SUPPLEMENTARY MATERIAL

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SUPPLEMENTARY METHODS

Next-generation sequencing (NGS) in the Caris dataset.

NGS was performed on genomic DNA isolated from FFPE samples using an NGS platform (Illumina, Inc., San Diego, CA). A custom-designed SureSelectXT assay (Agilent Technologies, Santa Clara, CA) was used to enrich 592 cancer-related whole-gene targets (Caris MI TumorSeek panel). All variants were detected with > 99% confidence based on allele frequency and amplicon coverage, with an average sequencing coverage depth of 750 and an analytic sensitivity of 5%. Identified genetic variants were analyzed by board-certified molecular geneticists and categorized as follows according to the American College of Medical Genetics and Genomics standards: “pathogenic,” “presumed pathogenic,” “variant of unknown significance,” “presumed benign,” or “benign.” When assessing mutation frequencies of individual genes, “pathogenic” and “presumed pathogenic” were counted as mutations, whereas “variant of unknown significance,” “presumed benign,” and “benign” were excluded.

Whole Transcriptome Sequencing (WTS) in the Caris dataset.

WTS uses a hybrid-capture method to pull down the full transcriptome from a FFPE tumor samples using the Agilent SureSelect Human All Exon V7 bait panel (Agilent Technologies, Santa Clara, CA) and the Illumina NovaSeq platform (Illumina, Inc., San Diego, CA). FFPE specimens underwent pathology review to diagnose percent tumor content and tumor size; a minimum of 10% of tumor content in the area for microdissection was required to enable enrichment and extraction of tumor-specific RNA. Qiagen RNA FFPE tissue extraction kit was used for extraction, and the RNA quality and quantity were determined using the Agilent TapeStation. Biotinylated RNA baits were hybridized to the synthesized and purified cDNA targets and the bait-target complexes were amplified in a post capture PCR reaction. The resultant libraries were quantified and normalized, and the pooled libraries were denatured, diluted and sequenced. Raw data was demultiplexed using the Illumina DRAGEN FFPE accelerator. FASTQ files were aligned with STAR aligner (Alex Dobin, release 2.7.4a github). A full 22,948-gene dataset of expression data was produced by the Salmon, which provides fast and bias-aware quantification of transcript expression(1). BAM files from STAR aligner were

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further processed for RNA variants using a custom detection pipeline. The reference genome used was GRCh37/hg19 and analytical validation of this test demonstrated ≥ 97% Positive Percent Agreement (PPA), ≥ 99% Negative Percent Agreement (NPA) and ≥ 99% Overall Percent Agreement (OPA) with a validated comparator method.

Immunohistochemical analysis in the Caris dataset.

Immunohistochemistry (IHC) was performed on full FFPE sections of glass slides. Slides were stained using automated staining techniques, per the manufacturer's instructions, and were optimized and validated per CLIA/CAO and ISO requirements. Staining was scored for intensity (0 = no staining; 1+ = weak staining; 2+ = moderate staining; 3+ = strong staining) and staining percentage (0–100%). A board-certified pathologist evaluated all IHC results independently.

PD-L1 expression was tested by IHC using SP142 antibody (Spring Biosciences), tumors with staining scores of 2+ or 3+ in > 5% of tumor cells were regarded as PD-L1 positive.

Assessment microsatellite instability (MSI) and mismatch repair (MMR) status in the Caris dataset.

A combination of multiple test platforms was used to determine the MSI or MMR status of the tumors profiled, including fragment analysis (FA, Promega, Madison, WI), IHC (MLH1, M1 antibody; MSH2, G2191129 antibody; MSH6, 44 anti-body; and PMS2, EPR3947 antibody [Ventana Medical Systems, Inc., Tucson, AZ, USA]) and NGS (for tumors tested with NextSeq platform, 7,000 target microsatellite loci were examined and compared to the reference genome hg19 from the University of California). The three platforms generated highly concordant results as previously reported (2) and in the rare cases of discordant results, the MSI or MMR status of the tumor was determined in the order of FA, IHC and NGS.

Assessment of the tumor mutational burden (TMB) in the Caris dataset.

TMB was measured by counting all nonsynonymous missense, nonsense, in-frame insertion/deletion and frameshift mutations found per tumor that had not been previously described as germline alterations in dbSNP151, Genome Aggregation Database (gnomAD) databases or benign variants identified by Caris

geneticists. A cutoff point of ≥ 10 mutations per MB was used based on the KEYNOTE-158 trial (3). Caris Life Sciences is a participant in the Friends of Cancer Research TMB Harmonization Project (4).

Gene Set Enrichment Analysis (GSEA), Ingenuity Pathway Analysis (IPA) and signature scores in the Caris dataset.

WTS data was used as input for pathway gene enrichment analyses using GSEA (5) and significantly enriched results were reported as having a $P < 0.05$ and FDR (q) of < 0.25.

Gene expression pathways were analyzed using Differential Gene Expression analysis (limma) and QIAGEN IPA (QIAGEN Inc.,https://digitalinsights.qiagen.com/IPA) (6).

WTS data was used to analyze both Interferon gamma signature (7) and T cell inflamed score (8).

RNA Sequencing in the CALGB/SWOG 80405 dataset.

In the CALGB/SWOG 80405, RNA-seq (Illumina HiSeq 2500) was performed using RNA isolated from FFPE samples. RNA was extracted using the High Pure FFPET RNA Isolation Kit (Roche). RNA libraries were prepared for sequencing with 250ng of input RNA using the Illumina TruSeq RNA Access target enrichment and library preparation protocol. Paired-end RNA-Seq was performed targeting 50 M reads with a read length of 2x100 bp per sample on the Illumina HiSeq 2500 instrument. RNA-seq data quality control and normalization were performed using MAPRSEQ 3.1 and Conditional Quantile Normalization (CQN) as previously published (9, 10).

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SUPPLEMENTARY TABLES

Table S1. Demographics and Clinical Characteristics of the Caris Cohort According to: (a). *CCR5* expression groups, (b). *CCL5* expression groups.

a.

	Total	*CCR5 Q1*	*CCR5 Q2*	*CCR5 Q3*	*CCR5 Q4*	P-value
Patient number	7604	1901	1901	1901	1901	
Median age (years)	62	61	62	63	63	< 0.0001
Sex **Male** **Female**	 4224 (56%) 3380 (44%)	 1045 (55%) 856 (45%)	 1102 (58%) 799 (42%)	 1063 (56%) 838 (44%)	 1014 (53%) 887 (47%)	NS
Location of tumor sampling **Primary** **Metastatic** **Unclear**	4134 (54%) 3458 (45%) 12 (0.2%)	1070 (56%) 827 (44%) 4 (0.2%)	1112 (58%) 787 (41%) 2 (0.1%)	1075 (57%) 823 (43%) 3 (0.2%)	877 (46%) 1021 (54%) 3 (0.2%)	< 0.0001
Primary tumor location **Left** **Right** **Unclear**	4160 (55%) 2310 (30%) 1134 (15%)	1067 (56%) 550 (29%) 284 (15%)	1108 (58%) 535 (28%) 258 (14%)	1033 (54%) 593 (31%) 275 (14%)	952 (50%) 632 (33%) 317 (17%)	< 0.001

b.

