

Supplementary Information

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

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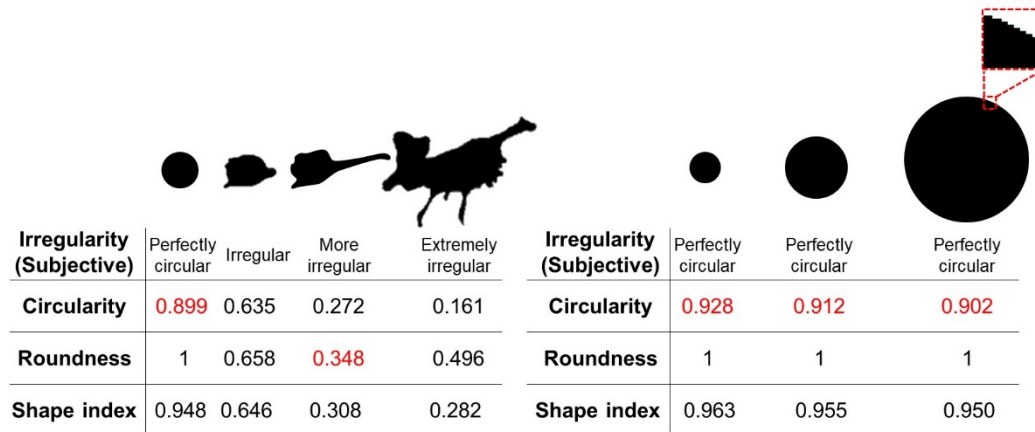
Supplementary Figure 4

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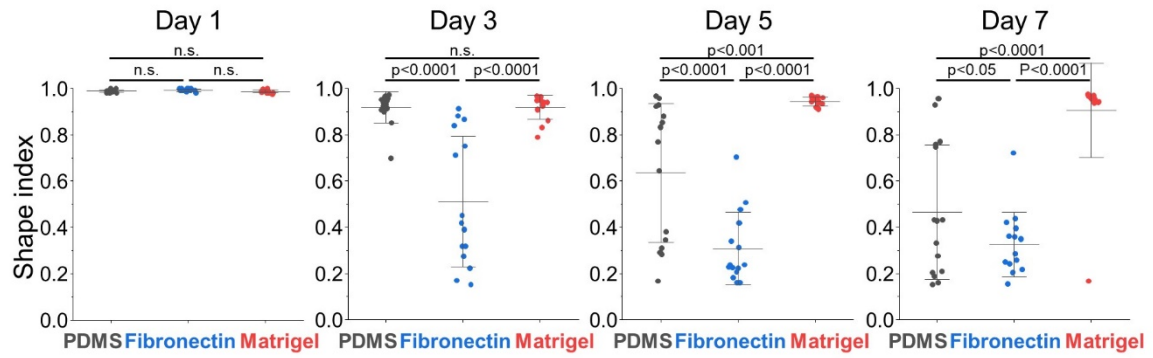
Captions for Supplementary Video 1-7

Supplementary Figure 1



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

Supplementary Figure 2

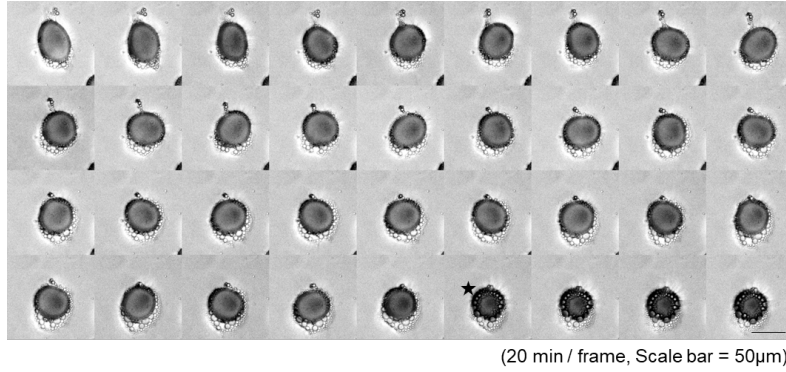


Supplementary Fig. 2. Shape index distributions of vWAT adipocytes over time. I-shaped bars represent mean \pm s.d. n=15 each. p-value from one-way ANOVA with Fisher's LSD *post hoc* test. t-value from two-tailed t-test v.s. Day n-2 data of the same group.

