A truncating mutation in the autophagy gene \textit{UVRAG} drives inflammation and tumorigenesis in mice

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Aberrant autophagy is a major risk factor for inflammatory diseases and cancer. However, the genetic basis and underlying mechanisms are less established. \textit{UVRAG} is a tumor suppressor candidate involved in autophagy, which is truncated in cancers by a frameshift (FS) mutation and expressed as a shortened \textit{UVRAGFS}. To investigate the role of \textit{UVRAGFS} in vivo, we generated mutant mice that inducibly express \textit{UVRAGFS} (\textit{iUVRAGFS}). These mice are normal in basal autophagy but deficient in starvation- and LPS-induced autophagy by disruption of the \textit{UVRAG-autophagy} complex. \textit{iUVRAGFS} mice display increased inflammatory response in sepsis, intestinal colitis, and colitis-associated cancer development through NLRP3-inflammasome hyperactivation. Moreover, \textit{iUVRAGFS} mice show enhanced spontaneous tumorigenesis related to age-related autophagy suppression, resultant \(\beta\)-catenin stabilization, and centrosome amplification. Thus, \textit{UVRAG} is a crucial autophagy regulator in vivo, and autophagy promotion may help prevent/treat inflammatory disease and cancer in susceptible individuals.

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Autophagy, an evolutionarily conserved catabolic process, is responsible for regulated degradation of intracellular components to maintain cellular homeostasis and adapt to environmental cues. During starvation, increased levels of autophagy allow cells to breakdown cytoplasmic materials and release essential metabolites as an energy source. Recent studies further link autophagy to lipid metabolism, whereby starvation-induced autophagy mediates lipid droplet degradation (lipophagy), which provides free fatty acids to maintain metabolic homeostasis. Furthermore, autophagy-mediated selective clearance of damaged mitochondria (mitophagy) attenuates mitochondrial stress upon infection or inflammation to prevent excessive mitochondrial ROS (mtROS) production, which otherwise perpetuates the inflammatory response, leading to immune pathology and tissue damage. A lack of sufficient autophagy has been implicated in inflammatory pathologies and immune-dysfunction such as sepsis, Crohn’s disease, and diabetes. Under unstressed conditions, chronic autophagy suppression is associated with decreased lifespan in animal models, such as age-related renal and cardiac deterioration and spontaneous tumorigenesis. However, the precise mechanisms that link autophagy activity to age-related diseases remain incompletely understood.

The exploding wave of autophagy investigation in the past decade has led to the discovery of a plethora of autophagy-related genes that govern its induction and execution. The ultraviolet (UV) irradiation resistance-associated gene (UVRAG) is an autophagy-related factor that forms a complex with Beclin1 and the lipid kinase Vps34 to promote autophagic membrane remodeling. Later, it was discovered that different UVRAG complexes exist and modulate the tightly regulated autophagosome progression to lysosomal degradation, as well as other membrane trafficking events that either intersect or converge with the autophagy pathway. Overexpression of UVRAG activates autophagy and suppresses tumor growth, whereas silencing of UVRAG causes failure of autophagy and uncontrolled cell proliferation. On the other hand, UVRAG also exerts autophagy-independent functions in DNA repair and organelle integrity. Notably, a frameshift (FS) mutation in UVRAG, which results in the expression of a truncated UVRAG (referred to as UVRAGFS, iUVRAGFS), is linked to susceptibility to different cancer types. In addition to losing the tumor suppressing properties of wild-type (WT) UVRAG, the truncated UVRAGFS overrides co-existing WT protein and acts as a dominant-negative mutant and autophagy suppressor in cell-based assays. However, some studies and models have shown that deletion of yeast Vps38p and Arabidopsis Vps38, which are considered as orthologs of mammalian UVRAG, impairs vacuolar protein sorting but has minimal effect on autophagy, raising the concern whether UVRAG regulates autophagy in mammals in vivo. Unfortunately, there is no genetic evidence in mice showing a role of UVRAG in autophagy largely because genetic inactivation of UVRAG results in early embryonic lethality. Hence, definitive evidence that the autophagic response succumbs to impaired UVRAG function and its impact on tissue homeostasis and disease propensity is lacking.

Herein, we generated a doxycycline (Dox)-inducible mouse model that does not affect basal autophagy but is significantly impaired in starvation- and Toll-like receptor (TLR)-induced autophagy by expression of the cancer-derived UVRAGFS (referred to as iUVRAGFS). Mice expressing UVRAGFS display an enhanced inflammatory response through hyperactivated NLRP3 and IL-1β secretion primed by mitochondrial signaling. Using iUVRAGFS mice, we furthermore demonstrate that long-term UVRAGFS expression leads to centrosome amplification, age-related autophagy suppression, and resultant β-catenin oncoprotein stabilization, leading to increased susceptibility to spontaneous tumorigenesis in multiple organs. To the best of our knowledge, our results provide the first genetic evidence connecting UVRAG suppression to autophagy regulation, inflammatory disorders, and cancer predisposition.

Results

UVRAGFS inhibits starvation-induced autophagy in vivo. To study the role of UVRAGFS in a temporal-specific manner, we generated a conditional Flag-tagged UVRAGFS, luciferase transgene under the control of a Dox-responsive element (designated TRE-UVRAGFS) (Supplementary Fig. 1a). These mice were crossed to ROSA26-rTA2-M2 mice to enable Dox-inducible expression of human UVRAGFS in a tightly regulated fashion (Supplementary Fig. 1a). This double transgenic strain is referred to as iUVRAGFS. Dox treatment induced UVRAGFS expression at the mRNA and protein levels without affecting endogenous UVRAG expression (Supplementary Fig. 1b, c). No UVRAGFS transgene expression was detected in Dox-treated WT littermate control (Supplementary Fig. 1b, c). Luciferase expression was not detected in untreated mice but strongly correlated with UVRAGFS expression, providing a visual biomarker for UVRAGFS expression (Supplementary Fig. 1d–f). The transgene expression was reversible, showing loss of luciferase and UVRAGFS expression 4 days following Dox withdrawal in different organs (Supplementary Fig. 1g–h). Dox-treated iUVRAGFS mice were of normal size and weight, and displayed normal histology in major organs (Supplementary Fig. 1h, i).

To explore the impact of UVRAGFS on autophagy in vivo, we bred WT control or iUVRAGFS mice to GFP-tagged LC3 transgenic mice that express a fluorescent marker of autophagosomes, on C57BL/6J background, and analyzed the tissues of resultant compound mice after starvation (Fig. 1a). In skeletal muscle, liver, heart, and colon, Dox-treated iUVRAGFS mice had significantly decreased numbers of GFP-LC3 puncta compared to controls and to Dox-untreated mice that showed a marked increase in GFP-LC3 puncta upon starvation (Fig. 1a). We further confirmed mice with UVRAGFS expression having suppressed starvation-induced autophagy by western blot analyses (Fig. 1b). There were decreased levels of autophagosome-associated lipidated LC3 (LC3-II) and increased levels of the autophagy substrate p62 in 48 h-starved iUVRAGFS mice on Dox, while Atg16 and Atg12 conjugation to Atg5 remained unaffected (Fig. 1b). The deficient starvation-induced autophagy in Dox-treated iUVRAGFS mice was not associated with increased cell death (Supplementary Fig. 1j). Using electron microscopy (EM), it was also observed that the numbers of autophagic vacuoles (autophagosome and autolysosome) were comparable at baseline in liver of Dox-untreated and -treated mice but failed to increase in Dox-treated iUVRAGFS mice following starvation (Fig. 1c). In parallel with compromised starvation-induced autophagy, Dox-treated iUVRAGFS mice showed massive enlargement and accumulation of lipid droplets (LD) in liver when compared to controls (Fig. 1c). Increased LDs were also observed in fasting WT mice, but to a much lesser extent, and LDs were mostly surrounded by autophagic vesicles that were not found in cells with UVRAGFS expression (Fig. 1c). The marked increase of LDs was further confirmed by Oil red O staining of liver sections in starved iUVRAGFS mice on Dox (Supplementary Fig. 1j), supporting previous findings on the potential role of autophagy in the clearance of hepatic LDs. Thus, UVRAGFS prevents starvation-induced autophagy activation in vivo, which may disturb metabolic adaptation of cells to nutrient deprivation and therefore increase the risk of metabolic disorders.

