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Supporting Information for

Compression drives diverse transcriptomic and phenotypic adaptations in melanoma

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Materials and Methods

Spheroid preparation

The YUMM spheroids were prepared following the standard spheroid preparation protocol. Briefly, 50 µl of boiled 20% agarose in 1X Phosphate Buffered Saline (PBS) mixture was pipetted to each well of a 96-well plate. The plate was left at room temperature to allow gel. 1000 YUMM cells in the culture medium were transferred into each well of the 96-well plates. The plate was then centrifuged at 400 relative centrifugal force (rcf) for 10 min to allow cell aggregation at the bottom of the gel. The spheroids were incubated at 37 °C for 4 days before being embedded in collagen gels.

Spheroid and single cell embedding in collagen gels

Double-distilled H_2O, 10X PBS, and acetic-acid-solubilized type I rat tail collagen (Corning, NY, USA) were mixed and neutralized by 0.5 N NaOH to reach a final collagen concentration of 1 mg/ml. Prepared YUMM spheroid or single B16F0 cell suspension was mixed with collagen and then transferred to a 24-well glass-bottomed cell culture plate (MatTek, Ashland, MA, USA) that is kept on ice. The plate with spheroids was then transferred to an incubator and flipped several times to locate the spheroid around the center of the gel during gelation. The fresh isotonic or osmotic medium was then added to each well. The culture plates were kept in an incubator at 37°C with 5% CO_2. The medium was replaced every 2-3 days.

Microfluidic device preparation

Standard photo- and soft-lithography techniques were used for the fabrication of microfluidic devices mentioned in previous studies (30, 31). Briefly, the photomask was made with Layout Editor. SU-8 (SU8-2015, Newton) was exposed under the photomask after being spin-coated on a silicon wafer to form a negative pattern. The wafer was then covered with mixed polydimethylsiloxane (PDMS) at a 10:1 weight ratio (Sylgard 184, Dow Corning). After baking, the PDMS mold was peeled and bonded to a glass coverslip for cell loading and imaging.

EdU assay staining

For EdU assay staining, an EdU Imaging Kit (Life Technologies, CA) was used to stain the cells following their assay protocol. Briefly, the compressed and uncompressed cells were stained with 1X working EdU solution at 10 µM for 1 day. The cells were then fixed with 3.7% paraformaldehyde in PBS for 15 minutes and permeabilized with 0.1% Triton X-100 for 10 minutes. After permeabilization, the cells were stained with a 1X Click-iT® EdU reaction cocktail and 5 µg/mL Hoechst for 30 minutes at room temperature, respectively.

Immunofluorescent staining

The compressed and uncompressed cells were fixed with 3.7% paraformaldehyde in PBS for 15 minutes and permeabilized with 0.1% Triton X-100 for 10 minutes in preparation for immunofluorescent labeling. After then, 3% BSA was used to block the cells for 1 hour. 1:200 PBS diluted YAP/TAZ (D24E4) or β-Catenin Rabbit mAb (Cell Signaling, MA) was used to stain the cells for 30 minutes before being rinsed with PBS and stained with a 1:500 PBS diluted anti-rabbit Alexa Fluor 488 dye (Abcam, UK). Finally, 5 µg/mL Hoechst 33342 solution and 1:2000 diluted Rhodamine Phalloidin (Invitrogen, USA) were added.

TMRM staining

For TMRM mitochondria membrane potential staining, cells were stained with TMRM in isotonic medium for 30 minutes, then the medium was replaced by osmotic medium with TMRM to introduce osmotic compression to the cells for confocal imaging.

Wound healing assay

The 2 well silicon inserts (ibidi) were placed in each well of a 24-well glass-bottomed cell culture plate (MatTek, Ashland, MA, USA). The desired amount of cells were then seeded inside the reservoir of the inserts and cultured overnight to allow cell spreading. The following day, the inserts were removed and three mild medium-wash cycles were applied to get rid of any suspended debris. The cells were imaged immediately (0 hours) and 18 hours after the removal of the inserts.

Transwell piston assay
The cells were cultured on a 6-well plate 0.4 μm transwell (Corning, NY). The transwells were soaked under an isotonic medium overnight to allow cell spreading. The cell monolayer was then gently covered with a thin layer of agarose gel and a bead holder. To prevent compression shocks, the beads were weighted to the desired weight before being added to the holder one by one.