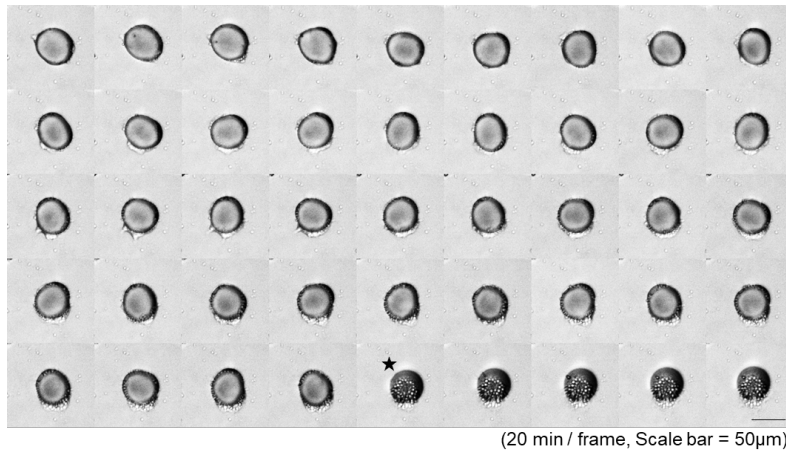
Supplementary Figure 3

(1) Secretion involving mechanical vibration

The lipid droplet vibrates vigorously before the secretion.



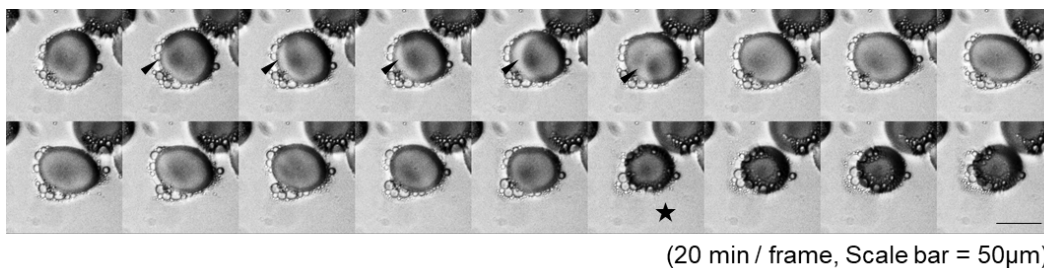
Supplementary Fig. 3.a. Mechanical vibration involved secretion, case 1 (Cell featured Fig.4)



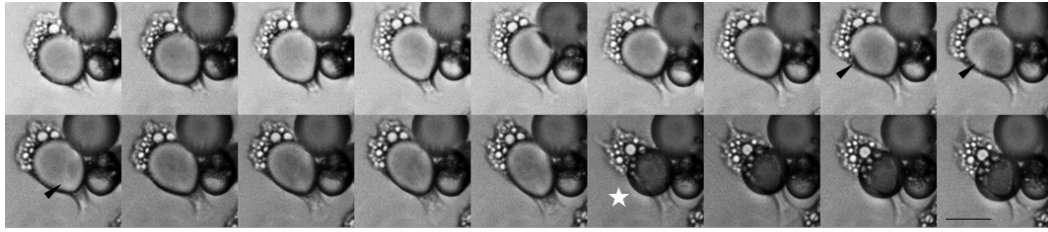
Supplementary Fig. 3.b. Mechanical vibration involved secretion, case 2

(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)

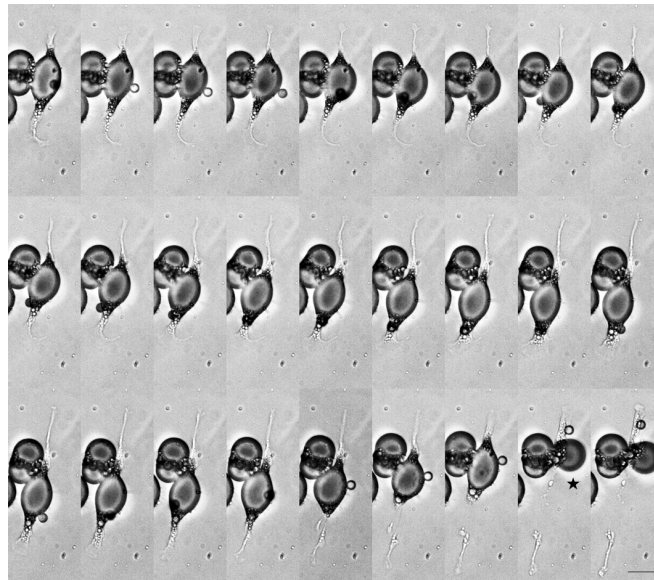


(20 min / frame, Scale bar = 50 μ m)

Supplementary Fig. 3.a.d. Crescent-shaped band structure involved secretion, case 2

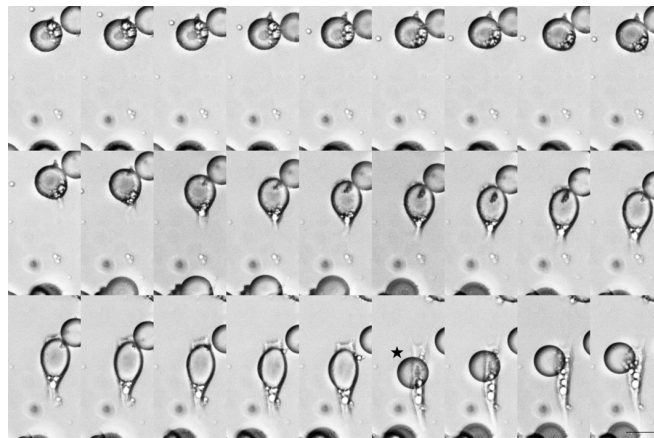
(3) Secretion involving sudden cellular elongation

Lipid droplets get extruded soon after the sudden elongation of the cell.



