# Differential Inhibition of *Arabidopsis* Superoxide Dismutases by Peroxynitrite-mediated Tyrosine Nitration

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## Supplemental Table S1: Oligonucleotides for cloning of superoxide dismutase nucleotide sequences and site-directed mutagenesis

All oligonucleotides were obtained from Eurofins MWG Operon (Martinsried, Germany), diluted in water (100 µM) and stored at -20 °C.

| Name | Sequence |
|---|---|
| **Oligonucleotides for amplification of superoxid dismutase nucleotide sequences** | |
| MnSOD_ for | 5´- ATGGCGATTCGTTGTGTAGC - 3´ |
| MnSOD_rev (with stop codon) | 5´- TCAGTTGTTTTCCTTCTCATAAACC - 3´ |
| MnSOD_rev (without stop codon) | 5´- GTTGTTTTCCTTCTCATAAACCTC - 3´ |
| FeSOD1_ for | 5´- ATGGCTGCTTCAAGTGCTG - 3´ |
| FeSOD1_rev (with stop codon) | 5´- TTAAGCAGAAGCAGCCTTGG - 3´ |
| FeSOD1_rev (without stop codon) | 5´- AGCAGAAGCAGCCTTGGC - 3´ |
| FeSOD2_ for | 5´- ATGATGAATGTTGCAGTGACAG - 3´ |
| FeSOD2_rev (with stop codon) | 5´- TTAGTCAACCTCAGATACATCG - 3´ |
| FeSOD2_rev (without stop codon) | 5´- GTCAACCTCAGATACATCGAT - 3´ |
| FeSOD3_ for | 5´- ATGAGTTCTTGTGTTGTGACG - 3´ |
| FeSOD3_rev (with stop codon) | 5´- TTAAGCGATTGGGATGTTGG - 3´ |
| FeSOD3_rev (without stop codon) | 5´- AGCGATTGGGATGTTGGG - 3´ |
| CuZnSOD1_ for | 5´- ATGGCGAAAGGAGTTGCAG - 3´ |
| CuZnSOD1_rev (with stop codon) | 5´- TTAGCCCTGGAGACCAATG - 3´ |
| CuZnSOD1_rev (without stop codon) | 5´- GCCCTGGAGACCAATGAT - 3´ |
| CuZnSOD2_ for | 5´- ATGGCTGCCACCAACACAA - 3´ |
| CuZnSOD2_rev (with stop codon) | 5´- TTAGAGCGGCGTCAAGC - 3´ |
| CuZnSOD2_rev (without stop codon) | 5´- GAGCGGCGTCAAGCCA - 3´ |
| CuZnSOD3_ for | 5´- ATGGAAGCTCCTAGAGGAAATC - 3´ |
| CuZnSOD3_rev (with stop codon) | 5´- CTATAGTTTAGCATCCGCAGAT - 3´ |
| CuZnSOD3_rev (without stop codon) | 5´- TAGTTTAGCATCCGCAGATGATT - 3´ |

## Oligonucleotides for GATEWAY-Cloning of superoxide dismutase nucleotide sequences

| | |
|---|---|
| attB1_MnSOD_for (with stop codon) | 5´- GGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCGATTCGTTGTGTAGC - 3´ |
| attB2_MnSOD_rev (with stop codon) | 5´- GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAGTTGTTTTCCTTCTCATAAACC - 3´ |
| attB1_MnSOD_for (without stop codon) | 5´-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGGCGATTCGTTGTGTAGC - 3´ |
| attB2_MnSOD_rev (without stop codon) | 5´- GGGGACCACTTTGTACAAGAAAGCTGGGTCGTTGTTTTCCTTCTCATAAACCTC - 3´ |
| attB1_FeSOD1_for (with stop codon) | 5´- GGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCTGCTTCAAGTGCTG - 3´ |
| attB2_FeSOD1_rev (with stop codon) | 5´- GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAAGCAGAAGCAGCCTTGG - 3´ |
| attB1_FeSOD1_for (without stop codon) | 5´- GGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGGCTGCTTCAAGTGCTG - 3´ |
| attB2_FeSOD1_rev (without stop codon) | 5´- GGGACCACTTTGTACAAGAAAGCTGGGTCAGCAGAAGCAGCCTTGGC - 3´ |
| attB1_FeSOD2_for (with stop codon) | 5´- GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGATGAATGTTGCAGTGACAG - 3´ |
| attB2_FeSOD2_rev (with stop codon) | 5´- GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGATGAA - 3´ |
| attB1_FeSOD2_for (without stop codon) | 5´- GGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGATGAATGTTGCAGTGACAG - 3´ |
| attB2_FeSOD2_rev (without stop codon) | 5´- GGGGACCACTTTGTACAAGAAAGCTGGGTCGTCAACCTCAGATACATCGAT - 3´ |
| attB1_FeSOD3_for (with stop codon) | 5´- GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGAGTTCTTGTGTTGTGACG - 3´ |
| attB2_FeSOD3_rev (with stop codon) | 5´- GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAAGCGATTGGGATGTTGG - 3´ |
| attB1_FeSOD3_for (without stop codon) | 5´- GGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGAGTTCTTGTGTTGTGACG - 3´ |
| attB2_FeSOD3_rev (without stop codon) | 5´- GGGACCACTTTGTACAAGAAAGCTGGGTCAGCGATTGGGATGTTGGG - 3´ |

