Supporting Information for

Post-thaw Culture and Measurement of Total Cell Recovery is Crucial in the Evaluation of New Macromolecular Cryoprotectants

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Additional methods

Non-frozen cell viability and plating efficiency

To determine the viability and plating efficiency of non-frozen A549 and SW480 cells after plating, cells were removed from culture by treatment with 0.25% trypsin plus 1 mM ethylenediaminetetraacetic acid (EDTA) in balanced salt solution for 5 min at 37 °C and then counted using the trypan blue exclusion assay. The cell solution was diluted to 1×10^5 cells·mL^{-1} in complete cell media and 500 µL was added to individual wells of a 24 well plate in triplicate for each timepoint. Plates were placed in an incubator at 37 °C, 5 % CO_2. After either 6 or 24 hours, the supernatant was collected and centrifuged at 180 × *g* for 5 minutes to pellet cells. The supernatant was removed and the pellet resuspended in 100 µL complete media, then cells were counted using the trypan blue exclusion assay to determine the number of non-attached cells. The adherent cells were washed with 250 µL sterile PBS then 300 µL trypsin-EDTA was added for 5 minutes and plates were incubated at 37 °C, 5 % CO_2. After 5 minutes, complete media was added to neutralise trypsin then samples were transferred to centrifuge tubes and centrifuged at 180 × *g* for 5 minutes. The supernatant was removed and cells were resuspended in 400 µL complete media, then the cell viability was determined using the trypan blue exclusion assay. Cell viability was calculated as the ratio of unstained (viable) cells to total cells. Plating efficiency was calculated as the ratio of unstained cells (viable) to the total number of cells initially plated.

ADDITIONAL DATA

Averaged recovery and viability data

Recovery and viability data for both A549 and SW480 cell lines was reported as biological repeats to allow clear visualisation of trends in the main paper. The total averaged and combined data for each experiment is combined in Figure S1 – S4.

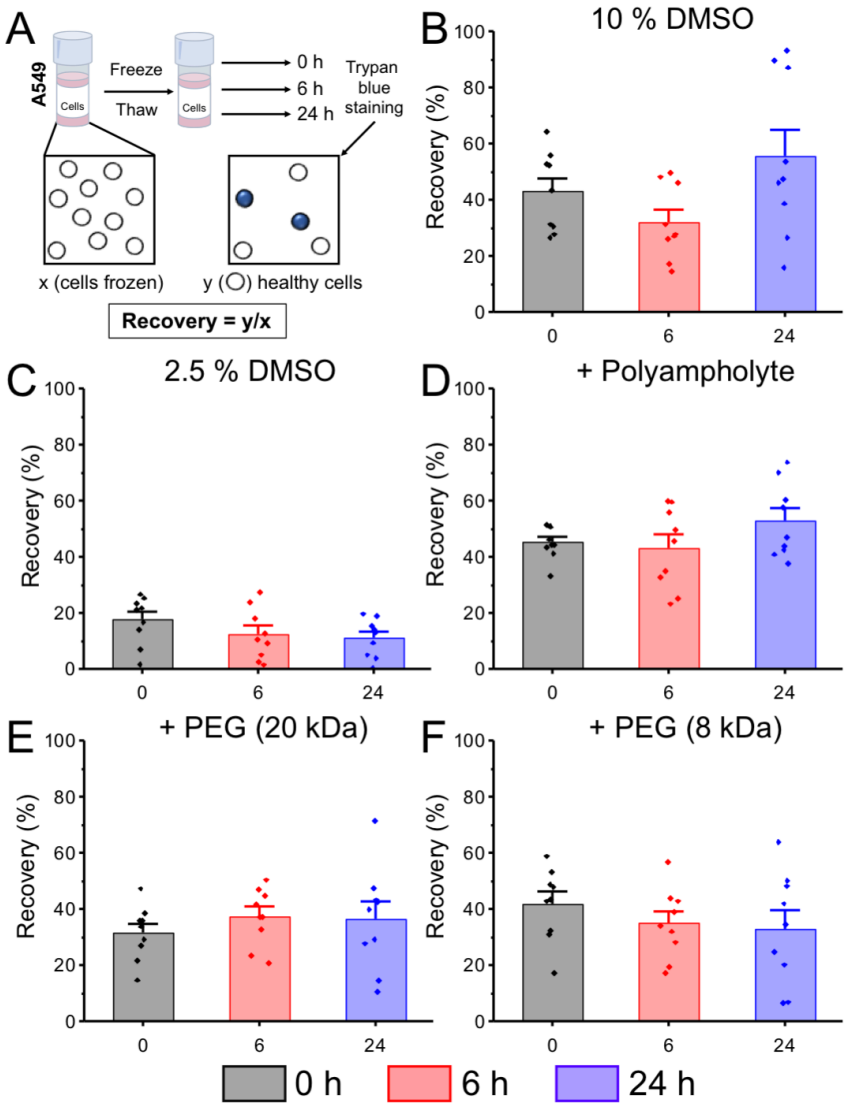

Figure S1. Average recovery data for A549 cells from 3 × biological × 3 technical repeats, calculated by trypan blue exclusion. Bars display mean recovery values (n=9), error bars are ±SEM. A549 cells were frozen in: B) 10 % DMSO; C) 2.5 % DMSO; D) 20 mg·mL^{-1} polyampholyte + 2.5 % DMSO; E) 20 mg·mL^{-1} poly(ethylene glycol) (20 kDa) + 2.5 % DMSO; F) 20 mg·mL^{-1} poly(ethylene glycol) (8 kDa) + 2.5 % DMSO.

