





The therapeutic potential of activators of two-pore domain ion channels.

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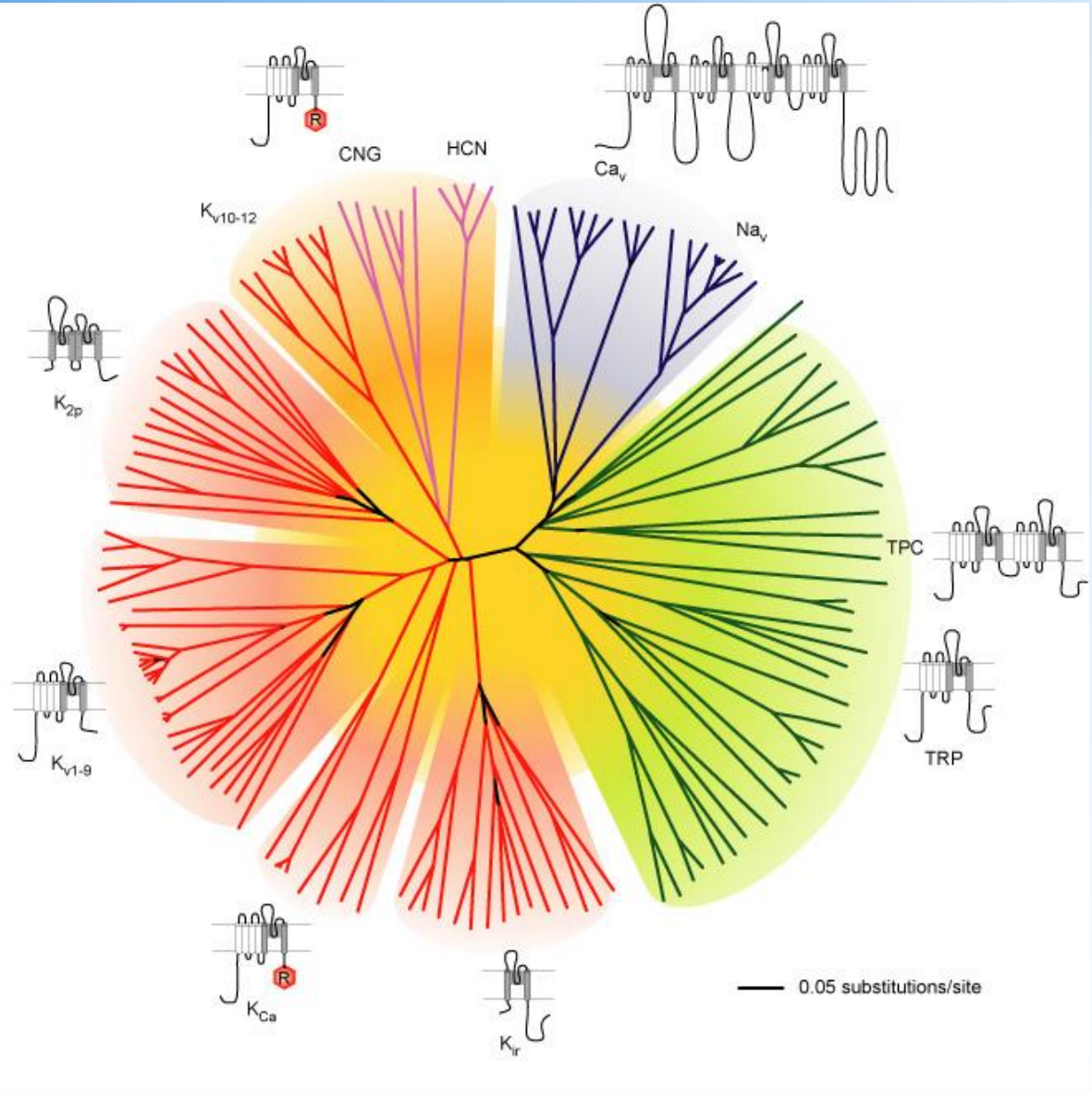
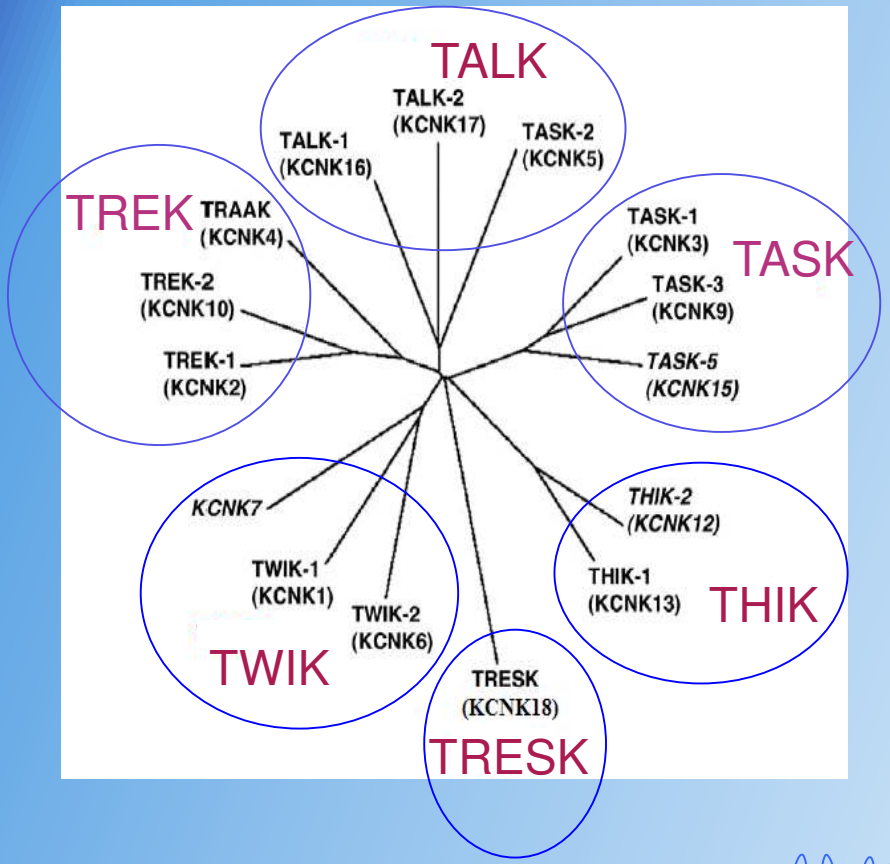
The voltage-gated-like (VGL) ion channel “chanome”

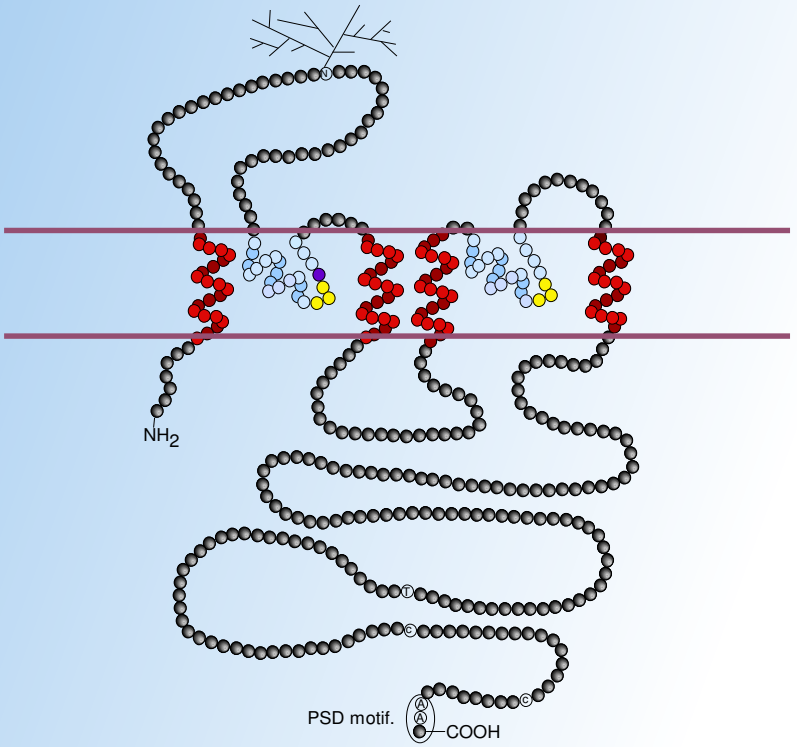


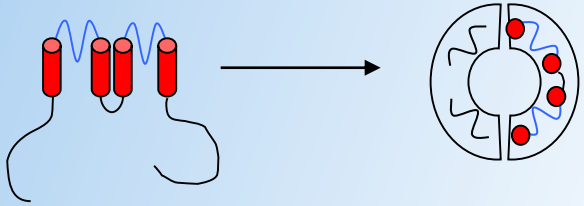
(from Yu & Catterall 2004, *Science STKE 253,* 15)

15 mammalian K2P channels in 6 families

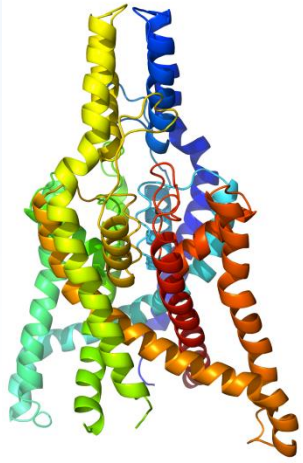
6 families of K2P channels







K2P channels are dimers



Seminar Themes

- KCNK9 Imprinting Syndrome
- KCNK3 in pulmonary hypertension
- K2P channels and pain
- Quantification and characterisation of K2P channel activators

Pathophysiology of K2P channels

Disease	Channel	Reference
Depression	TREK1 (KCNK2)	Heurteaux et al (2006) Nature Neurosci 9: 1134
Migraine	TRESK (KCNK18)	Lafreniere et al (2010) Nature Medicine 16: 1157
Cancer	TASK3 (KCNK9)	Sun et al (2016) Nature Commun 7: 10339
Deafness	TASK2 (KCNK5)	Cazals et al (2015) Nature Commun 6: 8780
KCNK9 Imprinting Syndrome	TASK3 (KCNK9)	Graham et al (2016) Am J Med Gen A 170:2632
Pulmonary Hypertension	TASK1 (KCNK3)	Antigny et al (2016) Circulation 133:1371

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KCNK9 imprinting syndrome

All affected individuals had moderate to severe mental retardation and were hyperactive.

Severe feeding difficulties at infancy.

Dysphagia of solid foods until near puberty.

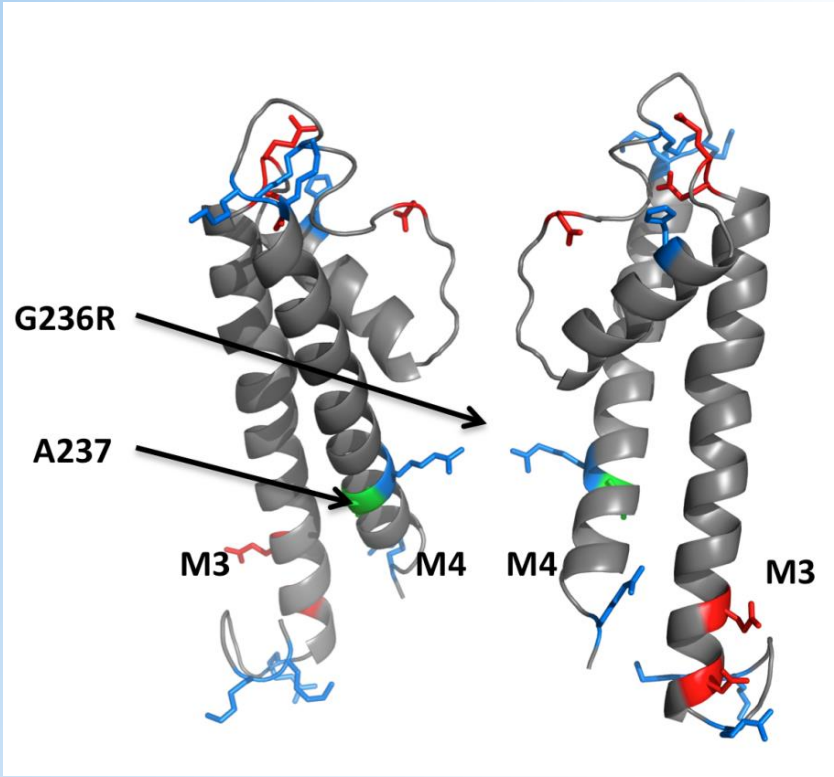
Generalized hypotonia at an early age followed by weakness of proximal muscles.

Barel et al (2008) Am J Hum Gen 83: 193-199

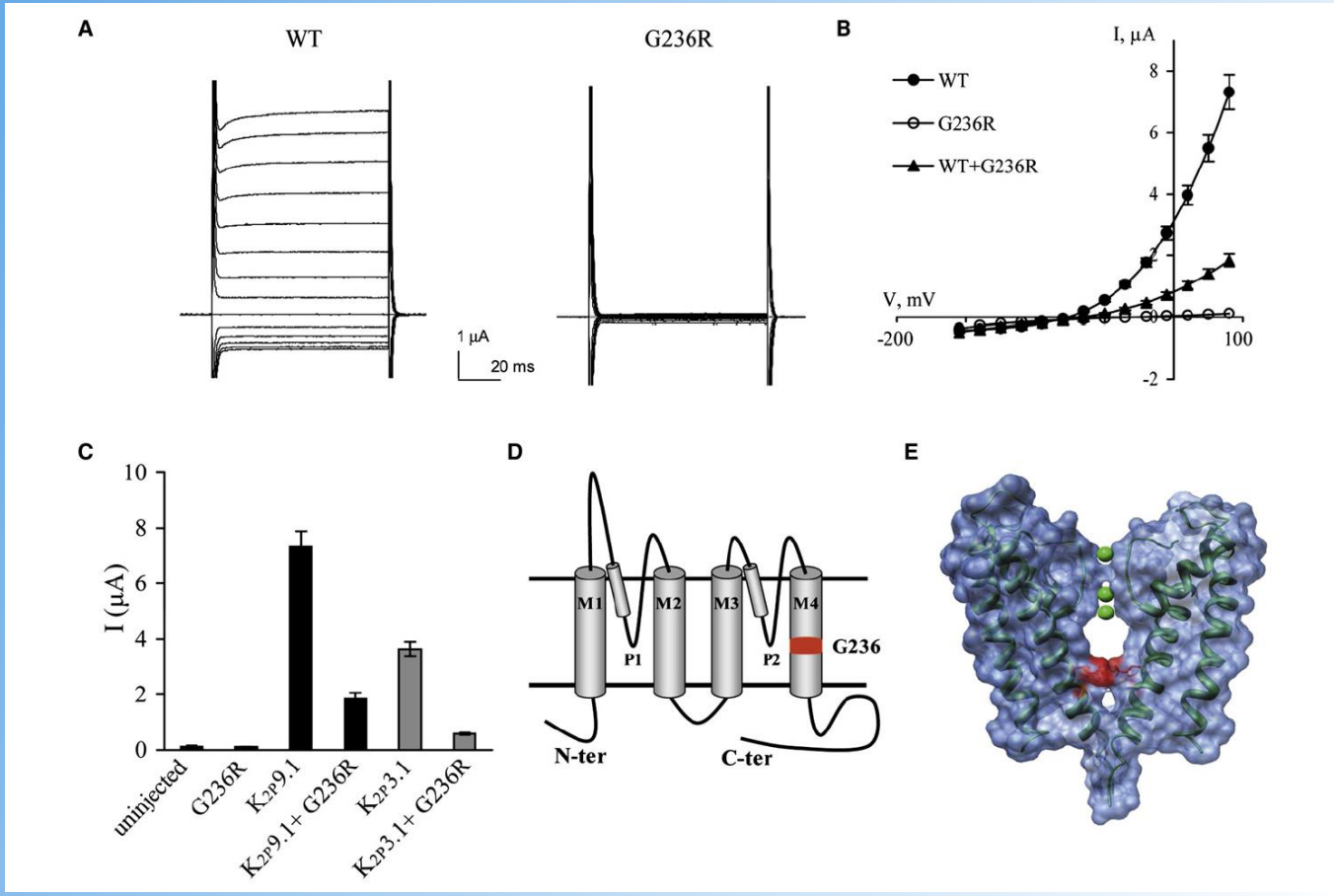
G236R in TASK3 underlies KCNK9 Imprinting Syndrome

