In vivo genome editing and organoid transplantation models of colorectal cancer and metastasis

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In vivo interrogation of the function of genes implicated in tumorigenesis is limited by the need to generate and cross germ-line mutant mice. Here we describe approaches to model colorectal cancer (CRC) and metastasis, which rely on in situ gene editing and orthotopic organoid transplantation in mice without cancer-predisposing mutations. Autochthonous tumor formation is induced by CRISPR-Cas9-based editing of the Apc and Trp53 tumor suppressor genes in colon epithelial cells and by orthopic transplantation of Apc-edited colon organoids. Apc/Δ71 KrasG12D/+; Trp53/Δ(AKP) mouse colon organoids and human CRC organoids engraft in the distal colon and metastasize to the liver. Finally, we apply the orthotopic transplantation model to characterize the clonal dynamics of Lgr5+ stem cells and demonstrate sequential activation of an oncogene in established colon adenomas. These experimental systems enable rapid in vivo characterization of cancer-associated genes and reproduce the entire spectrum of tumor progression and metastasis.

Recent tumor sequencing studies have identified a large number of candidate genes that are mutated in CRCs and may contribute to carcinogenesis, tumor phenotype, and treatment responses in subsets of patients1,2. Traditionally, functional assessment of putative cancer-associated genes in vivo has required the development of genetically engineered mouse models (GEMMs) of CRC through extensive intercrossing or de novo generation of mice with desired genetic mutations, which is expensive and time-consuming. Most GEMMs of CRC such as the ApcMin mouse3,4 are also limited by delayed tumor onset (i.e., 2–4 months) and high tumor burden (i.e., 30–100 polyps) in the small intestine, which is a rare location for human intestinal tumors and precludes study of tumor progression beyond early adenomas or longitudinal studies using colonoscopy5,6. Tumorigenesis can be localized to the colons of Apc0/– mice with either colon-specific promoters driving Cre recombinase, which are limited by slow tumor growth (i.e., 4–6 months)7–9, or rectal enema of adenoviral Cre recombinase, which requires colonic injury and/or time-consuming surgery10,11.

In addition to GEMMs, human and mouse cell lines are used to model CRC in vivo. Typical sites of cell transplantation are the mouse flank or kidney capsule, which do not recapitulate the native stroma of the colon mucosa6. Several groups have sought to orthotopically deliver tumor cell lines into the mouse colon, either surgically into the cecal serosa12 (which is not the relevant tissue layer for CRC development) or into the mucosa via rectal enema14, injury15, electrocoagulation16, or colonoscopy-guided mucosal injection17. However, these orthotopic models are limited by the use of mouse or human cell lines that are not genetically defined and poorly recapitulate the histology of CRC.

The CRISPR–Cas9 nuclease editing system offers the ability to somatically mutate one or more genes in wild-type mice to assess their role in tumorigenesis18. We and others have demonstrated the feasibility of inducing tumorigenesis in the lung by lentiviral delivery of CRISPR–Cas9 components19,20. CRISPR-Cas9 gene editing has been applied to engineer human CRC three-dimensional cultures, or organoids; mutations in APC, KRAS, TP53, SMAD4, and/or PIK3CA were required for successful engraftment at ectopic sites in mice21,22.

Here we describe CRISPR-Cas9-based somatic gene editing and orthotopic organoid transplantation approaches that employ colonoscopy-guided mucosal injection for primary and metastatic tumor induction in mice without cancer-predisposing mutations. We first optimized a colonoscopy-guided mucosal injection system to produce a mucosal bubble that localizes the injection to the lamina propria of the distal 4 cm of the mouse colon (Supplementary Fig. 1a and Supplementary Video 1), based on previous reports17,23. Using this...
Massively parallel sequencing of the genomic region flanking the sgRNA target site revealed clonal CRISPR-Cas9-mediated frameshift insertions or deletions in Apc. Notably, we observed distinct Apc-inactivating mutations arising in equal biallelic proportions, which suggests that these tumors originated from two cells (Supplementary Fig. 4a). Thus, we demonstrate the application of CRISPR–Cas9 for tracing the clonal origins of cancer, consistent with a recent report of organismal lineage tracing using CRISPR–Cas9 (ref. 30). This approach is useful for determining the types of mutations that are most potent in transforming cells and thereby conferring growth advantage within a multiclonal tumor.

To increase viral titer, we generated a lentivirus containing the Apc sgRNA without Cas9 (U6::sgApC-CMV::Cre, or pUSCC; genome size ~4.4 kb), which produced viral titers of ~10,000 TU/μl. Mucosal injection of pUSCC-sgApc into Rosa26SL-Cas9-eGFP/+ mice resulted in tumor formation in 92% of mice (Fig. 1b and Table 1). Having shown that mucosal injection of lentivirus results in widespread stromal cell infection (Supplementary Fig. 1c), we sought to restrict CRISPR–Cas9 gene editing to colon epithelial cells for cancer modeling. We administered tamoxifen to Rosa26SL-Cas9-eGFP/+;VillinCreER mice to express Cas9 specifically in intestinal epithelial cells. Mucosal delivery of a U6::sgApC-EFS::turboRFP (pUSET; genome size ~3.8 kb) lentivirus resulted in efficient tumorigenesis characterized by infection of stromal and epithelial cells and CRISPR-mediated Apc editing only in Cas9-positive epithelial cells (Fig. 1c and Table 1). Consistent with a tenfold increase in viral titer compared to the U6::sgApC-EFS::Cas9-P2A-GFP lentivirus, we detected multiple frame-shifting Apc mutations present in similar proportions, indicating polyclonal tumors that arose from greater than ten cells of origin (Supplementary Fig. 4b).

