

# Ethaverine and Papaverine target CDK5 and inhibit lung cancer cell proliferation and migration.

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**KEYWORDS:** CDK5; Kinase; small molecule inhibitor; lung cancer; proliferation; migration

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## SUPPLEMENTARY

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### MATERIALS & METHODS

**Protein Kinase Inhibitors:** Roscovitine was purchased from Selleckchem. Papaverine hydrochloride and Ethaverine hydrochloride were purchased from Key Organics. Stock solutions were prepared at 10 mM in DMSO and stocked at -20°C. Inhibitors were freshly diluted in PBS to desired concentration prior to use.

**CDKCONF5 and CDKCONF2 assays :** screening conditions with the CDKCONF5 biosensor were described previously in Peyressatre et al [14]. Fluorescence emission of 10 nM Cy3-labelled CDKCONF5 Biosensor in 50 mM KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>PO<sub>4</sub>, pH 6.5, 500 mM NaCl, was measured at 570 nm following excitation at 550 nm after 5 hours incubation with 10<sup>-5</sup> M final concentration small molecules. ATP, Roscovitine and Dinaciclib were used as negative controls. Relative increase of fluorescence emission was determined relative to the basal fluorescence of Cy3-labelled CDKCONF5 biosensor alone. A molecule was considered a hit when it was not autofluorescent, yet induced changes in Cy3-labelled CDKCONF5 biosensor fluorescence 3 times or greater than the standard deviation of CDKCONF5 biosensor fluorescence alone. CDKCONF2 biosensor was used as described previously [17]

**Protein expression and purification of GST-CDK5.** Recombinant GST-CDK5 was expressed in *E. coli* following induction with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 5 h at 25°C (GST-CDK5) and purified by FPLC as described previously [18], first by affinity chromatography on a GST-Trap HP column (GE Healthcare) followed by size exclusion chromatography on Hiload 16/600 Superdex 75 prepgrade column (GE Healthcare) equilibrated in TBS buffer (50 mM TRIS-HCl, pH 7.4, 150 mM NaCl).

**Fluorescence Titration experiments.** Fluorescence titration assays were performed in 96-well plates using a Clariostar™ spectrofluorimeter (BMG) in 200 μL PBS (Sigma). Fluorescence emission of Cy3-labelled CDKCONF5 or CDKCONF2 biosensors was acquired at 570 nm following excitation at 544 nm either alone, or following incubation with negative controls (ATP, Roscovitine), or small molecules Ethaverine and Papaverine. Data analysis was performed using the GraFit 7 Software (Erathicus Ltd). Experiments were performed in triplicate, and error bars indicate standard deviation from average. Substrat binding experiments were performed like the fluorescence titration assays, in 96-well plates using a Clariostar™ spectrofluorimeter (BMG) in 200 μL PBS (Sigma). Data analysis was

performed using the GraFit 7 Software (Erathicus Ltd). Experiments were performed in triplicate, and error bars indicate standard deviation from average.

**Kinase Activity Assays:** Kinase enzymatic activities were assayed in 384-well plates using the ADP-Glo™ assay kit (Promega, Madison, WI) according to the recommendations of the manufacturer. Recombinant human CDK5/p25 (expressed in bacteria) was assayed in buffer A (10 mM MgCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT, 25 mM Tris-HCl pH 7.5, 50 µg/mL heparin) with 37.2 µM of histone H1 as substrate. In order to determine the half maximal inhibitory concentration (IC<sub>50</sub>), the enzymatic reactions were carried out in a final volume of 6 µl for 30 min at 30°C in the presence of 10µM or 200µM of ATP and in the absence or presence of increasing doses of the tested compounds, (R)-Roscovitine, Ethaverine or Papaverine. The transmitted signal was measured using the Envision (PerkinElmer, Waltham, MA) microplate luminometer and expressed in Relative Light Unit (RLU). Kinase activities are expressed in % of maximal activity, i.e. measured in the absence of inhibitor.

**Cell Culture, cell proliferation and cell extract preparation.** Cell culture media, serum and antibiotics were purchased from Sigma. A549 cells were cultured in RPMI + Glutamax, supplemented with 10% FBS, 100 units/mL (0.168 mM) penicillin (G sodium salt) and 100 µg/mL (0.172 mM) streptomycin at 37°C in an atmosphere containing 5% CO<sub>2</sub>. Cell extracts were prepared in PBS lysis buffer containing PBS (Sigma) 0.2 % NP40, 1mM EDTA, 2 mM PMSF, Complete™ protease inhibitors (Roche), and normalized following spectrophotometric dosage at 280 nm. For cell proliferation/viability assays, 4000 cells in 100 µL medium / well were seeded in 96-well plates. 24 h later, cells were treated in triplicate or quadruplicate with different concentrations of small molecule inhibitors (from 10 nM to 20 µM). Sequential treatments (“\_Seq”) were performed every 24h hours with single drugs or combinations. Stock solutions of drugs were prepared in DMSO and freshly diluted in PBS to the desired concentration prior to use, then added to unsynchronized cells cultured to subconfluency (60-70%), which were then further incubated for 24, 48 or 72 h. Cell proliferation was evaluated by crystal violet staining as described previously [14,17]. Cells were washed with PBS, fixed with 3.7% formaldehyde for 10 min and then incubated with 0.1% crystal violet dye for 30 minutes. After rinsing, crystals were dissolved in 10% acetic acid and viability was determined by measuring absorbance at 595 nm.

**siRNA treatment :** A549 were treated with 100nM or 200nM siRNA from Santa Cruz (sc-29263) complexed with the peptide vector CADY at 1:40 molar ratio as described previously [19]. For experiments where multiple siRNA treatments were combined with Roscovitine/Ethaverine treatments, A549 cells were treated with 100nM siRNA 24h after seeding (siRNA 1), then again another 24h later (siRNA 1 + 2) and again 48h later (siRNA 1+2+3) alone or combined with Roscovitine/ Ethaverine.

**Cellular Extract thermal-shift profiling assays.** Sample preparation was adapted from the protocol described in [20]. A549 cells were cultured for 48h in Petri dishes (100 mm ø) with 5 x 10<sup>5</sup> cells per dish. Cells were washed with PBS (Sigma) and collected by scrapping before centrifugation at 13,300 rpm for 10 min at 4°C. Cell pellets were resuspended in 500 µL PBS supplemented with protease inhibitor cocktail Complete™ (Roche) and treated with 9 µM Roscovitine, 11 µM Ethaverine or 8 µM Papaverine for 1h at 37°C with rotation. Each sample was equally separated into 8 fractions of 50 µL for thermal profiling. Fractions were heated using a PCR (Biometra T Personal Thermocycler) for 3 min at different temperatures (37°C, 41°C, 45°C, 49°C, 55°C, 60°C, 63°C, and 69°C). Fractions were lysed adding 50 µL PBS lysis buffer containing PBS (Sigma) 0.2 % NP40, 1mM EDTA, 2 mM PMSF, Complete™ protease inhibitors (Roche) for 1h at 4°C by gentle rotation. Fractions were then centrifuged at 13,300 rpm for 15 min at 4°C and 30 µl supernatants were collected, and boiled in 30 µL Laemmli buffer for 5 min at 95°C prior to SDS-PAGE and Western blotting.

