

SUPPLEMENTARY MATERIAL FOR THE PAPER

MOLECULAR BASIS FOR THE REDUCED CATALYTIC ACTIVITY OF THE NATURALLY OCCURRING T560M MUTANT OF HUMAN 12/15-LIPOXYGENASE THAT HAS BEEN IMPLICATED IN CORONARY ARTERY DISEASE

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Running head: mutant 12/15-LOX

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Table S1 Partial sequence alignment of LOX isoforms

Species and isoform (if known or predicted)	T560 (or S)	Q294	N287
human 12/15LOX	SWVPNAPCTMRLP	-CSQCHLAAP	QAQLEKELEGGTLFEADFSLLDGIKAN----VIL
rattus 12/15LOX	YWVPNAPCTMRLP	-CSQCYLAAP	QAQLNKELQKGTLFADFFLLDGIKAN----VIL
mus musculus 12/15LOX	YWVPNAPCTMRLP	-CSQCYLAAP	QAQLDEELKKGTLFEADFFLLDGIKAN----VIL
pan troglod. 12/15LOX	SWVPNAPCTMRLP	-CSQCHLAAP	QAQLEKELEGGTLFEADFSLLDGIKAN----VIL
pongo 12/15LOX	SWVPNAPCTMRLP	-CSQCHLAAP	QAQLEKELEGGTLFEADFSLLDGIKAN----VIL
macaca 12/15LOX	TWVPNAPCTMRLP	-CSQCYLAAP	QAQLEKELEGGTLFEADFSLLDGIKAN----VIL
sus scrofa 12/15LOX	TWVPNAPCTMRLP	-CSQCYLAVP	QAQLEKELQGGTLFEADFSLLDGIKAN----VIL
bos taurus 12/15LOX	SWVPNAPCTMRLP	-CTQCYVAAP	QAELKELQGGTLFEADFSLLMGIKAN----VIL
equus 12/15LOX	AWVPNAPCTMRLP	-CSQCYLAAP	QAQLEKELQGGMLFEADFSLLDGIKAN----VIL
canis 12/15LOX	TWVPNAPCTMRLP	-CSQCYLAAP	QIQLEQELQGGRLFADFFVLLDGIKAN----VIL
oryctolagus 12/15LOX	TWVPNAPCTMRLP	-YCQCYLAAP	QAQLEKELKAGTLFEADFALLDNIKAN----VIL
oryctolagus 12LOX	TWVPNAPCTMRLP	-YCQCYLAAP	QAQLEKELKAGTLFEADFALLDNIKAN----VIL
mus musculus 12LOX	GWVPNAPCTMRMP	-GEPQCYLAAP	QAQLEKELKNGSLFEADFILLDGIIPAN----VIR
rattus 12LOX	GWVPNAPCTMRMP	-GEPQCYLAAP	QAQLEKELKDGCLFEADFILLDGIIPAN----VIR
human 12LOX	AWVPNAPCTMRMP	-GEPQCYLAAP	QAQLEKELQNGSLFEADFILLDGIIPAN----VIR
pan troglod. 12LOX	AWVPNAPCTMRMP	-CRNSDIAWP	RAQLEKELQ-----VPLLPLPGTVLS----LVV
canis 12LOX	AWVPNAPCTMRMP	-GEPQCYLAAP	RAQLERELQNGSLFEADFILLDGLPTV----VIR
bos taurus 12LOX	AWVPNAPCTMRMP	-GEPQCYLAAP	RAQLERELQNGSLFEADFILLDGIIPAN----VIR
danio rerio 12LOX	AWVPNTFCTMRHP	-NSPQCYIAAP	TTTLNBEELKKGNI FLVDHAILDGIIPAN----VIR
Danio rerio5	AWVPNTFCTMRHP	-NSPQCYIAAP	TTTLNBEELKKGNI FLVDHAILDGIIPAN----VIR
rattus 15LOX2	VWMPNLPSPMQLP	-GKPCFSAAP	GTSLQAELEKGSFLVLDHGILSGVQTN----VIN
mus musculus 8LOX	VWMPNLPSPMQLP	-GKPCFSAAP	GTSLQAELEKGSFLVLDHGILSGVHTN----ILN
pan troglod. 15LOX2	AWMPNLPSPMQLP	-GKPCFSAAP	GTSLQAELEKGSFLVLDHGILSGIQTN----VIN
human 15LOX2	AWMPNLPSPMQLP	-GKPCFSAAP	GTSLQAELEKGSFLVLDHGILSGIQTN----VIN
macaca 15LOX2	AWMPNLPSPMQLP	-GKPCFSAAP	GTSLQAELEKGSFLVLDHGILSGIHTN----VIN
equus 15LOX2	AWMPNLPSPMQLP	-GRPCFSAAP	GTSLQAELEKGSFLVLDHGILSGVHTN----VIN
bos taurus 15LOX2	AWMPNLPSPMQLP	-GRPCFSTAP	GTSLQAELEKGSFLVDYDLLAGVRTN----VIN
canis 15LOX2	VWMPNLPSPMQLP	-GKPCFSAAP	GTSLQAELEKGSFLVLDHGILCGVRTN----LIN
human 12RLOX	AWMPNFPASMRNP	-GRKHHCAP	GTCLQAELEKGNIFLADYRIMEGIPTV----ELS
pan troglodyt. 12RLOX	AWMPNFPASMRNP	-GRKHHCAP	GTCLQAELEKGNIFLADYRIMEGIPTV----ELS
canis 12RLOX	AWMPNFPSSMRNP	-GRKHHCAP	GTCLQAELEKGNIFLADYRILEGIPTI----ELN
bos taurus 12RLOX	AWMPNFPSSMRNP	-GQKCYHCAP	GTCLQAELEKGNIFLADYRILEGIPTI----ELN
mus musculus 12RLOX	SWMPNFPSSMRNP	-GQKHHCAP	GTCLQAELEKGNIFLADYRILDGIPTV----ELN
rattus 12RLOX	SWMPNFPSSMRNP	-GQKHHCAP	GTCLQAELEKGNIFLADYRILDGIPTV----ELN
equus eLOX3	AWMPNAPSSMRQP	-GSQCYVAAP	ATCLQTELERGNIFLADYWILAEVPPVH----CLN
bos taurus eLOX3	AWMPNAPSSMRQP	-GHPQCYVAAP	GTCLQTELERGNIFLADYWILAEVPPVH----CLN
human eLOX3	AWMPNAPSSMRQP	-GRQCYVAAP	DTCLQTELERGNIFLADYWILAEAPTH----CLN
canis eLOX3	AWMPNTPSSMRQP	-GRPCYVAAP	NTCLQTELERGHIFLADYWILEEAPVH----CLN
mus musculus eLOX3	AWMPNAPSSMRQP	-GLQCYVTAP	GTCLQTELERGHIFLADYWILAEAPVH----CLN
rattus eLOX3	AWMPNAPSSMRQP	-GRQCYVTAP	GTCLQTELERGHIFLADYWILAEAPVH----CLN
gallus eLOX3	AFLPNAPSSMRHP	-GRQCYVAAP	GTTLDKEMKEGRIFIVDYEVLLQGIPTV----TIH
Xenopus tropicalis1	AWMPNPGPSTMRKP	-GERQCYLAAP	STTLQAELEKGNIFLADYKILEGIPTV----VIN
Xenopus tropicalis2	AWMPNPGPSTMRKP	-GERQCYLAAP	STTLQAELEKGNIFLADYKILEGIPTV----VIN
Xenopus laevis2	AWMPNAPSTMRKP	-GERQCYIAAP	STTLQAELEKGNIFLADYKILEGIPTV----VIN
Xenopus laevis1	SWMPNPGPSTMRKP	-GETQCYLAAP	STTLQAELEKGNIFLADYKILEGIPTV----VIN
Ambystoma mexicanum	SWMPNPGPSTMRQP	-GKQCYMAAP	STSLEKELQKGNIFIVDHKMMEGLRAN----VLN
Salmo salar2	GWMPNTPFISLQQP	-RKKQCYMAAP	SSSLLRELEQSGTIFLLDYKNLDGLKAN----VIN

