Evasion of apoptosis is a hallmark of cancer, which is frequently mediated by upregulation of the antiapoptotic BCL-2 family proteins. In colorectal cancer (CRC), previous work has highlighted differential antiapoptotic protein dependencies determined by the stage of the disease. While intestinal stem cells (ISCs) require BCL-2 for adenoma outgrowth and survival during transformation, ISC-specific MCL1 deletion results in disturbed intestinal homeostasis, eventually contributing to tumorigenesis. Colon cancer stem cells (CSCs), however, no longer require BCL-2 and depend mainly on BCL-XL for their survival. We therefore hypothesized that a shift in antiapoptotic protein reliance occurs in iSCs as the disease progresses from normal to adenoma to carcinoma. By targeting antiapoptotic proteins with specific BH3 mimetics in organoid models of CRC progression, we found that BCL-2 is essential only during ISC transformation while MCL1 inhibition did not affect adenoma outgrowth. BCL-XL, on the other hand, was crucial for stem cell survival throughout the adenoma-to-carcinoma sequence. Furthermore, we identified that the limited window of BCL-2 reliance is a result of its downregulation by miR-17-5p, a microRNA that is upregulated upon APC-mutation driven transformation. Here we show that BCL-XL inhibition effectively impairs adenoma outgrowth in vivo and enhances the efficacy of chemotherapy. In line with this dependency, expression of BCL-XL, but not BCL-2 or MCL1, directly correlated to the outcome of chemotherapy-treated CRC patients. Our results provide insights to enable the rational use of BH3 mimetics in CRC management, particularly underlining the therapeutic potential of BCL-XL targeting mimetics in both early and late-stage disease.
ABT-199 [17]. In the colon, loss of BCL-2 or BCL-XL does not impair intestinal homeostasis while recent data suggest that MCL1 deletion causes aberrant cell death, thereby prompting Wnt-dependent proliferation that eventually leads to tumor formation, indicating potential detrimental side-effects of targeting this protein [14, 17, 18].

To better assess the role of antiapoptotic proteins in CRC progression, we made use of a panel of genetically-engineered colon organoids that reflect the classical progression pathway of CRC [19–21]. Clonogenic assays with specific BH3 mimetics revealed that ISCs require BCL-2 activity solely during transformation and that this dependence is lost quickly after, while MCL1 inhibition does not affect ISC clonogenicity and BCL-XL is essential for ISC survival throughout CRC progression. Here we show that loss of sensitivity to BCL-2 inhibition is apparent after acquisition of an APC mutation, which is mediated by the upregulation of microRNA-17-5p that targets and downregulates BCL-2. Furthermore, we find that BCL-XL inhibition impairs adenoma outgrowth in vivo and augments chemotherapy-induced cell death in tumor-derived organoids. Our results provide the mechanism behind transformation-driven changes in antiapoptotic protein dependence and highlight the therapeutic potential of BCL-XL inhibition in CRC.

RESULTS
BCL-2 and BCL-XL are essential for ISC survival during transformation
Previously we have shown BCL-2 to be critical for stem cell survival following loss of Apc [17]. We confirmed the importance of BCL-2 for ISC transformation by performing a clonogenic assay with small intestinal organoids from Lgr5CreERT2Apcfl/fl mice. This assay measures survival of the stem cell compartment, which is crucial for the clonogenic potential of the cultures (Fig. 1a). Treatment with BCL-2 specific inhibitor ABT-199 simultaneously with tamoxifen-induced Apc loss reduced outgrowth of Apc−/− cells, while non-induced organoids remained unaffected by BCL-2 inhibition (Fig. 1b). In the same study, BCL-XL inhibitor WEHI-539 did not affect outgrowth of Apc−/− organoids, thus indicating that BCL-XL is nonessential for adenoma survival [17]. However, BH3 profiling has recently shown that WEHI-539 is only weakly efficient in inducing apoptosis in BCL-XL dependent cells while another higher affinity inhibitor, A-1155463, is far more potent [22, 23].

Analyzing both BCL-XL inhibitors confirmed that WEHI-539 did not impair adenoma outgrowth while A-1155463 clearly reduced Apc−/− organoid survival during transformation (Fig. 1c). The observed decrease in organoid clonogenicity was not a result of toxicity as wild-type organoids were insensitive to the combination of tamoxifen and BH3 mimetics (Supplementary Fig. 1a). MCL1 deletion has been shown to drastically affect several cell types including the colon epithelium, however specific MCL1 inhibitors seem to be well tolerated in several preclinical cancer models [18, 24, 25]. To assess the role of MCL1 in ISC transformation, we tested a specific inhibitor, AZD5991, and found that MCL1 targeting did not impair Apc−/− organoid outgrowth (Fig. 1d). Taken together, our results provide evidence that BCL-2 and BCL-XL, but not MCL1, are crucial for Apc-mutant stem cell survival during transformation.

In contrast to the dependency observed during transformation, we have shown that tumor-derived CSCs are solely BCL-XL-dependent, pointing to a loss of the initial BCL-2 dependence [16]. We confirmed this finding with ABT-199, AZD5991, and A-1155463, where only BCL-XL inhibition induced significant cell death in Wnt-high CSCs, measured by the percentage of cells with active caspase-3 (Supplementary Fig. 1b) [26]. To ensure that the difference in ABT-199 sensitivity between early and late stage disease was not due to a difference between small intestine (SI) and colon-derived cultures, we also tested the sensitivity of Lgr5CreERT2Apcfl/fl colon-derived organoids to BCL-2 and BCL-XL inhibition during transformation and observed strong dependency on both (Fig. 1e), while MCL1 inhibition again had no impact on adenoma outgrowth (Fig. 1f). Our results therefore indicate that while BCL-2 is essential for Apc-mutant stem cell survival during transformation, this dependence is reduced at a certain stage during CRC progression.

BCL-2 dependence is lost immediately after transformation
The most frequently mutated genes in the classical CRC progression pathway, namely APC, KRAS, P53, and SMAD4, were previously altered in human colon-derived organoids using CRISPR-Cas9-mediated genome editing [20]. We employed this panel of organoids to assess changes in antiapoptotic protein dependence of ISCs. Clonogenic outgrowth of normal colon organoids was unaffected by treatment with either ABT-199 or A-1155463 (Fig. 2a, b) while in APC knock-out (APC−/−) organoids, ABT-199 treatment had no effect (Fig. 2c) and A-1155463 treatment strongly impaired organoid clonogenicity (Fig. 2d). To corroborate this finding, we analyzed activation of apoptosis by cleavage of caspase-3 and found clear induction only in APC−/− organoids treated with A-1155463, indicating that apoptosis is induced in these organoids upon BCL-XL, but not BCL-2, inhibition (Fig. 2e). Similarly, mouse Apc−/− adenomas were also insensitive to ABT-199 treatment while A-1155463 clearly impaired adenoma outgrowth, indicating that this is not a difference between mouse and human-derived cultures (Supplementary Fig. 2a, b). To exclude that A-1155463 induced apoptosis could arise from off-target effects, shRNA knockdown of BCL-XL was performed. Strikingly, BCL-XL knockdown severely impaired survival of APC−/− organoids, while knockdown with a control shRNA had no effect (Fig. 2f and Supplementary Fig. 2c).

