Table S1

Drugs	Manufacturer	Concentrations		
Drugs in screen		**MM1.s**	**OPM-2**	**L363**
Melphalan	Sigma-Aldrich	2.5 µM	5 µM	10 µM
Vincristine	Selleck Chemicals	20 nM	3 nM	3 nM
Dexamethasone	Sigma-Aldrich	100 nM	12.5 nM	100 nM
Bortezomib	Tebu Bio	4 nM	4 nM	8 nM
Panobinostat (LBH589)	Selleck Chemicals	5 nM	5 nM	20 nM
BCL-2i ABT-199 / Venetoclax	LKT Laboratories	100 nM	100 nM	100 nM
MCL-1i S63845	Servier	100 nM	10 nM	10 nM
BCL-XLi A-1155463	MedChemExpress	100 nM	100 nM	100 nM
Other drugs in study				
Rapamycin (Sirolimus)	Selleck Chemicals			
P70S6K1 inhibitor (PF-4708671)	Selleck Chemicals			
Copanlisib (BAY 80-6946)	Selleck Chemicals			
Prednisolone	Sigma-Aldrich			

Supplemental Table 1. Overview of drugs. Overview of drugs incorporated in this study, with specified concentrations used per HMCL for the lethality screen.

Table S2

Characteristics	MM1	MM2	MM3	MM4	MM5	MM6	MM7	MM8	MM9
Age at diagnosis, y	53	52	59	66	80	55	51	68	48
Sex	female	male	male	male	male	male	male	male	male
Plasma cells, %	38	70	67	70	20	75	34	36	37
Immunoglobulin type	IgG	IgG	nd	IgG	IgA	IgA	IgG	nd	IgG
Light chain type	lambda	kappa	lambda	lambda	kappa	lambda	kappa	lambda	lambda
ISS stage at diagnosis	1	1	3	1	1	2	3	1	3
Cytogenetics									
hyperdiploidy	no	yes	no	yes	yes	nd	no	no	no
amplifications / deletions	$1q^{+}$,$13q^{-}$	no	$1q^{+}$,$13q^{-}$	no	$13q^{-}$	$1q^{+}$, $13q^{-}$	$13q^{-}$	no	no
translocations	t(14;20)	no	no	no	no	t(4;14)	t(14;16)	no	no

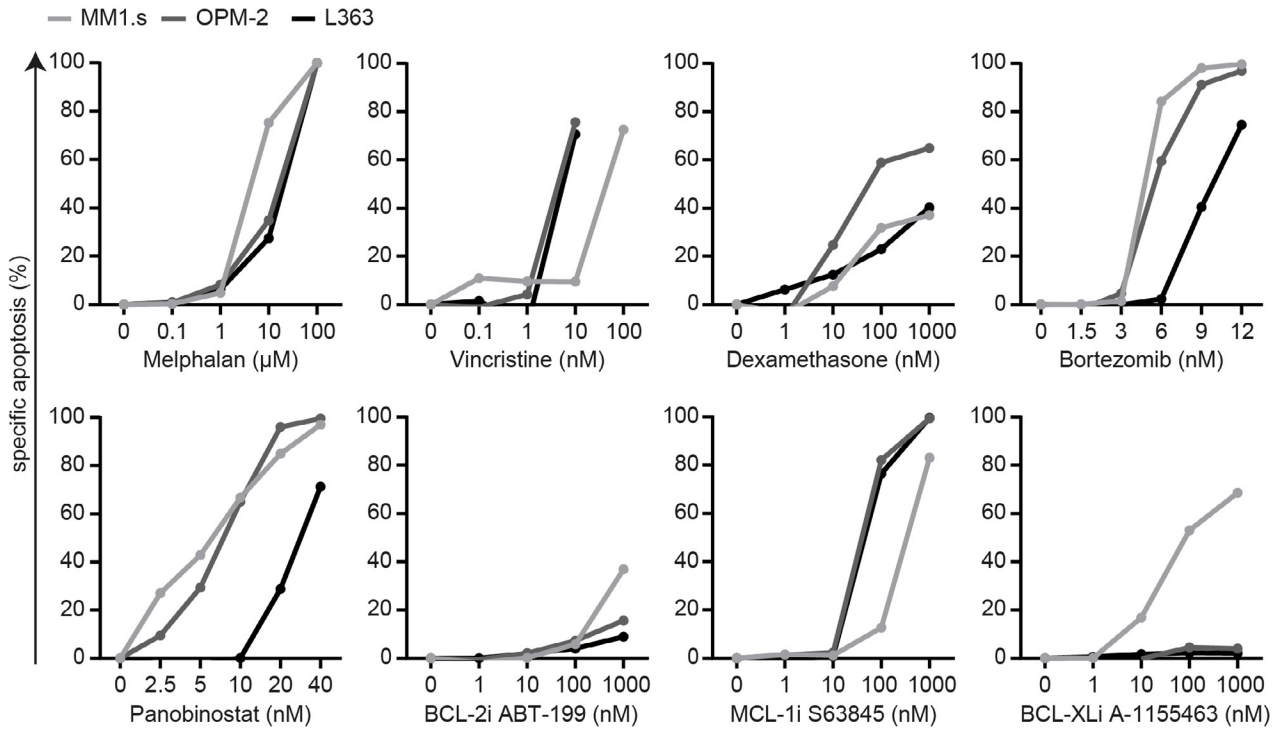
Supplemental Table 2. Overview of clinical characteristics and cytogenetics of MM patients. MM1-MM9 indicate the 9 unique MM patients included in this study. ISS, international staging system; y, year; nd, no data.

Table S3

Antibody	Clone	Manufacturer
rabbit anti-MCL-1	Y37	Abcam
mouse anti-BCL-2	124	Cell Signaling
rabbit anti-BCL-XL	54H6	Cell Signaling
rabbit anti-FOXO1	C29H4	Cell Signaling
rabbit anti-FOXO3a	D19A7	Cell Signaling
rabbit anti-pGSK3β Ser9	D85E12	Cell Signaling
rabbit anti-4E-BP1	53H11	Cell Signaling
rabbit anti-p4E-BP1 Thr37/46	236B4	Cell Signaling
rabbit anti-P70S6K	49D7	Cell Signaling
rabbit anti-pP70S6K Thr389	108D2	Cell Signaling
mouse anti-S6	54D2	Cell Signaling
rabbit anti-pS6 Ser235/236	D57.2.2E	Cell Signaling
rabbit anti-pS6 Ser240/244	D68F8	Cell Signaling
rabbit anti-pAkt Ser473	D9E	Cell Signaling
mouse anti-Akt	40D4	Cell Signaling
rabbit anti-BIM	C34C5	Cell Signaling
rabbit anti-pBad Ser136	D25H8	Cell Signaling
mouse anti-NOXA	114C307,1	Novus Bio
mouse anti-α-tubulin	DM1A	Cell Signaling
mouse anti-lamin B1		Cusabio
mouse anti-GAPDH	GA1R	Invitrogen

