13th Annual

ION CHANNEL RETREAT 2015

Share Knowledge. Exchange Ideas. Establish Partnerships.

July 7th, 2015

Structure, Function and Engineering of Ion Channels

Targeting voltage-gated K^+ channels in cancer reveal new biochemical pathways and therapeutic opportunities

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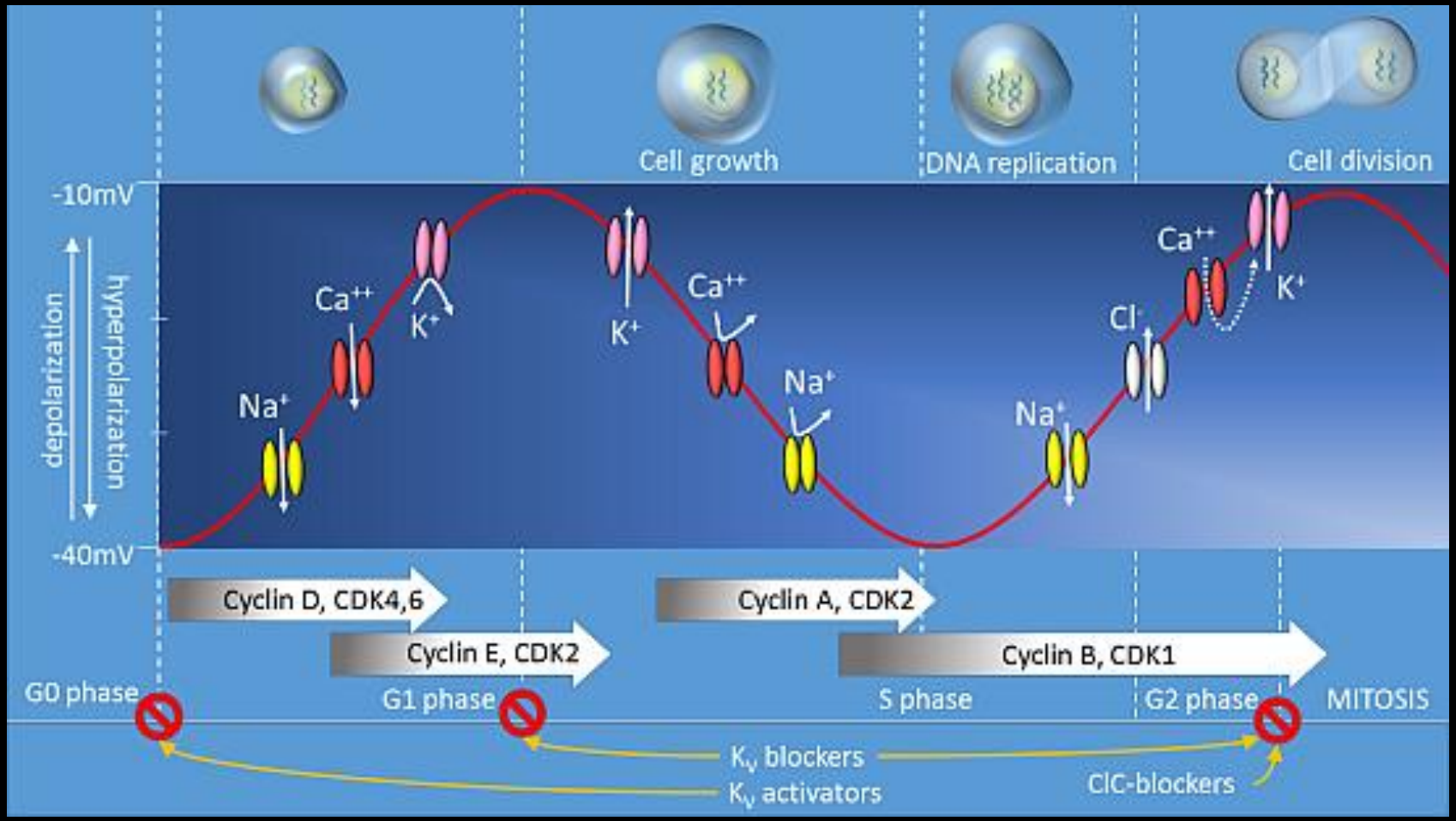
Department of Molecular Pharmacology & Therapeutics

Loyola University, Chicago

Voltage-Gated Ion Channels in Cancer Cell Proliferation

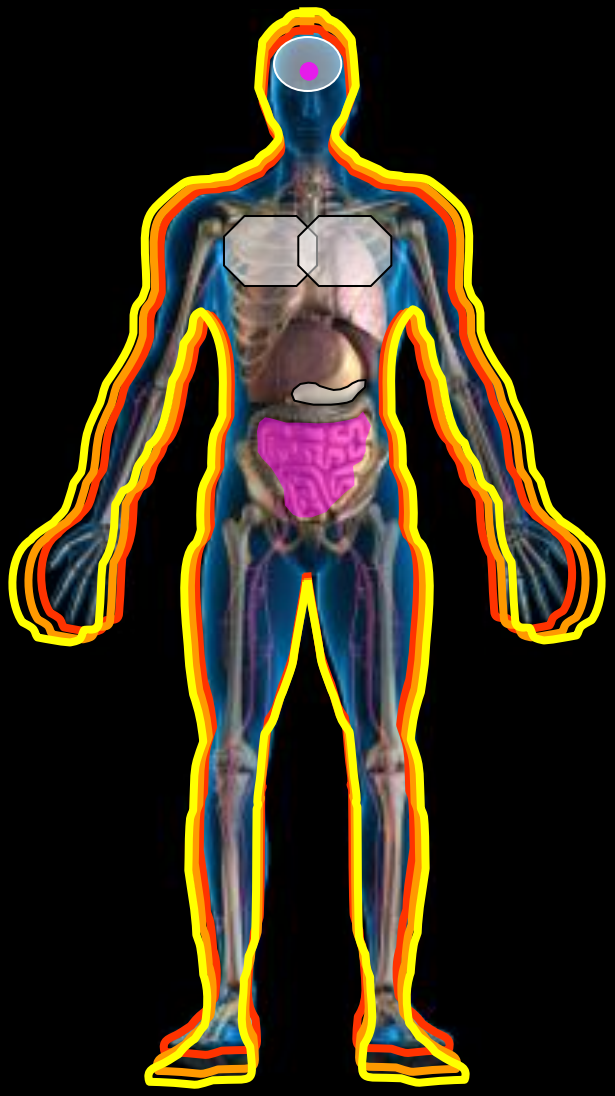
Vidhya R. Rao ,Mathew Perez-Neut ,Simon Kaja and Saverio Gentile

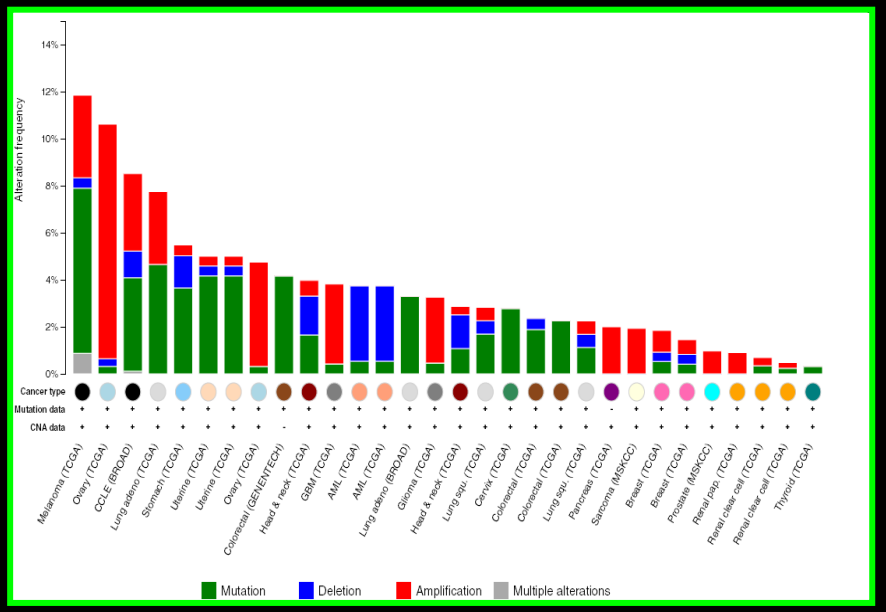
Cancers 2015, *7*(2), 849-875



Potassium channels have been found overexpressed in a variety of tumors of different histogenesis but absent in healthy cells from which the respective tumor are derived