	Total	*CCL5 Q1*	*CCL5 Q2*	*CCL5 Q3*	*CCL5 Q4*	P-value
Patient number	7604	1901	1901	1901	1901	
Median age (years)	62	60	62	62	63	< 0.01
Sex **Male** **Female**	 4224 (56%) 3380 (44%)	 1045 (55%) 856 (45%)	 1117 (59%) 784 (41%)	 1056 (56%) 845 (44%)	 1006 (53%) 895 (47%)	NS
Location of tumor sampling **Primary** **Metastatic** **Unclear**	4134 (54%) 3458 (45%) 12 (0.2%)	1008 (53%) 890 (47%) 3 (0.2%)	1081 (57%) 819 (43%) 1 (0.1%)	1071 (56%) 826 (43%) 4 (0.2%)	974 (51%) 923 (49%) 4 (0.2%)	NS
Primary tumor location **Left** **Right** **Unclear**	4160 (55%) 2310 (30%) 1134 (15%)	1106 (58%) 509 (27%) 286 (15%)	1112 (58%) 521 (27%) 268 (14%)	1042 (55%) 586 (31%) 273 (14%)	900 (47%) 694 (37%) 307 (16%)	< 0.0001

Transverse colon tumor location grouped with right and rectal tumors grouped with left.

P-values reflect Q1 vs Q4 comparison.

Table S2. Significant GSEA Results in pMMR/MSS CRC (*P* < 0.05 and *q* < 0.25).

CCL5 Q1 vs Q4	size	es	nes	p.values	q.values
HALLMARK_ADIPOGENESIS	191	-0.74	-1.30	0.03	0.15
HALLMARK_ALLOGRAFT_REJECTION	194	-0.77	-1.30	0.02	0.16
HALLMARK_APOPTOSIS	159	-0.75	-1.30	0.03	0.21
HALLMARK_INTERFERON_GAMMA_RESPONSE	198	-0.79	-1.30	0.01	0.15
HALLMARK_KRAS_SIGNALING_DN	193	-0.63	-1.30	0.03	0.15
HALLMARK_PI3K_AKT_MTOR_SIGNALING	104	-0.78	-1.28	0.045	0.17

Negative enrichment score indicates that these pathways were enriched in Q4 (*CCL5* high expressed tumors).

No pathways in GSEA met statistical significance for *CCR5*.

Table S3. Demographics and Clinical Characteristics of CALGB/SWOG 80405 Patients According to *CCR5* and *CCL5* Expression (by tertile).

		CCR5				*CCL5*			
	Overall (N=429)	**T1 (N=144)**	**T2 (N=143)**	**T3 (N=142)**	***P*-value***	**T1 (N=146)**	**T2 (N=144)**	**T3 (N=139)**	***P*-value***
Age									
Median (Min, Max)	60.1 (24.0, 83.4)	59.3 (24.0, 78.8)	58.9 (26.0, 83.1)	63.2 (30.6, 83.4)	**0.044**	60.2 (34.4, 78.8)	58.5 (24.0, 80.2)	62.8 (26.0, 83.4)	0.16
Sex									
Male	269 (63 %)	91 (63 %)	93 (65 %)	85 (60 %)	0.66	99 (68 %)	84 (58 %)	86 (62 %)	0.24
Female	160 (37 %)	53 (37 %)	50 (35 %)	57 (40 %)		47 (32 %)	60 (42 %)	53 (38 %)	
ECOG performance status									
0	251 (59 %)	90 (62 %)	78 (55 %)	83 (58 %)	0.4	94 (64 %)	86 (60 %)	71 (51 %)	0.07
1	178 (41 %)	54 (38 %)	65 (45 %)	59 (42 %)		52 (36 %)	58 (40 %)	68 (49 %)	
Primary tumor sidedness									
Left	256 (60 %)	87 (60 %)	91 (64 %)	78 (55 %)	0.32	95 (65 %)	94 (65 %)	67 (48 %)	**0.004**
Right or transverse	173 (40 %)	57 (40 %)	52 (36 %)	64 (45 %)		51 (35 %)	50 (35 %)	72 (52 %)	
Number of metastatic sites									
1	221 (52 %)	78 (54 %)	74 (52 %)	69 (49 %)	0.46	81 (55 %)	73 (51 %)	67 (48 %)	**0.031**
2	147 (34 %)	51 (35 %)	49 (34 %)	47 (33 %)		53 (36 %)	52 (36 %)	42 (30 %)	
3+	61 (14 %)	15 (10 %)	20 (14 %)	26 (18 %)		12 (8 %)	19 (13 %)	30 (22 %)	
Treatment arm									
Bevacizumab	223 (52 %)	77 (53 %)	73 (51 %)	73 (51 %)	0.91	78 (53 %)	74 (51 %)	71 (51 %)	0.91
Cetuximab	206 (48 %)	67 (47 %)	70 (49 %)	69 (49 %)		68 (47 %)	70 (49 %)	68 (49 %)	
Backbone chemotherapy									
FOLFIRI	109 (25 %)	43 (30 %)	32 (22 %)	34 (24 %)	0.32	40 (27 %)	32 (22 %)	37 (27 %)	0.55

		CCR5				*CCL5*			
	Overall (N=429)	**T1 (N=144)**	**T2 (N=143)**	**T3 (N=142)**	***P*-value***	**T1 (N=146)**	**T2 (N=144)**	**T3 (N=139)**	***P*-value***
FOLFOX	320 (75 %)	101 (70 %)	111 (78 %)	108 (76 %)		106 (73 %)	112 (78 %)	102 (73 %)	
RAS status									
Wildtype	318 (74%)	98 (68 %)	111 (78 %)	109 (77 %)	0.39	104 (71 %)	103 (72 %)	111 (80 %)	0.31
Mutant	80 (19%)	33 (23 %)	23 (16 %)	24 (17 %)		29 (20 %)	32 (22 %)	19 (14 %)	
Unknown	31 (7%)	13 (9 %)	9 (6 %)	9 (6 %)		13 (9 %)	9 (6 %)	9 (6 %)	
KRAS status									
Wildtype	362 (84 %)	116 (81 %)	123 (86 %)	123 (87 %)	0.32	121 (83 %)	121 (84 %)	120 (86 %)	0.72
Mutant	67 (16 %)	28 (19 %)	20 (14 %)	19 (13 %)		25 (17 %)	23 (16 %)	19 (14 %)	
NRAS status									
Wildtype	376 (88%)	120 (83 %)	131 (92 %)	125 (88 %)	0.24	124 (85 %)	124 (86 %)	128 (92 %)	**0.049**
Mutant	14 (3%)	5 (3 %)	3 (2 %)	6 (4 %)		4 (3 %)	9 (6 %)	1 (1 %)	
Unknown	39 (9%)	19 (13 %)	9 (6 %)	11 (8 %)		18 (12 %)	11 (8 %)	10 (7 %)	
BRAF status									
Wildtype	330 (77 %)	108 (75%)	118 (83 %)	104 (73 %)	0.075	112 (77 %)	121 (84 %)	97 (70 %)	**0.0032**
Mutant	60 (14 %)	17 (12 %)	16 (11 %)	27 (19 %)		16 (11 %)	12 (8 %)	32 (23 %)	
Missing	39 (9%)	19 (13%)	9 (6%)	11 (8%)		18 (12 %)	11 (8 %)	10 (7 %)	
MSI status from PCR									
MSI-H	29 (7 %)	4 (3 %)	11 (8 %)	14 (10 %)	0.11	2 (1 %)	6 (4 %)	21 (15 %)	**8.2e-05**
MSI-L	19 (4 %)	7 (5 %)	8 (6 %)	4 (3 %)		10 (7 %)	5 (3 %)	4 (3 %)	
MSS	328 (76 %)	110 (76%)	111 (78 %)	107 (75 %)		111 (76 %)	120 (83 %)	97 (70 %)	
Missing	53 (13%)	23 (16%)	13 (9%)	17 (12%)		23 (16 %)	13 (9 %)	17 (12 %)	

**P*-values were generated with Kruskal–Wallis tests and Fisher's exact tests for continuous variables and categorical variables, respectively.

