Thawed cryopreserved synovial mesenchymal stem cells show comparable effects to cultured cells in the inhibition of osteoarthritis progression in rats

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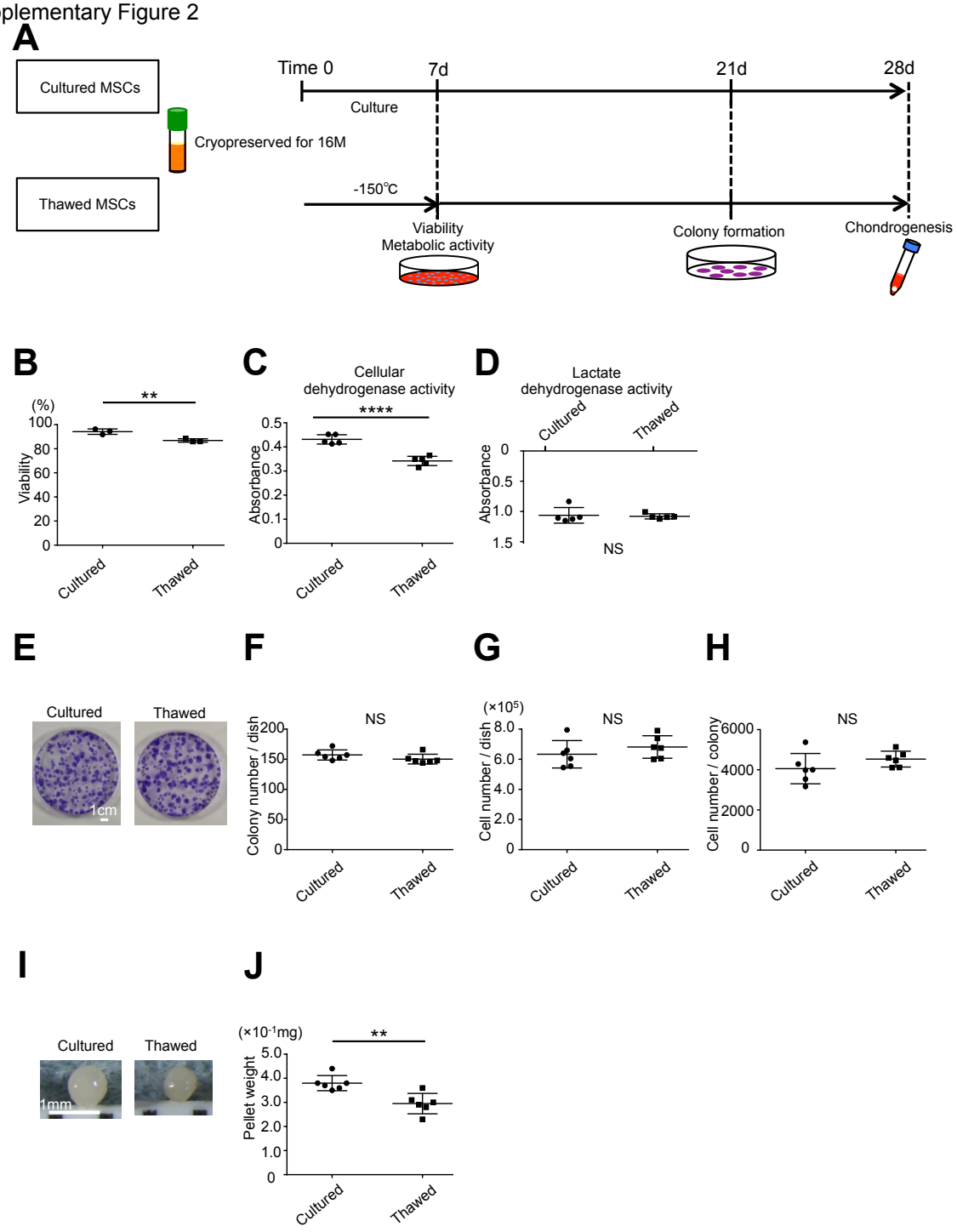
SUPPLEMENTARY INFORMATION

Additional supporting information may be found in the online version of this article:

Supplementary Figure 2



Supplementary Figure 2. Effects of 16 months of cryopreservation on the in vitro viability and properties of rat synovial MSCs. (A) Scheme: A sample of 1×10^6 synovial MSCs suspended in 1000 μL preservation fluid containing 95% FBS with 5% DMSO was cryopreserved at −150 °C for 16 months and then used as thawed MSCs. Thawed MSCs were also cultured for 1 week for use as cultured MSCs. The cells were analyzed for viability and metabolic activity. A 0.5 μL volume of cell suspension (containing 500 cells, including living and dead cells) was allocated to a 60 cm^2 dish and cultured for colony formation. A 250 μL volume of cell suspension (containing 2.5×10^5 cells, including living and dead cells) was allocated to a 15 mL tube and cultured for chondrogenesis. (B) Viability was assessed by trypan blue staining. The average with SD is shown (n=3). (C) Cellular dehydrogenase activity was used to confirm live cell metabolic activity (n=5). (D) Lactate dehydrogenase activity was used as a marker of dead cells (n=5). (E) Colony formation: colonies were stained with crystal violet. (F) Colony number per dish (n=6). (G) Cell number per dish (n=6). (H) Cell number per colony (n=6). (I) Cartilage pellets. (J) Cartilage pellet weight (n=6). ND, not detected; NS, not significant; **p < 0.01, ****p < 0.0001 by Student's t-test (Fig. 1B, C and D) or Mann-Whitney's U test (Fig. 1F, G, H and J).