Aurora Ion Channel Retreat
2015

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(–)-Englerin A as a novel potent activator of TRPC4 and TRPC5 channels

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David Beech Lab
School of Medicine
University of Leeds

Introduction to TRP channels

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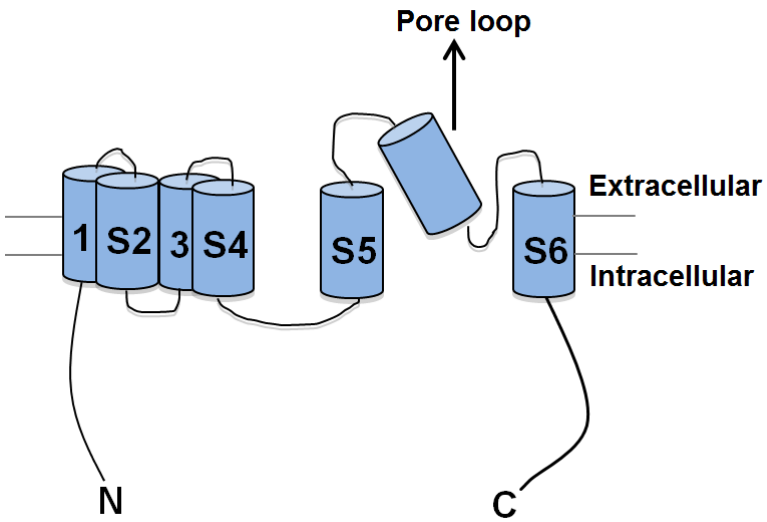
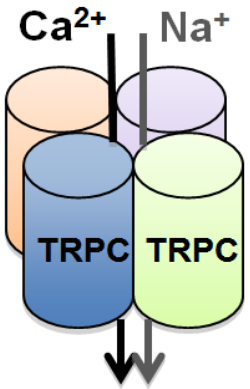
- Non-selective cation channels
- Ubiquitously expressed throughout the human body
- 7 families of TRP channels
  - C = ‘canonical’
- 6 membrane spanning domains with the pore loop between S5 and S6
- Often expressed as heteromeric channels
- Lack of specific small molecule modulators





TRP channel activation by natural chemicals – TRPC?

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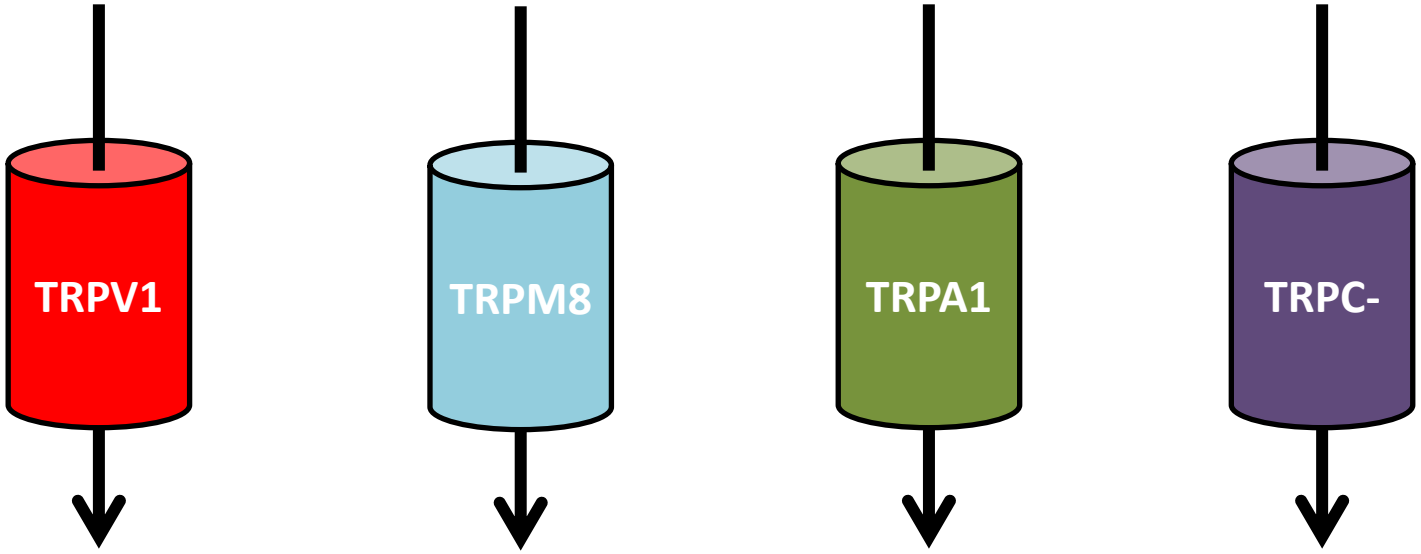
capsaicin
heat

menthol
cold

wasabi
mustard oil
cinnamon

?



Ca^{2+}/Na^{+} Ca^{2+}/Na^{+} Ca^{2+}/Na^{+} Ca^{2+}/Na^{+}

Inhibition of TRPC5 by an omega-3 fatty acid

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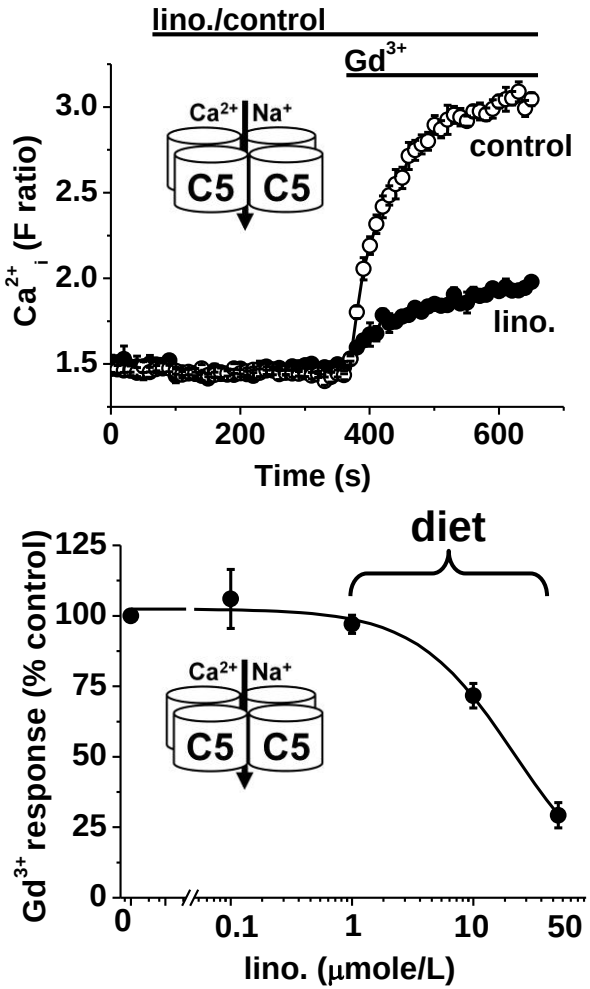
α-Linolenic acid (ALA) is an essential omega-3 fatty acid found in seeds, nuts and many common vegetable oils.



