

1 **Supplementary Information**

2 **Table S1** Primers used for gene amplification. Restriction sites are underlined.

Gene	Direction	Sequence
<i>casI</i> (ro05822)	Forward	CCGGCATATGGAGTTGGAACGCACCACGATCG
	Reverse	GCGCAAGCTTCACACGTGCAGGAATCGTTGCCG
<i>casG</i> (ro05820)	Forward	CGCCATATGACTGCCCTACAGATCCAC
	Reverse	GGCCAAGCTTCAGACGGTGATGTCCCGGCGG
<i>fadE34</i> (rv3573c)	Forward	GGGCCATATGGTAGCGACCGTCACCGACGAAC
	Reverse	CGGCAAGCTTCAACGCGGCAGACCCAGCAGC
<i>casC</i> (ro05816)	Forward	GGCGCATATGACAATGGGATTGACCGAAGAAGAGCG
	Reverse	CCGCAAGCTTCACGCTCCCTTTCCGGGCTCGGG

3

4 **Table S2** Primers used for site-directed mutagenesis. Substituted codons/bases are bolded.

Mutation	Direction	Sequence 5' to 3'
FadE29E241Q	Forward	CTCAACAACCAACGGGTAATGCTCGGCCCGGCCGGGC
	Reverse	ATTACCCGTTGGTTGTTGAGTTGGGTGGTGATCAGCCGCC
FadE28R227A	Forward	GGCGAACCGCGAAGCAGTTCGGTAAGCCGCTGTCAACCTTCCAG
	Reverse	GAAGTCTTCGCGTTCGCCACGTAGTCGGCGGTCAGCCGC
FadE34E581Q	Forward	CCAACCAGCGGGTCGCGATGGCCACC
	Reverse	GACCCGCTGGTTGGCTAACGTGGTGCGCGC
FadE34R236A	Forward	CCAAGGTGGCAGAGCAATTCGGCAAGCCG
	Reverse	CGAATTGCTCTGCAACCTTGGCGTAGGCAACCG
CasCE598Q	Forward	GCCAACAACGGGTGTCGCTGTCGCACG
	Reverse	CCGTTGGTTGGCGAGGGTCGTGCGGG
CasCR239A	Forward	CAACGTAGCTCATCAGTTCGGCCGCCCATC
	Reverse	CTGATGAGCTACGTTGGCGTAGTCGGCGGCG
<i>casC</i> sacI5	Forward	CTCCTGGTGGGCGAACCGGGCGACGGCTGGGAGCTCGC
<i>casC</i> sacI3	Reverse	CGTTGGCGAGGGTCGTGCGGGCGAGCTCCCAGCCGTC

5

Table S3 Sequence similarities/identities among classical and steroid degrading ACADs.

Percent similarities and identities between pairs of sequences are indicated at the top and bottom of each cell, respectively. The following ACADs are compared : short chain acyl-CoA dehydrogenase from *Megasphaera elsdenii* (SCAD_Melsdenii), pig medium chain acyl-CoA dehydrogenase (MCAD_Pig), human isovaleryl-CoA dehydrogenase (IVD_human), rat long chain acyl-CoA dehydrogenase (rat_LCAD), human isobutyryl-CoA dehydrogenase (IBH_human), FadE26 (FadE26_Mtb), FadE27 (FadE27_Mtb), FadE28 (FadE28_Mtb), FadE29 (FadE29_Mtb), N-terminal ACAD domain of FadE34 (FadE34Nterm_Mtb), C-terminal ACAD domain of FadE34 (FadE34Cterm_Mtb) from *M. tuberculosis* H37Rv, N-terminal ACAD domain of CasC (CasCNterm_Mtb) and C-terminal ACAD domain of CasC (CasCterm_Mtb) from *R. jostii* RHA1.

	FadE26_Mtb	FadE27_Mtb	FadE28_Mtb	FadE29_Mtb	CasCNterm_Rjostii	CasCCterm_Rjostii	FadE34Nterm_Mtb	FadE34Cterm_Mtb
FadE26_Mtb	-	31% 14%	26% 13%	62% 45%	26% 13%	40% 24%	27% 15%	39% 25%
FadE27_Mtb	-	-	41% 25%	26% 12%	32% 22%	22% 12%	29% 19%	24% 13%
FadE28_Mtb	-	-	-	26% 14%	31% 19%	24% 13%	29% 17%	24% 12%
FadE29_Mtb	-	-	-	-	25% 14%	41% 25%	27% 16%	41% 26%
CasCNterm_Rjostii	-	-	-	-	-	20% 10%	52% 40%	19% 10%
CasCCterm_Rjostii	-	-	-	-	-	-	23% 10%	69% 49%
FadE34Nterm_Mtb	-	-	-	-	-	-	-	22% 12%
IVH_Human	43% 20%	37% 22%	29% 16%	41% 22%	33% 19%	32% 17%	30% 16%	33% 16%
LCAD_rat	36% 20%	30% 14%	28% 14%	36% 19%	28% 13%	29% 15%	26% 11%	29% 17%
IBH_human	38% 20%	31% 14%	30% 14%	39% 23%	31% 16%	32% 19%	30% 15%	34% 19%
SCAD_Melsdenii	38% 20%	36% 21%	32% 21%	38% 21%	33% 18%	35% 18%	32% 17%	33% 17%
MCAD_Pig	39% 21%	33% 16%	30% 16%	37% 20%	32% 17%	34% 19%	29% 18%	35% 20%

15 **Figure S1** Multiple sequence alignment of acyl-CoA dehydrogenases.