U6::sgApC-EFS::turboRFP tumors that progressed for 1 year demonstrated high-grade dysplasia on histology but no local invasion or liver metastasis (Supplementary Fig. 5a,b and data not shown). In comparison to 6-week-old tumors, 1-year-old U6::sgApC-EFS::turboRFP tumors exhibited dominance of 1–2 Apc frame-shifting mutations. These mutations most likely provided a growth advantage to the initiating cells (Supplementary Fig. 4b).

To demonstrate the application of our in vivo gene editing system for modeling cancer-associated genes, we performed mucosal editing of the tumor suppressor Trp53 alone (i.e., pUSCC-sgTrp53 into Apcfl/fl;Rosa26SL-Cas9-eGFP/+ mice) or in combination with Apc (i.e., hU6::sgApC-sU6::sgTrp53-EFS::turboRFP into Rosa26SL-Cas9-eGFP/+;VillinCreER mice treated with tamoxifen) (Supplementary Fig. 5c,d and Table 1). Massively parallel sequencing of the genomic region flanking the sgTrp53 target site revealed multiple frame-shifting mutations in these tumors (Supplementary Fig. 6a,b). Together, these studies demonstrate the utility and multiplexability of in vivo somatic CRISPR–Cas9 editing for CRC modeling and assessment of gene function in mice without germline cancer-predisposing mutations.

We sought to use our mucosal injection approach to develop an in vivo model of intestinal organoid function in which cultured intestinal organoids are grown in the native colon environment of a host mouse. We derived intestinal and colon organoids from Rosa26SL-tdTomato/+, pUSCC-sgApc, and data not shown). In comparison to intestinal organoid and assessment of gene function in mice without germline cancer-predisposing mutations.

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transplantation by rectal enema into mice, our approach does not require colitis or mechanical injury.

We exploited our mucosal injection technique to model CRC by orthotopically transplanting cancer cells. Syngeneic transplantation of an AKP murine CRC cell line derived from a genetically engineered mouse tumor resulted in reproducible engraftment and invasive tumor formation (Supplementary Fig. 8a and Table 1). However, these tumors did not reproduce the glandular histopathology of human colon adenocarcinoma.

We hypothesized that Apc-deficient intestinal organoids would engraft and form tumors in the distal colon after orthotopic transplantation. Intestinal and colon organoids were infected with U6: sgApc-EFS: Cas9-P2A-GFP lentivirus, and then selected for Apc loss by culturing in media without the exogenous Wnt ligands Wnt3a and R-spondin-1 (Supplementary Fig. 8b). These organoids exhibited CRISPR-Cas9-mediated editing at the sgApc target locus and activation of the Wnt signaling pathway by quantitative real-time (qRT) PCR for Wnt target genes (Supplementary Figs. 8c, d, 9a, and Supplementary Table 1). Orthotopic xenograft and syngeneic transplantation of CRISPR-Cas9-edited Apc-null intestinal organoids into immunodeficient mice and C57BL/6 mice, respectively, resulted in adenomas that extended to the epithelial surface with nuclear β-catenin localization (Fig. 2a, Supplementary Fig. 8e, and Table 1).

Analysis of mutations in these tumors at the sgApc binding site revealed substantial intratumoral and intertumoral heterogeneity (Supplementary Fig. 9b). We did not find local invasion or liver metastasis in Apc-null orthotopic tumors in immunodeficient (N = 16) and syngeneic recipients (N = 20) that progressed for 24 weeks (data not shown). No tumors formed upon transplantation of Apc-null intestinal organoids into the flanks of syngeneic mice, which suggests that the colon mucosa is a more permissive environment for intestinal organoid engraftment than the subcutaneous space (N = 5, data not shown). Our mucosal injection system complements the enema model of organoid orthotopic transplantation described by O'Rourke et al.34.