Reciprocally, BCL-XL overexpression induced significant resistance to A-1155463 in APC−/− organoids (Fig. 2g and Supplementary Fig. 2d), thus confirming that impaired clonogenicity upon A-1155463 treatment was a direct result of BCL-XL inhibition. In addition, we tested MCL1 dependency with AZD5991, which had no effect on the clonogenicity of both wild-type and APC−/− organoids (Supplementary Fig. 2e, f). This pattern of sensitivity to BCL-XL inhibition and absence of BCL-2 and MCL1 dependency in transformed ISCs was maintained throughout the progression panel of organoids with increasing mutation loads (Fig. 2h and Supplementary Fig. 2g). Altogether these results indicate that BCL-2 exerts its pro-survival functions during transformation alone while BCL-XL is required for apoptosis evasion throughout the adenoma-to-carcinoma sequence. Intriguingly, triple (APC−/−KRASG12DPS3Ko) and quadruple (APC−/−KRASG12DPS3KoSMAD4Ko) mutant organoids did show slightly increased resistance to A-1155463, which is consistent with the role of P53 in apoptosis induction [27]. In agreement, shRNA-mediated knockdown of Smad4 in mouse Apc−/−KrasG12DPS3Ko organoids did not induce increased resistance to A-1155463, while quadruple mutant organoids were again more resistant (Supplementary Fig. 2h). This P53 mutation induced resistance to A-1155463 is likely due to the observed downregulation of its known proapoptotic targets such as PUMA and BAX [27, 28] (Supplementary Fig. 7e, f).

BCL-XL is crucial for stem cell survival in patient-derived adenoma and tumor organoids
To ascertain the clinical relevance of the above findings, we tested the response of organoids derived from familial adenomatous polyposis (FAP) and CRC patients to antiapoptotic protein inhibition. Tubular adenomas (TA) derived from FAP patients represent precursor lesions that have an increased likelihood of developing into carcinomas and are normally the result of loss or mutation of the wild-type Apc allele [19]. All four tested TA cultures were insensitive to increasing doses of BCL-2 inhibition.
with ABT-199 and treatment did not induce cleavage of caspase-3, similar to the APCKO human organoids (Fig. 3a, c and Supplementary Fig. 3a, c). Conversely, BCL-XL inhibition strongly impaired adenoma outgrowth and A-1155463 treated TAs showed elevated expression of cleaved caspase-3 (Fig. 3b, c and Supplementary Fig. 3b, c). Also in this setting, MCL1 inhibition did not impair adenoma clonogenicity, in agreement with the minimal impact of AZD5991 on APCKO organoids (Fig. 3d).

### Fig. 1 BCL-2 and BCL-XL are essential for ISC survival during transformation.

**a** Overview of the organoid clonogenic assay. **b–d** Graphs depict relative outgrowth (number of organoid structures relative to the number prior to treatment and reseeding) of proximal small intestine (SIP)-derived organoid structures quantified 3 days after passaging for the indicated genotype, either without treatment or upon treatment with 1 µM **b** ABT-199, **c** A-1155463 and WEHI-539, or **d** AZD5991, at the time of induction. Each dot represents a replicate (minimal \( n = 7 \) per condition, \( n = 3 \) independent experiments), error bars indicate s.e.m. ***\( p < 0.001 \), ****\( p < 0.0001 \), student’s t-test. **e, f** Graphs depict relative outgrowth of colon-derived organoid structures quantified 3 days after passaging for the indicated genotype, upon treatment with 1 µM **e** ABT-199 and A-1155463 or **f** AZD5991, at the time of induction. Each dot represents a replicate (minimal \( n = 5 \) per condition, \( n = 2 \) independent experiments), error bars indicate s.e.m. *\( p < 0.05 \), **\( p < 0.01 \), ordinary one-way ANOVA.
Tumor organoids established from patient-derived CRC biopsies have been shown to closely reflect several characteristics of the original tumor [21]. We tested four of these tumor organoids (p6T, p9T, p16T, and p24aT), all of which were previously confirmed to have alterations in APC, KRAS, and P53, with p16T also carrying a SMAD4 mutation [21]. As expected, all four tumor organoids were more sensitive to BCL-XL inhibition as the IC50s for A-1155463 were much lower than for ABT-199 and AZD5991 (Fig. 3e), confirming that classical CRC precursor and carcinoma lesions depend on BCL-XL for survival while BCL-2 and MCL1 remain nonessential in this context.
BCL-2 expression is decreased upon transformation

In a study that assessed determinants of BH3 mimetic efficacy, sensitivity to BCL-2 inhibition could be predicted by expression levels of the protein, where BCL-2 expressing cells respond better to ABT-199 than non-expressers [28]. Having established BCL-2 inhibition to be ineffective after ISC transformation, we therefore determined whether this relates to its expression pattern during CRC progression by analyzing four publicly-available expression datasets. This revealed a significant decrease in BCL-2 expression in adenomas compared to healthy tissue and a further decrease in CRC tumors, which was also confirmed in the TCGA COREAD cohort (Fig. 5a and Supplementary Fig. 5a). Conversely, BCL-XL expression increased upon progression from normal to adenoma and furthermore in the carcinoma stage (Fig. 5b and Supplementary Fig. 5b). At the protein level, BCL-2 was detected in normal crypts, however its expression was nearly absent in adjacent tumor tissue, while BCL-XL showed strong positive staining in both normal and tumor tissue (Fig. 5c, d). Independent confirmation with a single-cell RNA sequencing dataset of normal and CRC tumor samples [30] revealed a significant decrease in BCL-2 and concomitant increase in BCL-XL, specifically in the epithelial tumor compartment (Fig. 5e, f). Based on these observations, we next ascertained whether the observed decrease in BCL-2 expression occurs already upon acquisition of an APC mutation, specifically in the stem cell compartment where BCL-2 is specifically expressed [17]. To do so, we made use of a validated colon stem cell marker, PTK7, and confirmed its function as a stem cell marker for APC KO human organoids as well [31] (Supplementary Fig. 5c, d). Analysis of BCL-2 expression in PTK7-high stem cells of normal and APC KO human organoids confirmed a decrease in stem cell BCL-2 expression upon loss of APC (Fig. 5g and Supplementary Fig. 5e, g). A similar analysis of BCL-XL expression showed no change in expression between normal and APC KO stem cells (Fig. 5h and Supplementary Fig. 5f, g). Our data thus indicates that transformation results in a decrease in BCL-2 expression, which explains the observed lack of impact of BCL-2 inhibition in APC KO and TA cultures.