16 Amino acid sequences of proteins are as follows: short chain acyl-CoA dehydrogenase from *Megasphaera*

17 *elsdenii* (SCAD_Melsdenii), pig medium chain acyl-CoA dehydrogenase (MCAD_Pig), human isovaleryl-CoA

18 dehydrogenase (IVD_human), rat long chain acyl-CoA dehydrogenase (rat_LCAD), human isobutyryl-CoA

19 dehydrogenase (IBH_human), FadE26 (FadE26_Mtb), FadE27 (FadE27_Mtb), FadE28 (FadE28_Mtb), FadE29

20 (FadE29_Mtb), N-terminal ACAD domain of FadE34 (FadE34Nterm_Mtb), C-terminal ACAD domain of

21 FadE34 (FadE34Cterm_Mtb) from *M. tuberculosis* H37Rv, N-terminal ACAD domain of CasC

22 (CasCNterm_Mtb) and C-terminal ACAD domain of CasC (CasCterm_Mtb) from *R. jostii* RHA1. Fully

23 conserved residues are highlighted in red while functional residues within classical ACADs that are conserved

24 in steroid degrading ACADs are highlighted as follows: residues highlighted in green are the catalytic glutamic

25 acid residues and residues highlighted in purple are the arginine residues that interact with the pyrophosphate

26 FAD. Residues that form side chain interactions with isoalloxazine and adenosine ribose of FAD are

27 highlighted in yellow and the arginine residues that interact with CoA of substrate are highlighted in cyan.

```

MCAD_Pig
SCAD_Melsdenii
IBH_human
LCAD_rat
IVH_Human
FadE26_Mtb
FadE27_Mtb
FadE28_Mtb
FadE29_Mtb
CasCterm_Rjostii
CasCterm_Rjostii
FadE34Nterm_Mtb
FadE34Cterm_Mtb

```

1 10 20

```

MCAD_Pig
SCAD_Melsdenii
IBH_human
LCAD_rat
IVH_Human
FadE26_Mtb
FadE27_Mtb
FadE28_Mtb
FadE29_Mtb
CasCterm_Rjostii
CasCterm_Rjostii
FadE34Nterm_Mtb
FadE34Cterm_Mtb

```

30 40 50 60 70

```

MCAD_Pig
SCAD_Melsdenii
IBH_human
LCAD_rat
IVH_Human
FadE26_Mtb
FadE27_Mtb
FadE28_Mtb
FadE29_Mtb
CasCterm_Rjostii
CasCterm_Rjostii
FadE34Nterm_Mtb
FadE34Cterm_Mtb

```

80 90 100 110 120

```

MCAD_Pig
SCAD_Melsdenii
IBH_human
LCAD_rat
IVH_Human
FadE26_Mtb
FadE27_Mtb
FadE28_Mtb
FadE29_Mtb
CasCterm_Rjostii
CasCterm_Rjostii
FadE34Nterm_Mtb
FadE34Cterm_Mtb

```

130 140 150 160 170 180

```

MCAD_Pig
SCAD_Melsdenii
IBH_human
LCAD_rat
IVH_Human
FadE26_Mtb
FadE27_Mtb
FadE28_Mtb
FadE29_Mtb
CasCterm_Rjostii
CasCterm_Rjostii
FadE34Nterm_Mtb
FadE34Cterm_Mtb

