

## *Supplementary Material*

# **Wnt Signaling Directs Human Pluripotent Stem Cells into Vascularized Cardiac Organoids with Chamber-like Structures**

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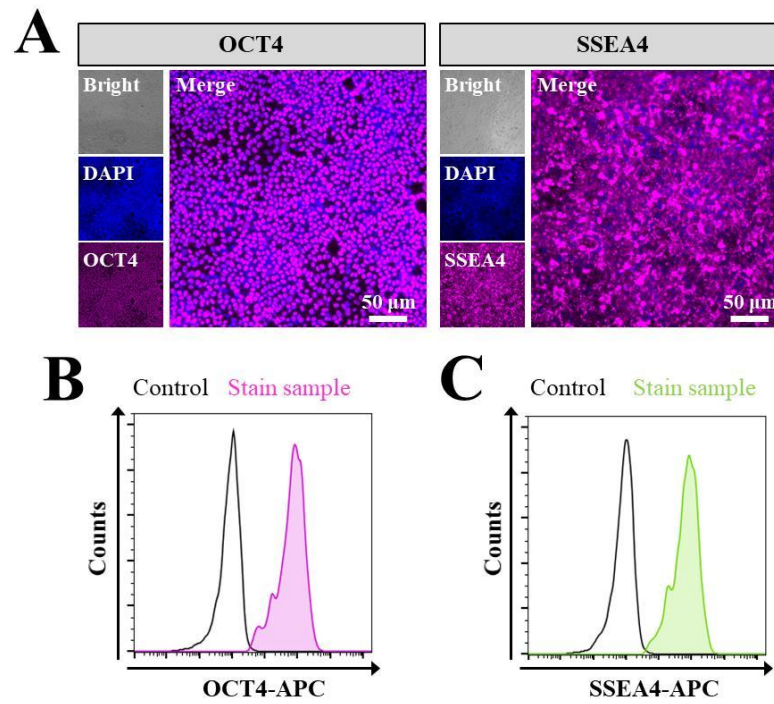
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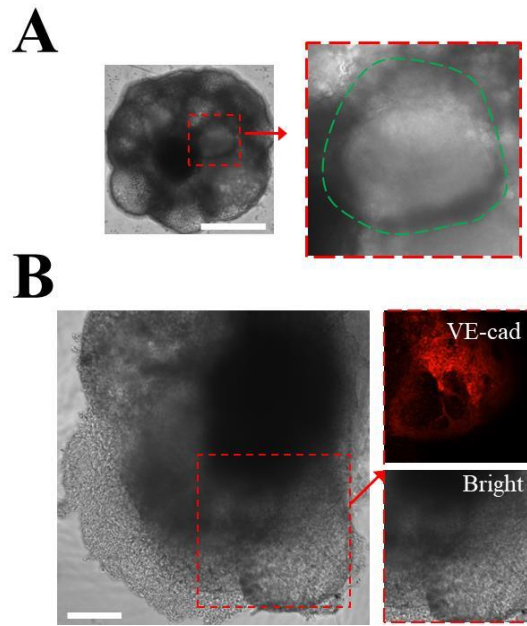
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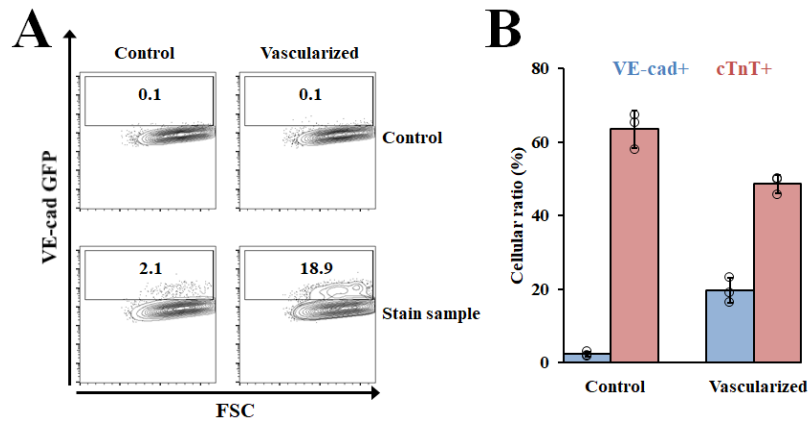
**#These authors contributed equally**



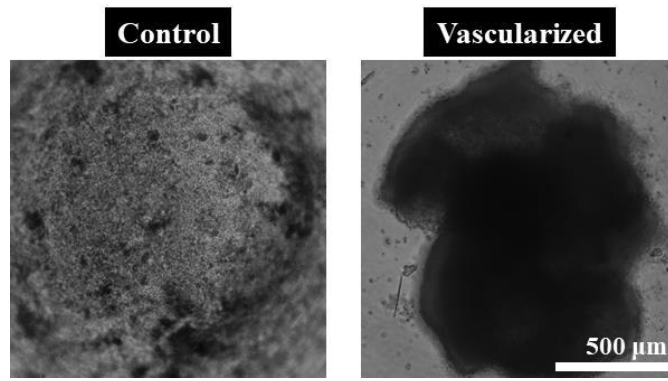
**Supplementary Figure 1.** Pluripotent stem cell marker characterization. (A) Fluorescence images of OCT4 and SSEA4 expression. Flow analysis of OCT4 (B) and SSEA4 (C) expression.



**Supplementary Figure 2.** Chamber characterization. (A) Brightfield images of cardiac organoids. (B) Representative fluorescence images of VE-cad expression inside cardiac organoids.



**Supplementary Figure 3.** Flow cytometry analysis of cardiac endothelial cells (CECs) and cardiomyocytes (CMs) in hPSC-derived organoids. (A) Representative flow cytometry analysis of VE-cad-GFP expression within the indicated cardiac organoids was shown. (B) The percentages of CECs and CMs within indicated cardiac organoids were quantified and shown.



**Supplementary Figure 4.** Brightfield images of different cardiac organoids after the treatment with Temozolomide.