Ion channels in cancer: an update

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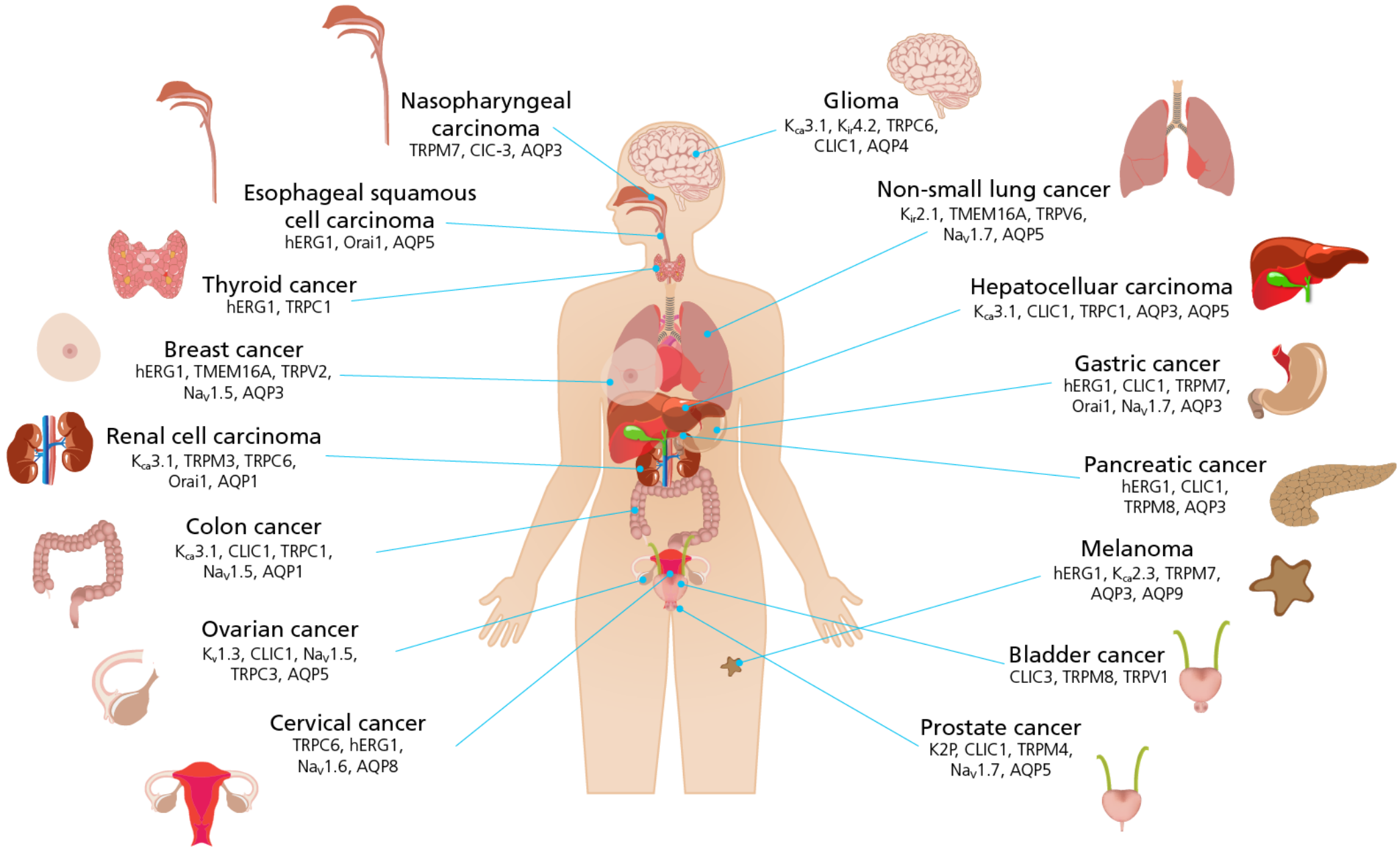
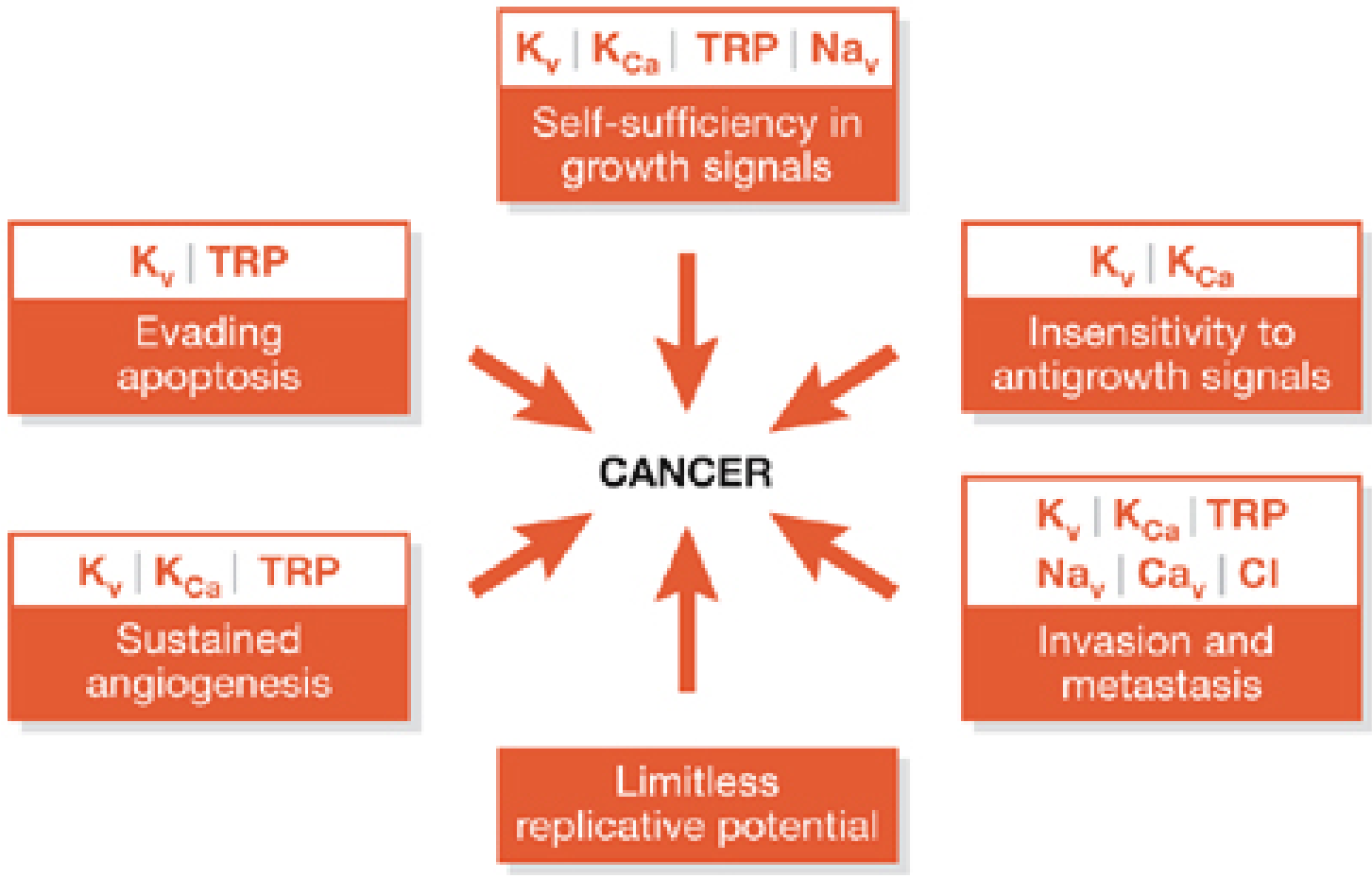
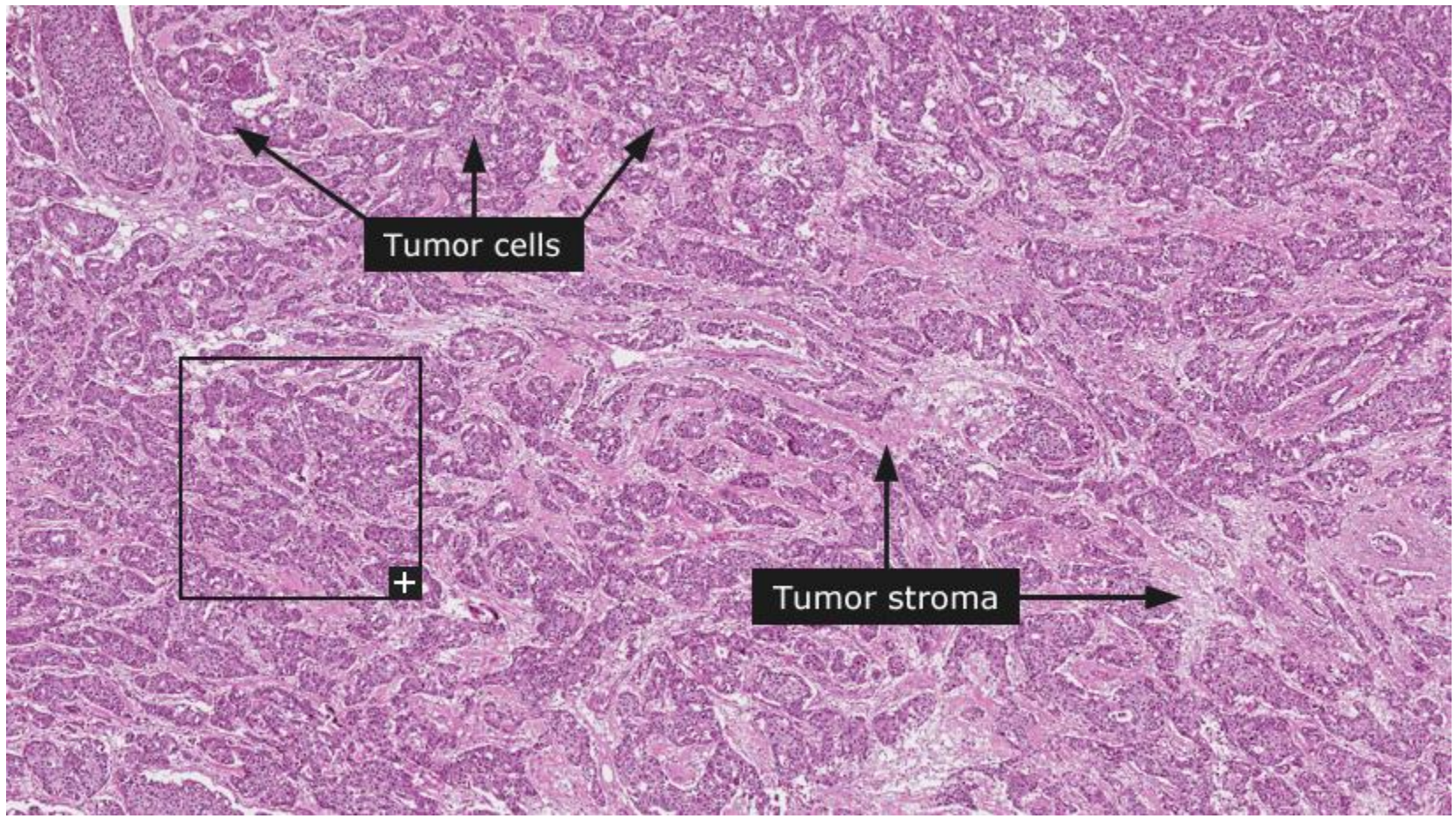
Ion channels in cancer

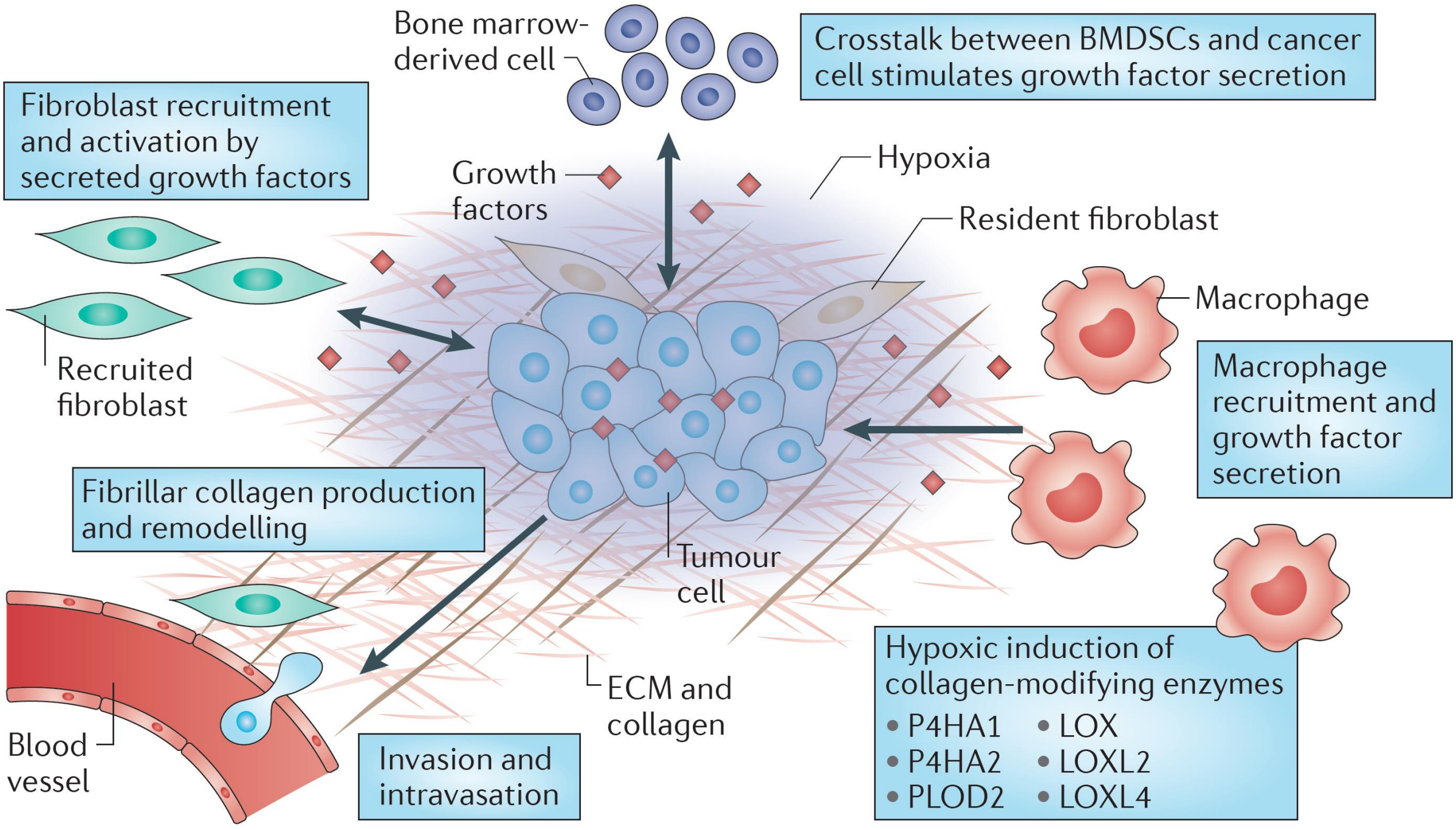


Ion channels in the cancer hallmarks

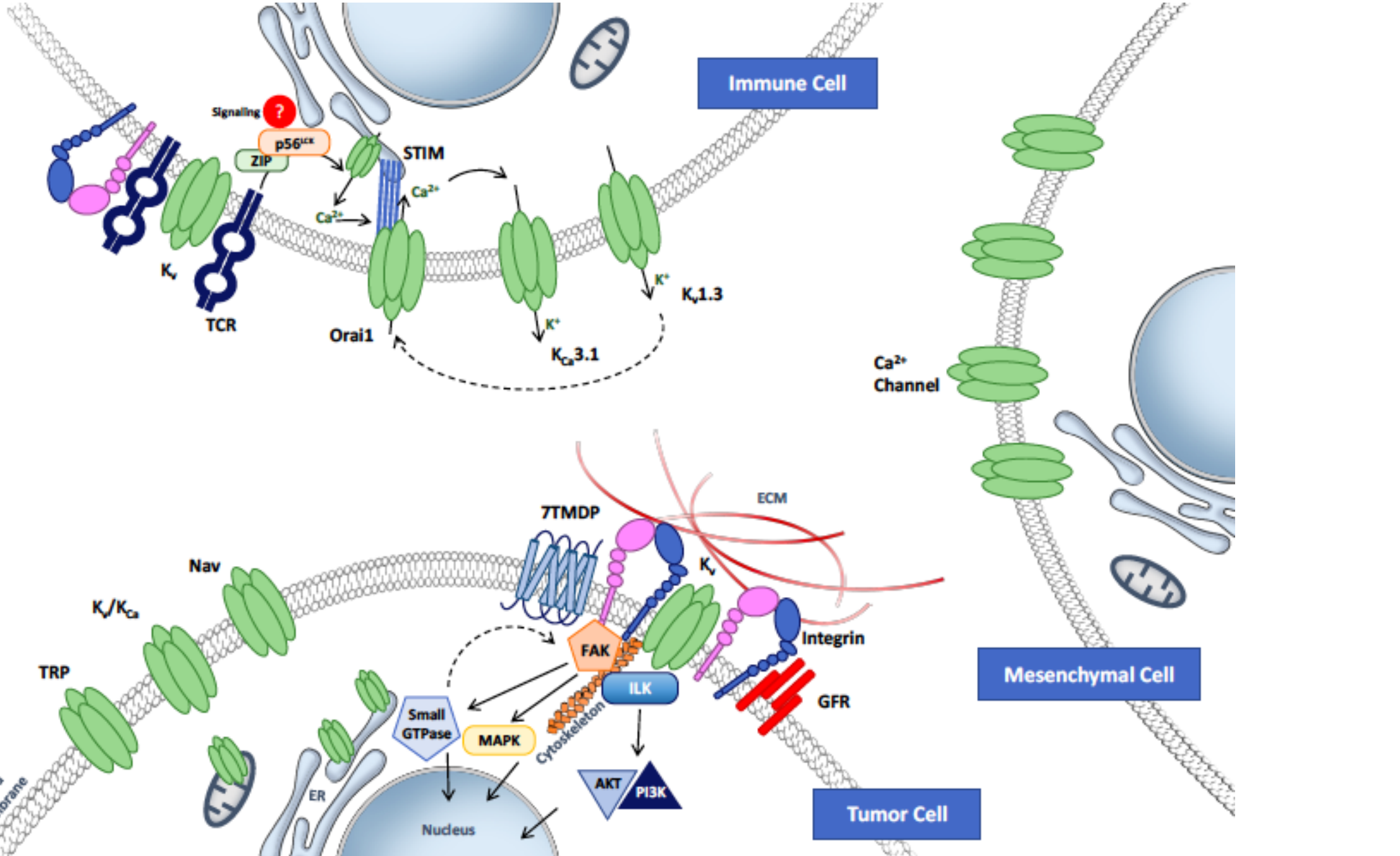


The cancer tissue




Bone marrow-derived cell
Crosstalk between BMDSCs and cancer cell stimulates growth factor secretion
Fibroblast recruitment and activation by secreted growth factors
Growth factors
Hypoxia
Resident fibroblast
Macrophage
Recruited fibroblast
Macrophage recruitment and growth factor secretion
Fibrillar collagen production and remodelling
Tumour cell
ECM and collagen
Blood vessel
Invasion and intravasation
Hypoxic induction of collagen-modifying enzymes
• P4HA1
• P4HA2
• PLOD2
• LOX
• LOXL2
• LOXL4

