Figure S1. Quantification of OSM expression in CRC cell lines. (A) Relative OSM expression levels in various CRC cell lines were measured via RT-qPCR. (B) OSM expression was measured via RT-qPCR in OSM overexpression cell line HCT116 and RKO. OSM, oncostatin M; CRC, colorectal cancer; RT-qPCR, reverse transcription-quantitative PCR.

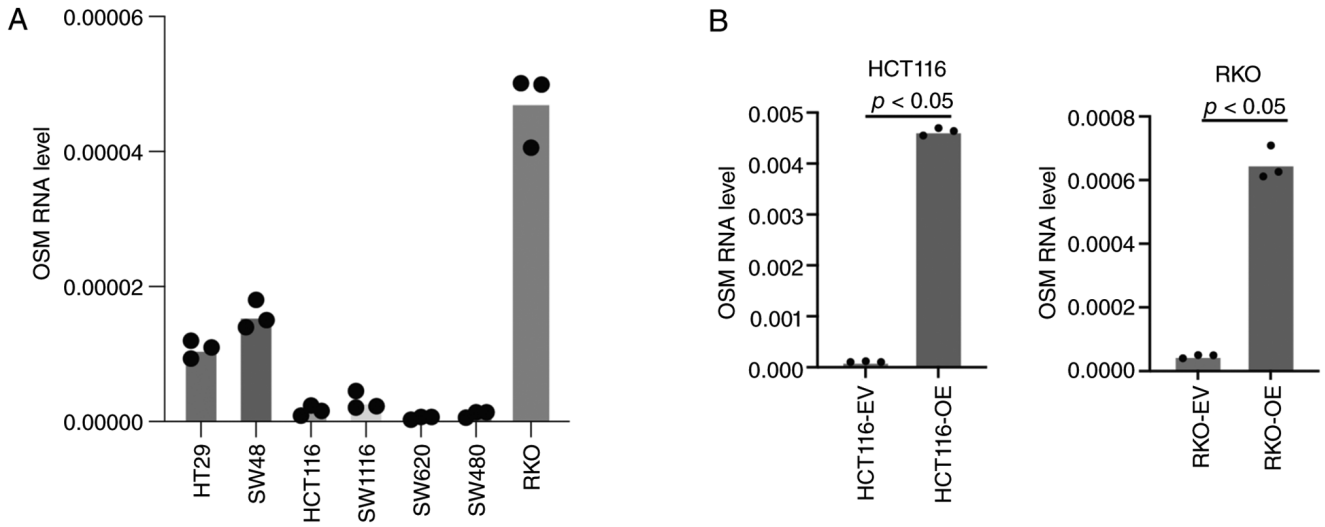
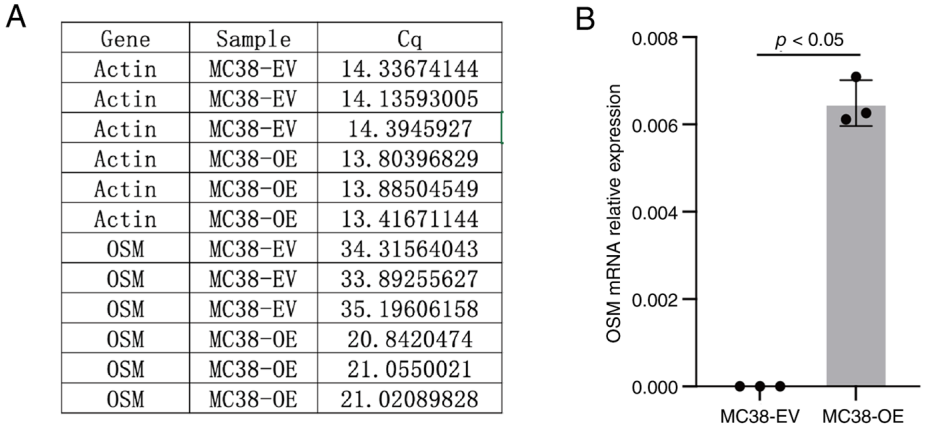
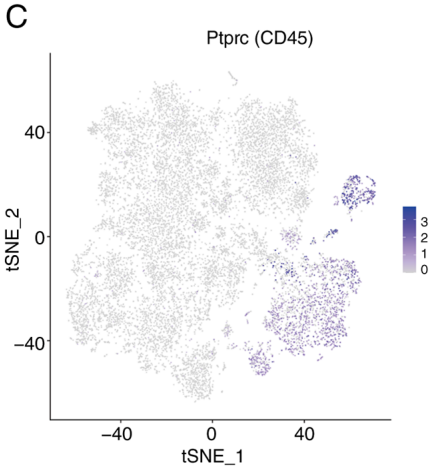


