

## SUPPLEMENTAL MATERIAL

Name	Nucleotide sequence
PtTrxClotfor	5' <u>CCCC<b>CCATGG</b></u> CTACAGTAAAGTTGGTGGATGCA3'
PtTrxClotrev	5'CCCC <b>GGATCC</b> TTAATTCCCAGAAACAAGGGC3'
PtTrxClotRTfor	5' <u>CCCC<b>CCATGG</b></u> CTACAGTAAAGTTGGTGGATGCA3'
PtTrxClotRTrev	5'CCCC <b>GGATCC</b> TTAATTCCCAGAAACAAGGGC3'
PtTrxClotGFPfor	5' <u>CCCC<b>GGATCC</b></u> TTCACAGAAACAAGGGC3'
PtTrxlike1RTfor	5'CCCC <b>CCATGG</b> GCTGAGGATAATACAAAGAAA3'
PtTrxlike1RTrev	5'CCCC <b>GGATCC</b> TTACGAATGCAACCACAAGCG3'
PtTrxlike1GFPfor	5'CCCC <b>CCATGG</b> GCTGAGGATAATACAAAG3'
PtTrxlike1GFPfor	5'CCCC <b>GGATCC</b> GAAATGCAACCACAAGCG3'
PtTrxlike2.1for	5'CCCC <b>CCATGG</b> GCTACCAGGCCAACCC3'
PtTrxlike2.1rev	5'CCCC <b>GGATCC</b> TTATACGAATTTCTGGATCAT3'
PtTrxlike2.1C45Sfor	5'TCTTGGTGCCGAAATCCATTTATTTGAAGCCA3'
PtTrxlike2.1C45Srev	5'TGGCTTCAAATAAATGGATTTCCGGCACCAAGA3'
PtTrxlike2.1R43G/K44Pfor	5'ATGGCTTCTTGGTGCGGGCCATGCATTTATTTGAAG3'
PtTrxlike2.1R43G/K44Pfor	5'CTTCAAATAAATGCATGGCCCGCACCAAGAAGCCAT3'
PtTrxlike2.1RTfor	5'CGTGACTTGTCAGTATTAAGT3'
PtTrxlike2.1GFPfor	5'CCCC <b>CCATGG</b> GCTTCTAAGATCTTACAGAAC3'
PtTrxlike2.1GFPfor	5'CCCC <b>GGATCC</b> CAAGGTACCCTCATTTC3'
PtTrxlike2.2RTfor	5'CGTGACTTGTCAGTATTAAGT3'
PtTrxlike2.2RTrev	5'CCCC <b>GGATCC</b> TCACAAGGTATCCTCATTTCATC3'
PtTrxlike2.3RTfor	5'AAGCATTTCCTTGTCAAACCC3'
PtTrxlike2.3RTrev	5'CCCC <b>GGATCC</b> TCACAAGGTATCCTCATTTCATC3'
PtTrxlilium1.1RTfor	5'TCAAACCCGGCAACAC3'
PtTrxlilium1.1RTrev	5'GGGTTTTCTCCTCTGAGTC3'
PtTrxlilium1.2for	5'CCCC <b>CCATGG</b> GCTTCGGTGTGGCTCAG3'
PtTrxlilium1.2rev	5'CCCC <b>GGATCC</b> TCATCTCCCCGAAATGGC3'
PtTrxlilium1.2RTfor	5'GTTTCTTCTAATGTTGG3'
PtTrxlilium2.1RTfor	5'ATAAGAGGATCTCTTTTC3'
PtTrxlilium2.1RTrev	5'GCAACGGGCTGTGTTGTG3'
PtTrxlilium2.2for	5'CCCC <b>CCATGG</b> GCTGAAACTGACCAACCG3'
PtTrxlilium2.2rev	5'CCCC <b>GGATCC</b> TTATGTAGATCCTGCTGT3'
PtTrxlilium2.2RTfor	5'TAAGAATTTATCCTCTTTATA3'
PtTrxlilium2.2RTrev	5'GCAACGGGCTGTGTTGTG3'
PtTrxlilium3for	5'CCCC <b>CCATGG</b> GCTAAGGCAAAATCCCCCTCT3'
PtTrxlilium3rev	5'CCCC <b>GGATCC</b> TTAGGTGTCTTGAGCTGC3'
PtTrxlilium3S87Pfor	5'CGCACTTCTTGTGGACCCTGCAAGTATATAGAG3'
PtTrxlilium3S87Pfor	5'CTCTATATACTTGCAGGGTCCACAAGAAGTGCG3'
PtTrxlilium3S84Wfor	5'GATTTTTATCGCACTTGGTGTGGAAGCTGCAAG3'
PtTrxlilium3S84Wrev	5'CTTGCAGCTTCCACACCAAGTGCGATAAAAATC3'
PtTrxlilium3S84W/S87Pfor	5'TATCGCACTTGGTGTGGACCATGCAAGTAT3'
PtTrxlilium3S84W/S87Pfor	5'ATACTTGCATGGTCCACACCAAGTGCGATA3'

