

# STEMRNA iPSC-DERIVED HUMAN VENTRICULAR CARDIAC TISSUE (iHCT) FOR SAFETY PHARMACOLOGY ASSESSMENT

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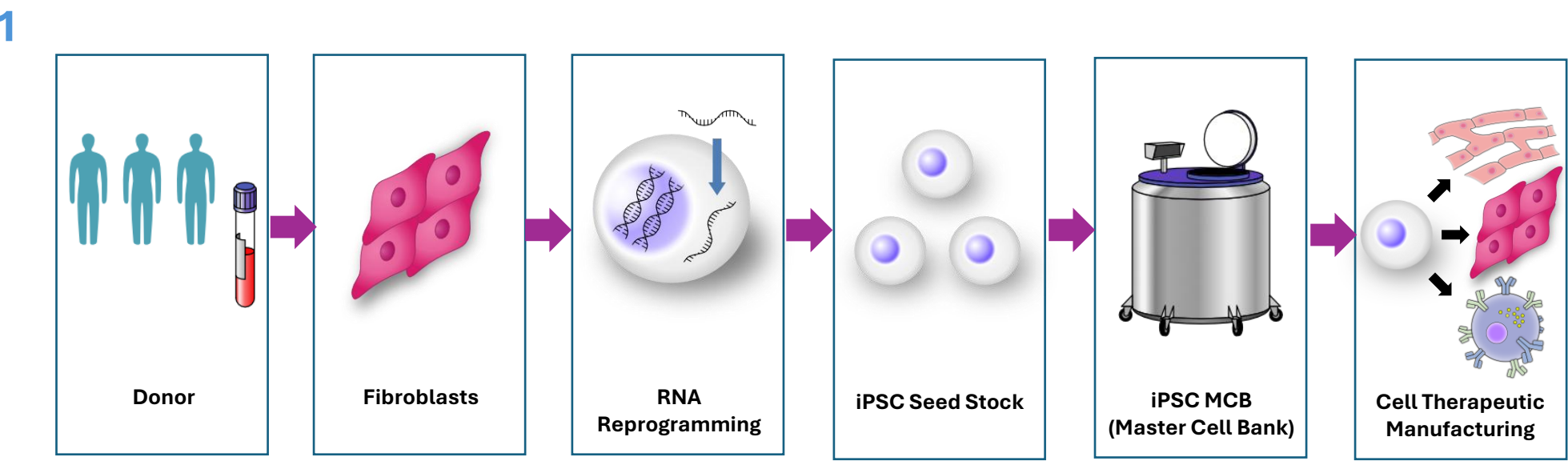
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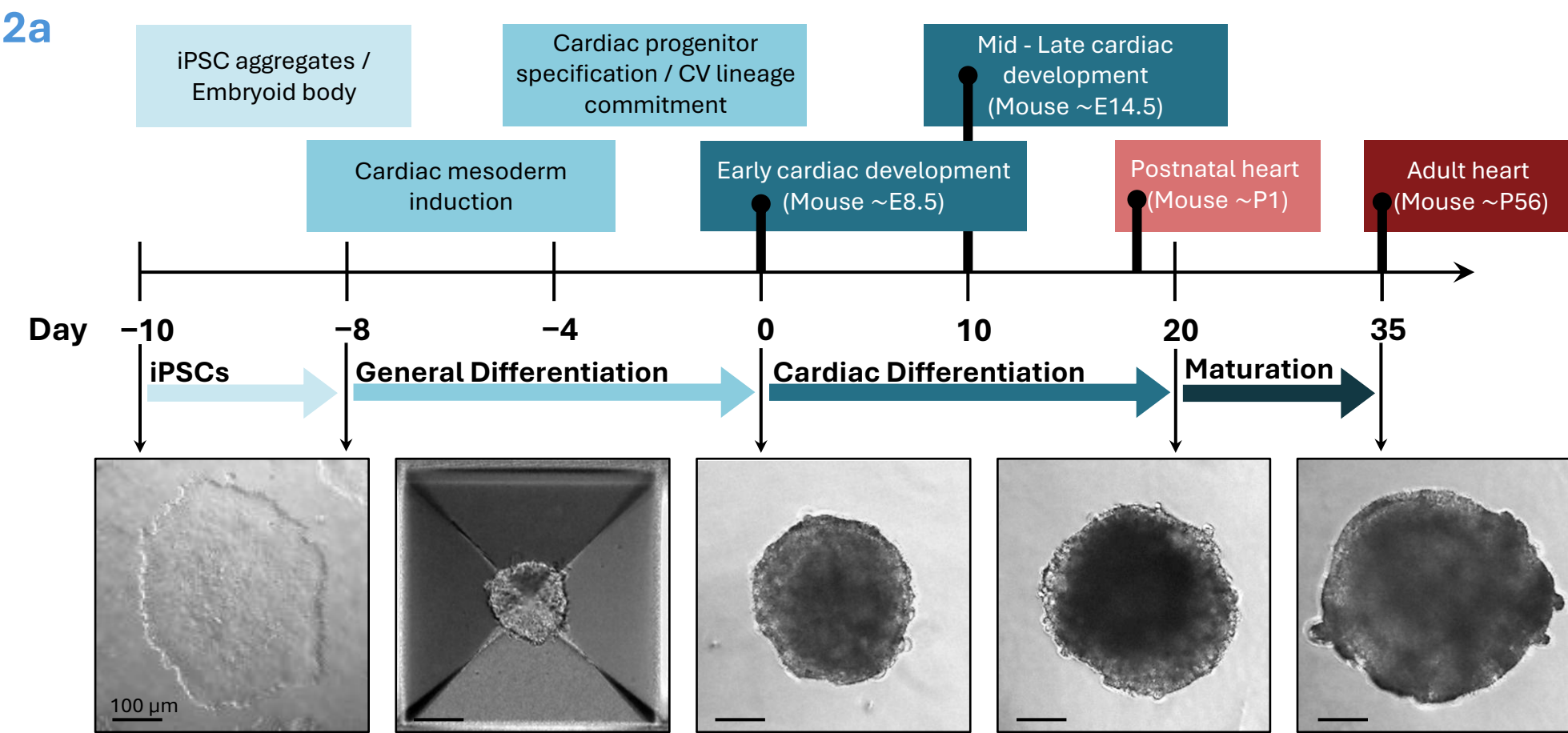
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The generation of synthetic human tissue necessitates mimicry of native tissue cell composition, architecture, and molecular fidelity. Many of these projects start with iPSCs (induced pluripotent stem cells). Here we detail the establishment of developmentally staged iPSC-derived human ventricular cardiac tissue (iHCT) from a clinical StemRNA™ iPSC and a hypoimmune iPSC line and an initial assessment of their suitability for the prediction of drug safety. These iPSC lines are created from ethically sourced starting material, they are integration-free because they are generated by reprogramming with mRNA. Both StemRNA iHCTs and iHCT's generated from the hypoimmune iPSCs are composed of all cell-types found in native human cardiac ventricles, with the concordant molecular, metabolic, structural, and physiologic characteristics. iHCTs exhibit vascularization, which is necessary for physiologic nutrient and oxygen distribution within the organoid proper, and for effective distribution of drugs and test compounds. By virtue of their self-organization and the composition of all relevant cardiac cell types, these iHCTs allow for more precise modelling and interrogation of drug effects on the heart compared to 2D monoculture systems typically containing only one relevant cardiac cell type. We assessed both iHCTs' response to a selection of reference cardiovascular drugs, for which drug effects and function are well established. In these studies, we successfully recapitulated the expected cardiac responses for 95% of compounds tested. Thus, cardiac tissue generated from StemRNA iPSC provides a clinically relevant method for reliable testing and detection of potential beneficial or cardiotoxic effects of new pharmaceutical compounds on the heart and its functionality. Taken together, the RNA iPSC iHCTs can facilitate accurate and precise drug testing to increase screening efficiency and better streamline drug development workflows.

