Supplemental Material

Supplemental Experimental Procedures

1. Evaluation of the combinations of amiloride with other anti-myeloma agents

Cells were treated for 24, 48 and 72 hours with double combinations at different doses of amiloride and other antimyeloma agents. Cell viability was analyzed by MTT assay and a constant ratio experimental design was used. The potency of each combination in different cell lines was quantified with the Calcusyn software (Biosoft, Ferguson, MO, USA), which is based in the Chou Talalay method and calculates a combination index (CI) with the following interpretation:

Combination index	Interpretation
<0.3,	strong synergism
0.3-0.7	synergism
0.7-0.85	moderate synergism
0.85-0.90	slight synergism
0.90-1.10	additive effect
>1.10	antagonism

2. Effect of MSCs on amiloride-induced growth inhibition

The methods for isolation and expansion of MSCs have been described by Garayoa *et al.* (1). Next, the MSCs were plated in 96-well culture dishes (8000/well) (3 biological replicates) and allowed to reach confluence during 48 hours. Then, medium was removed and 20,000 luciferase-expressing myeloma cells (MM1S-luc) in RPMI 1640 containing 10% fetal bovine serum were plated on top of the MSCs and treated for 48 hours with different concentrations of amiloride. After the incubation period, luciferin substrate (Caliper Life Sciences, Hopkinton, MA) at a final concentration of 150 g/mL was added for 10 minutes and bioluminescence (photons/sec) was analyzed in a Xenogen IVIS Imaging System 50 Series (Caliper Life Sciences).

3. MM xenograft murine model

To fit the tumor growth curves, we used the exponential model y = A*ekx resorting to the EXFIT option of the SIMFIT package (www.simfit.org.uk). The k parameter corresponds to the tumor growth rate and the A parameter to the initial tumor size. The k parameter together with its standard error (Sk) were extracted for each condition to compare the different drug experiments. The regression parameters comparison was carried out using a t-test for unequal variances. The area under the curve (AUC) for each growth curve was calculated by cubic splines fitting using the COMPARE option of SIMFIT. Differences between each pair of AUCs were tested, inside the x-overlap window of both curves, using COMPARE under the formula 200(A0)/(B1+B2), with A0 being the integral of the absolute difference between curves 1 and 2, B1 and B2 being the AUC of both curves considering a zero-base line for the y values. Differences between curves were expressed as percentages"

4. Immunoblotting

Whole cell lysates were collected using RIPA buffer (Santa Cruz Biotechnology) containing protease and phosphatase inhibitors (Roche). Protein samples were subjected to SDS-PAGE electrophoresis and transferred to 0.45 µm polyvinylidene fluoride (PVDF) membrane using iBlot® Dry Blotting System (Invitrogen). The antibodies used for immunoblotting were: anti-p53 protein (Cell Signaling) and anti-β-actin-HRP (Sigma-Aldrich) as control for protein loading. The chemiluminescence was detected using Clarity Western ECL Substrate (BioRad).

5. RNA sequencing

Twelve total RNAs were measured for quantity and quality using Agilent 2100 Bioanalyzer (Agilent Technologies). All samples reached the minimum standards, concentrations higher than 200 ng/µl and RIN values over 8.0. Libraries were constructed following a TruSeq Stranded mRNA Sample Preparation Guide (Illumina). Briefly, mRNA was purified from 4 µg of total RNA using oligo (dT) magnetic beads. The purified mRNA was fragmented into small pieces using fragmentation buffer in

combination of heat treatment (94ºC, 8 min). Taking these short fragments as templates, first-strand cDNA was synthesized using reverse transcriptase and random hexamer primers. The addition of Actinomycin D prevents spurious DNA-dependent synthesis, while allowing RNA-dependent synthesis, improving strand specificity detection. Second-strand cDNA synthesis was followed using RNase H, which removes the RNA template, and a DNA polymerase I which synthesizes a replacement strand, incorporating dUTP in place of dTTP to generate ds cDNA. The incorporation of dUTP quenches the second strand during amplification, because the next polymerase does not incorporate past this nucleotide in further amplification. At the end of this process a blunt-ended cDNA is obtained. Indexes and sequencing adapters were ligated to short cDNA fragments after purification with Ampure XP beads (Beckman Coulter), and which were used to distinguish different sequencing samples.