ION CHANNELS IN THE TUMOR MICROENVIRONMENT



Agenda of the session

- A. Arcangeli (Ion channels in Cancer: from molecular devices totherapeutic targets)
- N. Verma (ion chennels in the immune cells of the tumor microenvironment)
- L. Leanza (intracellular (mitochondrial) channels as novel antineoplastic targets)
- Anna Borgstroem (Investigation of TRPM4 and its Role in Cell Migrationand Proliferation of Prostate Cancer Cells)

Ion channels in cancer: from molecular devices to therapeutic targets

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Agenda

- Novel achievements:

- K^+ channels networking (Kca 3.1 and hERG1)

- Targeting specific hERG1 conformational states

The networking of Potassium channels

FULL PAPER

BJC

British Journal of Cancer (2017), 1–13 | doi: 10.1038/bjc.2017.392

Keywords: Riluzole; SKA-31; E4031; Cisplatin uptake; preclinical mouse models

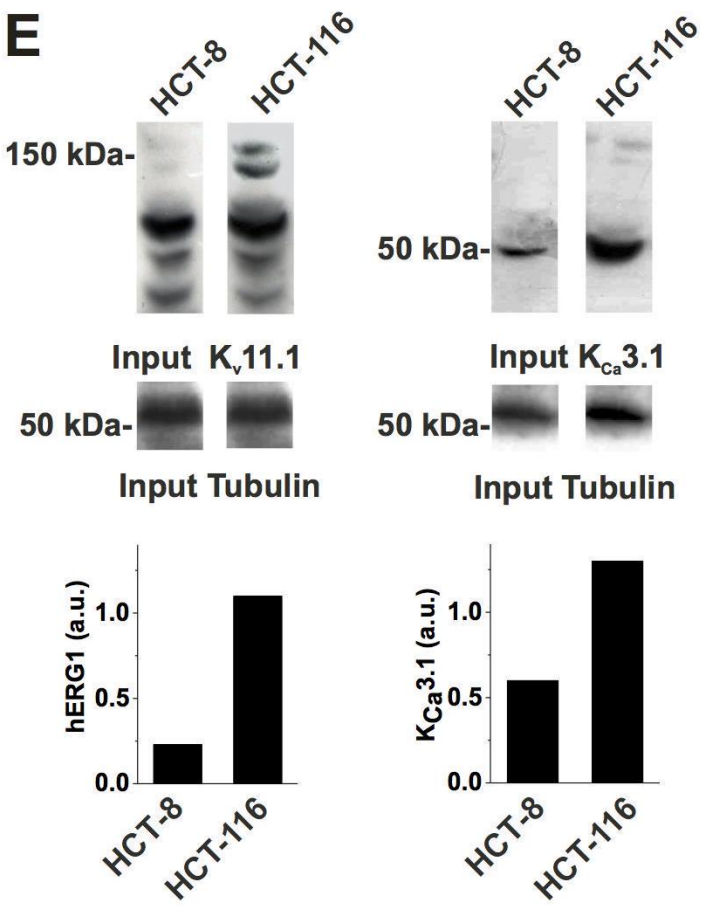
The combined activation of $K_{Ca}3.1$ and inhibition of $K_v11.1$/hERG1 currents contribute to overcome Cisplatin resistance in colorectal cancer cells

Serena Pillozzi[1,9], Massimo D'Amico[2,9], Gianluca Bartoli[1], Luca Gasparoli[1], Giulia Petroni[1], Olivia Crociani[1], Tiziano Marzo[3,4], Angela Guerriero[1], Luigi Messori[3], Mirko Severi[5], Roberto Udisti[5], Heike Wulff[6], K George Chandy[7], Andrea Becchetti[8] and Annarosa Arcangeli*[,1]

D

	HCT-116		HCT-8	
	Ct value	Score of expression (a.u.)	Ct value	Score of expression (a.u.)
KCNH1	36.33±1.29	0	36.47±1.24	0
KCNH2	25.31±0.66	2	27.57±0.93	1
KCNA3	31.79±0.36	0	29.76±0.28	0
KCNMA1	31.38±1.15	0	32.04±0.53	0
KCNN3	28.93±0.57	0	35.03±2.04	0
KCNN4	22.46±0.44	2	30.04±1.53	0
SLC31A1	22.61±0.26	2	22.31±0.32	2
SLC31A2	32.26±0.71	1	30.54±0.39	0
ATP7A	26.09±0.98	1	23.45±0.29	2
ATP7B	26.71±0.48	1	24.69±0.28	2
LRRC8A	27.15±0.34	1	28.39±0.45	0
LRRC8D	26.38±0.58	1	28.04±0.09	0

E



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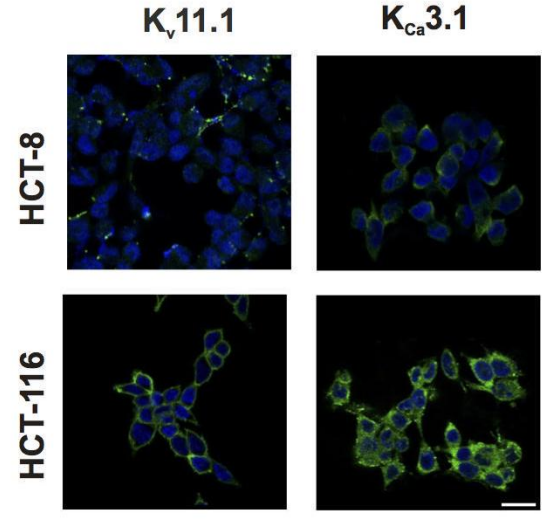


Cisplatin-resistant CRC cells express higher levels of KCa3.1 and Kv11.1 channels compared with Cisplatin-sensitive cells

In resistant cells, KCa3.1 activators (SKA-31) and Kv11.1 inhibitors (E4031) had a synergistic action with Cisplatin in triggering apoptosis and inhibiting proliferation.

Table 1A. IC_{50} values and effects on apoptosis and cell cycle distribution of Cisplatin, Riluzole, SKA-31, TRAM-34 and E4031 in HCT-116 and HCT-8 cells

			Apoptosis		Cell cycle		
	IC_{50} (μM)	Concentration of the drug (μM)	Early apoptosis (%)	Late apoptosis (%)	G0/G1 (%)	S (%)	G2/M (%)
HCT-116							
Control	—		1.0 ± 0.6	0.8 ± 0.5	27.7 ± 7.7	55.5 ± 3.7	16.9 ± 6.4
Cisplatin	25.2 ± 2.1	25	5.9 ± 1.0	5.3 ± 1.5	48.0 ± 4.5	39.2 ± 2.5	12.8 ± 3.3
			$P = 0.001$	$P = 0.009$	$P = 0.009$	$P = 0.018$	
Riluzole	9.5 ± 1.0	10	13.6 ± 3.7	10.3 ± 4.1	51.6 ± 6.0	26.1 ± 8.8	22.3 ± 3.1
			$P = 0.004$	$P = 0.028$	$P = 0.004$	$P = 0.021$	
SKA-31	5.3 ± 0.3	5	7.0 ± 0.9	3.5 ± 0.9	55.3 ± 2.4	31.4 ± 7.7	13.3 ± 5.7
			$P = 0.000$	$P = 0.015$	$P = 0.006$	$P = 0.028$	
TRAM-34	24.4 ± 1.8	25	9.6 ± 2.1	6.6 ± 0.9	50.5 ± 3.2	34.2 ± 5.0	15.4 ± 7.6
			$P = 0.001$	$P = 0.000$			
E4031	6.6 ± 1.6	7	5.2 ± 1.2	4.1 ± 1.0	51.4 ± 4.3	26.4 ± 4.1	22.3 ± 6.1
			$P = 0.005$	$P = 0.010$	$P = 0.010$	$P = 0.012$	
HCT-8							
Control	—		0.8 ± 0.3	1.2 ± 0.3	30.7 ± 2.4	53.8 ± 3.3	15.5 ± 1.9
Cisplatin	8.7 ± 1.4	9	5.5 ± 1.4	13.4 ± 7.2	46.9 ± 1.3	43.4 ± 1.9	9.7 ± 3.1
			$P = 0.008$	$P = 0.018$	$P = 0.019$	$P = 0.002$	$P = 0.041$
Riluzole	12.9 ± 0.7	13	3.6 ± 0.9	4.0 ± 1.2	8.5 ± 3.4	6.7 ± 4.3	84.6 ± 5.6
			$P = 0.008$	$P = 0.035$	$P = 0.003$	$P = 0.000$	$P = 0.000$
SKA-31	46.9 ± 1.4	45	3.0 ± 0.4	10.2 ± 5.0	47.5 ± 3.4	39.9 ± 8.7	12.6 ± 10.0
			$P = 0.001$	$P = 0.011$	$P = 0.021$	$P = 0.046$	
TRAM-34	20.1 ± 1.1	20	3.2 ± 1.2	2.7 ± 1.0	62.9 ± 3.2	28.9 ± 4.5	8.2 ± 7.6
			$P = 0.012$	$P = 0.019$	$P = 0.026$	$P = 0.026$	
E4031	13.3 ± 1.3	13	2.8 ± 1.9	2.8 ± 0.7	25.2 ± 0.4	57.1 ± 2.2	17.7 ± 2.4
				$P = 0.015$	$P = 0.012$		

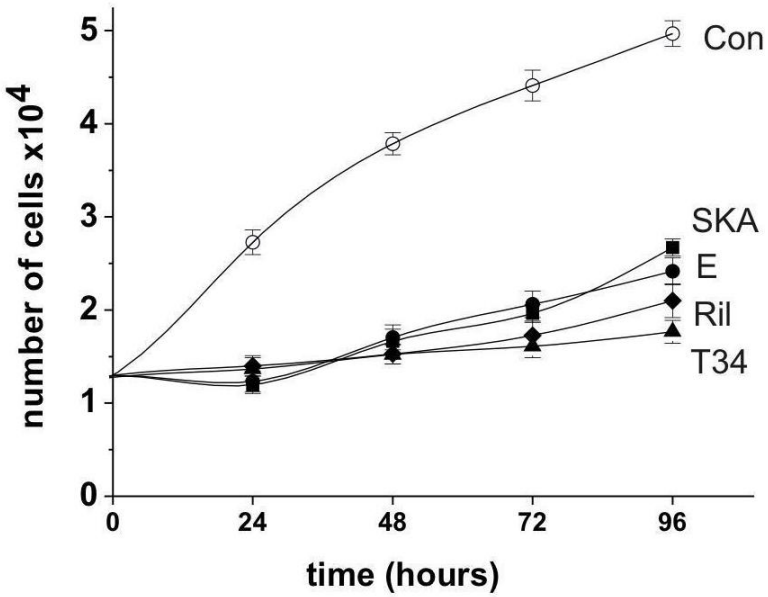
IC_{50} values were determined after 24 h of treatment by the Trypan Blue exclusion test, using the Origin Software. Apoptosis and cell cycle distributions were evaluated by treating the cells with the drug concentrations indicated in the third column for 24 h. The percentage of cells in early (Annexin +/PI − cells) and late apoptosis (Annexin +/PI + cells) was determined by Annexin/PI assay as detailed in the Materials and Methods section. Cell cycle distribution was assessed by flow cytometry after staining the cells with propidium iodide (PI) and is indicated as the percentage of cells in the different cell cycle phases. Data are means ± s.e.m. of three independent experiments, each carried out in triplicate. For statistical analysis, Student's *t*-test was applied.

Table 1B. Combination index and percentage (%) of apoptotic HCT-116 cells after different treatment combinations

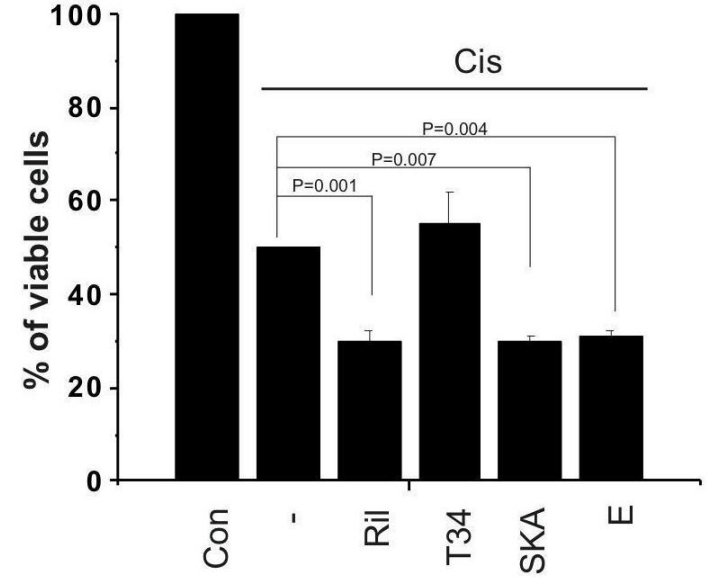
			Apoptosis	
Drug (concentration μM)	Combination index at IC_{50}	Effect	Early apoptotic cells (%)	Late apoptotic cells (%)
Cisplatin (25)	—		5.9 ± 1.0	5.3 ± 1.5
Cisplatin (25) + Riluzole (10)	0.70 ± 0.08	S	10.6 ± 1.3 $P = 0.021$	17.6 ± 3.3 $P = 0.016$
Cisplatin (25) + SKA-31 (5)	0.64 ± 0.11	S	12.5 ± 3.9	10.1 ± 2.4
Cisplatin (25) + TRAM-34 (25)	2.66 ± 0.78	A	13.8 ± 3.6 $P = 0.016$	8.7 ± 1.6
Cisplatin (25) + E4031 (7)	0.68 ± 0.07	S	8.0 ± 0.3	13.2 ± 3.4 $P = 0.042$
Cisplatin (25) + Riluzole (10) + E4031 (7)	0.47 ± 0.05	S	ND	ND
Cisplatin (25) + SKA-31 (5) + E4031 (7)	0.69 ± 0.14	S	ND	ND
Oxaliplatin (60) + Riluzole (10)	0.98 ± 0.01	S	ND	ND
Oxaliplatin (60) + SKA-31 (5)	0.71 ± 0.05	S	ND	ND
Oxaliplatin (60) + TRAM-34 (25)	3.36 ± 0.34	A	ND	ND
Oxaliplatin (60) + E4031 (7)	0.83 ± 0.01	S	ND	ND

Abbreviation: ND = not determined. CI > 1, antagonism (A); CI = 1, additivity (Ad); CI < 1, synergy (S). HCT-116 cells were exposed to Cisplatin or Oxaliplatin in combination with Riluzole, SKA-31, TRAM-34 and E4031 for 24 h as described in Pillozzi *et al*, 2011. All the drugs were used at drug concentrations indicated in the first column. Data are means ± s.e.m. of three independent experiments, each carried out in triplicate. CI values were calculated using the Calcusyn software Version 2 (Biosoft). For statistical analysis, Student's *t*-test was applied.

