ATR-101 inhibits cholesterol efflux and cortisol secretion by ATP-binding cassette transporters, causing cytotoxic cholesterol accumulation in adrenocortical carcinoma cells

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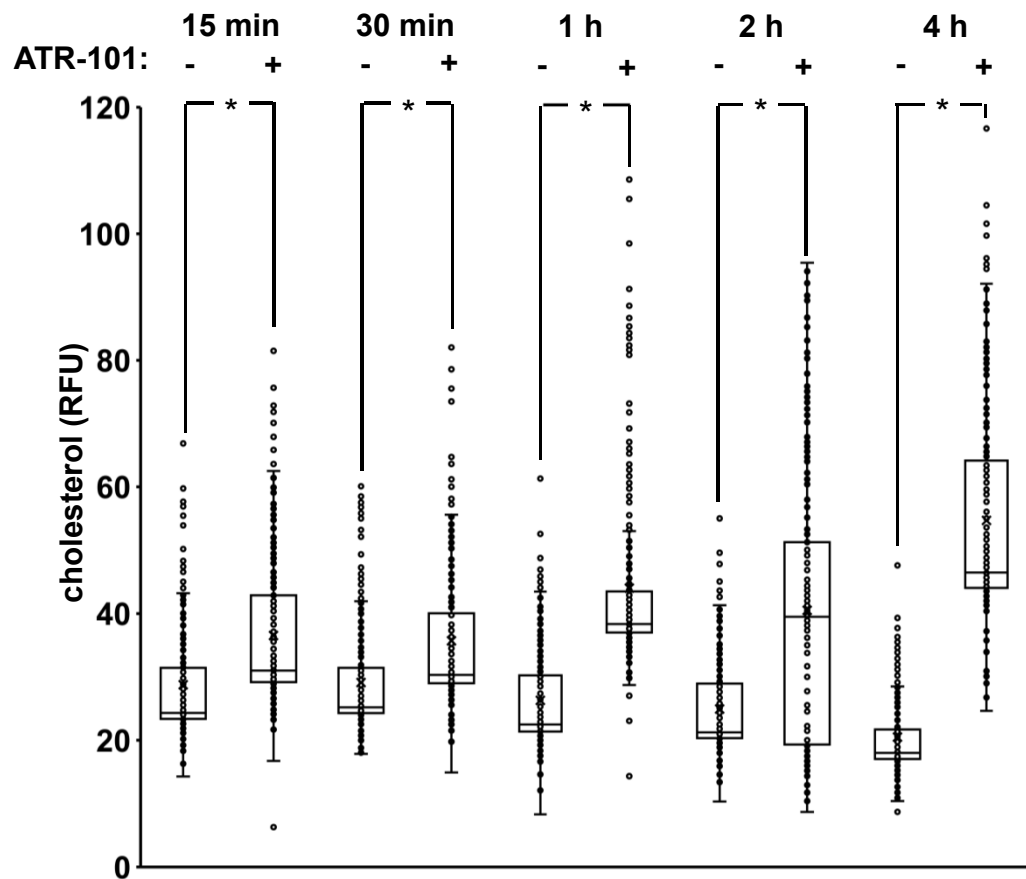
Table of Contents for Supporting Information

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Figure S1

D

E

DMSO

1 μM ATR-101

1 μM PD129337

Fig. S1. Effects of ATR-101 compared with PD129337 on the cholesterol levels, ATP levels, caspase 3/7 activities, and cholesterol esterification, at different times in H295R cells.

A. Quantitation of the time-dependence of the change in cholesterol levels in H295R cells after ATR-101 addition. H295R cells were cultured with DMSO vehicle (-) or with 60 μM ATR-101 (+) for the indicated times. The cells were fixed and stained with filipin III. Filipin III fluorescence was visualized by fluorescence microscopy using a 4X objective. The mean fluorescence intensities and areas of 220 to 310 individual H295R cell clusters (approximately 5500-7800 cells) for each time and condition were quantified using ImageJ v1.50i software. To quantify the areas and intensities of the clusters, manual fluorescence intensity thresholding was used to divide each image into signal comprising the cell clusters and background. The intensity divided by the area was plotted for each cell cluster. The mean, quartiles and ±2SD were plotted at each time after ATR-101 addition. The statistical significance of the differences in fluorescence intensity after ATR-101 addition were evaluated by using unpaired two-tailed Student's *t*-tests (Cells cultured with ATR-101 *versus* corresponding control cells; *$P < 0.05$). The data are representative of two experiments.

ATR-101 caused an increase in cholesterol accumulation within 15 minutes after addition to H295R cells. There was a wide range of filipin III intensities among different cell clusters, but the majority of cell clusters had a narrower range of intensities as indicated by the quartiles shown.

B. Effects of different concentrations of ATR-101 *versus* PD129337 on the ATP levels and on the caspase 3/7 activities in H295R (upper graphs) and BD140C (lower graphs) cells. The cells were cultured with the indicated concentrations of ATR-101 (cl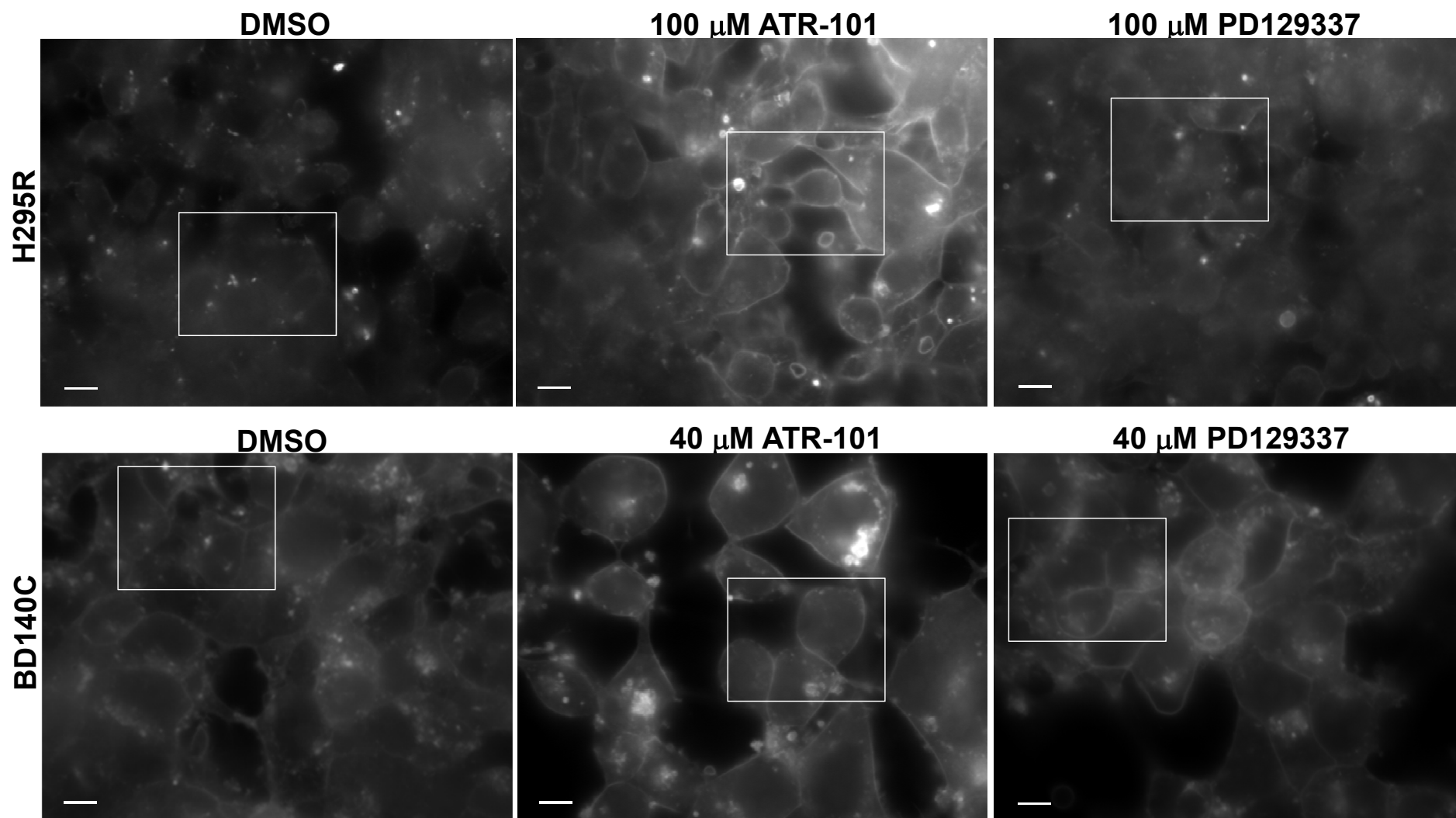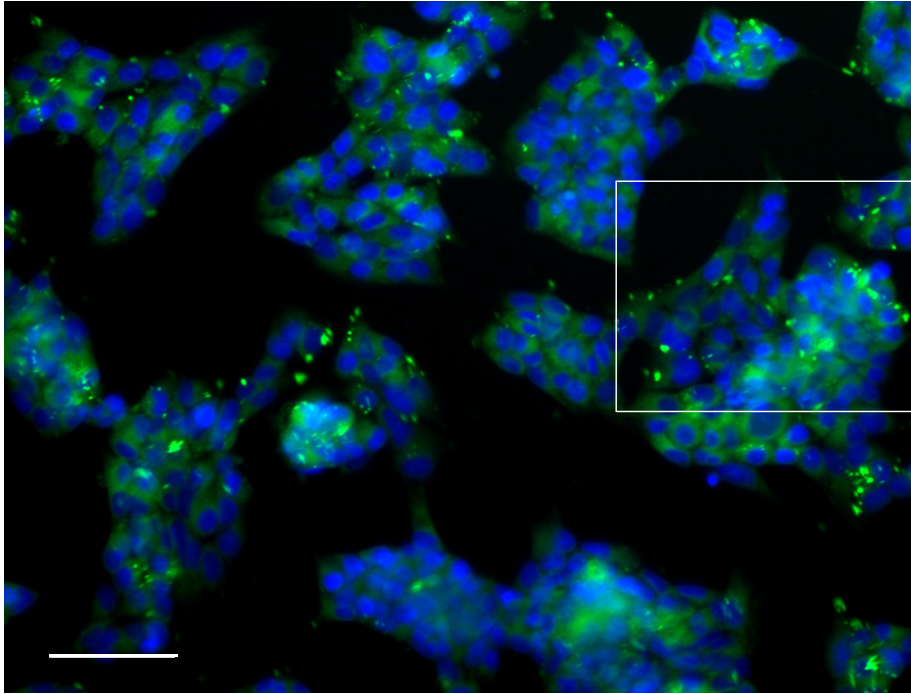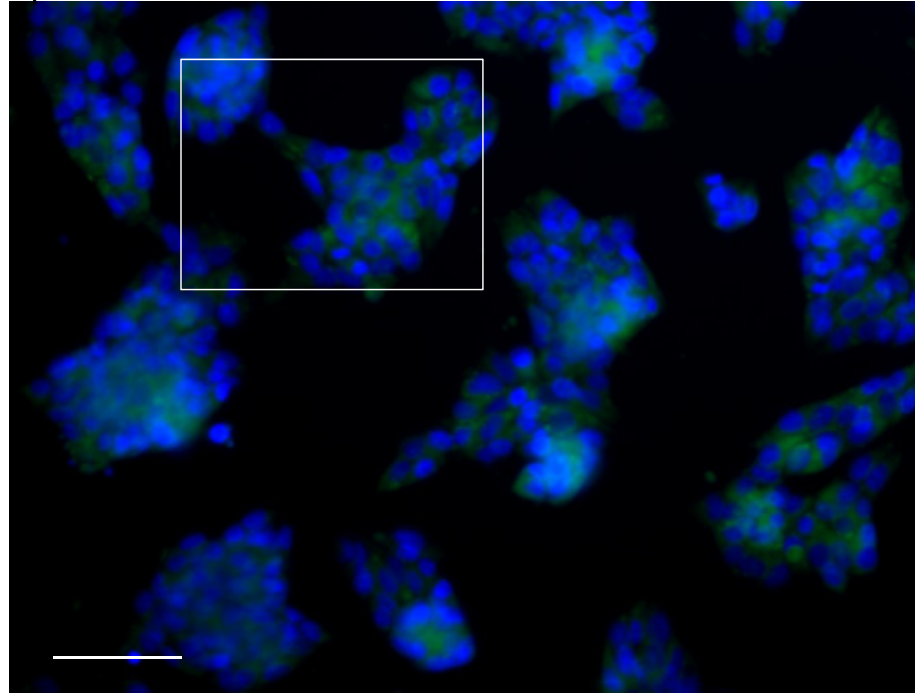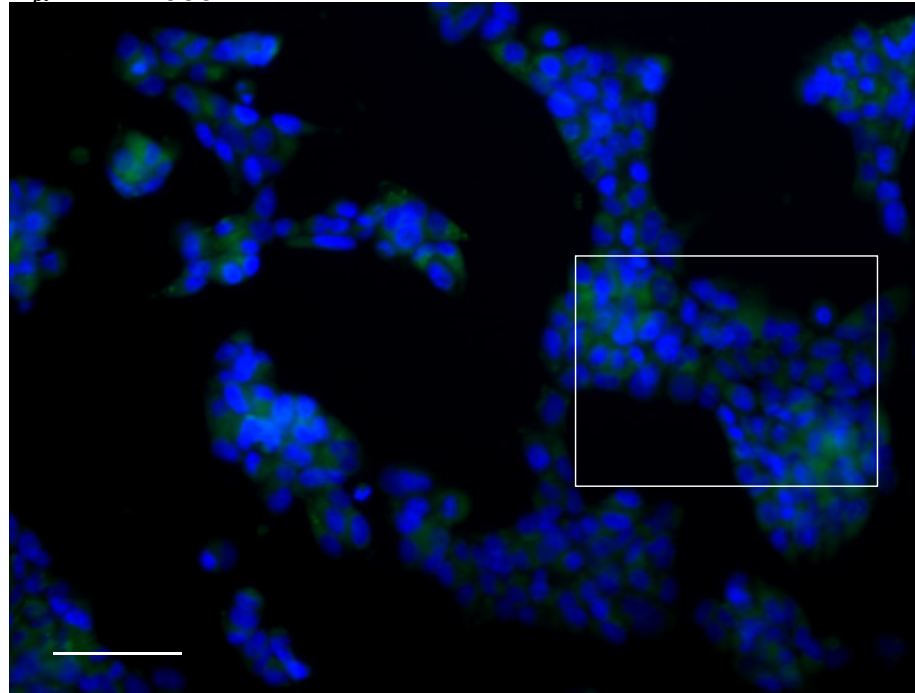osed triangles) or PD129337 (open squares) for 24 h. The ATP levels (left graphs) and the caspase 3/7 activities (right graphs) were measured in cells that were cultured in parallel. The graphs show the means and the standard deviations of six samples from three experiments. The statistical significance of the differences in ATP levels and caspase 3/7 activities in cells that were cultured with each concentration of ATR-101 or PD129337 were evaluated by using two-way analysis of variance followed by Sidak's *post hoc* tests (ATR-101 *vs.* PD129337, *$P < 0.05$).

ATR-101 reduced the ATP levels and increased the caspase 3/7 activities in H295R and BD140C cells. The concentrations of ATR-101 that were required for ATP depletion and for caspase 3/7 activation were similar in each of the cells lines, but they were slightly different between H295R and BD140C cells. PD129337 had no detectable effect on the ATP levels or the caspase 3/7 activities in these cells. ACAT inhibition was therefore not sufficient to cause ATP depletion or caspase 3/7 activation in these cells.

C. Effects of different concentrations of ATR-101 versus PD129337 on NBD-cholesterol esterification in H295R cells. The cells were incubated with the indicated concentrations of ATR-101 or PD129337 for 2 h, followed by 2 h with added NBD-cholesterol (1 μg/ml). The images show NBD (green) and Hoechst (blue) fluorescence captured with a 20X objective and are representative of two independent experiments. The concentrations of ATR-101 and

PD129337 that inhibited cholesterol esterification in H295R and BD140C cells were similar to the concentrations that inhibit ACAT enzyme activity in vitro (Trivedi *et al.*, 1993; Trivedi *et al.*, 1994). The scale bars denote 30 μm.

We compared the effects of ATR-101 and of PD129337 on cholesterol esterification in ACC-derived cells by imaging NBD-cholesterol accumulation in lipid droplets. PD129337 inhibited NBD-cholesterol accumulation more effectively than ATR-101. The inhibition of NBD-cholesterol esterification by low concentrations of ATR-101 and by PD129337 does not correlate with cholesterol accumulation or with ATP depletion or caspase 3/7 activation in H295R cells. The inhibition of cholesterol esterification as well as other effects of PD129337 in H295R cells (Fig. S7A) indicate that PD129337 accessed the same locations as ATR-101 in cells.

D. The entire fields from which the images in Figure 1C were cropped are shown for H295R (upper images) and for BD140C (lower images) cells. H295R and BD140C cells were cultured with DMSO vehicle or with the indicated concentrations of ATR-101 or of PD129337 for 4 h. After 4 h, the media was removed from the cells and immediately replaced with 4% paraformaldehyde and fixed at room temperature for 20 min. Cells were washed twice in PBS. Prior to staining, a stock solution of filipin III was prepared in DMSO (10 mg/ml). The filipin III stock solution was diluted 100X in PBS for a final concentration of 100 μg/ml and added directly to cells. Cells were incubated with filipin III at 37C in the dark for 2 h, washed twice in PBS. Filipin III fluorescence was visualized by confocal fluorescence microscopy using an excitation wavelength of 387/11 nm with a 60X oil objective. The images show filipin III fluorescence and are representative of two independent experiments for each cell line. The scale bars denotes 10 μm.

ATR-101 caused an increase in cholesterol accumulation in the plasma membrane. The filipin III staining intensities of individual cells in a cluster varied, and the overall filipin III staining intensities of H295R cells that were cultured with ATR-101 were significantly different from control cells and cells that were cultured with PD129337.

E. The entire fields from which the images in Figure 1E were cropped are shown. H295R cells were incubated with 1 μM of ATR-101 or PD129337 for 2 h, followed by 1.5 h with added NBD-cholesterol (1 μg/ml). After 1.5 h, Hoechst 33342 was added at a final concentration of 3 μg/ml. After 30 min, the media was removed and replaced with fresh media. The cells were visualized using confocal fluorescence microscopy using excitation wavelengths of either 485/20 nm (NBD) or 387/11 nm (Hoechst) with a 20X objective. The images show NBD (green) and Hoechst (blue) fluorescence and are representative of images collected in five independent experiments for each cell line. The scale bars denote 30 μm.

NBD-cholesterol produced a variable number of foci with intense fluorescence and a diffuse fluorescence of variable intensity in control H295R cells. ATR-101 and PD129337 at low concentrations eliminated both the intense foci and the diffuse fluorescence.