To understand how UVRAGFS suppresses starvation-induced autophagy in vivo, we examined WT UVRAG association with
Beclin1 and Vps34, which are parts of an autophagic-specific class III phosphatidylinositol-3-OH kinase (PI3KC3) complex that has a key role in autophagosome formation. In the 48-h-starved tissue of Dox-induced iUVRAGFS mice, there was a marked reduction in UVRAG co-immunoprecipitation with Beclin1 and Vps34, concomitant with an increase in its binding to Rubicon, a negative regulator of the PI3KC3 complex. Reduced assembly of the UVRAG-containing PI3KC3 complex was not due to alteration of UVRAG S497 phosphorylation (S498 in human UVRAG) in iUVRAGFS mice, which, mediated by mTORC1, was previously shown to decrease the autophagy activity of UVRAG in vitro. Rather, reduced assembly was associated with the dominant-negative effect of UVRAGFS that bound to and sequestered UVRAG from the PI3KC3 complex, leading to autophagy suppression. These results indicate that UVRAGFS inhibits starvation-induced autophagy by acting...
**Enhanced inflammatory responses by UVRAG<sub>F5</sub>** In parallel with impaired LPS-induced autophagy in Dox-treated iUVRAG<sup>F5</sup> mice, there was increased vulnerability to intraperitoneally injected LPS (Supplementary Fig. 3a). The survival of UVRAG<sub>F5</sub>-expressing mice in septic shock was significantly lower than that of controls (no Dox) (Supplementary Fig. 3a). Notably, Dox-treated iUVRAG<sub>F5</sub> mice had similar levels of serum TNF-α, IL-6, and IFN-β after LPS stimulation as control mice (Fig. 3a). Characterization of NF-κB activation in BMDM from control and UVRAG<sub>F5</sub>-expressing mice revealed no discernible differences in the phosphorylation and degradation of the NF-κB inhibitor IκBα (Supplementary Fig. 3b) and in nuclear translocation of p65 (Supplementary Fig. 3c). Consistently, the expression of Tnf-α, Il-6, and Ifnb mRNA in spleens was comparable between LPS-stimulated UVRAG<sub>F5</sub>-expressing mice and control mice (Supplementary Fig. 3d). Unlike TNF-α, whose synthesis and secretion are inflammasome-independent, production of IL-1β and IL-18, which requires inflammasome-dependent caspase-1 activation<sup>4,36</sup>, was more abundant in the serum of Dox-induced iUVRAG<sub>F5</sub> mice than littermate control in sepsis (Fig. 3a). However, their mRNA synthesis was not increased accordingly (Supplementary Fig. 3d). To determine whether UVRAG<sub>F5</sub> plays a role in LPS-induced inflammasome activation, we stimulated control and UVRAG<sub>F5</sub>-expressing BMDM with LPS and the NLRP3 inflammasome activator ATP and observed more than two-fold increase in mature IL-1β production, but not pro-IL-1β levels, by Dox-induced iUVRAG<sub>F5</sub> macrophages compared with control cells, suggesting that enhanced IL-1β secretion is likely related to increased inflammasome activation (Fig. 3b, c). As expected, there was a notable increase in caspase-1 activation (indicated by cleaved caspase-1) in LPS + ATP-treated iUVRAG<sub>F5</sub> BMDM on Dox, whereas activation of the inflammasome-independent proinflammatory caspase, caspase-11, by LPS was similar in UVRAG<sub>F5</sub>-expressing BMDM relative to its activation in control cells (Fig. 3b, c). Elevated caspase-1 activation and IL-1β production correlated with increased rate of macrophage death (Supplementary Fig. 3e) and IL-1β-inducible chemokines CXCL1, CXCL2, and CCL2 expression in spleens (Supplementary Fig. 3f). Treatment of iUVRAG<sub>F5</sub> BMDM with Z-YVAD-FMK, a caspase-1 inhibitor, ablated enhanced IL-1β secretion by UVRAG<sub>F5</sub> (Fig. 3d), confirming that UVRAG<sub>F5</sub>-associated increased levels of IL-1β was the consequence of increased caspase-1 activation. Thus, caspase-1 overactivation contributes to excessive inflammation in Dox-treated iUVRAG<sub>F5</sub> mice after LPS stimulation.

Caspase-1 is activated when it is recruited and incorporated into inflammasomes, which contains a Nod-like sensor protein such as NLRP3 and the adapter molecule ASC<sup>38</sup>. We observed that UVRAG<sub>F5</sub>-expressing BMDM demonstrated a marked increase in NLRP3 and ASC co-immunoprecipitation with pro-caspase-1 than in control BMDM, suggesting increased NLRP3 inflammasome assembly upon UVRAG<sub>F5</sub> expression (Fig. 3b).
Despite NF-κB-dependent upregulation of NLRP3 by LPS + ATP, no differences in NLRP3 inflammasome protein expression were observed in control versus UVRAGFS-expressing cells (Fig. 3b), suggesting that increased NLRP3 inflammasome assembly by UVRAGFS is not due to altered protein expression. Administration of MCC950, a small-molecule inhibitor of NLRP3 in inflammasome assembly, decreased the levels of IL-1β in serum and improved the survival rate of mice treated with LPS (Fig. 3e,f), suggesting that aberrant activation of NLRP3 inflammasome directly contributes to UVRAGFS-associated massive inflammation during sepsis. To examine whether the observed increase in NLRP3 inflammasome assembly/activation is due to specific effects of UVRAGFS, or due to defective autophagy, we conducted the same experiment in macrophages from mice that were deficient for the autophagy gene Atg5 (Atg5−/−), an essential autophagy protein that associates with Atg12 and Atg16 to...
As seen with UVRAG FS expression, tamoxifen abrogated the similarity of the phenotypes of cells from UVRAGFS mice and atg5−/− deficient mice, in agreement with previous studies demonstrating that autophagy, which selectively labels impaired mitochondria, on Tom20-labeled mitochondria. 

Additionally, dox-treated iuVRAGFS mice exhibited increased mitochondrial membrane potential (∆Ψm) loss (Supplementary Fig. 3h), cytosolic mitochondrial DNA (mtDNA) release (Supplementary Fig. 3i), and mtROS production (Fig. 3g) in LPS + ATP-treated BMDM. This increase was associated with impaired mitochondrial homeostasis, as indicated by increased aggregation of mitophagy marker protein p62 and Parkin, which selectively labels impaired mitochondria, on Tom20-labeled mitochondria (Fig. 3h and Supplementary Fig. 3j). Excess mtROS also resulted in increased genomic instability, as suggested by higher levels of γ-H2AX in dox-treated iuVRAGFS BMDM (Fig. 3b). Treatment of cells with the mitochondrial-specific antioxidant MitoTO, which eliminated not only mtROS but also prevented mtDNA release (Fig. 3g and Supplementary Fig. 3i), abrogated the increase of both caspase-1 activation and IL-1β production in dox-treated iuVRAGFS BMDM in response to LPS + ATP (Fig. 3i). These data are in agreement with previous studies demonstrating that autophagy, particularly mitophagy, prevents perpetuated inflammasome activation by eliminating inflammasome activators such as mtROS or mtDNA. Notably, autophagy is also found to suppress cytosolic DNA-induced AIM2 inflammasome activation by promoting its lysosomal turnover. However, UVRAGFS-expressing BMDM responded normally to poly(A)dt treatment, when compared to control cells (Supplementary Fig. 3k), suggesting that UVRAGFS-suppressed autophagic degradation might not play a dominant role in the modulation of AIM2 inflammasome. Nevertheless, these results indicate that the impaired PIs-induced autophagy activation is responsible for aberrant NLRP3-inflammasome activation and increased septic shock in iuVRAGFS mice.

**UVRAGFS exacerbates inflammatory responses in colitis.** Abnormal inflammasome activation is implicated in inflammatory bowel diseases (IBD)48,49. We next examined whether UVRAGFS suppression of autophagy exacerbates intestinal inflammation induced by oral administration of dextran sodium sulfate (DSS), an epithelial irritant. Dox-treated cohorts of WT and iuVRAGFS mice were fed 5% DSS (w/v) in their drinking water for 6 days, followed by 4 days of regular drinking water. Expression of UVRAGFS made mice more susceptible to DSS-induced colitis (Fig. 4a). In addition, UVRAGFS-expressing mice exhibited increased disease severity, as demonstrated by elevated disease activity indices (DAI) and colonic shortening at necropsy on days 6 and 10 (Fig. 4b, c). Enhanced colitis corresponded with an increase in all indices of histopathological changes, including crypt damage and inflammatory infiltrates (Fig. 4d, e). This increased susceptibility of UVRAGFS-expressing mice to colitis is not due to inherent differences at baseline, because no difference in colon length and crypt structure were observed between two genotypes (Supplementary Fig. 4a, b). The number of bacteria in the feces of both cohorts was also comparable (Supplementary Fig. 4c). To assess whether UVRAGFS suppression of autophagy contributes to DSS-induced intestinal inflammation, we analyzed autophagy activity in DSS-treated colons. As expected, DSS treatment led to elevated levels of autophagy, as measured by increased LC3-II conversion and p62 turnover, in control colon lysates, which was suppressed in dox-treated iuVRAGFS mice (Fig. 4f, g). Deficient autophagy was associated with increased NLRP3 inflammasome assembly and culminated caspase-1 activation as observed in sepsis, enabling enhanced cleavage of pro-IL-1β and pro-IL-18 into their bioactively active forms IL-1β and IL-18 in DSS-treated UVRAGFS mice colons (Fig. 4f–h). By contrast, formation of other colitis-relevant inflammasomes such as AIM2, NLRP6, and NLRC4 by DSS was comparable in colons between control and UVRAGFS-expressing mice (Fig. 4i, f), highlighting a critical role of the NLRP3 inflammasome in UVRAGFS, associated colitis. In accord, amounts of proinflammatory cytokines IL-1β and IL-18, but not IL-6 and TNF-α, produced by colonic tissue were increased in DSS-fed UVRAGFS-expressing mice versus control mice (Fig. 4i). Supporting this, UVRAGFS-expressing mice had elevated induction of mRNA expression for chemotactic chemokines including CXCL1, CXCL2, CXCL3, CCL2, CCL3, and CXCCL10 that can be induced by IL-1β and IL-18 in colon (Supplementary Fig. 4d) and enhanced neutrophil infiltration (Fig. 4d). As observed in sepsis, UVRAGFS did not alter the response of mice to DSS with respect to NF-κB activation in colon (Supplementary Fig. 4e).

