ATR addiction in multiple myeloma: synthetic lethal approaches exploiting established therapies

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Supplemental Methods

Cell culture and reagents

MM cell lines MM1.S, H929, KMS20, RPMI 8226, LP1, OPM2, U266, and KMS34 were kindly provided by researchers or purchased from American Type Culture Collection (ATCC). All cell lines were cultured in RPMI 1640 medium (Euroclone, Pero, Italy) supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS, Euroclone), 2 mM of L-glutamine and 1% of penicillin and streptomycin antibiotics (Euroclone). HeLa, U2OS and HEK923T cells were purchased from ATCC and grown in DMEM (Euroclone) supplemented as above. Cultures were maintained in a humidified tissue culture incubator at 37°C in 5% CO_2 for no more than 4 weeks after thawing. Cell lines were authenticated by STR analysis (Cell ID™ System, Promega, Madison, WI, USA) and routinely tested for the presence of mycoplasma contamination.

MM1.S-Luc and U266-Luc cells stably expressing luciferase were generated by transduction with a 3rd generation lentiviral vector carrying the luciferase gene. pLenti PGK V5-LUC Neo (w623-2) was a gift from Eric Campeau (Addgene plasmid # 21471).

Constitutive knockdown of CDC25A expression was achieved by cloning short hairpin sequences targeting human CDC25A into the lentiviral vector pLKO.1. The gene-specific constructs derive from the RNAi Consortium (TRC) shRNA Library (https://portals.broadinstitute.org/gpp/public/), while the non-mammalian shRNA control plasmid was purchased from Sigma-Aldrich (St. Louis, MO, USA). The shRNAs sequences were as follow:

CTRL (non-targeting): CAACAAGATGAAGAGCACCAA

sh#704: CCCGTCGTGAAGGCGCTATTT

sh#112: ACAACCGATGCAAGCTGTTTG

Lentiviral production and cell infection were performed as already published.[1,2]

Primary MM cells were collected from Bone Marrow (BM) aspirates through positive selection with anti-CD138 coated magnetic nanoparticles (Robosep, Stemcell Technologies, Vancouver Canada) as in [3]. Bone Marrow (BM) Stromal Cells (BMSCs) were obtained by culturing CD138-negative mononuclear cells in DMEM medium with FBS, antibiotics and 2mM L-glutamine. Samples from patients were obtained upon written informed consent. This study was carried out in accordance with protocols approved by the Institutional Review Board, and the procedures followed were in accordance with the Declaration of Helsinki of 1975, as revised in 2000.

The ATM inhibitor KU-55933 and the ATR inhibitors VX-970, VE-821 and AZD6738 were purchased from Selleckchem (Houston, TX, USA) and dissolved in DMSO. Hydroxyurea was from Sigma-Aldrich, doxorubicin and melphalan were obtained from San Raffaele Hospital and dissolved in PBS.

Compounds treatment, proliferation and apoptosis assays

Cells were seeded in 96-well flat bottom white plates at the density of 5,000 cells per well in 50 µl medium and left at room temperature for 30 min before incubating at 37°C for 24 h. Compounds were serially diluted in DMSO or PBS and further diluted to 2x final concentration in medium before adding 50 µl compound to the cells. Final DMSO concentration was 0.1%. Treatments were performed in technical triplicates. Cells were incubated with

the compounds at 37°C for 72 h with no further changes of media or compounds refresh. Cell viability was measured with CellTiter-Glo (Promega) according to manufacturer's instructions. Briefly, plates were allowed to equilibrate at room temperature for 20 min, and an equal volume of CellTiter-Glo reagent was added directly to the wells. Plates were incubated at room temperature for 30 min on a shaker and luminescence was measured on a Mithras LB 940 microplate reader (Berthold Technologies, Bad Wildbad, Germany). Luminescence reading was expressed as percentage relative to the DMSO or PBS-treated control cells. Unless otherwise specified, experiments were repeated al least 3 times. The experimental data (percentage of viable cells compared to control) were analyzed independently using the Combenefit software, a tool enabling the visualization, analysis and quantification of drug combination effects.[4] For each combination experiment, we applied the most widely used effect-based methodology (Bliss independence model), and dose-effect-based strategy (Loewe additivity).[5] The degree of interactions was visualized as a colour scale ranging from blue (synergy) to red (antagonism). Only combinations giving consistent results with both models were taken into consideration.[5,6] A combination giving a synergy score value lower than -10 was considered to be antagonistic, from -10 to 10, additive, larger than 10 synergistic.

For apoptosis determination, cells were stained with fluorescin isothiocyanate (FITC)-labeled Annexin V and Propidium Iodide (PI) using a FITC AnnexinV Apoptosis detection kit (BD Pharmingen, San Diego, CA, USA) according to the manufacturer's istructions. Annexin V and PI positive

cells were quantified using a Cytoflex S Flow Cytometer (Beckman Coulter, Brea, CA) and FCS Express 6 Flow Cytometry Software (De Novo Software Glendale, CA)

Immunoblots and antibodies

Whole-cell extracts were obtained by lysis in sodium dodecyl sulfate (SDS) buffer (50 mM of Tris HCl pH 6.8, 10% glycerol, 2% SDS). Proteins were separated by SDS-polyacrylamide gel electrophoresis, blotted onto an Immobilon-P Polyvinylidene difluoride membrane (Merck KGaA, Darmstadt, Germany), and probed with the indicated antibodies. ECL Detection System (GE Healthcare, Hercules, CA, USA) and Clarity Western ECL Blotting Substrate (Bio-Rad, Hercules, CA, USA) were used for the chemiluminescent reaction.

The antibodies used were as follow: phospho RPA 32 (S4/S8) (A300-245A), phospho RAD17 (S645) (A300-153A), RAD17 (A305-788A) from Bethyl (Montgomery, TX, USA); phospho CHK1 (S345) (#2348), phospho CHK2 (T68) (#2197), CHK1 (#2360), CHK2 (#2662), phospho P53 (S15) (#9284), PARP (#9542) and cleaved Caspase-3 (#9661) from Cell Signaling (Leiden, The Netherlands); RPA32/RPA2 (ab2175) from Abcam (Cambridge, UK); P53 (DO-1) (sc-126) from Santa Cruz (Dallas, TX, USA); anti-GAPDH (GTX627408) from GeneTex (Irvine, CA, USA); anti-Vinculin (V9131) from Sigma-Aldrich; HRP-conjugated anti-mouse and anti-rabbit secondary antibodies from GE Healthcare.

Imaging flow cytometry

Cells were fixed with 1% Paraformaldehyde (Sigma-Aldrich), washed with PBS containing 2% FBS, permeabilizated for 20min with 1x Permeabilitazion Buffer (Thermofisher, Waltham, MA), then sequentially incubated with γH2AX (clone 2F3) from BioLegend (San Diego, California) at a dilution of 1:500 and a secondary antibody AlexaFluor-647 (A-31571, Thermofisher) at a dilution of 1:1000, each for 1 h at 4°C. Incubation for 5 min with 5 µg/ml DAPI (Thermofisher), preceded a final set of washes with PBS containing 2 mM EDTA.

γH2AX intensity was analysed by imaging flow cytometry using ImagestreamX MarkII System (Amnis, Merck). The instrument is equipped with 3 lasers (405nm, 488nm and 642nm), 6-channels CCD camera, Multimag option but no EDF (Extended Depth of Field) element. Excitation laser settings were 405 nm (9 mW) and 642 nm (90 mW). At least 30,000 events were collected for each sample with the 60X_0.9NA objective, at low speed, and the images were analyzed using IDEAS 6.2 software. Single-stained samples were acquired with the identical laser settings of the samples and used for compensation. First, cells were gated for cells in-focus using the gradient root mean square feature (RMS) and then single cells were identified using area and aspect ratio features on the brightfield image. DAPI positive cells were gated for intensity of DAPI channel (Ch01) and intensity of side scattering (Ch06), and healthy cells were then further gated for intensity and max pixel intensity of side scattering (Ch06). The intensity of γH2AX staining was quantified in the nuclear region. For the identification of γH2AX foci a first spot

mask was used to select the spots with a size ≥ 2 pixels and a spot to cell background ratio ≥ 10. A threshold feature of 70% was then applied and a further peak function with spot to cell background ratio of 10 was then used to extract foci from the background. As a final step the γH2AX foci mask was combined with the nuclear mask in order to ensure that the foci identified were inside the nucleus. Spot Count feature was finally used to count the number of foci per cell.

RNA extraction, reverse transcription and Real Time PCR

Total RNA was isolated using RNeasy Plus Mini Kit (Qiagen, Hilden, DE) according to the manufacturer's instructions. cDNA was synthesized with random primers using the Promega Reverse Transcription System (Promega). Quantitative Real Time PCR was performed using SYBR Green Mastermix (Thermofisher) in combination with specific primer pairs on the ViiA™7 Real Time PCR System (Thermofisher). Primer pairs were as follow:

TBP forward GCTGGCCCATAGTGATCTTT

TBP reverse CTTCACACGCCAAGAAACAGT

CDC25A forward TCTGAAGAATGAGGAGGAGACC

CDC25A reverse AAACAGCTTGCATCGGTTGT.

Mice, bioluminescent imaging and pharmacological treatments

All mice were housed and bred in the institutional pathogen-free animal facility, treated in accordance with the European Union guidelines and with the approval of the San Raffaele Scientific Institute Institutional Ethical Committee. $Rag2^{-/-}\gamma c^{-/-}$ mice on BALB/c background were kindly provided by CIEA (Central Institute for Experimental Animals, Kawasaki, Japan) and

Taconic (Rensselaer, NY, USA).

Eight to ten-week-old $Rag2^{-/-}\gamma c^{-/-}$ mice were injected intravenously with 5 x 10^6 luciferase expressing cells in 200 µl of PBS. Mice were monitored for myeloma progression by bioluminescent imaging (BLI) using the IVIS SpectrumCT System (Perkin Elmer, USA). The system is composed of a low noise, back-thinned, back-illuminated CCD camera cooled at -90°C with a quantum efficiency in the visible range above 85%. Each mouse received an intra-peritoneal injection of 150 mg luciferin/kg body weight 10 min before BLI. During image acquisition, the animals were kept at 37°C and under gaseous anesthesia (2–3% isoflurane and 1 l/min oxygen). Dynamic BLI was performed by acquiring a set of images every 2 min from 10 to 20 min after luciferin injection to detect the highest BLI signal. The images were obtained using the following IVIS settings: exposure time=auto, binning=8, f=1 and a field of view equal to 22 cm (field D). No emission filters were used during BLI acquisitions. BLI image analysis was performed by placing Region of Interests (ROI) and by measuring the total flux (photons/seconds) within the ROI. Images were acquired and analyzed using Living Image 4.5 (Perkin Elmer).

Treatment commenced when the tumour burden became detectable, 4 and 8 weeks after inoculation for mice injected with MM1S-Luc and U266-Luc cells, respectively. Mice were assigned into 4 treatment groups of 5 animals each: VX-970, melphalan, VX-970/melphalan and vehicle. Treatment cycles consisted of 5 days of treatment followed by 2 days rest. A total of 3 treatment cycles were given. VX-970, 60 mg/kg in a solution in 10% Vitamin E Tocopheryl Polyethylene Glycol Succinate (VitE TPGS, Sigma-Aldrich), was

administered by oral gavage once a day continuously for 5 days. Melphalan (2 mg/kg in PBS) was administered by intraperitoneal injection once a day on day 1, 3 and 5 of each treatment cycle. The control group was treated with vehicle (10% VitE TPGS in water) and PBS. Body weight was assessed twice a week. Mice were imaged at day 5 of each treatment cycle and one week after the end of the treatment in order to assess tumour burden during and post-therapy. Mice were euthanized by CO_2 inhalation when they became detectably ill and developed hind limb paralysis.

Immunohistochemistry on human BM biopsies

4 μm thick sections were obtained from Bouin-fixed, paraffin-embedded tissue blocks of bone marrow biopsies of untreated patients with a diagnosis of MM. The antibodies used were as follow: γH2AX (clone 2F3) from BioLegend (San Diego, CA, USA) at a dilution of 1:200 and phospho CHK1 (S317) (A304-673A) from Bethyl at a dilution of 1:200. Sections underwent antigen retrieval with CC1 solution (Roche). For both antibodies immunohistochemistry was performed with an automated immunostainer (Ventana Benchmark Ultra; Tucson, Arizona), according to the manufacturer's instructions; Optiview Universal DAB detection kit (Roche) was used for detection. The expression of γH2AX and phospho Chk1 was evaluated as nuclear staining in positive cells and reported as mean percentage compared to the total amount neoplastic cells. A Zeiss Axioskope 40 microscope was used for slide evaluation and microphotographs were taken with a Zeiss MRc Axiocam (Zeiss GmbH, Germany).

3D culture and response to drugs of human primary MM cells

3D dynamic culture was performed using the RCCS^{TM} bioreactor RCCS-1 (Synthecon Inc., Houston TX, USA), as in [3]. Scaffold discs were cut from Spongostan gelatin sheets (Ethicon Inc., Somerville NJ, USA), pre-seeded with BMSC (100,000/scaffold) and then seeded with MM cells (200,000/scaffold) isolated from BM biopsies. Resulting constructs were kept in parallel cultures in the bioreactor (2 constructs/vessels) and treated with either VX-970 (0.3 μM) or melphalan (1.2 μM) or a combination of both for 72 h. At the end of the culture period, cells were recovered from scaffolds by means of liberase (25 mg/ml, (Roche Diagnostic, Mannheim, Germany) and stained with PC7-conjugated anti-CD38 (#560677) and FITC-conjugated Annexin V (#556547), both from BD Pharmingen (San Diego, CA, USA) before flow cytometric (FACS) analysis (FC500, Beckman Coulter)[3]. In selected experiments, scaffolds retrieved at the end of the culture were fixed for immunohistochemical analyses. Serial 5 μm thick sections were stained using anti-CD138 (760-4248, Roche, Basel, CH) and anti-CD73 (ab124725, Abcam) antibodies.[3]

Statistical analysis

Statistical analyses were performed using the GraphPad software, (Prism 7.0 software) unless otherwise specified. For proliferation and combination assays statistical analysis was performed using two-way ANOVA followed by Dunnett correction for multiple comparisons and multiple t tests with statistical significance determined using the Holm-Sidak method. Statistical analysis of the Kaplan-Meier survival curves was done using the log-rank test.

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Supplementary figure legends

Supplementary Figure 1. **Basal levels of DNA damage in MM cells**. (A) Exponentially growing MM cells were harvested and analysed by immunoblot for the expression of the indicated proteins. GAPDH was used as loading control. (B) Cells as in (A) were harvested, fixed and processed for imaging flow cytometry to detect γH2AX signal. Results are presented as the mean of the γH2AX signal for individual nucleus, averaged from a minimum of 2 independent experiments (mean ± SEM).

Supplementary Figure 2. **γH2AX levels in MM cell lines, before and after treatment with HU, melphalan and VX-970.** MM1.S, H929, KMS20 and OPM2 cells were left untreated (NT) or treated with 0.15 μM VX-970 (VX) before the addition of HU (2 mM) or melphalan (50 μM) for 3 h. At the end of the treatment, cells were fixed and processed for imaging flow cytometry to detect the γH2AX signal for individual nucleus (left Y axis). Mean of γH2AX signal (right Y axis) for each treatment is reported. Results are representative of two independent experiments.

Supplementary Figure 3. **The VX-970-analog VE-821 and the structurally unrelated ATR inhibitor AZD6738 are less effective that VX-970 in reducing MM cells viability**. MM cells were seeded in 96-well plates and treated for 72 h with DMSO (as control) or with the indicated concentrations of the VX-970-analog VE-821 or the structurally unrelated ATR inhibitor ADZ6738. Cell viability was assessed using CellTiter-Glo assay. Results are presented as the mean percentage of viable cells in treated samples, relative to DMSO control cells averaged from 3 independent experiments (mean ±

SEM) each with 3 replicates per condition. Results of statistical analysis are reported in Supplementary Table 1.

Supplementary Figure 4. Loewe synergy matrices in MM cells treated with melphalan and VX-970. The relative drug synergy scores were calculated analyzing the experimental data of Figure 2 A using the Combenefit software. The colored areas are indicative of the degree of synergy between the drug combinations. Different color codes (black, blue and red) are indicative of the concentrations of melphalan used to treat the cells depending on their sensitivity to the drug. NaN, not a number, as resulted from the combination file.

Supplementary Figure 5. Combination of ATR inhibition by VX-970 and melphalan treatment induces apoptosis in MM cells. Exponentially growing MM cells were treated with melphalan (MM1.S and H929 0.625 μM; RPMI and KMS20 1.25 μM; LP1 and OPM2 10 μM) either alone or in combination with increasing concentrations of VX-970 (0.075, 0.15 and 0.3 μM). After 48 h, cells were harvested and stained with Annexin V and PI before FACS analysis. Results are representative of 3 independent experiments.

Supplementary Figure 6. γH2AX levels in MM cell lines, before and after treatment with doxorubicin and KU-55933. Exponentially growing MM1.S, H929 and RPMI 8226 cells were either left untreated (NT) or treated with VX-970 (VX, 0.15 μM) or KU-55933 (KU, 10 μM) before the addition of doxorubicin (0.5 μM) for 3 h. At the end of the treatment, cells were fixed and processed for imaging flow cytometry to detect the γH2AX signal for individual

nucleus (left Y axis). Mean of γH2AX signal (right Y axis) for each treatment is reported. Results are representative of two independent experiments.

Supplementary Figure 7. ATM inhibition does not increase the apoptosis induced by doxorubicin in MM cells. Exponentially growing MM cells were left untreated (NT) or treated with KU-55933 (KU, 10 μM) and doxorubicin (Doxo, 0.5 μM) either alone or in combination (KU/Doxo). After 72 h, cells were harvested and stained with Annexin V and PI before FACS analysis. Results are representative of 3 independent experiments.

Supplementary Figure 8. Loewe synergy matrices in MM cells treated with doxorubicin and KU-55933. Loewe synergy matrices and the relative drug synergy scores calculated analyzing the experimental data of Figure 3 B using the Combenefit software. The colored areas are indicative of the degree of synergy between the drug combinations. Different color codes (red, blue and black) are indicative of the concentrations of doxorubicin used to treat the cells depending on their sensitivity to the drug.

