
Optogenetic control of transiently transfected hiPSC-derived cardiomyocytes for the assessment of drug related cardiotoxicity

Event

SoT - Nanion Technologies and Ncardia Sponsored Session

Presenter

Greg Luerman, PhD

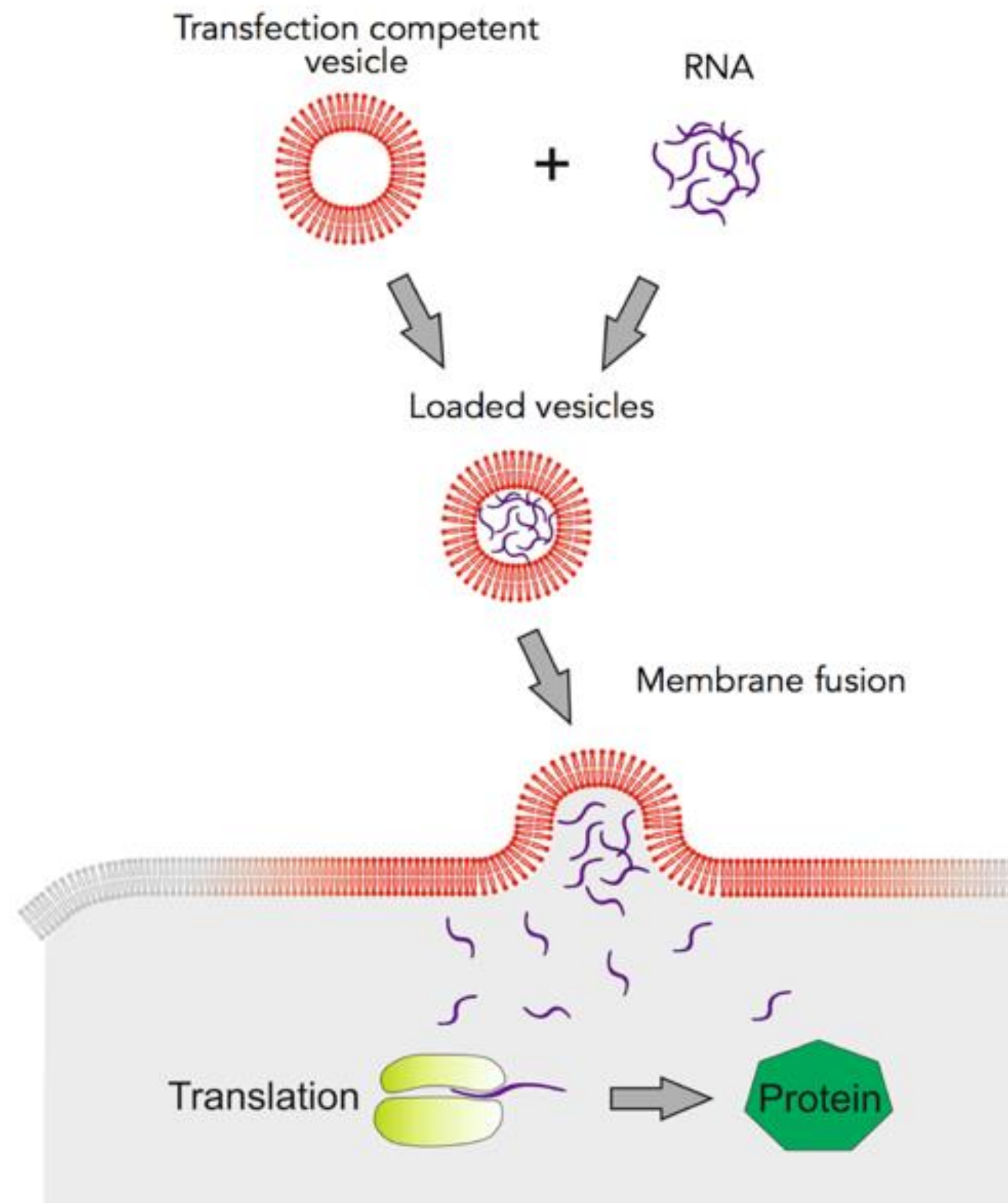
Function

Technical Director, North America

Date

March 12, 2018

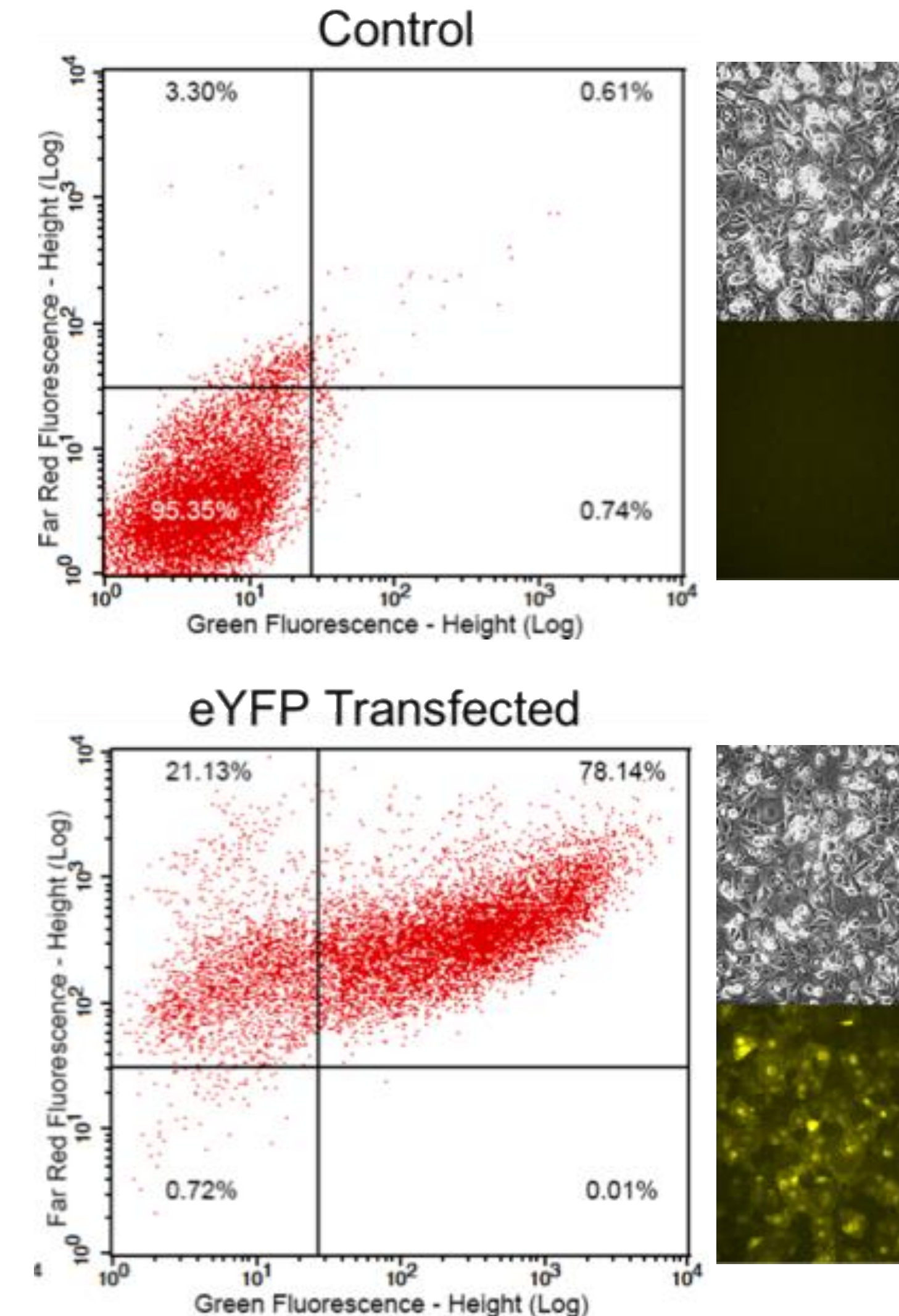
Introducing Xpress.4U™



- Transfection competent vesicles are formed around RNA cargo
- Loaded vesicles fuse with plasma membrane of target cells
- Cargo is directly released into cytosol, bypassing the endo- / lysosomal pathway
- Instant bioavailability of cargo molecules in cytosol
- Fluorescent tracer molecule in vesicles allows for verification of successful transfection or cell sorting in flow cytometry

