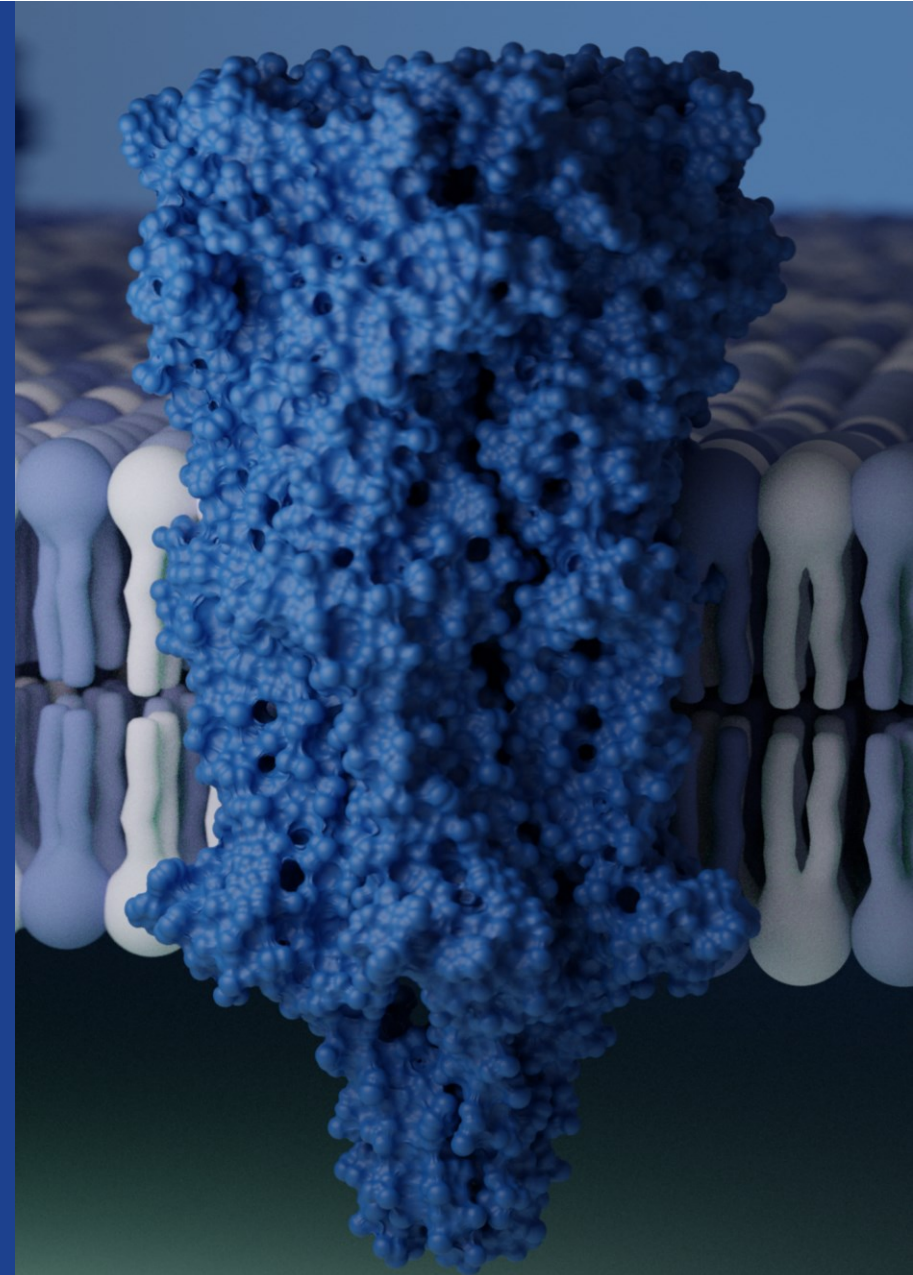


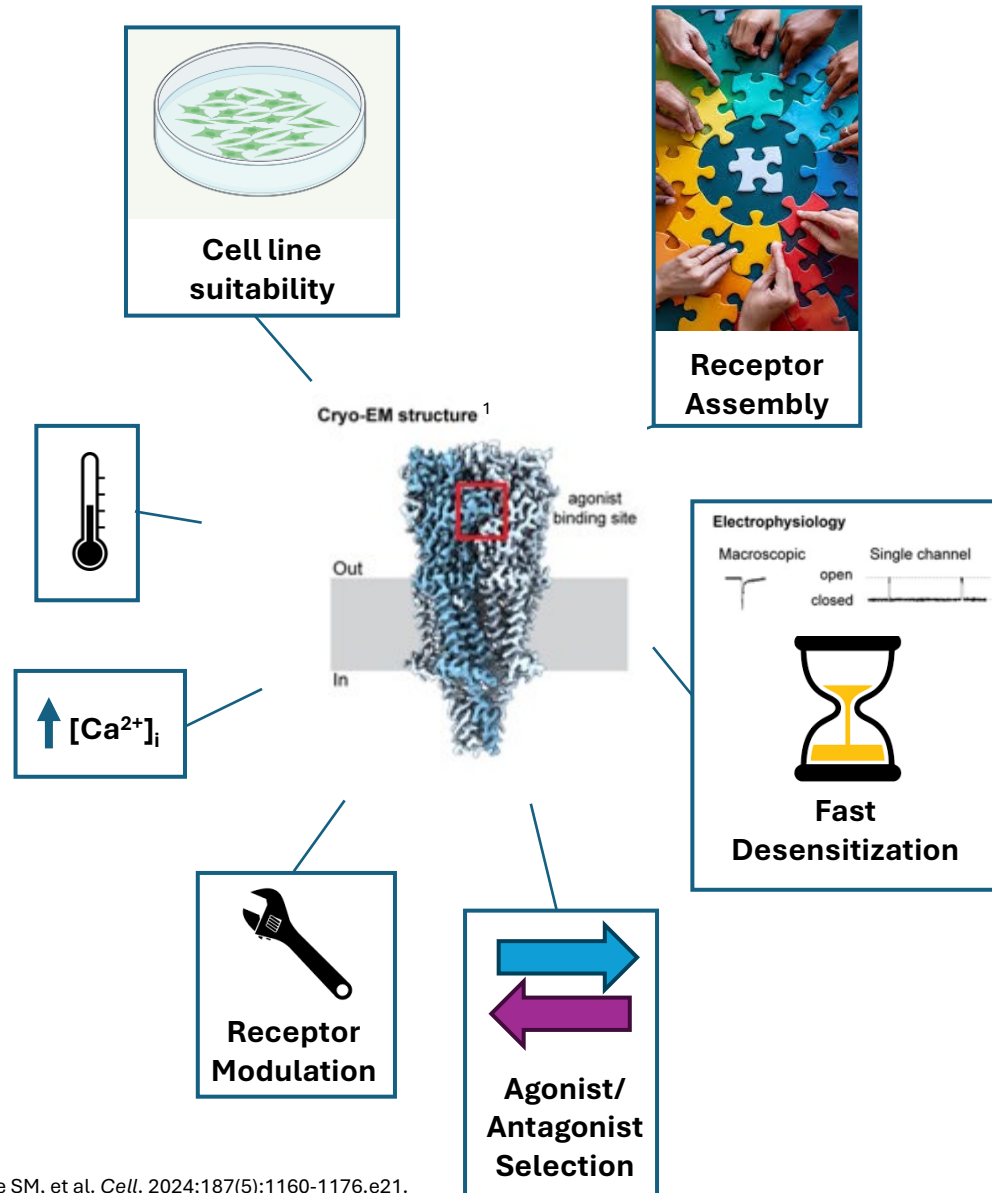
Achieving speed and precision: Reliable activation of nAChR $\alpha 7$

Tim Strassmeier
Director of Scientific Operations

Webinar
November 21, 2024



nAChR α 7 assay: Challenges and Typical Solutions



¹Burke SM, et al. *Cell*. 2024;187(5):1160-1176.e21.

Consumables can drive new applications

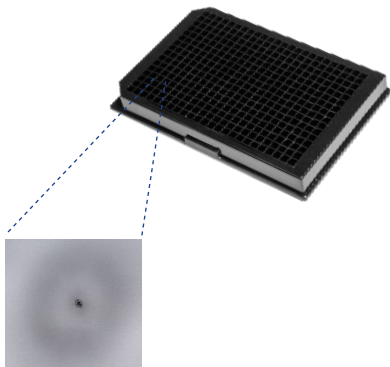


SyncroPatch 384

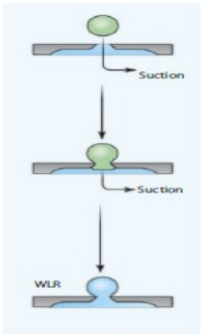
384 Patch Module



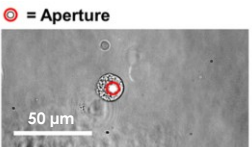
Glass Chip with 384 Patch holes



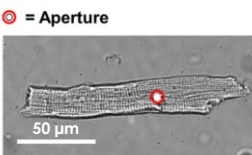
Planar Patch



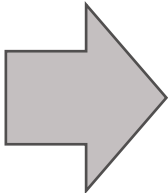
Single Well, one Patch hole



hiPSC-CM



Native-CM



1x L-Type



1x 3.0 µm

1x S-Type



1x 2.0 µm

4x S-Type



4x 2.0 µm

8x S-Type



8x 2.0 µm

1st Generation – Old Standard – Release of SyncroPatch 384

2nd Generation - New Standard with improved Performance

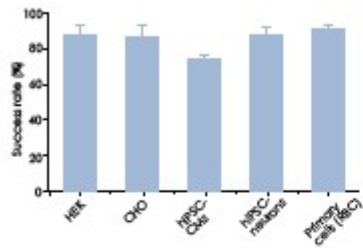
3rd Generation – FF Chips, Recording in Absence of internal Fluoride
Rapedius et al., Front Mol Neuro, 22 August 2022; 15:982316.

4th Generation – Adjusted Positioning for mechanical Stimulation of PIEZO1
Murciano et al., JGP, 4 December 2023; 155 (12): e202213132.

5th Generation – Chips for organellar Recordings and Patch Clamp of small cells

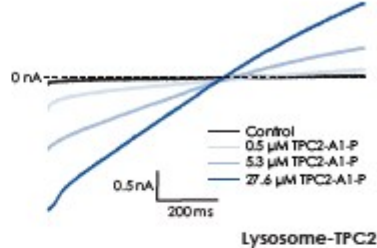
NPC-384 chips

For all your patch clamp needs



Standard experiments

Excellent success rates for cell lines, iPSCs and primary cells.



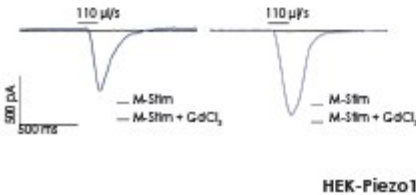
Recording organelles

Lysosomes, mitochondria or other intracellular organelles.



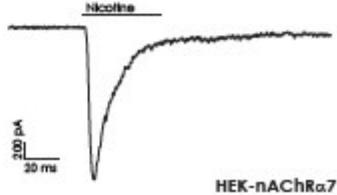
Mechano-activated ion channels

Mechanical activation of piezo (or other) channels.

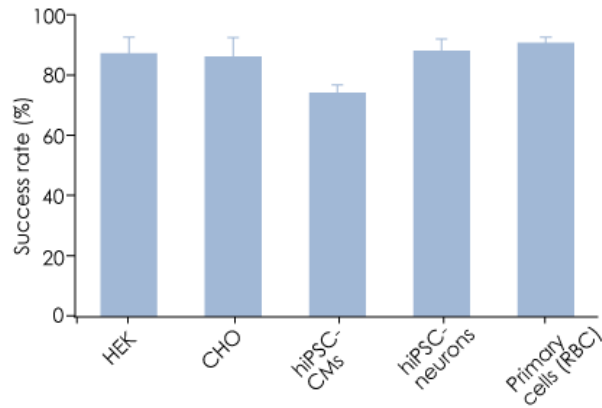


Fast ligand-gated ion channels

Reliably record fast desensitizing ligand-gated ion channels.

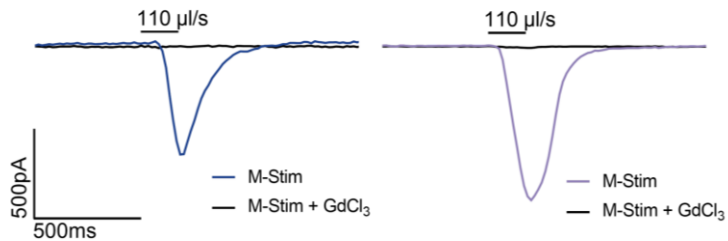


- 1st Generation – Old Standard – Release of SyncroPatch
- 2nd Generation - New Standard with improved Performance
- 3rd Generation – FF Chips, Recording in Absence of internal Fluoride
Rapedius et al., *Front Mol Neuro*, 22 August 2022; 15:982316.
- 4th Generation – Adjusted Positioning for mechanical Stimulation of PIEZO1
[Murciano et al., JGP, 4 December 2023; 155 \(12\): e202213132.](#)
- 5th Generation – Chips for organellar Recordings and Patch Clamp of small cells



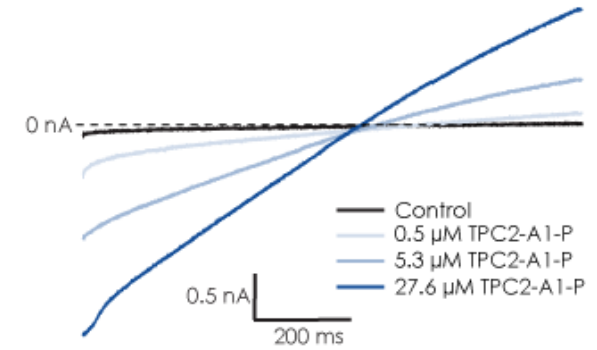
Standard experiments

Excellent success rates for cell lines, iPSCs and primary cells.



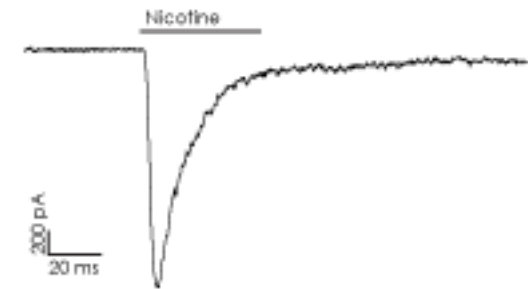
Mechano-activated ion channels

Mechanical activation of Piezo (or other) channels.



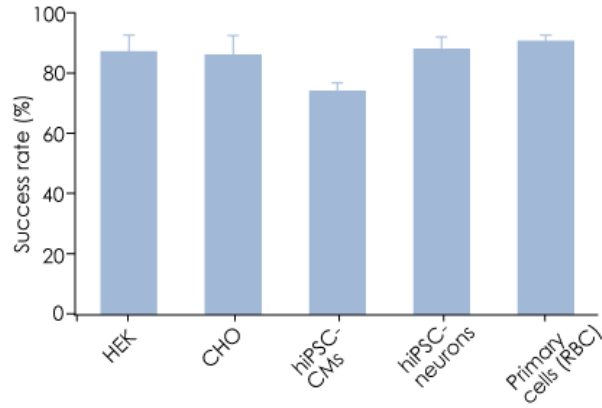
Recording organelles

Lysosomes, mitochondria or other intracellular organelles.



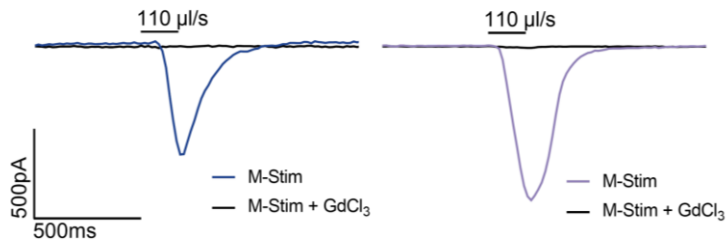
Fast ligand-gated ion channels

Reliably record fast desensitizing ligand-gated ion channels.



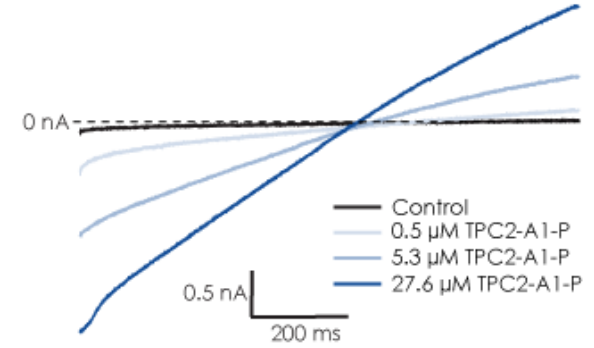
Standard experiments

Excellent success rates for cell lines, iPSCs and primary cells.



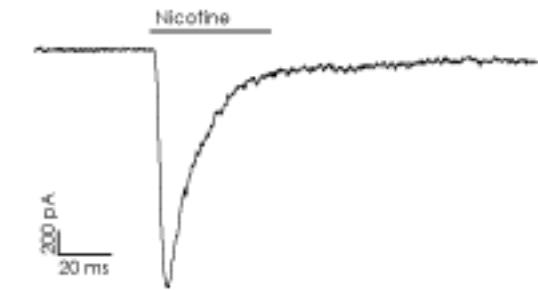
Mechano-activated ion channels

Mechanical activation of Piezo (or other) channels.



Recording organelles

Lysosomes, mitochondria or other intracellular organelles.



Fast ligand-gated ion channels

Reliably record fast desensitizing ligand-gated ion channels.

] Webinar [

Achieving speed and precision: Reliable activation of nAChR $\alpha 7$



Ilka Rinke-Weiß
Senior scientist
Nanion Technologies



James Costantin
SciMD Manager
Eurofins DiscoverX

Eurofins DiscoverX PrecisiON™ Ion Channel Products: Positive Allosteric Modulation of the nACh $\alpha 7$ /ric3 Channel

November 21st, 2024

James Costantin, Ph.D.