Melanin assay
A fluorimetric melanin assay kit was used to perform the standard melanin assay (AAT Bioquest, Sunnyvale, CA). Cells that have been osmotically compressed for 5 days are trypsinized and lysed at a concentration of 2 million cells per milliliter. 50 μL of the cell lysate was introduced into each well of a solid black 96-wells microplate. Then, 50 μL of signal enhancer provided by the kit was added to each well. The plates were incubated at room temperature for 30 minutes. The fluorescence intensity was measured with a fluorescence plate reader at Ex/Em= 470/550 nm with cutoff= 515 nm. The data was normalized by the mean value of the uncompressed B16F0 cells.

Live and live/dead staining
For live-cell cytoplasm labeling, the cells were either stained with a cell tracker Green CMFDA Dye (Invitrogen, USA) for 1 hour prior to confocal imaging. For live/dead cell nucleus labeling, a LIVE/DEAD cell Imaging kit (488/570) (Invitrogen, USA) was used to validate cell viability. The cells were stained for 1 hour prior to confocal imaging.

Image analyses
Confocal microscopy-based cell volume reconstruction has been previously validated using high-resolution structured illustration microscopy (showing < 10% measurement uncertainty) (17). Imaris software was used to reconstruct the single cell 3D volume indicated in Fig. 1A and Fig. 1D in order to determine cell volume, 3D surface area and sphericity.

For live/dead imaging analysis, the images were first projected on the XY plane using a maximum projection method implemented in ImageJ. The number of live/dead positive cells was binarized and quantified using the ImageJ plug-in "Analyze Particles".

For the immunofluorescent imaging analysis, we applied a maximum projection method in ImageJ to project each fluorescent channel of the 3D images onto the XY plane. We generated a mask of the entire cell by combining the images of the Phalloidin and Hoechst channels. We then used the Hoechst channel to create a mask of the cell nucleus and subsequently obtained a mask of the cell cytoplasm by subtracting the nucleus mask from the full cell mask. To map out the signals inside and outside the cell nucleus for the marker of interest channel (β-catenin or YAP/TAZ), we applied the nucleus mask and cytoplasm mask to the marker channel, respectively. The sum fluorescence intensity (SFI) was then calculated for the signals inside the cell nucleus and cytoplasm, respectively. Furthermore, the cytoplasmic mean fluorescence intensity (MFI) was calculated by dividing the cytoplasmic SFI by the cytoplasmic mask area (Fig. S4).

For TMRM cluster measurement, a custom ImageJ macro script was used to outline individual cells, followed by the isolation of 3D images for each cell. Clustered mitochondria were identified by applying a universal threshold to the TMRM channel of each Z slide. The "Analyze Particles" plugin in ImageJ was used to determine the size of each mitochondrial cluster. To calculate the cluster size and TMRM volume of a cell, the averaged size and summed size of all clusters were analyzed within each cell, respectively. the results of each cell are normalized against the mean of the ISO condition, resulting in an average of 100% for both "Normalized fragment (cluster) size" and "Normalized TMRM volume" for ISO, as demonstrated in the figure.

Plots
All heatmaps were created with Qlucore software. The pathway plots were created with GSEA_4.2.3. The prognosis plots were created with GEPIA2 (http://gepia2.cancer-pku.cn/). All schematics were created with Biorender.com. All other data plots are created with GraphPad Prism 9.

