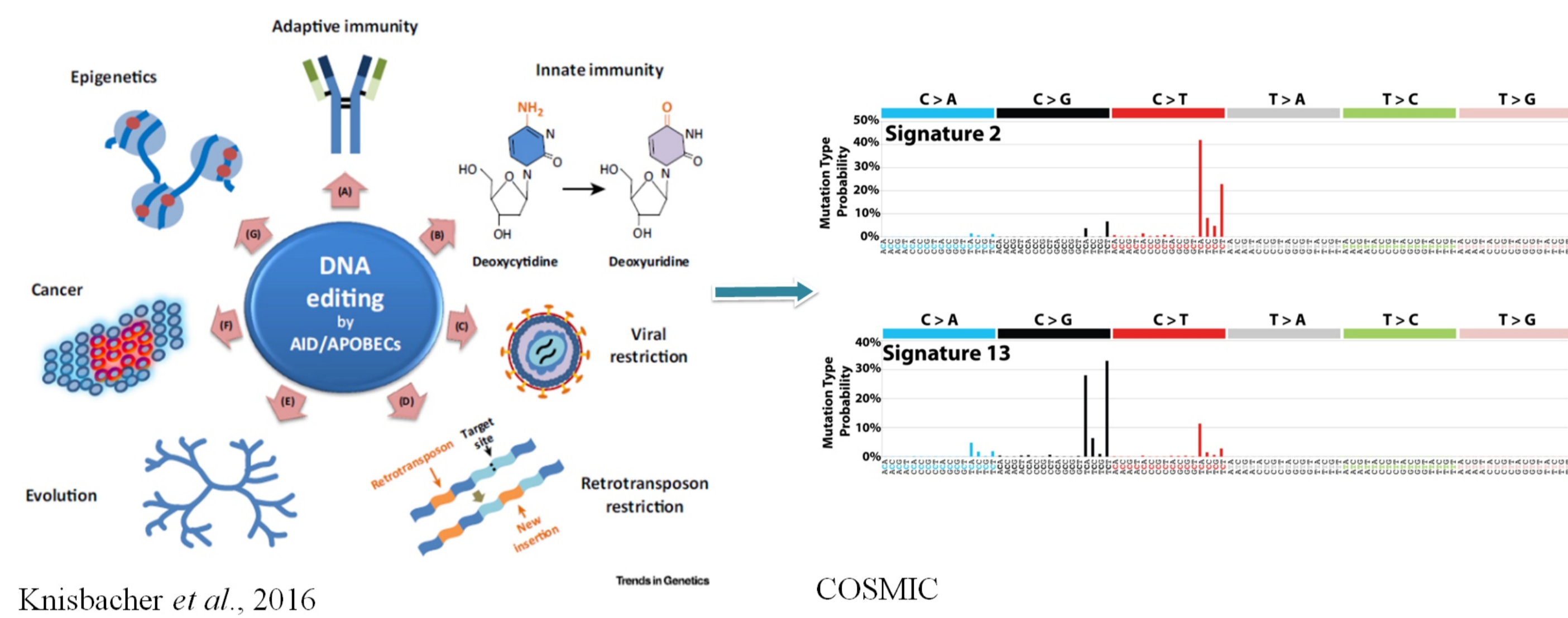


MARINA CHIANELLO NICOLAU FAGUNDES<sup>1</sup>, PAULO THIAGO SANTOS<sup>2</sup>, MARIANA BORONI<sup>3</sup>, PEDRO NICOLAU NETO<sup>1</sup>, TATIANA DE ALMEIDA SIMÃO<sup>4</sup>, LUIS FELIPE RIBEIRO PINTO<sup>1,4</sup>, SHEILA COELHO SOARES LIMA<sup>1</sup>

<sup>1</sup>Programa de Carcinogênese Molecular-CPQ - Instituto Nacional de Câncer; <sup>2</sup>Laboratório de Hanseníase - Instituto Oswaldo Cruz (IOC/Fiocruz); <sup>3</sup>Laboratório de Bioinformática e Biologia Computacional - Instituto Nacional de Câncer; <sup>4</sup>Departamento de Bioquímica - IBRAG - Universidade do Estado do Rio de Janeiro.