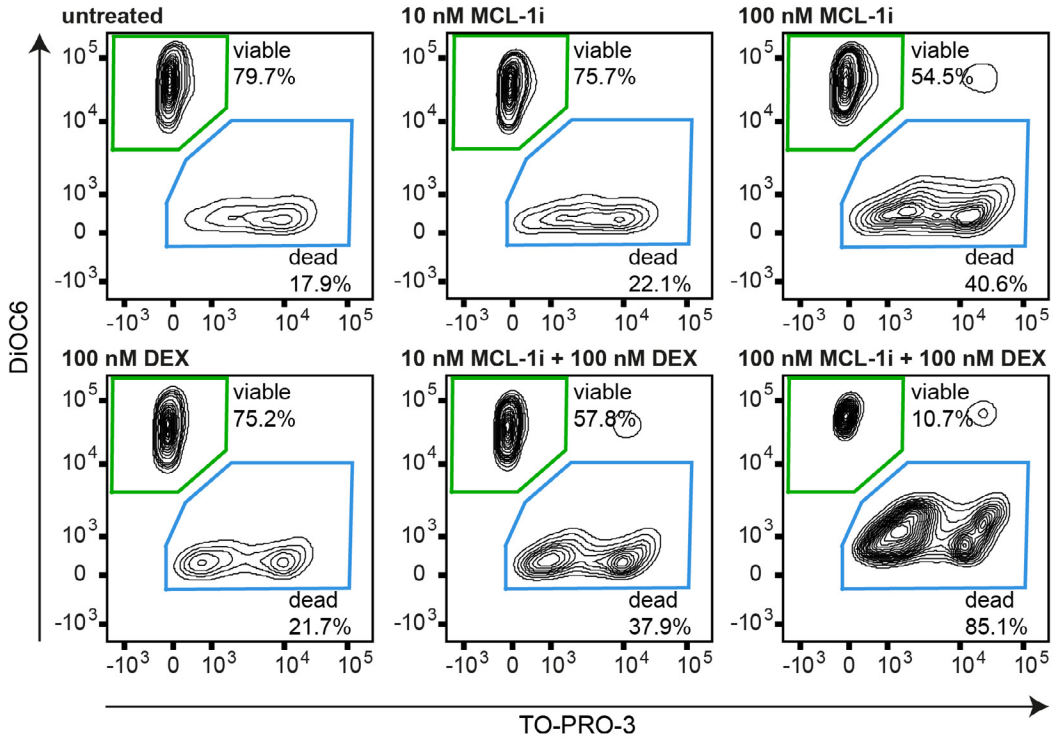
Supplemental Table 3. Overview of primary antibodies used for western blots in the study.

Figure S1



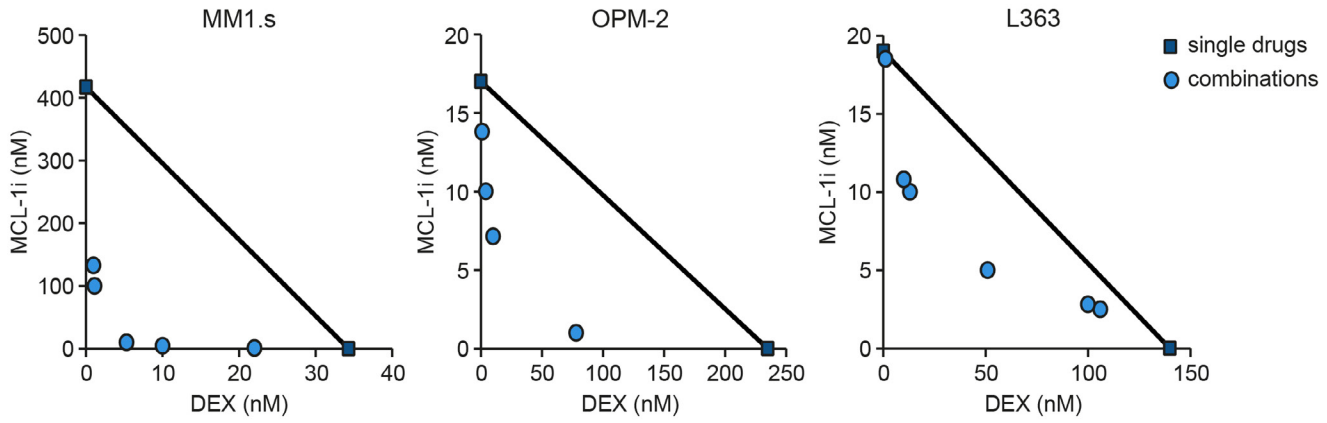
Supplemental Figure 1. Specific apoptosis induced by increasing concentrations of indicated drugs in HMCLs. The HMCLs MM1.s, OPM-2 and L363 were exposed to indicated concentrations of melphalan, vincristine, dexamethasone, bortezomib and panobinostat for 48 hours. In addition, HMCLs were exposed to indicated concentrations of BH3-mimetics BCL-2 inhibitor ABT-199, MCL-1 inhibitor S63845 and BCL-XL inhibitor A-1155463 for 24 hours.

Figure S2



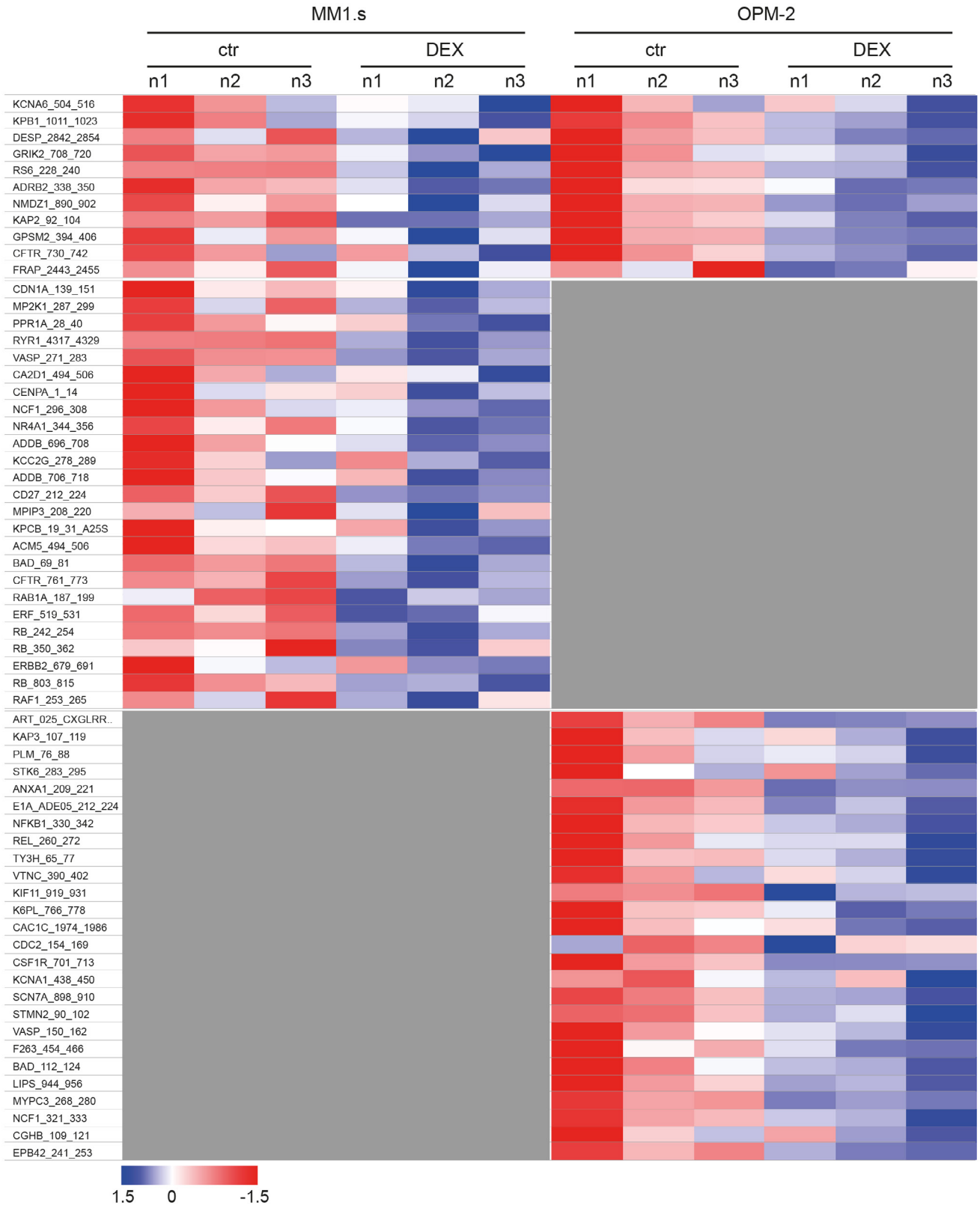
Supplemental Figure 2. Induction of apoptosis by dexamethasone and MCL-1i. Representative flow cytometric analysis plots of apoptosis induction in HMCLs after 48 hours of exposure to the indicated concentrations of dexamethasone (DEX) or MCL-1i. Gates represent viable ($DiOC6^+$/$TO\text{-}PRO\text{-}3^-$) and dead ($DiOC6^-$/$TO\text{-}PRO\text{-}3^+$) MM1.s cells.

