

Original Research

## Metastatic colorectal cancer cells upregulate SMLR1 that interacts with tumor-associated macrophages in the liver

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## Supplementary Materials

**Figure S1** Enhanced expression of membranous proteins among CRC tissues.

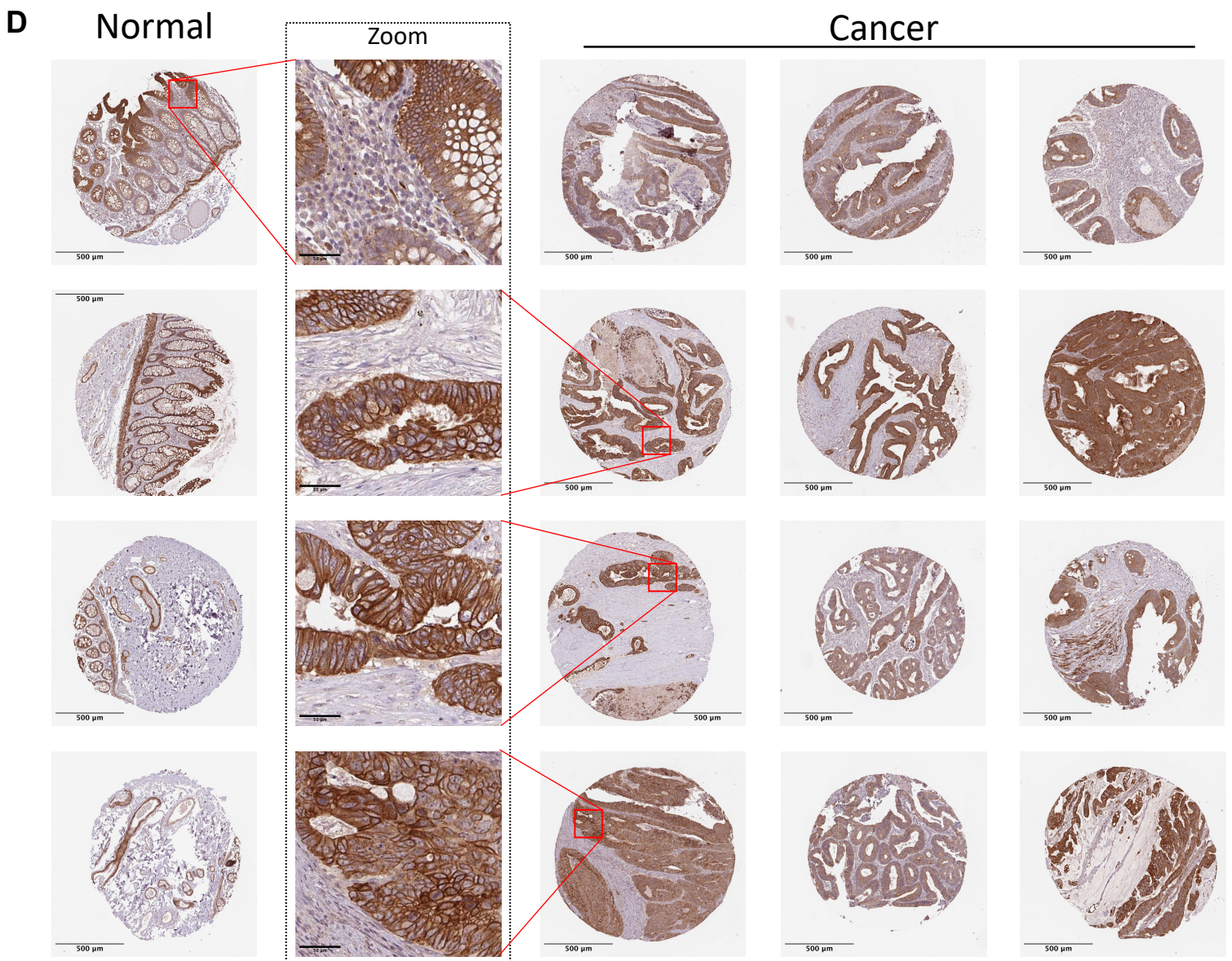
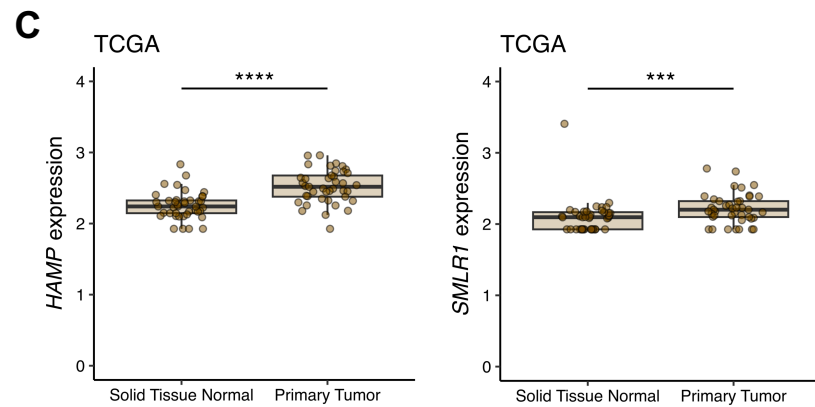
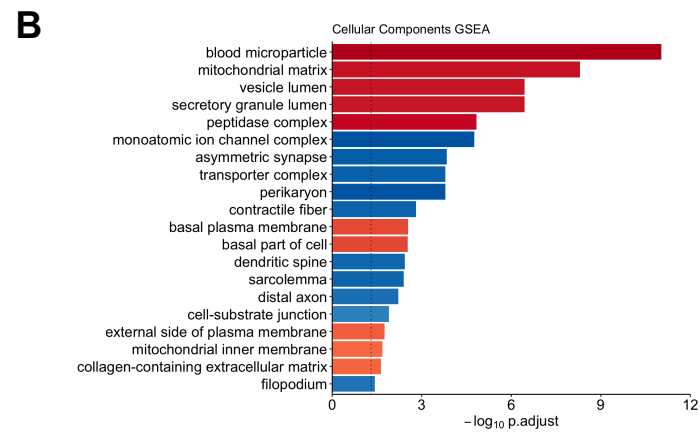
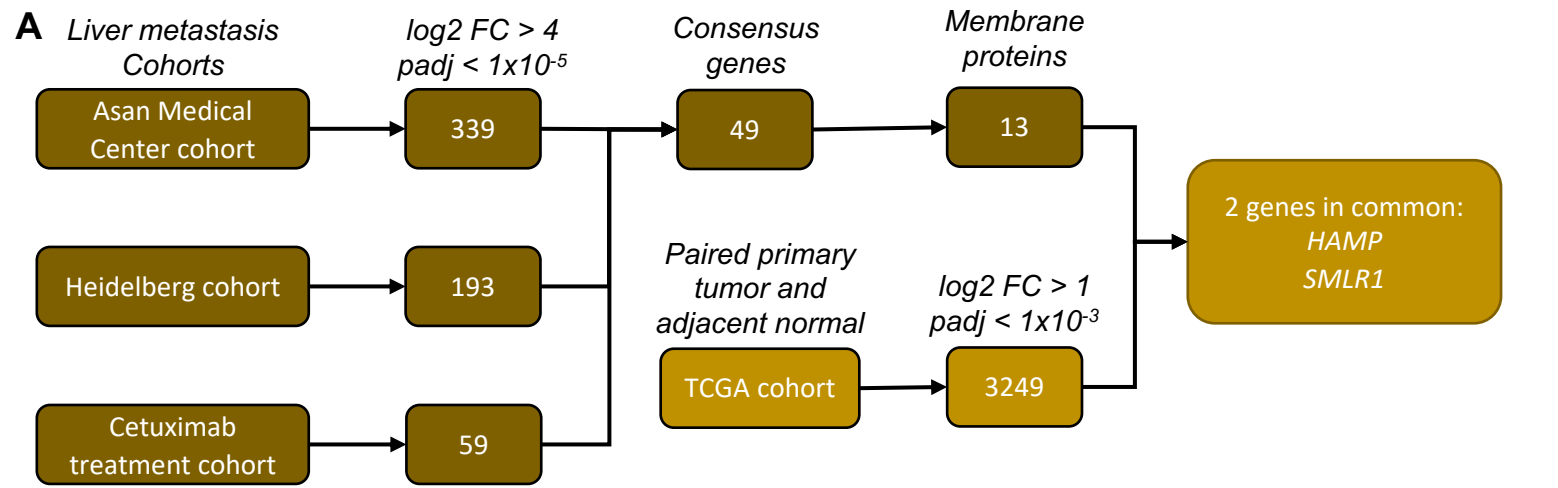
**Figure S2** *SMLR1* expression in colon cancer models.

