Supporting Information (Captions of Movies S1-S3 and Figures S1-S11 and on pages S3-S13)

Predehydration and Ice Seeding in the Presence of Trehalose Enable Cell Cryopreservation

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Movie captions

Movie S1. Significant IIF induced by extracellular ice recrystallization (IR) during warming observed by real-time cryomicroscopy. The boxed region in the movie corresponds to Fig.3a.

Movie S2. Without ice seeding (IS) at a high sub-zero temperature, significant extracellular ice recrystallization (IR) during warming leads to extensive hemolysis

Movie S3. With ice seeding (IS) at a high sub-zero temperature, extracellular IR can be effectively inhibited to minimize hemolysis during warming

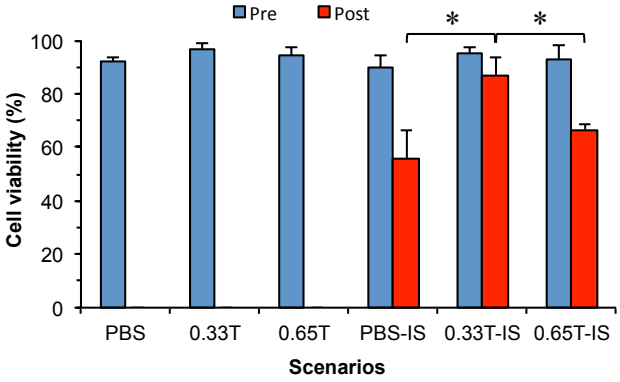

Figure S1. Viability of NIH 3T3 fibroblasts after cooling and warming. The viability was assessed after cooling and warming under the six different conditions shown in Fig. 1a.

