1 **Interleukin-11 is a Marker for Both Cancer- and Inflammation-Associated**

2 **Fibroblasts that Contribute to Colorectal Cancer Progression**

3

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42 SUMMARY

43 Interleukin (IL)-11 is a member of the IL-6 family of cytokines and involved in multiple
 44 cellular responses, including tumor development. However, the origin and functions of
 45 IL-11-producing (IL-11⁺) cells are not fully understood. To characterize IL-11⁺ cells *in*
 46 *vivo*, we generated *Il11* reporter mice. IL-11⁺ cells appeared in the colon of three murine
 47 tumor models, and a murine acute colitis model. *Il11ral* or *Il11* deletion attenuated the
 48 development of colitis-associated colorectal cancer. IL-11⁺ cells expressed fibroblast
 49 markers, and genes associated with cell proliferation and tissue repair. IL-11 induced
 50 STAT3 phosphorylation in colonic fibroblasts, suggesting the activation of IL-11⁺
 51 fibroblasts. Analysis using the human cancer database revealed that genes enriched in
 52 IL-11⁺ fibroblasts were elevated in human colorectal cancer, and correlated with
 53 reduced disease-free survival. Together, our results suggested that tumor cells induced
 54 IL-11⁺ fibroblasts, and that a feed-forward loop between IL-11 and IL-11⁺ fibroblasts
 55 might contribute to tumor development.

56 (149 words)

57

58 **KEYWORDS:** cancer-associated fibroblasts, colitis, colitis-associated colorectal cancer,
59 dextran sulfate sodium, inflammation-associated fibroblasts; interleukin-11, IL-11
60 receptor, reporter mice, STAT3.
61

62 INTRODUCTION

63 Maintenance of intestinal homeostasis involves a variety of cell types, including
64 epithelial, immune, and stromal cells^{1,2,3}. Within the intestinal lamina propria, stromal
65 cells include fibroblasts, α smooth muscle actin (α SMA)-positive myofibroblasts,
66 endothelial cells, and pericytes^{4,5}. These stromal cells organize the tissue architecture,
67 and have recently been revealed to play crucial roles in regulating immune responses,
68 tissue repair, and tumor development^{3,4,5}. Recent studies have focused on fibroblasts
69 that can support tumor growth, termed cancer-associated fibroblasts (CAFs)^{6,7,8}. In a
70 very recent study, single-cell RNA-sequencing (scRNA-seq) was performed to analyze
71 colon biopsies from healthy individuals and ulcerative colitis (UC) patients. The results
72 revealed that UC patients' colon samples included a unique subset of fibroblasts, termed
73 inflammation-associated fibroblasts (IAFs), with high expression of *IL11*, *IL24*,
74 *IL13RA2*, and *TNFSFR11B*⁹. Another prior study demonstrated that CD10 and GPR77
75 might be markers of CAFs that mediate chemotherapy resistance in human cancer
76 through IL-6 and IL-8 production¹⁰. Combined these findings together, stromal
77 fibroblasts play a crucial role in the development of cancer and colitis, the full picture of

78 fibroblast function and heterogeneity remains unclear.

79 Interleukin (IL)-11 is a member of the IL-6 family, and exhibits pleiotropic
80 functions, including hematopoiesis, bone development, tissue repair, and tumor
81 development^{11, 12}. The IL-11 receptor comprises IL-11R α 1, which binds IL-11, and
82 gp130, which transmits signals to the nucleus via Janus kinase (JAK) activation¹³. JAKs
83 phosphorylate STAT3, and phosphorylated STAT3 enters the nucleus where it activates
84 the transcription of various target genes associated with cell proliferation and apoptosis
85 suppression^{14, 15, 16, 17}. IL-11 production is regulated by several cytokines, including
86 TGF β , IL-1 β , IL-17A, and IL-22^{18, 19, 20, 21}. We previously demonstrated that IL-11
87 production is induced by reactive oxygen species (ROS) and the electrophile
88 1,2-naphthoquinone, which, in turn, promotes liver and intestine tissue repair^{22, 23}.
89 While IL-11 is reportedly produced in various cell types (including stromal,
90 hematopoietic, and epithelial cells) in response to different stimuli^{16, 17, 24, 25}, the cellular
91 sources of IL-11 *in vivo* are not fully understood.

92 Colorectal cancers exhibit increased IL-11 expression in human and mice.

93 Moreover, deletion of the *Il11ral* gene attenuates colitis-associated colorectal cancer

94 (CAC) development in mice treated with azoxymethane (AOM) and dextran sulfate
 95 sodium (DSS), or in adenomatous polyps of mice harboring mutation of the
 96 *Adenomatous polyposis coli (Apc)* gene (*Apc^{Min/+}*)¹⁷. On the other hand, *IL11* gene
 97 polymorphism is associated with increased ulcerative colitis (UC) susceptibility in
 98 human patients²⁶. *IL11* expression is increased in patients with mild UC, but decreased
 99 in patients with severe UC²⁷. These findings suggest that IL-11 may promote colorectal
 100 cancer development, but could also attenuate colitis under certain conditions. Moreover,
 101 recent studies report that IL-11 plays a crucial role in fibrosis development in various
 102 organs, including the lung, heart, liver, and kidney^{28, 29}.

103 In the present study, we aimed to characterize IL-11-producing cells *in vivo*. We
 104 generated *IL11-Enhanced green fluorescence protein (Egfp)* reporter mice, and detected
 105 IL-11⁺ cells in the colonic tumor tissues of a CAC mouse model and in *Apc^{Min/+}* mice.
 106 Deletion of *Il11ra* or *Il11* attenuated CAC development in mice. When tumor organoids
 107 were transplanted into the colon, IL-11⁺ cells appeared in the tumor tissues. Moreover,
 108 IL-11⁺ cells rapidly appeared in the colon of mice after DSS treatment. IL-11⁺ cells
 109 expressed stroma cell markers and genes associated with cell proliferation and tissue

110 repair, suggestive of colonic fibroblasts. IL-11 induced robust STAT3 phosphorylation
 111 in colonic fibroblasts, but not in colonic epithelial organoids. Using the human cancer
 112 database, we found that the genes enriched in IL-11⁺ fibroblasts were elevated in human
 113 colorectal cancer, and that high expression of several of these genes was correlated with
 114 reduced disease-free survival among CRC patients. Together, our present results
 115 demonstrated that tumor cells induced IL-11⁺ fibroblasts, and that a feed-forward loop
 116 between IL-11 and IL-11⁺ fibroblasts may contribute to tumor development.
 117

118 **RESULTS**

119 **Characterization of IL-11⁺ Cells in CAC Using *Il11-Egfp* Reporter Mice**

120 To characterize IL-11-producing (IL-11⁺) cells *in vivo*, we generated transgenic mice in
 121 which *Egfp* expression was under control of the *Il11* gene promoter using a bacterial
 122 artificial chromosome (BAC) vector (Supplementary Fig. 1a). *Egfp* cDNA and a polyA
 123 signal were inserted in-frame in the second exon of the *Il11* gene (Supplementary Fig.
 124 1a). As expected, *Il11* mRNA expression was correlated with *Egfp* mRNA expression in
 125 various tissues (Supplementary Fig. 1b, c). Notably, *Il11* mRNA expression was highest
 126 in the testis, and was very low in other tissues in mice under normal conditions
 127 (Supplementary Fig. 1b).

128 Although *Il11* mRNA expression is elevated in colorectal cancer among mice
 129 and human^{16, 17}, the origin of IL-11⁺ cells remains controversial. We used the *Il11-Egfp*
 130 reporter mice to monitor the appearance of IL-11⁺ cells in a CAC model. AOM
 131 administration followed by repeated exposure to DSS causes CAC development in
 132 mice^{30, 31, 32, 33}. On day 77 after AOM/DSS administration, the *Il11-Egfp* reporter mice
 133 developed large tumors in the colon (Fig. 1a, b). At this time, we isolated tumor and

134 nontumor tissues, and examined the *Il11* mRNA expression by qPCR. *Il11* and *Egfp*
135 mRNA expressions were elevated in tumor tissues compared to nontumor tissues from
136 mice with AOM/DSS-induced CAC (Fig. 1c).

137 We next isolated and characterized IL-11⁺ cells from tumors of *Il11-Egfp*
138 reporter mice. We observed increased numbers of EGFP (IL-11)⁺ cells in tumor tissues
139 compared to nontumor tissues from the colon of AOM/DSS-treated *Il11-Egfp* reporter
140 mice (Fig. 1d). While the majority of IL-11⁺ cells expressed stroma cell markers, such
141 as Thy1.2 and podoplanin, numerous IL-11⁺ cells expressed CD45.2 or EpCAM (Fig.
142 1e). Immunohistochemistry (IHC) revealed IL-11⁺ cells in stroma tissues surrounding
143 tumor cells (Fig. 1f). IL-11⁺ cells also expressed vimentin, collagen I, and collagen IV,
144 but not α SMA (Supplementary Fig. 1d), suggesting that these cells were fibroblasts but
145 not myofibroblasts. Notably, a few E-cadherin-positive tumor cells exhibited weak
146 EGFP expression (Fig. 1g). Overall, these results suggest that the IL-11⁺ cells
147 constituted heterogenous cell populations, which might explain previous apparently
148 inconsistent results showing that IL-11⁺ cells were derived from hematopoietic,
149 epithelial, or stromal cells^{17,24}.

