Mechanisms of Inflammatory and Neuropathic Pain

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Department of Neurosurgery

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Disclosures

MJC was an inventor on a now-expired patent (United States Patent #6,335,180) on TRPV1 and TRPV2 that was licensed through UCSF and Merck, and was entitled to royalties on that patent (> 4 yrs ago).

Pachyonychia Congenita Project (travel to annual meeting, gift to collaborator > 5 yrs ago)

Hydra Biosciences (SAB member > 5 yrs ago)

Two Approaches to Understanding Pain at the Molecular Level

1) **Start with a Molecule:**

Role of TRPV1 phosphorylation in inflammatory Pain

Two Approaches to Understanding Pain at the Molecular Level

1) **Start with a Molecule:**

Role of TRPV1 phosphorylation in inflammatory Pain

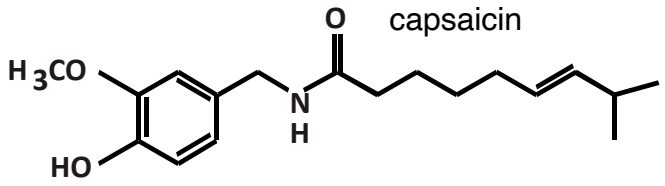
2) **Start with a Pain Condition:**

Pain in Hereditary Skin Diseases

Start with a Molecule (and a phenomenal mentor and team)
San Francisco, California 1997

David Julius

A much younger me



Cloning of Transient Receptor Potential Vanilloid 1 (TRPV1)

Dorsal Root Ganglion
cDNA Library
(~3 Million clones)

↓

HEK293 Cells

↓

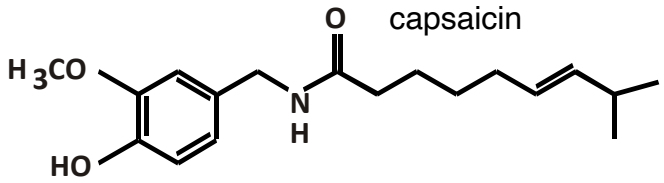
Load with Fura-2

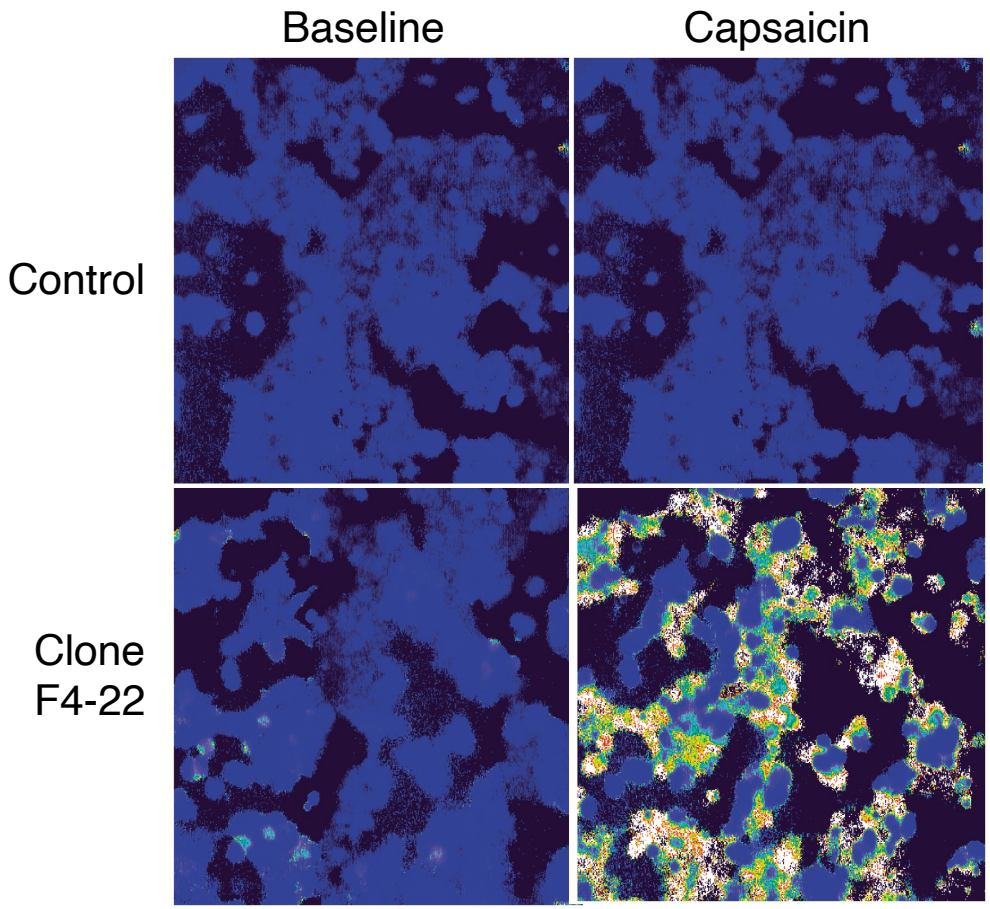
↓

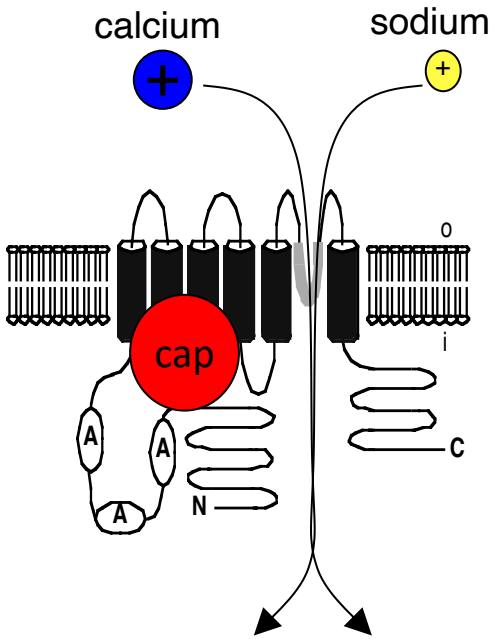
Add Capsaicin

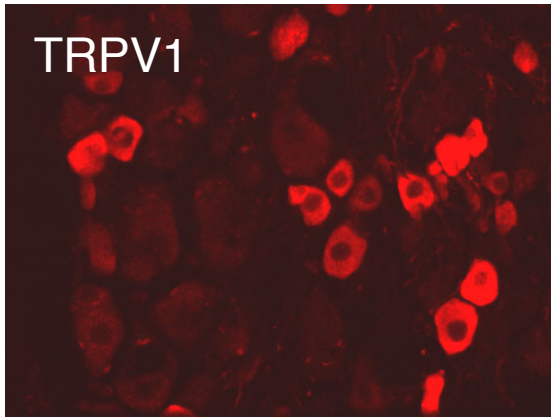
↓

Image
Intracellular Ca^{2+}



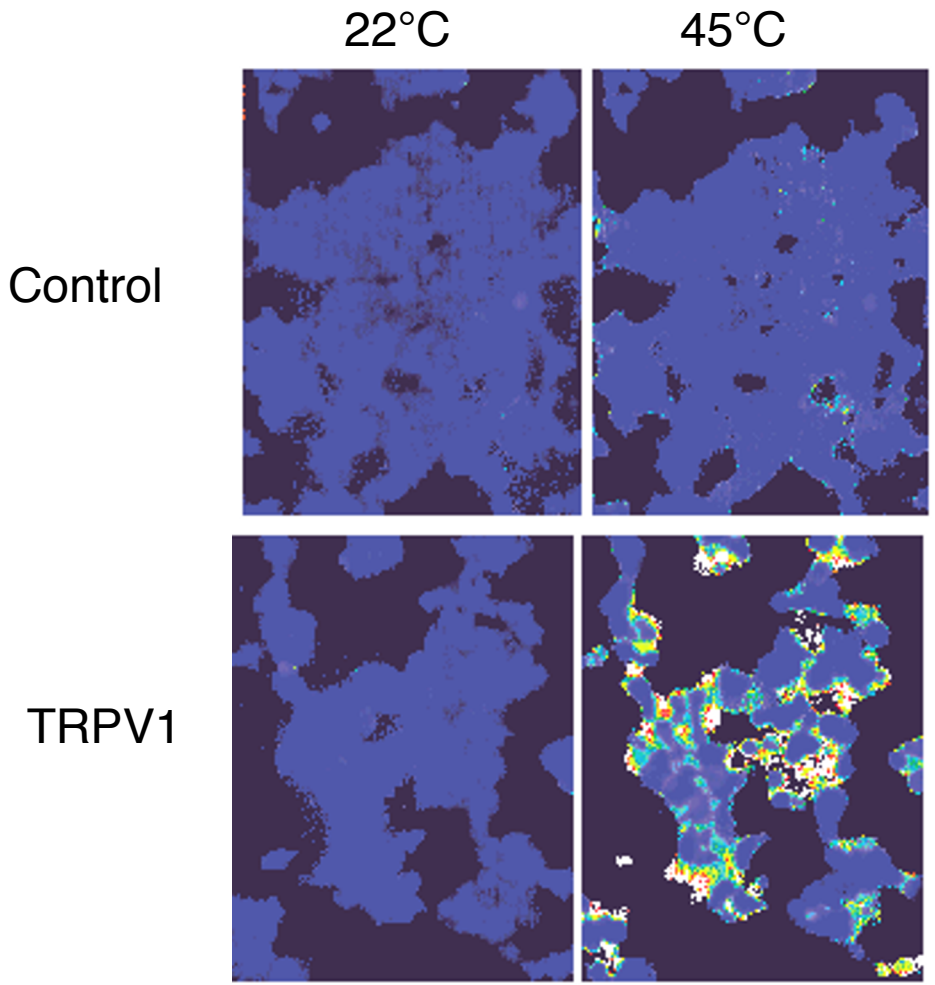




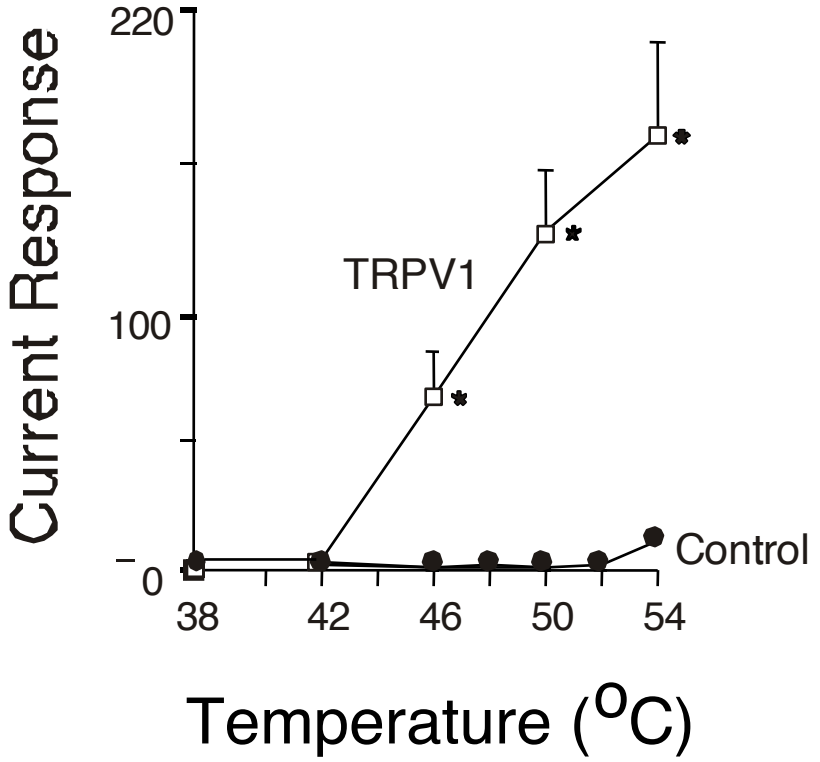


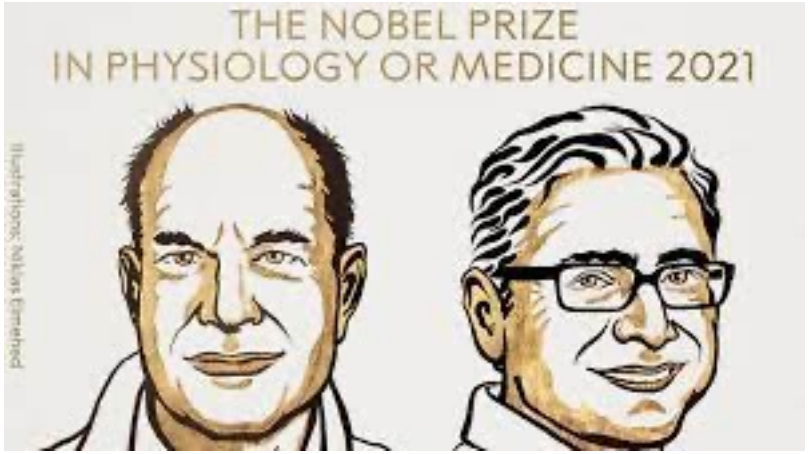
TRPV1 can alternatively be activated by painfully hot temperatures

