Literature Report

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Literature Source

Developmental Cell

Seipin Facilitates Triglyceride Flow to Lipid Droplet and Counteracts Droplet Ripening via Endoplasmic Reticulum Contact

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Introduction

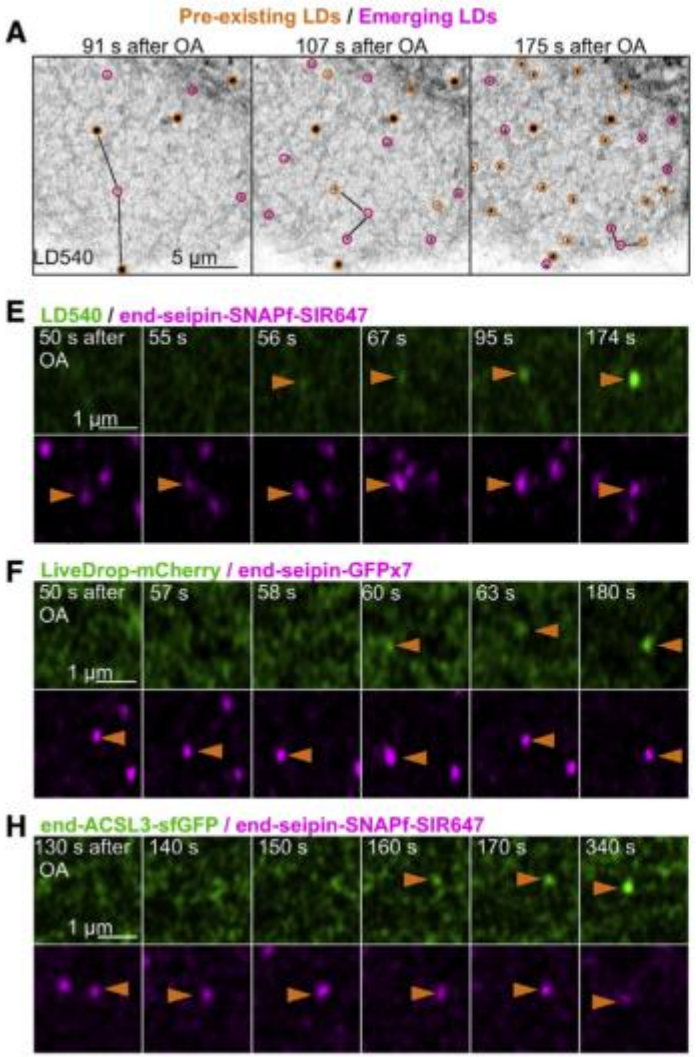

Figure 1. Seipin at LD Formation Sites.

(A) Cells with seipin tagged endogenously with SNAPf were delipidated for 3 days and imaged by Airyscan microscopy, starting 50 s after OA loading. Orange circles, pre-existing LDs; magenta circles, LDs forming between time points.

(B) Analysis of (A). n = 16 cells, 4 experiments.

(C) Distances of newly formed LDs to two nearest neighboring LDs were measured and compared with simulated images. Bars: mean ± SEM, n = 15 cells 4 experiments. ***p < 0.0005 (Mann-Whitney test) .

(D) Growth rate of individual LDs, pixel size 34.5 nm.

(E) Insets of video in (A). Orange arrowheads, immobilized SNAPf-seipin and subsequent LD540 accumulation at nascent LD.

(F). End-seipin-GFPx7 cells stably expressing BFP-KDEL (not shown) and LiveDrop-mCherry were delipidated for 1 day and imaged after OA loading. Orange arrowheads, immobilized seipin and subsequent LiveDrop accumulation at nascent LD.

(G) Analysis of (F). Tracking of LD-forming and not LD-forming seipins upon OA addition. Motility of seipins and LiveDrop/KDEL intensity ratio at seipin foci, ±SEM, n = 15–20 seipins from 6 cells, 3 experiments. **p < 0.005 (unpaired t test).

(H) Cells with sfGFP engineered to endogenous ACSL3 locus and SNAPf to endogenous seipin locus were delipidated for 3 days and imaged after OA loading.Orange arrowheads, immobilized seipin and subsequent ACSL3 accumulation at nascent LDs.