Supporting Information

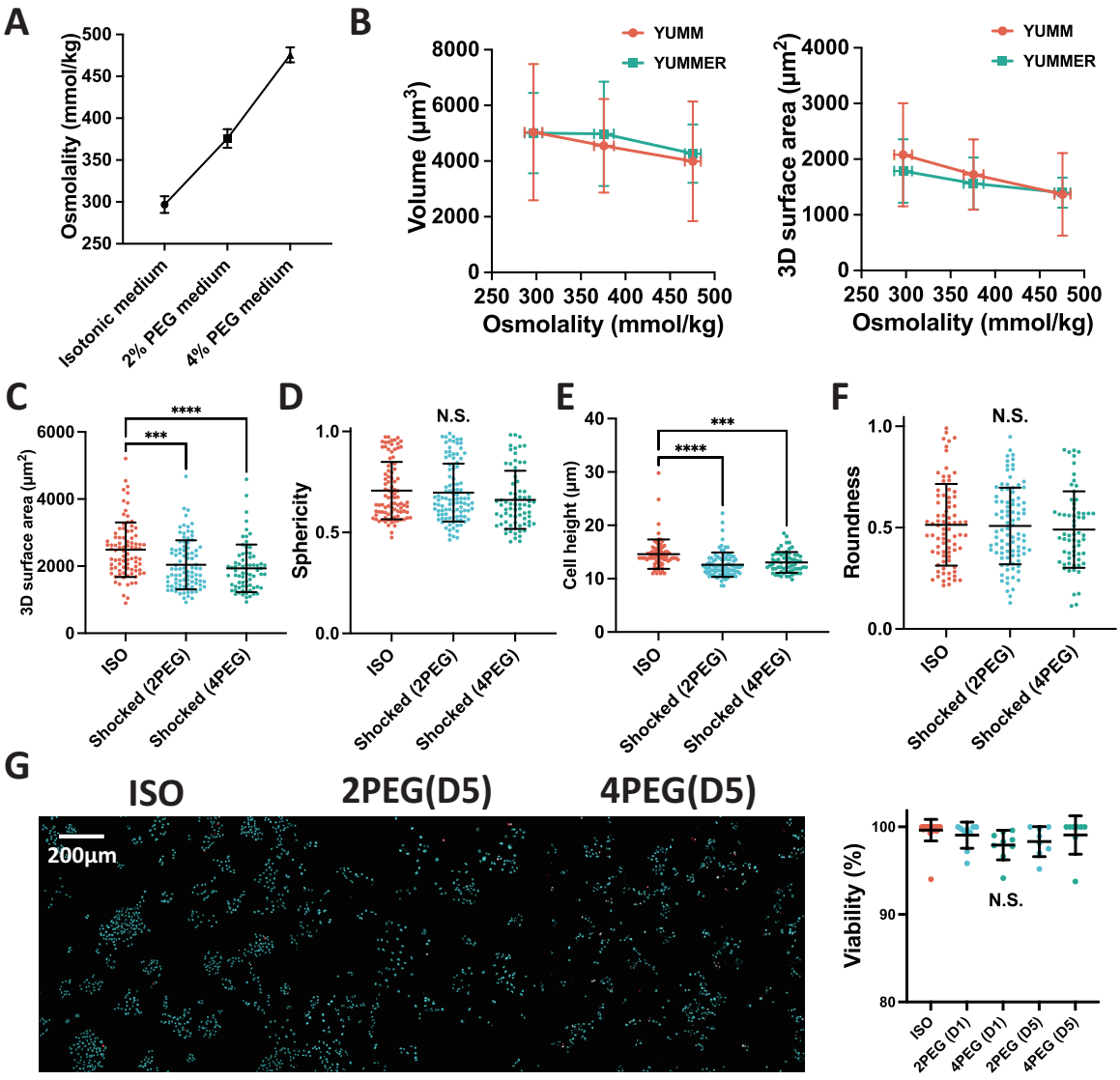

Figure S1. (A) The plot shows the osmolality of the media (n=5 for each condition, from one experiment). (B) Quantifications depict the volume (left) and 3D surface area (right) of the YUMM and YUMMER cells shocked in different medium conditions (n≥19 for each condition, from one experiment). (C-F) The plots show the 3D surface area (C), 3D sphericity (D), height (E) and 2D roundness (F) of B16F0 cells shocked in different medium conditions (n≥71 for each condition in C, D, n≥54 for each condition in E, F, from two independent experiments). (G) Left: Images of live/dead staining of uncompressed and 5-day compressed B16F0 cells. The dead cells are colored red. Right: The plot depicts the viability of adherent uncompressed and compressed B16F0 cells at day 1 and day 5 (n≥8 wells for each condition, from two independent experiments). All error bars are mean with standard deviation. Details of statistical analyses used can be found in Materials and Methods-statistical analysis.