Tetraodon nigroviridis2	GWPNSPISLQOP	-GKQYLMAP	VAICLGFAQKGNIFLCLDYKNLDGIKA	----TIN
Danio rerio2	LCIPNTPATMSRP	-GKQTYLSAP	GSSLDQEMEKGRVYLLDYEVLDQLPA	----TVN
Danio rerio3	LCIPNTPATMSRP	-GKQTYLSAP	GSSLDQEMEKGRVYLLDYEVLDQLPA	----TVN
Danio rerio4	LCIPNTPATMSRP	-GKQTYLSAP	GSSLDQEMEKGRVYLLDYEVLDQLPA	----TVN
Danio rerio1	LCIPNTPATMSRP	-GKQTYLSAP	GSSLDQEMEKGRVYLLDYEVLDQLPA	----TVN
bos taurus 5LOX	SWIPNAPPTMRAP	-CTLOFLAAP	QLTLEQEIEQGNIFIVDFKLLDGDIDAN	----KTD
sus scrofa 5LOX	SWIPNAPPTMRAP	-CTLOFLAAP	QLTLEQEVEQGNIFIVDFELLDGDIDAN	----KTD
human 5LOX	SWIPNAPPTMRAP	-CTLOFLAAP	QLSLEQEVQGNIFIVDFELLDGDIDAN	----KTD
macaca 5LOX	SWIPNAPPTMRAP	-CTLOFLAAP	QLSLEQEVQGNIFIVDFELLDGDIDAN	----KTD
equus 5LOX	SWIPNAPPTMRAP	-CTLOFLAAP	-----QGNIFIVDFELLDGDIDAN	----KTD
mus musculus 5LOX	SWIPNAPPTMRAP	-CTLOFLAAP	QLSLEQEVQGNIFIVDYELLDGDIDAN	----KTD
rattus 5LOX	SWIPNAPPTMRAP	-CTLOFLAAP	QLSLEQEVQGNIFIVDYELLDGDIDAN	----KTD
Mesocricetus auratus	SWIPNAPPTMRAP	-CTLOFLAAP	HLSLEQEVQGNIFIVDYELLDGDIDAN	----KTD
Xenopus tropicalis3	SWIPNSPPTMRQP	-CTMOYIAAP	NLTLEQEIEKGNIFIVNYELLDGISAN	----KTD
danio rerio 5LOX	SWIPNSPPTMRKP	-CTLOFLAAP	GLTLEELKEGNIFIADYEIMDGVTA	----STD
Salmo salar1	SWIPNAPPTMRKP	-CTLOFLAAP	EMTLEEEIKAGNIYLADYELMEDISP	----STD
Danio rerio6	AWVPNTPSTMRKP	-STROYLAAP	GTTLQEELQAGNIYIADYEILEDVQPN	----DTD
Danio rerio7	AWIPNTPSTMRKP	-STROYLAAP	GTTLQEELKAGNIYIADYEILEDVQPN	----DTD
Tetraodon nigroviridis1	SWVPNTPSTMRRP	-RTQOYIAAP	GLTLEEEIQAGNIYMADFELLDGVKPN	----DTD
Tetraodon nigroviridis3	SILPNYSLLLRKP	-GKPLHVTGP	GSSLQDEMOKGNIFLFDKAIMDGAPVR	----DYD
Branchiostoma floridae8	GFVFNAPLSLRP	-GNFYFAAPL	--TLSEIEIAGHIYIVDCGILDGLQON	----TSR
Branchiostoma floridae10	SFIPNAPWALMQP	-NVPYFTT--	--TLRQAVVAGNVYLYADYAILDGLIAG	----NVN
Branchiostoma floridae9	TCLPNMPLALRQY	-TEVFYHA--	GWSLRQEIAGHMYVDCSLMKGLSPN	----VLG
Clavularia viridis	GFTPNAPAILRQP	EADVRYCA--	--TLKEEIEDGHIYLADFKILEGARC	----GGP
Gersemia fruticosa2	GFTPNAPSILRQP	NADVRYCA--	--TLQEEIKDGNIYLADFEQLVGA	----GGP
Plexaura homomalla1	GFTPNAPAVLRHP	EADIRYCA--	--NLDEEIKDGHYIVDFKVLVGA	----GGP
Gersemia fruticosa1	GPHNSPFLMRQP	-EDKRYIT--	--TLEKAMKEGLIFITDYKILEGIPTM	----DTP
Nematostella vectensis2	LFMPNAPCCLRHP	KDKERYMA--	--SLYREMKDGHYIVDYKILEEIPRH	----MOG
Myxococcus xanthus	AFVFNAPLSMRRE	VEERRWAP--	--TLARALDAKRIFLIDFEILDDIRMY	----RKV
Branchiostoma floridae7	AFPPNYPAFMHGE	-----MTAP	-----LKNVYKKNLGGPPK	-----
Nematostella vectensis1	AFPPNYPAFMRGK	----RLISKP	GMTLEEAIAKRIYICNLITLSDIVCR	----FN
Branchiostoma floridae1	GFPPNYPAYLHGQ	---GRQLCCP	GRSLDDALQANRIFMVNYKVMEDLDLE	----TSD
Trichoplax adhaerens1	AFPPNYPFMRGK	---BKLVNPN	GFTLDEALAKKRLIFICDLTLMLE	----IPV
Trichoplax adhaerens3	GFPPNYPAFMNGK	---DKELVHP	GYTLDDAVSKNKIFLCLDLSMLHSV	----PLV
Branchiostoma floridae2	GFVFNMPLILEGN	---PRPMCAP	GLTLQKAIHDKRLYIVDHTIMKGT	----SFANTAH
Branchiostoma floridae3	GFVFNMPLILEGN	---PRPMCAP	GLKLDKAIKKRRLYIVDHSIMKITS	----SFGNKTA
Branchiostoma floridae5	GFVFNMPLVLEGE	---PRPMCAP	GLKLEDALQKSRLYYVDHTIMGIT	----SMANKRT