Sukumar et al 2012 Circ Res

Inhibition of TRPC5 by a chemical extract from ginger

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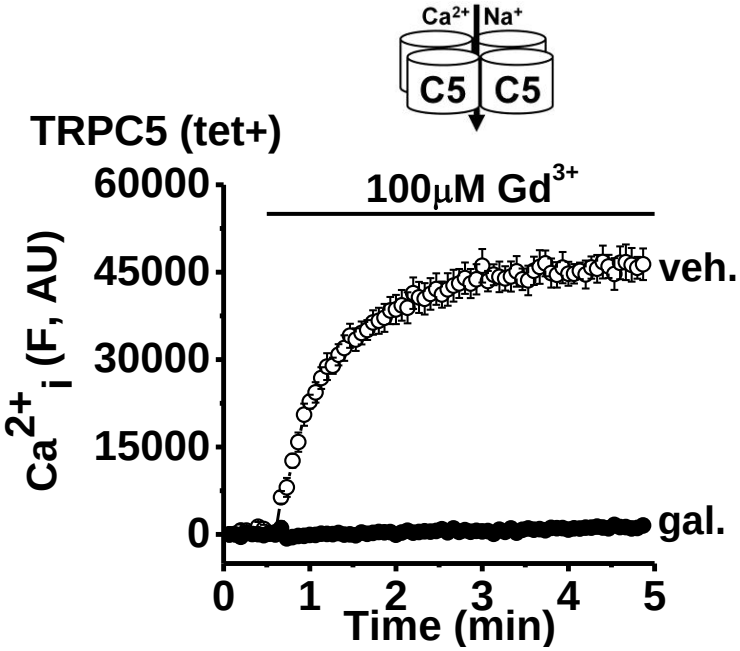
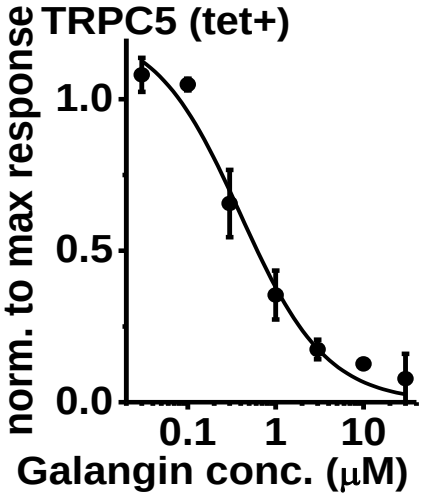
A traditional Chinese medicine for cardiovascular complaints





Naylor et al - In preparation

The discovery of (-)-Englerin A

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(-)-Englerin A is an extract from ***Phyllanthus engleri***

Heinrich Engler (1844-1930)
German botanist

(-)-Englerin A

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ORGANIC LETTERS

2008
Vol. 11, No. 1
57–60

Englerin A, a Selective Inhibitor of Renal Cancer Cell Growth, from *Phyllanthus engleri*

Angewandte Chemie

DOI: 10.1002/anie.200905032

Natural Product Synthesis

Total Synthesis and Absolute Configuration of the Guaiane Sesquiterpene Englerin A**

*Matthieu Willot, Lea Radtke, Daniel Könning, Roland Fröhlich, Viktoria H. Gessner, Carsten Strohmann, and Mathias Christmann**

Renal cell carcinoma

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61,560 new cases in USA (2015)

The most common type of kidney cancer

7th most common cancer in UK

Resistant to chemotherapy

Often asymptomatic until it spreads to other organs

Naveen Vasudev
Consultant in Medical Oncology

Sebastian Trainor
Registrar in Medical Oncology

The ‘Englerin team’ Synthesis and target identification

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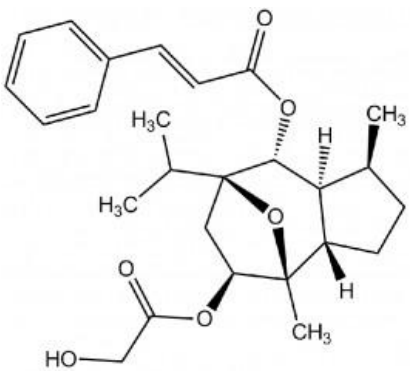
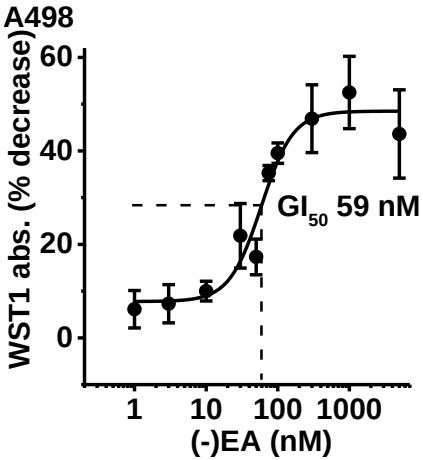
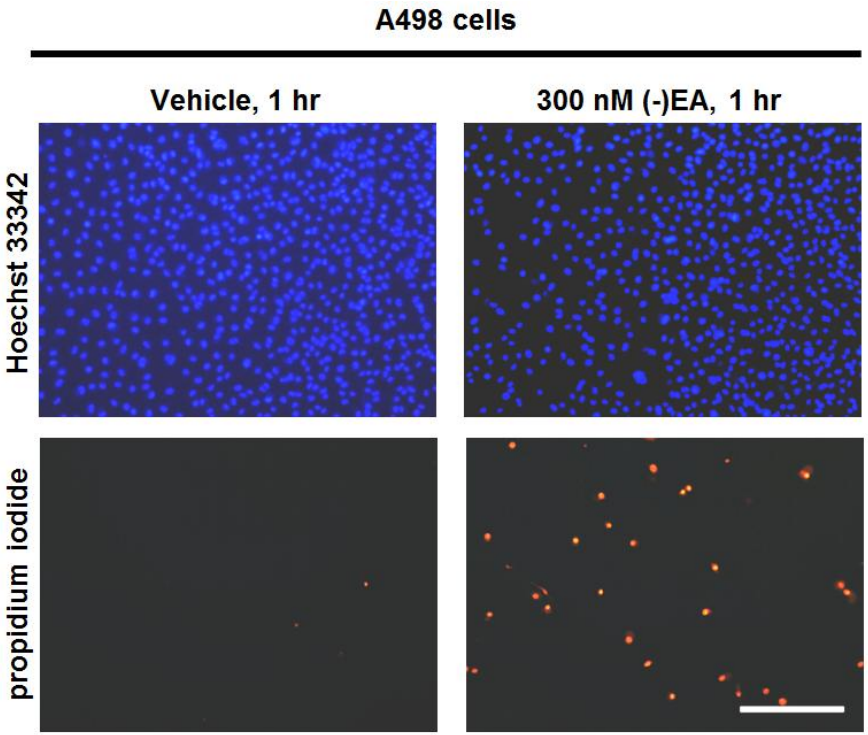
A498 = Renal cell carcinoma cell line