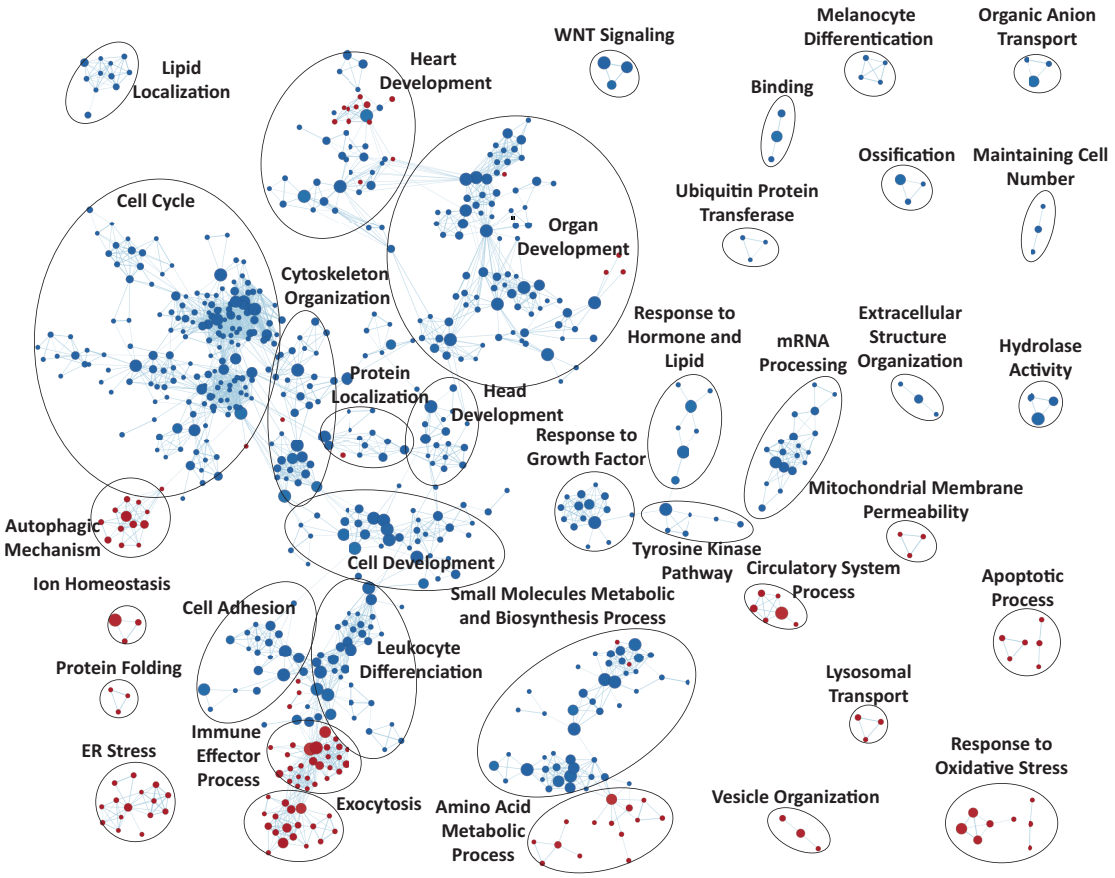

Figure S2. The network map reveals the clusters of activated (red) and suppressed (blue) biological process GO terms in 5-day compressed (2PEG and 4PEG combined) B16F0 cells (P<0.05, Q<0.5). The clusters are categorized and labeled based on the largest term in each cluster.

