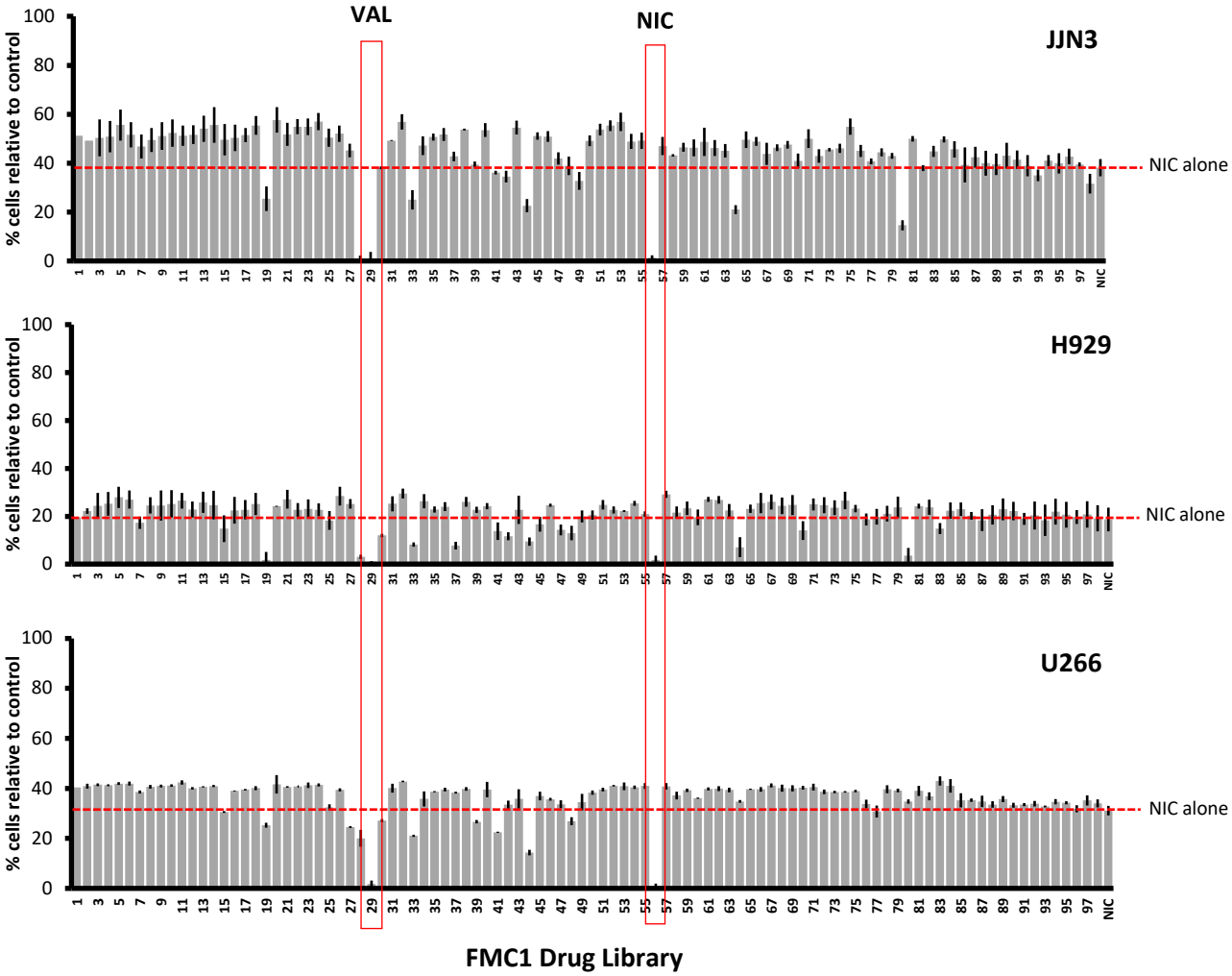
Supplementary Figures

Jiang et al, VaN in Myeloma

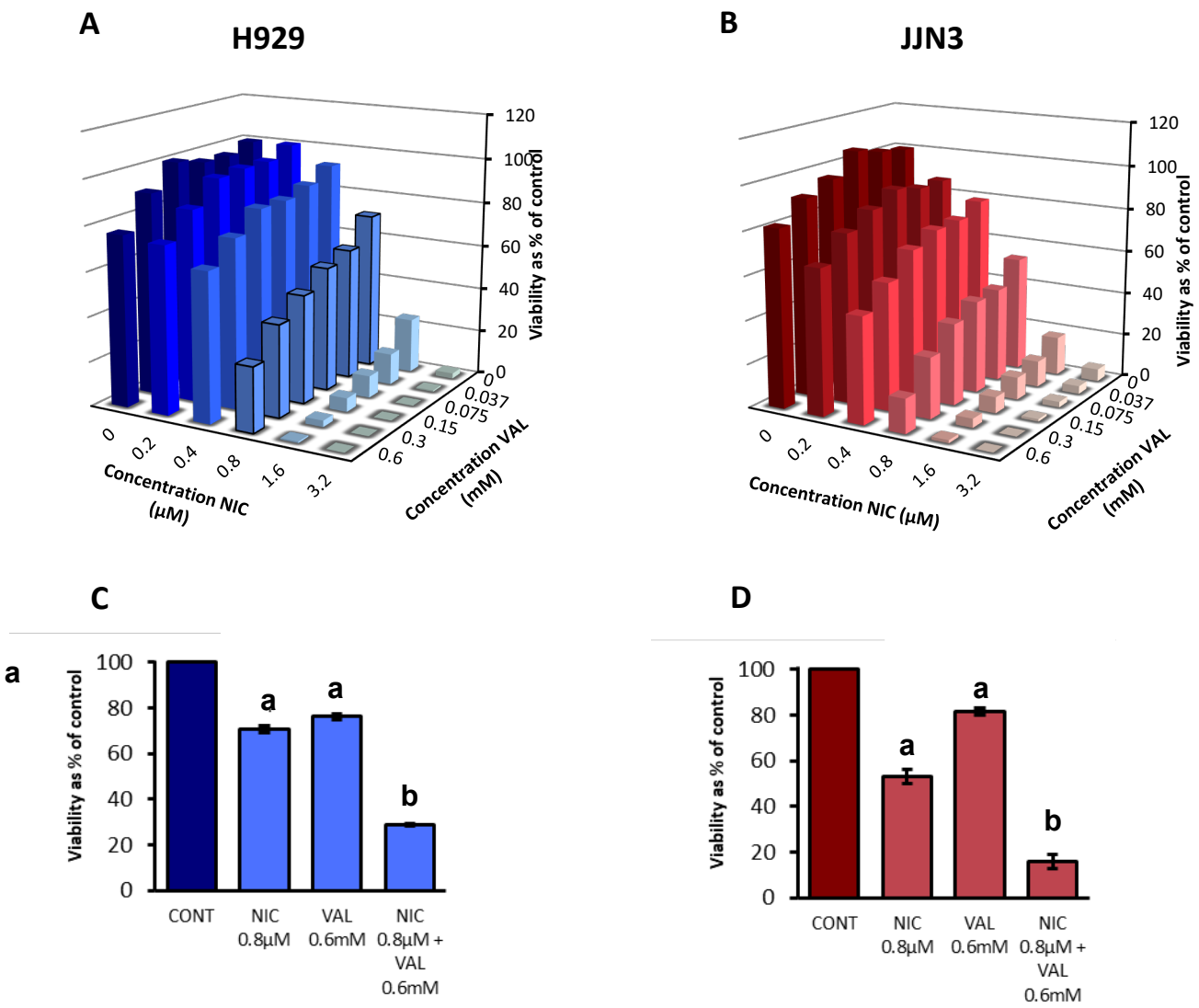
Supplementary Figure S1



Supplementary Figure S1. Screening the FMC drug repurposing library for adjuncts for NIC identifies Valproate.