We modeled more advanced CRCs by infecting Apc<sup>fl/fl</sup>, Kras<sup>G12D/+</sup> colon organoids with Ad5CMV: Cre, and then culturing them in media that contained nutlin-3 and lacked Wnt pathway agonists to generate AKP tumor organoids. These organoids developed invasive tumors with a desmoplastic stromal reaction after orthotopic, syngeneic transplantation, a cardinal feature of invasive human CRC (Supplementary Fig. 8f and Table 1). 12 weeks following orthotopic engraftment into NSG (no-obese diabetic/severe combined immunodeficient gamma) mice, AKP organoids invaded the muscularis propria, local vasculature, and metastasized to the liver in 33% of recipient mice (Fig. 2b, c, and Table 1). All mice with liver metastases exhibited primary colon tumors with invasion of the muscularis propria. These results indicate that orthotopic transplantation of murine tumor organoids can be used to model the entire spectrum of human CRC, including distant-organ metastasis.
Figure 1 CRISPR-Cas9-based in situ Apc editing in the colon epithelium induces adenoma formation. (a) Tumors in wild-type mice following mucosal injection with 1,000 transforming units (TU) per µl of U6-øsgApc-EFS-øCas9-P2A-GFP lentivirus. Tumors are indicated with white light colonoscopy, brightfield necropsy, GFP fluorescence necropsy, hematoxylin and eosin (H&E) staining, and β-catenin immunohistochemistry. Note patchy GFP expression in tumors (arrow). (b) Tumors in Rosa26SL-Cas9-eGFP+/ mice after mucosal delivery of a lentivirus encoding an sgRNA against Apc and Cre recombinase (U6-øsgApc-CMV-øCre, 10,000 TU/µl) (white light colonoscopy, brightfield necropsy, and GFP fluorescence necropsy; H&E immunohistochemistry and GFP/β-catenin immunofluorescence). GFP tumor fluorescence indicates Cre-induced expression of Cas9 and eGFP from the Rosa26 locus. (c) Tumorigenesis in Rosa26SL-Cas9-eGFP+/;VillinCreER+ mice treated with tamoxifen and then injected with lentiviruses encoding an sgRNA against Apc and turboRFP (U6-øsgApc-EFS-øturboRFP, 10,000 TU/µl) into the colon mucosa (white light, GFP/turboRFP fluorescence colonoscopy and brightfield/GFP/turboRFP fluorescence necropsy; GFP/turboRFP immunofluorescence). Arrowheads indicate turboRFP expression in stromal cells. Arrows point to GFP/turboRFP dual-positive tumor cells. Histology images are 20x and insets are 60x (scale bar, 200 µm). Dotted lines indicate tumors. tRFP: turboRFP; R26: Rosa26; N: normal; T: tumor.
Figure 2 Orthotopic transplantation models of mouse- and patient-derived colorectal cancer. (a) Tumors in the distal colons of NSG mice following orthotopic transplantation of wild-type colon organoids infected with U6::sgApc-EFS::Cas9-P2A-GFP lentivirus. Tumors are visualized with white light/GFP fluorescence colonoscopy, white light/GFP fluorescence necropsy, and GFP immunofluorescence. (b) Tumors induced in NSG mice by orthotopic transplantation of ApcΔ/Δ;KrasG12D/+;Trp53Δ/Δ (AKP) colon organoids. Tumors are imaged with colonoscopy, necropsy, hematoxylin and eosin (H&E) staining, β-catenin immunohistochemistry, and CDX2 immunohistochemistry. (c) Local invasion and liver metastases of engrafted AKP colon organoid tumors; as indicated, H&E and LYVE1 immunohistochemistry of primary colon tumors, necropsy, and CDX2 immunohistochemistry of liver metastases. Arrows indicate invasion of the muscularis propria and arrowheads demonstrate tumor invasion of a LYVE1-positive lymphatic vessel. (d) Tumors in NSG mice following orthotopic transplantation of patient-derived CRC organoids. Tumors in NSG mice following orthotopic transplantation of patient-derived CRC organoids. Tumors are demonstrated with colonoscopy, necropsy, H&E staining, β-catenin immunohistochemistry, and CDX2/human Keratin20 immunohistochemistry. (e) Local invasion and liver metastases of patient-derived organoid orthotopic tumors are demonstrated by H&E staining, LYVE1 immunohistochemistry, liver necropsy, and CDX2/human Keratin20 immunohistochemistry. Arrows denote invasion of the muscularis, and arrowheads indicate tumor invasion of a LYVE1-positive vessel. Histology images are 20× and insets are 60×. Scale bars, 200 µm. Dotted lines indicate tumors. T: tumor; N: normal colon; LYVE1: lymphatic vessel endothelial hyaluronan receptor 1; hKeratin20: human Keratin 20.
Finally, we asked whether in vitro CRISPR-Cas9-based editing of organoids, followed by orthotopic transplantation, can be applied to assess gene function in vivo. We infected colon organoids derived from Apc6/6 Rosa26LSL-Cas9-eGFP/+ mice with U6::sgTrp53-CMV::Cre lentivirus, cultured them in media without Wnt pathway agonists, and then in media with nutlin-3 to select for ApcΔ/Δ Trp53-null organoids. We then orthotopically engrafted Trp53-null tumor organoids into NSG mice to model TP53-mutant CRC (Supplementary Figs. 8g, 10a,b, and Table 1).

