Villeneuve et al., https://doi.org/10.1083/jcb.201705047

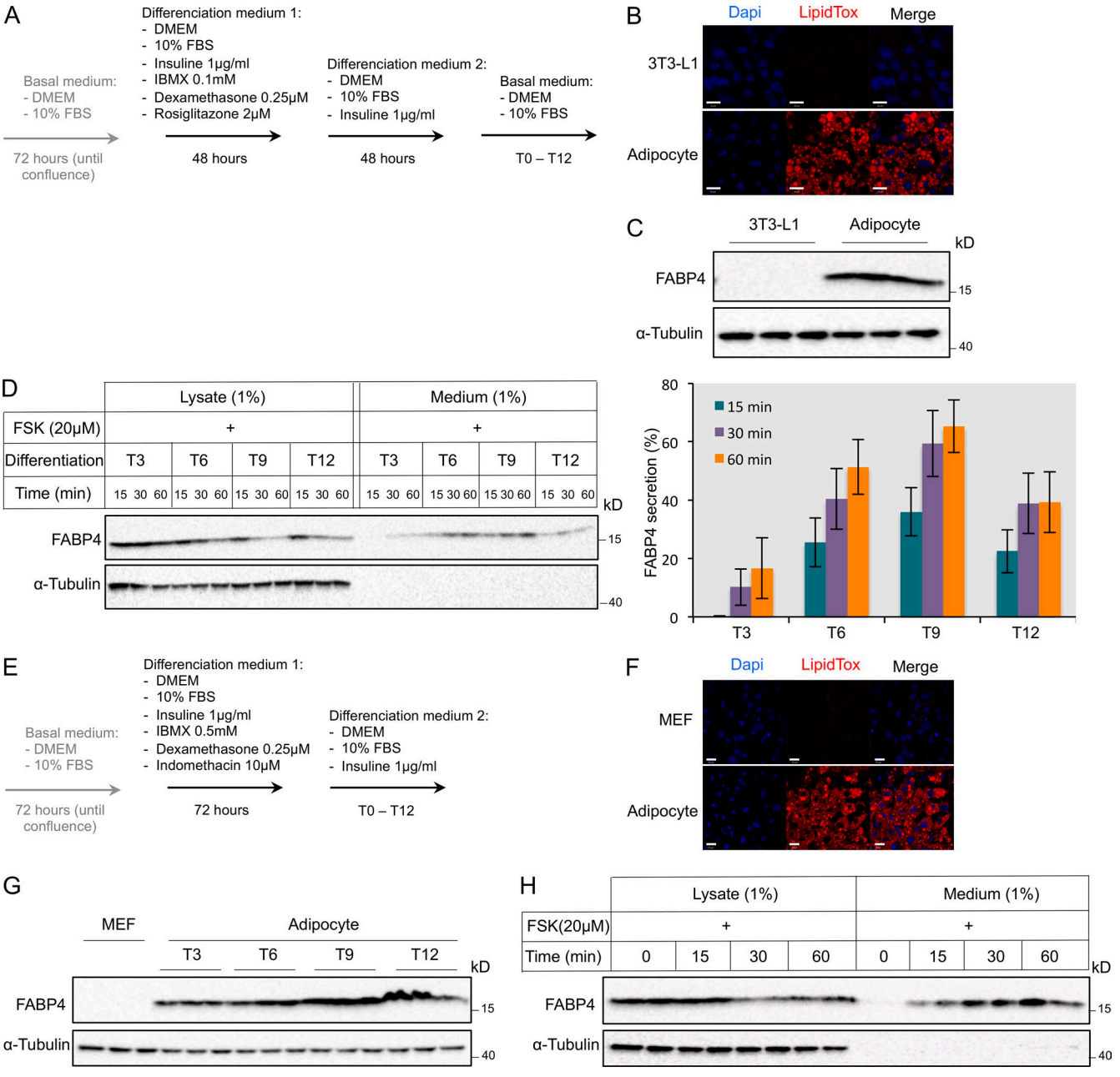

Figure S1. **3T3-L1 and MEF cells differentiated into adipocyte express and secrete FABP4. (A and E)** Schematic representation of the procedure used to induce 3T3-L1 and MEF cells differentiation into adipocytes, respectively. **(B and F)** Analysis by fluorescence microscopy using LipidTox neutral lipid staining of cells differentiated into adipocytes. Bar, 20 µm. **(C)** 3T3-L1 cells and 3T3-L1 cells differentiated into adipocytes at T9 were lysed, and total cell lysates were analyzed by Western blotting with anti-FABP4 and anti–α-tubulin antibodies. **(D)** Left: Adipocytes at different time points of differentiation were incubated with 20 µM FSK, and medium was collected and cells lysed. For each condition, 1% of total cell lysate and medium was analyzed by Western blotting with anti-FABP4 and anti–α-tubulin antibodies. Right: Quantification of FABP4 secretion. For each time point, FABP4 secretion was calculated as a percentage of the signal detected in the medium compared with the total amount (the sum of FABP4 in both medium and lysate). Results are shown as the mean ± SD of three independent experiments. **(G)** MEF cells and MEF cells differentiated into adipocytes at different time points were lysed, and total cell lysates were analyzed by Western blotting with anti-FABP4 and anti–α-tubulin antibodies. **(H)** MEF cells differentiated into adipocytes at T9 were incubated with 20 µM FSK, and medium was collected and cells lysed at the indicated time point. For each condition, performed in duplicate, 1% of total cell lysate and medium was analyzed by Western blotting with anti-FABP4 and anti–α-tubulin antibodies.

