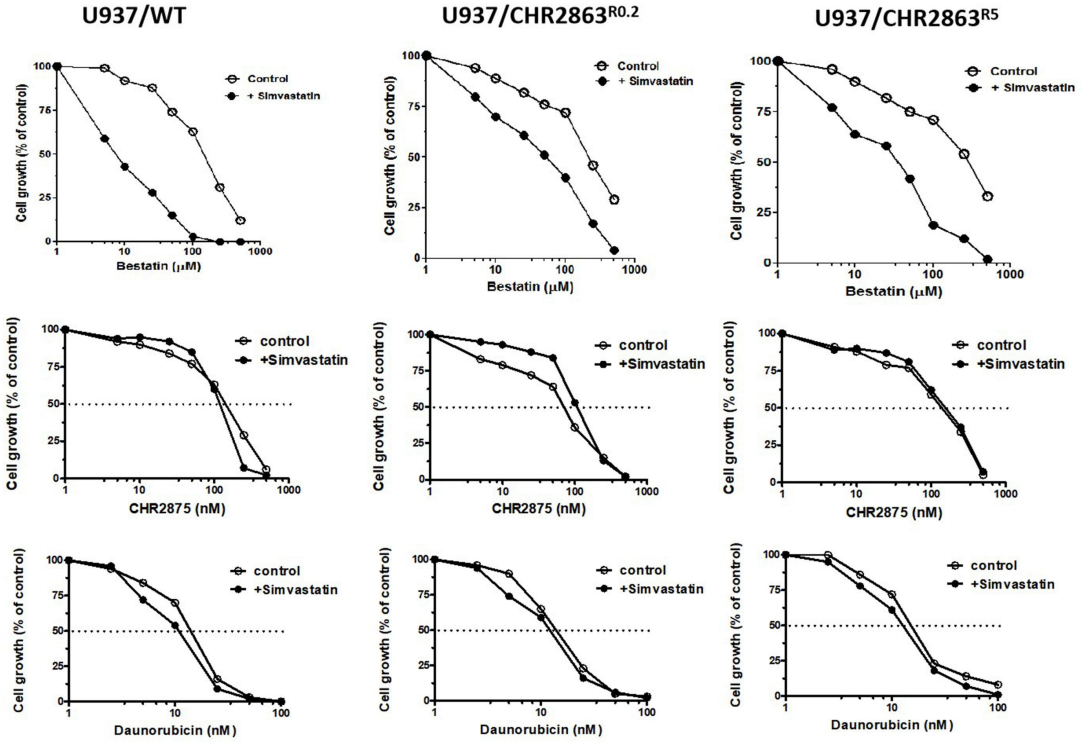
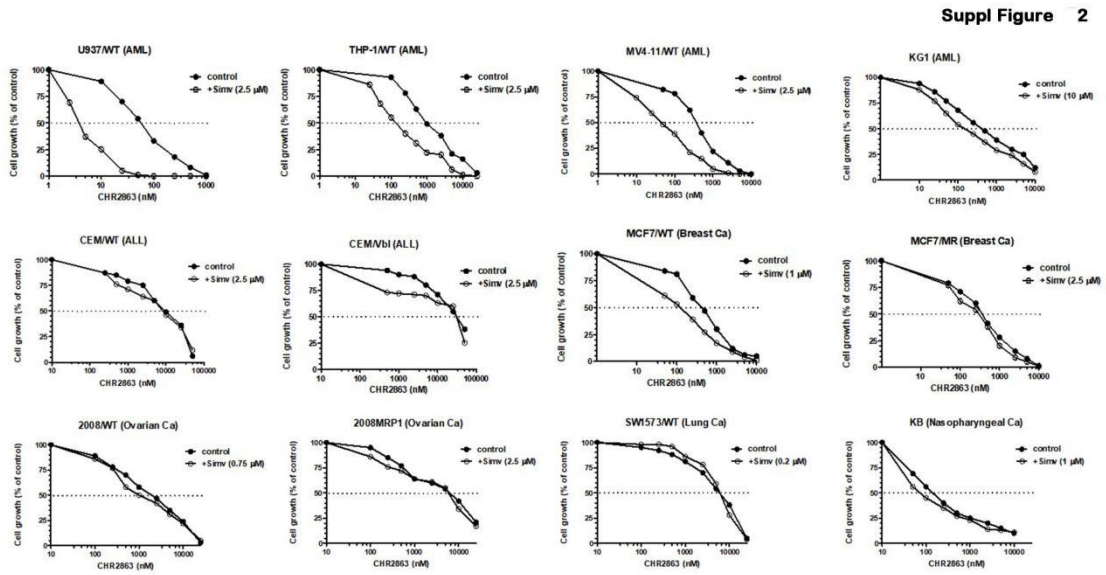
Supplementary Materials

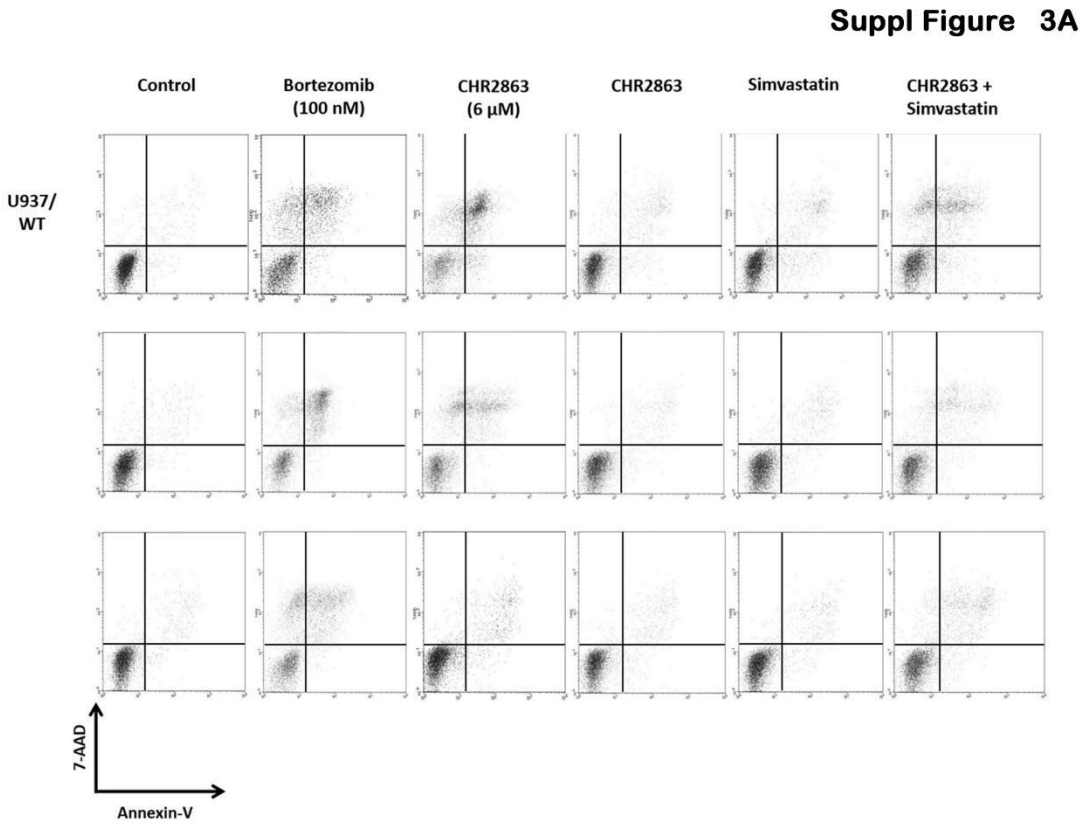
Suppl Figure 1



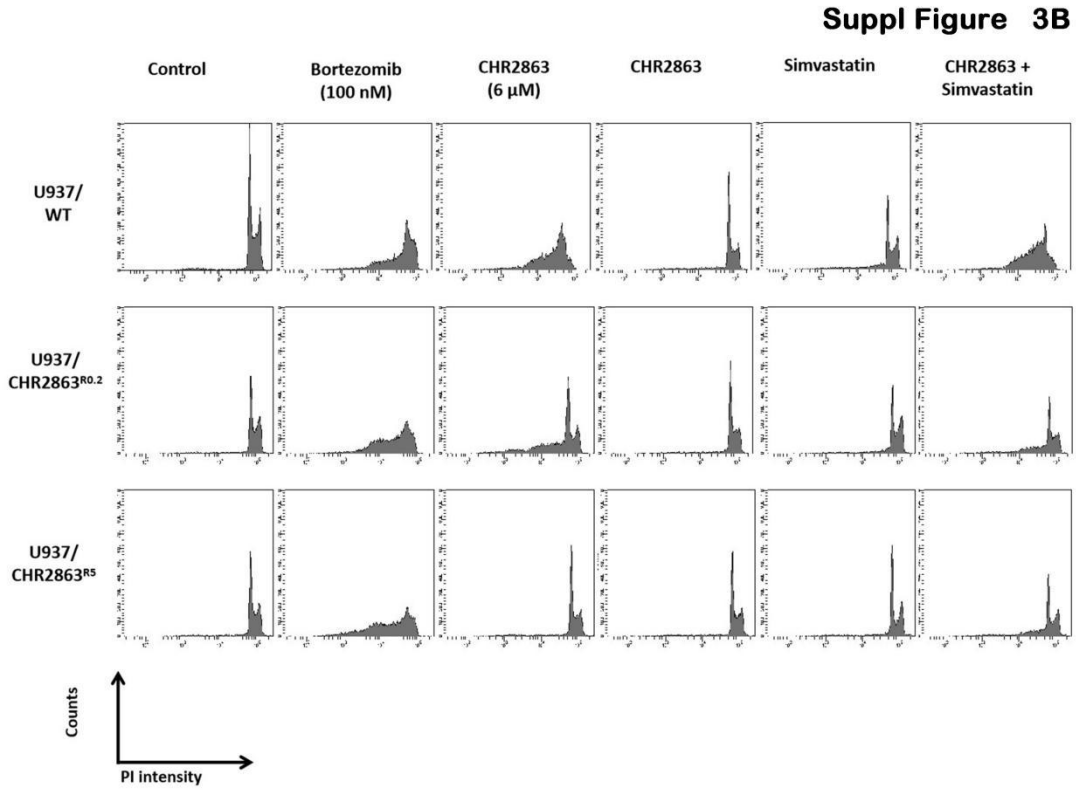
Supplementary Figure 1. Growth inhibitory effects of Bestatin (top row), CHR2875 (middle row) and Daunorubicin (bottom row) for U937/WT cells (left lane), U937/CHR2863$^{R0.2}$ cells (middle lane) and U937/CHR2863^{R5} cells (right lane) in the absence and presence of maximal in vitro non-toxic concentrations of simvastatin (2 µM, 2.5 µM and 2.5 µM, respectively). Cell growth inhibition was determined after 72 h of drug exposure. Results depicted are the mean of two separate experiments (for Bestatin) and four experiments for CHR2875 and Daunorubicin.



