Supplemental Data

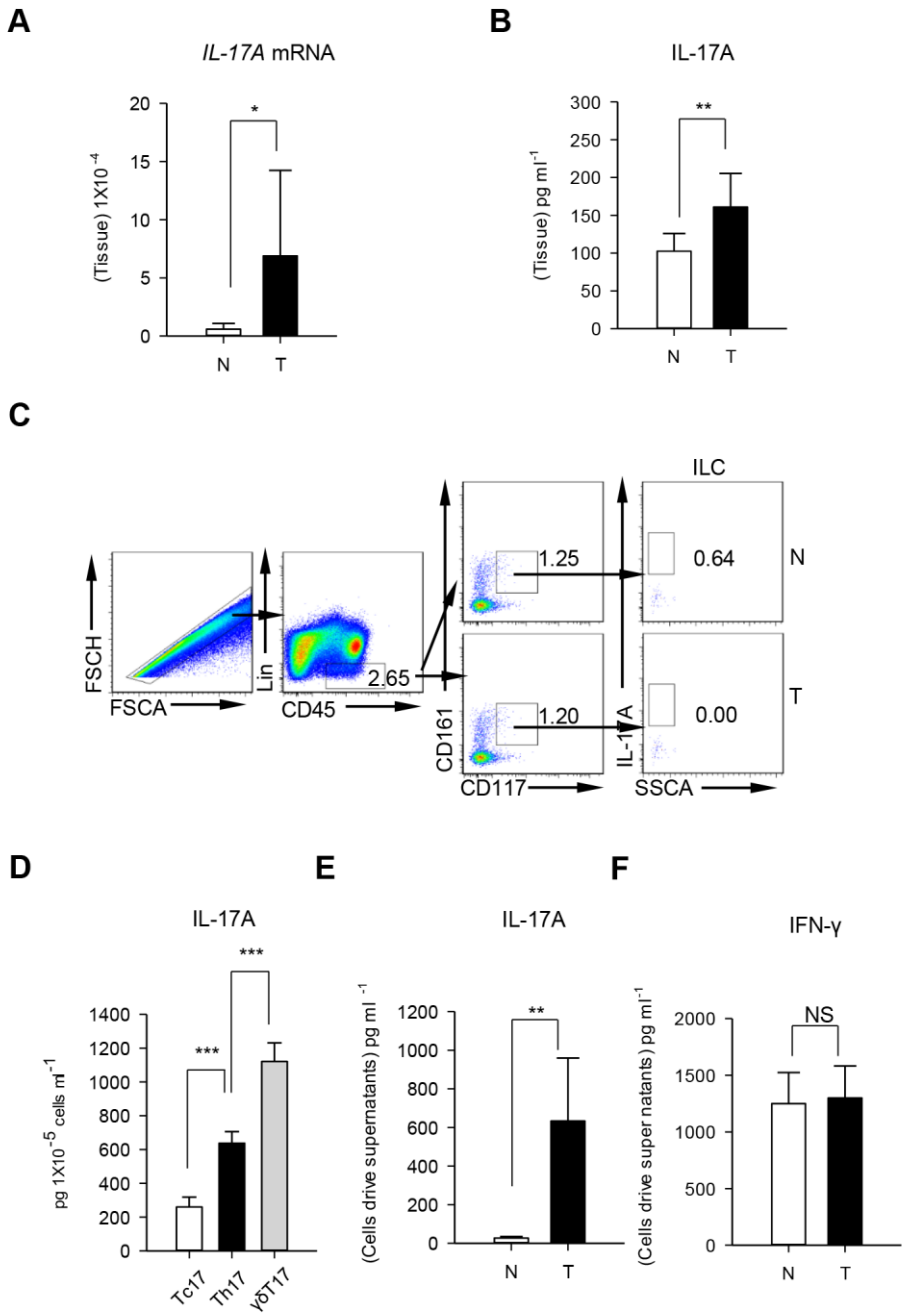

Figure S1. IL-17A Is Elevated in Tumor Tissues and Mainly from Tumor-infiltrating γδT Cells, but not from Tumor-infiltrating ILCs. Related to Figure 1.

(**A**) The relative mRNA levels of *IL-17A* in tumor and paired normal tissues determined by qRT-PCR and normalized to *GAPDH*. N: normal tissue; T: tumor. Data are shown as mean ± SEM; n=20; *p < 0.05.

(**B**) IL-17A protein levels in tumor and paired normal tissues-derived supernatants detected by ELISA. N: normal tissue; T: tumor. Data are shown as mean ± SEM; n=6; **p < 0.01.

(**C**) ILCs gating strategy and representative flow cytometric analysis of IL-17A production in tumor and paired normal tissues. One of 6 independent experiments is shown. N: normal tissue; T: tumor.

(**D**) IL-17A protein levels in sorted tumor-infiltrating Tc17, Th17 and γδT17 cells-derived supernatants detected by ELISA. Data are shown as mean ± SEM; n=6; ***p < 0.001.

(**E** and **F**) IL-17A and IFN-γ protein levels in sorted tumor-infiltrating γδT cells-derived supernatants detected by ELISA. N: normal tissue; T: tumor. Data are shown as mean ± SEM; n=6; NS: no statistical significance; **p < 0.01.