E

	+MβCD	+cholesterol
DMSO		
ATR-101		

Figure S2

H

0.6 mM MβCD: cholesterol   0.9 mM MβCD: cholesterol   1.2 mM MβCD: cholesterol

I

J   + MβCD

K   + ethanol   + cholesterol
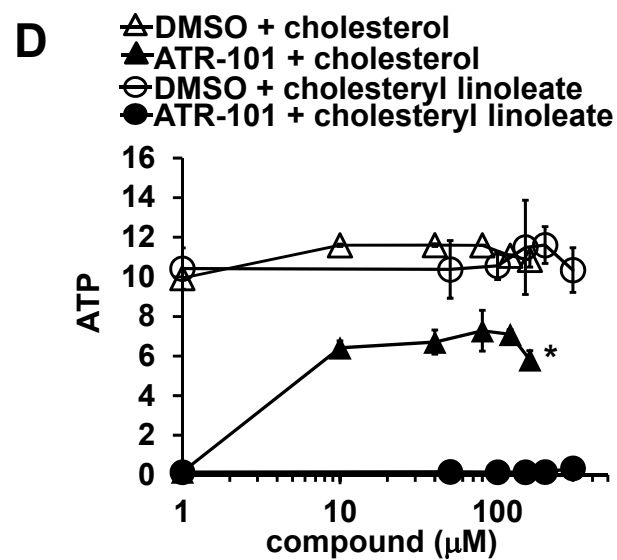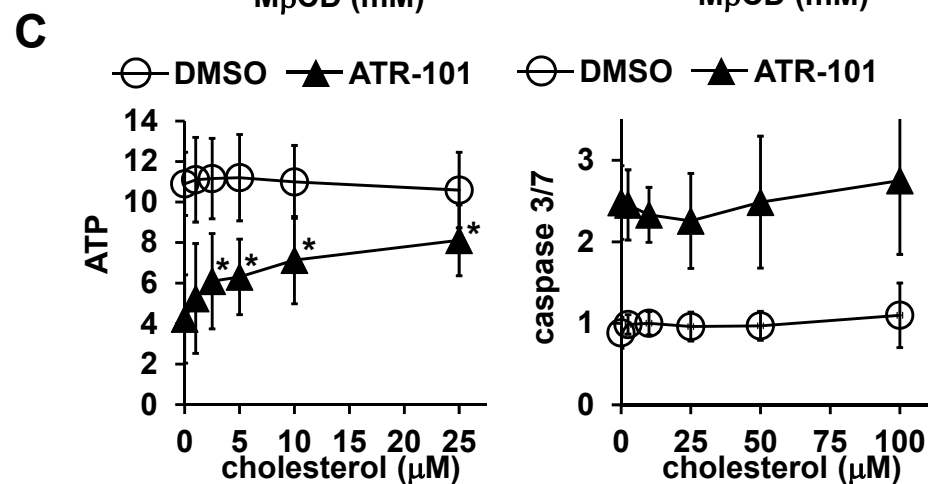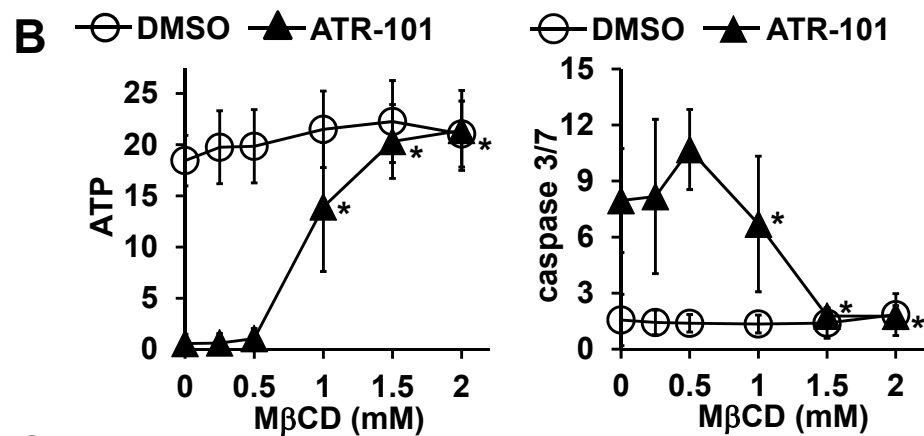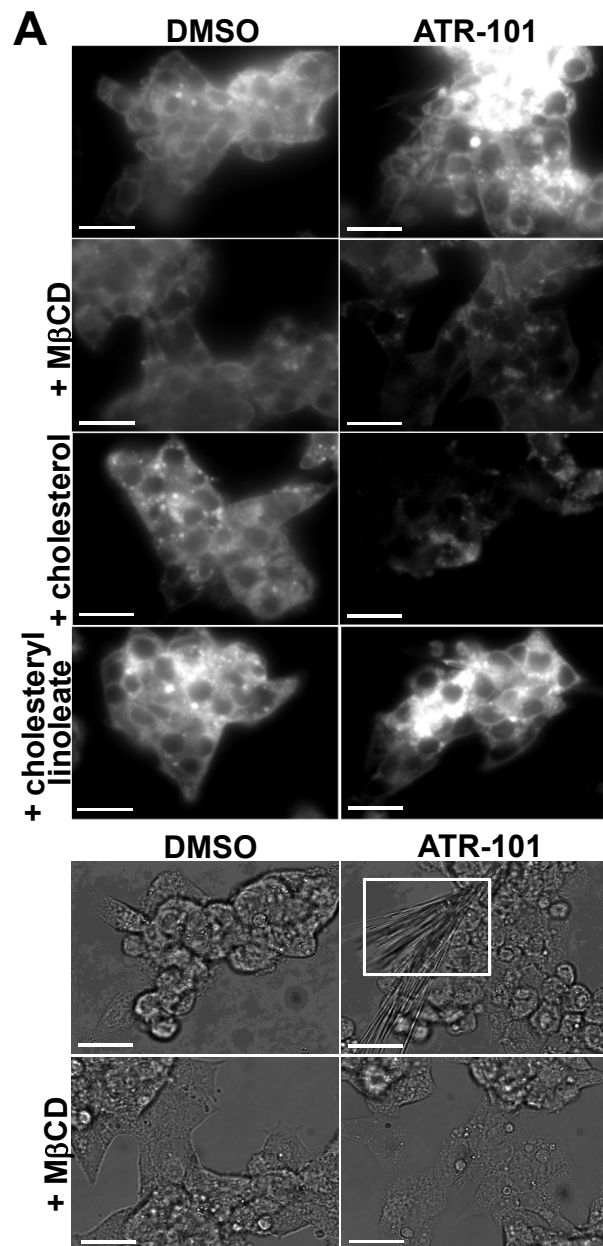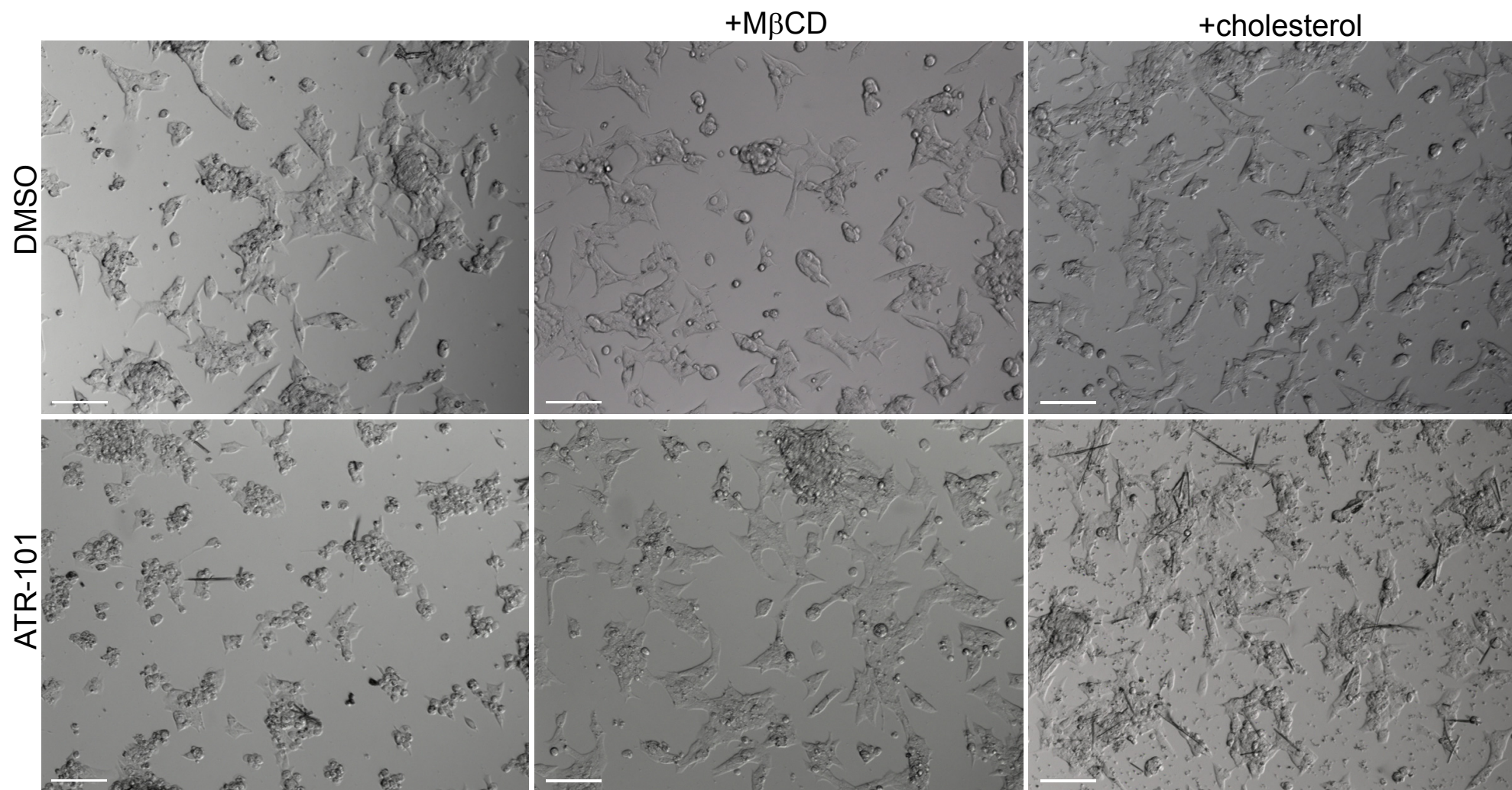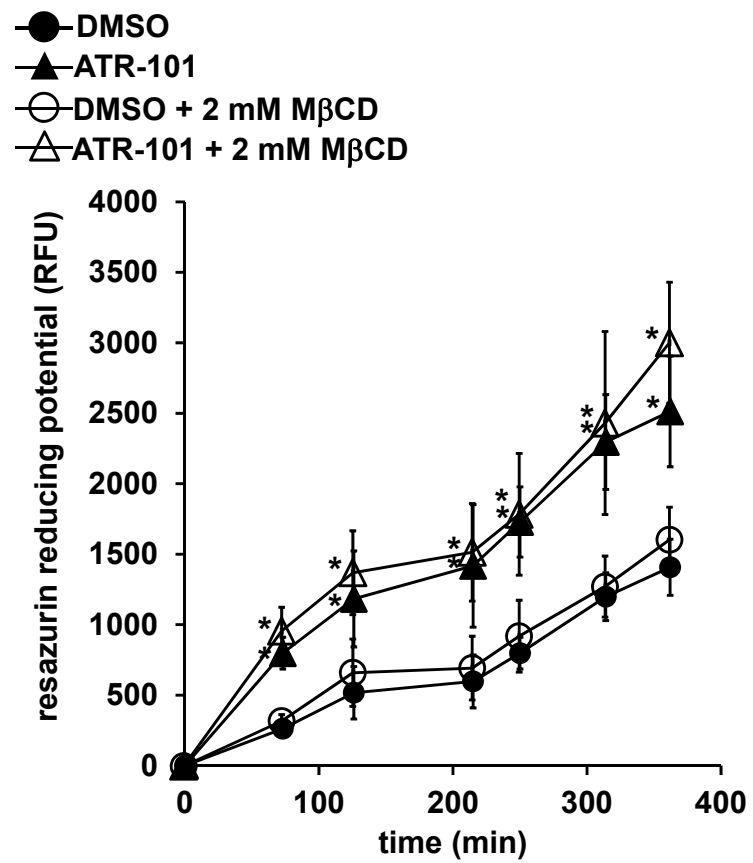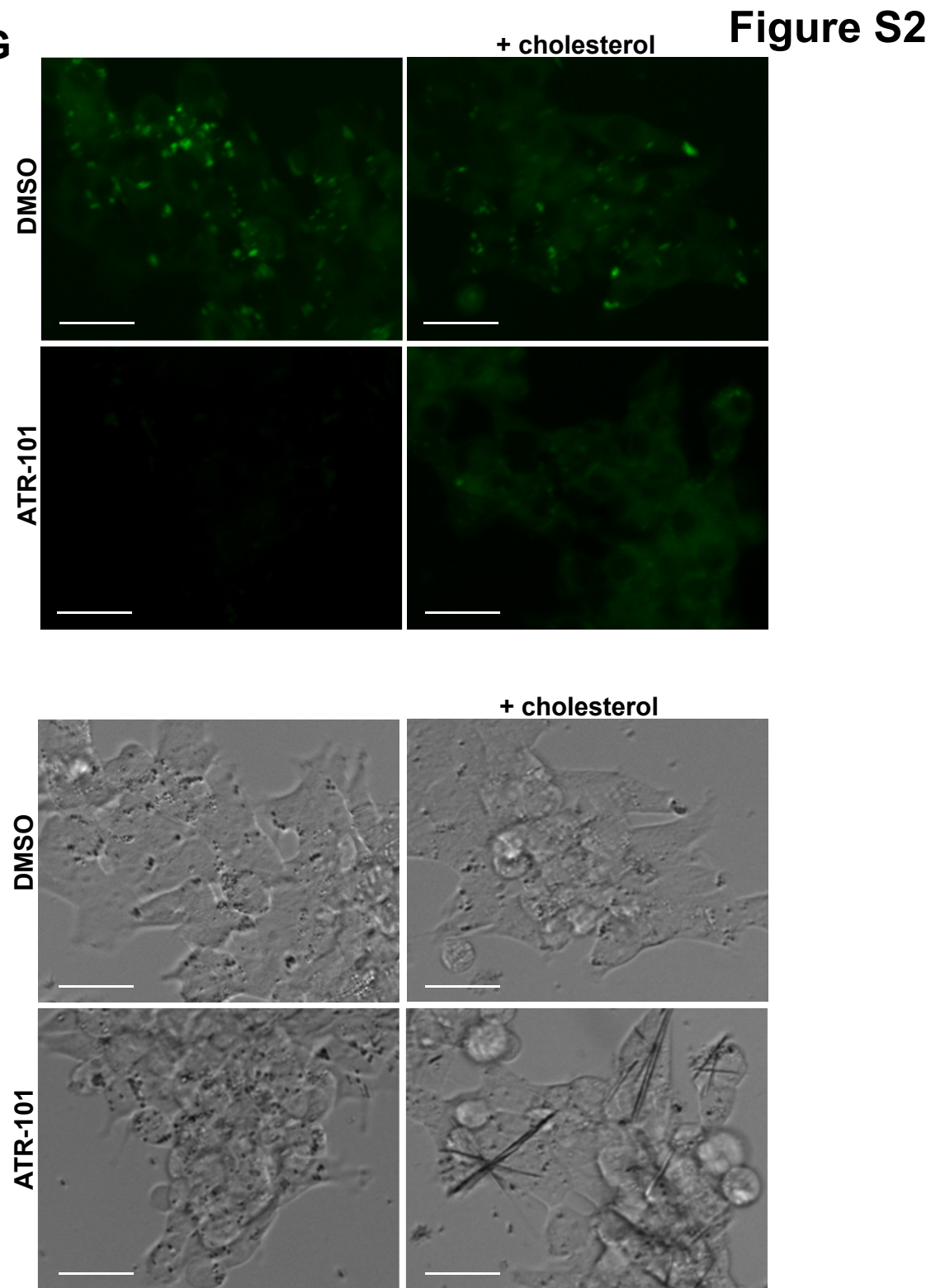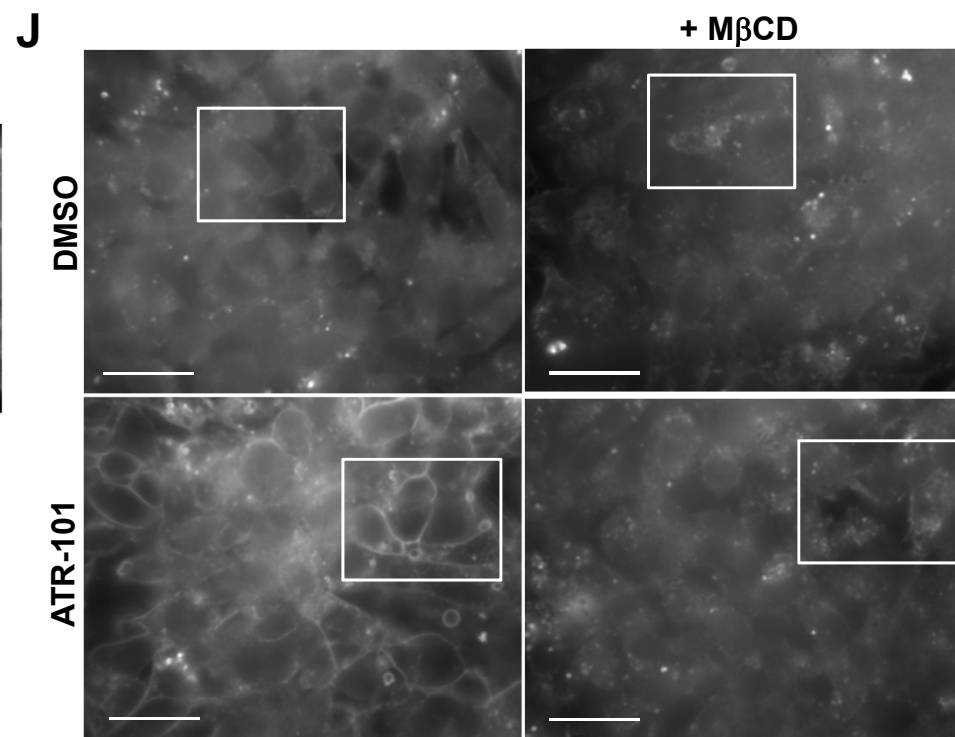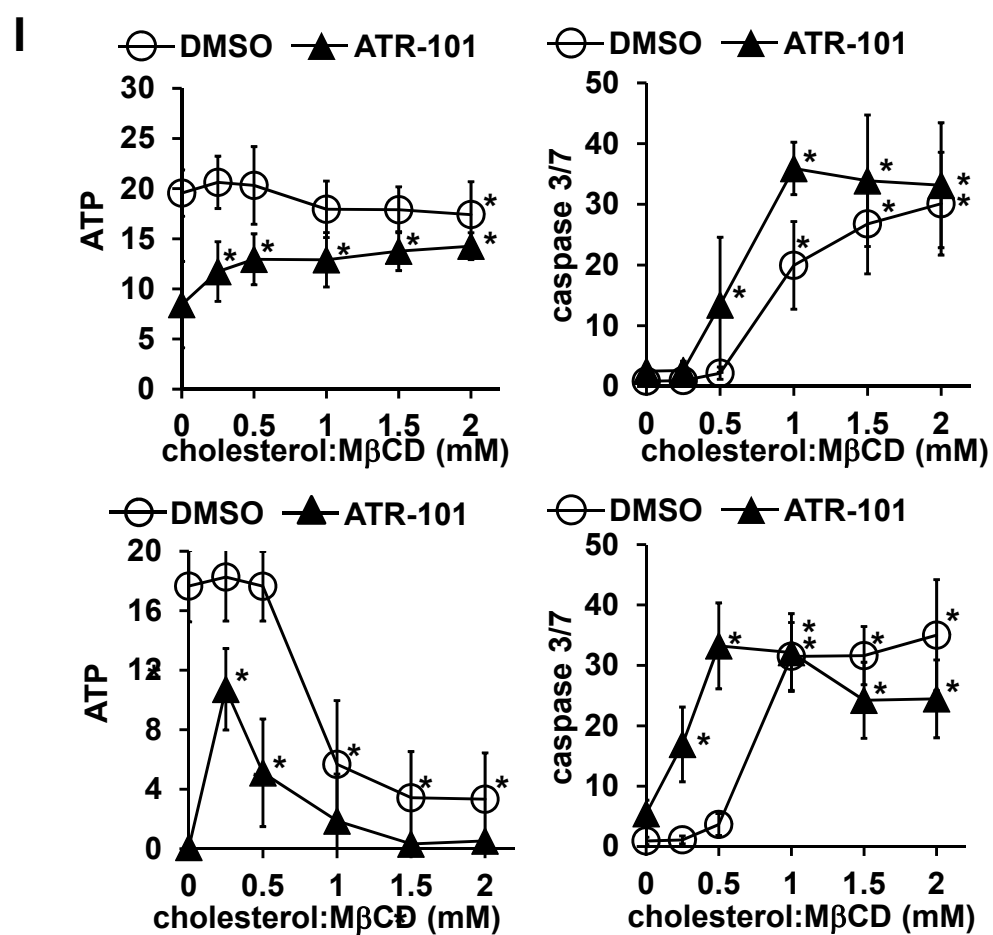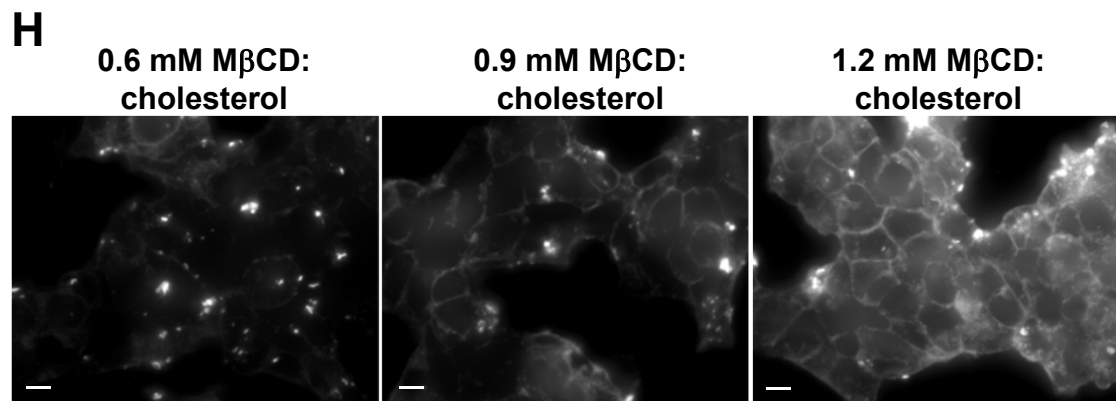
Figure S2. Effects of ATR-101 in combination with MβCD or with exogenous cholesterol on the cholesterol levels, ATP levels, caspase 3/7 activities, and cholesterol crystallization.

A. Effects of ATR-101 in combination with MβCD or with exogenous cholesterol on the cholesterol levels of H295R cells. The cells were cultured with DMSO vehicle (left images) or with 40 μM ATR101 (right images) alone (top row), together with 2 μM MβCD (second row), 160 μM cholesterol (third row), or 160 μM cholesterol linoleate (fourth row) for 24 h. MβCD is a cholesterol-binding compound that removes unesterified cholesterol from cell membranes (Yu *et al.*, 2005; Le Goff *et al.*, 2006) (Mahammad and Parmryd, 2008). Cholesterol was visualized using filipin III. The images show filipin III fluorescence (upper set of images) and phase contrast (lower set of images), and are representative of two independent experiments. The scale bars denote 30 μm.

ATR-101 caused an increase in intracellular cholesterol and in cholesterol crystallization at the plasma membrane. MβCD blocked the effects of ATR-101 on cholesterol accumulation and on cholesterol crystallization at the plasma membrane.   The cholesterol crystals were not visualized by filipin III, suggesting that the crystalline cholesterol is either inaccessible to filipin III binding, or is dislodged during the staining procedure. Exogenous cholesterol linoleate did not prevent the accumulation of cellular cholesterol in response to ATR-101.

B. Effects of MβCD on ATP depletion and caspase 3/7 activation by ATR-101 in H295R cells. The cells were cultured with the indicated concentrations of MβCD together with DMSO vehicle or 50 μM ATR-101 for 24 h. The ATP levels (left graph) and the caspase 3/7 activities (right graph) we measured in cells that were grown in parallel. The graphs show the means and the standard deviations of six samples from three experiments. The statistical significance of the differences in ATP levels and caspase 3/7 activities in cells that were cultured with each concentration of MβCD were evaluated by using one-way analysis of variance followed by Dunnett's *post hoc* tests (cells cultured with MβCD *vs.* corresponding controls, *$P < 0.05$).

MβCD suppressed the depletion of ATP and caspase 3/7 activation by ATR-101 in a concentration-dependent manner after 4 h.

C. Effects of exogenous cholesterol on ATP depletion and the caspase 3/7 activation by ATR-101 in H295R cells. The cells were incubated with DMSO or with 50 μM ATR-101 together with the indicated concentrations of exogenous cholesterol for 4 h. The ATP levels (left graph) and the caspase 3/7 activities (right graph) we measured in cells that were grown in parallel. The graphs show the means and the standard deviations of six samples from three experiments. The statistical significance of the differences in ATP levels and caspase 3/7 activities in cells that were cultured with each concentration of exogenous cholesterol were evaluated by using one-way analysis of variance followed by Dunnett's *post hoc* tests (cells cultured with exogenous cholesterol *vs. vs.* corresponding controls, *$P < 0.05$).

Exogenous cholesterol reduced ATP depletion, but did not affect caspase 3/7 activation by ATR-101 after 4 h. The differential effects of exogenous cholesterol on ATP depletion versus

caspase 3/7 activation by ATR-101 demonstrate that ATP depletion and caspase 3/7 activation by ATR-101 are independent.

D. Comparison of the effects of exogenous cholesterol and exogenous cholesterol linoleate on ATP depletion by ATR-101. H295R cells were cultured with DMSO or with 40 μM ATR-together with the indicated concentrations of exogenous cholesterol or exogenous cholesterol linoleate for 24 h and the ATP levels were measured. The graphs show the means and the standard deviations of two samples from one experiment and are representative of two experiments. The statistical significance of the difference in ATP levels in cells that were cultured with ATR-101 and cholesterol or ATR-101 and cholesterol linoleate was evaluated by using unpaired two-tailed Student's *t*-test (n=6, *$P < 0.05$). The ATP levels that are shown in Fig. S2D were measured in parallel with the visualization of cholesterol by filipin III binding in Fig. S2A.

Exogenous cholesterol but not exogenous cholesterol linoleate inhibits ATP depletion by ATR-101 after 24 h. The distinct effects of the exogenous cholesterol versus the cholesterol linoleate as well as the accumulated cellular cholesterol indicate that the exogenous cholesterol suppressed ATR-101 cytotoxicity by acting through a mechanism or at a location that was not accessible to the cholesterol that was generated inside cells that were cultured with ATR-101.
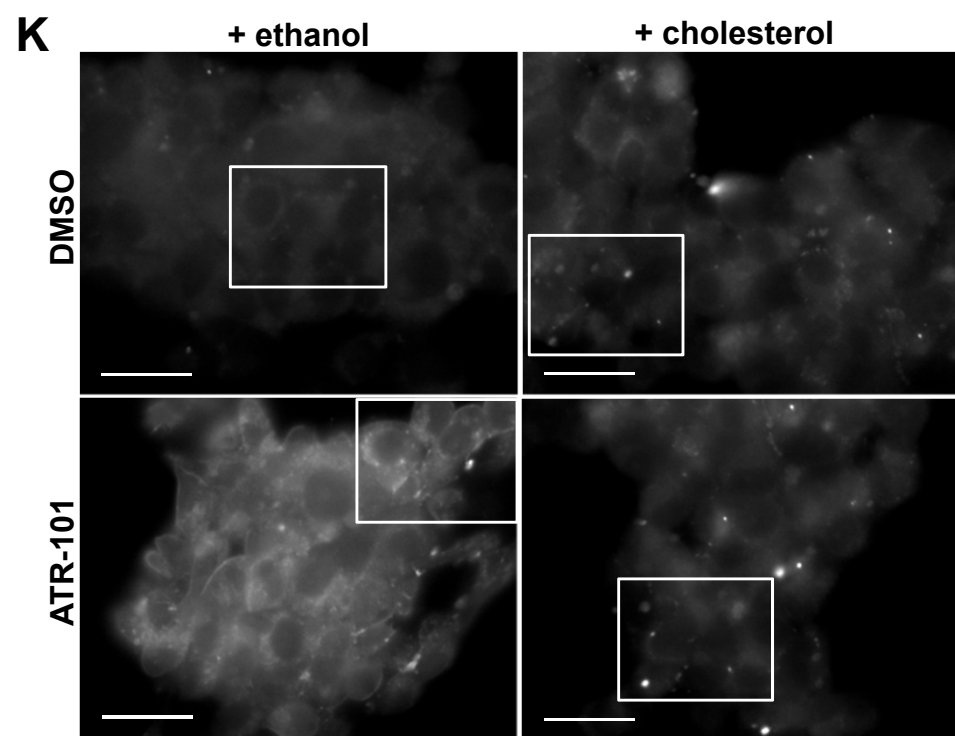
E. Visualization of the effects of ATR-101 alone and in combination with MβCD or with exogenous cholesterol on cell morphology after 30 h. The cells were cultured with DMSO vehicle (upper images) or with 40 μM ATR-101 (lower images) alone (left images), or in combination with 1.5 mM MβCD (middle images), or in combination with 40 μM cholesterol (right images).   Cell morphology was visualized by phase contrast microscopy using a 10X objective. The images are representative of 5 fields under each set of culture conditions. The scale bars denote 100 μm.

H295R cells incubated with ATR-101 were small, rounded and weakly attached to the plate after 30 h.   These changes in morphology are consistent with the loss of cell viability. The effects of ATR-101 on cell morphology were prevented in cells that were cultured with ATR-101 in combination with either MβCD or cholesterol.

F. Effect of ATR-101 on resazurin reducing potential in H295R cells.   The cells were cultured with DMSO vehicle or with 32 μM ATR-101 alone, or together with 2 mM MβCD. Resazurin fluorescence intensity was measured using a microplate reader at the indicated times at 37 C. The background signal of wells without resazurin was subtracted. The graph shows the means and the standard deviations of six samples from three experiments. The statistical significance of the differences in resazurin reducing potential at each time after ATR-101 addition in either the absence or the presence of MβCD were evaluated by using two-way analysis of variance followed by Sidak's *post hoc* tests (ATR-101 *vs.* DMSO control, *$P < 0.05$).

ATR-101 caused a sustained increase in resazurin fluorescence 1 h after addition to the culture medium. The increase in resazurin fluorescence caused by ATR-101 was not inhibited by MβCD, indicating that it was independent of cholesterol accumulation or ATP depletion by ATR-101.

G. Effects of ATR-101 in combination with exogenous cholesterol on NBD-cholesterol esterification and on extracellular cholesterol crystal formation in H295R cells. H295R cells were incubated with DMSO or with 100 μM ATR-101 alone or together with 40 μM of exogenous cholesterol for 2 h, followed by 2 h with added NBD-cholesterol (1 μg/ml). The images show NBD (green) fluorescence (left images) and phase contrast (right images) captured with a 60X objective. The scale bars denote 30 μm.

ATR-101 inhibits NBD-cholesterol esterification in the absence and in the presence of exogenous cholesterol. This indicates that exogenous cholesterol did not prevent ATR-101 entry into cells, or the inhibition of cholesterol esterification by ATR-101.

Exogenous cholesterol in combination with ATR-101 increased the amount of extracellular cholesterol crystals and prevented intracellular cholesterol accumulation. Abundant crystals were observed at cells that were cultured with ATR-101 together with exogenous cholesterol for 4 h, whereas cholesterol crystals were not visible at cells that were cultured with ATR-101 or exogenous cholesterol separately for 4 h. We hypothesize that the exogenous cholesterol nucleates cholesterol crystallization at the plasma membrane. The increase in cholesterol crystallization at cells that were cultures with ATR-101 and exogenous cholesterol was corroborated by measurement of the amount of cell-associated cholesterol that was associated with the cells (Fig. 2F). The amount of cholesterol that was released into the wash medium during the 30 second wash is a minimum estimate of the extracellular cholesterol that was associated with the cells since a larger amount of cholesterol was released during a longer incubation with the wash medium. It is unlikely that the extracellular cholesterol was released by cell lysis since cells that were cultured with ATR-101 together with exogenous cholesterol had a higher ATP level and a lower caspase 3/7 activity than cells that were cultures with ATR-101 alone. This indicates that the protection from ATR-101 cytotoxicity by exogenous cholesterol correlates with cholesterol crystal formation and an increase in the amount of extracellular cholesterol that is associated with the cells.

H. Effects of cholesterol:MβCD on the cholesterol levels of H295R cells. The cells were cultured with the indicated concentrations of cholesterol:MβCD for 4 h. The images show filipin III fluorescence and are representative of images from two separate experiments. The scale bars denote 10 μm.

We investigated the effects of cholesterol accumulation independently of ATR-101 by culturing H295R cells in the presence of cholesterol:MβCD. Cholesterol:MβCD concentrations that were 10-100 fold higher than the concentrations of exogenous cholesterol increased the intracellular cholesterol levels of H295R cells. At moderate cholesterol:MβCD concentrations

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(0.6 mM), the cholesterol was localized mainly to intracellular foci, and at the highest cholesterol:MβCD concentrations (1.2 mM), the cholesterol accumulated mainly in the plasma membrane. We were unable to visualize the cholesterol in cells that were cultured with cholesterol:MβCD in combination with ATR-101 since the cells that were cultured under these conditions did not adhere to slides under the conditions that are required to visualize filipin III binding.

I. Effects of ATR-101 in combination with cholesterol:MβCD on the ATP levels and the caspase 3/7 activities of H295R cells. The cells were cultured with the indicated concentrations of cholesterol:MβCD together with DMSO vehicle or 50 μM ATR-101 for 4 h (upper graphs) or 24 h (lower graphs), followed by measurement of the ATP levels (left graph) and caspase 3/7 activities (right graph). The graphs show the means and the standard deviations of six samples from three experiments. The statistical significance of the differences in ATP levels and caspase 3/7 activities in cells that were cultured with each concentration of cholesterol:MβCD were evaluated by using two-way analysis of variance followed by Dunnett's *post hoc* tests (cells cultured with cholesterol:MβCD vs. corresponding controls, *$P < 0.05$)

Moderate cholesterol:MβCD concentrations (≤0.5 mM) reduced ATP depletion by ATR-101 at 4 h and 24 h after addition to H295R cells, consistent with the reduction in ATR-101 dependent ATP depletion by exogenous cholesterol (Fig. 2C). The highest cholesterol:MβCD concentrations (≥1 mM) reduced ATP depletion by ATR-101 after 4 h, but they caused ATP depletion both alone and in combination with ATR-101 after 24 h. Cholesterol:MβCD increased the caspase 3/7 activity both alone and in combination with ATR-101 both at 4 h and at 24 h after addition to H295R cells. Cholesterol:MβCD therefore had effects in combination with ATR-101 that were distinct from the effects of MβCD and of exogenous cholesterol separately. The differences between these effects are likely to be due to the distinct activities of the low concentrations of exogenous cholesterol alone and the high concentrations of cholesterol complexed with MβCD

J.   The full fields from which the images in Fig. 2A (white rectangles) were taken. The scale bars denote 30 μm. H295R cells that were cultured with ATR-101 have higher levels of filipin III binding to the plasma membrane than control cells. Cells that were cultured with ATR-101 together with MβCD did not have a higher level of filipin III binding to the plasma membrane.

K. The full fields from which the images shown in Fig. 2C (white rectangles) were taken. The scale bars denote 30 μm. H295R cells that were cultured with ATR-101 have higher levels of filipin III binding to the plasma membrane than control cells. Cells that were cultured with ATR-101 together with exogenous cholesterol did not have a higher level of filipin III binding to the plasma membrane.

A

DMSO

glibenclamide

verapamil

ATR-101

B

+ MβCD
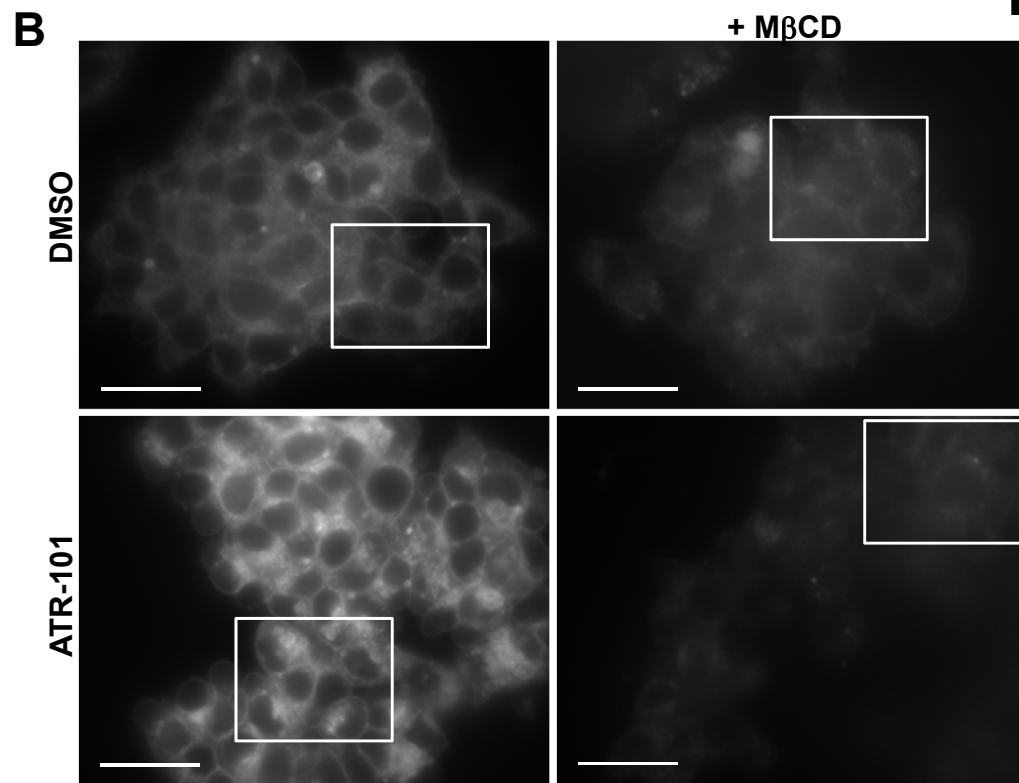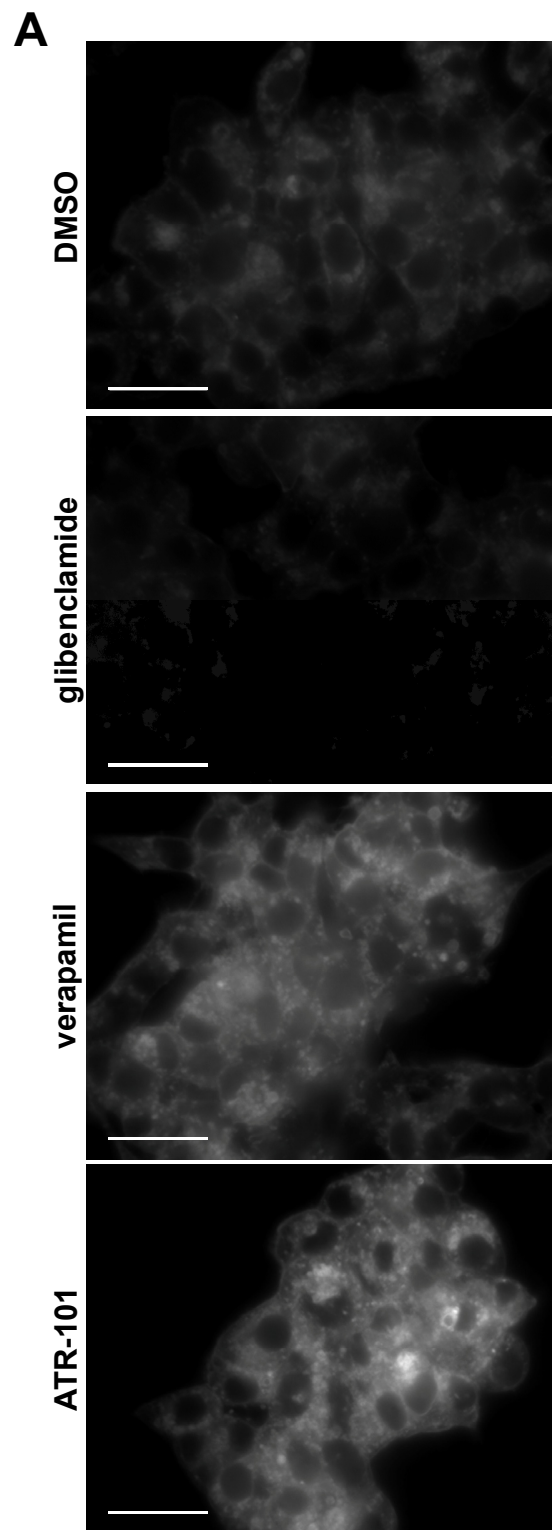
DMSO
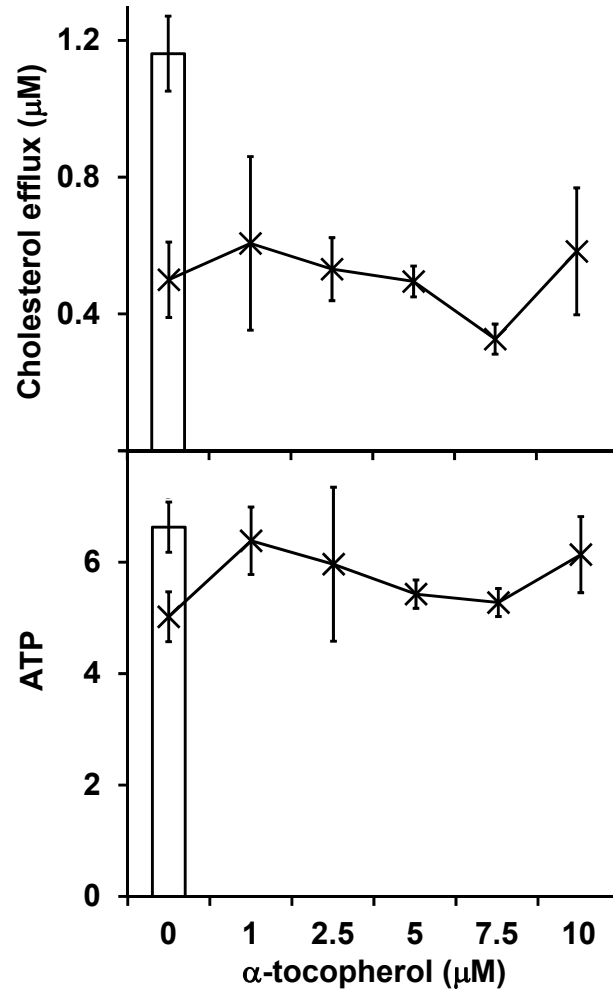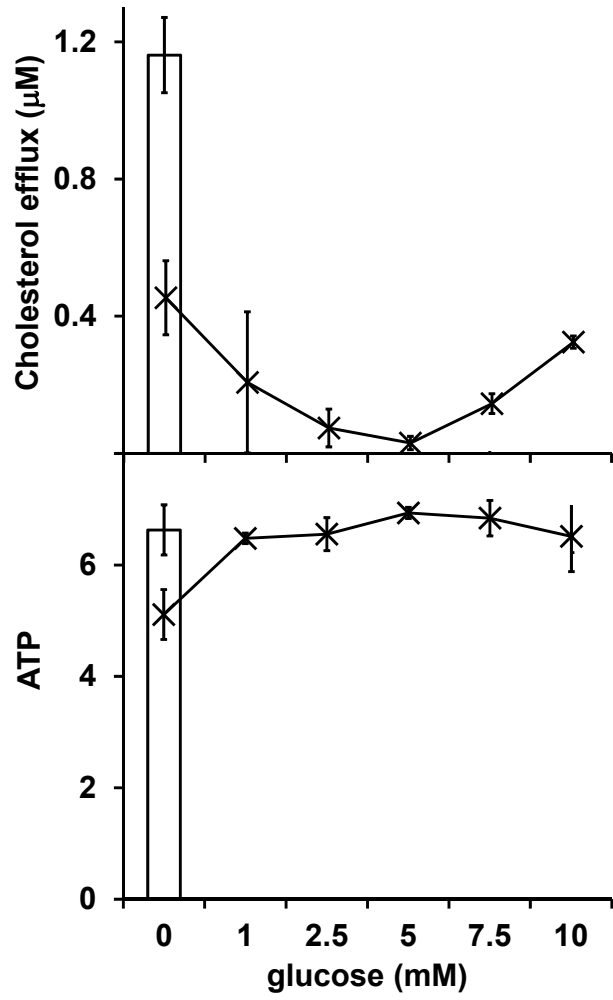
ATR-101

Figure S3

Figure S3. Effects of ATR-101 and of ABC transporter inhibitors on the cholesterol levels of H295R cells that were cultured in serum-free medium for 4 h.

