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

Supporting Information

A novel human arterial wall-on-a-chip to study endothelial inflammation and vascular smooth muscle cell migration in early atherosclerosis

Chengxun Su[1,2], Nishanth Venugopal Menon[1], Xiaohan Xu[1], Yu Rong Teo[1], Huan Cao[3], Rinkoo Dalan[4,5], Chor Yong Tay[3], and Han Wei Hou[1,4]*

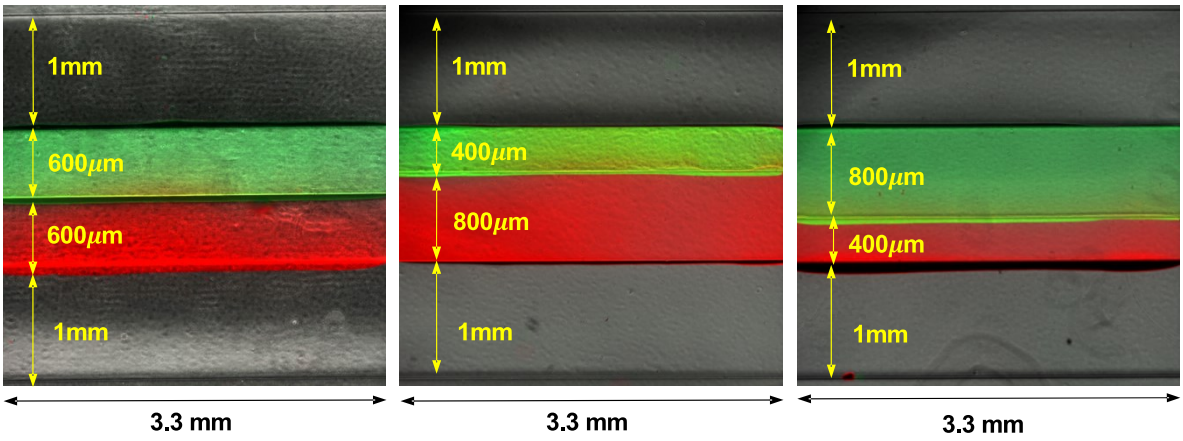

Figure S1. Pillar-free two-lane hydrogel confinement for devices with different channel width. Brightfield overlaid with fluorescence image of the device (top view) after gel loading. (green – first hydrogel (subendothelial layer), red – second hydrogel (SMC layer)).

