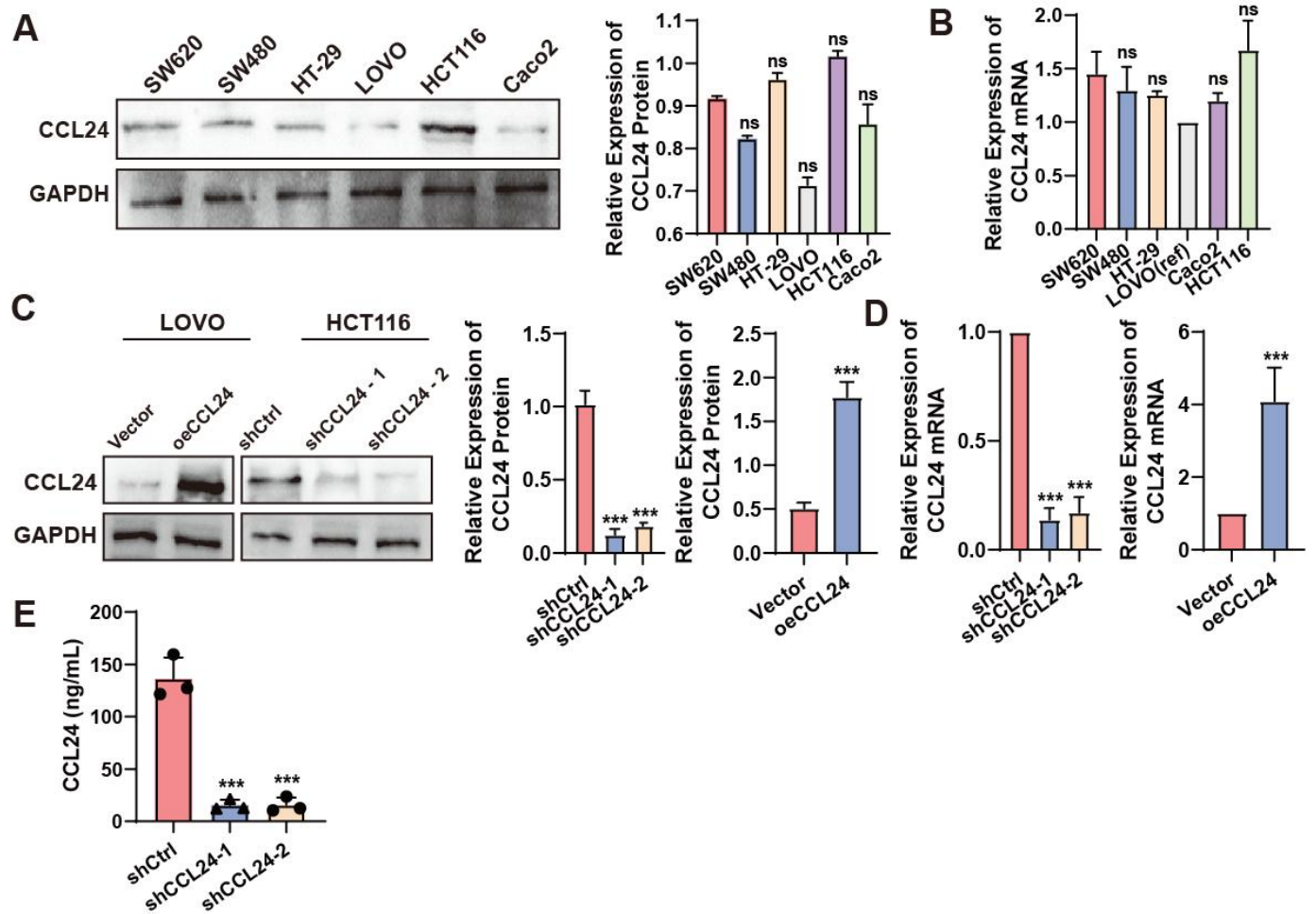


## Supporting Information

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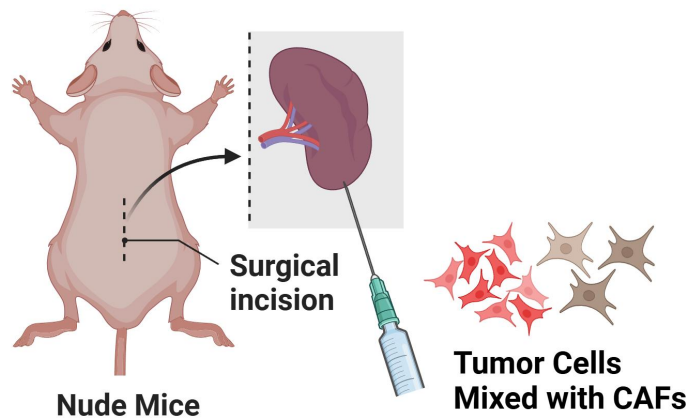
Bioengineered Tumor-Derived Extracellular Vehicles