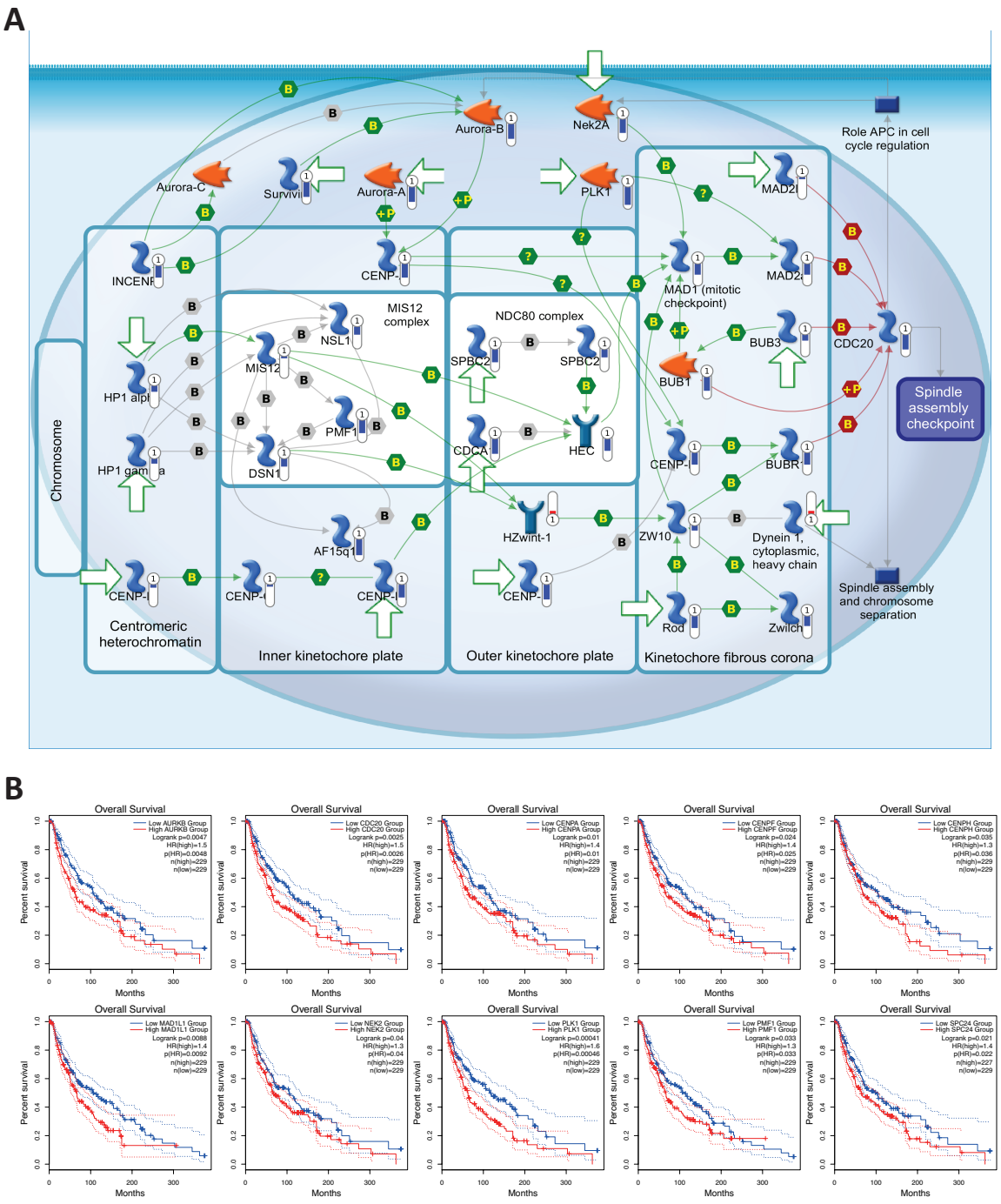

Figure S3. (A) The metaphase checkpoint pathway of the transcriptomic data of 4PEG vs ISO using the pathway map analysis in MetaCore. (B) The survival plots of TCGA cutaneous melanoma patients are divided by the proliferation-related genes identified in the compressed B16F0 cells. Details of statistical analyses used can be found in Materials and Methods-statistical analysis.

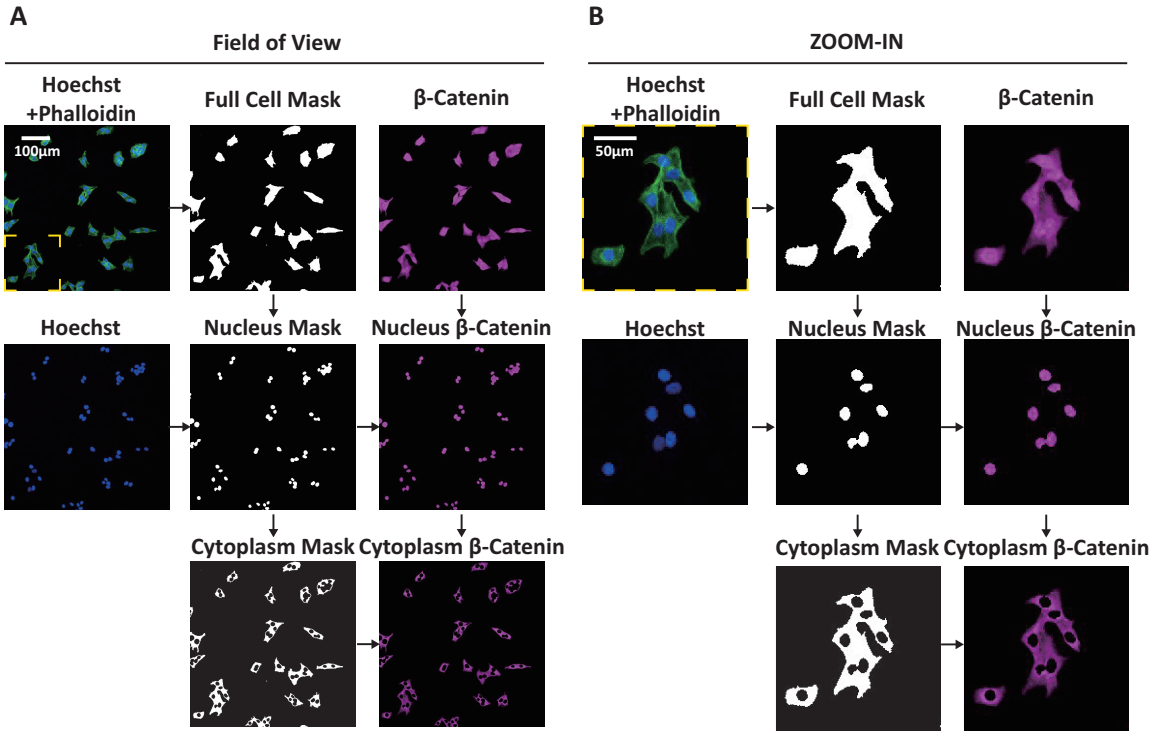

Figure S4. (A) The image analysis pipeline of a representative FOV in the ISO condition is presented to demonstrate the derivation of the full cell, nucleus, and cytoplasm masks. The masks were further applied to the marker channel (β-catenin) to derive the nucleus and cytoplasm marker intensity. (B) The corresponding zoom-in view of the lower-left corner of each image in (A).