Branchiostoma floridae4	GFVFNMPLMLEGN	---NRPMCAP	GLSLDGAIAKKRRLYIVDHTDMKIAS	----FAS
Branchiostoma floridae6	AFVFNMPLLEGN	-----MCA	-----	-----
Aspergillus flavus1	---AASGSPRSA	---RWTAA	---LAVLHQEGRFLANHSYQAAYPKTPG	-----
Aspergillus flavus2	ARGVGIPSPERGA	---RWTAA	---LAVLHQEGRFLANHSYQAAYPKTPG	-----
Magnaporthe	-----	---RYSA	-----	-----
Botryotinia fuckeliana	AFVFNKPSALFAP	---RFGCAS	---EGER--QGGLYVQDCSYFRK	----FAGVEEGEPLV
Sclerotinia sclerotiorum	AFIPNKPPALFTS	---RFACAS	---EGER--DGGLYVQDCSFRK	----FAGVEEGEPLV
Aspergillus fumigatus1	SFVVNKPPCLYTE	---HRYGCAS	---DLRNSCRKSLYMQDYSYFRKA	----AAGLDPTAVIK
Aspergillus fumigatus2	SFVVNKPPCLYTE	---HRYGCAS	---DLRNSCRKSLYMQDYSYFRKA	----AAGLDPTAVIK
Neosartorya fischeri	SFVVNKPPCLYAE	---YRYGCAS	---DLRNSCRGSLYMQDYSYFREA	----AAGLDPTAVIK
Aspergillus ochraceus	-----	-----	-----	-----
Nectria haematococca	SFVPAKPPALCTA	GKAERYACAP	---SDK-----DILIQDYSYFRKAT	----GLKDEENFI
Podospira anserina	VFVIAKPPCLCSP	-----WGCAA	---TIDP---ATLYVQDARNIRRS	----LGVENEILF
Pseudomonas aeruginosa1	TYAPAICAMSAAP	LTGTGFAYAP	--SLLEAAASRRLYLLDYAELGKLAPSG	-----
Pseudomonas aeruginosa3	TYAPAICAMSAAP	LTGTGFAYAP	--SLLEAAASRRLYLLDYAELGKLAPSG	-----
Pseudomonas aeruginosa5	TYAPAICAMSAAP	LTGTGFAYAP	--SLLEAAASRRLYLLDYAELGKLAPSG	-----
Pseudomonas aeruginosa2	TYAPAICAMSAAP	LTGTGFAYAP	--SLLEAAASRRLYLLDYAELGKLAPSG	-----
Pseudomonas aeruginosa4	TYAPAICAMSAAP	LTGTGFAYAP	--SLLEATASRRLYLLDYAELGKLAPSG	-----
Burkholderia thailandensis	TYAPALSALLSAP	LTGIGYSYAP	--NLVSAANRRLYLLDYVNDGLLATS	-----SR
Shewanella denitrificans	SFAPALGTANWCE	QAVDKRVFAP	--SLAQALKDKRLYIVDYKELSVLVDH	-----
Photobacterium profundum	SFAPAVTGAIWCK	QTPEKQVFAP	--SLAEALAESRVFLLDYHELKVLVDN	-----
Plesiocystis pacifica1	SFTPAMPLASYAP	PAGRKYVSPG	---LADALAQRVYVADWAPLTA	----TAMTPG
Acaryochloris marina1	TYIPNLPLAGYRP	-DAQKYVYAP	-DSLQKAGAEGRLYITDYAVLNLL	----LEPG
Acaryochloris marina3	TFVPMPLAGFNE	REIPKYRYAP	-ESLQALNDGRVYLYADYQILEE	----IDAG
Acaryochloris marina2	VYTPGMPLAGYQP	PDQKQYISAP	-DSLQIAITEGRLYIADYAILLAGA	----ING
Nostoc punctiforme	GYAAAIPLAGYLP	PHEQKYLYAP	-DSLAAAGIEGRLYLADYKILDGA	----ING
Synechococcus sp	SFAPAMPTAGYLP	PEQKQYLYAA	-DDLERAITEGRLYAVDYALLESV	----IEGGT
Cyanothece sp1	TYVPGFPLARYSP	LVQKQYICAP	VDSLAEVIESQRLYVSDYGILNSLVNG	-----
Plesiocystis pacifica2	AYAPAFPLAQYAP	PAAQKYISAP	GDTLAAALAEGRFTCDYAGAE	----GIVAG
Cyanothece sp2	AFIPNMPLATYAT	VQTKTIAAP	--TLAEATAQKGLFITNYKGLAELS	----PGRY
Cyanothece sp3	AFIPNMPLATYAT	VQTKTIAAP	--TLAEATAQKGLFITNYKGLAELS	----PGRY
Microcystis aeruginosa	AFSPNIPLAGYQS	---MKTVTAP	--TIASEAAKGNLFLADYAPLHHL	----TLTG
Plesiocystis pacifica3	SFIPSVSGTAYKA	FGRQRYLSAP	-LTLEQAVSAKRLYVCDYSMFAG	----IEAGE
Shewanella woodyi	SYLPSVSGTLYAK	HEKQRYPHAP	-LTLSQAADQGLLYVCDYAMFAG	----LKGSE
Nematostella vectensis3	AYTSPMPTKIYDD	R---MWWPKSF	-APVSMIINDGSVFCVQPEFDNL	----PTVPD
Plexaura homomalla2	GFVFNLPKTYLDD	RREMRGRSAP	-STFSEMIDAGRVYIVEFSYLYD	----LSNVED
Ostreococcus lucimarinus	SYCPNRPLIMRKP	---QGYCVEN	GKTLSELAAGHIFMVYELLEDIKRG	-----