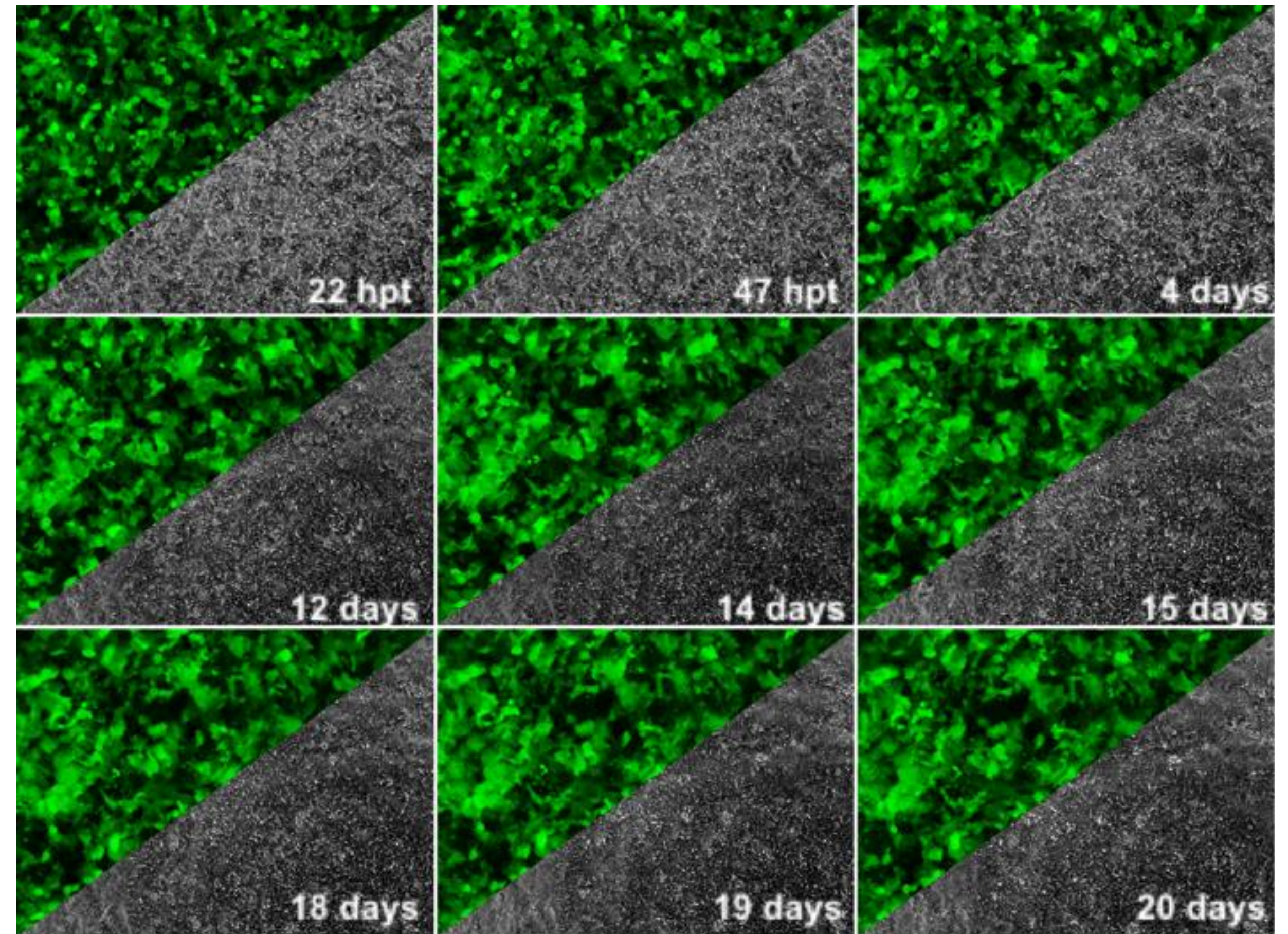
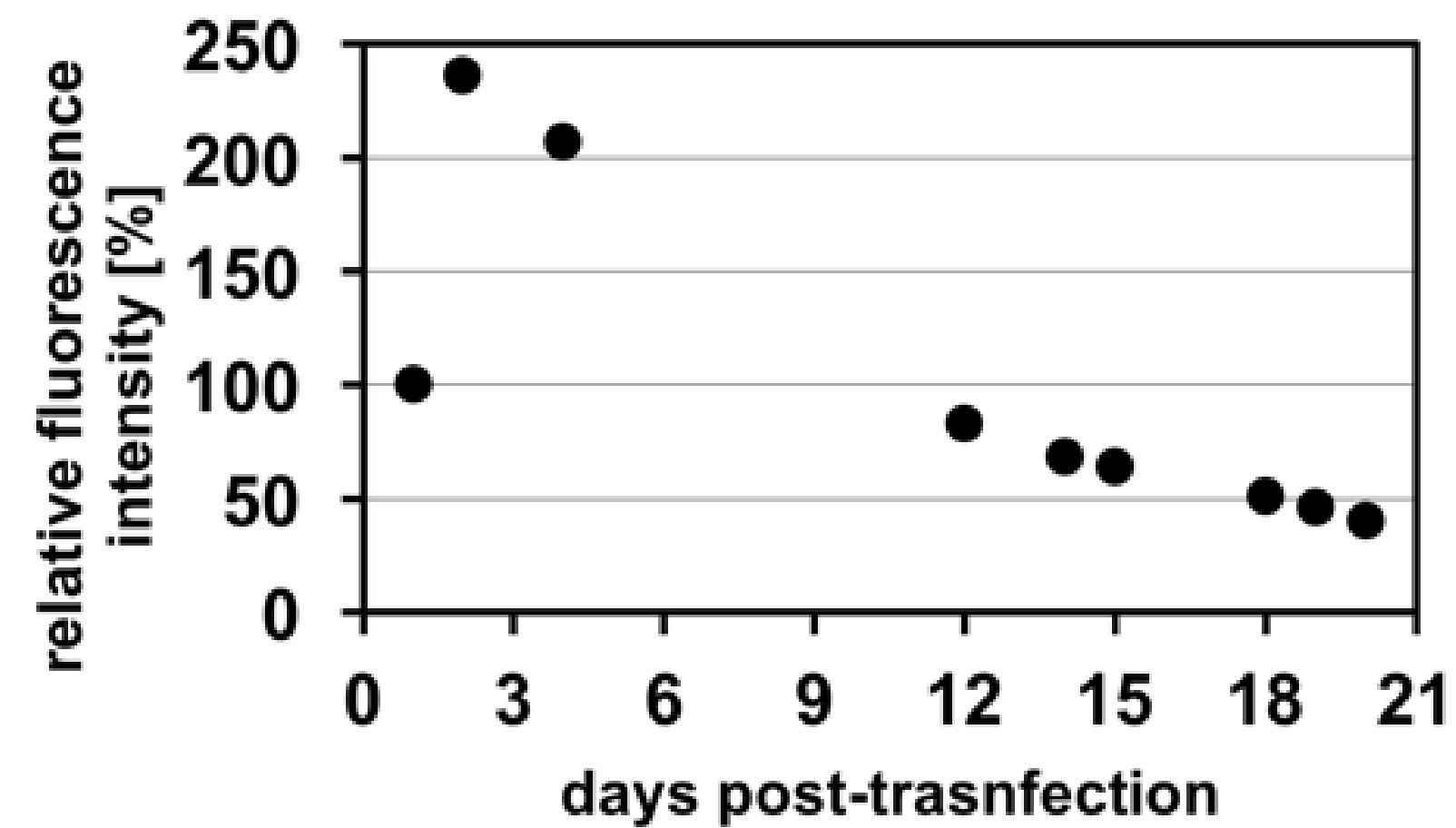
Xpress.4U™ Efficiency

