mTORC1-mediated translational elongation limits intestinal tumour initiation and growth

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Inactivation of APC is a strongly predisposing event in the development of colorectal cancer1,2, prompting the search for vulnerabilities specific to cells that have lost APC function. Signalling through the mTOR pathway is known to be required for epithelial cell proliferation and tumour growth3–5, and the current paradigm suggests that a critical function of mTOR activity is to upregulate translational initiation through phosphorylation of 4EBP1 (refs 6, 7). This model predicts that the mTOR inhibitor rapamycin, which does not efficiently inhibit 4EBP1 (ref. 8), would be ineffective in limiting cancer progression in APC-deficient lesions. Here we show in mice that mTORC1 activity is absolutely required for the proliferation of Apc-deficient (but not wild-type) enterocytes, revealing an unexpected opportunity for therapeutic intervention. Although APC-deficient cells show the expected increases in protein synthesis, our study reveals that it is translation elongation, and not initiation, which is the rate-limiting component. Mechanistically, mTORC1-mediated inhibition of eEF2 kinase is required for the proliferation of APC-deficient cells. Importantly, treatment of established APC-deficient adenomas with rapamycin (which can target eEF2 through the mTORC1–S6K–eEF2K axis) causes tumour cells to undergo growth arrest and differentiation. Taken together, our data suggest that inhibition of translation elongation using existing, clinically approved drugs, such as the rapalogs, would provide clear therapeutic benefit for patients at high risk of developing colorectal cancer.

The ability of the intestinal epithelium to regenerate after challenge has been well described9–11. We have shown that this is a Wnt-driven process that mimics the proliferation observed after Apc deletion11,12 and is a valuable model of the early stages of intestinal cancer. However, the underlying mechanisms controlling these processes are largely unknown. The serine/threonine kinase mTOR, particularly as part of mTORC1, is a known mediator of cell growth and proliferation13. Previous studies have suggested that mTORC1 may be important in both the intestinal stem-cell niche and for intestinal tumorigenesis14–16. We therefore queried the role of mTORC1 in intestinal proliferation after Wnt activation. Following Apc deletion there was an increase in the phosphorylation status of the mTORC1 effectors RPS6 and 4EBP1 that was dependent on MYC expression. Increased phosphorylation of these proteins was also seen during crypt regeneration (Fig. 1a–c and Extended Data Fig. 1a). Importantly, the mTOR inhibitor rapamycin blocked intestinal regeneration, demonstrating that mTORC1 signalling is required for this process (Fig. 1d, e). Given that rapamycin did not affect apoptosis or proliferation in the normal intestine (Extended Data Fig. 1b, c), these data suggest that there may be a potential therapeutic window, between normal intestinal enterocytes and those with a high level of Wnt activity. Therefore, we deleted raptor (Rptor; an essential component of mTORC1) in APcfl/fl mice to see if we could eliminate this window and determine if rapamycin, given specifically to Apcfl/fl mice, causes a loss of proliferative potential in vivo. As shown in Figure 1f, g and h, treatment of Apcfl/fl mice with rapamycin specifically disrupted intestinal regeneration in Apcfl/fl mice, whereas administration of rapamycin to wild-type mice did not significantly decrease intestinal regeneration. Whiskers show maximum and minimum, black line shows median (n = 6 biological replicates per group).

**p value < 0.02, Mann–Whitney U test. e. Representative haematoxylin and eosin (H&E) staining of regenerating intestines 72 h after exposure to 14 Gy γ-irradiation. Arrowheads indicate regenerating crypts (representative of 6 biological replicates). f. Representative H&E staining 96 h after Apc loss, showing that 10 mg kg⁻¹ rapamycin treatment or Rptor deletion prevent Wnt-driven proliferation (representative of 6 biological replicates). Treatment began 24 h after Apc deletion. Red bar is graphical representation of crypt size. Scale bars, 100 μm.

