## Supplemental Methods

**Cell lines and cell culture.**

All cell lines were obtained from the American Type Culture Collection (ATCC), and cultured in ATCC-recommended media (Gibco) supplemented with fetal bovine serum (FBS) and antibiotics (penicillin and streptomycin). We confirmed that all cells were not contaminated after blasting their genomes to the ATCC short tandem repeat (STR) database and detecting mycoplasma-specific sequence signatures in supernatant via PCR-based standard procedures.

**Differentiation of THP-1 to M2-like macrophages and transwell co-culture system.**

THP-1 cells were treated with phorbol 12-myristate 13-acetate (PMA) (10 ng/ml) for 24 h and then cultured with IL-4 and IL-13 (25 ng/ml) for 48 h to generate THP-1-derived M2-like macrophages. Co-culture of M2-like macrophages and CSF1R overexpressing DLD1 or HCT116 cells was performed using a transwell system with a 0.4 μm pore polyester membrane. The transfected cells on transwells were cultured in a complete medium supplemented with CSF1 or IL34 for 3 h and co-cultured with M2-like macrophages for 1 h.

**Vector constructions and lentivirus generation.**

Full-length human (NM_005211.3) and mouse (NM_001037859.2) pLV-CSF1R plasmids are available in commerce (Sino Biological), and their sequences were further confirmed by independent third-party sequencing. We co-transfected pLV-CSF1R plasmids or empty vectors with matched psPAX2 and pMD2G plasmids into 293T cells to produce lentiviruses. CSF1R stably overexpressed DLD1, HCT116, and CT26 cell lines were generated by infecting lentivirus and filtering out positive colonies via puromycin screening.

**Cell proliferation assay.**

The real-time confluence of cells in 96-well plates was monitored by an IncuCyte system. A commercial Cell Counting Kit (CCK)-8 assay kit was used to evaluate the cell viability according to the manufacturer's instructions. The half-maximal inhibitory concentration ($IC_{50}$) was analyzed in

GraphPad Prism software.

**Genomic DNA/RNA extraction.**

The extraction of DNA and RNA was performed using a DNA extraction kit and spin-column RNA purification kit. RNA derived from tissue samples was isolated with a Trizol reagent.

**Quantitative RT-PCR and Western blotting.**

Quantitative RT-PCR was performed using an SYBR Green Mix system following the manufacturer's instructions. The expression of the targeted genes was calculated by the Ct method relative to the control sample after normalized to a loading control GAPDH. The sequences of primers are described in the online supplemental table 1.

Proteins were separated by SDS-PAGE for Western blotting following classical experimental protocols. Primary antibodies were used to recognize target proteins, which detailed information was presented in the online supplemental table 1. We used ImageJ software to quantify all protein bands and nearby backgrounds from western blotting films. The relative expression of targeted proteins with background subtraction was normalized to matched internal GAPDH and control groups.

**Immunohistochemistry (IHC).**

IHC staining was performed on formalin-fixed paraffin-embedded (FFPE) tissue specimens. In simple terms, tissue sections were heated to ablate wax and then treated with EDTA antigen repair solution and sheep serum blocking solution. Biomarkers were recognized by primary antibodies targeting F4/80 (1:500), CD206 (1:800), CSF1R (1:50), CD163 (1:600), CD4 (1:200) and CD8α (1:800), and then treated with secondary antibodies, followed by staining process using DAB and hematoxylin. Sections were scanned by a Slide Scanning Image System (Shenzhen, China) for whole slide scanning. In each tissue, ten different regions with 100× magnification were randomly selected for qualification, and positive cells in an area of 400× magnification among the upon regions were counted using ImageJ.

**Caspase 3/7 activity assay.**

The transfected DLD1, HCT116, and CT26 cells (10000 cells per well) were seeded in white-walled 96-well plates for 24 h, and the Caspase-Glo 3/7 Reagent was added to detect the caspase 3/7 activities by reading luminescence according to the kit's protocols.

**ELISAs.**

The transfected DLD1 and HCT116 cells seeded in 24-well plates were cultured with a complete medium supplementing with CSF1 and IL34 for 3 h. The CSF1 and IL34 levels were detected using ELISA kits following their instructions.

