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"Improved production of Humira antibody in the genetically engineered *Escherichia coli* SHuffle, by co-expression of human PDI-GPx7 fusions."

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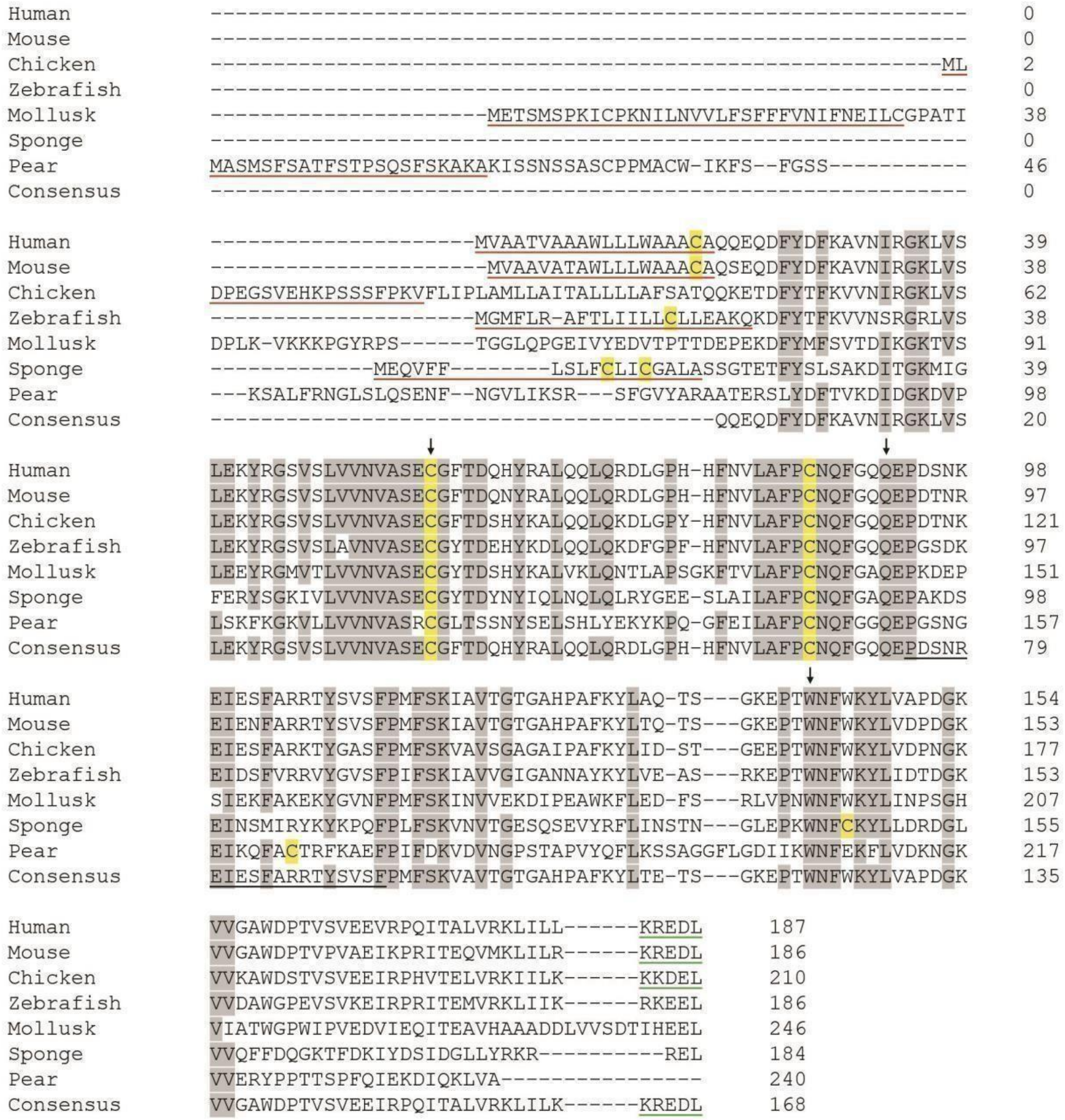

