



CXCR7/CXCR4 heterodimer-induced histone demethylation: a new mechanism of colorectal tumorigenesis

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Abstract

Both chemokine receptors (CXCRs) 7 and 4 can facilitate immune cell migration and mediate a vast array of physiological and pathological events. Herein we report, in both human and animal studies, that these two CXCRs can form heterodimers in vivo and promote colorectal tumorigenesis through histone demethylation. Compared with adjacent non-neoplastic tissue, human colorectal cancer (CRC) tissue showed a significant higher expression of CXCR4 and CXCR7, which was colocalized in the cancer cell epithelium. The CXCR7/CXCR4 heterodimerization was associated with increased histone demethylase JMJD2A. Villin-CXCR7-CXCR4 transgenic mice demonstrated a greater degree of exacerbated colitis and tumorigenesis than villin-CXCR7 and villin-CXCR4 mice. The CXCR7/CXCR4 heterodimerization also promoted APC mutation-driven colorectal tumorigenesis in APC^{Min/+}/villin-CXCR7-CXCR4 mice. Further analysis showed that the CXCR7/CXCR4 heterodimer induced nuclear β arr1 recruitment and histone demethylase JMJD2A, leading to histone demethylation and resulting in transcription of inflammatory factors and oncogenes. This study uncovered a novel mechanism of colorectal tumorigenesis through the CXCR7/CXCR4 heterodimer-induced histone demethylation. Inhibition of CXCR7/CXCR4 heterodimer-induced histone demethylation could be an effective strategy for the prevention and treatment of colorectal cancer.

Introduction

Colorectal cancer (CRC) is the third most common malignancy and fourth most common cause of cancer-related

deaths worldwide [1]. CRC includes hereditary, sporadic, and inflammatory bowel disease (IBD)-associated cancers. Pathologically, CRC arising in the patients with IBD typically affects young individuals, tends to be multifocal, and often progresses to invasive adenocarcinoma. Moreover, colitis-associated cancer (CAC) is difficult to treat, and has high mortality. More than 20% of IBD patients develop CAC within 20–30 years of disease onset, and >50% of these patients will die from CAC [2]. Although immune-mediated pathogenesis naturally link IBD and CAC [3], the mechanisms of colorectal tumorigenesis remains poorly understood, which further limits its therapeutic and preventive options.

CXCR7, a heptahelical G protein-coupled receptor, and its ligand CXCL12 have received attention in colorectal tumorigenesis due to their enhanced expression in the pathological processes of colitis [4]. Although accumulating reports suggest that CXCL12-driven CXCR7 play an important role in promoting IBD [5, 6], the mechanism of CXCR7-driven tumorigenesis is still unclear. Interestingly, CXCL12 binding can induce CXCR7 internalization but CXCR7/CXCL12 complex does not completely activate the canonical Gi-mediated signaling network [7–9]. Further

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studies found that CXCR7 is an atypical chemokine receptor regarded as “intercepting receptor” that can bind to a broader spectrum of chemokines. Although CXCL12 can bind both CXCR4 and CXCR7 in T lymphocytes, it has a 10-fold higher affinity to CXCR7 than to CXCR4 [9]. Recently, the reports suggest that CXCR7 could cooperate with CXCR4 to form heterodimers. CXCR7/CXCR4 heterodimer might impair CXCR4-mediated Gi activation and calcium responses in T lymphocytes [10]. CXCR7/CXCR4 heterodimer could recruit nuclear beta-arrestin 1 (β arr1) to enhance cancer cell migration [11]. However, it is not yet known whether the CXCR7/CXCR4 heterodimer can form in vivo, and if it does, the functional difference between CXCR7 monomer and CXCR7/CXCR4 heterodimer.

In the current study, we first showed that CXCR7 and CXCR4 are widely expressed and that their expression was colocalized in human CRC tissue. We then demonstrated the roles of CXCR7/CXCR4 heterodimer in colitis development and colitis-driven CRC. To unveil the mechanism of CXCR7/CXCR4 heterodimer in tumorigenesis, we analyzed the downstream enzymes and molecules of CXCR7/CXCR4 heterodimer. Our study provides evidence that CXCR7 and CXCR4 could form a heterodimer in vivo and that CXCR7/CXCR4 heterodimer drive tumorigenesis through promoting histone demethylation.

Results

Human CRC tissue has increased expression of CXCR7 and CXCR4, CXCR7/CXCR4 colocalization, and elevated histone demethylase JMJD2A than the adjacent non-neoplastic tissue

We analyzed expression levels of CXCR7, CXCR4, CXCR7/CXCR4 heterodimer and JMJD2A in the 20 matched CRC specimens and their adjacent non-neoplastic tissue. CRC epithelium showed a strong and diffuse membranous and cytoplasmic staining for both CXCR7 (Fig. 1a) and CXCR4 (Fig. 1b). Immunoreactivity for CXCR7 was determined in 19 (95%) of the 20 CRC specimens and 1 (5%) in the paracancerous tissues. Immunoreactivity for CXCR4 was observed in 14 (70%) of the 20 CRC specimens and 1 (5%) in the paracancerous tissues. In the 19 specimens that were immunoreactive for CXCR7, 14 specimens also had CXCR4 staining. Of the 20 CRC specimens, only one specimen stained negatively for both CXCR4 and CXCR7. The Spearman correlation analysis shows a positive correlation between CXCR7 and CXCR4 (Supplementary Table S1).

Immunofluorescence microscopy analyzed the link between CXCR7 and CXCR4. CXCR7 and CXCR4 were colocalized in cancer cell membrane and cytoplasm (Fig.

1c), suggesting the formation of CXCR7 and CXCR4 heterodimers. Thirteen cancer tissues showed colocalization of CXCR7 with CXCR4 in the 20 patients (65%) studied.

Histone demethylase JMJD2A staining was present in both cytoplasm and nucleus in 14 of the 20 CRC specimens (70%) (Fig. 1d). Interestingly, of the 13 patients with CXCR7 and CXCR4 colocalization, 11 specimens expressed high nuclear JMJD2A (84.6%) (Supplementary Table S2).

Western blot analysis showed higher CXCR7 and CXCR4 in colonic adenocarcinoma (T) than the corresponding adjacent non-neoplastic tissues (N) (Fig. 1e). We analyzed the expression levels of JMJD2A and demethylation sites trimethyl-histone H3 at lysine 9 (H3K9me3) and trimethyl-histone H3 at lysine 36 (H3K36me3). Cancer tissues demonstrated higher JMJD2A levels than their non-neoplastic tissues. These cancer tissues correspondingly expressed lower levels of nucleic trimethyl H3K9me3 and trimethyl H3K36me3 than their adjacent non-neoplastic tissues (Fig. 1f). These results suggest a link between the CXCR7/CXCR4 heterodimer and histone demethylation in human CRC tissues.

Increased expression of CXCR7 in the colorectal epithelia promotes chronic inflammation in mice

To investigate the role of CXCR7 in colitis development, we exposed villin-CXCR7 mice and their wild-type (WT) littermates to 1% dextran sulfate sodium (DSS) and then analyzed the severity of colorectal inflammation. Both WT and villin-CXCR7 mice developed colitis, showing diarrhea, hair bristling, and body weight loss. Villin-CXCR7 mice demonstrated more severe symptoms of colitis with higher disease activity index (DAI) than their WT littermates (Figure S1A and S1B). Villin-CXCR7 mice showed shorter colorectal length than WT mice (Figure S1C). Microscopic examination showed increased exacerbated colonic damage with massive inflammatory cells in villin-CXCR7 mice (Figure S1D-iv). The scores of inflammation in two groups of mice are shown in Figure S1D. Enzyme-linked immunosorbent assay (ELISA) analysis showed higher levels of M-CSF, GM-CSF, tumor necrosis factor alpha (TNF- α), and interleukin-6 (IL-6) in villin-CXCR7 mice than in WT mice (Figure S1E).

Increased CXCR7 promotes CAC and APC mutation-driven CRC

To investigate the role of CXCR7 in colorectal tumorigenesis, villin-CXCR7 mice and their WT littermates were exposed to azoxymethane (AOM)/DSS as indicated in Fig. 2a. Villin-CXCR7 mice showed more severe colitis than WT mice as indicated by body weight loss, increased DAI,

