Supplementary Information

Structure of a human intramembrane ceramidase explains enzymatic dysfunction found in leukodystrophy

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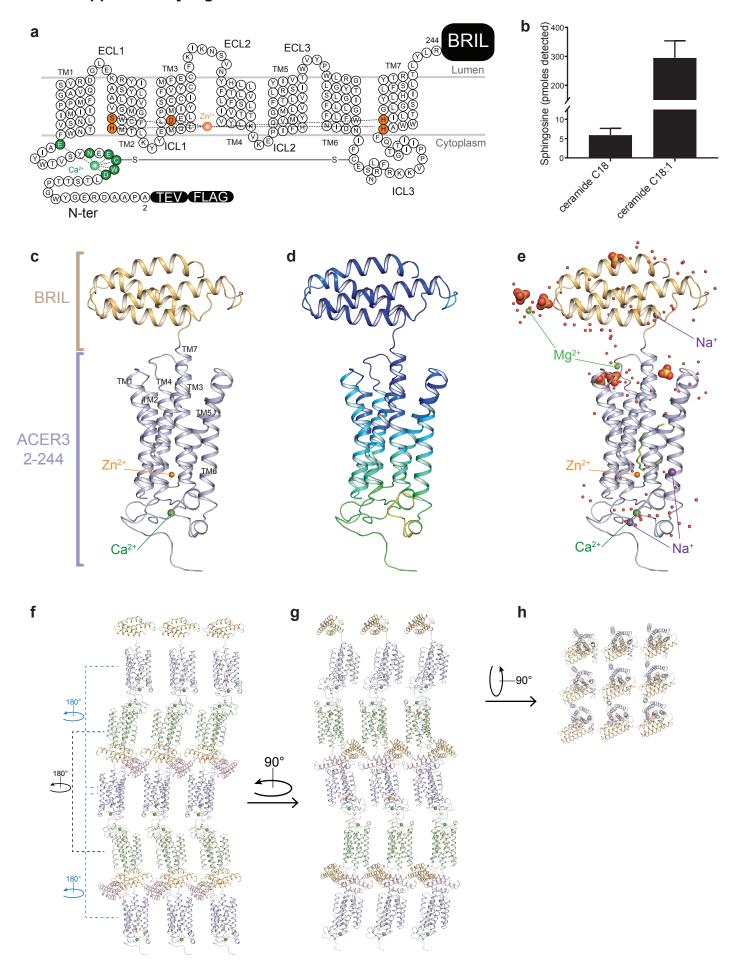
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Supplementary Table 1. List of primers used for site directed mutagenesis of ACER3-BRIL

Mutant	Forward Primer (5'-3')	Reverse Primer (5'-3')
D19G	CAACGTTGGGTTGGTGTGAAGAGAAC	ATGTGGTAGGTCCCCAATA
E22G	CAACGTTGGATTGGTGTGGAGAGAAC	Same as for D19G mutant
N24G	GTGTGAAGAGGGCTATTCAGTGACTTGG	CAATCCAACGTTGATGTG
E33G	GTATATTGCCGGATTCTGGAATAC	CAAGTCACTGAATAGTTCTC
S99A	GATTTACGCTTGCTGCATCTTCG	ATCGGTAACTCATCCAGTAAC
Y149A	CAAGTCATGGCCGGAATGCTCGTG	GTGGAATATGGGCTCTTTGA
S228A	GACTGGTCTTGGTGCCTATCTCCACA	AGTATGTGCCACCATGCGTG

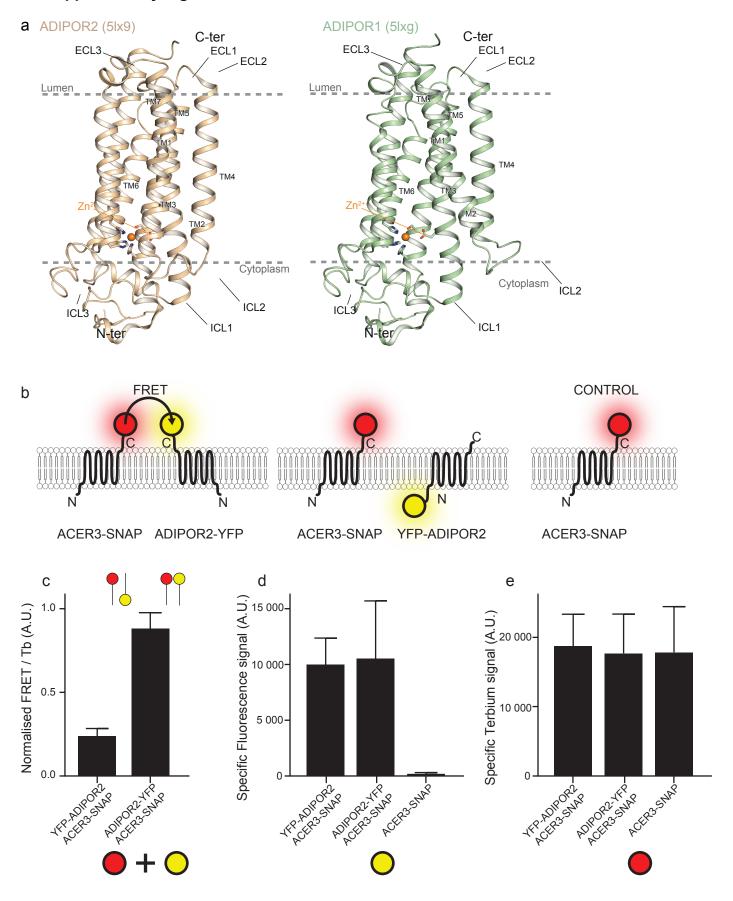
Supplementary Figure 1.



Supplementary Figure 1. ACER3-BRIL crystal structure.

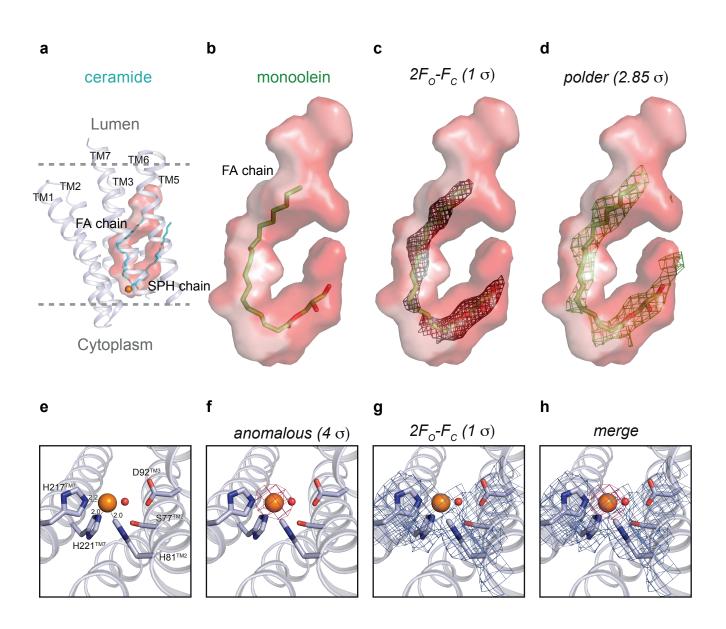
(a) Snake plot presenting the crystallized ACER3-BRIL construct and highlighting the residues coordinating Ca^{2+} and Zn^{2+} (green and orange circles, respectively). The BRIL module fused to the C-teminus as well as N-terminal Flag-tag and TEV cleavage site are shown as black rectangulars. 23 residues at the C-terminal end of ACER3 were truncated to get well diffracting crystals. (b) Detected sphingosine in LC-MS/MS analysis revealed that ACER3-BRIL hydrolyzes C18:1 ceramide substrate in a preferred manner over C18:0 ceramide in detergent micelles. Relative sphingosine values are represented as the mean ± SD of three independent measurements performed in six replicates. (c,e) ACER3-BRIL crystal structure viewed within the membrane plane with ACER3 and BRIL coloured in light blue and wheat, respectively. (d) Cartoon representation of ACER3-BRIL structure coloured according to the crystallographic B factors revealing increased flexibility at the Nterminus of ACER3. Regions of lowest and highest B-factor are shown in blue and yellow, respectively. Lattice packing of ACER3-BRIL crystals viewed within the membrane plane (f, g) and extracellular side (h). The ACER3-BRIL fusion protein crystallized with an anti-parallel arrangement of the molecules. ACER3 and BRIL are coloured in light blue and wheat or in light green and light purple, respectively.

Supplementary Figure 2.



Supplementary Figure 2. Comparisons of the 7TM fold and topology of ACER3 and ADIPORs.

(a) Crystal structures of ADIPOR1 (PDB 5LXG) and ADIPOR2 (PDB 5LX9) shown as light green and dark yellow, respectively, view from the membrane side. This view shows the similar 7TM fold and topology between ACER3 and AdipoRs. (b) Schematic representation of the different transfection conditions highlighting the positions of the fluorescent tags used to monitor the topology of ACER3 and ADIPOR2 by Fluorescence Resonance Energy Transfer (FRET) in living cells. (c) Graph representing the quantification (as described in the methods section) of the specific FRET normalized by the Terbium signal alone. Signal is higher when ACER 3 and ADIPOR2 have tags in the same orientation. Quantification of specific fluorescence and Terbium signals are represented in (d) and (e), respectively, and do not show changes between conditions. The results shown are the mean ± sem of three independent experiments performed in triplicate.



Supplementary Figure 3. Calculated electron density maps and tentatively modeled ligands.