Figure S3



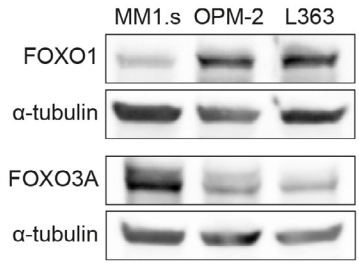
Supplemental Figure 3. Dexamethasone synergizes with MCL-1i to induce apoptosis of HMCLs. Representative isobolograms of indicated HMCLs exposed to combinations of dexamethasone (DEX) and MCL-1i for 48 hours. Blue dots indicate the drug combination that induced 50% specific apoptosis (IC_{50}) for MM1.s and OPM-2 or 25% specific apoptosis (IC_{25}) for L363. The blue squares indicate the IC_{50} (MM1.s and OPM-2) or IC_{25} (L363) of the single drugs. The black lines connecting both single drug data points indicate an exact additive effect with a combination index (CI) of 1. All data points to the left of this line have a CI < 1, indicating synergy for the drug combination.

Figure S4



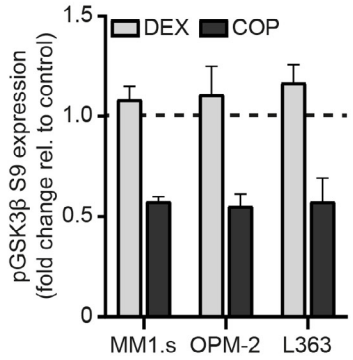
Supplemental Figure 4. Dexamethasone significantly reduces peptide phosphorylation by STKs in HMCLs. Heatmap showing peptides with significantly reduced phosphorylation by serine/threonine kinases (STK) in MM1.s and OPM-2 exposed for 4 hours to 1 µM dexamethasone (DEX) or DMSO control (ctr). Per treatment group 3 technical replicates were included. Statistical analysis was performed by paired t-tests. Grey fields indicate no statistically significant results for the specific peptides in the indicated HMCL.

Figure S5



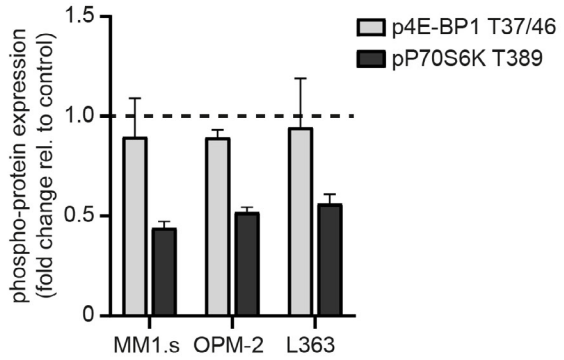
Supplemental Figure 5. FOXO protein expression in HMCLs. Western blot showing FOXO1 and FOXO3a protein expression in untreated HMCLs. Alpha-tubulin was used as a loading control.

Figure S6



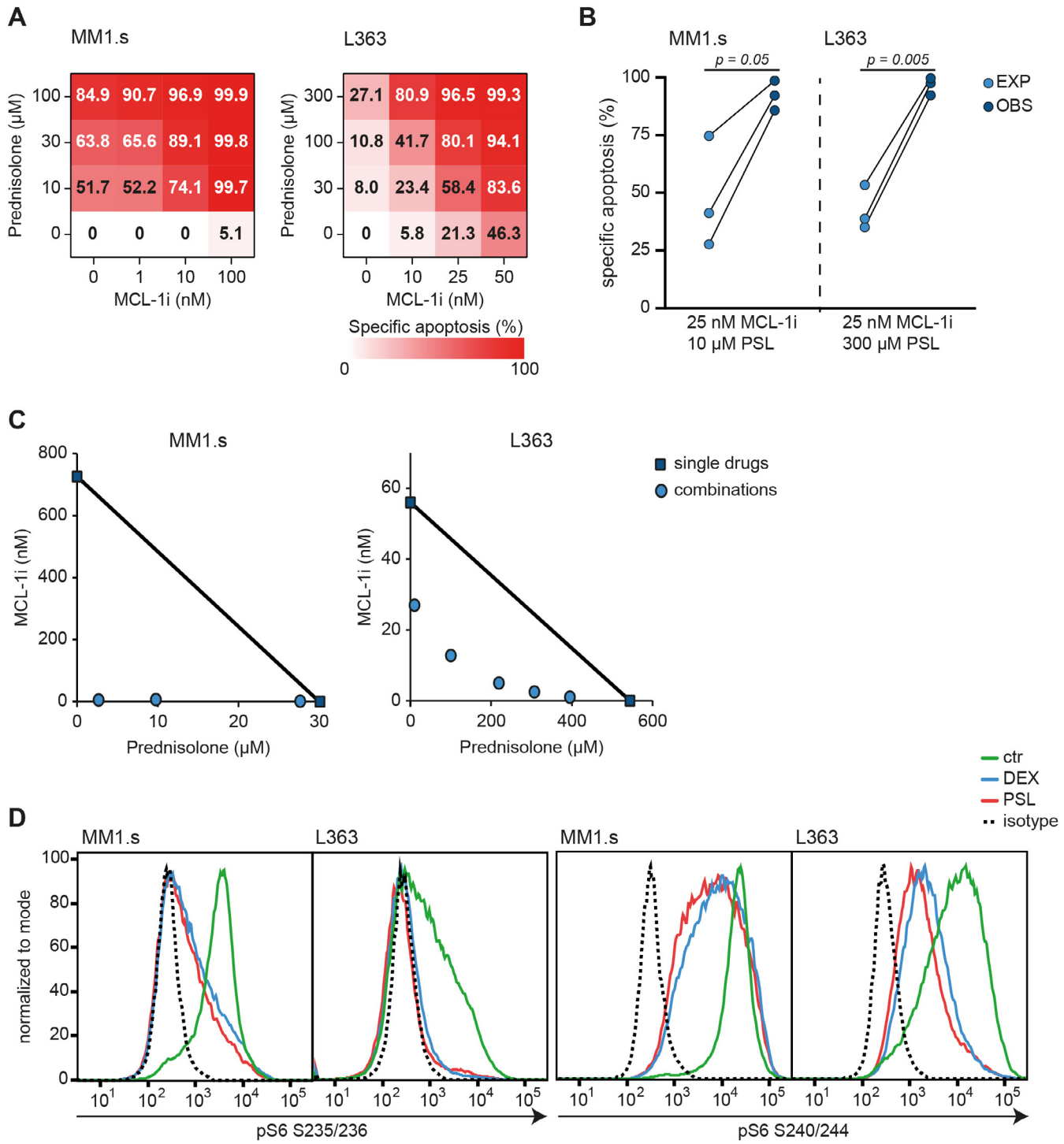
Supplemental Figure 6. Exposure of HMCLs to dexamethasone does not reduce inhibitory GSK3β phosphorylation. Quantification of GSK3β phosphorylation on serine residue 9 (pGSK3β S9) in indicated HMCLs exposed to 1 μM dexamethasone (DEX) or 100 nM copanlisib (COP) for 4 hours. Quantification was performed on western blots of 2 individual experiments, normalized to α-tubulin for loading control and relative to untreated control cells, indicated by the dashed line.

