

Supplementary Information for

Structural Basis for Expanded Substrate Specificities of Human Long Chain Acyl-CoA Dehydrogenase and Related Acyl- CoA Dehydrogenases.

Beena Narayanan, Chuanwu Xia, Ryan McAndrew, Anna L. Shen, and Jung-Ja P. Kim

Supplementary Figure Legends.

Figure S1. ACAD structural comparison. Overlay of human LCAD structure (Mol A, green) with those of rat SCAD (1JQI), shown in blue; pig MCAD (3MDE), shown in magenta; and human VLCAD N-terminal domain (3B96, residues 54-445), shown in cyan. C12-CoA is shown as sticks and FAD as gold sticks.

Figure S2. ACAD sequence alignment. Alignment of LCAD protein sequence with other eucaryotic ACADs and related *M. tuberculosis* ACADs. Alignments were carried out using COBALT (Papadopoulos JS and Agarwala R (2007) COBALT: constraint-based alignment tool for multiple protein sequences, *Bioinformatics* 23:1073-79). Residues corresponding to LCAD residues 127-139 were manually aligned according to structural comparisons of LCAD, SCAD, MCAD, VLCAD, *MtbChsE4*, and ACAD11. Percent identity of each sequence to LCAD is indicated in parentheses. Conserved residues are shown in red. Positions where <50% of the sequences contain gaps are shown in gray lower case. Positions where >50% of the sequences contain gaps are shown in gray upper case. The catalytic Glu or Asp residues are highlighted in green. The position of the conserved LCAD Pro132 is highlighted in yellow. Putative LCAD lysine residues deacetylated by SIRT3 are shown in bold. Gray bars highlight residues homologous to MCAD ETF-interacting residues. Glu residues interacting with Arg249 of ETF are indicated by bold underline. Accession numbers are LCAD, NP_001599.1; *MtbCNF74574*, CNF74574; IVD, NP_002216.3; SCAD, AAD00552.1; IBD, NP_055199.1; MCAD, NP_001120800.1; ACAD9, NP_054768; VLCAD, BAA29057.1; *Mtb4HR3_A*, 4HR3_A; *MtbChsE4*, I6YCA3; *MtbChsE2*, P71858.2; ACAD10, NP_001130010; ACAD11, NP_115545; Acad12, NP_848914.2.

Figure S3. A stereo figure for the electron density of acetoacetyl-CoA bound to the wild-type LCAD, showing hydrogen bonding interactions that stabilize CoA binding. Hydrogen bonding interactions between LCAD and acetoacetyl CoA are shown as dotted lines. The 2Fo-Fc map of acetoacetyl-CoA at 1.0 σ level is shown in gray mesh.

Figure S4. LCAD-ETF complex. LCAD monomers are shown in green, magenta, cyan and blue ribbons. The ETF alpha subunit is shown in gold and the beta subunit in gray. FAD is shown as gold sticks. The arrows point to ETF β Leu195, shown as gray sticks. The view shown in (a) is rotated 90° along the Y axis to produce the view shown in (b).