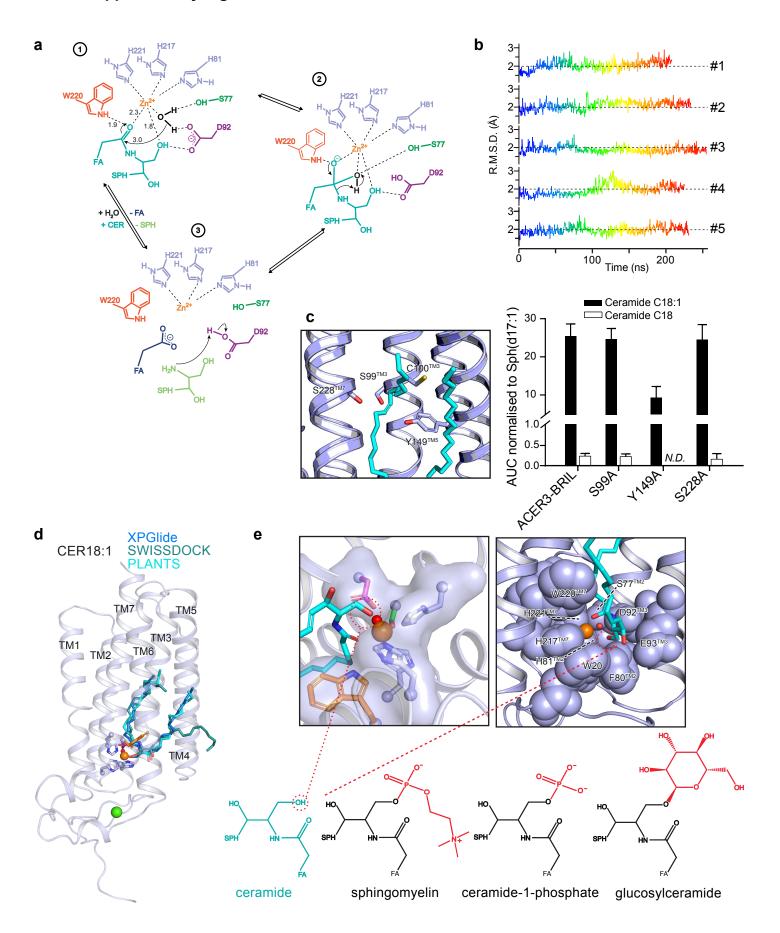
(a) Representation of the hook-shaped cavity within the ACER3 7TM with the ceramide binding pose. (b) (c) Position of the modeled monoolein and representation of the calculated 2Fo-Fc map contoured at 1 σ (b) as well as the polder OMIT map (ref 21 in the main text) contoured at 2.85 σ (c). (e) Close-up view of the Zn²⁺ binding site in ACER3 with the anomalous difference maps of ACER3 calculated from the data set collected at Zn-edge (i.e., λ =1.281 Å) at a resolution of 3 Å and contoured at 4 σ (f), the 2Fo-Fc map contoured at 1 σ (g) and the superposition of both maps (h).

Supplementary Figure 4.