Supplementary Figure 9. ATM inhibition by KU-55933 has limited activity on MM cells *per se* but is synergic with ATR inhibition by VX-970. (A) MM cells were seeded in 96-well plates and treated for 72 h with DMSO (as control, NT) or increasing concentrations of KU-55933 either alone or in combination with the indicated doses of VX-970. Cell viability was assessed using CellTiter-Glo assay. Results are presented as the mean percentage of viable cells in treated samples, relative to DMSO control cells averaged from a minimum of 3 independent experiments (mean ± SEM) each with 3 replicates per condition. Proliferation curves for each cell line were generated using

GraphPad Prism and presented on the left panels of the figure. The dotted vertical line indicates the response of MM cells to increasing concentrations of KU-55933 alone. Filled black dots indicate the cellular response to VX-970 alone. Results of statistical analysis are reported in Supplementary Table 4. (B) For each cell line, the Bliss and the Loewe synergy matrices were obtained analyzing the experimental data with the Combenefit software. The colored areas are indicative of the degree of synergy between the drug combinations.

Supplementary Figure 10. Effect of ATR and ATM inhibition on γH2AX intensity and foci in MM cells. Exponentially growing MM1.S, H929, RPMI 8226 and U266 cells were left untreated (NT) or treated with 0.15 μM VX-970 and 10 μM KU-55933 either alone or in combination for 4 h. At the end of the treatment, cells were fixed and processed for imaging flow cytometry. Results are representative of two independent experiments. (A) γH2AX intensity for individual nucleus (left Y axis) and the mean of γH2AX signal (right Y axis) for each treatment are reported. (B) The number of γH2AX foci for individual nucleus (left Y axis) and the mean of γH2AX foci (right Y axis) for each treatment are reported.

Supplementary Figure 11. Combination of ATR inhibition by VX-970 and melphalan treatment induces apoptosis in U266 cells without ongoing DNA damage. (A) U266-melphalan resistant cells were treated with melphalan either alone or in combination with increasing concentrations of VX-970 (0.075, 0.15 and 0.3 μM). After 48 h, cells were harvested, and immunoblotted for the indicated antibodies. The levels of cleaved PARP and

caspase-3 served as indicators of apoptosis. GAPDH was used as loading control. (B) Cells were treated as in (A). After 48 h, cells were harvested, and stained with Annexin V and PI before FACS analysis. Results are representative of 3 independent experiments.

Supplementary Figure 12. ATM inhibition by KU-55933 alone or in combination with doxorubicin does not restrain proliferation in cells without ongoing DNA damage. (A) Exponentially growing U266 cells were left untreated (NT) or treated with VX-970 (VX, 0.15 μM) or KU-55933 (KU, 10 μM). Treatment with both compounds initiated 1 h before the addition of doxorubicin (0.5 μM). After 3 h, cells were fixed and processed for imaging flow cytometry to detect the γH2AX signal for individual nucleus (left Y axis). Mean of γH2AX signal (right Y axis) for each treatment is reported. Results are representative of two independent experiments. (B) As in (A), cells were either left untreated (NT) or treated with increasing concentrations of KU-55933 (KU, 5 and 10 μM) and VX-970 (VX, 0.15 μM). At the end of the treatment cells were harvested and analyzed by immunoblot for the expression of the indicated proteins. GAPDH was used as loading control. (C) Cells were seeded in 96-well plates and treated for 72 h with DMSO (as control, NT) or increasing concentrations of KU-55933 either alone or in combination with the indicated doses of doxorubicin. Cell viability was assessed using CellTiter-Glo assay. Results are presented as the mean percentage of viable cells in treated samples, relative to DMSO control cells averaged from a minimum of 3 independent experiments (mean ± SEM), each with 3 replicates per condition. Proliferation curves were generated using

GraphPad Prism. The dotted vertical line indicates the response of the cells to increasing concentrations of KU-55933 alone. Filled black dots indicate the cellular response to doxorubicin alone. The Bliss and the Loewe synergy matrices with the relative drug synergy scores calculated using the Combenefit software are reported on the right of the proliferation profile. The colored areas are indicative of the degree of synergy between the drug combinations. Results of statistical analysis are reported in Supplementary Table 3. (D) Exponentially growing U266 cells were left untreated (NT) or treated with KU-55933 (KU, 10 μM) and doxorubicin (Doxo, 0.5 μM) either alone or in combination (KU/Doxo). After 72 h, cells were harvested and stained with Annexin V and PI before FACS analysis. Results are representative of 3 independent experiments.

Supplementary Figure 13. ATR inhibition by VX-970 sensitizes U266 cells to melphalan in vivo. $Rag2^{-/-}\gamma c^{-/-}$ mice were injected intravenously with $5x10^6$ U266-Luc cells. (A) Treatment was performed according to the schedule reported, starting 8 weeks after injection, when the tumour burden became evident by BLI (week 8). BLI was performed every week during the treatment and one week after the stop of the treatment (week 12). (B) The graph shows the tumour burden increase quantified as total flux measured from bioluminescent images during the treatment period. Data represent the mean ± SD of 5 mice in each treatment arm. Statistical analysis was performed using the two-way ANOVA and Tukey's multiple comparison test (*$p<0.05$).

Supplementary Figure 14. ATR inhibition by VX-970 sensitizes HeLa and U2OS cells to melphalan. (A) HeLa and U2OS cells were seeded in 96-well plates and treated for 72 h with DMSO (as control, NT) or increasing concentrations of VX-970 either alone or in combination with the indicated doses of melphalan. Cell viability was assessed using CellTiter-Glo assay. Results are presented as the mean percentage of viable cells in treated samples, relative to DMSO control cells averaged from a minimum of 3 independent experiments (mean ± SEM), each with 3 replicates per condition. Proliferation curves for each cell line were generated using GraphPad Prism. The dotted vertical line indicates the response of the cells to increasing concentrations of VX-970 alone. Filled black dots indicate the cellular response to melphalan alone. For each cell line the Bliss synergy matrices and the relative drug synergy scores were calculated using the Combenefit software. The colored areas in the matrix are indicative of the degree of synergy between the drug combinations. Different color codes (black, blue and red) are indicative of the concentrations of melphalan used to treat the cells depending on their sensitivity to the drug. (B) Cells were treated with the indicated concentrations of VX-970 and melphalan either alone or in combination. Representative pictures were taken after 48 h of treatment.

Supplementary Figure 15. CDC25A interference in MM cells. RPMI 8226 and HeLa cells were transduced with two different lentiviral constructs targeting CDC25A, sh#704 and sh#112. Non-targeting scramble shRNA was used as negative control (CTRL). (A) Knock down efficiency was verified by PCR analysis. CDC25A RNA levels were normalized to TBP mRNA levels.

Results are presented as fold relative to CTRL. (B) Cells expressing CTRL and shCDC25A constructs were seeded in 96-well plates left untreated (NT) or treated with the indicated concentrations of VX-970 and melphalan either alone or in combination for 72 h. Cell viability was assessed using CellTiter-Glo assay. Results are presented as the mean percentage of viable cells in treated samples, normalized to the relative DMSO control cells and are representative of 2 (for HeLa cells) and 3 (for RPMI 8226) independent experiments each with 3 replicates per condition.

Supplementary table legends

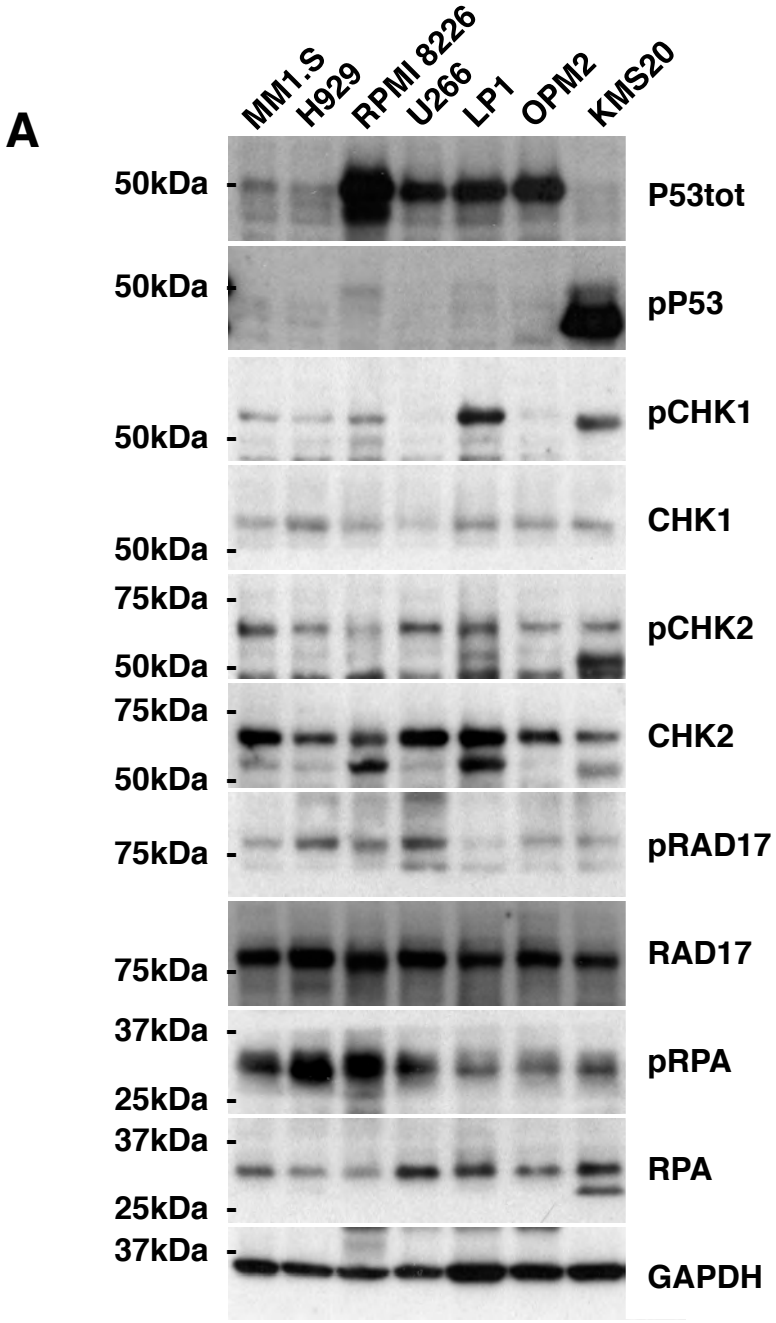
Supplementary Table 1. Comparison of the effects of ATR inhibitors, VX-970, VE-821 and AZD6738 in different MM cell lines. # Statistical analysis was performed using two-way ANOVA followed by Dunnett correction for multiple comparisons. ## Multiple t tests were performed and statistical significance determined using the Holm-Sidak method, with alpha=5.000%. Only significant results are shown.

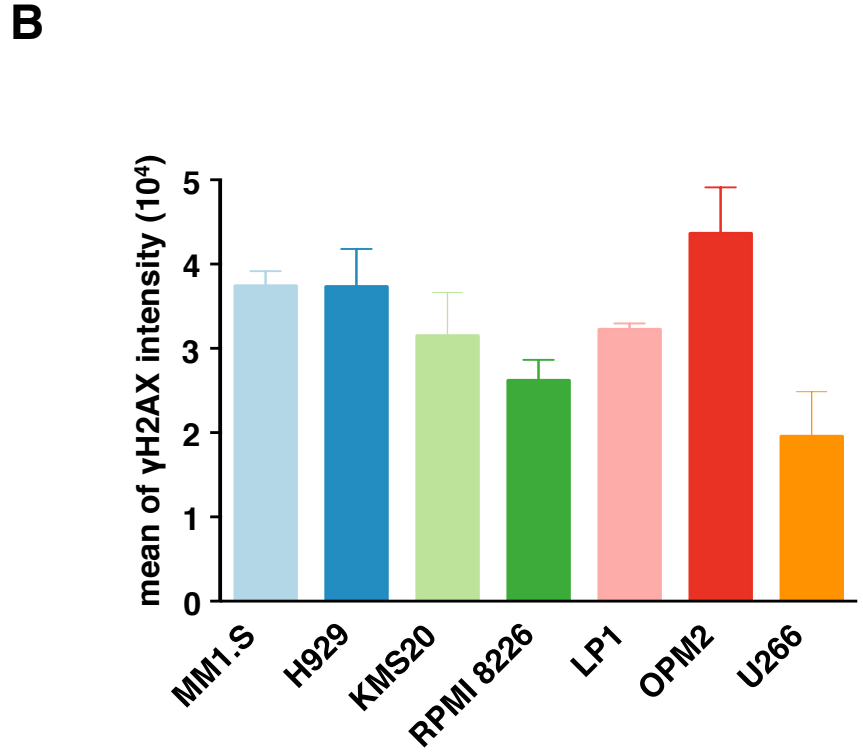
Supplementary Table 2. Comparison of the effects of VX-970 alone or in combination with Melphalan in different MM cell lines. # Statistical analysis was performed using two-way ANOVA followed by Dunnett correction for multiple comparisons. ## Multiple t tests were performed and statistical significance determined using the Holm-Sidak method, with alpha=5.000%. Only significant results are shown.

Supplementary Table 3. Comparison of the effects of KU-55933 alone or in combination with Doxorubicin in different MM cell lines. Statistical

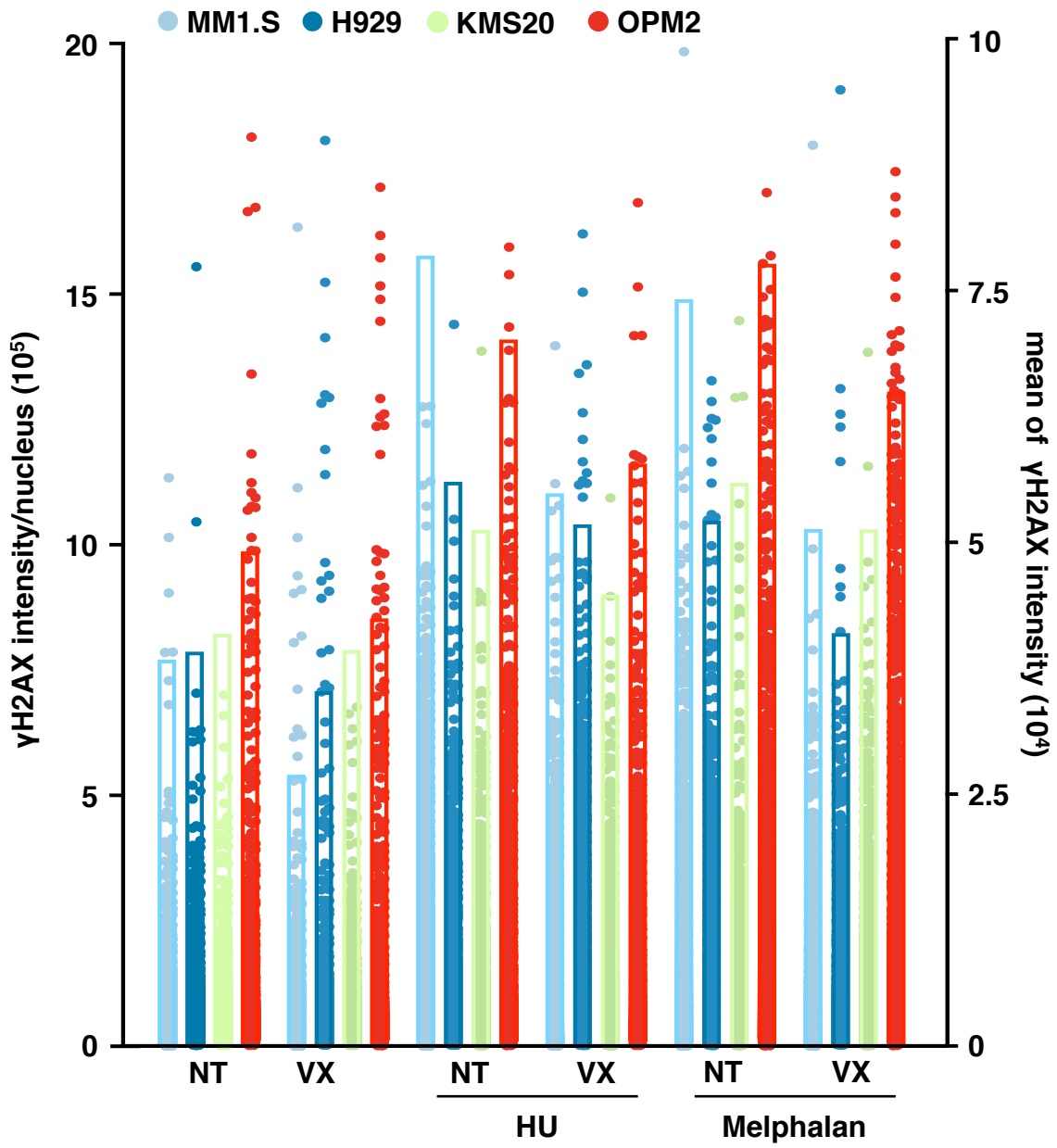
analysis was performed using two-way ANOVA followed by Dunnett correction for multiple comparisons. ## Multiple t tests were performed and statistical significance determined using the Holm-Sidak method, with alpha=5.000%. Only significant results are shown.

Supplementary Table 4. Comparison of the effects of VX-970 alone or in combination with KU-55933 in different cell lines. # Statistical analysis was performed using 2 way ANOVA followed by Dunnett correction for multiple comparisons. ## Multiple t tests were performed and statistical significance determined using the Holm-Sidak method, with alpha=5.000%. Only significant results are shown.





