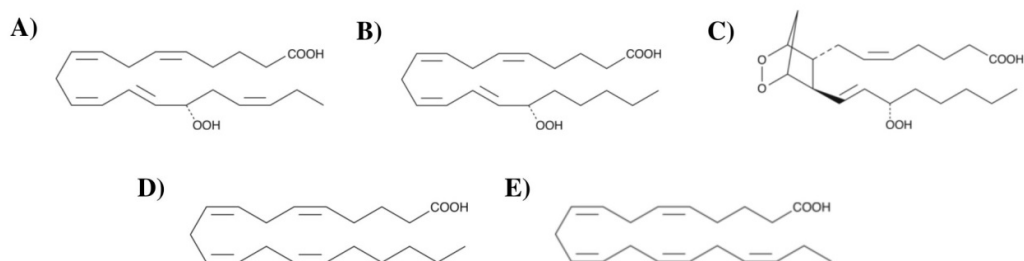
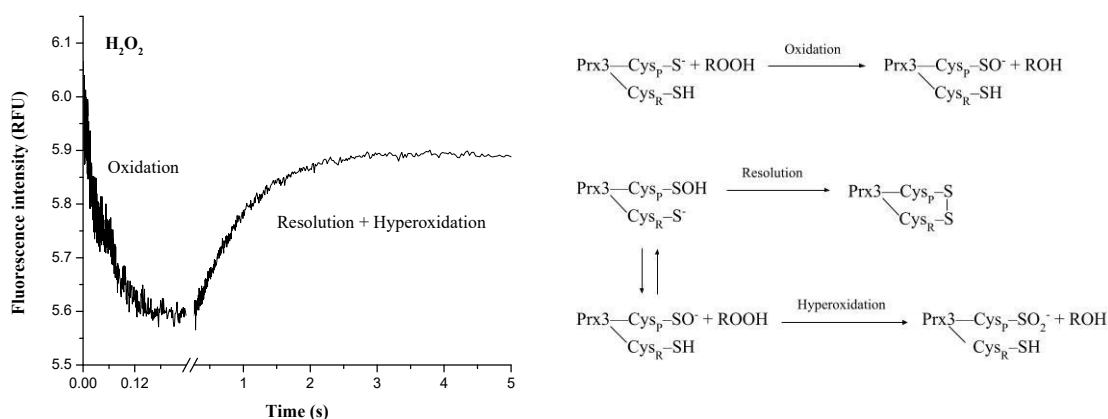


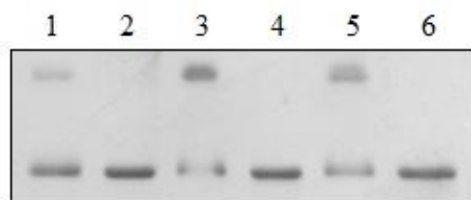
## Supplementary Materials



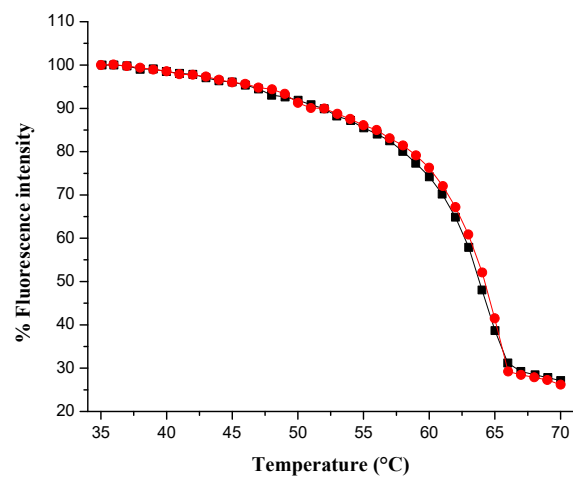
**Supplementary Figure S1.** Molecular representations of rFA-OOH and FA used throughout this work. (A) 15S-hydroperoxy-5Z,8Z,11Z,13E,17Z-eicosapentaenoic acid (15(S)-HpEPE); (B) 15S-hydroperoxy-5Z,8Z,11Z,13E-eicosatetraenoic acid (15(S)-HpETE); (C) 9 $\alpha$ ,11 $\alpha$ -epidioxo-15S-hydroperoxy-prosta-5Z,13E-dien-1-oic acid (PGG<sub>2</sub>); (D) arachidonic acid (AA) and (E) eicosapentaenoic acid (EPA).



**Supplementary Figure S2.** Intrinsic fluorescence changes in *HsPrx3* caused by Cys<sub>P</sub> oxidation, resolution and hyperoxidation. Oxidation (from thiol to sulfenic acid) and hyperoxidation (from sulfenic acid to sulfinic acid) in *HsPrx3* are bimolecular processes while resolution is intramolecular and depends on a change from the so-called fully folded to a locally unfolded conformation followed by the condensation reaction between the sulfenic acid in Cys<sub>P</sub> and the thiolate in Cys<sub>R</sub> from another protein subunit. In the case of reaction with H<sub>2</sub>O<sub>2</sub>, in which the rate constant of hyperoxidation is  $1.1 \times 10^3 \text{ M}^{-1}\text{s}^{-1}$ , hyperoxidation does not compete with resolution ( $2 \text{ s}^{-1}$  at pH 7.8 and 14°C) at  $<50 \text{ }\mu\text{M}$  oxidant concentration. Adapted from [13].



**Supplementary Figure S3.** Effect of 15(S)-HpETE *versus* H<sub>2</sub>O<sub>2</sub> on the distribution of HsPrx3 between monomeric and disulfide-bonded dimeric species. SDS-PAGE of reduced HsPrx3 (5 μM) without treatment (lane 1 w/o β-ME, lane 2 with β-ME); exposed to H<sub>2</sub>O<sub>2</sub> (500 μM) (lane 3 w/o β-ME, lane 4 with β-ME); exposed to 15(S)-HpETE (10 μM) (lane 5 w/o β-ME, lane 6 with β-ME).



**Supplementary Figure S4.** Effect of ethanol on the heat-induced unfolding of *HsPrx3*. Heat unfolding transitions following the intrinsic fluorescence intensity ( $\lambda_{\text{ex}} = 295 \text{ nm}$ ,  $\lambda_{\text{em}} = 335 \text{ nm}$ ) of the tryptophans of *HsPrx3* ( $4 \mu\text{M}$ ) in the absence (■) and presence of the same volume of ethanol (●) that was used to solubilize AA.

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VTQHAPYFKGTAVVNGEFKDLSDDFKGKYLVLFFYPLDFTFVCPTEIVAFSDKANEFHD
+ + AP FK TAVV+G FK++ L D+KGKY+VLFFYPLDFTFVCPTEI+AFS++A +F
IGKPAPDFKATAVVDGAFKEVKLSDYKGKYVVLFFYPLDFTFVCPTEIIAFSNRAEDFRK

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+ CEV+ VSVDS F+HLAWINTPRK GGLG +NI LL+D+T+++S DYGVL G+A R
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GLFIIDPNGVIKHLVNDLPVGRSVEETLRLVKAFQYVETHGEVCPANWTPDSPTIKPSP
GLFIID GV++ ++VNDLPVGRSV+E LRLV+AFQY + HGEVCPA W P S TIKP+
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AASKEYFQKVN
SKEYF K N
DDSKEYFSKHN

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**Supplementary Figure S5.** Sequence alignment of mature *HsPrx3* (black) and *HsPrx2* (blue). Residues that make up the hydrophobic patch near Cys<sub>p</sub> in *HsPrx3* are conserved in *HsPrx2* (indicated in yellow).