Regulation of Plant ER Oxidoreductin 1 Activity for Efficient Oxidative Protein Folding

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Table S1

Second structure contents of recombinant GmERO1a (WT) and its cysteine-mutants. Second structure contents were estimated from the vacuum ultraviolet circular dichroism (VUVCD) spectrum by SERCON3 program. Second structer contents of Human ERO1α was obtaind from PDB # 3AHR.

Protein	Second structure contents (%)				Numbers of			
	α-helix	β-strand	turn	coil	α-helix residues	α-helix segments	β-strand residues	β-strand segments
GmERO1a								
WT	51.1	5.5	13.7	31.1	193.7	20	20.8	4
C113A	52.4	8.2	13.5	29.8	198.6	20	31.1	7
C118A	50.8	7.9	13.6	30.3	192.5	20	29.9	7
C121A	43.8	10.5	17.5	31.5	166.0	17	39.8	9
C123A	43.2	10.0	18.9	30.2	163.7	16	37.9	9
C146A	42.2	10.2	17.2	31.1	159.9	17	38.7	9
C113/146A	50.9	7.9	13.4	32.0	192.9	20	29.9	8
C121/146A	53.1	8.7	12.6	30.1	201.2	20	33.0	7
C123/146A	55.6	3.5	15.2	29.0	210.7	18	13.3	3
C118/121A	50.6	7.3	11.9	32.7	191.8	20	27.7	8
Human ERO1α	48.9	6.4	6.9	-	185.3	16	24.3	9