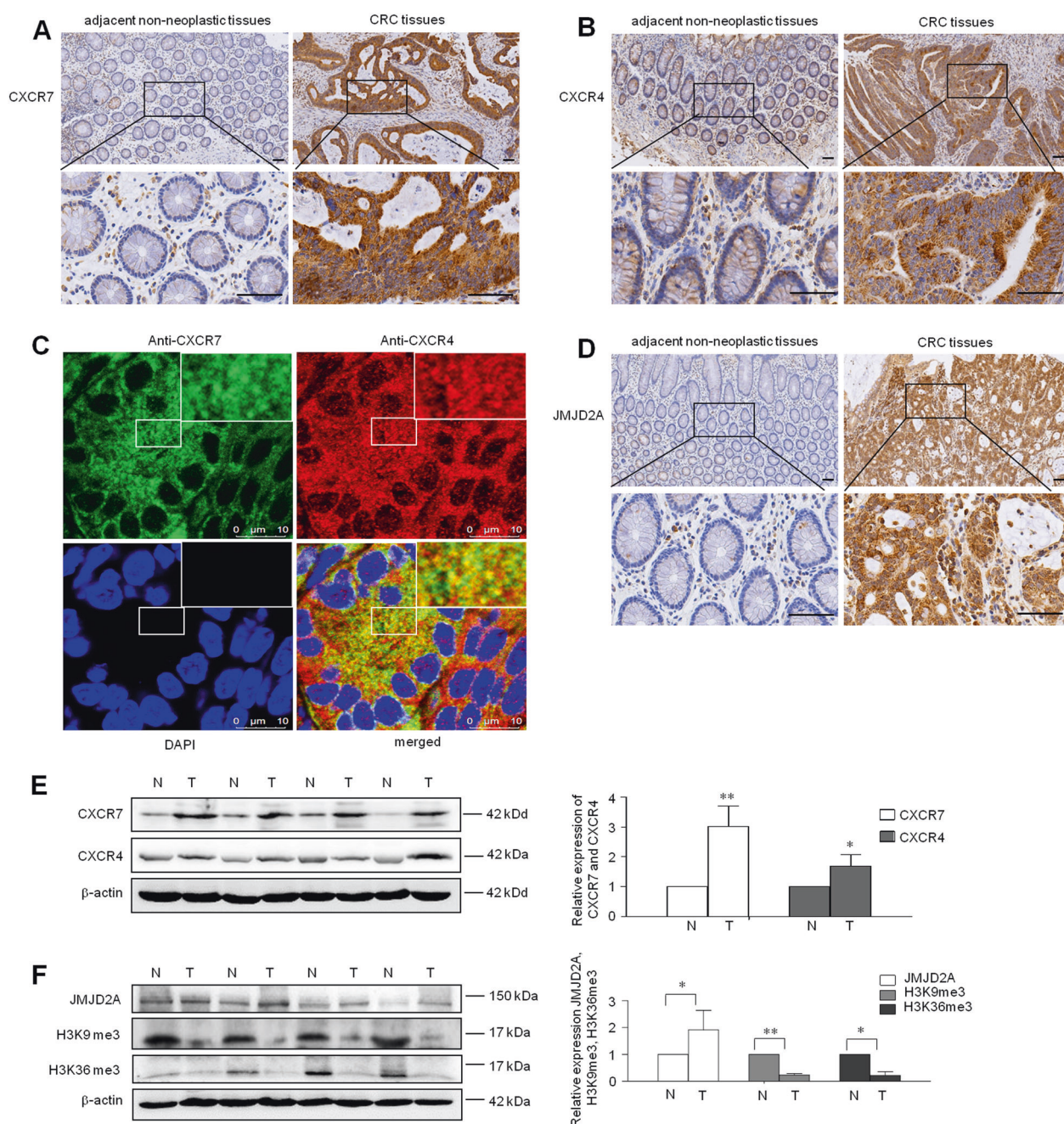


Fig. 1 Expression of CXCR7, CXCR4, and JMJD2A in cancer tissues of CRC patient and their corresponding adjacent non-neoplastic tissues. **a** Expression of CXCR7 in a representative ($n = 20$) CRC tissues (marked by T) and adjacent non-neoplastic tissues (marked by N). 20 samples. Immunoperoxidase upper images $10\times$, lower images $40\times$. **b** Expression of CXCR4 in a representative ($n = 20$) CRC tissues (marked by T) and adjacent non-neoplastic tissues (marked by N). Immunoperoxidase upper images $10\times$, lower images $40\times$. **c** Immunofluorescent staining of CXCR7 (green), CXCR4 (red), DAPI (blue) and the merged image in a representative sample of CRC tissue ($n = 20$). The CXCR7/CXCR4 colocalization were observed in human CRC tissues. Immunofluorescence scale bar: $10\mu\text{m}$. **d** Expression of JMJD2A in a representative ($n = 20$) human CRC tissues (marked by T) and the adjacent non-neoplastic tissues (marked by N). Immunoperoxidase upper images $40\times$, lower images $100\times$. The boxed region

in each top panel is magnified and shown in the corresponding bottom panel. **e** Western blot analysis of CXCR7 and CXCR4 in the representative ($n = 20$) CRC tissue (marked by T) and its adjacent non-neoplastic tissue (marked by N). Each T and N represented a sample of cancer tissue and its adjacent non-neoplastic tissue. Protein level was determined by densitometry and normalized to β -actin (right). **f** Western blot analysis of JMJD2A, H3K9me3, and H3K36me3 in the representative ($n = 20$) human CRC tissues (marked by T) and adjacent non-neoplastic tissues (marked by N). Each T and N represented a sample of cancer tissue and its adjacent non-neoplastic tissue. Protein level was determined by densitometry and normalized to β -actin (right). $*p < 0.05$, $**p < 0.01$ between cancer tissues and their corresponding adjacent non-neoplastic tissues. Statistics were obtained from two-tailed Student's *t*-test

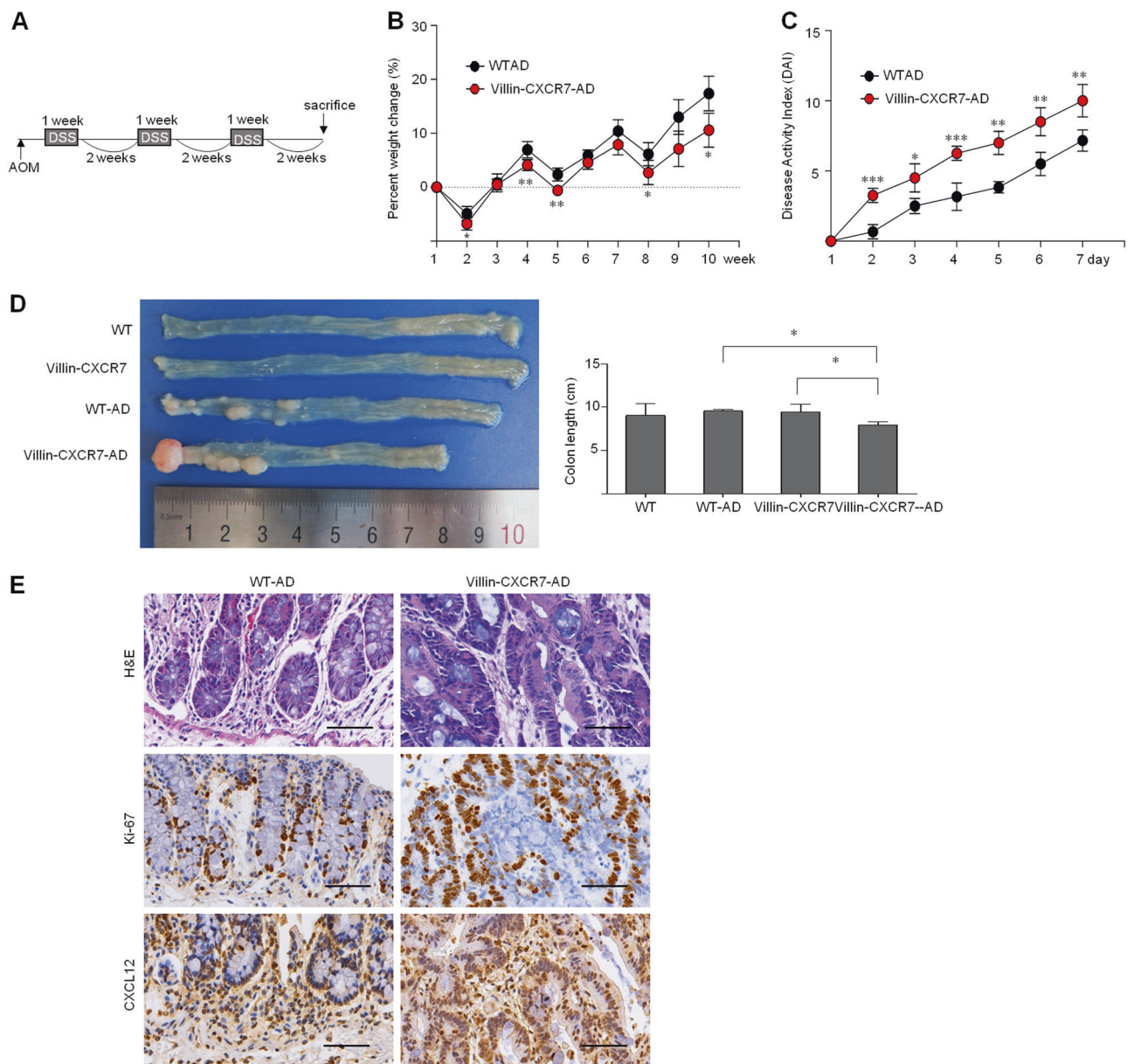


Fig. 2 High CXCR7 increases the AOM/DSS-induced CRC in villin-CXCR7 mice. **a** The schematic regimen of colitis-associated cancer model by treatment with AOM/DSS. WT and villin-CXCR7 mice were injected intraperitoneally with 10 mg/kg azoxymethane (AOM) and after 7 days, received drinking water containing 1.0% dextran sulfate sodium (DSS). **b** Mice body weight changes in WT (black) and villin-CXCR7 mice (red). **c** Disease activity index (DAI) scores for WT (black) and villin-CXCR7 mice (red) during the week of third DSS cycle. **d** Representative image of CRC and length of colon. Colorectal tissue length was significantly shorter in villin-CXCR7

mice than WT mice. **e** Representative images of colorectal tissues from WT and villin-CXCR7 mice. Increased tumorigenesis in villin-CXCR7 mice as compared with WT mice (upper panel, H&E 20×). High levels of Ki-67 (middle panel, immunoperoxidase, 20×) and CXCL12 (lower panel, immunoperoxidase, 20×) in colorectal epithelium of villin-CXCR7 mice as compared with WT mice. $n = 4$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ between villin-CXCR7 and WT mice. Statistics were obtained from two-tailed Student's t -test. WT-AD wild mice exposed to AOM/DSS, Villin-CXCR7-AD villin-CXCR7 mice exposed to AOM/DSS

and shortened colorectal length (Figs. 2b–d). AOM/DSS-induced colorectal tumor by 100% in both villin-CXCR7 and WT mice. However, tumors in villin-CXCR7 mice developed larger size (Fig. 2d) with higher dysplasia than in WT mice (Fig. 2e, top row). Ki-67 staining of tumor tissues indicated higher cell proliferation rate in villin-CXCR7

mice than in WT mice (Fig. 2e, second row). As a ligand for CXCR7, high CXCL12 might drive CXCR7 to promote colitis and tumorigenesis. We observed a higher level of CXCL12 in villin-CXCR7 mice than in WT mice (Fig. 2e, third row). $APC^{min/+}$ /villin-CXCR7 mice also developed more colorectal tumors than $APC^{min/+}$ mice (Figure 2SA–C).