a		
Q9NUN7 ACER3_HUMAN		VSNLIMI-IPPMFGA-VQSVRDGLE <mark>K</mark> RYIASYLALTV <mark>VG</mark> MG <mark>S</mark> WCFHMTLKYEMQ
Q8TDN7 ACER1_HUMAN		F SNI PFFIFGPLMMLLMHPYAQKRSRYIYVVWVLFMIIGLFSMYFHMTLSFLGQ
Q5QJU3 ACER2_HUMAN Q8R4X1 ACER1_MOUSE	MHVPGTRAKM	ISNVLFFILPPICMCLFRQYATCFNSGIYLIWTLLVVVGIGSVYFHATLSFLGQ FSNVFFLIFGPLMMFLMHPYAQKRT <mark>R</mark> CFYGVSVLFMLIGLFSMYFHMTLSFLGQ
Q8VD53 ACER2_MOUSE	MGAPHWWDHLRAGSSEVDWCEDNYTIVPAIAEFYNT	ISNVLFFILPPICMCLFRQYATCFNSGIYLIWTLLVVVGIGSVYFHATLSFLGQ
Q9D099 ACER3_MOUSE M0R603 ACER1_RAT	MYAPSTRAKMSSIFAYQSSEVDWCESNFQHSELVAEFYNT	VSNLIMI-IPPIFGA-IQGIRDRLEKRYIAAYLALTVVGMGSWCFHMTLKYEMQ FSNVFFLIFGPLMMFLMHPYAQKRS <mark>W</mark> GIYGVSVLFMVIGLFSMYFHMTLSFVGQ
D3ZNW4 ACER2_RAT	MGAPH <mark>WW</mark> DQLRAGSSEVDWCEDNYTIVPAIAEFYNT	ISNVLFFILPPICMCLFRQYATCFNSGIYLIWTLLVVVGIGSVYFHATLSFLGQ
F1M6P3 ACER3_RAT F1MGH9 ACER1_BOVIN	WAPAVDRKGYWGPTTSTLDWCEENYVVTLFVAEFWNT VEMPSVFAYOSSEVDWCEPNFOYSOLVAEFYNT	VSNLIMI-IPPIFGA-IQGFRDRLE <mark>K</mark> RYIAAYVALTVVGMGSWCFHMTLKYEMQ FSNVTFFIF <mark>GP</mark> LMTFLMRPYIQQRS <mark>R</mark> YLYVLFVLFTVTGLFSMYFHMTLSFLGQ
E1B8N2 ACER2_BOVIN	MSAPHWWDOLRAGSSEVDWCEDNYTIVPAIAEFYNT	ISNVLFFILPPICMCLFRQYATCFNSGIYLIWTLLVVVGIGSVYFHATLSFLGQ
A7MBH7 ACER3_BOVIN F1NLL6 ACER1_CHICK	MAPAGDREGYWGPTTSTLDWCEENYAVTWYIAEFWNT	V SNLIMI-LPPIFGA-MQSVRSGLEKRYIASYLALTVVGMGSWCFHMTLKYEMQ ISNVCFFILSAALLHLNRQYCQQRTVPMYFISGLLLCVGIFSMYFHMTLSYVGQ
E1C990 ACER2_CHICK	MGAMLWWDOLRAGSSEVDWCEDNYTIVPAIAEFYNT	ISNVLFFVLPPICMCLFRQYATCFNSGIYLIWTLLVVVGIGSVYFHATLSFLGQ
E1C413 ACER3_CHICK A0A1S3T5X1 ACER1_SALSA	MAPAADREGYWGPPTSTLEWCEENYAVSYYIAEFWNT	V _{S N L} IFI-LPPIYGA-IRTYKDGLE <mark>K</mark> RYLAAYLCLTAVGLGSWCFHMTLKYEMQ MSSLFFFVISPIMLYLLHPYAR <mark>ERNL</mark> AVHLVWIMMIFVGLFSAYFHMTLSFMGQ
A0A1S3RGK9 ACER2_SALSA		ISNILFFVLPPILMCLFRQYATHFNSGIYLIWILLVVIGIGSTYFHATLSFLGQ
A0A1S3LR05 ACER3_SALSA Q56812 ACER1_DANRE	MAPSADBLGYWGBPTSTLDWCEENYVVSFYIAEFWNT	VSNLIMI-LPPIYGA-IQTYKDGLE <mark>F</mark> RYVYSFLGLAA <mark>VGIGSWCFHMTLQYEMQ</mark> MSSFIFFVISPIMLYLLHPYAK <mark>ERNL</mark> AVHLVWIMMVF <mark>V</mark> GIFSMYFHMTLSFMGQ
E7FCF4 ACER2_DANRE	MMMMDAHLWDHLQAGSSEVDWCEGNYLIYPGIAEFYNT	ISNVLFFVLPPILMCLFRQYATHFNSGIYLIWTLLVVVGIGSTYFHATLSFLGQ
E9QEA6 ACER3_DANRE	MAPAADRPGYWGTPTSTLDWCEENYVVSYYIAEFWNT	VSNLIMI-LPPIYGA-IQTCRDGLEVRYVWSFLGLAAVGIGSWSFHMTLQYEMQ
Q9VIP7 ACER_DROME P38298 YPC1_YEAST	MGIFRWNYPESSVPGVWGETTSTIDWCEENYVVSPYIAEWSNT	FSNFLFILLPPVLIMLFKEYGRFVT <mark>P</mark> GIHVIWVLLIVVGLSSMYFHATLSLIGQ LTNSVFI-LSAIYTT-YSAYKNKLE <mark>K</mark> RFLLIGFGYGL <mark>VGVGSWLFHMTLKYRFQ</mark>
Q02896 YDC1_YEAST	MLFSWPYPEAP <mark>IE</mark> GYWGKP <mark>T</mark> SLIDWCE <mark>ENYVVSPYIAEW</mark> SNT	ITNSIFL-MTAFYST-YSAWRNKLE <mark>T</mark> RYILIGMGFSL <mark>VG</mark> IG <mark>SWLFHMTLQ</mark> YRYQ
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Q9NUN7 ACER3_HUMAN Q8TDN7 ACER1_HUMAN	LIDETATINLLCSCYSTWMPRCYFPS-FLCCNRSOFTPL	LFTLVLFSLIVTTVYLKVKEPIFHQVMYGMLVFTLVLRSIYIVTWVYPW VFITTVVSTLLSFLRPTVNAYALNSIALHILYIVCQE-YRKTSNK
Q5QJU3 ACER2_HUMAN	MLDELAVLWVLMCALAMWFPRRYLPK-IFRNDRGRFKVV	VSVLSAVTTCLAFVKPAINNISLMTLGVPCTALLIAE-LKRCDNM
Q8R4X1 ACER1_MOUSE Q8VD53 ACER2 MOUSE	LLDEISILWLLASGYSVWLPRCYFPK-FVKGNRFYFSCL MLDELAILWVLMCALAMWFPRRYLPK-IFRNDRGRFKAV	VSVLSAVTTCLAFVKPAINNISLMTLGVPCTALLIAE-LKRCDNM VTITTIISTFLTFVKPTVNAYALNSIAIHILYIVRTE-YKKIRDD VCVLSAITTCLAFIKPAINNISLMILGUPCTALLVAE-LKRCDNV
Q9D099 ACER3_MOUSE	LIDDLPMIXSCCIFVYCMFECFKTKSSINXHL	LFTLFLISLIVTIIIILKVKEPIPHQVMIGMLVPILVLKSIIIVTWVIPW
MOR603 ACER1_RAT D3ZNW4 ACER2_RAT	LLDEISILWLLASGYSVWLPRCYFPK-FIKGSRFYFSCL MLDELAILWVLMCALAMWFPRRYLPK-IFRNDRGRFKAV	VIMTTIISTFLTFVKPTVNAYALNSIAIHILYIVRKE-YKKTSNR VCVLSAVTTCLAFVKPAINNISLMILGVPCTALLIAE-LKRCDNV
F1M6P3 ACER3_RAT	LLDELPMIYSCCIFVYCMFECFKTKSSINYHL	LFTLVLFSLIVTTIYLKVKEPIFHQVMYGMLVFALVLRSIYIVTWVYPW
F1MGH9 ACER1_BOVIN	MLDEIAILWLLASGYSIWLPRCYFPA-FLGQNRSRYSSL	IIIISLVSTFLSFLRPTINAYALNAIGLHIIYIVVQE-YKKTKNK VCVLSAVTTCLAFVKPAINNISLMTLGVPCMALLIAE-LRRCDNV
E1B8N2 ACER2_BOVIN A7MBH7 ACER3_BOVIN	LLDELPMIYSCCIFVYCMFECFKMKKSVNYHL	LFTLVLFSLIVTMVYLKVKEPIFHQVMYGMLVFTLVVRSIYIVTWVYPW
F1NLL6 ACER1_CHICK	LLDELSILWTLAVAYSFWYPRAHFPK-CIKSRKH-FYWL	GGVTTVITTVMSFIKPSINAYALNCIAFHLLYLTWRE-LKKCNDQ VGVLSGVTTCLAFIKPAINNISLMTLGVPCTALLIAE-LKRCENL
E1C990 ACER2_CHICK E1C413 ACER3_CHICK	LLDELPMIYSCCVFVYCLYECFKY KNT V NYPM	LFILITYSFIVSIVYLKLKOPVFHOIMYGTLVSVIVLRSVYIVLWVYPW
A0A1S3T5X1 ACER1_SALSA	MLDELSILWVLGLCYGLWFPRRLFPS-FIKDRTT-FSRL	VMMITVVTTLCSFVKPTANAYLLNCFALHIIYSLGLE-MRTCTDP IGILSGITTGLAFVKPVVNSVSLMTLGIPCTVLLITE-LKRCENP
A0A1S3RGK9 ACER2_SALSA A0A1S3LR05 ACER3_SALSA	LLDELPMIYSTCVFVYCLYECFKQQNTLGVFP	IMLLFIFSVSVVYLQWKEPVFHQVMYGALVACLVMRSIFIVTWVYPW
Q56812 ACER1_DANRE	MLDELSILWVLAIGYSLWFPRKHFPS-FVKDRTS-FARL	IMLLFIFSVSVSVVYLQWKEPVFHQVMYGALVACLVMRSIFIVTWVYPW VLTITIISTLSSFVKPTANAYALNCFAIHILYSLFVE-LKSCTDE
E7FCF4 ACER2_DANRE E9QEA6 ACER3_DANRE		'IGVLSGITTCLAFIKPAINSITLMTLGIPCTALLITE-LKRCDNL IILLLTFSIIVSVIILLWKEPVFHQVMYAVLVAFLVIRSVFIVTWVYPW
Q9VIP7 ACER_DROME	LLDELAILWVFMAAFSLFYPKRYYPK-FVKNDRKTFSWL	MLLSAIAATGLSWWKPIV <mark>NA</mark> FVLMF <mark>-</mark> MSVPTMVMLYTE-LQRVSDQ
P38298 YPC1_YEAST Q02896 YDC1_YEAST		GVIIGLAVTTASILYVIYKNVDIHQILFGVQIVVVAATAGSLTYRYVHDPLAKR SFIMCGIVTILTWIYVVVQKPAIFQVLYGILTLLVVVLSGWLTYYHVHDSFAKK
		* *
Q9NUN7 ACER3_HUMAN	-LRGLGYTSLGIFLLGFLFWNIDNIFCESLRNFRK-KVPPIIGITT	QFHAWWHILTGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPVILFEPL
Q8TDN7 ACER1_HUMAN Q5QJU3 ACER2 HUMAN	ELRHLIEVSVVLWAVALTSWISDRLLCSFWQRIHFF	YLHSIWHVLISITFPYGMVTMALVDANYEMPGETLKVRYWPRDSWPV- YLHCMWHILICLAAYLGCVCFAYF <mark>DAA</mark> SEIPE <mark>O</mark> GPVIKFWPNEKWAFI
Q8R4X1 ACER1_MOUSE	DLRHLIAVSVVLWAAALTSWISDRVLCSFWQRIHFY	YLESIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVHYWPRDSWVI-
Q8VD53 ACER2_MOUSE		
09D099IACER3 MOUSE	RVFKLGLFSGLWWTLALFCWISDQAFCELLSSFHFP	YLHCVWHILICLASYLGCVCFAYF <mark>DAA</mark> SEIPEQGPVI RFWPSEKWAF I OFHAWWHILFGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPAVMFEPO
Q9D099 ACER3_MOUSE M0R603 ACER1_RAT	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAALTSWVSDRVLCSFWQRIQFF	QFHAWWHILTGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVHYWPRDSWLI-
M0R603 ACER1_RAT D3ZNW4 ACER2_RAT	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAALTSWVSDRVLCSFWQRIQFF RVFKLGFSGLWWTLALFCWISDRAFCFLSSFHFP	QFH_AWWHILWGLGSYLHILFSLYRTLYLRYRPKVKFLFGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVHYWPRDSWLI- VIHCVWHILWCLAXYLGCVCFAYFDAASEIPEGGPVIRFWPSEKWAFI
M0R603 ACER1_RAT D3ZNW4 ACER2_RAT F1M6P3 ACER3_RAT F1MGH9 ACER1_BOVIN	-LRGLGYTSLTVFLLCFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHIIAVSVILWAAATTSWVSDRVLCSFWQRIQFF RVFKIGLFSGLWWTLAIFCWISDRAFCELLSSFHFP LRGLGYTSLTVFLLCFLLWNVDNIFCDSLRNFRK-TVPPVLGVAT	QFHAWWHILWGLGSYLHILFSLYRTLYLR-YRPKVKFLFGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVFHYWPRDSWLI- YLHCVWHILICLAAYLGCVCFAYFDAASEIPEQGPVIRFWPSEKWAFI QFHAWWHILTGLGSYLHILFSLYRTLYLR-YRPKVKFLFGIWPMVMFEPQ YLHSIWHVLHSFFFPYGWVILALVDSEYEMPNKTLKVRYWPRDTWPM-
MOR603 ACER1_RAT D3ZNW4 ACER2_RAT F1M6P3 ACER3_RAT F1M6H9 ACER1_BOVIN E1B8N2 ACER2_BOVIN	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAALTSWVSDRVLGSFWQRIQFF RVFKLGLFSGLWWTLALFCWISDRAFCELLSSFHFP LRGLGYTSLTVFLLGFLLWNVDNIFCDSLRNFRK-TVPPVLGVAT ELQHLIEVSTVIWALAFTSWISDRLLGSFWQWINFS RVFKLGLFSGLWWTLAFTCWITDRAFCELLSSVHFP	QFHAWWHILWGLGSYLHILFSLYRTLYLRYRPKVKFLFGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVHYWPRDSWLI- YLHCVWHILICLAAYLGCVCFAYFDAASEIPEQGPVIRFWPSEKWAFI QFHAWWHILTGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPMVMFEPQ YLHSIWHVLISFTFPYGMVILALVDSEYEMPNKTLKVRYWPRDTWPM- YLHCVWHILICLAAYLGCVCFAYFDAASEIPEQGPVIRYWPRDTWPM- JLHCVWHILIGLGSYLHILFSLYRTLYLRYRPKVKFLFGIWPVILFEPL
MOR603 ACER1_RAT D3ZNW4 ACER2_RAT F1M6P3 ACER3_RAT F1MGH9 ACER3_BOVIN E1B8N2 ACER2_BOVIN A7MBH7 ACER3_BOVIN F1NLL6 ACER1_CHICK	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHIIAVSVILWAAATTSWVSDRVLGSWQRIQFF RVFKLGLFSGLWWTLALFCWISDRAFCELLSSFHFP -LRGLGYTSLTVFLLGFLWNVDNIFCDSLRNFRK-TVPPVLGVAT ELQHLIEVSTVIWALAFTSWISDRLLCSFWQWINFS RVFKLGLFSGLWWTLALFCWITDRAFCELLSSVHFP	QFHAWWHILWGLGSYLHILFSLYTRTLYLR-YRPKVKFL-FGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVHYWPRDSWLI- YLHCVWHILICLAAYLGCVCFAYFDAASEIPEQGPVIRFWPSEKWAFI QFHAWWHILTGLGSYLHILFSLYTRTLYLR-YRPKVKFLFGIWPMVMFEPQ YLHSIWHVLISFTFPYGMVILALVDSEYEMPNKTLKVRFWPRDTWPM- YLHCVWHILICLAAYLGCVCFAYFDAASEIPEQGPVI
MOR603 ACER1_RAT DZ2NW4 ACER2_RAT F1M6P3 ACER3_RAT F1M6P9 ACER1_BOVIN E1B8N2 ACER2_BOVIN A7MBH7 ACER3_BOVIN F1NLL6 ACER1_CHICK E1C990 ACER2_CHICK	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHIIAVSVILWAAAITSWVSDRVLGFWQRIQFF RVFKLGLFSGLWWTLALFCWISDRAFCELLSSFHFP	QFHAWWHILIGLGSYLHILFSLYTRTLYLR-YRPKVKFL-FGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVFGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVRFWPSEKWAFI QFHAWWHILIGLGSYLHILFSLYTRTLYLR-YRPKVKFL-FGIWPMVMFEPQ YLHSIWHVLISFTFPYGMVILALVDSEYEMPNKTLKV
MOR603 ACER1_RAT D32NW4 ACER2_RAT F1M6P3 ACER3_RAT F1M6H9 ACER1_BOVIN E1B8N2 ACER2_BOVIN A7MBH7 ACER3_BOVIN F1NLL6 ACER1_CHICK E1C413 ACER3_CHICK E1C413 ACER3_CHICK	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAALTSWVSDRVLCSFWQRIQFF RVFKLGLFSGLWWTLALFCWISDRAFCELLSSFHFP -RGLGYTSLTVFLLGFLLWNVDNIFCDSLRNFRK-TVPPVLGVAT ELQHLIEVSTVIWALAFTSWISDRLLCSFWQWINFS RVFKLGLFSGLWWTLALFCWITDRAFCELLSSVHFP	QFHAWWHILWGLGSYLHILFSLYRTLYLRYRPKVKFLFGIWPAVMFEPQ YLHGVWHILIGLGSYLHILFSLYRTLYLRYRPKVKFLFGIWPAVMFEPQ YLHGVWHILIGLGSYLHILFSLYRTLYLRYRPKVKFLFGIWPMVMFEPQ YLHGVWHILIGLGSYLHILFSLYRTLYLRYRPKVKFLFGIWPMVMFEPQ YLHGVWHILIGLGSYLHILFSLYRTLYLRYRPKVKFLFGIWPVILFEPL YFHSFWHVLIAVSLYCFPLVMYPDVTYEMPAFKPKLKFWPSERWAFI YFHSFWHVLIAVSLYCFPLVMYPDVTYEMPAFKPKL
MOR603 ACER1_RAT D32NW4 ACER2_RAT F1M6P3 ACER3_RAT F1M6H9 ACER1_BOVIN E1B8N2 ACER2_BOVIN A7MBH7 ACER3_BOVIN F1NLL6 ACER1_CHICK E1C413 ACER3_CHICK AOA1S3T5X1 ACER1_SALSA AOA1S3T5X1 ACER1_SALSA	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHIIAVSVILWAAATTSWVSDRVLGFWQRIQFF RVFKLGLFSGLWWTLALFCWISDRAFCELLSSFHFP	QFHAWHILIWGLGSYLHILFSLYTRTLYLR-YRPKVKFL-FGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVFGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVRFWPSEKWAFI QFHAWHILIGLGSYLHILFSLYTRTLYLR-YRPKVKFLFGIWPMVMFEPQ YLHSIWHVLISFTFPYGMVILALVDSEYEMPNKTLKVKFWPSERWAFI QFHAWHILIGLGSYLHILFSLYTRTLYLR-YRPKVKFLFGIWPVILFEPL YFHSFWVLIAVSLLYCFPLVMYFDVTYEMPAFKPKL
MOR603 ACER1_RAT D32NW4 ACER2_RAT F1M6P3 ACER3_RAT F1M6H9 ACER1_BOVIN E1B8N2 ACER2_BOVIN A7MBH7 ACER3_BOVIN F1NLL6 ACER1_CHICK E1C413 ACER3_CHICK E1C413 ACER3_CHICK	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHIIAVSVILWAAATTSWVSDRVLGFWQRIQFF RVFKLGLFSGLWWTLALFCWISDRAFCELLSSFHFP	QFHAWHILIWGLGSYLHILFSLYTRTLYLR-YRPKVKFL-FGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVFGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVRFWPSEKWAFI QFHAWHILIGLGSYLHILFSLYTRTLYLR-YRPKVKFLFGIWPMVMFEPQ YLHSIWHVLISFTFPYGMVILALVDSEYEMPNKTLKVKFWPSERWAFI QFHAWHILIGLGSYLHILFSLYTRTLYLR-YRPKVKFLFGIWPVILFEPL YFHSFWVLIAVSLLYCFPLVMYFDVTYEMPAFKPKL
