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The functional role of ion channels in cancer: New developments!

Prof. Dr. İnci Özer

5 February 1942 – 16 September 2011

Nav channels and cancer (metastatic disease)!

1. **Introduction**
- Principles
- Hypothesis
2. **Role of Nav's in metastatic cell behaviours**
3. **Systems (patho)biology of VGSC expression in cancer**
4. ***In vivo* evidence**
5. **Clinical potential: Can cancer Nav (nNav1.5) be inhibited specifically?**
6. **Conclusions!**

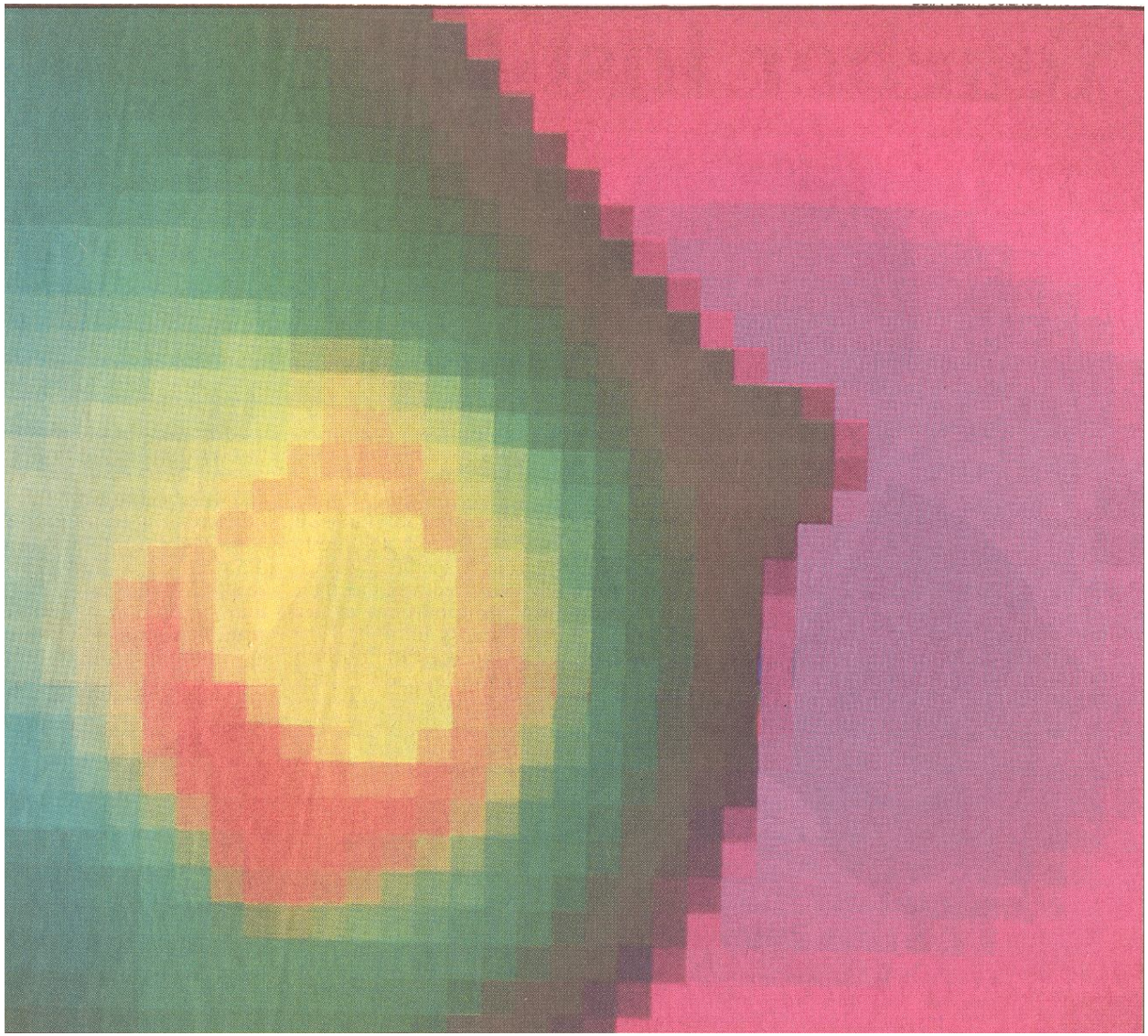
Problems in clinical management of cancer :

1. Definitive / functional diagnosis
&
2. Effective therapy
(long-lasting and non-toxic)

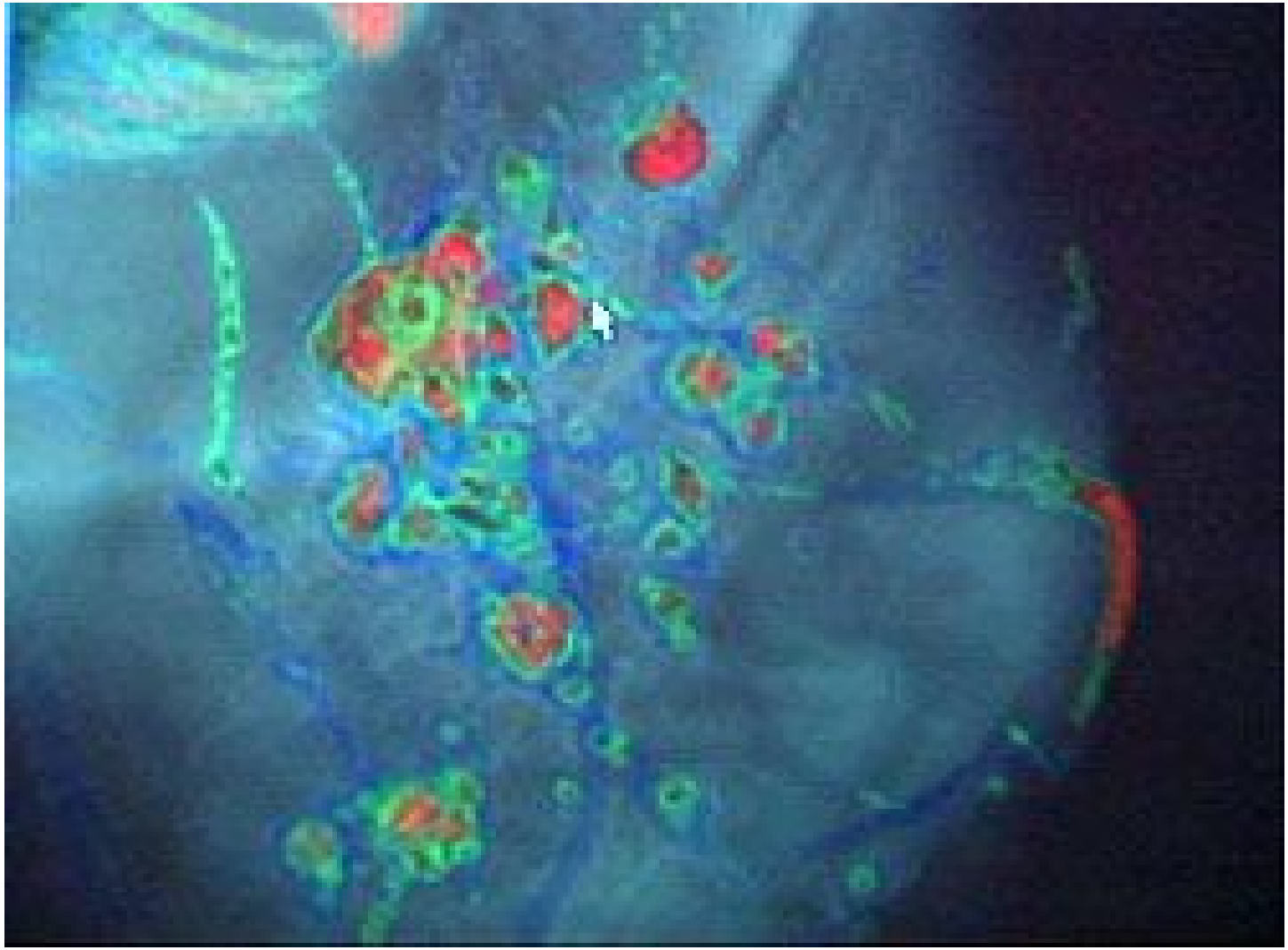
PRINCIPLES

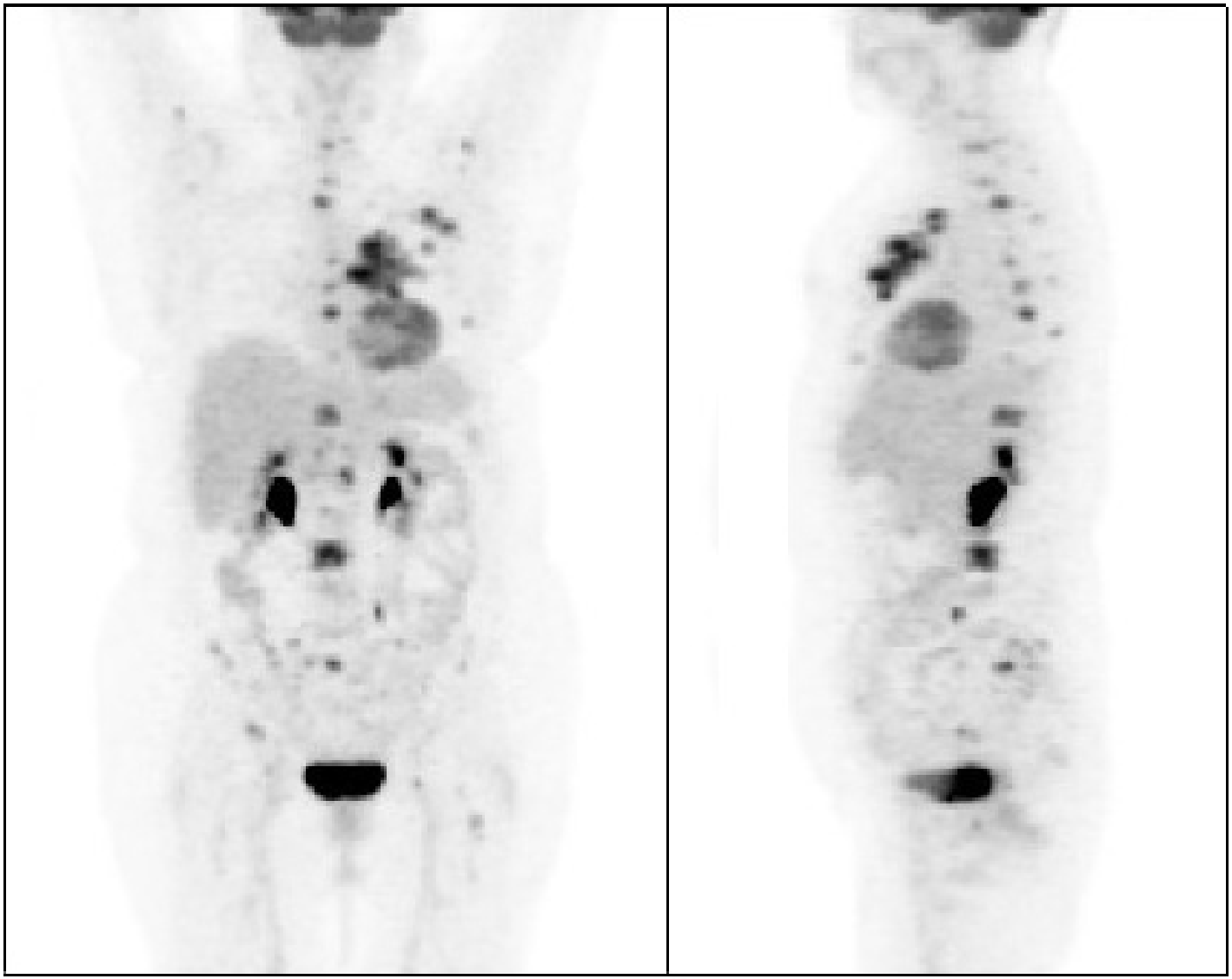
1. Cancer is a complex process (nb so is brain physiology).
2. What kills most people with cancer is metastasis (secondary tumorigenesis).
3. Primary tumorigenesis (proliferation) and secondary tumorigenesis (metastases) may be programmed differently, even independently!
4. Metastasis involves a range of basic cellular behaviours.

PRIMARY TUMOURIGENESIS



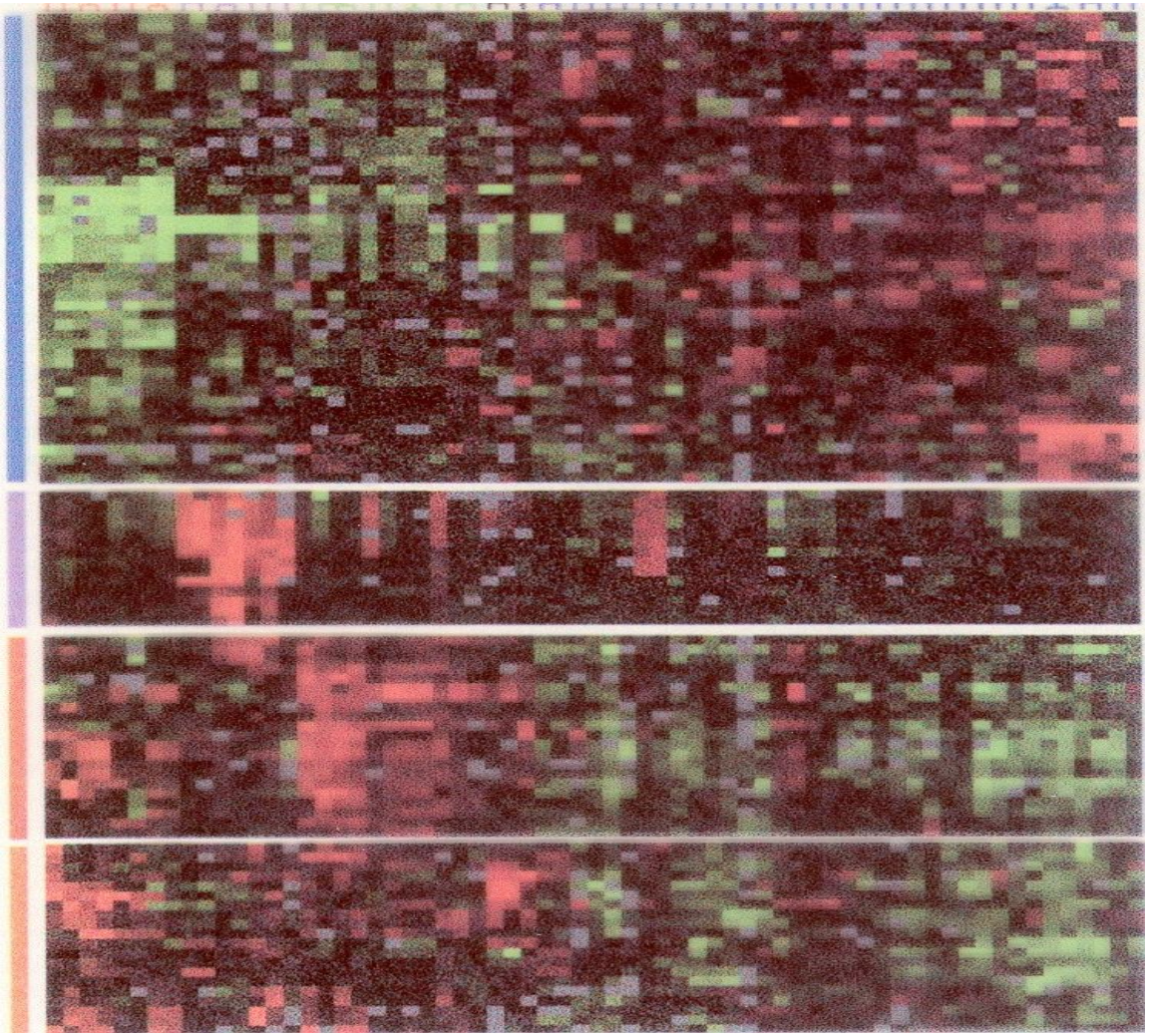
Invasive BCa





PET (positron emission tomography) scan
of a patient with metastatic breast cancer

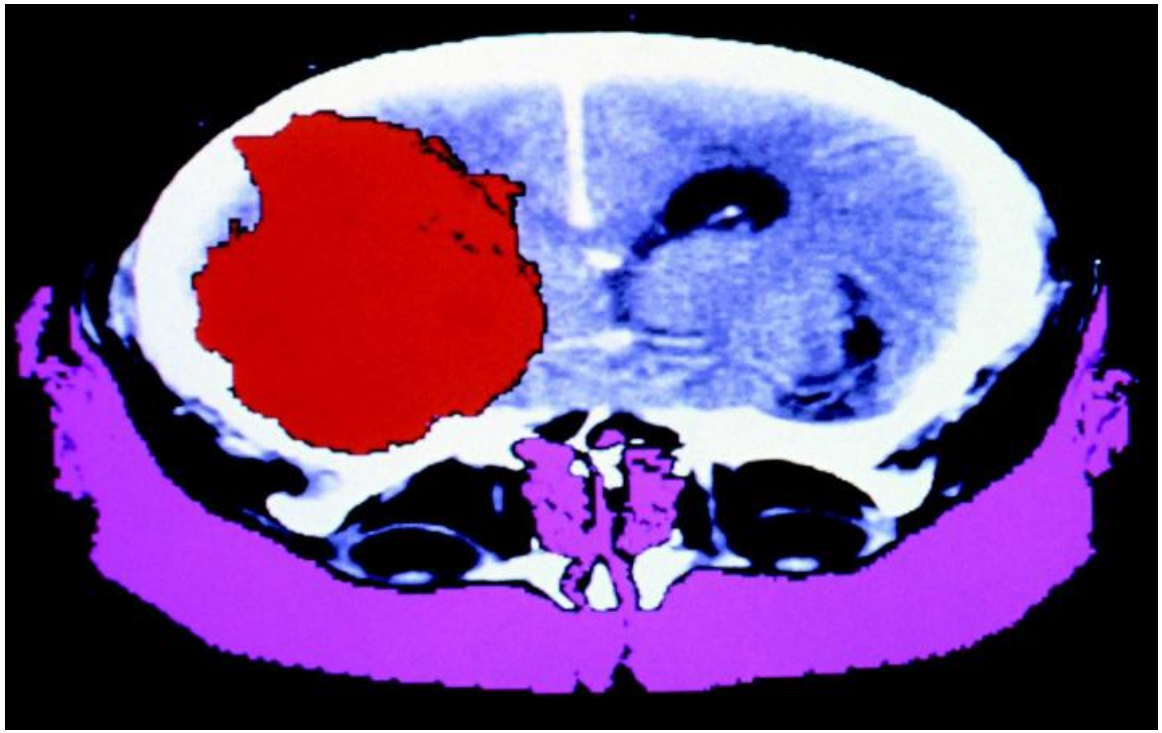
Genes and Cancer



NEWS NATURE (2008) Vol 455, p.148 (11 September 2008)

Cancer complexity slows quest for cure!

Hopes that large studies of cancer genomics will justify their high cost by offering a fast-track to cures have been dealt a blow by a series of papers.



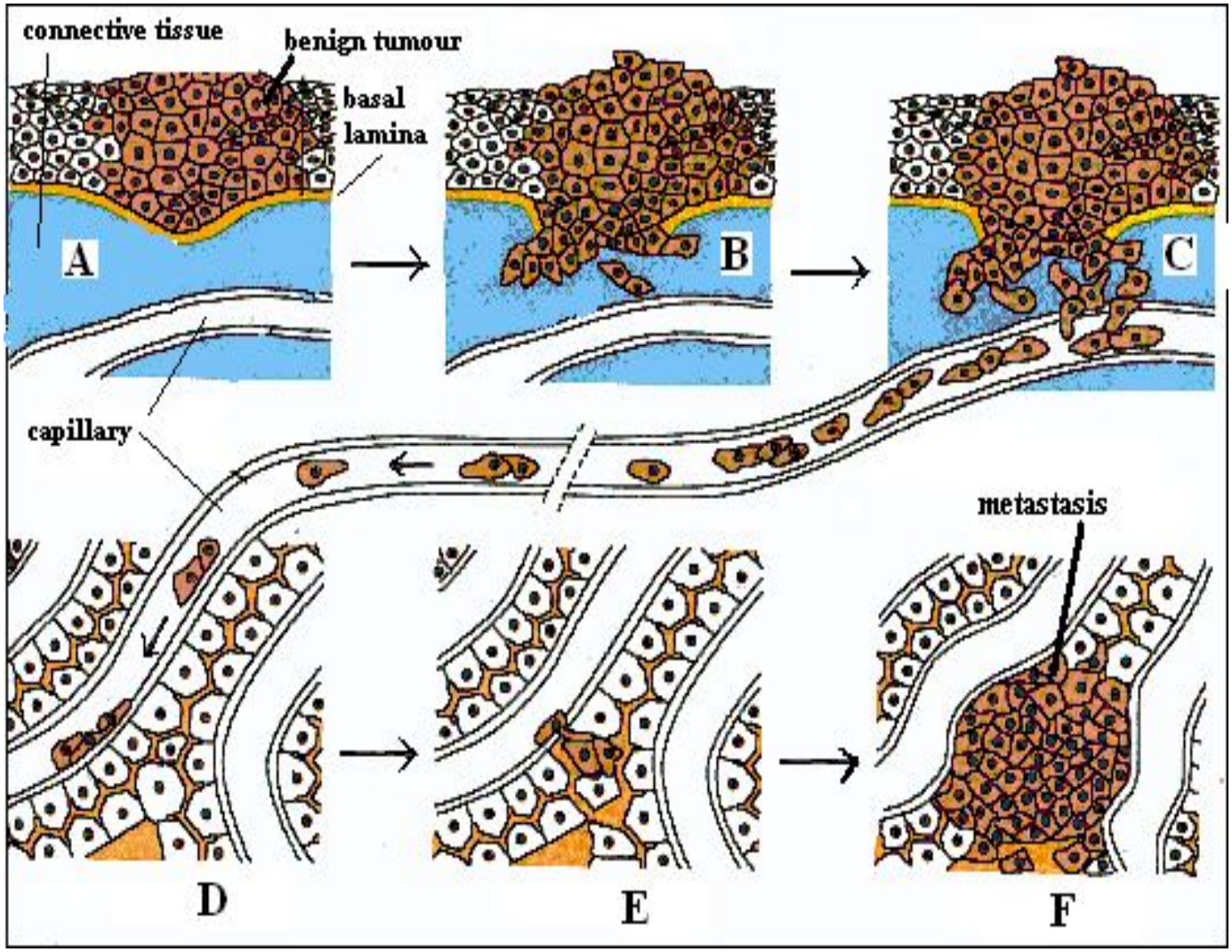
Solid tumours such as glioblastoma (red) can be caused by multiple genes in different patients.

