Supplemental Data

Human exportin-1 is a target for combined therapy of HIV and AIDS related lymphoma

Eline Boons[1$], Els Vanstreels[1$], Maarten Jacquemyn[1$], Tatiane C Nogueira[1], Jasper E Neggers[1], Thomas Vercruysse[1], Joost van den Oord[2], Sharon Tamir[3], Sharon Shacham[3], Yosef Landesman[3], Robert Snoeck[1], Christophe Pannecouque[1], Graciela Andrei[1] and Dirk Daelemans[1,*]

[1]KU Leuven, Department of Microbiology and Immunology, Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, B-3000 Leuven, Belgium
[2]KU Leuven, Department of Imaging and Pathology, Translational Cell & Tissue Research, B-3000 Leuven, Belgium
[3]Karyopharm Therapeutics, Newton 02459 MA, USA

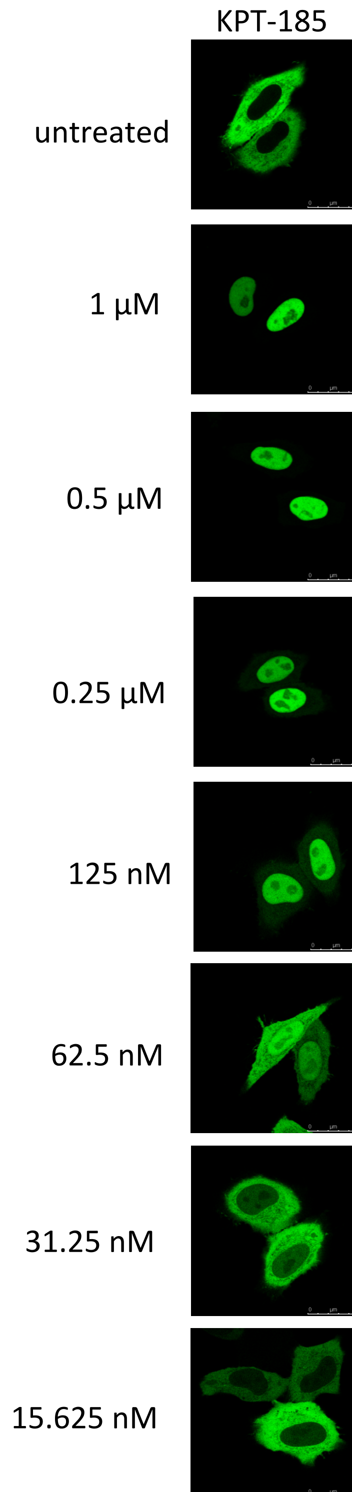

Figure S1. Dose-dependent inhibition of the RevM5-GFP transport to the cytoplasm by KPT-185. HeLa cells were transfected with RevM5-GFP and incubated with different concentrations of compound and analyzed by confocal microscopy after 3 hours of treatment.