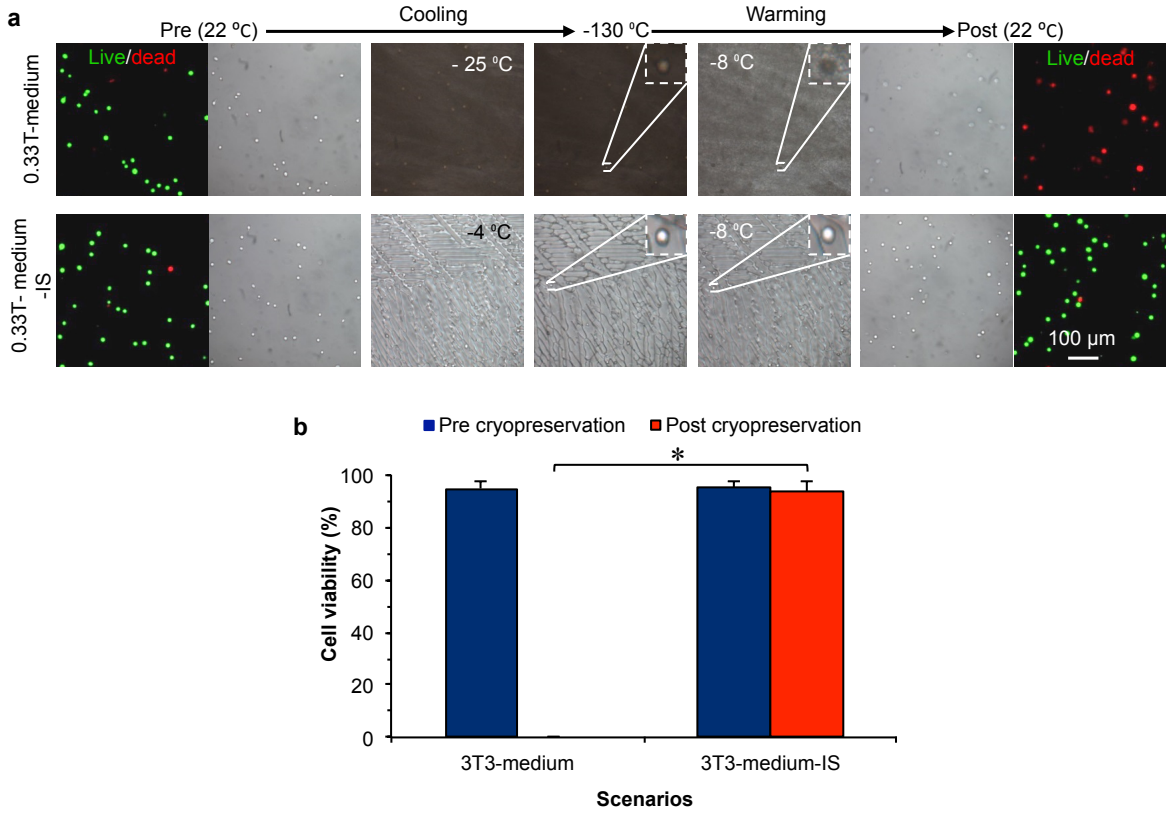

Figure S2. Cryomicroscopy studies of NIH 3T3 fibroblasts cooled to -130 °C. The cells were suspended in medium during the studies. a) Phase and fluorescence images of the cell suspension before (Pre) cooling, during cooling and warming, and after (post) warming. b) Viability of the fibroblasts before and after the cooling and warming procedure with and without ice seeding (IS) at -4 °C. *: $p < 0.05$ and $n = 3$.

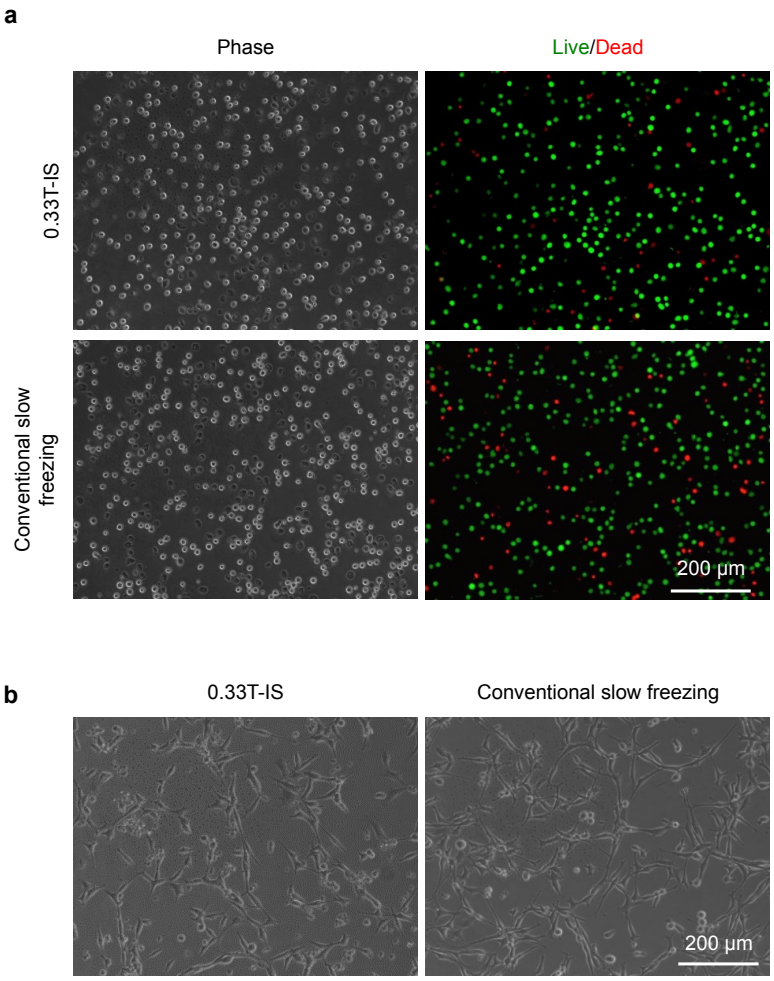

Figure S3. Morphology and viability of NIH 3T3 fibroblasts after cryopreservation. a) Phase and fluorescence images of the cells post cryopreservation using the conventional slow-freezing method versus the new pCPA-free approach. b) Phase images of the cells cultured for 20 hours post cryopreservation using the two different approaches.

