Table S1: Association of monocyte percentages, sCD163 levels and TAM subsets with clinicopathological characteristics of CRC patients. A. sCD163 levels in serum. B. Subset distribution of circulating monocytes. C. Expression of CD163 on circulating monocyte subsets. D. Density of sTAMs in primary colorectal tumors. E. Subset distribution of sTAMs in primary colorectal tumors. F. Density of ieTAMs in primary colorectal tumors. G. Subset distribution of ieTAMs in primary colorectal tumors. Statistically significant P-values (≤0.05) are indicated in bold. Abbreviations: CRC (colorectal cancer), ieTAM (intraepithelial TAM), MFI (median fluorescence intensity), sCD163 (soluble CD163), SD (standard deviation), sTAM (stromal TAM), TAMs (tumor-associated macrophages), TNM (Tumor, Node, Metastasis).

| Parameters                        | sCD163 (mg/l) | No. | Mean | SD | P-value |
|-----------------------------------|---------------|-----|------|----|---------|
| TNM stage (Spearman)             |               |     |      |    | 0.141   |
| TNM stage (ANOVA)                |               |     |      |    | 0.215   |
| Stage 0/I                        |               | 15  | 1.9  | 0.7|         |
| Stage II/III                     |               | 43  | 2.4  | 1.1|         |
| Stage IV                         |               | 6   | 2.7  | 1.1|         |
| Tumor location                   |               |     |      |    | 0.626   |
| Colon                             |               | 52  | 2.3  | 1.1|         |
| Rectum                           |               | 12  | 2.2  | 0.7|         |
| Tumor differentiation grade      |               |     |      |    | 0.729   |
| Well/moderate                    |               | 48  | 2.3  | 1.0|         |
| Poor                              |               | 13  | 2.4  | 1.0|         |
| Tumor lymph node invasion        |               |     |      |    | 0.355   |
| Yes                               |               | 26  | 2.4  | 1.2|         |
| No                                |               | 37  | 2.2  | 0.9|         |
### Table: CD163 Expression in Colorectal Cancer

| Parameters                        | Total monocytes (% of CD45+ PBMCs) | Classical monocytes (% of total monocytes) | Intermediate monocytes (% of total monocytes) | Nonclassical monocytes (% of total monocytes) |
|-----------------------------------|-----------------------------------|------------------------------------------|---------------------------------------------|---------------------------------------------|
| No.                               | Mean SD  | P-value | Mean SD  | P-value | Mean SD  | P-value | Mean SD  | P-value |
| TNM stage (Spearman)              | 0.004    | 0.219   | 0.179   | 0.682   | 0.641   |
| TNM stage (ANOVA)                 | 0.060    | 0.215   | 0.165   | 0.682   | 0.641   |
| Stage 0/I                         | 14 23 13 | 89 6 3 | 7 4 2 |
| Stage II/III                      | 25 28 12 | 88 6 5 | 6 4 2 |
| Stage IV                          | 8 34 11 | 91 6 3 | 2 4 4 |
| Tumor location                    | 0.874    | 0.874   | 0.961   | 0.826   | 0.826   |
| Colon                             | 35 28 14 | 89 7 4 | 6 4 3 |
| Rectum                            | 12 26 8 | 89 4 4 | 4 4 2 |
| Tumor differentiation grade       | 0.055    | 0.039   | 0.948   | 0.886   | 0.886   |
| Well/moderate                     | 34 25 12 | 88 7 5 | 7 4 3 |
| Poor                              | 11 34 15 | 92 3 2 | 1 4 2 |
| Tumor lymph node invasion         | 0.011    | 0.254   | 0.465   | 0.877   | 0.877   |
| Yes                               | 17 40 13 | 89 8 5 | 7 4 3 |
| No                                | 30 24 11 | 89 5 4 | 4 4 2 |
CD163 as a biomarker in colorectal cancer: the expression on circulating monocytes and tumor-associated macrophages, and the soluble form in the blood

| Parameters                          | CD163 (MFI) | CD163 (MFI) | CD163 (MFI) | CD163 (MFI) |
|-------------------------------------|-------------|-------------|-------------|-------------|
|                                     | Total monocytes | Classical monocytes | Intermediate monocytes | Nonclassical monocytes |
|                                     | No. | Mean | SD | P-value | Mean | SD | P-value | Mean | SD | P-value | Mean | SD | P-value |
| TNM stage (Spearman)                | 0.968 | 0.894 | 0.813 | 0.161 |
| TNM stage (ANOVA)                   | 0.837 | 0.896 | 0.485 | 0.286 |
| Stage 0/I                           | 14 | 10805 | 7656 | 11376 | 8560 | 17282 | 10209 | 4014 | 1949 |
