Expanded View Figures

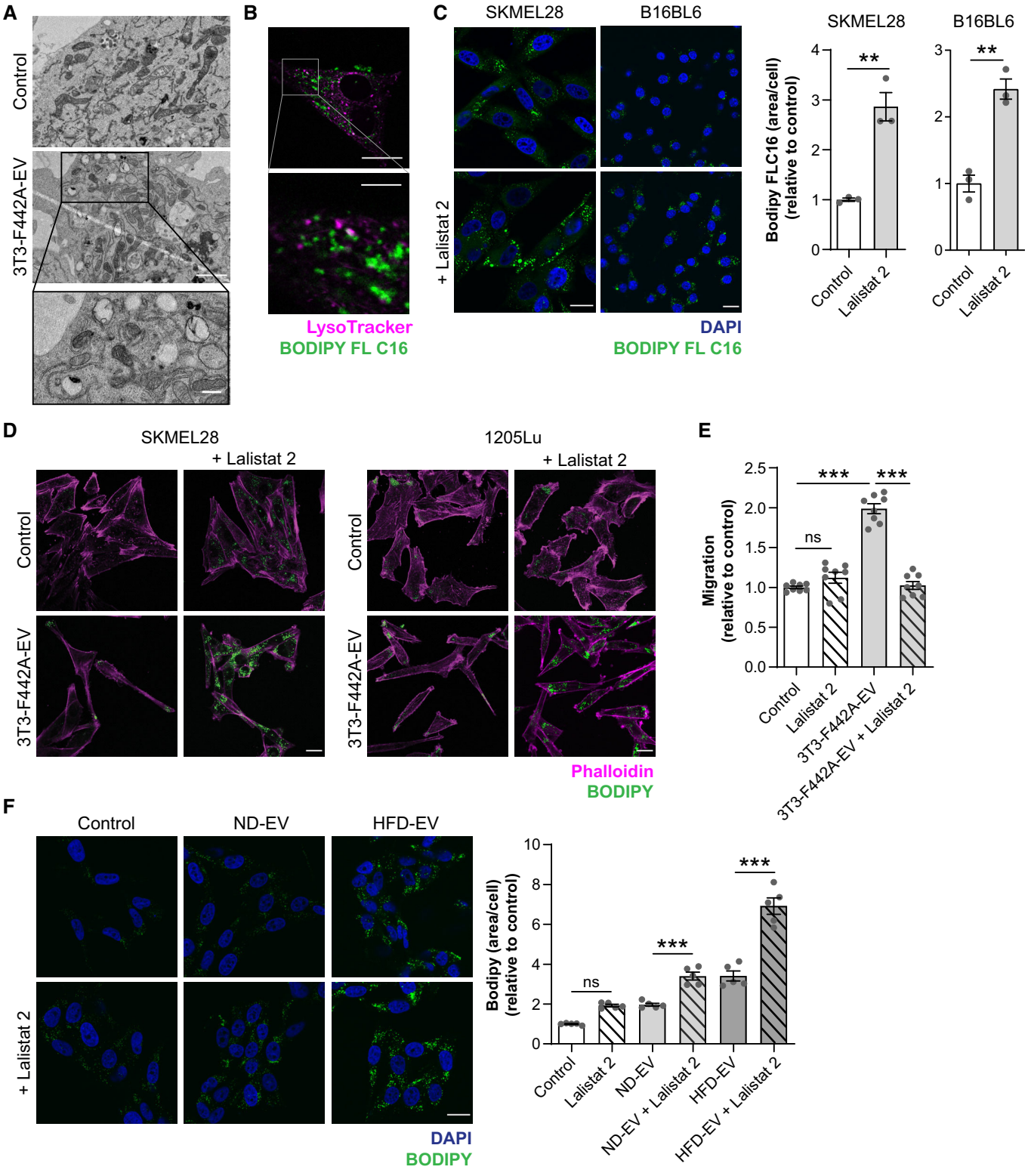

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Figure EV1. FA transferred from adipocytes to melanoma cells by EV are released from lipid droplets by lipophagy.