Table S4. Demographics and Clinical Characteristics of CALGB/SWOG 80405 Patients According to CCR/L5 Composite Biomarker Expression (by tertile).

	T1 (N=143)	T2 (N=144)	T3 (N=142)	*P*-value*
Age				
Median (Min, Max)	59.4 (31.5, 78.8)	59.0 (24.0, 81.7)	63.2 (26.0, 83.4)	0.13
Sex				
Male	92 (64 %)	90 (62 %)	87 (61 %)	0.87
Female	51 (36 %)	54 (38 %)	55 (39 %)	
ECOG performance status				
0	91 (64 %)	82 (57 %)	78 (55 %)	0.29
1	52 (36 %)	62 (43 %)	64 (45 %)	
Primary tumor sidedness				
Left	93 (65 %)	92 (64 %)	71 (50 %)	**0.017**
Right or transverse	50 (35 %)	52 (36 %)	71 (50 %)	
Number of metastatic sites				
1	75 (52 %)	78 (54 %)	68 (48 %)	0.13
2	54 (38 %)	48 (33 %)	45 (32 %)	
3+	14 (10 %)	18 (12 %)	29 (20 %)	
Treatment arm				
Bevacizumab	78 (55 %)	71 (49 %)	74 (52 %)	0.67
Cetuximab	65 (45 %)	73 (51 %)	68 (48 %)	
Backbone chemotherapy				
FOLFIRI	40 (28 %)	33 (23 %)	36 (25 %)	0.62
FOLFOX	103 (72 %)	111 (77 %)	106 (75 %)	
RAS status				
Wildtype	100 (70 %)	109 (76 %)	109 (77 %)	0.74
Mutant	31 (22 %)	25 (17 %)	24 (17 %)	
Unknown	12 (8 %)	10 (7 %)	9 (6 %)	
KRAS status				
Wildtype	116 (81 %)	123 (85 %)	123 (87 %)	0.41
Mutant	27 (19 %)	21 (15 %)	19 (13 %)	
NRAS status				
Wildtype	121 (85 %)	130 (90 %)	125 (88 %)	0.46
Mutant	4 (3 %)	4 (3 %)	6 (4 %)	
Unknown	18 (13 %)	10 (7 %)	11 (8 %)	
BRAF status				
Wildtype	111 (78 %)	118 (82 %)	101 (71 %)	**0.024**
Mutant	14 (10 %)	16 (11 %)	30 (21 %)	
Unknown	18 (13 %)	10 (7 %)	11 (8 %)	
MSI status from PCR				

	T1 (N=143)	**T2 (N=144)**	**T3 (N=142)**	***P*-value***
MSI-H	1 (1 %)	9 (6 %)	19 (13 %)	**0.001**
MSI-L	9 (6 %)	5 (3 %)	5 (4 %)	
MSS	113 (79 %)	115 (80 %)	100 (70 %)	
Unknown	20 (14 %)	15 (10 %)	18 (13 %)	

**P*-values were generated with Kruskal–Wallis tests and Fisher's exact tests for continuous variables and categorical variables, respectively.

Table S5. Patient Outcome According to Tumor Sidedness in the pMMR/MSS Caris CODEai Cohort.

	LEFT		RIGHT	
HAZARD RATIO (*P*-values)	**CCL5 HIGH vs. LOW|MSS**	**CCR5 HIGH vs. LOW|MSS**	**CCL5 HIGH vs. LOW|MSS**	**CCR5 HIGH vs. LOW|MSS**
REGARDLESS OF TREATMENT	1.015 (0.844)	0.96 (0.589)	1.101 (0.188)	0.983 (0.812)
OXALIPLATIN	1.057 (0.531)	0.896 (0.212)	0.911 (0.343)	**0.789 (0.016)**
IRINOTECAN	1.055 (0.631)	0.93 (0.497)	0.988 (0.929)	0.799 (0.079)
CETUXIMAB	0.946 (0.824)	0.74 (0.226)	0.667 (0.229)	0.691 (0.283)
BEVACIZUMAB	0.96 (0.666)	**0.818 (0.042)**	0.924 (0.461)	**0.806 (0.044)**

HR provided for Q4 vs Q1.

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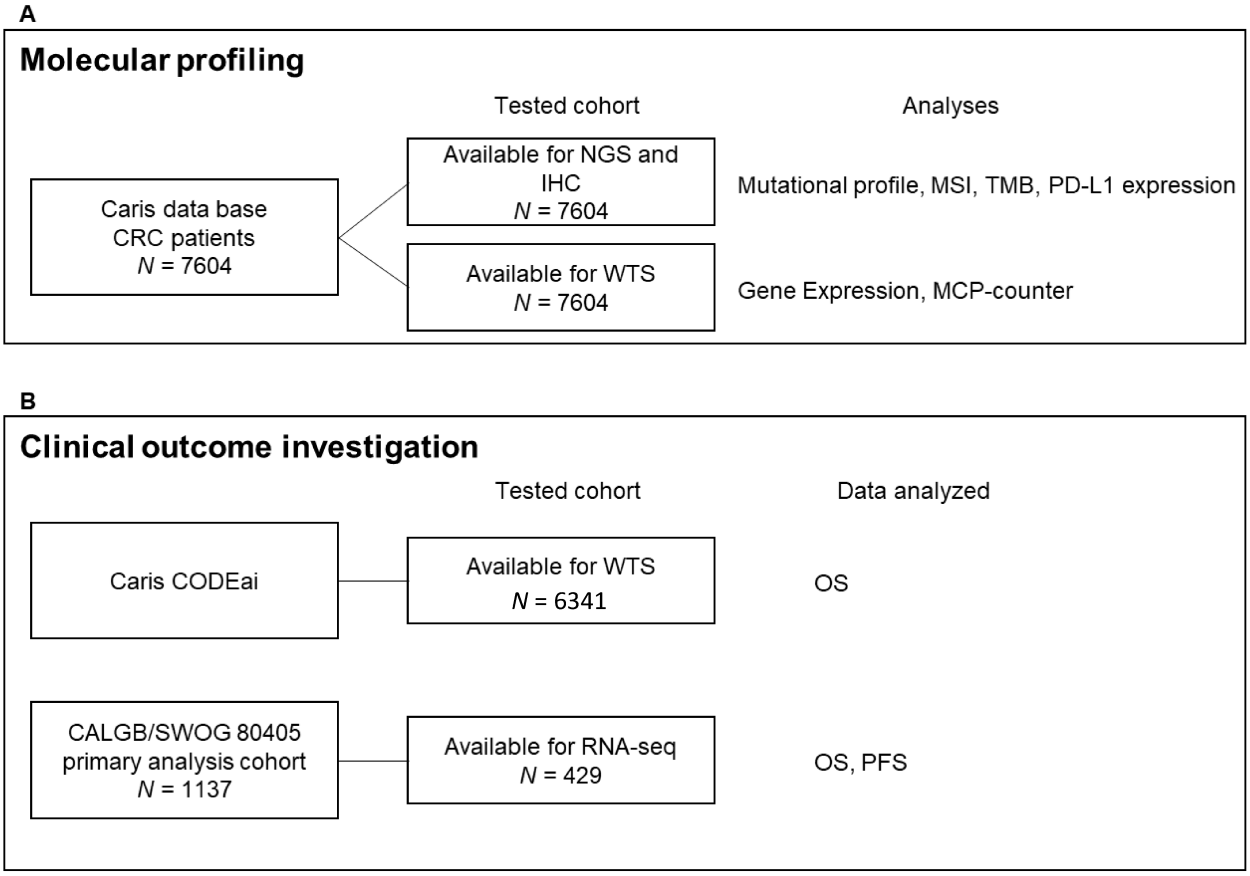
SUPPLEMENTARY FIGURES

Figure S1. Patient Cohorts Flow Diagrams.