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LETTER

Figure 1 | mTORC1 is essential for Wnt-driven proliferation in a MYC-dependent manner. a, b, Representative immunohistochemistry (IHC) of phospho-RPS6 (pS6) and phospho-4EBP1 (p4EBP1) showing increased staining 96 h after Apc deletion. Rptor deletion caused a loss of positivity in both, whereas 10 mg kg⁻¹ rapamycin treatment (beginning at 24 h) specifically disrupts RPS6 phosphorylation (representative of six biological replicates). c, Representative IHC of phospho-RPS6 96 h after Cre induction showing that Wnt-driven RPS6 phosphorylation is MYC dependent (representative of 3 biological replicates). d, Mice were exposed to 14 Gy γ-irradiation and intestinal regeneration was measured 72 h later by counting the number of viable crypts and multiplying that by the average size of the regenerating crypts. Boxplot shows that 10 mg kg⁻¹ rapamycin treatment and Rptor deletion significantly decrease intestinal regeneration. Whiskers show maximum and minimum, black line shows median (n = 6 biological replicates per group).

**p value < 0.02, Mann–Whitney U test. e. Representative haematoxylin and eosin (H&E) staining of regenerating intestines 72 h after exposure to 14 Gy γ-irradiation. Arrowheads indicate regenerating crypts (representative of 6 biological replicates). f. Representative H&E staining 96 h after Apc loss, showing that 10 mg kg⁻¹ rapamycin treatment or Rptor deletion prevent Wnt-driven proliferation (representative of 6 biological replicates). Treatment began 24 h after Apc deletion. Red bar is graphical representation of crypt size. Scale bars, 100 μm.
Given that rapamycin treatment and Rptor deletion had similar effects, we examined whether rapamycin treatment was sufficient to modify intestinal tumorigenesis, either prophylactically or chemotherapeutically. First we assessed whether rapamycin could suppress a model of intestinal tumorigenesis, in which Apc deletion is targeted to Lgr5-positive stem cells using Lgr5CreER (Lgr5CreER Apcre). Mice were treated starting when mice showed signs of intestinal disease, and lasted for the duration of the experiment (Extended Data Fig. 2a, b). Next we treated mice (Apcre or Lgr5CreER Apcre) with established adenomas. Remarkably, the mice lost their clinical symptoms of disease and survived significantly longer than controls (Fig. 2c, d and Extended Data Fig. 3c). We next analysed the tumours from these mice over a time course after rapamycin treatment. Treatment caused a loss of proliferation specifically within the tumours by 72 h, and an increase in the number of lysozyme-positive Paneth cells (Fig. 2e and Extended Data Fig. 3d, e). By 30 days, most tumours had shrunk considerably to small non-proliferative lesions that no longer contained Paneth cells (Fig. 2f and Extended Data Fig. 3f). Within the normal intestine there are two main cell populations that show high levels of Wnt signalling: the label-retaining/progenitor population and the Paneth cell population15. Our data suggest that treatment of mice with rapamycin causes the differentiation of the tumour’s Wnt-high progenitor cells into the other Wnt-high fate in the intestine: namely non-proliferative Paneth-like cells. The cell-cycle arrest in these cells was examined by staining for p12, p16 and p53. No increase in these markers was observed, suggesting that a classical cell-cycle arrest pathway had not been engaged (Extended Data Fig. 4a). We reasoned that if mice were removed from rapamycin the tumours would regain proliferative capacity. Indeed, when rapamycin treatment was halted, signs of intestinal neoplasia were observed approximately 40–60 days later (Extended Data Fig. 3c). This suggested that intestinal adenoma stem cells were still present. Tumours from Lgr5CreER Apcre mice were stained to detect LGR5–GFP positivity. We found that, after rapamycin treatment, numerous LGR5-positive cells were still present, indicating that, although rapamycin treatment causes a regression of the lesions, the tumour-initiating cells remain (Extended Data Fig. 4b).