Supplementary Figure 1



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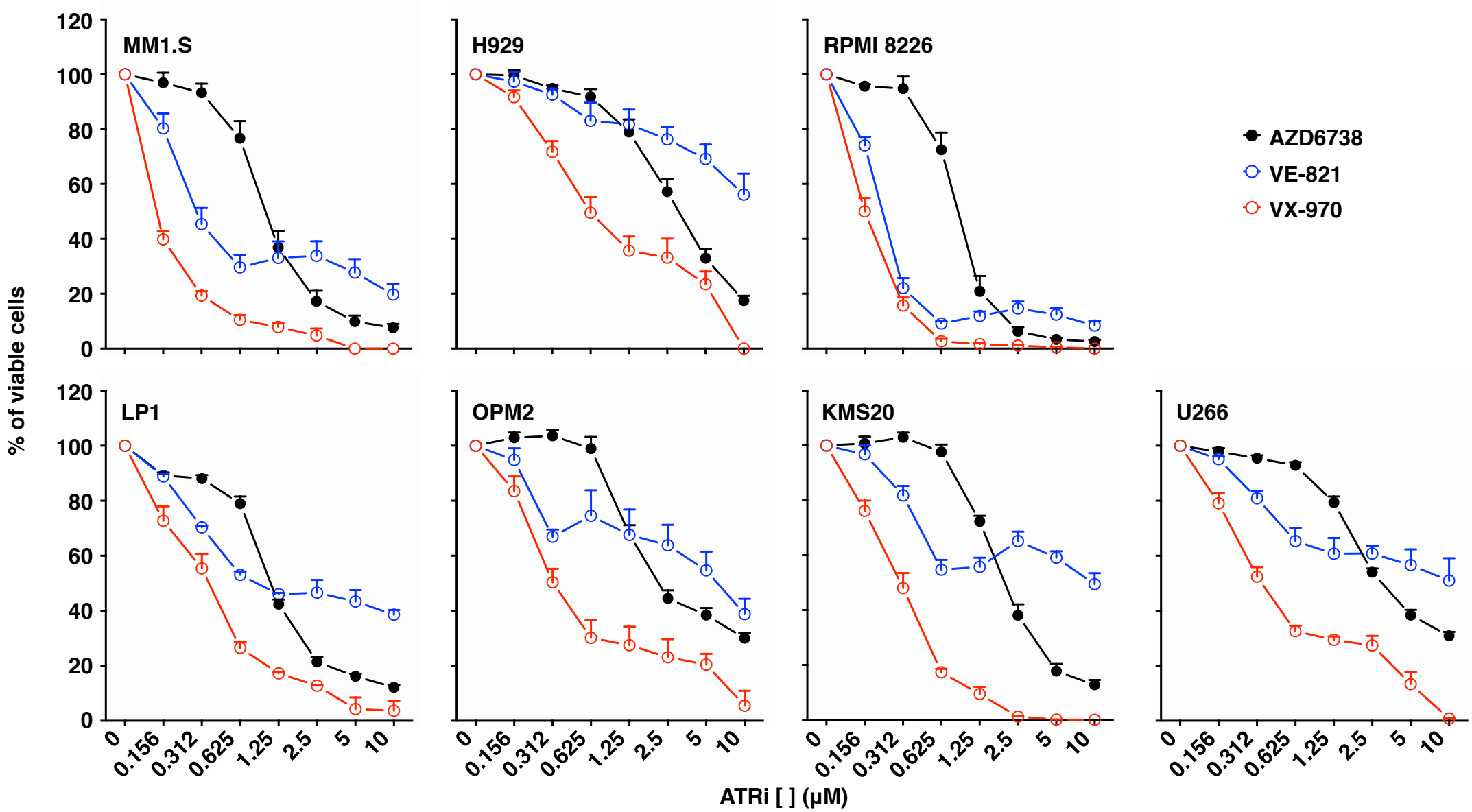
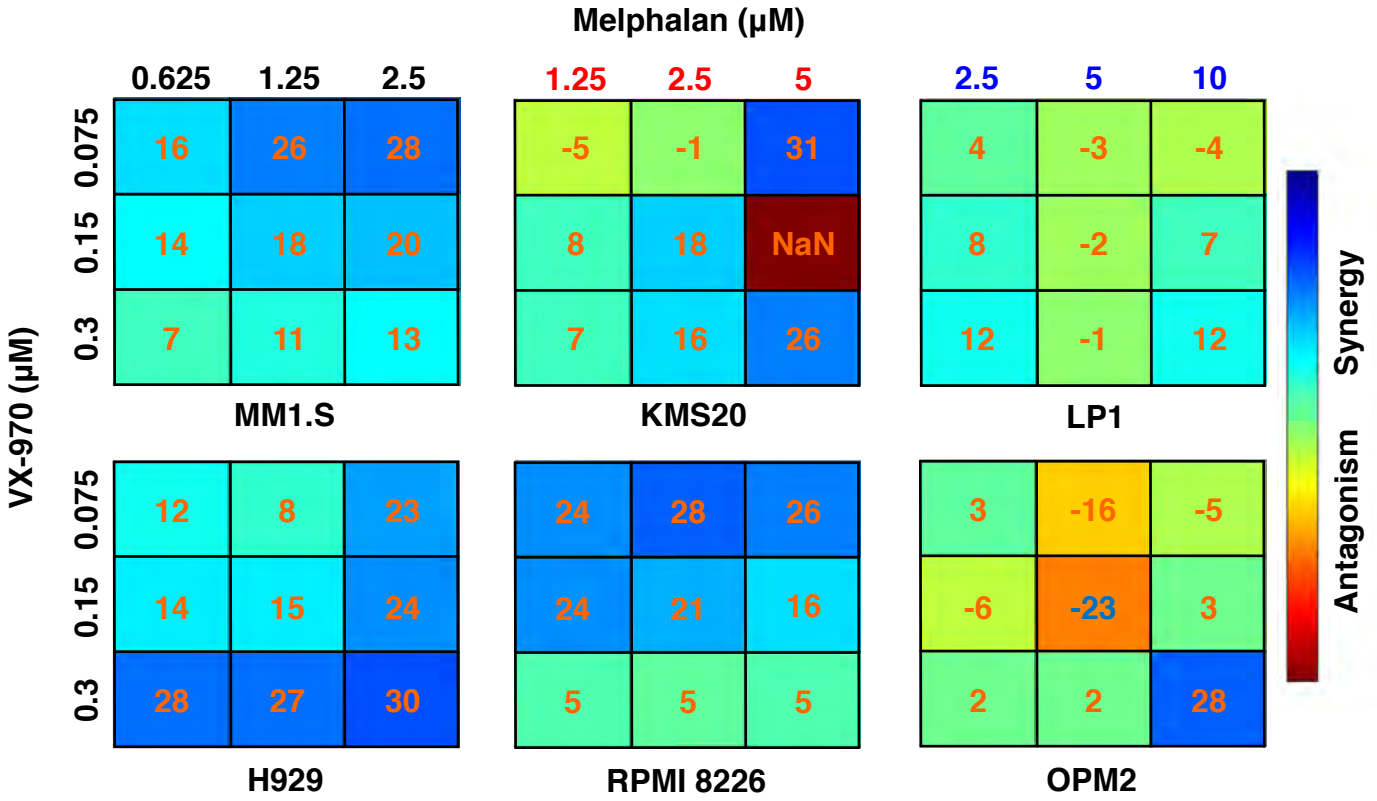
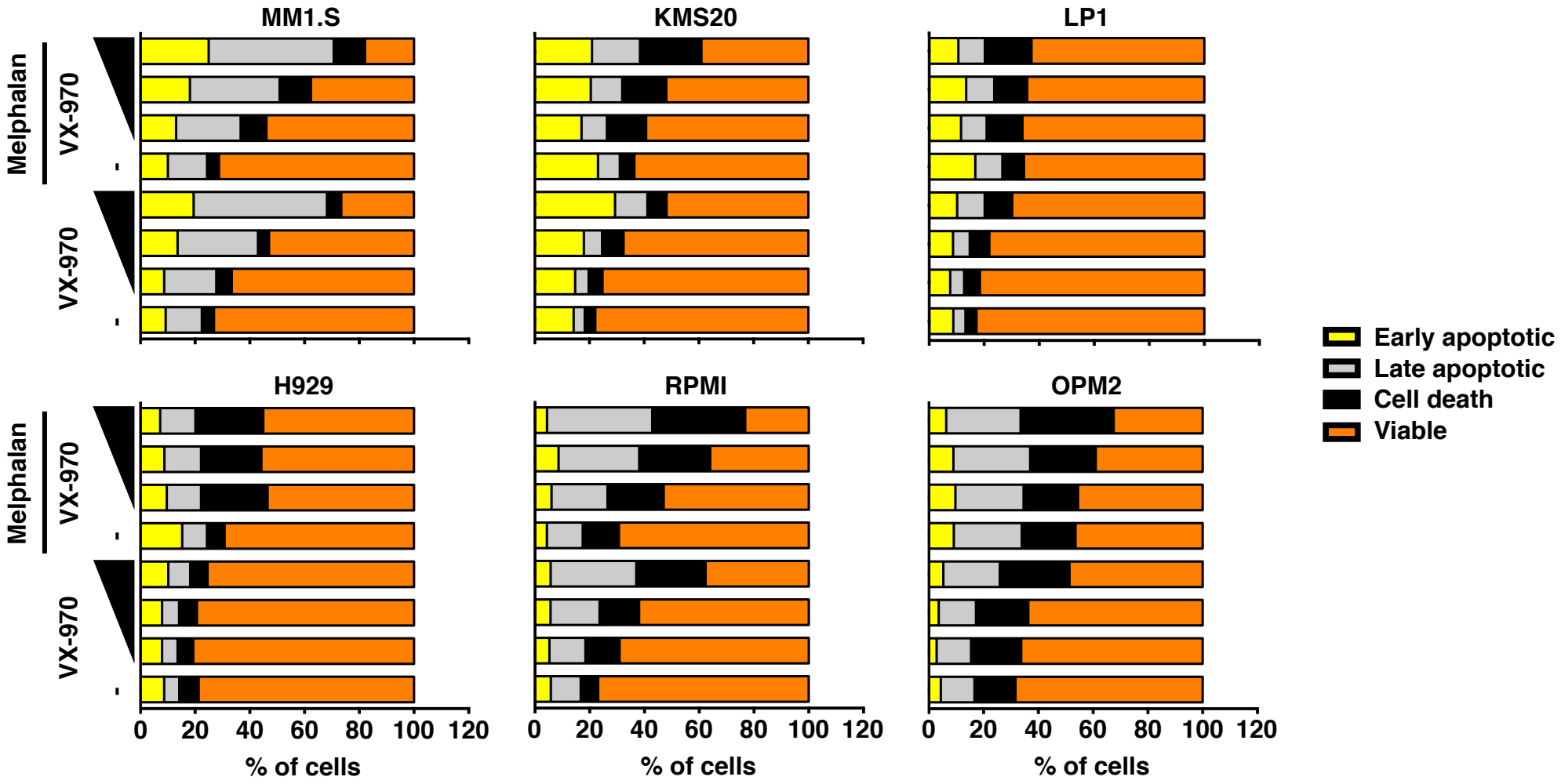