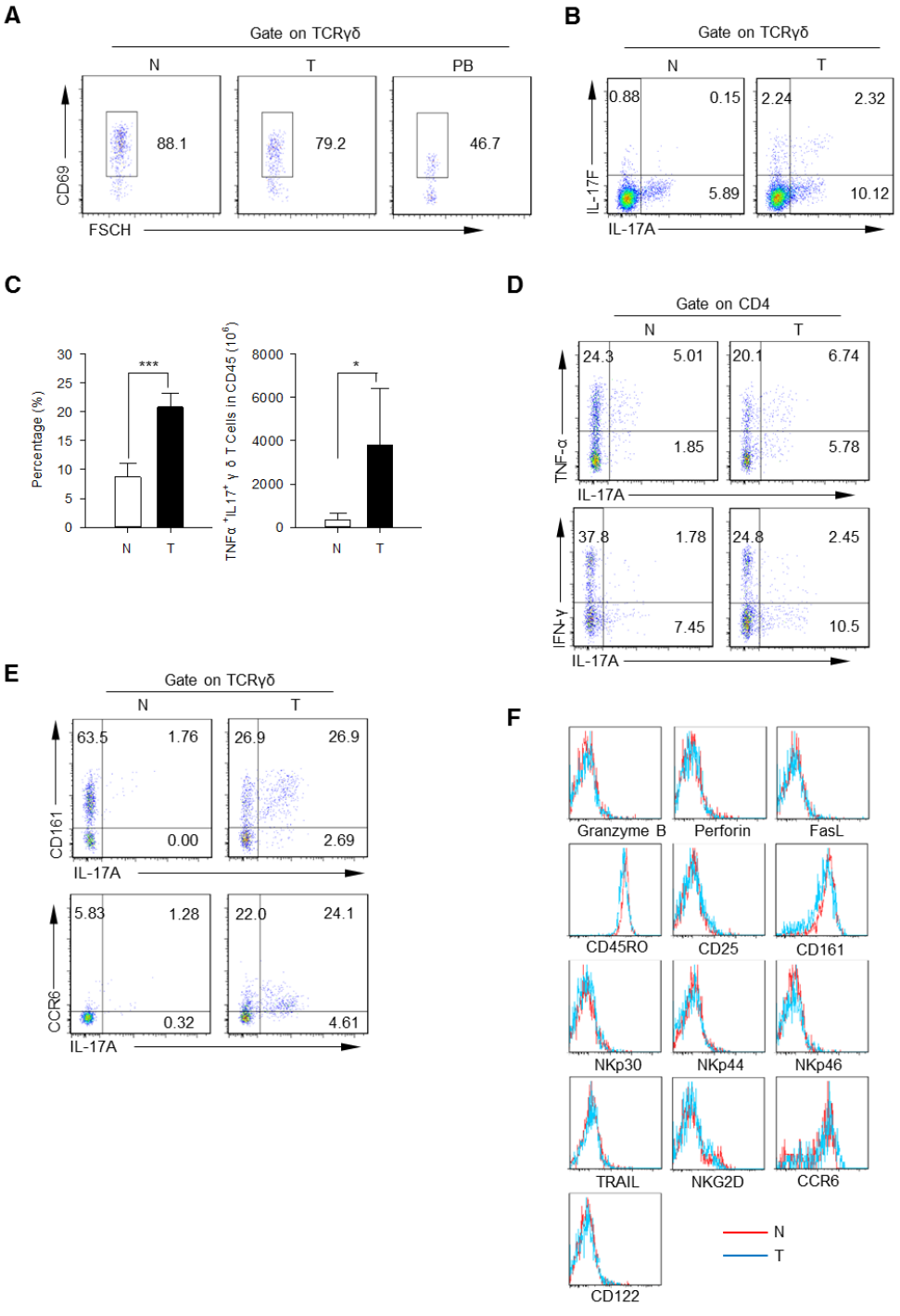

Figure S2. CD69, CD161, CCR6 and IL-17F Detection on γδT Cells and Intracellular Cytokines Assayed on Th17 Cells. Single-cell suspensions were prepared from tumor, paired normal tissues and autologous peripheral blood (PB). **Related to Figure 2.**

(**A**) CD69 expression on γδT cells was determined by flow cytometry (FCM). Representative flow cytometric analysis shows that CD69 is highly expressed on γδT cells isolated from normal and tumor tissues but not PB. One of 3 independent experiments is shown. N: normal tissue; T: tumor; PB: peripheral blood.

(**B**) IL-17F and IL-17A production in γδT cells was determined by FCM. One of 3 independent experiments is shown. N: normal tissue; T: tumor.

(**C**) Bar diagram summarizes the percentages (left) of IL-17A$^+$ TNF-α$^+$ subpopulations present in the γδTCR$^+$ T cells, and absolute numbers (right) of IL-17A$^+$ TNF-α$^+$ γδT cells in CD45$^+$ cells (1×10^6). N: normal tissue; T: tumor. Data are shown as mean ± SEM; n=10; *$p < 0.05$; ***$p < 0.001$.

(**D**) Cytokines production in tumor-infiltrating Th17 cells were detected by FCM. Representative flow cytometric analysis shows that IL-17A$^+$ TNF-α$^+$ Th17 cells and IL-17A$^+$ IFN-γ$^+$ Th17 cells are not significantly increased in tumor tissues. One of 3 independent experiments is shown. N: normal tissue; T: tumor.

(**E**) CCR6 and CD161 expression on γδT17 cells was detected by FCM. Representative flow cytometric analysis shows that CCR6 and CD161 are candidate markers of γδT17 cells in tumor tissues. γδT cells in paired normal tissues express minimal CCR6 corresponding to low IL-17 production. One of 6 independent experiments is shown. N: normal tissue; T: tumor.

(**F**) Comparative FCM analyses of γδT17 cells isolated from the tumor and paired normal tissue. Flow plots were gated on CD45$^+$ CD3$^+$ TCRγδ$^+$ IL-17A$^+$ cells. One of 3 independent experiments is shown.