**Figure S3** Gene set enrichment analysis of genes correlated with *SMLR1* mRNA expression.

**Figure S4** Gating strategy of liver resident cells.

**Figure S5** Characterization of SIGLEC1+MRC1+ cells in CRLM tissues.

**Figure S6** Cancer cell-macrophage interaction among patients and in vitro models.



## Figure S1 Enhanced expression of membranous proteins among CRC tissues

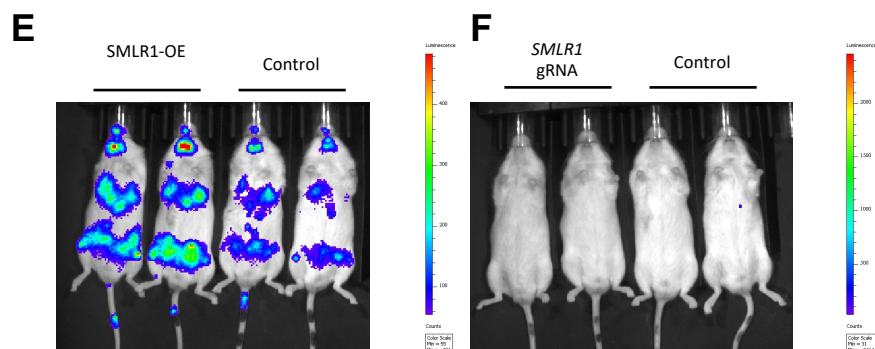
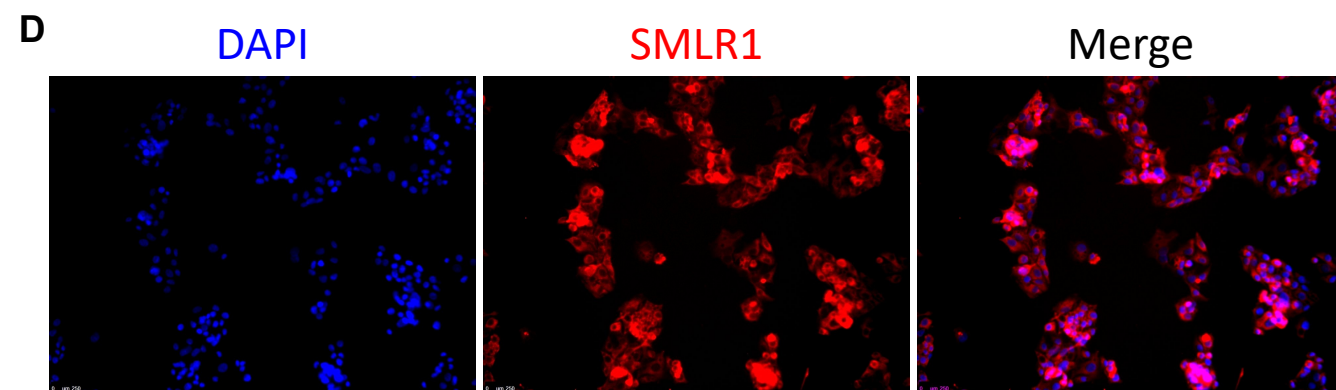
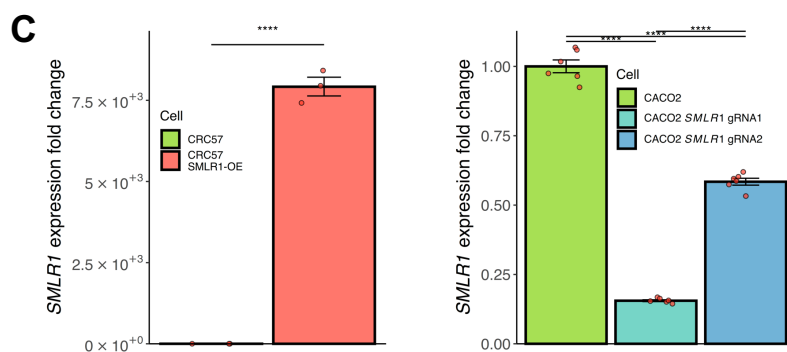
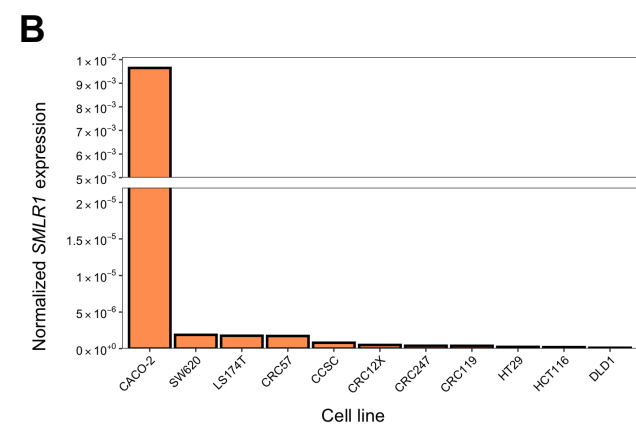
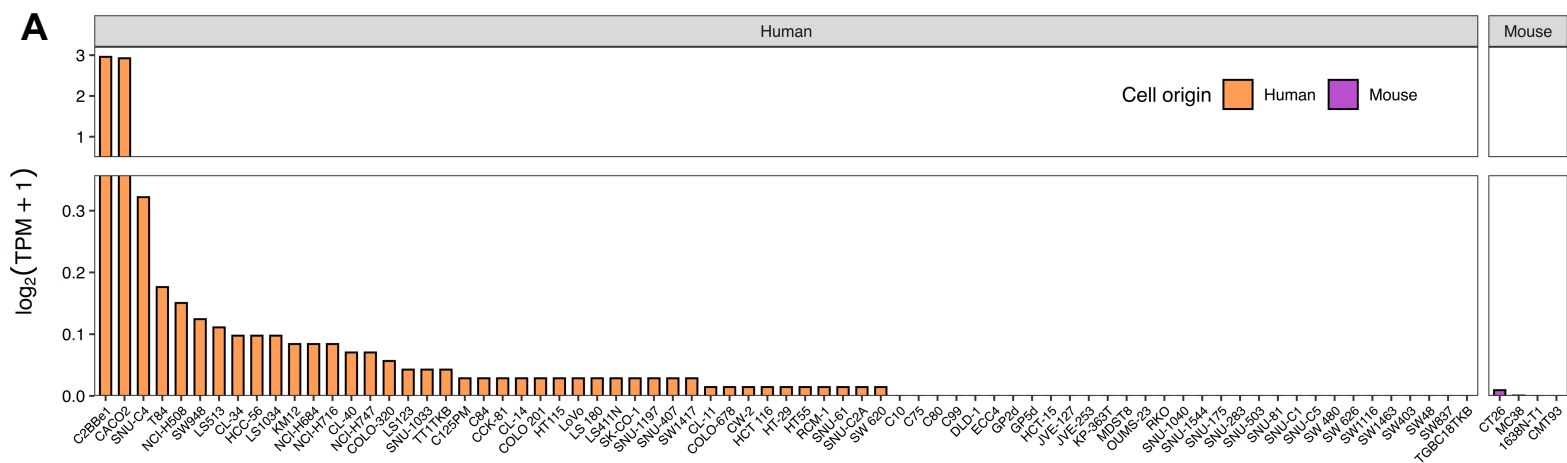
A Schematic diagram showing the gene candidate screening design and results.

