APPENDIX

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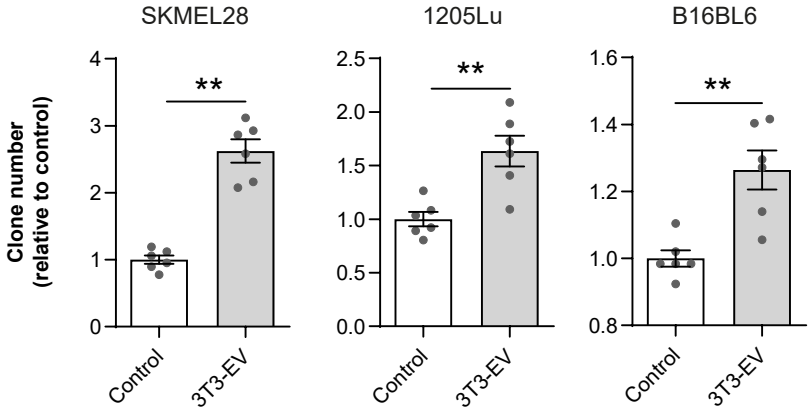
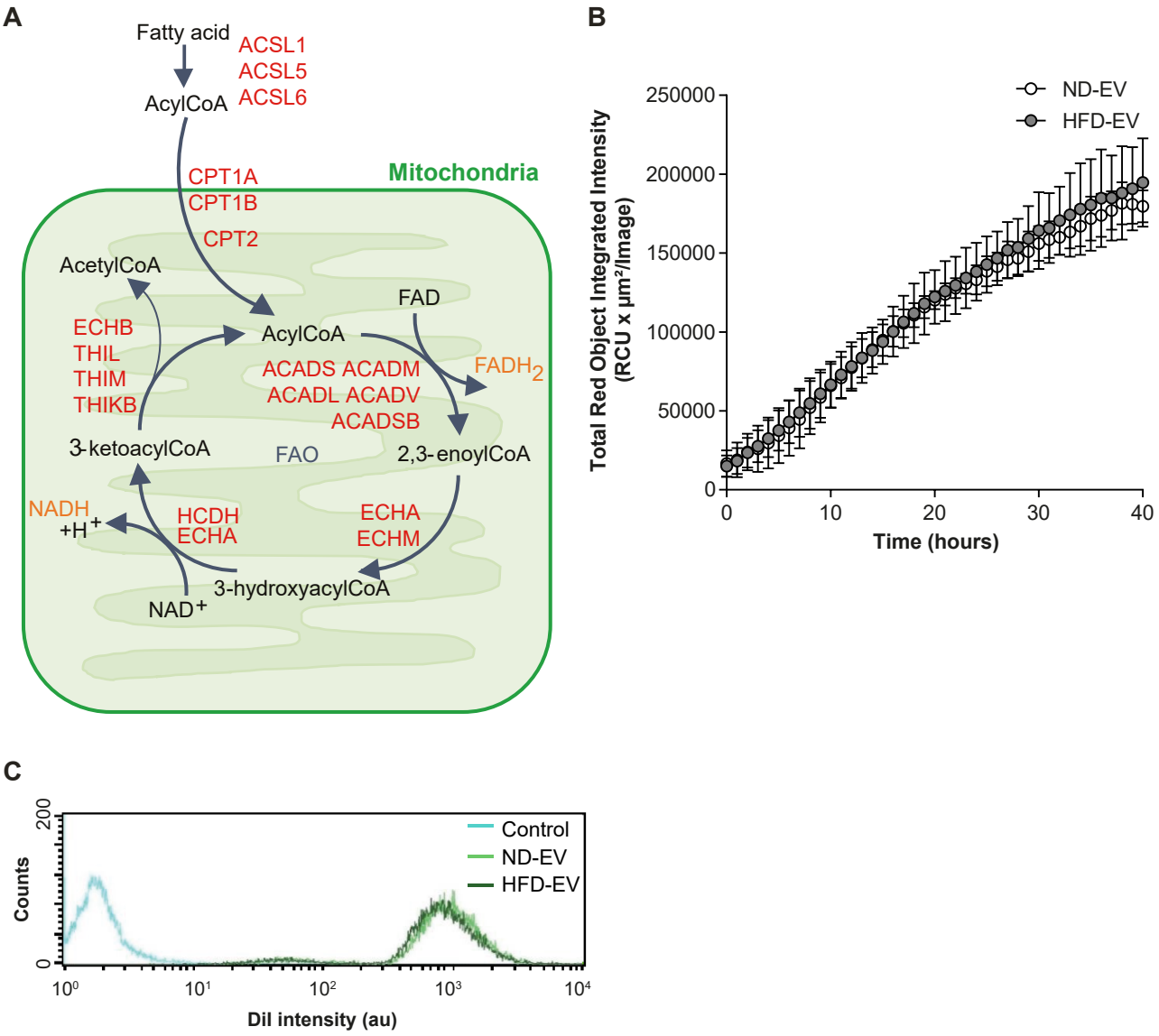


Appendix Figure S1: Adipocyte EV promote the clonogenic potential of melanoma cell lines
Colony formation assay with the indicated melanoma cell lines exposed, or not, to EV secreted by 3T3-F442A adipocytes (3T3-EV) (n=6). Bars and error flags represent means ± SEM; statistically significant by Student's t-test **P < 0.005.



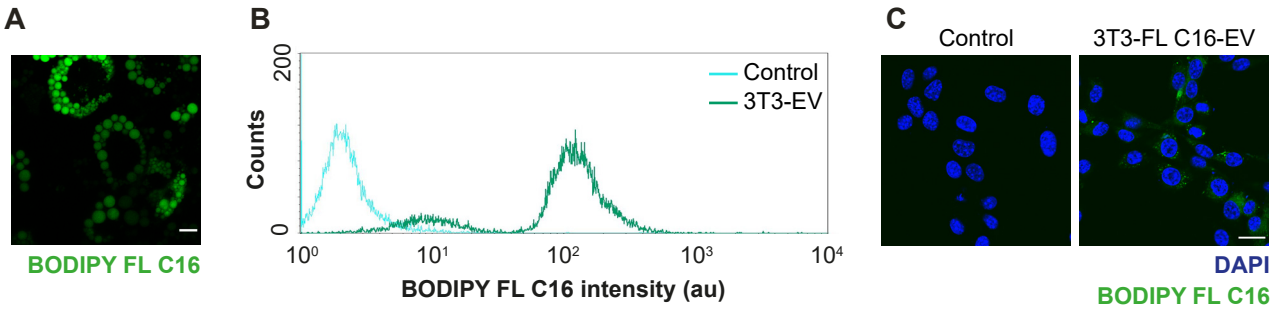
Appendix Figure S2: FAO proteins are not upregulated in obese adipocyte EV, and these EV are not preferentially taken up by melanoma cells

A) KEGG pathway analysis using DAVID v6.7 database (http://david.abcc.ncifcrf.gov/) of the proteins that are significantly neither up-, nor downregulated in EV from obese adipocytes revealed a strong association with the FA degradation pathway [p-value 7.4x10-9 and corrected (Benjamini-Hochberg) 1.7x10-9]. Such proteins identified in equal abundance in obese and lean adipocyte EV are indicated in red on the diagram.

