
Supplementary Information

Cytochrome P450 surface domains prevent the β -carotene monohydroxylase CYP97H1 of *Euglena gracilis* from acting as a dihydroxylase.

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Table S1. Protein sequences of the redox partner and the cytochrome P450 variants, truncated forms and the chimeras. The *E. coli* codon optimized sequences were cloned on the corresponding plasmid pTLXX.

FER1 Ferredoxin *S. oleracea*

MAAYKVTLVTPTGNVEFQCPDDVYILDAAEEEGIDLPYSCRAGSCSSCAGKLKTGSLNQDDQSFLDDDQIDEGWVL
TCAAYPVSDVTIETHKEEELTA*

Ferredoxin reductase *S. oleracea*

MQIASDVEAPPPAPAKVEKHSKKMEEGITVNKFKPKTPYVGRCLLNTKITGDDAPGETWHMVFSHEGEIPYREGQS
VGVIPDGEDKNGKPHKLRLYSIASSALGDFGDAKSVSLCVKRLIYTNDAGETIKGVCSNFLCDLKPGEVKTGPVG
KEMLMKDPNATIIMLTGTGTGIAPFRSFLWKMFEEKHDDYKFNGLAWLFLGVPTSSSLYKEEFKMKKEKAPDNFRL
DFAVSREQTNEKGEKMYIQTRMAQYAVELWEMLKKDNTFYMCGLKGMEKGIDDIMVSLAAAEIDWIEYKRQL
KKAQWNVVEVY*

pTL45: bov-CYP97H1

MALLAVFLGLSCLLLSLWGSRRKTPNRSQCLLAFTGEREGLRHQELVPIRNLDINCHGVKTSCTQLQATVEPSPEQ
ESQLPRAEDMAIGVTAQEYVTHYIQNVAQFFVPMKWEDNIPVVSVDIFKWGAVDIPMERLLQSKLTDVFTGGFQDI
TGVPVFILLHRYMALSPIYKLCIGPRSVVVISDAVAVKHILRSEVGKYDKGILAEVLKPIMGKGLIPADTITWLTRRRQL
KPAFHQKWLHDQLTLYSTVGNRLVAFLAARPGQTIDMQERFCSASLDIIGKAVFNIEFGSITRESPVIQAVYAVMRE
AERRASSIVPYWQLPGGTREFDQHMKVLDLDTSLVEQCVQQVSTEEDEEPQKGNNSLLRFLVEARGQDVTNQQL
RDDLMTMLIAGHETTAATLTWALHELTKPENRDFLKRKVAEVDVSLGLRDFITLDDVKQMPLVRYSLVEALRLYP
PPMLIRCLKEDHLTGVPFSGAGMTIKPGQDVMLATWSLNRDQRLWGPADKYNPLRFYTAVHGSPEYKAAGWA
GFDPARVRGLYPDENAADFGFIPFGGGGRKCMGDQFAILESSVLLSMLLRDFSFEAADTVTLGMGATIFAKEGLMM
KVTARPPQPPDQDEASPVAVASDALLSSVA*

pTL68: full-length CYP97H1

MDGDSIACTPFLRAWHWHHGLILVASSMLTIVIINAQAQQLTISSPLKSSTRSTTSVVLGGTQRYRLPPTAGTAVGTV
VAHPNNANPSKARLHGMLVNKAIFLPLLLPIVAGIAWLRRKTPNRSQCLLAFTGEREGLRHQELVPIRNLDINCHG
VKTSTCTQLQATVEPSPEQESQLPRAEDMAIGVTAQEYVTHYIQNVAQFFVPMKWEDNIPVVSVDIFKWGAVDIP
ERLLQSKLTDVFTGGFQDITGVPVFILLHRYMALSPIYKLCIGPRSVVVISDAVAVKHILRSEVGKYDKGILAEVLKPI
GKGLIPADTITWLTRRRQLKPAFHQKWLHDQLTLYSTVGNRLVAFLAARPGQTIDMQERFCSASLDIIGKAVFNIEF
GSITRESPVIQAVYAVMREAERRASSIVPYWQLPGGTREFDQHMKVLDLDTSLVEQCVQQVSTEEDEEPQKGNNSL
LRFLVEARGQDVTNQQLRDDLMTMLIAGHETTAATLTWALHELTKPENRDFLKRKVAEVDVSLGLRDFITLDDVK
QMPLVRYSLVEALRLYPAPPMLIRCLKEDHLTGVPFSGAGMTIKPGQDVMLATWSLNRDQRLWGPADKYNPLR
FYTAVHGSPEYKAAGWAGFDPARVRGLYPDENAADFGFIPFGGGGRKCMGDQFAILESSVLLSMLLRDFSFEAADT
VTLGMGATIFAKEGLMMKVTARPPQPPDQDEASPVAVASDALLSSVA*

