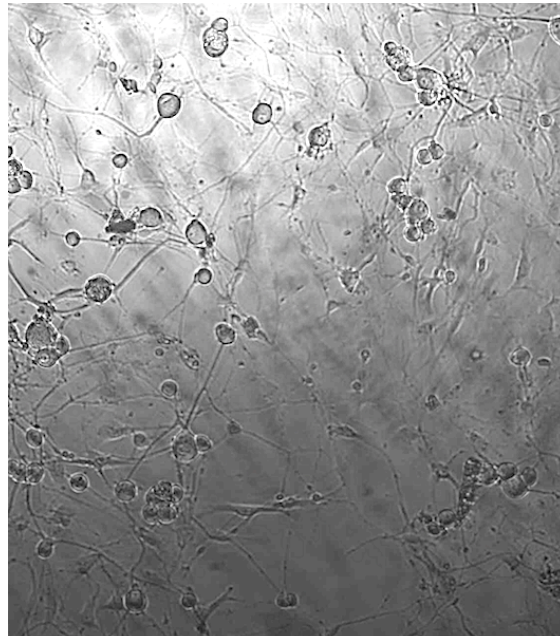
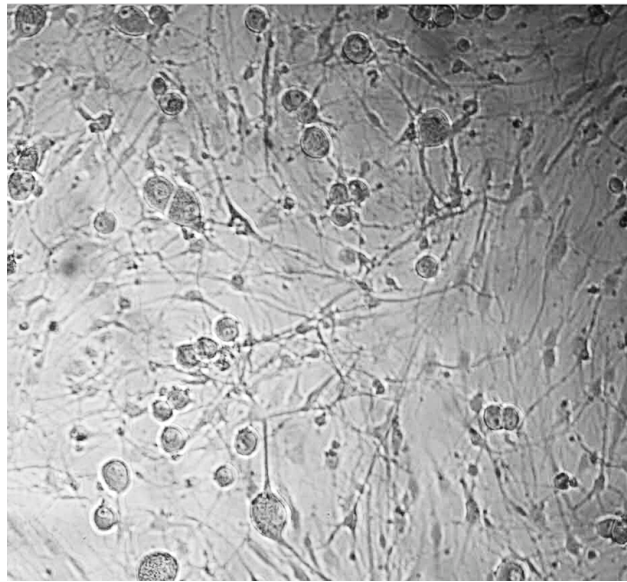


The Secretomes of Painful Versus Nonpainful Human Schwannomatosis Tumor Cells Differentially Influence Sensory Neuron Gene Expression and Sensitivity

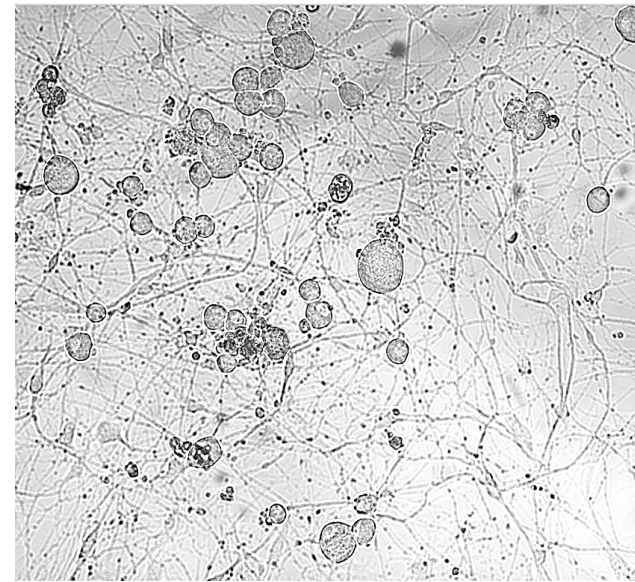
Kimberly Laskie Ostrow<sup>1,5\*</sup>, Katelyn J. Donaldson<sup>1</sup>; Randy Rubright<sup>1</sup>; Michael J. Caterina<sup>2,3,4,5</sup>; Allan Belzberg<sup>2,5</sup> Ahmet Hoke<sup>1</sup>



