



Supplementary Figure 1. The major molecular species of PI in mammals (A) and C. elegans (B).



Supplementary Figure 2. A procedure for determining *sn*-2-acyl LPIAT activity. A scheme of the procedure is shown in (A). After the *in vitro* acyltransferase assay, the lipids were extracted and separated by TLC as described in *Materials and Methods*. The resulting PI fractions were re-extracted from the TLC plates and treated with phospholipase A₂. The distribution of radioactivity among the reaction products (free fatty acid and lysoPI) was assessed following TLC (B, C). Radiolabeled free fatty acid and lysoPI were indicated as gray bars and black bars, respectively (C). [¹⁴C]stearoyl-CoA was used as an acyl donor. The membrane fraction of transgenic worms expressing ACL-10 showed increased stearoyl-CoA:*sn*-2-acyl LPIAT activity (C, compare black bars). "I", "FA" and "FFA" in panel A indicate inositol, fatty acid, and free fatty acid, respectively.

Gene	Sequence name	Human homologue
acl-1	F59F4.4	AGPAT1, AGPAT2
acl-2	T06E8.1	AGPAT1, AGPAT2
acl-3	ZK809.2	Tafazzin
acl-4	F49H12.6	GPAT3, GPAT4
acl-5	R07E3.5	GPAT3, GPAT4
acl-6	F08F3.2	GPAT1, GPAT2
acl-7	Y46G5A.21	DHAPAT
acl-8	T05H4.1	LYCAT
acl-9	ZK40.1	LYCAT
acl-10	F55A11.5	LYCAT
acl-11	F28B3.9	AGPAT5
acl-12	C01C10.3	LPGAT1
acl-13	F08G5.2	LPGAT1
acl-14	K07B1.5	LPGAT1

Supplementary Table 1 *C*.*elegans* AGPAT family



Supplementary Figure 3. Genomic structures of *acl-8*, *acl-9* and *acl-10*. The *acl-8*, *acl-9* and *acl-10* genes are located on chromosomes V. *acl-8* and *acl-9* are adjacent to each other. Gray and white boxes correspond to coding exons and UTRs, resepctively. The positions of the predicted conserved lysophospholipid acyltransferase motifs (motif I~IV), (Lewin *et al.*, 1999; Beigneux *et al.*, 2006), characteristic of AGPAT family members, are shown in red. The *tm2290* allele lacks the acyltransferase motif III and IV of *acl-8* and the entire *acl-9* genomic region, suggesting that this mutation is null allele of both *acl-8* and *acl-9*. The *tm1045* allele lacks all acyltransferase motifs of *acl-10*.