- Rhabdomyosarcoma
- Leukemia
- Melanoma
- Neuroblastoma
- Breast cancer
- Pancreatic cancer
- Intestine





hERG1 channel expression pattern in tumors

Breast cancer facts sheet

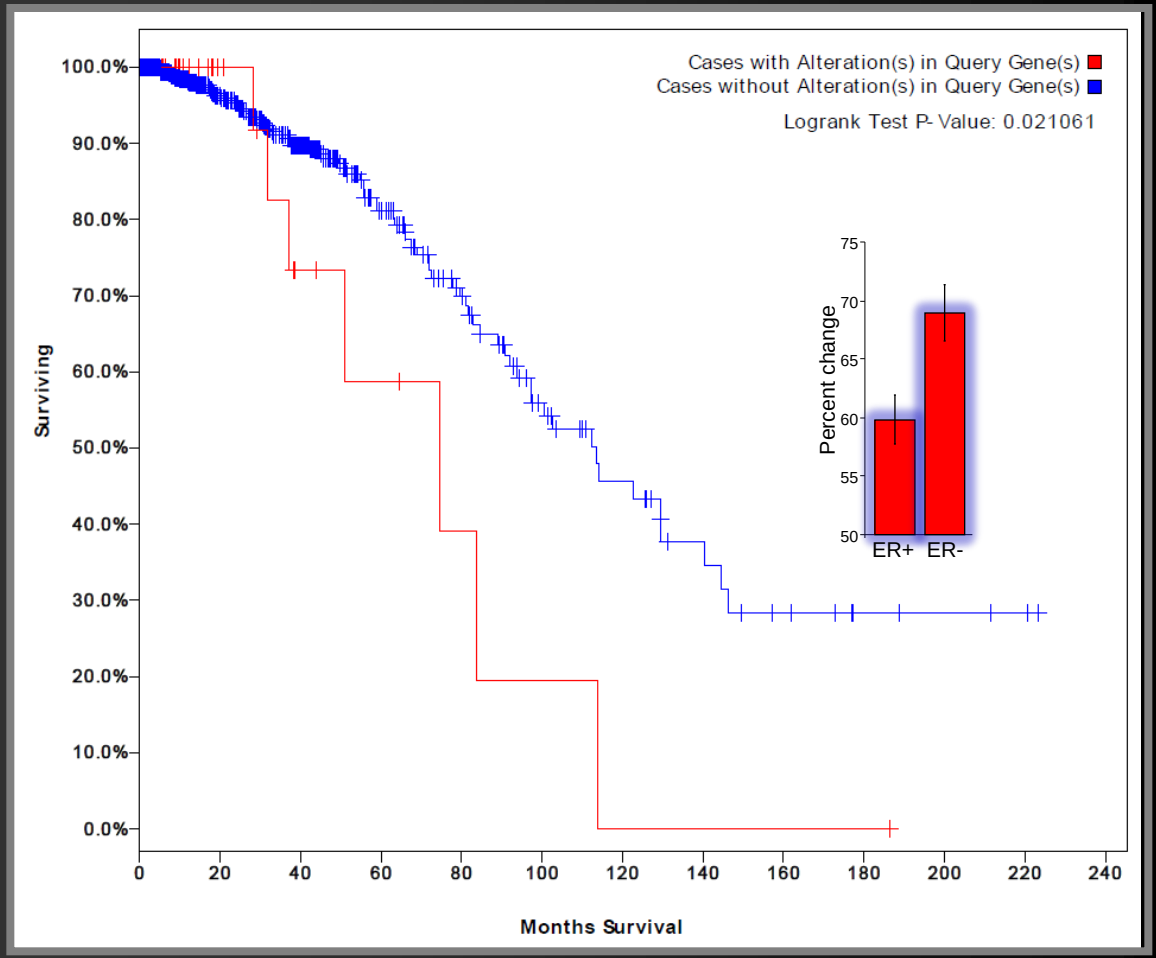
- 1 in 8 women will be diagnosed with breast cancer in their lifetime.

- 1 in 33 women will die from breast cancer.

- Men have the possibility of developing breast cancer as well.

- hERG1 expression is associated with higher mortality

- hERG1 is more abundantly expressed in ER-neg



Overall Survival Kaplan-Meier Estimate
www.cbioportal.org/public-portal

➢Can we manipulate hERG1 to fight breast cancer ?

Previous investigation have shown that blocking hERG1 current activity leads to cancer cell death.
However, because of the deleterious effects of hERG1 channel blockers on heart performance
these drugs cannot be used in cancer therapy

Recently, a group of structurally different hERG1 activa

It has been shown that mammalian hea
They exert less dramatic side effects co

The therapy was successful
but the patient died

NS1643

N,*N*'-*Bis*[2-hydroxy-5-(trifluoromethy-l)phenyl]urea

PD 118057

2-[[4-[2-(3,4-Dichlorophenyl)ethyl]-phenyl]amino]benzoic acid

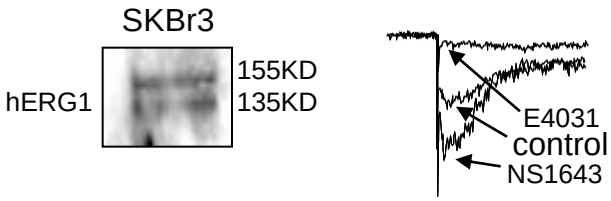
Q1- What are the consequences of hERG1 potassium channel stimulation in breast cancer cells?

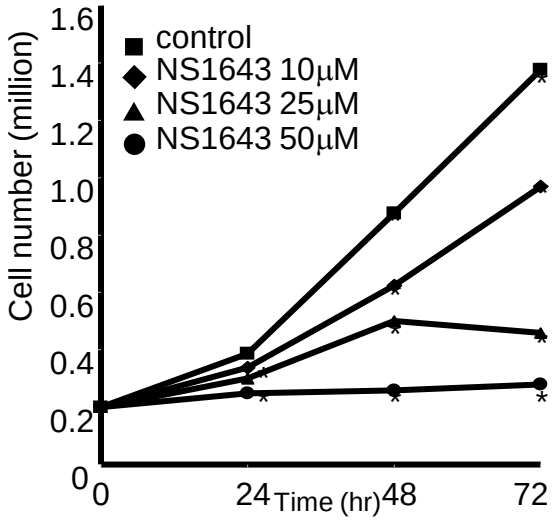
Q2- What is the biochemical signaling activated by stimulation of hERG1 potassium channels in breast cancer cells?

hERG1 agonists NS1643 inhibit proliferation in breast cancer cells

Kate Lansu

Lansu et al.
Cell death and Disease 2013

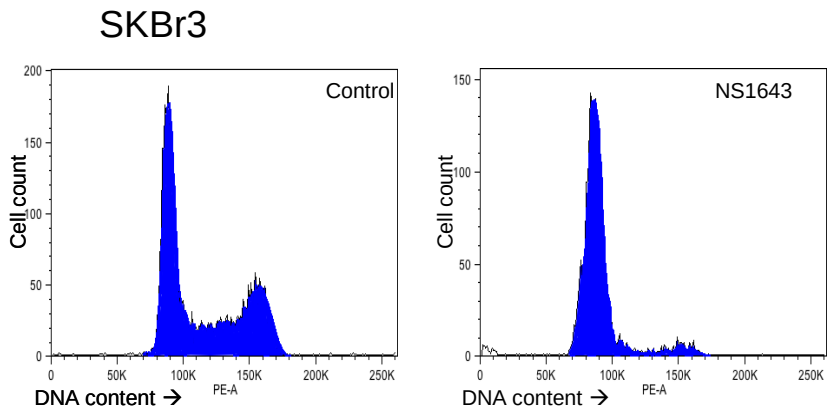


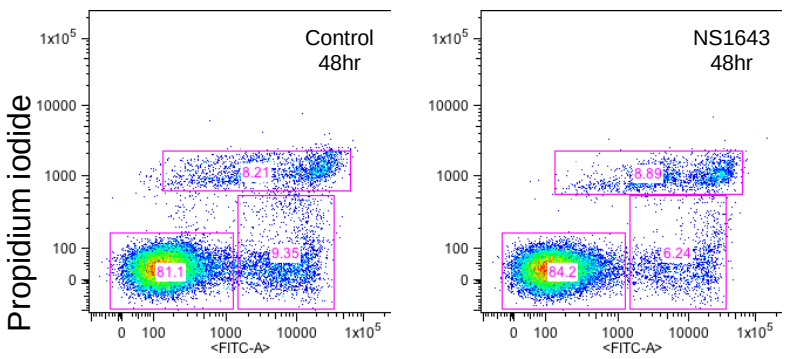


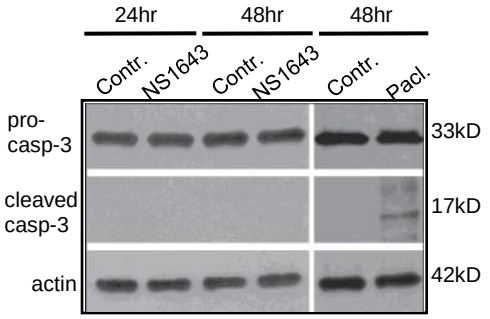
A&D) hERG1 channel expressed in ER-neg breast cancer cell line SKBr3 and CHO cells.
B&D) Effects of the NS1643 (50μM) on hERG1 currents.
C&F) Effects of NS1643 on proliferation rate of hERG1 negative cells.

