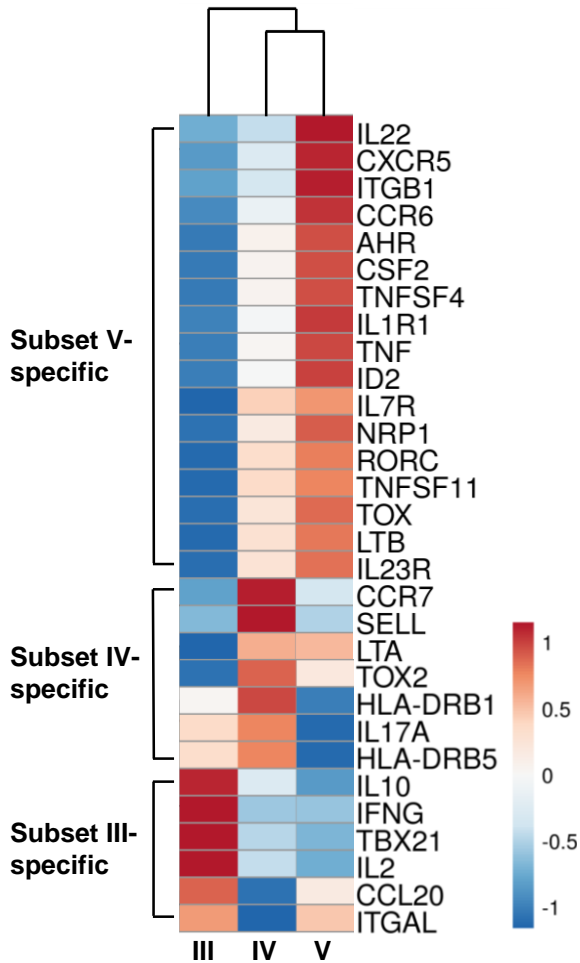


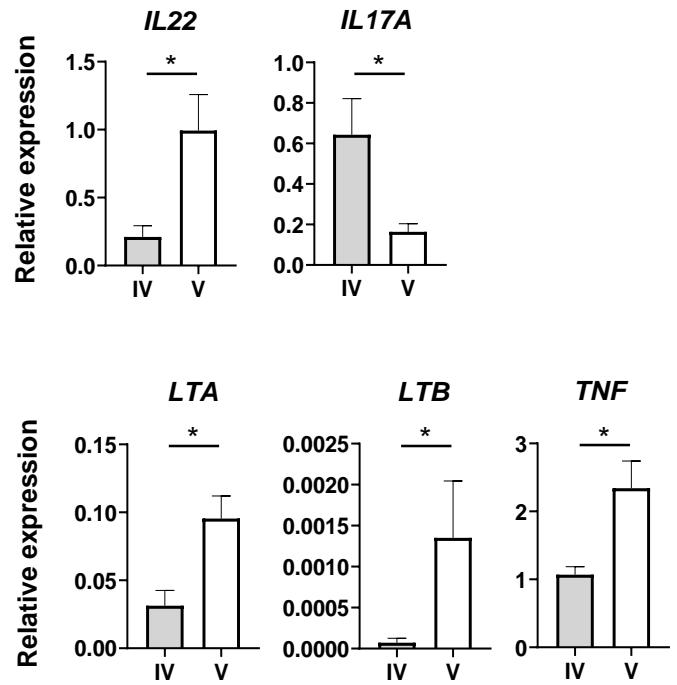
### Supplementary Figure S1: Characterization of human ILC subsets in normal colon.