(-)-Englerin A





Akbulut Gaunt et al 2015 Angewandte Chemie Int Ed

Potent stereo-selective activator of TRPC4

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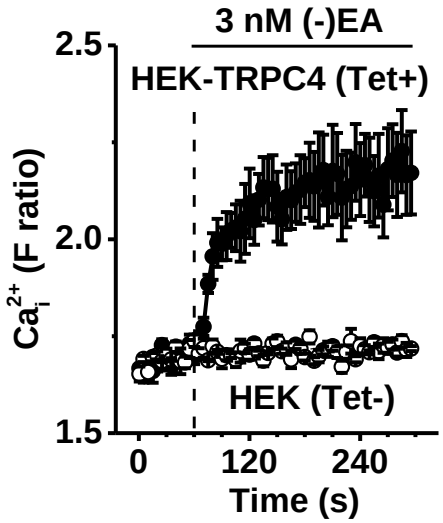

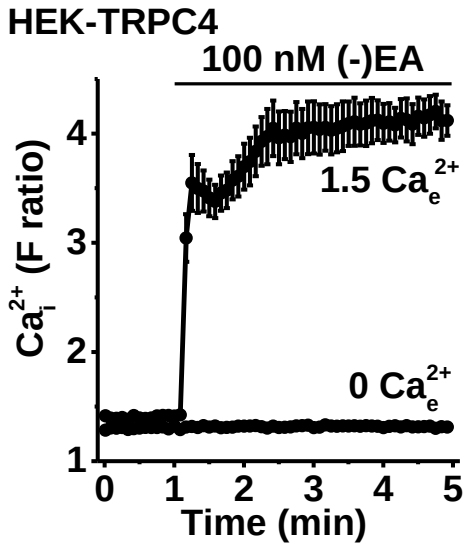

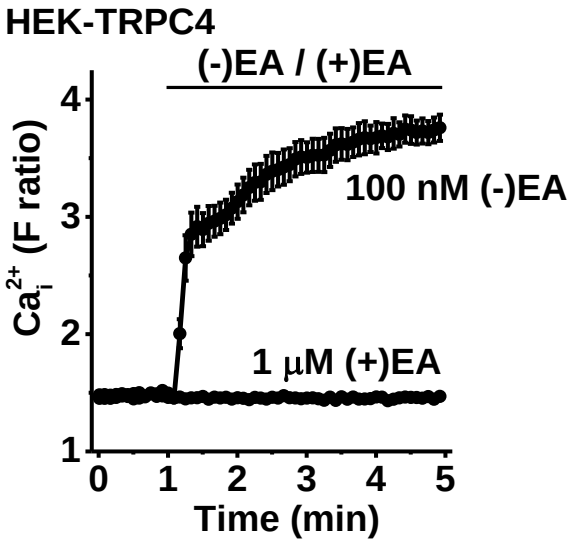

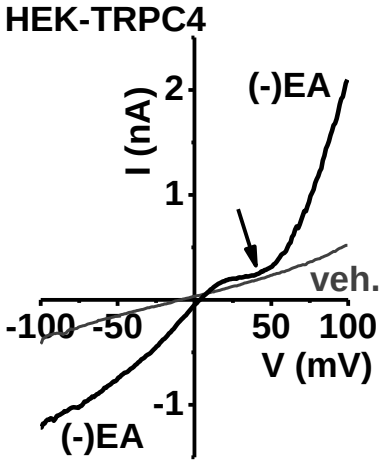

(-)-Englerin A evokes calcium entry in HEK cells overexpressing TRPC4

Extremely efficacious activator

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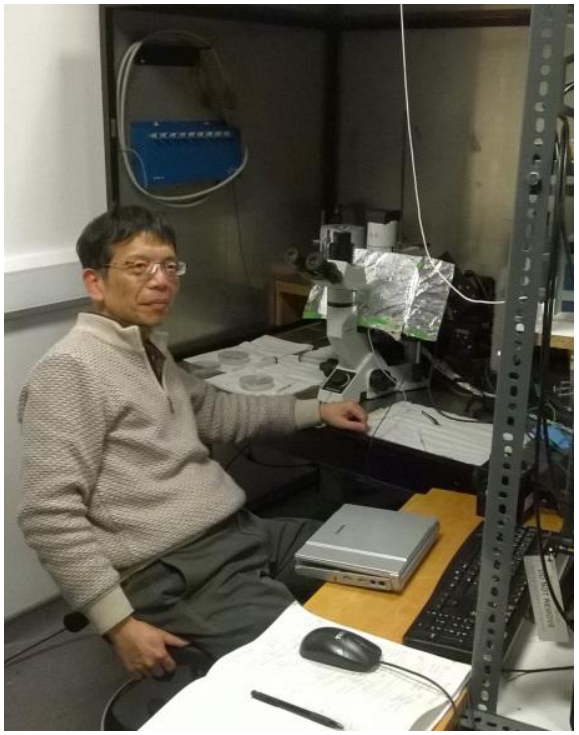
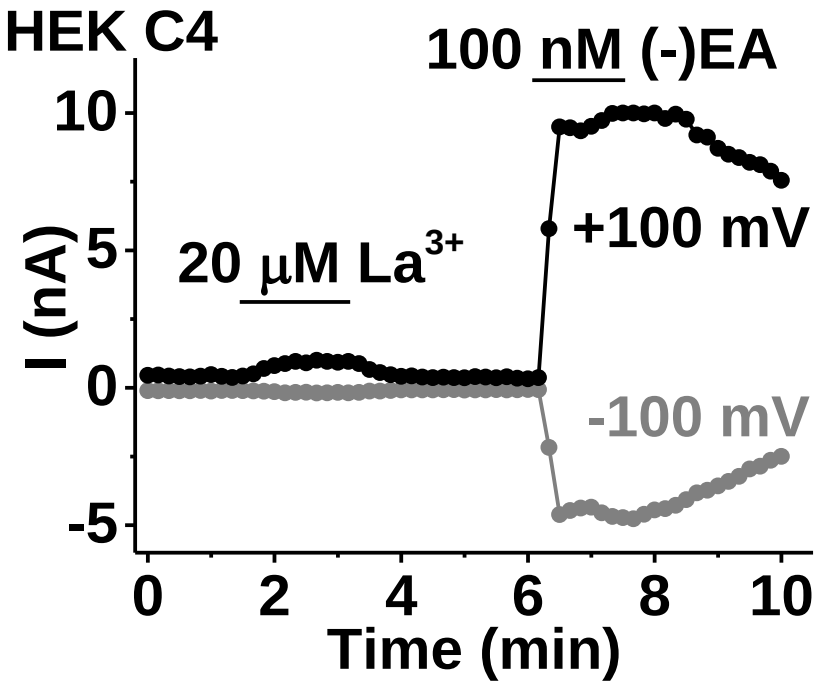