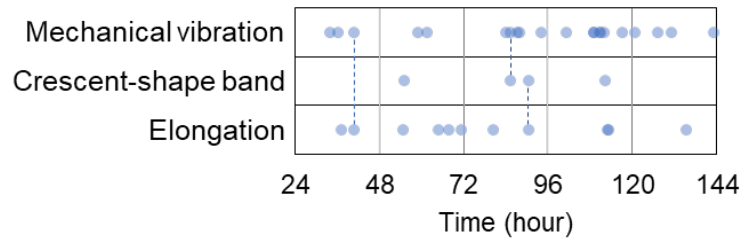
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Supplementary Fig. 3.e. Elongation-driven secretion, case 1



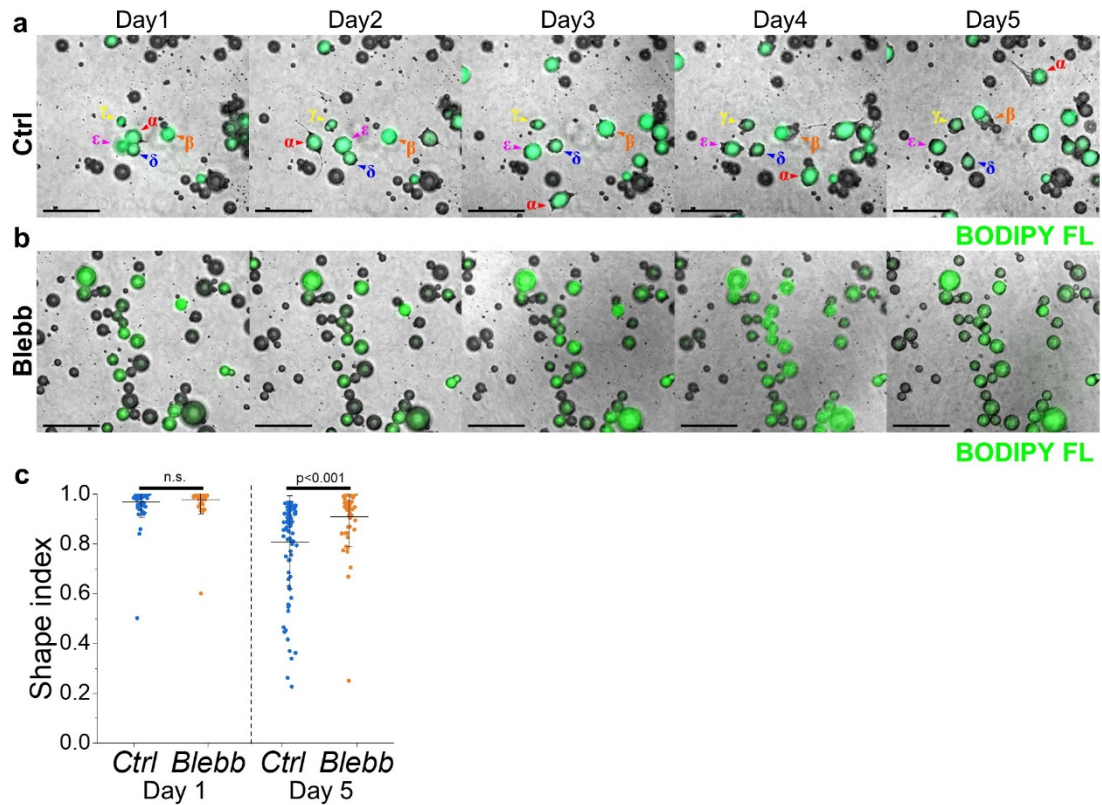
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Supplementary Fig. 3.f. Elongation-driven secretion, case 2



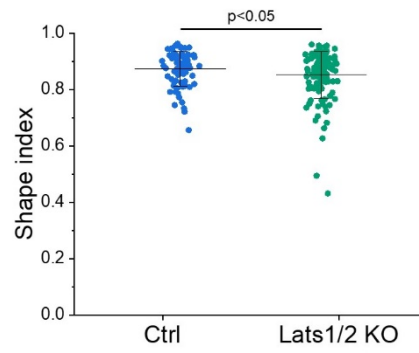
Supplementary Fig. 3.g. Timepoints of liposecretion events
 (Two points connected with the dotted line indicate a single event)

