Supplemental Figures

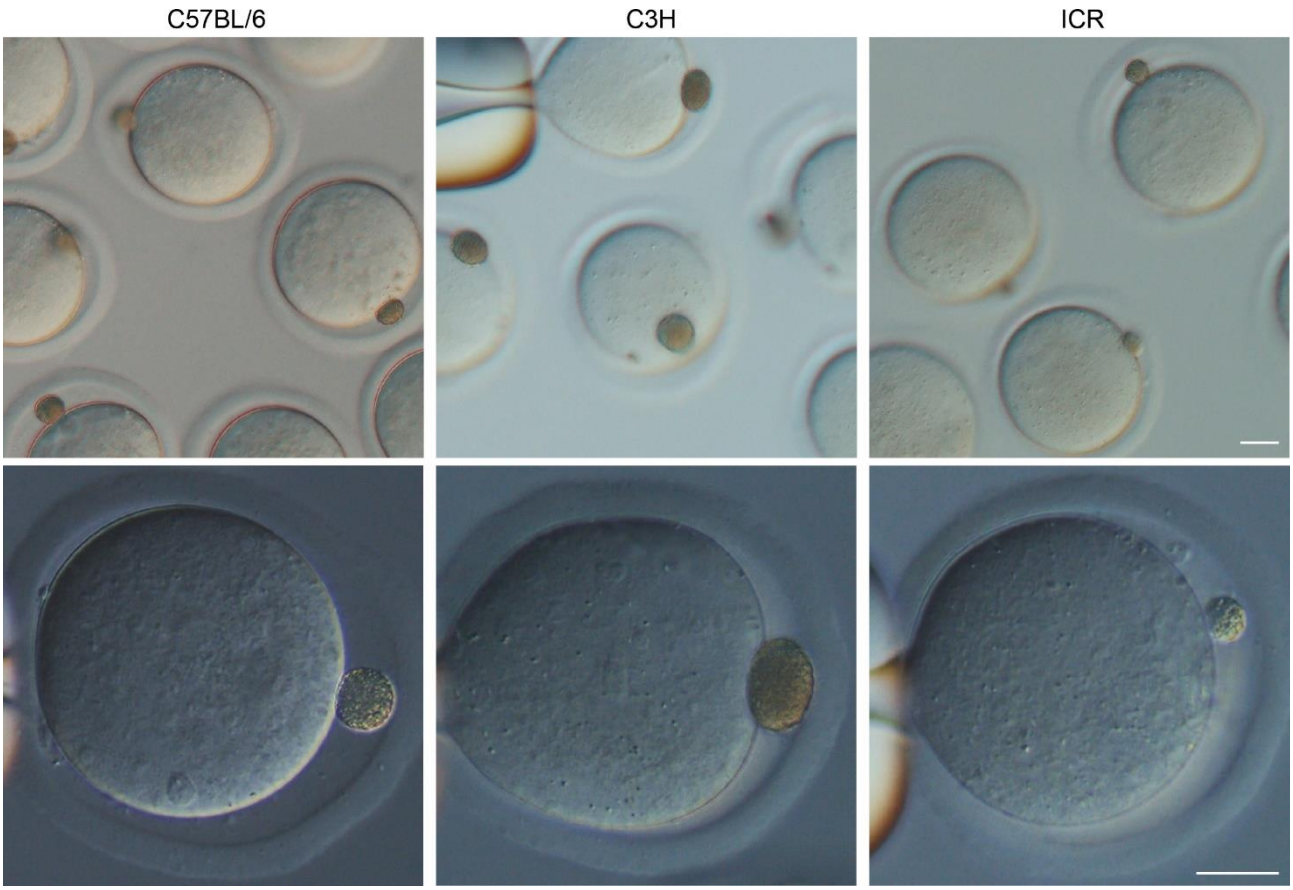


Fig. S1. Strain difference of LD morphology after two-step centrifugation.
Representative images of MII oocytes collected from C57BL/6 (left), C3H (middle), and ICR (right) females and subjected to two-step centrifugation. Higher-magnification images are shown at the bottom. Notably, the size of LDs released into the PV space differed among mouse strains. Scale bars, 20 µm.