MIR-17-5p regulates BCL-2 expression in APC-mutant organoids

Next, we assessed the mechanism by which BCL-2 expression levels decrease upon transformation. We first evaluated dependency status by analyzing the TCGA COAD dataset on cBioPortal, where miR-17-5p was not downregulated by any alterations in the gene (Fig. 6a). Evaluation of promoter methylation in the same dataset showed an absence of methylation in the BCL-2 promoter region (Fig. 6a), further confirmed by treatment of APC KO organoids with the demethylating agent decitabine, which did not affect BCL-2 mRNA levels (Fig. 6b). The BCL-2 gene is located on 18q and could therefore be lost as a consequence of loss of heterozygosity (LOH) of this chromosomal region, which frequently occurs in CRC, albeit at a later stage of the disease [32–34]. However, karyograms of APC KO human organoids did not show loss of 18q [20]. Similarly, single-cell karyotype sequencing to quantify copy number alterations revealed no 18q LOH in any of the TA cultures, thereby excluding LOH as the underlying mechanism of decreased BCL-2 expression in adenomas (Fig. 6c and Supplementary Fig. 6a).

Several microRNAs show aberrant expression in colon adenoma and carcinoma samples in comparison to normal tissue [35]. We examined the most commonly upregulated microRNAs in early adenomas and found several of them to have binding sites on the BCL-2 promoter region, which frequently occurs in CRC, albeit at a later stage of the disease [32–34]. Of these, the miR-17-92 family was of particular interest as it is a direct target of β-catenin, upregulated upon mutation in the APC gene [37]. Several members of this microRNA family have high miRmap scores for binding in the 3′ UTR of BCL-2 (Supplementary Table 1) [35, 36]. Of note, miR-17 and miR-18a have previously been shown to target BCL-2 in a luciferase reporter assay and one or more binding sites for miR-17-5p and miR-18a-3p are present on the BCL-2 3′ UTR [38] (Supplementary Fig. 6b, c). We therefore examined the expression of these microRNAs in our APC KO and TA organoids and found that both miR-17-5p and miR-18a-3p were increased in APC KO organoids and in the majority of TA cultures (Fig. 6d, e). A significant negative correlation between the protein levels of BCL-2 and the expression of both miR-17-5p and miR-18a-3p was also observed in these organoids (Fig. 6f, g). To assess if these microRNAs mediate the downregulation of BCL-2, we transduced APC KO organoids with lentiviral microRNA inhibitors that target either miR-17-5p or miR-18a-3p. Protein expression of BCL-2 was found to be significantly upregulated upon miR-17-5p inhibition, but not upon miR-18a-3p inhibition (Fig. 6h and Supplementary Fig. 7a). We confirmed the efficacy of miR-17-5p inhibition with the observed upregulation of BIM, a known target of miR-17 (Supplementary Fig. 7b) [39]. MYC, another mir-17 target [40], was not altered at the mRNA level and...
was lowly expressed in these cells (Supplementary Fig. 7c, d). Importantly, BCL-XL, which is not a miR-17 target, remained unaffected (Supplementary Fig. 7b). Furthermore, we observed a significant negative correlation between miR-17 and BCL-2 levels in the TCGA dataset of micro-satellite stable (MSS) CRC tumors (Fig. 6i). Our results thus confirm that APC-driven transformation results in upregulation of mir-17-5p, which mediates repression of BCL-2.

Altogether, our data explain why ISC transformation results in a rapid decrease in BCL-2 expression and as a consequence, a decrease in BCL-2 dependency. However, to elucidate why transformation results in an increased sensitivity to A-1155463 while normal ISCs are resistant, we profiled the expression of key members of the BCL-2 family and assessed if transformation-driven changes to the apoptotic threshold could explain this shift in BCL-XL dependency [41, 42]. Surprisingly, mRNA and protein levels of several proapoptotic proteins were decreased upon transformation. Nevertheless, we also observed a dramatic decrease in MCL1 levels, which, together with the above described repression of BCL-2, results in evident reliance on BCL-XL for survival, thereby making it a key vulnerability in transformed ISCs (Supplementary Fig. 7e, f).

### BCL-XL expression is predictive for chemotherapy response

Our data show that BCL-XL plays a critical role in CRC survival, yet the in vivo use of A-1155463 was ineffective in impairing preexisting adenoma growth (Supplementary Fig. 4c, d). We therefore determined if BCL-XL inhibition could enhance the efficacy of chemotherapy on patient-derived tumor organoids. Matrix titration of A-1155463 and Oxaliplatin revealed that the
efficacy of the two compounds is strongly synergistic (Fig. 7a–c and Supplementary Fig. 8a–c). Intriguingly, studies have shown that the proximity of tumor cell mitochondria to the apoptotic threshold is also predictive of chemotherapy response in patients [43]. We therefore analyzed if the changes we detected in antiapoptotic protein reliance have clinical relevance in terms of outcome in chemotherapy-treated nonmetastatic CRC patients. To exclude variation in chemotherapy response due to CRC subtype differences, we made use of the Marisa dataset and selected the microsatellite stable epithelial subtypes CMS2 and CMS3 (n = 119), which almost invariably carry an APC mutation and are therefore representative of the observed APC-driven decrease in BCL-2 expression. Kaplan–Meier analysis for relapse-free survival was performed with a cut-off based on median expression of BCL-2, MCL1, or BCL-XL. While BCL-2 and MCL1 expression did not differentiate survival probability (Fig. 7d, e), patients with low BCL-XL expression and therefore a lower apoptotic threshold displayed a more favorable response to adjuvant chemotherapy in comparison to the high BCL-XL expressors (Fig. 7f), thus suggesting that the extent of BCL-XL-driven protection from mitochondrial apoptosis is predictive for response to chemotherapy.

**DISCUSSION**

The development of BH3 mimetics has greatly facilitated our understanding of the role of antiapoptotic proteins in normal and disease settings. In particular, their specificity for individual BCL-2 family members allows for dissecting the antiapoptotic vulnerabilities of cancer cells and thereby define enhanced therapeutic strategies. By employing these powerful tools, we show that while BCL-2 is only essential during ISC transformation, BCL-XL is critical for ISC survival throughout CRC progression. Previous work has shown that transforming B lymphoid cells present with an increased sensitivity to MCL1 loss [44]. However, we did not find that AZD5991 treatment does not impair ISC transformation in our models, even though MCL1 deletion was shown to severely disrupt intestinal homeostasis [18]. Earlier observations indicate that MCL1 deletion is not directly comparable to its pharmacological inhibition, which is often well tolerated at therapeutically effective doses [18, 24]. We and others have shown that while most solid tumors including CRC do not respond to MCL1 inhibition, combined inhibition of MCL1 and BCL-XL is very potent, suggesting that high levels of BCL-XL deter the efficacy of MCL1 inhibition alone [24, 25, 29, 45]. Overall, our data indicates that BCL-2 and MCL1 expression is rapidly lost upon transformation and that this results in a shift towards increased BCL-XL dependency. Our data further solidifies BCL-XL as a key factor for apoptosis resistance in CRC and moreover, emphasizes its importance in early stages of the disease. Patient-derived organoids have been used as a model to predict patient response to chemotherapy [46] and our results highlight the therapeutic potential for BCL-XL inhibition in CRC as A-1155463 treatment effectively impairs viability of organoids derived from FAP and CRC patients.