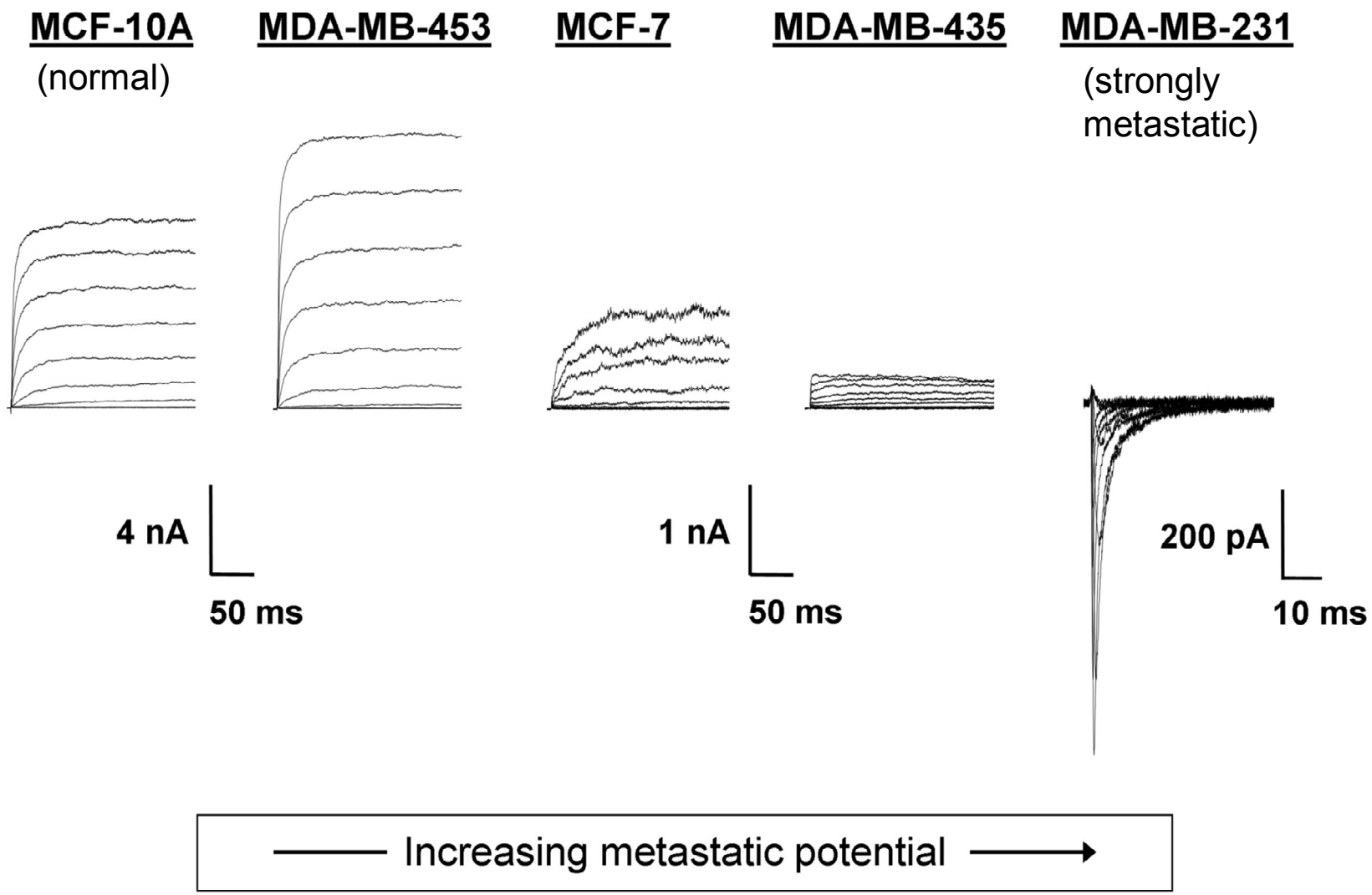
gene → protein → signal → function
(→ disease)

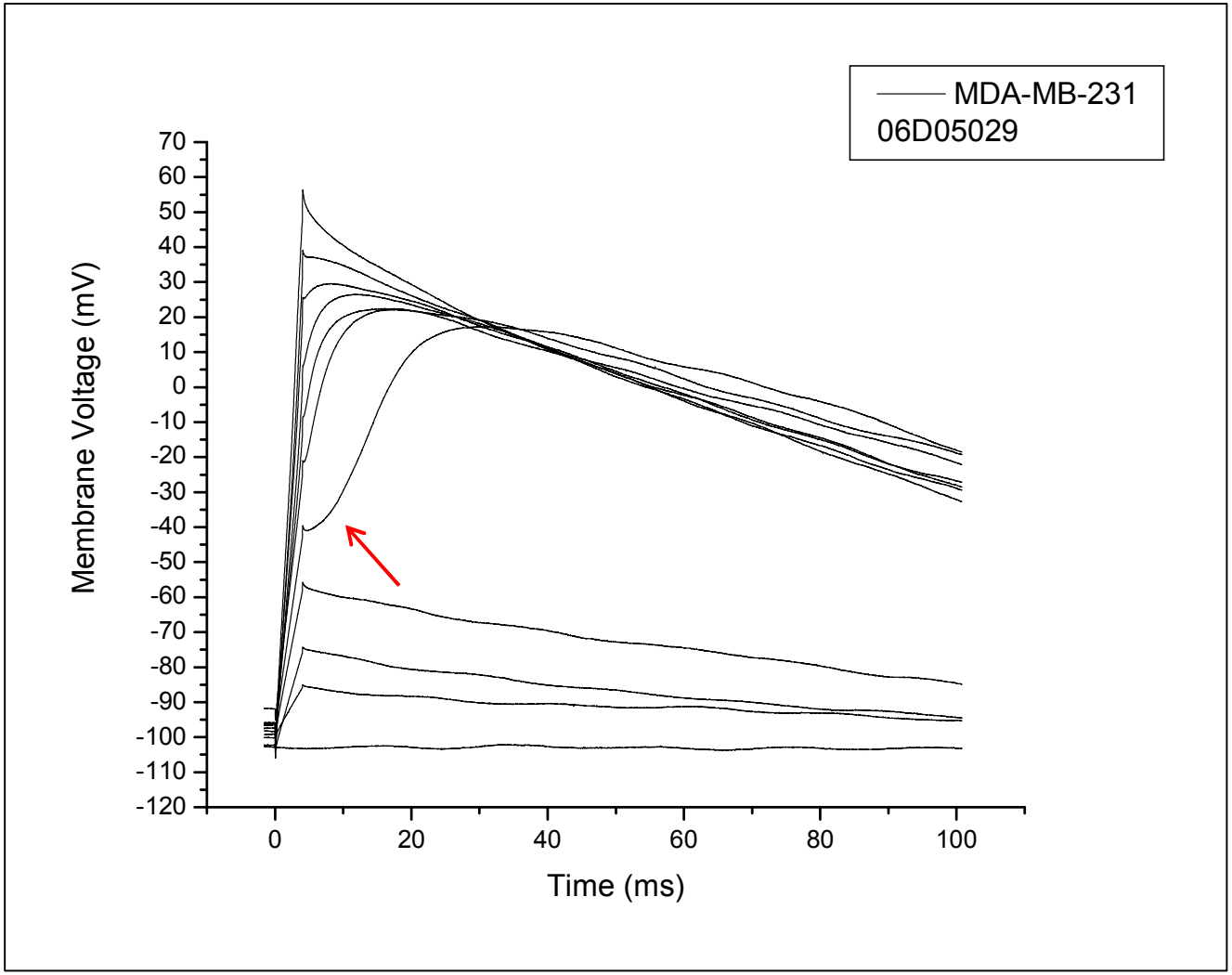
-

ELECTRO-PHYSIOLOGICAL SIGNALLING!

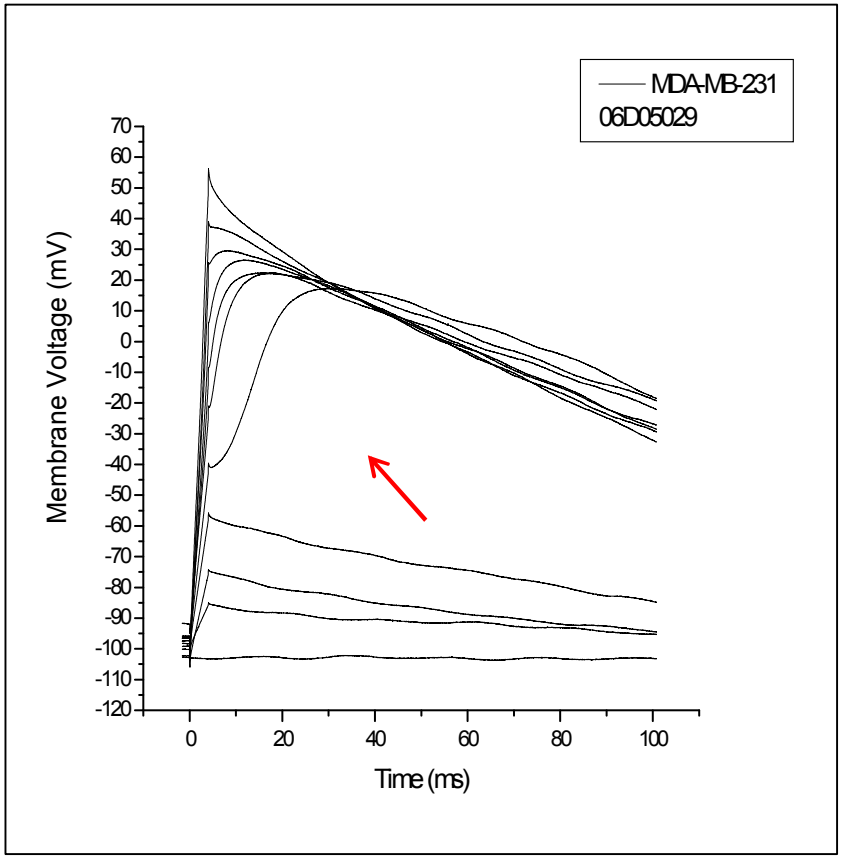
Metastatic cascade



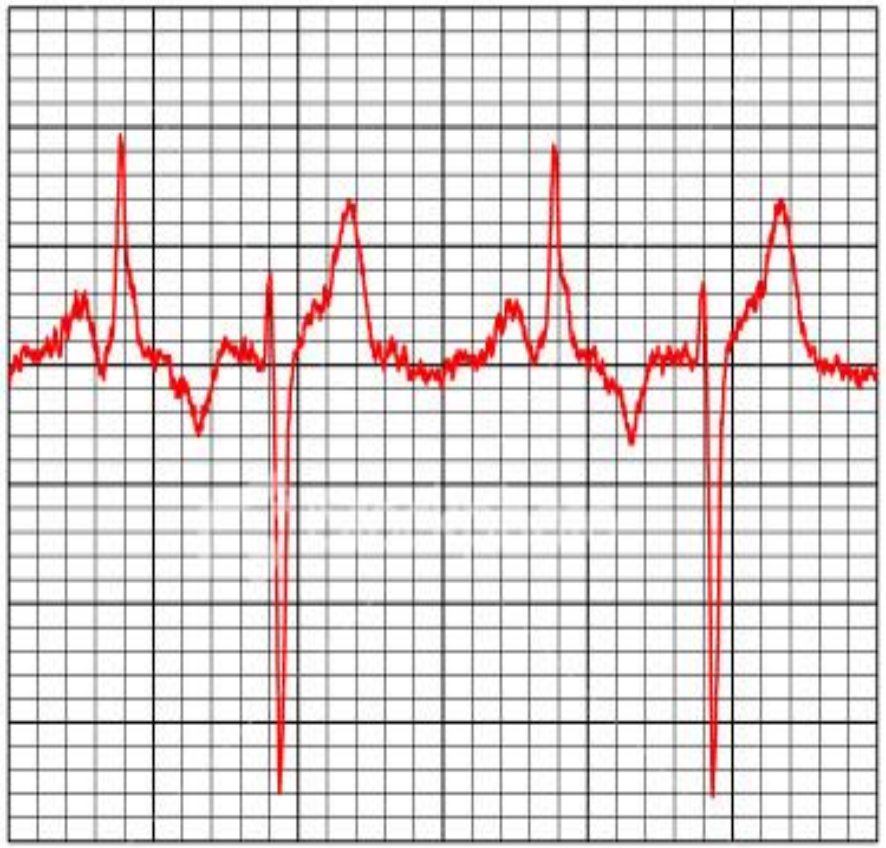

MCF-10A
(normal)
MDA-MB-453
MCF-7
MDA-MB-435
MDA-MB-231
(strongly
metastatic)
4 nA
50 ms
1 nA
50 ms
200 pA
10 ms
Increasing metastatic potential


MDA-MB-231
06D05029
Membrane Voltage (mV)
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
0
20
40
60
80
100
Time (ms)

Cellular excitability!



breast cancer cell –
action potential

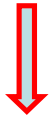


heart ECG

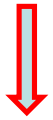
CELEX Hypothesis :

$I_{in}\ (I_{Na})\uparrow + I_{out}\ (I_K)\downarrow$

membrane excitability
(regenerative activity)



cellular hyperactivity
('unsociable' behaviour)



Metastatic potential ↑

VGSC upregulation

(+ VGPC downregulation

where studied) found in:

- Breast cancer
- Prostate cancer
- Lung cancer (SCLC, NSCLC and mesothelioma)
- Cervical cancer
- Ovarian cancer
- Colon cancer
- ++

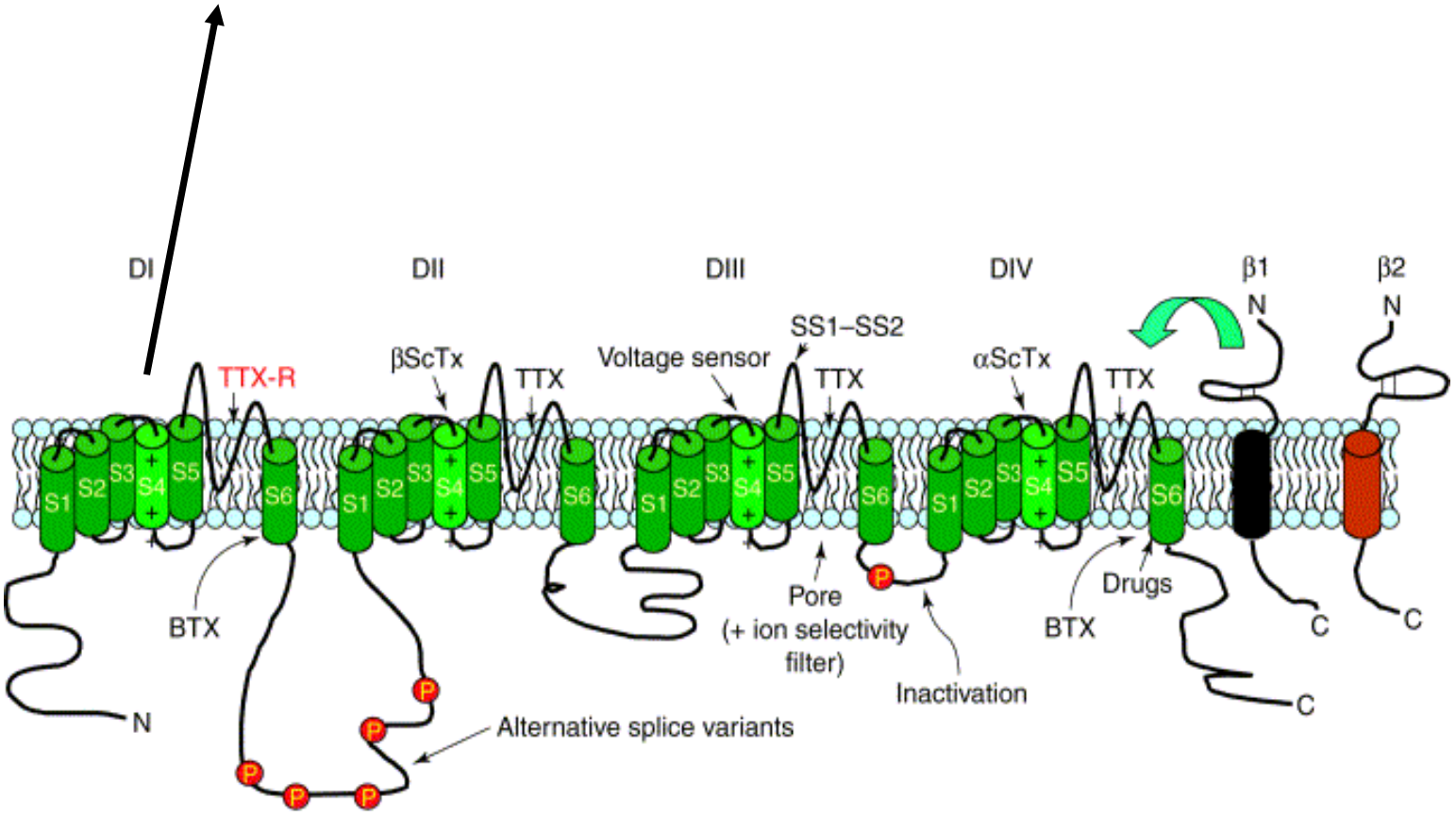
In vitro evidence (TTX, siRNA and pAb)

- Lateral motility
- Transverse migration
- Electrotaxis
- Adhesion
- NO production
- Endocytic membrane activity (secretion)
- Invasion
- Angiogenesis!

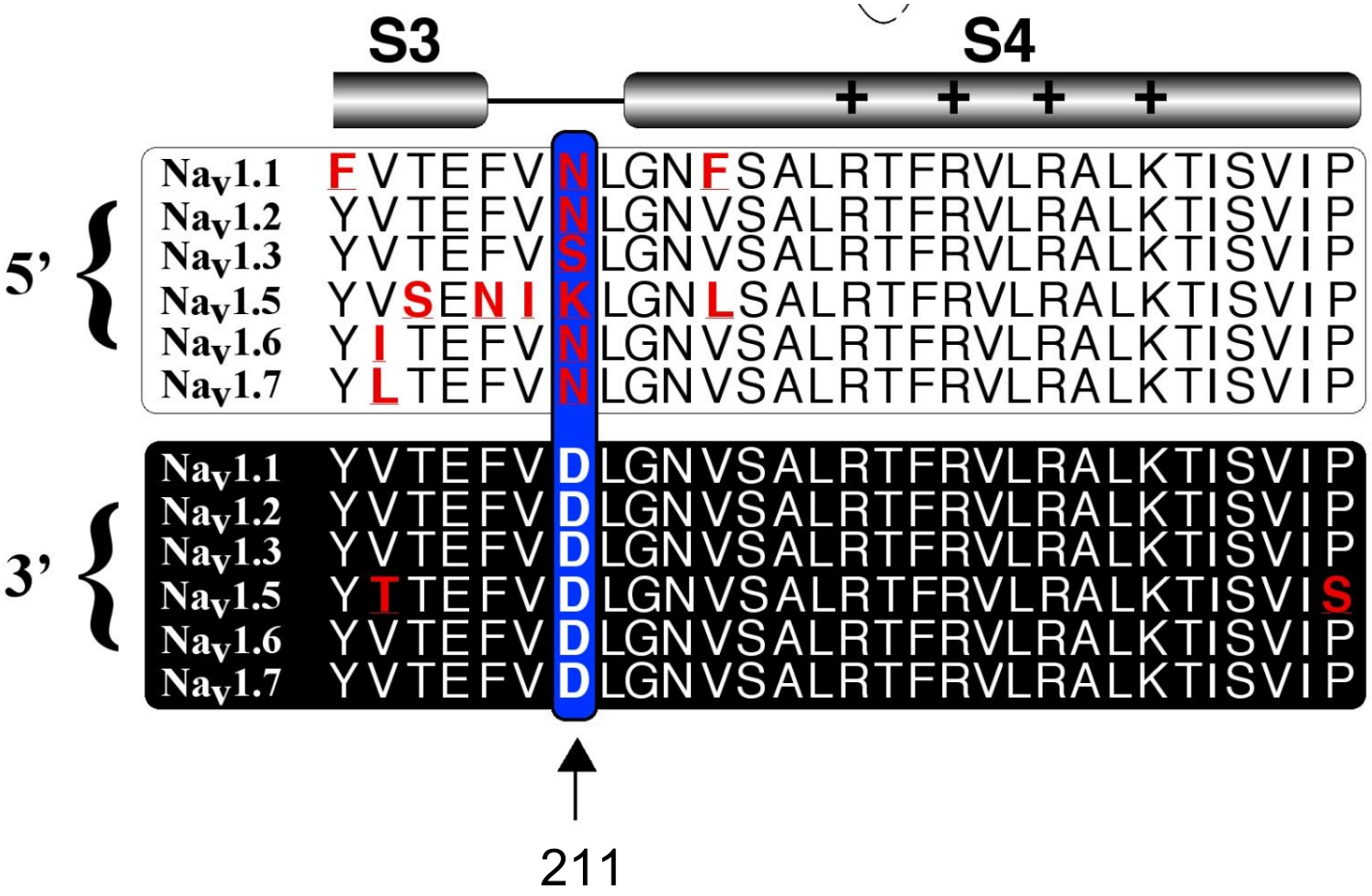
What is the molecular nature of the culprit VGSC in metastatic BCa?

Consensus : Y V T E F V X L G N V S A L R T F R V L R A L K T I S V I P

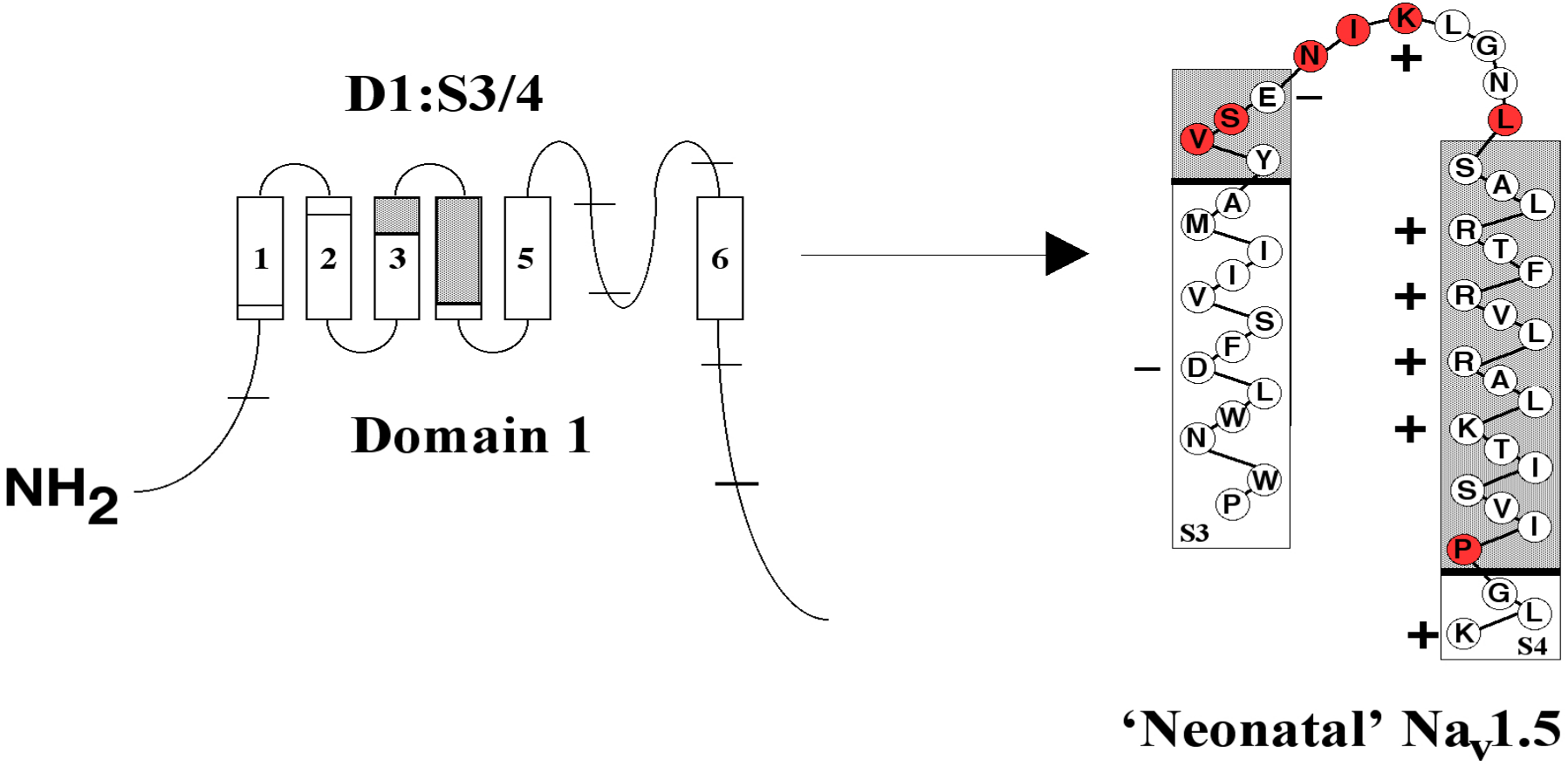
Nav1.5 (N) : . . S . N I K . . . L . . . .
Nav1.5 (A) : . T . . . . D . . . . S



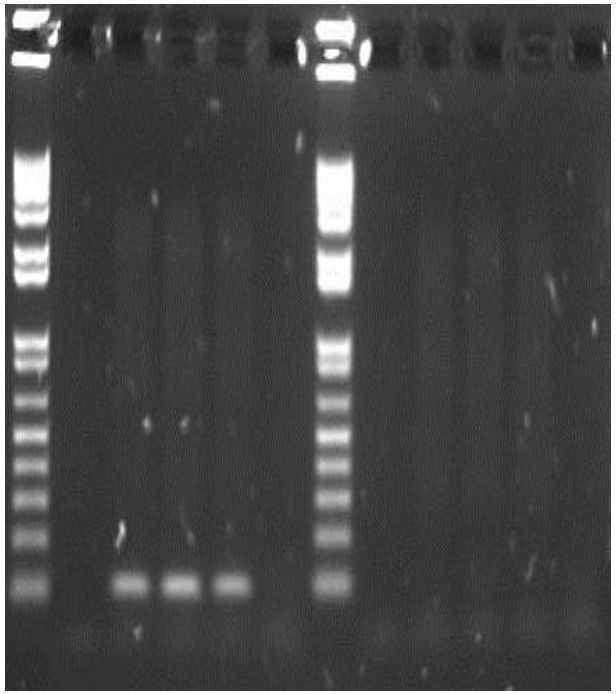
nNav1.5 D1:S3 is unique among VGSCα subunits!