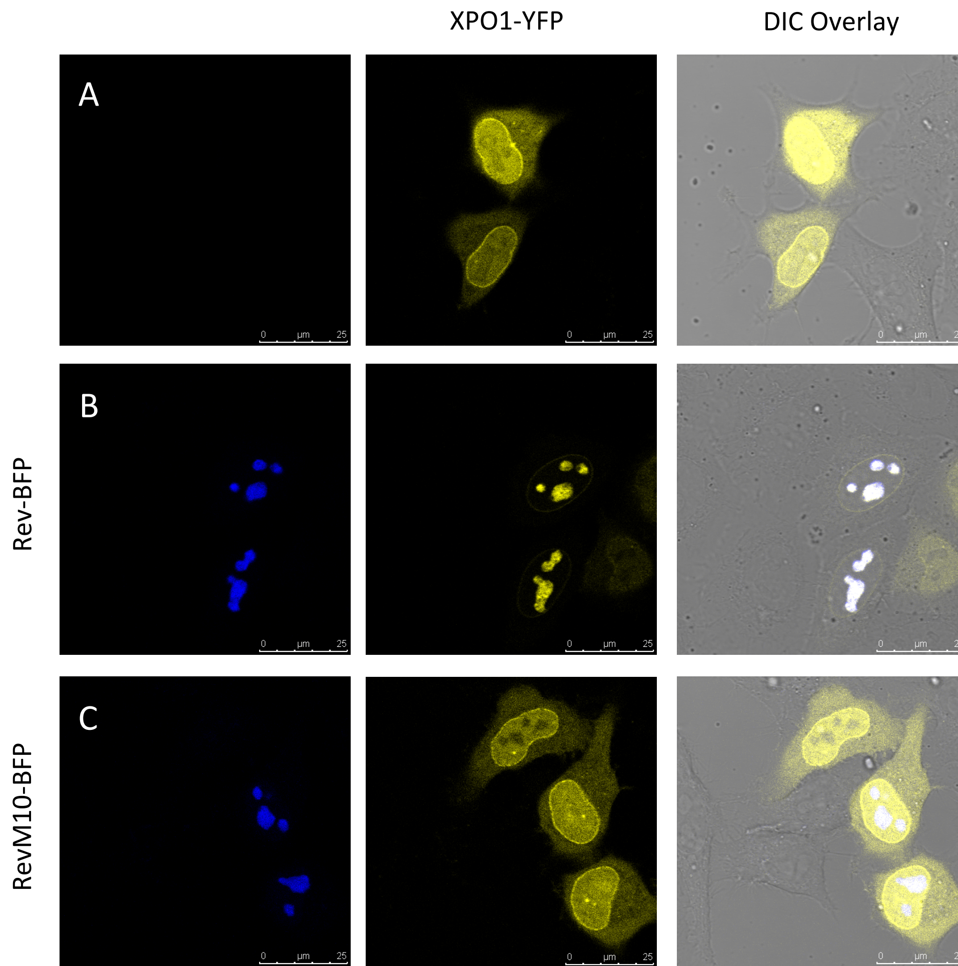

Figure S2. XPO1-YFP co-localizes only weakly with RevM10-BFP as compared to with wild-type Rev-BFP. XPO1-YFP localizes mainly to the nuclear membrane and is excluded from the nucleoli (A). When co-expressed with wild-type Rev-BFP, XPO1-YFP predominantly co-localizes with Rev in the nucleoli of the cells (B), while only very little XPO1-YFP can be found co-localized with RevM10-BFP in the nucleoli (C). RevM10 is a transdominant negative mutant of Rev containing the $L_{78}E_{79}$ to DL mutation in its NES (Malim *et al*. *Cell* 58(1):205-14 (1989)).

