

SUPPLEMENTARY MATERIAL FIG. 1

<i>R. trifolii</i> matB	-----Msnhlfdamraaapgnap	18
<i>B. japonicum</i> matB	-----Mnraananlfsrlfdgl--ddpkrla	24
<i>S. coelicolor</i> matB	-----Msslfpal-spaptgap	16
<i>H. sapiens</i> ACSF3	mp-phvltfrrlgcalascrlapahhgsgllhtapvarsdrsapvfttra-lafgdria	58
<i>B. taurus</i> ACSF3	mplpyvgmalrrlawavascrlapwtlqasgplhsapaarsdcsapvfira-pafgdrla	59
<i>H. sapiens</i> ACSM2	mhwlrvvgglctlwgtqmssrtlyinsqgl---vslqwgqhgevpakfnfa-sdvldhwa	55
<i>M. musculus</i> ACSM2	mhhlwkiprlftlwgneiscrtfhnnikkl---ipiqwgqhgeapakfnfa-sdvidhwa	55
<i>R. trifolii</i> matB	firid---ntrt-----wtyddafalsgriasamdal-----girpgdr	54
<i>B. japonicum</i> matB	iethd---gari-----sygdliaragqmanvlvar-----gvpkgdr	59
<i>S. coelicolor</i> matB	adrpalrfgers-----ltyaelaaaagatagrigger-----g-----r	50
<i>H. sapiens</i> ACSF3	lvdqh---grh-----tyrelrsrslrlsgeicrlcgcvggdreer	97
<i>B. taurus</i> ACSF3	lidqh---grh-----tykdlylrsrlrlsreicqlracadgdlreer	98
<i>H. sapiens</i> ACSM2	dmeka---gkrlpspalwvvnkgkelmwnfrelsensqqaanilsga--c--glqrgdr	108
<i>M. musculus</i> ACSM2	sveka---gkrssgpalwvnmngsgkeikwsfrelseaskqtanvlsga--c--glhrgdr	108
<i>R. trifolii</i> matB	vavqveksaealilylaclrsgavylplntaytlaeldyfigdaeprrlvvassaragve	114
<i>B. japonicum</i> matB	vavqveksvanivlylatvragavylplntaytlneldyfigdaepsllvcdpskaegla	119
<i>S. coelicolor</i> matB	vavwatpametgvavvaallagvaavplnpgsgdkelahilsdsapslvlappdael---	107
<i>H. sapiens</i> ACSF3	vsflcandasyvvaqwaswmsggvavplyrkhpaaqleyvicdsqssvvlvsqeylells	157
<i>B. taurus</i> ACSF3	vsllcsndvsfvvaqwaawmsggvavplyrkhpaaqleyfiqdsrsvvlagpehvells	158
<i>H. sapiens</i> ACSM2	vavmlprvpewwlvilgciraglifmpgtiqmkstdilyrlqmskakaivagdeviqevd	168
<i>M. musculus</i> ACSM2	vavvlpripewwlmilgcmrtglvfmpgtiqmrssdilyrlqaskaraivagdevaqevd	168
<i>R. trifolii</i> matB	tiak-----prgaivetldaagsgsl--ldlardepadvdasrsaddlaailytsgttg	167
<i>B. japonicum</i> matB	piaa-----kvkagvetlpgdgkgsll--teaadkassafttvprenddlaailytsgttg	172
<i>S. coelicolor</i> matB	-----ppalgalervdvdrargavpedgaddgdpalvvytsgttg	148
<i>H. sapiens</i> ACSF3	pvvr-----klgvpllpptpaiytga---veepaevpvpeqgwrnkq--amiiytsgttg	207