Trichoplax_adhaerens2	TFLPNMPGKIYNV	GRTMMLMSP	SNTFVDAVQDGRVYVSEFPELGRGIRTSDFEELP
Dictyostelium_discoideum	GFTPNVPGALYMD	KKRGRYISSP	--SNFQKMLDNGNLYMVDYSIFDDVNYYES-----
Branchiostoma_floridae1	GYPNRPPEMLKPK	--EGKYFYAP	--TVLELISERRLFIVDYNILRKIPQQ-----
Phytophthora_infestans	AAPFSAPALYNST	-NKTESKYAP	DPTVDAAISNHRVVFVQDFSSMGQYTDSE-----
Acaryochloris_marina4	GDVSYSTIGLWDT	PGKLN RV TGH	DPDLPELGITGALDLMGTSVVSYPNLTTRTRLD

Fig. S1. Expression of human ALOX15 mutants. ALOX15 species were expressed as his-tag fusion proteins in *E. coli*. After cell disruption the lysis supernatant was purified on an open bed Ni-TED column, LOX protein was eluted with 200 mM imidazole and five fractions (E1, E2...) were collected. Aliquots of the fractions were analyzed by immunoblotting using an anti-his-tag antibody and densitometrically quantified. The factors for achieving the same amount of wild-type enzyme are given.