To further determine the contribution of hematopoietic UVRAGFS versus non-hematopoietic UVRAGFS in driving exacerbated colitis, we generated UVRAGFS bone marrow chimeras by reconstituting both control and UVRAGFS-expressing recipients with bone marrow harvested from either control or UVRAGFS-expressing donors, as previously described, and confirmed UVRAGFS expression in these chimeras (Supplementary Fig. 4f). We observed that both sets of chimeric mice were more hypersensitive to DSS-induced colitis compared with the control→control animals, as indicated by increased colon length reduction.
and histological indices (Supplementary Fig. 4g–i). Histopathological analysis of colons isolated from the chimeric mice revealed that all of the UVRAGFS-expressing animals (both donor and recipient) exhibited increased crypt damage, extensive areas of ulceration, and enhanced inflammation compared with the control mice (Supplementary Fig. 4h, i). Thus, UVRAGFS functions through both hematopoietic and non-hematopoietic compartments in inflamasome activation. Administration of the NLRP3 inhibitor MCC950 attenuated the clinical and histological signs of colitis in Dox-treated iUVRAGFS mice, including weight loss, colon length reduction, histological indices, tissue injury, and inflammatory infiltrates (Supplementary Fig. 4j–n; Fig. 4j–l). Although inflammatory cytokines IL-6 and TNF-α were unaffected, colonic IL-1β was significantly lower after MCC950.
treatment (Fig. 4i). These results indicate that heightened NLRP3 activation with subsequently increased production of proinflammatory cytokines account for increased colitis in iUVRAGFS mice.

**UVRAGFS predisposes mice to colitis-associated CRC.**

We next examined whether autophagy restriction of inflammation-associated activation confers a protective role from colitis-associated neoplastic process in iUVRAGFS mice, using an established azoxymethane (AOM)-DSS model of colorectal cancer (CRC)52. No difference in body weight was observed between Dox-treated iUVRAGFS and control littermates (Supplementary Fig. 5b). However, we observed a more than two-fold increase in the incidence of tumors in Dox-treated iUVRAGFS mice compared with controls, and the average tumor size and tumor load was elevated upon UVRAGFS expression (Fig. 5a–d). Immunohistochemistry analyses revealed increased cell proliferation (Ki67 staining) and decreased apoptosis, as indicated by reduced active caspase-3 staining, on day 60 post-treatment, in the colon of Dox-treated iUVRAGFS versus controls (Fig. 5e). Furthermore, there were decreased levels of the epithelial cell markers, Keratin 20 (Krt20) and E-cadherin, but increased levels of the mesenchymal markers, N-cadherin and vimentin, in colons with UVRAGFS expression in the course of tumorigenesis, while the epithelial-to-mesenchymal transition (EMT)-related Wnt/β-catenin pathway remained unaffected (Fig. 5e and Supplementary Fig. 5c). The hyperproliferation and blocked differentiation by UVRAGFS is consistent with early onset of colitis-associated CRC in Dox-treated iUV- RAGFS mice.

In concordance with increased colonic tumorigenesis, Dox-treated iUVRAGFS mice colon displayed severe crypt disruption and inflammatory infiltration after AOM-DSS treatment (Fig. 5f, g). There were increased caspase-1 activation and IL-1β production in the colon of Dox-treated iUVRAGFS as compared to control mice, which correlated with suppressed autophagy, as measured by LC3 conversion and p62 turnover (Fig. 5e, h), supporting the concept that disturbed autophagy by UVRAGFS has a mechanistic role in unresolved inflammation-associated activation and inflammation-associated tumor susceptibility.

**Promotion of spontaneous tumorigenesis by UVRAGFS.**

Despite the oncogenic feature of UVRAGFS in vitro16, it remained untested whether UVRAGFS was sufficient to initiate tumorigenesis in mammals. To this end, we aged cohorts of iUVRAGFS mice and WT littermates that were fed Dox starting from 2 month of age and explored the impact of UVRAGFS on spontaneous tumorigenesis. Dox-treated iUVRAGFS mice succumbed to the development of spontaneous tumors starting at 30 weeks (Fig. 6a). By 18 months of age, approximately 90% of UVRAGFS-expressing mice as compared with 32% of Dox-fed wild-type mice had malignancies (Supplementary Fig. 6a). The tumors in iUVRAGFS mice on Dox comprised mainly of lymphomas and adenocarcinomas. We observed massive spleen and lymph node enlargement in most (70–80%) of UVRAGFS, expressing mice autopsied at 6–18 months of age, which was histologically confirmed as lymphomas (Fig. 6b, c). By contrast, few lymphomas were observed in Dox-treated WT mice (Fig. 6c and Supplementary Fig. 6b). The lymphomas invaded a variety of tissues including the liver and lungs (Fig. 6b). The predominant type of lymphomas developed in UVRAGFS-expressing mice was diffuse large B cell lymphoma (DLBCL), which showed strong immunoreactivity for the B cell marker, CD20, but were negative for the germinal center marker Bcl-6 (Fig. 6d). Some Dox-induced iUVRAGFS mice also developed low grade B cell lymphoma, suggestive of extranodal marginal zone B cell lymphoma with crystal storing histiocytes, positive for monotypic kappa (Fig. 6d). In addition to frank lymphomas, there was increased prevalence of splenic, nodal, and extranodal lymphoproliferative disease (LPD) associated with UVRAGFS expression (Fig. 6c). Moreover, Dox-treated iUVRAGFS mice had lung lesions in earlier stages of neoplasia, including poorly differentiated adenocarcinomas with nuclear immunoreactivity for TTF-1, a tissuespecific transcription factor in lung epithelial cells53 (Fig. 6d). All malignancies had detectable UVRAGFS expression in tumor cells (Fig. 6d). Thus, expression of this cancer-derived UVRAGFS results in increased susceptibility to spontaneous malignancies.

**UVRAGFS promotes proliferation and β-catenin activation.**

Although colon tumors were not observed in our cohort of aged mice, histopathologic examination of colons from Dox-treated iUVRAGFS mice revealed hyperplastic changes compared with those from controls (Fig. 7a). Crypt length was increased by ~1.3-fold along the entire length of the colon in Dox-treated iUVRAGFS mice along with cytoplasmic expression of UVRAGFS at the colonic crypts (Fig. 7a, b). No significant differences were detected in the number of cleaved caspase-3-positive cells in the colon of control versus UVRAGFS-expressing mice (Fig. 7c). However, there was increased Ki67 staining throughout the crypts in UVRAGFS-expressing mice (Fig. 7c, d). The increased proliferation in colons from Dox-treated iUVRAGFS mice was accompanied by a notable increase in genomic instability, as measured by levels of γ-H2AX44 (Fig. 7c, d), providing in vivo support for a putative defect in DNA damage repair in cells with UVRAGFS expression46. We next asked whether the proproliferative effect of UVRAGFS in the colon of aged mice might also involve aberrant inflammation-associated tumor susceptibility. After extensive searching for mechanistic role in unresolved inflammation-associated tumor susceptibility.
(2-month-old) and older (18-month-old) mice in either genotype (Supplementary Fig. 7a). Likewise, no discernable change was observed in the expression of inflammasome proteins (i.e. ASC, NLRP3, NLRP6, AIM2, and NLRC4) in these mice colons (Supplementary Fig. 7a). Similar results were obtained in splenocytes that gave rise to increased spontaneous malignancies (Supplementary Fig. 7b). These data indicate that UVRAGFS affects cell proliferation and spontaneous tumorigenesis independently of inflammasome activation.