- Channelrhodopsin-2 eYFP transfected Cor.4U® cardiomyocytes were examined 17 hours post-transfection.
- Control cells show double-negative signals for both eYFP and far red (lipid label)
- Xpress.4U™ treated cells show nearly 100% positive infrared staining and almost 80% of ChR2-eYFP expression



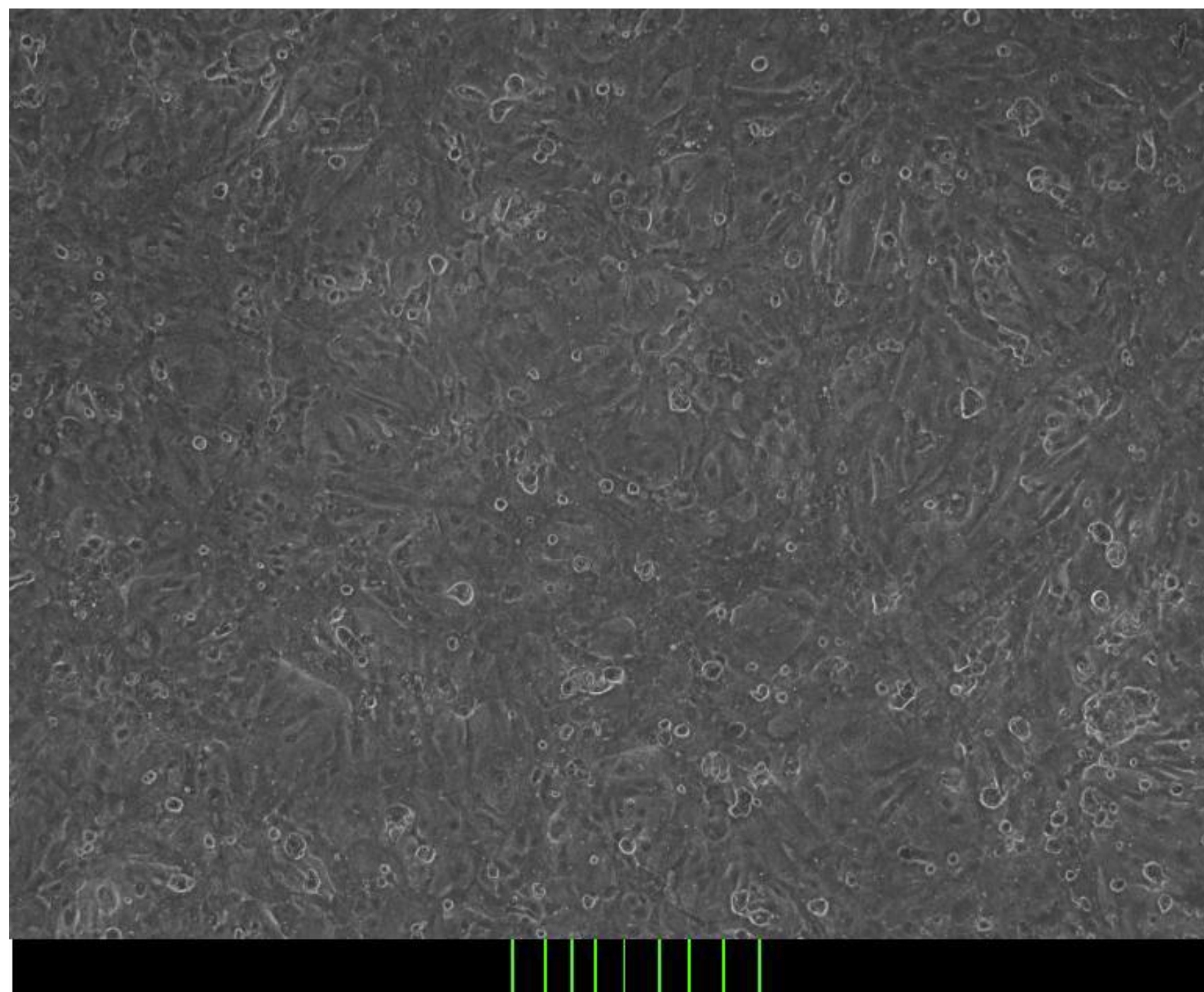
Xpress.4U™ Stability

eGFP expression > 21 days



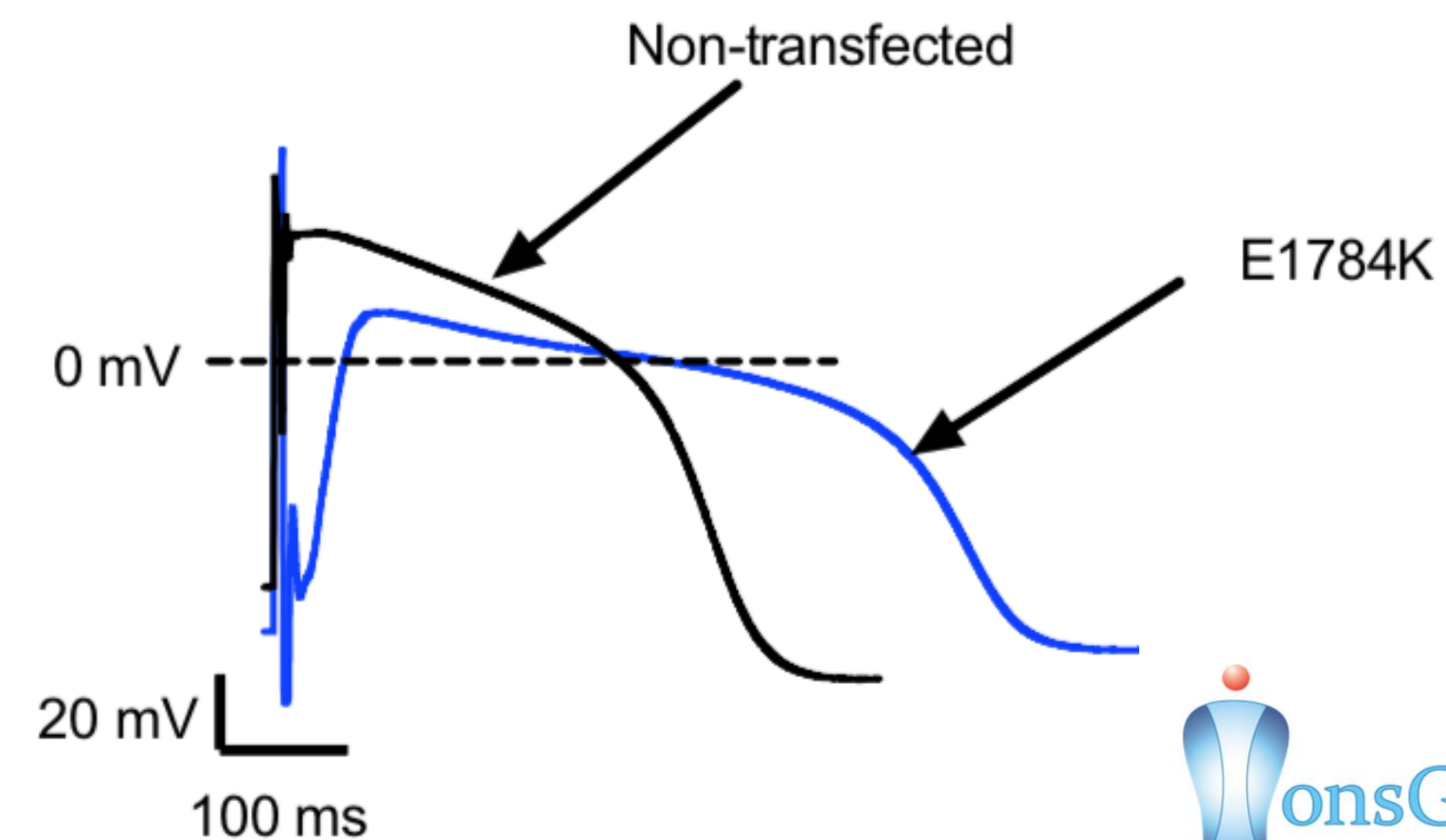
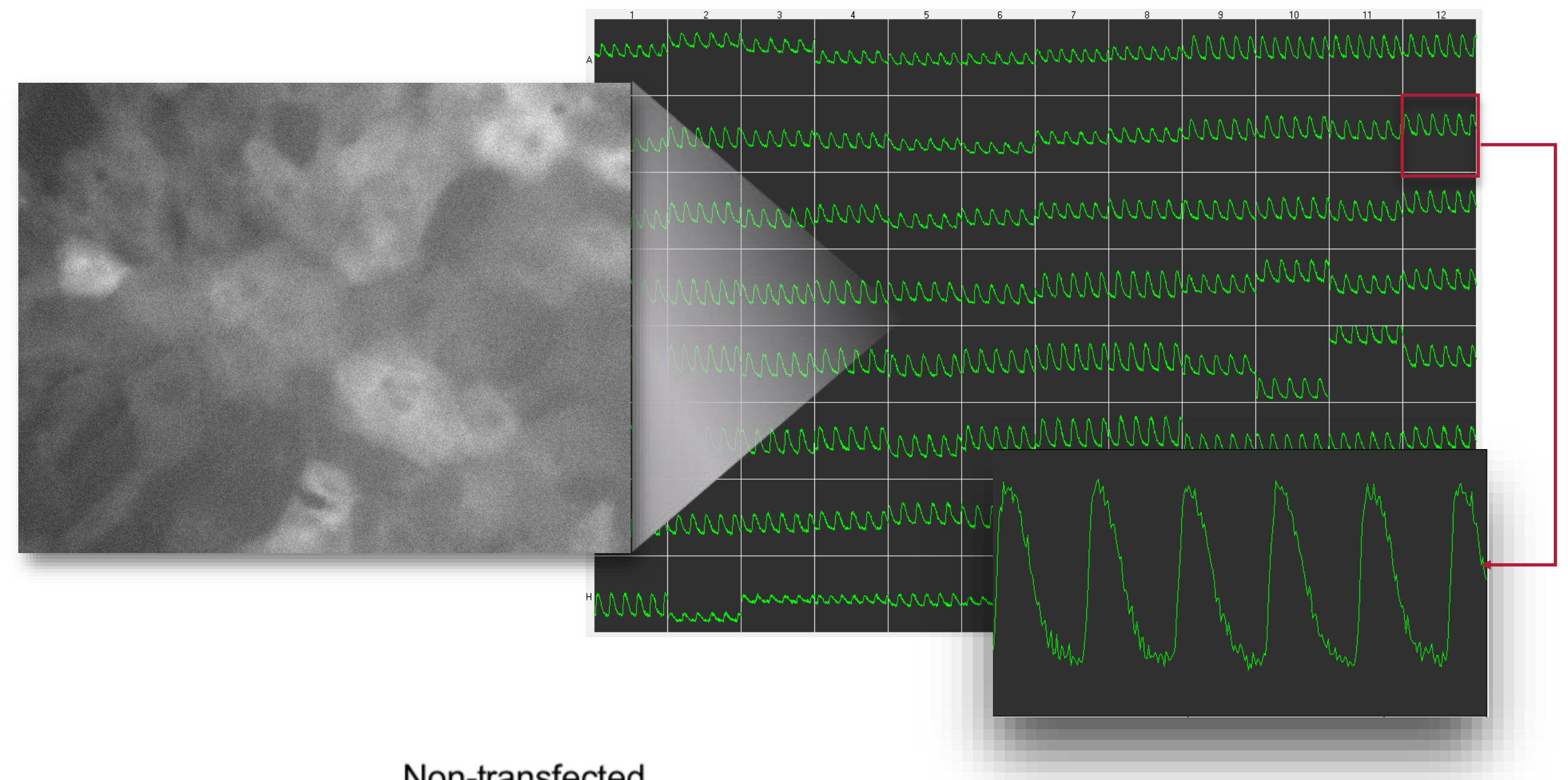