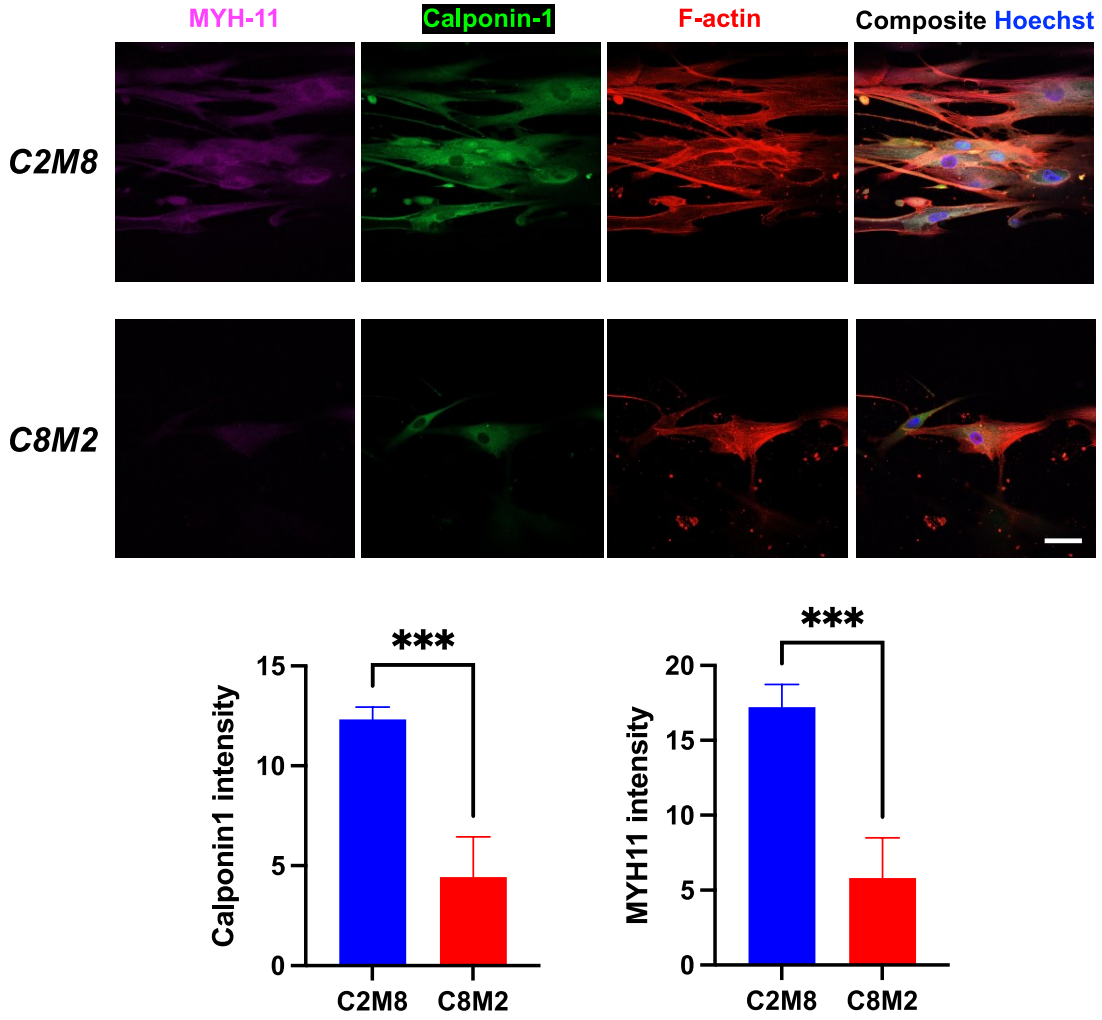

Figure S2. Fluorescence image and quantification of Calponin1 intensity and MYH11 intensity of SMC cultured under C2M8 and C8M2 condition. (magenta – MYH11, green – Calponin1, red – F-actin, blue – nuclei). Scale bar: 50 μm (n = 3 chips). Results were expressed as mean ± SD. Data were analyzed with unpaired student's t-test. (***p < 0.001)

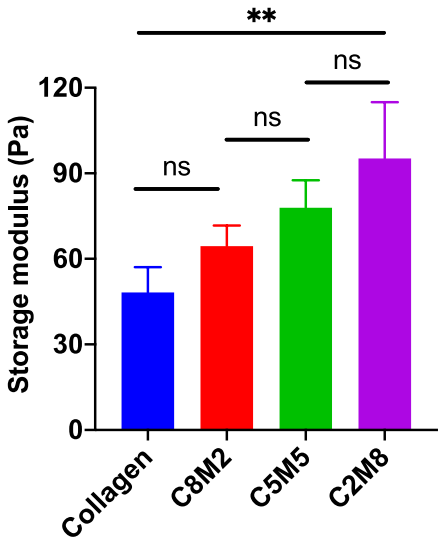

Figure S3. Storage modulus of collagen I, C8M2, C5M5 and C2M8 measured by rheometer (n = 3) Results were expressed as mean ± SD. Data were analyzed with one-way ANOVA with Tukey's multiple comparisons test (**p<0.01, "ns" – not significant).