А

ACL-0 T MSSINEPPIDE AGOUIGTERELGUEERATEGNETUTEGENERGENEN ACL-0 T MRIPCLERPIDGWEEGECIEFATEGNETUTEGENERGENERGENERGENERGENEN Mouse 45 RLVATWUTLPVALLETMEGVEVITGDAFVPGERSVIINNERTROWMELWNCHME Mouse 45 RLVATWUTLPVALLETMEGVEVITGDAFVPGERSVIINNERTROWMELWNCHME ACL-8 45 RLVATWUTLPVALLETMEGVEVITGDAFVPGERSVIINNERTROWMELWNCHME ACL-8 45 RLVATWUTLPVALLETMEGVEVITGDAFVPGERSVIINNERTROWMELWNCHME ACL-8 45 RLVATWUTLPVALLETMEGVEVITGDAFVPGERSVIINNERTROWMELWNCHME ACL-8 45 RLVATWUTLPVALLETMEGVEVITGDAFVPGERSVIINNERTROWMELWNCHME ACL-8 46 RLVGFWUTEGCSIIEWVFGANVEVIGOGFIPGERSVIINNERTROWMELWNCHME ACL-8 47 RLVGFWUTEGCSIIEWVFGANVEVIGOGFIPGERSVIINNERTROWMELWNCH ACL-9 47 RLVGIWVIMGGALCNYIFGANTRIKGDFINHEGPATUINNERTROWMELWNCH ACL-9 47 RLVGIWVIMGGALCNYIFGANTRIKGDFINHEGPATUINNERTROWMELWOCLUN ACL-9 47 RLVGIWVIMGGALCNYIFGANTRIKGDFINHEGPATUINNERTROWMEWN ACL-0 51 RATSYWMITMGLSIKSUPGFGWANQAAFVFINHENDENTWERTROWMEWN ACL-0 51 RATSYWMITPMGLLEFIMGVRIRVSGEIFFGSPAN MMUMHRTRIDWWMWWCALYQI Human 103 VLRVEKICLKASLKGVPGFGWANQAAFVFINHEMENDUN ACL-8 103 VLRVEKICLKASLKSUPGFGWANQAAFVFINHEMENDUN MUMHRTRIDWWMWWCALYQI ACL-8 103 VLRVEKICLKASLKSUPGFGWANQAAFVFINHEMENDUN ACL-8 103 VLRVEKICLKASLKSUPGFGWANQAAFVFINHEMENDUN ACL-8 103 VLRVEKICLKAALKSUPGFGWANQAAFVFINHEMENDUN ACL-8 103 VLRVEKICLKAALKSUPGFGWANQAAFVFINHEMENDUN ACL-8 103 VLRVEKICLKAALKSUPGFGWANQAAFVFINHEMENDUN ACL-8 103 VLRVEKICLKAALKSUPGAGWANGSGYIFIORNEENDUN ACL-8 104 VLNOCKILKAALKSUPGAGWANGSGYIFIORNEENDUN ACL-8 105 FPECTDUTINKSRSN AFAEKNGLQKYEVVUNPRTIGFFVVDRREEGKNUDAKNYU ACL-9 111 LITSNKISLKAQUKKUPGAGGAANQAAFVFIN ACL-8 104 FPECTDUTINKSRSN AFAEKNGLQKYEVVUNPRTIGFFVVDRREEGKNUDAKNYU ACL-9 104 FFECTDUTINKSRSN AFAEKNGLQKYEVVUNPRTIGFFVVDRREEGKNUDAKNYU ACL-10 111 LITSNKISLKAQUKKUPGAGGAANGAANGEVYLUPRTIGFFVVDRREEGKNUDAKNYU ACL-10 111 LITSNKISLKAQUKKUPGAGGAANGAANGEVYLUPRTIGFFVVDRREEGKNUDAKNYU ACL-10 111 LITSNKISLKAQUKKUPGAGGAANQAAFVFU ACL-8 104 FFECTDUTINKSRSN AFAEKNGLQKYEVVUNPRTIGFFU ACL-9 104 FFECTDUTINKSRSN AFAEKNGLQKYEVVUNPRTIGFU ACL-10 111 FFESSUND ACL-10 111 FFESSUND ACL-10 111 FFESSUND A	MLMD 6 5 S 11 S	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Human 45 R LVATWLTLPVALLETMFGVKVTITGDAFVPGERSVILMNHRTRNDVMFLWNCLMRY Mouse 45 R LVATWLTLPVALLETMFGVKVTTGDAFVPGERSVILMNHRTRNDVMFLWNCLMRY Chicken 45 RIVATWLTLPVALLETMFGVKVTTGDGFPGERSVILMNHRTRNDVMFLWNCLMRY Ach-8 45 RIVATWLTLPVALLELVCVVTGDGFPGERSVILMNHRTRNDVMFLWNCLMRY Ach-8 45 RIVATWLTLPVALLELVCVVVTGDGFPGERSVILMNHRTRLDWMFLWNCLMRY Ach-8 45 RIVGFWLTPPCSLFEWVFGVNFRVTGDLERDEPATULMNHRTRLDWMFWCLUNCLMRY Ach-9 67 RLVGFWLTPPCSLFEWVFGVNFRVTGDLERDEPATULMNHRTRLDWMFWWCLUNCLMRY Ach-9 67 RLVGFWLTPPCSLFEWVFGVNFRVTGDLERDEPATULMNHRTRLDWMFWWCLUNCLMRY Ach-9 67 RLVGFWLTPPCSLFEWVFGVNFRVTGDLERDEPATULMNHRTRLDWMFWWCLUNCLMRY Ach-9 67 RLVGFWLTPPCSLFEWVFGVNFRVTGDLERDEPATULMNHRTRLDWMFWWCLUNCLMRY Muman 103 VLRLEKICLKSSLVSVPGFGWAMQVAF FTH RKWKDDXSHFEDMIDVFC01HEPLC Muman 103 VLRLEKICLKSSLKSVPGFGWAMQVAF FTH RKWKDDXSHFEDMIDVFC01HEPLC Chicken 103 VLRLEKICLKSSLKSVPGFGWAMQVAF FTH RKWKDDXSHFEDMIDVFC01HEPLC Ach-8 105 LTTEKISLKAFUKKVPGAGWAMQAASYTHDNKENKUKVVKSHFEDMIDVFC01HEPLC Ach-8 105 LTTEKISLKAFUKKVPGAGWAMASSGYTHDNFENDKVVLERIVVKVYSGSEKKVQ	S h (S h (S h) (S h	02 02 02 02 04 20 10 62 62 62 62 62 62 62 62 70 22 22
Mouse 45 R LVATWLTLPVALLETWFGVRVVITGDGFVPGERSVIIMMHRTRVDWFLWNCLMRX Chicken 45 R LVATWLTLPVALLETWFGVRVVTGDGFIPGERSVIIMMHRTRVDWFLWNCLMRX Zebraish 45 R LVGFWLTFPCSLITEWFGVNFVTGDGFIPGERSVIIMMHRTRVDWFLWNCLMRX ACL-8 45 R LVGFWLTFPCSLITEWFGVNFNVTGDLFERDEPALLIMMHRTRLDWMFLWQCLLRX ACL-8 45 R LVGFWLTFPCSLITEWFGVNFNVTGDLFERDEPALLIMMHRTRLDWMFLWQCLLRX ACL-8 45 R LVGFWLTFPCSLITEWFGVNFNVTGDLFERDEPALLIMMHRTRLDWMFLWQCLLRX ACL-8 45 R LVGFWLTFPCSLITEWFGVNFNVTGDLFERDEPALLIMMHRTRLDWMFLWQCLLRX ACL-10 51 R ATSYWMTIPMGLLEFLMGVRIRWSGDEFFGSPAMUMALAK ACL-10 51 R ATSYMMTIPMGLLEFLMGVRIRWSGDEFFGSPAMUMALAF Mouse 103 Y LR LEKICLKASLKGVPGFGWAMQVAFFIFLORKWKDDKSHFEDMIDYFCDIHEPUG Chicken 103 Y LR LEKICLKASLKSUPGFGWAMQVAFFIFLORKWKDDKSHFEDMIDYFCSIREW ACL-8 105 L RTKSKSKSK NAFAEKNQVPGGGWAMQYAFFIFLORKWKDDKSHFEDMIDYFCSIREW ACL-8 103 Y LR LEKICLKASLKGVPGFGWAMQYAFFIFLORKWKDDKSHFEDMIDYFCSIREW REDMIDYFCSIREW Chicken 103 Y LR LEKICLKASLKGVPGFGWAMQYAFFIFLORKWKDDKSHFEDMIDYFCSIREW REDMIDYFCSIREW REDMIDYFCSIREW ACL-10 111 L TSNKJSKAR NDFAEMAWAQKAFYF		02 02 04 20 10 62 62 62 62 64 80 70 22 22
Chicken 45 RIVATWLTLPVALLE LVEGAKUVTGDGFIPGERSVIIMMHRTRMDTWFLWCLLER ACL-8 45 RIVATWLTLPVALLE LVEGVKUVTGDGFIPGERSVIIMMHRTRLDTWFLWCLLER ACL-8 45 46 RLVGFWLTFPCSLIEWVFGVNFRVTGDLIFRDEPATLIMMHRTRLDTWFFLWCLLER ACL-8 61 47 RIVGFWLTFPCSLIEWVFGVNFRVTGDLIFRDEPATLIMMHRTRLDTWFFLWALYKM ACL-10 51 48 RLVGFWLTFPCSLIEWVFGVNFRVTGDLIFRDEPATLIMMHRTRLDTWFFLWALYKM ACL-10 51 49 RLVGFWLTFPCSLIEWVFGVNFRVTGDLIFRDEPATLIMMHRTRLDTWFVGUFFWALYKM ACL-10 51 40 92 YLRLEKICLKASLKGVPGFGWAMQAAA YIFHRWWKDDXSHFEDMIDVFGDIHEPUG TWAALYKM 400000 92 YLRLEKICLKSSLKSVPGFGWAMQVAAFIFIQRKWEDDSHFEDMIDVFGDIHEPUG Chicken 103 401 92 YLRLEKICLKSSLKSVPGFGWAMQVAAFIFIQRKWEDDSHFEDMIHVFCDIHEPUG Chicken 103 402 92 YLRLEKICLKSSLKSVPGFGWAMQVAAFIFIQRKWEDDSHFEDMIHVFCCAHFPUG RALSSLKAQUKKUPGAGWAMSSGSYIFLDRSHDDSHKUDNSWFENMIHVFCCAHPUG RALSSLKAQUKKUPGAGWAMASSGSYIFLDRSHDDSHKUDNINWYAETEVYG ACL-10 111 40000 92 112 111 112 111 112 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111		02 02 04 20 10 62 62 62 64 80 70 22 22
