The human immunodeficiency virus-1 protease inhibitor nelfinavir impairs proteasome activity and inhibits the proliferation of multiple myeloma cells *in vitro* and *in vivo*

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Online Supplementary Design and Methods

Flow cytometry

Apoptosis was analyzed by flow cytometry (Becton Dickinson FACS Calibur / BD Bioscience) using recombinant human (rh) annexin V-fluorescein isothiocyanate (FITC) staining (AbCys®, Paris, France). CellQuest Pro software (BD Bioscience) was applied for flow cytometry analyses. Data are presented as dot plots of at least 50 000 counted events per sample. Cells (1×10^6) were incubated with nelfinavir (for 17 h) without fetal calf serum. The cells were then washed twice and dissolved in 1x binding buffer for annexin V-FITC before being incubated for 10 min at room temperature with rh-annexin V-FITC. Propidium iodide was added just before the FACS analysis.

For flow cytometry analysis of AKT phosphorylation, 10^6 cells were serum-starved for 4 h, incubated with insulin-like growth factor (IGF-1) (100 ng/mL) for 30 min then cultured for 2 h with nelfinavir (5 μM). For analysis of STAT-3 and ERK1/2 phosphorylation, 10^6 cells were serum-starved for 4 h, incubated with nelfinavir (5 μM and 20 μM) for 2 h before interleukin-6 stimulation (100 ng/mL) for 30 min. Cells were fixed with PFA-4% for 10 min at room temperature and permeabilized for 30 min in methanol 50% at 4°C. Cells were then washed in phosphate-buffered saline (PBS) and incubated with specific antibodies for 1 h at room temperature. Antibodies recognizing P-AKT, P-STAT3 and P-ERK1/2 were purchased from Cell Signaling and BD Bioscience, respectively.

Western blot and antibodies

Cells were lysed with RIPA buffer, samples (30 μg of protein) were boiled, sheared and clarified by centrifugation, before being separated on an 8-10% sodium dodecylsulfate-polyacrylamide gel and blotted onto nitrocellulose membranes. Membranes were blocked in PBS containing 0.1% Tween 20 and 5% bovine serum albumin before adding the primary antibody. Antibody binding was detected using the ECL chemiluminescence kit (Amerham, Arlington Heights, IL), and digitized using an Arcus II scanner. The antibodies for CHOP, ubiquitin, AKT, P-AKT(ser473) and caspase-3 were from Cell Signaling, those for ATF4 (H-290) and actin were from Santa Cruz. The rabbit polyclonal antibody for P-PERK was from Biolegend. The horseradish peroxidase anti-mouse, goat and rabbit antibodies were from Santa Cruz.

Generation of U226-luc cells

Infectious non-replicative retroviral vector supernatants were produced in 293T cells by co-transfection of three plasmids using the liposomal method according to the manufacturer's instructions (Effectene® Transfection Reagent – Qiagen). 293T cells were seeded in a 10 cm tissue culture dish 24 h prior to transfection in Dulbecco's modified Eagle's medium to obtain semi-confluent cells the day of transfection. For the production of retroviral particles, co-transfection of the pMSCV/Luc plasmid driving the luciferase gene and neomycin resistance (G418 at 1 mg/mL) was achieved with the pMD.G envelope plasmid containing the VSV-g envelope protein and a plasmid expressing gag and pol genes. The U266 cells were incubated for 48 h with the supernatant of the retroviral producer with FCS (10%) and polybrene (2 μg/mL). The U266-luc cells were then selected on the basis of their neomycin resistance.

