Preclinical evidence for an effective therapeutic activity of FL118, a novel survivin inhibitor, in patients with relapsed/refractory multiple myeloma

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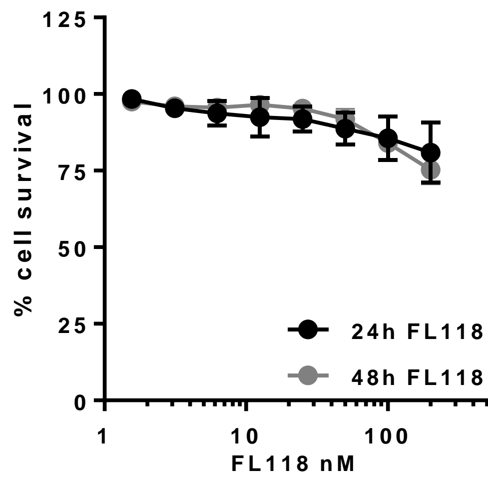
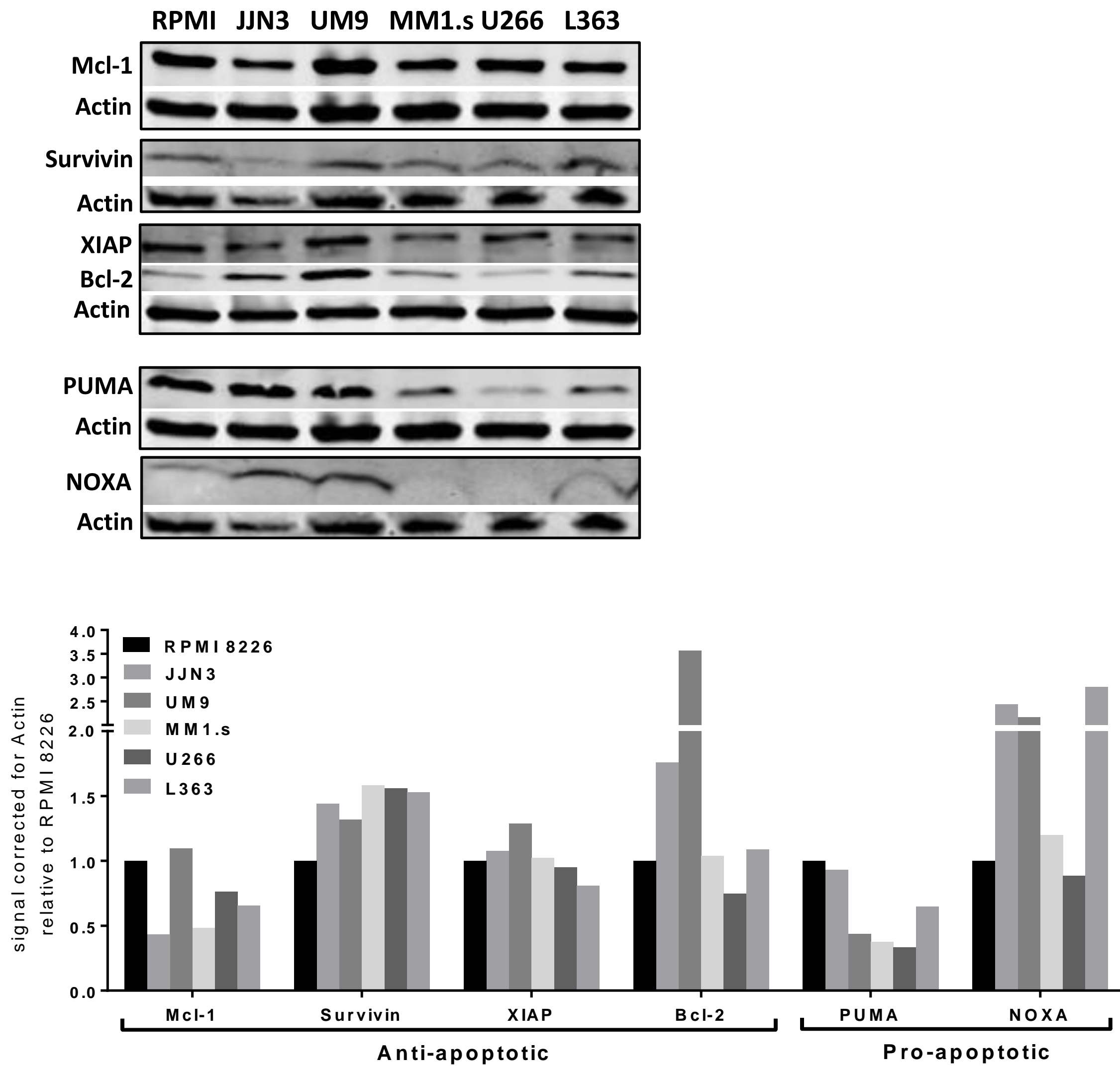
P53 status	Cell line	P53 mutation type	Genomic description	Protein description	Effect
Wildtype	MM1.s	wild-type			
LEO*	JJN3	wild-type			
Mutated	RPMI 8226	point mutation	g.14522G>A	p.E285K	missense
	U266	point mutation	g.13160G>A	p.A161T	missense
	L363	point mutation**	g.14109G>C	p.R261T	splice
	UM9	deletion***	g.12021_14754del	p.S33_Q331del	nonsense

