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Supplementary Materials for

Collagen hydrogel viscoelasticity regulates MSC chondrogenesis in a ROCK-dependent manner

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Figs. S1 to S9

Supplementary Materials

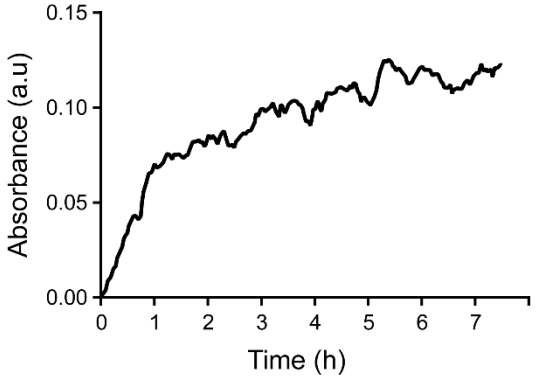

Fig S1. Turbidity curve of collagen solution obtained at 313 nm and 4 °C. Collagen concentration: 3 mg/ml.

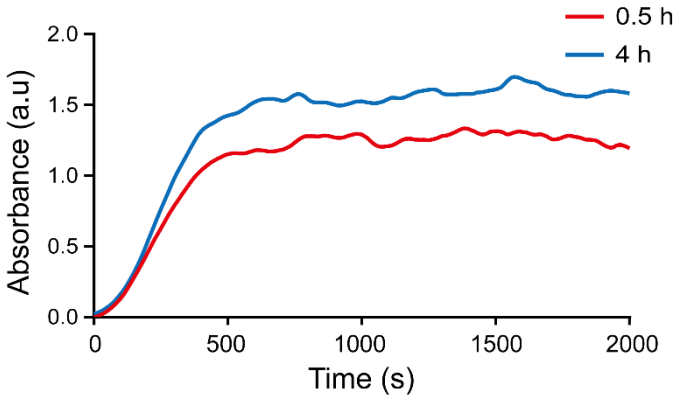

Fig S2. Turbidity curves of collagen solution obtained at 313 nm and 37 °C after low-temperature incubation for 0.5 and 4 h. Collagen concentration: 3 mg/ml.

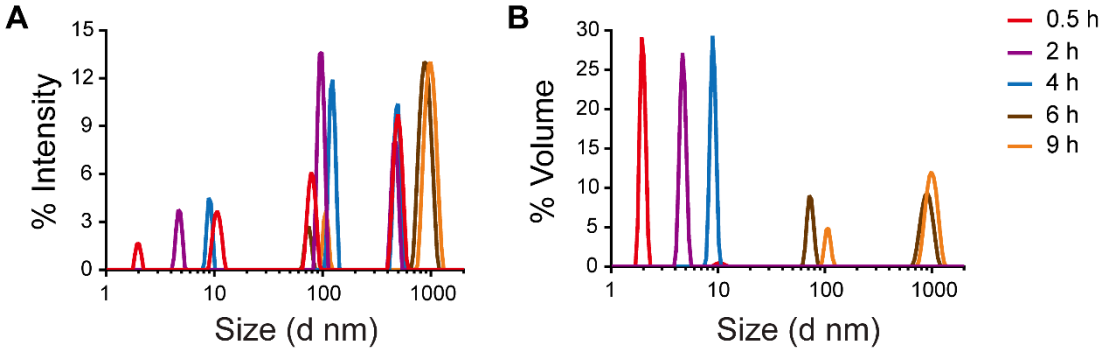

Fig S3. The size distribution of collagen fibers in collagen solutions with different low-temperature incubation time. (A) Light intensity particle size distribution of collagen fibers. **(B)** Volume particle size distribution of collagen fibers. Collagen concentration: 3 mg/ml.