The use of BCL-XL inhibitors in the clinic is hampered by their toxicity to platelets, whose lifespan is determined by BCL-XL expression [47]. However, this could be circumvented with altered dosing strategies, particularly by administering lower doses in combination with other chemotherapeutics. Another possibility for circumventing platelet toxicity and still achieving CRC tumor killing would be to use BCL-XL inhibitors with reduced oral bioavailability, which could particularly work in the context of intestinal tumors. We therefore used A-1155463 instead of its more orally bioavailable counterpart A-1331852 in our in vivo experiments, which showed considerable efficacy in reducing adenoma outgrowth with no observed toxicity, indicating potential for less orally bioavailable BCL-XL inhibitors in CRC therapy. However, oral A-1155463 treatment on its own did not affect the growth of established adenomas in mice, which could be due to inadequate dosing of A-1155463 as transformed cells are not as sensitive to its inhibition as transforming cells. In this case, using higher doses of less orally bioavailable BCL-XL inhibitors would offer potential to improve treatment efficacy but more so, identifying kinase targets whose inhibition could synergize with BCL-XL targeting could provide novel strategies to target CRC tumors. Here we show that BCL-XL inhibition enhances oxaliplatin-induced apoptosis, while earlier studies also observed synergy with inhibitors of kinases such as MEK [48, 49].
Fig. 5  BCL-2 expression is decreased upon transformation. a, b mRNA expression of a BCL-2 and b BCL-XL in a compiled dataset of colon normal, adenoma and tumor samples. c, d Immunohistochemical analysis of c BCL-2 and d BCL-XL expression in normal and adjacent tumor tissue. Scale bars, 200 µm. e, f Gene expression (raw mean counts) of e BCL-2 and f BCL-XL in a single cell RNA-Seq dataset of normal and tumor samples, separated by cell type. Data were represented as mean ± SE and node size indicates the number of cells per sample. Samples in different cell types are shown in the same order. p < 0.005 for the fold change in BCL-2 and BCL-XL expression in normal vs tumor epithelial cells. g, h Intracellular FACs staining of g BCL-2 and h BCL-XL in the PTK7 high stem cell fraction of normal and APC^−/− human organoids. (n = 3 independent experiments). Error bars indicate s.e.m. **p < 0.01, student’s t-test.
Previously, we have shown BCL-2 inhibition with ABT-199 to effectively reduce adenoma burden in vivo [17] while in our study this effect is no longer apparent. However, in our in vivo experiment tumor formation was localized to the colon rather than the SI as was the case in the previous study and so we reason that the orally bioavailable ABT-199 is likely absorbed through the SI and fails to reach the tumor in the colon. The limited window of ABT-199 efficacy that we observe in our organoid progression panel is supported by the decrease in BCL-2 expression detected upon transformation. In the context of tumorigenesis, such a
**MATERIALS AND METHODS**

**Mouse organoid isolation and culture**

Intestinal crypts were derived from the SI and colon of a wild-type and Lgr5Cre+Apca−/− mouse. Crypts were isolated and cultured as described previously [58]. Briefly, the intestine was isolated and separated into SI and colon pieces. After flushing with PBS, the intestines were opened longitudinally and villi were scraped off the SI. Both sections were then cut into small pieces of ~0.5 cm and washed thoroughly with cold PBS supplemented with antibiotics (hereon referred to as PBS + antibiotics). These pieces were then incubated with 2 mM (SI) and 25 mM (colon) EDTA (Thermo Fischer Scientific, Landsmere, The Netherlands) in PBS at 4 °C on a roller for 30 min. Crypts were isolated by washing the pieces with PBS + antibiotics and vigorously shaking to release crypts into the supernatant containing crypts was transferred to a new tube and this step was repeated three times. The crypts were then spun down, pooled, and seeded in Matrigel. Normal colon organoids were maintained in Advanced DMEM/F12 supplemented with N2 and B27 supplement, antibiotic/antimycotic, gentamicin (Thermo Fischer Scientific), 2 mM GlutaMAX-1, 10 mM HEPES, 2 mg/ml heparin (Sigma-Aldrich), 10 mg/ml nicotinamide (Sigma-Aldrich), 500 mM A83-01 (Tocris, Abingdon, UK), 3 µM SB202190 (Sigma-Aldrich), 50% WNT3A conditioned medium, 50 ng/ml human EGF (Peprotech, London, UK), 20% Rspo1 conditioned medium, 10% Noggin conditioned medium, 10 mM PGE2 (Santa Cruz Biotechnology, Heidelberg, Germany), and 10 µM ROCK inhibitor (Sigma-Aldrich). Normal organoids were dissociated every 7–10 days and medium was refreshed every 2/3 days.

**Human organoid isolation and culture**

Normal colon samples (WT1 and WT2; WT1 is the normal organoid source of the CRISPR-Cas9 generated mutant organoids) were obtained from a piece of normal mucosa in the resection specimens of two CRC patients, at a distance of at least 10 cm from the cancerous tissue. Normal organoids were isolated as previously described [59]. In short, the submucosal layer was removed and the mucosal piece was washed with PBS + antibiotics. The tissue was then cut into small pieces using a scalpel and washed thoroughly with PBS + antibiotics. The cut pieces were incubated in 8 mM EDTA in PBS at 4 °C on a roller for 1 h. The EDTA solution was replaced with PBS + antibiotics and crypts were released by vigorous shaking. The supernatant containing crypts was transferred to a new tube and this step was repeated three times. The crypts were then spun down, pooled, and seeded in Matrigel. Normal colon organoids were maintained in Advanced DMEM/F12 supplemented with N2 and B27 supplement, antibiotic/antimycotic, gentamicin (Thermo Fischer Scientific), 2 mM GlutaMAX-1, 10 mM HEPES, 2 mg/ml heparin (Sigma-Aldrich), 10 mg/ml nicotinamide (Sigma-Aldrich), 500 mM A83-01 (Tocris, Abingdon, UK), 3 µM SB202190 (Sigma-Aldrich), 50% WNT3A conditioned medium, 50 ng/ml human EGF (Peprotech, London, UK), 20% Rspo1 conditioned medium, 10% Noggin conditioned medium, 10 mM PGE2 (Santa Cruz Biotechnology, Heidelberg, Germany), and 10 µM ROCK inhibitor (Sigma-Aldrich). Normal organoids were dissociated every 7–10 days and medium was refreshed every 2/3 days.