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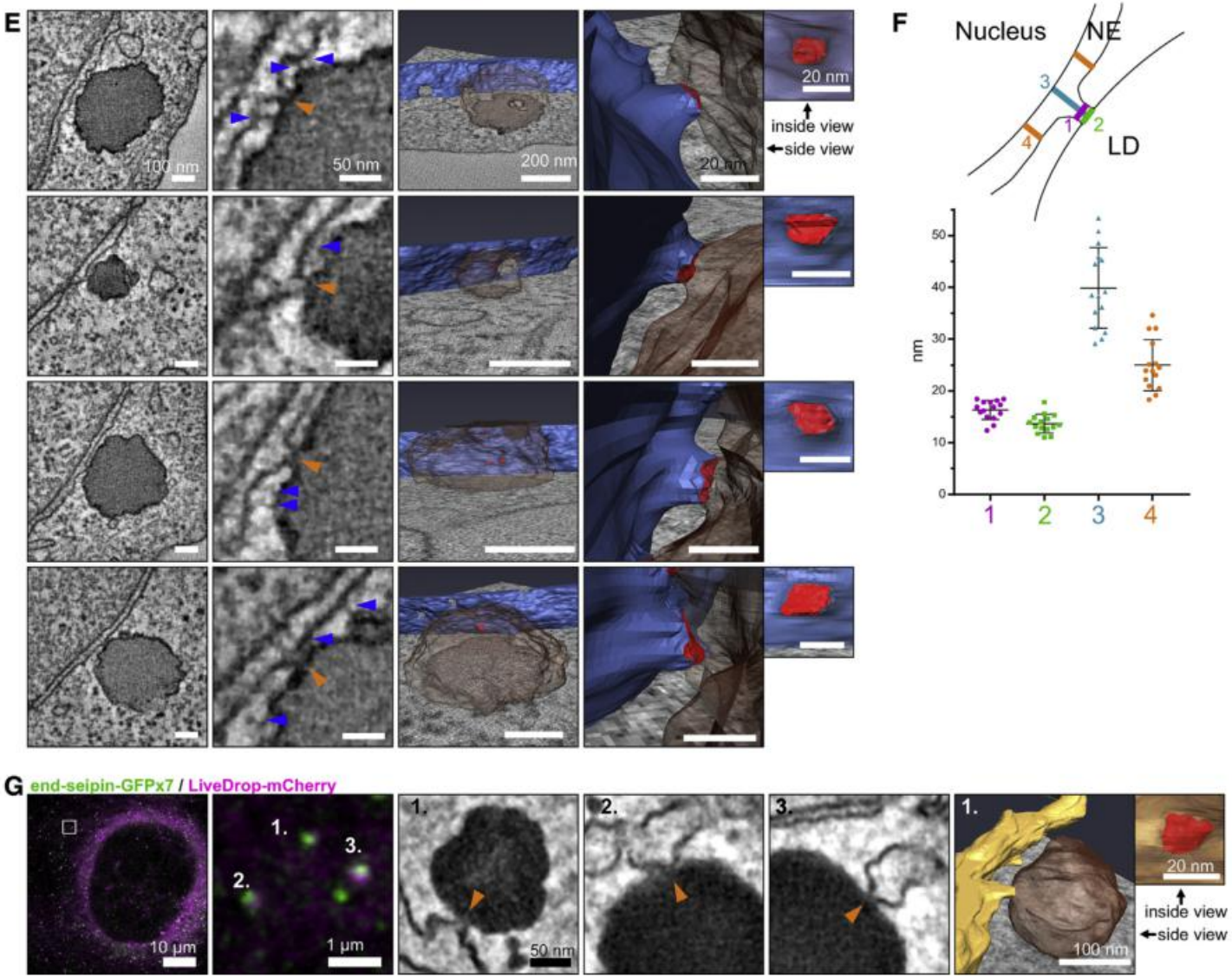

Figure 2. Seipin Relocalizes LD Formation and Seipin-LD Contacts Have Uniform Membrane Architecture.

(E) Stableseipin-NE-trapped cells were delipidated for 3 days, treated with OA for 1 h and processed for ET. Single 2-nm-thick tomogram slices and models of 3D reconstructions of NE-associated LDs (brown), NE (blue), and NE-LD contacts (red). Each row depicts an LD and its contact. Orange and blue arrowheads indicate NE-LD contacts and fiber-like connections between NE and LDs, respectively.

(F) Analysis of (E). Dimensions of NE-LD contacts from tomogram slices. n = 15 contacts from 14 LDs, 2 experiments.