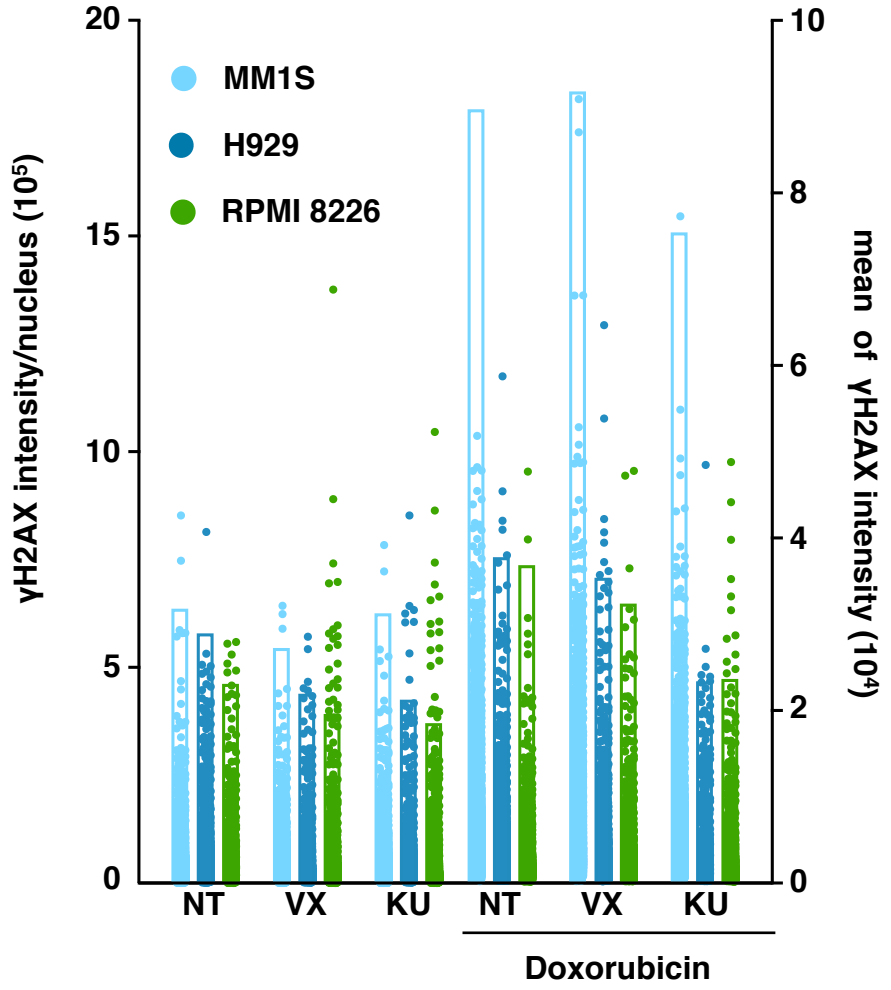
Supplementary Figure 3



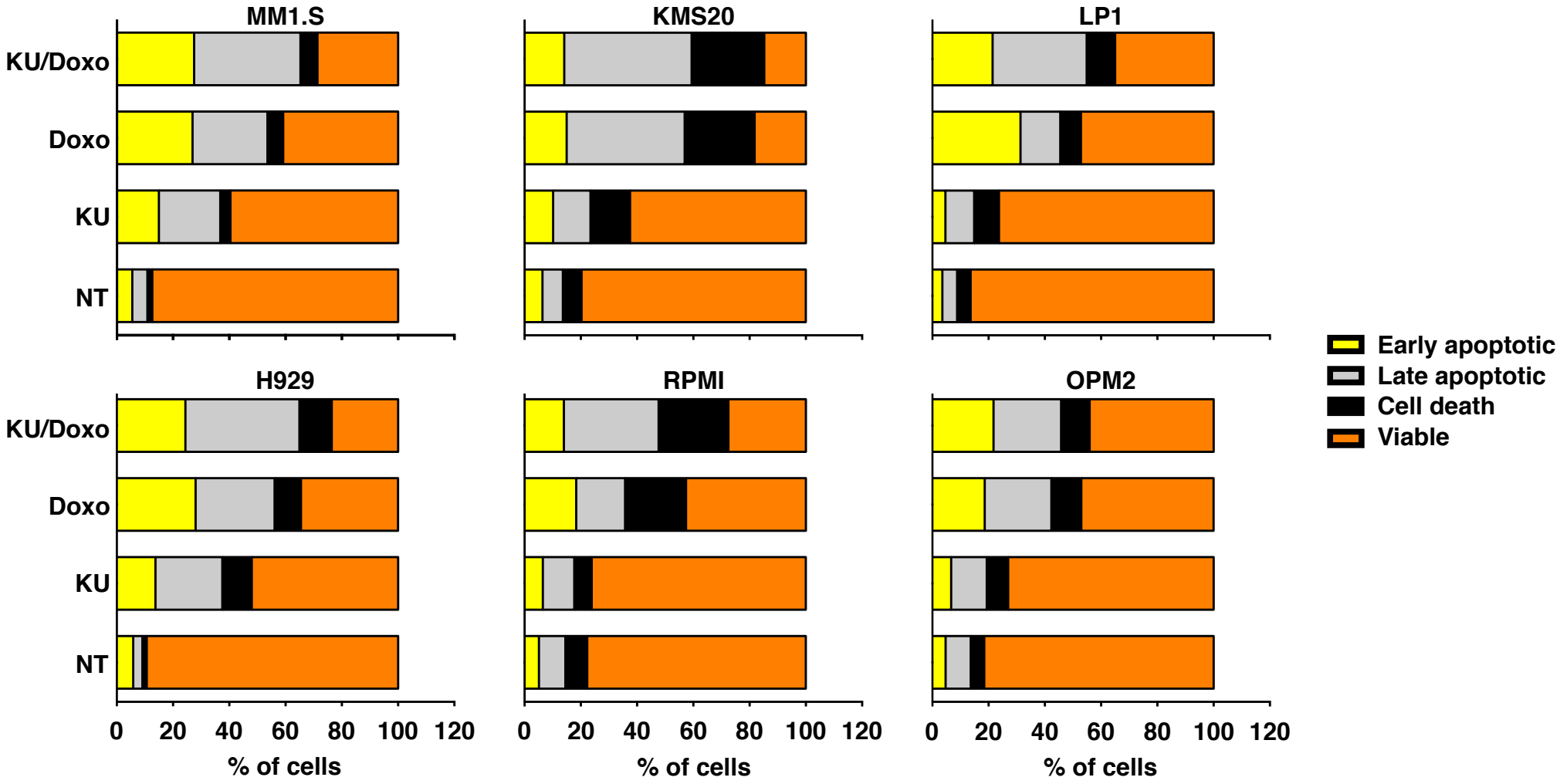
Supplementary Figure 4



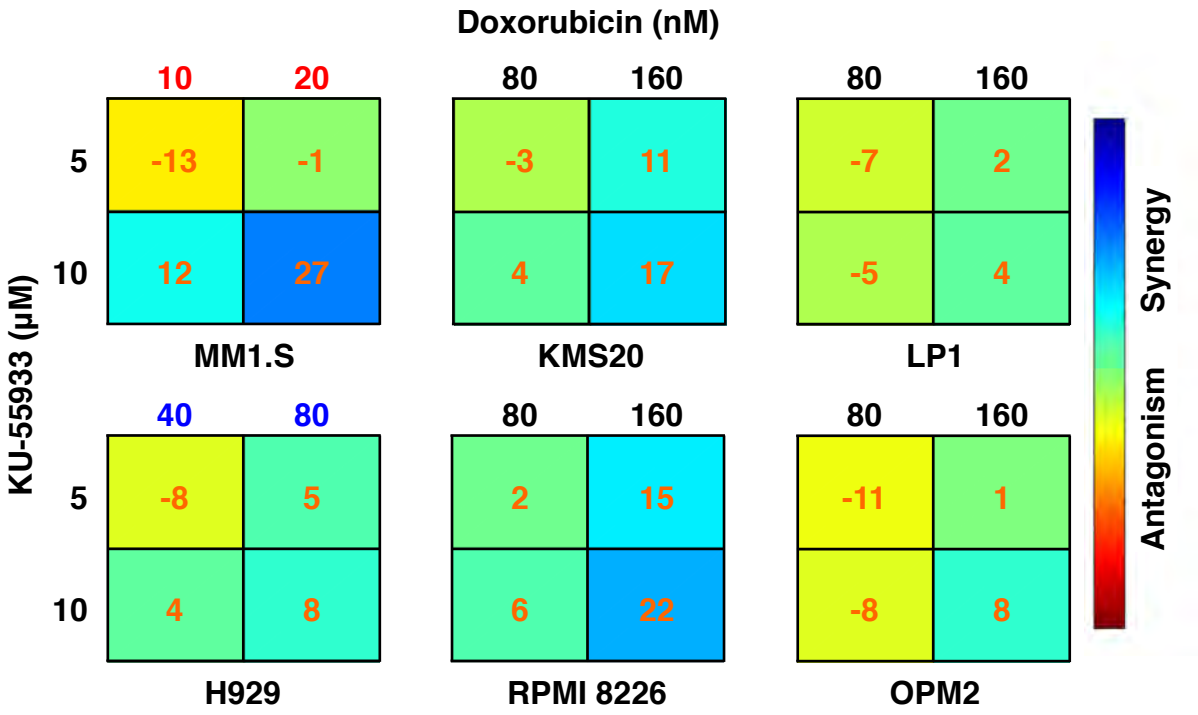
Supplementary Figure 5



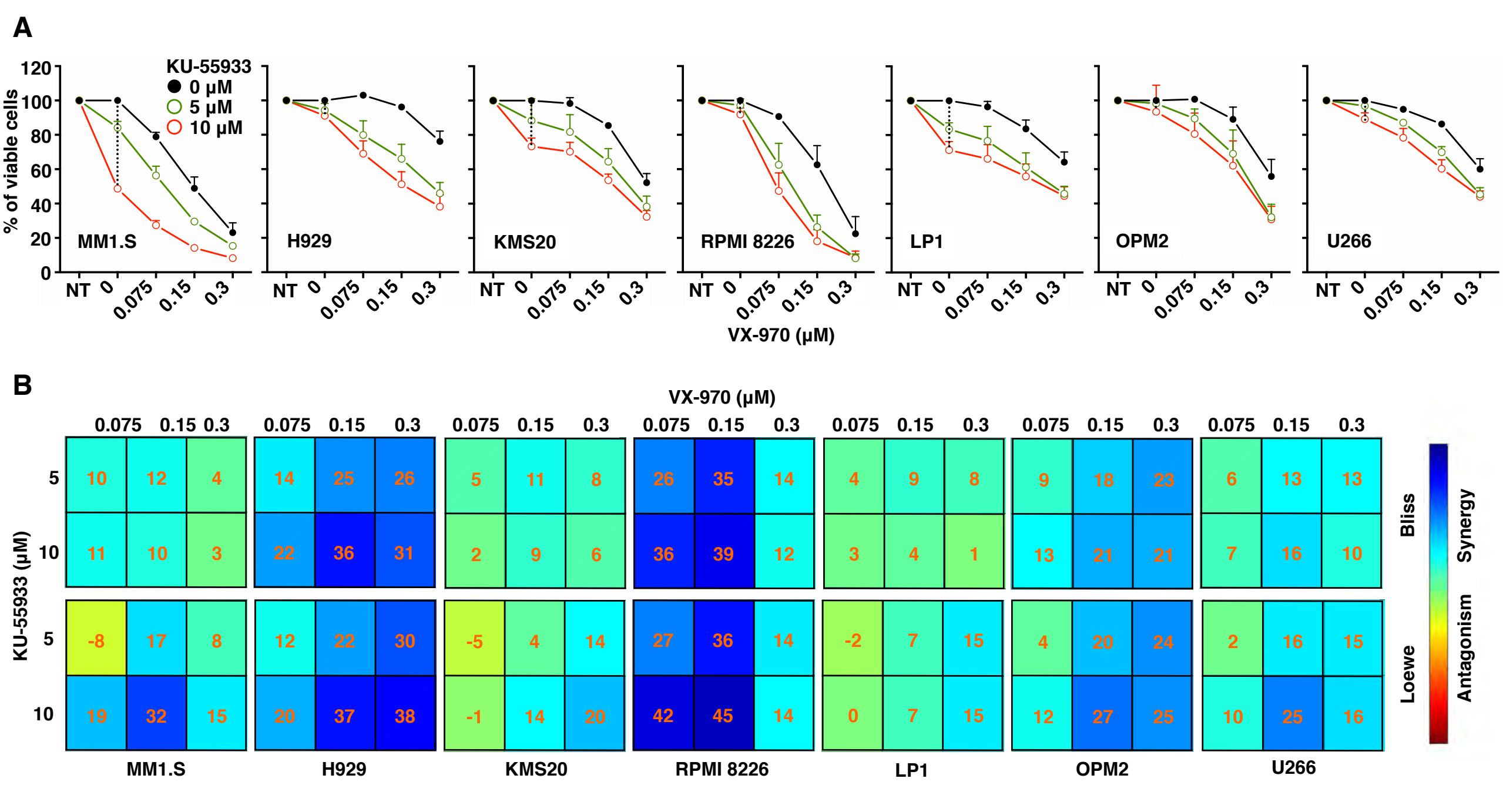
Supplementary Figure 6



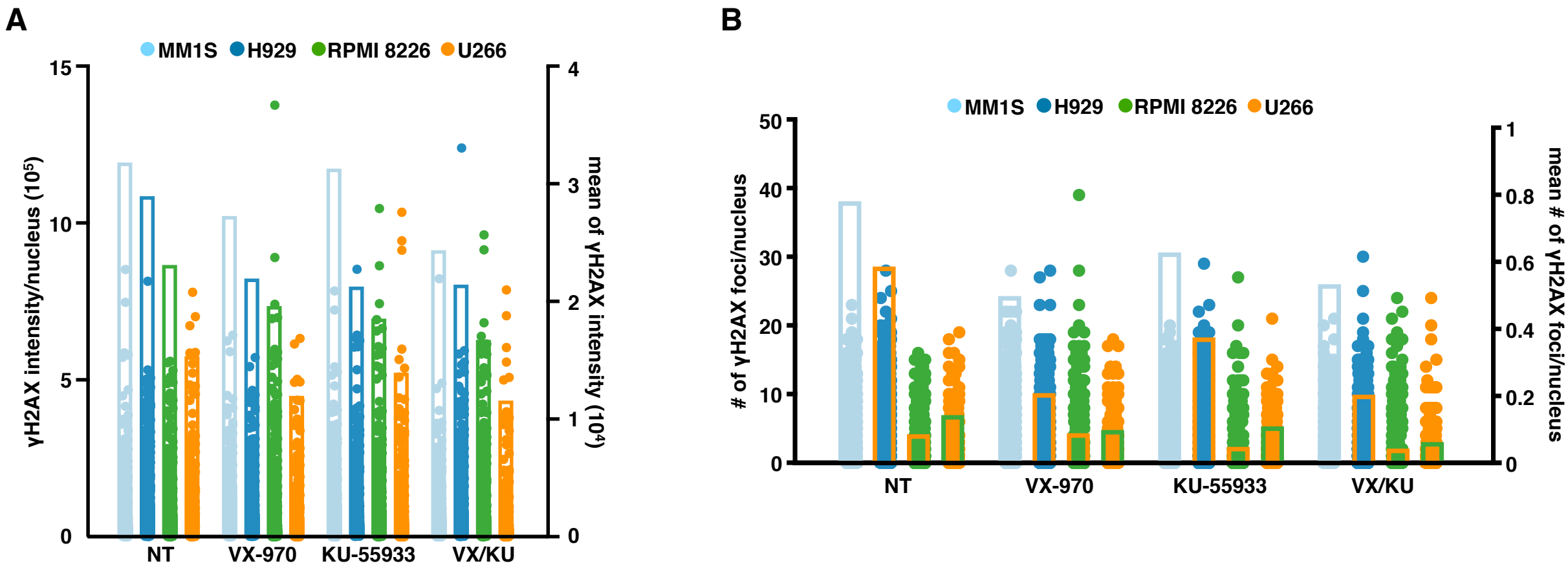
Supplementary Figure 7



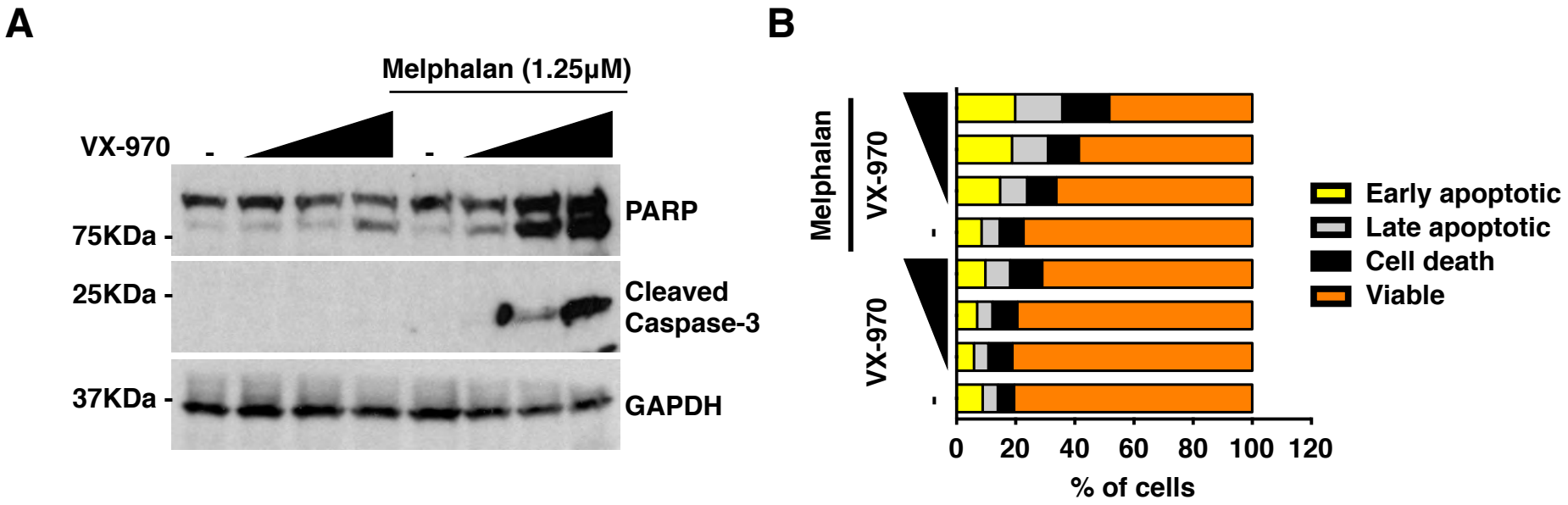
Supplementary Figure 8



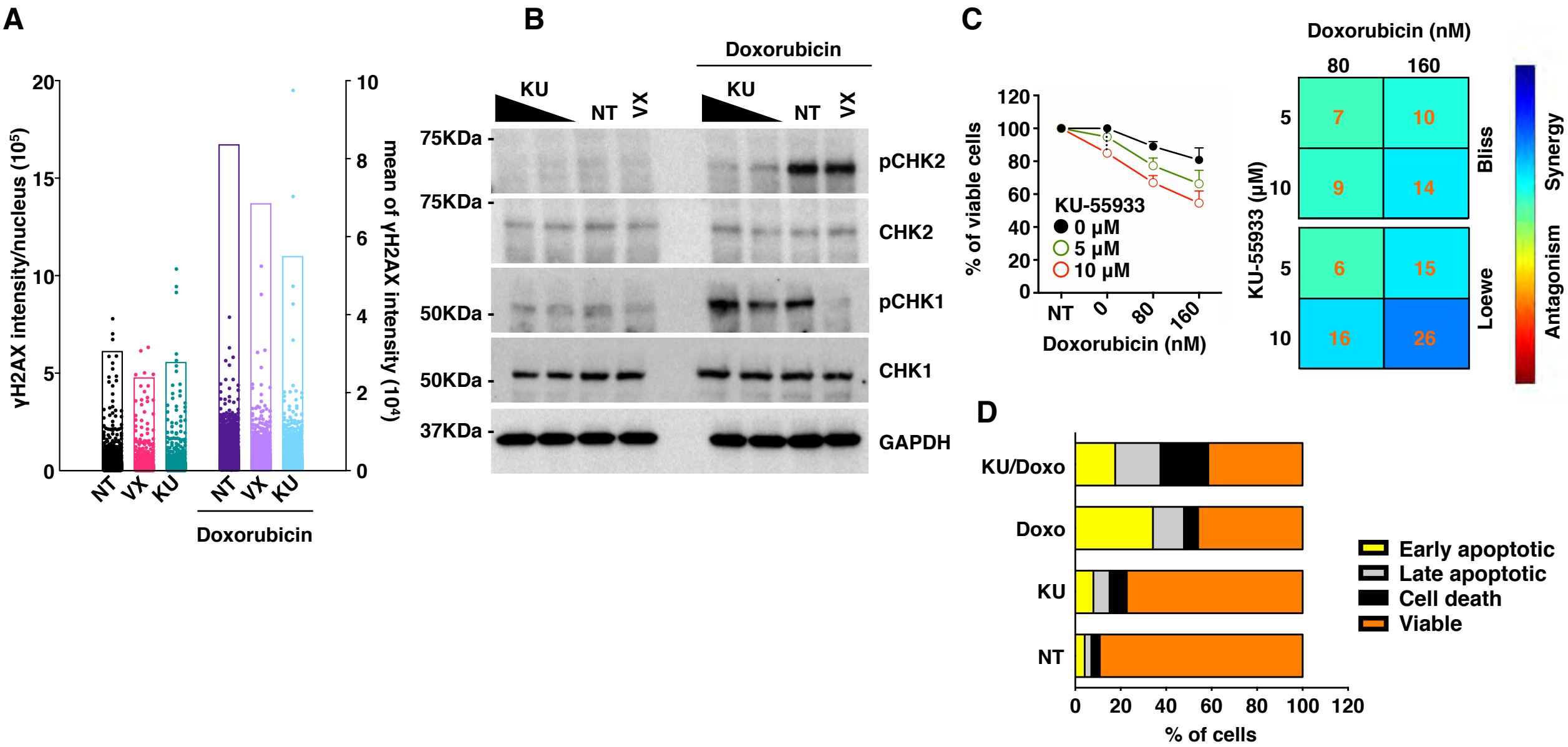
Supplementary Figure 9



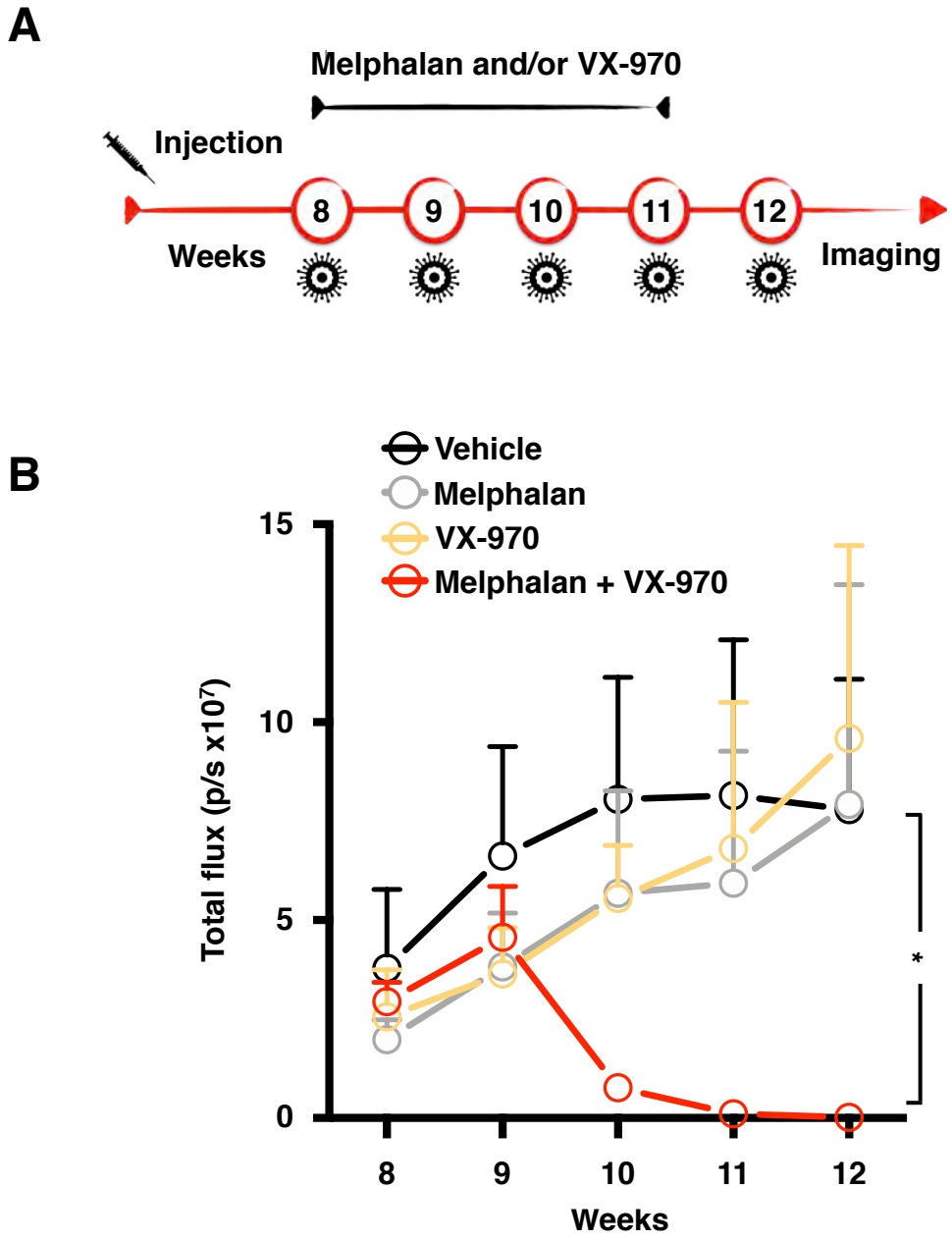
Supplementary Figure 10



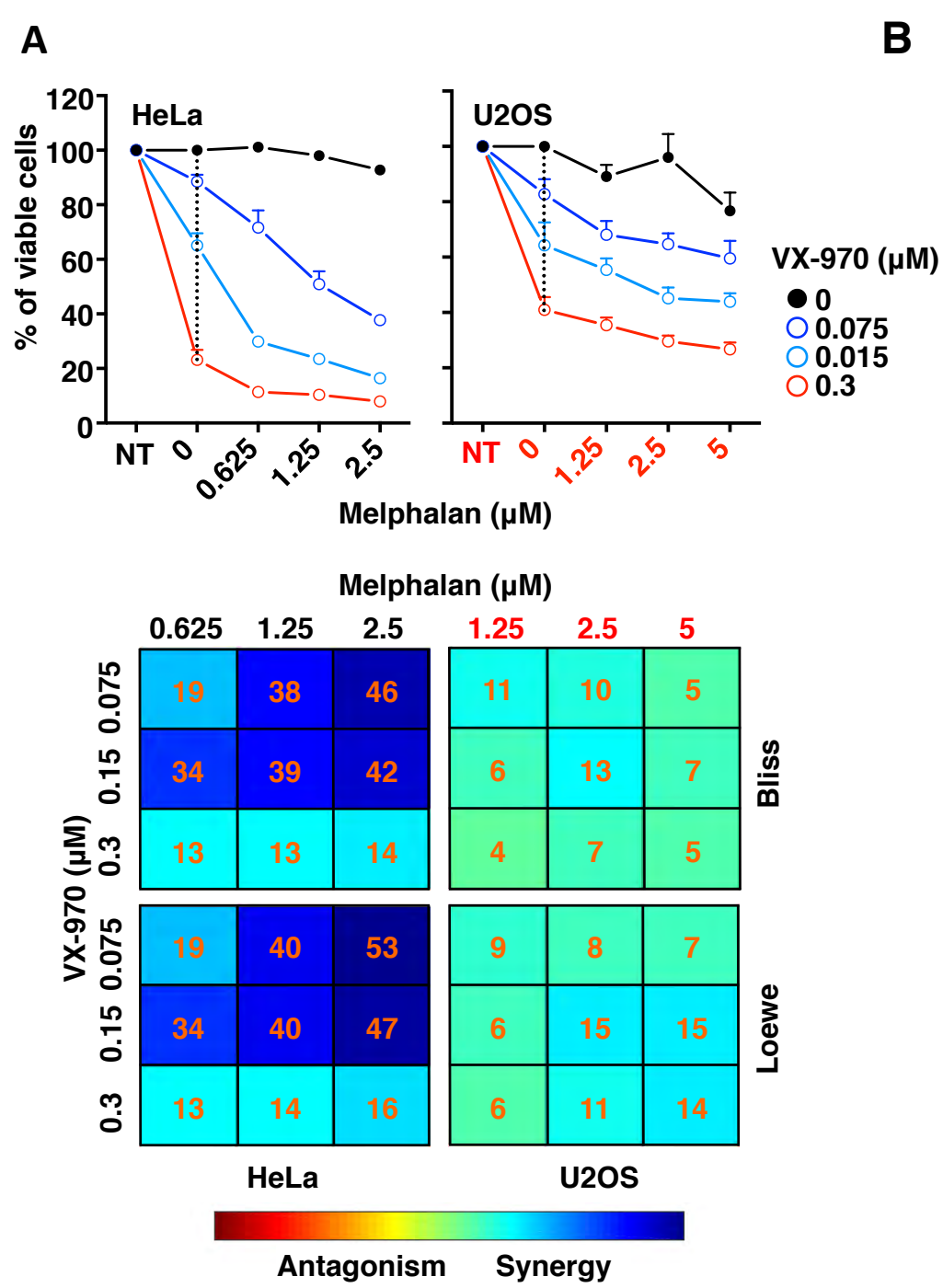
Supplementary Figure 11

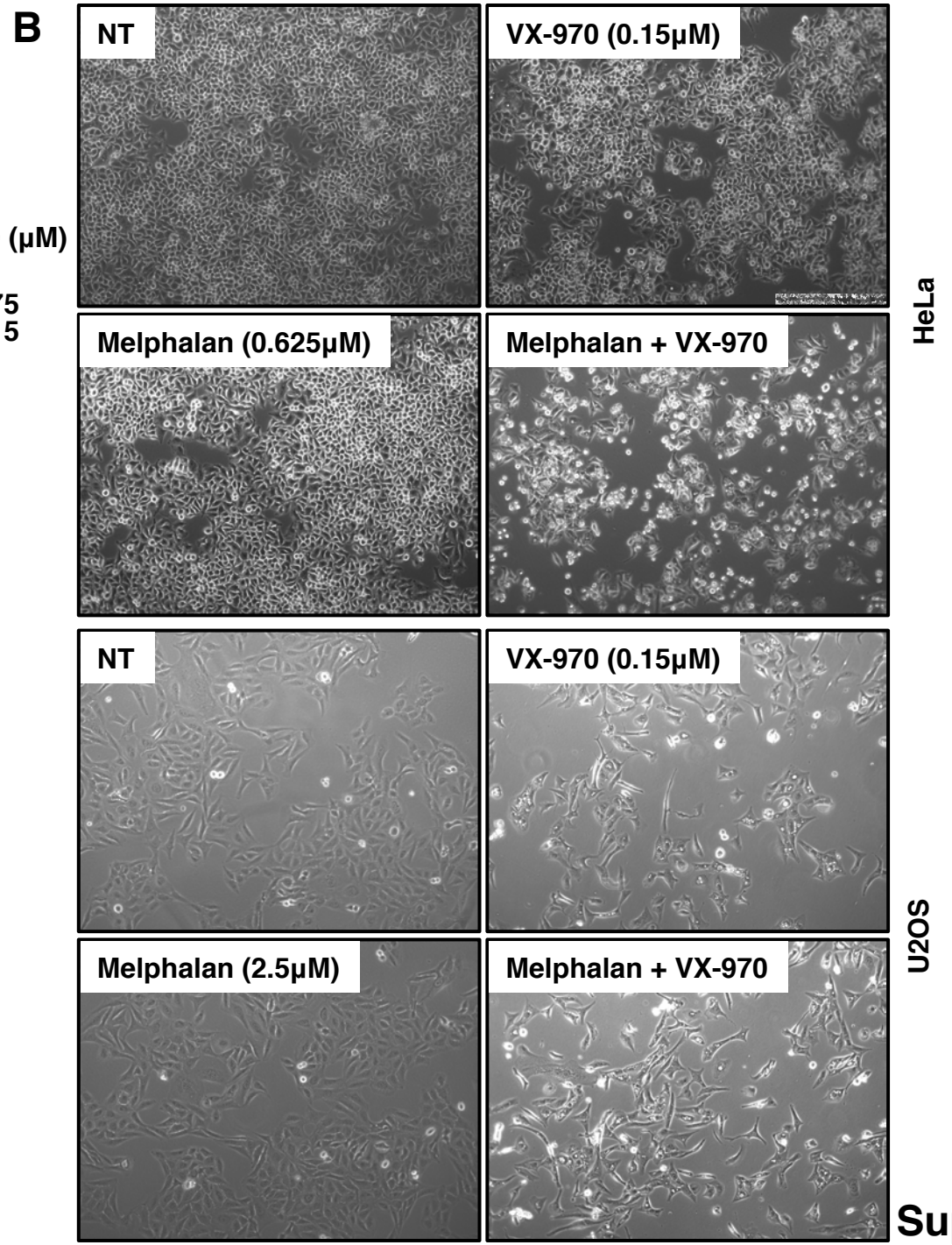


Supplementary Figure 12

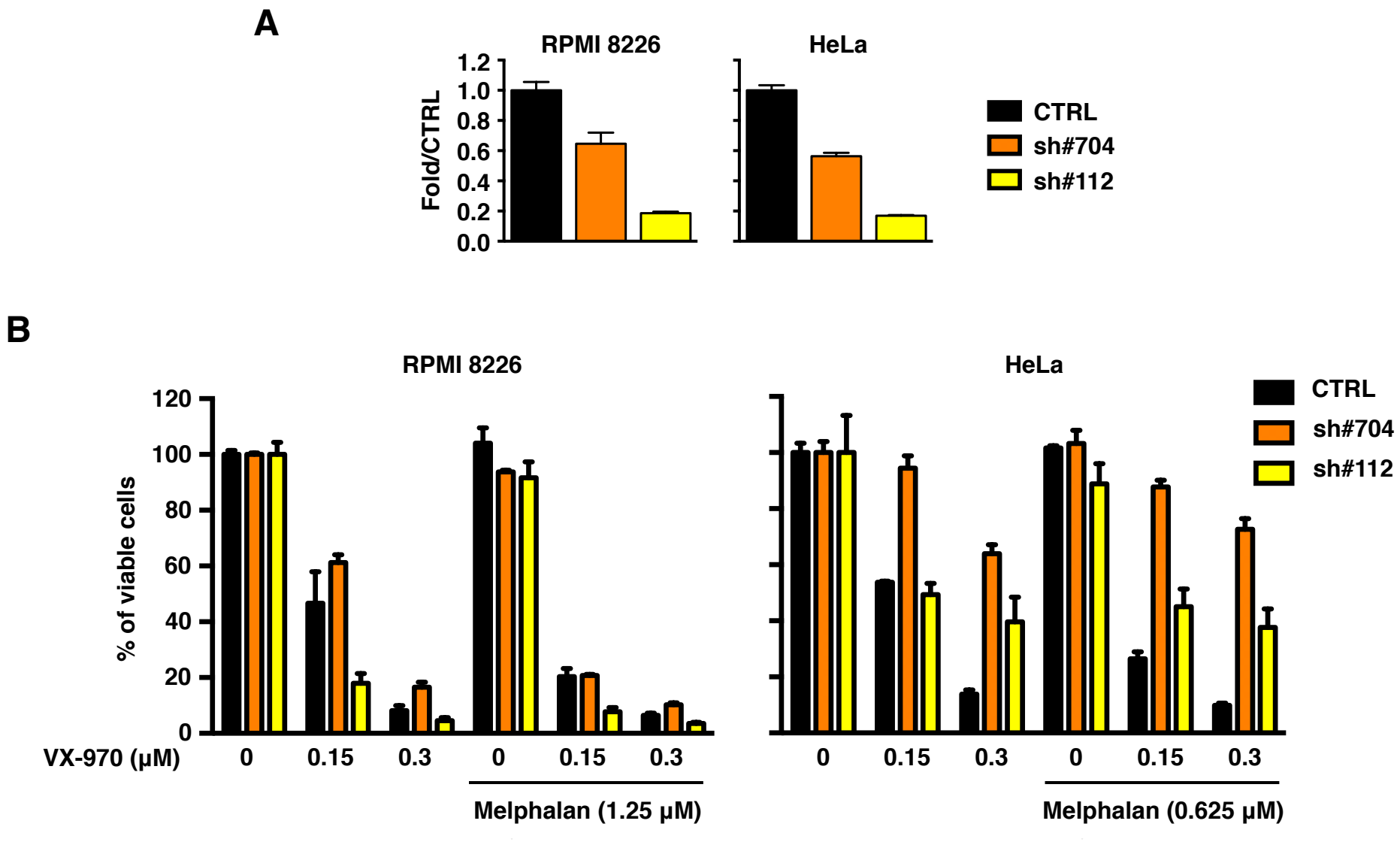


Supplementary Figure 13





Supplementary Figure 14



Supplementary Figure 15

Supplementary Table 1

Comparison of the effects of different ATR inhibitors, VX-970, VE-821 and AZD6738 in different MM cell lines using the GrapPad software

Statistical analysis was performed using two-way ANOVA followed by Dunnett correction for multiple comparisons.

Multiple t tests were performed and statistical significance determined using the Holm-Sidak method, with alpha=5.000% Only significant results are shown.

Dunnett's multiple comparisons test#				
MM1.S	Mean Diff	95% CI of diff	Significant?	Summary
0μM				
VX-970 vs.VE-821	0	-8,926 to 8,926	No	ns
VX-970 vs.AZD6738	0	-9,563 to 9,563	No	ns
0.15μM				
VX-970 vs.VE-821	-40,4	-50,91 to -29,89	Yes	****
VX-970 vs.AZD6738	-57,06	-66,69 to -47,43	Yes	****
0.3μM				
VX-970 vs.VE-821	-26,05	-35,68 to -16,41	Yes	****
VX-970 vs.AZD6738	-73,95	-83,59 to -64,32	Yes	****
0.6μM				
VX-970 vs.VE-821	-19,12	-30,51 to -7,741	Yes	***
VX-970 vs.AZD6738	-66,18	-78,07 to -54,29	Yes	****
1.2μM				
VX-970 vs.VE-821	-25,21	-37,82 to -12,60	Yes	****
VX-970 vs.AZD6738	-29,03	-41,64 to -16,43	Yes	****
2.5μM				
VX-970 vs.VE-821	-29,05	-41,66 to -16,44	Yes	****
VX-970 vs.AZD6738	-12,5	-25,11 to 0,1118	No	ns
5μM				
VX-970 vs.VE-821	-27,68	-40,29 to -15,07	Yes	****
VX-970 vs.AZD6738	-9,831	-22,44 to 2,778	No	ns
10μM				
VX-970 vs.VE-821	-19,7	-33,43 to -5,971	Yes	**
VX-970 vs.AZD6738	-7,588	-21,32 to 6,139	No	ns

Multiple t test##							
MM1.S	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
VX-970 vs.VE-821							
0.15μM	1,04985E-13	39,8454	80,2478	-40,4024	4,64241	8,70289	94