The final cDNA library was generated with an amplification step and purification process, then libraries were first quantified and validated with Agilent 2100 Bioanalyzer (Agilent Technologies). Next, libraries were normalized and pooled for clustering generation and sequencing using Illumina HiSeq™ 2500 with a configuration of 100 cycles Paired-end Reads, following manufacturer's instructions (Illumina), at Life sequencing S.L. (Valencia, Spain).

6. RNA sequencing analysis

All sequencing software tools were run with the default or recommended settings. RNA-Seq analysis was carried out in eight cores and 32 GB of RAM computer. The operating system was Ubuntu 14.04.2 LTS using version of X_86 64 bits. Paired-end FASTQ files for 12 samples were used in the RNA-Seq analyses. Adapter and quality trimming was done by the cutadapt tool version v1.4 (http://dx.doi.org/10.14806/ej.17.1.200) removing all bases with a Phred quality average threshold < 20 (Q < 20). FASTQ files quality was assessed using FASTQC (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/) before and after the trimming process. The trimmed reads were mapped against the Homo sapiens genome

GRCh37 assembly obtained from Ensembl (http://www.ensembl.org/data/ftp/index.html) using in a first step the Bowtie2 aligner version 2.2.6 (2), in order to find the mean insert size to calculate the mean mate inner distance for each sample. The reads were then re-aligned using the TopHat v2.0.11 aligner (3). TopHat writes read data in the BAM format which is the binary version of the Sequence Alignment/Map (SAM) files, these files were used for downstream analyses at gene and isoform levels.

Regarding the gene level analyses, HTSeq version 0.6.0 was used to count the number of reads per gene from BAM files (4) considering the intersection-strict model whereby the whole read must fall entirely inside the feature to be counted. Reads that align multiple features are deleted to avoid double counting, and using GRCh37.82.gtf file from Ensembl as gene model. Low expression genes (those whose sums of reads were ≤ 1 in all samples) were removed. After the filtering process, the remaining genes were normalized dividing the counts by the sample-specific size factors, estimated using the median ratio method (5). Based on normalized count data, DESeq2 R package version 1.12.3 (6) (http://doi.org/10.1186/s13059-014-0550-8) modelled gene expression according a negative binomial distribution and generated a list of genes ranked based on the false discovery rate (FDR) using the Benjamini-Hochberg procedure.

The alternative splicing isoforms were detected using cufflinks version 2.2.1 following the Tuxedo pipeline (7). The isoforms were analyzed following 2 sequential approaches. First, a global analysis of isoforms was carried out considering all the isoforms with an absolute value of fold change (|FC|) ≥ 2 as differentially expressed between the contrasted conditions and using them for further analysis. Second, only the isoforms with a |FC| ≥ 2 between contrasted conditions corresponding to genes without expression modifications were considered. These genes were those not identified as a significant (FDR > 0.05 and |FC| < 2) by the DESeq2 package. The resulting isoforms were also used for downstream analysis.

Differential alternative splicing events were detected using rMATS version 3.0.9 (8), which identifies the number of observed reads that unambiguously support the presence or absence of each splicing event. Most of the significant AS events (FDR < 0.05) were classifying into five main types of pattern: patterns: skipped exon (SE), alternative 5' splice site (A5SS), alternative 3' splice site (A3SS), mutually exclusive exons (MXE) and retained introns (RIs). rMATS also calculates the difference in the ratio of these events between two conditions, calculating a false discovery rate.

The enrichment analysis was conducted through the Webgestalt web tool (9) in all analysis levels, using as data sources the Gene Ontology and the KEGG databases.

Supplemental tables

Supplemental Table 1. Molecular classification of human myeloma cell lines (HMCLs) as previously described (10–12). OD: oligomerization domain; DBD: DNA-binding domain.