Suppressed Colorectal Cancer Liver Metastasis and Bevacizumab Resistance

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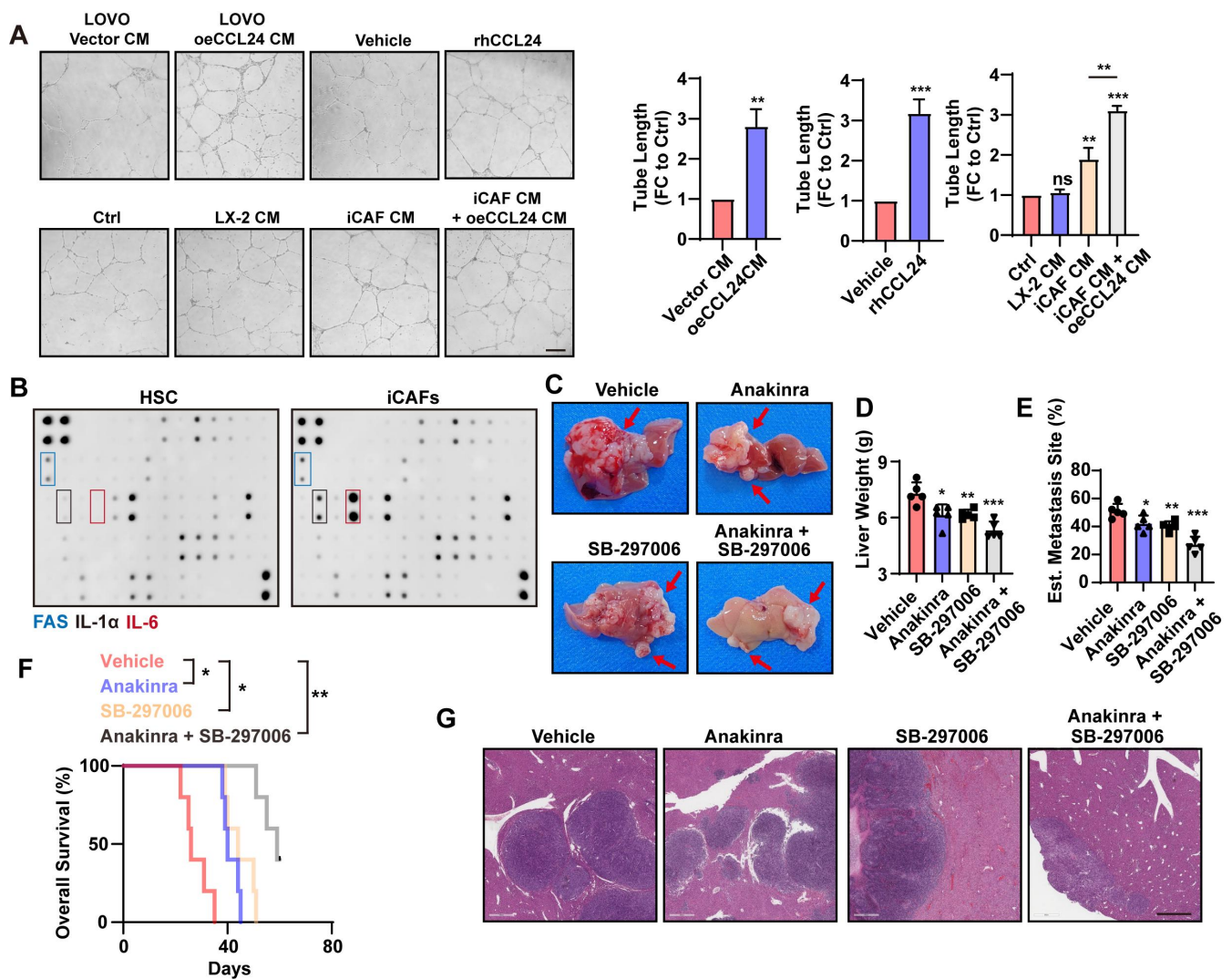
**Figure S1 | Knockdown of CCL24 in tumor cell lines.**