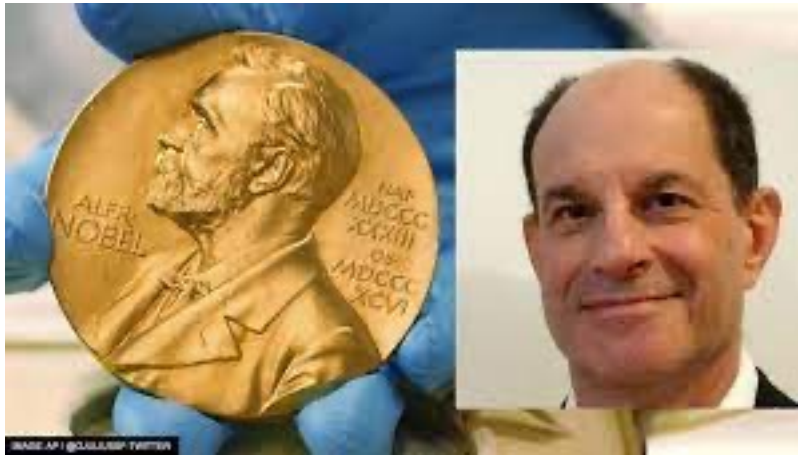
Calcium Imaging

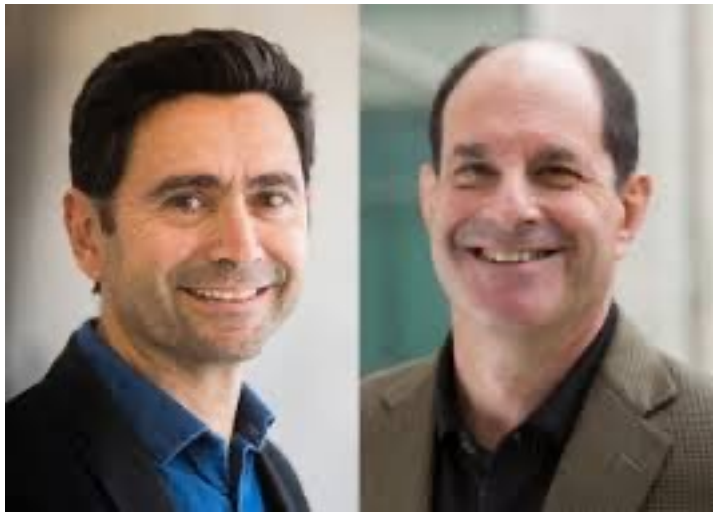


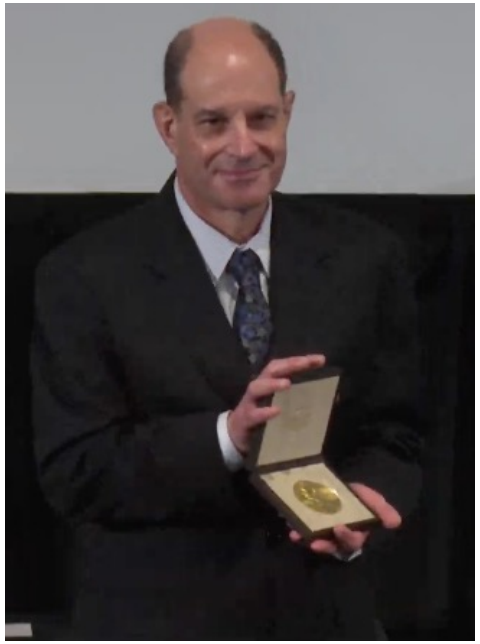
Voltage Clamp




THE NOBEL PRIZE
IN PHYSIOLOGY OR MEDICINE 2021

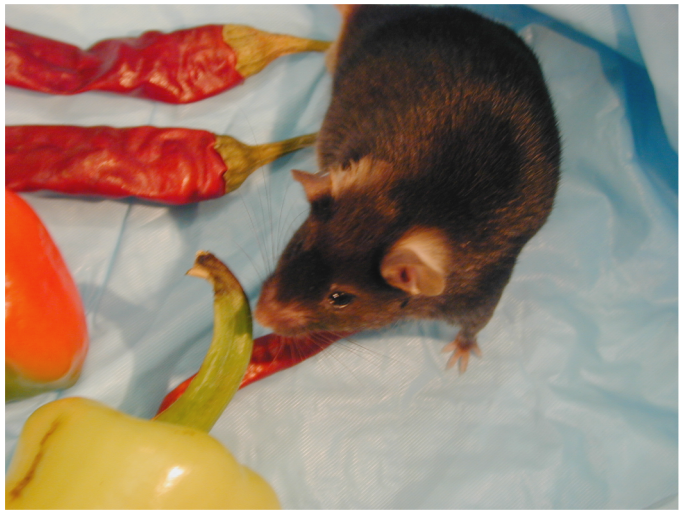

ALFR
NOBEL

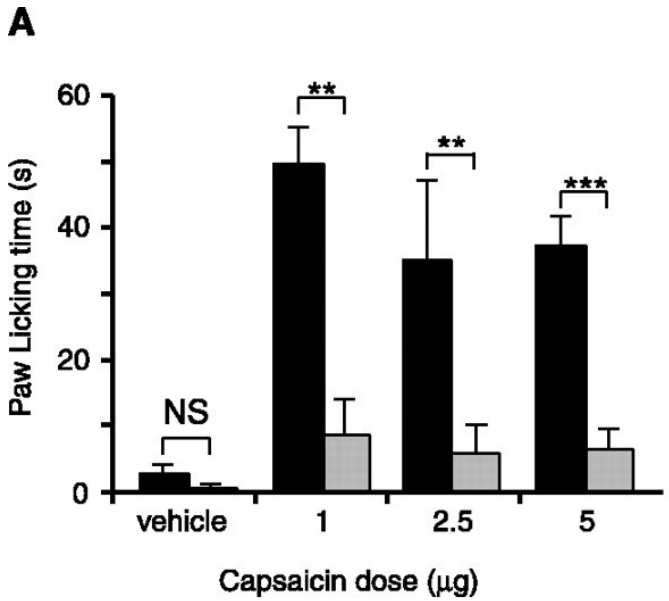





ALFR
NOBEL
David Julius, Ardem Patapoutian
win Nobel Prize in Medicine

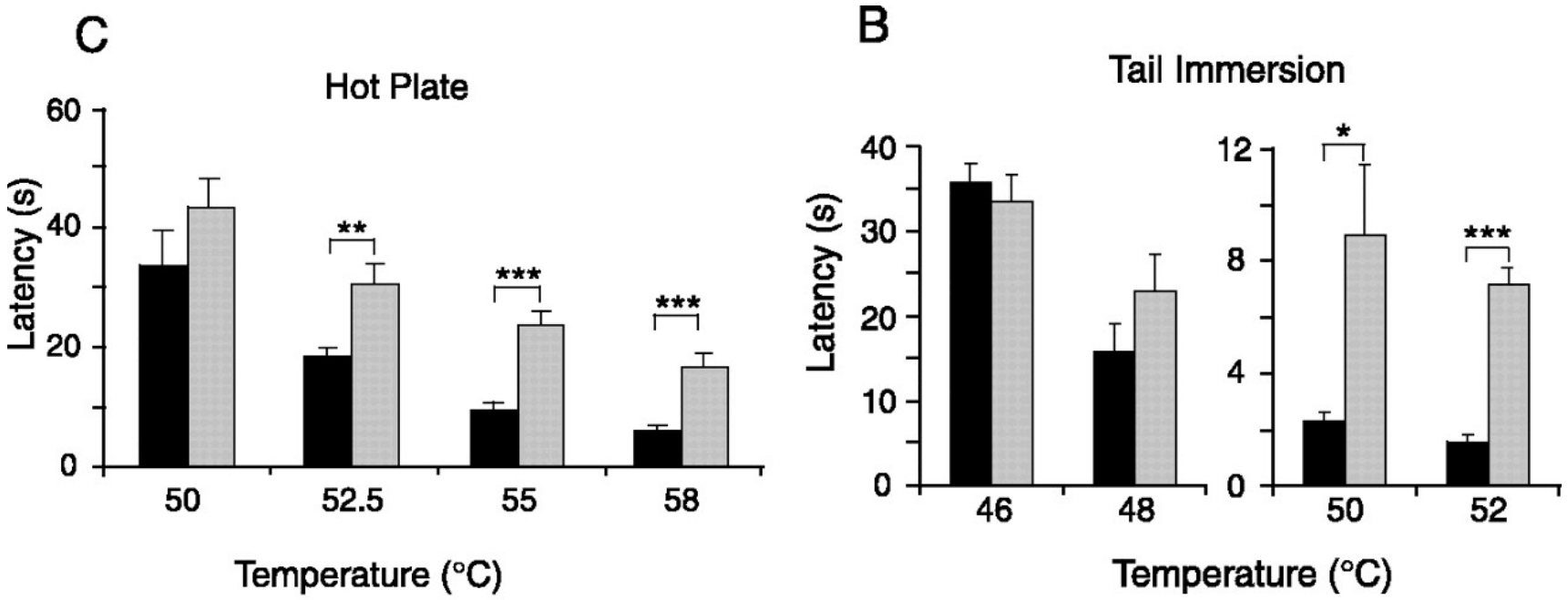
Mice lacking TRPV1 do not respond to capsaicin





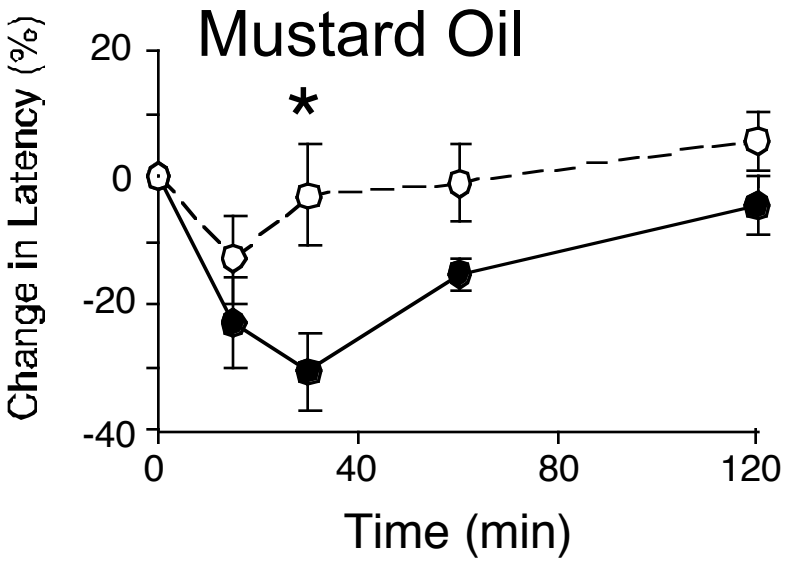
Caterina et al. (2000)

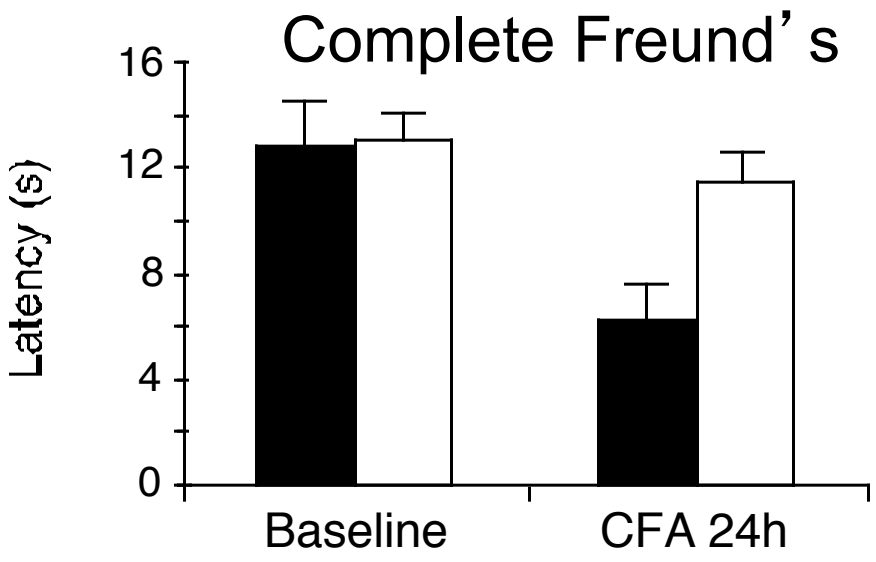
Mice lacking TRPV1 show partially impaired heat pain



Caterina et al. (2000)

TRPV1 null mice are deficient in inflammation evoked heat hyperalgesia



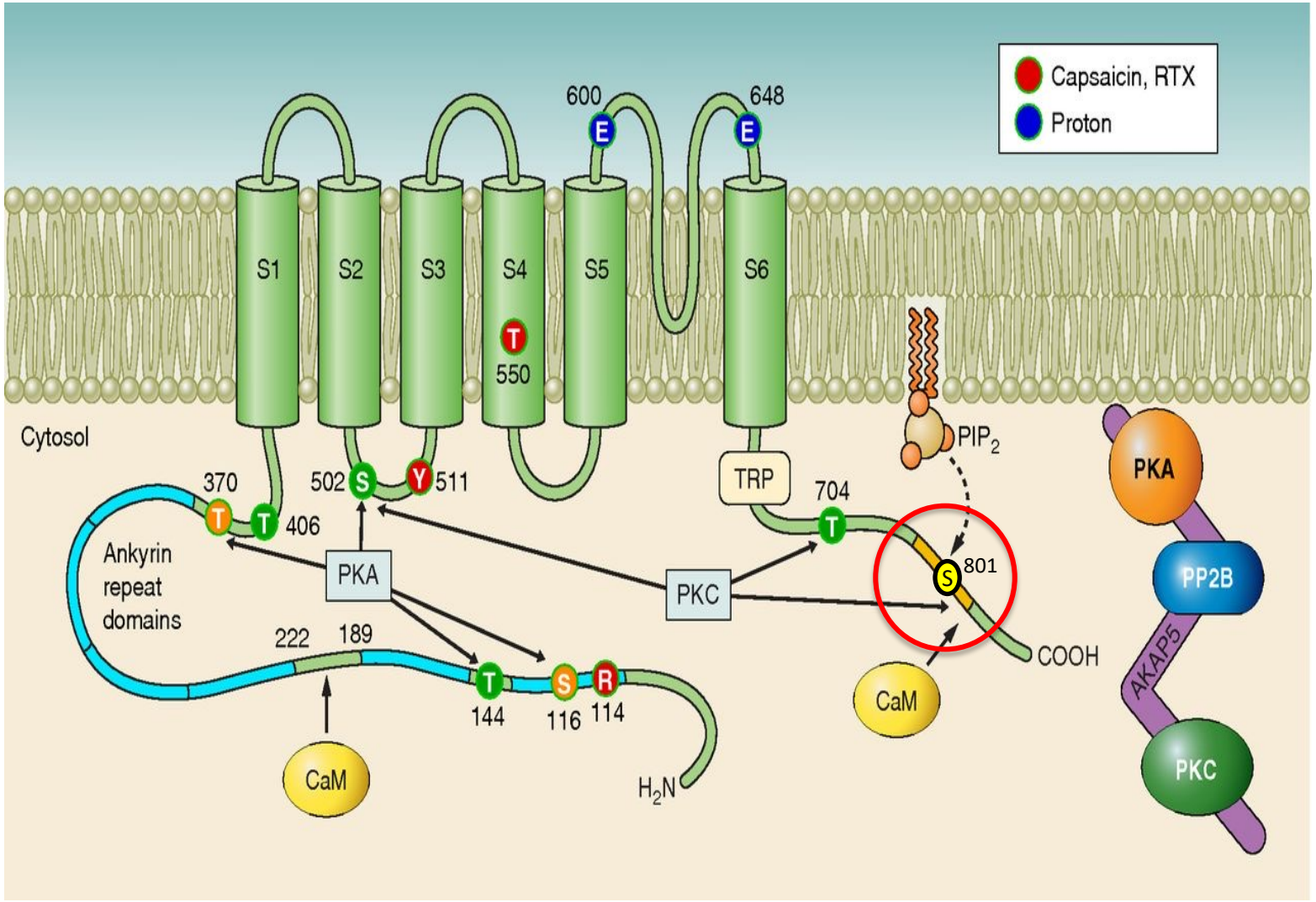


● Wild-type

○ TRPV1 -/-

Caterina et al. 2000

TRPV1 is subject to extensive posttranslational modulation

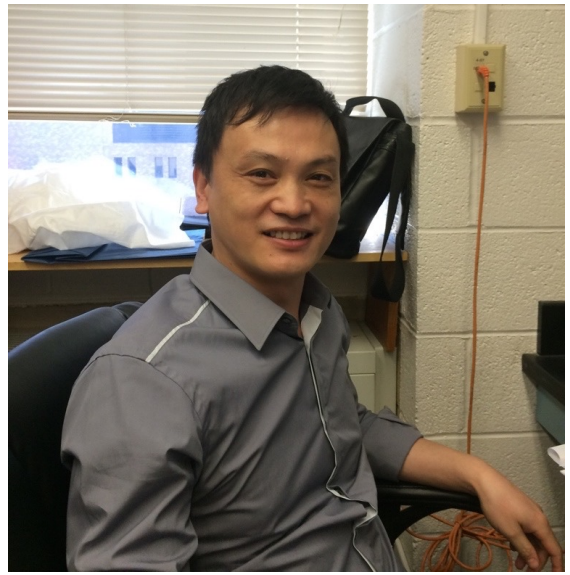


Bourinet *et al.*, *Physiological Reviews* (2014)