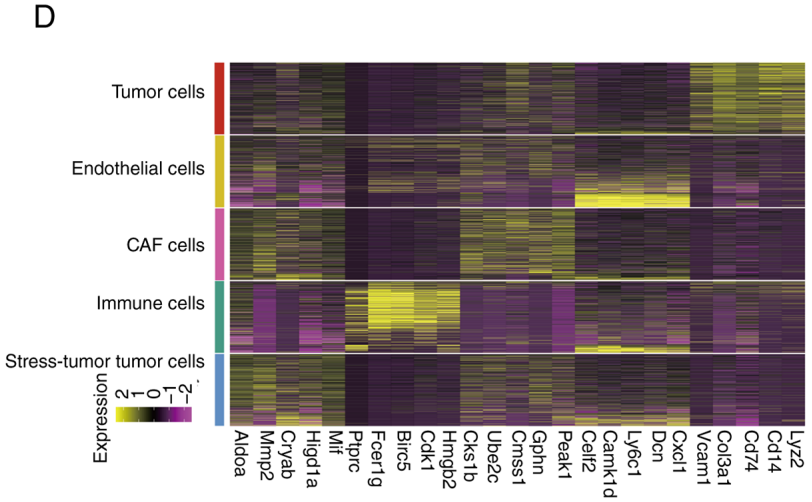
Figure S2. Validation of OSM overexpression in MC38 cells and annotation of cell populations in the scRNA-seq dataset. OSM overexpression cell line was constructed in MC38 cell line and scRNA-seq was performed in subcutaneous tumor model in C57/B6 mouse. Then, cell cluster analysis was performed. (A) Raw quantification cycle (Cq) values for Osm and the reference gene Actin in MC38 cells transduced with the empty vector (MC38-EV) or the OSM-overexpression construct (MC38-OE). (B) Relative Osm mRNA expression in MC38-EV and MC38-OE cells was determined by reverse transcription-quantitative PCR. Data are presented as the mean ± SD; n=3 replicates. Statistical significance was assessed using a two-sided unpaired Student's t-test. (C) t-SNE feature plot showing the expression distribution of Ptprc (CD45) across cells in the scRNA-seq dataset. (D) Heatmap showing the scaled expression of representative marker genes used to identify the major tumor, stromal, Ly6c1-expressing stromal, immune, and stress-associated tumor-cell populations. (E) Dot plot showing the expression of representative marker genes across the annotated cell populations and tumor-cell subclusters. Dot size represents the percentage of cells expressing the indicated gene, and color intensity represents the scaled average expression level. OSM, oncostatin M; scRNA-seq, single cell RNA sequencing.

