Supplementary Figure 1

A

RD

RH30

B

RMS13

TE381.T

C

RD

RH30

GSK690

JNJ

Ex917

JNJ
Supplementary Figure 1

D

**DNA fragmentation [%]**

**PI-positive cells [%]**

E

**Control**

**GSK690 + JNJ**

**RD**

**RH30**

**Cell viability [% of Ctrl]**

**GSK690**

**JNJ**

**GSK690 + JNJ**
Supplementary Figure 2

A

![Graph A with cell viability data for GSK960 and GSK690 on RD and RH30.

B

![Graph B with cell viability data for Ex917 on RD and RH30.]}
Supplementary Figure 3

A

![Bar chart showing cells per phase of total cells with GSK690 and JNJ treatments.]

B

![Histograms showing PI fluorescence intensity with GSK690 and JNJ treatments.]

Control GSK690

Control JNJ

Control GSK690 + JNJ
Supplementary Figure 4

A

B

C

DNA fragmentation [%]

DNA fragmentation [%]

DNA fragmentation [%]

\[
\begin{array}{ccc}
\text{GSK690} & \text{JNJ} & \text{Ex917} \\
- & + & - \\
- & - & + \\
\end{array}
\]

\[
\begin{array}{ccc}
\text{GSK690} & \text{JNJ} & \text{SAHA} \\
- & + & - \\
- & - & + \\
\end{array}
\]
Supplementary Figure 5

A

RD

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B

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RH30

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C

RD

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Supplementary Figure 6

A

RD

DNA fragmentation [%]

Time [h]

Ctrl

GSK690+JNJ

Ctrl+zVAD

GSK690+JNJ+zVAD

**

* 

RH30

DNA fragmentation [%]

Time [h]

Ctrl

GSK690+JNJ

Ctrl+zVAD

GSK690+JNJ+zVAD

**

* 

B

RD

DNA fragmentation [%]

GSK690

JNJ

Necrostatin-1

+ +

- -

+ +

- -

- +

+ -

+ +

RH30

DNA fragmentation [%]

GSK690

JNJ

Necrostatin-1

+ +

- -

+ +

- -

- +

+ -

+ +
Supplementary Figure 7

A

BIM
-23kD

NOXA
-11kD

β-Actin
-42kD

B

RD

GSK690  -  +  -  +  
JNJ    -  -  +  +  
BCL-2  -  -  23kD  
β-Actin -  -  42kD  
BCL-xL -  -  28kD  
β-Actin -  -  42kD  
MCL-1  -  -  43kD  
β-Actin -  -  42kD  

RH30

GSK690  -  +  -  +  
JNJ    -  -  +  +  
BCL-2  -  -  23kD  
β-Actin -  -  42kD  
BCL-xL -  -  28kD  
β-Actin -  -  42kD  
MCL-1  -  -  43kD  
β-Actin -  -  42kD  

Supplementary Figure 7
Supplementary Figure 8

Cell viability [% of Ctrl]

RD

RH30

GSK690

JNJ

EV

mBCL-2 OE

*
Supplementary Figure Legends

Supplementary Figure 1: GSK690/SAHA combination and Ex917/JNJ-26481585 combination induce cell death in RMS cell lines

A RD cells were treated with 1 µM GSK690 and/or 15 nM JNJ-26481585 and RH30 cells were treated with 10 µM GSK690 and/or 15 nM JNJ-26481585 for 72 hours. Cell death was measured by fluorescence-based microscope analysis of PI uptake using Hoechst 33342 and PI double-staining. B, Cells were treated with 10 µM GSK690 (RMS13) or 1 µM GSK690 (TE381.T) and/or 15 nM JNJ-26481585 for 72 hours. Cell death was determined by flow cytometric analysis of DNA fragmentation of PI-stained nuclei. C and D, Cells were treated for 72 hours with 10 µM Ex917 and/or 15 nM JNJ-26481585 (C) or 1 µM GSK690 (RD cells) or 10 µM GSK690 (RH30 cells) and/or 2 µM SAHA (D). Cell death was determined by flow cytometric analysis of DNA fragmentation of PI-stained nuclei (RD cells) or fluorescence-based microscope analysis of PI uptake using Hoechst 33342 and PI double-staining (RH30 cells). E, Cells were treated with 1 µM GSK690 (RD) or 10 µM GSK690 (RH30) and 15 nM JNJ-26481585 for 120 hours and cell viability was determined by crystal violet assay. In A-E, mean and SD of three independent experiments performed in triplicate are shown; *P<0.05; **P<0.01; ***P<0.001.