20 Years Later: What is the specific function of TRPV1 S800 phosphorylation in vivo?

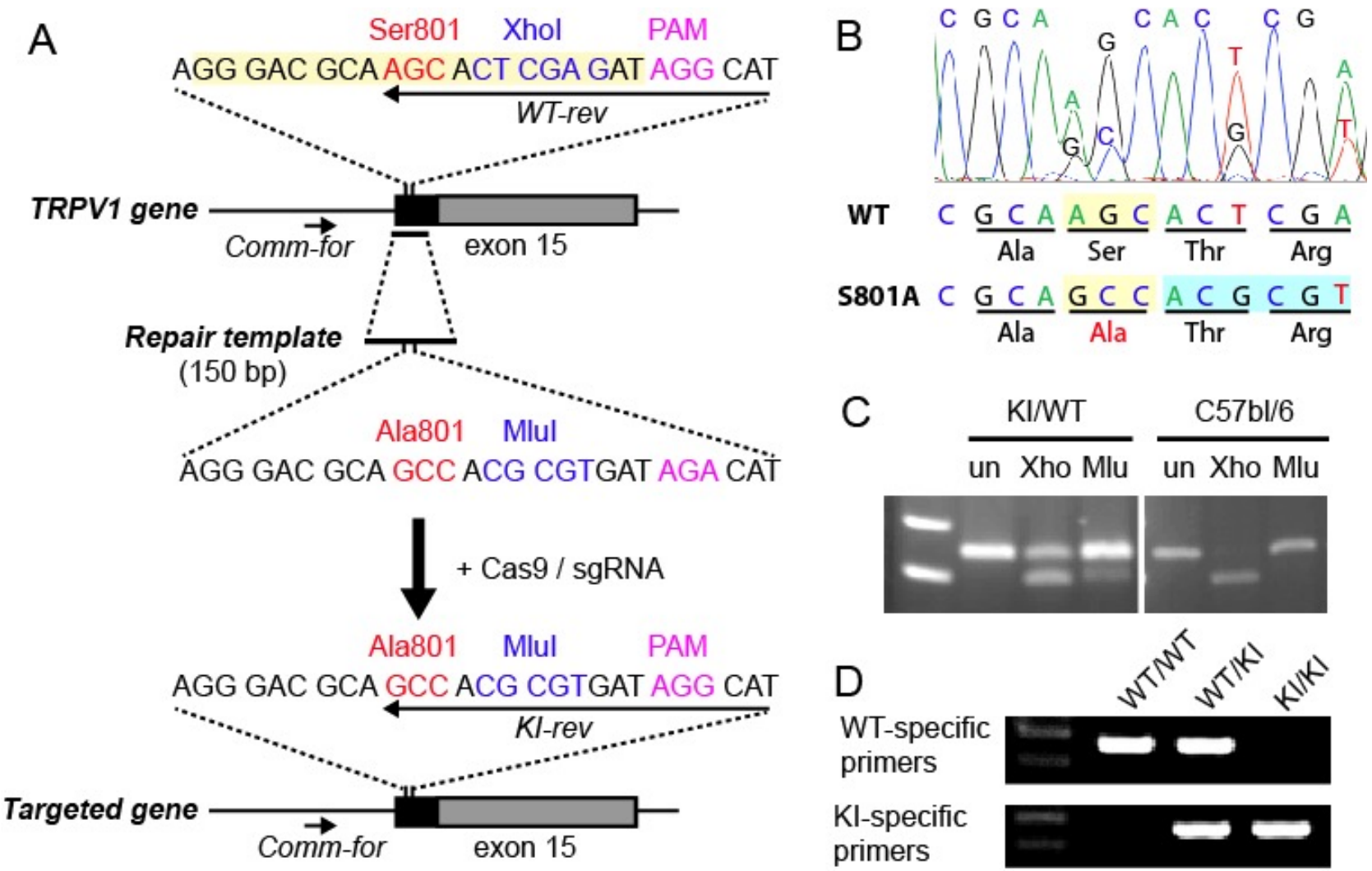
Man-Kyo Chung
(U Maryland)

Jonathan Joseph
(U Maryland)



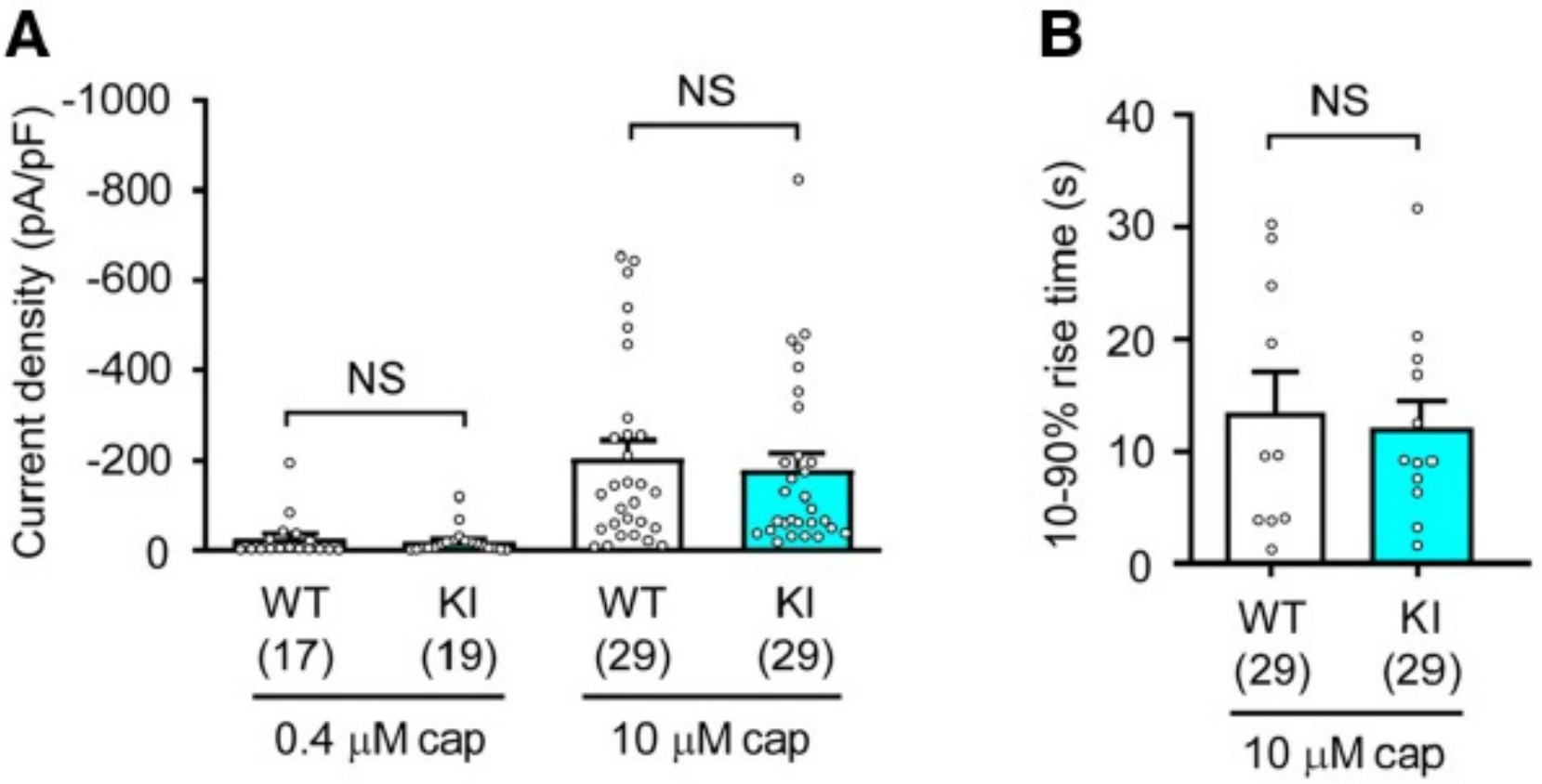
Lintao Qu
(Johns Hopkins)