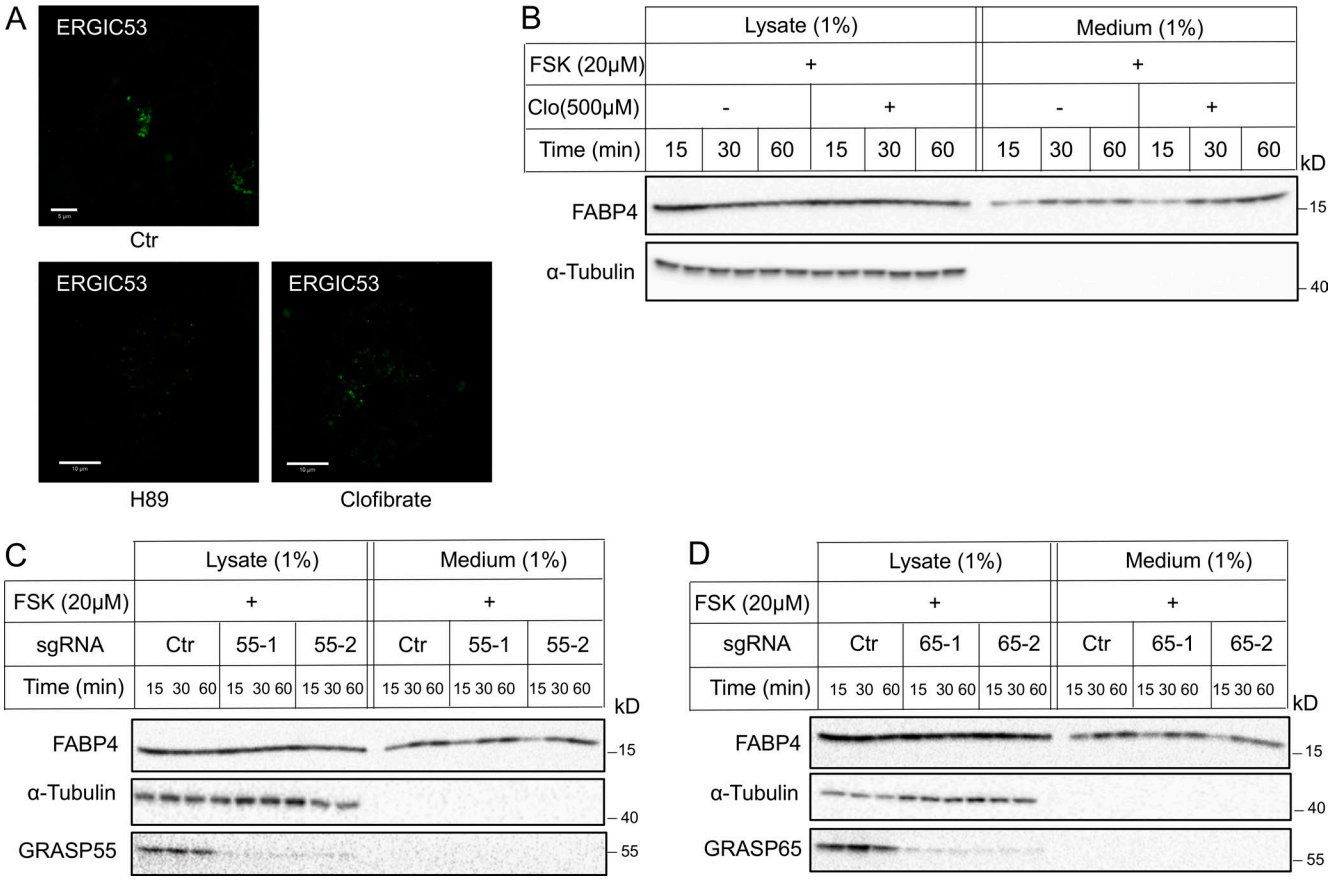

Figure S2. **ERGIC dispersal and GRASP55 and GRASP65 knockout do not affect FSK-induced FABP4 secretion. (A)** Adipocytes were incubated in Ctr or in the presence of 100 µM H89 or 500 µM Clo for 60 min, and the localization of ERGIC53 was monitored by immunofluorescence microscopy. Ctr, bar, 5 µm. Conditions in presence of H89 or Clo, bars, 10 µm. **(B)** Adipocytes were incubated with 20 µM FSK in the presence or absence of 500 µM Clo, and at indicated times, medium fractions were collected and cells lysed. For each condition, performed in duplicate, 1% of total cell lysate and medium fractions was analyzed by immunoblotting with anti-FABP4 and anti–α-tubulin antibodies. Results obtained using adipocytes incubated with 100 µM H89 are shown in Fig. 2 H. **(C and D)** Wild-type adipocytes and adipocytes depleted for GRASP55 (C) and GRASP65 (D) using the CRISPR/Cas9 system were incubated with 20 µM FSK, and at indicated times, medium was collected and cells lysed. For each condition, 1% of total cell lysate and medium was analyzed by Western blotting with anti-FABP4 and anti–α-tubulin antibodies. The knockout efficiency was validated by Western blotting with anti-GRASP55 (A) and anti-GRASP65 antibodies.

