

SUPPLEMENTARY INFORMATION

Supplementary Figure 1: Gating strategy used for FACS analysis of hiPSC-ECs. (a) Forward and side scatter dot plots showing the starting cell population, (b) the gating to discriminate single cells and (c) the gating to isolate CD43-APC positive cells with the help of cells stained with isotype control (APC). The percentage of the relevant cell populations are provided. Cells were acquired with a MACSQuant VYB (Miltenyi Biotech) and analyzed with FlowJo Software.

Supplementary Figure 2: Gating strategy used for FACS analysis of hiPSC-CMs. (a) Forward and side scatter dot plots showing the starting cell population, (b) the gating to discriminate single cells and (c) the gating to isolate cTnT-VioBlue positive cells with the help of unstained cells (mBEL) or cells stained with isotype control (VioBlue). The percentage of the relevant cell populations are provided. Cells were acquired with a MACSQuant VYB (Miltenyi Biotech) and analyzed with FlowJo Software.

Supplementary Figure 3: Equipment used for electrical pacing of cardiac MTs. (a) Pulse stimulator connected to custom-made electrodes fitting the V-shaped 96-wells and (b) details of custom-made electrodes.

Supplementary Video 1: Beating monolayer hiPSC-CMs after 21 d of differentiation using LI-BPEL protocol with lactate purification

Supplementary Video 2: Beating monolayer hiPSC-CMs after 21 d of differentiation using mBPEL protocol

Supplementary Video 3: Time-lapse movie of the first 72 h of MT formation (time frame: 15 min)

Supplementary Video 4: Movie of representative MT showing CD31 positive endothelial cells organized in a vessel-like structure (in red) and cardiomyocytes stained with ACTN2 (in green) (15 f.p.s.)

Supplementary Video 5: Contracting MT paced at 1 Hz at after 21 d in culture

Supplementary Video 6: Contracting MT loaded with Fluo-4, paced at 1.5 Hz after 21 d in culture

Supplementary Table 1: Raw data about morphological, contraction and single-cell electrophysiological parameters measured in LUMC0099iCTRL04 and LUMC0020iCTRL06 hiPSC lines, relative to Table 3.