**Western blotting.** Normalized cell lysates were loaded on a 15% SDS-PAGE gels and transferred to PVDF membranes (Immobilon-P, Merck Millipore) for 1h15 h at 100 V using a liquid BioRad system surrounded by ice. Membranes were first blocked with PBS-Tween 0.1% / BSA 5% for 1 h at room temperature, then incubated at 4°C with the monoclonal mouse anti-CDK5 antibody (Santa-Cruz SC-249, 1:1000); monoclonal rabbit anti-SOD1 (Sigma-Aldrich HPA001401, 1:2500). Western Blots of cell cycle proteins were performed with antibodies against cyclin A (Cell Signalling 4656S), cyclin B (Cell Signalling 12231S), phosphor-cyclin B (Cell Signalling 4131S), phosph-Histone H3 (Cell Signalling 9706S). Western Blots of apoptotic markers were performed with antibodies against cleaved caspase 3, (Cell Signalling 9664S caspase 8 (Cell Signalling 4790S), caspase 9 (Cell Signalling) 9502S and cleaved PARP (Cell Signalling 9541S).

Membranes were washed three times for 10 min with PBS-Tween 0.1% and incubated for 1h at room temperature with mouse or rabbit IgG horseradish peroxidase-conjugated secondary antibody (Amersham NXA31V or NA9340V, 1:10000). Membranes were washed again three times for 10 min with PBS-Tween 0.1% and then were visualized using Amersham ECL prime Western blotting detection reagents (Cytiva, RPN2232). Signals were detected with an Amersham Imager 680 (GE Healthcare) and quantification of band intensity were measured with ImageJ software.

**Flow cytometry studies (FACS).** A549 cells grown in 100mm diameter dishes, treated with different drugs for 48h were trypsinized from dishes and washed with PBS, centrifuged and fixed through resuspension in 300µl cold PBS and 700µl absolute ethanol, then stored at 4°C. Cells were centrifuged and rinsed in PBS, resuspended in 1ml PBS, 1% BSA, then treated with 50µg RNase A for 15 min at 37°C and finally stained with 50µg propidium iodide for 30min at room temperature, then stored at 4°C protected from light. Samples were analyzed on a Novocyte 451160621613 flow cytometer and data were analyzed using the NovoExpress 1.6.0 software.

**Time-lapse live cell imaging experiments.** 35000 A549 cells were seeded into each well of a 6-well plate and grown for 48h prior to treatment with drugs, then placed in an incubation chamber at 37°C containing 5% CO<sub>2</sub>. Phase-contrast microscopy images were automatically acquired with an Olympus IX83 inverted microscope equipped with a 40X LUCPLFLN 0.6NA PH2 objective. For each well, different fields were imaged (6 minimum) every 10 min for 24h and scans were acquired using MetaMorph software with a scMOS ZYLA 4.2MP camera 2048 x 2048 pixel resolution and saved in .TIF format. ImageJ software was used to build stack of images and to analyse the number of cell division manually using Cell Counter plugin.

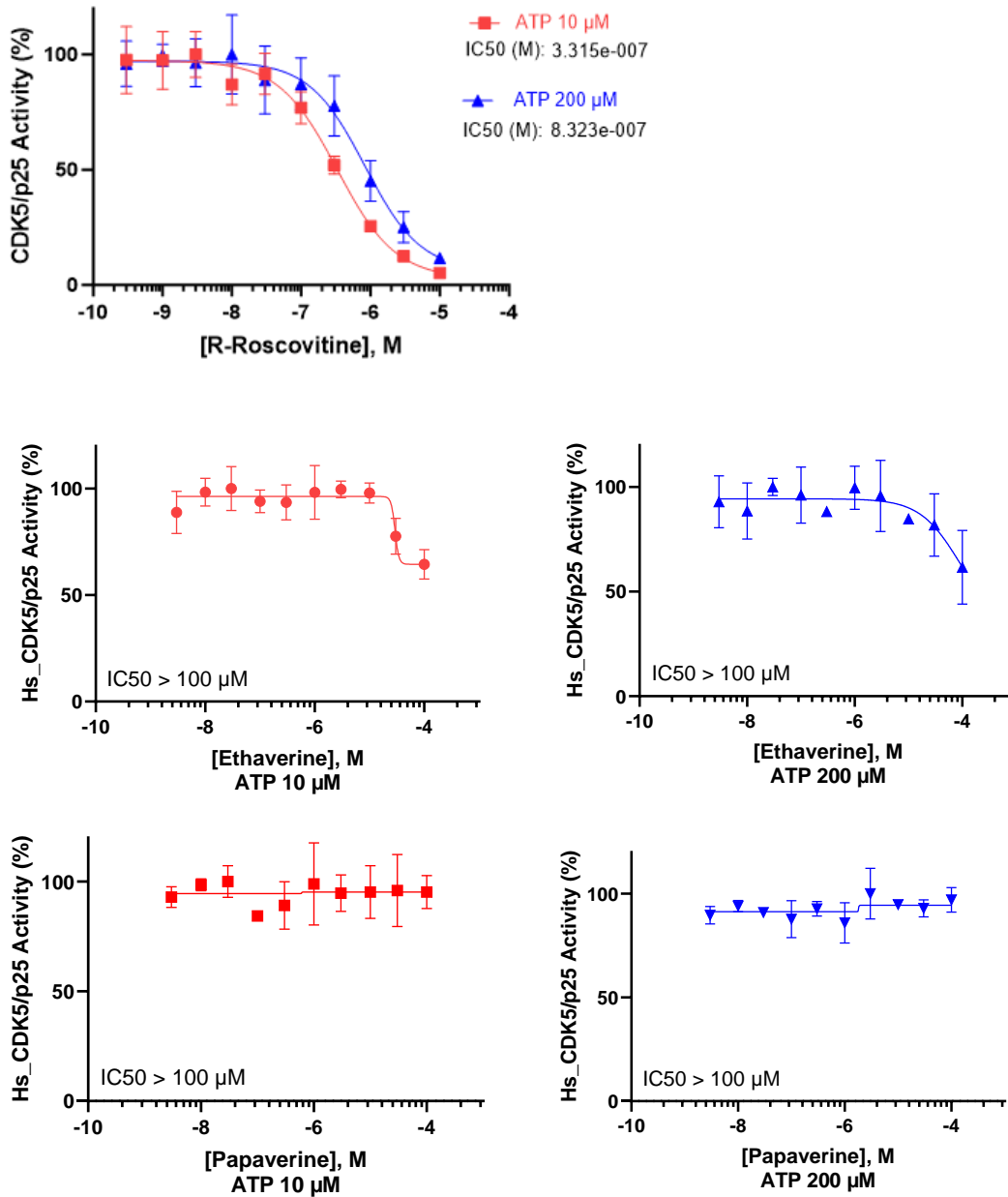
**Cell migration assays.** Cell migration assays were adapted from “scratch” assays described in [21]. 200 000 A549 cells were seeded in each well of 6-well plates and grown for 48h at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. Linear wounds were made using a sterile 200 µL pipette tip scratched across the diameter of the well. Wells were washed 5 times with PBS in order to remove debris resulting from the scratch and plates were incubated in fresh medium containing different drugs at desired concentrations. Phase-contrast microscopy images were automatically acquired with an Olympus IX83 inverted microscope equipped with a 10X UPFLN 0.3NA PH1 objective. For each well, three different fields were imaged every 30 min for 48h and scans were acquired using MetaMorph software with a scMOS ZYLA 4.2MP camera 2048 x 2048 pixel resolution and saved in .TIF format. ImageJ software was used to build stacks of images and analyses were done using Wound healing Tool plugin [22].