M4

TREK1	281	G----A	286
TRESK	347	G----F	352
TWIK1	256	G----L	261
TASK3	231	G----G	236

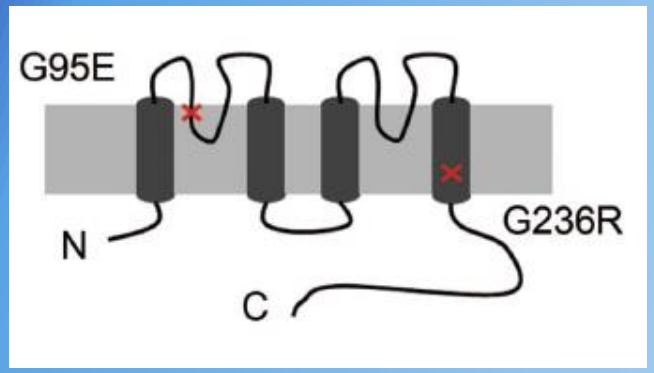


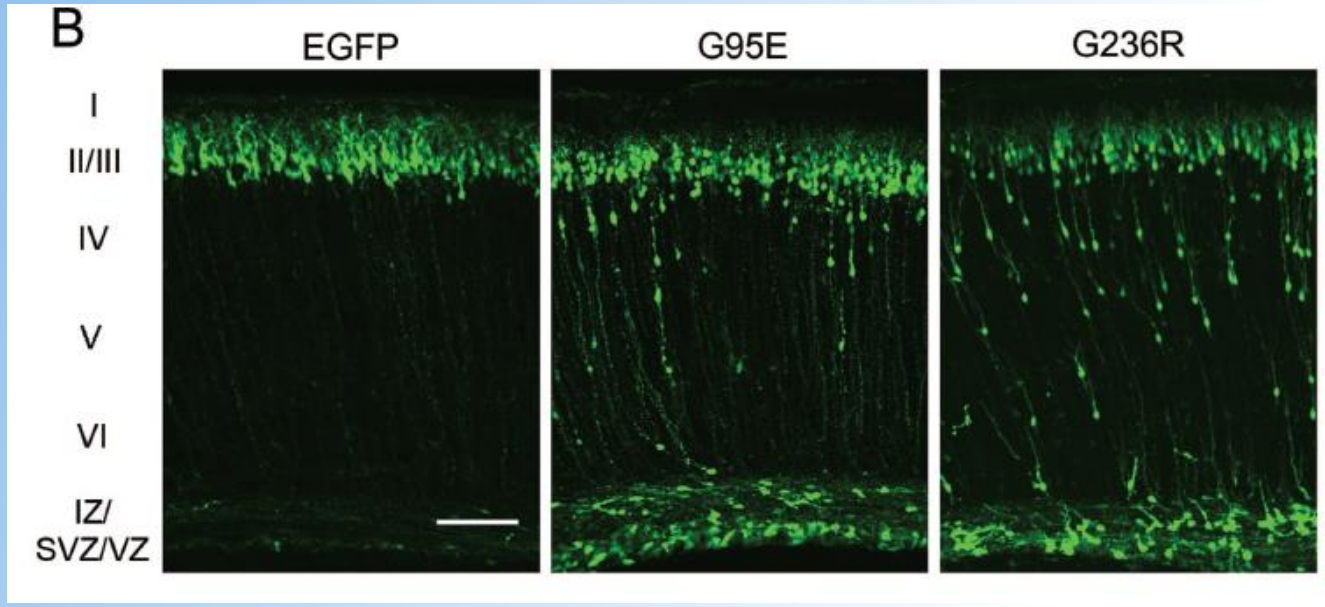
TASK3 G236R channels described as non-functional



Barel et al (2008) Am J Hum Gen 83: 193-199

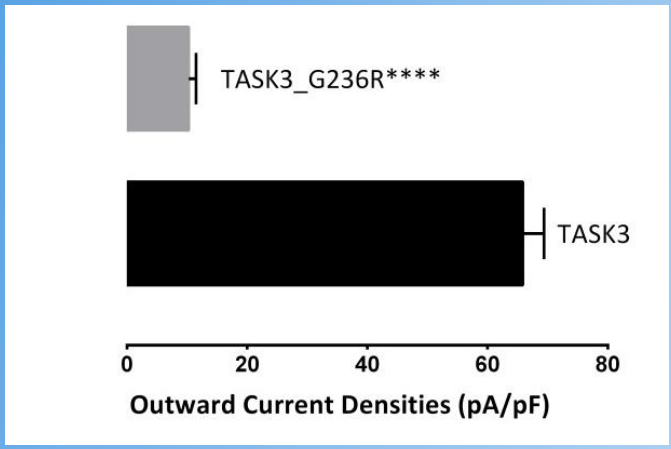
Dysfunction of TASK3 channels impairs neuronal migration

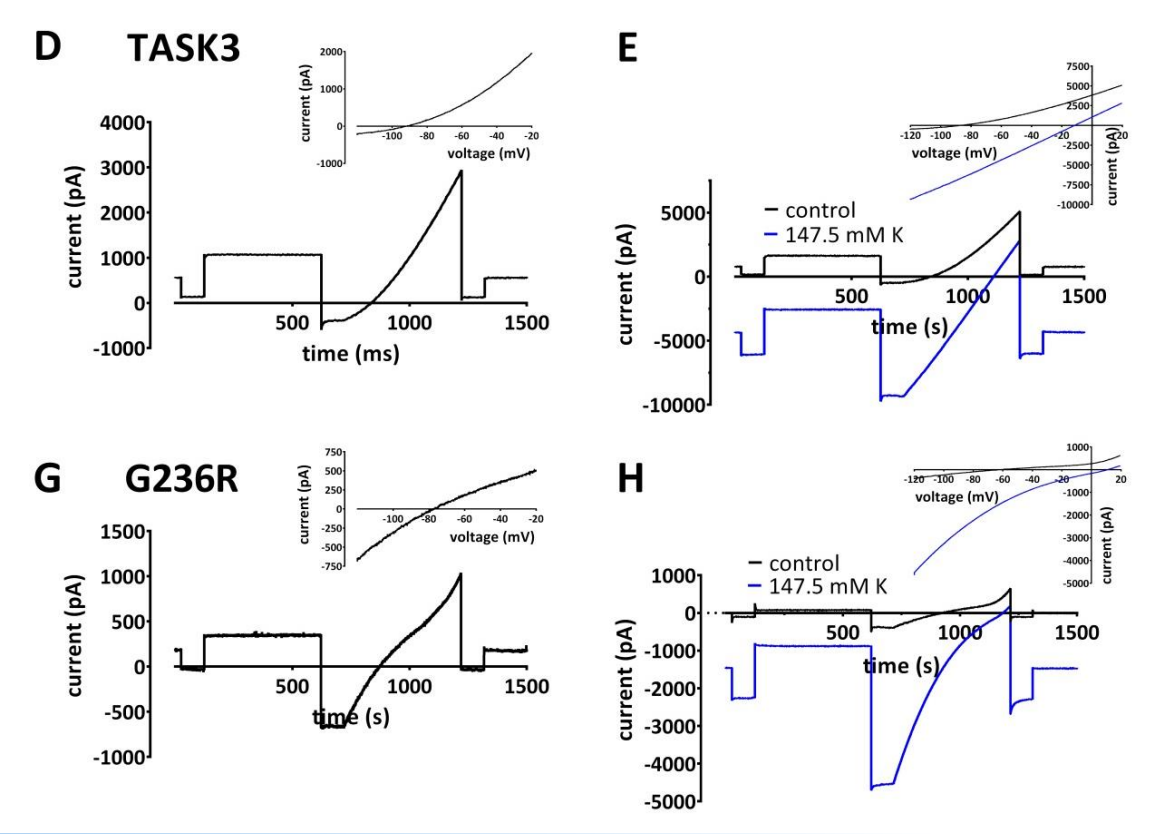




Bando et al (2014) Cerebral Cortex 24: 1017-1029

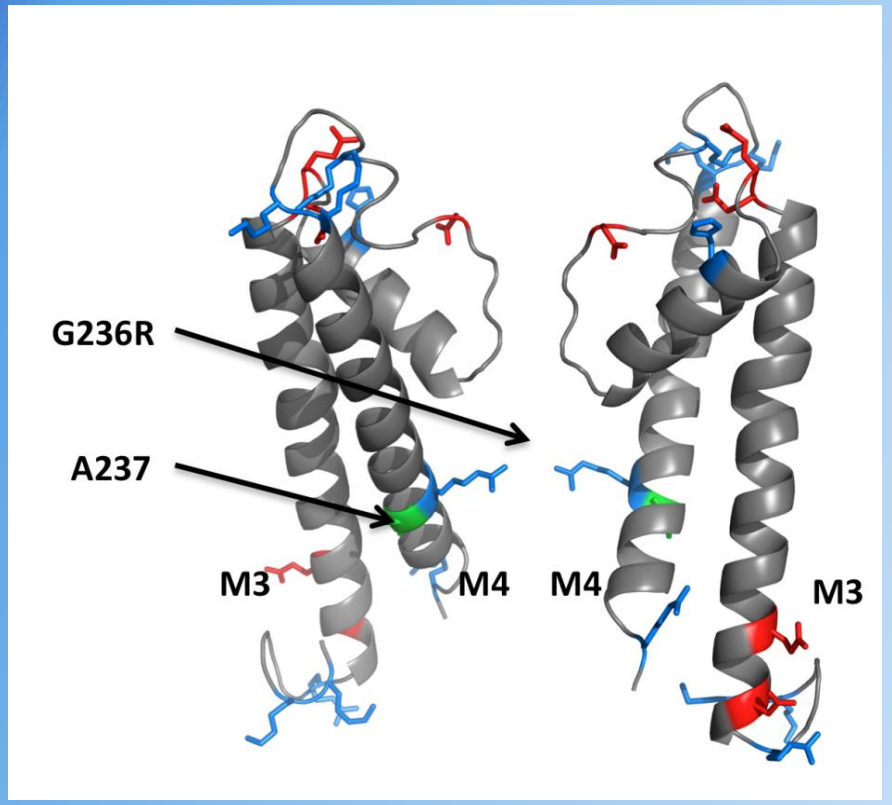
G236R gives small but detectable currents that inwardly rectify

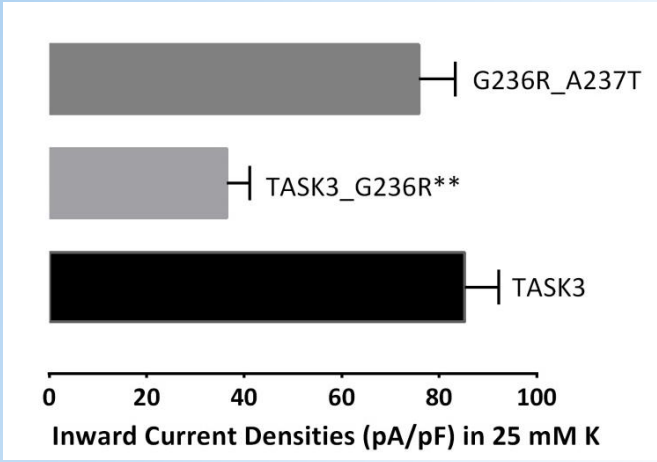


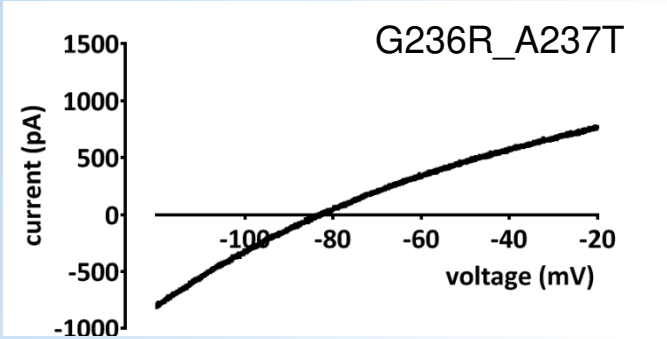


Veale et al (2014) Mol Pharm 85: 397-407

Gain of function mutation A237T can reverse effect of G236R on TASK3 current density

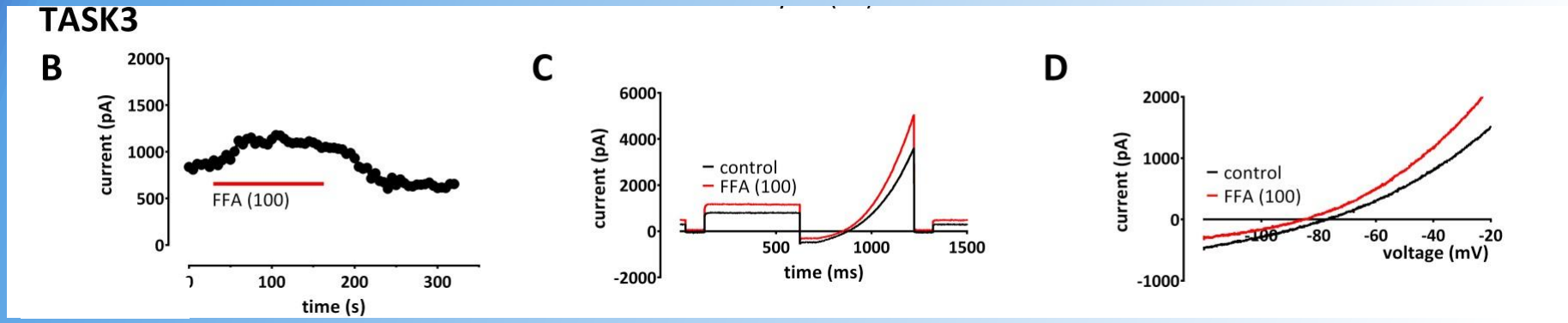






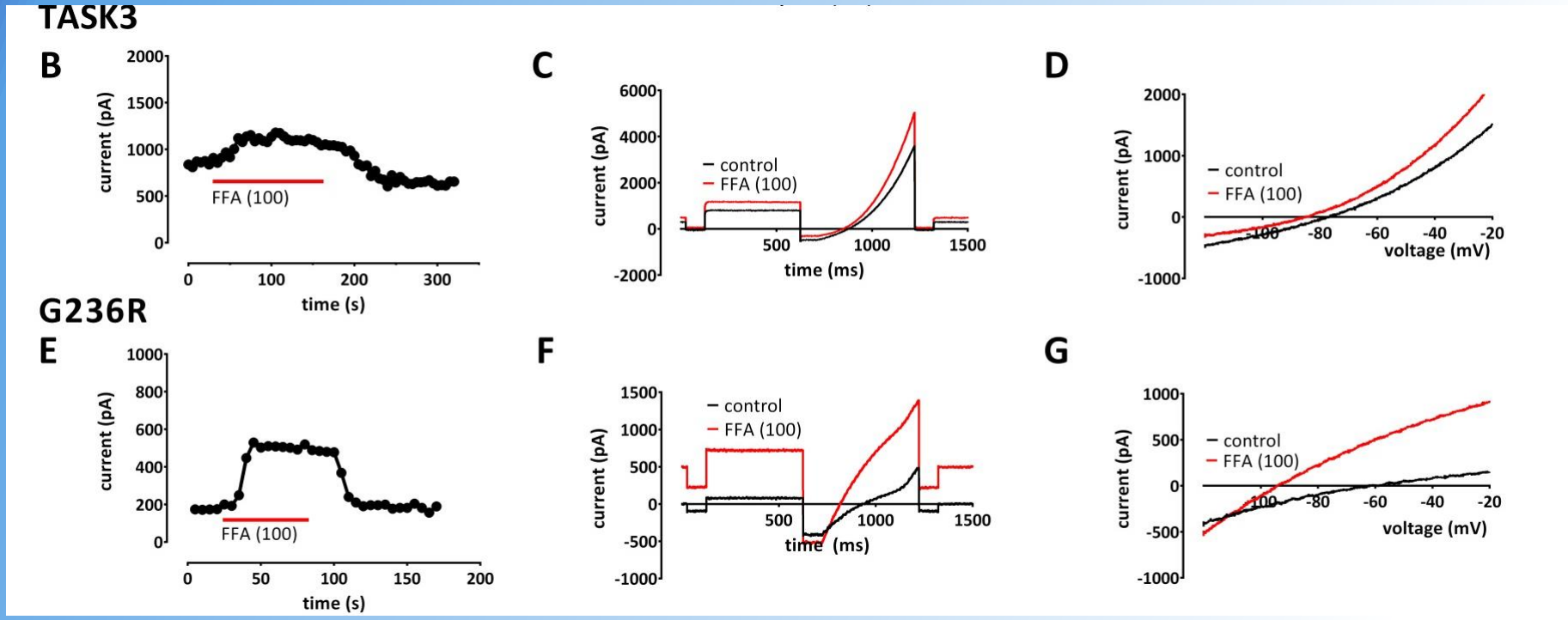
Veale et al (2014) Mol Pharm 85: 397-407

Flufenamic acid moderately enhances TASK3 current



Veale et al (2014) Mol Pharm 85: 397-407

Flufenamic acid partially restores current through G236R channels



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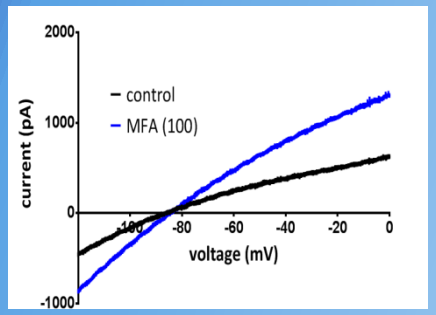
KCNK9 Imprinting Syndrome summary

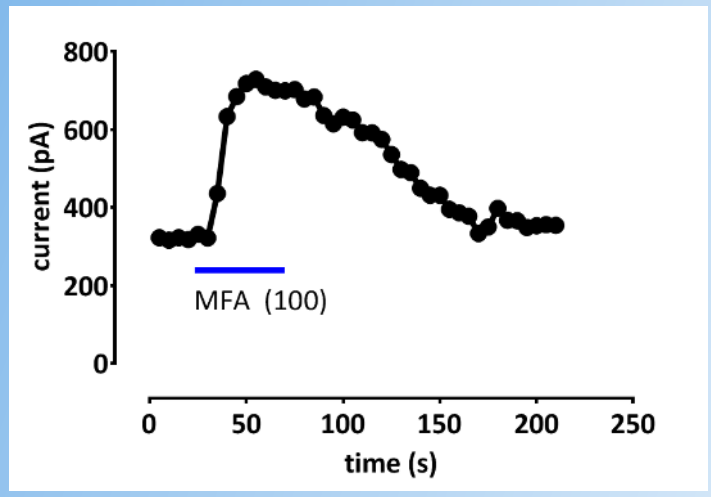
G236R mutated TASK3 channels give rise to KCNK9 Imprinting Syndrome and this mutation impedes neural migration during development.