	HMCLs Name	IGH translocations	*TP53* status	Mutation	Type of mutation	Domain of p53
1	NCI-H929	t(4;14)	wild type (WT)	-	-	-
2	JJN3	t(14;16)	deletion (DEL)	-	-	-
3	KMS12-BM	t(11;14)	mutated (MUT)	R337L	missense	OD
4	KMS12-PE	t(11;14)	mutated (MUT)	R337L	missense	OD
5	U-266	CCND1 insertion	mutated (MUT)	A161T	missense	DBD
6	MM1S	t(14;16)	wild type (WT)	-	-	-
7	RPMI-8226	t(14;16)	mutated (MUT)	E285K	missense	DBD

Supplemental Table 2. Patients' characteristics

Code	Patient No.	Age	Gender	Disease status	Treatment	FISH 17p	Experiments
P1	42394	55	M	Newly-diagnosed		normal	Citotoxicity of amiloride on PC from total BM sample
P2	42532	87	M	Newly-diagnosed		normal	Citotoxicity of amiloride on PC from total BM sample
P3	42598	87	F	Newly-diagnosed		normal	Citotoxicity of amiloride on PC from total BM sample
P4	42869	83	M	Newly-diagnosed		deletion	Citotoxicity of amiloride on PC from total BM sample
P5	50393	83	F	Newly-diagnosed		normal	Citotoxicity of amiloride on PC from total BM sample
P6	43449	63	M	Refractory/Progression	VTD/VDL-PACE/pembrolizumab-LD	deletion	Citotoxicity of amiloride on PC from total BM sample
P7	43226	63	M	Refractory	VTD-ASCT/ixazomib maintenance/KRD	deletion	Citotoxicity of amiloride on PC from total BM sample
P8	32585	72	M	Relapse	VMP	normal	Citotoxicity of amiloride on PC from total BM sample
P9	50616	76	M	Newly-diagnosed		normal	Citotoxicity of amiloride on PC from total BM sample
P10	47687	73	M	Refractory	VMP	normal	Citotoxicity of amiloride on PC from total BM sample
P11	50150	84	F	Newly-diagnosed		normal	Cell viability and RNA isolation of CD138+ fraction treated with amiloride
P12	50161	61	F	Newly-diagnosed		normal	Cell viability and RNA isolation of CD138+ fraction treated with amiloride
P13	50160	46	M	Newly-diagnosed		normal	Cell viability and RNA isolation of CD138+ fraction treated with amiloride
P14	40420	81	M	Relapse	VMP/LD	normal	Cell viability and RNA isolation of CD138+ fraction treated with amiloride
P15	50204	83	F	Newly-diagnosed		normal	Cell viability and RNA isolation of CD138+ fraction treated with amiloride
P16	50207	69	F	Relapse	VMP	normal	Cell viability and RNA isolation of CD138+ fraction treated with amiloride
P17	48886	58	F	Refractory	VTD	normal	Cell viability and RNA isolation of CD138+ fraction treated with amiloride
P18	50007	84	F	Newly-diagnosed		normal	Cell viability and RNA isolation of CD138+ fraction treated with amiloride

Supplemental Table 3. Amiloride-induced transcript isoforms expression changes of genes which encode proteins involved in spliceosome complex and its regulation. Only common transcript isoforms in both cell lines are shown. The direction of the change upon amiloride treatment is indicated (FC ≥ 2: upregulation or FC ≤ -2: downregulation), and the proteins are classified according to the spliceosome complexes to which they belong. The transcript isoforms with a log2 (FC) = 99 were those transcripts only expressed in amiloride treated-cells; and the transcript isoforms with log2 (FC) = -99 were those transcripts only expressed in control cells.

		KMS12-BM	JJN3		
Gene Symbol	**Transcript**	**log2 (FC)**	**log2 (FC)**	**Protein**	**Spliceosome complex**
SNRPC	ENST00000244520	7.14	1.22	159 aa	U1 snRNP
SF3A2	ENST00000586396	-1.21	-1.69	135 aa	U2 snRNP
U2AF1	ENST00000464750	6.22	99.00	75 aa	U2 snRNP-related
EIF4A3	ENST00000576547	-9.93	-8.82	125 aa	EJC/TREX complex
SRSF1	ENST00000258962	16.04	1.10	248 aa	common component (SR protein)
SRSF10	ENST00000259043	7.78	7.64	127 aa	common component (SR protein)
HNRNPC	ENST00000556513	4.44	7.33	231 aa	common component (hnRNP protein)
HNRNPC	ENST00000553753	1.01	1.49	288 aa	
HNRNPC	ENST00000420743	1.78	99.00	306 aa	
HNRNPC	ENST00000555215	99.00	4.14	187 aa	
HNRNPC	ENST00000557768	3.22	1.99	54 aa	
TXNL4A	ENST00000269601	99.00	99.00	142 aa	U5 snRNP and U4/U6-U5 tri-snRNP complexes
TXNL4A	ENST00000588162	-99.00	-99.00	57 aa	