Scientific Market Development Manager – Ion Channels

Eurofins DiscoverX, Fremont, CA

Eurofins DiscoverX[®] Company Introduction

Ion Channel Product Portfolio

Validation Data for nACh $\alpha 7/\text{ric}3$

- Stable Cell Lines
- Ready-to-Assay Frozen Cells

High-Quality Ion Channel Products for Target Discovery, Hit Screening, Lead Optimization, and Safety Studies



DiscoverX was founded in 2000 and became the products business of Eurofins Discovery in 2017

Provide > 1000 functional assays available for popular target classes

- GPCRs
- Kinases
- Cytokine/Interleukin Receptors
- Checkpoints Receptors
- Nuclear Proteins
- Epigenetic Proteins
- Cell Signaling Pathway Targets
- Ion Channel Products



Headquarters based in Fremont, CA

Suitable for target discovery, hit screening, lead optimization, and safety studies

- PrecisION™ Stable Cell Lines
- PrecisION Ready-to-Assay (RTA) Frozen Cells
- PathHunter® Cell-based Trafficking Assays for pharmacochaperone discovery
- Custom Development Capabilities

PrecisION ion channel stable lines history

- Cytomyx begins the collection of PrecisION cell lines
- From Cytomyx to Serologicals to Millipore to Merck Millipore
- Eurofins acquires Merck Millipore
- DiscoverX acquired by Eurofins in 2017

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-ND7-23

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
K_{ir}2.1-HEK
K_{ir}6.2/SUR2A-HEK
KNCQ1/hminK-CHO

HCN Channels

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

Ca²⁺ Channels

Ca_v1.2 α_{1C}/β_{2a}/α_{2δ}₁
Ca_v2.2-HEK
Ca_v3.2-HEK

Other Channels

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

Ligand-Gated Channels

nAChR α₁/β₁/δ/ε-HEK
nAChR α₃/β₄-HEK
nAChR α₄/β₂-HEK
nAChR α₇/ric3-HEK
GABA_A α₁/β₃/γ₂-HEK
GABA_A α₂/β₃/γ₂-HEK
GABA_A α₃/β₃/γ₂-HEK
GABA_A α₄/β₃/γ₂-HEK
GABA_A α₅/β₃/γ₂-HEK
GABA_A α₆/β₃/γ₂-HEK
GlyRA1-HEK
GluR6-HEK

Coming Soon

GABA_A α1/β3/γ2-HEK
TRPM8

Stable Cell Line Validation for nACh α -7/ric3

300 μ M Acetylcholine

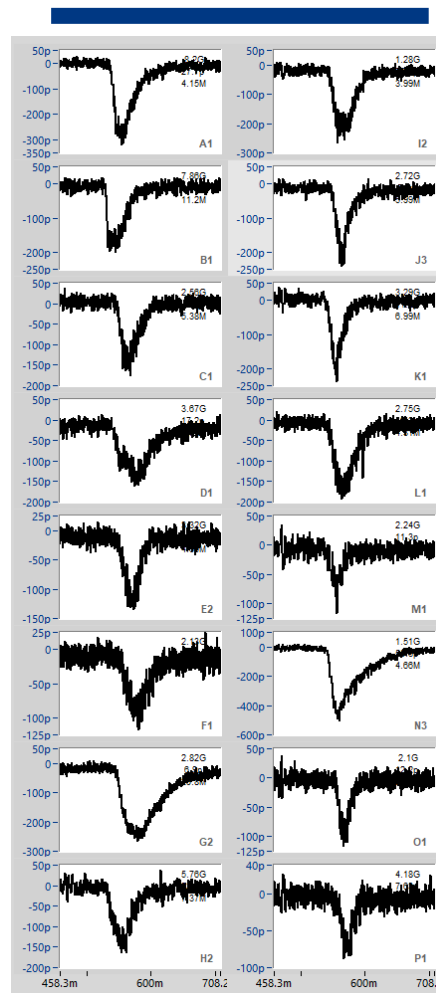
First Run (no optimization)

- Seals
 - 95.8% over 500M
- Expression
 - 86% < -75pA
 - 76% < -100 pA
 - 31% < -200 pA

Ligand "puff" technique:

- 10 μ l of ligand added (40 μ l/s)
- 50 μ l quickly removed
- 1500 ms exposure

1-Hole/well Recording Plate



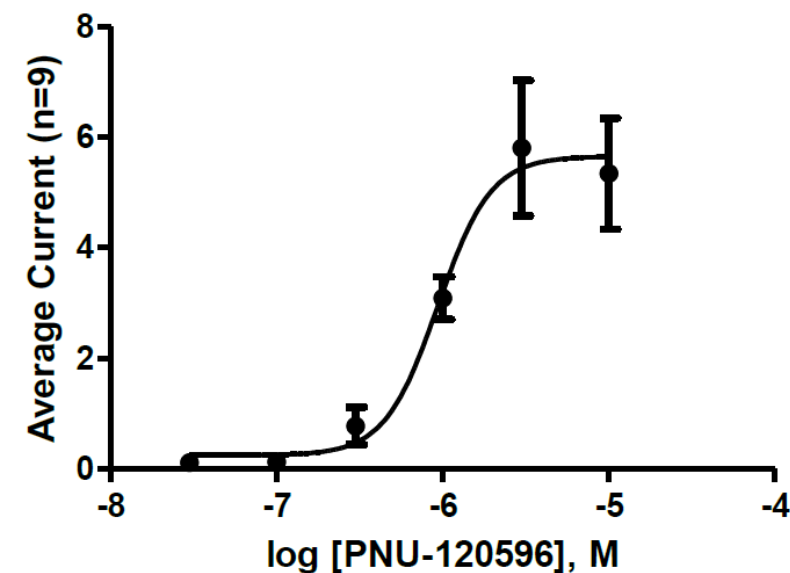
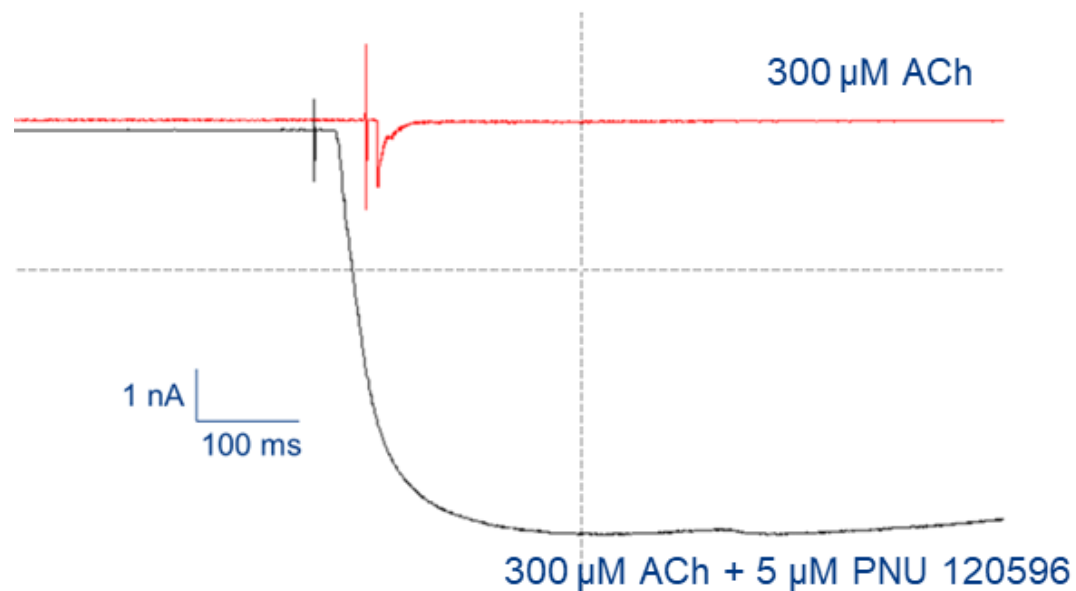
Catalog #: CYL3097
(Stable Cell Line)

Robust expression of rapidly desensitizing currents

SynroPatch 384i Data, Oct 11, 2022

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

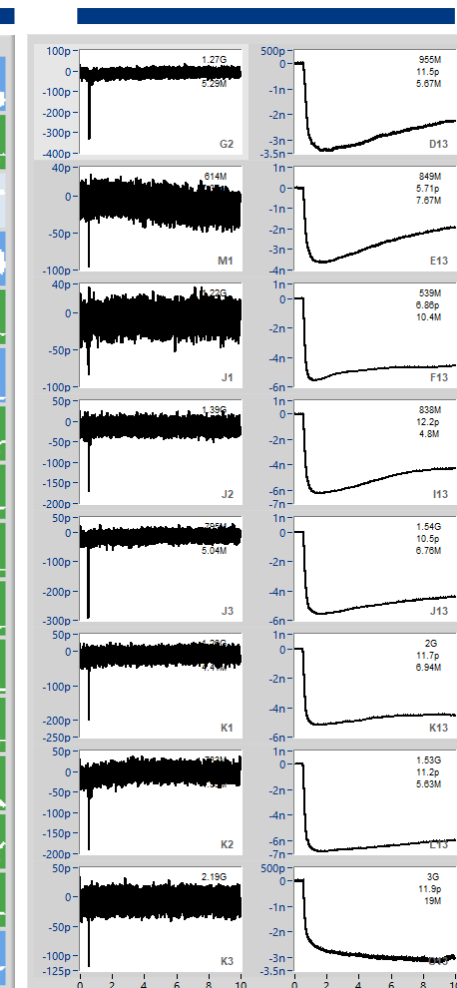
Reference: Hurst RS et al (2005), J Neurosci., 25(17): 4396 – 4405

PAM Modulation of Stable nAChR $\alpha 7$ /ric3 Cell Line

Potentiation of ACh currents by 5 μ M PNU-120596

Ligand “puff” technique:
10 μ l of ligand added (40 μ l/s)
50 μ l quickly removed
1500 ms exposure

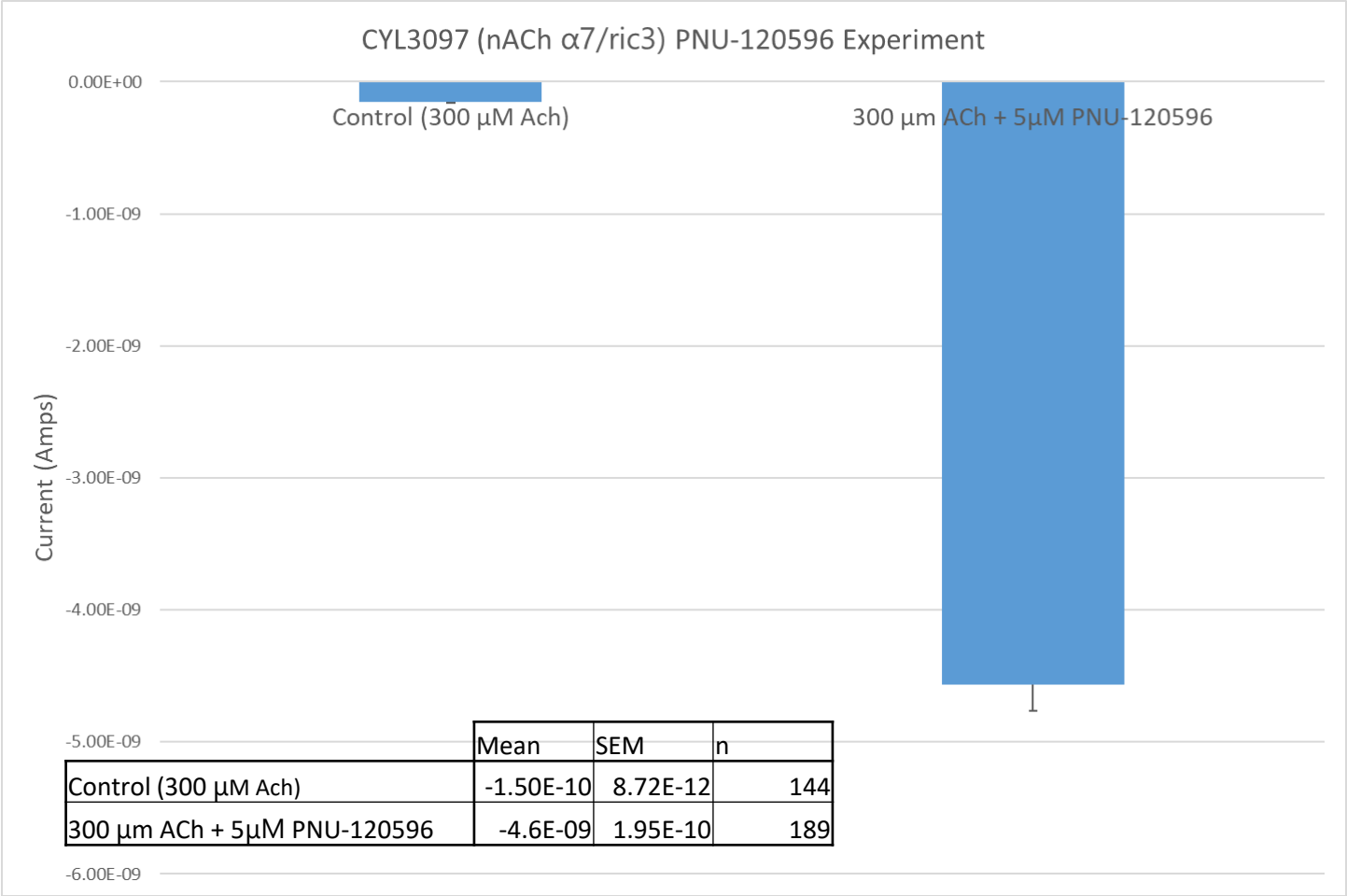
1-Hole/well Recording Plate



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

5 μ M PNU-120596
potentiates ACh
currents by over 30-
fold

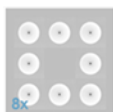
1-Hole/well Recording Plate



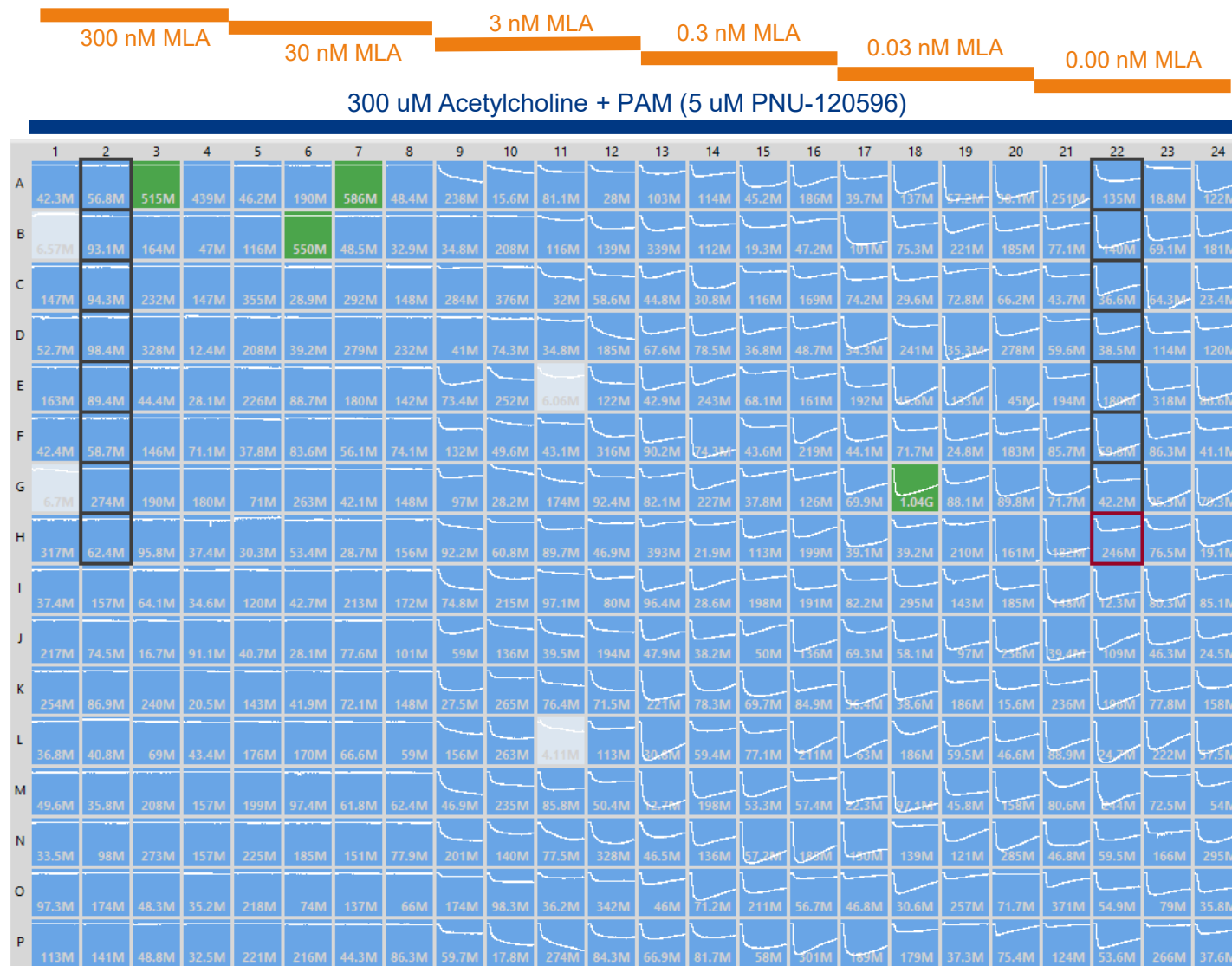
Antagonist* Dose Response Curve of nAChR $\alpha 7$ /ric3