B Gene set enrichment analysis of consensus genes against the cellular component (CC) gene ontology.

C Gene expression of *HAMP* (left) and *SMLR1* (right) in adjacent normal tissues and primary tumor among TCGA dataset.

D SMLR1 protein expression among human colon, rectum, and colorectal cancer tissue. Images credit: Human Protein Atlas (v23.proteinatlas.org).

Data information: For box-plot: center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range. \*\*\*p-value < 0.001, and \*\*\*\*p-value < 0.0001.



## Figure S2 *SMLR1* expression in colon cancer models

A *SMLR1* gene expression among human (from CCLE) and mouse (from TISMO) colon cancer cell lines.

B *SMLR1* gene expression in selected cancer cell lines derived from qPCR. *SMLR1* expression was normalized to *Actin* expression.

C *SMLR1* gene expression in engineered cancer cell lines derived from qPCR. *SMLR1* expression was normalized to *Actin* expression and data were shown as fold-change normalized to the vector controls.

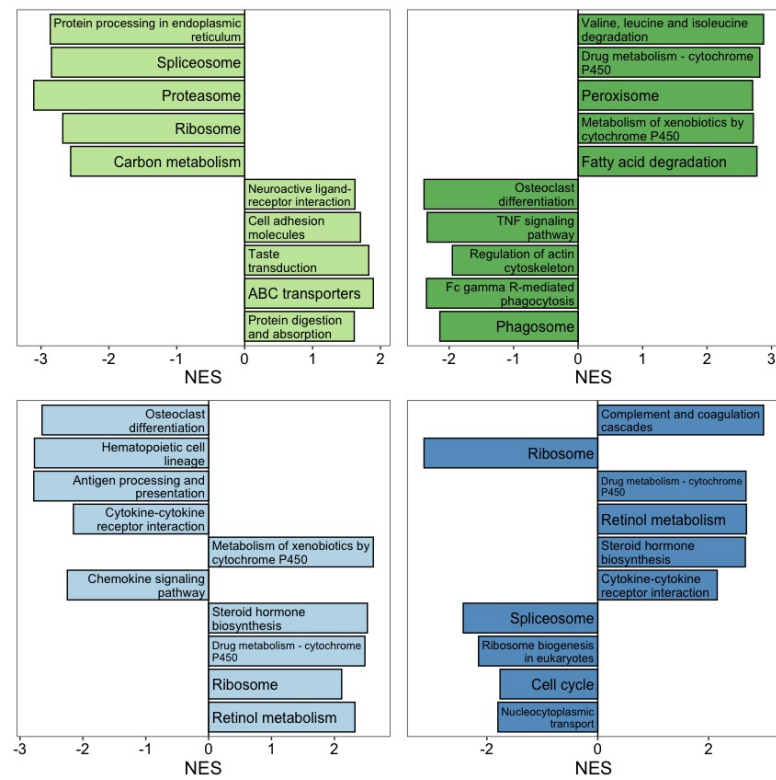
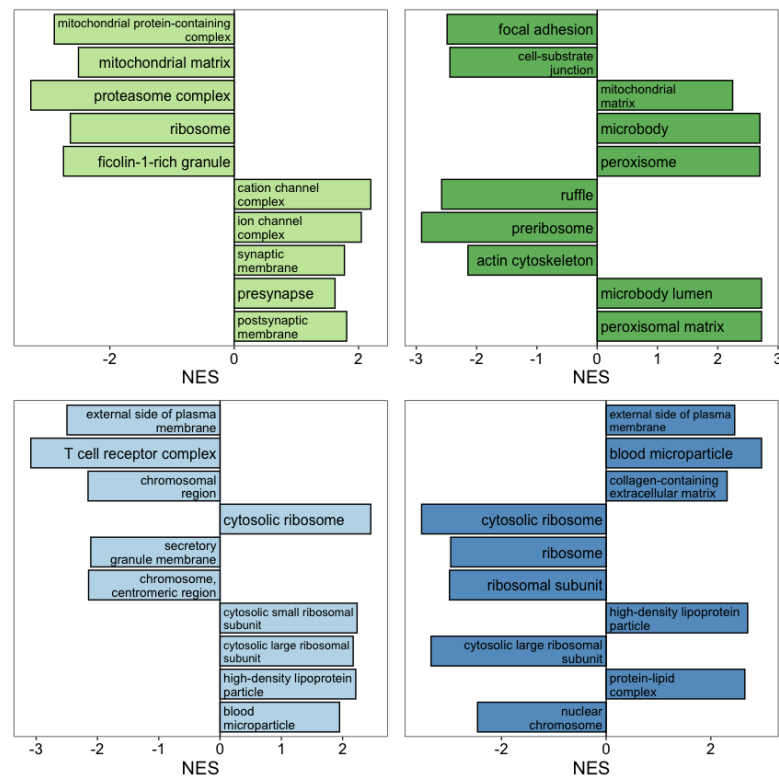
D Representative image of *SMLR1* protein expression in CACO2 cell in immunocytochemistry experiments.