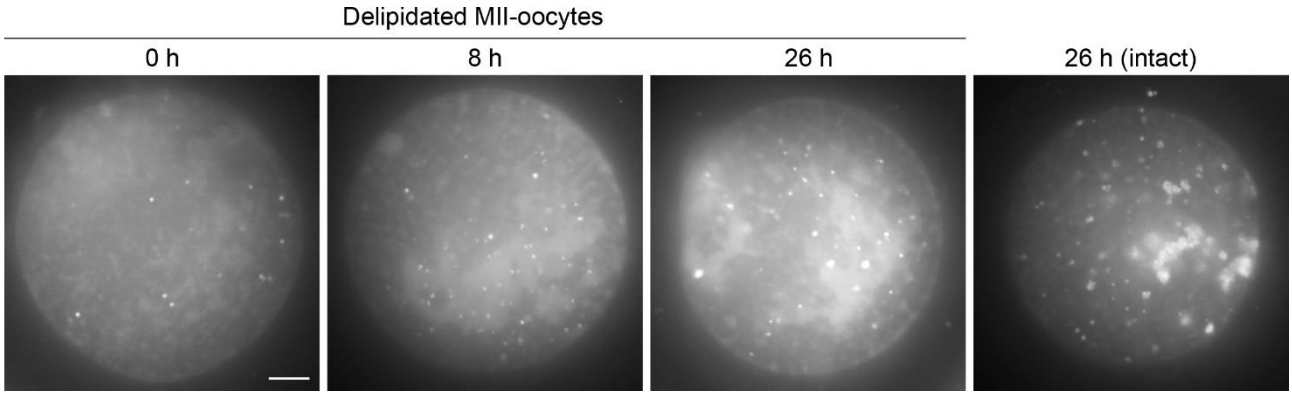


Fig. S2. Production of new LDs under BSA-free conditions.
Representative images of delipidated MII oocytes cultured in BSA-free medium (FHM-PVP) and stained with BODIPY 493/503 to visualize LDs at the indicated times after delipidation. As a control, non-delipidated (intact) MII oocytes were cultured in FHM-PVP for 26 h and stained with BODIPY 493/503. Scale bars, 10 µm.