## INTRODUCTION

- The AID/APOBEC protein family is involved in DNA editing, with APOBEC3s being an enzymatic source of endogenous mutations in tumors<sup>1</sup>.
- The APOBEC signatures are present in tumor genomes as C-to-T and C-to-G transitions in TpC context<sup>2,5</sup>;
- In esophageal squamous cell carcinoma (ESCC), the presence of APOBEC-associated mutational signature in 50% of samples suggests that the deamination catalyzed by these enzymes may play a pivotal role in its development<sup>6</sup>;
- Head and Neck squamous cell carcinoma (HNSCC) also shows high *APOBEC3B* mRNA levels and displays the putative APOBEC mutational signature<sup>7</sup>;
- Both tumors are frequent among men in Brazil<sup>8,9</sup> and share similarities in morphology and etiologic factors<sup>10,11</sup>.



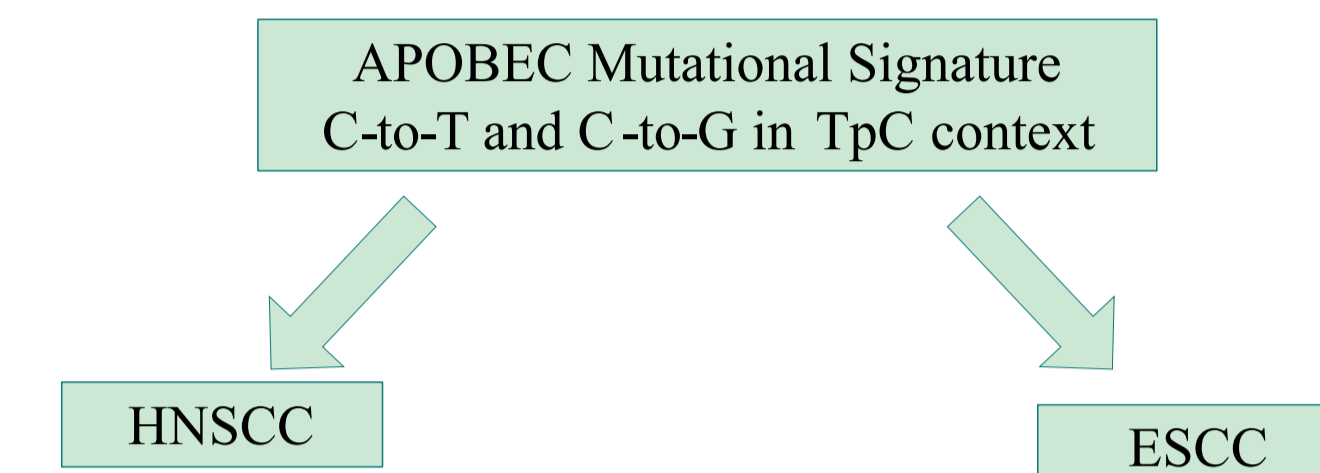
Knisbacher *et al.*, 2016

COSMIC

## OBJECTIVE

To evaluate the contribution of APOBECs to the mutational burst in HNSCC and ESCC.

