Supplemental Experimental Procedures

Oil Red O staining- Lipid droplets were stained with Oil Red O. Briefly, cells were rinsed three times in PBS and then fixed in 10% (v/v) Formalin Neutral Buffer Solution (Wako) for 15 min. Fixed cells were rinsed twice in deionized water, stained with a working solution of Oil Red O for 4 h at 37 °C, and rinsed three times with deionized water. The cells were counterstained with Hematoxylin for 30 s for nuclear staining and finally rinsed three times with deionized water.

Supplemental Results

Lipin 1 induced by sterol depletion modulates lipogenesis in adipocyte cells. We used the human liposarcoma SW872 cell line for this experiment. SW872 cells were used in many studies as a human adipocyte cell model (1–4). Compared with mouse 3T3-L1 adipocytes, SW872 cells have the advantage of being of human origin and of not requiring an incubation cocktail (e.g., dexamethasone, insulin, and isobutylmethylxanthine) to differentiate into mature adipocytes. SW872 cells, when initially plated, have an immature adipocyte phenotype, and they constitutively express important adipocyte marker proteins such as PPARγ, lipoprotein lipase, CD36, and adipsin (4).

We examined whether lipin 1 was regulated by intracellular sterol in SW872 cells as well as human Huh7 hepatoblastoma cells. To differentiate into adipocytes, SW872 cells were cultured for 8 days at confluence. At day 8, we added sterols or statin to the cells and incubated for 6 days (from day 8 to day 14). We then examined lipin 1 protein levels using immunoblots. Lipin 1 protein levels increased in the cells at lower levels of cellular sterols compared with those in cells containing higher levels of cellular sterols (Supplemental Fig. S5A). These results indicate that the expression of *LPIN1* is regulated by sterol depletion in SW872 cells.

To determine whether the lipin 1 induced by sterol depletion affects PAP1 activity, we measured the PAP1 activity in SW872 cells treated with sterols or statin. As shown in Supplemental Fig. S5B, PAP1 activity was induced approximately 3-fold in statin-containing medium relative to those in sterol-containing medium. These results suggest that PAP1 activity is regulated in a sterol-dependent manner in adipocyte cells.

Finally, to investigate whether the cellular triglyceride contents were increased after treatment with statin, we evaluated lipid droplets content by Oil Red O staining. After 6 days incubation in statin-containing medium, SW872 cells are assumed to be mature adipocytes and large lipid droplets are accumulated (Supplemental Fig. S5C). In contrast, SW872 cells after treatment with sterols also accumulated lipid droplets, but these were small in size and sparsely scattered. These results suggest that lipin 1 induced by sterol depletion modulates triglyceride

accumulation in adipocyte cells.

Supplemental References

1. Wassef, H., Bernier, L., Davignon, J., and Cohn, J. S. (2004) *J. Nutr.* **134**, 2935-2941
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3. Izem, L., and Morton, R. E. (2001) *J. Biol. Chem.* **276**, 26534-26541
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Supplemental Figure Legends

Supplemental Fig. S1. SRE and NF-Y-binding sites of the *LPIN1* promoter are necessary for the activation of *LPIN1* transcription by sterol depletion. *A*. Scheme representing the luciferase reporter gene construct containing the *LPIN1* promoter sequence. Wild-type and mutated SRE or NF-Y-binding site sequences are shown. *B*. HepG2 cells were transfected with luciferase reporter constructs containing the mutated SRE and/or NF-Y-binding sites of the *LPIN1* promoter, together with pcDNA3 and pSV–β-galactosidase. Luciferase activities were measured, as described in Experimental Procedures. Values are expressed as fold induction of the value when LPIN1–2571 was transfected with sterols, set at 1.

