Supplementary Figures

Supplementary Figure 1: Characterization of BEZ235 effects on MYC protein stability and cell proliferation.

A. Half-life of total MYC and pT58 MYC in SW480, SW620 cells treated with BEZ235 (200nM, 24h) and HCT116 (WT and *FBXW7*-deficient) cells. Half-life was calculated from immunoblots as shown in Figure 1A and 1C. Note that half-life estimates in HCT116 wild type cells are less precise due to longer time points.

B. Levels of total MYC and pT58 MYC measured in control SW620 cells and in cells treated with 200nM BEZ235 for 24h.

C. Effect of BEZ235 on 4E-BP1 in colorectal cancer cell lines. Cells were treated with BEZ235 (200nM, 48h). Immunoblots were probed with the indicated antibodies (n=2) (left panel). 4E-BP1 mRNA levels were assessed by RQ-PCR (n=2) (right panel).

D. Growth curves of CACO2, HCT116, Ls174T and SW620 cells treated with BEZ235 (200nM) or solvent control. Error bars indicate SD of triplicates (n=3).

E. FACS analysis of cell cycle phases of SW480 cells in response to BEZ235 (200nM, 24h) or solvent control. Error bars indicate SD of triplicate biological replicates (n=3).

F. Growth curve of SW480 cells treated with BEZ235 (200nM) for two days ("once BEZ") or permanently treated. Error bars indicate SD of triplicate biological replicates (n=3).

Supplementary Figure 2: Effect of BEZ235 on MYC-dependent gene expression in colorectal cancer cell lines.

A. SW620 cells were transfected with indicated siRNAs. 48h after transfection cells were treated with BEZ235 (200nM, 24h). Isolated RNA was subjected to microarray analysis. Left panel: Immunoblot of indicated proteins. Right panel: MYC mRNA levels were assessed by RQ-PCR.

B. GSEA plots comparing BEZ235-treated and non-treated situation in control cells.

C. Boxplots of well-characterized gene sets in response to BEZ235 treatment, MYC knockdown or a combination of both. Gene sets contain upregulated MYC target genes (left panel) and genes involved in translation (right panels). p-values were calculated using Mann Whitney U test.

D. Summary of GSEA analysis of up- or downregulated gene sets upon BEZ235 treatment. First column indicates rank in regulated gene sets, second column indicates name of the gene sets, third column indicates nominal p-value, fourth column the FDR q-value.

Supplementary Figure 3: Effect of BEZ235 on MAP-kinase signaling.

A. SW620 cells were treated with BEZ235 (500nM, 24h) and lysates were immunoblotted with the indicated antibodies (n=2).

B. The indicated cell lines were transfected with siRNA targeting p110alpha subunit of PI3K (+) or a control siRNA. 72h after transfection indicated protein levels were determined by immunoblotting (n=2).

C. RQ-PCR expression analysis of FOXO3A target genes upon BEZ235 treatment (200nM, 24h) of SW480 cells (n=2).

D. Expression of mRNAs encoding the indicated tyrosine kinases. SW480 cells were transfected with siRNA targeting FOXO3A or a non-targeting control. 48h after transfection

cells were treated with BEZ235 (200nM, 24h). Total RNA was isolated and subjected to RQ-PCR analysis (n=2).

Supplementary Figure 4: Characterization of the 4E-BP1(4A) allele and eIF4A inhibitors.

A. SW480 4E-BP1(4A) cells were treated with BEZ235 (200nM) or increasing concentration of DOX for 48h. Cell lysates were probed for the indicated proteins.

B. Ls174T 4E-BP1(4A) cells were incubated with DOX. Upper panel shows FACS analysis for cell cycle phase in response to DOX (24h) or solvent control (n=3). Lower panel shows colony assay stained with crystal violet 5 days after induction.

C. SW480 cells were treated with BEZ235 (200nM) and the indicated concentrations of Rocaglamide. Cells were harvested after 48h and immunoblots probed with the indicated antibodies (n=2).

D. Growth curve of SW480 cells treated with Silvestrol (25nM) or solvent control. Error bars indicate SD of biological triplicates (n=3).

E. FACS analysis showing cell cycle distribution of SW480 and Ls174T cells in response to Silvestrol (25nM, 24h) or solvent control. Error bars indicate SD of triplicate biological replicates (n=3).

F. Cell cycle analysis of SW480 cells in response to MYC knockdown, silvestrol treatment or a combination of both.

Supplementary Figure 5: Additional characterization of translation in colon carcinoma.

A. Polysome fractionation of SW480 cells (Figure 5B) treated with BEZ235 (200nM), DOX, Silvestrol (25nM) or solvent control for 24h. RNA was isolated from the indicated fractions and relative mRNA content of *PFN2* was measured by RQ-PCR (n=2).

