

Tannic acid-serum albumin (TASA) complex maintains cell viability and protects from oxidative damage during hypothermic preservation

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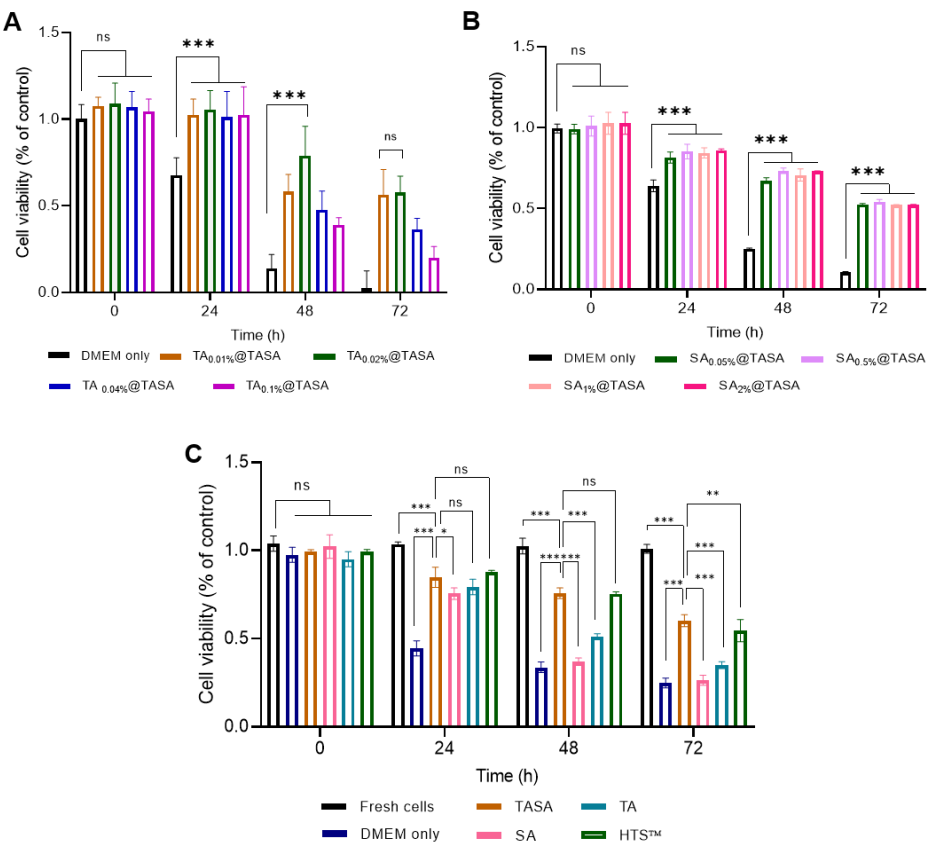
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49 **Figure S1. Optimizing the composition of tannic acid-serum albumin as a preserving solution. (A)** Optimization

50 of tannic acid (TA) concentration and **(B)** serum albumin (SB) concentration on mesenchymal stem cells (MSCs)

51 using the Cell Counting Kit-8 (CCK-8) assay. **(C)** Effect of TASA on MSCs cell viability. Hypothermolsol™ (HTS™)

52 is a commercially available hypothermic preservation solution. Data are expressed as mean ± s.d. A one-way ANOVA

53 with Tukey's multiple comparison test was used for statistical analysis. **p* < 0.05, ***p* < 0.01, and ****p* < 0.001; ns,

54 not significant.

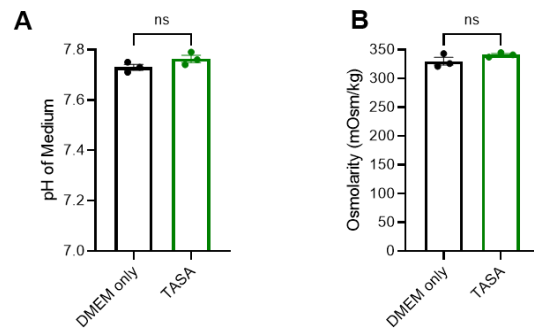
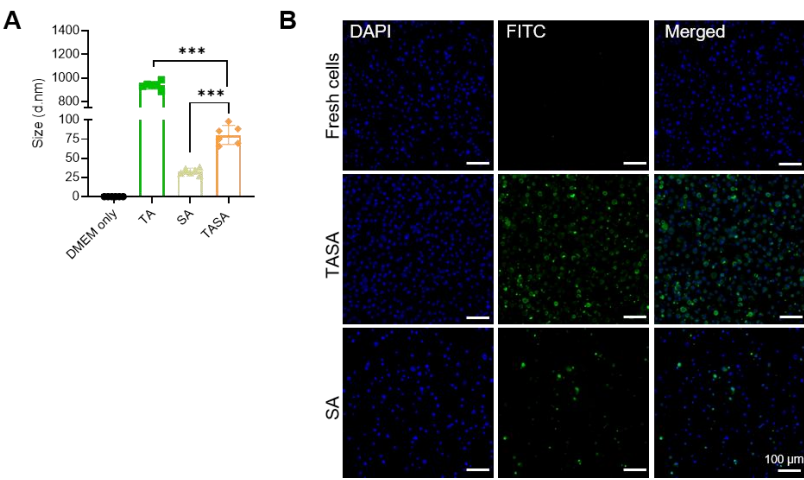