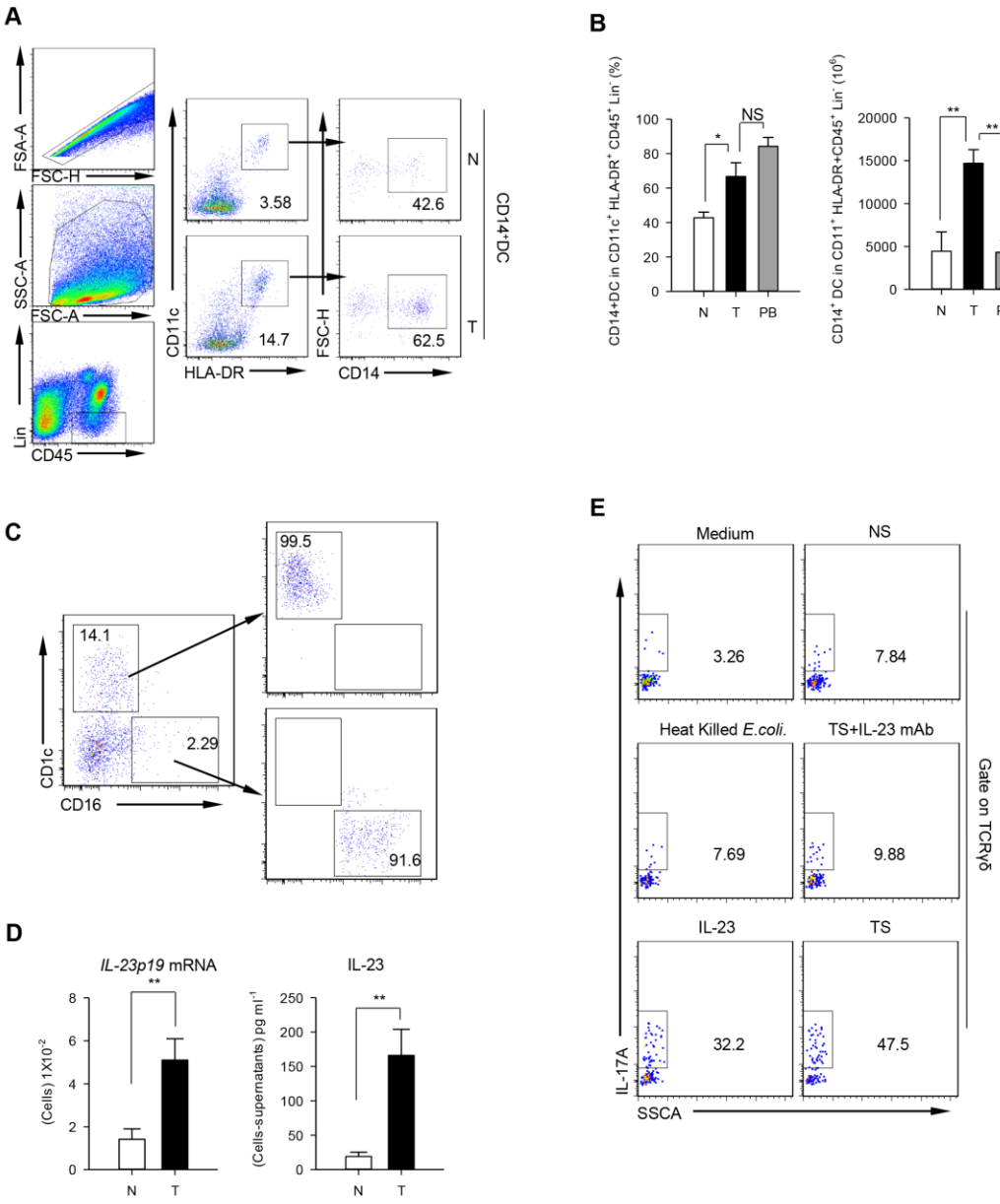

Figure S3. DC Subpopulation and Surface Markers of Inf-DCs and Inf-Mϕ Detected by FCM in the Tumors. Single-cell suspensions were prepared from tumor, paired normal tissues and autologous PB. Total DCs, $CD14^+$ DCs, inf-DCs and inf-Mϕ were detected by FCM. Expression levels of *IL-23mRNA* and IL-23 Protein in sorted inf-DCs and inf-Mϕ were measured. The impact of tumor tissue containing IL-23 on γδT17 was assayed *in vitro*. **Related to Figure 3.**

(**A**) Gating strategy and representative FCM dot plots of total DCs and $CD14^+$ DCs in the tumor and paired normal tissues. One of 6 independent experiments is shown. N: normal tissue; T: tumor; PB: peripheral blood.

(**B**) Summary of percentages and absolute numbers of $CD14^+$ DC in tumor, paired normal tissues and PB. N: normal tissue; T: tumor; PB: peripheral blood. Data are shown as mean ± SEM; n=6; NS: no statistical significance; *$p < 0.05$; **$p < 0.01$.

(**C**) Representative flow cytometric analysis of the purity of sorted inf-DCs and inf-Mϕ by FACS.

(**D**) *IL-23p19* mRNA expression levels in inf-DCs were determined by qRT-PCR and normalized to *GAPDH.* IL23 protein expression levels in supernatants were detected by ELISA. N: normal tissue; T: tumor. Data are shown as mean ± SEM; n=6; **p < 0.01.

(**E**) γδT cells isolated from normal tissues were incubated in conditioned medium (Heat Killed *E.coli.*, NS, TS, IL-23, TS with IL-23 neutralizing antibody or TS with control mAb, respectively) as indicated for 14 days, and the percentages of γδT17 cells were detected by FCM. One of 6 independent experiments is shown. NS: normal tissue-derived supernatants; TS: tumor-derived supernatants.