Mutated channels carry significantly less current than that through normal TASK3 channels and show inward rectification.

Current can be restored, at least in part, either by further gain-of-function mutation of the channel or by drugs such as flufenamic acid.

KCNK9 Imprinting Syndrome





Mefenamic acid (Ponstel in USA)

"……has been on MFA for one year, and his development and responsiveness is clearly better while on MFA than while off it during treatment rest periods."

Graham et al (2016) Am J Med Genet 170:2632

KCNK9 Imprinting Syndrome (30 individuals)

30 individuals have clinical symptoms consistent with KCNK9 Imprinting Syndrome and have undergone genetic screening.

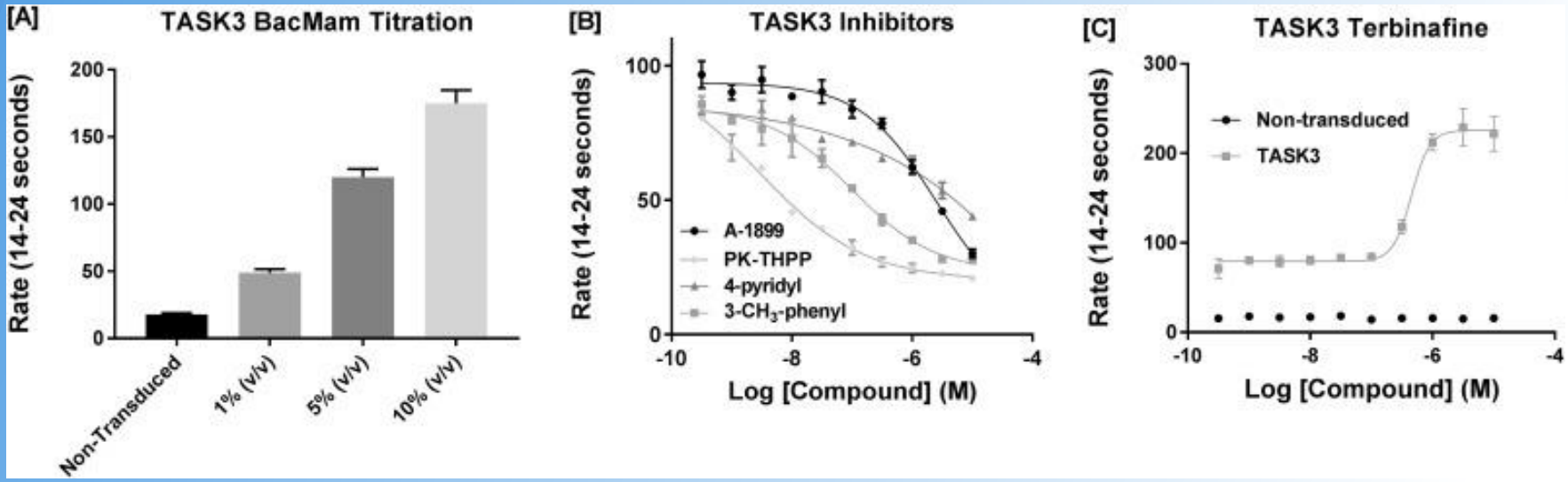
24 (80%) have G236R mutation on KCNK9

6 (20%) have a different mutation on KCNK9 .

For these other mutations, current is not reduced nor does it show inwardly rectification. However, regulation of current is often altered.

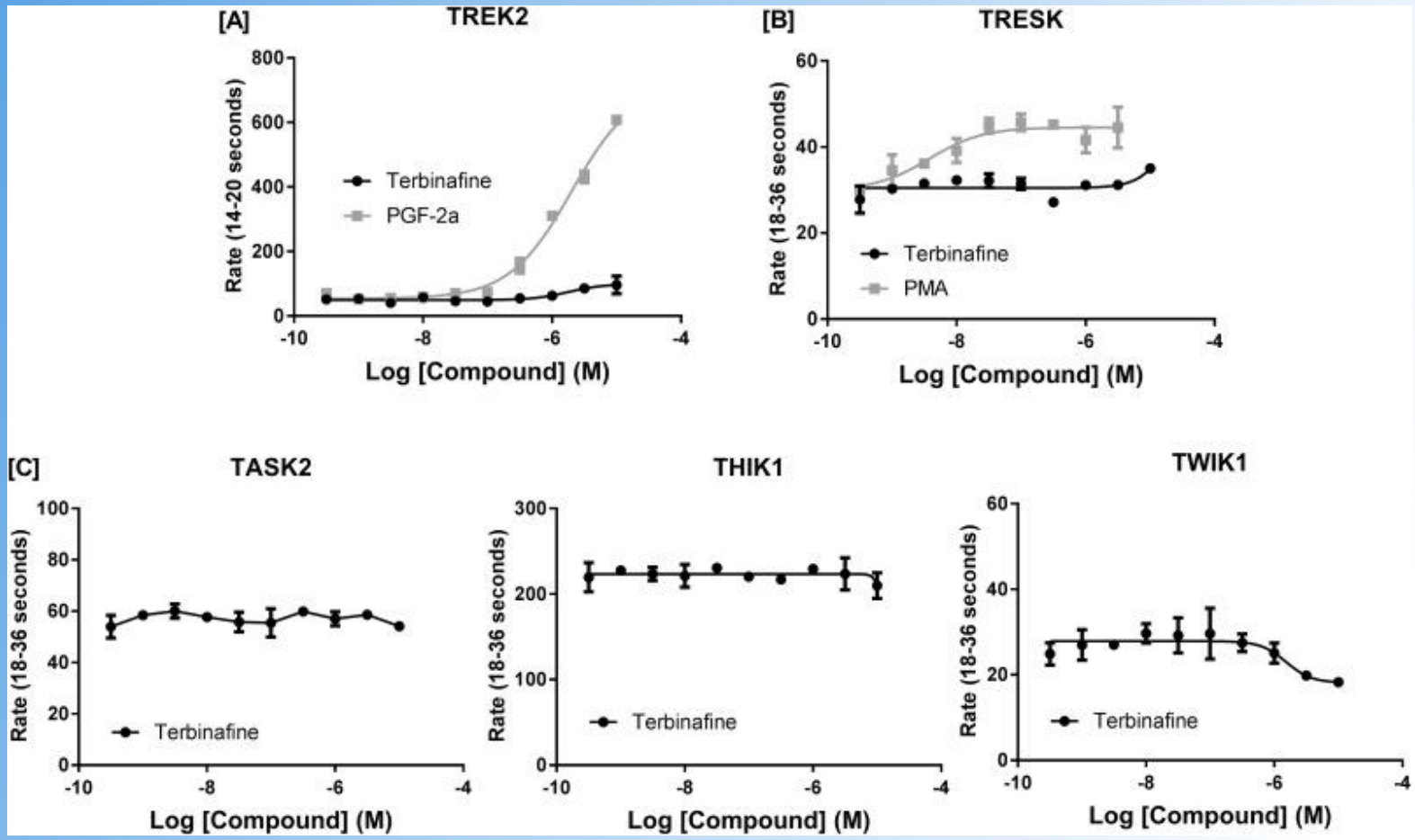
Veale, Graham et al unpublished

Terbinafine enhances Thallium flux through TASK3 channels



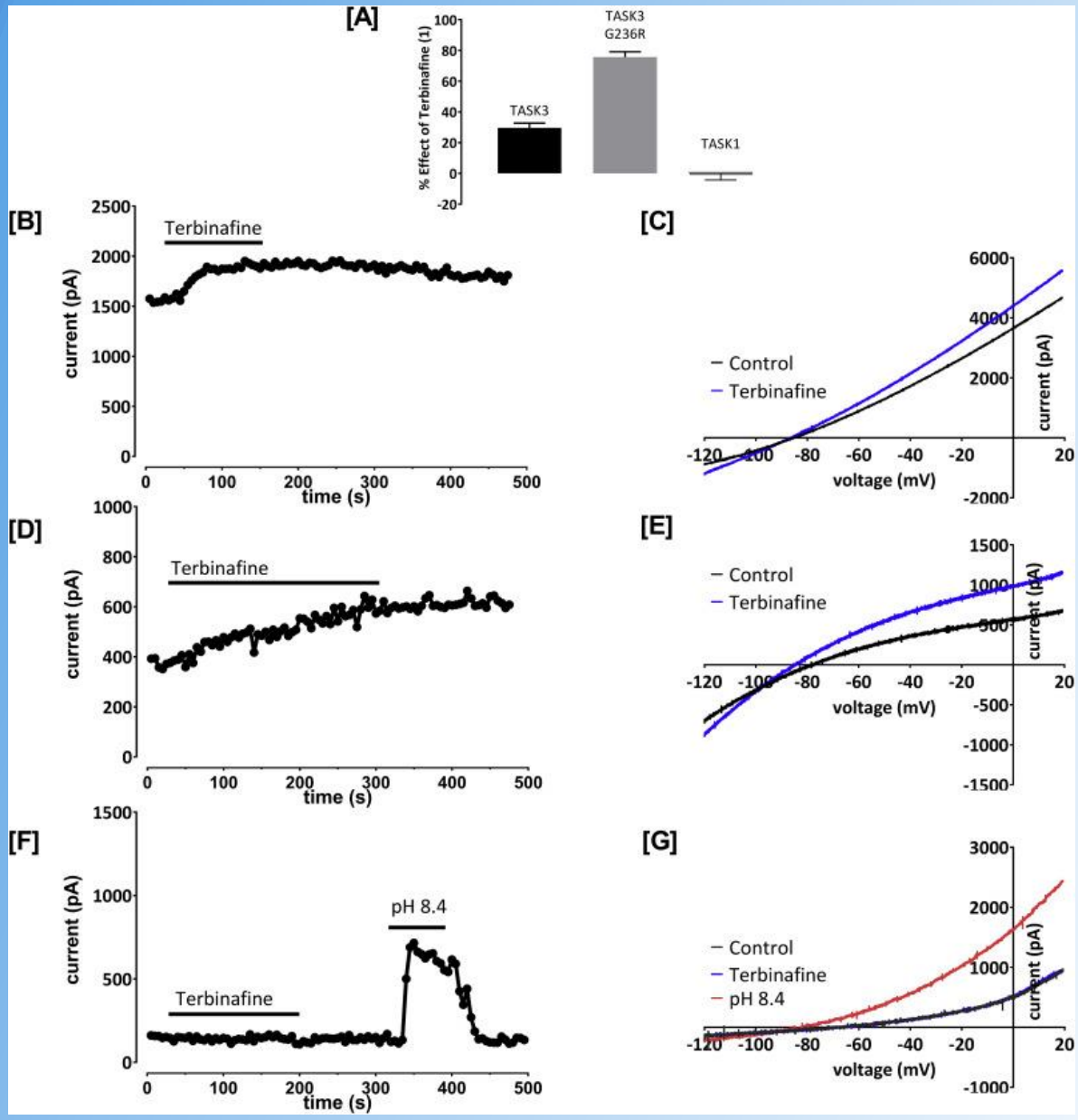
Wright et al 2017 BBRC

Terbinafine does not alter Thallium flux through other K2P channels



Wright et al 2017 BBRC

Terbinafine enhances TASK3 current



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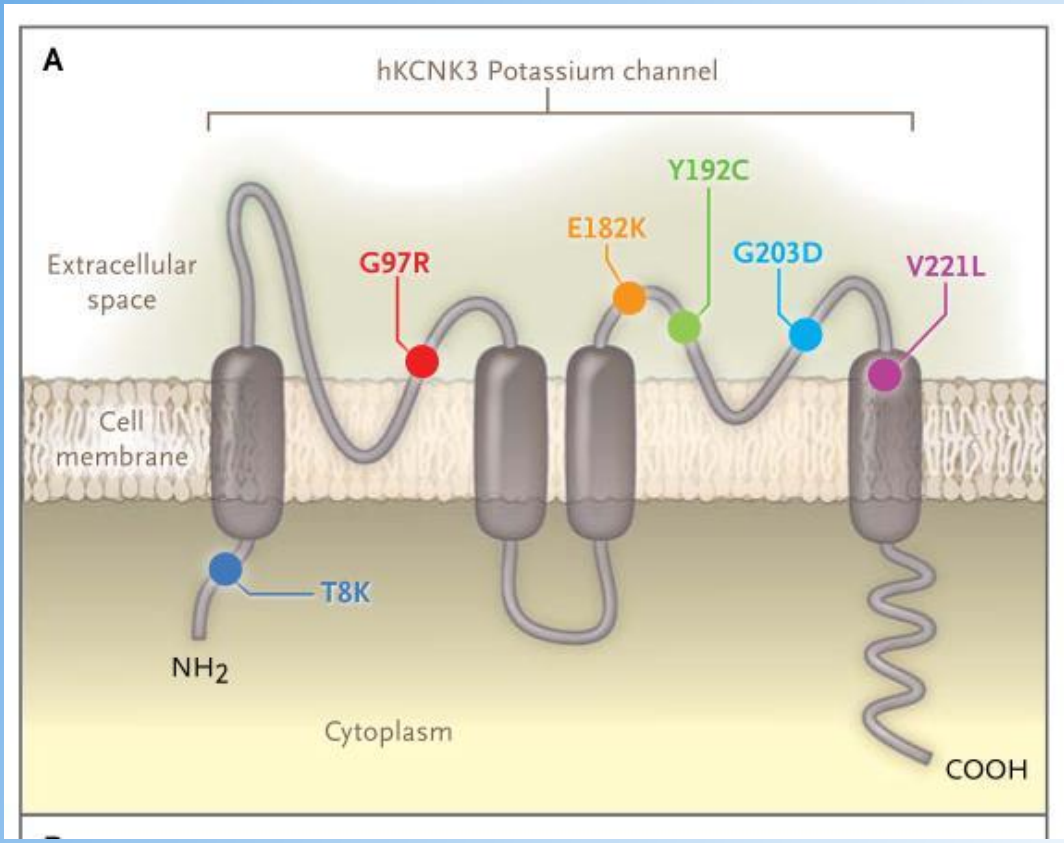
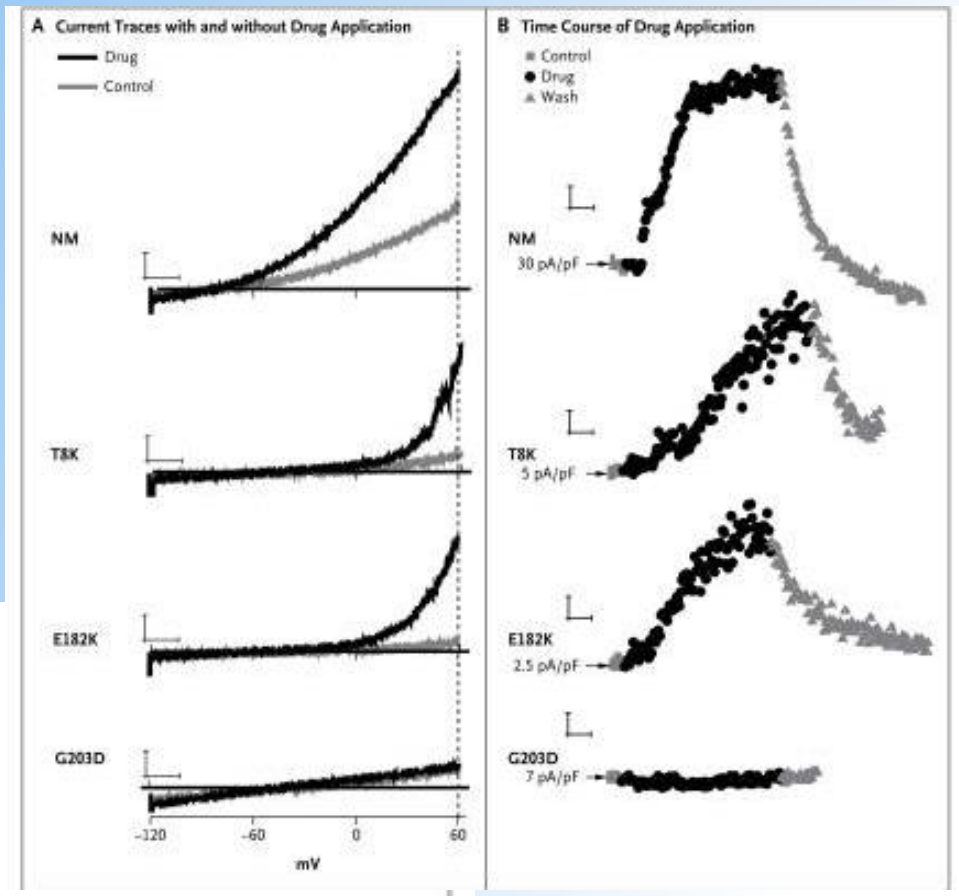
KCNK3 mutations in patients with pulmonary hypertension