Figure S2. Analysis of (A) pH and (B) osmolarity of TASA-containing medium. Data are expressed as mean $\pm$ s.e.m. An unpaired *t*-test was applied for statistical analysis. ns, not significant.

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70 **Figure S3. Size and distribution of tannic acid-serum albumin complex (TASA).** (A) Size distribution analysis of
71 solutions: DMEM only, tannic acid (TA), serum albumin (SA), and TASA. Data are expressed as mean $\pm$ s.e.m. (B)
72 Localization and distribution of TASA and SA. Scale bar: 100 μ m. A one-way ANOVA with Tukey's multiple
73 comparison test was used for statistical analysis. *** $p < 0.001$; ns, not significant.

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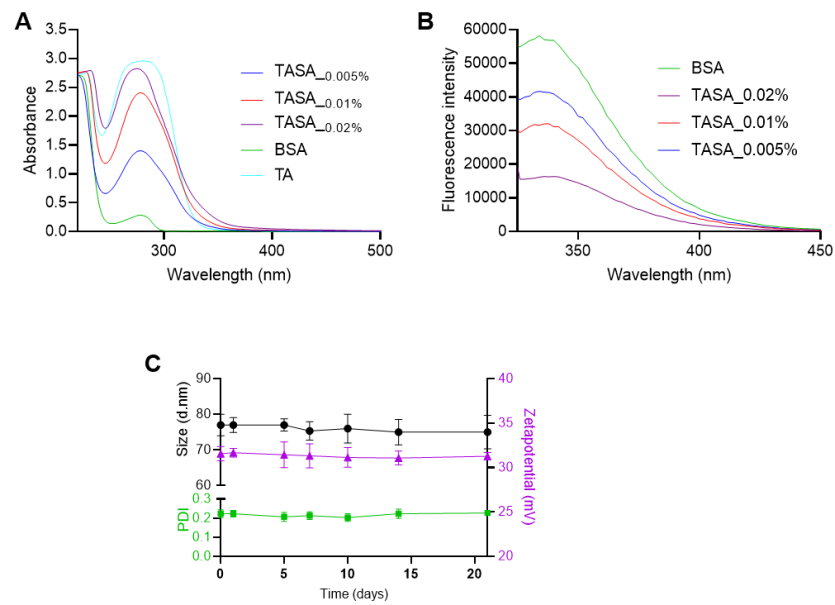
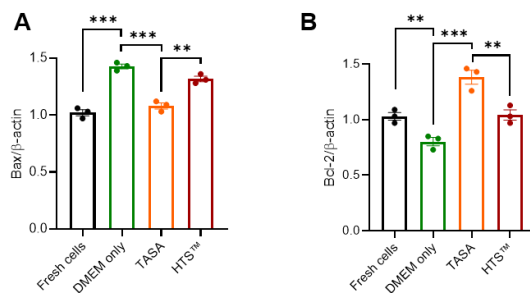


Figure S4. Characterization of the TASA complex. (A) Concentration-dependent analysis of the TASA complex using a UV-visible spectrophotometer. (B) Fluorescence quenching assay using a Synergy Neo2 Multi-Mode Plate Reader. Serum albumin conjugated with FITC was used to make the TASA complex. (C) Long-term stability of the TASA complex at 4 °C. Data are expressed as mean $\pm$ s.e.m.

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88 **Figure S5. Relative protein expression analyzed through Western blot analysis (From Figure 3C).** (A) Bax
 89 protein (n = 3) and (B) Bcl-2 protein (n = 3). β-actin was used as a loading control protein. Data are expressed as mean
 90 ± s.e.m. A one-way ANOVA with Tukey's multiple comparison test was used for statistical analysis. ** $p < 0.01$
 91 and *** $p < 0.001$.

