Supplementary Information:

Bioinformatics:

NCBI and Celera databases were used for the sequence analysis. Multiple protein alignment and phylogenetic tree were created using Vector NTi suites (InforMax, Bethesda, MD).

Genomic localization of human AGPAT10/GPAT3 and gene-structure:

The human cDNA for AGPAT10 was blasted against the genomic databases (NCBI) and UCSC Genome Browser which identified the gene located on chromosome 4q21.23 (nucleotides 84,676,677 to 84,746,049, Human assembly released March 2006).

Human AGPAT10/GPAT3 genomic organization:

A search against the human genomic databases localized the human gene on chromosome 4q21.23. Based on the predicted and the amplified sequences, the exon-intron boundaries were determined. The human gene spans over 69 kb and consists of 13 exons with the last exon having a long untranslated region (**Suppl Table 2**). All the exon and intron junctions obeyed the AG-GT dinucleotide acceptor-donor rule.

Cloning of human AGPAT10/GPAT3:

The search for additional gene encoding for an AGPAT was performed by homologue comparison with known AGPATs deposited in the various databases including NCBI, Celera and ESTs. One of the hits, NM_032717, showed significant homology to the known AGPATs, including the conserved motifs, suggesting that this predicted sequence might have the AGPAT activity.

Sequence Data Bases were searched for the protein sequences which have good homology for the AGPAT domains. The NM_032717 which appeared promising were amplified from the omental adipose tissue RNA. The amplified sequences showed that the start of the ORF for human *AGPAT10/GPAT3* was in good context for the Kozak rule in initiating protein translation (Kozak 1984) predicting a protein of 434 residues and the predicted molecular weight of 48,702 kDa. Alignment of AGPAT10/GPAT3 from several species showed that this isoform was highly conserved and is also present in invertebrates like worms (*C. elegans*) and in plants (*Arabidopsis*). The putative conserved motifs, NHX_4D and EGTR, seen in almost all proteins of the glycerophospholipid acyltransferase family were conserved; however, arginine in motif EGTR was replaced with cysteine.

Amplification of human AGPAT10/GPAT3 cDNA:

To determine if the sequences are expressed in human tissues, we initially designed primers 1 and 2 (**Suppl. Table 1**) within the coding region to amplify the mRNA from the adipose tissue in the 5'-region using the human adipose tissue Marathon Ready cDNA (Clontech, Palo Alto, CA). The amplified PCR product was purified and the sequence was found to be identical to the predicted open reading frame (ORF). To amplify additional ORF, primers were then designed in the 3'- region (primers 3 and 4; **Suppl Table 1**). The entire ORF was then amplified by mixing the two overlapping fragments, using primer pair 1 and 4 (**Suppl table 1**). PCR product was gel purified and sequenced directly using the amplification

primers and cloned in TA cloning vector pDrive-AGPAT10/GPAT3 (pCR2.1; Invitrogen, Carlsbad, CA) for further sequencing and cloning steps.

Generation of wild type AGPAT10/GPAT3 expression plasmid:

To construct the AGPAT10/GPAT3 expression plasmid, the pDrive-AGPAT10/GPAT3 was used as the template and primer pair designed to only amplify the ORF (primers 5 and 6; **Suppl Table 1**). The restriction sites *BamHI* and *XhoI* were incorporated into the primers for ease in subsequent cloning into the mammalian expression vector; pcDNA3.1(+)neo. The amplified product and the vector were digested with *XhoI* and *BamHI* and ligated to the same sites. The expression plasmid was restricted with appropriate enzymes to ascertain the orientation of the insert in relation to the promoter. This expression plasmid was named pcDNA3.1-AGPAT10/GPAT3.