The expression of CCL24 chemokines in CRC cell lines detected by Western-blotting (A) and PCR (B). The HCT116 cell line exhibited a higher level of CCL24 expression, while LOVO demonstrated lower expression of CCL24. (C) The stable knockdown of CCL24 by siRNAs and plasmids. The expression of CCL24 was detected by Western-blotting and PCR analysis. (E) The supernatant of HCT116 culture medium indicated that the CCL24 significantly decreased after the slicing CCL24 mRNA. ns, not significant; \*\*\*,  $P < 0.001$  in the statistics analysis when compared to the control groups.



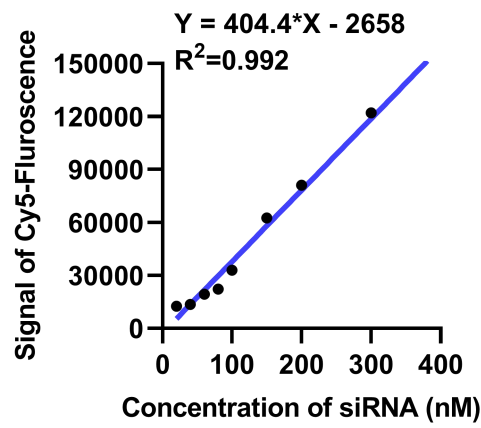
**Figure S2 | The construction model of liver metastasis CRC mice model.**

The human tumor cell lines and human CAF cells were injected into spleen to construct the CRC liver metastasis mice model.



**Figure S3 | HSCs differentiated as iCAFs via CCL24/CCR3/NF- $\kappa$ B pathway.**

(A) Exogenous addition of oeCCL24 LOVO cell culture supernatant to LX-2 cells shows activation of the NF- $\kappa$ B signaling pathway, and the CCR3 receptor blocker SB-297006 can antagonize the activation of the NF- $\kappa$ B signaling pathway induced by CCL24. The tube formation assay implied that the tumor cell originated CCL24 promote vessel formation. (B) In the tumor microenvironment with elevated iCAFs proportion, the levels of cytokines such as IL-1 $\alpha$  and IL-6 are significantly increased. IL-1 $\alpha$  and tumor-derived CCL24 bind to IL-1R and CCR3 receptors on the surface of HUVEC cells, respectively, promoting tumor-associated angiogenesis and antagonizing the therapeutic effect of Bev, mediating resistance to Bev treatment; The in vivo experiments suggest that the use of IL-1R and CCR3 receptor inhibitors significantly improves the therapeutic effect of Bev in mCRC. (C) The representative image, the weight (D), estimated metastasis site of resected liver (E). (F) The combination of SB-297006 and Anakinra significantly improve the therapy effect in mCRC mice model. (G) The representative HE staining of the resected metastatic tumor tissue, Scale bar = 500  $\mu$ m. ns, not significant; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$  when compared to the control groups.