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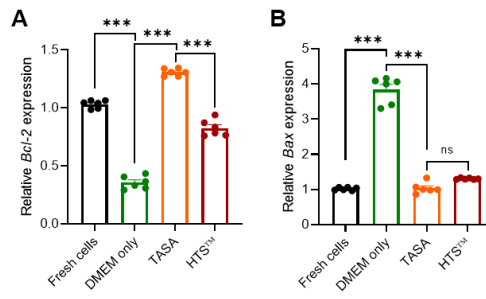


Figure S6. Relative gene expression was analyzed by PCR analysis. (A) Bcl-2 and (B) Bax mRNA expression. *Gapdh* was used as a housekeeping gene. Data are expressed as mean $\pm$ s.e.m. A one-way ANOVA with Tukey's multiple comparison test was used for statistical analysis. *** $p < 0.001$; ns, not significant.

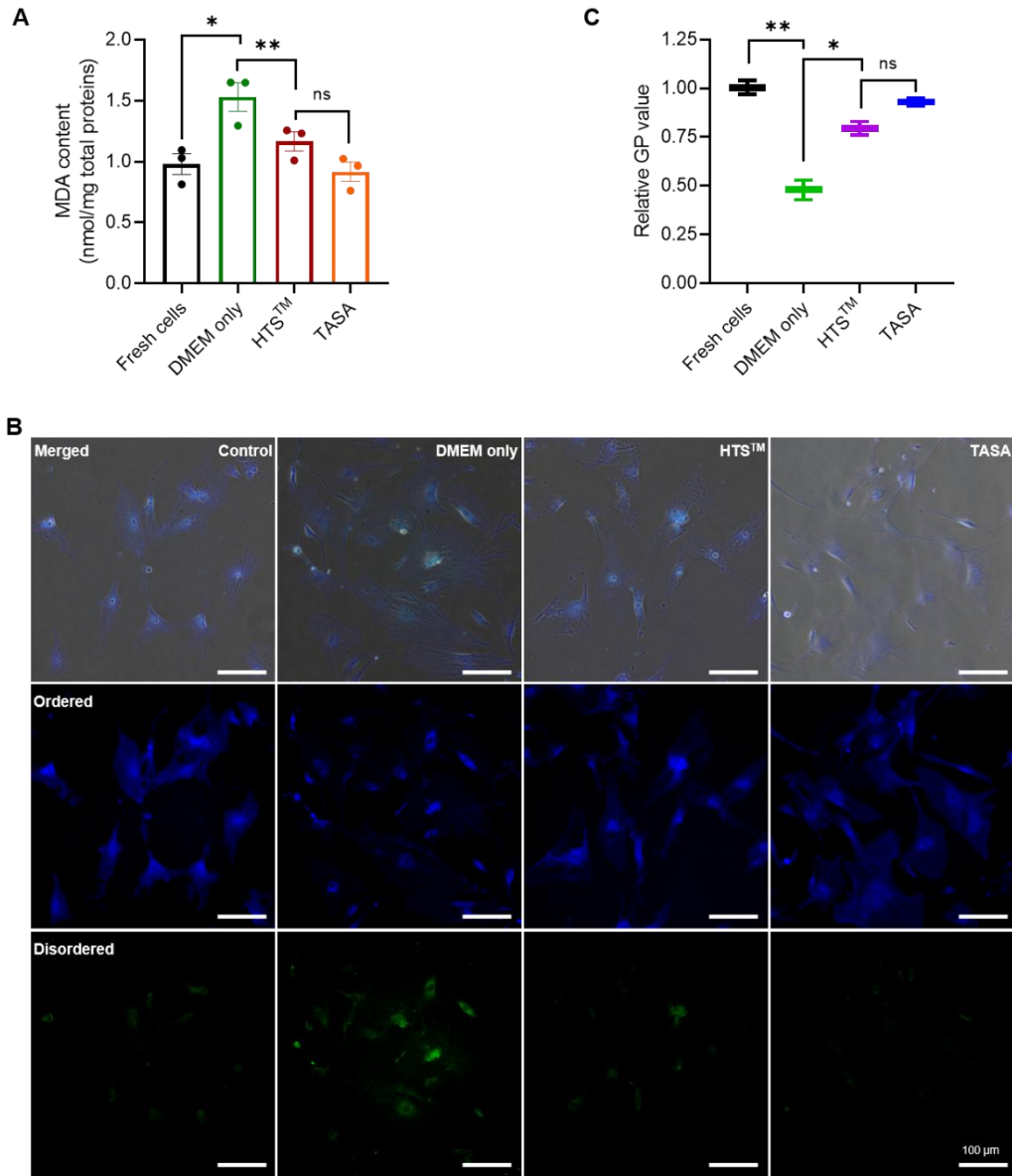


Figure S7. Effect of TASA on lipid peroxidation and fluidity during hypothermic storage. (A) Analysis of lipid peroxidation using a Malondialdehyde (MDA) assay kit. (B–C) Evaluation of cell membrane fluidity using C-Laurdan dye and its relative quantification. Blue color represents ordered lipid on the cell membrane, while green color represents disordered lipid on the cell membrane. Scale bar: 100 μ m. Data are expressed as mean $\pm$ s.e.m. A one-way ANOVA with Tukey's multiple comparison test was used for statistical analysis. * p < 0.05 and ** p < 0.01; ns, not significant.