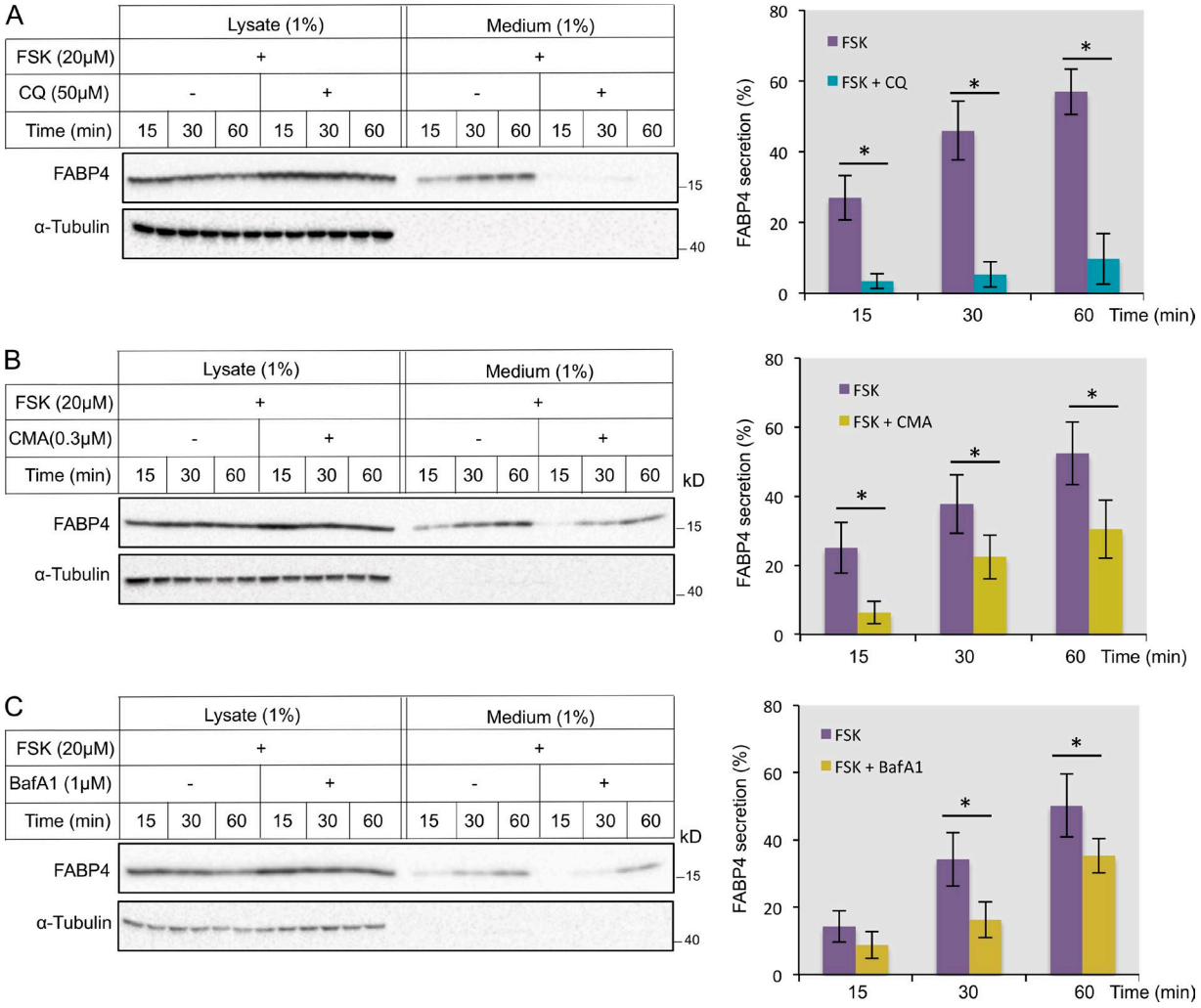

Figure S3. **FABP4 secretion is inhibited by CQ, concanamycin A, and bafilomycin A1. (A)** Left: MEF cells differentiated into adipocytes were incubated with 20 μM FSK in the presence or absence of 50 μM CQ, and at indicated times, medium was collected and cells lysed. **(B and C)** Left: 3T3-L1 cells differentiated into adipocytes were incubated with 20 μM FSK in the presence or absence of 0.3 μM concanamycin A (CMA; B) or 1 μM bafilomycin A1 (C), and at indicated times, medium was collected and cells lysed. For each condition, performed in duplicate, 1% of total cell lysate and medium was analyzed by Western blotting with anti-FABP4 and anti–α-tubulin antibodies. Right: Quantitation of FABP4 secretion. For each condition, FABP4 secretion was calculated as a percentage of the signal detected in the medium compared with the total amount (the sum of FABP4 in both medium and lysate). Results are shown as the mean ± SD of three independent experiments. *, $P < 0.05$.

