Supplemental Materials and Methods

Immunofluorescence

Cytospins were washed with PBS-Tween (0.1%). After incubation with BSA for 30 minutes, cytospins or slides were incubated with the primary antibodies, RASSF4 and alpha-tubulin (respectively Novus Biologicals and Bioké), overnight at 4°C. Detection was performed using a secondary antibody (anti-rabbit or anti-mouse) coupled with a fluorescence dye. Cytospins or slides were mounted using ProLong Gold Antifade Mountant with DAPI (ThermoFisher Scientific). Pictures were taken by a Nikon Eclipse 90i microscope with a Nikon DS-Ri1 camera.

Human phospho-kinase array kit

The human phospho-kinase array kit (R&D systems) was used according to manufacturer's instructions. Pixel densities of indicated proteins were quantified by Adobe Photoshop CS6 and presented as the fold change compared to reference.

RNA sequencing analysis

The RNA sequencing (RNA-seq) library preparation was done with 150ng of input RNA using the Illumina TruSeq Stranded mRNA Library Prep Kit. Paired-end RNA-seq was performed with illumina NextSeq sequencing instrument (Helixio, Clermont-Ferrand, France). RNA-seq read pairs were mapped to the reference human GRCh37 genome using the STAR aligner [(1)]. The expression level of each gene was summarized and normalized using DESeq2 R/Bioconductor package [(2)]. The raw gene-wise read counts were deposited in the GEO database as a private data set (GSE85020). Differential expression analysis was performed using DESeq2 pipeline. P values were adjusted to control the global FDR across all comparisons with the default option of the DESeq2 package. Genes were considered differentially expressed if they had an adjusted p-value of 0.05 and a fold change of 1.5. Pathway enrichment analyses were performed using the R package ReactomePA [(3)].

Supplemental references

1. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S *et al.* STAR: ultrafast universal RNA-seq aligner. Bioinformatics 2013;**29**:15-21.
2. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014;**15**:550.
3. Yu G, He QY. ReactomePA: an R/Bioconductor package for reactome pathway analysis and visualization. Mol Biosyst 2016;**12**:477-479.

Supplemental Figure Legends

Supplemental Figure S1: Prognostic value of the classical RASSF proteins RASSF1, RASSF3, RASSF5 and RASSF6 in MM patients. The prognostic value of the classical RASSF proteins RASSF1 **(A)**, RASSF3 **(B)**, RASSF5 **(C)** and RASSF6 **(D)** was determined using the HM, UAMS TT2 and UAMS TT3 cohort. Maxstat analysis was used to calculate the optimal separation of the previously untreated patients in terms of overall survival based on a cut-off value. The following probesets were used: 204346_s_at (RASSF1), 230466_s_at (RASSF3), 223322_at (RASSF5) and 229147_at (RASSF6). Data was analyzed through genomicscape (http://genomicscape.com/).

Supplemental Figure S2: Cellular localization of RASSF4. Cellular localization of RASSF4 in non-dividing (upper panel) and dividing (lower panel) XG-7 cells. Cytospins were made and immunofluorescence staining for DAPI, α-tubulin and RASSF4 was performed. Scale bar = 4 μM. One experiment representative of 3 is shown.

Supplemental Figure S3: Inducible doxycycline-dependent overexpression of human RASSF4. A) Scheme depicting the experimental set-up. Transduced cell lines were cultured with doxycycline (dox, 1 mg/ml). Cells were refreshed on day 3 and 7 and dox was added on day 0, 3, 5 and 7. **B-E)** Overexpression of huRASSF4 was confirmed on mRNA **(B)** and protein **(C-E)** level 3 days after adding dox (D) or not (control (C)) using qRT-PCR and western blot or immunofluorescence staining respectively. Immunofluorescence staining for DAPI and huRASSF4 was performed on cytospins. Actin was used as reference gene in qPCR analysis and tubulin was used as loading control for western