Plate view of MLA dose response

8-Holes/well Population Patch Plate

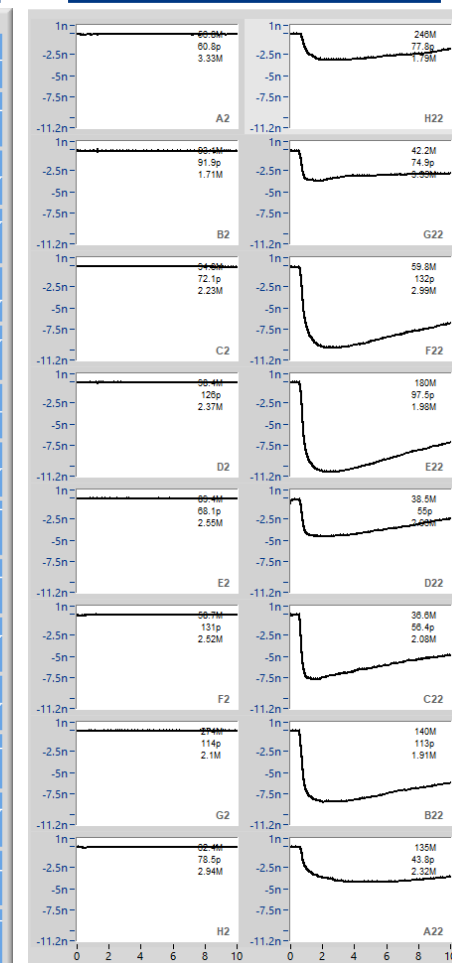


SyncroPatch 384i Data, Dec 27, 2022



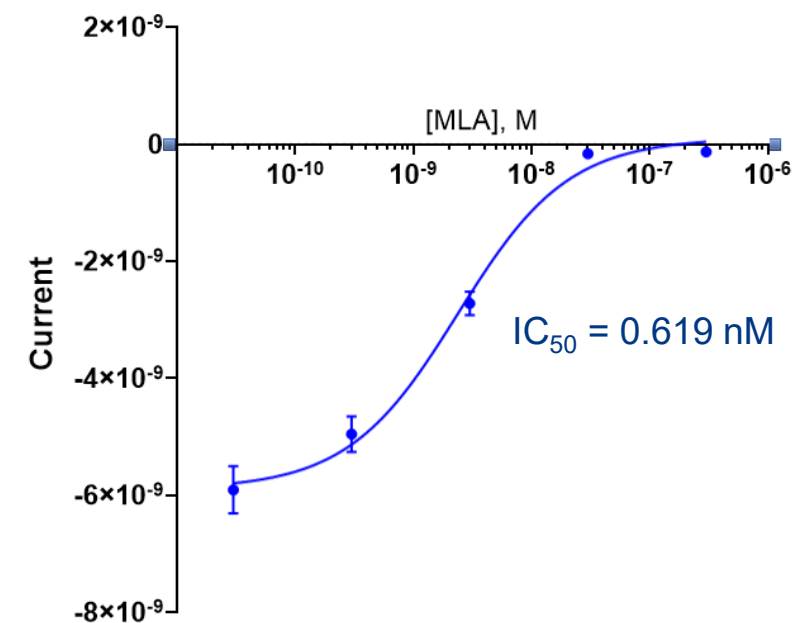
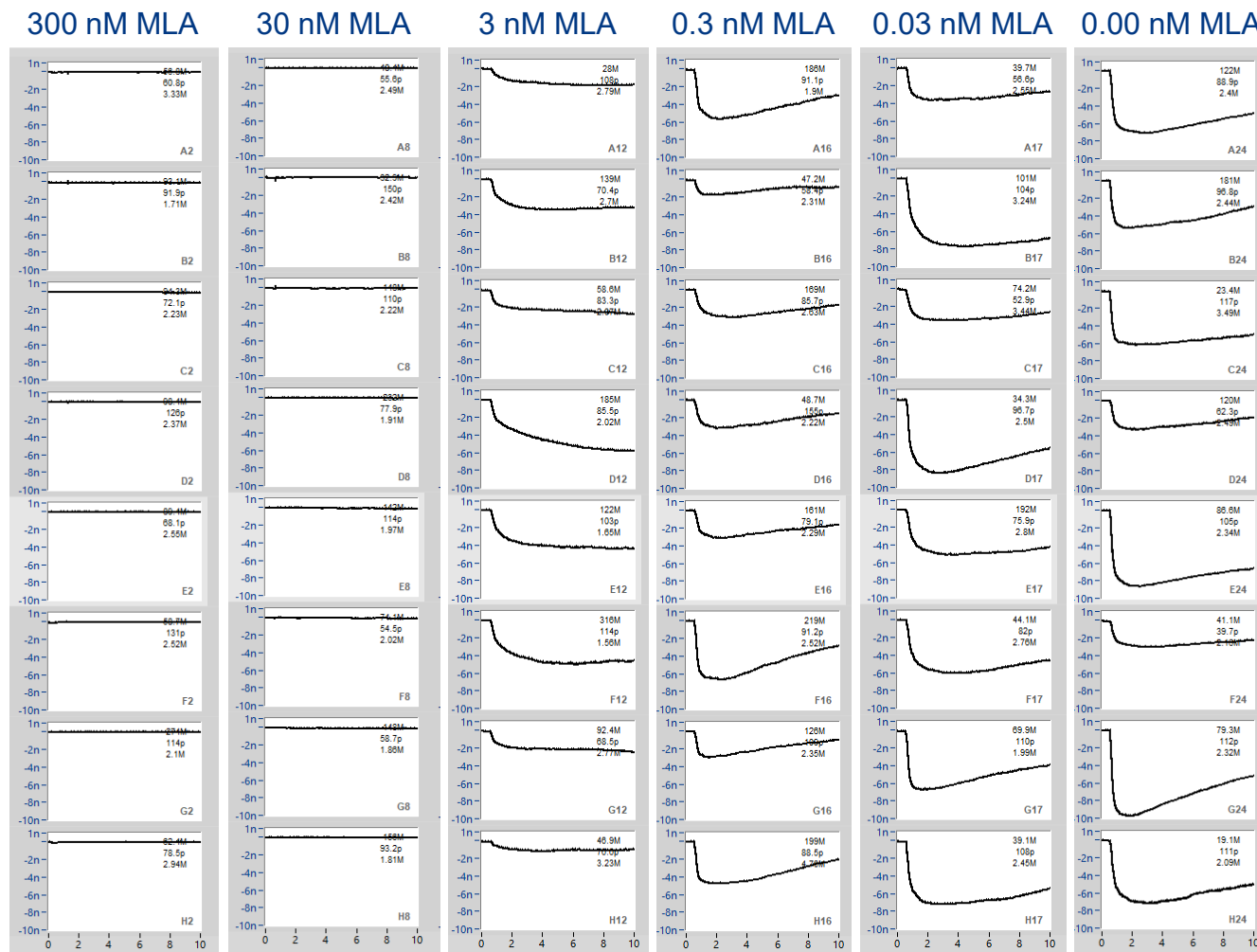
* By Methyllycaconitine (MLA)

300 nM MLA

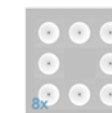


Antagonist* Dose Response Curve of nAChR $\alpha 7/\text{ric3}$

*Methyllycaconitine (MLA)



8-Holes/well Population Patch Plate



SyncroPatch 384i Data, Dec 27, 2022

Ready-to-Assay (RTA) version of nACh $\alpha 7$ /ric3 cell line

- Vials contained 4M cells each, 3 vials run per experiment
 - Finished RTA product will contain 6M cells/vial
- At Nanion's suggestion "ligand puff" parameters were changed
 - Ligand addition decreased from 10 μ l to 5 μ l
 - Dispense speed increased from 40 μ l/s to 50 μ l/s
 - Exposure time reduced from 1500 ms to 500 ms

Acetylcholine peak current magnitudes in the absence of PAM's increased by 7.2-fold

Catalog #: CYL3097RTA
(RTA Frozen Cells)

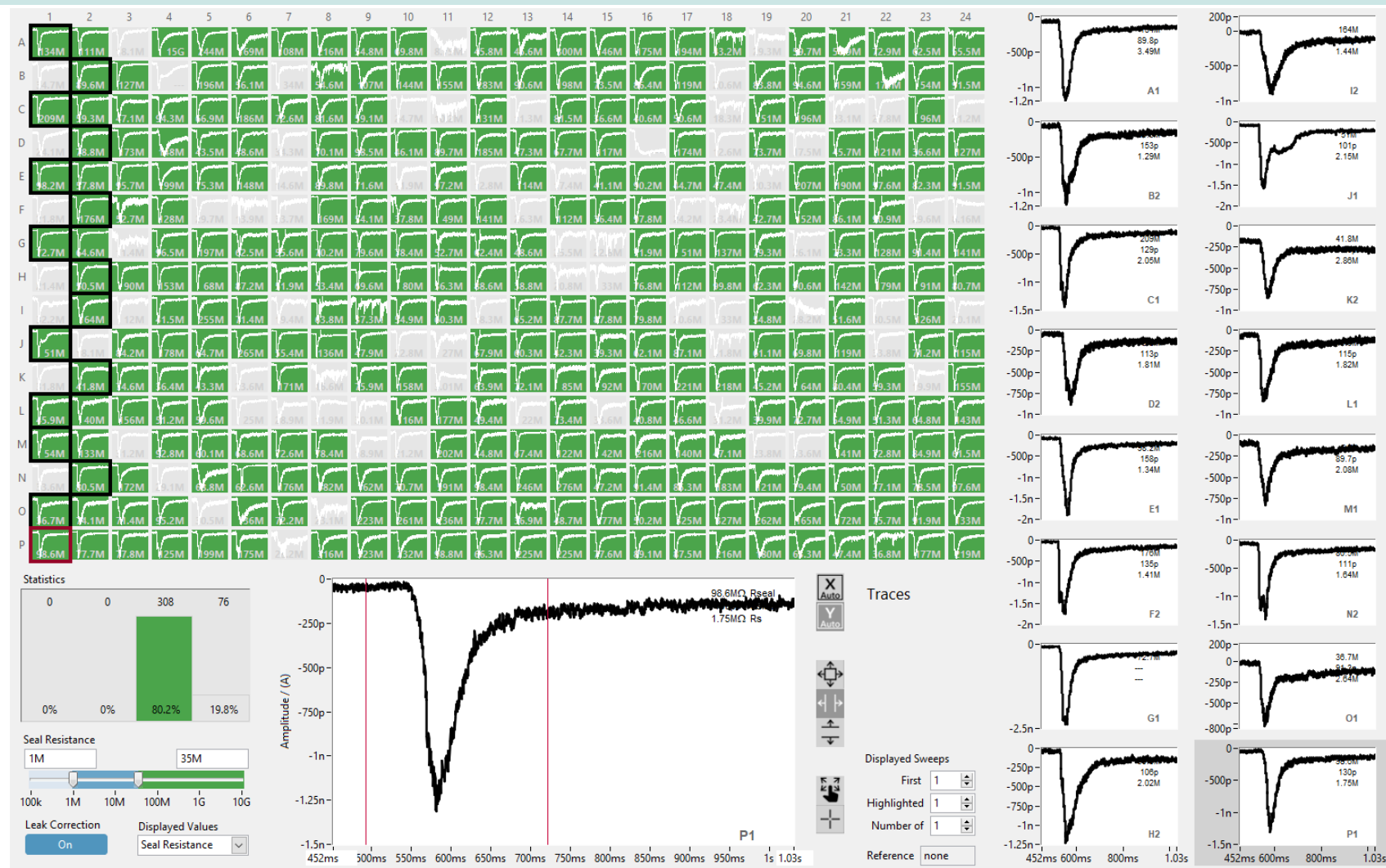
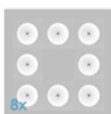
nACh $\alpha 7$ /ric3-RTA Validation

300 μ M Acetylcholine

Ligand “puff” technique:
5 μ l of ligand added (50ul/s)
50 μ l quickly removed
500 ms exposure

Cells run too dilute for 8x plates (0.25 x 10^6 /ml), compromised seals and success rates

8-Holes/well Population Patch Plate



Robust expression of rapidly desensitizing currents SyncroPatch 384i Data, Oct 15, 2024

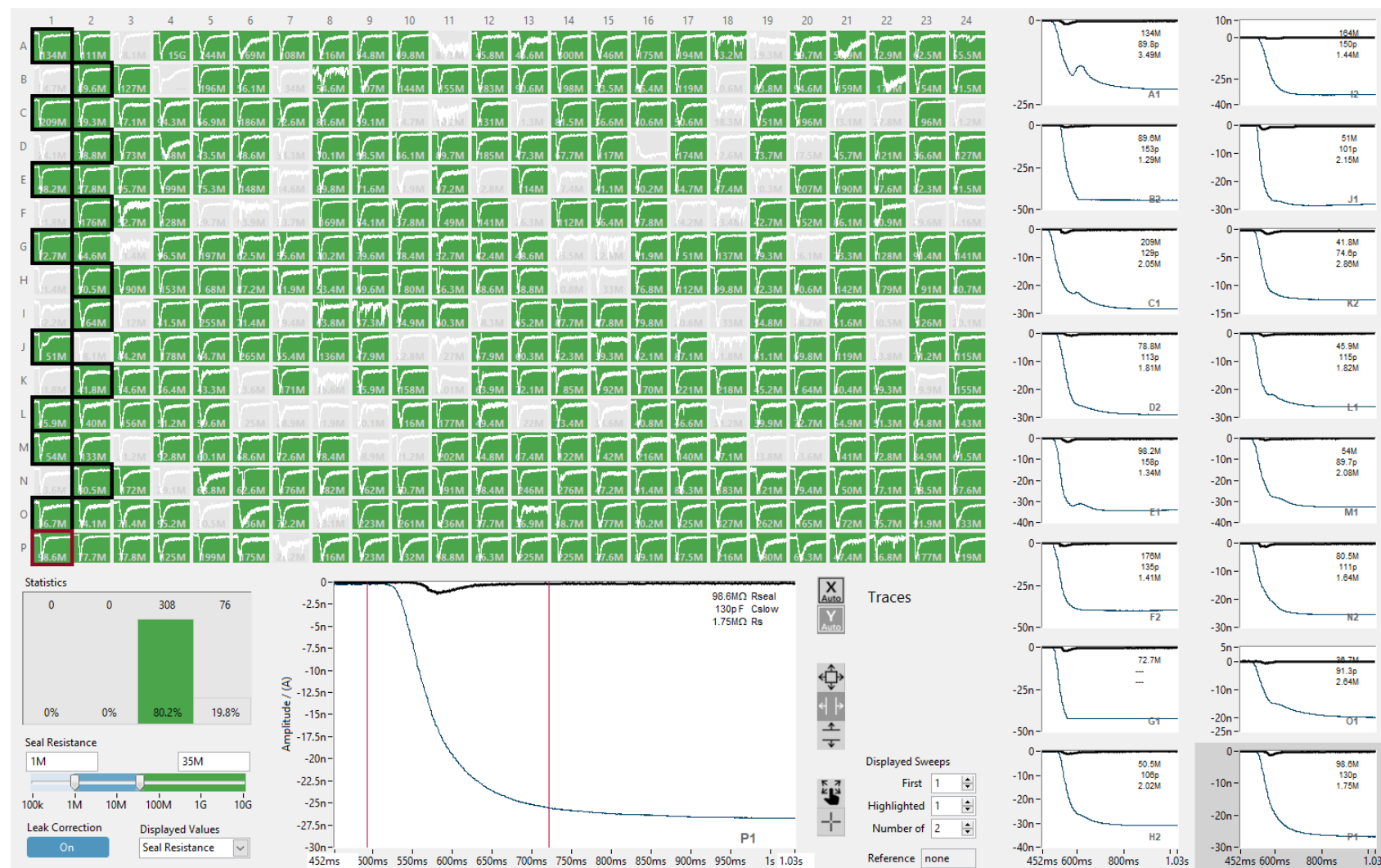
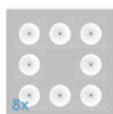
nACh $\alpha 7$ /ric3-RTA Validation

Potentiation of 300 μ M
ACh currents by 5 μ M
PNU-120596

Ligand “puff” technique:

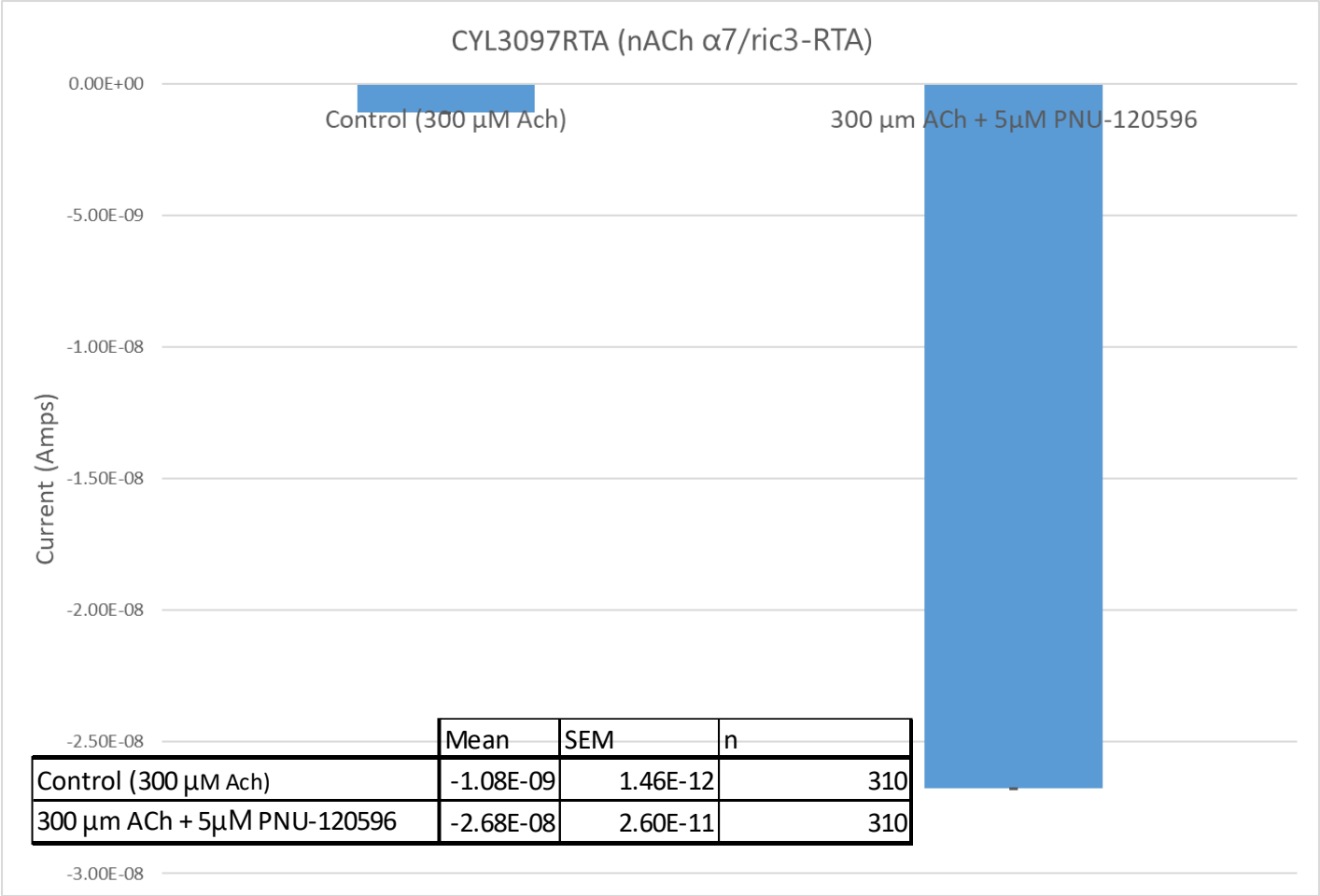
- 5 μ l of Ligand added (50ul/s)
- 50 μ l quickly removed
- 500 ms exposure

Cells run too dilute for 8x plates (0.25 x 10⁶/ml), compromised seals and success rates
8-Holes/well Population Patch Plate



SyncroPatch 384i Data, Oct 15, 2024

5 μ M PNU-120596
potentiates ACh
currents by 25-fold



SyncroPatch 384i Data, Oct 15, 2024

nACh $\alpha 7$ /ric3-RTA Validation

300 μ M Acetylcholine

Success Rate = 82%

Ligand “puff” technique:

5 μ l of Ligand added (50 μ l/s)