The increased cell proliferation in iUVRAGFS mice suggested that an UVRAGFS-dependent oncogenic signaling or mitogen might be involved in spontaneous tumorigenesis. Given the
Fig. 4 UVRAGFS exacerbates intestinal colitis in vivo. a Kaplan–Meier curve showing survival of control and iUVRAGFS mice (n = 10 per group) fed with 5% DSS in drinking water for 6 days. **P < 0.01 (Log-rank test). b Disease activity index (DAI) of colitis in control and iUVRAGFS mice in a. The DAI value was calculated as described in Methods. c Colon lengths of DSS-treated mice with indicated genotypes were assessed at the time of necropsy (days 6 and 10) with representative macroscopic images (left). d H&E-stained sections of colon from DSS-treated mice with indicated genotypes on day 10 after DSS. Inset magnification highlights inflammation and crypt damage. Arrows indicate neutrophil infiltration. Scale bars, 300 μm. e Histopathology scores as indicated in the colon of mice with indicated genotypes after DSS-induced colitis. n = 5–6 per genotype. f Co-IP of inflammasome complex assembly as indicated in colon tissues of mice with indicated genotypes before/after DSS. WB of indicated protein expression in colon homogenates are shown (lower panel). g Histopathology scores (j) and H&E-stained sections (k) of colons from mice with indicated genotypes treated with MCC950 during DSS-induced colitis. n = 3–6 per genotype. Scale bars, 100 μm. iELISA of IL-1β, IL-6, and TNFα (left panel) and IL-18 (right panel) in colons of mice with indicated genotypes after DSS treatment. n = 3–5 per genotype. j, k Histopathology scores (j) and H&E-stained sections (k) of colons from mice with indicated genotypes treated with MCC950 during DSS-induced colitis. n = 3–6 per genotype. Scale bars, 100 μm. l ELISA of cytokines in colons of mice with indicated genotypes. j, k, n = 3–5 per genotype. Data in f are from one experiment that is representative of three independent experiments. For all quantifications, data (mean ± SD) were analyzed with Student’s t-test or two-way ANOVA. n.s., not significant; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. Source data are provided as a Source Data file. See Supplementary Fig. 9 for uncropped data of f.

The essential role of the Wnt/β-catenin pathway in intestinal homeostasis and tumorigenesis, we tested whether UVRAGFS regulates β-catenin expression. UVRAGFS expression resulted in an increase in overall β-catenin levels and in their nuclear accumulation in colon (Fig. 7e, f). Immunoblotting analysis of colon tissues of mice with indicated genotypes after DSS treatment. WB of indicated protein expression in colon homogenates are shown (lower panel). Densitometric quantification of the ratios of LC3-II/LC3-I and p62/actin (g) and the relative production of cleaved caspase-1, IL-1β, and IL-18 to their precursors (h) in the colon tissues of mice with indicated genotypes after DSS treatment. Analogue results were obtained in splenocytes (Supplementary Fig. 7c). Notably, in the colon of young mice, no genotype-specific differences were observed in any of the β-catenin-related factors assessed (Fig. 7g), indicating that the enhanced β-catenin activation in older UVRAGFS-expressing mice genuinely reflects age-related changes.

The increase in β-catenin protein without a concomitant increase in mRNA level in iUVRAGFS mice suggested that β-catenin is subjected to post-translational regulation. Canonically, cytoplasmic β-catenin protein levels are controlled by the Axin1-APC-GSK3 destruction complex (DC), and mutations in DC components stabilize and activates β-catenin, resulting in cancer, mostly notably in the colon. Exon sequencing of Axin1, APC, and GSK3 in colon DNA from the 18-month-old Dox-treated iUVRAGFS mice did not reveal any genetic changes. Both the young and older Dox-treated iUVRAGFS mice and controls expressed comparable levels of DC components and the β-catenin DC complex assembly (Supplementary Fig. 7d). These results point to a role for UVRAGFS in β-catenin activation in older mice through a mechanism independently of the β-catenin DC properties, which induces transcription of its target oncogenes and increases cancer susceptibility.

UVRAGFS activates β-catenin via autophagy suppression. Autophagy was recently implicated in the regulation of Wnt/β-catenin pathway. We postulated that UVRAGFS exerts its role in β-catenin activation by autophagy suppression. Although UVRAGFS did not readily affect basal autophagy in young mice, it exacerbated age-related decline in autophagic function in 18-month-old mice in conjunction with increased β-catenin activity in vivo (Fig. 7g and Supplementary Fig. 7c). Inhibition of autophagy by 3-methyladenine (3-MA), chloroquine (CQ), or by depletion of Beclin1 and UVRAG, increased β-catenin protein levels in immortalized fetal human colon epithelial cells (IFHC) and in SW480 CRC cells (Fig. 7i, j and Supplementary Fig. 7e, f), indicating a steady-state turnover of β-catenin through the autophagy-lysosome pathway. We therefore tested whether UVRAGFS expression is sufficient to stabilize β-catenin protein in colon epithelial cells. Concomitant with the dose-dependent decrease of autophagy in UVRAGFS-expressing SW480 cells, there was an increase in β-catenin protein levels and the transcriptional output of the Wnt pathway, accordingly, while β-catenin mRNA levels remained unaffected (Fig. 7k, l). UVRAGFS expression also promoted the G1/S transition and cell cycle progression as expected (Fig. 7m). Indeed, elevated β-catenin proteins correlated with a marked decrease in β-catenin association with the autophagy marker proteins LC3-II (Fig. 7k).

Conversely, overexpression of WT UVRAG or treating cells with Torin 1, which reverses the decreased autophagy induced by UVRAGFS, promoted β-catenin association with LC3-II and reduced β-catenin levels to those observed in control cells (Supplementary Fig. 7g). A similar reduction in β-catenin turnover and consequent upregulated β-catenin target-gene expression were also observed in SW480 cells with UVRAG knockdown (Supplementary Fig. 7h, i), suggesting that UVRAG suppression, rather than other potential effects of UVRAGFS, contributes to β-catenin regulation. Consistently, activation of β-catenin by UVRAG suppression was associated with an increase in clonogenic survival of SW480 cells (Supplementary Fig. 7j), which was reversed by iCRT14, an inhibitor of β-catenin-TCF binding. Hence, the inhibited clonogenic growth by iCRT14 supported the regulatory role of β-catenin on tumorigenesis associated with UVRAG suppression and suggest that decreased autophagy is a major contributor to aberrant β-catenin activation in non-challenged conditions. We confirmed that these effects of autophagy suppression in vitro on β-catenin regulation also occurred in vivo. In 18-month-old iUVRAGFS mice, induced UVRAGFS expression decreased autophagy and resulted in increased levels of β-catenin (Fig. 7n and Supplementary Fig. 7k). The reduced association of β-catenin with autophagosomes in these mice was accompanied by increased interaction with TCF4, leading to upregulation of the Wnt/β-catenin signaling (Fig. 7n and Supplementary Fig. 7k). These results indicate that UVRAGFS stabilizes β-catenin by inhibiting it autophagic degradation, a mechanism that may contribute to spontaneous tumorigenesis in UVRAGFS-expressing mice.

Centrosome amplification associated with UVRAGFS. Because the role of UVRAGFS in cancer is also linked to its ability to promote centrosome amplification (CA) and chromosomal instability in vitro, we further investigated the effect of UVRAGFS on centrosome homeostasis in vivo. There was a significant increase in the incidence and degree of supernumerary centrosomes in the tissues of 18-month-old Dox-treated iUVRAGFS mice as compared to the age-matched control mice (Fig. 8a–c). This phenotype was most pronounced in the colon and spleen of iUVRAGFS versus control mice (Fig. 8a, b), where high rates of proliferation was observed (Figs. 6 and 7c). In young mice.
**Fig. 5** UVRAG<sup>FS</sup> predisposes mice to colitis-associated colon cancer. 

**a** Representative macroscopic images of the colon from AOM-DSS-treated control (Ctrl) and UVRAG<sup>FS</sup> mice in the presence/absence of Dox at the time of necropsy (day 60). Red arrows highlight colonic tumors. 

**b–d** The number (b), size (c), and overall load (d) of colonic tumors in AOM-DSS-treated mice in a. Tumor load is evaluated by totaling the diameters of all tumors in AOM-DSS-treated mice in a. *n* = 5–12 mice per genotype. 