Zebraish 45 RIVATWLTLPVALLELVLGVKWVFGDGEIPGERSVIINNHRTRLDWMFLWCCLEX ACL-8 45 RLVGFWLTFPCSLTEWVFGVNFRVTGDLIERDEPATTIMNHRTRLDWMFLWCCLEX ACL-10 51 RATSYWMTIPMGLEFLMGVRIRVSGDLIERDEPATTIMNHRTRLDWMFKWNLYKN ACL-10 51 RATSYWMTIPMGLEFLMGVRIRVSGDLIERDEPATTIMNHRTRLDWMFKWNLYKN Mouse 103 YLRLEKICLKASLKGVPGFGWAMQAAAYTIFIHRKWKDDKSHFEDMIDVFGDIHEPLG Mouse 103 YLRLEKICLKSSLKSVPGFGWAMQAAAYTIFIHRKWKDDKSHFEDMIDVFGDIHEPLG Chicken 103 YLRUEKICLKSSLKSVPGFGWAMQVAAFIFIQRKWEDDRSHFEDMIDVFGDIHEPLG Zebraish 103 YLRUEKICLKASLKGVPGFGWAMQVAAFIFIQRKWEDDRSHFEDMIDVFGDIHEPLG Zebraish 103 YLRUEKICLKASLKGVPGFGWAMQVAFFIFURR RKWDDRSHFEDMIDVFGCAIHEPLG ACL-8 105 LUTTEKISLKAPUKKSVPGFGWAMQVAFFIFURR RKWDDRSHFEDMIDVFGCAIHEPLG ACL-8 105 LUTTEKISLKAPUKKSVPGFGWAMQVAFFIFURR RKWDDRSHFEDMIDVFGCAIHEPLG Mouse 103 YLROEKICLKASLKSVPGFGWAMQVAFSIFUOR RKWDDRSHFEDMIDVFCDIHEPLG ACL-8 105 LUTTEKISLKGMLKVPGAGWAMSGSYTFUOR RKWDDRSHFEDMIDVFCDIHEPLG ACL-10 111 LITSKSSRNAFAEKNGLQKYVYUHPRTTGFTVVDRLERVKNLVKNLOENDAWDD FPEGTDLTENSKSSRNAFAEKNGLQKYYVUHPRTTGFTFVVDRLERVKNLOAUNDAVHDD ACL-10	(S 7) (DPW 7) (NPW 7) (NP	02 04 20 10 62 62 62 62 62 62 62 62 62 62 70 22 22
ACL-8 45 REVGFWILTEPCSLIEWVFGVNFRVTGDLIERDEPALTIMNHETRLDWLFFWNALYKN ACL-9 61 RAISYWMTTPGALCNYTGDLIERDEPALTIMNHETRLDWLFFWNALYKN ACL-10 51 RAISYWMTTPGALCNYTGGALENTYSGDEIFFGSPAMTVMHETRLDWLFFWNALYKN Muman 103 YLRVEKICLKSSLKSVPGFGWAMQAAAYTFTHRKWKDDKSHFEDMIDYFCDTHEPLG Mouse 103 YLRVEKICLKSSLKSVPGFGWAMQAAAYTFTHRKWKDDKSHFEDMIDYFCATHEPLG Chicken 103 YLRVEKICLKSSLKSVPGFGWAMQAAAYTFTHRKWKDDKSHFEDMIDYFCATHEPLG Zebralish 103 YLRVEKICLKSSLKSVPGFGWAMQAAAYTFTHRKWKDDKSHFEDMIDYFCATHEPLG Zebralish 103 YLRQEKICLKAALKSVPGFGWAMQAAAYTFTHRKWKDDKSHFEDMIDYFCATHEPLG Zebralish 103 YLRQEKICLKAALKSVPGFGWAMQAAAYTFTHRKWKDDKSHFEDMIDYFCATHEPLG ACL-8 105 LLTTEKISKKGKMLKYPGAGWAMQAAAYTFTHRKWKDDKSHFENMUYFCATHEPLG ACL-10 111 LTTEKISKKGMLKYPGAGWAMQAAAYTFTHRKWKDDKKSHFENMUYFYSGSEKKYC Mouse 163 FPEGTDLTENKSSSSRNAFAEKNGLQKYEYVLHPRTIGFTFVVDRLREGKNLDAVHDT Mouse 164 FPEGTDLTENKSSSSSNAFAEKNGLQKYEYVLHPRTIGFTFVVDRLREGKNLDAVHDT Mouse 163 FPEGTDLTENKARSNDFAEKNGLQKYEYVLHPRTIGFTFVVDRLREGKNLDAVHDT Mouse 163 FPEGTDLTENKARSNDFAEKNGLQKYEYVLHPRTIGFTFVVDRLREGKNLDAVHDT Mouse 163	IDPW 1 IDPW 1 INPW 1 ILLI 1 ILLI 1 ILLI 1 ILL 1	04 20 10 62 62 62 62 64 80 70 22 22
ACL-9 61 REVGINVINGGALCNYIFGANIRIKGDFINNEGPANIVMNHETRLDWLFFWNALVKM ACL-10 51 RAISYWMTIPMGLEFLMGVRIRVSGDEIFFGSPAMIVMNHETRLDWLYMWGALYQI Human 103 YLRLEKICLKASLKGVPGFGWAMQAAYIFIARKWKDDKSHFEDMIDYFCAIHEPLC Mouse 103 YLRLEKICLKASLKGVPGFGWAMQAAYIFIARKWKDDKSHFEDMIDYFCAIHEPLC Chicken 103 YLRQEKICLKSSLKSVPGFGWAMQVAAFIFIQRKWEDDKSHFEDMIDYFCAIHEPLC Chicken 103 YLRQEKICLKSSLKSIPGFGWAMQVAAFIFIQRKWEDDRSHFEDMIDYFCAIHEPLC Chicken 103 YLRQEKICLKASLKSIPGFGWAMQVAAFIFIQRKWEDDRSHFEDMIDYFCAIHEPLC Chicken 103 YLRQEKICLKAALKSVPGFGWAMQVAAFIFIQRKWEDDRSHFEDMIDYFCAIHEPLC Chicken 103 YLRQEKICLKAAPLKKIPGAGWAMQAASYIFLDRSFDTDKFVLGRKWYSSEKKYC ACL-9 121 LCTTEKISKGMLKYVPGAGWAMQAASYIFLDRSFDTDKTKLDNILNYYAETEYKYC ACL-10 111 LITSNKISLKAQLKKLPGAGFGMAAAGFYFLERNAEVDKRSFDDAIDAIDYFKNIDKKYC Muman 163 FPEGTDLTENNKARSNDFAEKNGLQKYYVLHPRTTGFTFVVCGRREGKNLDAVHDI Chicken 163 FPEGTDLTENNKARSNDFAEKNGLQKYYVLHPRTTGFTFVVCGRREGKNLDAVHDI Chicken 163 FPEGTDLTENNKARSNDFAEKNGLQKYYVLHPRTTGFTFVVCGRREGKNLDAVHDI Chicken 163 FPEGTDLTENNKARSNDFAEKNGLQKYYVLHPRTTGFTFVVGGTREGKNLDAVHDI Chicken 164	ILLI 1/ ILL 1/ ILL 1/ ILL 1/ ILL 1/	20 10 62 62 62 62 62 64 80 70 22 22
ACL:10 51 NATSYWMTTPMGLLEFEMGVRIRVSGDETEFGSPAMLVMNHRTKLDWMYMWGALYQI Imman 103 YLRLEKICLKASLKGVPGFGWAMQAAYTIFHRWKDDKSHFEDMIDYFCATHEPUG Mouse 103 YLRLEKICLKASLKGVPGFGWAMQAAYTIFHRWKDDKSHFEDMIDYFCATHEPUG Chicken 103 YLRLEKICLKSSLKSVPGFGWAMQAAFTFIQRKWEDDKSHFEDMIDYFCATHEPUG Chicken 103 YLRLEKICLKSSLKSVPGFGWAMQVAAFTFIQRKWEDDKSHFENMLHYFCDTHEPUG Chicken 103 YLRLEKICLKSSLKSVPGFGWAMQVAAFTFIQRKWEDDKSHFENMLHYFCDTHEPUG Chicken 103 YLRQEKTCLKAALKSVPGFGWAMQVAAFTFIQRKWEDDKSHFENMLHYFCDTHEPUG ACL:8 103 YLRQEKTCLKAALKSVPGFGWAMQVAAFTFIQRKWEDDKSHFENMLHYFCDTHEPUG ACL:8 103 YLRQEKTCLKAALKSVPGFGWAMQVAAFTFIQRKWEDDKSHFENMLHYFCDTHEPUG ACL:9 121 LCTTEKTSLKAPLKKUPGAGWAMGAASYTFLORNFENDKYVUSKSEKKYG ACL:10 111 LTSNKTSLKAQLKKUPGAGEGMAAAQFVFLORSFDTDKTKLDNILNYAETEYKYG Mouse 163 FPEGTDLTENNKARSNDFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDT Mouse 163 FPEGTDLTENNKARSNDFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDT ACL:8 165 FPEGTDLTENKARSNDFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDT ACL:8 165 FPEGTDLTENKARSNDFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDT ACL:8 165 FP	INPW 1 ILI 1 ILI 1 ILI 1 ILI 1 ILI 1 ILL 1<	10 62 62 62 62 62 62 62 62 62 62 62 62 70 22 22