Prof. Katsuhiko Muraki
Aichi Gakuin University



Activates TRPC4 and TRPC5 with nanomolar potency

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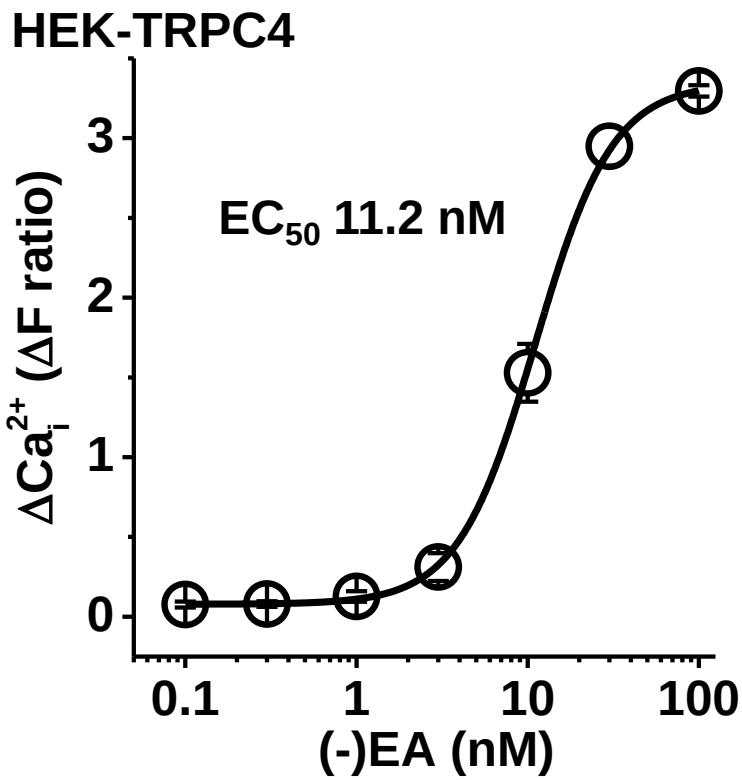

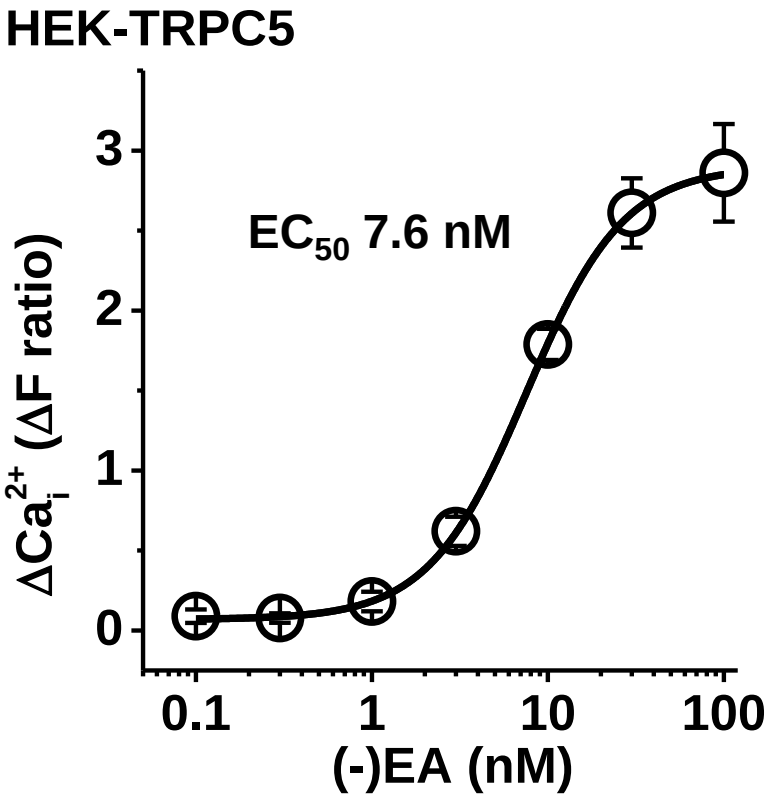

Specific TRPC4/5 channel activator

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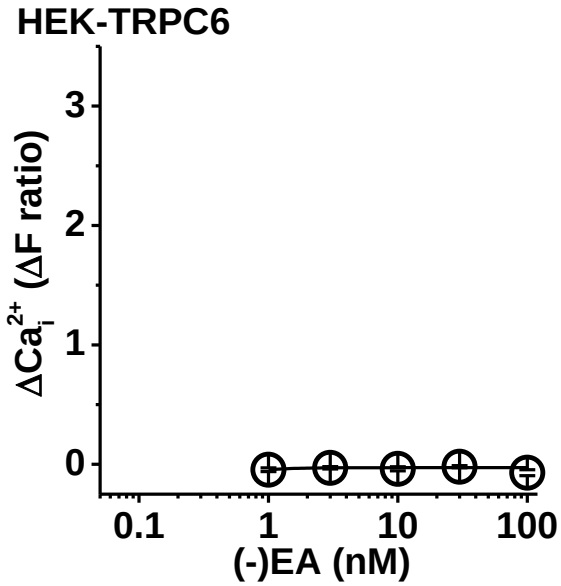

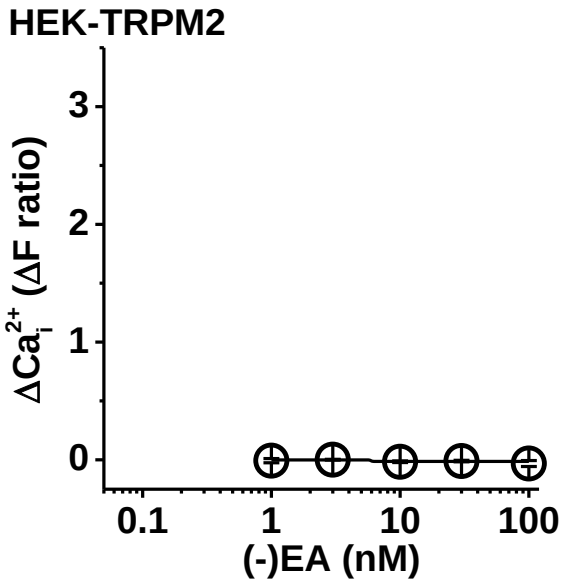

No effect on

TRPC6
TRPM2
TRPC3
TRPV4
Orai1 SOCE
Orai3
$Na_V1.5$
$K_V4.3$
$K_V1.5$
KCNQ
hERG
$Ca_V1.2$
Kir2.1
HCN4