Supplemental Table 4. Amiloride-induced transcript expression changes of genes involved in p53 pathway in KMS12-BM cell line. The direction of the change upon amiloride treatment is indicated (FC ≥ 2: upregulation or FC≤ -2: downregulation). The transcript isoforms with a log2 (FC) = 99 were those transcripts only expressed in amiloride treated-cells; and the transcript isoforms with log2 (FC) = -99 were those transcripts only expressed in control cells.

Gene Symbol	Transcript	log2 (FC)	Biotype	Protein
CDK4	ENST00000547853	99.00	Protein coding	2 aa
CDK4	ENST00000550419	1.06	Nonsense mediated decay	201 aa
CDK4	ENST00000546489	-99.00	Protein coding	208 aa
CDK4	ENST00000552254	1.70	Protein coding	203 aa
CDK4	ENST00000549606	3.88	Protein coding	40 aa
CDK4	ENST00000547281	-1.21	Protein coding	186 aa
CDK4	ENST00000552862	3.26	Protein coding	115 aa
CDK4	ENST00000551800	-99.00	Protein coding	132 aa
CDK4	ENST00000312990	1.01	Protein coding	111 aa
CDK4	ENST00000257904	-4.83	Protein coding	303 aa
CDK4	ENST00000551888	6.00	Processed transcript	no protein
CDK4	ENST00000552388	-1.48	Protein coding	170 aa
CDK4	ENST00000551706	3.52	Retained intron	no protein
TP53	ENST00000514944	99.00	Protein coding	155 aa
TP53	ENST00000505014	99.00	Retained intron	no protein
TP53	ENST00000269305	4.17	Protein coding	393 aa
CHEK1	ENST00000532669	1.08	Protein coding	192 aa
CHEK1	ENST00000438015	-2.05	Protein coding	476 aa
CHEK1	ENST00000544373	-7.36	Protein coding	382 aa
CHEK1	ENST00000532449	-99.00	Protein coding	442 aa
CHEK1	ENST00000278916	99.00	Protein coding	432 aa
CHEK1	ENST00000498122	-99.00	Nonsense mediated decay	89 aa
CHEK1	ENST00000427383	-99.00	Protein coding	492 aa
CHEK1	ENST00000528761	99.00	Processed transcript	no protein
CHEK1	ENST00000524737	-99.00	Protein coding	476 aa
SHISA5	ENST00000417841	99.00	Protein coding	112 aa
SHISA5	ENST00000466424	-99.00	Processed transcript	no protein
SHISA5	ENST00000426002	-4.64	Protein coding	137 aa
SHISA5	ENST00000460758	1.62	Retained intron	no protein
CCNB1	ENST00000506572	-2.40	Protein coding	401 aa
CCNB1	ENST00000508407	99.00	Protein coding	244 aa
CCNB1	ENST00000507798	1.87	Protein coding	185 aa
CCNB1	ENST00000505500	-10.47	Protein coding	396 aa
BID	ENST00000473439	-1.53	Processed transcript	no protein
BID	ENST00000399765	2.57	Protein coding	99 aa
SESN1	ENST00000520364	5.51	Retained intron	no protein

