

Materials and methods

1 Cell lines and cell culture

The human multiple myeloma cell lines ARP-1, CAG, U266, AMO1, LP-1, and OPM2 were kindly donated by Dr. Qing Yi (Center of Hematologic Malignancy Research Institute, Houston Methodist, Houston, USA) and were cultured in RPMI-1640 medium containing 1% L-glutamine (Corning Cellgro, Corning, NY, USA) and 10% fetal bovine serum (Corning Cellgro) at 37 °C incubator with 5% CO₂.

All primary myeloma cells from MM patients were isolated using CD138 beads (Stemcell Technologies, Canada), and were cultured in RPMI-1640 medium containing 1% L-glutamine (Corning Cellgro) and 20% fetal bovine serum (Corning Cellgro) at 37 °C incubator with 5% CO₂, then treated with the indicated concentrations of CUDC-101.

2 Western blot analysis

In order to detect the effects of the drugs on protein levels, MM cells (1×10^6 /group) were seeded in 10 cm Petri dishes and treated with the indicated concentrations of CUDC-101 or Bortezomib for 24 h. Then, cells were collected and washed with PBS twice and lysed with RIPA containing 1× protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific, Waltham, MA, USA). After incubating for 30 min on ice, the lysate was centrifuged at 12000g for 10 min at 4 °C and the supernatants were collected. Next, a BCA Protein Assay Kit (Pierce, Rockford, USA) was used to detect the concentration of the extracted proteins. Total proteins were added with 4× sodium dodecyl sulfate loading buffer (Invitrogen) and boiled at 95 °C for 20 min, then equal amounts of protein (20–60 µg) was separated by 4%–12% or 4%–20% SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Merck Millipore, Darmstadt, Germany). Subsequently, the membranes were blocked with 5% non-fat milk (BD) or 5% bovine serum albumin for 1–2 h at room temperature, then incubated with specific primary antibodies overnight at 4 °C. The next day, the membranes were washed three times with Tris-buffered saline containing Tween 20 (TBST) and incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit or anti-mouse antibodies at room temperature for 1 h, then the membranes were washed three times with TBST. Finally, the protein bands were imaged using a ChemiDocTMMP Imaging System (Bio-Rad) and an Enhanced Chemiluminescence Detection kit (Biological Industries, Kibbutz Beit Haemek, Israel).

3 Reagents and antibodies

CUDC-101 and Bortezomib were obtained from Selleck Chemicals LLC (Houston, USA). Primary antibodies against GAPDH, PARP, Bcl-xl, BAX, and PI3K were purchased from Abcam (Cambridge, UK). Primary antibodies against caspase-3, caspase-9, EGFR, pEGFR, AKT, pAKT, ERK, pERK, mTOR, pmTOR, HDAC3, HDAC4, HDAC7, cyclinB1, pCDC2, CDC2, P21, and P27 were purchased from Cell Signaling Technology (MA, USA).

4 Cell proliferation assay

Cell counting kit-8 assays (Dojindo, Kuamoto, Japan) were used to assess the proliferation of MM cells. MM cells (1×10^4 /well) were seeded in a 96-well plate and cultured in a 37 °C, 5% CO₂ incubator, treated with different concentrations of drugs for 24 h or 48 h, added with 10 µL CCK-8 reagent to the 96-well plate, and incubated for 2 h. Then, the absorbance was read at 450 nm with a microplate reader (Bio-Rad, Model 680, California, USA).

5 Apoptosis

Annexin V-FITC and PI (Dojindo) was used to stain MM cells according to the manufacturer's instructions to analysis of apoptosis. CD138Ab (Biolegend, CA, USA) staining was used to extract

primary MM cells for analysis. All experiments were detected using a flow cytometer (BD Biosciences, San Diego, USA) and analyzed by FlowJo 10.4.

6 Immunohistochemistry

Tumor samples from NOD-SCID mice, which had been 4% paraformaldehyde-fixed and paraffin-embedded, were used for immunohistochemistry staining to analyze cleaved PARP and cleaved caspase-3.

7 TdT-mediated dUTP Nick-End Labeling

2×10^6 cells were seeded in a 10 cm Petri dish and treated with different concentrations of CUDC-101 for 24 h, then collected and washed with PBS twice for analysis with the TUNEL assay. TdT-mediated dUTP Nick-End Labeling (TUNEL assay, Vazyme, China) was employed to stain MM cells according to the manufacturer's instructions to quantify the apoptosis of cells, as detected by flow cytometer and analysis using FlowJo 10.4.