```

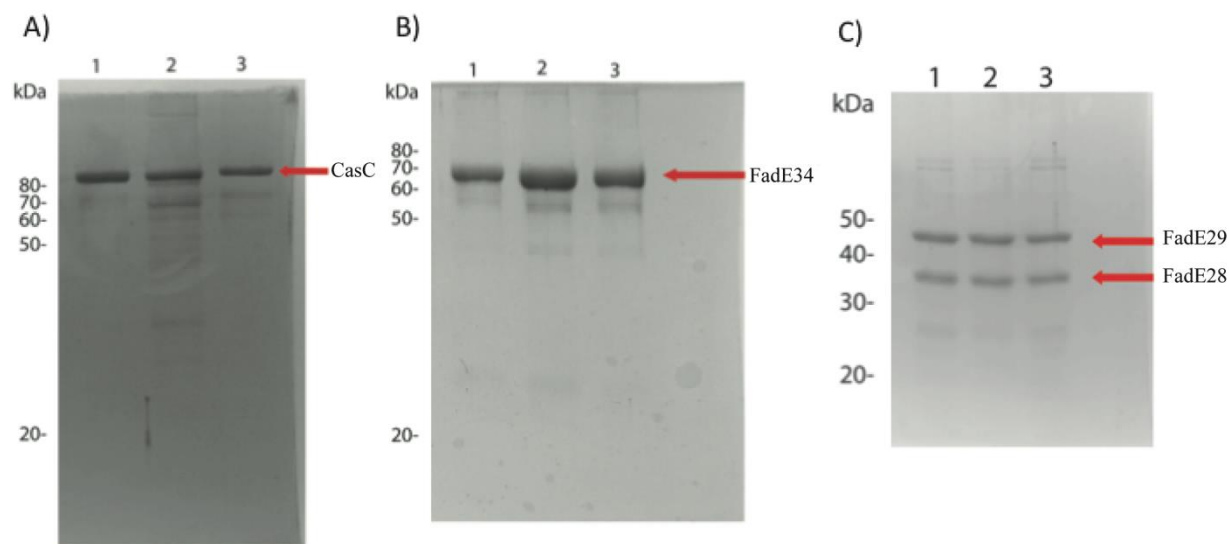
190 200 210 220 230 240

	250	260	270	280	290	300
MCAD_Pig	IAMGTFDKT	RPVVAAGAVGLAQRALDEATKYALE	R	KTFGKLLAEHQGISFLLADMAMKVE		
SCAD_Melsdenii	IAMMTLDGG	RIGVAAQALGIAEAAALADAVEYSKQ	R	VQFGKPLCKFQSFKFLADMAMKQIE		
IBH_human	IAVRGLNGG	RINIASCSLGAHAASVILTRDHLNVR	K	QFGEPLASNQYLQFTLADMATRLV		
LCAD_rat	YLMQELPQ	ERLLIADLAISACEFMFEETRNYVRQ	R	KAFGKTVAHQTVQHKLAELKTNIC		
IVH_Human	VLMSGLDL	ERLVLAGGPLGLMQAVLDHTIPYLHV	R	EAFGQKIGHFQLMQGKMDMYTRLM		
Fade26_Mtb	LVTNQLNH	ERVALVSPAP		IFGCLREVREWAQNTKDAGGTRLIDSEWVQLNLARVHAKAE		
Fade27_Mtb	LG	TLSTLSRTAFQLGVLERGLQMTAEYART	R	EQFDRPIGSPQAVGQRLADGYIDVK		
Fade28_Mtb		QLALAVMGAYADGLVAGALRLTADYVAN	R	KQFGKPLSTFQTVAQAALAEVYIASR		
Fade29_Mtb	LITTQLNN	ERVMLGPAGR		FASIYDRVHAWAS		VPGGNGVTPIDHDDVKRALGEIRAIWR
CasCterm_Rjostii		RVRDLAATLFAAEASGVADWAVTTADYANV	R	HQFGRPIGQFGVQKRVARMLCLAE		
CasCterm_Rjostii	LARTTLAN	ERVSLSHDSS		LGSGGEALLTLAGELPGIDEEQLT		VLGKVLCDAQ
Fade34Nterm_Mtb		RVEDLAATVLAEEAAGVARWTLDTAVAYAKV	R	EQFGKPIGSPQAVKHLCAQMLCRAE		
Fade34Cterm_Mtb	LARTTLAN	ERVAMATGTA		LGNPMEELLKVLGDME		LDVAQQDRLGRILLIAQ

	310	320	330	340	350
MCAD_Pig	LARLSYQRAAWE	IDS	GRR	NTYYASIAKAYAADIANQLATDAVQVFG	
SCAD_Melsdenii	AARNLVYKAAACKKQE		GKP	FTVDAAIAKRVASDVAMRVTTAVQIFG	
IBH_human	AARLMVRNAAVALQE			ERKDAVALCSMAKLFATDECFAICNQALQMHG	
LCAD_rat	VTRAFVD	SCLQLHE		TKRLDSASASMAKYWASELQNTVAQCQVQLHG	
IVH_Human	ACROYVYNVAKACDE			GHC	TAKDCAGVILYSACATQVALDGIQCFG
Fade26_Mtb	VLKLINWELASSQSGP			KDAGPSPADASA	AKVFGTELATEAYRLLMEVLG
Fade27_Mtb	GLRLTLTQA	AARVAEDSLASR		ECPQPADIDVATAGFWAAEAGHRVAHTIVHVHG	
Fade28_Mtb	TIDLVAKSVIWR	LAED		LDAGDDLGVVLYGWWVTSQAPPAMQICHHLHG	
Fade29_Mtb	INELLNWQVASAG			EDINMADAATAKVFGERVQRAGRLAAEIVG	
CasCterm_Rjostii	QARVCAWDAARAMNP	GSSARAATAVPRCSAPEASLAAAVAGATAPDAFAVTKDCIQVVG			
CasCterm_Rjostii	SGGLMGLR	TTLRTIA		GGQPGAESSVAKLLGVEHIQVWVEVAMDWAG	
Fade34Nterm_Mtb	QADVAAAADAARAAAAD			SDGTQLSIAAAVAASIGIDAAKANAKDCIQVVG	
Fade34Cterm_Mtb	AGALLDRRIAELAVG			GQDPGAQSSVRKLI	GVRYRQALAEYLMVEVSD