We next examined the mechanism of mTORC1 requirement after Apc loss. mTORC1 is known to regulate protein synthesis on multiple levels and most research has focused on two downstream effectors: 4EBP1 and S6K. A number of studies have suggested that translation initiation, via the 4EBP1–eIF4E axis, is the critical effector of mTOR in cancer6,16. However, it has been shown that rapamycin preferentially inhibits the phosphorylation of S6K over 4EBP1 (ref. 8), suggesting that 4EBP1-mediated inhibition of translation initiation may not be limiting in the context of Apc loss. To assess the changes in translational control in response to mTORC1 inhibition, we measured the polysomal distribution in wild-type, Apc-deficient and Apc/Rptor-deficient intestinal epithelial cells 4 days after gene deletion. Apc deletion resulted in a decrease in the number of polysomes, whereas Apc/Rptor co-deletion reversed this effect (Fig. 3a). The decrease in the number of polysomes after Apc deletion could suggest either reduced translation initiation (and, consequently, a lower overall level of translation) and/or a faster rate of translational elongation. Global translation rates were measured using an in vitro intestinal crypt culture model17. The Apc-deficient cells were shown to have increased 35S-labelled methionine/cysteine incorporation compared with wild type, showing higher overall levels of protein synthesis (Fig. 3b). Unfortunately, Rptor deletion prevented the growth of crypts in vitro so this could not be assayed (Extended Data Fig. 5).

To measure the rate of translational elongation, an in vitro harringtonine run-off assay was performed18, as described in Methods. There was a >2.5-fold increase in ribosome run-off in crypts with Apc deletion compared with wild type (Fig. 3c and Extended Data Fig. 6a–d). This suggests that, after Wnt activation, elongation, rather than initiation, is rate limiting for protein synthesis and that mTORC1 must be activated to overcome this.

Cycloheximide (an inhibitor of elongation19) reduced proliferation associated with Apc deletion to a similar level to rapamycin (Extended Data Fig. 6e, f). While cycloheximide is acknowledged to inhibit elongation19, it must be emphasized that 72 h treatment could result in broad alterations in protein synthesis. However, the Apc-specific loss of proliferation observed here provides ‘proof of principle’ to demonstrate that the modulation of protein synthesis may be useful as a chemotherapeutic strategy.

As most previous work has suggested that translation initiation downstream of 4EBP1 is limiting to cancer20, it was important to probe known effectors of mTORC1 in this system. Given the alteration of elongation rates, Ef2k, a known target of S6K21,22 was of particular interest.
is a negative regulator of eEF2, giving mTORC1 the ability to promote translational elongation via S6K (ref. 23). Using multiple mouse knock- out and knock-in alleles, we further dissected the downstream effectors of mTORC1 in intestinal regeneration. S6K1/2 (also known as Rps6kb1 and Rps6kb2, respectively) knockout decreased intestinal regeneration, while knock OUT of Eif4ebp1/2 (coding 4EBP1 and 4EBP2, respectively) had no effect. The 4EBP proteins are negative regulators of eIF4E, an increase (rather than a decrease) in regeneration may have been predicted, but this was not found. Moreover these intestines were still sensitive to rapamycin, demonstrating that rapamycin was acting via the mTORC1–S6K branch rather than the 4EBP1–eIF4E branch (Extended Data Fig. 7).

We then used an Eef2k-null mouse and showed that after irradiation and treatment with rapamycin, these mice were now resistant to mTORC1 inhibition, confirming the importance of translational elongation (Fig. 4a). To ensure that S6K was not also acting through its more established effector, rpS6, we used an Rps6 phospho-mutant that cannot be phosphorylated by S6K. This was unable to phenocopy Rptor deletion (Fig. 4a), showing that Wnt-driven regeneration requires increased translational elongation, mediated through mTORC1.

To prove that inhibition of eEF2K by S6K is required to allow increased eEF2 activity after Apc loss, we intercrossed VillinCreER Apcfl/fl mice to Eef2k−/− mice and treated these with rapamycin. In contrast to VillinCreER Apc−/− mice, these intestines were now resistant to the growth inhibitory effects of rapamycin (Fig. 4b, c). Tellingly, these mice no longer show an increase in the inhibitory phosphorylation of eEF2 after rapamycin treatment (Extended Data Fig. 8).