Stimulation of hERG1 channel causes cell cycle arrest in G_0/G_1

Lansu et al.
Cell death and Disease 2013

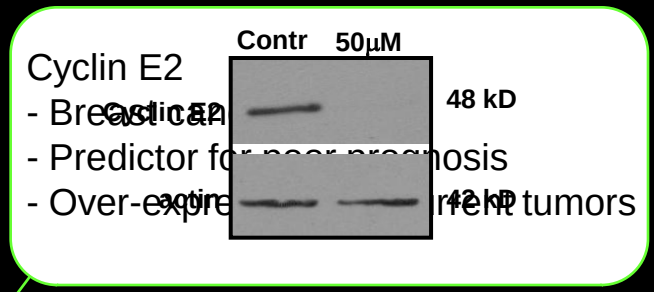


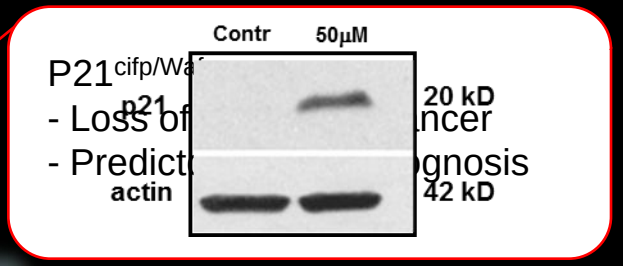




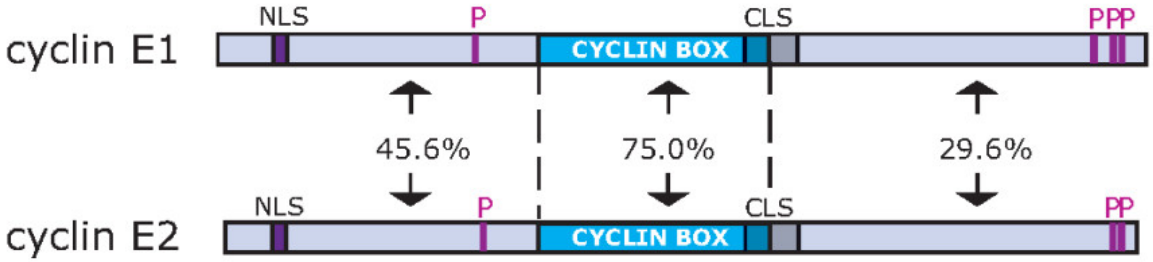
A, B & D) Cell cycle phase distribution of SKBr3 before and after NS1643 or NS1643+E4031 (herg1 blocker) treatment
F,G & H) Cell cycle phase distribution of MDA-MD-231 before and after NS1643 treatment.