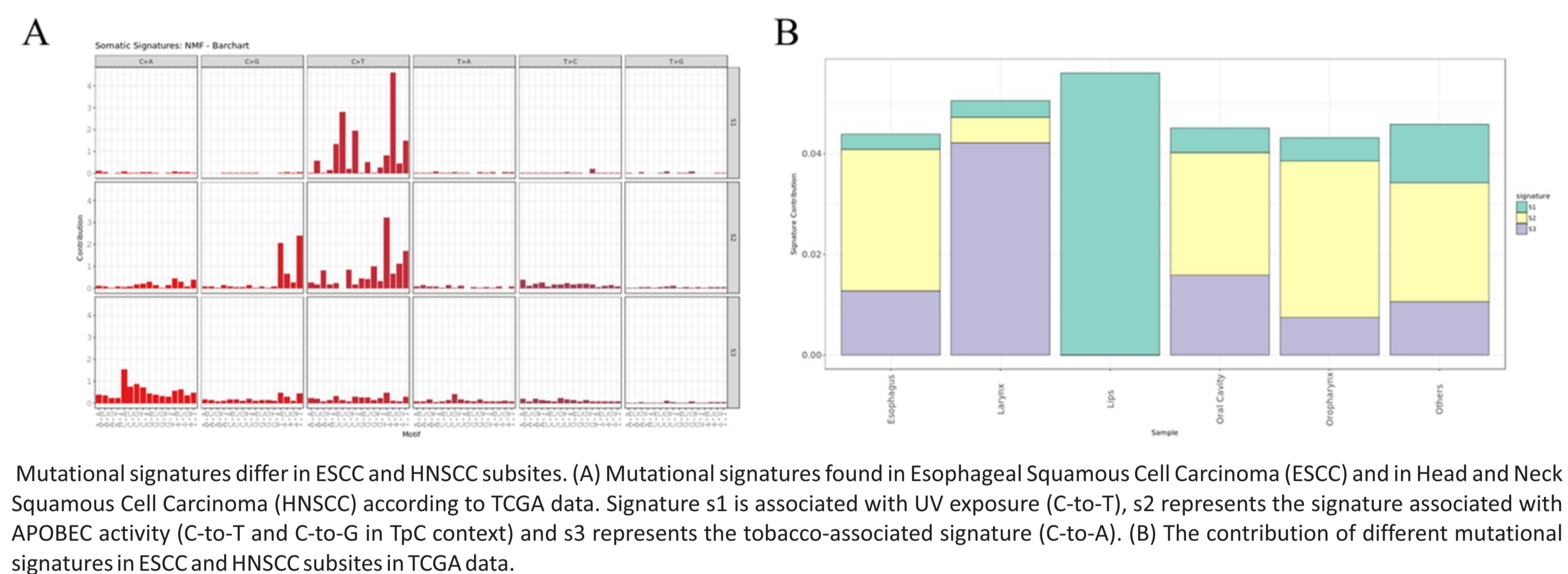
## HYPOTHESIS



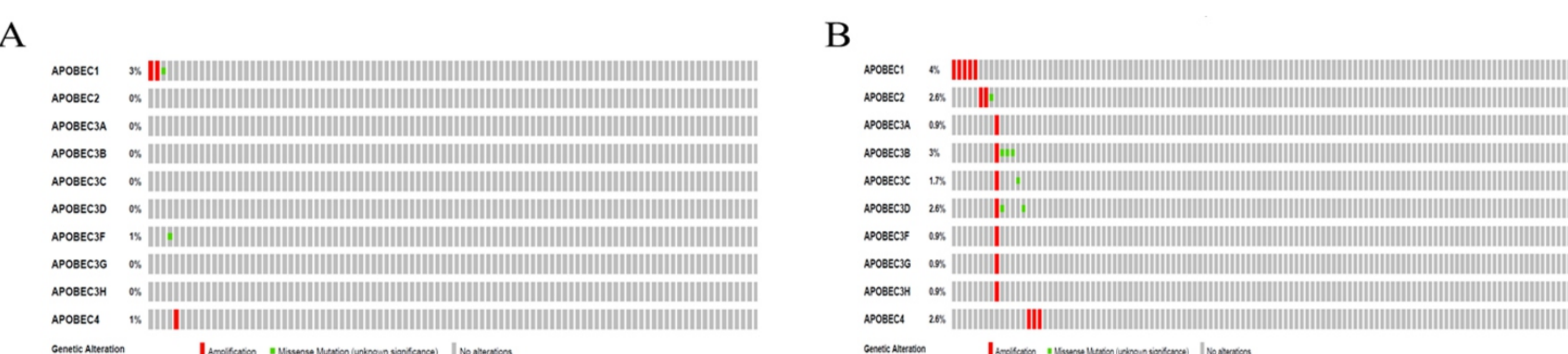
## METHODOLOGY

- Data regarding exome sequencing were obtained from TCGA database;
- Mutational and gene expression profile of *APOBECs* in laryngeal squamous cell carcinoma (LSCC) and ESCC and matched surrounding non-tumor tissue of LSCC and ESCC patients from Instituto Nacional de Câncer, INCA/RJ, were evaluated by RNA-seq;
- Using RT-qPCR, we validated the RNAseq data on *APOBECs* expression in ESCC;
- Bioinformatics analyses were performed using R software;
- All differences were considered statistically significant when  $p < 0.05$ .

## RESULTS



Mutational signatures differ in ESCC and HNSCC subsites. (A) Mutational signatures found in Esophageal Squamous Cell Carcinoma (ESCC) and in Head and Neck Squamous Cell Carcinoma (HNSCC) according to TCGA data. Signature s1 is associated with UV exposure (C-to-T), s2 represents the signature associated with APOBEC activity (C-to-T and C-to-G in TpC context) and s3 represents the tobacco-associated signature (C-to-A). (B) The contribution of different mutational signatures in ESCC and HNSCC subsites in TCGA data.