| Stage II/III                        | 25 | 1188 | 5021 | 12343 | 5491 | 17525 | 7839 | 3733 | 1976 |
| Stage IV                            | 8 | 10912 | 4857 | 11571 | 5858 | 14009 | 3353 | 2817 | 1140 |
| Tumor location                      | 0.762 | 0.806 | 0.542 | 0.188 |
| Colon                               | 35 | 11239 | 6000 | 11785 | 6772 | 16010 | 9411 | 3436 | 1778 |
| Rectum                              | 12 | 11836 | 5370 | 12327 | 5754 | 19316 | 9639 | 4318 | 2030 |
| Tumor differentiation grade         | 0.704 | 0.700 | 0.969 | 0.277 |
| Well/moderate                       | 34 | 11366 | 6131 | 11915 | 6886 | 16375 | 8161 | 3760 | 1990 |
| Poor                                | 11 | 10614 | 3749 | 11060 | 4192 | 17005 | 7109 | 3121 | 1449 |
| Tumor lymph node invasion           | 0.942 | 0.900 | 0.947 | 0.528 |
| Yes                                 | 17 | 11308 | 4373 | 11763 | 5191 | 16151 | 4160 | 3372 | 1419 |
| No                                  | 30 | 11439 | 6534 | 12014 | 7178 | 17252 | 9644 | 3825 | 2079 |
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| Parameters                        | M0 sTAM density (cells/mm²) | M1 sTAM density (cells/mm²) | M2 sTAM density (cells/mm²) | M3 sTAM density (cells/mm²) |
|-----------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                                   | No. | Mean | SD  | P-value | Mean | SD  | P-value | Mean | SD  | P-value | Mean | SD  | P-value |
| TNM stage (Spearman)              |     |      |     | 0.563   |      |     | 0.998   |      |     | 0.423   |      |     | 0.947    |
| TNM stage (ANOVA)                 |     |      |     | 0.876   |      |     | 0.621   |      |     | 0.239   |      |     | 0.835    |
| Stage 0/I                         | 14  | 407  | 520 | 93      | 97   | 1153 | 675   | 220  | 294 |
| Stage II/III                      | 49  | 387  | 329 | 125     | 133  | 1092 | 621   | 174  | 225 |
| Stage IV                          | 9   | 335  | 161 | 64      | 44   | 1418 | 618   | 143  | 131 |
| Colon                             | 60  | 398  | 380 | 107     | 123  | 1166 | 655   | 154  | 190 |
| Rectum                            | 12  | 316  | 179 | 134     | 111  | 1036 | 503   | 305  | 353 |
| Tumor differentiation grade       |     |      |     | 1.000   |      |     | 0.800   |      |     | 0.112   |      |     | 0.894    |
| Well/moderate                     | 58  | 400  | 384 | 117     | 127  | 1087 | 609   | 189  | 243 |
| Poor                              | 13  | 336  | 187 | 94      | 83   | 1414 | 707   | 146  | 163 |
| Tumor lymph node invasion         |     |      |     | 0.553   |      |     | 1.000   |      |     | 0.802   |      |     | 0.508    |
| Yes                               | 30  | 391  | 322 | 107     | 104  | 1178 | 677   | 150  | 174 |
| No                                | 42  | 379  | 381 | 114     | 132  | 1121 | 604   | 200  | 262 |
| Parameters                                | M0 sTAMs (% of total sTAMs) | M1 sTAMs (% of total sTAMs) | M2 sTAMs (% of total sTAMs) | M3 sTAMs (% of total sTAMs) |
|-------------------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                                           | No. | Mean | SD   | P-value | Mean | SD   | P-value | Mean | SD   | P-value | Mean | SD   | P-value |
| TNM stage (Spearman)                      |     |      |      |         |      |      |         |      |      |         |      |      |         |
| TNM stage (ANOVA)                         |     |      |      | 0.952   | 0.970 | 0.419 | 0.731   | 0.814 | 0.598 | 0.287   | 0.940 | 0.174 | 0.311   |
| Stage 0/I                                 | 14  | 23   | 16   | 5       | 5    | 62   | 15      | 10   | 10   |         | 0.629 | 0.174 | 0.311   |
| Stage II/III                              | 49  | 22   | 15   | 8       | 9    | 61   | 19      | 9    | 9    |         | 0.629 | 0.174 | 0.311   |
| Stage IV                                  | 9   | 17   | 5    | 4       | 3    | 71   | 8       | 8    | 5    |         | 0.629 | 0.174 | 0.311   |
| Tumor location                            |     |      |      | 0.405   | 0.582 | 0.093 | 0.582   | 0.405 | 0.582 | 0.093   | 0.582 | 0.582 | 0.582   |
| Colon                                     | 60  | 22   | 14   | 7       | 8    | 63   | 18      | 8    | 8    |         | 0.615 | 0.173 | 0.173   |
| Rectum                                    | 12  | 19   | 12   | 8       | 7    | 59   | 14      | 14   | 12   |         | 0.615 | 0.173 | 0.173   |
| Tumor differentiation grade               |     |      |      | 0.405   | 0.582 | 0.093 | 0.582   | 0.405 | 0.582 | 0.093   | 0.582 | 0.582 | 0.582   |