Painful Tumor 1 CM



Painful Tumor 2 CM

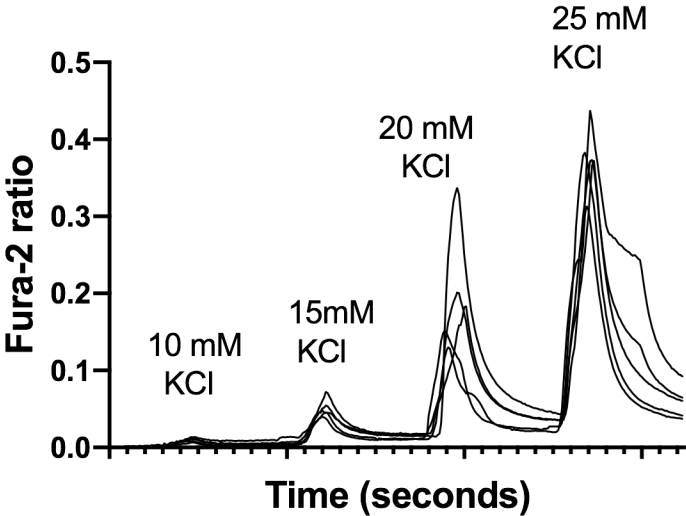


Non-Painful Tumor 2 CM

Supplemental Figure 1: Brightfield images of CM-treated DRGs prior to Calcium imaging. Neurite sprouting can be visualized in DRGs after 48 hours of incubation with SWN tumor CM regardless of pain status.

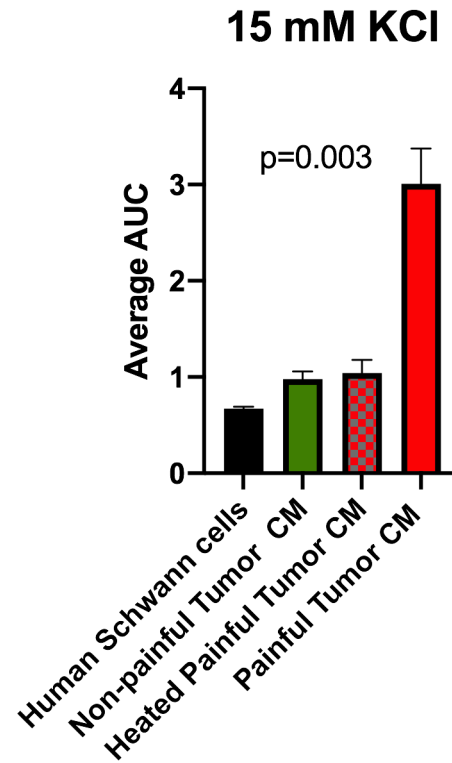
# Dose response to KCl After treatment with NP Tumor CM

— Non-painful Tumor 1      — Non-painful Tumor 2      — Non-painful Tumor 3  
 — average non-pain      — Human Schwann cells (control)

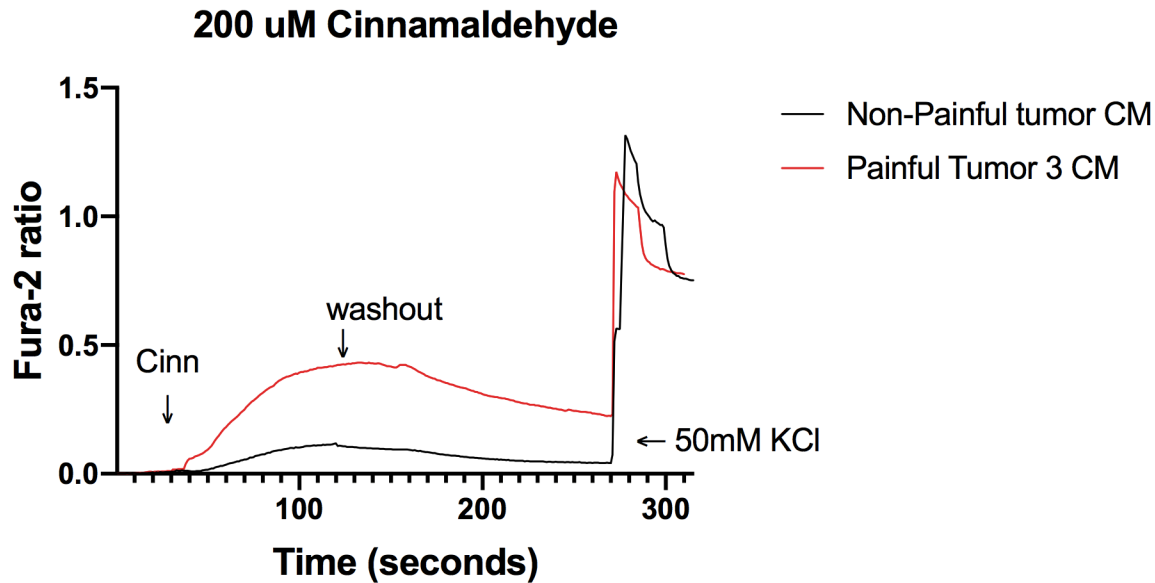


Supplemental Figure 2:  
 KCL dose response after pre-treatment with 3 separate non-painful tumor CMs. AUC was not statistically significant between groups.