### Supplemental Table I. Primers used for cloning, RT PCR and site-directed mutagenesis.

The *NcoI* and *BamHI* restriction sites are underlined and the mutagenic bases are in bold characters. The primers used for expression studies are indicated RT (for RT PCR experiments). Primers used for GFP experiment are indicated GFP (for green fluorescent protein experiment). The primers used for cloning possess restriction sites underlined.



D

PtTrx-lilium1.1 -MTEVLSKTNLFSSGNYQTRQHNTISVFTKSCRLKGFPSRVKPOGLRSQISRSSSSCSDFY 59  
 AtTrx-lilium1.1 -MTEVLSKTSFLGACGNHHRVDDFDFSPVSPVSGGFLKKSFSCLKKSQKPLR---SVFY 56  
 OsTrx-lilium1.1 -MAEALCSGSVASP-CGEVGVGFAAGLVRGAAAAAALAESVPIGGYSS-----KSTFP 51  
 OsTrx-lilium1.2 -MAATAAQAVAVKG-SVAVPPCGSRGRRRGAVASVRMAAAAATSALRIGR-----RSPFL 53  
 AtTrx-lilium1.3 MATDSFKLNPISFNRRARFDLRFAGISPKSISLCCISPR---LIS-----CNHFS 49  
 AtTrx-lilium1.2 MDAISSLGTNCVLSGVSPFSQENQSKSLSPFMSLDLKEHP----MAS-----ADFT 48  
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PtTrx-lilium1.1 GKRVVHVGNQSKPRRGYLPQASVVALTGLKLYAKKWWEKGLQPNMREVTSAQDLVDSL 119  
 AtTrx-lilium1.1 GKQIVFGDSQDESFR---RSSAITAQTTLRIGTAQKWWEKGLKDNMREISSAQELVDSL 113  
 OsTrx-lilium1.1 SGRVALTERKARPLR--NLEAAHGQMNLTIGKAMRWWEKCLQPNMREIESAQLDADSL 109  
 OsTrx-lilium1.2 GGRLAVGPRRSRPVPR--NLVAVP-QMNLAFKATKWWEKGLQPNMREVESAQDLVDSL 110  
 AtTrx-lilium1.3 PRTLISGENGNILFSKIKIPAFVRCQTSLGIGRNQKWWEKELKPNMKSVTSPQDLVSLR 109  
 AtTrx-lilium1.2 NQTLTAFSSSS-----ASPQAKTSSIGMSRGRMWEKSTNHNMLEIQSANHLVDSL 101  
 : : : : : \* \* \* \* \* : \* \* : \* : \* \* \*