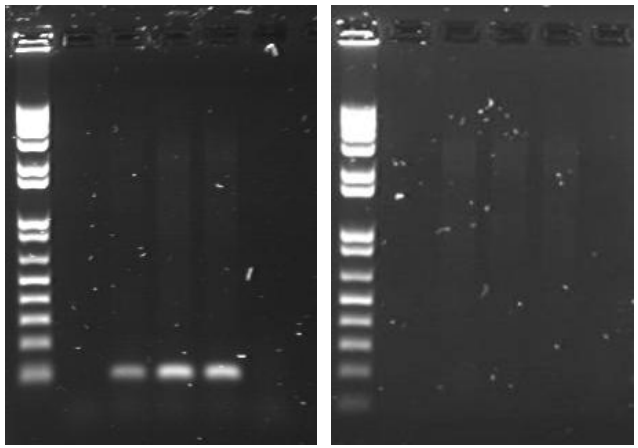
The unique sequence of Nav1.5 D1:S3/4 splice variant (Blast analysis)



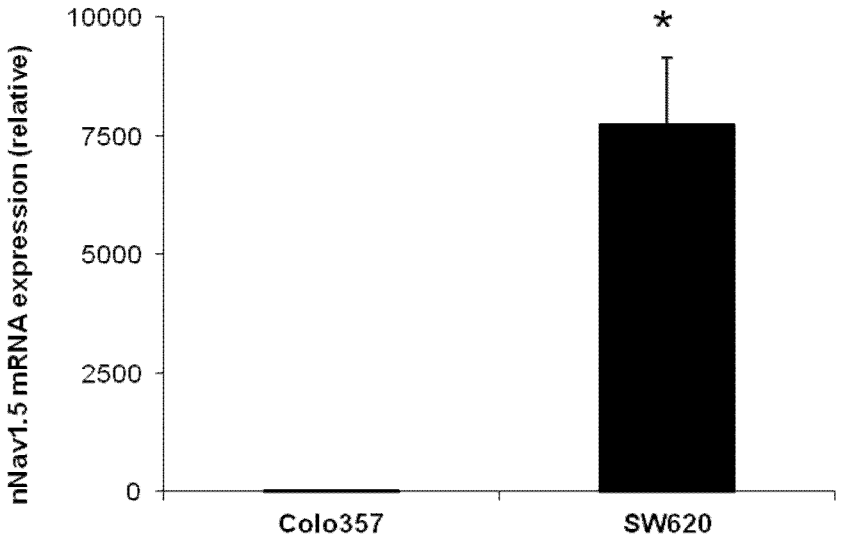
SW-620 Colo-357

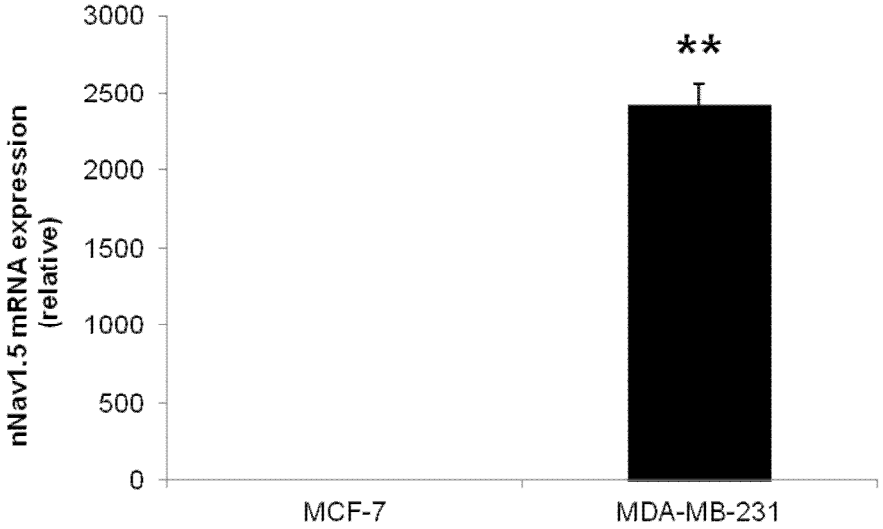


MDA-MB-231 MCF-7



Selective expression of nNav1.5 mRNA in human colon metastatic cancer cells of strong metastatic potential

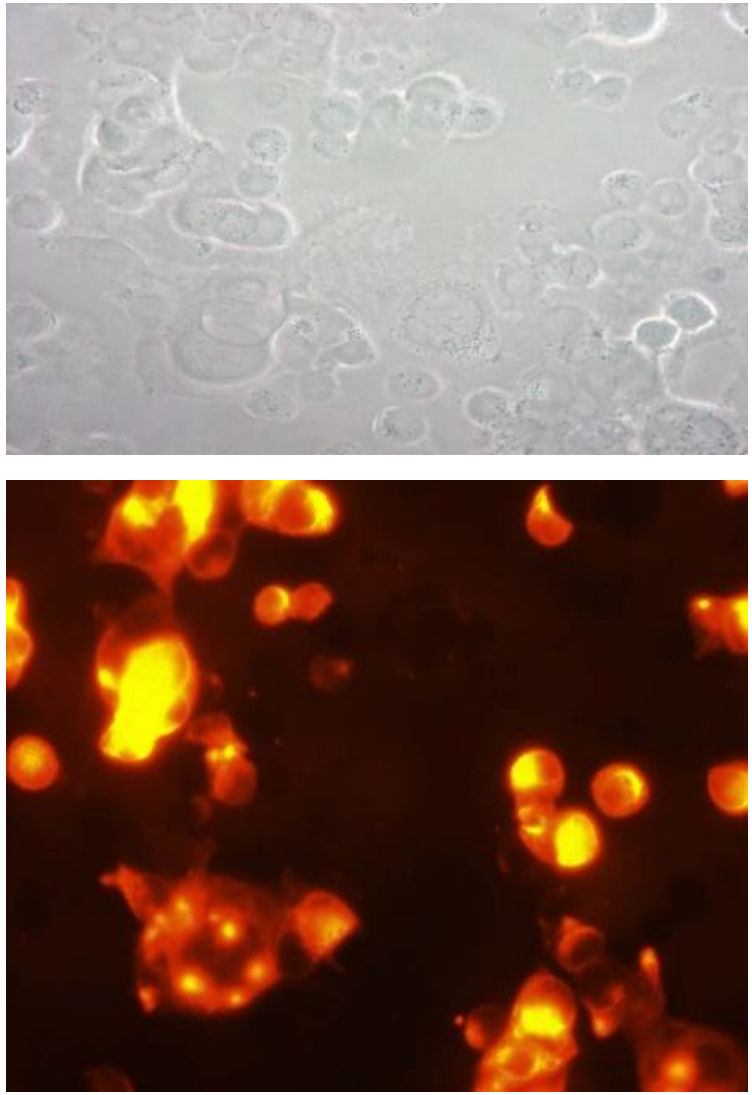




Comparison of sequences of PCR product from SW-620 cells and nNav1.5


```
nNav1.5      662  GGACCAAGTATGTCGAGTACACCTTCACCGCCATTTACACCTTTGAGTCTCTGGTCAAGA  721
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Seq.Res.     6    GGACCAAGTATGTCGAGTACACCTTCACCGCCATTTACACCTTTGAGTCTCTGGTCAAGA  65

nNav1.5      722  TTCTGGCTCGAGGCTTCTGCCTGCACGCGTTCACTTTCCTTCGGGACCCATGGAACTGGC  781
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Seq.Res.     66   TTCTGGCTCGAGGCTTCTGCCTGCACGCGTTCACTTTCCTTCGGGACCCATGGAACTGGC  125

nNav1.5      782  TGGACTTTAGTGTGATTATCATGGCGTATGTATCAGAAAATATAAAACTAGGCAATTTGT  841
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Seq.Res.     126  TGGACTTTAGTGTGATTATCATGGCGTATGTATCAGAAAATATAAAACTAGGCAATTTGT  185

nNav1.5      842  CGGCTCTTCGAACTTTCAGAGTCCTGAGAGCTCTAAAAACTATTTC-AGTTATCCCAGGG  900
                  |||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Seq.Res.     186  CGGCTCTTCGAACTTTCAGAGTCCTGAGAGCTCTAAAAACTATTTCTAGTTATCCCAGGG  245

nNav1.5      901  CTGAAGACCATCGTGGGG  918
                  ||||||||||||||||||
Seq.Res.     246  CTGAAGACCATCGTGGGG  263
```

ICC of SW-620 human colon cancer cells with NESOpAb (NP)



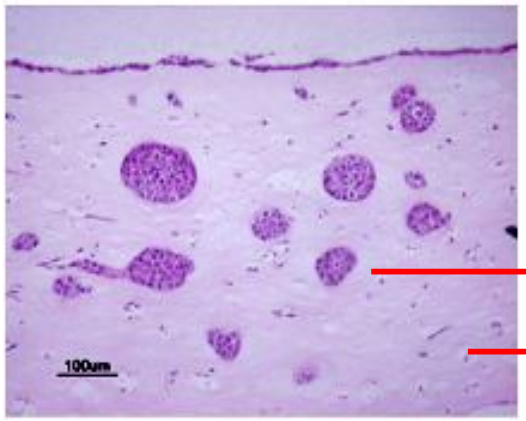
30 μm

→ VGSC expression (upregulation) in metastatic disease is an epigenetic, *oncofetal* phenomenon!

Role of VGSC in metastasis :

Tumour cell invasion

3D organotypic culture (BCa) - H & E staining



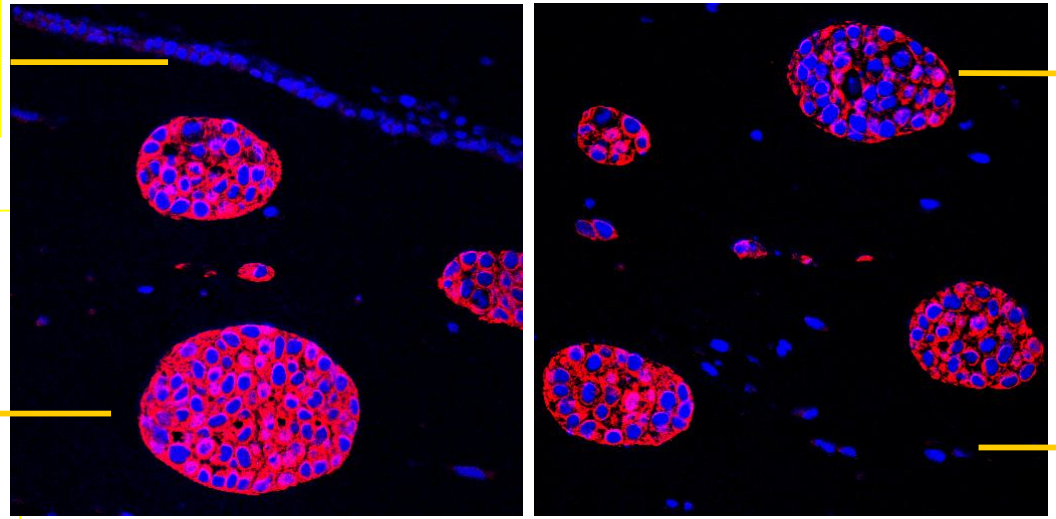
Cancer cells seeded on the top of the organotypic

cancer cells invading into the stroma

Stroma with fibroblasts

NESO staining (red) & DAPI staining (blue)

Non-invading cancer cells not stained with NESO



Group of cancer cells invaded into the stroma have a very strong NESO staining

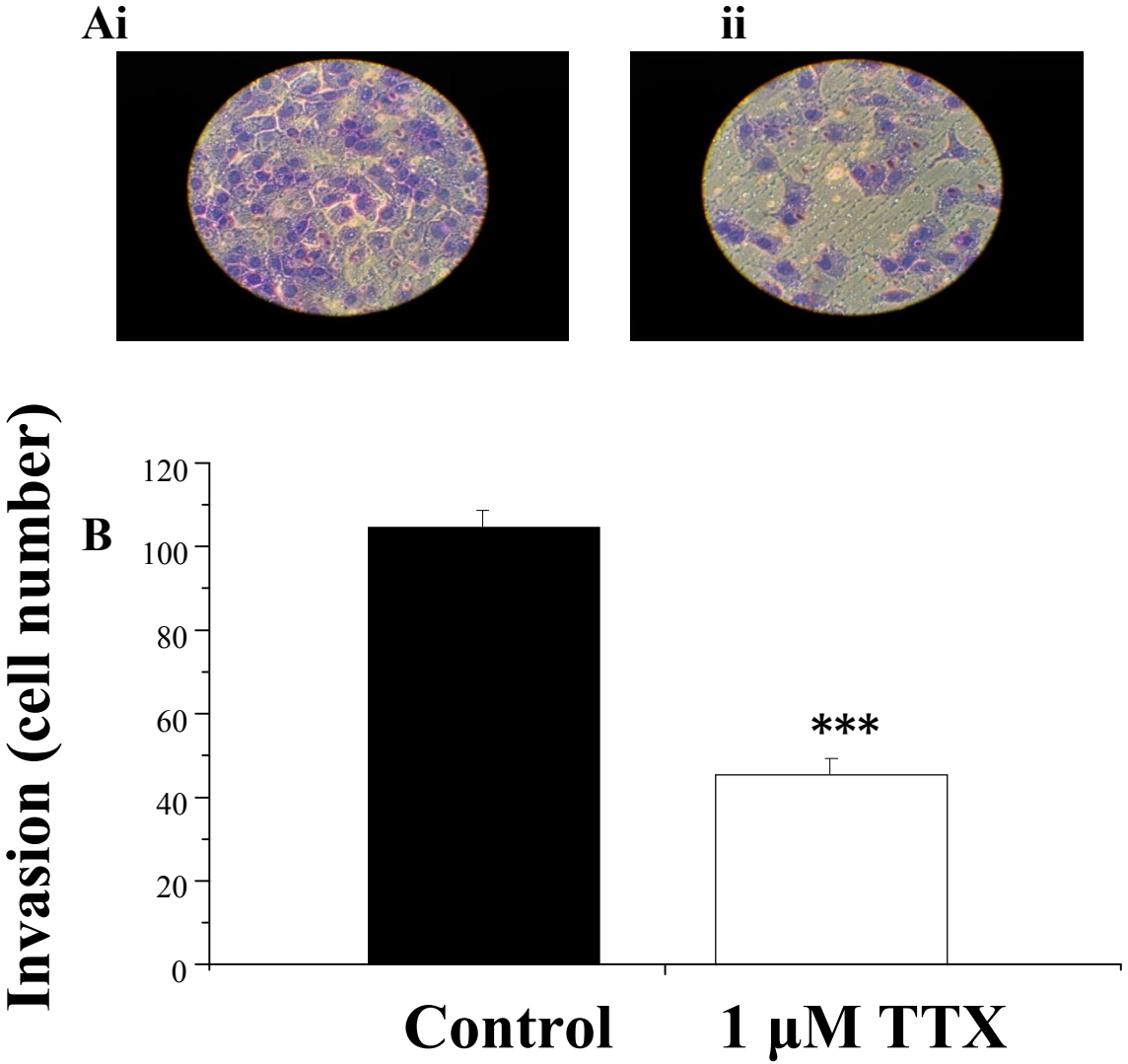
cancer cells invaded into the stroma are stained with NESO

Fibroblasts cells not stained with NESO

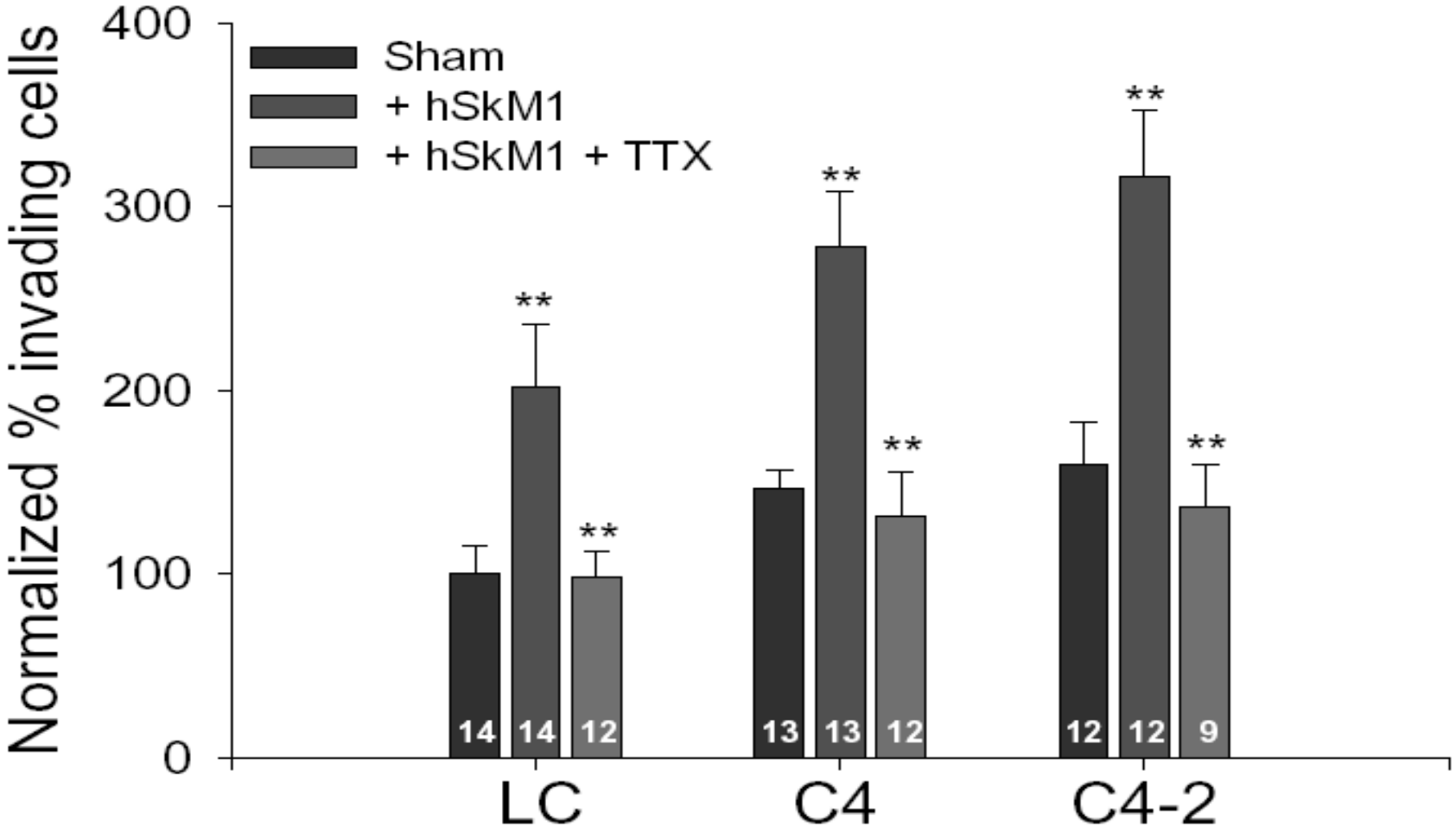
Courtesy of A-M. Chioni and R. Grose
Institute of Cancer, Barts and The London

Effect of tetrodotoxin (TTX) on invasion of SKOV3 cells *in vitro*.

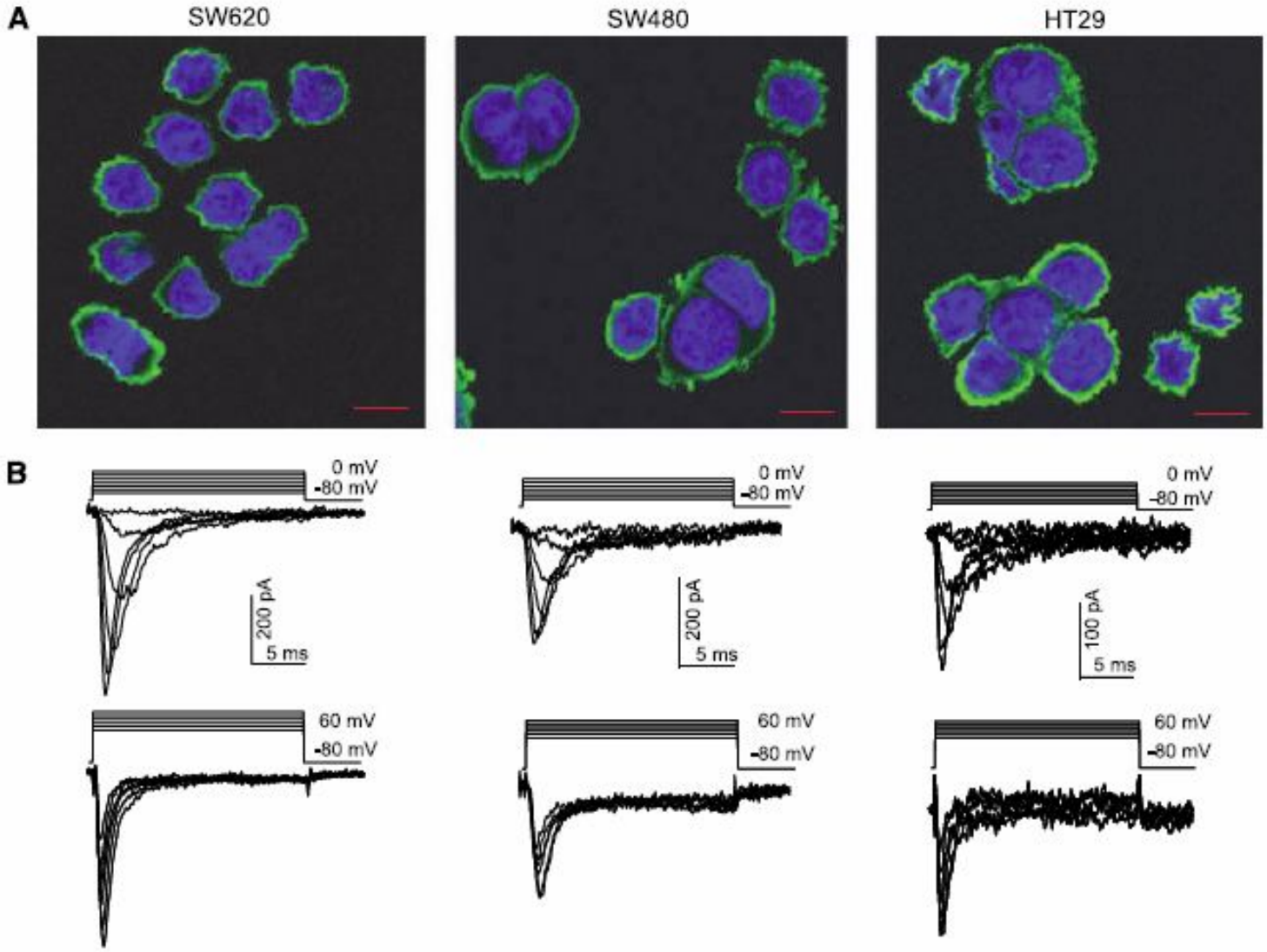
(A) Representative images showing SKOV3 cells having invaded a Matrigel® invasion chamber under (i) control conditions and (ii) following treatment with 1μM TTX. (B) Histogram indicating the reduction in the number of SKOV3 cells invading following treatment with 1μM TTX for 24 h in comparison to control conditions. (n=4; *** indicates $P < 0.01$)

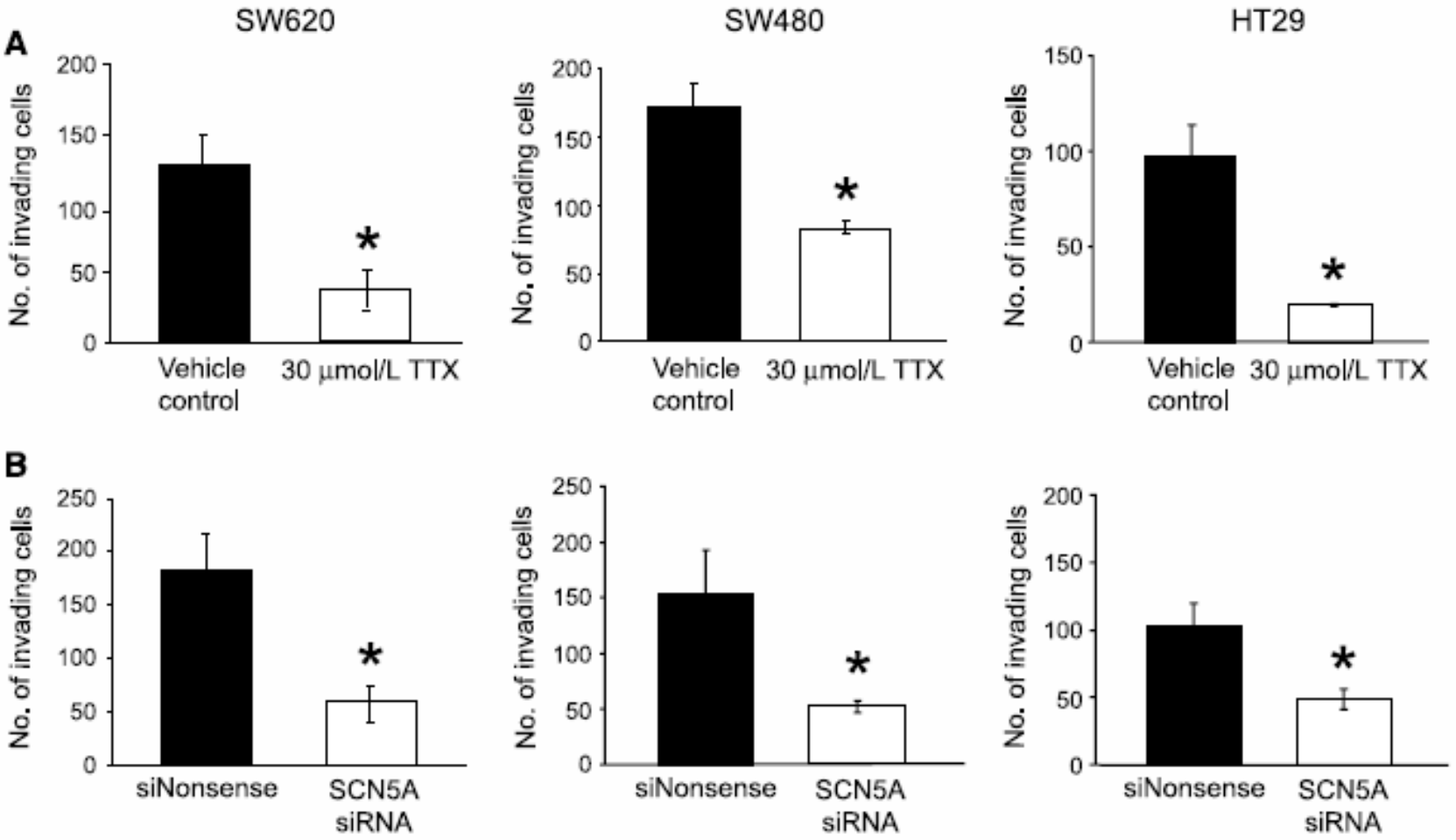


Bennett et al. (2004) – human prostate cancer



House et al. (2010) – human colon cancer




A
SW620
SW480
HT29
No. of invading cells
200
150
100
50
0
150
100
50
0
*
Vehicle control
30 μmol/L TTX
B
250
200
150
100
50
0
siNonsense
SCN5A siRNA

