## **Supplementary Materials and Methods**

## Reagents and antibodies

DMEM and FCS were purchased from GIBCO-Thermo Fisher Scientific. Etomoxir, trimetazidine, BSA and insulin were obtained from Sigma Aldrich. Matrigel was purchased from Corning. Bodipy® 493/503, DAPI and Alexa Fluor® 488 Phalloidin were obtained from Molecular Probes-Thermo Fisher Scientific. The Lowry biochemical assay kit was attained from Biorad. [1-14C]palmitate and [U-14C]D-glucose were obtained from Perkinelmer. The Lactate FS kit was purchased from Diagnostic System. For Nano-LC MS/MS analysis, modified sequencing grade trypsin was obtained from Promega and C18 columns were purchased from Dionex. Antibodies were obtained from the following sources: ALIX, TSG101 and FLOT1 (Santa Cruz Biotechnology, used at 1/200), ECHA and HCDH (Invitrogen-Thermo Fisher Scientific, used at 1/1000).

## Cell lines

All melanoma cells (except A375M and 1205Lu) are established lines derived from resected human melanomas. 501mel, Lyse, Daju and SKMEL28 cell lines originated from metastatic lymphonodal lesions (respectively ([1](#_ENREF_1)); ([2](#_ENREF_2)); ([2](#_ENREF_2)); ([3](#_ENREF_3))). A375M were obtained by clonal selection of the human metastatic cell line A375 ([4](#_ENREF_4)). 1205Lu were derived from the vertical growth phase (VGP) primary lesion-derived cell line WM793 by serial passage through athymic mice and selection of cells from lung metastases ([5](#_ENREF_5)). All were provided by Dr Lionel Larue (Institut Curie, Orsay, France). The human prostate tumor cell line PC-3 (provided by Dr Olivier Cuvillier, IPBS, Toulouse, France) is derived from human prostate carcinoma bone metastases ([6](#_ENREF_6)).

The murine 3T3-F442A preadipocyte cell line (obtained from the European Collection of Cell Cultures) was grown and differentiated into mature adipocytes as follows. 9.104 cells were seeded in 6-well plates and 3 days later, once cells had reached confluence, differentiation was induced by supplementing medium with 50nM insulin for 14 days. The term “adipocyte” refers to mature cells that were differentiated for at least 14 days.

All cell lines were cultured in DMEM supplemented with 10% FCS (Fetal Calf Serum), 125mg/mL streptomycin, and 125UI/mL penicillin and maintained at 37oC in a humidified atmosphere with 5% CO2. Cells were used within 2 months after resuscitation of frozen aliquots and regularly tested for mycoplasma contamination.

## Migration and invasion assays

One hundred thousand cells were plated in 6-well plates before adding exosomes for 48h incubation. After serum depletion for 12h, equal numbers of cells (1.5.105 for SKMEL28, 501mel, Lyse and A375M cells and 1.105 for 1205Lu and PC-3 cells) in serum-free DMEM were added to the upper compartments of transwell chambers (ThinCerts®, 12 wells, 8µm pores, Greiner Bio-One). DMEM supplemented with 10% FCS was used as a chemoattractant, and cells were then incubated at 37°C for 6h, 12h and 24h for 1205Lu, PC-3 and other melanoma cell lines respectively. Migrating cells were evaluated as previously described ([7](#_ENREF_7)). For invasion assays, Matrigel was added to Boyden chambers prior to tumor cell seeding as described elsewhere ([8](#_ENREF_8)).

## Bodipy staining

3T3-F442A preadipocytes were seeded on glass coverslips in 6-well plates (9.104 cells per well) and at different stages of differentiation, lipid content was assessed by bodipy staining. For this, cells were fixed with 3.7% paraformaldehyde for 15min at room temperature and permeabilized with 0.2% Triton X-100 during 5 min and blocking with 10% FCS and 2% BSA in PBS for 30min. Then, cells were incubated with the bodipy lipid probe for 15min (used at 1μg/ml) and DAPI for 5min (used at 1μM).

