Transplantation of engineered organoids enables rapid generation of metastatic mouse models of colorectal cancer

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Colorectal cancer (CRC) is a leading cause of death in the developed world, yet facile preclinical models that mimic the natural stages of CRC progression are lacking. Through the orthotopic engraftment of colon organoids we describe a broadly usable immunocompetent CRC model that recapitulates the entire adenoma–adenocarcinoma–metastasis axis in vivo. The engraftment procedure takes less than 5 minutes, shows efficient tumor engraftment in two-thirds of mice, and can be achieved using organoids derived from genetically engineered mouse models (GEMMs), wild-type organoids engineered ex vivo, or from patient-derived human CRC organoids.

In this model, we describe the genotype and time-dependent progression of CRCs from adenocarcinoma (6 weeks), to local disseminated disease (11–12 weeks), and spontaneous metastasis (>20 weeks). Further, we use the system to show that loss of dysregulated Wnt signaling is critical for the progression of disseminated CRCs. Thus, our approach provides a fast and flexible means to produce tailored CRC mouse models for genetic studies and preclinical investigation.

CRC is the second-leading cause of cancer-related deaths in developed countries, yet there remain few effective therapies for treating advanced disease. CRC develops in a stepwise fashion, whereby loss-of-function mutations in the adenomatous polyposis coli (APC) tumor suppressor drive the formation of precancerous benign adenomas, and subsequent mutations in KRAS and TP53 support malignant progression1,2.

Unlike many other cancer types, it has been difficult to develop GEMMs of CRC that accurately recapitulate advanced-stage disease in the correct anatomical location. This is because traditional GEMMs harboring germline APC mutations frequently develop small intestinal, rather than colonic lesions, and overall tumor burden limits the time whereby malignant progression can occur3–5. Tissue-restricted Cre/loxP-based strategies can drive cancer-predisposing lesions in the colon, but require multi-allelic intercrossing and still rarely show disease progression, thus limiting their utility for genetic and preclinical studies6–9. Although tumors produced by transplantation of human colon cells, grown as 3-dimensional tissue-forming “organoid” cultures10,11, provide one platform to produce more efficient CRC models, to date, these models have been used only to study tumors at ectopic sites and in immunocompromised recipients. Thus, no one system accurately recapitulates human CRC progression, that is also simple, fast, and flexible enough to produce tailored preclinical models at a reasonable cost.

We set out to build a modular and rapid approach to generate CRC preclinical models in the context of a physiologically accurate tissue environment. We envisaged an ideal model would: (1) develop focal tumors in the colon; (2) enable longitudinal analysis of disease progression and regression using endoscopy; (3) permit all stages of CRC progression; (4) allow rapid and iterative genetic manipulation; and (5) be broadly adaptable to laboratories that lack advanced surgical expertise. Given recent success in the isolation, culture, and genetic manipulation of colon organoids in vitro10–17, we hypothesized that genetically engineered organoids engrafted into the mucosal layer of the mouse colon would satisfy the above requirements.

First, to examine the feasibility of this approach, we isolated organoids from a GEMM that allows short hairpin RNA (shRNA)-mediated Apc silencing (shApc), linked to GFP expression18. In this system, expression of the reverse Tet-transactivator (rtTA) enables doxycycline-dependent Apc silencing and polyp growth, which is reversed upon doxycycline withdrawal (Apc restoration)18. Initial use of this model facilitated tracking of engrafted cells by GFP fluorescence, and it allowed us to benchmark the fidelity of the orthotopic engraftment approach against a well-characterized transgenic system18. We therefore generated colon organoid cultures from CAGs-LSL-rtTA3/TRE-GFP-shApc (hereafter, shApc) transgenic mice, induced