**e** IHC staining of Ki67, cleaved caspase 3, p62, keratin 20, E-cadherin, N-cadherin, and vimentin in the colons from AOM-DSS-treated mice of indicated genotypes. Data are from one animal that is representative of 5–12 animals in each group. The levels of Ki67 staining (top right) and tumor apoptosis (bottom right) in the indicated colon were quantified. Arrows (red) denote cells undergoing apoptosis. Scale bars, 100 μm. 

**f** H&E-stained sections of the colon from AOM-DSS-treated mice in a. Data are from one animal that is representative of 5–12 animals in each group. Scale bars, 100 μm. 

**g** Histological scores for crypt damage (top) and total histological score (bottom) of colons from mice in a were quantified at day 60 as described in the Methods. *n* = 10 mice per group. 

**h** WB analysis of caspase-1 cleavage, IL-1β production, LC3-I/II, and p62 levels in colon tissues of Dox-treated control and UVRAG<sup>FS</sup> mice after AOM-DSS treatment. Data in h are from one experiment that is representative of three independent experiments. For all quantifications, data (mean ± SD) were from the indicated number of independent experiments and analyzed with one-way ANOVA. n.s., not significant; *P* < 0.05; ***P* < 0.001; ****P* < 0.0001. Source data are provided as a Source Data file. See Supplementary Fig. 9 for uncropped data of h.
Taken together, these data indicate that CA induced by UVRAGFS promotes spontaneous tumorigenesis in mice. a Kaplan-Meier plot of time to development of any malignancy in Dox-treated iUVRAGFS mice (n = 25) versus Dox-treated control mice (n = 25). Malignancy is determined by complete histologic survey of all major internal organs. ***P < 0.001 (Log-rank test). b Representative images of DLBCL in different organs that developed in Dox-treated iUVRAGFS mice. c Percentage of control (n = 16) and UVRAGFS-expressing (n = 12) mice with spontaneous tumors, including lymphoproliferative disease (LPD), lymphomas, and non-lymphoid malignancies. d H&E and IHC analysis of the most frequently observed neoplastic lesions from Dox-treated iUVRAGFS mice using the antibodies as indicated. Scale bars, 100 μm.

(2-month-old) mice, despite a modest elevation in the level of CA upon UVRAGFS expression, no genotype-specific differences were observed with statistical significance in any of the tissues assessed (Supplementary Fig. 8a, b). This indicates that the exacerbated centrosome pathology in older UVRAGFS-expressing mice also reflects an increase in age-related changes in these organs. Consistent with the consensus that CA causes erroneous chromosomal segregation60, we detected a greater than 3-fold increase in aneuploidy in UVRAGFS-expressing splenocytes from both 18-month-old mice and 2-month-old mice to lesser extent (Fig. 8d). In parallel, genome-wide copy number variation (CNV) analysis61 of tumors developed in Dox-treated iUVRAGFS mice revealed marked variability and heterogeneity with more chromosomal amplifications and deletions than the control (Fig. 8e). Taken together, these data indicate that CA induced by UVRAGFS may play a role in UVRAGFS-associated chromosomal aneuploidies that potentially favor tumor formation in UVRAGFS-expressing mice.

Discussion

Herein, we demonstrate that a transgenic frameshift mutation in a key autophagy regulatory gene, Uvrag, which disrupts the UVRAG autophagy-regulatory complex in mice, results in impaired starvation- or endotoxin-induced autophagy activation, increased inflammatory response and associated pathologies, as well as age-related spontaneous malignancies. Thus, UVRAG has previously undescribed essential roles in the in vivo regulation of stimulus-induced autophagy as well as inflammatory disorders. Furthermore, our discovery indicates that time-dependent autophagy suppression by UVRAGFS and ensuing β-catenin stabilization/activation may serve as one tumor-promoting mechanism underpinning age-related cancer susceptibility.

Due to the embryonic lethality of Uvrag knockout mice, the exact in vivo role of mammalian UVRAG in autophagy regulation remained largely unaddressed. Using the GFP-LC3 reporter to track autophagy and an inducible Uvrag mutant mouse model, we demonstrated that the cancer-derived dominant negative mutant of UVRAG blocks starvation-induced autophagy because Dox-induced iUVRAGFS mice accumulated fewer autophagosomes upon starvation and displayed reduced rates of autophagic flux. We also found that lipophagy was impaired in UVRAGFS mice. Notably, our observation does not exclude a role for UVRAGFS and by extension autophagy inhibition in dysregulating other processes of lipid consumption (e.g. lipolysis or lipid oxidation), which merits further investigation. Likewise, we cannot definitely exclude other anti-autophagy effects of the UVRAGFS mutant besides UVRAG inhibition. Our data with iUVRAGFS mice also reveal that the effect of UVRAGFS on basal autophagy was not as strong as that induced by starvation, implying that the UVRAG-associated PI3KC3 activity is either not essential, or alternatively, other PI3KC3 complexes, for instance one that assembled with other PI3KC3 complexes, for instance one that assembled with Atg14, is sufficient for the basal levels of autophagy.

It is worth noting that Dox-induced iUVRAGFS mice are also impaired in endotoxin-induced autophagy and are susceptible to death after LPS injection. This increased susceptibility was associated with NLRP3 inflammasome hyperactivation and increased inflammatory cytokine production. Primary BMDM from Dox-induced iUVRAGFS mice, primed with LPS and treated with ATP, showed increased mtROS, NLRP3 inflammasome assembly and
pro-caspase-1 cleavage, and hypersecretion of IL-1β. These phenotypes in UVRAGfs mice are consistent with those observed in previous studies of mice with deficiency of Atg16, LC3, or Beclin14,28. It is difficult to conclude whether UVRAG functions directly through mitophagy or indirectly through other cellular factors in inflammatory response. However, the diminished recruitment of Beclin1 to the TLR4-receptor complex and the decreased UVRAG-Beclin1-Vps34 assembly/activity in Dox-induced iUVRAGFS mice suggest that the autophagy function of UVRAG is intrinsically required for mitochondrial homeostasis and adequate regulation of inflammasome activation during sepsis. We also provide data showing that uncontrolled inflammation in iUVRAGFS mice was associated with increased tumor growth and de-differentiation in colitis-associated colon cancer.

In addition to tumor-promoting inflammation, increased cancer incidence is probably the most well-known phenotype of cells that are deficient in autophagy-related gene function62,63. However, the precise mechanisms underlying autophagy-related tumor prevention remains elusive. Although knockout of UVRAG is embryonic lethal in mice, the inactivation of UVRAG by the
expression of UVRAGFS causes the premature onset of age-related neoplasia, particularly spontaneous lymphoma, in iUVRAGFS mice, independently of its role in inflammasome regulation. Previous work has shown that human cancers, especially those with microsatellite instability, show a global expression of UVRAGFS compared with normal tissues. Our work confirmed that this mutant promotes tumorigenesis in vivo. Intriguingly, UVRAGFS enhanced age-related decline of autophagy, which stabilized and thus increased the abundance of the β-catenin oncoprotein. These findings correlate age-related β-catenin accumulation with increased tumorigenesis in iUVRAGFS mice. Notably, overexpression/activation of β-catenin has been clinically correlated to the pathogenesis of DLBCL, a major tumor type developed in aged UVRAGFS mice, representing a new target for DLBCL lymphomagenesis. More studies are needed to determine whether this age-dependent regulation of β-catenin by UVRAGFS can be generalized to other autophagy-related tumor models. Furthermore, considering the pleiotropic effects of UVRAG in cellular homeostasis, we cannot rule out other pro-tumorigenic effects caused by UVRAG inhibition other than the autophagy-Wnt/β-catenin pathway that may influence the tumorigenic phenotype in iUVRAGFS mice. For instance, we have provided genetic proof of the previously described roles for UVRAGFS in the regulation of CA and ensuant chromosomal instability, which may improve cancer cell fitness during tumor progression. Nevertheless, the emerging facet of oncogene-autophagy interactions highlight a functional role for autophagy, more likely selective autophagy, in age-related diseases including cancer.

