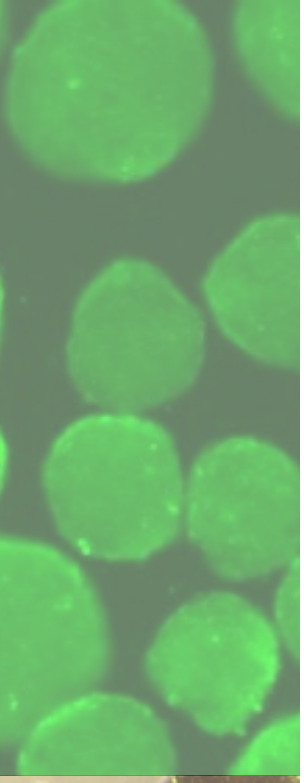
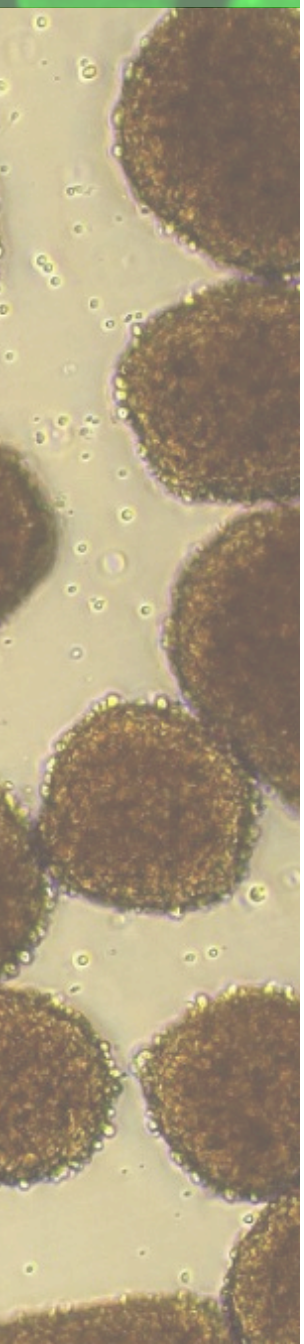




GREENSTONE BIOSCIENCES

# Greenstone Biosciences Cardiovascular New Approach Method (NAM) Portfolio:

## Modeling Cardiac Arrhythmias and Cardiomyopathies *In Vitro*

# Cardiovascular hiPSC Derived NAMs

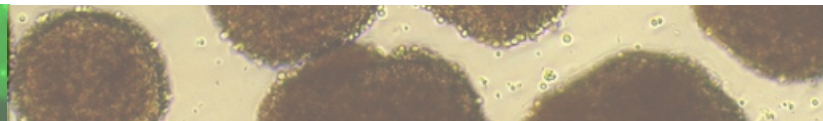
Heart disease remains the number one cause of death in the US. Despite decades of drug and therapy development there is still an unmet need for new medicines to treat heart disease. Animal models of heart disease do not fully recapitulate the complex human genetic and phenotypic responses to cardiovascular injury or medications. Recently the FDA has announced an initiative to reduce the use of animals in the drug discovery and development process, aiming to reduce the time and money spent to develop new cardiovascular drugs. Our hiPSC biobank contains many heart disease patient specific induced pluripotent stem cell (iPSC) lines that can be used for discovery and development of new therapies in vitro.

Cardiotoxicity of new medicines is a major concern for drug discovery and development. This toxicity can be missed in pre-clinical studies using animals. The FDA Modernization Act 2.0/3.0 has paved the way for use of human based in vitro assays called NAMs (New Approach Methods) for detection of cardiotoxicity. Our hiPSC Biobank can be mobilized for development of human cardiac NAMs for pre-clinical safety screening.

## Contents





# Greenstone Biosciences (GSB) hiPSC Atrial Cardiomyocytes

The most common cardiac arrhythmia is atrial fibrillation (AF). In this condition, the upper chambers of the heart begin to beat irregularly due to electrical abnormalities of the tissue. Patients with AF may have palpitations or be asymptomatic; AF raises the risk of blood clots, stroke, and other cardiovascular issues. AF mechanisms include abnormal electrophysiological function of the individual atrial cardiomyocytes.

GSB-Atrial Cardiomyocytes are a research tool for creating in vitro models of AF to discover and test new therapies and medications. GSB-Atrial Cardiomyocytes are also useful for high-throughput safety pharmacology screening of drugs in development to determine drug-induced cardiotoxicity (DICT).

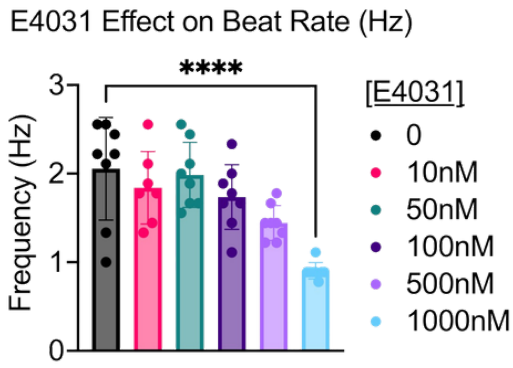
This is a significant New Approach Method (NAM) to reduce, refine, and replace the use of animals for preclinical cardiac safety screening.