0.3μM	2,1284E-08	19,3363	45,3818	-26,0455	4,25484	6,12137	94
2.5μM	1,08775E-06	4,7678	33,8223	-29,0545	5,57089	5,21541	94
5μM	3,00559E-06	0,0268766	27,7112	-27,6843	5,57089	4,96945	94
1.25μM	1,76004E-05	7,84621	33,0584	-25,2122	5,57089	4,52569	94
0.6μM	0,00025411	10,4875	29,6112	-19,1237	5,02868	3,80292	94
10μM	0,00161167	0,0147958	19,7139	-19,6991	6,06482	3,24809	94
VX-970 vs.AZD6738							
0.3μM	1,77106E-34	19,3363	93,2912	-73,9548	3,80923	19,4147	93
0.15μM	1,59325E-26	39,8454	96,9024	-57,057	3,80923	14,9786	93
0.6μM	9,00775E-25	10,4875	76,6699	-66,1823	4,70221	14,0747	93
1.2μM	8,24985E-08	7,84621	36,8809	-29,0347	4,98745	5,82156	93

Dunnett's multiple comparisons test#				
H929	Mean Diff	95% CI of diff	Significant?	Summary
0μM				
VX-970 vs.VE-821	0	-10,22 to 10,22	No	ns
VX-970 vs.AZD6738	0	-13,20 to 13,20	No	ns
0.15μM				
VX-970 vs.VE-821	-5,604	-19,06 to 7,854	No	ns
VX-970 vs.AZD6738	-7,904	-21,36 to 5,554	No	ns
0.3μM				
VX-970 vs.VE-821	-20,81	-34,27 to -7,351	Yes	**
VX-970 vs.AZD6738	-23,1	-36,56 to -9,645	Yes	***
0.6μM				
VX-970 vs.VE-821	-33,56	-45,94 to -21,18	Yes	****
VX-970 vs.AZD6738	-42,29	-56,75 to -27,84	Yes	****
1.2μM				
VX-970 vs.VE-821	-46,08	-59,79 to -32,36	Yes	****
VX-970 vs.AZD6738	-43,26	-58,88 to -27,65	Yes	****
2.5μM				
VX-970 vs.VE-821	-43,16	-58,09 to -28,23	Yes	****
VX-970 vs.AZD6738	-24,08	-40,77 to -7,388	Yes	**
5μM				
VX-970 vs.VE-821	-45,68	-61,30 to -30,07	Yes	****
VX-970 vs.AZD6738	-9,478	-26,17 to 7,215	No	ns
10μM				
VX-970 vs.VE-821	-56,1	-71,71 to -40,48	Yes	****
VX-970 vs.AZD6738	-17,47	-34,17 to -0,7816	Yes	*

Multiple t test##							
H929	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
VX-970 vs.VE-821							
10μM	1,41301E-10	0,0262067	56,1227	-56,0965	7,46253	7,51708	70
1.2μM	1,10063E-09	35,6946	81,773	-46,0784	6,55442	7,03013	70
5μM	4,76792E-08	23,4623	69,147	-45,6847	7,46253	6,12187	70
2.5μM	6,42024E-08	33,1412	76,3037	-43,1625	7,13555	6,04894	70
0.6μM	2,94059E-07	49,5049	83,0629	-33,5581	5,91648	5,67196	70
0.3μM	0,00185613	71,7572	92,5669	-20,8097	6,4319	3,23538	70
VX-970 vs.AZD6738							
0.6μM	1,34963E-09	49,5049	91,7987	-42,2938	5,89187	7,17834	59
1.2μM	5,93901E-09	35,6946	78,9569	-43,2623	6,36395	6,79803	59
0.3μM	8,77507E-05	71,7572	94,8607	-23,1034	5,48503	4,21208	59
2.5μM	0,000789056	33,1412	57,2223	-24,0811	6,80335	3,53959	59