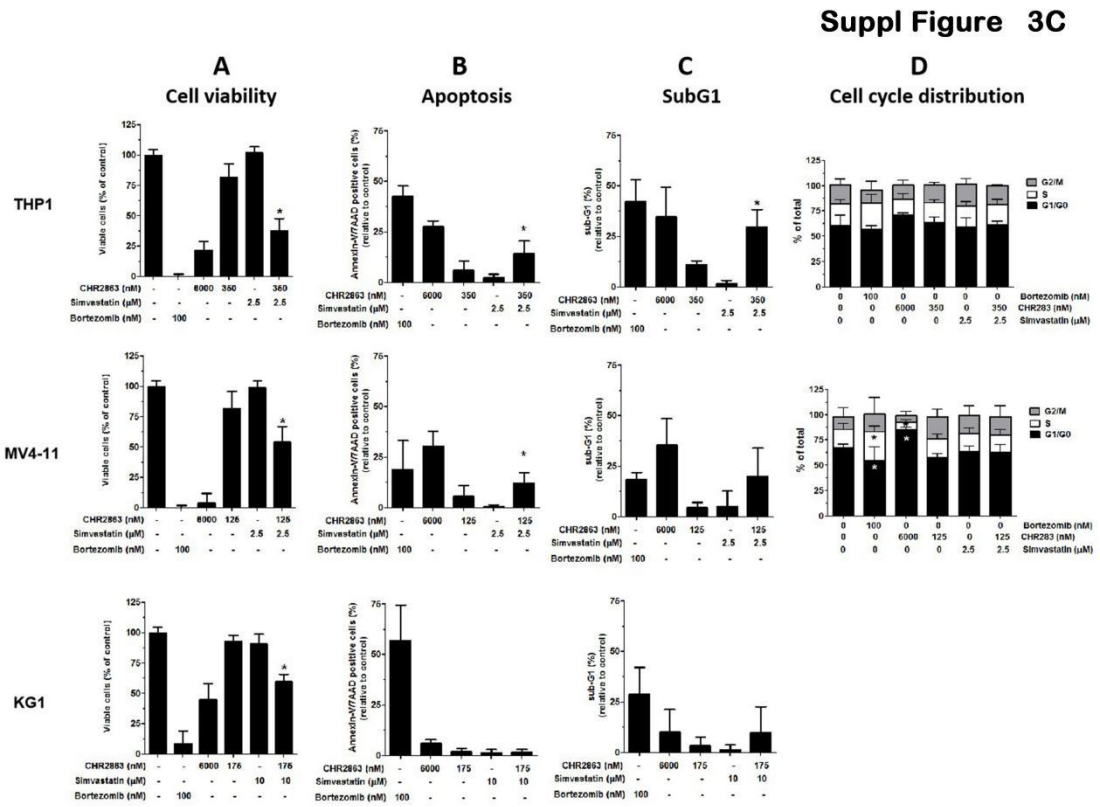
Supplementary Figure 2. Growth inhibition curves for selected AML cell lines (U937, THP1, MV4-11 and KG1), ALL cell lines (CEM, CEM/Vbl) and solid tumor cell lines (MCF7/WT, MCF7/MR, 2008, 2008/MRP1, SW1573 and KB) in the absence and presence of maximum in vitro non-toxic concentration of simvastatin (indicated for each cell line). Cell growth inhibition was determined after 72 h of drug exposure. Results depicted are the mean of 3-4 separate experiments.



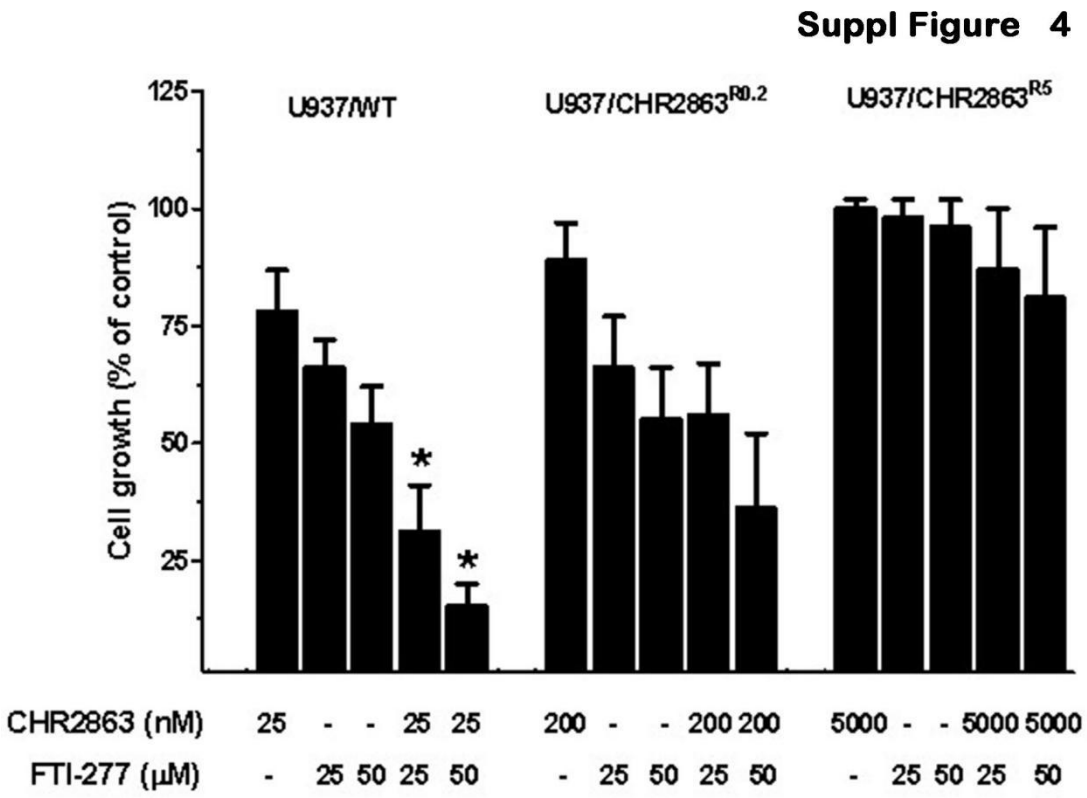
Supplementary Figure 3A. Representative FACS analysis plots of apoptosis induction in U937/WT, U937/CHR2863$^{R0.2}$ and U937/CHR2863^{R5} cells following 