- **No effect on 14 other ion channels or 59 GPCRs**
- **No binders detected in unbiased proteomic screening**

Powerful activation independent of G-proteins

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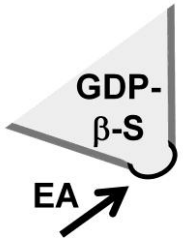


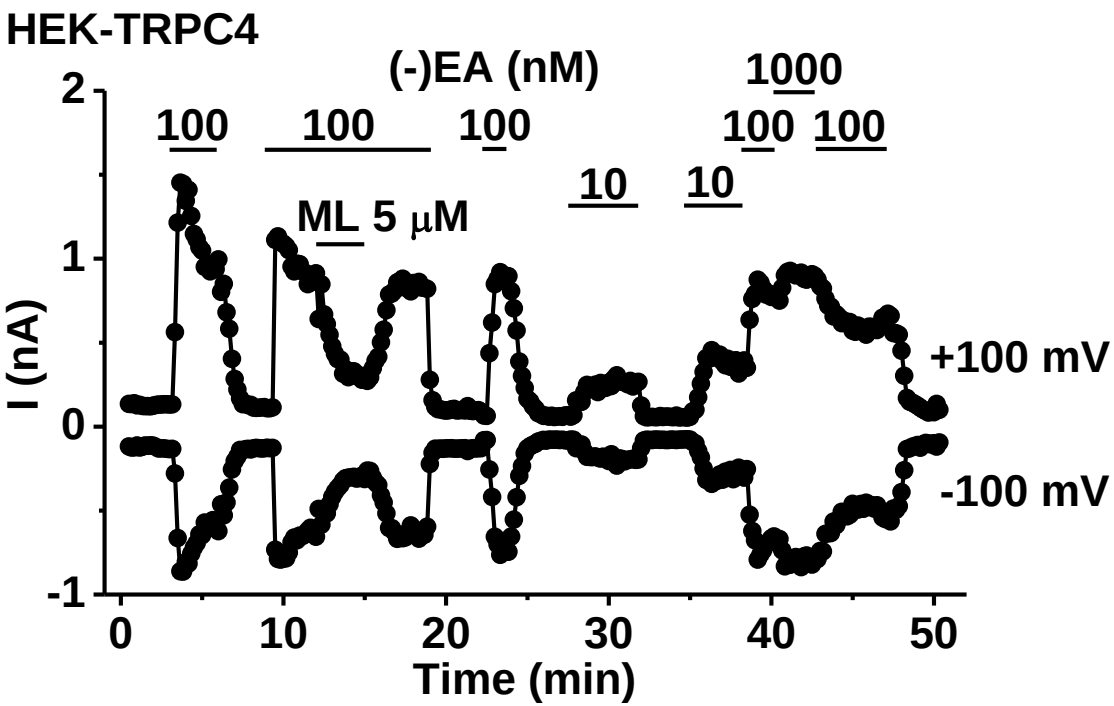
Outside-out patch configuration

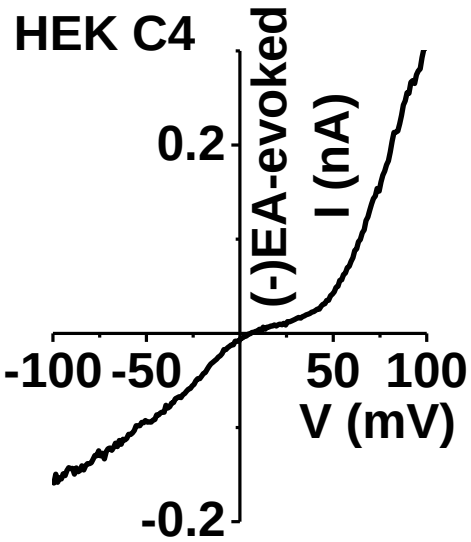
ML204

TRPC4/5 channel inhibitor

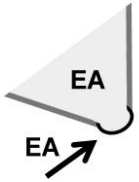
TRPC4 IC_{50} = 0.99 μM

TRPC5 IC_{50} = 9.2 μM

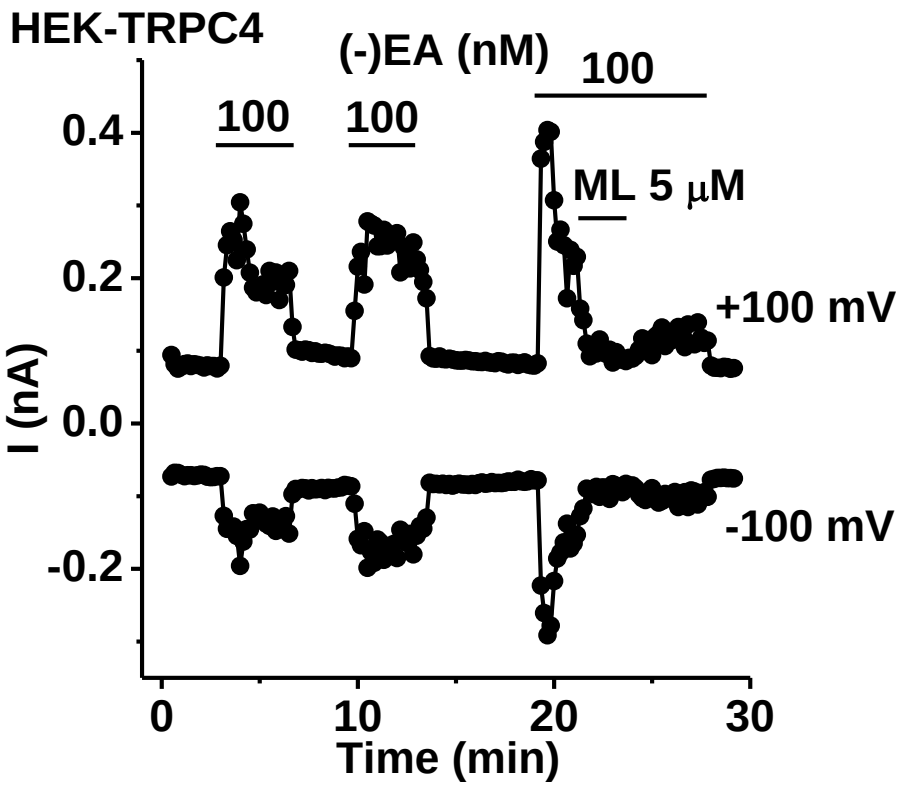


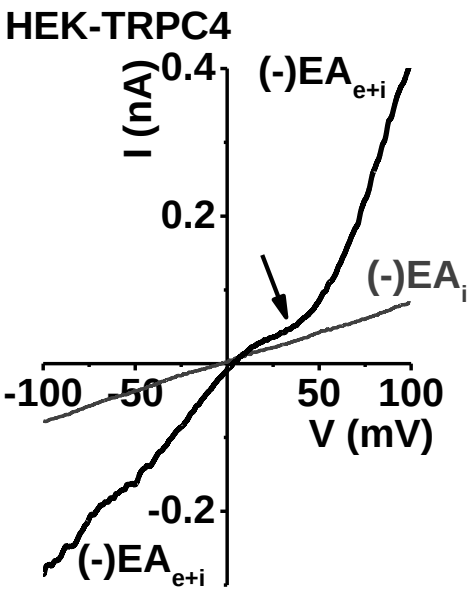


Extracellular effect



Outside-out patch configuration
(-)-Englerin A present in the intracellular pipette solution throughout