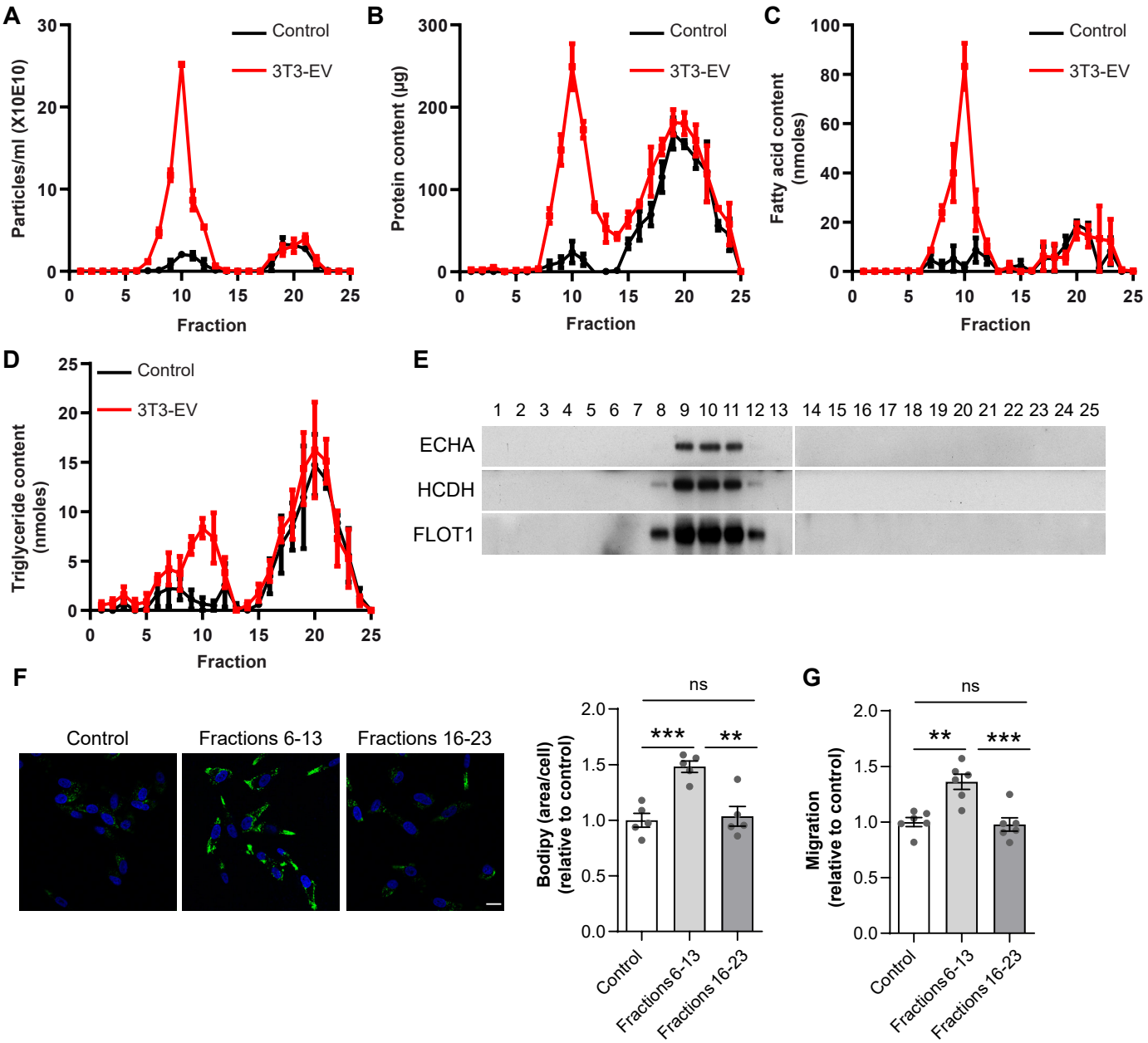
B-C) Adipocyte EV from lean (ND) and obese (HFD) mice were fluorescently labeled using the lipophilic DiI probe and added to SKMEL28 cells.

B) Fluorescent EV accumulation was evaluated using an Incucyte Zoom system. Bars and error flags represent means ± SEM.

C) After 48h, cells were harvested and fluorescence was evaluated by flow cytometry (a representative graph is shown). au: arbitrary units.



Appendix Figure S3: 3T3-F442A adipocytes accumulate fluorescent palmitate that is transferred to melanoma cells by EV
A) 3T3-F442A mature adipocytes were loaded with BODIPY FL C16 then fixed and observed by confocal microscopy. Scale bar: 20µm.
B) SKMEL28 cells exposed to EV secreted by 3T3-F442A adipocytes (3T3-EV) loaded with BODIPY FL C16 were analyzed by flow cytometry (a representative graph is shown). au: arbitrary units.
C) B16BL6 cells were exposed to EV secreted by 3T3-F442A adipocytes loaded with BODIPY FL C16 (3T3-FL C16-EV). Then, cells were fixed and counterstained with DAPI for confocal microscopy observation.
Scale bar: 20µm.



Appendix Figure S4: Only EV isolated from adipocyte conditioned medium using differential centrifugation transfer FA to melanoma cells and increase their migration