**Organoid transduction**

For the shRNA knockdown experiment, an equal number of APCΔK organoids were transduced with lentiviral shRNA constructs against either control (SCH002, MISSION shRNA, Merck) or BCL-XL (TRCN0000033500, Mission shRNA, Merck) by spin transduction. Briefly, organoids were collected and trypsinized using TrypLE Express (Thermo Fischer Scientific) for 3 min at 37 °C. Following dissociation, cells were washed, counted, and

**Fig. 6** MiR-17-5p regulates BCL-2 expression in APC-mutant organoids. (a) BCL-2 mutation and methylation status in CRC tumors of the TCGA COAD dataset, analyzed on.cbioportal. (b) mRNA expression of BCL-2 in decitabine treated APCΔK organoids (n = 2 independent experiments). Error bars indicate s.e.m. (c) Single cell karyotype-seq showing the ploidy in individual cells of TA1 and TA5. Graphs show individual cells (horizontal lines) and colors indicate copy number state for a given chromosome (columns). (d, e) qRT-PCR analysis of (d) miR-17-5p and (e) miR-18a-3p expression in human normal, APCΔK and TA cultures, normalized to U6 snRNA expression. Data represent mean s.e.m. (f) Correlation of f miR-17-5p and g miR-18a-3p expression data from (d) and (e), respectively to BCL-2 protein levels measured by intracellular FACS. P values are determined by two-tailed Pearson’s correlation. In Intracellular FACS staining of BCL-2 protein levels in human APCΔK organoids transduced with control, miR-17-5p, or miR-18a-3p anti-sense (AS) microRNA inhibitors (n = 3 independent experiments). Error bars indicate s.e.m. **p < 0.01, student’s t-test. (i) Correlation of miR-17 and BCL-2 expression in the TCGA COAD dataset.
Fig. 7  BCL-XL expression is predictive for chemotherapy response. a Phase-contrast images of p16T and p24aT human CRC organoids treated for 72 h with 10 µM oxaliplatin alone and in combination with 30 nM A-1155463. Scale bars, 250 µm. b, c 6 × 7 dose matrices of b p16T and c p24aT human CRC organoids treated with oxaliplatin in combination with A-1155463 for 5 days. Percentage inhibition was calculated from viability data measured by cell titer blue, after normalizing to control. Data were the average of two independent experiments. Bliss synergy scores were calculated for each dose combination and positive scores indicate synergistic effects. d-f Kaplan–Meier analysis of the relapse free survival probability of micro-satellite stable CM2 and CM3 patients in the Marisa dataset who received adjuvant chemotherapy (n = 119), based on a cut-off of median expression of d BCL-2, e MCL-1, or f BCL-XL. P values are based on the log-rank test. Adj Chemo patients who received adjuvant chemotherapy, MSS microsatellite stable, CMS2/3 consensus molecular subtype 2/3.
seeded into 48-well plates in organoid medium (see above) containing 10 µM ROCK inhibitor (Sigma-Aldrich), 8 µg/ml polybrene (Sigma-Aldrich), and 50 µL concentrated virus (AMICON Ultra-15 100k filters, Merck, Schiphol-Rijk, The Netherlands). Plates were spun down at 32 °C for 1 h at 1800 rpm. After ON incubation, cells were collected by washing with PBS and spun down and either processed for RNA extraction or seeded into Matrigel (Corning) for measuring outgrowth. APCΔCβ organoids were transduced with lentiviral pHEFTIR-EV (empty vector) or pHEFTIR-BCL-XL (overexpressor) constructs [16] and selected by sorting for the GFP positive population. APCΔCβ organoids were transduced with lentiviral microRNA inhibitors (Merck) targeting hsa-mir-17-5p (HLTUD2064), hsa-mir18a-3p (HLTUD2092), and a negative control cel-mir-243-3p (HLTUD002C). Transduced cells were selected with 4 µg/ml puromycin (InvivoGen, Toulouse, France) for 7 days.

Colon cancer spheroid culture

The colon cancer spheroid culture Co01 expressing the TCF/LEF reporter TOP-GFP was generated as previously described [26]. The spheroid culture was maintained in ultralow adhesive flasks (Corning) and cultured in advanced DMEM/F12 supplemented with N2 supplement, 2 mM L-glutamine, 0.15% v-v glucose, 100 µM β-mercaptoethanol, trace elements B and F, and B27 (1:5000), 10 µg/ml insulin, 50 ng/ml epidermal growth factor, and 10 ng/ml basic fibroblast growth factor (Tebu-Bio). Cultures were confirmed to be mycoplasma negative on a monthly basis.

Inhibitors

ABT-199 and WEHI-539 were purchased from Selleck Chemicals (Breda, The Netherlands), A-1155463 and AZD5991 from Chemietek (Indianapolis, USA), S-Aza-2-deoxycytidine (Dactebide) and Oxaliplatin were purchased from Sigma Aldrich. All compounds were dissolved in DMSO to a stock of 20 mM, except Oxaliplatin, which was dissolved in water to a stock of 12.5 mM.

Clonogenic assay

The clonogenic assay on Lgr5CreER/Flp(Apc/Cre) derived SI and colon organoid cultures was performed as previously described [58]. Briefly, organoids were plated in 48-well plates at a density of 25–50 crypt structures per well. After 3 days, the organoids were counted and treated with 1 µM (Z)-4-hydroxytamoxifen (24 h) to induce Apc loss and simultaneously treated with 1 µM BH3 mimetics for 72 h. To assess the clonogenic potential of the organoids, each well was passaged and the resulting organoid outgrowth was quantified after 3 days and normalized to the pretreatment count per well and compared to untreated controls.

The clonogenic assay on the human organoids was performed by seeding dissociated organoids in 48-well plates, following which they were treated after 3 days with the indicated dose of BH3 mimetics for 24 h. Each well was then passaged to a new well and the clonogenic capacity of organoids was assessed by microscope after 3 days and compared to untreated controls. All clonogenic assays were performed as two or three independent experiments and include at least three technical replicates per run.

Flow cytometry

Co01 expressing TOP-GFP was dissociated with Trypsin and stained for activated caspase-3 after 24 h of treatment with 1 µM ABT-199 or A-1155463. Staining was performed using RED-DEVD-FMK (CaspaTag caspase activated caspase-3 after 24 h of treatment with 1 µM ABT-199 or A-

RNA and microRNA isolation and quantitative real-time PCR

Organoids cultures were collected for RNA extraction using cell recovery solution to dissolve the Matrigel, following which the cells were pelleted and RNA was extracted using the NucleoSpin RNA II kit (Macherey-Nagel, Leiden, The Netherlands). For isolation of microRNA, Matrigel was dissolved in cell recovery solution and organoids were pelleted and resuspended in TRIzol (Thermo Fischer Scientific). Chloroform was added and the mixture was centrifuged as per the manufacturer’s instructions. The aqueous phase was carefully collected in a new tube, mixed with 100% ethanol (1.5x volume of aqueous phase) and RNA was isolated using the NucleoSpin RNA II kit (Macherey-Nagel) following manufacturer’s instructions.

