Supplementary material:

Bioinformatics:

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

Genomic localization of human AGPAT11 and gene-structure:

The human cDNA for AGPAT11 was isolated from the genomic databases located at NCBI and UCSC Genome Browser which identified the gene as located on chromosome 16q13 (NT_006576.15 based on human assembly released March 2006).

Cloning of human AGPAT11:

The search for additional genes encoding for an AGPAT was performed by homologue comparison with known AGPAT deposited in the various databases including NCBI, Celera and ESTs. One of the hits, **NM_017839**, showed significant homology to the known acyltransferases, including the conserved motifs, suggesting that this predicted sequence might have AGPAT activity.

Amplification of human AGPAT isoform 11:

To amplify AGPAT11, we designed primers based on the sequences available for **NM_017839** from the GeneBank. Our initial effort to amplify the **NM_017839** isoform from the human skeletal muscle and liver failed. However, only a faint band was observed in the adipose tissue indicating poor expression in this tissue. In order to clone this isoform, we then employed the human cDNA panel with primer pair 3 and 4 (**Supplementary Table 1**). The expression of **NM_017839** is relatively higher in the lung, spleen and small intestine compared to other tissues like heart and skeletal muscle;

which had very low expression as noted above. The PCR product when sequenced showed the predicted sequences.

To amplify the entire open reading frame (ORF), additional primers were designed in the 5'- and 3'-untranslated regions. The overlapping fragments were amplified with the following primer pairs – fragment I with 5 and 6 and fragment II with primers 1 and 7 (**Supplementary Table 1**). The amplified products were mixed and amplified with primer pair 1 and 7, which yielded the desired size fragment, which was cloned in TA cloning vector for further cloning and sequencing (pDrive-017839-short). A multiple protein sequence alignment from various species indicated that we had missed the 5' end of the protein. We then amplified the 5' sequences with primer pair 8 and 9 (**Supplementary Table 1**) and sequenced for PCR errors. The PCR fragment generated above and those amplified as NM_017839-short were used to amplify with primer pair 8 and 7 (**Supplementary Table 1**) to generate the ORF conforming to the predicted sequences (pDrive-017839-long). We designated this clone as AGPAT11.

Generation of AGPAT11 mammalian expression vector:

To construct the eukaryotic expression vector for AGPAT11, the ORF was amplified from pDrive-017839-long in two fragments with primer pairs 10 and 11 and primer pairs 12 and 13 (**Supplementary Table 1**). The PCR fragments were mixed and amplified with primers 10 and 13 which contain *BamHI* and *XhoI* restriction sites for ease in cloning. The amplified product was cloned in TA cloning vector (pDrive from Qiagen) and sequenced. The pcDNA3.1 (+) neo and pDrive-017839 (now designated as AGPAT11) were restricted with *BamHI* and *XhoI* and AGPAT11 was cloned in pcDNA3.1 (+) neo.

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 325 bp probe generated with primer pair 9 and 10 (**Supplementary Table 1**). 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 for 50 hours at – 80° C**.** The blot was stripped and re-probed with the β-actin probe as a control as suggested by the manufacturer.

Results

Cloning of the human AGPAT11:

Blasting of the known human *AGPATs* identified a sequence, GenBank entry **NM_017839**, which carries the putative acyltransferase domain. Employing a human tissue cDNA panel, we were able to amplify this acyltransferase from a number of tissues which upon sequencing showed the predicted sequence. The human ORF for AGPAT11 is in good context for the Kozak rule in initiating the protein translation. This predicts the protein of 544 residues and molecular weight of 60.2 kDa. The protein has two conserved motifs seen in the glycerophospholipid acyltransferase family of proteins, NHX_4D and EGTR. However, the arginine in the EGTR motif is substituted by cystine.

Human AGPAT11 genomic organization:

Search against the human genomic databases located the human AGAPT11 gene on chromosome 16q13. Based on the predicted sequences and amplified sequences the exon-intron boundaries were determined. The human gene consists of 14 exons, the last exon having a long untranslated region **(Supplementary Table 2).** All the exon and intron junctions obey the AG-GT dinucleotide acceptor-donor rule.

Supplementary Figure Legend:

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 *AGPAT11* sequences. The blot was hybridized and washed as described in methods. The blot was exposed for 50 h 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 h.

