Supplemental Figure 1. LN dissection numbers according to tumor location.

LN dissection numbers according to tumor location (right vs. left) are summarized in 21 NCCHE CRC cohort (A) and 130 SCC MSI-H/dMMR CRC cohort (B), respectively. Means and SDs are shown, and statistical analyses were performed using the t-tests. ns, not significant.
Supplemental Figure 2. Clustering with T cell phenotypes in TILs.

Twenty-two TILs from 21 CRC patients were examined with flow cytometry as in Figure 1. Clustering with T cell phenotypes in the TME with clinicopathological features (A), and the frequencies of eTreg cells in the TME according to tumor location, CD4+ T cells and CD8+ T cells in the TME according to pStage, and CD4+ T cells and CD8+ T cells in the TME according to MMR status (B) are shown. Means and SDs are shown, and statistical analyses were performed using the t-tests.
Supplemental Figure 3. Clustering with T cell phenotypes in LNLs.

Twenty-two LNLs of proximal LNs from 21 CRC patients were examined with flow cytometry as in Figure 1. Clustering with T cell phenotypes in proximal LNs with clinicopathological features (A), and the frequencies of eTreg cells in LNLs according to tumor location and effector memory CD8+ T cells in LNLs according to MMR status (B) are shown. Means and SDs are shown, and statistical analyses were performed using the t-tests. EM, effector memory; ns, not significant.
Supplementary Figure 4. Analyses of TCGA datasets of CRCs.

(A) Volcano plots according to microsatellite status. TCGA datasets of CRCs were examined, and differentially expressed genes between MSS and MSI-H were extracted. FC, fold change; FDR, false discovery rate. (B) CD8+ T cell infiltration according to microsatellite status. TCGA datasets were analyzed with CIBERSORTx. Statistical analysis was performed using the t-tests.
Supplemental Figure 5. The proportion of CD4+ T cells and CD8+ T cells in PBLs, LNLs and TILs.

PBLs, LNLs and TILs from 21 CRC patients were examined with flow cytometry as in Figure 1. Summaries for the frequencies of CD4+ T cells and CD8+ T cells are shown. Means and SDs are shown, and statistical analyses were performed using the one-way ANOVA tests with Bonferroni corrections. PB, peripheral blood; dLN, distal LN; pLN, proximal LN; ns, not significant.
Supplemental Figure 6. Comparison of immunological phenotypes between tonsils and LNs.

Tonsils from 5 patients with chronic tonsillitis who received surgical resection of tonsils were minced like tumor tissues, and immunological phenotypes were examined with flow cytometry. T cell fractions indicated were compared between tonsils and LNs. Means and SDs are shown, and statistical analyses were performed using the one-way ANOVA tests with Bonferroni corrections. dLN, distal LN; pLN, proximal LN; ns, not significant.
PBLs, LNLs and TILs from 21 CRC patients were examined with flow cytometry as in Figure 1. Summaries for frequencies of T-bet and Eomes expressing cells in CD4$^+$ T cells in PBLs, LNLs and TILs are shown. Means and SDs are shown, and statistical analyses were performed using the one-way ANOVA tests with Bonferroni corrections. PB, peripheral blood; dLN, distal LN; pLN, proximal LN; ns, not significant.
Supplemental Figure 8. The expression of Treg cell-related molecules by each FOXP3+CD4+ T cell subpopulation.

PBLs and LNLs of proximal LN from CRC patients were examined with flow cytometry as in Figure 1. Summaries for frequencies of PD-1, CTLA-4, CD39, GITR and ICOS expressing cells in total CD4+ T cells, naive Treg cells, eTreg cells and FOXP3low non-Treg cells in PBLs, LNLs and TILs are presented. Means and SDs are shown, and statistical analyses were performed using the one-way ANOVA tests with Bonferroni
corrections. ns, not significant.
Supplemental Figure 9. The proportion of FOXP3-CD4+ T cell subpopulation.

PBLs, LNLs and TILs from 21 CRC patients were examined with flow cytometry as in Figure 1. Summaries for frequencies of CD45RA-FOXP3-CD4+ T cells and CD45RA+FOXP3-CD4+ T cells in CD4+ T cells are shown. Means and SDs are shown, and statistical analyses were performed using the one-way ANOVA tests with Bonferroni corrections. PB, peripheral blood; dLN, distal LN; pLN, proximal LN; ns, not significant.
Supplemental Figure 10. TCR analyses according to MMR status and pathological staging.