Northern blot analysis:

To determine the tissue expression pattern and transcript size, a multiple tissue total RNA blot was obtained from Clontech (Palo Alto, CA) and was hybridized with the probe generated with primer pair 9 and 10 (325 bp fragment). Briefly, the blot was hybridized with ^{32}P labeled probe generated from the above fragment overnight at 60° C. The blot was washed with decreasing concentration of sodium salt at 65° C. The final wash was at 0.1X SSC; 0.1% SDS for 30 min at 65° C. The blot was exposed either overnight or for seven days at – 80° C**.** The blot was stripped and re-probed with the β-actin probe as a control as suggested by the manufacturer**.**

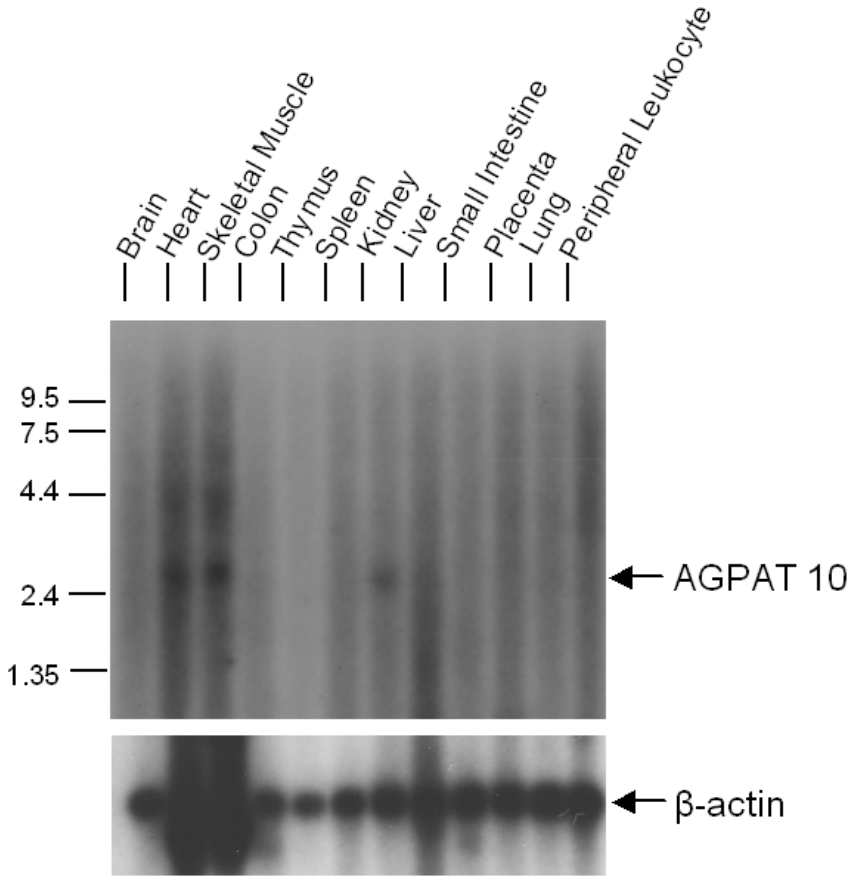
Comparison of amino acid sequence with other known AGPATs:

Multiple sequence alignment of the known human acyltransferases revealed that *AGPA10/GPAT3* belongs to the family of AGPATs. Previous studies have revealed that AGPATs 1-5 and 8-9 have the same two conserved motifs, NHX_4D and EGTR. As more AGPATs are cloned and sequenced, there appears to be flexibility in these conserved motifs. Substitution of arginine to cysteine in EGTR motif is tolerated for the enzymatic activity.