### Human multiple myeloma xenograft model, imaging process

For imaging, mice were injected intraperitoneally with 150 μg/g luciferin (Xenogen) and then anesthetized with isoflurane (Baxter). Ten minutes after the injection of luciferin, the mice were imaged using a charge-coupled device camera in the IVIS imaging system to evaluate the bioluminescence of the cancer cells in the animal. A gray-scale image of the mice was captured followed by an overlay of a bioluminescence map representing the spatial distribution of photons detected from the cleaved luciferin in the cancer cells expressing luciferase. The signal intensity was quantified using a version of IGOR Pro version 4.06A Software (WaveMetrics, Inc., Lake Oswego, OR, USA) called Living Image Version 2.50.2 (Xenogen).
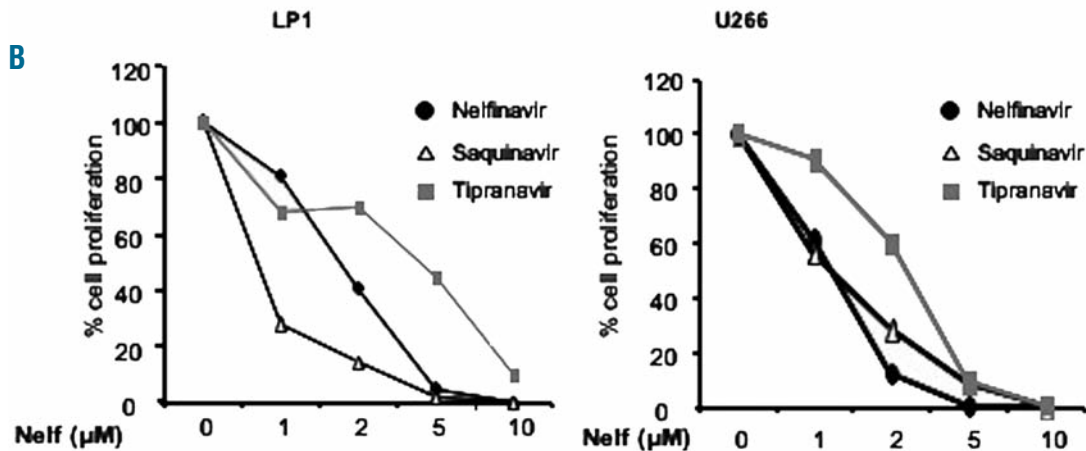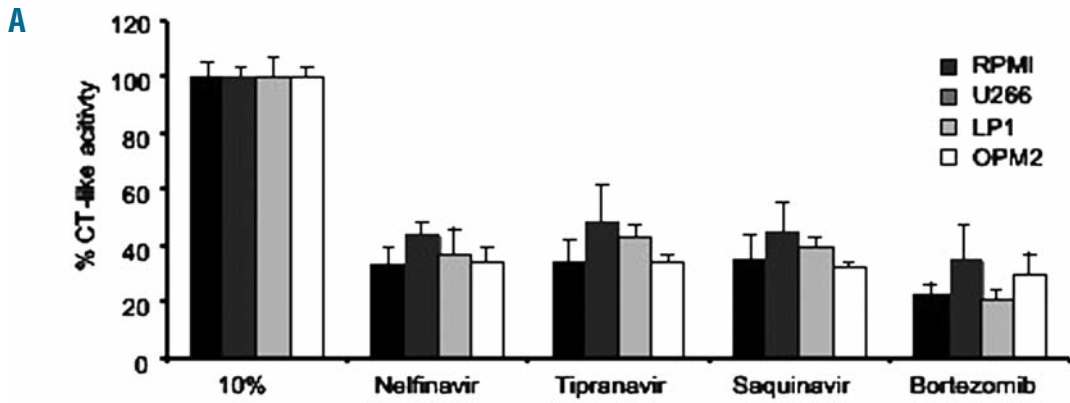
Online Supplementary Figure S1. (A) Bar graphs show the effects of HIV protease inhibitors nelfinavir, tipranavir and saquinavir on the CT-like activity of RPMI, U266, LP1 and OPM2 cells. The