**A**, Flow cytometric analysis of LPCs from normal human colon. Viable CD45<sup>+</sup> Lin<sup>-</sup> (CD3<sup>-</sup> CD11c<sup>-</sup> CD14<sup>-</sup> CD19<sup>-</sup> CD20<sup>-</sup>) cells were divided into two subsets: CD127<sup>-</sup> cells (I) and CD127<sup>+</sup> ILCs (II). CD127<sup>+</sup> ILCs (II) were further analyzed for the expression of CD117 and NKp44, identifying three populations: CD117<sup>-</sup> NKp44<sup>-</sup> cells (III), CD117<sup>+</sup> NKp44<sup>-</sup> cells (IV), and CD117<sup>+</sup> NKp44<sup>+</sup> cells (V). **B**, Frequencies of CD127<sup>-</sup> cells (I) and CD127<sup>+</sup> ILCs (II) among CD45<sup>+</sup> Lin<sup>-</sup> cells. **C**, Percentage of CD117<sup>-</sup> NKp44<sup>-</sup> cells (III), CD117<sup>+</sup> NKp44<sup>-</sup> cells (IV), and CD117<sup>+</sup> NKp44<sup>+</sup> cells (V) in population II. Data in **B** and **C** are presented as mean  $\pm$  SEM of 32 independent experiments. \*\* $P < 0.01$ , \*\*\*\* $P < 0.0001$ . **D**, Expression of *TBX21* and *RORC* mRNA in four CD45<sup>+</sup> Lin<sup>-</sup> cell subsets purified from normal colon. Data are presented as mean  $\pm$  SEM,  $n = 5$  or  $7$  in each group. \* $P < 0.05$ . **E**, Flow cytometric analysis of T-bet and RORyt expression in three CD127<sup>+</sup> ILC subsets purified from normal colon.