Figure S7



Supplemental Figure 7. Dexamethasone reduces phosphorylation of mTORC1 substrates 4E-BP1 and P70S6K. Quantification of 4E-BP1 phosphorylation on threonine 37/46 residues (p4E-BP1 T37/46) and P70S6K phosphorylation on threonine residue 389 (pP706SK T389) in indicated HMCLs exposed to 1 μM dexamethasone for 4 hours. Quantification was performed on western blots of 2 individual experiments, normalized to total 4E-BP1 or total P70S6K protein expression for loading control and relative to untreated control cells, indicated by the dashed line.

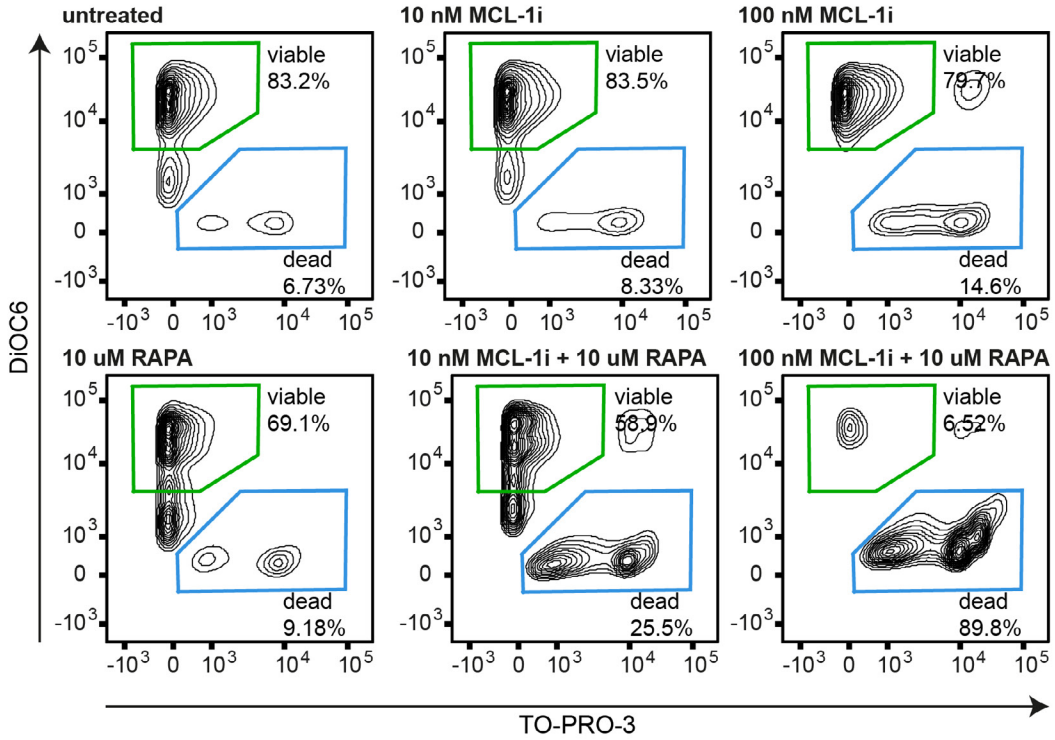
Figure S8



Supplemental Figure 8. Prednisolone synergizes with MCL-1i to induce apoptosis of HMCLs and reduces phosphorylation of S6. (A) Heatmaps showing specific apoptosis of indicated HMCLs induced by serial dilutions of prednisolone and MCL-1i, individual or combined. Viability was analyzed after 48 hours of drug exposure, values represent the mean of 3 individual experiments. (B) Plots comparing EXP to OBS specific apoptosis induced by prednisolone (PSL) and MCL-1i combinations. Per HMCL the drug combination that resulted in the highest average OBS-EXP ratio was selected from the data obtained in panel A. The 3 connected data points show the data obtained from 3 individual experiments. Statistical analysis was performed by paired t-tests. (C) Representative isobolograms of indicated HMCLs exposed to combinations of prednisolone and MCL-1i for 48 hours. Blue dots indicate the drug combination that induced 50% specific apoptosis (IC_{50}), the blue squares indicate the IC_{50} of the single drugs. The black lines connecting both single drug data points indicate an exact additive effect with a CI of 1. All data points to the left of this line have a CI < 1,

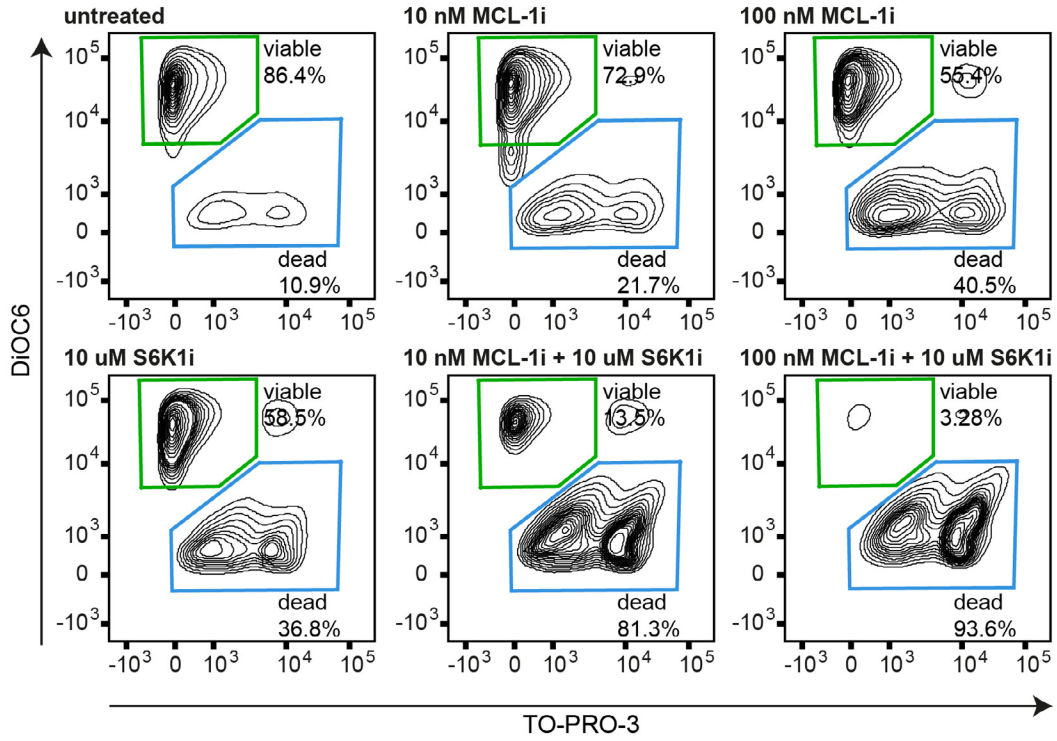
indicating synergy for the drug combination. (D) Histograms comparing expression of S6 phosphorylation on serine residues 235/236 (pS6 S235/236) and 240/244 (pS6 S240/244) in indicated HMCLs after 4 hours of exposure to 1 μM dexamethasone (DEX), 100 μM PSL for MM1.s or 300 μM PSL for L363, determined by flow cytometric analysis. Expression was relative to untreated control cells (ctr) and corrected for isotype control, indicated by the dashed line.