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To implant shApc organoids in the colon, we adapted a protocol established for the engraftment of wild-type colon organoids
(Online Methods, Supplementary Video 1, Supplementary Fig. 1, and Fig. 1a). This approach relies on short-term treatment with dextran sodium sulfate (DSS) to induce transient colonic injury and establish a niche for cell engraftment. Following transplant, macroscopic GFP+ lesions were first identifiable using fluorescence endoscopy at 10 d and persisted up to a year after transplantation (Supplementary Fig. 1). As occurs in Apc-mutant GEMMs, mice receiving shApc organoids also develop benign tumors—however, in this setting they are restricted to the colon, rather than the small intestine. One-year post-engraftment, polyps remained embedded within normal host mucosa as benign tubular adenomas (Fig. 1b). That these single-mutant (shApc only) lesions never show submucosal invasion up to a year after engraftment indicates that the DSS procedure does not in itself enable tumor progression.

Oncogenic KRAS and TP53 mutations are associated with malignant disease progression in human CRC. Accordingly, transplantation of shApc organoid cultures harboring oncogenic KrasG12D and Trp53R172H- Foxp3mut tissues produced frank carcinomas with submucosal invasion (Fig. 1c). Consistent with studies in germline transgenic animals, we showed that restoration of Apc expression in benign shApc and invasive shApc/KrasG12D/p53mut-engrafted tumors induced cell differentiation, tumor regression, and the reestablishment of crypt-villus homeostasis (Fig. 1b,d and Supplementary Figs. 1–3). Hence, orthotopic engraftment of colonic organoids enabled the rapid production of focal colorectal tumors while retaining the histopathology and physiology of gold-standard autochthonous models.

In principle, the organoid engraftment approach described above could be used to produce cohorts of mice from pre-existing engineered organoids in a fraction of the time and expense of traditional GEMMs. Having credentialed the first-generation model, we set out to establish a generalizable platform in which the tumor genotype or tissue context could be easily manipulated and the resulting tumors studied in a physiologically accurate microenvironment. To do this, we generated organoid cultures harboring the most common CRC mutant alleles in a C57BL/6 background, which would allow subsequent engraftment into syngeneic recipient mice. We reasoned that studying CRCs with Apc, Kras, and Trp53 mutations was the most relevant context because they represent the three most-frequent genetic alterations in human CRC. To engineer organoids with this configuration, we isolated colon stem cells from LSL-KrasG12D/p53flox/flox C57BL/6 mice and engineered biallelic loss-of-function mutations in Apc by CRISPR-Cas9-mediated genome editing with an sgApc-Cas9-Cre (sgApc-C/C vector (Fig. 2a)16. This construct also expresses Cre recombinase to simultaneously activate Kras and delete Trp53, which we confirmed by genomic PCR, DNA sequencing, and functional selection (Supplementary Fig. 4). Of note, although the genes we modified permit rapid selection by altering growth factor dependencies (e.g., RSPO/Wnt) or sensitivity to pathway inhibitors (e.g., Nutlin-3), it is possible to manually isolate edited clones for those endpoints (Supplementary Fig. 4).

For engraftment in syngeneic mice, we treated cohorts of C57BL/6j animals with 3% DSS and transplanted 300,000 Apcmut/KrasG12D/p53mut

cells per mouse, as disassociated organoid fragments. The mice in this treatment regimen had a 97% survival rate, associated with minimal and transient weight loss (Supplementary Table 1). Further, comprehensive immunophenotyping and complete blood count (CBC) analysis of non-engrafted animals confirmed that this DSS pretreatment regimen had no significant long-term effects on the immune system or peripheral blood counts (Supplementary Fig. 5). We observed tumor engraftment in 62% of viable DSS-treated mice, and an average of one

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We were unable to detect any signs of dysplasia or aberrant tissue architecture, and lymphovascular invasion (Supplementary Fig. 7). Advanced signs of disease, including dysplasia, loss of tissue architecture in host mucosa examined at these later time points (Fig. 2c). At 16 weeks after transplant, two of three mice that were staged radiographically by MRI showed locally invasive disease with tumor mass extending past the serosal lining and infiltrating the pericolorectal adipose tissue (stage II (T3)) (Supplementary Fig. 7). Notably, we never observed disease progression past stage I in transgenic mice of a similar genotype, likely owing to the reduced survival of GEMMs harboring multiple primary lesions throughout the small and large intestine (Supplementary Fig. 7).