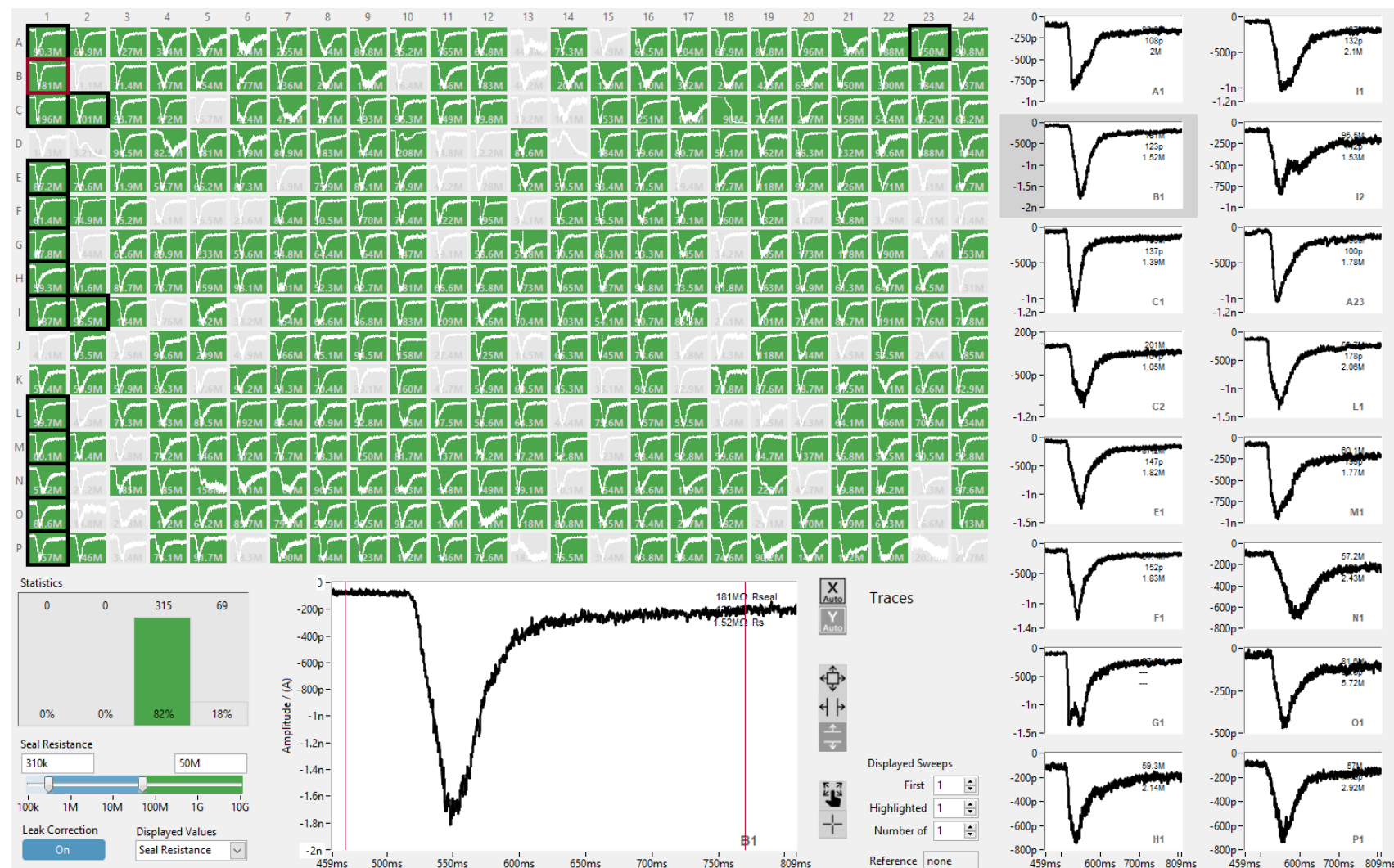
50 μ l quickly removed

500 ms exposure

Cells run @ (0.36 x 10⁶/ml)

Seal Filter = Excludes wells <50 M Ω

Current Filter = Excludes wells >-200 pA



Robust expression of rapidly desensitizing currents

SyncroPatch 384i Data, Oct 22, 2024

nACh $\alpha 7$ /ric3-RTA Validation

300 μ M Acetylcholine
+ 3.5 μ M NS-1738

Success Rate = 82%

Ligand “puff” technique:

5 μ l of Ligand added (50 μ l/s)

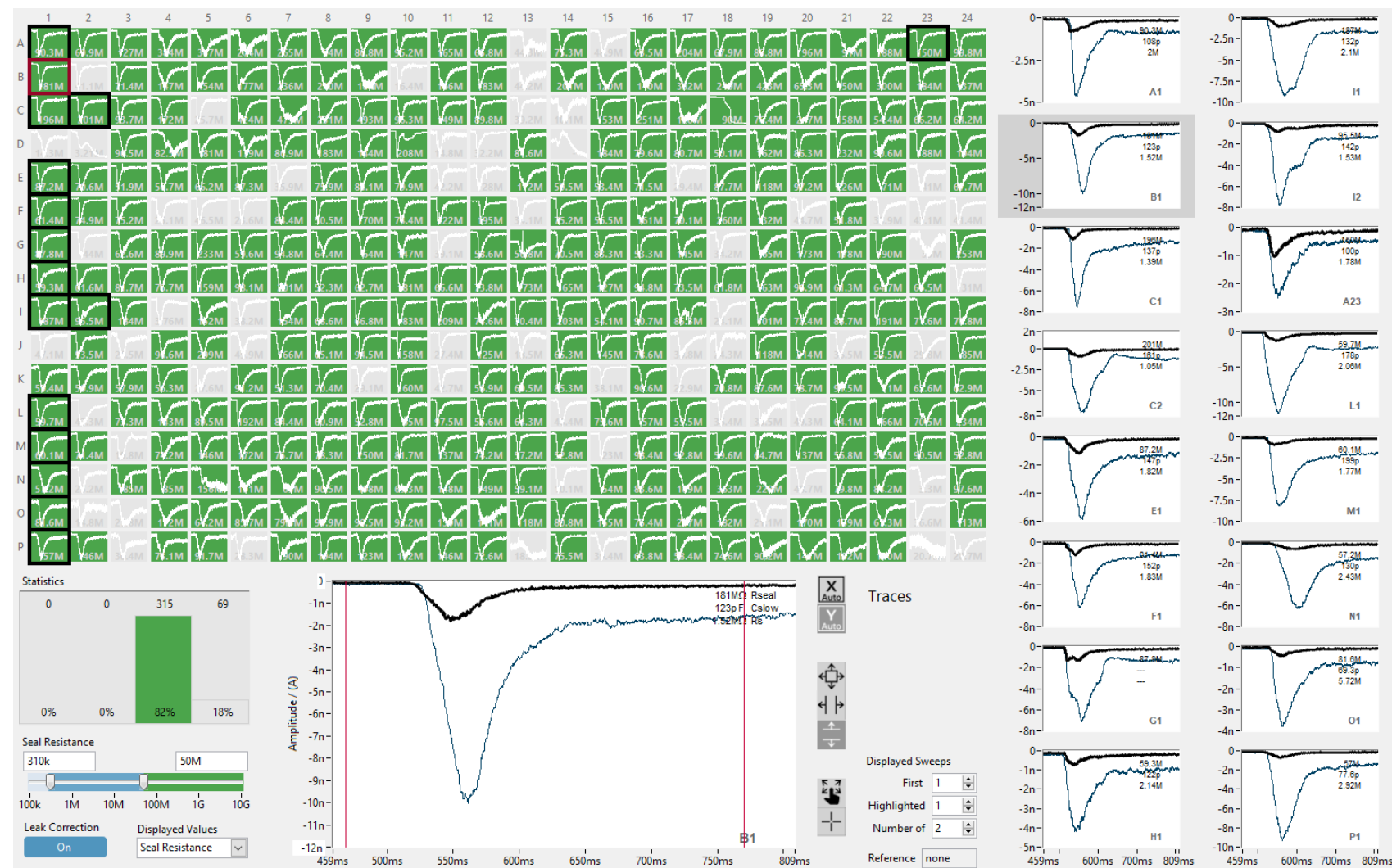
50 μ l quickly removed

500 ms exposure

Cells run @ (0.36 x 10⁶/ml)

Seal Filter = Excludes wells <50 M Ω

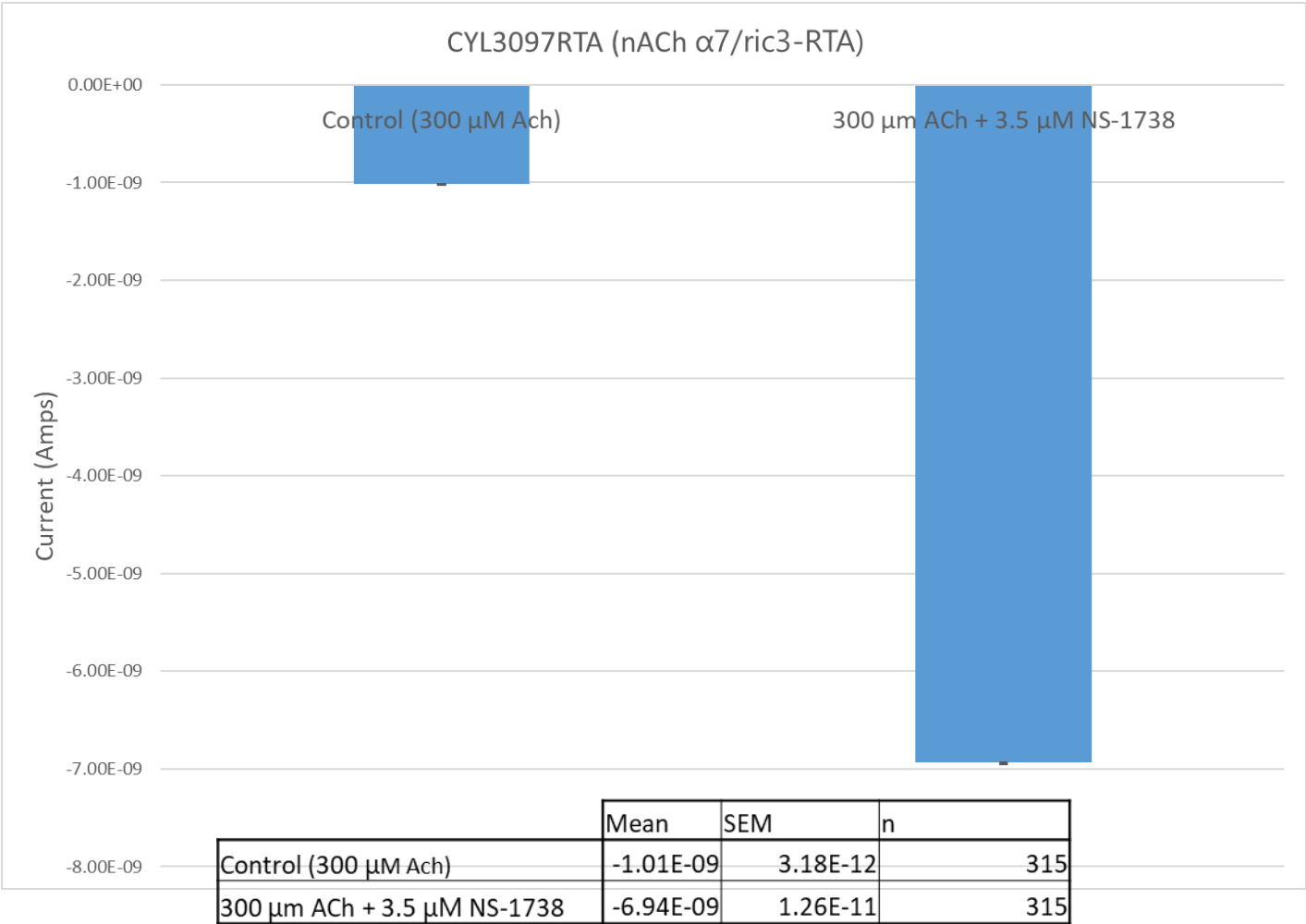
Current Filter = Excludes wells >-200 pA



NS-1738 increases peak currents, desensitization still occurs

SyncroPatch 384i Data, Oct 22, 2024

3.5 μ M NS-1738
potentiates ACh
currents by 6.8-fold



SyncroPatch 384i Data, Oct 22, 2024

nACh $\alpha 7$ /ric3-RTA Validation

300 μ M Acetylcholine

Success Rate = 83%

Ligand “puff” technique:

5 μ l of Ligand added (50 μ l/s)

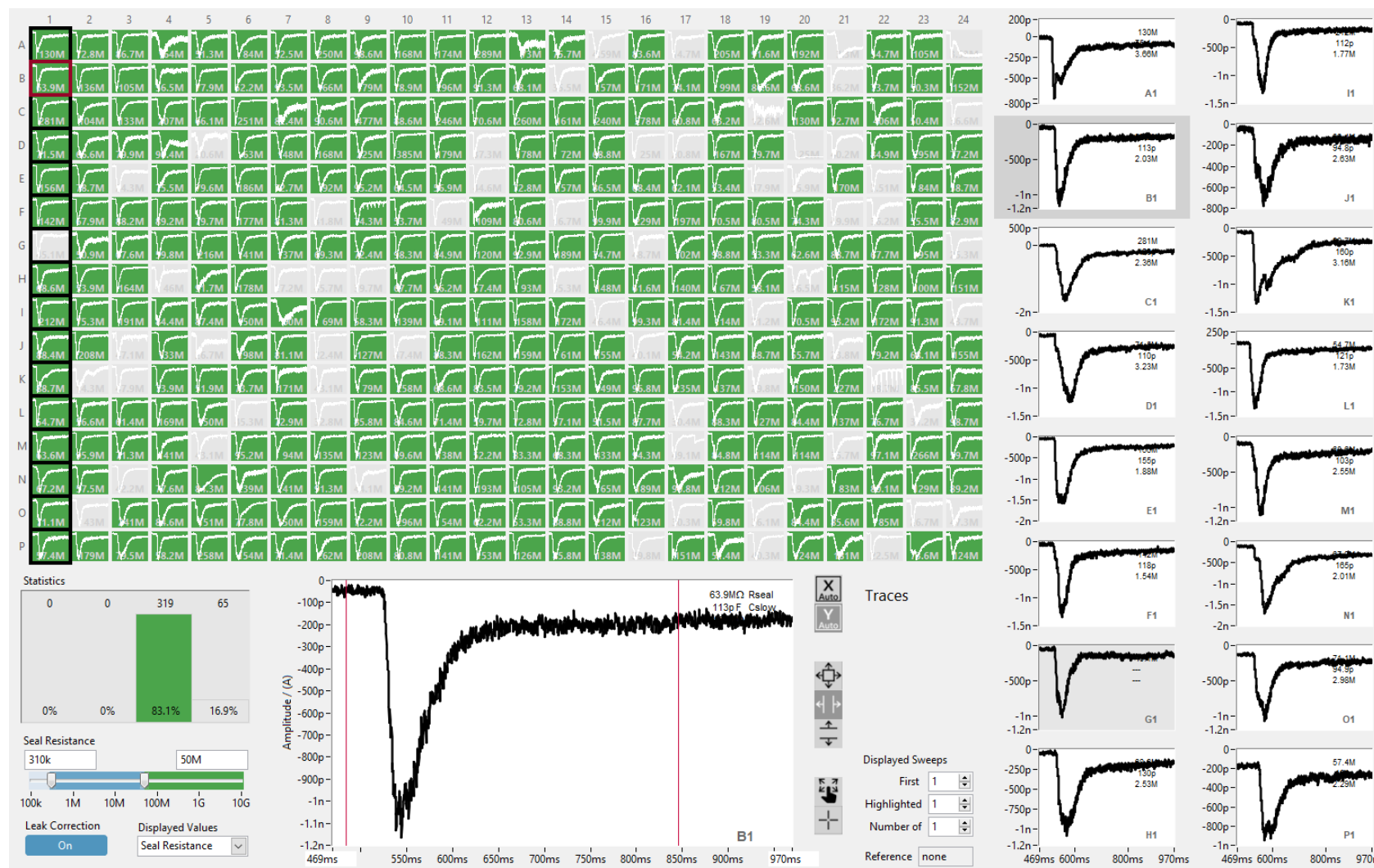
50 μ l quickly removed

500 ms exposure

Cells run @ (0.36 x 10⁶/ml)

Seal Filter = Excludes wells <50 M Ω

Current Filter = Excludes wells >-200 pA



Robust expression of rapidly desensitizing currents

nACh $\alpha 7$ /ric3-RTA Validation

300 μ M Acetylcholine
+ NS-1738 Dose
Response Curve

Success Rate = 83%

Ligand “puff” technique:

5 μ l of Ligand added (50 μ l/s)

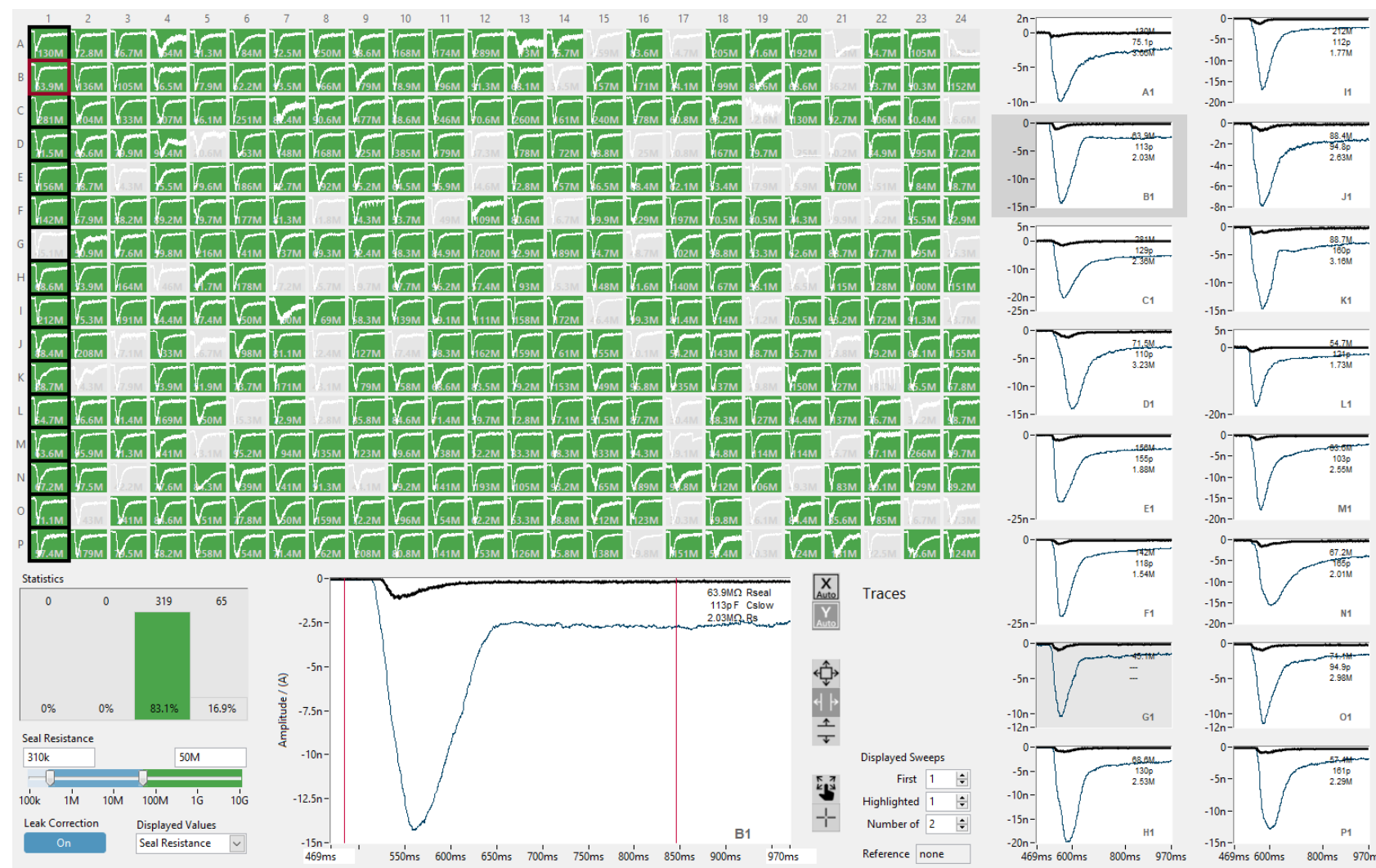
50 μ l quickly removed

500 ms exposure

Cells run @ (0.36 x 10⁶/ml)