(-)-Englerin A acts potently to evoke calcium entry in A498 cells

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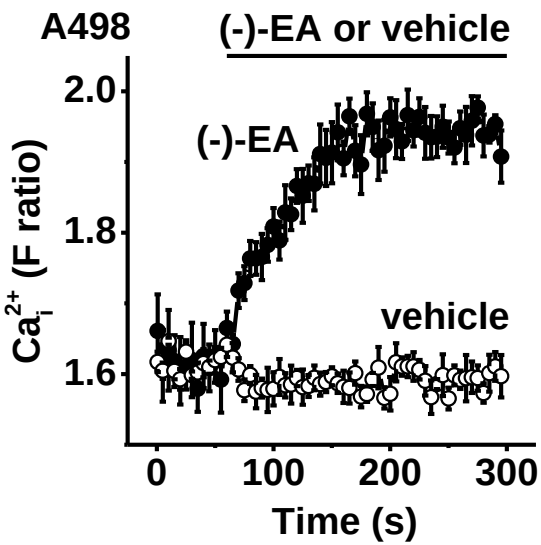

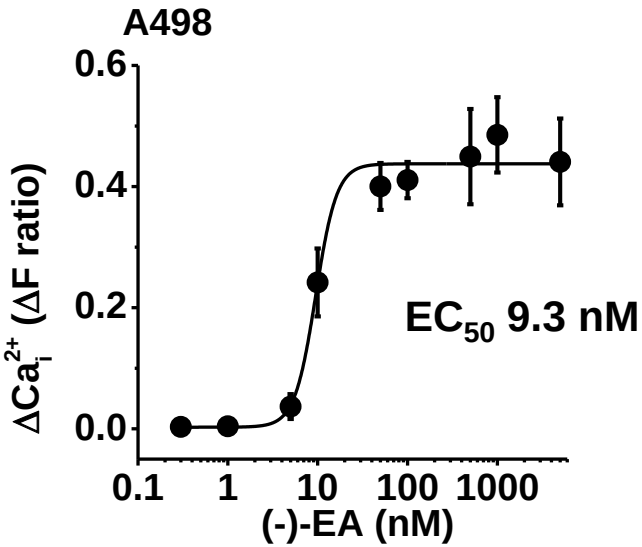

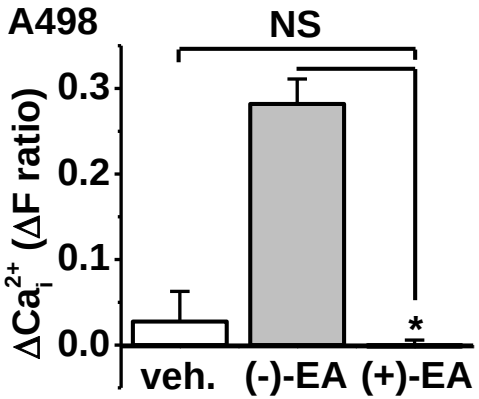

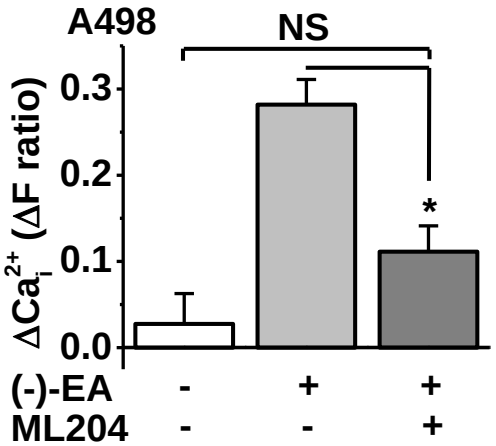

The native channels act remarkably like heteromers

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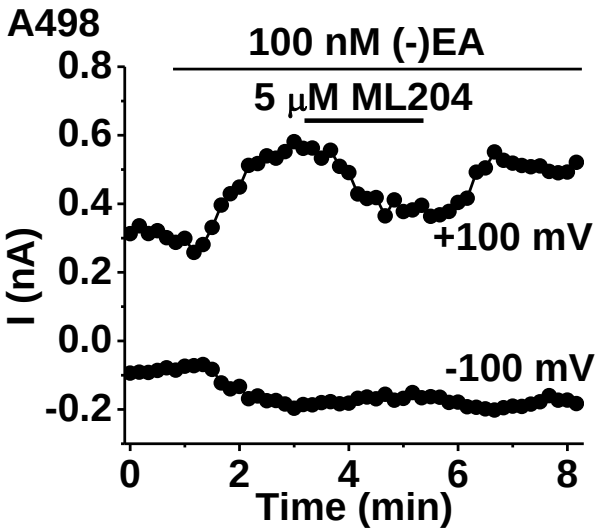