A Transmission electron micrographs of SKMEL28 cells exposed, or not, to 3T3-F442A EV. Scale bar: 1 μm. A zoomed crop of the area with autophagic structures containing lipids is shown. Scale bar: 0.5 μm.

B SKMEL28 cells were incubated with EV secreted by 3T3-F442A adipocytes previously loaded with BODIPY FL C16. Then, cells were washed and stained with the LysoTracker probe, and live cells were observed by confocal microscopy. A zoomed crop is shown below (scale bar: 5 μm).

C Indicated melanoma cells were exposed to 3T3-F442A EV and treated, or not, with Lalistat 2. Then, cells were fixed, stained with BODIPY, and counterstained with DAPI for confocal microscopy observation. Quantification of BODIPY FL C16 staining area per cell is shown on the right (n = 3).

D SKMEL28 and 1205Lu cells were exposed to 3T3-F442A EV in the presence, or not, of Lalistat 2. Cells were then fixed and stained with BODIPY and Phalloidin before observation by confocal microscopy.

E SKMEL28 cells were exposed to 3T3-F442A EV and treated, or not, with Lalistat 2. Cell migration was then evaluated in Boyden chamber assays (n = 8).

F SKMEL28 cells were exposed, or not, to adipocyte EV from lean (ND) or obese (HFD) mice with, or without, Lalistat 2. Cells were then fixed, stained with BODIPY, and counterstained with DAPI before observation by confocal microscopy. Quantification of BODIPY staining per cell is shown on the right (n = 5).

Data information: Unless indicated otherwise, on confocal images, scale bars represent 20 μm. Bars and error bars represent means ± SEM; statistically significant by Student's t-test (C) or by one-way ANOVA with *post hoc* Tukey's test (E and F), $^{**}P < 0.01$, $^{***}P < 0.001$, ns: non-significant.

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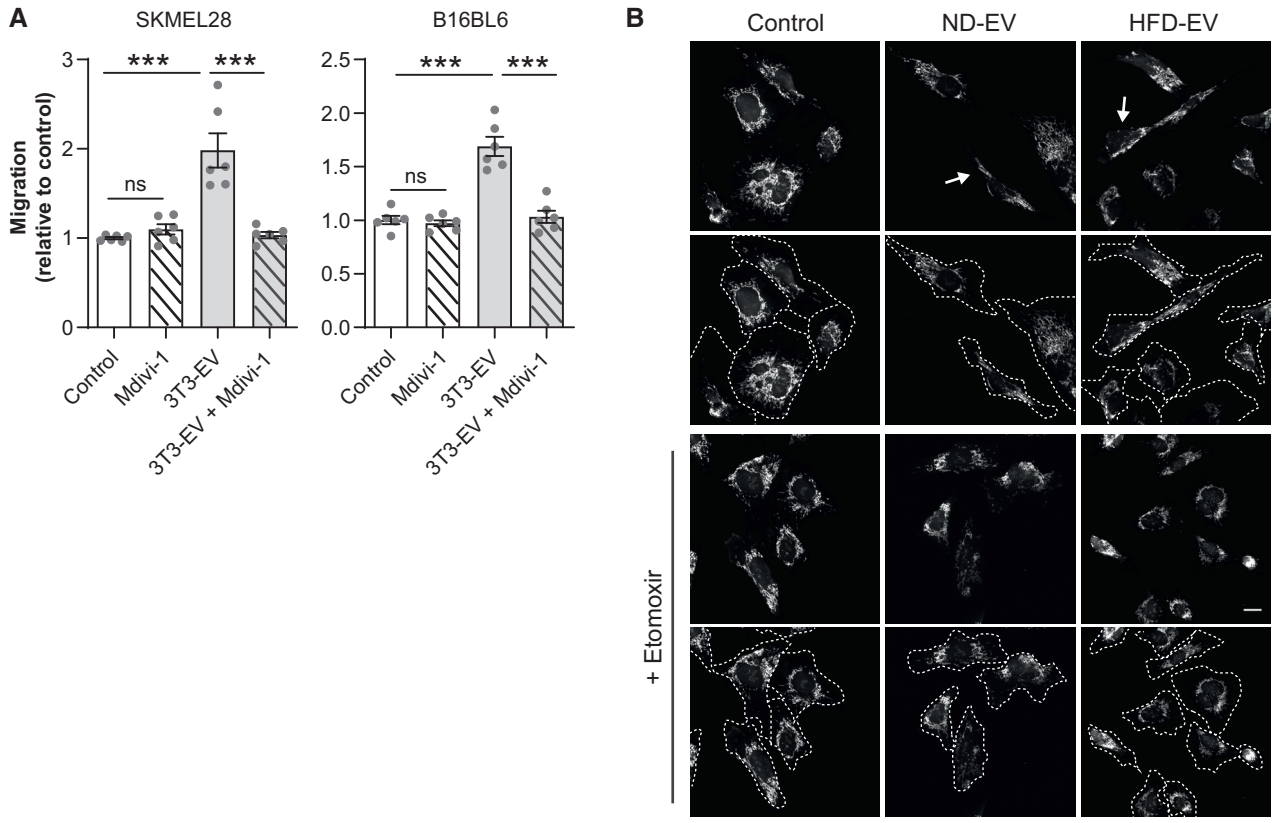

