oligodendrocytes, astrocytes, kidney epithelial cells, and, to a limited extent, B lymphocytes. In vitro, the virus can only be efficiently propagated in primary human fetal glial cells or in human fetal glial cell lines such as POJ and SVG (*29*–*31*). This restricted tropism is due to the presence or absence of cell-type-specific transcription and replication factors and to the presence of specific virus receptors. HeLa cells are completely refractory to infection by JCV but will support early viral gene expression when transfected with JCV DNA. HeLa cells express the JCV receptor–type sialic acid (α 2-6 SA) and bind virus as well as permissive glial cells, suggesting that sialic acid is not sufficient for mediating virus infection (*32*). Our ability to rescue JCV infection in receptor-negative HeLa cells by transiently or stably introducing the $5HT_{2A}$ receptor demonstrates that $5HT_{2A}R$ is a functional entry receptor for JCV. The breadth of other serotonergic receptors that might also function as JCV receptors has not been thoroughly investigated, but preliminary data have ruled out the 5HT1, 5HT3, and 5HT7 families.

Neurons express abundant levels of serotonin receptors but are generally refractory to infection by JCV. However, neurons do not express the receptor-type sialic acid for JCV, which indicates that infection of cells requires both components of the JCV receptor (*9*). Oligodendrocytes, astrocytes, B lymphocytes, and kidney epithelial cells all express both the alpha 2-6–linked sialic acid component of the JCV receptor and $5HT_{2A}$ receptors (*9*, *33*–*39*).

5HT2-family receptors are highly expressed on brain microvasculature, on astrocytes at the blood-brain barrier, and in brain regions lacking the blood-brain barrier, such as the area postrema and the choroid plexus. This raises the possibility that JCV may directly traffic to the CNS via the blood under viremic conditions, as occurs during severe and prolonged immunosuppression.

Finally, serotonin receptor agonists and antagonists are widely used to treat a variety of neurological and psychiatric disorders. Drugs that have been developed to treat PML have all been hampered by poor bioavailability in the CNS, a problem not inherent to serotonergic inhibitors. Prophylactic treatment of HIV-infected patients with serotonergic antagonists may prevent the spread of JCV to the CNS and the development of PML. Aggressive therapeutic treatment of patients with PML may reduce viral spread within the CNS and prevent additional episodes of demyelination.

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Materials and Methods

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Fat Mobilization in Adipose Tissue Is Promoted by Adipose Triglyceride Lipase

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Mobilization of fatty acids from triglyceride stores in adipose tissue requires lipolytic enzymes. Dysfunctional lipolysis affects energy homeostasis and may contribute to the pathogenesis of obesity and insulin resistance. Until now, hormone-sensitive lipase (HSL) was the only enzyme known to hydrolyze triglycerides in mammalian adipose tissue. Here, we report that a second enzyme, adipose triglyceride lipase (ATGL), catalyzes the initial step in triglyceride hydrolysis. It is interesting that ATGL contains a "patatin domain" common to plant acyl-hydrolases. ATGL is highly expressed in adipose tissue of mice and humans. It exhibits high substrate specificity for triacylglycerol and is associated with lipid droplets. Inhibition of ATGL markedly decreases total adipose acyl-hydrolase activity. Thus, ATGL and HSL coordinately catabolize stored triglycerides in adipose tissue of mammals.

Animals, seed plants, and fungi commonly store excessive amounts of energy substrates in the form of intracellular triglyceride (TG) deposits. In mammals, TGs are stored in adipose tissue, where they provide the primary source of energy during peri-

ods of food deprivation. Whole-body energy homeostasis depends on the precisely regulated balance of lipid storage and mobilization. Mobilization of stored fat is mediated by lipolytic enzymes, which degrade adipose TGs and release nonesterified fatty acids (FAs) into the circulation. Dysregulation of TG-lipolysis has been linked to variation in the concentration of circulating FA, an established risk factor for the development of insulin resistance in type 2 diabetes and related disorders (*1–4*).

