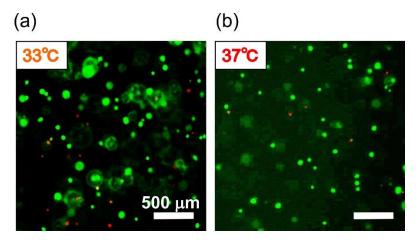
## Supplementary Materials: Mesenchymal Stem Cell Culture on Poly(*N*-isopropylacrylamide) Hydrogel with Repeated Thermo-Stimulation

Aya Mizutani Akimoto, Erika Hasuike Niitsu, Kenichi Nagase, Teruo Okano, Hideko Kanazawa, and Ryo Yoshida

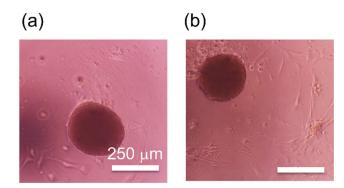
## LIVE/DEAD Assay

The cytotoxicity of the PNIPAAm gel was evaluated using the LIVE/DEAD Viability/Cytotoxicity Kit (L3224). The PNIPAAm gel was synthesized by mixing poly(NIPAAm-co-NAPMAm), pNHS-octa-PEG, and RGDS peptides in the presence of hbmMSCs ( $2.0 \times 10^5$  cells/mL) to encapsulate cells. After seven days of culture in a humidified atmosphere with 5.0% CO $_2$  at  $37^{\circ}$ C, the encapsulated cells were double-stained with an acetomethoxy derivative of calcein (for live cells) and ethidium homodimer I (for dead cells) according to the general procedure.



**Figure S1.** Fluorescence images showing the results of LIVE/DEAD assay for hbmMSCs encapsulated inside the PNIPAAm gel after seven days of culture at (a) 33 °C and (b) 37 °C.

## hbmMSC Culture Using Normal Cell Culture Media



**Figure S2.** Phase-contrast images of hbmMSCs on the PNIPAAm gel at (a) 33°C and (b) 37°C after four days in culture using MSCBM as cell culture media.

Although the large cell aggregation was observed, all other hbmMSCs showed elongated shapes.