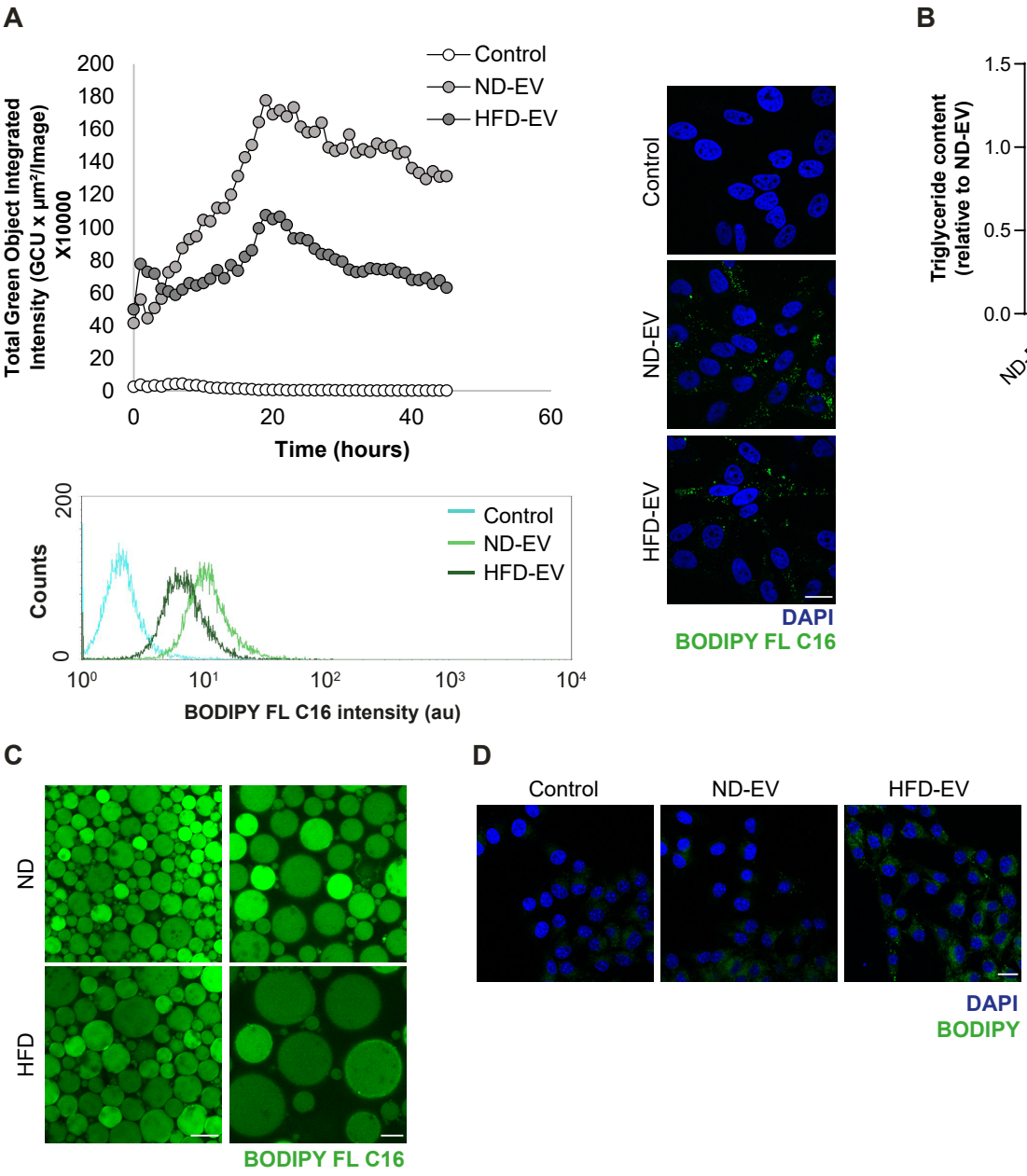
A to E) 3T3-F442A adipocyte conditioned medium was subjected to differential centrifugations as described in the material and methods section. The 100,000 g pellet (3T3-EV) was resuspended in 500µl of ultracentrifugated complete DMEM and fractioned using size exclusion chromatography into 25 fractions of 500µl. These fractions were analyzed to determine A) particle concentration and B) protein content. Then, an MTBE extraction was performed on each fraction and C) FA and D) triglyceride content were measured. In E), the presence of FAO enzymes was analyzed by western blot. FLOT1 was used as a control for EV fractions. Ultracentrifugated DMEM, processed in the same way, was used as a control. Bars and error flags represent means ± SEM; n=3.

F and G) 1205Lu melanoma cells were treated with pooled fractions 6-13 or 16-23 of 3T3-EV.

F) Cells were fixed and stained with BODIPY to assess neutral lipid stores and DAPI was used to counterstain nuclei. Left panel, images obtained using confocal microscopy. Right panel, quantification of BODIPY staining area (n=5).

G) Cell migration was evaluated in a Boyden chamber assay (n=6).

Bars and error flags represent means ± SEM; statistically significant by one-way ANOVA with post hoc Tukey's test, *P < 0.05, **P < 0.01, ***P < 0.001, ns: non-significant.



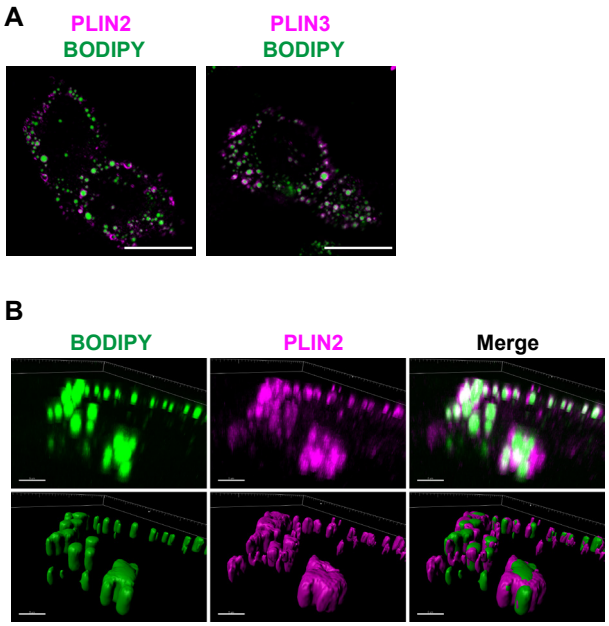
Appendix Figure S5: Primary adipocytes accumulate fluorescent palmitate that is transferred to melanoma cells by EV

A) SKMEL28 cells exposed to EV secreted by lean (ND) and obese (HFD) adipocytes previously loaded with BODIPY FL C16. Top left panel, cell fluorescence was assessed using an Incucyte Zoom. Bottom left and right panel, after 24h incubation, cells were harvested and fluorescence was analyzed by flow cytometry (a representative graph is shown) (left) or were fixed and counterstained with DAPI for confocal microscopy observation (right). Scale bar: 20µm. au: arbitrary units.

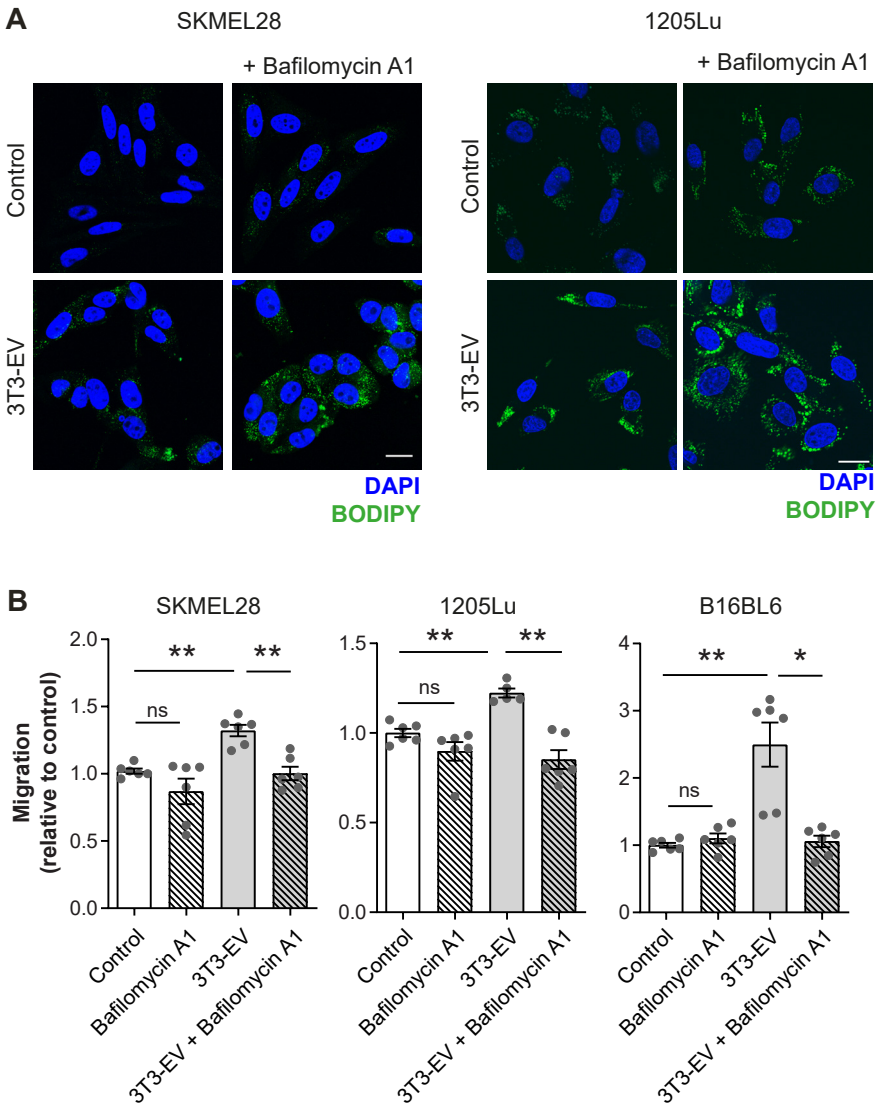
B) Lipids were extracted from EV secreted by adipocytes from lean (ND) and obese (HFD) mice (n=4) and triglyceride content was measured. Bars and error flags represent means ± SEM; statistically significant by unpaired Student's t-test, ns: non-significant.