**5-Aza-2′-deoxycytidine (5-AZA) treatment.**

Cells were treated with 5-AZA (5 or 10 μM) for 48 h and refreshed the medium every 12 h. The cells' DNA and RNA were extracted, respectively, to detect DNA methylation status and mRNA expression.

## Supplemental Material

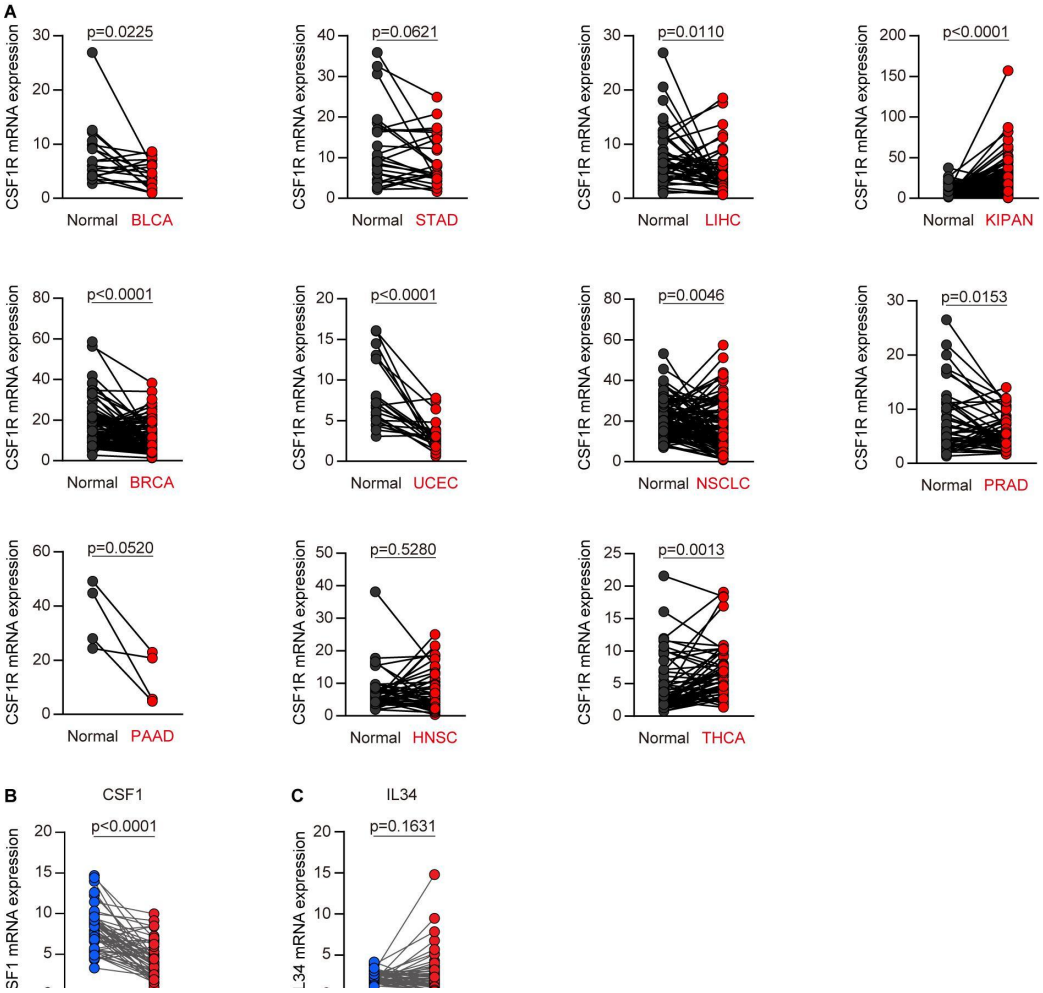

**Figure S1** Expression of CSF1R, CSF1, and IL34 in malignant tissues and normal tissues. (A) The analysis of CSF1R mRNA expression in common malignancies and their parental normal tissues in the TCGA cohort, including bladder urothelial carcinoma, stomach adenocarcinoma, hepatocellular carcinoma, pan-kidney cohort, breast invasive carcinoma, uterine corpus endometrial carcinoma, non-small-cell lung cancer, prostate adenocarcinoma, pancreatic adenocarcinoma, head and neck squamous cell carcinoma, thyroid carcinoma tissues. (B, C) CSF1 (B) and IL34 (C)mRNA expression in colorectal cancer (n=50 pairs) versus adjacent normal tissues using the TCGA datasets. *P<0.05, **P<0.01, and ***P<0.001 by two-sided paired Student t-test. Data are expressed as mean ± SD.