Figure EV2. Adipocyte EV modify melanoma cell mitochondrial dynamics, a process that is dependent on FAO and promotes aggressiveness.

A Human SKMEL28 and murine B16BL6 cells were exposed to 3T3-F442A EV (3T3-EV) and treated, or not, with Mdivi-1. Cell migration was then evaluated in Boyden chamber assays. Bars and error bars represent means ± SEM (n = 6); statistically significant determined by one-way ANOVA with *post hoc* Tukey's test, ***$P < 0.001$, ns: non-significant.

B 1205Lu cells were exposed to adipocyte EV from lean (ND) or obese (HFD) mice for 48 with, or without, Etomoxir for the last 24 h. Cells were then stained with a MitoTracker probe, fixed, and observed by confocal microscopy. Arrows indicate the presence of mitochondria in membrane protrusions. Underneath each original image, an image depicting the outline of cells in dotted lines is shown. Scale bar: 20 μm.

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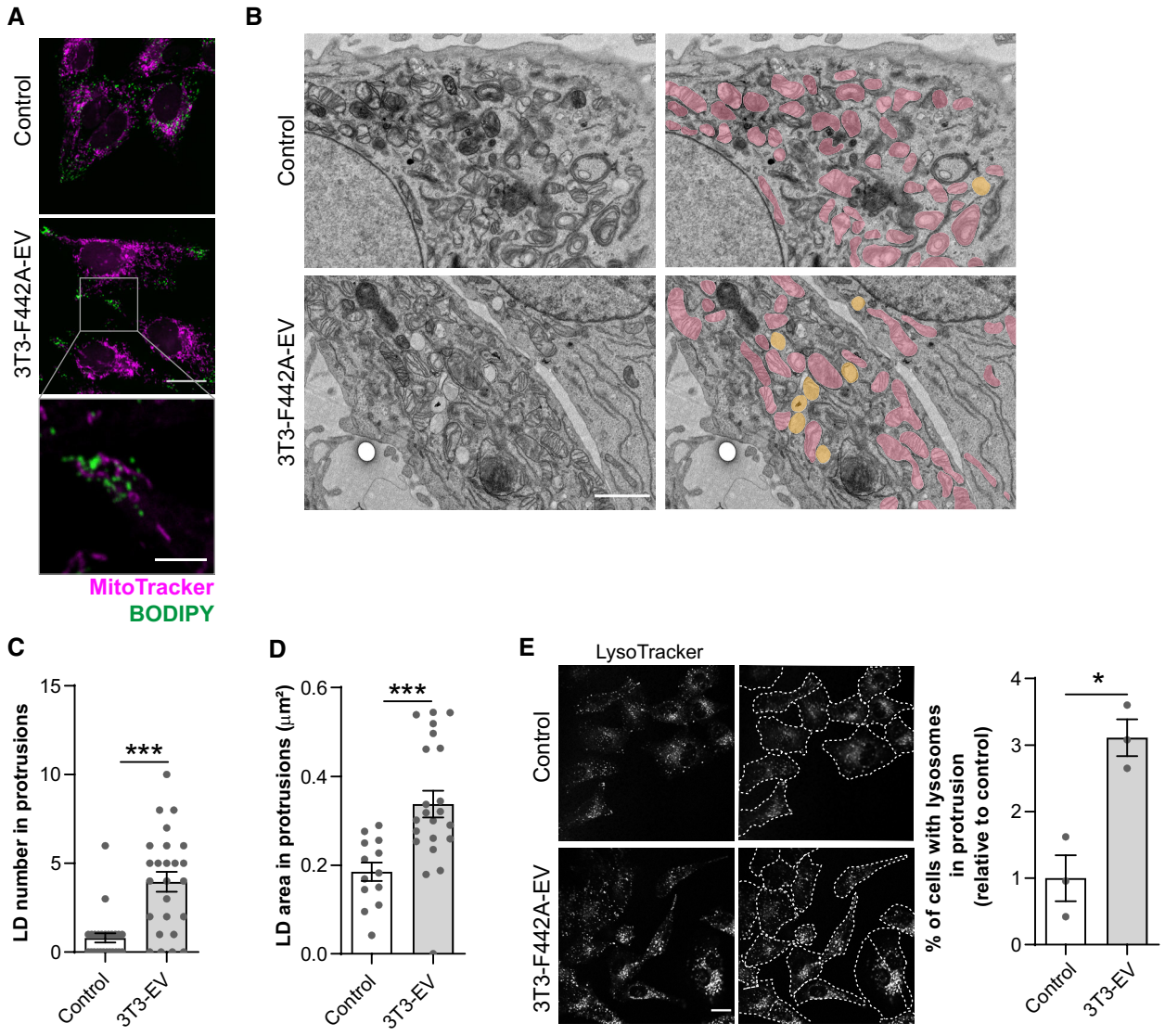

Figure EV3. Lipid droplets and lysosomes are found in membrane protrusions, at proximity to mitochondria, in melanoma SKMEL28 cells exposed to adipocyte EV.

A SKMEL28 cells exposed to 3T3-F442A EV were stained with a MitoTracker probe. Then, cells were fixed, stained with BODIPY, and observed by confocal microscopy. Scale bar: 20 µm. A zoomed crop of the area containing mitochondria and lipid droplets in a membrane protrusion is shown. Scale bar: 5 µm.

B Transmission electron microscope observations of SKMEL28 cells exposed, or not, to 3T3-F442A EV. Mitochondria are colored in pink, and lipid droplets are colored in yellow on images on the right. Scale bar: 1 µm.

C Number of lipid droplets (LD) found within membrane protrusions in transmission electron microscopy images of SKMEL28 cells exposed, or not, to 3T3-F442A EV (3T3-EV) (n = 25).

D Area of lipid droplets (LD) found within membrane protrusions in transmission electron microscopy images of SKMEL28 cells exposed, or not, to 3T3-F442A EV (3T3-EV) (n = 13 for control and n = 22 for 3T3-EV).

E Left panel, SKMEL28 melanoma cells were exposed, or not, to 3T3-F442A EV. Then, live cells were stained with LysoTracker and observed by confocal microscopy. Beside each original image, an image depicting the outline of cells in dotted lines is shown. Right panel, quantification of the percentage of cells presenting lysosomes within membrane protrusions (n = 3). Scale bar: 20 µm.

Data information: Bars and error bars represent means ± SEM; statistically significant by Student's t-test, *P < 0.05, ***P < 0.001, ns: non-significant.

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