## Cell cycle analysis

Cells were incubated or not with ad-exos for 48h. Cells were then harvested, washed in PBS and treated with 25µg/ml of propidium iodide, 0.1% W/V of trisodium citrate dehydrate, 10% V/V of RNase (with 1mg/mL RNase A, 0.5mM EDTA), 0.1% V/V Triton X-100 for 30 minutes at 4°C. Cell cycle was then assessed by flow cytometry using a FACScan flow cytometer (Becton Dickinson, Franklin Lanes, NJ).

Nano-LC MS/MS analysis

50µg of proteins were reduced with 25mM DTT in 25mM Tris-HCl pH6.8, 5% glycerol, 1% SDS for 30min at 56°C, alkylated by addition of 90mM iodoacetamide 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 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 with 400ng of modified sequencing-grade trypsin solution in 50mM ammonium bicarbonate (overnight at 37°C). The resulting peptides were extracted from the gel slices by three incubations (15min at 37°C) under shaking: a first incubation in 50mM ammonium bicarbonate and two subsequent incubations in 10% formic acid, ACN (1:1). The three successive extractions were pooled with the initial digestion supernatant, vacuum-dried in a Speed-Vacuum and re-suspended in 50µL of 2% ACN, 0.05% trifluoroacetic acid (TFA).

Peptide mixtures were analyzed by nano-LC-MS/MS using an Ultimate3000 system (Thermo Scientific Dionex, Sunnyvale, CA, USA) coupled to an LTQ-Orbitrap Velos mass spectrometer (ThermoFisher Scientific, Bremen, Germany) operating in positive mode. Five microliters of each sample were loaded onto a C18 precolumn (300µm inner diameter x 5mm) 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 x 15cm; PepMap 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 by using a 5-25% gradient of solvent B for 75min then a 25-50% gradient of solvent B for 40min at a flow rate of 300nL/min. The LTQ-Orbitrap Velos was operated in data-dependent acquisition mode with the Xcalibur software (version 2.0 SR2, Thermo Fisher Scientific). Survey scan MS spectra were acquired in the Orbitrap on the 300–2000 m/z (mass to charge ratio) range with the resolution set to a value of 60 000 at m/z 400. Up to ten of the most intense multiply charged ions (2+ and 3+) per survey scan were selected for CID fragmentation, and the resulting fragments were analyzed in the linear trap (LTQ). Dynamic exclusion was used within 60s to prevent repetitive selection of the same peptide.

## Database search and data analysis

The Mascot Daemon software (version 2.3.2, Matrix Science, London, UK) was used to perform database searches in batch mode. The following parameters were set for creation of the peak lists: parent ions in the mass range 400-4500, no grouping of MS/MS scans and threshold at 1000. A peak list was created and Mascot searches were performed. Data were searched against *Mus musculus* entries in the SwissProt database. Cysteine carbamidomethylation was set as a fixed modification; methionine oxidation and protein N-terminal acetylation were set as variable modifications. Other post-translational modifications have not been taken into account. Two missed trypsin cleavage sites were allowed. The mass tolerances in MS and MS/MS were set to respectively 5ppm and 0.6Da and the instrument setting was specified as ‘ESI-Trap’. To calculate the False Discovery Rate (FDR), the search was performed using the ‘decoy’ option in Mascot. Mascot results were analyzed with the in-house developed software Mascot File Parsing and Quantification (MFPaQ) version 4.0.0 (<http://mfpaq.sourceforge.net>) ([9](#_ENREF_9)). Peptide identifications extracted from Mascot result files were validated at a final peptide FDR<1%. FDR was calculated at the protein level: FDR = number of validated decoy hits/(number of validated target hits + number of validated decoy hits) x100. Unambiguous protein identification is provided by a proteotypic peptide, i.e. a peptide with a unique amino acid sequence among all proteins. Five biological replicates were analyzed in order to minimize potential experimental deviations. Only proteins identified in at least two independent experiments with an FDR<0.1% or three experiments with an FDR<1% were validated.

The identified proteins were grouped according to their biological function through literature investigation and function analysis using by DAVID (Database for Annotation, Visualization and Integrated Discovery) v6.7 database <http://david.abcc.ncifcrf.gov/> and UniProt <http://www.uniprot.org/>. To analyze ad-exos protein specificity, we used exocarta database ([10](#_ENREF_10)).