C) Lean (ND) and obese (HFD) murine adipocytes were loaded with BODIPY FL C16 and observed using confocal microscopy. Scale bars: 100µm (left) or 40µm (right).

D) B16BL6 murine cells were exposed, or not, for 24h to primary adipocyte EV from lean mice fed a normal diet (ND) or obese mice fed a high fat diet (HFD). Then, cells were fixed, stained with BODIPY and counterstain with DAPI. Scale bar: 20µm.



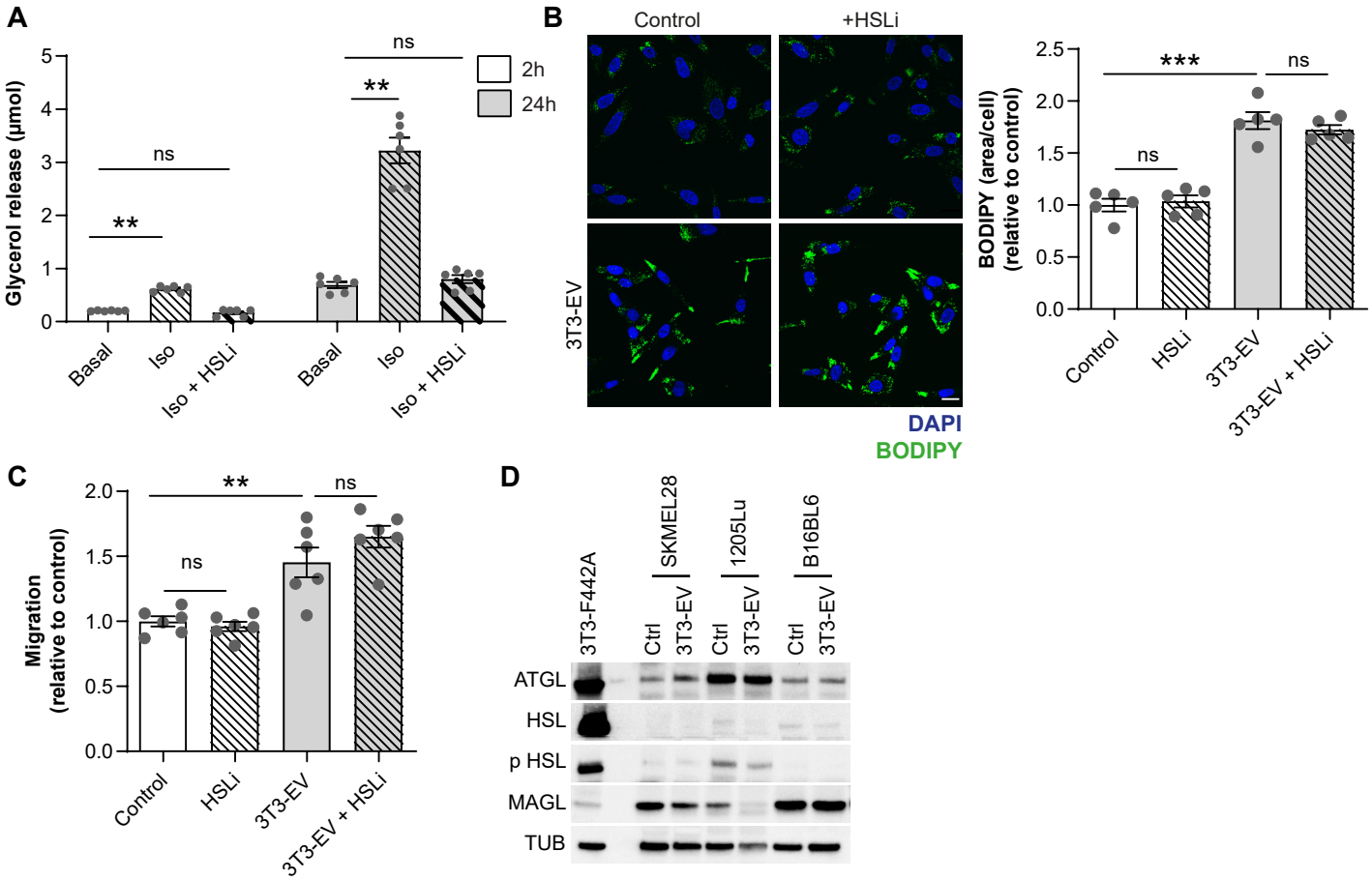
Appendix Figure S6: BODIPY-positive subcellular structures are lipid droplets
A) 1205Lu cells were exposed to primary adipocyte EV from obese mice for 24h. Then, cells were fixed, and immunofluorescence assays were performed using antibodies targeting PLIN2 (left panel) or PLIN3 (right panel). Then, cells were stained with BODIPY and by observed by confocal microscopy. Scale bar: 20µm.
B) A 3D reconstruction (upper panel), and corresponding iso-surface rendering (lower panel) showing that BODIPY positive droplets are covered by PLIN (here, PLIN2 is shown). Scale bar: 5µm.



Appendix Figure S7: Autophagy is required for lipid degradation and cell migration in response to adipocyte EV

A) SKMEL28 and 1205Lu cells were exposed to 3T3-F442A EV (3T3-EV) and treated, or not, with Bafilomycin A1. Then, cells were fixed, stained with BODIPY and counterstained with DAPI for observation by confocal microscopy. Scale bar: 20µm.

B) Indicated melanoma cells were exposed to 3T3-F442A EV (3T3-EV) and treated, or not, with Bafilomycin A1. Cell migration was then evaluated in Boyden chamber assays. Bars and error flags represent means ± SEM (n=6 except for 1205Lu treated with 3T3-EV: n=5); statistically significant by one-way ANOVA with post hoc Tukey's test, *P < 0.05, **P < 0.01, ns: non-significant.



Appendix Figure S8: Cytosolic lipases are not responsible for fatty acid mobilization in melanoma cells treated by adipocyte EV

A) 3T3-F442A adipocytes were treated with isoproterenol (Iso) for indicated times in the presence (HSLi) or absence (Iso) of HSL inhibitor (100µM). Then, lipolysis was evaluated by measuring glycerol release. Basal lipolysis (basal), in the absence of isoproterenol, was also measured. This result demonstrates the efficacy of HSLi (n=6).