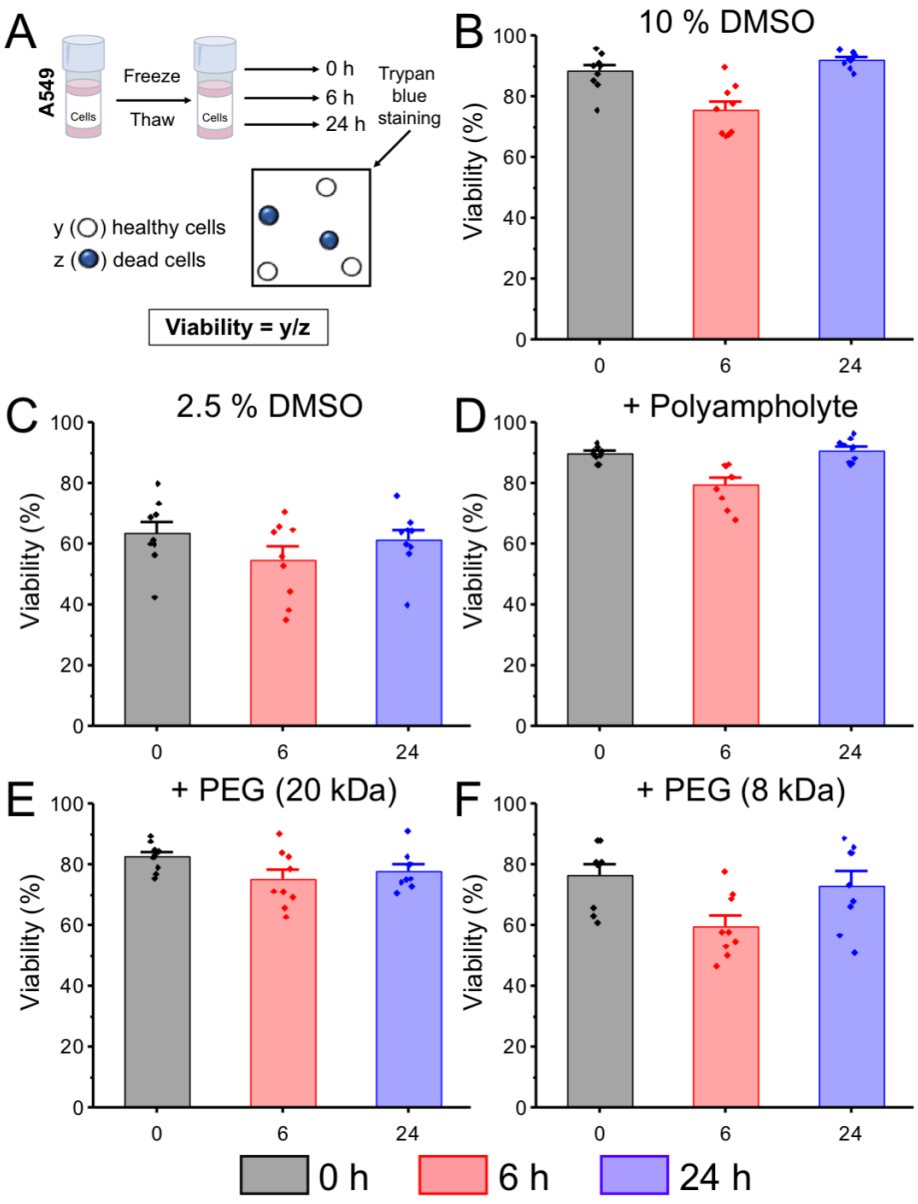

Figure S2. Average viability data for A549 cells from 3 × biological × 3 technical repeats calculated by trypan blue exclusion. Bars display mean recovery values (n=9), error bars are ±SEM. A549 cells were frozen in: B) 10 % DMSO; C) 2.5 % DMSO; D) 20 mg·mL^{-1} polyampholyte + 2.5 % DMSO; E) 20 mg·mL^{-1} poly(ethylene glycol) (20 kDa) + 2.5 % DMSO; F) 20 mg·mL^{-1} poly(ethylene glycol) (8 kDa) + 2.5 % DMSO.