EGFP-positive cells were not detected in the colon of *Il11-Egfp* reporter mice before AOM/DSS treatment (data not shown), possibly suggesting that small numbers of resident IL-11⁺ cells might proliferate and expand *in situ*. Alternatively, IL-11⁻ cells could be converted into IL-11⁺ cells after AOM/DSS treatment. To discriminate these two possibilities, we investigated whether IL-11⁺ cells expressed the cell-proliferating antigen Ki67. The majority of IL-11⁺ cells did not express Ki67 (Fig. 1h), supporting the possibility that IL-11⁺ cells were derived from IL-11⁻ cells during tumor development.

Attenuated CAC Development in *Il11ra1*^{-/-} and *Il11*^{-/-} Mice

A previous study reported attenuated CAC development in *Il11ra1*^{-/-} mice¹⁷. Consistently, our present data confirmed the attenuation of AOM/DSS-induced CAC in *Il11ra1*^{-/-} mice compared to wild-type mice (Supplementary Fig. 2a). To further substantiate that the IL-11/IL-11R-dependent signaling pathway contributed to CAC development, we investigated *Il11*^{-/-} mice. As we previously reported³⁴, *Il11*^{-/-} mice showed no abnormalities. When wild-type and *Il11*^{-/-} mice were treated with

166 AOM/DSS, we observed attenuated CAC development in *Il11*^{-/-} mice compared to
 167 wild-type mice (Supplementary Fig. 2b). Moreover, reciprocal BM transfer
 168 experiments revealed that *Il11* expression in non-hematopoietic cells might be
 169 primarily responsible for the increased tumor numbers and tumor load in the colon
 170 (Supplementary Fig. 2c, d), although *Il11* expression in hematopoietic cells also partly
 171 contributed the increased tumor area in the colon (Supplementary Fig. 2c, d). We
 172 focused our subsequent analyses on IL-11⁺ colonic fibroblasts, which are hereafter
 173 referred to as IL-11⁺ colon cancer-associated fibroblasts (CAFs).

174

175 IL-11⁺ CAFs Appear in Tumor Tissues in the Absence of Inflammation

176 The above-described results suggested that tumor cells may have educated the
 177 surrounding IL-11⁻ cells to become IL-11⁺ CAFs. An alternative possibility is that
 178 IL-11⁻ cells might cell-autonomously become IL-11⁺ CAFs within the setting of an
 179 inflammatory milieu triggered by DSS administration. To discriminate these
 180 possibilities, we used two different inflammation-independent murine tumor models:
 181 adenomatous polyps in *Apc*^{Min/+} mice³³ and transplantation of tumor organoids into

182 wild-type mice. In *Apc*^{Min/+} mice, *Il11* and *Egfp* mRNA expression levels were elevated
 183 in colon tumors compared to nontumor tissues (Fig. 2a), and IL-11⁺ CAFs appeared in
 184 stroma tissues surrounding tumor cells in the colon (Fig. 2b). Consistent with the results
 185 of IHC, we found increased numbers of IL-11⁺ CAFs in the tumor tissues compared to
 186 non-tumor tissues from the colon of *Apc*^{Min/+} mice (Fig. 2c). Moreover, most IL-11⁺
 187 CAFs expressed podoplanin and Thy1.2, and small numbers of IL-11⁺ cells expressed
 188 hematopoietic and epithelial cell markers (Fig. 2d). Again, we observed weak EGFP
 189 expression in some E-cadherin-positive tumor cells themselves (Fig. 2e). Notably, most
 190 IL-11⁺ CAFs were not Ki67⁺ (Fig. 2f). IL-11⁺ CAFs expressed vimentin, collagen I, and
 191 collagen IV, but not αSMA (Fig. 2g).

192 We previously generated tumor organoids from the intestines of AKTP mice,
 193 which exhibit mutations of *Apc*, *Kras*, *Tgfr2*, and *Tp53* in intestinal epithelial cells³⁵.
 194 These tumor organoids were transferred into the colon of *Il11-Egfp* reporter mice, and
 195 we examined whether IL-11⁺ CAFs appeared along with tumor development
 196 (Supplementary Fig. 3a). On day 30 post-transplantation, the tumor organoids had
 197 developed into large tumors in the colon and, importantly, IL-11⁺ CAFs appeared in the

198 tumor tissues (Figure Supplementary Fig. 3b). Moreover, these IL-11⁺ CAFs expressed
199 podoplanin and vimentin, but not CD45 or E-cadherin (Supplementary Fig. 3c).
200 Together, these results strongly support that tumor cells instructed IL-11⁻ cells to
201 become IL-11⁺ CAFs in the absence of inflammation.

202

203 **IL-11⁺ Cells Appear in the Colon of DSS-Treated Mice and Express Stromal Cell** 204 **Marker**

205 To test whether inflammation alone induced IL-11⁺ cell development, we treated
206 *Il11-Egfp* reporter mice with only DSS. *Il11* and *Egfp* expressions were very low in the
207 colon of untreated *Il11-Egfp* reporter mice, and were elevated in the colon of *Il11-Egfp*
208 reporter mice on day 7 after DSS treatment (Fig. 3a). IHC revealed that on day 6 after
209 DSS treatment, large numbers of IL-11⁺ cells appeared in the subepithelial tissues,
210 where intestinal epithelial cells were detached due to severe inflammation (Fig. 3b).
211 Moreover, we detected the rapid appearance of IL-11⁺ cells just one day after DSS
212 treatment (Fig. 3c). For a more detailed comparison of the phenotypes of IL-11⁺ CAFs
213 in tumor tissues and the IL-11⁺ cells that appeared in colitis, we used flow cytometry to

214 analyze the expressions of various cell surface markers. After DSS treatment, we
215 observed increased numbers of IL-11⁺ cells in the colon (Fig. 3d). These IL-11⁺ cells
216 expressed stroma cell markers, including Thy1.2, podoplanin, CD29, PDGFR- α ,
217 ICAM-1, VCAM-1, and Sca-1, but not CD45.2, EpCAM, CD31, or Ter119 (Fig. 3e).
218 IL-11⁺ cells were also positive for vimentin, collagen I, and collagen IV, but not α SMA
219 (Fig. 3f), suggesting that these cells were fibroblasts, but not myofibroblasts. Moreover,
220 these IL-11⁺ cells did not incorporate 5-bromo-2'-deoxyuridine (BrdU), a hallmark of
221 cell proliferation, suggesting that they did not proliferate *in situ* (Fig. 3g). Together,
222 these findings suggest that inflammation alone was sufficient to induce IL-11⁺ cell
223 development.

224

225 **IL-11⁺ Cells Express Genes Associated with Cell Proliferation and Tissue Repair**

226 To further characterize the IL-11⁺ cells that appeared in colitis, we used a cell sorter to
227 isolate IL-11⁺ cells as EGFP⁺ cells from the colon of DSS-treated *Il11-Egfp* reporter
228 mice. These sorted EGFP⁺ cells were subjected to transcriptome analysis, and their gene
229 expression profiles were compared with those of EGFP⁻ cells. Heat-map analysis

230 revealed different gene expression patterns in these two populations (Fig. 4a). A volcano
 231 plot showed significantly elevated expressions of several genes, including *Il11*, *Mmp3*,
 232 and *Timp1*, in IL-11⁺ cells compared to IL-11⁻ cells (Fig. 4b). Gene Ontology (GO)
 233 enrichment analysis revealed that EGFP⁺ cells exhibited enriched expressions of genes
 234 associated with cell proliferation, organ morphogenesis, angiogenesis, and wound
 235 healing (Fig. 4c). qPCR confirmed that IL-11⁺ cells showed elevated levels of cytokines
 236 (*Il6* and *Il11*), chemokines (*Ccl2* and *Ccl11*), and genes associated with organ
 237 development (*Hgf* and *Tnfsf11*) (Fig. 4d). We also detected elevated expression levels of
 238 genes associated with colorectal cancer susceptibility loci (e.g., *Grem*, *Bmp2*, and
 239 *Bmp4*) and tumor development and invasion (e.g., *Wnt5a*, *Ereg*, *Mmp3*, *Mmp13*, and
 240 *Timp1*) (Figure 4D).

241 Of note, two unique subsets of fibroblasts termed inflammation-associated
 242 fibroblasts (IAFs)⁹ and cancer-associated fibroblasts (CAFs) with mediating
 243 chemotherapy resistance¹⁰ have been recently reported in ulcerative colitis (UC)
 244 patients and chemotherapy-resistant cancer patients, respectively. To investigate the
 245 relationship between IL-11⁺ fibroblasts and IAFs or CAFs with mediating

246 chemotherapy resistance, we examined their hallmark gene expressions. We found
247 elevated expressions of *Il13ra2* and *Tnfrsf11b* (markers of IAFs), but not *Cd10* or
248 *Gpr77* (markers of CAFs with mediating chemotherapy resistance) in IL-11⁺ cells
249 compared to IL-11⁻ cells (Fig. 4e). These findings indicated that the IL-11⁺ cells
250 appearing in the colon of DSS-treated mice were phenotypically similar to the IAFs
251 observed in UC patients, but not CD10⁺GPR77⁺ CAFs. Thus, these cells were referred
252 to as IL-11⁺ IAFs.