### **Statistical analyses**

All data were analyzed and reported as the mean ± SD, except for proliferation assays reported as the mean ± SEM, from at least two or three representative and separate experiments or more whenever indicated. Statistical analyses were performed using PRISM 8 software (GraphPad, San Diego, California, USA). P-values were determined by One-way ANOVA test, after normality tests were passed. All statistical comparisons are reported relative to the Mock. Statistical significance is defined as: \*p < 0.0332, \*\*p < 0.0021, \*\*\*p < 0.002 and \*\*\*\*p < 0.0001.

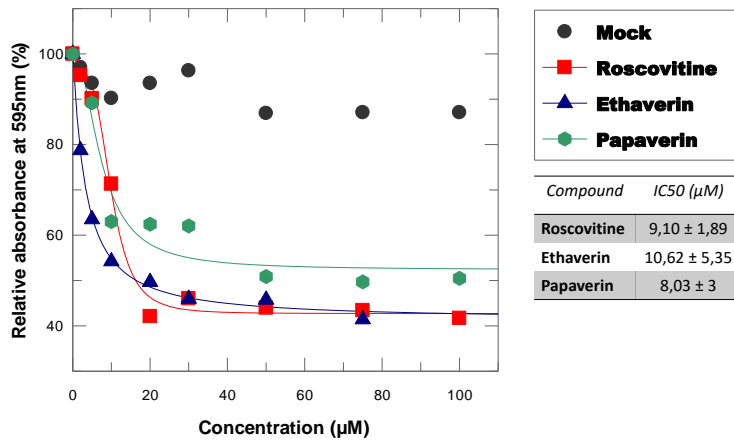
SUPPLEMENTARY FIGURES

**Supplementary Figure S1. CDK5/p25 Kinase Activity Inhibition by Roscovitine, Ethaverine and Papaverine.** Determination of the IC<sub>50</sub> values of (R)-Roscovitine, Ethaverine and Papaverine, against human recombinant CDK5/p25 at two ATP concentrations, 10 and 200 μM. Kinase activities are expressed in percentage of maximal CDK5/p25 kinase activity, i.e., measured in the absence of inhibitor. Mean percentages are reported +/- SD. Kinase assays were performed in quadruplicates. Only the IC<sub>50</sub> values for (R)-Roscovitine were determined using GraphPad Prism.

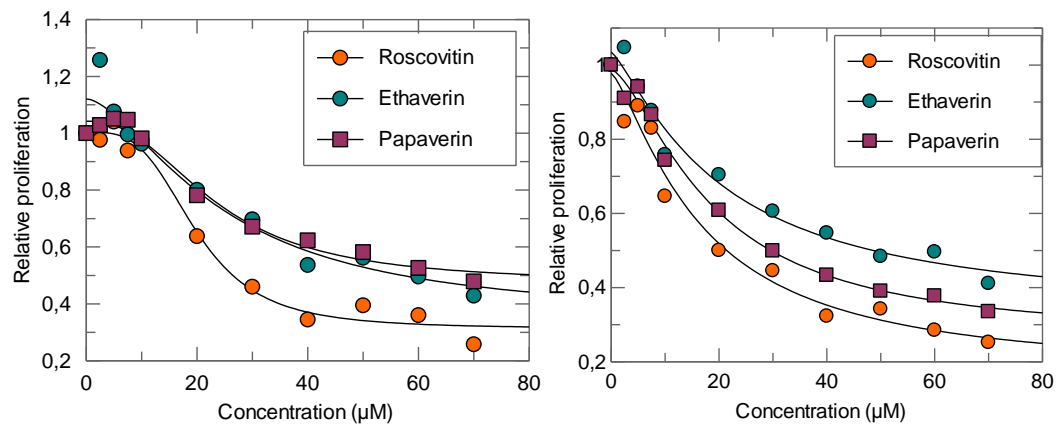


**Supplementary Figure S2. Determination of IC50 values for Roscovitine, Ethaverine and Papaverine in A549 cells**

IC50 values were determined for each compound in the A549 cell line after 24h treatment with drugs (0µM to 100µM) in proliferation assays (Crystal Violet). The graph corresponds to a representative experiment. The table represents the mean of IC50 from 3 independent experiments



IC50 values were determined for each compound in the PC9 cell line (left panel) and H1299 cell line (right panel) after 24h treatment with drugs in proliferation assays (Crystal Violet). The graph shows a representative experiment and the corresponding IC50 values below each graph