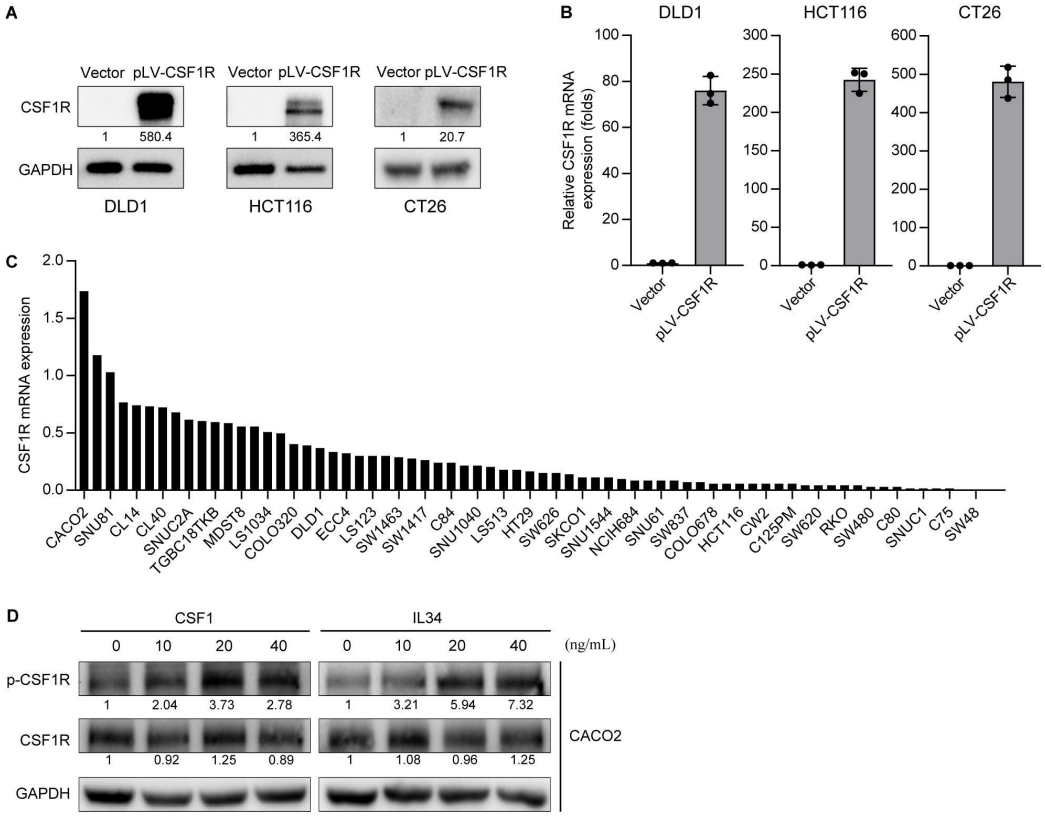


**Figure S2** The analysis of CSF1R expression in transfected CRC cells and a panel of CRC cell lines. (A, B) Validation of CSF1R stably overexpressing DLD1, HCT116, and CT26 cells using Western Blotting (A) and RT-PCR (B). (C) The data derived from the CCLE datasets were accessed to filter the

CSF1R expression in CRC cell lines. (D) The activation of the CSF1R (phospho-Tyr723) pathways in CACO2 cells treated with CSF1 and IL34 was detected using Western blotting.