253 We next examined whether the gene expression profiles were similar between
254 IL-11⁺ IAFs and IL-11⁺ CAFs. The genes enriched in IL-11⁺ IAFs, including *Ccl2*,
255 *Osmr*, *Wnt5a*, *Ereg*, *Mmp13*, *Timp1*, and *Tnfrsf11b*, were also elevated in
256 AOM/DSS-induced tumor tissues (Supplementary Fig. 4). Although we did not examine
257 the gene expression profiles of IL-11⁺ CAFs from tumor tissues, our findings suggest
258 that IL-11⁺ IAFs may be phenotypically similar to IL-11⁺ CAFs.

259

260 **The MEK/ERK Pathway is Involved in *Il11* Upregulation in Tumor Tissues**

261 Since IL-11⁺ CAFs might promote tumor development, it is crucial to investigate the

mechanisms by which inflammation or tumor cells induce IL-11 expression. We previously reported that oxidative stress induces *Il11* mRNA expression in an ERK/Fra-1-dependent manner^{22, 23}. Thus, we tested whether DSS treatment induced oxidative stress in the colon. We observed enhanced oxidative stress in colonic cells after DSS treatment, and found that an antioxidant, N-acetyl cysteine (NAC) administration attenuated this DSS-induced oxidative stress (Fig. 5a). Additionally, DSS-induced oxidative stress was ameliorated by administration of antibiotics (Abx) (Fig. 5b), suggesting that bacterial infection induces oxidative stress. Consistent with these findings, both an antioxidant, NAC and Abx blocked the DSS-induced upregulation of *Il11* mRNA (Fig. 5c, d). Furthermore, DSS induced ERK phosphorylation, which was blocked by administration of the MEK inhibitor trametinib or NAC, accompanied by downregulation of *Il11* expression (Fig. 5e–g). These results indicated that oxidative stress-dependent ERK activation might contribute to *Il11* expression in the colon. Although several studies report that TGF β induces IL-11 production in various cells^{20, 21}, we found that neutralization of TGF β signals using anti-TGF β antibody did not block *Il11* expression in the colon after DSS treatment (Fig.

278 5h, i).

279 We next investigated whether *Il11* expression was induced in the tumor
280 microenvironment in a manner similar to those in DSS-induced colitis. Administration
281 of trametinib, but not Abx, NAC, or neutralizing antibody against TGF β , decreased *Il11*
282 expression in CAC after AOM/DSS treatment (Fig. 5j–o). Moreover, we observed a
283 similar inhibitory effect of Trametinib, but not Abx, NAC, or neutralizing antibody
284 against TGF β in colon tumors in *Apc^{min/+}* mice (Supplementary Fig. 5a–f). Together,
285 these results suggested that *Il11* expression was induced in a MEK/ERK-dependent
286 manner, although the upstream signals that induce activation of the MEK/ERK pathway
287 could differ between cases of inflammation versus tumors. Of note, induction of *Il11* in
288 the colon might be independent of TGF β in the tumor microenvironment at least under
289 our experimental conditions.

290

291 **IL-11 Preferentially Induces Signals to Fibroblasts**

292 Previous studies report that IL-11 induces signals in colonic epithelial cells and
293 fibroblasts^{17, 29}. To determine which cells were the primary targets of IL-11, we

294 examined the *Il1ral* expression on colonic fibroblasts and normal colonic epithelial
 295 organoids. Since IL-22 induces signals in intestinal epithelial cells³⁶, we also examined
 296 the IL-22 receptor expression on both cell types. Colonic epithelial organoids showed
 297 high expression of *Il22r*, but not *Il1ral*, while colonic fibroblasts exhibited high
 298 expression of *Il1ral*, but not *Il22r* (Fig. 6a). Consistently, robust STAT3
 299 phosphorylation in colonic fibroblasts was induced by IL-11, but not IL-22 (Fig. 6b). To
 300 further characterize IL-11-inducible genes *in vivo*, we injected IL-11R agonist into
 301 wild-type mice²², which induced upregulation of many genes in the colon (Fig. 6c–e),
 302 some of which were enriched in IL-11⁺ IAFs (Fig. 6e). We performed qPCR to verify
 303 the induction of these genes (including *Hgf*, *Osmr*, *Rspo3*, *Mmp3*, *Timp1*, and *Pdpr*) in
 304 the colon after injection of IL-11R agonist (Supplementary Fig. 6). It is likely that IL-11
 305 released from colonic fibroblasts might activate IL-11⁺ IAFs or CAFs in an autocrine or
 306 paracrine manner. Overall, our findings suggested that expression of IL-11-stimulated
 307 genes in IL-11⁺ CAFs might further modulate the tumor microenvironment.

308

309 **Genes Enriched in IL-11⁺ IAFs Show Elevated Expression in Human Colorectal**

310 **Cancer**

311 Assuming that the appearance of IL-11⁺ CAFs was correlated with CAC development in
312 mice (Fig. 1, 2), we hypothesized that the genes enriched in IL-11⁺ IAFs might also be
313 elevated in human colorectal cancer. We focused on genes with over two-fold greater
314 expression in IL-11⁺ IAFs compared to IL-11⁻ cells (Fig. 4), and extracted 17 genes
315 matching our criteria from the human cancer database (GSE33133). Intriguingly,
316 subsets of genes elevated in IL-11⁺ IAFs, including *HGF*, *TNFSF11*, *MMP3*, *MMP13*,
317 and *TIMP1*, were significantly upregulated in colon cancer tissues compared to normal
318 mucosa (Supplementary Fig. 7a).

319 Since many of the genes enriched in IL-11⁺ IAFs were also upregulated in tumor
320 tissues (Fig. 4d, e, Supplementary Fig. 7a), we hypothesized that the genes elevated in
321 IL-11⁺ IAFs, along with *IL11*, might critically affect the prognosis of cancer patients.
322 Thus, we compared disease-free survival of colorectal cancer patients according to the
323 expression levels of genes enriched in IL-11⁺ IAFs. We found that reduced disease-free
324 survival time was associated with higher expression of *IL6*, *MMP13*, and *TIMP1*, but
325 not of *IL6/IL6R*, *IL11*, or *IL11/IL11R* (Supplementary Fig. 7b). Notably, higher

326 expression of *IL6/IL6R/IL11/IL11R/GP130* was associated with significantly diminished
327 disease-free survival time. These results suggested that prognosis of human colorectal
328 cancer patients may be critically determined by IL-11⁺ IAFs and, possibly, by IL-11⁺
329 CAFs, but not by IL-11.

330

331 **IL-11 Expression is Correlated with Progression of Human Cancers**

332 To characterize IL-11⁺ cells in human colon tumor samples, we performed staining with
333 anti-human IL-11 antibody. First, we verified that anti-human IL-11 antibody detected
334 endogenous IL-11, using lysates of the human breast cancer cell line MDA-MB-231, in
335 the absence or presence of siRNA against human *IL11* (Fig. 7a). IL-11⁺ cells were
336 scarcely detected in normal colonic tissues, but were numerous in adenomas and in
337 early and advanced colorectal cancer tissues (Fig. 7b). Intriguingly, the relative area of
338 IL-11⁺ cells and the IL-11 signaling intensity were increased in advanced colon cancers
339 compared to normal tissues (Fig. 7c, d). Moreover, positive staining for IL-11 was
340 observed in vimentin-positive stromal cells, E-cadherin-positive tumor cells, and
341 CD45-positive hematopoietic cells (Fig. 7e). Thus, IL-11⁺ cells comprised

342 heterogeneous cell populations in the tumor microenvironment, although the majority
343 were fibroblasts.

344 **DISCUSSION**

345 In the present study, we generated *Il1l-Egfp* reporter mice to characterize IL-11⁺ CAFs
346 or IL-11⁺ IAFs in different murine tumor and colitis models. We found that IL-11⁺ cells
347 in tumor tissues constituted heterogeneous cell populations, including stromal, epithelial,
348 and hematopoietic cells, and that *Il1lra*^{-/-} or *Il1l*^{-/-} deletion attenuated CAC
349 development in mice. Reciprocal BM transfer experiments revealed that stromal cells
350 critically contributed to tumor progression; thus, we focused on IL-11⁺ CAFs. IL-11
351 activated IL-11⁺ colonic fibroblasts. Transcriptome analysis showed that IL-11⁺ IAFs
352 expressed genes associated with tissue repair and cell proliferation. Moreover, some of
353 the genes enriched in IL-11⁺ IAFs were elevated in human colorectal cancer tissues, and
354 their high expression was associated with poor prognosis of human colorectal cancer.
355 Thus, our results suggest that a feed-forward loop between IL-11 and IL-11⁺ CAFs may
356 contribute to tumor development.