The cell cycle





E-type cyclins




```
P24864 CCNE1_HUMAN 1   MPRERRERDAKERDTM----KEDGGAEFSARSRKRKANVTVFLQDPDEEMAKIDRTARDQ 56
O96020 CCNE2_HUMAN 1   MSRRSSRLQAKQQPQPSQTESPQEAQIIQAKKRKTTQ----DVKKRREEVTKKHQYEIRN 56
                           * *.  . :**::       . : .  :.*:.** .      ::.  **::* .:    :
P24864 CCNE1_HUMAN 57  CGSQPWDN-NAVCADPCSLIPTPDKEDDDRVYPNSTCKPRIIAPSRGSPLPVLSWANREE 115
O96020 CCNE2_HUMAN 57  C----WPPVLSGGISPCIIIETPHKEIGTSDFSRFTNYRFKNLFINPSPLPDLSWGCSKE 112
                           *    *    :   .** :* **.**     : . *         . **** ***.  :*
P24864 CCNE1_HUMAN 116 VWKIMLNKEKTYLRDQHFLEQHPLLQPKMRAILLDWLMEVCEVYKLHRETFYLAQDFFDR 175
O96020 CCNE2_HUMAN 113 VWLNMLKKESRYVHDKHFEVLHSDLEPQMRSILLDWLLEVCEVYTLHRETFYLAQDFFDR 172
                           **  **:**. *::*:**   *  *:*:**:******:******.***************
P24864 CCNE1_HUMAN 176 YMATQENVVKTLLQLIGISSLFIAAKLEEIYPPKLHQFAYVTDGACSGDEILTMELMIMK 235
O96020 CCNE2_HUMAN 173 FMLTQKDINKNMLQLIGITSLFIASKLEEIYAPKLQEFAYVTDGACSEEDILRMELIILK 232
                           :* **::: *.:******:*****:****** ***::********** ::** ***:*:*
P24864 CCNE1_HUMAN 236 ALKWRLSPLTIVSWLNVYMQVAYLNDLHEVLLPQYPQQIFIQIAELLDLCVLDVDCLEFP 295
O96020 CCNE2_HUMAN 233 ALKWELCPVTIISWLNLFLQVDALKDAPKVLLPQYSQETFIQIAQLLDLCILAIDSLEFQ 292
                           ****.*.*:**:****:::**  *:*  :****** *: *****:*****:* :*.***
P24864 CCNE1_HUMAN 296 YGILAASALYHFSSSELMQKVSGYQWCDIENCVKWMVPFAMVIRETGSSKLKHFRGVADE 355