IC50 Roscovitine / PC9 : 19.8 +/- 1.6

IC50 Ethaverine / PC9 : 24.7 +/- 7.6

IC50 Papaverine / PC9 : 23.4 +/- 2.4

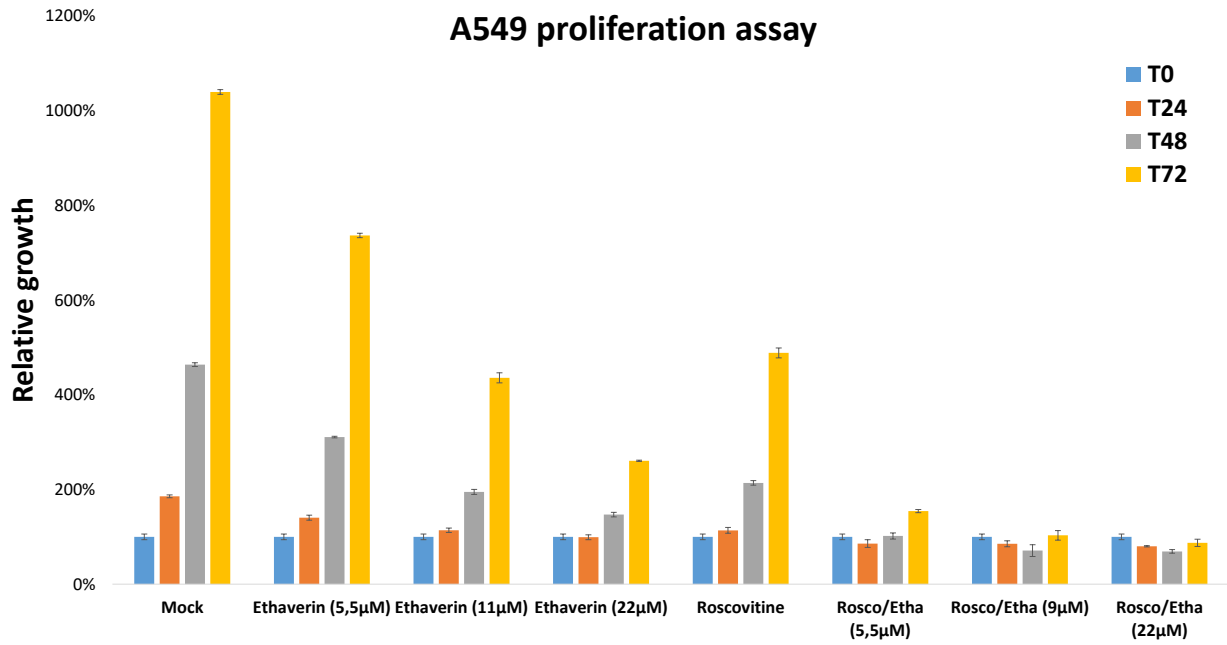
IC50 Roscovitine / H1299 : 16.6 +/- 4.8

IC50 Ethaverine / H1299 : 20.3 +/- 7.3

IC50 Papaverine / H1299 : 18.5 +/- 3

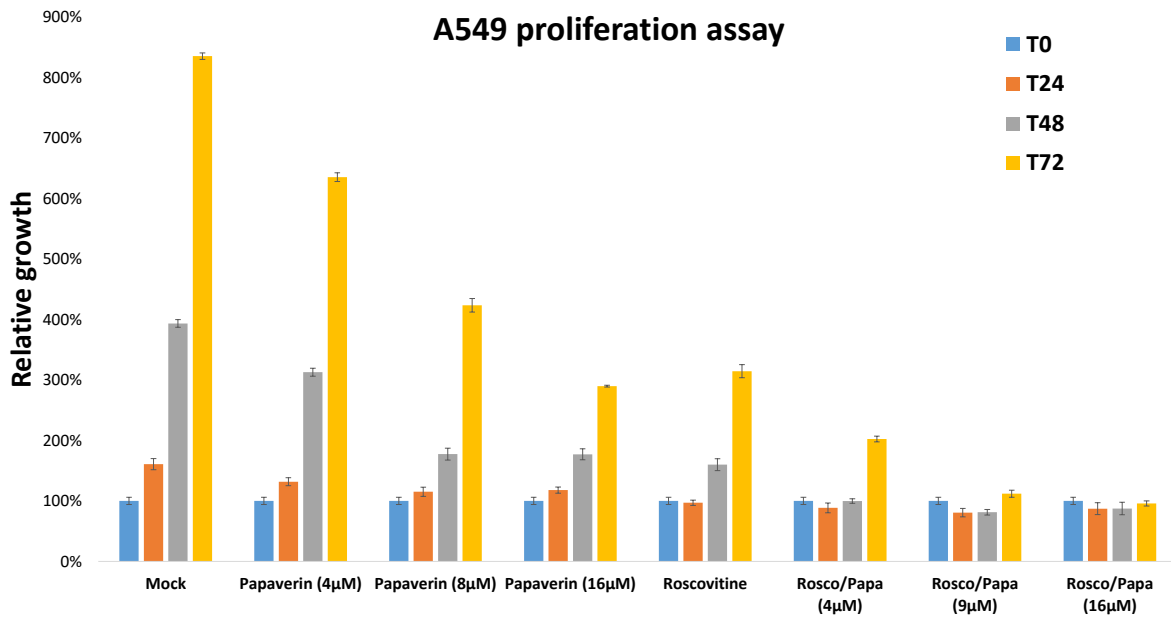
Supplementary Figure S3. A549 proliferation assay following treatment with Ethaverine and Roscovitine/Ethaverine

Proliferation assays (Crystal Violet).

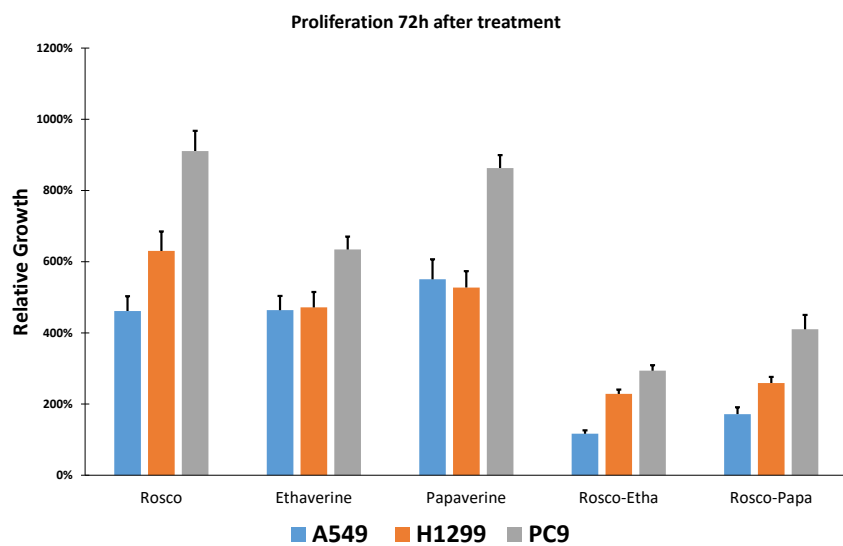
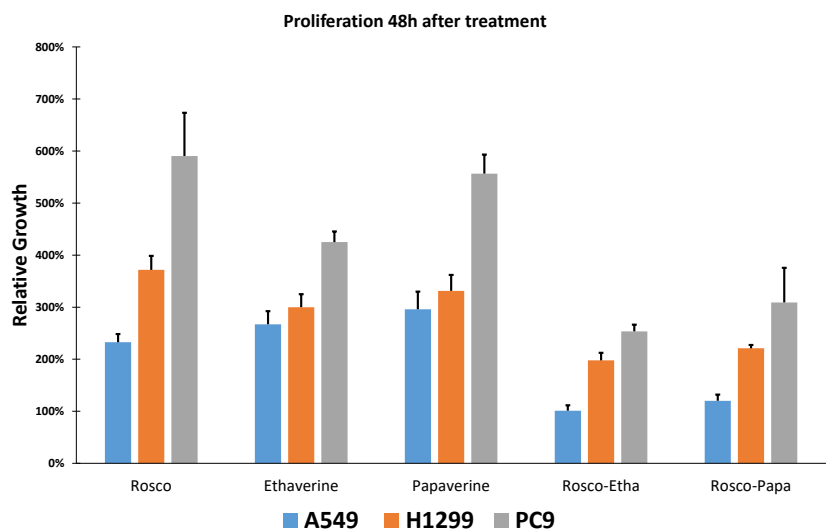
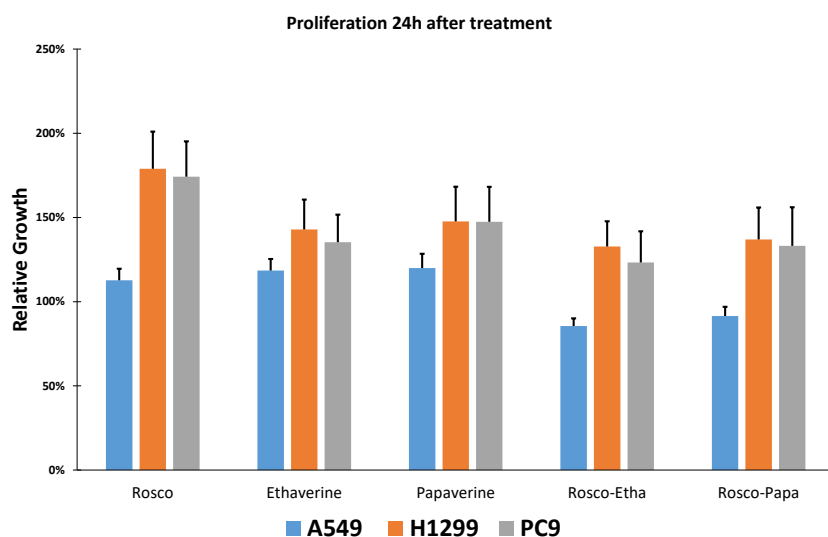