PtTrx-lilium1.1 NAGDQLVVVDFSPGCGGCKALHPKICQLAEMNPDVQFLQVNYEEHKSMCYSLNVHVLFP 179  
 AtTrx-lilium1.1 NAGDKLVVDFSPGCGGCKALHPKICQFAEMNPDVQFLQVNYEEHKSMCYSLGVHVLFP 173  
 OsTrx-lilium1.1 NAGDKLVVDFSPGCGGCRALHPKICQLAEMNPEVFLQVNYEKHKSMCYSLHVVHVLFP 169  
 OsTrx-lilium1.2 NAGDNLVIVDFSPGCGGCRALHPKICQIAEQNPVDFLQVNYEEHKSMCYSLHVVHVLFP 170  
 AtTrx-lilium1.3 NAGDKLVVDFSPGCGGCKALHPKICKIAEKNPEVEFLQVNYEHRSLCQSLNIHVLFP 169  
 AtTrx-lilium1.2 NAGDRLVVDLDFYSPGCGGCKSLHPKICQLAETNPVMFVKVQEEELRMTCHGLNVHVLFP 161  
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PtTrx-lilium1.1 FRFYRGAHGRLCSFSCTNATIKKFKDALAKHTPERCSLGPCKGLEEKELVALAANKDLSF 239  
 AtTrx-lilium1.1 FRFYRGSQGRVCSFSCTNATIKKFRDALAKHGPDRCGLGPKGLEEKELVALAANKELNF 233  
 OsTrx-lilium1.1 FRFYRGAQGRVSSFSCTNATIKKFKDALAKHGPDRCGLGPAKGLEESELMALAINRDLNF 229  
 OsTrx-lilium1.2 FRFYRGAQGRVCSFSCTNATIKKFRDALAKHGPDRCGLGPTRGLEEESELLALANNDLQF 230  
 AtTrx-lilium1.3 FRFYRGSQGRVCSFSCTNATIRKFKALEKHGREQCSIGETKGLEEKELVAMAANKDLSF 229  
 AtTrx-lilium1.2 FKFYRGAEGKVCFSCTIATINKFKKALDKHGSEKSLGDAKGLDEKELAAALASVVELK 221  
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PtTrx-lilium1.1 TYTPKVPQAPVPAEEVAPTAGPSHSDRGLPLPLPITSSKSAQDSEKTLVSSGR--- 295  
 AtTrx-lilium1.1 TYTPK-----PVPVEKEAATPD----SNPSLPVPLPSMSS-----NDEKTLVSAGR--- 275  
 OsTrx-lilium1.1 TYTPNQ---DLVPIADALLKEAA---APGGPWLPLPATATQLFIQGSSENSLLSSGR--- 279  
 OsTrx-lilium1.2 NYTKKP---ELVPSGECRSCQEL---DCGTTTF-LPRKPCQQV----- 266  
 AtTrx-lilium1.3 DYKPTS-----CGNIQEQKKE-----IFLPKSPTFNKQKEVEHSLLVSPAPA 273  
 AtTrx-lilium1.2 NSLTMH----QASNIGYKTEEQY-----QTMVL----- 245

E

PtTrx-lilium2.1 MADVVG--LPSFRSLRVPSYLL---ASNNSTSLQPLLSHNQINFSDKRIS-----L 46  
 PtTrx-lilium2.2 MAVVVGL-LPSFRSLGFPSSLLTSFASSNPI SLQPLLSHKHISFSDIRIYPLLYSSAATA 59  
 AtTrx-lilium2 MAGVRLTTTSVQAIRVSSSFSSFATALNP--LQPCLPPNSNLNSDKRLRLLSSSPSCSS 58  
 OsTrx-lilium2 MAEALLP-LPRRLVVTASTPACSSASSSTSPSPHCLLSRANPR-----P 43  
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PtTrx-lilium2.1 SRFSSS-----PRDQFLSFKVHATVAET-DQPKWERNAGPNMIDIHSTDEFLS 94  
 PtTrx-lilium2.2 SRFSSS-----RRKQLLSFKVHATVAET-DQPKWERNAGPNMIDIHSTEEFLR 107  
 AtTrx-lilium2 SHYHPSGLGSHLPLRRPKSQVVRVKVDENVAET-EPPKWVERNA-PNMVDIHSTEEFLS 116  
 OsTrx-lilium2 PRLAAP-----SPPRRRLKAHAASDKSEQPKWERNAGPNMIDIHSTQEFLD 92  
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PtTrx-lilium2.1 ALSQAEDRLVIVEFYGTWCASCALFPKLCRTAEDHPEILFLKVNFDENKPMCKSLNVKV 154  
 PtTrx-lilium2.2 ALSEAGDRLVIVEFYGTWCASCALFPKLCRTAEEHPEILFLKVNFDENKPMCRSLNVKV 167  
 AtTrx-lilium2 ALSGAGERLVIVEFYGTWCASCALFPKLCRTAVEHPDIVFLKVNFDENKPMCKSLNVRV 176  
 OsTrx-lilium2 ALRDAGDRLVIVEFYGTWCASCALFPRLCRTAVENPDILFLKVNFDENKPMCKRLNVKV 152  
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PtTrx-lilium2.1 LPYFHFYRGAHQLESFSCSLAKFQKIKDSIEMHNTARCSIGPPKGVGELTLESVSASQD 214  
 PtTrx-lilium2.2 LPYFHFYRGAHQLESFSCSLVKFQKIKDAIEMHNTARCSIGPPKAVGELTLESISAPQD 227  
 AtTrx-lilium2 LPFFHFYRGADGQLESFSCSLAKFQKIKDAIQLHNTDRCSLGPVPEGLTLA----- 229  
 OsTrx-lilium2 LPYFHFYRGADGQLEAFSCSLAKFQKIKDAI AVHNTARCSIGPPVGVGDVLDL----PEE 208  
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PtTrx-lilium2.1 KPVGST-- 220  
 PtTrx-lilium2.2 KTAGST-- 233  
 AtTrx-lilium2 KPAASS-- 235  
 OsTrx-lilium2 KPAEASPR 216  
 \* : :

