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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	LE-MA900 FP-Cell Sorter Software (SONY) for flow cytometry data collection Tecan i-control 2.0 software (TECAN, Austria) for 96 well plate reader absorbance and fluorescence. NIS-Elements Imaging Software (NIKON) for confocal microscopy data collection.
Data analysis	LE-MA900 FP-Cell Sorter Software (SONY) for flow cytometry data collection and analysis. FlowJo 10.5.3 for flow cytometry data analysis and plotting of final flow cytometry plots. NIS-Elements AR5.21.03 Imaging Software (NIKON) for confocal microscopy data collection and analysis. ImageJ 1.52a (National Institute of Health, USA) public software for counting number of forming colonies in the clonogenic assay. Microsoft Office Home and Student 2016 (excel and power point) for data analysis and figures. Perkin Elmer ChemDraw Professional 16.0 for drawing chemical structures. VESTA version 3.5.7 for three-dimensional visualization of electronic chemical structures.

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 source data used during this study are uploaded to the Zenodo database, accessible at: https://doi.org/10.5281/zenodo.8271482. The datasets generated during and/or analyzed during the current study are available from the corresponding author on request.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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	Sample size calculation was performed. Expected mean differences between the control and the treatment group and expected standard deviations were obtained from previous tumor growth delay experiments in our research group. For therapeutic significance, in the B16-F10 tumor model we expected to observe a mean value of the tumor size in the control group at 400 mm3 and standard deviation of 220 mm3 versus a mean value of 50 mm3 and standard deviation of 50 mm3 in the treatment group. Selecting a power of 80% (probability of finding an effect) and two-tailed significance level of 5% (p = 0.05, confidence interval = 95%), we calculated 4 mice per group. Considering 10% of attrition factor (death/euthanasia due to ulcerations, moribund, other reasons) during the study, then we placed DMSO+Light (n=5), Cy7.5-amine+Light (n=5, one was deceased during the study, n=4 final) and Cy7.5-amine (n=4). The attrition factor for correcting mice numbers was justified since we have observed attrition in previous studies using light activated molecular machines (MM) in the MM+Light treatment group in B16-F10 model possibly by a triggered strong immune-and-anti-inflammatory response. No attrition was expected in the control group treated with Cy7.5-amine based on previous preliminary experiments, then n=4 was justified. For therapeutic significance, in the A375 tumor model study we increased the significance level (two-tailed, p = 0.001, confidence interval = 99.9%) and power of 90%. The expected mean values in the control group were 400 mm3 and standard deviation of 220 mm2 versus 50 mm3 and standard deviation of 50 mm3 in the treatment group. Using these parameters, we predicted 9 mice per group. Then correcting by 10% of attrition factor. Then we placed 10 mice per group.
Data exclusions	No data was excluded
Replication	The flow cytometry analyses were repeated multiple times (n = 3 or larger) at a single concentration and the results were reproducible. Then, when the concentration response curves were measured, one sample was analyzed per concentration, but 7 or more concentrations were analyzed per molecule. The flow cytometry analysis were very reproducible even when the experiments were done on different repetitions on different days. Sometimes in flow cytometry a baseline of high cell death population was presented, but we learn to fix that by washing the culture plate several times with PBS buffer to wash off any death cell (which typically easily detach) and then harvest the healthy cells only, with this trick we got typically ~98-99% viable cell population measured using DAPI at the beginning of the treatments. The ROS measurement were very reproducible, even when the experiments were done at different repetitions on different days. The temperature measurements on the media varied relatively depending on the temperature of the room, so I have to do these measurements on the same day for better reproducibility since the temperature exchange in the solution will depend on the room temperature. In this way have similar initial temperatures at time zero of irradiation. The singlet oxygen measurements using DPBF as a probe, it will be varied from the condition of DPBF, always fresh solution was prepared for