Systems (patho)biology of VGSC expression in cancer

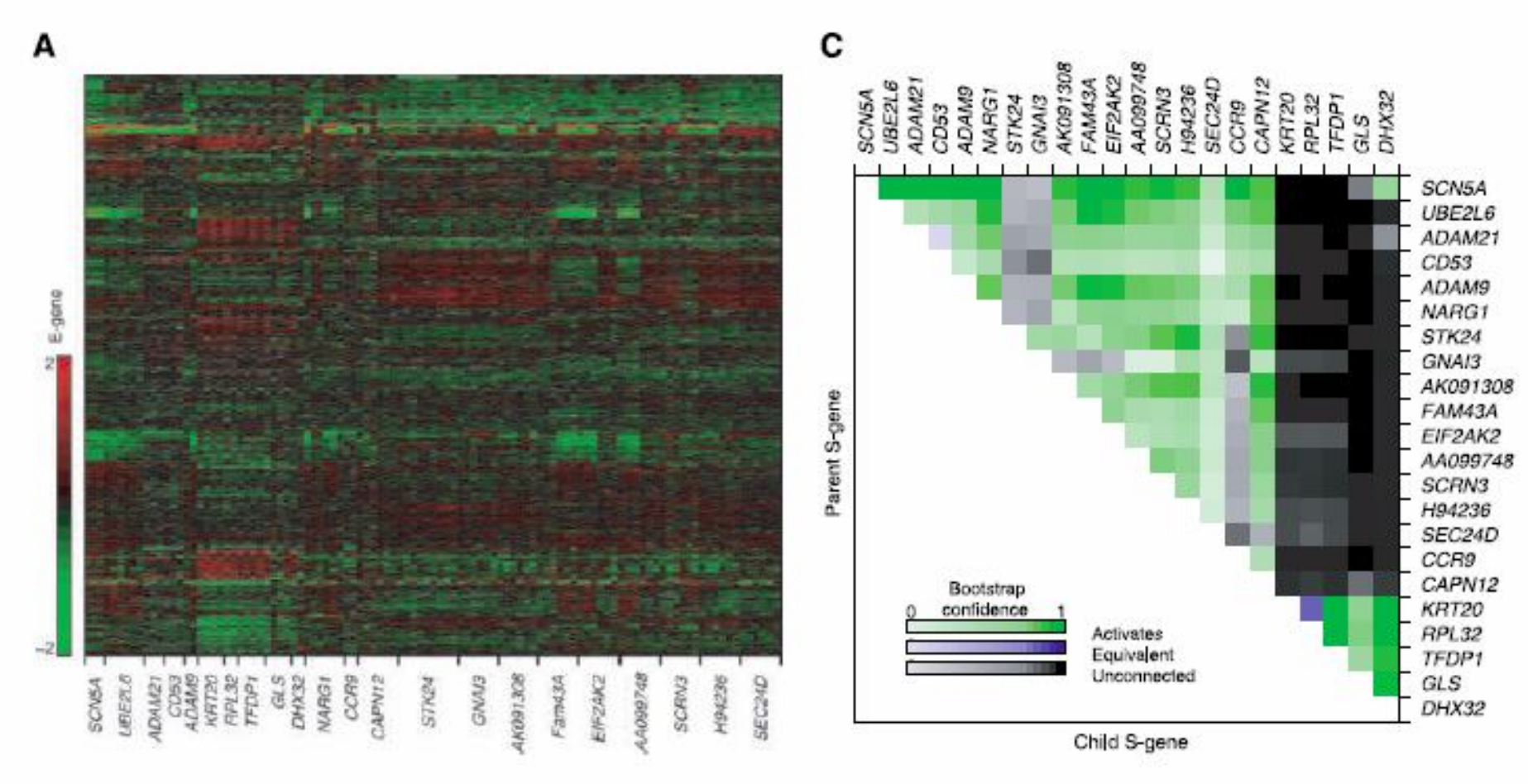
Tumor and Stem Cell Biology

Cancer Research

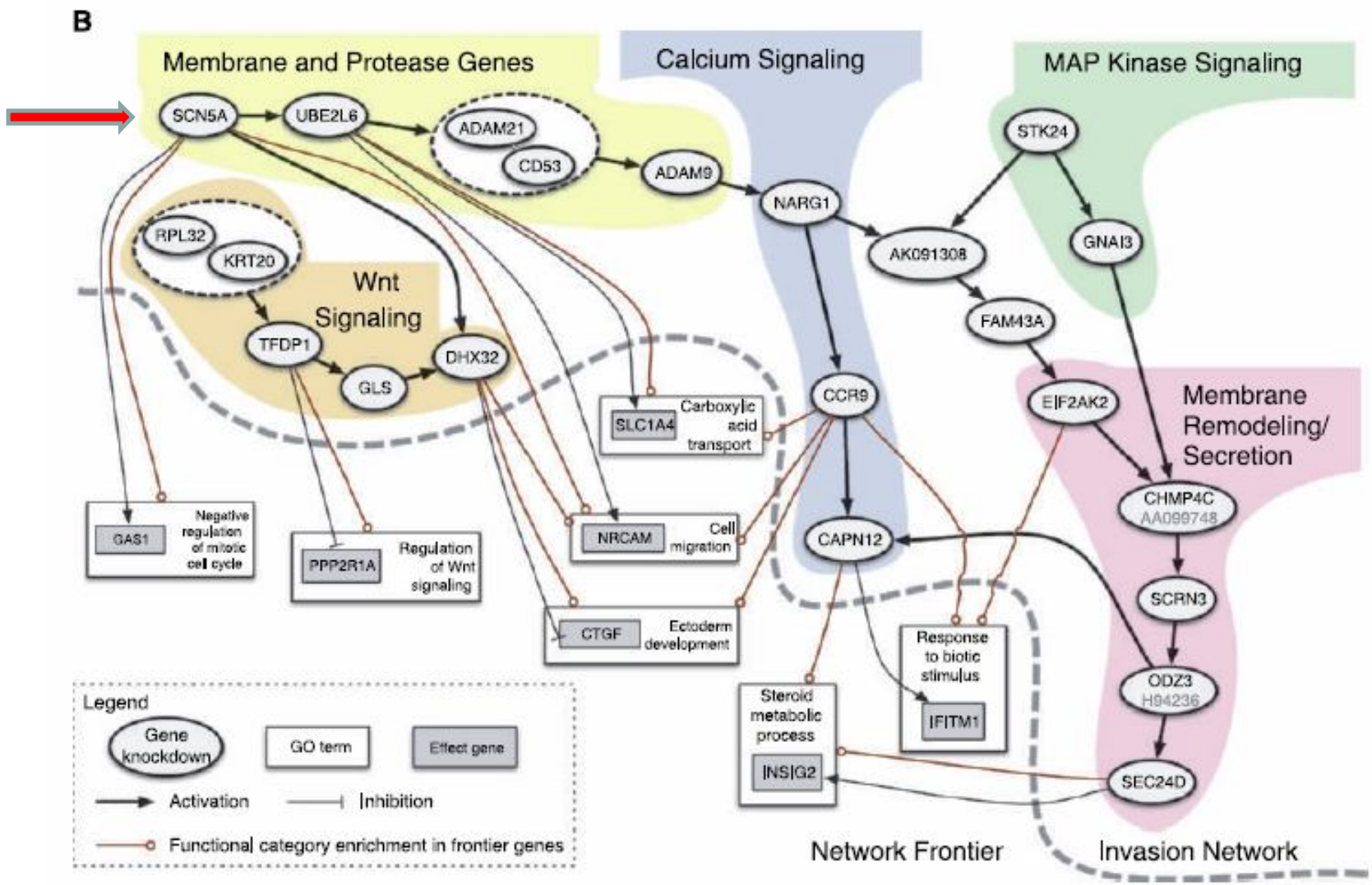
Voltage-Gated Na^+ Channel *SCN5A* Is a Key Regulator of a Gene Transcriptional Network That Controls Colon Cancer Invasion

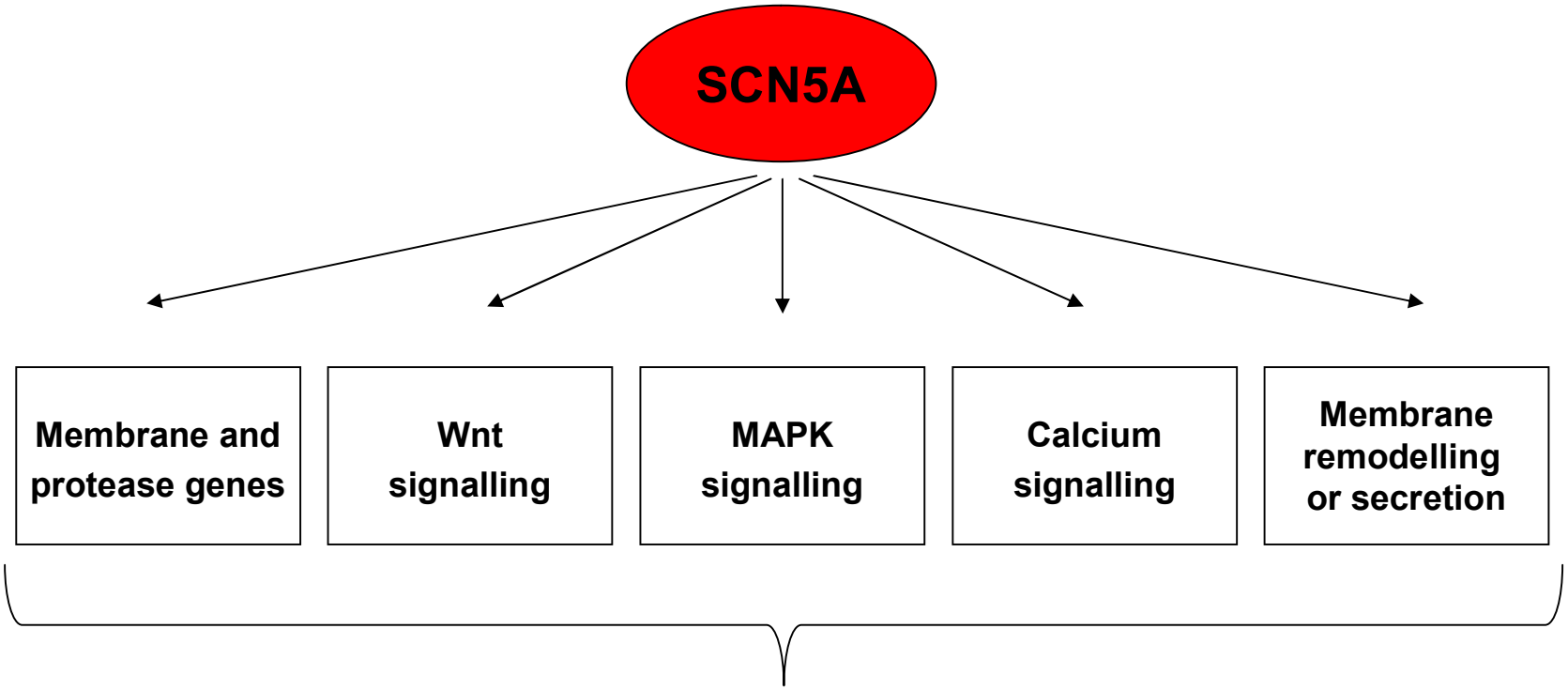
Carrie D. House[1], Charles J. Vaske[3], Arnold M. Schwartz[2], Vincent Obias[2], Bryan Frank[1], Truong Luu[1], Narine Sarvazyan[1], Rosalyn Irby[4], Robert L. Strausberg[5], Tim G. Hales[1], Joshua M. Stuart[3], and Norman H. Lee[1]

Control of gene expression by Nav1.5 activity in human colon cancer



Nav1.5 as a potential transducer of the invasion machinery in human colon cancer




SCN5A
Membrane and protease genes
Wnt signalling
MAPK signalling
Calcium signalling
Membrane remodelling or secretion
Tumour cell invasion

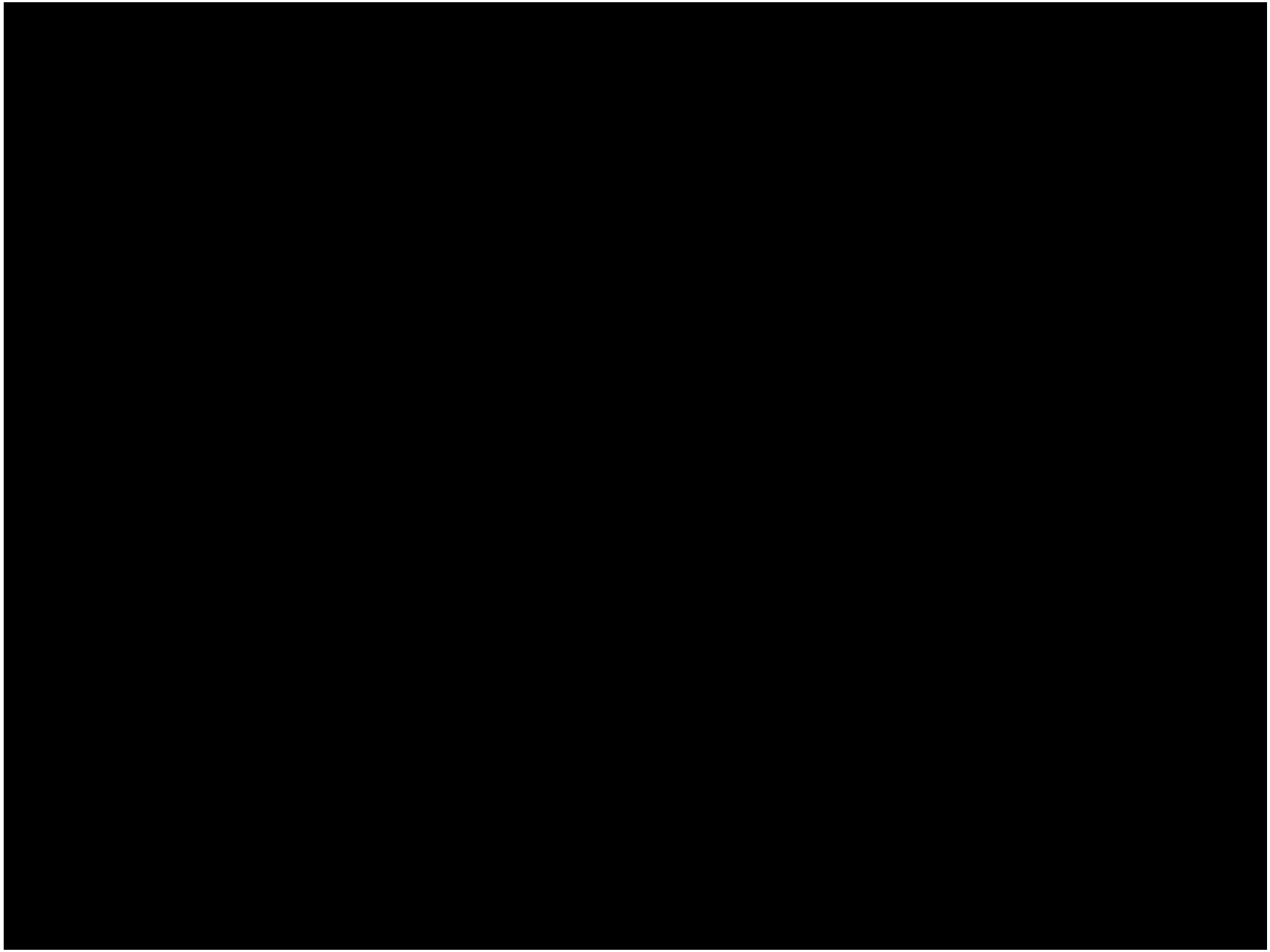
VGSC downstream signalling :

Intracellular Ca^{2+}

(Fluo-4AM, λ_{ex} = 488 nm)

-

Dr Rüstem Őnkal &
Dr Nahit Rizaner
(Uluslararasi Kibris Universitesi)



Role of VGSC in metastasis *in vivo*

Previous in vivo evidence

- **IHC (human)**
- **SQT-PCR re LNM (human)**
- **Tissue electrolyte imaging**

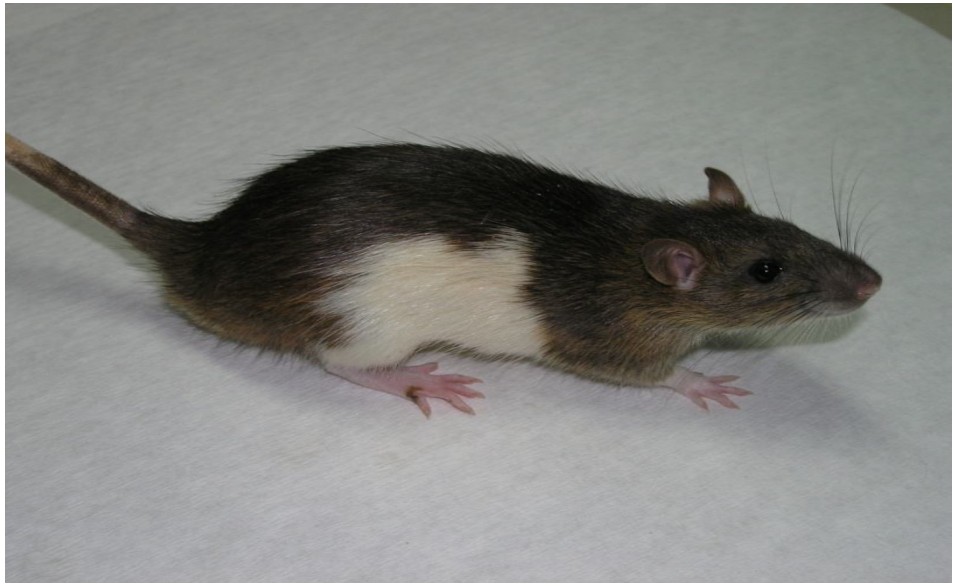
Copenhagen (Dunning) model of rat prostate cancer:

Effect of intra-tumour blockage of VGSC activity on lung metastasis

-

Prof Dr Seyhan Altun
(Dr) Senay Yildirim
(Istanbul Universitesi)

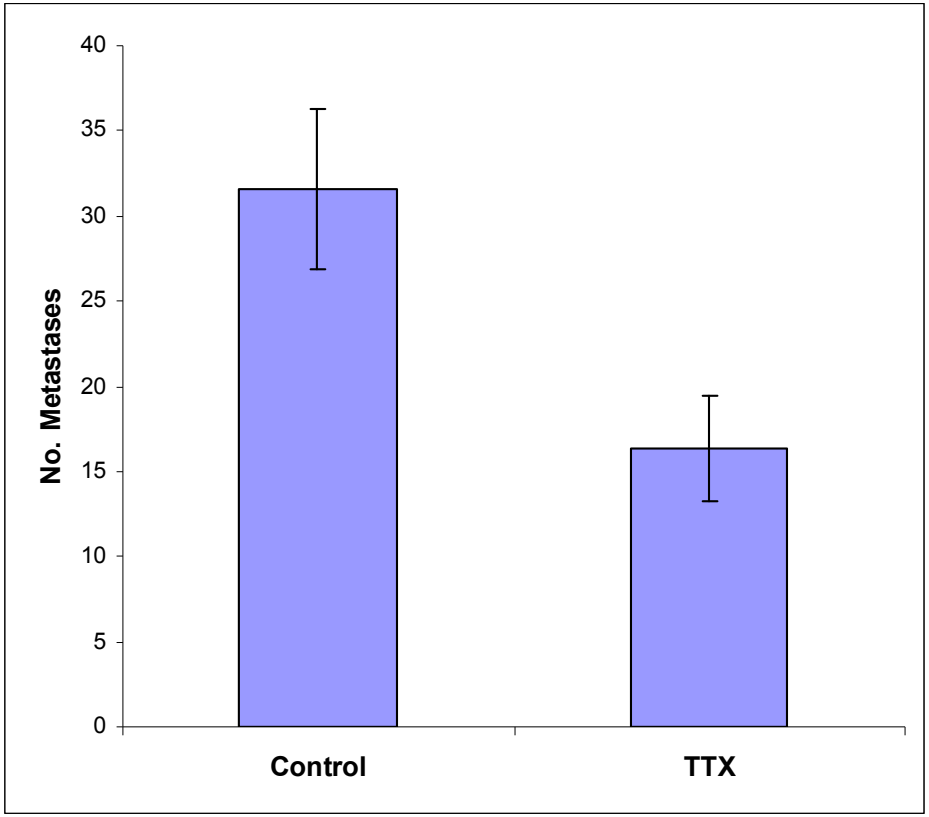
In vivo anti-metastatic effect of TTX :
Copenhagen Rat - PCa Model



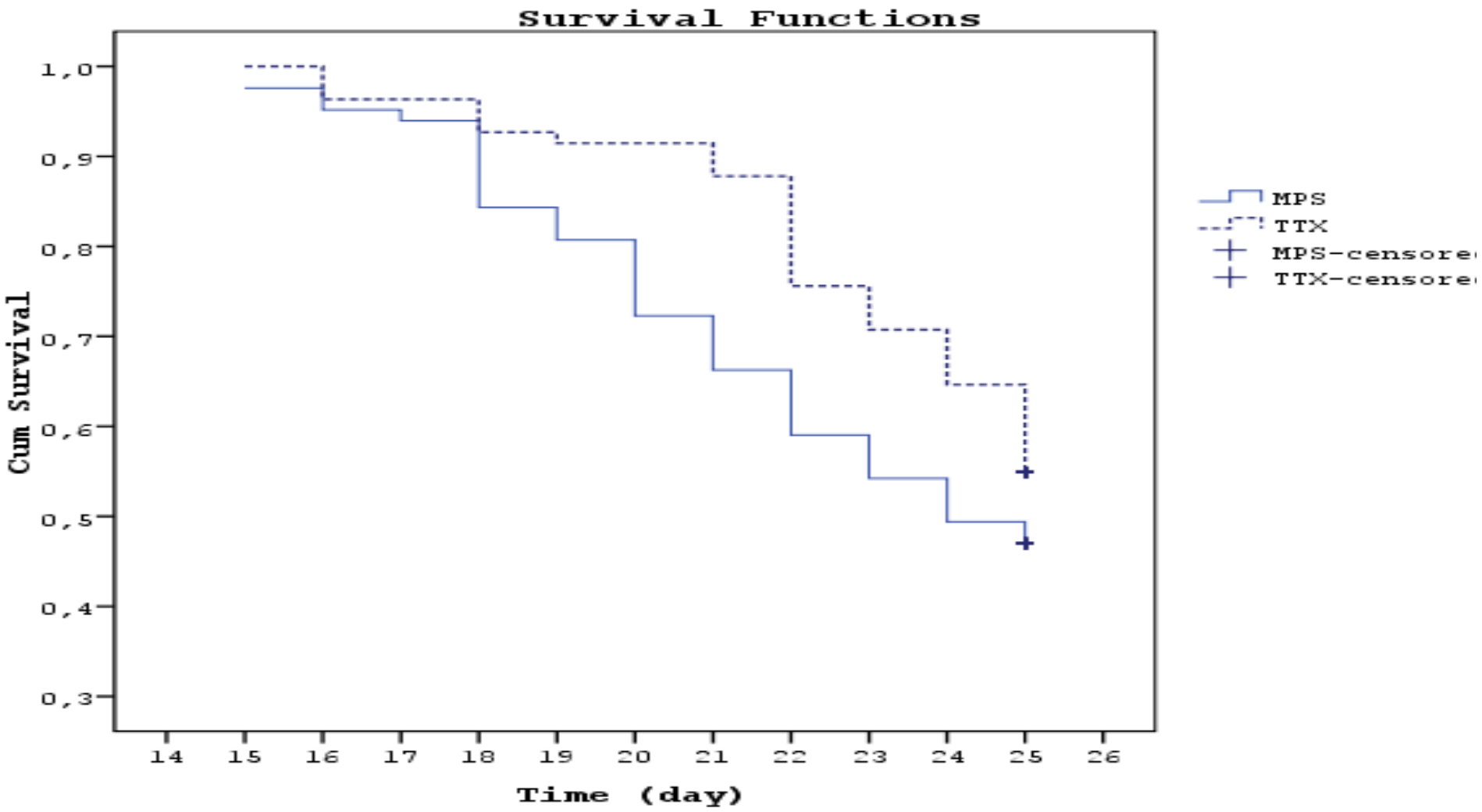
Normal

Primary tumour induced by Mat-LyLu cell inocculation (200,000 cells, day 21)