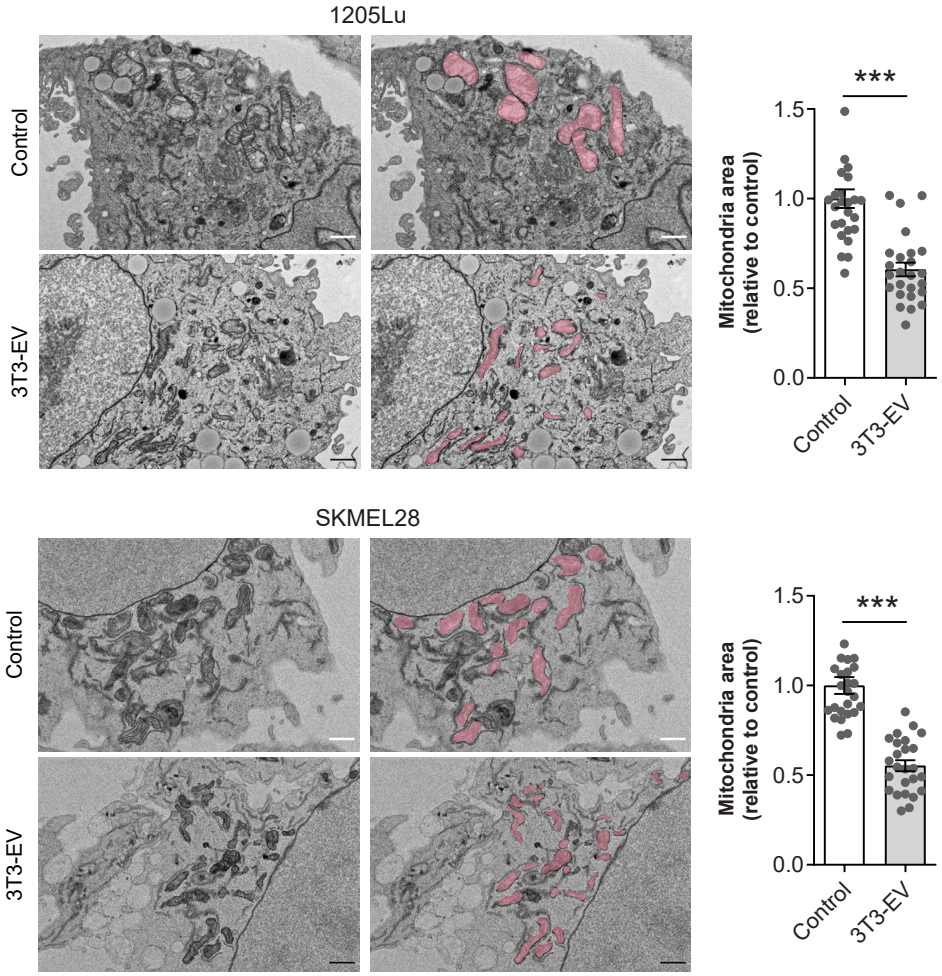
B-C) 1205Lu cells were exposed to 3T3-F442A EV (3T3-EV) treated, or not, with HSLi.

B) Then, cells were fixed, stained with BODIPY and counterstained with DAPI for observation by confocal microscopy. Scale bar: 20µm. Left panel, images obtained using confocal microscopy. Right panel, quantification of BODIPY staining area (n=5).

C) Cell migration was evaluated in a Boyden chamber assay (n=6).

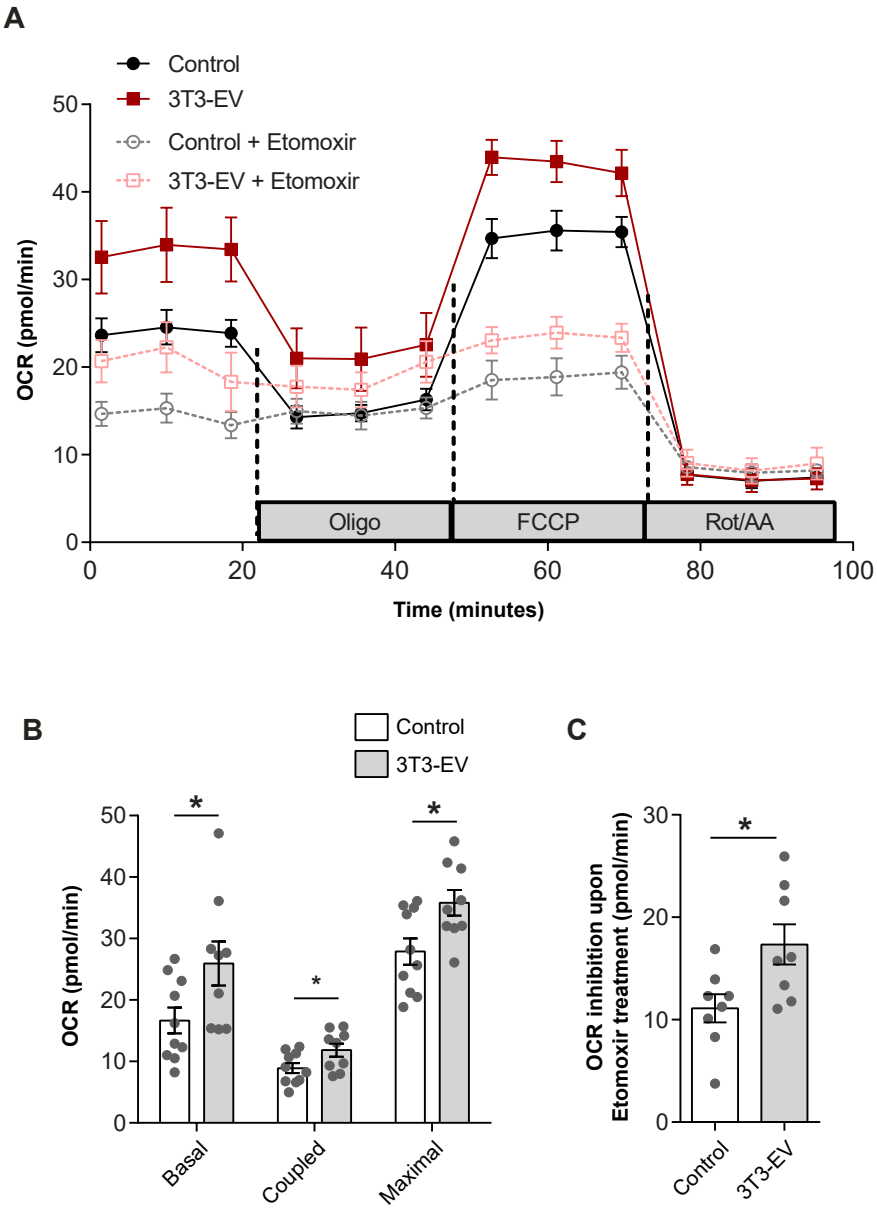
D) Western blot analysis of the indicated cytosolic lipases in melanoma cell lines exposed or not to 3T3-F442A EV (3T3-EV). Tubulin (TUB) is used as a loading control. 3T3-F442A adipocytes (3T3-F442A) are used as a positive control for lipase expression. pHSL: phosphoHSL; Ctrl: control; 3T3-EV: 3T3-F442A EV.