Transplanted C57Bl/6 mice had a maximum survival of 21.4 weeks (compared to 10.1 weeks for the GEMM model), providing more time for local dissemination to regional lymph nodes, and metastatic progression (Fig. 3a and Supplementary Figs. 7 and 8). We identified macroscopic liver metastasis in one-sixth of animals examined 16 weeks after transplant (Fig. 3b and Supplementary Figs. 8 and 9). Consistent with metastatic human CRC, this metastasis contained moderately differentiated gland-forming adenocarcinoma, regions of stratified nuclei, and intracellular Mucin. Immunohistochemical staining for colon-specific markers (Krt20 and Villin) and genotyping by PCR confirmed the lesion was derived from transplanted organoids (Supplementary Figs. 8 and 9). These studies demonstrate that orthotopic engraftment of \( Apc^{mut}/Kras^{G12D}/p53^{mut} \) organoids in syngeneic recipients can recapitulate the entire adenoma–carcinoma–metastasis progression sequence in vivo (Supplementary Fig. 10). Since distant metastasis does not occur in every animal, this platform provides an ideal context for interrogating the additional genetic and environmental factors that enhance metastatic progression from the primary site.

Previous efforts to develop orthotopic transplant models have relied on tumor-derived cell lines cultured on plastic, rather than as
organoids in a three-dimensional (3D) protein matrix. To assess any differences between these approaches, we derived isogenic two-dimensional (2D) cultures from our engineered organoids and transplanted cells exactly as described for 3D cultures. In contrast to the glandular and well-differentiated tumors derived from organoids, 2D cells engrafted as poorly differentiated, flat lesions that invaded the muscle wall and formed distant metastases in as little as 5 weeks (Supplementary Fig. 11). Thus, although the model is aggressive, the engraftment of 2D cell lines in this context does not recapitulate the histopathology or stepwise tumor progression characteristic of organoid engraftment and human CRC.

The stepwise tumor progression model described above effectively mimics all stages of human CRC, including the stochastic timing of metastatic spread. However, robust pre-clinical studies for treatment of metastatic disease rely on the generation of large cohorts of mice with synchronous disease onset and progression. Given that...
engineered organoids can accurately model disease following orthotopic engraftment, we asked whether it was possible to produce a histologically accurate model of experimental metastasis using splenic, intravenous, or direct liver injection methods.

To test this, we injected engineered organoids as dissociated single cells into either the tail or splenic veins, or directly into the liver, of C57Bl/6 host mice. We observed tumor formation using either triple-mutant C57Bl/6 organoids (Fig. 4a and Supplementary Figs. 12 and 13) or organoids that had been further engineered to model Smad4 loss, which has been shown to promote more aggressive disease progression in animal models24,25 (Supplementary Fig. 14). Tumor foci in the liver were histologically indistinguishable from spontaneous metastases in orthotopically engrafted mice, containing cells with stratified nuclei, high Ki67 positivity, and little to no expression of Krt20 (Fig. 4a). Similarly, organoids showed robust colonization and growth of histologically accurate metastatic CRC foci in the lungs following intravenous injection (Supplementary Figs. 12 and 13). Thus, engineered organoids can be used to rapidly derive genetically defined and histologically accurate models of metastatic CRC, either by orthotopic transplantation to faithfully recapitulate disease progression, or by surrogate ‘vessel injection’ to create large cohorts of predictable size- and age-matched tumors for interventional studies.