Applications of Xpress.4U™

ChannelRhodopsin2 for
optogenetic pacing



spontaneous | light pulses | recovery

GCaMP6f+ for HTS calcium flux



Disease
Modeling

Xpress.4U™ LightPace

Xpress.4U™ LightPace Kit

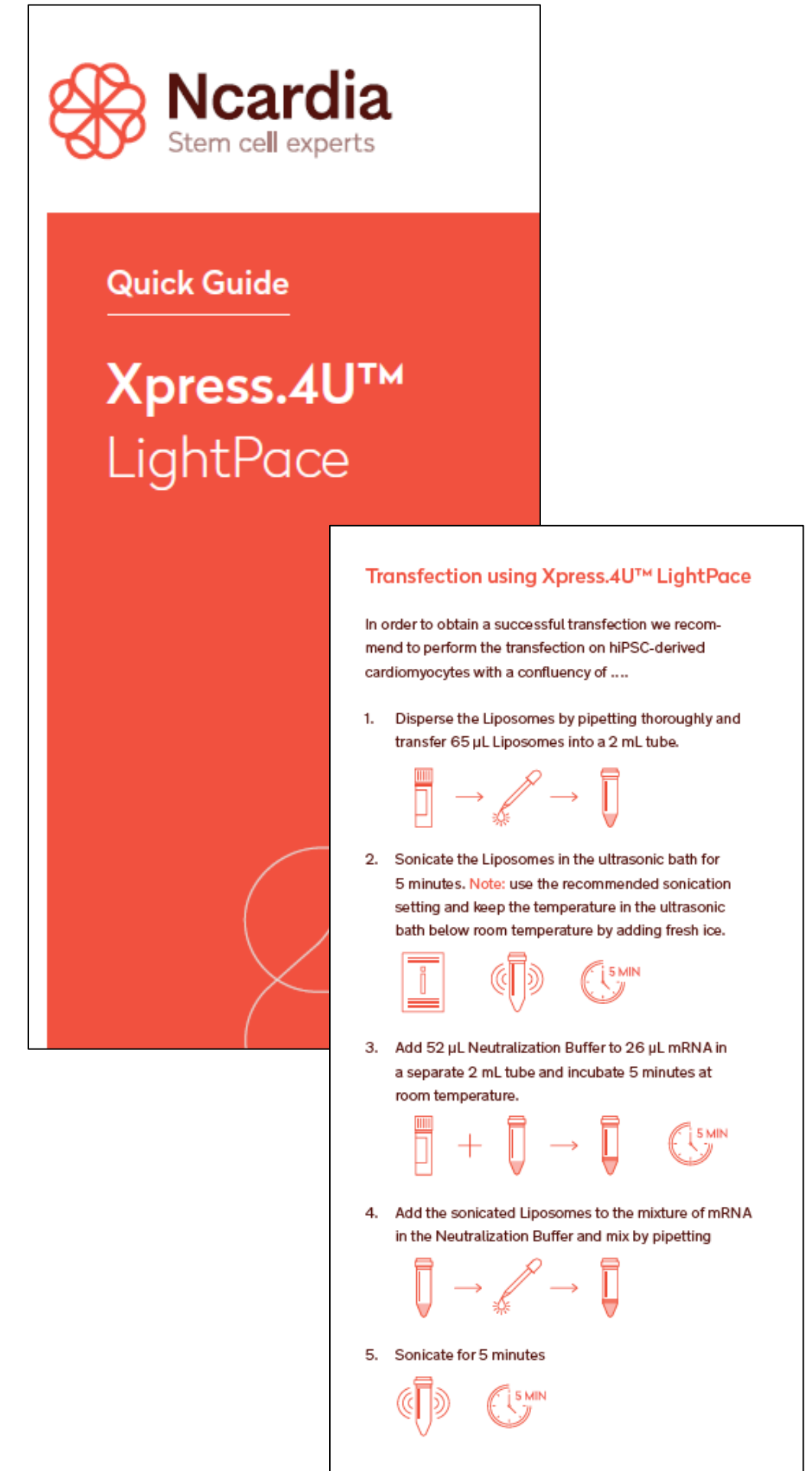
- ChannelRhodopsin2 mRNA
- Proprietary reagents for fusion reaction
- Strict QC
- Fully optimized and validated protocol
- Included pacing protocol
- Quick Guide for the bench side
- Application note on assessment of compound responses