Figure S9



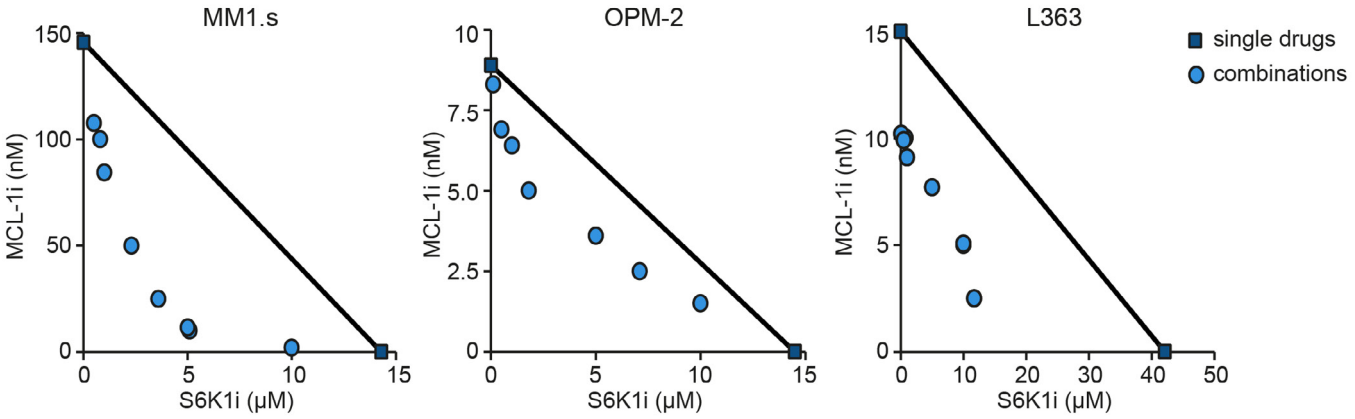
Supplemental Figure 9. Induction of apoptosis by rapamycin and MCL-1i. Representative flow cytometric analysis plots of apoptosis induction in HMCLs after 48 hours of exposure to the indicated concentrations of rapamycin (RAPA) and MCL-1i. Gates represent viable ($DiOC6^{+}$/$TO\text{-}PRO\text{-}3^{-}$) and dead ($DiOC6^{-}$/$TO\text{-}PRO\text{-}3^{+}$) MM1.s cells.

Figure S10



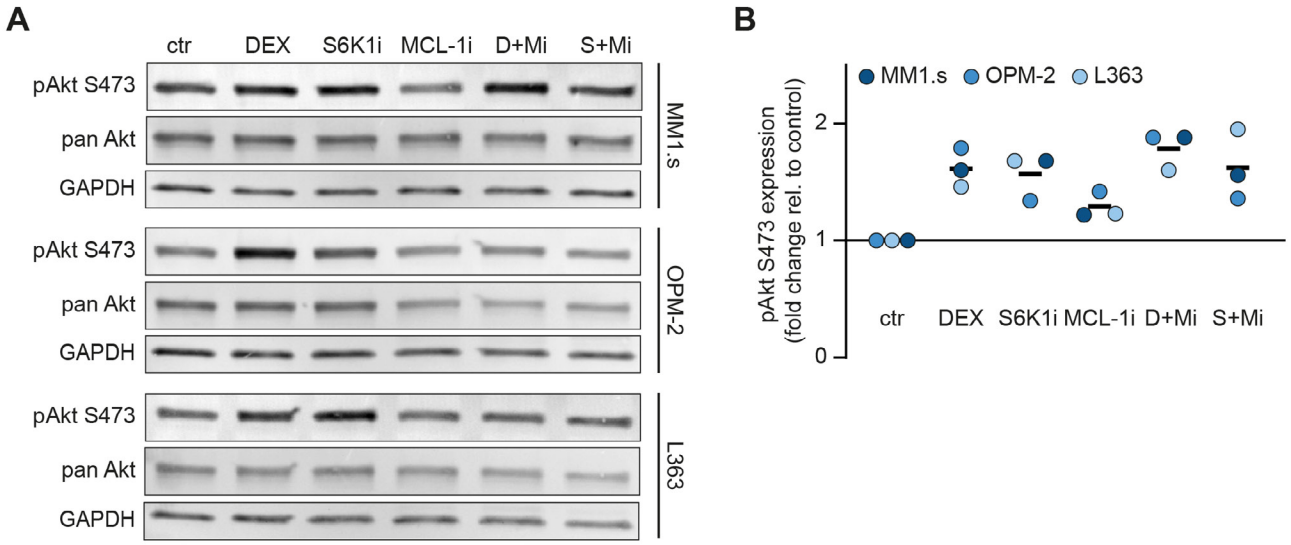
Supplemental Figure 10. Induction of apoptosis by S6K1i and MCL-1i. Representative flow cytometric analysis plots of apoptosis induction in HMCLs after 48 hours of exposure to the indicated concentrations of S6K1i and MCL-1i. Gates represent viable ($DiOC6^+/TO\text{-}PRO\text{-}3^-$) and dead ($DiOC6^-/TO\text{-}PRO\text{-}3^+$) MM1.s cells.

Figure S11



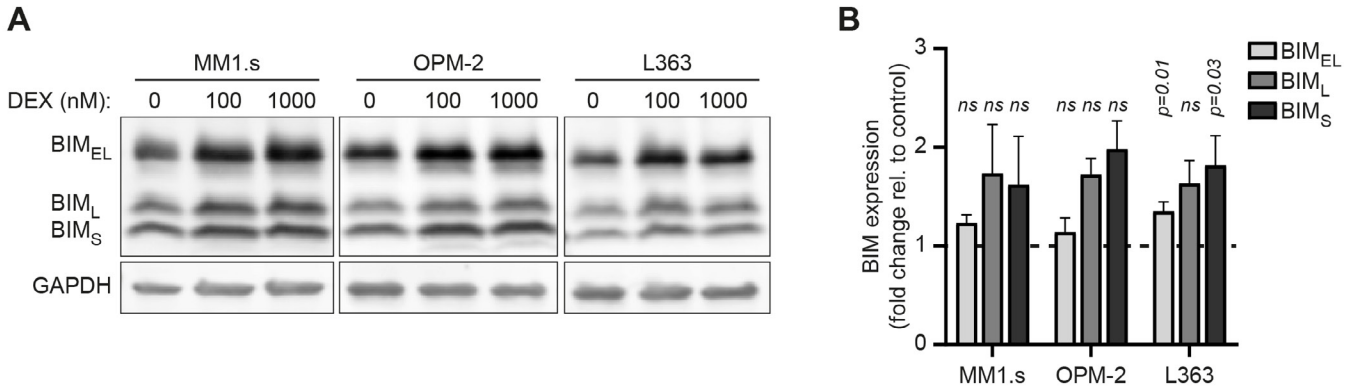
Supplemental Figure 11. S6K1i synergizes with MCL-1i to induce apoptosis of HMCLs. Representative isobolograms of indicated HMCLs exposed to combinations of S6K1i and MCL-1i for 48 hours. Blue dots indicate the drug combinations that induced 50% specific apoptosis (IC_{50}). The blue squares indicate the IC_{50} of the single drugs. The black lines connecting both single drug data points indicate an exact additive effect with a CI of 1. All data points to the left of this line have a CI < 1, indicating synergy for the drug combination.