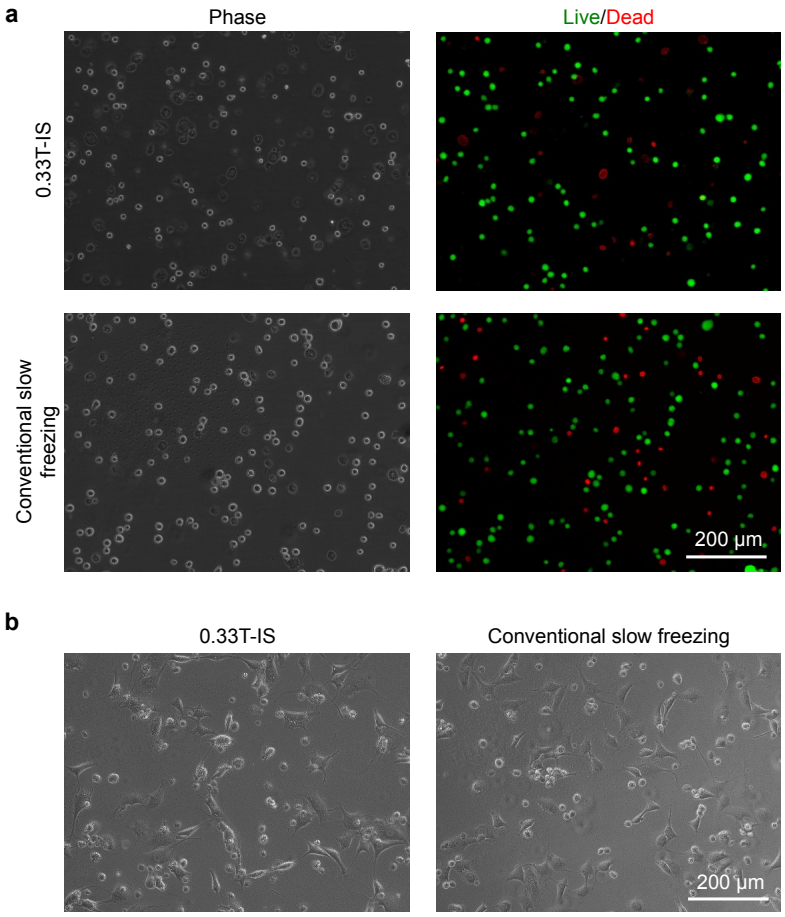

Figure S4. Morphology and viability of C3H10T1/2 mesenchymal stem cells. The cells were with cryopreservation. a) Phase and fluorescence images of the cells post cryopreservation using the conventional slow-freezing method versus the new pCPA-free approach. b) Phase images of the cells cultured for 20 hours post cryopreservation using the two different approaches.

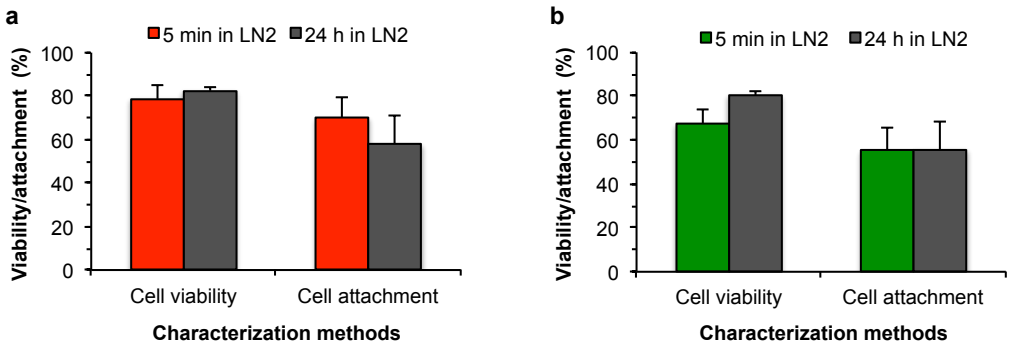

Figure S5. Effect of storage time in liquid nitrogen on cell viability and attachment. a) NIH 3T3 fibroblasts and b) C3H10T1/2 mesenchymal stem cells were cryopreserved by the new method with pre-dehydration using 0.33 M trehalose and ice seeding at -4 °C in the presence of 0.33 M trehalose (0.33T-IS). The data show that the storage time (5 min *versus* 24 h) in liquid nitrogen does not significantly affect the post-cryopreservation viability and attachment of both types of cells.