pTL70: bov-capless-CYP97H1

MALLLAVFLGLSCLLLSLWGSSPIYKLCIGPRSVVVISDAVAVKHILRSEVGKYDKGILAEVLKPIMGKGLIPADTITWL
LTRRRQLKPAFHQKWLHDQLTLYSTVGNRLVAFLAARPGQTIDMQRFCASLDIIGKAVFNIEFGSITRESPVIQAV
YAVMREAERRASSIVPYWQLPGGTREFDQHMKVLDLDTSLVEQCQVQVSTEEDEEPQKGNNSLRLFLVEARGQD
VTNQQLRDDLMTMLIAGHETTAATLTWALHELTKPENRDFLKRKVAEVDVSLGLRDFITLDDVKQMPLVRYSLVE
ALRLYPAPPMLIRRLCKEDHLTGVGPFSAGMTIKPGQDVMLATWSLNRDQRLWGPADADKYNPLRFYTAVHGSPEY
KAAGWAGFDPARVRGLYPDENAADFGFIPFGGGGRKCMGDQFAILESSVLLSMLLRDFSFEAADTVTLGMGATIFA
KEGLMMKVITARPPQPPDQDEASPVAVASDALLSSVA*

pTL71: supertruncated-CYP97H1

MSPIYKLCIGPRSVVVISDAVAVKHILRSEVGKYDKGILAEVLKPIMGKGLIPADTITWLTRRRQLKPAFHQKWLHDQ
LTLTLYSTVGNRLVAFLAARPGQTIDMQRFCASLDIIGKAVFNIEFGSITRESPVIQAVYAVMREAERRASSIVPYWQL
PGGTREFDQHMKVLDLDTSLVEQCQVQVSTEEDEEPQKGNNSLRLFLVEARGQDVTNQQLRDDLMTMLIAGHET
TAATLTWALHELTKPENRDFLKRKVAEVDVSLGLRDFITLDDVKQMPLVRYSLVEALRLYPAPPMLIRRLCKEDHLT
GVGPFSAGMTIKPGQDVMLATWSLNRDQRLWGPADADKYNPLRFYTAVHGSPEYKAAGWAGFDPARVRGLYPDE
NAADFGFIPFGGGGRKCMGDQFAILESSVLLSMLLRDFSFEAADTVTLGMGATIFAKEGLMMKVITARPPQPPDQDE
ASPVAVASDALLSSVA*

pTL72: tCYP97H1

MGSRRKTPNRSQCLLAFTGEREGLRHQELVPIRNLINCHGVKTSCTQLQATVEPSPEQESQLPRAEDMAIGVTAQE
YVTHYIQNVAQFFVPMKWEDNIPVVSVDIFKWGAVDIPMERLLQSKLTDVFTGGFQDITGVPVFILLHRYMALSPIY
KLCIGPRSVVVISDAVAVKHILRSEVGKYDKGILAEVLKPIMGKGLIPADTITWLTRRRQLKPAFHQKWLHDQLTLYS
TVGNRLVAFLAARPGQTIDMQRFCASLDIIGKAVFNIEFGSITRESPVIQAVYAVMREAERRASSIVPYWQLPGGT
REFDQHMKVLDLDTSLVEQCQVQVSTEEDEEPQKGNNSLRLFLVEARGQDVTNQQLRDDLMTMLIAGHETTAAT
LTWALHELTKPENRDFLKRKVAEVDVSLGLRDFITLDDVKQMPLVRYSLVEALRLYPAPPMLIRRLCKEDHLTGVGP
FSAGMTIKPGQDVMLATWSLNRDQRLWGPADADKYNPLRFYTAVHGSPEYKAAGWAGFDPARVRGLYPDENAAD
FGFIPFGGGGRKCMGDQFAILESSVLLSMLLRDFSFEAADTVTLGMGATIFAKEGLMMKVITARPPQPPDQDEASPV
VASDALLSSVA*