48 h of exposure to simvastatin and CHR2863 combinations (as described in Figure 5) and then analyzed for Annexin-V/7AAD staining. Cells treated for 24 h with 0.1 µM bortezomib served as apoptosis induction control. Lower and upper right quadrant represents early apoptotic and late apoptotic cells, respectively.



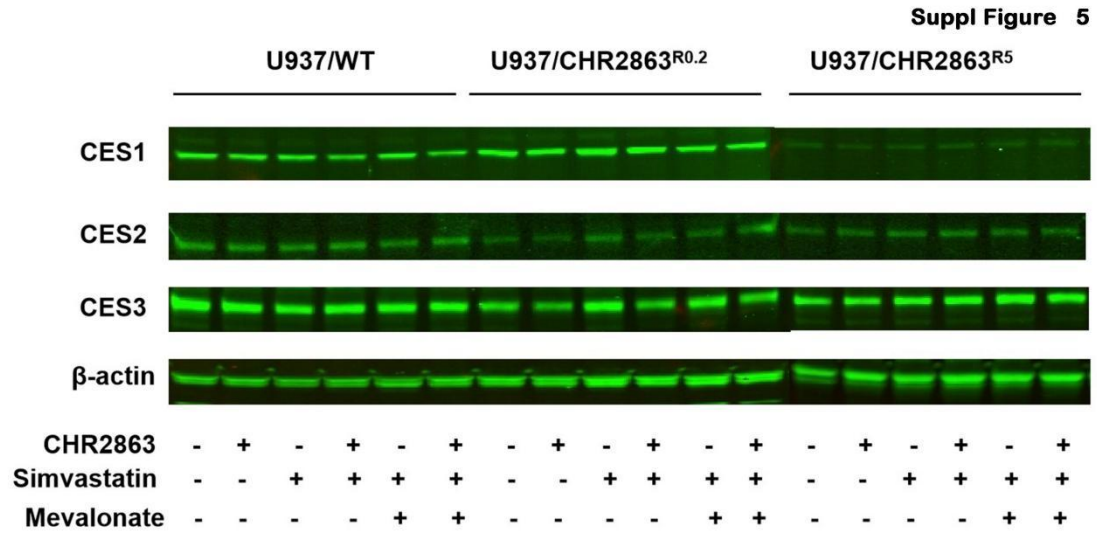
Supplementary Figure 3B. Representative alterations in cell cycle distribution of U937/WT, U937/CHR2863$^{R0.2}$ and U937/CHR2863^{R5} cells following 48 h of exposure to simvastatin and CHR2863 combinations (as described in Figure 5) and then analyzed for PI staining. Cells treated for 24 hr with 0.1 µM bortezomib served as control.