## Product Specifications

CM Purity Markers:
>90% cTnT+ cells

Functional Characterization:
Atrial CM APD<<Ventricular CM APD

Shipping Conditions:
Cryopreserved cells; Shipped on Dry Ice;
Store in liquid nitrogen upon arrival



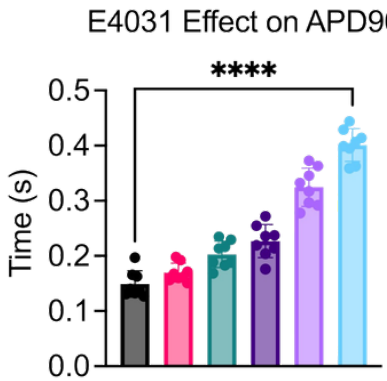
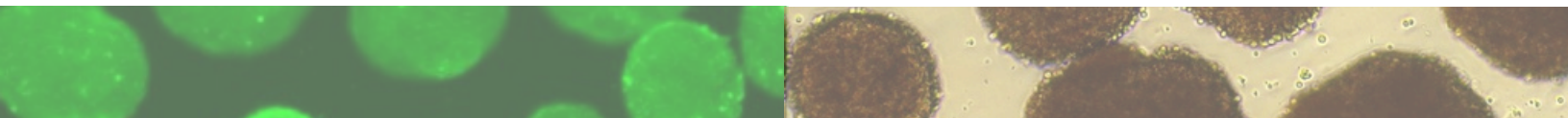




**Figure 1.** GSB Atrial Cardiomyocytes beat spontaneously and respond to drugs including hERG channel blockers. E4031 causes dose dependent APD90 prolongation, indicating proarrhythmia potential of hERG Channel blockade.





# Greenstone Biosciences (GSB) hiPSC Ventricular Cardiomyocytes

Human induced pluripotent stem cell–derived ventricular cardiomyocytes (hiPSC-ventricular CMs) are electrically and mechanically active human heart cells generated in vitro that recapitulate key structural, electrophysiological, and pharmacological properties of native ventricular myocardium, including expression of ion channels such as hERG (IKr), Nav1.5, and L-type Ca channels. These cells are now widely used in high-throughput in vitro cardiotoxicity screening to assess drug-induced effects on repolarization, conduction velocity, calcium handling, and contractility, enabling early detection of proarrhythmic liabilities such as QT prolongation and torsades de pointes risk. Under the U.S. Food and Drug Administration's regulatory framework, safety pharmacology data generated using hiPSC-CMs are accepted to support Investigational New Drug (IND) applications. GSB hiPSC Ventricular CMs are highly enriched for cTnT+ cells.

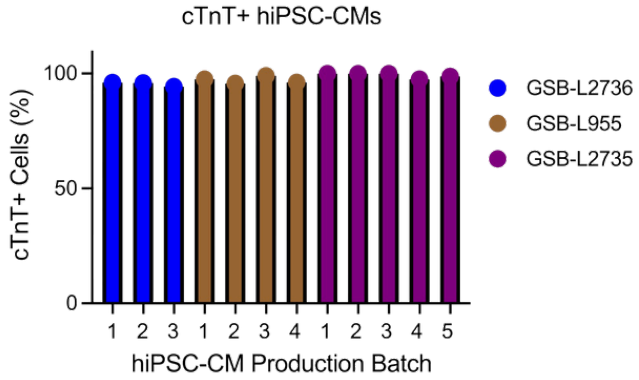
## Product Specifications

CM Purity Markers:
>90% cTnT+ cells

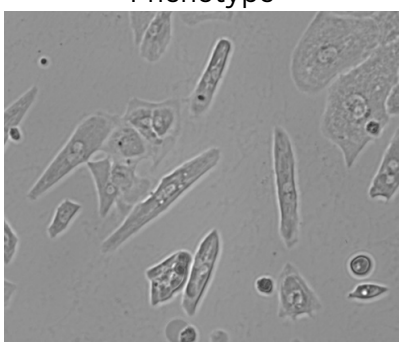
Functional Characterization:
Ventricular CM APD>>Atrial CM APD

Shipping Conditions:
Cryopreserved cells; Shipped on Dry Ice

Mycoplasma:
Negative




Mature hiPSC-CM Phenotype



Fetal hiPSC-CM Phenotype

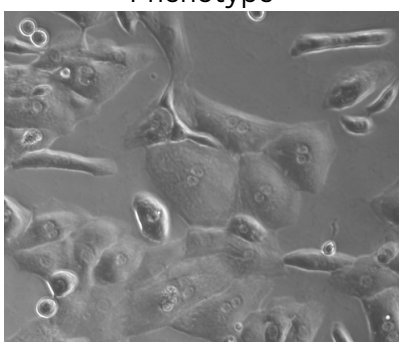
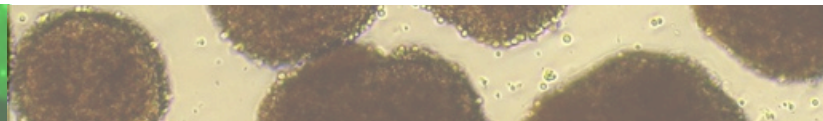


**Figure 2.** GSB Ventricular Cardiomyocytes thawed and replated for maturation to adult-like phenotypes (left) or to study fetal-like cardiomyocytes (right). Greenstone offers healthy control or disease specific hiPSC-ventricular CMs.