During periods of increased energy demand, lipolysis in adipocytes is activated by hormones, such as catecholamines. Hormone interaction with G protein–coupled receptors results in increased adenylate cyclase activity, increased adenosine 3′,5′-monophosphate (cAMP) levels, and the activation of cAMP-dependent protein kinase (protein kinase A, PKA) (*5*). PKA phosphorylates two important proteins with established functions in lipolysis: HSL, an enzyme that catabolizes adipose tissue TGs, and perilipin A, an abundant structural protein located on the surface of lipid droplets. These modifications induce the translocation of HSL from the cytoplasm to the lipid droplet, where efficient TG hydrolysis occurs (*6*). Current models depict HSL as the rate-limiting enzyme in TG mobilization. However, the nonobese phenotype of HSL knock-out (HSL-KO) mice (*7–9*) and the accumulation of diglycerides (DGs) in their adipose tissue (*10*) suggest that there may be one or more additional lipases in adipose tissue that preferentially hydrolyzes the first ester bond of the TG molecule.

To search for such TG lipases, we screened gene and protein databases for murine and human proteins with structural homologies to known lipases, i.e., the GXSXG motif for serine esterases and α/β hydrolase folds. Candidates were analyzed for TG-hydrolase activity and expression in mouse adipose tissue. Only one previously undescribed enzyme fulfilled these requirements, and we named it "adipose triglyceride lipase" (ATGL).

The murine gene for ATGL (NCBI nucleotide entry AK031609) encodes a 486–amino acid protein (BAC27476) with a calculated molecular mass of 54 kD. The amino acid sequences of murine ATGL and two closely related proteins, NP_473429 (annotated as adiponutrin) and XP_128189 are shown in fig. S1. The human ATGL gene, also designated TTS-2.2, encodes a 504–amino acid protein (NP_065109) with 86% identity to the mouse enzyme. The N-terminal regions of ~260 residues in both the murine and the human enzyme contain a "predicted esterase of the α/β hydrolase fold" domain (COG1752) (*11*), as well as a GXSXG site with a putative active serine (amino acid 47). Moreover, a "patatin" domain (Pfam01734) can be detected in the same region (*12*). Patatin domain–containing proteins are commonly found in plant storage proteins such as the prototype patatin, an abundant protein of potato tubers (*13*). These proteins have been shown to have acyl-hydrolase activity on phospholipid, monoglyceride, and DG substrates. Patatin-domains are also present in TGL3, a TG-lipase of *Saccharomyces cerevisiae* (*14*), and human cytosolic phospholipase A2 (*15*).

For ATGL, mRNA is expressed at high levels in murine white and brown adipose tissue (WAT and BAT, Fig. 1A) and to a lesser degree in testis, cardiac muscle, and skeletal muscle. Highest expression of human ATGL (TTS-2.2) mRNA is also found in adipose tissue (fig. S2). ATGL mRNA expression was first detected 4 days after induction of differentiation of murine 3T3-L1 adipocytes, and maximum expression was observed at day 6 (Fig. 1B). To investigate whether ATGL hydrolyzes neutral lipids, we transfected simian

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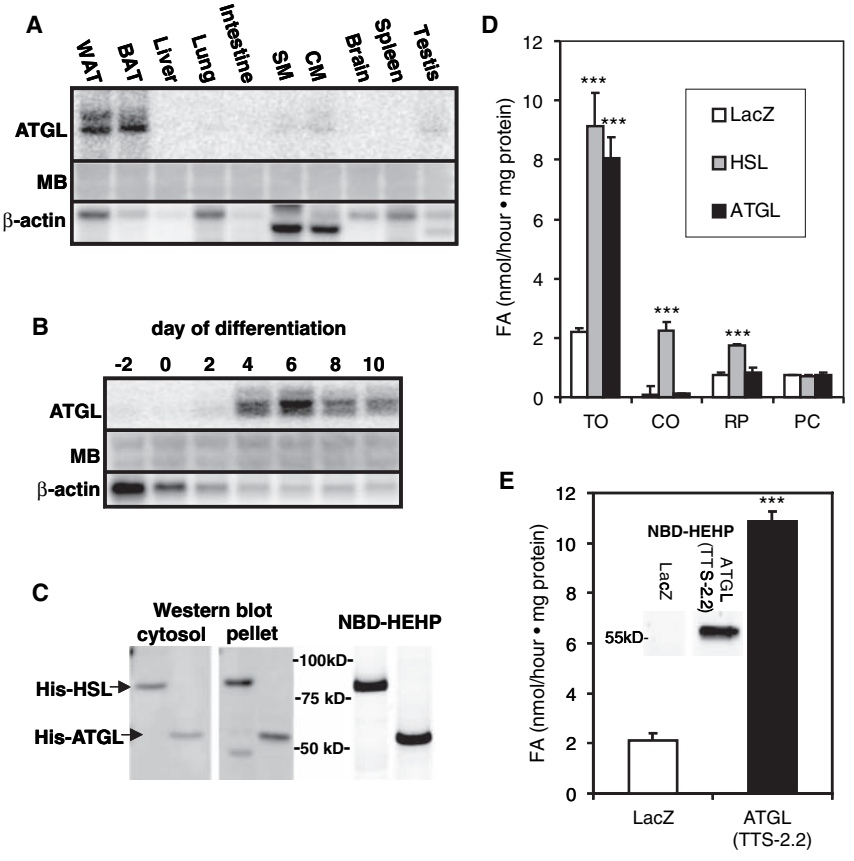