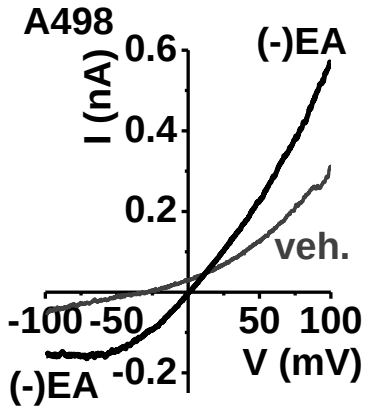

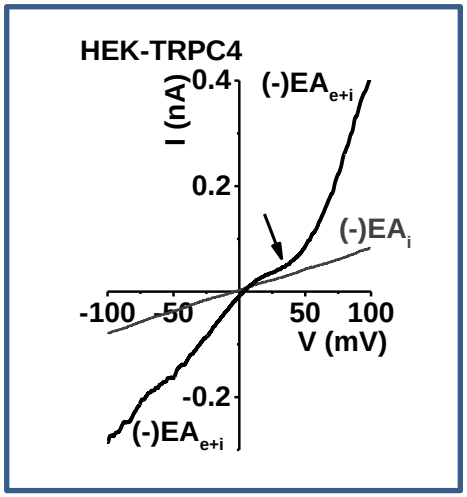

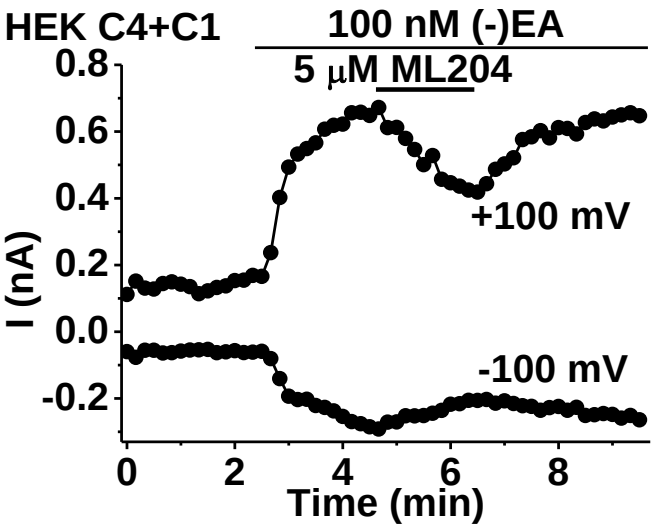

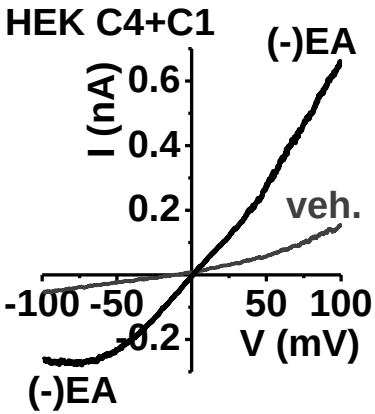

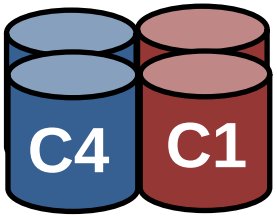

TRPC1 and TRPC4 are involved in the mechanism of cell death

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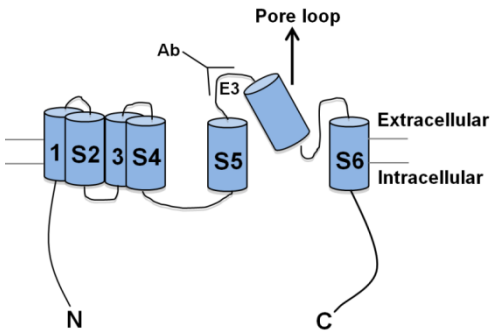
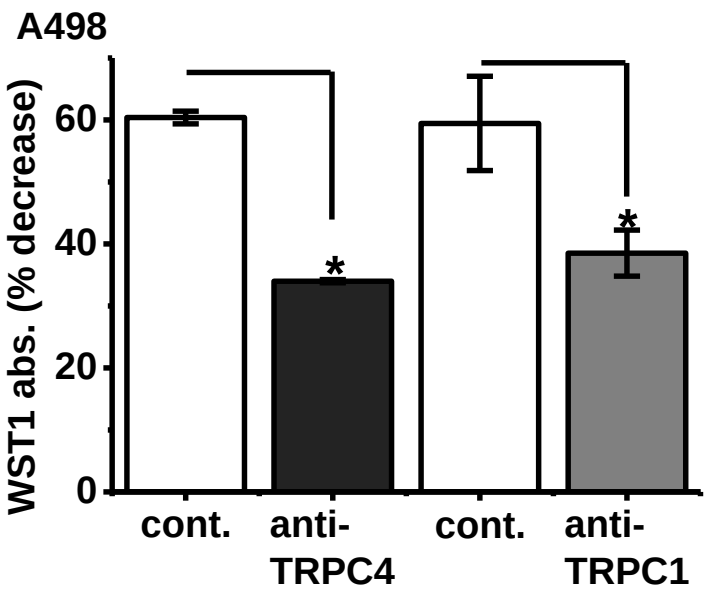
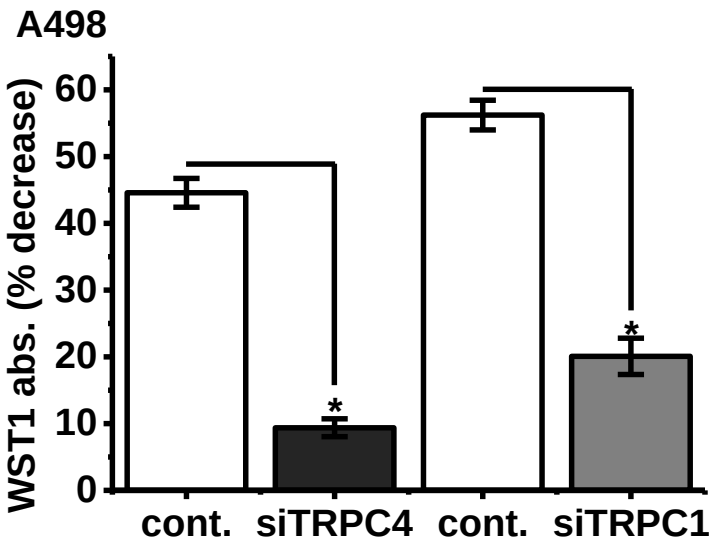
Blocking antibodies