(G) End-seipin-GFPx7 cells stably expressing LiveDrop-mCherry were delipidated for 2 days, treated with OA for 120 s, fixed, imaged by Airyscan microscopy, and processed for ET. Insets: three LiveDrop puncta with seipin association and corresponding tomograms of ER-LD contacts (orange arrowheads) and a 3D model of a reconstruction of an LD (brown), nearby ER (yellow), and its ER-LD contact (red). Same orientation of LDs in light microscopy images and tomograms.

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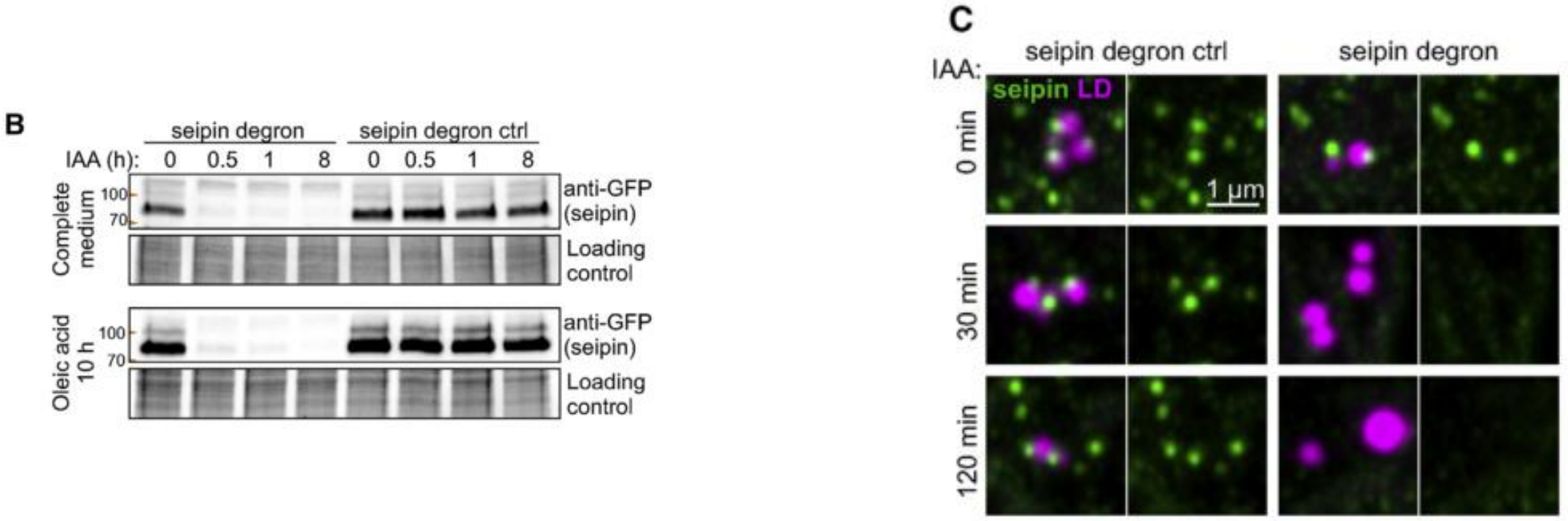

Figure 3. Effect of Acute Seipin Removal on LD Formation and Maintenance.
(B) Immunoblots of seipin degron-A and seipin degron control (ctrl) cells treated with IAA.
(C) OA-loaded seipin degron ctrl and seipin degron-A cells were treated with IAA, fixed, and stained with Lipid TOXDeepRed. Airyscan z-stack maximum intensity projections.