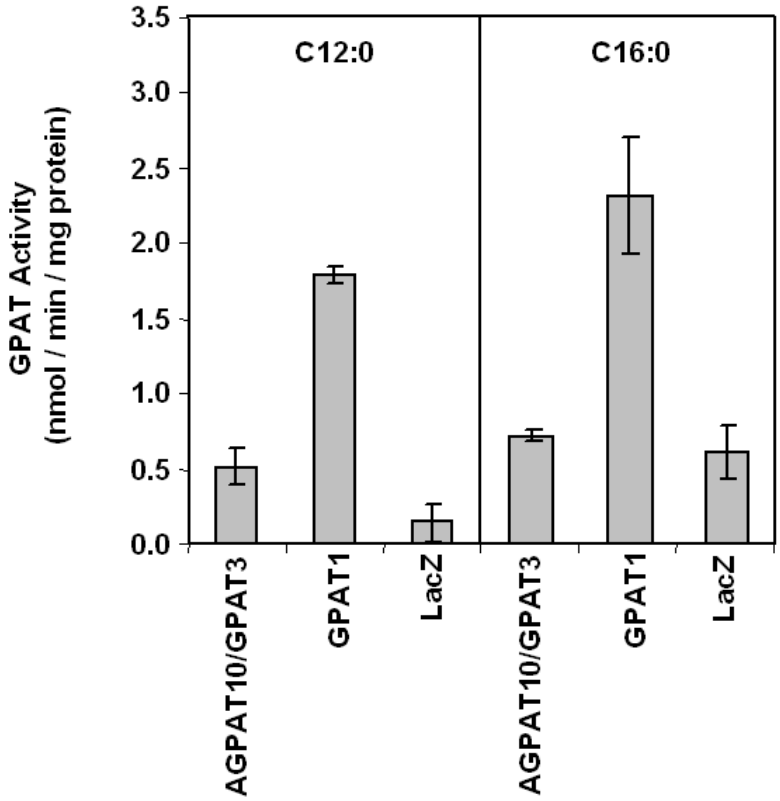
Generation of Huh-7-shRNA-AGPAT2 Knockdown cell:

The construction of knockdown cell line was achieved as mentioned in the materials and methods. Packaging of various viral shRNA construct in 293T cells resulted in construction of multiplication deficient Lenti viral particles in 293T cells. The viral supernatant was collected separately and stored at -80°C. Viral titer determination by qRT-PCR kit showed that the viral titre of the various constructs ranged from 10^6 -10^8. Huh7 cells were infected with this purified virus and selected for puromycin resistance at a predetermined concentration of 1 μg/mL. The cells were further propagated and stored. The transcripts for AGPAT2 levels in Huh7 knock down cells were determined by quantitative as well semi-quantitative PCRs.