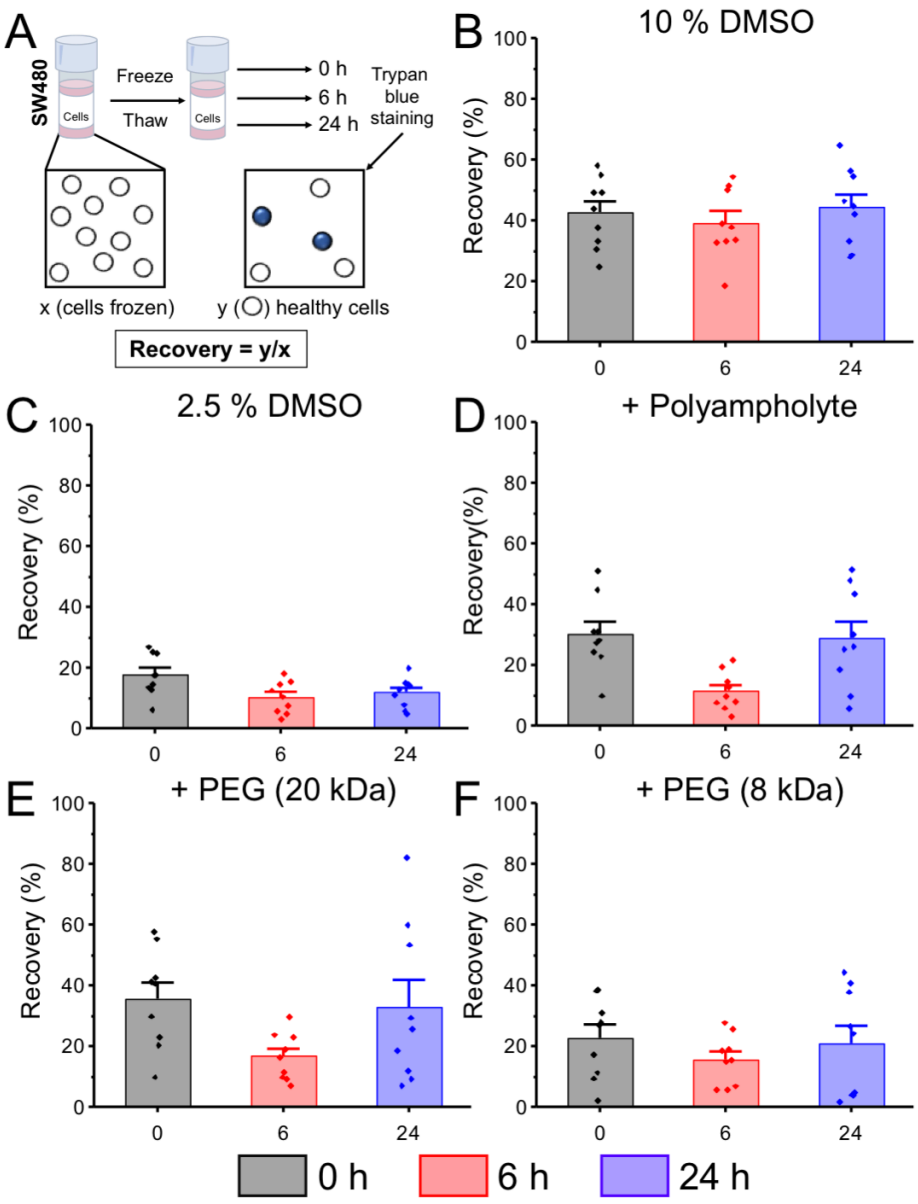

Figure S3. Average recovery data for SW480 cells from 3 × biological × 3 technical repeats calculated by trypan blue exclusion. Bars display mean recovery values (n=9), error bars are ±SEM. SW480 cells were frozen in: B) 10 % DMSO; C) 2.5 % DMSO; D) 20 mg·mL^{-1} polyampholyte + 2.5 % DMSO; E) 20 mg·mL^{-1} poly(ethylene glycol) (20 kDa) + 2.5 % DMSO; F) 20 mg·mL^{-1} poly(ethylene glycol) (8 kDa) + 2.5 % DMSO.