better reproducibility to avoid using long-stored-autoxidixed BPBF in solution. And always fresh solution prepared of all compounds.
Experiment on confocal microscopy photoactivation of molecular jackhammers (Fig. 4) was repeated in an independent experiment (several months later) and the result were reproducible.
In vivo, therapeutic efficacy study in F16-F10 tumor model was performed once (n=4 per per group, this is 4 repetitions). But we justified change to work with A375 tumor model since B16-F10 produces high levels of melanin which interferes with the vibronic-driven-action photoactivation (written in the main text). Then A375 tumor model was conducted with a larger repetition number (n=10 mice per group). The therapeutic effect by VDA was successfully observed in both studies in vivo.
The binding of MJH into the A375 cells and the effect of acid in the media (lower pH) and its quantification (Extended Data Figure 3) was performed twice and the replications were successful (10,0000 cells were analyzed).
Localization of molecular machines with confocal microscope (Extended Data Fig 4), two experiments were performed independently, the second experiment was performed several months later, and the results were successfully replicated.
The experiment of photoactivation of Cy7-amine using 680 nm Light versus 730 nm light (Extended Data Fig 5) was performed once. However, several concentrations were tested to measure the concentration response and 10,000 cells cere analyzed per each concentration.
The effects of ROS scavengers on the cell permeabilization (Extended Data Fig 6) were performed 3 times independently, and in all attempts the replication were successful.
Clonogenic assay and crystal violet assay were repeated 3 and 4 times independent samples, and in all the attempts the replications were successful.
The time-course VDA treatment of DPhPC GUVs were replicated n=7 independent experiments. Not every experiment was successful because it required localization of the GUVs immobilized on the PVA matrix. Such matrix likely was formed during the synthesis. This PVA matrix possibly is a mixture of free phospholipids and PVA. When the GUVs were immobilized and localized sitting on the surface of this matrix, the experiments were successful. When the GUVs were encapsulated too deep within the matrix it was more difficult to image them and it was more difficult to disassemble them due to the matrix physically could prevent them from breaking. In this last case, not every experiment was successful.

Randomization

Mice were randomly allocated. Mouse cages were allocated randomly, injected with tumor cells. Next, mice were randomly placed in new cages to reduce any cell injection bias per cage. For experiments other than animal experiments, the covariates were controlled by keeping covariates constant among experimental groups, for example: incubation temperature, time of incubation, times of exposure, room temperature (conduct the experiment at similar room temperatures for the time-course temperature measurements), times between sample preparation and measurement, prepare fresh samples of DPBF every time for the ROS experiment to avoid differences due to chemical decomposition, measure and calibrate the light doses frequently, etc.

Blinding

A staff investigator (AC) who was blinded to the group allocation performed the measurements and data collection. Other member of the team (RR) conducted the initial data analysis. And (CAO) finalized the data analysis and final plots. This approach reduced any bias to the expected results.

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

Methods

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

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Mouse melanoma B16-F10 cells were obtained from the ATCC (CRL-6475). Originally was isolated from skin tissue of a mouse with melanoma. B16F10 is a murine melanoma cell line from the C57BL/6J mouse. It is a subclone of the B16 tumor line, generated by injecting mice with B16 tumor cells, collecting and culturing secondary tumor growths, then injecting them into fresh mice, a total of 10 times. B16F10 cells are highly metastatic and can form tumors and metastases post implantation into syngenic C57BL/6 mice or immunocompromised mice.
Human melanoma A375 cells were obtained from ATCC (CRL-1619). Originally was isolated from the skin of a 54-year-old, female patient with malignant melanoma.

Authentication

ATCC source. Cell morphology coincided with the reported at ATCC and literature such as the expected rate of tumor grow and presence of melanin.

Mycoplasma contamination

ATCC source. Quality control specifications = mycoplasma no detected.

Commonly misidentified lines (See ICLAC register)	Our cell lines F16-F10 and A375 were searched against commonly misidentified cell lines data base, https://iclac.org/databases/cross-contaminations/ , and they were not found.

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	In our studies we used 7-8 weeks old female C57BL/6J mice (Jackson Laboratories, strain #000664) or 7-8 weeks old athymic female nude (nu/nu) mice from Envigo/Harlan labs. The housing conditions of the mouse were: Temperature 72 oF (high 74 oF, Low 70 oF), humidity 45% (high 55%, low 40%) and 12 h light/dark cycle.
Wild animals	Study did not involve wild animals
Reporting on sex	The murine B16F10 and human A345 melanoma cells generate tumors in male and female C57BL/6J mice and nude mice respectively. In this study we selected females because males tend to fight and sometimes, they injured their subcutaneous tumors, these will affect the outcome of our experiments.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal studies were approved (protocol number 00000950-RN03) by the Institutional Animal Care and Use Committee (IACUC) of the University of Texas MD Anderson Cancer Center (Houston, TX).