Supplementary Figure S4. A549 proliferation assay following treatment with Papaverine and Roscovitine/Papaverine

Proliferation assays (Crystal Violet).



Supplementary Figure S5. Statistical differences between cell lines following treatment with drugs



## One-Way ANOVA Test

	Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
<b>T24 Rosco</b>	A549 vs. H1299	-66.36	-111.9 to -20.86	Yes	**	0.0031
	A549 vs. PC9	-61.67	-114.3 to -9.026	Yes	*	0.0189
	H1299 vs. PC9	4.683	-54.57 to 63.94	No	ns	0.9794
	Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
<b>T24 Ethaverine</b>	A549 vs. H1299	-24.30	-65.59 to 16.99	No	ns	0.3251
	A549 vs. PC9	-16.74	-63.90 to 30.42	No	ns	0.6561
	H1299 vs. PC9	7.556	-43.38 to 58.49	No	ns	0.9281
	Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
<b>T24 Papaverine</b>	A549 vs. H1299	-27.64	-77.10 to 21.83	No	ns	0.3615
	A549 vs. PC9	-27.37	-83.87 to 29.12	No	ns	0.4615
	H1299 vs. PC9	0.2617	-60.76 to 61.28	No	ns	>0.9999
	Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
<b>T24 Rosco-Ethaverine</b>	A549 vs. H1299	-47.25	-87.11 to -7.381	Yes	*	0.0182
	A549 vs. PC9	-37.70	-82.72 to 7.309	No	ns	0.1124
	H1299 vs. PC9	9.541	-37.20 to 56.29	No	ns	0.8667
	Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
<b>T24 Rosco-Papaverine</b>	A549 vs. H1299	-45.40	-95.17 to 4.360	No	ns	0.0782
	A549 vs. PC9	-41.61	-97.80 to 14.58	No	ns	0.1748
	H1299 vs. PC9	3.797	-54.56 to 62.15	No	ns	0.9855
	Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value

## One-Way ANOVA Test

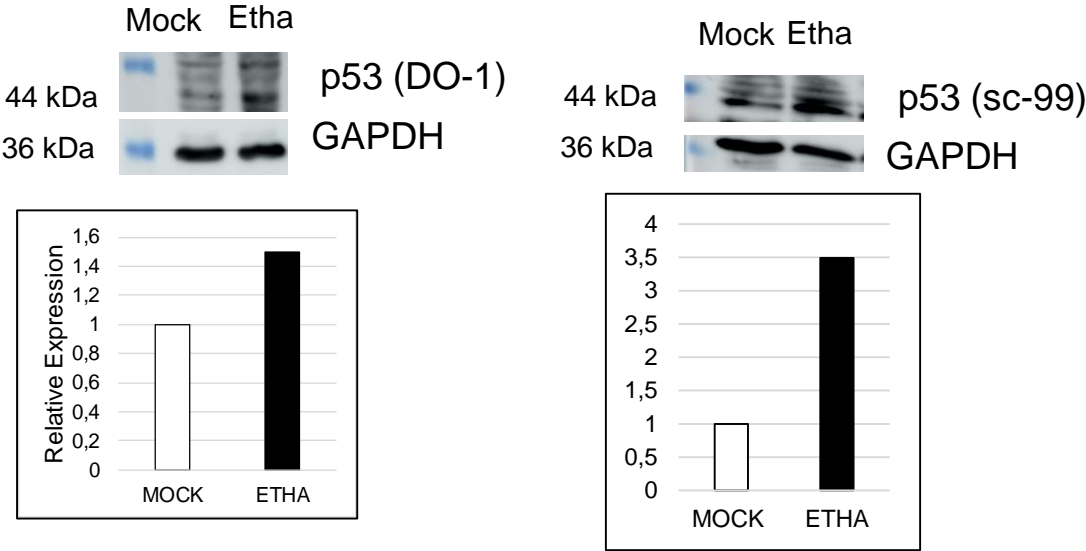
	Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
<b>T48 Rosco</b>	A549 vs. H1299	-138.9	-234.6 to -43.23	Yes	**	0.0033
	A549 vs. PC9	-357.6	-476.5 to -238.8	Yes	****	<0.0001
	H1299 vs. PC9	-218.7	-350.6 to -86.81	Yes	***	0.0009
	Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
<b>T48 Ethaverine</b>	A549 vs. H1299	-32.86	-119.9 to 54.18	No	ns	0.6216
	A549 vs. PC9	-158.0	-257.4 to -58.58	Yes	**	0.0015
	H1299 vs. PC9	-125.1	-232.5 to -17.75	Yes	*	0.0200
	Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
<b>T48 Papaverine</b>	A549 vs. H1299	-35.42	-152.5 to 81.69	No	ns	0.7354
	A549 vs. PC9	-260.6	-394.4 to -126.9	Yes	***	0.0001
	H1299 vs. PC9	-225.2	-369.7 to -80.78	Yes	**	0.0018
	Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
<b>T48 Rosco-Ethaverine</b>	A549 vs. H1299	-96.79	-138.9 to -54.71	Yes	****	<0.0001
	A549 vs. PC9	-152.4	-199.9 to -104.9	Yes	****	<0.0001
	H1299 vs. PC9	-55.61	-105.0 to -6.276	Yes	*	0.0252
	Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
<b>T48 Rosco-Papaverine</b>	A549 vs. H1299	-100.8	-179.6 to -21.94	Yes	*	0.0108
	A549 vs. PC9	-188.8	-283.5 to -94.20	Yes	***	0.0001
	H1299 vs. PC9	-88.03	-185.9 to 9.822	No	ns	0.0831
	Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value