A

Gene	Sample	Cq
Actin	MC38-EV	14.33674144
Actin	MC38-EV	14.13593005
Actin	MC38-EV	14.3945927
Actin	MC38-OE	13.80396829
Actin	MC38-OE	13.88504549
Actin	MC38-OE	13.41671144
OSM	MC38-EV	34.31564043
OSM	MC38-EV	33.89255627
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OSM	MC38-OE	20.8420474
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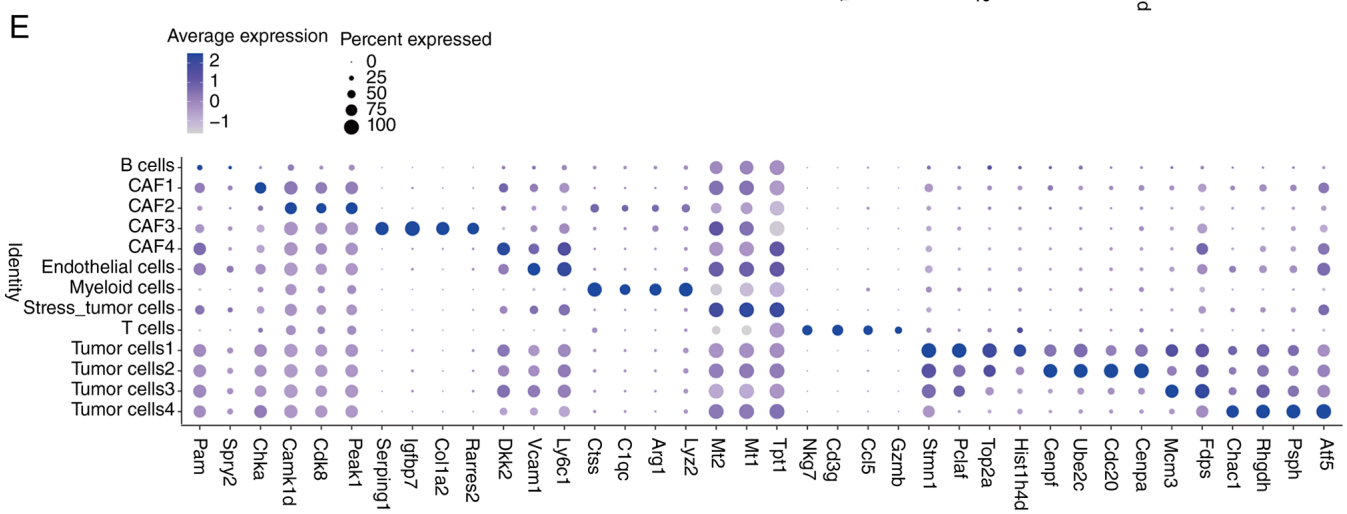


Figure S3. OSM effects the TME of colorectal cancer based on scRNA-seq data analysis. TME was further analyzed after OSM overexpression based on scRNA-seq data. (A) Bubble chart of gene expression in myeloid cell subsets. (B) Heatmap of high expression genes in identified clusters. OSM, oncostatin M; TME, tumor microenvironment; scRNA-seq, single cell RNA sequencing; MDSC, myeloid-derived suppressor cell.

