma-Aldrich | C7880 |
| Sodium thiosulfate | Sigma-Aldrich | 72049 |
| Sodium sulfide | Sigma-Aldrich | 407410 |
| Potassium cyanide | Sigma-Aldrich | 60178 |
| Potassium thiocyanate | Sigma-Aldrich | P2713 |
| Sodium sulfite | Sigma-Aldrich | S0505 |
| Glutathione, reduced | Sigma-Aldrich | G6529 |
| Beta-mercaptoethanol | Sigma-Aldrich | M3148 |
| Bovine serum albumin, fatty acid free | Sigma-Aldrich | A8806 |
| D-cysteine hydrochloride monohydrate | Sigma-Aldrich | C8005 |
| D-alanine | Sigma-Aldrich | A7377 |
| Monobromobimane | Sigma-Aldrich | B4380 |
| 4-chloro-7-nitrobenzofurazan | Sigma-Aldrich | 163260 |
| DAz-2 | Cayman Chemicals | 13382 |
| Cyanine5 alkyne | Lumiprobe | B30B0 |
| Copper(II)-TBTA complex | Lumiprobe | 21050 |
| Neocuproine | Sigma-Aldrich | N1501 |
| IGEPAL | Sigma-Aldrich | I3021 |
| Iodoacetamide | Sigma-Aldrich | I5161 |
| Trifluoroacetic acid | Biosolve | 202341 |
| Acetonitrile | Carl-Roth | 7330.1 |

| | | |
|-------------------------------------|---------------|----------|
| Triethylammonium bicarbonate buffer | Sigma-Aldrich | T7408 |
| Ni ²⁺ -Sephacrose beads | Cytiva | 17526801 |
| PreScission Protease | Cytiva | 27084301 |
| GST-sepharose | Cytiva | 17527901 |
| Streptavidin (SA) sepharose beads | Cytiva | 17511301 |

Supplementary Table 2: Plasmids

| Plasmid | Reference |
|-------------------------------|-----------|
| pQE60-roGFP2-His | 1 |
| pETHisSUMO-Tum1-roGFP2 | This work |
| pETHisSUMO-Tum1(C259S)-roGFP2 | This work |
| pETHisSUMO-Tum1 | This work |
| pETHisSUMO-Tum1(C259S) | This work |
| pET-SBP-HisSUMO-Tum1 | This work |
| p415TEF-roGFP2 | 2 |
| p415TEF-Tum1-roGFP2 | This work |
| p415TEF-Tum1(C259S)roGFP2 | This work |
| p415TEF-Su9roGFP2 | This work |
| p415TEF-Su9Tum1-roGFP2 | This work |
| p415TEF-Su9Tum1(C259S)-roGFP2 | This work |
| p416TEF-Su9DAAO | This work |
| pLPCx roGFP2 | This work |
| pLPCx MPST-roGFP2 | This work |
| pLPCx MPST(C248S)-roGFP2 | This work |

Supplementary Table 3: Primers

| Name | Sequence | Purpose |
|---------------------------------|--|--|
| p415TEF(NcoI)-Tum1 | AAGTTTTCTAGAGGATCCACAT GCCATTATTTGATCTTATTTTC | Amplify Tum1 to assemble with p415TEF(NcoI digested) and linker-roGFP2 |
| linker.roGFP2-Tum1 | CACCACCTGAACCACCATCTCT GTTTTCAAGCAATC | Amplify Tum1 to assemble with p415TEF(NcoI digested) and linker-roGFP2 |
| Tum1-linker.roGFP2 | AAACAGAGATGGTGGTTCAGG TGGTGGTGGTTCAGGTGGTGG TGGTTCAGGTGGAGGAGGATC AGGAGGAGGAGGATCAGGAG GAGGAGGATCAGGAGGAGCTA GCGAATTCTCAAAG | Amplify linker-roGFP2 to assemble with Tum1 and p415TEF (HindIII digested) |
| p415TEF(HindIII)-linker.roGFP2 | TGACTCGAGGTCGACGGTATC GATATTATTTATACAATTCATCC ATACCC | Amplify linker-roGFP2 to assemble with Tum1 and p415TEF (HindIII digested) |
| Tum1_C259S_fw | CCAACTATTTGCTCTTCTGGAA CTGGCGTTTCAGG | Site-directed mutagenesis of Tum1 catalytic Cys 259 to serine |
| Tum1_C259S_rev | CCTGAAACGCCAGTTCAGAA GAGCAAATAGTTGG | Site-directed mutagenesis of Tum1 catalytic Cys 259 to serine |
| p415TEFSu9(BamHI)-Tum1-roGFP2 | GAAGCGCGCCTACTCTTCCGG ATCCCCATTATTTGATCTTATTT CTCC | Amplify Tum1-roGFP2 to assemble with p415TEF + Su9 signal peptide for mitochondrial localization (BamHI/HindIII digested) |
| p415TEFSu9(HindIII)-Tum1-roGFP2 | CGAGGTCGACGGTATCGATAT TATTTATACAATTCATCCATACC | Amplify Tum1-roGFP2 to assemble with p415TEF + Su9 signal peptide for mitochondrial localization (BamHI/HindIII digested) |
| p416TEFSu9(BamHI)-Su9 | TAAGTTTTCTAGAACTAGTGGA TCCATGGCCTCCACTCGTGTC | Amplify F ₀ -ATPase (Su9) from <i>Neurospora crassa</i> to assemble with p416TEF (BamHI/HindIII digested) and DAAO. |
| DAAO-Su9 | TCTGGCTGTGGGATCCGGAAG AGTAGGC | Amplify F ₀ -ATPase (Su9) from <i>Neurospora crassa</i> to assemble with p416TEF (BamHI/HindIII digested) and DAAO. |