O96020 CCNE2_HUMAN 293 YRILTAAALCHFTSIEVVKKASGLEWDSISECVDWMVPFVNVVKSTSPVKLKTFKKIPME 352
                           * **:*:** **:* *:::*.** :* .*.:**.*****. *::.*.  *** *: :  *
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O96020 CCNE2_HUMAN 353 DRHNIQTHTNYLAMLEEVNYINTFRKGGQLSPVCNGGIMTPPKSTEKPPGKH---      404
                           * ****** : * :*::..  ::: .  : :    .*::***:* :*  .
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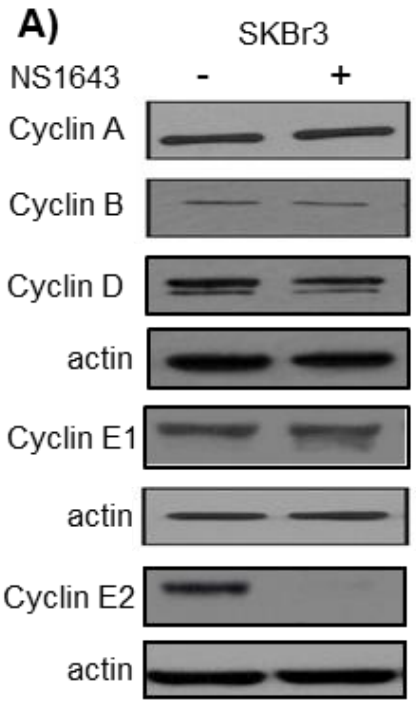
hERG1 stimulation selectively targets cyclin E2 for degradation

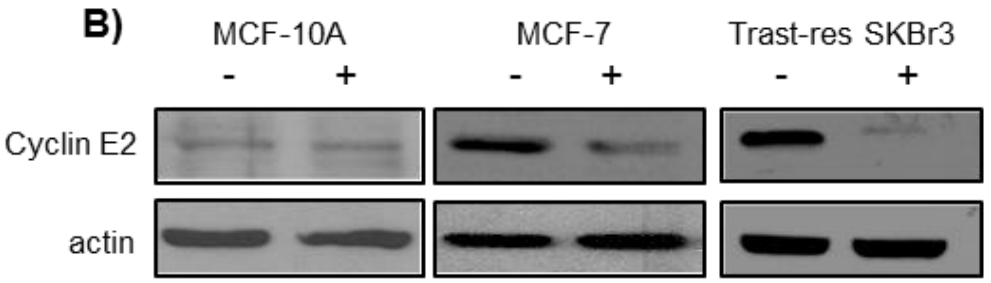
Mathew Perez-Neut

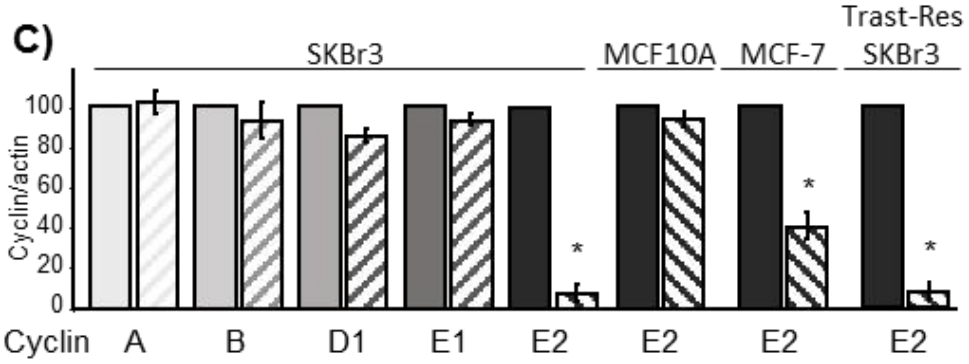
Perez et al.
Oncotarget Jan 2015

(2 hours)

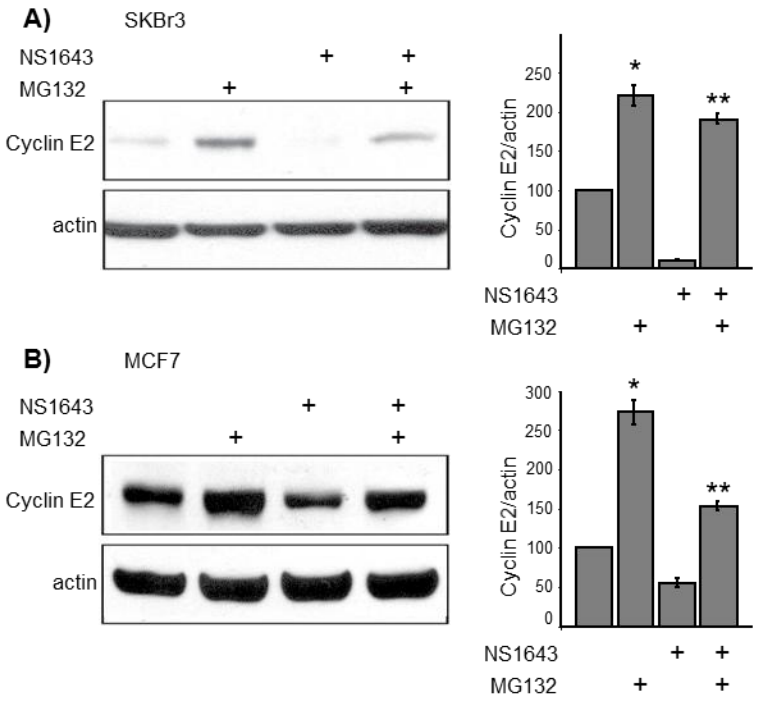


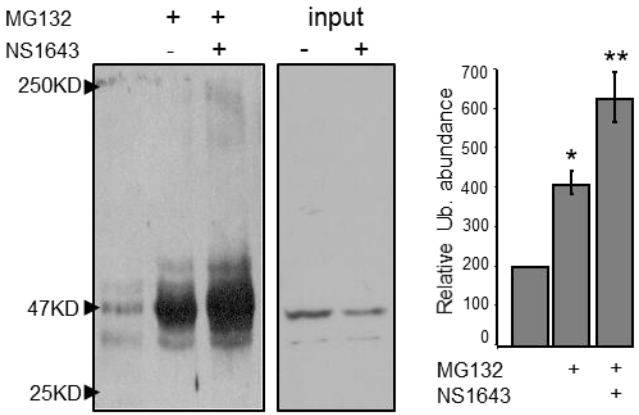
Kv11.1 positive



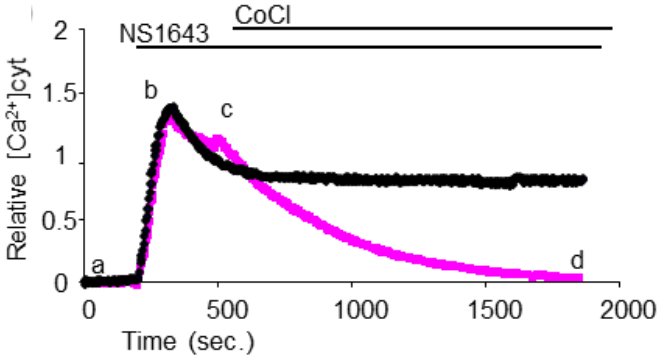


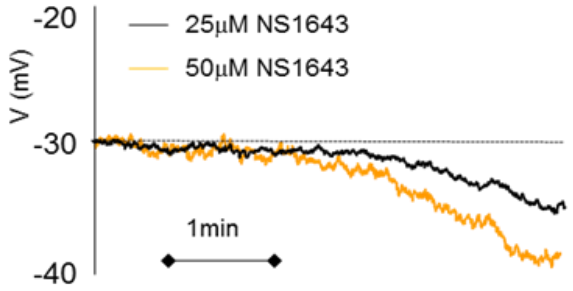
hERG1 stimulation selectively targets cyclin E2 for degradation

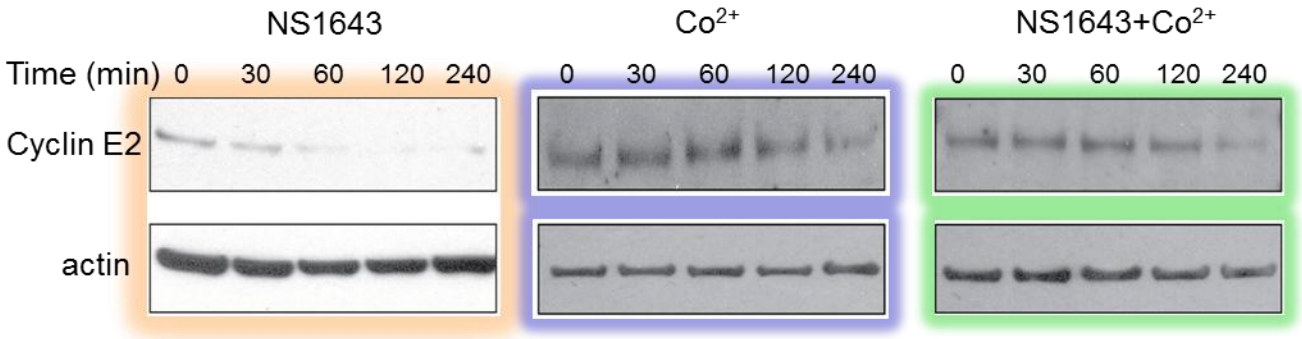


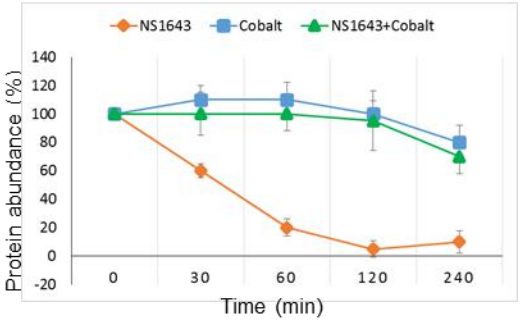


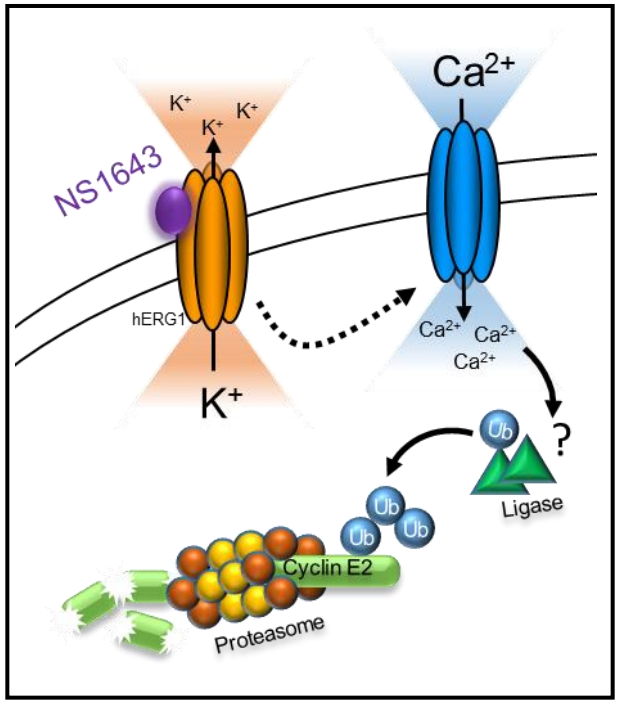
Stimulation of hERG1 leads to Ca^{2+}-dependent cyclin E2 degradation







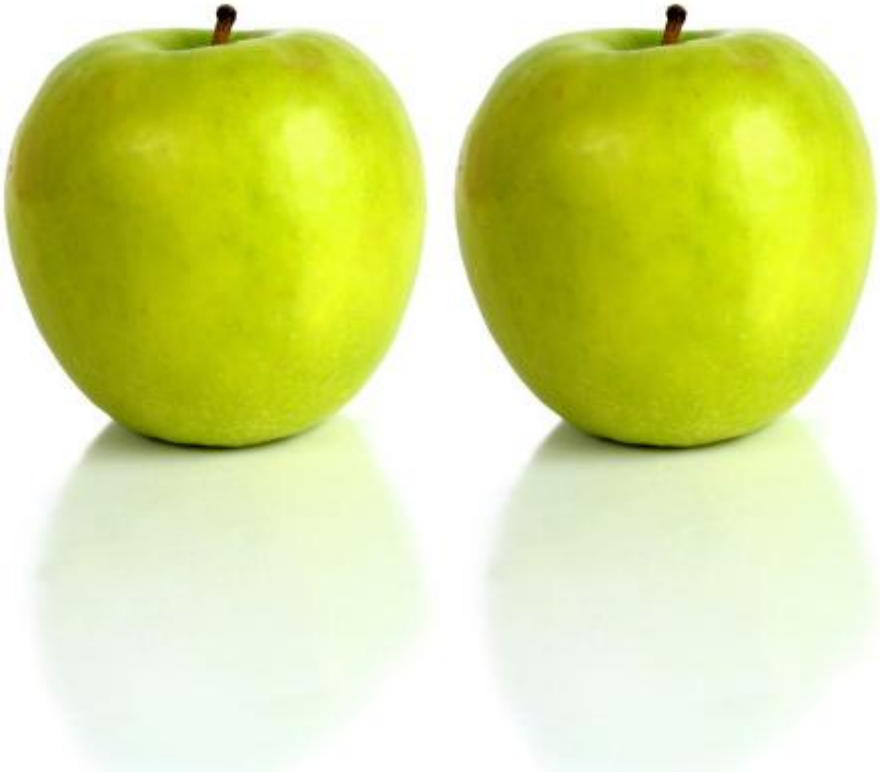




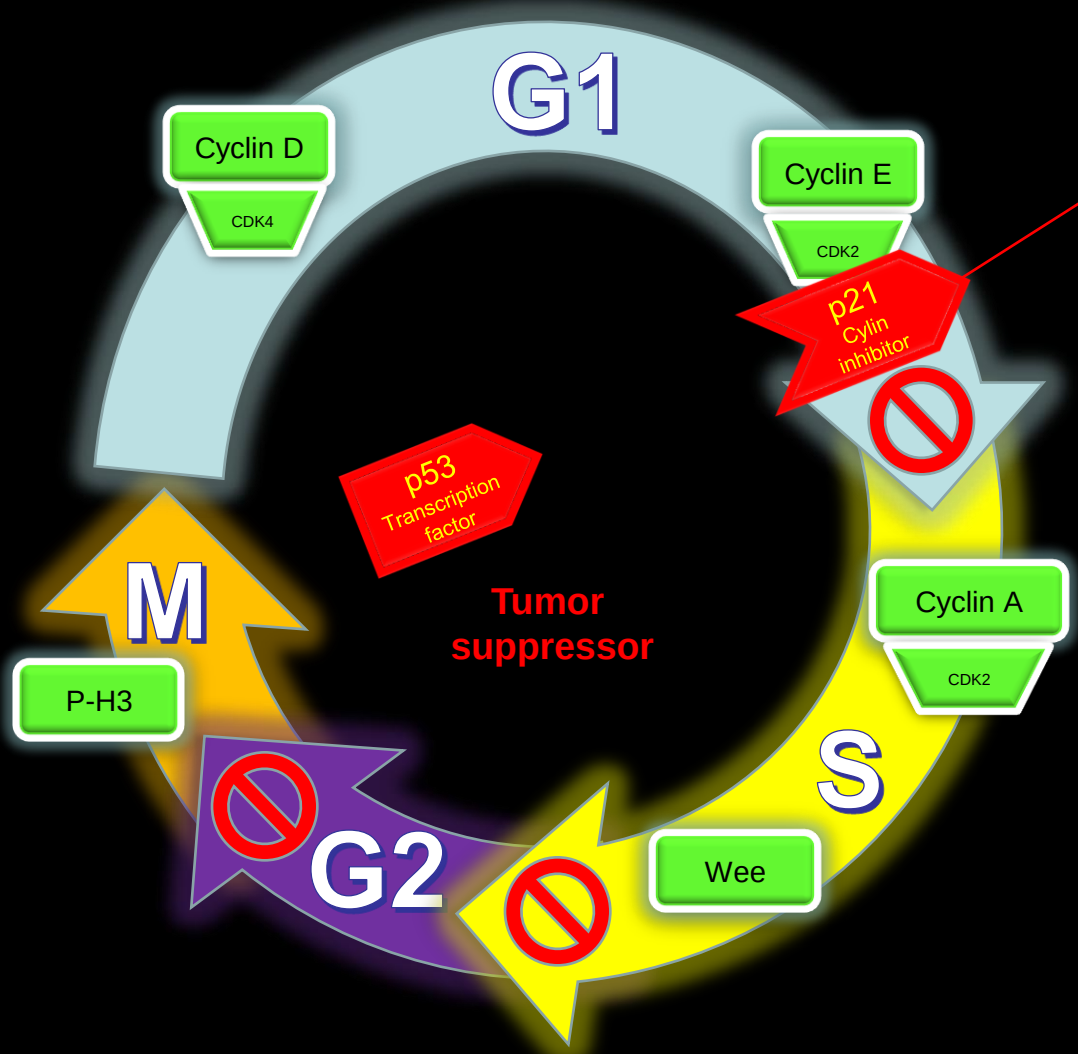
Perez et al. "Stimulation of hERG1 channel activity promotes a calcium-dependent degradation of cyclin E2, but not cyclin E1, in breast cancer cells" Jan. 2015 - Oncotarget

Cyclin E1

Cyclin E2

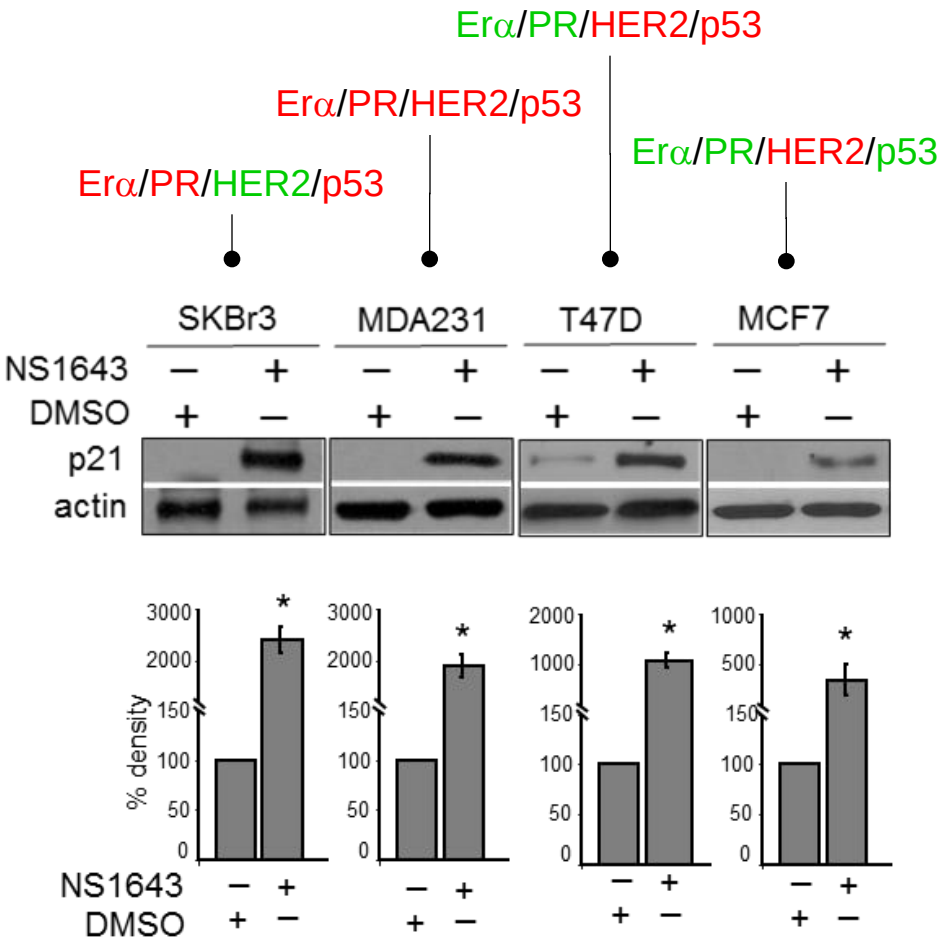


p21 cip/WAF

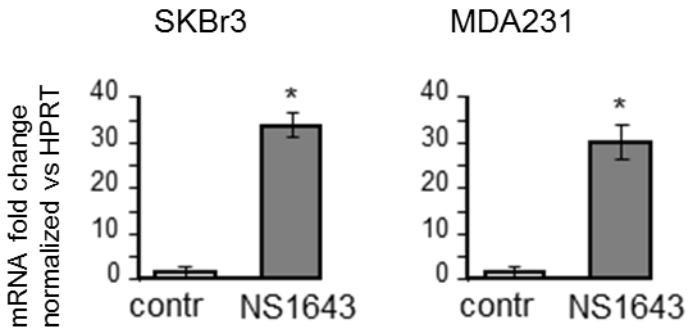