Fig. S1

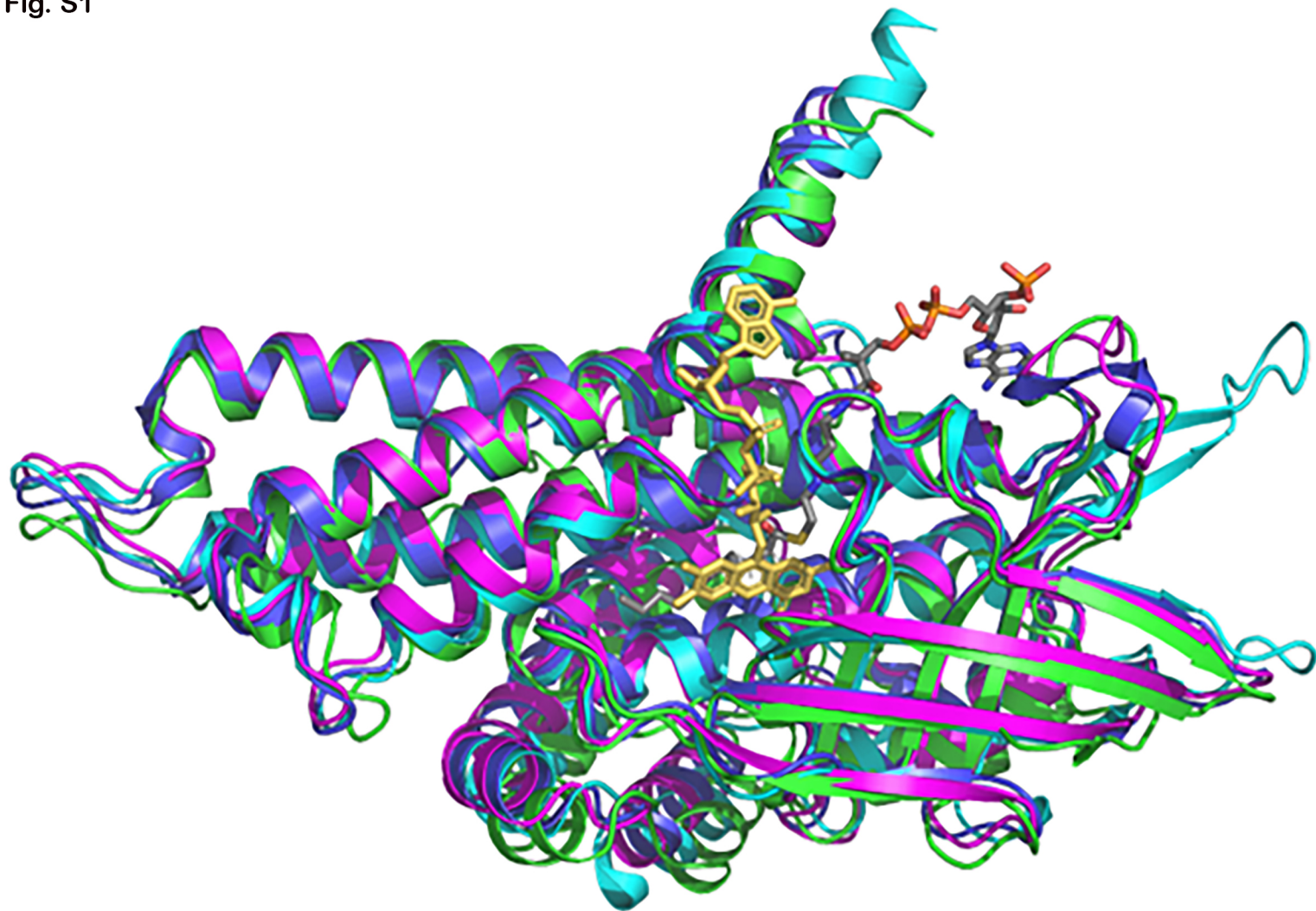


Fig. S2

LCAD	1	MAARLLRGSLRVLGG [4]	RQLPAA [4]	SGGEERLETPsAKKLTDIGIRRI	FSP	EHDI	FRKSVRKFFQEEVIP	74
<i>Mtb</i> CNF74574 (52)	1	MG-----	-----	-----	-----	RDIYTDEHDA	FRDMVRSFIAKEAAP	27
IVD (34)	1	MATATRLLGWRVASW	RLRPPL [2]	FVSQRAHSL-----	PVDDAINGL	SEEQRQ	LRQTMAKFLQEHLP	63
SCAD (33)	1	MAAALLAR---ASG	PARRAL [2]	RAWRQLHTIY-----	QSVE---	LPETHQM	LLQTCRDFAEKELFP	55
IBD (33)	1	MLWSGCRRFGARLG-	-----	[3] GGLRVLVQTGhRSLTSCIDPSMGL	NEEQKE		FQKVAFDFAAREMAP	62
MCAD (32)	1	MAAGFGRCCR---CSL	QVLRSI [2]	FHWSQHTKANrQREPGLGFSFEF	TEQQKE		FQATARKFAREEIIP	66
ACAD9 (31)	1	[12] -AARACRGLVVSTAN [4]	RTSPPV	RAFAKELFLGkIKKKEVFPFPEVS	QDELNE [3]		FLGPVEKFFTEEVDS	84
VLCAD (29)	1	[42] GAAQLALDKSDSHPS [4]	RKKPAK [3]	KSFAVGMFKGqLTDDQVFPYPSVL	NEEQTQ [4]		LVEPVSRFFEEVNDP	119
<i>Mab</i> 4HR3_A (28)	1	-----	-----	-----	-----	GPGSMDFAP---SARAAE	LIAAVREFIDAEMP	30
<i>Mtb</i> ChsE4 (27)	1	-----	-----	-----	-----	MRISYTPQQEE	LRRELRSYFATLMTP	26
<i>Mtb</i> ChsE2 (26)	1	-----	-----	-----	-----	MFIDLTPQRQ	LQAEIRQYFSNLISP	26
ACAD10 (26)	1	[652] ARPQSQCPTGSRYS [4]	EASPAH	-----	TSRGGLVISPELS	SPPVRE	LYHRLKHFMQRVYP	711
ACAD11 (26)	1	[356] -----	RTF [4] ---PQI	-----	DTTGQLFVQ----	TRKGQE	VLIKVKHFMKQHILP	396
ACAD12 (25)	1	[112] AGPRSPRTPKGVGRH [4]	AASPSH	-----	EAKGGLVISPEGL	SPAVRK	LYEQLVQFIEQKVYP	171
LCAD	75	HHSEWEKA	GEV--S---REVWEKAGKQGLLVNIAEHLG	GIGGD-LYSAAIVWEEQAYS-N-CS-GPG---	FSIHSG			139