MOR603 ACER1_RAT D32NW4 ACER2_RAT F1MGP3 ACER3_RAT F1MGP3 ACER1_BOVIN E1B8N2 ACER2_BOVIN A7MBH7 ACER3_BOVIN F1NLL6 ACER1_CHICK E1C990 ACER2_CHICK A0A1S3T5X1 ACER1_SALSA A0A1S3TGS4 ACER2_SALSA A0A1S3TGS5 ACER2_SALSA Q56812 ACER1_DANRE E7FCF4 ACER2_DANRE	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAALTSWVSDRVLGSFWQRIQFF RVFKLGLFSGLWWTLALFCWISDRAFCELLSSFHFP -LRGLGYTSLTVFLLGFLLWNVDNIFCDSLRNFRK-TVPPVLGVAT ELQHLIEVSTVIWALAFTSWISDRLLCSFWQWINFS -LRGLGYTSLGVFLLGFLFWNIDNIFCDSLRNFRK-KVPPVIGVTT RVHRMAKMVVWVLATSSWISDRAFCELSSVHFP	QFHAWWHILWGLGSYLHILFSLYRTLYLRYRPKVKFLFGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVHYWPRDSWLI- QFHAWWHILTGLAYYGCVCFAYFDAASEIPEQGPVIRFWPSEKWAFI QFHAWWHILTGLGSYLHILFSLYRTLYLRYRPKVKFLFGIWPMVMFEPQ YLHSIWHVLISFTFPYGMVILALVDSEYEMPNKTLKVRYWPRDTWPM- LHCVWHILICLAYYGCVCFAYFDAASEIPEQGPVI
MOR603 ACER1_RAT D32NW4 ACER2_RAT F1MGP3 ACER2_RAT F1MGP3 ACER2_BOVIN A7MBH7 ACER3_BOVIN A7MBH7 ACER3_BOVIN F1NLL6 ACER1_CHICK E1C990 ACER2_CHICK A0A1S3T5X1 ACER1_SALSA A0A1S3TGK9 ACER2_SALSA A0A1S3LR05 ACER3_SALSA Q56812 ACER1_DANRE	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHIIAVSVILWAAATTSWVSDRVLGSFWQRIQFF RVFKLGLFSGLWWTLALFCWISDRAFCELLSSFHFP	QFHAWWHILIGLGSYLHILFSLYTRTLYLR-YRPKVKFLFGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYYMPDKTLKVFHWPRDSWLI- YLHCVWHILICLAAYLGCVCFAYFDAASEIPEQGPVIRFWPSEKWAFI QFHAWWHILTGLGSYLHILFSLYTRTLYLR-YRPKVKFLFGIWPMVMFEPQ YLHSIWHVLISFTFPYGMVILALVDSEYEMPNKTLKV
MOR603 ACER1_RAT D32NW4 ACER2_RAT F1MGP3 ACER2_RAT F1MGP3 ACER2_BOVIN A7MBH7 ACER3_BOVIN A7MBH7 ACER3_BOVIN F1NLL6 ACER1_CHICK E1C490 ACER2_CHICK A0A1S3T5X1 ACER1_SALSA A0A1S3T6X1 ACER1_SALSA A0A1S3LR05 ACER2_SALSA Q56812 ACER1_DANRE E7FCF4 ACER2_DANRE E9QEA6 ACER3_DANRE E9QEA6 ACER3_DANRE E938298 YPC1_YEAST	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAALTSWVSDRVLCSFWQRIQFF 	QFHAWHILIGLGSYLHILFSLYRTLYLRYRPKVKFLFGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVRFWPSEKWAFI QFHAWWHILTGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPMVMFEPQ YLHSIWHVLISTTFPYGMVILALVDSEYEMPNKTLKVRFWPSEKWAFI QFHAWWHILIGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPVILFEPL YFHSFWHVLIAVSLLYCFPLVMYPDVTYEMPAFKPKL
MOR603 ACER1_RAT D32NW4 ACER2_RAT F1MGP3 ACER2_RAT F1MGP3 ACER2_BOVIN E1B8N2 ACER2_BOVIN A7MBH7 ACER3_BOVIN F1NLL6 ACER1_GHICK E1C910 ACER2_CHICK E1C413 ACER3_CHICK A0A1S3T5X1 ACER1_SALSA A0A1S3T5X1 ACER2_SALSA Q56812 ACER3_CHICK E7CF4 ACER2_DANRE E7CF4 ACER2_DANRE E9QEA6 ACER3_DANRE Q9VIP7 ACER_DROME	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAALTSWVSDRVLCSFWQRIQFF 	QFHAWWHILIGLGSYLHILFSLYIRTLYLR-YRPKVKFLFRUWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVRFWPSEKWAFI QFHAWWHILTGLAYLGCVCFAYFDAASEIPEQGFVI
MOR603 ACER1_RAT D32NW4 ACER2_RAT F1MGP3 ACER2_RAT F1MGP3 ACER2_BOVIN A7MBH7 ACER3_BOVIN A7MBH7 ACER3_BOVIN F1NLL6 ACER1_CHICK E1C490 ACER2_CHICK A0A1S3T5X1 ACER1_SALSA A0A1S3T6X1 ACER1_SALSA A0A1S3LR05 ACER2_SALSA Q56812 ACER1_DANRE E7FCF4 ACER2_DANRE E9QEA6 ACER3_DANRE E9QEA6 ACER3_DANRE E938298 YPC1_YEAST	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAALTSWVSDRVLCSFWQRIQFF 	QFHAWHILIGLGSYLHILFSLYRTLYLRYRPKVKFLFGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVRFWPSEKWAFI QFHAWWHILTGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPMVMFEPQ YLHSIWHVLISTTFPYGMVILALVDSEYEMPNKTLKVRFWPSEKWAFI QFHAWWHILIGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPVILFEPL YFHSFWHVLIAVSLLYCFPLVMYPDVTYEMPAFKPKL
MOR603 ACER1_RAT D32NW4 ACER2_RAT F1M6P3 ACER3_RAT F1M6P3 ACER3_RAT F1M6P3 ACER3_RAT F1M6P3 ACER3_RAT F1M16 ACER1_BOVIN F1M16 ACER1_BOVIN F1M16 ACER1_CHICK E1C90 ACER3_CHICK A0A1S3T5X1 ACER1_SALSA A0A1S3T5X1 ACER1_SALSA A0A1S3LR05 ACER3_SALSA Q56812 ACER1_DANRE E7FCF4 ACER2_DANRE E9QEA6 ACER3_DANRE E9QEA6 ACER3_DANRE Q9VIP7 ACER3_DANRE P38298 YPC1_YEAST Q0896 YDC1_YEAST Q9NUN7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAATTSWVSDRVLGSFWQRIQFF RVFKLGLFSGLWWTLALFCWISDRAFCELLSSFHFP -LRGLGYTSLTVFLLGFLLWNVDNIFCDSLRNFRK-TVPPVLGVAT ELQHLIEVSTVIWALAFTSWISDRLLCSFWQWINFS	QFHAWWHILIGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPAVMFEPQ YLHSIWHYLISITFPYGIVTMALVDAKYEMPDKTLKVRFWPSEKWAFI QFHAWWHILTGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPMVMFEPQ YLHSIWHYLISTTFPYGMVILALVDSEYEMPNKTLKVRFWPSEKWAFI QFHAWWHILIGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPWVMFEPQ YLHSIWHYLISTTFPYGMVILALVDSEYEMPNKTLKV
MOR603 ACER1_RAT D32NW4 ACER2_RAT F1MGP3 ACER3_RAT F1MGP3 ACER1_BOVIN A7MBH7 ACER3_BOVIN A7MBH7 ACER3_BOVIN F1NLL6 ACER1_CHICK E1C990 ACER2_GHICK E1C413 ACER3_CHICK A0A1S3T5X1 ACER1_SALSA A0A1S3T5X1 ACER1_SALSA A0A1S3RG5 ACER2_SALSA Q56812 ACER1_DANRE E9QEA6 ACER3_DANRE E9QEA6 ACER3_DANRE P38298 YPC1_YEAST Q02896 YPC1_YEAST Q9NUN7 ACER3_HUMAN	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAALTSWVSDRVLCSFWQRIQFF RVFKLGLFSGLWWTLALFCWISDRAFCELLSSFHFP -LRGLGYTSLTVFLLGFLLWNVDNIFCDSLRNFRK-TVPPVLGVAT ELQHLIEVSTVIWALAFTSWISDRLLCSFWQWINFS	QFHAWWHILIGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPAVMFEPQ YLHSIWHYLISITFPYGIVTMALVDAKYEMPDKTLKVRFWPSEKWAFI QFHAWWHILTGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPMVMFEPQ YLHSIWHYLISTTFPYGMVILALVDSEYEMPNKTLKVRFWPSEKWAFI QFHAWWHILIGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPWVMFEPQ YLHSIWHYLISTTFPYGMVILALVDSEYEMPNKTLKV
M0R603 ACER1_RAT D32NW4 ACER2_RAT F1MGP3 ACER2_RAT F1MGP3 ACER2_BOVIN A7MBH7 ACER3_BOVIN A7MBH7 ACER3_BOVIN F1NLL6 ACER1_CHICK E1C990 ACER2_GHICK A0A1S3T5X1 ACER1_SALSA A0A1S3T5X1 ACER1_SALSA A0A1S3T65 ACER2_SALSA Q56812 ACER1_DANRE E7FCF4 ACER2_DANRE E9QEA6 ACER3_DANRE Q9VIP7 ACER3_DANRE Q9VIP7 ACER3_HUMAN Q8T0N7 ACER3_HUMAN Q50JU3 ACER2_HUMAN Q50JU3 ACER2_HUMAN Q8TAX1 ACER1_MUSE Q8VD53 ACER2_MOUSE	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAATTSWVSDRVLGSFWQRIQFF RVFKLGLFSGLWWTLALFCWISDRAFCELLSSFHFP -LRGLGYTSLTVFLLGFLLWNVDNIFCDSLRNFRK-TVPPVLGVAT ELQHLIEVSTVIWALAFTSWISDRLLCSFWQWINFS -RGLGYTSLGVFLLGFLFWNIDNIFCDSLRNFRK-KVPPVIGVTT RVFKLGLFSGLWWTLAFTSWISDRWLCWLCQAINFP -LRGLGYTSLTVFLMGFFLWNVDNIFCDSLRNFRK-KVPPVIGVTT RVHRMAKMVVWVLATSWISDRWLCWLCQAINFP	QFHAWWHILIGLGSYLHILFSLYTRTLYLRYRPKVKFLFIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVFIWPQDSWLI- GFHAWWHILTGLAYLGCVCFAYFDAASEIPEQGPVIRFWPSEKWAFI QFHAWWHILTGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPMVMFEPQ YLHSIWHVLISFTFPYGMVILALVDSEYEMPNKTLKV
MOR603 ACER1_RAT D32NW4 ACER2_RAT F1M6P3 ACER2_RAT F1M6P3 ACER1_BOVIN E1B8N2 ACER3_BOVIN A7MBH7 ACER3_BOVIN F1NLL6 ACER1_BOVIN E1C900 ACER2_CHICK E1C413 ACER3_CHICK AOA1S3T5X1 ACER1_SALSA AOA1S3T5X1 ACER1_SALSA Q56812 ACER1_DANRE E7PCF4 ACER2_DANRE E9QEA6 ACER3_DANRE Q9VIP7 ACER_DROME P38298 YPC1_YEAST Q0896 YDC1_YEAST Q9NUN7 ACER3_HUMAN Q8TDN7 ACER1_HUMAN Q8QU3 ACER2_HUMAN Q8AX1 ACER1_MOUSE	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAALTSWVSDRVLCSFWQRIQFF RVFKLGLFSGLWWTLALFCWISDRAFCELLSSFHFP -LRGLGYTSLTVFLLGFLLWNVDNIFCDSLRNFRK-TVPPVLGVAT ELQHLIEVSTVIWALAFTSWISDRLLCSFWQWINFS -LRGLGYTSLGVFLGFLFWNIDNIFCDSLRNFRK-KVPPVIGVTT RVFRMAKAMVVWVLAITSWLSDRWLCWLCQAINFP	QFHAWWHILIGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVRYWPRDSWLI- QFHAWWHILTGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPMVMFEPQ YLHSIWHVLISFTFPYGMVILALVDSEYEMPNKTLKVRYWPRDTWPM- YLHCVWHILICLAAYLGCVCFAYFDAASEIPEQGPVI
MOR603 ACER1_RAT D32NW4 ACER2_RAT F1MGP3 ACER3_RAT F1MGP3 ACER3_BOVIN A7MBH7 ACER3_BOVIN A7MBH7 ACER3_BOVIN F1NLL6 ACER1_CHICK E1C900 ACER2_CHICK A0A1S3T5X1 ACER1_SALSA A0A1S3T5X1 ACER1_SALSA A0A1S3T6X1 ACER1_SALSA Q56812 ACER1_DANRE E7FCF4 ACER2_DANRE E9GEA6 ACER3_DANRE Q9UF7 ACER_DROME P38298 YPC1_YEAST Q02896 YDC1_YEAST Q9NUN7 ACER3_HUMAN Q8TDN7 ACER1_HUMAN Q8TDN7 ACER1_HUMAN Q8QJ3 ACER2_HUMAN Q8VD53 ACER2_HUMAN Q8VD53 ACER2_MOUSE Q9D099 ACER3_MOUSE Q9D099 ACER3_MOUSE Q9D099 ACER3_RAT D32NW4 ACER2_RAT	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAALTSWVSDRVLGSFWQRIQFF RVFKLGLFSGLWWTLALFCWISDRAFCELLSSFHFP -LRGLGYTSLTVFLLGFLLWNVDNIFCDSLRNFRK-TVPPVLGVAT ELQHLIEVSTVIWALAFTSWISDRLCSFWQWINFS -LRGLGYTSLGVFLLGFLFWNIDNIFCDSLRNFRK-KVPPVIGVTT RVHRMAKAMVVWVLATSWISDRWLGWLCQAINFP RVFKLGLFSGLWWTLAFCWISDRAFCELWSSFNFP RVFKLGLFSGLWWLALFCWISDKAFCEIWSSFNFP	QFHAWWHILIGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVRYWPRDSWLI- QFHAWWHILTGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPMVMFEPQ YLHSIWHVLISFTFPYGMVILALVDSEYEMPNKTLKVRYWPRDTWPM- YLHCVWHILICLAAYLGCVCFAYFDAASEIPEQGPVI
MOR603 ACER1_RAT D32NW4 ACER2_RAT F1MGP3 ACER2_RAT F1MGP3 ACER3_BOVIN A7MBH7 ACER3_BOVIN A7MBH7 ACER3_BOVIN F1NLL6 ACER1_BOVIN F1NLL6 ACER1_CHICK E1C413 ACER3_CHICK A0A1S3T5X1 ACER1_SALSA A0A1S3T5X1 ACER1_SALSA A0A1S3TC5 ACER2_SALSA A0A1S3IR05 ACER2_SALSA A0A1S3IR05 ACER3_SALSA Q56812 ACER1_DANRE E7PCF4 ACER2_DANRE E9QEA6 ACER3_DANRE E9QEA6 ACER3_DANRE Q9XUN7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8QJU3 ACER2_HUMAN Q8R4X1 ACER1_HUMAN Q8R4X1 ACER1_MOUSE Q9NUS9 ACER3_MOUSE MOR603 ACER1_RAT	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAALTSWVSDRVLGSFWQRIQFF RVFKLGLFSGLWWTLALFCWISDRAFCELLSSFHFP -LRGLGYTSLTVFLLGFLLWNVDNIFCDSLRNFRK-TVPPVLGVAT ELQHLIEVSTVIWALAFTSWISDRLLCSFWQWINFS -LRGLGYTSLGVFLLGFLFWNIDNIFCDSLRNFRK-KVPPVIGVTT RVHRMAKAMVVWVLATSWLSDRWLCWLCQAINFP RVFKLGLFSGLWWTLALFCWISDKAFCELWSSFNFP	QFHAWWHILIGLGSYLHILFSLYTRTLYLRYRPKVKFLFIWPACWFFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVRFWPSEKWAFI QFHAWWHILTGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPMVMFEPQ YLHSIWHVLISTTFPYGMVILALVDSEYEMPNKTLKVRFWPSEKWAFI QFHAWWHILTGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPVILFEPL YFHSFWHVLIAVSLYCFFLVMYPDAASEIPEQGPVI
MOR603 ACER1_RAT D32NW4 ACER2_RAT F1MGP3 ACER2_RAT F1MGP3 ACER3_BOVIN A7MBH7 ACER3_BOVIN A7MBH7 ACER3_BOVIN F1NL16 ACER1_BOVIN F1NL16 ACER1_CHICK E1C413 ACER3_CHICK A0A1S3T5X1 ACER1_SALSA A0A1S3T5X1 ACER1_SALSA A0A1S3T6X ACER2_SALSA A0A1S3T6X ACER2_SALSA A0A1S3LR05 ACER2_SALSA A0A1S3LR05 ACER3_SALSA Q56812 ACER1_DANRE E7PCF4 ACER2_DANRE E9QEA6 ACER3_DANRE E9QEA6 ACER3_DANRE Q9VID7 ACER3_DANRE Q9VID7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8TDN7 ACER1_HUMAN Q8TDN7 ACER1_HUMAN Q8R4X1 ACER1_MOUSE Q9D099 ACER3_MOUSE MOR603 ACER1_RAT D32NW4 ACER2_RAT F1MGP3 ACER1_BOVIN E1B8N2 ACER2_BOVIN	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAALTSWVSDRVLCSFWQRIQFF RVFKLGLFSGLWWTLALFCWISDRAFCELLSSFHFP	QFHAWWHILIGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVRFWPSEKWAFI QFHAWWHILTGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPMVMFEPQ YLHSIWHVLISFTFPYGMVILALVDSEYEMPNKTLKVRFWPSERWAFI QFHAWWHILTGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPVULFEPL YHSFWHVLIAVSLLYCFPLVWYFDVTYEMPAFKPKL