- **A p53 loss-of-function mutation is the most frequent mutation leading to cancer.**
- **p21 acts as a master effector of multiple tumor suppressor pathways**
- **p21 expression is tightly controlled by the tumor suppressor p53**

Stimulation of hERG1 leads to an increase of the tumor suppressor p21

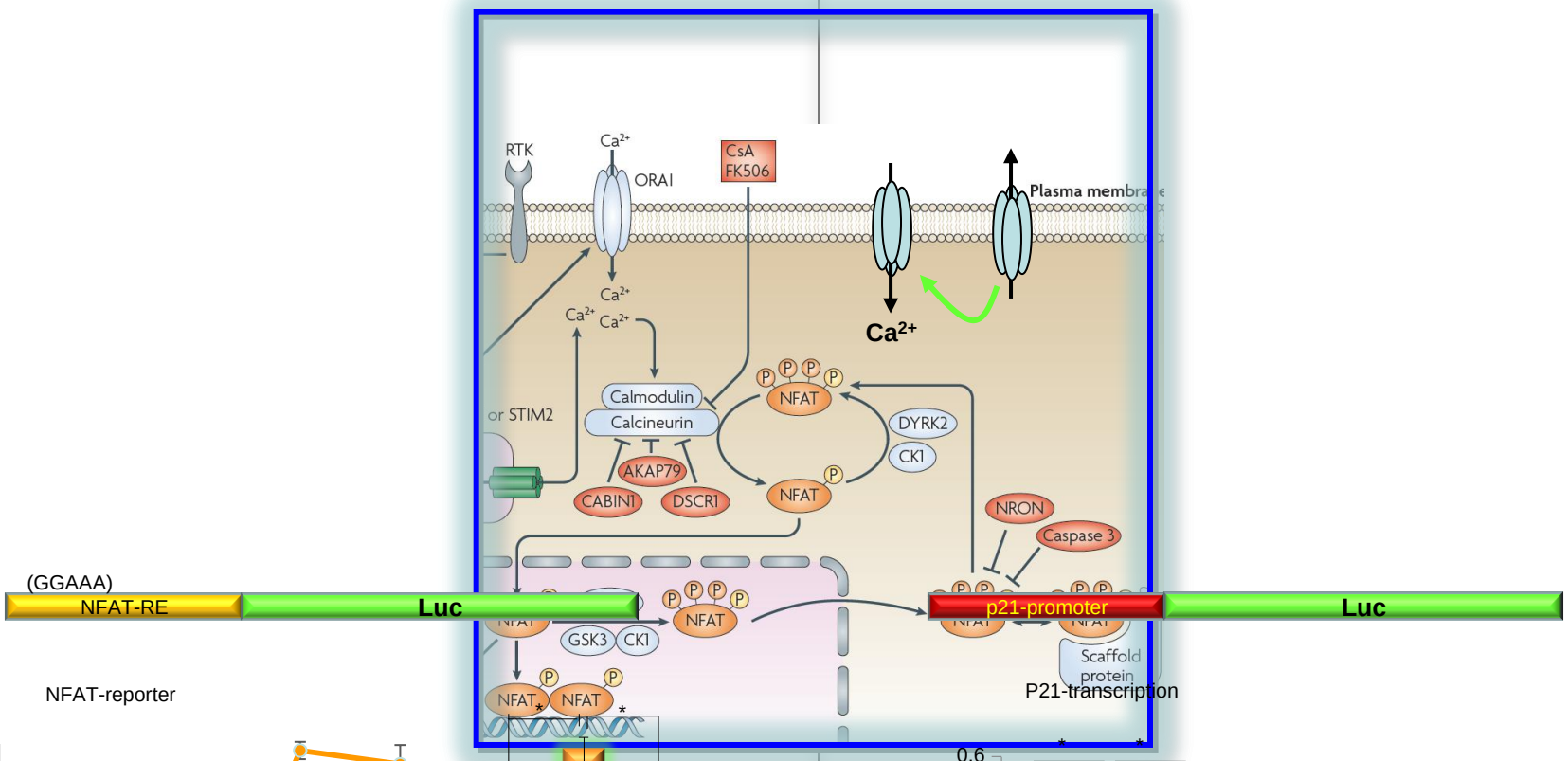


- Erα: Estrogen Receptor alpha
- PR: Progesterone Receptor
- HER2: EGFR2
- p53: →p21

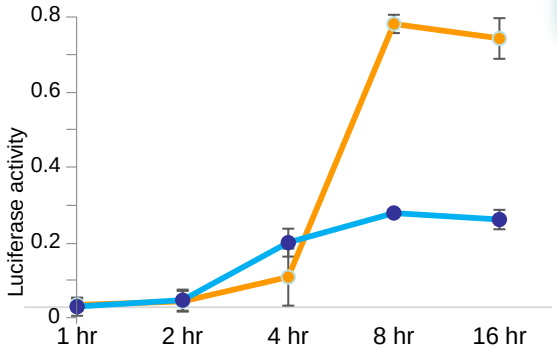


Perez et al.
Oncotarget Apr 2015

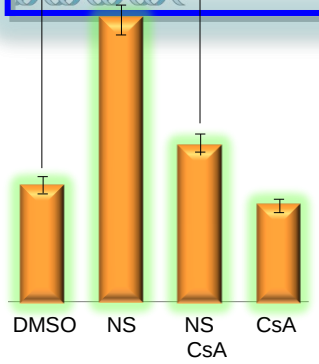
Stimulation of hERG1 activates transcription of the tumor suppressor p21



NFAT-reporter

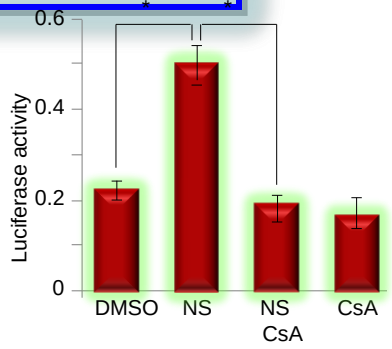


Time course (Hr) of the effect NS1643 on NFAT-dependent transcription in SKBr3 cells.

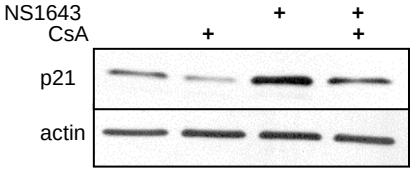


Effect NS1643 on NFAT-dependent transcription (at 8hr)

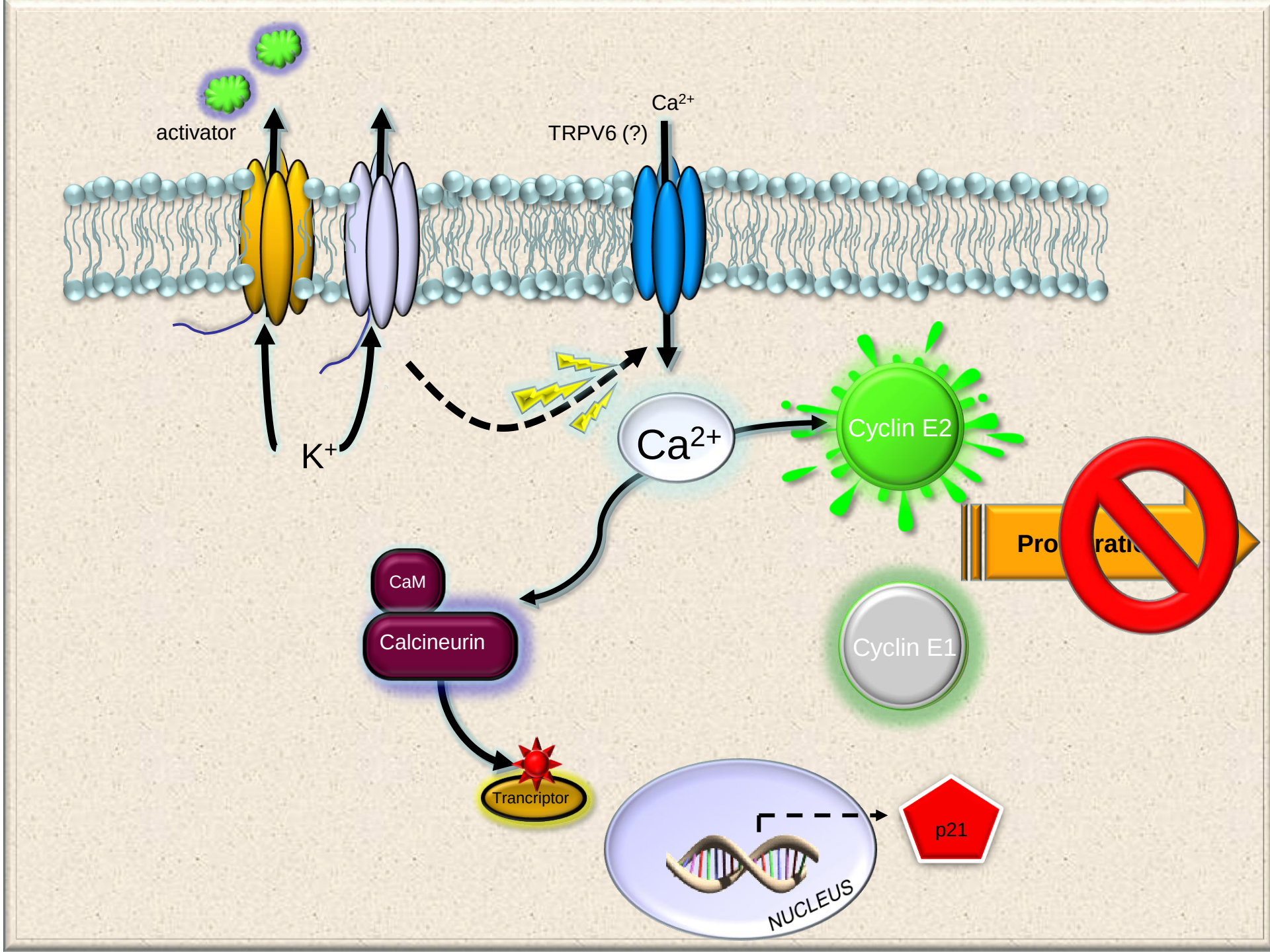
P21-transcription



Effect NS1643 on p21-transcription (at 8hr)

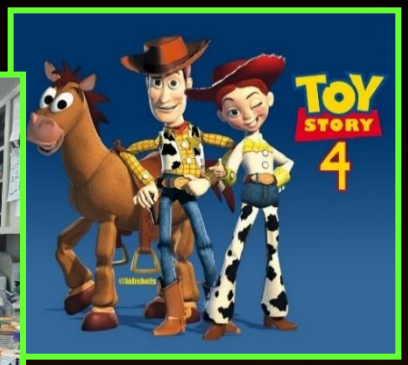


Effect NS1643 on p21 protein expression (at 24hr)


activator
Ca2+
TRPV6 (?)
K+
Ca2+
Cyclin E2
Pro rati
CaM
Calcineurin
Cyclin E1
Trancriptor
p21
NUCLEUS

Thanks to:

Katherine Lansu
(2011-2013)
now Ph.D. candidate UNC



The bunch…

Vidhya Rao
(Post-doc 2014-present)

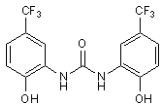
Mathew (one t) Perez
Psychedelic Tech 2013-present
now Ph.D. candidate U.of C.

Richard Miller
(Northwestern Univ.)

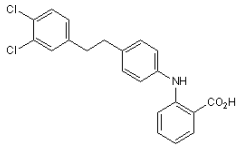