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Human       ------------------------------------------------------------   0
Mouse       ------------------------------------------------------------   0
Chicken     ----------------------------------------------------------ML   2
Zebrafish   ------------------------------------------------------------   0
Mollusk     ----------------------METSMSPKICPKNILNVVLFSFFFVNIFNEILCGPATI   38
Sponge      ------------------------------------------------------------   0
Pear        MASMSFSATFSTPSQSFSKAKAKISSNSSASCPPMACW-IKFS--FGSS-----------   46
Consensus   ------------------------------------------------------------   0

Human       ---------------------MVAATVAAAWLLLWAAACAQQEQDFYDFKAVNIRGKLVS   39
Mouse       ----------------------MVAAVATAWLLLWAAACAQSEQDFYDFKAVNIRGKLVS   38
Chicken     DPEGSVEHKPSSSFPKVFLIPLAMLLAITALLLLAFSATQQKETDFYTFKVVNIRGKLVS   62
Zebrafish   ---------------------MGMFLR-AFTLIILLCLLEAKQKDFYTFKVVNSRGRLVS   38
Mollusk     DPLK-VKKKPGYRPS------TGGLQPGEIVYEDVTPTTDEPEKDFYMFSVTDIKGKTVS   91
Sponge      -------------MEQVFF--------LSLFCLICGALASSGTETFYSLSAKDITGKMIG   39
Pear        ---KSALFRNGLSLQSENF--NGVLIKSR---SFGVYARAATERSLYDFTVKDIDGKDVP   98
Consensus   ----------------------------------------QQEQDFYDFKAVNIRGKLVS   20
                             ↓                                   ↓
Human       LEKYRGSVSLVVNVASECGFTDQHYRALQQLQRDLGPH-HFNVLAFPCNQFGQQEPDSNK   98
Mouse       LEKYRGSVSLVVNVASECGFTDQNYRALQQLQRDLGPH-HFNVLAFPCNQFGQQEPDTNR   97
Chicken     LEKYRGSVSLVVNVASECGFTDSHYKALQQLQKDLGPY-HFNVLAFPCNQFGQQEPDTNK   121
Zebrafish   LEKYRGSVSLAVNVASECGYTDEHYKDLQQLQKDFGPF-HFNVLAFPCNQFGQQEPGSDK   97
Mollusk     LEEYRGMVTLVVNVASECGYTDSHYKALVKLQNTLAPSGKFTVLAFPCNQFGAQEPKDEP   151
Sponge      FERYSGKIVLVVNVASECGYTDYNYIQLNQLQLRYGEE-SLAILAFPCNQFGAQEPAKDS   98
Pear        LSKFKGKVLLVVNVASRCGLTSSNYSELSHLYEKYKPQ-GFEILAFPCNQFGGQEPGSNG   157
Consensus   LEKYRGSVSLVVNVASECGFTDQHYRALQQLQRDLGPH-HFNVLAFPCNQFGQQEPDSNR   79
                                                           ↓
Human       EIESFARRTYSVSFPMFSKIAVTGTGAHPAFKYLAQ-TS---GKEPTWNFWKYLVAPDGK   154
Mouse       EIENFARRTYSVSFPMFSKIAVTGTGAHPAFKYLTQ-TS---GKEPTWNFWKYLVDPDGK   153
Chicken     EIESFARKTYGASFPMFSKVAVSGAGAIPAFKYLID-ST---GEEPTWNFWKYLVDPNGK   177
Zebrafish   EIDSFVRRVYGVSFPIFSKIAVVGIGANNAYKYLVE-AS---RKEPTWNFWKYLIDTDGK   153
Mollusk     SIEKFAKEKYGVNFPMFSKINVVEKDIPEAWKFLED-FS---RLVPNWNFWKYLINPSGH   207
Sponge      EINSMIRYKYKPQFPLFSKVNVTGESQSEVYRFLINSTN---GLEPKWNFCKYLLDRDGL   155
Pear        EIKQFACTRFKAEFPIFDKVDVNGPSTAPVYQFLKSSAGGFLGDIIKWNFEKFLVDKNGK   217
Consensus   EIESFARRTYSVSFPMFSKVAVTGTGAHPAFKYLTE-TS---GKEPTWNFWKYLVAPDGK   135