# Cardiovascular Disease Patient Selection: Clinical Trial in a Dish (CTiD)

The Greenstone Bioscience Biobank contains >2000 unique patient hiPSC lines. Many of our hiPSC lines are derived from patients' diagnosed with inherited arrhythmia syndromes like Long QT or inherited cardiomyopathy such as Hypertrophic Cardiomyopathy (HCM). Cells are also available from oncology, neuromuscular, metabolic and rare disease patients. Table 1 displays a sample of some of the cardiac diseases available in Greenstone's Biobank.

| Disease | Gene (Variant) | | | | |
|---|---|---|---|---|---|
| Atrial fibrillation | *ABCC9* (p.S1402C) | *ALMS1* (p.I486V) | *ANK2* (p.R2506Q) | *DMD* (p.G2609D) | *KCNQ1* (p.R231H) |
| Brugada syndrome | *DSP* (p.H1679Y) | *SCN5A* (p.R27H) | *SCN5A* (p.R367H) | *SCN5A* (p.R620H) | *SCN5A* (p.P701L) |
| Dilated cardiomyopathy | *ACTN2* (p.Q860*) | *LMNA* (p.R133Q) | *MYH7* (p.A26V) | *TNNT2* (p.R183W) | *TTN* (p.E3497K) |
| Hypertrophic cardiomyopathy | *MYBPC3* (p.R502W) | *MYH7* (p.R663H) | *RYR2* (p.A77V) | *TNNI3* (p.S199N) | *TPM1* (p.M281T) |
| Long QT syndrome | *KCNH2* (p.R328C) | *KCNQ1* (p.R190Q) | *KCNQ1* (p.A341V) | *KCNQ1* (p.G269S) | *SCN5A* (p.T512I) |
| LVNC | *CASQ2* (p.S113N) | *NEB* (c.4109A>G) | *PLN* (p.R14Δ) | *TBX20* (p.Y317*) | *TNNT2* (p.R139H) |

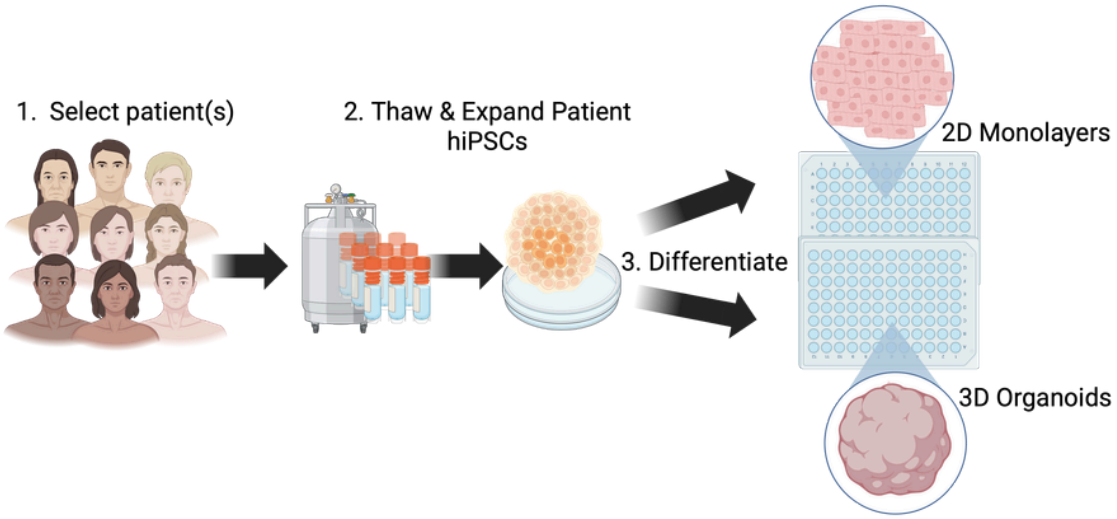
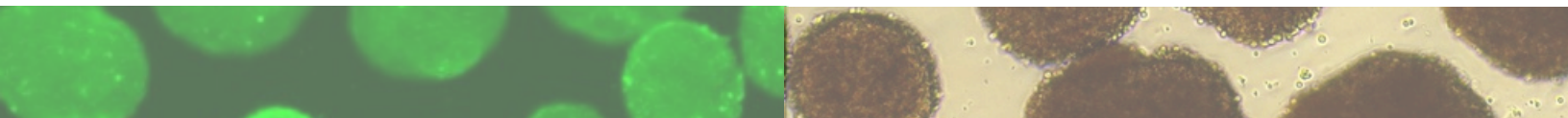



**Figure 3.** Greenstone Biosciences offers a complete beginning to end Clinical Trial in a Dish service. Step 1. choose patient(s) to include for in vitro CTiD. Step 2. GSB scientists retrieve, thaw and expand patient hiPSCs. Step 3. GSB scientists differentiate patient hiPSCs to cardiac myocytes for use in 2D monolayer and/or 3D organoid assays.





# Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC)

Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) is an inherited cardiomyopathy characterized by progressive fibrofatty replacement of right ventricular myocardium, most commonly caused by pathogenic variants in desmosomal genes such as PKP2, DSG2, and DSP that impair mechanical coupling between cardiomyocytes. This structural remodeling disrupts cell–cell adhesion and gap junction integrity, leading to slowed conduction, regional conduction block, and reentrant ventricular arrhythmias that typically originate in the right ventricle. ARVC patients are at elevated risk for malignant ventricular arrhythmias and sudden cardiac death, particularly in young individuals and athletes, new therapies are required to treat ARVC.

ARVC patients are also highly sensitive to drug induced cardiotoxicity (Figure 4). ARVC patients' hiPSC-CM monolayers represent an important in vitro cardiac safety screening paradigm.

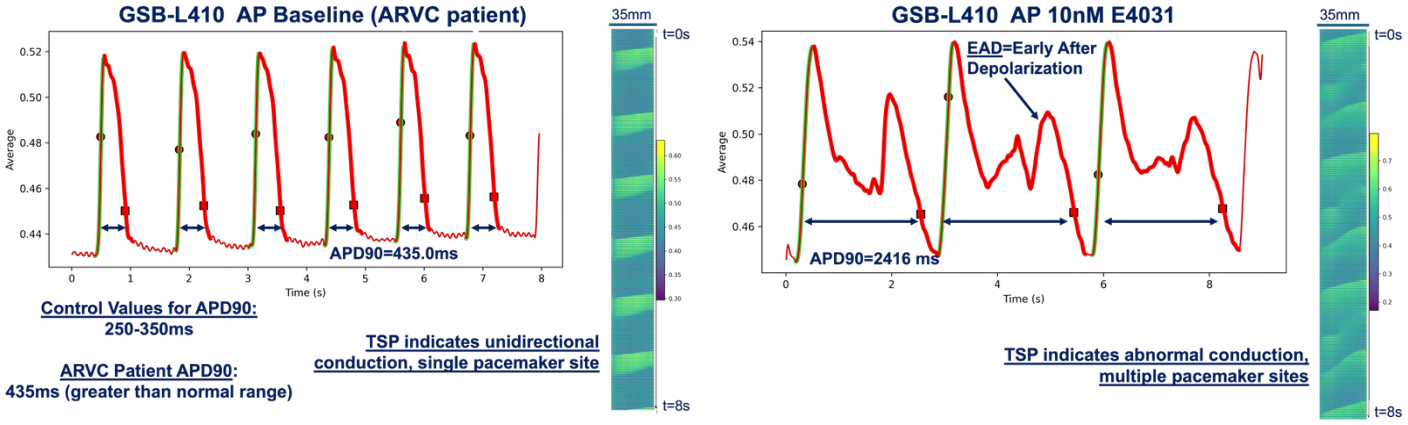
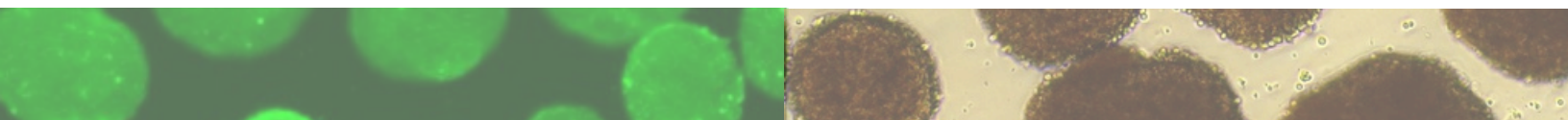



**Figure 4.** Greenstone Biosciences hiPSC Biobank includes ARVC patient cell lines (e.g., GSB-L410). This hiPSC line was generated by reprogramming the patient's peripheral blood mononuclear cells (PBMCs) to hiPSCs using Sendai Virus. The patient (white, female, 34yo) was found to have a pathogenic PKP2 gene variant that causes a premature stop codon (p.Arg79Stop (c.235 C>T)). GSB-L410 ventricular hiPSC-CMs have prolonged action potential duration (APD90) at baseline and severe arrhythmia phenotype upon hERG K channel block (10nM E4031). These hiPSC-CMs are ideally suited for disease modeling and cardiac safety screening of new therapies.