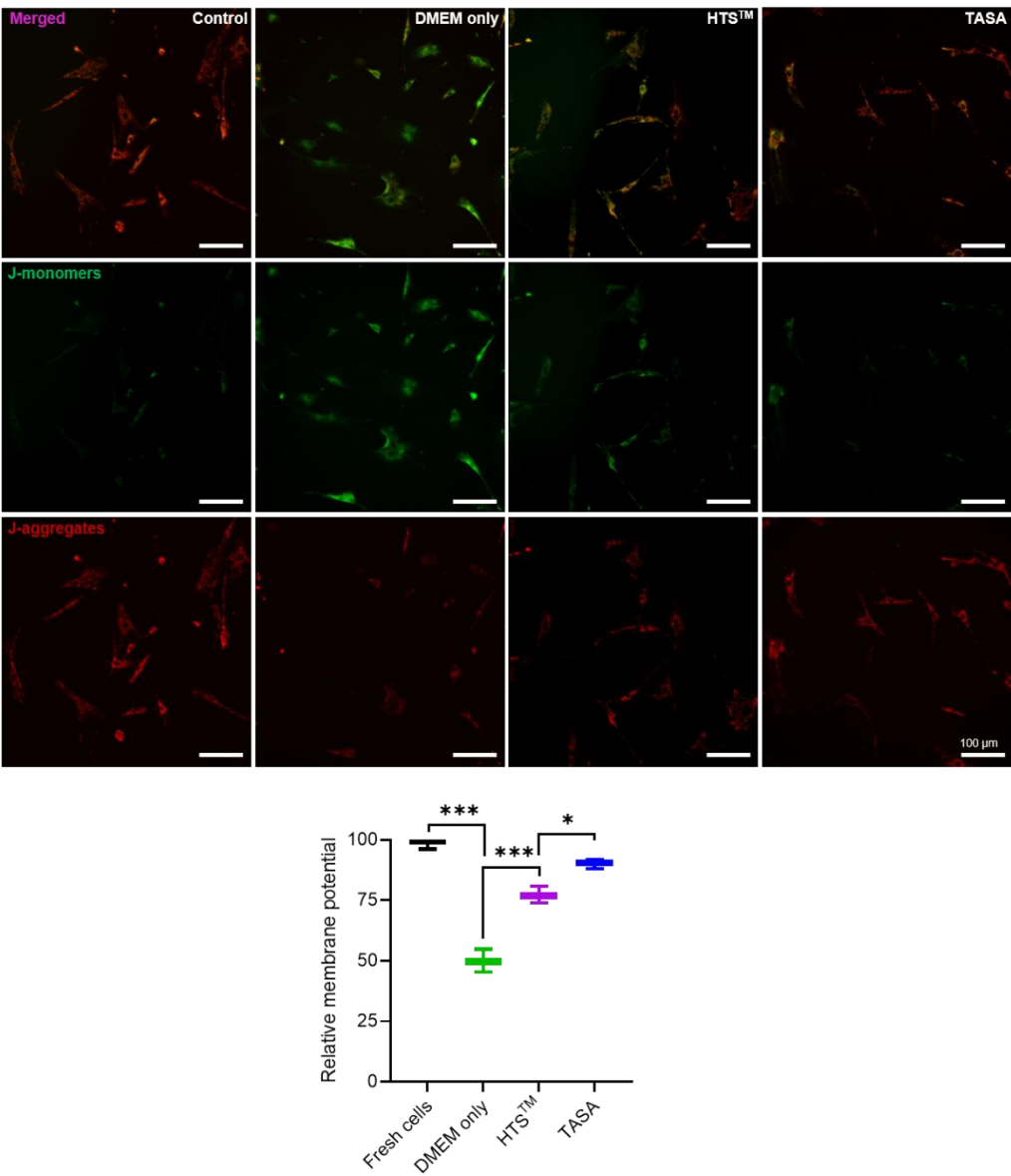


Figure S8. Membrane potential analysis using JC-1 staining after hypothermic preservation. Scale bar: 100 μm . Data are expressed as mean $\pm$ s.e.m. A one-way ANOVA with Tukey's multiple comparison test was used for statistical analysis. * $p < 0.05$ and *** $p < 0.001$.

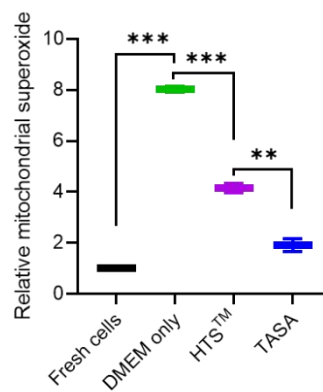