For gene expression analysis using quantitative real-time PCR (qRT-PCR), RNA was reverse transcribed to cDNA using Superscript III as per the manufacturer’s instructions (Thermo Fischer Scientific). SYBR green (Roche, Woerden, The Netherlands) was used to perform qRT-PCR following the manufacturer’s instructions on a Roche Light Cycler 480 II. All obtained values were normalized to the expression of RPLP0 and all primer sequences are provided in supplementary Table 2. For analysis of microRNA expression levels using qRT-PCR, RNA was reverse transcribed to cDNA using the reverse transcription enzyme of the miRCURY LNA RT kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The miRCURY SYBR Green PCR kit (Qiagen) was used to perform qRT-PCR with the following miRCURY LNA miRNA PCR assays: hsa-mir-17-5p and hsa-mir-18a-3p, as per the manufacturer’s instructions (Qiagen). Data shown have been normalized to the expression of U6 snRNA.

Immunohistochemistry

Matrigel cultures were embedded in paraffin as previously described [58]. Briefly, organoids in Matrigel were fixed with 4% paraformaldehyde (PFA, Klinipath, Duiven, The Netherlands) over night at 4 °C. PFA was then replaced with 70% ethanol for 30 min following which dehydration was carried out with 100% ethanol and then xylene, both twice for 30 min at RT. After dehydration, the Matrigel pieces were incubated in paraffin at 60 °C twice for 30 min and finally embedded in paraffin. Four micrometer sections were cut from paraffin embedded blocks using the microtome. Slides were stained for cleaved caspase-3 (1:200, 9661, Cell Signaling Technology), BCL-2 (15071 S, 1:100, Cell Signaling Technology), and BCL-XL (2764, 1:500, Cell Signaling Technology) using standard procedures according to the manufacturer’s protocols.

Immunoblotting

Analysis of protein expression was done as previously described [45], organoids were collected in cell recovery solution (BD Biosciences) at 4 °C for 30 min to dissolve Matrigel and then lysed using 1x RIPA Lysis and Extraction buffer (Thermo Scientific) containing Halt protease and phosphatase inhibitor cocktail (1:100, Thermo Fischer Scientific). Protein samples were quantified using the Pierce BCA protein assay kit (Thermo Fischer Scientific) as per the manufacturer’s instructions. About 1 µg protein was loaded per well into 4–15% precast gels (Bio-Rad, Lunteren, The Netherlands) and then transferred to PVDF membranes using the Trans-Blot Turbo transfer system (Bio-Rad) according to the manufacturer’s instructions using the mixed molecular weight transfer settings. Membranes were blocked for 1 h in 5% bovine serum albumin (BSA) in Tris-buffered saline and Tween-20 (1x) and stained with primary antibody overnight at 4 °C. The following primary antibodies were tested: MCL1 (1:1000, #4572, Cell Signaling), BCL-XL (1:1000, #2764, Cell Signaling), BIM (1:500, ADI-AAP-330-E, Enzo life sciences), c-Myc (1:1000, #9402, Cell Signaling), BAX (1:1000, #2774, Cell Signaling), BAK (1:1000, #3814, Cell Signaling), and BID (1:1000, #2002, Cell Signaling), all diluted in 5% BSA in TBS-T. After washing the blots four times for 20 min each with TBS-T, the secondary antibody anti-rabbit-horseradish peroxidase (1:5000, #40500, Southern Biotech, Uden, The Netherlands) or anti-mouse-horseradish peroxidase (1:5000, #1031-05, Southern Biotech) was added for 2 h at room temperature. Following another round of 4 x 20 min washes, the membranes were developed using the Lumilight Western blotting substrate (Sigma-Aldrich) and imaged on the ImageQuant LAS4000 (GE Healthcare Life Sciences, Rijswijk, The Netherlands). GAPDH was used as a loading control, a representative GAPDH is shown as multiple blots were

were performed on the FACS Canto (BD biosciences) and PTk7 sorting of APCΔCβ organoids was performed on the SH800S Cell Sorter (Sony, Hoofddorp, The Netherlands). All stainings were performed as three independent experiments.
run at the same time for proteins of the same molecular weights (1:5000, MAB374, Sigma-Aldrich).

Cell viability assay
Tumor organoids were isolated using cell recovery solution (BD Biosciences) at 4 °C for 30 min to dissolve Matrigel. Organoid structures were then spun down and trypsinized using TrypLE Express (Thermo Fischer Scientific) for 3 min at 37 °C. Following dissociation, cells were washed, counted, and seeded in 96-well plates at a density of 4000 cells per well in 6 µL Matrigel drops. After overnight incubation, cells were treated in a titration with BH3 mimetics and/or Oxalipatin using the HP D300 digital dispenser (Hewlett-Packard, Amstelveen, The Netherlands) for 5 days. Organoids were imaged using the EVOS cell imaging system (Thermo Fischer Scientific) and cell viability was assessed using CellTiterBlue (Promega, Leiden, The Netherlands) according to the manufacturer’s instructions. All viability assays were performed as two or three independent experiments. Synergy was calculated using the Synergy Finder web tool [60] and the Bliss independence model was employed to calculate the Bliss score for the most synergistic three-by-three dose window in the dose-response matrices, positive bliss scores are considered indicative of synergistic interactions.

Animal experiments
All experiments were performed according to UK Home Office regulations (Project Licence 70/8664 and 70/9112), adhered to ARRIVE guidelines and were reviewed by local animal welfare and the ethical review committee at the University of Glasgow. Intracolic inductions in 12–20-week-old male and female VillinCreERT2/ApcMin C57BL/6 mice were performed under general anesthesia wherein a single 70 µl 100 µM dose of 4-hydroxy tamoxifen (H7904-5MG from Sigma) was injected into the colonic submucosa via a colonicoscop (Karl Storz TELE PACK VET X LED endoscopic video unit). Mice were treated with ABT-199 (n = 4), A-1155463 (n = 5), or vehicle-only (n = 6) at a dose of 100 mg/kg by oral gavage. Drugs were dissolved in 60% Phosal 50PG (Lipoid, Ludwigshafen, Germany), 30% polyethylene glycol 400, and 10% ethanol. For the transformation experiment, treatment was started 2 days prior to tamoxifen induction and continued afterwards on every other day for 28 days. To assess efficacy in preexisting tumors, colonic adenomas were allowed to grow out for 2 weeks after which mice were treated with either vehicle (n = 4) or A-1155463 (n = 4), five times a week for 28 days. Tumor growth in the colon was monitored weekly, for 3 weeks by colonoscopy. Tumor size was measured relative to lumen size with ImageJ. For the transformation study, mice were randomly assigned with ImageJ. For the transformation study, mice were randomly assigned to treatment groups and scoring of tumor burden was blinded. For the transformation study, mice were treated with ABT-199 (n = 5), or vehicle-only (n = 4) as a control. The single cell sample size calculation was performed as described above.