<i>Mtb</i> CNF74574	28	HHQQWEKD	GIV--S---REVWLAAGRAGLLGIDMPEEYG	GGGNDdYRYVIFNEELAKA-G-VH-GPG---	FAVHND			93
IVD	64	KAQEIDRS	NEFKNL---REFWKQLGNLGLVGITAPVQYG	GSGLG-YLEHVLVMEIISRA-S-GAVGLS---	YGAHSN			131
SCAD	56	IAAQVDKE	HLF--P---AAQVKMGGLGLLAMDVPEELG	GAGLD-YLAYAIAMEEISRGCA-ST-GVI---	MSVNNS			121
IBD	63	NMAEWDQK	ELF--P---VDVMRKAQGLFGGVYIQTDTV	GSGLS-RLDTSVIFEALATG-C-TS-TTA---	YISIHN			127
MCAD	67	VAAEYDKT	GEY--P---VPLIRRAWELGLMNTHIPENC	GLGLG-TFDACLISEELAYGCT-GV-QTA---	IE-GNS			131
ACAD9	85	--RKIDQE	GKI--P---DETLEKLKSLGLFGLQVPEEYG	GLGFS-NTMYSRLGEIISM--D-GSITVT---	LAHQQA			147
VLCAD	120	--AKNDAL	EMV--E---ETTQQLKELGAFGLQVPSELG	GVGLC-NTQYARLVEIVGMHDL-GV-GIT---	LGHQQS			183
<i>Mab</i> 4HR3_A	31	VERAVLAH [13]	ELWHVPpelDSLKAKARAAGLWNLFPLDPE- [1]	GGGLS-NSEYAPLAEQMGRS-LfAPT	VFN---CNAPDS			115
<i>Mtb</i> FaDE26	27	ERREALSS [2]	GEYGVGnvYRETIAQMGRDGWLALGWPKEYG	GQGRS-AMDQLIFTDEAAIA---G-APV---	PFLTIN			96
<i>Mtb</i> FaDE29	27	DERTEMEK	DRHGPA--yRAVIRRMGRDGRLGVGWPKFEG	GLGFG-PIEQQIFVNEAHRA-D---VPL---	PAVTLQ			92
ACAD10	712	AEPELQSH [4]	ARWSPSpliEDLKEKAKAEGLWNLFPLEAD [5]	GAGLT-NVEYAHLCCELMGTS-LyA---	PEVCNCSAPDT			792
ACAD11	397	AEKEVTEF [8]	DKWGKPlviDKLKEMAKVEGLWNLFPAVS-	--GLS-HVDYALIAEETGK--C-Ff-AP	PDVFNCQAPDT			473
ACAD12	172	LEPELQRH [4]	NRWSPSpliEDLKEKAKAEGLWNLFPLETD [5]	GAGLT-NVEYAHLCCEVMGMS-LyASEIFN---	CSVPDM			252
