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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☒	☐	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	TEM: iTEM – analySIS Image Processing (Olympus); Bioanalyzer: 2100 Expert (Agilent); ExoView®: nScan 2.8.10 (NanoView Biosciences) (for acquisition); NanoViewer (NanoView) (for analysis) and BD FACSSuite™ softwer for flow cytometry data acquisition.
Data analysis	GraphPad Prism 8 was used to analyze the data; Adobe InDesign 2020 was used to make illustrations and FlowJo 7.6.5 to analyze flow cytometry data, Proteome Discoverer version 2.4 (Thermo Fisher Scientific) using the MASCOT algorithm (Matrixscience) for proteomic data analysis and Qlucore Omics Explorer for unsupervised hierarchical clustering.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The full-length, unprocessed blots and gels of the western blot results shown in Extended data figure 5 to the figshare.com web site (https://doi.org/10.6084/m9.figshare.12854744.v1). The complete MS proteomics data are available via ProteomeXchange with identifier PXD021694

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	As minimum, three samples were analyzed. Exceptions are: TEM evaluation of large and small LD and HD EVs (N = 2), measurement of proteins in EVs isolated using protocol 2 (N = 2), Western blot (N = 2) performed to validate mass spectrometry results and ExoView™ analysis showed in Figure 3C (N = 1). Three spots were measured for all samples investigated by ExoView™, however if the program recognized a problem with one spot it was removed and the two remaining spots were then shown. The results are presented as the mean from 3 different spots on the chip (technical replicates).
Data exclusions	No data were excluded.
Replication	The protocol has been replicated several time by different researchers to isolate EVs from different type of tissues.
Randomization	During EV isolation and characterization every sample is randomized.
Blinding	No blinding was necessary for this protocol.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Antibodies used for Western blot analysis: calnexin (clone C5C9, Cell Signaling Technology; cat. no. 2679S); flotillin-1 (clone EPR6041, Abcam); CD63 (clone H5C6, BD Biosciences; cat. no. 556019); CD9 (clone MM2/57, Millipore; cat.no. CBL162); CD81 (clone M38, Abcam); mitofilin (clone AB-2547893, Invitrogen; cat. no. PA5-30419) and ADAM10 (clone 163003, R&D System; cat. no. MAB1427-SP) Antibodies used for flow cytometry analysis: PE-labelled anti-CD9 (clone M-L13, BD Bioscience, cat. no. 341647), anti-CD63 (clone H5C6, BD Bioscience, cat. no. 557305), anti-CD81 (clone JS-81, BD Bioscience, cat. no. 561957) . Antibodies used for ExoView™ experiments were provided in the kit.
Validation	Before performing the experiments, each antibody has been tested using the positive control sample indicated by manufacturer.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HMC1 (Dr Joseph Butterfield Mayo Clinic, Rochester, MN)
Authentication	Cell line was authenticated by commercial.
Mycoplasma contamination	Cells have not been tested for mycoplasma contamination. Cells were only used for minor validation experiment and the

Mycoplasma contamination	main protocol was performed on tissues.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cells line is used in this study.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Melanoma: laboratory animal: C57BL/6J, male, 6 weeks old. The tumor was induced by injection of B16F10 cells. Colon cancer: laboratory animal: BALB/c, male, 6 weeks old. The tumor was induced by injection of CT26 cells.
Wild animals	*Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.*
Field-collected samples	Mice were maintained in microisolator cages, exposed to a 12 h light/12 h dark cycle, and provided with standard feed and water.
Ethics oversight	The study was approved by the local Animal Ethics Committee in Gothenburg, Sweden (permit no. 1624-19) and was carried out according to institutional animal use and care guidelines (Directive 2010/63/EU on the protection of animals used for scientific purposes).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics	Melanoma: tumor samples were retrieved from patients undergoing surgery at Sahlgrenska University Hospital after signed informed consent. Tumor tissues were collected prospectively in patients undergoing surgery for melanoma. Colon cancer and colon mucosa: tumor samples were retrieved from patients undergoing surgery at Sahlgrenska University Hospital after signed informed consent. Tumor tissue and non-cancer tissue were collected prospectively in patients undergoing surgery for colon cancer.
Recruitment	Melanoma: in total 26 samples from 21 patients were retrieved. There was one sample from an advanced primary tumor (stage II), 15 samples from in-transit or lymph nodes metastasis (stage III) and 10 samples from distant subcutaneous, small bowel or liver metastasis (stage IV). Colon cancer and colon mucosa: tissues from 3 patients were retrieved (3 colon cancer samples and 3 colon mucosa tissues). Tumors were at different stages: two at stage III and one at stage IIA.
Ethics oversight	In this study all samples were collected and processed in alignment with the Regional Ethical Review Board at the University of Gothenburg, Sweden (melanoma metastases #096-12 and #995-16; colon cancer and colon mucosa #118-15).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	HMC-1 cells were pelleted at 300 × g for 10 minutes and resuspended in PBS and incubated with collagenase D (2 mg/ml, Roche, Basal, Switzerland) and DNase I (40 U/ml, Roche) or with equal volume of PBS (negative control) at 37 °C for 30 minutes with gentle agitation. After centrifugation at 300 × g for 10 minutes to remove enzymes, cells were resuspended in 50µl of human IgG (Sigma-Aldrich) and incubated for 15 minutes at RT, before being washed twice more. Cells were incubated with anti-CD9, anti-CD63, anti-CD81, or the corresponding isotype control (and 5 µl of the vital dye 7-Amino-Actinomycin (7-AAD) for 40 minutes at RT and washed twice. Vesicles isolated from HMC-1 cells (large and small vesicles) were incubated with anti-CD63-coated beads (Thermo Fisher Scientific) overnight at 4°C with gentle agitation (10µg EV protein/50,000 beads/antibody). Each sample was divided in two and half of the sample was treated with collagenase D (2 mg/ml, Roche) and DNase I (40 U/ml, Roche) or with equal volume of PBS (negative control). After incubation at 37 °C for 30 minutes with gentle agitation, the bead-EV complexes were washed twice with 1% EV-depleted FBS in PBS, incubated with human IgG (Sigma-Aldrich) for 15 minutes at 4°C, washed twice, and incubated with the same PE antibodies as the cells or the corresponding isotype control for 40 minutes at RT under agitation. The samples were washed twice before analysed.
Instrument	BD FACSVerse™ flow cytometry

Software	BD FACSSuite™ software for flow cytometry data acquisition; FlowJo 7.6.5 for analysis.
Cell population abundance	Cells were gated in FSC and SSC as shown in Extended Fig 2. 64.2-94.6% of the total events collected ended up in this gate for all the samples analyzed. Both large and small EVs were bound to anti-CD63 beads. In FSC and SSC gate was used for the single bead population as shown in Extended Fig 2. 70.4-96.2% of all events collected ended up in this gate. 10 000 events were collected both for cells and EV-bead complexes.
Gating strategy	Cells were gated in FSC and SSC as shown in Extended Fig 2. 64.2-94.6% of the total events collected ended up in this gate for all the samples analyzed. Both large and small EVs were bound to anti-CD63 beads. In FSC and SSC gate was used for the single bead population as shown in Extended Fig 2. 70.4-96.2% of all events collected ended up in this gate. 10 000 events were collected both for cells and EV-bead complexes. The expression of CD9, CD63, and CD81 as well as 7-AAD positivity was is presented as histograms. As this most clearly shows the comparison between the treated and none-treated samples.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.