A. Comparison of the effects of glibenclamide, verapamil, and ATR-101 on the cholesterol levels in H295R cells that were cultured in serum-free medium. The medium of cells that were cultured under standard conditions was replaced with serum-free media containing apoA-I with either DMSO vehicle, 50 µM glibenclamide, 50 µM verapamil, or 50 µM ATR-101 for 4 h. The cholesterol in the cells was visualized by filipin III binding. The images show filipin III fluorescence and are representative of two independent experiments. The scale bars denote 30 µm.

 Verapamil and ATR-101 but not glibenclamide increased the level of cholesterol in intracellular membranes of H295R cells that were cultured in serum-free medium. The lack of cholesterol accumulation in cells that were cultured with glibenclamide indicates that the inhibition of cholesterol efflux was not sufficient to cause cholesterol accumulation in H295R cells. The increase in cholesterol in cells that were cultured with verapamil indicates that the inhibition of cholesterol efflux was not necessary for cholesterol accumulation. The increase in cholesterol efflux caused by verapamil under these same conditions is an independent indicator of the increase in intracellular cholesterol caused by verapamil (Fig. 3D). The increase in cholesterol efflux caused by verapamil likely represents a compensatory mechanism in response to MDR1 inhibition by verapamil.

B. The full fields from which the images in Fig. 3A (white rectangles) were taken. The scale bars denote 30 µm.

 ATR-101 increased the cholesterol levels in H295R cells that were cultured in serum-free medium. MβCD suppressed the cholesterol accumulation that was caused by ATR-101 in serum-free medium. MβCD also reduced the basal level of cholesterol in H295R cells that were cultured in serum-free medium.

 The rate of cholesterol efflux in the absence of ATR-101 corresponded to 5% of the total amount of cholesterol and cholesterol esters per hour in H295R cells. The inhibition of cholesterol efflux alone was therefore unlikely to account for the accumulation of cholesterol in cells that were cultured with ATR-101. Additional activities of ATR-101 likely contributed to cholesterol accumulation and cytotoxicity.

C. Effects of glucose and α-tocopherol on cholesterol efflux versus ATP levels in H295R cells cultured with ATR-101. The levels of cholesterol in the medium (upper graphs) and of cellular ATP (lower graphs) were measured in the same cultures 4 h after replacing the standard culture medium with serum-free media containing apoA-I and DMSO vehicle (white bars) or 100 µM ATR-101 (line graphs) and the indicated concentrations of glucose (left) or α-tocopherol (right).

 ATR-101 inhibited cholesterol efflux under conditions in which the ATP levels were restored in the presence of either glucose or α-tocopherol.
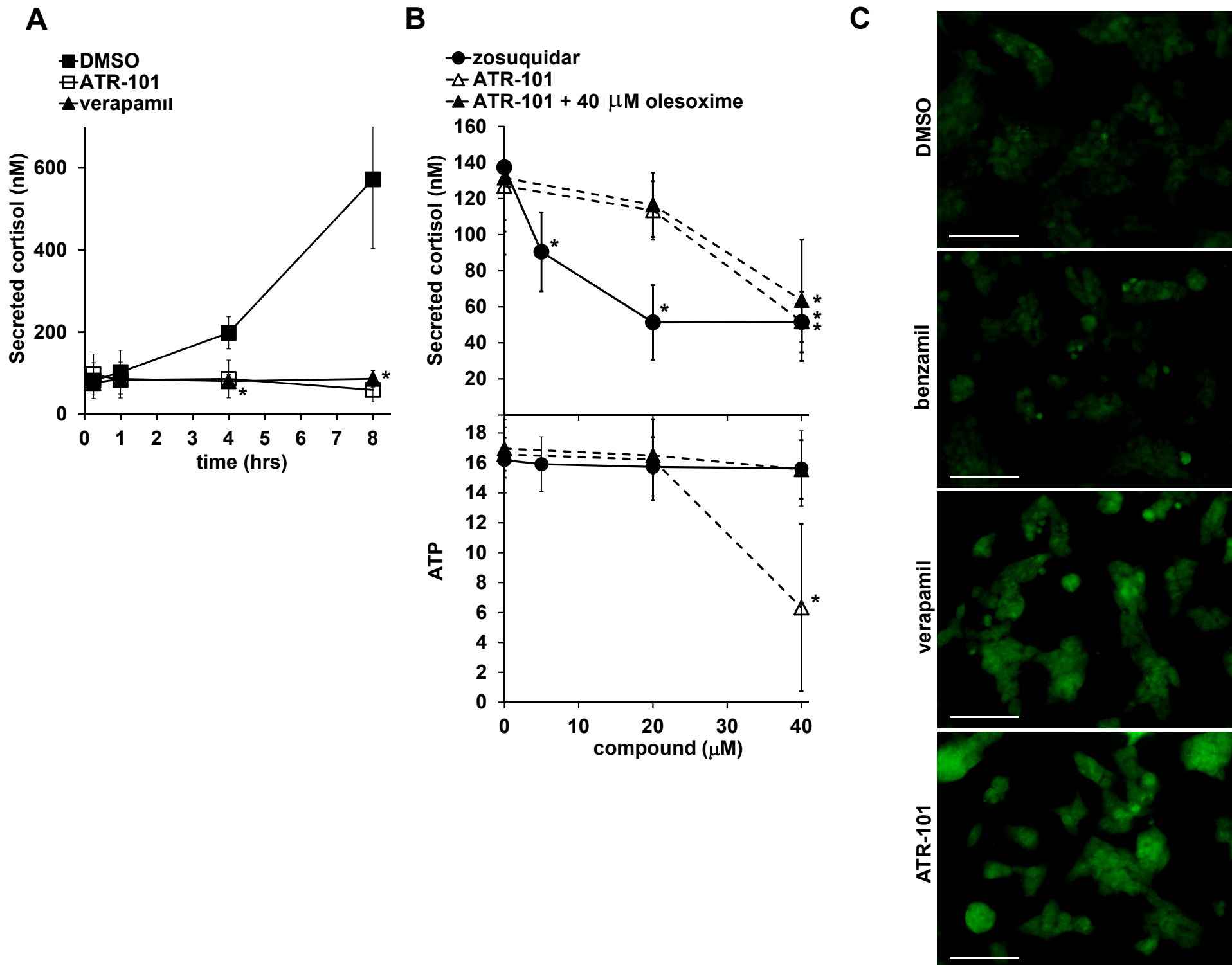
Figure S4

Figure S4. Effects of ATR-101 and of MDR1 inhibitors on cortisol secretion and on doxorubicin accumulation.

A. Comparison of the effects of ATR-101 and verapamil on cortisol secretion. The cells were switched to media with DMSO vehicle, 100 µM ATR-101, or 100 µM verapamil. The levels of cortisol secreted into the media were measured at the indicated times. The graph shows the means and the standard deviations of five samples from two experiments. The statistical significance of the differences in the cortisol concentrations in the medium at each time after verapamil addition were evaluated by using two-way analysis of variance followed by Sidak's *post hoc* tests (verapamil *vs.* DMSO controls, *$P < 0.05$).

       ATR-101 and verapamil inhibited cortisol secretion from H295R cells with similar efficiencies. The inhibition of cortisol secretion by verapamil suggests that MDR1 is required for the cortisol secretion that is detected in the ACC-derived cells.   Cortisol secretion from H295R cells was increased by forskolin, consistent with the induction of corticosteroid biosynthesis by cAMP signaling (Rainey *et al.*, 1993).

B. Comparison of the effects of zosuquidar, ATR-101, and ATR-101 together with olesoxime on cortisol secretion and on the ATP levels of H295R cells. The cells were switched to media containing indicated concentrations of zosuquidar, ATR-101, or ATR-101 together with 40 µM olesoxime. The levels of cortisol secreted into the media (upper graph) and the cellular ATP levels (bottom graph) were measured after 4 h. The graphs show the means and the standard deviations of five samples from two experiments.   The statistical significance of the differences in the cortisol concentrations in the medium and the ATP levels in the cells that were cultured with the indicated concentrations of the compounds were evaluated by using one-way analysis of variance followed by Dunnett's *post hoc* tests (clls cultured with ATR-101 or zosuquidar *vs.* corresponding controls, *$P < 0.05$).

       Zosuquidar inhibited cortisol secretion from H295R cells. Zosuquidar selectively inhibits MDR1 and does not inhibit the closely related MRP1, MRP2, or BCRP ABC transporters (Shepard *et al.*, 2003) . An 8-fold higher concentration of of zosuquidar did not cause ATP depletion. MDR1 inhibition was therefore not sufficient for ATP depletion.

       Perturbations to mitochondrial functions can affect steroidogenesis and cholesterol efflux (Midzak *et al.*, 2011b; Graham, 2015). ATR-101 inhibited cholesterol efflux (Fig. 3E) and cortisol secretion (Fig. S4B) in the absence and in the presence of the mitoprotective compound olesoxime (Bordet *et al.*, 2010) to the same extent. Olesoxime reduced ATP depletion by ATR-101 (Fig. 3E, S4B). ATR-101 therefore inhibited cholesterol efflux and cortisol secretion by mechanisms that did not require full ATP depletion.

C. Effects of different ABC transporter inhibitors on doxorubicin accumulation in H295R cells. The cells were cultured in the presence of 25 µM doxorubicin together with DMSO vehicle, 20 µM benzamil, 20 µM verapamil, or 20 µM ATR-101. The levels of doxorubicin in the cells

were imaged after 2 h by fluorescence microscopy using a 20X objective. The images show doxorubicin fluorescence and are representative of images from two independent experiments. The scale bars denote 100 μm.

A low level of doxorubicin fluorescence was detected in H295R cells that were cultured with doxorubicin in the absence of MDR1 inhibitors. ATR-101 and verapamil increased doxorubicin accumulation suggesting that they inhibited MDR1 activity. Benzamil did not increase doxorubicin accumulation, consistent with the export of doxorubicin primarily by MDR1 and the lack of MDR1 inhibition by benzamil.
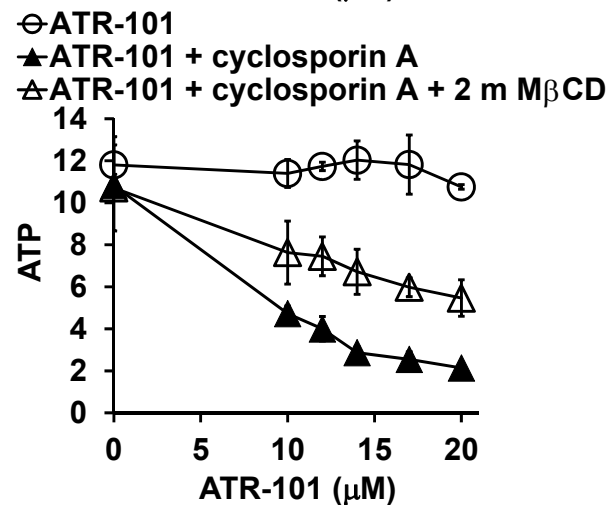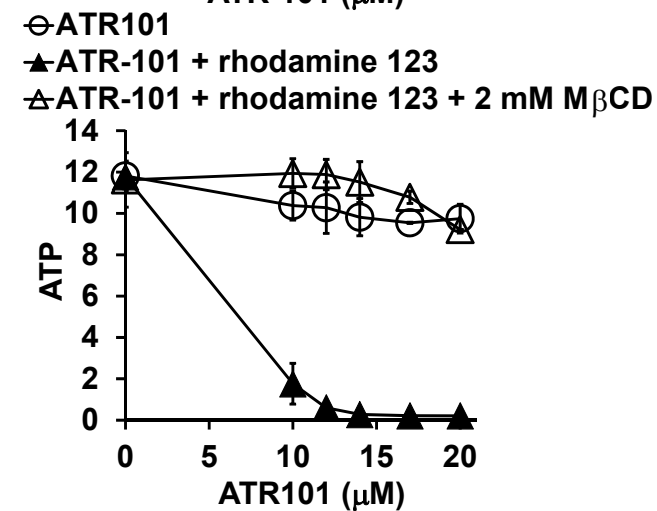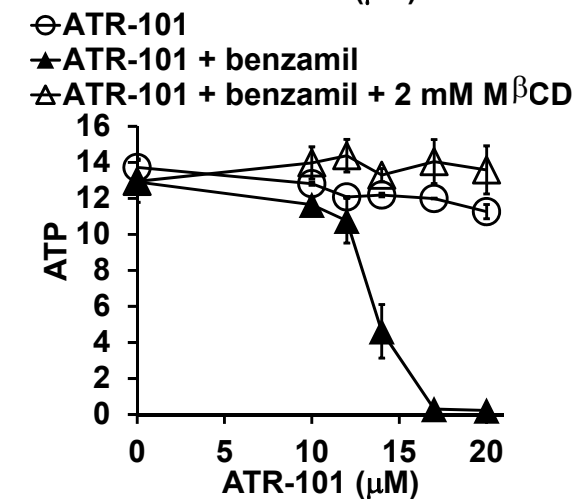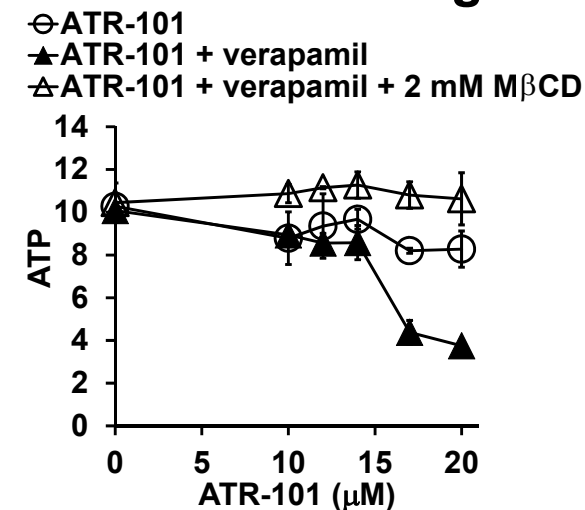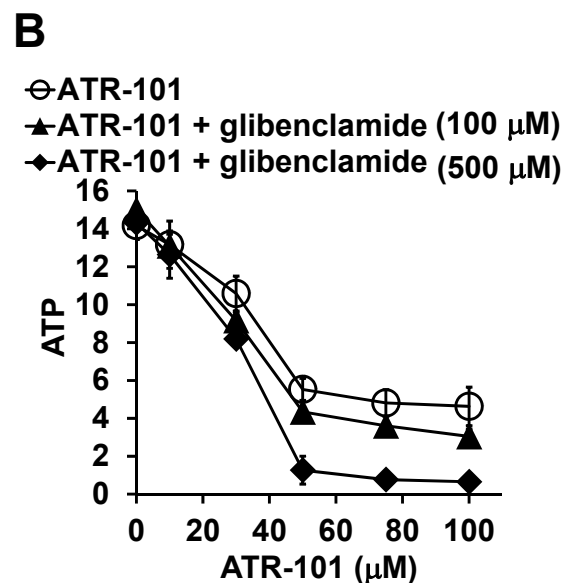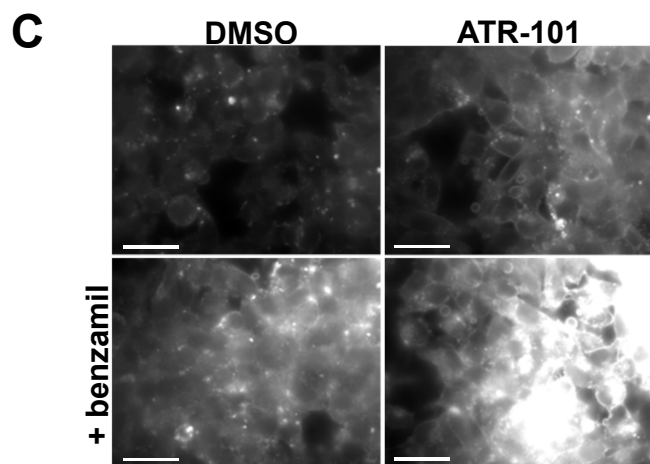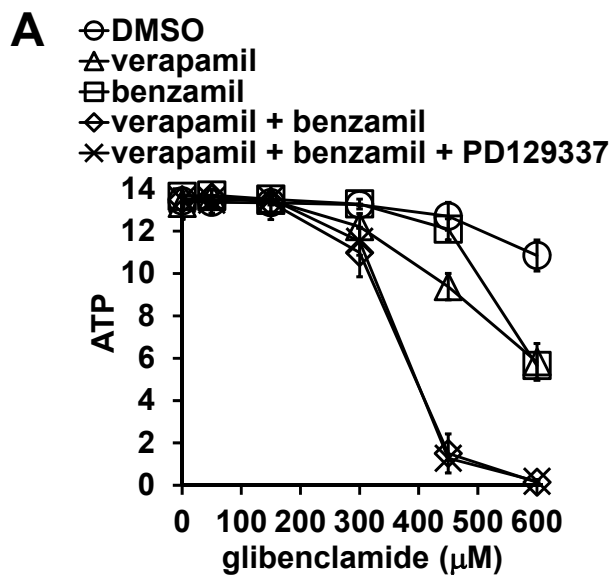
Figure S5

Figure S5. Combined effects of ABC transporter inhibitors with each other and with ATR-101 on the ATP levels of H295R cells.

A. Effects of different combinations of ABC transporter inhibitors on the ATP level of H295R cells. The cells were cultured with the indicated concentrations of glibenclamide in combination with DMSO vehicle or with verapamil (50 µM), benzamil (50 µM), and PD129337 (1 µM). The ATP levels of the cells were measured 4 h after addition of the compounds. The data show the means and standard deviations of two cultures of cells with each concentration of each combination of inhibitors, and are representative of the results from two experiments.

       Inhibitors of ABCA1, ABCG1 and MDR1 had a synergistic effect on ATP depletion in H295R cells. ATP depletion by these ABC inhibitors was not enhanced by ACAT inhibition.

B. Effects of ATR-101 in combination with selective ABC transporter inhibitors on H295R cells. H295R cells were cultured with the indicated concentrations of ATR-101 together with DMSO vehicle, glibenclamide (100 µM or 500 µM), benzamil, rhodamine 123, or cyclosporine A (20 µM each), in the absence and in the presence of 2 mM MβCD. The ATP levels of the cells were measured after 4 h. The graphs show the means and the standard deviations of two cultures of cells with each concentration of each combination of inhibitors, and are representative of the results from two experiments. The data for the graphs shown with different ABC transporter inhibitors were obtained in separate experiments.

       ATR-101 in combination with ABCG1 (benzamil) and MDR1 (cyclosporin A, verapamil) inhibitors caused larger than additive reductions in the ATP levels of cells. ATR-101 in combination with an ABCA1 inhibitor (glibenclamide) did not cause a larger than additive reduction in the ATP levels of cells. The combined effects of ATR-101 with selected ABC transporter inhibitors on ATP depletion were suppressed by MβCD, indicating that the combined cytotoxicity required cholesterol accumulation. ATR-101 in combination with the mitochondrial inhibitor and MDR1 substrate rhodamine-123 also caused larger than additive reductions in the ATP levels of cells. These result suggest that the potency of ATR-101 was enhanced when it was used in combination with inhibitors of ABCG1, MDR1, or mitochondrial functions.

C. Effects of ATR-101 and benzamil on the cholesterol level of H295R cells. The cells were cultured with DMSO vehicle or 20 µM ATR-101 and 5 µM benzamil, separately and in combination for 1 h. The cells were fixed and the cholesterol was visualized using filipin III. The images show filipin III fluorescence captured with a 60X oil objective and are representative of two independent experiments. The scale bars denote 30 µm.

       Benzamil and ATR-101 in combination caused a larger than additive increase in cholesterol accumulation.

A

○ DMSO   ◆ 20 μM ATR-101
■ 35 μM ATR-101 ▲ 50 μM ATR-101

ATP vs abiraterone acetate (μM)

ATP vs ketoconazole (μM)

ATP vs anastrozole (μM)

ATP vs trilostane (μM)

ATP vs metyrapone (μM)

B

Figure S6

cholesterol → ATP loss

ketoconazole ⊣ CYP11A1

P5 → P4 → DOC

CYP11B2 → CORT

metyrapone ⊣ CYP11B2

abiraterone acetate, ketoconazole ⊣ CYP17A1

3β-HSD

17P5 → 17P4 → S → cortisol

abiraterone acetate, ketoconazole ⊣ CYP17A1

DHEA → A4 → CYP19 → E1

trilostane ⊣ 3β-HSD

anastrozole ⊣ CYP19

Figure S6. Effects of ATR-101 in combination with inhibitors of steroidogenesis on the ATP levels of H295R cells.

A. Effects of different inhibitors of steroidogenesis on ATP depletion by ATR-101. H295R cells were cultured with DMSO vehicle or the indicated concentrations of ATR-101 together with the indicated concentrations of abiraterone acetate, ketoconazole, anastrozole, trilostane, or metyrapone for 4 h and the ATP levels were measured. The graphs show the means and the standard deviations of two cultures of cells with each concentration of each combination of inhibitors shown, and are representative of results from two experiments. The data for the graphs shown were obtained from parallel cultures with all inhibitors.

The diagram on the right shows the principal targets of abiraterone acetate, ketoconazole, anastrozole, trilostane, and metyrapone in the major branches of adrenocortical steroidogenesis. The enzymes are indicated inside the rectangles and the rectangles are superimposed on the reactions (arrows) that they catalyze. Only a subset of the intermediates are shown.   Pregnenolone (P5); 17-hydroxypregnenolone (17-OHP), dihydroepiandrostenedione (DHEA), dihydroepiandrostenedione sulfate (DHEAS), progesterone (P4), 17-hydroxyprogesterone (17-OHP4), androstenedione (A4), corticosterone (DOC), 11-deoxycortisol (S), estrone (E1), corticosterone (CORT).

Low concentrations of many different inhibitors of steroidogenesis reduced ATP depletion by ATR-101. The concentrations of the inhibitors that reduced ATP depletion by ATR-101 were consistent with their inhibitory coefficients for different steroidogenic enzymes (Takahashi *et al.*, 1990; Johansson *et al.*, 1998; Garrido *et al.*, 2014). High concentrations of some of these inhibitors enhanced ATP depletion both alone and in combinatioin with ATR-101. The concentrations of ketoconazole and abiraterone acetate that enhanced ATP depletion were consistent with the concentrations that inhibit MDR1 (Siegsmund *et al.*, 1994; Benoist *et al.*, 2016).

Exogenous steroids and synthetic androgen derivatives can influence cholesterol trafficking and metabolism (Liscum and Faust, 1989; Butler *et al.*, 1992; Debry *et al.*, 1997; Lange *et al.*, 1997; Hartgens *et al.*, 2004; Lucken-Ardjomande *et al.*, 2008; Midzak *et al.*, 2011a; Garevik *et al.*, 2012; Midzak *et al.*, 2012).   These results suggest that steroid accumulation can contribute to ATR-101 cytotoxicity through the inhibition of cholesterol trafficking.

A △ATR-101 ⊖PD129337

B
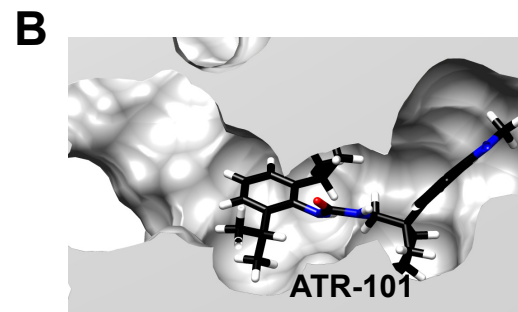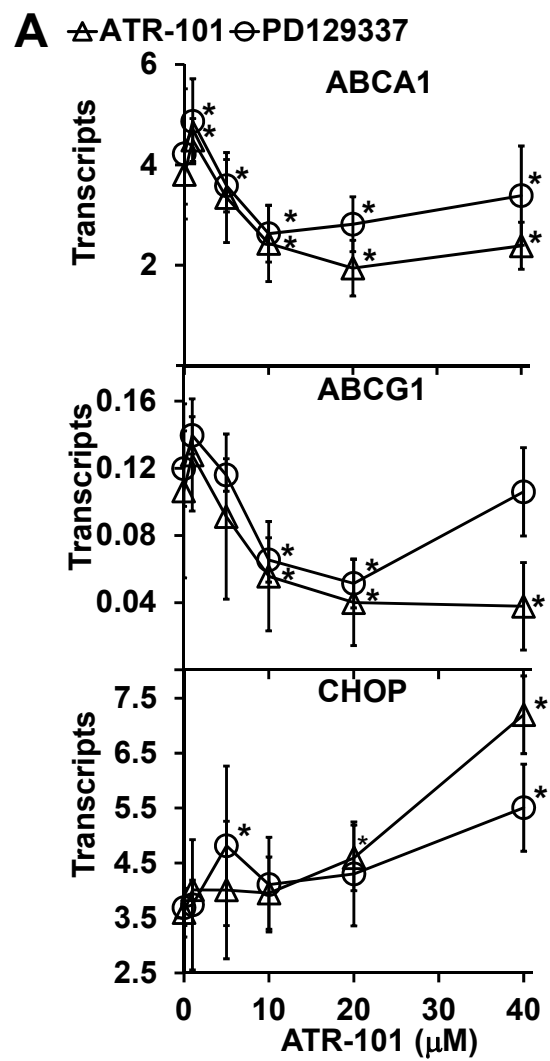
ATR-101

Figure S7. Effects of ATR-101 compared with PD129337 on transcript levels in H295R cells.

A. Comparison of the effects of ATR-101 and PD129337 on transcription of ABCA1, ABCG1 and CHOP. The levels of the transcripts indicated in each graph are plotted in cells that were cultured with the concentrations of ATR-101 or PD129337 indicated at the bottom of the figure for 4 h. The transcript levels were normalized by the RPL9 transcript levels. The graphs show the means and the standard deviations of five samples from four experiments. The statistical significance of the differences in transcript levels in cells that were cultured with each concentration of ATR-101 or PD129337 were evaluated by one-way analysis of variance followed by Dunnett's *post hoc* tests (cells cultured with ATR-101 or PD129337 *vs.* cells cultured with DMSO, *$P < 0.05$).