CXCR7 increases the severity of inflammation by promoting the infiltration of monocytic myeloid-derived suppressor cells (MDSCs) and M2-like macrophages into colorectal epithelia and lamina propria

In mice, immature myeloid MDSCs, defined as $CD11b^+Gr-1^+$ cells, are divided into two subsets, polymorphonuclear MDSC (G-MDSCs defined by $CD11b^+Ly6G^+$) and monocytic MDSC (M-MDSCs defined by $CD11b^+Ly6C^+$) [12]. AOM/DSS exposure induced a greater increase of M-MDSCs in the colorectal tissue of villin-CXCR7 than in WT mice (Fig. 3a, $p < 0.01$ between WT-AD and villin-CXCR7-AD). No difference was observed in G-MDSCs between these two groups of mice (Fig. 3b, $p > 0.05$ between WT-AD and villin-CXCR7-AD). The profiles of M-MDSCs and G-MDSCs in the blood of villin-CXCR7 and WT mice were shown in Figure S3A,B. Villin-CXCR7 mice demonstrated lower levels of M1-like macrophages in the colonic tissues than WT mice ($F4/80^+CD86^+$, Fig. 3c, $p < 0.01$ between WT-AD and villin-CXCR7-AD), whereas higher levels of M2-like macrophages were observed in villin-CXCR7 mice than in WT mice ($M\Phi$, $F4/80^+CD206^+$, Fig. 3d, $p < 0.05$ between WT-AD and villin-CXCR7-AD). Further, a greater increase of Treg cells ($CD4^+CD25^+FoxP3^+$) was detected in villin-CXCR7 mice than in WT mice (Fig. 3e and Figure S3C, $p < 0.05$ between WT-AD and villin-CXCR7-AD). No statistically significant changes of dendritic cells (DCs, $CD86^+CD11c^+$) were observed in villin-CXCR7 and WT mice (Fig. 3f and Figure S3D). Immunohistochemistry (IHC) showed an higher level of $CD8^+$ T cells in WT mice, whereas villin-CXCR7 mice demonstrated lower levels of $CD8^+$ T cells in colorectal and cancer tissue and the blood (Fig. 3g, upper panel; Figure S3E). No statistically significant reduction of $CD4^+$ T cells was detected in both WT and villin-CXCR7 mice (Fig. 3g, bottom panel; Figure S3F).

During colitis and tumorigenesis, inflamed mucosa and tumor cells produce a large amount of inflammatory mediators. Villin-CXCR7 mice demonstrated higher levels of GM-CSF, M-CSF, IL-6, and TNF- α (Fig. 4a), and TNF- α and IL-1 β (Fig. 4b) than those of WT mice. High CXCR7 also activated the Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) (JAK2/STAT3) pathway (Fig. 4c), which further prompted inflamed mucosal cells and tumor cells to produce inflammatory mediators [13].

CXCR7/CXCR4 heterodimer exacerbated CXCR7- or CXCR4-induced CRC

To investigate the role of CXCR7/CXCR4 heterodimer in tumorigenesis, villin-CXCR7 and villin-CXCR4 mice were

crossed to generate villin-CXCR7-CXCR4 mice. Villin-CXCR7-CXCR4 mice developed more advanced tumors than villin-CXCR7 and villin-CXCR4 mice (Fig. 5a-i). $APC^{min/+}$ /villin-CXCR7-CXCR4 mice also demonstrated more colorectal tumors than $APC^{min/+}$ /villin-CXCR7 mice (Figure S4A-C). AMD3100 (CXCR4 antagonist) strongly prevented AOM/DSS-induced tumors in villin-CXCR7-CXCR4 mice. AMD3100 alone did not induce colorectal tumorigenesis in WT mice (Fig. 5a-ii).

Pathological analysis showed advanced colorectal tumors in villin-CXCR7-CXCR4 mice, followed by villin-CXCR7 and villin-CXCR4 mice (Fig. 5b, top row). IHC analysis indicated a strong diffuse Ki-67 staining in the dysplastic mucosa of villin-CXCR7-CXCR4 mice, whereas AMD3100-treated mice showed only scattered positivity in the basal cells of crypt (Fig. 5b, second row).

Immunofluorescence microscopy determined the link of CXCR7 with CXCR4 in villin-CXCR7-CXCR4 mice. High intensity of CXCR7 and CXCR4 were seen and the colocalization of these CXCRs was observed in merged sections (Fig. 5c). Co-Immunoprecipitation (Co-IP) assay showed that CXCR7 was detected in the anti-CXCR4-incubated protein sample, but not in the IgG-incubated protein sample from villin-CXCR7-CXCR4 mice (Fig. 5d-i), which provides further evidence of CXCR7/CXCR4 heterodimerization. Similar result was observed in $APC^{Min/+}$ /villin-CXCR7-CXCR4 mice (Fig. 5d-ii). These results suggest the formation of CXCR7/CXCR4 heterodimer in villin-CXCR7-CXCR4 mice.

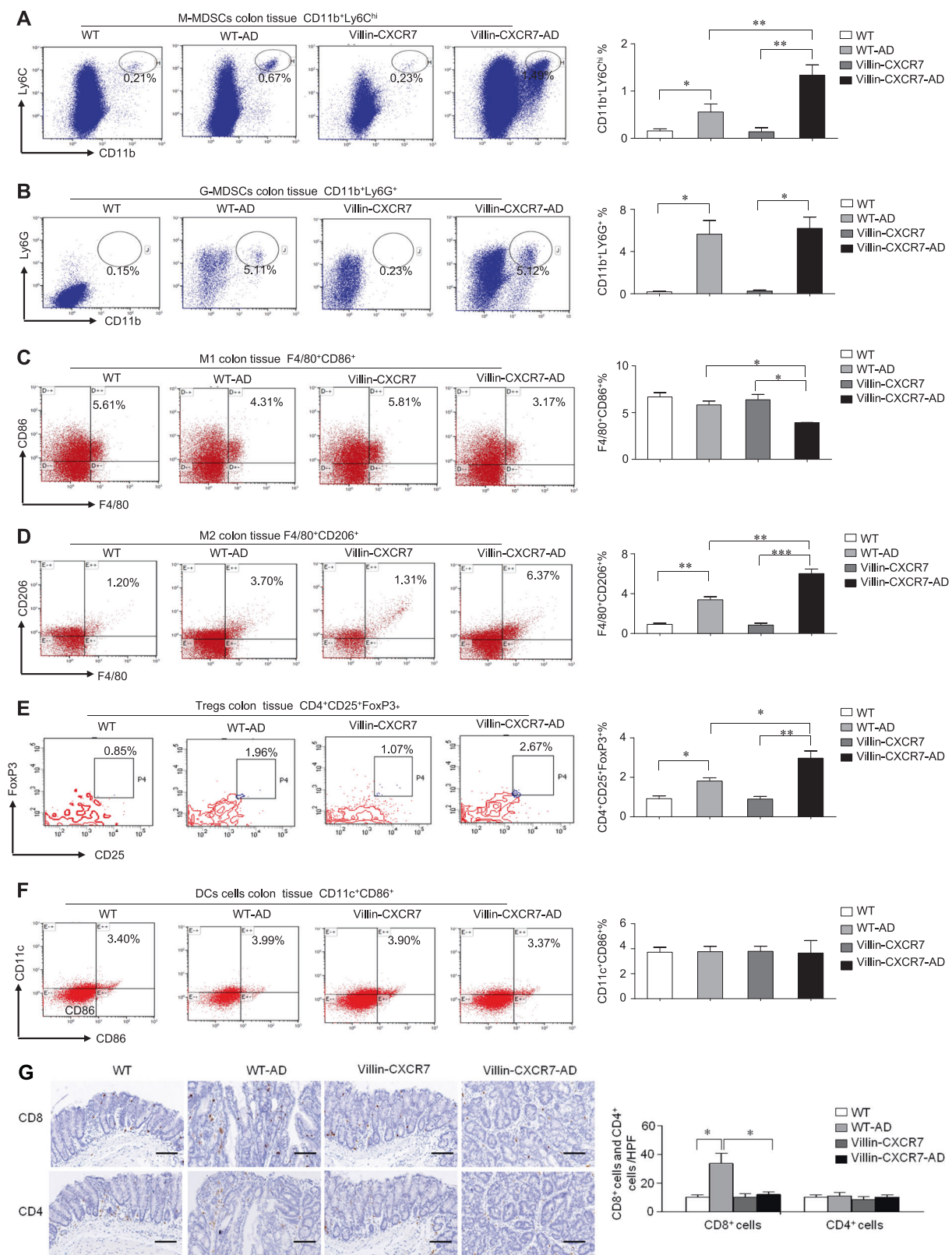
AOM/DSS induced more M-MDSCs and M2-like macrophages in villin-CXCR7-CXCR4 mice than villin-CXCR7 and villin-CXCR4 mice ($p < 0.05$). AMD3100 strongly inhibited the infiltration of M-MDSCs ($p < 0.01$, Fig. 5e) and M2-like macrophages ($p < 0.05$, Fig. 5f) in colorectal tissue of villin-CXCR7-CXCR4 mice.

Our results further illustrated that CXCR7/CXCR4 heterodimer induced a greater increase of Treg cells but not DCs in colorectal tissue in villin-CXCR7-CXCR4 mice than in villin-CXCR7 mice and villin-CXCR4 mice (Figure S5A for Treg cells, $p < 0.05$; Figure S5B for DCs, $p > 0.05$).

As expected, AMD3100 significantly inhibited CXCR7/CXCR4 heterodimer-induced higher JAK2/STAT3/nuclear factor- κ B (NF- κ B) cascades in villin-CXCR7-CXCR4 mice (Figure S5C).

CXCR7/CXCR4 heterodimer induces β arr1 recruitment to nucleus to enhance JMJD2A activity in the demethylation of histones

Western blot analysis of nuclear protein showed higher level of β arr1 in villin-CXCR7 mice than in villin-CXCR4 mice. Villin-CXCR7-CXCR4 mice demonstrated highest level of β arr1. AMD3100 inhibited nuclear β arr1 in villin-



CXCR7-CXCR4 mice (Fig. 6a). Further, higher levels of JMJD2A were observed in villin-CXCR7-CXCR4 mice than in villin-CXCR7 and villin-CXCR4 mice ($p < 0.01$),

and a lower level of JMJD2A in AMD3100-treated mice (Fig. 6b, $p < 0.01$ vs. untreated mice).