Seal Filter = Excludes wells <50 M Ω

Current Filter = Excludes wells >-200 pA

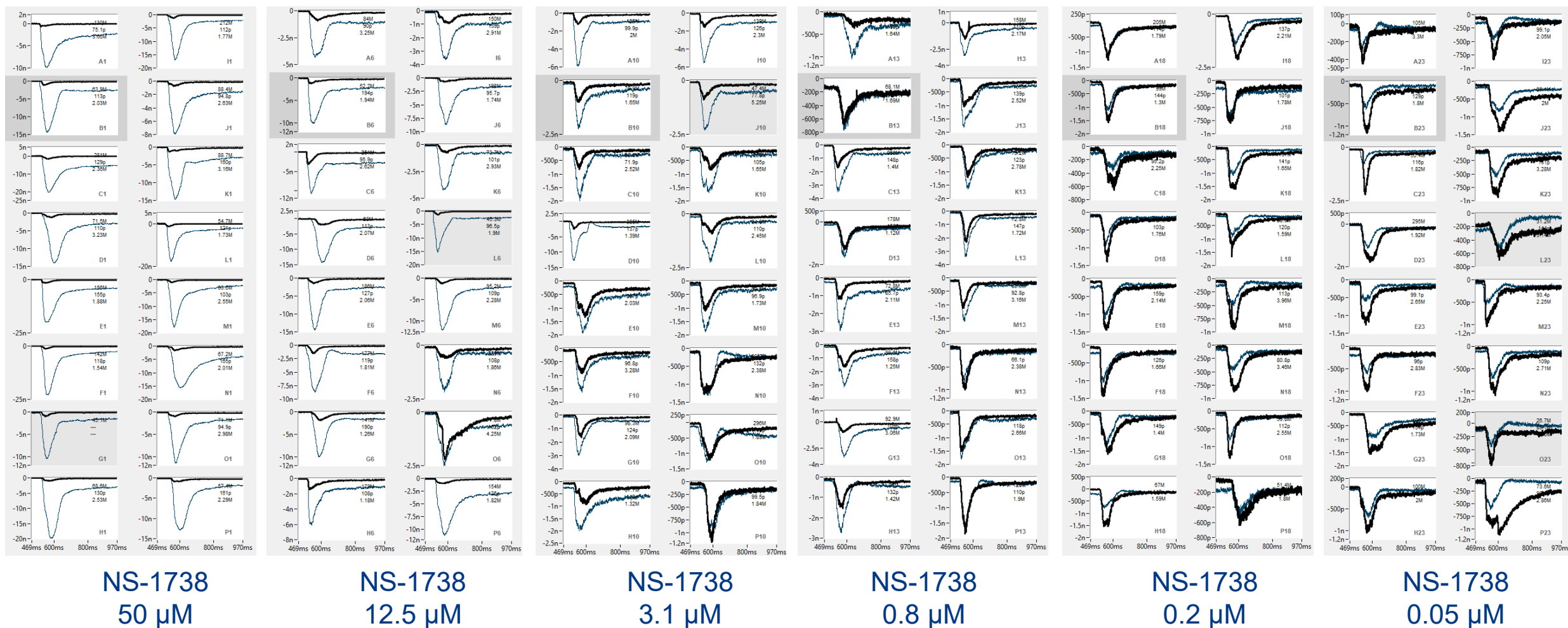


Potentiation by highest dose of NS-1738 (50 μ M)

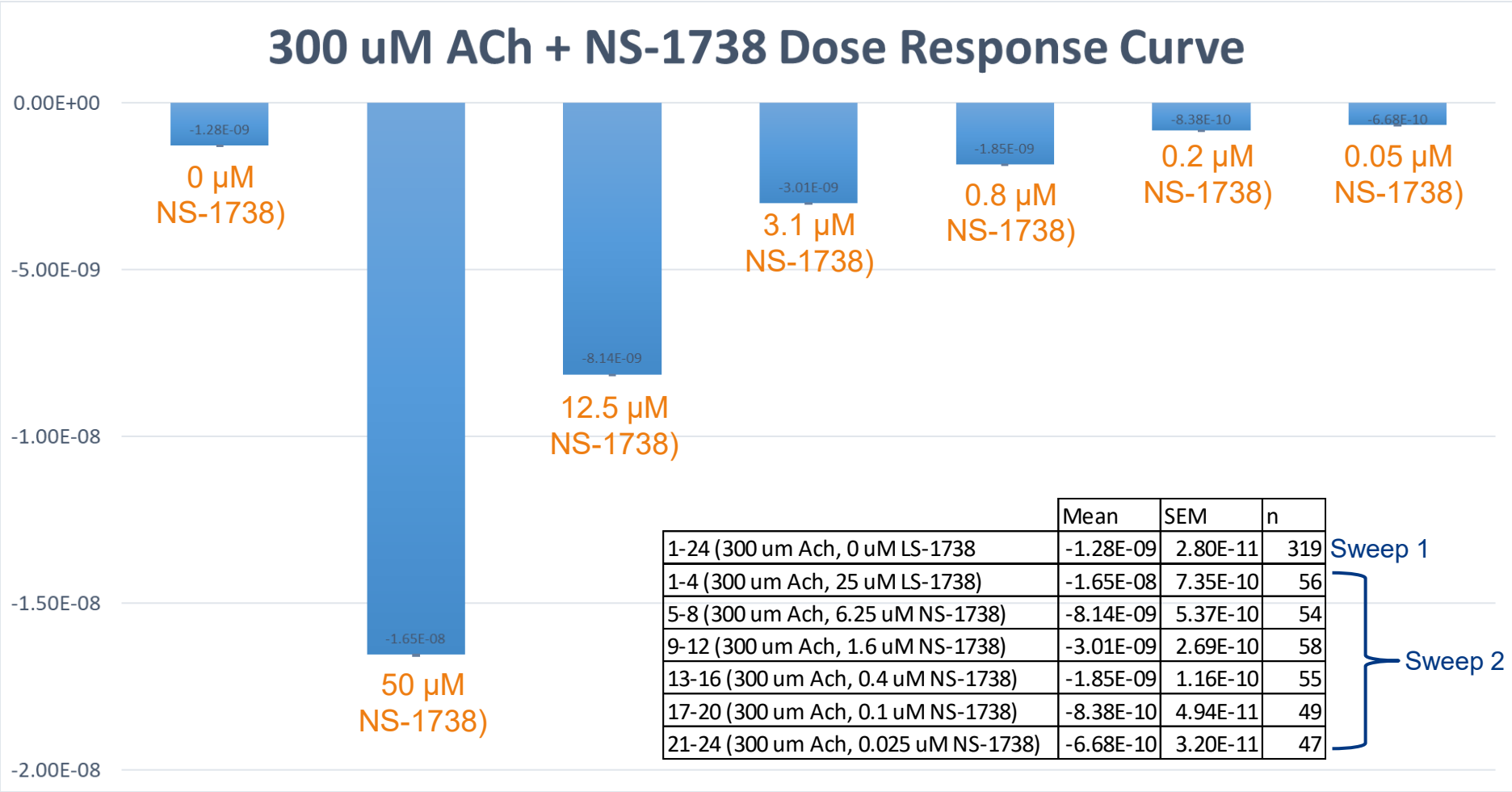
SyncroPatch 384i Data, Oct 23, 2024

nACh $\alpha 7$ /ric3-RTA Validation

300 μ M Acetylcholine + (NS-1738 Dose Response Curve)



SyncroPatch 384i Data, Oct 23, 2024



SyncroPatch 384i Data, Oct 23, 2024

Eurofins DiscoverX[®] Ready-to-Assay (RTA) Cell Lines

We have begun converting our Cardiac Safety Panel and Pain Panel Cell Lines into RTA format

Custom requests available to convert any of our stable cell lines into RTA format

Cardiac Safety Panel RTA Cells

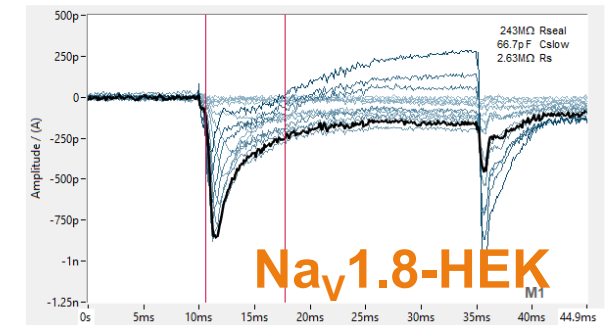
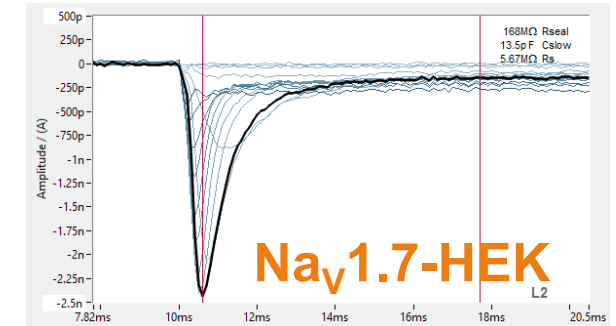
hERG-CHO
 $\text{Ca}_v1.2 \alpha_{1C}/\beta_{2a}/\alpha_2\delta_1$
 $\text{Na}_v1.5$ -HEK

Pain Panel RTA Cells

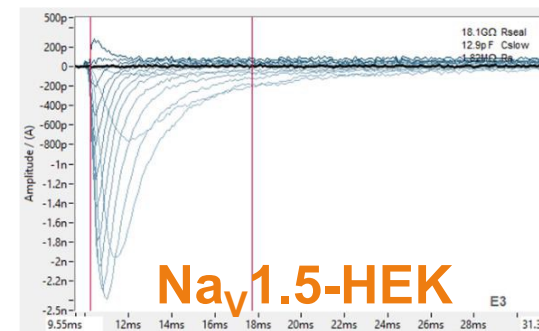
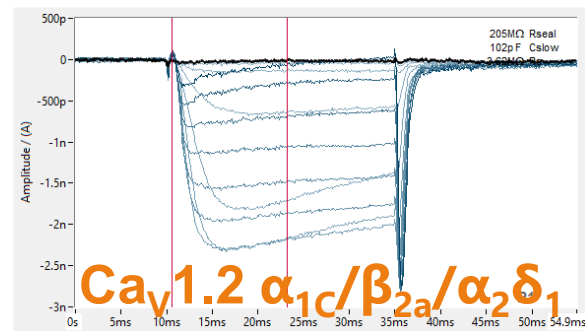
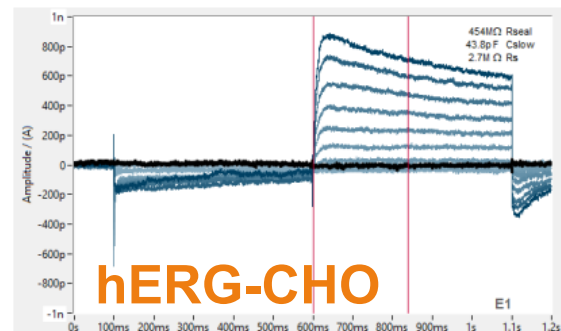
$\text{Na}_v1.7$ -HEK
 $\text{Na}_v1.8/\beta 1$ -HEK

Coming Soon

nACh $\alpha 7/\text{ric}3$



All RTA Cells are of Human Origin

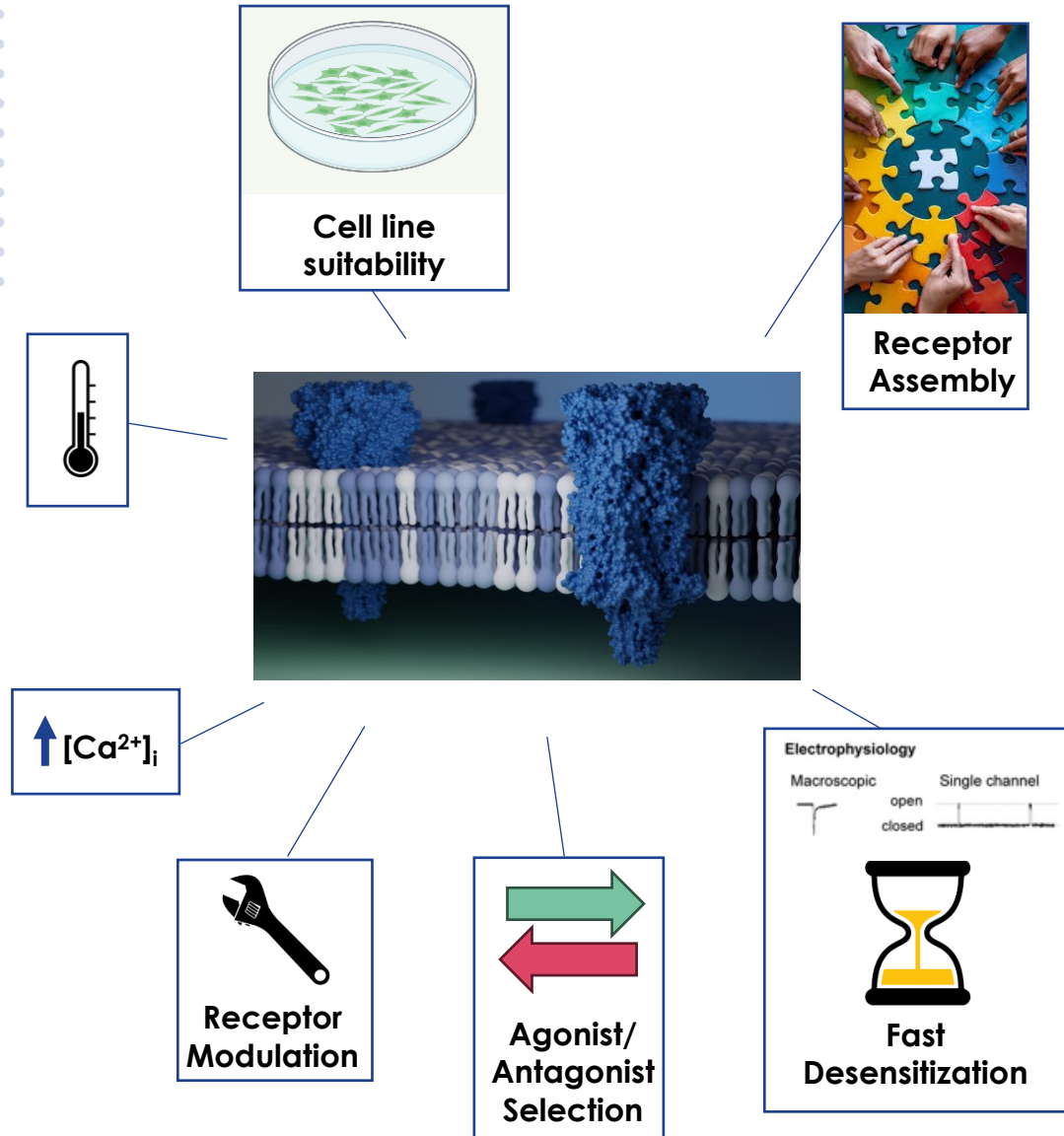


Achieving Speed and Precision: Reliable Activation of nAChR $\alpha 7$ with NPC-384T F/M STIM Chips on the SyncroPatch 384