Supplementary Figure 2: GSK690 reduces cell viability

A and B RD and RH30 cells were treated with indicated concentrations of GSK690 or Ex917 for 72 hours. Cell viability was determined by MTT assay. In A and B, mean and SD of three independent experiments performed in triplicate are shown.
Supplementary Figure 3: GSK690/JNJ-26481585 cotreatment arrests cells in G2/M phase

RD cells were treated with 1 µM GSK690 and/or 15 nM JNJ-26481585 for 24 hours. DNA content of fixed and PI-stained nuclei was determined by flow cytometry and analyzed with FlowJo software. For A, Mean and SD of three independent experiments performed in triplicate are shown; *P<0.05; **P<0.01; ***P<0.001. In B, the histogram of an exemplarily experiment is shown.

Supplementary Figure 4: GSK690/JNJ-26481585 combination treatment does not affect non-malignant C2C12 myoblasts

A-C, C2C12 myoblast cells were treated 1 µM GSK690 and/or 15 nM JNJ-26481585 (A), 10 µM Ex917 and/or 15 nM JNJ-26481585 (B) or 1 µM GSK690 and/or 2 µM SAHA (C) for 72 hours. Cell death was measured by flow cytometric analysis of DNA fragmentation of PI-stained nuclei. In A-C, mean and SD of three independent experiments performed in triplicate are shown.

Supplementary Figure 5: LSD1 and HDAC inhibitors are target specific for the respective histone modification

A and B, Cells were treated with HDAC (15 nM JNJ-26481585; 2 µM SAHA) and LSD1 inhibitors (1 µM GSK690 (RD); 10 µM GSK690 (RH30); 10 µM Ex917) for 3 hours. Histone acetylation and H3K4 dimethylation were detected by Western blotting. Histone H3 and β-Actin served as loading controls. C, RD Cells were treated with 1 µM GSK690 and/or 15 nM JNJ-26481585 for 1 hour. Histone acetylation was detected by Western blotting. Histone H3 and β-Actin served as loading controls.
Supplementary Figure 6: Rescue with zVAD.fmk at early time points for GSK690/JNJ-26481585 combination treatment

A. Cells were treated for indicated time points with 1 µM GSK690 (RD) or 10 µM GSK690 (RH30) and/or 15 nM JNJ-26481585 in the presence or absence of 50 µM zVAD.fmk. Cell death was measured by flow cytometric analysis of DNA fragmentation of PI-stained nuclei (RD) or by fluorescence-based microscope analysis of PI uptake using Hoechst 33342 and PI double-staining (RH30). B. Cells were treated for 72 hours with 1 µM GSK690 (RD) or 10 µM GSK690 (RH30) and 15 nM JNJ-26481585 in the presence or absence of 50 µM Necrostatin-1. Cell death was measured by flow cytometric analysis of DNA fragmentation of PI-stained nuclei. In A and B, mean and SD of three independent experiments performed in triplicate are shown; *P<0.05; **P<0.01; ***P<0.001.

Supplementary Figure 7: Expression levels of BCL-2 proteins in RMS cells

A. Constitutive protein levels of NOXA and BIM of untreated RD and RH30 cells were assessed by Western blotting, β-Actin was used as loading control. B, Cells were treated with 1 µM GSK690 (RD) or 10 µM GSK690 (RH30) and/or 15 nM JNJ-26481585 for 21 hours (RD) and 15 hours (RH30). Protein levels of BCL-2, BCL-xL and MCL-1 were detected by Western blotting, β-Actin served as loading control.

Supplementary Figure 8: Overexpression of BCL-2 partially rescues GSK690/JNJ-26481585-induced reduction of cell viability

Cells were treated with 1 µM GSK690 (RD) or 10 µM GSK690 (RH30) and/or 15 nM JNJ-26481585 for 36 hours (RD) and 24 hours (RH30). Cell viability was determined with crystal violet assay. Mean and SD of three independent experiments performed in triplicate are shown; *P<0.05; **P<0.01; ***P<0.001.
Supplementary Materials and Methods

Determination of cell viability
For crystal violet staining cells were stained for 10 minutes in crystal violet solution (0.5% crystal violet, 30% ethanol, and 3% formaldehyde), washed with tap water and air-dried. For colorimetric measurement crystal violet was resolved in 1% SDS and absorbance at 550 nM was quantified by microplate reader (Infinite M100, Tecan, Männedorf, Switzerland).

Cell cycle analysis
DNA content of fixed and PI-stained nuclei was determined by flow cytometry and analyzed by FlowJo software (Tree Star Inc., Ashland, OR, USA) according to the manufacturer's instructions.
Supplementary Table T1: Synergistic induction of cell death by GSK690 and JNJ-26481585

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Combination index was calculated by CalcuSyn software as described in Materials and Methods for data on GSK690- and/or JNJ-26481585-induced cell death as shown in Fig. 1A; CI<0.9 indicates synergism, 0.9-1.1 additivity and CI>1.1 antagonism.
## Supplementary Table 2: List of Primers

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