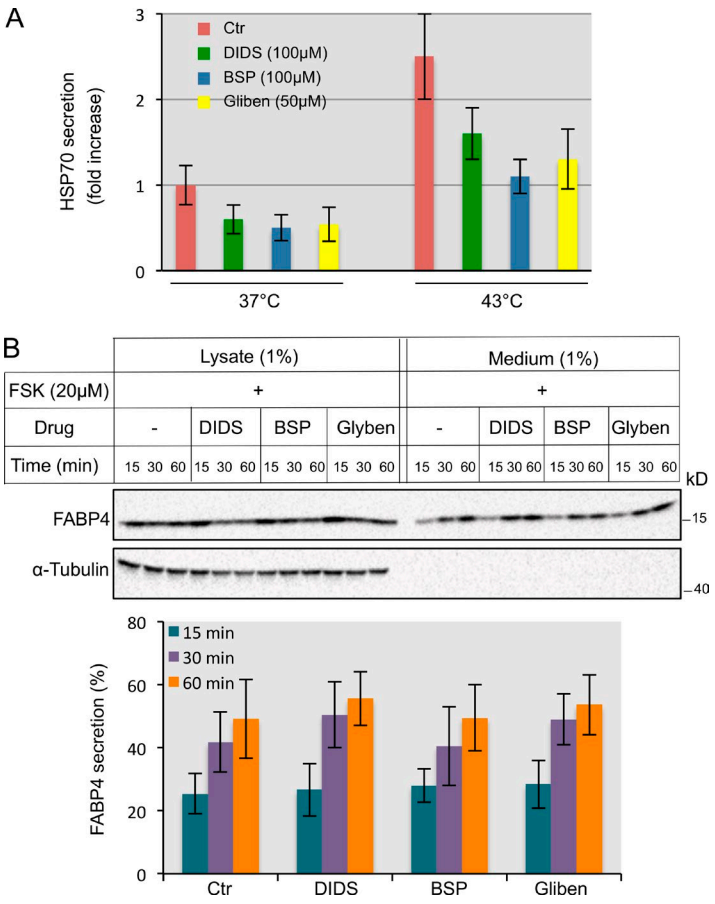

Figure S4. **FABP4 secretion is not impaired by ABC transport inhibitors. (A)** HSP70 secretion is inhibited by ABC transporter inhibitors. PC-3 cells were pretreated for 30 min with 100 µM DIDS, 100 µM BSP, or 50 µM glibenclamide and incubated at 37°C or 43°C for 30 min. After heat treatment, cells were incubated 1 h at 37°C. Then media were harvested and tested for the presence of HSP70 using an ELISA kit from Enzo Life sciences. **(B)** Top: Adipocytes were incubated with 20 µM FSK in the presence or absence of 100 µM DIDS, 100 µM BSP, or 50 µM glibenclamide, and at indicated times, medium was collected and cells lysed. For each condition, 1% of total cell lysate and medium was analyzed by Western blotting with anti-FABP4 and anti–α-tubulin antibodies. Bottom: Quantitation of FABP4 secretion as described in A. Results are shown as the mean ± SD of three independent experiments.

