SUPPLEMENTAL MATERIAL

Afik et al., http://dx.doi.org/10.1084/jem.20151193

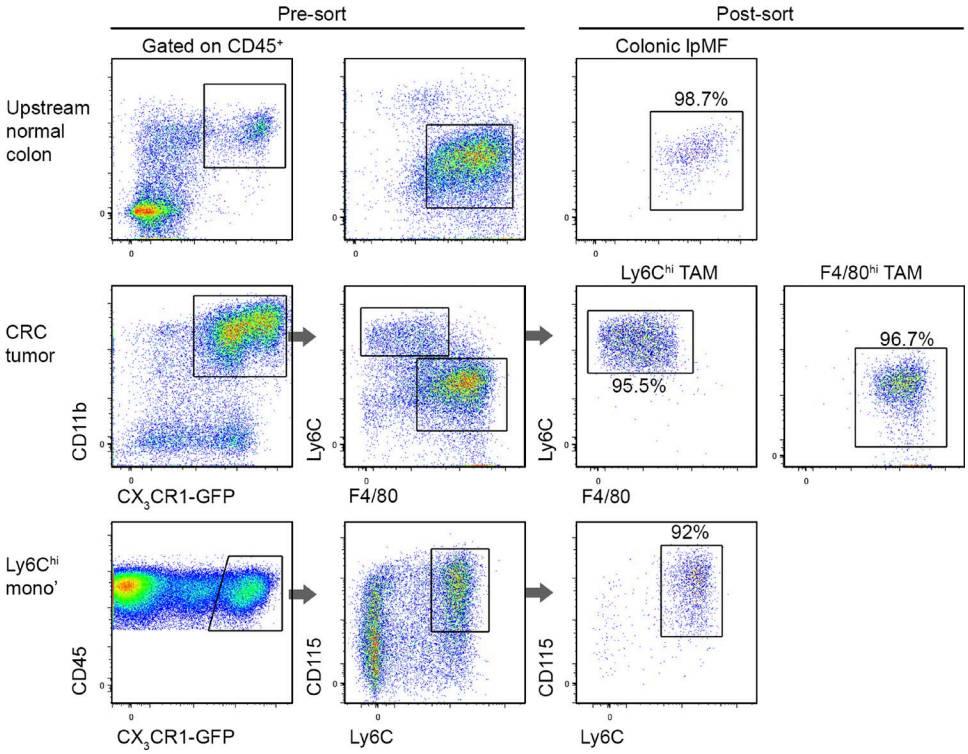

Figure S1. **Sorting strategy of Ly6C^{hi} monocytes, colonic lpMFs, and CRC TAMs.** Representative flow cytometry images showing the strategy that was implemented for the sorting of colonic lpMFs (top), Ly6C^{hi} and F4/80^{hi} TAM subsets from CRC tumors (middle), and of Ly6C^{hi} monocytes (mono') from the spleen (bottom). Purity degree is mentioned in percentage for each cell population.