PCa Metastases to Lung


40
35
30
25
20
15
10
5
0
No. Metastases
Control
TTX

Kaplan-Meier Analysis

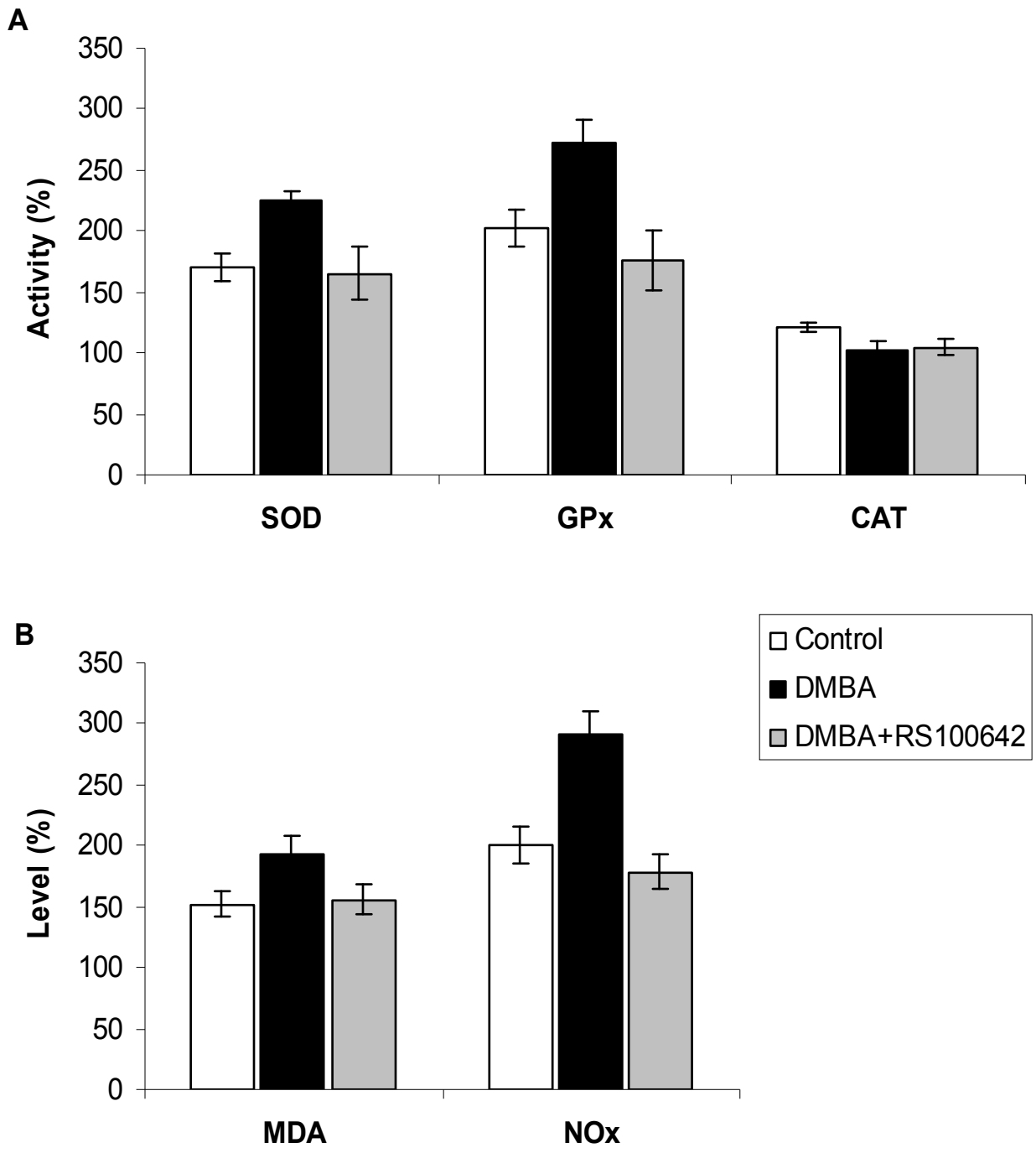


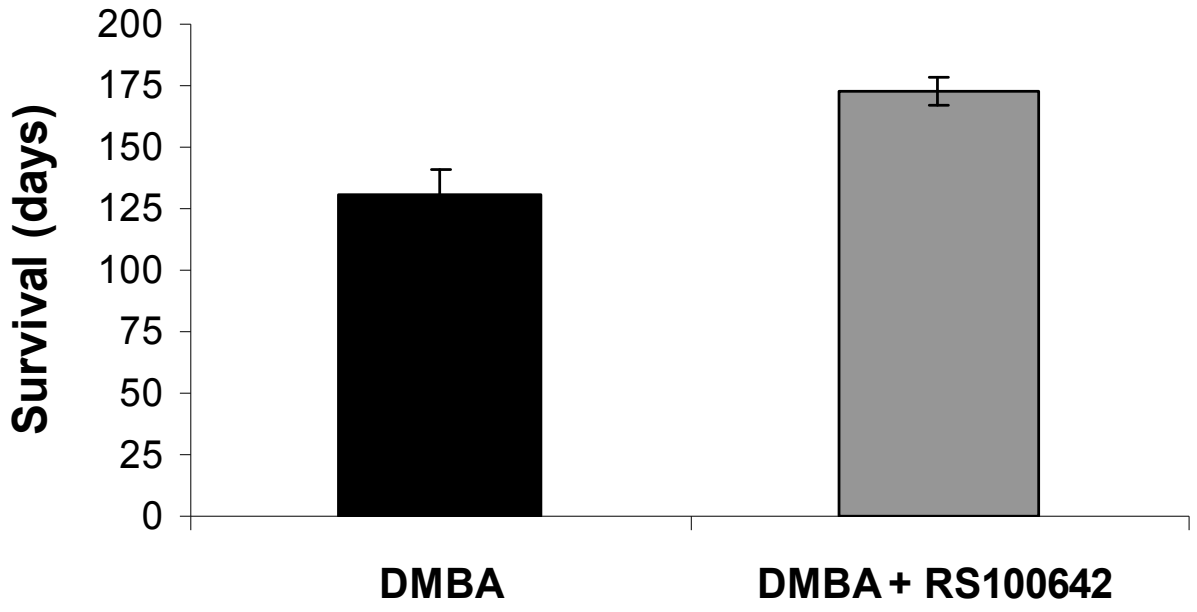
DMBA model of rat breast cancer:

Effect of systemic application of a clinical blocker of VGSC activity tumour burden (antioxidant response)

-

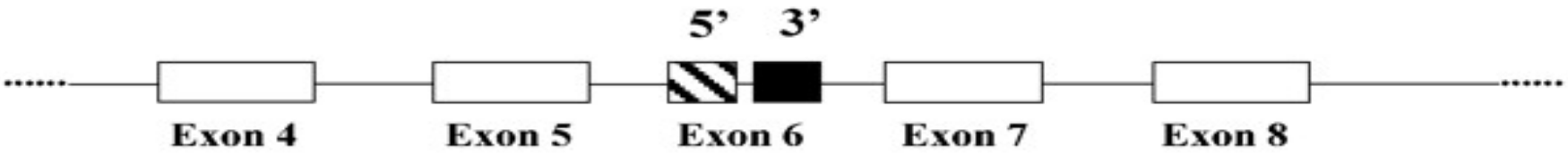
Dr Kadir Batcioglu
(Malatya Universitesi)


A
350
300
250
200
150
100
50
0
Activity (%)
SOD
GPx
CAT
B
Level (%)
MDA
NOx
Control
DMBA
DMBA+RS100642


Survival (days)
200
175
150
125
100
75
50
25
0
DMBA
DMBA + RS100642

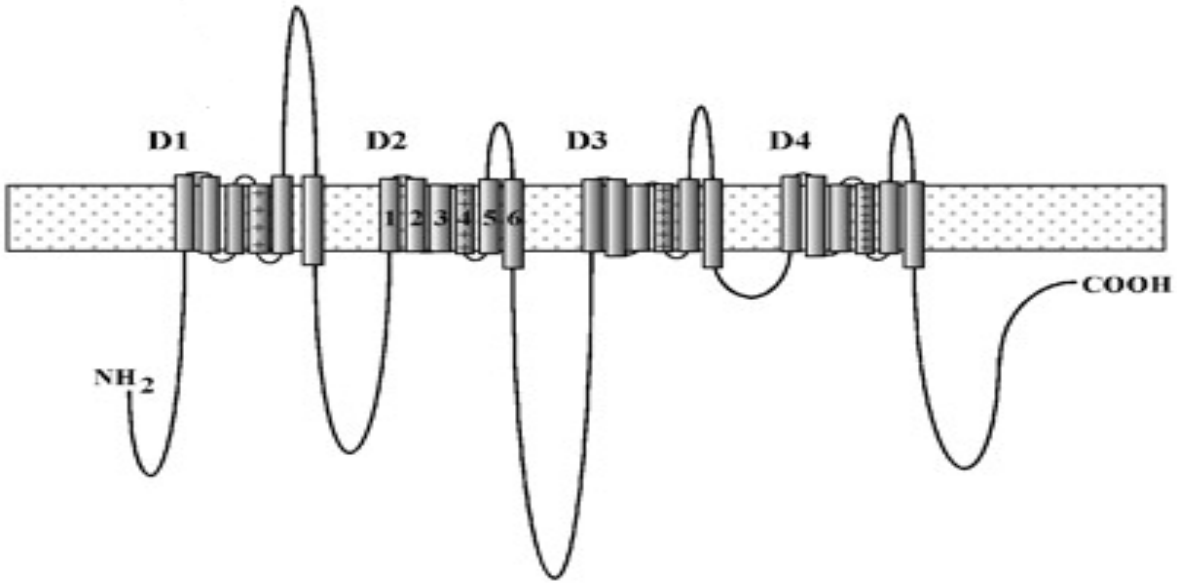
Can nNav1.5 be inhibited specifically?

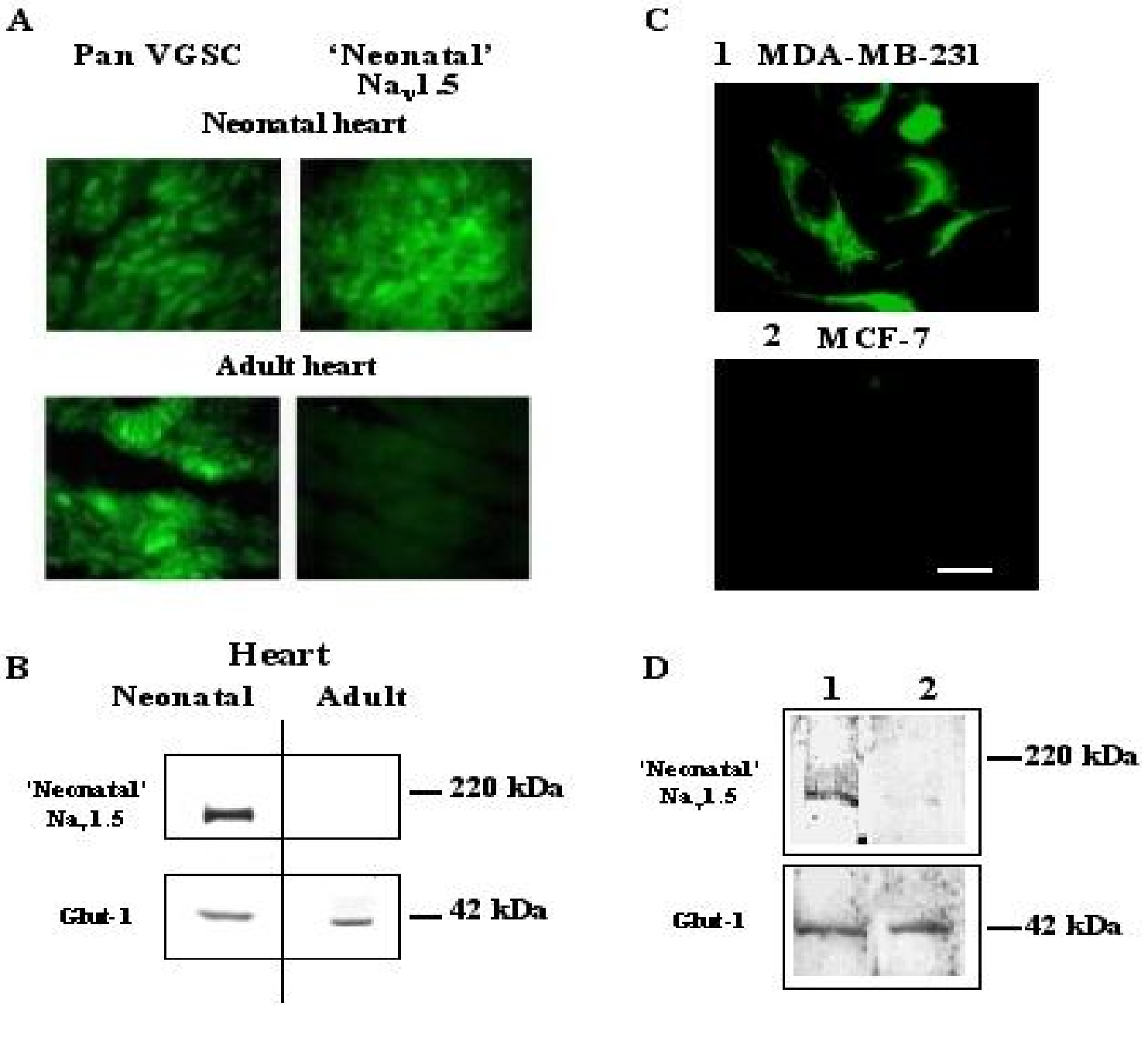
1. A nNav1.5-specific blocking antibody: NESOpAb!

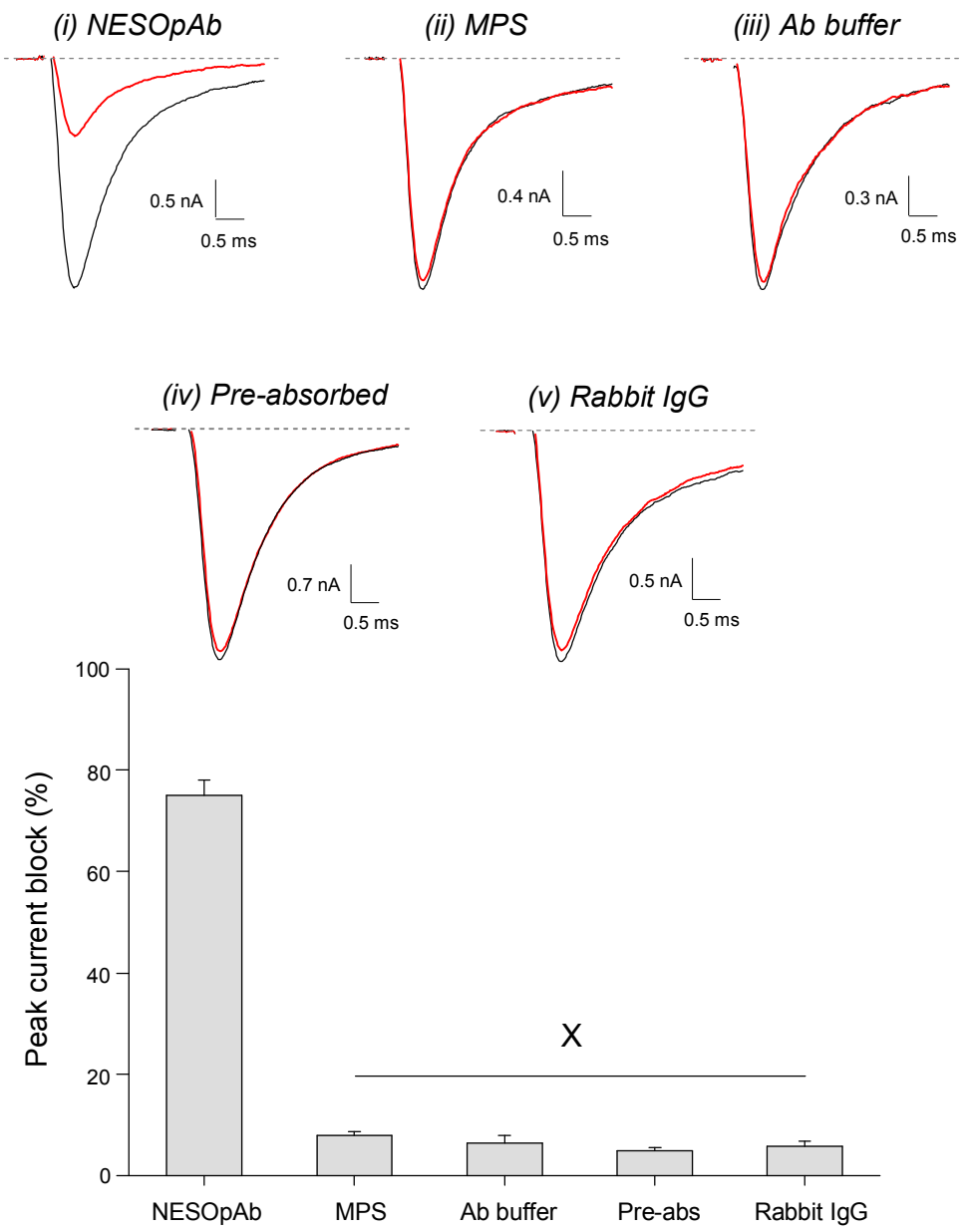


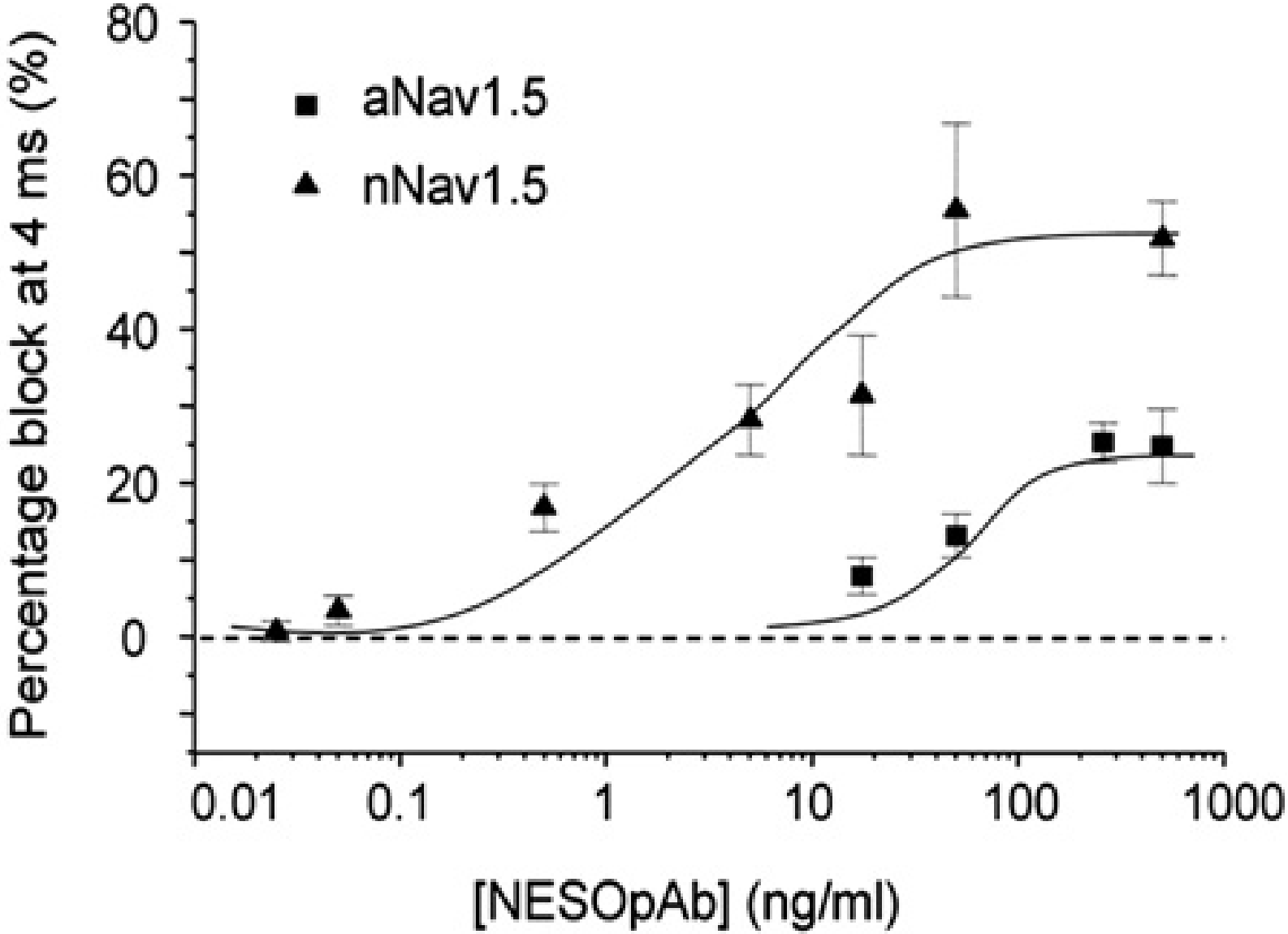
neonatal adult

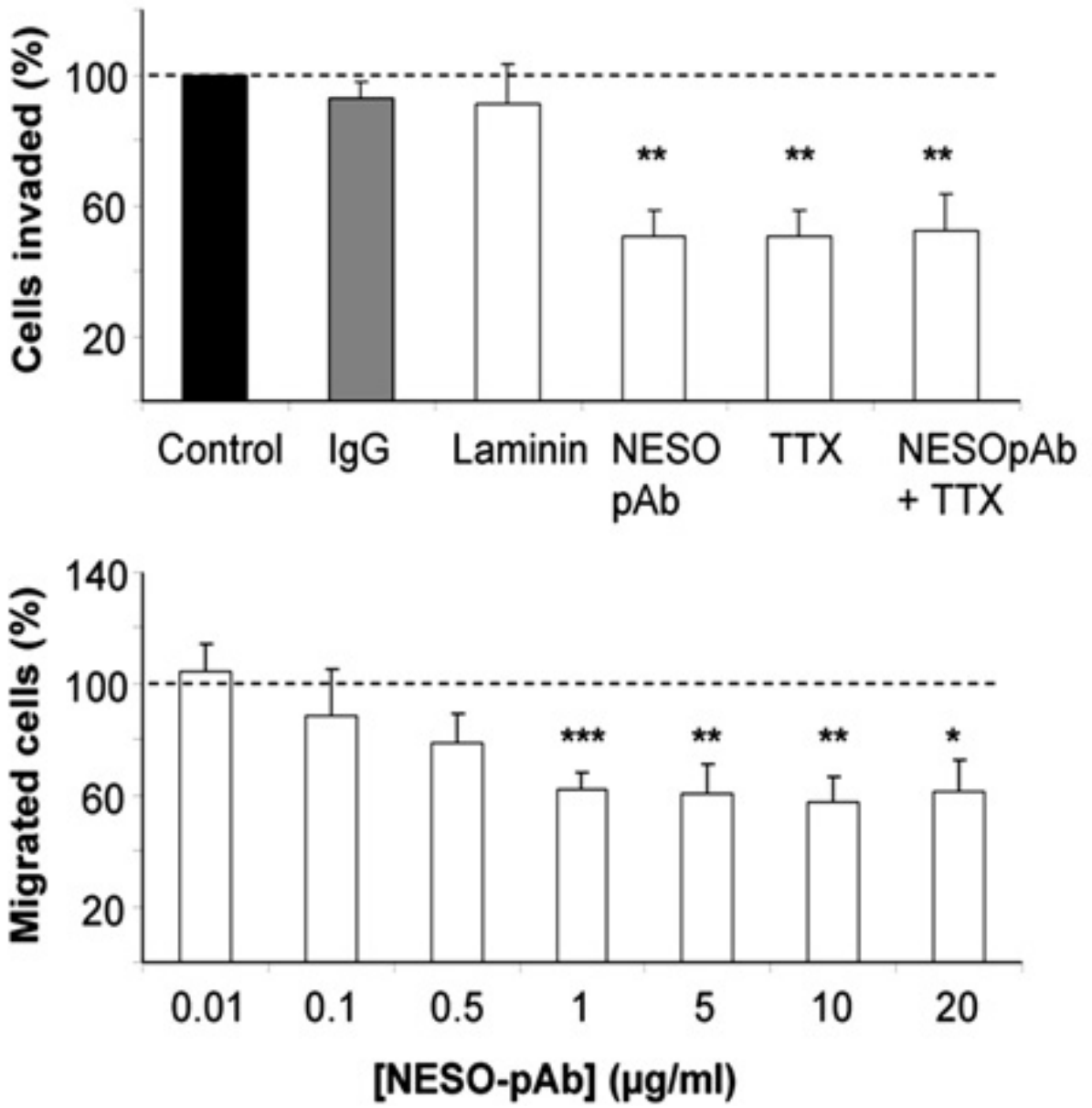
VGSCα Consensus	Y V T E F V X L G N V S A L R T F R V L R A L K T I S V I P
Nav1.5 (5’ splice)	• • S • N I K • • • L •
Nav1.5 (3’ splice)	• T • • • • D • S

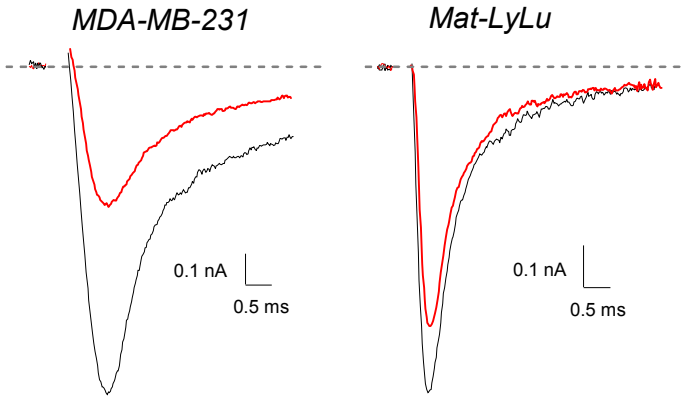


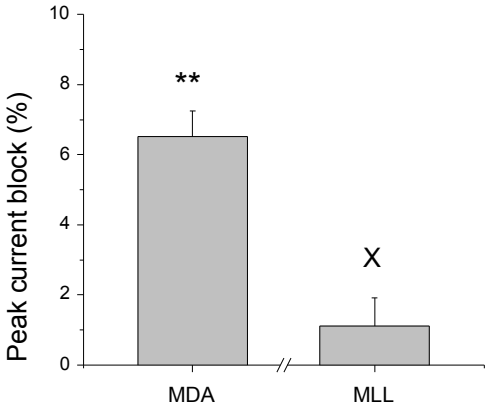

A
Pan VGSC
'Neonatal' $Na_v1.5$
Neonatal heart
Adult heart
C
1 MDA-MB-231
2 MCF-7
B
Heart
Neonatal
Adult
'Neonatal' $Na_v1.5$
220 kDa
Glut-1
42 kDa
D
1
2
'Neonatal' $Na_v1.5$
220 kDa
Glut-1
42 kDa


(i) NESOpAb
0.5 nA
0.5 ms
(ii) MPS
0.4 nA
0.5 ms
(iii) Ab buffer
0.3 nA
0.5 ms
(iv) Pre-absorbed
0.7 nA
0.5 ms
(v) Rabbit IgG
0.5 nA
0.5 ms
Peak current block (%)
100
80
60
40
20
0
X
NESOpAb
MPS
Ab buffer
Pre-abs
Rabbit IgG