pTL73: capless CYP97H1

MDGDSIACTPFLRAWHWHHGLILVASSMLTIVIINAQAQQLTISSPLKSSTRSTTSVVLGGTQRYRLPPTAGTAVGTV
VAHPNANANPSKARLHGMLVNKAIFLPLLLPIVAGIAWLSPIYKLCIGPRSVVVISDAVAVKHILRSEVGKYDKGILAE
VLKPIMGKGLIPADTITWLTRRRQLKPAFHQKWLHDQLTLYSTVGNRLVAFLAARPGQTIDMQRFCASLDIIGKA
VFNIEFGSITRESPVIQAVYAVMREAERRASSIVPYWQLPGGTREFDQHMKVLDLDTSLVEQCQVQVSTEEDEEPQ
KGNNSLRLFLVEARGQDVTNQQLRDDLMTMLIAGHETTAATLTWALHELTKPENRDFLKRKVAEVDVSLGLRDFI
TLDDVKQMPLVRYSLVEALRLYPAPPMLIRRLCKEDHLTGVGPFSAGMTIKPGQDVMLATWSLNRDQRLWGPAD
KYNPLRFYTAVHGSPEYKAAGWAGFDPARVRGLYPDENAADFGFIPFGGGGRKCMGDQFAILESSVLLSMLLRDFS
FEAADTVTLGMGATIFAKEGLMMKVITARPPQPPDQDEASPVAVASDALLSSVA*

pTL79: M182-CYP97H1

MAIGVTAQEYVTHYIQNVAQFFVPMKWEDNIPVVSVDIFKWGAVDIPMERLLQSKLTDVFTGGFQDITGVPVFILL
HRYMALSPIYKLCIGPRSVVVISDAVAVKHILRSEVGKYDKGILAEVLKPIMGKGLIPADTITWLTRRRQLKPAFHQK
WLHDQLTLYSTVGNRLVAFLAARPGQTIDMQRFCASLDIIGKAVFNIEFGSITRESPVIQAVYAVMREAERRASSI
VPYWQLPGGTREFDQHMKVLDLDTSLVEQCQVQVSTEEDEEPQKGNNSLRLFLVEARGQDVTNQQLRDDLMTML
LIAGHETTAATLTWALHELTKPENRDFLKRKVAEVDVSLGLRDFITLDDVKQMPLVRYSLVEALRLYPAPPMLIRRL
CKEDHLTGVGPFSAGMTIKPGQDVMLATWSLNRDQRLWGPADADKYNPLRFYTAVHGSPEYKAAGWAGFDPARV
RGLYPDENAADFGFIPFGGGGRKCMGDQFAILESSVLLSMLLRDFSFEAADTVTLGMGATIFAKEGLMMKVITARPP
QPPDQDEASPVAVASDALLSSVA*

pTL80: M206-CYP97H1

MKWEDNIPVVSVDIFKWGAVDIPMERLLQSKLTDVFTGGFQDITGVPVFILLHRYMALSPIYKLCIGPRSVVVISDAV
AVKHILRSEVGKYDKGILAEVLKPIMGKGLIPADTITWLTRRRQLKPAFHQKWLHDQLTLYSTVGNRLVAFLAARPG
QTIDMQRFCASLDIIGKAVFNIEFGSITRESPVIQAVYAVMREAERRASSIVPYWQLPGGTREFDQHMKVLDLDT
SLVEQCQVQVSTEEDEEPQKGNNSLRLFLVEARGQDVTNQQLRDDLMTMLIAGHETTAATLTWALHELTKPENRD
FLKRKVAEVDVSLGLRDFITLDDVKQMPLVRYSLVEALRLYPAPPMLIRRLCKEDHLTGVGPFSAGMTIKPGQDVML
ATWSLNRDQRLWGPADADKYNPLRFYTAVHGSPEYKAAGWAGFDPARVRGLYPDENAADFGFIPFGGGGRKCMG
DQFAILESSVLLSMLLRDFSFEAADTVTLGMGATIFAKEGLMMKVITARPPQPPDQDEASPVAVASDALLSSVA*

pTL81: tCYP97H1-fullAt: entire F'-G' loop 26 residues from CYP97A3At

MGSRRKTPNRSQCLLAFTGEREGLRHQELVPIRNLDINCHGVKTSCTQLQATVEPSPEQESQLPRAEDMAIGVTAQE
YVTHYIQNVAQFFVPMKWEDNIPVVSVDIFKWGAVDIPMERLLQSKLTDVFTGGFQDITGVPVFILLHRYMALSPIY
KLCIGPRSVVVISDAVAVKHILRSEVGKYDKGILAEVLKPIMGKGLIPADTITWLTRRRQLKPAFHQKWLHDQLTLYS
TVGNRLVAFLAARPGQTIDMQUERFCSASLDIIGKAVFNIEFGSITRESPVIQAVYAVMREAERRSVSPVWDIPIWKD
ISPRQRKVAQHMKVLDDVLTSLVEQCQVQVSTEEDEEPQKGNNLSLLRFLVEARGQDVTNQQLRDDLMTMLIAGHE
TTAATLTWALHELTKPENRDFLKRKVAEVDVSLGLRDFITLDDVKQMPLVRYSLVEALRLYPAPPMLIRRLCKEDHL
TGVGPFSAAGMTIKPGQDVMLATWSLNRDQRLWGPADADKYNPLRFYTAVHGSPEYKAAGWAGFDPARVRGLYPDE
NAADFGFIPFGGGGRKCMGDQFAILESSVLLSMLLRDFSFEAADTVTLGMGATIFAKEGLMMKV TARPPQPPDQDE
ASPVAVASDALLSSVA*