SESN1	ENST00000523632	-5.86	Retained intron	no protein
SESN1	ENST00000356644	1.30	Protein coding	492 aa
SESN1	ENST00000368971	-5.92	Retained intron	no protein
CDK1	ENST00000519078	1.04	Protein coding	189 aa
CDK1	ENST00000373809	2.74	Protein coding	240 aa
CDK1	ENST00000395284	-99.00	Protein coding	297 aa
CCND1	ENST00000535993	-99.00	Retained intron	no protein
CCND1	ENST00000542367	-99.00	Retained intron	no protein
RFWD2	ENST00000367667	11.86	Nonsense mediated decay	113 aa
RFWD2	ENST00000367666	-1.58	Protein coding	566 aa
RRM2B	ENST00000519125	-5.73	Retained intron	no protein
RRM2B	ENST00000519317	1.17	Protein coding	139 aa
BAX	ENST00000345358	-99.00	Protein coding	192 aa
BAX	ENST00000515540	-1.81	Nonsense mediated decay	41 aa
GTSE1	ENST00000491863	6.92	Retained intron	no protein
ATR	ENST00000383101	-1.74	Protein coding	2580 aa
PERP	ENST00000421351	3.78	Protein coding	193 aa
CDKN2A	ENST00000361570	99.00	Protein coding	170 aa
CDKN2A	ENST00000498628	-1.15	Protein coding	105 aa

References

1. Garayoa M, Garcia JL, Santamaria C, Garcia-Gomez A, Blanco JF, Pandiella A, et al. Mesenchymal stem cells from multiple myeloma patients display distinct genomic profile as compared with those from normal donors. Leukemia. 2009;23:1515–27.
2. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9:357–9.
3. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol. 2013;14:R36.
4. Anders S, Pyl PT, Huber W. HTSeq--a Python framework to work with high-throughput sequencing data. Bioinforma Oxf Engl. 2015;31:166–9.
5. Anders S, Huber W. Differential expression analysis for sequence count data. Genome Biol. 2010;11:R106.
6. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15:550.
7. Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, et al. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat Protoc. 2012;7:562–78.
8. Shen S, Park JW, Lu Z, Lin L, Henry MD, Wu YN, et al. rMATS: robust and flexible detection of differential alternative splicing from replicate RNA-Seq data. Proc Natl Acad Sci U S A. 2014;111:E5593-5601.
9. Wang J, Duncan D, Shi Z, Zhang B. WEB-based GEne SeT AnaLysis Toolkit (WebGestalt): update 2013. Nucleic Acids Res. 2013;41:W77-83.
10. Surget S, Descamps G, Brosseau C, Normant V, Maïga S, Gomez-Bougie P, et al. RITA (Reactivating p53 and Inducing Tumor Apoptosis) is efficient against TP53abnormal myeloma cells independently of the p53 pathway. BMC Cancer. 2014;14:437.
11. Moreaux J, Klein B, Bataille R, Descamps G, Maïga S, Hose D, et al. A high-risk signature for patients with multiple myeloma established from the molecular classification of human myeloma cell lines. Haematologica. 2011;96:574–82.
12. Teoh PJ, Chung TH, Sebastian S, Choo SN, Yan J, Ng SB, et al. p53 haploinsufficiency and functional abnormalities in multiple myeloma. Leukemia. 2014;28:2066–74.

Supplemental Figure Legends

Figure S1. (A) Protein expression of p53 in MM cell lines. Basal protein level of p53 was analyzed by Western blot in 7 MM cell lines. β-actin was used as loading control. **(B) Cell viability of isolated CD138+ cells from MM patients.** Cells were treated with the indicated doses of amiloride for 24 h and cell viability was analyzed by CellTiter-Glo luminescent assays. The average of luminescent values of control untreated samples was taken as 100%. The statistically significant differences between untreated and treated cells were determined with Student's t-test and represented as ***$p < 0.001$ (C) BM cells obtained from four donors were treated *ex vivo* with increasing concentrations of amiloride for 48 h. After the incubation period, cells were stained with the combination of annexin-V-FITC and five monoclonal antibodies (CD38-APC-H7, CD45-OC515, CD56-PE, CD19-PECy7 and CD3-APC) for the analysis of apoptosis in plasma cells and T lymphocytes (CD3+), B lymphocytes (CD19+), NK cells (CD56+/CD3-) and granulocytes (SSChigh/CD45+dim). Results are presented as the percentage of Annexin V-positive cells (Mann–Whitney U test).