$10x10^3$ JJN3 and H929 cells and $20x10^3$ U262 cells were incubated with our in-house FMC custom drug repurposing library in the presence of 1μM niclosamide (NIC) for 72 hours and cell viability determined as % of viable cells compared to untreated control wells using Cell Titer blue assay. Results shown are mean± SEM of N=3 for JJN3 and N=4 for H929 and U262 repeats. The dotted line highlights the effects of 1μM NIC alone on each cell line. Red boxes highlight NIC and valproate (VAL) in the library.

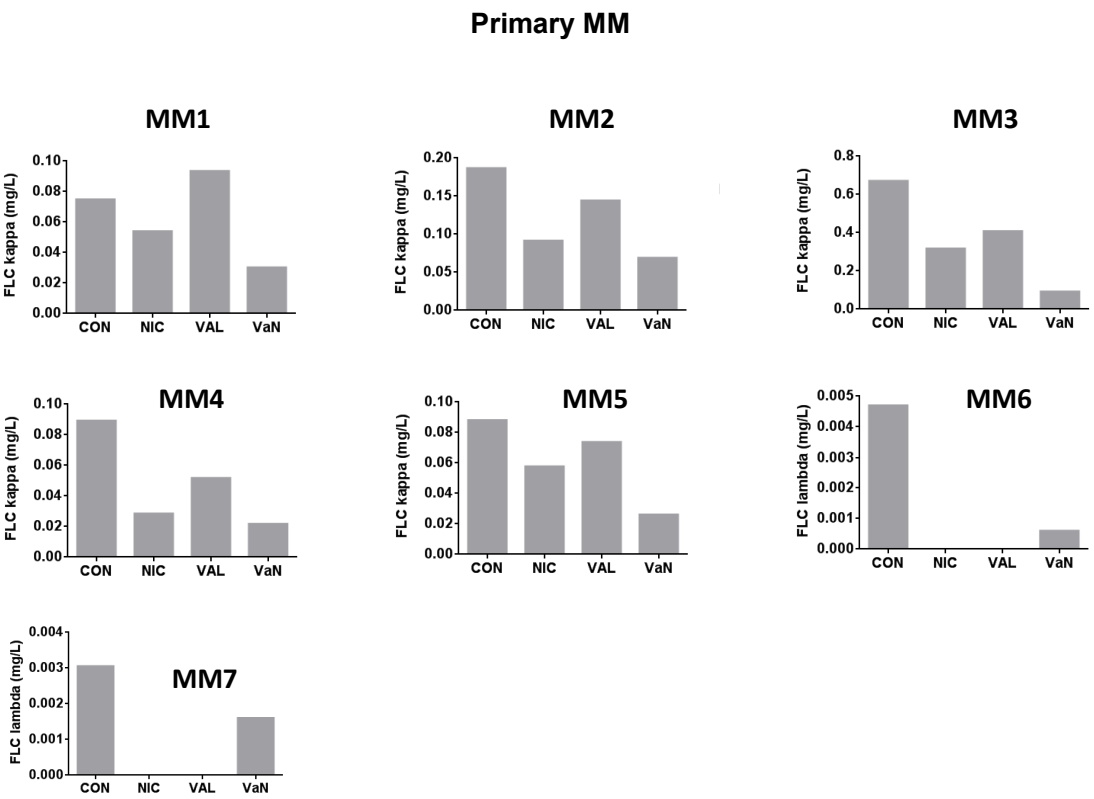
Supplementary Figure S2



Supplementary Figure S2. Combination activity of niclosamide and valproate against H929 and JJN3.

Cells were incubated with serum doubling dilutions of niclosamide (NIC) and valproate (VAL) up to peak serum concentrations for 72 hours and cell viability determined as % of viable cells compared to untreated control wells using Cell Titer blue assay. Results shown are mean values of experiments (A) H929 n=4, (B) JJN3 n=3 ±SEM. (C) and (D) shows viability data for 0.8mM NIC and o.6mM VAL. $p<0.05$ using one-way ANOVA, significance is denoted by a or b. Same letter indicates no difference between treatments and different letters denote significance between treatments.

