Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary figures and legends:

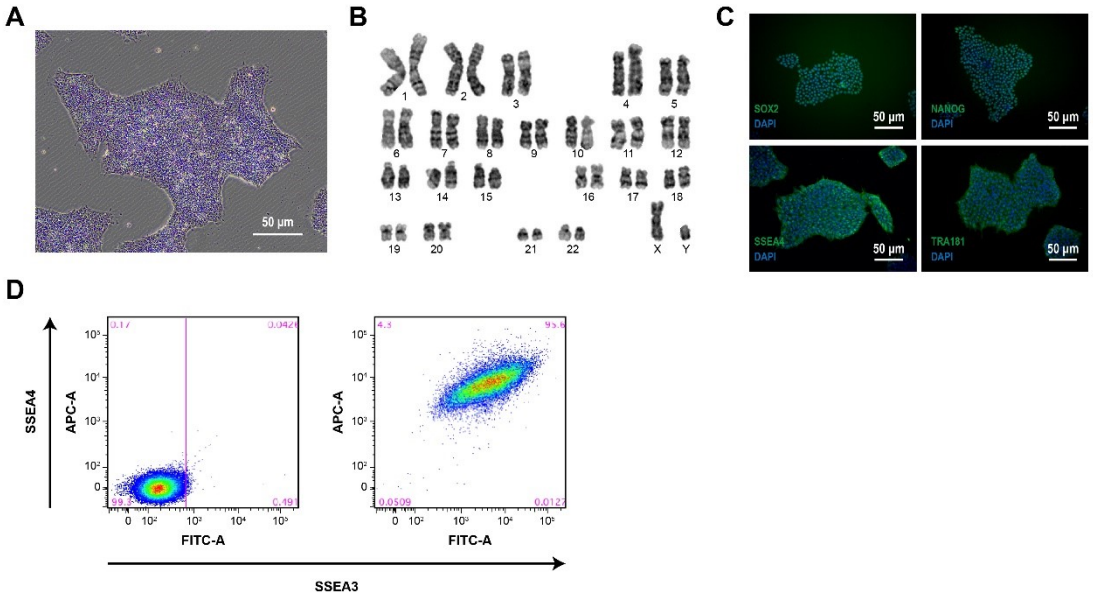

Figure S1: The characterization of hiPSCs

(A) Phase-contrast image of hiPSCs. (B) The karyotype of hiPSC line. (C) Immunofluorescence staining analyses of pluripotent markers. SOX2, NANOG, SSEA4 and TRA181, Alexa 488 (Green); nuclei, DAPI (blue). (D) Flow cytometry analyses of pluripotent markers (SSEA3 and SSEA4). the left hand side FACS plot was isotype control, the right hand side FACS plot was stained hiPSCs.

Scale bar: A, B and C=50µm.