We subsequently aimed to develop patient-derived orthotopic mouse models of CRC. Engraftment of human CRC cell lines efficiently

**Figure 3** Lgr5 cell lineage tracing and sequential mutagenesis in established orthotopic colon adenomas. (a) Colon organoids derived from Lgr5CreER/+, Rosa26LSL-tdTomato/+ mice were subjected to CRISPR-Cas9-based Apc editing with U6::sgApc-EFS::Cas9-P2A-GFP infection, and then orthotopically transplanted into NSG mice to generate Apc-null tumors in vivo. Tumor-bearing mice received one dose of tamoxifen to label Lgr5+ cells and their progeny with tdTomato and were evaluated 2 d, 3 weeks, or 6 weeks later. Proliferating cells were marked with a 5-ethyl-2′-deoxyuridine (EdU) pulse 4 hours before euthanasia. (b) In vivo tumor imaging by white light, GFP fluorescence, and tdTomato fluorescence colonoscopy (dotted lines) at 2 d, 3 weeks, and 6 weeks after cell labeling. (c) GFP, tdTomato, and EdU immunofluorescence images of orthotopic tumors. Arrowhead indicates cell labeling with tdTomato at 2 d after labeling (white arrowhead). Arrow points to GFP-negative tumor areas that suggest mosaic lentiviral silencing. (d–f) Immunofluorescence images were analyzed for tdTomato+ tumor area relative to total GFP+ tumor area (d); average tdTomato+ clone size (i.e., total tdTomato+ area/total clone number) (e); and proportion of EdU+–proliferating/tDTomato+GFP+ tumor cells vs. EdU+–proliferating/tDTomato–GFP– tumor cells at 6 weeks after labeling (f). *P < 0.005 (one-way ANOVA), **P = 0.01 (Student’s t-test). R26: Rosa26; tdTom: tdTomato.
formed tumors that extended to the surface of the epithelium and invaded the muscularis propria, but did not reflect the histology of human CRC (Supplementary Fig. 11a,b and Table 1). In contrast, patient-derived orthotopic organoid xenografts produced tumors that accurately recapitulated the epithelial and stromal histology of the patient tumor. In addition, patient-derived organoid transplants formed invasive colon tumors that metastasized to the liver in 25% of mice with primary tumors at 8 weeks and in 45% of mice at 12 weeks after transplantation (Fig. 2d,e, Supplementary Fig. 12a,b,d,e, Table 1, and Supplementary Table 2). Patient-derived CRC organoids transplanted into the mouse flank formed histologically similar tumors but did not metastasize (N = 5 tumors; Supplementary Fig. 12c and data not shown). Engraftment of patient-derived orthotopic xenografts, in which CRC tissue was digested and directly transplanted into the distal colon without exposure to tissue culture conditions, also engendered tumors that exhibited histological features of human CRC (Supplementary Fig. 11c, Table 1, and Supplementary Table 2). These findings demonstrate that orthotopic transplantation of patient-derived CRC organoids robustly models primary and metastatic human CRC.

Microsatellite unstable CRC is associated with prominent lymphocytic infiltration and improved survival with anti-programmed death 1 (PD-1) immune checkpoint inhibition25. However, xenograft models of patient-derived CRC lack a human immune system and therefore have limited utility for studying tumor immunology or immune checkpoint blockade. We derived organoids from a microsatellite instability-high (MSI-H) CRC from a patient with Lynch syndrome (patient B; see Supplementary Table 2). We then orthotopically transplanted the MSI-H organoids into NSG mice with a reconstituted human immune system, which elicited a human lymphocytic infiltrate that is similar to what is observed in MSI-H patient tumors. (Supplementary Fig. 12f)26.

Lgr5 (leucine-rich repeat containing G-protein-coupled receptor 5) marks a subpopulation of small intestinal adenoma stem cells that generate new adenoma cells26. However, the mouse models previously used for Lgr5 cell labeling and retraceing do not model colon adenomas and do not permit gene mutation in established adenomas. We used our orthotopic transplantation system to overcome these limitations and trace the lineage of Lgr5 cells in established colon adenomas. We generated Lgr5\textsuperscript{CreER\textsuperscript{flox}}, Rosa26\textsuperscript{LSL-tTomato\textsuperscript{flox}} mice, derived colon organoids, inactivated Apc by \textit{in vivo} lentiviral infection (i.e., U6::sgApc-EFS::Cas9-P2A-GFP), and orthotopically engrafted these tumor organoids into NSG mice to generate tumors in vivo that were visualized by colonoscopy 2 weeks post-injection. Tumor-bearing mice were then administered a tamoxifen pulse to label Lgr5 cells with tdTomato (Fig. 3a). Tumors were visualized with fluorescence colonoscopy and immunofluorescence 2 days, 3 weeks, and 6 weeks after labeling (Fig. 3b,c). Lgr5 cell-derived populations increased in proportion to the total tumor area and in average clone size at 3 and 6 weeks after labeling compared to labeling at 2 days (Fig. 3d,e). Notably, tdTomato\textsuperscript{+} areas contained more proliferating EdU\textsuperscript{+} cells compared to tdTomato\textsuperscript{-} areas of the tumors, indicating that clones derived from Lgr5\textsuperscript{+} cells harbored increased proliferative potential (Fig. 3f). Finally, we activated oncogenic Kras in Lgr5\textsuperscript{+} tumor cells by administering tamoxifen to Apc-null Lgr5\textsuperscript{CreER\textsuperscript{flox}}, Rosa26\textsuperscript{LSL-tTomato\textsuperscript{flox}}, and Kras\textsuperscript{LSL-G12D}\textsuperscript{+} orthotopic tumors (Fig. 3g,h). These results reveal cancer stem cell activity for Lgr5 tumor cells in colon adenomas and demonstrate the application of our orthotopic transplantation model for sequential mutagenesis in established colon tumors.