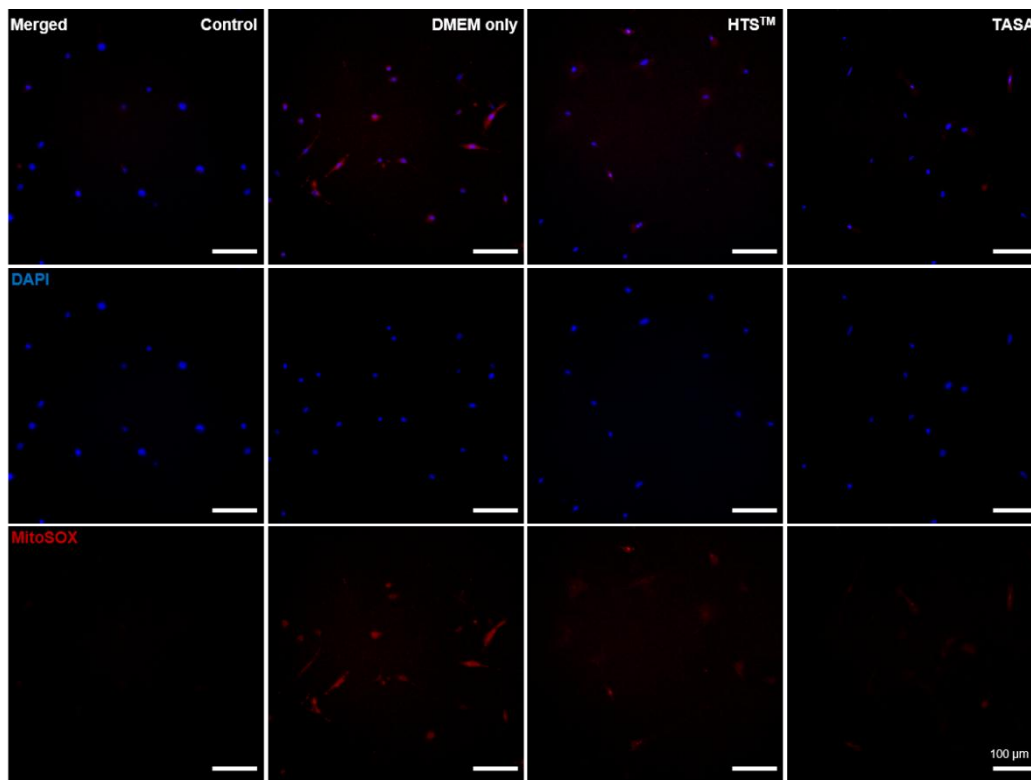


Figure S9. Mitochondrial ROS analysis using MitoSOX™ staining after hypothermic preservation. Scale bar: 100 μ m. Data are expressed as mean $\pm$ s.e.m. A one-way ANOVA with Tukey's multiple comparison test was used for statistical analysis. ** $p < 0.01$ and *** $p < 0.001$.