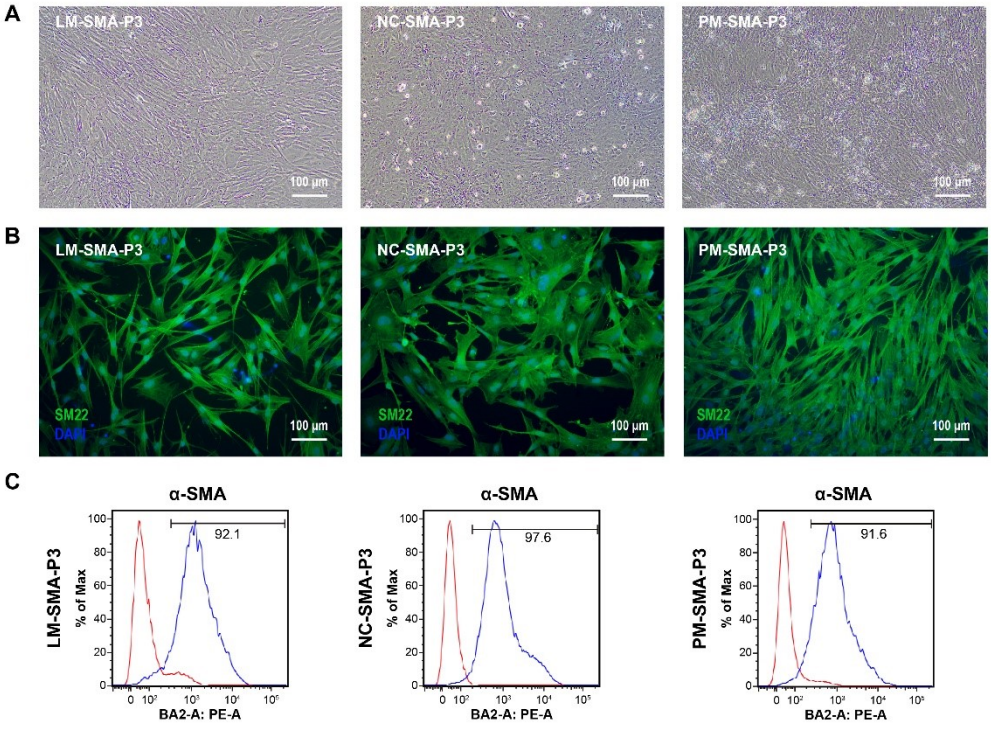

Figure S2: The characterization of expanded hiPSC-SMCs lineages

(A) Phase-contrast images of expanded hiPSC-SMCs lineages. (B) Immunofluorescence staining analyses of the expression of SM22. SM22, Alexa 488 (Green); nuclei, DAPI (blue). (C) Flow cytometry analyses of vascular smooth muscle marker α-SMA in expanded indicated hiPSC and hiPSC-SMCs. Scale bar: A, B and C=100μm.

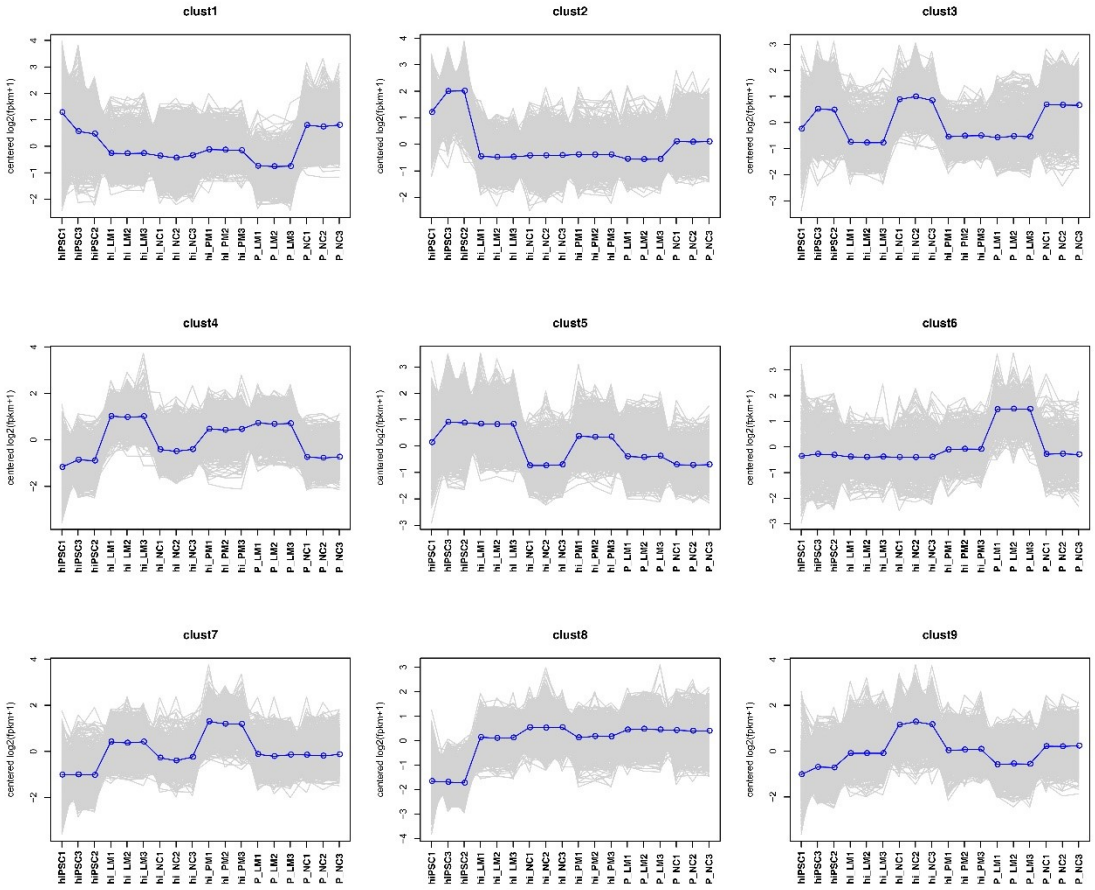

Figure S3: The distribution kinetics of differential gene clusters in hiPSCs, Primary SMCs and hiPSC-SMCs lineages

The distribution curve showing the enriched gene clusters in hiPSCs, Primary SMCs and hiPSC-SMCs lineages.

