Supplementary data

Supplementary Tables

Table S1: Detailed characteristics of proteasome inhibitors used in the study.

i.v. = intravenous infusion, s.c. = subcutaneous administration

Proteasome inhibitor	Chemical entity	Route of administration	Clinical Status	Target in the proteasome complex
Bortezomib	boronate	i.v. infusion or s.c.	Approved for MM	β5i and β5c (in higher dose β1i and β1c)
Carfilzomib	epoxyketone	i.v. infusion	Approved for MM	β5i and β5c (in higher dose β2i and β2c)
LU005i	epoxyketone	i.v.	preclinical	β5i, β2i and β1i [34]
LU035i	epoxyketone	i.v.	preclinical	β5i [34]
LU102	vinylsulfone	i.v.	preclinical	β2i and β2c [48]
LU025c	epoxyketone	i.v.	preclinical	β5c [46]

Table S2: Basic biological and clinical characteristics of B-CLL cohort.

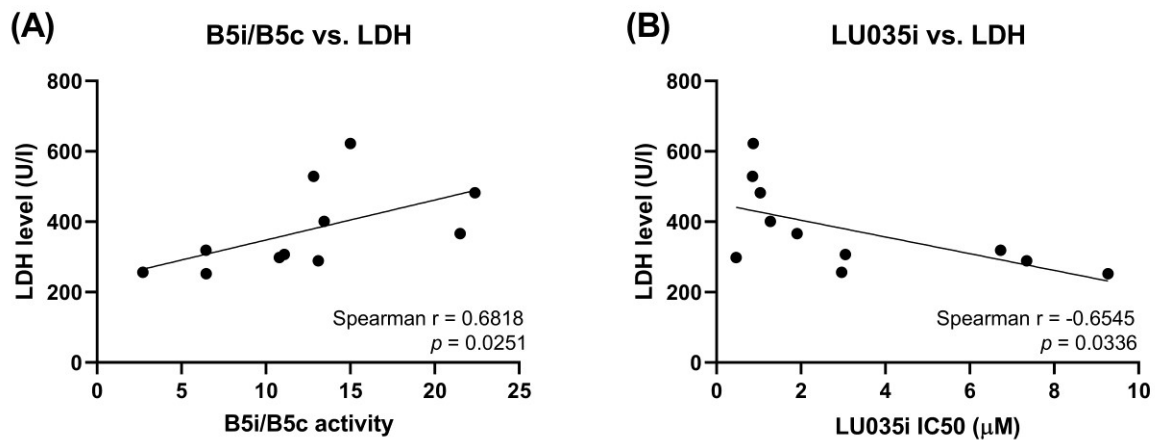|	B-CLL
Nr of patients	17
Sex: male-female (%)	11 (65%) - 8 (35%)
Age (median; min-max)	69 (54-81)
Sample characteristics	
diagnosis (%)	1 (5.9%)
relapse (%)	16 (94.1%)
Cytogenetic aberrations	
TP53: del/mut/normal/NA	3/3/7/4
ATM: del/normal/NA	3/8/6
13q14: del/normal/NA	8/3/6
IGHV status: mutated/unmutated/NA	3/7/7
Biochemical characteristics	
Hemoglobin, g/l (median; min-max)	125.5 (95-144)
Leukocytes, g/l (median; min-max)	81.9 (19-334)
Bilirubin, µmol/l (median; min-max)	9.5 (5-34)
LDH, U/l (median; min-max)	319 (252-622)

NA = non available; del = deletion; mut = mutation

Figure S1: Correlation of LDH with proteasome activity and cytotoxicity of the immunoproteasome selective inhibitor in B-CLL primary samples. A) Correlation of LDH with the activity ratio of proteasome β5i/β5c. **B)** Correlation of LDH with the cytotoxicity of LU035i, an immunoproteasome β5i selective inhibitor (presented as the IC_{50} values). Spearman correlation coefficient and *p* value are presented for bot analyses.
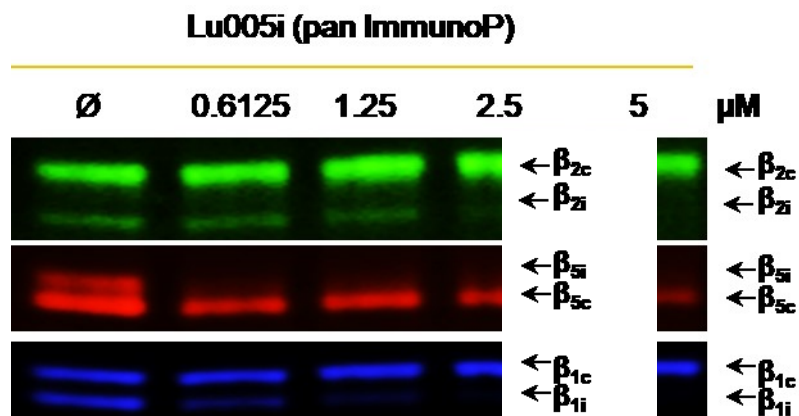