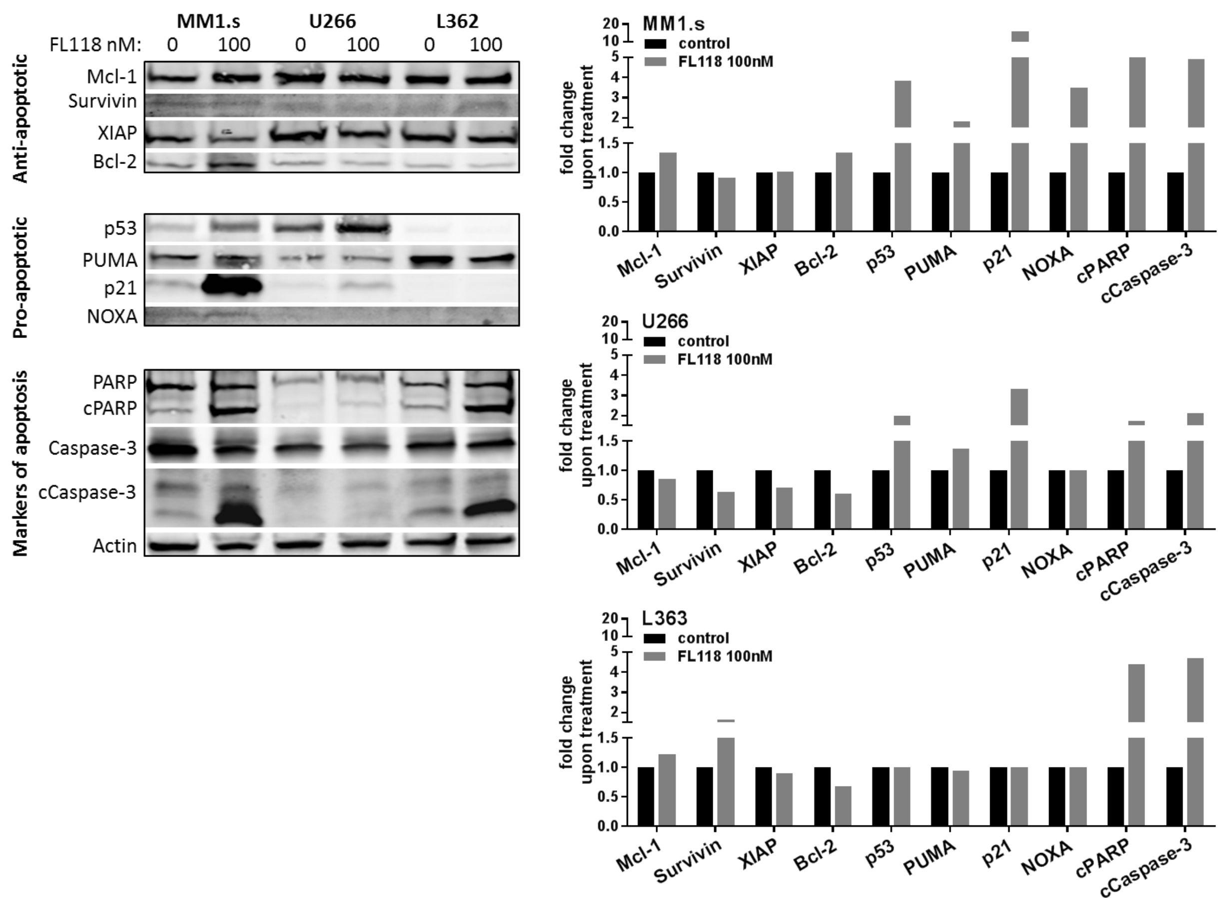
Supplementary table S1. P53 status of MM cell lines. The P53 status of each cell line was validated using BigDye Direct Cycle Sequencing Kit (Life technologies). Sequencing was performed on Applied Biosystems 3500 Series Genetic Analyzer (Life Technologies) and analysis was done using database version R12.[1] * JJN3 cells displayed wild-type P53 DNA but LEO (loss of gene expression without gene mutation). ** L363 cells displayed a point mutation in exon 7/intron 7 splice site (14109G>C) impairing splicing of intron 7. *** Amplified DNA products of P53 exons 4-9 of UM9 cells could not be detected on agarose gel, nor correctly analyzed after sequencing.