Human       VVGAWDPTVSVEEVRPQITALVRKLILL------KREDL   187
Mouse       VVGAWDPTVPVAEIKPRITEQVMKLILR------KREDL   186
Chicken     VVKAWDSTVSVEEIRPHVTELVRKIILK------KKDEL   210
Zebrafish   VVDAWGPEVSVKEIRPRITEMVRKLIIK------RKEEL   186
Mollusk     VIATWGPWIPVEDVIEQITEAVHAAADDLVVSDTIHEEL   246
Sponge      VVQFFDQGKTFDKIYDSIDGLLYRKR----------REL   184
Pear        VVERYPPTTSPFQIEKDIQKLVA----------------   240
Consensus   VVGAWDPTVSVEEIRPQITALVRKLILK------KREDL   168
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Figure S1. Sequence alignment of the candidate GPx7 proteins. The alignment was obtained using ClustalW and manually edited. Amino acids whose identify threshold is above 80% are shaded in gray. Cysteines are highlighted in yellow, signal peptide is underlined in red, dimer interface is underlined in black, arrows point at catalytic residues determined from Conserved Domain Database (https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) and the classical C-terminal ER retention signal sequence KDEL for lumen bound proteins is underlined in green.

Table S1. Primers used in construction of plasmids.

Name	Sequence (5’ to 3’)
NK1012-flag-r	caagcttgGTCGACTTActtgtcatcgtcatccttgtaatcgatgtcatg
NK1013-c-f	GAAAAAACGTGAAGACCTGgactacaaagaccatgacggtgattataaagat
NK1014-pxb-f	CTGGTTGCGGCGgactacaaagaccatgacggtgattataaagat
NK1015-aqu-f	GCGCCGTGAACTGgactacaaagaccatgacggtgattataaagat
NK1016-lak-f	CATTCACGAGGAGCTGgactacaaagaccatgacggtgattataaagat
NK1017-dre-f	CGCAAGGAAGAGCTGgactacaaagaccatgacggtgattataaagat
NK1018-gga-f	CCTGAAGAAGAAAGATGAACTGgactacaaagaccatgacggtgattataaagat
NK1019-mmu-f	GTAAGCGTGAAGACCTGgactacaaagaccatgacggtgattataaagat
NK1020-has-f	GAAACGTGAGGACCTGgactacaaagaccatgacggtgattataaagat
5’-nsGpx7 (NcoI)	ggtCCATGGccCAGCAGGAGCAGGACTTC
3’-nsGpx7 (HindIII)	ggtAAGCTTATAAGTCTTCTCGCTTCAGTAG

Table S2. Construction of plasmids used in this study.

Plasmid	Template	Primer pair	Cloning method
pACYC Duet - PDI-hsa Gpx7 - Flag	pACYC Duet - PDI-hsa Gpx7*	NK1012-FLAG-r NK1020-has-f	NEBuilder® HIFI DNA (NEB, cat. No. E5520)
pACYC Duet - PDI-mmu Gpx7 - Flag	pACYC Duet - PDI-mmu Gpx7*	NK1012-FLAG-r NK1019-mmu-f	
pACYC Duet - PDI-lak Gpx7 - Flag	pACYC Duet - PDI-lak Gpx7*	NK1012-FLAG-r NK1016-lak-f	
pACYC Duet - PDI-aqu Gpx7 - Flag	pACYC Duet - PDI-aqu Gpx7*	NK1012-FLAG-r NK1015-aqu-f	
pACYC Duet - PDI-dre Gpx7 - Flag	pACYC Duet - PDI-dre Gpx7*	NK1012-FLAG-r NK1017-dre-f	
pACYC Duet - cPDI-Gpx7 - Flag	pACYC Duet - cPDI-Gpx7*	NK1012-FLAG-r NK1013-c-f	
pACYC Duet - PDI-gga Gpx7 - Flag	pACYC Duet - PDI-gga Gpx7*	NK1012-FLAG-r NK1018-gga-f	
pACYC Duet - PDI-pxb Gpx7 - Flag	pACYC Duet - PDI-pxb Gpx7*	NK1012-FLAG-r NK1014-pxb-f	