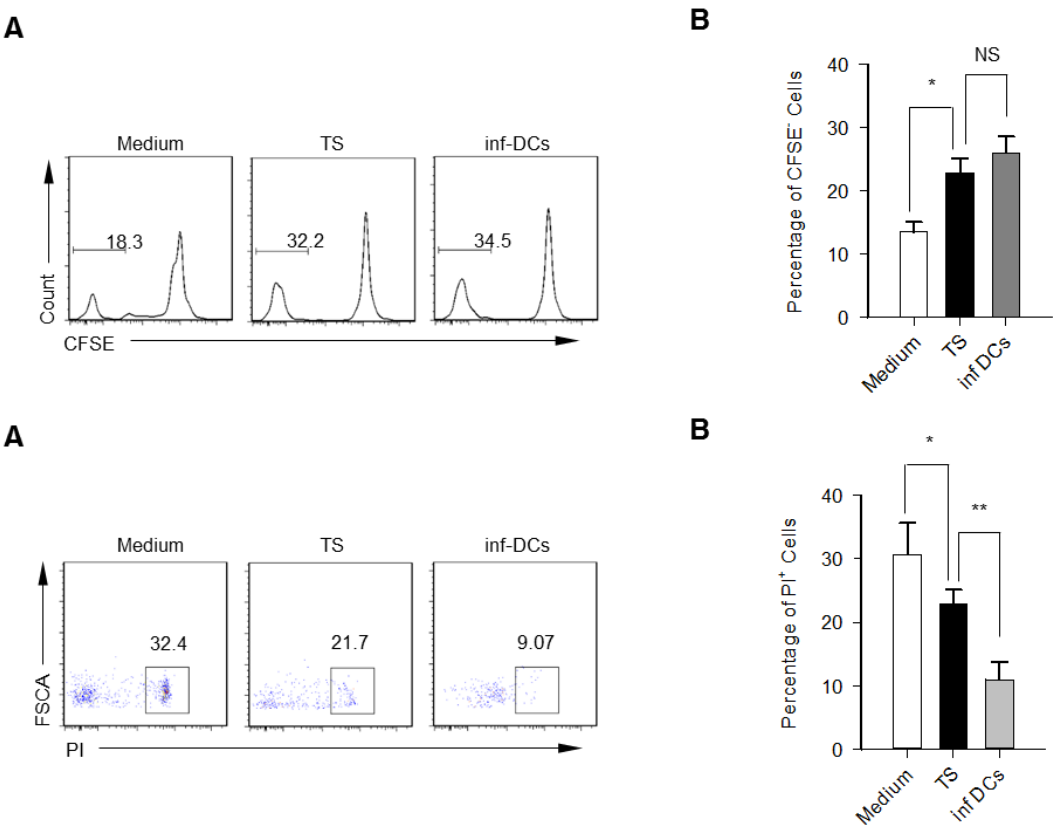

Figure S4. Tumor-derived Supernatants and Inf-DCs Promote the Proliferation and Survival of γδT17 *In Vitro*. The sorted CD161$^+$ CCR6$^+$ γδT cells from normal tissues were co-cultured with tissue-derived supernatants or pre-activated inf-DCs (treated with Heat Killed *E.coli.* for 3 days) sorted from paired tumor tissues for 2 weeks *in vitro*. Percentages of CFSE$^-$ and PI$^+$ cells in IL-17A$^+$ γδT cells were determined by FCM. **Related to Figure 3.**

(**A** and **B**) Sorted CD161$^+$ CCR6$^+$ γδT cells were labeled with CFSE and co-cultured with tumor-derived supernatants or pre-activated inf-DCs for 2 weeks *in vitro*. IL-17A$^+$ CFSE^{low} γδT cells were determined by FCM. One of 3 independent experiments is shown (**A**). Bar diagram summarizes the percentages of proliferated cells (CFSE^{low}) in IL-17A$^+$ γδT cells (**B**). TS: tumor-derived supernatants. Data are shown as mean ± SEM; n=3; NS: no statistical significance; *p < 0.05.

(**C** and **D**) Sorted CD161$^+$ CCR6$^+$ γδT cells were co-cultured with tumor-derived

supernatants or pre-activated inf-DCs for 2 weeks *in vitro*. PI was added in the co-cultured medium for the last 15 min, PI^+ $IL\text{-}17A^+$ γδT cells were examined by FCM. One of 3 independent experiments is shown (**C**). Bar diagram summarizes the percentages of PI^+ $IL\text{-}17A^+$ γδT cells (**D**). TS: tumor-derived supernatants. Data are shown as mean ± SEM; n=3; *p < 0.05; **p < 0.01.