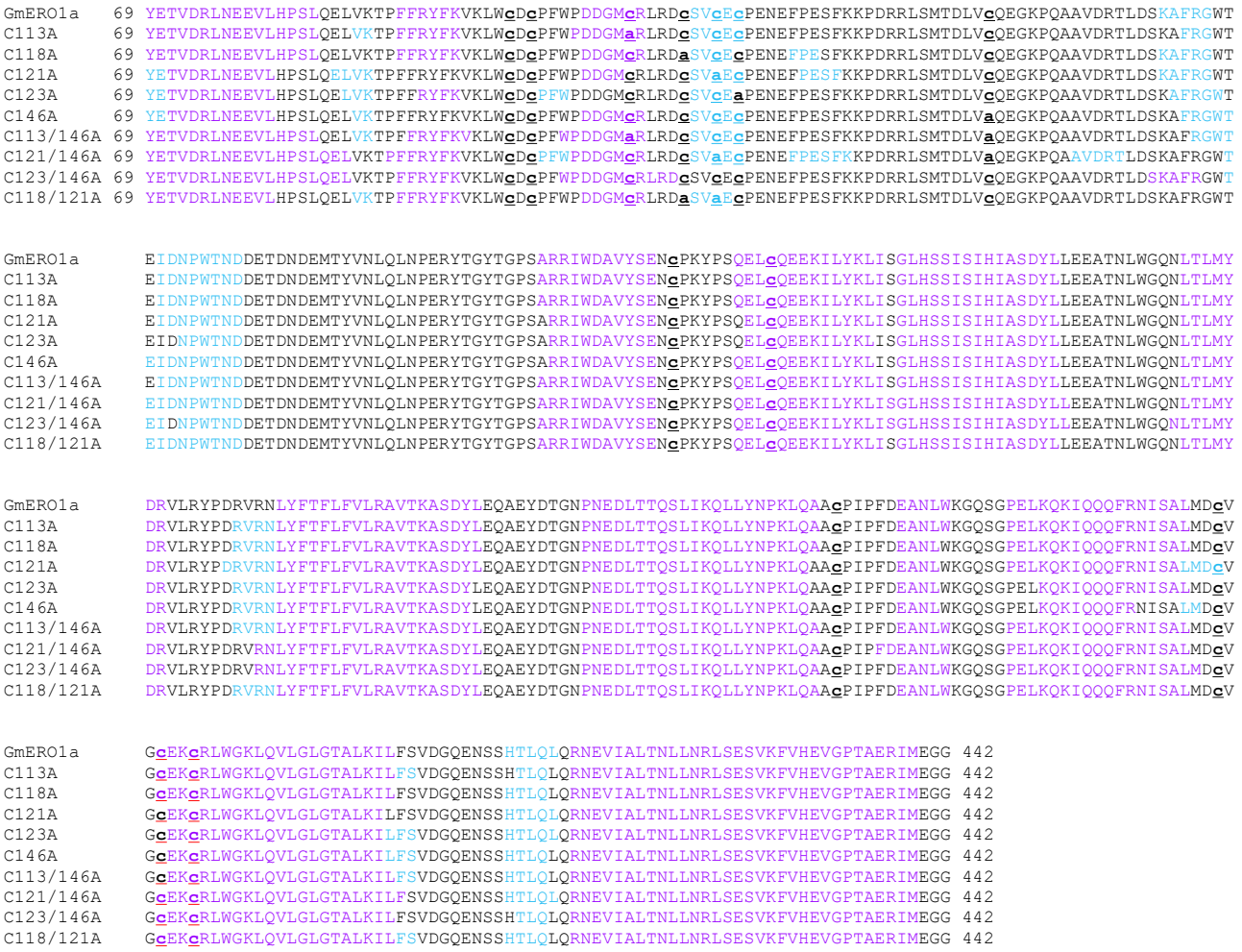


Figure S1. Alignment of amino acid sequences of wild type GmERO1a and its cysteine-mutants. Second structures of GmERO1a and its cysteine-mutants were predicted from VUVCD spectrum. Purple, light blue and black amino acid residues in the amino acid sequences are included in helix, β structures and turn or coil, respectively.

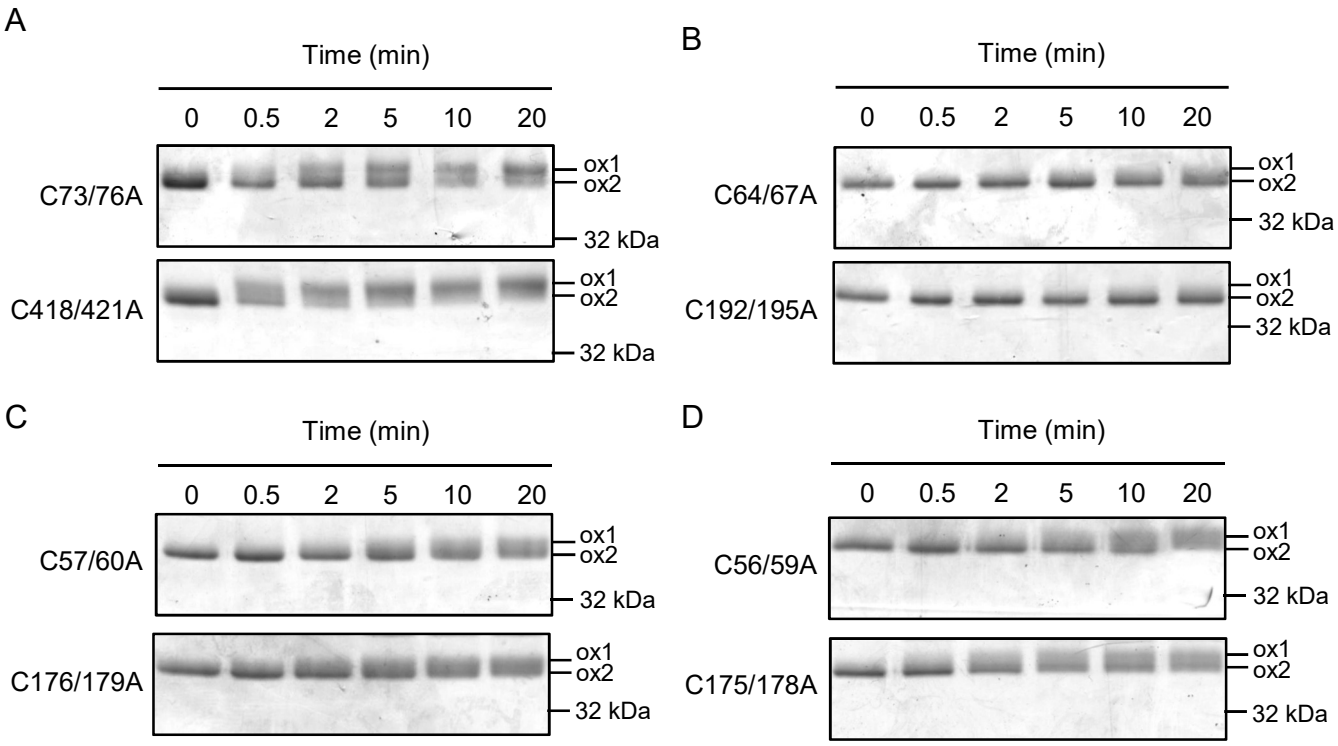
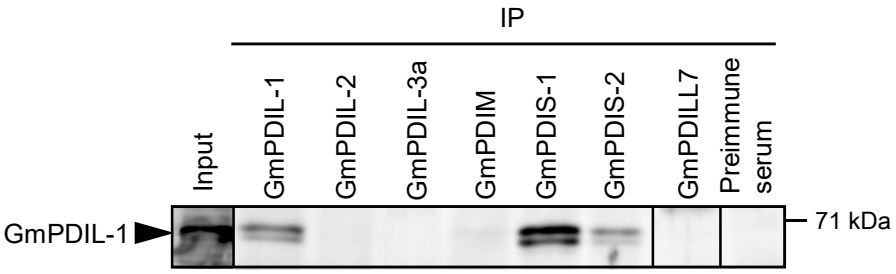