Hannah Gaunt

siRNA



Melanie Ludlow

Emerging correlation

TRPC1/4 expression, cell death and ionic current

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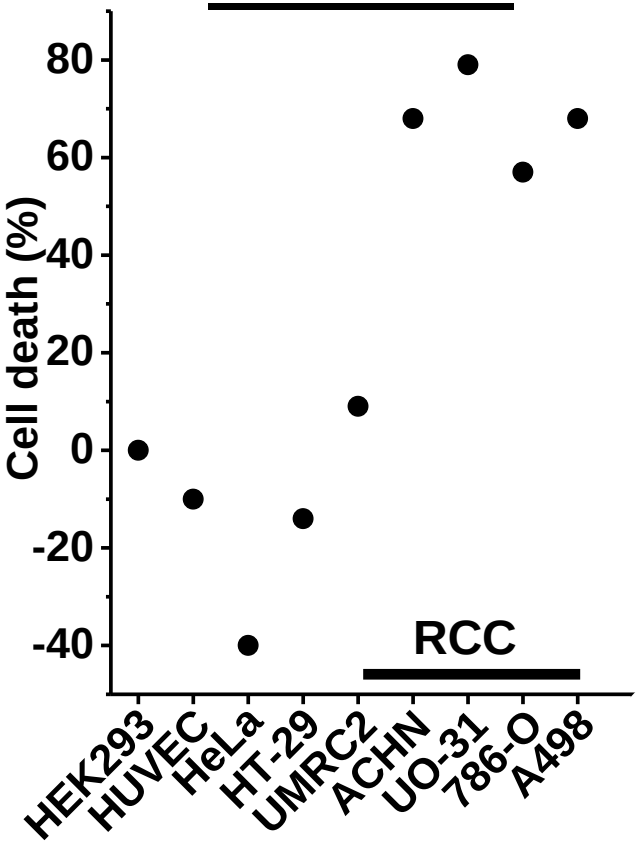
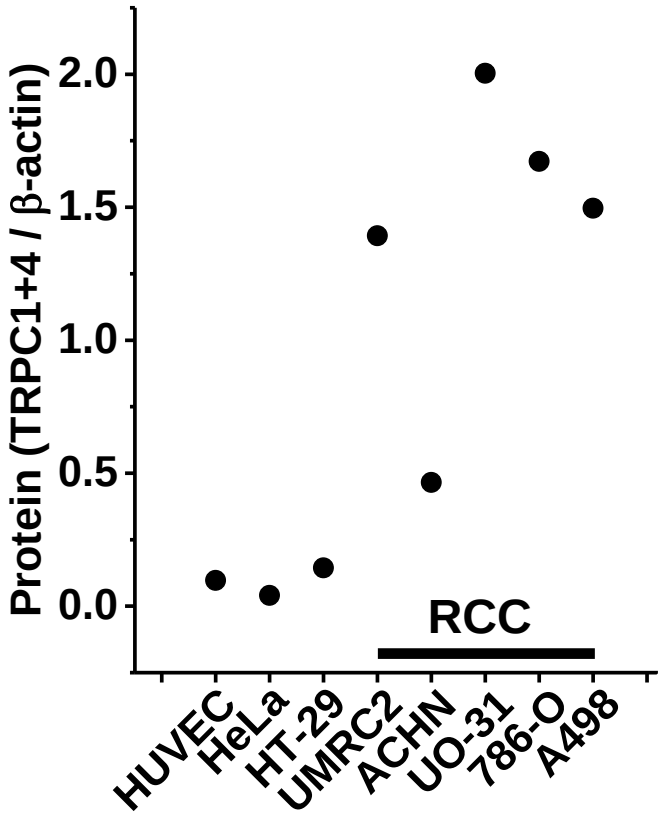
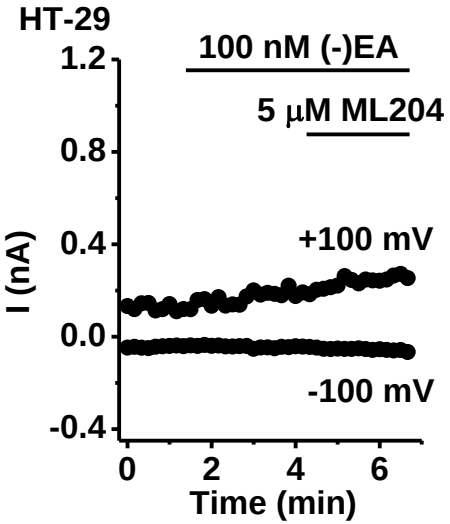
Cell death



Protein



Ionic current



HT-29 colorectal cancer cell line

Melanie Ludlow, Hannah Gaunt, Katsuhiko Muraki

In-vivo

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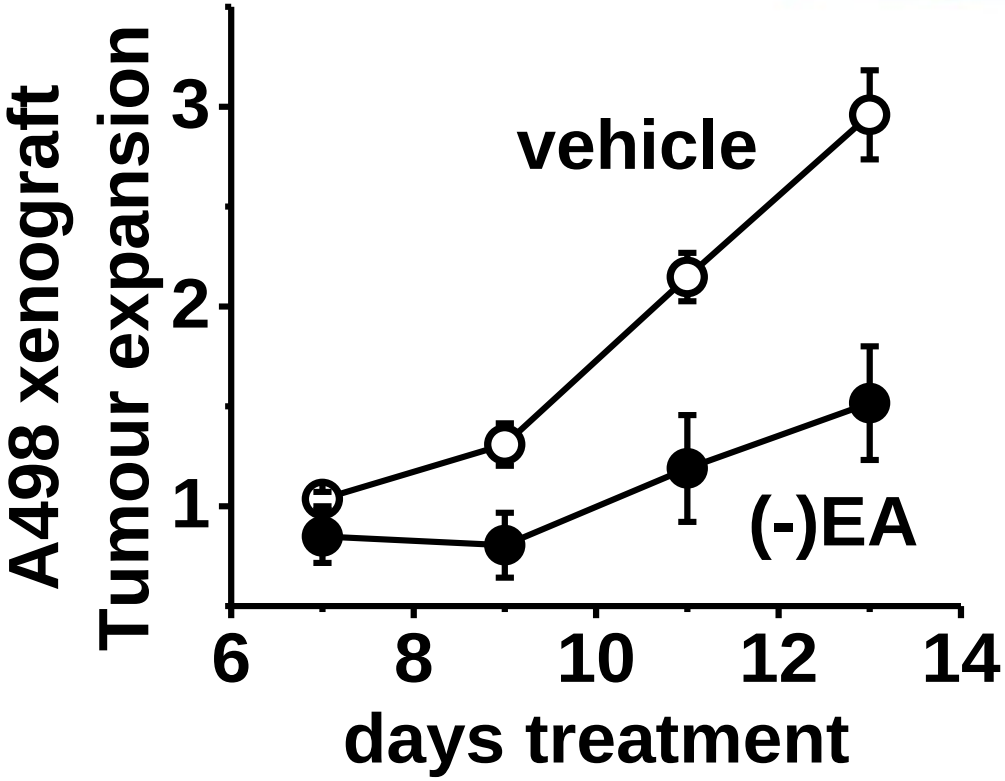

- A498 cells were subcutaneously implanted on the left flanks of 8 week old SCID beige mice
- 2 mg / kg (-)-EA or DMSO treatment (n=4 each)
- Intraperitoneal injection daily

Naveen Vasudev, Hannah Gaunt

Summary

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- (-)-Englerin A **selectively** activates TRPC4 and TRPC5 channels with **high potency and efficacy**
- Acts on the **extracellular** side of the membrane independent of G-protein activation
- TRPC1/4 protein expression **correlates** with the susceptibility to (-)-Englerin A evoked cell death
- (-)-Englerin A activated **TRPC1/4 channels** are involved in its mechanism of cell death

Implications:

1. A potential route to a renal cell carcinoma therapy
2. A channel activator in screens for TRPC4/5 inhibitors
3. For understanding TRPC4/5 channel biology

Thank you!

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The University of Leeds, UK
David Beech
Katsuhiko Muraki
Naveen Vasudev
Melanie Ludlow
Mohamed Amer
Alexander Bruns
Sebastian Trainor
Nicholas Moss

The Max Plank Institute, Dortmund, Germany
Herbert Waldmann
Yasemin Akbulut
Slava Ziegler

Freie Universität Berlin Germany
Mathias Christmann

MRC Medical Research Council

MAX-PLANCK-GESELLSCHAFT

Alexander von Humboldt Stiftung/Foundation

erc European Research Council

British Heart Foundation

Cardiovascular Research Leeds UK

wellcome trust

Out of Africa:
a natural born killer

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TRPC4 activators as selective chemotherapy "magic bullets" for renal cell carcinoma !?

Hannah Gaunt
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