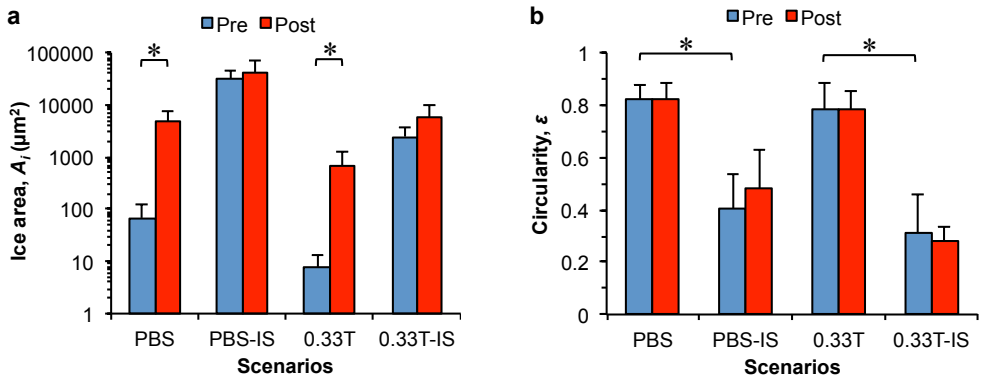

Figure S6. Effect of annealing during warming on the morphology of ice crystals. The annealing was done at -6 °C for 15 min. a) Area A_i; and b) circularity ε of ice crystals formed during cooling under various experimental scenarios before and after annealing during warming. *: $p < 0.05$ and $n = 3$.