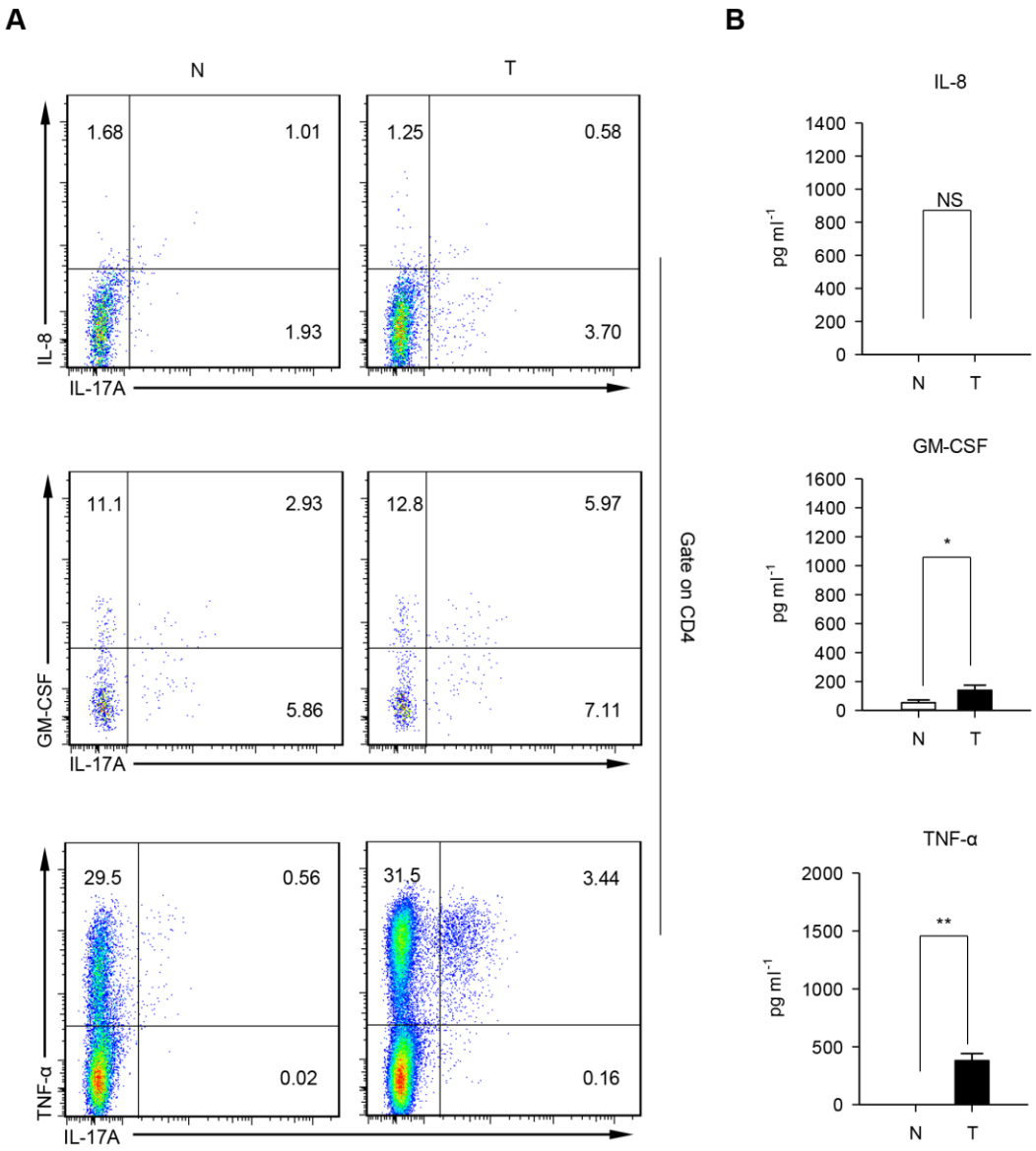

Figure S5. Th17 Cells Secrete Low Levels of TNF-α, IL-8, and GM-CSF. Single-cell suspensions were prepared from the tumor and paired normal tissues. **Related to Figure 5.**

(**A**) Representative flow cytometric analysis of intracellular cytokines production by Th17 cells shows that Th17 cells in the tumor tissues produce low levels of TNF-α, GM-CSF and IL-8. One of 6 independent experiments is shown. N: normal tissue; T: tumor.

(**B**) The production of TNF-α, IL-8, and GM-CSF in sorted $CD161^+$ $CCR6^+$ $CD4^+$ Th17

cells culture medium was detected by ELISA. N: normal tissue; T: tumor; Data are shown as mean ± SEM; n=3; NS: no statistical significance; *p < 0.05; **p < 0.01.

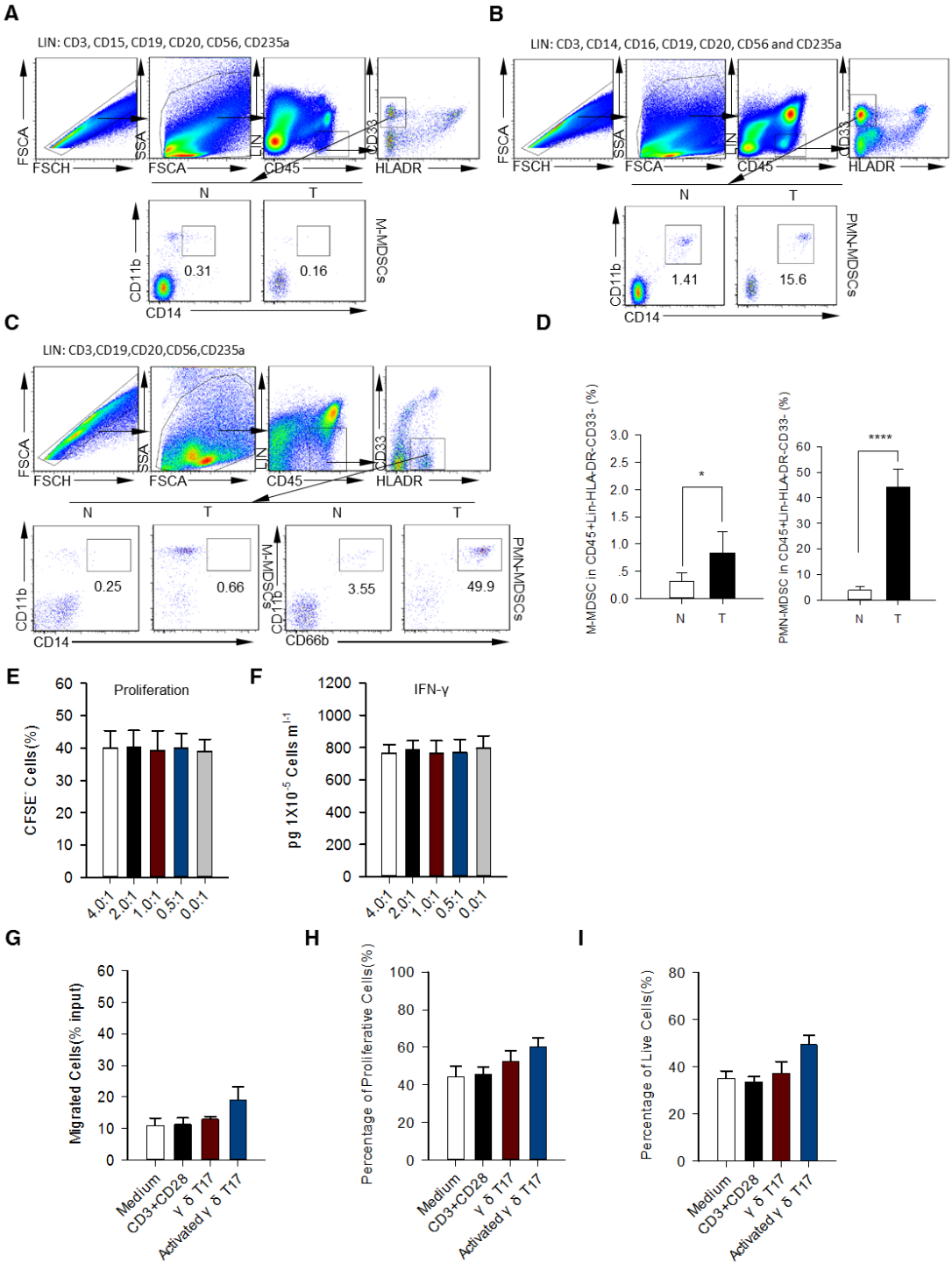

Figure S6. MDSC Detection in Human CRC. Single-cell suspensions were prepared from the tumor and paired normal tissues. M-MDSCs and PMN-MDSCs were detected by FCM. N: normal tissue; T: tumor. **Related to Figure 6.**

(**A**) Representative FCM dot plots show the gating strategy of M-MDSCs in the paired tumor and normal tissues.