| | |
|---|---|
| attB1_CuZnSOD1_for (with stop codon) | 5´- GGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCGAAAGGAGTTGCAG - 3´ |
| attB2_CuZnSOD1_rev (with stop codon) | 5´- GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAGCCCTGGAGACCAATG - 3´ |
| attB1_CuZnSOD1_for (without stop codon) | 5´- GGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGGCGAAAGGAGTTGCAG - 3´ |
| attB2_CuZnSOD1_rev (without stop codon) | 5´- GGGACCACTTTGTACAAGAAAGCTGGGTCGCCCTGGAGACCAATGAT - 3´ |

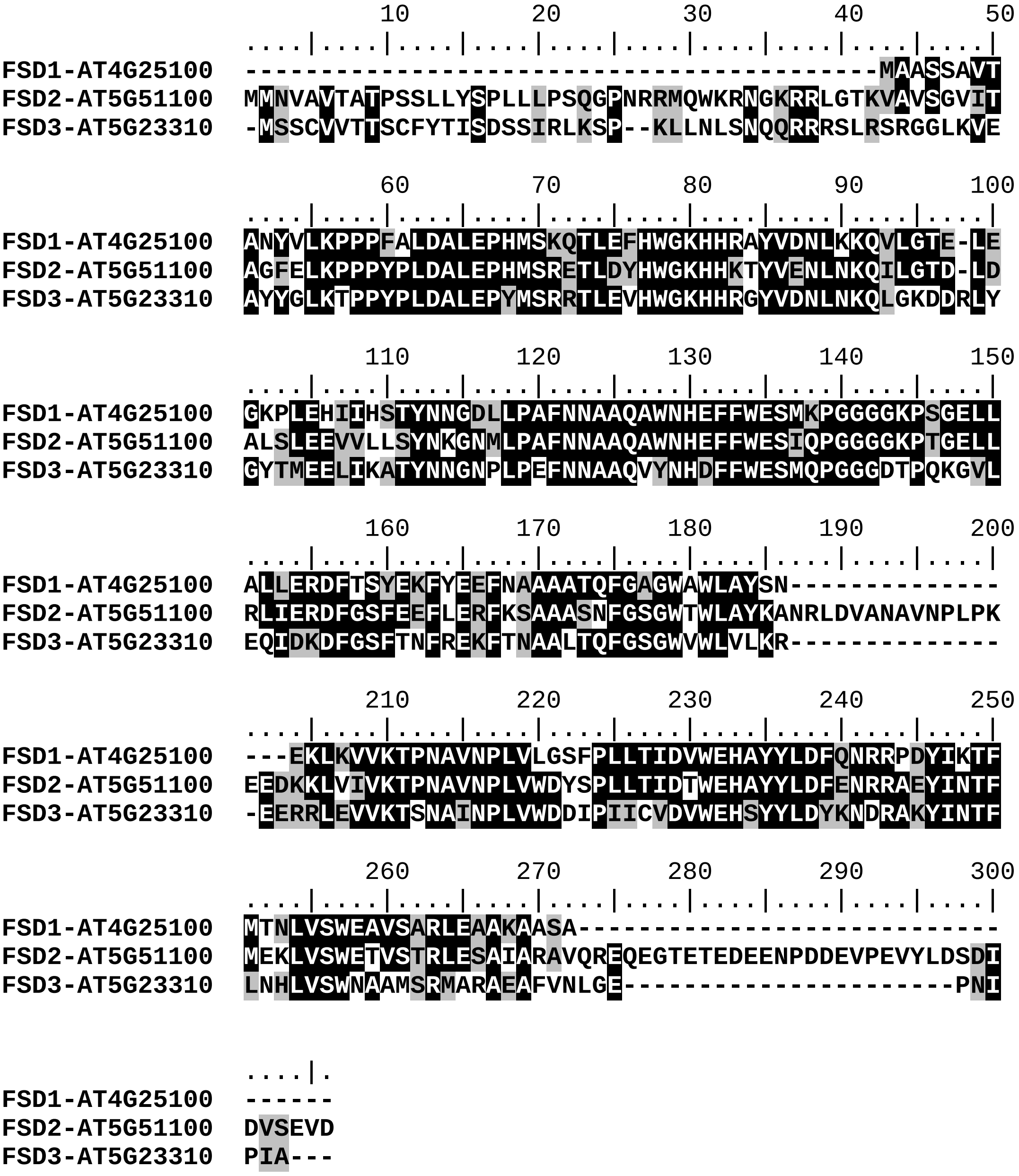
| | |
|---|---|
| attB1_CuZnSOD2_for (with stop codon) | 5´- GGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGTTTCCGCGGCGAAGAAGGC - 3´ |
| attB2_CuZnSOD2_rev (with stop codon) | 5´- GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAGAGCGGCGTCAAGC - 3´ |
| attB1_CuZnSOD2_for (without stop codon) | 5´- GGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGGTTTCCGCGGCGAAGAAGGC - 3´ |
| attB2_CuZnSOD2_rev (without stop codon) | 5´- GGGACCACTTTGTACAAGAAAGCTGGGTCGAGCGGCGTCAAGCCA - 3´ |