| Well/moderate                              |      |      |      |         |      |      |         |      |      |         |      |      |         |
| Poor                                      | 58  | 23   | 15   | 7       | 8    | 61   | 18      | 10   | 10   |         | 0.615 | 0.173 | 0.173   |
| Poor                                      | 13  | 18   | 7    | 5       | 5    | 70   | 11      | 7    | 6    |         | 0.615 | 0.173 | 0.173   |
| Tumor lymph node invasion                 |     |      |      | 0.405   | 0.582 | 0.093 | 0.582   | 0.405 | 0.582 | 0.093   | 0.582 | 0.582 | 0.582   |
| Yes                                       | 30  | 21   | 12   | 7       | 7    | 63   | 17      | 9    | 9    |         | 0.615 | 0.173 | 0.173   |
| No                                        | 42  | 21   | 15   | 7       | 8    | 62   | 18      | 10   | 9    |         | 0.615 | 0.173 | 0.173   |
### CD163 as a biomarker in colorectal cancer: the expression on circulating monocytes and tumor-associated macrophages, and the soluble form in the blood

| Parameters                              | M0 ieTAM density (cells/mm²) | M1 ieTAM density (cells/mm²) | M2 ieTAM density (cells/mm²) | M3 ieTAM density (cells/mm²) |
|-----------------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
|                                         | No.  | Mean | SD | P-value | No.  | Mean | SD | P-value | No.  | Mean | SD | P-value | No.  | Mean | SD | P-value |
| TNM stage (Spearman)                    |      |      |    |         |      |      |    |         |      |      |    |         |      |      |    |         |
|                                         | 0.743 | 0.293 | 0.901 | 0.092 |
| TNM stage (ANOVA)                       |      |      |    |         |      |      |    |         |      |      |    |         |      |      |    |         |
|                                         | 0.763 | 0.164 | 0.816 | 0.329 |
| Stage 0/I                               | 14   | 128  | 71 |         | 197  | 177  | 56 | 35 | 91   | 109 |
| Stage II/III                            | 46   | 155  | 114|         | 192  | 195  | 60 | 56 | 50   | 59 |
| Stage IV                                | 8    | 132  | 80 |         | 73   | 41   | 61 | 34 | 26   | 23 |
| Tumor location                          |      |      |    |         |      |      |    |         |      |      |    |         |      |      |    |         |
| Colon                                   | 58   | 148  | 106|         | 183  | 177  | 62 | 52 | 59   | 74 |
| Rectum                                  | 10   | 137  | 80 |         | 156  | 221  | 47 | 34 | 34   | 49 |
| Tumor differentiation grade             |      |      |    |         |      |      |    |         |      |      |    |         |      |      |    |         |
| Well/moderate                           | 55   | 137  | 90 |         | 186  | 193  | 49 | 40 | 54   | 71 |
| Poor                                    | 12   | 197  | 141|         | 160  | 134  | 105| 68 | 64   | 76 |
| Tumor lymph node invasion                |      |      |    |         |      |      |    |         |      |      |    |         |      |      |    |         |
| Yes                                     | 27   | 146  | 84 |         | 178  | 203  | 53 | 39 | 41   | 50 |
| No                                      | 41   | 147  | 114|         | 180  | 170  | 64 | 56 | 65   | 81 |
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| Parameters                        | M0 ieTAMs (% of total ieTAMs) | M1 ieTAMs (% of total ieTAMs) | M2 ieTAMs (% of total ieTAMs) | M3 ieTAMs (% of total ieTAMs) |
|-----------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
|                                   | No.  | Mean | SD  | P-value | Mean | SD  | P-value | Mean | SD  | P-value | Mean | SD  | P-value |
| TNM stage (Spearman)              |      | 0.076 |     |         | 0.430 |     |         | 0.205 |     |         | 0.137 |     |         |
| TNM stage (ANOVA)                 |      | 0.207 |     |         | 0.252 |     |         | 0.067 |     |         | 0.441 |     |         |
| Stage 0/I                        | 14   | 34   | 23  |         | 37   | 18  |         | 13   | 9   |         | 17   | 18  |         |
| Stage II/III                     | 46   | 37   | 21  |         | 38   | 20  |         | 14   | 11  |         | 11   | 10  |         |
| Stage IV                         | 8    | 45   | 12  |         | 26   | 9   |         | 21   | 6   |         | 9    | 9   |         |
| Tumor location                   |      | 0.315 |     |         | 0.860 |     |         | 0.768 |     |         | 0.153 |     |         |