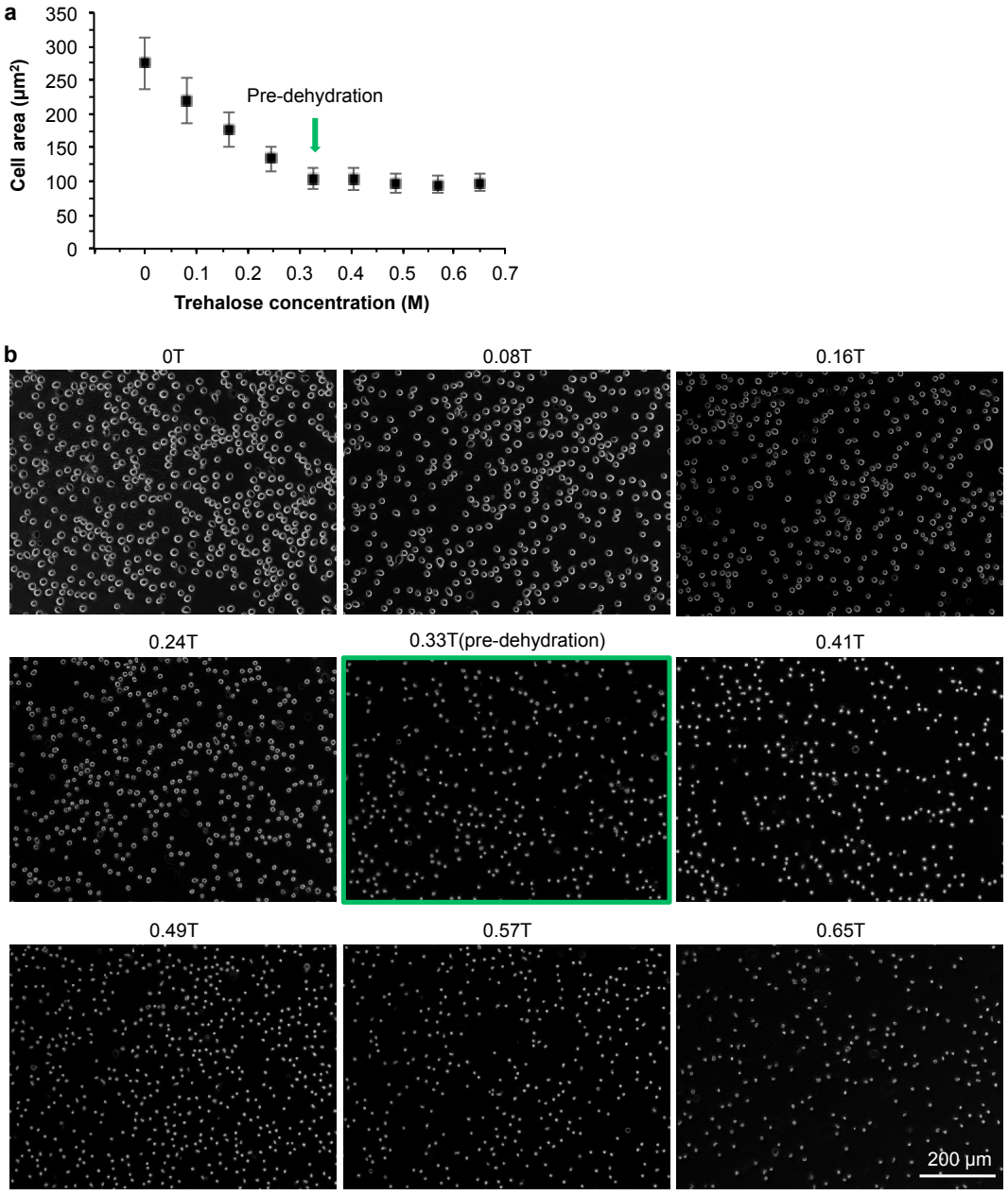

Figure S7. Pre-dehydration of NIH 3T3 fibroblasts. The pre-dehydration was done with various concentrations of trehalose in their culture medium. a) Cell area at equilibrium after pre-dehydration with various concentrations of trehalose for at least 10 minutes. b) Corresponding phase images of cells post pre-dehydration. The green arrow and box indicate the optimal/lowest concentration (0.33 M) for pre-dehydrating the cells to their minimal volume with minimized osmotically active water.

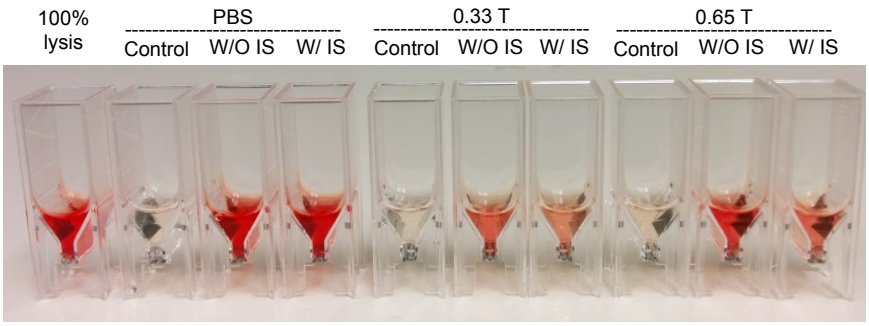

Figure S8. Analysis of human red blood cells after cryopreservation without pCPA. Supernatant collected from the samples of cells after cryopreservation under six different conditions together with the negative (100% lysis) and positive (in PBS, 0.33 M trehalose, and 0.65 M trehalose without cryopreservation) controls. "W/" and "W/O" stand for with and without, respectively.