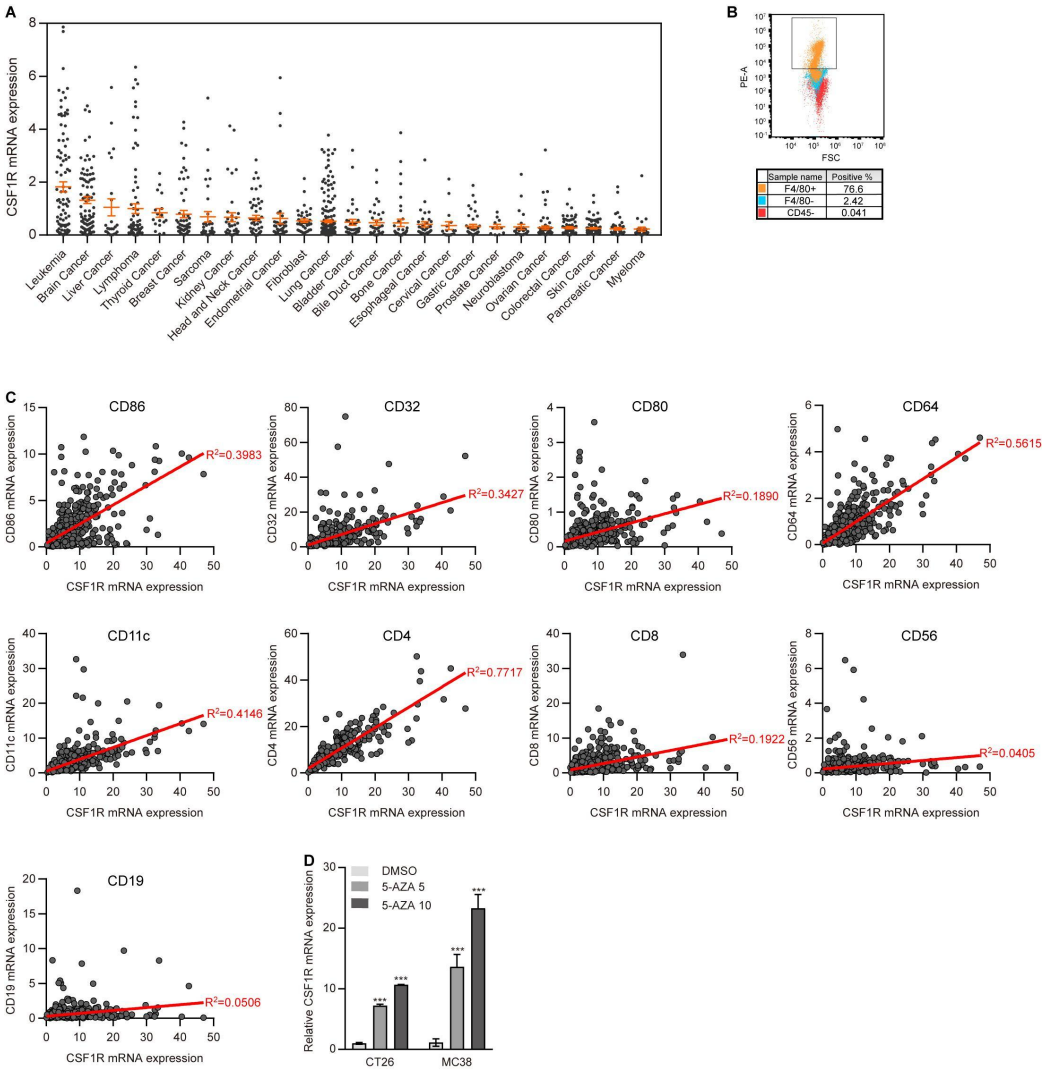


**Figure S3** (A) CSF1R mRNA expression on multiple types of cell lines was shown after filtering the available data from the CCLE datasets. (B) Flow cytometry was used to verify the purity of sorted cells in magnetic bead-based sorting. (C) Spearman's rank correlation coefficient was used to assess statistical relationships between CSF1R mRNA expression and CD86 ($R^2$=0.3983), CD32 ($R^2$=0.3427), CD80 ($R^2$=0.1890), CD64 ($R^2$=0.5615), CD11c ($R^2$=0.4146), CD4 ($R^2$=0.7717), CD8

($R^2$=1922), CD56 ($R^2$=0.0405), and CD19 ($R^2$=0.0506) mRNA expression from TCGA datasets. (D) CSF1R mRNA expression in CT26 and MC38 cells treated with or without 5-AZA were measured using RT-PCR.