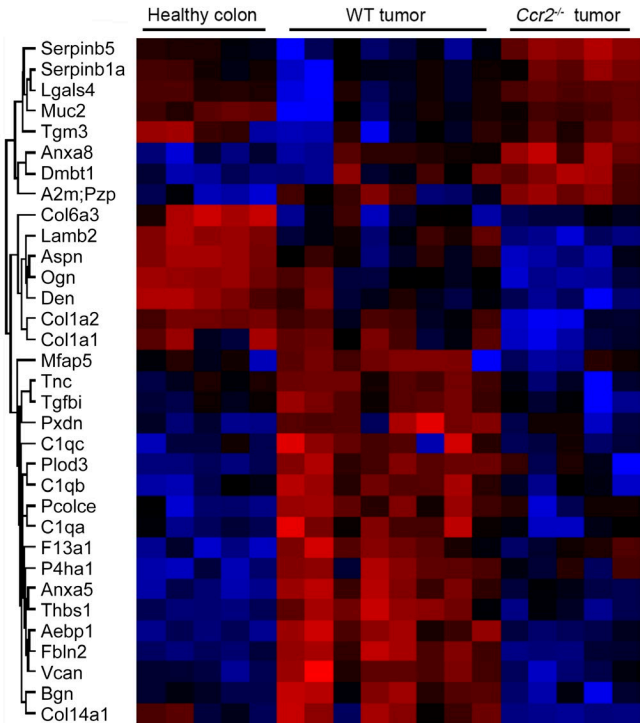

Figure S2. **CRC tumors acquire a unique ECM protein signature in comparison with healthy upstream colon that is altered in the absence of TAMs.** Color-coded heat map showing 31 ECM-related proteins that were differentially expressed between healthy upstream colon and CRC tumors, as well as between WT and *Ccr2*$^{-/-}$ tumors. Of note, collagens I α1 and α2 were not significantly changed between healthy colon and WT tumors but were added into this heat map. Data were analyzed by unpaired, two-tailed Student's *t* test analysis (pFDR = 0.05) and were z scored. Each column represents a biological repeat ($n = 5$ for normal colon, $n = 8$ for WT tumor, and $n = 5$ for *Ccr2*$^{-/-}$ tumors).

A ECM Regulators

Gene Symbol	Ly6C(hi) spleen monocytes	Ly6C(hi) TAM	F4180(hi) TAM	Resident colonic lpMF
Adamdec1	6.2	6.1	7.2	10.6
Loxl2	6.3	6.6	6.4	7.1
Mmp15	6.6	7.2	7	9
Mmp13	6.9	9.2	11	12.6
Leprel1	7	8.4	7.5	11.1
Timp3	7.1	8.2	7.4	9.6
Mmp12	7.2	10.3	11	11.2
Mmp25	7.4	8.4	8.7	8.3
Tgm3	7.4	8.1	7.7	11.5
Loxl3	7.5	8.3	8.6	9.6
Serpinb6a	7.6	9.4	10.1	11.3
Serpine1	7.7	11.2	9.9	11.1
Egln3	7.7	10.5	9.6	8.9
Timp1	7.8	11.1	9.2	8.5
Mmp9	8	8.6	9.2	9.1
Adam19	8.5	8.8	8.3	11
Adamtsl4	8.6	10.6	10.2	9.7
Mmp14	8.6	11.8	11.9	12.4
Tgm1	9	9.7	9	8.7
Tgm2	9.9	13	12.2	11.8
Adam8	9.9	12.1	12.2	9.7
Mmp19	10.2	11.3	10.3	10
Ctsl	10.2	12.4	12.9	12.2
Plod1	10.3	10.8	11.6	10.7
Ctsev	10.3	9.1	8.6	9.7
Ctso	10.5	10.3	10.9	10.2
P4ha1	10.5	11.2	11	10.5
Timp2	10.6	11.1	11.8	11.9
Adam9v	11.1	11.8	12	11.6
Adam10	11.1	11.4	11.3	10.7
Adam17	11.2	11.2	11.3	11.1
Mmp8	11.3	10.6	7.9	5.1
Adam15	11.5	11.5	11.7	11.3
Ctsd	11.6	12.6	13	12.5
Cstb	11.6	12.4	12.1	11.8
Plod3	11.8	11.2	10.8	10.2
F10	11.9	11.6	9.5	7.2
Ctsh	12	12	12.2	12
F13a1	12.2	11.8	11.6	10.5
Hpse	12.4	11.1	10.8	9.5
Ctsz	12.7	12.9	13.2	12.9
Ctss	12.8	12.9	12.9	12.7
Ctsc	12.8	13.2	13.3	13.3
Ctsa	12.8	12.7	13	12.8
Ctsb	12.9	12.9	13.2	13.2
Cst3	13.5	13.4	13.5	13.2

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B Collagens

Gene Symbol	Ly6C(hi) spleen monocytes	Ly6C(hi) TAM	F4180(hi) TAM	Resident colonic lpMF
Col14a1	7.4	8.7	10.8	9.7
Col6a2	8.2	9.3	8.8	8.7
Col3a1	8.5	9.7	8.9	9.4
Col6a1	8.6	9.7	9.2	9
Col1a2	8.6	9.6	9	9.3
Col1a1	9.1	10.2	9.5	9.9

C Proteoglycans

Gene Symbol	Ly6C(hi) spleen monocytes	Ly6C(hi) TAM	F4180(hi) TAM	Resident colonic lpMF
Aspn	5.1	9.2	8	8.3
Ogn	6.1	9.7	8.5	8.9
Prelp	7.4	8.1	7.5	9.3
Den	7.4	12	11.3	12.1
Bgn	7.7	12.1	11.5	11.8
Vcan	12.3	11.9	9.5	7.7
Srgn	13.4	13.7	13.4	13.4