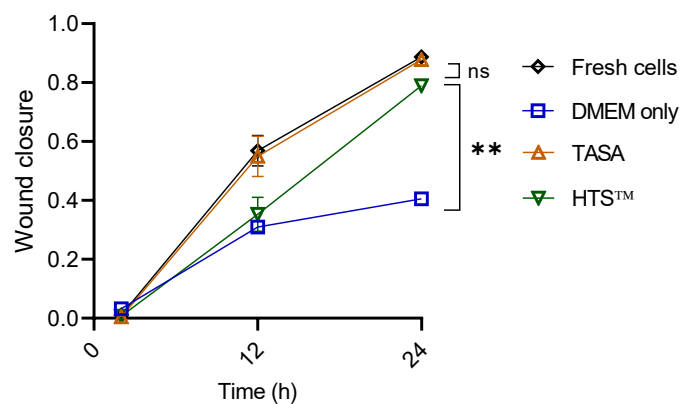


Figure S10. Relative quantification of wound closure analysis from Figure 5A. Data are expressed as mean $\pm$ s.e.m. A one-way ANOVA with Tukey's multiple comparison test was used for statistical analysis. $**p < 0.01$; ns, not significant.

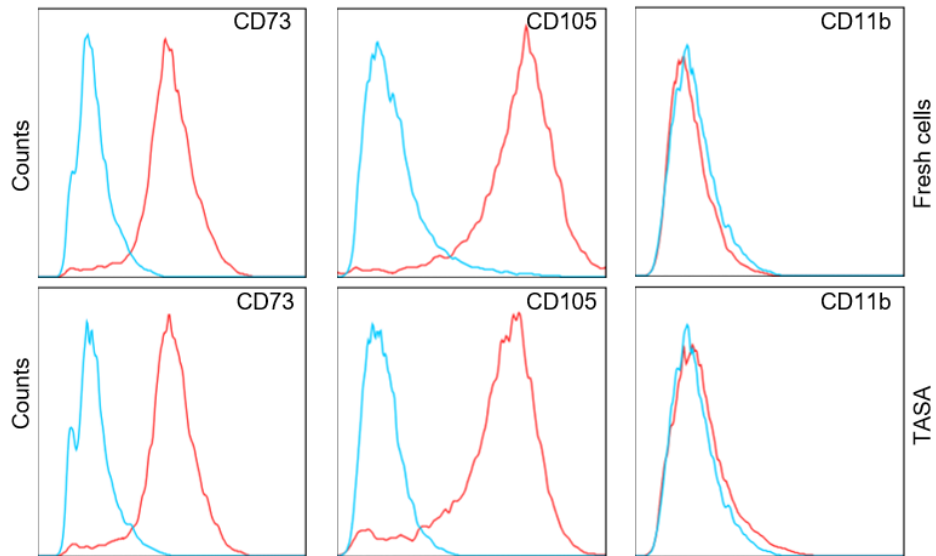


Figure S11. Positive surface markers (CD73 and CD105) and a negative surface marker (CD11b) analysis of mesenchymal stem cells (MSCs) were analyzed in normal MSCs (Fresh MSCs) and TASA-preserved MSCs using flow cytometry.

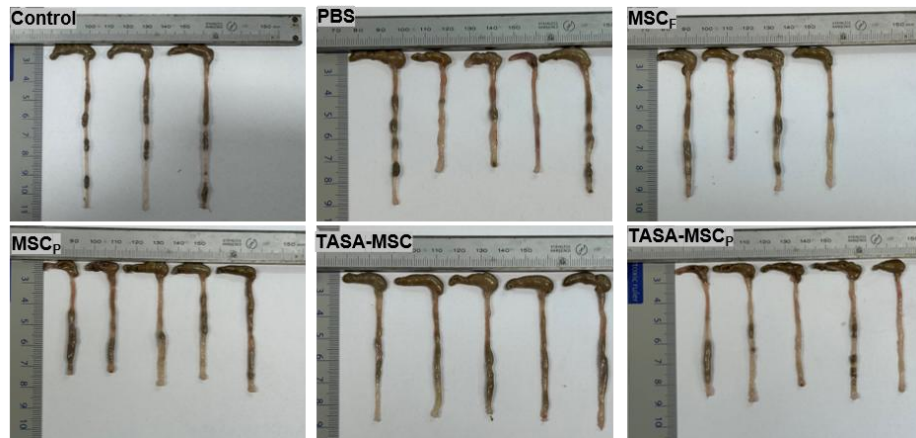


Figure S12. Images of colon retrieved from the DSS-induced colitis model. Control (n = 3), PBS (n = 5, DSS + PBS), Fresh MSC (MSC_F; n = 4, DSS + MSC_F), preserved-MSC in DMEM only (MSC_P; n = 5, DSS + MSC_P), 1 h-TASA-treated MSCs (TASA-MSC; n = 5, DSS + TASA-MSC), and TASA-preserved MSCs (TASA-MSC_P; n = 5, DSS + TASA-MSC_P).