Bars and error flags represent means ± SEM; statistically significant by one-way ANOVA with post hoc Tukey's test (for A and B) or Kruskall-Wallis with post hoc Dunn's test (C), **P < 0.01, ***P < 0.001, ns: non-significant.



Appendix Figure S9: Mitochondria size is decreased in melanoma cells exposed to adipocyte EV

Left panels, transmission electron microscope observations of 1205Lu and SKMEL28 cells exposed, or not, to 3T3-F442A EV (3T3-EV). Scale bar: 1µm. Mitochondria are colored in pink on images on the right. Right panels, measurements of mitochondria area (n=25; except for 1205Lu control: n=26). Bars and error flags represent means ± SEM; statistically significant by one-way ANOVA with post hoc Tukey's test, ***P < 0.001.



Appendix Figure S10: Adipocyte EV promote FAO-dependent cell respiration in melanoma cells

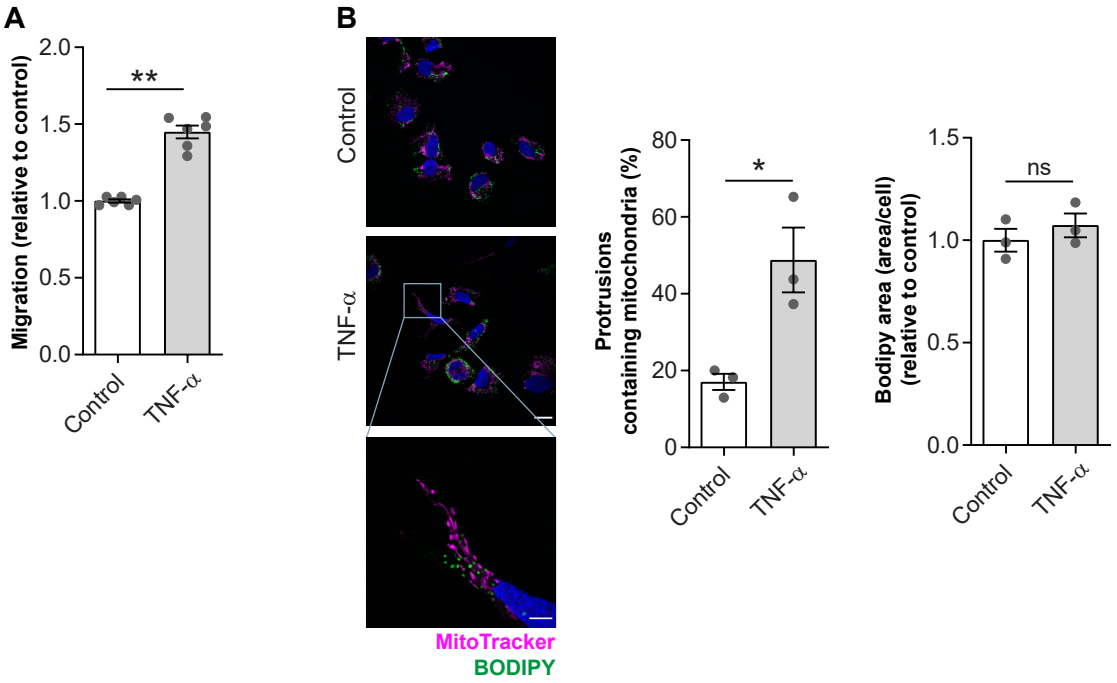
1205Lu cells were incubated with 3T3F442A adipocyte EV (3T3-EV) for 48h with, or without, Etomoxir for the last 24h. Mitochondrial respiration was evaluated by measuring the oxygen consumption rate (OCR) under basal conditions (assay medium with or without Etomoxir) and after sequential injection of oligomycin (Oligo), FCCP and rotenone/antimycin (Rot/AA).

A) Curve representation of data (n≥8).

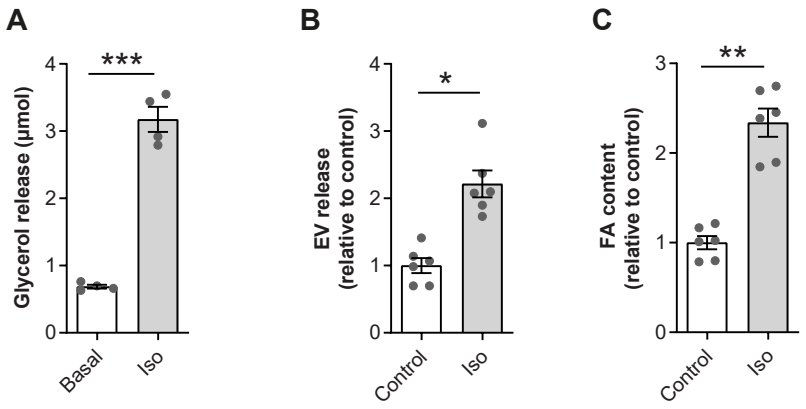
B) Bar representation of basal, coupled and maximal respiration (control: n=10; 3T3-EV: n=9), calculated as described in Appendix Supplementary Material and Methods.

C) Bar representation of the inhibition in OCR induced by Etomoxir (n=8), calculated as described in Appendix Supplementary Material and Methods.

Bars and error flags represent means ± SEM; statistically significant by unpaired Student's t-test, *P < 0.05.



Appendix Figure S11: TNF-α stimulates melanoma migration and mitochondrial redistribution but not lipid accumulation
1205Lu cells were treated with 100ng/mL of TNF-α for 48h.
A) Cell migration was then evaluated in Boyden chamber assays (n=6). Our results show that TNF-α promotes melanoma cell migration.
B) Left panel, cells were stained with a MitoTracker probe then fixed and stained with BODIPY and DAPI for observation by confocal microscopy. Scale bar: 20µm. A zoomed crop of a membrane protrusion is shown. Scale bar: 5µm. Middle panel, the number of protrusions containing mitochondria were counted (n=3). Right panel, quantification of BODIPY staining is shown (n=3).
Bars and error flags represent means ± SEM; statistically significant by unpaired Student's t-test, *P < 0.05, **P < 0.01, ns: non-significant.



Appendix Figure S12: Stimulated lipolysis promote adipocyte EV shedding and increases fatty acid content in EV
3T3-F442A adipocytes were treated, or not, with isoproterenol (Iso) for 24 h. Then, conditioned medium was recovered.
A) Lipolysis was evaluated by measuring glycerol release (n=4).
B) The number of EV present in conditioned medium was determined using nanoparticle tracking analysis (n=6).
C) EV were isolated, submitted to MTBE extraction and FA content was analyzed (n=6).
Bars and error flags represent means ± SEM; statistically significant by unpaired Student's t-test, *P < 0.05, **P < 0.01, ***P < 0.001.