| | | |
|---------------------------|---|---|
| Su9-DAAO | TTCCGGATCCCACAGCCAGAA GAGGGTG | Amplify DAAO to assemble with p416TEF (BamHI/HindIII digested) and F ₀ -ATPase (Su9) from <i>N. crassa</i> . |
| p416TEFSu9(HindIII)-DAAO | CGAGGTCGACGGTATCGATAA GCTTTTAGCTCTCCCTAGCTGC | Amplify DAAO to assemble with p416TEF (BamHI/HindIII digested) and F ₀ -ATPase (Su9) from <i>N. crassa</i> . |
| pETHisSUMO_fw | AGACAAGCTTAGGTATTTATTC G | Amplify linearized pETHisSUMO |
| pETHisSUMO-rv | TGGACCCTGGAACAGAAC | Amplify linearized pETHisSUMO |
| pETHisSUMO-Tum1-roGFP2-fw | AAGTTCTGTTCCAGGGTCCAAT GCCATTATTTGATCTTATTTTC | Amplify Tum1-roGFP2 to assemble with linearized pETHisSUMO vector |
| pETHisSUMO-Tum1-roGFP2-rv | ATAAATACCTAAGCTTGTCTTT ATTTATACAATTCATCCATACC C | Amplify Tum1-roGFP2 to assemble with linearized pETHisSUMO vector |
| roGFP2_C148S_fw | GGAGTACAACACTACAACAGCCA CAACGTCTATATC | Mutagenesis cys148 from roGFP2-His expressed in pQE60 vector to serine |
| roGFP2_C148S_rv | GATATAGACGTTGTGGCTGTT GTAGTTGTACTCC | Mutagenesis cys148 from roGFP2-His expressed in pQE60 vector to serine |
| roGFP2_C205S_fw | ACCACTACCTGAGCACCAGCT CCGCCCTGAGCAAA | Mutagenesis cys205 from roGFP2-His expressed in pQE60 vector to serine |
| roGFP2_C205S_rv | TTTGCTCAGGGCGGAGCTGGT GCTCAGGTAGTGGT | Mutagenesis cys205 from roGFP2-His expressed in pQE60 vector to serine |
| pETHisSUMO-Tum1_fw | AAGTTCTGTTCCAGGGTCCAAT GCCATTATTTGATCTTATTTTC | Amplify Tum1 (no roGFP2) to anneal to linearized pETHisSUMO |
| pETHisSUMO-Tum1_rv | ATAAATACCTAAGCTTGTCTTT AATCTCTGTTTTTCAGCAATC | Amplify Tum1 (no roGFP2) to anneal to linearized pETHisSUMO |
| MPST_C248S_fw | CTGGTTGCTACAAGCGGTTCA GGCG | Mutagenesis cys248 from human MPST to serine |
| MPST_C248S_rv | CGCCTGAACCGCTTGTAGCAA CCAG | Mutagenesis cys248 from human MPST to serine |

Supplementary references

1. Gutscher, M. *et al.* Real-time imaging of the intracellular glutathione redox potential. *Nat Methods* 5, 553–559 (2008)
2. Morgan, B. *et al.* Multiple glutathione disulfide removal pathways mediate cytosolic redox homeostasis. *Nat Chem Biol* 9, 119–125 (2013)