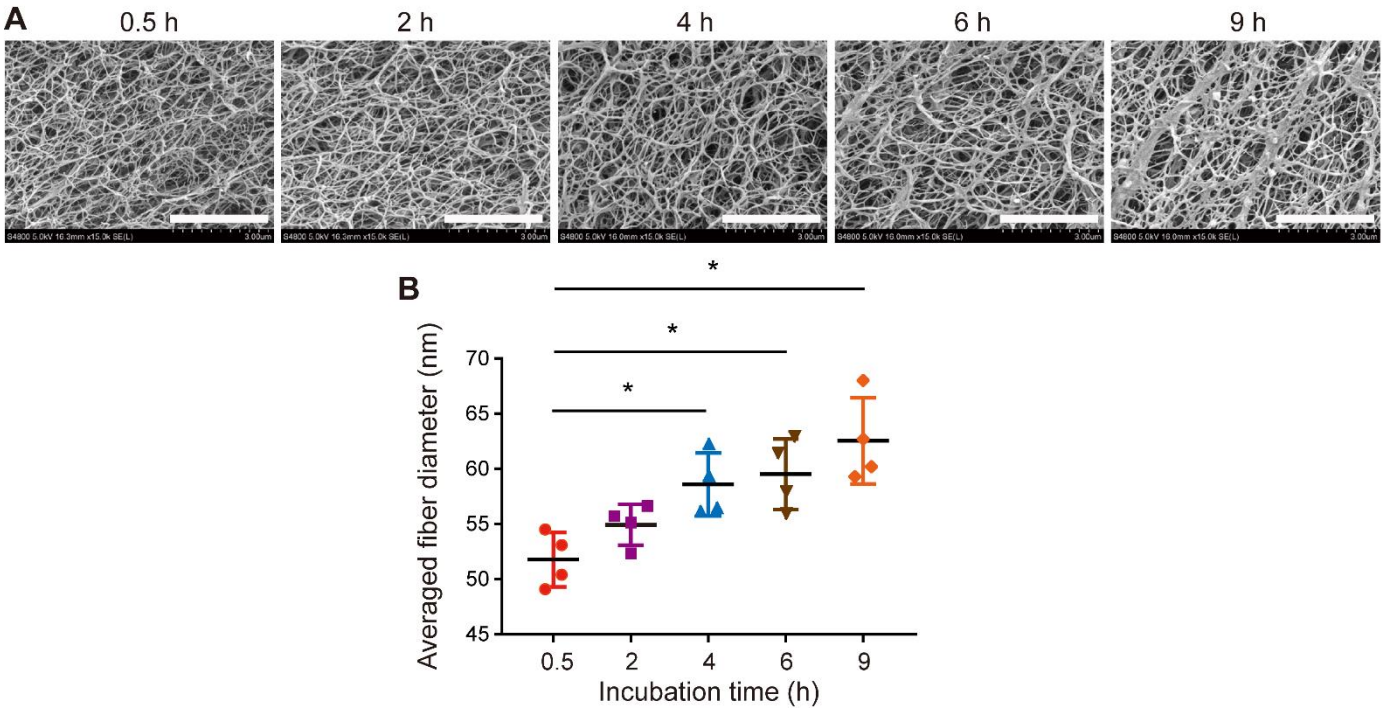

Fig S4. SEM characterization of collagen fibers with different low-temperature incubation times. (A) SEM images of critical point-dried collagen hydrogels with various low-temperature incubation time. Scale bars, 3 μm. **(B)** Quantification of the diameter of collagen fibers (averaged from four regions; $*P < 0.05$). Collagen concentration: 3 mg/ml.