<i>B. taurus</i> ACSF3	pvaq-----klgvpllpptvyhg---aedpeeglvlernwrdrg--amiiytsgttg	208
<i>H. sapiens</i> ACSM2	tvasecplrikllvsekscdgwlnf--kklneastthhcvetgsqeaaiyftsgtsg	226
<i>M. musculus</i> ACSM2	avapdcsflkikllvsensregwlnf--kallkeastihqcvetesresaiyftsgtsg	226
<i>R. trifolii</i> matB	rskgamlthgnllsnaltlrdfwr-vtagdrlihalpifhthglfvatnvtllagasmf-	225
<i>B. japonicum</i> matB	rskgamlthdnlasnslslvgywr-ftdkdvlihalpiyhthglfvatnvtlfsrasmi-	230
<i>S. coelicolor</i> matB	ppkgaviprralattldaladawq-wtgedvlvqglplfhvhgvlvgilgplrrggsvr-	206
<i>H. sapiens</i> ACSF3	rpkgvlsthqnravvtglvhkwa-wtkddvilhvlplhhvhgvvnallcplwv gatcv-	265
<i>B. taurus</i> ACSF3	rpkgvlsthqnravvtglvhkwa-wtkddvilhvlplhhvhgvvnallcplwv gatcv-	266
<i>H. sapiens</i> ACSM2	lpkmaehsysslgkakmdag-wtqlgasdimwtisdtgwilnilgsllswtlgactfv	285
<i>M. musculus</i> ACSM2	ppkmaehshcslgikakmdaaswtglstsdiiwtisdtawimmilgaflepwlvacifv	286
<i>R. trifolii</i> matB	-llskfdpe-eilslm-----pqatmlmgvptfyvrlq-----sprldkqavan----	268
<i>B. japonicum</i> matB	-flpkldpd-liiklm-----aratvlmgvptfytrllq-----naalsrettrh----	273
<i>S. coelicolor</i> matB	-hlgrfstegaareln-----dgatemlfgvptmyhriae-----tlpadpelaka----	250
<i>H. sapiens</i> ACSF3	-mmpefspq-qvwekflssetprinvmavptiytklmeydrhftqphaqdfvravcee	323
<i>B. taurus</i> ACSF3	-mlpefsaq-lvwekflsseapqinvfmapvtiysklmdyydkhftqphvqdfvravcee	324
<i>H. sapiens</i> ACSM2	hllpkfdpl-vilktl---ssypiksmmgapivyrmlq-----qdlssykfphl----	331
<i>M. musculus</i> ACSM2	hllpkfdsq-tvlkvl---ssypintlvgapiiyrmlq-----qdlssykfphl----	332
<i>R. trifolii</i> matB	-i---rlfisgsapllaethtefgartghailetygmtetnmntsnpyek-riagtvgf	323
<i>B. japonicum</i> matB	-m---rlfisgsapllaethrewsartghavletygmtetnmntsnpydge-rvpavvgf	328
<i>S. coelicolor</i> matB	-lagarllvsgsaalpvhdheriaaatgrrrvieetygmtetlmtsvradge-pragtvgv	308
<i>H. sapiens</i> ACSF3	ki---rlmvsgsaalplpvlekwnitghtlletygmtetigmalsgplttamrlpgsvgt	380
<i>B. taurus</i> ACSF3	ki---rlmvsgsaalplpvlekwnitghtlletygmtetigmalsnpltaa-rlpgsvgt	380
<i>H. sapiens</i> ACSM2	-----qncлагgesllpetlenwraqtgdirefygqetgltcmvsktmk-ikpgymgt	385
<i>M. musculus</i> ACSM2	-----hscfsggetllpetlenwkaktgleireiygqetglicrvsrtmk-vkpgylgt	386