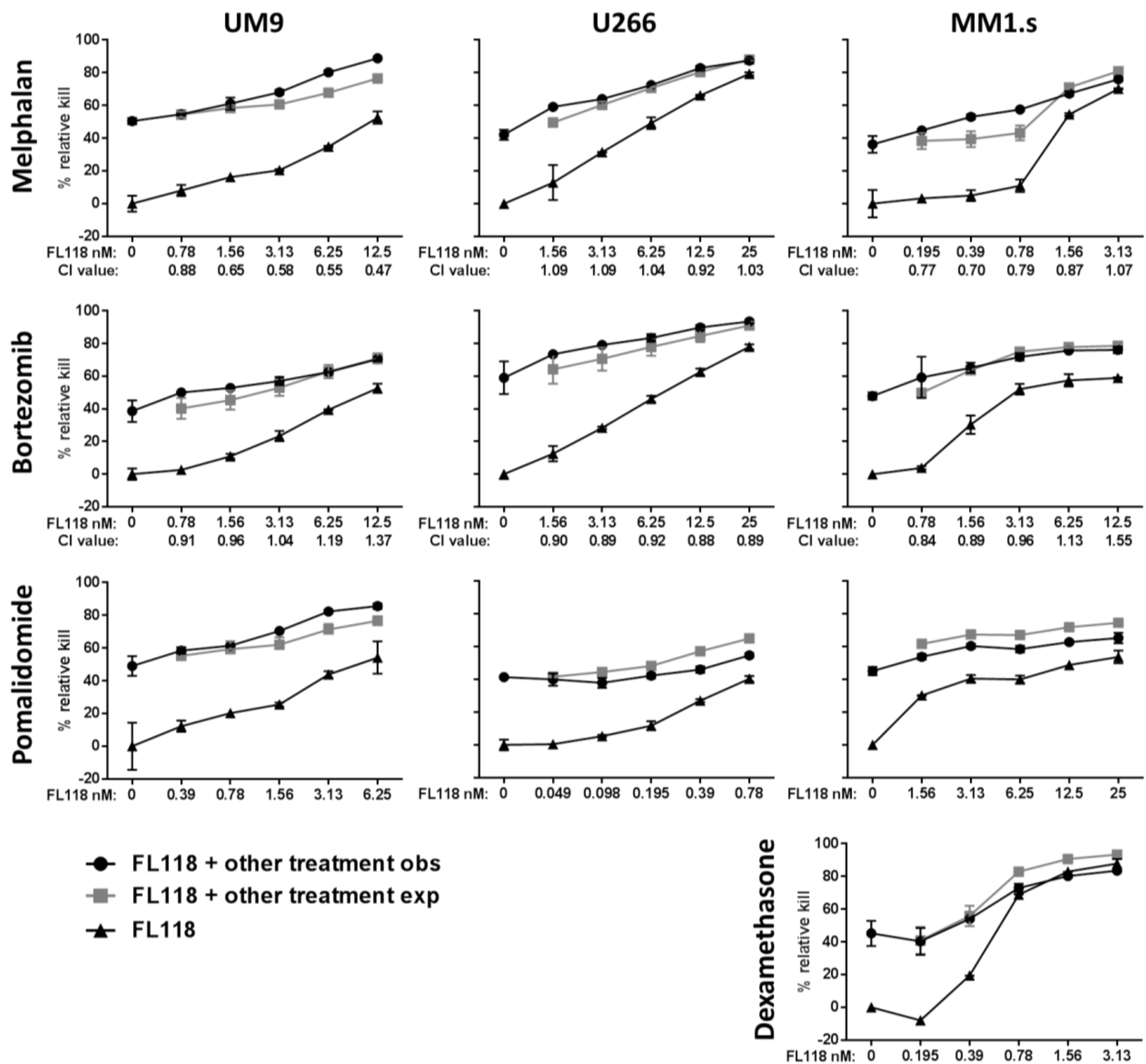
Supplementary figure S1. Minimal toxicity of FL118 on BMMSCs. A pool (n=12) of newly diagnosed-MM patient derived BMMSCs were treated with serial concentrations of FL118. Cell viability was determined by Cell TiterGlo after indicated period of time. Error bars represent the SD of three independent experiments.