**F**

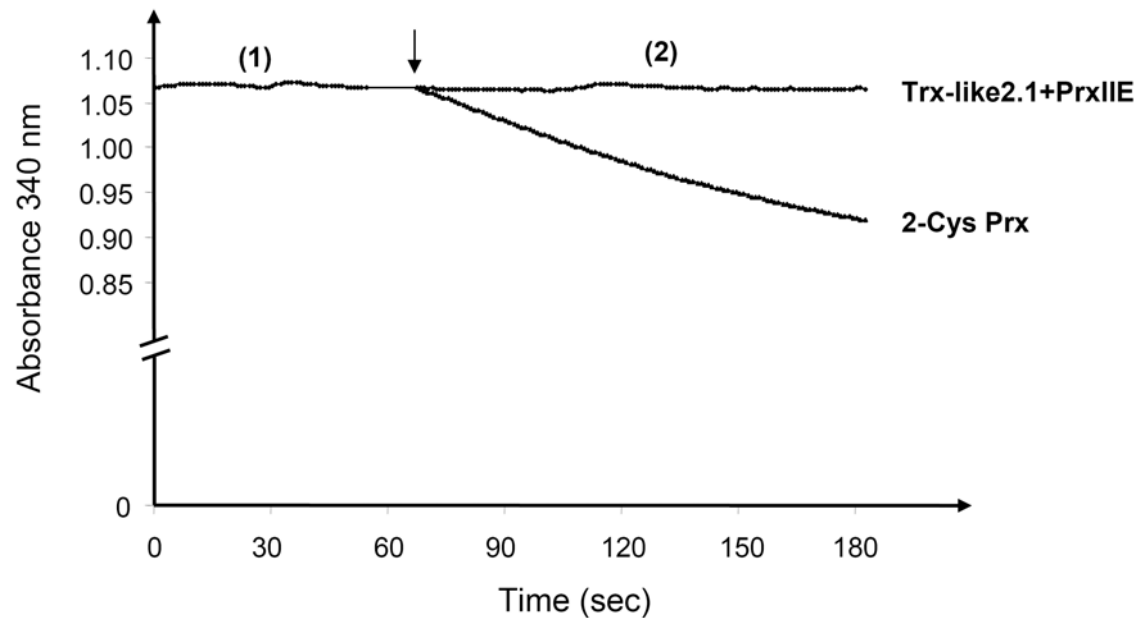
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PtTrx-lilium3      MEKARTFLLQLQQHIDCGGSLNVVMQKQNILYSKASFG-FGRNPDRQLDCRIPCVPMSL 59
AtTrx-lilium3      ----MTPLLWF-----CFKILIFDSNLQISHCSYHGYSGLTSR---GGINTVENHRW 45
OsTrx-lilium3      -----
                                     :   :   :
PtTrx-lilium3      PCRGNEKSCLMKAKFPSITKYAGLNFPPKNNVASRPGRIKAVVDENPGELSDEDDDLCPVD 119
AtTrx-lilium3      VWHNNG----VRLSFP--RAESSINITMGCTLQR-GIAKSLSQENLVELSDENDDLCPVE 98
OsTrx-lilium3      PLRRHR-----HLSSSSSSASSTESDGGGGSTNGSLPGLP---PVVVEEEEEFCPVE 83
                                     :   :   :   *   :   :   :****:
PtTrx-lilium3      CVREFKTDEEFLRILEKAKETDSLIVVVDFFYRTSCGSKYIEQGFALCKKQSGDQEAAVTF 179
AtTrx-lilium3      CVTEFKTDELLSVLEKSKETNSLVVVDFFYRTACGSKYIEQGFSLCKKQSGDQEAAPVIF 158
OsTrx-lilium3      CVTEFKTEELARVLERAKATGALVVVDFFRFPSCGSKYIEQGFMKLCKKQSGDHGSSVVF 143
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PtTrx-lilium3      LKHNVIDEYDEQSEVAERLRIKTVPLFHFYKKGVLVESFPTRDKERILGAILKYTSPAAQ 239
AtTrx-lilium3      LKHNVVDEYDEQSEVAERLRIKAVPLFHFYKNGVLLESFATRDKERIDAAILKYTSSSES- 217
OsTrx-lilium3      LKHNVIDEYDEQSEVADRLRIKVVPLFHFYKNGVLEAFATRDKERIIAAIQKYTAPSSP 203
*****:*****:***** *****:*****: * ***** ** * :
PtTrx-lilium3      DT----- 241
AtTrx-lilium3      -----
OsTrx-lilium3      PAESEEPSQEG 214