To illustrate the use of this experimental metastasis model to evaluate therapeutic strategies, we examined the requirement for sustained Apc loss and Wnt hyperactivation in metastatic lesions, which had not been possible in previous GEMM models owing to early morbidity and limited disease progression. To test whether triple-mutant (TRE-shApC/Kras/p53/MNIL-Control, hereafter AKP) or quadruple-mutant (TRE-shApC/Kras/p53/MNIL-shSmad4, hereafter AKPS) murine CRC tumors rely on hyperactive Wnt signaling when growing at distant tissue sites, we used the splenic injection method. Ten weeks following liver seeding of luciferase-expressing tumors via splenic injection, histological examination of livers revealed multiple metastatic foci ranging in size from <1–7 mm at their widest point (Fig. 4b, top panel, and Supplementary Fig. 14).

In both AKP and AKPS tumors, restoration of Apc expression during this period induced a marked drop in luciferase signal (Supplementary Fig. 14). Similar to what we observed in either transgenic or transplanted primary tumors, Apc restoration drove Wnt pathway suppression and tumor cell differentiation, but now also formed large areas of fibrosis or necrosis in the liver (Fig. 4b, bottom panel). We also observed large glands of post-mitotic colon epithelium that appeared atrophic and flattened. Notably, some glandular structures stained positive for the enterocyte marker Keratin 20, while others expressed a canonical marker of differentiated intestinal goblet cells, Muc2 (Fig. 4b, bottom panel), which we also confirmed by qRT PCR analysis on tumor-derived organoids (Supplementary Fig. 14). Thus, aggressive CRC tumor cells growing in the liver remain dependent on Wnt hyperactivation for survival, and retain their intrinsic differentiation program in the absence of local microenvironmental cues. Long-term follow-up of tumors with Apc expression restored was complicated by the development of severe ascites, perhaps due to the presence of ectopic, functional colon epithelium in the liver of these mice. Similarly, restoration of Apc expression in CRC lung lesions led to labored breathing and endobronchial accumulation of colonic Mucin (data not shown). From a translational perspective, this implies that Wnt pathway inhibitors may be effective in treating metastatic CRC, but additional consideration needs to be paid to the effects of colonic cell differentiation in distant tissue sites.

Our studies illustrate that use of ex vivo-engineered mouse organoids provides a flexible, fast, and low-cost platform to model all stages of CRC (Fig. 4c). The approach is also easily adapted for the orthotopic engraftment of patient-derived CRC organoids for the production of histopathologically accurate pre-clinical human cancer models (Supplementary Fig. 15). In this context, our work is complementary to previous efforts to build genetically defined human organoid models10,11 and ‘living biobank’ repositories26, as well as direct injection of organoids or CRISPR–Cas9 constructs to the colonic submucosa27. Combined, these tools provide an opportunity platform to create genetically tractable and orthotopic human CRC models. Moreover, the resulting mice are mosaic, in that engrafted cells are surrounded by genetically normal tissue as occurs during human cancer disease progression.

This broadly adaptable strategy does not require complicated surgical expertise or special equipment (Supplementary Video 1), and has a throughput and versatility that substantially exceeds previous efforts to model this disease. It facilitates the rapid production of immunocompetent mice harboring primary or metastatic CRC, allows longitudinal studies on the natural process of CRC progression, and enables the production of large cohorts of mice with metastatic disease for preclinical studies. As the system produces genetic chimeras, it is possible to engraft benign or malignant organoid models into genetically modified hosts, enabling an examination of tumor–stroma interactions likely to be important in disease progression and therapy response. Collectively these models will foster an expanded understanding of the genetic factors mediating CRC behavior, and help drive the development of targeted small molecules, immunotherapies, and/or diagnostic tests for detection and treatment of metastatic CRC.