	360	370	380	390	
MCAD_Pig	GNGFNTEYPVEKLMR	DAK	IYQIY	GTAGIQR	IIAREHIGRYKN
SCAD_Melsdenii	GYGYSEYYPVARHMR	DAK	ITQIY	GTNEV	QLMVTGGALLR
IBH_human	GYGYLKDYAVQQYVR	DSR	VHQIL	GSNE	VMRILISRLLQE
LCAD_rat	GWGYMWEYPIAKAYV	DAR	VQPIYGG	TNE	IMKELIARQIVSDS
IVH_Human	GNGYINDFPMPGRFLR	DAK	LYEIGAG	TS	EVRRLVIGRAFNA DFH
Fade26_Mtb	TAATLRQNSPGALLRGRVERMHRACLILTFGGG	TNE	VQRDI	IGMVALGLPRANR	
Fade27_Mtb	GVGVDTDHPVHRYPFLAAKQTE		FALGGA	TGQLRR	IGRELAETPA
Fade28_Mtb	GMGMDVTPMHRYYSTIKDLT		RLLGGP	SHRLELLGARCSLT	
Fade29_Mtb	KYGNPAEPDTAELLR	WLDQAQTKRNLVITFGGGVNE	VMRE	MIAASGLKVPVPR	
CasCterm_Rjostii	GIGYTWEHDAHLYLR	RAQS	LRILLGS	TAS	WRRRVARATLG
CasCterm_Rjostii	PRALLGDQPRTSATQMFLNSQ		CMSIAGG	TN	QNLNIGERLLGLPRDPEPGKGA
Fade34Nterm_Mtb	GIGCTWEHDAHLYLR	RAHG	IGGF	LGG	SGRWLRRV
Fade34Cterm_Mtb	GGGLVENR	AVYDFL	NTR	CLTIAGG	TEQILLTVAERLLGL

28 **Figure S2** Coomassie blue stained SDS-PAGE gels of purified wild-type and variants of CasC,
29 FadE34 and FadE28-FadE29. A) CasC B) FadE34 C) FadE28-FadE29. Positions of molecular
30 weight markers are shown beside each gel. The wild-type proteins in each gel (CasC, FadE34
31 and FadE28-FadE29) are in lane 1, the arginine to alanine variants (CasCR239A, FadE34R236A,
32 FadE28R227A) are in lane 2, and the glutamate to glutamine variants (CasCE598Q,
33 FadE34E581Q, FadE29E241Q) are in lane 3.
34



35
36
37
38
39
40

41 **Figure S3** Multiple sequence alignment of CasC, FadE34 and other 2 domain ACADs.
42 Amino acid sequences of proteins are as follows: FadE34 from *M. tuberculosis* H37Rv
43 (FadE34_Mtb), CasC from *R. jostii* RHA1 (CasC_Rjostii), and homologs of FadE34/CasC from
44 *Mycobacterium smegmatis* JS623 (Mycsm00272_MsmegJS623, GI:505117216), *Mycobacterium*
45 *rhodesiae* NBB3 (MycrhN2240_MrhodNBB3, GI:503976649), *Rhodococcus erythropolis* PR4
46 (RER51020_ReryPR4, GI:527108386), *Rhodococcus equi* 103S (REQ43690_Requi103S,
47 GI:503182764), *Gordonia polyisoprenivorans* VH2 (GPOLc41540_GpolyVH2, GI:504129432)
48 and *Comamonas testosteroni* CNB-2 (CtCNB1299_CtestCNB2, GI:264677435). Fully
49 conserved residues are highlighted in red while the positions of the catalytic glutamic acid
50 residues and the arginine residues that interact with the pyrophosphate FAD are indicated with
51 blue asterisks.

52

53

54

55

56

57

58

59

60

61

62

63

64

65

66

67

68

69

FadE34_Mtb VVATVTEQSAARELVRGWARTAAASGAAATAAVRDMVEYGFEEGNADAWRPVFAAGL**AGL**CLFGVAVF**EDC**
CasC_Rjostii MTMGLTTEERDLRDSVRGWA...TRVTPAVLREAVE...AKTEQRPLFWKSL**ADL**GLVGLHLF**EDV**
Mycsm00272_Msmeg MPIAITSPEHNDLADSVQSLV...ARVAPSEVLHEALE...TPVSNPPPYWKAA**EQ**QLQGVHLS**ESV**
MycrhN2240_MrhodNBB3 MSATITDEQFAARELVRSWA...AASGAAA...AARDVE...QGQPDARAAAYDGL**AGL**GIFGVAIF**EEQ**
RER51020_ReryPR4 MTIGLSEEDRDLRDSVRGWA...ARHATPTAIREAVE...AKVETRPQLWSGL**EQ**GLLGLHVA**EEF**
REQ43690_RequiMIREAVE...AKTETRPGYWGS**LEL**GMLGLHLF**EEF**
GPOLc41540_GpolyVH2 MTLATSSEQLAVCEAIAAWS...ARVGTTDIARAQID...KPGEDWLGLFHQL**AEF**CLFAAVLP**ESK**
CtCNB1299_CtestCNB2 MNFDSDDDQNALRNEIRKFL...TRESPLTQARALLE...GEGHHAQDVWSGM**AGL**GVSSLMFL**EDC**