Fig. 1. Analysis of ATGL gene expression and enzyme activity. Total RNA (10 μg) from tissues of fasted mice (**A**) and 3T3-L1 adipocytes at various stages of differentiation (**B**) were subjected to Northern blot analysis. Specific mRNAs were detected with a radiolabeled mouse ATGL cDNA probe. Blots were stained for RNA by methylene blue (MB). A radiolabeled β-actin cDNA probe served as positive hybridization control. Abbreviations: WAT, white adipose tissue; BAT, brown adipose tissue; SM, skeletal muscle; CM, cardiac muscle. (**C**) His-tagged murine HSL and ATGL were detected by Western blotting in cytosolic extracts (100,000*g* supernatant) and membrane fractions (100,000*g* pellet) of transiently transfected COS-7 cells by using a monoclonal antibody against His. In addition, His-tagged murine HSL and ATGL were analyzed by binding of the fluorescent lipase inhibitor NBD-HEHP. Cytoplasmic extracts were preincubated with NBD-HEHP, subjected to SDS-PAGE. NBD-HEHP-labeled proteins were visualized by a BioRad FX Pro Laserscanner (*17*). (**D**). Lipid-hydrolase assays of cytosolic extracts of COS-7 cells expressing murine His-tagged ATGL, HSL or β-galactosidase (LacZ) using substrates containing radiolabeled triolein (TO), cholesteryl-oleate (CO), retinyl-palmitate, (RP), or phosphatidylcholine (PC) (*17*). (**E**) TG-hydrolase assay of cytosolic extracts of COS-7 cells expressing human ATGL (TTS-2.2), or β-galactosidase (LacZ) using a radiolabeled triolein substrate. Inset: The fluorescent lipase inhibitor NBD-HEHP binds to human ATGL (TTS-2.2) expressed in transfected COS-7 cells. Data are presented as means ± SD and represent at least three independent experiments. (***$P < 0.001$)

virus-40–transformed monkey kidney cells (COS-7) with cDNA clones expressing either murine histidine (His)–tagged ATGL or murine His-tagged HSL. Both enzymes were detected in the cytosolic supernatant and the membrane pellet fraction of transfected COS-7 cells by Western blotting (Fig. 1C). When extracts from transfected cells were preincubated with a fluorescent lipase inhibitor (NBD-HEHP) (*16*) and subsequently subjected to SDS–polyacrylamide gel electrophoresis (SDS-PAGE) analysis and fluorography (*17*), fluorescent signals were observed in positions corresponding to the expected molecular mass of ATGL (54 kD) and HSL (84 kD), (Fig. 1C). This fluorescent probe only reacts with enzymatically active Ser-lipases (*16*) which indicates that ATGL is enzymatically active in transfected COS-7 cells. To confirm this, we performed activity assays with radiolabeled lipid substrates (Fig. 1D) (*17*). In accordance with previous data (*18*, *19*), the cytosolic fractions of HSL-transfected cells exhibited increased TG-hydrolase (4.2-fold), cholesteryl ester hydrolase (23-fold), and retinyl ester hydrolase (2.3-fold) activities compared with LacZ-transfected cells. In contrast, cytosolic fractions of ATGL- transfected COS-7 cells hydrolized only the TG substrate (3.7-fold increase). Thus, ATGL is a TG-hydrolase, but in contrast to HSL, it does not hydrolyze cholesteryl or retinyl ester bonds. Similarly to murine ATGL, human ATGL (TTS-2.2) also exhibited marked lipolytic activity against a radiolabeled TG substrate and bound the lipase inhibitor NBD-HEHP (Fig. 1E).