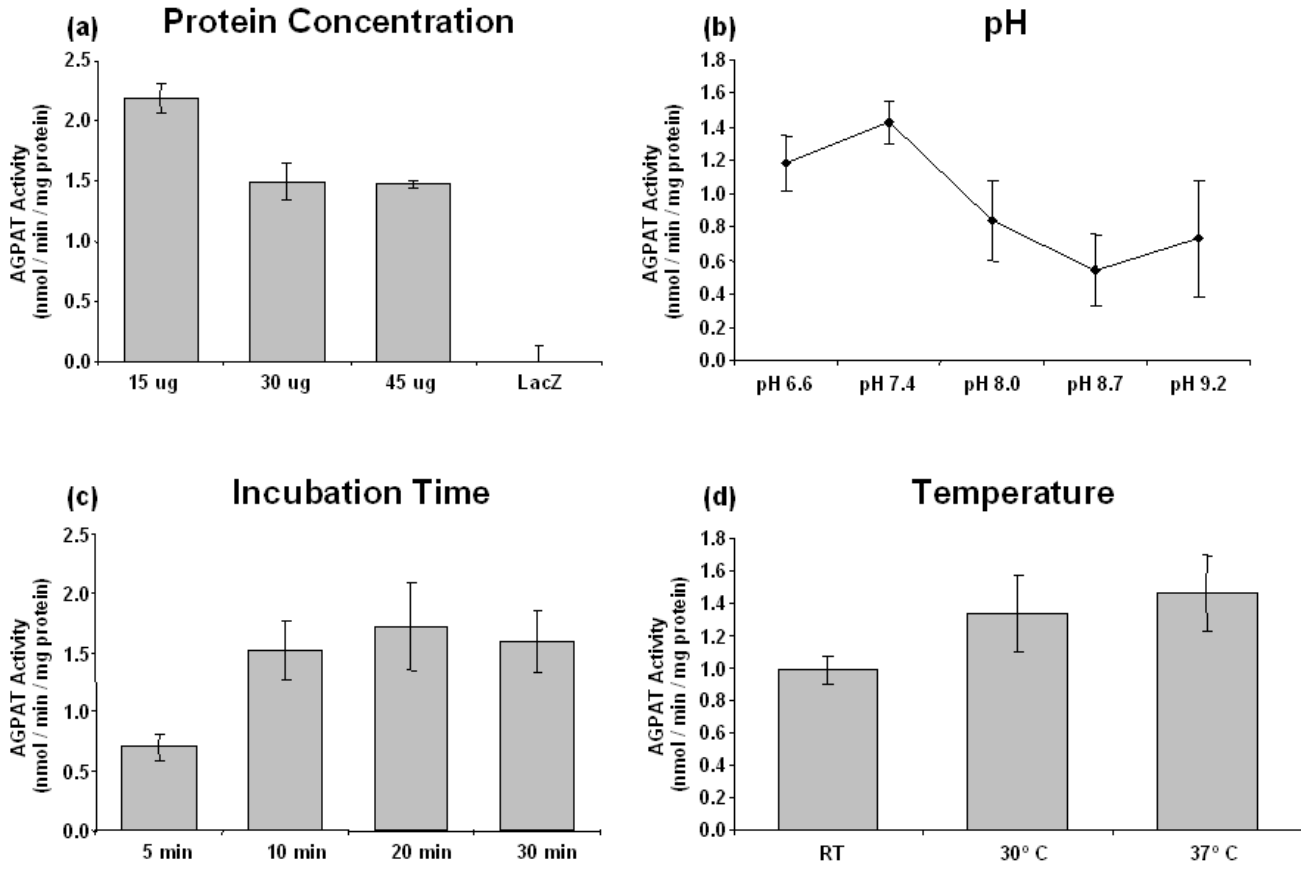
Suppl Fig. 1:
Northern blot analysis. Human multiple tissue blot containing 10 μg of poly(A)$^+$ RNA for each tissue was hybridized with ^{32}P-cDNA for human *AGPAT10/GPAT3* sequences. The blot was hybridized and washed as described in methods. The blot was exposed for four days at -80° C to detect any weak signal. The blot was stripped and re-probed with β-actin cDNA as an internal control and exposed for 16 hrs.



