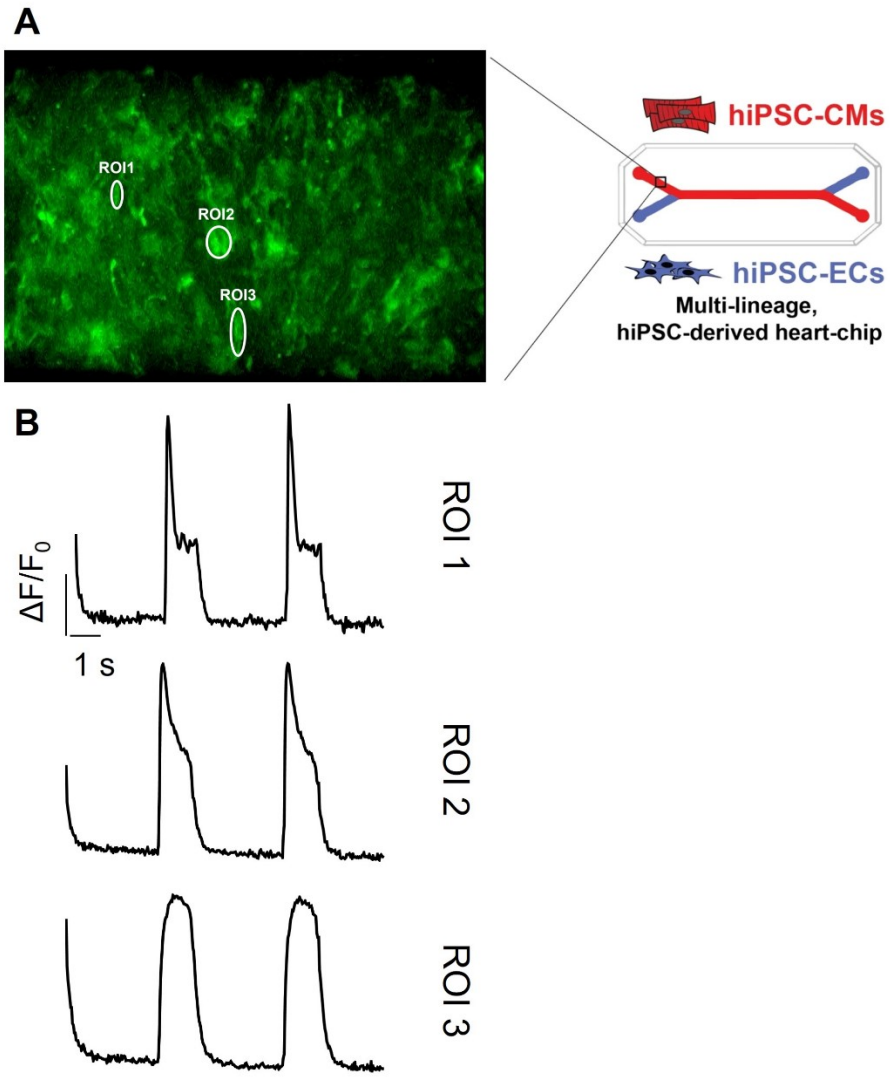
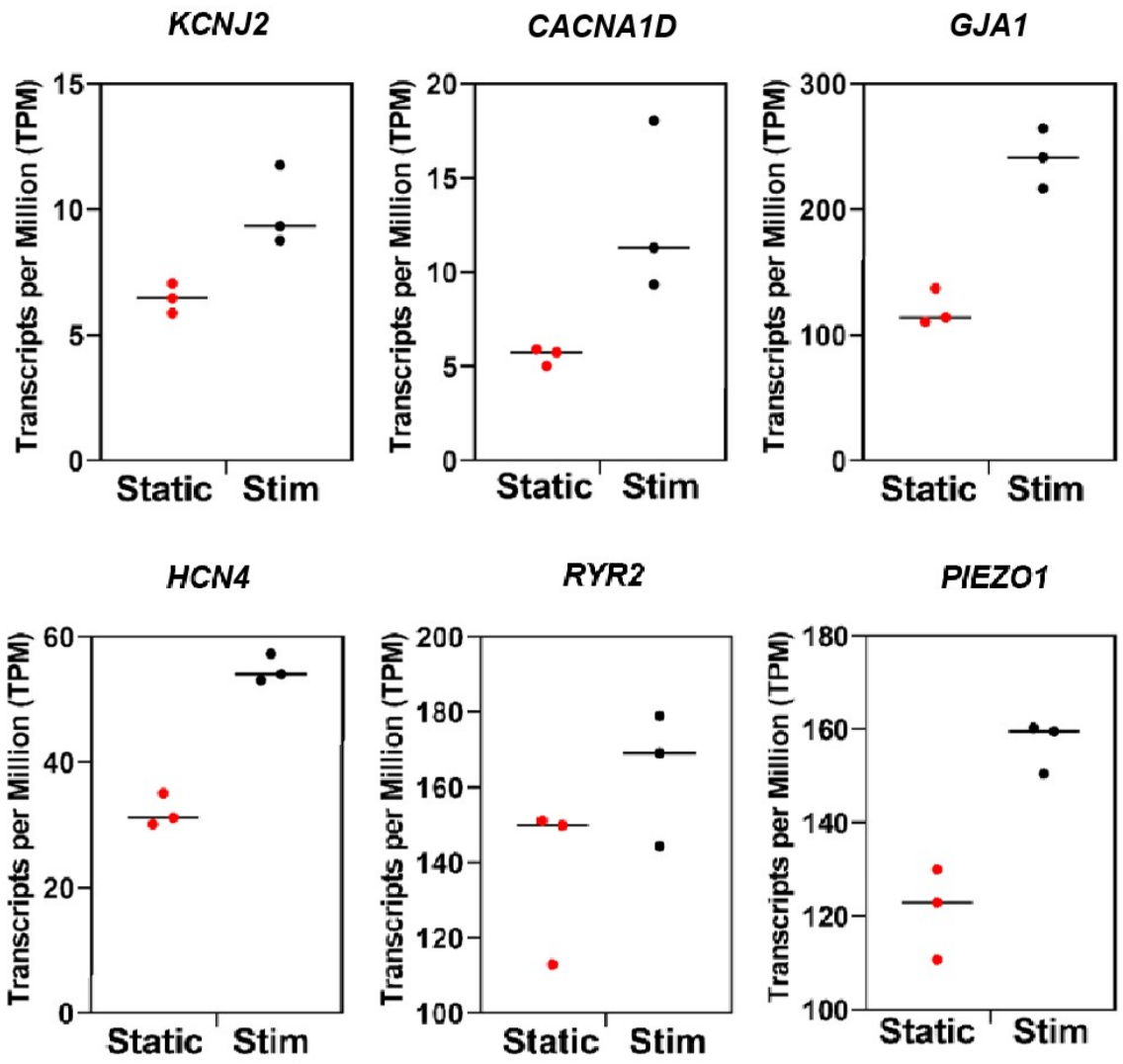
Electronic Supplementary Material (ESI) for Lab on a Chip.