Many TG lipases can hydrolyze more than one ester bond within the TG molecule, which results in the formation of monoglycerides or glycerol. For example, HSL cleaves TGs and DGs; however, its specific activity for DGs is 10 times that for TGs (*20*). In contrast, ATGL exhibited only very weak activity against a radiolabeled DG substrate compared with the TG substrate (Fig. 2A). Low DG-hydrolase activity of ATGL was also confirmed in experiments in which we measured the relative abundance of lipolytic reaction products (Fig. 2B) (*17*). Compared with control extracts of LacZ-transfected COS-7 cells, extracts from ATGL and HSL-transfected cells showed higher acyl-hydrolase activities (FA release) by factors of 7.5 and 10, respectively, when a [9,10-^{3}H(*N*)]-labeled triolein–substrate was used. In the presence of ATGL, the accumulation of DGs was increased 21-fold, which suggests that the enzyme predominantly hydrolyzed the first ester bond of TGs. In contrast, no DG accumulation was observed in lipolysis assays with cytosolic extracts from HSL-transfected cells. Monoglyceride levels were slightly increased in both ATGL- and HSL-transfected cells. From the molar ratios of DG and MG accumulation versus FA release we calculated that ~90% of the FA molecules released by the action of ATGL originate from the hydrolysis of TGs in the first ester bond. In contrast, in the presence of HSL, most FA originated from all three ester bonds resulting in glycerol formation. Thus, ATGL and HSL have different substrate-specificities within the lipolytic cascade, which suggests that they might act coordinately in the catabolism of TGs.

This hypothesis was confirmed by the product profiles generated in triolein hydrolysis assays using combined extracts of LacZ-, ATGL-, or HSL-transfected cells. Relative to extracts from LacZ-transfected cells, the acyl-hydrolase activity was increased in equal volume mixtures of HSL/LacZ extracts (4.8-fold), ATGL/LacZ extracts (4-fold), and ATGL/HSL extracts (16-fold) (Fig. 2C). The accumulation of DGs was increased 12.5-fold when LacZ/ATGL extracts were used and reduced to basal levels with ATGL/HSL extracts. We speculate that during the lipolytic breakdown of TGs, ATGL is predominantly responsible for the initial step of TG hydrolysis and provides DG substrate for the subsequent action of HSL, namely, the conversion of DGs into monoglycerides. In support of this model, the total acyl-hydrolase activity (FA release) in extracts containing ATGL/HSL was nearly 2 times the sum of the individual activities (Fig. 2C). During the final step of lipolysis, monoglycerides are converted to FA and glycerol by monoglyceride lipase (*21*).

The expected intracellular localization of a lipase involved in TG mobilization would be on lipid droplets. To determine whether this is true for ATGL, we constructed an adenovirus vector encoding His-tagged mouse ATGL and used it to infect 3T3-L1 adipocytes at day 8 of differentiation. Western blotting analysis revealed that the majority of ATGL protein (~50%) was present in the cytoplasm (Fig. 3A). However, a distinct fraction of ATGL (~10%) was found