| | |
|---|---|
| attB1_CuZnSOD3_for (with stop codon) | 5´- GGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGAAGCTCCTAGAGGAAATC - 3´ |
| attB2_CuZnSOD3_rev (with stop codon) | 5´- GGGGACCACTTTGTACAAGAAAGCTGGGTCCTATAGTTTAGCATCCGCAGAT - 3´ |
| attB1_CuZnSOD3_for (without stop codon) | 5´- GGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGGAAGCTCCTAGAGGAAATC - 3´ |
| attB2_CuZnSOD3_rev (without stop codon) | 5´- GGGGACCACTTTGTACAAGAAAGCTGGGTCTAGTTTAGCATCCGCAGATGATT - 3´ |

## Oligonucleotides for site-directed mutagenesis

| | |
|---|---|
| MnSODY63F_for | 5´- CATCACCAGGCTTTTGTTACTAATTAC - 3´ |
| MnSODY63F_rev | 5´- GTAATTAGTAACAAAAGCCTGGTGATG - 3´ |

*Arabidopsis* FSDs



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                         10        20        30        40        50
                ....|....|....|....|....|....|....|....|....|....|
FSD1-AT4G25100  ------------------------------------------MAASSAVT
FSD2-AT5G51100  MMNVAVTATPSSLLYSPLLLPSQGPNRRMQWKRNGKRRLGTKVAVSGVIT
FSD3-AT5G23310  -MSSCVVTTSCFYTISDSSIRLKSP--KLLNLSNQQRRSLRSRGGLKVE

                         60        70        80        90       100
                ....|....|....|....|....|....|....|....|....|....|
FSD1-AT4G25100  ANYVLKPPPFALDALEPHMSKQTLEFHWGKHHRAYVDNLKKQVLGTE-LE
FSD2-AT5G51100  AGFELKPPPYPLDALEPHMSRETLDYHWGKHHKTYVENLNKQILGTD-LD
FSD3-AT5G23310  AYYGLKTPPYPLDALEPYMSRRTLEVHWGKHHRGYVDNLNKQLGKDDRLY

                        110       120       130       140       150
                ....|....|....|....|....|....|....|....|....|....|
FSD1-AT4G25100  GKPLEHIIHSTYNNGDLLPAFNNAAQAWNHEFFWESMKPGGGGKPSGELL
FSD2-AT5G51100  ALSLEEVVLLSYNKGNMLPAFNNAAQAWNHEFFWESIQPGGGGKPTGELL
FSD3-AT5G23310  GYTMEELIKATYNNGNPLPEFNNAAQVYNHDFFWESMQPGGGDTPQKGVL

                        160       170       180       190       200
                ....|....|....|....|....|....|....|....|....|....|
FSD1-AT4G25100  ALLERDFTSYEKFYEEFNAAAATQFGAGWAWLAYSN--------------
FSD2-AT5G51100  RLIERDFGSFEEFLERFKSAAASNFGSGWTWLAYKANRLDVANAVNPLPK
FSD3-AT5G23310  EQIDKDFGSFTNFREKFTNAALTQFGSGWVWLVLKR--------------