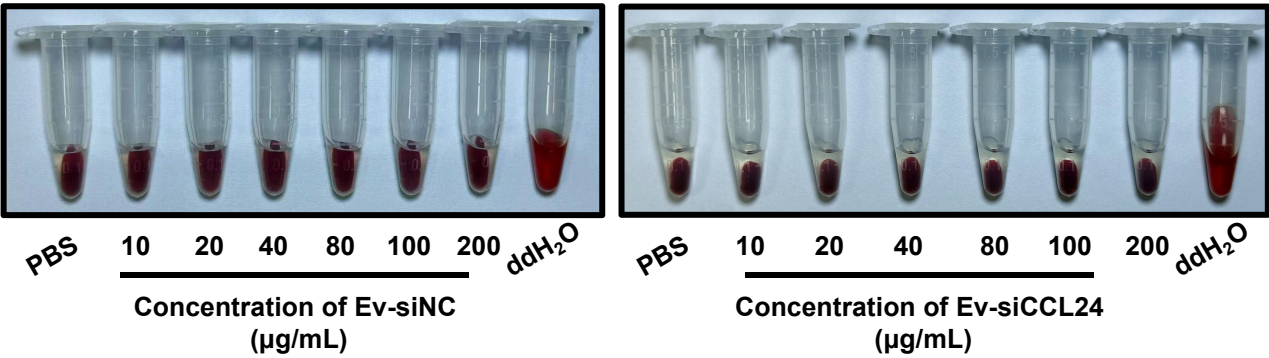


**Figure S4 | The standard curves of siRNA concentration and signal intensity of Cy5 fluorescence.**



**Figure S5 | The biodistribution analysis of the bioengineered EVs in PDX model.**

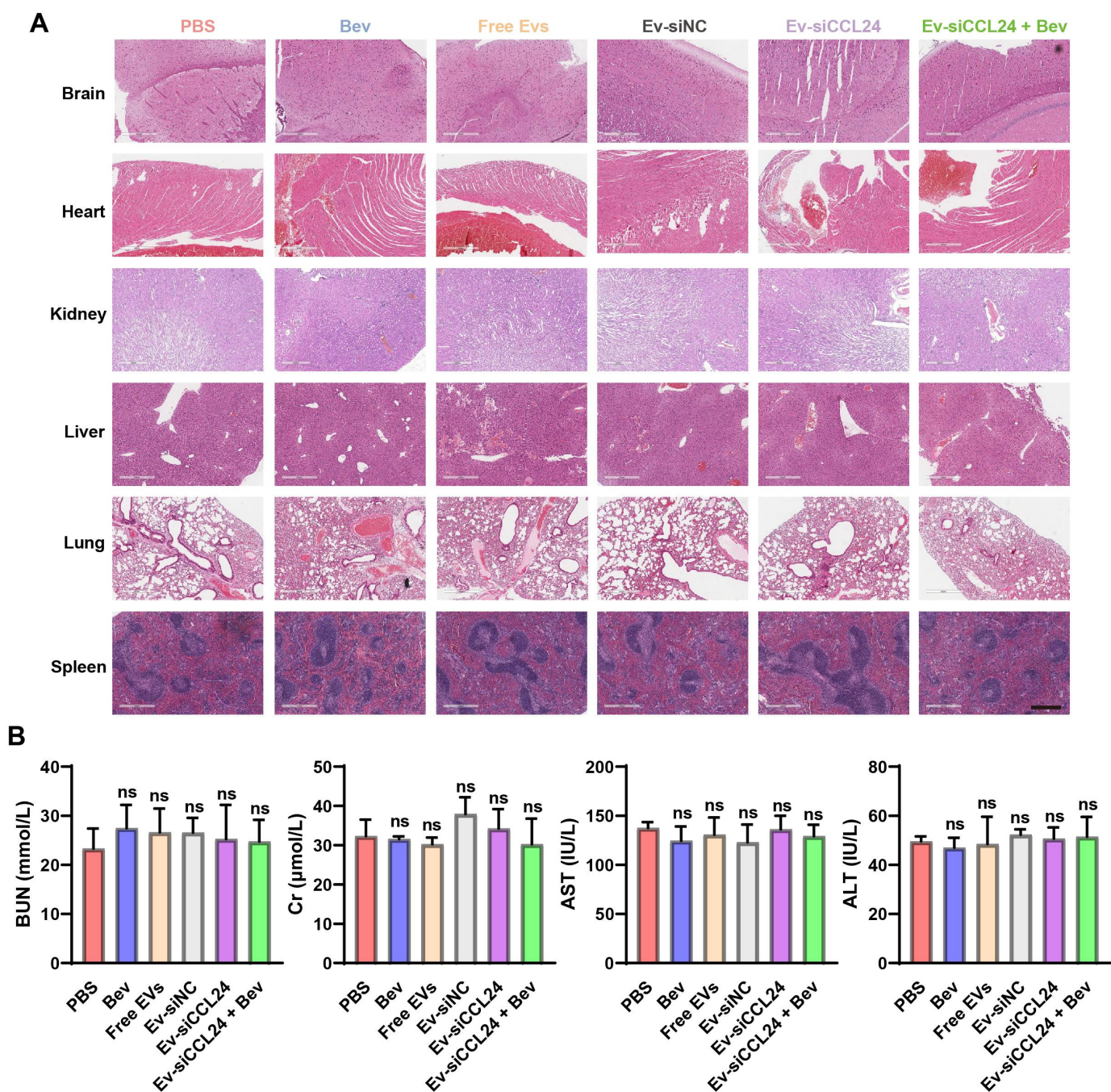
At 4 h after i.v. injection of EV-siNC-Cy5 or free siNC-cy5, fluorescence