◀ **Fig. 3** High CXCR7 regulates the immunocytes in colorectal tissues. WT and villin-CXCR7 mice with or without AOM/DSS were sacrificed. Immunocytes isolated from intraepithelial tissues and lamina propria were analyzed by flow cytometry assay. **a** AOM/DSS increased more M-MDSCs in villin-CXCR7 mice than in WT mice ($p < 0.05$). **b** Villin-CXCR7 and WT mice demonstrated similar profiles of increase of G-MDSCs in colorectal tissues. $n = 3$. **c, d** High CXCR7 significantly inhibited M1-like macrophages (**c**) and increased M2-like macrophages $n = 3$ (**d**) in colorectal tissues. $n = 4$. **e** AOM/DSS increased more Treg cells in villin-CXCR7 mice than WT mice ($n = 3$, $p < 0.05$). **f** DCs did not show significant changes between villin-CXCR7 mice than WT mice ($n = 3$, $p > 0.05$). **g** Representative images ($n = 3$) shows that CD8⁺ T cells were increased in colorectal tissues of WT mice but not villin-CXCR7 mice (upper panel). CD4⁺ T cells did not show significant changes in both WT and villin-CXCR7 mice (bottom panel). Immunoperoxidase 40 $\times$. Statistics were obtained from two-tailed Student's *t*-test. * $p < 0.05$, ** $p < 0.01$ between WT and WT-AD or villin-CXCR7 and villin-CXCR7-AD or WT-AD and villin-CXCR7-AD; *** $p < 0.001$ villin-CXCR7 and villin-CXCR7-AD. WT-AD wild mice exposed to AOM/DSS, Villin-CXCR7-AD villin-CXCR7 mice exposed to AOM/DSS

To investigate the link between β arr1 and JMJD2A, human colon cancer SW480 cells was silenced by β arr1 small interfering RNA (siRNA). β arr1 deficiency significantly reduced JMJD2A expression (Fig. 6c). These results suggest an association of β arr1 with JMJD2A. However, we have not detected a direct interaction of β arr1 with JMJD2A using the Co-IP assay (Fig. 6d).

JMJD2A is a histone demethylase with specificity toward H3K9me3 and H3K36me3 [14]. We used antibodies against trimethyl H3K9me3 to bind nuclear H3K9me3 and H3K36me3 in CRC cells. Lower levels of anti-trimethyl H3K9me3 and anti-trimethyl H3K36me3 were determined in villin-CXCR7-CXCR4 mice as compared with villin-CXCR7 and villin-CXCR4 mice (Fig. 6e), which indicated the removal of methyl groups from histones H3K9me3 and H3K36me3. Further, we used AMD3100 to block CXCR4. High levels of anti-trimethyl H3K9me3 and anti-trimethyl H3K36me3 were observed in villin-CXCR7-CXCR4 mice (Fig. 6e). These results suggest that CXCR4-CXCR7 heterodimer increase JMJD2A expression, which promotes histone demethylation.

CXCR7/CXCR4 heterodimer-induced demethylation of histones H3K9 and H3K36 promotes the transcription of inflammatory genes and oncogenes

To investigate the roles of demethylated H3K9 and H3K36 in promoting target genes, we performed chromatin immunoprecipitation (ChIP) assay with antibodies to H3K9me3 and H3K36me3. Villin-CXCR7/CXCR4 mice demonstrated higher levels of transcriptions in GM-CSF, IL-6, TNF- α , and C-Myc than those of villin-CXCR7 mice and villin-CXCR4 mice. There was a significant decrease in the binding of H3K9me3 with IL-6 (Fig. 7a) and TNF- α

(Fig. 7a), and with GM-CSF (Fig. 7b) and C-Myc (Fig. 7b); and a decrease in the binding of H3K36me3 with IL-6 (Fig. 7c) and TNF- α (Fig. 7c), and with GM-CSF (Fig. 7d) and C-Myc (Fig. 7d). The CXCR7/CXCR4 heterodimer-induced transcription of these demethylated histone target genes is further supported by the results of western blotting (Fig. 7e) and ELISA (Fig. 7f) assays. These results suggest that the CXCR7/CXCR4 heterodimer-induced demethylation of histones H3K9 and H3K36 promotes the transcription of pro-inflammatory genes and oncogenes.

Inhibition of JMJD2A prevents AOM/DSS-induced colitis and CRC in villin-CXCR7-CXCR4 mice

We used JIB-04 (100 mg/kg by intraperitoneal injection for 63 days), JMJD2A inhibitor, to treat villin-CXCR7-CXCR4 mice. Mice were then exposed to AOM/DSS. JIB-04 strongly prevented large sized (2–6 mm) tumors (100%). Smaller colorectal polyps sized 1–2 mm and <1 mm were inhibited by 87.5% and 80%, respectively (Figs. 8a, b). Consistent with the effects of JIB-04, histological analysis showed normal colorectal tissue structures (Fig. 8c, top). Ki-67 staining intensity was strongly inhibited (Fig. 8c, bottom). JIB-04 treatment did not induce colorectal tumor in WT mice (Fig. 8d). Flow cytometry analysis showed that JIB-04-treated villin-CXCR7-CXCR4 mice demonstrated lower levels of M-MDSCs (Fig. 8e), M2-like macrophages (Fig. 8f), and Treg cells (Fig. 8g) in colorectal tissues. JIB-04 alone did not significantly impact on DCs in colonic tissue (Fig. 8h). The demethylated histone-induced high IL-6, TNF- α , C-Myc, and GM-CSF were consequently inhibited by JIB-04 (Figs. 8i–k). These results further proved that CXCR7/CXCR4 heterodimer-induced histone demethylation is responsible for colitis and colitis-driven tumorigenesis.

Discussion

Although a link between colitis and CAC has been established, the roles of chemokines and their receptors in colitis and CAC development remains largely unknown. In this study, using both human CRC tissue and transgenic mice models, we demonstrate that CXCR7 and CXCR4 can form heterodimer in vivo. Importantly, the CXCR7/CXCR4 heterodimer can promote colitis and CAC development. Furthermore, our study showed that the CXCR7/CXCR4 heterodimer can recruit β arr1 to nucleus to promote JMJD2A demethylase and histone demethylation. Histone demethylation lead to the transcription of an array of inflammatory genes and oncogenes. Knockdown of β arr1 or inhibition histone lysine demethylase JMJD2A inhibited colitis and colitis-driven tumorigenesis. These results open a new window for the development of novel therapeutic and preventive strategies against CAC.

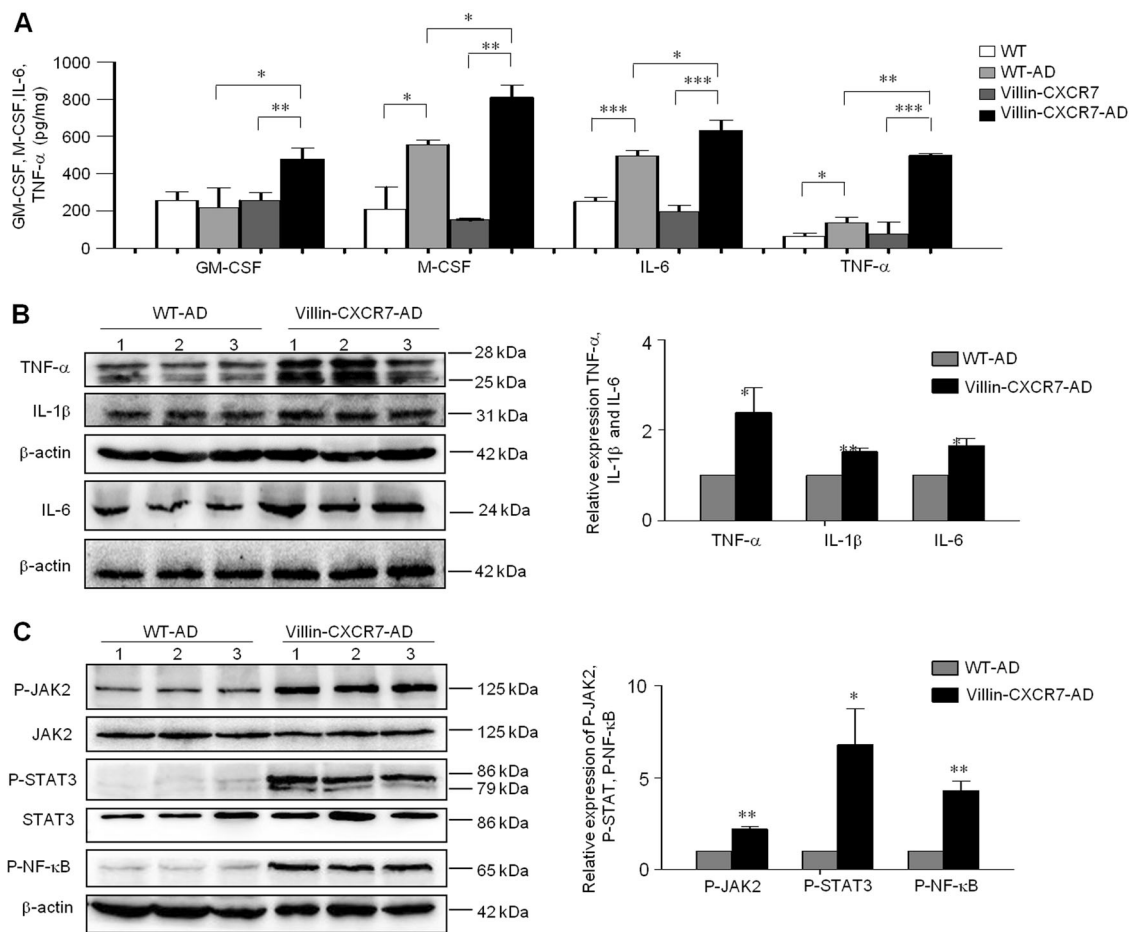


Fig. 4 High CXCR7 increases pro-inflammatory mediators in colorectal mucosal tissues. **a** ELISA assays showed higher levels of GM-CSF, M-CSF, IL-6, and TNF-α in villin-CXCR7 mice than in WT mice. $n = 3$. **b** Western blot assay further demonstrated higher levels of IL-6, TNF-α, and IL-1β in villin-CXCR7 than in WT mice. 1, 2, 3 represented the samples from three mice. $n = 3$. **c** Western blot analyzed the JAK2/STAT3/NF-κB cascades in villin-CXCR7 and WT mice. Higher levels of the JAK2/STAT3/NF-κB cascades were

detected in villin-CXCR7 than those of WT mice. 1, 2, 3 represented samples from three mice. $n = 3$. * $p < 0.05$, ** $p < 0.01$ between villin-CXCR7 and WT mice. Statistics were obtained from two-tailed Student's *t*-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ between WT and WT-AD or villin-CXCR7 and villin-CXCR7-AD or WT-AD and villin-CXCR7-AD. WT-AD wild mice exposed to AOM/DSS, Villin-CXCR7-AD villin-CXCR7 mice exposed to AOM/DSS