B. SW480 cells were incubated with BEZ235 (200nM), Cymarin (100nM) or both. Cells were harvested after 48h and immunoblots probed with the indicated antibodies (n=2),

C. Boxplots of PDCD4-, eIF4A-, eIF4E-, 4E-BP2- and 4E-BP1-expression in colorectal tissue (n=24) and colorectal carcinoma (n=36). Data are taken from (49). p-values were calculated using Student´s t-test.

D. Tissue from colon cancer and normal mucosa was stained with indicated antibodies. The section was digitalized with a 200x magnification. Each panel shows a representative staining at cancer to normal mucosa junction (black line) (n=10).

Supplementary Figure 6: BEZ235 does not alter proliferation or MYC levels in wild type or APC deficient intestinal enterocytes.

A. Representative H&E staining of BEZ235-treated versus control wild type or APC-deficient intestines. Please note that the controls in this Figure show the same pictures as in Figure 5 as mice were dosed at the same time.

B. Representative BrdU staining of proliferation in BEZ235-treated versus control wild type or APC deficient intestines. Although APC deficiency conferred the expected increase in proliferation ($p=0.04$, Mann Whitney), BEZ235 did not significantly alter proliferation in wild type or APC deficient intestines. The number of BrdU positive nuclei per crypt-villus axis was scored in 30 full crypts in at least 3 mice.

C. Representative Ki67 staining showing BEZ235 did not alter proliferation in wild type or APC deficient intestines.

D. MYC staining of BEZ235-treated versus control wild type or APC-deficient intestines.

E. CDKN1A staining showing an increase in CDKN1A positivity in APC deficient intestines following BEZ235 treatment. (Upper row: magnification 200x; lower line magnification 400x of the villus / crypt junction).

Supplementary Figure 7: Additional characterization of silvestrol and BEZ235 effects *in vivo*.

A. *In situ* hybridization of *Myc* mRNA in silvestrol, BEZ235 and control treated wild type or APC deficient intestines. The panel shows three representative pictures for each condition.

B. Quantification of MYC protein levels in the lower half of the crypt after silvestrol and BEZ235 treatment.

Supplementary Table 1: Reagents used in this study.

A. List of antibodies.

B. Primer sequences.

A

Company	Ordering number	Protein
Cell signaling	9401	pMYC T58
	9272	AKT
	9275	pAKT T308
	9315	GSK3
	9336	p-GSK3 S9
	2212	S6
	2215	p-S6 S240/244
	2972	mTOR
	2971	p-mTOR S244
	9464	pFOXO 1/3A
	9452	4E-BP1
	9455	p-4E-BP1 T70
	9451	p-4E-BP1 S65
	2855	p-4E-BP1 t37/46
	4249	PI3K
	9101	p-ERK1/2 T202/204
	9121	p-MEK S217/221
	2067	eIF4E
	2013	eIF4A
	2498	eIF4G
Abcam	AB3207	MYC
	AB51156	p-MYC S62
Santa Cruz	13136	CDK2
	11351	FOXO3A
	154	ERK1/2
	764	MYC
Sigma	A5441	Beta-ACTIN
	V9131	VINCULIN
	HPA001032	PDCD4
BD Biosciences	347580	anti-BrdU
Dako	M7202	CDKN1A
Thermo	RM-9106	KI-67
others	9E10	MYC

B

Oligo	Sequence
B2MG forward	GTGCTCGCGCTACTCTCT
B2MG reverse	GTCAACTTCAATGTCGGAT
MYC forward	CACCAGCAGCGACTCTGA
MYC reverse	GATCCAGACTCTGACCTTTTGC
P21 forward	CGAAGTCAGTTCCTTGTGGAG
P21 reverse	CATGGGTTCTGACGGACAT
MUC2 forward	CATGGGTTCTGACGGACAT
MUC2 reverse	GAACACGGTGGTCCTCTTGT
HER3 forward	CACAATGCCGACCTCTCC
HER3 reverse	CACGAGGACATAGCCTGTCA
IR forward	ACACGATGAATTCCAGCAACT
IR reverse	CGATGGTCTTCTCGCCTTC
IGF1R forward	AAAAACCTTCGCCTCATCCT
IGF1R reverse	TGGTTGTCGAGGACGTAGAA
CCND1 forward	GCTGTGCATCTACACCGACA
CCND1 reverse	TTGAGCTTGTTCACCAGGAG
P27 forward	CCGGCTAACTCTGAGGACAC
P27 reverse	GGCCCCAAACACATTCTATG
GADD45 forward	GAGAGCAGAAGACCGAAAGG
GADD45 reverse	TGACTCAGGGCTTTGCTGA
PFN2 for	GGCAGAGCTGGTAGAGTCTTG
PFN2 rev	TAGCAGCTAGAACCCAGAGTCTC
TUBB3 for	GCAACTACGTGGGCGACT
TUBB3 rev	ATGGCTCGAGGCACGTACT
B-ACTIN for	CCAACCGCGAGAAGATGA
B-ACTIN rev	TCCATCACGATGCCAGTG