LCAD	140	IVMSYITNHGSEEQIKHFIPQMTAGKCIGAIAMTEPG-AGSDLQGIKTNAK--	KDGS	DWILNGSKVFISNGSLSDVVIVV				216
<i>Mtb</i> CNF74574	94	INGGYLRQLCTEEQKRRWFPGYCSGEIITAIAMTEPA-AGSDLQGIKTAV--	KDGD	DYVLNGSKTFISNGILADLVIVV				170
IVD	132	LCINQLVRNGNEAQKEKYLPKLISGEYIGALAMSEPN-AGSDVVSMLKAE--	KKGN	HYILNGNKFWITNGPDADVLIVV				208
SCAD	122	LYLGPILKFGSKEQKQAVVTPFTSGDKIGCFALSEPG-NGSDAGAATTAR--	AEGD	SWVLNGTKAWITNAWEASA	AAVVF			198
IBD	128	MCAWMIDSFGNEEQRHKFCPLCTMEKFASYCLTEPG-SGSDAASLLTSAK--	KQGD	HYILNGSKAFISGAGESDIYVVM				204
MCAD	132	LGQMPIIIAGNDQQKKYLGRMTTEPLMCAYCVTEPG-AGSDVAGIKTKAE--	KKGDE	YIINGQKMWITNGGKANWYFLL				208
ACAD9	148	IGLKGIIILAGTEEQKAKYLPKLASGEHIAAFCLTEPA-SGSDAASIRSRA	tl	EDKKHYILNGSKVWITNGGLANIFTVF				226
VLCAD	184	IGFKGILLFCTKAQKEKYLPKLASGETVAAFCLTEPS-SGSDAASIRTSAP	ps	PCGKYITLNGSKLWISNGGLADIFTVF				262
<i>Mab</i> 4HR3_A	116	GNMEVLHRYGSQEKEVWLEPLLEGDIRSAFCMTEPDvASSDATNMAATAV--	VEGDE	VVINGRKWWSTGVGHPDCKVII				193
<i>Mtb</i> FaDE26	97	SVAPTIMAYGTDEQKRFFLPRIAAGDLHFSIGYSEPG-AGTDLANLRTTAV--	RDGD	DYVVGQKMWTSLIQYADYVWLA				173
<i>Mtb</i> FaDE29	93	TVGPTLQAHGSELQKKKFLPAIAGEAHFAIGYTEPE-AGTDLASLRTTAV--	RDGD	HYIVNGQKVFTTGAHDADYIWL				169
ACAD10	793	GNMELLVRYGTEAQKARWLIPLLEGKARSCFAMTEPQvASSDATNIEASIR--	EEDS	FYVINGHKWWITGILDPRCQLCV				870
ACAD11	474	GNMEVLHLYGSEEQKKQWLEPLLQGNITSCFCMTEPDvASSDATNIECSIQ--	RDED	SYVINGKKWSSGAGNPKCKIAI				551
ACAD12	253	GTMEILVRYGTEEQKARWLVPILLEGRIHSCFAMTDRKvASSDASNIEASIK--	EEDN	SYVINGHKWWTSGILHPHCKLCV				330

