

## SUPPLEMENTARY INFORMATION

**Title: HERG1A potassium channel is the predominant isoform in head and neck squamous cell carcinomas: evidence for regulation by epigenetic mechanisms**

### **AUTHORS:**

Sofía Tirados Menéndez<sup>1‡</sup>, María Ángeles Villaronga<sup>1‡</sup>, Juan P. Rodrigo<sup>1</sup>, Saúl Álvarez-Teijeiro<sup>1</sup>, Rocío G. Urduñigo<sup>2</sup>, Mario F. Fraga<sup>2,3</sup>, Carlos Suárez<sup>1</sup>, Juana María García-Pedrero<sup>1</sup>.

<sup>1</sup> Servicio de Otorrinolaringología, Hospital Universitario Central de Asturias and Instituto Universitario de Oncología del Principado de Asturias, Oviedo, Spain.

<sup>2</sup> Unidad de Epigenética del Cáncer, Instituto Universitario de Oncología del Principado de Asturias, Universidad de Oviedo, Spain.

<sup>3</sup> Department of Immunology and Oncology, National Center for Biotechnology, CNB-CSIC, Cantoblanco, Madrid E-28049, Spain

‡These authors contributed equally to this work.

### **\* Correspondence:**

Juana María García-Pedrero, PhD

Hospital Universitario Central de Asturias

Laboratorio 2 ORL - IUOPA

33006 Oviedo, Spain

Phone: +34 985107937

E-mail: [juanagp@ficyt.es](mailto:juanagp@ficyt.es)

**Table S1.** Correlation between HERG1A (NT) and HERG1 pan (CT) immunostaining (N=123)

Characteristic	No.	HERG1A protein expression	
		Negative	Positive
<b>HERG1 protein expression</b>			
Negative	15	8	7
Positive	108	3	105

<sup>†</sup>Kendall's Tau = 0.580; *P* < 0.001

**Table S2.** Associations between HERG1A protein expression and clinicopathological findings, site, relapse and disease outcome

Characteristic	No.	HERG1A protein expression (%)	<i>P</i>
<b>- pT classification</b>			
T1-T2	35	29 (83)	0.203 <sup>#</sup>
T3	45	42 (93)	
T4	45	42 (93)	
<b>- pN classification</b>			
N0	40	32 (80)	0.018 <sup>†</sup>
N1-3	85	81 (95)	
<b>- Disease stage</b>			
I-II	15	9 (60)	<0.001 <sup>#</sup>
III	24	22 (92)	
IV	86	82 (95)	
<b>- Pathological grade</b>			
Well differentiated	35	28 (80)	0.048 <sup>#</sup>
Moderately differentiated	56	53 (95)	
Poorly differentiated	34	32 (94)	
<b>- Site</b>			
Pharynx	61	58 (95)	0.128 <sup>†</sup>
Larynx	64	55 (86)	
<b>- Tumour Recurrence‡</b>			
No	35	29 (80)	0.028 <sup>†</sup>
Yes	75	72 (96)	
<b>- Distant Metastasis</b>			
No	60	52 (87)	0.038 <sup>†</sup>
Yes	50	49 (98)	
<b>- Disease status</b>			
Alive without disease	29	24 (83)	0.045 <sup>†</sup>
Dead of index cancer	70	67 (96)	
Died of other causes	14	13 (85)	
<b>Total Cases</b>	125	113 (90)	

<sup>#</sup> Chi-square and <sup>†</sup> Fisher's exact tests.

<sup>‡</sup> 15 patients who died from causes not related to the index tumour before 36 months of follow-up were excluded from the recurrence analysis.