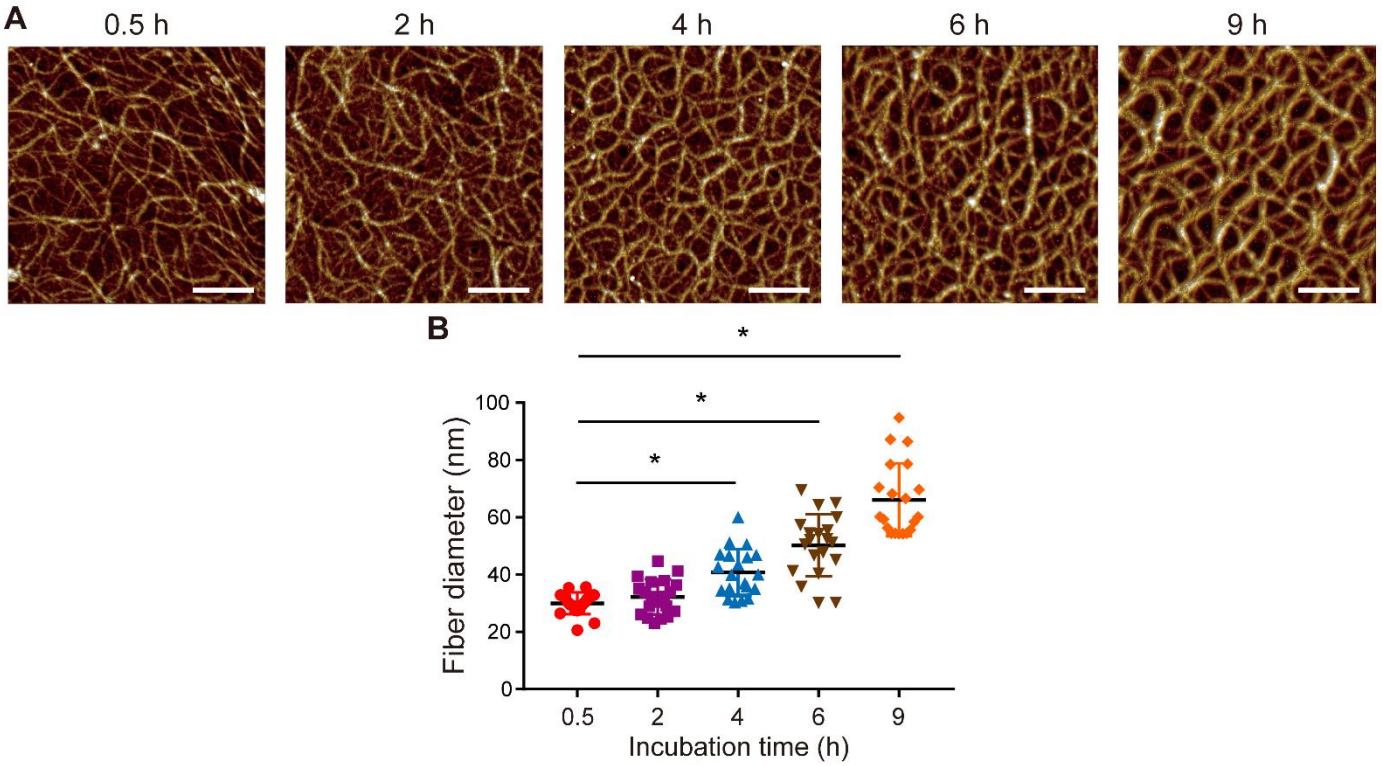

Fig S5. AFM characterization of collagen fibers with different low-temperature incubation times. (A) AFM images of dried collagen hydrogels with various low-temperature incubation time. Scale bars, 500 nm. **(B)** Diameter of individual collagen fibers with various low-temperature incubation time (n = 21 single fibers from three regions per each condition; **P* < 0.05). Collagen concentration: 3 mg/ml.