A

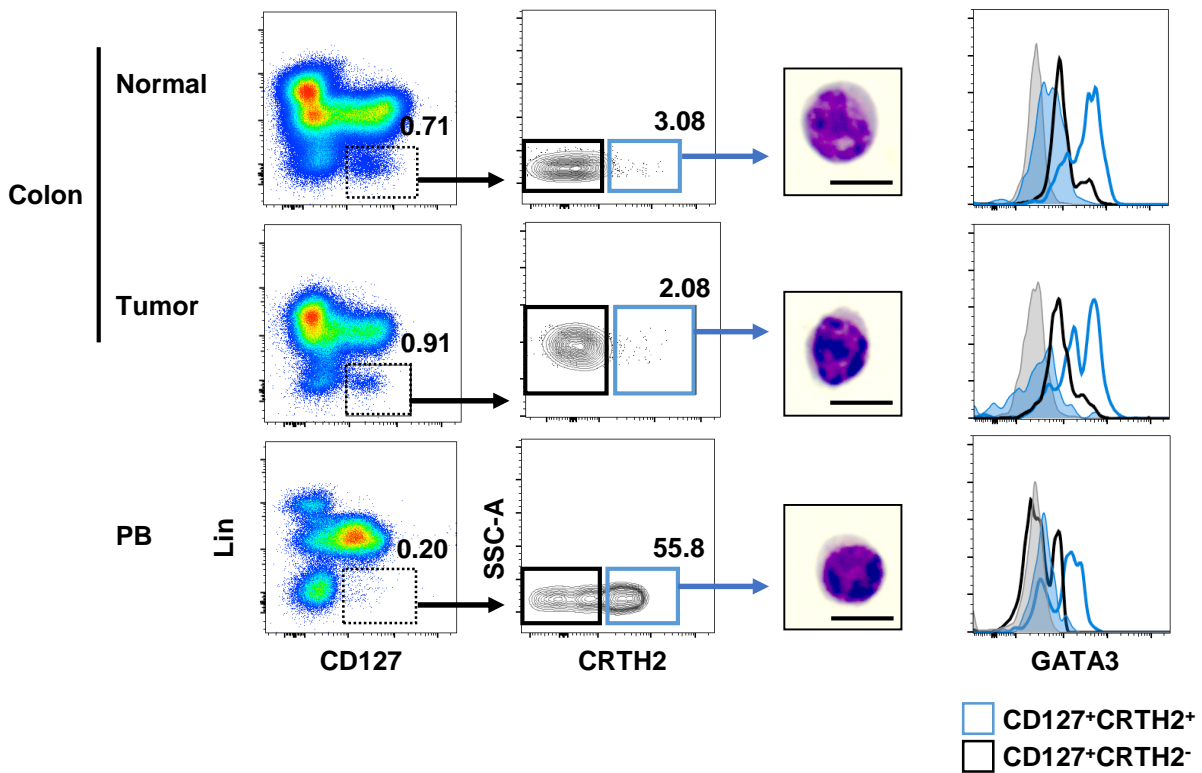


B



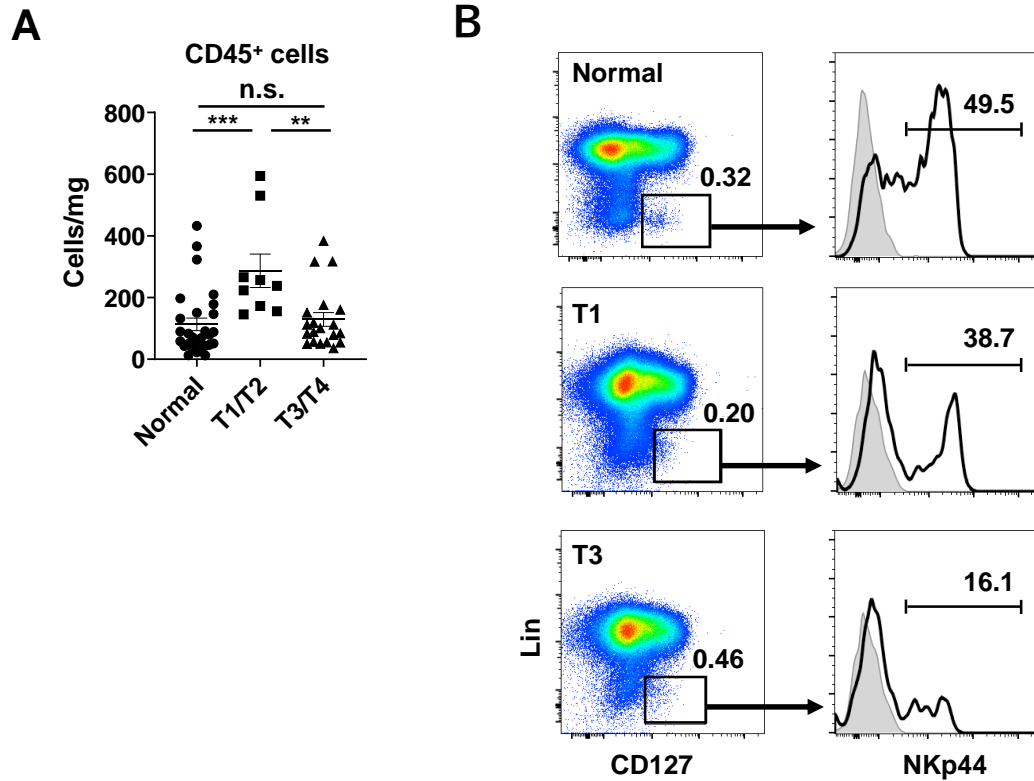
**Supplementary Figure S2: Gene expression profiles of ILC subsets in normal human colon.**

**A**, Heat map analysis of the ILC-related genes in the RNA-seq analysis of subsets III, IV, and V from normal mucosa. **B**, Expression of *IL22*, *IL17A*, *LTA*, *LTB*, and *TNF* in subsets IV and V isolated from normal colon. Data are presented as mean  $\pm$  SEM.  $n = 5$  or  $7$  in each group.  $*P < 0.05$ .



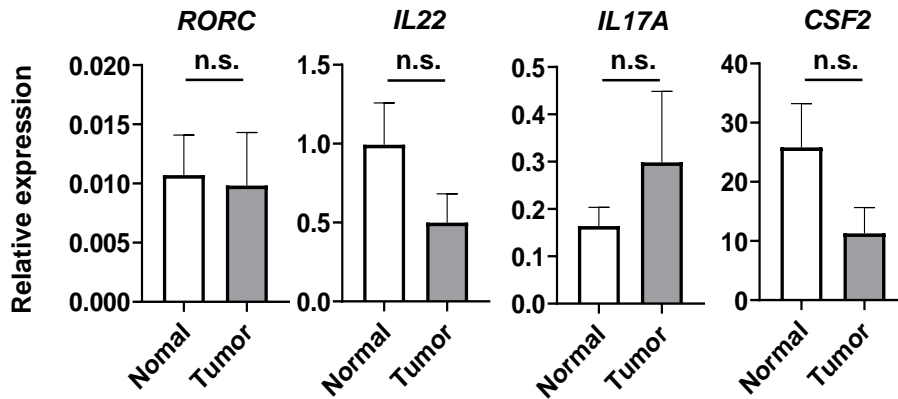
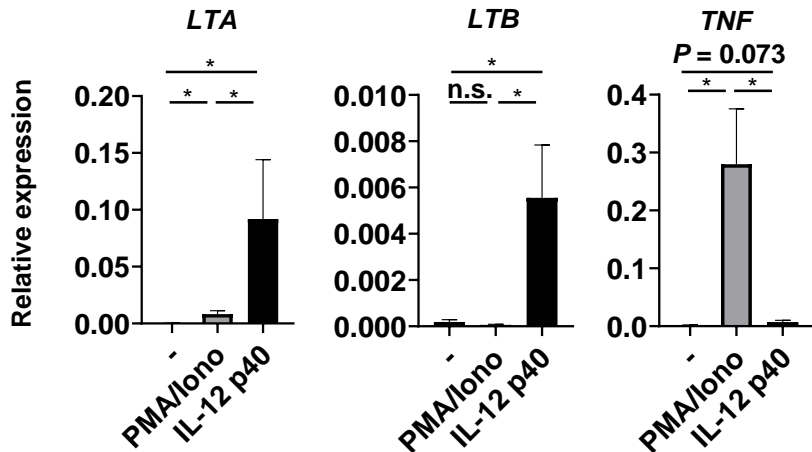
**Supplementary Figure S3: ILC2s in normal colonic mucosa, colon tumor tissue, and peripheral blood.**