To assess whether the increased elongation after Apc deletion had differential effects on cell-cycle-regulating proteins, RNA and protein levels of several key cell-cycle regulators were tested (Fig. 4d, e). This analysis revealed that while there were increased RNA and protein levels of cyclin D1, cyclin D2, CDK4 and CDK6, cyclin D3 had increased protein levels in the absence of increased messenger RNA levels. Cyclin D3 protein levels were sensitive to rapamycin exposure, and this sensitivity depends on eEF2K (Extended Data Fig. 9). Additionally, ribosomes were shown to elongate approximately four times faster on cyclin D3 messages in Apc-deficient cells than in wild-type cells. No differences were detected in other messages tested (Extended Data Fig. 10). Taken together, these data suggest that cyclin D3 is translationally regulated at the level of elongation, consistent with previous reports. The contribution of cyclin D3 to the proliferative phenotype remains to be elucidated.

We report that mTORC1 is an essential downstream effector of Wnt signalling in the intestine. We show that intestinal proliferation associated with Wnt signalling requires the mTORC1–S6K–eEF2K axis in Wnt-driven proliferation. Mice were exposed to 14 Gy γ-irradiation, and intestinal regeneration was calculated 72 h after exposure by examining the number and size of regenerating crypts relative to wild-type regenerating intestines. KO, knockout. Whiskers show maximum and minimum, black line shows median (n = 6 per group). *P value ≤ 0.05, Mann–Whitney U test. NS, not significant.
importance of elongation in this context has been suggested in a small number of publications, but this study provides key in vivo evidence26–28. Finally, we have also shown that targeting mTOR and translational control may be a viable strategy for chemoprevention of colorectal carcinoma in high-risk patients, and treatment of early stage disease. Indeed, recent studies have suggested that the chemopreventative agents aspirin and mesalazine also target mTOR29,30.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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METHODS

Mouse colonies. All experiments were performed according with UK Home Office regulations (license 60/1483) which undergoes local ethical review at Glasgow University. Outbred male mice from 6 to 12 weeks of age were used. The majority of the work was performed on C57BL/6 mice: AhCre, ApoE<sup>−/−</sup>, ApoM<sup>−/−</sup> and Rptor<sup>−/−</sup> Sk6/l2 knockout and Rps6<sup>mut</sup> mice were all C57BL/6. Some treatment experiments were performed on mice from three generations C57BL/6 (Lgsr<sup>Cre</sup>E<sup>AP</sup>)<sup>−/−</sup>.

The alleles used were as follows: VillinCre<sup>−/−</sup> (ref. 31). AhCre (ref. 11), Lgsr<sup>Cre</sup>E<sup>AP</sup> (ref. 32). ApoE<sup>−/−</sup> (ref. 33), ApoM<sup>−/−</sup> (ref. 34), Mys<sup>−/−</sup> (ref. 35), Rpto<sup>−/−</sup> (ref. 36), RoSA- tdRFP (ref. 37), Efpelf1 knock out (ref. 38), Efpelf2 knock out (ref. 39), S6k1 knock out (ref. 40), S6k2 knock out (ref. 41), Efp2 knock out (ref. 42), Rps6<sup>−/−</sup> (ref. 43). Recombination by VillinCre<sup>−/−</sup> was induced with one intraperitoneal (i.p.) injection of 80 mg kg<sup>−1</sup> tamoxifen on day 0 and day 1. Analysis of VillinCre<sup>−/−</sup>-induced mice was at day 4 after induction. Red fluorescent protein (RFP) analysis was carried out by inducing recombination by AhCre using a single i.p. injection of 80 mg kg<sup>−1</sup> β-naphthoflavone. RFP visualization was carried out on day 4. Mice carrying the Lgsr<sup>Cre</sup>E<sup>AP</sup> transgene were given one i.p. injection of 120 mg kg<sup>−1</sup> tamoxifen.

For regeneration experiments, mice were exposed to γ-irradiation from caesium-137 sources. This delivered γ-irradiation at 0.423 Gy min<sup>−1</sup>. Rapamycin treatment was performed using a daily i.p. injection of 10 mg kg<sup>−1</sup> (refs 43, 44) in 5% Tween80 and 5% polyethylene glycol in PBS. Cycloheximide was used for downstream analysis.