pTL82: tCYP97H1-6At: 6 missing counterpart residues from CYP97A3At

MGSRRKTPNRSQCLLAFTGEREGLRHQELVPIRNLDINCHGVKTSCTQLQATVEPSPEQESQLPRAEDMAIGVTAQE
YVTHYIQNVAQFFVPMKWEDNIPVVSVDIFKWGAVDIPMERLLQSKLTDVFTGGFQDITGVPVFILLHRYMALSPIY
KLCIGPRSVVVISDAVAVKHILRSEVGKYDKGILAEVLKPIMGKGLIPADTITWLTRRRQLKPAFHQKWLHDQLTLYS
TVGNRLVAFLAARPGQTIDMQUERFCSASLDIIGKAVFNIEFGSITRESPVIQAVYAVMREAERRASSIVPYWQLPGGK
GYQSPSREFDQHMKVLDVLTSLVEQCQVQVSTEEDEEPQKGNNLSLLRFLVEARGQDVTNQQLRDDLMTMLIAGH
ETTAATLTWALHELTKPENRDFLKRKVAEVDVSLGLRDFITLDDVKQMPLVRYSLVEALRLYPAPPMLIRRLCKEDH
LTGVGPFSAAGMTIKPGQDVMLATWSLNRDQRLWGPADADKYNPLRFYTAVHGSPEYKAAGWAGFDPARVRGLYPD
ENAADFGFIPFGGGGRKCMGDQFAILESSVLLSMLLRDFSFEAADTVTLGMGATIFAKEGLMMKV TARPPQPPDQD
EASPVAVASDALLSSVA*

pTL83: tCYP97A3At

MSFPSTVKNGLSKIGIPSNVLDPMFDWTGSDQDYPKVPEAKGSIQAVRNEAFFIPLYELFLTYGGIFRLTFGPKSFLIVS
DPSIAKHILKDNAKAYSKGILAEILDFVMGKGLIPADGEIWRRRRRRAIVPALHQKYVAAMISLFGESDRLCQKLDA
AALKGEEVEMESLFSRLTLDIIGKAVFNIDFDSL TNDTGVI EAVYTVLREAEDRSVSPVWDIPIWKDISPRQRKVATS
LKLINDTLDDLIATCKRMVEEEELQFH E EYMNERDPSILHFLASGDDVSSKQLRDDLMTMLIAGHETSAAVLTWTF
YLLTTEPSVVAKLQEEVDSVIGDRFPTIQDMKKLKYTTRVMNESLRLYPQPPVLIRRSIDNDILGEYPIKRGEDIFISVW
NLHRSPLHWDDAEKFNPERWPLDGPNPNETNQNF SYLPFGGGPRKCIGDMFASFENVVAIAMLIRRFNFQIAPGAP
PVKMTTGATIHTEGLKLTVTKRTPKPLDIPSPILPMDTSRDEVSSALS*

pTL84: Nterm97H1-CYP97A3At

MGSRRKTPNRSQCLLAFTGEREGLRHQELVPIRNLDINCHGVKTSCTQLQATVEPSPEQESQLPRAEDMAIGVTAQE
YVTHYIQNVAQFFVPMKWEDNIPVVSVDIFKWGAVDIPMERLLQSKLTDVFTGGFQDITGVPVFILLHRYMALSFPS
TVKNGLSKIGIPSNVLDPMFDWTGSDQDYPKVPEAKGSIQAVRNEAFFIPLYELFLTYGGIFRLTFGPKSFLIVSDPSIAK
HILKDNAKAYSKGILAEILDFVMGKGLIPADGEIWRRRRRRAIVPALHQKYVAAMISLFGESDRLCQKLDA AALKGE
EVEMESLFSRLTLDIIGKAVFNIDFDSL TNDTGVI EAVYTVLREAEDRSVSPVWDIPIWKDISPRQRKVATSLKLIND
TLDDLIATCKRMVEEEELQFH E EYMNERDPSILHFLASGDDVSSKQLRDDLMTMLIAGHETSAAVLTWTFYLLTTE
PSVVAKLQEEVDSVIGDRFPTIQDMKKLKYTTRVMNESLRLYPQPPVLIRRSIDNDILGEYPIKRGEDIFISVWNLHRSP
LHWDDAEKFNPERWPLDGPNPNETNQNF SYLPFGGGPRKCIGDMFASFENVVAIAMLIRRFNFQIAPGAPPVKMTT
GATIHTEGLKLTVTKRTPKPLDIPSPILPMDTSRDEVSSALS*