Advantages of Xpress.4U™ LightPace

- No protocol optimization needed
- No toxicity
- Validated on different instruments
- Strictly QCed manufacturing
- No viral biosafety requirements
- No stimulation artifacts, no beat rate correction needed

→ Enables detection of (reverse) use dependency,
isolate repolarization effects, etc



Ncardia
Stem cell experts

Quick Guide

Xpress.4U™
LightPace

Transfection using Xpress.4U™ LightPace

In order to obtain a successful transfection we recommend to perform the transfection on hiPSC-derived cardiomyocytes with a confluency of ...

1. Disperse the Liposomes by pipetting thoroughly and transfer 65 µL Liposomes into a 2 mL tube.

2. Sonicate the Liposomes in the ultrasonic bath for 5 minutes. *Note:* use the recommended sonication setting and keep the temperature in the ultrasonic bath below room temperature by adding fresh ice.

3. Add 52 µL Neutralization Buffer to 26 µL mRNA in a separate 2 mL tube and incubate 5 minutes at room temperature.

4. Add the sonicated Liposomes to the mixture of mRNA in the Neutralization Buffer and mix by pipetting

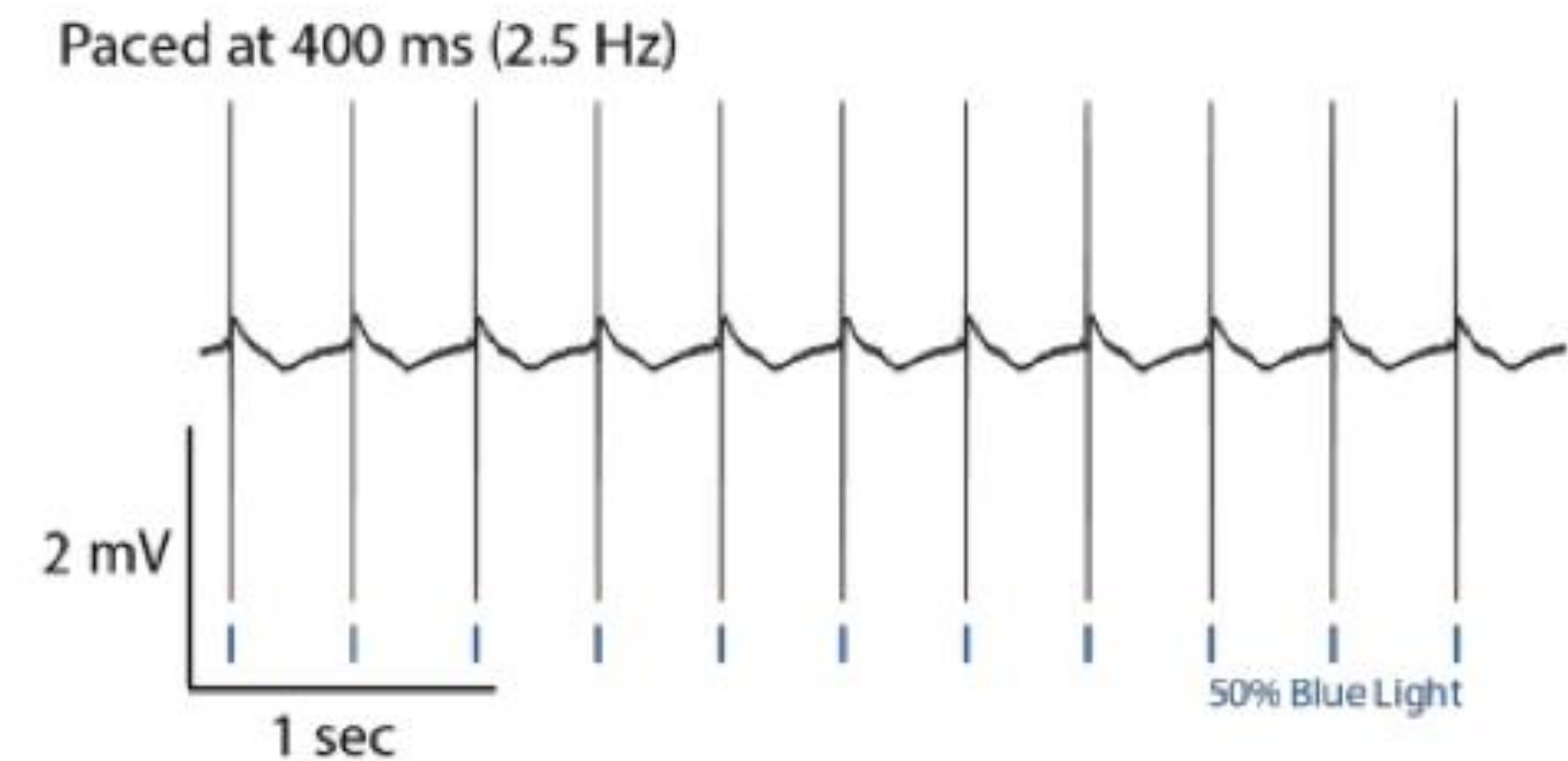
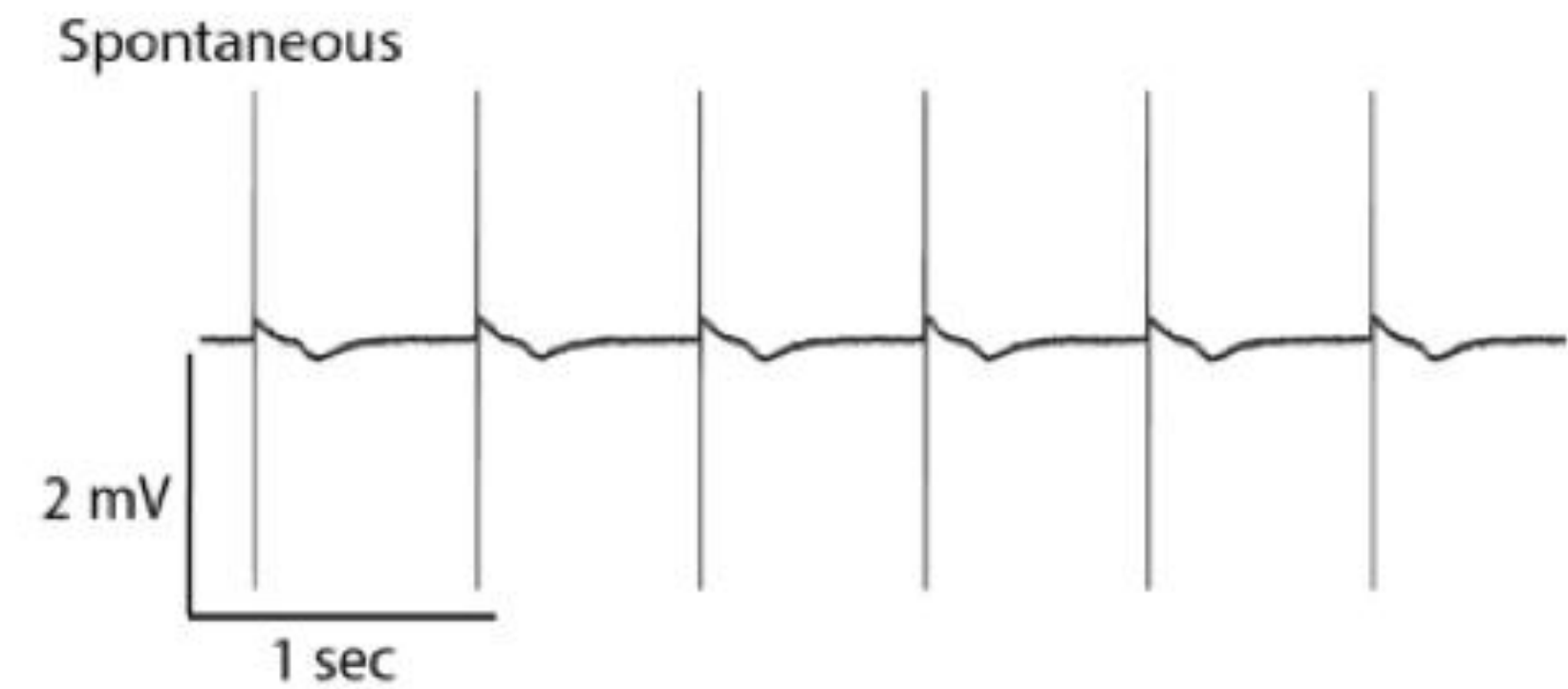
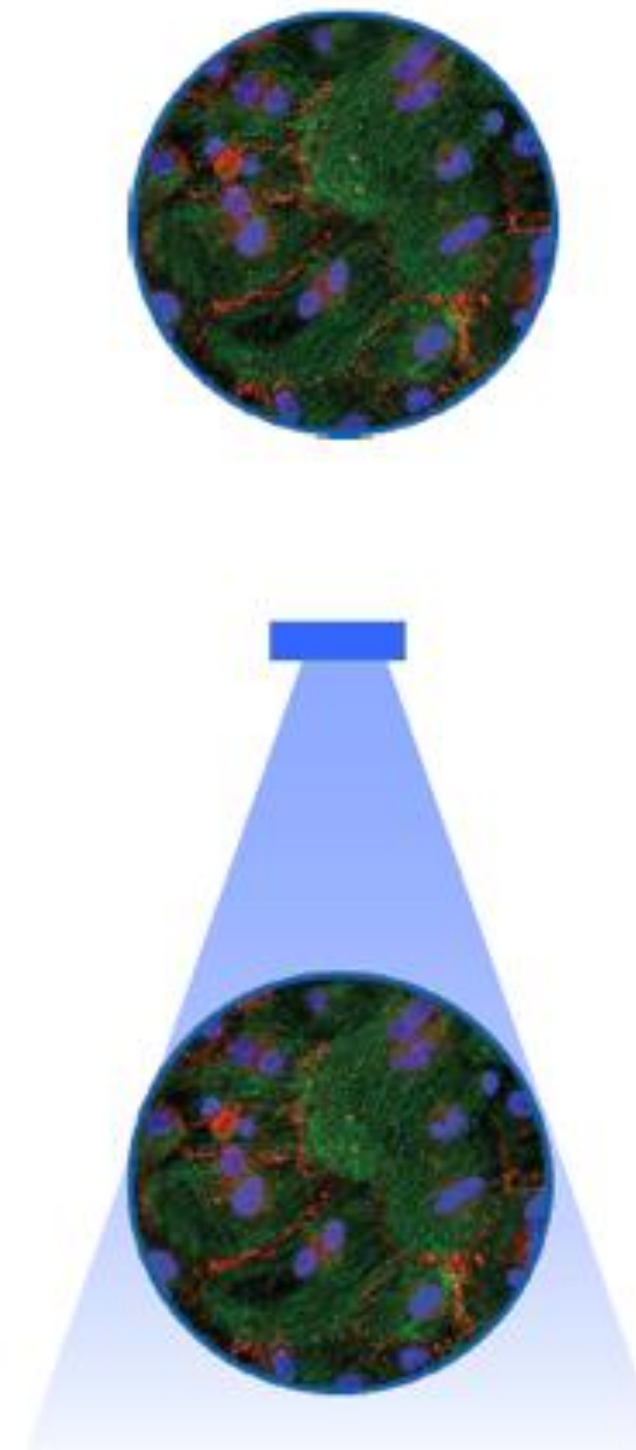
5. Sonicate for 5 minutes