Our \textit{in situ} epithelial gene editing and orthotopic transplantation systems provide considerable advances over standard mouse models of CRC: (1) tumors are located in the appropriate tissue compartment (i.e., the colon) and correct tissue layer (i.e., lamina propria); (2) organoids require only Apc loss for orthotopic engraftment, instead of multiple additional oncogenic mutations that are required for engraftment at other sites\textsuperscript{21,22}; (3) tumors form within a few weeks; (4) tumors are seen in almost all experimental mice; (5) tumors are longitudinally monitored with colonoscopy; (6) tumors with advanced mutations reproduce key pathological features of human CRC, including progression from primary cancer, invasion of the muscularis propria, and liver metastasis\textsuperscript{5,37,38}; (7) customized viral vectors reduce the cost and time required to functionally interrogate cancer-associated genes; (8) tumors are quickly induced with defined CRISPR-Cas9-based genetic alterations \textit{in vivo} or in organoids without the time-consuming need to generate mutations in the germline; and (9) orthotopic transplantation of Apc-edited organoids permits lineage tracing and sequential mutagenesis in established adenomas.

An important goal of cancer modeling is to reproduce primary and metastatic disease \textit{in vivo} for preclinical research and for clinical applications. Patient-derived CRC organoids have been shown \textit{in vitro} to recapitulate molecular, genetic, and pathological features of the original tumors, but to date have been engrafted only into ectopic sites. Organoid-based models of metastatic CRC are limited to seeding of distal organs from the kidney capsule or spleen\textsuperscript{39,40}. We demonstrate the use of patient-derived organoids to model tumor formation in the native colon environment with tumor and stromal histology that accurately reflects the patient’s disease. Finally, we apply our orthotopic transplantation system to model patient-derived primary cancer, local tumor invasion, and liver metastasis.

\section*{METHODS}

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

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\section*{AUTHOR CONTRIBUTIONS}

J.R. and T.T. performed all experiments and participated in their design and interpretation with T.J. and O.H.Y. J.R. and O.H.Y. developed and optimized the colonoscopy mucosal injection technique, with assistance from D.K. and P.K. J.R. wrote the paper with support from T.T. and O.H.Y. N.M.C. contributed to study design, plasmid design, lentivirus production, and mucosal injections. F.J.S.-R. contributed to plasmid and study design, and performed massively parallel...
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Recurrent R-spondin fusions in colon cancer.

Rapid colorectal adenoma formation initiated by conditional

sequencing, M.A., Y.K.P., R.N., R.A., X.L., D.K., K.W., S.R., and A.A. assisted with
mucosal injections, mouse and human organoid derivation, molecular biology,
and immunohistochemistry. A.R. assisted with humanized mouse experiments.
M.A.O. designed and synthesized lipid nanoparticles for mRNA encapsulation.
G.E., E.T.S., M.S.T., A.I.R., Y.S., J.Y., L.C., V.D., and L.Z. assisted with human
CRC specimen collection. S.B. performed organoid qRT-PCR. A.B. performed
bioinformatics analysis. R.L., J.L., J.C., P.N.T., R.O.H., and T.J. participated in
interpretation of results. Ö.H.Y. supervised all aspects of the study.

Competing financial interests

The authors declare no competing financial interests.

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ONLINE METHODS
Mice. Mice were housed in the animal facility at the Koch Institute for Integrative Cancer Research at MIT. All animal studies described in this study were approved by the MIT Institutional Animal Care and Use Committee. ApoE<sup>−/−</sup> (ref. 41), Katrl<sub>LSL-G12D</sub> (<ref. ref. 42), Villin<sup>Cre</sup> (<ref. ref. 43), Lgr5<sup>Cre</sup> (<ref. ref. 27), Lgr5<sup>ΔNKR</sup> (ref. 44), and Rosa26<sup>LSL-Cas9</sup>-GFP (ref. 31) mice were maintained on pure C57BL/6 or mixed C57BL/6 x 129SvJbgb1Sv genetic background. Orthotopic transplantation experiments with C57BL/6 organs were performed on syngeneic C57BL/6 or NOD SCID gamma (NSG)<sup>−</sup> recipient mice. Mice were maintained on a mixed C57BL/6J x 129SvJ genetic background. ORK inhibitor Y-27632 dihydrochloride monohydrate (APIExBO, catalog # A3008) to prevent cell death by anoikis.