Chemokine receptors belong to a superfamily of heptahelical G protein-coupled receptors (GPCRs) located on cell surface where they sense extracellular chemokines and transmit signals that are important for exerting their inflammatory functions [15]. In inflamed mucosal cells and cancer cells, the roles of chemokine receptors expand from the regulation of leukocyte attraction to promotion of cell survival, proliferation, and dissemination [16]. CXCR4 and ligand CXCL12 have attracted more attention due to their roles in immune responses [17]. However, recent studies indicate that previous described monogamous CXCR4/CXCL12 signaling axis might have been oversimplified to account for its roles in the inflammation and tumorigenesis [12]. In fact, adjunct functions of CXCR4 in colitis and tumorigenesis have been suggested in recent reports [18, 19]. CXCL12 was found binding to CXCR7 with greater

affinity than to CXCR4 ($K_d = 0.4$ nM vs. 3.6 nM). The underlying mechanism of CXCR7 in the pathological inflammation and tumorigenesis has not been well understood. Previous studies described CXCR7 as a “scavenger” or “decoy” chemokine receptor in the pathological inflammation [20]. In this study, when exposed to AOM/DSS villin-CXCR7 mice showed more severity of colitis and tumorigenesis than villin-CXCR4 mice. Thus, it is conceivable to speculate that if coupled with CXCR7, CXCR4 might be more effective in promoting in colitis and colitis-driven tumorigenesis.

The formation of CXCR7/CXCR4 heterodimer and its roles in colorectal tumorigenesis has recently attracted attention. Chemokine receptors formed heterodimers as efficiently as homodimers. The CXCR7/CXCR4 heterodimer could augment CXCL12-mediated signals, thus

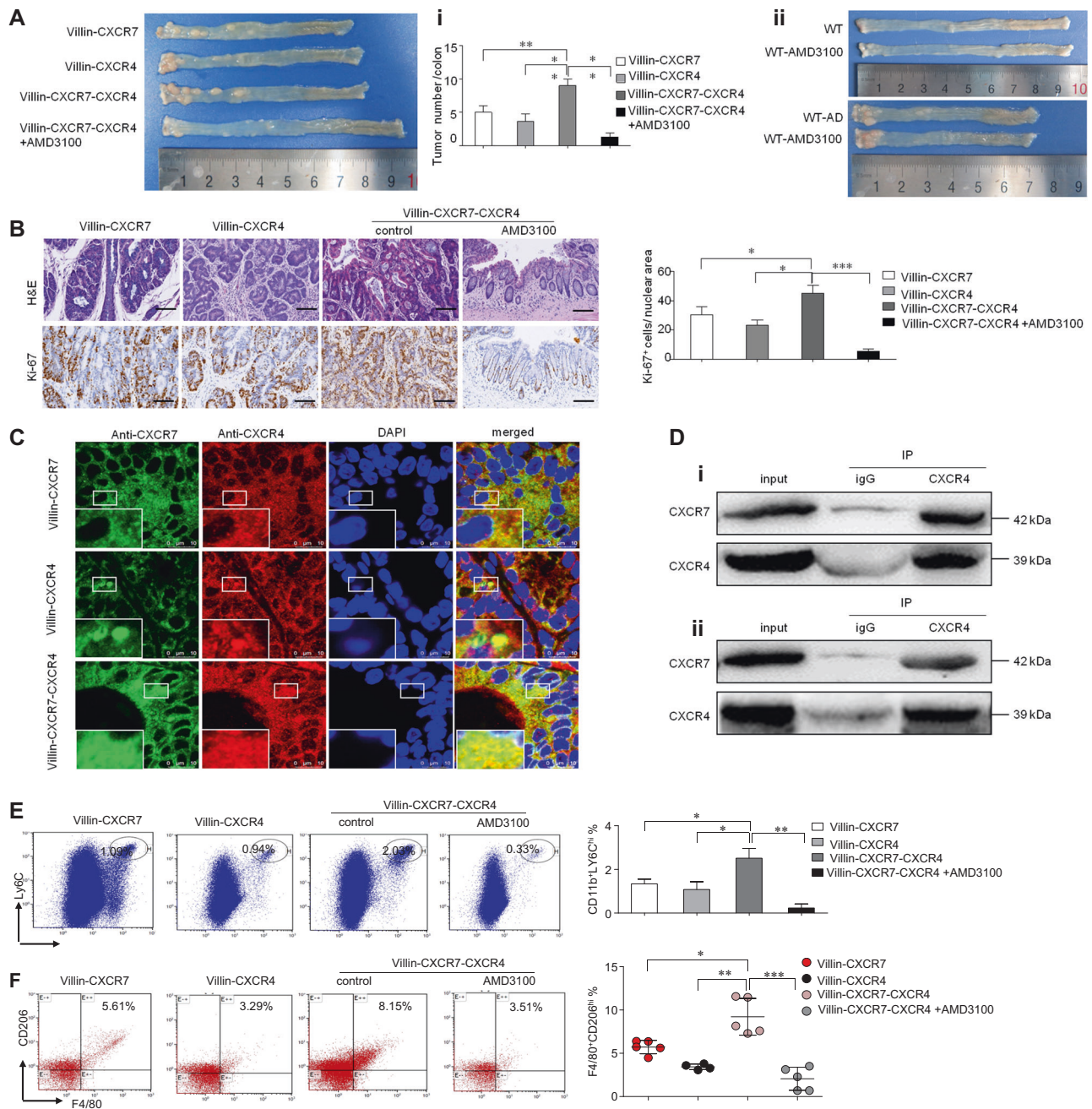


Fig. 5 CXCR7/CXCR4 heterodimer intensifies CXCR7-induced CRC. **a–i** Gross appearance and tumor count of colorectal cancer in villin-CXCR7, villin-CXCR4, villin-CXCR7-CXCR4 mice, and AMD3100-treated villin-CXCR7-CXCR4 mice. AMD3100 strongly inhibited colorectal cancer. **a–ii** AMD3100 alone did not induce colon tumorigenesis, and impact on AOM/DSS-induced colon tumorigenesis in WT mice. $n = 3$. **b** H&E-stained colonic tissues (upper 20x) and IHC analysis of Ki-67 expression (lower 20x) in colorectal cancer tissues. $n = 3$. **c** Immunofluorescent staining of CXCR7 (green), CXCR4 (red), DAPI (blue) and merged images in colorectal cancer of villin-CXCR7, villin-CXCR4, and villin-CXCR7-CXCR4 mice. The CXCR7/CXCR4 colocalization was observed in colorectal cancer of villin-CXCR7-CXCR4 mice. Immunofluorescence scale bar: 10 μ m. **d** Co-IP analysis of the CXCR7/CXCR4 heterodimer in colorectal cancer of villin-

CXCR7-CXCR4 mice (**i**) and *APC^{Min/+}*/villin-CXCR7-CXCR4 mice (**ii**). Anti-CXCR4 antibody was used for Co-IP assay. High CXCR7/CXCR4 heterodimer was clearly observed in colorectal cancer of villin-CXCR7-CXCR4 and *APC^{Min/+}*/villin-CXCR7-CXCR4 mice. **e** Flow cytometric analysis of M-MDSCs in colonic tissues of villin-CXCR7, villin-CXCR4, and villin-CXCR7-CXCR4 mice. The increased M-MDSCs were reduced by AMD3100 in villin-CXCR7-CXCR4 mice. $n = 3$. **f** Flow cytometric analysis of M2-like macrophages in colonic tissues of villin-CXCR7, villin-CXCR4, and villin-CXCR7-CXCR4 mice. The increased M2-like macrophages were reduced by AMD3100 in villin-CXCR7-CXCR4 mice. $n = 4$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ between villin-CXCR4 or villin-CXCR7 and villin-CXCR7-CXCR4 mice. Statistics were obtained from two-tailed Student's *t*-test