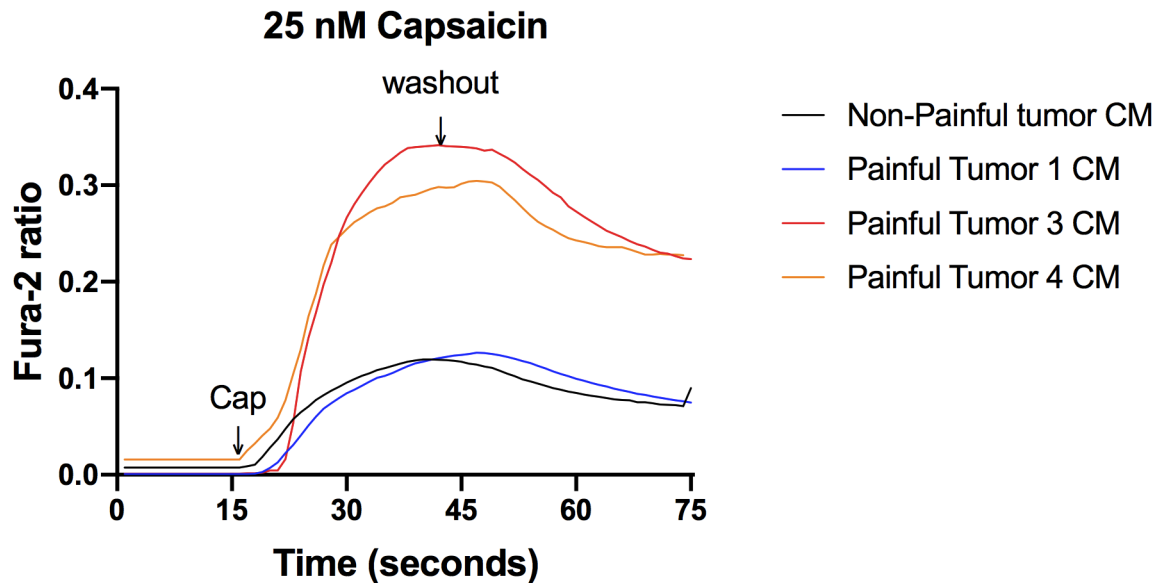
KCL Dose	Average AUC NP Tumor 1	Average AUC NP Tumor 2	Average AUC NP Tumor 3	p value
10 mM	0.243 (+/- 0.07)	0.120 (+/- 0.08)	0.221 (+/- 0.06)	p=0.37
15 mM	0.946 (+/- 0.12)	0.85 (+/- 0.24)	1.14 (+/- 0.16)	p=0.4
20 mM	3.12 (+/- 0.51)	2.86 (+/- 0.53)	4.2 (+/- 0.59)	p=0.2
25 mM	6.45 (+/- 1.68)	6.92 (+/- 1.58)	6.94 (+/- 0.74)	p=0.9



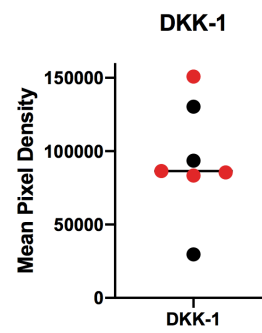
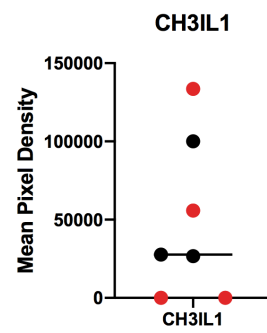
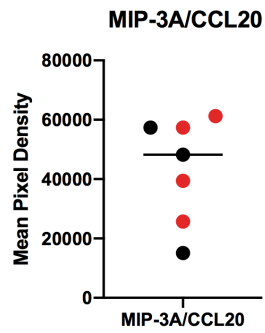
Supplemental Figure 3: Effect of Heating painful Tumor CM. Heating the painful CM at 80C for 30 min (**red patterned**) reduced responsiveness to that of non-painful CM (green)