imaging showed accumulation in the tumor with the EVs transporter. Without the EVs, the no fluorescence was detected in the major organs.



**Figure S6 | The bio-compatibility test of bioengineered EVs in blood circulation.**

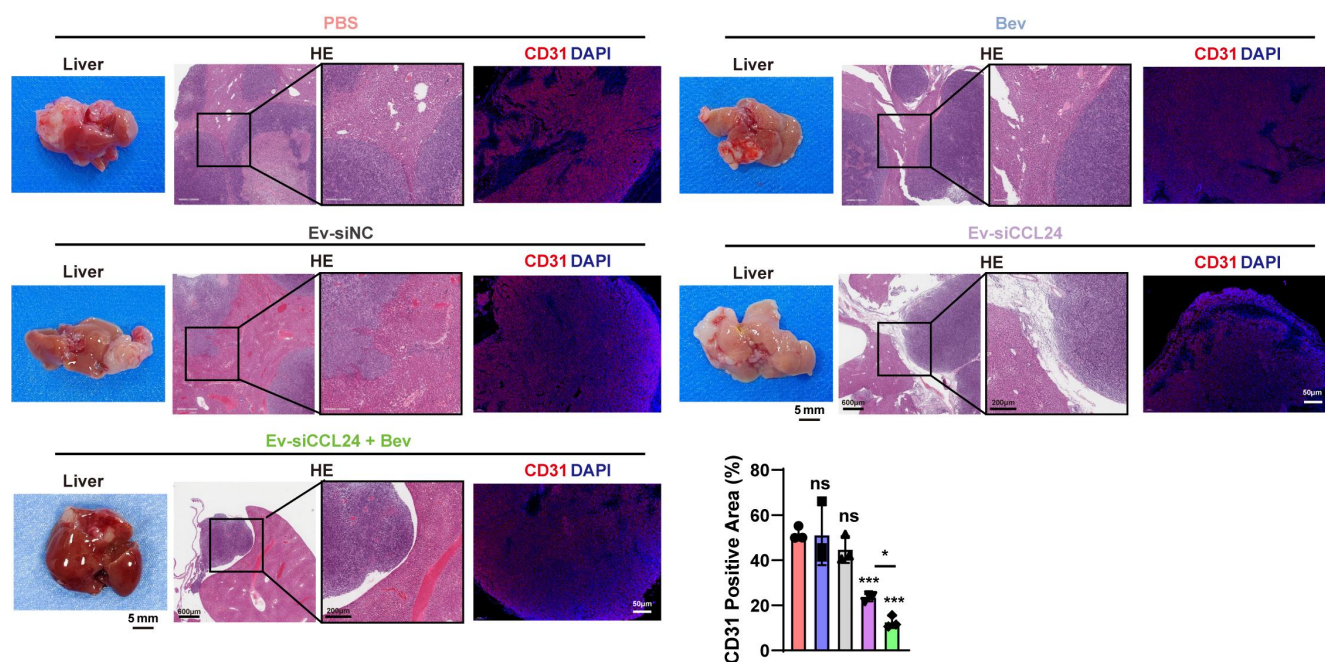
The bioengineered EVs populations did not cause hemolytic reaction *in vitro*.