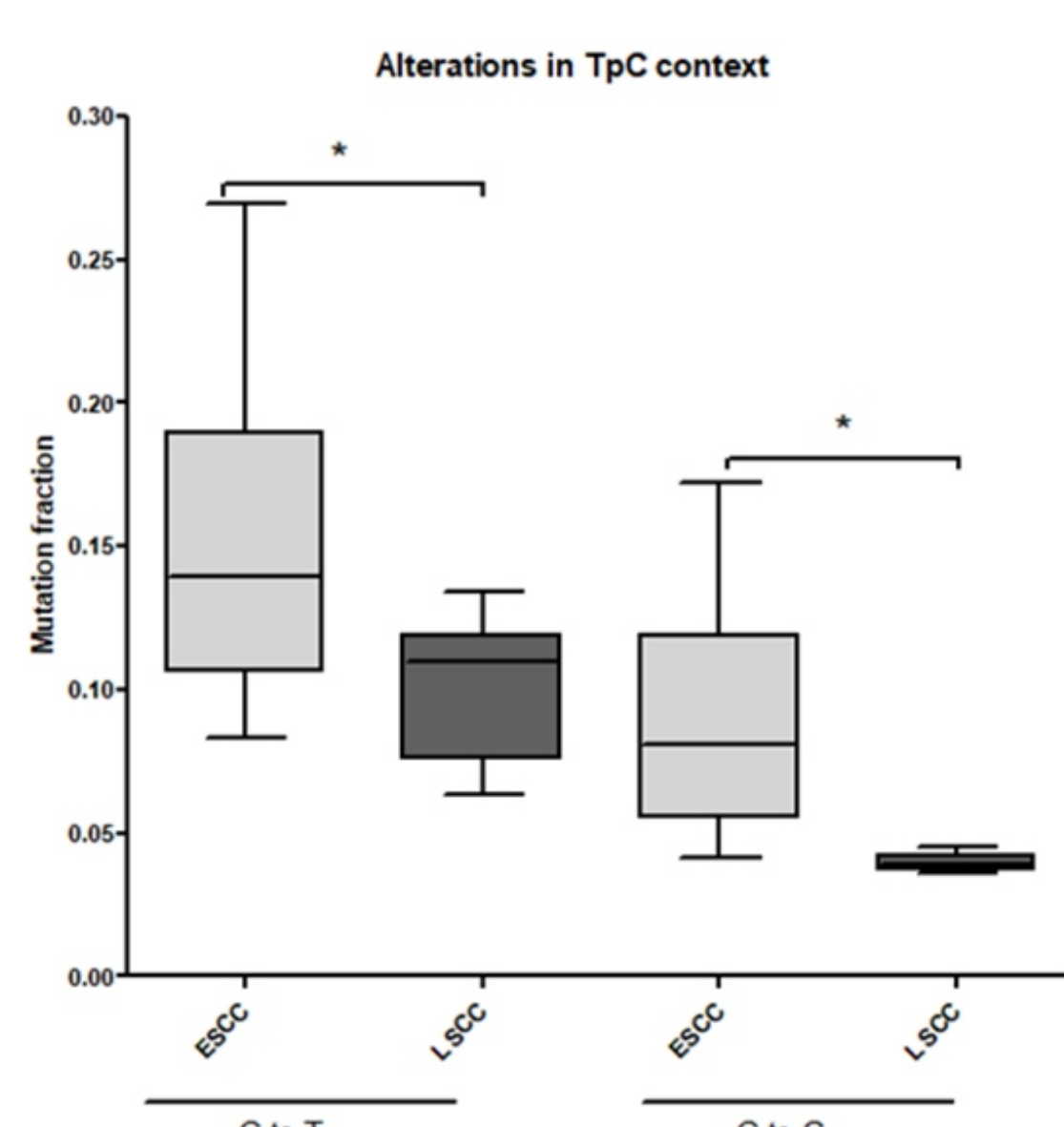


Main *APOBEC* Genetic Alterations found in ESCC patients (A) and in LSCC patients (B) from TCGA database.