Supplementary Figure S3



Supplementary Figure 4. VaN reduces FLC secretion from primary MM cells

Primary bone marrow aspirates from MM patients were washed and treated with CON, NIC (1uM), VAL (0.6mM) or the combination VaN for 18 hours, cell numbers allowing. Where samples were small, CON and VaN treatments were set up. Supernatant were collected after 18 hours. ELISA plates (Corning, USA) were coated with capture antibody (anti-lambda or anti-kappa light chain monoclonal antibody) overnight at 4°C. After blocking with 2% BSA for 1 hour, cell supernatants were added and incubated for 1 hour at room temperature (RT). Plates were washed and incubated with the detection antibody (HRP-labelled anti-kappa or anti-lambda polyclonal antibody) for 1 hour at RT and developed with TMB substrate. The reaction was stopped by addition of 15% H_2SO_4 and absorbance read at 450nm.

Supplementary Figure S4

qRT-PCR primer sequences

Igκ

Forward primer: 5'-CACACTGGCCTCCGATCAC-3'

Reverse primer: 5'-TGCAGCCACAGTTCGTTTAATC-3'

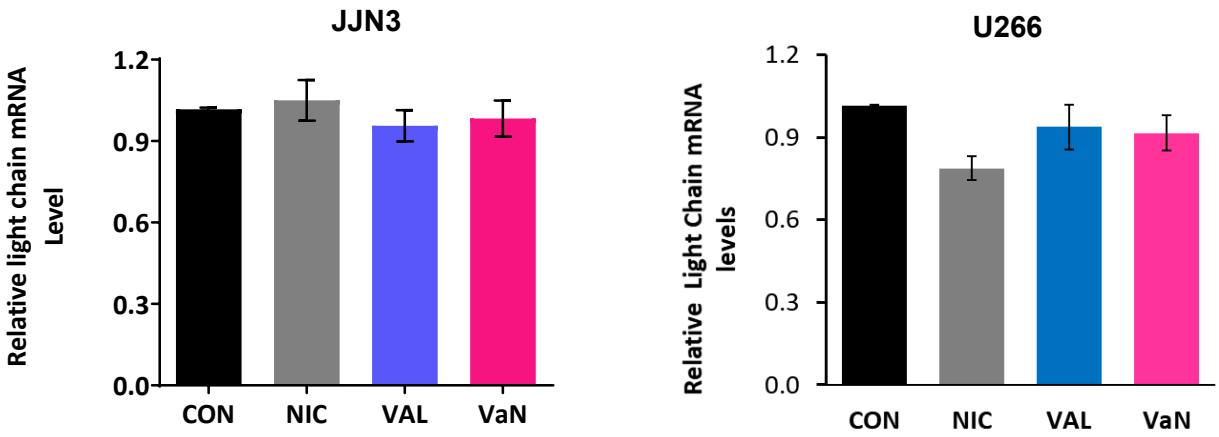
Probe: 6-FAM-TTCGGCCAAGGGACACGACTG-TAMRA

Igλ

Forward primer: 5'-CCACCAAACCCTCCAAACAG-3'

Reverse primer: 5'-GGGCGTCAGGCTCAGGTA-3'

Probe: 6-FAM-CAACAACAAGTACGCGGCCAGCAG-TAMRA.