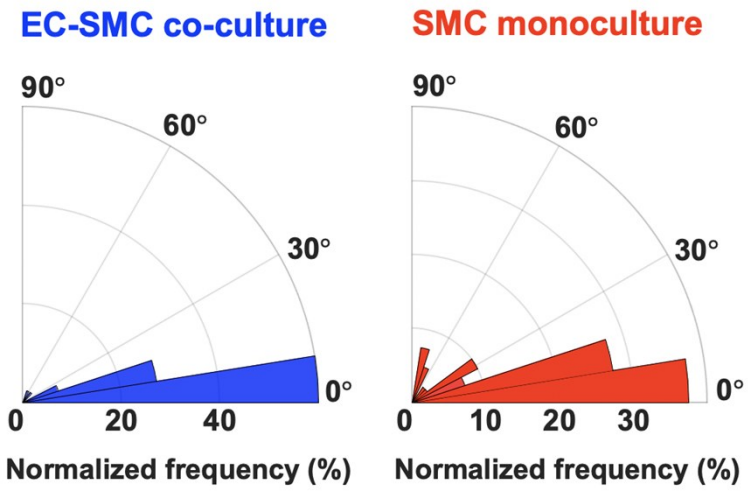

Figure S4. Angle orientation distribution of co-cultured SMC (non-migrated) and monocultured SMC (non-migrated) at day 3 (~40-50 cell measurements from n=3 chips from 3 independent experiments).

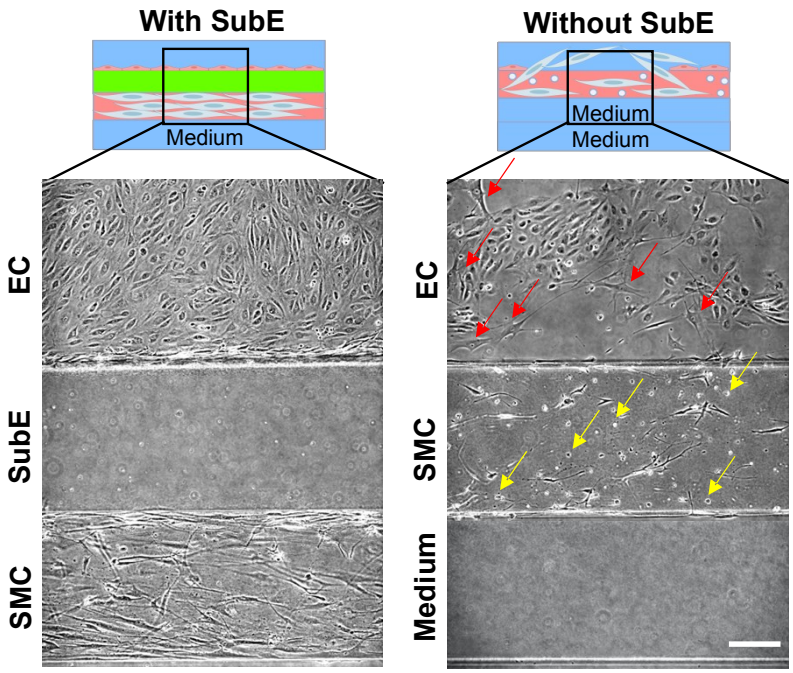

Figure S5. Brightfield image of EC-SMC co-culture with subendothelial layer (left) and without subendothelial layer (right) at day 5. Red arrow indicates SMC that migrated into the EC channel; yellow arrow indicates SMC that remained rounded-up. Scale bar: 200 μm.