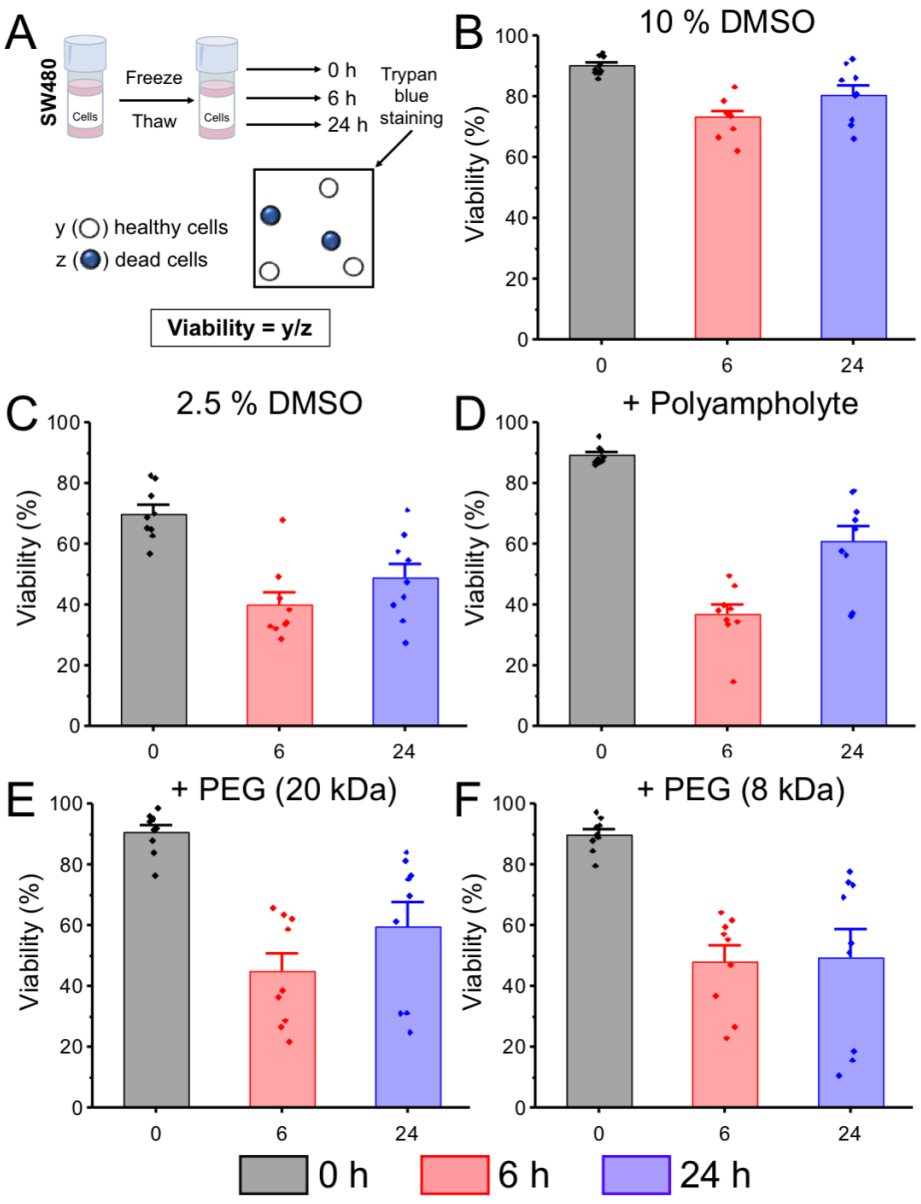

Figure S4. Average viability data for SW480 cells from 3 × biological × 3 technical repeats calculated by trypan blue exclusion. Bars display mean recovery values (n=9), error bars are ±SEM. SW480 cells were frozen in: B) 10 % DMSO; C) 2.5 % DMSO; D) 20 mg·mL^{-1} polyampholyte + 2.5 % DMSO; E) 20 mg·mL^{-1} poly(ethylene glycol) (20 kDa) + 2.5 % DMSO; F) 20 mg·mL^{-1} poly(ethylene glycol) (8 kDa) + 2.5 % DMSO.

Non-frozen cell viability

The viability and plating efficiency of non-frozen A549 and SW480 cells was measured at 6 and 24 hours after treatment with trypsin-EDTA, centrifugation and re-plating and analysed using the trypan blue assay. This was to ensure that cell processing conditions did not impact on the assays used here.