Murine intestinal organoids were infected with lentivirus based on a protocol previously described for organoid retroviral infection<sup>31</sup>. Briefly, 2–3 days following culture of intestinal crypts, media was changed for culture media plus 10 mM nicotinamide (Sigma-Aldrich, catalog # N3376). At this point, organoids were cystic or round in appearance, which indicates the presence of healthy stem cells. The following day, organoids were disrupted mechanically by pipetting up and down 30 to 50 times with a 1,000-µl pipette. At this stage, the organoids were ideally small clumps of fewer than ten cells. If mechanical dissociation was not sufficient, then organoids were gently dissociated enzymatically with 1x TrypLE Express (Life Technologies, catalog # 12604-021) at 37 °C for 1–5 min. Organoids were then resuspended in a 24-well plate with culture media supplemented with 10 µM Y27632, 10 mM nicotinamide, and 8 µg/ml Polybrene (Sigma-Aldrich, catalog # TR-1003). The plate was centrifuged for 60 min at 600g at 32 °C (spinoculation), and then incubated at 37 °C for 6 h. Infected organoids were then embedded in Matrigel and cultured with culture media plus 10 µM Y27632 and 10 mM nicotinamide. For sgApc CRISPR-Cas9 viral infections, organoids were maintained in conditioned media for 7 d to permit Cas9-mediated editing of Apc, then in media without Wnt3α or R-spondin-1 (Advanced DMEM/F12 (Life Technologies, catalog # 12634-028) supplemented with 1× N2 (Life Technologies, catalog # 17502-048) and 1× B27 (Life Technologies, catalog # 17504-044)) for selection of App-deficient organoids.

AKP colon organoids were generated by culturing colon organoids from App<sup>−/−</sup>, Katrl<sub>LSL-G12D</sub> (<ref. ref. 42), R26<sub>LSL-Cas9</sub>-GFP (<ref. ref. 20), or lentivirus were resuspended in OptiMEM, and then delivered by an assistant to form a large bubble (Supplementary Video 1). 2-3 injections were performed per mouse.

For orthotopic organoid transplantations, organoids were gently mechanically dissociated, resuspended in 90% minimal media (Advanced DMEM supplemented with N2 and B27) and 10% Matrigel, and then transplanted into recipient mice. Mice underwent colonoscopy 4–8 weeks following lentiviral injection or organoid transplantation to assess tumor formation. Colonoscopy videos and images were saved for offline analysis. Tumor size was quantified using ImageJ, as previously described<sup>23</sup>. Following euthanasia, the distal colon mucosa of recipient mice (ideally 6-10 weeks old) by optical colonoscopy using a custom injection needle (Hamilton Inc., 33 gauge, small Hub RN NDL, 16 inches long, point 4, 45-degree bevel, like part number 7803-05), a syringe (Hamilton Inc., part number 7656-01), a transfer needle (Hamilton Inc., part number 7770-02), and a colonoscope with integrated working channel (Richard Wolf 1.9 mm/9.5 French pediatric urethroscope, part number 8626.431). For each injection, the tip of the injection needle was first placed into the colon mucosa. 50-100 µl of media was then quickly delivered by an assistant to form a large bubble (Supplementary Video 1). 2-3 injections were performed per mouse.

For murine colonoscopy and mucosal injection. Optical and fluorescence colonoscopy was performed using the Image 1H3-Z.Spies HD Camera System (part TH1000, Image 1 HUB CCU (parts TC200, TC300), 175 Watt D-Light Cold Light Source (part 20133701-1), AIDA HD capture system, Hopkins Telescope (part 64301A), and fluorescent filters in the tTomato (emission 554 nm) and GFP channels (emission 509 nm) (all from Karl Storz).

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transiently with 0.3% Triton-X100. Primary antibodies were detected with the appropriate Alexa 488, 594 or 647 secondary antibodies (ThermoFisher Scientific). Tissues were post-fixed in 1% paraformaldehyde and mounted in Vectashield with DAPI (VectorLabs, catalog # H1200). Immunofluorescence images were captured using a Nikon A1R confocal microscope using 10× or 20× air objectives and multichannel scanning in frame mode (pinhole 1.2 Airy units). For identification of proliferating cells, mice harboring orthotopic tumors were injected intraperitoneally with 1 mg of 5-ethynyl-2-deoxyuridine (EdU; Setareh Biotech, catalog # 61135-33-9) 4 hours before euthanasia. EdU was detected in cryosections using the Click-IT Edu Alexa Fluor 488 Imaging Kit (ThermoFisher Scientific, catalog # C10337) according to the manufacturer's protocol. Each antibody has been validated for use in mouse intestinal tissues, as detailed on the manufacturers’ websites.

**Bioluminescence imaging.** Colonies of wild-type mice were injected with lipid nanoparticles containing firefly luciferase mRNA or firefly luciferase mRNA alone. Rosa26SLE-mTomato/EGFP/Luciferase mice were injected with Ad5CMV::Cre under colonscopy guidance. Two days later, mice were injected intraperitoneally with 100 mg/kg d-luciferin, which was allowed to circulate for 10 min, followed by euthanasia and dissection of colons for bioluminescence imaging (IVIS, Caliper Life Sciences).