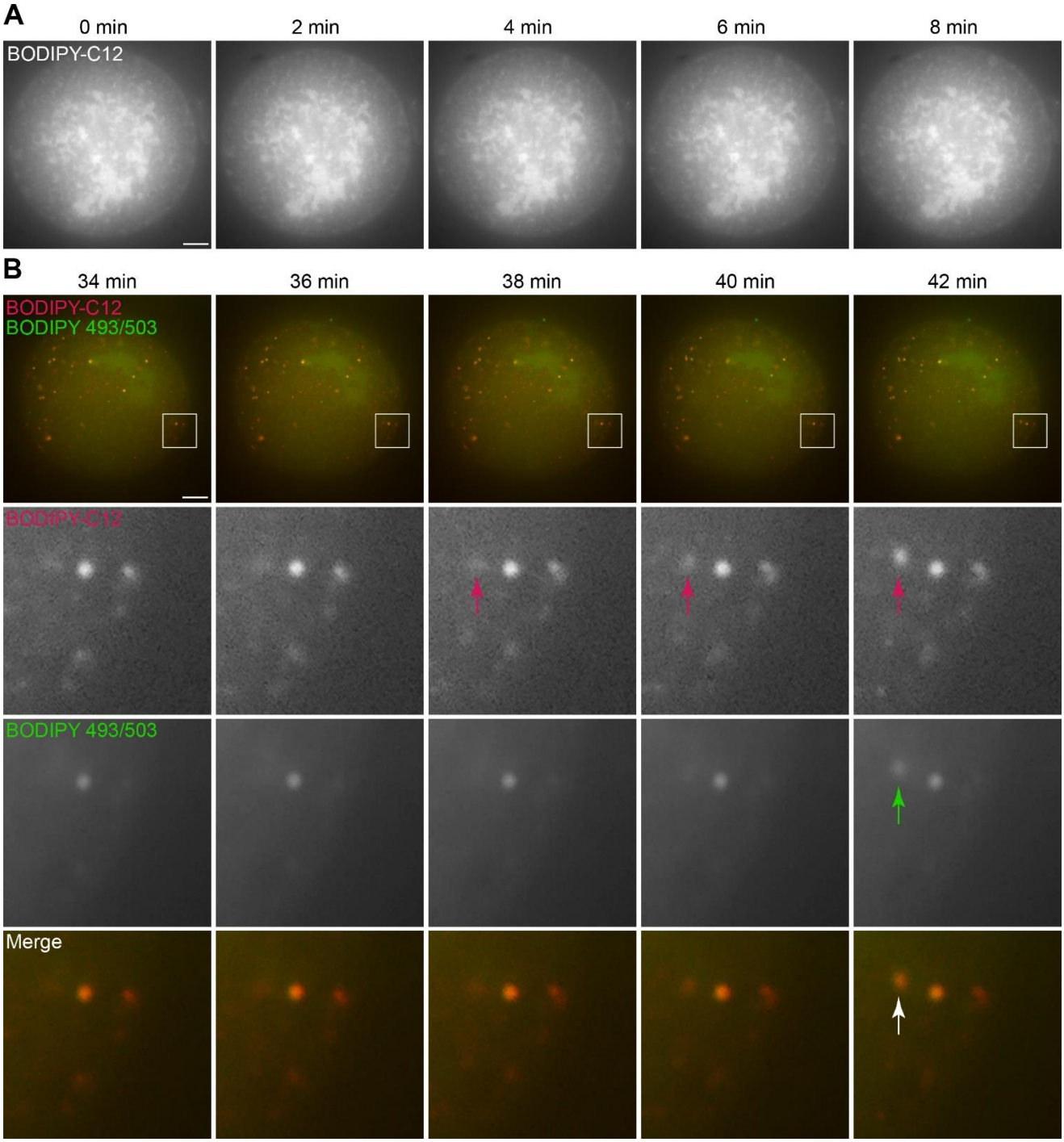


Fig. S3. Visualization of emergence of new LDs after delipidation.
(A) MII oocytes were delipidated and stained with BODIPY-C12 for 30 min immediately before the movie was taken; images were acquired at 2-min intervals for 30 min. Time indicates minutes from beginning of imaging.
(B) MII oocytes were delipidated and stained with BODIPY-C12 (red) followed by BODIPY 493/503 staining (green) before the movie was taken; images were acquired at 2-min intervals for 60 min. Selected time frames (from beginning of imaging) are shown. Higher-magnification images of the boxed region (in upper panels) are shown at the bottom as either GFP or RFP channels or overlay of GFP and RFP channels. Red arrows, BODIPY-C12 positive (emerging) LDs; green arrow, BODIPY 493/503 positive (mature) LDs; white arrow, BODIPY-C12 and BODIPY 493/503 double positive LD. Scale bars, 10 µm (A,B).