E Representative luminescent images of NSG mice abdomen among mice tail vein injected with either *SMLR1*-OE or control vector expressing CRC57 for 1 week.

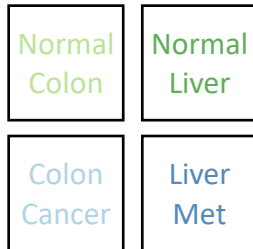
F Representative luminescent images of NSG mice abdomen among mice tail vein injected with either *SMLR1*-gRNA or control vector expressing CACO2 for 3 weeks.

Data information: For the bar chart: data are presented as mean  $\pm$  SEM. \*\*\*\*p-value < 0.0001.



**A****B**

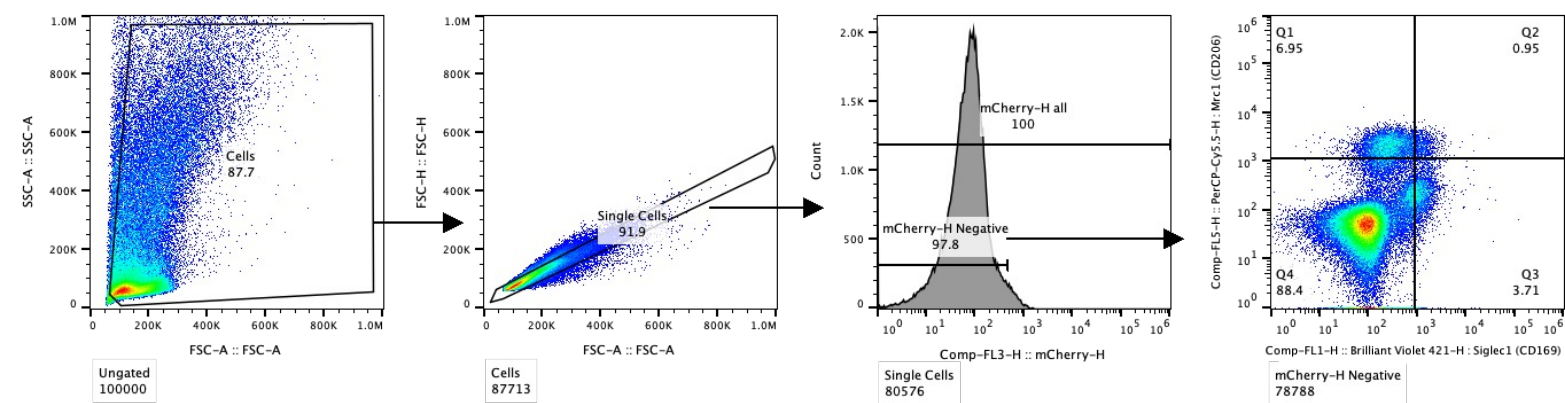
Legend:

**Figure S3 Gene set enrichment analysis of genes correlated with *SMLR1* mRNA expression**

A Gene set enrichment analysis results based on the KEGG database among tissues.