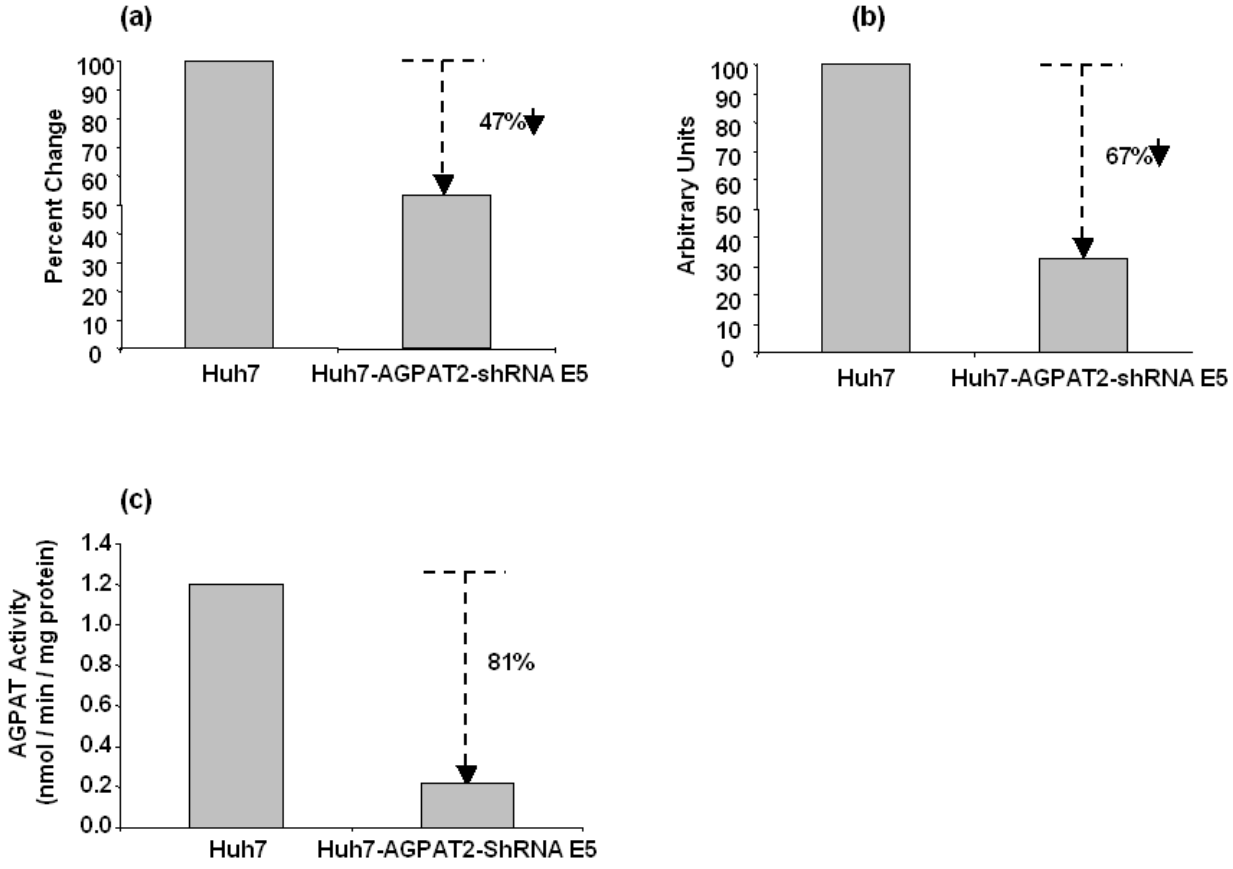
Suppl Fig. 2:
Glycerol-3-phosphate acyltransferase enzymatic activity of AGPAT10/GPAT3 in the HEK-293 cell lysate. The GPAT activity was determined by incubating [^{14}C]-glycerol-3-phosphate with C12:0-acyl-CoA and C16:0-acyl-CoA. Recombinant adenovirus expressing human GPAT1 was included as a positive control. The recombinant adenovirus β-galactosidase was used as a control.



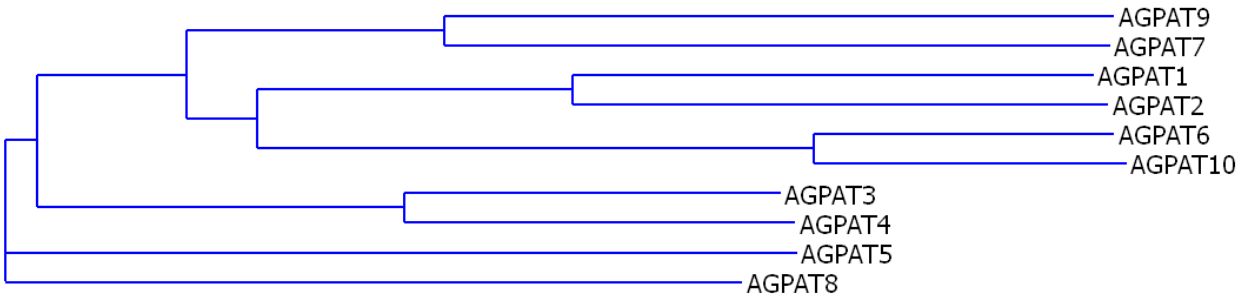
Suppl Fig. 3:
Preliminary characterization for AGPAT activity of human AGPAT10/GPAT3 for protein concentration, pH, reaction time and temperature. Shown is the mean from one experiment carried out in triplicate.