D ECM Glycoproteins

Gene Symbol	Ly6C(hi) spleen monocytes	Ly6C(hi) TAM	F4180(hi) TAM	Resident colonic lpMF
Dpt	5.5	6.9	6.2	9.9
Postn	6.6	8.4	7.5	10.6
Thbs4	7.2	8.4	8	11.3
Pxdn	7.3	8.6	7.8	8.1
Dmbt1	7.7	10.1	9.1	13.9
Spp1	7.9	12.9	13	9.4
Nid1	7.9	9.6	8.7	9.1
Aebp1	8	9.4	8.6	8.4
Lrg1	8	9.2	7.8	6.9
Fbln1	8.2	8.5	8.1	9.5
Lamc1	8.4	9.8	9.4	9.1
Pcolce	8.5	10.5	9.9	9.6
Sparc	8.6	12	11.3	12.2
Ltbp3	8.7	9.9	10.1	9.8
Spon1	8.9	10.7	10.9	8.9
Efemp2	8.9	9.8	10	9.9
Mfap1a	10.1	9.8	9.8	9.6
Ecm1	10.7	12.9	12.6	12.9
Mfap3	11.1	10.8	10.7	10.3
Vwa5a	11.3	11	10.9	10.9
Fg12	11.4	11.7	11.6	11.8
Thbs1	11.4	13.6	13.1	13.2
Emilin2	12.3	11.8	11.2	9.8
Tgfbi	13.4	13.7	13.5	13.2
Fn1	14.1	13.6	13.1	10.3

E ECM-affiliated proteins

Gene Symbol	Ly6C(hi) spleen monocytes	Ly6C(hi) TAM	F4180(hi) TAM	Resident colonic lpMF
Sdc2	6.4	9	8.1	9.2
Muc3	6.7	8.8	8	13.5
Muc13	6.9	8.4	7.8	12.1
Muc2	7.8	8.7	8.3	13.5
Lgals4	8.3	10.1	9.5	13.7
Sdc1	8.4	9.8	8.6	10
C1qa	8.4	11.5	12.8	13.3
Sdc4	8.5	11.3	11.3	12.1
C1qc	9.4	12.5	13.4	13.9
C1qb	10.3	13	13.5	14
Sdc3	11.3	11.9	12.2	11.4
Anxa1	12.3	11.6	11	10.6
Lgals1	12.6	13.3	13.2	12.6
Lgals3	12.9	12.9	12.7	12.8
Anxa5	13.1	12.9	12.9	12.9

Figure S3. **TAMs express a unique signature of ECM-related genes.** (A–E) Affymetrix gene array heat map analyses showing the differential raw expression level of ECM-related genes of sorted Ly6C^{hi} and F4/80^{hi} TAMs (day-14 tumors) in comparison with their Ly6C^{hi} monocyte precursors (splenic reservoir) and colonic lpMFs sorted from upstream normal colonic mucosa. ECM genes are categorized as ECM regulators (A), collagens (B), proteoglycans (C), glycoproteins (D), and ECM-affiliated proteins (E). Values are presented as ($\log_2$) gene expression average of two biological repeats, each performed from a pool of mice ($n = 5$ for splenic monocytes, and $n \geq 10$ for TAMs and colonic lpMFs).