Dunnett's multiple comparisons test#				
RPMI 8226	Mean Diff	95% CI of diff	Significant?	Summary
0µM				
VX-970 vs.VE-821	0	-11,03 to 11,03	No	ns
VX-970 vs.AZD6738	0	-9,846 to 9,846	No	ns
0.15µM				
VX-970 vs.VE-821	-24,06	-35,28 to -12,85	Yes	****
VX-970 vs.AZD6738	-45,66	-56,87 to -34,44	Yes	****
0.3µM				
VX-970 vs.VE-821	-6,356	-17,57 to 4,860	No	ns
VX-970 vs.AZD6738	-79,1	-90,31 to -67,88	Yes	****
0.6µM				
VX-970 vs.VE-821	-6,492	-20,55 to 7,568	No	ns
VX-970 vs.AZD6738	-69,8	-83,86 to -55,74	Yes	****
1.2µM				
VX-970 vs.VE-821	-10,32	-24,38 to 3,742	No	ns
VX-970 vs.AZD6738	-19,3	-33,36 to -5,242	Yes	**
2.5µM				
VX-970 vs.VE-821	-13,52	-27,58 to 0,5439	No	ns
VX-970 vs.AZD6738	-5,171	-19,23 to 8,889	No	ns
5µM				
VX-970 vs.VE-821	-11,86	-25,92 to 2,199	No	ns
VX-970 vs.AZD6738	-2,865	-16,93 to 11,19	No	ns
10µM				
VX-970 vs.VE-821	-8,393	-21,54 to 4,759	No	ns
VX-970 vs.AZD6738	-2,562	-15,71 to 10,59	No	ns

Multiple t test##							
RPMI 8226	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
VX-970 vs.VE-821							
0.15µM	2,65657E-05	50,0105	74,0739	-24,0634	5,28	4,55747	59
VX-970 vs.AZD6738							
0.3µM	2,56731E-21	15,6963	94,7939	-79,0976	5,44279	14,5325	60
0.6µM	8,94622E-15	2,67292	72,4768	-69,8039	6,8229	10,2308	60
0.15µM	1,06866E-11	50,0105	95,6668	-45,6563	5,44279	8,38841	60
1.25µM	0,00634152	1,59807	20,8995	-19,3015	6,8229	2,82893	60

Dunnett's multiple comparisons test#				
U266	Mean Diff	95% CI of diff	Significant?	Summary
0µM				
VX-970 vs.VE-821	0	-9,951 to 9,951	No	ns
VX-970 vs.AZD6738	0	-11,15 to 11,15	No	ns
0.15µM				
VX-970 vs.VE-821	-16	-27,23 to -4,764	Yes	**
VX-970 vs.AZD6738	-18,76	-30,00 to -7,530	Yes	***
0.3µM				
VX-970 vs.VE-821	-28,5	-39,74 to -17,27	Yes	****
VX-970 vs.AZD6738	-43,08	-54,31 to -31,84	Yes	****
0.6µM				
VX-970 vs.VE-821	-32,74	-45,05 to -20,44	Yes	****
VX-970 vs.AZD6738	-60,31	-72,62 to -48,01	Yes	****
1.2µM				
VX-970 vs.VE-821	-31,29	-45,50 to -17,08	Yes	****
VX-970 vs.AZD6738	-49,96	-64,18 to -35,75	Yes	****
2.5µM				
VX-970 vs.VE-821	-33,43	-47,64 to -19,22	Yes	****
VX-970 vs.AZD6738	-26,61	-40,83 to -12,40	Yes	***
5µM				
VX-970 vs.VE-821	-43,28	-57,49 to -29,07	Yes	****
VX-970 vs.AZD6738	-25,09	-39,30 to -10,88	Yes	***
10µM				
VX-970 vs.VE-821	-50,15	-64,36 to -35,94	Yes	****
VX-970 vs.AZD6738	-30,07	-44,29 to -15,86	Yes	****

Multiple t test##							
U266	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
VX-970 vs.VE-821							
10µM	1,04985E-13	39,8454	80,2478	-40,4024	4,64241	8,70289	94
5µM	2,1284E-08	19,3363	45,3818	-26,0455	4,25484	6,12137	94
0.6µM	1,08775E-06	4,7678	33,8223	-29,0545	5,57089	5,21541	94
0.3µM	3,00559E-06	0,0268766	27,7112	-27,6843	5,57089	4,96945	94
2.5µM	1,76004E-05	7,84621	33,0584	-25,2122	5,57089	4,52569	94
1.25µM	0,00025411	10,4875	29,6112	-19,1237	5,02868	3,80292	94
0.15µM	0,00161167	0,0147958	19,7139	-19,6991	6,06482	3,24809	94
VX-970 vs.AZD6738							
0.6µM	2,23922E-16	32,5544	92,8681	-60,3138	5,46032	11,0458	63
0.3µM	2,70822E-12	52,3848	95,4603	-43,0755	4,98456	8,64177	63
1.25µM	4,83381E-11	29,4229	79,3878	-49,9649	6,30503	7,92461	63
10µM	1,13172E-05	0,81522	30,8898	-30,0746	6,30503	4,76994	63
2.5µM	7,95189E-05	27,3965	54,011	-26,6145	6,30503	4,22115	63
5µM	0,000181072	13,3286	38,4199	-25,0913	6,30503	3,97956	63
0.15µM	0,000368874	79,0938	97,8587	-18,7648	4,98456	3,76459	63

Dunnett's multiple comparisons test#				
LP1	Mean Diff	95% CI of diff	Significant?	Summary
0µM				
VX-970 vs.VE-821	0	-12,33 to 12,33	No	ns
VX-970 vs.AZD6738	0	-12,33 to 12,33	No	ns
0.15µM				
VX-970 vs.VE-821	-16,13	-28,61 to -3,640	Yes	**
VX-970 vs.AZD6738	-16,52	-29,01 to -4,038	Yes	**
0.3µM				
VX-970 vs.VE-821	-14,94	-27,42 to -2,450	Yes	*
VX-970 vs.AZD6738	-32,67	-45,16 to -20,19	Yes	****
0.6µM				
VX-970 vs.VE-821	-26,54	-41,83 to -11,25	Yes	***
VX-970 vs.AZD6738	-52,45	-67,74 to -37,16	Yes	****
1.2µM				
VX-970 vs.VE-821	-28,74	-44,03 to -13,44	Yes	***
VX-970 vs.AZD6738	-25,14	-40,43 to -9,843	Yes	***
2.5µM				
VX-970 vs.VE-821	-33,85	-49,14 to -18,55	Yes	****
VX-970 vs.AZD6738	-8,637	-23,93 to 6,657	No	ns
5µM				
VX-970 vs.VE-821	-39,12	-54,41 to -23,83	Yes	****
VX-970 vs.AZD6738	-11,88	-27,17 to 3,414	No	ns
10µM				
VX-970 vs.VE-821	-34,92	-50,22 to -19,63	Yes	****
VX-970 vs.AZD6738	-8,526	-23,82 to 6,767	No	ns

Multiple t test##							
LP1	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
VX-970 vs.VE-821							
5µM	5,02977E-06	4,27118	43,391	-39,1198	7,66914	5,10094	51
10µM	3,30477E-05	3,6543	38,5763	-34,922	7,66914	4,55358	51
2.5µM	5,28966E-05	12,7423	46,5901	-33,8478	7,66914	4,4135	51
1.25µM	0,000457107	17,2604	45,996	-28,7356	7,66914	3,74691	51
0.6µM	0,00109878	26,502	53,0407	-26,5387	7,66914	3,46046	51
0.15µM	0,0129561	72,7038	88,8303	-16,1265	6,26183	2,57536	51
0.3µM	0,0208123	55,3816	70,3187	-14,9371	6,26183	2,38542	51
VX-970 vs.AZD6738							
0.6µM	6,21446E-09	26,502	78,9526	-52,4506	7,53217	6,96354	51
0.3µM	2,39179E-06	55,3816	88,0536	-32,672	6,14999	5,31253	51
1.25µM	0,00158553	17,2604	42,3966	-25,1362	7,53217	3,33718	51
0.15µM	0,00971127	72,7038	89,2284	-16,5246	6,14999	2,68694	51

Dunnett's multiple comparisons test#				
OPM2	Mean Diff	95% CI of diff	Significant?	Summary
0µM				
VX-970 vs.VE-821	0	-13,54 to 13,54	No	ns
VX-970 vs.AZD6738	0	-17,12 to 17,12	No	ns
0.15µM				
VX-970 vs.VE-821	-11,31	-29,04 to 6,412	No	ns
VX-970 vs.AZD6738	-19,39	-37,12 to -1,668	Yes	*
0.3µM				
VX-970 vs.VE-821	-16,58	-34,30 to 1,147	No	ns
VX-970 vs.AZD6738	-53,26	-70,98 to -35,53	Yes	****
0.6µM				
VX-970 vs.VE-821	-44,37	-61,60 to -27,14	Yes	****
VX-970 vs.AZD6738	-68,79	-88,41 to -49,18	Yes	****
1.2µM				
VX-970 vs.VE-821	-40,1	-57,33 to -22,87	Yes	****
VX-970 vs.AZD6738	-40,33	-59,94 to -20,71	Yes	****
2.5µM				
VX-970 vs.VE-821	-40,74	-59,50 to -21,98	Yes	****
VX-970 vs.AZD6738	-21,42	-42,39 to -0,4448	Yes	*
5µM				
VX-970 vs.VE-821	-34,22	-53,84 to -14,60	Yes	***
VX-970 vs.AZD6738	-18,01	-38,98 to 2,960	No	ns
10µM				
VX-970 vs.VE-821	-33,28	-52,90 to -13,66	Yes	***
VX-970 vs.AZD6738	-24,53	-45,50 to -3,559	Yes	*

Multiple t test##							
OPM2	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
VX-970 vs.VE-821							
0.6µM	2,25056E-06	30,1569	74,5311	-44,3741	8,46661	5,24107	59
1.25µM	1,41093E-05	27,4725	67,5709	-40,0984	8,46661	4,73606	59
2.5µM	4,29146E-05	23,0739	63,8163	-40,7424	9,21728	4,42022	59
5µM	0,000763831	20,4447	54,6655	-34,2208	9,63966	3,55	59
10µM	0,00103316	5,48769	38,7698	-33,2821	9,63966	3,45262	59
VX-970 vs.AZD6738							
0.6µM	6,52957E-13	30,1569	98,9517	-68,7948	7,08329	9,71226	48
0.3µM	7,22128E-11	50,3407	103,598	-53,2572	6,39981	8,32168	48
1.25µM	7,35175E-07	27,4725	67,7988	-40,3263	7,08329	5,69316	48
10µM	0,00217653	5,48769	30,0184	-24,5308	7,57236	3,23951	48
0.15µM	0,00392937	83,5144	102,907	-19,3927	6,39981	3,03021	48
2.5µM	0,00680788	23,0739	44,4909	-21,4169	7,57236	2,82831	48
5µM	0,0214008	20,4447	38,457	-18,0123	7,57236	2,37869	48

Dunnett's multiple comparisons test#				
KMS20	Mean Diff	95% CI of diff	Significant?	Summary
0μM				
VX-970 vs.VE-821	0	-11,35 to 11,35	No	ns
VX-970 vs.AZD6738	0	-10,23 to 10,23	No	ns
0.15μM				
VX-970 vs.VE-821	-20,6	-31,95 to -9,259	Yes	***
VX-970 vs.AZD6738	-24,56	-34,79 to -14,34	Yes	****
0.3μM				
VX-970 vs.VE-821	-33,76	-45,11 to -22,42	Yes	****
VX-970 vs.AZD6738	-54,94	-65,16 to -44,71	Yes	****
0.6μM				
VX-970 vs.VE-821	-37,35	-51,25 to -23,46	Yes	****
VX-970 vs.AZD6738	-80,32	-93,32 to -67,32	Yes	****
1.2μM				
VX-970 vs.VE-821	-46,35	-60,24 to -32,45	Yes	****
VX-970 vs.AZD6738	-62,9	-75,90 to -49,91	Yes	****
2.5μM				
VX-970 vs.VE-821	-64	-77,89 to -50,10	Yes	****
VX-970 vs.AZD6738	-36,92	-49,92 to -23,92	Yes	****
5μM				
VX-970 vs.VE-821	-59,03	-72,92 to -45,13	Yes	****
VX-970 vs.AZD6738	-17,69	-30,69 to -4,692	Yes	**
10μM				
VX-970 vs.VE-821	-49,43	-63,32 to -35,53	Yes	****
VX-970 vs.AZD6738	-12,77	-25,77 to 0,2274	No	ns

Multiple t test##							
KMS20	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
VX-970 vs.VE-821							
2.5μM	2,76632E-12	1,19025	65,1875	-63,9973	6,98187	9,1662	50
5μM	3,32802E-11	0,0957113	59,121	-59,0253	6,98187	8,45408	50
10μM	4,50361E-09	0,021346	49,4509	-49,4295	6,98187	7,07969	50
1.25μM	2,20876E-08	9,41481	55,763	-46,3482	6,98187	6,63836	50
0.3μM	2,88507E-07	48,0559	81,8195	-33,7637	5,70068	5,92275	50
0.6μM	2,19746E-06	17,3529	54,705	-37,3521	6,98187	5,34987	50
0.15μM	0,000698663	76,1872	96,7915	-20,6043	5,70068	3,61436	50
VX-970 vs.AZD6738							
0.6μM	6,52957E-13	30,1569	98,9517	-68,7948	7,08329	9,71226	48
0.3μM	7,22128E-11	50,3407	103,598	-53,2572	6,39981	8,32168	48
1.25μM	7,35175E-07	27,4725	67,7988	-40,3263	7,08329	5,69316	48
2.5μM	0,00217653	5,48769	30,0184	-24,5308	7,57236	3,23951	48
0.15μM	0,00392937	83,5144	102,907	-19,3927	6,39981	3,03021	48
5μM	0,00680788	23,0739	44,4909	-21,4169	7,57236	2,82831	48

Supplementary Table 2

Comparison of the effects of VX-970 alone or in combination with Melphalan in different MM cell lines using the GrapPad software

Statistical analysis was performed using two-way ANOVA followed by Dunnett correction for multiple comparisons.

Multiple t tests were performed and statistical significance determined using the Holm-Sidak method, with alpha=5.000% Only significant results are shown.

Dunnett's multiple comparisons test#				
MM1.S	Mean Diff	95% CI of diff	Significant?	Summary
VX-970_0µM vs.VX-970_ 0.075µM	45,96	39,66 to 52,25	Yes	****
VX-970_0µM vs.VX-970_ 0.15µM	63,15	56,86 to 69,45	Yes	****
VX-970_0µM vs.VX-970_ 0.3µM	73,84	67,55 to 80,13	Yes	****
VX-970_0µM				
Melph_0µM vs. Melph_0.625µM	9,058	-3,198 to 21,31	No	ns
Melph_0µM vs. Melph_1.25µM	17,31	5,056 to 29,57	Yes	**
Melph_0µM vs. Melph_2.5µM	44,46	32,20 to 56,72	Yes	****
VX-970_0.075µM				
Melph_0µM vs. Melph_0.625µM	29,42	17,16 to 41,67	Yes	****
Melph_0µM vs. Melph_1.25µM	48,9	36,64 to 61,16	Yes	****
Melph_0µM vs. Melph_2.5µM	63,4	51,14 to 75,65	Yes	****
VX-970_0.15µM				
Melph_0µM vs. Melph_0.625µM	19,85	7,596 to 32,11	Yes	***
Melph_0µM vs. Melph_1.25µM	28,28	16,03 to 40,54	Yes	****
Melph_0µM vs. Melph_2.5µM	35,5	23,24 to 47,75	Yes	****
VX-970_0.3µM				
Melph_0µM vs. Melph_0.625µM	7,726	-4,530 to 19,98	No	ns
Melph_0µM vs. Melph_1.25µM	12,61	0,3501 to 24,86	Yes	*
Melph_0µM vs. Melph_2.5µM	16,17	3,912 to 28,42	Yes	**

Multiple t test##							
MM1.S	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
Melph_0µM vs. Melph_0.625µM							
VX-970_0.075µM	2,51258E-06	70,4036	40,9868	29,4168	4,53318	6,48922	20
VX-970_0.15µM	0,000290038	39,8013	19,9501	19,8512	4,53318	4,37908	20
Melph_0µM vs. Melph_1.25µM							
VX-970_0.075µM	5,32075E-09	70,4036	21,5032	48,9004	5,04435	9,69409	20
VX-970_0.15µM	1,73255E-05	39,8013	11,5185	28,2828	5,04435	5,60682	20
VX-970_0µM	0,00264031	99,9999	82,6887	17,3112	5,04435	3,4318	20
VX-970_0.3µM	0,0212806	18,2371	5,63147	12,6057	5,04435	2,49896	20
Melph_0µM vs. Melph_2.5µM							
VX-970_0.075µM	3,4225E-10	70,4036	7,00439	63,3992	5,56846	11,3854	20
VX-970_0µM	1,2019E-07	99,9999	55,5405	44,4595	5,56846	7,98416	20
VX-970_0.15µM	3,20799E-06	39,8013	4,30194	35,4993	5,56846	6,37507	20
VX-970_0.3µM	0,00878786	18,2371	2,06987	16,1673	5,56846	2,90336	20

Dunnett's multiple comparisons test#				
H929	Mean Diff	95% CI of diff	Significant?	Summary
VX-970_0µM vs.VX-970_ 0.075µM	13,97	5,429 to 22,51	Yes	***
VX-970_0µM vs.VX-970_ 0.15µM	21,06	12,51 to 29,60	Yes	****
VX-970_0µM vs.VX-970_ 0.3µM	33,95	25,41 to 42,49	Yes	****
VX-970_0µM				
Melph_0µM vs. Melph_0.625µM	2,954	-15,23 to 21,14	No	ns
Melph_0µM vs. Melph_1.25µM	13,34	-2,405 to 29,09	No	ns
Melph_0µM vs. Melph_2.5µM	30	13,30 to 46,71	Yes	***
VX-970_0.075µM				
Melph_0µM vs. Melph_0.625µM	23,43	5,248 to 41,62	Yes	**
Melph_0µM vs. Melph_1.25µM	31,93	16,18 to 47,68	Yes	****
Melph_0µM vs. Melph_2.5µM	53,17	36,47 to 69,88	Yes	****
VX-970_0.15µM				
Melph_0µM vs. Melph_0.625µM	31,51	13,32 to 49,69	Yes	***
Melph_0µM vs. Melph_1.25µM	33,7	17,95 to 49,45	Yes	****
Melph_0µM vs. Melph_2.5µM	48,35	31,65 to 65,05	Yes	****
VX-970_0.3µM				
Melph_0µM vs. Melph_0.625µM	27,65	9,462 to 45,83	Yes	**
Melph_0µM vs. Melph_1.25µM	26,78	11,03 to 42,53	Yes	***
Melph_0µM vs. Melph_2.5µM	36,21	19,51 to 52,92	Yes	****