MOR603 ACER1_RAT D32NW4 ACER2_RAT F1MGP3 ACER2_BAT F1MGP3 ACER3_BOVIN A7MBH7 ACER3_BOVIN A7MBH7 ACER3_BOVIN F1NLL6 ACER1_CHICK E1C409 ACER2_CHICK A0A1S3T5X1 ACER1_SALSA A0A1S3T5X1 ACER1_SALSA A0A1S3T6X1 ACER1_SALSA A0A1S3T6X1 ACER1_SALSA Q56812 ACER1_DANRE E7FCF4 ACER2_DANRE E9GEA6 ACER3_DANRE G9VID7 ACER3_DANRE 938298 YPC1_YEAST Q02896 YDC1_YEAST Q9NUN7 ACER3_HUMAN Q8TDN7 ACER1_HUMAN Q8TDN7 ACER1_HUMAN Q8TDN7 ACER1_HUMAN Q8TDX7 ACER2_MOUSE Q9D099 ACER3_MOUSE Q9D099 ACER3_RAT F1MGP3 ACER3_BOVIN E1B8N2 ACER3_BOVIN A7MBH7 ACER3_BOVIN	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAALTSWVSDRVLCSFWQRIQFF RVFKLGLFSGLWWTLALFCWISDRAFCELLSSFHFP	QFHAWWHILIGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVRFWPSEKWAFI QFHAWWHILTGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPMVMFEPQ YLHSIWHVLISTTFPYGMVILALVDSEYEMPNKTLKV
MOR603 ACER1_RAT D32NW4 ACER2_RAT FIMGP3 ACER3_RAT FIMGP3 ACER3_BOVIN E1B8N2 ACER3_BOVIN A7MBH7 ACER3_BOVIN FINL6 ACER1_GHICK E1C490 ACER2_CHICK E1C413 ACER3_CHICK A0A1S3T5X1 ACER3_CHICK A0A1S3T5X1 ACER3_SALSA A0A1S3GK9 ACER2_SALSA A0A1S3GK9 ACER2_SALSA A0A1S3IR05 ACER2_SALSA A0A1S3IR05 ACER2_SALSA Q56812 ACER1_DANRE E9QEA6 ACER3_DANRE E9QEA6 ACER3_DANRE E9QEA6 ACER3_DANRE 29VIP7 ACER3_BUNRE P38298 YPC1_YEAST Q02896 YDC1_YEAST Q02896 YDC1_YEAST Q02896 YDC1_YEAST Q9NUN7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8QJU3 ACER2_HUMAN Q8QJU3 ACER2_MOUSE Q9D099 ACER3_MOUSE M0R603 ACER1_RAT D32NW4 ACER2_RAT FIMGP9 ACER3_BOVIN E1B8N2 ACER2_BOVIN A7MBH7 ACER3_BOVIN FINL16 ACER1_CHICK	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAALTSWVSDRVLCSFWQRIQFF RVFKLGLFSGLWWTLALFCWISDRAFCELLSSFHFP -LRGLGYTSLTVFLLGFLLWNVDNIFCDSLRNFRK-TVPPVLGVAT ELQHLIEVSTVIWALAFTSWISDRLLCSFWQWINFS -LRGLGYTSLGVFLCFLFWNIDNIFCDSLRNFRK-KVPPVIGVTT RVFKLGLFSGLWWTLALFCWISDRAFCELWSSFNFP	QFHAWWHILIGLGSYLHILFSLYRTLYLRYRPKVKFLFGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVRFWPRDSWLI- QFHAWWHILTGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPMVMFEPQ YLHSIWHVLISTTFPYGMVILALVDSEYEMPNKTLKVRFWPSEKWAFI QFHAWWHILTGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPVILFEPL YFHSFWHVLIAVSLLYCFPLVMYDVTYEMPAFKPKL
MOR603 ACER1_RAT D32NW4 ACER2_RAT F1MGP3 ACER2_RAT F1MGP3 ACER3_BOVIN A7MBH7 ACER3_BOVIN A7MBH7 ACER3_BOVIN F1NL16 ACER1_BOVIN F1NL16 ACER1_CHICK E1C413 ACER3_CHICK A0A1S3T5X1 ACER1_SALSA A0A1S3T5X1 ACER1_SALSA A0A1S3TC5 ACER2_SALSA A0A1S3TC5 ACER2_SALSA A0A1S3LR05 ACER2_SALSA A0A1S3LR05 ACER3_SALSA Q56812 ACER1_DANRE E7PCF4 ACER2_DANRE E9QEA6 ACER3_DANRE E9VET7 ACER_DANRE E92EA6 ACER3_BONRE Q9VID7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8TDN7 ACER1_HUMAN Q8DVD53 ACER2_HUMAN Q8R4X1 ACER1_MOUSE Q9D099 ACER3_MOUSE MOR603 ACER1_RAT D32NW4 ACER2_RAT F1MGP3 ACER3_BOVIN A7MBH7 ACER3_BOVIN A7MBH7 ACER3_CHICK E1C413 ACER1_CHICK	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAALTSWVSDRVLCSFWQRIQFF RVFKLGLFSGLWWTLALFCWISDRAFCELLSSFHFP -LRGLGYTSLTVFLLGFLLWNVDNIFCDSLRNFRK-TVPPVLGVAT ELQHLIEVSTVIWALAFTSWISDRLLCSFWQWINFS -LRGLGYTSLGVFLCFLFWNIDNIFCDSLRNFRK-KVPPVIGVTT RVFKLGLFSGLWWTLALFCWISDRAFCELWSSFNFP	QFHAWWHILIGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPAVMFEPQ YLHGVWHILIGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPAVMFEPQ QFHAWWHILTGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPMVMFEPQ YLHGVWHILIGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPWVMFEPQ YLHGVWHILIGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPVILFEPL YFHSFWHVLAVSLLYCFPLVMYFDVYYEMPAFKPKLGYWPSERWAFI QFHAWWHILTGLGSYLHILSLYTRTLYLRYRPKVKFLFGIWPVILFEPL YLHGVWHILIGLGSYLHILSLYTRTLYLRYRPKVKFL
MOR603 ACER1_RAT D32NW4 ACER2_RAT FIMGP3 ACER3_RAT FIMGP3 ACER3_RAT FIMGP3 ACER3_RAT FIMGP3 ACER3_RAT FIML6 ACER1_BOVIN AMBH7 ACER3_BOVIN FINLL6 ACER1_BOVIN FINLL6 ACER1_CHICK E1C413 ACER3_CHICK AOA1S3TGX1 ACER1_SALSA AOA1S3TGX1 ACER1_SALSA AOA1S3TGX1 ACER1_SALSA AOA1S3TGX1 ACER1_SALSA AOA1S3TGX1 ACER1_SALSA AOA1S3TGX1 ACER1_SALSA AOA1S3TGX1 ACER1_SALSA Q56812 ACER3_DANRE E9QEA6 ACER3_DANRE E9QEA6 ACER3_DANRE E9QEA6 ACER3_DANRE Q9VIP7 ACER3_DANRE Q9VIP7 ACER3_DANRE Q9VIP7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8GA11 ACER1_HUMAN Q8GA12 ACER2_MOUSE Q9D099 ACER3_MOUSE MOR603 ACER1_GAT FIMGP3 ACER3_RAT FIMGP3 ACER3_RAT FIMGP3 ACER3_BOVIN AMBH7 ACER3_CHICK E1C990 ACER3_CHICK E1C990 ACER2_CHICK E1C413 ACER2_SALSA AOA1S3TGX9 ACER2_SALSA	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAALTSWVSDRVLGSFWQRIQFF RVFKLGLFSGLWWTLALFCWISDRAFCELLSSFHFP -LRGLGYTSLTVFLLGFLLWNVDNIFCDSLRNFRK-TVPPVLGVAT ELQHLIFVSTVIWALAFTSWISDRLLCSFWQWINFS -LRGLGYTSLGVFLGFLFWNIDNIFCDSLRNFRK-KVPPVIGVTT RVFRMAKAMVVWVLAITSWLSDRWLCWLCQAINFF	QFHAWWHILIGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVRFWPRDSWLI- QFHAWWHILTGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPMVMFEPQ YLHSIWHVLISTTFPYGMVILALVDSEYEMPNKTLKV
MOR603 ACER1_RAT D32NW4 ACER2_RAT F1MGP3 ACER2_RAT F1MGP3 ACER3_BAT F1MGP3 ACER3_BOVIN A7MBH7 ACER3_BOVIN F1NLL6 ACER1_BOVIN F1NLL6 ACER1_CK E1C413 ACER3_CHICK A0A1S3T5X1 ACER2_SALSA A0A1S3T5X1 ACER3_SALSA Q56812 ACER1_DANRE E79CF4 ACER2_DANRE E90EA6 ACER3_DANRE E90EA6 ACER3_DANRE E90EA6 ACER3_DANRE E90EA6 ACER3_DANRE 202896 YCC1_YEAST Q0896 YCC1_YEAST Q0896 YCC1_YEAST Q0896 YCC1_YEAST Q0895 ACER2_MOUSE Q8UD53 ACER2_MOUSE Q9DU75 ACER3_RAT F1MGF3 ACER3_RAT F1MGF3 ACER3_RAT F1MGF3 ACER3_BOVIN E1B8N2 ACER2_BOVIN F1NLL6 ACER1_CHICK E1C990 ACER3_CHICK A0A1S3T5X1 ACER2_SALSA A0A1S3T6X1 ACER2_SALSA A0A1S3T6X1 ACER2_SALSA A0A1S3T6X1 ACER2_SALSA A0A1S3T6X1 ACER2_SALSA A0A1S3T6X1 ACER2_SALSA A0A1S3T6X5 ACER2_SALSA	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAALTSWVSDRVLGSFWQRIQFF RVFKLGLFSGLWWTLALTSWISDRLCSFWQWINFS -LRGLGYTSLTVFLLGFLLWNVDNIFCDSLRNFRK-TVPPVLGVAT ELQHLIFVSTVIWALAFTSWISDRLLCSFWQWINFS -LRGLGYTSLGVFLGFLFWNIDNIFCDSLRNFRK-KVPPVIGVTT RVFRMAKAMVVWVLAITSWLSDRWLCWLCQAINFFP	OF HAWMELD GLGSYLHILFSLYTRTLYLR YRPKVKFL FGIW PAVMFEPQ YLHSIWHYLISITFPYGUVTALVDAKYEMPDKTLKV FRW PRDSKWAFI QFHAWWHILTGLGSYLHILFSLYTRTLYLR YRPKVKFL FGIW PNW MFEPQ YLHCVWHILTGLGSYLHILFSLYTRTLYLR YRPKVKFL FGIW PNW PM- YLCWHILTGLGSYLHILFSLYTRTLYLR YRPKVKFL FGIW PNW PM- YLGWHILTGLGSYLHILFSLYTRTLYLR YRPKVKFL FGIW PNW PM- YLCWHILTGLGSYLHILFSLYTRTLYLR YRPKVKFL FGIW PNW PM- YLGWHILTGLGSYLHILFSLYTRTLYLR YRPKVKFL FGIW PNW PM- YLGWHILTGLGSYLHILFSLYTRTLYLR YRPKVKFL FGIW PNW PNM- YLGWHILTGLGSYLHILFSLYTRTLYLR YRPKVKFL FGIW PNW PNM- YLGWHILTGLGSYLHILFSLYTRTLYLR YRPKVKFL FGIW PSEWAFI YLGWHILTGLGSYLHILFSLYTRTLYLR YRPKVKFL FGIW PSEWAFI YLGWHILTGLGSYLHILSSLYTRTLYLR YRPKVKFL FGIW PSEWAFI YLGWHILTGLGSYLHILSSLYTRTLYLR YRPKVKFL FRWN PSEWAFI YLGWHILTGLGSYLHILSSLYTRTLYLR YRPKVKFL FRWN PSEWAFI YLGWHILTGLGSYLHILSSLYTRTLYLN YSWN YNNAI YLGWHILTGLGSYLHILFSLYTNYLVYN YN YNNAI YLGWHILTGLGSYLHILSSLYTRTLYLN YSCNYQ FIWN WANFE YLGWHILTGLGSYLHILSSLYTRTLYLN YSCNYQ FIWN WANFE YLGWHILTGLGSYLHUNGH
MOR603 ACER1_RAT D32NW4 ACER2_RAT FIMGP3 ACER3_RAT FIMGP3 ACER3_RAT FIMGP3 ACER3_RAT FIMGP3 ACER3_RAT FIMGP3 ACER2_BOVIN A7MBH7 ACER3_BOVIN FINLL6 ACER1_CHICK E1C990 ACER2_CHICK E1C413 ACER3_CHICK A0A1S3TSX1 ACER1_SALSA A0A1S3TSX1 ACER1_SALSA A0A1S3TR05 ACER2_SALSA A0A1S3TR05 ACER2_SALSA Q58012 ACER1_DANRE E79CF4 ACER2_DANRE E9QEA6 ACER3_DANRE Q9VIP7 ACER_DROME P38298 YPC1_YEAST Q0806 YDC1_YEAST Q9NUN7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8TA12 ACER1_RAT D32NW4 ACER2_MOUSE Q9D099 ACER3_RAT FIMGP3 ACER3_RAT FIMGP3 ACER3_BOVIN A7MBH7 ACER3_BOVIN FINLL6 ACER3_CHICK E1C413 ACER3_CHICK A0A1S3TSX1 ACER1_SALSA A0A1S3TSX1 ACER1_SALSA A0A1S3RG9 ACER2_BALSA A0A1S3RG9 ACER2_DANRE E7FCF4 ACER2_DANRE	-LRGLGYTSLTVFLLGFLLWNIDNIFGDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAALTSWVSDRVLGSFWQRIQFF -RVFKLGLFSGLWWTLALTSWISDRVLGSFWQWINFS -LRGLGYTSLTVFLLGFLLWNVDNIFGDSLRNFRK-TVPPVLGVAT ELQHLIFVSTVIWALAFTSWISDRLLGSFWQWINFS	QFHAWWHILIGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPAVMFEPQ YLHSIWHVLISITFPYGIVTMALVDAKYEMPDKTLKVRFWPRDSWLI- QFHAWWHILTGLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPMVMFEPQ YLHSIWHVLISTTFPYGMVILALVDSEYEMPNKTLKV
MOR603 ACER1_RAT D32NW4 ACER2_RAT FIMGP3 ACER3_RAT FIMGP3 ACER3_RAT FIMGP3 ACER3_RAT FIMGP3 ACER3_ROVIN E188N2 ACER2_BOVIN A7MBH7 ACER3_BOVIN FINIL6 ACER1_CHICK E1C90 ACER2_CHICK E1C913 ACER3_CHICK A0A1S3TGK3 ACER1_SALSA A0A1S3TGK3 ACER1_SALSA A0A1S3TGK3 ACER2_SALSA Q56812 ACER1_DANRE E7FCF4 ACER2_DANRE E9QEA6 ACER3_DANRE Q9VIP7 ACER_DANRE E9QEA6 ACER3_DANRE Q9VIP7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8QJU3 ACER2_MUSE Q9D099 ACER3_MOUSE MOR603 ACER1_RAT D3ZNW4 ACER2_MOUSE Q9D099 ACER3_MOUSE MOR603 ACER1_GAT FIMGP3 ACER1_BOVIN E1B8N2 ACER2_BOVIN A7MBH7 ACER3_CHICK A0A1S3TSX1 ACER1_SALSA A0A1S3TGK9 ACER2_SALSA A0A1S3TGK9 ACER2_SALSA A0A1S3TGK9 ACER3_SALSA Q56812 ACER3_DANRE E7FCF4 ACER2_DANRE E7FCF4 ACER2_DANRE E7FCF4 ACER2_DANRE E7FCF4 ACER2_DANRE E7FCF4 ACER2_DANRE E9QEA6 ACER3_DANRE E9QEA6 ACER3_DANRE	-LRGLGYTSLTVFLLGFLLWNIDNIFCDSLRNFRK-RVPPVLGVTT DLRHIIAVSVILWAAALTSWVSDRVLGSFWQRIQFF 	OF HAWHILD GLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPAVMFEPQ YLHSIWHULTSITFPYGUVTALVDAKYEMPDKTLKVRFWPSEKWAFI QFHAWHILTGLGSYLHILFSLYTRTLYLRVRPKVKFLFGIWPMVMFEPQ YLHSIWHULTGLGSYLHILFSLYTRTLYLRVRPKVKFLFGWPRDWWRMFEPQ YLHSIWHULTGLGSYLHILFSLYTRTLYLRVRPKVKFLFGWPRDWWRMFEPQ YLHSIWHULTGLGSYLHILFSLYTRTLYLRVRPKVKFLFGWPRDWPM- YLHSWHILTGLGSYLHILFSLYTRTLYLRVRPKVKFLFGWPRDWPM- YLHSWHILTGLGSYLHILFSLYTRTLYLRVRPKVKFLFGWPSDSWPI- YHSFWHULAVSLYCFPLVMYPDVTYEMPARKKPKLKFWPSEWAFI YHSFWHULAVSLYCFPLVMYPDVTYEMPARKKPKLKFWPSEWAFI YHSFWHULTGLGSYLHILFSLYTRTLFLK
MOR603 ACER1_RAT D32NW4 ACER2_RAT FIMGP3 ACER3_RAT FIMGP3 ACER3_RAT FIMGP3 ACER3_RAT FIMGP3 ACER3_RAT FIMGP3 ACER2_BOVIN A7MBH7 ACER3_BOVIN FINLL6 ACER1_CHICK E1C990 ACER2_CHICK E1C413 ACER3_CHICK A0A1S3TSX1 ACER1_SALSA A0A1S3TSX1 ACER1_SALSA A0A1S3TR05 ACER2_SALSA A0A1S3TR05 ACER2_SALSA Q58012 ACER1_DANRE E79CF4 ACER2_DANRE E9QEA6 ACER3_DANRE Q9VIP7 ACER_DROME P38298 YPC1_YEAST Q0806 YDC1_YEAST Q9NUN7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8TDN7 ACER3_HUMAN Q8TA12 ACER1_RAT D32NW4 ACER2_MOUSE Q9D099 ACER3_RAT FIMGP3 ACER3_RAT FIMGP3 ACER3_BOVIN A7MBH7 ACER3_BOVIN FINLL6 ACER3_CHICK E1C413 ACER3_CHICK A0A1S3TSX1 ACER1_SALSA A0A1S3TSX1 ACER1_SALSA A0A1S3RG9 ACER2_BALSA A0A1S3RG9 ACER2_DANRE E7FCF4 ACER2_DANRE	-LRGLGYTSLTVFLLGFLLWNIDNIFGDSLRNFRK-RVPPVLGVTT DLRHLIAVSVILWAAALTSWVSDRVLGSFWQRIQFF -RVFKLGLFSGLWWTLALTSWISDRVLGSFWQWINFS -LRGLGYTSLTVFLLGFLLWNVDNIFGDSLRNFRK-TVPPVLGVAT ELQHLIFVSTVIWALAFTSWISDRLLGSFWQWINFS	OF HAWHELD-GLGSYLHILFSLYTRTLYLRYRPKVKFLFGIWPAVMFEPQ YLHSIWHULTSITFPYGUVTALVDAKYEMPDKTLKVRFWPSEKWAFI QF HAWHELTGLGSYLHILFSLYTRTLYLRYRPKVKFLRFWPSEKWAFI QF HAWHELTGLGSYLHILFSLYTRTLYLRYRPKVKFLRFWPSEKWAFI QF HAWHELTGLGSYLHILFSLYTRTLYLRYRPKVKFL