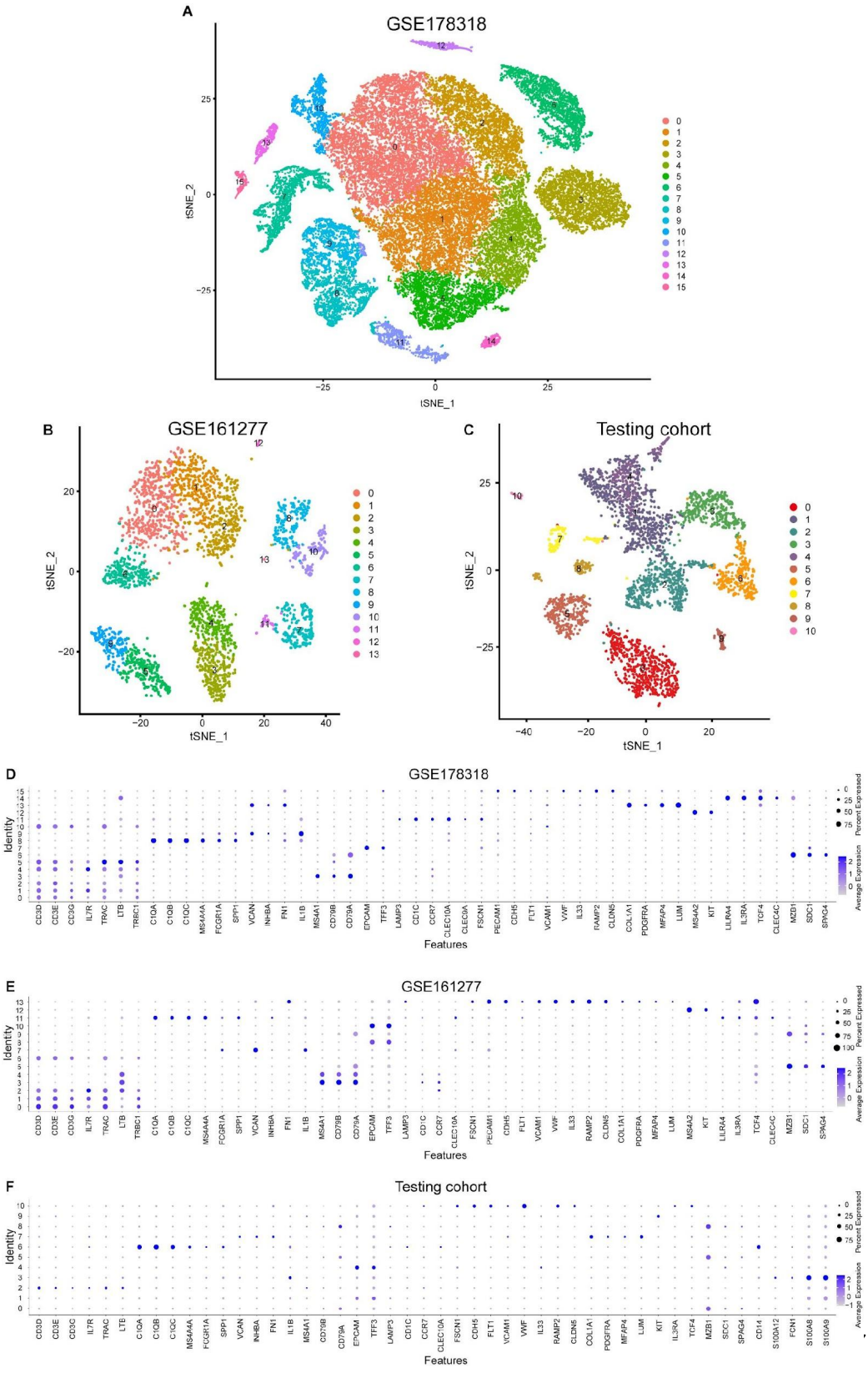
A
GSE178318
tSNE_1
tSNE_2
B
GSE161277
C
Testing cohort
D
GSE178318
E
GSE161277
F
Testing cohort
Identity
Features
Percent Expressed
Average Expression

**Figure S4** Cell type constitution of CRC tissues. (A, B, C) t-SNE plots of cells from GSE178318 (A), GSE161277 (B) and testing cohort (C). Colors represent cell types, and each dot represents a single cell. (D, E, F) Log-normalized expression levels of typical marker genes for cell types in GSE178318 (D), GSE161277 (E) and testing cohort (F). Colors represent the average expression value within a cluster, and circle size represents the percentage of cells expressing the gene.