<i>R. trifolii</i> matB	plpdvtvrvtddpatg--lalpp-----eqtgmieik-gp----nvf	357
<i>B. japonicum</i> matB	plpgvslrvtdpetg--kelpr-----eeigmievk-gp----nvf	362
<i>S. coelicolor</i> matB	plpgvelrlveedgtpiaaldg-----esvgeiqvr-gp----nlf	344
<i>H. sapiens</i> ACSF3	plpgvqvrvsvsenpq--reacsytihaegdergtkvtpgfefekegellvr-gp----svf	433
<i>B. taurus</i> ACSF3	plpgvevrvsvsenpq--kdsspylihaegseentkvtpgfefekegellvr-gp----svf	433
<i>H. sapiens</i> ACSM2	aascydvqviddkgn---vlpp-----gtegdigirvkipirpigif	423
<i>M. musculus</i> ACSM2	afahydvqvideqgn---vlpp-----gkegdiairvkipiwpigmf	424
<i>R. trifolii</i> matB	kgywrmpektaaeftadgffisgdlgkidrdgyvhivgr-gkdlvisggyniypkevege	416
<i>B. japonicum</i> matB	kgywrmpektkaefrpdgffitgdlgkidgkgyvhilgr-gkdlvisggfnvypkeiese	421
<i>S. coelicolor</i> matB	teylnrpddataaafteedgffrtgdmavrdpdgyvrvgrkatdliksggykigageiena	404
<i>H. sapiens</i> ACSF3	reywnkpeetksaftldgwfktgdtvfvk-dgqywirgrtsvdiiktgykvsalevewh	492
<i>B. taurus</i> ACSF3	reywdkpeetkaafstdgwfktgdtvfvk-dgqywirgrtsvdiiktgykvsaleverl	492
<i>H. sapiens</i> ACSM2	sgyvenpdktaanirgd-fwllgdrigkdedgyfqfmgr-addiinssgyrigpsevena	481
<i>M. musculus</i> ACSM2	sgyvdpnkktqdnirgd-fwlmgdrigkdpgeyfhfigr-sddiinssgyrigpsevena	482
<i>R. trifolii</i> matB	idqiegvesavigvphpdfgegvtavvvrkp-----gaaldekaivsalqdrlyrkq	470
<i>B. japonicum</i> matB	idampgvvesavigvphadfggvtavlvcnk-----gaevseasvlkaldgrlakfkm	475
<i>S. coelicolor</i> matB	llehpvreaavtgepdplgerivawivpadp-----aappalgtladhvaarlaphkr	459
<i>H. sapiens</i> ACSF3	llahpsitdvavigvdpmtwggrvta-vvtlre-----ghslshrelkewarnvlapyav	546
<i>B. taurus</i> ACSF3	llahpsitdvavigvdpmtwggrvta-vvtlqe-----ghslshrelkewargvlapyav	546
<i>H. sapiens</i> ACSM2	lmkhpavvetavisspdpsrgevka-fvilasqflshdpeqltkelqghvksvtapyky	540
<i>M. musculus</i> ACSM2	lmehpavsetavisspdpsrgevka-fvvlapeflshdrdqtkvlqehvksvtapyky	541
<i>R. trifolii</i> matB	pkriifaedlprntmgkvqknilrqgyadlytrt	504
<i>B. japonicum</i> matB	pkrvfvvdelprntmgkvqknvlrdtykdiyakk	509
<i>S. coelicolor</i> matB	prvvryldavprndmgkimkralnrd	485
<i>H. sapiens</i> ACSF3	pselvlveeiprnqmgkidkkalirh---fhps	576
<i>B. taurus</i> ACSF3	pselllveeiprnqmgkvnkrdlvrq---lyphekgapeagsq	586
<i>H. sapiens</i> ACSM2	prkiefvlnlpktvtgkiqrklrdk---ewkms---gkaraq	577
<i>M. musculus</i> ACSM2	prkvefvldlpktvtgkieraklrak---ewkts---gra	575

Supplementary FIG. 1. **Multiple sequence alignments of prokaryotic malonyl-CoA synthetases (matB) and mammalian acyl-CoA synthetases.** Alignments were performed using KALIGN at EMBL-EBI with the default parameters. Acidic residues are colored blue, basic residues magenta, hydrophobic residues red, others green. The mammalian proteins all have predominantly basic aminoterminal extensions typical of mitochondrial targeting sequences. Both ACSF3 and ACSM2 mammalian proteins exhibit approximately 60% sequence similarity with prokaryotic malonyl-CoA synthetases (matB). However, only ACSF3 proteins have the positionally conserved motif **eRygmte** motif found in the prokaryotic matB proteins. Since this study was completed, a crystal structure was solved for the *S. coelicolor* matB that showed the Arg283, within this motif plays a key role in substrate recognition by forming a salt bridge with the 3-carboxylate of malonate (1). Significantly, a second residue involved in malonate binding, Ser261, is also universally conserved in matB and ACSF3 proteins (shaded yellow), but not in the ACSM2 proteins.

REFERENCE

- Hughes, A. J. and Keatinge-Clay, A. (2011) *Chem Biol* **18**, 165-176