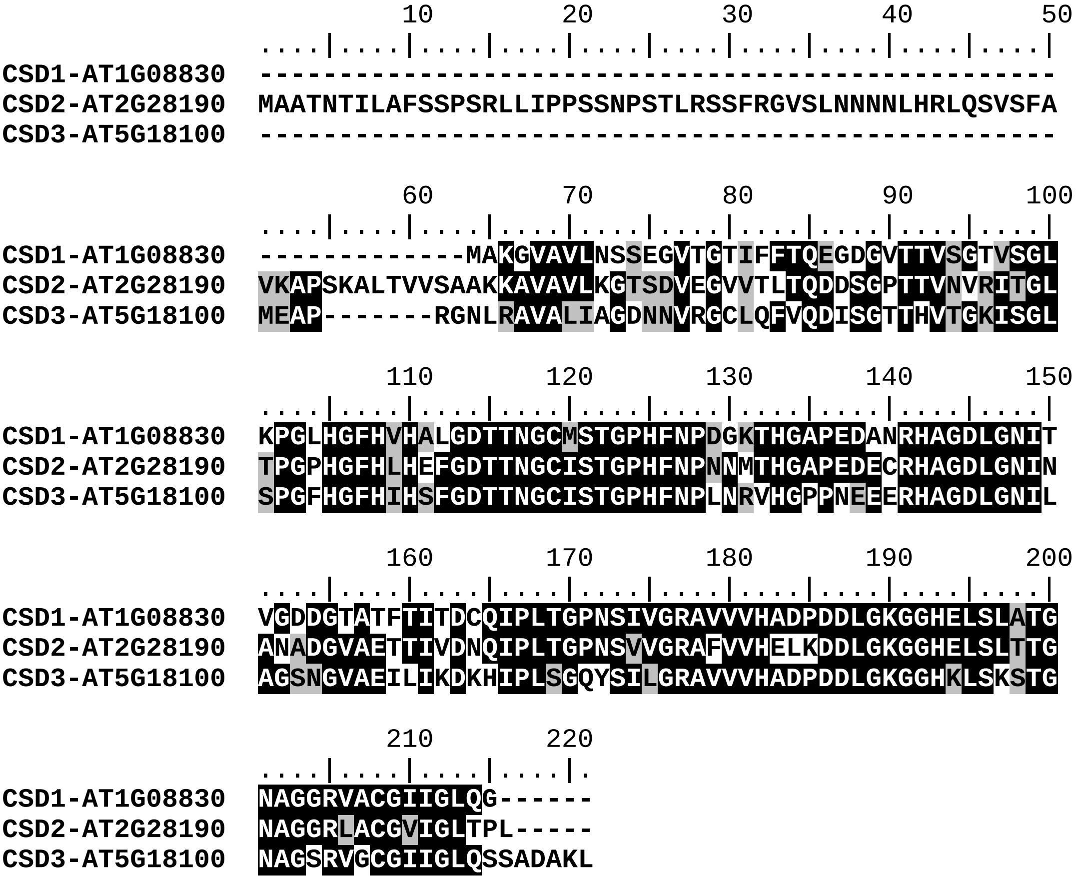
                        210       220       230       240       250
                ....|....|....|....|....|....|....|....|....|....|
FSD1-AT4G25100  ---EKLKVVKTPNAVNPLVLGSFPLLTIDVWEHAYYLDFQNRRPDYIKTF
FSD2-AT5G51100  EEDKKLVIVKTPNAVNPLVWDYSPLLTIDTWEHAYYLDFENRRAEYINTF
FSD3-AT5G23310  -EERRLEVVKTSNAINPLVWDDIPIICVDVWEHSYYLDYKNDRAKYINTF

                        260       270       280       290       300
                ....|....|....|....|....|....|....|....|....|....|
FSD1-AT4G25100  MTNLVSWEAVSARLEAAKAASA----------------------------
FSD2-AT5G51100  MEKLVSWETVSTRLESAIARAVQREQEGTETEDEENPDDEVPEVYLDSDI
FSD3-AT5G23310  LNHLVSWNAAMSRMARAEAFVNLGE----------------------PNI