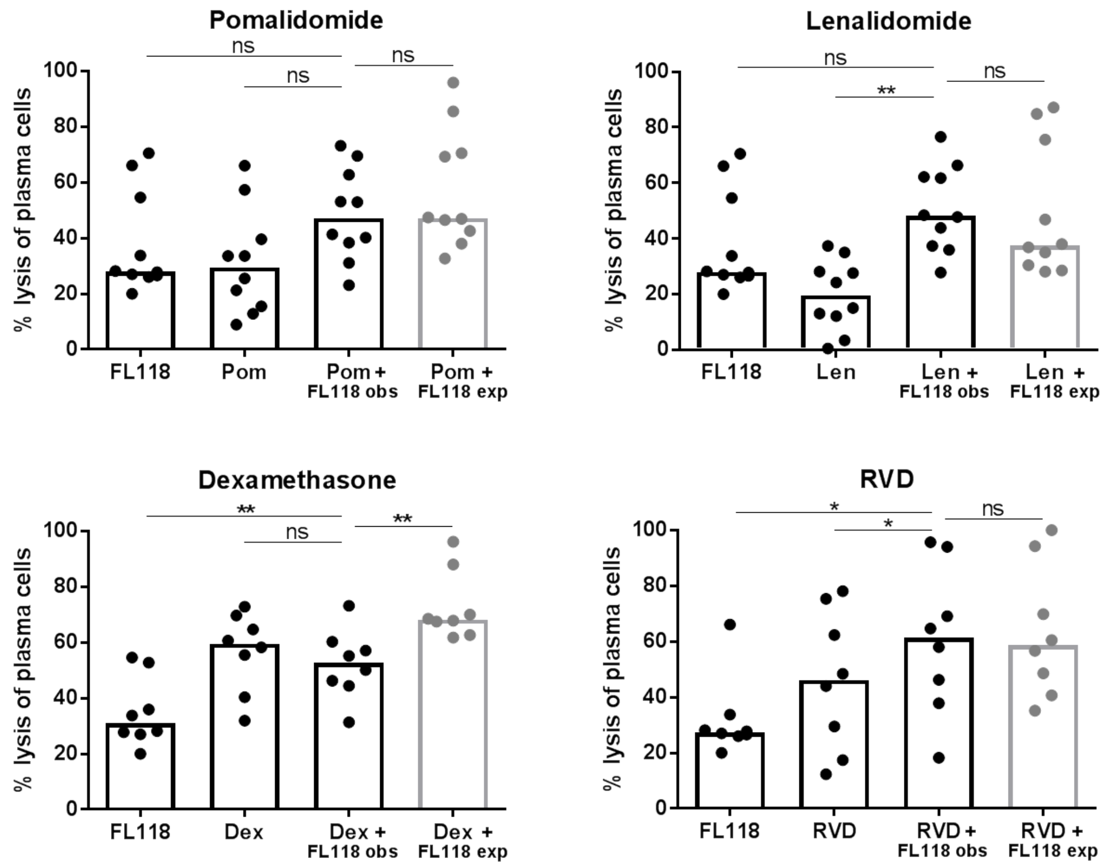
Supplementary figure S2. Protein expression levels of FL118 target genes in MM cell lines. Untreated MM cells were lysed and analyzed using Western blots with corresponding antibodies as shown. Actin was used as internal controls. Independent Western blots were performed at least twice.