Figure S12



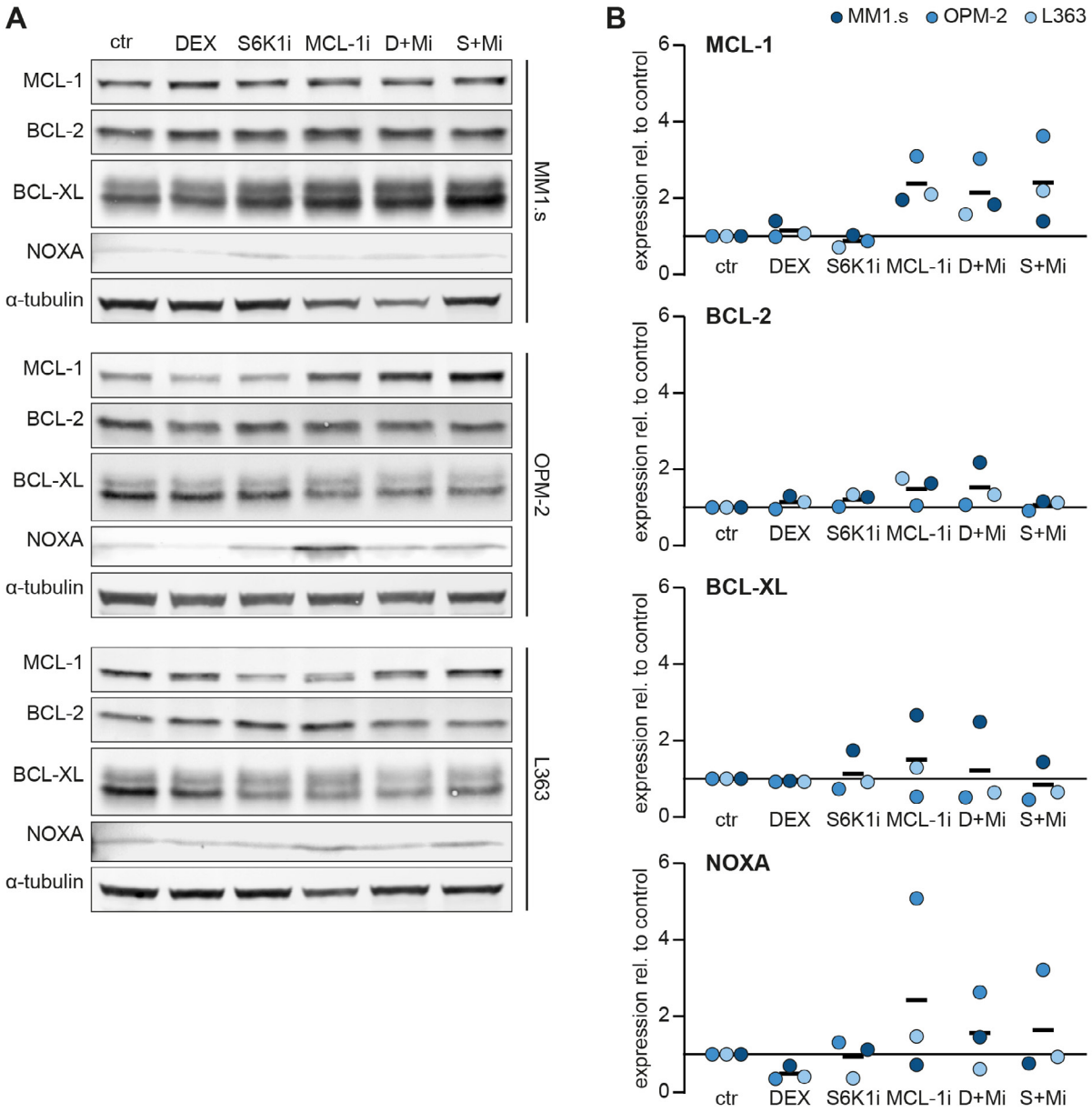
Supplemental Figure 12. Dexamethasone and S6K1i have similar effects on Akt kinase activity. (A) Representative western blot showing phosphorylation of Akt on the activating serine 473 residue (pAkt S473) in indicated HMCLs after 4 hours of exposure to 100 nM dexamethasone (DEX; D), 1 μM S6K1i (S), MCL-1i (Mi; 100 nM for MM1.s and 25 nM for OPM-2 and L363) and combinations thereof, relative to pan Akt and GAPDH as a loading control. Ctr indicates untreated control cells. (B) Quantification of pAkt S473 expression, normalized to pan Akt protein expression and relative to untreated control cells (ctr). All datapoints show the mean quantification that was performed on western blots of 2 individual experiments as described for panel A. The black lines indicate the mean value of the 3 indicated HMCLs.

Figure S13



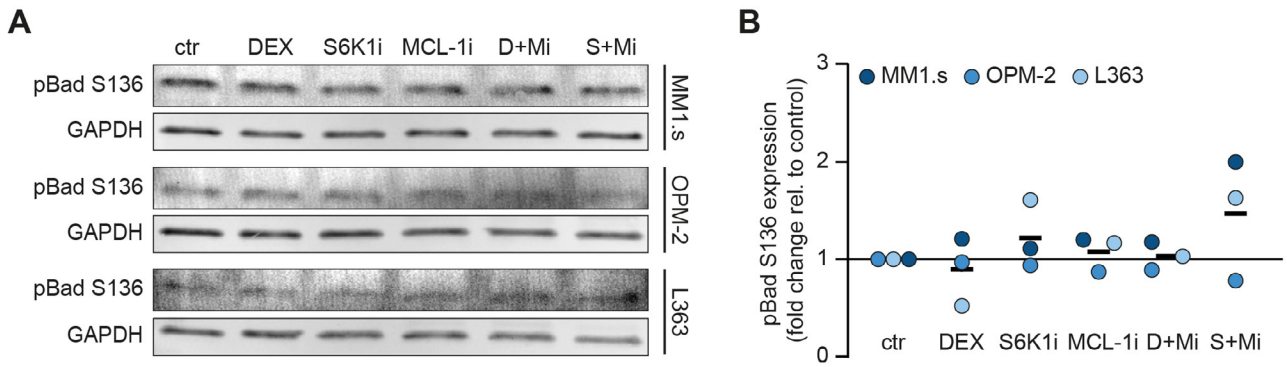
Supplemental Figure 13. Exposure of HMCLs to dexamethasone increases BIM protein expression in a dose-dependent manner. (A) Representative western blot showing protein expression of 3 BIM isoforms in indicated HMCLs exposed to indicated concentrations of dexamethasone (DEX) for 24 hours. (B) Quantification of 3 BIM isoforms protein expression in indicated HMCLs exposed to 1 μM dexamethasone for 24 hours. Quantification was performed on western blots of 3 individual experiments, normalized to GAPDH for loading control and relative to untreated control cells, indicated by the dashed line. Statistical analysis was performed by paired t-tests; ns, not significant.