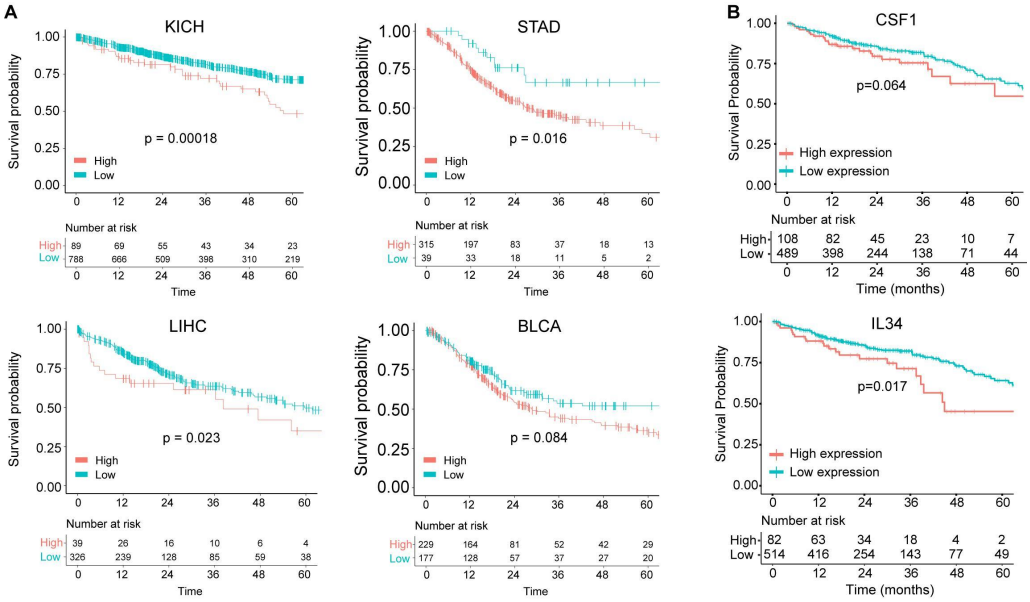


**Figure S5** CSF1R expression is associated with prognosis in several tumor types. (A) The Kaplan Meier curves show the high CSF1R expression is related to poor prognosis in the TCGA cohort, including KICH (high: n=89; low: n=788; *p*=0.00018, log-rank test), STAD (high: n=315; low: n=39; *p*=0.016, log-rank test), LIHC (high: n=39; low: n=326; *p*=0.023, log-rank test) and BLCA (high: n=229; low: n=177; *p*=0.084, log-rank test). (B) Kaplan Meier curves plot the high and low (most significant split) expression of CSF1 (high: n=108; low: n=489; *p*=0.064, log-rank test) and IL34 (high: n=82; low: n=514; *p*=0.017, log-rank test) against overall survival of patients with colorectal cancer in the TCGA cohort.

