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## Supplementary Information

## Human induced pluripotent stem cell-derived cardiac muscle rings for biohybrid selfbeating actuator

Tomohiro Morita, Minghao Nie, Shoji Takeuchi

Graduate School of Information Science and Technology, The University of Tokyo



**Figure S1. Setup for measuring the contractile force of a cardiac ring.** *Scale bar; 2 mm.* 



**Figure S2. Spontaneous contractile property with applied strain.** (a) *A functional evaluation of cardiac muscle rings, with antagonistic muscle structures added after removal from the substrate, and those that continued cultivation within the substrate.* (b) *Spontaneous contractile frequency with applied strain. All scale bars; 1.5 mm.* 



Figure S3. Presence and absence of tension balance by antagonistic pair structure. (a) Images of driving parts of the biohybrid self-beating actuators with and without antagonistic structure on the 5<sup>th</sup> day of culture. To observe the muscle shortening phenomenon, we verified the thickness of the PDMS body to 800, 300, and 150  $\mu$ m. (b) Distance between the anchors vs body thickness. All scale bars; 500  $\mu$ m.