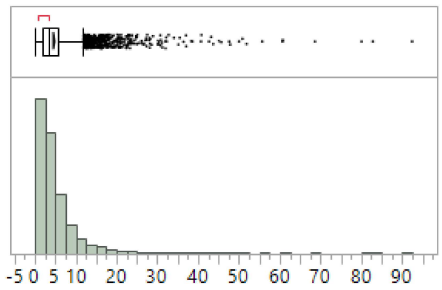
The flow diagrams show which patient cohort was tested for each analysis in this study for molecular profiling (A) and clinical outcome investigation (B).

Abbreviations: CRC, colorectal cancer; IHC, immunohistochemistry; MSI, microsatellite instability; NGS, next-generation sequencing; OS, overall survival; PFS, progression-free survival; RNA-seq, RNA-sequencing; TMB, tumor mutational burden; WTS, whole transcriptome sequencing.

Figure S2. Correlation between *CCR5* and *CCL5* Expression in CRC and Expression Levels in Primary Tumors vs Metastatic Sites.

a.

CCR5

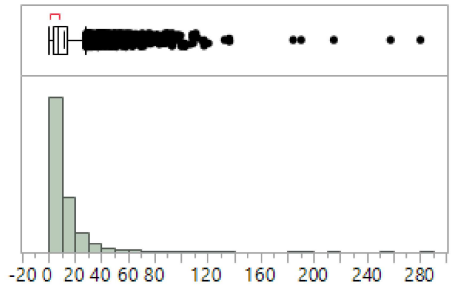


Quantiles		
100.0%	maximum	92.4789
99.5%		30.744335
97.5%		17.2310875
90.0%		9.590635
75.0%	quartile	5.776735
50.0%	median	3.20205
25.0%	quartile	1.7240575
10.0%		0.9120605
2.5%		0.387119
0.5%		0.00053775
0.0%	minimum	0

Summary Statistics	
Mean	4.6349225
Std Dev	5.0870464
Std Err Mean	0.0583371
Upper 95% Mean	4.7492793
Lower 95% Mean	4.5205658
N	7604

b.

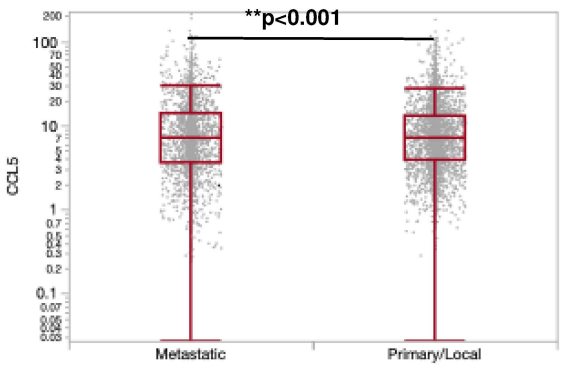
CCL5



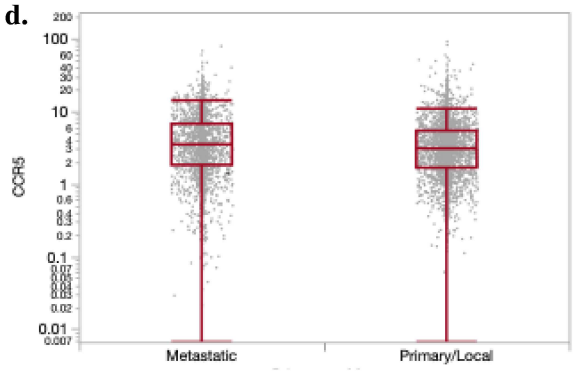
Quantiles		
100.0%	maximum	280.651
99.5%		90.834555
97.5%		46.0946875
90.0%		24.91185
75.0%	quartile	13.5456
50.0%	median	7.216445
25.0%	quartile	3.7515875
10.0%		2.0384
2.5%		0.985666875
0.5%		0.312925475
0.0%	minimum	0

Summary Statistics	
Mean	11.394999
Std Dev	14.186239
Std Err Mean	0.1626845
Upper 95% Mean	11.713905
Lower 95% Mean	11.076092
N	7604

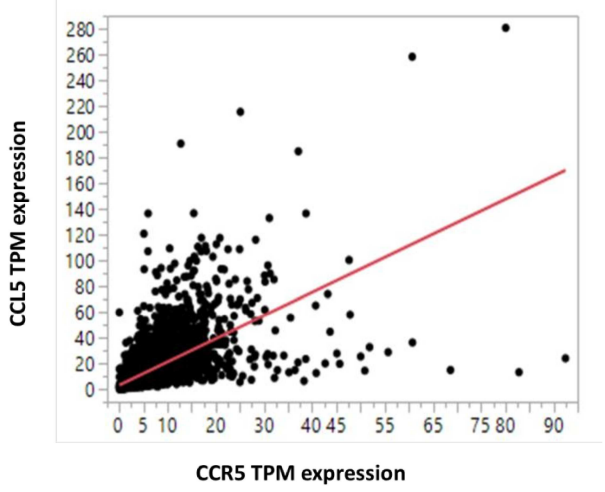
c.



d.



e.



Linear Fit

CCL5 = 3.0243059 + 1.8060049*CCR5

Summary of Fit

RSquare	0.419406
RSquare Adj	0.41933
Root Mean Square Error	10.81016
Observations (or Sum Wgts)	7604

Analysis of Variance

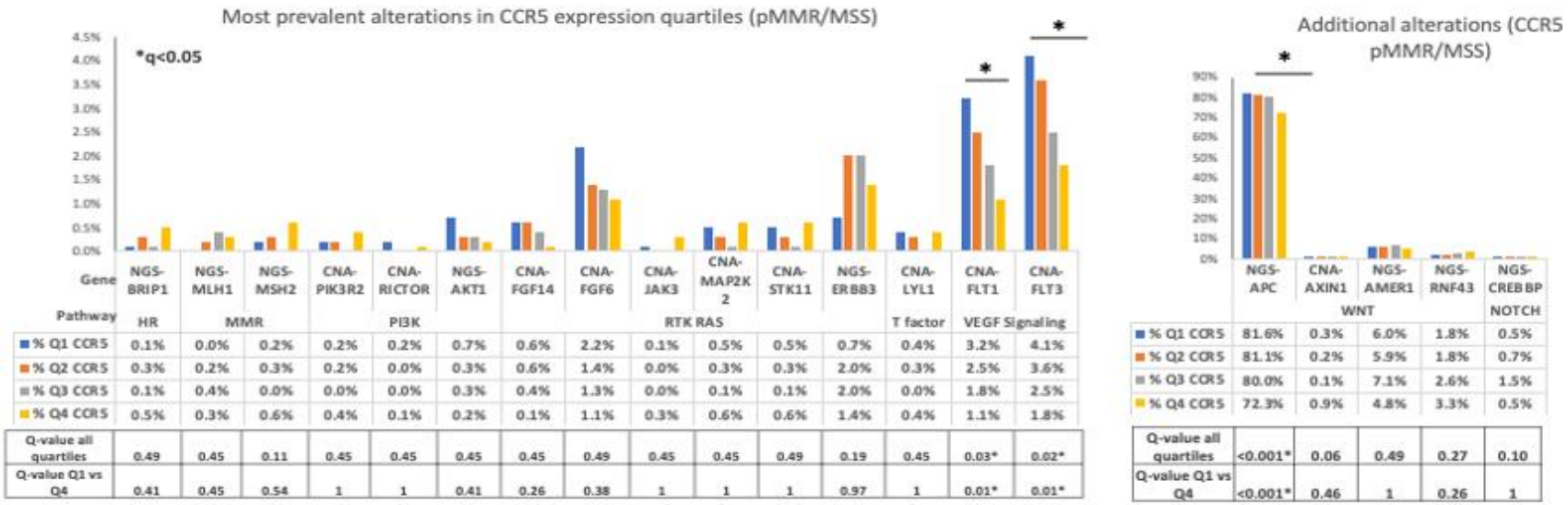
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	641732.8	641733	5491.489
Error	7602	888366.1	117	Prob > F
C. Total	7603	1530099.0		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	3.0243059	0.167713	18.03	<.0001*
CCR5	1.8060049	0.024371	74.10	<.0001*