Muman 103 YLR LEKICLKASLKGVPGFGWAMQAAAYIFIHRKWKDDKSHFEDMIDYFCDTHEPLU Mouse 103 YLR VEKICLKASLKGVPGFGWAMQAAAYIFIHRKWKDDKSHFEDMIDYFCDTHEPLU Chicken 103 YLR VEKICLKSSLKSTPGFGWAMQVAAFIFIQRKWEDDKSHFEDMIDYFCDTHEPLU Zebrafish 103 YLR VEKICLKASLKGVPGFGWAMQVAAFIFIQRKWEDDKSHFEDMIDYFCDTHEPLU Zebrafish 103 YLR VEKICLKASLKSVPGFGWAMQVAAFIFIQRWEDDKSHFEDMULYYYSGESKKYC ACL-8 105 LTTEKISLKAPUKKIPGAGWAMSSGSYIFIDRSFDTDKTKLDNILNYYSESEKKYC ACL-10 111 LTEKISLKAPUKKIPGAGWAMGAASYIFIDRSFDTDKTKLDNILNYYAETEYKYC ACL-10 111 LTEKISLKAQLKKUPGAGWAMGAASYIFIDRSFDTDKTKLDNILNYYAETEYKYC Mouse 163 FPEGTDLTENSKSRSNAFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI Mouse 163 FPEGTDLTENSKSRSNAFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI Chicken 163 FPEGTDLTENSKSRSNAFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI ACL-8 165 FAEGTDLTENSKSRSNAFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI ACL-8 165 FAEGTDLKGERATRUSDAFADKNGLPRYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI ACL-9 141 FPEGTDLKGERATRUSDAFADKNGLPRYEYVLHPRTTGFTFVVDLRKKENYTKYMYDI ACL-10 171 FPEGTDLKGERATRUSDAFADKNGLPRYEYVLHPRTTGFTFVVDLRKKENYTKYMYDI ACL-10	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	62 62 62 64 80 70 22 22
Human 103 VERVERTCLERSSLRSVPGFGWAMQVAAFIFINRWEDDSSHFEDMIDVFCATHEPL Mouse 103 VLRVERTCLERSSLRSVPGFGWAMQVAAFIFIQRWEDDKSHFEDMIDVFCATHEPL Chicken 103 VLRVERTCLERSSLRSVPGFGWAMQVAAFIFIQRWEDDKSHFEDMILVFCATHEPL Chicken 103 VLRVERTCLERSSLRSVPGFGWAMQVAAFIFIQRWEDDKSHFEDMILVFCATHEPL CacLas 103 VLRVERTCLERSSLRSVPGFGWAMQVAAFIFIQRWEDDRSHFEDMILVFCATHEPL CacLas 103 VLRVERTCLERSSLRSVPGFGWAMQVAAFIFIQRWEDDRSHFEDMILVFCATHEPL CacLas 103 VLRVERTCLERSSLRSVPGFGWAMQVAAFIFIQRWEDDRSHFEDMILVFCATHEPL ACL-8 105 LCTTEKTSLKGMLKVPGAGWAMQAASYIFLDRSFDVDKPLERVVCPGRTREGKNLDAVHDI Mouse 163 FPEGTDLTENNKARSNDFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI Mouse 163 FPEGTDLTENNKARSNDFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI Mouse 163 FPEGTDLTENNKARSNDFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI ACL-8 165 FAEGTDLKGERATRLSDAFADKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI ACL-9 181 FPEGTDLTENTRARSDEFAEKNGLQKYEYVLHPRTTGFTFIVDTLRGGDNLDAVHDI ACL-9 181 FPEGTDLTENTRARSDEFAEKNGLQKYEYVLHPRTTGFTFIVDTLRGFTVVVELLEGNNDAVHDI ACL-9 181 FPEGTDLTENTRARSDEFAEKNGLQKYEYVLHPRTTGFTFIVDTLRGFTFIVDTLRGGDNLDAVHDI ACL-9	TVA 2	62 62 62 64 80 70 22 22
Muman 223 YPHNIPQSEKHLUQG-DFPKEIHFHVHRYPIDTLPTSKEDLQLWCHKRWEEKERER Human 223 YPHNIPQSEKHLUQG-DFPKEIHFHVHRYPIDTLPTSKEDLQLWCHKRWEEKERER Human 223 YPHNIPQSEKHLUQG-DFPKEIHFHVHRYPIDTLPTSKEDLQLWCHKRWEEKERER Human 223 YPHNIPQSEKHLUQG-DFPKEIHFHVHRYPIDTLPTSKEDLQLWCHKRWEEKERER Human 223 YPHNIPQSEKHLUQG-DFPKEIHFHVQRYPISKELDLWRFTGFTSKEDLQLWCHKRWEEKERER Human 223 YPHNIPQSEKHLUQG-DFPKEIHFHVQRYPISKELDLWRFTGFTSKELDLWWRRANNIKYTYD Human 223 YPHNIPQSEKHLUQG-DFPKEIHFHVQRYPISKELDLWWRTGFTSKELDLWWRRANNIKYTYD Human 223 YPHNIPQSEKHLUQG-DFPKEIHFHVQRYPISKELDLWWRTGFTSKELDLWWRRANNIKYTYD Human 223 YPHNIPQSEKHLUQG-DFPKEIHFHVQRYPISKELDLWWRTGFTSKELDLWWRRANNIKYTYD Human 223 YPHNIPQSEKKHLUQG-DFPKEIHFHVQRYPISTYPISKELDLWWRTGFTSKELDLWWRRANNIKYTYD Human 223 YPHNIPQSEKKHLUQG-DFPKEIHFHVQRYPISTYPISKELDLWWRHRANNIKYTYDY ACL-10 171 FPEGTDLKSEWTTLKSREFAKKGLWHRYPISTYPISKELDLWWRHRANNIKYTYDY Human 223 YPHNIPQSEKHLUQG-DFPKEIHFHVQRYPISTYPISKELDLWWCHKKRWEEKERR KEKERRY YYYDIFTTGFTSKELDLWWCHKRWEEKERR YYYDIFTTGFTSKELDLWWCHKRWEEKERR KIKKSEWTTLKSREFAKKGLWHYYPISTYPISKELDLWWWCHKKRWEEKERR YYYNIFYSKELYYWYPISKELDLWWCHKKRWEEKERR <		62 62 64 80 70 22 22
Zebrafish 103 Y L R Q E K T C L K A A L K S V P G F G W A M Q V A S F T F I Q R R W E D D R T H M S N M L Q Y F C R I R E P V A ACL-8 105 L L T T E K I S L K A P L K K I P G A G W A M Q A A S Y I F L D R S F D T D K T K L D N I L N Y A E T E Y K Y A ACL-9 121 L C T T E K I S L K A D L K K I P G A G W A M Q A A S Y I F L D R S F D T D K T K L D N I L N Y A E T E Y K Y A ACL-10 111 L I T S N K I S L K A O L K K I P G A G F G M A A A C F V F L E R N A E V D K R S F D D A I D Y F K N I D K K Y I Mouse 163 F P E G T D L T E N S K S R S N A F A E K N G L Q K Y E Y V L H P R T T G F T F V V D R L R E G K N L D A V H D I Mouse 163 F P E G T D L T E N S K S R S N A F A E K N G L Q K Y E Y V L H P R T T G F T F V V D R L R E G K N L D A V H D I Chicken 163 F P E G T D L T E N N K A R S N D F A E K N G L Q K Y E Y V L H P R T T G F T F V V D R L R E G K N L D A V H D I ACL-8 163 F P E G T D L T E N N K A R S N D F A E K N G L Q K Y E Y V L H P R T T G F T F I V D T L R G G D N L D A V H D I ACL-8 164 F A E G T D K G R A T R L S D A F A E K N G L Q K Y E Y V L H P R T T G F T F I V D T L R G G D N L D A V H D I ACL-9 181 F P E G T D L T E N T R A R S D E F A E K N G L Q K Y E Y V L H P R T T G F T F I V D T L R G G D N L D A V H D I ACL-8 223 Y P H N I P Q S E K H L L Q G - D F P K E I H F H V Q R Y P A D S L P T S K E D L Q L W C K R		62 64 80 70 22 22