inhibitory activity of bortezomib is also indicated. (B). Proliferation of LP1 (Left) and U266 (Right) cells treated with increasing concentrations of the HIV protease inhibitors nelfinavir, saquinavir and tipranavir.
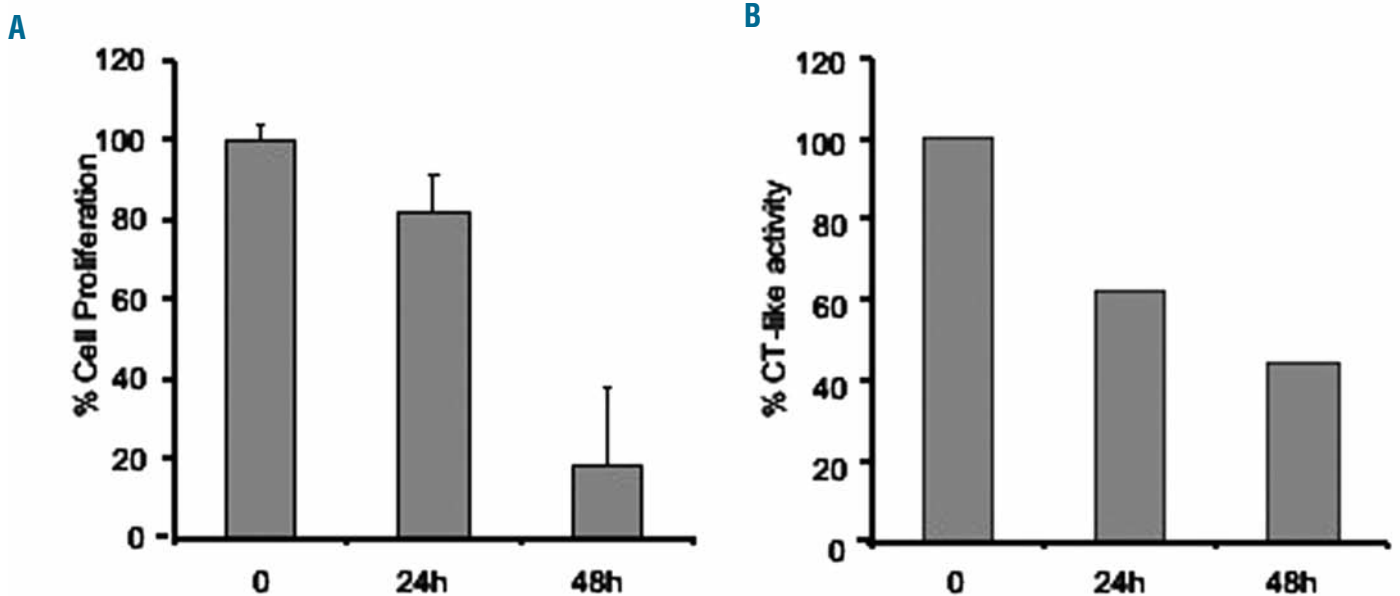
Online Supplementary Figure S2. (A) Histograms showing the kinetics of bortezomib (10 nM) inhibition of U266 cell proliferation. (B). The corresponding kinetics of CT-like activity in bortezomib-treated U266 cells.
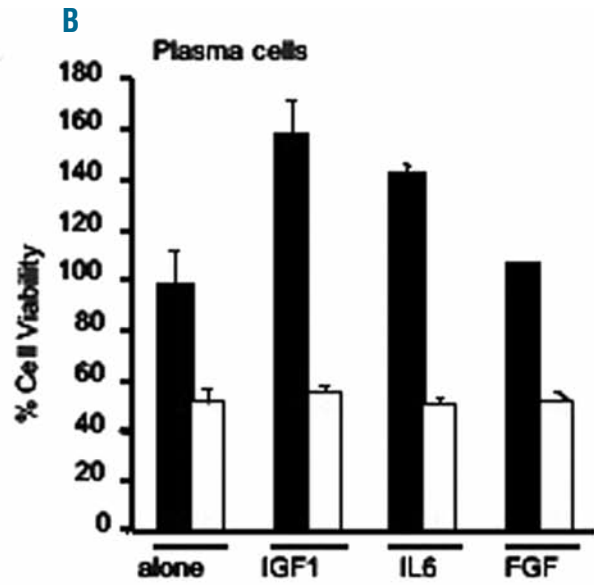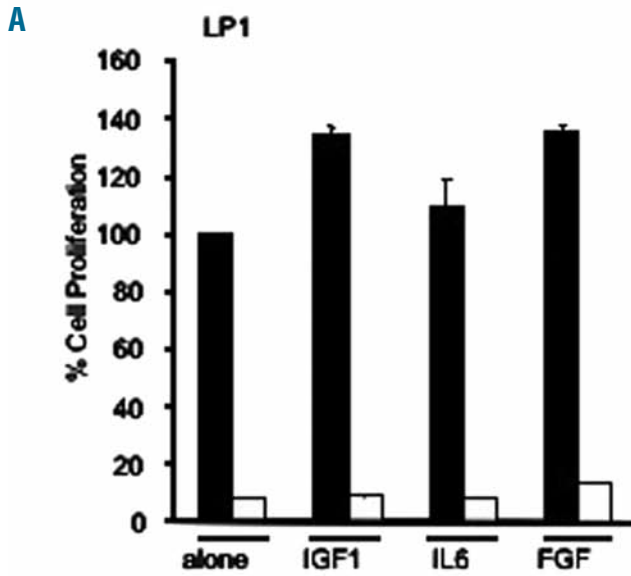
Online Supplementary Figure S3. (A) Histograms showing the proliferation of LP1 cells treated (filled) or not (open) with 5 μM nelfinavir in the presence of IGF-1 (100 ng/mL), IL-6 (100 ng/mL) or FGF (100 ng/mL) + heparin (100 μg/mL). (B). Histograms showing the viability of plasma cells from a patient treated (filled) or not (open) with 10 μM of nelfinavir in the presence of IGF-1 (100 ng/mL), IL-6 (100 ng/mL) or FGF (100 ng/mL) + heparin (100 μg/mL). Error bars correspond to the standard deviation.