Figure S3. Association between *CCR5* and *CCL5* Expression and Tumor Molecular Characteristics in pMMR/MSS CRC.

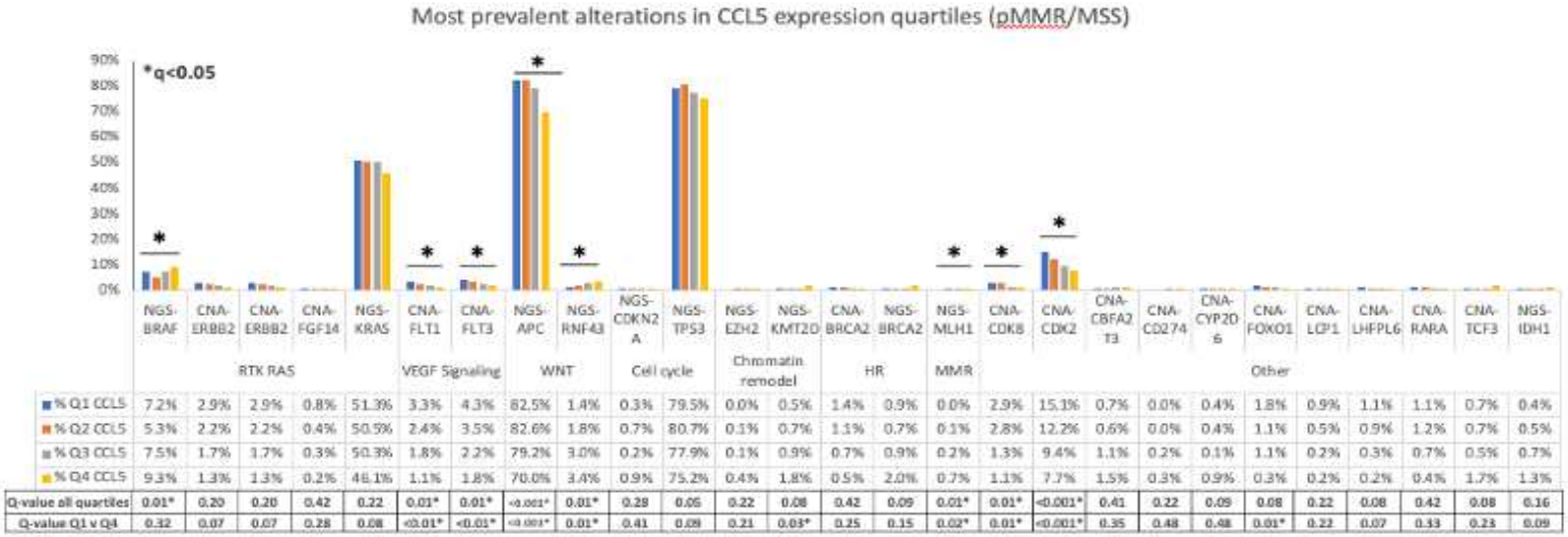
a.



Gene	NGS-BRIP1	NGS-MLH1	NGS-MSH2	CNA-PIK3R2	CNA-RICTOR	NGS-AKT1	CNA-FGF14	CNA-FGF6	CNA-JAK3	CNA-MAP2K2	CNA-STK11	NGS-ERBB3	CNA-LYL1	CNA-FLT1	CNA-FLT3
Pathway	HR	MMR		PI3K			RTK RAS						T factor	VEGF Signaling	
% Q1 CCR5	0.1%	0.0%	0.2%	0.2%	0.2%	0.7%	0.6%	2.2%	0.1%	0.5%	0.5%	0.7%	0.4%	3.2%	4.1%
% Q2 CCR5	0.3%	0.2%	0.3%	0.2%	0.0%	0.3%	0.6%	1.4%	0.0%	0.3%	0.3%	2.0%	0.3%	2.5%	3.6%
% Q3 CCR5	0.1%	0.4%	0.0%	0.0%	0.0%	0.3%	0.4%	1.3%	0.0%	0.1%	0.1%	2.0%	0.0%	1.8%	2.5%
% Q4 CCR5	0.5%	0.3%	0.6%	0.4%	0.1%	0.2%	0.1%	1.1%	0.3%	0.6%	0.6%	1.4%	0.4%	1.1%	1.8%
Q-value all quartiles	0.49	0.45	0.11	0.45	0.45	0.45	0.45	0.49	0.45	0.45	0.49	0.19	0.45	0.03*	0.02*
Q-value Q1 vs Q4	0.41	0.45	0.54	1	1	0.41	0.26	0.38	1	1	1	0.97	1	0.01*	0.01*

Gene	NGS-APC	CNA-AXIN1	NGS-AMER1	NGS-RNF43	NGS-CREBBP
Pathway	WNT				NOTCH
% Q1 CCR5	81.6%	0.3%	6.0%	1.8%	0.5%
% Q2 CCR5	81.1%	0.2%	5.9%	1.8%	0.7%
% Q3 CCR5	80.0%	0.1%	7.1%	2.6%	1.5%
% Q4 CCR5	72.3%	0.9%	4.8%	3.3%	0.5%
Q-value all quartiles	<0.001*	0.06	0.49	0.27	0.10
Q-value Q1 vs Q4	<0.001*	0.46	1	0.26	1

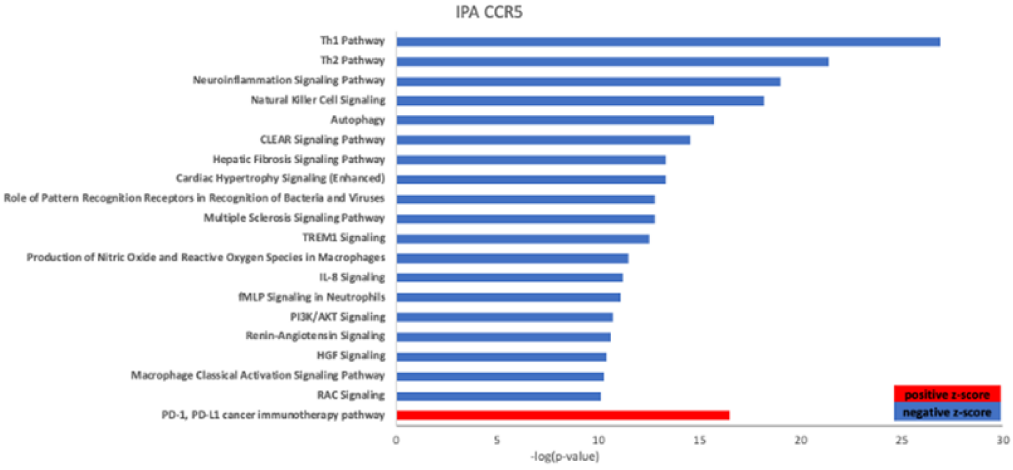
b.