METHODS

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

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

K.P.O’R., L.E.D., and S.W.L. conceived the project. K.P.O’R., designed, performed and analyzed experiments, and wrote the paper. E.L., G.L., E.M.S., T.B., E.M., J.S., P.R., B.L., T.H., C.P., H.B., and M.A.R. provided reagents, performed or analyzed experiments. L.E.D. and S.W.L. supervised experiments, analyzed data, and wrote the paper.
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1. Fearon, E.R. & Vogelstein, B. A genetic model for colorectal tumorigenesis. Cell 61, 759–767 (1990).
2. Markowitz, S.D. & Bertagnolli, M.M. Molecular origins of cancer: molecular basis of colorectal cancer. N. Engl. J. Med. 361, 2449–2460 (2009).
3. Taketo, M.M. & Edelmann, W. Mouse models of colon cancer. Gastroenterology 136, 780–798 (2009).
4. Heijstek, M.W., Kranenburg, O. & Borel Rinkes, I.H.M. Mouse models of colorectal cancer and liver metastases. Dig. Surg. 22, 16–25 (2005).
5. Oh, B.Y., Hong, H.K., Lee, W.Y. & Cho, Y.B. Animal models of colorectal cancer with liver metastasis. Cancer Lett. 387, 114–120 (2017).
6. Hinoi, T. et al. Mouse model of colonic adenoma-carcinoma progression based on somatic Apc inactivation. Cancer Res. 67, 9721–9730 (2007).
7. Byun, A.J. et al. Colon-specific tumorigenesis in mice driven by Cre-mediated inactivation of Apc and activation of mutant Kras. Cancer Lett. 347, 191–195 (2014).
8. Xue, Y., Johnson, R., Desmet, M., Snyder, P.W. & Fleet, J.C. Generation of a transgenic mouse for colorectal cancer research with intestinal cre expression limited to the large intestine. Mol. Cancer Res. 8, 1095–1104 (2010).
9. Tetteh, P.W. et al. Generation of an inducible colon-specific Cre enzyme mouse line for colon cancer research. Proc. Natl. Acad. Sci. USA 113, 11859–11864 (2016).
10. Matano, M. et al. Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids. Nat. Med. 21, 256–262 (2015).
11. Drost, J. et al. Sequential cancer mutations in cultured human intestinal stem cells. Nature 521, 43–47 (2015).
12. O’Rourke, K.P., Dow, L.E. & Lowe, S.W. Immunofluorescent staining of mouse intestinal stem cells. Journal Bio. Protoc. 6, e1732 (2016).
13. O’Rourke, K.P., Ackerman, S., Dow, L.E. & Lowe, S.W. Isolation, culture, and maintenance of mouse intestinal stem cells. Bio. Protoc. 6, e1733 (2016).
14. Sato, T. et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 459, 262–265 (2009).
15. Sato, T. et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett’s epithelium. Gastroenterology 141, 1762–1772 (2011).
16. Dow, L.E. et al. Inducible in vivo genome editing with CRISPR-Cas9. Nat. Biotechnol. 33, 390–394 (2015).
17. Koo, B.-K. et al. Controlled gene expression in primary Lgr5 organoid cultures. Nat. Methods 9, 81–83 (2011).
18. Dow, L.E. et al. Apc restoration promotes cellular differentiation and reestablishes crypt homeostasis in colorectal cancer. Cell 161, 1539–1552 (2015).
19. Schwick, G. et al. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell 13, 653–658 (2013).
20. Onuma, K. et al. Genetic reconstitution of tumorigenesis in primary intestinal cells. Proc. Natl. Acad. Sci. USA 110, 11127–11132 (2013).
21. Yui, S. et al. Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5+ stem cell. Nat. Med. 18, 618–623 (2012).
22. Brannon, A.R. et al. Comparative sequencing analysis reveals high genomic concordance between matched primary and metastatic colorectal cancer lesions. Genome Biol. 15, 454 (2014).
23. Martin, E.S. et al. Development of a colon cancer GEMM-derived orthotopic transplant model for drug discovery and validation. Clin. Cancer Res. 19, 2929–2940 (2013).
24. Kainzinger, T. et al. SMAD4-deficient intestinal tumors recruit CCR1+ myeloid cells that promote invasion. Nat. Genet. 39, 467–475 (2007).
25. Takaku, K. et al. Intestinal tumorigenesis in compound mutant mice of both Dpc4 (Smad4) and Apc genes. Cell 92, 645–656 (1998).
26. van de Wetering, M. et al. Prospective derivation of a living organoid biobank of colorectal cancer patients. Cell 161, 933–945 (2015).
27. Roper, J. et al. In vivo genome editing and organoid transplantation models of colorectal cancer and metastasis. Nat. Biotechnol. http://dx.doi.org/10.1038/nbt.3836 (2017).
Organoid transfection. Prior to transfection, organoids were cultured in complete growth media (ENRW) supplemented with 10 µM Y-27632 (EMD Millipore, 688001) for 2 d, 2 µg of vector DNA was mixed with 6 µl of Lipofectamine 2000 (Thermo-Fisher Scientific, 11668-027) in 50 µl of Opti-MEM Reduced Serum Medium (Thermo-Fisher Scientific, 31980-070) and allowed to incubate for 20 min at room temperature. Organoids, containing approximately 100,000 cells, were digested in 0.25% trypsin/0.02% EDTA in PBS at 37 °C for exactly 7 min, and then mechanically dissociated using a 200 µm pipette, then 10 µl of DMEM (+penicillin/streptomycin +10% FBS) was mixed with the solution and centrifuged at 1,500 r.p.m. for 5 min. The pellet was resuspended in 450 µl of ENRW + Y-27632 and mixed with the 50 µl of lipid:DNA complex, plated in a 24-well plate and spun at 600 g for 60 min at 32 °C, and then placed in an incubator for 4 h at 37 °C. The cells were then collected, spun at 1,500 r.p.m. for 5 min, and replated in Matrigel as normal. pMaxGFP (Lonza) was used as a positive control and cells were checked for viability and GFP expression 24 h after transfection. Transfected organoids were allowed to recover for 5 d in ENRW+Y-27632 before being passaged into selective media conditions.