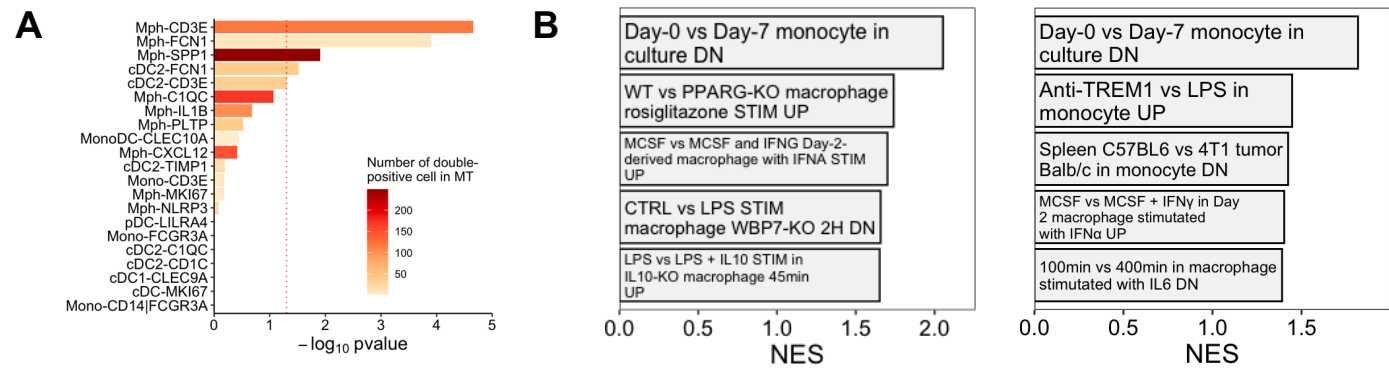
B Gene set enrichment analysis results based on cellular component gene ontology database among tissues.

Top-left (light green): Normal colon; Top-right (dark green): Normal liver; Bottom-left (light blue): Primary colon cancer; Bottom-right (dark blue): Liver metastasis of colon cancer.



**Figure S4 Gating strategy of liver resident cells**

The gating strategy that had been applied to all samples. Cancer cells were engineered and labeled with mCherry.