Clodia Osipo
Loyola Cancer Center

…it is not just hERG1

hERGs activator

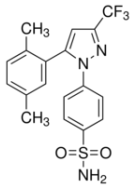


NS1643



PD118057

KCNQ5 activator



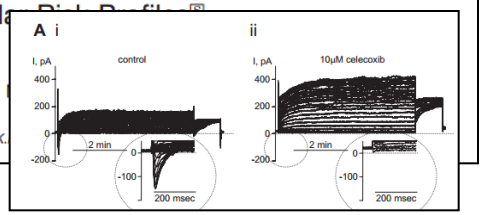
- Dymethylcelecoxib
- Celebrex®

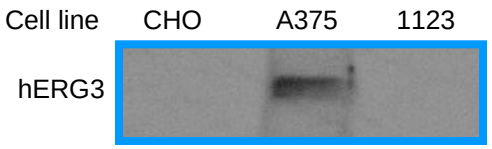
Celecoxib: A Specific COX-2 Inhibitor With Anticancer Properties

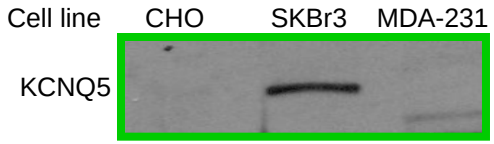
Differential Effects of Selective Cyclooxygenase-2 Inhibitors on Vascular Smooth Muscle Ion Channels May Account for Differences in Cardiovascula

Lioubov I. Brueggemann, Alexander R.
Kenneth L. Byron

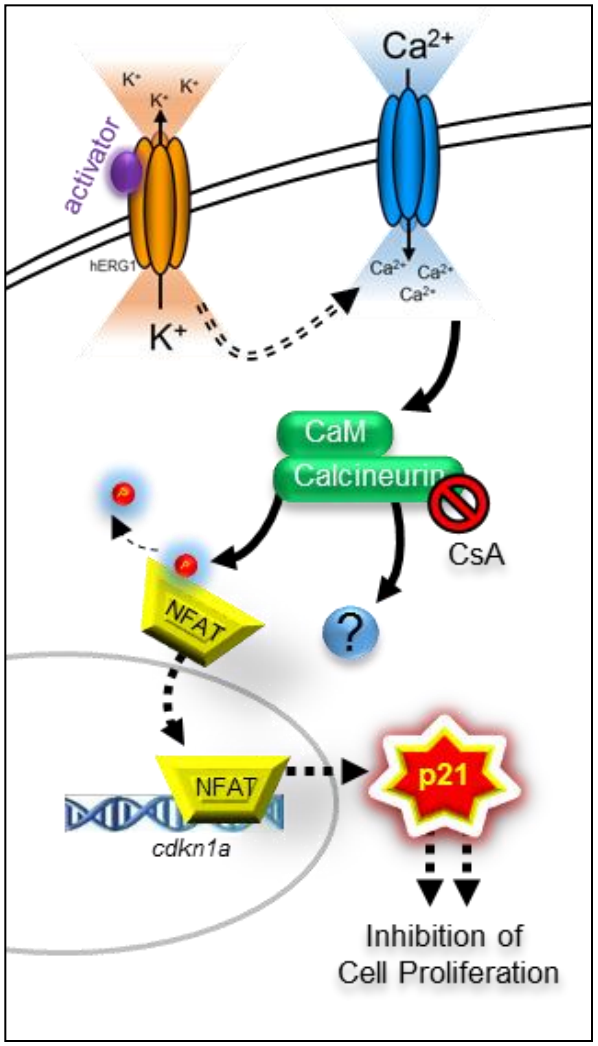
*Department of Pharmacology (L.I.B., A.R.M., B.K.M., K.
Maywood, Illinois*

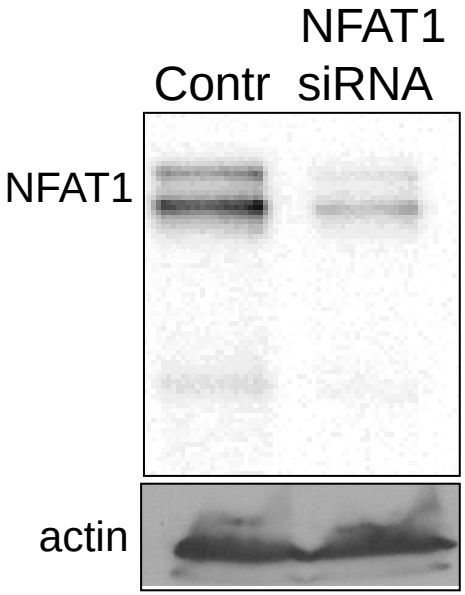


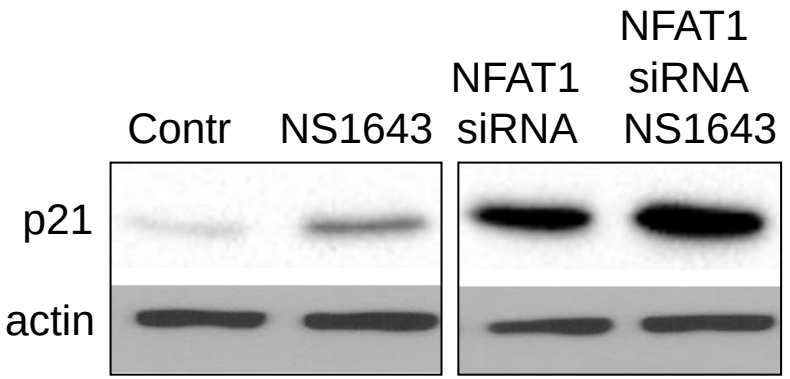




	hERGs activators			KCNQ5 activator
	SKBr3 (Herg1)	MDA-231 (Herg1)	Melanoma (Herg3)	SKBr3 KCNQ5
Proliferation	Inhibition	Inhibition	Inhibition	Inhibition
Cell cycle	Stop G1/S	Stop G1/S	Stop G2/M	Stop G1/S
Cell death	No	No	No(?)	No(?)
Autophagy	No	No	N.D.	N.D.
Senescence	Yes	Yes	Yes	Yes







Perez et al. "hERG1Kv11.1 activation stimulates transcription of p21wafcip in breast cancer cells via a calcineurin-dependent mechanism" Apr. 2015 - Oncotarget

- Ion channels are pore-forming membrane proteins whose functions include establishing electrical signals by gating the flow of ions across the cell membrane.

- Ion channels are key components in a wide variety of biological processes that involve both rapid and slow changes in cells

