

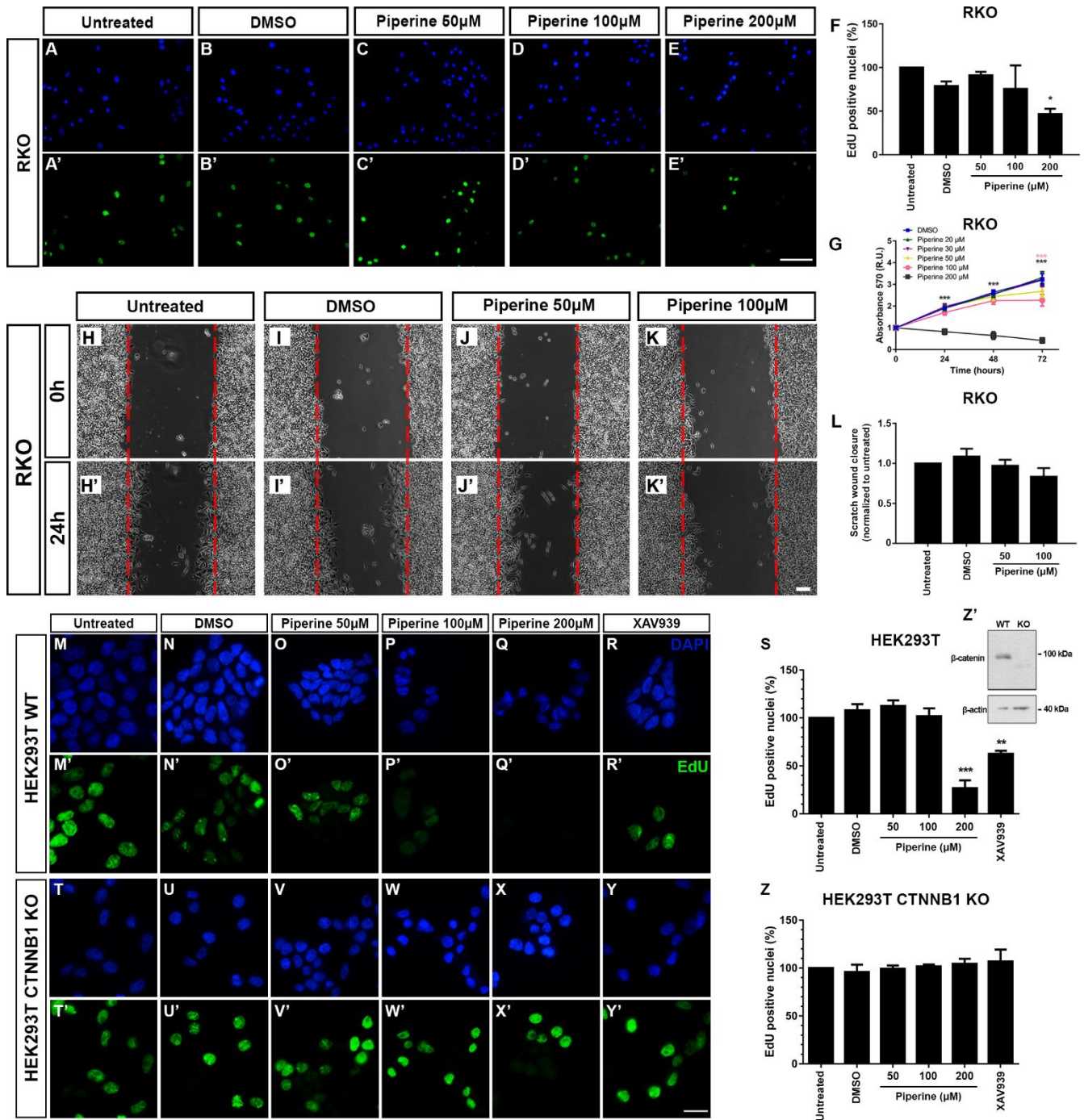
## Supplementary Info File

Title: Piperine suppresses the Wnt/ $\beta$ -catenin pathway and has anti-cancer effects on colorectal cancer cells

Authors: Gracielle C. de Almeida<sup>1</sup>, Luiz F. S. Oliveira<sup>1</sup>, Danilo Predes<sup>1</sup>, Harold H. Fokoue<sup>2,3</sup>, Ricardo M. Kuster<sup>4</sup>, Felipe L. Oliveira<sup>1</sup>, Fabio A. Mendes<sup>1\*</sup>, Jose G. Abreu<sup>1\*</sup>.