**Methods**

**Mouse models.** For generation of iUVRAGFS transgenic mice, a Flag-tagged human UVRAGFS mutant was PCR amplified and subcloned into pMUC1 and Not I restriction sites of pTRE-Tight (631059, Clontech), which contained a T- responsive Pgst1 promoter and a SV40 poly(A). The IRES-luciferase (Luc) cDNA fragment (5’-flanked into Not I site of pTRE-Tight). The resulting plasmid, pTRE-fgh-Flag-UVRAGFS-Luc, was digested with Xho I to release the transgenic cassette. The gel-purified cassette was injected into the pronuclei of fertilized 1-cell stage embryos (B6D2F1 background) with standard procedure. Injected embryos were cultured in M16 medium (M6111, Cytoscreen) at 37 °C under 5% CO2 overnight. All the two-cell stage embryos were then transferred into oviducts of the pseudopregnant CD-1 female mice at 0.5 dpc by Norris Comprehensive Cancer Center Transgenic Mice Core Facility (USC). Integration of the construct was confirmed by PCR (Supplementary Table 1). Two independent founder lines were identified and back-crossed for more than 20 generations to C57BL/6 mice (Jackson Laboratories). Flag-UVRAGFS-Luc transgenic mice were crossed with Rosa26-rtTA*M2 mice (Jackson Laboratories) in a pure C57BL/6 background to generate the double-transgenic mice (Rosa26-rtTA*M2Flag-UVRAGFS-Luc), denoted as iUVRAGFS. Animals were maintained on the C57BL/6 background. To turn on the expression of UVRAGFS, iUVRAGFS mice were administered a doxycycline (Dox) diet (TD.01306, Envigo) beginning at 22 days of age. iUVRAGFS mice and its wild-type littermate control mice were further crossed with GFP-LC3 transgenic mice on the C7BL/6 background and tissues of offspring were used for autophagy analyses in vivo. Both male and female control and iUVRAGFS mice were used in the studies.

Atg5Crelox mice were a gift from Dr. Noboru Mizushima (Tokyo Medical and Dental University, Tokyo, Japan). Atg5Crelox mice bred to Rosa26CreER(T2) mice (004847; Jackson Laboratories) to generate Atg5/−/− mice upon administration of tamoxifen.

In vivo bioluminescence imaging, in vivo bioluminescence imaging was performed using the IVIS Lumina LT Series III in vivo imaging system (CLSI13633, PerkinElmer). Images were captured and analyzed with Living Image 3.1 software. Signal intensity was measured over the region of interest and quantified as flux (photons per cent per s).

For generation of bone marrow chimeric mice, bone marrow in recipient mice was ablated by lethal irradiation with 550 Gy (twice, 24 h apart) before transplantation. Bone marrow were flushed from the femurs and tibias from wild-type control and iUVRAGFS donor mice on Dox and washed twice with warm PBS.

In all, 1 × 107 BM cells per mouse were infused intravenously into the tail veins of recipient mice. Mice were housed in microisolator cages for 6 weeks for full reconstitution and recovery before induction of DSS-colitis. Mice were provided with water containing gentamicin sulfate (0.2 mg/ml) for the first two weeks after transplantation. To assess BM reconstitution, peripheral blood was collected from chimeric mice and RNA was isolated using the Mouse RiboPure® Blood RNA Isolation Kit according to the manufacturer’s protocol (AM1951, Invitrogen) for RT-PCR with primers for mouse luciferase (Supplementary Table 2).

For LPS sepsis model, 8–10-week-old and sex-matched iUVRAGFS mice were injected intraperitoneally with 20 mg kg−1 body weight of lipopolysaccharides (LPS) from E. Coli (L8724, Sigma). Tissues and blood were collected 3 h post injection. Survival after LPS challenge was assessed every 12 h for 3 days. All survived mice were euthanized at the end of the third day. To inhibit the NLRP3 inflammasome activation, control and iUVRAGFS mice on Dox were treated i.p. with 20 mg kg−1 MCO9509 or saline vehicle 1 h before LPS administration and then daily for 3 days. Mice were euthanized 24 h after the last treatment on day 3.

For induction of DSS-induced colitis and treatment studies, experimental colitis was induced by adding DSS (5% wt/vol) to the drinking water for 6 days, followed by a 4-day recovery period with water. Mice were weighed daily and monitored for clinical signs of colitis (e.g., weight loss, stool consistency, and rectal bleeding). To inhibit the NLRP3 inflammasome activation in vivo, control and iUVRAGFS mice on Dox were treated i.p. with 20 mg kg−1 MCO9509 or saline vehicle daily on days 0–5 of DSS administration. Mice were euthanized 24 h after the last treatment on day 6. A colitis disease activity index (DAI) was calculated for each mouse daily based on the following criteria: weight loss from baseline (0, no weight loss; 1, 1–3% weight loss; 2, 3–6% weight loss; 3, 6–9% weight loss; 4, >9% weight loss); stool consistency (0, normal; 2, loose stool; 4, diarrhea); and fecal blood (0, none; 2, blood visible in stool; 4, gross bleeding). Gross bleeding was defined as fresh perianal blood with obvious hematocrit. Upon necropsy, colon length was measured.

For induction of colitis-associated tumorigenesis, colitis-associated colon tumorigenesis was induced according to the literature. Briefly, Dox-treated/untreated control and iUVRAGFS mice were administered AOM (10 mg kg−1 body weight) intraperitoneally on day 0, followed by three cycles of DSS (2.5%, wt/vol) in the drinking water for five days with a 14-day water interval between each DSS cycle. The animals were weighed daily and sacrificed on day 60. All mice were maintained in a pathogen-free facility with ad libitum access to food and water. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Southern California and performed in accordance with IACUC guidelines for animal care and use.

**Cell culture and transfection.** 293T cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) (5796, Sigma). Primary fetal human colon epithelial cells (FHC) (CRL-1831, ATCC) were cultured in DMEM:F-12 medium, supplemented with 25 mM HEPES, 10 ng ml−1 cholera toxin, 5 μg ml−1 insulin, 5 μg ml−1 transfected with increasing amounts of Flag-UVRAGFS. WCL were used for IP with anti-β-catenin (Jackson Laboratories). 1 Quantitative RT-PCR of indicated gene expression in cells. n = 3. m Cell cycle analyses of SW480 cells stably expressing vector or UVRAGFS: n = 3. 1 Co-IP of β-catenin with LC3 and TCF4 in spleens from 18-month-old mice. Data in b-f are from 10 to 20 HPP pooled from three independent experiments. Data in g n are from two randomly chosen samples per group with similar results observed in all ten samples per genotype. For all quantifications, data (mean ± SD) were analyzed with Student’s t-test (b, d, f), two-way ANOVA (h, m) or one-way ANOVA (k, i, m), n.s., not significant; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. Scale bars, 100 μm. Source data are provided as a Source Data file. See Supplementary Fig. 9 for uncorrected data of g, i, j, k, n and Supplementary Fig. 10 for raw data of m.
**Fig. 8** UVRAG<sup>FS</sup> promotes centrosome amplification in tissues. 

**a** Quantification of the level of centrosome amplification in indicated tissues from Dox-treated 18-month-old control (n = 4) and iUVRAG<sup>FS</sup> mice (n = 4).

**b** Quantification of centrosome numbers in indicated tissues from 18-month-old Dox-treated control (n = 4) and iUVRAG<sup>FS</sup> mice (n = 4). C. centrosome.

**c** Representative confocal images of centrosomes in tissues from Dox-treated iUVRAG<sup>FS</sup> mice or control animals. Centrosomes were immunostained for γ-Tubulin (red). Nuclei were stained with DAPI (blue). Data are from one animal that is representative of four animals in each group. Scale bar, 10 μm.

**d** Representative images of metaphase spread from splenocytes of control (n = 5) and iUVRAG<sup>FS</sup> mice (n = 5) treated with Dox for 2 or 18 months (left). The percentage of cells with abnormal karyotype (aneuploidy) was quantified (bottom). In all, 50–100 cells were evaluated per mice per experiment. Scale bars, 25 μm.

**e** Heatmap of copy number alteration (CNA) profiles shows heterogeneous amplifications (red) and deletions (blue) among different tumors (n = 13) from Dox-treated iUVRAG<sup>FS</sup> mice. CNA profiles are created using the mouse genome (mm9) as reference. Copy number is displayed as the ratio to the median. For all quantification, data represents the mean ± SD derived from the indicated number of independent experiments. Source data are provided as a Source Data file. *P < 0.05; **P < 0.01; ****P < 0.0001 (Student’s t test).
transferrin, 100 ng/ml of Dox on the 3rd day after plating. For in vitro treatments, 100 μl of medium from L929 mouse fibroblasts. To induce UVRAGFS expression, cells were transfected with plasmid constructs. Focus Biomolecules) for 1 h and followed by stimulation with 1 mM ATP (1 mM) for 1 h, or transfected with vehicle or poly(dA:dT) (1 μg/ml) for 6 h using LypOvec® (Ivec-2, InvivoGen). For infiltration, BMDMs were primed with 100 ng/ml of LPS for 6 h followed by treatment with 10 μM Z-YVAD-FMK (ALX-260-154-R020, Enzo Life Sciences) or 1 μM of MitoQ (10-1363-0005, Focus Biomolecules) for 1 h and followed by stimulation with 1 mM ATP treatment for 1 h.

**Plasmid constructs**

The mRFP-EGFP-LC3 plasmid and p40(phox)-PX-EGFP plasmid constructs were kindly provided by Drs. Jae U Jung (University of Southern California) and S. Field (University of California, San Diego), respectively. All constructs were confirmed by sequencing using an ABI PRISM 377 automatic DNA sequence (Applied Biosystems).