| Colon                            | 58   | 36   | 20  |         | 36   | 19  |         | 15   | 11  |         | 13   | 12  |         |
| Rectum                           | 10   | 43   | 19  |         | 35   | 18  |         | 14   | 8   |         | 7    | 9   |         |
| Tumor differentiation grade      |      | 0.327 |     |         | 0.095 |     |         | 0.004 |     |         | 0.630 |     |         |
| Well/moderate                    | 55   | 36   | 21  |         | 38   | 20  |         | 13   | 10  |         | 12   | 12  |         |
| Poor                             | 12   | 42   | 19  |         | 28   | 15  |         | 20   | 7   |         | 10   | 11  |         |
| Tumor lymph node invasion        |      | 0.151 |     |         | 0.739 |     |         | 0.826 |     |         | 0.205 |     |         |
| Yes                              | 27   | 41   | 20  |         | 35   | 20  |         | 14   | 8   |         | 10   | 11  |         |
| No                               | 41   | 35   | 21  |         | 37   | 19  |         | 15   | 12  |         | 13   | 13  |         |
Table S2: Flow cytometry antibody panel used for the identification of monocytes in peripheral blood of CRC patients. Abbreviations: AF (alexa fluor), APC (allophycocyanin), BV (brilliant violet), FITC (fluorescein isothiocyanate), nIR (near-infrared), PE (phycoerythrin), PE-Cy7 (phycoerythrin-cyanine7), PerCP (peridinin chlorophyll protein complex), V500 (violet500).

| Marker | Fluorochrome | Clone   | Source          | Staining concentration (µg/ml) |
|--------|--------------|---------|-----------------|------------------------------|
| CD3    | BV605        | SK7     | BD Biosciences  | 1.25                         |
| CD4    | PE-Cy7       | SK3     | BD Biosciences  | 0.03                         |
| CD8    | AF700        | RPA-T8  | BD Biosciences  | 0.32                         |
| CD14   | V500         | Møp9    | BD Biosciences  | 0.25                         |
| CD16   | PerCP-eFluor710 | 3G8    | eBioscience     | 0.75                         |
| CD25   | APC          | BC96    | eBioscience     | 0.75                         |
| CD45   | FITC         | HI30    | BD Biosciences  | 0.3                          |
| CD127  | BV421        | HIL-7R-M21 | BD Biosciences | 1.5                          |
| CD163  | PE           | Mac2-158 | Trillium        | 0.5                          |
| Live/dead | nIR     | -       | Life Technologies | recommended                   |
Flow cytometry gating strategy

Figure S1: Flow cytometry gating strategy used for the identification of circulating monocyte subsets. A. Mononuclear cells (excluding CD45 cells and SSC$^{\text{high}}$ cells). B. Viable mononuclear cells (excluding dead cells). C. Single mononuclear cells (excluding doublets). D. CD14$^+$ and/or CD163$^+$ monocytes (excluding CD14 CD163 lymphocytes present in the red gate). E. Classical (CD14$^{++}$CD16$^{-}$), intermediate (CD14$^{+}$CD16$^{+}$) and nonclassical (CD14$^{+}$CD16$^{++}$) monocytes. Abbreviations: FSC (forward scatter), nIR (near-infrared), PBMC (peripheral blood mononuclear cells), SSC (side scatter).
Figure S2: Association between circulating monocytes and Tregs in CRC patients. Correlation between the percentage of total circulating CD14⁺ and/or CD163⁺ monocytes and CD127<low>CD25⁺ Tregs in CRC patients (N=47). Abbreviations: CRC (colorectal cancer), Tregs (regulatory T cells).
**Figure S3**: Association between sCD163 levels and CD163 expression by monocytes in CRC patients. Correlation between sCD163 levels and CD163 expression on circulating monocytes in CRC patients (N=38). Abbreviations: CRC (colorectal cancer), MFI (median fluorescence intensity).
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Figure S4: Association between circulating Tregs and M2 TAMs in CRC patients. Correlation between total circulating CD127^low^CD25^+^ Tregs and CD68^+^iNOS^CD163^+^ M2 sTAM density (N=42) and ieTAM density (N=39) in CRC patients. Abbreviations: CRC (colorectal cancer), ieTAM (intraepithelial TAM), iNOS (inducible nitric oxide synthase), sTAM (stromal TAM), TAM (tumor-associated macrophage), Tregs (regulatory T cells).
Figure S5: Association between sCD163 levels and CD163 expression by TAMs in CRC patients. Correlation between sCD163 levels and CD68\(^+\)iNOS\(^+\)CD163\(^+\) M2 sTAM density (N=60) and iE TAM density (N=56) in CRC patients. Abbreviations: CRC (colorectal cancer), iNOS (inducible nitric oxide synthase), iE TAM (intraepithelial TAM), sTAM (stromal TAM), TAM (tumor-associated macrophage).