LCAD	217	AVT---NH-EAPSPA	HGISLFLVENG	MKGFIKGRKL	HKMGLKAQ---	DTAELFFEDIRLPASALLGEENKGFYYIMKELP	289			
MtbCNF74574	171	AKT---DP-SAG--	AKGVSLLAVERG	MDGFERGRNL	DKVGMHAQ---	DTAELFFDNVRVPKANLLGEEGMGFIYLMTNLA	241			
IVD	209	AKT---DL-AAVPAS	RGITAFIVEK	GMPGFSTSKKL	DKLGMRGS---	NTCELIFEDCKIPAANILGHENKGVYVLMSSGLD	281			
SCAD	199	AST---DR-AL--	QNGISAF	LVPMPTPGLTLGKKE	DKLGIRGS---	STANLIFEDCRIPKDSILGEPGMGFKIAMQTL	269			
IBD	205	CRT---GG-PGP---	KGISCIVVEK	GTPGLSFGKKE	EKKVGWNSQ---	PTRAVIFEDCAVPVANRIGSEGQGFLLI	274			
MCAD	209	ARS---DPdP	KAPANKAFTGFIVEAD	TPIGIQIGRKE	ELNMQRCS---	DTRGIVFEDVKVPKENVLIGDGAGFKVAMGAFD	282			
ACAD9	227	AKTEVvD--	SDGSVKDKITAFIVERD	FGGVTNGKPE	DKLGIRGS---	NTCEVHFENTKIPVENILGEVGDGFKVAMNIN	301			
VLCAD	263	AKTPVtDP-AT	GAVKEKITAFVVERG	FGGITHGPP	EKKMGIKAS---	NTAEVFFDGVVRVPSENVLGEVSGFKVAMHILN	338			
Mab4HR3_A	194	FMGLT-DP--	NAHRYARHSMVL	VPMDTPGITVERML	PTMGFYDE-pg	GHGVVSFDNVRLPADAFIAGPGKGFEIAQGRLG	269			
MtbFaDE26	174	VRTNPeSS--	GAKKHRGISVL	LIVPTTAEGFS	WTPV-HTMAGPDT	-----SATYYS	SDVRVPVANRVGEENAGWKLVTNQLN	245		
MtbFaDE29	170	CRT---DP--	NAAKHKGISIL	IVDTKDPGYS	WTPITLADGAHHT	-----NATYYND	VVRVPVDMVLVGKENDGWRLITTQLN	239		
ACAD10	871	FMGKT-DP--	HAPRHRQQSVLL	VPMDTPGIKI	IRPLTVYGLEDA-pg	GHGEVRF	FEHVVRVPKENMVLGPGRGFEIAQGRLG	946		
ACAD11	552	VLGRTqNT--	SLSRHKQHSMIL	VPMNTPGVKI	IRPLSVFGYTDN	fhgGHFEI	HFNQVRVPATNLI	LGEGRGFEISQGRLG	629	
ACAD12	331	FMGKT-DP--	QAPRHQQQSMLL	VPMDSPGITVIRPL	SVFGLED	pGpGFAEV	QFKDVRVPKENI	ILGPGRAFEIAQGRLG	406	
LCAD	290	QERLLIADVAIS	ASEFMFEET	TRNYV-KQRKA	FGKTV	AHLQTVQHKL	AELKTHICVTRA	FVDNCLQLHEA-KRLD-SAT-A	365	
MtbCNF74574	242	RERLSIGATAMAA	AEDAFERTLEYC	-RTREAFGRPI	GKFGQHN	RFTLAEMKTELT	VARSF	DECILKEGG-GELT-ADE-A	317	
IVD	282	LERLVL	AGGPLGLMQAVL	DHTIPYL-HVRE	AFGQKIGHF	QQLMQGMAD	MYTRLMACRQ	YVYNVAKACDE-GHCT-AKD-C	357	
SCAD	270	MGRIGIASQALG	IAQTALDCAVNYA	-ENRMAFGAP	LTKLQVIQFK	LADMALALESAR	LLTWRAAMLKDN-KKPF-IKE-A	345		
IBD	275	GGRINIASCSLGA	AHASVILTRDHL	-NVRKQFGE	PLASNQYLQFT	LADMATRLVAAR	LMVRNA	AAVALQE-ERKD-AVALC	351	
MCAD	283	KTRPVVAAGAV	GLAQRALDEATKYA	-LERKTFGKLL	VEHQAI	SFMLAEMAMK	VELARMSYQRAA	WEVDS-GRRN-TYY-A	358	
ACAD9	302	SGRFSMGSVVAG	LLKRLIEMTAEYA	-CTRKQFNKRL	SEFGLIQEK	FALMAQKAYV	MESMTYLTAG	MLDQPGFPD-CSIEA	379	