Data analysis and visualization
BCL-2 and BCL-XL expression graphs were generated from a collection of three public Affymetrix datasets (GSE20916, GSE8671, and GSE4183), which were rma normalized and merged using quantile normalization in R. P values were calculated using the Mann–Whitney test: RNA-sequencing (RNA-Seq) data from the TCGA cohort was normalized from raw counts to FPKM (log2 transformed). The single-cell RNA-Seq data and cell type annotations were previously published [30] and available from NCBI GEO with accession GSE132465 and GSE144735. The raw counts per sample (separate tumor vs normal per patient) and major cell types were summarized into the mean and standard error, visualized as forest plots and statistically tested using the R package acdx (https://github.com/pwirapati/acdx). Fold-change and bootstrap p value calculation (200 replicates) is based on random-effects gamma GLM model.

Data availability
The authors declare that all data supporting the findings of this study are available within the article and its Supplementary Information files. Data used to generate Fig. 5a, b, e, f are publically available (GSE20916, GSE8671, GSE4183, GSE132465, and GSE144735), as is the Marisa dataset (GSE39582). The publically available TCGA COAD dataset was used to generate Fig. 6i and supplementary Fig. 5a, b.

REFERENCES
1. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cojzinjsen M, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature. 2007;449:1003–7.
2. Ramesh P, Medema JP. BCL-2 family deregulation in colorectal cancer: potential for BH3 mimetics in therapy. Apoptosis. 2020;25:305–20.
3. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100:57–70.
4. Bock FJ, Tait SWG. Mitochondria as multifaceted regulators of cell death. Nat Rev Mol Cell Biol. 2020;21:85–100.
5. Rampino N, Yamamoto H, Ionov Y, Li Y, Sawai H, Reed JC, et al. Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. Science. 1997;275:867–90.
6. Campbell KJ, Tait SWG. Targeting BCL-2 regulated apoptosis in cancer. Open Biol. 2018;8:180002.
7. Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, et al. The landscape of somatic copy-number alteration across human cancers. Nature. 2010;463:899–905.
8. Souers AJ, Leveson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med. 2013;19:202–8.
9. Merino D, Kelly GL, Lessene G, Wei AH, Roberts AW, Strasser A. BCL-2-mimetic drugs: blazing the trail for new cancer medicines. Cancer Cell. 2018;34:879–91.
10. Vaillant F, Merino D, Lee L, Breslin K, Pal B, Ritchie ME, et al. Targeting BCL-2 with the BH3 mimetic ABT-199 in estrogen receptor-positive breast cancer. Cancer Cell. 2013;24:120–9.
11. Zhang H, Xue J, Hessler P, Tahir SK, Chen J, Jin S, et al. Genomic analysis and selective small molecule inhibition identifies BCL-X(L) as a critical survival factor in a subset of colorectal cancer. Mol Cancer. 2015;14:126.
12. Maurer CA, Friess H, Bühler SS, Wahl BR, Graber H, Zimmermann A, et al. Apoptosis inhibiting factor Bcl-xl might be the crucial member of the Bcl-2 gene family in colorectal cancer. Dig Dis Sci. 1998;43:2641–8.
13. Krajewska M, Mass SF, Krajewski S, Song K, Holt PR, Reed JC. Elevated expression of Bcl-X and reduced Bak in primary colorectal adenocarcinomas. Cancer Res. 1996;56:2422–7.
14. Scherr AL, Gdynia G, Salou M, Radhakrishnan P, Duglova K, Heller A, et al. Bcl-xl is an oncogenic driver in colorectal cancer. Cell Death Dis. 2016;7:e2342.
15. Zhang YL, Pang LQ, Wu Y, Wang X, Wang CQ, Fan Y. Significance of Bcl-xl in human colon carcinoma. World J Gastroenterol. 2008;14:3069–73.
16. Colak S, Zimmerlin CD, Fessler E, Hogdal L, Prasetyanti PR, Grandela CM, et al. Decreased mitochondrial priming determines chemoresistance of colon cancer stem cells. Cell Death Differ. 2014;21:1170–7.
17. van der Heijden M, Zimmerlin CD, Nicholson AM, Colak S, Kemp R, Meijer SL, et al. Bcl-2 is a critical mediator of intestinal transformation. Nat Commun. 2017;8:10916.
18. Healy ME, Boege Y, Hodder MC, Böhm F, Malehmir M, Scherr AL, et al. MCL1 is required for maintenance of intestinal homeostasis and prevention of carcinoma in mice. Gastroenterology. 2020;159:183–99.
19. Fessler E, Drozd J, van Hooff SR, Linnekom JF, Wang X, Jansen M, et al. TGFβ signaling directs serrated adenomas to the mesenchymal colorectal cancer subtype. EMBO Mol Med. 2016;8:745–60.
20. Tao ZF, Hasvold L, Wang L, Wang X, Petros AM, Park CH, et al. Discovery of a BH3 pro
21. van de Wetering M, Francis HE, Francis JM, Colak S, Zimberlin CD, Fessler E, Hogdal L, et al. The MCL1 inhibitor S63845 is tolerable and effective in diverse cancer models. Nature. 2021;583:477–82.
22. Tron AE, Belmonte MA, Adam A, Aquila BM, Boise LH, Chiarparin E, et al. Discovery of BCL-2 inhibitors with potent activity in vitro and in vivo. ACS Chem Biol. 2014;9:1088–93.
23. Kotschy A, Salavik Z, Murray J, Davidson J, Maragno AL, Le Tommelin-Braizat G, et al. The MCL1 inhibitor S63845 is tolerable and effective in diverse cancer models. Nature. 2021;583:477–82.
24. Vermeulen L, De Sousa EMF, van der Heijden M, Cameron K, de Jong JH, Borovski P, et al. Ongoing chromosomal instability and karyotype evolution in human colorectal cancer. Cancer Res. 2019;80:3521–31.
25. Zhang L, Ramesh P, Medema JP. BH3 mimetic sensitivity of colorectal cancer cell lines in correlation with molecular features identifies predictors of response. Int J Mol Sci. 2021;22:3811.
26. Smit WL, Spaan CN, Johannes de Boer R, Ramesh P, Martins Garcia T, Meijer BJ, et al. Distinct thresholds govern Myc expression and disease progression. Carcinogenesis. 2019;2019:79:4882–95.
27. Meijer GA, Hermsen MA, Baak JP, van Diest PJ, Böhm F, Malehmir M, Scherr AL, et al. Increased expression of miR-17-92 cluster through PTK7 is required for maintenance of intestinal homeostasis and prevention of carcinoma in mice. Gastroenterology. 2020;159:183–99.