In vivo TRPV1 S801A mutagenesis using CRISPR-Cas9



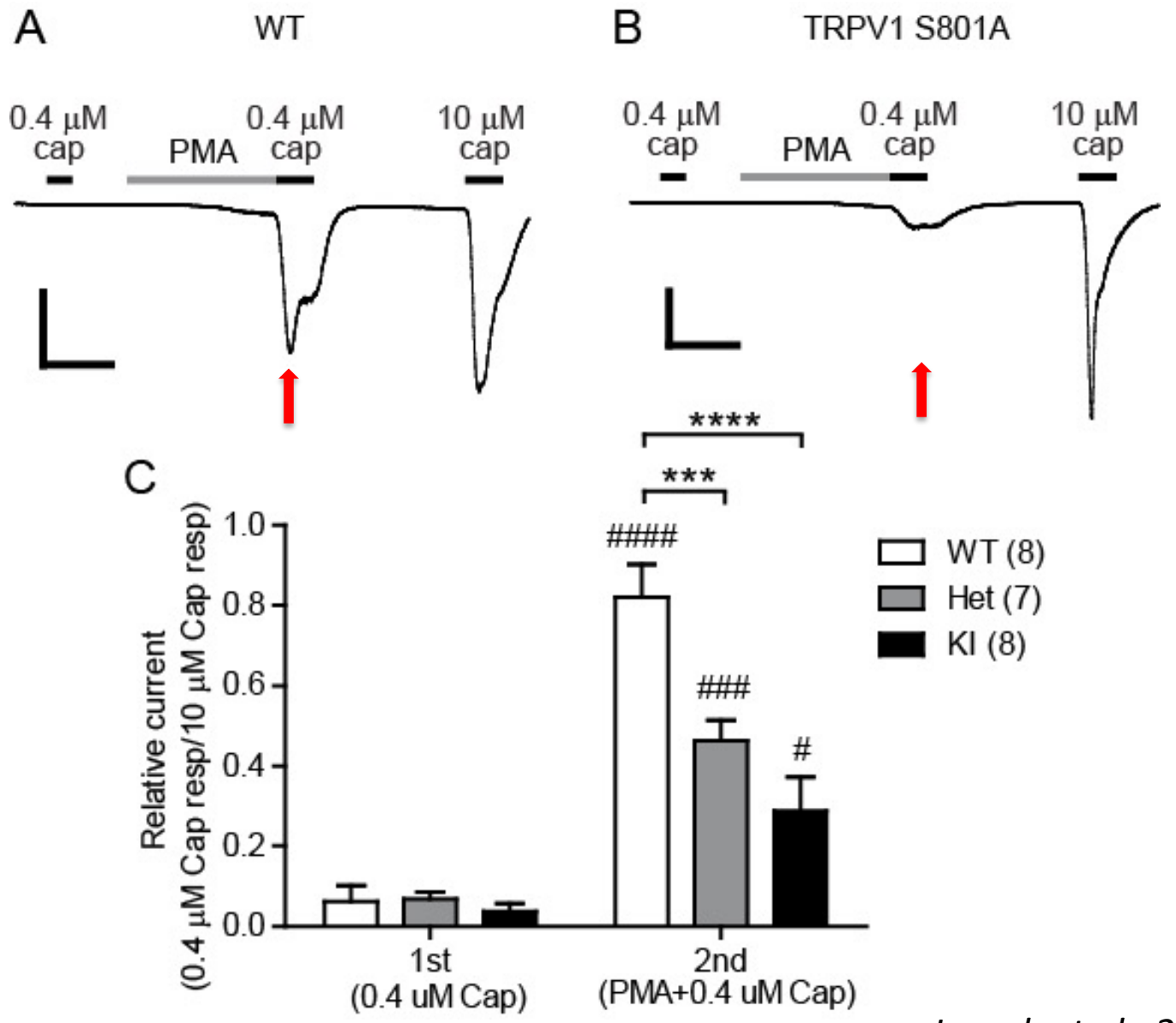
Joseph et al., 2019

Sensory neurons from TRPV1 S801A mice show normal capsaicin-evoked currents



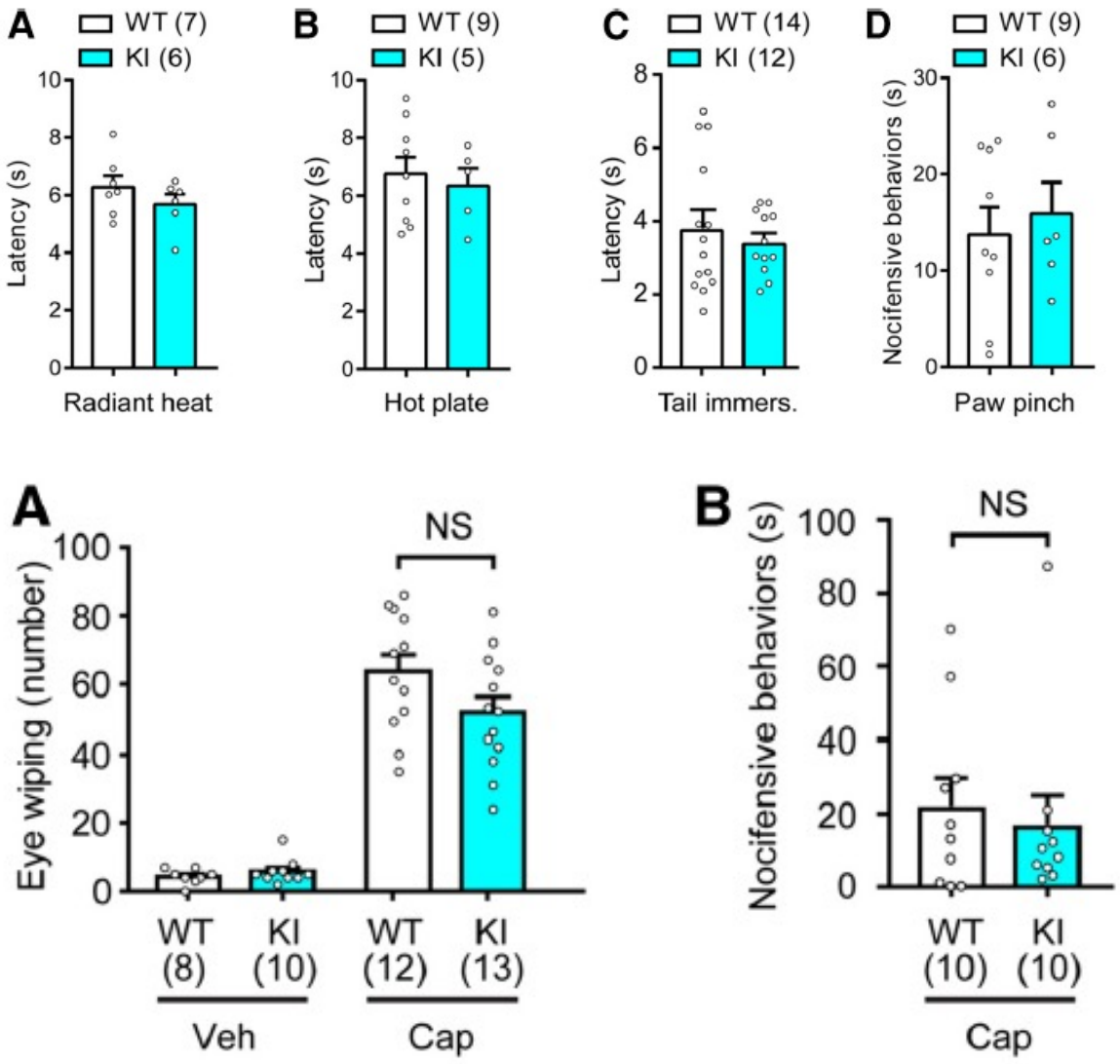
Joseph et al., 2019

Sensory neurons from TRPV1 S801A mice show impaired sensitization by phorbol esters



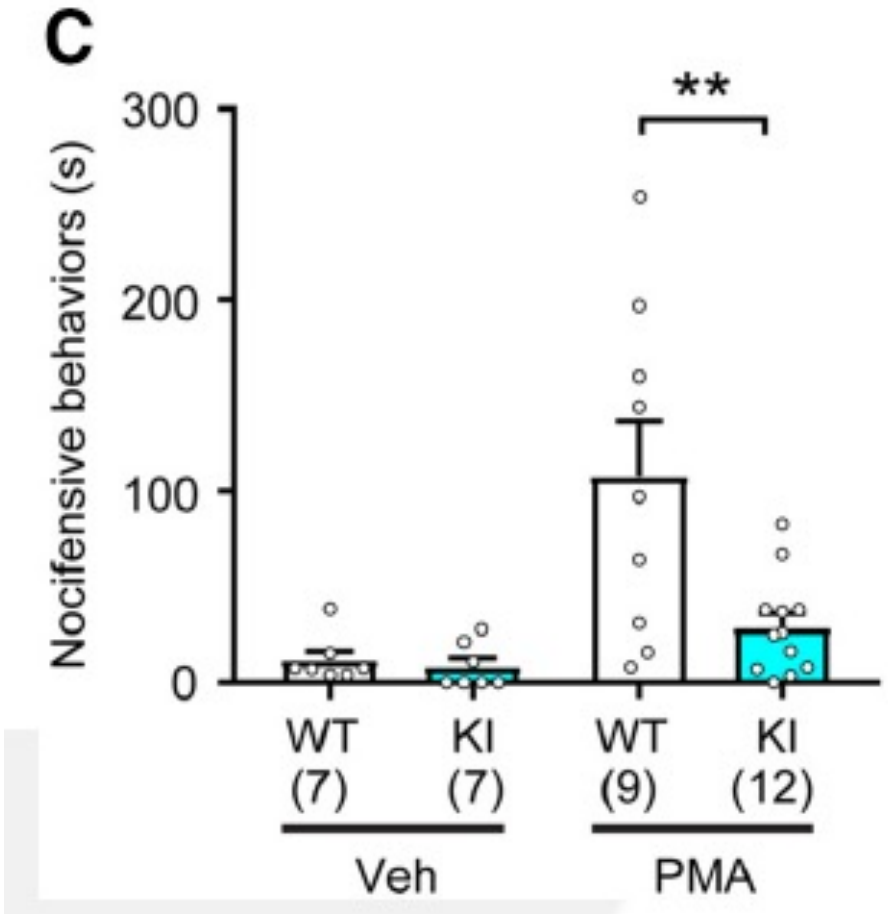
Joseph et al., 2019

TRPV1 S801A mice show normal behavioral responses to heat and capsaicin



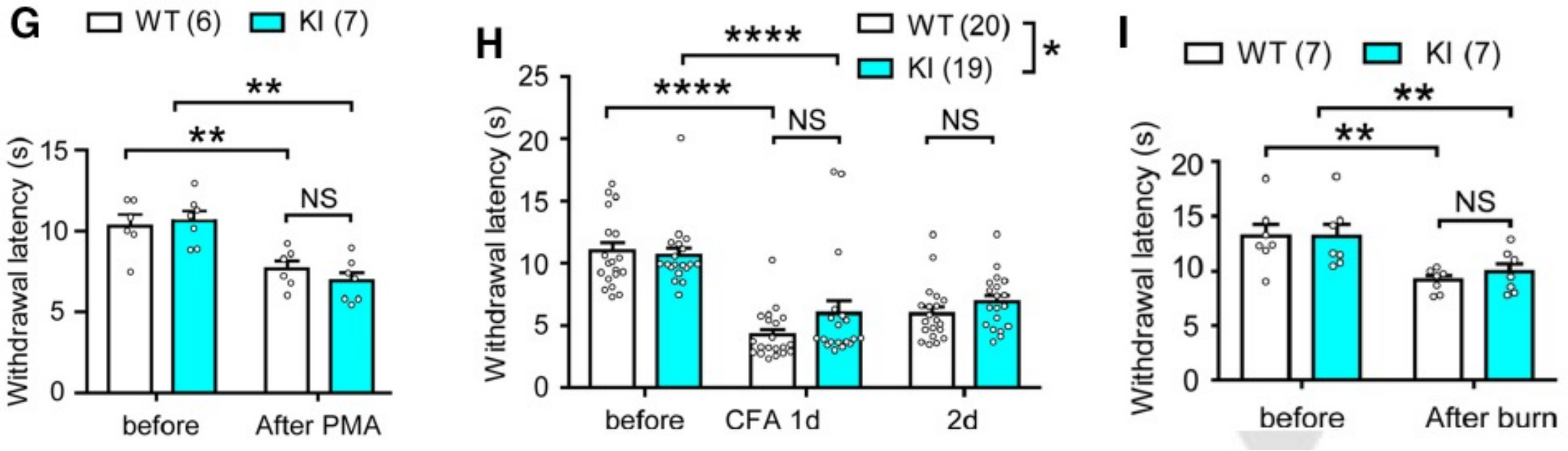
Joseph et al., 2019

TRPV1 S801A mice show impaired PMA-induced nocifensive behaviors



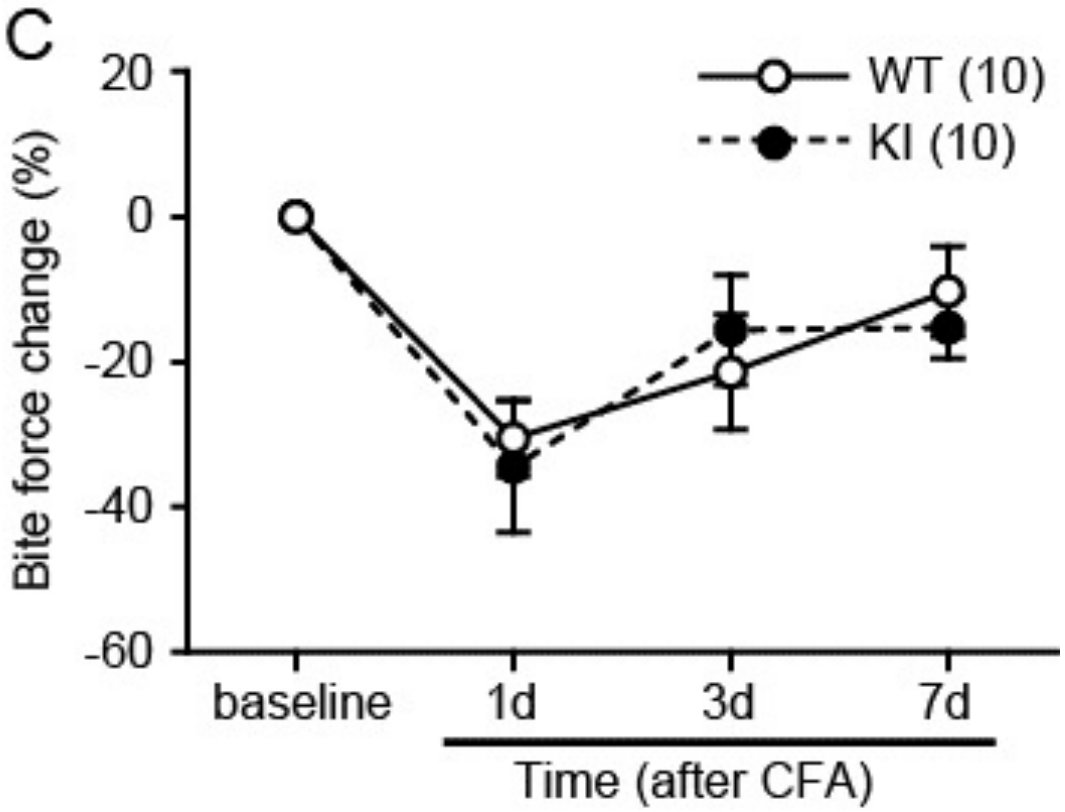
Joseph et al., 2019

TRPV1 S801A mice show normal thermal hyperalgesia following skin treatment with PMA, CFA, or mild burn



Joseph et al., 2019

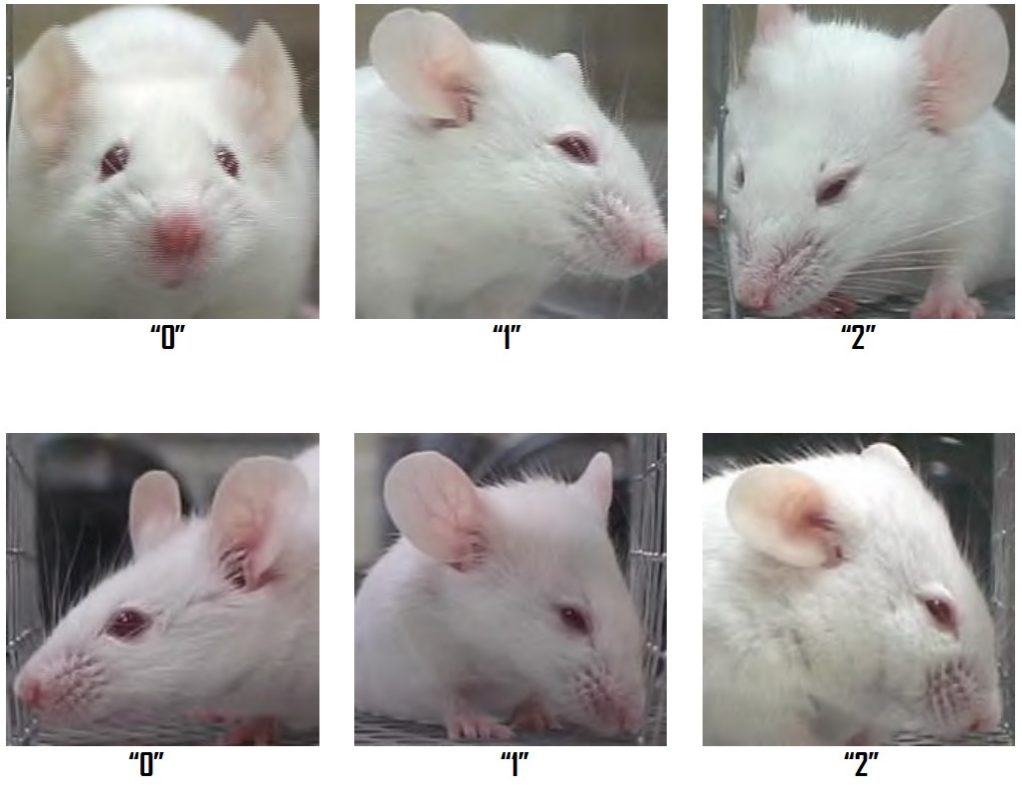
TRPV1 S801A mice show normal change in bite force following masseter muscle inflammation with CFA