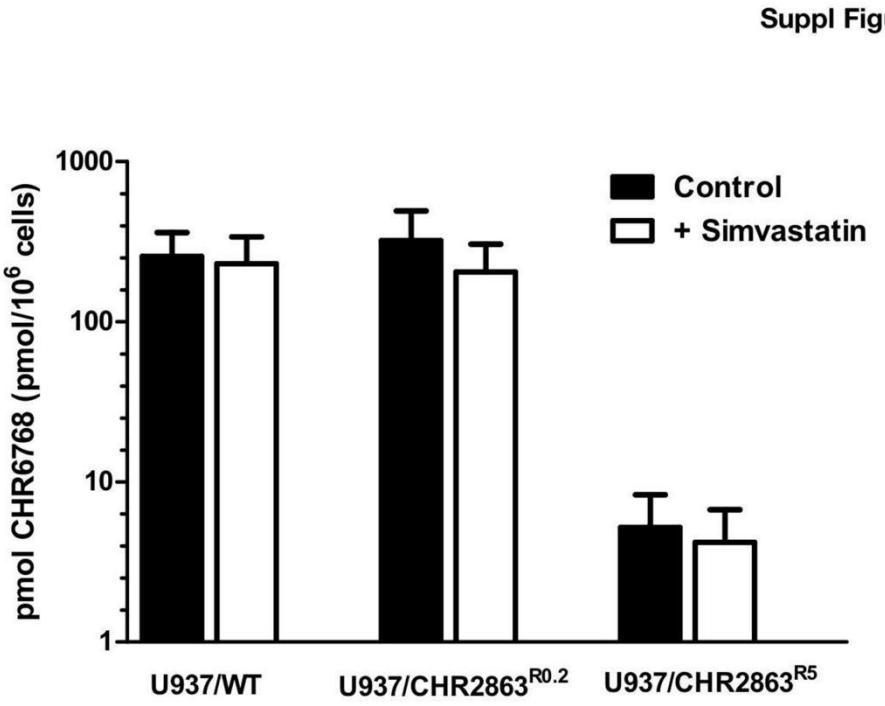
Supplementary Figure 3C. Effect of simvastatin and CHR2863 combinations on cell viability, apoptosis induction and cell cycle distribution in AML cell lines THP1, MV4-11 and KG1 cells. Cells were incubated for 48 h with the indicated concentrations simvastatin, CHR2863 and their combination and assessed for their impact on (A) cell viability, (B) apoptosis induction, (C) sub-G1 fraction and (D) cell cycle distribution. Cells incubated for 24 h with bortezomib or 48 h with 6 μM CHR2863 served as the control for cell growth inhibition and apoptosis induction. Percentages of apoptotic cells in control untreated THP1, MV4-11 and KG1 cells were: 7.2% ± 1.1%, 9.8% ± 3.8% and 7.9% ± 3.7%, respectively. Sub-G1 fraction in control untreated THP1, MV4-11 and KG1 cells was 9.8% ± 3.8%, 10.4% ± 2.1% and 11.4% ± 5.2%, respectively. Results depicted are the mean ± SD of 4-5 independent experiments. *Combination statistically significant ($P < 0.05$) different compared to single drugs.