Affiliations: <sup>1</sup> Instituto de Ciências Biomédicas, Programa de Biologia Celular e do Desenvolvimento, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil; <sup>2</sup> Instituto Nacional de Ciência e Tecnologia de Fármacos e Medicamentos (INCT-INOVAR; <http://www.inct-inovar.ccs.ufrj.br/>), Laboratório de Avaliação e Síntese de Substâncias Bioativas (LASSBio®, <http://www.lassbio.icb.ufrj.br>) Universidade Federal do Rio de Janeiro, CCS, Cidade Universitária, Rio de Janeiro, RJ, Brazil. <sup>3</sup> Programa de Pós-Graduação em Farmacologia e Química Medicinal, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Avenida Carlos Chagas Filho, 373, Ilha do Fundão, 21941-912, Rio de Janeiro, RJ, Brazil. <sup>4</sup> Instituto de Pesquisas em Produtos Naturais, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

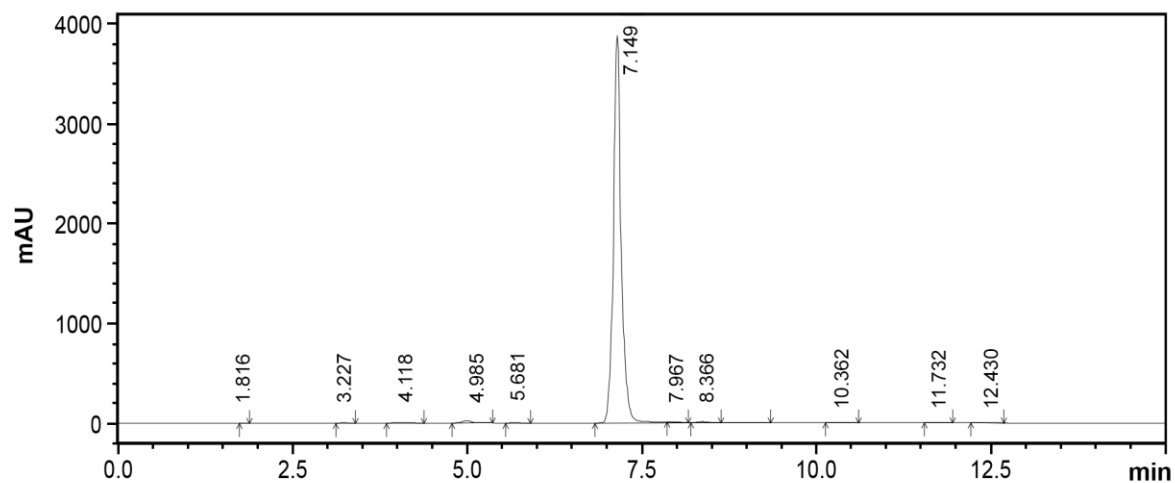
Correspondence to: Fabio A. Mendes, [mendes@icb.ufrj.br](mailto:mendes@icb.ufrj.br) and Jose G. Abreu, [garciajr@icb.ufrj.br](mailto:garciajr@icb.ufrj.br)