ATR-101 and PD129337 inhibited liver X receptor target genes (ABCA1 and ABCG1) and activated the ER-stress response gene (CHOP) within 4 h after drug exposure. The effects of PD129337 on these transcripts levels indicate that they are not a result of cytotoxicity.

B. Model for ATR-101 binding to LXRα. A web-based docking program (http://swissdock.eu) (Grosdidier *et al.*, 2007) was used to simulate ATR-101 binding to LXRα (PDB ID: 1UHL). The LXRα ligand binding pocket is displayed in surface area representation. ATR-101 is displayed in stick representation. The ATR-101 docking shown is representative of 24 dockings inside of the ligand binding pocket out of a total of 256 dockings.

Supporting Materials and Methods

Reagents

ATR101, PD129337 (Sigma #PH001507), glibenclamide (Abcam #ab120267), zosuquidar (Sigma #SML1044), benzamil (Sigma #B2417), cyclosporin A (Cayman #12088), rhodamine 123 (Cayman #16672), olesoxime (ToCris #2906), doxorubicin (Cayman #15007), ketoconazole (Sigma #K1003), abiraterone acetate (Cayman #15148), metyrapone (Cayman #14994), trilostane (Cayman #14164), anastrozole (Cayman #11987), and U18666A (Sigma #U3633) were dissolved in DMSO at concentrations ranging from 50 to 250 mM. The final concentration of DMSO for all samples within each experiment was the same and ranged from 0.1 to 0.4% for all experiments. NBD-cholesterol (Molecular Probes #N1148), cholesterol (Sigma #C3045), cholesterol linoleate (Sigma #C0289), and α-tocopherol (Sigma #T3251) were dissolve in ethanol at concentrations ranging from 20 to 240 mM. The final concentration of ethanol for all samples within each experiment was the same and ranged from 0.02 to 0.2% for all experiments. Verapamil (Sigma #V4629), methyl-β-cyclodextrin (Sigma #C4555), and cholesterol:methyl-β-cyclodextrin (Sigma #C4951) were dissolved in the cell culture media.

Cell Culture

The H295R adrenocortical carcinoma cell line was obtained from ATCC. The cells were tested and confirmed to be free of mycoplasma by Radil Inc. The BD140C adrenocortical carcinoma cell line was kindly provided by Dr. Kimberly Bussey (TGen, Pheonix, Arizona). The cell lines were cultured in DMEM/F12 media (Gibco #11330) supplemented with 10% FBS (Atlanta Biologicals #S11595, lots E12069, H1030), and 1% penicillin-streptomycin (Gibco #15140). 7-9 days before each experiment the cells were passed 2 times in DMEM without glucose (Gibco#11966) supplemented with 10 mM galactose, 5% FBS (Atlanta Biologicals # S11595, lots E12069, H1030), 1% penicillin-streptomycin (Gibco #15140), 1% L-glutamate (Gibco #25030), 5 mM sodium HEPES, and 1 mM sodium pyruvate. The total serum cholesterol concentration in the culture medium was 42.7 μM. The cells were allowed to adhere to the tissue culture plates for 48 h after the second passage before the start of each experiment.

Visualization of cholesterol in cells

The cells were seeded in 96-well ibiTreat μ-Plates (Ibidi #89626) in 100 μl at a density of 50,000 cells per well. After 48 h, 20-80 μl media was removed from each well and replaced with 20 μl of each compound diluted to 6X of the final concentration in the culture medium to produce a final volume of 120 μl. The cells were incubated at 37 C in 5% CO2 atmosphere for a time ranging from 15 min to 24 h. The medium was removed and immediately replaced with 100 μl of 4% paraformaldehyde and the cells were fixed at room temperature for 20 min. The cells were washed twice with 200 μl PBS. The freshly prepared filipin III (Cayman #70440) stock solution (10 mg/ml in DMSO) was diluted 100X in PBS for a final concentration of 100 μg/ml and added directly to cells. The fixed cells were incubated with filipin III at 37C in the

dark for 2 h. The cells were washed twice with 100 μl PBS, and the bound filipin III was visualized by fluorescence microscopy using 377±11 nm excitation and 447±60 nm emission wavelengths with a 60X oil objective.

Visualization of cholesterol esterification in cells
The cells were seeded in 96-well ibiTreat μ-Plates (Ibidi #89626) in 100 μl at a density of 50,000 cells per well. After 48 h, 20-80 μl media was removed from each well and replaced with 20 μl of each compound diluted to 6X of the final concentration in the culture medium to produce a final volume of 120 μl. The cells were incubated at 37 C in 5% CO_2 atmosphere for either 2 or 22 h. 5 μl of NBD-cholesterol was added to each well to produce a final concentration of 1 μM. When NBD-cholesterol is esterified and localized to cytoplasmic lipid droplets, it produces bright fluorescent foci in the cell. The inhibition of ACAT activity prevents fluorescent focus formation by NBD-cholesterol (Lada *et al.*, 2004). After 1.5 h, 5 μl of Hoechst 33342 was added to each well to produce a final concentration of 3 μg/ml. After 30 min, the medium was replaced with fresh medium. NBD-cholesterol esterification was visualized by fluorescence microscopy using either 485/20 nm (NBD) or 387/11 nm (Hoechst) excitation wavelengths and images were captured using either a 60X oil objective or a 20X objective.

Cellular ATP level
The cells were seeded in 96-well tissue culture plates (Corning #3585) in 100 μl at a density of 25,000 cells per well. After 48 h, 20-80 μl of medium was removed from each well and replaced with 20 μl of each compound diluted to 6X of the final concentration in the culture medium to produce a final volume of 120 μl. The cells were incubated at 37 C in 5% CO_2 atmosphere for a time ranging from 15 min to 24 h. The medium was removed and immediately replaced with 50 μl of CellTiter-Glo luminescence cell viability assay reagent diluted in buffer according to the manufacturer's protocol (Promega #G7572). The cells were lysed by agitation at room temperature for 20 min in the dark. The luminescence was measured using a SpectraMax M5 microplate reader (Molecular Devices) with a 0.5 s acquisition time. The luminescence (RLU) values for control cells ranged from 5000 to 20000 RLU in all experiments, and were scaled by a factor of 0.001 to plot all graphs.

Caspase 3/7 activity
The cells were seeded in 96-well tissue culture plates (Corning #3585) in 100 μl at a density of 25,000 cells per well. After 48 h, 20-80 μl of medium was removed from each well and replaced with 20 μl of each compound diluted to 6X of the final concentration in the culture medium to produce a final volume of 120 μl. The cells were incubated at 37 C in 5% CO_2 atmosphere for a time ranging from 15 min to 24 h. The medium was removed and immediately replaced with 50 μl of Apo-ONE homogenous caspase-3/7 assay reagent diluted in

buffer according to the manufacturer's protocol (Promega #G7790). The samples were incubated for 18 h at room temperature in the dark. The fluorescence was measured using a SpectraMax M5 microplate reader with a 0.5 s acquisition time. The fluorescence values (RFU) for control cells ranged from 500 to 1000 RFU in all experiments and were scaled by a factor of 0.001 to plot all graphs.

Extracellular cholesterol associated with cells

The cells were seeded in 96-well tissue culture plates (Corning #3585) in 100 µl at a density of 100,000 cells per well. After 48 h, 60 µl of medium was removed from each well and replaced with 10 µl of each compound diluted to 6X of the final concentration in the culture medium to produce a final volume of 60 µl. The cells were incubated at 37 C in 5% CO_2 atmosphere for 4 h. The medium was removed and replaced with 50 µl serum-free medium supplemented with 5 µg/mL apoA-I (without or with 50 µM glibemclamide to detect ABCA1 transporter-dependent efflux) for either 30 sec or 1 h at 37 C in 5% CO_2 atmosphere. After the indicated time, the supernatant, was transferred to a new 96-well plate and a fluorometric-based cholesterol detection reagent (Cayman #10007640) was added.

Cholesterol efflux

The cells were seeded in 96-well tissue culture plates (Corning #3585) in 100 µl at a density of 100,000 cells per well. After 48 h, the medium was removed and replaced with 50 µl serum-free medium supplemented with 5µg/mL apoA-I. After the indicated time, the supernatant containing the effluxed cholesterol, was transferred to a new 96-well plate. A cholesterol detection reagent (Cayman #10007640) consisting of cholesterol assay buffer, cholesterol assay detector (10-acetyl-3,7-dihydroxyphenoxazine [ADHP]), horseradish peroxidase, cholesterol oxidase, and cholesterol esterase was added, and the reactions were incubated for 30 min at 37 C. The resazurin fluorescence intensities were measured using 555 nm excitation and 590 nm emission wavelengths. The cholesterol concentrations were calculated by interpolation between the values produced by cholesterol standards that were analyzed in parallel.

To measure the ATP levels in the same cells that were used to measure cholesterol efflux, 100 µl of CellTiter-Glo luminescence cell viability reagent (Promega #G7572) was added, and the cells were lysed by agitation at room temperature for 20 min in the dark. The luminescence was measured using a SpectraMax M5 microplate reader (Molecular Devices) with a 0.5 s acquisition time. The luminescence (RLU) values for control cells ranged from 5000 to 20000 RLU in all experiments, and were scaled by a factor of 0.001 to plot all graphs.

Cortisol secretion

The cells were seeded in 96-well tissue culture plates (Corning #3585) in 100 µl at a density of 100,000 cells per well. After 48 h, the medium was removed and replaced with 50 µl of fresh medium. After the indicated incubation time, aliquots of the supernatant ranging from 0.5 to 10 µl were diluted in 50 µl of medium were used to measure the amount of cortisol secreted.

Cortisol detection and quantification were performed by indirect ELISA according to the manufacturer's protocol (Arbor Assays #K003).

Doxorubicin clearance

The cells were seeded in 96-well ibiTreat μ-Plates (Ibidi #89626) in 100 μl at a density of 50,000 cells per well. After 48 h, 40-60 μl of medium was removed from each well and replaced with 20 μl of 150 μM doxorubicin and the indicated compounds diluted to 6X of the final concentration in the culture medium, to produce a final volume of 120 μl (final doxorubicin concentration of 25 μM). The cells were incubated at 37 C in 5% CO_2 atmosphere until significant doxorubicin accumulation could be detected in the ATR-101 treated samples relative to control samples (2 h). The medium was removed and immediately replaced with 100 μl of fresh media and intracellular doxorubicin fluorescence was visualized by fluorescence microscopy using a 20X objective.

Transcript measurement

H295R cells were seeded in 6-well tissue culture plates (Corning #3506) at a density of 5 X10^5 per well. After 48 h, the compound(s) indicated were added, and the cells were cultured for the indicated time. The cells were harvested in 1.35 ml culture medium with trypsin. The cells were collected by centrifugation and lysed in 350 μl of RLT buffer (Qiagen) with β-mercaptoethanol. mRNA extraction and DNase treatment were performed according to the manufacturer's protocol (Qiagen). All RNA samples had 260/280 ratios greater than 1.5. The same amount of RNA ranging from 0.1 to 0.5 μg was used for reverse transcription using the Roche Transcriptor First Strand cDNA synthesis kit (Roche #04897030001) using the manufacturer's protocol. SYBR Green I-based real-time qPCR assays were performed using a Roche LightCycler480 instrument. The levels of transcripts in different samples were normalized by the levels of RPL9 transcripts.

Molecular docking simulations

A web-based docking engine (http://swissdock.eu) was used to simulate ATR-101 and PD129337 binding to LXRα (PDB ID: 1UHL). ChemBioOffice was used to create mol2 files of the compounds for docking. The UCSF Chimera dockprep plugin was used to prepare PDB files for docking.

Supporting Table

Table S1. *Primer sequences used for qPCR*

gene	forward primer	reverse primer
ABCA1	ACAATCCTGCAGTGCTTCCT	GGCAGGTACAGCGTGAAGTAG
ABCG1	TGCTTCCACACTGTTGTCCT	CTTGACCATTTCCCTTCTGC
IDOL	CGAGGACTGCCTCAACCA	TGCAGTCCAAAATAGTCAACTTCT
ACTHR	CATGGGCTATCTCAAGCCAC	GAGATCTTCCTGGTGTGGGATC
CYP17A1	GCATCATAGACAACCTGAGCAA	GGGTTTTGTTGGGGAAAATC
SULT2A1	AAGCTGATCTGCCTGTAGCTG	TGGTGTGAGGGTTTCAACTG
HSD3B2	CCAGTAGCATAGAGGTAGCC	TCAGATTCCACCCGTTAGC
CYP21A2	TTGTGGACATGATTCCCTTTC	CTGCTTCTCCTCGTTGTGGT
CHOP	TGTTCAAGAAGGAAGTGTATCTTCA	TGATGCCTGTTTTTGTAGGTAAAG

Table Analyzed   1B. ATP level vs. time, +ATR-101 or PD129337

Two-way ANOVA   Ordinary

Alpha   0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	15.5	<0.0001	****	Yes
Row Factor	41.23	<0.0001	****	Yes
Column Factor	40.78	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	48890256	4	12222564	F (4, 40) = 62.25	P<0.0001
Row Factor	130027513	4	32506878	F (4, 40) = 165.6	P<0.0001
Column Factor	128634753	1	128634753	F (1, 40) = 655.2	P<0.0001
Residual	7853620	40	196340		

Number of missing values   0

Compare each cell mean with the other cell mean in that row

Number of families   1

Number of comparisons per family   5

Alpha   0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
DMSO - ATR-101					
0.25	382.7	-372.9 to 1138	No	ns	0.6285
0.5	2192	1436 to 2947	Yes	****	<0.0001
1	3196	2441 to 3952	Yes	****	<0.0001
2	3886	3130 to 4642	Yes	****	<0.0001
4	6383	5627 to 7139	Yes	****	<0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
DMSO - ATR-101								
0.25	10294	9911	382.7	280.2	5	5	1.366	40
0.5	10067	7876	2192	280.2	5	5	7.82	40
1	9578	6382	3196	280.2	5	5	11.41	40
2	9006	5120	3886	280.2	5	5	13.87	40
4	8582	2199	6383	280.2	5	5	22.78	40

Table Analyzed   1B. Caspase activity vs. time, +ATR-101 or PD129337

Two-way ANOVA   Ordinary

Alpha   0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	5.289	0.0019	**	Yes
Row Factor	30.3	<0.0001	****	Yes
Column Factor	54.14	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	445165	4	111291	F (4, 40) = 5.149	P=0.0019
Row Factor	2550285	4	637571	F (4, 40) = 29.5	P<0.0001
Column Factor	4557453	1	4557453	F (1, 40) = 210.9	P<0.0001
Residual	864565	40	21614		

Number of missing values   0

Compare each cell mean with the other cell mean in that row

Number of families   1

Number of comparisons per family   5

Alpha   0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
DMSO - ATR-101					
0.25 h	-361.3	-612 to -110.6	Yes	**	0.0019
0.5 h	-433.5	-684.3 to -182.8	Yes	***	0.0002
1 h	-599.5	-850.2 to -348.8	Yes	****	<0.0001
2 h	-772	-1023 to -521.3	Yes	****	<0.0001
4 h	-852.7	-1103 to -602	Yes	****	<0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
DMSO - ATR-101								
0.25 h	2080	2441	-361.3	92.98	5	5	3.886	40
0.5 h	2116	2550	-433.5	92.98	5	5	4.663	40
1 h	2193	2792	-599.5	92.98	5	5	6.448	40
2 h	2353	3125	-772	92.98	5	5	8.303	40
4 h	2417	3270	-852.7	92.98	5	5	9.17	40

2way ANOVA of 1D. H295R ATP level vs. ATR-101 or PD129337, 4 h

Table Analyzed	1D. H295R ATP level vs. ATR-101 or PD129337 concentration, 4 h

Two-way ANOVA	Ordinary
Alpha	0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	32.49	<0.0001	****	Yes
Row Factor	40.57	<0.0001	****	Yes
Column Factor	14.63	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	118800799	6	19800133	F (6, 98) = 43.11	P<0.0001
Row Factor	148380193	6	24730032	F (6, 98) = 53.84	P<0.0001
Column Factor	53508617	1	53508617	F (1, 98) = 116.5	P<0.0001
Residual	45010766	98	459294		

Number of missing values	0

Compare each cell mean with the other cell mean in that row

Number of families	1
Number of comparisons per family	7
Alpha	0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
ATR-101 - PD129337					
0	363.3	-565.2 to 1292	No	ns	0.9057
5	107	-821.5 to 1035	No	ns	>0.9999
10	215.6	-712.9 to 1144	No	ns	0.9946
20	51.4	-877.1 to 979.9	No	ns	>0.9999
40	-1404	-2333 to -476	Yes	***	0.0005
60	-4101	-5029 to -3172	Yes	****	<0.0001
100	-4909	-5837 to -3980	Yes	****	<0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
ATR-101 - PD129337								
0	11084	10721	363.3	338.9	8	8	1.072	98
5	10650	10543	107	338.9	8	8	0.3156	98
10	10777	10561	215.6	338.9	8	8	0.6361	98
20	10676	10625	51.4	338.9	8	8	0.1517	98
40	9018	10422	-1404	338.9	8	8	4.145	98
60	6367	10467	-4101	338.9	8	8	12.1	98
100	5338	10247	-4909	338.9	8	8	14.49	98

2way ANOVA 1D. H295R caspase activity vs. ATR-101 or PD129337, 4h

Table Analyzed	1D. H295R caspase activity vs. ATR-101 or PD129337 concentration, 4 h

Two-way ANOVA	Ordinary
Alpha	0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	32.05	<0.0001	****	Yes
Row Factor	31.17	<0.0001	****	Yes
Column Factor	34.46	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	11845810	6	1974302	F (6, 98) = 225.5	P<0.0001
Row Factor	11519967	6	1919994	F (6, 98) = 219.3	P<0.0001
Column Factor	12736858	1	12736858	F (1, 98) = 1455	P<0.0001
Residual	857839	98	8753		

Number of missing values	0

Compare each cell mean with the other cell mean in that row

Number of families	1
Number of comparisons per family	7
Alpha	0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
ATR-101 - PD129337					
0	22.67	-105.5 to 150.8	No	ns	0.999
5	52.03	-76.15 to 180.2	No	ns	0.8882
10	57.67	-70.51 to 185.8	No	ns	0.8253
20	409.6	281.4 to 537.8	Yes	****	<0.0001
40	1125	997.2 to 1254	Yes	****	<0.0001
60	1382	1254 to 1510	Yes	****	<0.0001
100	1672	1543 to 1800	Yes	****	<0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
ATR-101 - PD129337								
0	1037	1014	22.67	46.78	8	8	0.4845	98
5	1091	1039	52.03	46.78	8	8	1.112	98
10	1110	1052	57.67	46.78	8	8	1.233	98
20	1423	1013	409.6	46.78	8	8	8.755	98
40	2136	1010	1125	46.78	8	8	24.06	98
60	2420	1038	1382	46.78	8	8	29.55	98
100	2687	1016	1672	46.78	8	8	35.73	98

2way ANOVA of 1D. BD140C ATP level vs. ATR-101 or PD129337, 4 h

Table Analyzed	1D. BD140C ATP level vs. ATR-101 or PD129337 concentration, 4 h

Two-way ANOVA	Ordinary
Alpha	0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	21.45	<0.0001	****	Yes
Row Factor	24.99	<0.0001	****	Yes
Column Factor	41.76	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	42381243	5	8476249	F (5, 60) = 21.81	P<0.0001
Row Factor	49384400	5	9876880	F (5, 60) = 25.41	P<0.0001
Column Factor	82532640	1	82532640	F (1, 60) = 212.4	P<0.0001
Residual	23318048	60	388634		

Number of missing values	0

Compare each cell mean with the other cell mean in that row

Number of families	1
Number of comparisons per family	6
Alpha	0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
ATR-101 - PD129337					
0	-144.8	-1124 to 834.4	No	ns	0.9991
5	-446.8	-1426 to 532.4	No	ns	0.7736
10	-1660	-2640 to -681.2	Yes	***	0.0001
20	-2716	-3695 to -1737	Yes	****	<0.0001
40	-3595	-4574 to -2616	Yes	****	<0.0001
100	-4285	-5264 to -3306	Yes	****	<0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
ATR-101 - PD129337								
0	6717	6862	-144.8	359.9	6	6	0.4023	60
5	6269	6716	-446.8	359.9	6	6	1.241	60
10	5115	6775	-1660	359.9	6	6	4.613	60
20	3901	6617	-2716	359.9	6	6	7.546	60
40	3084	6679	-3595	359.9	6	6	9.988	60
100	2378	6663	-4285	359.9	6	6	11.91	60

2way ANOVA of 1D. BD140C caspase activity vs. ATR-101 or PD129337, 4h

Table Analyzed	1D. BD140C caspase activity vs. ATR-101 or PD129337 concentration, 4 h

| Two-way ANOVA | Ordinary |
| Alpha | 0.05 |

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	31.43	<0.0001	****	Yes
Row Factor	31.32	<0.0001	****	Yes
Column Factor	34.97	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	5574417	4	1393604	F (4, 50) = 172.8	P<0.0001
Row Factor	5553960	4	1388490	F (4, 50) = 172.2	P<0.0001
Column Factor	6202466	1	6202466	F (1, 50) = 769	P<0.0001
Residual	403263	50	8065		

Number of missing values	0

Compare each cell mean with the other cell mean in that row

Number of families	1
Number of comparisons per family	5
Alpha	0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
ATR-101 - PD129337					
0	13.28	-125.2 to 151.7	No	ns	0.9997
5	65.31	-73.12 to 203.7	No	ns	0.6993
10	458	319.6 to 596.4	Yes	****	<0.0001
20	1090	951.9 to 1229	Yes	****	<0.0001
40	1588	1450 to 1727	Yes	****	<0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
ATR-101 - PD129337								
0	1021	1008	13.28	51.85	6	6	0.2561	50
5	1082	1017	65.31	51.85	6	6	1.26	50
10	1498	1040	458	51.85	6	6	8.833	50
20	2105	1015	1090	51.85	6	6	21.03	50
40	2602	1014	1588	51.85	6	6	30.63	50

Table Analyzed	2B. ATP level vs. MBCD concentration, 4h, +ATR-101				
Data sets analyzed	A : 0	B : 0.25	C : 0.5	D : 1	E : 1.5

ANOVA summary

F	97.79
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.9532

Brown-Forsythe test

F (DFn, DFd)	0.5473 (5, 24)
P value	0.7387
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test

Bartlett's statistic (corrected)	1.416
P value	0.9226
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	477591874	5	95518375	F (5, 24) = 97.79	P<0.0001
Residual (within columns)	23443645	24	976819		
Total	501035519	29			

Data summary

Number of treatments (columns)	6
Number of values (total)	30
Number of families	1
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
0 vs. 0.25	172.4	-1512 to 1857	No	ns	0.9985	B	0.25
0 vs. 0.5	-910.2	-2595 to 774.7	No	ns	0.468	C	0.5
0 vs. 1	-6437	-8122 to -4752	Yes	****	0.0001	D	1
0 vs. 1.5	-8231	-9916 to -6546	Yes	****	0.0001	E	1.5
0 vs. 2	-9369	-11053 to -7684	Yes	****	0.0001	F	2

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 0.25	3416	3244	172.4	625.1	5	5	0.2758	24
0 vs. 0.5	3416	4327	-910.2	625.1	5	5	1.456	24
0 vs. 1	3416	9853	-6437	625.1	5	5	10.3	24
0 vs. 1.5	3416	11648	-8231	625.1	5	5	13.17	24
0 vs. 2	3416	12785	-9369	625.1	5	5	14.99	24

Table Analyzed	2B. Caspase activity vs. MBCD concentration, 4h, +ATR-101				
Data sets analyzed	A : 0	B : 0.25	C : 0.5	D : 1	E : 1.5

ANOVA summary

F	93.22
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.951

Brown-Forsythe test

F (DFn, DFd)	1.107 (5, 24)
P value	0.383
P value summary	ns

Are SDs significantly different (P < 0.05)? No

Bartlett's test
Bartlett's statistic (corrected)   18.9
P value   0.002
P value summary   **
Are SDs significantly different (P < 0.05)? Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	39075912	5	7815182	F (5, 24) = 93.22	P<0.0001
Residual (within columns)	2012102	24	83838		
Total	41088014	29			

Data summary
Number of treatments (columns)   6
Number of values (total)   30

Number of families   1
Number of comparisons per family   5
Alpha   0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?
0 vs. 0.25	-154.4	-648 to 339.2	No	ns	0.8659 B	0.25
0 vs. 0.5	1101	607.9 to 1595	Yes	****	0.0001 C	0.5
0 vs. 1	2132	1638 to 2625	Yes	****	0.0001 D	1
0 vs. 1.5	2547	2053 to 3040	Yes	****	0.0001 E	1.5
0 vs. 2	2626	2132 to 3120	Yes	****	0.0001 F	2

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 0.25	3632	3787	-154.4	183.1	5	5	0.8433	24
0 vs. 0.5	3632	2531	1101	183.1	5	5	6.015	24
0 vs. 1	3632	1501	2132	183.1	5	5	11.64	24
0 vs. 1.5	3632	1086	2547	183.1	5	5	13.91	24
0 vs. 2	3632	1006	2626	183.1	5	5	14.34	24

Ordinary one-way ANOVA of 2D. ATP level vs. cholesterol concentration, 24 h, +AT

Table Analyzed   2D. ATP level vs. cholesterol concentration, 24 h, +ATR-101
Data sets analyzed   A : 0   B : 1   C : 2.5   D : 10   E : 20

ANOVA summary
F   83.04
P value   <0.0001
P value summary   ****
Significant diff. among means (P < 0.05)? Yes
R square   0.9454

Brown-Forsythe test
F (DFn, DFd)   0.0787 (5, 24)
P value   0.9949
P value summary   ns
Are SDs significantly different (P < 0.05)? No

Bartlett's test
Bartlett's statistic (corrected)   1.448
P value   0.919
P value summary   ns
Are SDs significantly different (P < 0.05)? No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	615675479	5	123135096	F (5, 24) = 83.04	P<0.0001
Residual (within columns)	35589813	24	1482909		
Total	651265291	29			

Data summary
Number of treatments (columns)   6
Number of values (total)   30

Number of families	1
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
0 vs. 1	-2316	-4392 to -240.1	Yes	*	0.0251	B	1
0 vs. 2.5	-8678	-10754 to -6602	Yes	****	0.0001	C	2.5
0 vs. 10	-11065	-13141 to -8989	Yes	****	0.0001	D	10
0 vs. 20	-11107	-13183 to -9031	Yes	****	0.0001	E	20
0 vs. 40	-11104	-13180 to -9028	Yes	****	0.0001	F	40

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 1	880.6	3197	-2316	770.2	5	5	3.007	24
0 vs. 2.5	880.6	9558	-8678	770.2	5	5	11.27	24
0 vs. 10	880.6	11946	-11065	770.2	5	5	14.37	24
0 vs. 20	880.6	11987	-11107	770.2	5	5	14.42	24
0 vs. 40	880.6	11985	-11104	770.2	5	5	14.42	24

Ordinary one-way ANOVA of 2D. Caspase activity vs. cholesterol concentration, 24

Table Analyzed	2D. Caspase activity vs. cholesterol concentration, 24 h, +ATR-101			
Data sets analyzed	A : 0	B : 1	C : 2.5	D : 10	E : 20

ANOVA summary
F	69.42
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.9353

Brown-Forsythe test
F (DFn, DFd)	1.273 (5, 24)
P value	0.3078
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test
Bartlett's statistic (corrected)	7.627
P value	0.178
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	92469004	5	18493801	F (5, 24) = 69.42	P<0.0001
Residual (within columns)	6394168	24	266424		
Total	98863172	29			

Data summary
Number of treatments (columns)	6
Number of values (total)	30

Number of families	1
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
0 vs. 1	-2774	-3654 to -1895	Yes	****	0.0001	B	1
0 vs. 2.5	-111.5	-991.4 to 768.4	No	ns	0.9964	C	2.5
0 vs. 10	1547	667.5 to 2427	Yes	***	0.0004	D	10
0 vs. 20	1877	997.4 to 2757	Yes	****	0.0001	E	20
0 vs. 40	2538	1658 to 3418	Yes	****	0.0001	F	40

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF

0 vs. 1	4391	7166	-2774	326.4	5	5	8.499	24
0 vs. 2.5	4391	4503	-111.5	326.4	5	5	0.3415	24
0 vs. 10	4391	2844	1547	326.4	5	5	4.74	24
0 vs. 20	4391	2514	1877	326.4	5	5	5.751	24
0 vs. 40	4391	1853	2538	326.4	5	5	7.776	24

Ordinary one-way ANOVA of 2F. Insoluble external cholesterol vs. compound, 4h

Table Analyzed	2F. Insoluble external cholesterol vs. compound, 4h
Data sets analyzed	A : DMSO B : ATR-101 C : choleste D : cholesterol+ATR-101

ANOVA summary

F	144.8
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.9645

Brown-Forsythe test

F (DFn, DFd)	1.841 (3, 16)
P value	0.1805
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test

Bartlett's statistic (corrected)	10.13
P value	0.0175
P value summary	*
Are SDs significantly different (P < 0.05)?	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	35.74	3	11.91	F (3, 16) = 144.8	P<0.0001
Residual (within columns)	1.316	16	0.08226		
Total	37.05	19			

Data summary

Number of treatments (columns)	4
Number of values (total)	20
Number of families	1
Number of comparisons per family	3
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
DMSO vs. ATR-101	-1.006	-1.477 to -0.5362	Yes	***	0.0001	B	ATR-101
DMSO vs. cholesterol	-1.319	-1.79 to -0.8491	Yes	****	0.0001	C	cholesterol
DMSO vs. cholesterol+ATR-101	-3.65	-4.12 to -3.179	Yes	****	0.0001	D	cholesterol+ATR-101