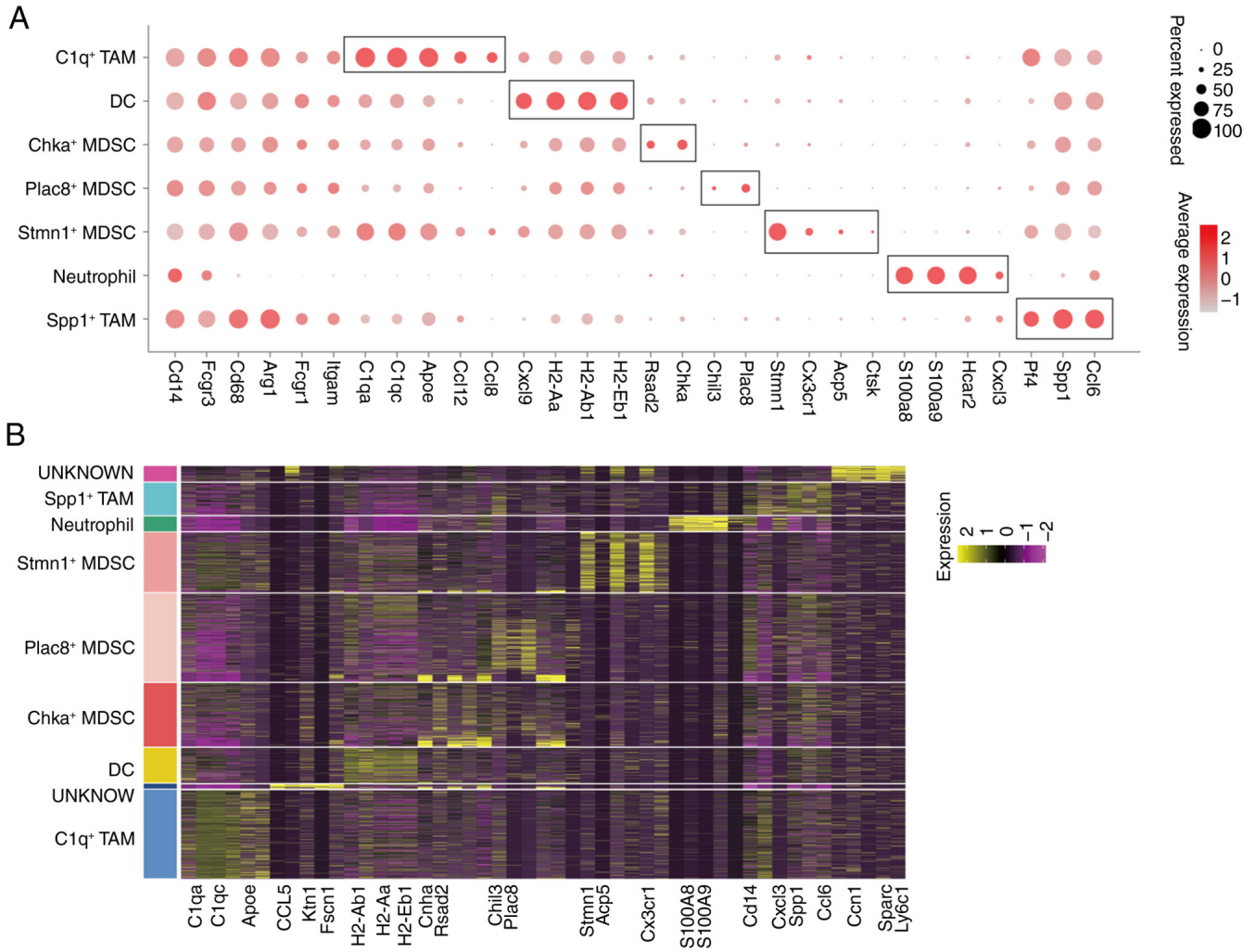


Figure S4. Transcriptional alterations in C1q⁺ TAMs following OSM overexpression. Myeloid cell analysis after OSM overexpression in MC38 cells based on scRNA-seq in subcutaneous tumor model of C57/B6 mouse. (A) Volcano plot showing differentially expressed genes in C1q⁺ TAMs between the OSM-OE and OSM-EV groups. Red and blue indicate upregulated and downregulated genes, respectively, and selected genes are labeled. (B) KEGG pathways enriched among genes upregulated in C1q⁺ TAMs from the OSM-OE group. Dot size represents the number of genes, and color represents the adjusted P-value. (C) KEGG pathways enriched among genes downregulated in C1q⁺ TAMs from the OSM-OE group. Dot size represents the number of genes, and color represents the adjusted P-value. (D) Dot plot showing the expression of selected Hallmark gene sets in C1q⁺ TAMs from the OSM-EV and OSM-OE groups. Dot size represents the percentage of cells expressing genes within the indicated signature, and color represents average scaled expression. (E) Gene set enrichment analysis of the HALLMARK_IL6_JAK_STAT3_SIGNALING gene set in C1q⁺ TAMs between the OSM-OE and OSM-EV groups. The running enrichment score and positions of genes in the ranked list are shown. (Because one pooled scRNA-seq library was available per condition, differential-expression and pathway-enrichment results were considered exploratory cell-level descriptions and were not interpreted as estimates of between-animal biological variability). TAMs, tumor-associated macrophages; OSM, oncostatin M; scRNA-seq, single cell RNA sequencing; KEGG, Kyoto Encyclopedia of Genes and Genomes; OE-, overexpressing; EV, empty vector.