ACL-8 105 LLTTEKISLKAPLKKIPGAGWAMSSGSYIFLDRNFENDKPVLERIVKYSGSEKKYG ACL-9 121 LCTTEKISLKAPLKKIPGAGWAMQAASYIFLDRSFDTDKTKLDNILNYAETEYKYG ACL-10 111 LITSNKISLKAPLKKIPGAGFGMAAAGFVFLERNAEVDKRSFDDAIDYFKNIDKKYG MCL-10 111 LITSNKISLKAPLKKIPGAGFGMAAAGFVFLERNAEVDKRSFDDAIDYFKNIDKKYG MCL-10 111 LITSNKISLKAPLKKIPGAGFGMAAAGFVFLERNAEVDKRSFDDAIDYFKNIDKKYG MOUSE 163 FPEGTDLTENSKSRSNAFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI Mouse 163 FPEGTDLTENSKSRSNAFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI Chicken 163 FPEGTDLTENSKSRSNAFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI ACL-8 165 FAEGTDLTENTRARSDEFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI ACL-9 181 FPEGTDLTENTRARSDEFAEKNGLQKYEYVLHPRTTGFTVDIVDRLREGKNLDAVHDI ACL-9 181 FPEGTDKGERATRLSDAFAEKNGLRHYYVULHPRTTGFKELMELMKKENYTKYVYDI ACL-9 181 FPEGTDKGERATRLSDAFAEKNGLRHYVYVULHPRTTGFKELMELMKKENYTKYVYDI Human 223 YPHNIPQSEKHLUQG-DFPREIHFHVURYPIDTLPTSKEDLQLWCHKRWEEKEERLR Mouse 223 YPHNIPQSEKHLUQG-DFPREIHFHVURYPIDTLPTSKEDLQLWCHKRWEEKEERLR ACL-10 121 FPEGTDKSEKHLUQG-DFPREIHFHVURYPIDTLPTSKEDLQLWCHKRWEEKEERLR ACL-24 25	TVA 2	64 80 70 22 22
ACL-9 121 LCTTEKISLKGMLKYVPGAGWAMQAASYIFLORSFDTDKTKLDNILNYYAETEYKYC ACL-10 111 LITSNKISLKAQLKKIPGAGFGMAAAQFVFLERNAEVDKRSFDDAIDYFKNTDKKYC Mouse 163 FPEGTDLTENSKSRSNAFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI Mouse 163 FPEGTDLTENSKSRSNAFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI Chicken 163 FPEGTDLTENSKSRSNAFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI ACL-8 163 FPEGTDLTENSKSRSNAFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI ACL-9 181 FPEGTDLTENSKSRSNAFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI ACL-9 181 FPEGTDLTENSKSRSNAFAEKNGLQKYEYVLHPRTTGFTVVDRLREGKNLDAVHDI ACL-9 181 FPEGTDLTENSKRSEFKENGLQKYEYVLHPRTTGFTVVDRLREGKNLDAVHDI ACL-9 181 FPEGTDLTANTKARSNDFAEKNGLRHYEYVLHPRTTGFTVDLWCRKRGGVNLDAVHDI ACL-9 181 FPEGTDLTANTKARSNDFAEKNGLRHYEYVLHPRTTGFTVDLWCRKRGGVNLDAVHDI ACL-9 181 FPEGTDLTANTKARSNDFAEKNGLRHYEYVLHPRTTGFTUVDTGFKELMELMKKENYTKYYDY ACL-10 171 FPEGTDLTANTKARSNDFAEKNGLRHYEYVLHPRTTGFTUVDTGFKELMELMKKENYTKYYDY ACL-10 171 FPEGTDLTANTKARSNDFAEKNGLRHYEYVLHOKKKGLVKYKKERGGVNLKENYTKYYDY Human 223 YPHNIPQSEXHLLQG-DFPREIHFHVNRYPTIDTLPTSKEDLQUWCHKRWEEKERRLC ACL-10	TVA 2	80 70 22 22
ACL-10 111 LITSNKJSLKAQLKKLPGAGFGMAAACFVFLERNAEVDKRSFDDAIDYFKNDDKKYC Mouse 163 FPEGTDLTENSKSRSNAFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI Mouse 163 FPEGTDLTENSKSRSNAFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI Chicken 163 FPEGTDLTENSKSRSNAFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI Zebrafish 163 FPEGTDLTENSKSRSNAFAEKNGLQKYEYVLHPRTTGFTFVVECLREGNNLDAVHDI Zebrafish 163 FPEGTDLTENTRARSDEFAEKNGLQKYEYVLHPRTTGFTFVVECLREGNLDAVHDI ACL-9 181 FPEGTDLTENTRARSDEFAEKNGLQKYEYVLHPRTTGFTFVVECLREGNLDAVHDI ACL-9 181 FPEGTDLTENTRARSDEFAEKNGLQKYEYVLHPRTTGFTVVECLREGNLDAVHDI ACL-9 181 FPEGTDLTENTRARSDEFAEKNGLQKYEYVLHPRTTGFTVVECLREGNLDAVHDI ACL-9 181 FPEGTDLTENTRARSDEFAEKNGLQKYEYVLHPRTTGFTVIVGTURGFTVHVQQAMRRANNIKYYYDD ACL-10 171 FPEGTDKSEWTTLKSREFAKKNGLRHLDYVLYPRTGFVHIVQAMRRANNIKYYYDD Human 223 YPHNIPQTEKHLLQG-DFPREIHFHVNRYPIDTLPTSKEDLQLWCHKRWEEKEERLR Chicken 223 YPQNIPQTEKHLLQG-DFPREIHFHVNRYPIDTLPTSKEDLQLWCHKRWEEKEERLR Chicken 223 YPQNIPQTEKHLLNG-NFPREIHFHVNRYPIDTLPTSKEDLQLWCHKRWEEKEERLR ACL-10 231 YPQNIPQTEKHLLNG-NFPREIHFHVNRYFTORFUTYSVALEDUWCQERWREKERRLC ACL-2	TVA 2	70 22 22
motif III motif IV Human 163 FPEGTDLTENSKSRSNAFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDJ Mouse 163 FPEGTDLTENSKSRSNAFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDJ Chicken 163 FPEGTDLTANTKARSNDFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDJ Zebrafish 163 FPEGTDLTANTKARSNDFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDJ ACL-8 165 FAEGTDLTANTKARSNDFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDJ ACL-9 181 FPEGTDLTANTKARSNDFAEKNGLQKYEYVLHPRTTGFTIVDTLRGGDNLDAVHDJ ACL-9 181 FPEGTDKCPKATERSRIHSEKKGLVHYCYVLHPRTTGFTIVDLWCHKRWEGKENYTKYVYDL ACL-10 171 FPEGTDKSEWTTLKSREFAKKNGLRHLTYVLYPTTGFLHLTNKMREQEYVEYTYDJ Human 223 YPHNIPQSEXHLLQG-DFPREIHFHVHRYPTDTLPTSKEDLQLWCHKRWEEKEERLR Mouse 223 YPNIPQTEKHLLQG-DFPREIHFHVQRYPADSLPTSKEDLQLWCHKRWEEKEERLR Chicken 223 YPQNIPQTEKHLLQG-VFPREIHFHVQRYPADSLPTSKEDLQUWCHKRWEEKERRLQ Chicken 223 YPQNIPQTEKHLLQG-VFPREIHFHVQRYPADSLPTSKEDLQUWCHKRWEEKERRLQ ACL-8 225 YSGTIVDTEAKLLAG-NFPEKEIHFHVQRYPASSVPAGAAGLQAWCQERWREKERRLQ ACL-8 225 YSGTIVDTEAKLLAG-NFPEKEIHFHVQRFTVASVPAGAAGLQAWCQERWREKERLQ ACL-8 225 YSGTIVDTEAKLLAG-NFPEKEIHFHVQRFTVASVPAGAAGLQAW	TVA 2	22 22
Human 163 FPEGTDLTENSKSRSNAFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDD Mouse 163 FPEGTDLTENSKARSNDFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDD Chicken 163 FPEGTDLTENSKARSNDFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDD Chicken 163 FPEGTDLTENSKARSNDFAEKNGLQKYEYVLHPRTTGFTFVVELLREGNNLDAVHDD Chicken 163 FPEGTDLTENSKARSNDFAEKNGLQKYEYVLHPRTTGFTFIVDTLREGKNLDAVHDD ACL-8 165 FAEGTDKGERATRLSDAFADKNGLPRYEYVLHPRTTGFTFIVDTLRGGDNLDAVHDD ACL-9 181 FPEGTDKCPKATERSRIHSEKKGLVHYQYVLHPRTTGFKFLMELMKKENYTKYVYDV ACL-10 171 FPEGTDKSEWTTLXSREFAKKNGLRHLDYVLYPRTTGFLHLUNKKENYTKYVYDD Human 223 YPHNIPQSEKHLUQG-DFPREIHFHVHRYPIDTLPTSKEDLQLWCHKRWEEKEERIK Mouse 223 YPHNIPQSEKHLUQG-DFPREIHFHVHRYPIDTLPTSKEDLQLWCHKRWEEKEERIK Mouse 223 YPHNIPQSEKHLLQG-VFPREIHFHVHRYPIDTLPTSKEDLQLWCHKRWEEKEERIK Mouse 223 YPHNIPQSEKHLLQG-VFPREIHFHVHRYPTIGTVPTSKEDLQLWCHKRWEEKEERIK Mouse 223 YPHNIPQSEKHLLQG-VFPREIHFHVHRYPTIGTVPTSKEDLQLWCHKRWEEKEERIK ACL-10 231 YPNNIPQTEKHLLAG-NFPREIHFHVQRYPTIGTVPTSKEDLQLWCQKRWEEKEERIK ACL-8 225 YSGTIVDTEAKLLAG-NFPREIHFHVQRFTIEAIPQTDEAGAAGLQAWCQERWREKERRLG ACL-8 2	TVA 2	22