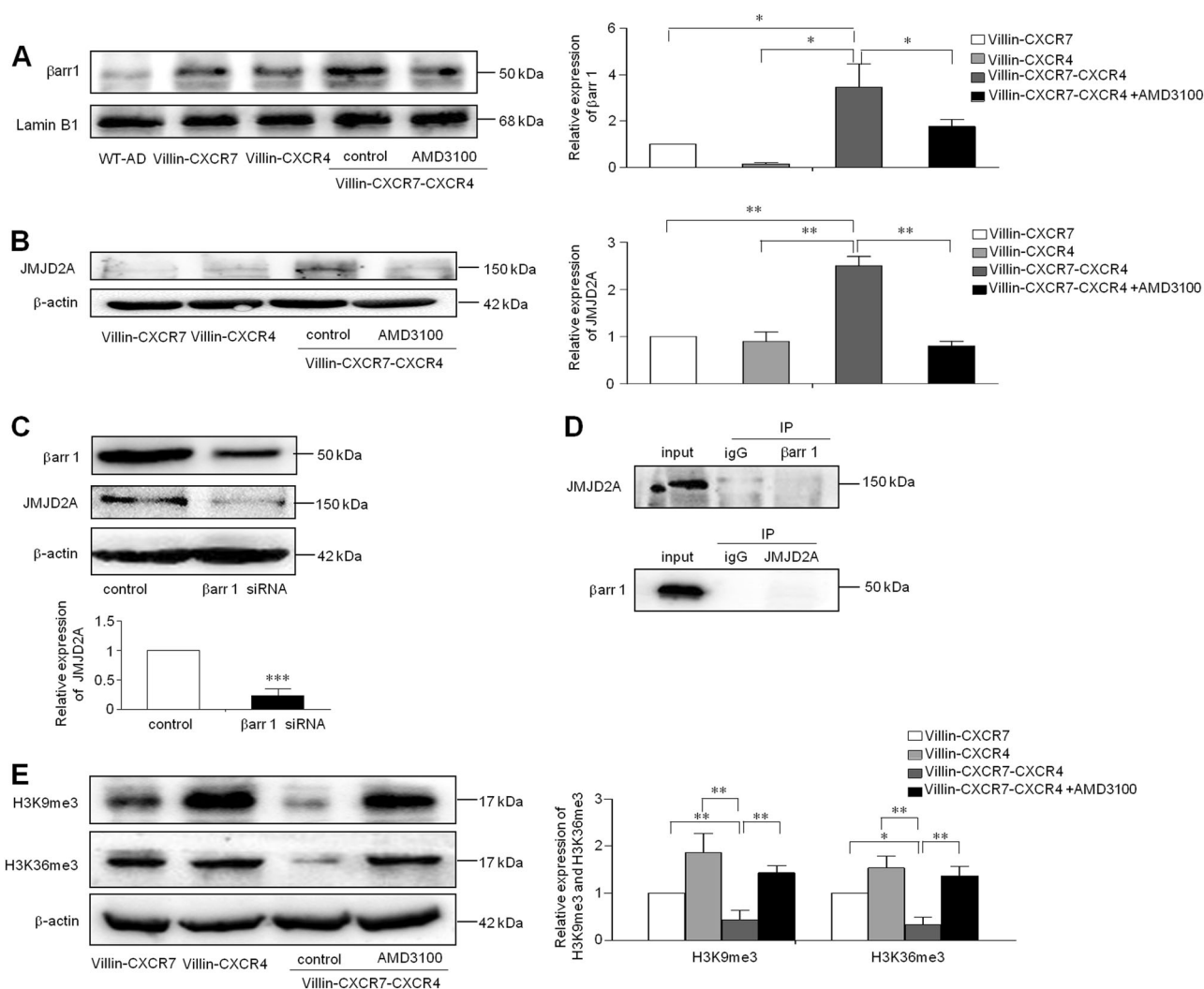


Fig. 6 CXCR7/CXCR4 heterodimer induces βarr1 translocation to the nucleus to promote the JMJD2A-mediated demethylation of histones. **a** Western blotting shows high nuclear βarr1 in colorectal cancer tissues of villin-CXCR4-CXCR7 mice as compared with villin-CXCR7 and villin-CXCR4 mice. AMD3100 significantly reduces CXCR4-CXCR7 heterodimer-induced nuclear βarr1. *n* = 3. Expression was quantified by densitometry and normalized to Lamin B1 (right). **b** Villin-CXCR4-CXCR7 mice showed higher levels of JMJD2A in colorectal cancer tissues as compared with villin-CXCR7 and villin-CXCR4 mice, and the increase was significantly attenuated in the AMD3100-treated villin-CXCR7-CXCR4 mice. *n* = 3. Expression

was quantified by densitometry and normalized to β-actin (right). **c** Knockdown of βarr1 by siRNA significantly reduced JMJD2A in colonic cancer SW480 cells. *n* = 3. **d** Co-IP analysis indicated a lack of interaction between βarr1 and JMJD2A. **e** Western blotting demonstrated reduced nuclear H3K9me3 and H3K36me3 in colorectal cancer tissues of villin-CXCR4-CXCR7 mice as compared with villin-CXCR7 and villin-CXCR4 mice, whereas AMD3100-treated villin-CXCR4-CXCR7 mice showed increased nuclear H3K9me3 and H3K36me3 expression. *n* = 3. **p* < 0.05, ***p* < 0.01, ****p* < 0.001 between villin-CXCR4 or villin-CXCR7 and villin-CXCR7-CXCR4 mice. Statistics were obtained from two-tailed Student's *t*-test

CXCR7/CXCR4 heterodimer might play more important roles than CXCR4 or CXCR7 monomer in colitis-driven tumorigenesis. In human cancer cells, CXCR7 cannot signal directly through the G protein-linked pathway, however, CXCR7/CXCR4 heterodimer is able to facilitate this signaling pathway. In human T lymphocytes, CXCR7 co-expression with CXCR4 was found to regulate the CXCL12-promoted cell migration [11]. CXCR7/CXCR4 heterodimer might constitutively recruit βarr1 and activate levels of the kinases of p38/mitogen-activated protein kinase (MAPK), stress-activated MAP kinases (SAPK), and

extracellular regulated protein kinases 1/2 (ERK1/2), leading to the migration of CXCR4-expressing breast cancer cells [10, 11].

Prior to our study, two important questions remained unanswered: Can CXCR7/CXCR4 heterodimers form *in vivo*? Do CXCR7 monomers and CXCR7/CXCR4 heterodimers signal differently? Herein, we showed that a high level of CXCR7/CXCR4 heterodimer is expressed in 65% of human CRC tissues, indicating the wide expression of the heterodimer in cancer tissues. We generated villin-CXCR4 and villin-CXCR7 mice models and crossed them

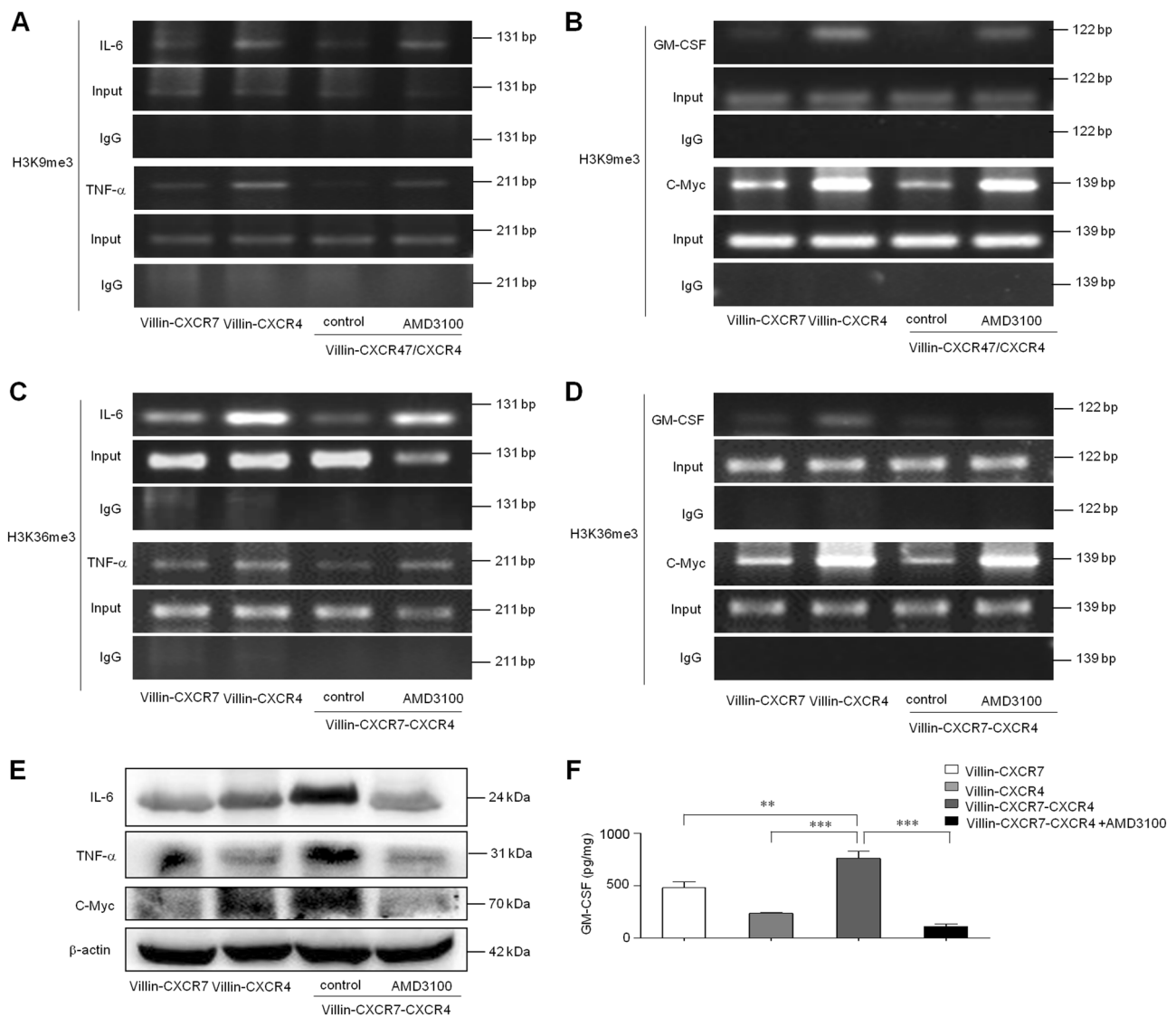


Fig. 7 ChIP assay analyzed the transcription of GM-CSF, M-CSF, IL-6, TNF- α , and C-Myc in villin-CXCR7, villin-CXCR4, villin-CXCR7-CXCR4, and AMD3100-treated villin-CXCR-CXCR4 mice using antibodies against H3K9me3 and H3K36me3. **a** PCR analyzed the transcription of IL-6 and TNF- α gene bound to H3K9me3 in cancer cells of villin-CXCR4, villin-CXCR7, and villin-CXCR-CXCR4 mice. **b** PCR analyzed the transcription of GM-CSF and C-Myc gene bound to H3K9me3 in cancer cells of villin-CXCR4, villin-CXCR7, and villin-CXCR-CXCR4 mice. **c** PCR analyzed the transcription of IL-6 and TNF- α gene bound to H3K36me3 in cancer cells of villin-CXCR4, villin-CXCR7, and villin-CXCR-CXCR4 mice. **d** PCR analyzed the transcription of GM-CSF and C-Myc gene bound to H3K36me3 in cancer cells of villin-CXCR4, villin-CXCR7, and villin-

CXCR-CXCR4 mice. **e** Western blotting analyzed IL-6, TNF- α , and C-Myc in villin-CXCR4, villin-CXCR7, and villin-CXCR-CXCR4 mice. Higher level of these pro-inflammatory mediators was determined higher levels in villin-CXCR4-CXCR7 mice as compared with villin-CXCR7 and villin-CXCR4 mice. AMD3100 strongly reduced the levels of IL-6, TNF- α , and C-Myc in villin-CXCR4-CXCR7 mice. **f** ELISA assay showed significantly higher levels of GM-CSF in villin-CXCR4-CXCR7 mice as compared with villin-CXCR7, and villin-CXCR4 mice. AMD3100 strongly reduced the levels of GM-CSF in villin-CXCR7-CXCR4 mice. $n = 3$. ** $p < 0.01$, *** $p < 0.001$ between villin-CXCR4 or villin-CXCR7 and villin-CXCR7-CXCR4 mice. Statistics were obtained from two-tailed Student's t -test

to produce villin-CXCR7-CXCR4 mice. CXCR7/CXCR4 heterodimer was confirmed in cancer tissues. Villin-CXCR7-CXCR4 mice demonstrated more advanced colitis and CRC than villin-CXCR4 and villin-CXCR7 mice. These results suggest that CXCR7/CXCR4 heterodimer does play an important role in colitis and colitis-driven tumorigenesis.