Supplementary Table 1: Primers for human *AGPAT11* cDNA amplification:

Primer #	5'--3' primer sequences
1	CAACATCAGGAGGAGAATGGCCC
2	TTCCACCAGCCCAGCTTCCATAG
3	GCATCTATTGCGAGTTCCTCAAAAGG
4	TGGCACGTCTGGAGGTCTAGGTATGT
5	GGCCATTTGCTGCAATTTCAACAGT
6	GGCAATGTTAGCTGTCCTGC
7	TGCCTTTACAATTTCAAGCAAAAGC
8	GTCTTCGGCTCAGTTTTGG
9	GGGCCATTCTCCTCCTGATGTTG
10	CGGATCCATGTTCTTTTCAATGGGATTTATAG
11	ATTTTCGGCTAATTTTAGTAAATTC
12	GAATTTACTAAAATTAGCCGAAAATTG
13	CGCTCGAGTCAGTCATCTTTTTTGTCTGAGGTAC
14	CGCTCGAGATGAGCCGGTGCGCCCAG
15	CGGATCCGTCATCTTTTTTGTCTGAG

Supplementary Table 2: The exon-intron boundary sequences for human *AGPAT11*. Intronic sequences are in lower case whereas exonic sequences are in uppercase letters. The splice site acceptor and donor dinucleotide are in bold.

exon	Length (bp)	cDNA position	Acceptor	Donor	Intron
1	311	1-311		AGGCGGGTCCAG**gt**gaggggctgtg	1
2	140	32-451	tttgactttc**ag**ATTGTCCTTCTT	GTTGGAGGAG**gt**aagaaataatt	2
3	218	452-669	atattttcaa**ag**GAAAATTACTC	TCTGATTGGCA**gt**aagtacttgtaa	3
4	113	670-782	tttgttttaa**ag**GACTGTTACGGG	GAATGGCCCCAG**gt**aaaacatggtaga	4
5	61	783-843	ttttttccca**ag**ATACTAGTTTTCCC	TTTTAAACCAG**gt**gagaaaaatt	5
6	59	844-902	aatgtggttgc**ag**GAGCCTTCATTCC	AAACAAGCTG**gt**aagcacagta	6
7	35	903-937	ttgttattat**ag**GATACTGTGACCT	GATATACATT**gt**aagtcacttat	7
8	55	938-992	ttttttctcttc**ag**CATTCAGCTTT	TAGAAGTTGAG**gt**aagtcattcaaa	8
9	83	993-1075	tttgtctttc**ag**TTTATGCCAG	TAATGGCAGA**gt**aagtgtctata	9
10	126	1076-1201	taatacttttt**ag**AGCTCTGGGAAT	GAAAATTGAA**gt**aagtgtatttt	10
11	154	1202-1355	ctttgtttgttt**ag**ATTAGATTGGG	CTCTTTGACAGG**gt**atgttaaaattta	11
12	99	1356-1454	cccatcctttcac**ag**AACCATGATGG	GGCATTTAAGgtact**gt**cagcccc	12
13	136	1455-1590	ttttattttcttc**ag**CTGTTTGACGTT	ATTTCCTATG**gt**gagtaggcaat	13
14	326	1591-1916	ctttctcttct**ag**AGGGAATTTAAAA (+)		

Supplementary Table 3: Normal and tumor tissue and the diagnosis used in the study

Tissues	QC Diagnosis	% tumor	% necrosis or fibrosis
Breast, normal	Breast w/ fibrocystic changes		
Breast, tumor	Infiltrating ductal carcinoma, border & DCIS	50	50% fib
Breast, tumor	DICS w/infiltrating ductal carcinoma	70	40% fib/nec
Breast, tumor	Invasive ductal carcinoma	100	60% nec/fib
Cervix, normal	Ectocervix (fibroids & abnormal uterine bleeding)		
Cervix, normal	Endocervix		
Cervix, tumor	SCC of cervix 15/30	15	30
Cervix, tumor	Poorly diff squamous cell carcinoma of cervix		
Cervix, tumor	SCC of cervix, recurrent, w/border of nl 90/70	90	70
Colon, normal	NI colon		
Colon, normal	NI colon (full thickness section)		
Colon, normal	Normal colon w/ edema		
Colon, tumor	Adenocarcinoma of colon	90	60
Colon, tumor	Invasive adenocarcinoma of colon	60	60% nec/fib
Colon, tumor	Adenocarcinoma of colon w/border nl colon	50	60% fibrosis

Supplementary Figure 1