Mouse 163 FPEGTDLTENKKARSNDFAEKNGLQKYEYVLHPRTTGFTFVVDRLREGKNLDAVHDI Chicken 163 FPEGTDLTENKKARSNDFAEKNGLRKYEYVLHPRTTGFTFVVECLREGNNLDAVHDI Zebrafish 163 FPEGTDLTENKARSNDFAEKNGLRKYEYVLHPRTTGFTFVVECLREGNNLDAVHDI Zabrafish 163 FFEGTDLTENKARSNDFAEKNGLQKYEYVLHPRTTGFTFVVECLREGNNLDAVHDI ACL-8 165 FAEGTDKGERATRLSDAFADKNGLPRYEYVLHPRTTGFTIVDTLRGGDNLDAVHDI ACL-9 181 FPEGTDKCPKATERSRIHSEKKGLVHYQYVLHPRTTGFLHLLNKMREGEYVEYIYDY ACL-10 171 FPEGTDKSEWTTLKSREFAKKNGLRHLTYVLYPRTTGFLHLLNKMREGEYVEYIYDI Human 223 YPHNIPQSEXHLLQG-DFPREIHFHVHRYPIDTLPTSKEDLQLWCHKRWEEKEERLF Mouse 223 YPHNIPQSEXHLLQG-DFPREIHFHVHRYPIDTLPTSKEDLQLWCHKRWEEKEERLF Chicken 223 YPHNIPQTEKHLLQG-VFPREIHFHVHRYPIDTLPTSKEDLQLWCHKRWEEKEERLF Zobrafish 223 YPHNIPQTEKHLLQG-VFPREIHFHVHRYPIDTLPTSKEDLQLWCHKRWEEKERLF Mouse 223 YPQNIPQTEKHLLQG-VFPREIHFHVQRYPADSLPTSKEDLQLWCHKRWEEKERLF Zobrafish 223 YPQNIPQTEKHLLQG-VFPREIHFHVQRYPIETVASVPAGAAGLQAWCQERWREKERER Zobrafish 223 YPQNIPQTERHLLAG-VFPREIHFHVQRFTASVPAGAAGLQAWCQERWREKERRLG ACL-8 225 YSGTIVDTEAKLLAG-NFPDKVHLDVKKYKLDEIP-TGEGCEKWLTDLWATKEKRLK ACL-9	a second s	22
Chicken 163 FPEGTDLTANTKARSNDFAEKNGLRKYEYVLHPRTTGFTFVVECLREGNNLDAIHDI Zebrafish 163 FPEGTDLTENTRARSDEFAEKNGLQKYEYVLHPRTTGFTFVVECLREGNLDAVHDI ACL4 165 FAEGTDKCPKATERSRIHSEKKGLVHYEYVLHPRTTGFTFVVECLREGNNLDAVHDI ACL9 181 FPEGTDKCPKATERSRIHSEKKGLVHYEYVLHPRTTGFTFVVECLREGNNLDAVHDI ACL-10 171 FPEGTDKCPKATERSRIHSEKKGLVHYEYVLHPRTTGFVHIVQAMRRANNIKYIYDV ACL-10 171 FPEGTDKSEWTTLKSREFAKKNGLRHLDYVLYPRTTGFLHLLNKMREQEYVEYIYDI Human 223 YPHNIPQSEKHLLQG-DFPREIHFHVHRYPIDTLPTSKEDLQLWCHKRWEEKEERIF Mouse 223 YPQNIPQTEKHLLLG-DFPKEIHFHVQRYPADSLPTSKEDLQLWCCHRWEEKEERLR Chicken 223 YPQNIPQTEKHLLLG-NFPKEIHFHVQRYPADSLPTSKEDLQLWCQKRWEEKEERLR Zebratish 223 YPQNIPQTEKHLLNG-NFPKEIHFHVQRYPADSLPTSKEDLQLWCQKRWEEKEERLR Chicken 223 YPQNIPQTEKHLLNG-NFPKEIHFHVQRYPADSLPTSKEDLQLWCQKRWEEKERERLG ACL-8 254 YSGTIVDTEAKLLAG-NFPCKVHLDVKKYKUR ACL-8 225 YSGTIVDTEAKLLAG-NFPCKVHLDVKKYKULDEIP-TGEGCEKWLTDLWATKEKRLK ACL-9 241 FGDAIVQSELDIFAHGVCPKEVFYQVIKYPTEAIPQTDALGQWLVNLWRNKEEKLK ACL-10 231 YPYNIVQSEIDLYLKGASPREVHFNIKIPISQVPLAEQDASRWLTDLWATKEEKLK MacL-10 241	IVA 2	
Zebrafish 163 FPEGTDLTENTRARSDEFAEKNGLQKYEYVLHPRTTGFTFIVDTLRGGDNLDAVHDT ACL-8 165 FAEGTDKGERATRLSDAFADKNGLPRYEYVLHPRTTGFKFLMELMKKENYTKYYDD ACL-8 167 FPEGTDKGERATRLSDAFADKNGLPRYEYVLHPRTTGFKFLMELMKKENYTKYYDD ACL-9 181 FPEGTDKGERATRLSDAFADKNGLPRYEYVLHPRTTGFKFLMELMKKENYTKYYDD ACL-10 171 FPEGTDKSEWTTLKSREFAKKNGLRHLTYVLYPRTTGFLHLINKMREQEYVEYIYDT Human 223 YPHNIPQSEKHLLQG-DFPREIHFHVHRYPIDTLPTSKEDLQLWCHKRWEEKEERLF Mouse 223 YPYNIPQTEKHLLUG-DFPREIHFHVHRYPIDTLPTSKEDLQLWCCHRRWEEKEERLF Chicken 223 YPQNIPQTEKHLLUG-DFPREIHFHVQRYPADSLPTSKEDLQLWCQKRWEEKEERLF Chicken 223 YPQNIPQTEKHLLAG-VFPREIHFHVQRYPIETVPTSKEELQLWCQKRWEEKEERLF Chicken 223 YPQNIPQTERHLLAG-VFPREIHFHVQRYPIETVPTSKEELQLWCQKRWEEKEERLF Zabrafish 223 YPQNIPQTERHLAG-NFPKEIHFHVQRYPIETVPTSKEELQLWCQKRWEEKEERLF Chicken 224 YPQNIPQTERHLAG-NFPCKVHVQRYPIETVPTSKEELQUWCQKRWEEKEERLF Chicken 225 YSGTIVDTEAKLLAG-NFPCKVHVQRYPIETVASVPAGAAGLQAWCQERWREKERRLG ACL-10 231 YPYNIVQSEIDUVKGASPREVHFNIRKIPISQVPLAEQDASRWLTDRWTIKEQLH Human 282 Q	TVA 2	22
ACL-8 165 FABE GTDKGERATRLSDAFADKNGLPRYEYVLHPRTTGFKELMELMKKENYTKYVYDI ACL-9 181 FPEGTDKCPKATERSRIHSEKKGLVHYGYVLHPRTTGFVHIVQAMRRANNIKYTYDY ACL-10 171 FPEGTDKSEWTTLKSREFAKKNGLRHLDYVLYPRTTGFLHLTNKMREQEYVEYTYDI Human 223 YPHNIPQSEKHLLQG-DFPREIHFHVHRYPIDTLPTSKEDLQLWCHKRWEEKEERLF House 223 YPNIPQTEKHLLQG-DFPREIHFHVHRYPIDTLPTSKEDLQLWCHKRWEEKEERLF Chicken 223 YPNIPQTEKHLLG-DFPKEIHFHVHRYPIDTLPTSKEDLQLWCHKRWEEKEERLF Chicken 223 YPQNIPQTEKHLLG-VFPKEIHFHVQRYPIETVPTSKEDLQLWCCKRWEEKEERLF Chicken 223 YPQNIPQTEKHLLGG-VFPREIHFHVQRYPIETVPTSKEDLQLWCCKRWEEKERLF Chicken 223 YPQNIPQTEKHLLGG-VFPREIHFHVQRYPIETVPTSKEDLQLWCCKRWEEKERLF Chicken 223 YPQNIPQTEKHLLGG-VFPREIHFHVQRYPIETVPTSKEDLQLWCCKRWEEKERLF Chicken 224 YPQNIPQTERHLLAG-VFPREIHFHVQRYFIEAIPQTGAAGLQAWCQERWREKERLC ACL-8 225 YSGTIVDTEAKLLAG-NFPDKVHLDVKKYKLDEIP-TGEGEKWUTDLWATKEKRLK ACL-9 241 FGDAIVQSELDIFAHGVCPKEVFQVIKYPTEAIPQTEAIPQTDEALGQWLVNLWRNKEEKLK ACL-10 231 YPYNIVQSEIDLVLKGASPREVHFHITKIPISQVPLANEQDASRWLTDRWTKEEKLK Human 282 Q	TVA 2	22
ACL-9 181 FPEGTDKCPKATERSRIHSEKKGUVHYOYVLHPRVTGFVHIVQAMRRANNIKYIYDA ACL-10 171 FPEGTDKSEWTTLKSREFAKKNGLRHLDYVLYPRTTGFLHLINKMREQEYVEYIYDI Human 223 YPHNIPQSEKHLLQG-DFPREIHFHVHRYPIDTLPTSKEDLQLWCHKRWEEKEERIF Chicken 223 YPHNIPQTEKHLLQG-DFPREIHFHVHRYPIDTLPTSKEDLQLWCHKRWEEKEERIF Chicken 223 YPNIPQTEKHLLQG-VFPREIHFHVQRYPADSLPTSKEDLQLWCHRRWEEKEERIF Chicken 223 YPQNIPQTEKHLLQG-VFPREIHFHVQRYPADSLPTSKEDLQLWCHRRWEEKEERIF Chicken 223 YPQNIPQTEKHLLAG-VFPREIHFHVQRYPADSLPTSKEDLQLWCQKRWEEKEERIF Chicken 223 YPQNIPQTEKHLLAG-NFPKEIHFHVQRYPIETVPTSKEDLQLWCQKRWEEKEERIF Cal-8 225 YSGTINDTEAKLLAG-NFPKEIHFHVQRFTVASVPAGAAGLQAWCQERWREKERRLC ACL-8 225 YSGTINDTEAKLLAG-NFPDKVHLDVKKYKLDEIP-TGEGCEKWLTDLWATKEKRLK ACL-9 241 FGDAIVQSELDIFAHGVCPKEVFYQVIKYPTEAIPQTDEALGQWLVNLWRNKEEKLK ACL-10 231 YPYNIVQSEIDLVLKGASPREVHENTRKIPISQVPLNEQDASRWLTDRWTIKEQLLH Human 282 Q	TIA 2	24
