

Supplemental material

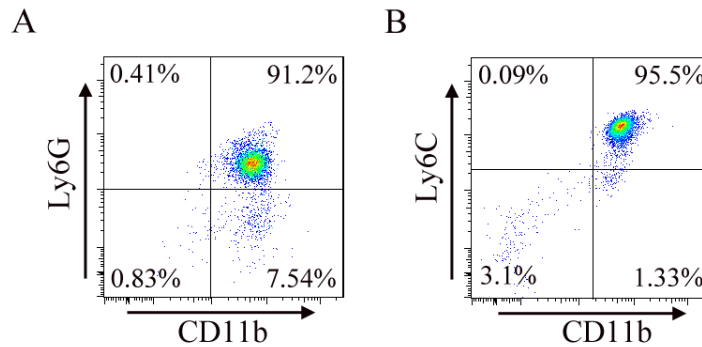


Figure S1. The purity of G-MDSCs or M-MDSCs were analyzed by FACS.

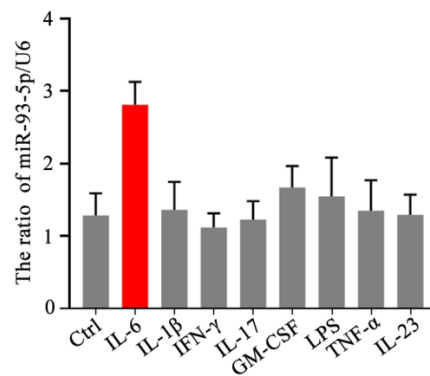


Figure S2. qRT-PCR analysis of the effect of inflammatory molecules on the expression of miR-93-5p in G-MDSCs. Splenic G-MDSCs were treated with various factors in the presence of GM-CSF. After 48 h incubation at 37°C, 5% CO₂, cells were collected and the levels of miR-93-5p were detected by qRT-PCR. Experiments were performed three times by using triplicate samples per group.

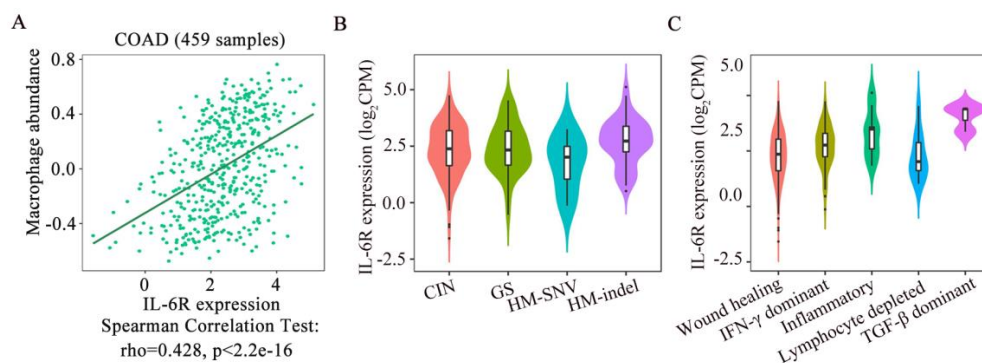


Figure S3. The correlations between IL-6R expression and risk factors for COAD were predicted with TISIDB database.

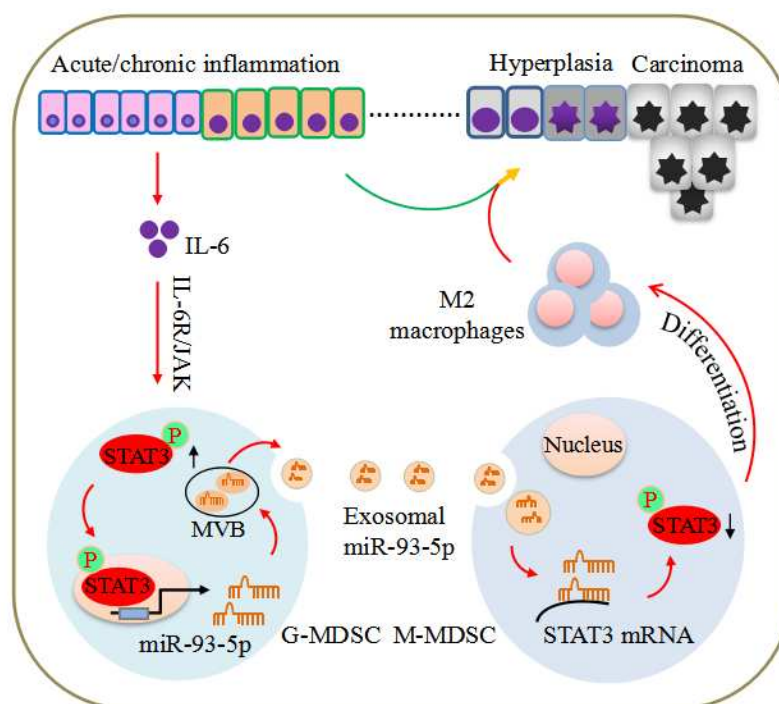


Figure S4. Schematic illustration of GM-Exo-mediated differentiation of M-MDSC into M2 macrophages to promote colitis-cancer transformation. During chronic inflammation, IL-6 promotes miR-93-5p synthesis in G-MDSCs by activating the JAK/STAT3 pathway via IL-6R and increases the abundance of miR-93-5p in GM-Exo. GM-Exo inhibits STAT3 activity in M-MDSCs via miR-93-5p, resulting in the differentiation of M-MDSC into M2 macrophages, which promotes the transformation of colitis to colorectal cancer.