FadE34_Mtb GAGGSIEDLCAMVDEAARALVPGPVAT.....TAVATLVVSDPK...LRALASGER..FAGVAIDG
CasC_Rjostii GAGCGLVELAVVTEELGRVLLPGPFLL.....TVLLGAVLHESGR.TGELSGVADGST..LGAVALQP
Mycsm00272_Msmeg QQGFGLLELAIVLAEFGYVAVPGFVP.....SAIASALISANDRDAKLNDLNASGEV..IAAYAIDS
MycrhN2240_MrhodNBB3 GADGTVEDLCAMVDEAGAALVAGPVAT.....TALATLLIDRAD...VLEPLIAGER..VAGVSLTS
RER51020_ReryPR4 GAGYGLVELAIVVEELGRSMVPGTFLL.....TVVVSVAVLSADGV.TAPLAGLVADGST..LGAVALQP
REQ43690_Requi GAGYGLVELAIVTEELGRAMVPGPFLL.....TAVSVTLREAGR.TAELEGLDAGSL..FGAVALQP
GPOLc41540_GpolyVH2 GLGAGFTDLAAMLEQCGRDLVPGPIGE.....TAATATALTMTDD..PRAEELAAV..VAGEAPGV
CtCNB1299_CtestCNB2 GIGLGAMEMCVVAEVRQLSPVPLASTMYLAVQAVLLSTLQEQSAQRRLQWLLPVSVGSIGLCAAAFTDQG

FadE34_Mtb G...VQVDPKSTASGTVGRVL.GGAPGGVVL**LP**ADGN.....WLLVDTACDEVVVEPLRAT**D**FSLP
CasC_Rjostii GSLRLVRDGGVVTLDGESYVL.GGQVGDVFL**LA**ATDGDG.....TVYVVVARDRLDVTDLP**SH**DVVR
Mycsm00272_Msmeg G.LTATRHDGLVIRGEVRAVP.AAAQAALV**LV**PAIDSG.....EEMVLDADQLEIEPAK**S**DPLRP
MycrhN2240_MrhodNBB3 D...VTFD..GGRASGTAEVVL.GADPAGV**LV**LPAGDK.....VVLIDAGAGVTIEPLKAT**D**FSRP
RER51020_ReryPR4 GSLKIARDGDTATLSGTSSQIL.GGHVGDVFL**LA**TDG.....FVLLGRDRLEVTLDL**AS**YDVVR
REQ43690_Requi GGLKLVRDGGKTTLSGTSSHIL.GGQVGDVFL**LA**ASDDDDAAEGGLSFVVVARDRLDV**T**ELPS**H**DVVR
GPOLc41540_GpolyVH2 V...PVFDG**PL**PSIGDRV**EL**VVPGY**DA**AV**LV**LP**TV**DG.....WRILAPAYGMER**RT**V**ET**VP**D**GATP
CtCNB1299_CtestCNB2 EPPSGLPLFDGKTLKGECP**LV**ADGMAAQ**GV**LA**RN**ETGAD.....VLVAF**RC**DYGS**MR**TK**KL**L**T**D**PS**KP

FadE34_Mtb LARMVLT...SAPVT..VLEVSG.ERVEDLAATVLAEEAA**G**VARWTLDTAVA**YAKVRE**Q**FG**K**PT**CS**F**OAV
CasC_Rjostii SATAAANRLAIAEVD..VLQ**IDS**.QRVRDLAATLFAAAEAS**G**VADWAVTAA**YANVR**HQ**FG**RP**IC**Q**F**OAV
Mycsm00272_Msmeg LAHVRANAVEGEDR..VLSHL**SRT**LARALMSTLLSAEA**I**GVARWATDTASA**YAKI**EQ**FG**RP**IC**Q**F**OAV
MycrhN2240_MrhodNBB3 LGRVVL...SAPAE..ALS**AST**.QRPTDVAATVLAEEAA**G**LARWALQ**TATE**YAK**VR**EQ**FG**K**PT**CS**F**OAV
RER51020_ReryPR4 NSEVTVDGLIADSE..ILT**IDT**.QRVLDIAATLFAAAEAS**G**LADWAVTTSAD**YAKV**Q**FG**RP**IC**Q**F**OAV
REQ43690_Requi NAEVTADGLELSDGD..VLE**LDS**.QRILDIAATLFAAAEAS**G**LADWATTTAA**YARV**Q**FG**RP**IC**Q**F**OAV
GPOLc41540_GpolyVH2 VTRVTIEGL.EPDAP..IVC**ADS**.PLVDGVLALARN**A**FAS**G**VCRWALDTAV**G**YAK**VR**Q**FG**RP**IC**Q**F**OAV
CtCNB1299_CtestCNB2 YAWLRF**FD**GTA**EQ**LDGAS**AA**QV**LA**RVRNRAAV**ML**A**FE**Q**L**GAADA**AL**EM**ACS**Y**ARE**R**K**A**FG**RP**IC**Q**F**OAV