Left, Flow cytometric analysis of CRTH2 expression on CD127<sup>+</sup> ILCs. Middle, May-Giemsa staining of CRTH2<sup>+</sup> ILCs isolated from the indicated tissue. Scale bars =10  $\mu$ m. Right, Flow cytometric analysis of GATA3 expression in CD127<sup>+</sup> CRTH2<sup>+</sup> cells and CD127<sup>+</sup> CRTH2<sup>-</sup> cells. The shaded histogram indicates isotype control.



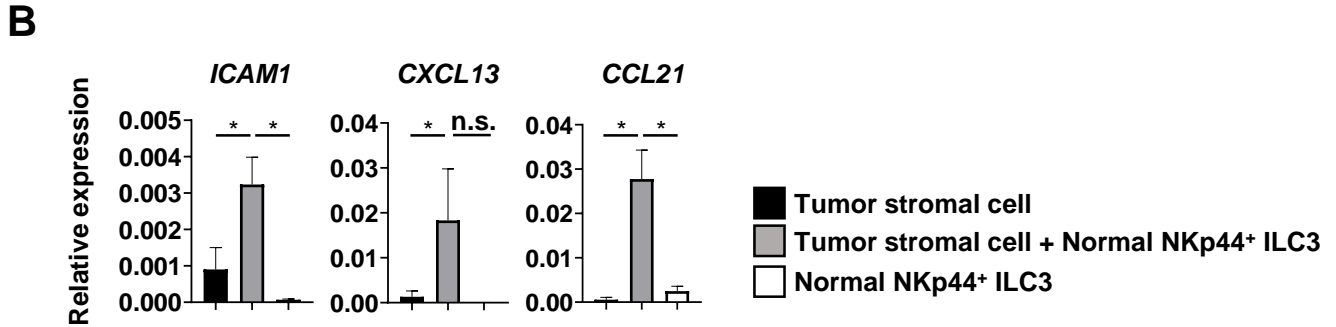
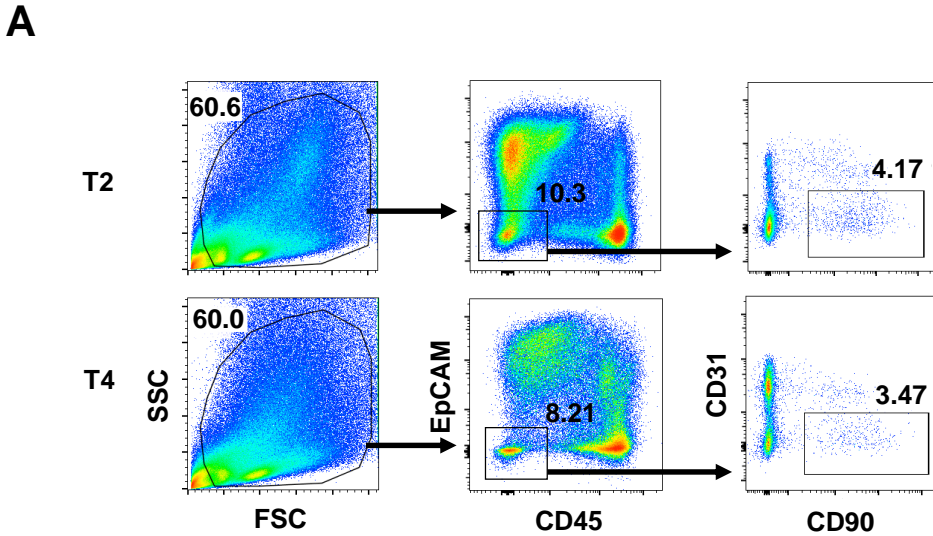
**Supplementary Figure S4: Number of CD45<sup>+</sup> cells and NKp44 expression on ILCs during tumor progression.**