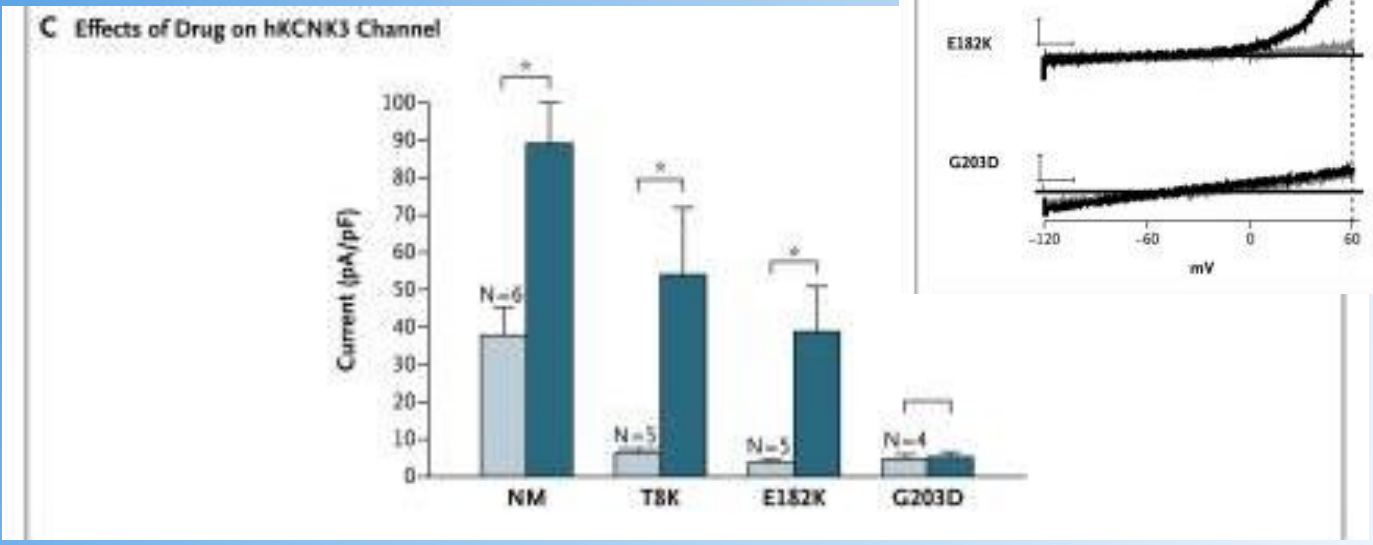


Ma et al (2013) A novel channelopathy in pulmonary arterial hypertension. *N Engl J Med* **369:** 351-361

Pharmacological recovery of KCNK3 function in mutated channels

In WT TASK1 channels and some mutated channels, current can be enhanced pharmacologically (by ONO-RS-082, 10 µM).





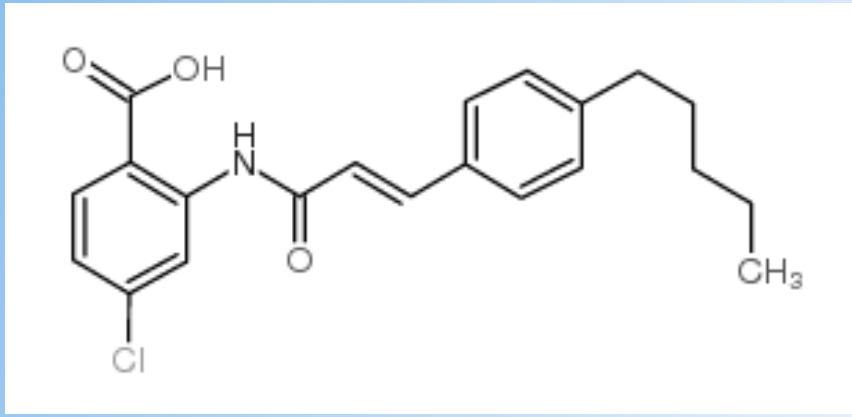
Ma et al (2016) NEJM

ONO-RS-082

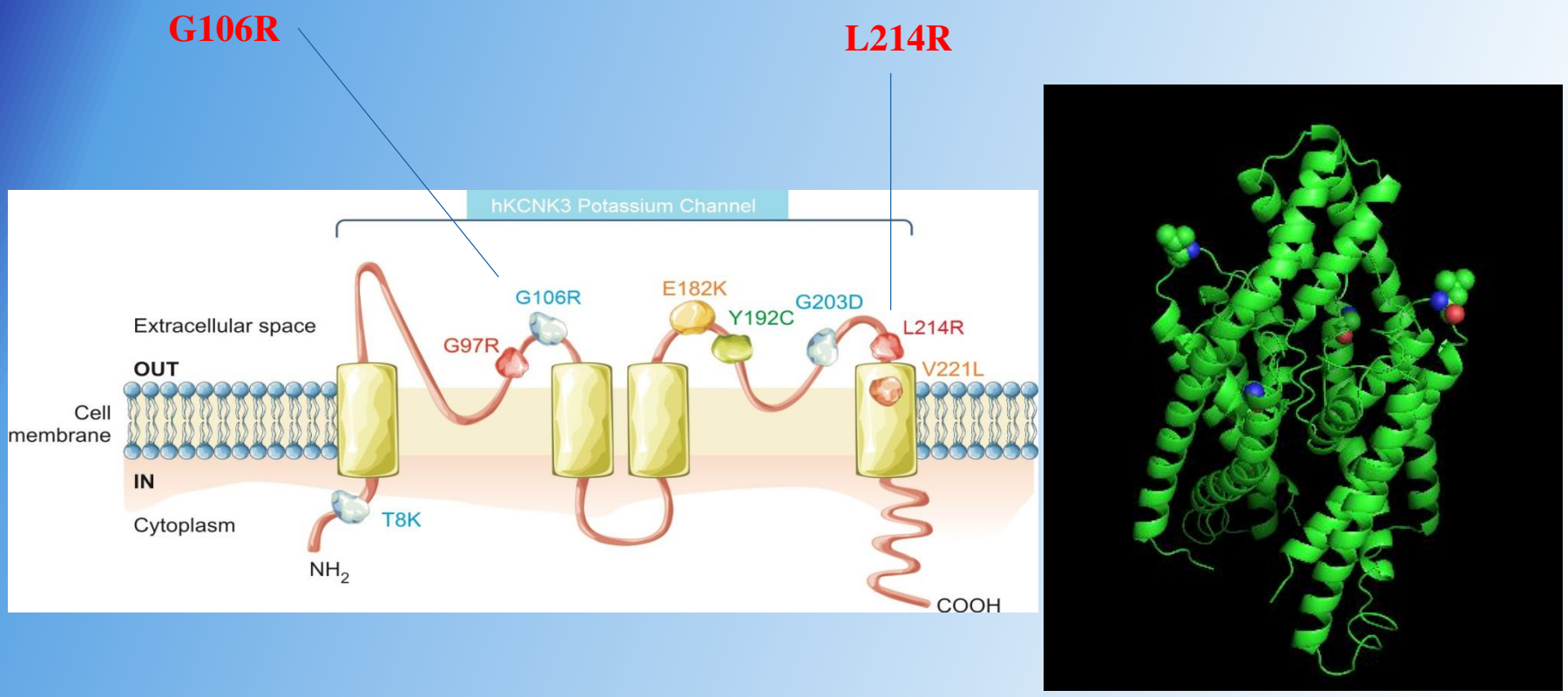
Sold as a phospholipase A2 inhibitor

IUPAC name:

4-Chloro-2-[[(*E*)-3-(4-pentylphenyl)prop-2-enoyl]amino]benzoic acid

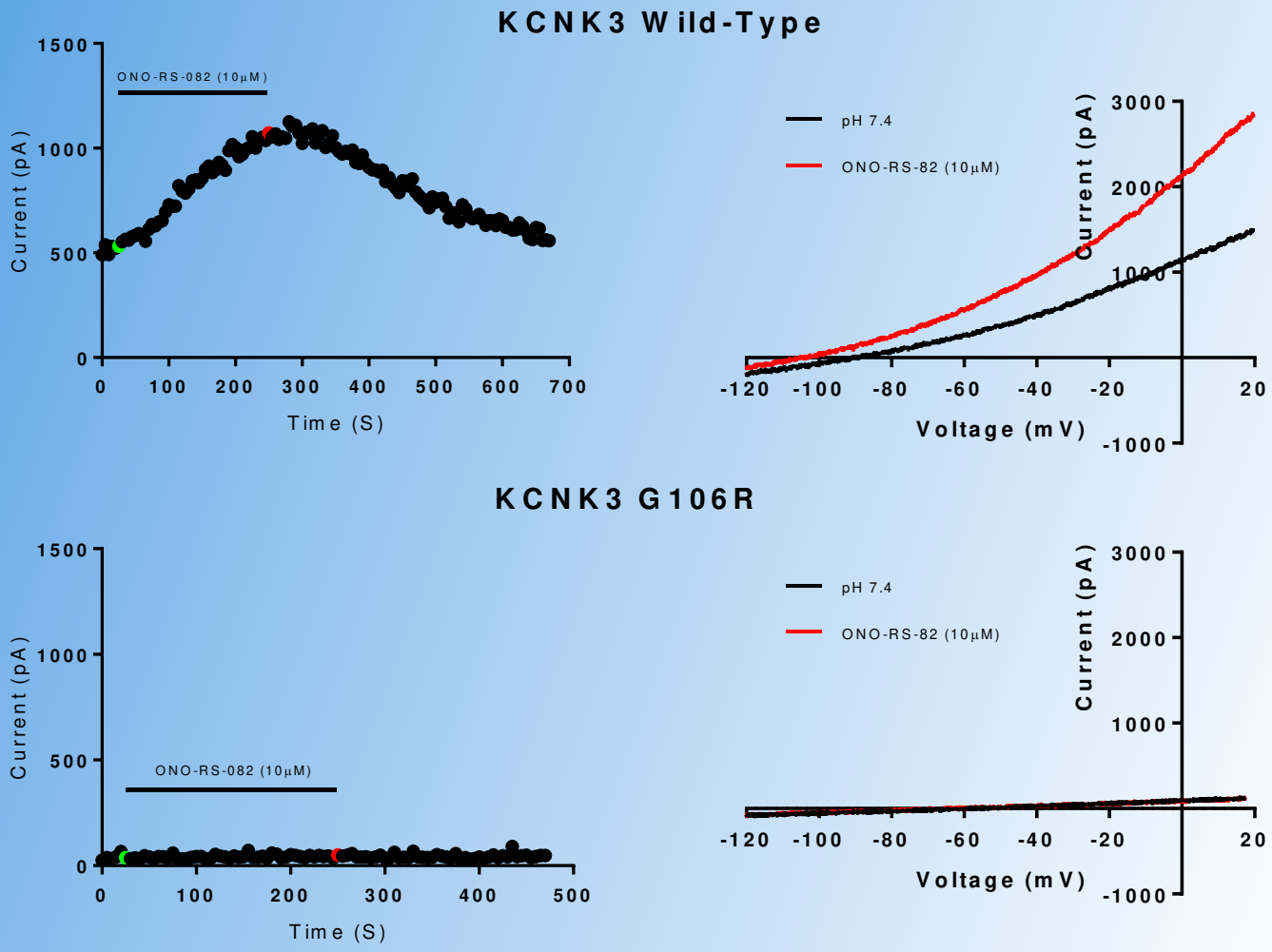


New KCNK3 mutations in Pulmonary Hypertension

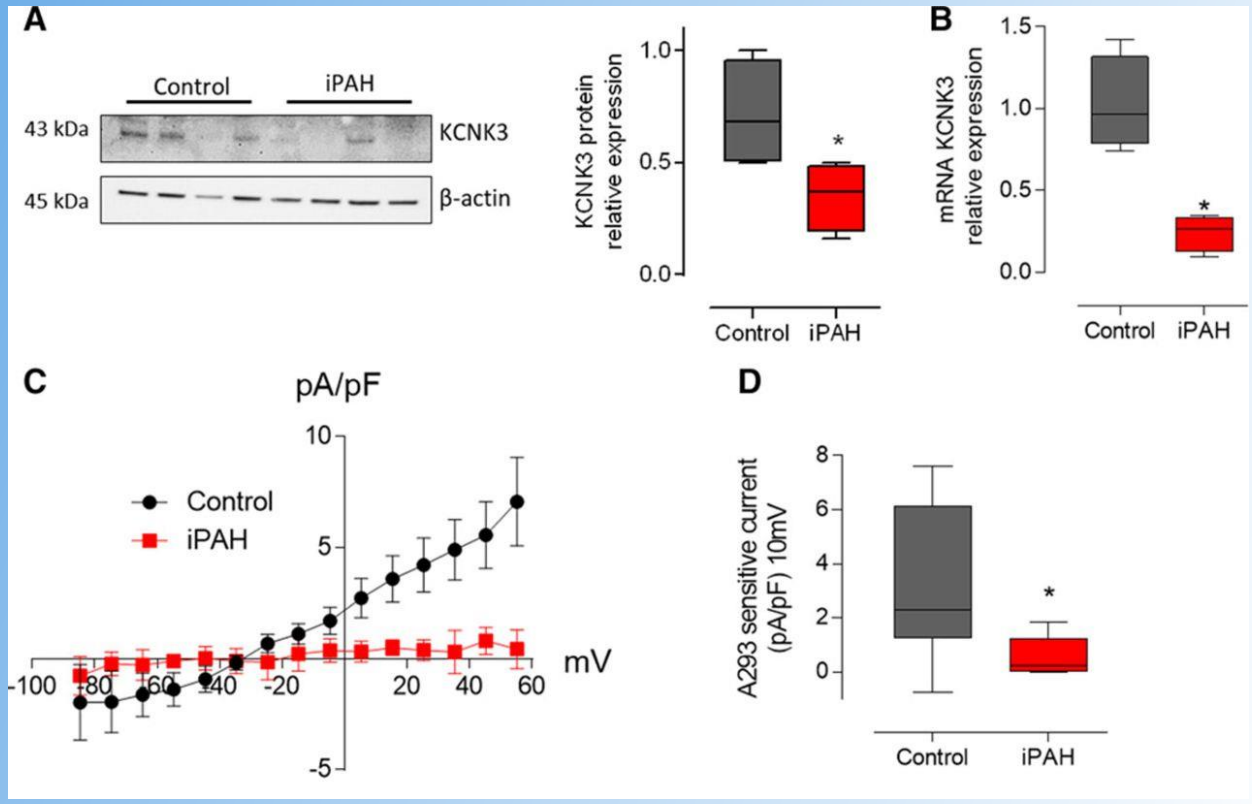


Navas Tejedor P, Tenorio Castaño J, Palomino Doza J, Arias P, Gordo G, López Meseguer M, Román Broto A, Lapunzina P, Escribano Subías P. (2016)
An homozygous mutation in KCNK3 is associated with an aggressive form of hereditary pulmonary hypertension.
Clin Genet. 2016 Sep 20. doi: 10.1111/cge.12869.

No pharmacological recovery of KCNK3 current in newly identified, mutated channels



Pulmonary expression of potassium channel subfamily K member 3 (KCNK3) is reduced in patients with pulmonary arterial (PA) hypertension (PAH).

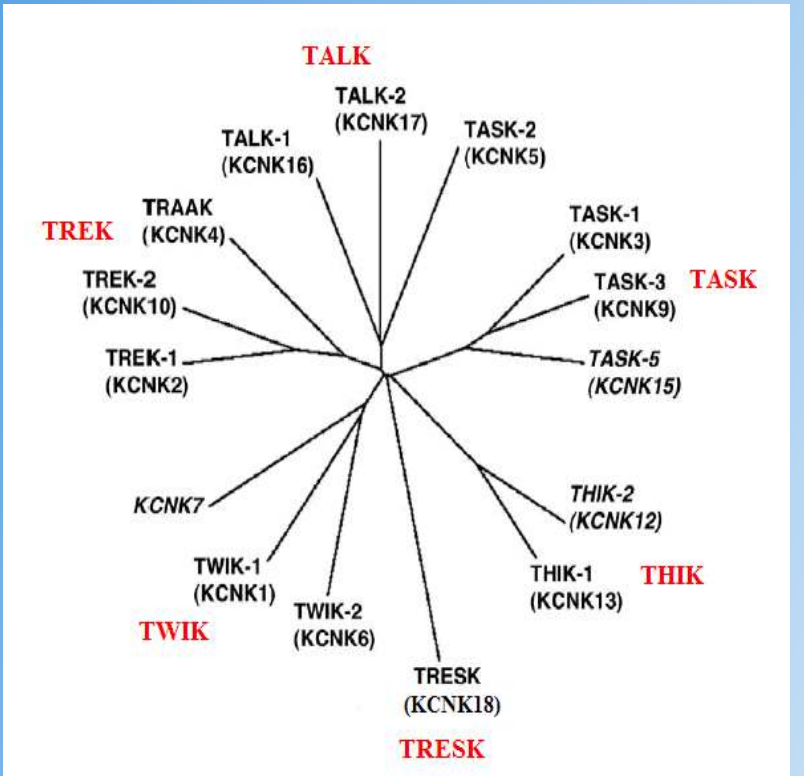


Loss of KCNK3 function is a key event in the pathogenesis of both heritable AND idiopathic pulmonary hypertension.