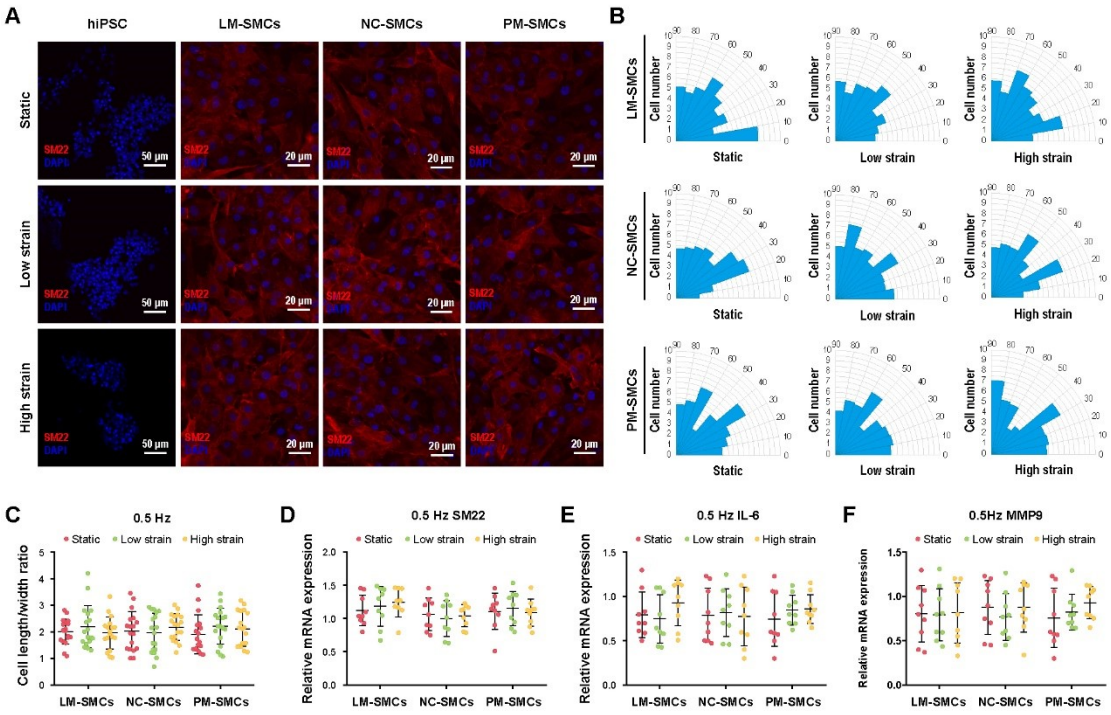

Figure S4: Effects of different cyclic strain in 0.5Hz on hiPSCs, LM-SMCs, NC-SMCs and PM-SMCs in the chip system.

(A) Immunofluorescence staining of SM22 in both the static and stretched hiPSCs, LM-SMCs, NC-SMCs and PM-SMCs (0.5Hz). (B) Alignments of LM-SMCs, NC-SMCs and PM-SMCs exposed to static, low or high strain for 24 hours (0.5Hz). (C) Length-to-width ratio of hiPSC-SMCs after exposuring to static, low or high strain for 24 hours (0.5Hz). (n=6, cells were measured in three fields per sample). (D-F) RT-qPCR analyses for the expression of SM22, IL-6 and MMP-9 (0.5Hz), (n=3). Data from three independent biological replicates each with two or four technical replicates. All the data are expressed as the means ± standard deviation. The *P* values between two groups were calculated using unpaired Student's t test or one-way analysis of variance (ANOVA). *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Scale bar=50μm, 20μm.