Multiple t test##							
H929	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
Melph_0µM vs. Melph_0.625µM							
VX-970_0.15µM	3,49867E-08	94,2863	62,7808	31,5055	3,96123	7,95348	24
VX-970_0.3µM	3,2329E-07	75,8187	48,1715	27,6473	3,96123	6,97946	24
VX-970_0.075µM	4,19449E-06	100,562	77,1294	23,4331	3,96123	5,91562	24
Melph_0µM vs. Melph_1.25µM							
VX-970_0.15µM	2,4252E-06	94,2863	60,59	33,6964	5,88807	5,72282	32
VX-970_0.075µM	5,80061E-06	100,562	68,6311	31,9314	5,88807	5,42307	32
VX-970_0.3µM	7,35922E-05	75,8187	49,0413	26,7774	5,88807	4,54773	32
VX-970_0µM	0,030338	100	86,6564	13,3436	5,88807	2,2662	32
Melph_0µM vs. Melph_2.5µM							
VX-970_0.075µM	1,14096E-08	100,562	47,3879	53,1746	6,68022	7,96001	28
VX-970_0.15µM	7,04272E-08	94,2863	45,9363	48,35	6,68022	7,23779	28
VX-970_0.3µM	8,78796E-06	75,8187	39,6062	36,2125	6,68022	5,42085	28
VX-970_0µM	0,00011124	100	69,9967	30,0033	6,68022	4,49137	28

Dunnett's multiple comparisons test#				
KMS20	Mean Diff	95% CI of diff	Significant?	Summary
VX-970_0µM vs.VX-970_ 0.075µM	20,76	8,997 to 32,52	Yes	***
VX-970_0µM vs.VX-970_ 0.15µM	38,34	26,58 to 50,10	Yes	****
VX-970_0µM vs.VX-970_ 0.3µM	62,87	51,11 to 74,63	Yes	****
VX-970_0µM				
Melph_0µM vs. Melph_1.25µM	-1,606	-25,27 to 22,06	No	ns
Melph_0µM vs. Melph_2.5µM	1,242	-21,07 to 23,56	No	ns
Melph_0µM vs. Melph_5µM	28,31	4,637 to 51,97	Yes	*
VX-970_0.075µM				
Melph_0µM vs. Melph_1.25µM	3,543	-20,13 to 27,21	No	ns
Melph_0µM vs. Melph_2.5µM	16,49	-5,827 to 38,80	No	ns
Melph_0µM vs. Melph_5µM	48,7	25,03 to 72,37	Yes	****
VX-970_0.15µM				
Melph_0µM vs. Melph_1.25µM	9,366	-14,30 to 33,03	No	ns
Melph_0µM vs. Melph_2.5µM	19,14	-3,173 to 41,46	No	ns
Melph_0µM vs. Melph_5µM	44,63	20,97 to 68,30	Yes	****
VX-970_0.3µM				
Melph_0µM vs. Melph_1.25µM	7,127	-16,54 to 30,80	No	ns
Melph_0µM vs. Melph_2.5µM	15,95	-6,365 to 38,26	No	ns
Melph_0µM vs. Melph_5µM	25,38	1,717 to 49,05	Yes	*

Multiple t test##							
KMS20	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
Melph_0µM vs. Melph_5µM							
VX-970_0.075µM	0,00017278	89,1531	40,4551	48,698	11,2483	4,32937	28
VX-970_0.15µM	0,000457728	72,7007	28,0666	44,6341	11,2483	3,96808	28
VX-970_0µM	0,0178635	99,9998	71,6943	28,3055	11,2483	2,51643	28
VX-970_0.3µM	0,0320154	42,5073	17,1225	25,3849	11,2483	2,25677	28

Dunnett's multiple comparisons test#				
RPMI 8226	Mean Diff	95% CI of diff	Significant?	Summary
VX-970_0µM vs.VX-970_ 0.075µM	26,53	20,24 to 32,82	Yes	****
VX-970_0µM vs.VX-970_ 0.15µM	62,99	56,70 to 69,28	Yes	****
VX-970_0µM vs.VX-970_ 0.3µM	88,04	81,75 to 94,33	Yes	****
VX-970_0µM				
Melph_0µM vs. Melph_1.25µM	-0,5573	-12,81 to 11,70	No	ns
Melph_0µM vs. Melph_2.5µM	0,8412	-11,41 to 13,09	No	ns
Melph_0µM vs. Melph_5µM	6,245	-6,008 to 18,50	No	ns
VX-970_0.075µM				
Melph_0µM vs. Melph_1.25µM	24,05	11,79 to 36,30	Yes	****
Melph_0µM vs. Melph_2.5µM	28,5	16,24 to 40,75	Yes	****
Melph_0µM vs. Melph_5µM	26,01	13,75 to 38,26	Yes	****
VX-970_0.15µM				
Melph_0µM vs. Melph_1.25µM	23,86	11,61 to 36,11	Yes	****
Melph_0µM vs. Melph_2.5µM	20,92	8,665 to 33,17	Yes	***
Melph_0µM vs. Melph_5µM	16,09	3,834 to 28,34	Yes	**
VX-970_0.3µM				
Melph_0µM vs. Melph_1.25µM	5	-7,253 to 17,25	No	ns
Melph_0µM vs. Melph_2.5µM	4,564	-7,689 to 16,82	No	ns
Melph_0µM vs. Melph_5µM	5,063	-7,191 to 17,32	No	ns

Multiple t test##							
RPMI 8226	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
Melph_0µM vs. Melph_1.25µM							
VX-970_0.075µM	3,79527E-07	90,0912	66,0443	24,0469	3,24907	7,40115	20
VX-970_0.15µM	4,26352E-07	49,5485	25,6892	23,8593	3,24907	7,34343	20
Melph_0µM vs. Melph_2.5µM							
VX-970_0.075µM	3,46019E-06	90,0912	61,5952	28,496	4,49473	6,33987	20
VX-970_0.15µM	0,000152913	49,5485	28,6301	20,9183	4,49473	4,65397	20
Melph_0µM vs. Melph_5µM							
VX-970_0.075µM	2,43033E-05	90,0912	64,0847	26,0065	4,76633	5,45629	20
VX-970_0.15µM	0,00300833	49,5485	33,461	16,0874	4,76633	3,37522	20

Dunnett's multiple comparisons test#				
LP1	Mean Diff	95% CI of diff	Significant?	Summary
VX-970_0µM vs.VX-970_ 0.075µM	5,814	-3,214 to 14,84	No	ns
VX-970_0µM vs.VX-970_ 0.15µM	21,36	12,33 to 30,39	Yes	****
VX-970_0µM vs.VX-970_ 0.3µM	40,99	31,96 to 50,02	Yes	****
VX-970_0µM				
Melph_0µM vs. Melph_2.5µM	1,221	-17,39 to 19,83	No	ns
Melph_0µM vs. Melph_5µM	-3,774	-20,76 to 13,22	No	ns
Melph_0µM vs. Melph_10µM	-3,959	-20,95 to 13,03	No	ns
VX-970_0.075µM				
Melph_0µM vs. Melph_2.5µM	4,175	-14,44 to 22,79	No	ns
Melph_0µM vs. Melph_5µM	-3,037	-20,03 to 13,95	No	ns
Melph_0µM vs. Melph_10µM	-4,063	-21,05 to 12,93	No	ns
VX-970_0.15µM				
Melph_0µM vs. Melph_2.5µM	7,851	-10,76 to 26,46	No	ns
Melph_0µM vs. Melph_5µM	-1,785	-18,78 to 15,20	No	ns
Melph_0µM vs. Melph_10µM	6,754	-10,24 to 23,74	No	ns
VX-970_0.3µM				
Melph_0µM vs. Melph_2.5µM	11,83	-6,779 to 30,44	No	ns
Melph_0µM vs. Melph_5µM	-1,327	-18,32 to 15,66	No	ns
Melph_0µM vs. Melph_10µM	12,09	-4,896 to 29,08	No	ns

Multiple t test##
LP1
not significant for all the comparisons

Dunnett's multiple comparisons test#				
OPM2	Mean Diff	95% CI of diff	Significant?	Summary
VX-970_0µM vs.VX-970_ 0.075µM	8,931	-1,417 to 19,28	No	ns
VX-970_0µM vs.VX-970_ 0.15µM	22	11,65 to 32,35	Yes	****
VX-970_0µM vs.VX-970_ 0.3µM	55,66	45,31 to 66,00	Yes	****
VX-970_0µM				
Melph_0µM vs. Melph_2.5µM	-1,05	-21,04 to 18,94	No	ns
Melph_0µM vs. Melph_5µM	-4,604	-24,60 to 15,39	No	ns
Melph_0µM vs. Melph_10µM	21,95	1,957 to 41,95	Yes	*
VX-970_0.075µM				
Melph_0µM vs. Melph_2.5µM	7,943	-12,05 to 27,94	No	ns
Melph_0µM vs. Melph_5µM	-0,4123	-20,41 to 19,58	No	ns
Melph_0µM vs. Melph_10µM	34,81	14,81 to 54,80	Yes	***
VX-970_0.15µM				
Melph_0µM vs. Melph_2.5µM	5,874	-14,12 to 25,87	No	ns
Melph_0µM vs. Melph_5µM	-0,3564	-20,35 to 19,64	No	ns
Melph_0µM vs. Melph_10µM	28,4	8,410 to 48,40	Yes	**
VX-970_0.3µM				
Melph_0µM vs. Melph_2.5µM	2,097	-17,90 to 22,09	No	ns
Melph_0µM vs. Melph_5µM	1,625	-18,37 to 21,62	No	ns
Melph_0µM vs. Melph_10µM	28,24	8,241 to 48,23	Yes	**

Multiple t test##							
OPM2	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
Melph_0µM vs. Melph_10µM							
VX-970_0.075µM	0,00179491	96,649	61,8409	34,808	9,91528	3,51055	24
VX-970_0.15µM	0,00853805	81,7755	53,3709	28,4046	9,91528	2,86473	24
VX-970_0.3µM	0,00888498	47,7005	19,4648	28,2357	9,91528	2,8477	24
VX-970_0µM	0,0365891	100	78,0489	21,9511	9,91528	2,21387	24

Dunnett's multiple comparisons test#				
U266	Mean Diff	95% CI of diff	Significant?	Summary
VX-970_0µM vs.VX-970_ 0.075µM	28,72	20,74 to 36,70	Yes	****
VX-970_0µM vs.VX-970_ 0.15µM	41,38	33,40 to 49,36	Yes	****
VX-970_0µM vs.VX-970_ 0.3µM	57,5	49,52 to 65,48	Yes	****
VX-970_0µM				
Melph_0µM vs. Melph_1.25µM	2,504	-11,58 to 16,59	No	ns
Melph_0µM vs. Melph_2.5µM	6,102	-10,35 to 22,55	No	ns
Melph_0µM vs. Melph_5µM	8,269	-8,181 to 24,72	No	ns
VX-970_0.075µM				
Melph_0µM vs. Melph_1.25µM	29,58	15,49 to 43,67	Yes	****
Melph_0µM vs. Melph_2.5µM	47,55	31,10 to 64,00	Yes	****
Melph_0µM vs. Melph_5µM	58,98	42,53 to 75,43	Yes	****
VX-970_0.15µM				
Melph_0µM vs. Melph_1.25µM	26,65	12,56 to 40,73	Yes	****
Melph_0µM vs. Melph_2.5µM	43,47	27,02 to 59,92	Yes	****
Melph_0µM vs. Melph_5µM	54,79	38,34 to 71,24	Yes	****
VX-970_0.3µM				
Melph_0µM vs. Melph_1.25µM	22,81	8,723 to 36,90	Yes	***
Melph_0µM vs. Melph_2.5µM	30,6	14,15 to 47,05	Yes	***
Melph_0µM vs. Melph_5µM	38,34	21,89 to 54,79	Yes	****

Multiple t test##							
U266	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
Melph_0µM vs. Melph_1.25µM							
VX-970_0.075µM	6,88764E-06	95,5053	65,9264	29,5789	5,62917	5,25457	36
VX-970_0.15µM	3,37968E-05	80,5295	53,8824	26,6471	5,62917	4,73376	36
VX-970_0.3µM	0,000258862	58,1051	35,295	22,8101	5,62917	4,05213	36
Melph_0µM vs. Melph_2.5µM							
VX-970_0.075µM	5,01546E-08	95,5053	47,9577	47,5476	6,451	7,37057	28
VX-970_0.15µM	2,56736E-07	80,5295	37,0559	43,4736	6,451	6,73905	28
VX-970_0.3µM	5,58421E-05	58,1051	27,5017	30,6035	6,451	4,74398	28
Melph_0µM vs. Melph_5µM							
VX-970_0.075µM	9,16051E-15	95,5053	36,5215	58,9838	3,98601	14,7977	28
VX-970_0.15µM	5,67874E-14	80,5295	25,7418	54,7877	3,98601	13,745	28
VX-970_0.3µM	2,2561E-10	58,1051	19,7662	38,3389	3,98601	9,61836	28
VX-970_0µM	0,047328	100	91,7305	8,26947	3,98601	2,07462	28

Supplementary Table 3

Comparison of the effects of KU-55933 alone or in combination with Doxorubicin in different MM cell lines using the GrapPad software

Statistical analysis was performed using 2 way ANOVA followed by Dunnett correction for multiple comparisons.

Multiple t tests were performed and statistical significance determined using the Holm-Sidak method, with alpha=5.000% Only significant results are shown.

Dunnett's multiple comparisons test#				
MM1.S	Mean Diff	95% CI of diff	Significant?	Summary
KU_0µM vs. KU_5µM	12,11	1,344 to 22,88	Yes	*
KU_0µM vs. KU_10µM	45,9	35,13 to 56,67	Yes	****
KU_0µM				
Doxo_0nM vs. Doxo_10nM	5,547	-13,11 to 24,20	No	ns
Doxo_0nM vs. Doxo_20nM	23,96	5,306 to 42,61	Yes	*
KU_5µM				
Doxo_0nM vs. Doxo_10nM	5,118	-13,54 to 23,77	No	ns
Doxo_0nM vs. Doxo_20nM	19,77	1,120 to 38,43	Yes	*
KU_10µM				
Doxo_0nM vs. Doxo_10nM	12,08	-6,572 to 30,73	No	ns
Doxo_0nM vs. Doxo_20nM	26,56	7,903 to 45,21	Yes	**

Multiple t test##							
MM1.S	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
Doxo_0nM vs. Doxo_20nM							
KU_10µM	0,0143944	57,1397	30,5828	26,5569	9,29056	2,85848	12
KU_0µM	0,024146	100	76,0404	23,9595	9,29056	2,57891	12

Dunnett's multiple comparisons test#				
H929	Mean Diff	95% CI of diff	Significant?	Summary
KU_0µM vs. KU_5µM	5,899	-0,05575 to 11,85	No	ns
KU_0µM vs. KU_10µM	15,48	9,521 to 21,43	Yes	****
KU_0µM				
Doxo_0nM vs. Doxo_40nM	17,22	6,908 to 27,53	Yes	**
Doxo_0nM vs. Doxo_80nM	52,37	42,06 to 62,69	Yes	****
KU_5µM				
Doxo_0nM vs. Doxo_40nM	11,68	1,371 to 22,00	Yes	*
Doxo_0nM vs. Doxo_80nM	48,31	37,99 to 58,62	Yes	****
KU_10µM				
Doxo_0nM vs. Doxo_40nM	11,7	1,383 to 22,01	Yes	*
Doxo_0nM vs. Doxo_80nM	38,57	28,25 to 48,88	Yes	****

Multiple t test##							
H929	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
Doxo_0nM vs. Doxo_40nM							
KU_0µM	1,04957E-05	100	82,7787	17,2213	2,38347	7,22532	12
KU_10µM	0,000361341	78,0805	66,3836	11,6969	2,38347	4,9075	12
KU_5µM	0,000364523	90,8992	79,2147	11,6845	2,38347	4,90231	12
Doxo_0nM vs. Doxo_80nM							
KU_0µM	1,784E-07	100	47,6252	52,3748	4,91228	10,662	12
KU_5µM	4,29185E-07	90,8992	42,5938	48,3054	4,91228	9,83361	12
KU_10µM	4,5548E-06	78,0805	39,5136	38,5669	4,91228	7,85112	12

Dunnett's multiple comparisons test#				
KMS20	Mean Diff	95% CI of diff	Significant?	Summary
KU_0µM vs. KU_5µM	12,51	-2,915 to 27,93	No	ns
KU_0µM vs. KU_10µM	23,02	7,593 to 38,44	Yes	**
KU_0µM				
Doxo_0nM vs. Doxo_80nM	18,08	-13,45 to 49,60	No	ns
Doxo_0nM vs. Doxo_160nM	52,31	18,27 to 86,36	Yes	**
KU_5µM				
Doxo_0nM vs. Doxo_80nM	18,46	-13,06 to 49,99	No	ns
Doxo_0nM vs. Doxo_160nM	48,2	14,16 to 82,25	Yes	**
KU_10µM				
Doxo_0nM vs. Doxo_80nM	12,3	-19,22 to 43,82	No	ns
Doxo_0nM vs. Doxo_160nM	39,4	5,351 to 73,45	Yes	*

Multiple t test##							
KMS20	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
Doxo_0nM vs. Doxo_160nM							
KU_0µM	0,00461973	100	47,6856	52,3144	15,7355	3,32461	15
KU_5µM	0,0078854	85,2294	37,0258	48,2036	15,7355	3,06337	15
KU_10µM	0,0243195	70,1094	30,7099	39,3995	15,7355	2,50386	15