What happens after CXCR7/CXCR4 heterodimer formation? We found that CXCR7/CXCR4 heterodimer increased the infiltration of M-MDSCs and M2-like macrophages in colonic tissues. Persistent increase of these inflammatory cells produces a favorable microenvironment that further promotes tumorigenesis [21–23]. In this microenvironment, MDSCs could inhibit M1-like

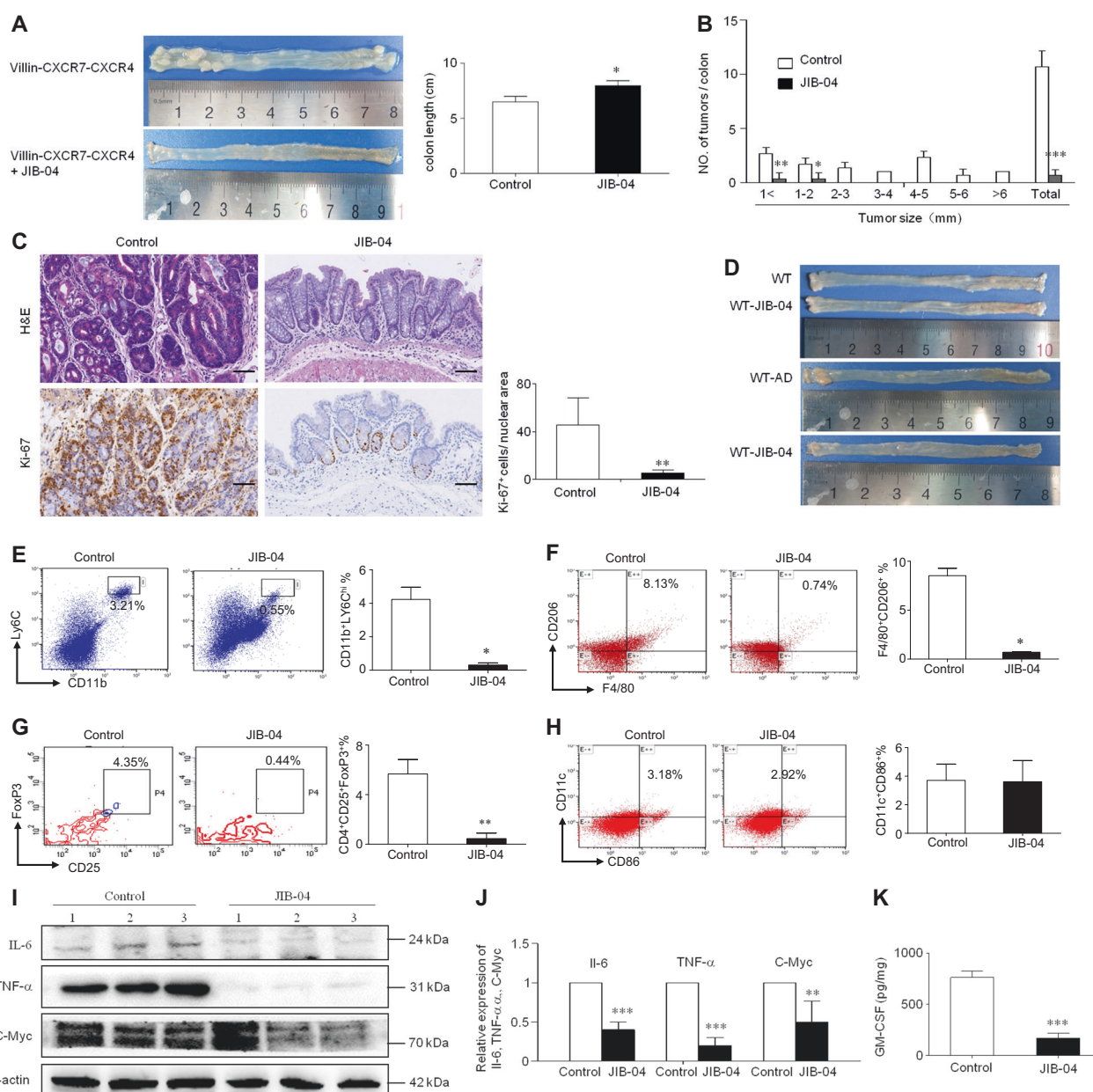


Fig. 8 Inhibition of JMJD2A prevents the CXCR7/CXCR4 heterodimer-induced colorectal cancer in mice. Villin-CXCR7-CXCR4 mice were exposed to AOM/DSS or in combination with JIB-04. **a** Gross appearance of colorectal cancer in villin-CXCR7-CXCR4 mice. JIB-04 treatment resulted in an increase of colorectal length. $n = 3$. **b** Analysis of tumor number and size distribution in colorectal tissues of villin-CXCR7-CXCR4 mice. $n = 3$. **c** Histology of colorectal cancer from villin-CXCR7-CXCR4 mice (upper left H&E 20 $\times$) and colonic tissues from JIB-04-treated villin-CXCR7-CXCR4 mice (upper right H&E 20 $\times$). $n = 3$. Ki-67 index in colorectal cancer from villin-CXCR7-CXCR4 mice (lower left, immunoperoxidase 20 $\times$) and colonic tissues from JIB-04-treated villin-CXCR7-CXCR4 mice (lower right, immunoperoxidase 20 $\times$). $n = 3$. Quantification of Ki-67-positive cells is shown on the far right. **d** JIB-04 did not induce colorectal tumorigenesis (upper), or impact on

AOM/DSS-induced colorectal tumorigenesis in WT mice (lower). **e–g** Flow cytometric analysis showed a significant decrease of M-MDSCs (**e**), M2-like macrophage (**f**), and Treg cells (**g**) in colon tissues of villin-CXCR7-CXCR4 mice treated with JIB-04. $n = 3$. **h** DCs did not show significant changes in villin-CXCR7-CXCR4 mice exposed to AOM/DSS, and JIB-04. $n = 3$. **i** JIB-04 significantly inhibited CXCR7/CXCR4 heterodimer-induced higher levels of IL-6, TNF- α , and C-Myc in villin-CXCR7-CXCR4 mice. 1, 2, 3 represented three mice. $n = 3$. **j** ELISA analysis shows lower levels of IL-6, TNF- α , C-Myc in JIB-04-treated mice than those in untreated control mice. **k** ELISA analysis shows a lower level of GM-CSF in JIB-04-treated mice than those untreated control mice. $n = 3$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ between villin-CXCR7-CXCR4 mice (control) and JIB-04-treated villin-CXCR7-CXCR4 mice (JIB-04). Statistics were obtained from two-tailed Student's t -test

macrophages and T-cell responses, and stimulate Treg cells expansion [24]. MDSCs also produce various inflammatory

cytokines, which further stimulate tumorigenesis [12, 13]. In our study, all villin-CXCR4, villin-CXCR7, and villin-

CXCR7-CXCR4 mice developed advanced colorectal tumorigenesis than WT mice. Villin-CXCR7-CXCR4 mice demonstrated the worst CRC. CXCR4 inhibitor AMD3100 strongly prevented the AOM/DSS-induced colorectal tumorigenesis in villin-CXCR7-CXCR4 mice. These results suggest an essential role of CXCR4 in the CXCR7/CXCR4 heterodimer-induced colorectal tumorigenesis.

β arrrs are cytosolic proteins. β arrrs function as scaffolds for multiple kinases that connect GPCRs to various cytoplasmic effector pathways, such as MAPK and protein kinase B/phosphatidylinositol-4,5-bisphosphate 3-kinase (Akt/PI3K). Activation of GPCRs can induce β arrrs translocation from cytoplasm to cell membrane to bind the phosphorylated receptors, and hence results in receptor endocytosis and attenuation of receptor signaling [25]. Recently, Kang et al. uncovered an important function of nuclear β arrr1 in cancer cells. β arrr1 in response to GPCRs signal could translocate to the nucleus, leading to β arrr1 enrichment at specific promoters such as that of p27 and c-fos, where it facilitates the recruitment of histone acetyltransferase p300, resulting in enhanced local histone H4 acetylation and transcription of these genes [26]. However, the roles of nuclear β arrr1 still remain largely unknown. β arrrs serve as scaffold multifunctional proteins that can interact with multifunctional molecules involved in signal transduction and cellular trafficking, facilitating interprotein interactions with formation of multiprotein complexes [27]. Our result showed that CXCR7/CXCR4 heterodimer translocated much more β arrr1 to the nucleus of cancer cells in villin-CXCR7-CXCR4 mice than in villin-CXCR7 and villin-CXCR4 mice. CXCR7/CXCR4 heterodimer-induced β arrr1 translocation then activated the histone lysine demethylase JMJD2A. β arrr1 silencing led to the inhibition of nuclear JMJD2A. However, a direct interaction of β arrr1 and JMJD2A was not seen in our identified study. These results suggest that the CXCR7/CXCR4 heterodimer-induced nuclear β arrr1 might regulate JMJD2A expression by multiprotein complexes. Here, we show that increased JMJD2A could increase demethylation of H3K9 and H3K36, resulting in transcription of TNF- α , IL-6, C-Myc, and GM-CSF. We thus suggest that CXCR7/CXCR4 heterodimer-induced β arrr1 might translocate to the nucleus and then selectively enrich the promoters of TNF- α , IL-6, C-Myc, where it might facilitate the recruitment of histone demethylase, resulting in enhanced local histone demethylation and transcription of these genes.