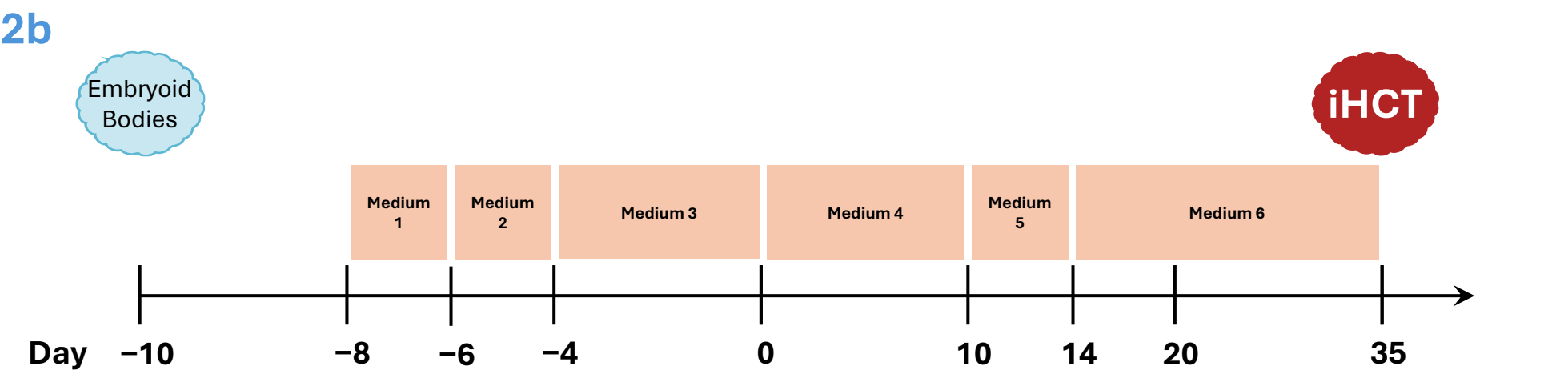
## StemRNA iPSC Seed Stock Clone Generation into Cell Therapy Product