milliseconds

- Neuronal transmission (270 miles/hr)
- Muscle contraction

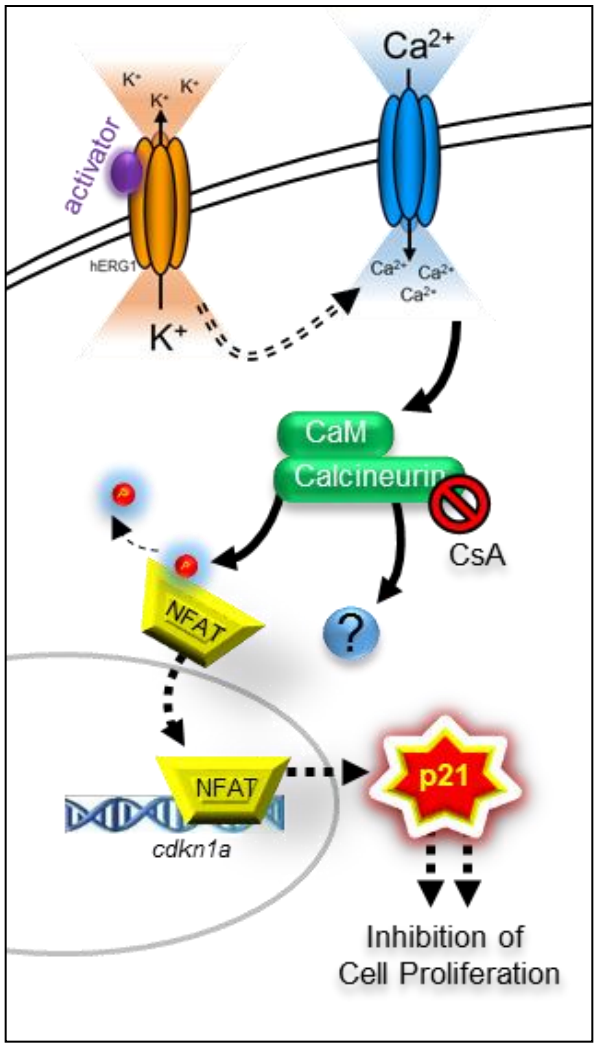
minutes

- Cell volume
- T-cell activation
- Transport of nutrients
- Secretion

hours

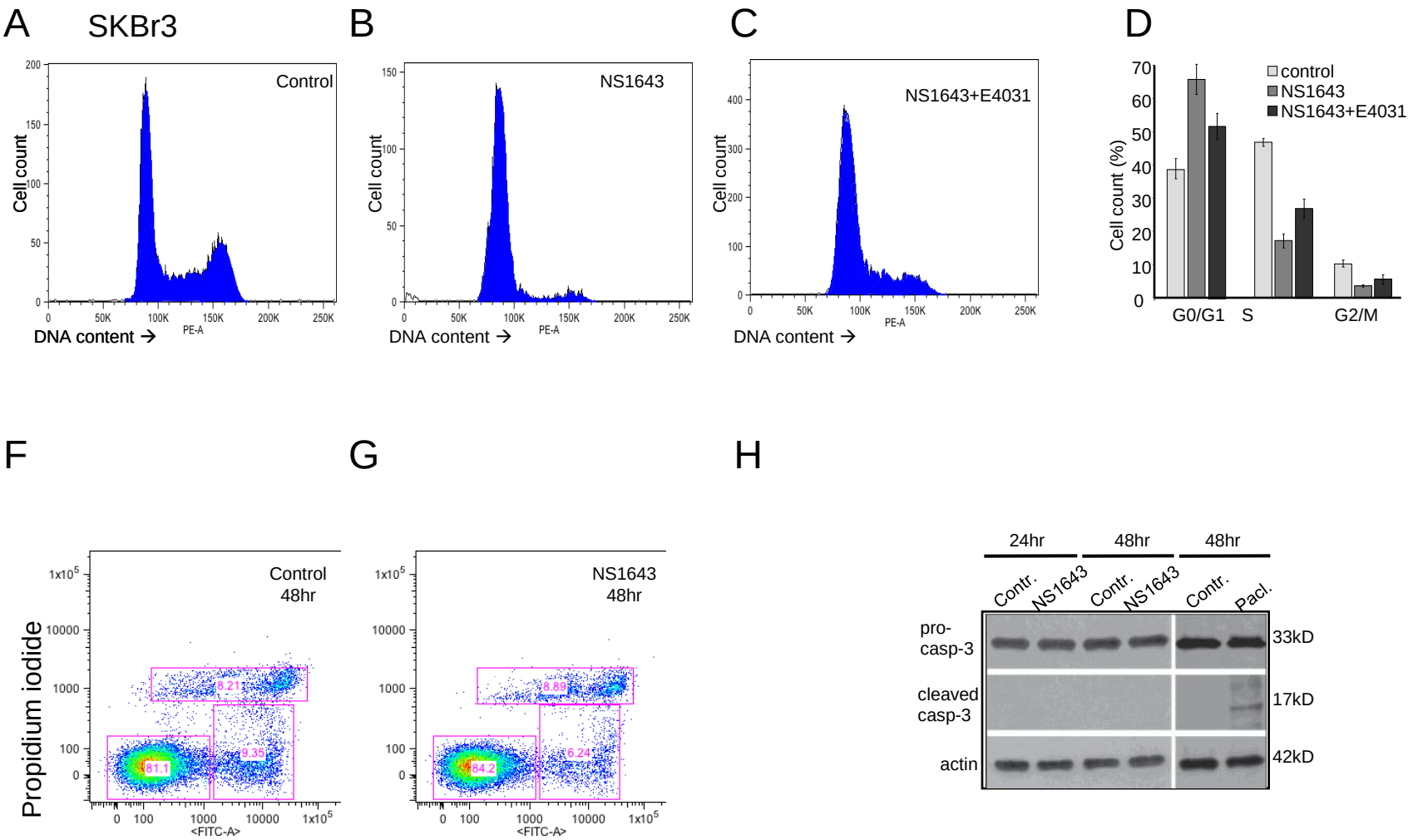
- Proliferation

CANCER



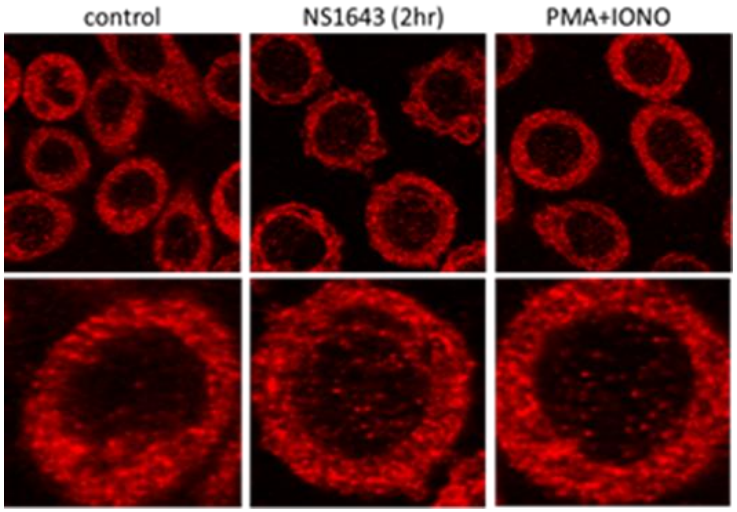
Perez et al. "hERG1Kv11.1 activation stimulates transcription of p21wafcip in breast cancer cells via a calcineurin-dependent mechanism" Apr. 2015 - Oncotarget

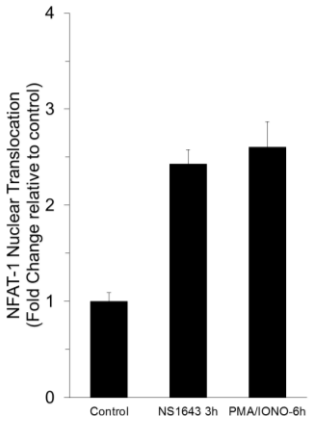
Stimulation of hERG1 channel causes cell cycle arrest in G_0/G_1

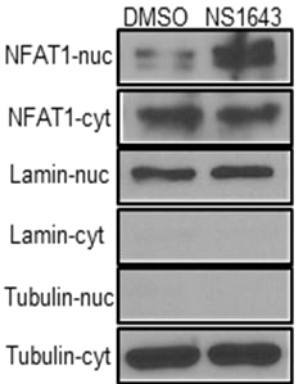


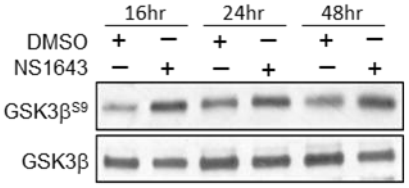
A, B & D) Cell cycle phase distribution of SKBr3 before and after NS1643 or NS1643+E4031 (herg1 blocker) treatment
F,G & H) Cell cycle phase distribution of MDA-MD-231 before and after NS1643 treatment.

NS1643 promotes NFAT nuclear translocation

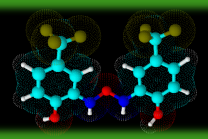


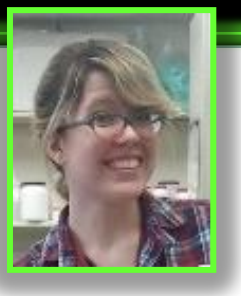




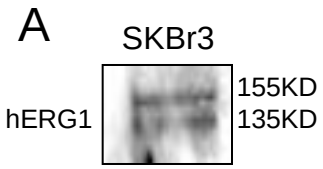


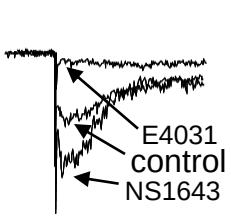
hERG1 agonists NS1643 inhibit proliferation in breast cancer cells

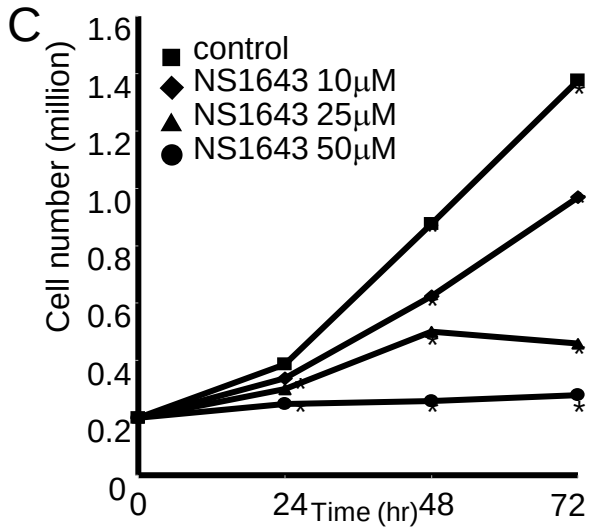




Kate Lansu







A&D) hERG1 channel expressed in ER-neg breast cancer cell line SKBr3 and CHO cells.
B&D) Effects of the NS1643 (50μM) on hERG1 currents.
C&F) Effects of NS1643 on proliferation rate of hERG1 negative cells.