# Dilated Cardiomyopathy (DCM)
## MYH7, MYBPC3, TTN, LMNA, FLNC Mutations

Dilated cardiomyopathy (DCM) is a myocardial disorder characterized by left ventricular dilation and systolic dysfunction, manifesting clinically as reduced contractility and decreased ejection fraction. Patients with DCM are at elevated risk for heart failure progression and life-threatening ventricular arrhythmias that can lead to sudden cardiac death. Inherited mutations in sarcomeric and cytoskeletal genes disrupt force generation, sarcomere integrity, or nuclear-cytoskeletal coupling. Human induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs) generated from DCM patients carrying these mutations retain patient-specific genetic backgrounds and recapitulate key disease phenotypes in vitro. These hiPSC-CM models commonly demonstrate reduced contractile force, abnormal calcium handling, sarcomere disorganization, and increased susceptibility to arrhythmogenic events, enabling mechanistic studies and therapeutic screening for DCM.

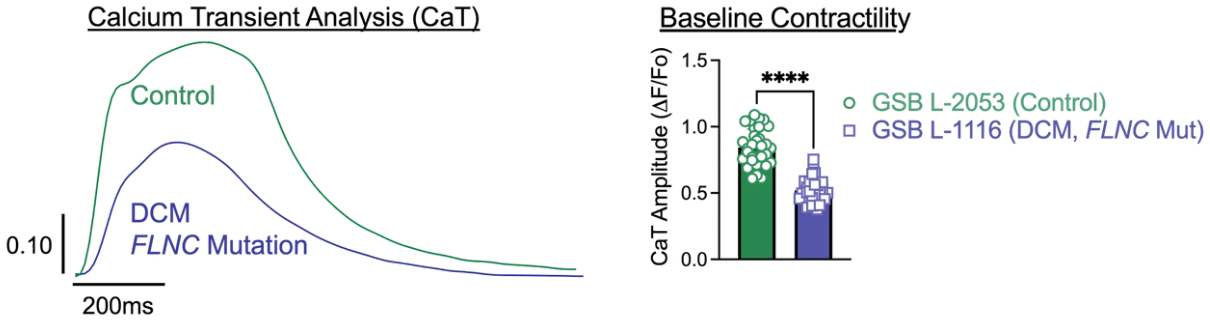
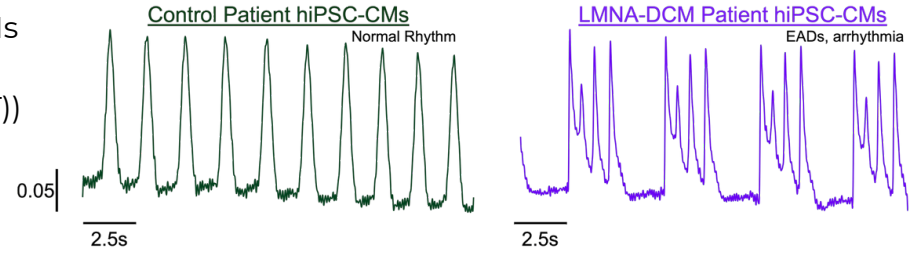
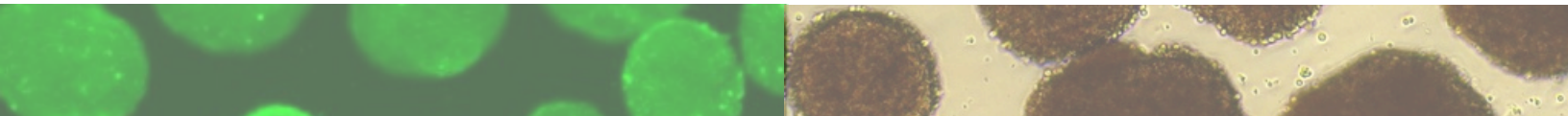



**Figure 5.** DCM patient hiPSC-CMs (GSB-L1116; FLNC mutation:p.Gly1891Valfs*62) have reduced contractility relative to control patient (GSB-L2053) hiPSC-CMs, assessed by calcium flux measurements.

**Figure 6.** DCM patient hiPSC-CMs (GSB-L387; LMNA mutation: p.Leu323GlyfsX7 (c.967_968delCT)) have baseline arrhythmias characterized by early after depolarizations (EADs).