Supplementary Figure 4. ConSurf color-coded multiple sequence alignment of ACER family.

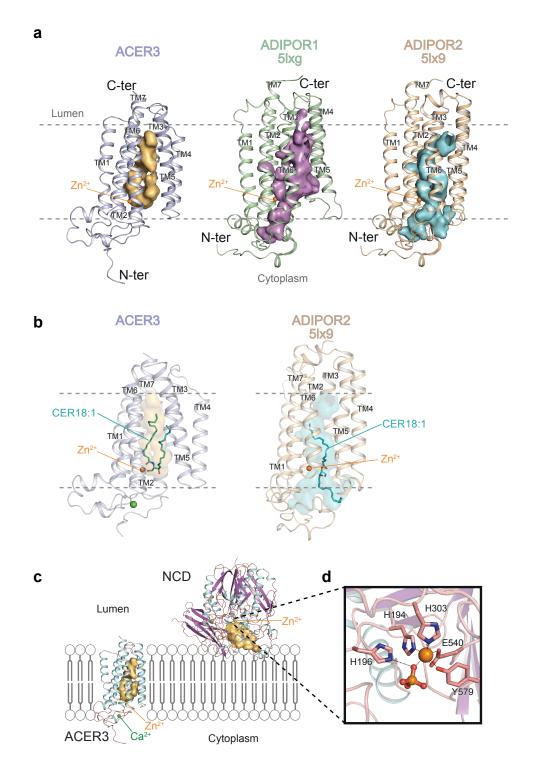
(a) Sequence alignment of alkaline ceramidases from human (Q9NUN7, Q8TDN7, Q5QJU3), mouse (Q8R4X1, Q8VD53, Q9D099), rat (M0R603, D3ZNW4, F1M6P3), bovine (F1MGH9, E1B8N2, A7MBH7), chicken (F1NNL6, E1C990, E1C413), salmon (A0A1S3T5X1, A0A1S3RGK9, A0A1S3LR05), zebrafish (Q568I2, E7FCF4, E9QEA6), fruit fly (Q9VIP7) and yeast (P38298, Q02896) created using Consurf server¹ (consurf.tau.ac.il). The color-coding bar shows coloring scheme according to degree of conservation ranging from not conserved, cyan to highly conserved, magenta. Residues coordinating Ca²⁺ (green asterisks) and Zn²⁺ (orange asterisks) ions are outstandingly conserved indicating the importance of Ca²⁺ and Zn²⁺ binding sites. (b) Representation of the phylogenetic tree created using Consurf server.