Determination of protein synthesis rates. Protein concentrations were determined using a BCA Protein Assay Kit (Pierce). Equal amounts of cellular protein (30 μg) were separated on a 4%–12% gradient gel (Novex) and subsequently transferred to a PVDF membrane (Amersham). Total protein was visualized with Ponceau (Sigma). After blocking the membranes in TBS containing 5% BSA (Sigma), 0.02% Triton X-100 for 1 h, primary antibodies were added in block solution at the following dilutions: CDK4 (Santa Cruz SC-260, 1:1,000), CDK6 (Cell Signaling 5136, 1:1,000), cyclin D1 (Cell Signaling 2926, 1:2,000), cyclin D2 (Cell Signaling 3741, 1:1,000) and β-actin (Sigma A2282, 1:5,000). After washing, the appropriate HRP-conjugated secondary goat antibodies (Dako) were added diluted 1:10,000 in block for 1 h. Antibody binding was detected using ECL Western Blotting Substrate (Pierce). Primary antibody incubations were carried out at 4 °C overnight. Remaining incubations were carried out at room temperature.

RNA isolation. Snap-frozen intestinal epithelial tissue was homogenized and RNA was extracted using the TRIzol method (Ambion). qPCR. One microgram of RNA was reverse transcribed to cDNA using a Quan-Trimite Reverse Transcription Kit (Qigen) in a reaction volume of 20 μl. qPCR was performed on each sample in triplicate in a 20 μl reaction mixture containing 10 μl of 2× D<sup>2</sup>DNaseo MS master mix (Thermo Scientific), 0.5 μM of each of the primers (detailed later) and 2 μl cDNA generated previously. The reaction mixture without a template was run in triplicate as a control. The reaction conditions were as follows: 95 °C for 15 min, followed by 40 cycles of three steps consisting of denaturation at 94 °C for 15 s, primer annealing at 60 °C for 30 s, and primer extension at 72 °C for 30 s. A melting curve analysis was performed from 70 °C to 95 °C in 0.3 °C intervals. Gpahd was used to normalize for differences in RNA input.

qRT–PCR primers. qRT–PCR primers were as follows. Cnd1 forward, 5′-GAGA AGTGTGGAATCCTACACTG-3′; Cnd1 reverse, 5′-AAATGAGACTCAGCTTCG-3′; Gtggc-3′; Cnd2 forward, 5′-CTACCAGCTCAAGTTG-3′; Cnd3 reverse, 5′-GTTTGGAGACATCCCCATGAC-3′; Gtcd4 forward, 5′-AAATTGTGAGTCTGCATA-3′; Gtcd4 reverse, 5′-AGAAGATGCTGATAGGATCTC-3′; Cdk6 forward, 5′-GGGCCATACCAAGACACATCA-3′; Cdk6 reverse, 5′-AGGTTAAGGCCACATCGA-3′; Cdk3 forward, 5′-CGGCTCCCTTGCAGCTG-3′; Cdk3 reverse, 5′-GCCAGCTGGATCGACTGTTG-3′; Rp32 forward, 5′-GGTCTACGAGTAGGCTTGG-3′; Rp32 reverse, 5′-CCTACACGTTCACCTGAGG-3′.