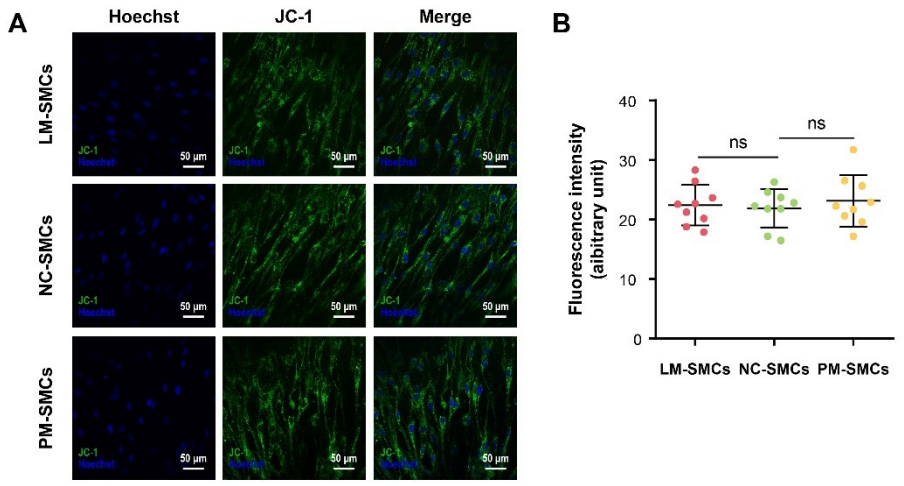

Figure S5: The rational coordination and consistency of the hiPSC-SMCs based organ-on-a-chip.

(A) Representative images of JC-1 staining of the mitochondrial membrane potentials in LM-SMCs, NC-SMCs and PM-SMCs exposed to suitable rhythm and strain for 24 hours. The scale bar=50μm. (B) Intensity of immunofluorescence staining of JC-1 (n=3, data from three independent biological replicates each with two or four technical replicates.) All the data are expressed as the means ± standard deviation. The *P* values between two groups were calculated using unpaired Student's t test or one-way analysis of variance (ANOVA). ns, not significant.

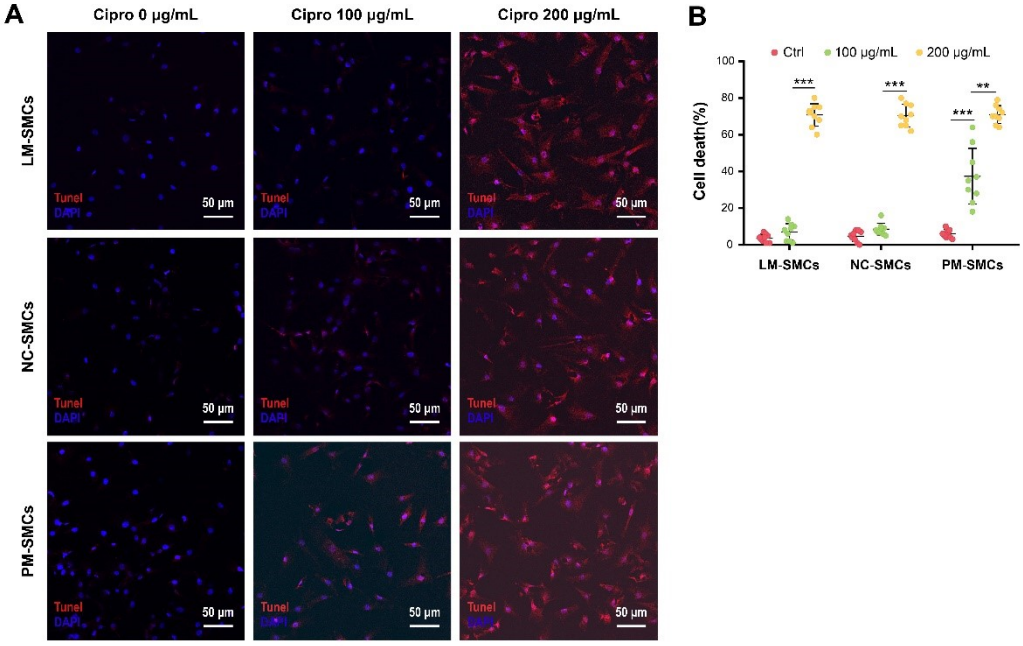

Figure S6. The effects of ciprofloxacin on apoptosis of hiPSC-SMCs. **(**A-B) Representative results of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) analyses showing that ciprofloxacin treatment enhanced the apoptosis in hiPSC-SMCs (n=6, cells were measured in three fields per sample). All the data are expressed as the means ± standard deviation. The *P* values between two groups were calculated using unpaired Student's t test or one-way analysis of variance (ANOVA). *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 1. Primers used for RT-qPCR.

Table 2. Reagents and antibodies used for western blot and immunohistochemistry.

Table 3. All hierarchical clusters in unstretched SMCs.

Table 4. Enriched gene in stretched SMCs versus unstretched SMCs.

Table 5. Enriched gene in stretched PM-SMCs comparing with stretched LM-SMCs and NC-SMCs.