## One-Way ANOVA Test

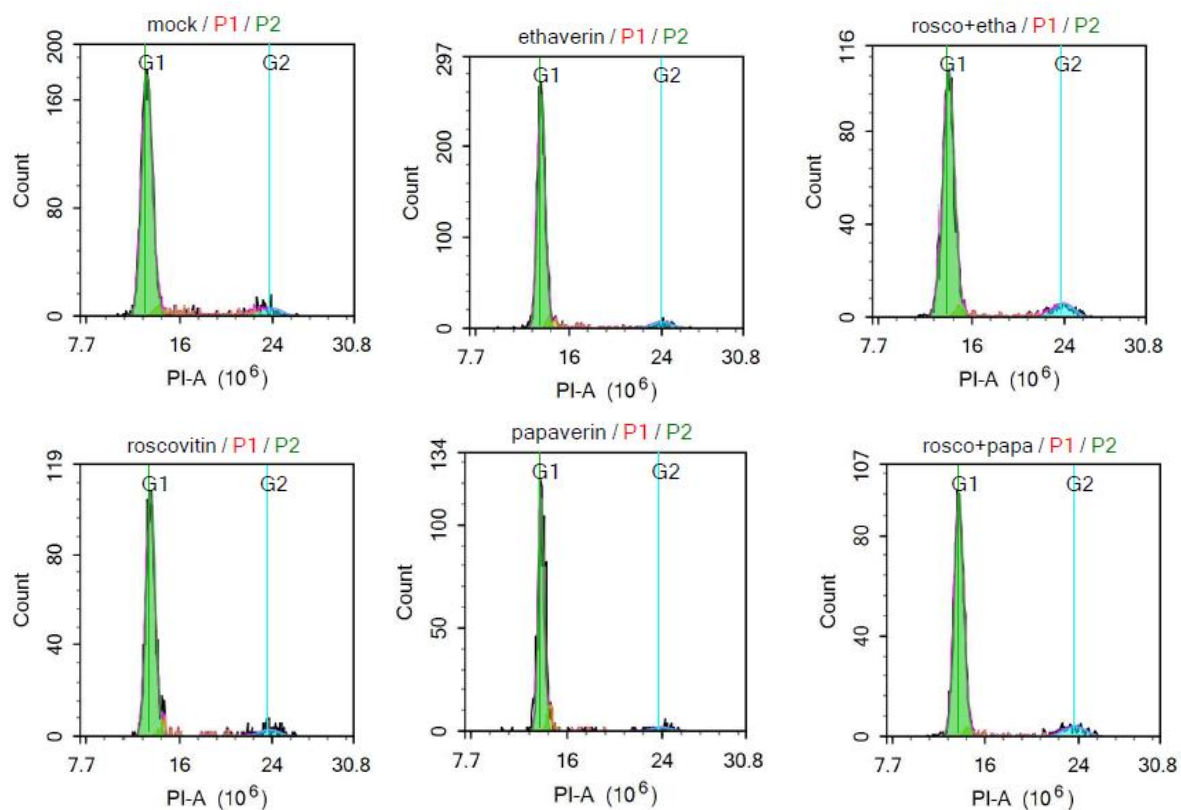
	Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
<b>T72 Rosco</b>	A549 vs. H1299	-168.6	-338.5 to 1.252	No	ns	0.0520
	A549 vs. PC9	-449.3	-645.9 to -252.7	Yes	****	<0.0001
	H1299 vs. PC9	-280.7	-502.0 to -59.45	Yes	*	0.0105
	Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
<b>T72 Ethaverine</b>	A549 vs. H1299	-7.756	-149.1 to 133.6	No	ns	0.9898
	A549 vs. PC9	-170.3	-331.7 to -8.873	Yes	*	0.0372
	H1299 vs. PC9	-162.5	-336.9 to 11.82	No	ns	0.0711
	Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
<b>T72 Papaverine</b>	A549 vs. H1299	23.09	-159.0 to 205.1	No	ns	0.9468
	A549 vs. PC9	-312.7	-520.6 to -104.8	Yes	**	0.0026
	H1299 vs. PC9	-335.8	-560.3 to -111.2	Yes	**	0.0027
	Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
<b>T72 Rosco-Ethaverine</b>	A549 vs. H1299	-112.1	-151.5 to -72.71	Yes	****	<0.0001
	A549 vs. PC9	-177.2	-221.7 to -132.7	Yes	****	<0.0001
	H1299 vs. PC9	-65.08	-111.3 to -18.88	Yes	**	0.0049
	Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
<b>T72 Rosco-Papaverine</b>	A549 vs. H1299	-87.25	-164.3 to -10.16	Yes	*	0.0248
	A549 vs. PC9	-238.4	-327.9 to -148.9	Yes	****	<0.0001
	H1299 vs. PC9	-151.1	-245.7 to -56.55	Yes	**	0.0017
	Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value



Supplementary Figure S6. Western blotting of p53 levels in A549 cells treated with Ethaverine



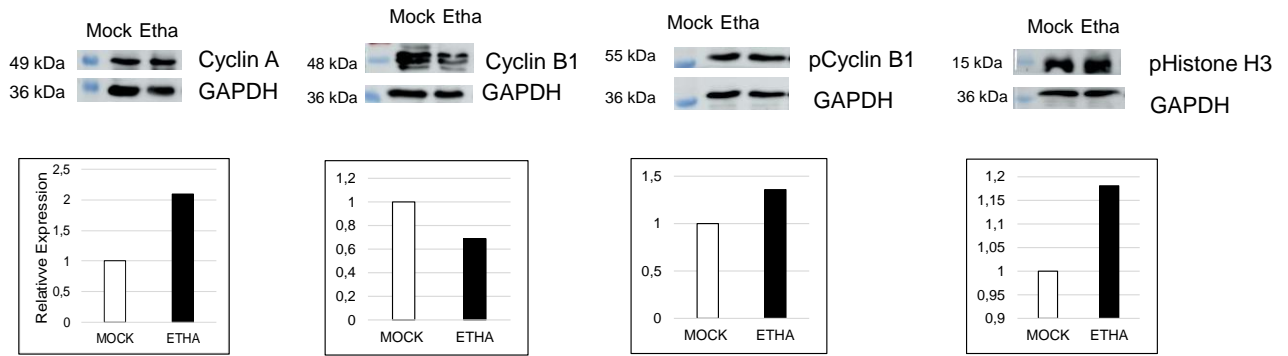
Supplementary Figure S7 - FACS Analysis of A549 cells treated with Roscovitine, Ethaverine and Papaverine