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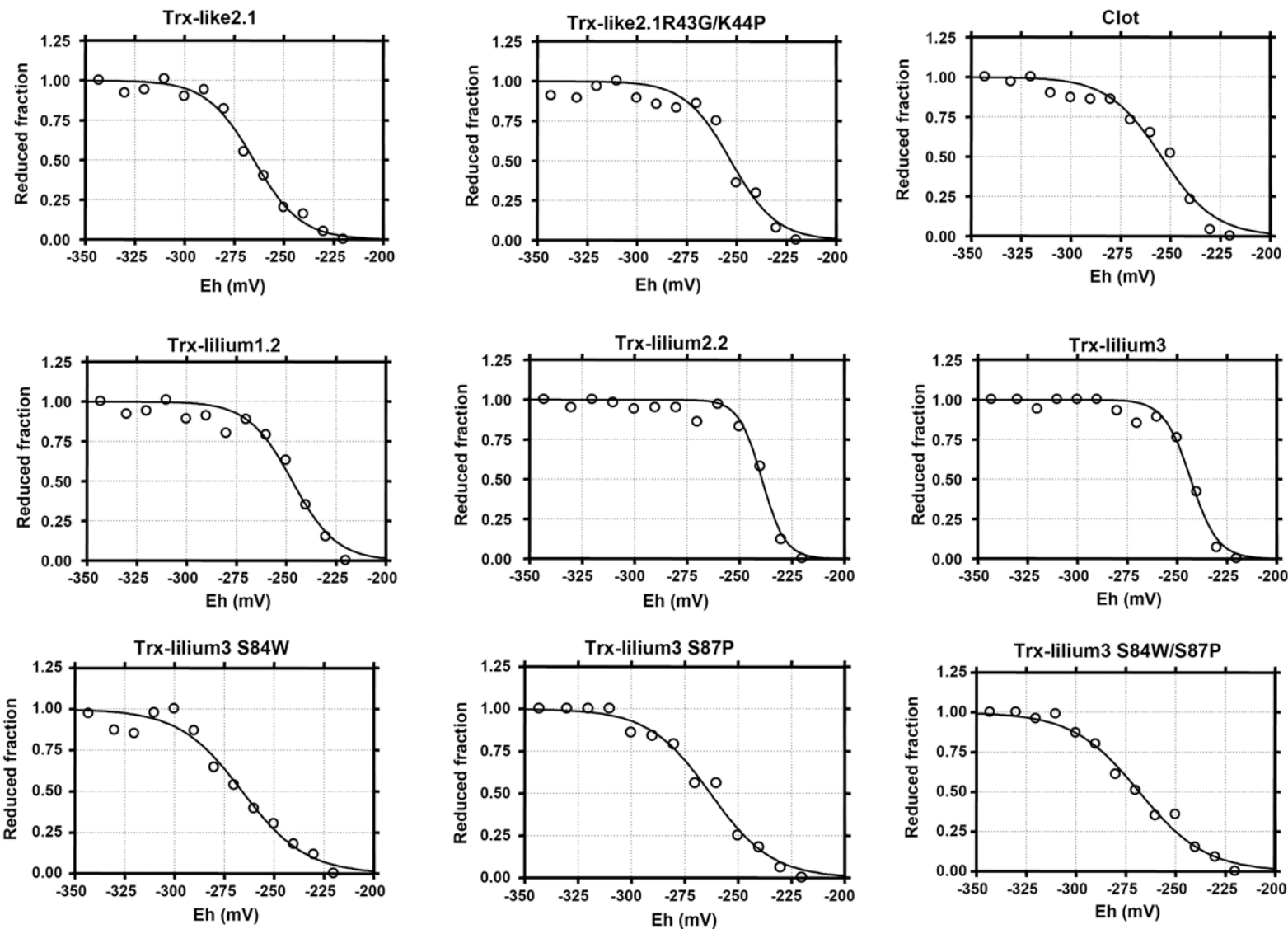
**Supplemental Figure 1: Amino acid sequence comparison of atypical Trx families.**