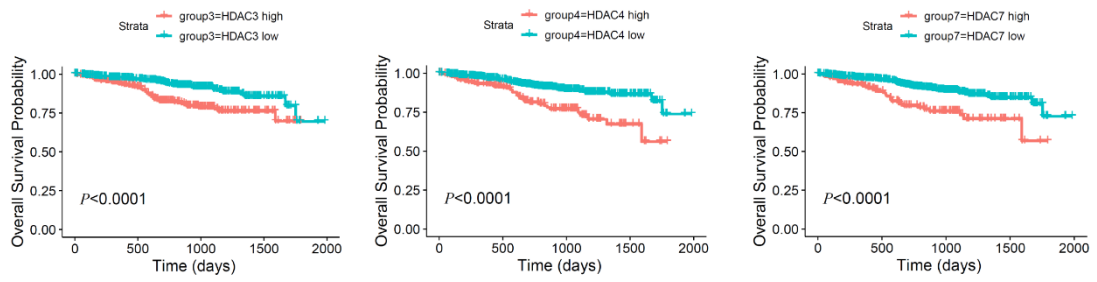
8 MM xenograft model

Four-week-old male NOD-SCID mice were purchased from the Model Animal Research Center of Nanjing University (Nanjing, China) and housed in the animal facility of Zhejiang University School of Medicine. After three days of acclimatization, ARP-1 (2×10^6 cells /mice) were injected subcutaneously into the right flanks of the mice. The mouse tumors were measured every two days. When the tumor volume reached approximately 100–130 mm³, the mice were randomly divided into groups and administered intraperitoneal injections of CUDC-101 (30 mg/kg daily) , and saline was used as control. The tumor's length and width were measured with a caliper before drug treatment, and the tumor volume was calculated as $1/2 \times L \times W^2$, where L is the tumor length and W is the tumor width. All experiments followed the procedures and protocols of the Animal Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine.

9 Statistical analysis

In this study, all data were obtained from at least three independent experiments and analyzed by GraphPad Prism 7.00 (GraphPad Software, CA, USA). The significant differences between the experimental groups were determined by two-tailed Student's t-tests. All *P* values less than 0.05 were considered as statistically significant.

a



b

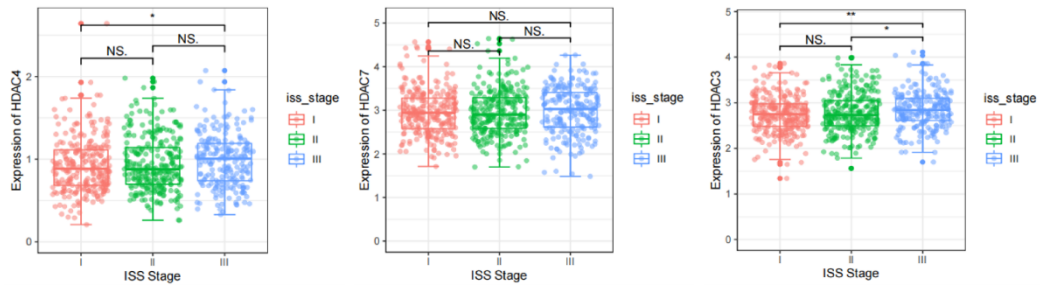


Fig. S1 Clinical significance of HDAC3, HDAC4 and HDAC7 in MM. (A) The overall survival analysis of patients with multiple myeloma in high-level and low-level groups of HDAC3, HDAC4, HDAC7 from the MMRF CoMMpass database. (B) HDAC3, HDAC4 and HDAC7 expression in plasma cells purified from different ISS stage from the MMRF CoMMpass database. NS means Non Significance, * $P < 0.05$, ** $P < 0.01$, * $P < 0.001$, **** $P < 0.0001$.**