                ....|.
FSD1-AT4G25100  ------
FSD2-AT5G51100  DVSEVD
FSD3-AT5G23310  PIA---
```

**Supplemental Figure S1: Alignment of amino acid sequences of *Arabidopsis* FSD isoforms.** Identical amino acids are highlighted in black, similar ones in grey. Dashes: Introduced gaps to maximize sequence similarity.

*Arabidopsis* CSDs



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                        10        20        30        40        50
                ....|....|....|....|....|....|....|....|....|....|
CSD1-AT1G08830  --------------------------------------------------
CSD2-AT2G28190  MAATNTILAFSSPSRLLIPPSSNPSTLRSSFRGVSLNNNNLHRLQSVSFA
CSD3-AT5G18100  --------------------------------------------------

                        60        70        80        90       100
                ....|....|....|....|....|....|....|....|....|....|
CSD1-AT1G08830  -------------MAKGVAVLNSSEGVTGTIFFTQEGDGVTTVSGTVSGL
CSD2-AT2G28190  VKAPSKALTVVSAAKKAVAVLKGTSDVEGVVTLTQDDSGPTTVNVRITGL
CSD3-AT5G18100  MEAP-------RGNLRAVALIAGDNNVRGCLQFVQDISGTTHVTGKISGL

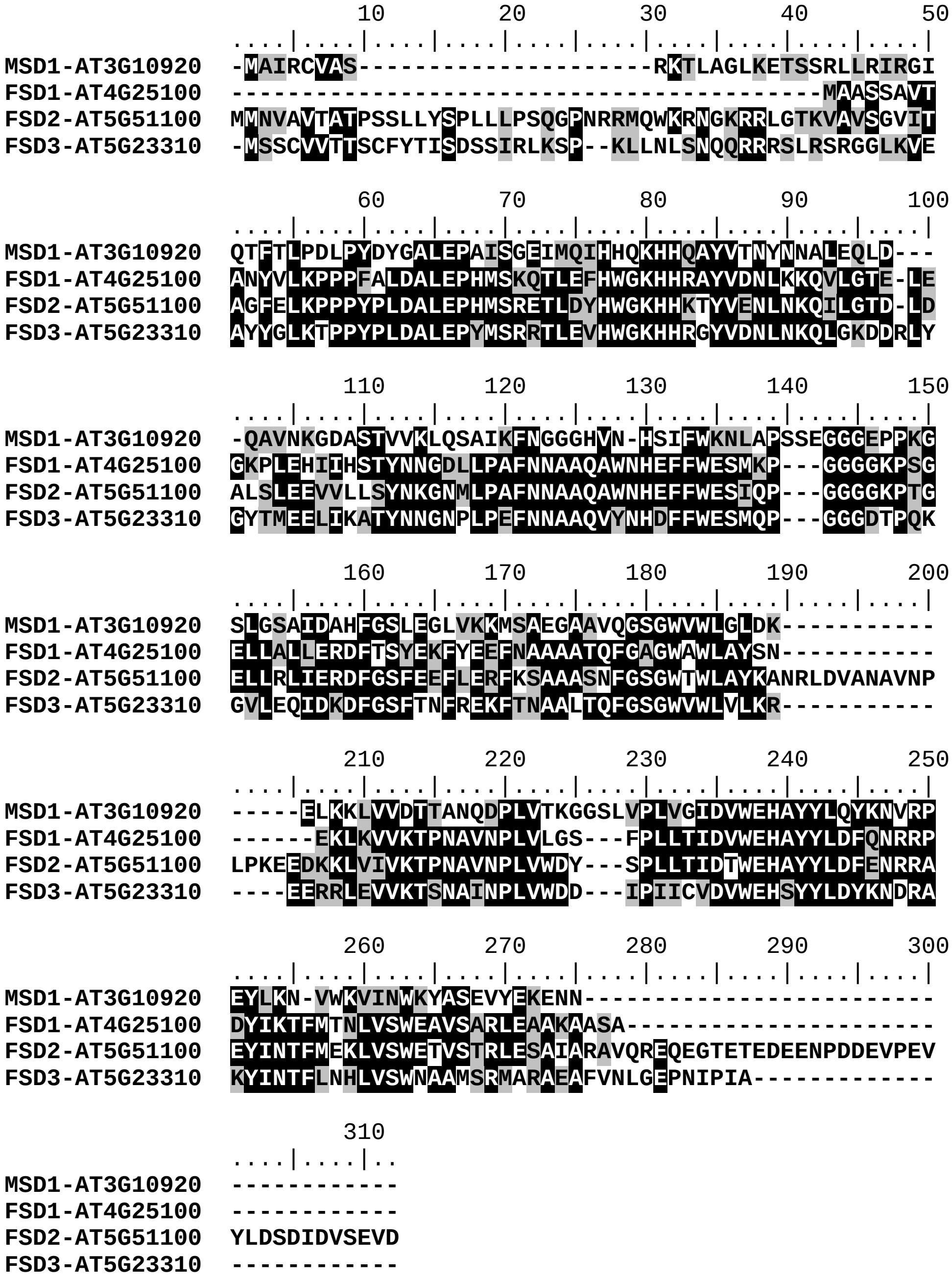
                       110       120       130       140       150
                ....|....|....|....|....|....|....|....|....|....|
CSD1-AT1G08830  KPGLHGFHVHALGDTTNGCMSTGPHFNPDGKTHGAPEDANRHAGDLGNIT
CSD2-AT2G28190  TPGPHGFHLHEFGDTTNGCISTGPHFNPNNMTHGAPEDECRHAGDLGNIN
CSD3-AT5G18100  SPGFHGFHIHSFGDTTNGCISTGPHFNPLNRVHGPPNEEERHAGDLGNIL

                       160       170       180       190       200
                ....|....|....|....|....|....|....|....|....|....|
CSD1-AT1G08830  VGDDGTATFTITDCQIPLTGPNSIVGRAVVVHADPDDLGKGGHELSLATG
CSD2-AT2G28190  ANADGVAETTIVDNQIPLTGPNSVVGRAFVVHELKDDLGKGGHELSLTTG
CSD3-AT5G18100  AGSNGVAEILIKDKHIPLSGQYSILGRAVVVHADPDDLGKGGHKLSKSTG

                       210       220
                ....|....|....|....|.
CSD1-AT1G08830  NAGGRVACGIIGLQG------
CSD2-AT2G28190  NAGGRLACGVIGLTPL-----
CSD3-AT5G18100  NAGSRVGCGIIGLQSSADAKL
```

**Supplemental Figure S2: Alignment of amino acid sequences of *Arabidopsis* CSD isoforms.** Identical amino acids are highlighted in black, similar ones in grey. Dashes: Introduced gaps to maximize sequence similarity.