The alignments of Clot (A), Trx-like1 (B), Trx-like2 (C), Trx-lilium1 (D), Trx-lilium2 (E) and Trx-lilium3 (F) protein families were performed with ClustalW from sequences retrieved in phytozome (<http://www.phytozome.net/>) for *Populus trichocarpa* (Pt), *Arabidopsis thaliana* (At) and *Oryza sativa* (Os). The strictly conserved amino acids are marked by an asterisk while other conservative amino acid changes are indicated by a colon. Accession numbers are as follows: PtClot (POPTR\_0001s23270), AtClot (At5g42850), OsClot (Os06g21550), PtTrx-like1 (POPTR\_0006s12500), OsTrx-like1 (Os04g47260), AtTrx-like1 (At3g53220), PtTrx-like2.1 (POPTR\_0016s05950), PtTrx-like2.2 (POPTR\_0010s23260), PtTrx-like2.3 (gw1.VIII.1056.1), AtTrx-like2.1 (At5g06690), AtTrx-like2.2 (At5g04260), OsTrx-like2.1 (Os02g53400), PtTrx-lilium1.2 (POPTR\_0013s05790), PtTrx-lilium2.1 (POPTR\_0006s15520), PtTrx-lilium1.1 (POPTR\_0019s04750), PtTrx-lilium2.2 (POPTR\_0018s07010), PtTrx-lilium3 (POPTR\_0014s02920), AtTrx-lilium1.1 (At1g08570), AtTrx-lilium1.2 (At5g61440), AtTrx-lilium1.3 (At2g33270), AtTrx-lilium2 (At4g29670), AtTrx-lilium3 (At1g07700), OsTrx-lilium1.1 (Os07g48510), OsTrx-lilium1.2 (Os03g21000), OsTrx-lilium2 (Os05g11090), OsTrx-lilium3 (Os02g35900). Note that for this alignment the poplar sequences used are those from the *Populus trichocarpa* sequenced genome, and that a few variations can exist between poplar species.