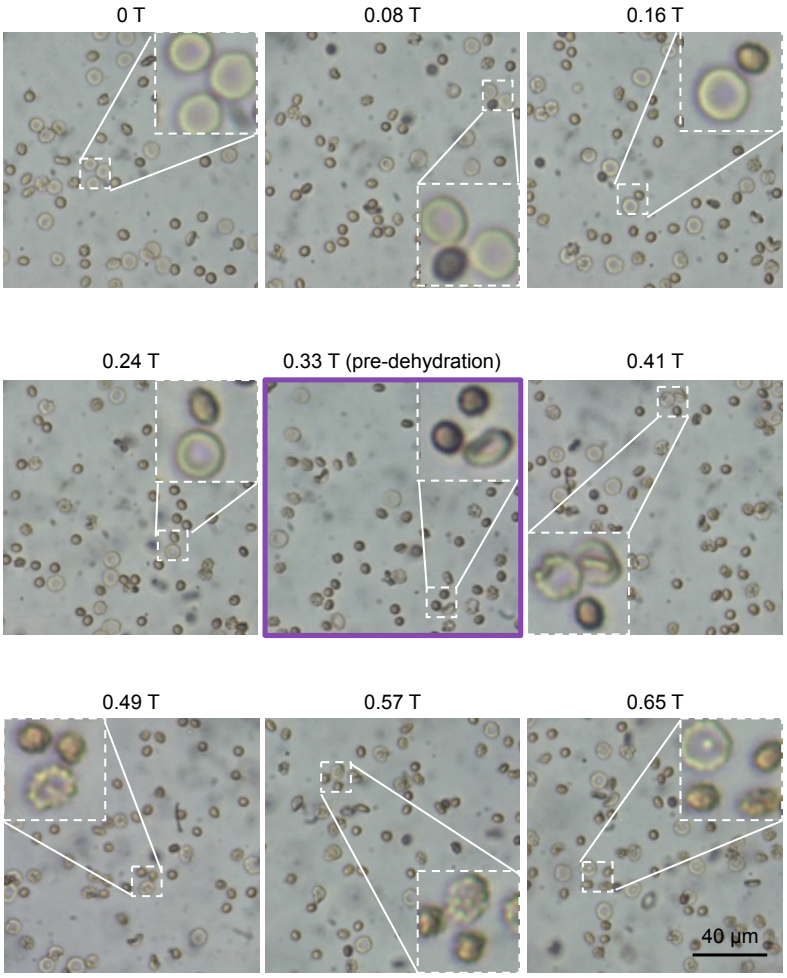

Figure S9. Pre-dehydration of hRBCs. The pre-dehydration was done with various concentrations of trehalose in their culture medium. The pre-dehydration was for at least 10 minutes to reach equilibrium. When the trehalose concentration is not lower than 0.41 M, the resultant crenated morphology of hRBCs indicates over-dehydration and cell injury. The 0.33 M trehalose can dehydrate the cells to their minimal volume without producing rough surface (surrounded by purple box). Therefore, is used as the optimal concentration for pre-dehydration of the hRBCs in this study.