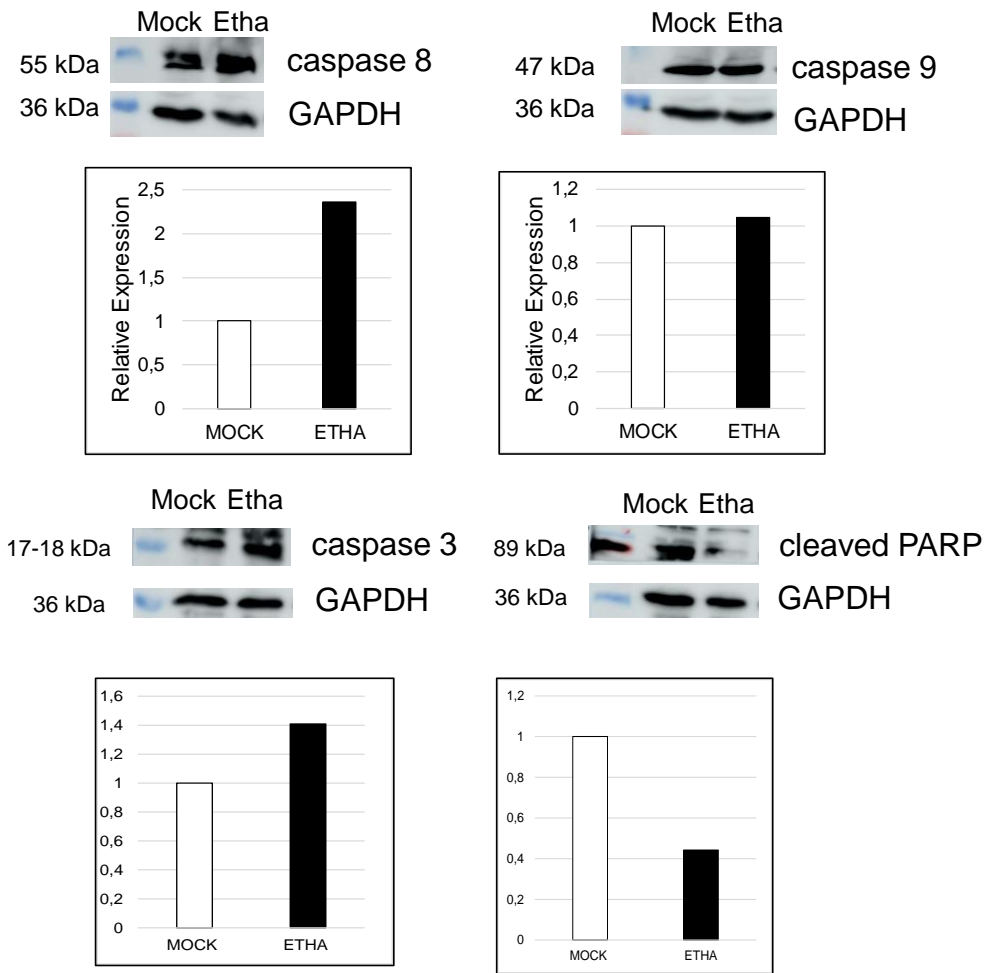
48h Treatment

	% G1	% S	% G2
Mock	84,60	9,77	5,35
Roscovitin	88,07	5,84	6,03
Ethaverin	84,76	7,78	7,04
Papaverin	83,71	9,16	5,72
Rosco + Etha	83,37	7,44	8,73
Rosco + Papa	84,59	6,22	8,49

**Supplementary Figure S8 Western Blotting of cell cycle markers**

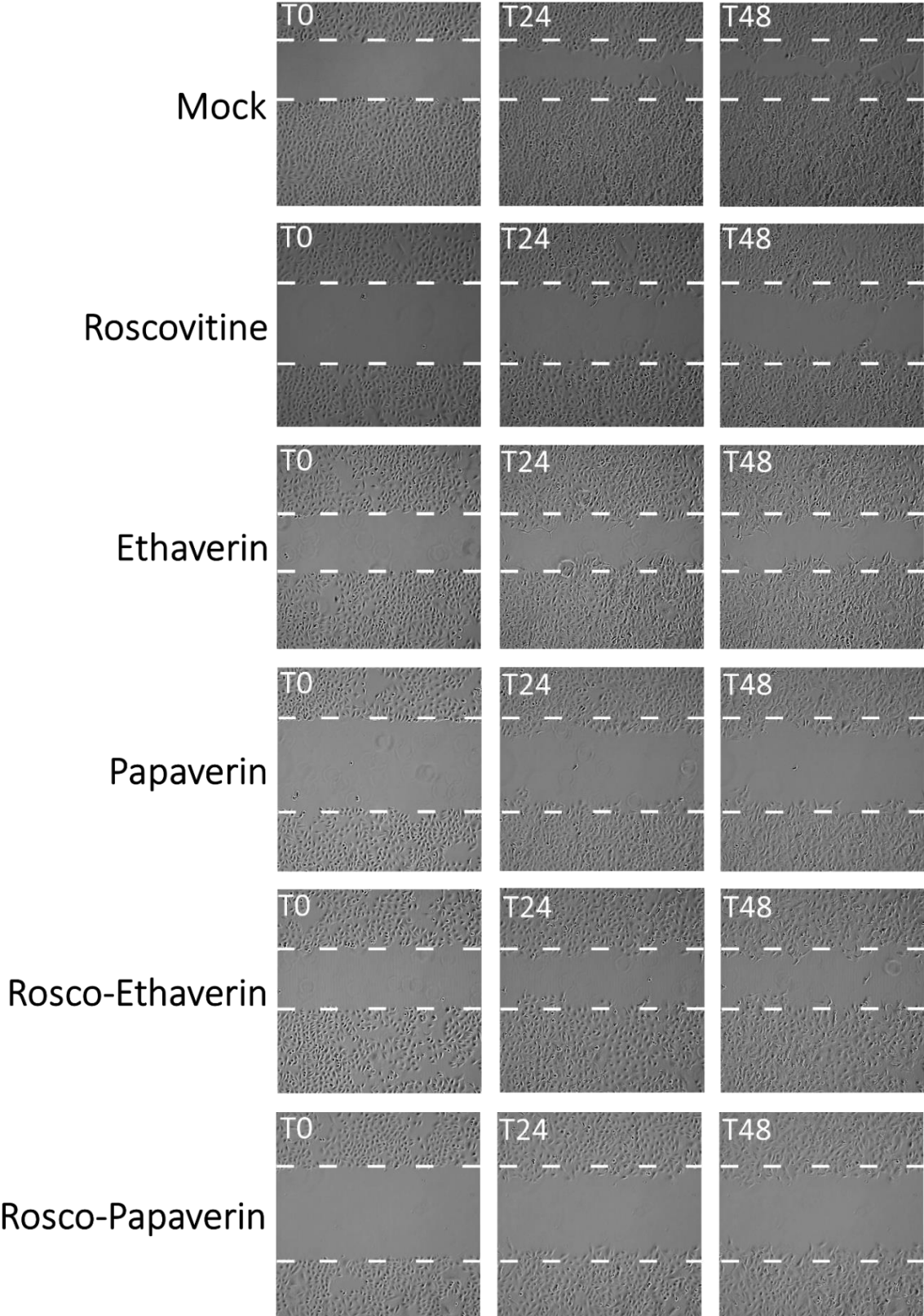


**Supplementary Figure S9. Western Blotting of apoptosis markers**



Supplementary Figure S10. Time-lapse imaging of A549 cell migration

A549 cell migration in a standard scratch assay



SUPPLEMENTARY TABLES

**Supplementary Table S1. Relative proliferation of A549 cells.**

The percentage of cell proliferation relative to the mock are represented as mean  $\pm$  SEM.