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	A375 cells were cultured as described in materials and methods. One day before the treatment, cells were inoculated at 5 million cells per dish (10 cm polystyrene tissue culture dish). The cells were harvested using 0.05% trypsin-EDTA (Gibco, 25-300-054), then the cells were counted and were adjusted to a cell density of 2x105 cells/mL in DMEM media with L-glutamine, 4.5 g/L glucose, and sodium pyruvate (Corning Inc. 10013CV) and supplemented with 10% FBS (Corning, 35010CV), 1X MEM vitamin solution (Gibco, 11120052), 1X MEM non-essential amino acid solution (Gibco, 11140050) and penicillin/streptomycin. 1 mL of this cell suspension containing 2x105 cells was used in each treatment. In a 1.5 mL Eppendorf tube, 1 µL of stock solution containing 2 mM Cy7.5-amine (or other cyanine molecule or other concentration) in DMSO (Fisher, 99.7%) was placed in the bottom of the tube, then 1 mL of the cell suspension was added into the tube to get final concentration of 2 µM of Cy7.5-amine containing 0.1% DMSO and 2x105 cells. The mixture was then incubated at 37 °C and 5% CO2 for 30 min. Then, 1 µM DAPI was added into the cell suspension. Then, the cells suspension was transferred to a 35 mL polystyrene tissue culture dish and immediately the cells were treated under the light beam of NIR light of 730 nm at 80 mW/cm2 (or adjusted powers down to 20 mW/cm2) for 10 min (or adjusted illumination times down to 30 s) using LED light source (Prizmatix, UHP-F-730, Israel) which covers the entire dish. The spectral intensity of the LED is shown in Fig. 1e. While the cells were treated, the dish was placed on top of an aluminum block painted black, so that the excess NIR light and that was not reflected back into the cell suspension while the aluminum block acts as a heat-sink, maintaining a constant temperature in the dish during the irradiation. The instrument for flow cytometry analysis (SONY, MA900 Multi-Application Cell Sorter using the LE-MA900 FP-Cell Sorter Software) was already set up and calibrated by the time the light treatment was finished. Therefore, as soon as the 10-min light treatment was completed, the cell suspension was rapidly transferred from the 35 mm dish to a flow cytometry tube and the cells were analyzed for DAPI permeabilization and Cy7.5-amine binding. It took ~30 s to load the sample and to start the analysis. Therefore, the permeabilization of cells was measured as DAPI positive cells and occured immediately due to the membrane permeabilization caused by Cy7.5-amine excitation with the 730 nm NIR light. The flow cytometry data was analyzed using FlowJo software. The light intensity was measured using an Optical Power Meter from Thorlabs, sensor model S302C and console model PM100D.
Instrument	SONY, MA900 Multi-Application Cell Sorter
Software	SONY, LE-MA900 FP-Cell Sorter Software
Cell population abundance	Typically the cell population of interest for the analysis was nearly ~95-99 %. This is the population of singlets within the cell population.
Gating strategy	Flow cytometry data processing and gating strategy. The flow cytometry data was analyzed using FlowJo software version

Gating strategy

10.5.3. The cells were plot using the forward scattering area (FSC-A) versus side scattering area (SSC-A) as shown in Supplementary Information Fig. 5a. Then, a wide polygonal gate was drawn as shown in Supplementary Information Fig. 5a since we were interested to detect any cell death after treatment, we were not only interested to measure healthy cell populations. Sometimes, we observed that some death cell population upon treatment could shift outside the gate if a narrow gate was used. Then, the singlet cells were selected by plotting the FSC-A versus the forward scattering height (FSC-H) as shown in Supplementary Information Fig. 5b. Then, the singlets were selected and the DAPI fluorescence intensity was plotted versus the SSC-A and the gate for DAPI positive cells was drawn as shown in Supplementary Information Fig. 5c and the analysis was applied to all samples in the group. In Supplementary Information Fig. 5d can be observed a DAPI positive population in the same analysis group. This analysis was applied to calculate the percentage of DAPI positive cells. The same gating analysis also was done in the SONY, LE-MA900 FP-Cell Sorter Software while collecting the data.

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