Antigny et al (2016) Circulation 133:1371

Therapeutic targeting of K2P channels in pain

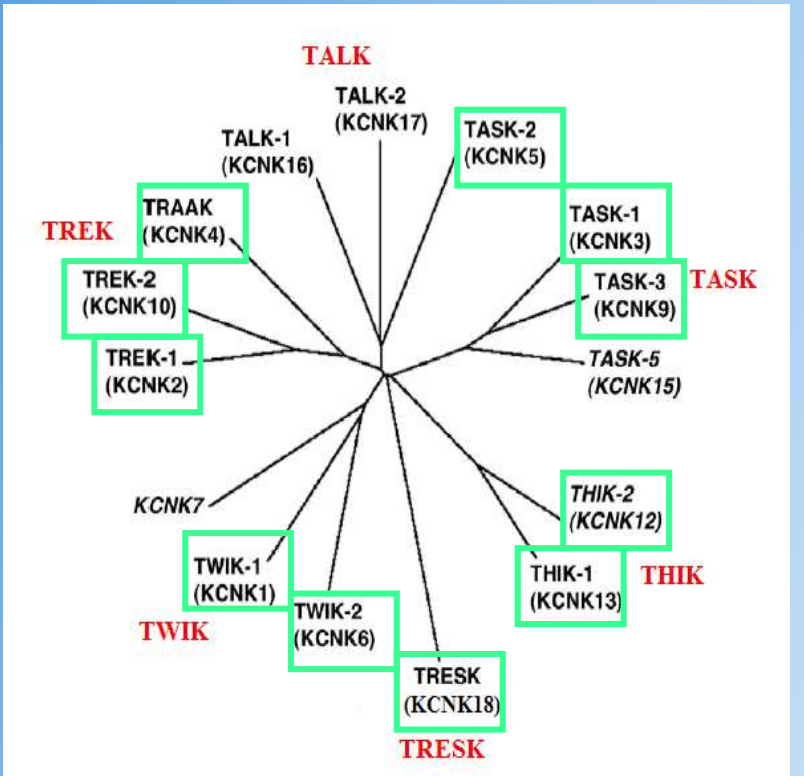
K2P channels in sensory neurons



There is evidence for expression of many different K2P channel subunits in nociceptive dorsal root ganglion neurons and trigeminal ganglion neurons.

Mathie & Veale 2015 Pflugers Archiv 467: 931

K2P channels in sensory neurons

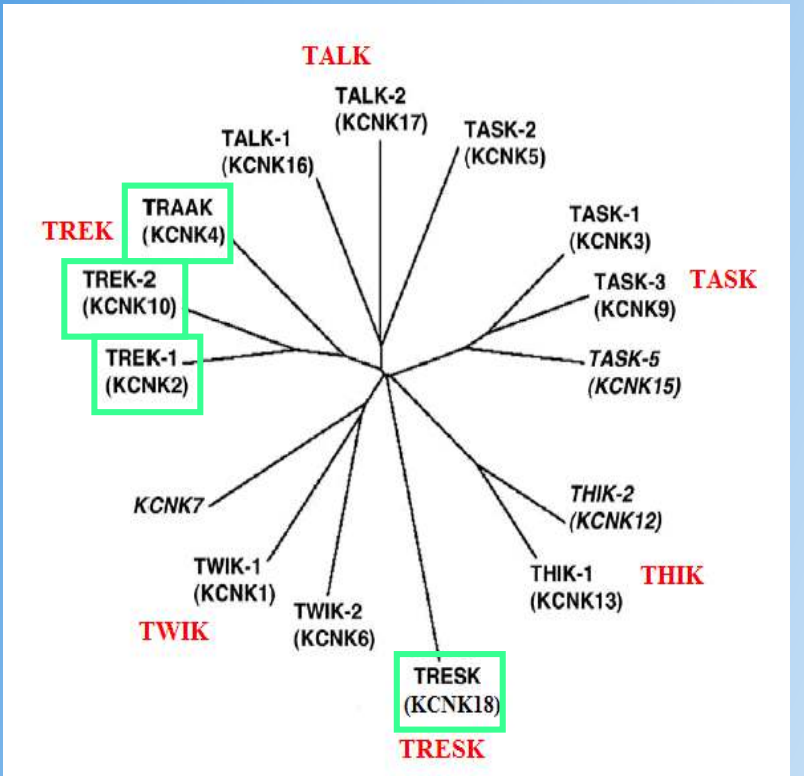


There is evidence for expression of many different K2P channel subunits in nociceptive dorsal root ganglion neurons and trigeminal ganglion neurons.

These include TREK1, TREK2, TRAAK, TRESK, TASK1, TASK3, TASK2, TWIK1, TWIK2, THIK1 and THIK2

Mathie & Veale 2015 Pflugers Archiv 467: 931

K2P channels in sensory neurons

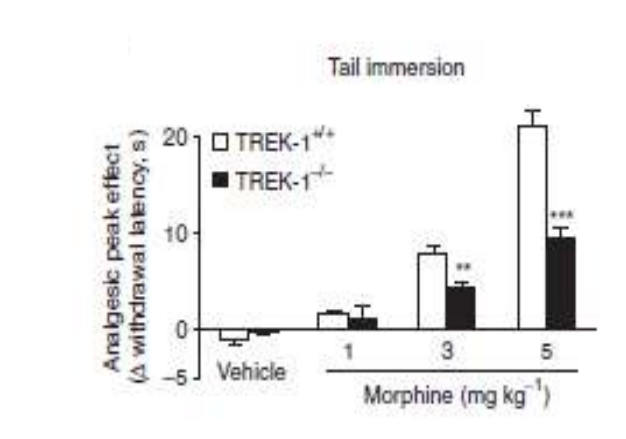


Of these, the strongest body of evidence from functional studies highlights the importance of TREK1, TRESK, TRAAK and recently, TREK2 channels.

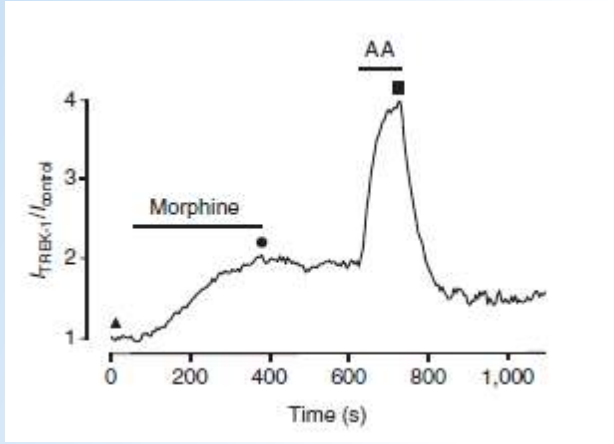
Mathie & Veale 2015 Pflugers Archiv 467: 931

TREK1 channels in pain

TREK1 knock out mice are more sensitive than wild type mice to a number of painful stimuli but less sensitive to morphine-induced analgesia.



Morphine stimulates TREK1 current

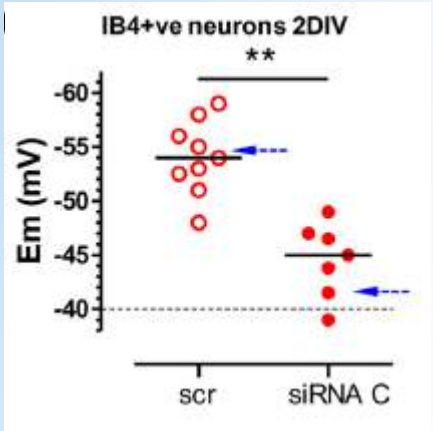


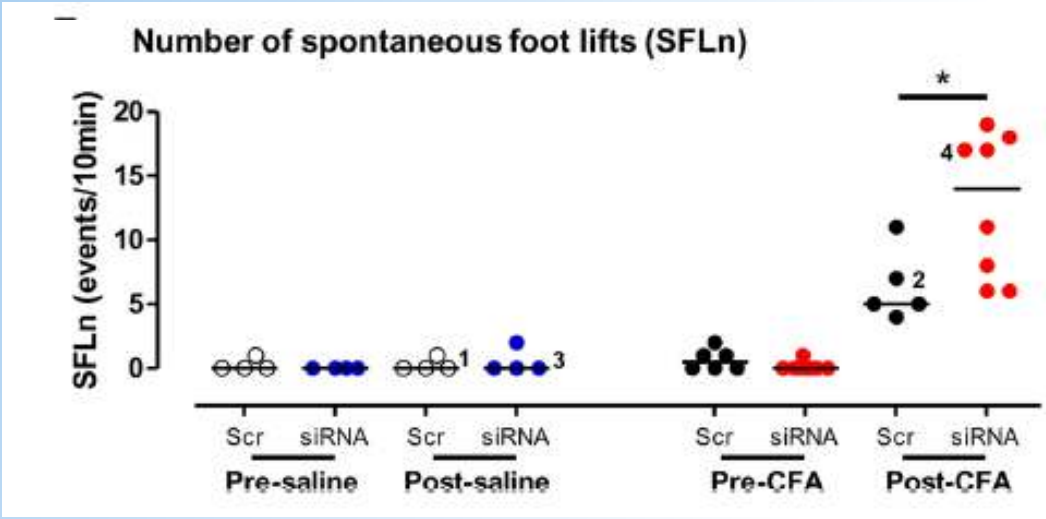
Devilliers et al. Nature Comm. 2013 4:2941

TREK2 channels in pain

TREK2 channels contribute to the resting membrane potential of IB4 binding C nociceptors.

TREK2 knock down animals are more sensitive in models of neuropathic pain



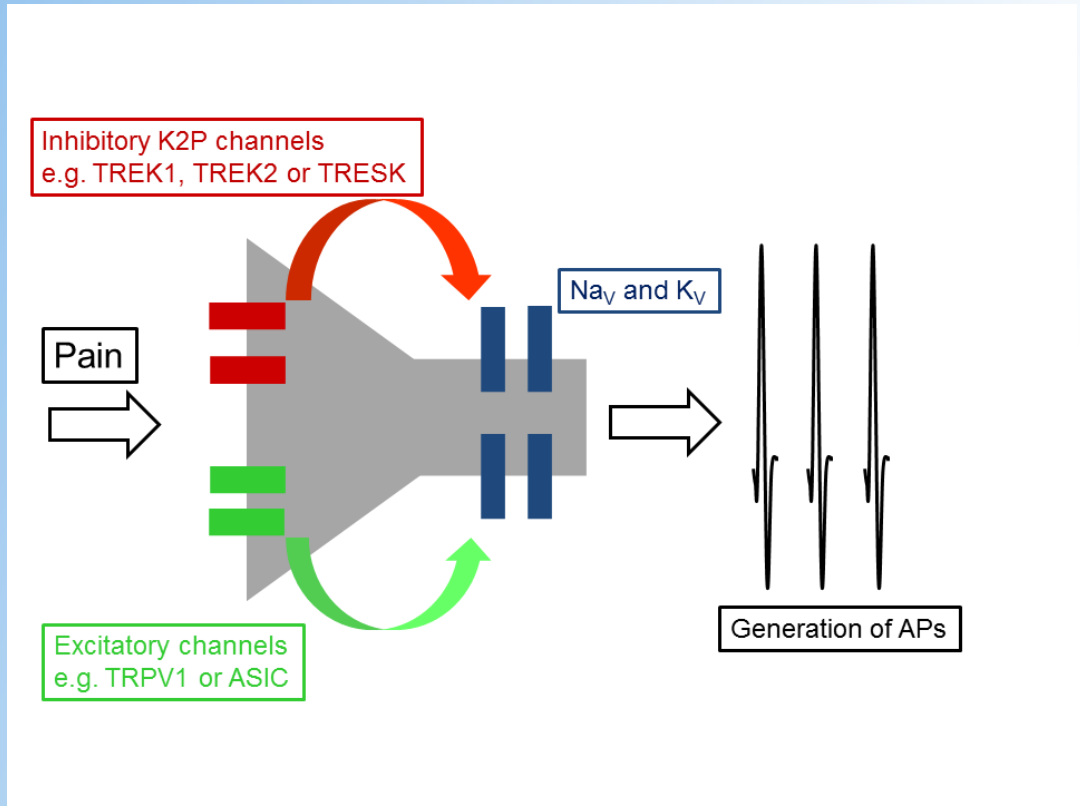


Acosta et al. J. Neurosci. 2014;34:1494

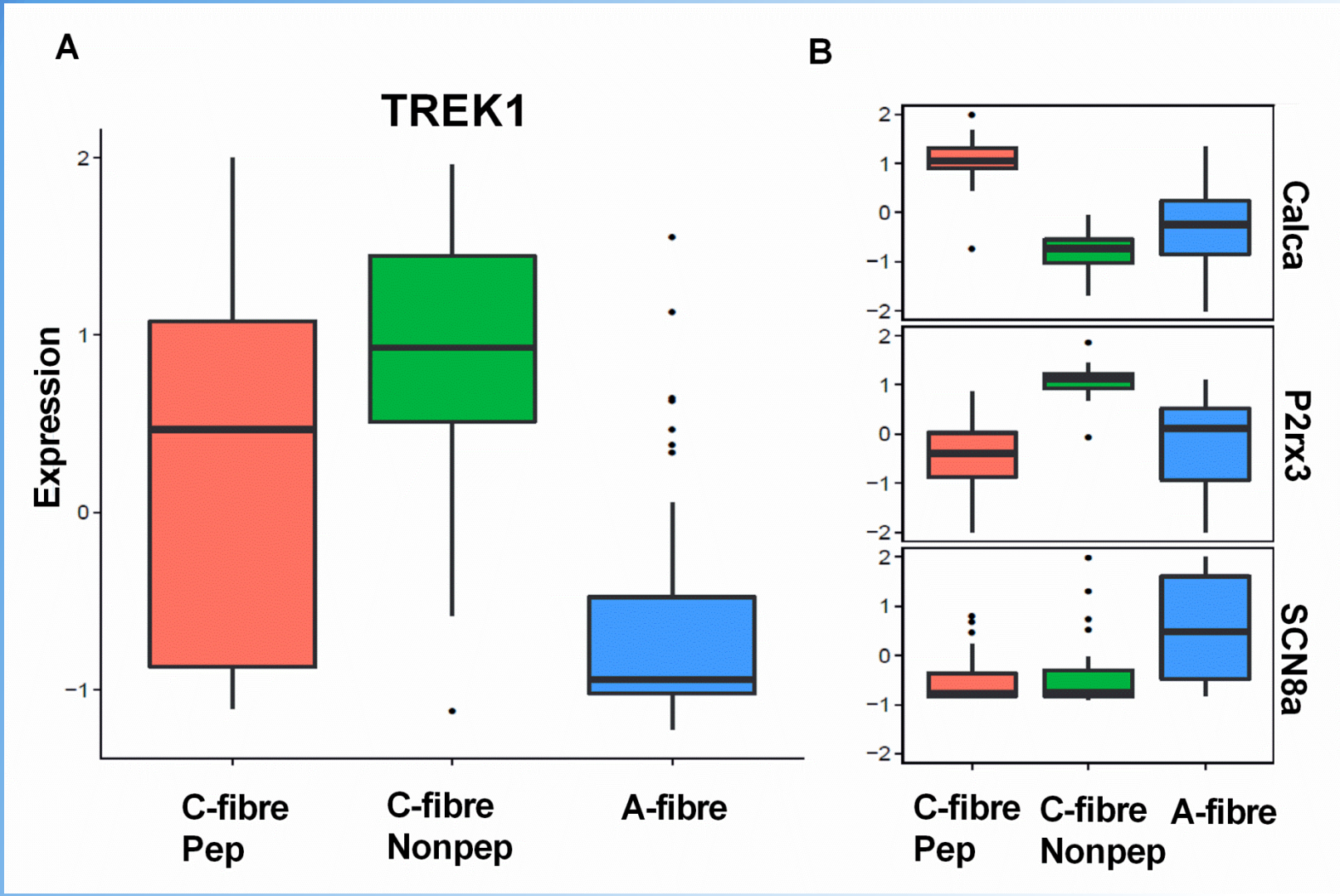
Therapeutic targeting of K2P channels in pain

Therapeutic strategy:

Enhance the activity of K2P channels to dampen excitability?