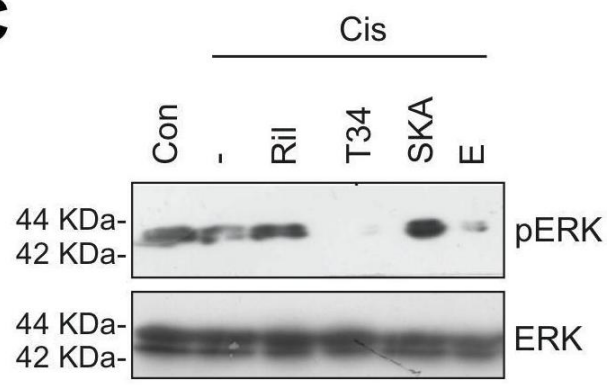
A

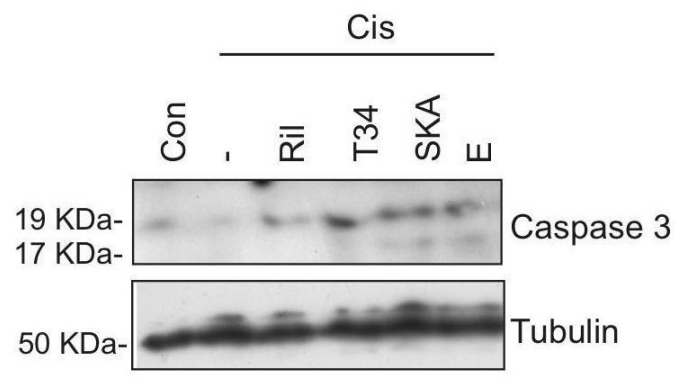

number of cells x10^4
time (hours)
Con
SKA
E
Ril
T34

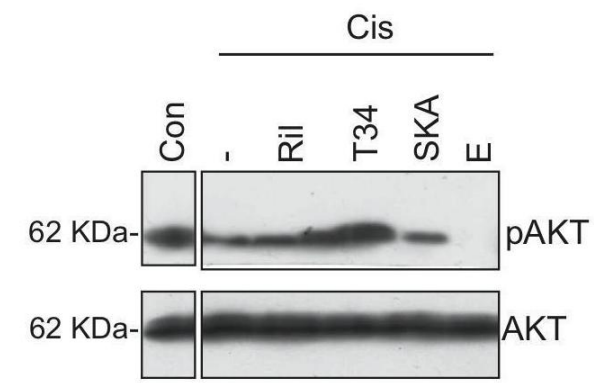
B

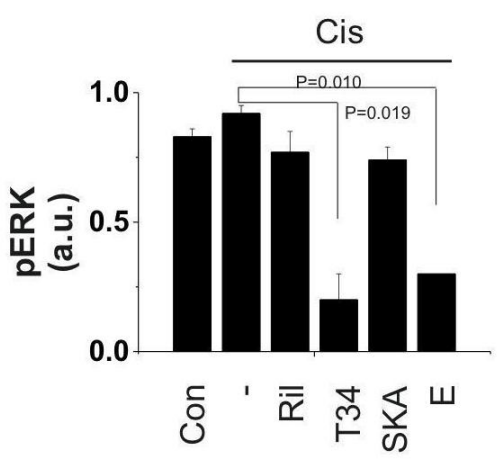

% of viable cells
Cis
P=0.004
P=0.007
P=0.001
Con
-
Ril
T34
SKA
E

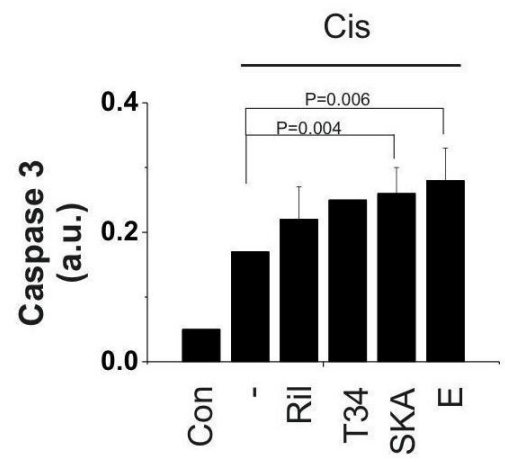
C

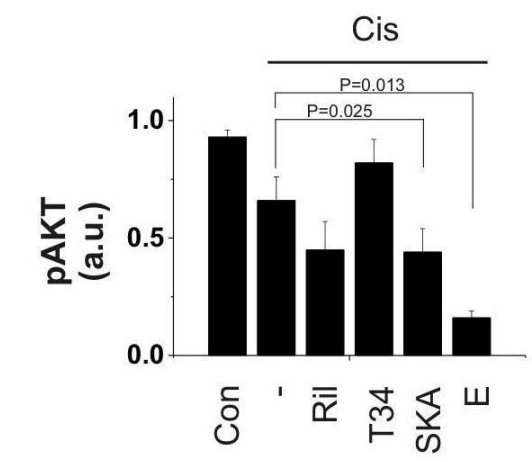

Cis
Con
-
Ril
T34
SKA
E
44 KDa-
42 KDa-
pERK
44 KDa-
42 KDa-
ERK


Cis
Con
-
Ril
T34
SKA
E
19 KDa-
17 KDa-
Caspase 3
50 KDa-
Tubulin

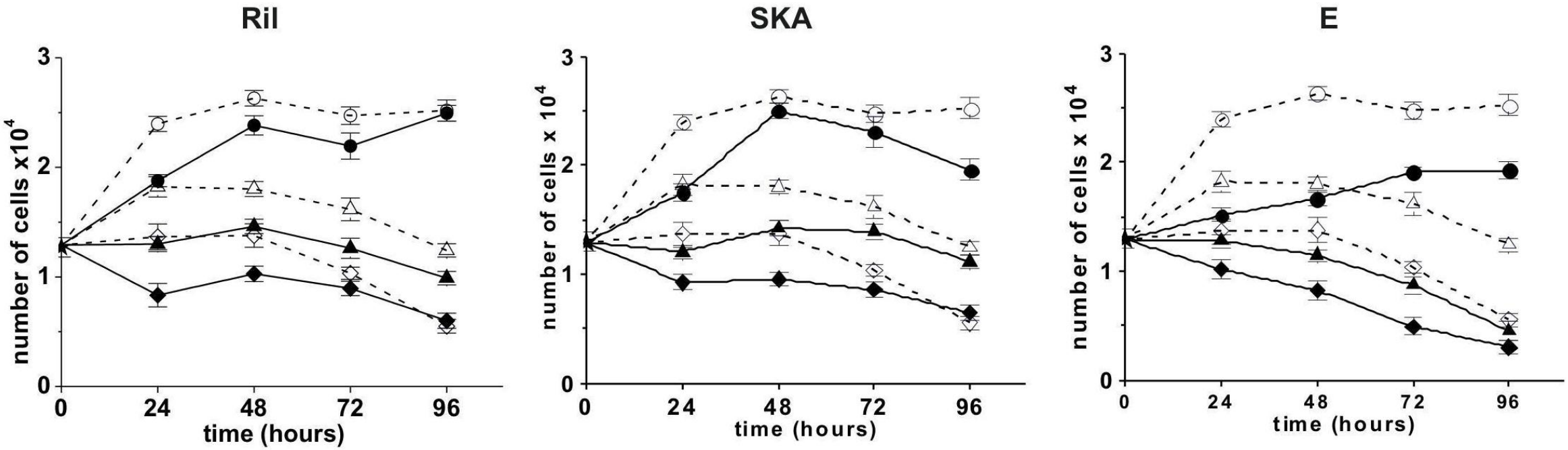

Cis
Con
-
Ril
T34
SKA
E
62 KDa-
pAKT
62 KDa-
AKT


Cis
P=0.010
P=0.019
pERK (a.u.)
Con
-
Ril
T34
SKA
E

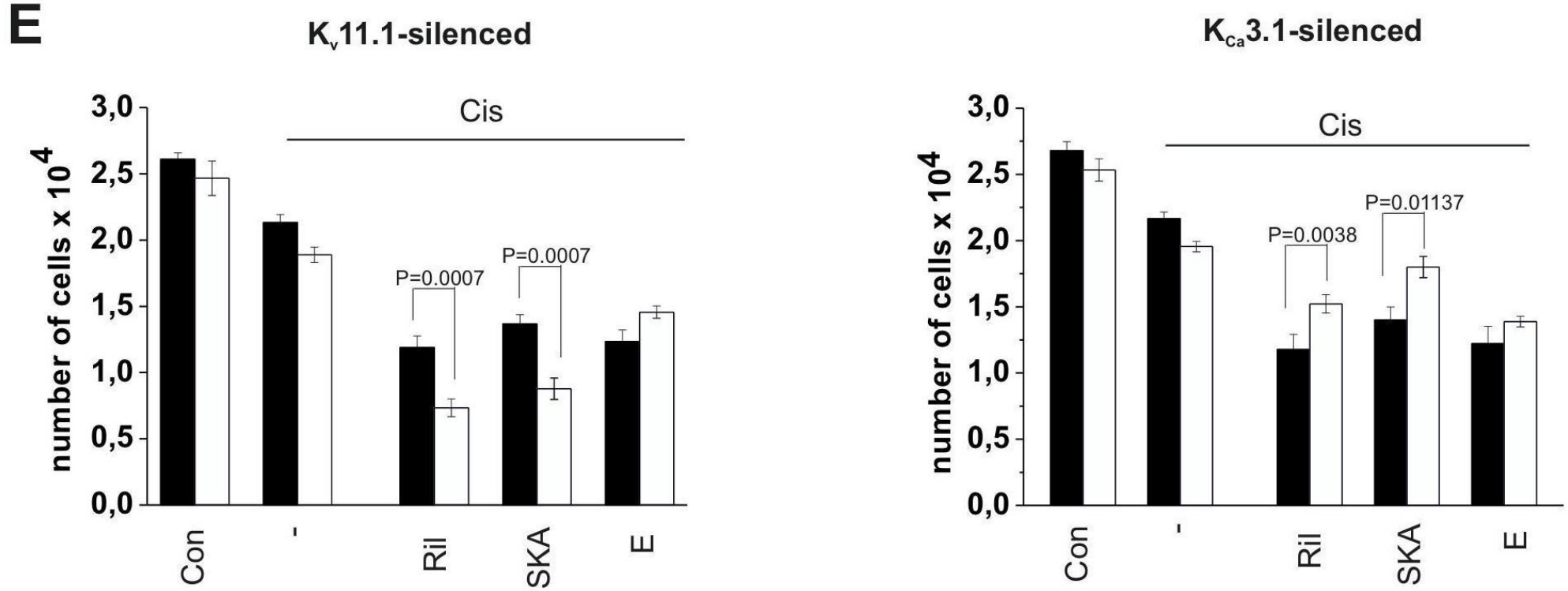

Cis
P=0.006
P=0.004
Caspase 3 (a.u.)
Con
-
Ril
T34
SKA
E


Cis
P=0.013
P=0.025
pAKT (a.u.)
Con
-
Ril
T34
SKA
E

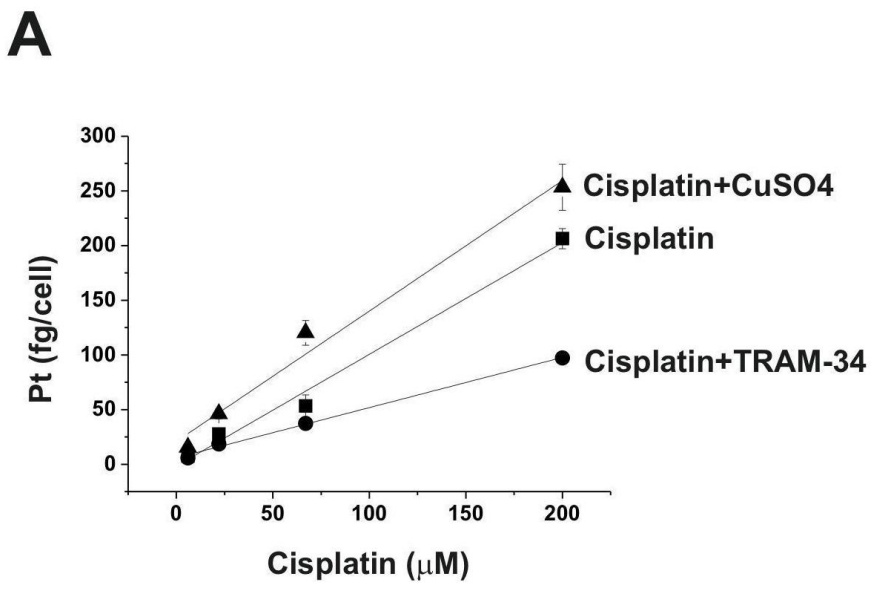
D

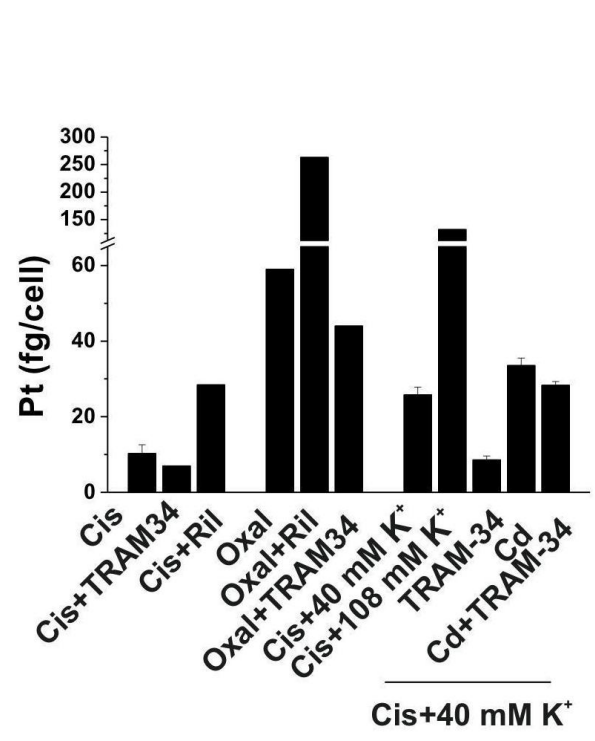

Ril
SKA
E
number of cells x10^4
number of cells x 10^4
time (hours)
0
24
48
72
96
1
2
3

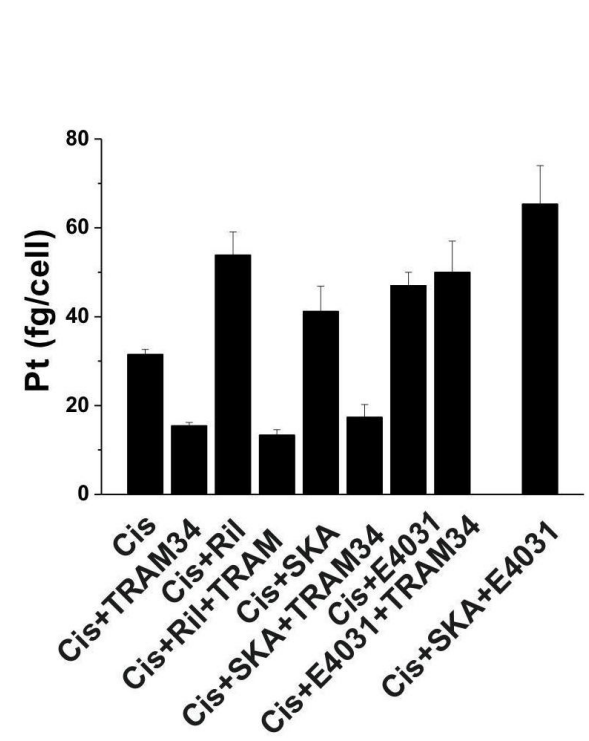
E

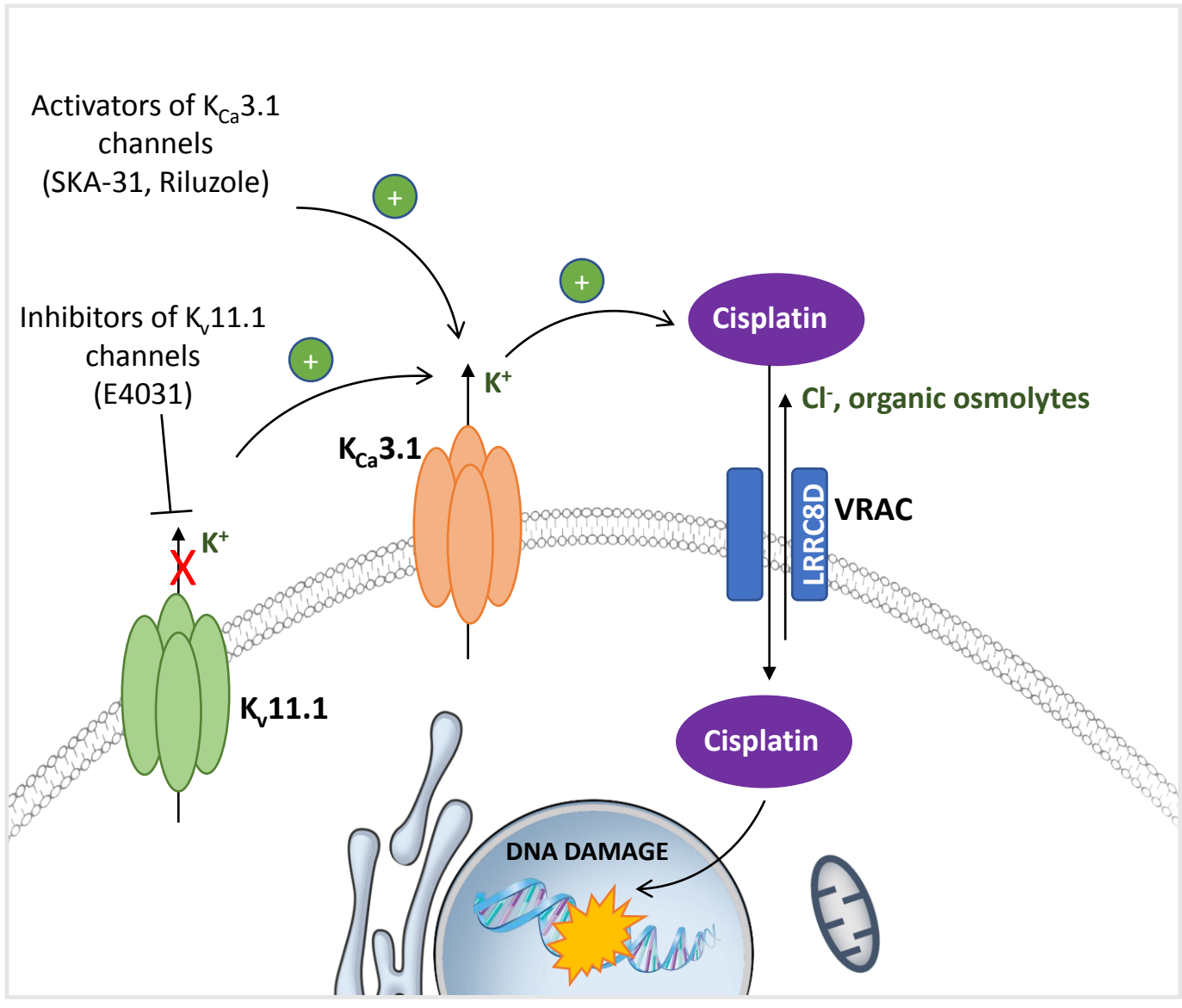

K_v11.1-silenced
K_Ca3.1-silenced
Cis
number of cells x 10^4
P=0.0007
P=0.0007
P=0.0038
P=0.01137
Con
-
Ril
SKA
E
0,0
0,5
1,0
1,5
2,0
2,5
3,0

Cisplatin uptake into resistant cells depended on KCa3.1 channel activity, as it was potentiated by KCa3.1 activators









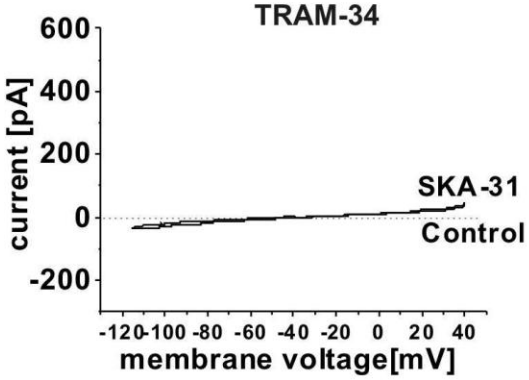
The activation of $K_{Ca}3.1$ modulates the VRAC-dependent uptake of Cisplatin (Jentsch et al, 2016).