**Figure S7 | Biosafety analysis of the bioengineered EVs in PDX model.**

(A) The HE staining analysis revealed that no obvious toxicity was found in the major organs and all the PDX mice survived at the end of the *in vivo* experiments, Scale bar = 600  $\mu\text{m}$ . (B) The serum tests of BUN, Cr, AST, ALT revealed that there were no obvious changes among the groups. ns, not significant



**Figure S8 | The images of the resected liver metastatic tumors and its corresponding HE staining, IF analysis .**

The HE staining and CD31 IF analysis revealed that the combination usage of Ev-siCCL24 and Bev significantly reduced the vessel formation in metastatic tumor tissue. The scale bar have been marked in the images. ns, not significant; \*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$  when compared to the control groups.

**Table S1** | Clinical Information on patients whose tumors were collected for RNA sequence.

Patient No.	Age	Gender	Primary Tumor Location	Pathology Type	Date of Primary Tumor Specimen Collection	Date of Metastasis Tumor Specimen Collection
#1	45	Male	Colon	Adenocarcinoma	2019.3	2021.1
#2	59	Female	Rectum	Adenocarcinoma	2019.5	2020.12
#3	53	Male	Colon	Adenocarcinoma	2020.1	2021.5
#4	52	Male	Colon	Adenocarcinoma	2021.5	NA
#5	66	Female	Colon	Adenocarcinoma	2021.6	NA
#6	71	Female	Colon	Adenocarcinoma	2021.5	NA

NA: Not available.

**Table S2** | Sequence of siRNAs sequences used in the research.

Gene	Sequence
siCCL24-1	Sense: 5'-AAUUCUCUUGGAAACAAAGAA-3'
	Anti-Sense: 5'-CUUUGUUUCCAAGAGAAUUCC-3'
siCCL24-2	Sense: 5'-AAGAAACAGGAAAAUUAGCUC-3'
	Anti-Sense: 5'-GCUAAUUUUCUGUUUCUUAG-3'
siNC	Sense: 5'-UUCUUCGAACGUGUCACGUTT-3
	Anti-Sense: 5'-ACGUGACACGUUCGGAGAATT-3'

