



Characterization of Two Eurofins DiscoverX's PrecisION[®] Nicotinic Cell Lines Using Automated Electrophysiology

Drug Discovery for Ion Channels XXIII Satellite Meeting

February 17th, 2023

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Scientific Market Development Manager – Ion Channels

Eurofins DiscoverX, Fremont CA



OUR EXPERTISE
IN YOUR HANDS.
DISCOVER
CONFIDENTLY.

Eurofins DiscoverX company introduction

Introduction to ion channel products

- PrecisION[®] stable cell lines
- PathHunter[®] cell-based trafficking assays
- Custom development capabilities

SyncroPatch 384i utility in workflows

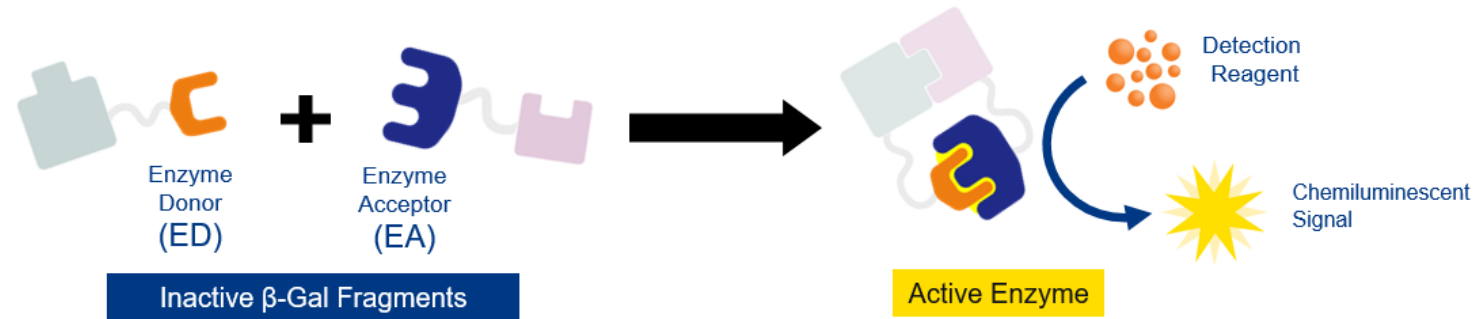
Nicotinic data on SyncroPatch

Summary and conclusions



Technology based on the Enzyme Fragment Complementation (EFC) platform

- Flexible assay platform based on a split β -galactosidase enzyme



Over 1000 functional assays available for popular target classes

- GPCRs
- Kinases
- Cytokines
- Checkpoints Receptors
- Nuclear Proteins
- Epigenetic Proteins
- Cell Signaling Pathway Targets
- Ion Channels



Headquarters based in Fremont, CA

High-quality cell lines for target discovery, hit screening, lead optimization, and safety studies

Eurofins acquired DiscoverX (founded in 2000) in 2017 and became part of Eurofins Discovery

Obtained PrecisiON ion channel stable cell lines portfolio through acquisitions

- Cytomyx begins the collection of PrecisiON cell lines
- Serologicals acquires Cytomyx
- Millipore acquires Serologicals
- Merck KGaA acquires Millipore
- Eurofins acquires Merck Millipore (EMD Millipore)

Human Origin Unless Otherwise Noted

hERG (K⁺)

hERG-CHO
hERG-HEK

Na⁺ Channels

Na_v1.1-HEK
Na_v1.2-CHO
Na_v1.3-CHO
Na_v1.4-HEK
Na_v1.5-HEK
Na_v1.6-HEK
Na_v1.7-HEK
Na_v1.8/β1-HEK
ratNa_v1.8-ND7

K⁺ Channels

K_v1.1-CHO
K_v1.2-CHO
K_v1.3-CHO
K_v1.4-CHO
K_v1.5-CHO
K_v1.6-CHO
K_v1.7-CHO
K_v1.8-CHO
K_v12.2-HEK
K_v2.1-CHO
K_v2.1/K_v9.2-CHO
K_v3.1-CHO
K_v3.2-CHO
K_v3.3-CHO
K_v4.2/KChIP2-CHO

K⁺ Channels

K_v4.3/KChIP1-CHO
K_v4.3/KChIP2-HEK
K_v7.2/K_v7.3-CHO
K_v7.3/K_v7.5-CHO
K_v7.4-HEK
K_v7.4/K_v7.5-HEK
Kir2.1HEK
Kir6.2/SUR2A-HEK
KNCQ1/hminK-CHO

HCN Channels

HCN1-HEK
HCN2-HEK
HCN3-HEK
HCN4-CHO

Ca²⁺ Channels

Ca_v2.2-HEK
Ca_v3.2-HEK

Other Channels

ASIC3-HEK
CFTR-HEK
TRPA1-HEK
TRPV1-HEK
TRPV3-HEK
TRPV4-HEK

Ligand-Gated

nAChR α1/β1/δ/ε-HEK
nAChR α3/β4-HEK
nAChR α4/β2-HEK
nAChR α7/ric3-HEK
GABAA α1/β3/γ2-HEK
GABAA α3/β3/γ2-HEK
GABAA α4/β3/γ2-HEK
GABAA α5/β3/γ2-HEK
GABAA α6/β3/γ2-HEK
GlyRA1-HEK
GluR6-HEK

PathHunter® Ion Channel Trafficking Assays

Identify pharmacochaperones that rescue disease relevant mutant membrane proteins

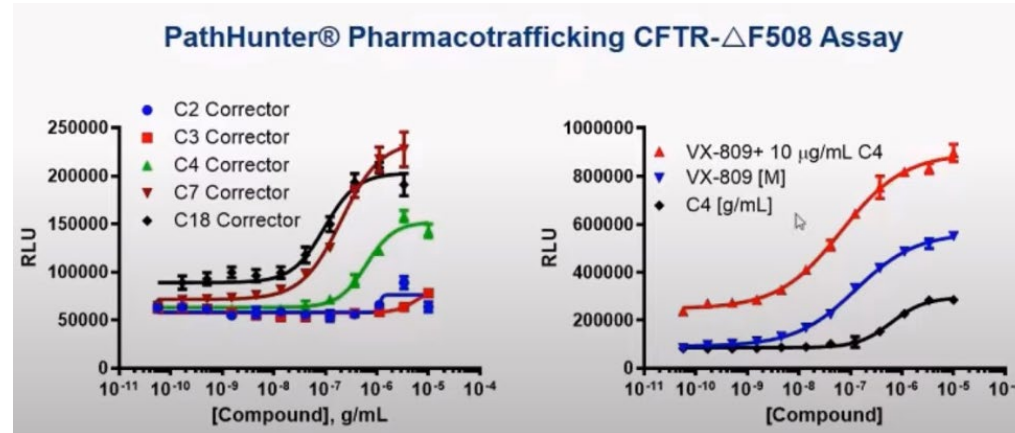
Currently two ion channel trafficking assays

- Contact customdevelopment@eurofins.com for additional assays

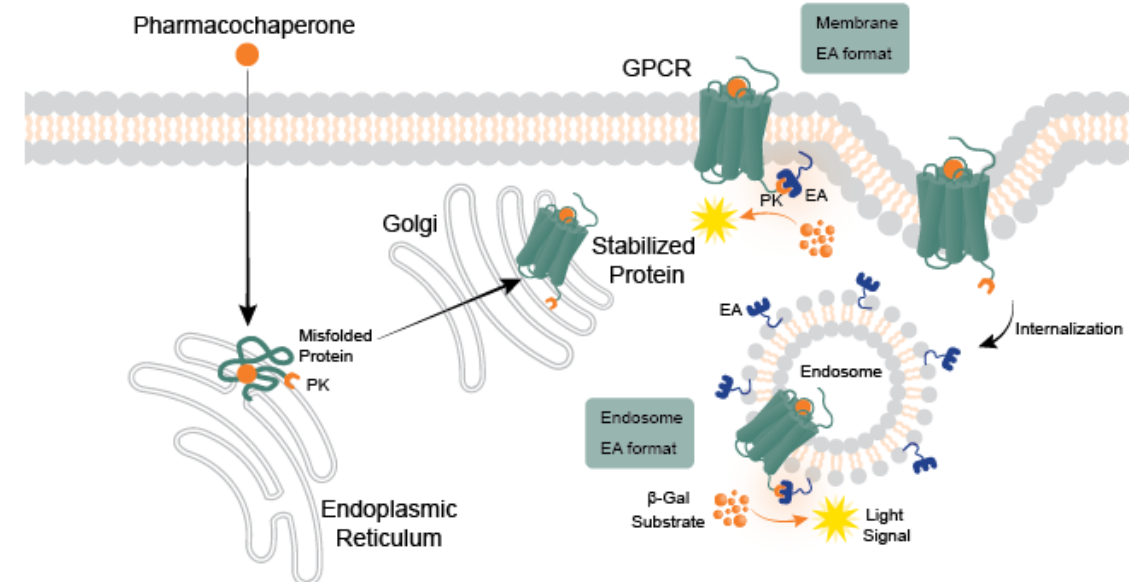
CFTR-ΔF508 trafficking assay (collaboration with Vertex)

- Compound rank order of correctors was the same as indicated in the literature
- Additive effect was revealed for VS-809 and C4 corrector

CFTR-ΔF508	Cystic fibrosis transmembrane conductance regulator	Cystic fibrosis
KCNH2(G601S)	Potassium voltage-gated channel, subfamily H (eag-related), member 2	Long QT syndrome (Cardiac arrhythmias)