Copy number profiling. Tumor DNA was isolated using the AllPrep DNA/RNA Micro Kit, (Qiagen, 80284) according to instructions provided with the kit. 1 µg of DNA was sonicated (17W, 75 s) using the E220 sonicator instrument (Covaris, Woburn, MA). Samples were subsequently prepared and indexed using Illumina TruSeq library prep system. Libraries were purified using AMPure XP magnetic beads (Beckman Coulter, Brea, CA). PCR enriched, and sequenced on an Illumina HiSeq instrument. Sequence reads were mapped using Bowtie with PCR duplicates removed. Uniquely mappable reads were further processed for copy number determination using the ‘varbin’ algorithm using 5,000 bins, allowing for a median resolution of ~600 kb. GC content normalization, segmentation, and copy number estimation was determined as described previously28. Tumor ploidy was confirmed via propidium iodide staining and cell cycle fluorescence-activated cell sorting (FACS) analysis.

Human organoid specimen procurement. All patient-derived fresh tissue samples were collected with informed patient consent according to protocols approved by the institutional review board of Weill Cornell Medicine (IRB # 1305013903). Fresh tissue biopsies or resection specimens were directly taken from the procedure rooms to the frozen section area in pathology. A board-certified pathologist collected the tumor tissue for sequencing and tumor organoid development. Time between harvesting fresh tissue specimens to placing them in transport media (Dulbecco’s modified Eagle medium (DMEM, Invitrogen) with Glutamax (1×, Invitrogen), 100 U/ml penicillin, 100 µg/ml streptomycin ( Gibco), primocin 100 µg/ml (InvivoGen), 10 µM Rock inhibitor Y-27632 (Selleck Chemical Inc.)) varied from 10 to 45 min. Sample size varied between biopsies (~0.2 to 0.3 mm²) and resection specimens (~0.5 mm² to 0.5 cm²).