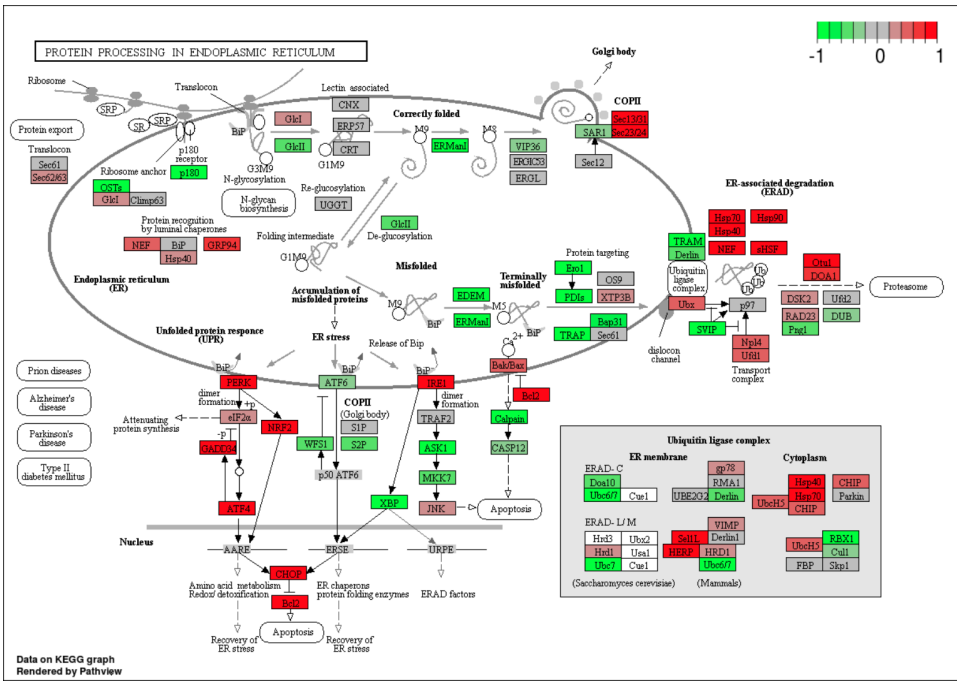


Supplementary Figure 5. VaN does not modulate Ig lambda or kappa mRNA levels

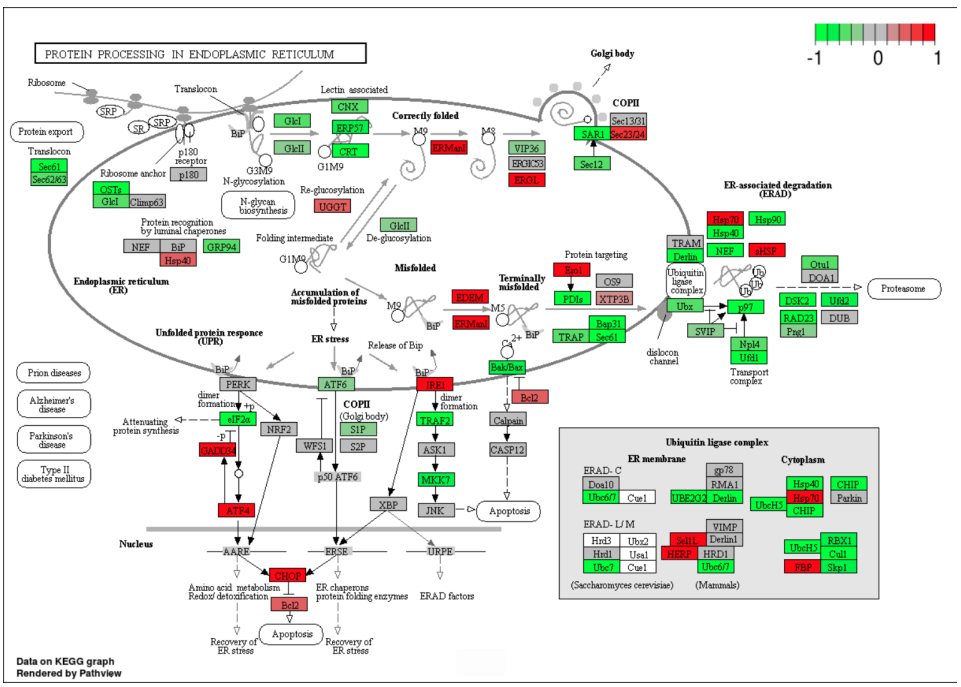
JJN3 and U266 cells were treated with CONT, NIC, VAL and VaN for 24hours. Total RNA was extracted, cDNA synthesised before QRT-PCR performed using gene-specific primers (Sigma, UK) and probes (Eurogentec Ltd, UK) for Igκ and Igl light chains **(A)** in a 20µl reactions contained 1 × Mastermix buffer (Bioline, UK), 900nM each of forward and reverse primers, 125nM gene-specific probe, 50nM internal standard control 18S ribosome primers, 200nM 18S probe (5'-VIC and 3'-TAMRA labelled; Applied Biosystems, UK) and 20ng cDNA. Reactions were performed using an Agilent AriaMAx Real-Time PCR System (Agilent Technologies, UK) cycling for 44 cycles with denaturation at 95 °C and annealing and elongation at 60 °C for 1 min. Light chain mRNA levels were calculated relative to CONT. Data shown is for N=3/4 experiments, each performed in triplicate, ±SEM.