Supplementary Figure 5. Proposed general acid-base catalytic mechanism, MD simulation of C18:1 ceramide and steric hindrance for ceramide derivatives.

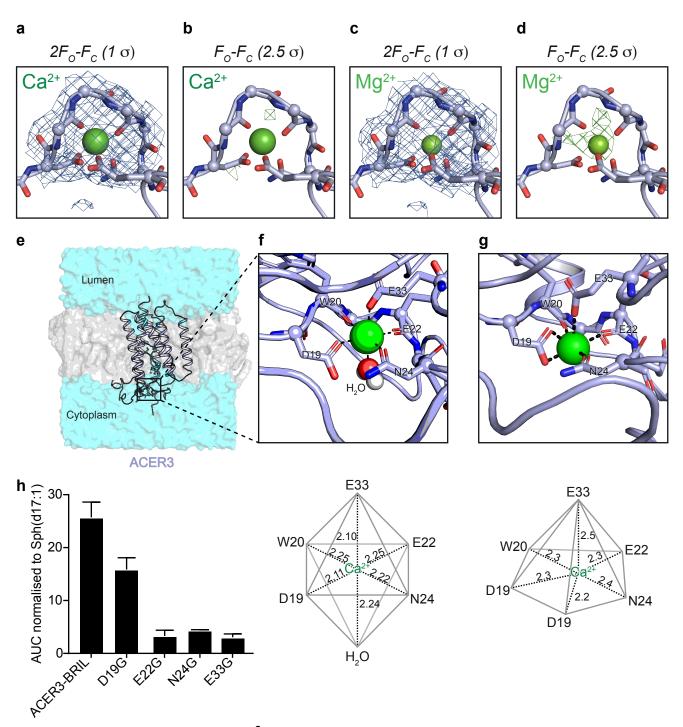
(a) Based on the interactions with docked ceramide C18:1 and the Zn²⁺ catalytic site architecture in the structure, we propose a general acid-base catalysis mechanism for the hydrolysis of the amide bond by ACER3. In this mechanism, the zinc ion activates a water molecule for nucleophilic attack of the amide carbon in which D92^{TM3} acts as a proton acceptor/donor (1 and 3). W220 side chain polarizes the amide carbonyl and, together with the Zn²⁺, stabilize the oxyanion formed in the tetrahedral transition state (2). (b) Representation of all-atom R.M.S.D. of the bound C18:1 ceramide in trajectory of five independent simulations. (c) Close up view of the S99, Y149, S228 domain (left panel) and enzymatic assays performed with the C18:1 (black bars) or C18 (grey bars) substrates showing the Area Under the Curve (AUC) sphingosine signal normalized to the internal standard (Sph d17:1) for ACER3-BRIL, ACER3-BRIL-S99A, ACER3-BRIL-Y149A and ACER3-BRIL-S228A mutants (right panel). The results shown are the mean \pm s.d. of two independent experiments performed in pentaplicate. (d) Top scoring C18:1 ceramide binding poses obtained using GlideXP, the SWISSDOCK webserver (in blind docking mode) or PLANTS. (e) Left panel: close up view of the catalytic site with key residues shown as sticks and coloured as in (a). Right panel: W20, F80, H81 and D92 are shown as light blue spheres and are involved in the steric hindrance close to the primary alcohol of ceramide, supporting a possible mechanism for substrate selectivity.