Assay Principle

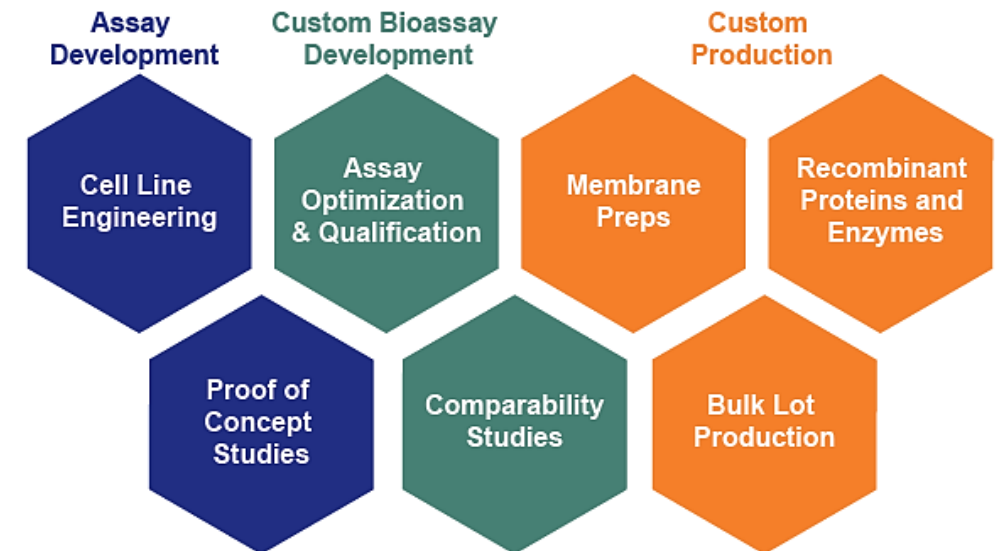


Your target biology, our expertise — building a better assay together

- **Development Expertise** — Decades of cell-based assay development, cell line engineering, and recombinant enzyme development expertise
- **Cell Line Engineering Capability** — Exogenous expression approaches (constitutive vs inducible) or gene editing (e.g. KO/KI with CRISPR/Cas9)
- **Collaborative** — Consultative assay development with regular updates through a dedicated project manager
- **Complete Solution** — Customized assay development with screening and profiling services within the same team



Custom Development Capabilities



Eurofins DiscoverX company introduction

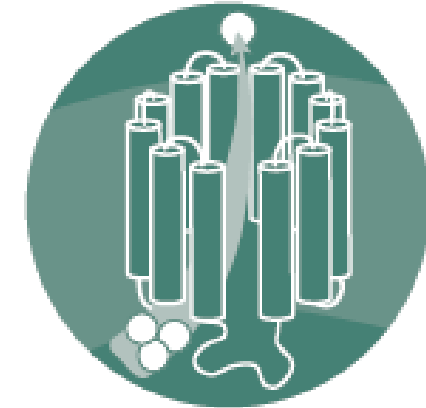
Introduction to ion channel products

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SyncroPatch 384i utility in workflows

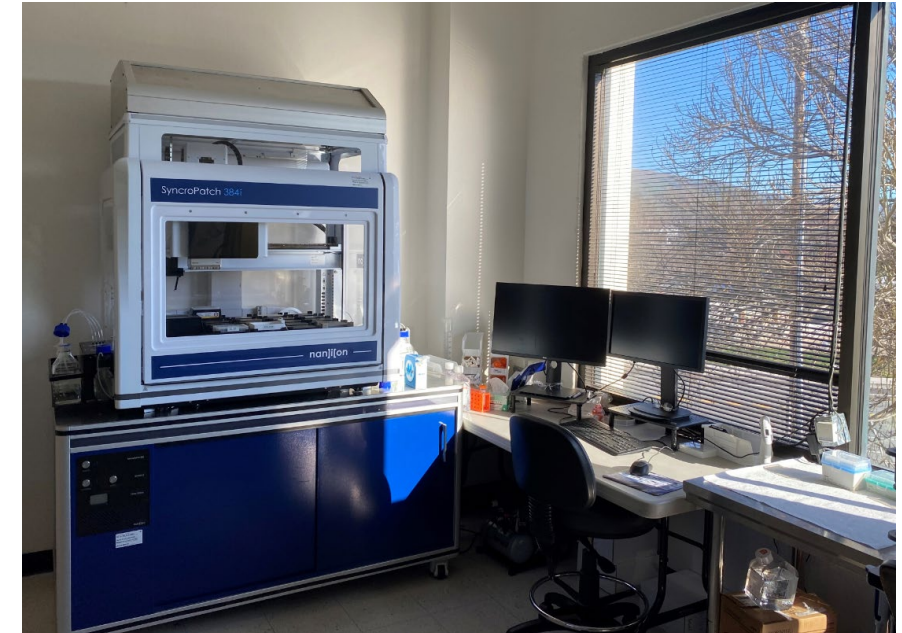
Nicotinic data on SyncroPatch

Summary and conclusions



Cell line production, marketing, and technical support

- Quality Control (QC) production of existing Eurofins DiscoverX PreciSION[®] cell lines at Fremont, CA site
- QC production of frozen ready-to-use (RTU) PreciSION channel cell lines and “panels” for therapeutic areas
- Validation of custom ion channel targets through Custom Development Capabilities program
- Expansion of our Custom Development Capabilities program into rare disease targets
 - Channelopathy variants and wild type controls



Ion channel CRO Services performed by Eurofins Discovery
in St. Charles, MO

SP 384i Functional Expression of nAChR $\alpha 4/\beta 2$

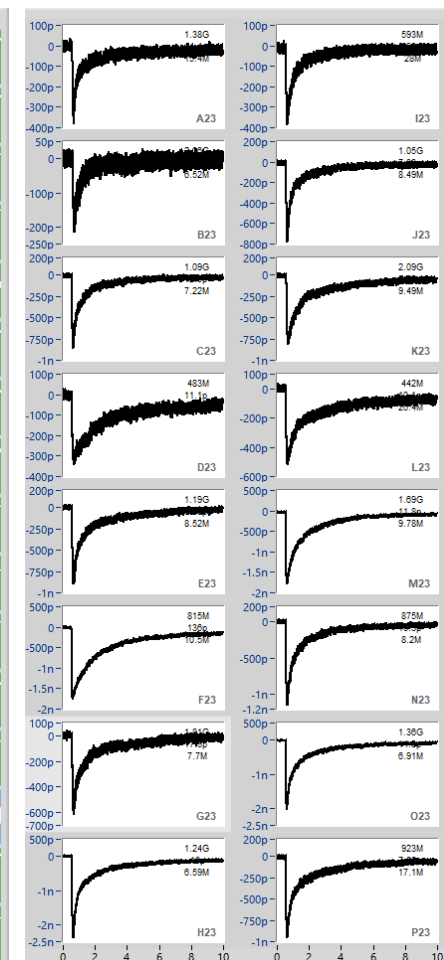
200 μ M Acetylcholine

First run (no optimization)

- Low seals
 - 40% over 500M
- Nice expression
 - 86% < -200 pA

All SP 384i data (except where noted)

- Single shot addition, ligand-puff
- Single-hole/well plate

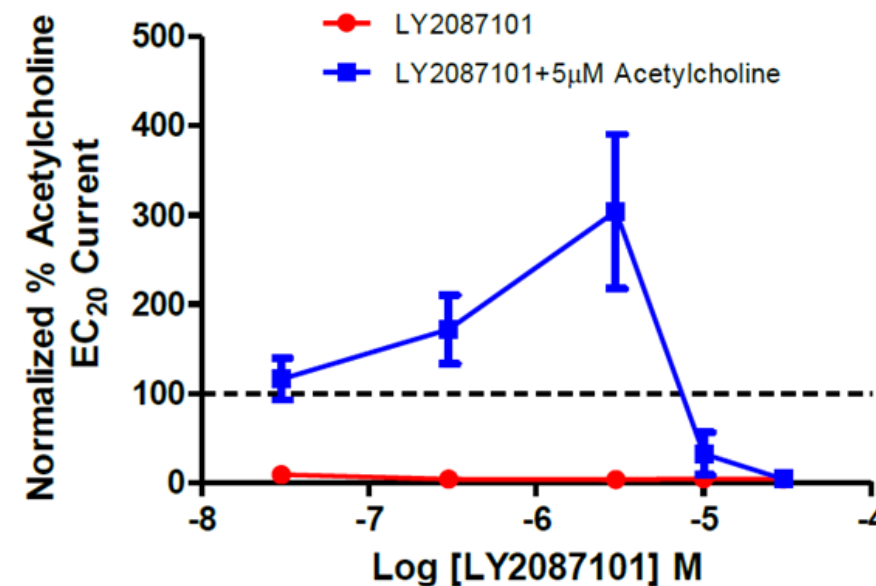
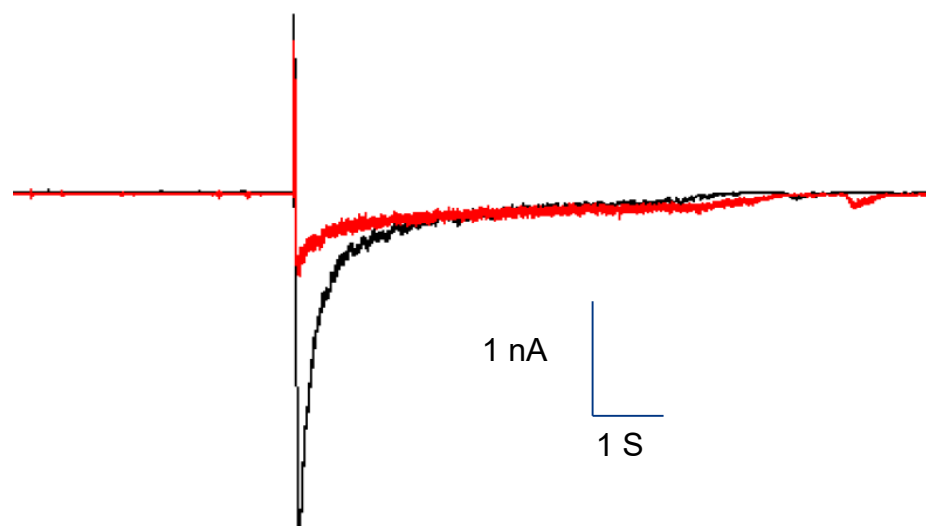