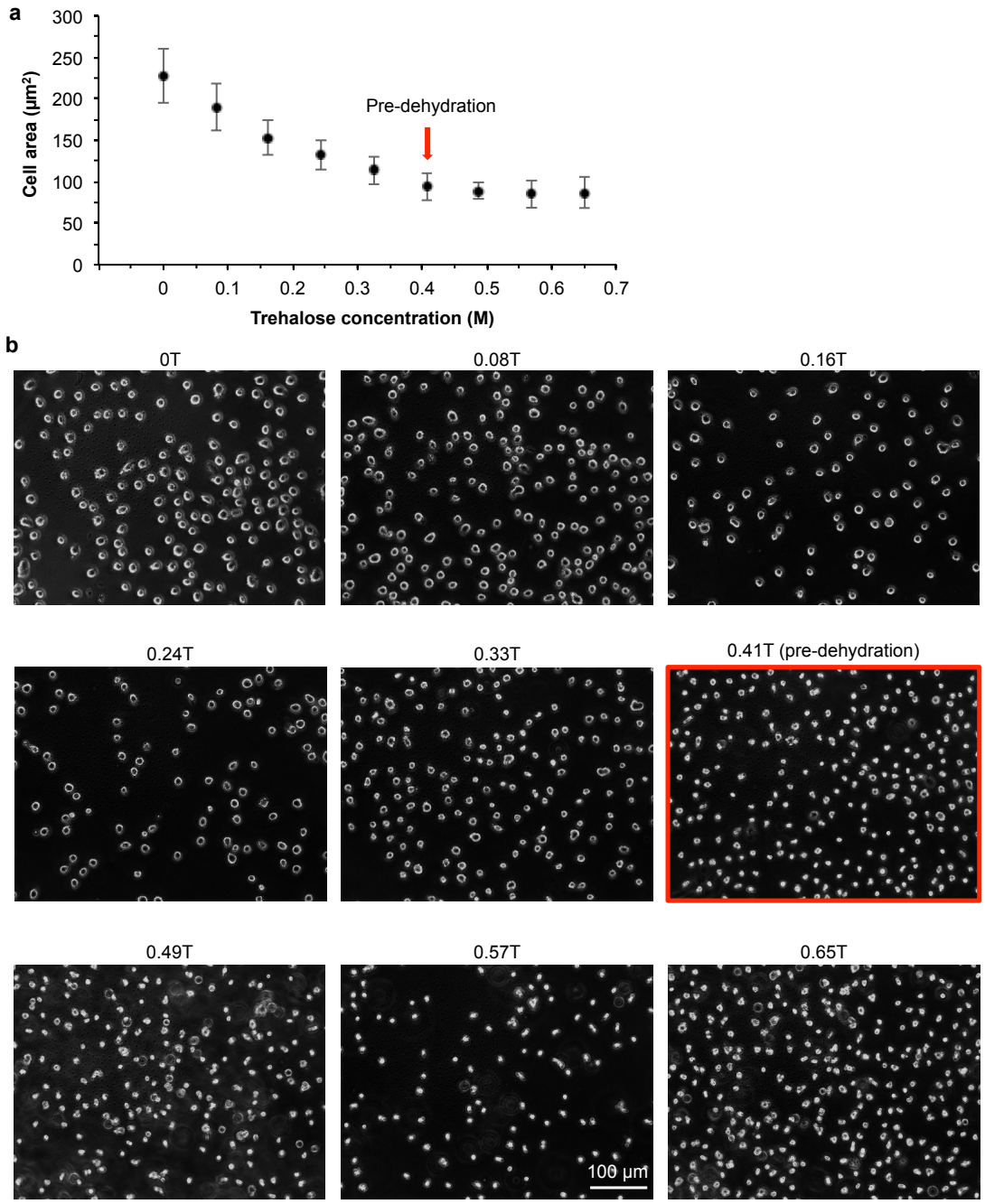

Figure S10. Pre-dehydration of C3H10T1/2 mesenchymal stem cells. The pre-dehydration was done with various concentrations of trehalose in their culture medium. a) Cell area at equilibrium after pre-dehydration with various concentrations of trehalose for at least 10 minutes. b) Corresponding phase images of cells post pre-dehydration. The red arrow and box indicate the optimal/lowest concentration (0.41 M) for pre-dehydrating the cells to their minimal volume with minimized osmotically active water.

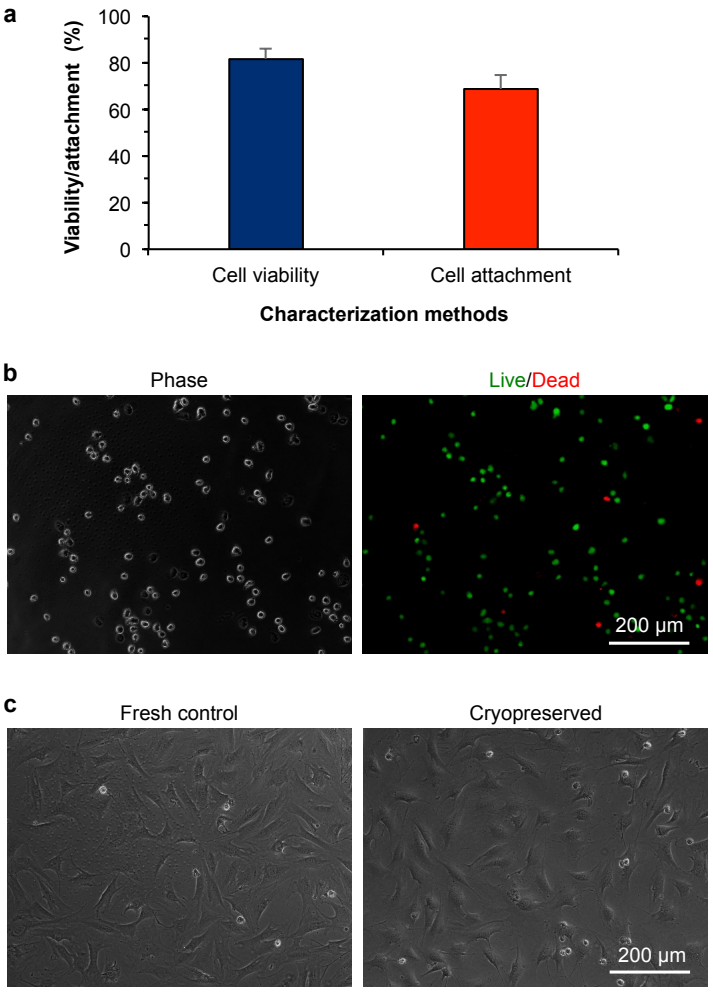

Figure S11. Cell viability and attachment of C3H10T1/2 mesenchymal stem cells. The cells were after cryopreservation with pre-dehydration using 0.41 M trehalose. a) Quantitative data of cell viability and attachment (n=4). b) Phase and fluorescence images of cells post cryopreservation with pre-dehydration using 0.41 M trehalose indicating high cell viability. c) Cell attachment images of fresh control (without cryopreservation or pre-dehydration) and experimental group (cryopreservation with pre-dehydration using 0.41 M trehalose).