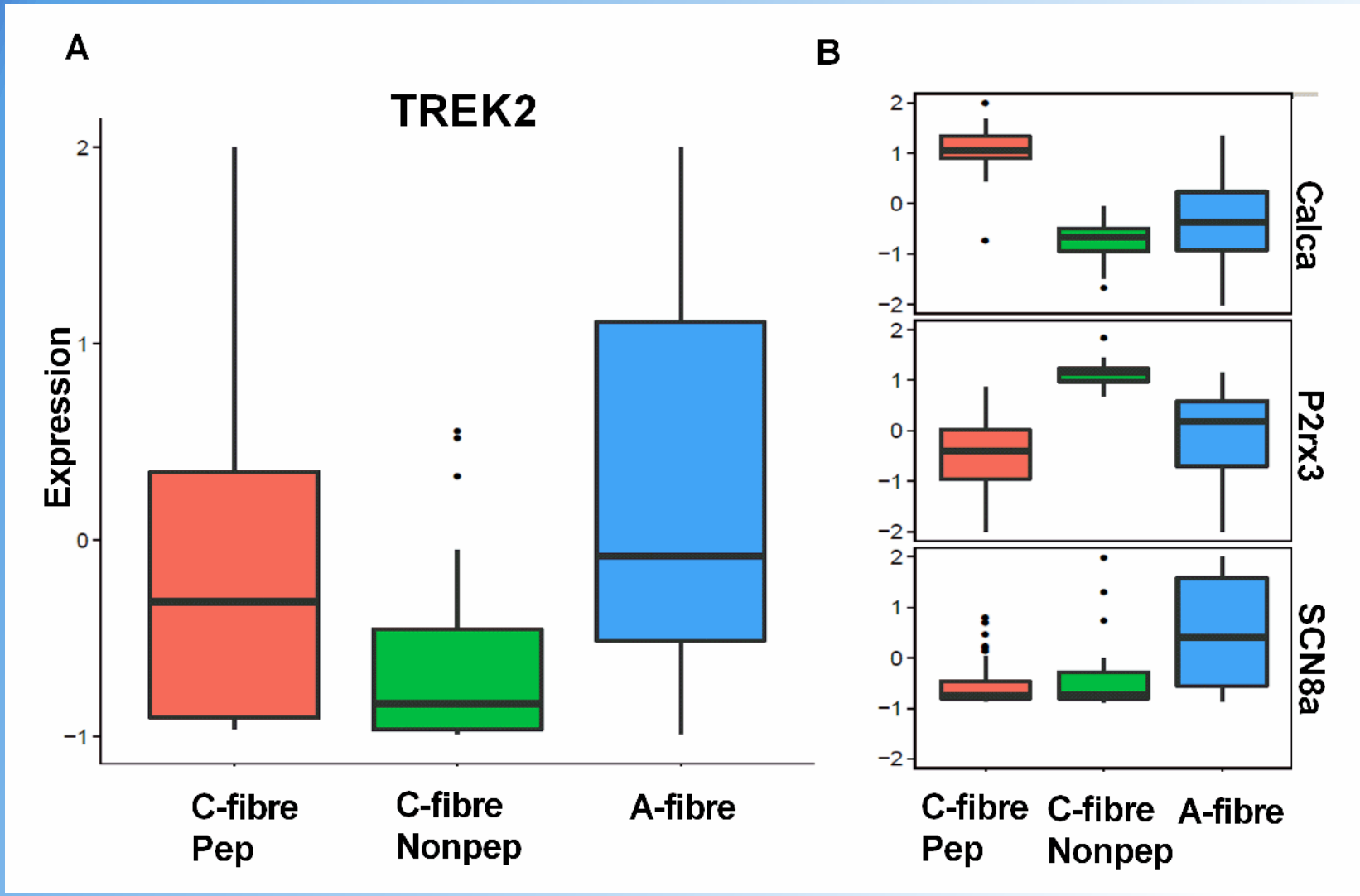
TREK1 expression in single DRG neurons



Single cell RNA sequencing from 120 rat DRG neurons

Loucif et al 2017 under review

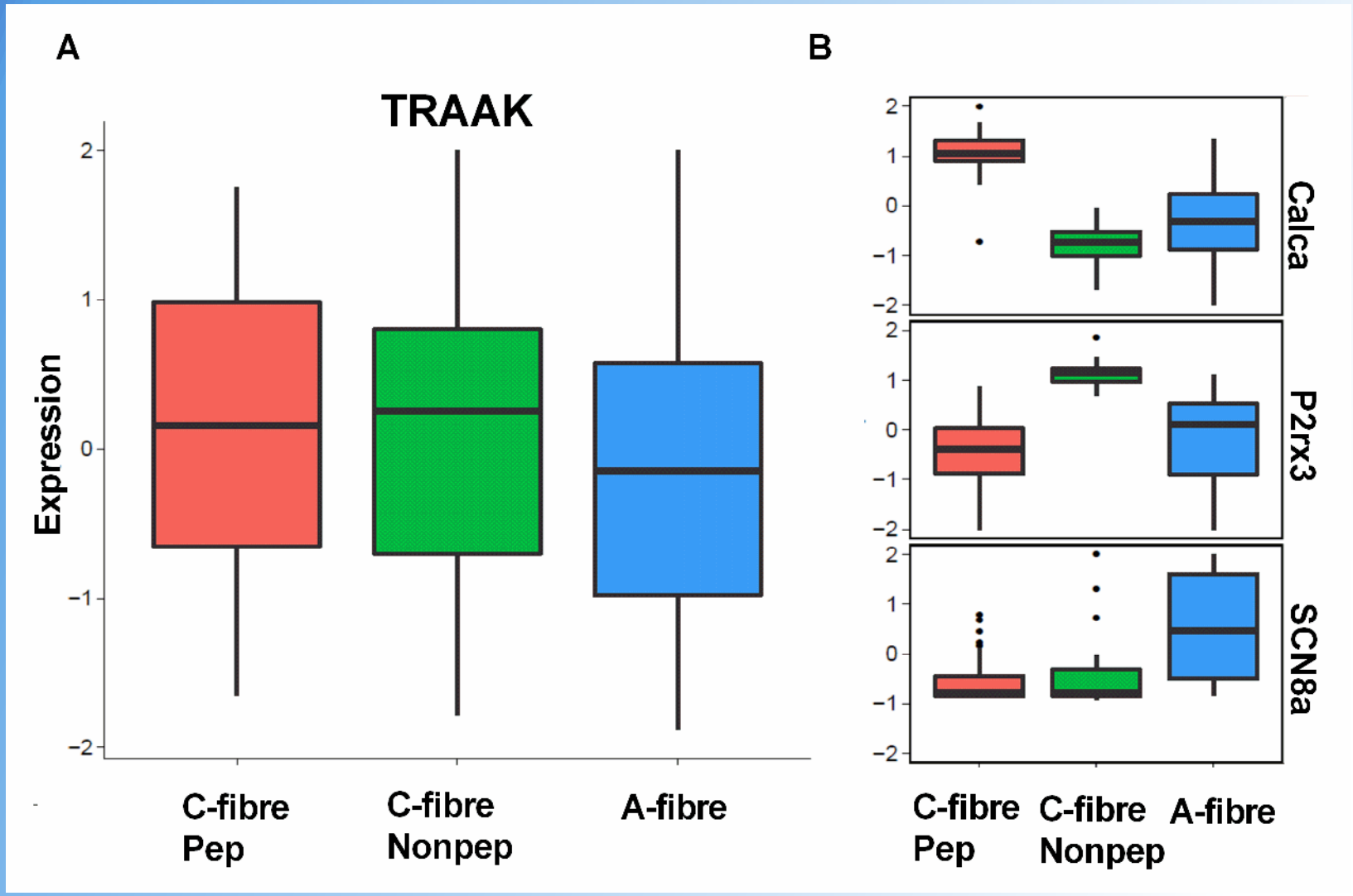
TREK2 expression in single DRG neurons



Single cell RNA sequencing from 120 rat DRG neurons

Loucif et al 2017 under review

TRAAK expression in single DRG neurons



Single cell RNA sequencing from 120 rat DRG neurons

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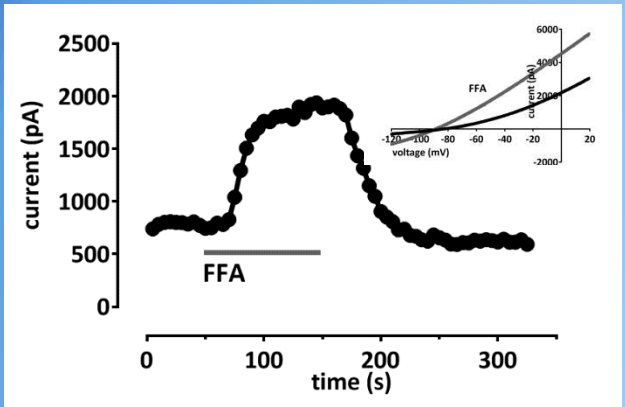
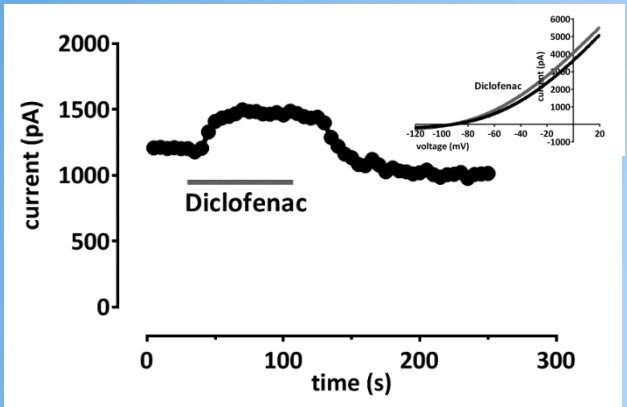
Enhancers of TREK1 channel activity

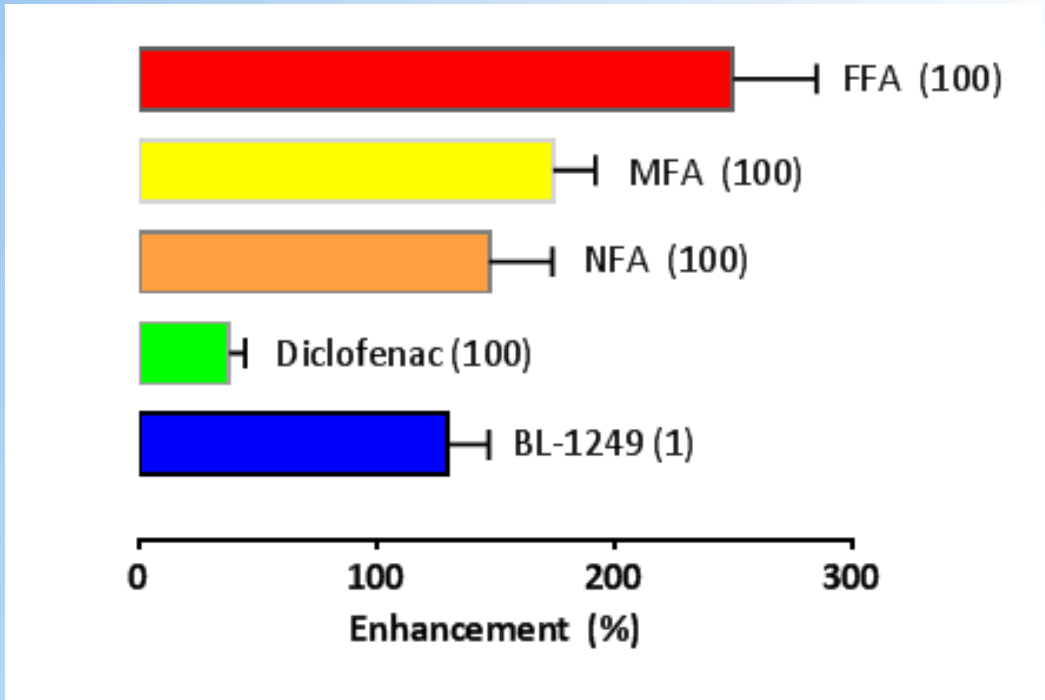
Compound	Enhancement of recombinant TREK1 channels	Other information	Reference
Riluzole	100 μM gives ~2 fold increase	Transient enhancement followed by inhibition through activation of PKA. Also enhances TRAAK	Duprat et al 2000
DEPC	0.1% gives ~3 fold increase (at pH 7.4)	Stops H ions binding to H126 and inhibiting current	Sandoz et al 2009 Veale et al 2010
CAPE	40 μM gives ~6 fold increase	Acts extracellulary	Danthi et al 2004
CDC	10 μM gives ~6 fold increase 20 μM gives 2.64 fold increase	Acts extracellularly	Danthi et al 2004 Rodrigues et al 2014
Compound 12U (CDC analogue)	20 μM gives 2.87 fold increase		Rodrigues et al 2014
Flufenamic Acid	100 μM gives ~3-4 fold increase	Other fenamates (such as niflumic acid, mefenamic acid) activate TREK1 but are less potent. Also enhances TREK2 and TRAAK	Takahira et al 2005 Veale et al 2014
BL-1249	EC_{50} of 1 μM which gives ~2 fold increase Maximum ~4 fold increase (100 mM)		Cao et al 2010 Veale et al 2014
ML67-33	EC_{50} of 36 μM in oocytes or 10 μM in HEK293 cells, which gives ~7 fold increase. Maximum ~11 fold increase (100 μM)	Most potent of a series of dihydroacridine analogues Also enhances TREK2 and TRAAK	Bagriantsev et al 2013

Mathie & Veale 2015 Pflugers Archiv 467: 931

Enhancement of TREK current by fenamates

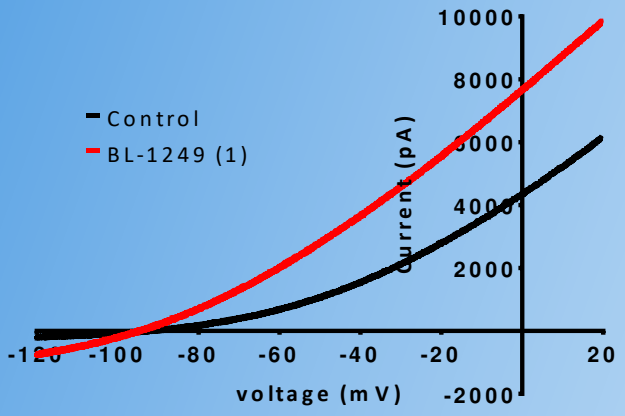


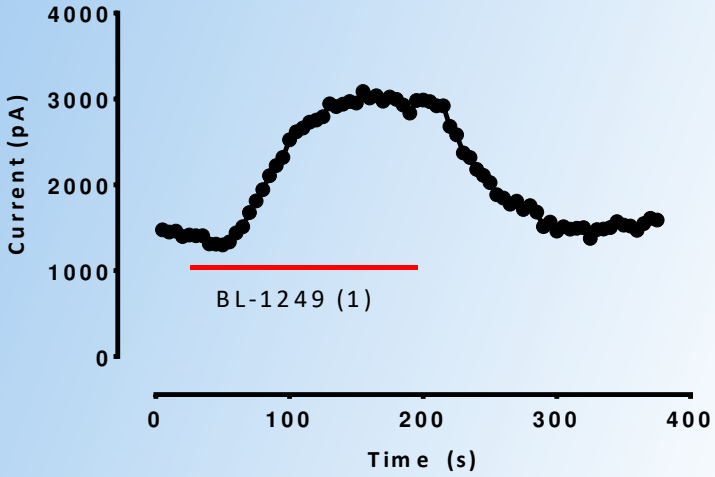




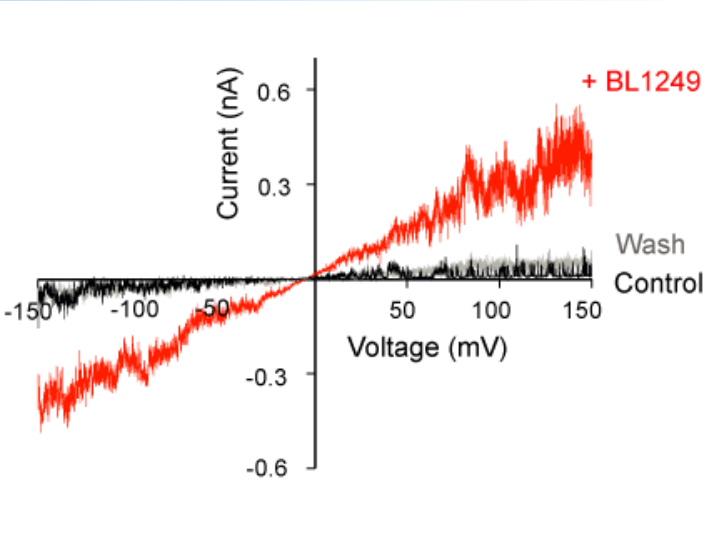
Veale et al (2014) Mol Pharm 85: 671-681