SyncoPatch (SP) 384i Data, Sept 16th 2022

Positive Allosteric Modulation (PAM) of nAChR $\alpha 4/\beta 2$

Previous work on this cell line showed the effect of the PAM LY2087101 in the presence of 5 μM ACh ($\sim\text{EC}_{20}$)

- Large potentiation by 3 μM LY2087101
- Inhibition at higher concentrations



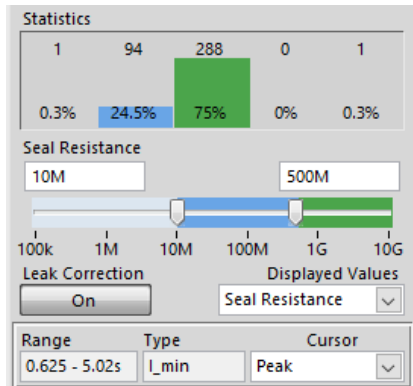
Source: Data Sheet for CYL3106 – PatchXpress® Data
Reference: Broad LM *et al* (2006). JPET 318(3):1108-1117.

Positive Allosteric Modulation (PAM) of nAChR $\alpha 4/\beta 2$

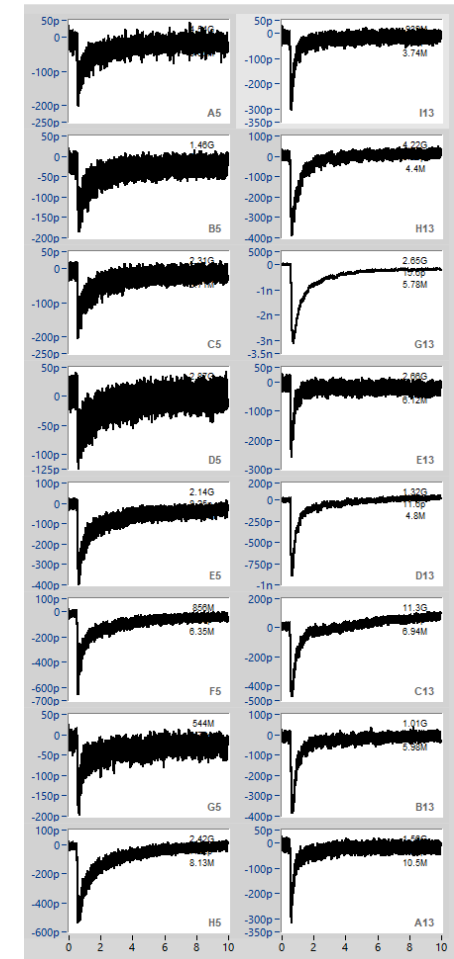
Robust enhancement of submaximal ACh currents (EC_{20}) by LY2087101

5 μ M Acetylcholine

3 μ M LY2087101

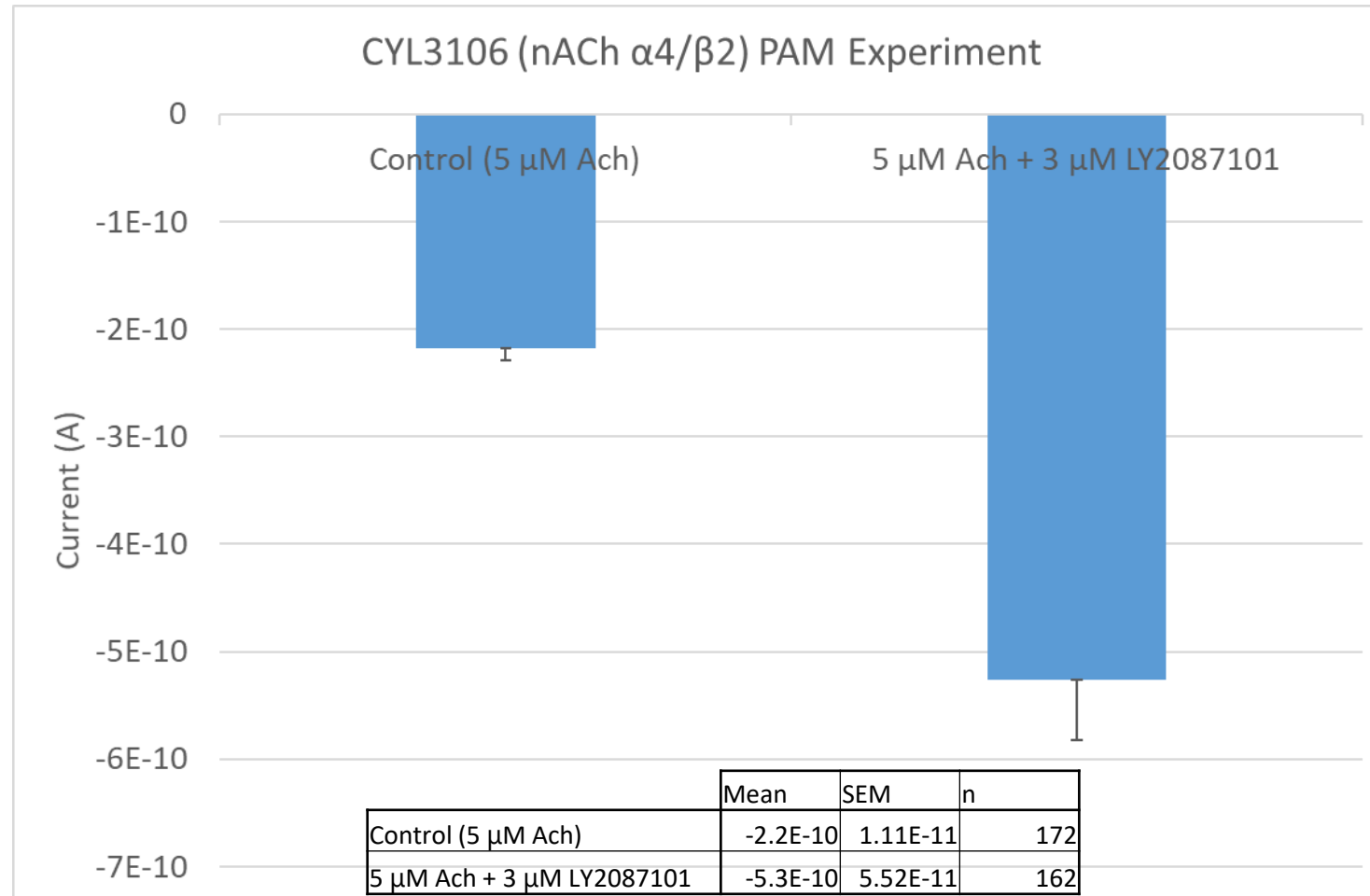


SyncroPatch 384i Data, Nov 02, 2022 (#3)



Positive Allosteric Modulation (PAM) of nAChR $\alpha4/\beta2$

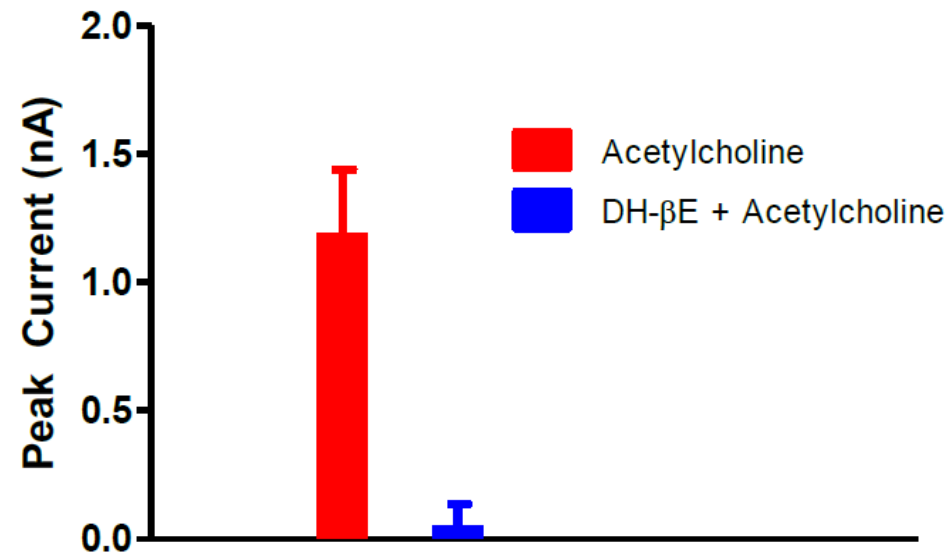
Robust enhancement of submaximal ACh currents (EC_{20}) by LY2087101



Antagonism of nAChR $\alpha4/\beta2$ by DH- β E

Previous work on this cell line showed the effect of the antagonist dihydro- β -erythroidine (DH- β E) in the presence of 100 μ M Ach

- Large Inhibition by 10 μ M DH- β E



Source: Data Sheet CYL3106 – PatchXpress® Data

References:

- Chavez-Noriega LE et al (1997), JPET 280(1): 346-356
- Yu R et al (2019). Br J Pharmacol. 2019;176:2750–2763

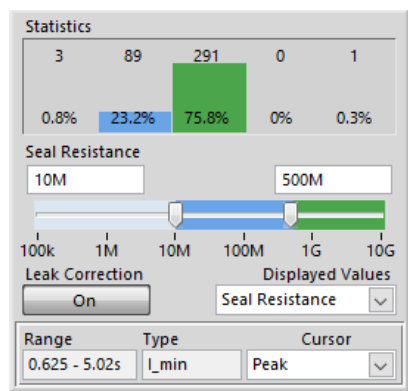
Antagonist* Data for nAChR $\alpha 4/\beta 2$

Inhibition of ACh currents by 10 μM DH- βE

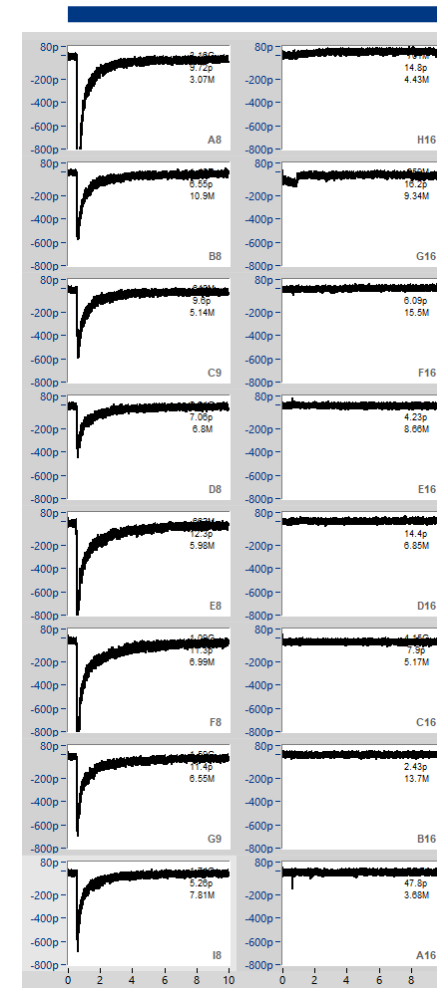
100 μM Acetylcholine

10 μM DH- βE

*dihydro- β -erythroidine

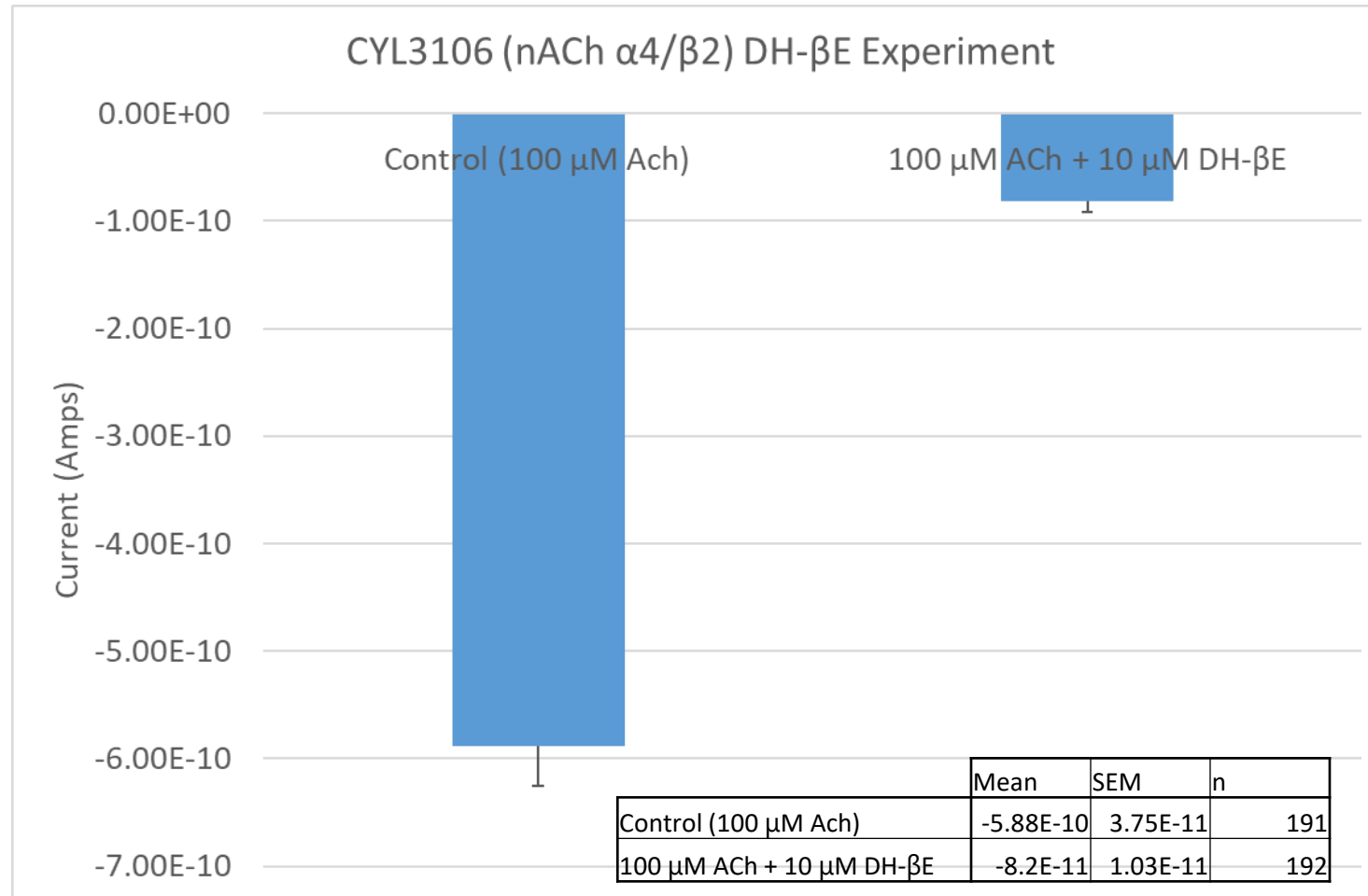


SynroPatch 384i Data, Nov 08, 2022



Antagonism of nAChR $\alpha 4/\beta 2$ by DH- β E:

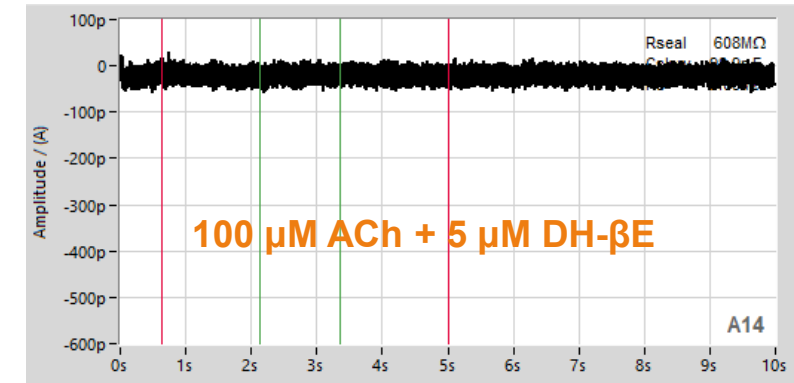
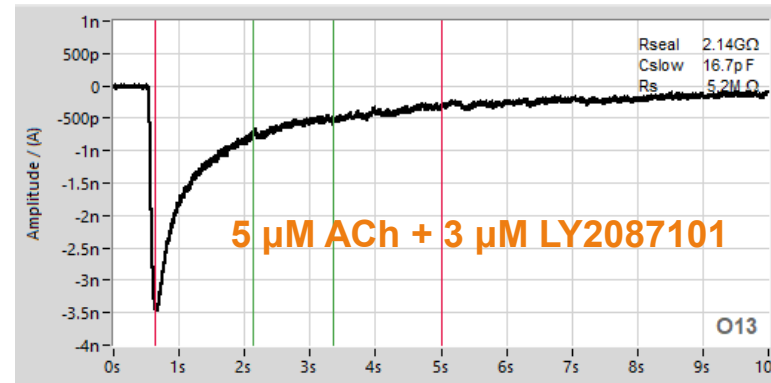
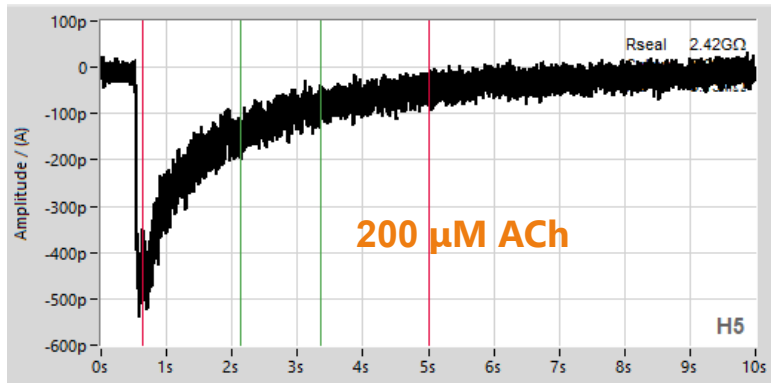
Complete inhibition
of ACh currents
down to the
baseline noise



SyncroPatch 384i Data, Nov 08, 2022

Functional Activity

- **Agonist Activation:** Currents were activated by 200 μM ACh, and partially activated with 5 μM ACh [EC_{20}]. PAM enhancement by 2.4 fold of the 5 μM ACh currents with 3 μM LY2087101
- **Antagonist Inhibition:** Inhibition by 10 μM dihydro- β -erythroidine (DH- β E) of currents activated by 100 μM ACh