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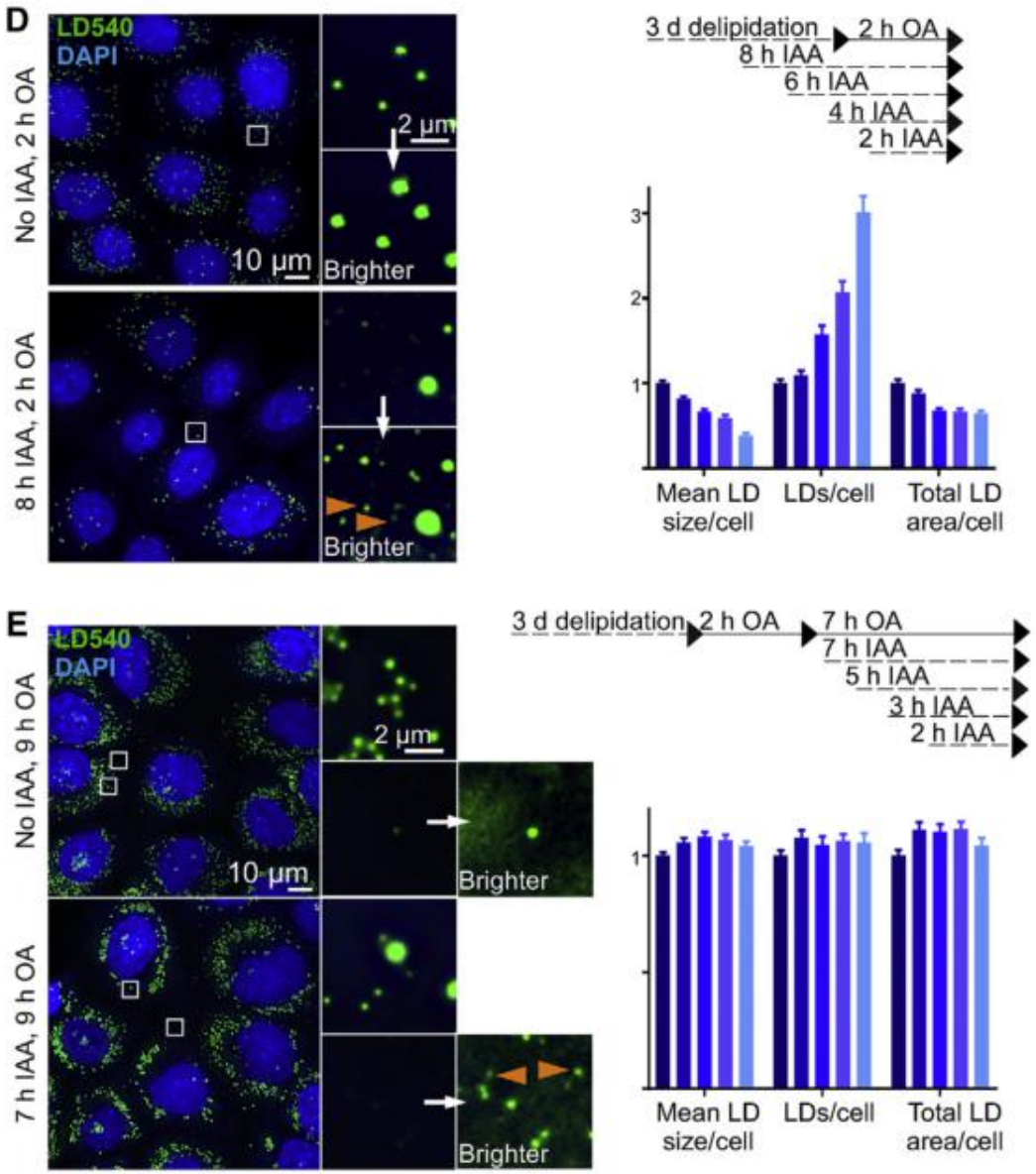

Figure 3. Effect of Acute Seipin Removal on LD Formation and Maintenance.

(D) Seipin degron-A cells were treated as indicated, fixed, stained, imaged by wide-field microscopy, and analyzed for LD sizes. Maximum intensity projections of deconvolved z-stacks. Orange arrowheads, tiny LDs in seipin-depleted cells. Bars: mean ± SEM, n = 197–237 cells, 2 experiments. IAA-treated cells are significantly different starting from 2 h IAA onward ($p < 0.005$, unpaired t test).

(E) Seipin degron-A cells were treated as indicated, imaged, and analyzed as in (D). Bars: mean ± SEM, n = 432–655 cells, 2 experiments. IAA-treated cells are significantly different starting from 3 h IAA onward ($p < 0.005$, unpaired t test).