BL-1249 enhances TREK1 current



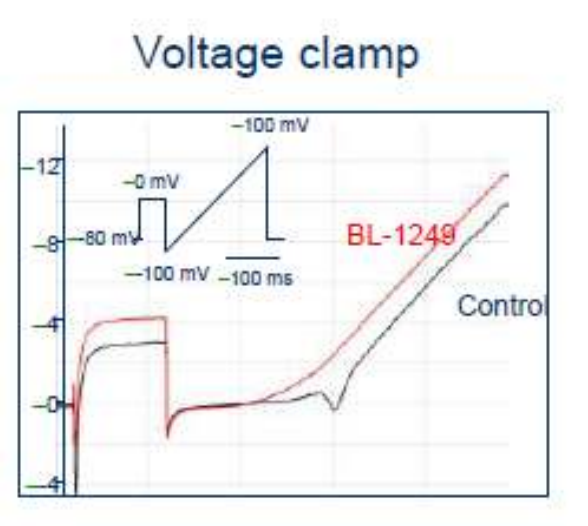


Purified TREK channels expressed in lipid bilayers retain sensitivity to BL1249

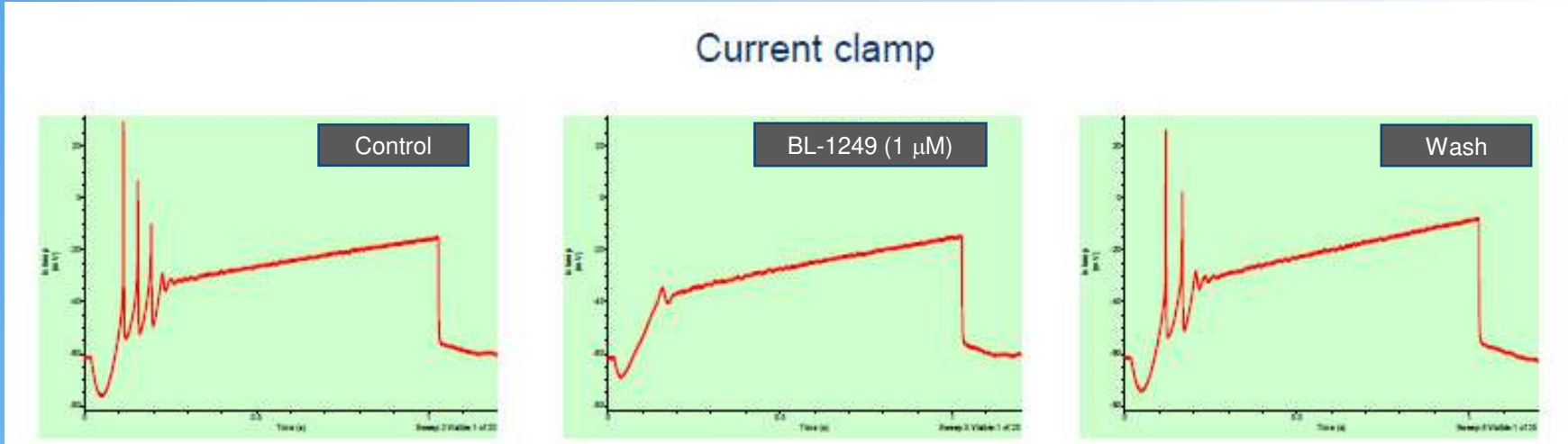


Experiments of Lishuang Cao
Published as supplement to Dong et al (2015) Science

BL-1249 dampens DRG excitability

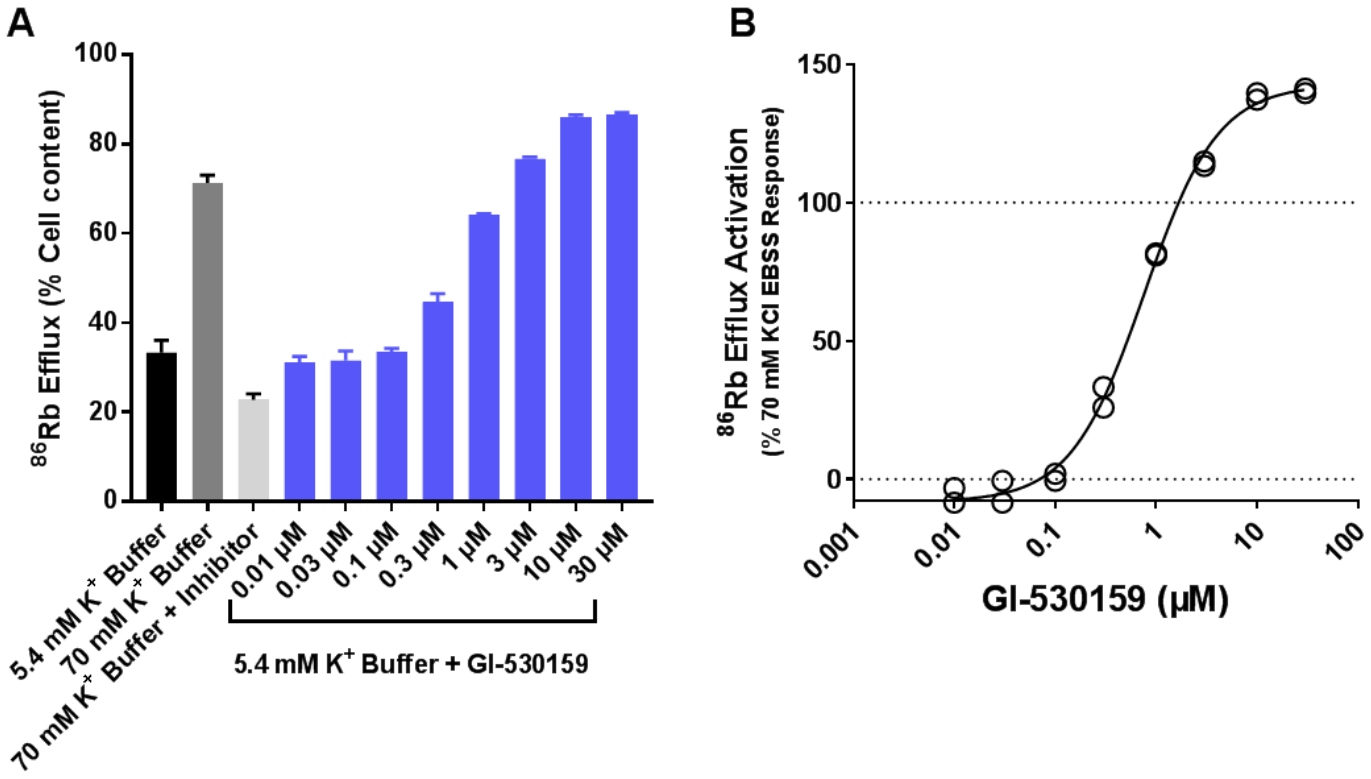


BL-1249 (1 μM) enhances outward current and inhibits action potential firing in dissociated rat dorsal root ganglion neurons



Cao et al unpublished

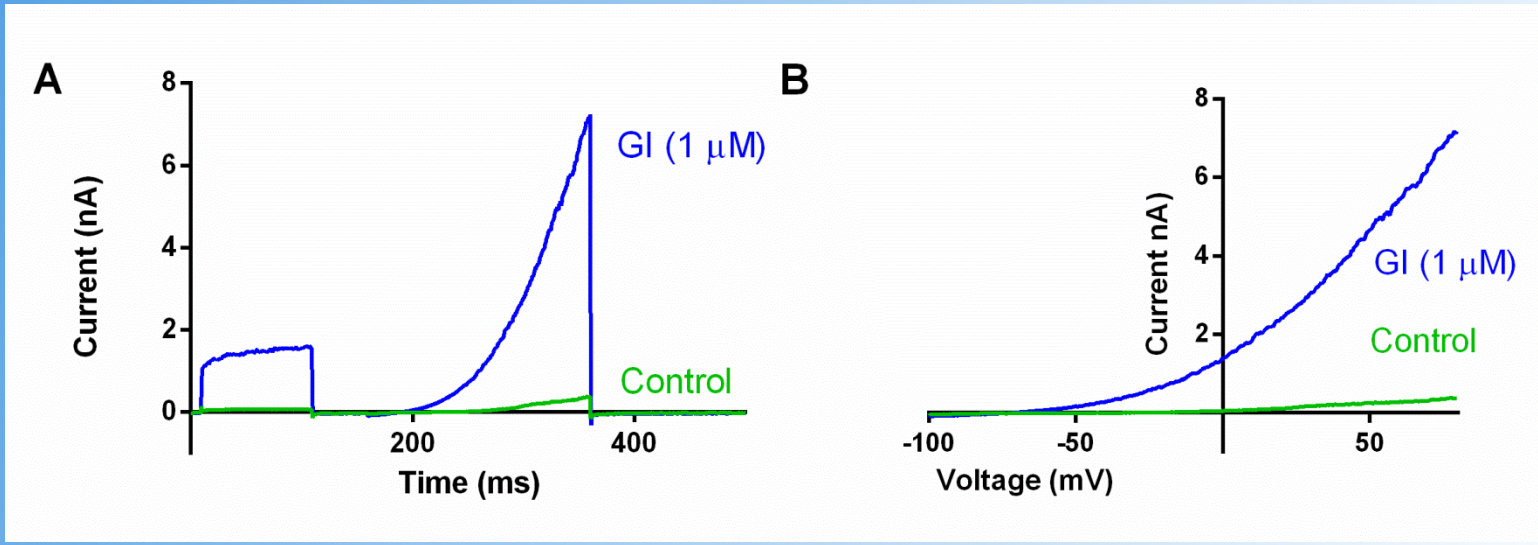
GI-530159 enhances Rb flux through TREK1 channels

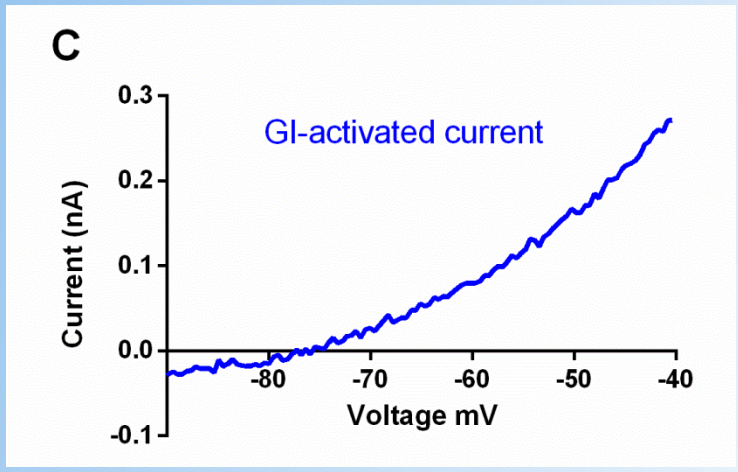


Loucif et al 2017 under review

GI-530159 enhances TREK1 current

in stably transfected HEK-293 cells





Loucif et al 2017 under review

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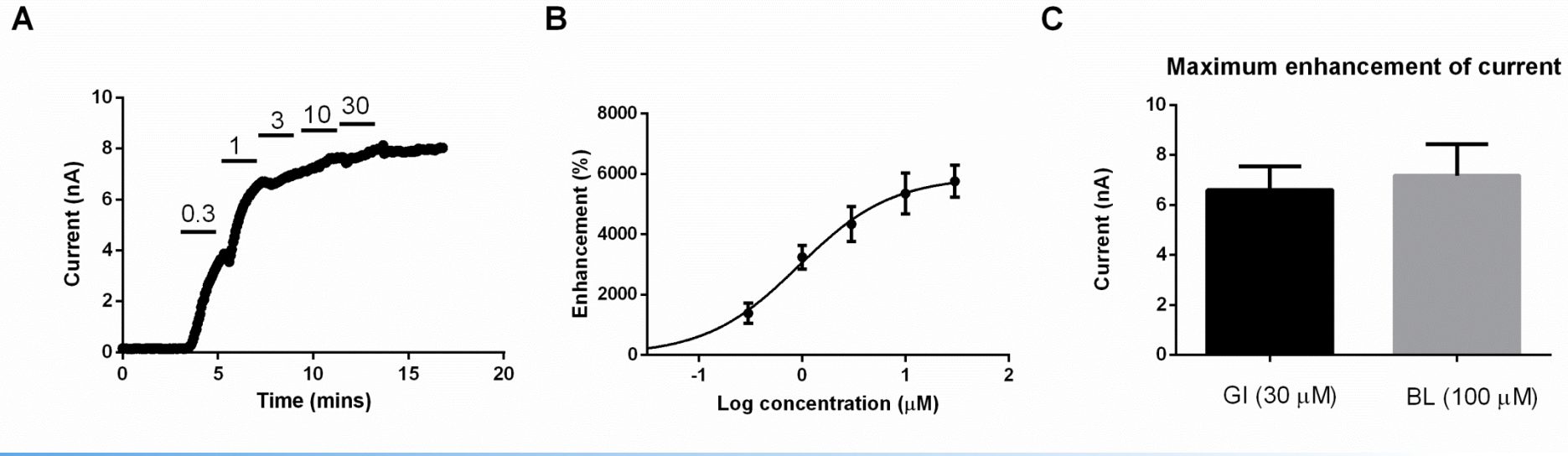


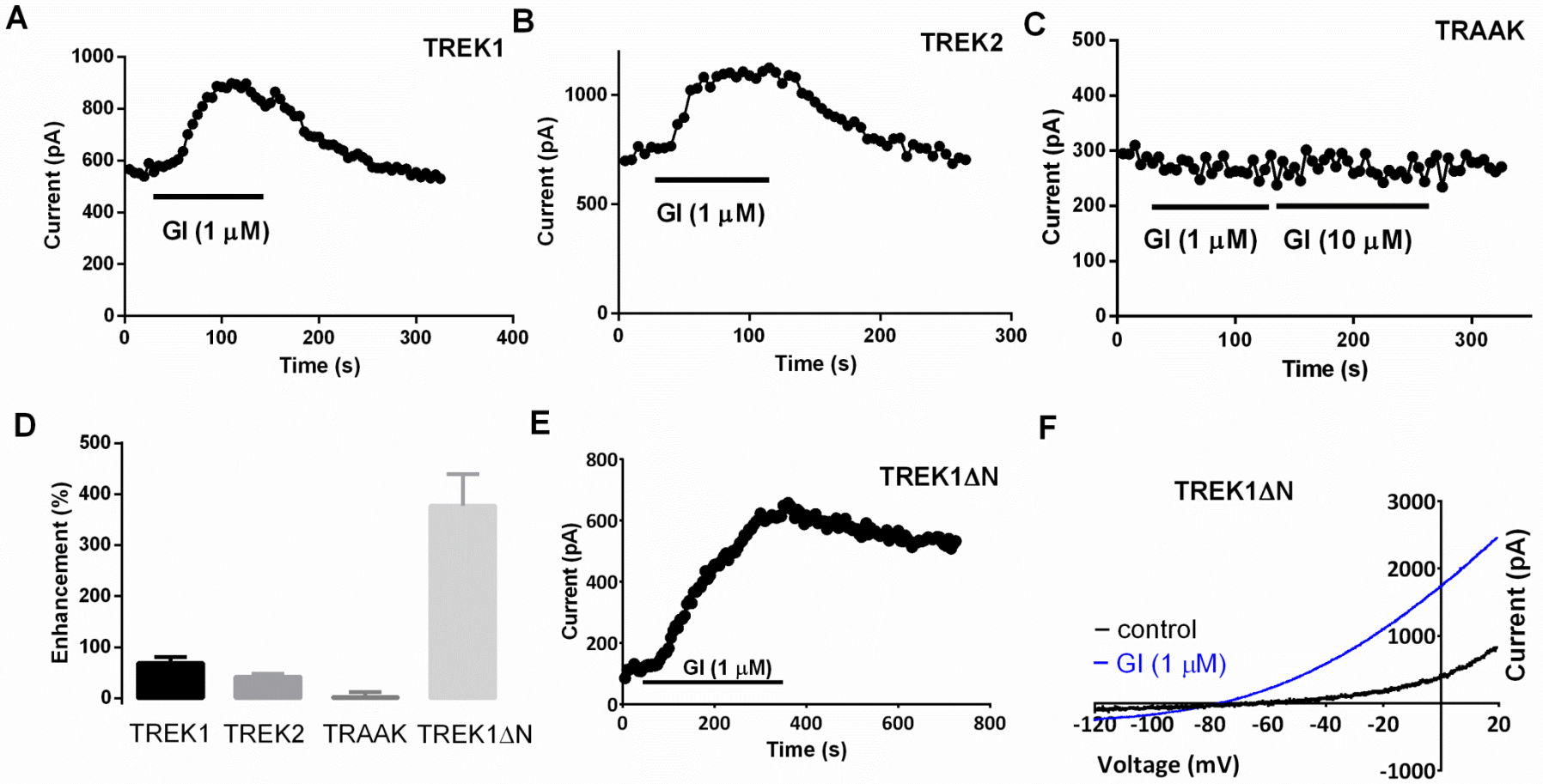
GI-530159 has no detectable effect on:

KCNQ1, KCNQ2, KCNQ3, BK, hERG
$Na_V1.2$, $Na_V1.7$, $Na_V1.8$
TRESK THIK1, TASK2, small activation of TASK3 channels