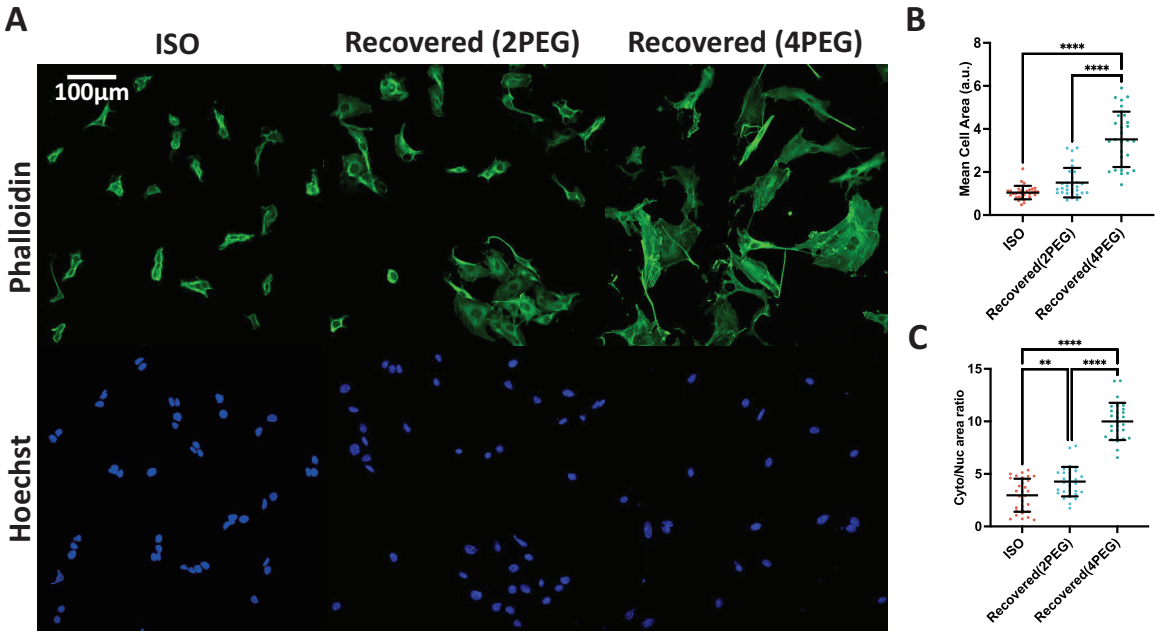

Figure S5. (A) Immunofluorescent images of the recovered B16F0 cells. (B, C) The normalized mean cell area (B) and cytoplasm area to nuclear area ratio (C) of the uncompressed and recovered cells are shown (n≥29 wells for each condition, from three independent experiments). All error bars are mean with standard deviation. Details of statistical analyses used can be found in Materials and Methods-statistical analysis.