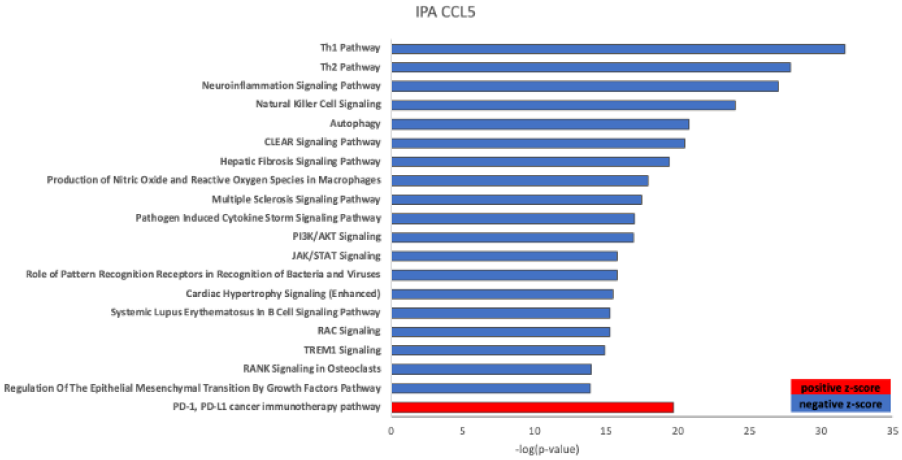
	NGS-BRAF	CNA-ERBB2	CNA-ERBB2	CNA-FGF14	NGS-KRAS	CNA-FLT1	CNA-FLT3	NGS-APC	NGS-RNF43	NGS-CDKN2A	NGS-TP53	NGS-EZH2	NGS-KMT2D	CNA-BRCA2	NGS-BRCA2	NGS-MLH1	CNA-CDK8	CNA-CDK2	CNA-CBFA2T3	CNA-CD274	CNA-CYP2D6	CNA-FOXO1	CNA-LCP1	CNA-LHFPL6	CNA-RARA	CNA-TCF3	NGS-IDH1
	RTK RAS					VEGF Signaling		WNT		Cell cycle		Chromatin remodel		HR		MMR	Other										
% Q1 CCL5	7.2%	2.9%	2.9%	0.8%	51.3%	3.3%	4.3%	82.5%	1.4%	0.3%	79.5%	0.0%	0.5%	1.4%	0.9%	0.0%	2.9%	15.1%	0.7%	0.0%	0.4%	1.8%	0.9%	1.1%	1.1%	0.7%	0.4%
% Q2 CCL5	5.3%	2.2%	2.2%	0.4%	50.5%	2.4%	3.5%	82.6%	1.8%	0.7%	80.7%	0.1%	0.7%	1.1%	0.7%	0.1%	2.8%	12.2%	0.6%	0.0%	0.4%	1.1%	0.5%	0.9%	1.2%	0.7%	0.5%
% Q3 CCL5	7.5%	1.7%	1.7%	0.3%	50.3%	1.8%	2.2%	79.2%	3.0%	0.2%	77.9%	0.1%	0.9%	0.7%	0.9%	0.2%	1.3%	9.4%	1.1%	0.2%	0.1%	1.1%	0.2%	0.3%	0.7%	0.5%	0.7%
% Q4 CCL5	9.3%	1.3%	1.3%	0.2%	46.1%	1.1%	1.8%	70.0%	3.4%	0.9%	75.2%	0.4%	1.8%	0.5%	2.0%	0.7%	1.1%	7.7%	1.5%	0.3%	0.9%	0.3%	0.2%	0.2%	0.4%	1.7%	1.3%
Q-value all quartiles	0.01*	0.20	0.20	0.42	0.22	0.01*	0.01*	<0.001*	0.01*	0.28	0.05	0.22	0.08	0.42	0.09	0.01*	0.01*	<0.001*	0.41	0.22	0.09	0.08	0.22	0.08	0.42	0.08	0.16
Q-value Q1 v Q4	0.32	0.07	0.07	0.28	0.08	<0.01*	<0.01*	<0.001*	0.01*	0.41	0.09	0.21	0.03*	0.25	0.15	0.02*	0.01*	<0.001*	0.35	0.48	0.48	0.01*	0.22	0.07	0.33	0.23	0.09

Figure S4. Ingenuity Pathway Analysis Results for *CCR5* and *CCL5* Q1 vs Q4 in pMMR/MSS CRC.

a.



b.



c.

Pathways Upregulated in CCR5 Q4 (Z score >=2)

Ingenuity Canonical Pathways	-log(p-value)	Ratio	z-score
PD-1, PD-L1 cancer immunotherapy pathway	16.5	0.566	3.983
PTEN Signaling	6.92	0.38	3.92
PPARα/RXRα Activation	4.79	0.323	3.781
PPAR Signaling	3.73	0.346	4.459
RHOGDI Signaling	3.37	0.291	5.032
Antioxidant Action of Vitamin C	2.11	0.295	5.196
Role of p14/p19ARF in Tumor Suppression	2.1	0.4	2.887
CDX Gastrointestinal Cancer Signaling Pathway	1.91	0.262	2.828
CTLA4 Signaling in Cytotoxic T Lymphocytes	1.09	0.219	5.964
Cyclins and Cell Cycle Regulation	0.839	0.247	2.496
LXR/RXR Activation	0.668	0.228	2.449
SPINK1 Pancreatic Cancer Pathway	0	0.15	3

d.

Pathways Upregulated in CCL5 Q4 (Z score >=2)

Ingenuity Canonical Pathways	-log(p-value)	Ratio	z-score
PD-1, PD-L1 cancer immunotherapy pathway	19.7	0.604	4.276
PTEN Signaling	10.7	0.433	4.621
PPAR Signaling	9.81	0.467	4.619
PPARα/RXRα Activation	7.26	0.359	4.371
Antioxidant Action of Vitamin C	5.17	0.375	5.657
RHOGDI Signaling	5.07	0.318	5.374
Endocannabinoid Cancer Inhibition Pathway	4.31	0.333	2.654
CTLA4 Signaling in Cytotoxic T Lymphocytes	3.69	0.253	6.917
CDX Gastrointestinal Cancer Signaling Pathway	3.52	0.297	2.782
Role of p14/p19ARF in Tumor Suppression	3.17	0.467	3.742
SPINK1 Pancreatic Cancer Pathway	0	0.15	3

Figure S4 (a) and (b) show top 20 significant results of IPA for *CCR5* and *CCL5*, respectively; (c) and (d) show upregulated pathways with Z score $\geq$ 2 for *CCR5* and *CCL5* Q4, respectively.

Figure S5. Comparison of Immune Cell Ratio in the TME According to *CCR5* and *CCL5* Expression (Q1 vs Q4) in pMMR/MSS CRC.

