Supplementary materials



Figure S1. Analysis of phosphorylated and total 4EBP1, GSK3 β and ERK1/2 protein expression by immunoblotting in SEM (**a**), RS4;11 (**b**), NALM-6 (**c**) and REH (**d**). Cells were incubated with the stated concentrations of MK-2206 for the indicated time periods. The displayed immunoblots are representative captions of at least two individual biological replicates. GAPDH was used as internal loading control. Blots were processed and cropped using Image Studio Lite 5.2 software and MS PowerPoint (2011) to improve clarity and conciseness.



Figure S2. Analysis of apoptosis induction by cleaved and total Caspase 3 expression via immunoblotting in SEM (**a**), RS4;11 (**b**), NALM-6 (**c**) and REH (**d**). Cells were incubated with the stated concentrations of MK-2206 for the indicated time periods. GAPDH was used as internal loading control. Blots were processed and cropped using Image Studio Lite 5.2 software and MS PowerPoint (2011) to improve clarity and conciseness.

Table S1. *P* values of SEM cells treated with increasing concentrations of MK-2206 compared to DMSO-treated control cells. Three to five individual biological replicates were considered for the statistical calculation of significance by student's t test after testing Gaussian distribution. Significance is defined by P < 0.05 (*), P < 0.01 (**) and P < 0.001 (***).

	Proliferation 48 h	Proliferation 72 h	Metabolic activity 48 h	Metabolic activity 72 h
0.01 µM	0.1110	0.2396	0.0448 *	0.5826
0.05 μM	0.0149 *	0.1865	0.0446 *	0.5267
0.1 μM	0.0015 **	0.1226	0.0141 *	0.0362 *
0.5 μΜ	0.0006 ***	0.0002 ***	0.0003 ***	0.0012 **
1.0 μM	0.0009 ***	0.0001 ***	0.0004 ***	0.0004 ***
5.0 μM	0.0002 ***	0.0000 ***	0.0001 ***	0.0000 ***
10.0 μM	0.0002 ***	0.0001 ***	0.0023 ***	0.0001 ***

Table S2. *P* values of SEM cells treated with increasing concentrations of MK-2206 compared to DMSO-treated control cells. Three to five individual biological replicates were considered for the statistical calculation of significance by student's t test after testing Gaussian distribution. Significance is defined by P < 0.05 (*), P < 0.01 (**) and P < 0.001 (***).

	Proliferation 48 h	Proliferation 72 h	Metabolic activity 48 h	Metabolic activity 72 h
0.01 μM	0.3819	0.7044	0.0264 *	0.7703
0.05 μM	0.6704	0.9160	0.0118 *	0.4330
0.1 µM	0.2955	0.0338 *	0.0136 *	0.1119
0.5 μΜ	0.0311 *	0.0100 *	0.0010 **	0.0012 **
1.0 µM	0.0101 *	0.0275 *	0.0008 ***	0.0002 ***
5.0 μM	0.0006 ***	0.0036 **	0.0000 ***	0.0000 ***
10.0 μM	0.0025 **	0.0008 ***	0.0003 ***	0.0000 ***

Table S3. *P* values of SEM cells treated with increasing concentrations of MK-2206 compared to DMSO-treated control cells. Three to five individual biological replicates were considered for the statistical calculation of significance by student's t test after testing Gaussian distribution. Significance is defined by P < 0.05 (*), P < 0.01 (**) and P < 0.001 (***).

	Proliferation 48 h	Proliferation 72 h	Metabolic activity 48 h	Metabolic activity 72 h
0.01 µM	0.4594	0.4237	0.1305	0.6046
0.05 μΜ	0.0464 *	0.1359	0.1320	0.8551
0.1 μΜ	0.0208 *	0.0094 **	0.0346 *	0.9930
0.5 μΜ	0.0126 *	0.0002 ***	0.0040 **	0.0038 **
1.0 μΜ	0.0046 **	0.0015 **	0.0030 **	0.0030 **
5.0 µM	0.0003 ***	0.0000 ***	0.0002 ***	0.0005 ***
10.0 µM	0.0000 ***	0.0000 ***	0.0000 ***	0.0000 ***

Table S4. *P* values of SEM cells treated with increasing concentrations of MK-2206 compared to DMSO-treated control cells. Three to five individual biological replicates were considered for the statistical calculation of significance by student's t test after testing Gaussian distribution. Significance is defined by P < 0.05 (*), P < 0.01 (**) and P < 0.001 (***).

	Proliferation 48 h	Proliferation 72 h	Metabolic activity 48 h	Metabolic activity 72 h
0.01 µM	0.5476	0.0302 *	0.6741	0.5963
0.05 μM	0.4013	0.0905	0.0607	0.0162 *
0.1 μM	0.0998	0.0274 *	0.0026 **	0.0023 **
0.5 μM	0.0022 **	0.0061 **	0.0014 **	0.0009 ***
1.0 μM	0.0412 *	0.0043 **	0.0015 **	0.0035 **
5.0 μM	0.0050 **	0.0060 **	0.0008 ***	0.0015 **
10.0 μM	0.0001 ***	0.0001 ***	0.0001 ***	0.0000 ***

Table 5. List of antibodies used for immunoblot analyses.

Antibody	Host	Clone	Dilution	Supplier
GAPDH	Mouse	ZG003	1:20,000	Invitrogen
Phospho AKT (Ser473)	Rabbit	polyclonal	1:500	Cell Signaling
AKT	Rabbit	polyclonal	1:1,000	Cell Signaling
Phospho 4EBP1 (Ser65)	Rabbit	174A9	1:1,000	Cell Signaling
4EBP1	Rabbit	polyclonal	1:1,000	Cell Signaling
Phospho GSK3β (Ser9)	Mouse	D2Y9Y	1:500	Cell Signaling
GSK3β	Mouse	3D10	1:1,000	Cell Signaling
Phospho-ERK (Tyr202/Tyr204)	Rabbit	polyclonal	1:1,000	Cell Signaling
ERK	Rabbit	137F5	1:1,000	Cell Signaling
Cleaved Caspase-3 (Asp175)	Rabbit	D175	1:500	Cell Signaling
Caspase-3	Rabbit	polyclonal	1:500	Cell Signaling
IRDye 680 goat anti-mouse	Goat		1:20,000	LI-COR
IRDye 800 goat anti-mouse	Goat		1:20,000	LI-COR
IRDye 680 goat anti-rabbit	Goat		1:20,000	LI-COR
IRDye 800 goat anti-rabbit	Goat		1:20,000	LI-COR