Joseph et al., 2019

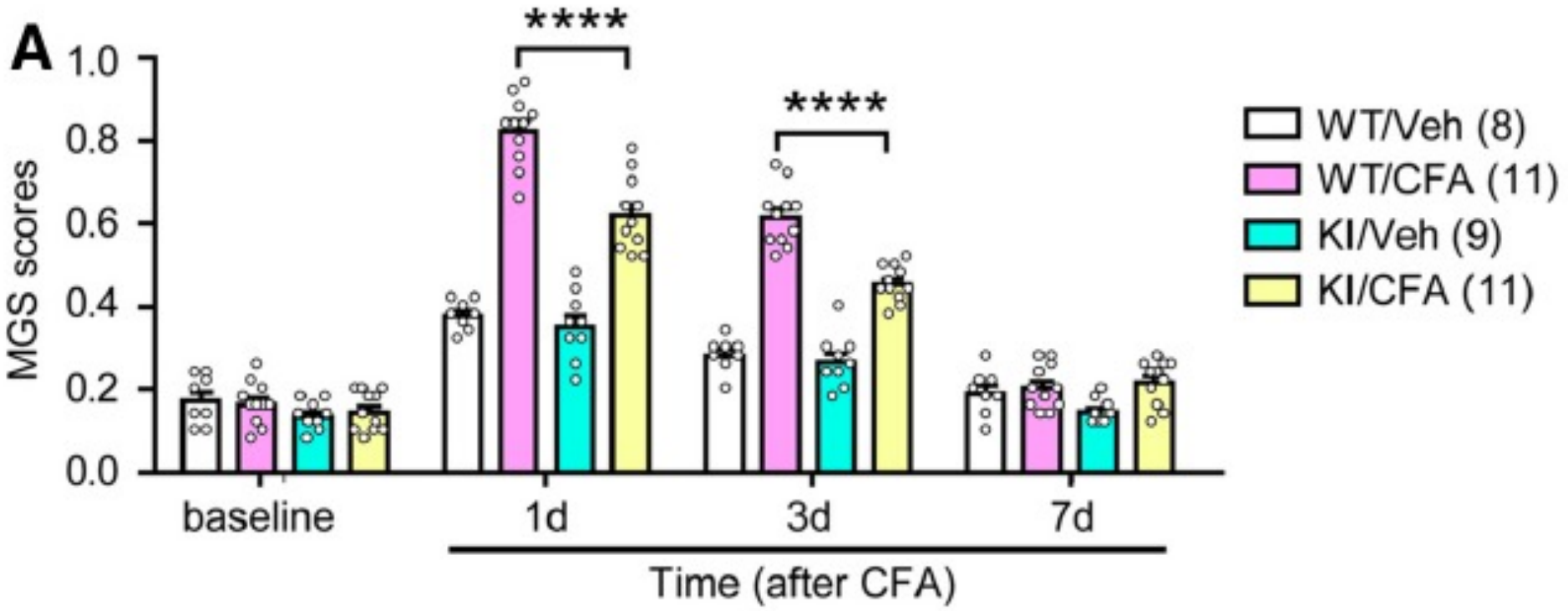
Spontaneous/ongoing pain assessment with Facial Grimace Scale

- **Orbital tightening**
- Nose Bulge
- Cheek Bulge
- **Ear Position**
- Whisker Change



Langford et al. Nature Methods 2010 Jun;7(6):447-9. *(Mogil lab)*

TRPV1 S801A mice show reduced spontaneous pain following muscle inflammation.



Joseph et al., 2019

Conclusions – Part 1

1. Prevention of TRPV1 phosphorylation at S801 reduces sensitization and reversal of desensitization by phorbol ester in neurons

2. TRPV1 phosphorylation at S801 appears to contribute to ongoing, but not evoked pain associated with masseter muscle inflammation.

3. These findings support the notion that stimulus-evoked and spontaneous pain sensitization are the products of distinct mechanisms

Two Approaches to Understanding Pain at the Molecular Level

1) Start with a Molecule:

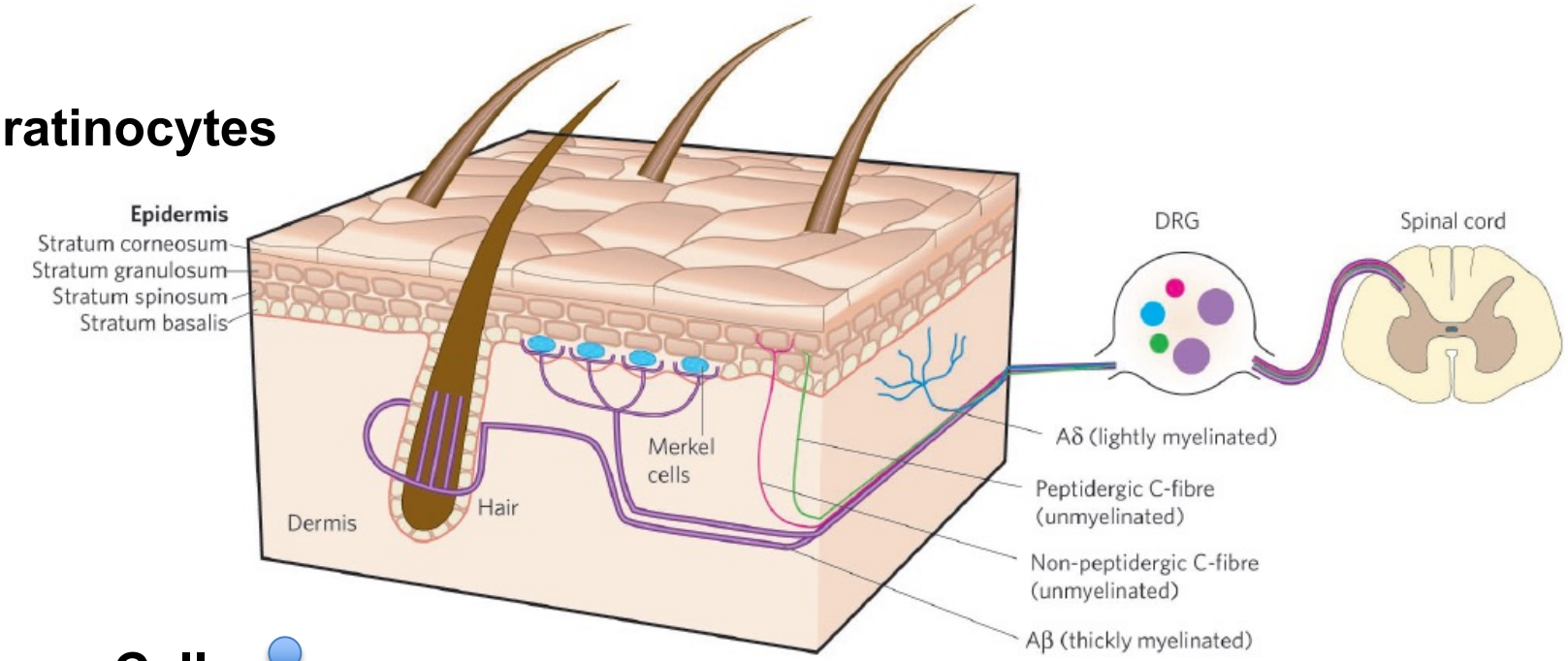
Role of TRPV1 phosphorylation in inflammatory Pain

2) Start with a Pain Condition:

Pain in Hereditary Skin Diseases

Multiple potential cellular contributors to cutaneous pain

Keratinocytes



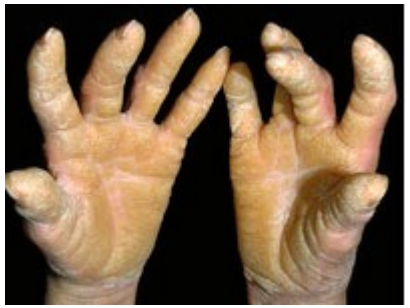
Immune Cells

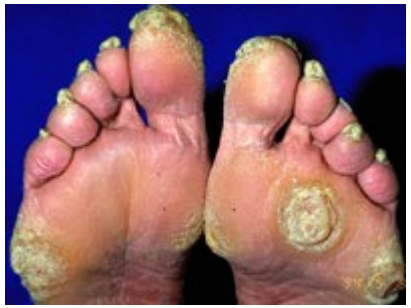
Macrophages
Lymphocytes
Mast Cells
Dendritic Cells
Neutrophils

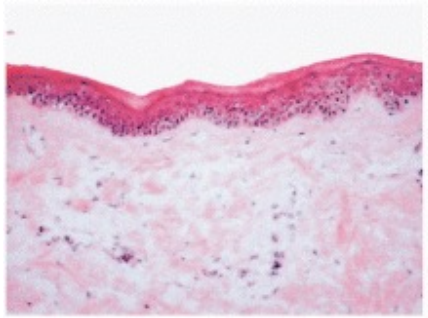
Sensory Neuron Subtypes (>10)

Glial Cells
Vascular Cells
Fibroblasts

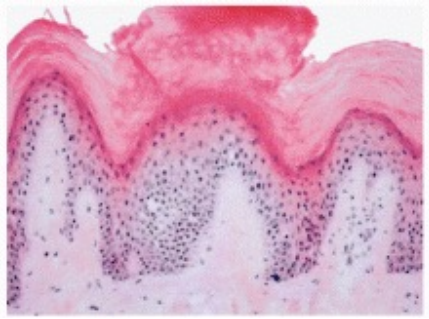
Pain in Hereditary Skin Diseases: Palmoplantar Keratodermas







McClean et al. JID 2011



DermNetNZ.org

Hereditary (25 genes) or Acquired (drugs, cancer)

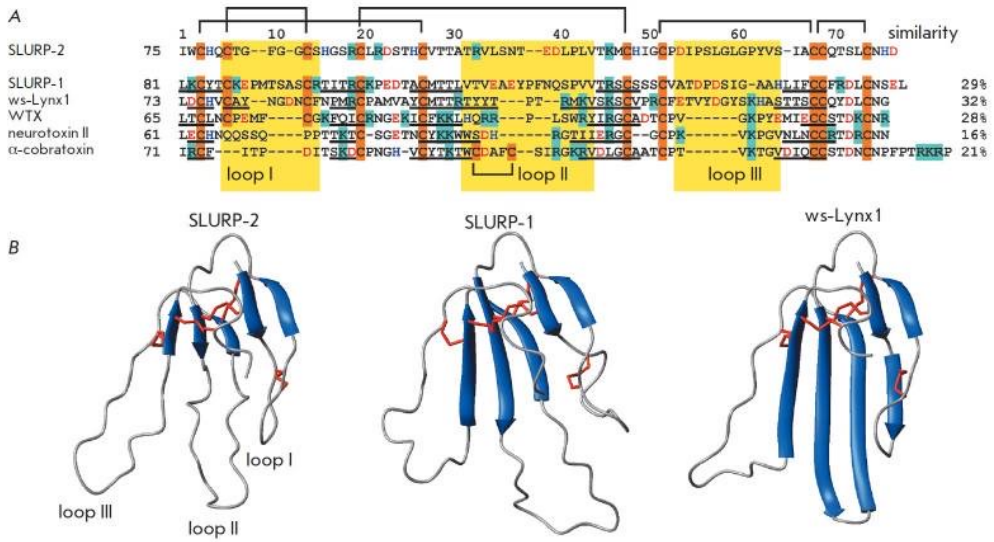
Despite similar overall histology, some forms consistently painful, others not

A Spectrum of Pain in Hereditary Palmoplantar Keratodermas

Disease (mutant gene)	Anatomical Changes	Sensory Changes
Pachyonychia Congenita (Keratin 6, 16, 17)	**Palmo-plantar keratoderma** Nail dystrophy Follicular hyperkeratosis Oral leukokeratosis Cysts	Painful calluses Changes with lesion severity Painful cysts “First bite syndrome”
Olmsted Syndrome (TRPV3, MBTPS2)	**Palmo-plantar keratoderma** Perioreficial lesions Alopecia Constricting Digit Bands	Warmth-induced pain Warmth-induced itch Erythromelalgia
Mal de Maleda (SLURP1)	**Palmo-plantar keratoderma** Nail abnormalities Brachydactyly Perioreficial lesions Hyperhidrosis	Pain in some patients
Epidermolytic PPK (Keratin 9)	**Palmo-plantar keratoderma** with epidermolysis	Pain in some patients

McClean et al. JID 2011, Perez et al. Am J Clin Dermatol 2016, McClean and Moore Hum Mol Gen 2011, Duchatelet et al JAMA Dermatol 2014)

SLURP1 or SLURP2 gene knockout produces palmoplantar keratoderma (model of the human disease Mal de Meleda)

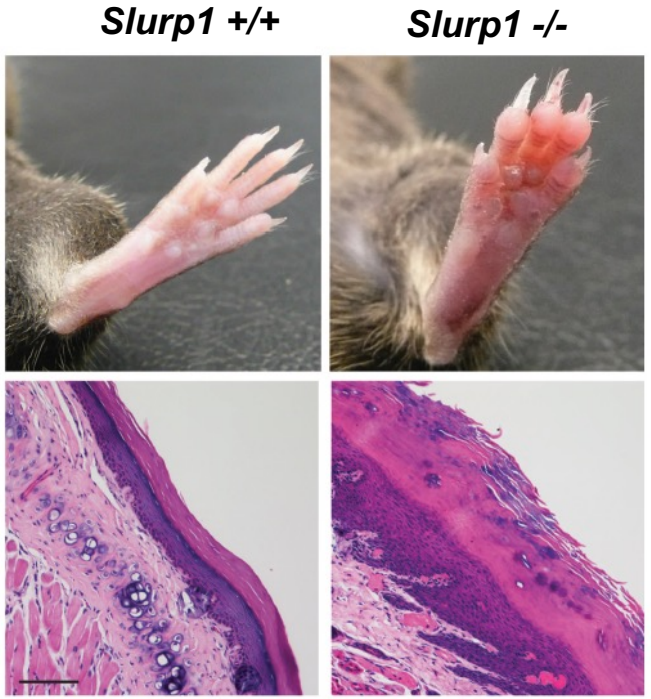


SLURP1 and **SLURP2**

Members of Ly6-PAR family of secreted molecules

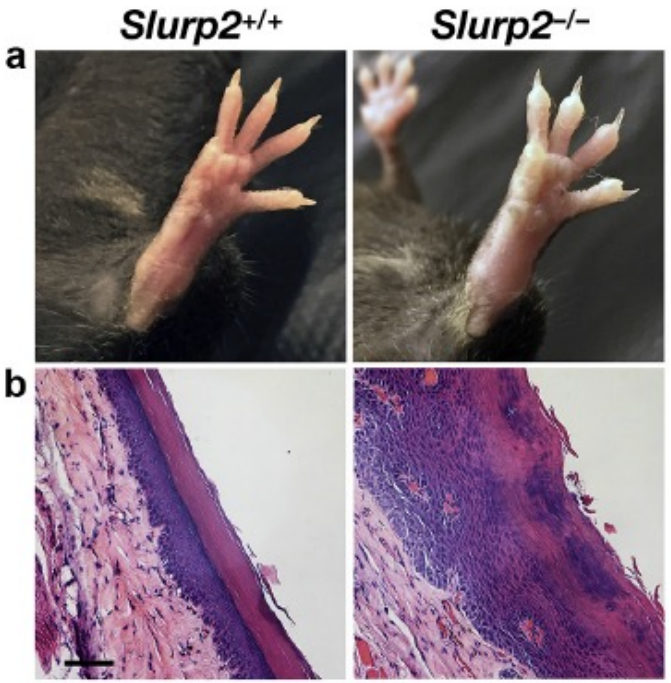
Modulate Nicotinic ACh receptors

Absence results in aberrant epidermal survival/differentiation



Adeyo et al. J. Invest. Dermatol 2014

(Young lab)