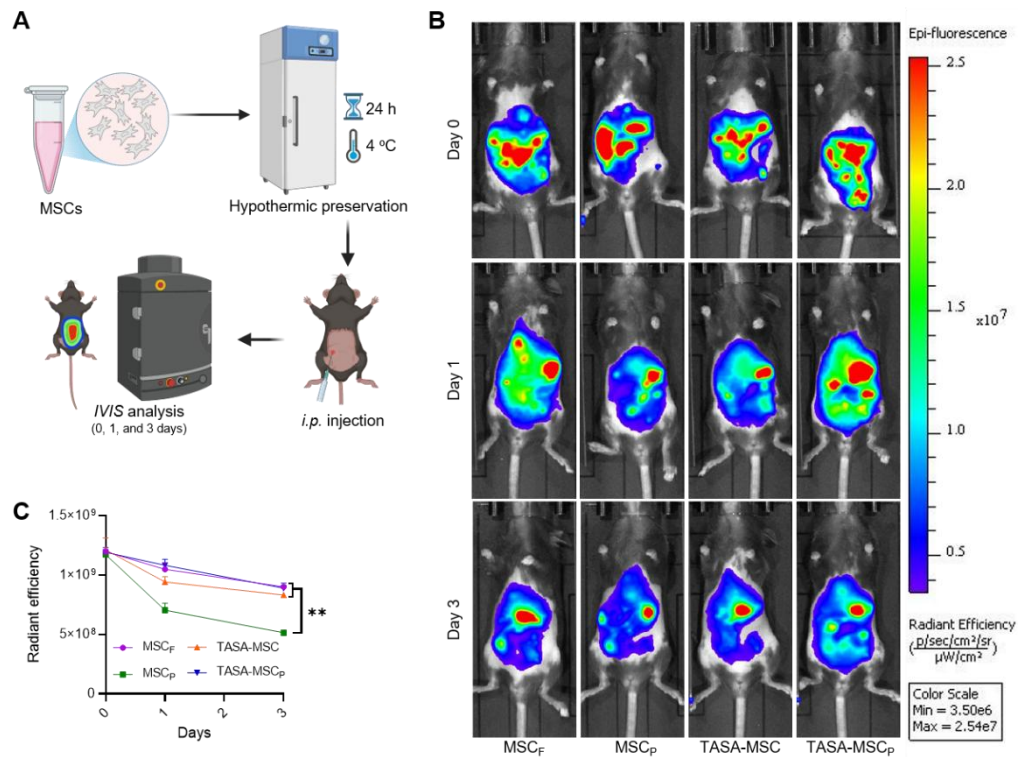


Figure S13. *In vivo* cell retention studies after hypothermic preservation. (A) Schematic illustration for the cell retention study. (B–C) MSCs (2×10^6 MSCs) from different groups; Fresh MSCs (MSC_F; $n = 3$), hypothermic groups: preserved MSCs in DMEM (MSC_P; $n = 3$), 1 h-TASA-treated MSCs (TASA-MSC; $n = 3$), and TASA-preserved MSCs (TASA-MSC_P; $n = 3$) were intraperitoneally administered, and fluorescence was analyzed using the *In Vitro* Imaging System Lumina. All cells were labelled with DiR dye. Data are expressed as mean $\pm$ s.e.m. A one-way ANOVA with Tukey's multiple comparison test was used for statistical analysis. ** $p < 0.01$.