357 The newly developed *Il1l-Egfp* reporter mice enabled us to characterize the
358 IL-11⁺ IAFs and CAFs that appeared in mouse models of colitis and CAC, respectively.
359 In the colitis model, IL-11⁺ IAFs exclusively expressed stromal cell markers, such as

360 Thyl and podoplanin. A recent cRNA-seq analysis of colon biopsies from healthy
361 individual and UC patients revealed that colon samples from UC patients exhibited a
362 unique subset of fibroblasts, termed IAFs, that express *IL11*, *IL24*, *IL13RA2*, and
363 *TNFSFR11B*⁹. Here we found elevated expression of these genes in IL-11⁺ IAFs
364 compared to IL-11⁻ cells. Thus, IL-11 is a hallmark of IAFs. Since IL-11⁺ IAFs
365 expressed genes associated with cell proliferation and tissue repair, IL-11⁺ IAFs might
366 contribute to colitis attenuation in human UC patients.

367 In contrast to in DSS-induced colitis, in tumor tissues, we detected numerous
368 IL-11⁺ cells expressing epithelial or hematopoietic cell markers, including EpCAM or
369 CD45.2. Consistently, previous studies report that IL-11⁺ cells are derived from stromal,
370 epithelial, or hematopoietic cells^{17, 24}. Our present results revealed that IL-11⁺, EpCAM⁺,
371 and E-cadherin⁺ epithelial cells might be tumor cells themselves, whereas we did not
372 fully investigate the nature and origin of CD45.2⁺ cells. Future scRNA-seq analysis of
373 IL-11⁺ cells will further elucidate the origin and characterization of heterogenous
374 populations of IL-11⁺ cells in the tumor microenvironment.

375 Tumors cells may support tumor growth by educating the surrounding

376 fibroblasts, which are referred to as CAFs^{8, 37, 38}. On the other hand, a recent study
377 reported that surface expression of CD10 and GPR77 may be markers of CAFs that
378 mediate chemotherapy resistance in human cancer through IL-6 and IL-8 production¹⁰.
379 CD10 and GPR77 expression were not elevated in IL-11⁺ IAFs or CAC in mice,
380 suggesting that IL-11⁺ IAFs and possibly IL-11⁺ CAFs might be different from human
381 CD10⁺GPR77⁺ CAFs.

382 Elucidation of the mechanisms underlying IL-11 production by stromal cells in
383 the tumor microenvironment may be crucial for understanding how tumor cells instruct
384 stromal cells. Previous studies demonstrate that TGFβ induces IL-11 upregulation in
385 various cell types^{20, 21}. Indeed, we found that TGFβ stimulation induced IL-11
386 production by colonic fibroblasts. Moreover, in murine xenograft models, human tumor
387 cell lines that ectopically express human TGFβ1 can elicit IL-11⁺ CAFs²⁴. However, in
388 our present study, anti-TGFβ antibody treatment did not downregulate *Il11* expression
389 in colonic adenomas of *Apc*^{min/+} mice, or in AOM/DSS-induced CAC. Furthermore,
390 culture supernatants of tumor organoids did not induce upregulated *Il11* expression by
391 colonic fibroblasts in the absence or presence of anti-TGFβ antibody. Overall, although

392 TGF β *per se* was able to induce IL-11 production, TGF β may not be involved in the
393 induction of IL-11⁺ CAFs in the tumor microenvironment, at least under our
394 experimental conditions. On the other hand, blockade of the ERK pathway attenuated
395 *Illi* expression in the colon of mice after DSS treatment, in colon adenomas of *Apc*^{min/+}
396 mice, and in AOM/DSS-induced CAC in mice. Thus, the signaling pathway(s) leading
397 to MEK/ERK activation might be critically involved in IL-11 production. Further
398 investigations are needed to address this subject.

399 Previous studies show that IL-11 and IL-22 induce proliferation and cell survival
400 of colonic epithelial cells through STAT3 activation^{15, 36}. Indeed, under our
401 experimental conditions in colonic epithelial organoids, IL-22 induced strong STAT3
402 phosphorylation, whereas IL-11 induced only weak STAT3 phosphorylation. In sharp
403 contrast, IL-11 induced robust phosphorylation of STAT3 in colonic fibroblasts. Thus, it
404 appears that colonic fibroblasts produced IL-11 in response to factors from tumor cells,
405 which, in turn, activated and induced STAT3 phosphorylation in colonic fibroblasts in
406 an autocrine or paracrine manner. This feed-forward loop between IL-11 and IL-11⁺
407 CAFs might contribute to tumor development.

408 CAC development was attenuated in *Il11ra1*^{-/-} and *Il11*^{-/-} mice treated with
409 AOM/DSS, but deletion of these genes did not dramatically affect tumor development
410 compared to in a previous study¹⁷. The reason for these discrepancies is currently
411 unknown. However, it is possible that the genetic background of the mice (C57/BL6 vs.
412 129/C57/BL6 mixed background) might affect the tumor cells' dependence on IL-11
413 signaling. On the other hand, our transcriptome analysis revealed that IL-11⁺ CAFs
414 expressed various genes associated with tissue repair and cell proliferation. Indeed, we
415 found that high expression of the genes enriched in IL-11⁺ IAFs (including *IL6*, *MMP3*,
416 and *TIMP1*, but not *IL11* itself) was associated with reduced disease-free survival of
417 CRC patients. Thus, it would be interesting to investigate whether depletion of IL-11⁺
418 CAFs might have profound effects on colorectal cancer progression.
419

420 **METHODS**

421 **Reagents**

422 Recombinant mouse IL-11 (R&D), IL-22 (Biolegend), mouse TGFβ1 (Biolegend),
 423 N-acetyl cysteine (NAC) (Nakalai), and Trametinib (LC Laboratories) were purchased
 424 from the indicated sources. The following antibodies used in this study were obtained
 425 from the indicated sources: anti-phospho-ERK (4370, CST), anti-Ki67 (ab16667,
 426 Abcam), anti-GFP (GFP-Go-Af1480 or GFP-Rb-Af2020, Frontier Institute), anti-BrdU
 427 (BU1/75, BIO-RAD), anti-IL-11 (LS-C408373, LSBio), anti-CD45 (13917, CST),
 428 anti-CD45 (IR751, Dako), anti-podoplanin (127403, BioLegend), anti-α-SMA (ab5694,
 429 Abcam), anti-collagen I (ab34710, Abcam), anti-collagen IV (ab6586, Abcam),
 430 anti-E-cadherin (560062, BD Biosciences), anti-E-cadherin (NCH-38, Dako),
 431 anti-vimentin (9856, CST), anti-phospho-STAT3 (9145, CST), anti-STAT3 (SC-482,
 432 Santa Cruz), anti-β-Actin (622102, Biolegend), and anti-tubulin (T5168,
 433 Sigma-Aldrich). Anti-horseradish peroxidase (HRP) -conjugated anti-rabbit IgG
 434 (NA934), HRP-conjugated anti-rat IgG (NA935), and HRP-conjugated anti-mouse IgG
 435 (NA931) antibodies were from GE Healthcare. Alexa Fluor 488-conjugated donkey
 436 anti-rabbit IgG (A21206), Alexa Fluor 594-conjugated donkey anti-rabbit IgG

437 (A21207), Alexa Fluor Plus 594-conjugated donkey anti-rabbit IgG (A32754), Alexa
438 Fluor 647-conjugated donkey anti-rabbit IgG (A31573), Alexa Fluor 594-conjugated
439 donkey anti-mouse IgG (A21203), and Alexa Fluor 488-conjugated donkey anti-goat
440 IgG (A11055) antibodies, and Alexa Fluor 594-conjugated streptavidin (S11227) were
441 purchased from Invitrogen.

442 Unless otherwise indicated, the following antibodies used for flow cytometry
443 were obtained from TONBO Biosciences; anti-CD11b (20-0112, clone M1/70),
444 anti-CD16/CD32-mAb (2.4G2) (made in house), anti-CD24a (Biolegend, 101813, clone
445 M1/69), anti-CD31 (eBioscience, 17-0311-82, clone 390), anti-CD34 (eBioscience,
446 13-0341-81, clone RAM34), anti-CD45.1 (35-0453, clone A20), anti-CD45.2 (20-0454,
447 clone 104), anti-EpCAM (BioLegend, 118214, clone G8.8), anti-Thy1.2 (20-0903,
448 clone 30-H12), anti-podoplanin (BioLegend, 127414, clone 8.1.1), anti-TER-119
449 (BioLegend, 116212, clone TER-119), anti-MHC Class II (Miltenyi Biotec,
450 130-102-139, clone M5/114.15.2), anti-ICAM-1 (BD Biosciences, 561605, clone 3E2),
451 anti-VCAM-1 (BioLegend, 105718, clone 429), anti-Sca-1 (BioLegend, 122512, clone

452 E13161.7), anti-Lyve1 (eBioscience, 50-0443-80, clone ALY7), anti-PDGFR α
453 (eBioscience, 17-1401-81, clone APA5), and Streptavidin APC (eBioscience).

454 The hybridoma cell line (1D11)³⁹ that produces neutralizing antibody against
455 all TGF β isoforms (β 1, β 2, and β 3) was purchased from ATCC, and anti-TGF β
456 antibody was produced in house. Control mouse IgG was purchased from
457 Sigma-Aldrich (I5381).