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
DMSO vs. ATR-101	0.4236	1.43	-1.006	0.1814	5	5	5.549	16
DMSO vs. cholesterol	0.4236	1.743	-1.319	0.1814	5	5	7.274	16
DMSO vs. cholesterol+ATR-101	0.4236	4.073	-3.65	0.1814	5	5	20.12	16

Ordinary one-way ANOVA of 2F. Soluble external cholesterol vs. time

Table Analyzed	2F. Soluble external cholesterol vs. time
Data sets analyzed	A : 0 B : 1 C : 2 D : 4 E : 6

ANOVA summary

F	65.3
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.9289

Brown-Forsythe test

F (DFn, DFd)	3.593 (4, 20)

P value		0.023		
P value summary	*			
Are SDs significantly different (P < 0.05)?	Yes			

Bartlett's test

Bartlett's statistic (corrected)		17.09	
P value		0.0019	
P value summary	**		
Are SDs significantly different (P < 0.05)?	Yes		

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	25.21	4	6.302	F (4, 20) = 65.3	P<0.0001
Residual (within columns)	1.93	20	0.0965		
Total	27.14	24			

Data summary

Number of treatments (columns)	5
Number of values (total)	25

Number of families	1
Number of comparisons per family	4
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
0 vs. 1	-0.389	-0.9099 to 0.1318	No	ns	0.1817	B	1
0 vs. 2	-0.8909	-1.412 to -0.37	Yes	***	0.0008	C	2
0 vs. 4	-1.957	-2.478 to -1.436	Yes	****	0.0001	D	4
0 vs. 6	-2.708	-3.229 to -2.188	Yes	****	0.0001	E	6

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 1	0.4231	0.8121	-0.389	0.1965	5	5	1.98	20
0 vs. 2	0.4231	1.314	-0.8909	0.1965	5	5	4.535	20
0 vs. 4	0.4231	2.38	-1.957	0.1965	5	5	9.961	20
0 vs. 6	0.4231	3.132	-2.708	0.1965	5	5	13.79	20

	Unpaired t test of 3B. ATP level vs. MBCD, serum-free media, +ATR-101	

Table Analyzed	3B. ATP level vs. MBCD, serum-free media, +ATR-101
Column B	ATR-101 + MBCD
vs.	vs.
Column A	ATR-101
Unpaired t test	
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=18.73 df=14
How big is the difference?	
Mean ± SEM of column A	5415 ± 164.5, n=8
Mean ± SEM of column B	13145 ± 378.4, n=8
Difference between means	7730 ± 412.6
95% confidence interval	6845 to 8615
R squared (eta squared)	0.9616
F test to compare variances	
F, DFn, Dfd	5.29, 7, 7
P value	0.043
P value summary	*
Significantly different (P < 0.05)?	Yes

	Unpaired t test of 3B. Caspase activity vs. MBCD, serum-free media, +ATR-101	

Table Analyzed	3B. Caspase activity vs. MBCD, serum-free media, +ATR-101
Column B	ATR-101 + MBCD
vs.	vs.
Column A	ATR-101
Unpaired t test	
P value	0.0002
P value summary	***
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=5.573 df=10
How big is the difference?	
Mean ± SEM of column A	2197 ± 65.23, n=6
Mean ± SEM of column B	1736 ± 50.83, n=6
Difference between means	-460.9 ± 82.7
95% confidence interval	-645.1 to -276.6
R squared (eta squared)	0.7565
F test to compare variances	
F, DFn, Dfd	1.647, 5, 5
P value	0.5975
P value summary	ns
Significantly different (P < 0.05)?	No

	2way ANOVA of 3C. Cholesterol efflux vs. time, +ATR-101 or DMSO	

Table Analyzed	3C. Cholesterol efflux vs. time, +ATR-101 or DMSO
Two-way ANOVA	Ordinary
Alpha	0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	30.16	<0.0001	****	Yes
Row Factor	41.15	<0.0001	****	Yes
Column Factor	22.61	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	3493071	4	873268	F (4, 50) = 62	P<0.0001
Row Factor	4765266	4	1191317	F (4, 50) = 84.58	P<0.0001
Column Factor	2618092	1	2618092	F (1, 50) = 185.9	P<0.0001
Residual	704234	50	14085		

Number of missing values   0

Compare each cell mean with the other cell mean in that row

Number of families   1
Number of comparisons per family   5
Alpha   0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
DMSO - ATR-101					
0.25	-25.72	-208.7 to 157.2	No	ns	0.9979
0.5	37.93	-145 to 220.9	No	ns	0.9873
1	175.4	-7.508 to 358.4	No	ns	0.0658
2	636.9	454 to 819.9	Yes	****	<0.0001
4	1264	1081 to 1447	Yes	****	<0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
DMSO - ATR-101								
0.25	-102.5	-76.76	-25.72	68.52	6	6	0.3754	50
0.5	-36.77	-74.7	37.93	68.52	6	6	0.5536	50
1	141.9	-33.5	175.4	68.52	6	6	2.56	50
2	573.4	-63.51	636.9	68.52	6	6	9.296	50
4	1310	45.91	1264	68.52	6	6	18.45	50

2way ANOVA of 3C. ATP level vs. time, +ATR-101 or DMSO

Table Analyzed   3C. ATP level vs. time, +ATR-101 or DMSO

Two-way ANOVA   Ordinary
Alpha   0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	14.26	<0.0001	****	Yes
Row Factor	42.94	<0.0001	****	Yes
Column Factor	20.48	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	92346410	4	23086603	F (4, 50) = 7.989	P<0.0001
Row Factor	278007030	4	69501757	F (4, 50) = 24.05	P<0.0001
Column Factor	132583040	1	132583040	F (1, 50) = 45.88	P<0.0001
Residual	144481981	50	2889640		

Number of missing values   0

Compare each cell mean with the other cell mean in that row

Number of families   1
Number of comparisons per family   5
Alpha   0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
DMSO - ATR-101					
0.25	-254.6	-2875 to 2366	No	ns	0.9997
0.5	1284	-1336 to 3905	No	ns	0.6654
1	3133	512.7 to 5753	Yes	*	0.0121
2	3594	973.4 to 6214	Yes	**	0.003
4	7108	4488 to 9729	Yes	****	<0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
DMSO - ATR-101								
0.25	17814	18068	-254.6	981.4	6	6	0.2594	50
0.5	18676	17392	1284	981.4	6	6	1.309	50
1	18080	14947	3133	981.4	6	6	3.192	50
2	16711	13117	3594	981.4	6	6	3.662	50
4	15834	8726	7108	981.4	6	6	7.243	50

Table Analyzed	3D. Cholesterol efflux vs. ATR-101 concentration				
Data sets analyzed	A : 0	B : 5	C : 10	D : 20	E : 40

ANOVA summary

F	67.06
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.92

Brown-Forsythe test

F (DFn, DFd)	7.456 (6, 35)
P value	<0.0001
P value summary	****
Are SDs significantly different (P < 0.05)?	Yes

Bartlett's test

Bartlett's statistic (corrected)	14.97
P value	0.0205
P value summary	*
Are SDs significantly different (P < 0.05)?	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	7175657	6	1195943	F (6, 35) = 67.06	P<0.0001
Residual (within columns)	624209	35	17835		
Total	7799866	41			

Data summary

Number of treatments (columns)	7
Number of values (total)	42
Number of families	1
Number of comparisons per family	6
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P \ A-?	
0 vs. 5	13.07	-194.9 to 221	No	ns	0.9997	B	5
0 vs. 10	46.01	-162 to 254	No	ns	0.9785	C	10
0 vs. 20	318.4	110.5 to 526.4	Yes	**	0.0012	D	20
0 vs. 40	502.4	294.4 to 710.3	Yes	****	0.0001	E	40
0 vs. 60	823.2	615.2 to 1031	Yes	****	0.0001	F	60
0 vs. 100	1152	943.8 to 1360	Yes	****	0.0001	G	100

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 5		1402	1389	13.07	77.1	6	6	0.1695	35
0 vs. 10		1402	1356	46.01	77.1	6	6	0.5967	35
0 vs. 20		1402	1083	318.4	77.1	6	6	4.13	35
0 vs. 40		1402	899.5	502.4	77.1	6	6	6.516	35
0 vs. 60		1402	578.8	823.2	77.1	6	6	10.68	35
0 vs. 100		1402	250.2	1152	77.1	6	6	14.94	35

Table Analyzed	3D. Cholesterol efflux vs. PD129337 concentration				
Data sets analyzed	A : 0	B : 5	C : 10	D : 20	E : 40

ANOVA summary

F	0.7695
P value	0.599
P value summary	ns
Significant diff. among means (P < 0.05)?	No
R square	0.1165

Brown-Forsythe test

F (DFn, DFd)	0.1936 (6, 35)
P value	0.9766
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test
Bartlett's statistic (corrected)   2.454
P value   0.8735
P value summary   ns
Are SDs significantly different (P < 0.05)? No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	41572	6	6929	F (6, 35) = 0.7695	P=0.5990
Residual (within columns)	315127	35	9004		
Total	356699	41			

Data summary
Number of treatments (columns)   7
Number of values (total)   42

Number of families   1
Number of comparisons per family   6
Alpha   0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P \ A-?	
0 vs. 5	-33.5	-181.3 to 114.3	No	ns	0.9759 B	5
0 vs. 10	-19.3	-167.1 to 128.5	No	ns	0.998 C	10
0 vs. 20	-92.71	-240.5 to 55.05	No	ns	0.3604 D	20
0 vs. 40	-66.35	-214.1 to 81.41	No	ns	0.6798 E	40
0 vs. 60	-7.596	-155.4 to 140.2	No	ns	0.9998 F	60
0 vs. 100	-14.82	-162.6 to 132.9	No	ns	0.9996 G	100

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 5	1353	1387	-33.5	54.78	6	6	0.6115	35
0 vs. 10	1353	1372	-19.3	54.78	6	6	0.3524	35
0 vs. 20	1353	1446	-92.71	54.78	6	6	1.692	35
0 vs. 40	1353	1419	-66.35	54.78	6	6	1.211	35
0 vs. 60	1353	1361	-7.596	54.78	6	6	0.1386	35
0 vs. 100	1353	1368	-14.82	54.78	6	6	0.2705	35

Ordinary one-way ANOVA of 3D. Cholesterol efflux vs. glibenclamide concentratio

Table Analyzed	3D. Cholesterol efflux vs. glibenclamide concentration				
Data sets analyzed	A : 0	B : 50	C : 150	D : 300	E : 450

ANOVA summary
F   817.7
P value   <0.0001
P value summary   ****
Significant diff. among means (P < 0.05)? Yes
R square   0.9927

Brown-Forsythe test
F (DFn, DFd)   2.284 (5, 30)
P value   0.0714
P value summary   ns
Are SDs significantly different (P < 0.05)? No

Bartlett's test
Bartlett's statistic (corrected)   7.631
P value   0.1778
P value summary   ns
Are SDs significantly different (P < 0.05)? No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	11451678	5	2290336	F (5, 30) = 817.7	P<0.0001
Residual (within columns)	84032	30	2801		
Total	11535710	35			

Data summary
Number of treatments (columns)   6
Number of values (total)   36

Number of families   1

Number of comparisons per family		5							
Alpha		0.05							

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary		Adjusted P \ A-?			
0 vs. 50		1408 1327 to 1489	Yes	****		0.0001 B		50	
0 vs. 150		1514 1433 to 1595	Yes	****		0.0001 C		150	
0 vs. 300		1502 1420 to 1583	Yes	****		0.0001 D		300	
0 vs. 450		1565 1484 to 1646	Yes	****		0.0001 E		450	
0 vs. 600		1549 1468 to 1630	Yes	****		0.0001 F		600	

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 50	1397	-10.35	1408	30.56	6	6	46.07	30
0 vs. 150	1397	-116.4	1514	30.56	6	6	49.54	30
0 vs. 300	1397	-104.3	1502	30.56	6	6	49.14	30
0 vs. 450	1397	-167.3	1565	30.56	6	6	51.21	30
0 vs. 600	1397	-151.9	1549	30.56	6	6	50.7	30

Ordinary one-way ANOVA of 3D. ATP levels vs. ATR-101 concentration, serum free m

Table Analyzed	3D. ATP level vs. ATR-101 concentration, serum-free media				
Data sets analyzed	A : 0	B : 5	C : 10	D : 20	E : 40

ANOVA summary
F	41.68
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.8772

Brown-Forsythe test
F (DFn, DFd)	2.223 (6, 35)
P value	0.0639
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test
Bartlett's statistic (corrected)	7.19
P value	0.3037
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	60144755	6	10024126	F (6, 35) = 41.68	P<0.0001
Residual (within columns)	8417575	35	240502		
Total	68562329	41			

Data summary
Number of treatments (columns)	7
Number of values (total)	42

Number of families	1
Number of comparisons per family	6
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary		Adjusted P \ A-?			
0 vs. 5		401.8 -361.9 to 1165	No	ns		0.5334 B		5	
0 vs. 10		763.3 -0.3403 to 1527	No	ns		0.0501 C		10	
0 vs. 20		1220 455.9 to 1983	Yes	***		0.0007 D		20	
0 vs. 40		2105 1341 to 2869	Yes	****		0.0001 E		40	
0 vs. 60		2556 1792 to 3320	Yes	****		0.0001 F		60	
0 vs. 100		3614 2850 to 4378	Yes	****		0.0001 G		100	

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 5	8589	8187	401.8	283.1	6	6	1.419	35
0 vs. 10	8589	7826	763.3	283.1	6	6	2.696	35
0 vs. 20	8589	7369	1220	283.1	6	6	4.307	35
0 vs. 40	8589	6484	2105	283.1	6	6	7.434	35

	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 60	8589	6033	2556	283.1	6	6	9.027	35
0 vs. 100	8589	4975	3614	283.1	6	6	12.76	35

Ordinary one-way ANOVA of 3D. ATP levels vs. PD129337 concentration, serum-free

Table Analyzed	3D. ATP level vs. PD129337 concentration, serum-free media				
Data sets analyzed	A : 0	B : 5	C : 10	D : 20	E : 40

ANOVA summary
F	0.6836
P value	0.6639
P value summary	ns
Significant diff. among means (P < 0.05)?	No
R square	0.1049

Brown-Forsythe test
F (DFn, DFd)	0.001965 (6, 35)
P value	>0.9999
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test
Bartlett's statistic (corrected)	0.1173
P value	>0.9999
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	231107	6	38518	F (6, 35) = 0.6836	P=0.6639
Residual (within columns)	1972087	35	56345		
Total	2203195	41			

Data summary
Number of treatments (columns)	7
Number of values (total)	42
Number of families	1
Number of comparisons per family	6
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P \ A-?	
0 vs. 5	120.1	-249.5 to 489.7	No	ns	0.8875 B	5
0 vs. 10	2.009	-367.6 to 371.6	No	ns	0.9999 C	10
0 vs. 20	-83.31	-452.9 to 286.3	No	ns	0.9766 D	20
0 vs. 40	109.6	-260 to 479.3	No	ns	0.9214 E	40
0 vs. 60	39.03	-330.6 to 408.7	No	ns	0.9996 F	60
0 vs. 100	136.3	-233.3 to 506	No	ns	0.8231 G	100

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 5	8465	8345	120.1	137	6	6	0.8764	35
0 vs. 10	8465	8463	2.009	137	6	6	0.01466	35
0 vs. 20	8465	8549	-83.31	137	6	6	0.6079	35
0 vs. 40	8465	8356	109.6	137	6	6	0.7999	35
0 vs. 60	8465	8426	39.03	137	6	6	0.2848	35
0 vs. 100	8465	8329	136.3	137	6	6	0.9948	35

Ordinary one-way ANOVA of 3D. ATP levels vs. glibenclamide concentration, serum-

Table Analyzed	3D. ATP level vs. glibenclamide concentration, serum-free media				
Data sets analyzed	A : 0	B : 50	C : 150	D : 300	E : 450

ANOVA summary
F	65.19
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.9157

Brown-Forsythe test
F (DFn, DFd)	0.002474 (5, 30)

P value	>0.9999						
P value summary	ns						
Are SDs significantly different (P < 0.05)?	No						

Bartlett's test

Bartlett's statistic (corrected)	0.2514
P value	0.9985
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	22703932	5	4540786	F (5, 30) = 65.19	P<0.0001
Residual (within columns)	2089635	30	69654		
Total	24793566	35			

Data summary

Number of treatments (columns)	6
Number of values (total)	36
Number of families	1
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P \ A-?	
0 vs. 50	-164.4	-569.3 to 240.4	No	ns	0.722 B	50
0 vs. 150	10.96	-393.9 to 415.8	No	ns	0.9999 C	150
0 vs. 300	-67.8	-472.7 to 337.1	No	ns	0.9893 D	300
0 vs. 450	583.5	178.7 to 988.4	Yes	**	0.0028 E	450
0 vs. 600	2104	1699 to 2508	Yes	****	0.0001 F	600

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 50	8515	8679	-164.4	152.4	6	6	1.079	30
0 vs. 150	8515	8504	10.96	152.4	6	6	0.0719	30
0 vs. 300	8515	8583	-67.8	152.4	6	6	0.445	30
0 vs. 450	8515	7931	583.5	152.4	6	6	3.829	30
0 vs. 600	8515	6411	2104	152.4	6	6	13.81	30

2way ANOVA of 3E. Cholesterol efflux vs. ATR-101 concentration, +olesoxime or DM

Table Analyzed	3E. Cholesterol efflux vs. ATR-101 concentration, +olesoxime or DMSO
Two-way ANOVA	Ordinary
Alpha	0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	0.5951	0.4837	ns	No
Row Factor	89.77	<0.0001	****	Yes
Column Factor	1.164	0.0116	*	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	43941	4	10985	F (4, 50) = 0.8782	P=0.4837
Row Factor	6628615	4	1657154	F (4, 50) = 132.5	P<0.0001
Column Factor	85927	1	85927	F (1, 50) = 6.869	P=0.0116
Residual	625448	50	12509		

Number of missing values	0

Compare each cell mean with the other cell mean in that row

Number of families	1
Number of comparisons per family	5
Alpha	0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
DMSO - Olesoxime					
0	-104.8	-277.2 to 67.56	No	ns	0.4439
12.5	-57.55	-230 to 114.9	No	ns	0.9062
25	12.09	-160.3 to 184.5	No	ns	>0.9999
50	-76.53	-248.9 to 95.87	No	ns	0.749

| 100 | | | -151.6 | -324 to 20.81 | No | | ns | | 0.1093 | |

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
DMSO - Olesoxime								
0	1802	1907	-104.8	64.57	6	6	1.624	50
12.5	1102	1160	-57.55	64.57	6	6	0.8913	50
25	982.9	970.9	12.09	64.57	6	6	0.1873	50
50	948.4	1025	-76.53	64.57	6	6	1.185	50
100	969.6	1121	-151.6	64.57	6	6	2.348	50

2way ANOVA of 3E. ATP level vs. ATR-101 concentration, +olesoxime or DMSO

Table Analyzed	3E. ATP level vs. ATR-101 concentration, +olesoxime or DMSO

Two-way ANOVA	Ordinary
Alpha	0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	7.125	<0.0001	****	Yes
Row Factor	85.49	<0.0001	****	Yes
Column Factor	4.682	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	87336244	4	21834061	F (4, 80) = 52.78	P<0.0001
Row Factor	1047881833	4	261970458	F (4, 80) = 633.3	P<0.0001
Column Factor	57392114	1	57392114	F (1, 80) = 138.7	P<0.0001
Residual	33092617	80	413658		

Number of missing values	0

Compare each cell mean with the other cell mean in that row

Number of families	1
Number of comparisons per family	5
Alpha	0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
DMSO - Olesoxime					
0	391.6	-406.1 to 1189	No	ns	0.6728
12.5	-1531	-2329 to -733.6	Yes	****	<0.0001
25	-5289	-6087 to -4491	Yes	****	<0.0001
50	-1267	-2065 to -469.2	Yes	***	0.0004
100	-289.8	-1088 to 507.9	No	ns	0.8767

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
DMSO - Olesoxime								
0	10492	10100	391.6	303.2	9	9	1.292	80
12.5	7367	8898	-1531	303.2	9	9	5.051	80
25	2786	8075	-5289	303.2	9	9	17.44	80
50	1563	2830	-1267	303.2	9	9	4.179	80
100	1188	1478	-289.8	303.2	9	9	0.9558	80

2way ANOVA of 4A. Cortisol secretion vs. time, +ATR-101 or DMSO, +DMSO					

Table Analyzed — 4A. Cortisol secretion vs. time, +ATR-101 or DMSO, +forskolin or DMSO

Two-way ANOVA — Ordinary
Alpha — 0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	41.3	<0.0001	****	Yes
Row Factor	31.73	<0.0001	****	Yes
Column Factor	23.06	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	442452	3	147484	F (3, 32) = 112.8	P<0.0001
Row Factor	339943	3	113314	F (3, 32) = 86.64	P<0.0001
Column Factor	247018	1	247018	F (1, 32) = 188.9	P<0.0001
Residual	41853	32	1308		

Number of missing values — 0

Compare each cell mean with the other cell mean in that row

Number of families	1
Number of comparisons per family	4
Alpha	0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
DMSO - ATR-101					
0.25	-15	-75.36 to 45.36	No	ns	0.9454
1	19.04	-41.32 to 79.39	No	ns	0.88
4	112.2	51.82 to 172.5	Yes	***	0.0001
8	512.5	452.1 to 572.8	Yes	****	<0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
DMSO - ATR-101								
0.25	82.15	97.15	-15	22.87	5	5	0.6559	32
1	102.7	83.65	19.04	22.87	5	5	0.8323	32
4	198.4	86.2	112.2	22.87	5	5	4.904	32
8	572	59.54	512.5	22.87	5	5	22.4	32

2way ANOVA of 4A. Cortisol secretion vs. time, +ATR-101 or DMSO, +forskolin					

Table Analyzed — 4A. Cortisol secretion vs. time, +ATR-101 or DMSO, +forskolin or DMSO

Two-way ANOVA — Ordinary
Alpha — 0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	37.52	<0.0001	****	Yes
Row Factor	34.43	<0.0001	****	Yes
Column Factor	25.18	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	1274022	3	424674	F (3, 32) = 139.7	P<0.0001
Row Factor	1169056	3	389685	F (3, 32) = 128.2	P<0.0001
Column Factor	855130	1	855130	F (1, 32) = 281.3	P<0.0001
Residual	97274	32	3040		

Number of missing values — 0

Compare each cell mean with the other cell mean in that row

Number of families	1
Number of comparisons per family	4
Alpha	0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value

DMSO+forskolin - ATR-101+forskolin

0.25	-9.815 -101.8 to 82.2	No	ns	0.9977	
1	20.46 -71.56 to 112.5	No	ns	0.963	
4	280 188 to 372	Yes	****	<0.0001	
8	879.1 787 to 971.1	Yes	****	<0.0001	

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
DMSO+forskolin - ATR-101+forskolin								
0.25	75.49	85.3	-9.815	34.87	5	5	0.2815	32
1	106.3	85.8	20.46	34.87	5	5	0.5869	32
4	376.4	96.37	280	34.87	5	5	8.03	32
8	946.2	67.12	879.1	34.87	5	5	25.21	32

2way ANOVA of 4B. Cortisol secretion vs. ATR-101 or PD129337 concentration

Table Analyzed	4B. Cortisol secretion vs. ATR-101 or PD129337 concentration
Two-way RM ANOVA	Matching: Stacked
Alpha	0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	44.81	<0.0001	****	Yes
Time	7.813	<0.0001	****	Yes
Column Factor	34.81	<0.0001	****	Yes
Subjects (matching)	6.469	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	106416	5	21283	F (5, 50) = 73.41	P<0.0001
Time	18558	5	3712	F (5, 50) = 12.8	P<0.0001
Column Factor	82673	1	82673	F (1, 10) = 53.81	P<0.0001
Subjects (matching)	15364	10	1536	F (10, 50) = 5.299	P<0.0001
Residual	14496	50	289.9		

Number of missing values	0

Within each column, compare rows (simple effects within columns)

Number of families	2
Number of comparisons per family	5
Alpha	0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
ATR-101					
0 vs. 1	-17.06	-43.31 to 9.187	No	ns	0.372
0 vs. 10	6.291	-19.96 to 32.54	No	ns	0.9759
0 vs. 20	36.26	10.01 to 62.51	Yes	**	0.0028
0 vs. 35	99.84	73.59 to 126.1	Yes	****	<0.0001
0 vs. 100	126.6	100.3 to 152.8	Yes	****	<0.0001
PD129337					
0 vs. 1	-3.142	-29.39 to 23.11	No	ns	0.999
0 vs. 10	-23.82	-50.07 to 2.428	No	ns	0.0917
0 vs. 20	-46.07	-72.31 to -19.82	Yes	***	0.0001
0 vs. 35	-47.13	-73.38 to -20.88	Yes	****	<0.0001
0 vs. 100	-69.67	-95.92 to -43.43	Yes	****	<0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
ATR-101								
0 vs. 1	195.3	212.3	-17.06	9.831	6	6	1.735	50
0 vs. 10	195.3	189	6.291	9.831	6	6	0.6399	50
0 vs. 20	195.3	159	36.26	9.831	6	6	3.689	50
0 vs. 35	195.3	95.44	99.84	9.831	6	6	10.16	50
0 vs. 100	195.3	68.69	126.6	9.831	6	6	12.88	50

PD129337

	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
0 vs. 1	189.4	192.6	-3.142	9.831	6	6	0.3196	50
0 vs. 10	189.4	213.2	-23.82	9.831	6	6	2.423	50
0 vs. 20	189.4	235.5	-46.07	9.831	6	6	4.686	50
0 vs. 35	189.4	236.6	-47.13	9.831	6	6	4.794	50
0 vs. 100	189.4	259.1	-69.67	9.831	6	6	7.087	50

2way ANOVA of 4B. ATP level vs. ATR-101 or PD129337 concentration

Table Analyzed   4B. ATP level vs. ATR-101 or PD129337 concentration

Two-way RM ANOVA   Matching: Stacked
Alpha   0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	46.09	<0.0001	****	Yes
Time	28.65	<0.0001	****	Yes
Column Factor	15.36	0.0022	**	Yes
Subjects (matching)	6.248	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	314667008	5	62933402	F (5, 40) = 101	P<0.0001
Time	195634263	5	39126853	F (5, 40) = 62.78	P<0.0001
Column Factor	104850250	1	104850250	F (1, 8) = 19.66	P=0.0022
Subjects (matching)	42657101	8	5332138	F (8, 40) = 8.555	P<0.0001
Residual	24931365	40	623284		

Number of missing values   0

Within each column, compare rows (simple effects within columns)

Number of families   2
Number of comparisons per family   5
Alpha   0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
ATR-101					
0 vs. 1	0.962	-1345 to 1347	No	ns	>0.9999
0 vs. 10	734.8	-611.5 to 2081	No	ns	0.5535
0 vs. 20	802	-544.4 to 2148	No	ns	0.4605
0 vs. 35	4141	2795 to 5487	Yes	****	<0.0001
0 vs. 100	11431	10085 to 12778	Yes	****	<0.0001
PD129337					
0 vs. 1	551.2	-795.1 to 1898	No	ns	0.8014
0 vs. 10	-125.4	-1472 to 1221	No	ns	0.9997
0 vs. 20	-429.9	-1776 to 916.4	No	ns	0.9185
0 vs. 35	-281.8	-1628 to 1065	No	ns	0.9862
0 vs. 100	-1296	-2642 to 50.84	No	ns	0.0642

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
ATR-101								
0 vs. 1	17173	17172	0.962	499.3	5	5	0.001927	40
0 vs. 10	17173	16438	734.8	499.3	5	5	1.472	40
0 vs. 20	17173	16371	802	499.3	5	5	1.606	40
0 vs. 35	17173	13032	4141	499.3	5	5	8.293	40
0 vs. 100	17173	5742	11431	499.3	5	5	22.89	40
PD129337								
0 vs. 1	16702	16151	551.2	499.3	5	5	1.104	40
0 vs. 10	16702	16827	-125.4	499.3	5	5	0.251	40
0 vs. 20	16702	17132	-429.9	499.3	5	5	0.861	40
0 vs. 35	16702	16984	-281.8	499.3	5	5	0.5644	40
0 vs. 100	16702	17997	-1296	499.3	5	5	2.595	40

Ordinary one-way ANOVA of 4C. Cortisol secretion vs. ATR-101 concentration

Table Analyzed	4C. Cortisol secretion vs. ATR-101 concentration				
Data sets analyzed	A : 0	B : 1	C : 10	D : 20	E : 35

ANOVA summary

F	62.64
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.(	Yes
R square	0.9126

Brown-Forsythe test

F (DFn, DFd)	1.456 (5, 30)
P value	0.2333
P value summary	ns
Are SDs significantly different (P < 0.(	No

Bartlett's test

Bartlett's statistic (corrected)	7.844
P value	0.1651
P value summary	ns
Are SDs significantly different (P < 0.(	No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	106057	5	21211	F (5, 30) = 62.64	P<0.0001
Residual (within columns)	10158	30	338.6		
Total	116215	35			

Data summary

Number of treatments (columns)	6
Number of values (total)	36
Number of families	1
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P	'A-?	
0 vs. 1	-17.99	-46.22 to 10.24	No	ns	0.3262	B	1
0 vs. 10	-12.99	-41.22 to 15.24	No	ns	0.6223	C	10
0 vs. 20	50.13	21.91 to 78.36	Yes	***	0.0003	D	20
0 vs. 35	104.1	75.91 to 132.4	Yes	****	0.0001	E	35
0 vs. 100	115.4	87.2 to 143.7	Yes	****	0.0001	F	100

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 1	172.4	190.4	-17.99	10.62	6	6	1.693	30
0 vs. 10	172.4	185.4	-12.99	10.62	6	6	1.223	30
0 vs. 20	172.4	122.3	50.13	10.62	6	6	4.719	30
0 vs. 35	172.4	68.25	104.1	10.62	6	6	9.802	30
0 vs. 100	172.4	56.96	115.4	10.62	6	6	10.86	30