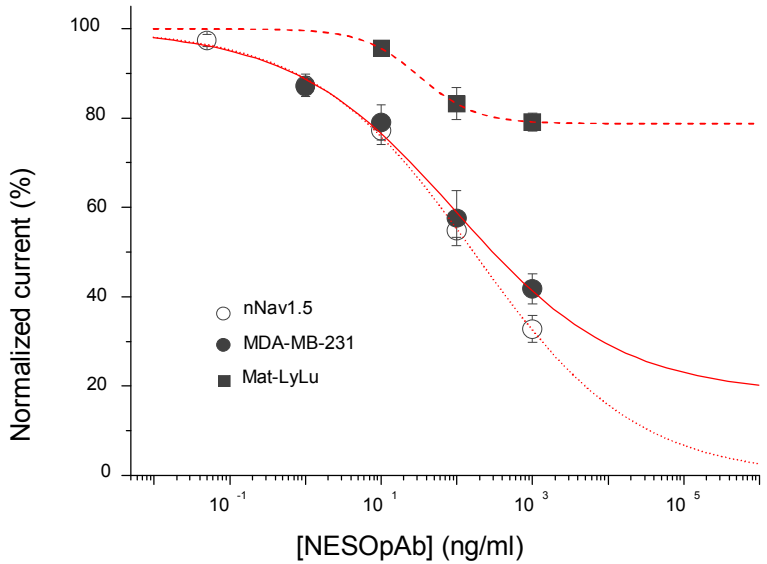

Percentage block at 4 ms (%)
80
60
40
20
0
aNav1.5
nNav1.5
0.01
0.1
1
10
100
1000
[NESOpAb] (ng/ml)


Cells invaded (%)
100
60
20
**
**
**
Control
IgG
Laminin
NESO pAb
TTX
NESOpAb + TTX
Migrated cells (%)
140
100
60
20
***
**
**
*
0.01
0.1
0.5
1
5
10
20
[NESO-pAb] (µg/ml)

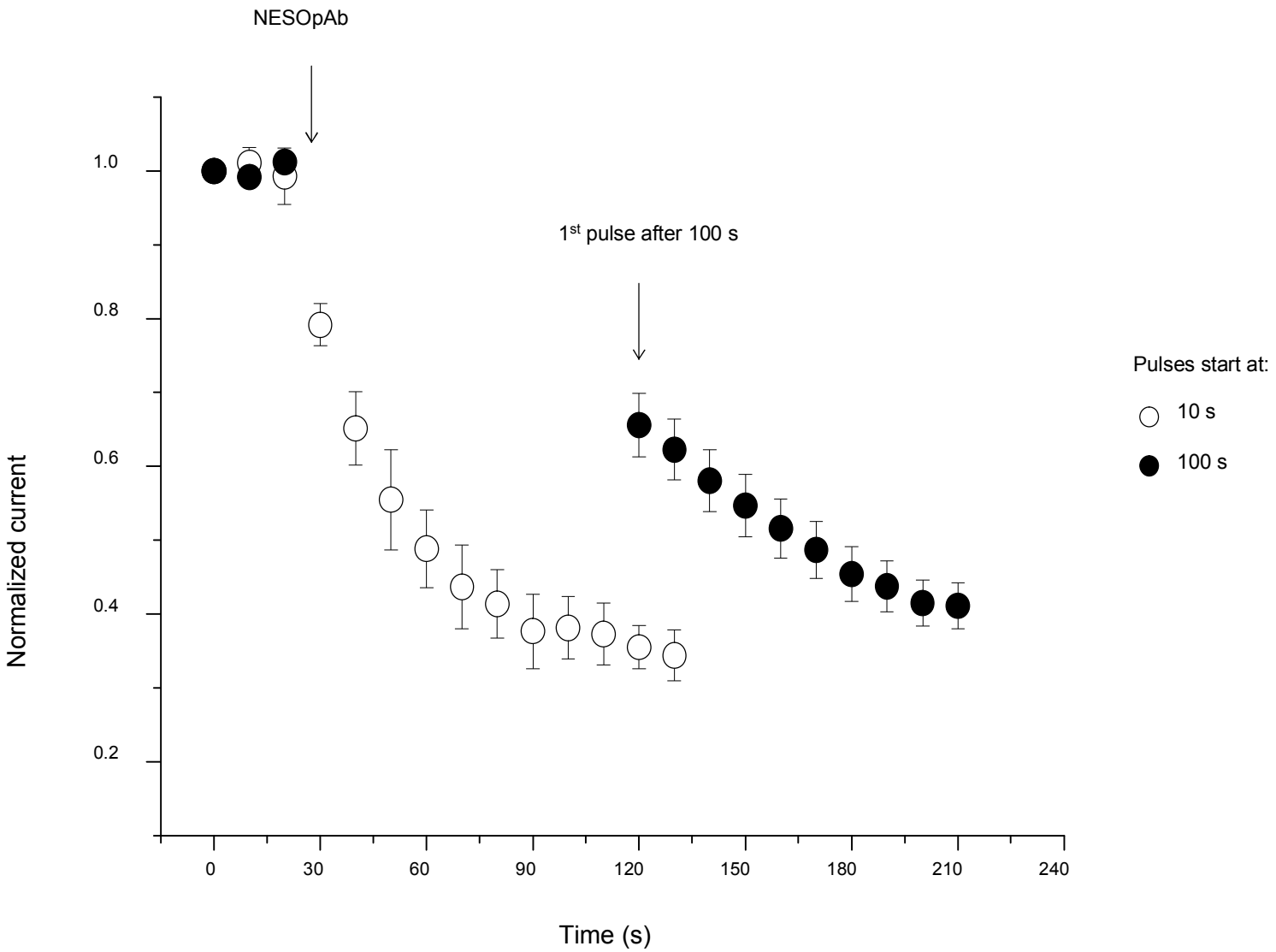




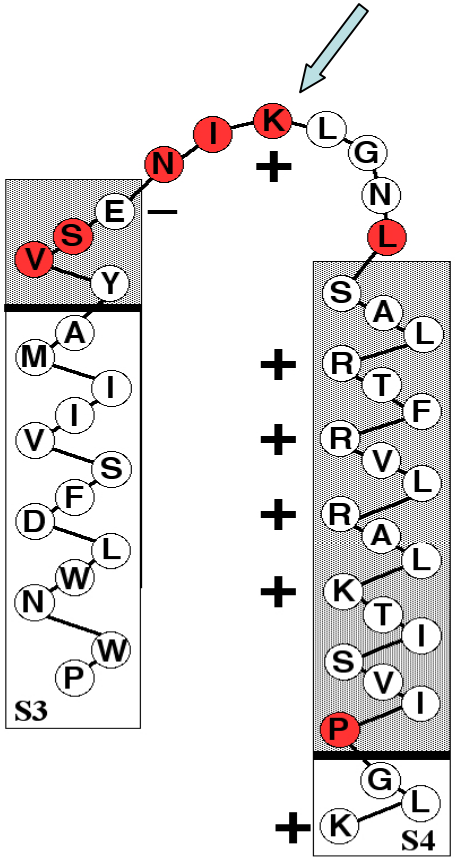
nNav1.5	205 Y**VS**E**NIK**LGN**L**SALRTFRVLRALKTISVI**P** 234
aNav1.5	205 YTTEFVDLGNVSALRTFRVLRALKTISVIS 234
nNav1.7	200 YLTEFVNLGNVSALRTFRVLRALKTISVIP 229



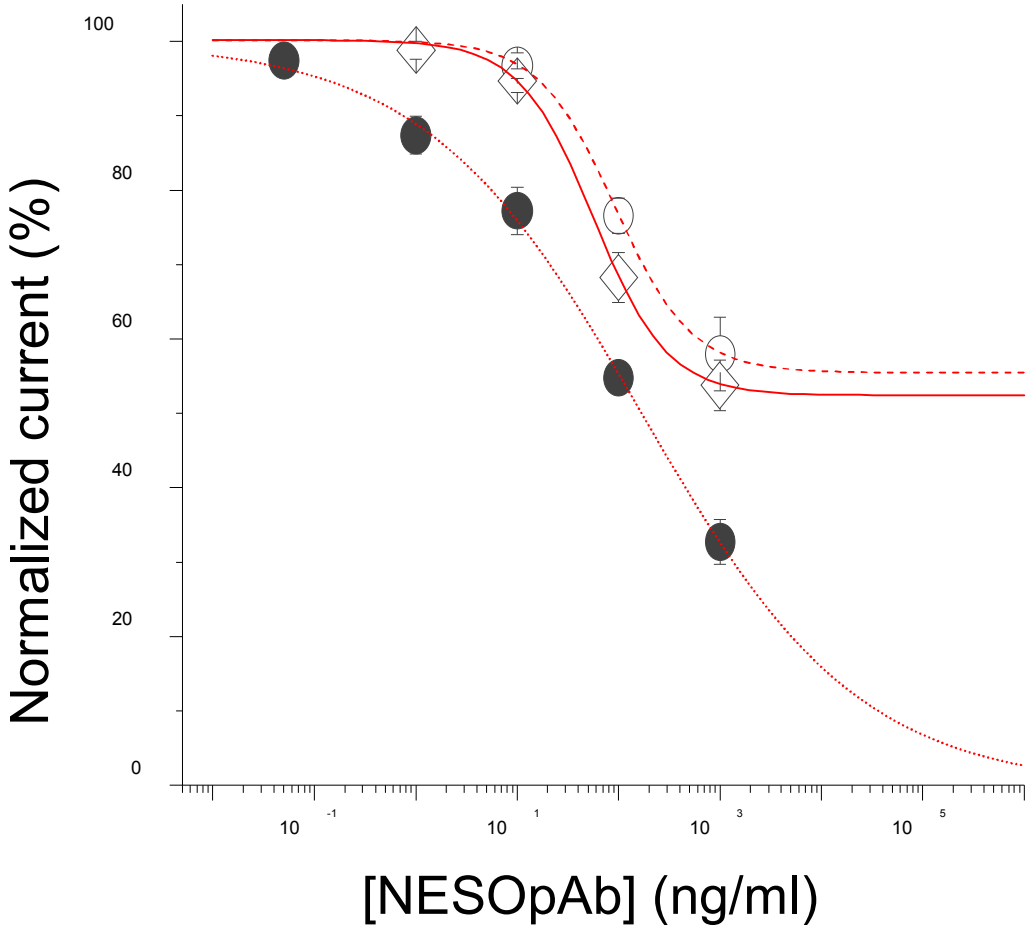
Use dependence of NESOpAb block



Epitope analysis of NESOpAb binding upon nNav1.5: Critical role of D211K



Lys^{211} is critical for NESOpAb binding



Can nNav1.5 be inhibited specifically?

2. Pharmacological distinction

nature Vol 456|13 November 2008|doi:10.1038/nature07473

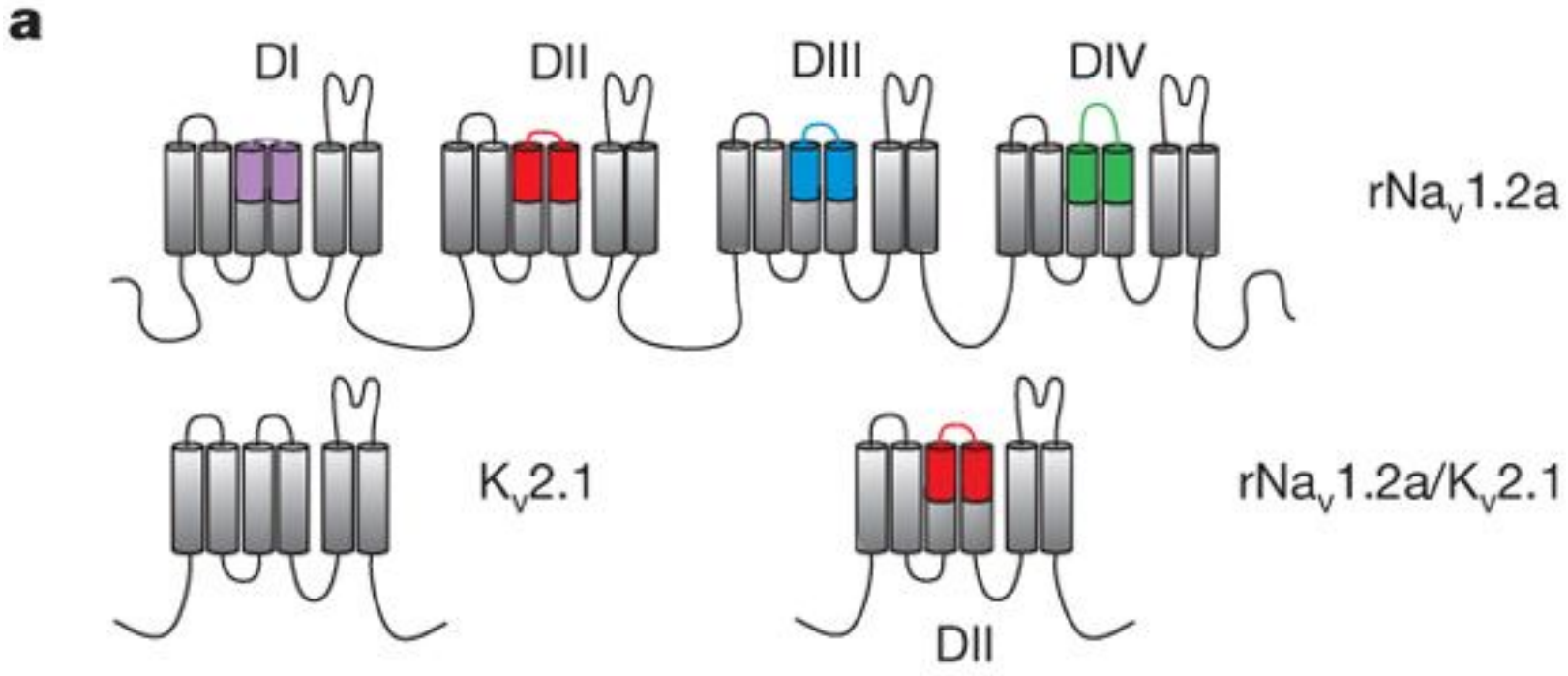
ARTICLES

Deconstructing voltage sensor function and pharmacology in sodium channels

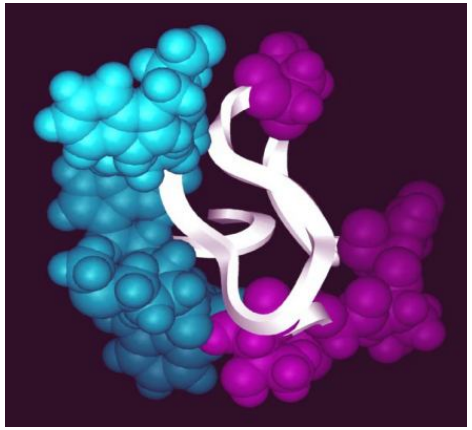
Frank Bosmans[1,2], Marie-France Martin-Eauclaire[3] & Kenton J. Swartz[1]

Voltage-activated sodium (Na_v) channels are crucial for the generation and propagation of nerve impulses, and as such are widely targeted by toxins and drugs. The four voltage sensors in Na_v channels have distinct amino acid sequences, raising fundamental questions about their relative contributions to the function and pharmacology of the channel. Here we use four-fold symmetric voltage-activated potassium (K_v) channels as reporters to examine the contributions of individual S3b–S4 paddle motifs within Na_v channel voltage sensors to the kinetics of voltage sensor activation and to forming toxin receptors. Our results uncover binding sites for toxins from tarantula and scorpion venom on each of the four paddle motifs in Na_v channels, and reveal how paddle-specific interactions can be used to reshape Na_v channel activity. One paddle motif is unique in that it slows voltage sensor activation, and toxins selectively targeting this motif impede Na_v channel inactivation. This reporter approach and the principles that emerge will be useful in developing new drugs for treating pain and Na_v channelopathies.

Transfer of the voltage sensor paddle motifs from rNav1.2a to Kv2.1.



ProTx-II



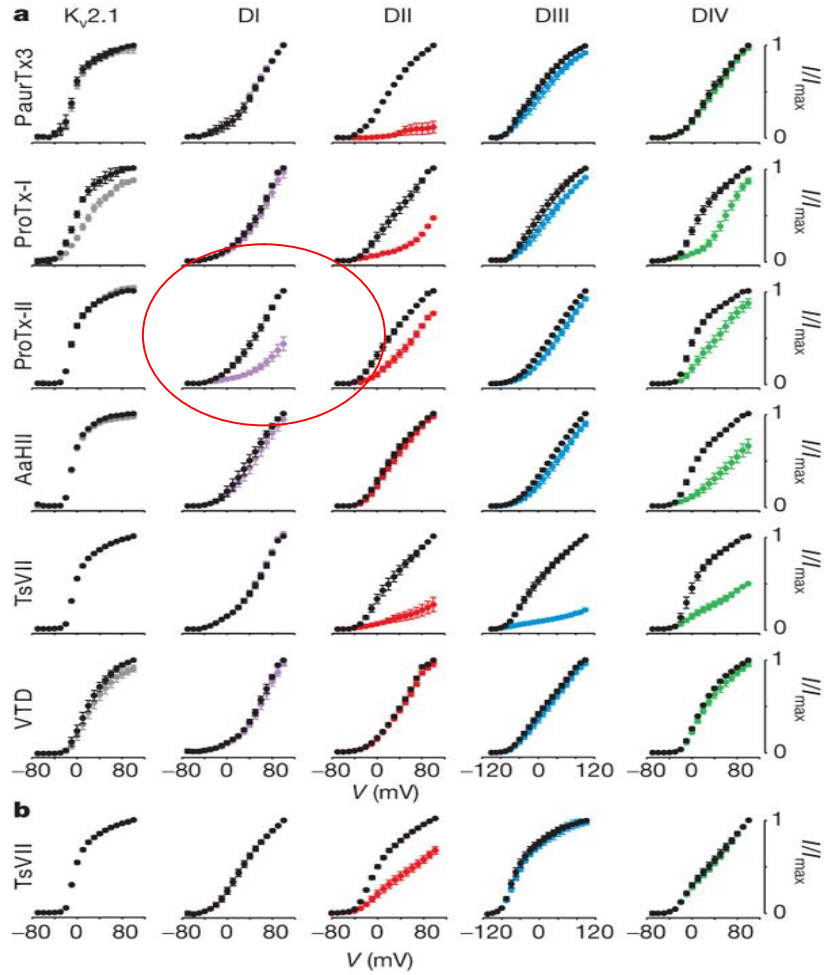
AA sequence (30):

Tyr-Cys^{2}-Gln-Lys-Trp-Met-Trp-Thr-Cys^{9}-Asp-Ser-Glu-Arg-Lys-Cys^{15}-Cys^{16}-Glu-Gly-Met-Val-Cys^{21}-Arg-Leu-Trp-Cys^{25}-Lys-Lys-Lys-Leu-Trp-OH

Formula: $C_{168}H_{250}N_{46}O_{41}S_{8}$
Molecular Weight: 3828.00 Da
Appearance: White lyophilized solid
Solubility: water and saline buffer

Neurotoxins are the only class of VGSC blockers that target extracellular S3-S4 linkers

Sensitivity of rNa$_v$1.2a paddle chimaeras to extracellular toxins.

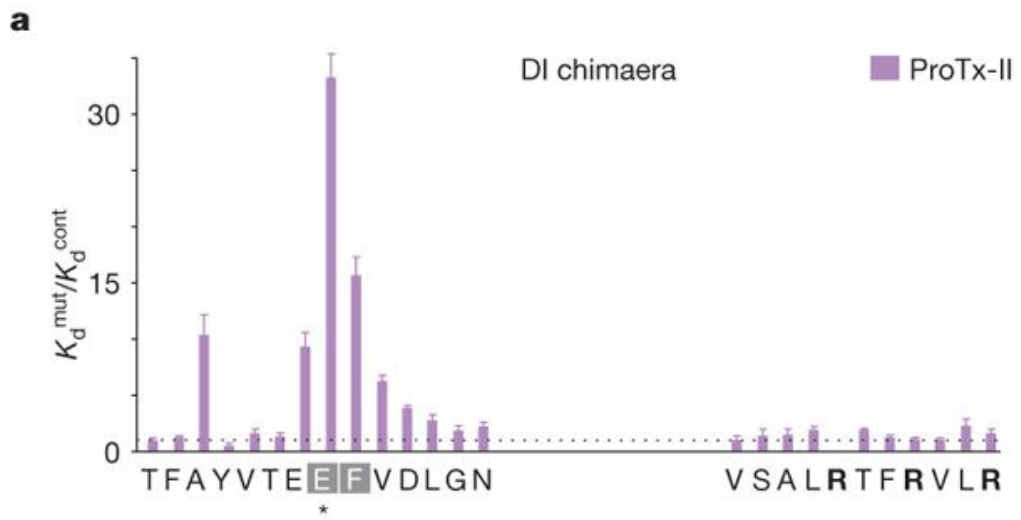


F Bosmans *et al.* *Nature* **456**, 202-208 (2008) doi:10.1038/nature07473

nature

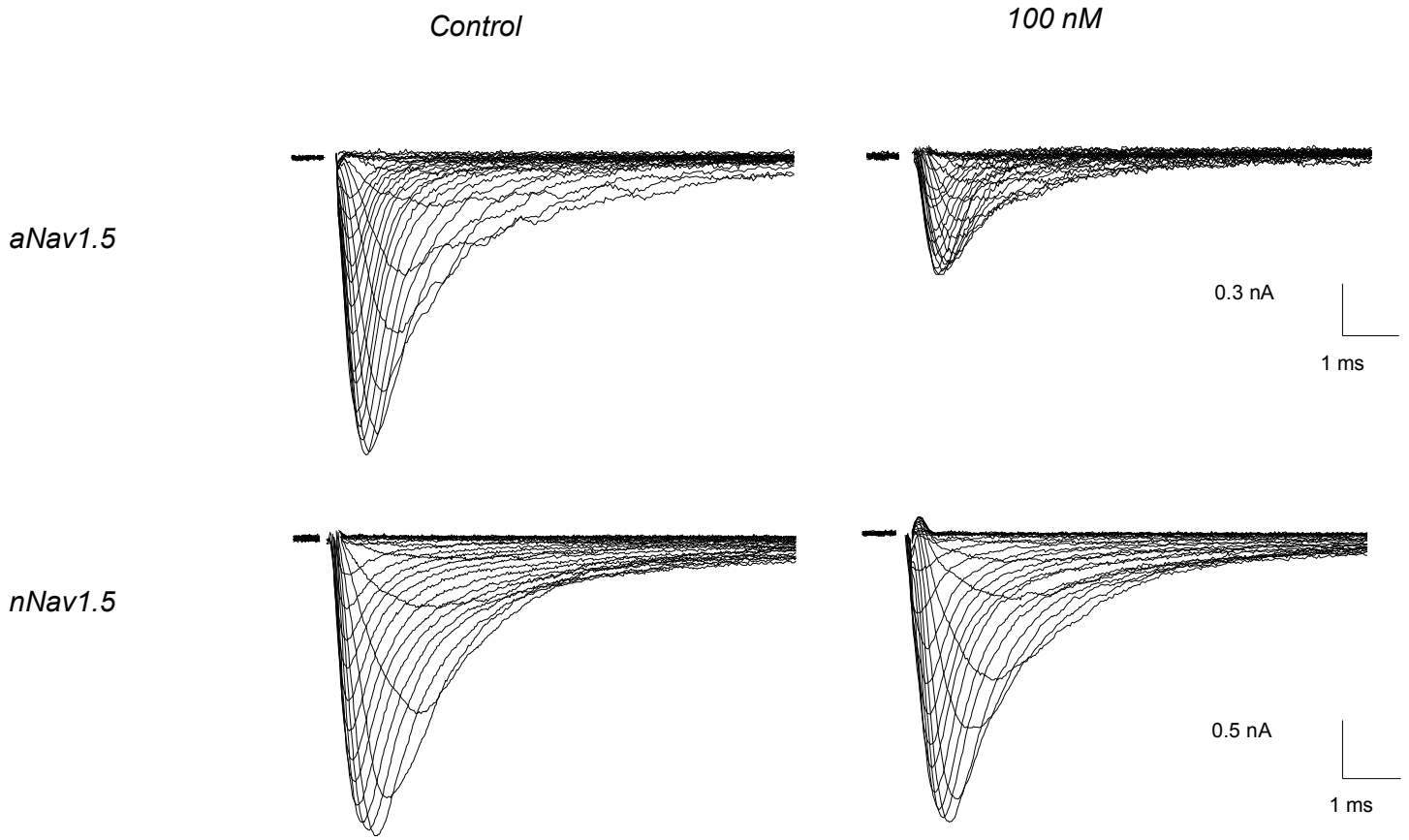
ProTxII is the only VGSC blocker neurotoxin that has been shown to target D1:S3-S4

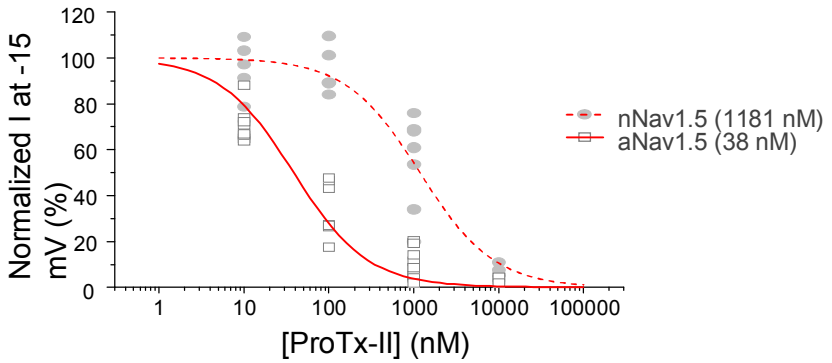
Scanning mutagenesis of Na_v channel paddle motifs.