**Figure 1: Generation of a cGMP Cell Therapy Product using StemRNA clinical iPSCs.**  
The donor is screened, the tissue is sourced, and the target cells are isolated and reprogrammed to iPSCs using clinical RNA technology to generate a clinical seed stock. In regenerative medicine projects, a cGMP iPSC MCB is manufactured from which the cell therapy product is generated.

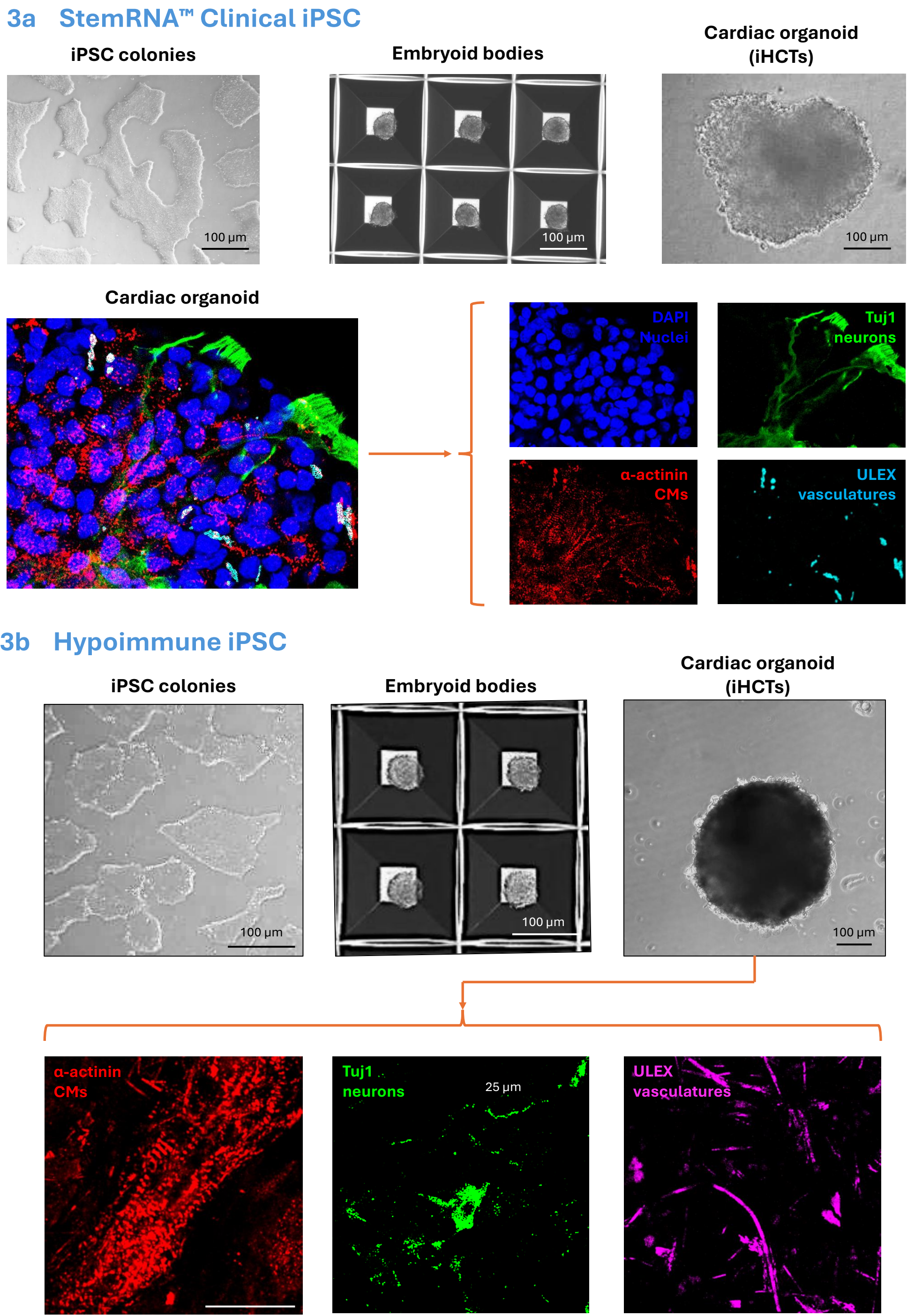


**Figure 2a: In vitro guided generation of human cardiac ventricular tissue.**  
Schematic representation of the differentiation timeline with corresponding brightfield images of iHCT growth, as benchmarked to equivalent stages of mouse heart development. Scale bar, 100 µm.