Ordinary one-way ANOVA of 4C. Coritsol secretion vs. ATR-101 concentration + cho

Table Analyzed	4C. Coritsol secretion vs. ATR-101 concentration + cholesterol				
Data sets analyzed	A : 0	B : 1	C : 10	D : 20	E : 35

ANOVA summary

F	104.9
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.(	Yes
R square	0.9459

Brown-Forsythe test

F (DFn, DFd)	1.098 (5, 30)
P value	0.382
P value summary	ns
Are SDs significantly different (P < 0.(	No

Bartlett's test

Bartlett's statistic (corrected)	12.92	
P value	0.0241	
P value summary	*	
Are SDs significantly different (P < 0.0	Yes	

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	144699	5	28940	F (5, 30) = 104.9	P<0.0001
Residual (within columns)	8274	30	275.8		
Total	152973	35			

Data summary

Number of treatments (columns)	6
Number of values (total)	36
Number of families	1
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P 'A-?	
0 vs. 1	-74.09	-99.57 to -48.62	Yes	****	0.0001 B	1
0 vs. 10	-34.69	-60.17 to -9.213	Yes	**	0.0048 C	10
0 vs. 20	11.13	-14.34 to 36.61	No	ns	0.6655 D	20
0 vs. 35	86.63	61.15 to 112.1	Yes	****	0.0001 E	35
0 vs. 100	106.3	80.87 to 131.8	Yes	****	0.0001 F	100

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 1	182.2	256.3	-74.09	9.588	6	6	7.727	30
0 vs. 10	182.2	216.9	-34.69	9.588	6	6	3.618	30
0 vs. 20	182.2	171.1	11.13	9.588	6	6	1.161	30
0 vs. 35	182.2	95.59	86.63	9.588	6	6	9.035	30
0 vs. 100	182.2	75.87	106.3	9.588	6	6	11.09	30

Ordinary one-way ANOVA of 4C. Cortisol secretion vs. ATR-101 concentration + a-t

Table Analyzed	4C. Cortisol secretion vs. ATR-101 concentration + a-tocopherol				
Data sets analyzed	A : 0	B : 1	C : 10	D : 20	E : 35

ANOVA summary

F	29.83
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.0	Yes
R square	0.8325

Brown-Forsythe test

F (DFn, DFd)	0.7591 (5, 30)
P value	0.5863
P value summary	ns
Are SDs significantly different (P < 0.0	No

Bartlett's test

Bartlett's statistic (corrected)	7.413
P value	0.1917
P value summary	ns
Are SDs significantly different (P < 0.0	No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	49720	5	9944	F (5, 30) = 29.83	P<0.0001
Residual (within columns)	10001	30	333.4		
Total	59721	35			

Data summary

Number of treatments (columns)	6
Number of values (total)	36
Number of families	1

| Number of comparisons per family | 5 |
| Alpha | 0.05 |

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P ' A-?	
0 vs. 1	-26.58	-54.59 to 1.432	No	ns	0.0675 B	1
0 vs. 10	-15.26	-43.27 to 12.74	No	ns	0.4697 C	10
0 vs. 20	-9.219	-37.23 to 18.79	No	ns	0.8497 D	20
0 vs. 35	47.54	19.53 to 75.55	Yes	***	0.0005 E	35
0 vs. 100	76.79	48.78 to 104.8	Yes	****	0.0001 F	100

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 1	185.3	211.8	-26.58	10.54	6	6	2.521	30
0 vs. 10	185.3	200.5	-15.26	10.54	6	6	1.448	30
0 vs. 20	185.3	194.5	-9.219	10.54	6	6	0.8745	30
0 vs. 35	185.3	137.7	47.54	10.54	6	6	4.51	30
0 vs. 100	185.3	108.5	76.79	10.54	6	6	7.285	30

Ordinary one-way ANOVA of 4C ATP level vs. ATR-101 concentration

Table Analyzed	4C ATP level vs. ATR-101 concentration				
Data sets analyzed	A : 0	B : 1	C : 10	D : 20	E : 35

ANOVA summary
F	247.1
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.(	Yes
R square	0.9763

Brown-Forsythe test
F (DFn, DFd)	0.7001 (5, 30)
P value	0.6276
P value summary	ns
Are SDs significantly different (P < 0.(	No

Bartlett's test
Bartlett's statistic (corrected)	10.45
P value	0.0635
P value summary	ns
Are SDs significantly different (P < 0.(	No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	550462132	5	110092426	F (5, 30) = 247.1	P<0.0001
Residual (within columns)	13364823	30	445494		
Total	563826955	35			

Data summary
Number of treatments (columns)	6
Number of values (total)	36

Number of families	1
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P ' A-?	
0 vs. 1	-385.6	-1409 to 638.3	No	ns	0.7739 B	1
0 vs. 10	-259.2	-1283 to 764.7	No	ns	0.9403 C	10
0 vs. 20	426.5	-597.4 to 1450	No	ns	0.703 D	20
0 vs. 35	3963	2939 to 4987	Yes	****	0.0001 E	35
0 vs. 100	10451	9427 to 11475	Yes	****	0.0001 F	100

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 1	13171	13557	-385.6	385.4	6	6	1.001	30
0 vs. 10	13171	13430	-259.2	385.4	6	6	0.6725	30

	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 20	13171	12745	426.5	385.4	6	6	1.107	30
0 vs. 35	13171	9208	3963	385.4	6	6	10.28	30
0 vs. 100	13171	2720	10451	385.4	6	6	27.12	30

Ordinary one-way ANOVA of 4C. ATP level vs. ATR-101 concentration + cholesterol

Table Analyzed	4C. ATP level vs. ATR-101 concentration + cholesterol				
Data sets analyzed	A : 0	B : 1	C : 10	D : 20	E : 35

ANOVA summary
F	13.88
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.0 Yes
R square	0.6982

Brown-Forsythe test
F (DFn, DFd)	0.3485 (5, 30)
P value	0.8791
P value summary	ns
Are SDs significantly different (P < 0.0 No

Bartlett's test
Bartlett's statistic (corrected)	2.37
P value	0.7959
P value summary	ns
Are SDs significantly different (P < 0.0 No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	36638761	5	7327752	F (5, 30) = 13.88	P<0.0001
Residual (within columns)	15837206	30	527907		
Total	52475967	35			

Data summary
Number of treatments (columns)	6
Number of values (total)	36
Number of families	1
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P	'A-?
0 vs. 1	-176.6	-1291 to 938	No	ns	0.9918 B	1
0 vs. 10	647.5	-467.1 to 1762	No	ns	0.4102 C	10
0 vs. 20	1607	492.2 to 2721	Yes	**	0.0028 D	20
0 vs. 35	1574	459.6 to 2689	Yes	**	0.0033 E	35
0 vs. 100	2709	1595 to 3824	Yes	****	0.0001 F	100

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 1	13724	13901	-176.6	419.5	6	6	0.421	30
0 vs. 10	13724	13077	647.5	419.5	6	6	1.543	30
0 vs. 20	13724	12117	1607	419.5	6	6	3.83	30
0 vs. 35	13724	12150	1574	419.5	6	6	3.753	30
0 vs. 100	13724	11015	2709	419.5	6	6	6.459	30

Ordinary one-way ANOVA of 4C. ATP levels vs. ATR-101 concentration + a-tocophero

Table Analyzed	4C. ATP levels vs. ATR-101 concentration + a-tocopherol				
Data sets analyzed	A : 0	B : 1	C : 10	D : 20	E : 35

ANOVA summary
F	9.863
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.0 Yes
R square	0.6218

Brown-Forsythe test
F (DFn, DFd)   0.2083 (5, 30)
P value   0.9564
P value summary   ns
Are SDs significantly different (P < 0.0 No

Bartlett's test
Bartlett's statistic (corrected)   1.838
P value   0.8711
P value summary   ns
Are SDs significantly different (P < 0.0 No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	28627428	5	5725486	F (5, 30) = 9.863	P<0.0001
Residual (within columns)	17414447	30	580482		
Total	46041875	35			

Data summary
Number of treatments (columns)   6
Number of values (total)   36

Number of families   1
Number of comparisons per family   5
Alpha   0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P	'A-?
0 vs. 1	57.76	-1111 to 1227	No	ns	0.9998 B	1
0 vs. 10	-620.7	-1789 to 548.1	No	ns	0.4938 C	10
0 vs. 20	-691.7	-1860 to 477	No	ns	0.393 D	20
0 vs. 35	-164.7	-1333 to 1004	No	ns	0.995 E	35
0 vs. 100	1982	813.7 to 3151	Yes	***	0.0005 F	100

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 1	13602	13544	57.76	439.9	6	6	0.1313	30
0 vs. 10	13602	14223	-620.7	439.9	6	6	1.411	30
0 vs. 20	13602	14294	-691.7	439.9	6	6	1.573	30
0 vs. 35	13602	13767	-164.7	439.9	6	6	0.3744	30
0 vs. 100	13602	11620	1982	439.9	6	6	4.507	30

Ordinary one-way ANOVA of 5A. ATP level vs. inhibitor combination					

Table Analyzed	5A. ATP level vs. inhibitor combination			
Data sets analyzed	A : DMSO B : GBZ	C : GBZP	D : b+GBZP	E : GBP

ANOVA summary

F	64.15
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.9182

Brown-Forsythe test

F (DFn, DFd)	1.202 (7, 40)
P value	0.3242
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test

Bartlett's statistic (corrected)	20.65
P value	0.0043
P value summary	**
Are SDs significantly different (P < 0.05)?	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	524547076	7	74935297	F (7, 40) = 64.15	P<0.0001
Residual (within columns)	46724343	40	1168109		
Total	571271419	47			

Data summary

Number of treatments (columns)	8
Number of values (total)	48
Number of families	1
Number of comparisons per family	7
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
DMSO vs. GBZ	4604	2900 to 6309	Yes	****	0.0001	B	GBZ
DMSO vs. GBZP	5865	4160 to 7569	Yes	****	0.0001	C	GBZP
DMSO vs. b+GBZP	-1625	-3330 to 78.78	No	ns	0.067	D	b+GBZP
DMSO vs. GBP	-608.8	-2313 to 1095	No	ns	0.8685	E	GBP
DMSO vs. GZP	806.7	-897.4 to 2511	No	ns	0.6628	F	GZP
DMSO vs. BZP	787.4	-916.8 to 2492	No	ns	0.685	G	BZP
DMSO vs. ATR-101	8278	6574 to 9983	Yes	****	0.0001	H	ATR-101

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
DMSO vs. GBZ	13282	8677	4604	624	6	6	7.379	40
DMSO vs. GBZP	13282	7417	5865	624	6	6	9.398	40
DMSO vs. b+GBZP	13282	14907	-1625	624	6	6	2.605	40
DMSO vs. GBP	13282	13890	-608.8	624	6	6	0.9756	40
DMSO vs. GZP	13282	12475	806.7	624	6	6	1.293	40
DMSO vs. BZP	13282	12494	787.4	624	6	6	1.262	40
DMSO vs. ATR-101	13282	5003	8278	624	6	6	13.27	40

Ordinary one-way ANOVA of 5A. Caspase activity vs. inhibitor combination	

Table Analyzed	5A. Caspase activity vs. inhibitor combination			
Data sets analyzed	A : DMSO B : GBZ	C : GBZP	D : b+GBZP	E : GBP

ANOVA summary

F	37.81
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.8687

Brown-Forsythe test
F (DFn, DFd)	1.88 (7, 40)
P value	0.0986
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test
Bartlett's statistic (corrected)	16.98
P value	0.0175
P value summary	*
Are SDs significantly different (P < 0.05)?	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	7413299	7	1059043	F (7, 40) = 37.81	P<0.0001
Residual (within columns)	1120390	40	28010		
Total	8533689	47			

Data summary
Number of treatments (columns)	8
Number of values (total)	48
Number of families	1
Number of comparisons per family	7
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
DMSO vs. GBZ	-932.2	-1196 to -668.3	Yes	****	0.0001	B	GBZ
DMSO vs. GBZP	-770.9	-1035 to -507	Yes	****	0.0001	C	GBZP
DMSO vs. b+GBZP	34.5	-229.4 to 298.4	No	ns	0.9994	D	b+GBZP
DMSO vs. GBP	97.52	-166.4 to 361.4	No	ns	0.8501	E	GBP
DMSO vs. GZP	-115.7	-379.6 to 148.2	No	ns	0.7304	F	GZP
DMSO vs. BZP	-280.1	-544 to -16.25	Yes	*	0.0334	G	BZP
DMSO vs. ATR-101	-785.2	-1049 to -521.3	Yes	****	0.0001	H	ATR-101

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
DMSO vs. GBZ	2441	3374	-932.2	96.63	6	6	9.647	40
DMSO vs. GBZP	2441	3212	-770.9	96.63	6	6	7.978	40
DMSO vs. b+GBZP	2441	2407	34.5	96.63	6	6	0.3571	40
DMSO vs. GBP	2441	2344	97.52	96.63	6	6	1.009	40
DMSO vs. GZP	2441	2557	-115.7	96.63	6	6	1.197	40
DMSO vs. BZP	2441	2722	-280.1	96.63	6	6	2.899	40
DMSO vs. ATR-101	2441	3227	-785.2	96.63	6	6	8.126	40

Ordinary one-way ANOVA of 5C. ATP level vs. glibenclamide + DMSO

Table Analyzed	5C. ATP level vs. glibenclamide + DMSO				
Data sets analyzed	A : 0	B : 50	C : 100	D : 250	E : 600

ANOVA summary
F	7.079
P value	0.0006
P value summary	***
Significant diff. among means (P < 0.05)?	Yes
R square	0.5311

Brown-Forsythe test
F (DFn, DFd)	0.0983 (4, 25)
P value	0.982
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test
Bartlett's statistic (corrected)	0.1022
P value	0.9987
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	33254311	4	8313578	F (4, 25) = 7.079	P=0.0006
Residual (within columns)	29359980	25	1174399		
Total	62614291	29			

Data summary

Number of treatments (columns)	5
Number of values (total)	30
Number of families	1
Number of comparisons per family	4
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
0 vs. 50	562.2	-1069 to 2193	No	ns	0.7802	B	50
0 vs. 100	1184	-446.8 to 2816	No	ns	0.2051	C	100
0 vs. 250	2010	378.5 to 3641	Yes	*	0.0126	D	250
0 vs. 600	2974	1343 to 4605	Yes	***	0.0003	E	600

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 50	12597	12035	562.2	625.7	6	6	0.8986	25
0 vs. 100	12597	11412	1184	625.7	6	6	1.893	25
0 vs. 250	12597	10587	2010	625.7	6	6	3.212	25
0 vs. 600	12597	9623	2974	625.7	6	6	4.753	25

Ordinary one-way ANOVA of 5C. ATP level vs. glibenclamide + ATR-101

Table Analyzed	5C. ATP level vs. glibenclamide + ATR-101			
Data sets analyzed	A : 0	B : 50	C : 100	D : 250	E : 600

ANOVA summary

F	30.76
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.8311

Brown-Forsythe test

F (DFn, DFd)	0.1577 (4, 25)
P value	0.9576
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test

Bartlett's statistic (corrected)	0.3589
P value	0.9857
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	143789123	4	35947281	F (4, 25) = 30.76	P<0.0001
Residual (within columns)	29215482	25	1168619		
Total	173004606	29			

Data summary

Number of treatments (columns)	5
Number of values (total)	30
Number of families	1
Number of comparisons per family	4
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
0 vs. 50	140.3	-1487 to 1768	No	ns	0.998	B	50
0 vs. 100	1270	-357.2 to 2897	No	ns	0.1586	C	100
0 vs. 250	1798	171.2 to 3426	Yes	*	0.0272	D	250

0 vs. 600		6007 4380 to 7634	Yes	****		0.0001 E		600

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 50	12186	12046	140.3	624.1	6	6	0.2248	25
0 vs. 100	12186	10916	1270	624.1	6	6	2.035	25
0 vs. 250	12186	10388	1798	624.1	6	6	2.881	25
0 vs. 600	12186	6179	6007	624.1	6	6	9.625	25

Ordinary one-way ANOVA of 5C. ATP level vs. benzamil + DMSO

Table Analyzed	5C. ATP level vs. benzamil + DMSO
Data sets analyzed	A : 0 B : 1 C : 2 D : 4 E : 7

ANOVA summary

F	0.3596
P value	0.8719
P value summary	ns
Significant diff. among means (P < 0.05)?	No
R square	0.05655

Brown-Forsythe test

F (DFn, DFd)	0.1157 (5, 30)
P value	0.988
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test

Bartlett's statistic (corrected)	0.2096
P value	0.999
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	2331827	5	466365	F (5, 30) = 0.3596	P=0.8719
Residual (within columns)	38902061	30	1296735		
Total	41233888	35			

Data summary

Number of treatments (columns)	6
Number of values (total)	36
Number of families	1
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
0 vs. 1	-293	-2040 to 1454	No	ns	0.9892	B	1
0 vs. 2	-526.6	-2273 to 1220	No	ns	0.8877	C	2
0 vs. 4	-567.4	-2314 to 1179	No	ns	0.856	D	4
0 vs. 7	-386.3	-2133 to 1361	No	ns	0.9649	E	7
0 vs. 10	-823.6	-2570 to 923.2	No	ns	0.6015	F	10

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 1	12838	13131	-293	657.5	6	6	0.4457	30
0 vs. 2	12838	13364	-526.6	657.5	6	6	0.8009	30
0 vs. 4	12838	13405	-567.4	657.5	6	6	0.863	30
0 vs. 7	12838	13224	-386.3	657.5	6	6	0.5875	30
0 vs. 10	12838	13661	-823.6	657.5	6	6	1.253	30

Ordinary one-way ANOVA of 5C. ATP level vs. benzamil + ATR-101

Table Analyzed	5C. ATP level vs. benzamil + ATR-101
Data sets analyzed	A : 0 B : 1 C : 2 D : 4 E : 7

ANOVA summary

F	172.8			
P value	<0.0001			
P value summary	****			
Significant diff. among means (P < 0.05)?	Yes			
R square	0.9664			

Brown-Forsythe test
F (DFn, DFd)	0.5589 (5, 30)
P value	0.7305
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test
Bartlett's statistic (corrected)	11.97
P value	0.0351
P value summary	*
Are SDs significantly different (P < 0.05)?	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	918620466	5	183724093	F (5, 30) = 172.8	P<0.0001
Residual (within columns)	31893816	30	1063127		
Total	950514283	35			

Data summary
Number of treatments (columns)	6
Number of values (total)	36
Number of families	1
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?
0 vs. 1	1799	217.2 to 3381	Yes	*	0.0214	B	1
0 vs. 2	2768	1186 to 4350	Yes	***	0.0003	C	2
0 vs. 4	10545	8963 to 12127	Yes	****	0.0001	D	4
0 vs. 7	11699	10117 to 13280	Yes	****	0.0001	E	7
0 vs. 10	12107	10525 to 13689	Yes	****	0.0001	F	10

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 1	12602	10803	1799	595.3	6	6	3.022	30
0 vs. 2	12602	9834	2768	595.3	6	6	4.65	30
0 vs. 4	12602	2057	10545	595.3	6	6	17.71	30
0 vs. 7	12602	903.5	11699	595.3	6	6	19.65	30
0 vs. 10	12602	495.1	12107	595.3	6	6	20.34	30

Ordinary one-way ANOVA of 5C. ATP level vs. cyclosporin A + DMSO

Table Analyzed	5C. ATP level vs. cyclosporin A + DMSO
Data sets analyzed	A : 0 B : 1 C : 2 D : 5 E : 10

ANOVA summary
F	14.62
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.709

Brown-Forsythe test
F (DFn, DFd)	0.1029 (5, 30)
P value	0.9908
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test
Bartlett's statistic (corrected)	0.5389
P value	0.9906
P value summary	ns

Are SDs significantly different (P < 0.05)?   No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	74234433	5	14846887	F (5, 30) = 14.62	P<0.0001
Residual (within columns)	30470366	30	1015679		
Total	104704798	35			

Data summary	
Number of treatments (columns)	6
Number of values (total)	36
Number of families	1
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
0 vs. 1	150.5	-1395 to 1697	No	ns	0.9987	B	1
0 vs. 2	177.1	-1369 to 1723	No	ns	0.9981	C	2
0 vs. 5	530.8	-1015 to 2077	No	ns	0.8282	D	5
0 vs. 10	599.3	-946.7 to 2145	No	ns	0.7547	E	10
0 vs. 20	4102	2556 to 5648	Yes	****	0.0001	F	20

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 1	13409	13259	150.5	581.9	6	6	0.2587	30
0 vs. 2	13409	13232	177.1	581.9	6	6	0.3043	30
0 vs. 5	13409	12878	530.8	581.9	6	6	0.9123	30
0 vs. 10	13409	12810	599.3	581.9	6	6	1.03	30
0 vs. 20	13409	9307	4102	581.9	6	6	7.051	30

Ordinary one-way ANOVA of 5C. ATP level vs. cyclosporin A + ATR-101

Table Analyzed	5C. ATP level vs. cyclosporin A + ATR-101			
Data sets analyzed	A : 0	B : 1	C : 2	D : 5	E : 10

ANOVA summary	
F	115.2
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.9505

Brown-Forsythe test	
F (DFn, DFd)	0.564 (5, 30)
P value	0.7267
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test	
Bartlett's statistic (corrected)	4.182
P value	0.5235
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	723545651	5	144709130	F (5, 30) = 115.2	P<0.0001
Residual (within columns)	37691953	30	1256398		
Total	761237604	35			

Data summary	
Number of treatments (columns)	6
Number of values (total)	36
Number of families	1
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?

0 vs. 1	-412 -2131 to 1307	No	ns		0.9517 B		1
0 vs. 2	517.1 -1202 to 2237	No	ns		0.8886 C		2
0 vs. 5	7192 5473 to 8912	Yes	****		0.0001 D		5
0 vs. 10	9610 7891 to 11330	Yes	****		0.0001 E		10
0 vs. 20	9697 7978 to 11417	Yes	****		0.0001 F		20

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 1	12346	12758	-412	647.1	6	6	0.6366	30
0 vs. 2	12346	11829	517.1	647.1	6	6	0.7991	30
0 vs. 5	12346	5154	7192	647.1	6	6	11.11	30
0 vs. 10	12346	2736	9610	647.1	6	6	14.85	30
0 vs. 20	12346	2649	9697	647.1	6	6	14.98	30

Ordinary one-way ANOVA of 5C. ATP level vs. rhodamine 123 + DMSO

Table Analyzed	5C. ATP level vs. rhodamine 123 + DMSO				
Data sets analyzed	A : 0	B : 2.5	C : 5	D : 10	E : 20

ANOVA summary
F	0.7731
P value	0.5767
P value summary	ns
Significant diff. among means (P < 0.05)?	No
R square	0.1141

Brown-Forsythe test
F (DFn, DFd)	0.1034 (5, 30)
P value	0.9907
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test
Bartlett's statistic (corrected)	0.5004
P value	0.9921
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	3882871	5	776574	F (5, 30) = 0.7731	P=0.5767
Residual (within columns)	30136736	30	1004558		
Total	34019608	35			

Data summary
Number of treatments (columns)	6
Number of values (total)	36
Number of families	1
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?
0 vs. 2.5	115.3	-1422 to 1653	No	ns	0.9997 B	2.5
0 vs. 5	-78.45	-1616 to 1459	No	ns	0.9998 C	5
0 vs. 10	-79.82	-1617 to 1458	No	ns	0.9998 D	10
0 vs. 20	316.9	-1221 to 1854	No	ns	0.9737 E	20
0 vs. 50	856.7	-680.8 to 2394	No	ns	0.449 F	50

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 2.5	11728	11613	115.3	578.7	6	6	0.1992	30
0 vs. 5	11728	11806	-78.45	578.7	6	6	0.1356	30
0 vs. 10	11728	11808	-79.82	578.7	6	6	0.1379	30
0 vs. 20	11728	11411	316.9	578.7	6	6	0.5476	30
0 vs. 50	11728	10871	856.7	578.7	6	6	1.48	30

Table Analyzed	5C. ATP level vs. rhodamine 123 + ATR-101				
Data sets analyzed	A : 0	B : 2.5	C : 5	D : 10	E : 20

ANOVA summary

F	215.6
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.9729

Brown-Forsythe test

F (DFn, DFd)	4.027 (5, 30)
P value	0.0065
P value summary	**
Are SDs significantly different (P < 0.05)?	Yes

Bartlett's test

Bartlett's statistic (corrected)	46.34
P value	<0.0001
P value summary	****
Are SDs significantly different (P < 0.05)?	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	585286204	5	117057241	F (5, 30) = 215.6	P<0.0001
Residual (within columns)	16289792	30	542993		
Total	601575996	35			

Data summary

Number of treatments (columns)	6
Number of values (total)	36
Number of families	1
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
0 vs. 2.5	4569	3439 to 5700	Yes	****	0.0001	B	2.5
0 vs. 5	8748	7618 to 9879	Yes	****	0.0001	C	5
0 vs. 10	10577	9447 to 11708	Yes	****	0.0001	D	10
0 vs. 20	10791	9660 to 11921	Yes	****	0.0001	E	20
0 vs. 50	10800	9670 to 11930	Yes	****	0.0001	F	50

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 2.5	11004	6435	4569	425.4	6	6	10.74	30
0 vs. 5	11004	2256	8748	425.4	6	6	20.56	30
0 vs. 10	11004	426.7	10577	425.4	6	6	24.86	30
0 vs. 20	11004	213.6	10791	425.4	6	6	25.36	30
0 vs. 50	11004	204.1	10800	425.4	6	6	25.39	30

Table Analyzed	6C. ATP levels with U18666A vs. ATR-101 concentration			

Two-way RM ANOVA	Matching: Stacked	
Alpha	0.05	

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	29.36	<0.0001	****	Yes
Time	25.7	<0.0001	****	Yes
Column Factor	40.9	<0.0001	****	Yes
Subjects (matching)	3.085	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	1230643094	20	61532155	F (20, 125) = 192.	P<0.0001
Time	1077394297	5	215478859	F (5, 125) = 673	P<0.0001
Column Factor	1714234197	4	428558549	F (4, 25) = 82.85	P<0.0001
Subjects (matching)	129320689	25	5172828	F (25, 125) = 16.1	P<0.0001
Residual	40021689	125	320174		

Number of missing values	0

Within each row, compare columns (simple effects within rows)

Number of families	6
Number of comparisons per family	4
Alpha	0.05

Dunnett's multiple comparisons tes	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
0					
0 vs. 5	461	-1053 to 1975	No	ns	0.8667
0 vs. 10	667.6	-846.5 to 2182	No	ns	0.6489
0 vs. 20	2059	544.7 to 3573	Yes	**	0.0038
0 vs. 35	2638	1123 to 4152	Yes	***	0.0001
0.1					
0 vs. 5	-72.67	-1587 to 1441	No	ns	0.9998
0 vs. 10	519.1	-995 to 2033	No	ns	0.8126
0 vs. 20	654.4	-859.7 to 2169	No	ns	0.6643
0 vs. 35	1933	418.6 to 3447	Yes	**	0.0073
1					
0 vs. 5	258.7	-1255 to 1773	No	ns	0.9808
0 vs. 10	476.8	-1037 to 1991	No	ns	0.8528
0 vs. 20	523.4	-990.7 to 2038	No	ns	0.8083
0 vs. 35	3432	1918 to 4946	Yes	****	0.0001
5					
0 vs. 5	29.32	-1485 to 1543	No	ns	0.9999
0 vs. 10	-147.7	-1662 to 1366	No	ns	0.9976
0 vs. 20	2082	568.1 to 3596	Yes	**	0.0033
0 vs. 35	12373	10859 to 13887	Yes	****	0.0001
10					
0 vs. 5	158.7	-1355 to 1673	No	ns	0.9969
0 vs. 10	-402.2	-1916 to 1112	No	ns	0.9125
0 vs. 20	11525	10011 to 13039	Yes	****	0.0001
0 vs. 35	12957	11443 to 14471	Yes	****	0.0001
25					
0 vs. 5	-147.2	-1661 to 1367	No	ns	0.9976
0 vs. 10	4250	2735 to 5764	Yes	****	0.0001
0 vs. 20	13082	11568 to 14596	Yes	****	0.0001
0 vs. 35	12962	11448 to 14476	Yes	****	0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF
0								
0 vs. 5	14865	14404	461	613.4	6	6	0.7515	150
0 vs. 10	14865	14197	667.6	613.4	6	6	1.088	150

0 vs. 20	14865	12806	2059	613.4	6	6	3.356	150
0 vs. 35	14865	12227	2638	613.4	6	6	4.3	150
0.1								
0 vs. 5	14031	14104	-72.67	613.4	6	6	0.1185	150
0 vs. 10	14031	13512	519.1	613.4	6	6	0.8463	150
0 vs. 20	14031	13377	654.4	613.4	6	6	1.067	150
0 vs. 35	14031	12098	1933	613.4	6	6	3.151	150
1								
0 vs. 5	13930	13671	258.7	613.4	6	6	0.4218	150
0 vs. 10	13930	13453	476.8	613.4	6	6	0.7772	150
0 vs. 20	13930	13407	523.4	613.4	6	6	0.8532	150
0 vs. 35	13930	10498	3432	613.4	6	6	5.595	150
5								
0 vs. 5	13644	13615	29.32	613.4	6	6	0.0478	150
0 vs. 10	13644	13792	-147.7	613.4	6	6	0.2407	150
0 vs. 20	13644	11562	2082	613.4	6	6	3.394	150
0 vs. 35	13644	1272	12373	613.4	6	6	20.17	150
10								
0 vs. 5	13375	13216	158.7	613.4	6	6	0.2587	150
0 vs. 10	13375	13777	-402.2	613.4	6	6	0.6556	150
0 vs. 20	13375	1850	11525	613.4	6	6	18.79	150
0 vs. 35	13375	417.9	12957	613.4	6	6	21.12	150
25								
0 vs. 5	13461	13608	-147.2	613.4	6	6	0.24	150
0 vs. 10	13461	9211	4250	613.4	6	6	6.927	150
0 vs. 20	13461	378.6	13082	613.4	6	6	21.33	150
0 vs. 35	13461	498.5	12962	613.4	6	6	21.13	150