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Extended Data Figure 1 | mTORC1 is activated following Wnt-signal and its inhibition does not affect homeostasis. a, Representative IHC of phospho-RPS6 (pS6) and phospho-4EBP1 (p4EBP1) show mTORC1 activity during intestinal regeneration, 72 h after 14 Gy γ-irradiation (representative of 5 biological replicates). b, c, Boxplots demonstrating that 72 h of 10 mg kg\(^{-1}\) rapamycin treatment does not alter mitosis or apoptosis in normal intestinal crypts. Whiskers show maximum and minimum, black line shows median (\(n = 4\) per group). NS, not significant, Mann–Whitney \(U\) test. d, Intestines imaged on OV100 microscope, 96 h after induction, for red fluorescent protein (RFP). Tissue without the ROSA-tdRFP reporter (Neg control) show no RFP positivity, while the positive control (Pos control) and Rptor–deleted intestines show high RFP positivity (representative of 3 biological replicates). e, f, Boxplot showing that Rptor deletion does not affect mitosis or apoptosis rates in intestinal crypts, 96 h after induction. Whiskers show maximum and minimum, black line shows median (\(n = 4\) per group). NS, not significant, Mann–Whitney \(U\) test. Scale bars, 100 μm.
Extended Data Figure 2 | Rptor deletion is maintained in the small intestine. a, Representative IHC of phospho-RPS6 (pS6) and phospho-4EBP1 (p4EBP1) shows maintained loss of mTORC1 signalling 400+ days after Rptor deletion. Arrows indicate unrecombined escaper crypts that still show active mTORC1 signalling (representative of 5 biological replicates). b, c, Boxplots showing that mitosis and apoptosis are unchanged 400+ days after Rptor deletion. Mitosis and apoptosis were counted on H&E sections and are quantified as percent mitosis or apoptosis per crypt. Whiskers show maximum and minimum, black line shows median (n = 5 per group). NS, not significant, Mann–Whitney U test. Scale bars, 100 μm.
**Extended Data Figure 3** | Wnt signalling is still active after Rptor deletion and rapamycin treatment causes regression of established tumours.

**a**, Representative IHC of MYC and β-catenin showing high MYC levels and nuclear localization of β-catenin 96 h after Apc and Apc/Rptor deletion, demonstrating active Wnt signalling. Nuclear staining (as opposed to membranous staining) of β-catenin is indicative of active Wnt signalling. Scale bar, 100 μm (representative of 3 biological replicates).

**b**, Representative IHC of MYC and β-catenin showing high MYC levels and nuclear localization of β-catenin 96 h after Apc and Apc/Rptor deletion, demonstrating active Wnt signalling. Nuclear staining (as opposed to membranous staining) of β-catenin is indicative of active Wnt signalling. Scale bar, 100 μm (representative of 3 biological replicates).

**c**, Kaplan–Meier survival curve of ApcMin/+ mice treated with rapamycin when showing signs of intestinal neoplasia. Rapamycin treatment (10 mg kg⁻¹) started when mice showed signs of intestinal disease, and was withdrawn after 30 days. Animals continued to be observed until signs of intestinal neoplasia. Death of animals in the rapamycin group almost always occurred after rapamycin withdrawal (n = 8 per group). ***P value ≤ 0.001, log-rank test.

**d**, Boxplot showing that 72 h 10 mg kg⁻¹ rapamycin treatment causes an increase in lysozyme-positive cells in tumours. Percentage lysozyme positivity within tumours was calculated using ImageJ software (http://imagej.nih.gov/ij/). Whiskers show maximum and minimum, black line shows median (10 tumours from each of 5 mice per group were measured). **P value ≤ 0.014, Mann–Whitney U test.

**e**, Boxplot showing that 72 h 10 mg kg⁻¹ rapamycin treatment causes a decrease in BrdU positivity within tumours. Percentage BrdU positivity within tumours was calculated using ImageJ software. Whiskers show maximum and minimum, black line shows median (10 tumours from each of 5 mice per group were measured). **P value ≤ 0.021, Mann–Whitney U test.

**f**, Representative IHC of lysozyme, showing a lack of lysozyme-positive paneth cells in remaining cystic tumours after 30 days of 10 mg kg⁻¹ rapamycin treatment. Scale bars, 100 μm (representative of 5 biological replicates).
**Extended Data Figure 4 | IHC after rapamycin treatment.**

**a**, Representative IHC of p21, p16 and p53 after 6 h and 72 h of 10 mg kg$^{-1}$ rapamycin treatment. Staining shows no induction of these proteins in tumours after rapamycin treatment (representative of 5 biological replicates).