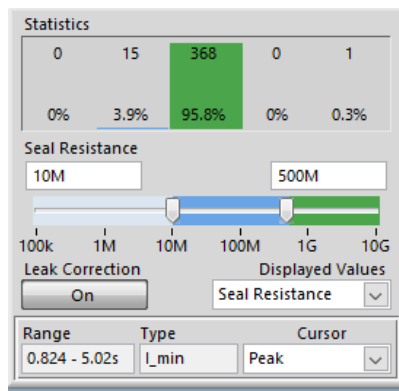


SP 384i Functional Expression of nAChR $\alpha 7$ /ric3

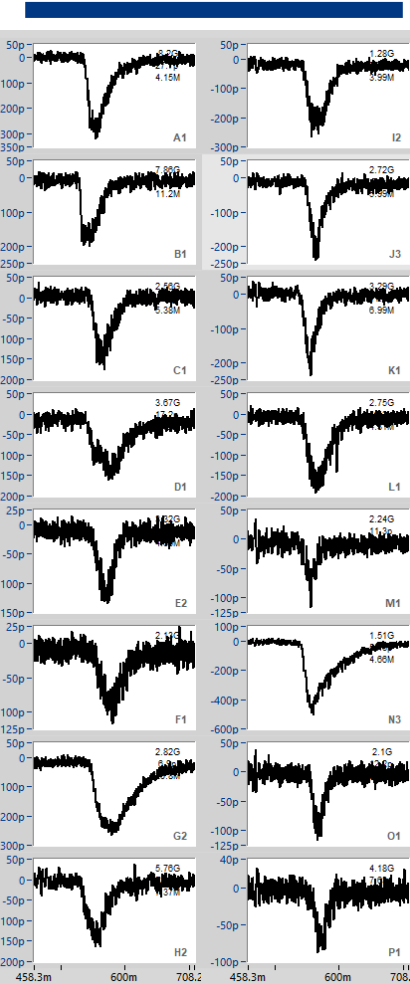
300 μ M Acetylcholine

First Run (no optimization)

- Good Seals
 - 95.8% over 500M
- Nice Expression
 - 86% < -75pA
 - 76% < -100 pA



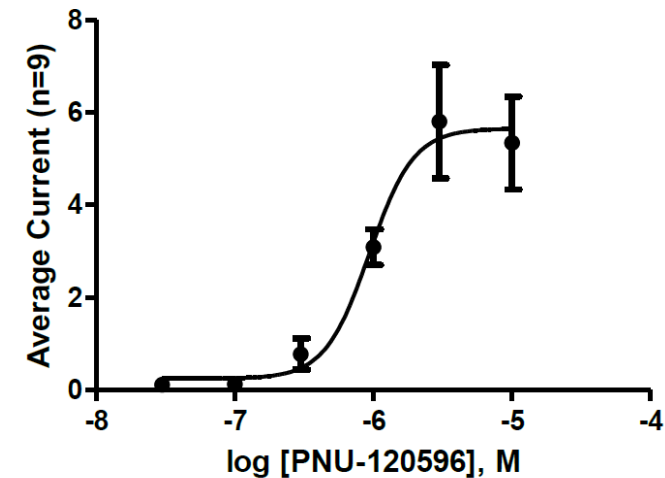
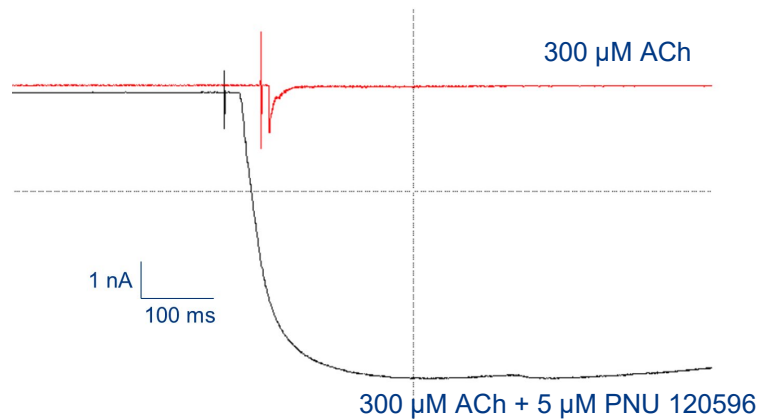
SyncroPatch 384i Data, Oct 11, 2022



Positive Allosteric Modulation (PAM) of nAChR $\alpha 7/\text{ric}3$

Previous work on this cell line showed the effect of the PAM PNU-120596 in the presence of 300 μM ACh

- Large potentiation by PNU-120596



Source: Data Sheet CYL3097 – PatchXpress® Data
Reference: Hurst RS et al (2005), J Neurosci., 25(17): 4396 – 4405

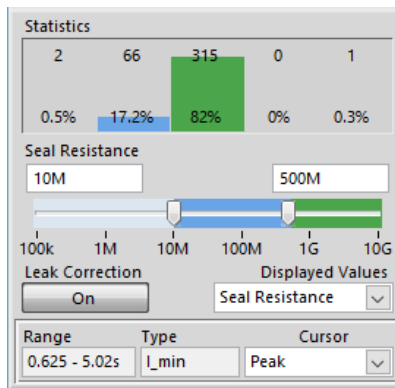
Positive Allosteric Modulation (PAM) of nAChR $\alpha 7/\text{ric}3$

300 μM ACh

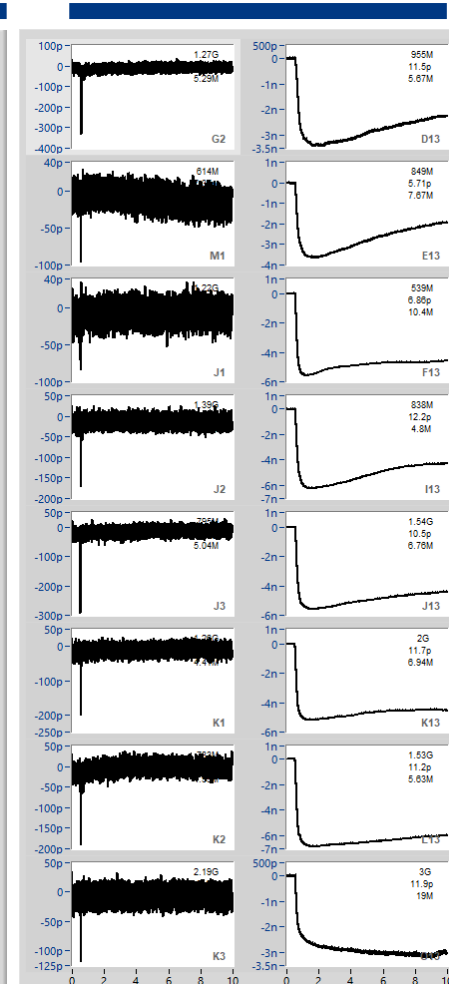
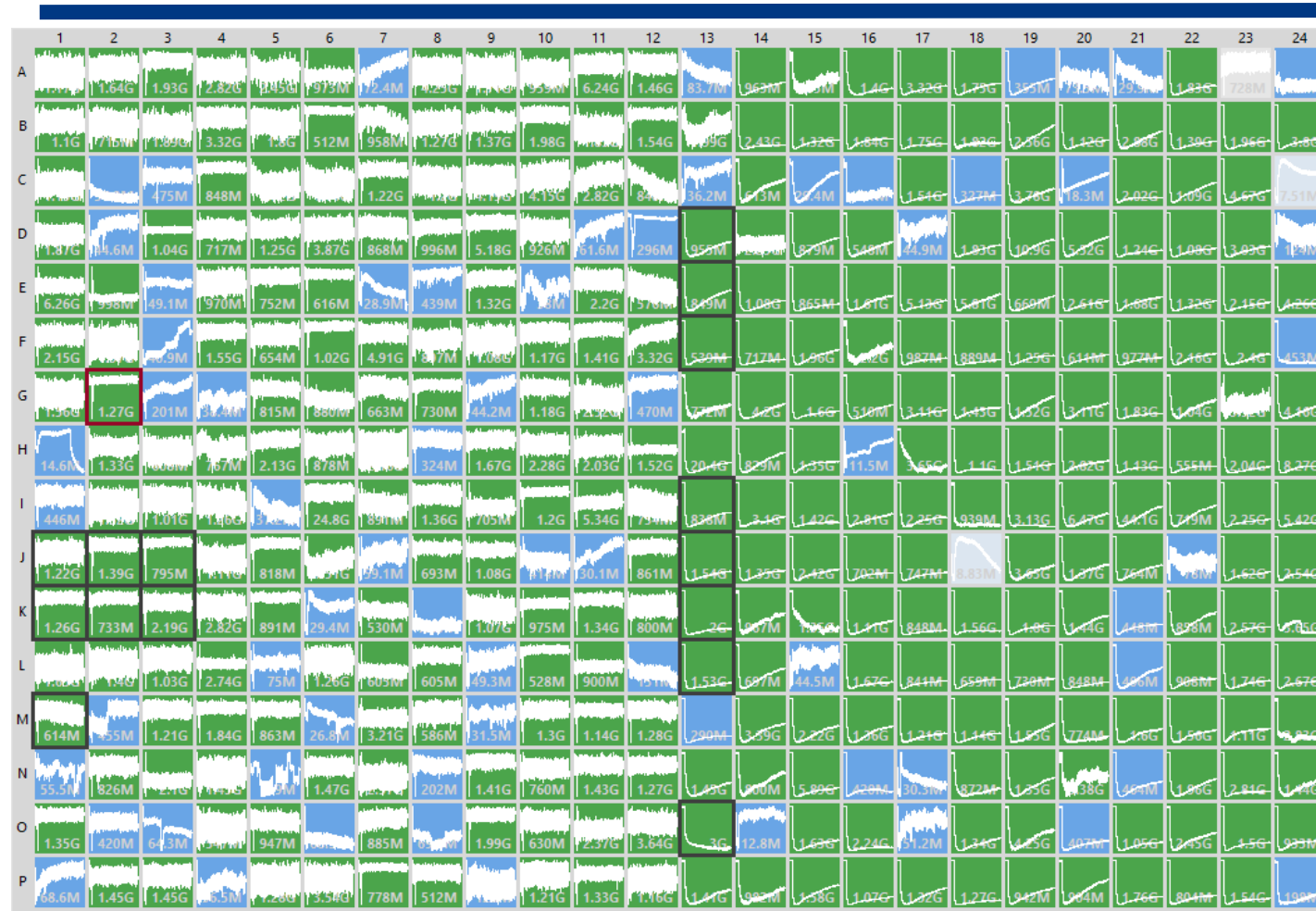
5 μM PNU-120596