28. Scherr M, Elder A, Bartmann K, Barzan D, Bomken S, Ricke-Hoch M, et al. Differential expression of miR-17–92 identifies BCL2 as a therapeutic target in BCR-ABL-positive B-lineage acute lymphoblastic leukemia. Leukemia. 2014;28:554–65.
29. Li Y, Choi PS, Casey SC, Dill DL, Felsher DW, MYC through miR-17–92 suppresses specific target genes to maintain survival, autonomous proliferation, and a neoplastic state. Cancer Cell. 2014;26:262–72.
30. Lee HO, Hong Y, Etlioglu HE, Cho YB, Pomella V, Van den Bosch B, et al. Lineage-
31. Jung P, Sommer C, Barriga FM, Buczacki SJ, Hernando-Momblona X, Sevillano M, et al. Isolation of human colon stem cells using surface expression of PTK7. Stem Cells. 2016;35:4558–68.
32. Montero J, Letai A. Why do BCL-2 inhibitors work and where should we use them for cancer therapy using BH3 mimetics - recent successes, current challenges and future promise. Febs J. 2016;283:3522–33.
33. van der Heijden M, Zimmerlin CD, Nicholson AM, Colak S, Zimberlin C, van Bokel R, Buijs A, et al. Sequential cancer mutations in human intestinal stem cells. Nature. 2021;552:43–7.
34. van de Wetering M, Francis HE, Francis JM, Colak S, Zimberlin CD, Fessler E, Hogdal L, et al. Prognostic value of a living organoid biobank of colorectal cancer patients. Cell. 2015;161:933–45.
35. Villalobos-Ortiz M, Ryan J, Mashaka TN, Opferman JT, Letai A. BH3 profiling discriminates on-target small molecule BH3 mimetics from putative mimetics. Cell Death Cell Differ. 2020;27:999–1007.
36. Kotschy A, Salavik Z, Murray J, Davidson J, Maragno AL, Le Tommelin-Braizat G, et al. The MCL1 inhibitor S63845 is tolerable and effective in diverse cancer models. Nature. 2021;583:477–82.
37. Tron AE, Belmonte MA, Adam A, Aquila BM, Bose LH, Chiarpinin E, et al. Discovery of Mcl-1-specific inhibitor AZD5991 and preclinical activity in multiple myeloma and acute myeloid leukemia. Nat Commun. 2018;9:3341.
38. Vermeulen L, de Sousa EMF, van der Heijden M, Cameron K, de Jong JH, Borovski P, et al. Ongoing chromosomal instability and karyotype evolution in human colorectal cancer. Cancer Res. 2019;80:3521–31.
39. Zhang L, Ramesh P, Medema JP. BH3 mimetic sensitivity of colorectal cancer cell lines in correlation with molecular features identifies predictors of response. Int J Mol Sci. 2021;22:3811.
40. O'Donnell KA, Wentzel EA, Zeller KL, Dang CV, Mendell JT. C-myc-regulated microRNAs modulate E2F1 expression. Nature. 2005;435:839–43.
41. Murphy DJ, Junttila MR, Pouyet L, Karnezis A, Shchors K, Bui DA, et al. Programmed anuclear cell death delimits platelet life span. Cell. 2007;128:1173–86.
42. Cragg MS, Jansen ES, Cook M, Harris C, Strasser A, Scott CL. Treatment of B-RAF mutant human tumor cells with a MEK inhibitor Bim and is enhanced by a BH3 mimetic. J Clin Invest. 2008;118:3651–9.
43. Kelly GL, Grabow S, Glaser SP, Fitzsimmons L, Aubrey BJ, Okamoto T, et al. Targeting of MCL1 kills MYC-driven mouse and human lymphomas even when they bear mutations in p53. Genes Dev. 2014;28:58–70.
44. Tao ZF, Hasvold L, Wang L, Wang X, Petros AM, Park CH, et al. Discovery of a BH3 pro
45. Zhang L, Ramesh P, Medema JP. BH3 mimetic sensitivity of colorectal cancer cell lines in correlation with molecular features identifies predictors of response. Int J Mol Sci. 2021;22:3811.
46. Dews M, Homayouni A, Yu D, Murphy D, Sevignani C, Wentzel E, et al. Augmentation of tumor angiogenesis by a Mcl-activated microRNA cluster. Nat Genet. 2006;38:1060–5.
47. Colak S, Fraser C, Mithalaglu N, Bhola PD, Chang W, McBrayer SK, et al. Developmental regulation of mitochondrial apoptosis by c-Myc governs age- and tissue-specific sensitivity to cancer therapeutics. Cancer Cell. 2017;31:142–56.
48. Montero J, Letai A. Why do BCL-2 inhibitors work and where should we use them in the clinic? Cell Death Differ. 2018;25:56–64.
49. Murphy DJ, Junttila MR, Pouyet L, Kamezis A, Shchors K, Bui DA, et al. Distinct thresholds govern Myc's biological output in vivo. Cancer Cell. 2008;14:547–57.
50. Ramesh P, Kirov AB, Huels DJ, Medema JP. Isolation, propagation, and cloning of intestinal stem cells. Methods Mol Biol. 2019;2002:61–73.
51. Sato T, Stange DE, Ferrante M, Vites RG, Van ESJ, Van den Brink S, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology. 2011;141:1762–72.
52. Jarvenpaa AS, He L, Anttioikko T, Teng J. SynergyFinder: a web application for analyzing drug combination dose-response matrix data. Bioinformatics. 2017;33:2413–5.
53. Bolhaqueiro ACF, Ponsioen B, Bakker B, Klaassen SJ, Kucukkose E, van Jaarsveld RH, et al. Ongoing chromosomal instability and karyotype evolution in human colorectal cancer organoids. Nat Genet. 2019;51:824–34.
54. R2 Database. The Department of Human Genetics in the Amsterdam Medical Centre (AMC), 2020.

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Conception and design of the study: P.R. and J.P.M. Experimental work: P.R., T.R.M.L., R.J., L.A.T., N.L., D.D., J.P., A.B.K, and S.M.V.N. Data analysis and interpretation: P.R., N.L.,
P.W., S.R.v.H., and J.P.M. Drafting of the manuscript: P.R. and J.P.M. Final manuscript contribution: P.R., T.R.M.L., R.J., L.A.T., N.L., P.W., S.R.v.H., D.D., J.P., A.B.K., S.M.v.N., S.T., G.J.P.L.K., O.J.S., and J.P.M.

**FUNDING**
This work was supported by Oncolead, KWF project 2015-7587 and 10150 funding to JPM lab, Cancer Research UK core funding to the CRUK Beatson Institute, A17196, and Cancer Research UK core funding to OJS lab, A21139. RJ was also funded by a Marie Skłodowska Curie Actions Individual Fellowship, 659666.

**CONFLICT OF INTEREST**
J.P.M. serves as an advisor to AbbVie on their CRC program.

**ADDITIONAL INFORMATION**
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41418-021-00816-w.

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