*Lactate dosage*

Lactate released in the medium was measured using the Lactate FS kit according to supplier’s instructions in cells treated or not with ad-exos for 48h. TruCal U was used as calibrator.

*RNA extraction and RT-q-PCR*

Total RNA was extracted from cells using the RNeasyExtraction Kit (Qiagen GmbH, Hilden, Germany). Each RNA sample wasreverse transcribed with the Superscript III First Strand Synthesis Kit (Invitrogen, Auckland, NZ) starting from 1µg of total RNA. Quantitative real-time PCR was performed on an ABIPrism 7000 (Applied Biosystems, Foster City, CA) using GAPDH and HPRT as internal standards to control variabilityin cDNA quantity. Primer sequences are available on request. Each 20μl reaction consisted of 5ng of cDNA, 1X SYBR Green TaqMan Universal PCR master mix (Applied Biosystems, Foster City, CA) and 500nM of primers. The amount of the target transcript was related to that of the reference genes by the Ct method. The cDNA samples were assayed in triplicate in three independent experiments. The changes in expression of the indicated genes were expressed relatively to the control (untreated cells).

## Mice diets

C57BL/6J mice (Janvier (Le Genest St Isle, France)) were assigned to a normal diet (ND) (PicoLab Rodent Diet 20, Purina Mills Inc., Brentwood, MO, USA) or high-fat diet (HFD) (Research Diets Inc., New Brunswick, New Jersey, USA). The energy contents of the diets were as follows: 16% protein, 80% carbohydrate, and 4% fat for the ND; and 20% protein, 20% carbohydrate, and 60% fat for the HFD. Mice (initially 8 weeks old) were fed a ND or a HFD for 15 weeks, after which average weight of ND mice was 29.9g (+/- 1.19g) and of HFD mice was 49.9g (+/-3.1g).

1. Topalian SL, Solomon D, Rosenberg SA. Tumor-specific cytolysis by lymphocytes infiltrating human melanomas. J Immunol. 1989;142:3714-25.

2. Dufour E, Carcelain G, Gaudin C, Flament C, Avril MF, Faure F. Diversity of the cytotoxic melanoma-specific immune response: some CTL clones recognize autologous fresh tumor cells and not tumor cell lines. J Immunol. 1997;158:3787-95.

3. Carey TE, Takahashi T, Resnick LA, Oettgen HF, Old LJ. Cell surface antigens of human malignant melanoma: mixed hemadsorption assays for humoral immunity to cultured autologous melanoma cells. Proceedings of the National Academy of Sciences of the United States of America. 1976;73:3278-82.

4. Kozlowski JM, Hart IR, Fidler IJ, Hanna N. A human melanoma line heterogeneous with respect to metastatic capacity in athymic nude mice. Journal of the National Cancer Institute. 1984;72:913-7.

5. Kath R, Jambrosic JA, Holland L, Rodeck U, Herlyn M. Development of invasive and growth factor-independent cell variants from primary human melanomas. Cancer research. 1991;51:2205-11.

6. Kaighn ME, Narayan KS, Ohnuki Y, Lechner JF, Jones LW. Establishment and characterization of a human prostatic carcinoma cell line (PC-3). Investigative urology. 1979;17:16-23.

7. Lazar I, Clement E, Ducoux-Petit M, Denat L, Soldan V, Dauvillier S, et al. Proteome characterization of melanoma exosomes reveals a specific signature for metastatic cell lines. Pigment cell & melanoma research. 2015;28:464-75.

8. Dirat B, Bochet L, Dabek M, Daviaud D, Dauvillier S, Majed B, et al. Cancer-associated adipocytes exhibit an activated phenotype and contribute to breast cancer invasion. Cancer research. 2011;71:2455-65.

9. Bouyssie D, Gonzalez de Peredo A, Mouton E, Albigot R, Roussel L, Ortega N, et al. Mascot file parsing and quantification (MFPaQ), a new software to parse, validate, and quantify proteomics data generated by ICAT and SILAC mass spectrometric analyses: application to the proteomics study of membrane proteins from primary human endothelial cells. Molecular & cellular proteomics : MCP. 2007;6:1621-37.

10. Mathivanan S, Simpson RJ. ExoCarta: A compendium of exosomal proteins and RNA. Proteomics. 2009;9:4997-5000.