**Lipid nanoparticle synthesis.** Lipid nanoparticles (LNPs) were synthesized as previously described. In brief, an ethanol phase containing the lipids and an aqueous phase containing the mRNA were mixed in a microfluidic chip device. The aqueous phase consisted of 300 µL of Cre mRNA (1 mg/mL in 10 mM TRIS-HCL, TriLink Biotechnologies), 155 µL of citrate (100 mM, pH 3), and 1,097 µL of water. The ethanol phase contained the ionizable lipid cck-E12 (3.0 mg, synthesized in our laboratory, as previously described, with 1,2-dioleoyl-sn-glycer-3-phosphoethanolamine (DOPE, 4.1 mg, Avanti Polar Lipids), cholesterol (3.3 mg, Sigma-Aldrich, catalog # C8667), and 1,2-dimyristoyl-sn-glycer-3-phosphoethanolamine-N-(methoxy(polyethylene glycol)-2000) (C14-PEG 2000, 1.4 mg, Avanti, catalog # K3468) was used for visualization.

For CDX2 (rabbit monoclonal CDX2; 1:1250, Abcam, catalog # ab76541), Cytokeratin 20 (rabbit monoclonal human specific Cytokeratin 20; 1:500, Abcam, catalog # ab76126), LVEY1 (rabbit polyclonal LVEY1; 1:200, Abcam, catalog # ab14917), and CD3 (rabbit monoclonal CD3; 1:200, Abcam, catalog # ab16669), sections were dried at 60°C for 1 hour, dewaxed and rehydrated before treatment with heat-induced heat-induced epitope retrieval (HIER). The sections were then incubated in 10 mM sodium-citrate (pH 6.0) or 10mM Tris (pH 9.0) buffered solutions containing 0.05% Tween and heated at 120°C for 2 min using a pressure cooker. To obtain consistent and reliable staining an automated staining system (LabVision Autostainer 360, ThermoFisher Scientific) was used. The sections were subsequently cooled and placed onto the Autostainer. To destroy all endogenous peroxidase and alkaline phosphatase activity in the tissue, the sections were pretreated using BLOXALL blocking solution (Vector Laboratories, catalog # SP-6000) for 10 minutes. After a blocking step with normal horse serum, the sections were incubated with the individual primary antibody for 1 hour followed by several washes and secondary ImmPRESS polymer detection systems (Vector Laboratories), according to the manufacturer’s protocols. The Vulcan Fast Red Chromogen Kit 2 (red staining; Biocare Medical, catalog # FR805) and the DAB Quanto Chromogen and Substrate (brown staining; ThermoFisher Scientific, catalog # TA-060-QHDX) were applied as substrates and hematoxylin used as counterstain. For multiple stainings, following HIER and blocking steps, the individual antibodies were incubated consecutively using the chromogens indicated above. Image documentation was performed using the Leica Aperio AT2 slide scanner system. Each antibody has been validated for use in mouse and human tissues, as detailed on the manufacturers’ websites.

**Immunofluorescence.** Immunofluorescence on cryosections was performed as previously described. Briefly, 7 µm cryosections of intestines were air-dried, fixed with cold acetone, washed with PBS and blocked with donkey immunomix (i.e., 5% normal donkey serum, 0.2% bovine serum albumin, 0.05% sodium azide, and 0.3% Triton-X100 in PBS). The following primary antibodies were used for immunostaining of mouse tissues: rabbit β-catenin (1:100, Abcam, catalog # ab25372), mouse β-catenin (1:100, BD Biosciences, catalog # 610154), rabbit polyclonal lysozyme (1:250, ThermoFisher Scientific, catalog # RR-372-A1), rat EpCAM-APC (1:500, Biolegend, catalog # 17-5971-82). Sections were washed with PBS containing 0.3% Triton-X100. Primary antibodies were detected with the appropriate Alexa 488, 594 or 647 secondary antibodies (ThermoFisher Scientific). Tissues were post-fixed in 1% paraformaldehyde and mounted in Vectashield with DAPI (VectorLabs, catalog # H1200). Immunofluorescence images were captured using a Nikon A1R confocal microscope using 10× or 20× air objectives and multichannel scanning in frame mode (pinhole 1.2 Airy units). For identification of proliferating cells, mice harboring orthotopic tumors were injected intraperitoneally with 1 mg of 5-ethynyl-2-deoxyuridine (EdU; Setareh Biotech, catalog # 61135-33-9) 4 hours before euthanasia. EdU was detected in cryosections using the Click-IT Edu Alexa Fluor 488 Imaging Kit (ThermoFisher Scientific, catalog # C10337) according to the manufacturer’s protocol. Each antibody has been validated for use in mouse intestinal tissues, as detailed on the manufacturers’ websites.

**Electron microscopy.** A cryogenic transmission electron microscopy (TEM) picture was taken of LNPs in a buffered solution on a lacey copper grid coated with a continuous carbon film. The grid was then mounted on a Gatan 626 cryo-holder equipped within a TEM column. The specimen and holder tip were continuously cooled by liquid nitrogen during transfer into the microscope and subsequent imaging. Imaging was performed using a JEOL 2100 FEG microscope using a minimum dose method that was essential to avoiding sample damage under the electron beam. The microscope was operated at 200 kV and with a magnification setting of 60,000 for assessing particle size and distribution. All images were recorded on a Gata 2x2k UltraScan CCD camera.