**Supplementary Figure S1. Piperine has less effect on a colorectal cell line without overactive Wnt signaling.**

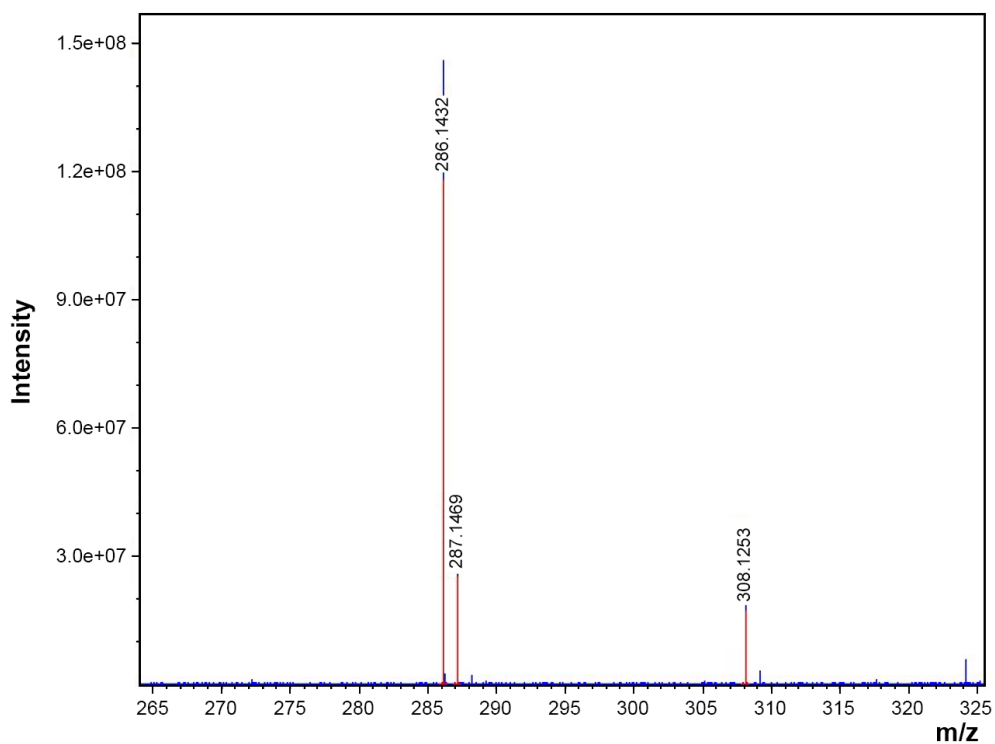
(A-F) Click-iT EdU assay of RKO cells untreated or treated with DMSO, 50, 100 or 200  $\mu$ M piperine. Scale bar = 50  $\mu$ m (F) Graph shows that 200  $\mu$ M piperine has effect on RKO cell lines proliferation. (G) MTT assay shows that RKO cells has reduced viability after piperine treatment. (H-L) Scratch assay of RKO cells untreated or treated with DMSO, 50, 100  $\mu$ M piperine. Scale bar = 140  $\mu$ m. (L) Graph shows that piperine has no effect on RKO cells migration after 24h treatment. (M-S) Click-iT EdU assay of HEK293T cell line untreated or treated with DMSO, 50,

100 or 200  $\mu$ M piperine. (T-Z) Click-iT EdU assay of HEK293T CTNNB1 KO cell line untreated or treated with DMSO, 50, 100 or 200  $\mu$ M piperine. Scale bar = 20  $\mu$ m (Z') Western blot assay showing absence of  $\beta$ -catenin band after CRISPR mediated knockout. Graphs shows mean and SEM. \* $p$ <0.05, \*\* $p$ <0.01, \*\*\* $p$ <0.001.