Supplementary Figure S5

H929



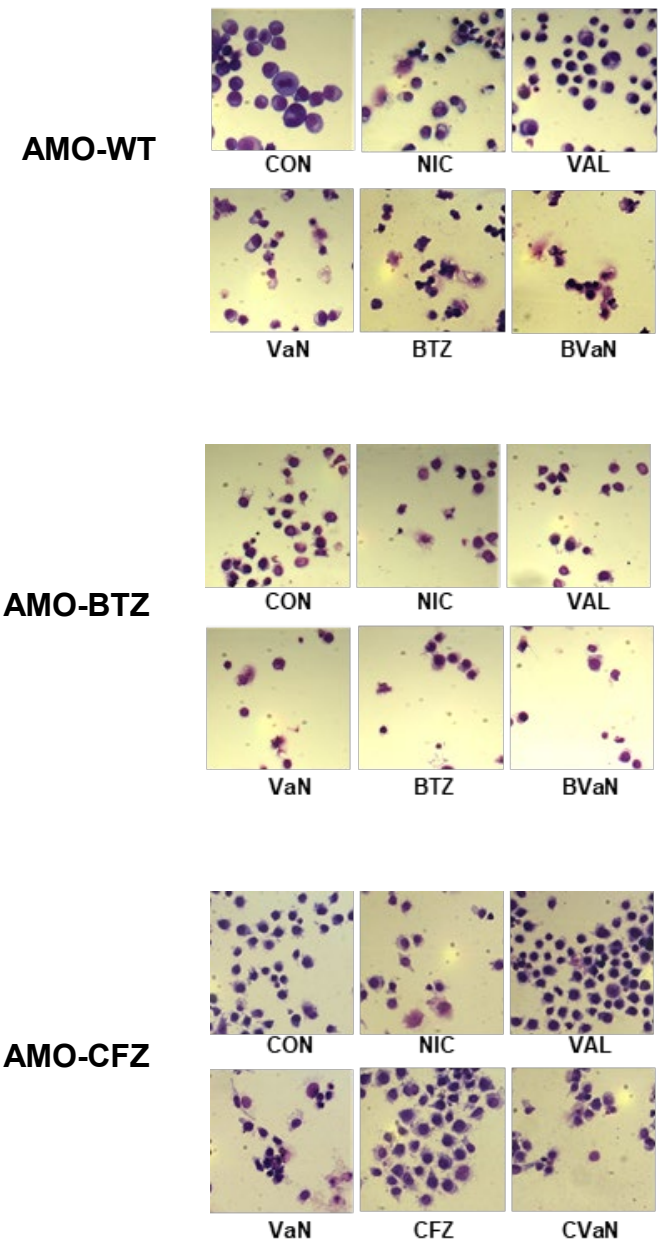
JJN3



Supplementary Figure 14. The changes in RNAseq data on unfolded protein response pathways.

Total RNASeq were performed on JJN3 and H929 cells after treated for 16 hours. KEGG pathway analysis of the RNASeq data further highlighted activation of the UPR with upregulation of key UPR mediators in both H929 and JJN3 cells. Genes highlighted in green were downregulated by VaN, and genes highlighted in red were upregulated by VaN.

Supplementary Figure S6



Supplementary Figure 15. Jenner-Giemsa image of AMO and derived PI-resistant cells.

AMO wild type cells (AMO-WT) and its bortezomib resistant(AMO-BTZ) and carfilzomib resistant(AMO-CFZ) cell lines were treated with vehicle control, niclosamide 1uM (NIC), valproate 0.6mM (VAL), the combination of nicalosamide and valproate(VaN), bortezomib 10nM(BTZ)/carfilzomib 10nM(CFZ) or the combination with proteasome inhibitor (BVaN/CVaN) for 24 hours. Representative images are shown of Jenner/Giemsa-stained cyto-spin slides after treatments.