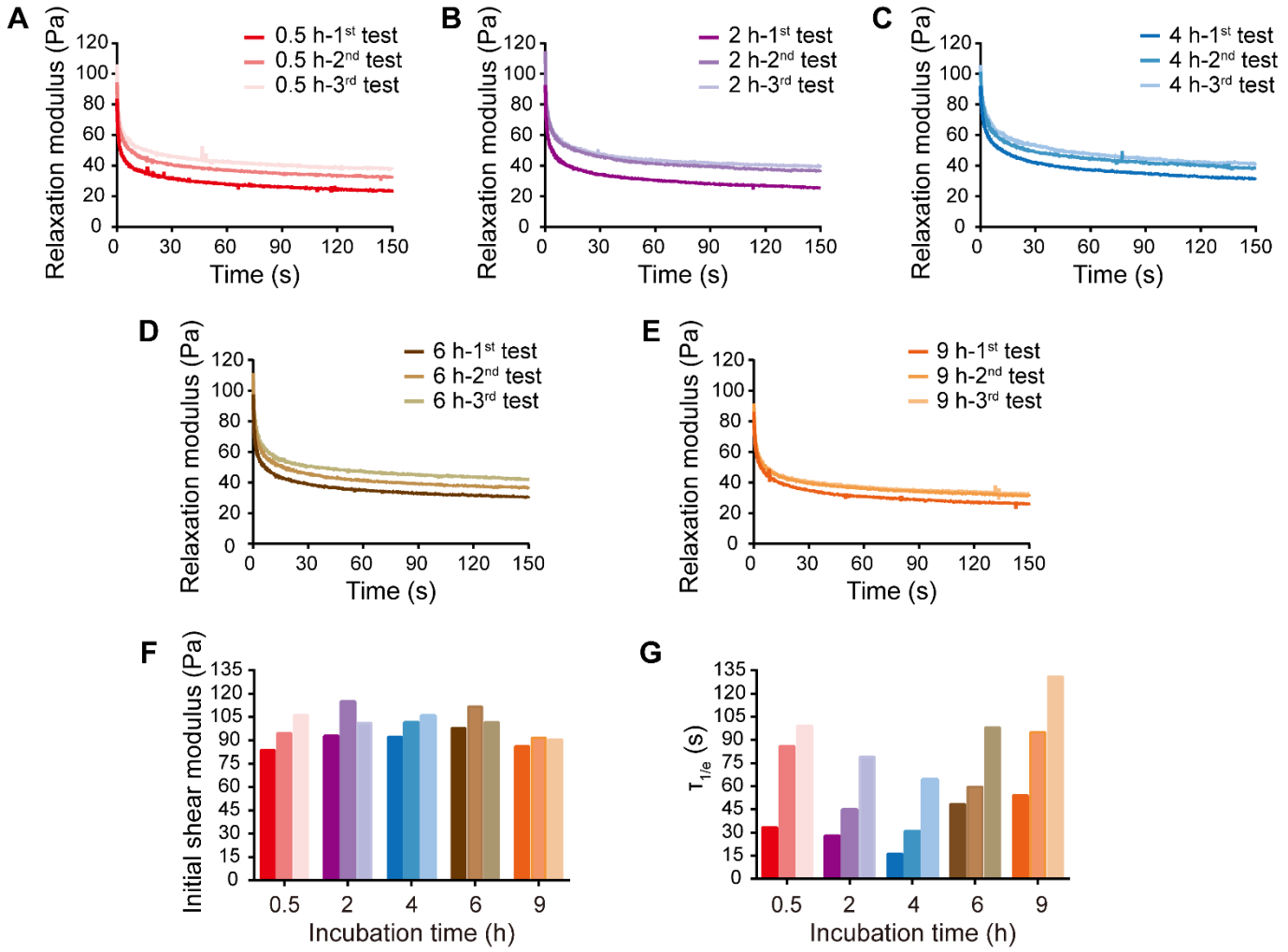

Fig S6. Repeated stress relaxation tests of collagen hydrogels with various low-temperature incubation times. (A-E) Representative repeated stress relaxation curves of collagen hydrogels with various low-temperature incubation time at a strain of 10%. **(F)** Initial shear modulus as determined by repeated stress relaxation tests of collagen hydrogels. **(G)** Time scale of repeated stress relaxation, $\tau_{1/e}$, for the different collagen hydrogels.

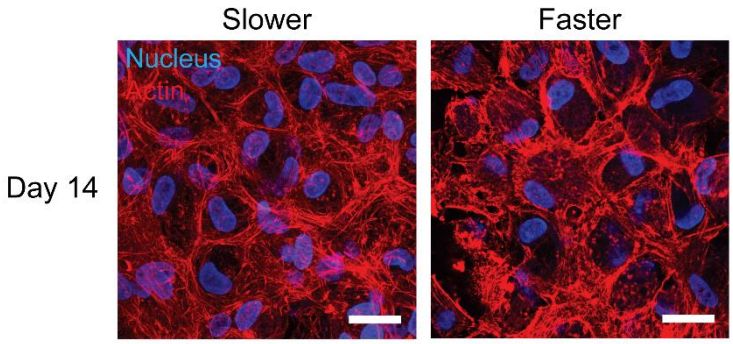

Fig S7. Representative images of fluorescence staining for F-actin of MSCs cultured in hydrogels for 14 days. Scale bars, 10 μm.

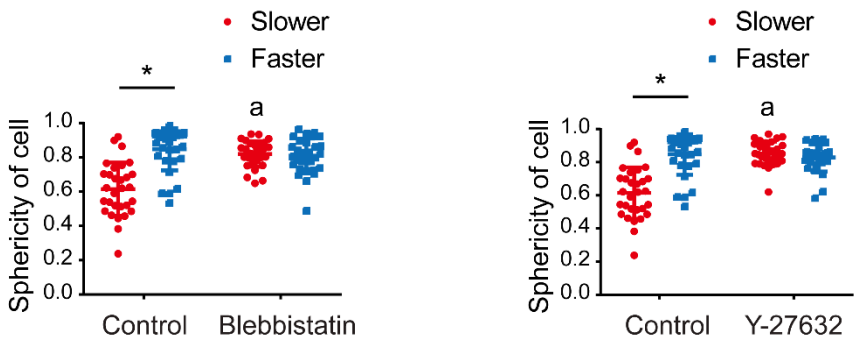

Fig S8. Quantification of sphericity of cells in the presence of blebbistatin or Y-27632 (n = 30 single cells from three biological replications per each condition; **P* < 0.05); a indicates a significant difference (**P* < 0.05) compared with the slower hydrogels in control group.

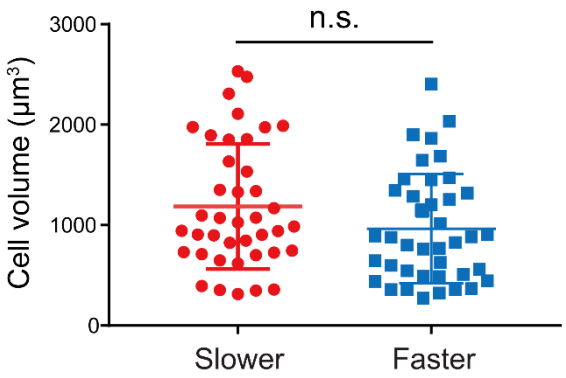

Fig S9. Quantification of cell volume in slower/faster-relaxing groups on day 7 (n = 30 single cells from three biological replications per each condition; $P = 0.4073$).