**A**, CD45<sup>+</sup> cells /mg (tissue weight) in normal colon (n = 28), T1/T2 tumor (n = 9), and T3/T4 tumor tissue (n = 20). Data are presented as mean  $\pm$  SEM. n.s., not significant. \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . **B**, Flow cytometric analysis of NKp44 expression on CD127<sup>+</sup> ILCs isolated from normal colon, T1 tumor, and T3 tumor tissue from the same patient. The shaded histograms indicate isotype control.

**A****B**

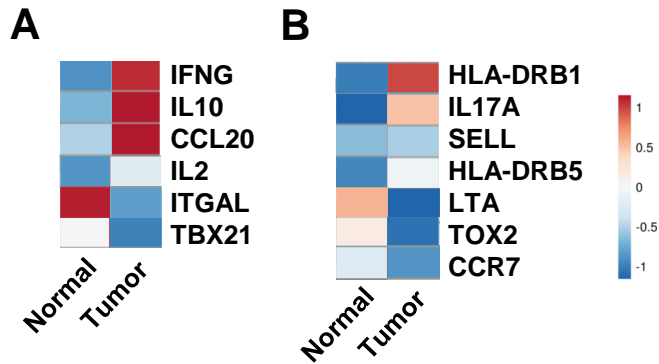
**Supplementary Figure S5: Gene expression patterns in NKp44<sup>+</sup> ILC3s from normal colon and T3/T4 tumor tissue.**

**A**, Expression of *RORC*, *IL22*, *IL17A*, and *CSF2* in NKp44<sup>+</sup> ILC3s isolated from the normal mucosa and tumor tissue of patients with stage T3/T4 CRC. Data are presented as mean  $\pm$  SEM of three independent experiments with five independent donors each. **B**, Expression of *LTA*, *LTB*, and *TNF* in T3/T4 tumor NKp44<sup>+</sup> ILC3s stimulated with PMA (50 ng/mL)/Ionomycin (1 µg/mL) for 3 h or IL-12 p40 (10 ng/mL) for 9 h. Data are presented as mean  $\pm$  SEM,  $n = 5$  in each group. n.s., not significant. \* $P < 0.05$ .



**Supplementary Figure S6: The effect of NKp44<sup>+</sup> ILC3s on stromal cells in the colon.**

**A**, Gating strategy for T2 and T4 stage CRC tumor stromal cells among viable cells. **B**, Expression of *ICAM1*, *CXCL13*, and *CCL21* in T3/T4 tumor stromal cells after co-culturing with colonic NKp44<sup>+</sup> ILC3s for 24 h. Data are presented as mean  $\pm$  SEM. n = 5 in each group. n.s., not significant. \**P* < 0.05.



**Supplementary Figure S7: Changes in the gene expression profiles of NKp44<sup>+</sup> ILC3s between normal mucosa and T3/T4 tumors.**

RNA-seq analysis was performed using NKp44<sup>+</sup> ILC3s isolated from normal mucosa (Normal) and T3/T4 tumor tissue (Tumor) from patients with CRC. **A**, Expression levels of ILC1-related genes (shown as Subset III-specific genes in Supplementary Figure S2A) in normal and tumor NKp44<sup>+</sup> ILC3s. **B**, Expression levels of NKp44<sup>-</sup> ILC3-related genes (shown as Subset IV-specific genes in Supplementary Figure S2A) in normal and tumor NKp44<sup>+</sup> ILC3s.