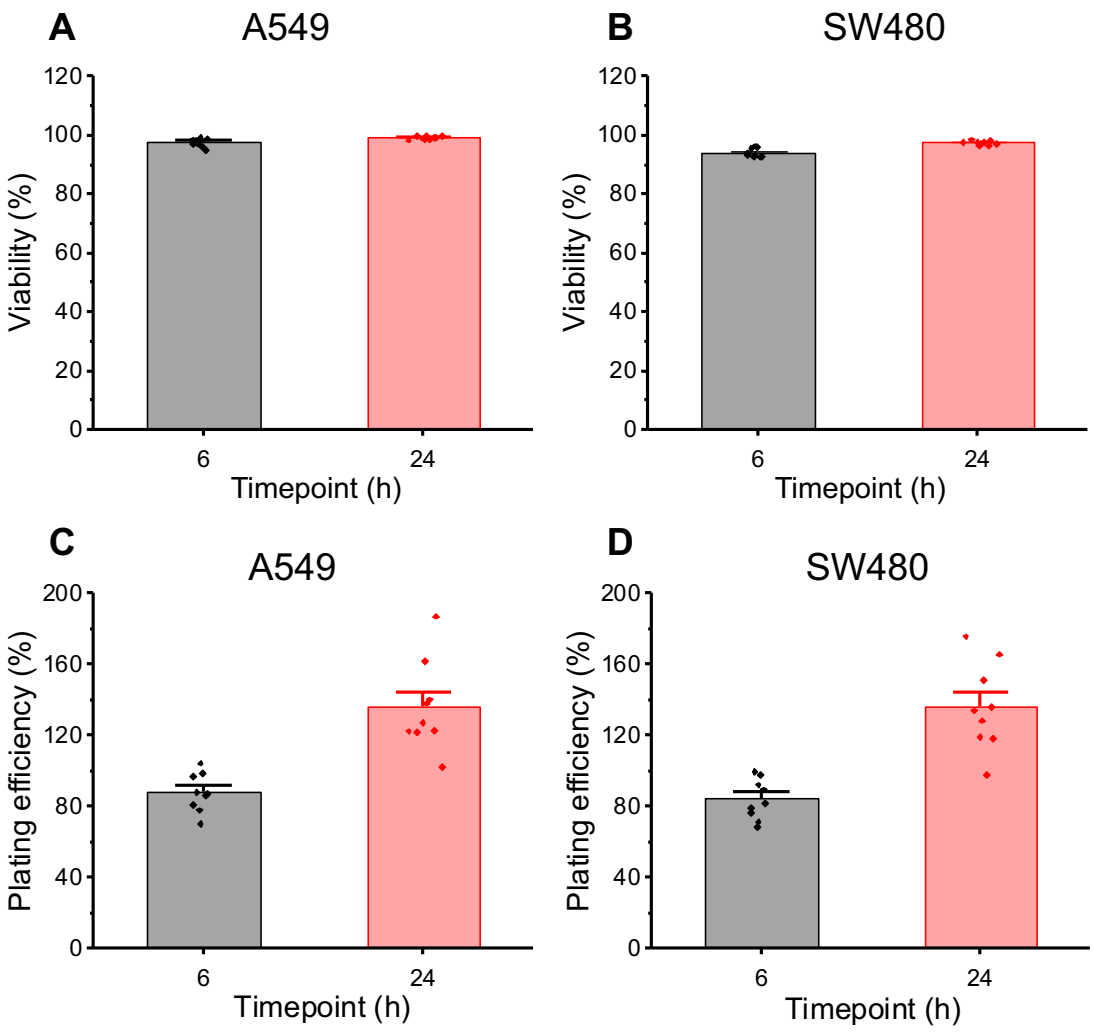

Figure S5. Viability of non-frozen (A) A549 and (B) SW480 cells and plating efficiency of non-frozen (C) A549 and (D) SW480 cells at 6 and 24 hours after treatment with trypsin-EDTA, centrifugation and re-plating, measured by trypan blue assay. Viability was calculated as the ratio of unstained cells to total cell number. Bars display mean values (n=6), error bars display ±SEM.

Apoptosis

Apoptosis was measured in A549 and SW480 cells using CellEvent™ Caspase-3/7 Green Detection Reagent at 6 and 24 hours post-thaw. Non-frozen cells (1×10^5 cells·mL^{-1}) were plated at the same time as frozen cells to serve as controls for normal culture and were either stained as described in the methods or left unstained as background controls. 1.6 mM H_2O_2 served as a positive control for apoptosis. Cells were frozen with either 10 % DMSO, 2.5 % DMSO, 2.5 % DMSO + 20 mg·mL^{-1} polyampholyte, 2.5 % DMSO + 20 mg·mL^{-1} PEG (20 kDa) or 2.5 % DMSO + 20 mg·mL^{-1} PEG (8 kDa). The following figures display phase contrast and the corresponding fluorescence microscopy images for cells stained with CellEvent™ Caspase-3/7 Green Detection Reagent. Green fluorescence indicates the presence of either caspase-3 or caspase-7 and strongly suggests that apoptosis was present in these samples. A lack of green staining strongly suggests that minimal apoptosis was present.