Human organoid tissue processing and cell culture conditions. Tissue samples were washed at least three times with transport media and placed in a sterile 3-cm petri dish (Falcon) for either total mechanical dissociation or dissection into smaller pieces (~2 mm diameter) before enzymatic digestion. Enzymatic digestion was done with 2/3 of 250 U/ml collagenase IV (Life Technologies) in combination with 1/3 of 0.05% trypsin-EDTA (Invitrogen) in a volume at least twenty times the tissue volume. Tubes were incubated on a shaker at 200 r.p.m. at a temperature of 37 °C until the digestion solution turned cloudy. The suspension was mixed with 10% FBS (Denville) enriched DMEM to inactivate the enzymes and centrifuged at an average of 1,000 r.p.m. for 5 min to pellet cells. The cell pellets were washed once with 10% FBS-enriched DMEM and once washed with DMEM only to wash away FBS residues. The cells were resuspended in a small volume of growth media (Advanced DMEM/F-12 (Invitrogen) with B27 supplement (Invitrogen), Glutamax (Invitrogen), penicillin/streptomycin (Invitrogen), HEPEs (Invitrogen), N-acetylcysteine (Sigma-Aldrich), (Leu15)-Gastrin I (Sigma-Aldrich), Recombinant EGF and Noggin, R-spondin, Wnt (all derived from in-house-conditioned media)) and mixed in a 1:2 volume of growth factor reduced Matrigel (Corning). Up to ten 50- to 125-µl drops of Matrigel/cell suspension were distributed into a 6-well cell suspension culture plate (SARSTEDT Ltd., Leicester, UK). The drops were solidified with a 15- to 30-min incubation in the
cell culture incubator at 37 °C and 5% CO₂. After solid drops formed 3–4 mL primary culture media was added. Each well was replaced with fresh culture media every 5 d until the organoid culture started growing and then every 2–4 d. Regular mycoplasma screening was performed using the MycoAlert kit (Lonza Inc.).

Human organoid maintenance. Organoid media was changed every 2–3 d, and they were passed approximately 1:4 every 6–8 d. To passage, the growth media was removed and the Matrigel was resuspended in 3 ml cold PBS, then transferred to a 15-ml Falcon tube. The organoids were mechanically disrupted using a p1,000 pipette by repeatedly pipetting, approximately 50 times. 8 ml of cold PBS was then added to the tube and the tubes were again pipetted, ~20 times, for further mechanical disruption. The cells were centrifuged at 1,000 r.p.m. for 4 min and the supernatant was aspirated. They were then resuspended in Matrigel and replaced as above. For freezing down organoid cell lines, the cells were resuspended in growth media containing 10% DMSO and 10% PBS after centrifugation above. They were stored in liquid nitrogen indefinitely.