Figure S2. (A) Melphalan resistant MM cell line RPMI-LR5 (panel left) and dexamethasone resistant MM cell line MM1R (panel right) were treated with the indicated doses of amiloride for 24, 48 and 72 h, and cell viability was analyzed by CellTiter-Glo luminescent assays. The average of luminescent values of control untreated samples was taken as 100%. Results are the means of three independent experiments. The statistically significant differences between untreated and treated cell lines were determined with Student's t-test. **(B,C,D)** MM cell lines were treated with the indicated double combinations of amiloride with dexamethasone or melphalan or **(E)** bortezomib. Cell viability was analyzed by MTT assay as represented in the graphs and the combination indexes (CI) were calculated with the Calcusyn software. CIs of < 0.3, 0.3-0.7, 0.7-0.85, 0.85-0.90, 0.9-1.10, and > 1.10 indicate strong synergism, synergism, moderate synergism, slight synergism, additive effect, and antagonism,

respectively. C: control; A: amiloride; D: dexamethasone; M: melphalan; B: bortezomib; d1, d2 and d3: drug concentrations used in the study; CR: constant ratio.

Figure S3. (A) MM1S-luc cells were treated for 48 hours with the indicated concentrations of amiloride in the presence or absence of MSCs derived from newly-diagnosed (ND) and relapsed/refractory (RR) MM patients, and proliferation was analyzed by bioluminescence (photons/sec). The statistically significant differences between untreated and treated cocultured cells were determined with Student's t-test and represented as $^{**}p < 0.01$ **(B)** MSCs were cultured with different doses of amiloride for 48 hours, and cell viability was analyzed by CellTiter-Glo luminescent assays. The statistically significant differences between untreated and treated cells were determined with Student's t-test and represented as $^{**}p < 0.01$

Figure S4. Amiloride induced apoptosis in MM cells. H929, KMS12-BM, JJN3 cells **(A)** and MM1S, U-266 and RPMI **(B)** were treated with increasing concentrations of amiloride for 48 h, and the induction of apoptosis was analyzed by flow cytometry after annexin-V/PI staining. Data are the means ± SD of three independent experiments. Statistically significant differences between untreated and treated cell lines are represented as $^{**}p < 0.01$ (Student's t-test).

Figure S5. Amiloride did not induce changes in the cell cycle profile of MM cells. The indicated MM cell lines were incubated with increasing concentrations of amiloride for 48 h and the cell cycle was analyzed by flow cytometry. (A) Data are the means ± SD of three independent experiments. (B) The histograms show the phases of the cell cycle, including the subG1 peak. Statistically significant differences between untreated and treated cell lines are represented as $^{***}p < 0.001$, $^{**}p < 0.01$ and $^{*}p < 0.05$ (Student's t-test).

Figure S6. Amiloride deregulated mitochondrial potential in MM cells. The indicated MM cell lines were incubated with increasing concentrations of amiloride for 24 h and the mitochondrial membrane depolarization was examined by flow cytometry after DilC1(5) staining (MitoStep). Dark grey color represented the depolarized cells

and grey color the cells without mitochondrial membrane depolarization. Data are the means ± SD of three independent experiments. Statistically significant differences between untreated and treated cell lines are represented as $**p < 0.01$ and $*p < 0.05$ (Student's t-test).

Figure S7. **Activity of amiloride through caspase-dependent and independent mechanisms. (A)** The activity of caspase 8, caspase 9 and caspase 3/7 was analyzed by luminescent caspase assays in different MM cell lines. **(B)** Pharmacologic inhibition of the activity of caspases with the pan-caspase inhibitor Z-VAD-FMK. MM1S and KMS12-BM cells were preincubated with the pan-caspase inhibitor (50 nM) for one hour and then were treated with amiloride (MM1S at 0.2 mM, and KMS12-BM at 0.1 mM) for 24 additional hours. The abrogated activity of caspase 3/7 was corroborated by luminescent caspase assay. Bortezomib (MM1S at 5 nM, and KMS12-BM at 10 nM) was used as positive control of caspase-dependent mechanism. Data are the means ± SD of three independent experiments. Statistically significant differences between untreated and treated cell lines are represented as $**p < 0.01$ and $*p < 0.05$ (Student's t-test).