pTL88: tCYP97H1 with substrate channel from CYP97AA1

MGSRRKTPNRSQCLLAFTGEREGLRHQELVPIRNLDINCHGVKTSCTQLQATVEPSPEQESQLPRAEDMAIGVTAQE
YVTHYIQNVAQFFVPMKWEDNIPVVSVDIFKWGAVDIPMERLLQSKLTDVFTGGFQDITGVPVFILLHRYMALSPIY
KLCIFGPRSFVVISDAVAVKHILRSEVGKYDKGILAEILKPIMGKGLIPADTITWLTRRRQLKPAFHQKWLHDQLTLYS
TVGNRLVAFLAARPGQTIDMQUERFCSASLDIIGKAVFNIEFGSITRESPVIQAVYAVMREAERRASSIIPYWQLPGGTR
EFDQHMKVLDVLTSLVEQCQVQVSTEEDEEPQKGNNLSLLRFLVEARGQDVTNQQLRDDLMTMLIAGHETTAATL
TWALHELTKPENRDFLKRKVAEVDVSLGLRDFITLDDVKQMPLVRYSLVEALRLYPAPPVLIRRLCKEDHLTGVGPF
SAGMTIKPGQDVFLATWSLNRDQRLWGPADADKYNPLRFYTAVHGSPEYKAAGWAGFDPARVRGLYPDENAADF
GFIPFGGGGRKCMGDQFAILESSVLLSMLLRDFSFEAADTVTLGMGATIFAKEGLMMKV TARPPQPPDQDEASPVAV
ASDALLSSVA*

pTL91: tCYP97H1 F105A

MGSRRKTPNRSQCLLAFTGEREGLRHQELVPIRNLDINCHGVKTSCTQLQATVEPSPEQESQLPRAEDMAIGVTAQE
YVTHYIQNVAQFFVPMKWEDNIPVVSVDIFKWGAVDIPMERLLQSKLTDVFTGGFQDITGVPVFILLHRYMALSPI
YKLCIGPRSVVVISDAVAVKHILRSEVGKYDKGILAEVLKPIMGKGLIPADTITWLTRRRQLKPAFHQKWLHDQLTLY
STVGNRLVAFLAARPGQTIDMQUERFCSASLDIIGKAVFNIEFGSITRESPVIQAVYAVMREAERRASSIVPYWQLPGGT

REFDQHMKVLDDVLTSLVEQCQVQVSTEEDEEPQKGNNSLRFLVEARGQDVTNQQLRDDLMTMLIAGHETTAAT
LTWALHELTKPENRDFLKRKVAEVD SVLGLRDFITLDDVKQMPLVRYSLVEALRLYPAPPMLIRRCLKEDHLTG VGP
FSAGMTIKPGQDVMLATWSLNRDQRLWGP DADKYNPLRFYTAVHGSPEYKAAGWAGFDPARVRGLYPDENAAD
FGFIPFGGGGRKCMGDQFAILESSVLLSMLLRDFSFEAADTVTLGMGATIFAKEGLMMKV TARPPQPPDQDEAS PVA
VASDALLSSVA*

pTL92: tCYP97H1 F108A

MGSRRKTPNRSQCLLAFTGEREGLRHQELVPIRNL DINCHGVKTSCTQLQATVEPSPEQESQLPRAEDMAIGVTAQE
YVTHYIQNVAQFFVPMKWEDNIPVVS VFDIAKWGAVDIPMERLLQSKLTDVFTGGFQDITGVPVFILLHRYMALSPI
YKLCIGPRSVVVISDAVAVKHILRSEVGKYDKGILAEVLKPI MGKGLIPADTITWLTRRRQLKPAFHQKWLHDQLTLY
STVGNRLVAFLAARPGQTIDMQERFCSASLDIIGKAVFN YEFGSITRESPVIQAVYAVMREAERRASSIVPYWQLPGGT
REFDQHMKVLDDVLTSLVEQCQVQVSTEEDEEPQKGNNSLRFLVEARGQDVTNQQLRDDLMTMLIAGHETTAAT
LTWALHELTKPENRDFLKRKVAEVD SVLGLRDFITLDDVKQMPLVRYSLVEALRLYPAPPMLIRRCLKEDHLTG VGP
FSAGMTIKPGQDVMLATWSLNRDQRLWGP DADKYNPLRFYTAVHGSPEYKAAGWAGFDPARVRGLYPDENAAD
FGFIPFGGGGRKCMGDQFAILESSVLLSMLLRDFSFEAADTVTLGMGATIFAKEGLMMKV TARPPQPPDQDEAS PVA
VASDALLSSVA*