Table S1. Primers for quantitative RT-PCR.

Gene	Sequence
<i>TNF-α</i>	F: 5'-GGTTCTGTCCCTTTCACCTCAC-3'
	R: 5'-TGCCTCTTCTGCCAGTTCC-3'
<i>iNOS</i>	F: 5'-CACCTTGGAGTTCACCCAGT-3'
	R: 5'-ACCACTCGTACTTGGGATGC-3'
<i>IL-12p40</i>	F: 5'-AGGTGCGTTCCTCGTAGAGA-3'
	R: 5'-AAAGCCAACCAAGCAGAAGA-3'
<i>IL-10</i>	F: 5'-TGCTAACCGACTCCTTAATGCA-3'
	R: 5'-TCATGGCCTTGTAGACACCTTG-3'
<i>TGF-β1</i>	F: 5'-TTGCTTCAGCTCCACAGAGA-3'
	R: 5'-TGGTTGTAGAGGGCAAGGAC-3'
<i>Arg-1</i>	F: 5'-ACAGAGAAGGTCTCTACATCAC-3'
	R: 5'-CGAAGCAAGCCAAGGTTAAAGC-3'
<i>CCL2</i>	F: 5'-CAGCAAGATGATCCCAATGA-3'
	R: 5'-GTAGGTTCTGATCTCATTTG-3'
<i>IL-6</i>	F: 5'-AGTTGCCTTCTTGGGACTGA-3'
	R: 5'-TCCACGATTCCCAGAGAAC-3'
<i>Mi-R93-5p</i>	F: 5'-ACACTCCAGCTGGGTCCTGTACT GACGTGCCC-3'
	R: 5'-CTCAACTGGTGTCTGTTGGA-3'
<i>Gapdh</i>	F: 5'-TGGATTGGACGCATTGGTC-3'
	R: 5'-TTTGCCTGGTACGTGTTGAT-3'
<i>U6</i>	F: 5'-ATTGG AACGATACAGAGAAGATT-3'

R: 5'-GGAACGCTTCACGAATTTG-3'

Reagents

Ly-6G-PE (RB6-8C5, Invitrogen). CD11b-FITC (M1/70, Invitrogen). Ly-6C-APC (1A8, Invitrogen). CD45-PE-Cy5.5 (30-F11, Invitrogen). CD68-PE (FA-11, Invitrogen). CD11b-APC (M1/70, Invitrogen). F4/80-PE (BM8, Invitrogen). CD206-PE (MR6F3, Invitrogen). p-STAT3-FITC (#4323, CST). CD3-PE (17A2, Invitrogen). CD8 α -APC (53-6.7, Invitrogen). The recombinant mouse IL-6 (50136-MNAE, SinoBiological). IL-1 β (50101-MNAE, SinoBiological). IFN- γ (50709-MNAH, SinoBiological). IL-17 (51065-MNAE, SinoBiological). GM-CSF (51048-MNAH, SinoBiological). LPS (#14011, CST). TNF- α (50349-MNAE, SinoBiological). IL-23 (CT028-M08H, SinoBiological). Anti mouse CD3 ϵ activated mAb (eBioscience). Anti mouse CD28 activated mAb (eBioscience). Mouse IL-6 ELISA Assay kit (Multi Sciences, Hangzhou, China). Mouse MDSC isolation kit (Miltenyi Biotec, Auburn, CA). PKH67 membrane dye (Sigma-Aldrich). siRNA Rab27a, miR-93-5p inhibitors, and the miDETECT A TrackTM miRNA qRT-PCR Starter Kit (C10712-2) were purchased from RiboBio Co. EntransterTM-R4000 (Engreen Biosystem Co). ExoELISA complete kit (CD63 detection) (EXOEL-CD63A-1, SBI). AOM (02180319, MPBiomedicals). Dextran Sulfate Sodium Salt (02160110, MPBiomedicals). qRT-PCR Kit (B639277, Sangon Biotech). Mouse CD4⁺ T Cell Isolation Kit (130-104-454, Miltenyi Biotec). Hoechst 33342 (B2261, Sigma-Aldrich). ExoQuick-TCTM Exosomes Kits (PM-EQPL10TC-1, SBI). Micro BCA Protein Assay Kit (C503061-1250, Sangon Biotech). Mouse IL-6 ELISA Kit (EK206/3-96, Multi Sciences). 8-well chamber slides (C7182, Sigma). Cucurbitacin I (JSI-124, Sigma Aldrich). Murine antibodies to IL-6 (MP5-20F3, Invitrogen). The primary antibodies for GAPDH (ab8245, Abcam), p-STAT3 (ab267373, Abcam), STAT3 (ab68153, Abcam), p-JAK1 (#74129, CST), JAK1 (ab133666, Abcam), p-JAK2 (ab32101, Abcam), JAK2 (ab108596, Abcam), NICD (ab52627, Abcam), HES1 (ab71559, Abcam), p38 (ab170099, Abcam), p-p38 (#4511, CST), p-ERK (ab229912, Abcam), ERK (ab32537, Abcam), p-PI3K (#17366, CST), PI3K (ab278545, Abcam), p-AKT (ab38449, Abcam), and AKT (ab8805, Abcam) were all commercially available. CellTraceTM CFSE (C34554, Invitrogen).