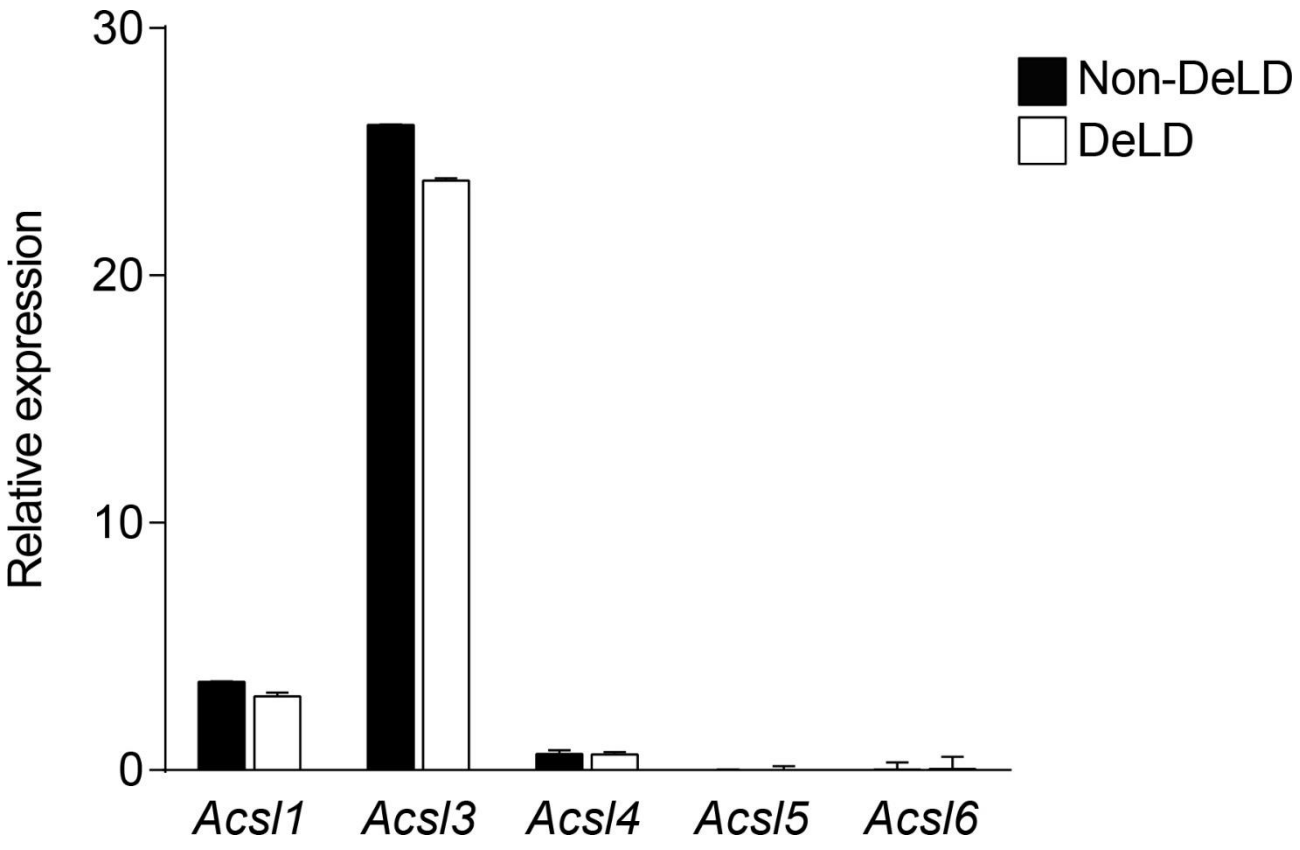


Fig. S4. RT-qPCR analysis of ACSL family in MII oocytes.
RT-qPCR analysis of *Acsl1, 3, 4, 5,* and *6* mRNA level in non-delipidated (Non-DeLD) or delipidated (DeLD) MII oocytes. Data are mean expression values relative to *β-actin*. Error bars represent SD of three biological replicates.