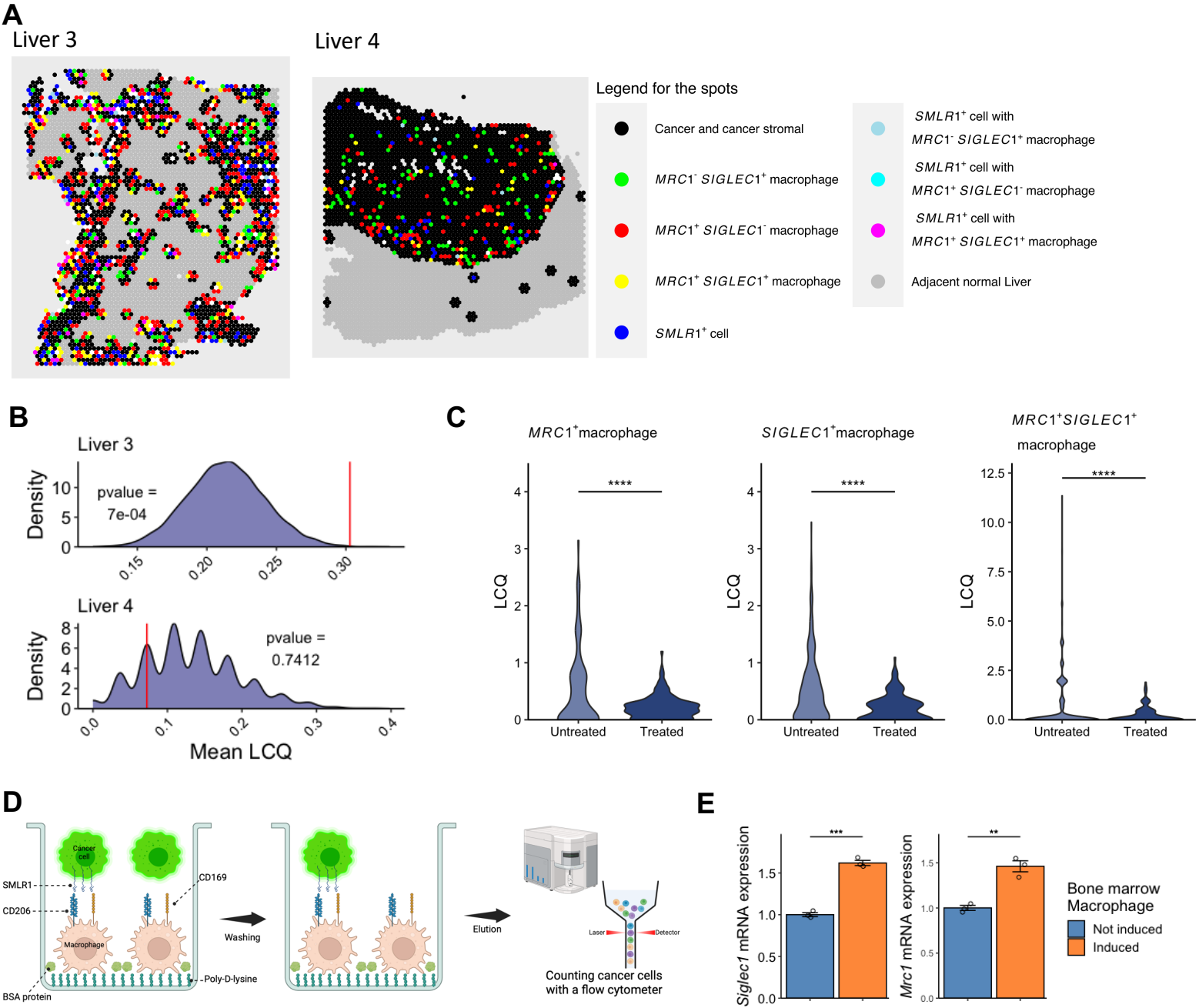


**Figure S5 Characterization of *SIGLEC1*<sup>+</sup>*MRC1*<sup>+</sup> cells in CRLM tissues**

A Comparison of the proportion of *SIGLEC1*<sup>+</sup>*MRC1*<sup>+</sup> cells between liver metastasis of colon cancer and adjacent normal liver by Fisher exact test.

B Gene set enrichment analysis (GSEA) of immune signatures that enriched in *SIGLEC1*<sup>+</sup>*MRC1*<sup>+</sup> cells compared with *SIGLEC1*<sup>-</sup>*MRC1*<sup>-</sup> cells (left) and GSEA of immune signatures that enriched in *SIGLEC1*<sup>+</sup>*MRC1*<sup>+</sup> cells compared with cells that are either *SIGLEC1*<sup>+</sup> or *MRC1*<sup>+</sup> (right).





**Figure S6 Cancer cell-macrophage interaction among patients and *in vitro* models**

A Spatial transcriptomics of colon cancer liver metastasis tissues from patients treated with XELOX before the tumor resection. Left section: Liver 3; Right section: Liver 4.

B Quantification of local colocation quotient (LCQ) between spots that contain *SMLR1*-expressing cancer cells and *SIGLEC1*-*MRC1* double positive macrophage. Simulated null mean LCQ were plotted as density plot and mean LCQ for Liver 3 (top) and Liver 4 (bottom) were indicated by the red vertical line.

C Quantification of *SMLR1*-expressing cancer cell and indicated cell types colocalization in colon cancer liver metastasis among neoadjuvant treated and untreated patients.

D Schematic diagram illustrating the steps of the cell retention assay.

E *Siglec1* and *Mrc1* gene expression among mouse bone marrow macrophages upon IL4 induction (n = 3).

Data information: Data are presented as mean  $\pm$  SEM. \*p-value < 0.05, \*\*p-value < 0.01, \*\*\*p-value < 0.001, and \*\*\*\*p-value < 0.0001.