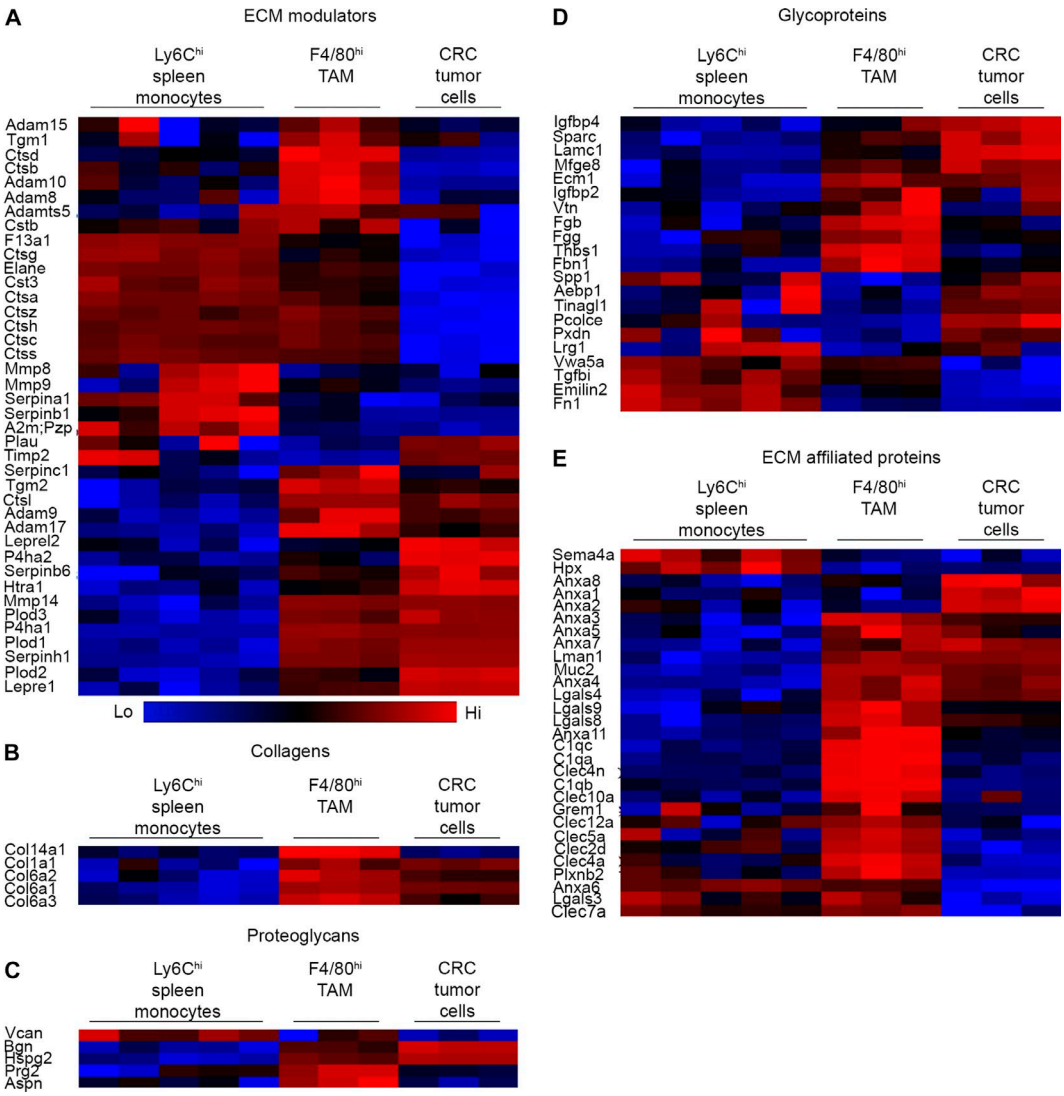

Figure S4. **Proteomic profiling of sorted F4/80^{hi} TAMs in comparison with their Ly6C^{hi} monocyte precursors and colocalizing CRC tumor cells.** (A–E) Color-coded heat maps presenting ECM-related proteins found by LC-MS/MS analysis to be significantly and differentially expressed in sorted F4/80^{hi} TAMs in comparison with their Ly6C^{hi} monocyte precursors and colocalizing colorectal tumor cells. For Ly6C^{hi} monocytes, five biological repeats were performed; each was extracted from a pool of three mice. For F4/80^{hi} TAMs and CRC tumor cells, three biological repeats were performed; each was extracted from a pool of five mice. Data were analyzed by unpaired, two-tailed Student's *t* tests (pFDR = 0.05), z scored, and divided into the following categories: ECM modulators (A), collagens (B), proteoglycans (C), glycoproteins (D), and ECM-affiliated proteins (E).

Table S1 is included as an Excel file and shows a GOEAST function enrichment analyses of the differentially expressed genes between Ly6C^{hi} TAMs and resident colonic lpMFs and between F4/80^{hi} TAMs and resident colonic lpMFs.

Table S2 is included as an Excel file and shows a DAVID function enrichment analyses of the differentially expressed genes between Ly6C^{hi} TAMs and resident colonic lpMFs and between F4/80^{hi} TAMs and resident colonic lpMFs.

Table S3 is included as an Excel file and shows an ingenuity pathway analysis of the differentially expressed genes between F4/80^{hi} TAMs and resident colonic lpMFs.