**Table S3** | Sequence of qRT-PCR Primers used in the research.

Gene	Primers	Sequence (5'-3')
CCL24	Forward	CTGTTACCTCCGGGTCCTTT
	Reverse	GGGGAAGCCTTCTTCTGCTT
ACTA2	Forward	GTTCCGCTCCTCTCTCCAAC
	Reverse	TAGTCCCGGGGATAGGCAAA
ACTG2	Forward	CGCCCTCGCCACCAGAT
	Reverse	CTGTCAGCAATGCCAGGGTA
COL1A1	Forward	TGACGAGACCAAGAACTGCC
	Reverse	GCACCATCATTTCCACGAGC
COL2A1	Forward	GTAGAGACCCGGACCCGC
	Reverse	ACTCTCCGAAGGGGATCTCA
IL1A (IL1 $\alpha$ )	Forward	TGGCGTTTGAGTCAGCAAAG
	Reverse	AGCACACCCAGTAGTCTTGC
IL1B (IL1 $\beta$ )	Forward	TCGCCAGTGAAATGATGGCT
	Reverse	TATCCTGTCCCTGGAGGTGG
IL6	Forward	CCTTCGGTCCAGTTGCCTT
	Reverse	AGCTGCGCAGAATGAGATGA
CXCL1	Forward	CTGGCGGATCCAAGCAAATG
	Reverse	GCCCCTTTGTTCTAAGCCAG
CXCL5	Forward	AAGTTCCCTCCCCACTCACA
	Reverse	GGCATCTAAAAAGCTCAGCAAT
GAPDH	Forward	TTCCAGTGGCTGCTGTTGTT
	Reverse	TTTCCGGGCGTAAAAGCACT



**Table S4 |** Antibodies and its dilutions.

Antigen	Host or Isotype	Manufacturer and Cat. No.	Dilution (x-fold) for Western-Blots	Dilution (x-fold) for IHC or IF	Dilution (x-fold) for Flow Cytometer
CD31	Rabbit	Proteintech; 11265-1-AP	Not available	200	Not available
CCL24	Rabbit	Proteintech; 22306-1-AP	1000	200	Not available
GAPDH	Rabbit	Abcam; ab9485	2000	Not available	Not available
Akt	Rabbit	Proteintech; 10176-2-AP	1000	Not available	Not available
p-Akt	Rabbit	Proteintech; 66444-1-Ig	1000	Not available	Not available
p65	Rabbit	Proteintech; 10745-1-AP	1000	Not available	Not available
p-p65	Rabbit	Proteintech; 82335-1-RR	1000	Not available	Not available
β-actin	Rabbit	Proteintech; 20536-1-AP	2000	Not available	Not available
Hsp70	Mouse	Abcam; ab2787	1000	Not available	Not available
TSG101	Mouse	Proteintech; 28283-1-AP	1000	Not available	Not available
CD63	Mouse	Abcam; ab21286	1000	Not available	Not available
PDPN	Mouse	Proteintech; 67432-1-Ig	Not available	200	Not available
IL-6	Rabbit	Proteintech; 83747-5-RR	Not available	200	Not available
Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594)	Goat	Abcam; ab150080	Not available	200	Not available
Donkey Anti-Mouse IgG H&L (Alexa Fluor® 488)	Donkey	Abcam; ab150105	Not available	200	Not available
APC anti-human CCR3	Mouse IgG1, κ	Biolegend; 310707	Not available	Not available	200
FITC anti-human PDPN	Rat IgG2b, κ	Biolegend; 337025	Not available	Not available	200
PE anti-human CCR3	Mouse IgG1, κ	Biolegend; 310705	Not available	Not available	200
APC Mouse IgG1, κ Isotype Ctrl	Mouse IgG1, κ	Biolegend; 400119	Not available	Not available	1000
FITC Rat IgG2b, κ Isotype Ctrl	Rat IgG2b, κ	Biolegend; 400605	Not available	Not available	1000
PE Mouse IgG1, κ Isotype Ctrl	Mouse IgG1, κ	Biolegend; 400112	Not available	Not available	1000
Goat Anti-Rabbit IgG H&L (HRP)	Goat	Abcam; ab97051	5000	Not available	Not available
Goat Anti-Mouse IgG H&L (HRP)	Goat	Abcam; ab6789	5000	Not available	Not available