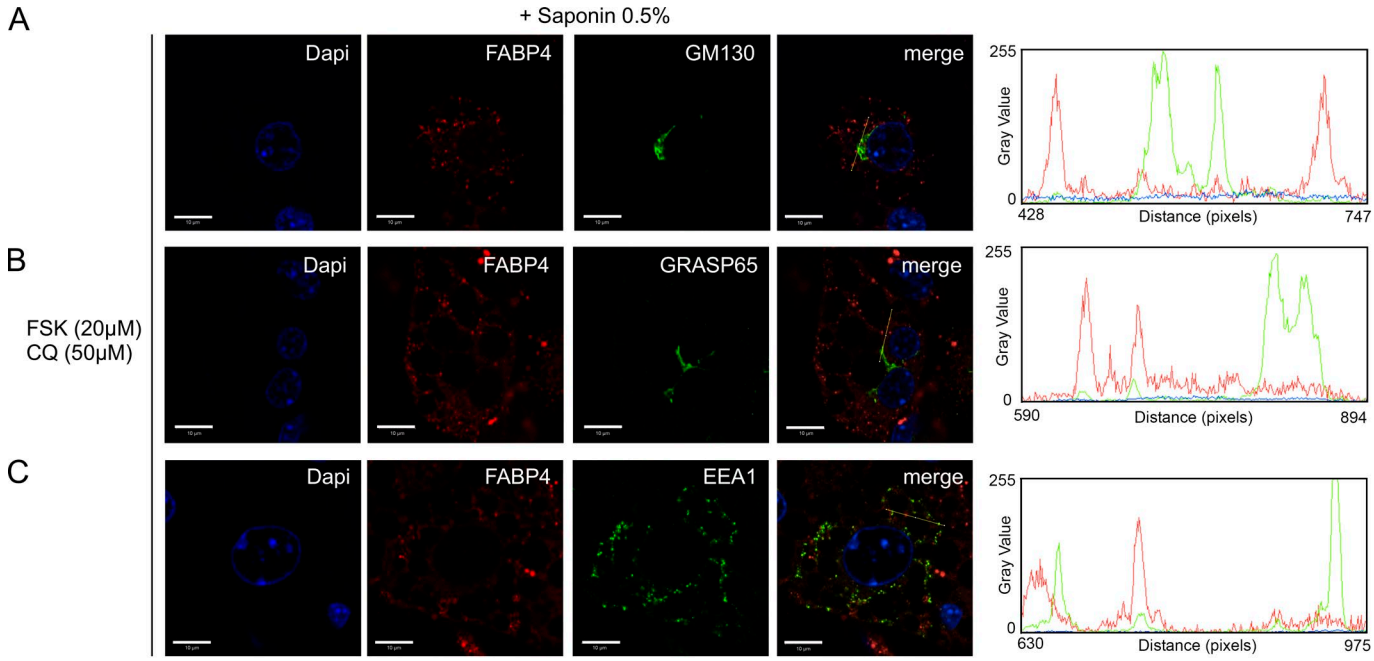

Figure S5. **Colocalization analysis of FABP4 with Golgi and early endosome markers.** Left: Adipocytes were incubated in the presence of 20 μM FSK with 50 μM CQ for 30 min, and cells were permeabilized with saponin before fixation. The colocalization of FABP4 with the indicated membrane markers was monitored by immunofluorescence microscopy. Bar, 10 μm. Pearson correlation coefficient (r) of colocalization, FABP4/GM130 r: 0.038 (A); FABP4/GRASP65 r: 0.055 (B); FABP4/EEA1 r: 0.121 (C); *n* = 10 for each colocalization analysis. Right: Intensity profile graphs of FABP4 colocalization with the indicated membrane markers.

Provided online is Table S1 in Excel showing pairs of oligonucleotides cloned in LentiCRISPRv2 for sgRNAs. Two pairs of oligonucleotides were designed to generate two different sgRNAs for each targeted gene.

Also included is a supplemental PDF showing replicate datasets for the experiments shown in Fig. 1 A; Fig. 2, C, E, and H; Fig. 3, A, B, E, and F; Fig. 6, A and B; Fig. S2 D; Fig. S3, B and C; and Fig. S4 B.