Figure S2. Conversion of ox-2 GmERO1a to the ox-1 form by reduced active center mutant of PDI family proteins. GmERO1a (5 μM) was incubated with 2 μM reduced cysteine-mutants of GmPDIL-1 (*A*), GmPDIM (*B*), GmPDIS-1 (*C*) or GmPDIS-2 (*D*) in the presence of 10 mM GSH at 25° C, then treated with N-ethylmaleimide and subjected to non-reducing SDS-PAGE. Proteins were stained with Coomassie Brilliant Blue G-250.

A



B

prey	(pmol)							bait
	80	40	20	10	5	2.5	1.25	
GmPDIS-1								GmPDIL-1
BSA								
GmPDIL-1								GmPDIS-1
BSA								

Figure S3. GmPDIL-1 associates with GmPDIS-1 in the ER. *A*, detection of PDI family protein complexes. Immunoprecipitation (IP). Soybean cotyledons (were frozen in liquid nitrogen and homogenized using a Dounce homogenizer at 4° C in 20 mM HEPES buffer (pH 7.2) containing 150 mM NaCl, 1% digitonin, and 1% protease inhibitor cocktail (Sigma-Aldrich). The homogenate was placed on ice for 1 h and centrifuged for 30 min at 10,000 × g at 4° C. Immunoprecipitation was performed at 4° C for 1 h with pre-immune serum or antiserum specific to PDI family proteins (39, 44, 51, 52). Immunoprecipitants were collected using protein A-conjugated Sepharose beads (Sigma-Aldrich), washed with 20 mM HEPES buffer (pH 7.2) containing 150 mM NaCl, and subjected to Western blot analysis using antiserum specific to GmPDIL-1 as primary antibodies. The cotyledon extracts (input) and resulting immunoprecipitants were subjected to Western blot with anti-GmPDIL-1 serum. *B*, Dot far-Western blot analysis of the association of GmPDIL-1 with GmPDIS-1. Indicated amounts of GmPDIS-1, GmPDIL-1, or bovine serum albumin (BSA) as prey were dot-blotted and incubated with GmPDIL-1 or GmPDIS-1 as bait. Bound GmPDIL-1 or GmPDIS-1 was immuno-stained.