Complete cohort, CCR5

CCR5 TME ratios	KW p value Q1 vs Q4	Median ratio Q1	Median ratio Q4
B cell/Lymphocytes	**p<0.0001	0.3833	0.3480
M1/M2	**p<0.0001	1.6890	1.3267
Neutrophil/Lymphocytes	**p<0.0001	0.7906	0.5178
NK cell/Lymphocytes	**p<0.0001	0.4083	0.2985
CD4+ T cell/Lymphocytes	**p<0.0001	0	0
CD8+ T cell/Lymphocytes	**p<0.0001	0	0.0318
T reg/Lymphocytes	**p<0.0001	0.1252	0.1866

Complete cohort, CCL5

CCL5 TME ratios	KW p value Q1 vs Q4	Median ratio Q1	Median ratio Q4
B cell/Lymphocytes	**p<0.0001	0.3885	0.3372
M1/M2	0.4172	1.5430	1.5055
Neutrophil/Lymphocytes	**p<0.0001	0.796	0.4708
NK cell/Lymphocytes	**p<0.0001	0.416	0.2888
CD4+ T cell/Lymphocytes	**p<0.0001	0	0
CD8+ T cell/Lymphocytes	**p<0.0001	0	0.0484
T reg/Lymphocytes	**p<0.0001	0.1193	0.1933

MSS cohort, CCR5

CCR5 TME ratios (MSS)	KW p value Q1 vs Q4	Median ratio Q1	Median ratio Q4
B cell/Lymphocytes	**p<0.0001	0.3864	0.3532
M1/M2	**p<0.0001	1.6469	1.2618
Neutrophil/Lymphocytes	**p<0.0001	0.8008	0.5366
NK cell/Lymphocytes	**p<0.0001	0.4104	0.3008
CD4+ T cell/Lymphocytes	**p<0.0001	0	0
CD8+ T cell/Lymphocytes	**p<0.0001	0	0.0253
T reg/Lymphocytes	**p<0.0001	0.1206	0.1823

MSS cohort, CCL5

CCL5 TME ratios (MSS)	KW p value Q1 vs Q4	Median ratio Q1	Median ratio Q4
B cell/Lymphocytes	**p<0.0001	0.3894	0.3458
M1/M2	0.0108	1.5358	1.3756
Neutrophil/Lymphocytes	**p<0.0001	0.8019	0.4814
NK cell/Lymphocytes	**p<0.0001	0.4170	0.2891
CD4+ T cell/Lymphocytes	**p<0.0001	0	0
CD8+ T cell/Lymphocytes	**p<0.0001	0	0.0410
T reg/Lymphocytes	**p<0.0001	0.1164	0.1875

Lymphocytes: NK, B cells, CD4+ T cells, CD8+ T cells and Treg total count.

Figure S6. Correlation between *CCR5* and *CCL5* Expression (a) and Association with TMB-H (b) and dMMR/MSI-H (c) in CALGB/SWOG 80405.

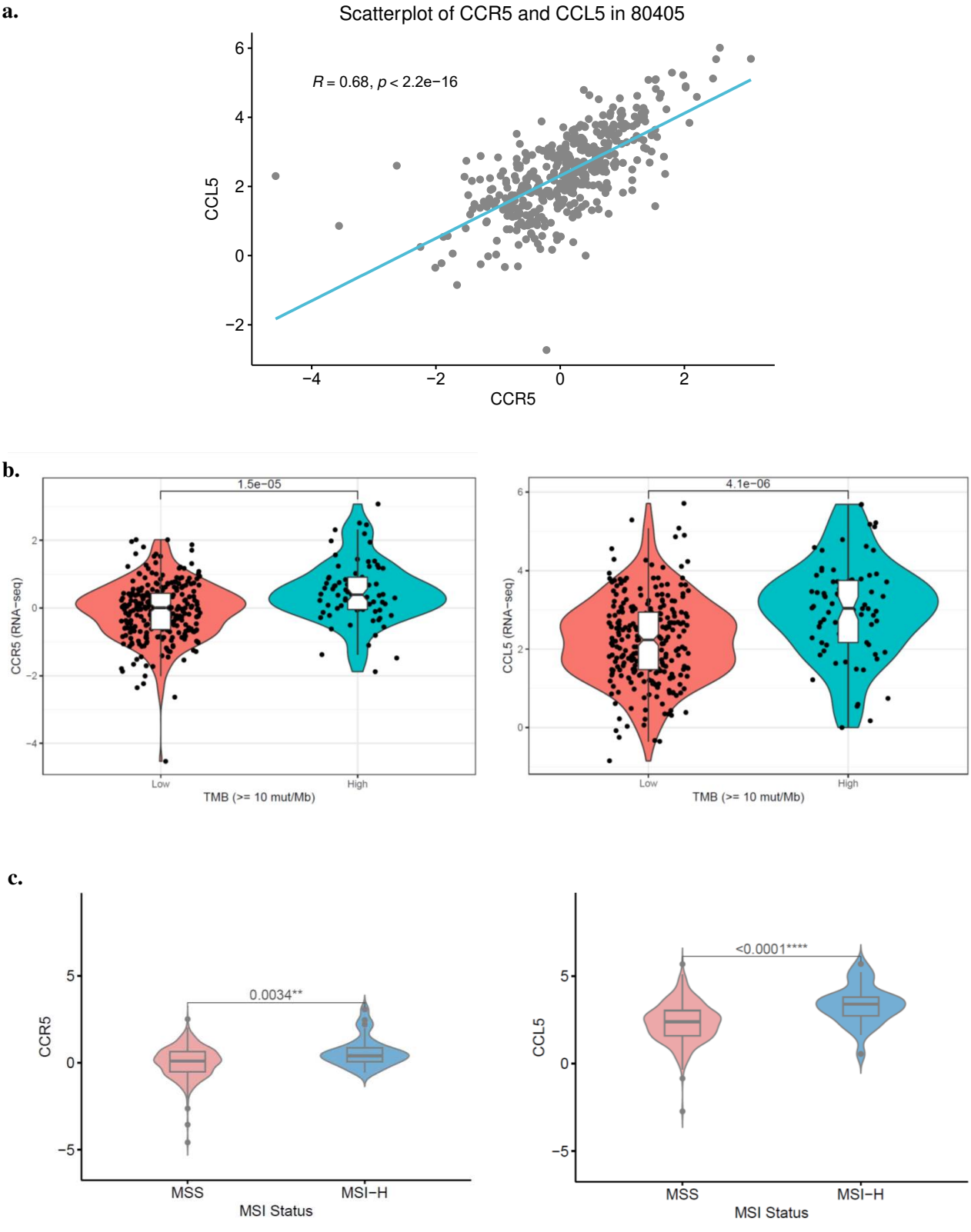
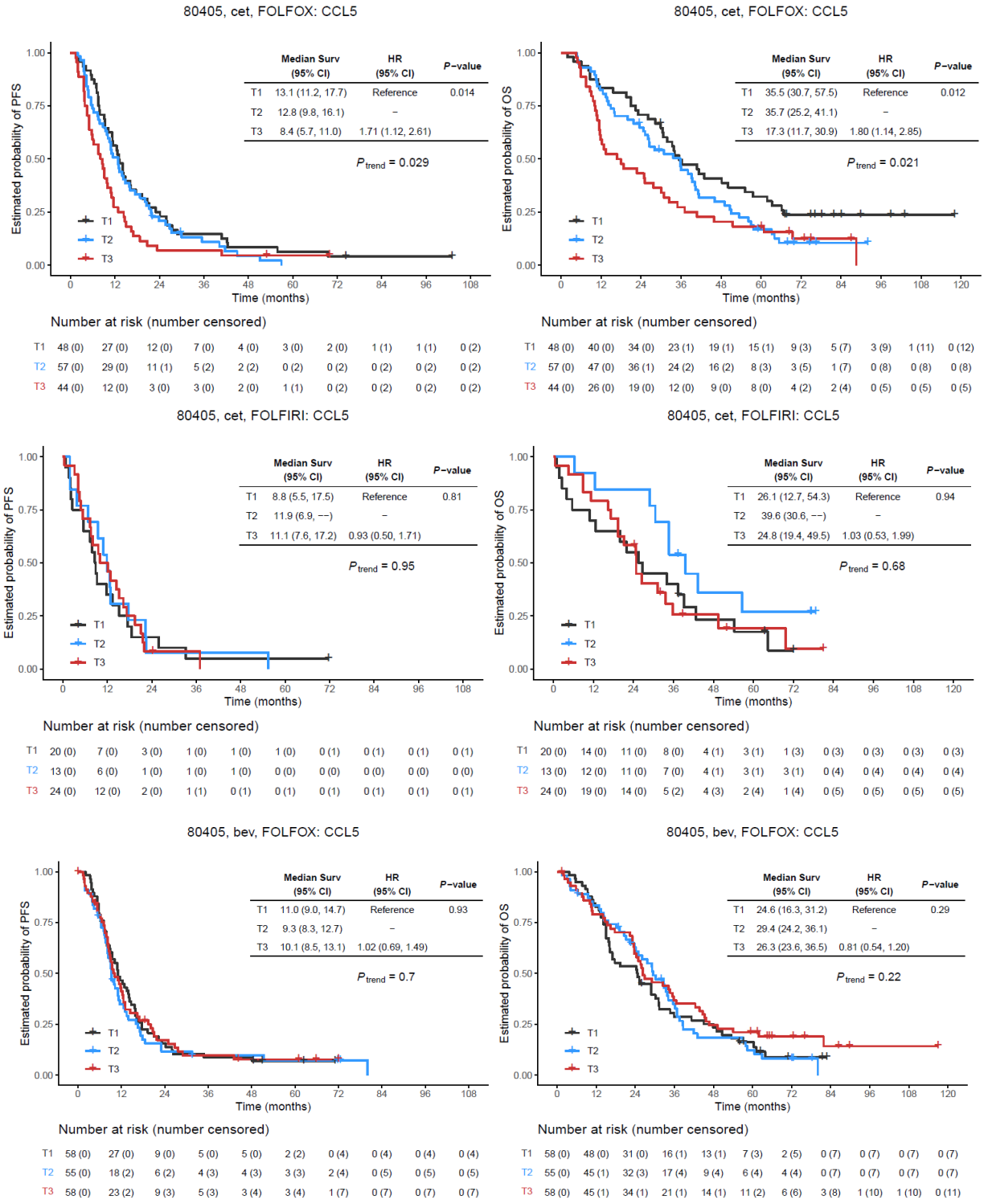