**b**, Representative IHC for LGR5–GFP showing high numbers of LGR5-positive cells after 7 and 30 days of 10 mg kg$^{-1}$ daily rapamycin treatment (representative of 5 biological replicates). Scale bars, 100 μm.
Extended Data Figure 5 | Rptor deletion in the intestinal crypt is lethal in vitro. a, Graph showing that Rptor deletion prevents intestinal crypts from growing ex vivo. Intestinal crypts were isolated and cultured as previously described\(^1\), 96 h after Cre induction. Number of viable organoids was counted by eye 72 h after crypt isolation. WT, wild type. Data are average ± standard deviation (n = 3 biological replicates per group).
Extended Data Figure 6 | Apc deletion increases translational elongation rates and cycloheximide treatment phenocopies rapamycin treatment.

a, Representative polysome profiles from wild-type ex vivo crypts incubated with harringtonine for 0 s (left) and 180 s (right) before harvest (n = 3 per time point). b, The areas under the sub-polysome (40S, 60S and 80S) and polysome sections as indicated by the dashed lines in a were quantified and expressed as a percentage of their sum. Data in the bar graph are the average ± s.e.m. (n = 3 per time point). c–d, Data are shown for Apc-deleted crypts, as for wild type in b and c (n = 3 biological replicates). e, Representative H&E staining showing that 35 mg kg⁻¹ cycloheximide treatment phenocopies rapamycin treatment 96 h after Apc deletion. Treatment began 24 h after induction (n = 3 biological replicates). f, Representative IHC for BrdU showing a loss of proliferation in tumours after 72 h of 35 mg kg⁻¹ cycloheximide treatment. (n = 3 biological replicates). Arrow highlights normal proliferating crypts. Scale bar, 100 μm.
Extended Data Figure 7 | S6k deletion decreases intestinal regeneration.

Graphical representation of findings, and boxplot showing that murine intestinal regeneration after irradiation is dependent on S6K. Animals were exposed to 14 Gy γ-irradiation, and intestinal regeneration was calculated 72 h after exposure by counting the number of viable crypts and multiplying that by the average size of the regenerating crypts. Relative regeneration was calculated by comparing each group to wild-type regeneration. The rapamycin treatment arm is reproduced from Fig. 4 for visual clarity. Whiskers show maximum and minimum, black line shows median (n = 4 per group). *P value = 0.034, Mann–Whitney U test.
Extended Data Figure 8 | Eef2k deletion drives resistance to rapamycin.

a, Representative IHC of phospho-eEF2 and phospho-RPS6 in wild-type (WT), Apc-deficient and Apc- and Eef2k-deficient mice (with or without 72 h 10 mg kg\(^{-1}\) rapamycin (rapa) treatment) shows that rapamycin is unable to induce eEF2 phosphorylation in the absence of eEF2K (\(n = 6\) biological replicates). KO, knockout. Scale bars, 100 \(\mu m\).
Extended Data Figure 9 | Cyclin D3 is regulated at the level of elongation.
a, Representative IHC of Apc-deleted intestines with or without Eef2k deletion. Antibodies to eEF2K, phospho-RPS6 and cyclin D3 are shown (representative of 3 biological replicates). After Eef2k knockout (KO), cyclin D3 levels are no longer decreased upon 10 mg kg\(^{-1}\) rapamycin (rapa) treatment. b, Boxplot showing the number of cyclin-D3-positive cells per crypt, 96 h after Apc deletion, with and without 10 mg kg\(^{-1}\) rapamycin treatment. Graph shows that in Eef2k knockout animals, rapamycin no longer reduced cyclin D3 levels \((n = 3\) biological replicates per group). *P value ≤ 0.05, Mann–Whitney U test. c, Western blot analysis of intestinal epithelial cells from Apc-deleted and Apc-deleted Eef2k knockout, with and without 10 mg kg\(^{-1}\) rapamycin. Antibodies to eEF2K, phospho-RPS6, cyclin D3 and β-actin are shown. Each well represents a different mouse from the relevant group. Cyclin D3 levels are no longer reduced after Eef2k deletion. Scale bar, 100 μm.
Extended Data Figure 10 | Ribosomes elongate faster on Ccnd3 after Apc deletion. The ribosome run-off rate of various messages was measured as in Fig. 3. Elongation of Ccnd3 was significantly increased, while Actb, Rps21, Rps6 and Ccnd1 remain unchanged. Data are average ± s.e.m. (n = 3 biological replicates per group). *P value ≤ 0.05, Mann–Whitney U test.