**Antibodies, fluorescent dyes, and other reagents.** The following antibodies were used in this study: polyclonal rabbit anti-UV Richard (C-term) (AP18585B, Abgent, 1:1000 for WB, 1:200 for IF), polyclonal rabbit anti-LC3B (2859T, Cell Signaling Technology, 1:1000 for WB), monoclonal mouse anti-p62 (814802, Biolegend, 1: 50 for WB, 1:200 for IP), monoclonal rabbit anti-Atg16 (8098T, Cell Signaling Technology, 1:1000 for WB), monoclonal rabbit anti-Atg5 (GT526061, GeneTex, 1:1000 for WB), polyclonal rabbit anti-Beclin-1 (110A6-1-AP, Proteintech, 1:1000 for WB, 1:200 for IF), polyclonal rabbit anti-Parkin (PRK8) (sc-32282, Santa Cruz Biotechnology, 1:1000 for WB), monoclonal mouse anti-Parkin (PRK8) (sc-32282, Santa Cruz Biotechnology, 1:1000 for WB), monoclonal mouse anti-p82 (814802, Biolegend, 1: 50 for WB), monoclonal rabbit anti-LC3B (2775S, Cell Signaling Technology, 1:1000 for WB), monoclonal rabbit anti-Caspase-1 (C-term) (AS2953, Panomics, 1:1000 for WB), monoclonal mouse anti-IκBα (sc-374615, Santa Cruz, 1: 1000 for WB), monoclonal rabbit NLRP3 (210-401-323, Rockland, 1:1000 for WB), monoclonal mouse anti-Caspase-11 (sc-4814T, Cell Signaling Technology, 1:1000 for WB), polyclonal rabbit anti-NFκB (8242S, Cell Signaling Technology, 1:500 for WB), monoclonal mouse anti-β-catenin (MA1-300, Thermo Fisher, 1:1000 for WB), polyclonal rabbit anti-APC (NBP2-15422, Novus Biologicals, 1:1000 for WB), monoclonal mouse anti-c-Myc (624802, Biolegend, 1:1000 for WB), polyclonal rabbit anti-c-Myc (10828-1-AP, Proteintech, 1:400 for IFC), polyclonal rabbit anti-Cd20 (PA5-16701, Thermo Fisher, 1:50 for IFC), polyclonal rabbit anti-Bcl-2 (21187-1-AP, Proteintech, 1:200 for IF), polyclonal rabbit anti-Kappa (44678-1-AP, Proteintech, 1:1000 for IF), polyclonal rabbit anti-phospho-β-catenin (Ser451/457, Cell Signaling Technology, 1:1000 for WB), monoclonal mouse anti-tubulin (GTX113286, GeneTex, 1:1000 for WB, 1:200 for IF), HRP-labeled or fluorocently labeled secondary antibody conjugates were purchased from Invitrogen. Purified rabbit anti-IgG was purchased from Pierce. Z-YVAD-fmk was from Enzo Life Sciences (ALX-260-154-R020; MitoOqueno (MitoQ) was from Focus Biomolecules (10-1363-0005). Torin1 was from Selleckchem, 3-MA was from Santa Cruz Biotechnology; MCC950 was from Adipogen; Adenovirus (Ad-LacZ, adenovirus); Dextran Sulfate Sodium was purchased from Sigma. For western blot analysis, frozen tissues were lysed in ice-cold RIPA lysis buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS) containing protease inhibitor cocktail (A132953, Pentafab) for 30 min at 4 °C. Lysates were centrifuged at 16,000 g for 10 min at 4 °C. Cleared lysates were diluted in 2X SDS-PAGE loading buffer and analyzed using antibodies as indicated.

**Immunochemistry and confocal microscopy**

Cells plated on coverslips were fixed with 4% paraformaldehyde (30 min at RT). After fixation, cells were permeabilized with 0.2% Triton X-100 for 5 min and blocked with 10% goat serum (G9032, Sigma) for 2 h at RT. Primary antibody staining was carried out using antisera or purified antibody in 1% goat serum for 1–2 h at RT or overnight at 4 °C. Secondary antibodies were then extensively washed with PBS, then stained with Alexa 488-, Alexa 546-, and/or Alexa 633-conjugated secondary antibodies in 1% goat serum for 1 h, followed by DAPI (4′, 6-diamidino-2-phenylindole) staining. Cells were mounted using Vectashield (Vector Laboratories, Inc.). Confocal images were acquired using a Nikon Eclipse C1 laser-scanning microscope (Nikon, PA), fluorescent staining was imaged sequentially in line-interface modes to eliminate crosstalk between the channels. The step size in the z-axis varied from 0.2 to 0.5 μm to obtain 16 slices/imaged plane. Immunochemistry (Immuno-Reagent Co., Ltd.), 1:1000 for WB), monoclonal mouse anti-Kappa (14678-1-AP, Proteintech, 1:500 for IF). N-cadherin (PA5-29570, Thermo Fisher, 1:1000 for IHC and 1:1000 for WB), polyclonal rabbit anti-PCNA (M08510, Novus Biologicals, 1:1000 for WB), monoclonal mouse anti-β-catenin (MA1-300, Thermo Fisher, 1:1000 for WB), polyclonal rabbit anti-phospho-β-catenin (Ser33/37/Thr41) (8814S, Cell Signaling Technology, 1:1000 for WB), polyclonal rabbit anti-APC (NB2-15422, Novus Biologicals, 1:1000 for WB), monoclonal mouse anti-c-Myc (626802, Biolegend, 1:1000 for WB), polyclonal rabbit anti-c-Myc (10828-1-AP, Proteintech, 1:400 for IHC), polyclonal rabbit anti-Cd20 (PA5-16701, Thermo Fisher, 1:50 for IHC), polyclonal rabbit anti-Bcl-2 (21187-1-AP, Proteintech, 1:200 for IF), polyclonal rabbit anti-Kappa (44678-1-AP, Proteintech, 1:1000 for IF), polyclonal rabbit anti-phospho-β-catenin (Ser33/37/Thr41) (8814S, Cell Signaling Technology, 1:1000 for WB), monoclonal mouse anti-Flag (F7425, Sigma; 1:1000 for IHC & WB), and polyclonal rabbit anti-γ-Tubulin (GTX113286, GeneTex, 1:1000 for IF). HRP-labeled or fluorocently labeled secondary antibody conjugates were purchased from Invitrogen. Purified rabbit anti-IgG was purchased from Pierce. Z-YVAD-fmk was from Enzo Life Sciences (ALX-260-154-R020; MitoOqueno (MitoQ) was from Focus Biomolecules (10-1363-0005). Torin1 was from Selleckchem, 3-MA was from Santa Cruz Biotechnology; MCC950 was from Adipogen; Adenovirus (Ad-LacZ, adenovirus); Dextran Sulfate Sodium was purchased from Sigma. Unless otherwise stated, all chemicals were purchased from Sigma.

**Autophagy analyses.** For assessment of autophagy in vivo, 6–8-week-old Dox-treated controlGFP-LC3 or iUVRAGCre-GFP-LC3 mice were either subjected to starvation for 48 h or challenged with 20 mg kg−1 LPS from Escherichia coli intraperitoneally. Mice were then perfused with 4% paraformaldehyde (PFA) in PBS with 0.1% w/v sodium azide. The brain was separated from the skull and fixed with 4% paraformaldehyde (PFA) for 24 h at 4 °C. After fixation, the brain was bleached in 30% hydrogen peroxide for 48 h. The brain was dehydrated in ascending grades of ethanol and then embedded in paraffin. Tissue sections were cut at 4 μm, deparaffinized in xylene and rehydrated in alcohol. Tissue sections were then routinely stained with hematoxylin and eosin. For negative controls, the primary antibody was excluded. For evaluation and scoring of immunohistochemical data, we randomly selected 10 fields within the tumor area under high power

**Immunohistochemistry.** Tissue sections were fixed in 10% neutral buffered formalin and embedded in paraffin. Tissue sections were routinely stained with hematoxylin and eosin. For immunohistochemistry staining, tissue slides were deparaffinized in xylene and rehydrated in alcohol. Endogenous peroxidase was blocked with 3% hydrogen peroxide. Antigen retrieval was performed by incubating the sections in boiling 10 mM sodium citrate pH 6.0 followed by incubation with the indicated primary antibody overnight at 4 °C. Antibody binding was detected with EnVision™ Dual Link System-HRP DAB kit (K-6010, Dako). Sections were counterstained with hematoxylin, dehydrated through ascending grades of alcohol, cleared in xylene, and mounted in DPX. The data obtained were statistically analyzed using the SPSS program. The significance of difference was calculated using the built-in colocalization analysis module of the NIS-Elements AR software. All experiments were independently repeated several times. The investigators conducted blind scoring for each quantification-related study.
**Histopathology analysis of colon.** Histopathology assessment was performed by an anatomical pathologist (A. H) from de-identified section slides. The features evaluated covered: acute and/or chronic inflammation, hyperplastic changes of the colon epithelium, and crypt distortion or damage, fibrosis, and neoplasia. Three independent parameters were measured: inflammatory cell infiltrate, extent of hyperplasia, and crypt distortion. The total histological score was calculated by summing of the three independent scores with a maximum score of 12. Inflammation was assessed using a scoring system from the literature: 0 = no inflammation; 1 = mild chronic mucosal inflammation; 2 = mild acute or moderate chronic mucosal and submucosal inflammation; 3 = severe acute or chronic mucosal, submucosal, and transmural inflammation. Hyperplastic changes were scored 0 = no increase in epithelial cell numbers in crypts relative to baseline; 1 = crypts lost; 2 = crypts present with flattened epithelium; 3 = only crypt epithelium present.