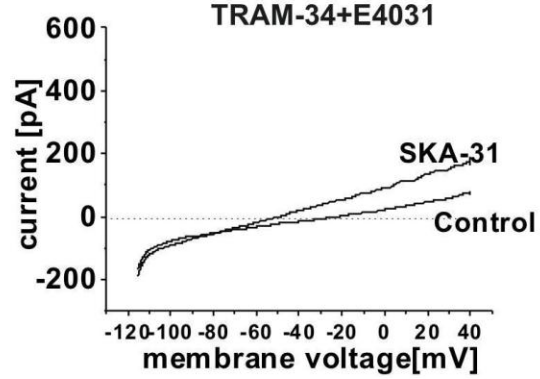
Blocking $K_v11.1$ increases the uptake of Cisplatin, which relies on the activity of $K_{Ca}3.1$ channels.

Kv11.1 blockade led to increased KCa3.1 expression and thereby stimulated Cisplatin uptake.

D

	Number of cells with active $K_{Ca}3.1$ current/total cells (%)	Slope fold variation	Current density (pA/pF)
Control	16/18 (89%)	3.4 ± 0.6	22.1 ± 2.4
E4031	14/18 (78%)	5.1 ± 0.6	42.2 ± 2.5
TRAM-34	0/11 (0%)	-	-
TRAM-34 +E4031	5/7 (71%)	1.7 ± 0.2	3.2 ± 0.8





E

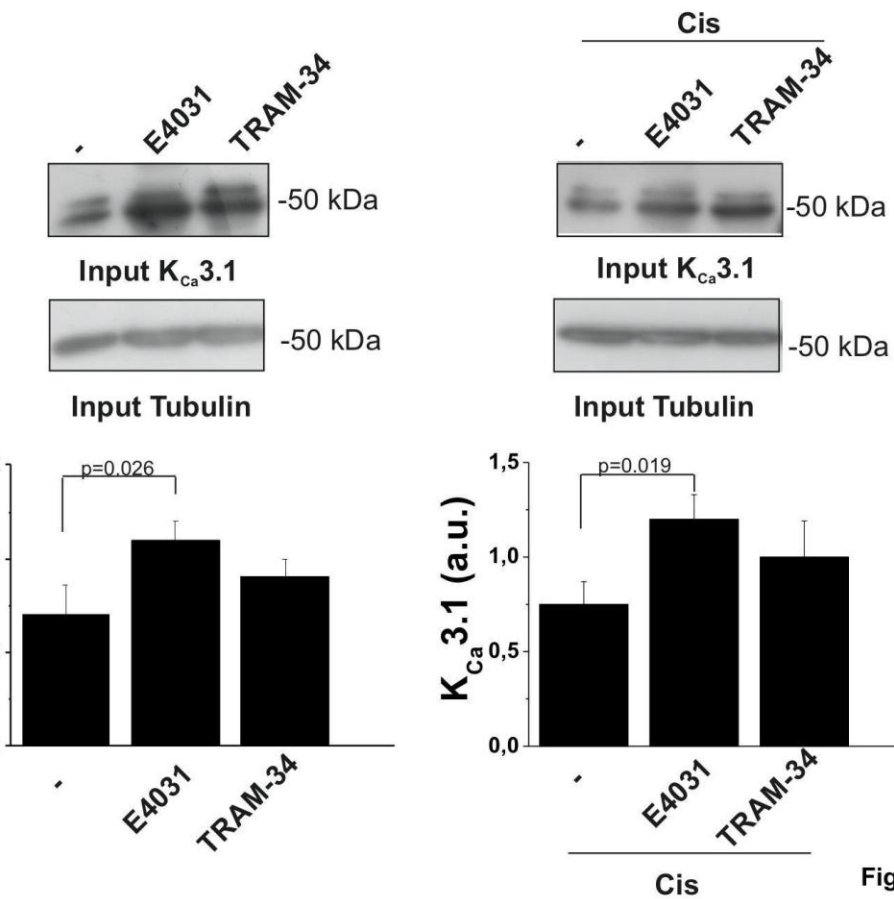
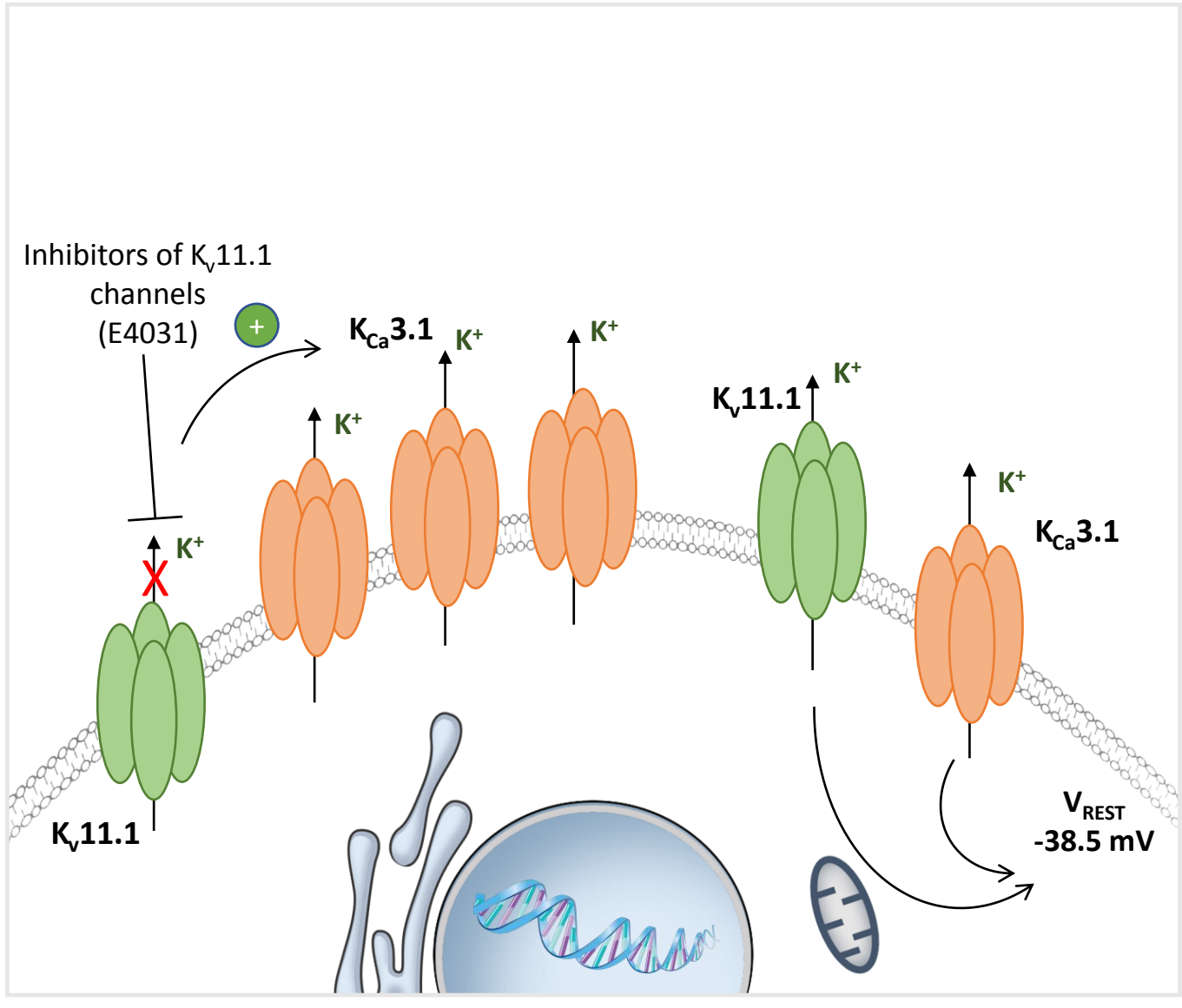


Figure 4



Cisplatin-resistant cells exhibit higher functional expression of K_{Ca}3.1 and K_v11.1 channels, compared with Cisplatin-sensitive cells.

The two channels are functionally related in these cells:

(1)they set VREST to more hyperpolarised values;

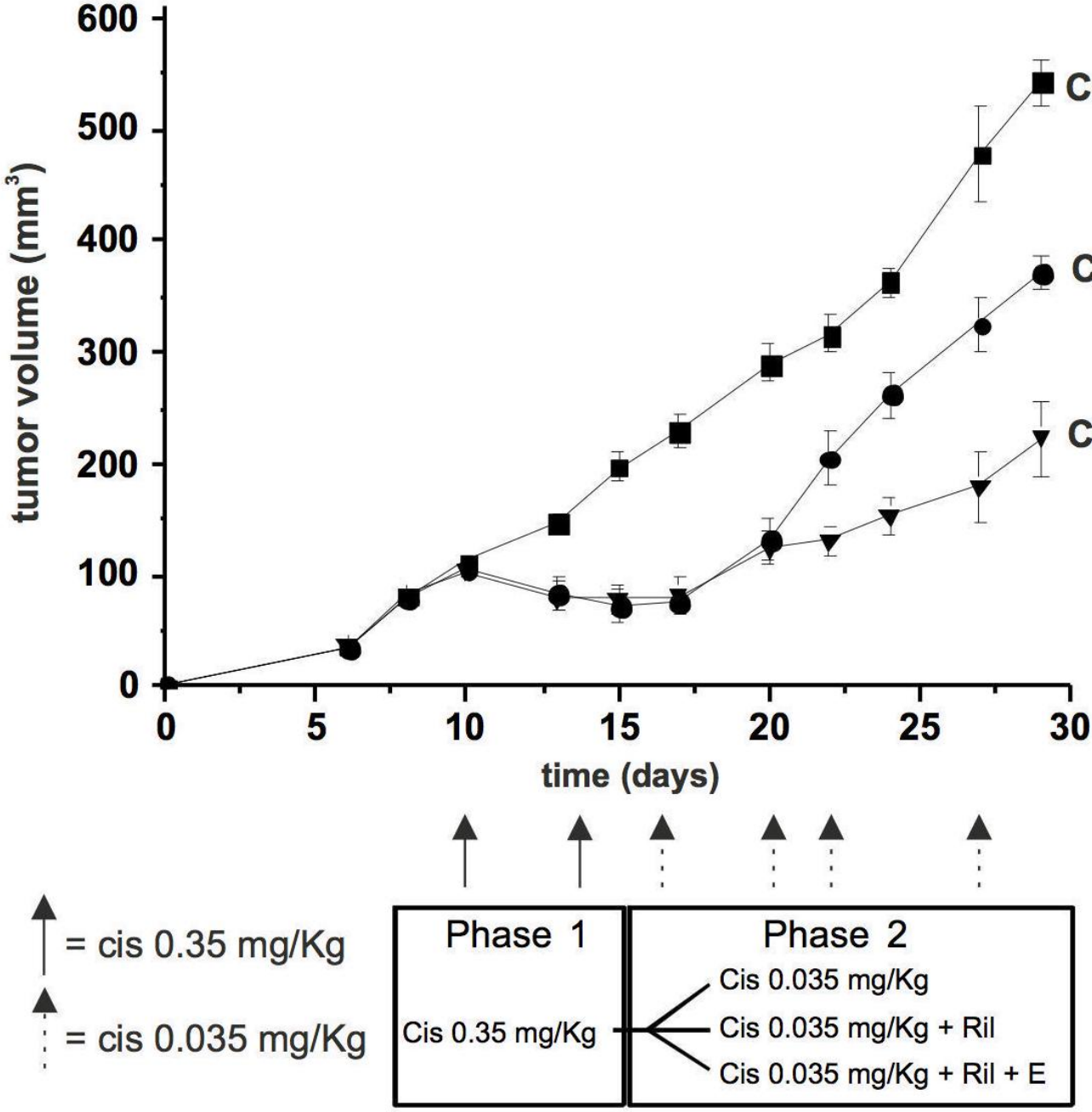
(2)their expression is coordinated, one compensating for the other: prolonged (24h) inhibition of Kv11.1 currents leads to upregulation of functional KCa3.1 channels.

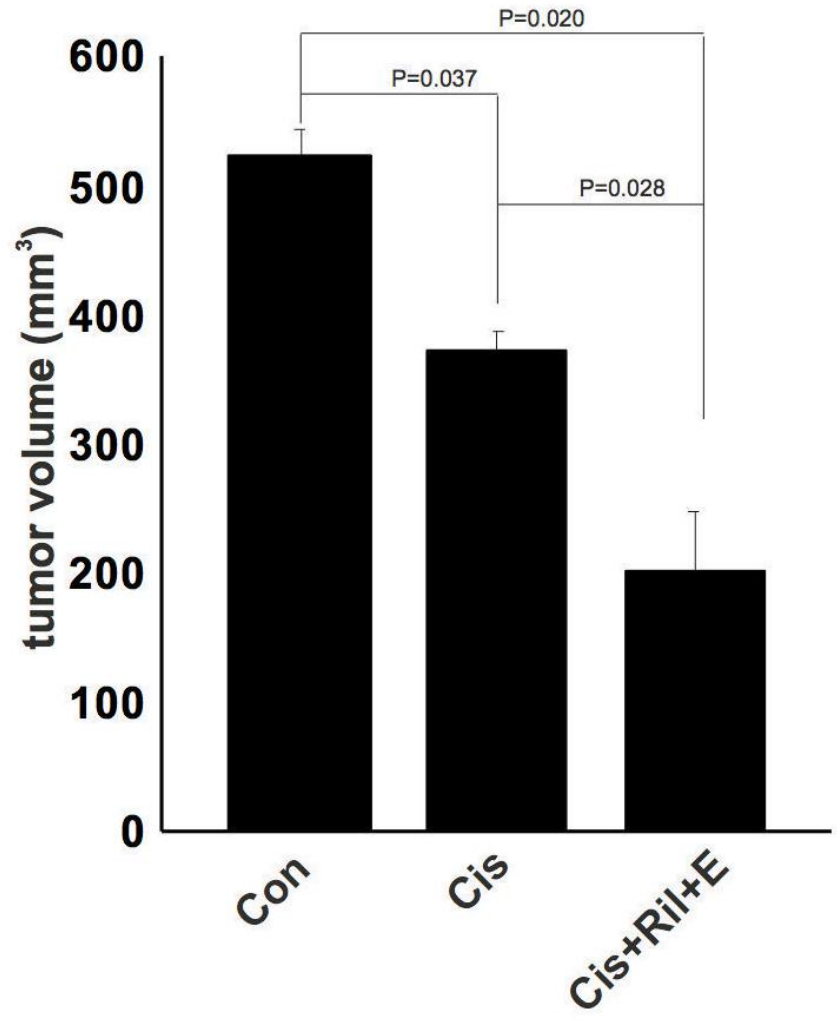
….a summary

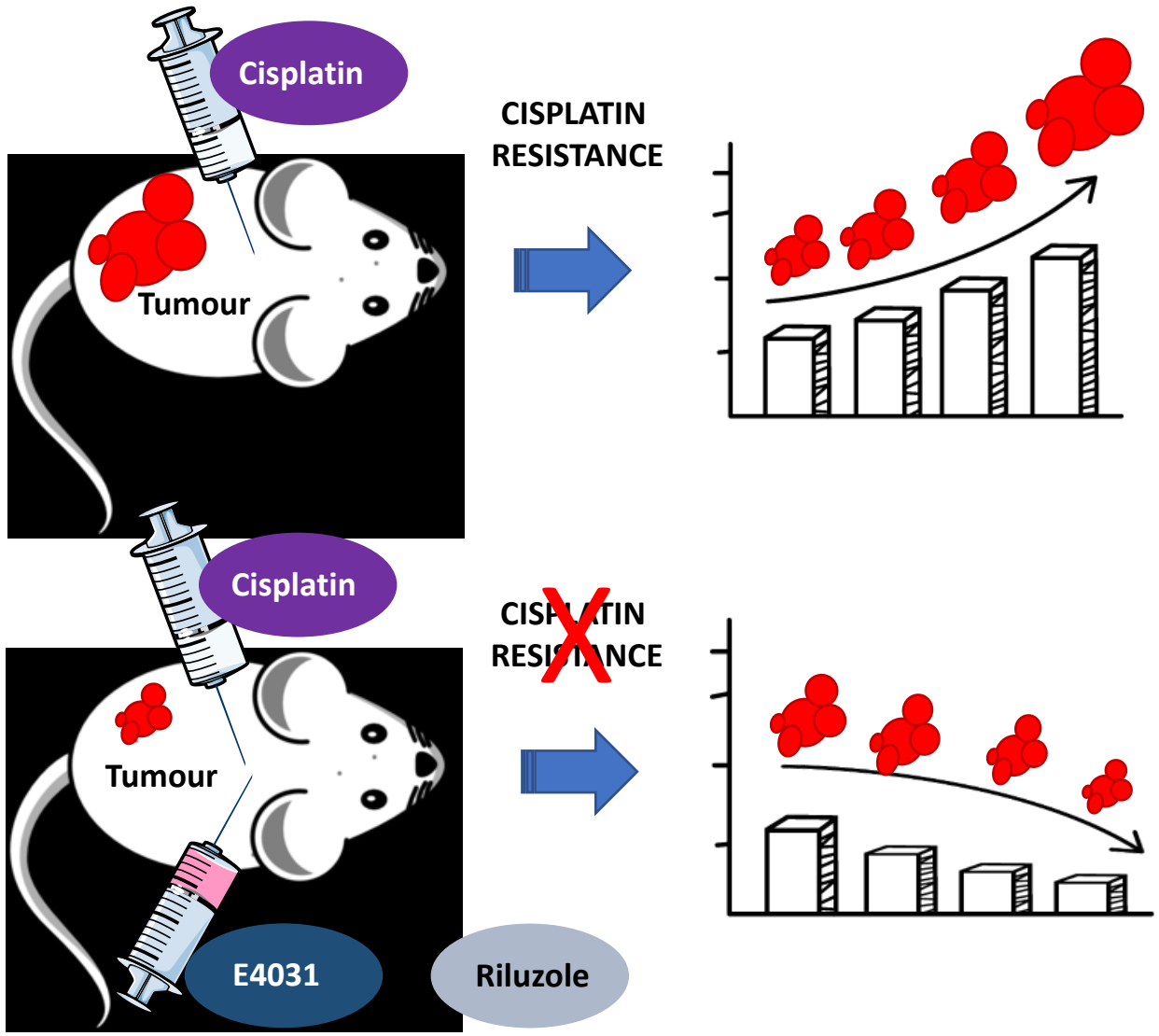
Table 2. Summary of the effects of K^+ channel modulators (Riluzole, SKA-31, TRAM-34 and E4031) on different biological processes of HCT-116 cells

