Supplementary figures

Supplementary Figure 1. Characterization of IL1R2 in tumor infiltrating Treg. (A) *Il1r2* expression and localization in $CD45^+$ TILs from MC38 tumor tissues (ArrayExpress: E-MTAB-8832). (B and C) B16 cells ($2x10^5$) were injected *i.d.* into the right flank of 6-week old female C57BL/6 mice (n=8) for 10 days, the spleens, TDLNs and tumor tissues were collected and the flow cytometry was used to analyze the IL1R2 expression on Tregs. (D and E) CT26 cells ($5x10^5$) were injected *i.d.* into the right flank of 6-week old female Balb/c mice (n=8) for 2 weeks, and the spleens (top), TDLNs (middle) and tumor tissues (low) were collected and the flow cytometry was used to analyze the IL1R2 expression on Tregs. $^{**}P<0.01$, $^{***}p<0.001$ and $^{****}p<0.0001$ Student's *t*-test.

Supplementary Figure 2. Establishment and identification of specific IL1R2 deletion in Tregs based on *Foxp3*^{Cre} mice. (A) Strategy of generation of *Il1r2*$^{fl/fl}$*Foxp3*^{Cre} mice. (B) Flow cytometry analysis confirmed specific deletion of IL1R2 expression on tumor infiltrating Tregs in MC38 tumors. (C, D and E) Establishment of B16-GVAX tumor model, and flow analysis of exhausted $CD8^+$ TILs. (C) B16 cells ($2x10^5$) were injected *i.d.* into the right flank of *Foxp3*^{Cre} and *Il1r2*$^{fl/fl}$*Foxp3*^{Cre} mice, and the lethally irradiated B16-GM-CSF cells ($2x10^5$) were injected *i.d.* into the left flank. αPD-1 mAbs or IgG treatment was performed on day 5 after tumor inoculation and again every 4 days for 4 times. Tumor sizes were monitored every two days. (D) The overall survival of different groups was monitored in *Foxp3*^{Cre} and *Il1r2*$^{fl/fl}$*Foxp3*^{Cre} mice challenging with αPD-1 mAbs and control IgG respectively. (Mice n=6 in each sub-group). (E) B16 tumor model without vaccination was established in IL1R2$^{fl/fl}$Foxp3^{Cre} and Foxp3^{Cre} mice in the treatment of αPD-1. The 6-8 week male mice of *Il1r2*$^{fl/fl}$*Foxp3*^{Cre} and *Foxp3*^{Cre} were used and divided into four groups *Foxp3*^{Cre}+IgG (n=5), *Il1r2*$^{fl/fl}$*Foxp3*^{Cre}+IgG (n=4), *Foxp3*^{Cre}+ ✔ PD-1 (n=3), *Il1r2*$^{fl/fl}$*Foxp3*^{Cre}+αPD-1 (n=3). $5x10^5$ B16 cells were inoculated subcutaneously in the

right flank, αPD-1 (J43, BP0033-2, BioXcell) and Hamster IgG (BE0091, BioXcell) were administrated at 200μg respectively on day 6, 9, 12. The tumor sizes were monitored and the tumor growth curves were charted. (F) Flow cytometry and statistical analysis of exhaust markers LAG3 and TIM3 on $CD8^+$ TILs from *Il1r2*$^{fl/fl}$*Foxp3*^{Cre} and *Foxp3*^{Cre} mice bearing MC38 tumor tissues. Data represent at least three independent experiments. $^*P<0.05$, $^{**}p<0.01$ and $^{****}p<0.0001$ by student t-test, two-way ANOVA analysis, or Logrank survival analysis.

Supplementary Figure 3. Verification of tumor growth, survival analysis, and flow analysis of TIL subsets by using sex-matched mice. (A and B) The replicated experiment was designed by using 6 week-*Il1r2*$^{fl/fl}$*Foxp3*^{Cre} mice and age-matched *Foxp3*^{Cre} female mice. (A) After MC38 cells ($2x10^6$/150μl) inoculation and tumor formation, αPD-1 (J43, BP0033-2, BioXcell) and Hamster IgG (BE0091, BioXcell) were administrated at 200μg respectively on day 5, 9, 13,17, the tumor growth curve was monitored 3 times per-week (n=6 for each group). (B) The survival of the mice was monitored and the survival analysis was performed by using Log-rank (n=6 for each group). (C, D, E and F) Gating strategy and flow analysis of TILs from MC38 tumor models treated by αPD-1 in *Il1r2*$^{fl/fl}$*Foxp3*^{Cre} or *Foxp3*^{Cre} male mice. The replicated experiment was designed by using 6 week-*Il1r2*$^{fl/fl}$*Foxp3*^{Cre} male mice and age-matched *Foxp3*^{Cre} male mice (n=6 for each group) for the MC38 tumor establishment and flow analysis. And after three times of αPD-1 or IgG treatment, the tumor tissues were collected and the TILs were analyzed by using FACS. Gating strategy for the flow analysis of TILs from MC38 tumor models treated by αPD-1 in *Il1r2*$^{fl/fl}$*Foxp3*^{Cre} or *Foxp3*^{Cre} male mice (C). The representative flow cytometry plots showed the percentages of total $CD45^+$ TILs (D), $CD8^+$ TILs in total $CD45^+$ TILs (E) and Treg in $CD4^+$ TILs (F) in different group mice. $^*P<0.05$ and $^{**}p<0.01$ and $^{***}p<0.001$ by student t-test, two-way ANOVA or Log-rank survival analysis.