(**B**) Representative FCM dot plots show the gating strategy of PMN-MDSCs in the paired tumor and normal tissues.
(**C** and **D**) Representative FCM dot plots show an alternative gating strategy of both M-MDSCs and PMN-MDSCs in the tumor and paired normal tissues (**C**). Percentages of M-MDSCs (left) and PMN-MDSCs (right) in tumor and paired normal tissues were summarized (**D**). N: normal tissue; T: tumor; Data are shown as mean ± SEM; n=6; *$p < 0.05$; ****$p < 0.0001$.
(**E** and **F**) Purified PMN from healthy donors were co-cultured with CFSE-labeled $CD3^+$ T cells isolated from autologous PB at different ratios (PMN-MDSC/$CD3^+$ T cells in 4:1; 2:1; 1:1; 0.5:1 and 0:1) in the presence of anti-CD3 and anti-CD28 mAbs. $CD3^+$ T cell proliferation was evaluated on day 6 by FCM (**E**). PMN of healthy donors were co-cultured with $CD3^+$ T cells from autologous PB for 12 hr. Concentrations of IFN-γ in the supernatants were detected by ELISA (**F**). Data are shown as mean ± SEM; n=3.
(**G, H** and **I**) Tumor-infiltrating PMN-MDSCs (upper well) and $CD161^+$ $CCR6^+$ γδT cells isolated from paired normal tissues (lower well) were co-cultured in transwell plate for 6hr, 24hr or 72hr, respectively. For migration assay (**G**), cells in the lower well were collected and counted. For proliferation assay (**H**), sorted tumor-infiltrating PMN-MDSCs were pre-labeled with CFSE, the proliferation of PMN-MDSCs was detected by FCM after 24 hr co-culture. For survival assay (**I**), PI was added in the co-cultured medium for the last 15 min, PI^+ PMN-MDSC cells were examined by FCM. Data are shown as mean ± SEM; n=3.

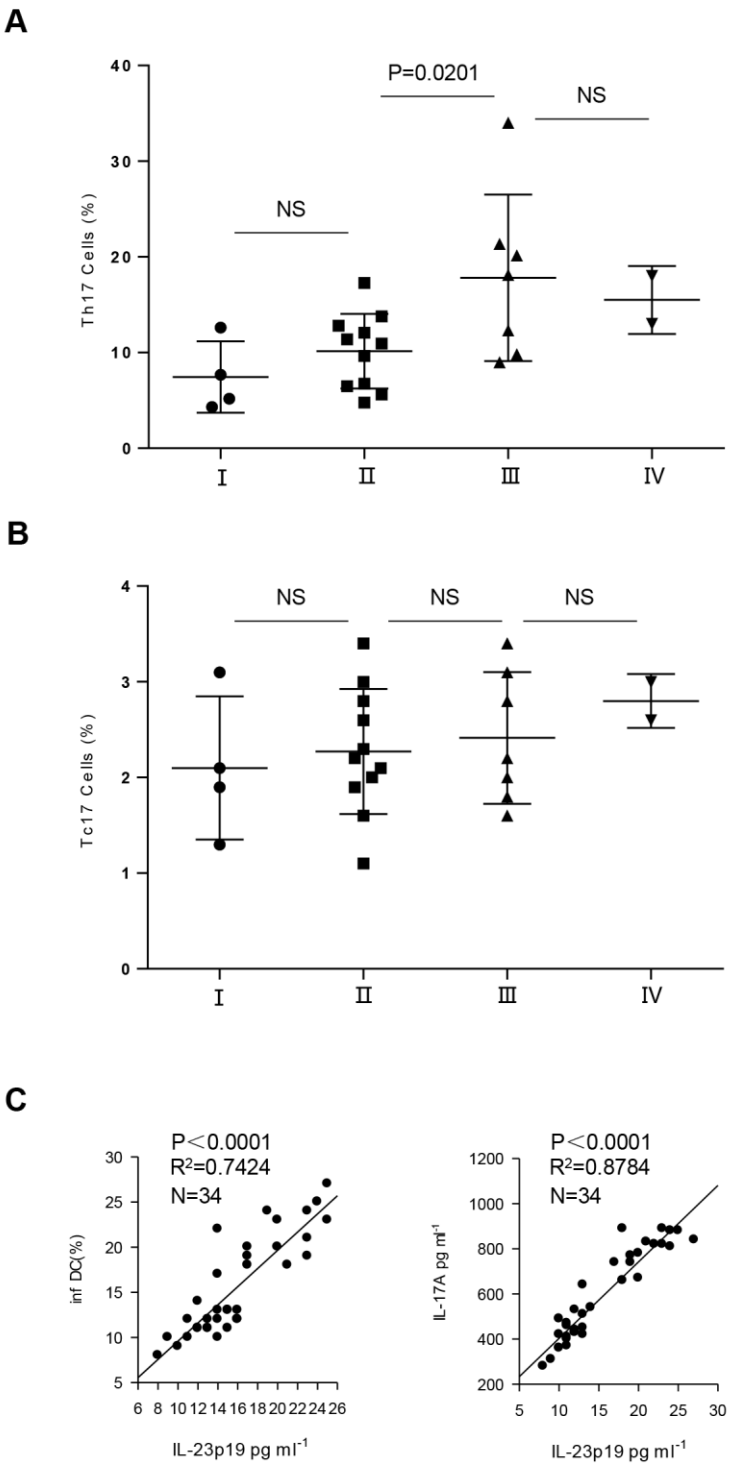

Figure S7. Correlations between Th17-Tc17 Cells and TNM Stages in Human CRC. Related to Figure 7.

(**A**) The percentages of Th17 cells were analyzed by FCM. The potential correlations between the percentages of Th17 cells and TNM stages were analyzed. Data are shown as mean ± SEM; n=24; NS: no statistical significance.