		Cisplatin			
Drug	**V_{REST}**	**Platinum uptake**	**Cell viability**	**Apoptosis**	**Cell cycle**
Riluzole	Hyperpolarisation	↑	↓ (S)	↑↑	↑↑ % of cells in G2/M
SKA-31	Hyperpolarisation	↑	↓ (S)	↑↑	↑ % of cells in G2/M
TRAM-34	Depolarisation	↓	(A)	↑↑	↑↑ % of cells in G2/M
E4031	Depolarisation	↑	↓ (S)	↑↑	↑ % of cells in G2/M

Abbreviations: ↓ = decrease, ↑ = increase, ↑↑ = strong increase, (A) = antagonism, (S) = synergy. V_{REST} was determined in cells treated with the single K^+ channel modulators alone; Platinum uptake, cell viability, apoptosis and cell cycle data are relative to treatments in combination with Cisplatin (25μM). Experimental data and concentrations used are from Table 1B, Figures 2–4 and Supplementary Table S7.


tumor volume (mm3)
600
500
400
300
200
100
0
0
5
10
15
20
25
30
time (days)
Con
Cis
Cis+Ril+E
= cis 0.35 mg/Kg
= cis 0.035 mg/Kg
Phase 1
Cis 0.35 mg/Kg
Phase 2
Cis 0.035 mg/Kg
Cis 0.035 mg/Kg + Ril
Cis 0.035 mg/Kg + Ril + E


P=0.020
P=0.037
P=0.028
tumor volume (mm3)
600
500
400
300
200
100
0
Con
Cis
Cis+Ril+E



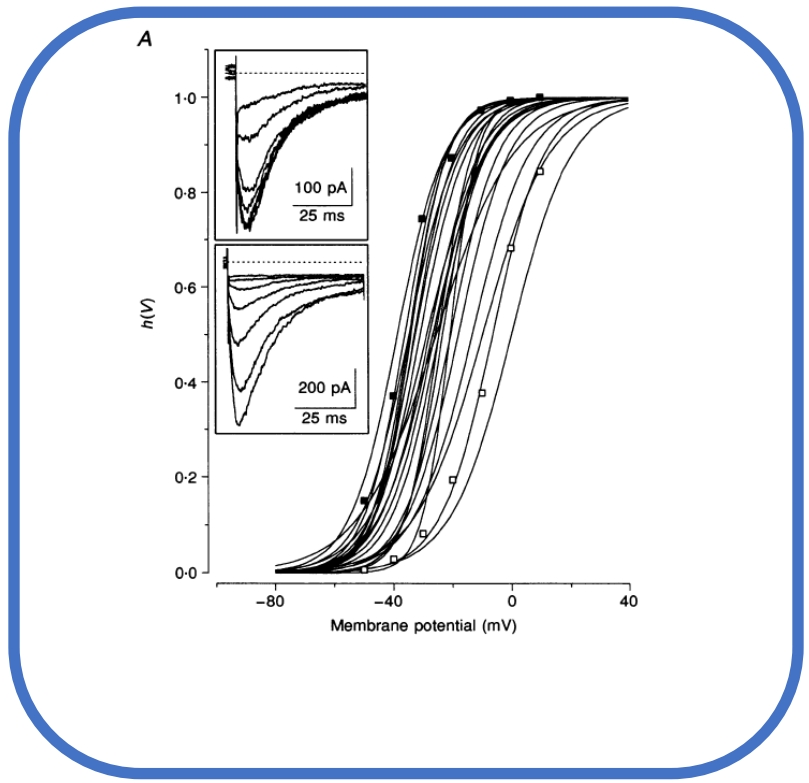
The concomitant activation of $K_{Ca}3.1$ and inhibition of $K_v11.1$ potentiates the pro-apoptotic activity of Cisplatin, both *in vitro* and *in vivo*, and contributes to overcome Cisplatin resistance.

hERG1

Journal of Physiology (1995), **489.2**, *pp.*455–471

A novel inward-rectifying K^+ current with a cell-cycle dependence governs the resting potential of mammalian neuroblastoma cells

Annarosa Arcangeli*, Laura Bianchi, Andrea Becchetti, Laura Faravelli, Marcella Coronnello†, Enrico Mini†, Massimo Olivotto* and Enzo Wanke‡



Strategies to target hERG1 in cancer

- ✓ ***Use of non cardiotoxic hERG1 blockers***

- ✓ ***Targeting the molecular differences between "tumour" and "cardiac" hERG1:***

CCR Translations

Clinical Cancer Research

hERG Channels: From Antitargets to Novel Targets for Cancer Therapy

Annarosa Arcangeli[1] and Andrea Becchetti[2]

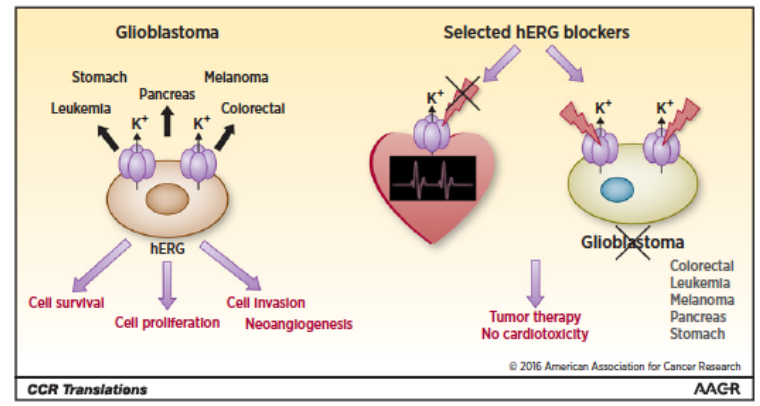


Figure 1.

Left, hERG is often overexpressed on the plasma membrane of different human cancer cells. It regulates tumor cell proliferation, survival, migration/invasiveness, and neoangiogenesis. Right, inhibiting hERG in different types of cancer cells (red lightning bolts) by using selective blockers that do not produce cardiac arrhythmia (as indicated by the black cross) is a possible strategy for anticancer therapy. The article by Pointer and colleagues (1) suggests that this is feasible in glioblastoma. Such a strategy may be effective in other cancers (shown in gray) in which hERG is overexpressed and has been shown to regulate neoplastic progression.

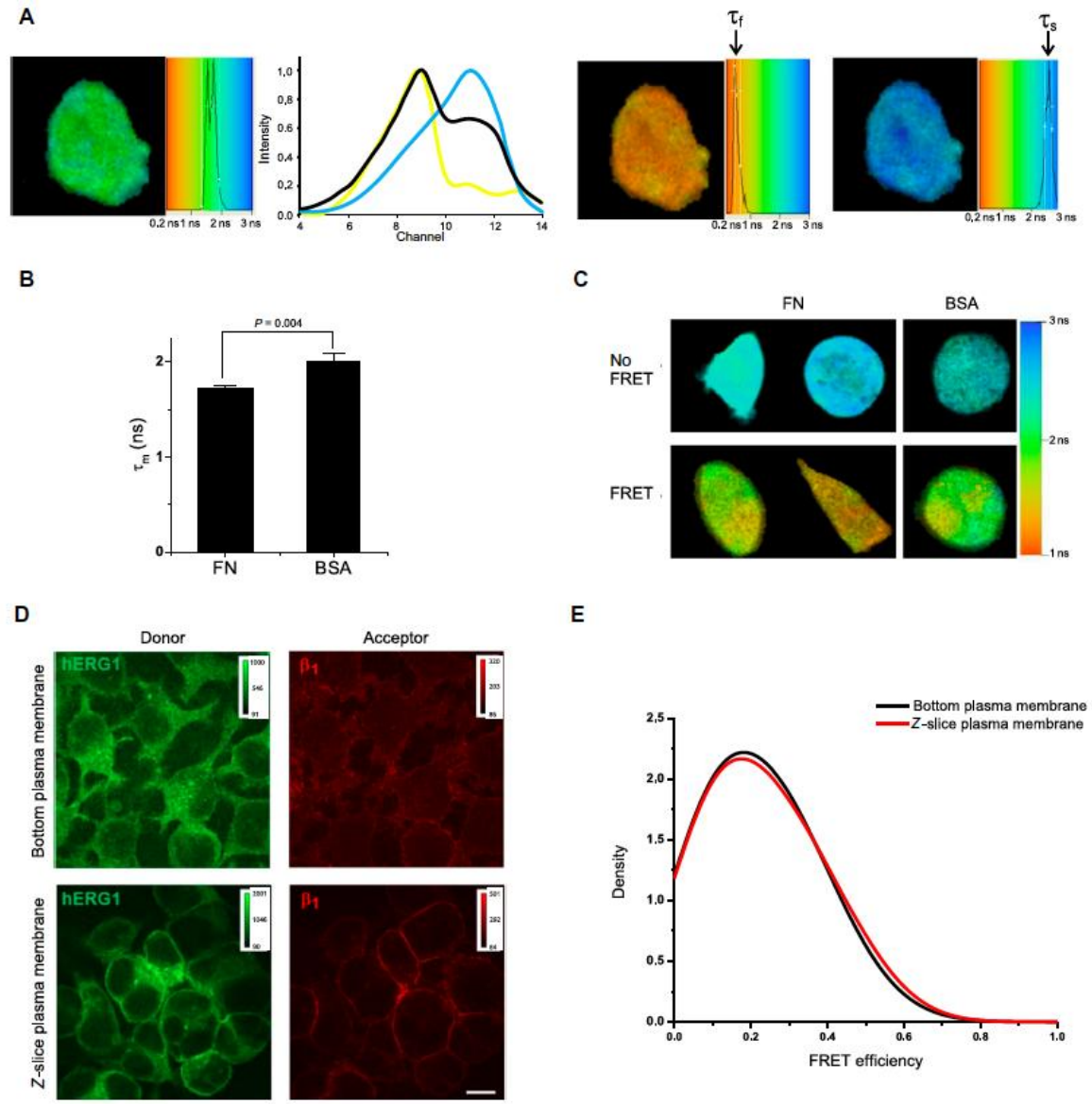
SCIENCE SIGNALING | RESEARCH ARTICLE

CANCER

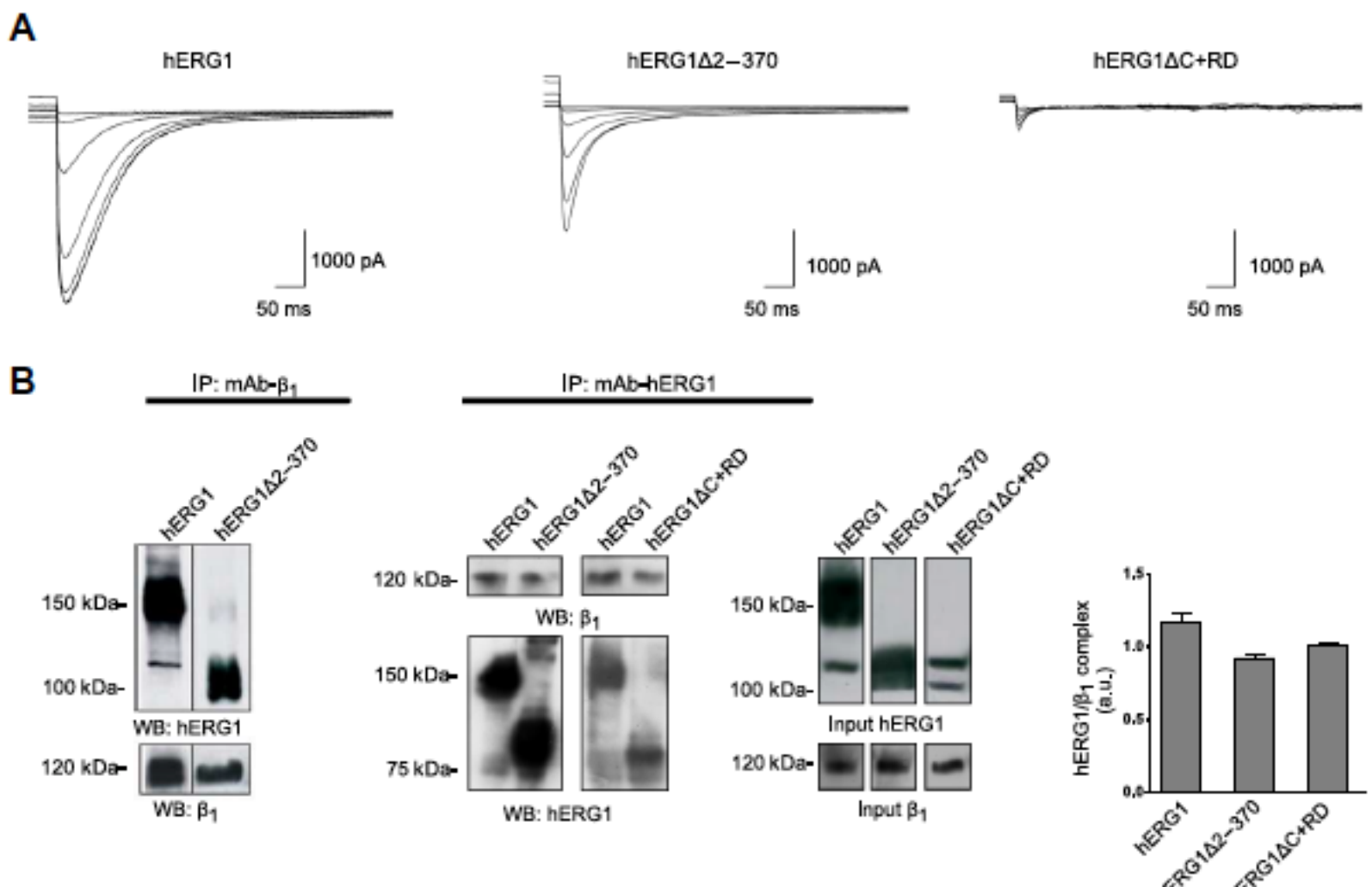
The conformational state of hERG1 channels determines integrin association, downstream signaling, and cancer progression

Andrea Becchetti,[1] Silvia Crescioli,[2] Francesca Zanieri,[2] Giulia Petroni,[2] Raffaella Mercatelli,[3] Stefano Coppola,[4] Luca Gasparoli,[2] Massimo D'Amico,[5] Serena Pillozzi,[2] Olivia Crociani,[2] Matteo Stefanini,[5] Antonella Fiore,[2] Laura Carraresi,[5] Virginia Morello,[6]* Sagar Manoli,[2] Maria Felice Brizzi,[7] Davide Ricci,[8] Mauro Rinaldi,[8] Alessio Masi,[2]† Thomas Schmidt,[4] Franco Quercioli,[3] Paola Defilippi,[4] Annarosa Arcangeli[2]‡

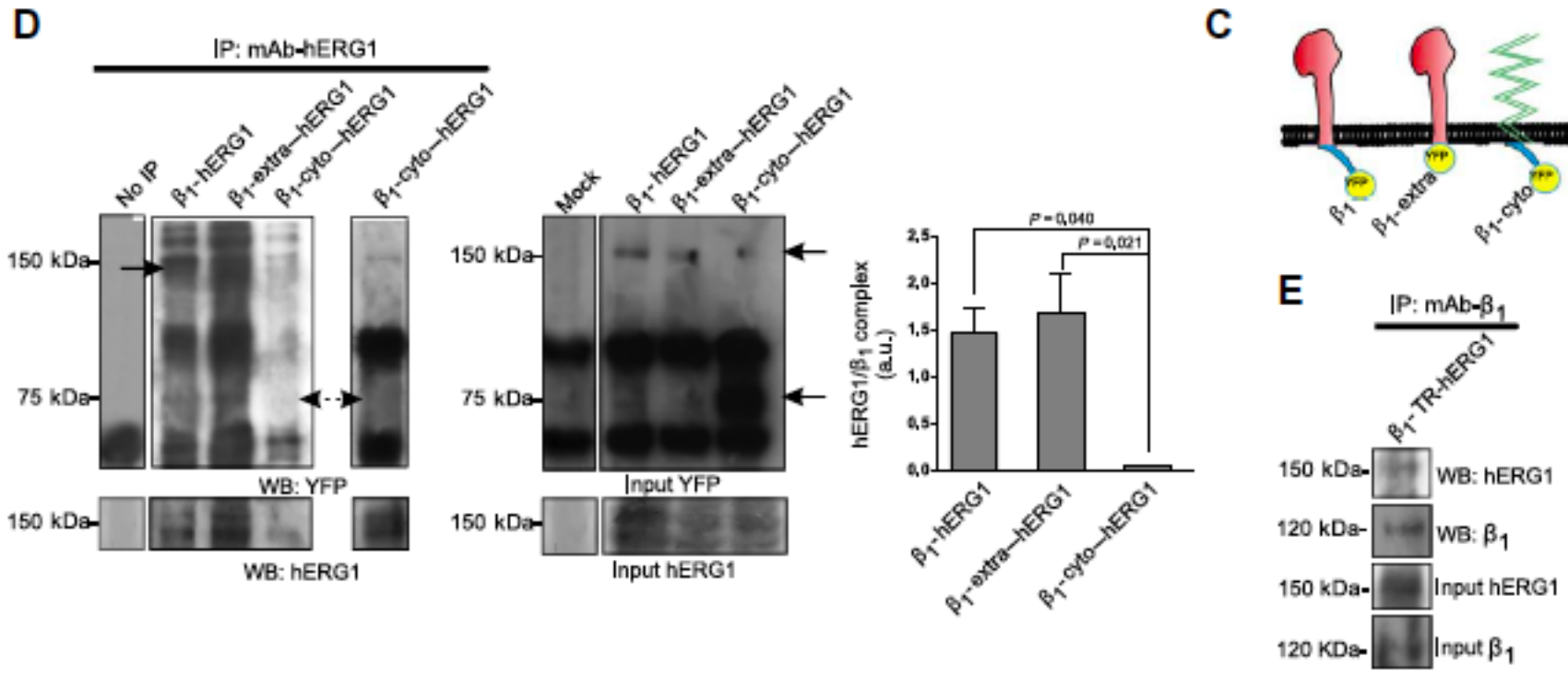
hERG1 and the beta1 integrin subunit are directly linked ……