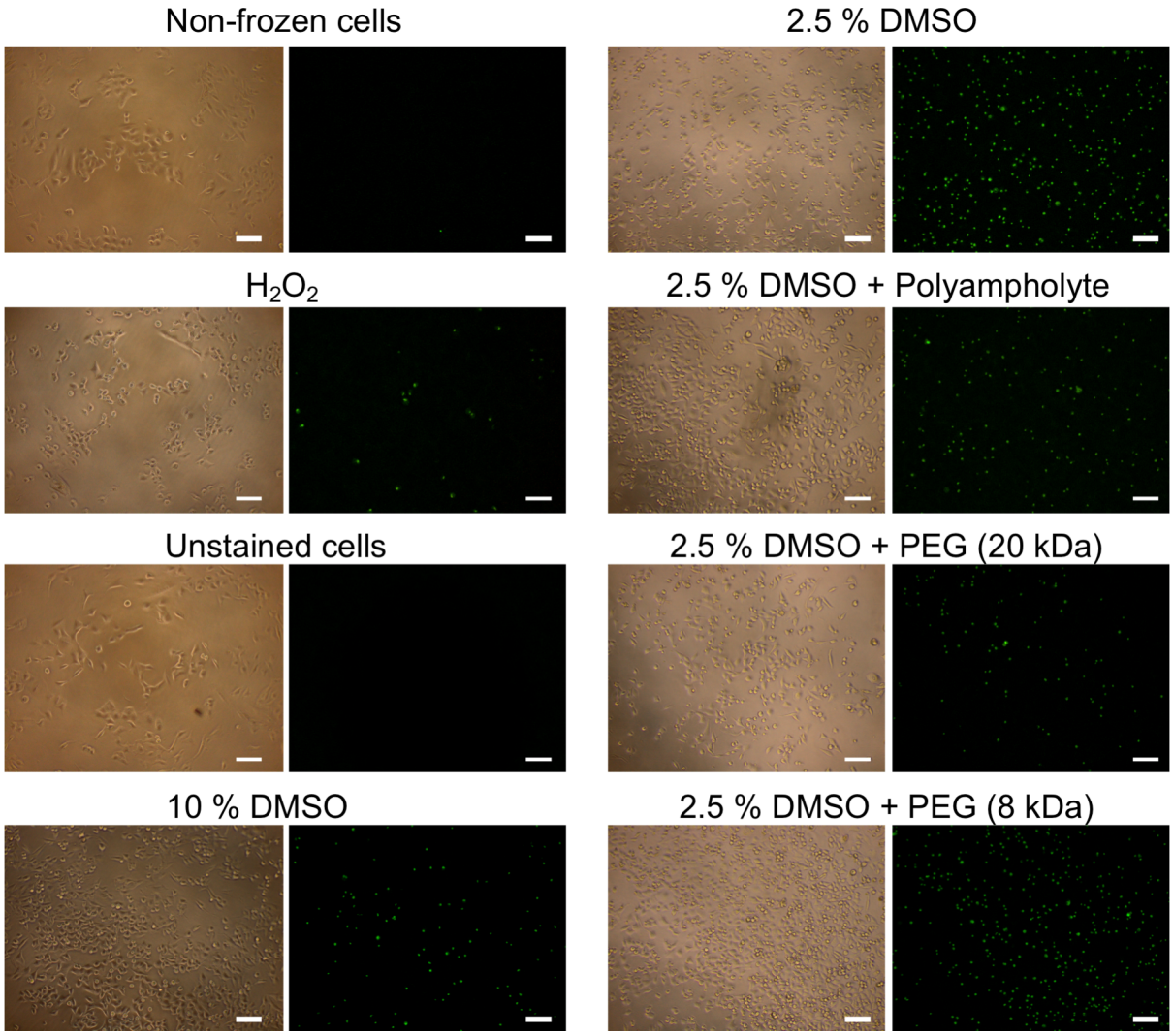

Figure S6. Light microscope and fluorescence images of A549 cells 6 hours post-thaw. Cells were cryopreserved, thawed and treated with 4 µM CellEvent™ Caspase-3/7 Green Detection Reagent for 6 hours at 37 °C, 5 % CO_2. Scale bar 100 µm.