Validated on multiple MEA+LED platforms

CardioExcyte96 SOL



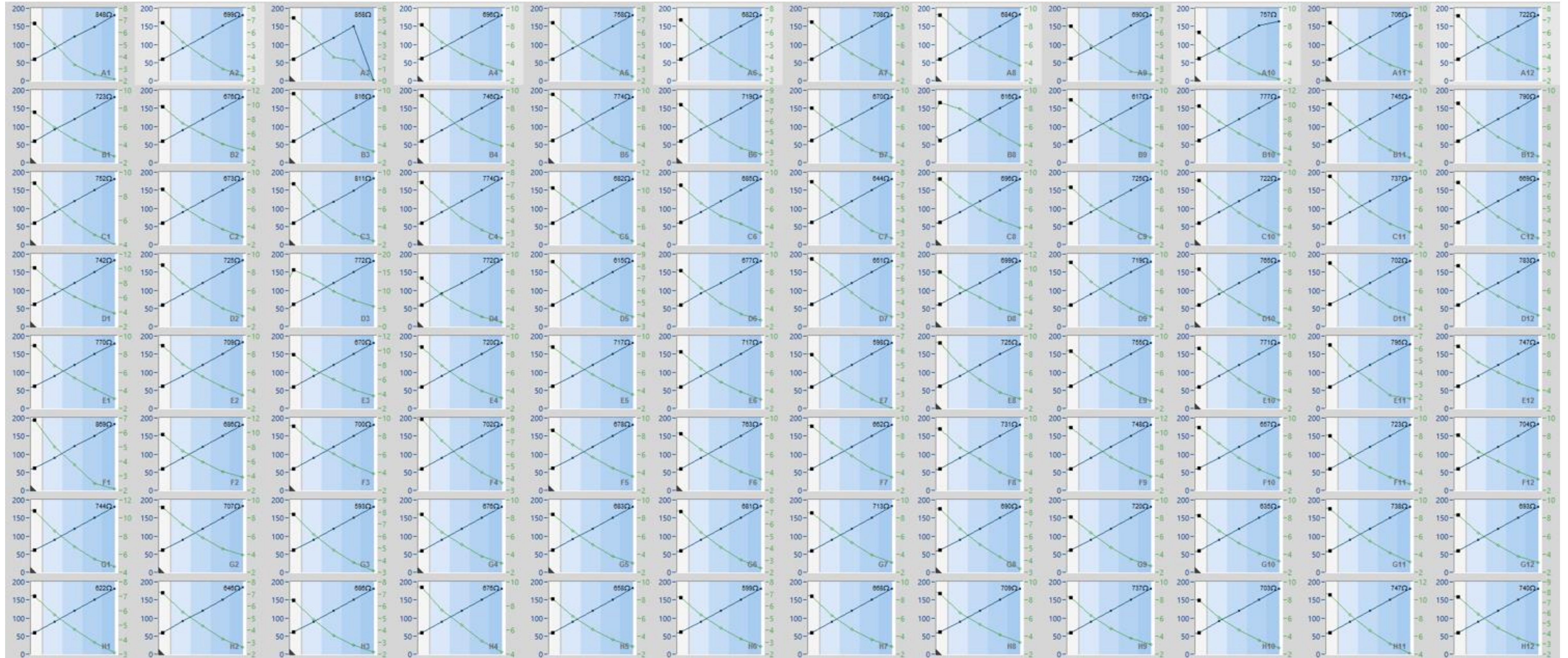
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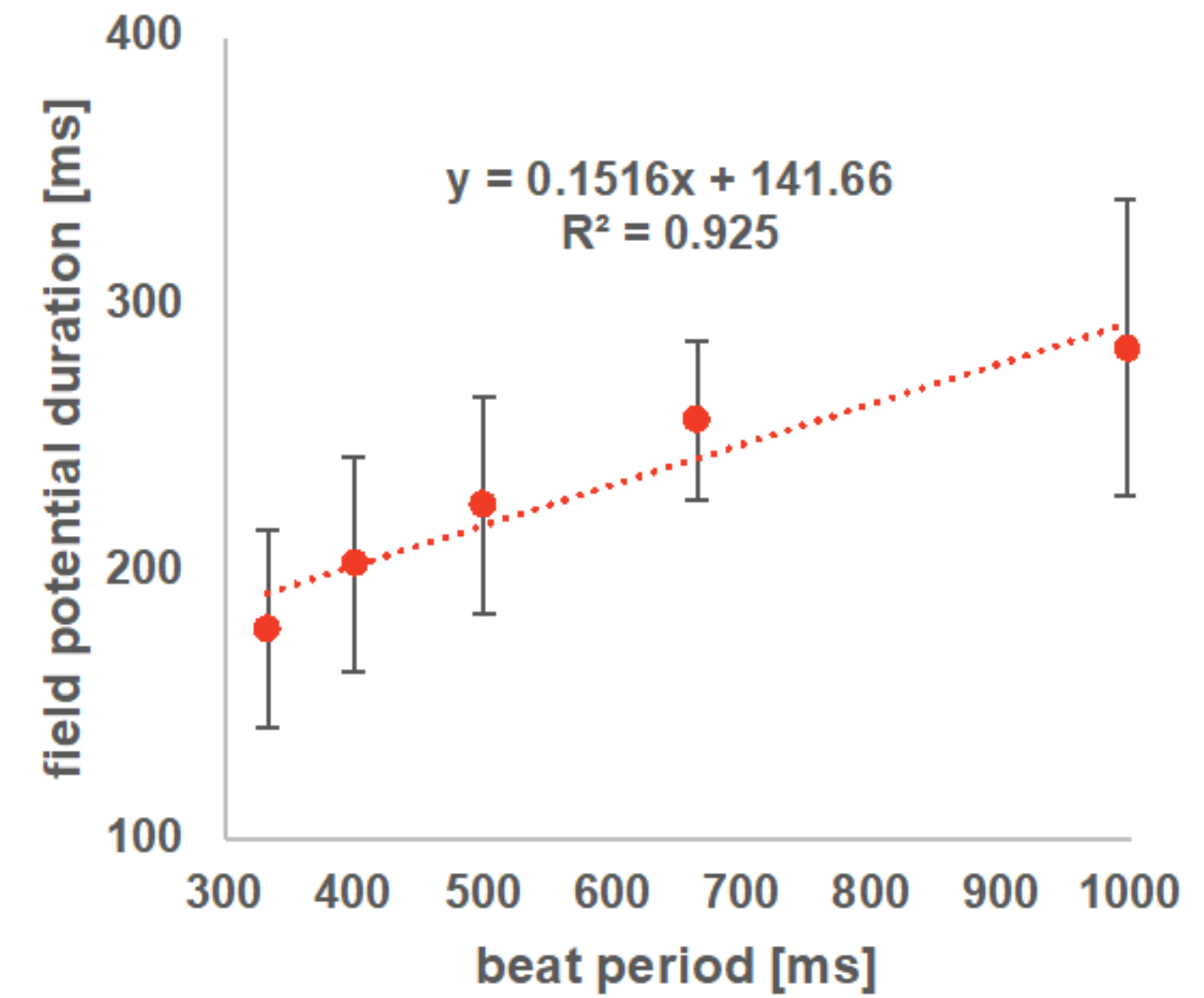
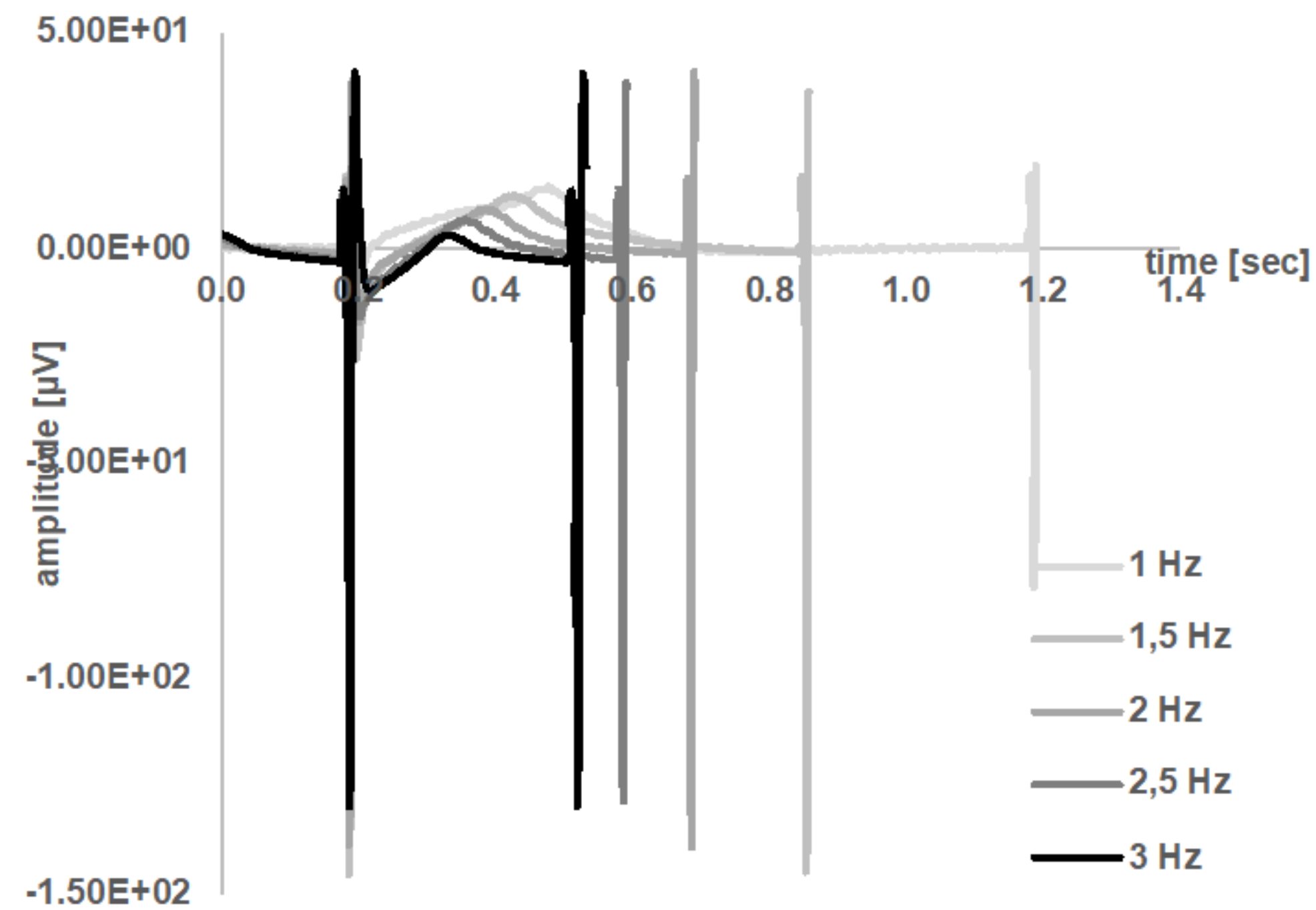
Xpress.4U™ LightPace in Cor.4U®