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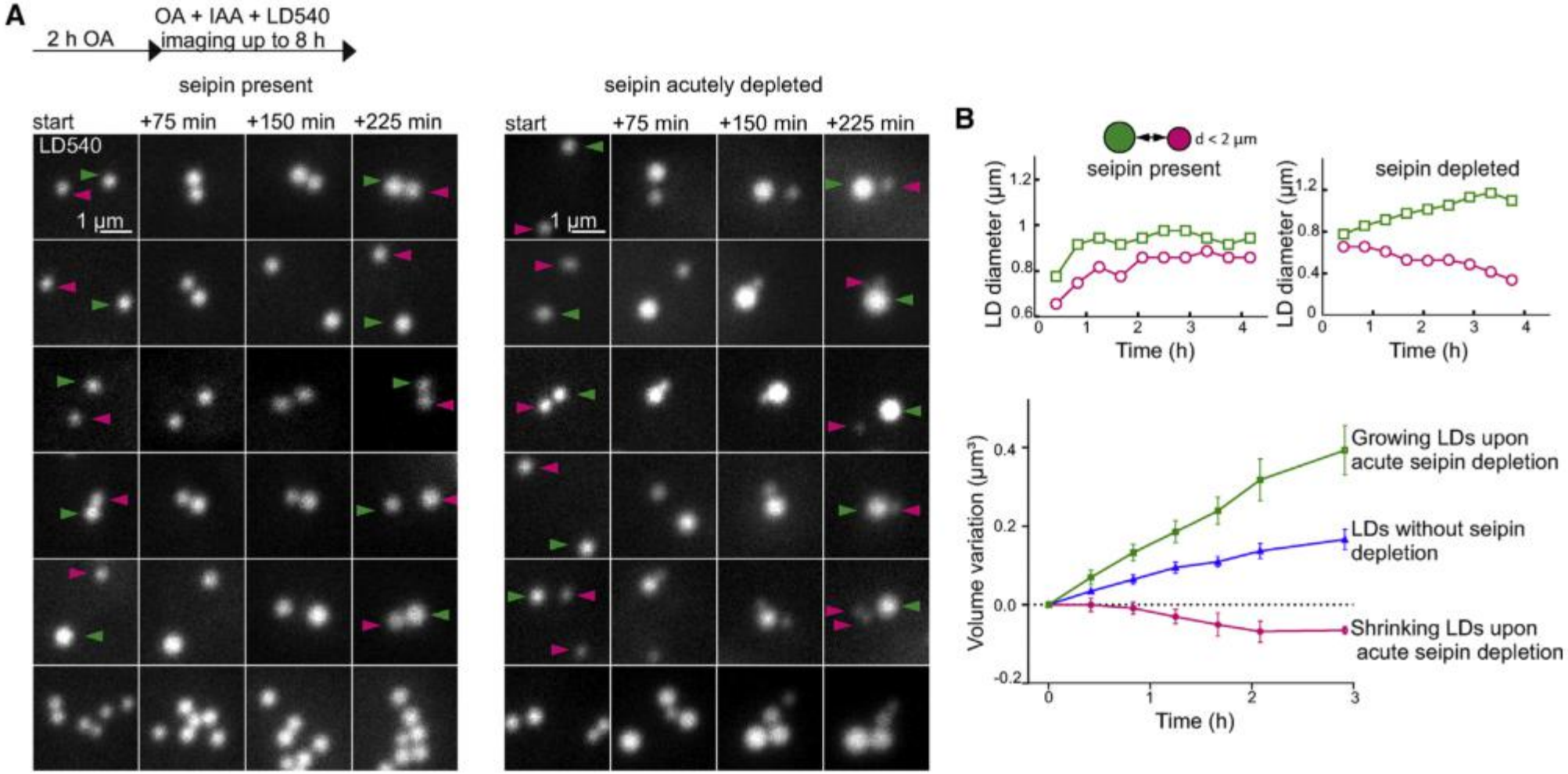

Figure 4. Seipin Removal from ER-LD Contacts Results in Inhomogeneous Neutral Lipid Partitioning to LDs.
(A) Seipin degron ctrl and seipin degron-A cells were treated as indicated and imaged live with wide-field microscopy. LD pairs and clusters, first frames are 25–50 min after IAA addition for degron, 25–240 min for ctrl examples. Colored arrowheads indicate the same LDs in the first and last panels.
(B)) Analysis of (A). Two nearby LDs were tracked over time and their sizes measured. Exemplary plots of nearby LD size changes and pooled data, n = 22–28 LDs, ± SEM, 2 experiments.

