nature portfolio

Corresponding author(s): Hadrien ALRIC

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Reporting Summary

Nature Portfolio 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 Portfolio 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	Microsoft excel
Data analysis	All tests were two-tailed with a significance level of p<0.05. All analyses were performed with R software version 3.6.0. or GraphPadPrism (GraphPad Software, La Jolla, USA).

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 Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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

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	We did not perform a sample size calculation because there are no clinical data in the literature evaluating the treatment tested for this indication. This is an exploratory study
Data exclusions	All analyses were conducted taking into account all the data.
Replication	We conducted multiple measurements for the biological variables to ensure their reproducibility.
Randomization	All rats were randomly assigned to each experimental treatment before the start of the experimentation (radiotherapy)
Blinding	It was not possible to administer the treatment in a blinded manner because it is a perioperative treatment that requires immediate preparation during surgery. However, the examiners were blinded during the collection of the final data (colonoscopy, autopsy, histopathology).

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used: CD90 (clone OX-7; BD Biosciences) and CD73 (clone 5F/B9; BD Biosciences) CD34 (clone ICO115, Santa Cruz Biotechnology) and CD45 (clone OX-1; BD Biosciences) markers. against CD63 (clone MX49.129.5, sc-5275 af647, Santa Cruz Biotechnology) and/or anti-CD9 (clone C-4, sc-13118 af647, Santa Cruz Biotechnology) and/or anti-CD81 (clone 5A6, sc-23962 af647, Santa Cruz Biotechnology)

Validation: https://www.biosciencegmbh.com/

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals: Forty Sragues-Dawley rats (7 weeks-old male rats of 250 to 300, Janvier-labs, France) were randomized into 4 groups of 10 rats.

Wild animals: no

Reporting on sex: male

Field-collected samples: They were housed in a temperature-controlled room (21+/-1 °C) and were exposed to a 12-hour light/dark cycle and fed with standard pellets with food and water available ad libitum. After an acclimatization period of a week, each rat received a fractioned irradiation of 3 x 12.5 Gray (Gy) in one week. Surgery was performed 3 weeks later with a low colo-colonic anastomosis during which the treatment of interest was applied (control, PF-127 alone, EVs alone or PF-127 containing EVs). Eight weeks after the end of the irradiation, colo-colic anastomoses were evaluated by colonoscopy. Immediately after colonoscopy, the rats were sacrificed and an autopsy was performed. The colonic anastomosis was then extracted for histological analysis (Figure 1). The physical condition of the rats was monitored on a daily basis. In case of reaching the ethical limit points defined in the protocol, the rats were euthanized under general anesthesia and an autopsy was performed.

Ethics oversight: All experiments were performed at the animal facilities of the Institut de Radioprotection et de Sûreté Nucléaire (Fontenay-aux-Roses, France, registry n°C92-032-01) in strict compliance with European directives (86/609/CEE) and were approved by local ethical committee of the Institute of Radioprotection and Nuclear Safety in Fontenay-aux-Roses. The experimental protocol was submitted to the French national authorization platform and after approval was registered under the APAFiS permit number #14843-201804241155405 v2 P18-03. We have complied with all relevant ethical regulations for animal use.

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

Plants

Seed stocks: *Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*

Novel plant genotypes: *Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*

Authentication: *Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

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	Extracellular vesicles
Instrument	Nano-flow cytometry analysis was performed utilizing the Flow NanoAnalyzer (NanoFCM Co., LTD) in accordance with the manufacturer's guidelines.
Software	The acquired data were processed using the NF Profession 1.0 software.
Cell population abundance	Prior to staining, samples were initially acquired to determine particle concentration and were subsequently diluted for optimal staining of EVs to a concentration of 5.109 particles/mL. Fluorochrome-conjugated antibodies were prepared for use by centrifuging at 10,000g for 15 min or filtering through a 0.22 µm syringe filter, and were diluted 10 folds for incubation with EVs. Before measurement, the samples underwent an additional 100-fold dilution in PBS. Blank controls included PBS and antibodies diluted 1000-fold in PBS. To establish the threshold between positive events and background noise in the fluorescence channel, signals from both unstained Extracellular Vesicles (EVs) and EVs incubated with the isotype control were considered. Each measurement was conducted in triplicate to ensure accuracy.
Gating strategy	Nano-flow cytometry analysis was performed utilizing the Flow NanoAnalyzer (NanoFCM Co., LTD) in accordance with the manufacturer's guidelines. To calibrate the system, a concentration standard (200nm PS QC beads, NanoFCM) was employed for measuring particle concentration, while a cocktail of silica beads (S16M-Exo, NanoFCM) was utilized to construct a calibration curve, facilitating the conversion of side scatter intensities to particle size. The laser configuration involved a 488 nm and 640nm laser at 20mW, 10% ss decay, with lens filters set at 525/40 and 670/30. The antibodies for staining were Alexa Fluor® 647-conjugated monoclonal mouse antibodies directed against CD63 (clone MX49.129.5, sc-5275 af647, Santa Cruz Biotechnology) and/or anti-CD9 (clone C-4, sc-13118 af647, Santa Cruz Biotechnology) and/or anti-CD81 (clone 5A6, sc-23962 af647, Santa Cruz Biotechnology) antibodies or isotype control (sc-516609, Santa Cruz Biotechnology). The staining process was carried out overnight at 4°C and isotype control antibodies were utilized at identical concentrations and incubation times. Labeled vesicles were then resuspended in 50µL of PBS and diluted 1:200 times. Positivity was defined using buffer alone (PBS), unstained vesicles, isotype controls, and auto-thresholding. Prior to staining, samples were initially acquired to determine particle concentration and were subsequently diluted for optimal staining of EVs to a concentration of 5.109 particles/mL. Fluorochrome-conjugated antibodies were prepared for use by centrifuging at 10,000g for 15 min or filtering through a 0.22 µm syringe filter, and were diluted 10 folds for incubation with EVs. Before measurement, the samples underwent an additional 100-fold dilution in PBS. Blank controls included PBS and antibodies diluted 1000-fold in PBS. To establish the threshold between positive events and background noise in the fluorescence channel, signals from both unstained Extracellular Vesicles (EVs) and EVs incubated with the isotype control were considered. Each measurement was conducted in triplicate to ensure accuracy. The acquired data were processed using the NF Profession 1.0 software.

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