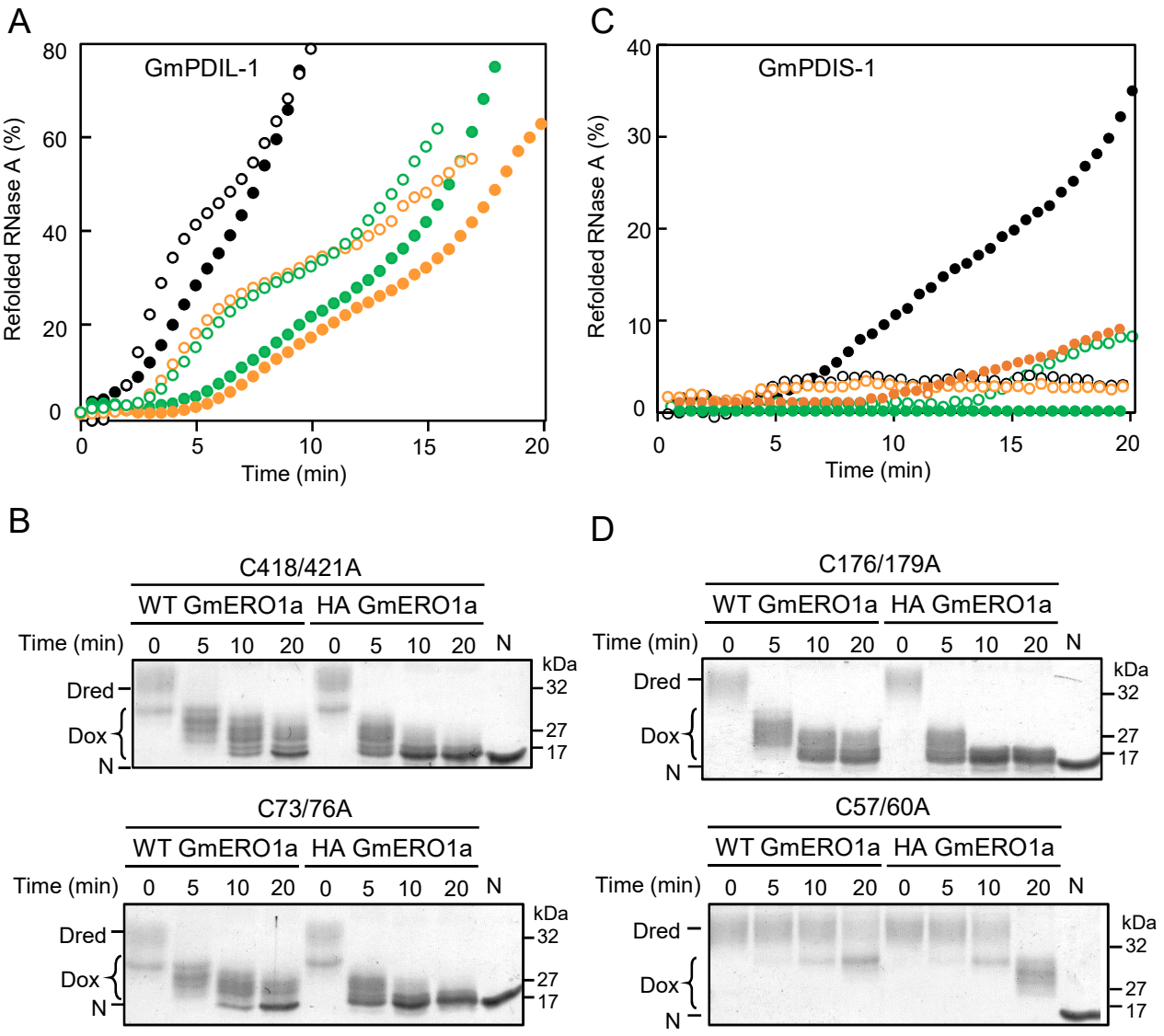


Figure S4. Oxidative refolding of RNase A catalyzed by GmPDIL-1, GmPDIS-1, or respective active-center cysteine mutants in the presence of wild-type (WT) GmERO1a or C121/146A hyperactive (HA) mutant. *A*, Reduced and denatured RNase A (8 μM) was incubated with 3 μM WT GmPDIL-1 (black), domain **a'** (C418/421A) (orange), or domain **a** (C73/76A) (green) active-center cysteine mutant in the presence of 1 μM WT (solid symbols) or HA GmERO1a (open symbols) at 25° C, after which the recovered RNase A activity was assayed. *B*, Formation of disulfide bonds in reduced and denatured RNase A during refolding catalyzed by the GmPDIL-1 C418/421A mutant (upper) or C73/76A mutant (bottom). Reactions were carried out as described in *A* above and quenched with 4'-acetamido-4'-maleimidylstilbene-2,2'-disulfonic acid. Proteins in the reaction mixture were analyzed by non-reducing SDS-PAGE. Dred, reduced and denatured RNase A; Dox, denatured RNase A with non-native disulfides; N, native RNase A. *C*, reduced and denatured RNase A was incubated with WT GmPDIS-1 (black) or domain **a'** active-center cysteine mutant (C176/179A) (orange) or domain **a** active-center cysteine mutant (C57/60A) (green) in the presence of WT (solid symbols) or HA GmERO1a (empty symbols) at 25° C, after which the recovered RNase A activity was assayed. *D*, formation of disulfide bonds in reduced and denatured RNase A during refolding catalyzed by the GmPDIS-1 C176/179A (upper) or C57/60A (bottom) mutant.