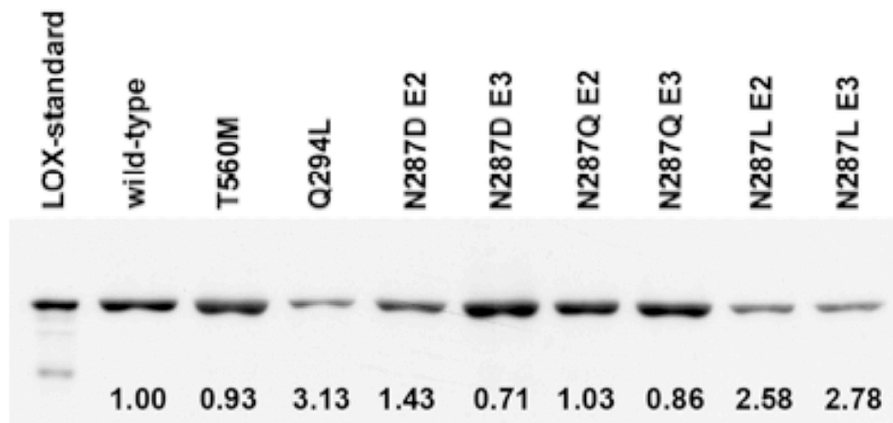


Fig. S2 Composition of the reaction products formed from arachidonic acid by the Q294L mutant of ALOX15. Enzyme preparation, activity assays and product analysis were carried out as described in Materials and Methods of the main ms. 4.6 μ g of purified enzyme were used for this activity assay.