Allan et al. J. Invest Dermatol 2016

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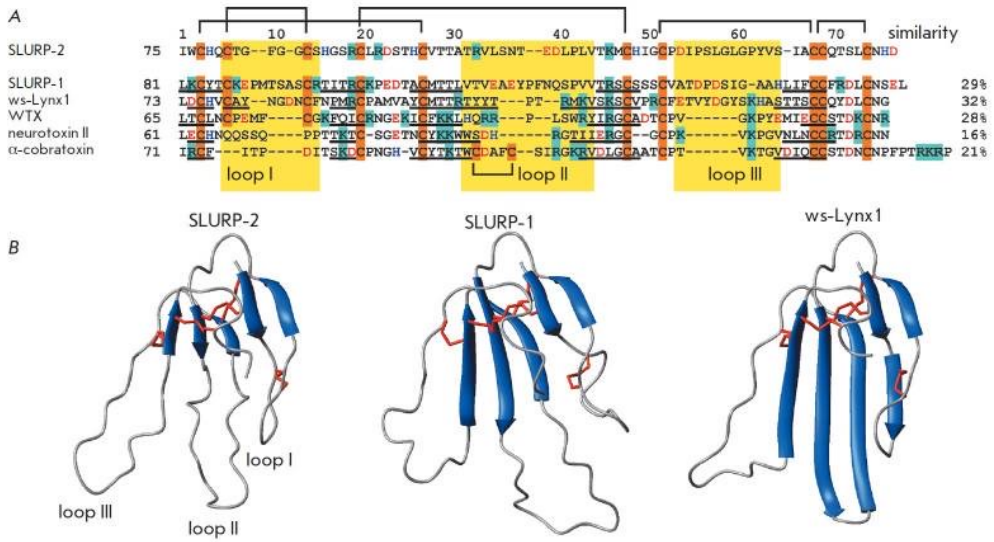

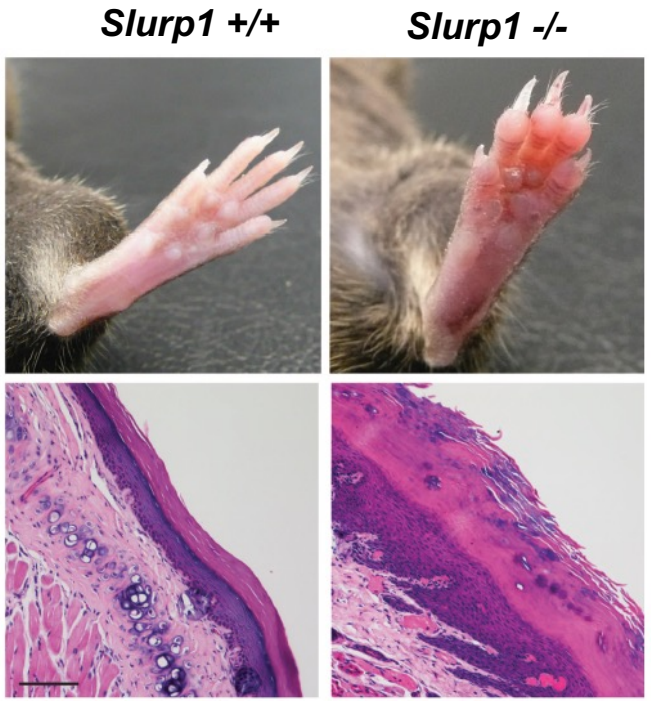

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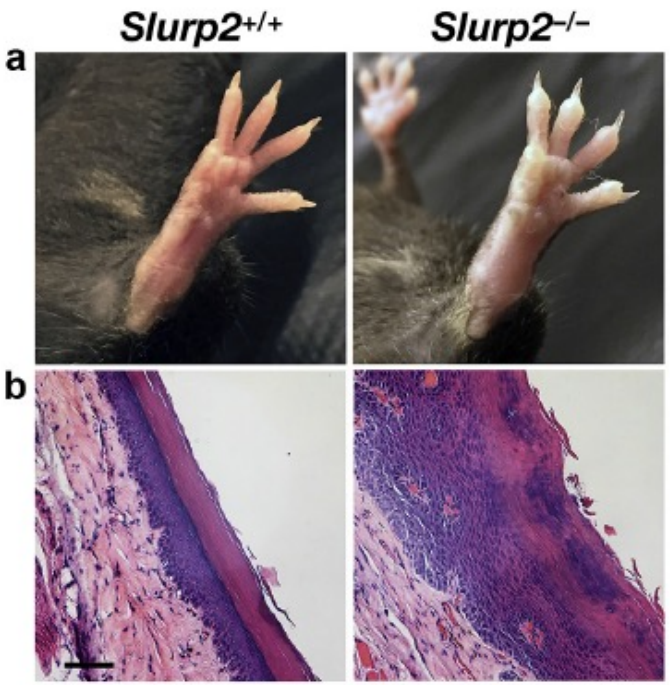

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(Young lab)

SLURP1 and **SLURP2**

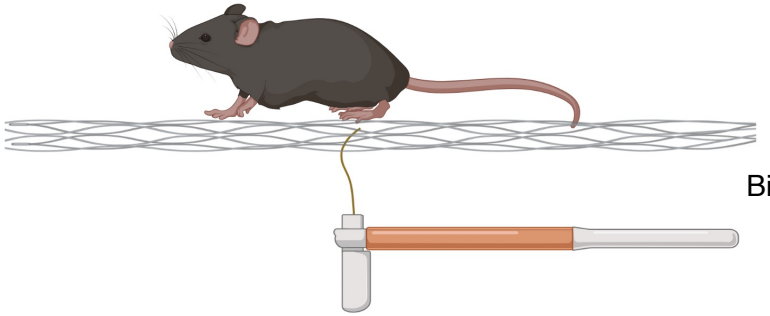
Members of Ly6-PAR family of secreted molecules

Modulate Nicotinic ACh receptors

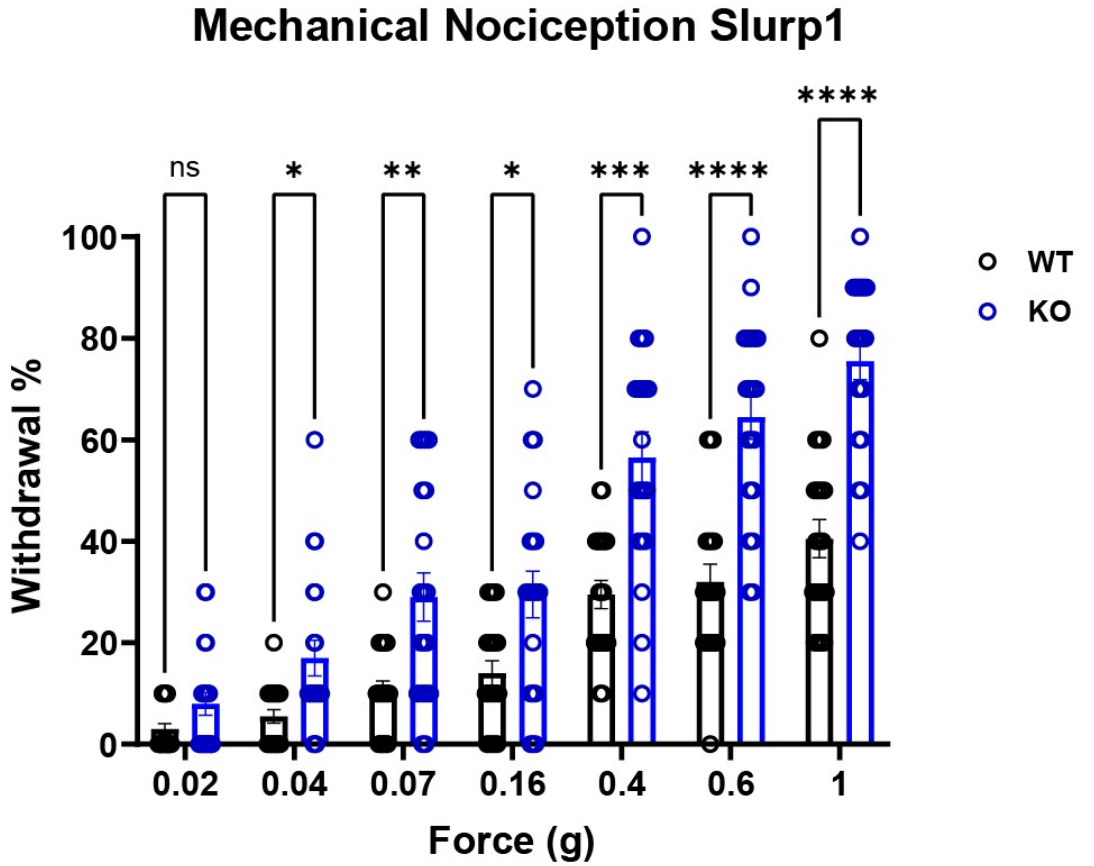
Absence results in aberrant epidermal survival/differentiation

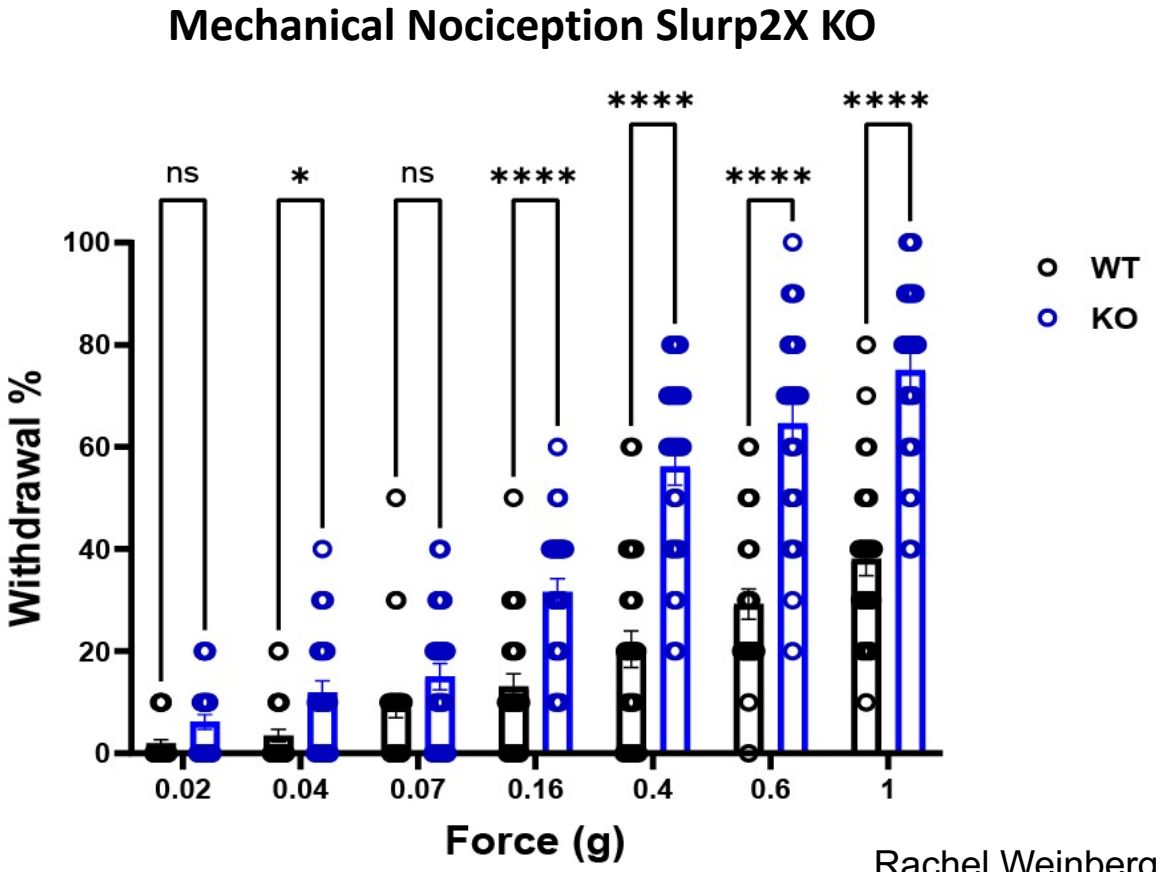
Do they exhibit sensory symptoms?