Supplementary figure S3. FL118 promotes pro-apoptotic signaling in FL118 high- and intermediate-susceptible MM cell lines MM1.s and U266, but not in the low-susceptible MM cell line L363. MM cell lines were treated with FL118 for 16 hours. Cells were then analyzed for FL118 target gene expression using western blots. Relative expression levels of indicated proteins upon treatment were plotted after correcting for the internal control Actin. Cleaved products of PARP and Caspase-3 are indicated as cPARP and cCaspase-3 respectively. Independent Western blots were performed at least twice.



Supplementary figure S4. FL118 enhances melphalan- and bortezomib-induced MM lysis in MM cell lines. LUC-transduced UM9, U266 and MM1.s MM cell lines were treated with FL118 and/or a currently used drug. MM cell viability was determined by BLI after 48 hour treatment with melphalan, bortezomib or dexamethasone combined with FL118, and after 72 hour treatment with pomalidomide combined with FL118. Results are representative of three independent assays. Error bars represent the SD. The observed lysis levels (obs) upon co-treatment were compared to the expected lysis levels (exp) as described in the legends of figure 2C. Combination Index (CI) values for the co-treatment of FL118 with melphalan or with bortezomib were quantified with the Chou-Talalay method. CI values could not be determined for pomalidomide or dexamethasone as we were unable to reach a dose-response curve for these drugs including data points above and below IC50.



Supplementary figure S5. FL118 does not enhance pomalidomide, lenalidomide, or dexamethasone-induced MM cell lysis but does enhance RVD- (currently applied combination treatment of bortezomib, dexamethasone, and lenalidomide) induced MM cell lysis. BMMNCs from MM patients were treated with predetermined suboptimal concentrations of FL118 (12.5-100nm) and/or with a predetermined suboptimal concentration of a currently used drug dexamethasone (0.5-3 µmol/L), lenalidomide (3 µmol/L), pomalidomide (2 µmol/L), or RVD: bortezomib (1 nmol/L), dexamethasone (1 µmol/L) and lenalidomide (1 µmol/L), for 48 hours. Ten BMMNC samples were used in pomalidomide, and lenalidomide treatment; eight BMMNC samples were used in dexamethasone and RVD treatment. Viable $CD138^{+}$ $CD38^{+}$ MM cells were enumerated via flow cytometry and % lysis was calculated relative to untreated samples. Bars represent the median. The observed lysis levels (obs) upon co-treatment were compared to the expected lysis levels (exp) as described in the legends of figure 2C. The statistical differences between treatments were tested using Wilcoxon matched-pairs rank test; * $P < 0.05$; ** $P < 0.01$; ns, not significant.

References

1. Petitjean A, Mathe E, Kato S, et al. Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. Hum Mutat. 2007;28(6):622-629.