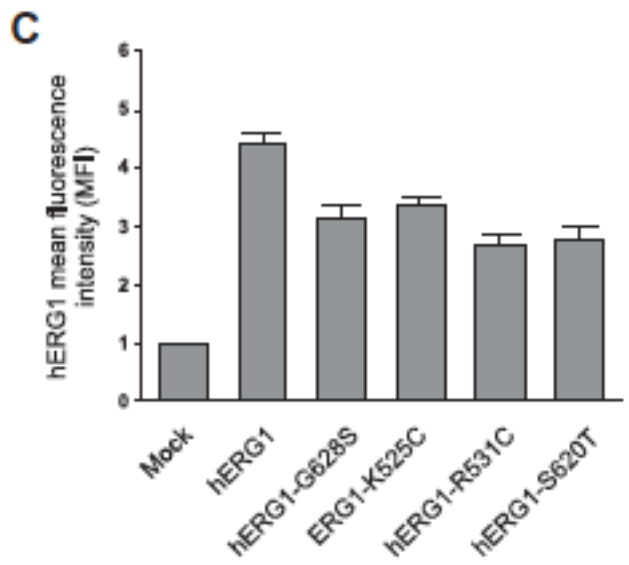
hERG1 and the beta1 integrin subunit are directly linked…..neither the N- or C-termini are involved

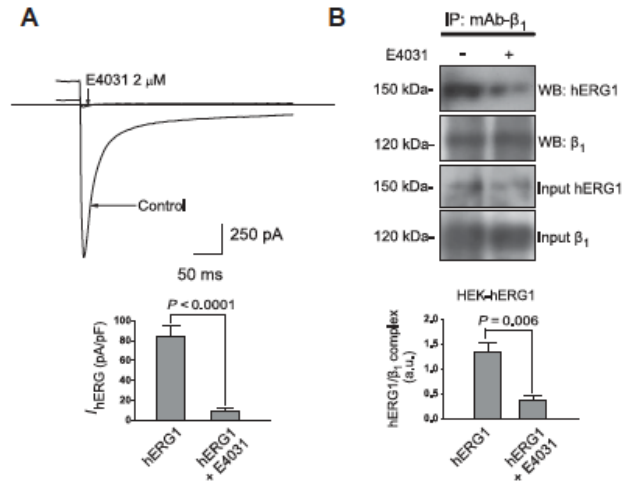


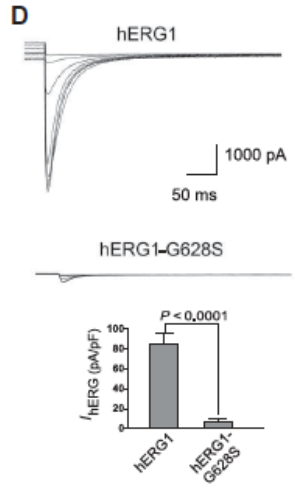
hERG1 and the beta1 integrin subunit are directly linked…..neither the N- or C-termini are involved

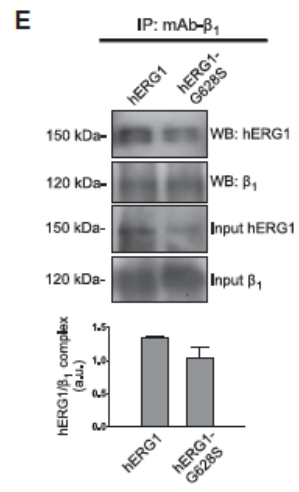


The hERG1 conformational state determines (the closed state favours) integrin association



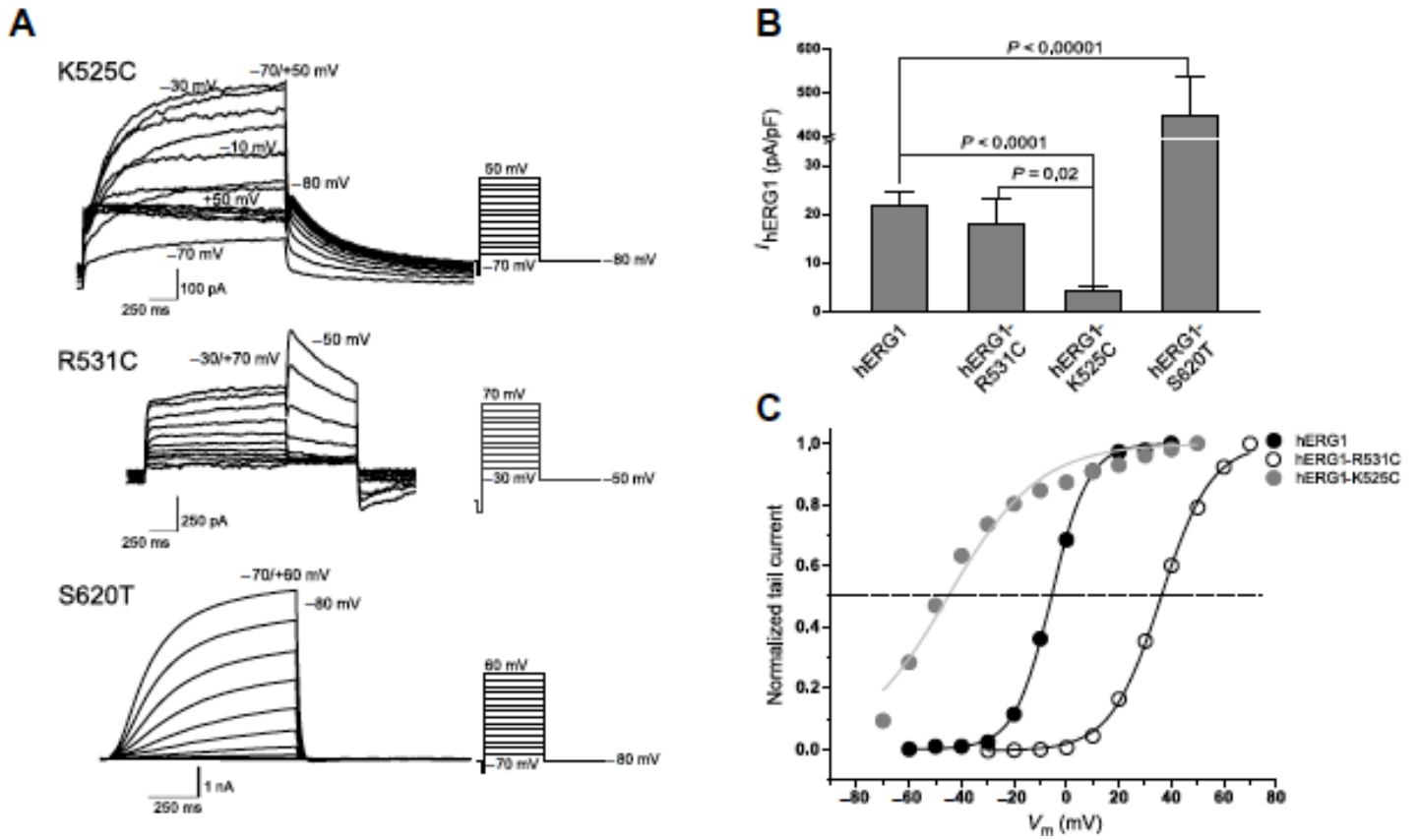




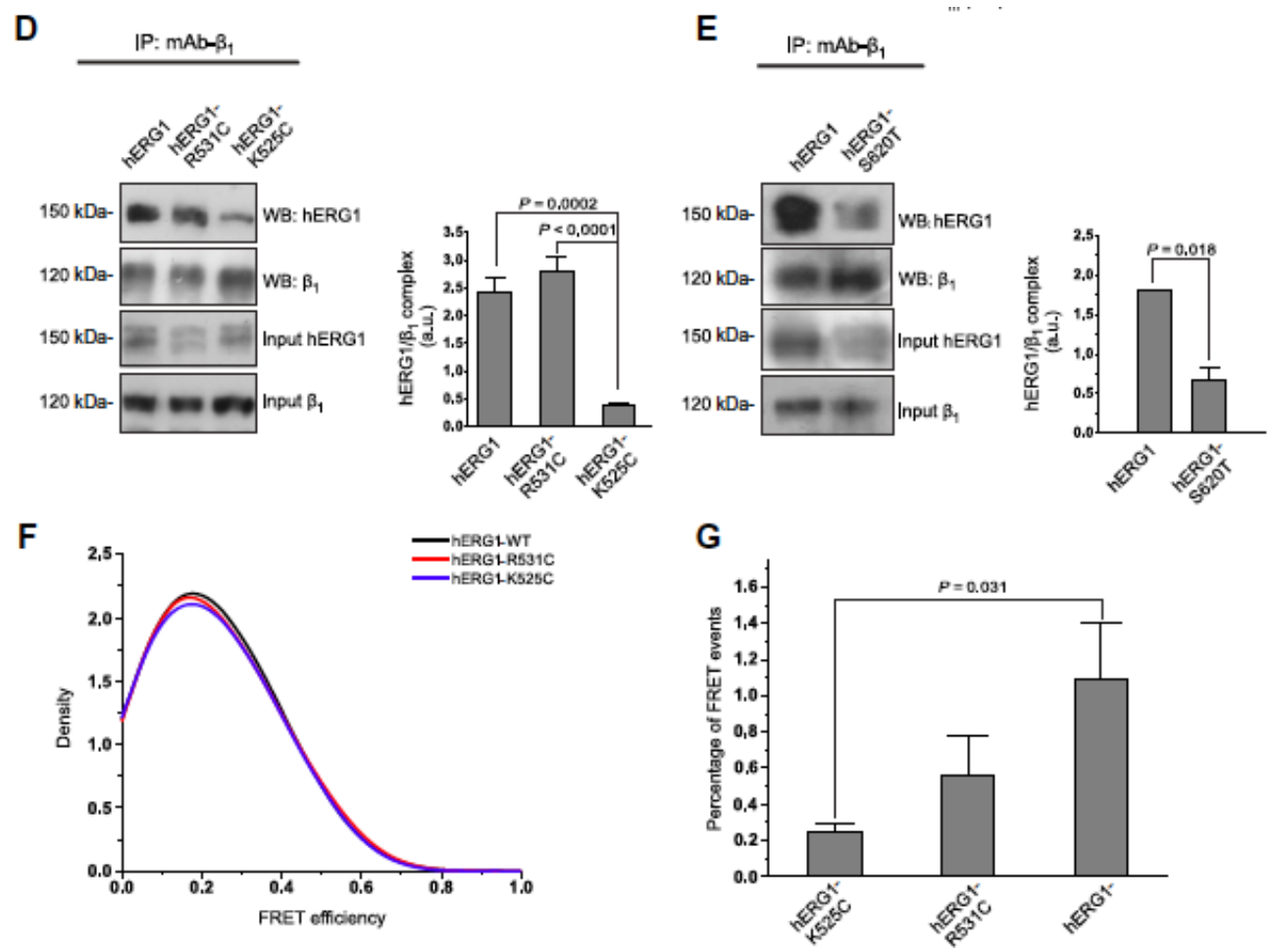


hERG1 mutants:
G628S: non conductive
S620T: non inactivating
*K525C: S4 (voltage sensor) mutant**
*R531C: S4 (voltage sensor) mutant**
**=alterations of gating*

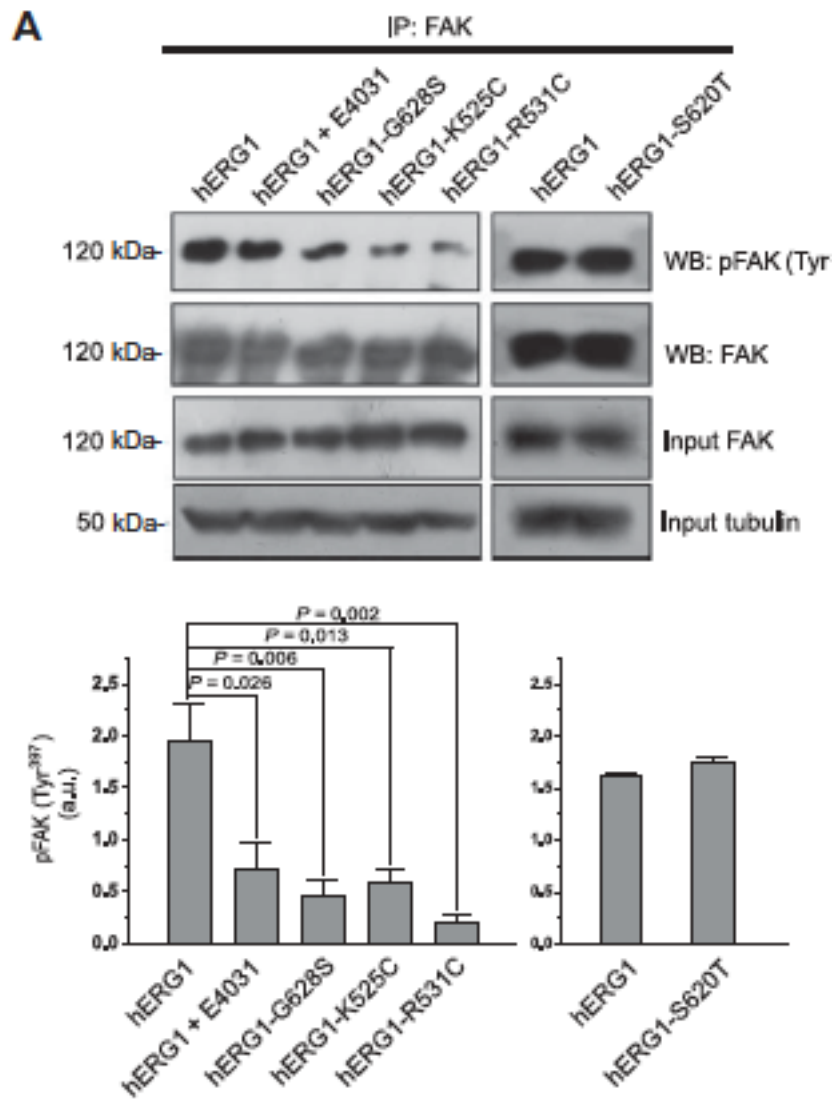
The hERG1 conformational state determines (the closed state favours) integrin association



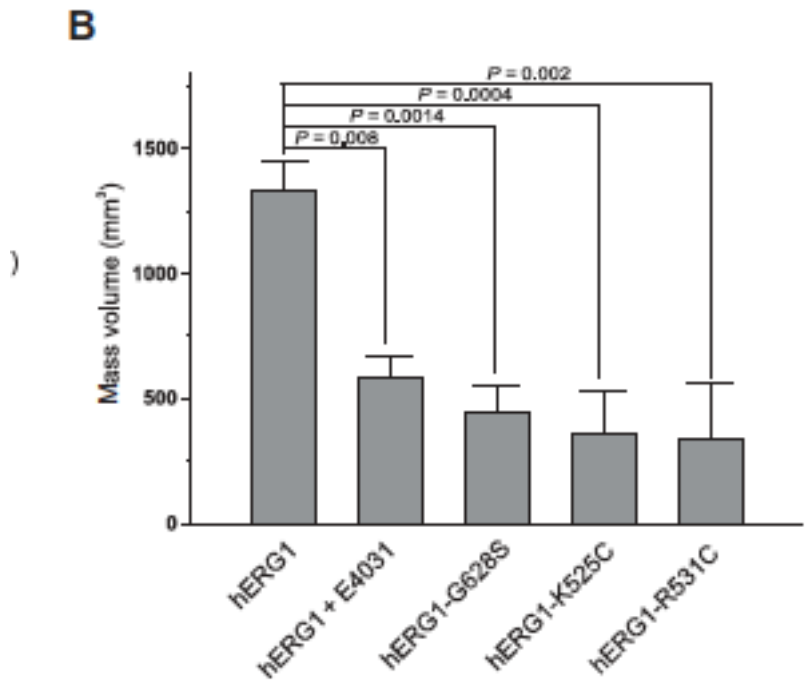
The hERG1 conformational state determines (the closed state favours) integrin association



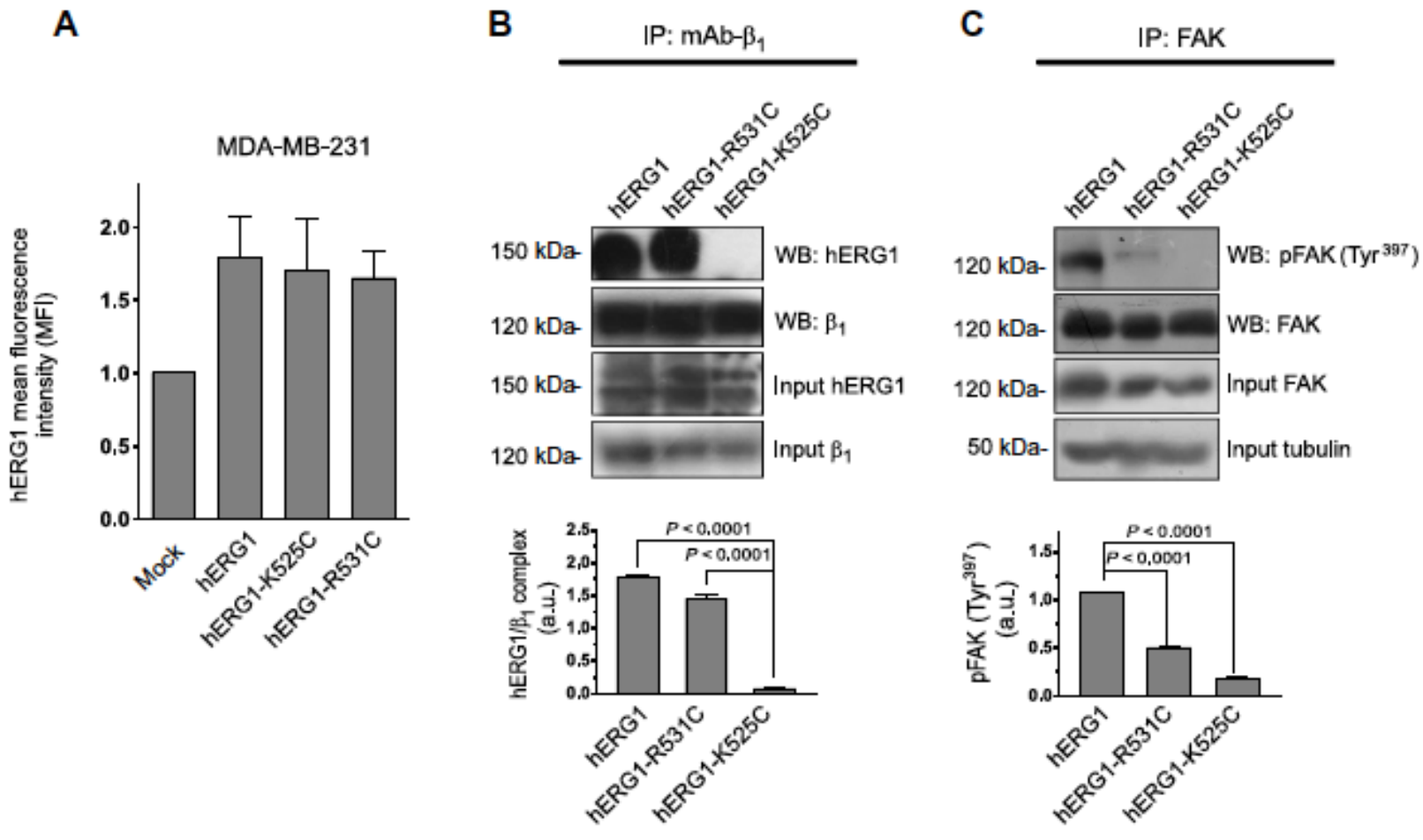
K^+ flux regulates integrin signaling (FAK phosphorylation)