PBLs, LNLs and TILs from 21 CRC patients who received surgical resection were prepared, and the TCR sequencing was performed with next-generation sequencing. If there were remaining samples, the TCR sequencing was performed for PBLs and LNLs of distal LNs. (A) Diversity of TCR repertoire according to MMR status. The diversity was evaluated with Shannon’s index. (B) Diversity of TCR repertoire and shared TCRs in TILs according to pathological staging. Shannon’s index (left) and the frequency of shared TCRs in TILs (right) of each sample are shown. Means and SDs are shown, and statistical analyses were performed using the t-tests. PB, peripheral blood; dLN, distal LN; pLN, proximal LN; ns, not significant.
Supplemental Figure 11. TMB of pMMR and dMMR CRCs.

TMB was analyzed with WES using next-generation sequencing if remaining tumor DNA samples were available. Comparison in non-synonymous single nucleotide variations (SNV) (left) and insertion or deletion (indel) (right) between pMMR and dMMR CRCs (A), the associations between TMB and TCR diversity in proximal LN (left) and tumors (right) (B) or between TMB and shared TCRs in LNLs and TILs (C) are presented. Means and SDs are shown, and statistical analyses were performed using the t-tests. ns, not significant.
Supplemental Figure 12. An ROC curve of LN dissection number for recurrence.

An ROC curve of LN dissection number for recurrence within 2 years after surgery was constructed to determine the cut-off value. ROC, receiver operating characteristic; AUC, area under curve.
Supplemental Table 1. Patient characteristics subjected to immunological analyses (NCCHE cohort).

| Characteristics | pMMR (n = 11, 11 samples) | dMMR (n = 10, 11 samples) | P |
|-----------------|---------------------------|---------------------------|---|
| Age (years)     |                           |                           |   |
| Average ± SD (median) | 60.2 ± 11.9 (61.5) | 65.6 ± 13.9 (67) | 0.022 |
| Sex             |                           |                           |   |
| Male            | 5                         | 7                         | 0.39 |
| Female          | 6                         | 3                         |   |
| Tumor location  |                           |                           |   |
| Right           | 5                         | 8                         | 0.39 |
| Left            | 6                         | 3                         |   |
| Differentiation |                           |                           |   |
| Well or moderate| 11                        | 6                         | 0.035 |
| Poor            | 0                         | 4                         |   |
| pStage          |                           |                           |   |
| I               | 1                         | 2                         |   |
| II              | 4                         | 5                         | 0.67* |
| III             | 5                         | 4                         |   |
| IV              | 1                         | 0                         |   |
| LN dissection number |                           |                           |   |
| Average ± SD (median) | 34.6 ± 14.4 (30.5) | 32.9 ± 14.7 (28) | 0.73 |

*pStage I or II vs. III or IV
| Characteristics         | n = 130                                      |
|-------------------------|---------------------------------------------|
| Age (years)             |                                            |
| Average ± SD (median)   | 64.8 ± 12.5 (66)                            |
| Sex                     |                                            |
| Male                    | 56                                          |
| Female                  | 74                                          |
| Tumor location          |                                            |
| Right                   | 39                                          |
| Left                    | 91                                          |
| Differentiation         |                                            |
| Well or moderate        | 31                                          |
| Poor                    | 99                                          |
| pStage                  |                                            |
| 0                       | 2                                           |
| I                       | 29                                          |
| II                      | 68                                          |
| III                     | 31                                          |
| Tumor diameter          |                                            |
| Average ± SD (median)   | 57.2 ± 34.9 (50)                            |
| Metastatic LN number    |                                            |
| Average ± SD (median)   | 0.76 ± 1.88 (0)                             |
| LN dissection number    |                                            |
| Average ± SD (median)   | 28.3 ± 15.8 (28)                            |
**Supplemental Table 3. Summary of antibodies used in flow cytometry analyses.**

| Tag  | Molecule | Clone   | Company              |
|------|----------|---------|----------------------|
| AF700| CD3      | UCHT1   | Thermo Fisher Science|
| V500 | CD4      | RPA-T4  | BD Biosciences       |
| BV785| CD8a     | RPA-T8  | BioLegend            |
| BV605| CCR7     | G043H7  | Biolegend            |
| BV711| CD45RA   | HI100   | BD Biosciences       |
| PE-Cy7| T-bet  | 4B10    | Thermo Fisher Science|
| FITC | Eomes    | WD1928  | Thermo Fisher Science|
| PE   | FOXP3    | 236A/E7 | Thermo Fisher Science|
| BV421| PD-1     | MIH4    | BD Biosciences       |
| APC  | CTLA-4   | L3D10   | Biolegend            |
| BV605| CD39     | TU66    | BD Biosciences       |
| PE-Cy7| GITR   | eVioAITR| Thermo Fisher Science|
| APC  | ICOS     | ISA-3   | Thermo Fisher Science|