ACL-10 171 FPEGTDKSEWTTLKSREFAKKNGLRHLUYVLYPRTTGELHLLNKMREQEYVEYIYD Human 223 YPHNIPQSEXHLLQG-DFPREIHFHVHRYPIDTLPTSKEDLQLWCHKRWEEKEERIF Mouse 223 YPYNIPQTEKHLLQG-DFPREIHFHVQRYPIDTLPTSKEDLQLWCHKRWEEKEERIF Chicken 223 YPQNIPQTEKHLLQG-NFPKEIHFHVQRYPIETVFTSKEDLQLWCQKRWEEKEERIF Zabratish 223 YPQNIPQTEKHLLAG-NFPKEIHFHVQRYPIETVASVPAGAAGLQAWCQERWREKERIF ACL-8 224 YSGTIVDTEAKLLAG-NFPREIHFHVQRFTVASVPAGAAGLQAWCQERWREKERRIF ACL-9 241 FGDAIVQSELDIFAHGVCPKEVFYQVIKYPTEAIPQTDALGQWLVNLWRNKEEKIK ACL-10 231 YPYNIVQSEIDLVLKGASPREVHENIRKIPISQVPLNEQDASRWLTDLWATKEKRIK Human 282 QGEKNFYFTGQSVIPPCKSELRVLVVKLLSILYWTLFSPAMC Mouse 282 QGEKNFHFTGQSTVPPCKSELRVLVVKLLSIVYWALFGSAMC Chicken 282 E	51G 2	40
Human 223 YPHNIPQSEKHLLQG-DFPREIHFHVHRYPIDTLPTSKEDLQLWCHKRWEEKEERLF Mouse 223 YPYNIPQTEKHLLLG-DFPKEIHFHVQRYPADSLPTSKEDLQLWCHKRWEEKEERLF Chicken 223 YPQNIPQTEKHLLLG-DFPKEIHFHVQRYPIETVPTSKEDLQLWCHKRWEEKEERLF Chicken 223 YPQNIPQTEKHLLLG-NFPKEIHFHVQRYPIETVPTSKEDLQLWCQKRWEEKEERLF Chicken 223 YPQNIPQTEKHLLAG-NFPKEIHFHVQRYPIETVPTSKEELQLWCQKRWEEKEERLF Cal-8 224 YSGTIVDTEAKLLAG-NFPCKVHLDVKKYKLDEIP-TGEGCEKWLTDLWATKEKRLK ACL-8 225 YSGTIVDTEAKLLAG-NFPCKVHLDVKKYKLDEIP-TGEGCEKWLTDLWATKEKRLK ACL-8 224 YSGTIVDTEAKLLAG-NFPCKVHLDVKKYKLDEIP-TGEGCEKWLTDLWATKEKRLK ACL-9 241 FGOAIVQSELDIFAHGVCPKEVFVQVIKYDIEAIPQTBALGQWLVNLWRNKEEKLK ACL-10 231 YPYNIVQSEIDLVLKGASPREVHFHTRKIPISQVPLNEQDASRWLTDRWTIKEQLLH Human 282 QGEKNFYFTGQSVIPPCKSELRVLVVKLLSILVWTLFSPAMC Mouse 282 Q	11A 2	30
Mouse 223 YPYNIPQTEKHLLLG - DFPKEIHFHVQRYPADSLPTSKEDLQLWCHRRWEEKEERLF Chicken 223 YPQNIPQTEKHLLAG - NFPKEIHFHVQRYPIETVPTSKEELQLWCQKRWEEKEERLF Zebratish 223 YPQNIPQTERHLLAG - NFPKEIHFHVQRFTVASVPAGAAGLQAWCQERWREKERRLC ACL-8 225 YSGTIVDTEAKLLAG - NFPDKVHLDVKKYKLDEIP - TGEGCEKWLTDLWATKEKRLK ACL-9 241 FGOALVQSELDIFAHGVCPKEVFYQVIKYPTEAIPQTDEALGQWLVDLWATKEKRLK ACL-10 231 YPYNIVQSEIDLVLKGASPREVHFHIRKIPISQVPLNEQDASRWLTDRWTIKEQLLF Human 282 QGEKNFYFTGQSVIPPCKSELRVLVVKLLSILYWTLFSPAMC Mouse 282 QGEKNFYFTGQSTVPPCKSELRVLVVKLLSIVYWALFCSAMC	SEV 2	R1
Chicken 223 YPQNIPQTEXHLLNG-NFPKEIHFHVQRYPIETVPTSKEELQLWCQKRWEEKERLF Zebratish 223 YPQNIPQTERHLLAG-VFPREIHFHVQRFTVASVPAGAAGLQAWCQERWREKERLC ACL-8 224 YSGTIVDTEAKLLAG-NFPDKVHLDVKKYKLDEIP-TGEGCEKWLTDLWATKEKRLK ACL-8 224 YSGTIVDTEAKLLAG-NFPDKVHLDVKKYKLDEIP-TGEGCEKWLTDLWATKEKRLK ACL-8 224 FGDAIVQSELDIFAHGVCPKEVFYQVIKYPTEAIPQTDEALGQWLVNLWRNKEEKLK ACL-9 241 FGDAIVQSELDIFAHGVCPKEVFYQVIKYPTEAIPQTDEALGQWLVNLWRNKEEKLK ACL-10 231 YPYNIVQSEIDLVLKGASPREVHFHIRKIPISQVPLNEQDASRWLTDRWTIKEQLLF Human 282 QGEKNFYFTGQSVIPPCKSELRVLVVKLLSILYWTLFSPAMC Mouse 282 QGEKNFHFTGQSTVPPCKSELRVLVVKLLSIVYWALFCSAMC Chicken 282 E	SEY 2	81
Zebratish 223 YPQNIPQTERHLLAG-VFPREIHFHVQRFTVASVPAGAAGLQAWCQERWREKERRLC ACL-8 225 YSGTIVDTEAKLLAG-NFPDKVHLDVKKYKLDEIP-TGEGCEKWLTDLWATKEKRLK ACL-9 241 FGDAIVQSELDIFAHGVCPKEVFYQVIKYPIEAIPQTDEALGQWLVNLWRNKEEKLK ACL-10 231 YPYNIVQSEIDLVLKGASPREVHFHIRKIPISQVPLNEQDASRWLTDRWTIKEQLL Human 282 QGEKNFYFTGQSVIPPCKSELRVLVVKLLSILYWTLFSPAMC Mouse 282 Q	REY 2	81
ACL-8. 225 YSGTIVDTEAKLLAG-NFPDKVHLDVKKYKLDEIP-TGEGCEKWLTDLWATKEKRL ACL-9. 241 FGDAIVQSELDIFAHGVCPKEVFYQVIKYPTEAIPQTDEALGQWLVNLWRNKEEKL ACL-10. 231 YPYNIVQSEIDLVLKGASPREVHFHIRKIPISQVPLNEQDASRWLTDRWTIKEQLL Human. 282 QGEKNFYFTGQSVIPPCKSELRVLVVKLLSILYWTLFSPAMC Mouse. 282 QGEKNFHFTGQSTVPPCKSELRVLVVKLLSIVYWALFCSAMC Chicken. 282 EGGKCFDETGQSIIPPCKSELRVLAVKCISLLYWTVPPMGTF	REY 2	81
ACL9 241 FGDATVQSELDIFAHGVCPKEVFYQVIKYPTEAIPQTDEALGQWLVNLWRNKEEKL ACL-10 231 YPYNIVQSEIDLVLKGASPREVHEHIRKIPISQVPLNEQDASRWLTDRWTIKEQLL Human 282 QGEKNFYFTGQSVIPPCKSELRVLVVKLLSILYWTLFSPAMC Mouse 282 QGEKNFHFTGQSTVPPCKSELRVLVVKLLSIVYWALFCSAMC Chicken 282 EGGKCFDETGQSIIPPCKSELRVLAVKCISLLYWTVPPMGTF	KFY 2	82
ACL-10 231 YPYNIVQSEIDLVLKGASPREVHENIRKIPISQVPLNEQDASRWLTDRWTIKEQLLF Human 282 QGEKNFYFTGQSVIPPCKSELRVLVVKLLSILYWTLFSPAMG Mouse 282 QGEKNFHFTGQSTVPPCKSELRVLVVKLLSIVYWALFGSAMG Chicken 282 EGGKCFDETGQSIIPPCKSELRVLAVKCISLLYWTVPPMGTF	RFY 3	00
Human 282 QGEKNFYFTGQSVIPPCKSELRVLVVKLLSILYWTLFSPAM(Mouse 282 QGEKNFHFTGQSTVPPCKSELRVLVVKLLSIVYWALFCSAM(Chicken 282 EGGKCFDETGQSIIPPCKSELRVLAVKCISLLYWTVPPMGTF	DFY 2	90
Muman 282 Q G G G K N FH FTG Q S G F V TPPCK SEL KVL VVKLLSL V W TFSFAM Mouse 282 Q G G K K N FH FTG Q S G F V PPCK SEL KVL VVKLLSL V W TFSFAM Chicken 282 E G G K C F D E TG Q S G F O E TG Q G F O E TG Q S G F O E TG Q S G F O E TG Q S G F O E TG Q S G F O E TG Q G F O E TG Q S G F O E TG Q S G F O E TG Q G F O		20
Chicken 282 EGGKCFDETGQSIIPPCKSELRVLAVKCISLLYWTVFPMGTF	LLL 3	26
GOICKERT 202 EGUKCEDETUQ3IIPPCKSELKVEAVKLISLEYWIVEPMGI		20
Zebralleh 202 ETVDPPEDAPAVEVCVPEPCCDSCOCVCVPPCVSECPVPTTLVASLIVUCVETTAAC	ALL 3	20
	AGAN O	20
ACL 9 201 EMPRN	IEME 3	46
AGL-10 291 SEEQPINROEPVERGDGVWRSWIKEPRICHEYVIKLTISLMFWTUVISECS	YHI 3	40
		1
Human 327 YLYSLVKWYFIITTIVIFVLQERIFGGLEIIELACYRLLHKQPHLNSKKNE 376		
Mouse 327 YLYSPVRWYFIISIVFFVLQERIFGGLEIIELACYRFLHKHPHLNSKKNE 376		
Chicken 327 YLYSFARWYFAAMJIIFVAQQKIFGGLELIELACHQYFKKQQKHDDTKMKKK 378		
Zebrafish 342 CLCPPAQFYFLFMVVFFLCQQRFTGGLELMELACHRYWSRRSADKQE 388		
ACL-8 325 MSLLWVKIMVSHAMIFYLASLRFYNGAFFVFLRWFEARRDCDKTSKKE 372		
AGL9 347 FESAFMFYWATUACVFYAAVHKFYGGLIFLATDRFNHSKQYGVVGQEPEVLNT 399		
AGL10 347 FEVRIEULGELYFFMISFYLSWRYNGIDRYIIFRWQESRMSLQRSPSSSSI 397		