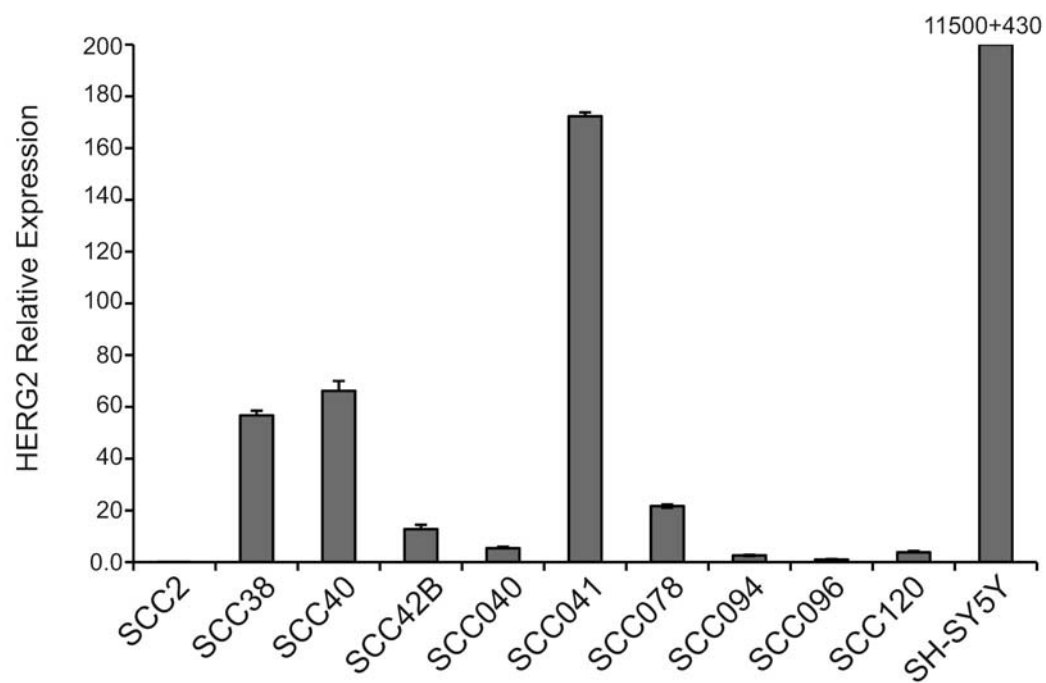
**Table S3.** Primers used in real-time RT-PCR and qChIP \*

GENE	FORWARD (5' -> 3')	REVERSE (5' -> 3')
HERG1A	ACCGTGAGATCATAGCACCTAA	ACAGGACCTGGGTGACCTTCT
HERG1B #	ACAGGACCTGGGTGACCTTCT	ACGTCGGCGCCCAGGGACA
HERG2	GGCCTTGGTTCCTATAGCCT	TCCAGGGGATGTAGAGGTGA
HERG3	CGGACCTCTTGACCATTCT	ATTGTCTTCGCTGGCATGTC
Kv 1.3	GCCAGTTCCCGTGATTGTTT	CGTTTTGAAAGGGGTCTGGG
KCa 3.1	GTTTCGTGGCCAAGCTTTACA	GCATTAACAGCCTGCCTCTC
Kv3.4	CGCTCTTCGAGGATCCCTACT	TGGTGATGGAGACCAGGATGA
HERG2 (KCNH6)*	ACGCTGCTGAGCCCG	CCTGCCCGGAGCCACA
HERG3 (KCNH7)*	GCGGGTGTCTAGAAGTGGTG	CTCTTTGTGGCAGAGCATCC
Kv 1.3 (KCNA3)*	GCAGCCTGACTCCCCGT	GCCCTCATGTTGTACCTCGG
KCa 3.1 (KCNN4)*	TGTTTGGGTGTGGCTCTTTG	GTCCACACACAGGCACAAAT
Kv 3.4 (KCNC4)*	TGCCTCCTCTTCGTCTCCTC	CTCCAACACCCCTCCCGAG
HERG1 (KCNH2)*	CCCGCAGTCCAGTCTTGG	CAGCACTAGGCTTCGGGTG
	<b>Probe</b> 6-FAM-CGCCCCGTGCTCGGCTTGG-TAMRA	

# Primer sequences taken from Pillozi *et al.* [13].

## Figure Legends

**Figure S1.** Analysis of HERG2 and HERG3 mRNA levels by real-time RT-PCR in HNSCC-derived cell lines. Data are expressed as the fold change in HERG2 or HERG3 levels in the HNSCC-derived cell lines normalised to L19 mRNA levels. The graphs represent the means + SD of at least two independent experiments performed in quadruplicate. SH-SY5Y cells served as a positive control.

**A****B**