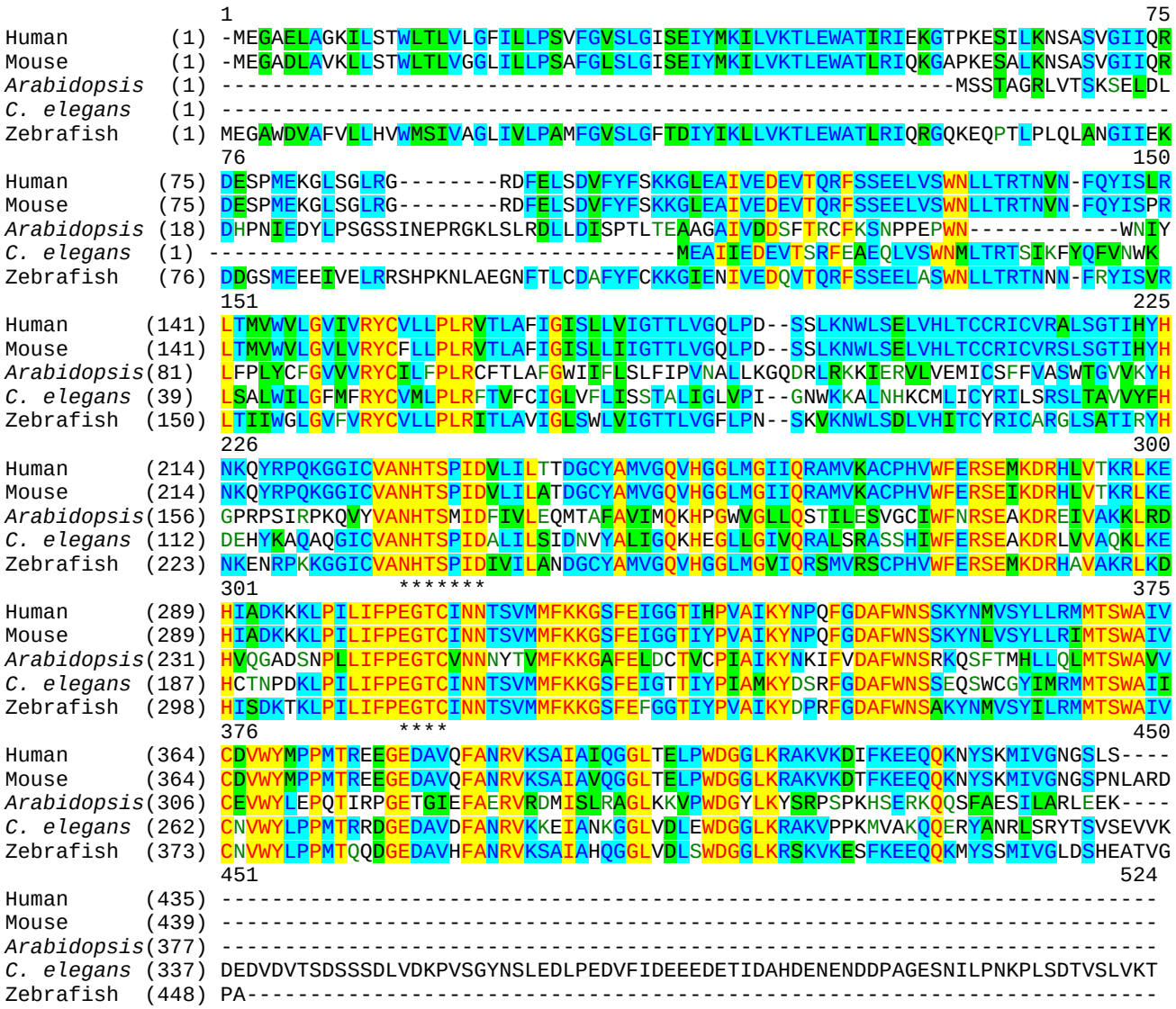
Suppl Fig 4:
Characterization of the Huh7-shRNA-AGPAT2-E5 cells for expression of AGPAT2 and total AGPAT activity. **A**, Shown is the mean for expression AGPAT2 transcript by real-time PCR which indicate a 47% down regulation. **B**, semi quantitative PCR also show 67% down regulation. **C**, Total AGPAT activity was reduced by about 80 % as compared to naïve Huh - 7 cells.