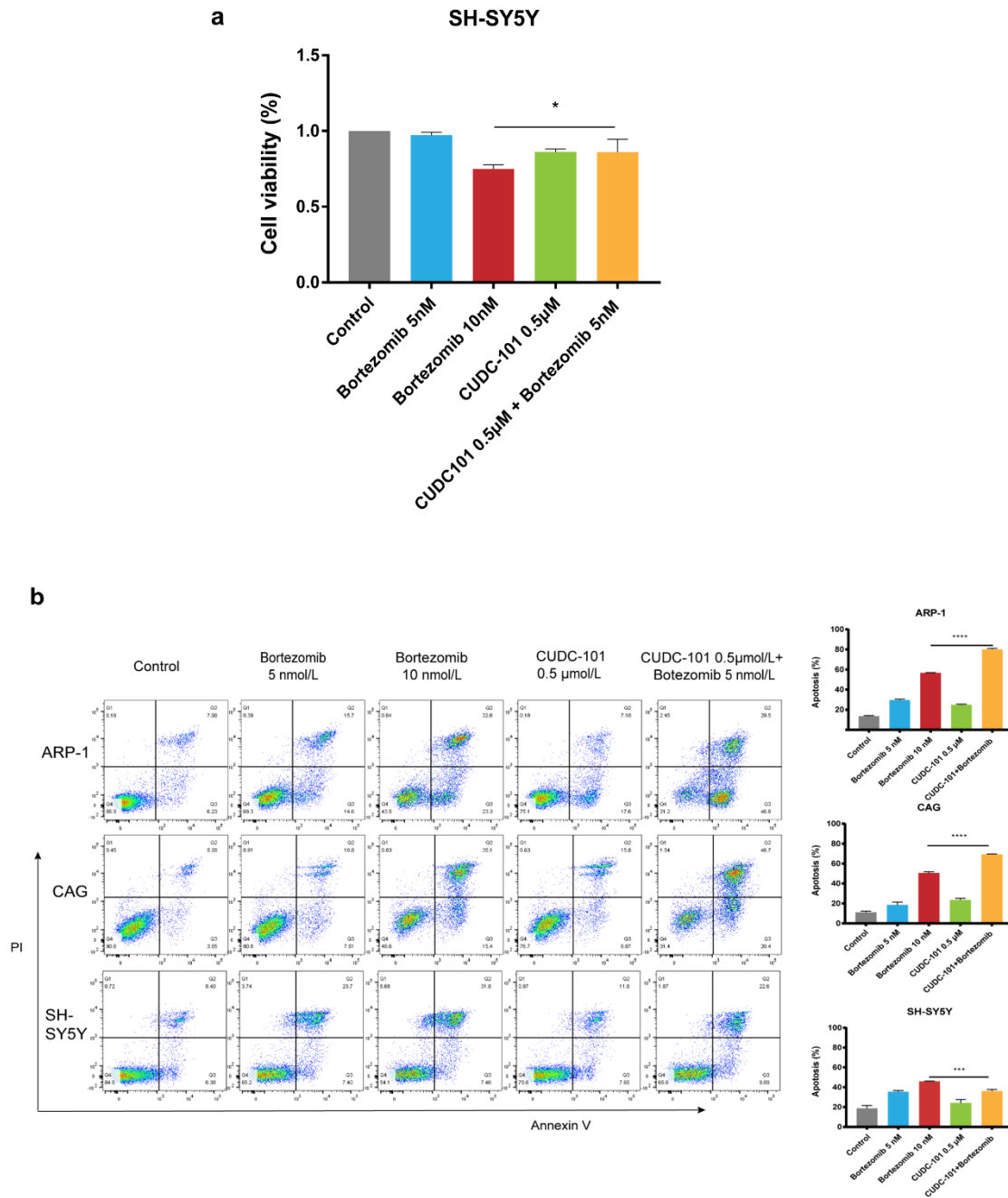


Fig. S2 CUDC-101 and Bortezomib has synergistical inhibit effect in MM cells rather than human neuroblastoma SH-SY5Y. (A) Cell proliferation assay detecting the inhibition effect of CUDC-101 and Bortezomib on human neuroblastoma SH-SY5Y after exposed 24 h. (B) ARP-1, CAG and SH-SY5Y cells were incubated with vehicle or CUDC-101 and Bortezomib for 24 h and then flow cytometry was performed to analyze the apoptosis using flow cytometer. All data are represented as mean±SD of at least three independent experiments. Student's t test A-B, * $P<0.05$, ** $P<0.01$, * $P<0.001$, **** $P<0.0001$.**