Unpaired t test of 6D. ATP levels with ATR-101 and U18666A vs. ATR-101, U18666A, MBCD

Table Analyzed	6D. ATP levels with ATR-101 and U18666A vs. ATR-101, U18666A, and MBCD
Column B	ATR-101+U18666A+MBCD
vs.	vs.
Column A	ATR-101+U18666A
Unpaired t test	
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=21.01 df=10
How big is the difference?	
Mean ± SEM of column A	1925 ± 393.9, n=6
Mean ± SEM of column B	16009 ± 542.4, n=6
Difference between means	14084 ± 670.3
95% confidence interval	12590 to 15577
R squared (eta squared)	0.9778
F test to compare variances	
F, DFn, Dfd	1.897, 5, 5
P value	0.4993
P value summary	ns
Significantly different (P < 0.05)?	No

Table Analyzed	7A. ABCA1 levels vs. ATR-101 concentration, 1 h			
Data sets analyzed	A : 0	B : 8	C : 16	D : 32

ANOVA summary

F	24.37
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.8205

Brown-Forsythe test

F (DFn, DFd)	0.08223 (3, 16)
P value	0.9687
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test

Bartlett's statistic (corrected)	0.08657
P value	0.9934
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

ANOVA table	SS	DF		MS	F (DFn, DFd)	P value
Treatment (between columns)	7.17E-07		3	2.39E-07	F (3, 16) = 24.37	P<0.0001
Residual (within columns)	1.569E-07		16	9.807E-09		
Total	8.739E-07		19			

Data summary

Number of treatments (columns)	4
Number of values (total)	20
Number of families	1
Number of comparisons per family	3
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
0 vs. 8	0.0004025	0.0002401 to 0.0005649	Yes	****	0.0001	B	8
0 vs. 16	0.0004713	0.0003089 to 0.0006336	Yes	****	0.0001	C	16
0 vs. 32	0.0004269	0.0002645 to 0.0005893	Yes	****	0.0001	D	32

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 8	0.001442	0.00104	0.0004025	0.00006263	5	5	6.426	16
0 vs. 16	0.001442	0.0009709	0.0004713	0.00006263	5	5	7.524	16
0 vs. 32	0.001442	0.001015	0.0004269	0.00006263	5	5	6.816	16

Table Analyzed	7A. ABCG1 levels vs. ATR-101 concentration, 1 h			
Data sets analyzed	A : 0	B : 8	C : 16	D : 32

ANOVA summary

F	409.6
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.9871

Brown-Forsythe test

F (DFn, DFd)	0.4793 (3, 16)
P value	0.7012
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test

Bartlett's statistic (corrected)	1.308
P value	0.7272
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

ANOVA table	SS	DF		MS	F (DFn, DFd)	P value
Treatment (between columns)	9.169E-09	3		3.056E-09	F (3, 16) = 409.6	P<0.0001
Residual (within columns)	1.194E-10	16		7.461E-12		
Total	9.288E-09	19				

Data summary	
Number of treatments (columns)	4
Number of values (total)	20
Number of families	1
Number of comparisons per family	3
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
0 vs. 8	0.0000343	2.982e-005 to 3.878e-005	Yes	****	0.0001	B	8
0 vs. 16	5.636E-05	5.188e-005 to 6.084e-005	Yes	****	0.0001	C	16
0 vs. 32	4.736E-05	4.288e-005 to 5.184e-005	Yes	****	0.0001	D	32

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 8	0.000075	0.0000407	0.0000343	0.000001728	5	5	19.85	16
0 vs. 16	0.000075	0.00001864	5.636E-05	0.000001728	5	5	32.63	16
0 vs. 32	0.000075	0.00002764	4.736E-05	0.000001728	5	5	27.42	16

Ordinary one-way ANOVA of 7A. IDOL levels vs. ATR-101 concentration, 1 h

Table Analyzed	7A. IDOL levels vs. ATR-101 concentration, 1 h			
Data sets analyzed	A : 0	B : 8	C : 16	D : 32

ANOVA summary	
F	56.87
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.9143

Brown-Forsythe test	
F (DFn, DFd)	3.557 (3, 16)
P value	0.0382
P value summary	*
Are SDs significantly different (P < 0.05)?	Yes

Bartlett's test	
Bartlett's statistic (corrected)	6.567
P value	0.0871
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

ANOVA table	SS	DF		MS	F (DFn, DFd)	P value
Treatment (between columns)	6.85E-06	3		2.283E-06	F (3, 16) = 56.87	P<0.0001
Residual (within columns)	6.424E-07	16		4.015E-08		
Total	7.492E-06	19				

Data summary	
Number of treatments (columns)	4
Number of values (total)	20
Number of families	1
Number of comparisons per family	3
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
0 vs. 8	0.00115	0.0008219 to 0.001479	Yes	****	0.0001	B	8
0 vs. 16	0.001487	0.001159 to 0.001816	Yes	****	0.0001	C	16
0 vs. 32	0.001332	0.001003 to 0.00166	Yes	****	0.0001	D	32

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 8	0.002498	0.001348	0.00115	0.0001267	5	5	9.078	16
0 vs. 16	0.002498	0.001011	0.001487	0.0001267	5	5	11.74	16

0 vs. 32	0.002498		0.001167	0.001332	0.0001267	5	5	10.51	16

Ordinary one-way ANOVA of 7A. CHOP levels vs. ATR-101 concentration, 1 h

Table Analyzed	7A. CHOP levels vs. ATR-101 concentration, 1 h			
Data sets analyzed	A : 0	B : 8	C : 16	D : 32

ANOVA summary
F   35.36
P value   <0.0001
P value summary   ****
Significant diff. among means (P < 0.05)? Yes
R square   0.8689

Brown-Forsythe test
F (DFn, DFd)   0.1769 (3, 16)
P value   0.9105
P value summary   ns
Are SDs significantly different (P < 0.05)? No

Bartlett's test
Bartlett's statistic (corrected)   0.1917
P value   0.9789
P value summary   ns
Are SDs significantly different (P < 0.05)? No

ANOVA table	SS	DF		MS	F (DFn, DFd)	P value
Treatment (between columns)	1.968E-05		3	6.561E-06	F (3, 16) = 35.36	P<0.0001
Residual (within columns)	2.969E-06		16	1.856E-07		
Total	2.265E-05		19			

Data summary
Number of treatments (columns)   4
Number of values (total)   20

Number of families   1
Number of comparisons per family   3
Alpha   0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
0 vs. 8	9.186E-05	-0.0006145 to 0.0007982	No	ns	0.9739	B	8
0 vs. 16	0.0001666	-0.0005398 to 0.0008729	No	ns	0.8734	C	16
0 vs. 32	-0.002201	-0.002907 to -0.001495	Yes	****	0.0001	D	32

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 8	0.005861	0.005769	9.186E-05	0.0002725	5	5	0.3372	16
0 vs. 16	0.005861	0.005694	0.0001666	0.0002725	5	5	0.6114	16
0 vs. 32	0.005861	0.008062	-0.002201	0.0002725	5	5	8.078	16

Ordinary one-way ANOVA of 7B. CYP17A1 transcripts vs. ATR-101

Table Analyzed	7B. CYP17A1 transcripts vs. ATR-101		
Data sets analyzed	A : DMSO	B : 16	C : 32

ANOVA summary
F   7.957
P value   0.0027
P value summary   **
Significant diff. among means (P < 0.05)? Yes
R square   0.4311

Brown-Forsythe test
F (DFn, DFd)   15.77 (2, 21)
P value   <0.0001
P value summary   ****
Are SDs significantly different (P < 0.05)? Yes

Bartlett's test
Bartlett's statistic (corrected)   11.22
P value   0.0037
P value summary   **

Are SDs significantly different (P < 0.05)? Yes

ANOVA table	SS	DF		MS	F (DFn, DFd)	P value
Treatment (between columns)	0.03325		2	0.01662	F (2, 21) = 7.957	P=0.0027
Residual (within columns)	0.04387		21	0.002089		
Total	0.07711		23			

Data summary
Number of treatments (columns)	3
Number of values (total)	24
Number of families	1
Number of comparisons per family	2
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?
DMSO vs. 16	0.0712	0.01703 to 0.1254	Yes	**	0.0099 B	16
DMSO vs. 32	0.08491	0.03074 to 0.1391	Yes	**	0.0024 C	32

Test details	Mean 1	Mean 2		Mean Diff.	SE of diff.	n1	n2	q	DF
DMSO vs. 16	0.2174		0.1462	0.0712	0.02285	8	8	3.116	21
DMSO vs. 32	0.2174		0.1325	0.08491	0.02285	8	8	3.715	21

Ordinary one-way ANOVA of 7B. SULT2A1 transcripts vs. ATR-101

Table Analyzed	7B. SULT2A1 transcripts vs. ATR-101
Data sets analyzed	A : DMSO B : 16 C : 32

ANOVA summary
F	17.79
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.6289

Brown-Forsythe test
F (DFn, DFd)	0.9893 (2, 21)
P value	0.3885
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test
Bartlett's statistic (corrected)	2.157
P value	0.3401
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

ANOVA table	SS	DF		MS	F (DFn, DFd)	P value
Treatment (between columns)	0.000072		2	0.000036	F (2, 21) = 17.79	P<0.0001
Residual (within columns)	4.249E-05		21	2.023E-06		
Total	0.0001145		23			

Data summary
Number of treatments (columns)	3
Number of values (total)	24
Number of families	1
Number of comparisons per family	2
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?
DMSO vs. 16	0.003669	0.001983 to 0.005355	Yes	****	0.0001 B	16
DMSO vs. 32	0.003679	0.001993 to 0.005365	Yes	****	0.0001 C	32

Test details	Mean 1	Mean 2		Mean Diff.	SE of diff.	n1	n2	q	DF
DMSO vs. 16	0.008212		0.004543	0.003669	0.0007112	8	8	5.159	21
DMSO vs. 32	0.008212		0.004533	0.003679	0.0007112	8	8	5.173	21

Table Analyzed	7B. HSD3B2 transcripts vs. ATR-101		
Data sets analyzed	A : DMSO B : 16		C : 32

ANOVA summary

F	6.162
P value	0.0078
P value summary	**
Significant diff. among means (P < 0.05)?	Yes
R square	0.3698

Brown-Forsythe test

F (DFn, DFd)	18.12 (2, 21)
P value	<0.0001
P value summary	****
Are SDs significantly different (P < 0.05)?	Yes

Bartlett's test

Bartlett's statistic (corrected)	6.82
P value	0.033
P value summary	*
Are SDs significantly different (P < 0.05)?	Yes

ANOVA table	SS	DF		MS	F (DFn, DFd)	P value
Treatment (between columns)	4.246E-05		2	2.123E-05	F (2, 21) = 6.162	P=0.0078
Residual (within columns)	7.234E-05		21	3.445E-06		
Total	0.0001148		23			

Data summary

Number of treatments (columns)	3
Number of values (total)	24
Number of families	1
Number of comparisons per family	2
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?
DMSO vs. 16	0.002954	0.0007546 to 0.005154	Yes	**	0.0085 B	16
DMSO vs. 32	0.002666	0.0004667 to 0.004866	Yes	*	0.017 C	32

Test details	Mean 1	Mean 2		Mean Diff.	SE of diff.	n1	n2	q	DF
DMSO vs. 16	0.006383		0.003429	0.002954	0.000928	8	8	3.184	21
DMSO vs. 32	0.006383		0.003717	0.002666	0.000928	8	8	2.873	21

Table Analyzed	7B. CYP21A2 transcripts vs. ATR-101		
Data sets analyzed	A : DMSO B : 16		C : 32

ANOVA summary

F	4.018
P value	0.0333
P value summary	*
Significant diff. among means (P < 0.05)?	Yes
R square	0.2767

Brown-Forsythe test

F (DFn, DFd)	2.41 (2, 21)
P value	0.1143
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test

Bartlett's statistic (corrected)	3.569
P value	0.1679
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

ANOVA table	SS	DF		MS	F (DFn, DFd)	P value
Treatment (between columns)	0.002716		2	0.001358	F (2, 21) = 4.018	P=0.0333

Residual (within columns)	0.007097	21	0.000338	
Total	0.009813	23		

Data summary

Number of treatments (columns)	3
Number of values (total)	24
Number of families	1
Number of comparisons per family	2
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
DMSO vs. 16	0.01356	-0.008223 to 0.03535	No	ns	0.2612	B	16
DMSO vs. 32	0.02605	0.00426 to 0.04784	Yes	*	0.0186	C	32

Test details	Mean 1	Mean 2		Mean Diff.	SE of diff.	n1	n2	q	DF
DMSO vs. 16	0.09619		0.08262	0.01356	0.009192	8	8	1.476	21
DMSO vs. 32	0.09619		0.07014	0.02605	0.009192	8	8	2.834	21

Table Analyzed   S1A. Mean fluorescence intensity of cell clusters vs. time after ATR-101 additio

Column B   0.25 h ATR-101
vs.   vs.
Column A   0.25 h DMSO

Unpaired t test
P value   <0.0001
P value summary   ****
Significantly different (P < 0.05)?   Yes
One- or two-tailed P value?   Two-tailed
t, df   t=9.345 df=617

How big is the difference?
Mean ± SEM of column A   28.76 ± 0.5096, n=313
Mean ± SEM of column B   36.52 ± 0.6587, n=306
Difference between means   7.761 ± 0.8306
95% confidence interval   6.13 to 9.392
R squared (eta squared)   0.124

F test to compare variances
F, DFn, Dfd   1.633, 305, 312
P value   <0.0001
P value summary   ****
Significantly different (P < 0.05)?   Yes

Table Analyzed   S1A. Mean fluorescence intensity of cell clusters vs. time after ATR-101 additio

Column D   0.5 h ATR-101
vs.   vs.
Column C   0.5 h DMSO

Unpaired t test
P value   <0.0001
P value summary   ****
Significantly different (P < 0.05)?   Yes
One- or two-tailed P value?   Two-tailed
t, df   t=7.284 df=532

How big is the difference?
Mean ± SEM of column C   29.34 ± 0.4887, n=313
Mean ± SEM of column D   35.71 ± 0.777, n=221
Difference between means   6.368 ± 0.8743
95% confidence interval   4.651 to 8.085
R squared (eta squared)   0.09068

F test to compare variances
F, DFn, Dfd   1.785, 220, 312
P value   <0.0001
P value summary   ****
Significantly different (P < 0.05)?   Yes

Table Analyzed   S1A. Mean fluorescence intensity of cell clusters vs. time after ATR-101 additio

Column F   1 h ATR-101
vs.   vs.
Column E   1 h DMSO

Unpaired t test
P value   <0.0001
P value summary   ****
Significantly different (P < 0.05)?   Yes
One- or two-tailed P value?   Two-tailed
t, df   t=19.86 df=617

How big is the difference?
Mean ± SEM of column E   26.29 ± 0.4407, n=306
Mean ± SEM of column F   44.54 ± 0.7997, n=313
Difference between means   18.25 ± 0.9187
95% confidence interval   16.44 to 20.05
R squared (eta squared)   0.39

F test to compare variances
F, DFn, Dfd   3.369, 312, 305
P value   <0.0001
P value summary   ****
Significantly different (P < 0.05)?   Yes

Unpaired t test of S1A. Mean fluorescence intensity of cell clusters vs. ATR-101 or DMSO, 2 h

Table Analyzed   S1A. Mean fluorescence intensity of cell clusters vs. time after ATR-101 additio

Column H   2 h ATR-101
vs.   vs.
Column G   2 h DMSO

Unpaired t test
P value   <0.0001
P value summary   ****
Significantly different (P < 0.05)?   Yes
One- or two-tailed P value?   Two-tailed
t, df   t=11.94 df=567

How big is the difference?
Mean ± SEM of column G   24.93 ± 0.452, n=266
Mean ± SEM of column H   40.5 ± 1.155, n=303
Difference between means   15.57 ± 1.304
95% confidence interval   13.01 to 18.13
R squared (eta squared)   0.2009

F test to compare variances
F, DFn, Dfd   7.443, 302, 265
P value   <0.0001
P value summary   ****
Significantly different (P < 0.05)?   Yes

Unpaired t test of S1A. Mean fluorescence intensity of cell clusters vs. ATR-101 or DMSO, 4 h

Table Analyzed   S1A. Mean fluorescence intensity of cell clusters vs. time after ATR-101 additio

Column J   4 h ATR-101
vs.   vs.
Column I   4 h DMSO

Unpaired t test
P value   <0.0001
P value summary   ****
Significantly different (P < 0.05)?   Yes
One- or two-tailed P value?   Two-tailed
t, df   t=33.46 df=546

How big is the difference?
Mean ± SEM of column I   20.51 ± 0.3551, n=303
Mean ± SEM of column J   54.69 ± 1.048, n=245
Difference between means   34.18 ± 1.021
95% confidence interval   32.17 to 36.19
R squared (eta squared)   0.6722

F test to compare variances
F, DFn, Dfd   7.04, 244, 302
P value   <0.0001
P value summary   ****
Significantly different (P < 0.05)?   Yes

2way ANOVA of S1B. H295R ATP level vs. ATR-101 or PD129337 concentration, 24 h

Table Analyzed	S1B. H295R ATP level vs. ATR-101 or PD129337 concentration, 24 h

Two-way ANOVA	Ordinary
Alpha	0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	29.17	<0.0001	****	Yes
Row Factor	31.81	<0.0001	****	Yes
Column Factor	35.53	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	352182736	6	58697123	F (6, 70) = 97.52	P<0.0001
Row Factor	383961538	6	63993590	F (6, 70) = 106.3	P<0.0001
Column Factor	428932710	1	428932710	F (1, 70) = 712.6	P<0.0001
Residual	42133227	70	601903		

Number of missing values	0

Compare each cell mean with the other cell mean in that row

Number of families	1
Number of comparisons per family	7
Alpha	0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
ATR-101 - PD129337					
0	42.94	-1195 to 1281	No	ns	>0.9999
5	-99.91	-1338 to 1138	No	ns	>0.9999
10	-517.7	-1756 to 720.1	No	ns	0.8686
20	-4149	-5387 to -2911	Yes	****	<0.0001
40	-7999	-9237 to -6761	Yes	****	<0.0001
60	-9318	-10556 to -8080	Yes	****	<0.0001
100	-9595	-10833 to -8357	Yes	****	<0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
ATR-101 - PD129337								
0	10729	10686	42.94	447.9	6	6	0.09587	70
5	10445	10544	-99.91	447.9	6	6	0.2231	70
10	9836	10353	-517.7	447.9	6	6	1.156	70
20	6291	10440	-4149	447.9	6	6	9.263	70
40	2197	10196	-7999	447.9	6	6	17.86	70
60	1146	10464	-9318	447.9	6	6	20.8	70
100	740.5	10336	-9595	447.9	6	6	21.42	70

2way ANOVA of S1B. H295R caspase level vs. ATR-101 or PD129337 concentration, 24

Table Analyzed	S1B. H295R caspase activity vs. ATR-101 or PD129337 concentration, 24 h

Two-way ANOVA	Ordinary
Alpha	0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	33.25	<0.0001	****	Yes
Row Factor	33.18	<0.0001	****	Yes
Column Factor	31.17	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	71369450	6	11894908	F (6, 70) = 162.1	P<0.0001
Row Factor	71216324	6	11869387	F (6, 70) = 161.7	P<0.0001
Column Factor	66894919	1	66894919	F (1, 70) = 911.4	P<0.0001
Residual	5137902	70	73399		

Number of missing values	0

Compare each cell mean with the other cell mean in that row

Number of families	1
Number of comparisons per family	7
Alpha	0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
ATR-101 - PD129337					
0	-6.626	-438.9 to 425.6	No	ns	>0.9999
5	95.48	-336.8 to 527.7	No	ns	0.9959
10	78.62	-353.6 to 510.9	No	ns	0.9988
20	762.1	329.9 to 1194	Yes	****	<0.0001
40	3076	2644 to 3508	Yes	****	<0.0001
60	4180	3748 to 4612	Yes	****	<0.0001
100	4308	3876 to 4740	Yes	****	<0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
ATR-101 - PD129337								
0	1011	1018	-6.626	156.4	6	6	0.04236	70
5	1122	1027	95.48	156.4	6	6	0.6104	70
10	1089	1010	78.62	156.4	6	6	0.5026	70
20	1779	1017	762.1	156.4	6	6	4.872	70
40	4085	1009	3076	156.4	6	6	19.67	70
60	5200	1020	4180	156.4	6	6	26.72	70
100	5323	1016	4308	156.4	6	6	27.54	70

2way ANOVA of S1B. BD140C ATP level vs. ATR-101 or PD129337 concentration, 24 h

Table Analyzed	S1B. BD140C ATP level vs. ATR-101 or PD129337 concentration, 24 h

| Two-way ANOVA | Ordinary |
| Alpha | 0.05 |

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	21.78	<0.0001	****	Yes
Row Factor	26.07	<0.0001	****	Yes
Column Factor	50.34	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	113202050	5	22640410	F (5, 60) = 144.6	P<0.0001
Row Factor	135468390	5	27093678	F (5, 60) = 173	P<0.0001
Column Factor	261609260	1	261609260	F (1, 60) = 1670	P<0.0001
Residual	9396613	60	156610		

Number of missing values	0

Compare each cell mean with the other cell mean in that row

Number of families	1
Number of comparisons per family	6
Alpha	0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
ATR-101 - PD129337					
0	-81.91	-703.5 to 539.7	No	ns	0.9995
5	-980.3	-1602 to -358.7	Yes	***	0.0004
10	-3584	-4205 to -2962	Yes	****	<0.0001
20	-5584	-6206 to -4963	Yes	****	<0.0001
40	-6396	-7018 to -5775	Yes	****	<0.0001
60	-6247	-6869 to -5626	Yes	****	<0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
ATR-101 - PD129337								
0	6752	6834	-81.91	228.5	6	6	0.3585	60
5	5738	6719	-980.3	228.5	6	6	4.291	60

10	3290	6874	-3584	228.5	6	6	15.69	60
20	1146	6731	-5584	228.5	6	6	24.44	60
40	160.6	6557	-6396	228.5	6	6	27.99	60
60	134.1	6381	-6247	228.5	6	6	27.34	60

2way ANOVA of S1B. BD140C caspase activity vs. ATR-101 or PD129337 concentration

Table Analyzed   S1B. BD140C caspase activity vs. ATR-101 or PD129337 concentration, 24 h

Two-way ANOVA   Ordinary
Alpha   0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	28.59	<0.0001	****	Yes
Row Factor	28.42	<0.0001	****	Yes
Column Factor	41.64	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	137215815	4	34303954	$F_{(4, 50)} = 264.3$	P<0.0001
Row Factor	136400350	4	34100088	$F_{(4, 50)} = 262.7$	P<0.0001
Column Factor	199824692	1	199824692	$F_{(1, 50)} = 1539$	P<0.0001
Residual	6490374	50	129807		

Number of missing values   0

Compare each cell mean with the other cell mean in that row

Number of families   1
Number of comparisons per family   5
Alpha   0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
ATR-101 - PD129337					
0	7.624	-547.8 to 563	No	ns	>0.9999
5	771.3	215.9 to 1327	Yes	**	0.0026
10	3240	2685 to 3796	Yes	****	<0.0001
20	7142	6587 to 7698	Yes	****	<0.0001
40	7088	6533 to 7643	Yes	****	<0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
ATR-101 - PD129337								
0	1014	1006	7.624	208	6	6	0.03665	50
5	1791	1020	771.3	208	6	6	3.708	50
10	4254	1014	3240	208	6	6	15.58	50
20	8148	1006	7142	208	6	6	34.34	50
40	8087	999.3	7088	208	6	6	34.07	50

Table Analyzed	S2B. ATP level vs. MBCD concentration, 24 h, +ATR-101				
Data sets analyzed	A : 0	B : 0.25	C : 0.5	D : 1	E : 1.5

ANOVA summary

F	206.9
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.9718

Brown-Forsythe test

F (DFn, DFd)	4.438 (5, 30)
P value	0.0038
P value summary	**
Are SDs significantly different (P < 0.05)?	Yes

Bartlett's test

Bartlett's statistic (corrected)	28.63
P value	<0.0001
P value summary	****
Are SDs significantly different (P < 0.05)?	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	3045702518	5	609140504	F (5, 30) = 206.9	P<0.0001
Residual (within columns)	88319546	30	2943985		
Total	3134022064	35			

Data summary

Number of treatments (columns)	6
Number of values (total)	36
Number of families	1
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
0 vs. 0.25	-58.33	-2690 to 2574	No	ns	0.9999	B	0.25
0 vs. 0.5	-491.5	-3124 to 2141	No	ns	0.9826	C	0.5
0 vs. 1	-13348	-15980 to -10716	Yes	****	0.0001	D	1
0 vs. 1.5	-19748	-22380 to -17116	Yes	****	0.0001	E	1.5
0 vs. 2	-20827	-23459 to -18195	Yes	****	0.0001	F	2

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 0.25	565.7	624	-58.33	990.6	6	6	0.05888	30
0 vs. 0.5	565.7	1057	-491.5	990.6	6	6	0.4961	30
0 vs. 1	565.7	13914	-13348	990.6	6	6	13.47	30
0 vs. 1.5	565.7	20314	-19748	990.6	6	6	19.94	30
0 vs. 2	565.7	21393	-20827	990.6	6	6	21.02	30

Table Analyzed	S2B. Caspase activity vs. MBCD concentration, 24 h, +ATR-101				
Data sets analyzed	A : 0	B : 0.25	C : 0.5	D : 1	E : 1.5

ANOVA summary

F	206.9
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.9718

Brown-Forsythe test

F (DFn, DFd)	4.438 (5, 30)
P value	0.0038
P value summary	**
Are SDs significantly different (P < 0.05)?	Yes

Bartlett's test

Bartlett's statistic (corrected)	28.63
P value	<0.0001

P value summary ****
Are SDs significantly different (P < 0.05)? Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	3045702518	5	609140504	F (5, 30) = 206.9	P<0.0001
Residual (within columns)	88319546	30	2943985		
Total	3134022064	35			

Data summary
Number of treatments (columns)	6
Number of values (total)	36
Number of families	1
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
0 vs. 0.25	-58.33	-2690 to 2574	No	ns	0.9999	B	0.25
0 vs. 0.5	-491.5	-3124 to 2141	No	ns	0.9826	C	0.5
0 vs. 1	-13348	-15980 to -10716	Yes	****	0.0001	D	1
0 vs. 1.5	-19748	-22380 to -17116	Yes	****	0.0001	E	1.5
0 vs. 2	-20827	-23459 to -18195	Yes	****	0.0001	F	2

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 0.25	565.7	624	-58.33	990.6	6	6	0.05888	30
0 vs. 0.5	565.7	1057	-491.5	990.6	6	6	0.4961	30
0 vs. 1	565.7	13914	-13348	990.6	6	6	13.47	30
0 vs. 1.5	565.7	20314	-19748	990.6	6	6	19.94	30
0 vs. 2	565.7	21393	-20827	990.6	6	6	21.02	30

Ordinary one-way ANOVA of S2C. ATP level vs. cholesterol concentration, 4 h, +AT

Table Analyzed	S2C. ATP level vs. cholesterol concentration, 4 h, +ATR-101				
Data sets analyzed	A : 0	B : 1	C : 2.5	D : 5	E : 10

ANOVA summary
F	9.344
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.609

Brown-Forsythe test
F (DFn, DFd)	0.3888 (5, 30)
P value	0.8525
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test
Bartlett's statistic (corrected)	1.162
P value	0.9485
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	56133808	5	11226762	F (5, 30) = 9.344	P<0.0001
Residual (within columns)	36045535	30	1201518		
Total	92179343	35			

Data summary
Number of treatments (columns)	6
Number of values (total)	36
Number of families	1
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
0 vs. 1	-1011	-2693 to 670.4	No	ns	0.3784	B	1
0 vs. 2.5	-1863	-3545 to -181.8	Yes	*	0.0258	C	2.5
0 vs. 5	-2079	-3760 to -397.2	Yes	*	0.0112	D	5

0 vs. 10		-2904 -4586 to -1223	Yes	***		0.0004 E		10
0 vs. 25		-3883 -5565 to -2202	Yes	****		0.0001 F		25

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 1	4228	5239	-1011	632.9	6	6	1.598	30
0 vs. 2.5	4228	6091	-1863	632.9	6	6	2.944	30
0 vs. 5	4228	6306	-2079	632.9	6	6	3.285	30
0 vs. 10	4228	7132	-2904	632.9	6	6	4.589	30
0 vs. 25	4228	8111	-3883	632.9	6	6	6.136	30

Ordinary one-way ANOVA of S2C. Caspase activity vs. cholesterol concentration, 4

Table Analyzed	S2C. Caspase activity vs. cholesterol concentration, 4 h, + ATR-101				
Data sets analyzed	A : 0	B : 2.5	C : 10	D : 25	E : 50

ANOVA summary

F	1.791
P value	0.145
P value summary	ns
Significant diff. among means (P < 0.05)?	No
R square	0.2299

Brown-Forsythe test

F (DFn, DFd)	0.747 (5, 30)
P value	0.5947
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test

Bartlett's statistic (corrected)	6.699
P value	0.244
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	864853	5	172971	F (5, 30) = 1.791	P=0.1450
Residual (within columns)	2897696	30	96590		
Total	3762548	35			

Data summary

Number of treatments (columns)	6
Number of values (total)	36
Number of families	1
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?
0 vs. 2.5	29.63	-447.1 to 506.4	No	ns	0.9997 B	2.5
0 vs. 10	151.7	-325.1 to 628.4	No	ns	0.8654 C	10
0 vs. 25	226.1	-250.7 to 702.8	No	ns	0.5964 D	25
0 vs. 50	-4.281	-481 to 472.5	No	ns	0.9999 E	50
0 vs. 100	-268.6	-745.3 to 208.2	No	ns	0.4387 F	100

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 2.5	2482	2453	29.63	179.4	6	6	0.1651	30
0 vs. 10	2482	2331	151.7	179.4	6	6	0.8453	30
0 vs. 25	2482	2256	226.1	179.4	6	6	1.26	30
0 vs. 50	2482	2487	-4.281	179.4	6	6	0.02386	30
0 vs. 100	2482	2751	-268.6	179.4	6	6	1.497	30