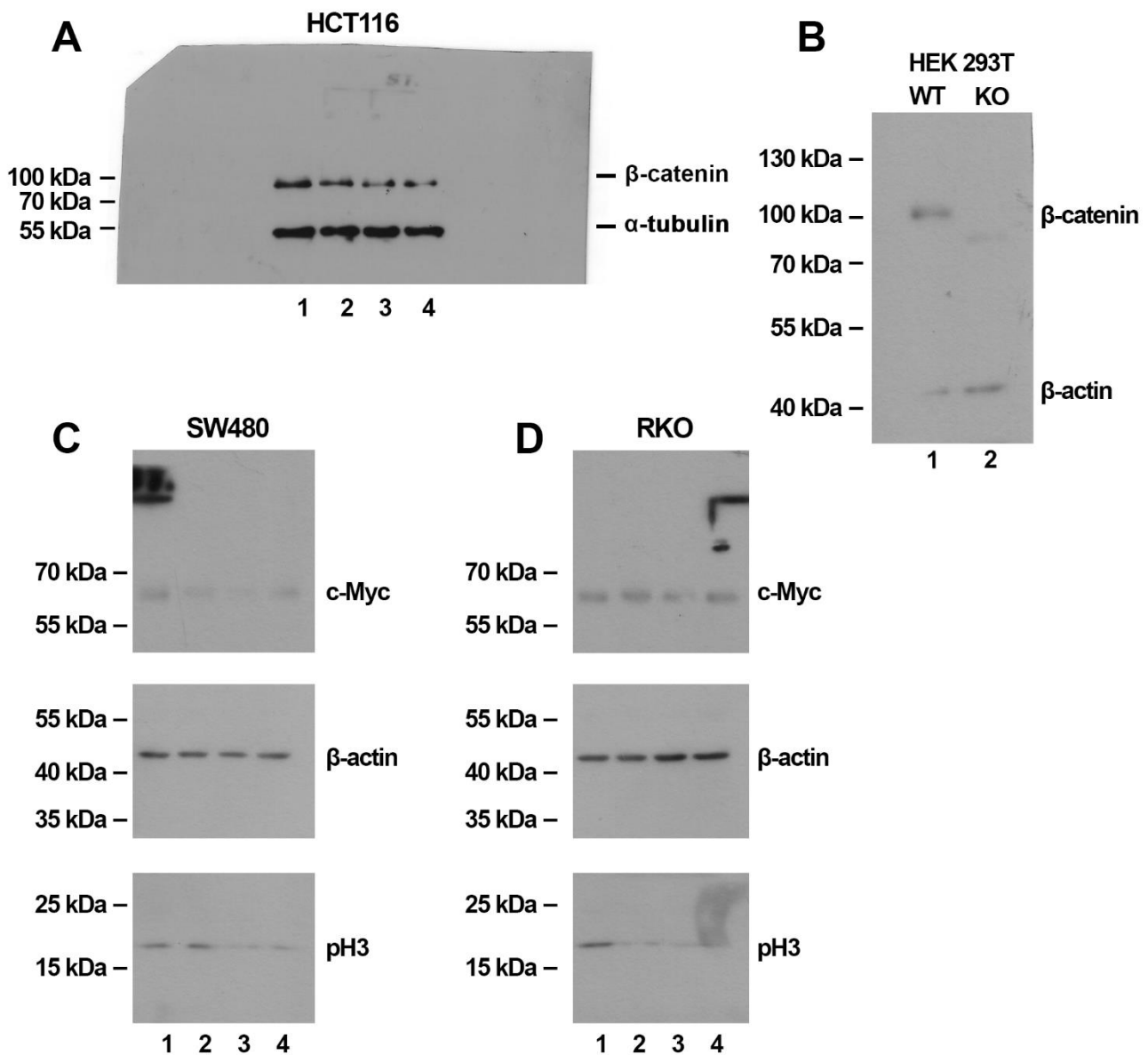
**Supplementary Figure S2: Piperine HPLC analysis.**

Data: UVvis ( $\lambda_{\max}$ ) = 345 nm , Retention time: 7.149 min; purity: 98.79%. (Mobile phase: Acetonitrile: 60% / Water: 40%).



**Supplementary Figure S3: Piperine high resolution electrospray ionization mass spectrometry analysis.**

HRESIMS: m/z calculated for  $C_{17}H_{21}NO_3 + [M+H]^+$  = 286.1438; found = 286.1432. m/z calculated for  $C_{17}H_{21}NNaO_3 + [M+Na]^+$  = 308.1257; found = 308.1253. m/z: Mass to charge ratio.



**Supplementary Figure S4: Raw immunoblot data.**

(A) Raw immunoblot data from HCT116 cell lysate shown in Figure 2. 1: Untreated; 2: DMSO; 3: 50 $\mu$ M piperine; 100 $\mu$ M piperine. (B) Raw immunoblot data from HEK293T cell lysate shown in Figure S1. 1: HEK293T WT, 2: HEK293T CTNNB1 KO. Raw immunoblot data from (C) SW480 or (D) RKO cell lysate shown in Figure 5: 1: DMSO; 2: 50 $\mu$ M piperine; 3: 100 $\mu$ M piperine; 4: 10 $\mu$ M XAV939. Anti- $\beta$ -catenin (1:1000, Sigma, C2206), anti- $\alpha$ -tubulin (1:1000, Sigma, T5168), anti-c-Myc (1:1000, CST, #5605), anti-phospho Histone-H3 (1:1000, CST, #3377), anti- $\beta$ -actin (1:2000, SCBT, sc-47778).

Peak n#	Retention Time (min)	Area	Height	Area (%)	Height (%)
1	1.816	1128	184	0.004	0.005
2	3.227	1607	198	0.005	0.005
3	4.118	19044	2089	0.062	0.053
4	4.985	204042	23941	0.666	0.613
5	5.681	1686	204	0.005	0.005
6	7.149	30283620	3868230	98.788	98.964
7	7.967	26178	2823	0.085	0.072
8	8.366	106734	10196	0.348	0.261
9	10.362	6370	486	0.021	0.012
10	11.732	1769	158	0.006	0.004
11	12.430	2877	205	0.009	0.005
Total		30655056	3908714	100.000	100.000

**Supplementary Table S1: Peak table of piperine HPLC analysis.**