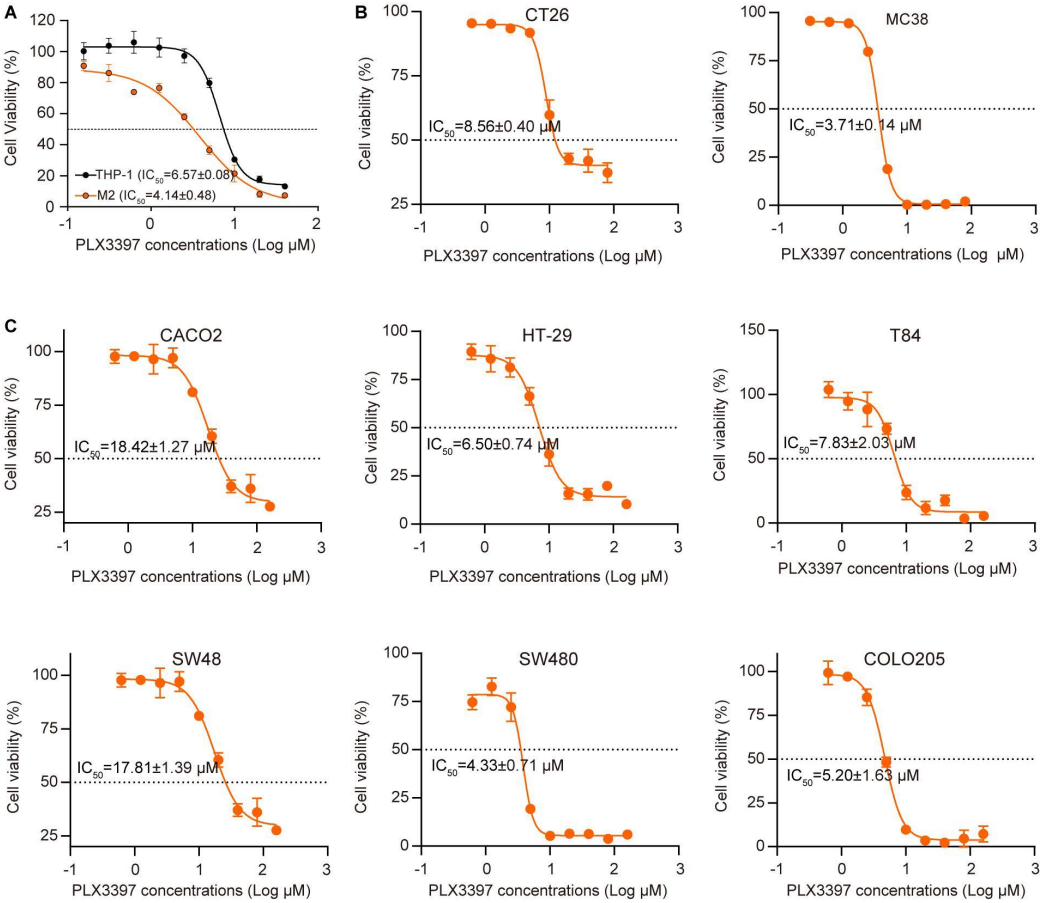


**Figure S6** The cytotoxicity of PLX3397 in multiple CRC cell lines. (A) The cytotoxicity of PLX3397 on M2-like macrophages and THP-1. Data are mean ± SD. (B, C) The cell viability of PLX3397-treated cells was normalized to agent-untreated control and was used to calculate the $IC_{50}$ in the GraphPad Prism.

**Table S1.** Detailed information about resources

| Reagent | Source | Catalog no. |
|---|---|---|
| *Antibodies* | | |
| F4/80 | Cell Signaling Technology | #70076 |
| CD206 | Cell Signaling Technology | #24595S |
| CD4 | Cell Signaling Technology | #25229 |
| CD8α | Cell Signaling Technology | #98941 |
| GAPDH | Proteintech Group | 60004-1-lg |
| Phospho-CSF1R (Tyr723) | Cell Signaling Technology | G1544 |
| CSF1R, Human | Abam | ab229188 |
| CSF1R, Mouse | ZENBIO | 160600 |
| CD163 | Abam | ab182422 |
| AKT | ZENBIO | 382804 |
| Phospho-AKT (Ser473) | ZENBIO | R22961 |
| Erk1/2 (137F5) | Cell Signaling Technology | #4695 |
| Phospho-Erk1/2 (Thr202/Tyr204) | Cell Signaling Technology | # 4370 |
| Anti-Rabbit IgG (H+L) | Proteintech Group | SA00001-2 |
| Anti-Mouse IgG (H+L) | Proteintech Group | SA00001-1 |
| InVivoMAb anti-mouse CSF1R (CD115) | Bio X Cell | BE0213 |
| InVivoMAb rat IgG2a isotype control, anti-trinitrophenol | Bio X Cell | BE0089 |
| InVivoMAb anti-mouse CTLA-4 (CD152) | Bio X Cell | BE0164 |
| InVivoMab anti-mouse PD-1 (CD279) | Bio X Cell | BE0146 |
| InVivoMAb mouse lgG2b isotype control | Bio X Cell | BE0086 |
| InVivoMAb rat lgG2a isotype control | Bio X Cell | BE0089 |
| CSF-1R/M-CSF-R (C-terminal) | Cell Signaling Technology | #3152 |
| CD45-Biotin | MiltenyiBiotec | 130-110-795 |
| CD45-PE | MiltenyiBiotec | 130-110-797 |
| Anti-F4/80-Biotin | MiltenyiBiotec | 130-116-514 |
| Anti-F4/80-PE | MiltenyiBiotec | 130-116-499 |
| *Chemicals and fluorescent probes* | | |
| 5-Aza-2′-deoxycytidine (5-AZA) | Sigma Aldrich | A3656 |
| Phorbol 12-myristate 13-acetate (PMA) | Sigma Aldrich | P8139 |
| Puromycin | InvivoGen | ant-pr-1 |
| Penicillin and streptomycin | Gibco | 15140122 |
| Basement membrane matrix | Corning | 354248 |
| Pexidartinib (PLX3397) | Selleck | S7818 |
| Ficoll-Paque PLUS | Cytiva | 17144002 |
| 5-Fluorouracil | Sigma Aldrich | F6627 |
| Luciferin | Promega | P1041 |
| Dimethyl sulfoxide (DMSO) | MedChemExpress | 196055 |
| Q-VD-Oph | Selleck | S7311 |
| MG-132 | Selleck | S2619 |
| *Commercial kits* | | |
| QIAamp DNA Mini Kit | QIAGEN | 51306 |
| EZ DNA Methylation Kit | Zymo Research | D5002 |
| HotStarTaq DNA Polymerase | QIAGEN | 203205 |
| CpGenome Universal Methylated DNA | Sigma Aldrich | S7821 |
| EpiTect Bisulfite Kit | QIAGEN | 59104 |