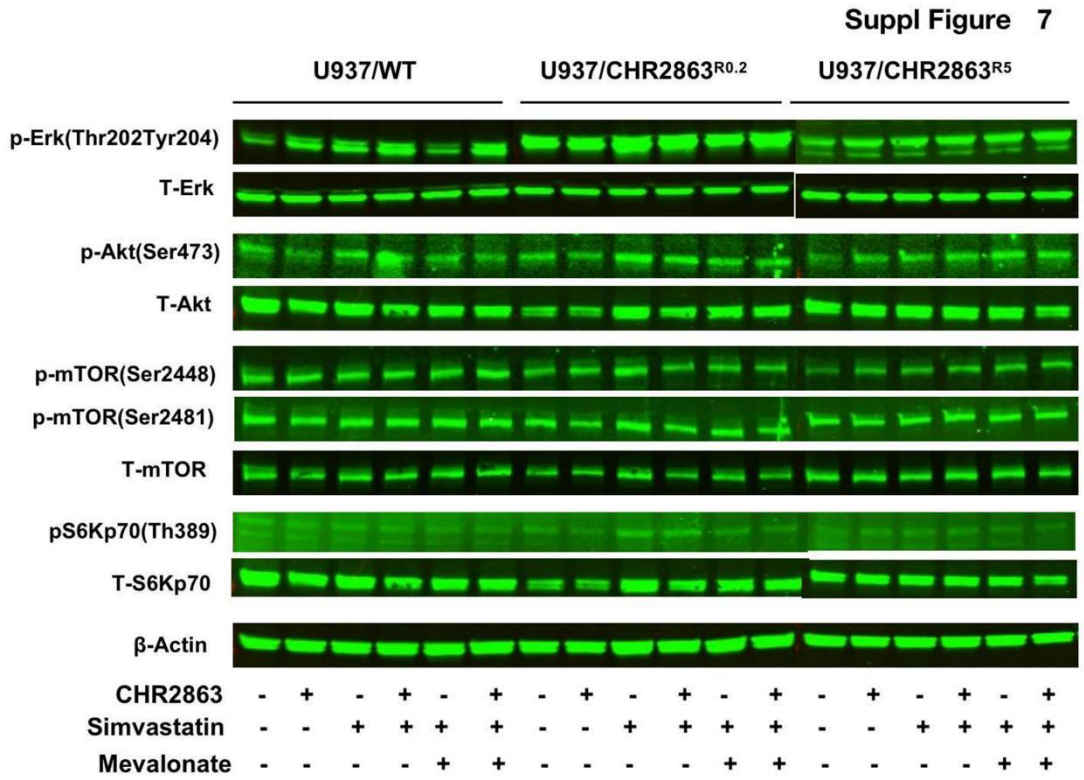
Supplementary Figure 4. Effect of farnesyltransferase inhibitor FTI-277 on CHR2863 activity. U937/WT, U937/CHR2863$^{R0.2}$ and U937/CHR2863^{R5} cells were incubated for 72 h with the indicated concentrations FTI-277, CHR2863 and their combination and assessed for the growth inhibitory effects. Results depicted are the mean ± SD of 4 separate experiments. *combination statistically significant ($P < 0.05$) different compared to single drugs.



Supplementary Figure 5. Effect of CHR2863, simvastatin and their combination on carboxyl esterase-1, -2 and 3 expression. Under conditions described in Figure 5, U937/WT, U937/CHR2863$^{R0.2}$ and U937/CHR2863^{R5} cells (3 × 10^5/ml in 10 mL medium) were incubated for 48 h with non-toxic concentrations simvastatin (2 μM, 2.5 μM and 2.5 μM, respectively), CHR2863 (25 nM, 250 nM and 5 μM, respectively) and 100 μM mevalonic acid (and in combination). Cell lysates were prepared and analyzed for carboxyl esterase (CES) 1, CES2 and CES3 expression. β-Actin expression served as loading control.



Supplementary Figure 6. Effect of simvastatin on the conversion of CHR2863 to its active metabolite CHR6768. U937/WT, U937/CHR2863$^{R0.2}$ and U937/CHR2863^{R5} cells (3 × 10^5/ml in 10 mL medium) were preincubated for 1 hour with simvastatin (2 μM, 2.5 μM and 2.5 μM, respectively) followed by incubation with 6 μM CHR2863 for 6 h at 37 °C. Then, cells were harvested and processed for LC/MS-MS analysis of CHR6768 metabolite formation. Results depicted are the mean ± SD of 6-8 separate experiments.



Supplementary Figure 7. Effect of simvastatin, CHR2863, mevalonic acid, and their combination on the levels of total and phosphorylated Erk, Akt mTOR and S6K. Under conditions described in Figure 5, U937/WT, U937/CHR2863$^{R0.2}$ and U937/CHR2863^{R5} cells (3 × 10^5/ml in 10 mL medium) were incubated for 48 h with non-toxic concentrations of simvastatin (2 μM, 2.5 μM and 2.5 μM, respectively), CHR2863 (25 nM, 250 nM and 5 μM, respectively) and 100 μM mevalonic acid as well as their combination. Cell lysates were prepared and analyzed for the levels of total and phosphorylated Erk, Akt, mTOR and S6K. β-actin expression served as the loading control.