Appendix Table S1: Antibodies used in this study

Antibody	Supplier	Reference	Assay	Dilution	Validation/citations
α-Tubulin (clone Ab-2)	THERMO FISCHER SCIENTIFIC	MS-581-P	WB	1/1000	More than 50 citations
ADFP (PLIN2)	abcam	ab108323	IF	1/1000	More than 10 citations
ATGL	Cell Signaling Technology	#2138	WB	1/1000	More than 50 citations
DNM1L (DRP1)	SIGMA Life Science	HPA039324	WB	1/1000	Enhanced validation
FIS1	SIGMA Life Science	HPA017430	WB	1/1000	Enhanced validation
FLOT1	SANTA CRUZ BIOTECHNOLOGY	sc-25506	WB	1/200	More than 50 citations
HADH (HCDH)	THERMO FISCHER SCIENTIFIC	PA5-31157	WB	1/1000	Advanced Verification
HADHA (ECHA)	THERMO FISCHER SCIENTIFIC	PA5-27348	WB	1/1000	Advanced Verification
HSL	Cell Signaling Technology	#4107	WB	1/1000	More than 50 citations
MAGL	SANTA CRUZ BIOTECHNOLOGY	sc-134789	WB	1/200	More than 10 citations
Phospho-HSL (Ser563)	Cell Signaling Technology	#4139	WB	1/1000	More than 50 citations
PLIN3	abcam	ab47639	IF	1/400	More than 10 citations

Appendix Supplementary Material and Methods:

Reagents and antibodies TNF-α was obtained from R&D system (Minneapolis, MN, USA), 2-(5,5-Dimethyl-1,3,2-dioxaborinan-2-yl) benzoic Acid Ethyl Ester (HSLi) from Santa Cruz Biotechnology (Dallas, TE, USA), Isoproterenol from Sigma Aldrich (Saint Louis, MO, USA). Bafilomycin A1 was purchased from Euromedex (Strasbourg, France). Seahorse XF assay medium containing 2 mM GlutaMAX and Seahorse XFe24 FluxPak were obtained from Agilent. Oligomycin, Rotenone, Antimycin, and FCCP (Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone) were obtained from Sigma Aldrich. Antibodies are described in Appendix Table S1.

Cell treatment For treatment of melanoma cells with EV in 6-well plates, for example, 1.10^5 melanoma cells were seeded and, 24h later, 5.10^{10} 3T3-F442A or primary adipocyte EV were added per well. Cells were incubated, or not, with EV for 24h for experiments to study lipid accumulation, 48h for experiments to study cell metabolism and mitochondrial dynamics and 72h for experiments to evaluate cell migration unless timepoints are specified otherwise. Where indicated, Bafilomycin A1 (5nM) was added 24h before the end of incubation with EV whereas HSLi (100µM in DMSO) was added from the beginning of EV treatment. Isoproterenol (10^{-6} M) and TNF-α (100 ng/mL) were added for indicated times.

Clonogenicity assay Cells were seeded in low concentrations (250 cells for 1205Lu and SKMEL28, and 200 cells for B16BL6) in 6 well plates in triplicate and treated, or not, with 3T3-F442A adipocyte EV. During this time, medium was refreshed twice a week, and contained or not fresh EV preparations. After 2 (B16BL6) or 3 weeks (1205Lu and SKMEL28), colonies were washed and fixed with methanol for 2min, then stained with 5% Toluidin blue (5% w/v) in Borax 0.1M (Sigma) for 5min to stain colonies**.** All colonies included in analysis consisted of at least 50 cells.

Stable isotope labeling with amino acids in cell culture (SILAC) After EV preparation as described in material and methods, 50µg of proteins were reduced with 25mM DTT in 40mM Tris-HCl pH6.8, 5% glycerol, 2% SDS for 15min at 65°C, alkylated by addition of 100mM chloroacetamide for 30min at room temperature in the dark and loaded on SDS-PAGE for a short run (0.5cm after entering the running phase of the gel). Proteins were stained using Coomassie Blue (Quick Coomassie Stain from Generon) and a single protein band was cut out and incubated three times on a shaker for 15min at 37°C in 100mM ammonium bicarbonate in 50% acetonitrile (ACN). The gel slices were then vacuum-dried and rehydrated in the presence of 1µg of modified sequencing-grade trypsin solution (ratio trypsin/protein 1/50) in 50mM ammonium bicarbonate (overnight at 37°C). The resulting peptides were extracted from the gel slices using two incubations (15min at 37°C) under shaking in 10% formic acid, ACN (1:1). The two successive extractions were pooled with the initial digestion supernatant, vacuum-dried. The peptidic fractions were resuspended in 50% ACN, 0.1% TFA, loaded on $ZipTip_{UC18}$

(Milipore) to avoid potential contaminant and eluted in 80% ACN, 0.1 TFA. Peptides were vacuum dried and re-suspended in 50µL of 2% ACN and 0.05% trifluoroacetic acid (TFA) to reach 1µg/µL.

Peptide mixtures were analyzed by nano-LC-MS/MS using an UltiMate 3000 RSLCnano system (Dionex, Amsterdam, The Netherlands) coupled to a Q-ExactivePlus mass spectrometer (ThermoScientific, Bremen, Germany) operating in positive mode. Two µg of each sample were loaded onto a C18 precolumn (300µm inner diameter x 5mm, Thermo Scientific) at 20µL/min in 2% ACN, 0.05% TFA. After 5min of desalting, the precolumn was switched on line with the analytical C18 column (75µm inner diameter × 50cm, in-house packed with Reprosil C18) equilibrated in 95% solvent A (5% ACN, 0.2% formic acid) and 5% solvent B (80% ACN, 0.2% formic acid). Peptides were eluted with two steps of solvent B gradient: a 5-25% gradient for 165min then a 25-50% gradient for 135min, at a flow rate of 300nL/min. The mass spectrometer was operated in data-dependent acquisition mode with Xcalibur software. MS survey scans were acquired in the Orbitrap on the 350–1500 *m/z* range, with the resolution set to 70,000. The 10 most intense ions per survey scan were selected for HCD fragmentation and resulting fragments were analyzed at a resolution of 17,500 in the Orbitrap. Dynamic exclusion was used within 30s to prevent repetitive selection of the same peptide. The Xcalibur raw files were processed with Proteome Discoverer software (version 2.1.1.21, Thermo Fischer Scientific) for database search with the Mascot search engine (version 2.6.0, Matrix Science, London, UK) combined with the Percolator algorithm (version 2.05) for peptide-spectrum matches (PSM) search optimization. The following parameters were set for creation of the peak list: parent ions in the mass range 350-5000 and no grouping of MS/MS scans. Peak list was searched against SP-mouse database released of 11th december 2018, 16979 entries. The search included methionine oxidation and protein N-terminal acetylation, SILAC labels Lys6 and Arg6 as variable modifications, and carbamidomethylation of cysteine as a fixed modification. Up to two missed trypsin/P cleavages were allowed. Mass tolerances in MS and MS/MS were set to 10 ppm and 25mmu, respectively. Mascot results were validated by the target-decoy approach using a reverse database at the same size. The Percolator algorithm was used to calculate a q-value for each peptide-spectrum matches (PSM), peptides and PSM were validated based on Percolator q-values at a False Discovery Rate (FDR) set to 1%.