Dr. Ilka Rinke-Weiß
Senior Scientist

Nanon Webinar
November 21st, 2024

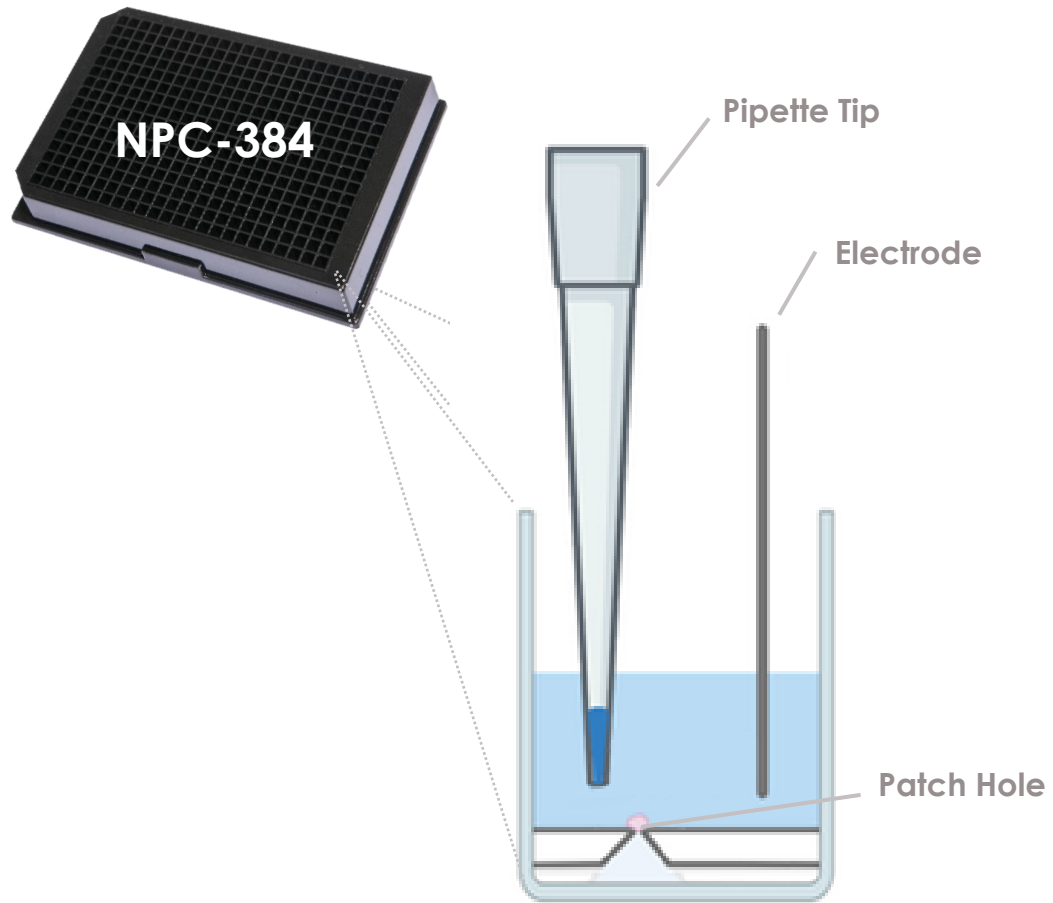




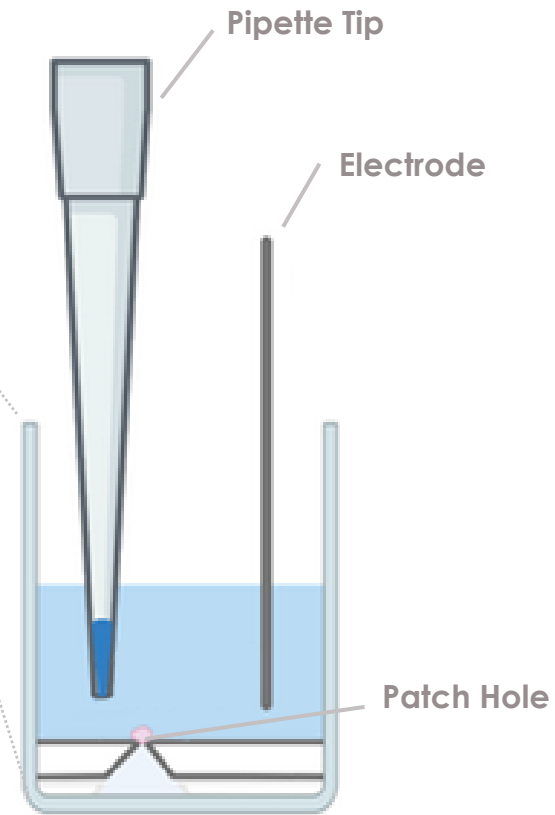
nAChR α 7 Assay - Challenges and Typical Solutions

- Careful cell line selection and involve chaperon proteins in heterologous systems
- Optimize exposure times and agonist concentrations
- Use selective agonists/antagonists
- Allosteric modulators to stabilize receptor function and prolong the open state.
- Use calcium chelators or replace calcium with other ions (e.g., barium) to reduce Ca²⁺-dependent effects.
- Consider measurements at different temperatures

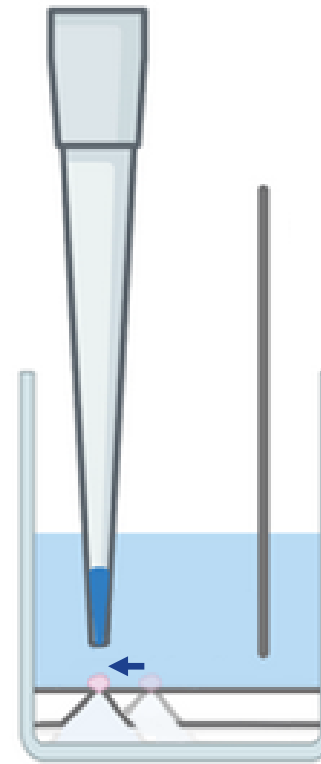
Gain Reliability by Adjusted Hole Positioning



Gain Reliability by Adjusted Hole Positioning



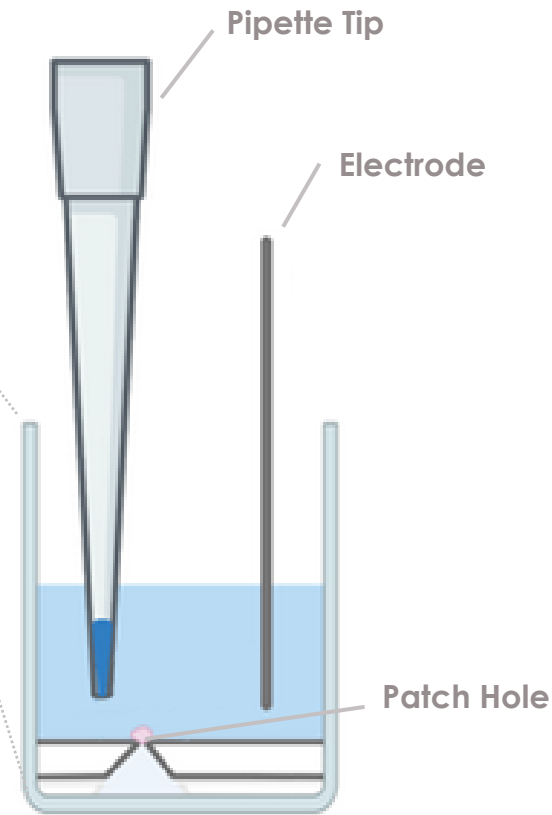
Standard Chip



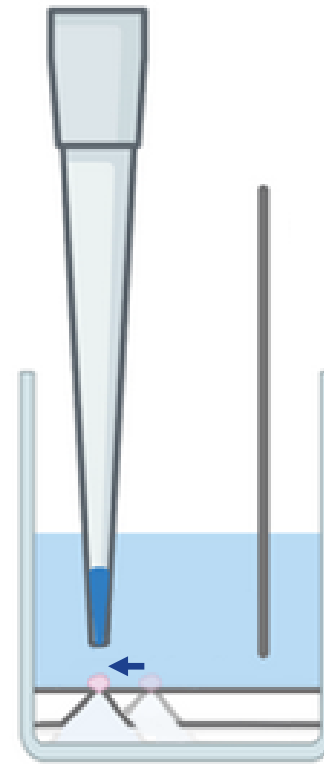
Adjusted Chip



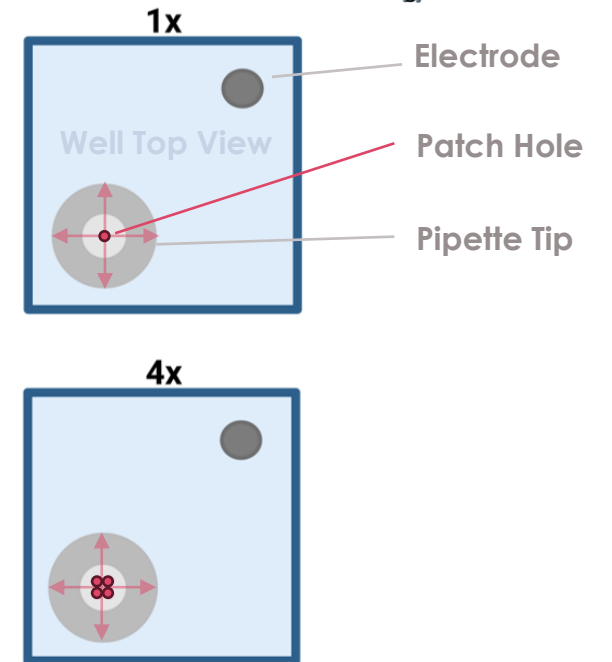
Gain Reliability by Adjusted Hole Positioning



Standard Chip



Adjusted Chip



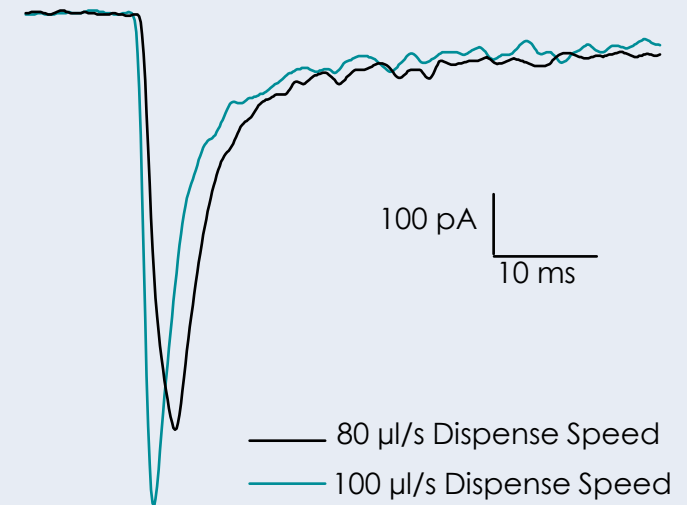
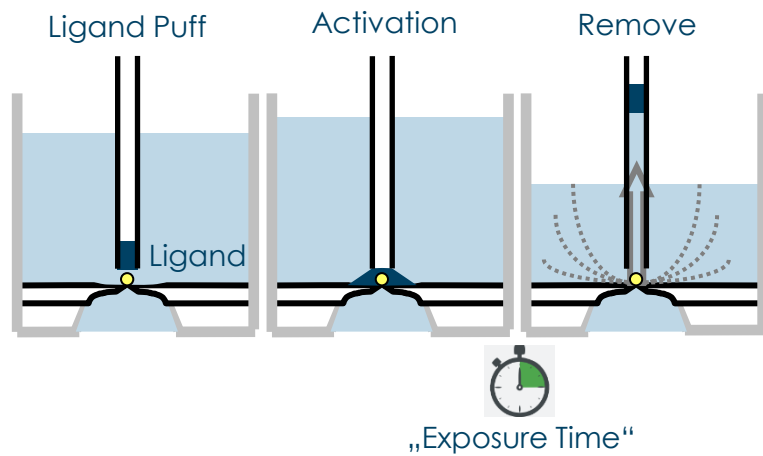
Adjusted Chip



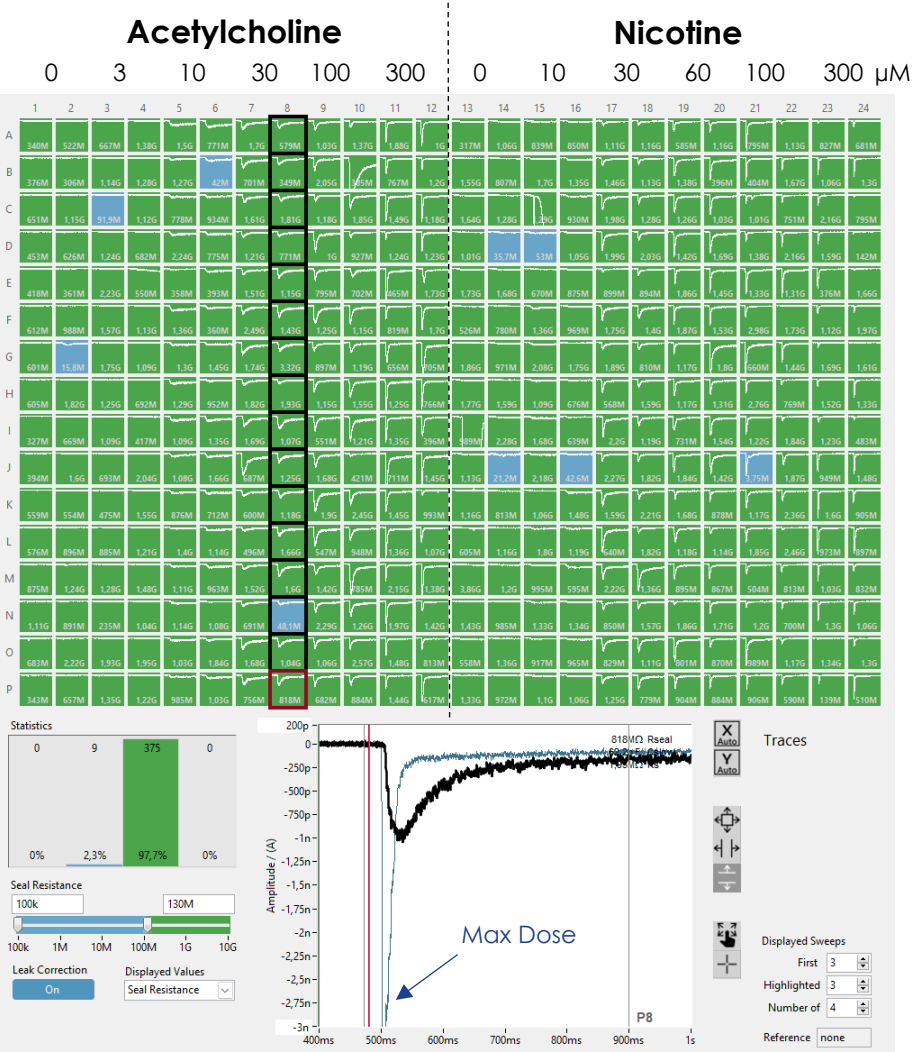


Liquid handling empowers best results

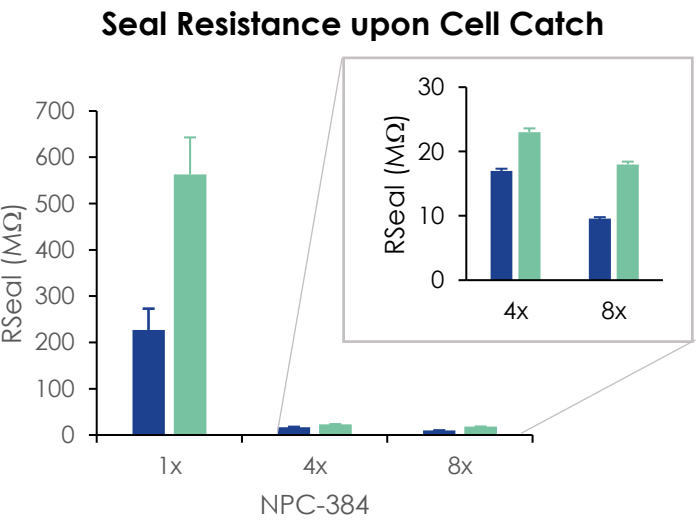
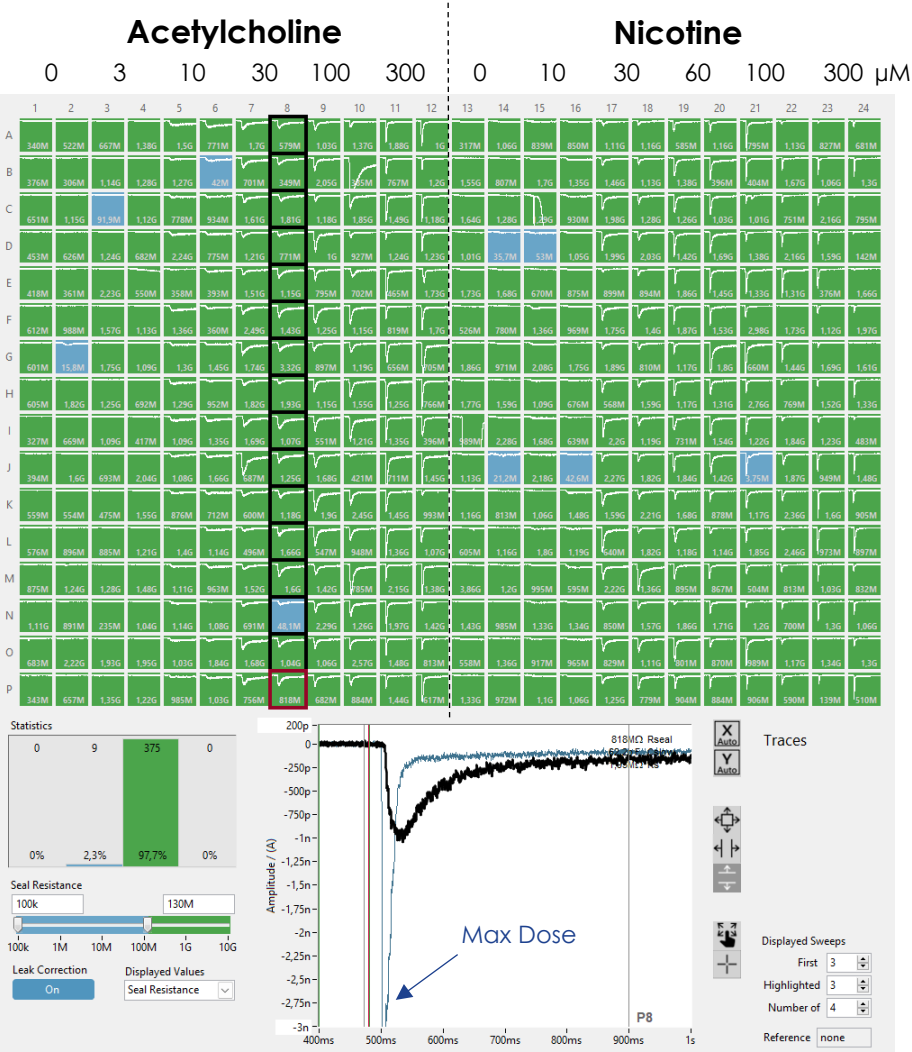
- Faster dispense speed enables faster kinetics of ligand gated ion channels, e.g. nAChR α 7, AMPA, P2X and others.