<b>Treatments</b>	<b>24h</b>	<b>48h</b>	<b>72h</b>
Mock	165,3 $\pm$ 9,6	441,7 $\pm$ 23,1	931,7 $\pm$ 29,7
Roscovitrine	112,7 $\pm$ 6,9	232,6 $\pm$ 15,8	461,5 $\pm$ 41,5
Roscovitrine_Seq	116,5 $\pm$ 16,3	134 $\pm$ 20	244,6 $\pm$ 29,9
Ethaverin	118,5 $\pm$ 6,9	267,1 $\pm$ 25,3	463,9 $\pm$ 39,7
Ethaverin_Seq	117,2 $\pm$ 13,3	227 $\pm$ 33	371,1 $\pm$ 39,1
Papaverin	120 $\pm$ 8,4	296,1 $\pm$ 33,8	550,5 $\pm$ 56,1
Papaverin_Seq	122,3 $\pm$ 18,7	296,1 $\pm$ 33,8	545,7 $\pm$ 73
Rosco-Etha	85,5 $\pm$ 4,6	101,1 $\pm$ 10,4	116,6 $\pm$ 9,7
Rosco-Etha_Seq	92,8 $\pm$ 6,8	65 $\pm$ 2,8	71,6 $\pm$ 6,2
Rosco-Papaverin	91,5 $\pm$ 5,5	120,2 $\pm$ 11,9	171,7 $\pm$ 19,5
Rosco-Papa_Seq	101,5 $\pm$ 6	72,9 $\pm$ 3,8	95,9 $\pm$ 3

**Supplementary Table S2. Relative proliferation and inhibition of A549 cells.**

The percentage of cell proliferation relative to the mock are represented as mean  $\pm$  SEM.

<b>Treatments</b>	<b>24h Proliferation (%)</b>	<b>48h Proliferation (%)</b>	<b>72h (Proliferation %)</b>
Mock	185,70 $\pm$ 2,95	463,81 $\pm$ 4,03	1039,85 $\pm$ 5,07
Ethaverin (5,5 $\mu$ M)	140,45 $\pm$ 5,33	310,77 $\pm$ 1,75	736,83 $\pm$ 4,62
Ethaverin (11 $\mu$ M)	113,89 $\pm$ 4,66	194,85 $\pm$ 5,19	435,97 $\pm$ 10,70
Ethaverin (22 $\mu$ M)	99,14 $\pm$ 5,24	146,93 $\pm$ 4,70	260,69 $\pm$ 1,29
Roscovitine (9 $\mu$ M)	113,63 $\pm$ 6,09	213,92 $\pm$ 4,81	488,59 $\pm$ 10,34
Rosco (9 $\mu$ M)/Ethaverin (5,5 $\mu$ M)	85,80 $\pm$ 8,02	101,81 $\pm$ 6,45	154,43 $\pm$ 3,32
Rosco (9 $\mu$ M)/Ethaverin (11 $\mu$ M)	85,41 $\pm$ 6,26	70,92 $\pm$ 12,43	103,21 $\pm$ 10,14
Rosco (9 $\mu$ M)/Ethaverin (22 $\mu$ M)	80,08 $\pm$ 1,26	69,15 $\pm$ 3,89	87,19 $\pm$ 7,63
<hr/>			
<b>Treatments</b>	<b>24h Inhibition (%)</b>	<b>48h Inhibition (%)</b>	<b>72h Inhibition (%)</b>
Ethaverin (5,5 $\mu$ M)	24,37 $\pm$ 3,80	33,00 $\pm$ 0,56	29,14 $\pm$ 0,63
Ethaverin (11 $\mu$ M)	38,67 $\pm$ 4,09	57,99 $\pm$ 2,66	58,07 $\pm$ 2,46
Ethaverin (22 $\mu$ M)	46,61 $\pm$ 5,28	68,32 $\pm$ 3,20	74,93 $\pm$ 0,49
Roscovitine (9 $\mu$ M)	38,81 $\pm$ 5,36	53,88 $\pm$ 2,25	53,01 $\pm$ 2,12
Rosco (9 $\mu$ M)/Ethaverin (5,5 $\mu$ M)	53,80 $\pm$ 9,34	78,05 $\pm$ 6,33	85,15 $\pm$ 2,15
Rosco (9 $\mu$ M)/Ethaverin (11 $\mu$ M)	54,00 $\pm$ 7,32	84,71 $\pm$ 17,52	90,07 $\pm$ 9,83
Rosco (9 $\mu$ M)/Ethaverin (22 $\mu$ M)	56,88 $\pm$ 1,57	85,09 $\pm$ 5,63	91,61 $\pm$ 8,76

**Supplementary Table S3. Relative proliferation of A549 cells.**

The percentage of cell proliferation relative to the mock are represented as mean  $\pm$  SEM.

Treatments	24h Prolife- ration (%)	48h Prolife- ration (%)	72h Prolife- ration (%)
Mock	160,79 $\pm$ 9,23	393,52 $\pm$ 6,41	835,59 $\pm$ 5,36
Papaverin (4 $\mu$ M)	131,81 $\pm$ 6,60	312,93 $\pm$ 6,59	635,40 $\pm$ 7,25
Papaverin (8 $\mu$ M)	115,16 $\pm$ 7,52	177,44 $\pm$ 9,74	423,51 $\pm$ 11,22
Papaverin (16 $\mu$ M)	117,95 $\pm$ 5,06	177,06 $\pm$ 9,09	289,80 $\pm$ 1,57
Roscovitine (9 $\mu$ M)	96,98 $\pm$ 4,39	160,03 $\pm$ 9,82	314,59 $\pm$ 10,81
Rosco (9 $\mu$ M)/Papa (4 $\mu$ M)	88,59 $\pm$ 8,13	99,78 $\pm$ 3,76	202,48 $\pm$ 4,77
Rosco (9 $\mu$ M)/Papa (8 $\mu$ M)	80,71 $\pm$ 6,97	81,35 $\pm$ 4,58	111,98 $\pm$ 5,83
Rosco (9 $\mu$ M)/Papa (16 $\mu$ M)	87,19 $\pm$ 9,81	87,45 $\pm$ 10,28	95,84 $\pm$ 4,31