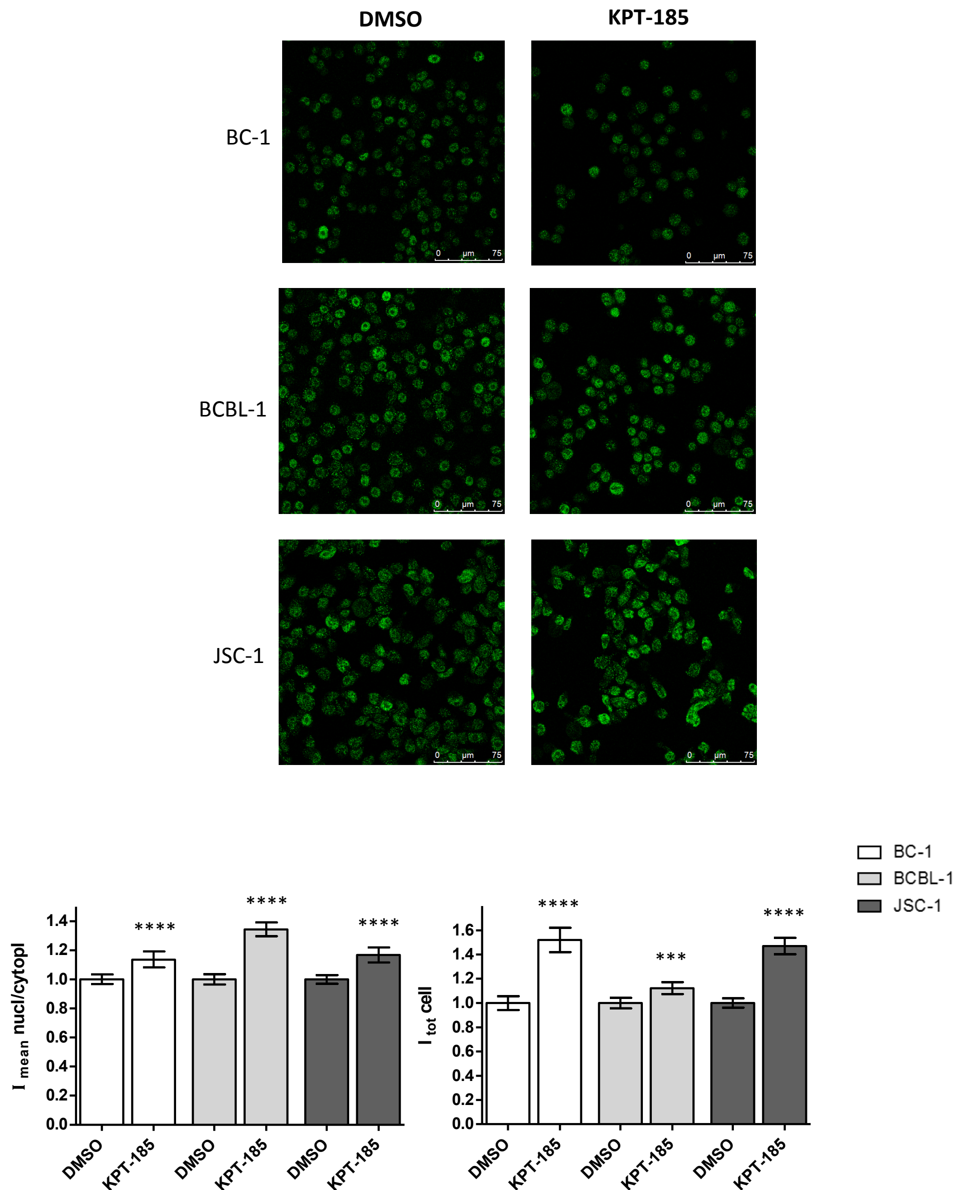

Figure S3. Immunostaining of p73 in PEL cells. Cells were treated with carrier (DMSO) or 1 µM KPT-185 for 16h, fixed, permeabilized and immunostained for p73. The fluorescence in the green channel was quantitated on a per cell basis employing CellProfiler image analysis software, nuclei were segmented based on the DAPI staining and the cytoplasm was defined by expanding the nuclei by a distance of N=8 pixels. A total of 200 to 600 cells were analyzed for each condition. For each cell the ratio of the mean pixel intensities in nucleus vs cytoplasm was calculated as well as the total intensity. Values were normalized relative to the DMSO control to be able to compare different experiments. Results were analyzed using a two-sided unpaired t-test with Welch's correction (**** $p<0.0001$ and ***$p<0.001$) and are depicted as mean +/- 95% CI.

In all 3 cell lines the nuclear as well as the total p73 intensities increased upon treatment with KPT-185.

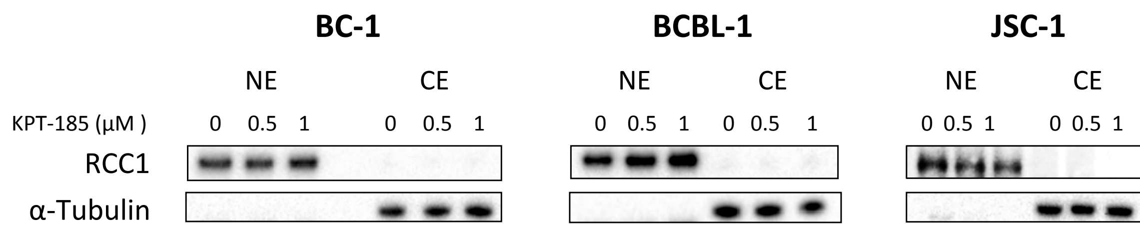

Figure S4. Control for nuclear/cytoplasmic separation. Nuclear (NE) and cytosolic (CE) fractions were analysed for the presence of RCC1 and α-tubulin.

Movie S1. Time-lapse movie of inhibition of XPO1-dependent nuclear export of RevM5-GFP. HeLa cells expressing RevM5-GFP were treated with 1µM KPT-185 and imaged over time. RevM5-GFP shuttles between the nucleus and cytoplasm using cellular import and export mechanisms, but under steady state conditions and before addition of compound, RevM5GFP localizes predominantly in the cytoplasm; upon treatment with KPT-185 the accumulation of RevM5-GFP can be observed as a result of inhibition of the XPO1-dependent nuclear export of the protein, while its import is unaffected.

Movie S2. Time-lapse movie of inhibition of XPO1-Rev interaction in living cells. Cells co-expressing Rev-GFP and XPO1-mRFP were treated with 1µM KPT-185 and imaged over time. Before addition of compound, XPO1-mRFP co-localizes with Rev-GFP cargo in the nucleoli of the cell resulting in a yellow color; upon treatment with KPT-185 the Rev-GFP/XPO1-mRFP interaction is disrupted and XPO1-mRFP detaches from its Rev-GFP cargo. Unbound XPO1-mRFP is found throughout the nucleus and at the nuclear envelope.