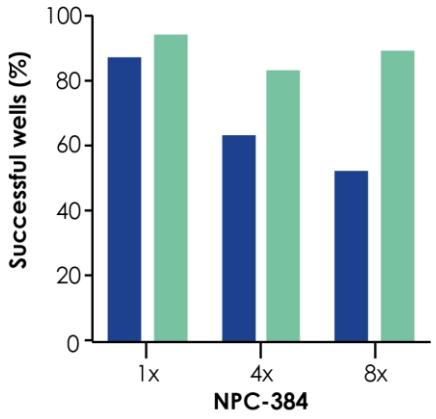
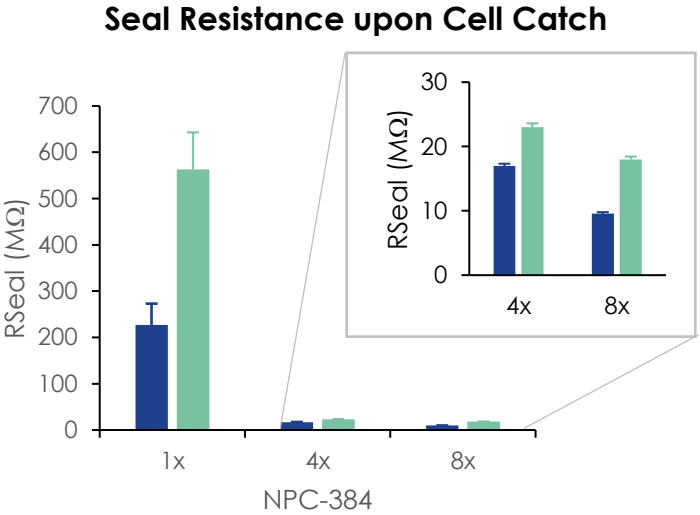
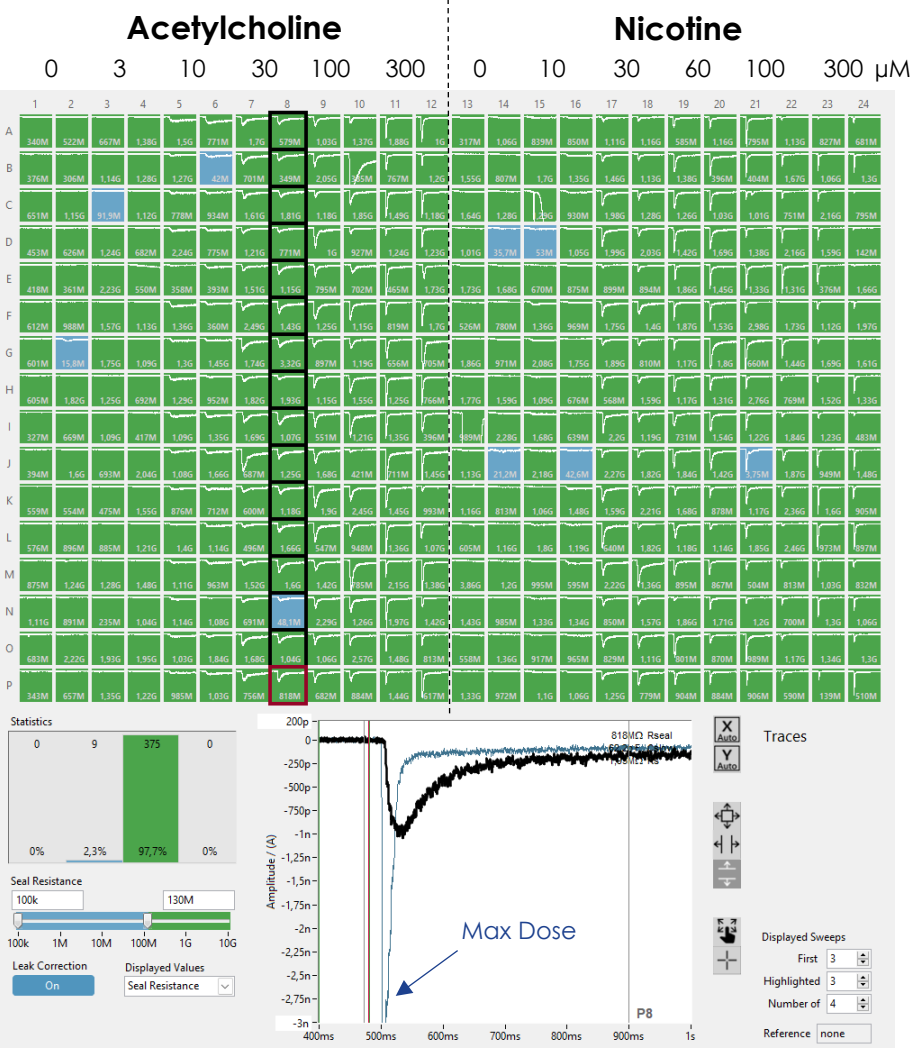
Adjusted Hole Position Improves Seal Resistance



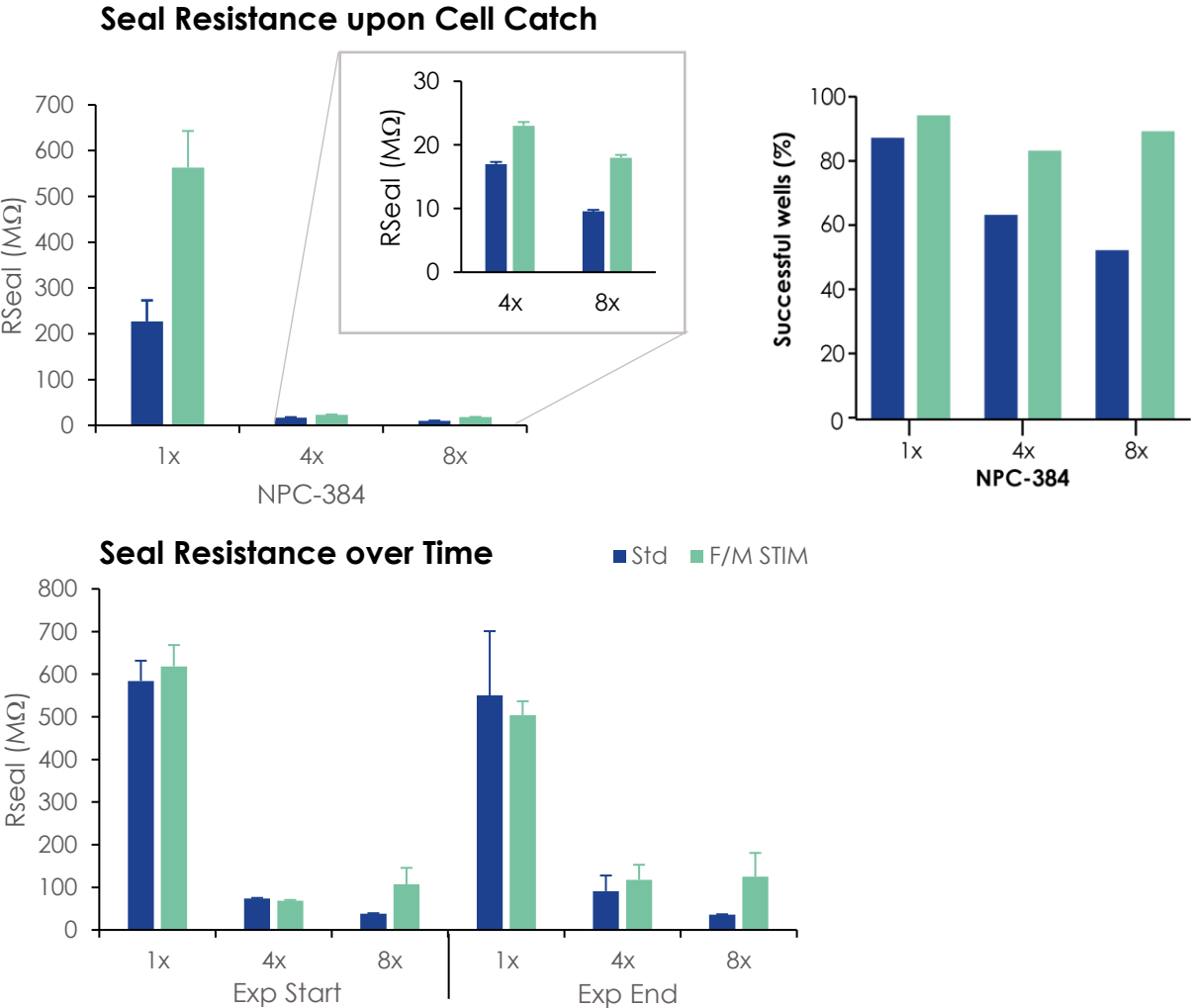
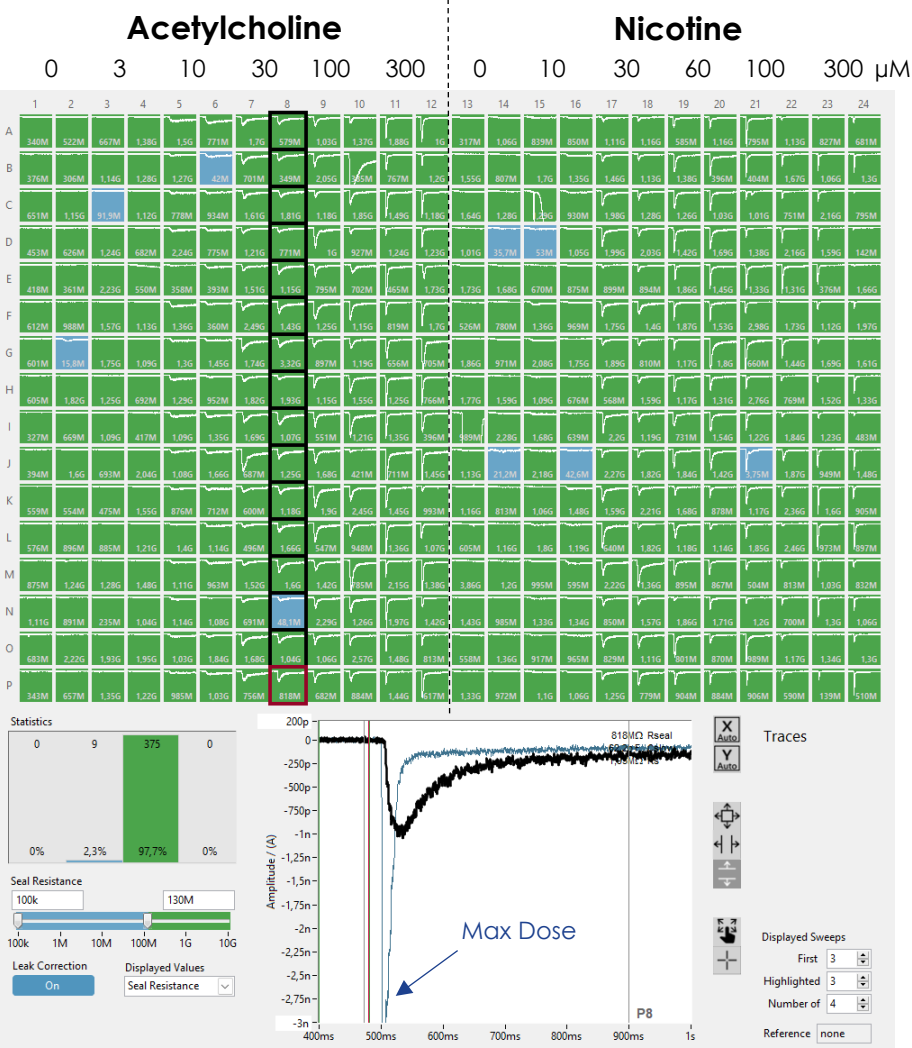
Adjusted Hole Position Improves Seal Resistance



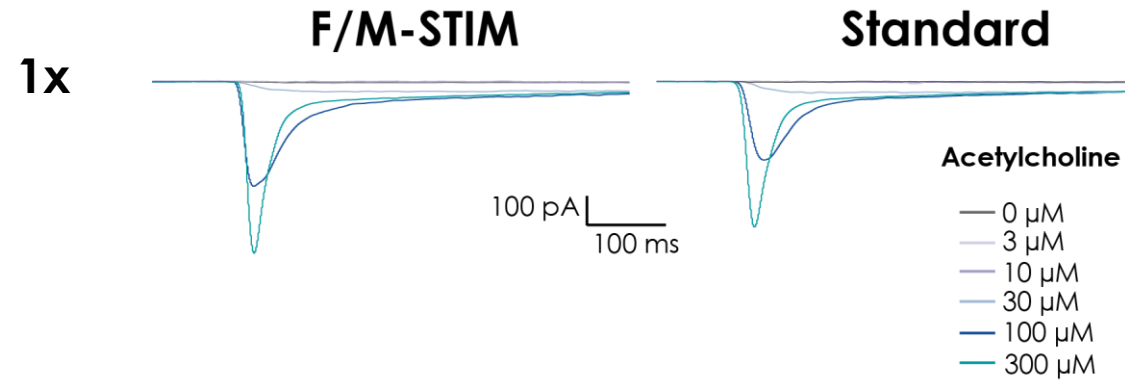
Adjusted Hole Position Improves Seal Resistance



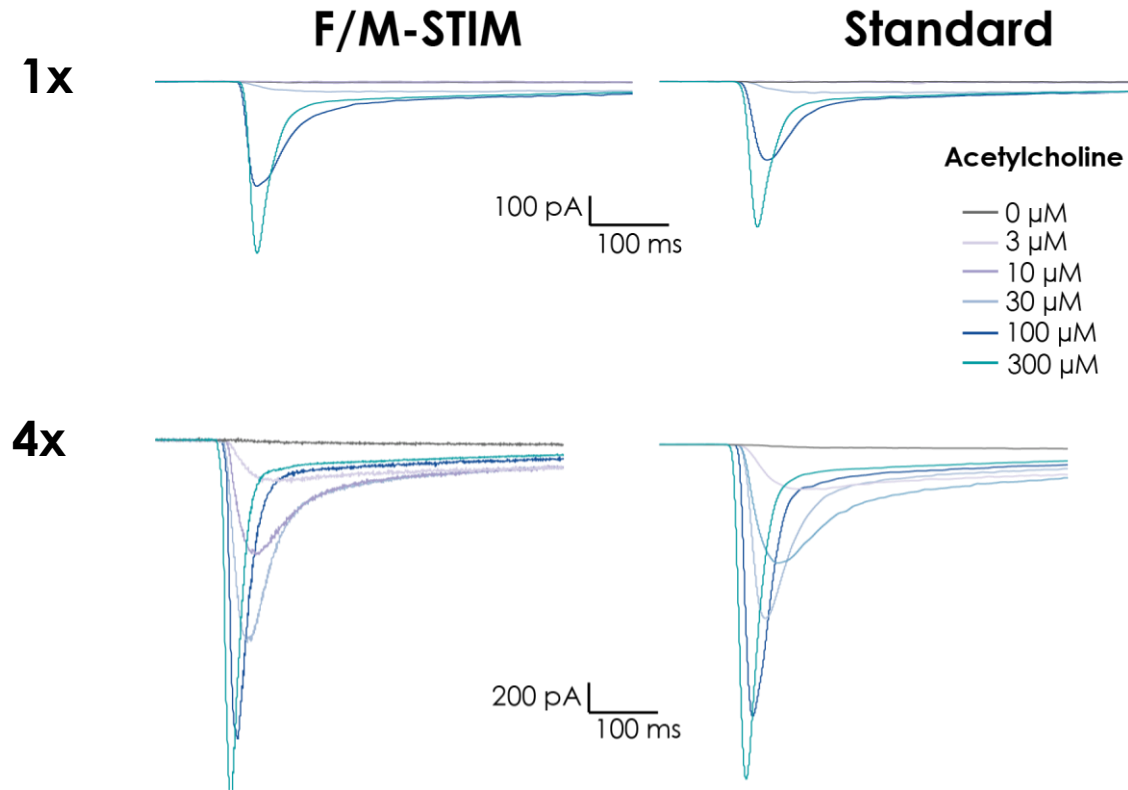
Adjusted Hole Position Improves Seal Resistance



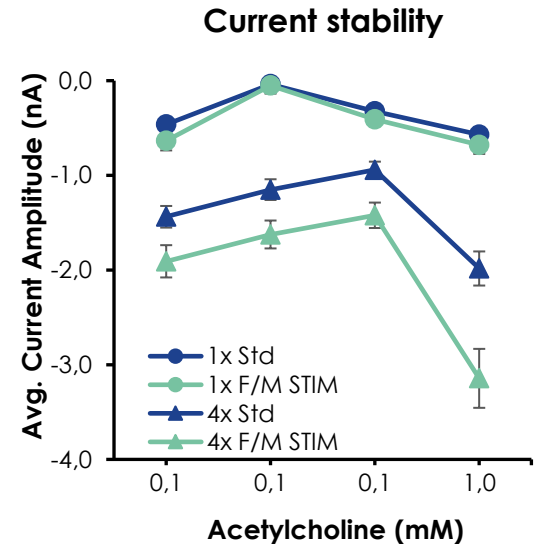
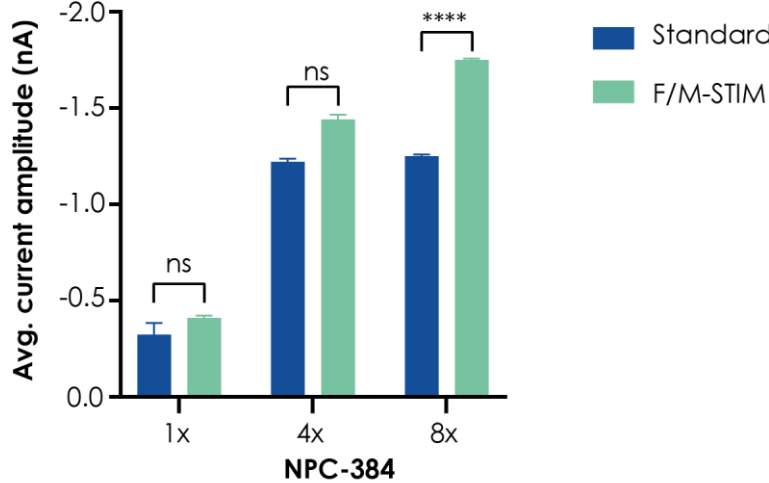
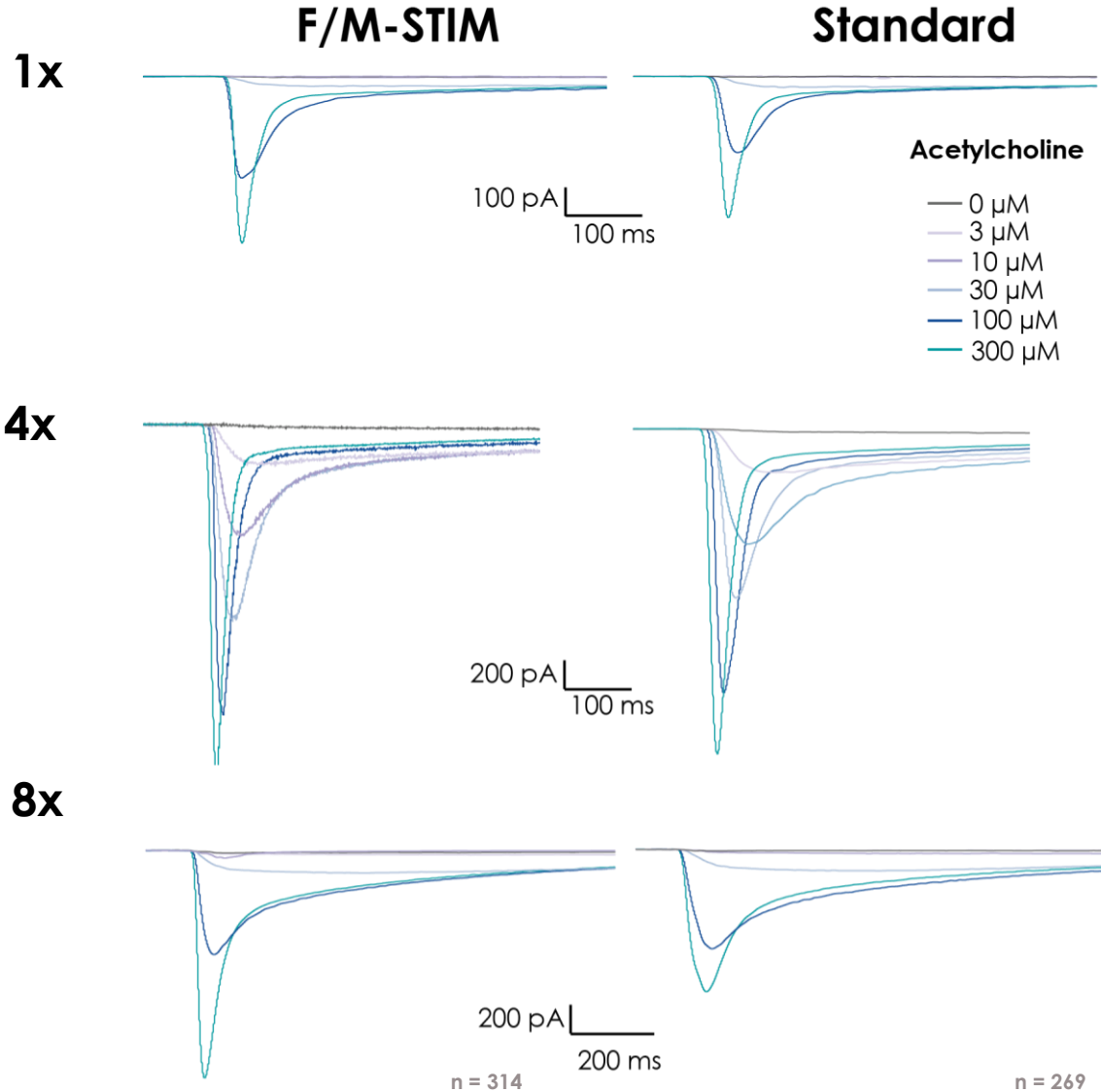
Gain Higher Current Amplitudes with Adjusted Chips