Loucif et al 2017 under review

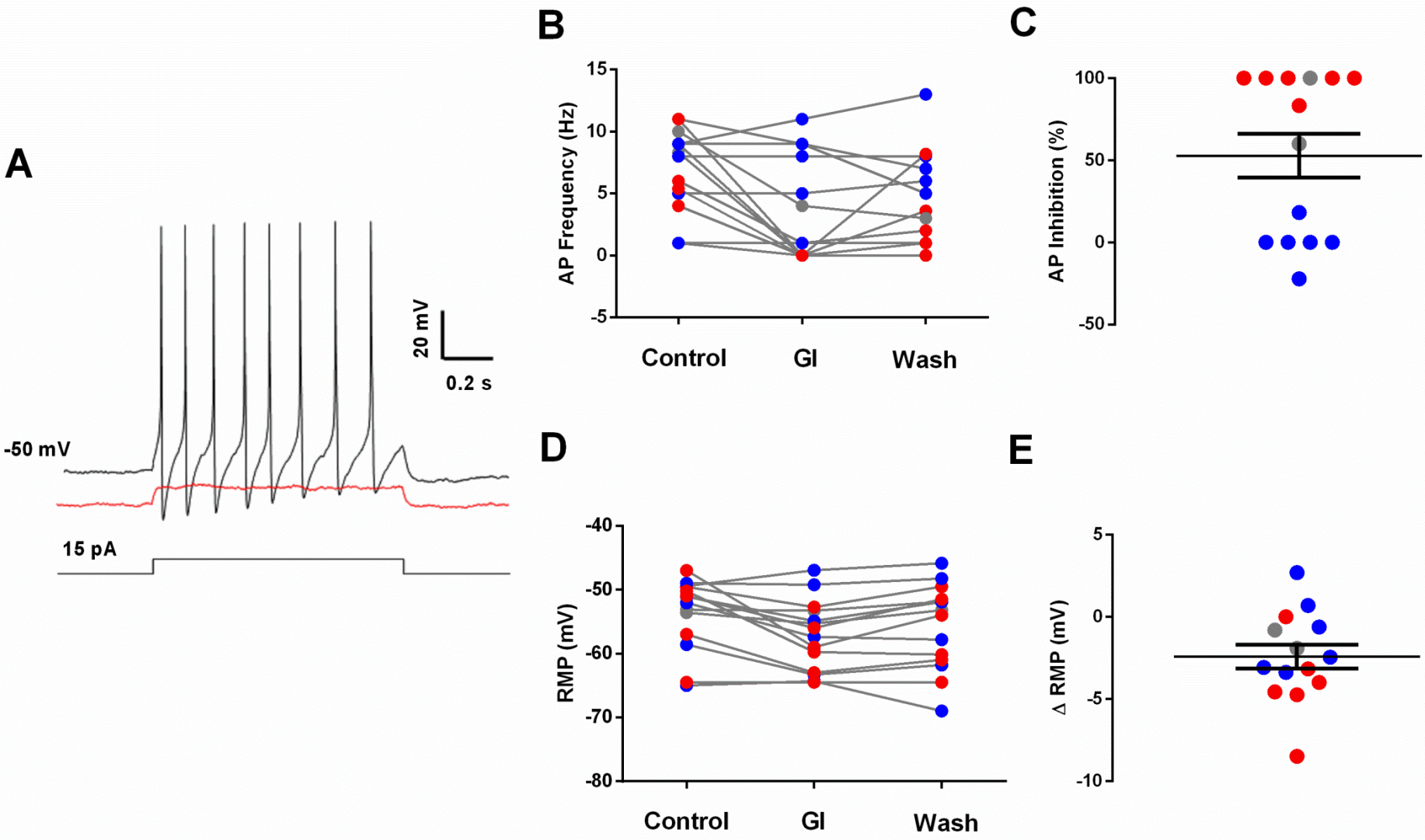
GI-530159 enhances TREK current

in transiently transfected tsA-201 cells



Loucif et al 2017 under review

GI-530159 decreases firing of DRG neurons



Loucif et al 2017 under review

Quantification and classification of K2P channel activators

- How do we best quantify activators of K2P channels?
  - EC_{50}? Fold increase in current? Relative to a standard compound?
- How do we deal with varying baseline (constitutively active) current across platforms
  - Flux assay versus patch-clamp? Mammalian cell versus oocyte?
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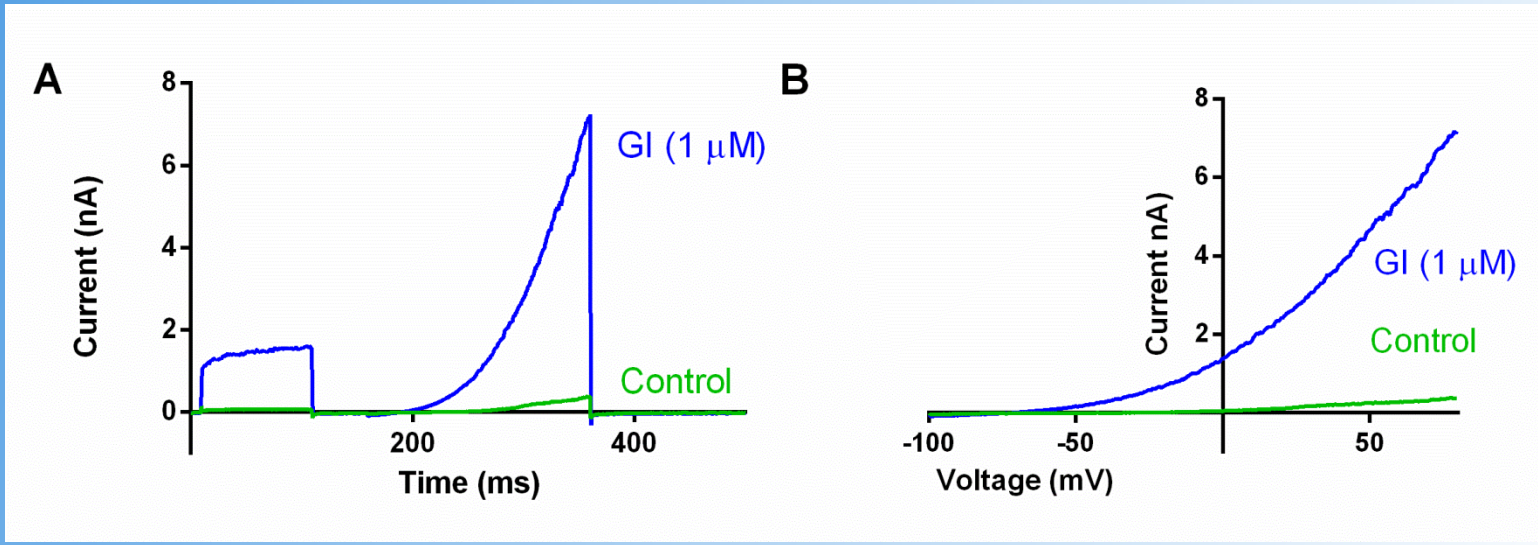
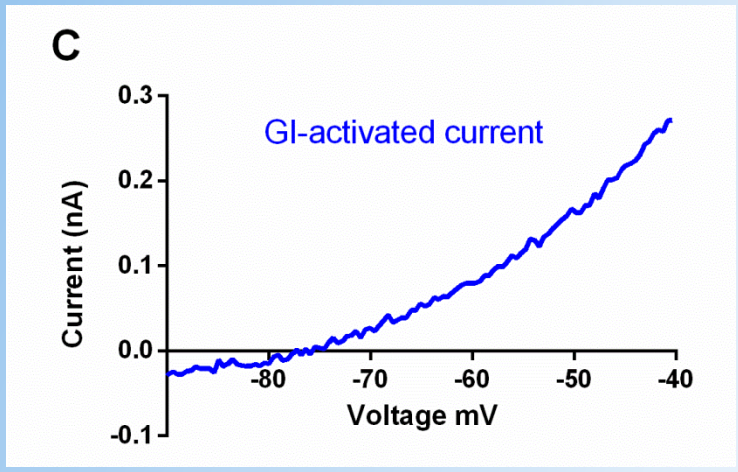
GI-530159 enhances TREK1 current

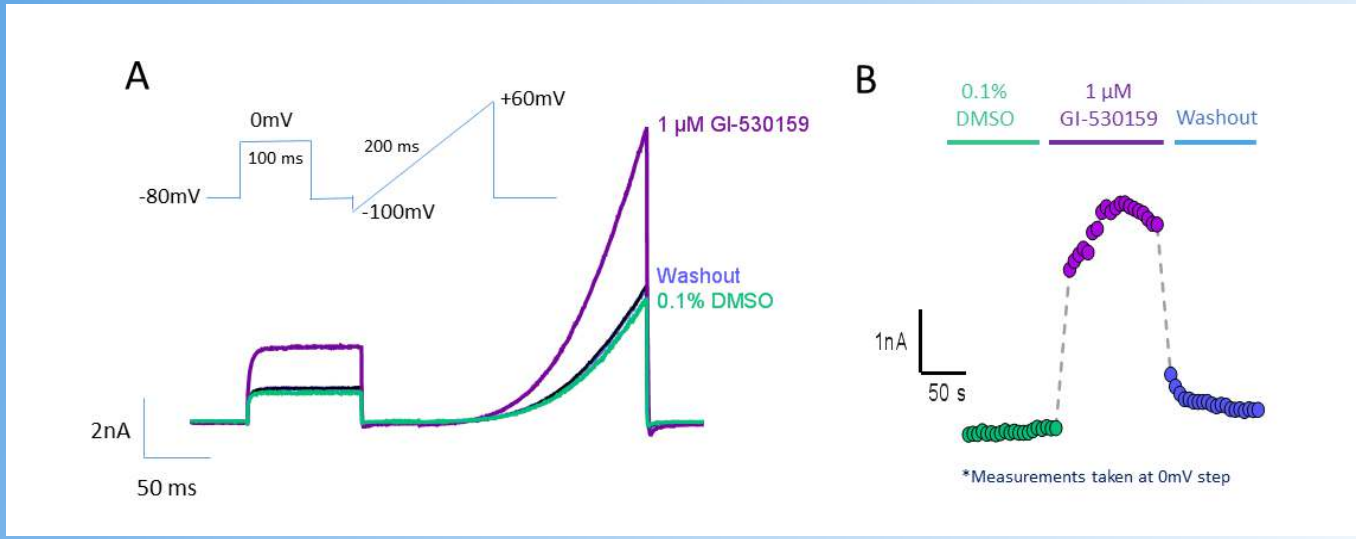
in stably transfected HEK-293 cells





Loucif et al 2017 under review

GI-530159 enhances TREK1 current
in CHO cells (Qpatch automated patch recording)



Loucif et al 2017 under review

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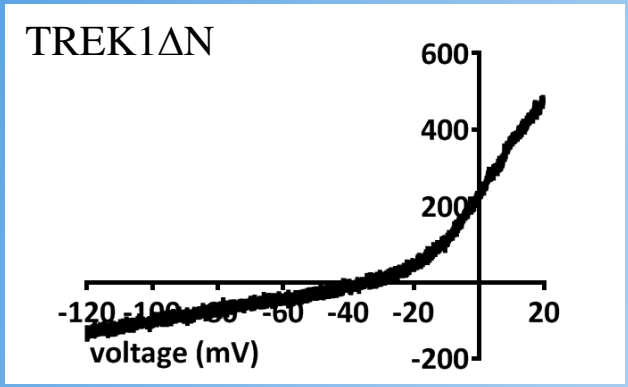
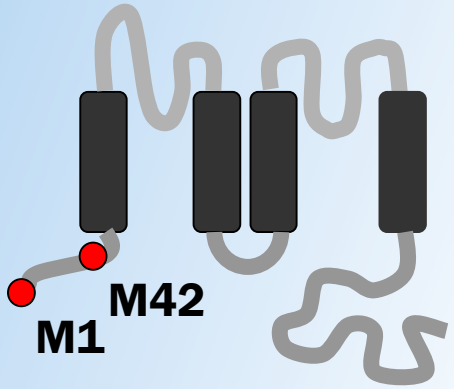
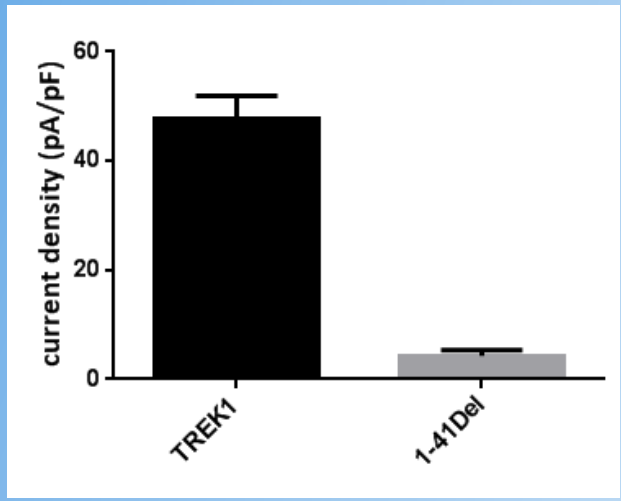
Alternative translation initiation gives a short form of TREK1 (TREK1ΔN) which is permeable to sodium

(Thomas et al 2008, Neuron 58:859)



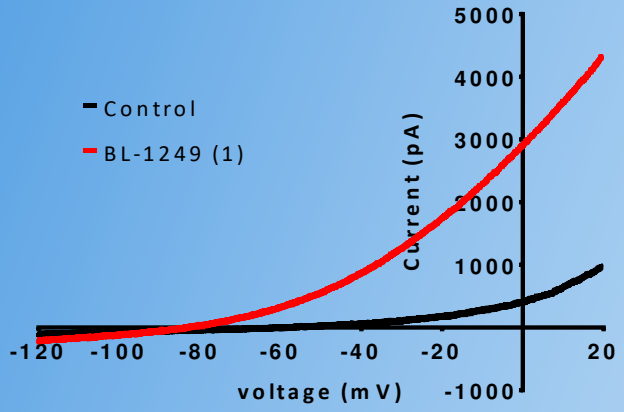


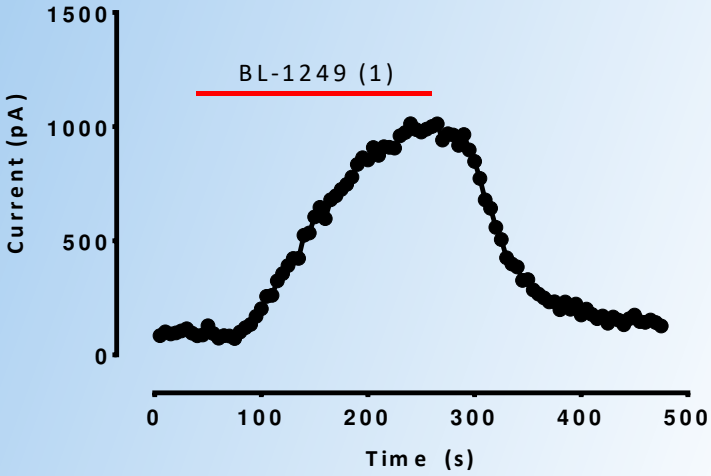


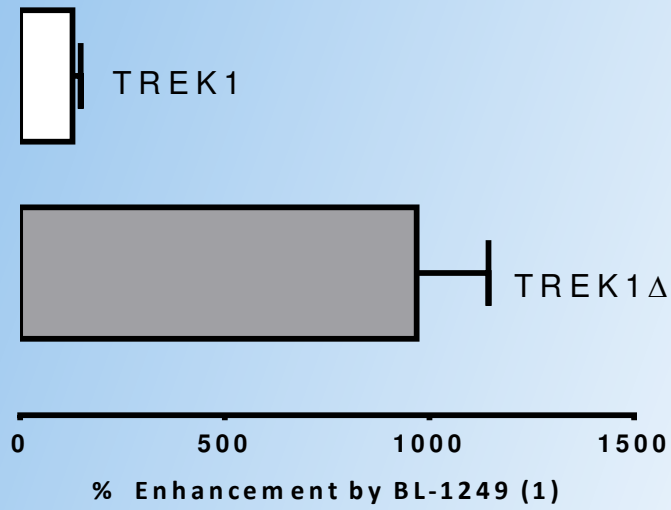
Channel	Vrev (mV)
TREK1	-85±1 (n=27)
TREK1ΔN	-47±5 (n=14)

Veale et al (2014) Mol Pharm 85: 671-681

BL-1249 enhances TREK1ΔN current







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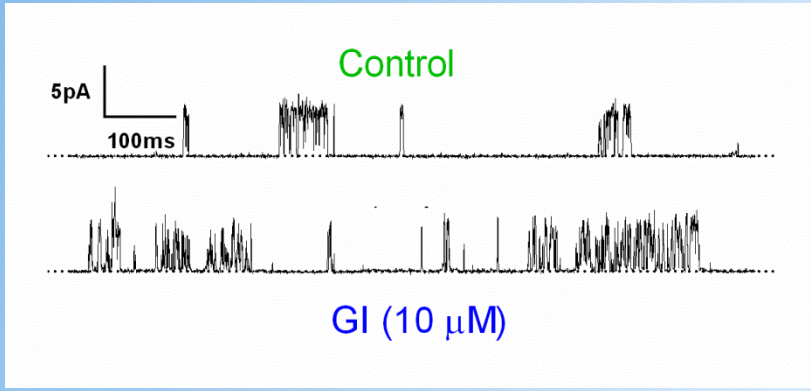
GI-530159 enhances TREK1 single-channel current open probability



Po increases from 0.01 to 0.03

Summary

- Role for selective KCNK9 activators in KCNK9 Imprinting Syndrome
- Role for selective KCNK3 activators in pulmonary hypertension
- Role for selective TREK activators in pain
- A Standard Operating Procedure for quantification and characterisation of K2P channel activators?

K2P channel colleagues

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Neil Castle, Brett Antonio, Shannon Zellmer, Katrina Yoger (Icagen)

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GI-530159 enhances TREK single-channel current open probability