Supplemental Fig. S2. *FAS* and *SCD* mRNA levels are regulated by intracellular sterols in Huh-7 cells, but *PPARα* and *PGC-1α* are not regulated. Huh-7 cells were cultured in medium containing sterols, LPDS, or statin for 24 h. Messenger RNA levels of lipid and energy metabolism-related genes were normalized to those of 36B4 mRNA and are expressed relative to the normalized values of cells in cultured sterol-containing medium, set at 1. All values are expressed as means ± SEM (n = 3).

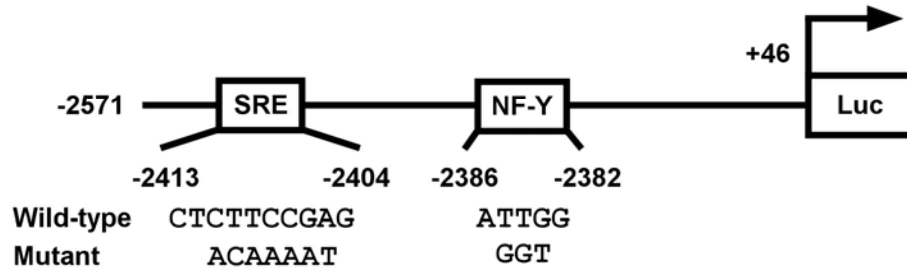
Supplemental Fig. S3. *LPIN1* mRNA levels decrease after transfection with *LPIN1* siRNA (no. 1 or no. 2) in Huh7 cells. Huh7 cells were transfected with either 25 nM control siRNA, *LPIN1* siRNA#1, or *LPIN1* siRNA#2. Total RNA was extracted for the analysis by real-time RT–PCR 48 h after transfection. All values are expressed as means ± SEM (n = 3). Target mRNA levels were normalized to cyclophilin A mRNA and are expressed relative to the values for cells transfected with control siRNA, set at 1.

Supplemental Fig. S4. *LPIN1* mRNA levels are regulated by T0901317, which is a compound that induces SREBP-1c. Huh-7 cells were cultured in DMEM, supplemented with 10% charcoal/dextran treated FBS. The cells were treated for 24 h with vehicle or 5 μM T0901317. *LPIN1* mRNA levels were normalized to those of cyclophilin A mRNA and are expressed relative to the normalized values of vehicle, set at 1. All values are expressed as means ± SEM (n = 3).

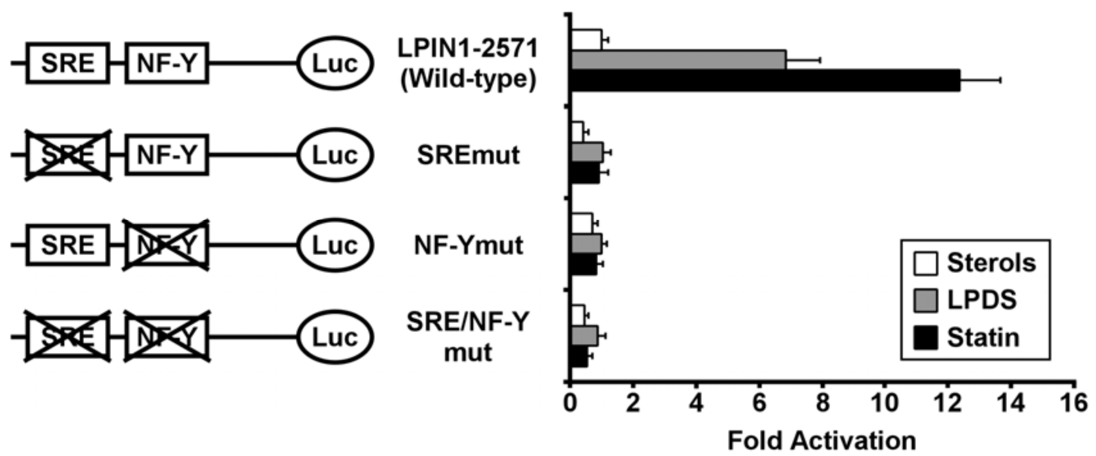
Supplemental Fig. S5. Lipin 1 induced by sterol depletion modulates triglyceride accumulation in SW872 cells. At d 0, cells were plated into tissue culture plates and a cultured in DMEM supplemented with heat-inactivated fetal bovine serum (FBS; 5%), 100 IU/mL penicillin, and 100 μg/mL streptomycin. At d 8, the cells were cultured in medium containing sterols or statin for 6 d (from day 8 to day 14). The medium was changed every 48 h. *A.* Cell extracts were prepared and aliquots (30 μg protein per lane) were subjected to SDS–PAGE. Immunoblots were probed with anti-lipin-1 antibody. The experiments were repeated three times. *B.* PAP1 activity was measured using the cytosolic fraction, as described in Experimental Procedures. Values are expressed as fold induction relative to sterols, set at 1. Values represent means ± SEM (n = 3). *C.* Oil Red O staining illustrates lipid accumulation in SW872 cells. The experiments were repeated three times.