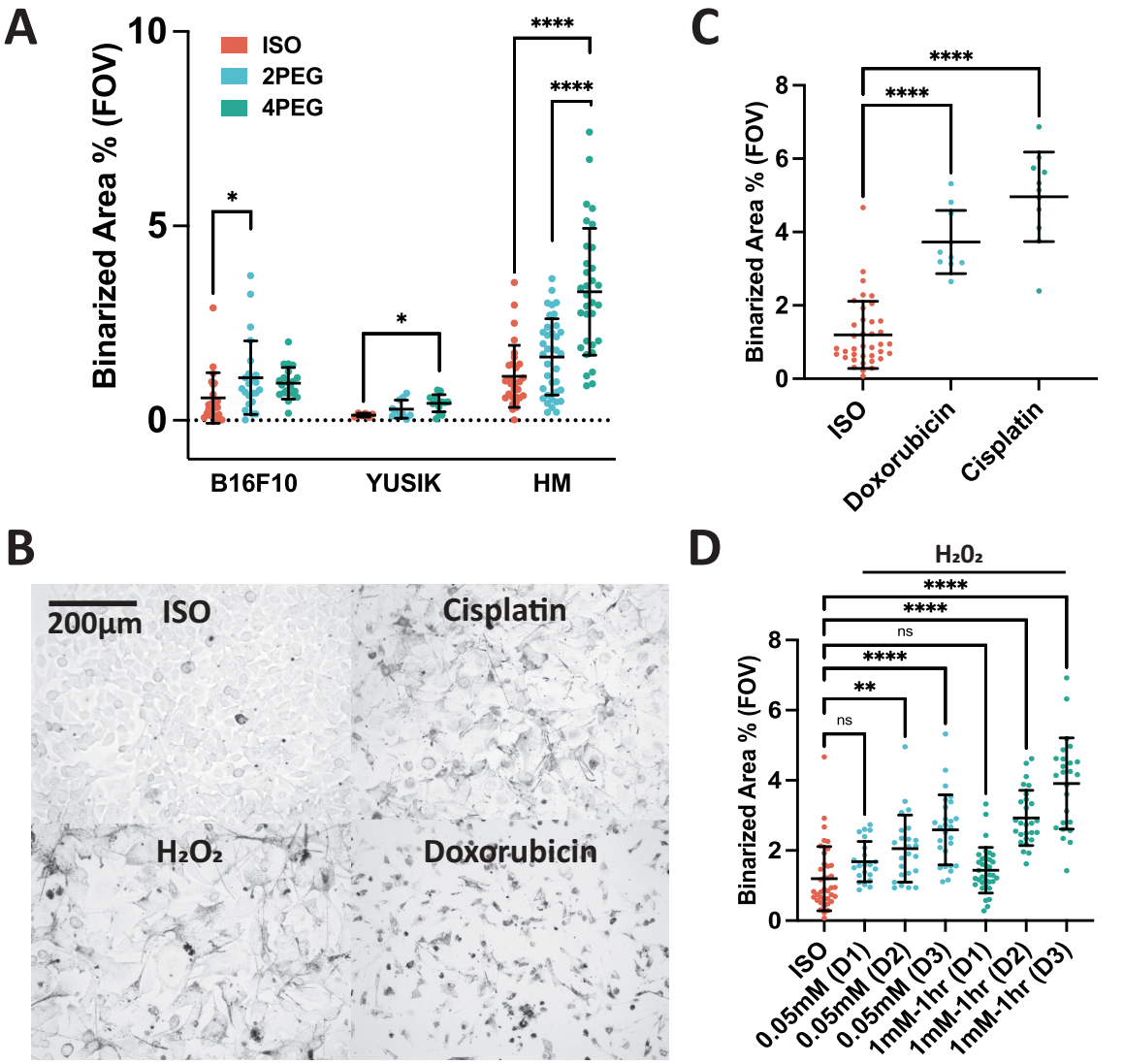

Figure S6. (A) Binarized melanin area percentage (FOV) of the uncompressed and 5-day compressed B16F10 cells, YUSIK cells and Human Melanocytes are plotted (n≥6 for each condition, human melanocytes data are from two independent experiments, all other data are from one experiment). (B) Representative images of B16F0 cells cultured with H_2O_2 (day 2, cells were treated with 5mM H_2O_2 for 1 hour at the beginning of the culture), cisplatin (day 5), or doxorubicin (day 5) without compression. (C) The binarized area percentage of the 5-day 10μM cisplatin and 0.5μM doxorubicin treated B16F0 cells are shown (n≥10, from one experiment). (D) The binarized area percentage on different days after the B16F0 cells were treated with H_2O_2 (0.05mM throughout the culture or 1mM of H_2O_2 for 1 hour at the beginning of culture) is shown (n≥22 for each condition, from two independent wells). All error bars are mean with standard deviation. Details of statistical analyses used can be found in Materials and Methods-statistical analysis.