pTL93: tCYP97H1 F129A

MGSRRKTPNRSQCLLAFTGEREGLRHQELVPIRNL DINCHGVKTSCTQLQATVEPSPEQESQLPRAEDMAIGVTAQE
YVTHYIQNVAQFFVPMKWEDNIPVVS VFDIFKWGAVDIPMERLLQSKLTDVATGGFQDITGVPVFILLHRYMALSPI
YKLCIGPRSVVVISDAVAVKHILRSEVGKYDKGILAEVLKPI MGKGLIPADTITWLTRRRQLKPAFHQKWLHDQLTLY
STVGNRLVAFLAARPGQTIDMQERFCSASLDIIGKAVFN YEFGSITRESPVIQAVYAVMREAERRASSIVPYWQLPGGT
REFDQHMKVLDDVLTSLVEQCQVQVSTEEDEEPQKGNNSLRFLVEARGQDVTNQQLRDDLMTMLIAGHETTAAT
LTWALHELTKPENRDFLKRKVAEVD SVLGLRDFITLDDVKQMPLVRYSLVEALRLYPAPPMLIRRCLKEDHLTG VGP
FSAGMTIKPGQDVMLATWSLNRDQRLWGP DADKYNPLRFYTAVHGSPEYKAAGWAGFDPARVRGLYPDENAAD
FGFIPFGGGGRKCMGDQFAILESSVLLSMLLRDFSFEAADTVTLGMGATIFAKEGLMMKV TARPPQPPDQDEAS PVA
VASDALLSSVA*

pTL94: tCYP97H1 F133A

MGSRRKTPNRSQCLLAFTGEREGLRHQELVPIRNL DINCHGVKTSCTQLQATVEPSPEQESQLPRAEDMAIGVTAQE
YVTHYIQNVAQFFVPMKWEDNIPVVS VFDIFKWGAVDIPMERLLQSKLTDVFTGGAQDITGVPVFILLHRYMALSPI
YKLCIGPRSVVVISDAVAVKHILRSEVGKYDKGILAEVLKPI MGKGLIPADTITWLTRRRQLKPAFHQKWLHDQLTLY
STVGNRLVAFLAARPGQTIDMQERFCSASLDIIGKAVFN YEFGSITRESPVIQAVYAVMREAERRASSIVPYWQLPGGT
REFDQHMKVLDDVLTSLVEQCQVQVSTEEDEEPQKGNNSLRFLVEARGQDVTNQQLRDDLMTMLIAGHETTAAT
LTWALHELTKPENRDFLKRKVAEVD SVLGLRDFITLDDVKQMPLVRYSLVEALRLYPAPPMLIRRCLKEDHLTG VGP
FSAGMTIKPGQDVMLATWSLNRDQRLWGP DADKYNPLRFYTAVHGSPEYKAAGWAGFDPARVRGLYPDENAAD
FGFIPFGGGGRKCMGDQFAILESSVLLSMLLRDFSFEAADTVTLGMGATIFAKEGLMMKV TARPPQPPDQDEAS PVA
VASDALLSSVA*

pTL95: K117-A127At-CYP97H1

MGSRRKTPNRSQCLLAFTGEREGLRHQELVPIRNL DINCHGVKTSCTQLQATVEPSPEQESQLPRAEDMAIGVTAQE
YVTHYIQNVAQFFVPMKWEDNIPVVK GSIQAVRNEAVFILLHRYMALSPIYKLCIGPRSVVVISDAVAVKHILRSEVG
KYDKGILAEVLKPI MGKGLIPADTITWLTRRRQLKPAFHQKWLHDQLTLYSTVGNRLVAFLAARPGQTIDMQERFCS
ASLDIIGKAVFN YEFGSITRESPVIQAVYAVMREAERRASSIVPYWQLPGGTREFDQHMKVLDDVLTSLVEQCQVQVS
TEEDEEPQKGNNSLRFLVEARGQDVTNQQLRDDLMTMLIAGHETTAATLTWALHELTKPENRDFLKRKVAEVD S
VLGLRDFITLDDVKQMPLVRYSLVEALRLYPAPPMLIRRCLKEDHLTG VGPFSAGMTIKPGQDVMLATWSLNRDQR
LWGP DADKYNPLRFYTAVHGSPEYKAAGWAGFDPARVRGLYPDENAADFGFIPFGGGGRKCMGDQFAILESSVLL
SMLLRDFSFEAADTVTLGMGATIFAKEGLMMKV TARPPQPPDQDEAS PVAVASDALLSSVA*