Comparison of *APOBECs* expression in ESCC and LSCC patients from INCA, using RNAseq

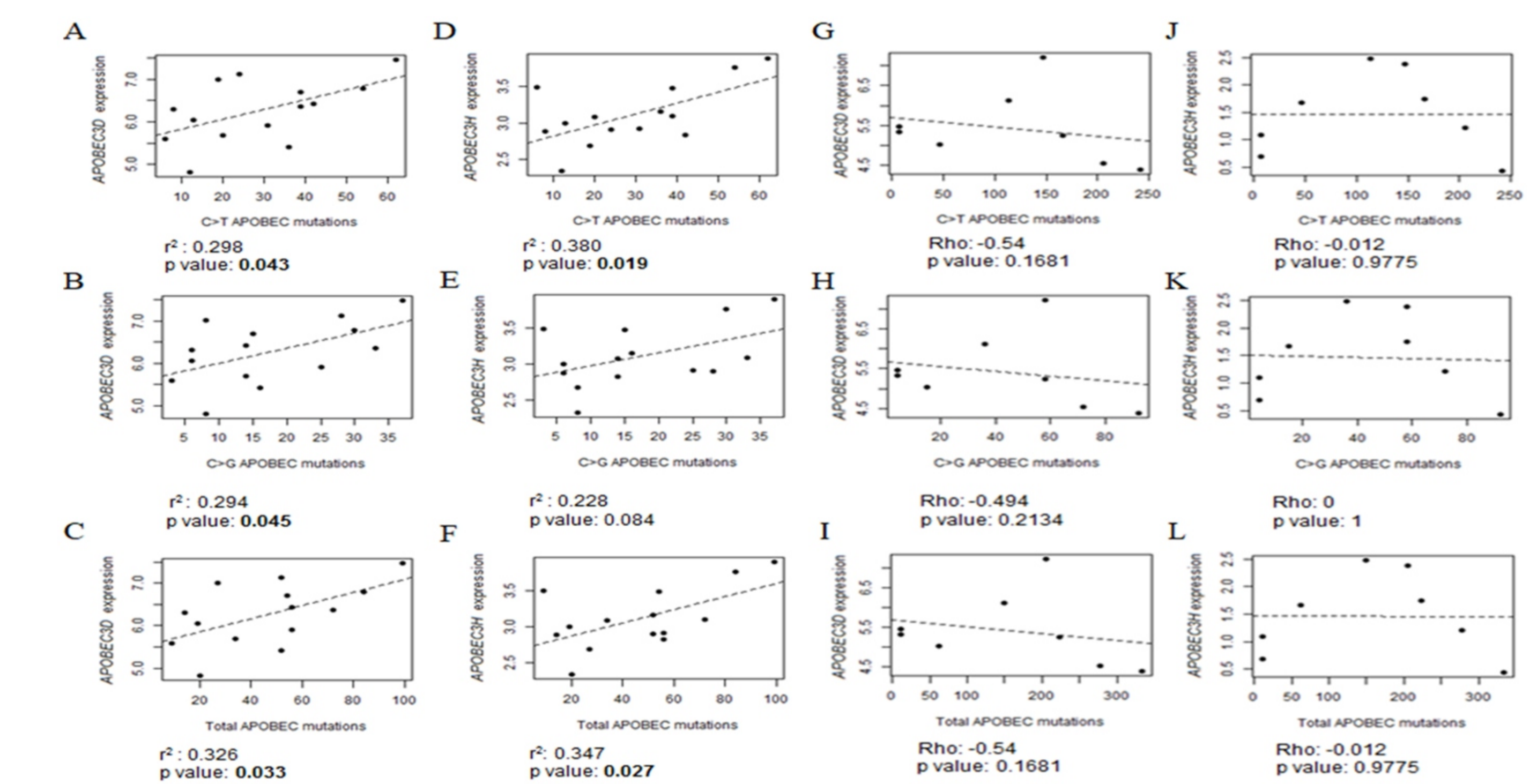
<i>APOBECs</i>	ESCC		LSCC	
	log2FoldChange	p Value	log2FoldChange	p Value
<i>APOBEC1</i>	ND	ND	ND	ND
<i>APOBEC2</i>	-0.94	0.074	-4.44	<b>0.00011</b>
<i>APOBEC3A</i>	3.37	<b>3.54E-10</b>	2.89	<b>0.00418</b>
<i>APOBEC3B</i>	2.95	<b>4.93E-17</b>	3.33	<b>3.8e-10</b>
<i>APOBEC3C</i>	0.34	0.058	0.34	0.31
<i>APOBEC3D</i>	1.81	<b>3.33E-08</b>	2.12	<b>0.00038</b>
<i>APOBEC3F</i>	1.54	<b>1.47E-10</b>	1.35	<b>0.00013</b>
<i>APOBEC3G</i>	1.34	<b>4.69E-09</b>	1.81	<b>0.00003</b>
<i>APOBEC3H</i>	0.99	<b>0.012</b>	0.67	0.607
<i>APOBEC4</i>	ND	ND	1.28	0.44