FadE34_Mtb **KH**L**CA**Q**ML**CR**AE**Q**AD**V**AA**DA**AR**AAA.....DSDGT**QL**SIAAAV**AS**IG**IDA**AK**AN**AK**DC**IQV
CasC_Rjostii **KH**RV**AR**ML**CL**AE**Q**AR**VC**AW**DA**AR**AN**MGSS**SARA**ATA**V**PRCS**AP**EA**SL**AAV**AG**AT**AD**PA**FA**V**T**K**DC**IQV
Mycsm00272_Msmeg **KH**K**CA**EM**I**AD**TER**ATA**AV**W**DA**AR**AI**DE**YS**.....ETGS**QD**S**AF**E**FA**AAV**AT**LA**PA**AA**LH**CT**QD**CIQV
MycrhN2240_MrhodNBB3 **KH**MC**AE**ML**LR**SE**Q**AS**V**AA**DA**AR**AV**S.....EDDER**QL**SIAV**AA**AG**IA**NA**AK**AN**AK**DCIQV
RER51020_ReryPR4 **KH**K**AR**ML**AL**AE**Q**AR**VC**AW**DA**AR**AL**TP.....ANGV**GA**EE**AS**LA**AA**V**AG**AT**AL**E**AG**FS**V**TR**DC**IQV
REQ43690_Requi **KH**K**AR**MF**SL**TE**Q**AR**VC**AW**DA**AR**AL**G.....EDVD**GT**E**AS**LA**AA**V**AG**AV**AL**E**SA**F**Q**V**T**K**DC**IQV
GPOLc41540_GpolyVH2 **KH**IC**AD**ML**CR**TE**Q**VE**AA**W**LD**AN**AL**DS.....AA**AD**DE**QL**SL**S**V**D**V**AD**IL**V**AD**LP**GA**V**TF**K**DC**V**QV
CtCNB1299_CtestCNB2 **KH**KL**AN**LY**TN**Q**L**AR**A**HC**Y**Y**GA**W**AL**T**AD**.....MAL**AD**GA**PE**LP**GA**AAA**AR**V**S**ST**Q**AL**SD**AA**Q**EN**L**HT

FadE34_Mtb LGGIGCT**WE**H**DA**HLYL**RR**AGIGGFLGGSGRWLRRV**T**ALT**QA**.....G**V**RRRL**GV**DL**LA**.
CasC_Rjostii LGGIGY**T**WE**H**DA**H**LYL**RR**A**Q**SLRILLGGSTAS**W**RRR**V**ARAT**LG**.....G**A**RRV**LG**IDL**PS**
Mycsm00272_Msmeg HGGIG**CT**WE**H**DA**H**LYL**RR**A**IM**LN**CF**RGAD**Y**P**Q**V**D**V**AT**S.....T**GL**R**K**ID**LD**LD**L**
MycrhN2240_MrhodNBB3 LGGIG**CT**WE**H**DA**H**LYL**RR**A**GI**E**H**FLGAG**Q**W**L**R**IA**T**L**T**Q**.....G**V**RR**LE**IDL**E**.
RER51020_ReryPR4 LGGIGY**T**WE**H**DA**H**LYL**RR**A**Q**SLRILLGGSTAS**W**RRR**V**AEL**T**IA.....G**T**RRV**LS**IEL**PP**
REQ43690_Requi LGGIG**CT**WE**H**DA**H**LYL**RR**A**Q**SLRILLGGSTAS**W**KRR**V**AN**L**T**ID**.....G**A**RRV**LS**IDL**PP**
GPOLc41540_GpolyVH2 LGGIG**CT**WE**H**DA**H**LYL**RR**S**AR**AR**AL**DAG**V**PAR**IS**L**S**Q**R**GLD.....G**GR**FE**IDL**S.
CtCNB1299_CtestCNB2 HGGIG**CT**WE**L**D**CH**LFY**RR**AR**Q**Q**AV**ELG**SI**H**AW**RE**Q**V**AV**EL**Q**K**RL**L**PA**K**ET**IG**Q**SS**AA**DR**Q**S**MD**F**ED**TP

FadE34_Mtb EVAGL**R**PE**IA**AA**VA**E**VA**AL**PE**EK**R**.....Q**VAL**AD**TC**LL**AP**H**W**P**AP**Y**C**.RGAS**PAE**
CasC_Rjostii DAED**I**RA**D**V**VA**EL**AP**AA**AM**GE**AE**R.....R**TYL**AE**K**GY**T**AP**H**L**P**T**PW**G.RGAG**A**VE
Mycsm00272_Msmeg DTE**K**L**RE**E**IR**AE**VA**AL**K**A**IP**RE**E**R.....T**VAL**AE**G**GW**V**P**H**L**P**K**P**W.GRAS**P**VE
MycrhN2240_MrhodNBB3 SVE**H**L**RE**PE**IA**S**AV**AE**VA**A**Q**PA**E**K**R**.....Q**VAL**AD**TC**LL**AP**H**W**P**RP**H.GEAG**S**PAE
RER51020_ReryPR4 EAE**A**T**RA**D**V**RA**EL**GP**AL**E**GA**EQ.....K**TYL**AE**K**GY**T**AP**H**L**P**TA**P**W.KSAD**A**VS
REQ43690_Requi EAE**Q**I**RA**D**V**RA**EL**GP**AL**E**GA**EQ.....K**AYL**AE**K**GY**T**SP**H**L**P**AP**W**.KGAD**AV**T
GPOLc41540_GpolyVH2 AVES**Q**RA**EV**RA**MA**E**K**V**RA**AP**E**SE**Q**.....R**RL**L**AE**S**G**Y**L**AP**H**W**P**PP**H**C.LGAD**PA**L
CtCNB1299_CtestCNB2 EE**AA**F**RA**EC**R**AW**L**Q**AN**A**E**PK**AS**AD**MY**FG**R**DM**T**AE**Q**R**ME**AA**R**L**W**Q**G**K**AA**AG**FG**A**IT**W**PK**ML**G**GR**GG**TP**M**Q