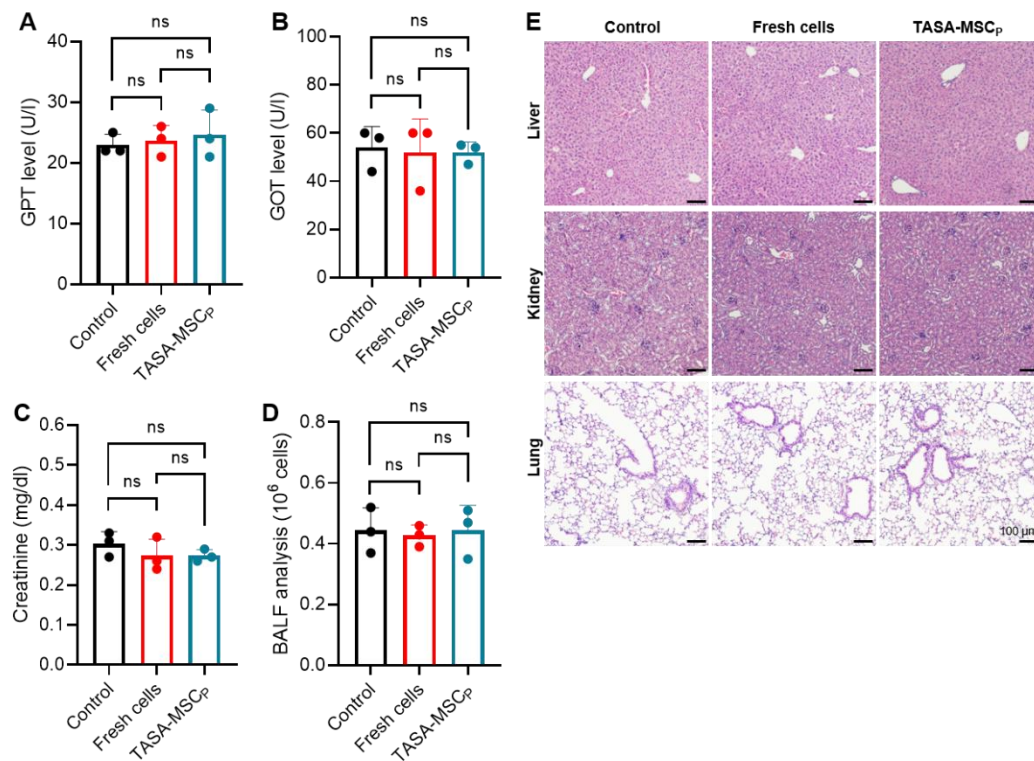


Figure S14. Biosafety evaluation of TASA-preserved mesenchymal stem cells (MSCs) after their intraperitoneal administration in healthy C57BL/6 mice. (A–B) Serum GPT and GOT were evaluated as liver injury markers. (C) Serum creatinine as a renal marker. (D) Total immune cells in bronchoalveolar lavage fluid (BALF). (E) H & E staining of liver, kidney, and lung. Scale bar: 100 μ m. Data are expressed as mean $\pm$ s.e.m. A one-way ANOVA with Tukey's multiple comparison test was used for statistical analysis. ns, not significant.

201 **Table S1.** List of mouse primer sequences.

Target gene	Primer	Nucleotide sequence
GAPDH	F	CATCACTGCCACCCAGAAGACTG
	R	ATGCCAGTGAGCTTCCCGTTCAG
Bax	F	AGGATGCGTCCACCAAGAAGCT
	R	TCCGTGTCCACGTCAGCAATCA
Bcl-2	F	CCTGTGGATGACTGAGTACCTG
	R	AGCCAGGAGAAATCAAACAGAGG
Cat	F	CGGCACATGAATGGCTATGGATC
	R	AAGCCTTCCTGCCTCTCCAACA
SOD2	F	TAACGCGCAGATCATGCAGCTG
	R	AGGCTGAAGAGCGACCTGAGTT
GPX1	F	CGCTCTTACCTTCCTGCGGAA
	R	AGTTCCAGGCAATGTCGTTGCG
p16	F	CACAAAGAGCCAGGCAGTGAAG
	R	TAGGAGGTCCACACCGTTCTTG
p21	F	TCGCTGTCTTGCACTCTGGTGT
	R	CCAATCTGCGCTTGGAGTGATAG
IL-6	F	TACCACTTCACAAGTCGGAGGC
	R	CTGCAAGTGCATCATCGTTGTTC
IL-10	F	CGGGAAGACAATAACTGCACCC
	R	CGGTTAGCAGTATGTTGTCCAGC
IL-22	F	GCTTGAGGTGTCCAACCTCCAG
	R	ACTCCTCGGAACAGTTTCTCCC
PD-L1	F	TGCGGACTACAAGCGAATCACG
	R	CTCAGCTTCTGGATAACCCTCG
NF-κB	F	GCTGCCAAAGAAGGACACGACA
	R	GGCAGGCTATTGCTCATCACAG
Zo-1	F	GTTGGTACGGTGCCCTGAAAGA

	R	GCTGACAGGTAGGACAGACGAT
Ocln	F	TGGCAAGCGATCATACCCAGAG
	R	CTGCCTGAAGTCATCCACACTC
Nrf-2	F	CACATCCAGTCAGAAACCAGTGG
	R	GGAATGTCTGCGCCAAAAGCTG
Vegf	F	CTGCTGTAACGATGAAGCCCTG
	R	GCTGTAGGAAGCTCATCTCTCC