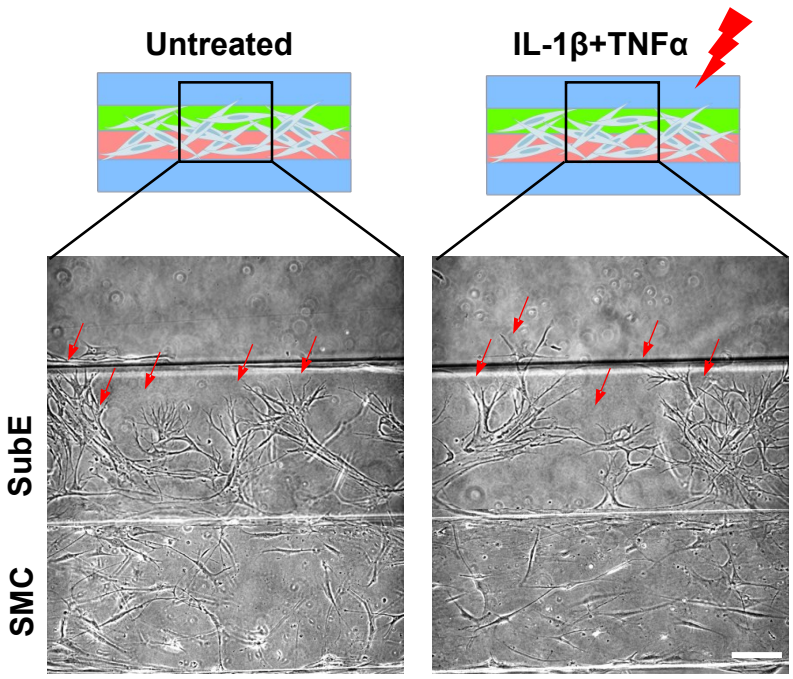

Figure S6. Inflammatory cytokine treatment on SMC monoculture. Brightfield image of untreated SMC monoculture (left) and IL-1β (1 ng/ml) + TNFα (1 ng/ml) treated SMC monoculture (right) at day 5. Red arrow indicates migrated SMC. Scale bar: 200 μm.

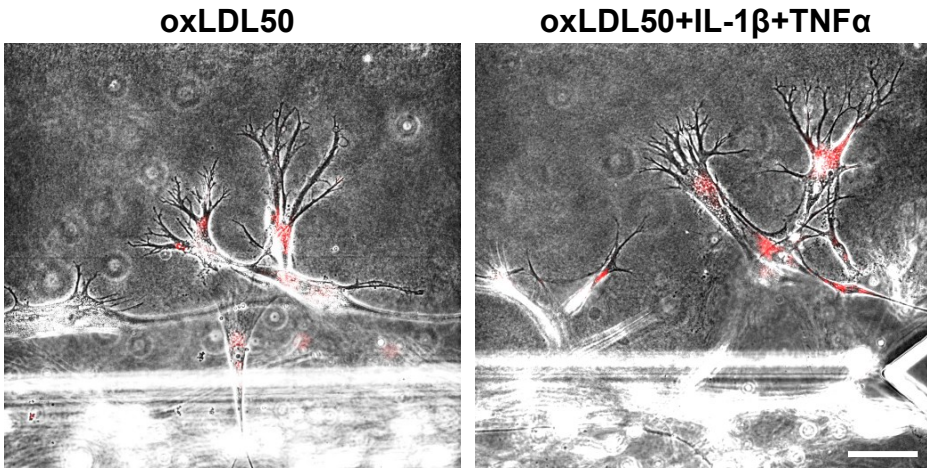

Figure S7. Representative fluorescence overlaid with brightfield image of oxLDL uptake by migrated SMC for chips treated with Dil-oxLDL (50 μg/ml) (left), and Dil-oxLDL (50 μg/ml) + IL-1β (1 ng/ml) + TNFα (1 ng/ml) (right) at day 5. Scale bar: 100 μm.

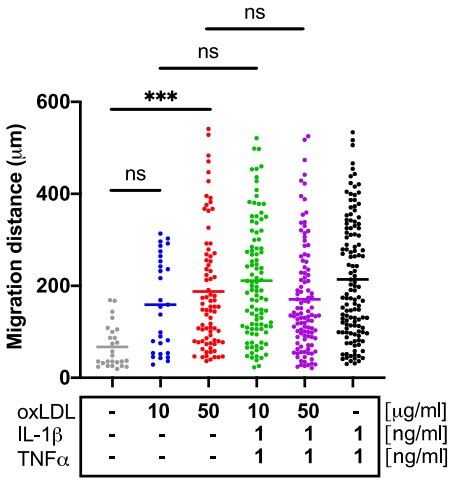

Figure S8. SMC migration distance into subendothelial layer for hyperlipidemia study (n = 3 chips). Results were expressed as scatter plot with a line at mean. Data were analyzed with one-way ANOVA with Tukey's multiple comparisons test (***p<0.001, "ns" – not significant).