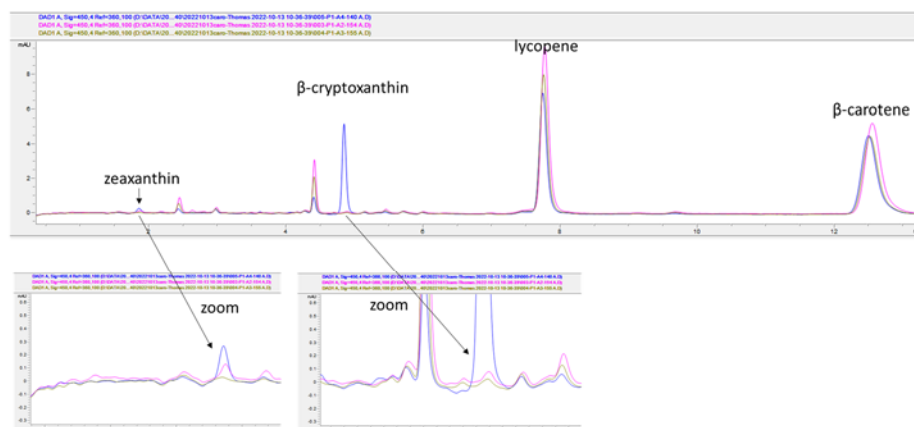
pTL96: M182-K117-A127At-CYP97H1

MAIGVTAQEYVTHYIQNVAQFFVPMKWEDNIPVVK GSIQAVRNEAVFILLHRYMALSPIYKLCIGPRSVVVISDAVAV
VKHILRSEVGKYDKGILAEVLKPI MGKGLIPADTITWLTRRRQLKPAFHQKWLHDQLTLYSTVGNRLVAFLAARPGQ
TIDMQERFCSASLDIIGKAVFN YEFGSITRESPVIQAVYAVMREAERRASSIVPYWQLPGGTREFDQHMKVLDDVLT S
LVEQCQVQVSTEEDEEPQKGNNSLRFLVEARGQDVTNQQLRDDLMTMLIAGHETTAATLTWALHELTKPENRDF
LKRKVAEVD SVLGLRDFITLDDVKQMPLVRYSLVEALRLYPAPPMLIRRCLKEDHLTG VGPFSAGMTIKPGQDVMLA
TWSLNRDQRLWGP DADKYNPLRFYTAVHGSPEYKAAGWAGFDPARVRGLYPDENAADFGFIPFGGGGRKCMGD
QFAILESSVLLSMLLRDFSFEAADTVTLGMGATIFAKEGLMMKV TARPPQPPDQDEAS PVAVASDALLSSVA*