FadE34_Mtb QLLID**Q**EL**AA**AK**VER**PD**L**VIG**W**.W**AV**PT**IL**EH**GT**PE**Q**IER**F**VP**AT**MR**GE**FL**WC**Q**L**F**SE**FP**GA**GS**DL**AS**LR**T
CasC_Rjostii QLVIA**E**EL**RA**AA**L**K**PH**DM**I**GN.W**V**VP**TL**IA**H**GS**EE**QL**Q**R**F**VP**PS**LR**GD**LV**WC**Q**L**F**SE**FP**GA**GS**DL**AS**LR**T
Mycsm00272_Msmeg QIIIA**Q**E**FT**T**G**K**V**RR**PN**MG**IA**.W**L**IP**SV**V**AF**GT**EE**Q**K**Q**R**FL**P**PT**LR**G**EM**I**WC**Q**L**F**SE**FP**GA**GS**DL**AS**LR**T
MycrhN2240_MrhodNBB3 QLLID**Q**EL**AK**T**D**V**VR**PD**L**VIG**W**.W**AV**PT**IL**EH**GS**PE**Q**IE**K**F**V**PA**T**LR**GE**FL**WC**Q**L**F**SE**FP**GA**GS**DL**AS**LR**T
RER51020_ReryPR4 QLVIA**E**EL**RA**AK**IK**PH**DM**I**GN**.W**V**IP**TL**IA**H**GN**EE**QL**A**K**F**VP**PS**LR**GD**LV**WC**Q**L**F**SE**FP**GA**GS**DL**AS**LR**T
REQ43690_Requi QLVIA**E**EL**RA**AA**L**V**PH**DM**I**GN.W**V**IP**TL**IA**H**GN**AE**Q**IE**R**F**VP**Q**SLR**GD**LV**WC**Q**L**F**SE**FP**GA**GS**DL**AS**LR**T
GPOLc41540_GpolyVH2 QLLID**A**E**FD**RA**GV**VR**PD**L**V**IG**W**.W**AV**PT**IL**EH**GT**DA**Q**R**DR**F**V**AP**T**LA**GD**LI**WC**Q**L**F**SE**FP**GA**GS**DL**AS**LR**T
CtCNB1299_CtestCNB2 ELI**WR**Q**E**GG**K**V**K**VP**T**GM**F**N**V**SL**GM**VL**PT**SM**A**H**AS**AE**VL**L**H**V**AP**AL**GG**KN**L**WC**Q**L**F**SE**FP**GA**GS**DL**GM**VR**T**