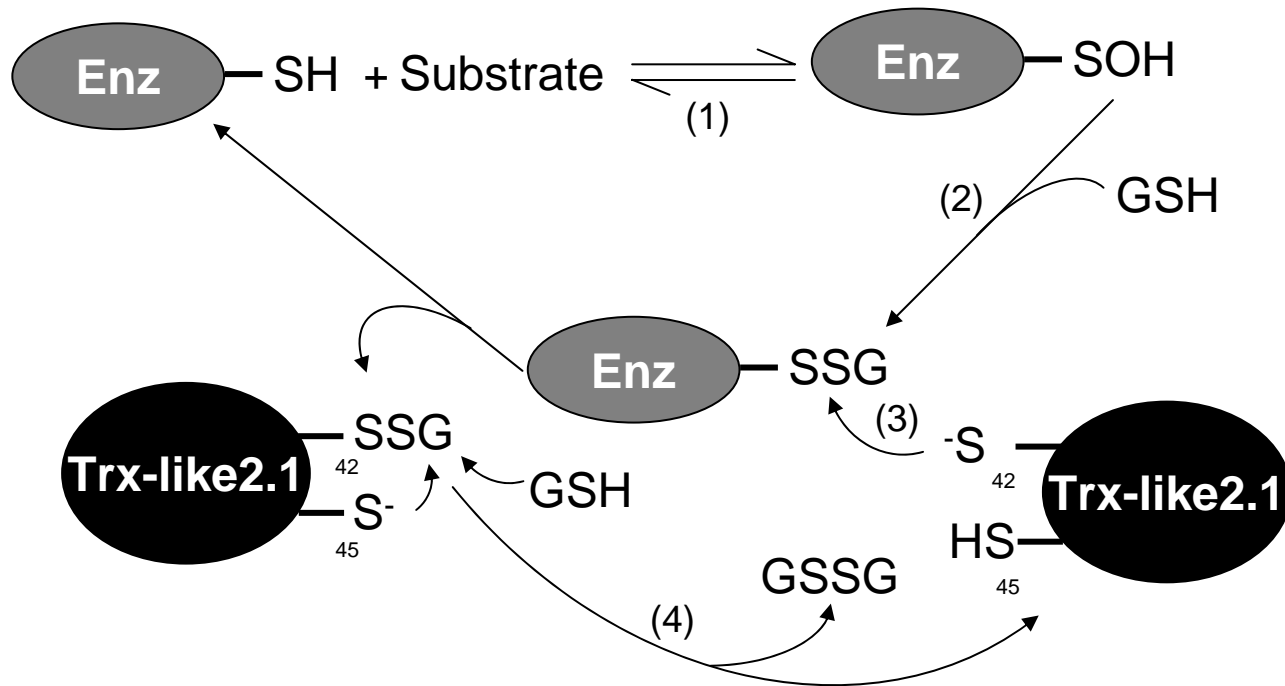
**Supplemental Figure 2. Assessment of poplar Trx-like2.1 reduction by Arabidopsis NTRc.**

The reduction of Trx-like2.1 by AtNTRc was evaluated using a coupled assay linked to the spectrophotometric detection of NADPH oxidation at 340 nm. NADPH, 1.5  $\mu$ M NTRc and 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> were initially incubated for 1 min (1). As indicated by the arrow, the *A. thaliana* 2-Cys Prx (2  $\mu$ M) used as a control, or a mixture of Trx-like2.1 (10  $\mu$ M) and PrxIIIE (2  $\mu$ M) have then been added to the cuvette (2). The consumption of NADPH concomitant to the reduction of hydrogen peroxide was followed as a function of time.



**Supplemental Figure 3. Redox midpoint potentials of Trxs-like and -lilium.**

The titrations were carried out using a total DTT concentration of 2 mM in the redox buffer and with a redox equilibration time of 2 h. Free protein thiols are labeled by mBBr. Values are the means  $\pm$  S.D. of three independent experiments.



**Supplemental Figure 4. Proposed mechanism for the glutathione- and Trx-like2.1-dependent regeneration of target proteins using a single catalytic cysteine.**

The first step consists of substrate reduction with the concomitant release of 1 mol of product and the formation of a stable sulfenic acid intermediate on the catalytic Cys (1). The sulfenic acid is then attacked by GSH leading to the release of one molecule of water and to the formation of a glutathione adduct (2), which is subsequently attacked by the catalytic cysteine of Trx-like2.1 (3). The glutathionylated form of Trx-like2.1 is likely reduced by another GSH molecule (4).