*Arabidopsis* MSD1-FSDs



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                        10        20        30        40        50
                ....|....|....|....|....|....|....|....|....|....|
MSD1-AT3G10920  -MAIRCVAS---------------------RKTLAGLKETSSRLLRIRGI
FSD1-AT4G25100  ------------------------------------------MAASSAVT
FSD2-AT5G51100  MMNVAVTATPSSLLYSPLLLPSQGPNRRMQWKRNGKRRLGTKVAVSGVIT
FSD3-AT5G23310  -MSSCVVTTSCFYTISDSSIRLKSP--KLLNLSNQQRRRSLRSRGGLKVE

                        60        70        80        90       100
                ....|....|....|....|....|....|....|....|....|....|
MSD1-AT3G10920  QTFTLPDLPYDYGALEPAISGEIMQIHHQKHHQAYVTNYNNALEQLD---
FSD1-AT4G25100  ANYVLKPPPFALDALEPHMSKQTLEFHWGKHHRAYVDNLKKQVLGTE-LE
FSD2-AT5G51100  AGFELKPPPYPLDALEPHMSRETLDYHWGKHHKTYVENLNKQILGTD-LD
FSD3-AT5G23310  AYYGLKTPPYPLDALEPYMSRRTLEVHWGKHHRGYVDNLNKQLGKDDRLY

                       110       120       130       140       150
                ....|....|....|....|....|....|....|....|....|....|
MSD1-AT3G10920  -QAVNKGDASTVVKLQSAIKFNGGGHVN-HSIFWKNLAPSSEGGGEPPKG
FSD1-AT4G25100  GKPLEHIIHSTYNNGDLLPAFNNAAQAWNHEFFWESMKP---GGGGKPSG
FSD2-AT5G51100  ALSLEEVVLLSYNKGNMLPAFNNAAQAWNHEFFWESIQP---GGGGKPTG
FSD3-AT5G23310  GYTMEELIKATYNNGNPLPEFNNAAQVYNHDFFWESMQP---GGGDTPQK

                       160       170       180       190       200
                ....|....|....|....|....|....|....|....|....|....|
MSD1-AT3G10920  SLGSAIDAHFGSLEGLVKKMSAEGAAVQGSGWVWLGLDK-----------
FSD1-AT4G25100  ELLALLERDFTSYEKFYEEFNAAAATQFGAGWAWLAYSN-----------
FSD2-AT5G51100  ELLRLIERDFGSFEEFLERFKSAAASNFGSGWTWLAYKANRLDVANAVNP
FSD3-AT5G23310  GVLEQIDKDFGSFTNFREKFTNAALTQFGSGWVWLVLKR-----------

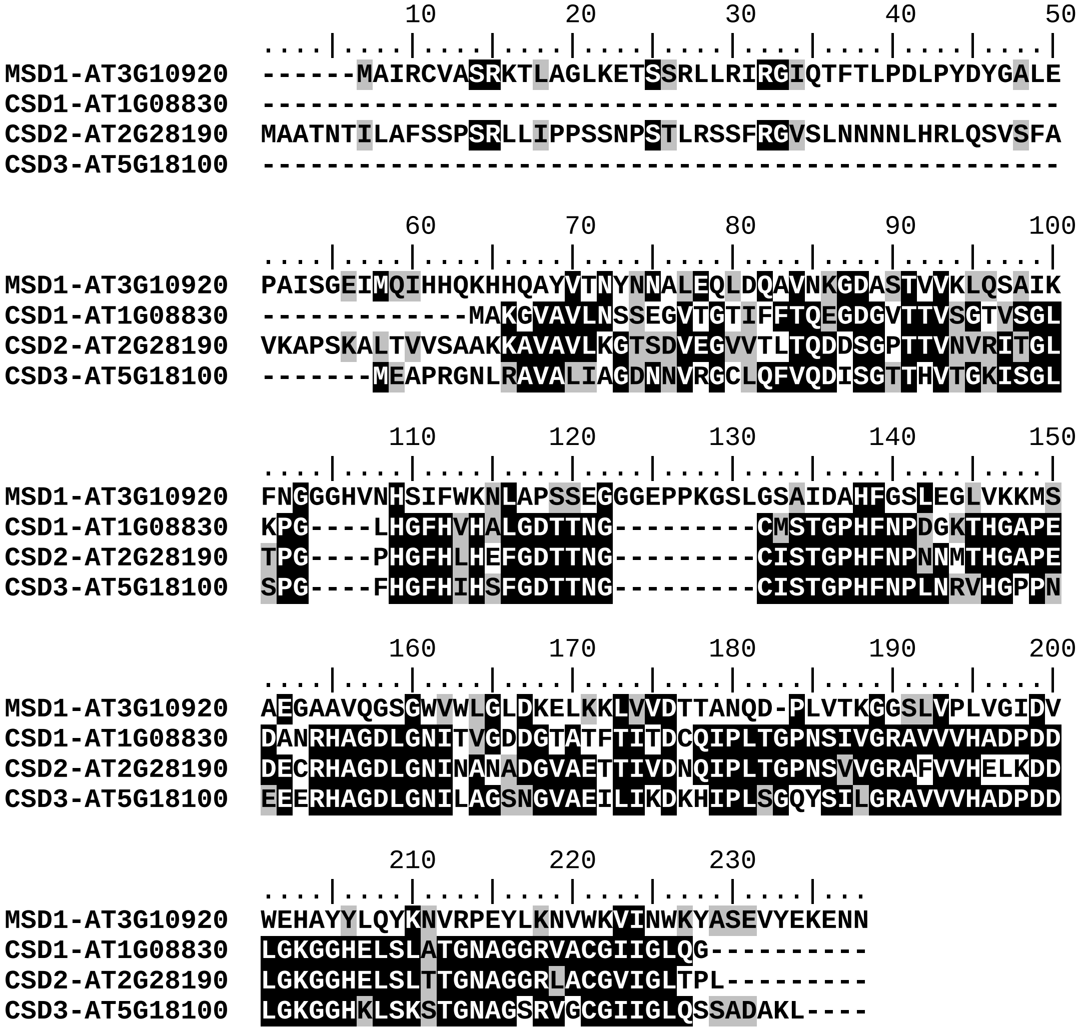
                       210       220       230       240       250
                ....|....|....|....|....|....|....|....|....|....|
MSD1-AT3G10920  -----ELKKLVVDTTANQDPLVTKGGSLVPLVGIDVWEHAYYLQYKNVRP
FSD1-AT4G25100  ------EKLKVVKTPNAVNPLVLGS---FPLLTIDVWEHAYYLDFQNRRP
FSD2-AT5G51100  LPKEEDKKLVIVKTPNAVNPLVWDY---SPLLTIDTWEHAYYLDFENRRA
FSD3-AT5G23310  ----EERRLEVVKTSNAINPLVWDD---IPIICVDVWEHSYYLDYKNDRA