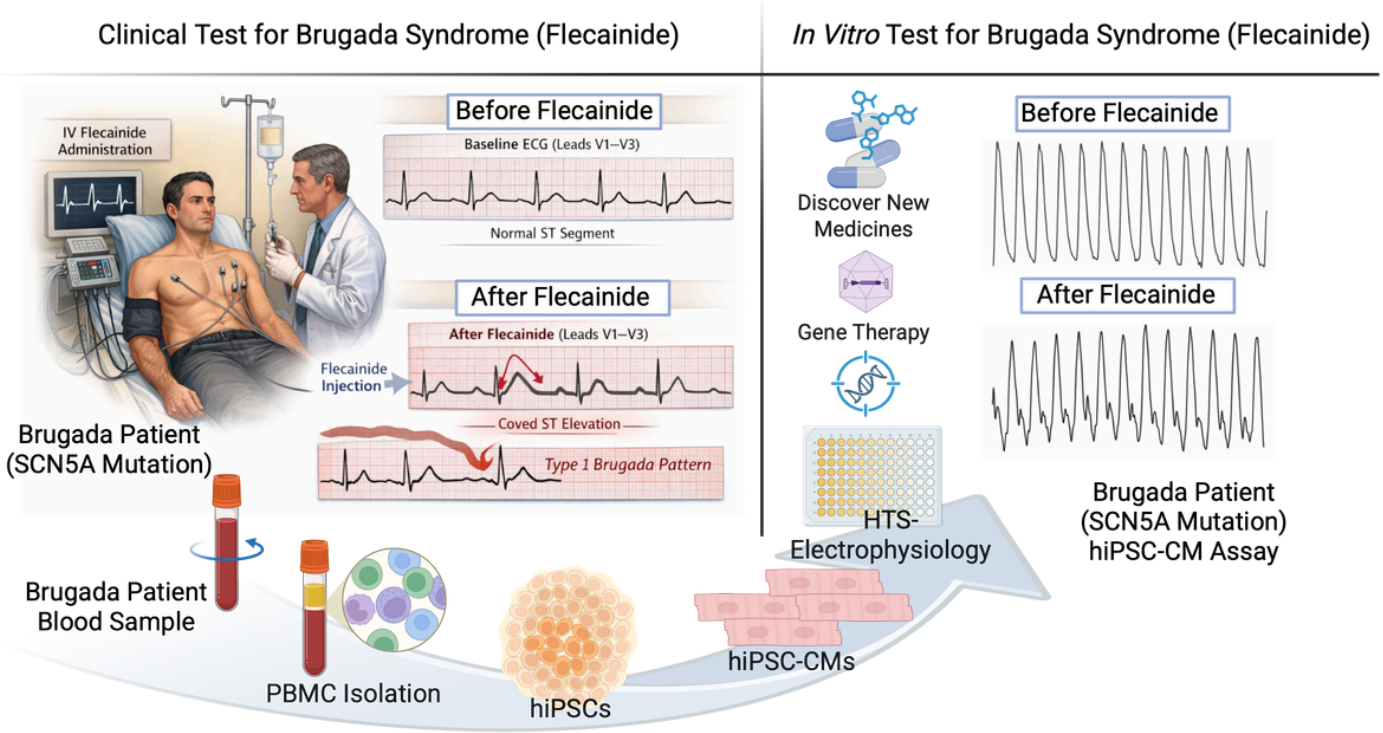


# Brugada Syndrome

Brugada syndrome is an inherited cardiac channelopathy characterized by a structurally normal heart but a high risk of ventricular tachyarrhythmias and sudden cardiac death. It is often considered a “silent” arrhythmia because affected individuals may have no symptoms until syncope or sudden cardiac arrest occurs. The disorder is most commonly caused by loss-of-function mutations in the SCN5A gene, which encodes the cardiac sodium channel. In patients with concealed disease, the diagnostic Type 1 ECG pattern can be uncovered by pharmacologic challenge with flecainide, which further suppresses sodium current and unmasks the characteristic ST-segment elevation in the right precordial leads (Figure 7). Brugada patient hiPSC-CMs recapitulate the clinical features of disease including response to flecainide.

**Figure 7.** Brugada patient hiPSC-CMs recapitulate clinical features in vitro (GSB-L322; SCN5A mutation: p.Glu1784Lys, (c.5350G>A)). Brugada patient hiPSC-CM monolayers have normal rhythm at baseline, but arrhythmia is uncovered with flecainde treatment.



# 3D Cardiac Organoids

Three-dimensional cardiac organoids provide a physiologically relevant in vitro model that more closely recapitulates native myocardial architecture, multicellular composition, and cell–cell interactions than traditional 2D monolayers. Their 3D structure supports improved maturation, force generation, and electrophysiologic coupling, enabling more accurate assessment of contractility, conduction, and arrhythmia susceptibility. Cardiac organoids derived from patient-specific hiPSCs can model genetic heart diseases and capture inter-individual variability in drug responses. As a result, they offer a scalable pre-clinical testing platform for evaluating efficacy, cardiotoxicity, and proarrhythmic risk early in the drug development pipeline.

