

## Supplementary Methods

### ***- Establishment of cell lines and cell culture***

Lymphoblastoid cell lines were established using standard procedures. Total RNA from lymphocytes was extracted with the RNA-PLUS (Quantum-Appligene) kit following the manufacturer's instructions. Human keratinocytes and fibroblasts were obtained from skin removed during routine plastic surgery of a normal individual. The skin sample was processed for primary keratinocyte culture and cells were grown according to the procedure described by Invitrogen Life Technologies using products from the company in serum-free keratinocyte medium supplemented with bovine pituitary extract (25 µg/ml) and recombinant epidermal growth factor (0.1 ng/ml). For primary fibroblast cultures we used DMEM (Dulbecco's Modified Eagle's Medium) with 10% fetal calf serum (FCS) and 2% L-glutamine. Cultures were allowed to proliferate for two passages and harvested when they reached 90% confluence.

### ***- Metabolic pulse-chase experiments with [<sup>3</sup>H]oleic acid and pyrenedecanoic acid***

For metabolic labeling experiments, fibroblasts were incubated in RPMI-1640 medium supplemented with 2% L-glutamine, 1% FCS and the labeled fatty acids, under the previously used conditions<sup>1,2</sup>. Briefly, cells were pulsed for 24h in 1% FCS medium containing [<sup>3</sup>H]oleic acid (10<sup>6</sup> dpm/ml, 20 nmol/ml), and were then chased in 10% FCS medium ([<sup>3</sup>H]oleic acid-free medium) for 48h. At the end of the chase period, cells were harvested, and lysed by sonication. Lipids were extracted by chloroform/methanol 2/1 (v/v) according to the method of Folch<sup>3</sup> and separated by thin layer chromatography on SilicaGel G plates developed using chloroform/methanol/water (100/42/6) as the solvent system under the previously used conditions<sup>1</sup>. Then the lipid spots were scraped off and counted by liquid scintillation spectrometry.

For fluorescence microscopy experiments, cells grown on glass coverslips were pulsed for 24h with the fluorescent fatty acid, pyrenedecanoic acid (P10) (33 nmol/ml), then chased in the standard medium containing 10% FCS. Cells were fixed in 4% formaldehyde in PBS for 15 min, the coverslip was attached on a glass slide with a drop of glycerol, and the cells were examined by fluorescence microscopy<sup>4</sup>.

**- Lipase assay**

The lipase activity was evaluated using the previously described assay for neutral cellular lipase<sup>2</sup>. Briefly, cells were lysed in distilled water by sonication (3 runs of 10 s, Soniprep 150 sonicator). The assay for determining neutral lipase activity contained 10 nmol/assay triolein (10,000 dpm [<sup>3</sup>H]triolein 0.1 % Triton X-100, 0.2 M citrate phosphate buffer pH 7.2 and enzyme solution (100 µg of protein) in a final volume 200 µl. The liberated oleic acid was extracted in a biphasic solvent system according to the method of Belfrage and Vaughan<sup>5</sup> and the radioactivity of the aqueous and organic phases was counted (Packard, Tricarb 2100 TR). The neutral lipase activity was expressed as nmol of fatty acid liberated

**- Isolation of lipid droplets**

Fibroblasts were incubated, under the pulsed conditions described above, for 24h with 20 nmol/ml oleic and 10 nmol/ml P10 which was used to detect the lipid-rich fraction. At the end of the pulse, cells were washed twice in PBS, scraped off, allowed to lyse for 30 min on ice in 0.5 ml of hypotonic lysis buffer (25 mM Tris-HCl buffer pH 7.4, containing 1 mM EDTA, 0.1 mM benzamidine, and 20 µM leupeptine, and 20 µM NaF) and homogenized with a glass homogenizer according to Egan *et al*<sup>6</sup>. After centrifugation (35,000 x g for 3h at 4°C), two fractions were collected, the floating fat cake fraction and the infranatant/membrane pellet, and used for determining lipase activity.

**- siRNA treatment**

siRNA targeting *ATGL* (SMARTPool *ATGL* - catalog number NM\_020376) and scrambled siRNA were purchased from Dharmacon (Lafayette, CO). Human fibroblasts (Mav1) were transfected with 20 µM double stranded siRNA in Optimem medium (Gibco) mixed with oligofectamine, using the supplier's instructions. Three hours after transfection, fibroblasts were incubated in RPMI containing 10% FCS for 72 h. Then fibroblasts were incubated for 12 h in RPMI containing 0.5% FCS, before treatment with oleic acid, under the pulse-chase conditions.

**- Transmission electron microscopy**

Cells or tissues were fixed in 1% glutaraldehyde in 0.1 M phosphate buffered saline (PBS) at 4°C for 2 h, and post-fixed with 1% osmium tetroxide in 0.1 M cacodylate buffer pH 7.2 for 1 h, and embedded in Epon. Ultrathin sections were stained with lead citrate and uranyl acetate, and were examined with a Hitachi 300 electron microscope.

### **- Mutation screening**

The sequences of *CGI-58/ABHD5* and *ATGL* were analyzed using the Ensembl (<http://www.ensembl.org>) and GenBank (<http://www.ncbi.nlm.nih.gov>) human genome databases. We amplified and sequenced the 7 coding exons and exon-intron boundaries of *CGI-58/ABHD5* with previously described primers<sup>7</sup>. We designed intronic oligonucleotide primers flanking the coding exons 2 to 10 for amplification and sequencing of the *ATGL* gene (Supplementary Table 2 online) using the Primer3 program ([http://www-genome.wi.mit.edu/genome\\_software/other/primer3.html](http://www-genome.wi.mit.edu/genome_software/other/primer3.html))<sup>8</sup>. The touch-down PCR reaction was carried out in a 25 µl volume containing 100 ng of genomic DNA (in 10 µl) with BD Advantage<sup>TM</sup>-GC Genomic Polymerase Mix (50x) or Ex-Takara Taq DNA polymerase : the initial denaturation step was performed at 95 °C for 3 min, followed by 5 cycles of amplification consisting of 40 s at 94 °C, 30 s at 68 °C, and a 30 s elongation step at 72 °C, followed by 34 cycles of 40 s at 94 °C, 30 s at optimal annealing temperature (55°C for exons 1-6, and 8-10; 60°C for exon 7 ), 30 s at 72 °C, and a 5 min terminal elongation step. Three µl of purified PCR products were added to 0.5 µl of sense or antisense primer (20 µM) and 1 µl of BigDye terminator mix (Applied Biosystems) in a 15 µl volume. The linear amplification consisted of an initial 5 min denaturation step at 96 °C, 20 cycles of 10 s of denaturation at 96 °C and a 4 min annealing/extension step at 56-60 °C. The reaction products were purified and sequenced on an Applied Biosystems Sequencer 3700. Forward or reverse strands from all patients and controls were sequenced for the entire coding region and the exon/intron boundaries. The sequences were analysed using the Phred Phrap program on Unix.

### **- RNA extraction**

Total RNA was isolated using the QIAamp RNA Mini Protocol for the isolation of total RNA from cultured cells (QIAGEN) following the manufacturer's instructions. The mRNA was isolated following the Oligotex direct mRNA protocol from the manufacturer (QIAGEN).

## **References**

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