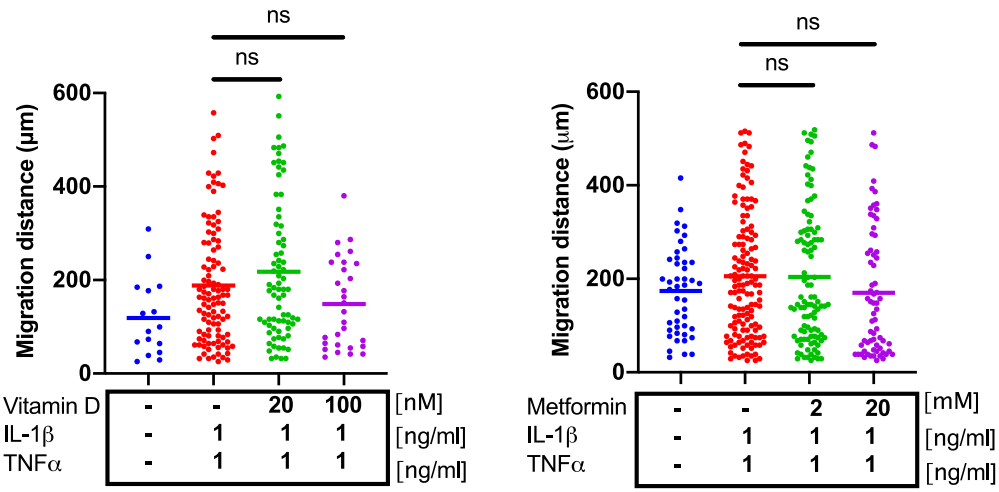

Figure S9. SMC migration distance into subendothelial layer for Vitamin D and Metformin studies (n = 4 chips). Results were expressed as scatter plot with a line at mean. Data were analyzed with one-way ANOVA with Tukey's multiple comparisons test ("ns" – not significant).

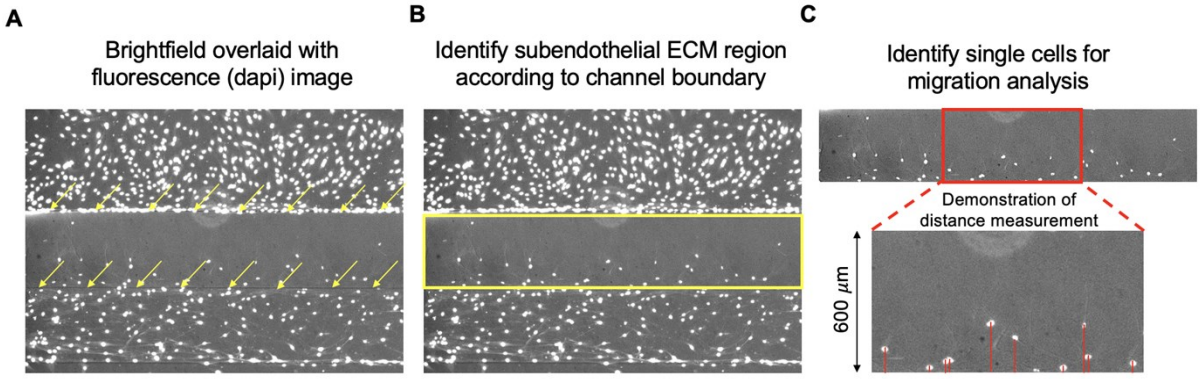

Figure S10. On-chip analysis of SMC migration. (**A**) Overlaid brightfield with fluorescence image of the chip. Cells were stained with Hoechst for nuclei visualization. Yellow arrows indicate the channel boundary of the subendothelial ECM region. (**B**) Identification of the subendothelial ECM region according to channel boundary. Yellow box indicates the subendothelial ECM region. (**C**) Identification and quantification of single SMC migration in the subendothelial ECM region. Red box indicates a magnified region to measure cell migrated distances (red lines).