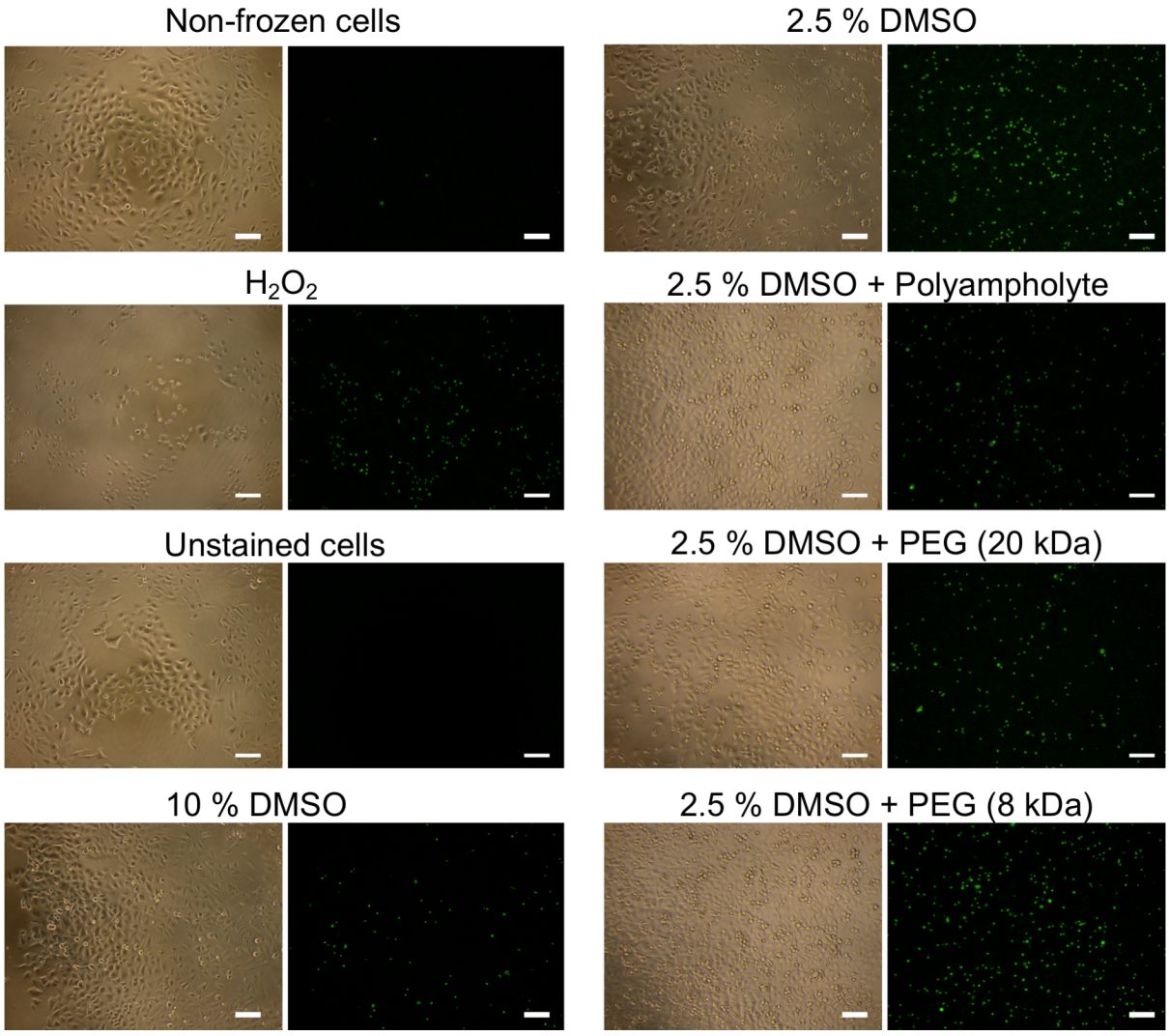

Figure S7. Light microscope and fluorescence images of A549 cells 24 hours post-thaw. Cells were cryopreserved, thawed and treated with 4 µM CellEvent™ Caspase-3/7 Green Detection Reagent for 24 hours at 37 °C, 5 % CO_2. Scale bar 100 µm.