Supplementary Figure 4



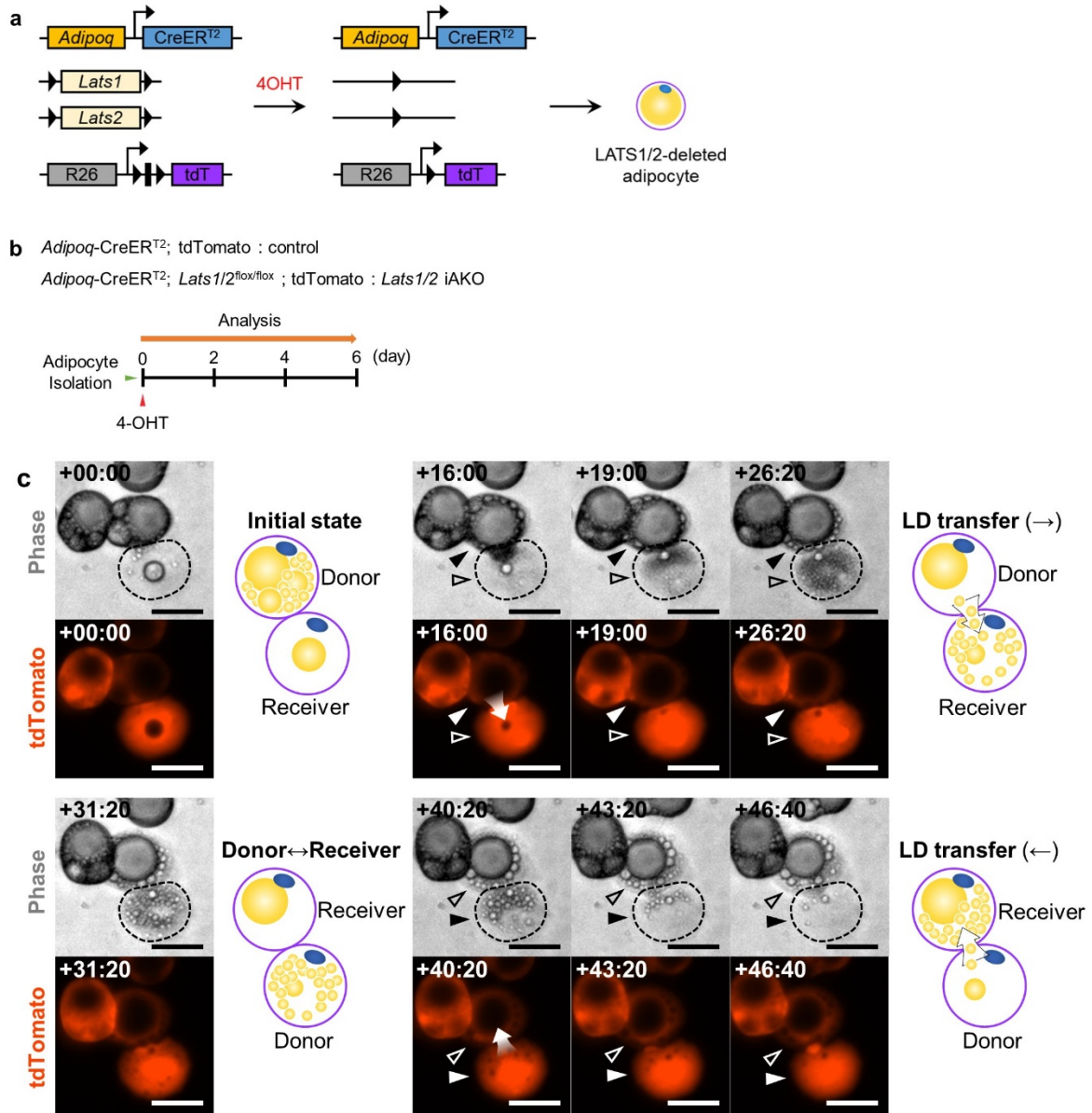
Supplementary Fig. 4. Blebbistatin suppresses LD secretion. **a** Representative image of dedifferentiation of the control group. Cells undergoing dedifferentiation moved around dynamically (scale bar = 200 μ m). **b** Representative images of 50 μ M blebbistatin treatment group. Cells are in a motionless state compared to the control group (scale bar = 200 μ m). **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 Figure 6



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^{T2}; *Lats1/2*^{fllox/fllox}; tdTomato) mice. **b** Experimental scheme for adipocyte isolation and deletion of LATS1/2 in adipocytes from control (*Adipoq*-CreER^{T2}; 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, 50 μ m.

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^{T2}; *Lats1/2*^{flox/flox}; tdTomato) mice. Relative time from 4d:18h:45m.