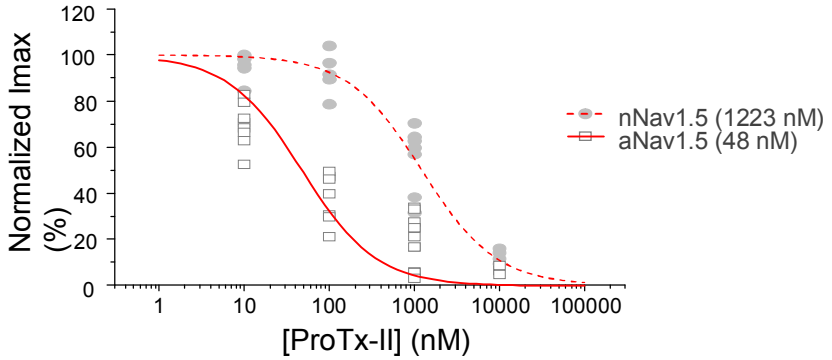


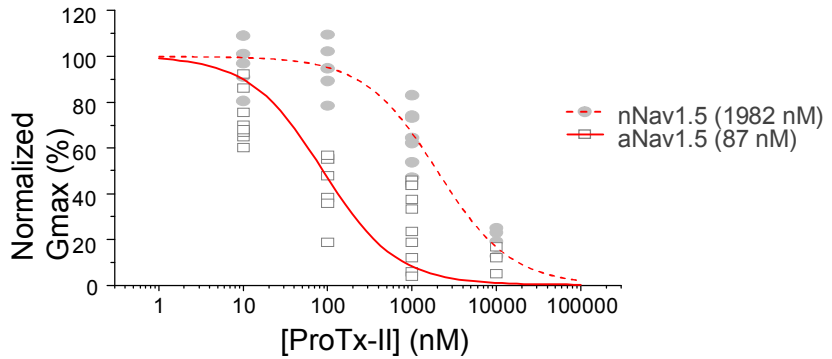
F Bosmans *et al.* *Nature* **456**, 202-208 (2008) doi:10.1038/nature07473

nature


Control
100 nM
aNav1.5
nNav1.5
0.3 nA
1 ms
0.5 nA
1 ms


Normalized I at -15 mV (%)
120
100
80
60
40
20
0
1
10
100
1000
10000
100000
[ProTx-II] (nM)
nNav1.5 (1181 nM)
aNav1.5 (38 nM)

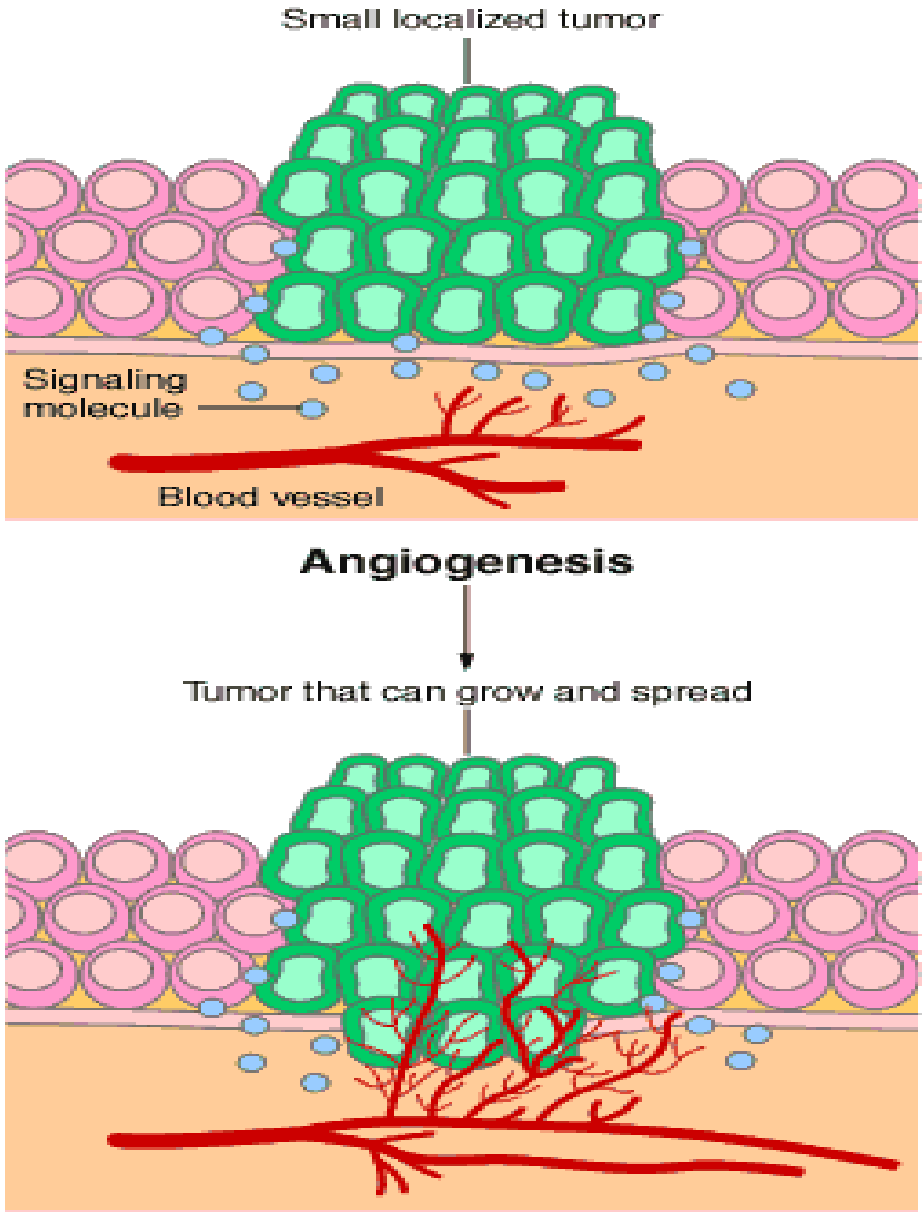

Normalized Imax (%)
120
100
80
60
40
20
0
1
10
100
1000
10000
100000
[ProTx-II] (nM)
nNav1.5 (1223 nM)
aNav1.5 (48 nM)

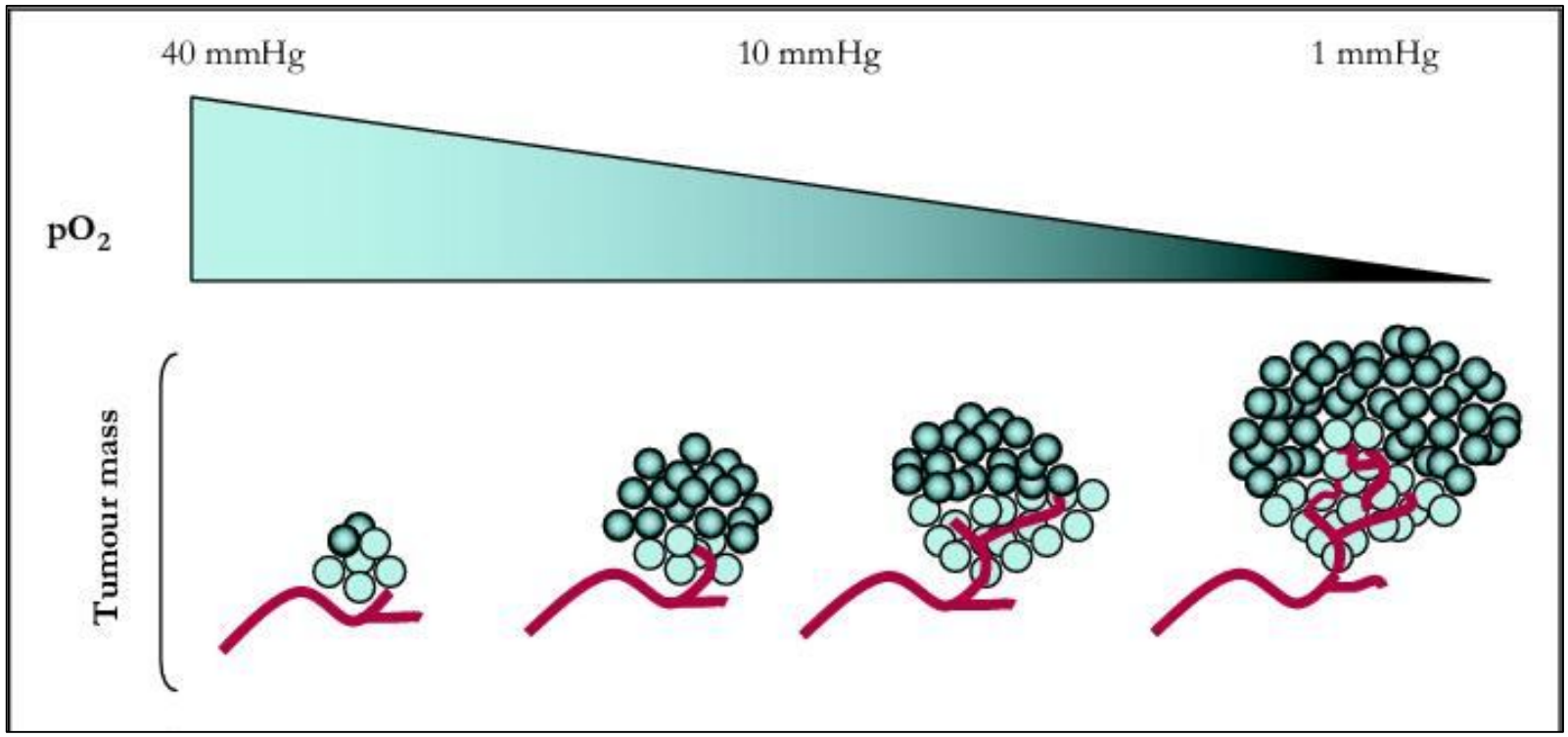

Normalized Gmax (%)
120
100
80
60
40
20
0
1
10
100
1000
10000
100000
[ProTx-II] (nM)
nNav1.5 (1982 nM)
aNav1.5 (87 nM)

Do we need to inhibit nNav1.5 specifically?

→ **VGSC persistent current!**

CANCER & HYPOXIA!


Small localized tumor
Signaling molecule
Blood vessel
Angiogenesis
Tumor that can grow and spread


40 mmHg
10 mmHg
1 mmHg
pO2
Tumour mass

Tumour hypoxia

Acute: functional changes in vasculature stability / aberrant shut down of a blood vessel.

Cycling: reoxygenation after acute hypoxia → 'hypoxia-reoxygenation injury' due to extensive production of reactive oxygen species (ROS).

Chronic: irregular distribution of tumour vessels that leads to limited O_2 diffusion within distances > 150-200 μM.

Different types can have differential effects at transcriptional (mainly through HIFs) and post-transcriptional levels. Hypoxic cells, in general, seek to acquire mechanisms that maintain their survival and promote migration/invasion away from stress stimuli (i.e. hypoxia). Such mechanisms are 'adoptive' in a tumour cell's perspective, but 'mal-adoptive' from an organismal perspective.

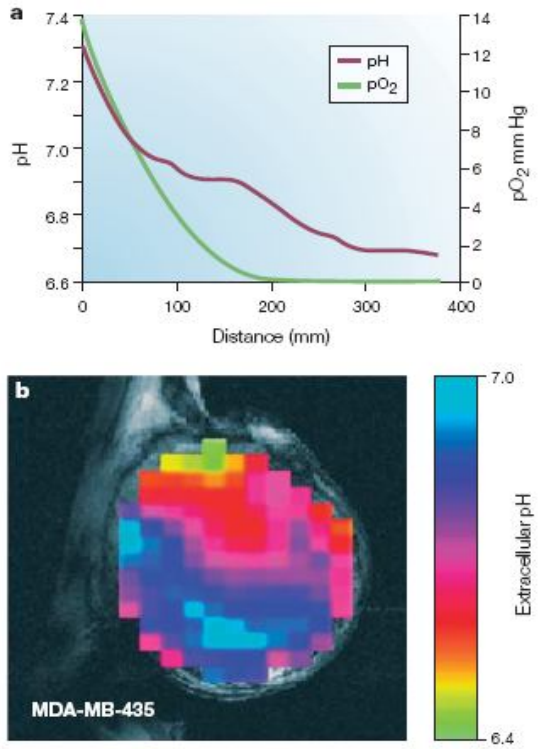
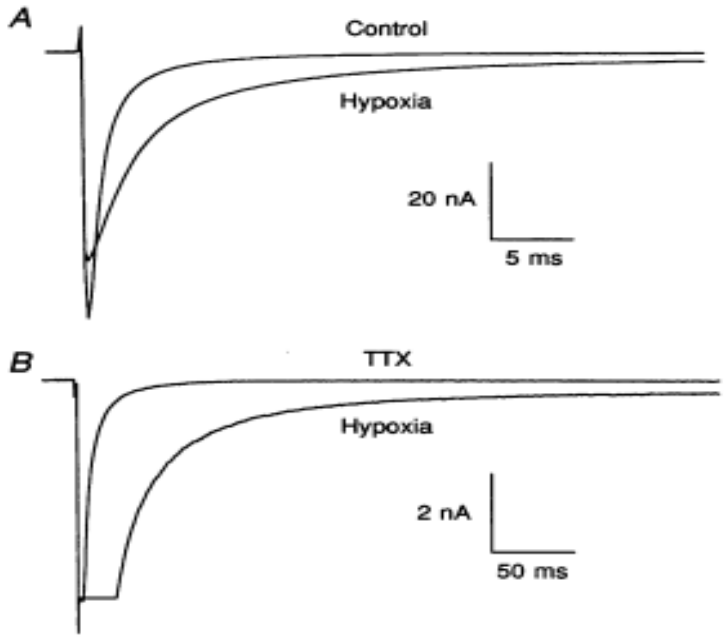


Figure 4 | **Hyperacidity of tumours.** These figures illustrate the micro- and macro-heterogeneity of pH. **a** | Tumour interstitial pH and partial pressure of oxygen (pO_2) are shown with distance from a vessel wall. These were measured *in vivo* in MCF-7 breast cancer cells using fluorescent ratio imaging. **b** | The extracellular pH of a MDA-MB-435 breast tumour in mice was imaged with the pH indicator IEPA and measured by ^{1}H magnetic-resonance spectroscopy. Part **a** reproduced with permission from REF. 30 © (1997) Nature Publishing Group. Part **b** reproduced with permission from REF. 26 © (2002) Wiley.

Gatenby & Gilles, 2005 (Nature Reviews Cancer)

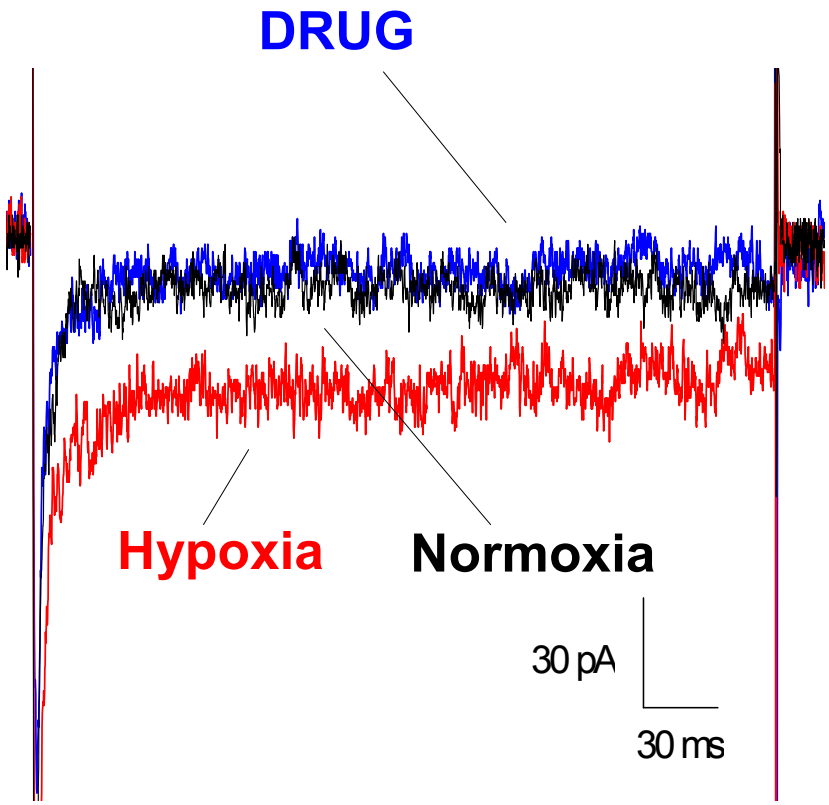
Hypoxia & VGSCs → I_{NaT} and I_{NaP}

1. Acute hypoxia (5-10 min) upregulates I_{NaP}, enhancing $[Na^+]_i$ (see below).
2. Chronic hypoxia (>24 h) upregulates VGSC current density (Fearon and Brown, 2004).
3. Mechanisms responsible for such effects are not known.
4. Hypoxic effects on VGSCs expressed in metastatic tumour cells have not been studied.

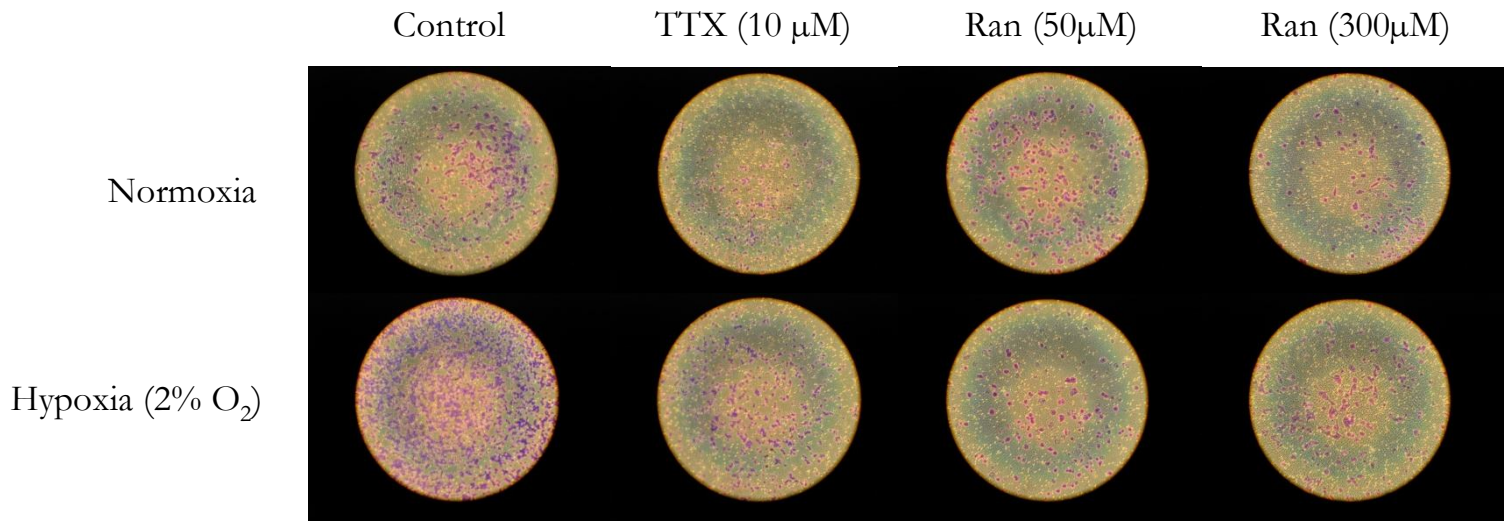


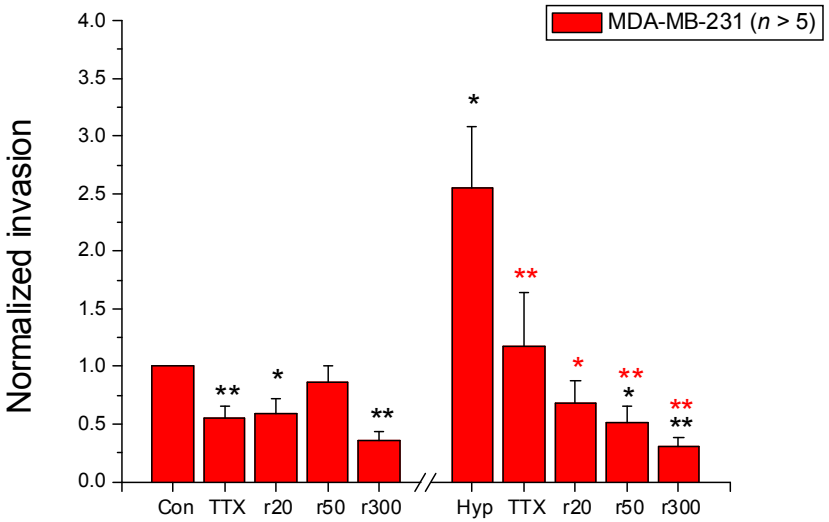
Ju et al., 1996 (J Physiol)

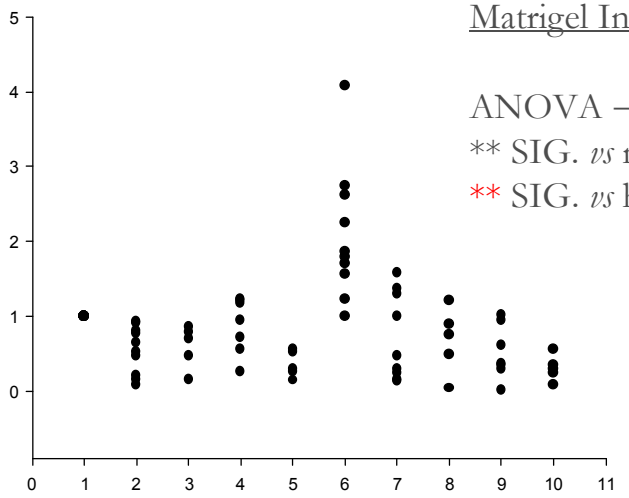
Pharmacology of I_{NaP}



Effects of drugs on invasiveness of MDA-MB-231 cells in normoxia and hypoxia







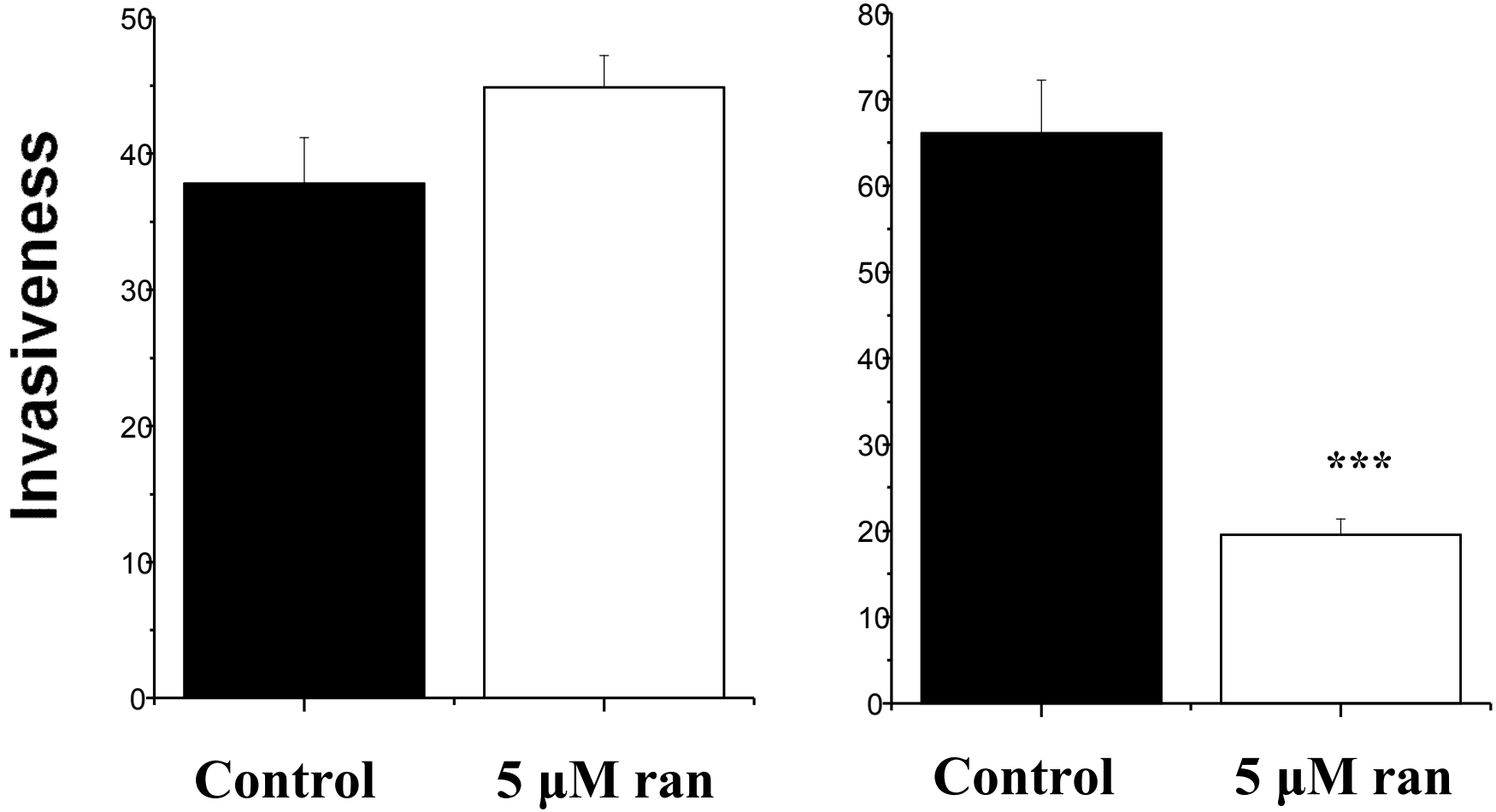
Matrigel Invasion– 24 h

ANOVA → *p*-value =0.000057

** SIG. *vs* normoxia control

** SIG. *vs* hypoxia control

Effect of 72 h preincubation on drug-induced inhibition of invasiveness (MDA-MB-231 cells) in therapeutic range



CONCLUSION:
Application of new technology
**(electrophysiology /
molecular pathophysiology):**

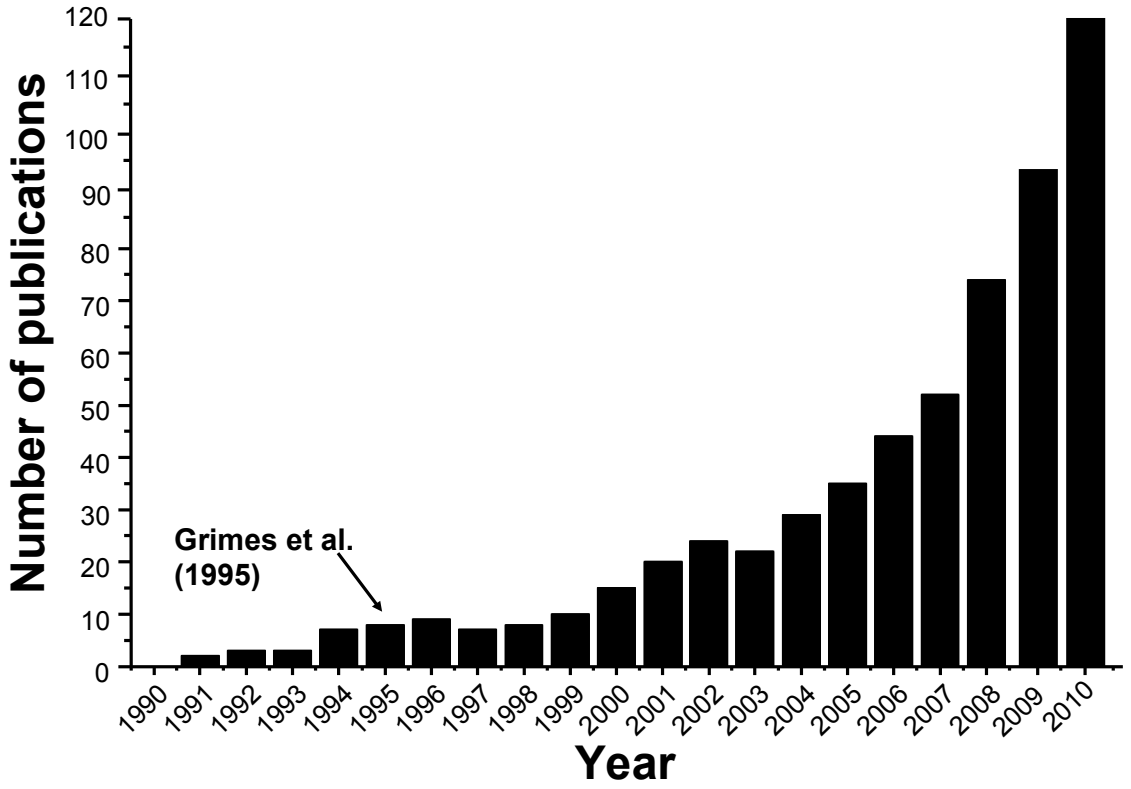
→ NEW CONCEPT
("CELEX Hypothesis")

→ NOVEL CLINICAL POTENTIAL
(New drugs;
improved existing drugs)!