(**B**) The percentages of Tc17 cells were analyzed by FCM. The potential correlations between the percentages of Tc17 cells and TNM stages were analyzed. Data are

shown as mean ± SEM; n=24; NS: no statistical significance.
(**C**) Tumor-infiltrating inf-DCs in $CD45^+$ Lin^- $CD11c^+$ $HLA\text{-}DR^+$ cells were defined with specific staining and analyzed by FCM. Potential correlations between inf-DCs and IL-23 concentration (left), IL-17A and IL-23 concentration (right) were evaluated.

Table S1 Clinicopathological Features of the CRC Patients. Related to Figure 7.

	All patients	**Patients with γδT17 cells analyses**	**Patients with IL-23, IL-17, inf-DCs, and PMN-MDSCs analyses**	**Patients with Th17 and Tc17 cells analyses**
	n=154	n=117	n=34	n=24
Age (range)	60.5(29-98)	63(35-98)	61(31-86)	60(33-79)
Male/female	90/64	72/45	21/13	14/10
tumor size				
≤5 cm	100	70	13	15
＞5 cm	54	47	21	9
TNM stage, No (%)				
I	31(20.13)	24(20.51)	7(20.59)	4(16.67)
II	58(37.66)	45(38.46)	13(38.23)	11(45.83)
III	50(32.47)	36(30.77)	11(32.35)	7(29.17)
IV	15(9.74)	12(10.26)	3(8.82)	2(8.33)
Differentiation, No (%)				
Diff	141(91.56)	110(94.02)	33(97.06)	22(91.67)
Undiff	13(8.44)	7(5.98)	1(2.94)	2(8.33)
Micro-invasive status, No (%)				
vascular invasion	47(30.52)	35(32.41)	10(29.41)	6(25)
Lymphatic invasion	51(33.12)	38(35.19)	12(35.29)	8(33.3)
Serum CEA levels				
≤5 ng ml^{-1}	106	81	24	16
＞5 ng ml^{-1}	48	36	10	8

TNM stage is according to AJCC cancer staging manual (6^{th} version). Diff (differentiation): Good and Moderate; Undiff (undifferentiation): Poor and undiff

Table S3 List of Primers Used for Q-RT-PCR

Target genes	**Primers**
IL-17A	Fwd: 5'-ACCAATCCCAAAAGGTCCTC-3'
	Rev: 5'-GGGGACAGAGTTCATGTGGT-3'
IL-23p19	Fwd: 5'-AGTGTGGAGATGGCTGTGACC-3'
	Rev: 5'-GCTGGGACTGAGGCTTGGAATCTG-3'
ZO-1	Fwd: 5'-CAAGATAGTTTGGCAGCAAGAGATG-3'
	Rev: 5'-ATCAGGGACATTCAATAGCGTAGC-3'

CLDN2	Fwd: 5'-GGCGGTAGCAGGTGGAGTC-3'
	Rev: 5'-CTTGGTAGGCATCGTAGTAGTTGG-3'
MUC2	Fwd: 5'-GACACCATCTACCTCACCCG-3'
	Rev: 5'-TGTAGGCATCGCTCTTCTCA-3'
OCLN	Fwd: 5'-TGTGGATCCCCAGGAGGCCA-3'
	Rev: 5'-AGGCACGTCCTGTGTGCCTG-3'
GAPDH	Fwd: 5'-GAAGGTGAAGGTCGGAGTC-3'
	Rev: 5'-GAAGATGGTGATGGGATTTC-3'

Supplemental Experimental Procedures

DNA Preparation and Bacteria Quantification by qPCR

Human tumor and normal tissue samples (20-100mg) were digested overnight in 0.7 ml molecular grade lysis buffer (100mM TrisHCl pH 8.5, 5 mM EDTA pH 8.0, 0.2% SDS, 200 mM NaCl, 1 mg/ml proteinase K) at 55 °C with rotation. The samples were centrifuged at 20,000g for 5min and then the liquid portion was moved to equal volume isopropanol. The precipitated DNA was recovered and resuspended in 0.4 mL TE buffer. An equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added, mixed by inversion and centrifuged at 20,000g for 5min. Then the aqueous phase was transferred to a fresh tube. If aqueous phase was milky, the phenol: chloroform: isoamyl alcohol step was repeated. 0.1 volume of 3M sodium acetate pH 5.2 and 2 volumes of 100% ethanol were added, incubated at -20 ºC for 1hr and centrifuged at 20,000g for 15min. The pellet was washed twice with 0.5 mlof 70% ethanol, centrifuging at 20,000g for 7min and discarding supernatant. The DNA pellet was resuspended in molecular grade water.

DNA (10ng) was used in each 20 µl KAPA SYBR FAST qPCR (Kapa Biosystems) reaction, which was performed in triplicate and analyzed on the Stratagene Mx3005P (Agilent Technologies). The universal eubacteria 16S primers were used (Fwd: 5'-GGTGAATACGTTCCCGG-3'; Rev: 5'-TACGGCTACCTTGTTACGACTT-3'). Relative abundance was calculated by the ΔC_T method.

Bacteria Fluorescent In Situ Hybridization

Bacteria fluorescent in situ hybridization was performed as previously described (Grivennikov et al., 2012). In brief, paraffin embedded slides were deparaffinized and hybridized to universal eubacterial or control probes labeled with Alexa594. Hybridization was performed at 48 ºC oven for 2 hr followed by washing and counter-stained by DAPI. The sequences of the probes are listed below: Eubacteria: GCTGCCTCCCGTAGGAGT; control: CGACGGAGGGCATCCTCA. For bacteria quantification, at low power (×100), the tissue sections were screened using an inverted microscope (model DM IRB; Leica), and the five most representative fields were selected.