.....and local tumor growth



The hERG1 conformational state determines (the closed state favours) integrin association: MDA-MB-231 breast cancer cells

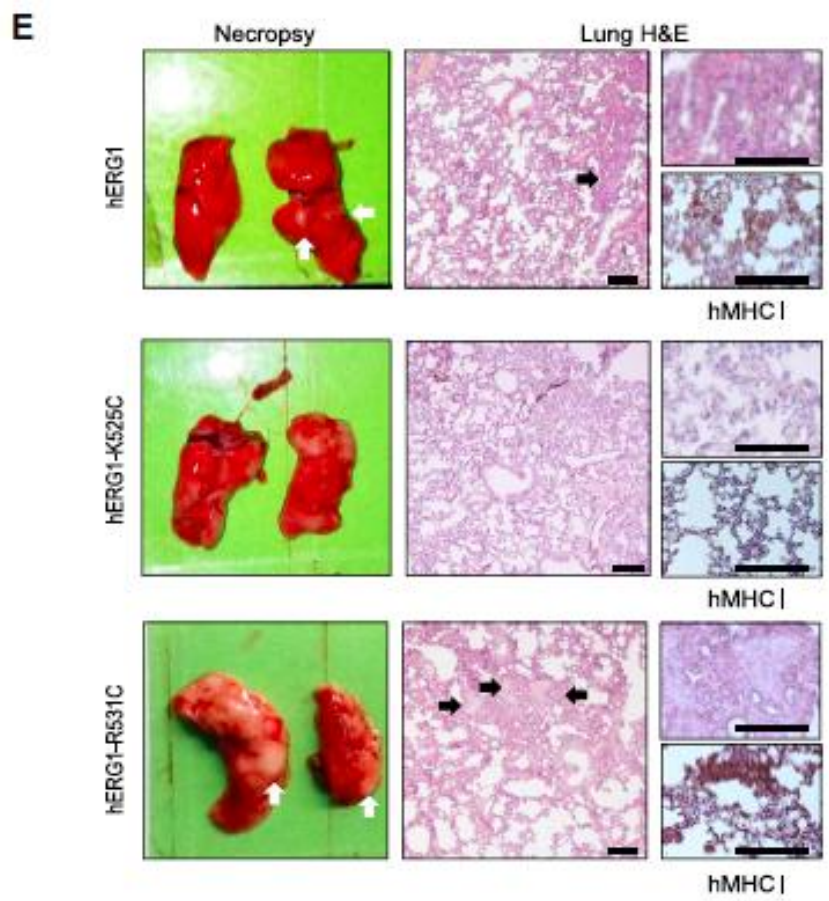


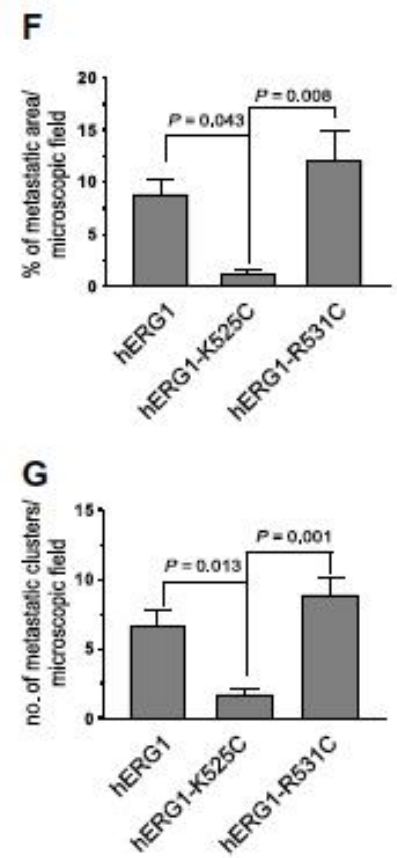
The hERG1 conformational state determines (the closed state favours) integrin association…..and tumor metastasis (MDA-MB-231 breast cancer cells)

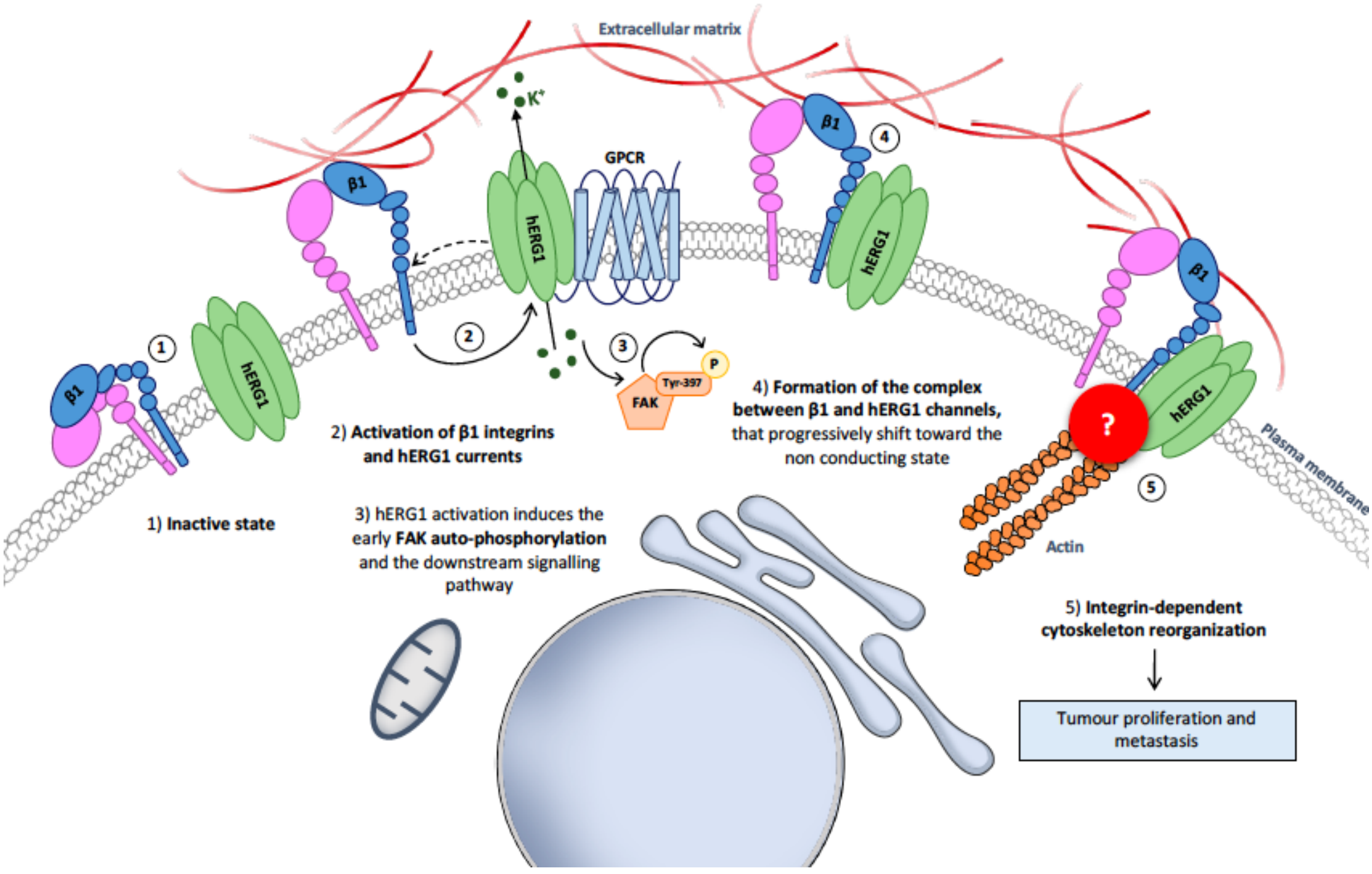
D

MDA-MB-231	hERG1	hERG1-K525C	hERG1-R531C
Local tumor growth			
Number of tumor masses (%)	9/10 (90%)	10/10 (100%)	9/10 (90%)
Median tumor volume (mm^3)	150 (19–300)	122 (33–300)	212 (33–300)
Metastases			
Inguinal lymph nodes Number of mice with macroscopic metastases (%)	2/5 (40%)	0/5 (0%)	3/5 (60%)
Lung Number of mice with macroscopic metastases (%)	2/5 (40%)	0/5 (0%)	4/5 (80%)

The hERG1 conformational state determines (the closed state favours) integrin association…...and tumor metastasis (MDA-MB-231 breast cancer cells)






Extracellular matrix
K+
GPCR
β1
hERG1
1
2
3
4
5
P
Tyr-397
FAK
?
Plasma membrane
Actin
1) Inactive state
2) Activation of β1 integrins and hERG1 currents
3) hERG1 activation induces the early FAK auto-phosphorylation and the downstream signalling pathway
4) Formation of the complex between β1 and hERG1 channels, that progressively shift toward the non conducting state
5) Integrin-dependent cytoskeleton reorganization
Tumour proliferation and metastasis

TUMOR

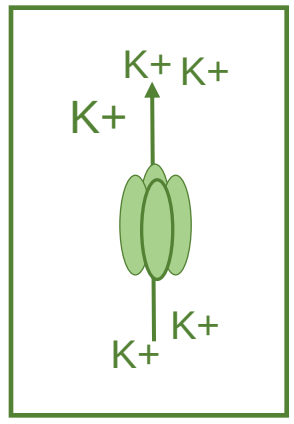
HEART

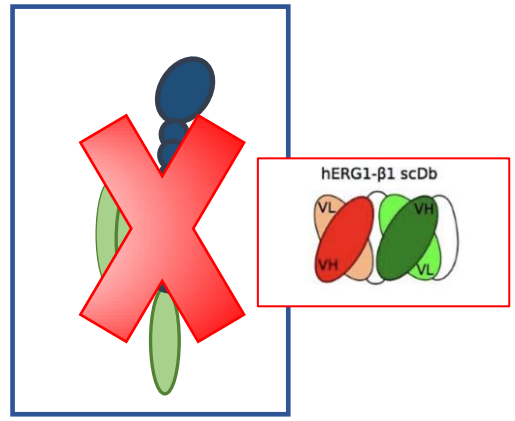
hERG1

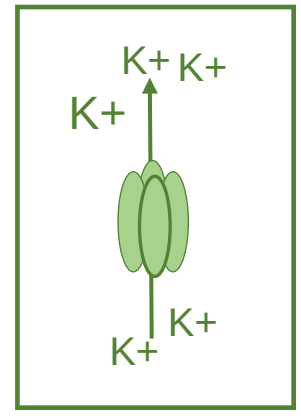
OPEN CHANNEL

CLOSED CHANNEL LINKED TO AN ACTIVE INTEGRIN

OPEN CHANNEL (ΔV)