Figure S8. The triple and double combination of dexamethasone and melphalan with amiloride displayed superior anti-MM activity and improved median survival compared with single agents and double combinations in a subcutaneous plasmacytoma model. CB17-SCID mice subcutaneously inoculated with 3 x 10^6 MM1S cells in the right flank were randomized to receive vehicle, amiloride (15 mg/kg, oral, daily), dexamethasone (0.5 mg/kg, i.p., 2 days weekly), melphalan (2.5 mg/kg, i.p., 2 days weekly) in monotherapy and the respective double and triple combinations (n = 5/group). **(A)** Evolution of tumor volumes of the plasmacytomas. Statistical differences between groups were evaluated fitting an exponential regression model and the regression parameters were compared using a *t*-test for unequal variances. and represented as $*p < 0.05$. Bars indicate standard errors of the mean. **(B)** Kaplan-Meier curves representing the survival of each treatment group. Mice were sacrificed

when their tumor diameters reached 2 cm or when they became moribund. Statistically significant differences were analyzed by the log-rank test, and represented as $**p < 0.01$ and $*p < 0.05$. **(C)** The toxicity profile of drugs *in vivo*, showed a lower body weight loss in all treated mice. It is represented as percentage of mouse body weight variation during the study in the xenograft plasmacytoma.

Figure S9. **Experimental design for RNA-Seq assay.** KMS12-BM and JJN3 cell lines, untreated or treated with amiloride (0.1 mM and 0.4 mM, respectively) for 24 h. The RNA was isolated and prepared for next-generation sequencing analysis.

Figure S10. Pathway enrichment analysis at gene level and differential transcript isoforms expression. (A) Summary of the biologically relevant pathways significantly enriched in KEGG analysis for amiloride-deregulated genes detected in KMS12-BM and JJN3 cell lines. Statistical significance of the enrichment was expressed as -log10 (Benjamini-Hochberg adjusted *p* - value). **(B)** Differential expression of transcript isoforms. Deregulates transcript isoforms were identified using Cufflinks following the Tuxedo pipeline when untreated and treated cell lines where compared. Only the isoforms that have a $|FC| \geq 2$ were considered as differentially expressed.

Figure S11. Validation of spliceosomal machinery alterations (A) CB17-SCID mice were subcutaneously inoculated into the right flank with $3x10^6$ MM1S (*TP53* WT; n = 6) cells or RPMI (*TP53* MUT; n = 4) cells in 100 µL of RPMI-1640 medium and 100 µL of Matrigel. After 11 weeks, when tumors had a large size, all mice were randomized into 2 groups, the control groups receiving the vehicle alone-PBS and the amiloride treatment groups receiving 20 mg/kg/day for two consecutive days, orally. Mice were sacrificed at third day and SC35-staining nuclear speckles detected by immunofluorescence assays. The figure shows the results corresponding to all mice. **(B)** CD138+ cells from one newly-diagnosed MM patient were treated with amiloride for 24 hours and SC35-staining nuclear speckles detected by immunofluorescence assay. **(C)** CD138+ cells from MM patients were treated with amiloride for 24 hours and the mRNA levels of spliceosome components were tested. The results are shown as the

magnitude of change between treated and untreated cells, using 18S rRNA as endogenous control. Results are combined to those from RNA-Seq results.

Figure S12. Validation of gene expression. The mRNA levels of *BBC3 (PUMA), BAX, BAK1, CDKN1A (p21), CDKN1B, TNFRSF10B* and *FAS (CD95)* were assessed by qRT-PCR. Results are the mean of three independent experiments. The direction of the fold change detected in the mRNA levels evaluated by both RNA-Seq data and qRT-PCR, concur.

Figure S13. Validation of p53 pathway activation in patient cells. CD138+ cells from MM patients were treated with amiloride for 24 hours and the mRNA levels of p53 signaling pathway were tested. The results are shown as the magnitude of change between treated and untreated cells, using 18S rRNA as endogenous control. Statistically significant differences between untreated and treated cell lines are represented as ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$ (Student's t-test).