Supplemental Figure S1

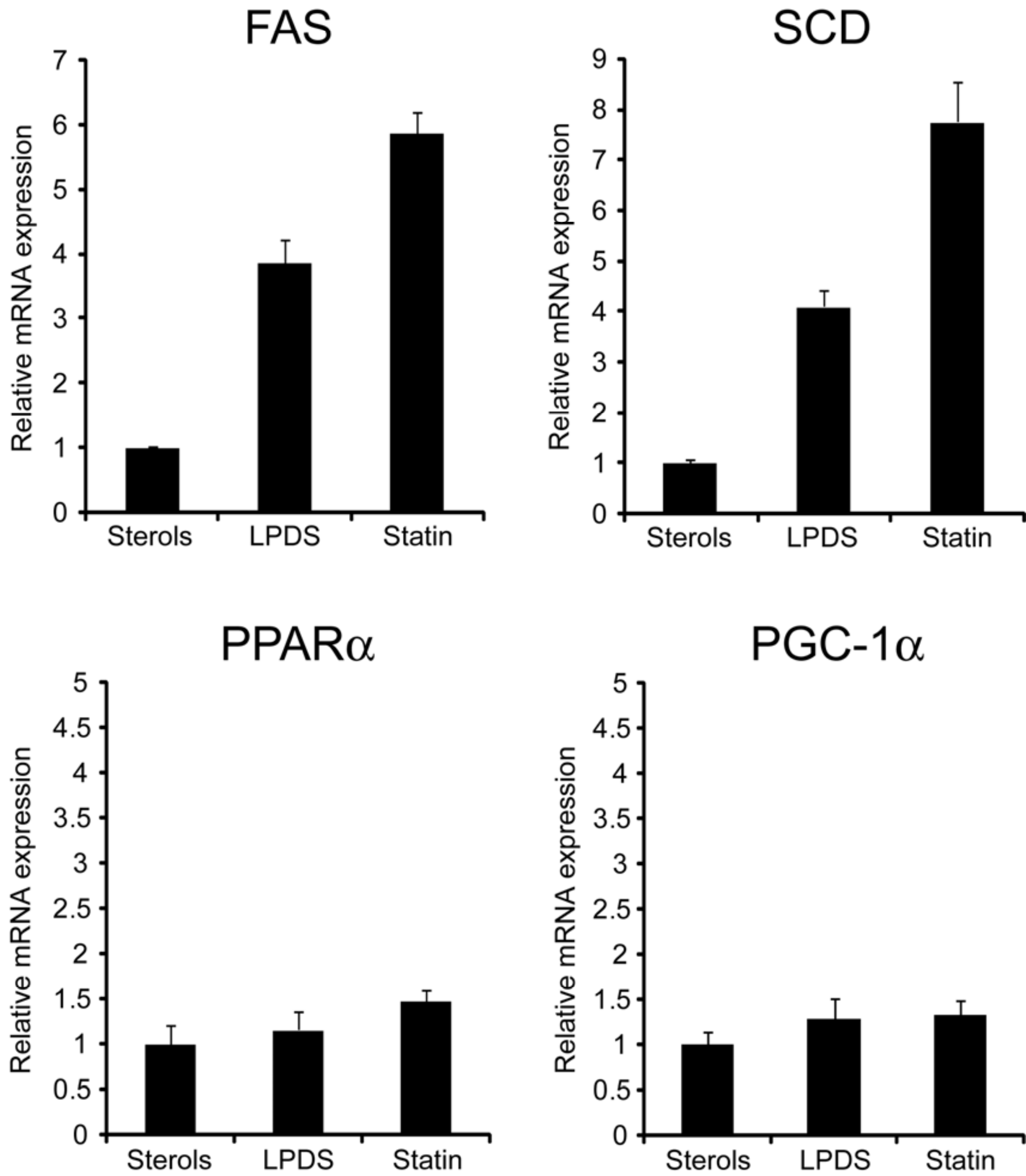
A



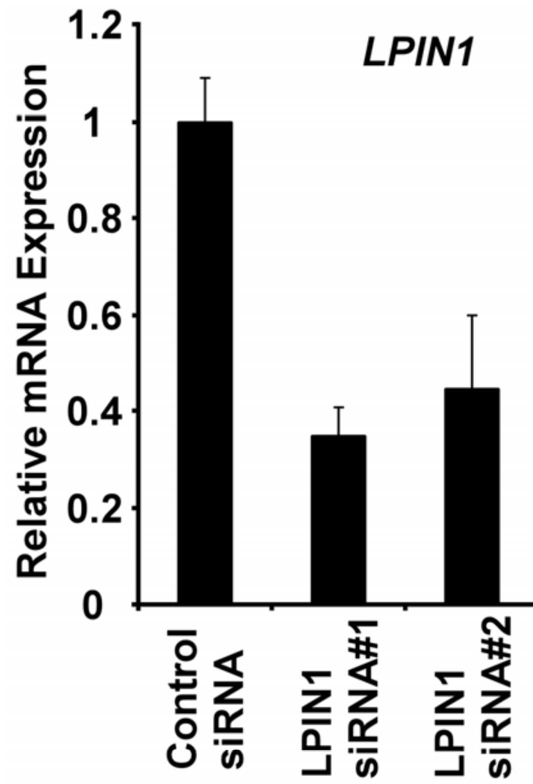
B



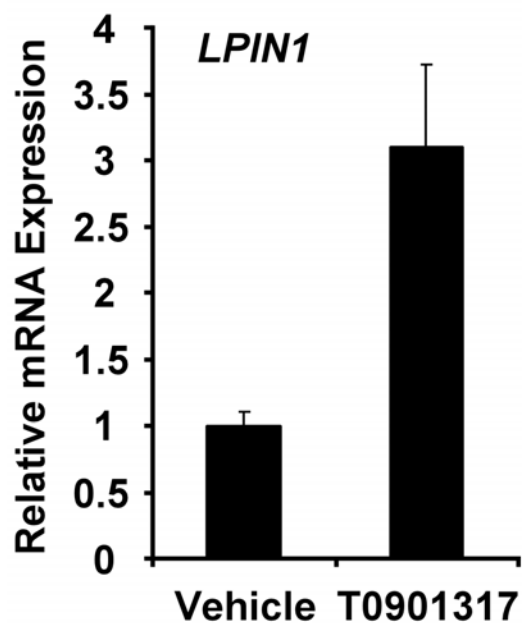