Increased Mechanical Nociception in Slurp KO mice



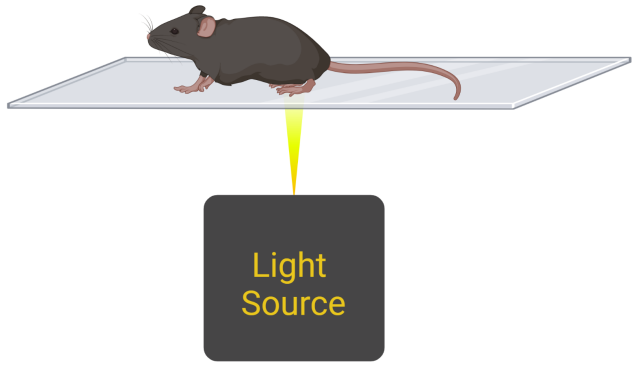
Biorender.com

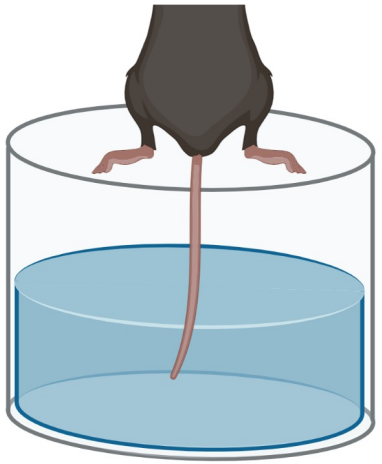




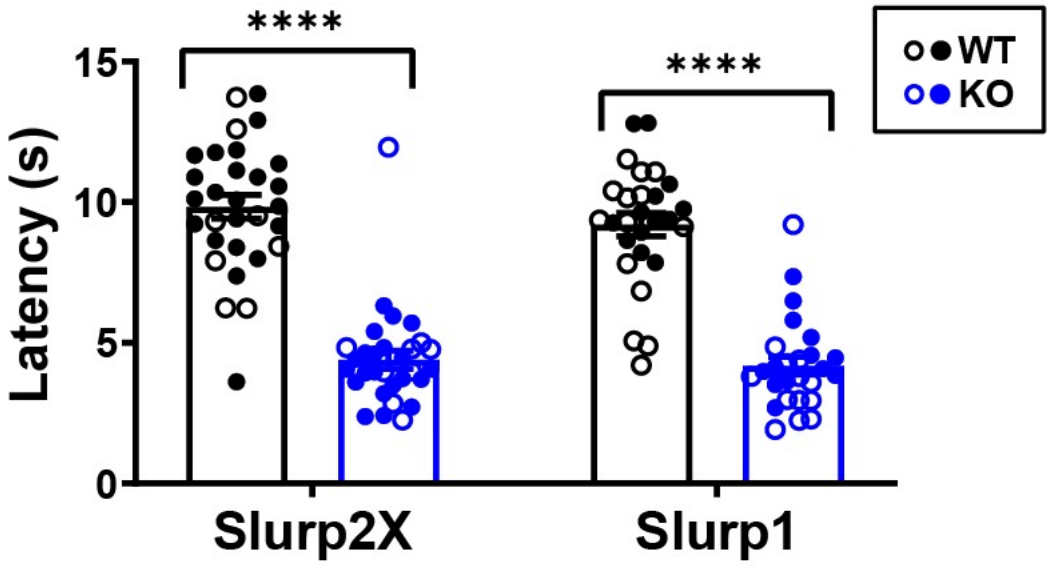
Rachel Weinberg

Increased Thermal Nociception in Slurp KO PPK-affected Skin

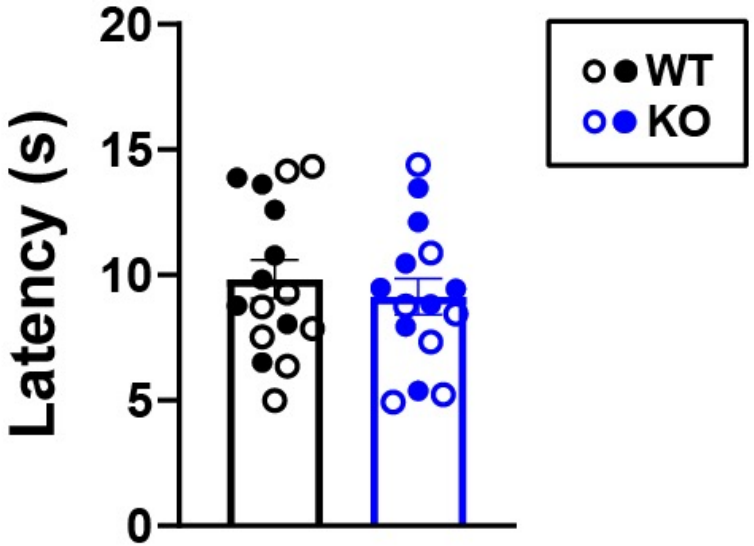




Hind Paw Thermal Sensitivity

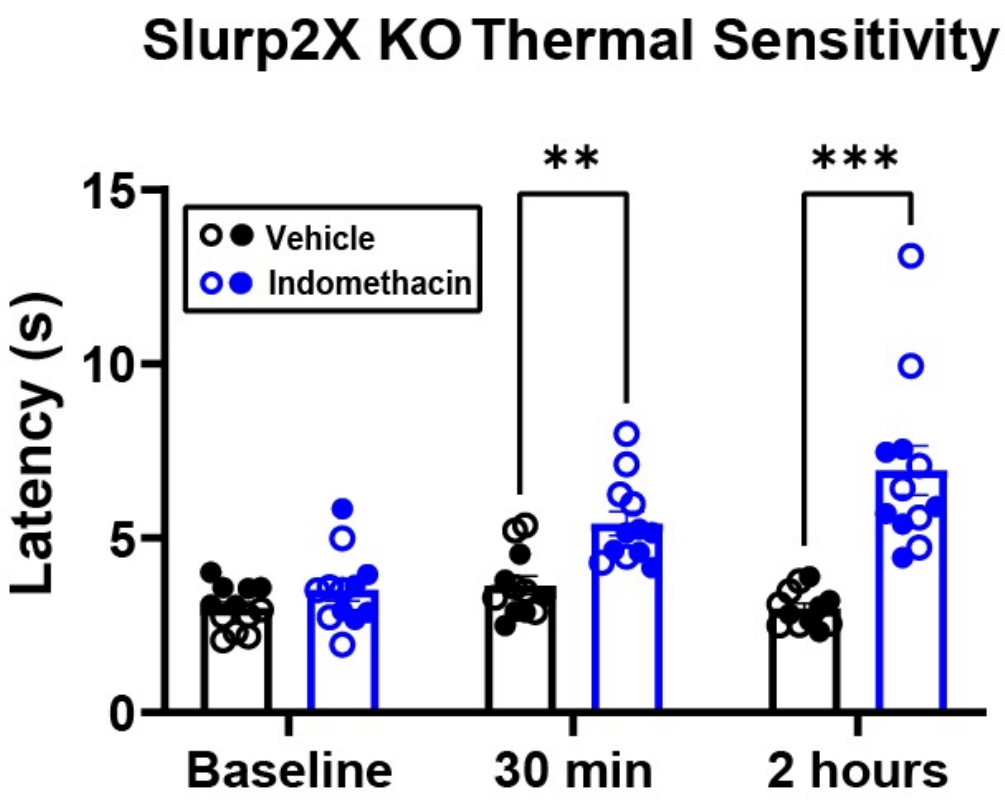


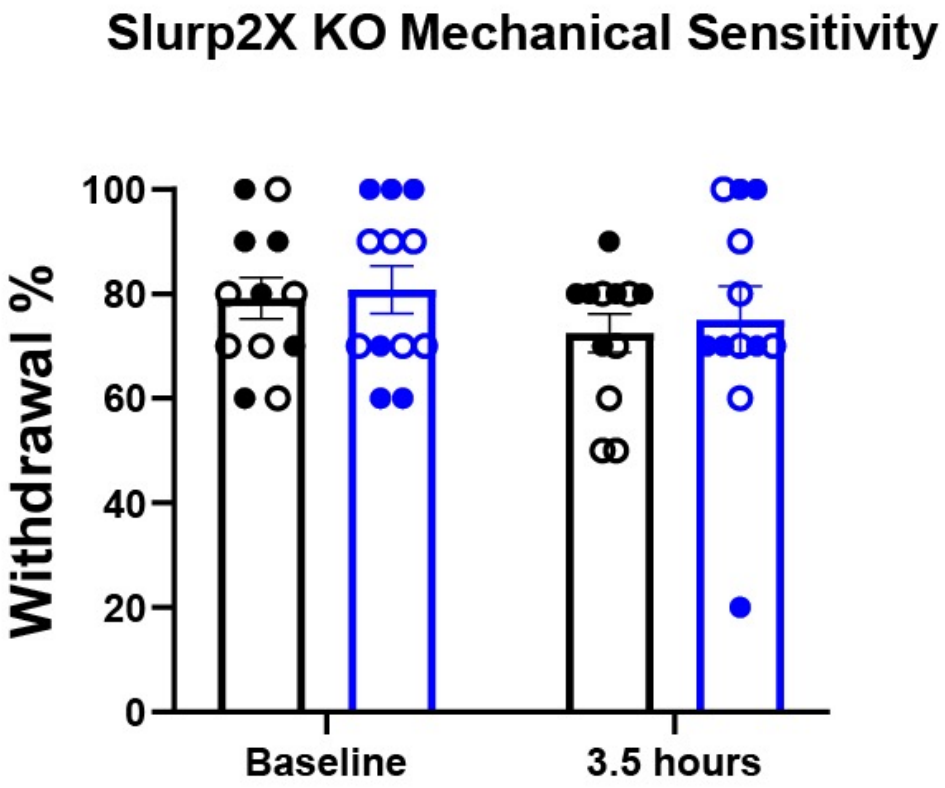
Tail Withdrawal from Heat



Rachel Weinberg

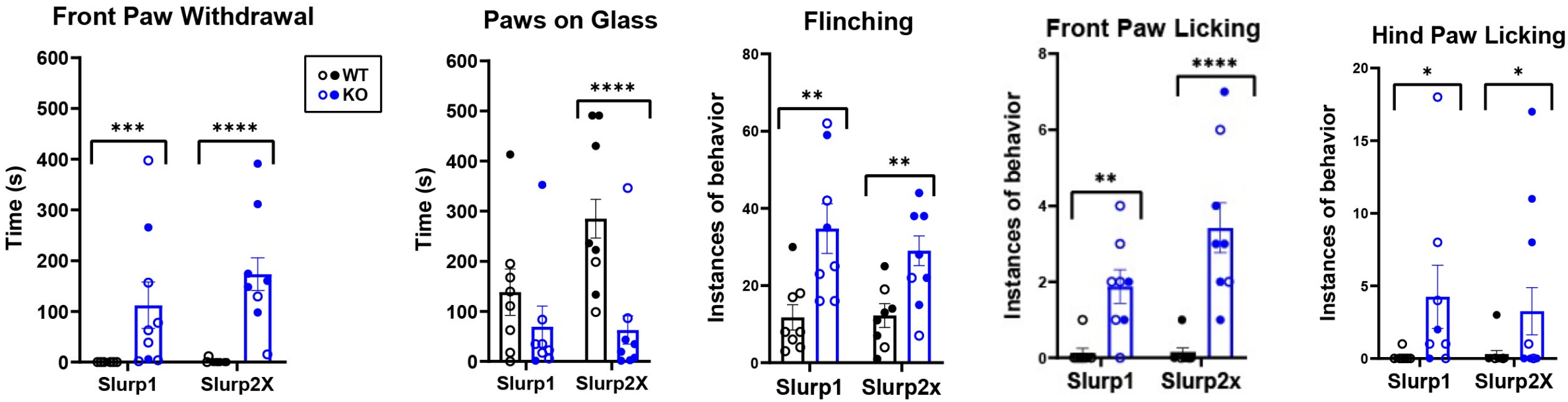
NSAIDs alleviate thermal hyperalgesia in Slurp KO mice





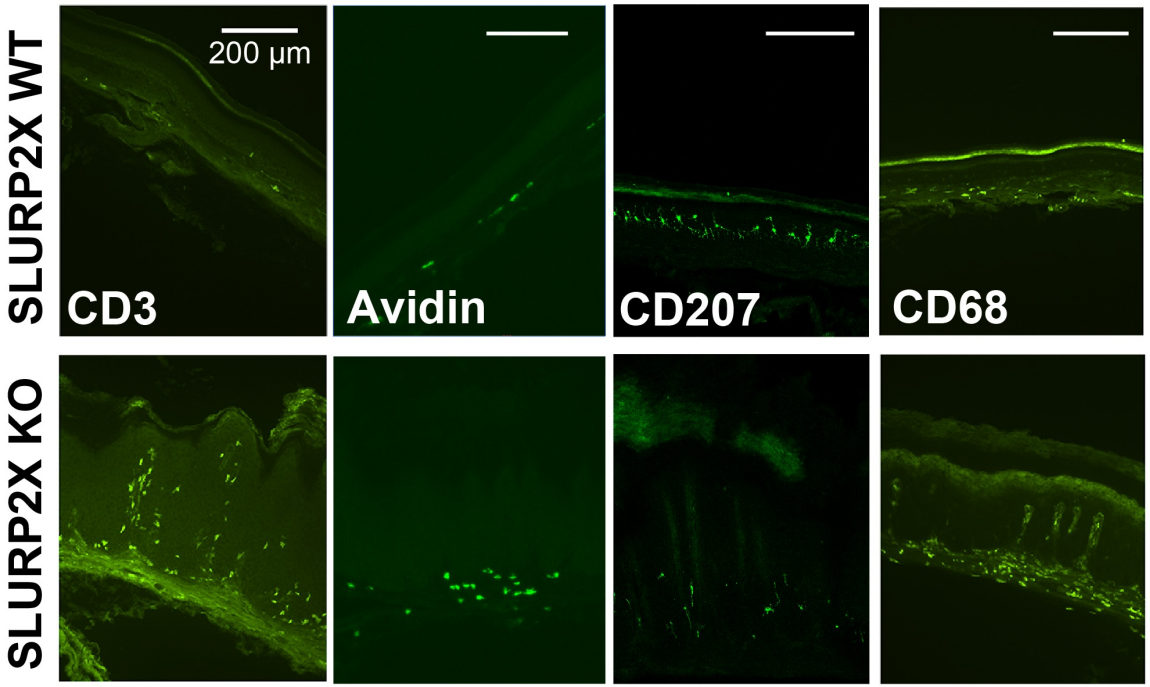
Rachel Weinberg

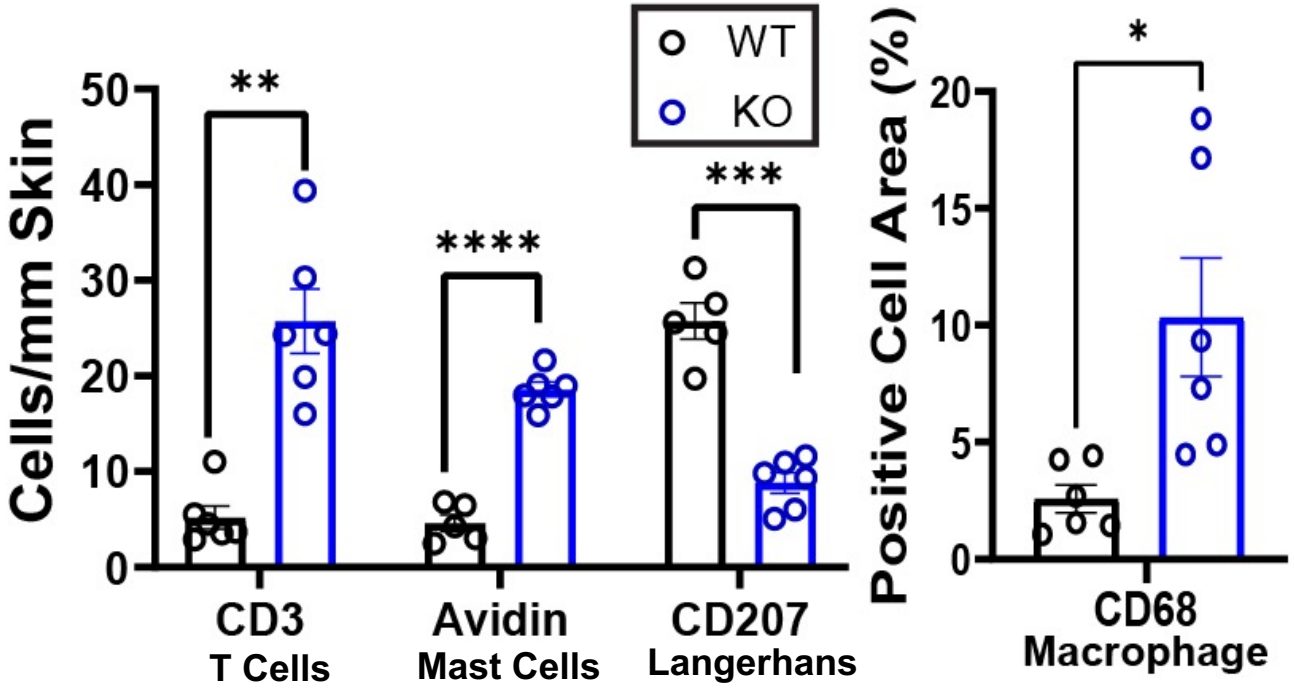
Increased Spontaneous Pain Behaviors in Slurp KO mice