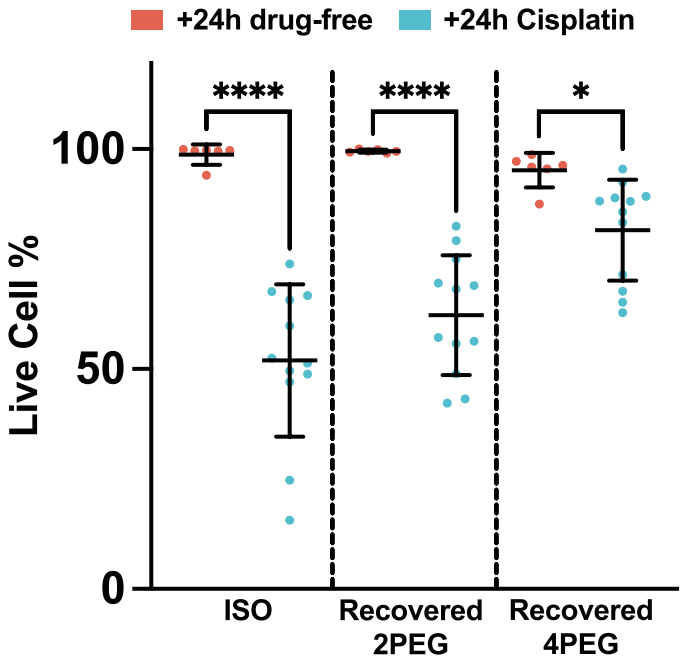

Figure S7. The percentage of live cells in each cisplatin treatment condition is compared to their matching cisplatin-free controls (n=6 wells for drug-free conditions, n=12 wells for cisplatin conditions, from three independent experiments). All error bars are mean with standard deviation. Details of statistical analyses used can be found in Materials and Methods-statistical analysis.

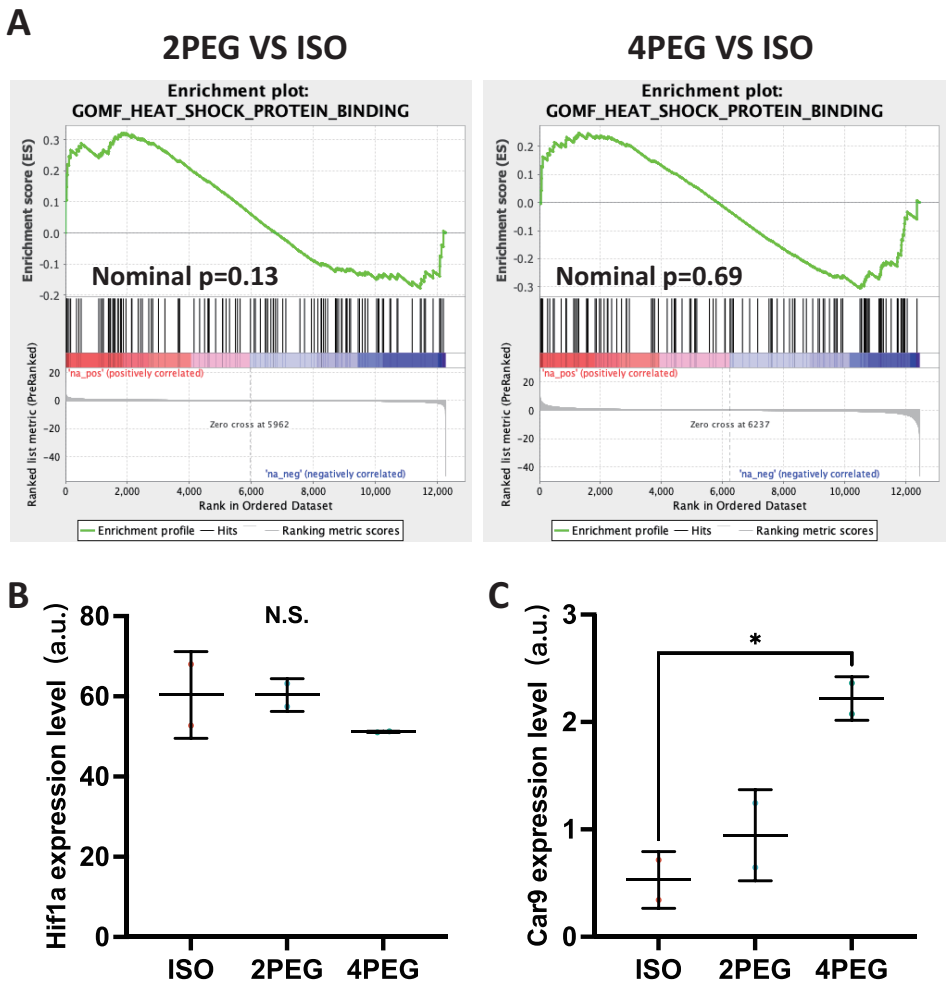

Figure S8. (A) The GSEA plots depict the pathway expression of 2PEG and 4PEG compared to ISO, respectively. (B) The plot shows the expression level of Hif1a across the uncompressed and compressed conditions. (C) The plot shows the expression level of Car9 across the uncompressed and compressed conditions. From two independent experiments. Error bars are mean with standard deviation. Details of statistical analyses used can be found in Materials and Methods-statistical analysis.