***In Vitro* Co-culture**

To investigate the role of bacterial products and CD40L in inf-DCs and inf-Mϕ for IL-23 production, inf-DCs (5×10^4) sorted from normal tissues were incubated for 24 hr in AIM V serum free medium in the presence or absence of heat killed *E.coli.* (10^5/ml), Pam3Csk4 (2µg/ml), LPS (2µg/ml), dimerized CD40-ligand (1µg/ml) or *E.coli.* DNA

(1μg/ml). To investigate the role of inf-DCs in γδT17 cells induction, tumor-infiltrating inf-DCs (5×10^4) were sorted and pre-activated with heat killed *E.coli.* (10^5/ml) for 4 hr. Cells were washed and co-cultured with equal number of autologous γδT cells from normal tissues. At day 7, cells were harvested and IL-17A$^+$ cells were detected by flow cytometry (FCM). To determine the role of inf-DCs in γδT17 cell cytokines production, sorted inf-DCs were pre-activated with heat killed *E.coli.* for 4 hr, washed and co-cultured with equal number of autologous normal γδT cells from normal tissues in 1μm transwell system for 3 days with or without IL-23 neutralizing antibody. After that, γδT cells were collected and incubated in AIM V serum-free medium for another 3 days, supernatants were collected for cytokine detection. To investigate the immunosuppression of tumor-infiltrating PMN-MDSCs, sorted PMN-MDSCs (1×10^5) were co-cultured with CFSE (Invitrogen) labeled autologous PB CD3$^+$ T cells in the presence of CD3 (Clone UCHT1, 10μg/ml) and CD28 (Clone 37407, 10μg/ml). After 48 hr, supernatants were collected for IFN-γ assay. At day 6, cultures were harvested and T cell proliferation was detected by FCM. To investigate the role of γδT17 cells in PMN-MDSC migration, proliferation and survival, activated γδT17 cells by inf-DCs or sorted tumor-infiltrating CD161$^+$ CCR6$^+$ γδT17 cells (1×10^5) were co-cultured with equal number of autologous CFSE labeled tumor-infiltrating PMN-MDSCs in 1μM or 3μM 96-transwell Boyden chambers (Corning Costar) with or without specific neutralizing antibody or neutralizing antibody cocktail as described in the previous study (Pelletier et al., 2010). After 6 hr, PMN-MDSCs in bottom wells were counted, and migration of PMN-MDSCs was determined as percentages of cells migrated in total cell input. For proliferation assay, PMN-MDSCs were pre-labeled with CFSE and then co-cultured with γδT17 cells for 24 hr. PMN-MDSCs proliferation were detected by FCM. For survival assay, PMN-MDSCs were co-cultured with γδT17 cells for 3 days. Cells were harvested and Propidium Iodide (PI, 1mg/ml) was added. PI$^+$ PMN-MDSCs were detected by FCM.

Inf-DCs-Induced γδT17 Cells Proliferation

γδT17 cells proliferation induced by inf-DCs was assessed using 96-transwell Boyden chambers (Corning Costar) of 1μM pore size. Sorted normal tissues-infiltrating γδT17 cells were pre-labeled with CFSE. After 14 days, CFSE$^-$ γδT17 cells were detected by FCM.

Inf-DCs-Induced γδT17 Cells Survival

Unlabeled γδT17 cells were co-cultured with inf-DCs as described in γδT17 cells proliferation experiment. After 14 days, 5 μl Propidium Iodide (1 mg/ml, Invitrogen) was added to the bottom wells and incubated for another 15 min, and PI$^+$ γδT17 cells were detected by FCM.

Inf-DCs-Polarized γδT17 Cells Preparation

γδT cells isolated from normal tissues were co-cultured with tumor-infiltrating inf-DCs in 1μM 96-transwell Boyden chambers (Corning Costar) for 14 days. γδT cells were collected for further use. To investigate the role of inf-DCs in cytokines production of γδT17 cells, γδT cells isolated from normal tissues were cultured in specific medium (medium only, medium containing equal tumor-infiltrating inf-DCs, medium containing equal heat killed *E.coli.* activated tumor-infiltrating inf-DCs or medium containing

equal heat killed *E.coli.* activated tumor-infiltrating inf-DCs and IL-23 neutralizing antibody (Clone 24901, 0.1 μg/mL, R&D Systems)) for 14 days in 1μM 96-transwell Boyden chambers. Then γδT cells were washed and incubated in AIM V serum free medium for another 3 days, and supernatants were collected for IL-17A, IL-8, TNF-α and GM-CSF detection.

γδT17 Cells-Induced PMN-MDSC Migration

PMN-MDSC migration induced by γδT17 cells was assessed using 96-transwell Boyden chambers (Corning Costar) of 3μM pore size. γδT17 cell suspensions (100μl) induced by inf-DCs (1×10^5/ml) were added to the bottom wells, whereas equal CSFE labeled PMN-MDSCs cell suspensions (100μl) were added to the top chambers. Then neutralizing antibody for IL-17A (Clone 41809, 1μg/ml, R&D Systems), IL-8 (Clone 6217, 0.4μg/ml, R&D Systems), TNF-α (Clone 6401, 0.06μg/ml, R&D Systems) and GM-CSF (Clone 3209, 0.5 μg/ml, R&D Systems), or neutralizing antibody cocktail containing the above four antibodies or their isotype-related antibodies (5μg/ml, BioLegend) was added to the bottom wells. After 6 hr, $CFSE^+$ PMN-MDSCs in bottom wells were counted by FCM, and migrated cells were calculated as ($CFSE^{hight}$/cells input) ×100%. Within 6 hr, the possibility of PMN-MDSC proliferation was eliminated by preliminary experiment.

γδT17 Cells-Induced PMN-MDSC Proliferation

PMN-MDSC proliferation induced by γδT17 cells was assessed using 96-transwell Boyden chambers (Corning Costar) of 1μM pore size. The following experimental procedure was same as PMN-MDSCs migration experiment. After 24 hr, $CFSE^-$ PMN-MDSCs were detected by FCM.

γδT17 Cells-Induced PMN-MDSC Survival

Unlabeled PMN-MDSCs were co-cultured with γδT17 cells as described in PMN-MDSC proliferation experiment. After 72hr, 5 μl Propidium Iodide (1 mg/ml, Invitrogen) was added to the bottom wells and incubated for another 15 min, and PI^+ PMN-MDSCs were detected by FCM.

Supplemental References

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