blotting. For qPCR analysis, data represent the mean ± SD of 3 independent experiments. For the western blot and immunofluorescent analysis, one experiment representative of 3 is shown. Scale bar = 40 µM. **F)** Effect of RASSF4 overexpression on caspase-3 cleavage. RASSF4 transduced cells were cultured with or without doxycycline (Dox) for indicated time points (days). The effect on caspase-3 cleavage was assessed using active caspase-3 FITC staining followed by flow cytometric analysis. Data represent the mean ± SD of 3 independent experiments. * indicates $p<0.05$. **G-H)** Effect of RASSF4 on OPM-2 cell viability, apoptosis and bortezomib sensitivity. **G)** Effect of RASSF4 on cell viability and apoptosis. Transduced cells were cultured with or without doxycycline (Dox) for indicated time points (days). The effect on viability was assessed using CellTiter-Glo® Luminescent Cell Viability Assay, while the effect on apoptosis was assessed using an annexinV-APC/7'AAD staining followed by flow cytometric analysis. The % apoptotic cells are the sum of the percentage annexinV (+) and annexinV(+)/7'AAD (+) cells. Data represent the mean ± SD of 3 independent experiments. **H)** Effect of forced RASSF4 expression on bortezomib drug sensitivity. Cells were treated with different concentrations of bortezomib (Bz, nM) 7 days after treatment with Dox for an additional 3 days. Apoptosis was determined by flow cytometry using an AnnexinV-FITC/7'AAD staining (n=1).

Supplemental Figure S4: Comparison of the RASSF4 expression levels of the primary MM cells (n=10) and a selected panel of HMCL (n=9) obtained through microarray analysis. The microarray data from the primary MM cells and HMCL was normalized in the same way.

Supplemental Figure S5: Effect of RASSF4 expression on tumor development. A-B) Tumor development upon injection AMO-1 transduced cells. **A)** Effect of RASSF4 expression on tumor volume. Tumor mass is encircled in red. **B)** Overexpression of huRASSF4 was confirmed on protein level using immunofluorescence staining for DAPI and huRASSF4. Scale bar = 100 µM. **C)** Confirmation of huRASSF4 overexpression in XG-7 transduced cells. Overexpression of huRASSF4 was confirmed on protein level using Western Blot analysis. Tubulin was used as loading control and one experiment representative of 3 is shown. 3 mice of each group are shown.

Supplemental Figure S6: Differentially expressed genes between all control and RASSF4 overexpressing human multiple myeloma cell lines. Differentially expressed genes were identified using DESeq2 R/Bioconductor package (adjusted p-value <= 0.05 and fold change >= 1.5). **A)** MA (Log ratio (M) versus mean average (A) expression) plot showing differentially expressed genes between HMCLs-Ctr (n=3) and HMCLs-RASSF4 (n=3). Significantly differentially upregulated genes in HMCLs-RASSF4 are represented by red dots, while significantly differentially downregulated genes are represented by blue dots. The top 20 significantly differentially expressed genes is shown. **B)** Pathway enrichment analysis of genes downregulated after RASSF4 overexpression. The R/Bioconductor package ReactomePA is used for functional annotation. The top 10 enriched pathways is shown (adjusted p-value <= 0.05).

Supplemental Figure S7: Anti-myeloma activity of trametinib in combination with the HDACi quisinostat or panobinostat. A) Effect on apoptosis. Cell lines were treated with trametinib and/or quisinostat (upper panel) or trametinib and/or panobinostat (lower panel) for 72 hours. Apoptosis was determined by flow cytometry using AnnexinV-FITC/7'AAD staining. Bars and error bars are mean ± SD of 3 independent experiments. P-values and combination indexes are shown in Supplemental Table S5. **B)** Effect of the HDACi panobinostat and/or the MEK1/2 inhibitor trametinib on RASSF4 mRNA expression. qPCR showing the effect of trametinib (100 nM) and/or panobinostat (15 nM) treatment on RASSF4 mRNA expression after 24 hours. Data represent the mean ± SD of 3 independent experiments. Actin was used as reference gene. *indicates $p<0.05$ compared to untreated conditions. $ indicates $p<0.05$ compared to both single agents.