Potentiation of ACh currents by 5 μM PNU-120596

- (X-axis full scale)



SyncroPatch 384i Data, Nov 2, 2022 (#1)



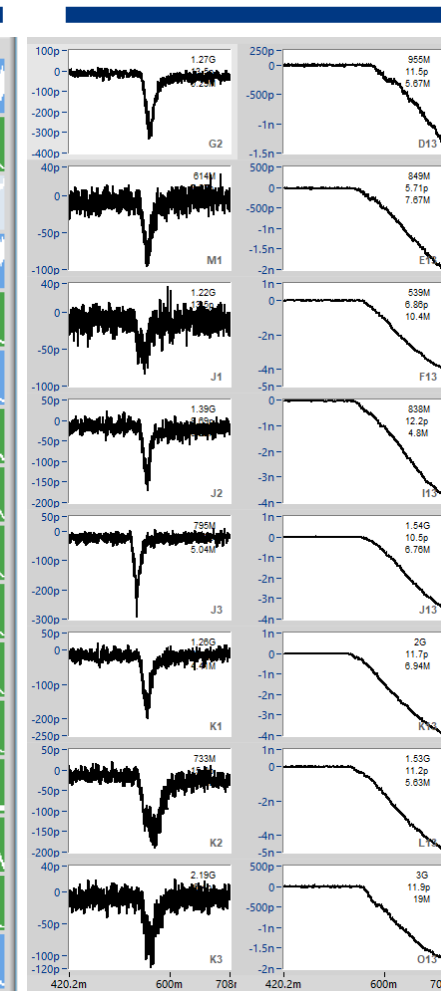
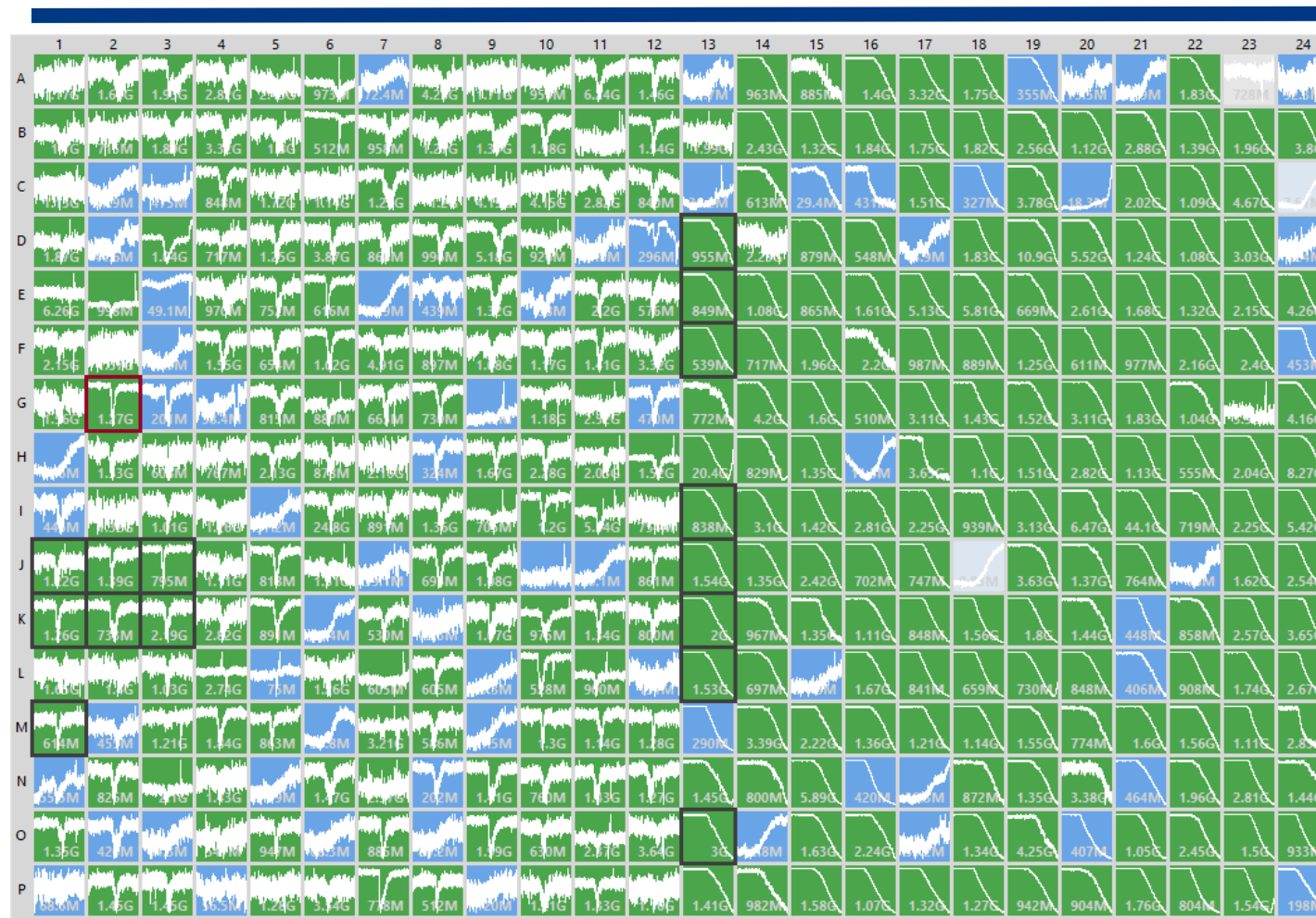
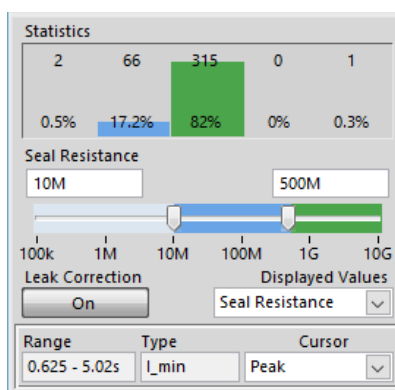
Positive Allosteric Modulation (PAM) of nAChR $\alpha 7/\text{ric}3$

300 μM ACh

5 μM PNU-120596

Potentiation of ACh currents by 5 μM PNU-120596

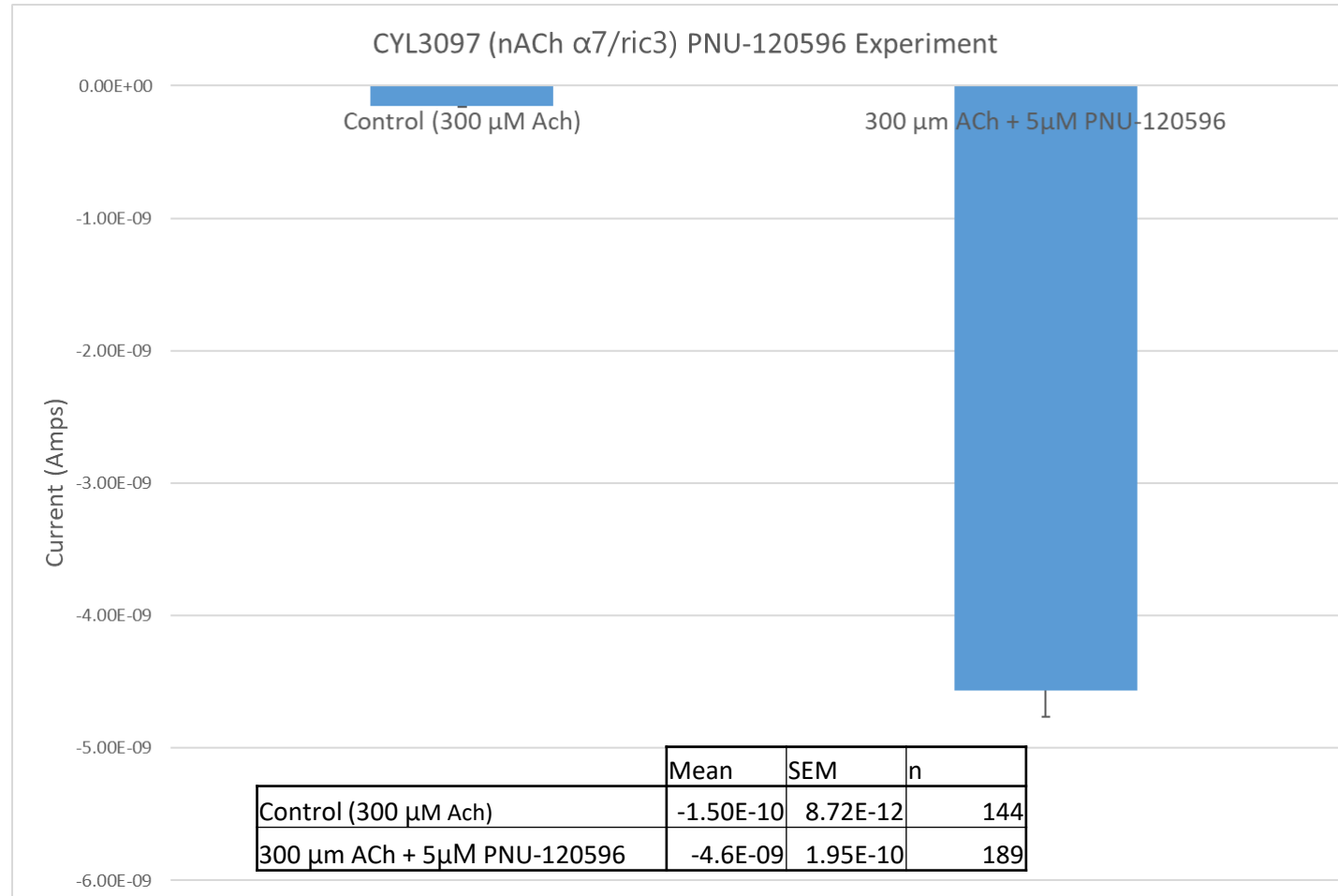
- (X-axis expanded to resolve 300 μM ACh currents)