JMJD2A is a histone 3 lysine 9 (H3K9) and lysine 36 (H3K36) trimethyl-specific lysine demethylase that catalyzes demethylation of di- and trimethylated Lys9 and Lys36 in histone H3 (H3K9me2/3 and H3K36me2/3) [28, 29]. Although this enzyme can demethylate di- and trimethylated residues at Lys9 and Lys36 of histone 3

(H3K9me2/3 and H3K36me2/3, respectively) *in vitro*, it cannot demethylate monomethylated residues *in vivo*. Thus, we used anti-H3K9me3 and H3K36me3 to analyze demethylated histones H3K9 and H3K36. Villin-CXCR7-CXCR4 mice demonstrated lower levels of trimethylated H3K9 and trimethylated H3K36 than villin-CXCR7 mice and villin-CXCR4 mice. These results indicate that the methyl groups of H3K9me3 and H3K36me3 are removed, suggesting that CXCR7/CXCR4 heterodimer could induce histone demethylation.

H3K9me3 demethylation could promote an open chromatin state, contributing to the transcription of promoter regions [30, 31], wherein JMJD2A activates the ribosomal RNA and activates rDNA transcription or directly acts as a transcriptional repressor or activator and regulates chromatin dynamics through its interactions with nuclear receptor co-repressor (N-CoR), retinoblastoma protein (pRB), histone deacetylases (HDACs), and histones 3 and 4 to transcribe activating protein 1 (AP-1), c-Jun and c-Fos, Ras, CCND1 and c-Jun, etc [32, 33]. However, functions of the JMJD2A-mediated demethylation of H3K36 are less clear. Some studies suggest that H3K36me3 might couple with RNA polymerase II (RNA Pol II) in the transcription elongation [34]. H3K36me3 might also be recognized by DNMT3A, thereby promoting DNA demethylation at nearby DNA regions [35]. Recently, the mechanism for epigenetic modification by Smyd2-mediated H3K36 dimethylation at TNF- α and IL-6 promoters has been proposed [36]. In our study, CXCR7/CXCR4 heterodimer induced high levels of JMJD2A, leading to demethylation of histones H3K9me3 and H3K36me3 than CXCR7 monomer and CXCR4 monomer. As a consequence, villin-CXCR7-CXCR4 mice demonstrated increased transcription of IL-6, TNF- α , GM-CSF, and C-Myc. JIB-04, a specific histone lysine demethylase JMJD2A inhibitor, strongly inhibited CXCR7/CXCR4 heterodimer-induced colorectal tumorigenesis. These results further support the notion that CXCR7/CXCR4 heterodimer drives colorectal tumorigenesis through increasing histone demethylation by activation of lysine demethylase.

In conclusion, based on clinical observations in human CRC tissues, we generated villin-CXCR7, villin-CXCR4, and villin-CXCR7-CXCR4 mice to explore the roles of CXCR7/CXCR4 heterodimer in colitis-driven tumorigenesis. Villin-CXCR7-CXCR4 mice demonstrated more advanced colorectal tumors than villin-CXCR7 and villin-CXCR4 mice. CXCR7/CXCR4 heterodimer induced nuclear β arrr1 to activate demethylase JMJD2A, which leads to the demethylation of histones H3K9me3 and H3K36me3. CXCR7/CXCR4 heterodimer-induced demethylation of histones is thus responsible for advanced colitis and colitis-driven tumorigenesis. This new mechanism of colorectal tumorigenesis opens a window for development of effective

strategies against colitis and colitis-driven tumorigenesis through inhibition of CXCR7/CXCR4 heterodimer-induced histone demethylation.

Materials and methods

Human colonic cancer specimens

The use of human clinical samples was approved by the Ethics Committee of Shandong Tumor Hospital. A total of 20 fresh colonic adenocarcinoma specimens and paired adjacent non-neoplastic tissues were collected from resected specimens between February and October of 2016.

Animals

Experiments related to animal were approved by Animal Welfare Committee of Capital Medical University. Mice were maintained under controlled room temperature and allowed unrestricted access to the standard mouse feed [37]. Villin-CXCR7-IREF and villin-CXCR4-IREF mice were generated by Cyagen Biosciences Inc. (Guangzhou, China). Genotyping was performed by polymerase chain reaction using the primers described in Supplementary Table S3. Villin-CXCR7 and villin-CXCR4 mice were crossed to generate villin-CXCR7-CXCR4 mice. *APC^{Min/+}* mice were obtained from Jackson Laboratory (Bar Harbor, ME). Villin-CXCR7 mice were crossed with *APC^{Min/+}* mice to generate *APC^{Min/+}/villin-CXCR7* mice. Genotyping of *APC^{Min/+}/villin-CXCR7* was performed by polymerase chain reaction using the primers described in Supplementary Table S3.

Cell line, cell culture, and gene silencing

Human CRC cell line SW480 was purchased from American Type Culture Collection (Manassas, Virginia). Cancer cells were maintained in medium supplemented with 10% (v/v) fetal bovine serum, in a humidified atmosphere (5% CO₂–95% air). Human *βarr1* siRNA (5'-AAAGCCUUCUGCGCGGAGAAU-3') was co-transfected into cells using Lipofectamine 2000 (Invitrogen). SiRNA-lipid complex was added to suspension and then incubated at 37 °C for 6–8 h. Medium was replaced and cells were incubated for 48 h before use.

Establishment of colitis and CAC models

Villin-CXCR7, villin-CXCR4, and villin-CXCR7-CXCR4 mice (8–10 weeks, *n* = 6 per group) were injected intraperitoneally with 10 mg/kg AOM (Sigma) and after 7 days, received drinking water containing 1.0% DSS (MP) as

shown in Fig. 2a. Their WT littermates were used for all experiments to ensure the same genetic background. The severity of colitis was scored with DAI as previously described [38].

Histopathology and IHC analysis

Routine hematoxylin–eosin staining of 4 μm thick sections were used for histological analysis of both human and mice tissue. The degree of colitis was graded as previously described [39]. IHC staining was performed using antibodies against CXCL12 (ab18919) (Abcam), JMJD2A (5328), and Ki-67 (12202) (Cell Signaling Technology). IHC scoring was obtained based on the staining intensity and percentage of stained cancer cells as previously described [40].

Flow cytometry analysis

Isolation of immunocytes from colonic tissues was performed using protocols as previously described [41]. Briefly, immunocytes were surface stained for antibodies for 1 h at 4 °C. The cells were washed with fluorescence-activated cell sorting buffer (2% fetal bovine serum–phosphate-buffered saline (PBS)). The data were acquired with an BD LSR Fortessa flow cytometer and analyzed using TreeStar Flowjo software. Information of antibodies used is presented in Supplementary Table S4.

Nuclear protein determination

Fresh colorectal epithelia or tumor tissues (100 mg) were homogenized in PBS (pH 7.2) at 4 °C. Total protein was extracted using a strong version of RIPA lysate (P0023B, Beyotime, China). Nuclear protein was isolated using NE-PER nuclear and cytoplasmic extraction reagent (P0028, Beyotime, China) according to manufacturer's protocols. In all, 1 mg tissue was washed with cold PBS and suspended in 1 ml of isolation buffer containing protease inhibitors and then lysed on ice for 10 min. After homogenization, a mix containing lysate was separated by centrifugation at 1500 × *g* for 10 min at 4 °C. Supernatant is a cytoplasmic protein, and precipitation is used to extract nuclear protein. Supernatant was centrifuged and protein was quantitatively determined using BAC 100 protein determination kit (Sigma).

ELISA

Fresh colorectal mucosal epithelia were homogenized in anti-protease buffer (PBS containing 1 mM EDTA, a protease inhibitor cocktail tablet, and 1 mM phenylmethanesulfonyl fluoride (PMSF)), and were then centrifuged 3000 × *g* for 10 min at 4 °C. TNF-α, IL-6 (Cayman

Chemical), GM-CSF (CSB-E04569m), and M-CSF (CSB-E04659m, CUSABIO) were quantified using ELISA kits according to manufacturer's protocols.

Western blot analysis

Western blotting was performed as previously described using antibodies against STAT3 (12640), P-STAT3 (9145), JAK2 (3230), P-JAK2 (3776), NF- κ B (8242), P-NF- κ B (3033), TNF- α (3707), IL-6 (12912), IL-1 β (12242), JMJD2A (5328), Lamin B1 (13435), c-Myc (9402) (Cell Signaling); CXCR4 (sc-53534 for Co-IP assay, Santa Cruz); CXCR4 (ab1670), CXCR7 (ab38089), β actin (ab32099), H3K9me3 (ab8898), and H3K36me3 (ab9050) (Abcam) antibodies [42].

Immunofluorescence analysis

Colorectal tissues were fixed in 4% paraformaldehyde, and then washed with PBS (pH 7.4) with a 3% dehydroxide solution. Tissues were cut into 5- μ m sections, blocked with 5% bovine serum albumin in PBS for 30 min and incubated with antibodies against CXCR7 (ab38089), CXCR4 (ab1670) (Abcam) at 4 °C overnight, followed by staining with Alexa488-labeled goat anti-rabbit (CA11008S, Invitrogen) or Alexa594-labeled chicken anti-goat (1387734, Invitrogen) for 1 h at room temperature. Cell nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI, Sigma). Images were acquired under confocal laser imaging system (TCS SP8, Leica).

ChIP-PCR assay

ChIP assay was conducted with a ChIP assay kit (Millipore) according to manufacturer's protocol, and then purified DNAs were measured using PCR. Primers are listed in Supplementary Table S5.

Statistical analysis

Data were described as mean $\pm$ S.D. Statistical analysis was done with SPSS/Win19.0 software (SPSS, Chicago, IL). Comparisons between transgenic and WT mice were conducted with a two-tailed Student's *t*-test. A *p*-value <0.05 was considered to be statistically significant. The Spearman correlation was used to determine relationships between CXCR7 and CXCR4, and CXCR7/CXCR4 heterodimer and JMJD2A in human CRC tissues.

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Author contributions X-JQ conceived and designed the study. Z-YS and FW performed experiments. Z-YS wrote the manuscript. S-XC performed statistical analysis. Z-HG provided intellectual inputs and edited the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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