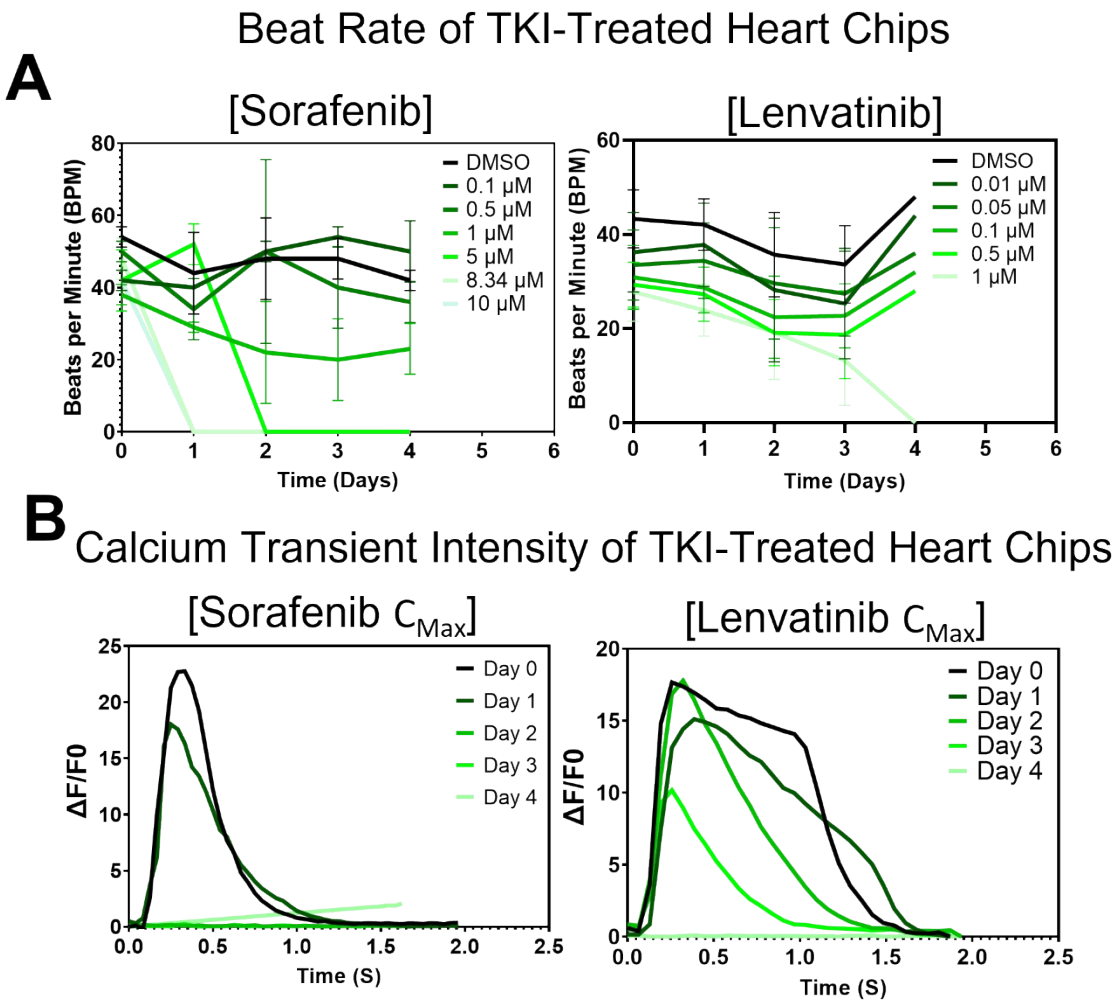
SUPPLEMENTAL FIGURES AND LEGENDS



Supplemental Figure 1: Calcium imaging of hiPSC-CMs on heart-chips. (A) Still frame from Supplemental Video 3 demonstrating GCaMP-hiPSC-CMs on heart-chips. Three regions of interest (ROI) are highlighted for analysis in (B). **(B)** Relative calcium spark intensity measurements of specific ROI were obtained using ImageJ software.



Supplemental Figure 2: Active fluid flow and stretch stimulation of heart-chips enhances hiPSC-CM transcriptional maturity associated with ion channel and mechanotransduction genes. *KCNJ2, CACNA1D, GJA1, HCN4,* and *RYR2* were all at a higher expression in transcripts per million in stim conditions compared to static chips. *KCNJ2* is known for encoding the inward rectifier potassium channel and its higher expression is linked to stabilization of the resting membrane potential in hiPSC-CMs. A more stabilized resting membrane potential is necessary for the harmonic conduction of calcium transients and action potentials in cardiomyocytes. *CACNA1D* is associated with L-type calcium channels and plays a key role in calcium handling in hiPSC-CMs as they mature. *GJA1* is responsible for encoding connexin 43, which is an essential protein for forming gap junctions that allow electrical coupling between hiPSC-CMs. *HCN4* encodes hyperpolarization-activated cyclic nucleotide-gated channels, crucial for pacemaker activity and spontaneous beating of hiPSC-CMs. *RYR2*, encoding the ryanodine receptor, is essential for the calcium-induced calcium release (CICR) phenomena in the sarcoplasmic reticulum, as a part of the excitation-contraction coupling in hiPSC-CMs. *PIEZO1* is the gene responsible for the transcription of a mechanosensitive ion channel in cardiomyocytes, that plays a critical role in sensing mechanical forces on the cell.



Supplemental Figure 3: Heart-chips depict a specific and sensitive platform for assessing cardiotoxicity of TKIs. (A) Beat rate variability profile of Sorafenib (left) and Lenvatinib (right) treated heart chips over 4 days of exposure. (n=3 chips per condition) **(B)** Calcium transient profile of Sorafenib (left) and Lenvatinib (right) treated heart chips over 4 days of exposure. (n=3 chips per condition)

Supplemental Video 1: Purified hiPSC-derived cardiomyocytes after differentiation and prior to replating on heart-chips.

Supplemental Video 2: hiPSC-CM channel on heart-chip showing synchronized contraction.

Supplemental Video 3: GCaMP-GFP hiPSC-CMs, used for calcium imaging, under static and stimulated conditions.

Supplemental Video 4: Three-dimensional reconstruction of CD144+ hiPSC-ECs under long-term active flow condition on heart-chip.