Supplementary Figure 6.



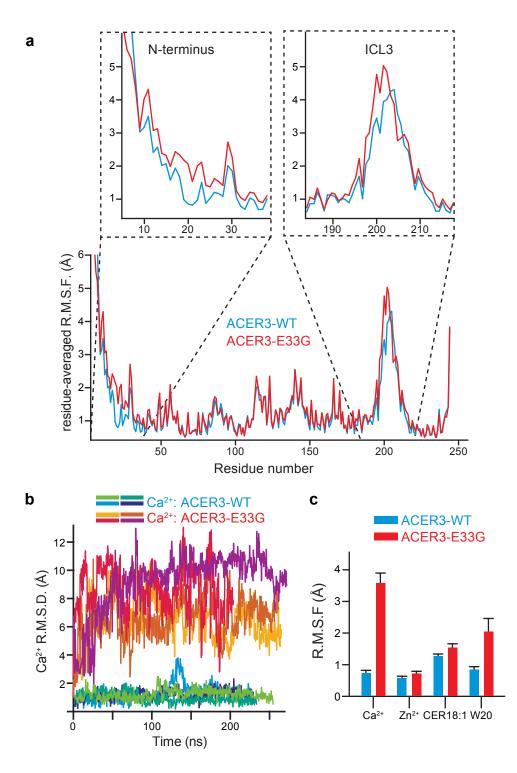
Supplementary Figure 6. Comparison of the structural features of ACER3, ADIPORs and neutral ceramidases.

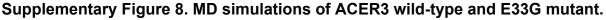
(a) Structures of ACER3, ADIPOR1 and ADIPOR2 viewed from within the membrane plane highlighting the distinct architecture on the intramembrane cavities (dark yellow, blue and purple for ACER3, ADIPOR2 and ADIPOR1, respectively). (b) Calculated ceramide C18:1 (coloured in cyan) binding modes in ACER3 and ADIPOR2. (c) Comparison of the ACER3 and neutral ceramidases structures coloured according to secondary structures (α -helices in cyan and β -sheet in purple) and showing the cavity accomodating the ceramide substrate located in the lipid bilayer for ACER3 (dark yellow) in contrast to the neutral ceramidase (NCD) which contains the ceramide binding pocket outside the lipid bilayer (dark yellow). (d) Zoom of the Zn²⁺ catalytic site of the NCD.



Supplementary Figure 7. Ca²⁺ coordination in the crystal structure and in MD simulations.

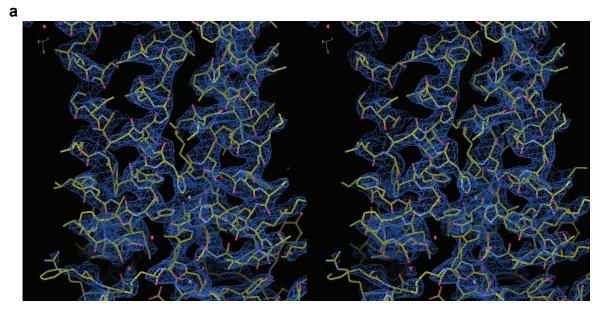
Comparison of 2Fo-Fc and Fo-Fc maps calculated either with Ca^{2+} (**a**, **b**) and Mg^{2+} (**c**, **d**). The positive signal indicated as a green mesh in (d) clearly indicates that the observed electron density cannot correspond to a Mg^{2+} ion. (**e**) Overall view of ACER 3 in a lipid bilayer (grey) surrounded with water (cyan). Zoom close to the Ca^{2+} ion (green spheres) illustrating the geometry of the Ca^{2+} binding site during MD simulations (octahedral) with the water molecule shown as white and red spheres (**f**) and in the crystal structure (incomplete pentagonal bipyramid) (**g**). (**h**) Enzymatic assays performed with the C18:1 substrate showing the Area Under the Curve (AUC) sphingosine signal normalized to the internal standard (Sph d17:1) for ACER3-BRIL, ACER3-BRIL-D19G, ACER3-BRIL-E22G, ACER3-BRIL-N24G and ACER3-BRIL-E33G mutants. The results shown are the mean \pm s.d. of two independent experiments performed in pentaplicate.





(a) Residue-averaged root mean square fluctuations (R.M.S.F.) of ACER3 wild-type (blue) and E33G mutant (red), highlighting the increase in flexibility observed in the N-terminus region and ICL3. A zoom of the N-terminus and ICL3 regions are shown in inset. (b) Comparison of the calcium ion R.M.S.D. in five independent wild-type and mutant MD trajectories. (c) R.M.S.F. of selected ligands/residues in wild-type (blue) and mutant (red) MD trajectories. The error bars correspond to the standard error calculated from five independent trajectories.

Supplementary Figure 9.



Supplementary Figure 9. Stereo images of a portion of the electron density map.

A portion of the 2Fo-Fc electron density map contoured at 1.5 σ .

Supplementary References

1 Ashkenazy, H. *et al.* ConSurf 2016: an improved methodology to estimate and visualize evolutionary conservation in macromolecules. Nucleic Acids Res **44**, W344-350, doi:10.1093/nar/gkw408 (2016).