458

459 **Mice**

460 *Il11*^{-/-} mice (generated in our lab) and *Il11ral*^{-/-} mice (provided by L. Robb) were
461 previously described^{34 40}. *Apc*^{min/+} (002020) were purchased from Jackson Lab.

462 C57BL/6 (CD45.2⁺) and C57BL/6-SJL (CD45.1⁺) mice were purchased from
463 Japan-SLC.

464 Mice in different cages or derived from different sources were cohoused for 2
465 weeks for normalization of the microbiota composition before experiments. All animals
466 were housed and maintained under specific pathogen-free conditions in the animal
467 facility at Juntendo University School of Medicine or Toho University School of

468 Medicine. All experiments were performed according to the guidelines approved by the
469 Institutional Animal Experiment Committee of Juntendo University School of Medicine
470 or Toho University School of Medicine (19-51-414, 19-51-411).

471

472 **Generation of *Il11-Egfp* reporter mice**

473 The *Egfp* reporter gene was introduced into the BAC clone (RP23-285B12) by two-step
474 Red/ET recombineering technology according to the manufacturer's protocol (Gene
475 Bridges). In the first step, a *rpsL-neo* cassette included in the kit was amplified by PCR
476 using a primer set (*Il11*-ET1-F2:
477 ACTCCCTCAGACCCAGAGTTTGGCCTGATTCTCCCTTCTGTCCACAGGTGG
478 CCTGGTGATGATGGCGGGATCG and *Il11*-ET1-R2:
479 ACGACTCTATCTGGCCAGAGGCTCAGCACACCAGGACCAGGCGACAAACT
480 CAGAAGAACTCGTCAAGAAGGCG) and inserted into the target region of the BAC
481 clone. In a second step, the *rpsL-neo* cassette in the modified BAC clone was replaced
482 with *Egfp-polyA* cassette which was amplified from *Egfp*-expression vector (pAWZ)
483 using a primer set (*Il11*-ET2-F2:

484 ACTCCCTCAGACCCAGAGTTTGGCCTGATTCTCCCTTCTGTCCACAGGTATG

485 GTGAGCAAGGGCGAG and *Il11-ET2-R2*:

486 ACGACTCTATCTGGCCAGAGGCTCAGCACACCAGGACCAGGCGACAAACct

487 CTAGTGGATCATTAACGCTTAC). A resultant clone designated as *Il11-Egfp* was

488 verified by restriction digestion of BAC DNA and by sequencing.

489 Intracytoplasmic sperm injection (ICSI) was performed as previously

490 described with slightly modifications⁴¹. The mixture of sperm and *Il11-Egfp* DNA was

491 diluted with Hepes-modified CZB containing 12% polyvinylpyrrolidone

492 (Sigma-Aldrich) before being used for ICSI. Injections were performed by

493 micromanipulators (Leica) with a PMM-150 FU piezo-impact drive unit (Prime Tech)

494 using a blunt-ended mercury-containing injection pipette. After discarding the midpiece

495 and tail, the head of spermatozoa was injected into an oocyte from C57/BL6 mice.

496 Oocytes matured into two-cell stage embryos at 24 hours after injection, and then

497 two-cell stage embryos were transferred to oviducts of pseudopregnant females.

498 Transgenic founders were backcrossed to C57/BL6J mice for several generations.

499 Among them, one line exhibited an intimate correlation of *Il11* and *Egfp* expressions

500 were selected and used for further experiments.

501

502 **Induction of DSS-induced colitis and colitis-associated cancer (CAC) in mice**

503 Nine- to fifteen-week-old wild-type or *Il11-Egfp* reporter mice received 1.5 % DSS

504 (MW: 36,000-50,000 D; MP Biomedicals) *ad libitum* in drinking water for 5 days,

505 which then was changed to regular water. To reduce gut commensal microflora, mice

506 received mixtures of antibiotics in drinking water containing ampicillin (1 g/L,

507 Sigma-Aldrich), kanamycin (0.4 g/L, Sigma-Aldrich), gentamicin (0.035 g/L,

508 Sigma-Aldrich), metronidazole (0.215 g/L, Sigma-Aldrich), vancomycin (0.18 g/L,

509 Sigma-Aldrich), and colistin (0.042 g/L, Sigma-Aldrich). Administration of antibiotics

510 into mice started at 4 weeks before DSS treatment and continued during DSS treatment.

511 To attenuate oxidative stress in DSS-treated mice, mice received NAC (10 g/L) along

512 with DSS in drinking water for 5 days. To neutralize TGF β in DSS-treated mice, mice

513 were intraperitoneally injected with anti-TGF β antibody (1D11) or control mouse IgGs

514 (5 mg/kg) on day 2 and day 4 after DSS treatment. To inhibit ERK activation in

515 DSS-treated mice, a MEK inhibitor, Trametinib (2 mg/kg) was administered into mice

516 by gavage at 30 and 6 hours before the start of DSS treatment at a fine suspension in
517 0.5% Hydroxypropyl Cellulose (Alfa Aesar) and 0.2 % Tween-80.

518 To induced CAC, 9- to 15-week-old mice were intraperitoneally injected with
519 10 mg/kg AOM (Sigma-Aldrich). One week later, mice received 1.5 % DSS *ad libitum*
520 in drinking water for 5 days, followed by 2 weeks of regular water, and this was
521 repeated for two additional cycles. To reduce numbers of commensal bacteria and
522 oxidative stress in AOM/DSS-treated mice or *Apc^{min/+}* mice, we administered mixtures
523 of antibiotics and NAC into the indicated mice for the last 8 weeks and 4 weeks just
524 before sacrifice, respectively. Trametinib (2mg/kg) (−6 and −30 hours) or anti-TGFβ
525 antibody (5 mg/kg) (on day −1, −3, −5) were administered into AOM/DSS-treated mice
526 or *Apc^{min/+}* mice at the indicated days just before sacrifice.

527

528 **Flow cytometry**

529 To isolate IL-11⁺ cells, the colon was removed from DSS-treated *Il11-Egfp* reporter
530 mice. Then, the colon was minced with scissors and digested in RPMI 1640 containing
531 100 U/mL Penicillin and 100 µg/mL Streptomycin, 1mg/mL Collagenase (Wako), 50

532 $\mu\text{g/mL}$ DNase (Roche), 0.5 mg/mL Dispase (Roche), and 2% (v/v) fetal bovine serum
533 (FBS, Gibco) for 60 min. Single cell suspensions were prepared, and cells were stained
534 with the indicated antibodies and analyzed by LSRFortessa X-20 (BD Biosciences) or
535 BD Verse (BD Biosciences). Data were processed by FlowJo software (FlowJo). To
536 further characterize IL-11⁺ cells, EGFP⁺ cells were sorted by MoFlo Astrios cell sorter
537 (Beckman Coulter) and subjected to microarray analysis.

538 To isolate IL-11⁺ cells from tumors in the colon of *Il11-Egfp* reporter mice
539 treated with AOM/DSS or *Apc^{min/+};Il11-Egfp* mice, tumor tissues were removed from
540 non-tumor tissues. Then, single cell suspension from tumor tissues was prepared as
541 described above. Cells were stained with the indicated antibodies and expression of
542 various cell surface markers on GFP⁺ cells were analyzed by flow cytometry.

543

544 **Generation of bone marrow chimeras**

545 After BM cells were prepared from *Il11^{+/+}* or *Il11^{-/-}* mice (CD45.2), 3-5 x 10⁶ BM cells
546 were transferred to 8-week-old recipient mice [C57BL/6-SJL mice (CD45.1)] that had
547 been exposed to lethal irradiation (9.0 Gray). In reciprocal BM transfer experiments,

548 BM cells from wild-type C57BL/6-SJL mice (CD45.1) were transferred to *Il1l^{+/+}* or
 549 *Il1l^{-/-}* mice (CD45.2) that had been exposed to lethal irradiation (9.0 Gray). At 2 to 3
 550 months after transfer, peripheral blood mononuclear cells were collected and stained
 551 with FITC-conjugated anti-CD45.1 and allophycocyanin (APC)-conjugated
 552 anti-CD45.2 antibodies. The chimerism of bone marrow cells was calculated by
 553 counting numbers of CD45.1⁺ and CD45.2⁺ by flow cytometry. Average chimerisms
 554 were more than 90 %.

555

556 Quantitative PCR (qPCR) Assays

557 Total RNAs were extracted from the indicated tissues of mice by using TRI Reagent
 558 (Molecular Research Center) or Sepasol II Super (Nacalai Tesque), and cDNAs were
 559 synthesized with the RevertraAce qPCR RT Kit (Toyobo). To remove residual DSS,
 560 mRNAs prepared from the colon of mice treated with DSS were further purified by
 561 LiCl precipitation as described previously ⁴². Quantitative polymerase chain reaction
 562 (qPCR) analysis was performed with the 7500 Real-Time PCR detection system with
 563 CYBR green method of the target genes together with murine *Hprt* an internal control

564 with 7500 SDS software (Thermo Fisher Scientific). The primers used in this study are
565 shown in Table S1.