Rachel Weinberg, Sandy Awad

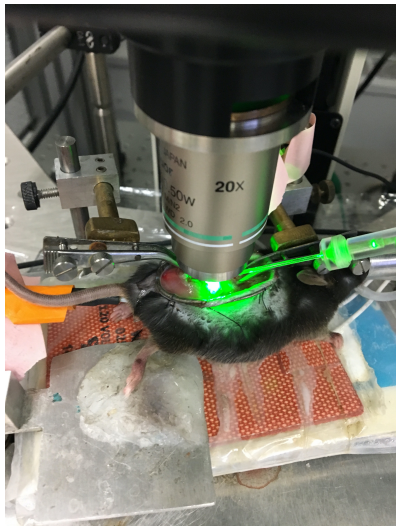
Mulitiple Inflammatory Cell Changes in PPK Skin of Slurp KO mice



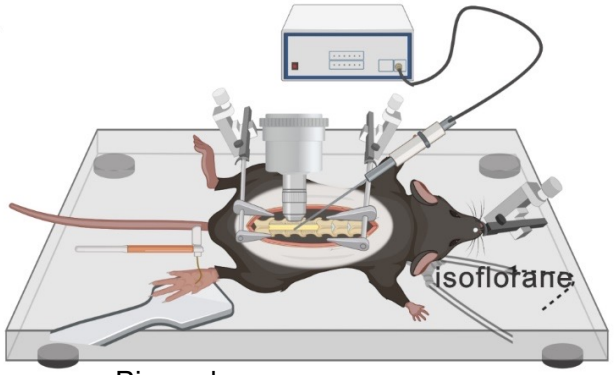


Rachel Weinberg, Leonie Bettin, Tyger Hanback

DRG Neurons from Slurp2x KO Mice are Hyperexcitable *in vivo*



A



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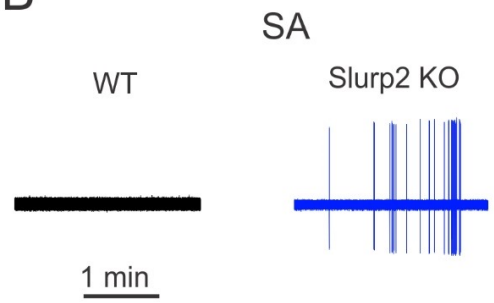
Spontaneous Activity

B



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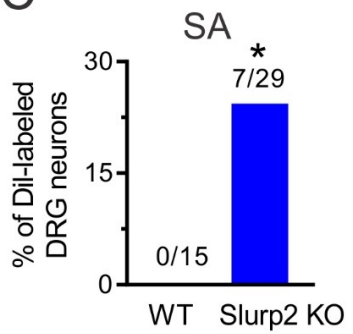

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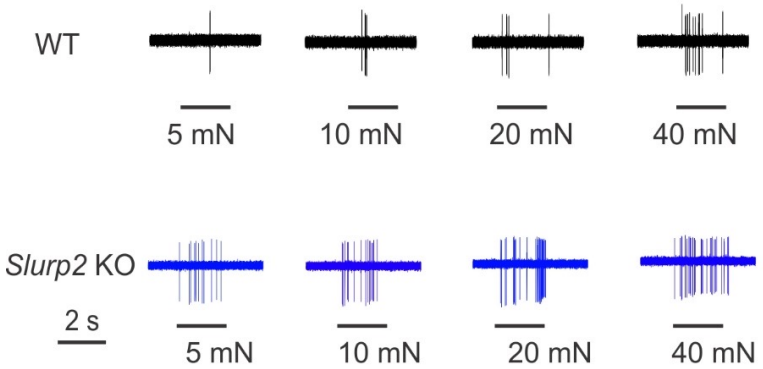

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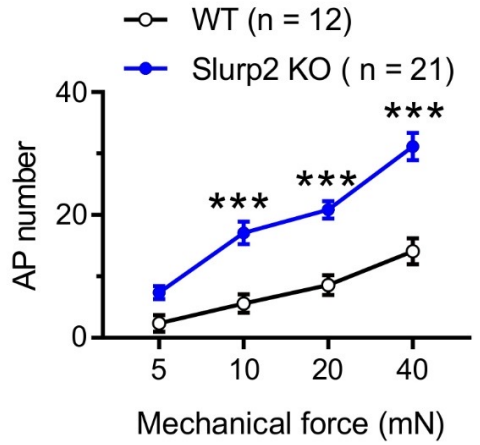

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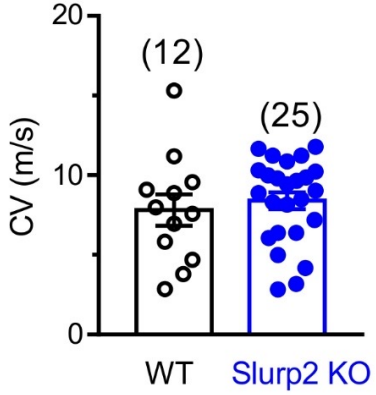
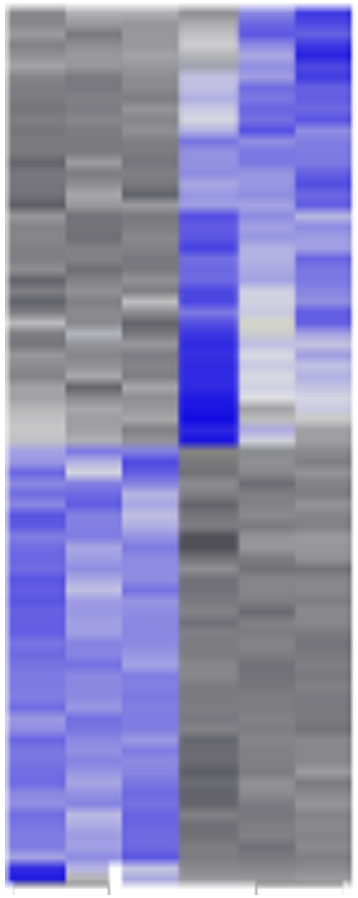


Lintao Qu

Transcriptomic Analysis of Skin and DRG in SLURP KO mice

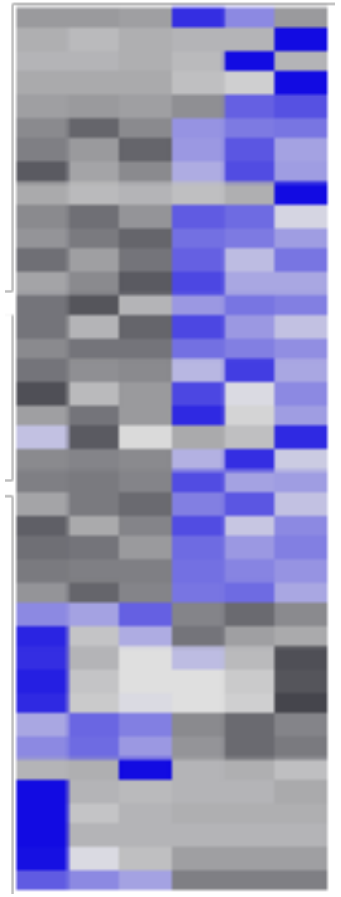
Paw Pad Skin
~5000 changes

WT SL1 KO



Lumbar DRG
~40 changes

WT SL1 KO



Gene Knockout, Knockdown in setting of SLURP KO ongoing

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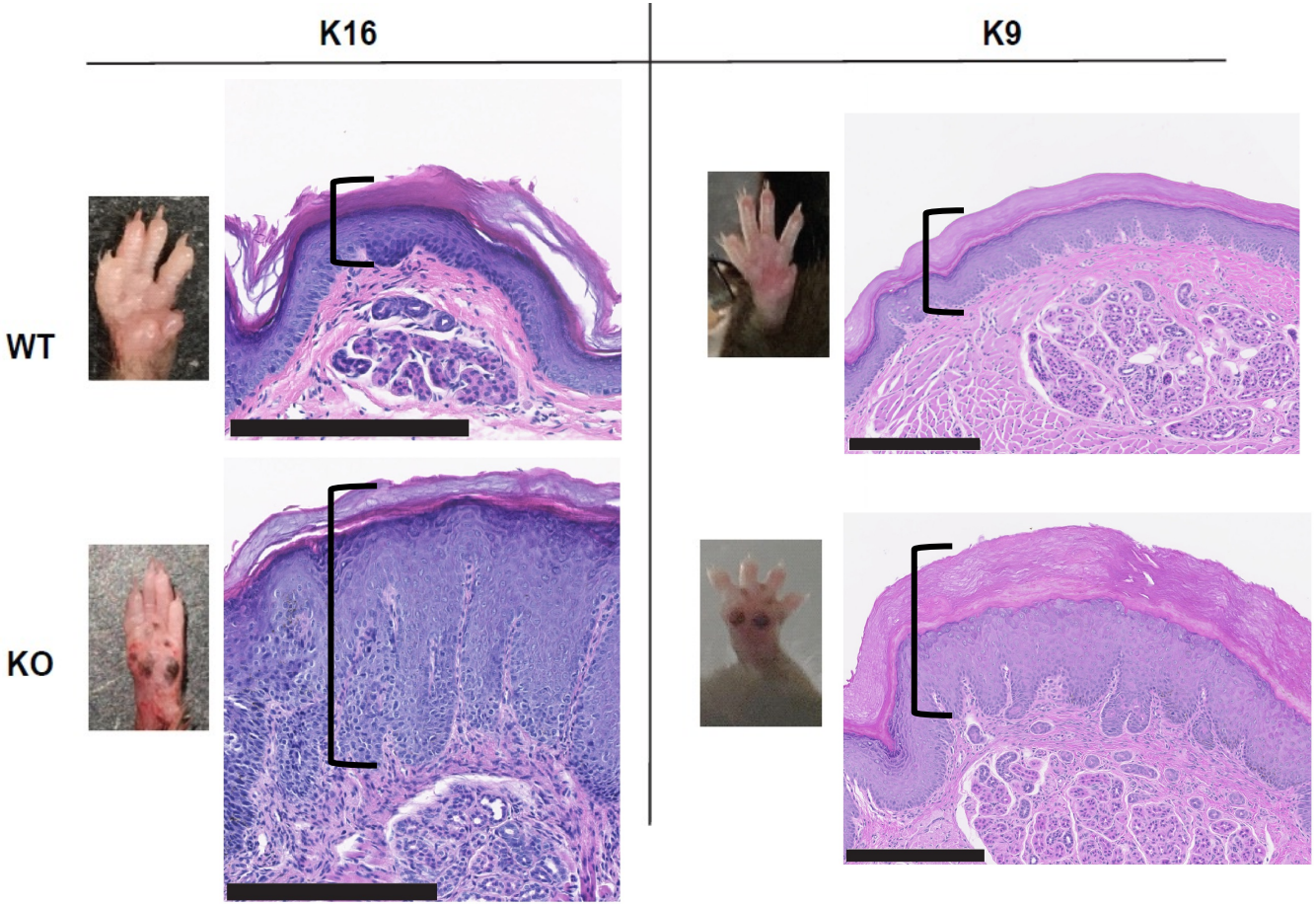
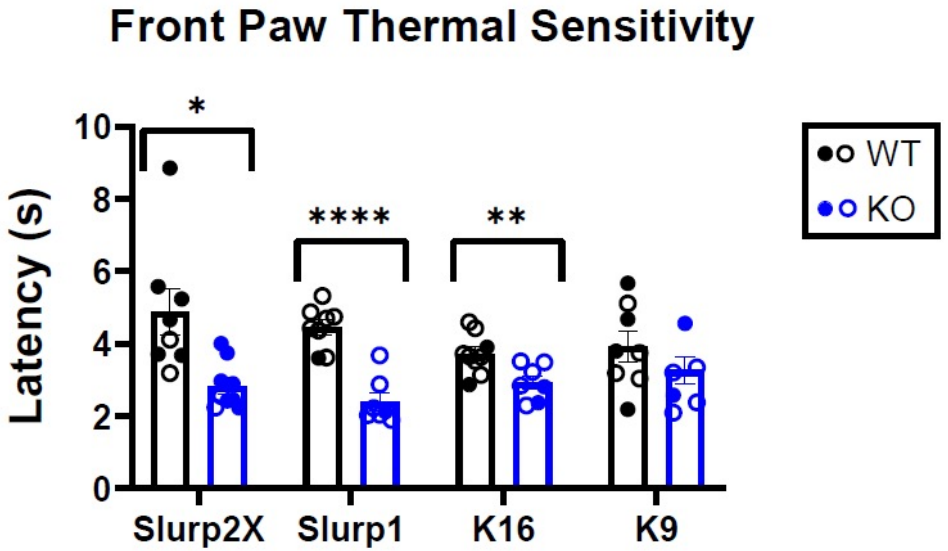
K16 KO and K9 KO models of PPK

Front Paws





Mice courtesy of Pierre Coulombe

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Conclusions – Part 2

1. SLURP1 KO and SLURP2X KO mouse models of Mal de Meleda show polymodal increase in pain sensitivity and spontaneous pain

2. Enhanced pain sensitivity is associated with a mixed immune cell infiltration

3. Enhanced pain sensitivity is associated with increased neuronal responsiveness and spontaneous firing

4. Enhanced pain sensitivity is associated with numerous gene expression changes in skin and sensory ganglia

5. A spectrum of mouse PPK models offers an excellent opportunity for comparative functional and molecular analyses to define heterogeneous mechanisms underlying pain in PPKs

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