| | | |
|---|---|---|
| Total RNA Purification Kit | Sangon | B518651 |
| RT Master Mix with gDNA Remover | TOYOBO | FSQ-301 |
| FastStart DNA Master SYBR Green I | Roche | 12239264001 |
| PCR Mycoplasma Detection Set | TaKaRa | 6601 |
| Human M-CSF ELISA Kit | ProteinTech | KE00184 |
| Human IL-34 ELISA Kit | ProteinTech | KE00151 |
| Dead Cell Removal Kit | MiltenyiBiotec | 130-090-101 |
| Streptavidin MicroBeads | MiltenyiBiotec | 130-048-101 |
| Cell Counting Kit-8（CCK-8） | APExBIO | K1018-500T |
| Caspase-Glo 3/7 Assay System | Promega | G8090 |
| *Commercial plasmids* | | |
| CSF1R, Human | Sino Biological | HG10161-UTLP |
| CSF1R, Mouse | Sino Biological | MG50059--UTLP |
| Empty vector | Sino Biological | LVCV-03 |
| *Recombinant proteins* | | |
| IL-34 | Sino Biological | 10948-H08S |
| IL-4 | Sino Biological | GMP-11846-HNAE |
| IL-13 | Sino Biological | 10369-HNAC |
| CSF1 | Cell Signaling Technology | #8929 |

| *Oligonucleotides* | | |
|---|---|---|
| Targeted Gene | Sequence | Source |
| CSF1R (qMSP) | For: TTATAGGTTGAGCGGAAATC<br>Rev: CCTCCGAAAAACTACCAAACG<br>Probe: FAM-GGGGTTGAGTTTGACGTTAAT-MGBNFQ | Invitrogen |
| AluC4 (qMSP) | For: GGTTAGGTATAGTGGTTTATATTTGTAATTTTAGTA<br>Rev: ATTAACTAAACTAATCTTAAACTCCTAACCTCA<br>Probe: NED-CCTACCTTAACCTCCC-MGBNFQ | Invitrogen |
| Human CSF1R (RT-PCR) | For: GACAGGAGAGAGCGGGACTA<br>Rev: AGCAGGTCAGGTGCTCACTA | Invitrogen |
| Mouse CSF1R (RT-PCR) | For: ACAAGTACAAGCAGAAGCCGA<br>Rev: AGGGCAACTGAGTAGGGTCA | Invitrogen |
| GAPDH (RT-PCR) | For: GGAGCGAGATCCCTCCAAAAT<br>Rev: GGCTGTTGTCATACTTCTCATGG | Invitrogen |