SynroPatch 384i Data, Nov 2, 2022 (#1)

Positive Allosteric Modulation (PAM) of nAChR $\alpha 7/\text{ric}3$

5 μM PNU-120596
potentiates ACh
currents by over 30
fold



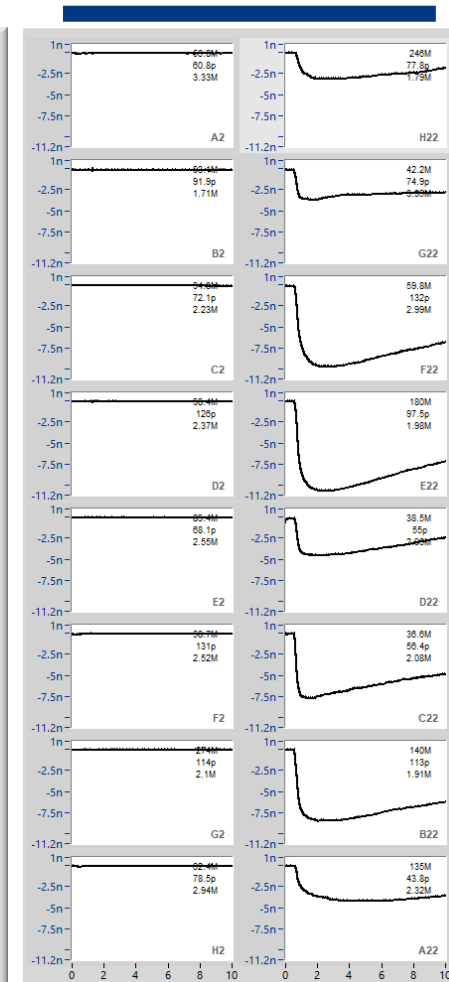
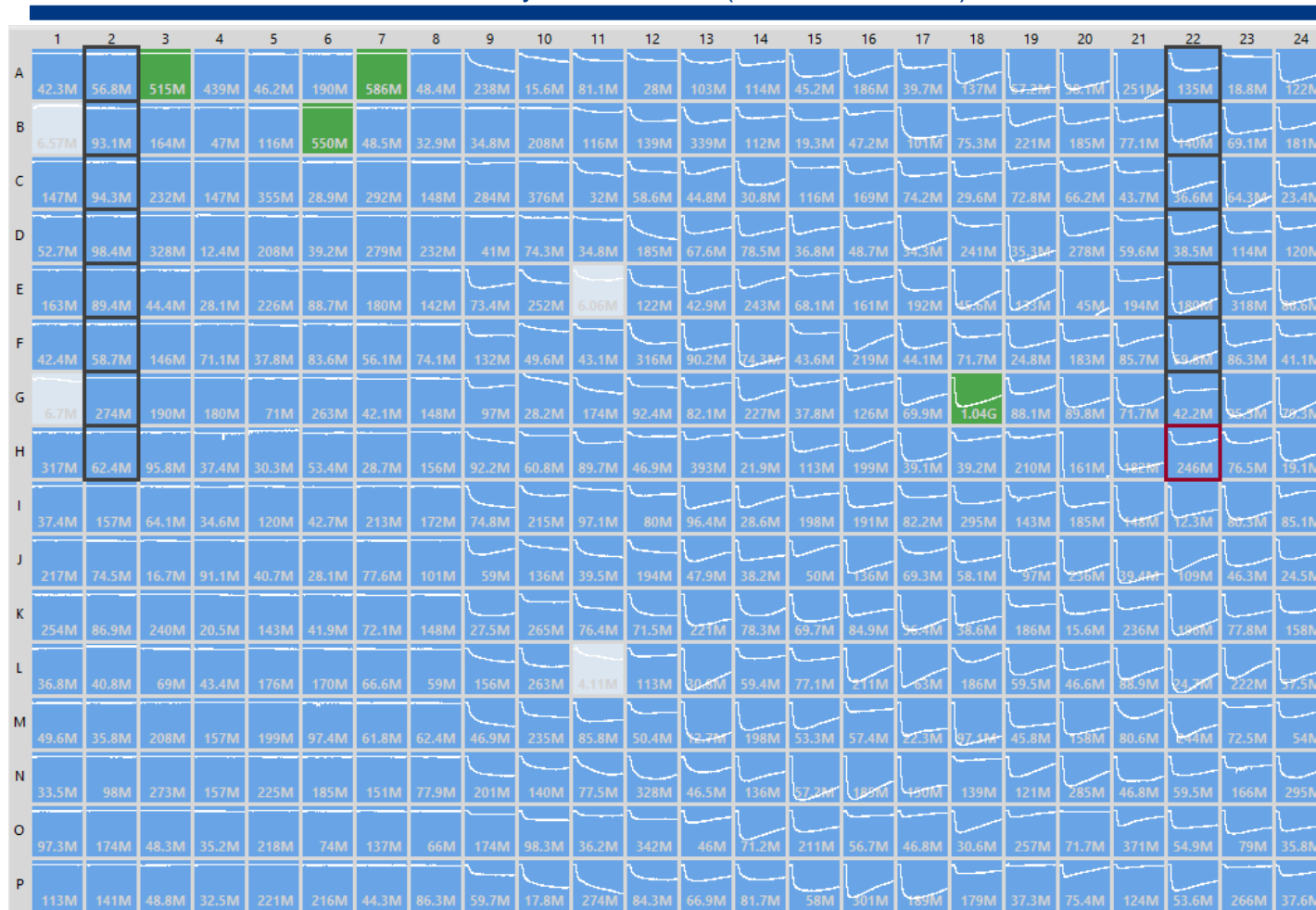
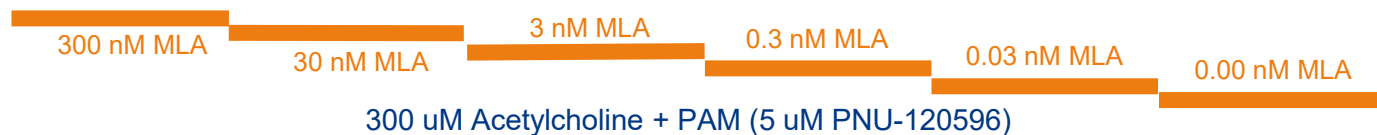
SyncroPatch 384i Data, Nov 2, 2022 (#1)

Antagonist* Dose Response Curve of nAChR $\alpha 7/ric3$

* By Methyllycaconitine (MLA)