FadE34_Mtb K**AV**RA**D**G.....G**W**LL**T**G**Q**K**V**W**T**S**A**A**H**K**AR**W**CV**L**AR**T.D**P**D**A**P**K**H**K**G**IT**Y**F**L**V**D**M**T**T**P**G**I**E**I**R**P
CasC_Rjostii R**AT**K**V**D**G**.....G**W**V**L**T**G**Q**K**V**W**T**S**A**A**R**S**D**AD**W**G**I**C**L**AR**T.D**G**S**V**P**K**H**K**G**IT**Y**F**L**D**M**S**G**S**D**G**L**D**I**R**P
Mycsm00272_Msmeg K**AT**K**V**D**G**.....G**W**R**I**T**G**Q**K**I**W**T**G**A**Q**L**S**Q**W**M**L**L**AR**T.D**P**S**A**P**K**H**N**G**IT**Y**F**L**D**M**K**S**E**G**VE**V**K**P
MycrhN2240_MrhodNBB3 K**AV**RA**E**G**V**S**AS**G**T**K**S**G**W**K**L**T**G**Q**K**V**W**T**S**A**A**H**K**A**AW**G**V**C**L**AR**T**.D**P**D**A**P**K**H**K**G**IT**Y**F**L**D**M**K**S**P**G**ID**I**R**P
RER51020_ReryPR4 K**AT**K**V**D**G**.....G**W**K**L**Q**G**Q**V**W**T**S**M**A**R**DA**H**W**G**I**C**L**AR**T.D**A**D**A**P**K**H**K**G**IT**Y**F**L**D**I**K**N**S**.G**L**D**I**R**P**
REQ43690_Requi K**AT**K**V**D**G**.....G**W**K**L**N**G**Q**K**V**W**T**S**M**A**RE**AD**W**G**I**C**L**AR**T.D**A**D**A**P**K**H**K**G**IT**Y**F**L**D**I**K**N**S**.G**L**D**I**R**P**
GPOLc41540_GpolyVH2 R**AT**R**T**D**G**.....G**W**M**L**T**G**Q**K**I**W**T**S**A**A**H**N**A**Q**W**G**I**C**L**AR**NG**D**G**T**P**K**H**K**G**IT**Y**F**L**V**D**M**AS**P**.G**ID**I**R**P
CtCNB1299_CtestCNB2 R**A**E**R**AT**D**.....G**R**E**W**L**V**N**G**Q**K**V**W**T**S**L**A**Q**F**A**Q**E**G**L**V**L**T**R**T**.N**P**Q**AS**K**FE**G**L**T**T**F**L**D**M**K**S**P.G**IT**V**R**P


```

540      550      560      570      580*      590      600
FadE34_Mtb      LREITGDSL FNEVFLDN VFVPEDEMVGAVNDGWRLAR TLANERVAMAT .GTALGNPMEELLKVLGDME .
CasC_Rjostii    LREITGEAL FNEVFLDD VFVPEDELLVGEFGDGWKLAR TLANERVSLSH .DSSLGSGGEALLTLAGELPG
Mycsm00272_Msmeg LREITGNAM FNEVFI DD VFVPEDDLVLGEVNRGWEVSR TLANERVSI GNSEAPFLANLNGFVEFIRDGH .
MycrhN2240_MrhodNBB3 LREITGDNLFNEVFFDD VFVPEDEMVGQVNDGWRLAR TLANERVAMAH .GTALGNPMEELLRTVAELE .
RER51020_ReryPR4 LREITGEAL FNEVFLDD VFVPEDECLVGEFGDGWKLAR TLANERVSLSN .DSSLGSGGEALLSLVDGLPG
REQ43690_Requi  LREITGEAL FNEVFLDD VFVPEDECLVAEFGDGWKLAR TLANERVSLSH .DSSLGSGGEALLDLAASIPG
GPOLc41540_GpolyVH2 LRELTGRAN FNEVFLDE VFVPEDCVVGVEVGGWWRLAR TLANERVAMG .GSLGKEMESLLHQISGMSR
CtCNB1299_CtestCNB2 IRQAGGESE FNEVFFED VFVPEDSQMVGKLGSGWKVTL TGLMAERLAIGGVMPAELWRTTAGLLADHRFDG

610      620      630      640      650      660      670
FadE34_Mtb      . . . . LDVAQQDR LGR L I L L A Q A G A L L D R R I A E L A V G G Q D P G A Q S S V R K L I G V R Y R Q A L A E Y L M E V S D G G G
CasC_Rjostii    G . . . I D E E Q L T V L G K V L C D A Q S G G L M G L R T T L R T I A G G Q P G A E S S V A K L L G V E H I Q V W E V A M D W A G P R A
Mycsm00272_Msmeg . . . . F D Q I D Q N R A G R L I A E G H A A K L L N M R S T L L T L A G D P M P A A A I S K L L S M R T G Q S Y A E F A V S S F G T D G
MycrhN2240_MrhodNBB3 . . . . L D S T G Q Q L G T L I L A A Q V G S L L D Q K I A E L L V G Q D P G P Q A S A R K L I G V R Y R Q A L S E L R M E L S P G G G
RER51020_ReryPR4 G . . . I D D E Q L T V L G K V L C D A Q S G G L L G L R T T L R S L T G A Q P G A E S S V A K L I G V E H I Q V W D V A M E W A G P S S
REQ43690_Requi  G . . . I D D E Q M T V L G K V L C D A Q S G G L M G L R T T L R S L T G A Q P G A E S S I A K L L G V E H I Q V W E V A M E W A G P S S
GPOLc41540_GpolyVH2 P . . . L E R A E Q A R L G R L V A D A H I G R V L D A R A V T R R L A G H D P G A L S N V R K L I G V A H R Q S V P E A A L D F L G L D A
CtCNB1299_CtestCNB2 R P A L Q D G R L R E R W A D L Y L K E Q A L W L L Q C R A L T A L S K G R Q P G P E M S G A K N V A A A A L Q S F S Y F A I D L L G E R G

680      690      700      710
FadE34_Mtb      LVEN . . . . . R A V Y D F L N T R C L T I A G G T E Q I L L T V A A E R L L G L P R . . . . .
CasC_Rjostii    L L G D Q P . . . R T S A T Q M F L N S Q C M S I A G G T T N V Q L N I I G E R L L G L P R D P E P G K G A . . . . .
Mycsm00272_Msmeg A I G D P D Q . . L Q G Q L A Q M L L A S R A T T I Y G G T S E V Q L N I I A E R L L G L P R D P . . . . .
MycrhN2240_MrhodNBB3 V V D N . . . . . K T V F D F L N T R C L T I A G G T E Q I L L T M A G E R L L G L P R . . . . .
RER51020_ReryPR4 L L G E Q H . . . R M S A T Q M F L N S Q C M S I A G G T T N V Q L N I I G E R I L G L P R D P E P G K . . . . .
REQ43690_Requi  L L G E Q H . . . R R S A T Q M F L N S Q C M S I A G G T T N V Q L N I I G E R I L G L P R D P D P G K . . . . .
GPOLc41540_GpolyVH2 V I A G . . . . . G A S D L A L Q N R C L S I A G G T T Q I L K T A A A E R I L G L P R A . . . . .
CtCNB1299_CtestCNB2 V L A A S E L G E R F A M V E R L W F G S A G M R I A G G T D E V V L N S I G E R V L G L A A E P R A D K D L P F C E L L A

```