Organoid transplantation. shApC organoids were engrafted into recipient Athymic-Nude-Foxn1 null (Envigo), which were pretreated with 2.5–3% DSS in their drinking water for 5 d and daily body weights were recorded on day 0, 3, 5, and 7. Notably, we observed no successful engraftment in Foxn1 null hosts treated with 1% or 2% DSS, and we also observed an increased rate of lethality when we treated males with 2.5–3% DSS compared to females. After the animals were allowed to recover for 2 d, mice were anesthetized with isoflurane (2%) and the colon was flushed with room temperature sterile PBS. After flushing, 2000 shApC organoid fragments in 50 µl of 5% Matrigel-PBS were instilled into the lumen of the colon by a p200 pipette enema, coated sparingly with petroleum jelly (Vaseline), over the course of 30 s. Organoid fragments were prepared identical as to passaging except that after centrifugation they were resuspended in 5% Matrigel-PBS and kept on ice until infusion. After infusion, the anal verge was sealed with 4 µl of Vetbond Tissue Adhesive (3M, 1469SB) to prevent luminal contents from being immediately excreted. They were then maintained on anesthesia for 5 min, and then allowed to recover in room air underneath a heat lamp. Six hours after the procedure the anal canal was inspected to make sure it was patent. For syngeneic transplants, C57Bl/6N Hsd (Envigo) and C57Bl/6 Jackson Labs animals were treated with 2.5–3% DSS for 5 d as above. Importantly, we noticed significant differences in DSS toxicity, as measured by weight loss and animal survival, among C57Bl/6 strains ordered from different vendors, and so we proceeded forward with C57Bl/6 animals ordered from Jackson Labs. Prior to transplantation, organoids were digested in 0.25% trypsin/0.02% EDTA in PBS at 37 °C for exactly 7 min, and then mechanically dissociated using a 200 pipette, then 10 ml of DMEM (+penicillin/streptomycin +10% FBS) was mixed with the solution and centrifuged at 1,500 r.p.m. for 5 min. They were inspected underneath the microscope to determine if they remained in clumps, and then resuspended to a final concentration of ~300 K cells/50 µl in 5% Matrigel-PBS. Importantly, Apcmut/KrasG12D/p53mut cells were grown from freshly thawed stock cells of cells that were cryogenically preserved at the same time they were characterized to be chromosomally stable and thus, genetically defined. Human tumor organoids were transplanted, exactly as above, into recipient Nod scid gamma mice and harvested at day 7, day 35 and day 70 after transplant. Untreated mice were collected as controls. Single-cell suspensions were derived from spleen through filtration (40 µm) and peripheral blood, while red blood cells were lysed with Ammonium-Chloride-Potassium Buffer (Quality Biological, 118-156-101). Cells were incubated with antibodies for 1 h on ice. All antibodies were purchased from eBioscience or BioLegend. We used the following fluorochrome anti-mouse antibodies: CD11b (M1/70), Gr-1 (RB6-8C5), CD3 (17A2), CD4 (GK1.5), CD8 (53-6.7), B220 (B114968). Stained cells were quantified using a BD Fortessa analyzer. FlowJo software (Tree Star) was used to generate FACS plots and tables, and to calculate the percentages of each population. The bar graphs were generated using Prism 6 and significance was determined using unpaired two-tailed t-test, without Welch correction as the variance was not significantly different. For the CBC analysis we used the Hemavet 950PS (Drew) hematology analyzer after collecting the peripheral blood in EDTA tubes (Sarstedt 20.1278.100). The CBC graphs were generated as described above using Prism 6.

Vector generation. The U6-sgRNA EF5-Cas9-P2A-Cre (sgRNA-CC) lentiviral vector was constructed based on the lentiCRISPR backbone29. We replaced PuroR with a Cre (PCR amplified from Puro.Cre empty vector30) and, subsequently, we destroyed the BsmBI restriction sites inside the Cre DNA using Quick-Change II Site Directed Mutagenesis (200523, from Agilent Technologies) according to instructions provided with the kit. The primers used were NhEl-Fw-Cre. 5’TCAGCGGTACGGGACCAGGGGCGCA CCAAACCTTCACGCTGCAGGCAGACCCGGCGAGAGAC CGGGCCTGTCTTATCTGAGCGCTACACC 3′ and MluR-Rv-Cre. 5′GATCGACGCTTACTGTCACTTGGCACTACGGAGGCAGCCC 3′. For sgRNA cloning, the sgRNA-CC vector was digested with BsmBI and ligated with BsmBI-compatible annealed ACG sgRNA oligos made previously15.

Data availability. Data are available from the corresponding authors upon reasonable request.

28. Baslan, T. et al. Optimizing sparse sequencing of single cells for highly multiplex copy number profiling. Genome Res. 25, 714–724 (2015).
29. Shalem, O. et al. Genome-scale CRISPR-Cas9 knockout screening in human cells. Science 343, 84–89 (2014).
30. Kumar, M. S. et al. Suppression of non-small cell lung tumor development by the let-7 microRNA family. Proc. Natl. Acad. Sci. USA 105, 3903–3908 (2008).