Unpaired t test of S2D. ATP level vs. cholesterol or cholesterol linoleate conce

Table Analyzed	S2D. ATP level vs. cholesterol or cholesterol linoleate concentration

Column B	cholesterol linoleate+ATR-101
vs.	vs.
Column A	cholesterol+ATR-101

Unpaired t test

P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=7.167 df=22

How big is the difference?	
Mean ± SEM of column A	5574 ± 753.1, n=12
Mean ± SEM of column B	173.3 ± 27.08, n=12
Difference between means	-5401 ± 753.6
95% confidence interval	-6964 to -3838
R squared (eta squared)	0.7002

F test to compare variances	
F, DFn, Dfd	773.2, 11, 11
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes

2way ANOVA of S2F. Resazurin reducing potential vs. time, +ATR-101 or DMSO

Table Analyzed	S2F. Resazurin reducing potential vs. time, +ATR-101 or DMSO
Two-way ANOVA	Ordinary
Alpha	0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	5.571	<0.0001	****	Yes
Row Factor	68.82	<0.0001	****	Yes
Column Factor	23.54	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	2695702	6	449284	F (6, 70) = 31.45	P<0.0001
Row Factor	33297777	6	5549630	F (6, 70) = 388.5	P<0.0001
Column Factor	11392473	1	11392473	F (1, 70) = 797.4	P<0.0001
Residual	1000056	70	14287		

Number of missing values	0

Compare each cell mean with the other cell mean in that row

Number of families	1
Number of comparisons per family	7
Alpha	0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
DMSO - ATR-101					
0	0	-190.7 to 190.7	No	ns	>0.9999
73	-536.5	-727.2 to -345.8	Yes	****	<0.0001
126	-665.8	-856.5 to -475.1	Yes	****	<0.0001
215	-819.9	-1011 to -629.2	Yes	****	<0.0001
250	-930.6	-1121 to -739.9	Yes	****	<0.0001
314	-1098	-1288 to -906.9	Yes	****	<0.0001
362	-1105	-1296 to -914.7	Yes	****	<0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
DMSO - ATR-101								
0	0	0	0	69.01	6	6	0	70
73	261.8	798.3	-536.5	69.01	6	6	7.774	70
126	516.8	1183	-665.8	69.01	6	6	9.648	70
215	597.5	1417	-819.9	69.01	6	6	11.88	70
250	798.2	1729	-930.6	69.01	6	6	13.49	70
314	1198	2296	-1098	69.01	6	6	15.9	70
362	1408	2513	-1105	69.01	6	6	16.02	70

2way ANOVA of S2F. Resazurin reducing potential vs. time, +ATR-101 DMSO, +MBCD

Table Analyzed	S2F. Resazurin reducing potential vs. time, +ATR-101 or DMSO
Two-way ANOVA	Ordinary
Alpha	0.05

Source of Variation	% of total variation	P value		P value summary	Significant?
Interaction	5.813	<0.0001		****	Yes
Row Factor	69.19	<0.0001		****	Yes
Column Factor	22.28	<0.0001		****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	4390725	6	731788	F (6, 70) = 24.99	P<0.0001
Row Factor	52260277	6	8710046	F (6, 70) = 297.5	P<0.0001
Column Factor	16831709	1	16831709	F (1, 70) = 574.8	P<0.0001
Residual	2049679	70	29281		

Number of missing values 0

Compare each cell mean with the other cell mean in that row

Number of families	1
Number of comparisons per family	7
Alpha	0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
MBCD+DMSO - MBCD+ATR-101					
0	0	-273 to 273	No	ns	>0.9999
73	-715.9	-988.9 to -442.9	Yes	****	<0.0001
126	-794.8	-1068 to -521.8	Yes	****	<0.0001
215	-920.9	-1194 to -647.9	Yes	****	<0.0001
250	-969	-1242 to -696	Yes	****	<0.0001
314	-1301	-1574 to -1028	Yes	****	<0.0001
362	-1566	-1839 to -1293	Yes	****	<0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
MBCD+DMSO - MBCD+ATR-101								
0	0	0	0	98.79	6	6	0	70
73	357.9	1074	-715.9	98.79	6	6	7.246	70
126	742.7	1537	-794.8	98.79	6	6	8.045	70
215	779.2	1700	-920.9	98.79	6	6	9.322	70
250	1033	2002	-969	98.79	6	6	9.809	70
314	1427	2728	-1301	98.79	6	6	13.16	70
362	1800	3366	-1566	98.79	6	6	15.85	70

2way ANOVA of S2I. ATP level vs. cholesterol:MBCD concentration, 4 h, +ATR-101

Table Analyzed S2I. ATP level vs. cholesterol:MBCD concentration, 4 h, +ATR-101

Two-way ANOVA	Ordinary
Alpha	0.05

Source of Variation	% of total variation	P value		P value summary	Significant?
Interaction	12.76	<0.0001		****	Yes
Row Factor	71.63	<0.0001		****	Yes
Column Factor	4.485	0.0009		***	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	140406209	5	28081242	F (5, 60) = 13.77	P<0.0001
Row Factor	787951020	1	787951020	F (1, 60) = 386.5	P<0.0001
Column Factor	49333479	5	9866696	F (5, 60) = 4.839	P=0.0009
Residual	122328663	60	2038811		

Number of missing values 0

Within each row, compare columns (simple effects within rows)

Number of families	2
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
DMSO					
0 vs. 0.25	-1069	-3198 to 1060	No	ns	0.5659
0 vs. 0.5	-760.6	-2890 to 1368	No	ns	0.8228
0 vs. 1	1615	-513.9 to 3744	No	ns	0.1954
0 vs. 1.5	1665	-463.8 to 3794	No	ns	0.1734

	Mean Diff.	95.00% CI of diff.	Significant?	Summary		Adjusted P Value
0 vs. 2	2149	20.03 to 4278	Yes	*		0.0471

ATR-101
0 vs. 0.25	-3297	-5426 to -1168	Yes	***		0.0008
0 vs. 0.5	-4525	-6654 to -2396	Yes	****		0.0001
0 vs. 1	-4474	-6603 to -2345	Yes	****		0.0001
0 vs. 1.5	-5336	-7465 to -3207	Yes	****		0.0001
0 vs. 2	-5825	-7954 to -3696	Yes	****		0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF
DMSO								
0 vs. 0.25	19555	20625	-1069	824.4	6	6	1.297	60
0 vs. 0.5	19555	20316	-760.6	824.4	6	6	0.9226	60
0 vs. 1	19555	17940	1615	824.4	6	6	1.959	60
0 vs. 1.5	19555	17890	1665	824.4	6	6	2.02	60
0 vs. 2	19555	17406	2149	824.4	6	6	2.607	60
ATR-101								
0 vs. 0.25	8429	11727	-3297	824.4	6	6	4	60
0 vs. 0.5	8429	12955	-4525	824.4	6	6	5.489	60
0 vs. 1	8429	12903	-4474	824.4	6	6	5.427	60
0 vs. 1.5	8429	13765	-5336	824.4	6	6	6.472	60
0 vs. 2	8429	14255	-5825	824.4	6	6	7.066	60

2way ANOVA of S2I. Caspase activity vs. cholesterol:MBCD concentration, 4 h, +AT

Table Analyzed   S2I. Caspase activity vs. cholesterol:MBCD concentration, 4 h, +ATR-101

Two-way ANOVA   Ordinary
Alpha   0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	3.473	<0.0001	****	Yes
Row Factor	5.602	<0.0001	****	Yes
Column Factor	86.17	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	513047069	5	102609414	F (5, 60) = 8.766	P<0.0001
Row Factor	827529992	1	827529992	F (1, 60) = 70.7	P<0.0001
Column Factor	12729423881	5	2545884776	F (5, 60) = 217.5	P<0.0001
Residual	702290283	60	11704838		

Number of missing values   0

Within each row, compare columns (simple effects within rows)

Number of families   2
Number of comparisons per family   5
Alpha   0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
DMSO					
0 vs. 0.25	-108.7	-5210 to 4993	No	ns	0.9999
0 vs. 0.5	-1326	-6427 to 3775	No	ns	0.9419
0 vs. 1	-19084	-24186 to -13983	Yes	****	0.0001
0 vs. 1.5	-25911	-31012 to -20809	Yes	****	0.0001
0 vs. 2	-29244	-34345 to -24142	Yes	****	0.0001
ATR-101					
0 vs. 0.25	-97.8	-5199 to 5004	No	ns	0.9999
0 vs. 0.5	-10919	-16021 to -5818	Yes	****	0.0001
0 vs. 1	-33389	-38490 to -28288	Yes	****	0.0001
0 vs. 1.5	-31364	-36466 to -26263	Yes	****	0.0001
0 vs. 2	-30623	-35724 to -25521	Yes	****	0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF
DMSO								
0 vs. 0.25	858.7	967.4	-108.7	1975	6	6	0.05504	60
0 vs. 0.5	858.7	2185	-1326	1975	6	6	0.6713	60
0 vs. 1	858.7	19943	-19084	1975	6	6	9.662	60

	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF
0 vs. 1.5	858.7	26770	-25911	1975	6	6	13.12	60
0 vs. 2	858.7	30102	-29244	1975	6	6	14.81	60
ATR-101								
0 vs. 0.25	2519	2617	-97.8	1975	6	6	0.04951	60
0 vs. 0.5	2519	13439	-10919	1975	6	6	5.528	60
0 vs. 1	2519	35908	-33389	1975	6	6	16.9	60
0 vs. 1.5	2519	33883	-31364	1975	6	6	15.88	60
0 vs. 2	2519	33142	-30623	1975	6	6	15.5	60

2way ANOVA of S2I. ATP level vs. cholesterol:MBCD concentration, 24 h, +ATR-101

Table Analyzed: S2I. ATP level vs. cholesterol:MBCD concentration, 24 h, +ATR-101

Two-way ANOVA: Ordinary
Alpha: 0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	15.64	<0.0001	****	Yes
Row Factor	32.34	<0.0001	****	Yes
Column Factor	48.85	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	541981972	5	108396394	F (5, 60) = 59.07	P<0.0001
Row Factor	1121055969	1	1121055969	F (1, 60) = 610.9	P<0.0001
Column Factor	1693093343	5	338618669	F (5, 60) = 184.5	P<0.0001
Residual	110105886	60	1835098		

Number of missing values: 0

Within each row, compare columns (simple effects within rows)

Number of families: 2
Number of comparisons per family: 5
Alpha: 0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
DMSO					
0 vs. 0.25	-609.4	-2629 to 1410	No	ns	0.8991
0 vs. 0.5	8.708	-2011 to 2029	No	ns	0.9999
0 vs. 1	11975	9955 to 13995	Yes	****	0.0001
0 vs. 1.5	14225	12205 to 16245	Yes	****	0.0001
0 vs. 2	14320	12300 to 16340	Yes	****	0.0001
ATR-101					
0 vs. 0.25	-10623	-12643 to -8604	Yes	****	0.0001
0 vs. 0.5	-5010	-7029 to -2990	Yes	****	0.0001
0 vs. 1	-1781	-3801 to 238.9	No	ns	0.1014
0 vs. 1.5	-227.6	-2248 to 1792	No	ns	0.9983
0 vs. 2	-432.1	-2452 to 1588	No	ns	0.9735

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF
DMSO								
0 vs. 0.25	17655	18264	-609.4	782.1	6	6	0.7792	60
0 vs. 0.5	17655	17646	8.708	782.1	6	6	0.01113	60
0 vs. 1	17655	5679	11975	782.1	6	6	15.31	60
0 vs. 1.5	17655	3430	14225	782.1	6	6	18.19	60
0 vs. 2	17655	3335	14320	782.1	6	6	18.31	60
ATR-101								
0 vs. 0.25	97.19	10721	-10623	782.1	6	6	13.58	60
0 vs. 0.5	97.19	5107	-5010	782.1	6	6	6.405	60
0 vs. 1	97.19	1878	-1781	782.1	6	6	2.277	60
0 vs. 1.5	97.19	324.8	-227.6	782.1	6	6	0.291	60
0 vs. 2	97.19	529.3	-432.1	782.1	6	6	0.5524	60

2way ANOVA of S2I. Caspase activity vs. cholesterol:MBCD concentration, 24 h, +A

Table Analyzed: S2I. Caspase activity vs. cholesterol:MBCD concentration, 24 h, +ATR-101

Two-way ANOVA: Ordinary
Alpha: 0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	26.56	<0.0001	****	Yes
Row Factor	4.148	<0.0001	****	Yes
Column Factor	65.78	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	3404583799	5	680916760	F (5, 60) = 90.77	P<0.0001
Row Factor	531838324	1	531838324	F (1, 60) = 70.9	P<0.0001
Column Factor	8433825739	5	1686765148	F (5, 60) = 224.9	P<0.0001
Residual	450078122	60	7501302		

Number of missing values	0

Within each row, compare columns (simple effects within rows)

Number of families	2
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
DMSO					
0 vs. 0.25	-177.2	-4261 to 3907	No	ns	0.9999
0 vs. 0.5	-2720	-6804 to 1364	No	ns	0.3016
0 vs. 1	-30563	-34647 to -26479	Yes	****	0.0001
0 vs. 1.5	-30691	-34775 to -26608	Yes	****	0.0001
0 vs. 2	-34079	-38163 to -29995	Yes	****	0.0001
ATR-101					
0 vs. 0.25	-11471	-15555 to -7387	Yes	****	0.0001
0 vs. 0.5	-27787	-31871 to -23703	Yes	****	0.0001
0 vs. 1	-26684	-30768 to -22600	Yes	****	0.0001
0 vs. 1.5	-18777	-22860 to -14693	Yes	****	0.0001
0 vs. 2	-19013	-23096 to -14929	Yes	****	0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF
DMSO								
0 vs. 0.25	926.3	1103	-177.2	1581	6	6	0.1121	60
0 vs. 0.5	926.3	3647	-2720	1581	6	6	1.72	60
0 vs. 1	926.3	31489	-30563	1581	6	6	19.33	60
0 vs. 1.5	926.3	31618	-30691	1581	6	6	19.41	60
0 vs. 2	926.3	35005	-34079	1581	6	6	21.55	60
ATR-101								
0 vs. 0.25	5445	16916	-11471	1581	6	6	7.254	60
0 vs. 0.5	5445	33232	-27787	1581	6	6	17.57	60
0 vs. 1	5445	32129	-26684	1581	6	6	16.87	60
0 vs. 1.5	5445	24222	-18777	1581	6	6	11.87	60
0 vs. 2	5445	24458	-19013	1581	6	6	12.02	60

Table Analyzed	S4A. Cortisol secretion vs. time, +DMSO or verapamil					
Two-way ANOVA	Ordinary					
Alpha	0.05					

Source of Variation	% of total variation	P value	P value summary	Significant?		
Interaction	35.88	<0.0001	****	Yes		
Row Factor	37.67	<0.0001	****	Yes		
Column Factor	23.09	<0.0001	****	Yes		

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	380052	3	126684	F (3, 32) = 113.7	P<0.0001
Row Factor	398945	3	132982	F (3, 32) = 119.4	P<0.0001
Column Factor	244514	1	244514	F (1, 32) = 219.5	P<0.0001
Residual	35645	32	1114		

Number of missing values 0

Compare each cell mean with the other cell mean in that row

Number of families	1
Number of comparisons per family	4
Alpha	0.05

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
DMSO - verapamil					
0.25	5.998	-49.71 to 61.7	No	ns	0.9976
1	16.38	-39.32 to 72.08	No	ns	0.904
4	117.6	61.92 to 173.3	Yes	****	<0.0001
8	485.5	429.8 to 541.2	Yes	****	<0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
DMSO - verapamil								
0.25	82.15	76.15	5.998	21.11	5	5	0.2842	32
1	102.7	86.3	16.38	21.11	5	5	0.776	32
4	198.4	80.75	117.6	21.11	5	5	5.572	32
8	572	86.53	485.5	21.11	5	5	23	32

Table Analyzed	S4B. Cortisol secretion vs. zosuquidar concentration			
Data sets analyzed	A : 0	B : 5	C : 20	D : 40

ANOVA summary	
F	65.36
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)? Yes	
R square	0.9246

Brown-Forsythe test	
F (DFn, DFd)	0.146 (3, 16)
P value	0.9308
P value summary	ns
Are SDs significantly different (P < 0.05)? No	

Bartlett's test	
Bartlett's statistic (corrected)	1.158
P value	0.7632
P value summary	ns
Are SDs significantly different (P < 0.05)? No	

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	24991	3	8330	F (3, 16) = 65.36	P<0.0001
Residual (within columns)	2039	16	127.5		
Total	27031	19			

Data summary	
Number of treatments (columns)	4

Number of values (total)		20						

Number of families		1						
Number of comparisons per family		3						
Alpha		0.05						

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P ' A-?			
0 vs. 5	46.85	28.34 to 65.36	Yes	****	0.0001 B			5
0 vs. 20	85.99	67.48 to 104.5	Yes	****	0.0001 C			20
0 vs. 40	85.77	67.26 to 104.3	Yes	****	0.0001 D			40

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 5	137.3	90.47	46.85	7.14	5	5	6.561	16
0 vs. 20	137.3	51.33	85.99	7.14	5	5	12.04	16
0 vs. 40	137.3	51.55	85.77	7.14	5	5	12.01	16

2way ANOVA of S4B. Cortisol secretion vs. ATR-101 concentration, -/+ olesoxime

Table Analyzed	S4B. Cortisol secretion vs. ATR-101 concentration, -/+ olesoxime

Two-way ANOVA	Ordinary
Alpha	0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	0.3187	0.7403	ns	No
Row Factor	0.9476	0.191	ns	No
Column Factor	86.17	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	105.9	2	52.97	F (2, 24) = 0.3045	P=0.7403
Row Factor	315	1	315	F (1, 24) = 1.81	P=0.1910
Column Factor	28642	2	14321	F (2, 24) = 82.32	P<0.0001
Residual	4175	24	174		

Number of missing values	0

Within each row, compare columns (simple effects within rows)

Number of families	2
Number of comparisons per family	2
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
ATR-101					
0 vs. 20	13.46	-6.139 to 33.06	No	ns	0.2056
0 vs. 40	75.06	55.46 to 94.66	Yes	****	0.0001
ATR-101+olesoxime					
0 vs. 20	14.88	-4.717 to 34.48	No	ns	0.1523
0 vs. 40	67.89	48.29 to 87.49	Yes	****	0.0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF
ATR-101								
0 vs. 20	126.9	113.5	13.46	8.342	5	5	1.614	24
0 vs. 40	126.9	51.86	75.06	8.342	5	5	8.997	24
ATR-101+olesoxime								
0 vs. 20	131.5	116.6	14.88	8.342	5	5	1.784	24
0 vs. 40	131.5	63.59	67.89	8.342	5	5	8.138	24

Ordinary one-way ANOVA of S4B. ATP level vs. zosuquidar concentration

Table Analyzed	S4B. ATP level vs. zosuquidar concentration			
Data sets analyzed	A : 0	B : 5	C : 20	D : 40

ANOVA summary	
F	0.47
P value	0.7074

P value summary	ns
Significant diff. among means (P < 0.05)?	No
R square	0.08099

Brown-Forsythe test

F (DFn, DFd)	0.09339 (3, 16)
P value	0.9626
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test

Bartlett's statistic (corrected)	0.4373
P value	0.9324
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	1610849	3	536950	F (3, 16) = 0.47	P=0.7074
Residual (within columns)	18279015	16	1142438		
Total	19889864	19			

Data summary

| Number of treatments (columns) | 4 |
| Number of values (total) | 20 |

Number of families	1
Number of comparisons per family	3
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P 'A-?	
0 vs. 5	777.9	-974.7 to 2530	No	ns	0.536 B	5
0 vs. 20	463.8	-1289 to 2216	No	ns	0.8334 C	20
0 vs. 40	559.5	-1193 to 2312	No	ns	0.7478 D	40

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
0 vs. 5	15190	14412	777.9	676	5	5	1.151	16
0 vs. 20	15190	14726	463.8	676	5	5	0.6861	16
0 vs. 40	15190	14630	559.5	676	5	5	0.8276	16

2way ANOVA of S4B. ATP level vs. ATR-101 concentration, -/+ olesoxime

| Table Analyzed | S4B. ATP level vs. ATR-101 concentration, -/+ olesoxime |

| Two-way ANOVA | Ordinary |
| Alpha | 0.05 |

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	27.87	<0.0001	****	Yes
Row Factor	17.24	<0.0001	****	Yes
Column Factor	44.53	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	131785155	2	65892577	F (2, 24) = 32.32	P<0.0001
Row Factor	81524118	1	81524118	F (1, 24) = 39.99	P<0.0001
Column Factor	210557403	2	105278702	F (2, 24) = 51.64	P<0.0001
Residual	48925847	24	2038577		

| Number of missing values | 0 |

Within each row, compare columns (simple effects within rows)

Number of families	2
Number of comparisons per family	2
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
ATR-101					
0 vs. 20	350.8	-1771 to 2472	No	ns	0.8966
0 vs. 40	10230	8108 to 12352	Yes	****	0.0001

ATR-101+olesoxime

0 vs. 20	463.3 -1658 to 2585	No	ns	0.8285	
0 vs. 40	1395 -727 to 3516	No	ns	0.2311	

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF
ATR-101								
0 vs. 20		16565	16215	350.8	903	5	5	0.3885	24
0 vs. 40		16565	6335	10230	903	5	5	11.33	24
ATR-101+olesoxime								
0 vs. 20		16955	16491	463.3	903	5	5	0.513	24
0 vs. 40		16955	15560	1395	903	5	5	1.544	24

Table Analyzed	S7A. ABCA1 levels vs. ATR-101 or PD129337 concentration, 4 h

Two-way ANOVA	Ordinary
Alpha	0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	2.73	0.0877	ns	No
Row Factor	7.556	<0.0001	****	Yes
Column Factor	76.95	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.000001437	5	2.875E-07	F (5, 48) = 2.054	P=0.0877
Row Factor	0.000003978	1	0.000003978	F (1, 48) = 28.42	P<0.0001
Column Factor	0.00004051	5	0.000008102	F (5, 48) = 57.89	P<0.0001
Residual	0.000006718	48	0.00000014		

Number of missing values	0

Within each row, compare columns (simple effects within rows)

Number of families	2
Number of comparisons per family	5
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
ATR-101					
0 vs. 1	-0.0006868	-0.001302 to -7.141e-005	Yes	*	0.0237
0 vs. 5	0.0004594	-0.000156 to 0.001075	No	ns	0.2049
0 vs. 10	0.001375	0.00076 to 0.001991	Yes	****	0.0001
0 vs. 20	0.001867	0.001251 to 0.002482	Yes	****	0.0001
0 vs. 40	0.00142	0.0008051 to 0.002036	Yes	****	0.0001
PD129337					
0 vs. 1	-0.0006488	-0.001264 to -3.346e-005	Yes	*	0.0354
0 vs. 5	0.0006339	1.854e-005 to 0.001249	Yes	*	0.0414
0 vs. 10	0.00159	0.0009748 to 0.002206	Yes	****	0.0001
0 vs. 20	0.001402	0.0007868 to 0.002018	Yes	****	0.0001
0 vs. 40	0.0008319	0.0002165 to 0.001447	Yes	**	0.0044

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF
ATR-101								
0 vs. 1	0.003808	0.004494	-0.0006868	0.0002366	5	5	2.903	48
0 vs. 5	0.003808	0.003348	0.0004594	0.0002366	5	5	1.942	48
0 vs. 10	0.003808	0.002432	0.001375	0.0002366	5	5	5.813	48
0 vs. 20	0.003808	0.001941	0.001867	0.0002366	5	5	7.89	48
0 vs. 40	0.003808	0.002387	0.00142	0.0002366	5	5	6.004	48
PD129337								
0 vs. 1	0.004218	0.004867	-0.0006488	0.0002366	5	5	2.742	48
0 vs. 5	0.004218	0.003584	0.0006339	0.0002366	5	5	2.679	48
0 vs. 10	0.004218	0.002628	0.00159	0.0002366	5	5	6.721	48
0 vs. 20	0.004218	0.002816	0.001402	0.0002366	5	5	5.926	48
0 vs. 40	0.004218	0.003386	0.0008319	0.0002366	5	5	3.516	48

Table Analyzed	S7A. ABCG1 levels vs. ATR-101 or PD129337 concentration, 4 h

Two-way ANOVA	Ordinary
Alpha	0.05

Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	7.819	0.0002	***	Yes
Row Factor	9.738	<0.0001	****	Yes
Column Factor	69.81	<0.0001	****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	6.462E-09	5	1.292E-09	F (5, 48) = 5.941	P=0.0002
Row Factor	8.048E-09	1	8.048E-09	F (1, 48) = 37	P<0.0001

Column Factor	5.77E-08	5	1.154E-08 F (5, 48) = 53.05 P<0.0001		
Residual	1.044E-08	48	2.175E-10		

Number of missing values   0

Within each row, compare columns (simple effects within rows)

Number of families   2
Number of comparisons per family   5
Alpha   0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
ATR-101					
0 vs. 1	-0.00002138	-4.564e-005 to 2.879e-00(	No	ns	0.1008
0 vs. 5	0.00001529	-8.975e-006 to 3.955e-00!	No	ns	0.3479
0 vs. 10	0.00005081	2.655e-005 to 7.507e-005	Yes	****	0.0001
0 vs. 20	0.00006651	4.225e-005 to 9.077e-005	Yes	****	0.0001
0 vs. 40	0.00006873	4.447e-005 to 9.299e-005	Yes	****	0.0001
PD129337					
0 vs. 1	-0.00001981	-4.407e-005 to 4.454e-00(	No	ns	0.1436
0 vs. 5	0.000003792	-2.047e-005 to 2.805e-00!	No	ns	0.9932
0 vs. 10	0.0000544	3.014e-005 to 7.866e-005	Yes	****	0.0001
0 vs. 20	0.00006837	4.411e-005 to 9.263e-005	Yes	****	0.0001
0 vs. 40	0.00001375	-1.051e-005 to 3.801e-00!	No	ns	0.447

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF
ATR-101								
0 vs. 1	0.0001065	0.0001279	-0.00002138	0.000009328	5	5	2.292	48
0 vs. 5	0.0001065	0.00009125	0.00001529	0.000009328	5	5	1.639	48
0 vs. 10	0.0001065	0.00005572	0.00005081	0.000009328	5	5	5.448	48
0 vs. 20	0.0001065	0.00004003	0.00006651	0.000009328	5	5	7.13	48
0 vs. 40	0.0001065	0.0000378	0.00006873	0.000009328	5	5	7.369	48
PD129337								
0 vs. 1	0.0001198	0.0001396	-0.00001981	0.000009328	5	5	2.123	48
0 vs. 5	0.0001198	0.000116	0.000003792	0.000009328	5	5	0.4065	48
0 vs. 10	0.0001198	0.00006539	0.0000544	0.000009328	5	5	5.832	48
0 vs. 20	0.0001198	0.00005142	0.00006837	0.000009328	5	5	7.329	48
0 vs. 40	0.0001198	0.000106	0.00001375	0.000009328	5	5	1.475	48

2way ANOVA of S7A. CHOP levels vs. ATR-101 or PD129337 concentration, 4 h

Table Analyzed   S7A. CHOP levels vs. ATR-101 or PD129337 concentration, 4 h

Two-way ANOVA   Ordinary
Alpha   0.05

Source of Variation	% of total variation	P value		P value summary	Significant?
Interaction	13.09	<0.0001		****	Yes
Row Factor	0.9174		0.0781	ns	No
Column Factor	72.41	<0.0001		****	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.000008642	5	0.000001728	F (5, 48) = 9.25	P<0.0001
Row Factor	6.056E-07	1	6.056E-07	F (1, 48) = 3.241	P=0.0781
Column Factor	0.0000478	5	0.000009559	F (5, 48) = 51.16	P<0.0001
Residual	0.000008969	48	1.868E-07		

Number of missing values   0

Within each row, compare columns (simple effects within rows)

Number of families   2
Number of comparisons per family   5
Alpha   0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
ATR-101					
0 vs. 1	-0.0004148	-0.001126 to 0.0002963	No	ns	0.4199

0 vs. 5	-0.0004114	-0.001122 to 0.0002997	No	ns	0.4277	
0 vs. 10	-0.000355	-0.001066 to 0.000356	No	ns	0.5661	
0 vs. 20	-0.0009965	-0.001708 to -0.0002855	Yes	**	0.003	
0 vs. 40	-0.003601	-0.004312 to -0.00289	Yes	****	0.0001	

PD129337

0 vs. 1	-0.00005224	-0.0007633 to 0.0006588	No	ns	0.9997	
0 vs. 5	-0.001126	-0.001837 to -0.0004153	Yes	***	0.0007	
0 vs. 10	-0.0004209	-0.001132 to 0.0002901	No	ns	0.406	
0 vs. 20	-0.000615	-0.001326 to 9.603e-005	No	ns	0.1106	
0 vs. 40	-0.00182	-0.002531 to -0.001109	Yes	****	0.0001	

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF
ATR-101								
0 vs. 1	0.003597	0.004012	-0.0004148	0.0002734	5	5	1.517	48
0 vs. 5	0.003597	0.004008	-0.0004114	0.0002734	5	5	1.505	48
0 vs. 10	0.003597	0.003952	-0.000355	0.0002734	5	5	1.299	48
0 vs. 20	0.003597	0.004593	-0.0009965	0.0002734	5	5	3.645	48
0 vs. 40	0.003597	0.007198	-0.003601	0.0002734	5	5	13.17	48
PD129337								
0 vs. 1	0.003687	0.003739	-0.00005224	0.0002734	5	5	0.1911	48
0 vs. 5	0.003687	0.004813	-0.001126	0.0002734	5	5	4.12	48
0 vs. 10	0.003687	0.004108	-0.0004209	0.0002734	5	5	1.54	48
0 vs. 20	0.003687	0.004302	-0.000615	0.0002734	5	5	2.25	48
0 vs. 40	0.003687	0.005507	-0.00182	0.0002734	5	5	6.658	48

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