Supplemental Figure S2

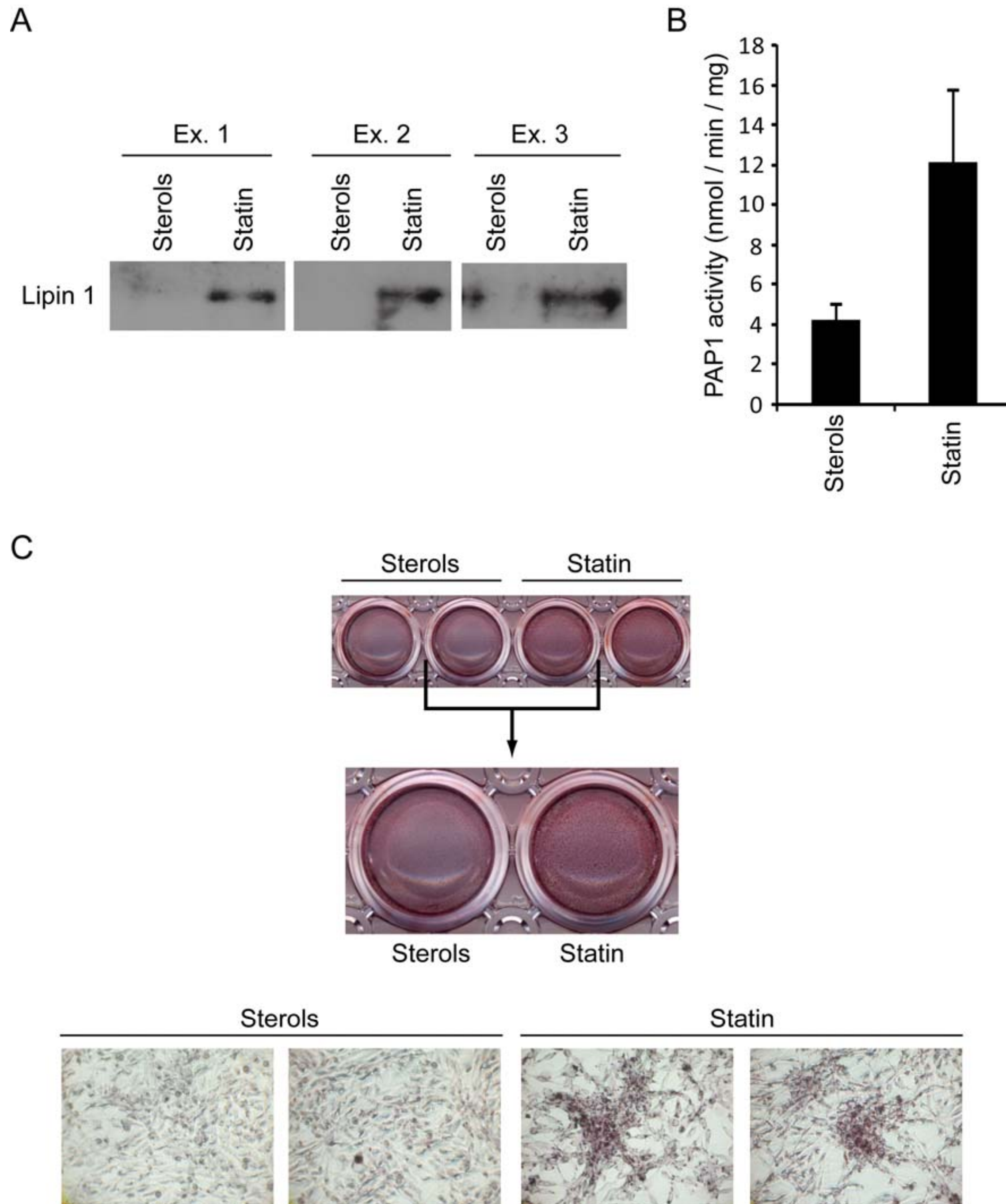


Supplemental Figure S3



Supplemental Figure S4




Supplemental Figure S5
A
Ex. 1
Ex. 2
Ex. 3
Sterols
Statin
Sterols
Statin
Sterols
Statin
Lipin 1
B
PAP1 activity (nmol / min / mg)
0
2
4
6
8
10
12
14
16
18
Sterols
Statin
C
Sterols
Statin
Sterols
Statin
Sterols
Statin