566

567 **Microarray analysis**

568 We compared gene expression profiles of RNAs from EGFP-positive and negative cells
569 from the colon of *Il11-Egfp* reporter mice. EGFP⁺ and EGFP⁻ cells were sorted from the
570 colon of *Il11-Egfp* mice (n = 3 mice) on day 7 after DSS administration, total RNAs
571 were extracted using TRI-reagent according to the manufacturer's instructions
572 (Molecular Research Center). Purified RNAs were subjected to a LiCl purification to
573 remove residual DSS. cDNA was synthesized with the Ambion WT Expression Kit
574 (Affymetrix) and labeled with GeneChip WT Terminal Labeling Kit (Affymetrix)
575 according to the manufacturer's protocol. Affymetrix GeneChip Hybridization wash
576 stain kit was used to hybridize samples to GeneChip Mouse Gene ST 1.0 ST Array
577 (Affymetrix). Signals were scanned with the Genechip Scanner 3000 7G, and obtained
578 data were analyzed with Affymetrix Expression Console software (Affymetrix). The
579 gene expression was analyzed using GeneSpring (Silicon Genetics). Functional

580 enrichment analysis of differentially expressed genes from the data of microarray
581 analysis was performed using DAVID Bioinformatics Resources^{43, 44}. Data were
582 deposited in NCBI as a GSE140411.

583 To identify target genes by IL-11 in the colon, we intravenously injected 10
584 µg of IL-11R agonist into wild-type mice (n=2-3 mice). Generation, purification, and
585 characterization of IL-11R agonist were described previously²². Mice were sacrificed at
586 3 hours after injection. Total RNAs were extracted from the colon of mice by using
587 Sepasol II Super. Labeled cRNA were prepared from total RNA using the Agilent's
588 Quick Amp Labeling Kit (Agilent). Following fragmentation, cRNA were hybridized to
589 SurePrint G3 Mouse Gene Expression 8x60K (Agilent) according to the manufacturer's
590 instruction. Raw data were extracted using the software provided by Agilent Feature
591 Extraction Software (v11.0.1.1). The raw data for same gene was then summarized
592 automatically in Agilent feature extraction protocol to generate raw data text file,
593 providing expression data for each gene probed on the array. Array probes that have
594 Flag A in samples were filtered out. Selected processed signal value was transformed by
595 logarithm and normalized by quantile method. Statistical significance of the expression

596 data was determined using fold change and LPE test in which the null hypothesis was
597 that no difference exists among 2 groups. Hierarchical cluster analysis was performed
598 using complete linkage and Euclidean distance as a measure of similarity.
599 Gene-Enrichment and Functional Annotation analysis for significant probe list was
600 performed using Gene Ontology (<http://geneontology.org>). All data analysis and
601 visualization of differentially expressed genes was conducted using R 3.0.2
602 (www.r-project.org). Data were deposited in NCBI as a GEO accession number
603 GSE141643.

604

605 Immunohistochemistry (IHC)

606 Tissues were fixed in 10% formalin and embedded in paraffin blocks.
607 Paraffin-embedded colonic sections were used for H&E staining, immunohistochemical,
608 and immunofluorescence analyses. For immunohistochemistry, paraffin-embedded
609 sections were treated with Instant Citrate Buffer Solution (RM-102C, LSI Medicine) or
610 Target Retrieval Solution (S1699, Dako) as appropriate to retrieve antigen. Then tissue
611 sections were stained with the indicated antibodies, followed by visualization of

612 Alexa-conjugated secondary antibodies, or biotin-conjugated secondary antibodies
613 followed by Streptavidin-HRP (Vector Laboratories).

614 In human tumor samples, tissue sections were preincubated with MaxBlock™
615 Autofluorescence Reducing Kit (MaxVision Biosciences) according to the
616 manufacturer's instructions. After blocking, tissue sections were stained with the
617 indicated antibodies as described above.

618 Pictures were obtained with an all-in-one microscope (BZ-X700, Keyence) or
619 BX-63 (Olympus) and analyzed with BZ-X Analyzer (Keyence) or cellSens (Olympus)
620 software. Confocal microscopy was performed on an LSM 880 (Zeiss) or A1R (Nikon).
621 Images were processed and analyzed using the ZEN software (Zeiss) or the
622 NIS-Elements (Nikon).

623

624 **Histological scoring of severity of colitis**

625 Severity of colitis was evaluated by the following criteria, including infiltration of
626 inflammatory cells and the degree of tissue damage as previously described ⁴⁵.

627 Infiltration of inflammatory cells; 0, the presence of rare inflammatory cells in the

628 colonic lamina propria; 1, increased numbers of inflammatory cells; 2, confluence of
629 inflammatory cells; 3, extending of inflammatory cells into the submucosa and
630 transmural extension of the inflammatory cell infiltrate. The degree of tissue damage; 0,
631 absence of mucosal damage; 1, discrete focal lymphoepithelial lesions; 2, mucosal
632 erosion and ulceration; 3, extensive mucosal damage and extension through deeper
633 structure of the intestinal tract wall. Total severity scores ranging from 0 to 6 represent
634 that 0 and 6 indicate no changes and extensive cell infiltration with tissue damage,
635 respectively.

636

637 **Organoid Culture and transplantation of tumor organoids**

638 Mouse colon organoids were established from isolated crypts of wild-type mice as
639 previously described with slight modification⁴⁶. Obtained colonic organoids were
640 cultured in Advanced DMEM/F12 (Thermo Fisher Scientific) containing 10 mM
641 HEPES (Thermo Fisher Scientific), 1 x GlutaMAX (Thermo Fisher Scientific), 1 x B27
642 (Thermo Fisher Scientific), N-acetyl cysteine (1 μ M), murine epidermal growth factor
643 (50 ng/mL, Peprotech), murine Noggin (100 ng/mL, Peprotech), 0.5 mM A83-01

644 (Tocris), 3 μ M SB202190 (Sigma), 1 μ M nicotinamide (Sigma), Afamin and
645 Wnt3A-condition medium (CM) (final concentration of 50%) which was provided by J.
646 Takagi⁴⁷ and R-spondin 1-CM (final concentration of 10%) (Trevigen).

647 The small intestinal tumor organoids from
648 *villin-CreER-Apc^{min/+}-Kras^{+LSL-G12D}-Tgfb β 2^{fllox/fllox}-Tp53^{+LSL-R270H}* (AKTP) mice were
649 described previously³⁵. AKTP organoids were cultured in Advanced DMEM/F12
650 containing 10 mM HEPES, 1 x GlutaMAX, 1 x B27, N-acetyl cysteine (1 μ M), murine
651 epidermal growth factor (50 ng/mL), murine Noggin (100 ng/mL).

652 For transplantation, AKTP organoids were mechanically dissociated, and 3 x
653 10⁵ organoid cells mixed in Matrigel were injected into the subepithelial tissues of the
654 rectum of *Il11-Egfp* reporter mice. At five weeks after transplantation, tumors were
655 removed and subjected to histological analysis.

656

657 Isolation and stimulation of colonic fibroblasts

658 To isolate colonic fibroblasts, single cell suspensions were prepared as described above
659 and cultured in DMEM containing 10% FBS, 1% GlutaMAX (Thermo Fisher

Scientific), 10 mM HEPES, 1% MEM Non-Essential Amino Acids Solution (100x) (Nacalai Tesque), 100 U/mL Penicillin, 100 µg/mL Streptomycin, and 2.5 µg/mL of Amphotericin B (Sigma). Culture medium was changed every day until colonic fibroblasts started to grow spontaneously.

To detect phosphorylation of STAT3 in colonic fibroblasts, colonic fibroblasts were stimulated with the indicated concentrations of IL-11 or IL-22 for 30 min. Total and phosphorylated STAT3 were analyzed by Western blotting.

Western Blotting

Cells were lysed in a RIPA buffer (50mM Tris-HCl, pH 8.0, 150mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, 25 mM β-glycerophosphate, 1mM sodium orthovanadate, 1mM sodium fluoride, 1 mM PMSF, 1 µg/ml aprotinin, and 1 µg/ml leupeptin). After centrifugation, cell lysates were subjected to SDS-PAGE and transferred onto polyvinylidene difluoride membranes (Millipore). The membranes were immunoblotted with the indicated antibodies. The membranes were developed with Super Signal West Dura extended duration substrate (Thermo Scientific) and analyzed by Amersham Biosciences imager 600 (GE Healthcare). In some experiments,

677 blots were quantified using freeware program Fiji.

678

679 **Enzyme-linked immunosorbent assay (ELISA)**

680 Concentrations of murine IL-11 in the culture supernatants were determined by ELISA

681 according to the manufacturers' instruction (R&D Systems).

682

683 **Knockdown of *IL11* by siRNAs**

684 A human breast cancer cell line, MDA-MB-231 cells were provided by T. Sakamoto.

685 MDA-MB-231 cells were maintained in DMEM containing 10% FBS. MDA-MB-231

686 cells were transfected with control or *IL11* siRNAs using Lipofectamine 2000

687 (Invitrogen) at a final concentration of 50 nM. At 36 h after transfection, knockdown

688 efficiency of IL-11 by siRNAs was analyzed by immunoblotting with anti-IL-11

689 antibody using cell lysates. Stealth RNAiTM siRNAs Negative Control, Med GC and

690 human *IL11*, NM_000641.3 (HSS179893, HSS179894, HSS179895) were purchased

691 from Thermo Fisher Scientific.