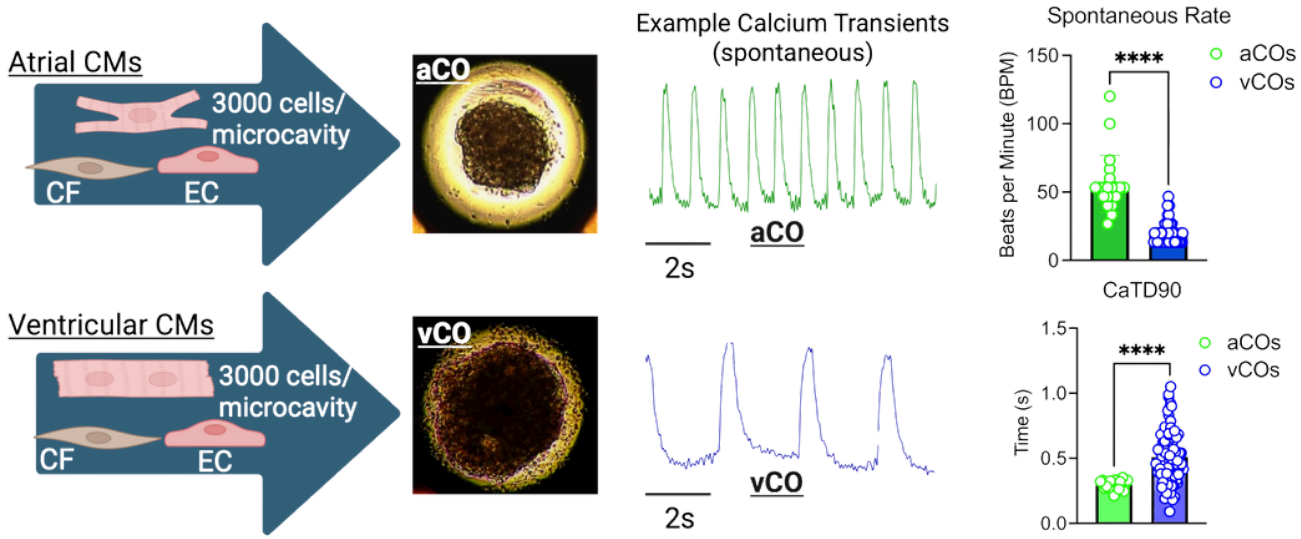
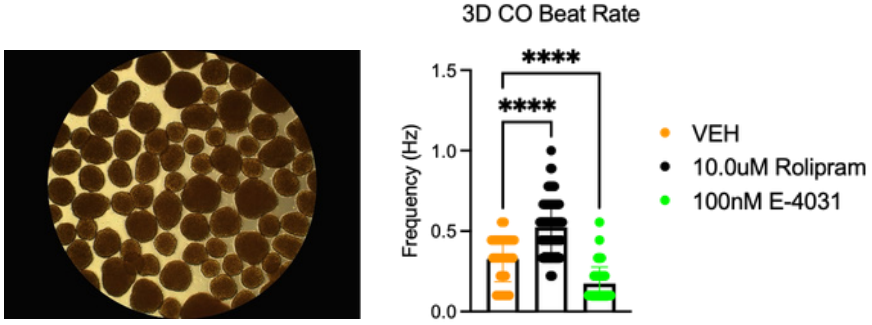
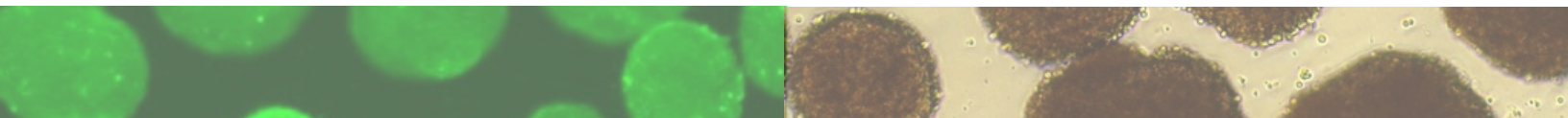



**Figure 8.** Atrial or Ventricular specific 3D cardiac organoids in vitro NAMs. Electrophysiological characterization demonstrates the expected chamber specific phenotypes. Cardiac organoids respond to drugs with expected effects, including hERG block (E-4031) and positive chronotropy (rolipram).