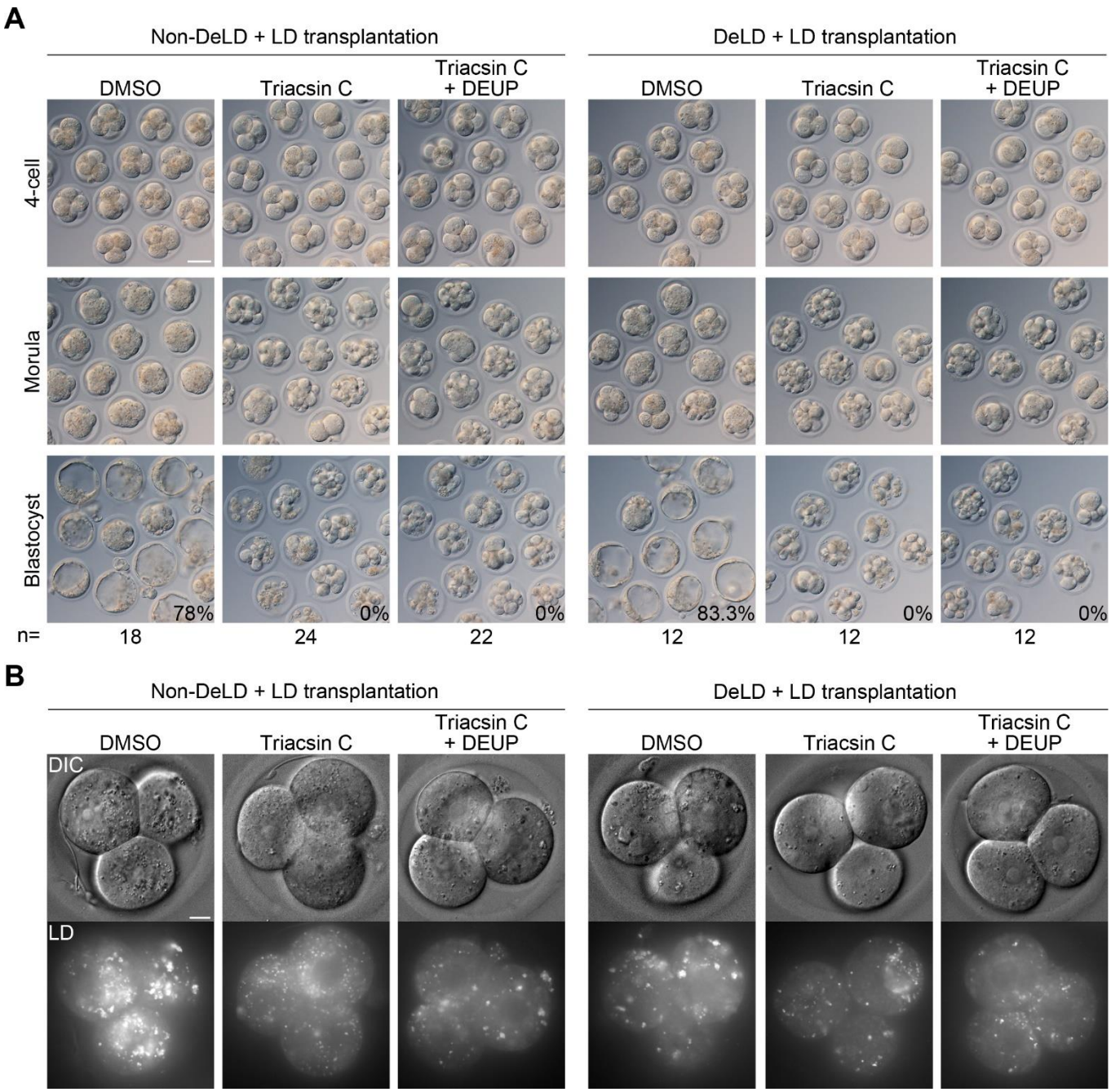
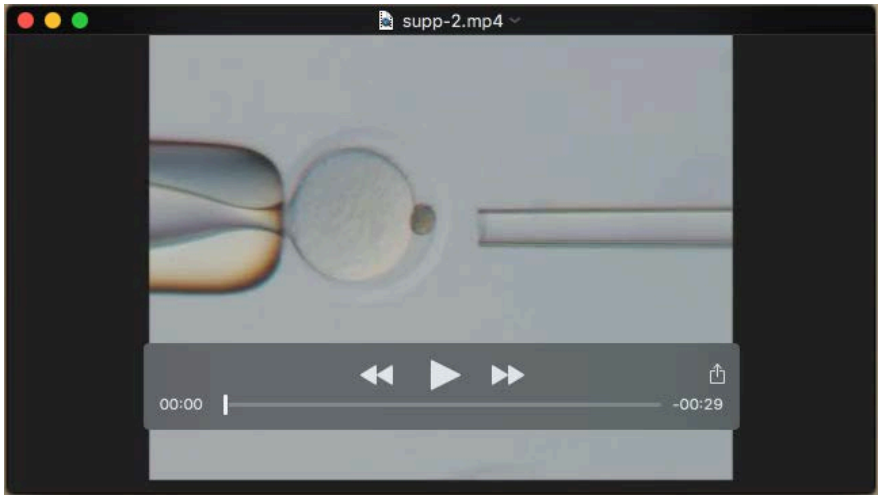
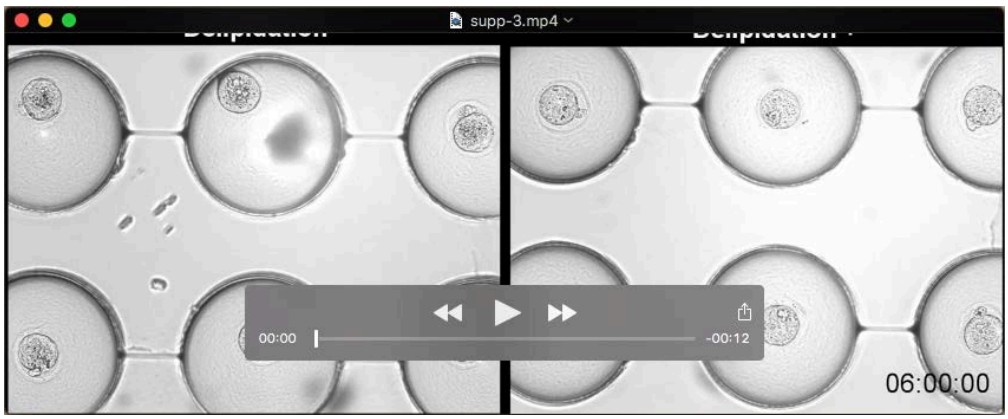