Figure S2: Inhibitory profile of pan-immunoproteasome selective inhibitor LU005i. AMO-1 cells were exposed to indicated doses of LU005i for 1h, then lysed and labelled with ABP labelling for 1h to visualize active proteasome β-subunits. Subsequently, the proteins were separated by SDS-PAGE.
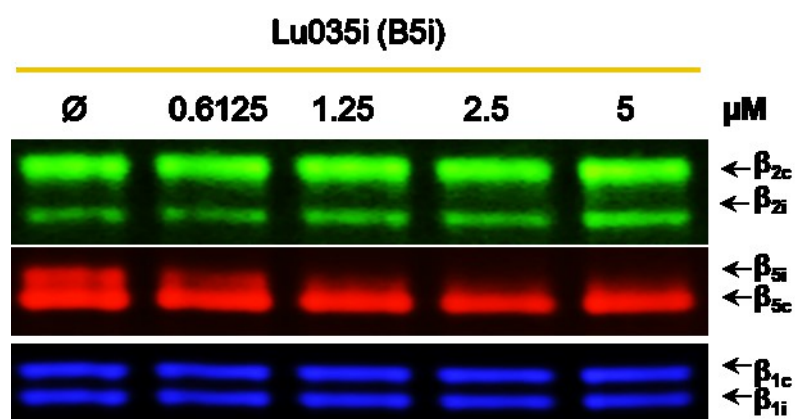
Figure S3: Inhibitory profile of β5-immunoproteasome selective inhibitor LU035i. AMO-1 cells were exposed to indicated doses of LU035i for 1h, then lysed and labelled with ABP labelling for 1h to visualize active proteasome β-subunits. Subsequently, the proteins were separated by SDS-PAGE.
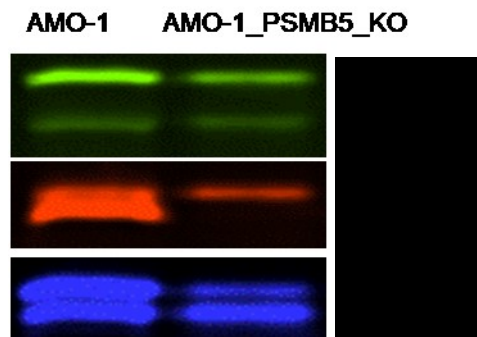

Figure S4: Profile of proteasome β-subunits activity in AMO-1 wild-type cells and in AMO-1 cells with PSMB5 knock-out. Cells were lysed and labelled with ABP labelling for 1h to visualize active proteasome β-subunits. Subsequently, the proteins were separated by SDS-PAGE.
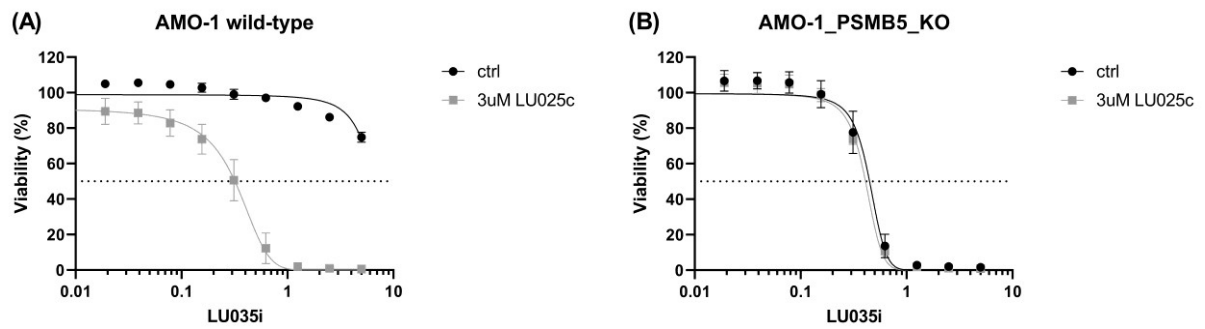
Figure S5: Dose-response curves of AMO-1 wild-type and AMO-1_PSMB5 knock-out cells to β5i inhibition in a presence or absence of the β5c inhibition. A) AMO-1wild-type and B) AMO-1_PSMB5_KO cells were exposed to increasing doses of LU035i in monotherapy or co-treated with 3 μM LU025c, viability was determined 48h after the treatment. Data represent mean ± SD of three repeats. LU035i = β5i selective inhibitor, LU025c = β5c selective inhibitor.