В

	motif I	motif II	motif III	motif IV
Human GPAT1	227LPVHRSHIDYLL	273GFFIRRR	³¹³ FLEGTRSRS	³⁴⁷ ILIIPVGISY
Human AGPAT1	¹⁰¹ VSNHQSSLDLLG	144VIFIDRK	176FPEGTRNHN	203VPIVPIVMSS
Human LYCAT	⁸² IMNHRTRMDWMF	130YIFIHRK	¹⁶³ FPEGTDLTE	¹⁹¹ YVLHPRTTGF
Mouse LYCAT	⁸² IMNHRTRVDWMF	¹³⁰ FIFIHRK	¹⁶³ FPEGTDLTE	¹⁹¹ YVLHPRTTGF
Chicken LYCAT	⁸² IMNHRTRMDWMF	130FIFIQRK	¹⁶³ FPEGTDLTA	¹⁹¹ YVLHPRTTGF
Zebrafish LYCAT	⁸² IMNHRTRLDWMF	¹³⁰ FIFIQRR	¹⁶³ FPEGTDLTE	¹⁹¹ YVLHPRTTGF
ACL-8	⁸² IMNHRTRLDWLF	132YIFLDRN	¹⁶⁵ FAEGTDKGE	193YVLHPRTTGF
ACL-9	98 IMNHRTRLDWLF	148YIFLDRS	¹⁸¹ FPEGTDKCP	209YVLHPRVTGF
ACL-10	88VMNHRTRLDWMY	¹³⁸ FVFLERN	¹⁷¹ FPEGTDKSE	200YVLYPRTTGF

Supplementary Figure 4. acl-9, acl-10 subfamily members in C. elegans are evolutionarily conserved. (A) Alignment of ACL-8, ACL-9, ACL-10 and their homologues (LYCAT) from human, mouse, chicken and zebrafish. Identical amino acids are shown on a black background and similar amino acids are on a grey background. The lysophospholipid acyltransferase motifs conserved in AGPAT family members are boxed. Accession numbers for the sequences used were as follows: human: NP 001002257; mouse: NP 001074540; chicken: NP 001026210; zebrafish: NP 998435; ACL-9: NP 504644; ACL-10: NP 505971. The amino acid sequence of ACL-8 was obtained from the sequence of acl-8 cDNA clone (yk569g12.5, kindly provided by Y. Kohara) and was different from that in the Wormbase database (http://www.wormbase.org/). The deduced ACL-8 amino acid sequence is: MKVIKGVTFIVLVFFSSLLGTVFLLFPLIPLAWFAPKLWRTCADRLVGF WLTFPCSLIEWVFGVNFRVTGDLIERDEPAILIMNHRTRLDWLFSWNALYKMDPWLLTTEKISLKAPLK KIPGAGWAMSSGSYIFLDRNFENDKPVLERIVKYYSGSEKKYQILLFAEGTDKGERATRLSDAFADKN GLPRYEYVLHPRTTGFKFLMELMKKENYIKYVYDLTIAYSGTIVDTEAKLLAGNFPDKVHLDVKKYK LDEIPTGEGCEKWLTDLWATKEKRLKKFYEOEERLEASGDRFEWPETTTGIGYYVAFAFWVLASLIWM GAIYSLLWVKIYVSIAIIFYLASLRFYNGAEFVFLRWFEARRDCDKTSKKE. (B) Comparison of amino acid sequences of motif I~IV. Numbers refer to amino acid residue position within each protein sequence. Red amino acids show consensus motifs that define AGPAT family. Blue amino acids are highly conserved in LYCAT proteins of various species, but not conserved in other AGPAT family members. Most of these "LYCAT signature amino acids" are conserved in C. elegans ACL-8, ACL-9 and ACL-10. Accession number. : human GPAT1; AAH30783, human AGPAT1; NP 006402.



acl-8 acl-9 acl-10 + ACL-10 (seam cell)



acl-8 acl-9 acl-10

+ mouse LYCAT





Supplementary Figure 5. Defects of vulval morphology and asymmetric cell division in *ipla-1* mutants, *acl-8 acl-9 acl-10* triple mutants and *acl-10* single mutants. (A-H) Vulval morphology. The protruding vulva phenotype (asterisk) observed in *ipla-1* mutants (B), *acl-8 acl-9 acl-10* mutants (C) and *acl-10* single mutants (E). Note that *acl-8 acl-9* double mutants show no defects in vulval morphology (D). The *dpy-7p::ipla-1::gfp* transgene fully rescue the vulval defects of *ipla-1* mutants (F). Both *acl-10p::acl-10::gfp* and *acl-10p::acl-10::mCherry* transgenes fully rescue the vulval defects of *acl-8 acl-9 acl-10* mutants (G, H). (I-M) Seam cells at the late L4 stage visualized by *scm::gfp*. Merged fluorescence and differential interference contrast images are shown. The letters (a), (b) and (c) correspond to those of Figure 3A. The asymmetry of the divisions is disrupted in *acl-8 acl-9 acl-10* mutants (L), indicating that *acl-10* cell-autonomously acts in seam cells. The expression of *acl-10* mutants (L), indicating that *acl-10* cell-autonomously acts in subfamily members. Scale bars are 20 μm.





Supplementary Figure 6. Negative ionization LC/ESI-MS spectra of PI molecular species of wild-type (upper), *ipla-1* mutants (middle) and *ipla-1;tbc-3* double mutants (lower). 'a' refers to alkyl ether linkage.



Supplementary Figure 7. *sn*-2-acyl LPIAT activities of wild-type and *acl-8 acl-9 acl-10* mutants were measured using [¹⁴C]stearoyl-CoA (18:0-CoA) or [¹⁴C]arachidonoyl-CoA (20:4n-6-CoA) as acyl donors. Each bar represents the mean \pm SEM of at least three independent experiments. ***P*<0.01.



Supplementary Figure 8. Intracellular localizations of IPLA-1 and ACL-10

Confocal images of transgenic worms expressing IPLA-1::mCherry and the ER marker ACS-20::EGFP (A) and ACL-10::mCherry and ACS-20::EGFP (B). Full genotypes of these strains are *acs-20;acs-22;xhEx3526[dpy-7p::ipla-1::mCherry];tmEx1920[acs-20p::acs-20::egfp]* (A) and *acs-20;acs-22;xhEx3529[acl-10p::acl-10::mCherry];tmEx1920[acs-20p::acs-20::egfp]* (B). The *acl-10p::acl-10::mCherry* transgene also rescued the phenotypes of *acl-8 acl-9 acl-10* mutants (Supplementary Figure 4H), indicating that this fusion protein is functional.



Supplementary Figure 9. Fatty acid composition of PI in *ipla-1; acl-8 acl-9 acl-10* quadruple mutants is similar to that in *ipla-1* mutants and *acl-8 acl-9 acl-10* mutants. (A) GC analysis of PI. Each bar represents the mean \pm SEM of at least three independent experiments. (B) Negative ionization LC/ESI-MS spectra of PI molecular species. 'a' refers to alkyl ether linkage.





Supplementary Figure 10. Proposed model for determination of the *sn*-1 fatty acid composition of PI. *ipla-1* and *acl-8*, -9, -10 subfamily members are the phospholipase A_1 and acyltransferases, respectively, which are involved in fatty acid remodeling of the *sn*-1 position of PI.



Supplementary Figure 11. (A) Negative ionization LC/ESI-MS spectra of PI molecular species of wild-type (upper) and *acl-10* mutants (lower). 'a' refers to alkyl ether linkage. (B) Quantitative RT-PCR analysis of *acl-8, acl-9* and *acl-10* expression. Expression of *acl-8, acl-9* and *acl-10* was normalized to that of *act-1*.



Supplementary Figure 12. Mouse LYCAT transfers stearic acid to the *sn*-1 position of PI as well as the *sn*-2 position of PI. HEK 293 cells were transfected with the mouse LYCAT expression plasmid. Expression of mouse LYCAT increased the LPIAT activities towards both *sn*-2-acyl lysoPI and *sn*-1-acyl lysoPI as acyl acceptors. [¹⁴C]stearoy-CoA was used as an acyl donor. Each bar represents the mean \pm SEM of at least three independent experiments. ***P*<0.01.

References

Beigneux, A. *et al.* (2006). Agpat6--a novel lipid biosynthetic gene required for triacylglycerol production in mammary epithelium. J. Lipid Res. 47, 734-744.

Lewin, T., Wang, P., and Coleman, R. (1999). Analysis of amino acid motifs diagnostic for the sn-glycerol-3-phosphate acyltransferase reaction. Biochemistry 38, 5764-5771.