Suppl Fig. 5:
Evolutionary dendrogram and conserved protein motifs among AGPATs. The dendrogram was computed from the aligned protein sequences using the ClustralW algorithm and VectorNTi's phylogenetic tree program with default settings. GenBank Accession numbers: AGPAT1: NP_006402; AGPAT2: NP_006403; AGPAT3: NP_064517; AGPAT4: NP_064518; AGPAT5: NP_060831; AGPAT6: NP_848934; AGPAT7: NP_705841; AGPAT8: NP_001074540; AGPAT9: NP_079106; AGPAT10: NM_032717, NP_116106 (and this study).



Suppl Fig. 6:
Multiple protein sequences alignment for AGPAT10/GPAT3 from various species. Protein alignment of human, mouse, *zebrafish*, *C. elegans* and *Arabidopsis* for AGPAT10/GPAT3 amino acid sequences. The identical residues are shaded in yellow and conserved residues are in blue and green. The two conserved motifs NHX_4D and EGTC are shown by asterisk. GenBank Accession numbers: Human AGPAT10/GPAT3; this study and NM_032717, NP_116106; Mouse: NM_172715, NP_766303; Zebrafish: NM_001002685, NP_001002685; C. elegans: NM_075979, NP_508380 and Arabidopsis; NM_125455, NP_568925.



Supplementary Tables

Suppl. Table 1: Primers for the cloning of the human AGPAT10/GPAT3

	Primer #	Primer name	Sequences 5'-3'
	1	288_S	AGAGCTGGCCGGGAAGATCCTTT
	2	840_AS	GTTTTTGAGGCTGCTGTCTGGCA
	3	830_S	GCCTCAAAAACTGGCTGAGTGAACTG
	4	1737_AS	CCTGTAGGTTCTGCCTCTGGCATAAA
	5	BamHI	CGGGATCCATGGAGGGCGCAGAGCTGGCCGG
	6	XhoI	CGCTCGAGTTAGCTGAGAGATCCATTGCCCAC
	7	XhoI-GFP	CGCTCGAGATGGAGGGCGCAGAGC
	8	BamHI-GFP	CGGATCCGCTGAGAGATCCATTGCC
	9	238_S	CCTCCACTGCGGACCTCTCCTGAGT
	10	543_AS	CCTTCCTCGTAGACCAGAGAGCCCTT

Suppl Table 2: Exon-intron structure for the human gene AGPAT10/GPAT3

Exon Number	Length (bp)	cDNA position	Acceptor	Donor	Intron
1	311	1-311		GAGGGCCTCC**gt**gagtcatctg	1
2	260	312-571	cttcccttcgc**ag**AGGTGAGTGC	AAAACTTTAGA**gt**gagtccggga	2
3	67	572-638	gtttttgaac**ag**TGGGCCACAA	GCTTCTGTTG**gt**gagttttcct	3
4	271	639-909	ctttcttttta**ag**GTATTATCCA	TGCCTCTGAG**gt**aagtcatatg	4
5	75	910-984	tttctttttc**ag**GGTTACCTTG	CAGACAGCAG**gt**gaaatgtttc	5
6	90	985-1074	tatttccttc**ag**CCTCAAAAAC	ATCATAACAA**gt**gagtatctgc	6
7	94	1075-1168	tttgtaattgc**ag**GCAGTACAGA	GTTATGCTATG**gt**aagagcagctc	7
8	116	1169-1284	tgctacttct**ag**GTTGGCCAGGTT	TTACTAAGAG**gt**aagcagtgaag	8
9	56	1285-1340	ttttttccat**ag**ACTAAAAGAA	TTTCCTGAAG**gt**aagaatgggc	9
10	86	1341-1426	acccccttgc**ag**GAACTTGCAT	TGCAATTAAG**gt**aaaacagata	10
11	129	1427-1555	ttctttttt**ag**TATAACCCTC	GACCAGAGAG**gt**attccttagctaa	11
12	80	1556-1635	tttttaaacc**ag**GAAGGAGAAG	AACTTCCCTG**gt**aagagaacttc	12
13	100	1636-1735(+)	ttctgtttgc**ag**GGATGGAGGA(+)		