VLCAD	339	NGRFGMAAALAG	TMRGIIAKAVDHA	-TNRTQFGE	KIHNFGLIQEK	LARMVMLQYV	TESMAYMVSAN	MDQ-GATD-FQIEA	415	
Mab4HR3_A	270	PGRVHHAMRLI	GLAEVALEHACRRG	-LDRTAFGKPL	VNLGGNRER	IAADARIAINQ	TRLLVLHAAW	LLDTVGIMG-ALSAV	347	
MtbFaDE26	246	HERVALVS--	PAPIFGCLREV	REWAqNTKDAG	GTRLIDSEWV	QLNLARVHAKA	EVVLKLINWEL	ASSQSGPKDAGpSPADA	323	
MtbFaDE29	240	NERVML--	GPAGRFASIY	DRVHAWA-SVPG	GNGVTPIDHDD	VKRALGEIRAI	WRINELLNWQ	VASAGEDINMA-----DA	311	
ACAD10	947	PGRIHCMRLIG	FSE	RALALMKARV-KSRL	AFGKPLVEQGT	VLADIAQSRVE	IEQARLLVLR	AAHLMDLAGNKA-AALDI	1024	
ACAD11	630	PGRIHCMRTV	GLAERALQIMCERA-TQRI	AFKKKLYA	HEVVAHWIAES	RIAIEKIRLL	TLKAAHSMDTLGSAG-AKKEI	707		
ACAD12	407	RARIHNSMRLI	GYSERALALMKTRV-MSRT	AFGKPLVEQGT	ILADIARS	RVEIEQARLLVLK	AAHLMDVAGNKA-AALEM	484		
LCAD	366	CMAKYWASELQNS	VAYDCVQLHGG	WGYMWEYP	IAKAYVD	ARVQPIYG	GTNEIMKELI	AREIVFDK---	430	
MtbCNF74574	318	AMLKWWNTELL	KRVVDRCVQLHGG	YGYMTEYP	IAKAYQD	VRIQTIFG	GTTEIMKEI	IGRGLV-----	380	
IVD	358	AGVILYSAECATQ	VALDGIQCFGG	NGYINDFP	MGRFLRD	AKLYEIGA	GTSEVRR	LVI	GRAFNADFH--	423
SCAD	346	AMAKLAASEAATA	ISHQAIQILGGM	GYVTEMP	AERHYRD	ARITEIYE	GTSEIQRL	VIAGHL	LSYRS-	412
IBD	352	SMAKLFATDECFA	ICNQALQMHGG	YGYLKDYA	VQQYVRD	SRVHQILE	GSNEVMRIL	ISRSLLQE----	415	
MCAD	359	SIAKAFAGDIANQ	LATDAVQILGG	NGFNTEYP	VEKLMRD	AKIYQIYE	GT	SQIQRLIVARE	HIDKYKN-	425
ACAD9	380	AMVKVFSSEAAW	QCVSEALQILGGL	GYTRDYP	YERILRD	TRILLIFE	GTNEILRM	YIALTGLQ	HAGRI [174]	621
VLCAD	416	AISKIFGSEAAW	KVTDECIQIMGG	MGMFKEPG	VERVLRD	LRIFRIFE	GTNDILRL	FVALQGC	MDKGKE [172]	655
Mab4HR3_A	348	SEIKVAAPNMAQQ	VIDMAIQIHGGG	GLSNDFP	LAAAWVN	ARALRLAD	GPDEVHRG	VVARIELAKY	AND	415
MtbFaDE26	324	SAAKVFGTE	LATEAYRLLM	EVLTGAATLRQNS [8]	VERMHRA	CLILTFGG	GTNEVQRDI	IGMVALGL	PRAN [1]	400
MtbFaDE29	312	AATKVFGTE	RVQRAGRLAEE	IVGKYGNPAEPD	TAELLRW [7]	NLVITFGG	GVNEVMRE	MIASGLK	VPRVP [1]	387
ACAD10	1025	AMIKMVAPS	MASRVIDRAIQ	AFGAAGLSSDYP	LAQFFTW	ARALRFAD	GPDEVHRAT	VAKLELKHRI--		1090
ACAD11	708	AMIKVAAPRAV	SKIVDWAIQVCGG	AGVSQDYP	LANMYAI	TRVLR	LADGPDEVHLS	AIATMELRDQAKR [5]		780
ACAD12	485	AMIKMVVPS	MANQVIDRAIQ	AFGAAGLSSDYP	LAQFFGW	AQTLRLGD	-----DOLK	VAKMELKNQ	SRL [8]	555

Fig. S3

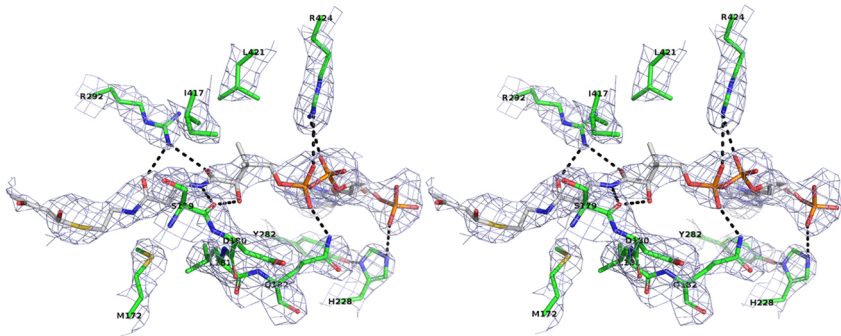


Fig. S4