692

693 **Comparison of the expression of enriched genes in IL-11⁺ IAFs between human**
 694 **normal mucosa and colon cancer tissues**

695 The publicly available data set (GSE33113) was obtained from the Gene Expression
 696 Omnibus (GEO). This data set contains gene expression data from 90 colon cancer
 697 patients and 6 healthy persons. The expression data of enriched genes in IL-11⁺ IAFs
 698 was retrieved from the data set (GSE33113) and their signaling intensities of each gene
 699 in normal mucosa and colon cancer tissues were compared.

700

701 **Survival analysis**

702 Two publicly available data sets (GSE17536 and GSE17537) were obtained from the
 703 Gene Expression Omnibus (GEO). These data sets contain gene expression data and
 704 disease-free survival information from 232 primary CRC patients. The expression levels
 705 of enriched genes in IL-11⁺ IAFs in these data sets was classified by the hierarchical
 706 clustering. Each enriched gene in IL-11⁺ IAFs was correlated with survival using the
 707 Kaplan-Meier method. Statistical significance was analyzed by Mantel-Cox log-rank
 708 test. R software was used for all statistical analysis.

709

710 **Human colorectal cancer tissues**

711 Human colon tumors and adjacent normal tissues were obtained from Toho University

712 Omori Medical Center and analyzed in accordance with approval by the Ethics

713 Committee of Toho University School of Medicine (A16111). Colon tumors included 10

714 cases of adenomas, 10 cases of early cancers, and 10 cases of advanced cancers.

715 Histological assessment of adenomas and adenocarcinomas was according to the

716 guidelines of the World Health Organization⁴⁸. Early and advanced colon cancers were

717 determined according to the TNM criteria (pT1, early cancer; pT2-4, advanced

718 cancers)⁴⁹.

719

720 **Statistical analysis**

721 Statistical significance was determined by the unpaired two-tailed Student's *t*-test,

722 Mann-Whitney U test, two-way ANOVA with Boniferroni's test, or one-way ANOVA

723 with Tukey's post-hoc test, Mantel-Cox log-rank test as indicated. **p* < 0.05 was

724 considered to be statistically significant. All statistical analysis was performed with

725 Graph Pad Prism 7 software (GraphPad Software).

726

727

728

729

730

731

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744

745 **AUTHOR CONTRIBUTIONS**

746 T.N. and H.N. designed research; T.N., Y.D., W.T., M.O., D.O., S.Y., M.K., E.N., Y.K.,
747 S.A-A., M.H., N.I., and N.T. performed research; M.N., M.O., H.Y., K.S., and T.M.

748 contributed to new reagents/analytical tools; T.N., Y.D., W.T., D.O., N.I., and H.N.

749 analyzed data; T.N., N.I., and H.N. wrote the paper.

750

751 **CONFLICT OF INTEREST**

752 The authors declare that they do not have competing financial interests.

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942 **FIGURE LEGENDS**

943 **Fig. 1.** Characterization of IL-11⁺ Cells in CAC Using *Il11-Egfp* Reporter Mice. **a**

944 Protocol for induction of AOM/DSS-induced CAC in mice. *Il11-Egfp* reporter mice

945 were intraperitoneally injected with AOM on day 0, followed by repeated DSS

946 administration. Colorectal cancer gradually develops on ~30 days after AOM injection.

947 Unless otherwise indicated, the following experiments used tumor and nontumor tissues

948 collected on day 98–105 after AOM/DSS treatment. **b** Representative image of tumor

949 and adjacent nontumor tissues in the colon of mice on day 77 after AOM injection.

950 Colonic sections were stained with hematoxylin & eosin (H&E). Scale bar, 200 μ m. **c**

951 Prepared mRNA samples from tumor and nontumor tissues were analyzed by qPCR to

952 determine expression of the indicated genes. Results are mean $\pm$ SE (n = 6–10 mice). **d**,

953 **e** From colonic tumor tissues of wild-type or *Il11-Egfp* reporter mice, single-cell

954 suspensions were prepared, and percentages of IL-11⁺ cells were determined.

955 Representative flow cytometry images are shown (**d**). Results are mean $\pm$ SE (n = 7

956 mice). Cells were stained with the indicated antibodies, and expression on EGFP⁺ cells

957 was analyzed by flow cytometry (**e**). Results are representative of four independent

958 experiments. **f** Nontumor and tumor tissue sections were stained with anti-GFP antibody.

959 Scale bars, 100 μ m. **g, h** Tumor sections were stained with the indicated antibodies (red)

960 and with anti-GFP antibody (green) ($n = 3$ mice). Right panels show enlarged images of

961 white boxes from left panels. White arrowheads indicate merged cells. Scale bars, 100

962 μ m, unless otherwise indicated. Statistical significance was determined by two-tailed

963 unpaired Student's *t*-test (**c, d**). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

964

965 **Fig. 2. IL-11⁺ Cells Appear in Tumor Tissues in the Absence of Inflammation. a**

966 Elevated *Il11* and *Egfp* expression in colonic tumors from *Apc^{min/+};Il11-Egfp* reporter

967 mice. Experiments were performed using tumor and nontumor tissues from the colon of

968 20- to 24-week-old *Apc^{min/+};Il11-Egfp* reporter mice. *Il11* and *Egfp* mRNA expression

969 was determined by qPCR. Results are mean $\pm$ SE ($n = 5$ mice). **b** Colonic tissue sections

970 from *Apc^{min/+};Il11-Egfp* reporter mice were stained with H&E (upper panels) or

971 anti-GFP antibody (lower panels). Middle and right panels, respectively, show

972 enlargements of the black and red boxes from the left panels. Red arrowheads indicate

973 tumor cells. Scale bar, 100 μ m. **c, d** IL-11⁺ cells express stromal cell markers and, to a

974 lesser extent, hematopoietic or epithelial cell markers. Colonic cells were prepared from
 975 tumors of *Apc^{min/+};Il11-Egfp* reporter mice, stained with the indicated antibodies, and
 976 analyzed by flow cytometry. Percentages of IL-11⁺ cells were determined (c). Results
 977 are mean ± SE (n = 3 mice). Representative histograms show expressions of the
 978 indicated markers on EGFP⁺ cells (d). Results are representative of three independent
 979 experiments. **e–g** Characterization of IL-11⁺ cells by IHC. Colonic tumor sections from
 980 *Apc^{min/+};Il11-Egfp* reporter mice were stained with the indicated antibodies (red) and
 981 anti-GFP antibody (green). White arrowheads indicate merged cells. Scale bar, 100 μm,
 982 unless otherwise indicated. Statistical significance was determined by two-tailed
 983 unpaired Student's *t*-test (a, c). **p < 0.01; ***p < 0.001.

984

985 **Fig. 3.** IL-11⁺ Cells Appear in the Colon of DSS-Treated Mice and Express Stromal Cell
 986 Marker. **a** *Il11-Egfp* reporter mice were treated with 1.5% DSS in drinking water for 5
 987 days, followed by a change to regular water. On day 7 after DSS treatment, *Il11* and
 988 *Egfp* mRNA expression in the colon was determined by qPCR. Results are mean ± SE
 989 (n = 9 mice). **b, c** Appearance of IL-11⁺ cells in submucosal tissues of the colon of

990 *Il11-Egfp* reporter mice on post-DSS treatment day 5 (b) or day 1 (c). Colonic tissue
 991 sections from untreated or DSS-treated *Il11-Egfp* reporter mice were H&E stained or
 992 immunostained with anti-GFP antibody. Right panels show enlargements of the boxes
 993 (b). Scale bar, 100 μ m. **d, e** Characterization of cell surface markers on IL-11⁺ cells.
 994 Colonic cells were prepared from the colon of *Il11-Egfp* reporter mice as in (b). We
 995 determined the percentages of EGFP⁺ (IL-11⁺) cells from the colon before and after
 996 DSS treatment (d). Cells were stained with the indicated antibodies, and marker
 997 expressions were analyzed on GFP-positive cells (e). Results are representative of three
 998 independent experiments. **f** Representative immunostaining of IL-11⁺ cells. Colonic
 999 tissue sections were prepared from *Il11-Egfp* reporter mice as in (b), and
 1000 immunostained with the indicated antibodies and anti-GFP antibody. Results are merged
 1001 images. Right panels are enlarged images from the boxes (n = 3–4 mice). White
 1002 arrowheads indicate merged cells. **g** IL-11⁺ cells do not proliferate *in situ*. *Il11-Egfp*
 1003 reporter mice were treated with DSS as in (a), and intraperitoneally administered BrdU
 1004 (40 mg/kg) on day 6. On day 7, colonic sections were prepared and stained with
 1005 anti-GFP and anti-BrdU antibodies. Results are representative images from three

independent experiments. Scale bars, 100 μ m, unless otherwise indicated. Statistical significance was determined by two-tailed unpaired Student's *t*-test (**a**). **p* < 0.05.