Comparative proteomic analysis of EV from lean and obese adipocytes

After preparation of EV proteins, 30µg of denaturated proteins were processed as described for the SILAC experiment. Then, peptides were analyzed by nano-LC-MS/MS using an UltiMate 3000 RSLCnano system (Dionex, Amsterdam, The Netherlands) coupled to an Orbitrap Velos Pro mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Five µg of each sample was loaded on a C18 precolumn (300µm inner diameter × 5mm, Thermo Scientific) in a solvent made of 2% ACN and 0.05% TFA, at a flow rate of 20µL/min. After 5min of desalting, the precolumn was switched online with the analytical C18 column (75µm inner diameter × 50cm, in-house packed with Reprosil C18) equilibrated in 95% solvent A (5% acetonitrile, 0.2% formic acid) and 5% solvent B (80% acetonitrile, 0.2% formic acid). Peptides were eluted using first a 5–25% gradient of solvent B for 75min, then a 25%-50% gradient for 30min, at a flow rate of 300nL/min. The LTQ Orbitrap Velos was operated in data-dependent acquisition mode with Xcalibur software. MS survey scans were acquired in

the Orbitrap on the 350–1800 *m/z* range, with the resolution set to 60,000. The 20 most intense ions survey scans were selected for fragmentation by collision-induced dissociation (CID), and the resulting fragments were analyzed in the linear trap. Dynamic exclusion was used within 60s to prevent repetitive selection of the same peptide.

For protein identification and quantification, raw MS files converted to the mzdb format and were processed with the mzdb-access library (https://github.com/mzdb) to generate peaklists. Data were searched with Mascot (version 2.6.1) against SP-mouse database released of 11th december 2018, 16979 entries. The search included methionine oxidation and protein N-terminal acetylation as a variable modification, and carbamidomethylation of cysteine as a fixed modification. Specificity of digestion was set for cleavage after lysine or arginine for trypsin-digested samples and two missed cleavages were allowed. The mass tolerance was set to 5 ppm for the precursor ion. It was set to 0.8 Da for fragment ions in CID mode. Validation and quantification of identifications were performed through a false-discovery rate set to 1% at protein and peptide-sequence match level, determined by target-decoy search using the in-house-developed software Proline 1.6 (http://proline.profiproteomics.fr/). Missing protein intensity values were replaced by a constant value calculated independently for each sample as the 5-percentile value of the protein population. A Welch t-test (two-tailed t-test, unequal variances) was then performed on log2 transformed values for each protein The thresholds for the significant proteins were set to 0.05 for the p-value and 2 for the fold change. The mass spectrometry proteomics data will be deposited to the ProteomeXchange Consortium via the PRIDE partner repositor.

EV labeling for uptake analysis EV were fluorescently labeled using DiI as previously described (Lazar et al, 2015). EV uptake by melanoma cells was analyzed by following cell fluorescence over time using the Incucyte Zoom System (Essen Bioscience, Hertfordshire, UK) or by analyzing cell fluorescence at the end of the incubation time by flow cytometry using a FACScan flow cytometer (Beckton Dickinson, Rutherford, NJ). The protocols are given below.

FA transfer by primary adipocyte EV Primary adipocytes from lean or obese animals were washed with prewarmed PBS with 0.1% BSA, then incubated with BODIPY FL C16 (used at 5µM) in DMEM with 2% FCS, 0.1% BSA and 50nM insulin for 4h. Then, adipocytes were washed twice with prewarmed PBS with 0.1% BSA and resuspended in ultracentrifugated DMEM with 50nM insulin overnight before collecting conditioned medium. EV were isolated from the medium as described in material and methods.

Incucyte analysis For incucyte analysis, the 10X objective was used and 9 planes of view were collected per well for experiments using 24-well plates or 3 planes of view for 96-well plates. Phase contrast, green channel (Ex: 440/80 nm; Em: 504/44 nm) and/or red channel (Ex: 565/05 nm; Em: 625/05 nm) were collected depending on the experiment in question.

Flow cytometry analysis After the indicated staining, cells were washed in PBS, harvested by trypsination, then resuspended in PBS, and immediately analyzed for fluorescence intensity by FACScan flow cytometer (Beckton Dickinson, Rutherford, NJ). Mean fluorescence intensity was quantified by CellQuest software (Becton Dickinson).

Size exclusion chromatography (SEC) EV were isolated from 180ml of 3T3-F442A conditioned medium as described in the material and methods section. The 100,000 g pellet was resuspended in 500µL of ultracentrifugated complete DMEM and fractioned using SEC into 25 fractions of 500µL as described in (Boing et al, 2014), except that Poly-Prep Chromatography columns were used. Protein concentration was determined using the DC™ Protein Assay from Biorad and particle concentration evaluated using Nanoparticle Tracking Analysis (NTA), as described in material and methods. Then, each fraction was concentrated using Amicon Ultra-0.5 Centrifugal Filter Unit 3KDa (Merck, Darmstadt, Germany) and submitted to MTBE extraction. Then, lipid and WB analyses were performed as described in material and methods or below (for triglycerides). For functional experiments, fractions 6-13 or 16-23 were pooled and concentrated (Amicon Ultra-4 Centrifugal Filter Unit 3KDa) before use.

Triglyceride extraction and dosage Lipid extraction was performed as described for FA. Triglycerides (contained in 10µL of buffer) were dosed using a colorimetric kit (Sigma).

PLIN staining Cells were seeded on glass coverslips and treated with HFD-EV. After 24h, cells were fixed with 3.7% paraformaldehyde for 15min at and permeabilized with 0.1% Triton X-100 for 5min. After blocking with 3% BSA in PBS for 45min, cells were incubated for 2h with the primary antibody directed against PLIN2 or PLIN3. After three washes in PBS, cells were incubated with the secondary antibody coupled to Alexa-594 from Invitrogen used at 1/1000 for 1h. Then, after 3 washes in PBS, BODIPY staining was performed before mounting slides with Vectashield mounting medium (Vector Laboratories, CA, USA). All steps are performed at room temperature. Z stack images were taken using a confocal microscope (Olympus, FV1000) with a 60X objective. Reconstruction of 3D images and iso surface rendering were performed with Imaris software (Bitplane).

Transmission electron microscopy Specimens were prepared as previously described (Lazar et al, 2015). Grids were examined with a transmission electron microscope (Jeol JEM-1400, Akishima, Tokyo, Japan) at 80kV. Mitochondria area was measured on these images using ImageJ software.

Measurement of oxygen consumption rate (OCR) OCR was measured using the XFe24 analyzer (Seahorse Bioscience). Five thousand cells were seeded in 24-well XFe24 cell culture plates and 24h later EV were added. Another 24h after, Etomoxir was added for the final 24h of incubation. At the end of the incubation time, medium was replaced with seahorse XF assay medium (Agilent) containing 2 mM GlutaMAX at pH 7.4,

supplemented with 2.5mM glucose and 0.5mM carnitine, and cells were placed at 37°C for 1h without CO_2. OCR was measured in the basal state (assay medium alone) and after consecutive treatment with: (i) 5 µmol/L oligomycin, (ii) 1µmol/L FCCP, and (iii) 5µmol/L rotenone/antimycin A. Three measurements were recorded after each injection, with each measurement consisting of 3min of mixing and 3min of measurement after a 2min waiting period. Results were normalized to cell confluence measured using Incucyte apparatus. To evaluate the proportion of coupled respiration, OCR in the presence of oligomycin was subtracted from basal OCR. Maximal respiration was evaluated after injection of FCCP. Etomoxir inhibition of OCR was calculated by subtracting Basal OCR with Etomoxir from basal OCR without Etomoxir.

Isoproterenol-induced lipolysis in adipocytes 3T3-F442A cells were treated with isoproterenol for the indicated times. Then, lipolysis was evaluated by free glycerol measurement in conditioned media using the Free Glycerol Reagent (Sigma).

References:

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