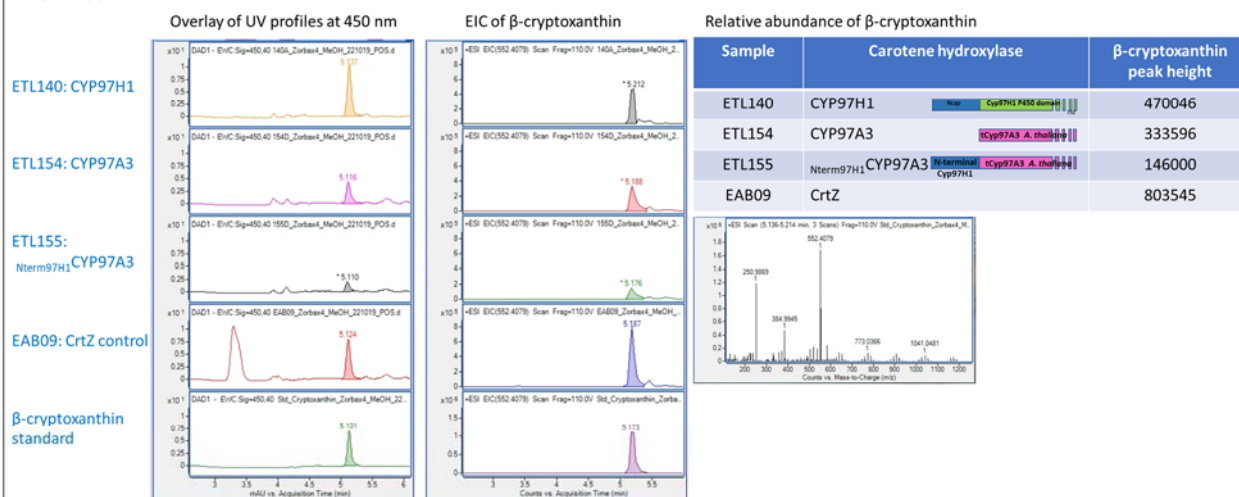
pTL98: MBP-CYP97A3At

MKIEEGKLVWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRFGGYAQSGL
LAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQ
EPYFTWPLIAADGGYAFKYENGKYDIKDVGVNDAGAKAGLTFLVDLIK NKHMNADTDYSIAEAAFNKGETAMTIN
GPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVA
LKSYYYELAKDPRIAATMENAQKGEIMPNIQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSSHHHHHHA
NSENLYFQGSFPSTVKNGLSKIGIPSNVLDFMFDWTGSDQDYPKVPEAKGSIQAVRNEAFFIPLYELFLTYGGIFRLTF
GPKSFLIVSDPSIAKHILKDNAKAYSKGILAEILDFVMGKGLIPADGEIWRRRRRRAIVPALHQKYVAAMISLFGASDR
LCQKLDAAALKGEEVEMESLFSRLTLDIIGKAVFNDFDSLNTDTGVIEAVYTVLREAEDRSVSPVWDIPIWKDISP
RQRKVATSLKLINDTLDDLIATCKRMVEEEELQFHEEYMNERDPSILHFLASGDDVSSKQLRDDLMTMLIAGHETS
AAVLTWTFYLLTTEPSVVAKLQEEVDSVIGDRFPTIQDMKKLKYTTRVMNESLRLYPQPPVLIRRSIDNDILGEYPIKR
GEDIFISVWNLHRSPLHWDDAEKFNPERWPLDGPNPNETNQNFSYLPFGGGPRKCIGDMFASFENVVAIAMLIRRF
NFQIAPGAPPVKMTTGATIHTEGLKLTVTKRTKPLDIPSPILPMDTSRDEVSSALS*

A: 450nm chromatogram



B: β -cryptoxanthin



C: Zeaxanthin

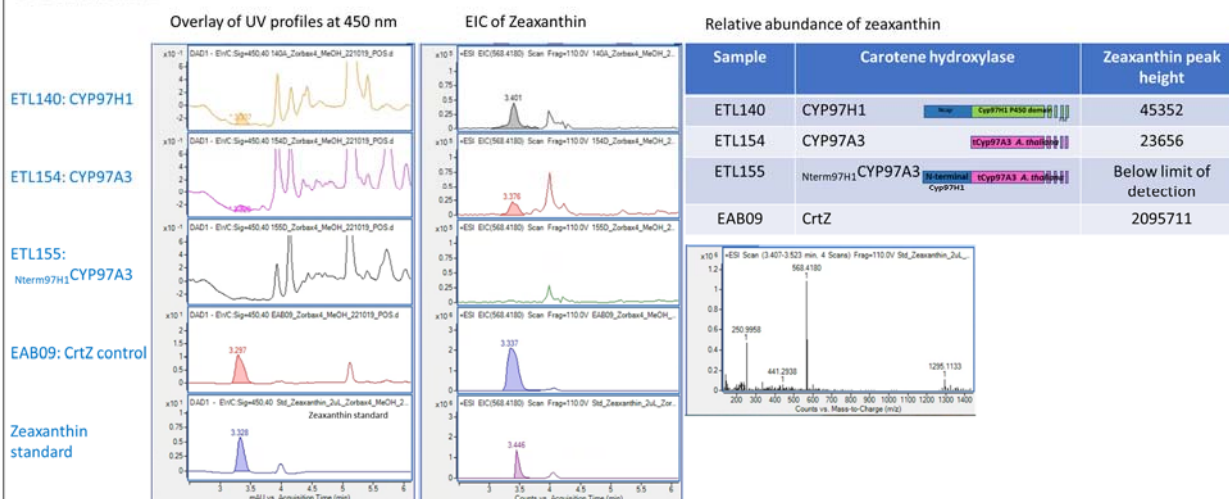


Figure S1. A: full 450nm chromatogram overlay of the acetone extracts from the strains ETL140: CYP97H1 (blue), ETL154: CYP97A3At (pink), ETL155: NtermCYP97H1 fused to CYP97A3At (gold), peaks at 2.4; 2.9 and 4.4 min are also present in the control strain ETL91 which does not express any hydroxylases. **B:** β -cryptoxanthin detection (visible spectra and mass spectrometry analysis). Same samples as in panel A, with the addition of EAB09 (strain expressing the non heme dihydroxylase CrtZ as zeaxanthin producer control). **C:** Zeaxanthin detection (visible spectra and mass spectrometry analysis). Same samples as in panel B.