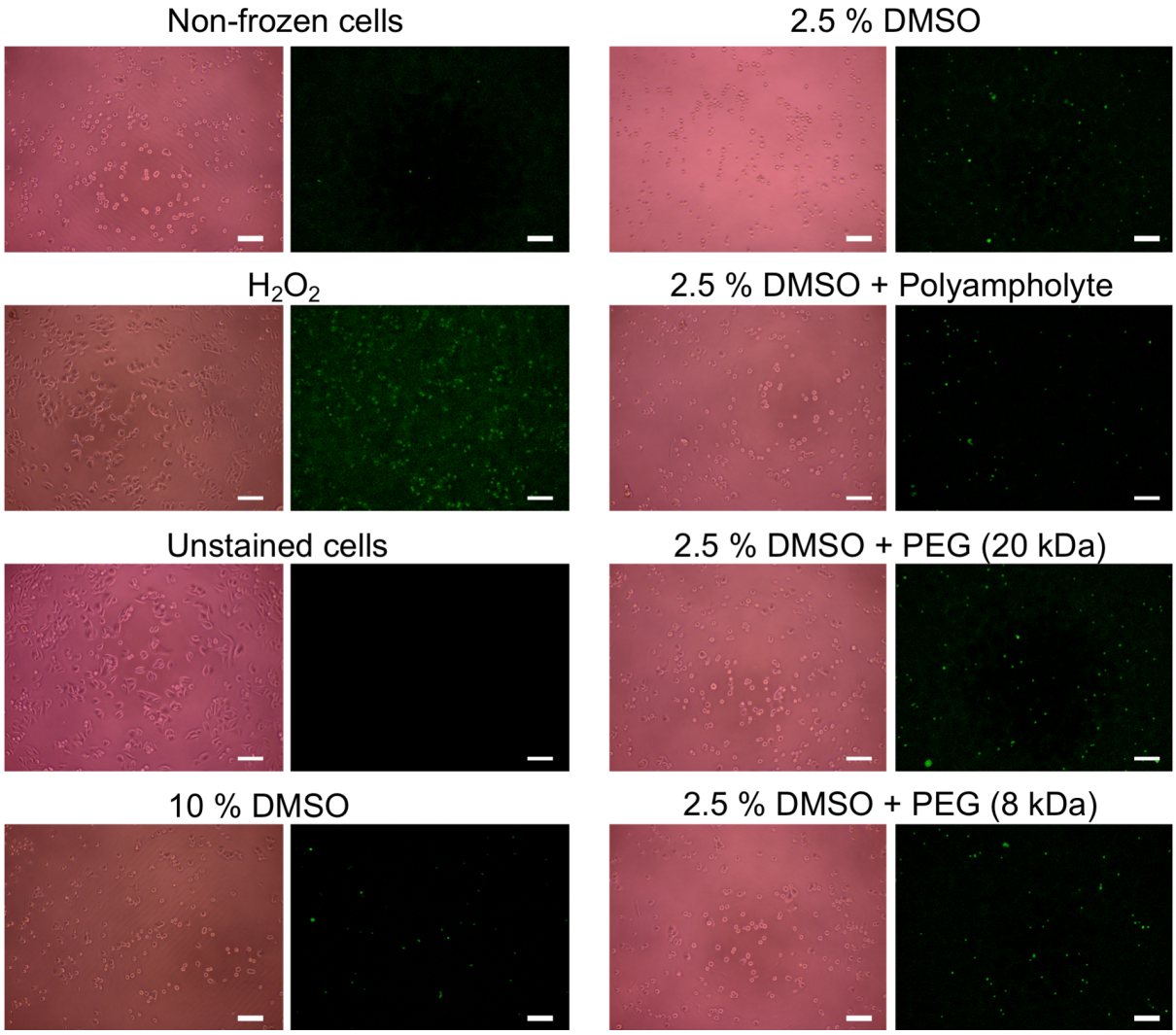

Figure S8. Light microscope and fluorescence images of SW480 cells 6 hours post-thaw. Cells were cryopreserved, thawed and treated with 4 µM CellEvent™ Caspase-3/7 Green Detection Reagent for 6 hours at 37 °C, 5 % CO_2. Scale bar 100 µm.

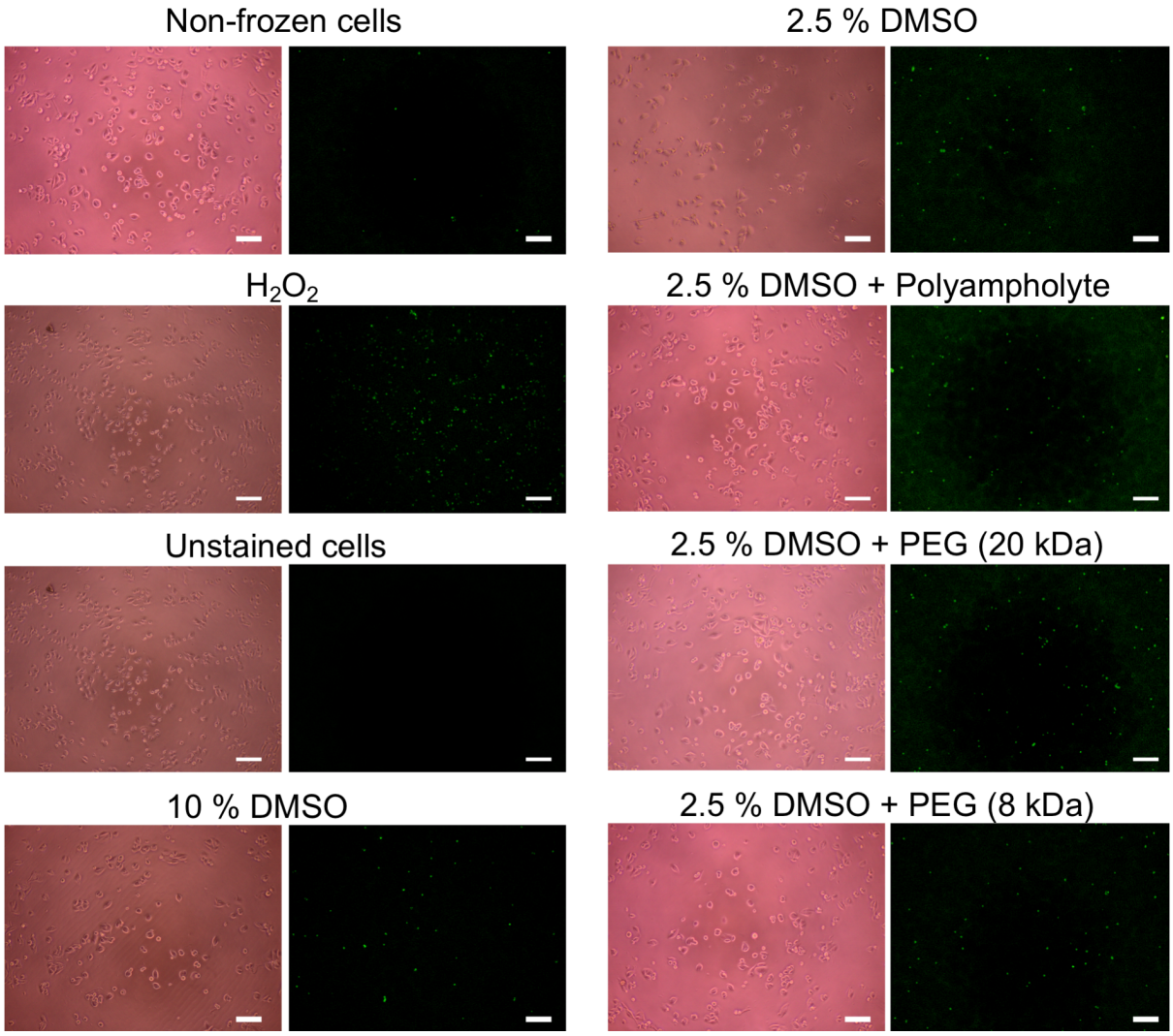

Figure S9. Light microscope and fluorescence images of SW480 cells 24 hours post-thaw. Cells were cryopreserved, thawed and treated with 4 µM CellEvent™ Caspase-3/7 Green Detection Reagent for 24 hours at 37 °C, 5 % CO_2. Scale bar 100 µm.