Figure S14



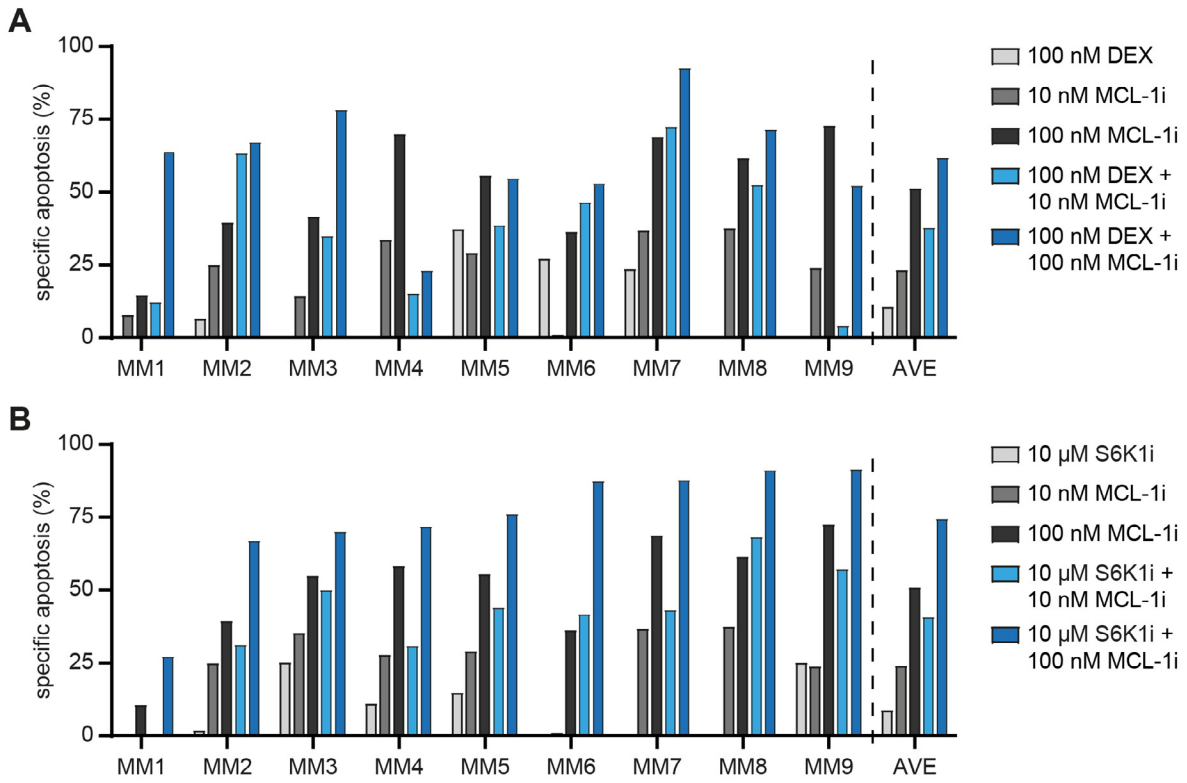
Supplemental Figure 14. Exposure of HCMLs to dexamethasone, S6K1i, and MCL-1i do not consistently alter protein expression of BCL-2 family members. (A) Representative western blot showing protein expression of pro-survival proteins MCL-1, BCL-2 and BCL-XL and BH3-only pro-apoptotic protein NOXA in indicated HMCLs exposed for 24 hours to 100 nM dexamethasone (DEX; D), 1 μM S6K1i (S), MCL-1i (Mi; 100 nM for MM1.s and 25 nM for OPM-2 and L363) and combinations thereof in the presence of 10 μM pan caspase inhibitor Q-VD-OPh, relative to α-tubulin as a loading control. Ctr indicates untreated control cells. (B) Quantification of MCL-1, BCL-2, BCL-XL and NOXA protein expression, normalized to α-tubulin protein expression and relative to untreated control cells (ctr). All datapoints show the mean quantification that was performed on western blots of 2 individual experiments as described for panel A. The black lines indicate the mean value of the 3 indicated HMCLs.

Figure S15



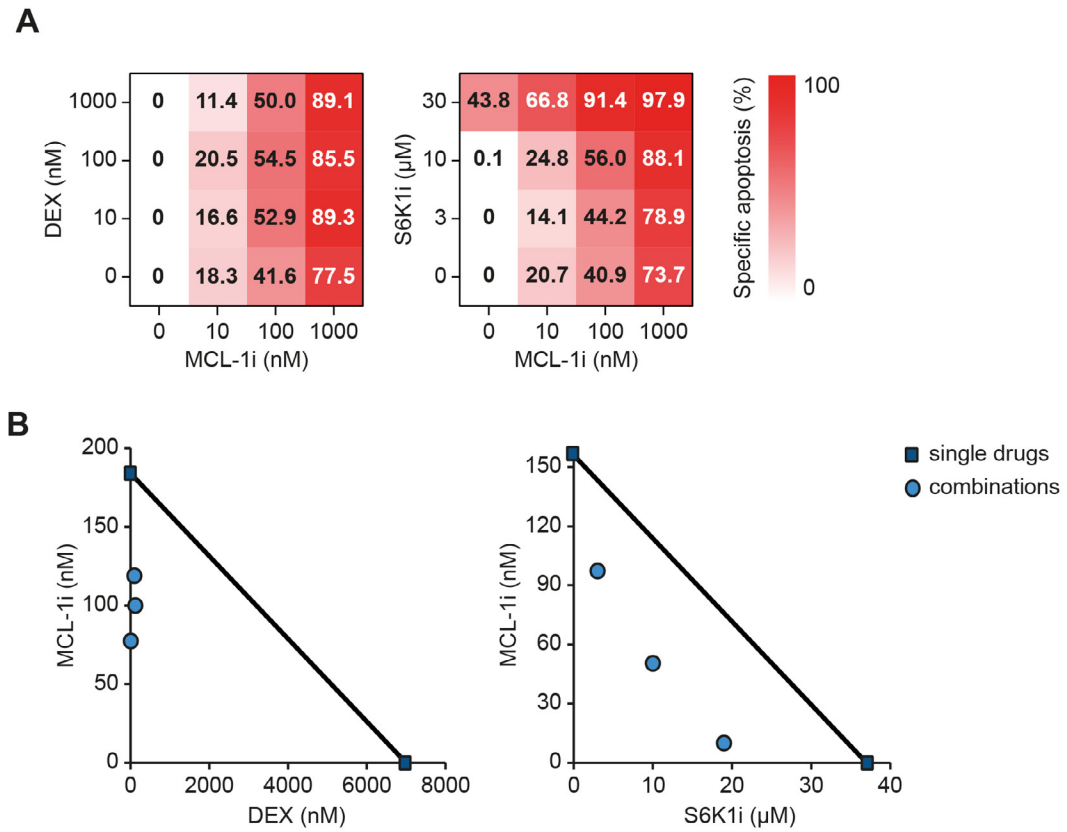
Supplemental Figure 15. Exposure of HCMLs to dexamethasone, S6K1i, and MCL-1i do not consistently alter pro-apoptotic Bad activity. (A) Representative western blot showing phosphorylation of Bad on the activating serine 136 residue (pBad S136) in indicated HMCLs exposed for 4 hours to 100 nM dexamethasone (DEX; D), 1 μM S6K1i (S), MCL-1i (Mi; 100 nM for MM1.s and 25 nM for OPM-2 and L363) and combinations thereof, relative to GAPDH as a loading control. Ctr indicates untreated control cells. (B) Quantification of pBad S136 protein expression, normalized to GAPDH protein expression and relative to untreated control cells. All datapoints show the mean quantification that was performed on western blots of 2 individual experiments as described for panel A. The black lines indicate the mean value of the 3 indicated HMCLs.