ESCC: esophageal squamous cell carcinoma; LSCC: laryngeal squamous cell carcinoma; log2FoldChange: log2 ratio of mRNA expression in tumors and non-tumor surrounding mucosa; ND: not detected; Bold p values highlight statistically significant differences ( $p < 0.05$ ).

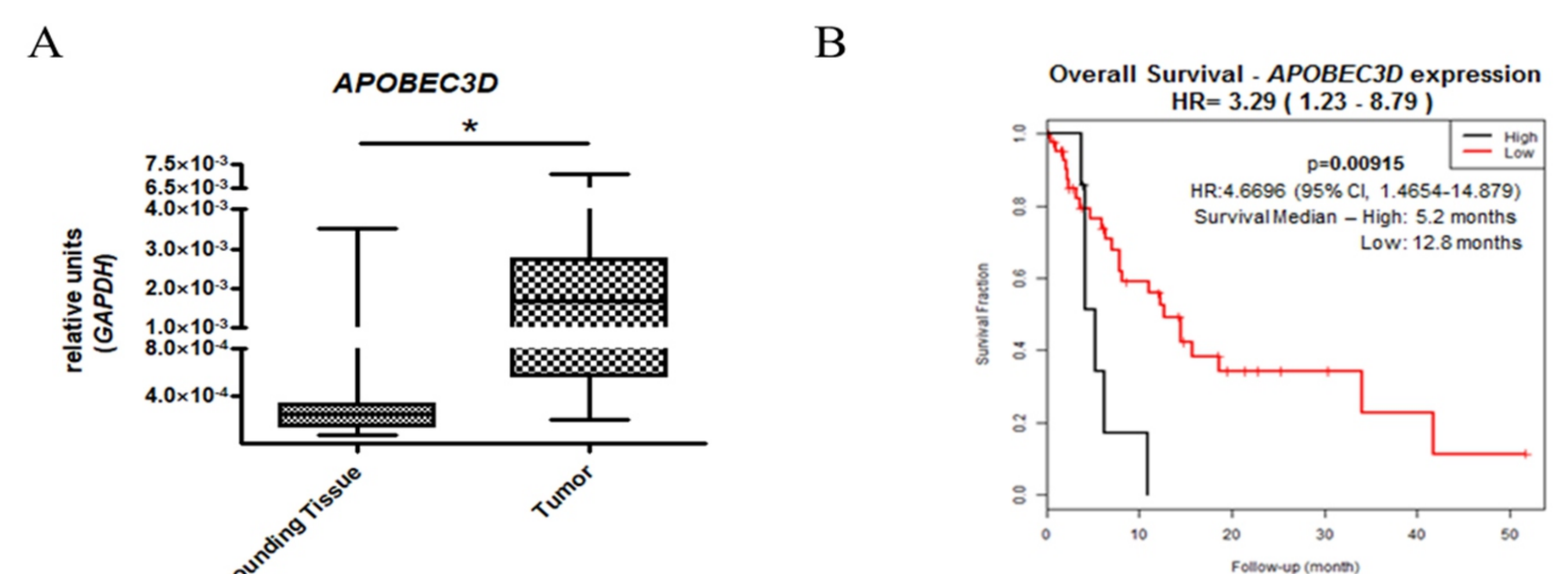


Comparison of the mutation fractions of C-to-T and C-to-G in TpC context in ESCC and LSCC samples using RNAseq, \* $p < 0.05$

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*APOBECs* mRNA expression correlates with C-to-T and C-to-G mutations in TpC context in ESCC patients (A-F) and in LSCC patients (G-L).



The expression profile of *APOBEC3D* (A) and the impact of this expression on overall survival by using multivariate analysis (B) in 49 samples of ESCC patients, \* $p < 0.05$ .

## CONCLUSION

The dysregulation of the APOBEC family of enzymes is a common feature in ESCC and LSCC, but these two tumors show different contributions of APOBEC mutational signature.

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