Dunnett's multiple comparisons test#				
RPMI 8226	Mean Diff	95% CI of diff	Significant?	Summary
KU_0µM vs. KU_5µM	9,667	-2,993 to 22,33	No	ns
KU_0µM vs. KU_10µM	15,58	2,916 to 28,24	Yes	*
KU_0µM				
Doxo_0nM vs. Doxo_80nM	26,09	0,8410 to 51,34	Yes	*
Doxo_0nM vs. Doxo_160nM	46,5	19,23 to 73,77	Yes	***
KU_5µM				
Doxo_0nM vs. Doxo_80nM	21,4	-3,852 to 46,64	No	ns
Doxo_0nM vs. Doxo_160nM	50,45	23,18 to 77,72	Yes	***
KU_10µM				
Doxo_0nM vs. Doxo_80nM	19,76	-5,485 to 45,01	No	ns
Doxo_0nM vs. Doxo_160nM	49,69	22,42 to 76,96	Yes	***

Multiple t test##							
RPMI 8226	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
Doxo_0nM vs. Doxo_80nM							
KU_0µM	1,40368E-05	100	73,9116	26,0884	4,42689	5,89316	18
KU_5µM	0,000133423	88,6919	67,2965	21,3954	4,42689	4,83305	18
KU_10µM	0,000299719	81,6641	61,9012	19,7629	4,42689	4,46428	18
Doxo_0nM vs. Doxo_160nM							
KU_5µM	0,0017728	88,6919	38,2382	50,4537	13,3061	3,79177	15
KU_10µM	0,00199331	81,6641	31,9729	49,6913	13,3061	3,73447	15
KU_0µM	0,00326152	100	53,5046	46,4954	13,3061	3,49429	15

LP1				
Dunnett's multiple comparisons test#	Mean Diff	95% CI of diff	Significant?	Summary
KU_0µM vs. KU_5µM	10,16	4,753 to 15,56	Yes	***
KU_0µM vs. KU_10µM	24,55	19,15 to 29,96	Yes	****
KU_0µM				
Doxo_0nM vs. Doxo_80nM	-1,078	-10,44 to 8,281	No	ns
Doxo_0nM vs. Doxo_160nM	0,942	-8,417 to 10,30	No	ns
KU_5µM				
Doxo_0nM vs. Doxo_80nM	-7,348	-16,71 to 2,011	No	ns
Doxo_0nM vs. Doxo_160nM	1,574	-7,786 to 10,93	No	ns
KU_10µM				
Doxo_0nM vs. Doxo_80nM	-4,606	-13,97 to 4,754	No	ns
Doxo_0nM vs. Doxo_160nM	3,538	-5,822 to 12,90	No	ns

Multiple t test##
LP1
not significant for all the comparisons

Dunnett's multiple comparisons test#				
OPM2	Mean Diff	95% CI of diff	Significant?	Summary
KU_0µM vs. KU_5µM	6,357	-2,885 to 15,60	No	ns
KU_0µM vs. KU_10µM	16,69	7,447 to 25,93	Yes	***
KU_0µM				
Doxo_0nM vs. Doxo_80nM	-1,849	-21,04 to 17,34	No	ns
Doxo_0nM vs. Doxo_160nM	34,15	14,96 to 53,34	Yes	***
KU_5µM				
Doxo_0nM vs. Doxo_80nM	1,208	-17,98 to 20,40	No	ns
Doxo_0nM vs. Doxo_160nM	29,47	10,28 to 48,66	Yes	**
KU_10µM				
Doxo_0nM vs. Doxo_80nM	2,347	-16,84 to 21,54	No	ns
Doxo_0nM vs. Doxo_160nM	26,52	7,327 to 45,71	Yes	**

Multiple t test##							
OPM2	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
Doxo_0nM vs. Doxo_160nM							
KU_0µM	0,00413561	100	65,8528	34,1471	10,1069	3,37858	15
KU_5µM	0,0106545	94,321	64,8543	29,4667	10,1069	2,91549	15
KU_10µM	0,0191732	84,3977	57,8805	26,5173	10,1069	2,62367	15

Dunnett's multiple comparisons test#				
U266	Mean Diff	95% CI of diff	Significant?	Summary
KU_0µM vs. KU_5µM	9,63	2,603 to 16,66	Yes	**
KU_0µM vs. KU_10µM	20,09	13,07 to 27,12	Yes	****
KU_0µM				
Doxo_0nM vs. Doxo_80nM	11	-3,008 to 25,02	No	ns
Doxo_0nM vs. Doxo_160nM	19,18	4,044 to 34,31	Yes	*
KU_5µM				
Doxo_0nM vs. Doxo_80nM	17,5	3,489 to 31,51	Yes	*
Doxo_0nM vs. Doxo_160nM	28,49	13,36 to 43,63	Yes	***
KU_10µM				
Doxo_0nM vs. Doxo_80nM	17,91	3,900 to 31,92	Yes	**
Doxo_0nM vs. Doxo_160nM	30,36	15,22 to 45,49	Yes	****

Multiple t test##							
U266	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
Doxo_0nM vs. Doxo_80nM							
KU_10µM	0,000515478	84,9152	67,0025	17,9127	4,24528	4,21944	18
KU_5µM	0,000639383	94,7972	77,296	17,5012	4,24528	4,12251	18
KU_0µM	0,0184037	100	88,996	11,004	4,24528	2,59207	18
Doxo_0nM vs. Doxo_160nM							
KU_10µM	0,000341644	84,9152	54,5598	30,3554	6,58781	4,60782	15
KU_5µM	0,000600489	94,7972	66,3031	28,4941	6,58781	4,32528	15
KU_0µM	0,0107441	100	80,8205	19,1795	6,58781	2,91136	15

Supplementary Table 4

Comparison of the effects of VX-970 alone or in combination with KU-55933 in different cell lines using the GrapPad software

Statistical analysis was performed using 2 way ANOVA followed by Dunnett correction for multiple comparisons.

Multiple t tests were performed and statistical significance determined using the Holm-Sidak method, with alpha=5.000% Only significant results are shown.

Dunnett's multiple comparisons test#				
MM1.S	Mean Diff	95% CI of diff	Significant?	Summary
VX-970_0μM vs.VX-970_ 0.075μM	23,42	16,39 to 30,44	Yes	****
VX-970_0μM vs.VX-970_ 0.15μM	46,74	39,72 to 53,76	Yes	****
VX-970_0μM vs.VX-970_ 0.3μM	62,06	55,04 to 69,08	Yes	****
VX-970_0μM				
KU_0μM vs. KU_5μM	15,86	4,461 to 27,26	Yes	**
KU_0μM vs. KU_10μM	51,33	39,93 to 62,72	Yes	****
VX-970_0.075μM				
KU_0μM vs. KU_5μM	22,57	11,17 to 33,97	Yes	***
KU_0μM vs. KU_10μM	51,46	40,06 to 62,86	Yes	****
VX-970_0.15μM				
KU_0μM vs. KU_5μM	19,34	7,942 to 30,74	Yes	**
KU_0μM vs. KU_10μM	34,7	23,30 to 46,10	Yes	****
VX-970_0.3μM				
KU_0μM vs. KU_5μM	7,786	-3,614 to 19,18	No	ns
KU_0μM vs. KU_10μM	14,88	3,479 to 26,28	Yes	*

Multiple t test##							
MM1.S	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
KU_0μM vs. KU_5μM							
VX-970_0.075μM	0,00109917	78,8644	56,2974	22,567	5,68434	3,97003	16
VX-970_0.15μM	0,00364053	48,8805	29,5392	19,3413	5,68434	3,40255	16
VX-970_0μM	0,0131052	100	84,1399	15,8601	5,68434	2,79014	16
KU_0μM vs. KU_10μM							
VX-970_0.075μM	1,23172E-08	78,8644	27,4017	51,4627	4,86001	10,589	16
VX-970_0μM	1,27893E-08	100	48,6743	51,3257	4,86001	10,5608	16
VX-970_0.15μM	2,3469E-06	48,8805	14,18	34,7005	4,86001	7,14	16
VX-970_0.3μM	0,00746105	23,1006	8,22289	14,8777	4,86001	3,06126	16

Dunnett's multiple comparisons test#				
H929	Mean Diff	95% CI of diff,	Significant?	Summary
VX-970_0μM vs.VX-970_ 0.075μM	11,15	-0,6293 to 22,94	No	ns
VX-970_0μM vs.VX-970_ 0.15μM	24,01	12,23 to 35,79	Yes	****
VX-970_0μM vs.VX-970_ 0.3μM	41,72	29,93 to 53,50	Yes	****
VX-970_0μM				
KU_0μM vs. KU_5μM	5,69	-13,44 to 24,82	No	ns
KU_0μM vs. KU_10μM	8,928	-10,20 to 28,06	No	ns
VX-970_0.075μM				
KU_0μM vs. KU_5μM	23,2	4,069 to 42,32	Yes	*
KU_0μM vs. KU_10μM	34,06	14,93 to 53,18	Yes	***
VX-970_0.15μM				
KU_0μM vs. KU_5μM	30,15	11,02 to 49,27	Yes	**
KU_0μM vs. KU_10μM	44,9	25,78 to 64,03	Yes	****
VX-970_0.3μM				
KU_0μM vs. KU_5μM	30,16	11,03 to 49,29	Yes	**
KU_0μM vs. KU_10μM	37,95	18,82 to 57,07	Yes	***

Multiple t test##							
H929	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
KU_0μM vs. KU_5μM							
VX-970_0.3μM	0,00121997	76,1121	45,9524	30,1597	7,69282	3,92049	16
VX-970_0.15μM	0,00122461	96,1352	65,9894	30,1458	7,69282	3,91869	16
VX-970_0.075μM	0,00821279	103,059	79,8623	23,1963	7,69282	3,01532	16
KU_0μM vs. KU_10μM							
VX-970_0.15μM	1,03771E-05	96,1352	51,231	44,9042	7,11635	6,31	16
VX-970_0.3μM	6,74133E-05	76,1121	38,167	37,9451	7,11635	5,33211	16
VX-970_0.075μM	0,000202062	103,059	69,0006	34,058	7,11635	4,78588	16

Dunnett's multiple comparisons test#				
KMS20	Mean Diff	95% CI of diff,	Significant?	Summary
VX-970_0µM vs.VX-970_ 0.075µM	3,806	-9,151 to 16,76	No	ns
VX-970_0µM vs.VX-970_ 0.15µM	19,38	6,419 to 32,33	Yes	**
VX-970_0µM vs.VX-970_ 0.3µM	46,29	33,33 to 59,24	Yes	****
VX-970_0µM				
KU_0µM vs. KU_5µM	11,63	-9,401 to 32,66	No	ns
KU_0µM vs. KU_10µM	26,66	5,632 to 47,70	Yes	*
VX-970_0.075µM				
KU_0µM vs. KU_5µM	16,56	-4,477 to 37,59	No	ns
KU_0µM vs. KU_10µM	28,07	7,037 to 49,10	Yes	**
VX-970_0.15µM				
KU_0µM vs. KU_5µM	21,03	,0007391 to 42,0	Yes	*
KU_0µM vs. KU_10µM	31,87	10,84 to 52,90	Yes	**
VX-970_0.3µM				
KU_0µM vs. KU_5µM	14	-7,035 to 35,03	No	ns
KU_0µM vs. KU_10µM	19,86	-1,168 to 40,90	No	ns

Multiple t test##							
KMS20	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
KU_0µM vs. KU_10µM							
VX-970_0.15µM	2,75347E-05	85,4936	53,6229	31,8707	5,50277	5,79176	16
VX-970_0.075µM	0,00010681	98,3041	70,2345	28,0696	5,50277	5,10101	16
VX-970_0µM	0,000178888	100	73,3354	26,6646	5,50277	4,84568	16
VX-970_0.3µM	0,00234951	52,2362	32,3717	19,8645	5,50277	3,60991	16

Dunnett's multiple comparisons test#				
RPMI 8226	Mean Diff	95% CI of diff,	Significant?	Summary
VX-970_0µM vs.VX-970_ 0.075µM	29,5	14,54 to 44,46	Yes	***
VX-970_0µM vs.VX-970_ 0.15µM	60,72	45,76 to 75,68	Yes	****
VX-970_0µM vs.VX-970_ 0.3µM	83,3	68,34 to 98,26	Yes	****
VX-970_0µM				
KU_0µM vs. KU_5µM	2,827	-21,46 to 27,12	No	ns
KU_0µM vs. KU_10µM	8,155	-16,13 to 32,44	No	ns
VX-970_0.075µM				
KU_0µM vs. KU_5µM	28,14	3,850 to 52,43	Yes	*
KU_0µM vs. KU_10µM	43,3	19,01 to 67,59	Yes	***
VX-970_0.15µM				
KU_0µM vs. KU_5µM	36,32	12,03 to 60,61	Yes	**
KU_0µM vs. KU_10µM	44,53	20,24 to 68,82	Yes	***
VX-970_0.3µM				
KU_0µM vs. KU_5µM	14,29	-9,998 to 38,58	No	ns
KU_0µM vs. KU_10µM	13,84	-10,45 to 38,13	No	ns

Multiple t test##							
RPMI 8226	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
KU_0µM vs. KU_5µM							
VX-970_0.15µM	0,00368704	62,5704	26,2494	36,321	10,6935	3,39653	16
KU_0µM vs. KU_10µM							
VX-970_0.15µM	0,000467293	62,5704	18,0379	44,5325	10,1698	4,37889	16
VX-970_0.075µM	0,000601258	90,6493	47,3478	43,3015	10,1698	4,25785	16

Dunnett's multiple comparisons test#				
LP1	Mean Diff	95% CI of diff	Significant?	Summary
VX-970_0µM vs.VX-970_ 0.075µM	5,185	-6,953 to 17,32	No	ns
VX-970_0µM vs.VX-970_ 0.15µM	17,99	5,849 to 30,12	Yes	**
VX-970_0µM vs.VX-970_ 0.3µM	33,36	21,22 to 45,50	Yes	****
VX-970_0µM				
KU_0µM vs. KU_5µM	16,56	-3,145 to 36,26	No	ns
KU_0µM vs. KU_10µM	28,77	9,067 to 48,48	Yes	**
VX-970_0.075µM				
KU_0µM vs. KU_5µM	19,87	0,1675 to 39,58	Yes	*
KU_0µM vs. KU_10µM	30,24	10,53 to 49,94	Yes	**
VX-970_0.15µM				
KU_0µM vs. KU_5µM	22,36	2,658 to 42,07	Yes	*
KU_0µM vs. KU_10µM	27,69	7,986 to 47,39	Yes	**
VX-970_0.3µM				
KU_0µM vs. KU_5µM	18,44	-1,267 to 38,14	No	ns
KU_0µM vs. KU_10µM	19,68	-0,02012 to 39,39	No	ns

Multiple t test##							
LP1	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
KU_0µM vs. KU_5µM							
VX-970_0.15µM	0,0117544	83,5873	61,2251	22,3623	7,86623	2,84282	16
KU_0µM vs. KU_10µM							
VX-970_0.075µM	0,00140167	96,4083	66,1703	30,238	7,84466	3,8546	16
VX-970_0µM	0,00207976	100	71,2286	28,7714	7,84466	3,66764	16
VX-970_0.15µM	0,00278246	83,5873	55,8969	27,6905	7,84466	3,52985	16
VX-970_0.3µM	0,0232386	64,2354	44,5514	19,684	7,84466	2,50923	16

Dunnett's multiple comparisons test#				
OPM2	Mean Diff	95% CI of diff	Significant?	Summary
VX-970_0µM vs.VX-970_ 0.075µM	6,997	-12,50 to 26,49	No	ns
VX-970_0µM vs.VX-970_ 0.15µM	23,89	4,394 to 43,38	Yes	*
VX-970_0µM vs.VX-970_ 0.3µM	57,69	38,20 to 77,18	Yes	****
VX-970_0µM				
KU_0µM vs. KU_5µM	1,79	-29,85 to 33,43	No	ns
KU_0µM vs. KU_10µM	6,581	-25,06 to 38,22	No	ns
VX-970_0.075µM				
KU_0µM vs. KU_5µM	11,28	-20,36 to 42,92	No	ns
KU_0µM vs. KU_10µM	20,21	-11,43 to 51,85	No	ns
VX-970_0.15µM				
KU_0µM vs. KU_5µM	20,07	-11,57 to 51,72	No	ns
KU_0µM vs. KU_10µM	26,99	-4,650 to 58,64	No	ns
VX-970_0.3µM				
KU_0µM vs. KU_5µM	23,75	-7,896 to 55,39	No	ns
KU_0µM vs. KU_10µM	25	-6,638 to 56,65	No	ns

Multiple t test##
OPM2
not significant for all the comparisons

Dunnett's multiple comparisons test#				
U266	Mean Diff	95% CI of diff	Significant?	Summary
VX-970_0µM vs.VX-970_ 0.075µM	8,569	1,275 to 15,86	Yes	*
VX-970_0µM vs.VX-970_ 0.15µM	23,16	15,86 to 30,45	Yes	****
VX-970_0µM vs.VX-970_ 0.3µM	45,51	38,22 to 52,80	Yes	****
VX-970_0µM				
KU_0µM vs. KU_5µM	3,233	-8,607 to 15,07	No	ns
KU_0µM vs. KU_10µM	10,9	-0,9412 to 22,74	No	ns
VX-970_0.075µM				
KU_0µM vs. KU_5µM	7,773	-4,068 to 19,61	No	ns
KU_0µM vs. KU_10µM	16,53	4,688 to 28,37	Yes	**
VX-970_0.15µM				
KU_0µM vs. KU_5µM	16,41	4,566 to 28,25	Yes	**
KU_0µM vs. KU_10µM	25,98	14,14 to 37,82	Yes	****
VX-970_0.3µM				
KU_0µM vs. KU_5µM	14,66	2,816 to 26,50	Yes	*
KU_0µM vs. KU_10µM	16,05	4,214 to 27,89	Yes	**

Multiple t test##							
U266	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df
KU_0µM vs. KU_5µM							
VX-970_0.15µM	0,00129254	86,2635	69,857	16,4066	4,21432	3,89306	16
VX-970_0.3µM	0,00310658	60,0163	45,3602	14,6561	4,21432	3,47768	16
KU_0µM vs. KU_10µM							
VX-970_0.15µM	0,000224758	86,2635	60,2818	25,9818	5,4886	4,73378	16
VX-970_0.075µM	0,00827946	94,8205	78,2919	16,5286	5,4886	3,01144	16
VX-970_0.3µM	0,00991086	60,0163	43,9616	16,0546	5,4886	2,92509	16