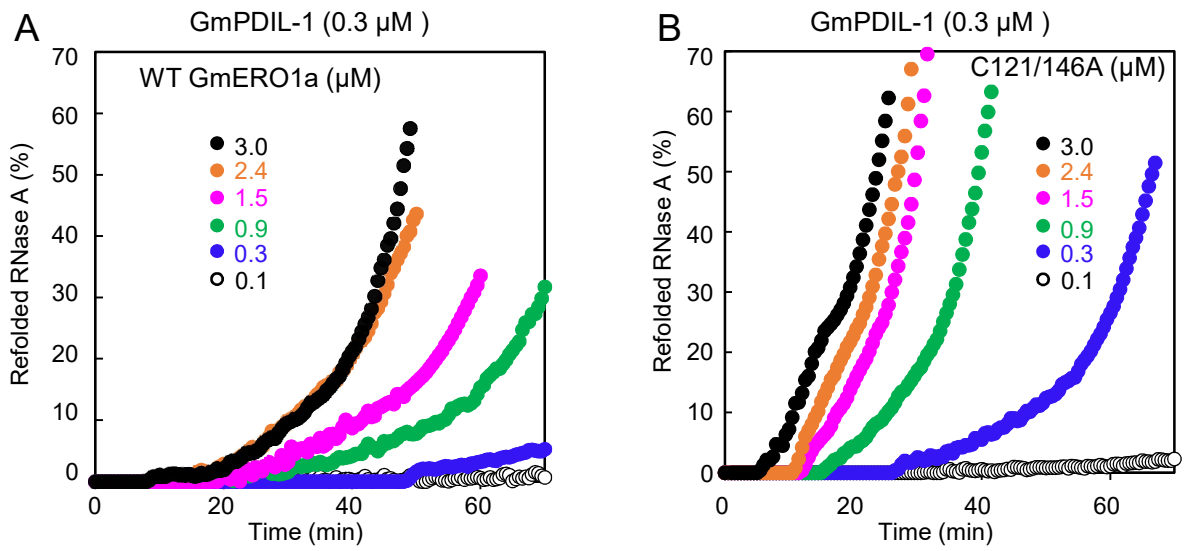


Figure S5. Effect of the loss of regulation of GmERO1a activity on refolding of RNase A catalyzed by GmPDIL-1. *A*, reduced and denatured RNase A (8 μM) was incubated with 0.3 μM GmPDIL-1 in the presence of GmERO1a at the indicated concentrations at 25° C, after which the recovered RNase A activity was assayed. *B*, reduced and denatured RNase A was incubated with GmPDIL-1 in the presence of the C121/146A hyperactive GmERO1a at the indicated concentrations, after which the recovered RNase A activity was assayed.

Table S2
List of primers and template plasmids for PCR of variant preparation.