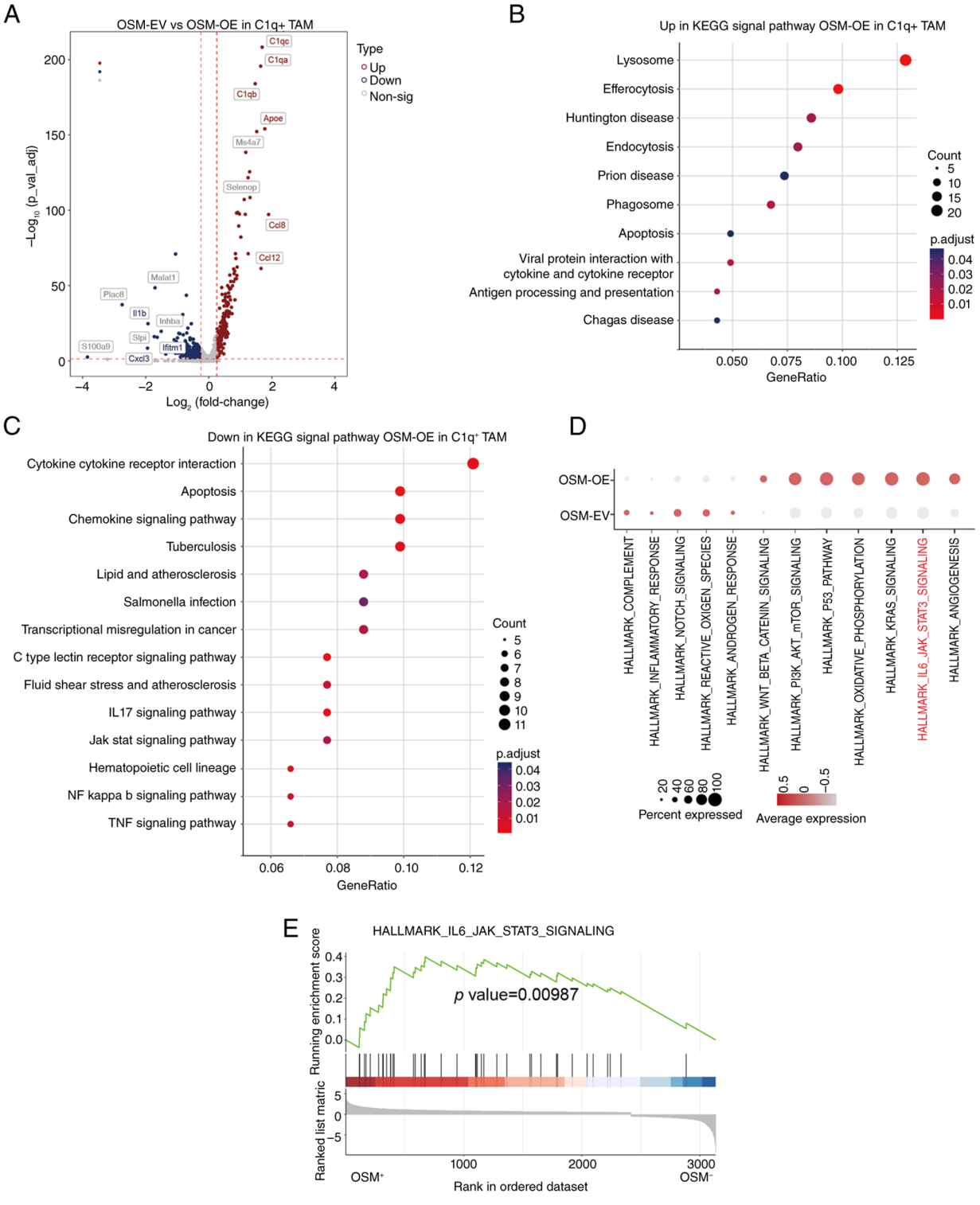


Figure S5. Flow cytometric assessment of tumor-infiltrating CD8⁺ T cells, exhaustion-associated markers, and tumor-cell PD-L1 expression following OSM overexpression. The efficiency verification result of OSM knocking down. (A) Representative flow cytometry plots showing the sequential gating strategy applied to single-cell suspensions from OSM-EV and OSM-OE MC38 tumors. (B) Descriptive comparison of the proportions of CD8⁺ T cells, PD-1⁺ CD8⁺ T cells, and TIM-3⁺ CD8⁺ T cells between the OSM-EV and OSM-OE tumors. CD8⁺ T-cell frequencies are expressed as percentages of the CD45⁺ population, whereas PD-1⁺ and TIM-3⁺ cells are expressed as percentages of the CD3⁺CD8⁺ T-cell population. (C) Original representative gating strategy for the assessment of PD-L1 expression in HCT116-EV and HCT116-OE cells (Fig. 6B). In MC38 cell line, the same control-based gating criteria were applied consistently to the OSM-EV and OSM-OE groups. Cells and singlets were sequentially identified, followed by selection of GFP/FITC-positive cells and measurement of PD-L1 fluorescence. The numbers indicate the events included in each gate and the median fluorescence intensity of PD-L1. (D) The efficiency verification result of OSM knocking down. (E) After RKO cells knocked down OSM and treated with IFNG, the phosphorylation level of STAT3 was verified by WB. (F) The phosphorylation level of STAT3 was verified by WB after RKO cells knocked down OSM. (G) The phosphorylation level of STAT3 in HCT116 cells was verified by WB after overexpression of OSM. PD-L1, programmed cell death 1 ligand 1; OSM, oncostatin M; OE-, overexpressing; EV, empty vector; WB, western blotting.