**Conventional electron microscopy.** Mice were euthanized, and liver and kidney were rapidly fixed overnight at 4 °C in 1/2 strength Karnovsky’s (2% paraformaldehyde and 2.5% glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.4). Tissues were post-fixed in 2% osmium tetroxide in 200 mM sodium cacodylate for 2 h at 4 °C and rinsed in 0.1 M cacodylate buffer. Samples were then block-stained, and immersed in 1% uranyl acetate overnight at 4 °C. The pellet was then rinsed with 0.1 M sodium acetate. Samples were dehydrated through a graded series of ethanol, and then infiltrated with Epon resin overnight at room temperature. They were then embedded in resin overnight at 60 °C. Thin sections were cut on a Leica Ultracut R, collected onto formvar-carbon coated slot grids. Sections were examined on a FEI 2100 transmission electron microscope. Images were recorded on film at ×5000 magnification.

**Metaphase spreads.** For metaphase spreads of splenocytes, freshly harvested spleens were minced and filtered through a 40 µm cell strainer into warm PBS. Cells were spun at 1000 x g for 5 min, resuspended in warm DMEM supplemented with 10% FBS, 100 U/mL penicillin,100 U/mL streptomycin. Cells were then treated with colcemid (KaryoMAX, GibCO) at 0.1 µg/ml for 1 h at 37 °C. Cells were swollen in prewarmed 75 mM KCl for 30 min at 37 °C, then carefully fixed in methanol: acetic acid (3:1) and kept at −20 °C. Metaphase spreads were prepared by dropping cells in the fixative onto Superfrost glass slides (Fisher Scientific) at 25 °C and 60% of humidity. After air dry, metaphase images were captured and analyzed using a 20X Nikon objective (PL APO, 1.4 NA). At least 10 metaphases from each tissue were scored for chromosomal aberration.

**Immunoblotting and immunoprecipitation.** For co-immunoprecipitation from tissue samples, frozen tissues were weighed and homogenized in ice-cold lysis buffer (20 mM Tris at pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100; 1 µl per 100 mg tissue) containing a complete protease inhibitor cocktail (A32953, Pierce). Lysates from each tissue were scored for chromosomal aberration.

**Bacteria colony forming units (c.f.u.).** Levels of c.f.u. in freshly isolated mice feces were determined by homogenization of feces in 0.01% Triton X-100 in PBS followed by serial dilution plating on non-selective Luria-Bertani agar plates.

**Cell viability.** Cell viability was determined using the CellTiter 96® non-radioactive cell proliferation assay (G4011, Promega) following the manufacturer’s instructions.

**Cell cycle analysis.** Cell cycle analysis was determined by flow cytometry (BD Biosciences, Franklin Lakes, NJ, USA). SW-480 cells were harvested, washed with phosphate-buffered saline (PBS), fixed with 70% cold ethanol overnight at 4 °C, and then incubated with a staining solution containing 100 µg/ml RNase and 50 µg/ml propidium iodide (PI; ab139418, Abcam) for 1 h at 37 °C before analysis by flow cytometry. A total of 10^4 nuclei were examined by a BD FACSScanto II flow cytometer. For analysis, first gate on the single cell population using pulse height vs. pulse area. Then apply this gate to the scatter plot and gate out debris. Gates are combined and applied to the PI histogram plot. DNA histograms were analyzed by FlowJo software (both from Becton- Dickinson, Mountain View, CA, USA). Results are presented as the percentage of cells in each phase.

**Measurement of mitochondrial membrane potential.** TMRM (AS-88065, AnaSpec) was used to measure mitochondrial membrane potential (Δψm). Briefly, Dox (1 µg/ml)-treated BMDMs were primed with LPS (100 ng/ml) for 6 h and stimulated with ATP (1 mM) for 30 min. The cells were then washed twice with PBS and incubated with 200 nM of TMRM and 25 nM of MitoTracker Green for 30 min at 37 °C and washed twice with PBS. The cells were resuspended in PBS and fluorescence intensity was measured with a FLUOstar Omega microplate reader (BMG Labtech). The ratio of TMRM to MTG fluorescence intensity was normalized to PBS controls.

**Mitochondrial ROS detection.** MitoSOX (M30088, Invitrogen) was used to measure mitochondrial ROS production. Briefly, Dox (1 µg/ml)-treated BMDMs were primed with LPS (100 ng/ml) for 2 h and stimulated with ATP (1 mM) for 30 min. The cells were then washed twice with PBS and incubated with 5 µM of MitosOX for 10 min and washed twice with PBS again. Fluorescence intensity was determined using a Synergy 2 multimode plate reader (BioTek Instruments), and the data were normalized to PBS controls.

**Measurement of cytosolic mitochondrial DNA (mtDNA).** Dox (1 µg/ml)-treated BMDMs were primed with LPS (100 ng/ml) for 2 h and stimulated with ATP (1 mM) for 30 min. MitoSOX was quantified using a mitochondrial isolation kit (98974, ThermoScientific) according to manufacturer’s instructions. Mitochondrial DNA encoding cytochrome c oxide 1 (COX1) was measured by quantitative qPCR (refer to Supplementary Table 2 for primers’ sequence) with the same volume of the DNA solution. Nuclear DNA encoding 18S ribosomal RNA was used for normalization.

**Clonogenic cell survival assay.** The log-phase cells were trypsinized, counted, and plated at appropriate dilutions in 6-well plates for colony formation. After 14 days of culture, colonies in the presence of cRT(4) (SMU 0203, Sigma) treatment (50 µM), colonies were fixed and stained with colony fixation-staining solution containing glutaraldehyde 6.0% (vol/vol) and crystal violet 0.5% (wt/vol) in H2O, and counted. Plating efficiency (PE) was determined for each individual cell line as described, and the surviving fraction (SF) was calculated based on the number of colonies that arose after treatment, expressed in terms of PE. Each experiment was repeated six times.

**Gene knockdown by shRNA and lentiviral gene delivery.** All shRNAs were purchased from Open Biosystem. Lentiviral-compatible shRNAs against Bcl2lin1 (sh1: V2LHS_241693, sense: TGTGTTGATCTACCTGACGC; sh2: V3LHS_332992, sense: TCGTACGAGCCATCTGCTG), UVRAG (sh1: V2LHS_197739, sense: ATTGATCTGACTGACCCAG; sh2: V3LHS_355751, sense: ATGCATCTAATCCTC), for lentivirus production, HER2K3T cells were transfected with the vector (e.g. pCDH-CMV-2E-FU-Puro or pGP2), pcMV-dR8.91 packaging plasmid, and pCMV-VSV-G envelope plasmid in a 5:1:4 ratio using the lentiviral transfer vector (e.g. pCDH-CMV-2E-FU-Puro or pGP2) and transduced cells were selected in 2 µg/ml-tetragonal (50 uM)-treated BMDMs were used for clonogenic survival analysis. The luciferase activity was measured using the luciferase assay kit (PierBio) following the manufacturer’s instructions.
null
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Author contributions
C.Q, Y.S., and H.G. performed research and analyzed the data. C.Q. conducted most mouse-related experiments and pathology analysis; Y.S. and H.G. conducted most biochemistry and molecular biology experiments. S.L., H.M., and N.S. helped with bone marrow chimera experiments. M.F., D.C., B.C., D.N., and N.W. contributed to the transgenic animals construction and characterization. S.R. and J.H. guided CNV analysis of mice tumors. S.E.M. and A.H. helped with IHC and pathological analyses. V.P., O.A., G.I., S.M.M., and H.C. participated in discussion. C.L. designed research, analyzed data, and wrote the paper. All authors read, contributed to, and approved the final manuscript.

Competing interests
The authors declare no competing interests.

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