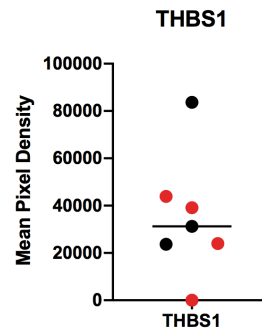
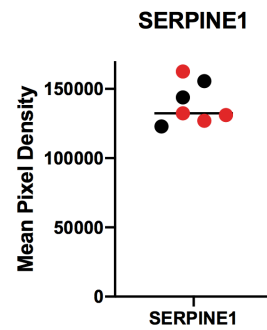
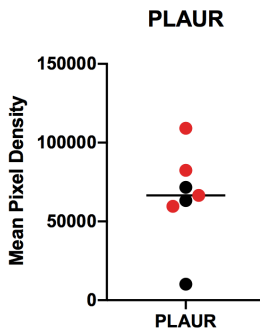
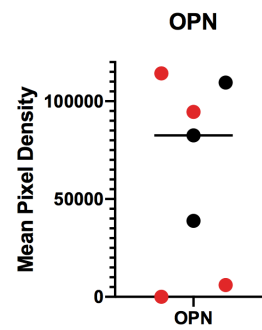
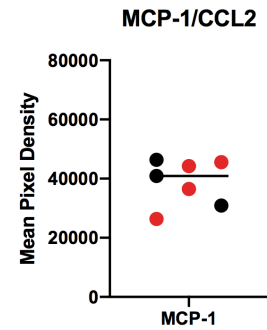
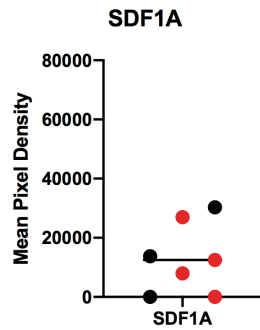
Supplemental Figure 4: DRG cells were perfused 200 uM Cinnamaldehyde for 120 sec with a washout with 3mM KCl buffer for and additional 2 minutes. Fura-2 ratio measurements were recorded at 2 second intervals. The datapoints were corrected for differing baseline readings of Fura-2. Area under the curve was calculated for each treatment. The effect of cinnamaldehyde started 30-60 seconds after treatment peaked at 120 seconds, and lasted for 4 minutes. Painful tumor 3 CM increased the responsiveness of the DRGs to cinnamaldehyde as calculated by area under the curve  $p=0.03$ .



Supplemental Figure 5: DRG cells were perfused 25nM capsaicin for 30sec with a washout with 3mM KCl buffer for 2 minutes. Fura-2 ratio measurements were recorded at 2 second intervals. The datapoints were corrected for differing baseline readings of Fura-2. Area under the curve was calculated for each treatment. The effect of capsaicin started immediately peaked after 30 seconds lasted for 1 minute. CM from painful tumors 3 and 4 CM increased the responsiveness of the DRGs to capsaicin as calculated by area under the curve  $p=0.0001$ . One way ANOVA with multiple comparisons was employed to determine statistical significance.



Supplemental Figure 6:  
Cytokines that were detected  
in CM that were similar  
between painful and Non-  
painful CM.



**Red** Painful CM  
**Black** Non-painful CM

NON-PAIN

PAINFUL

2014  
0

NP1

2014  
F  
NP3  
cell

PT  
1

2017-005  
NP2

2014-002  
2017-005

PT  
2

2017-004  
NP3  
semi

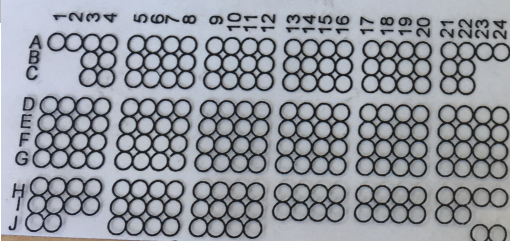
16-104

P  
3

17-002

PT  
4

Human XL Cytokine Array  
Transparency Overlay



Supplemental Figure 7: Human cytokine array blots after 2 min exposure to X-ray film. Positions A 1,2 A23,24 and J 23, 24 are positive controls used for normalizing protein levels between blots.