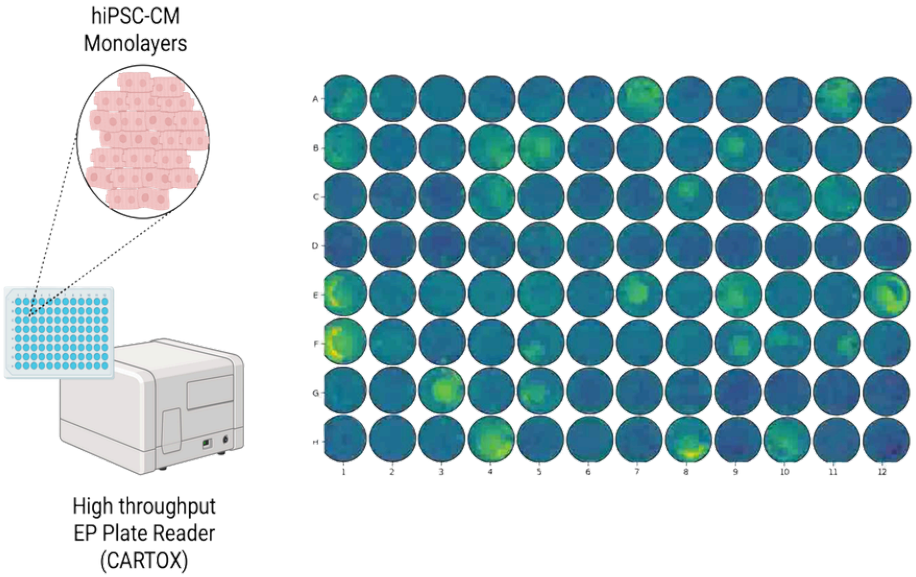



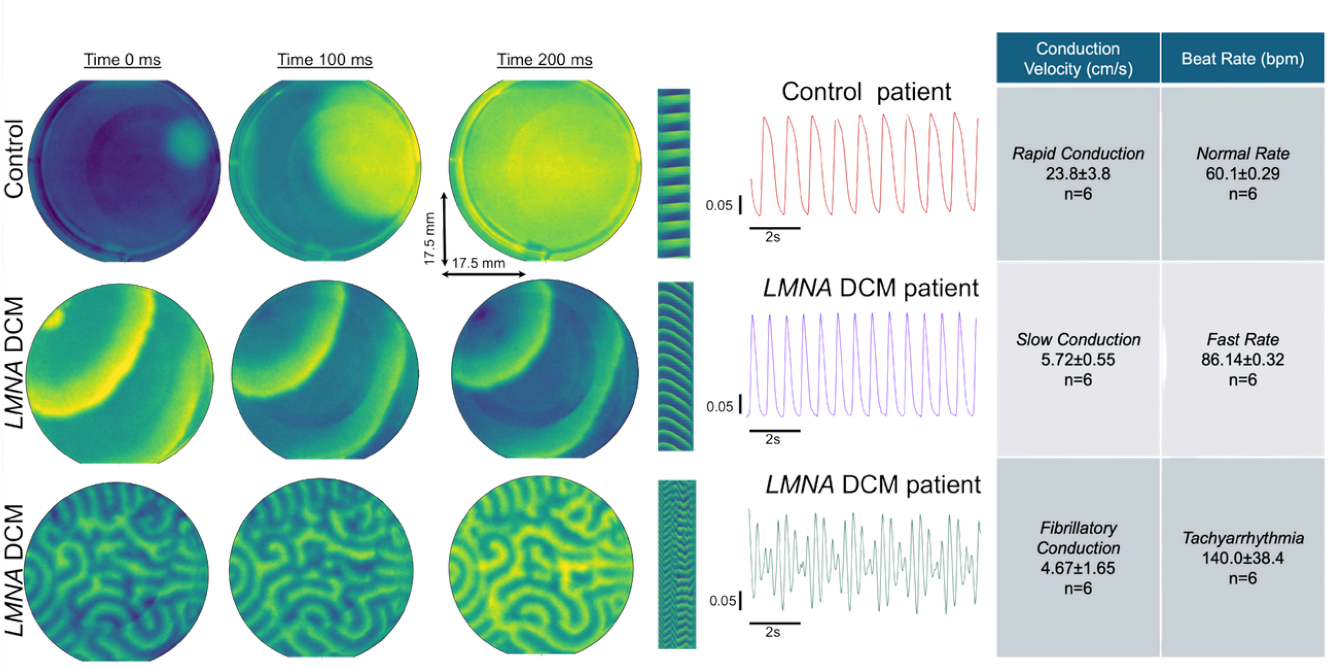
# Electrophysiology Screening Services

The FDA and ICH guidelines have adopted the use of hiPSC-CM monolayers as a cardiac safety screening assay. Greenstone's EP service can detect specific ion channel block, including Na, Ca and K (hERG).

**Figure 9.** High throughput medication screening using CARTOX Optical Mapping Reader. Greenstone provides cardiac efficacy and safety screening with detection of hiPSC-CM action potentials and calcium transients. In this assay we can detect ion channel block including:

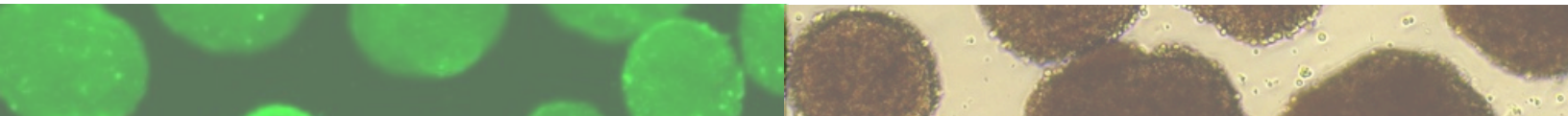
- hERG Channel
- Na Channel
- Ca Channel
- APD-Action Potential Duration
- Conduction Velocity
- Conduction patterns




| Conduction Velocity (cm/s) | Beat Rate (bpm) |
|---|---|
| *Rapid Conduction* 23.8±3.8 n=6 | *Normal Rate* 60.1±0.29 n=6 |
| *Slow Conduction* 5.72±0.55 n=6 | *Fast Rate* 86.14±0.32 n=6 |
| *Fibrillatory Conduction* 4.67±1.65 n=6 | *Tachyarrhythmia* 140.0±38.4 n=6 |





# Contact Us


+1 (650) 714-7060

info@greenstonebio.com

https://greenstonebio.com

Alexandria Center for Life Sciences
At Stanford Research Park
3160 Porter Drive, Suite 140
Palo Alto, CA 94304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