Gain Higher Current Amplitudes with Adjusted Chips

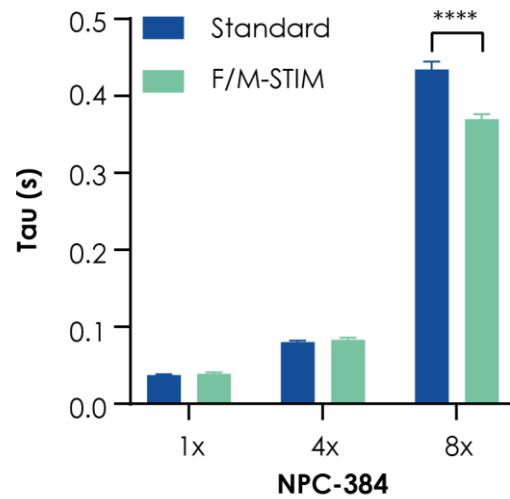
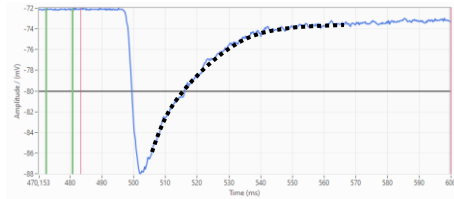


Gain Higher Current Amplitudes with Adjusted Chips



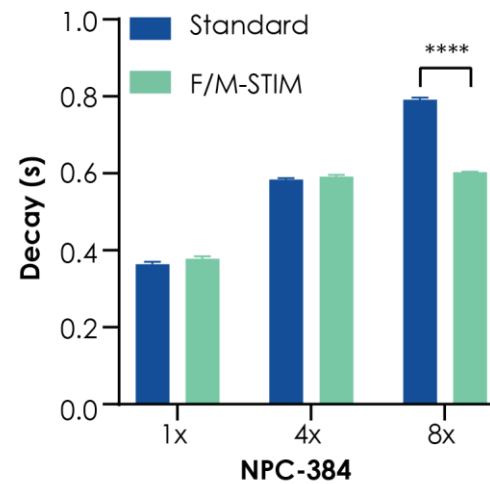
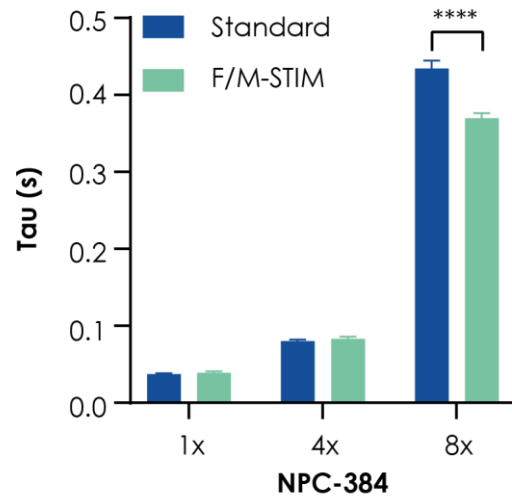
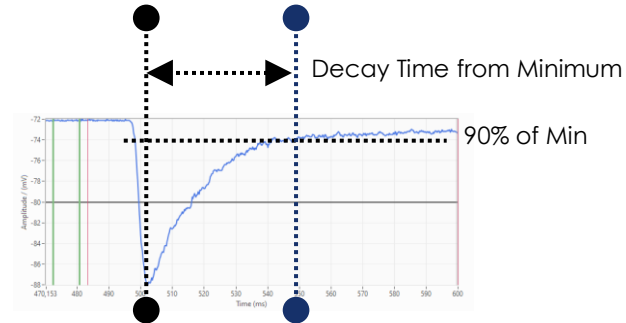
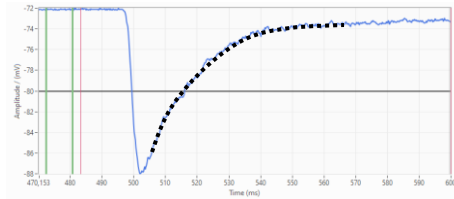
Gain Faster Kinetics with Adjusted Hole Position

Current Kinetics Analysis



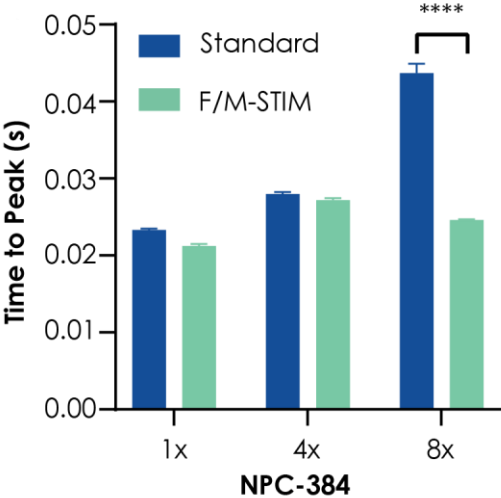
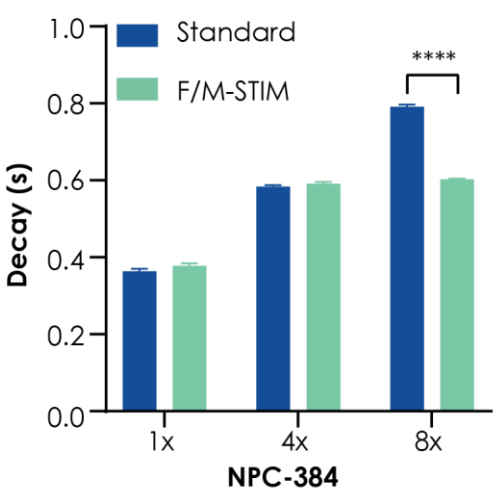
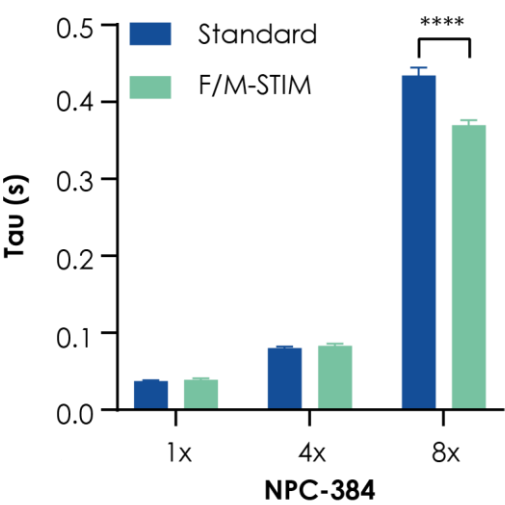
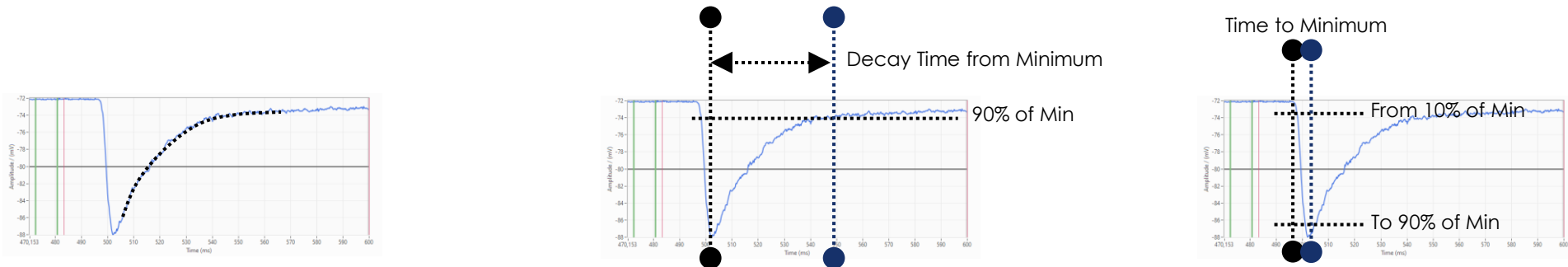
Gain Faster Kinetics with Adjusted Hole Position

Current Kinetics Analysis



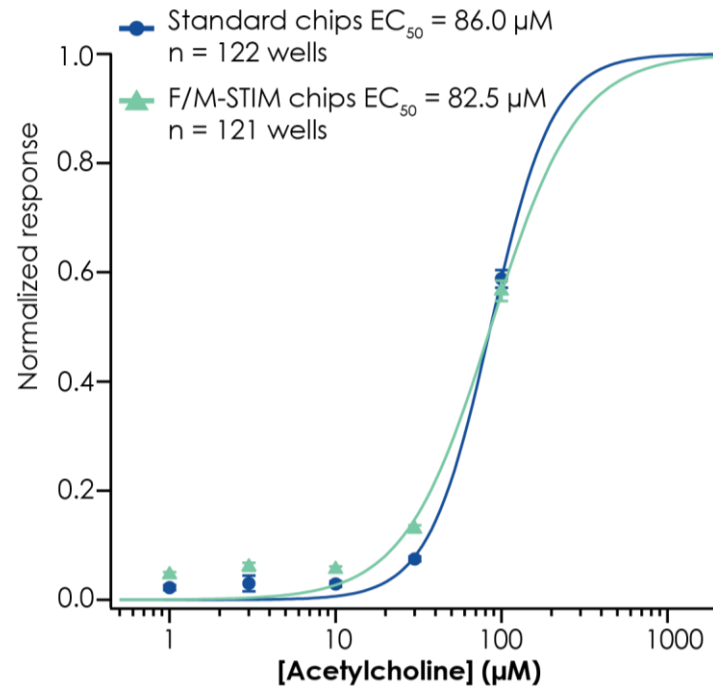
Gain Faster Kinetics with Adjusted Hole Position

Current Kinetics Analysis

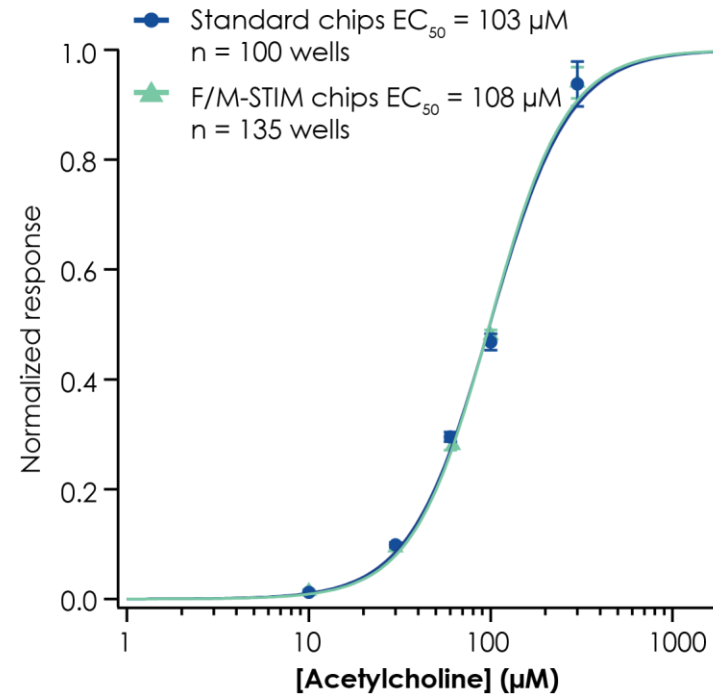


Maintain Compound Concentration Response Results

Single-hole chips



Multi-hole chips



Run with

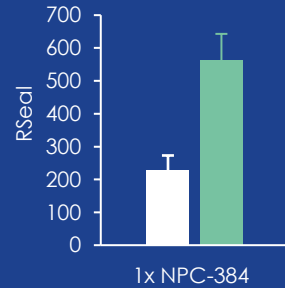


Run with



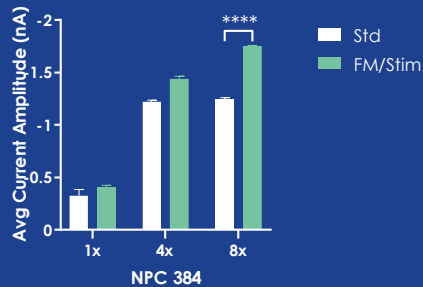
Summary

1



Initial higher Seal Resistance

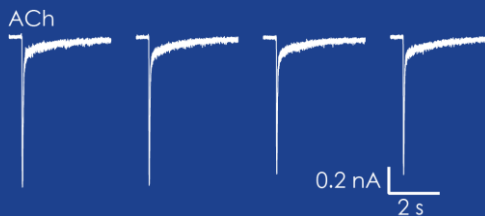
2



Bigger currents and faster kinetics

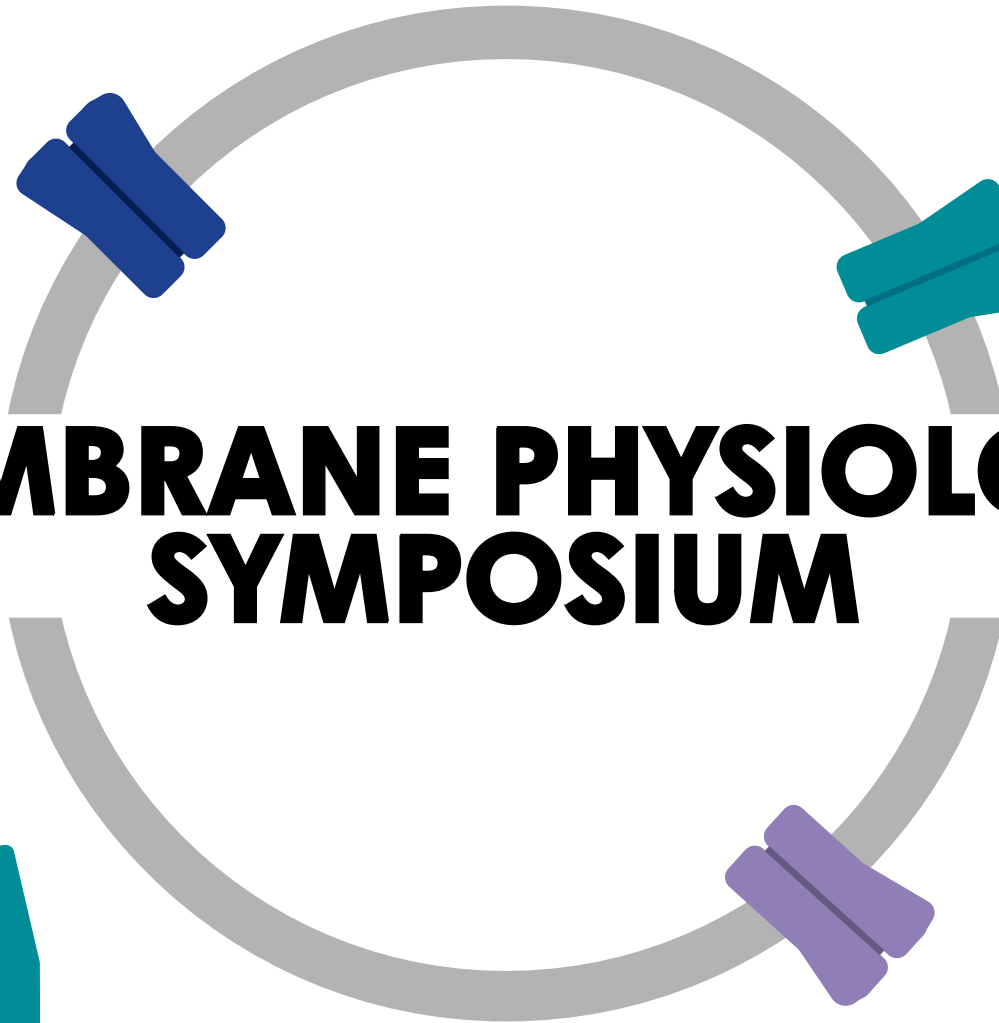
In conclusion, the results highlight the reliability, robustness and accuracy of the F/M STIM chip system.

3



Reproducible currents over multiple ligand additions.

Discussion: Q&A session



MEMBRANE PHYSIOLOGY SYMPOSIUM

Napa Valley, California

April 30th – May 1st, 2025

Science
Networking
& a little wine



research ins]i[ghts

cardiac safety
symposium 2025

Safety pharmacology

Cardiotoxicity

Ion Channels

When: May 21st, 2025

Where: Virtual symposium



Thank you

Office

Nanion Technologies

Address

Nanion Technologies GmbH
Ganghoferstr. 70a
80339 Munich
Germany

Contact

Team.marketing@nanion.de

<https://www.nanion.de/>