                       260       270       280       290       300
                ....|....|....|....|....|....|....|....|....|....|
MSD1-AT3G10920  EYLKN-VWKVINWKYASEVYEKENN-------------------------
FSD1-AT4G25100  DYIKTFMTNLVSWEAVSARLEAAKAASA----------------------
FSD2-AT5G51100  EYINTFMEKLVSWETVSTRLESAIARAVQREQEGTETEDEENPDDEVPEV
FSD3-AT5G23310  KYINTFLNHLVSWNAAMSRMARAEAFVNLGEPNIPIA-------------

                       310
                ....|....|..
MSD1-AT3G10920  ------------
FSD1-AT4G25100  ------------
FSD2-AT5G51100  YLDSDIDVSEVD
FSD3-AT5G23310  ------------
```

**Supplemental Figure S3: Alignment of amino acid sequences of *Arabidopsis* FSD isoforms and MSD1.** Identical amino acids are highlighted in black, similar ones in grey. Dashes: introduced gaps to maximize sequence similarity.

*Arabidopsis* MSD1-CSDs



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                             10        20        30        40        50
                    ....|....|....|....|....|....|....|....|....|....|
MSD1-AT3G10920     ------MAIRCVASRKTLAGLKETSSRLLRIRGIQTFTLPDLPYDYGALE
CSD1-AT1G08830     --------------------------------------------------
CSD2-AT2G28190     MAATNTILAFSSPSRLLIPPSSNPSTLRSSFRGVSLNNNNLHRLQSVSFA
CSD3-AT5G18100     --------------------------------------------------

                             60        70        80        90       100
                    ....|....|....|....|....|....|....|....|....|....|
MSD1-AT3G10920     PAISGEIMQIHHQKHHQAYVTNYNNALEQLDQAVNKGDASTVVKLQSAIK
CSD1-AT1G08830     -------------MAKGVAVLNSSEGVTGTIFFTQEGDGVTTVSGTVSGL
CSD2-AT2G28190     VKAPSKALTVVSAAKKAVAVLKGTSDVEGVVTLTQDDSGPTTVNVRITGL
CSD3-AT5G18100     -------MEAPRGNLRAVALIAGDNNVRGCLQFVQDISGTTHVTGKISGL

                            110       120       130       140       150
                    ....|....|....|....|....|....|....|....|....|....|
MSD1-AT3G10920     FNGGGHVNHSIFWKNLAPSSEGGGEPPKGSLGSAIDAHFGSLEGLVKKMS
CSD1-AT1G08830     KPG----LHGFHVHALGDTTNG---------CMSTGPHFNPDGKTHGAPE
CSD2-AT2G28190     TPG----PHGFHLHEFGDTTNG---------CISTGPHFNPNNMTHGAPE