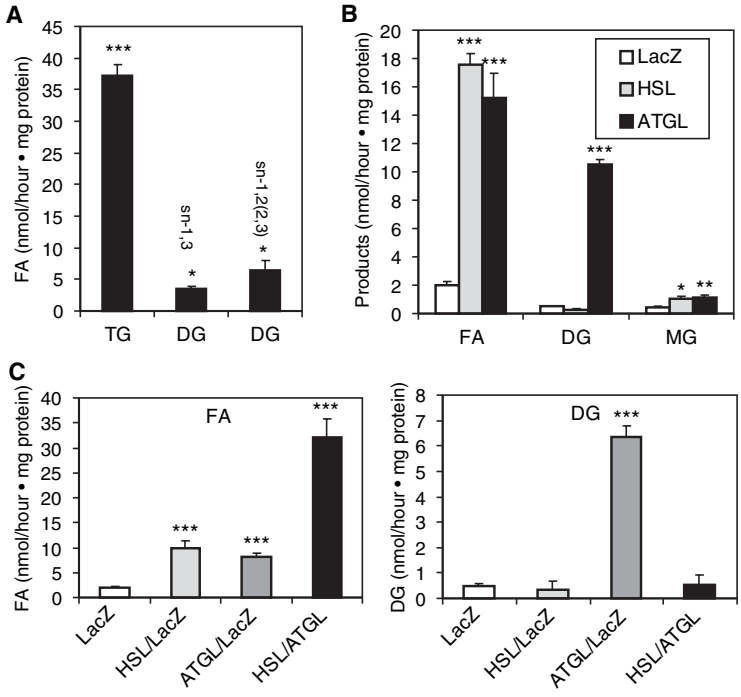

Fig. 2. Role of ATGL within the TG hydrolysis cascade. (**A**) Cytosolic extracts of HepG2 cells infected with an adenovirus construct expressing murine His-tagged ATGL (ATGL-Ad), or LacZ (LacZ-Ad) were incubated with radiolabeled TG or DG substrates. ATGL-mediated acyl-hydrolase activity was normalized by the activity measured in LacZ-Ad–infected cells. (**B**) Accumulation of reaction products during ATGL and HSL-mediated lipolysis. Cytosolic extracts of COS-7 cells transiently transfected with His-tagged LacZ, ATGL, or HSL were incubated with radiolabeled triolein. Lipids were extracted and separated by TLC, and the accumulation of free fatty acids (FA), digylcerides (DG), and monoglycerides (MG) was determined by liquid scintillation counting (*17*). (**C**) Effect of combined fractions of ATGL and/or HSL on acyl-hydrolase activity (FA) and diglyceride (DG) accumulation. Cytosolic extracts of COS-7 cells expressing LacZ were mixed 1:1 with extracts from cells expressing murine ATGL or HSL (ATGL/LacZ and HSL/LacZ) and compared with extracts prepared from a mixture of ATGL and HSL expressing cells (ATGL/HSL). Data are presented as means ± SD and represent three independent experiments. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

tightly associated with lipid droplets of adipocytes even after extensive purification of the droplets. The amount of lipid droplet-associated ATGL was not affected by the stimulation of lipolysis with isoproterenol. In addition, fluorescence microscopy revealed that a green fluorescent protein-ATGL fusion protein localizes to the lipid droplet when expressed in 3T3-L1 adipocytes (fig. S3).

Further proof for a functional role of ATGL as a TG-hydrolase was provided by the fact that adenovirus-infected 3T3-L1 cells expressing ATGL released higher levels of FA (5-fold) and glycerol (1.8-fold) compared with LacZ-infected cells under basal conditions (Fig. 3B). After isoproterenol stimulation, FA release was increased 1.8-fold and glycerol release 2.9-fold. Additionally, ATGL-overexpression caused an increase in the cellular steady-state levels of DGs (fig. S4). Thus, ATGL in adipocytes can markedly augment both basal and isoproterenol-stimulated lipolysis. In contrast, silencing ATGL gene expression by siRNA (Fig. 3C) or antisense-RNA (fig. S5) markedly decreased the release of FA and glycerol from stimulated and nonstimulated 3T3-L1 adipocytes.

We next tested the effect of a rabbit polyclonal antibody against mouse ATGL (ATGL-IgG) on the enzyme activity of adipose tissue extracts from wild-type and HSL-deficient mice. In comparison with rabbit nonimmune IgG (NI-IgG), ATGL-IgG inhibited the cytosolic acyl-hydrolase activity in white and brown fat of wild-type mice by 64% and 71%, respectively (Fig. 3D). In white and brown adipose tissue of HSL-deficient mice, the activity was decreased by 75% and 74%, respectively. Thus the combined deficiency of HSL and ATGL in adipose tissue causes a loss of more than 90% of the acyl-hydrolase activity observed in wild-type adipose tissue. Compared with wild-type adipose tissue, the ATGL-mediated acyl-hydrolase activity and ATGL mRNA levels were not up-regulated in HSL-deficient adipose tissue (*22*).

Considering the central role of PKA in the regulation of fat cell lipolysis we tested whether ATGL is a target for PKA-mediated phosphorylation. As described in SOM (fig. S6), ATGL can be phosphorylated, but in contrast to HSL, this modification is not mediated by PKA.