A pacing experiment on the CardioExcyte 96 SOL

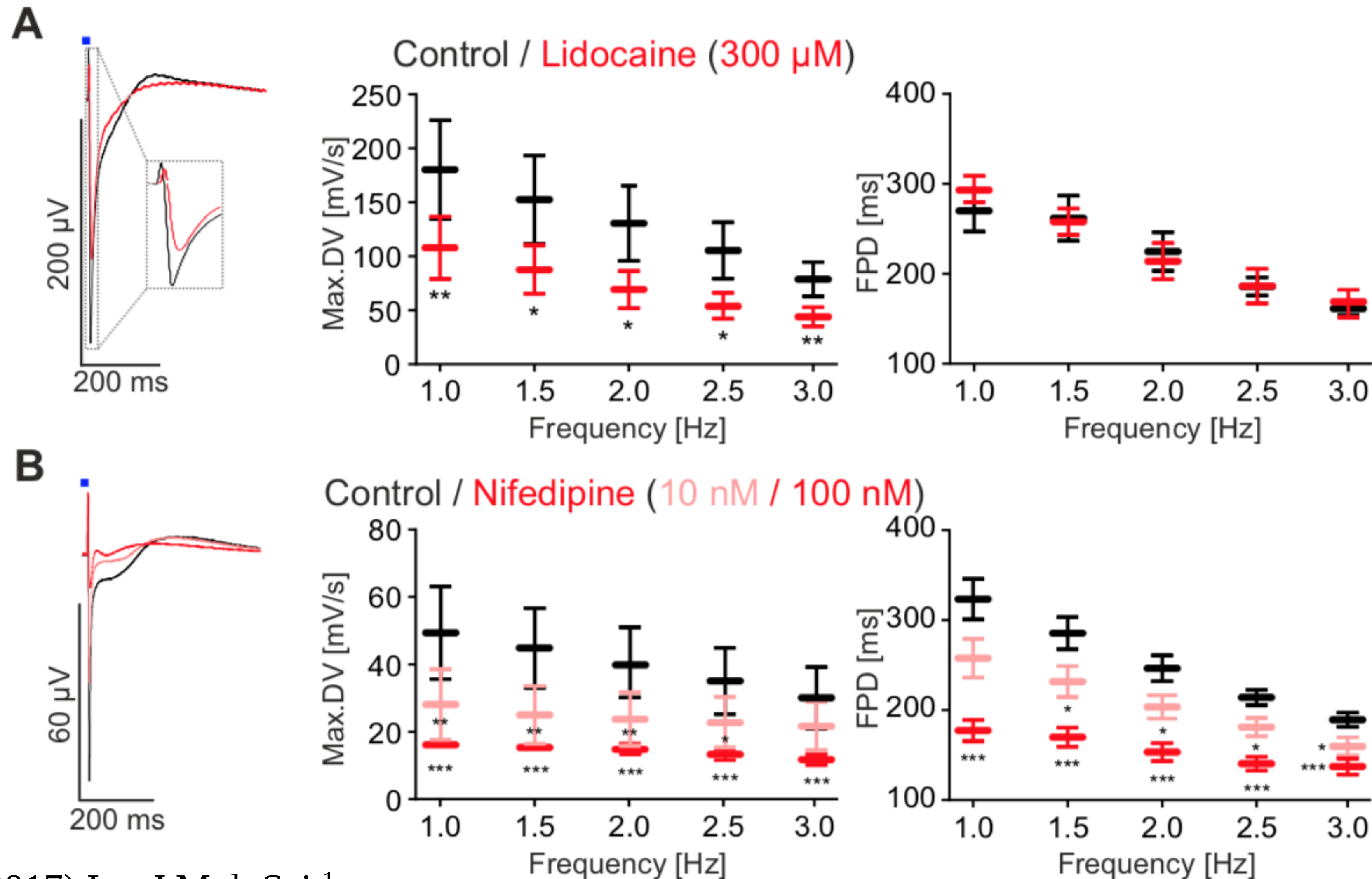
Well-to-well reproducibility: perfection?



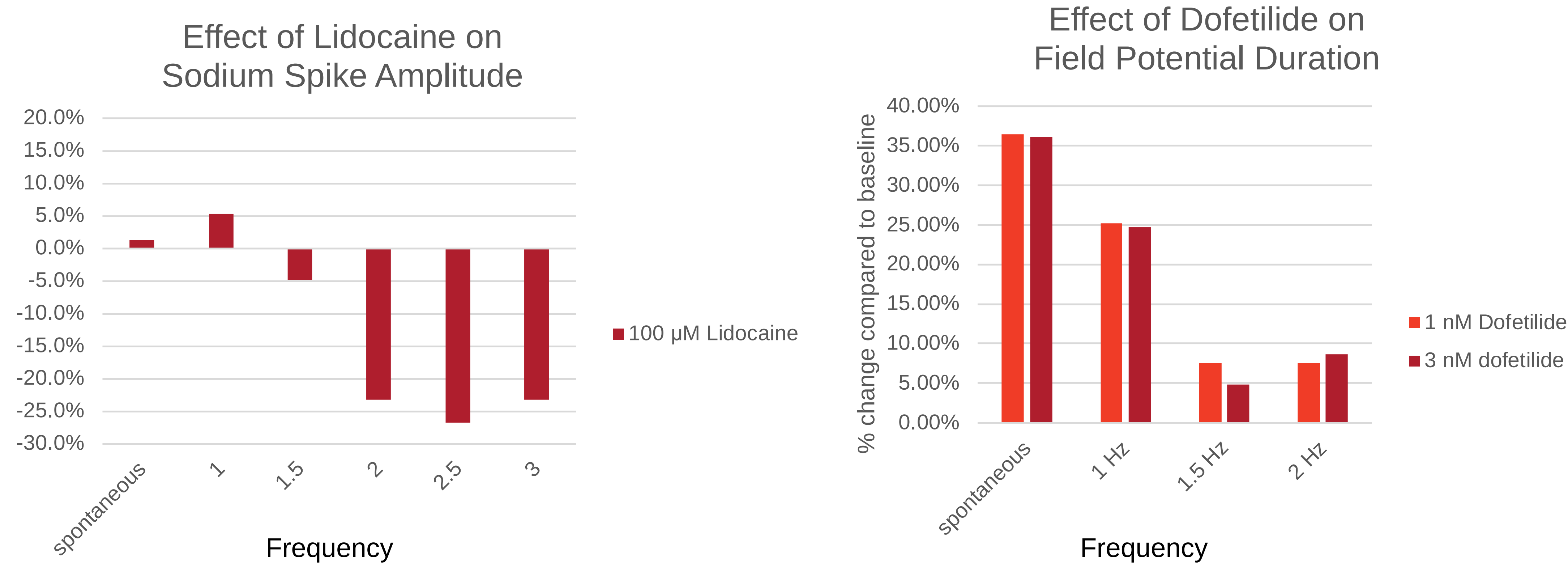
Correlation FPD – Beat period



Mechanistic insight with MEA + optogenetics



Use dependence and reverse-use dependence of Lidocaine and Dofetilide, respectively



Xpress.4U™ Advantages

- Virus-free, non-toxic
- No genomic integration, transient transfection of mRNA, siRNA
- Modified mRNAs allow for high stability and prolonged protein expression
- Optimized and validated protocol
- Easy to use
- Integrated fluorescent tracers help illuminate successfully transfected cells
- Functionalize cells with integrated biosensors and optogenetic tool

Thank you!