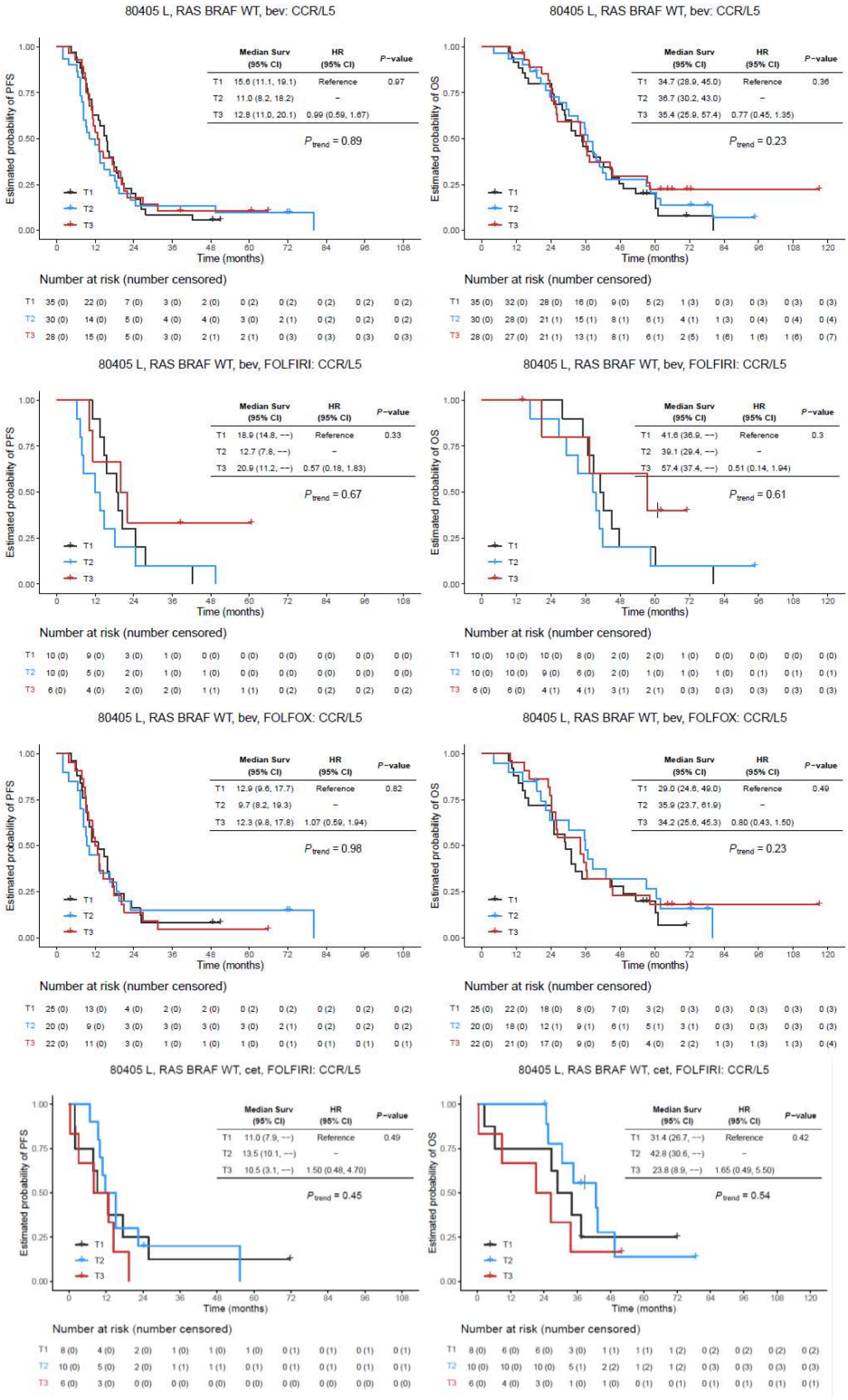
Figure S7. Association between *CCL5* Expression and Patient Outcomes in the CALGB/SWOG 80405 Trial.



The *P*-value corresponds to the statistical comparison for T3 vs T1 within each treatment.

The P_{trend} corresponds to the statistical test result by evaluating gene expression as a continuous variable.

Figure S8. Association Between CCR/L5 Combined Expression and Patient Outcomes in the CALGB/SWOG 80405 Trial for *RAS/BRAF* Wild-type Left-sided Tumors for Bevacizumab-based Treatment and FOLFIRI-based Treatments.



The *P*-value corresponds to the statistical comparison for T3 vs T1 within each treatment.
The P_{trend} corresponds to the statistical test result by evaluating gene expression as a continuous variable.