CSD3-AT5G18100     SPG----FHGFHIHSFGDTTNG---------CISTGPHFNPLNRVHGPPN

                            160       170       180       190       200
                    ....|....|....|....|....|....|....|....|....|....|
MSD1-AT3G10920     AEGAAVQGSGWVWLGLDKELKKLVVDTTANQD-PLVTKGGSLVPLVGIDV
CSD1-AT1G08830     DANRHAGDLGNITVGDDGTATFTITDCQIPLTGPNSIVGRAVVVHADPDD
CSD2-AT2G28190     DECRHAGDLGNINANADGVAETTIVDNQIPLTGPNSVVGRAFVVHELKDD
CSD3-AT5G18100     EEERHAGDLGNILAGSNGVAEILIKDKHIPLSGQYSILGRAVVVHADPDD

                            210       220       230
                    ....|....|....|....|....|....|....|...
MSD1-AT3G10920     WEHAYYLQYKNVRPEYLKNVWKVINWKYASEVYEKENN
CSD1-AT1G08830     LGKGGHELSLATGNAGGRVACGIIGLQG----------
CSD2-AT2G28190     LGKGGHELSLTTGNAGGRLACGVIGLTPL---------
CSD3-AT5G18100     LGKGGHKLSKSTGNAGSRVGCGIIGLQSSADAKL----
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**Supplemental Figure S4: Alignment of amino acid sequences of *Arabidopsis* CSD isoforms and MSD1.** Identical amino acids are highlighted in black, similar ones in grey. Dashes: Introduced gaps to maximize sequence similarity.

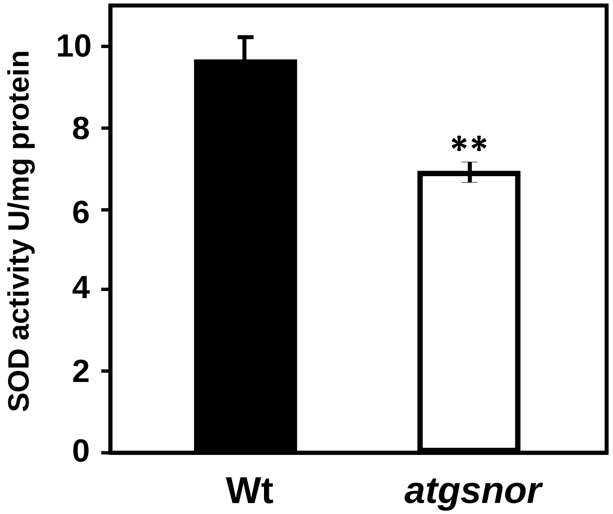

**Figure S5: Total SOD activity in *Arabidopsis* WT and GSNOR knock-out plants.** Proteins of 0,5 g leaf material of four week old plants were extracted with 1 ml extraction buffer. Total SOD activity was determined by monitoring reduction of cytochrome *c*. One unit is defined as enzyme amount necessary for 50% inhibition of cytochrome c reduction. Asterisks (**) indicate a significant difference between both lines (t-test, $p \leq 0.01$).

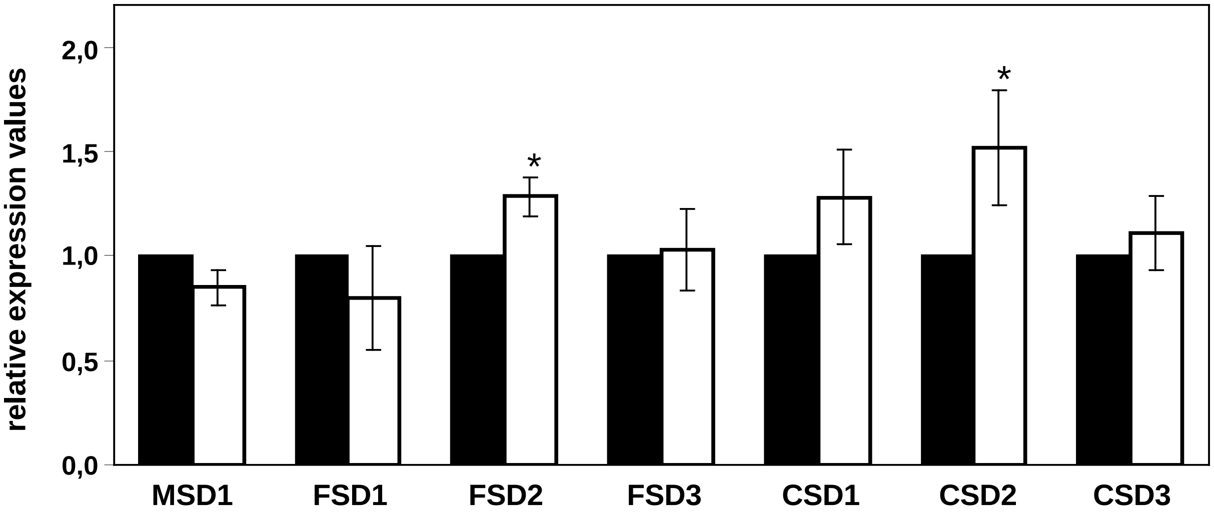

**Figure S6: Expression analysis of *Arabidopsis* SODs.**
Transcripts of *Arabidopsis* SODs were analysed by real-time quantitative PCR. Data represent the means ±SD of three experiments per lines. Normalization of real-time quantitative RT-PCR data was done by geometric averaging of multiple internal control genes (Ubiquitin 9 [At5g18380] and S16 [At5g18380 and At2g09990]).