FAK

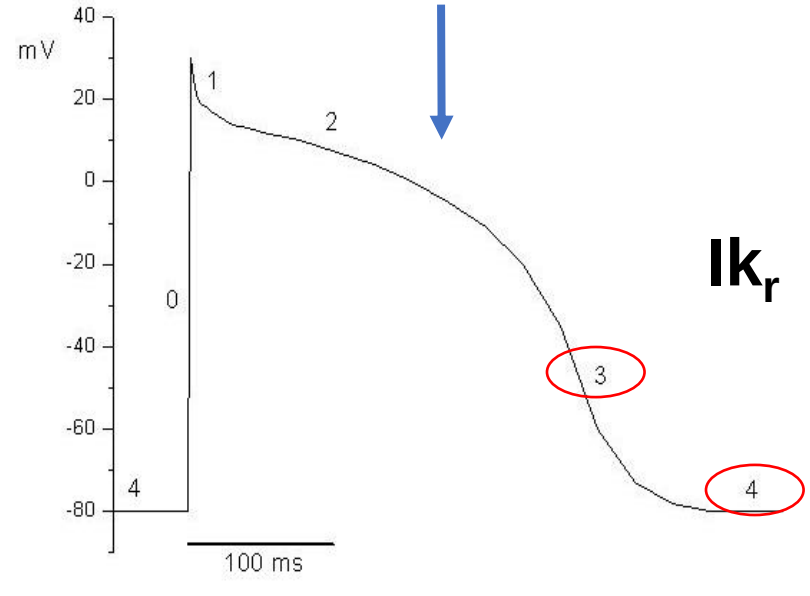
ERK p

AKT

HIFα p

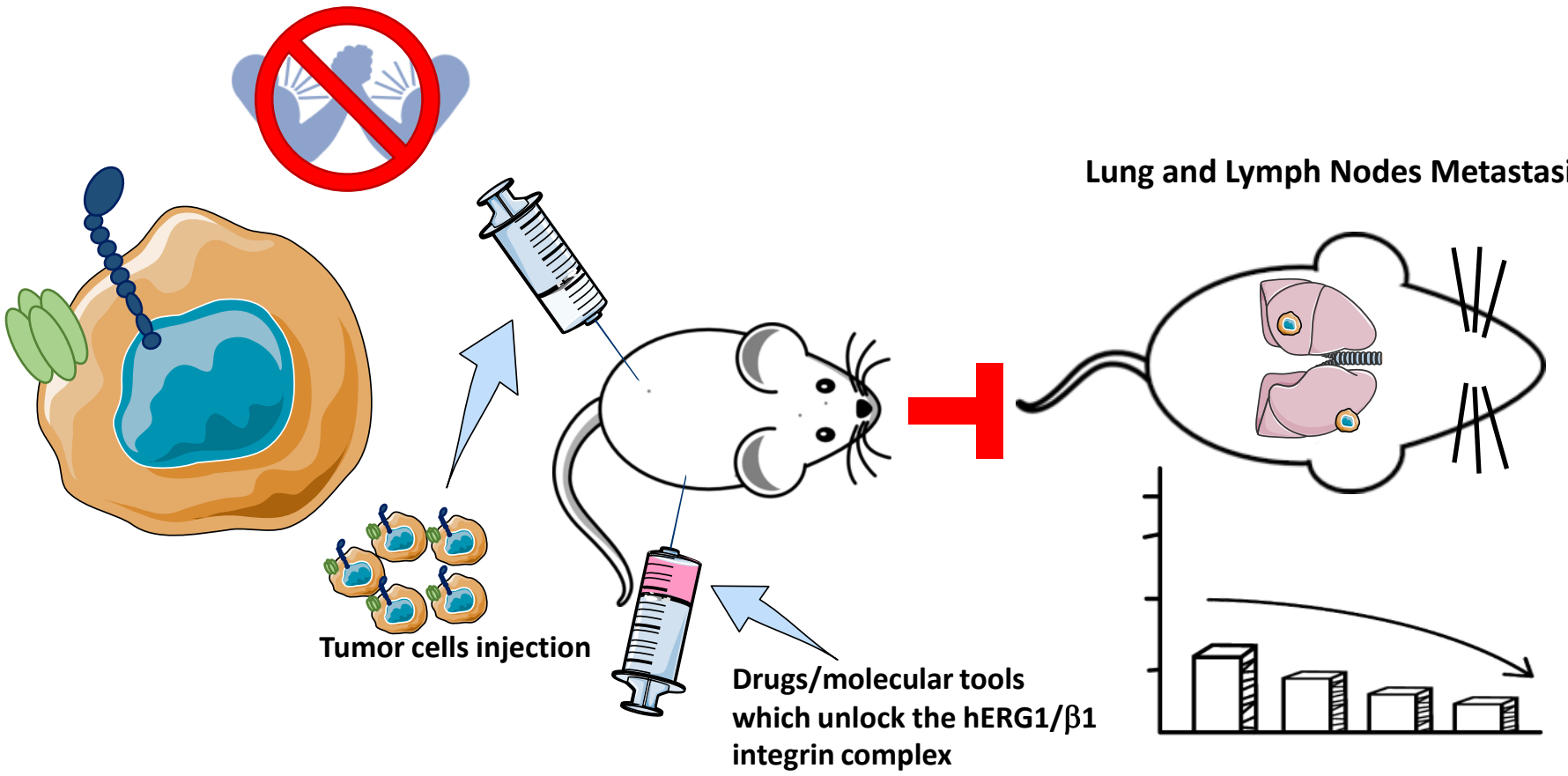
Proliferation
Survival

Invasiveness,
Angiogenesis
Metastasis

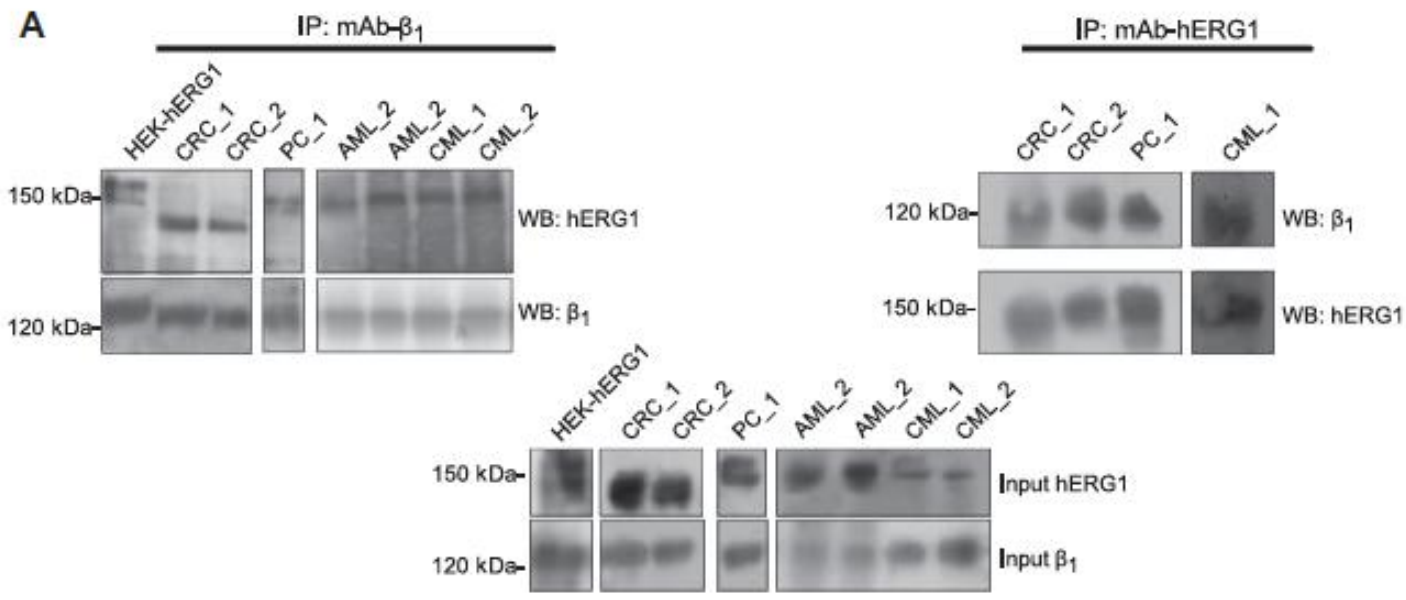


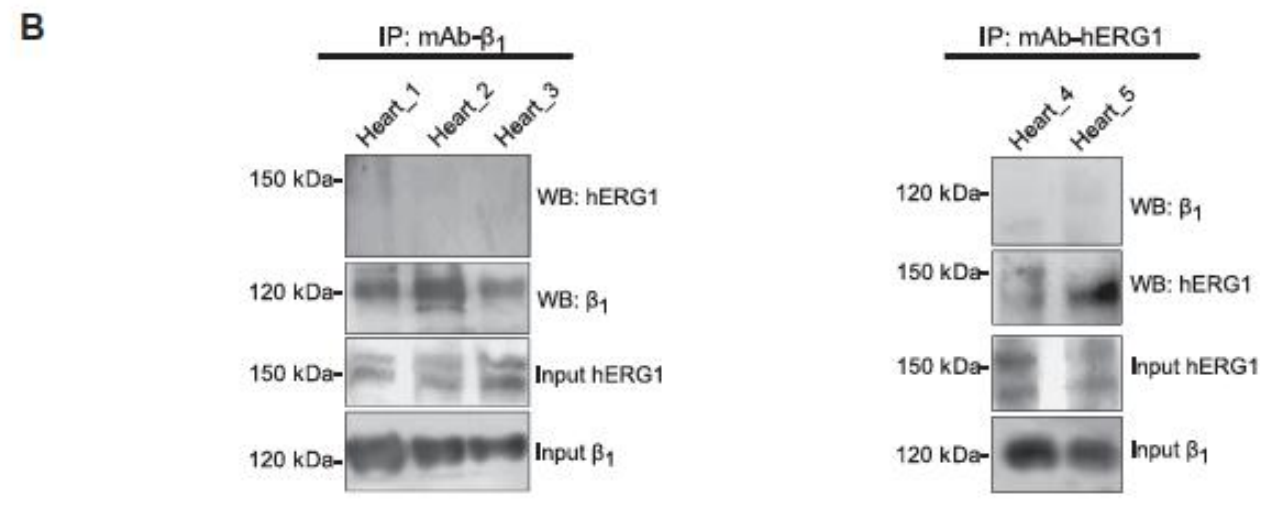
Ik_r

Disrupting the hERG1/β1 integrin complex inhibits tumor metastasis

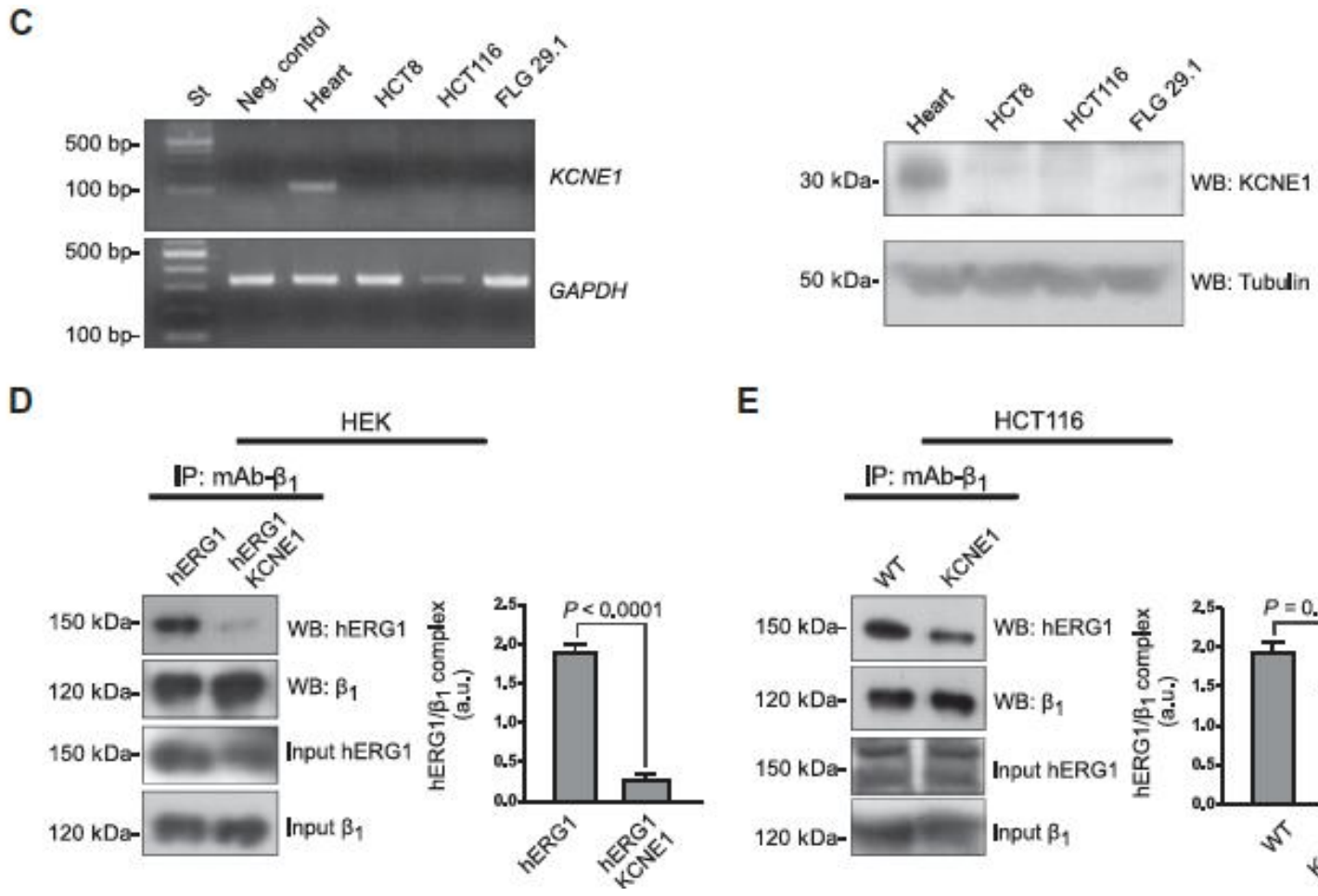


The hERG1/β1 complex occurs in tumour cells, but not in the heart



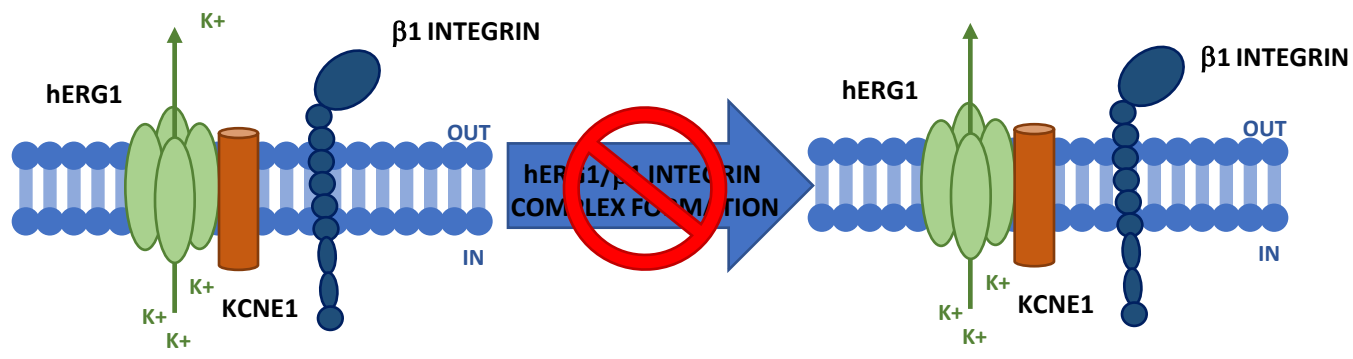


… because tumour cells do not express “canonical” (KCNE1) beta subunits

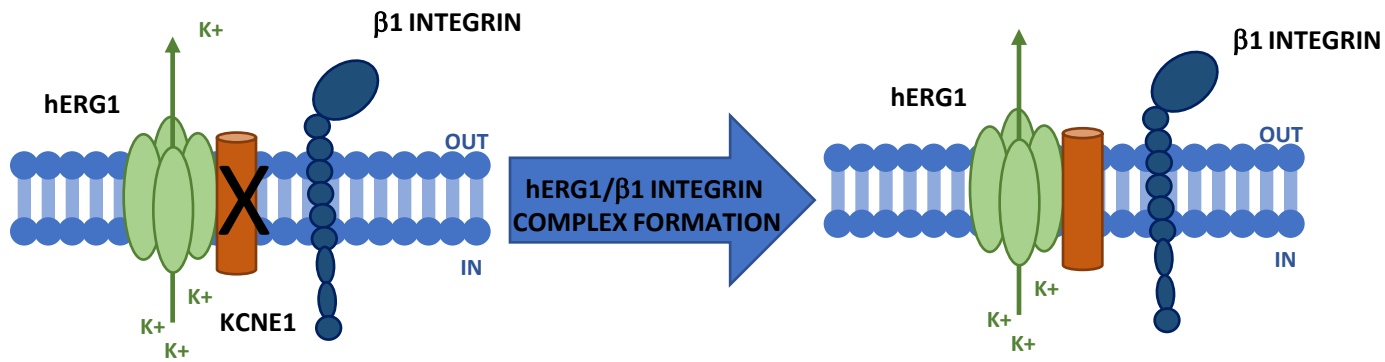


hERG1 and β1 integrin associate in human cancer tissue but not cardiac tissue.

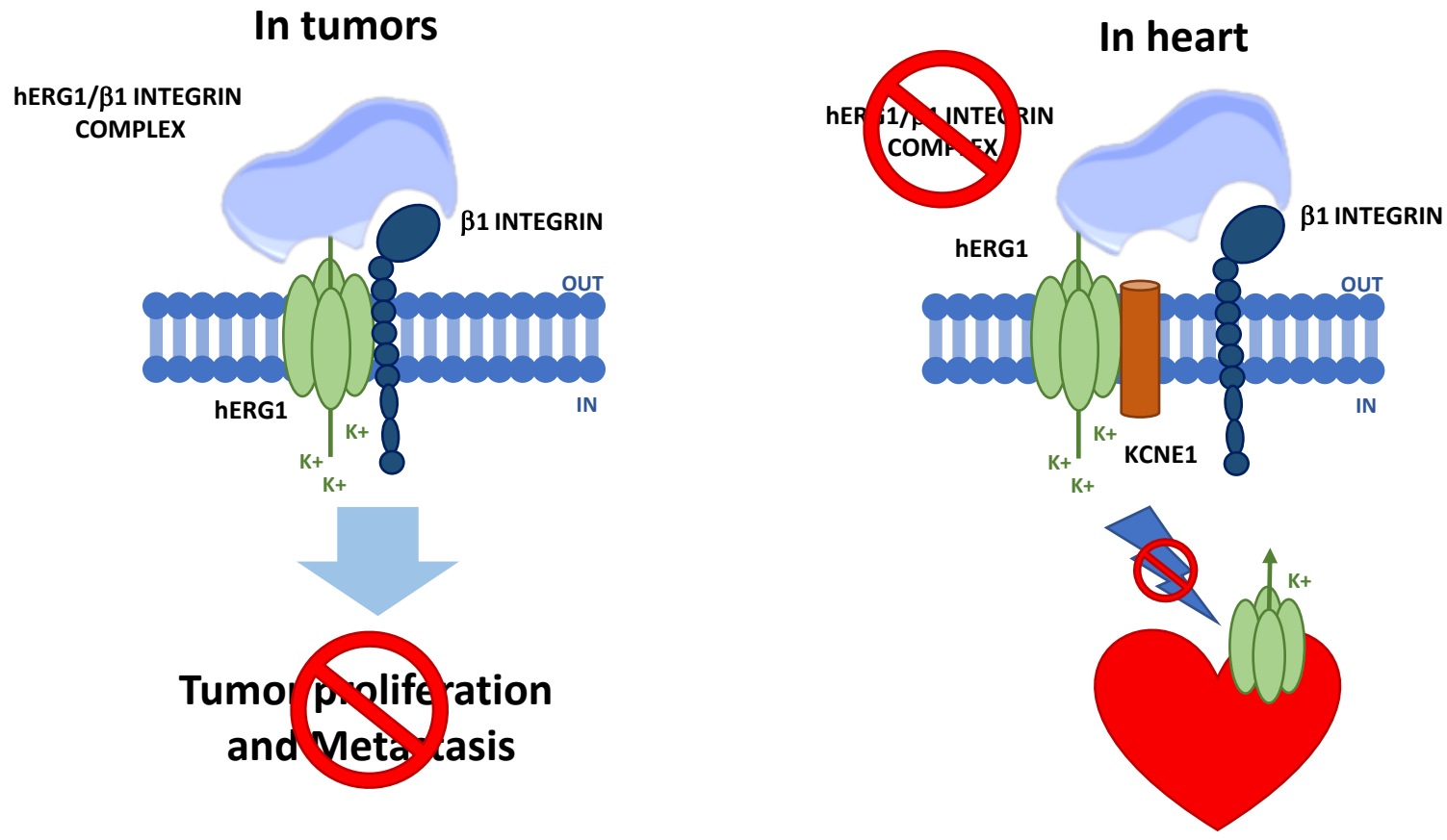
In heart



In tumors

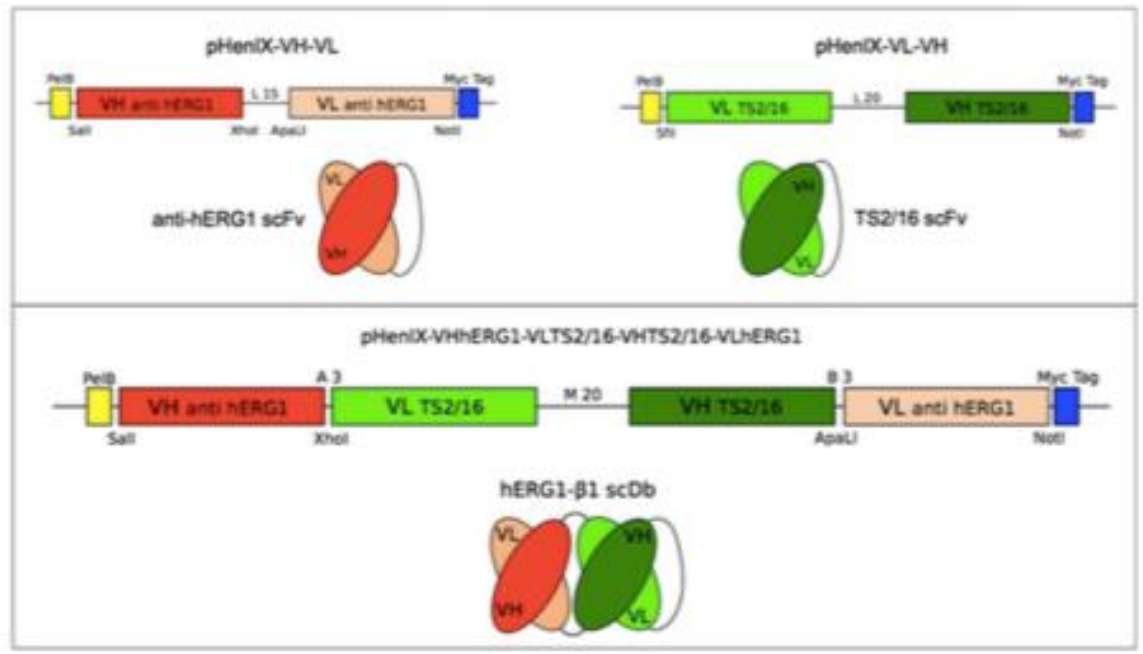


Future …. Blockade of the hERG1/β1 integrin complex



hERG1/integrin-based immunotherapy: bifunctional antibodies

MCK THERAPEUTICS



Patent(deposit n. 102017000083637)

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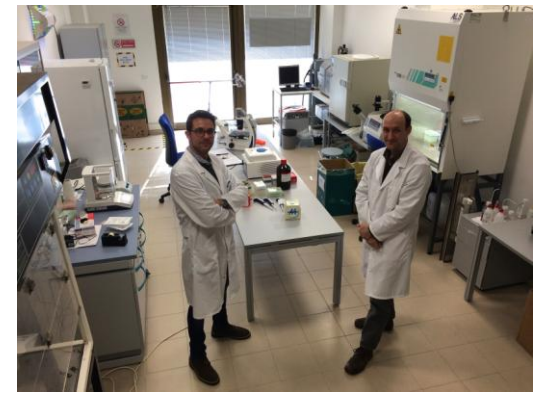

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Con la ricerca, contro il cancro

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