VOLTAGE-GATED SODIUM CHANNEL UPREGULATION AS A NOVEL PROGNOSTIC MARKER IN METASTATIC DISEASE (BCa and PCa)

- Upregulated mRNA by >1000-fold in strongly vs weakly metastatic cells; also *in vivo.*
- Upregulation maintained at protein and signal levels.
- Gene / protein in neonatal splice form, containing in BCa a unique sequence of 7 amino acids, targetable by antibody.
- Functional contribution to metastatic cascade.
- Biology compatible with expression early in metastasis.
- Under activity-dependent regulation (positive feedback)!
- Also of therapeutic potential (possible application of existing VGSC drugs, and novel Ab) – both to suppress and turn off channel activity / expression!

Number of publications citing 'ion channel' and 'cancer' per year from 1990 to 2010. Information from ISI Web of Knowledge[sm]



- The ion channels & cancer field is highly vibrant and destined to grow.
  - Conceptually and technically, we are the leaders!
- Neonatal Nav1.5 is a major target for colon as well as breast cancer.

Thank you!

The Wellcome Trust

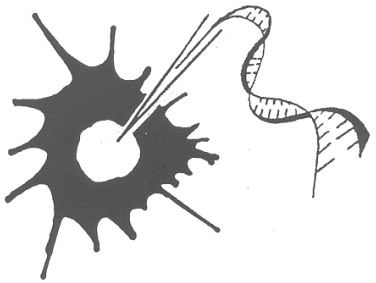
Prostate UK
research • information • training

MRC Medical Research Council

South of England
Prostate Cancer Collaborative

THE PROSTATE CANCER CHARITY

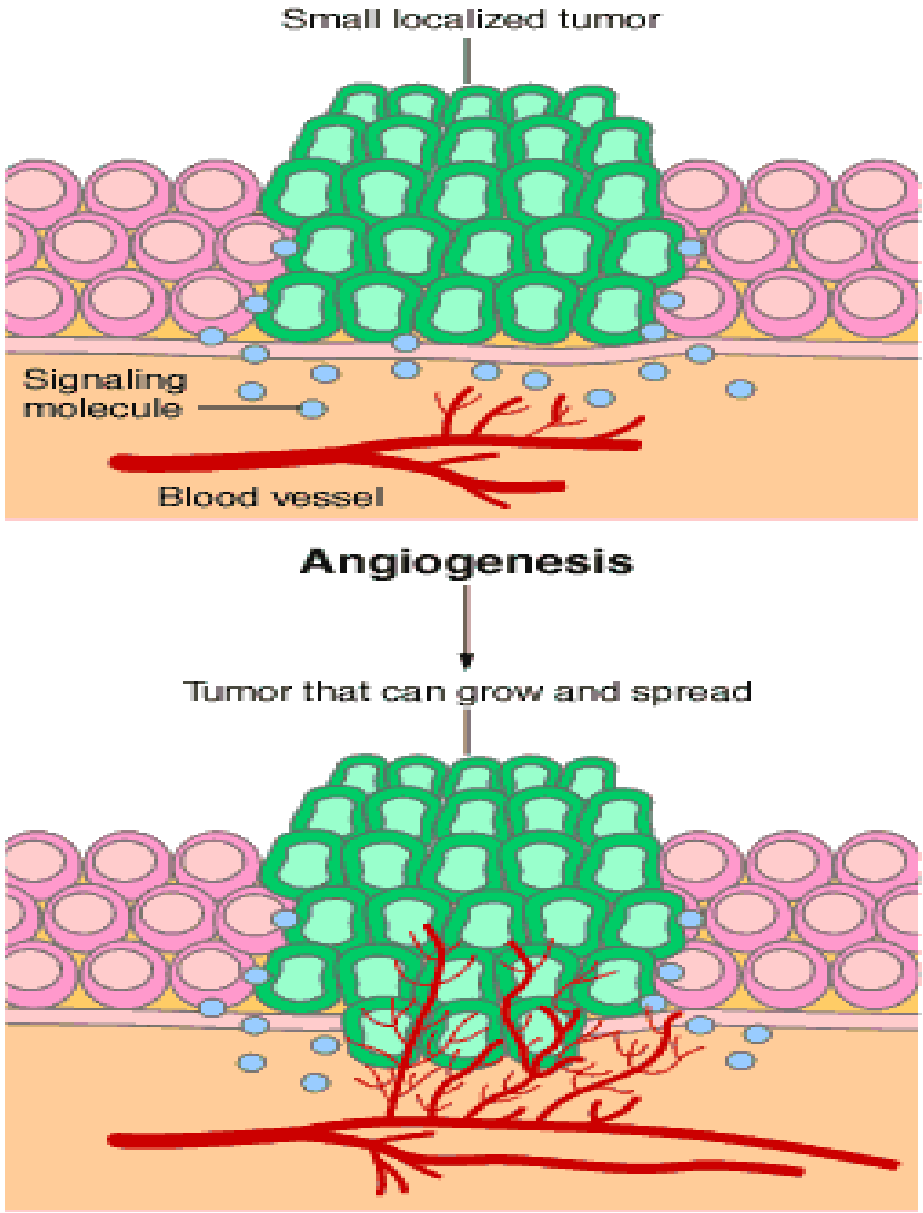
PCaSO

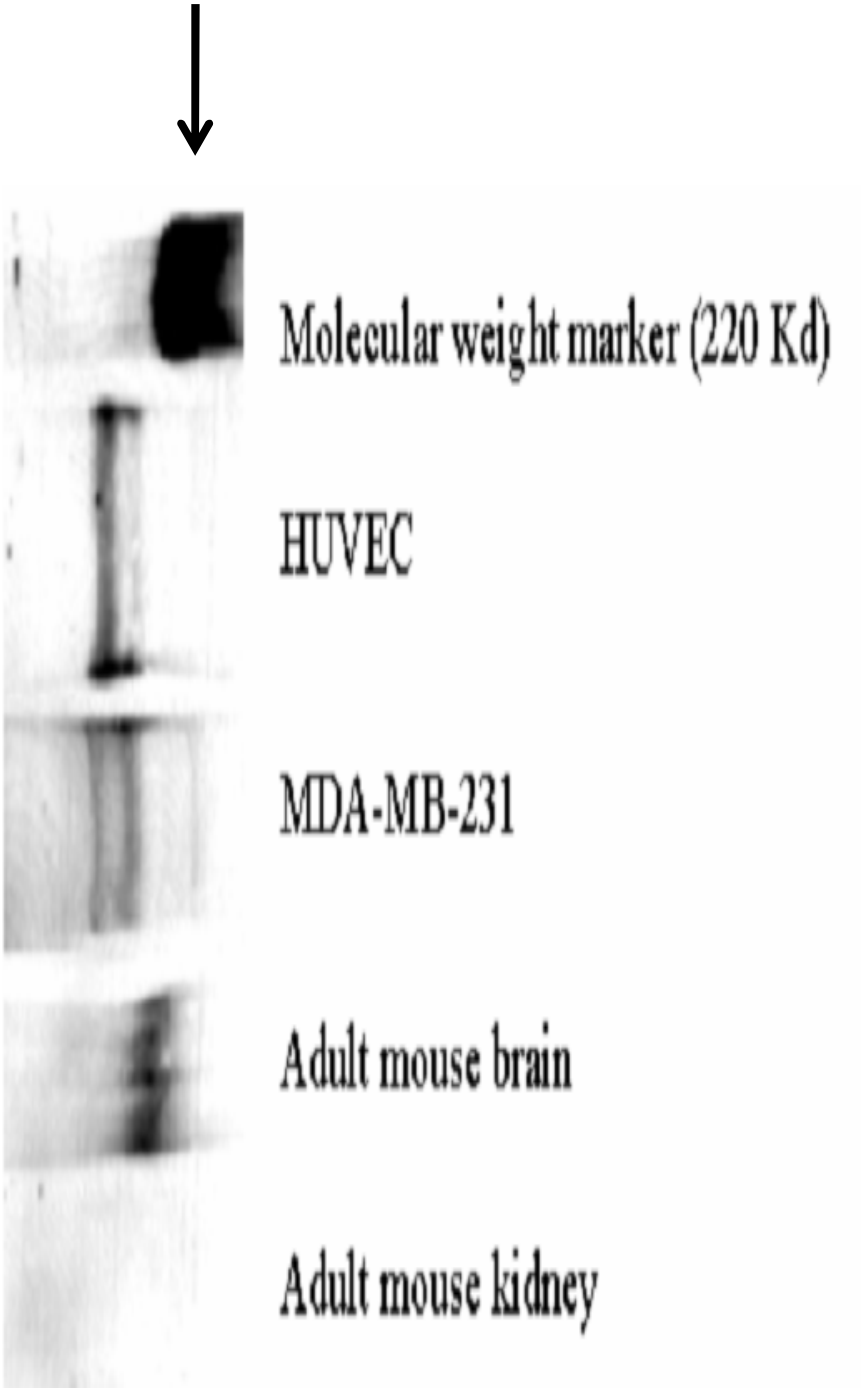


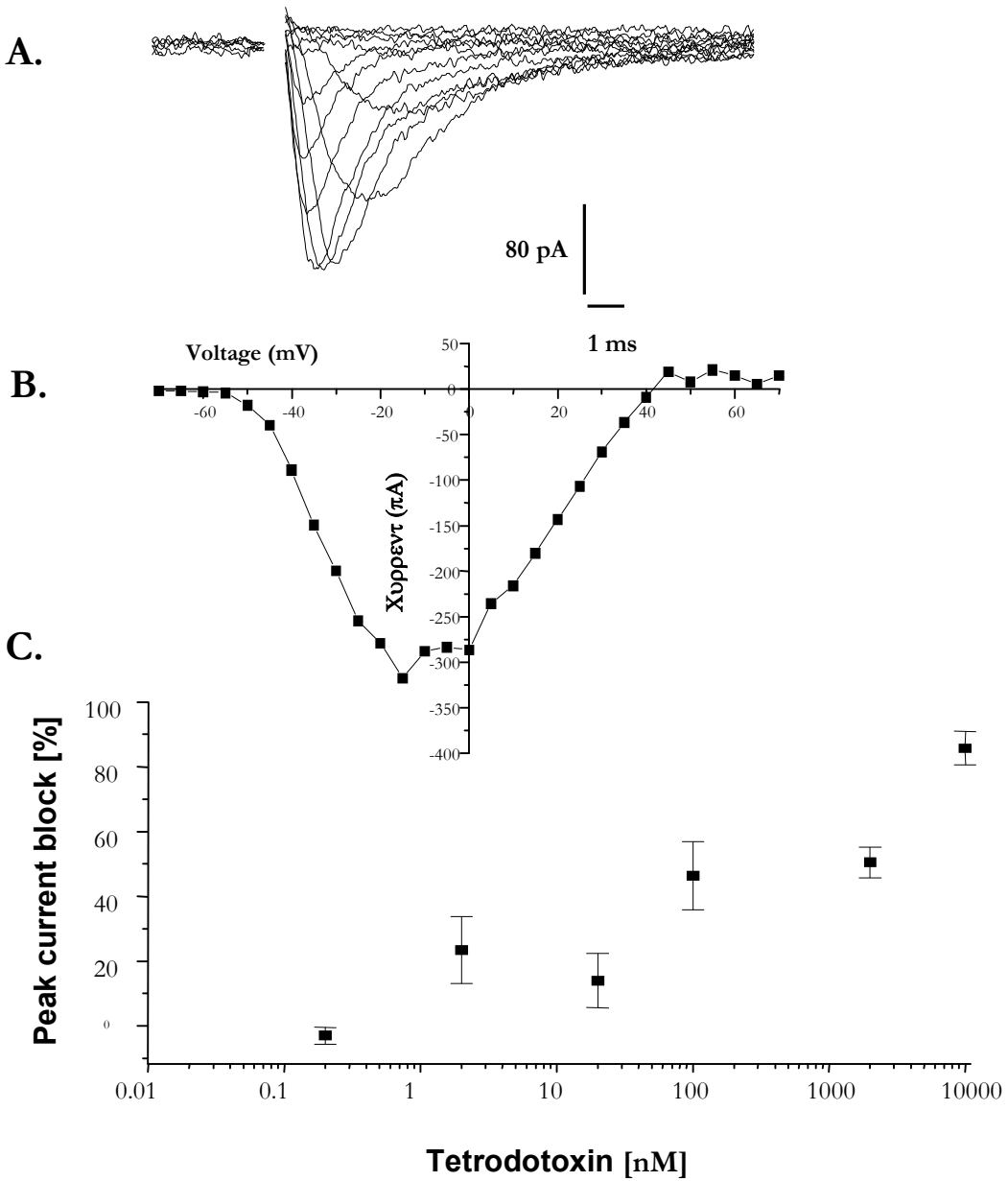
Pro Cancer Research Fund

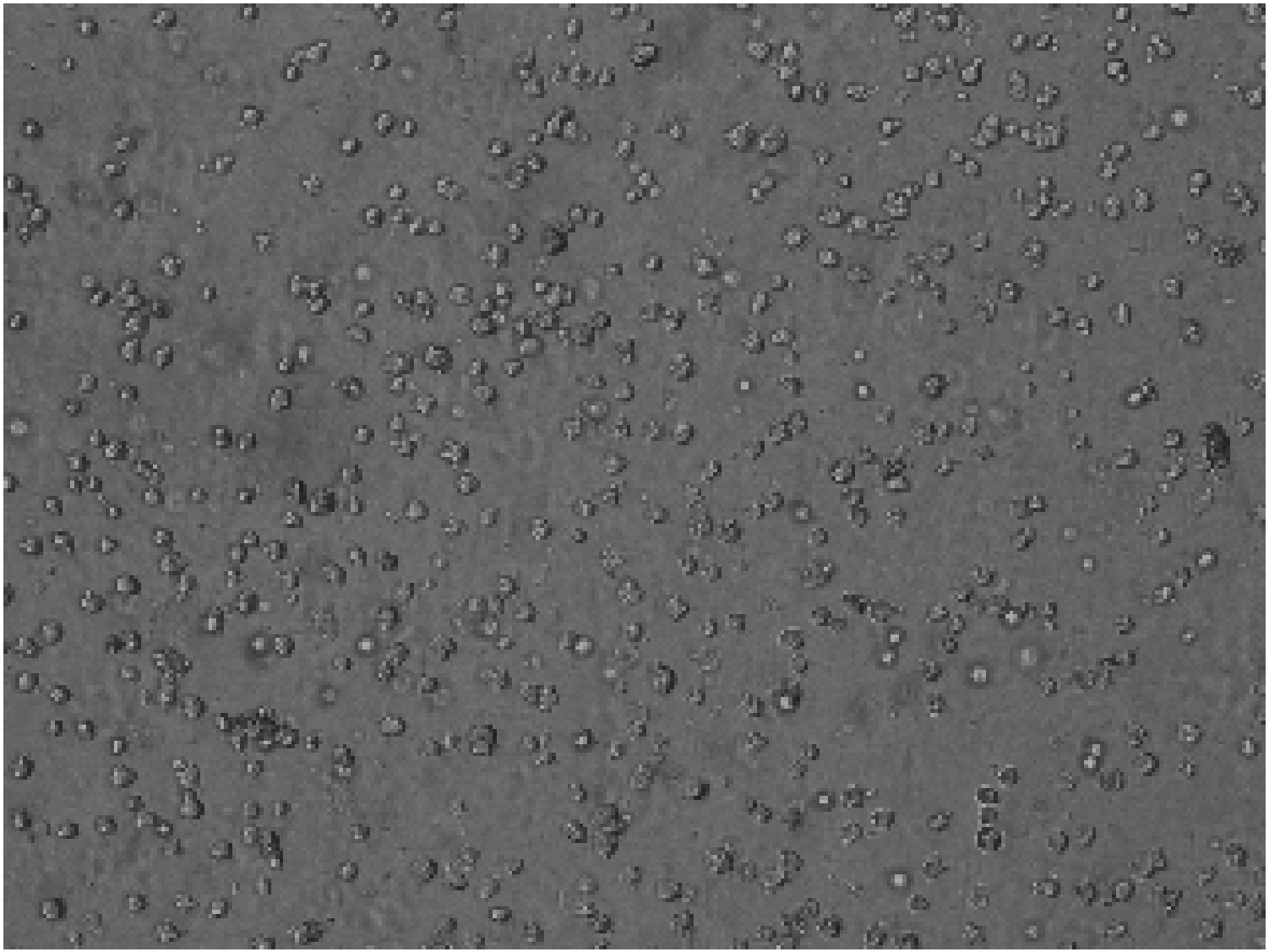
Role of VGSC in metastasis:

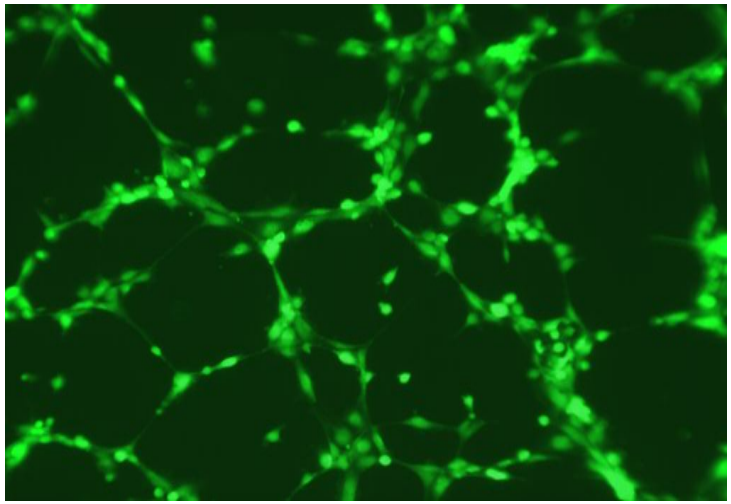
Angiogenesis

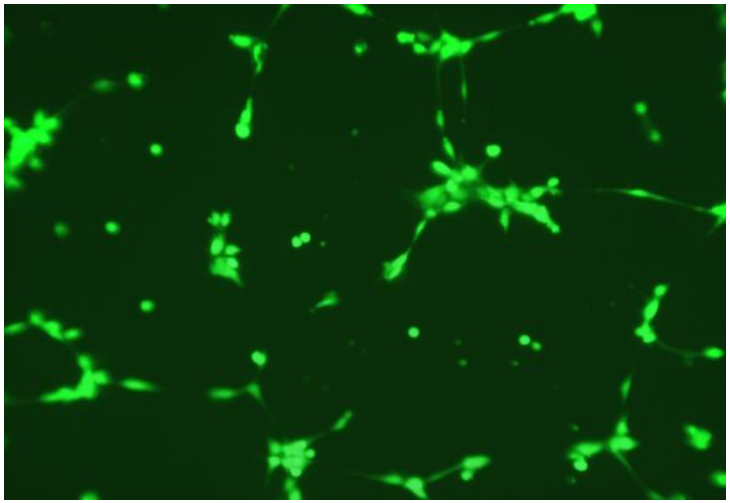

Small localized tumor
Signaling molecule
Blood vessel
Angiogenesis
Tumor that can grow and spread

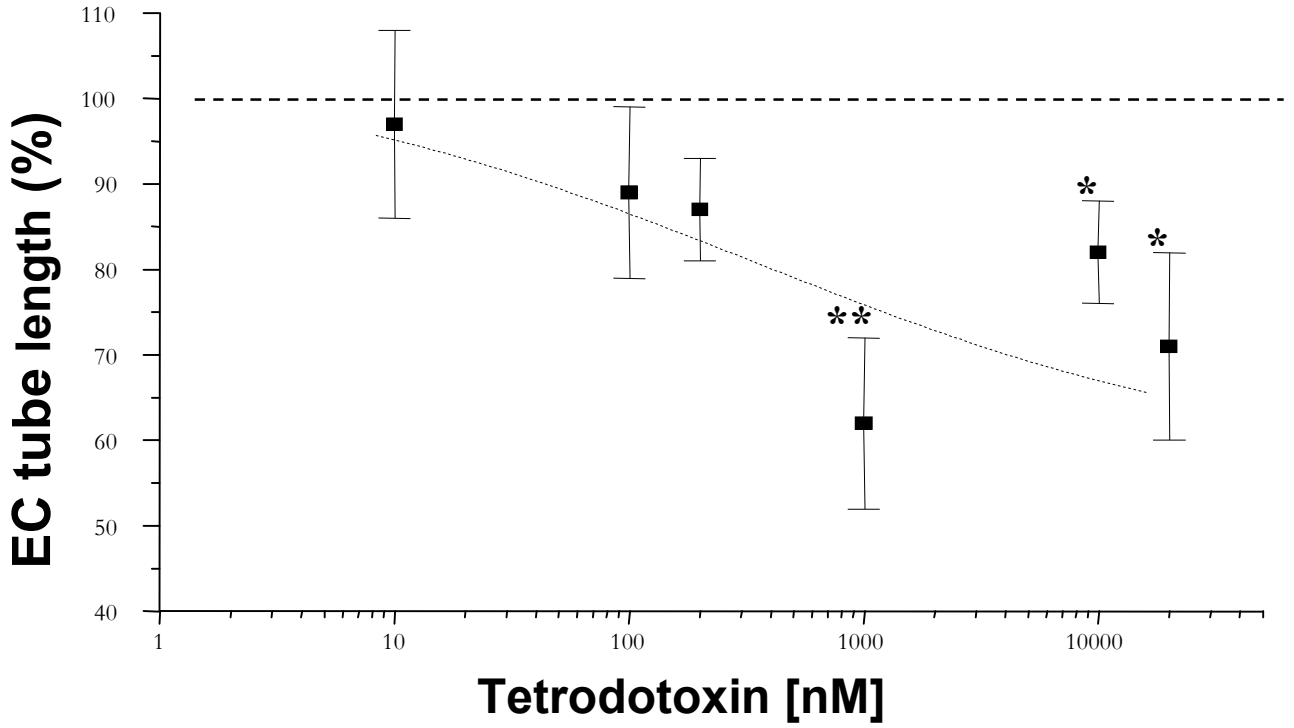

Molecular weight marker (220 Kd)
HUVEC
MDA-MB-231
Adult mouse brain
Adult mouse kidney


A.
80 pA
1 ms
B.
Voltage (mV)
Χυρρεντ (πA)
C.
Peak current block [%]
Tetrodotoxin [nM]








EC tube length (%)
110
100
90
80
70
60
50
40
1
10
100
1000
10000
Tetrodotoxin [nM]
*
**
*

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 286, NO. 19, pp. 16846–16860, May 13, 2011

Angiogenic Functions of Voltage-gated Na^+ Channels in Human Endothelial Cells

MODULATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) SIGNALING[*S]

Received for publication, September 22, 2010, and in revised form, February 15, 2011 Published, JBC Papers in Press, March 8, 2011, DOI 10.1074/jbc.M110.187559

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