Fig. S5. LD transplantation did not rescue the developmental arrest induced by Triacsin C.
(A) Preimplantation development of LD-transplanted embryos. LDs isolated from MII oocytes were transplanted into individual blastomeres of 2-cell embryos developed from non-delipidated or delipidated MII oocytes, and the resultant embryos were further cultured in the presence of DMSO, Triacsin C, or both. Percentages of embryos developing into blastocysts is indicated. n, numbers of embryos analyzed.
(B) LD-transplanted embryos developed from non-delipidated or delipidated MII oocytes, and cultured in the indicated condition, were stained with BODIPY 493/503 at the 4-cell stage. DIC, differential interference contrast. Scale bars, 50μm (A) and 10μm (B).



Movie 1. Removal of LDs from MII oocytes.
A two-step centrifugation pushes aggregated LDs into the PV space, and the resultant LDs were easily aspirated using a micropipette. The first and third MII oocytes show typical examples of successful LD removal, whereas the second MII oocyte retained some LDs even after centrifugation (mp4; 1.0 MB).



Movie 2. Delipidation does not affect embryonic development.
MII oocytes with or without delipidation were fertilized *in vitro* and imaged every 15 min. Time shows hours, minutes, and seconds from 6 h after IVF (mp4; 4.3 MB).

Movie 3. Triacsin C treatment impairs preimplantation embryonic development.
Preimplantation development of embryos developed from non-delipidated (Delipidation-) or delipidated (Delipidation+) MII oocytes subjected to IVF and cultured in the presence or absence of Triacsin C. Treatment of embryos with DMSO was used as a control. Images were acquired every 15 min. Time indicates hours, minutes, and seconds from 6 h after IVF (mp4; 4.4 MB).

Movie 4. LD transplantation.
LDs isolated from MII oocytes were divided into two pieces, and each LD was injected into individual blastomeres of 2-cell embryos developed from non-delipidated (this movie) or delipidated MII oocytes (mp4; 2.2 MB).