In summary, our findings suggest that ATGL is an important component of the lipolytic process and the mobilization of lipid stores in mammals. It is responsible for the initial step in TG catabolism. Accordingly, the inhibition of ATGL offers a potential therapeutic approach to control FA release from adipose tissue in patients with insulin resistance.

Note added in proof: Two manuscripts in press present findings that the hormonally and nutritionally regulated protein desnutrin (*23*) and the TG-hydrolace inducible phospholipase-A2-ζ (*24*) are identical to ATGL.

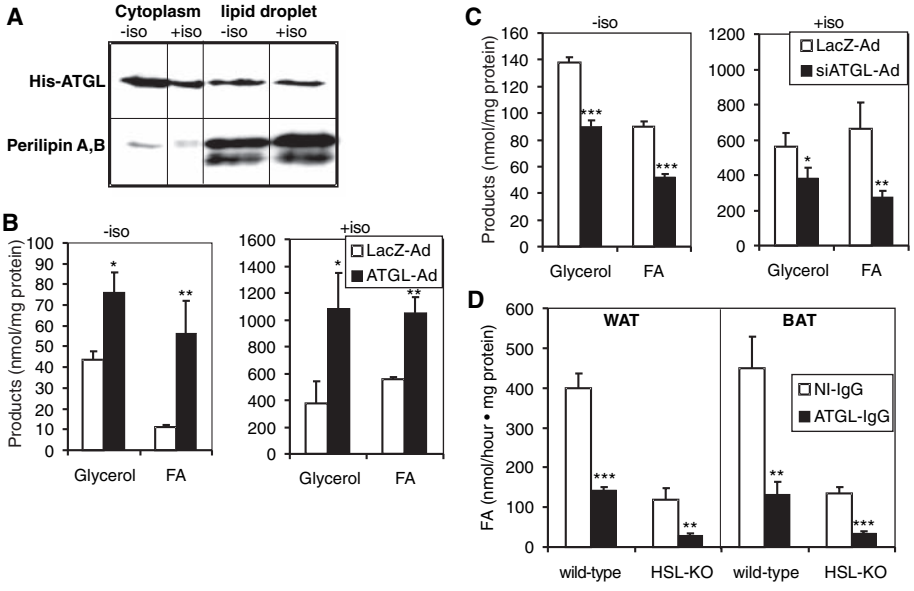

Fig. 3. Cellular localization and physiological function of ATGL in 3T3-L1 adipocytes. Recombinant adenovirus coding for LacZ (LacZ-Ad), His-tagged murine ATGL (ATGL-Ad) or a short interferring RNA for murine ATGL (siATGL-Ad) were used to infect 3T3-L1 adipocytes on day 8 of differentiation, and experiments were performed 2 days after infection (*17*). Before harvesting, cells were incubated in Dulbecco's minimum essential medium (DMEM)/2% FA-free bovine serum albumin (BSA) in the absence or presence of 10 μM isoproterenol (–/+ iso) for 1 hour (A) or 2 hours (B and C). (**A**) ATGL-protein was detected in the cytoplasmic fraction (10 μg of total protein) and in isolated lipid droplets (2 μg of total protein) of ATGL-Ad–infected 3T3-L1 adipocytes with an anti-His monoclonal antibody. Purification of lipid droplets was monitored by the enrichment of perilipin (>70-fold) using a rabbit polyclonal antibody against perilipin A and B. Release of glycerol and FA into the culture medium of 3T3-L1 adipocytes infected with ATGL-Ad (**B**) or siATGL-Ad (**C**). LacZ-Ad–infected cells were used as a control. Data are presented as means ± SD and represent three independent experiments. (**D**) Inhibition of cytosolic acyl-hydrolase activity in WAT and BAT by a polyclonal antibody against mouse ATGL (ATGL-IgG) measured by using radiolabeled triolein as substrate. The activity in cytosolic extracts of adipose tissue from wild-type and HSL-KO mice was determined in the presence of rabbit nonimmune IgG (NI-IgG) or ATGL-IgG. Data are presented as means ± SD and represent two independent experiments. WAT and BAT were obtained from three HSL-KO and three wild-type mice in each experiment. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

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Supporting Online Material

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Figs. S1 to S6
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Fat Mobilization in Adipose Tissue Is Promoted by Adipose Triglyceride Lipase

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