GmERO1a			
Variant	Forward primer	Reverse primer	Template plasmids
C113A	GGCCTGATGATGGCATGGCTCGGTTGCGGG	CCCGCAACCGAGCCATGCCATCATCAGGCC	GmERO1a WT / pGEX6p-2
C118A	GGTTGCGGGACGCTAGTGTG	CACACTAGCGTCCCGCAACC	GmERO1a WT / pGEX6p-2
C121A	GGTTGCGGGACTGTAGTGTGGCTGAATGCCCTGAAAATGAATTCC	GGAATTCATTTTCAGGGCATTCAGCCACACTACAGTCCCGCAACC	GmERO1a WT / pGEX6p-2
C123A	GGGACTGTAGTGTGTGTGAAGCCCCTGAAAATGAATTCCC	GGGAATTCATTTTCAGGGGCTTCACACACACTACAGTCCC	GmERO1a WT / pGEX6p-2
C146A	CGCCTTTCAATGACTGATCTTGTTGCCCAAGAAGGAAAACC	GGTTTTCCTTCTTGGGCAACAAGATCAGTCATTGAAAGGCG	GmERO1a WT / pGEX6p-2
C113/146A	CGCCTTTCAATGACTGATCTTGTTGCCCAAGAAGGAAAACC	GGTTTTCCTTCTTGGGCAACAAGATCAGTCATTGAAAGGCG	GmERO1a C113A / pGEX6p-2
C121/146A	CGCCTTTCAATGACTGATCTTGTTGCCCAAGAAGGAAAACC	GGTTTTCCTTCTTGGGCAACAAGATCAGTCATTGAAAGGCG	GmERO1a C121A / pGEX6p-2
C123/146A	GGGACTGTAGTGTGTGTGAAGCCCCTGAAAATGAATTCCC	GGGAATTCATTTTCAGGGGCTTCACACACACTACAGTCCC	GmERO1a C121/146A / pGEX6p-2
C121/123/146A	GGGACTGTAGTGTGGCTGAAGCCCCTGAAAATGAATTCCC	GGGAATTCATTTTCAGGGGCTTCAGCCACACTACAGTCCC	GmERO1a C123/146A / pGEX6p-2
C118/121A	GGTTGCGGGACGCTAGTGTG	CACACTAGCGTCCCGCAACC	GmERO1a C121A / pGEX6p-2
GmPDIL-1			
Variant	Forward primer	Reverse primer	Template plasmids
C73/76A	CCATGGGCTGGCCACGCTAAGAAGCTTGCTCCCGAGTAT	CTTCTTAGCGTGGCCAGCCCATGGAGCGTAGAACTCGACGACGATGAAA	GmPDIL-1 WT / pET46Ek/LIC
C418/421A	CCCTGGGCTGGTCATGCCAAACAGTTGGCTCCAATATTG	CTGTTTGGCATGACCAGCCCAGGGAGCATAAAACTCCAGCAGAACATTC	GmPDIL-1 WT / pET46Ek/LIC
GmPDIS-1			
Variant	Forward primer	Reverse primer	Template plasmids
WT(full)	CGCGTACGAATTCCCGACGACGTCGTTGTG	CGCGCATCTCGAGCTCAAGCCGCATATGTC	GmPDIS-1 full / pET46Ek/LIC
C57/60A	TATGCTCCTTGGGCTGGACACGCCAAAAAGCTTGCTCCAGAATATGAAAAG CTTGGTAGC	GGAGCAAGCTTTTTGGCGTGTCCAGCCCAAGGAGCATAGAACTCAACGAGAGCTC C	GmPDIS-1 full / pGEX6p-2
C176/179A	TATGCACCCTGGGCTGGACATGCCAAAAGTCTTGCTCCTACTTACGAGAAA GTTGCC	GGAGCAAGACTTTTGGCATGTCCAGCCCAGGGTGCATAAAACTCAACCAAGACAT C	GmPDIS-1 full / pGEX6p-2
GmPDIS-2			
Variant	Forward primer	Reverse primer	Template plasmids
WT(full)	CGAATCGGATCCGACGACGTCGTTGCAC	CGCGCATCTCGAGCTCAAGCAAAGATAGATAAG	GmPDIS-2 full / pET46Ek/LIC
C56/59A	TACGCTCCCTGGGCTGGACACGCCAAAAGGCTTGCCCCGGAGTACGAACA GCTC	GGCAAGCCTTTTGGCGTGTCCAGCCCAGGGAGCGTAAAACTCAACGAGAGCGGC	GmPDIS-2 full / pGEX6p-2
C175/178A	TATGCACCATGGGCTGGTCATGCCAAGGCCCTTGCCCCTATTTATGAAAAA GTTGC	GGCAAGGGCCTTGGCATGACCAGCCCATGGTGCATAGAATTCCACCAGAACATC	GmPDIS-2 full / pGEX6p-2

	Forward primer	Reverse primer
E. coli Trx1	GACGACGACAAGATGAGCGATAAAATTATTCACC	GAGGAGAAGCCCGGTTACGCCAGGTTAGCGTCGAG