Fig. 4. IL-11⁺ Cells Express Genes Associated with Cell Proliferation and Tissue Repair.

On day 7 after DSS treatment, cells were isolated from the colon of *Il11-Egfp* reporter mice, and IL-11⁺ cells (EGFP⁺) were sorted by flow cytometry. We isolated mRNA from IL-11⁻ and IL-11⁺ cells, and analyzed the gene expression by microarray analysis (n = 3). **a** Heat map of microarray gene expression of IL-11⁻ and IL-11⁺ cells. Legend on right shows gene-expression color normalized by Z-score transformation. **b** Volcano plot of whole genes. Horizontal line indicates genes differentially regulated in IL-11⁺ cells compared to IL-11⁻ cells, shown in log2. Vertical line indicates p values of statistical significance, shown in -log10. Significantly upregulated and downregulated genes are indicated by red and blue dots, respectively. Several upregulated genes are plotted. **c** Gene Ontology (GO) terms that were significantly enriched in IL-11⁺ cells compared to IL-11⁻ cells. **d, e** Gene expressions were analyzed by qPCR. Results are mean $\pm$ SE (n = 4 mice). Statistical significance was determined by two-tailed unpaired

1022 Student's *t*-test (**c, d**). **p* < 0.05; ***p* < 0.01; ****p* < 0.001; ns, not significant.

1023

1024 **Fig. 5.** The MEK/ERK Pathway is Involved in *Il11* Upregulation in Tumor Tissues. **a, b**

1025 Wild-type mice were treated with DSS with or without NAC (**a**) or Abx (**b**). Colonic

1026 cells were prepared and stained with CellRox-green, and ROS accumulation was

1027 analyzed by flow cytometry. Left panels show representative histograms of ROS levels

1028 in colonic cells. Right panels show percentages of CellRox-green-positive cells of an

1029 individual mouse. Results are mean ± SE (n = 3–4 mice). **c** Wild-type mice were treated

1030 as in A. Colonic expressions of *16S rRNA*, *Hmox1*, and *Il11* mRNA were determined by

1031 qPCR. Results are mean ± SE (n = 11). **d** Abx blocked *Il11* mRNA upregulation in the

1032 colon of DSS-treated mice. Wild-type mice were untreated or treated with DSS in the

1033 absence or presence of Abx. On day 7 after DSS treatment, qPCR was performed to

1034 determine the expression of bacterial *16S rRNA* and *Il11* mRNA in the colon. Results

1035 are mean ± SE (n = 10). **e, f** Trametinib inhibits ERK phosphorylation and *Il11* mRNA

1036 expression in the colon of DSS-treated mice. Wild-type mice were treated with DSS in

1037 the absence or presence of trametinib, and then colonic sections were prepared and

1038 stained with anti-pERK antibody (e). Scale bars, 100 μ m. *Il11* mRNA expression was
1039 determined by qPCR (f). Results are mean $\pm$ SE (n = 13–14). **g** NAC inhibits ERK
1040 phosphorylation in the colon of DSS-treated mice. Wild-type mice were treated with
1041 DSS in the absence or presence of NAC in the drinking water for 5 days. Colonic
1042 sections were stained with anti-pERK antibody. Results are representative of three
1043 independent experiments. Scale bar, 100 μ m. **h** *Tgfb3* expression in the colon of
1044 DSS-treated wild-type mice. Wild-type mice were treated with DSS as in Fig. 4a. On
1045 day 7 after DSS treatment, qPCR was performed to determine *Tgfb1*, *Tgfb2*, and *Tgfb3*
1046 mRNA expression in the colon. Results are mean $\pm$ SE (n = 8–10 mice). **i** Treatment
1047 with anti-TGF β antibody does not downregulate *Il11* mRNA expression in the colon of
1048 DSS-treated mice. Mice were intraperitoneally administered 100 μ g anti-TGF β
1049 antibody on days 2 and 4 after DSS administration, and sacrificed on day 5. *Il11* mRNA
1050 expression was determined by qPCR. Results are mean $\pm$ SE (n = 11–16 mice). **j**
1051 Schema of administration of various inhibitors in AOM/DSS-treated mice. Mice were
1052 treated with Abx for 8 weeks, NAC for 4 weeks, trametinib at –6 and –30 hours, or
1053 anti-TGF β antibody on day –1, –3, –5 (just before sacrifice). **k–m, o** Mice were treated

1054 as in Figure 1A, and then treated with Abx (**k**) (n = 7–9 mice), NAC (**l**) (n = 7–11 mice),
 1055 trametinib (**m**) (n = 10–12 mice), or anti-TGFβ antibody (n = 6–7 mice) (**o**) as in (**j**). On
 1056 day 98–105 after AOM injection, mRNA was extracted from tumor and non-tumor
 1057 tissues, and *Il11* expression was determined by qPCR. Results are mean ± SE. **n** Mice
 1058 were treated as in Fig. 1a. *Tgfb1*, *Tgfb2*, and *Tgfb3* expressions in tumor and nontumor
 1059 tissues were determined by qPCR. Results are mean ± SE (n = 7 mice). Statistical
 1060 significance was determined using the unpaired two-tailed Student's *t*-test (**c**, **h**),
 1061 Mann-Whitney U test (**f**, **i**), two-way ANOVA with Bonferroni's test (**d**, **k**, **l**, **m**, **o**), or
 1062 one-way ANOVA with Tukey's post-hoc test (**a**, **b**). **p* < 0.05; ***p* < 0.01; ****p* <
 1063 0.001; ns, not significant.

1064

1065 **Fig. 6** IL-11 Preferentially Induces Signals to Fibroblasts. **a** Relative expressions of
 1066 *Il11ra1*, *Il22ra1*, and *Il6st* in colonic epithelial organoids and colonic fibroblasts.
 1067 Colonic epithelial organoids and fibroblasts were established from wild-type mice as
 1068 described in the methods. Expressions of the indicated genes were determined by qPCR.
 1069 Results are mean ± sd of triplicate samples and representative of two independent

1070 experiments. **b** Colonic epithelial organoids and fibroblasts were unstimulated or
 1071 stimulated for 30 min with IL-11 (10 or 100 ng/mL) or IL-22 (10 or 100 ng/mL). Total
 1072 STAT3 and phosphorylated STAT3 (pSTAT3) were analyzed by Western blotting.
 1073 STAT3 and pSTAT3 signaling intensities were calculated by Fiji, and the relative ratio
 1074 of pSTAT3/STAT3 is shown. Results are representative of two independent experiments.

1075 **c** Administration of IL-11R agonist induces expression of the genes expressed in IL-11⁺
 1076 cells. We injected 8-week-old wild-type mice with 10 µg IL-11R agonist. At 3 hours
 1077 after injection, mRNA was isolated from the colon, and gene expressions were analyzed
 1078 by microarray analysis (n = 2 for untreated samples; n = 3 for injected samples). Heat
 1079 map of microarray gene expression in the colon of untreated and treated mice (**c**).
 1080 Legend on the right shows gene-expression color normalized by Z-score transformation.

1081 **d** Volcano plot of whole genes. Horizontal line indicates differentially regulated genes
 1082 in the colon after IL-11R agonist injection compared to before injection, shown in log₂.
 1083 Vertical line indicates p values of statistical significance, shown in -log₁₀. Significantly
 1084 upregulated genes are indicated by red dots. Several upregulated genes are plotted. **e**
 1085 Venn diagram of genes elevated in IL-11⁺ IAFs compared to IL-11⁻ cells, and genes

1086 elevated in the colon with IL-11R agonist treatment compared to untreated colon.
1087 Overlapping area includes 18 genes. Statistical significance was determined by
1088 two-tailed unpaired Student's *t*-test (**a**). **p* < 0.05; ***p* < 0.01; *****p* < 0.0001.

1089

1090 **Fig. 7.** IL-11 Expression is Correlated with Progression of Human Cancers. **a**

1091 Specificity of anti-IL-11 antibody used in the study. MDA-MB-231 cells were treated
1092 with control or three different siRNAs against human *IL11*, and IL-11 expression in cell
1093 lysates was analyzed by Western blotting with anti-IL-11 and anti-tubulin antibodies.

1094 Results are representative of two independent experiments. **b-d** Adenomas (n = 10),
1095 early (n = 10) and advanced (n = 10) colorectal cancers were stained with anti-IL-11
1096 antibody (**b**). Representative staining of respective tumors and adjacent normal tissues.

1097 Scale bars, 100 μm. Areas (**c**) and intensities (**d**) of IL-11⁺ signals were calculated as in
1098 the methods. Results are mean ± SE. **e** Tissues of advanced colorectal cancers were

1099 stained with anti-vimentin, anti-CD45, or anti-E-cadherin antibodies along with
1100 anti-IL-11 antibody (n = 3). Right panels are enlarged images of the white box in the

1101 left panels. Signaling intensities stained with the indicated antibodies on the white

1102 arrows were calculated and plotted in the right panels. Scale bars, 100 μ m. Statistical
 1103 significance was determined by Mann-Whitney U test (**c**, **d**). ** $p < 0.01$; ns, not
 1104 significant.

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NishinaFig1

Nishina Fig3

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