**Massively parallel sequencing.** A genomic region containing the sgApc target sequence was amplified using Herculase II Fusion DNA polymerase and gel purified (Forward primer 5′ to 3′: AAGACCGAGAAGCCTTGTTG; Reverse primer 5′ to 3′: GCTTTGGTCTCCTGTTACTCC). Sequencing libraries were prepared from 50 ng of PCR product using the Nextera DNA Sample Preparation Kit (Illumina) according to the manufacturer’s instructions and sequenced on Illumina MiSeq sequencers to generate 150-base pair (bp) paired-end reads. The sgTrp53 target sequence was amplified and sequenced using the above methods and the following primers: Forward primer 5′ to 3′: ATTCGGCCGCTGTCGAGGTC; Reverse primer 5′ to 3′: GGGCGGACTCTGGAGAACAGAA.

**Bioinformatic analysis of target loci.** Illumina MiSeq reads (150-bp paired-end) were trimmed to 120 bp after reviewing base quality profiles, in order
Murine CRC cell lines were derived from AKP genetically Adenomatous polyposis coli (APC) is required for normal hybridization http://www.R-project.org mice during laparotomy to restrict adenoviral delivery to the 5 6 Rapid discovery of potent siRNA-containing lipid nanoparticles © 2021 Nature America, Inc., part of Springer Nature. All rights reserved.

Kit at 1:1,000 dilution (Leica Biosystems, catalog # AR9551). Human CDX2 Retrieval Solution 1 (Leica Biosystems, catalog # AR9961), followed by the FFPE tissue consisted of a 10-min incubation at 95 °C in Bond Epitope Probe Hybridization. With these settings, the RNA unmasking conditions for eZ-l Detection 1-plex (Red) protocol; ViewRNA Dewax1 Preparation protoc...ing to hematoxylin counterstaining. Briefly, 5 mm-thick sections of formalin-digested with collagenase Type 1 (200 units in 5 ml PBS) (Worthington, 10 50. 10.1038/nbt.3836; Trp53 l) was delivered by rectal enema to C57BL/6 Apc fl/fl (l) was delivered by rectal enema to C57BL/6; 2602–2614 (2013). Vidigal, J.A. & Ventura, A. Rapid and efficient one-step generation of paired gRNA CRISPR-Cas9 libraries. Nat. Commun. 6, 8083 (2015). Miyoshi, H. & Stappenbeck, T.S. In vitro expansion and genetic modification of gastrointestinal stem cells in spheroid culture. Nat. Protoc. 8, 2471–2482 (2013). Koo, B.-K., Sasselli, V. & Clevers, H. Retroviral gene expression control in primary organoid cultures. Curr. Protoc. Stem Cell Biol. 27, SA.6 (2013). Roger, J. et al. The dual PI3K/mTOR inhibitor NVP-BEZ235 induces tumor regression in a genetically engineered mouse model of PIK3CA wild-type colorectal cancer. PLoS One 6, e25132 (2011). Yilmaz, Ö.H. et al. mTORC1 in the Paneth cell niche couples intestinal stem-cell function to calorie intake. Nature 486, 490–495 (2012). Tammela, T. et al. Angiopoietin-1 promotes lymphatic sprouting and hyperplasia. Blood 105, 4624–4648 (2005). Dong, Y. et al. Rapid discovery of potent siRNA-containing lipid nanoparticles enabled by controlled microfluidic formulation. J. Am. Chem. Soc. 134, 6948–6951 (2012). Dong, Y. et al. Lipopeptide nanoparticles for potent and selective siRNA delivery in rodents and nonhuman primates. Proc. Natl. Acad. Sci. USA 111, 3955–3960 (2014). Abouelhoda, M.I., Kurtz, S. & Ohlebusch, E. Replacing suffix trees with enhanced suffix arrays. J. Discrete Algorithms 2, 53–86 (2004). Smith, T.F. & Waterman, M.S. Identification of common molecular subsequences. J. Mol. Biol. 147, 195–197 (1981). Wang, K., Li, M. & Hakonarson, H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 38, e164 (2010). Döring, A., Weese, D., Rausch, T. & Reinert, K. SeqAn an efficient, generic C++ library for sequence analysis. BMC Bioinformatics 9, 11 (2008). Zhao, M., Lee, W.-P., Garrison, E.P. & Marth, G.T. SSSW library: an SIMD Smith-Waterman C/C++ library for use in genomic applications. PLoS One 8, e82138 (2013). Thovrildsdottir, H., Robinson, J.T. & Mesirov, J.P. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. Brief. Bioinform. 14, 178–192 (2013).
Corrigendum: In vivo genome editing and organoid transplantation models of colorectal cancer and metastasis

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In the version of this article initially published, the initial “J” was omitted from an author’s name, which should appear as Francisco J Sánchez-Rivera. The error has been corrected in the HTML and PDF versions of the article.