Supplementary Information

Ceiling culture chip reveals dynamic lipid droplet transport during adipocyte dedifferentiation via actin remodeling

Authors

Jiwon Kim, a† Kun-Young Park, b† Sungwoo Choi, b Ung Hyun Ko, a Dae-Sik Lim, c,d Jae Myoung Suh, b and Jennifer H. Shin a

a Department of Mechanical Engineering, Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea

b Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea

c National Creative Research Center for Cell Plasticity, Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea

d Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea

† These authors contributed equally to this work.

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Supplementary Figure 1

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Supplementary Fig. 1. Comparison of various indices for morphological quantification.
Supplementary Figure 2

Supplementary Fig. 2. Shape index distributions of vWAT adipocytes over time. I-shaped bars represent mean ± s.d. n=15 each. p-value from one-way ANOVA with Fisher’s LSD post hoc test. t-value from two-tailed t-test v.s. Day n-2 data of the same group.
Supplementary Figure 3

(1) Secretion involving mechanical vibration
The lipid droplet vibrates vigorously before the secretion.

Supplementary Fig. 3.a. Mechanical vibration involved secretion, case 1 (Cell featured Fig.4)

(2) Secretion involving crescent-shaped band structure
A rope-like structure, shown as a crescent-shaped band, appears and squeezes the LD to suddenly extrude it from the cell (Supplementary Video 3). Band structure is indicated by solid arrowheads.

Supplementary Fig. 3.c. Crescent-shaped band structure involved secretion, case 1
(Cell featured Supplementary Video 3)
(3) Secretion involving sudden cellular elongation

Lipid droplets get extruded soon after the sudden elongation of the cell.
**Supplementary Fig. 3.g.** Timepoints of liposecretion events
(Two points connected with the dotted line indicate a single event)
Supplementary Figure 4

Supplementary Fig. 4. Blebbistatin suppresses LD secretion. 

a Representative image of dedifferentiation of the control group. Cells undergoing dedifferentiation moved around dynamically (scale bar = 200μm).

b Representative images of 50μM blebbistatin treatment group. Cells are in a motionless state compared to the control group (scale bar = 200μm).

c Shape indices of control group and blebbistatin treatment group. On day 5, the shape indices of the blebbistatin treatment group are significantly higher than those of the control group. t-value from two-tailed t-test. n=76, 50, 87, and 54 for Ctrl Day 1, Blebb Day 1, Ctrl Day 5, and Blebb Day 5 respectively.
Supplementary Figure 5

Supplementary Fig. 5. Shape index distributions of control mT/mG mice adipocytes and Lats1/2 iAKO mice adipocytes at Day 5. t-value from two-tailed t-test. n=82 and 124 for Ctrl and Lats1/2 KO respectively.
Supplementary Fig. 6. Adipocyte-specific suppression of the Hippo pathway induces a unique mode of LD secretion into adjacent adipocytes. 

a) Schematic diagram for 4-hydroxytamoxifen (4OHT)-inducible Lats1/2 knockout and labeling primary adipocytes from Lats1/2 iAKO (Adipoq-CreER<sup>12</sup>; Lats1/2<sup>flox/flox</sup>; tdTomato) mice. 

b) Experimental scheme for adipocyte isolation and deletion of LATS1/2 in adipocytes from control (Adipoq-CreER<sup>12</sup>; tdTomato) or Lats1/2 iAKO mice and their analysis for 6 days. 

c) Time-lapse imaging of intercellular lipid exchange between adipocytes isolated from Lats1/2 iAKO mice. Relative time from 4d:18h:45m. Scale bar, 50μm.
Supplementary Video 1
Adipocyte undergoing dedifferentiation is making pseudopod structures towards the moving direction.

Supplementary Video 2
Abrupt distortion of cellular morphology before the whole lipid droplet secretion.

Supplementary Video 3
Ring-like structure is observed to sweeping the lipid droplet.

Supplementary Video 4
Dedifferentiated cell which is still expressing the GFP signal is dividing into two daughter cells.

Supplementary Video 5
Lipid contents of cells are being transferred to neighboring cell. Meanwhile, the sizes of donor cells are decreasing.

Supplementary Video 6
Lipid content of WT BODIPY+ cell is being transferred to neighboring mTmG cell. Meanwhile, the sizes of donor cells are decreasing. Relative time from 1d:4h:30m

Supplementary Video 7
Adipocyte-specific activation of YAP/TAZ induces a unique mode of liposecretion into adjacent adipocytes A Schematic diagram for 4-hydroxytamoxifen (4OHT)-inducible LATS1/2 knockout and labeling of primary adipocytes from Lats1/2 iAKO (Adipoq-CreER<sup>T2</sup>; Lats1/2<sup>flox/flox</sup>; tdTomato) mice. Relative time from 4d:18h:45m.