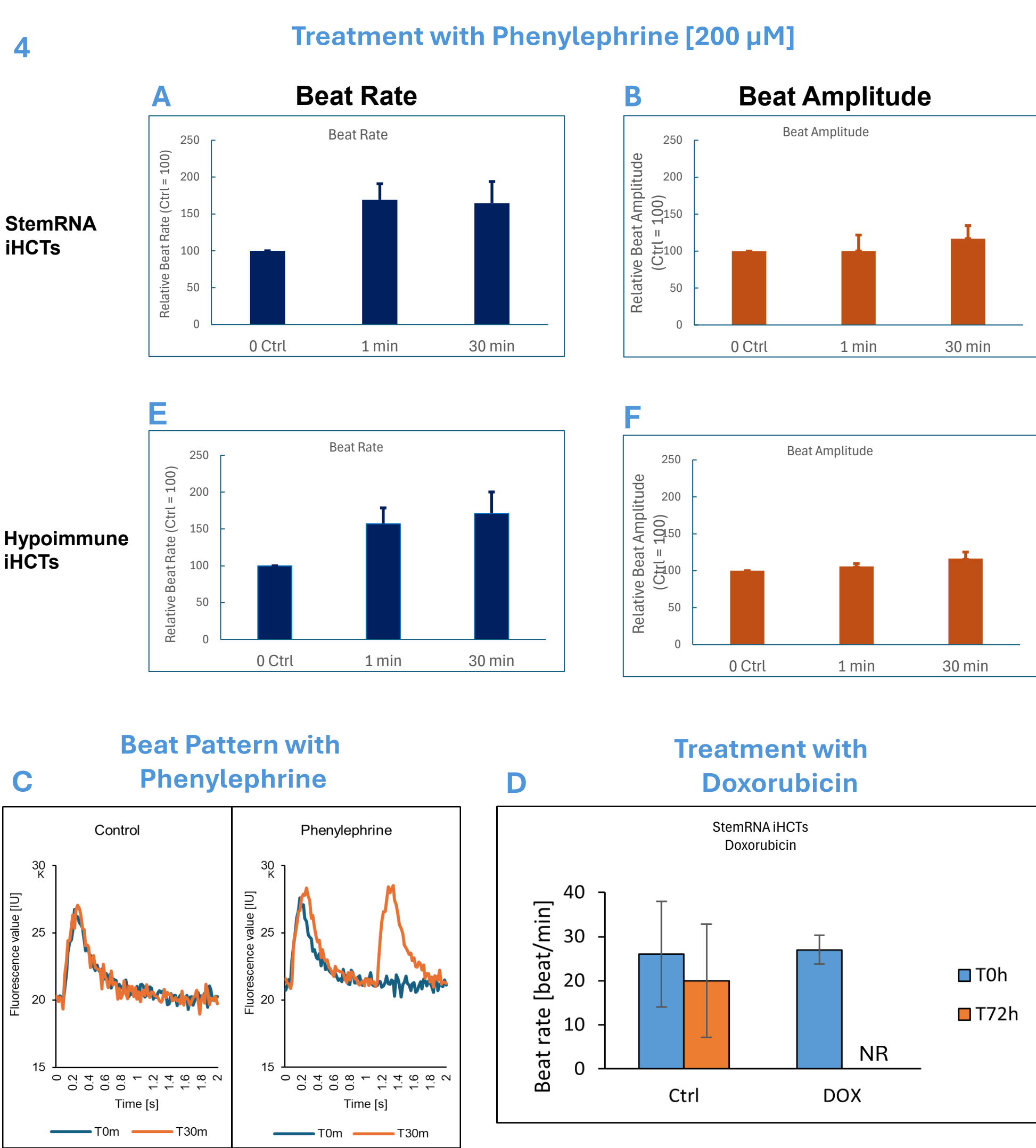
**Figure 2b: Timelines, media composition and corresponding cell lineage development of human cardiac ventricular tissue.**  
Schematic representation of iHCT development timeline and media switches at each step of the differentiation and maturation process.

## Cardiac organoid differentiation of a StemRNA™ Clinical iPSC and a hypoimmune B2M/CIITA KO iPSC line



**Figure 3: Successful differentiation of a clinical StemRNA iPSCs and a hypoimmune research line into developmentally staged iPSC-derived human ventricular cardiac tissue (iHCT).**  
3a) iPSCs of a female clinical StemRNA iPSC line and 3b) iPSCs of a male hypoimmune iPSC line (B2M/CIITA Knockout). For both iPSC lines embryoid body formation and cardiac organoid formation are shown. The iHCTs were stained with nuclear marker (DAPI – blue), endothelial marker (ULEX), neuronal marker (TUJ1) and cardiac marker (α-Actinin).

## Response of iHCTs from a StemRNA™ Clinical iPSC and a hypoimmune B2M/CIITA KO iPSC line to reference drugs



**Figure 4: Response of iHCTs to cardiovascular reference drugs.**  
iHCTs from StemRNA Clinical iPSCs (Panel A-D) and Hypoimmune iPSCs (Panel E-F) were treated with phenylephrine [200 µM] or doxorubicin [10 µM], and the beat rate and amplitude were measured by Calcium Transient Assay. iHCTs from both iPSC lines show a response in beat rate (Panel A, E), but not amplitude (Panel B, F), that is consistent with clinical results, and this response is sustained over at least 30 min. The Beat Pattern (Panel C) shows an increase in beat frequency following a 30 min exposure to phenylephrine [200 mM]. Doxorubicin treatment [10 µM] of StemRNA iHCTs (Panel D) causes a complete abrogation of beating after 72 hr treatment ("NR" = no response), consistent with its clinical findings as a cardiotoxic agent.

**Conclusions:**

- iHCTs can be generated in bulk from iPSCs using a well-defined protocol.
- StemRNA iPSC and B2M/CIITA K.O. lines can be successfully differentiated to iHCTs with little difference in morphology or cell type distribution.
- iHCTs from StemRNA iPSCs show expected responses to standard drugs such as phenylephrine (increased beat rate, unchanged amplitude) and doxorubicin (stop beating).