Supplementary Figure 4. Identification of *Il1r1* and marker genes in CAFs. (A and B) *Il1r1* expression in B16 melanoma public database (ArrayExpress: E-MTAB-7427, E-MTAB-7417) and our scRNA-seq data. (C) Expression of *Col1a1* and *Col3a1* on fibroblasts. (D) Expression of MHC-II molecules *H2-Aa* and *H2-Ab1* split in CAFs from *Foxp3*^{Cre} and *Il1r2*$^{fl/fl}$*Foxp3*^{Cre} mice. (E) Dot plot showed DEGs between iCAFs and apCAFs.

Supplementary Figure 5. Mechanism of MHC-II regulation on CAFs. (A and B) ssGSEA analysis of genes expressed in CAFs from *Foxp3*^{Cre} and *Il1r2*$^{fl/fl}$*Foxp3*^{Cre} mice. (C) UMAP analysis showing expression of transcription factors in two CAFs (apCAFs and iCAFs). (D) Schematic graph indicating transcription factors, *Irf5*, *Rel* and *Irf8,* and their targets - MHC-II genes.

Supplementary Figure 6. IFN-γ induced but IL-1 down-regulated MHC-II expression on fibroblast. (A) Images of primary fibroblasts on day 3, 6 and 10, and also the fibroblast cell line NIH 3T3. (B) Flow cytometry identified expression level of IL1R1 on NIH 3T3 (top) and primary fibroblasts (low). (C) The NIH 3T3 cells were treated with IFN-γ (10ng/mL), IL-1β (1ng/ml) and IFN-γ (10ng/mL) plus IL-1β (1ng/ml) respectively for 24h, both MHCII and PD-L1 were quantified by flow cytometry. Data represent at least three independent experiments. $^{*}P<0.05$, $^{***}p<0.001$ and $^{****}p<0.0001$ by student t-test.

Supplementary Figure 7. Functional analysis of tumor infiltrating Tregs. (A, B and C) Developmental trajectory analysis of tumor infiltrating Tregs (A. monocle, B. monocle3, C. slingshot). (D) UMAP showed detailed clusters of Tregs split in two groups of mice. (E) Expansion averages of four clusters of Tregs in two groups of mice. (F) Violin plots displayed negative immune checkpoint genes on Tregs.

Supplementary Figure 8. Developmental trajectory analysis of CD8^{+} TILs in in two groups of mice. (A) UMAP analysis showing all sub-populations of CD8^{+} TILs. (B, C and D)

Developmental trajectory analysis of tumor infiltrating $CD8^+$ TILs (B. monocle, C. monocle3, D. slingshot).

Supplementary Figure 9. Characteristic genes in $CD8^+$ and $CD4^+$ TILs. (A) UMAP analysis showing expression of markers genes in $CD8^+$ TILs. (B, C and D) Dot plot showing DEGs of $CD4^+$ TILs from *Foxp3*^{Cre} and *Il1r2*$^{fl/fl}$*Foxp3*^{Cre} mice.

Supplementary Figure 10. Flow cytometry validation of the changes of the main immune cell sub-populations based on the scRNA-seq. The MC38 tumor model was established in *Foxp3*^{Cre} mice and *Il1r2*$^{fl/fl}$*Foxp3*^{Cre} mice (6-8 weeks, female), and then treated by αPD-1 (200μg) or αCTLA-4 (200μg) from day 5 and for 3 times every 4 days. (A) The IFN-γ$^+$CD8$^+$TILs as well as GZMB$^+$CD8$^+$TILs were increased in the MC38 tumors from *Il1r2*$^{fl/fl}$*Foxp3*^{Cre} mice (n=5) in contrast to *Foxp3*^{Cre} mice (n=4) (P<0.05 respectively). (B) The IFN-γ$^+$CD8$^+$TILs were increased in the MC38 tumors from *Il1r2*$^{fl/fl}$*Foxp3*^{Cre} mice (n=5) in contrast to *Foxp3*^{Cre} mice (n=4) (P<0.05), while the GZMB$^+$CD8$^+$TIL percentage trended higher in the MC38 tumors from *Il1r2*$^{fl/fl}$*Foxp3*^{Cre} mice in contrast to *Foxp3*^{Cre} mice (P=0.1625). (C) Gating strategy for infiltrating MDSC, DC, Macrophages in MC38 tumors established in *Foxp3*^{Cre} mice and *Il1r2*$^{fl/fl}$*Foxp3*^{Cre} mice (6-8 weeks, female) treated by αPD-1 (200μg) or IgG control. (D) Statistical analysis for different myeloid cells populations, and we did not find any significant changes of different myeloid cells sub-populations.