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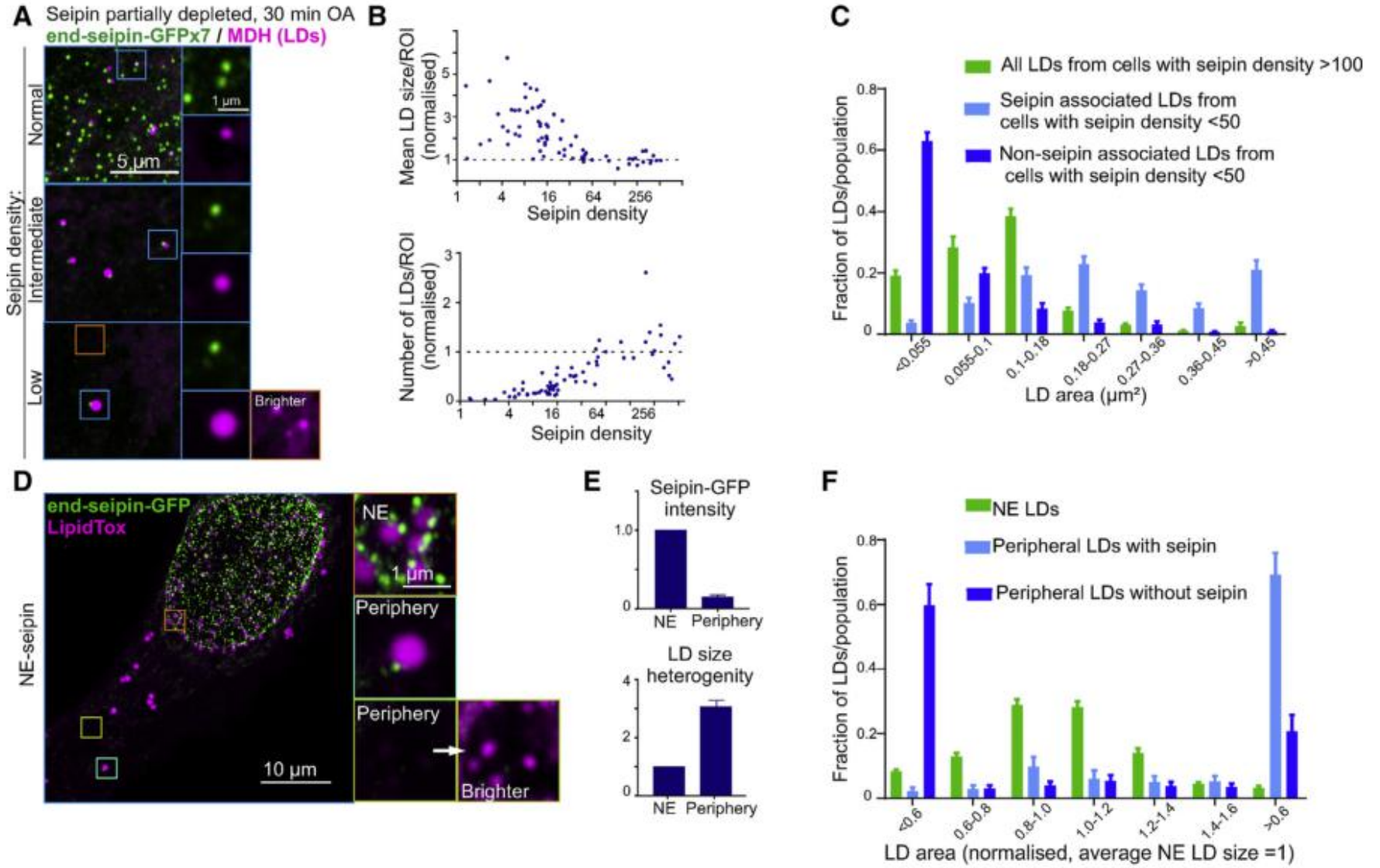

Figure 5. Seipin Functions Droplet Autonomously to Promote Triglyceride Delivery to LDs.

(A) End-seipin-GFPx7 cells were treated with seipin siRNAs for 3 days in delipidation conditions, last 18 h with DGATi, followed by 5 min washout of DGATi and 30-min OA loading. Cells were fixed, LDs stained, and imaged by Airyscan microscopy. Maximum intensity projections of z stacks.

(B) Analysis of (A). Seipin density: number of seipins/400 mm 2 ROI. LD data normalized to ROIs with seipin density >100. n = 73 ROIs, 2 experiments.

(C) Analysis of (A). Bars: mean ± SEM, n = 73 ROIs, 2 experiments.

(D) Seipin-NE-trapped cells were delipidated for 3 days, OA loaded for 2h, fixed, stained, and imaged with Airyscan microscopy. Maximum intensity projection of a z-stack from a seipin NE-trapped cell.

(E) Analysis of (D). Bars: mean ± SEM, n = 12 cells. LD size heterogeneity is the SD of LD sizes/population.

(F) Analysis of (D). Bars: mean ± SEM, n = 12 cells.