Figure S16



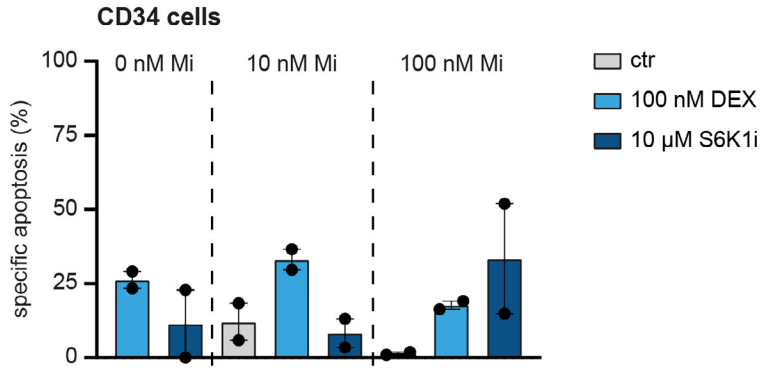
Supplemental Figure 16. Induction of apoptosis by dexamethasone, S6K1i and MCL-1i in individual primary MM samples. Specific apoptosis of $CD38^{+}$ primary MM cells after 48 hours of drug exposure. (A) Treatment with 100 nM dexamethasone (DEX), 10 nM MCL-1i, 100 nM MCL-1i or DEX-MCL-1i drug combinations. (B) Treatment with 10 µM S6K1i, 10 nM MCL-1i, 100 nM MCL-1i or S6K1i-MCL-1i drug combinations. Mean values of the 9 primary MM patient samples (AVE) are represented by bars separated by the dashed line.

Figure S17



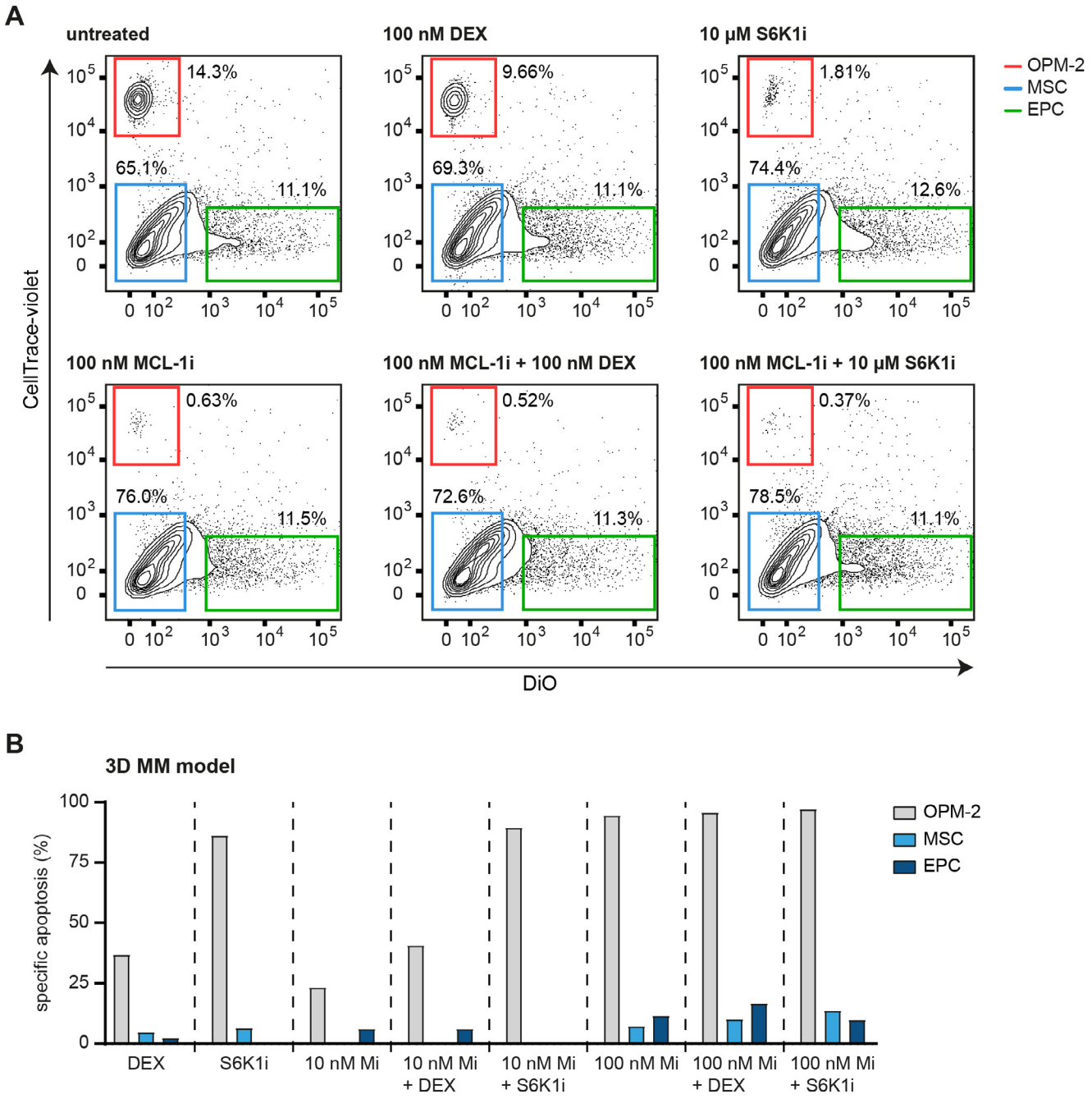
Supplemental Figure 17. Dexamethasone and S6K1i synergize with MCL-1i to induce apoptosis of primary MM cells. (A) Heatmaps showing specific apoptosis induced by single and combined serial dilutions of dexamethasone (DEX) and MCL-1i (left) or single and combined serial dilutions of S6K1i and MCL-1i (right). Viability of $CD38^{+}$ primary MM cells was analyzed after 48 hours of drug exposure, values represent the mean of 3 unique MM patient samples. (B) Isobolograms of the indicated drug combinations as described for panel A. Blue dots indicate the drug combination that induced 50% specific apoptosis (IC_{50}), the blue squares indicate the IC_{50} of the single drugs. For dexamethasone the IC_{50} was > 7000 nM, but it was set to 7000 nM to be able to calculate CI values. The resulting CI (<1) therefore underestimates the synergistic effect with MCL-1i. All datapoints show the mean values of the 3 unique MM patient samples included, as in panel A.

Figure S18



Supplemental Figure 18. Dexamethasone and S6K1i induce limited toxicity in combination with MCL-1i in healthy donor CD34$^+$ stem and precursor cells. Specific apoptosis induced by 100 nM dexamethasone (DEX) or 10 µM S6K1i as single drug dosages or in combination with 10 nM and 100 nM MCL-1i (Mi), relative to untreated control cells. Viability of CD34$^+$ cells was analyzed after 48 hours of drug exposure by negative TO-PRO-3 staining. The bars show the mean of the 2 unique healthy donor umbilical cord blood samples that were included, represented by the individual data points.

Figure S19



Supplemental Figure 19. Dexamethasone-MCL-1i and S6K1i-MCL-1i combinations kill myeloma cells but induce minimal toxicity of stem and progenitor cells in a pre-clinical 3D MM model. (A) Representative flow cytometric analysis plots showing relative contribution of OPM-2 (labeled with CellTrace-violet), EPC (labeled with DiO) and MSC (unlabeled) to the viable population of cells (pre-gated on TO-PRO-3 negative cells) after 48 hours of exposure to 100 nM dexamethasone (DEX), 10 µM S6K1i, and 100 nM MCL-1i as single drug dosages or combinations thereof. (B) Quantification of specific apoptosis induced in OPM-2, MSC and EPC analyzed as described for panel A after 48 hours of exposure to DEX, S6K1i, and 10 nM or100 nM MCL-1i (Mi), relative to untreated control cells. The plot combines the results of 2 individual experiments.