Plate view of MLA dose response

- Fixed Y-scaling (no-autoscaling)
- 8-hole Population Chip



Statistics

4	376	4	0	0
1%	97.9%	1%	0%	0%

Seal Resistance

10M 500M

100k 1M 10M 100M 1G 10G

Leak Correction On

Displayed Values Seal Resistance

Range 0.625 - 5.02s

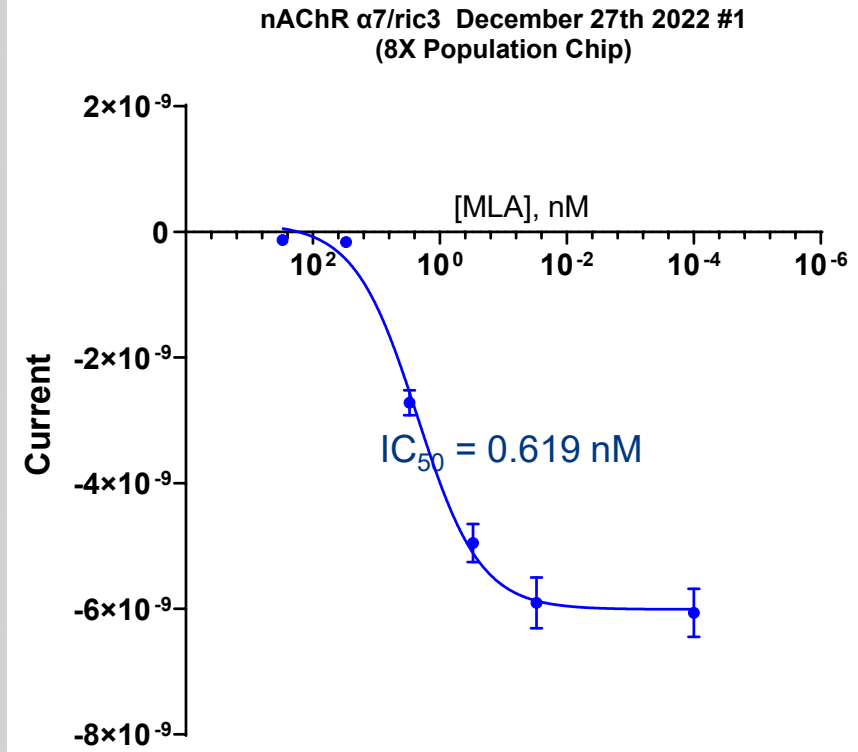
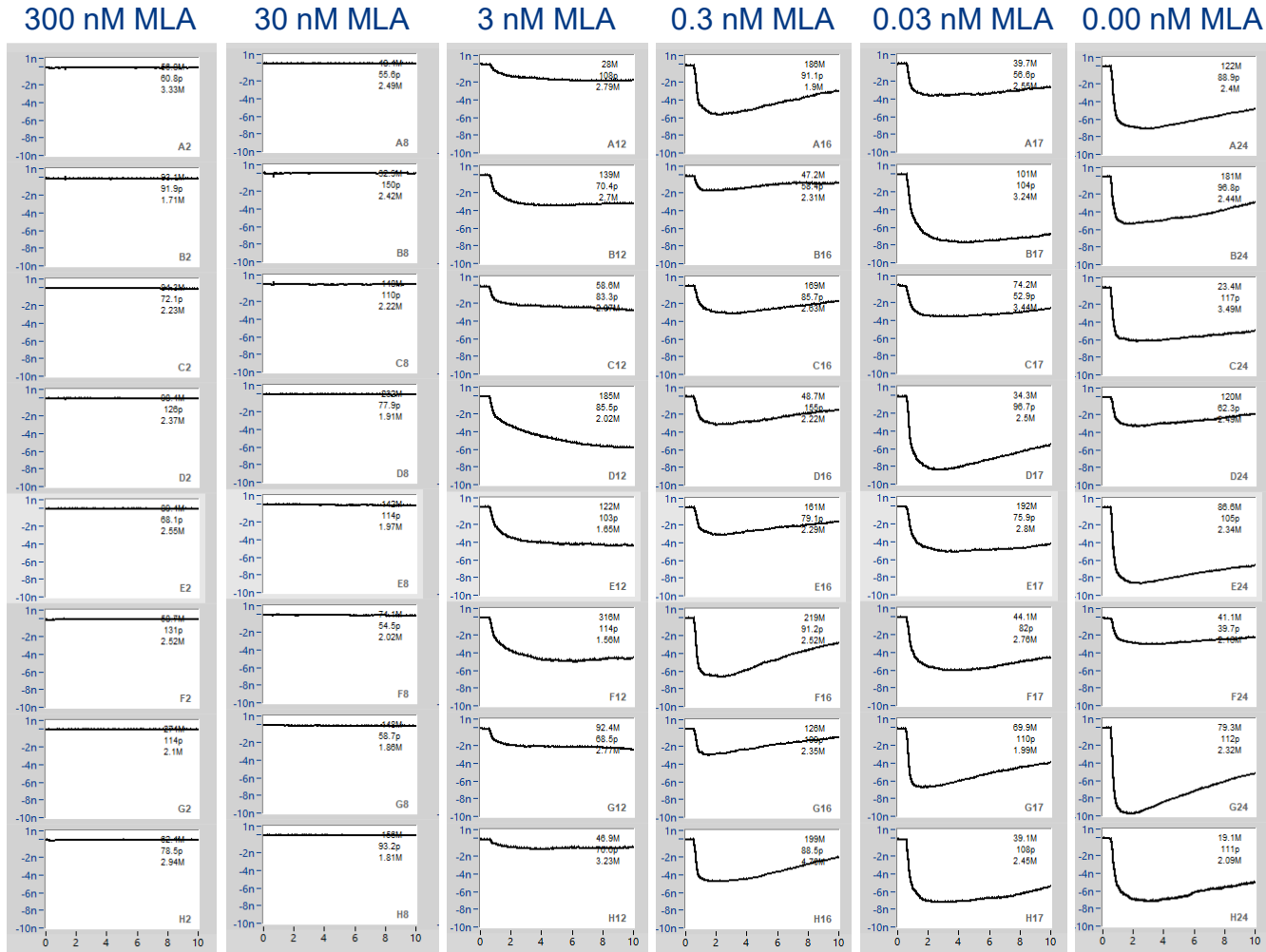
Type I_min

Cursor Peak

SyncoPatch 384i Data, Dec 27, 2022

Antagonist* Dose Response Curve of nAChR $\alpha 7/\text{ric}3$

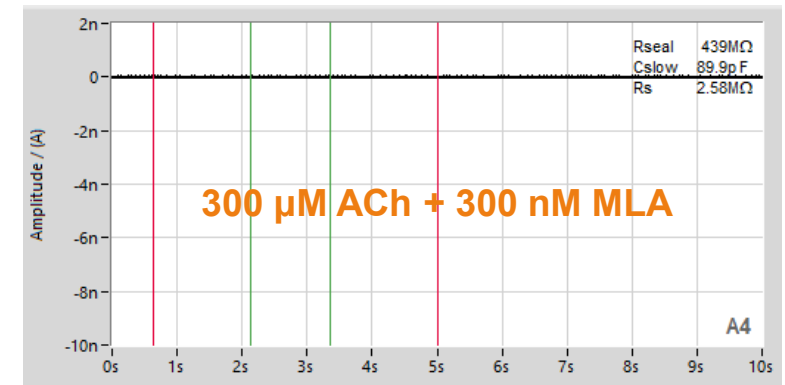
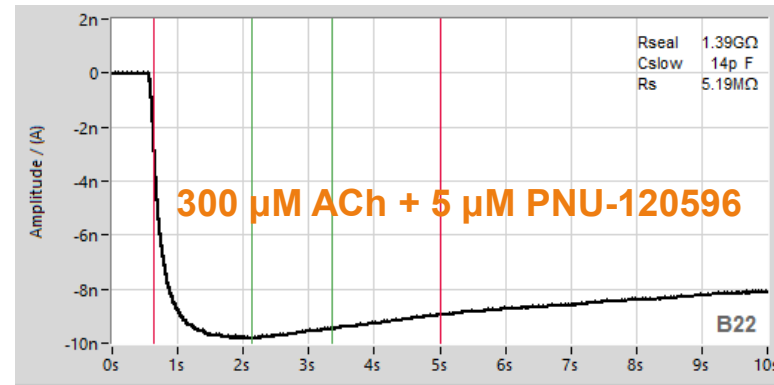
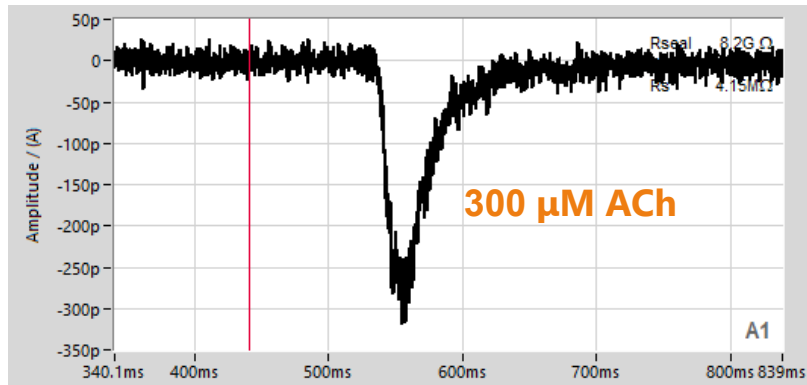
*Methyllycaconitine (MLA)



SyncroPatch 384i Data, Dec 27, 2022

Functional Activity

- **Agonist Activation:** Currents were activated by 300 μM ACh. The 300 μM ACh currents were enhanced with the PAM 5 μM PNU-120596
- **Antagonist Dose Response Curve:** MLA dose response curves measured using 8-hole/well plates in the absence of within well normalization (single shot 384-pipette addition)



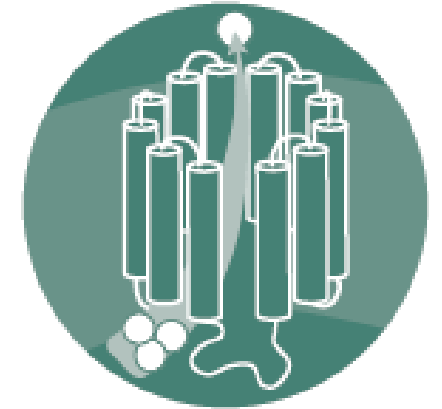
Our cell production team is actively expanding and re-validating our master cell banks into working cell bank (product) stocks in Fremont, CA. We are also providing Technical Support for our current product portfolio of PrecisION[®] stable cell lines out of our Fremont office

- We look forward to supporting your ion channel cell line needs!

The Custom Development Capabilities program is available for exclusive or non-exclusive development of new ion channel targets

We strongly encourage new requests to our Custom Development Capabilities program, especially for channelopathy variants, and corresponding wild-type ion channels

- This is an exciting rapidly expanding field of research and do hope to be directly involved in helping develop therapeutics toward channelopathies by providing stable cell lines and ion channel trafficking assays



Visit discoverx.com/ion-channels to learn more