**Supplementary information**

**Tet2 inactivation enhances the anti-tumor activity of tumor-infiltrating lymphocytes**

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**Figure S1.** **Tet2-deficient TILs show enhanced anti-tumor efficacy in vivo.**

A. The breeding strategy and representative genotyping results for the indicated transgenic mice.

B. Quantification of individual tumor size measurement in four experimental groups: WT control (CTL) (black), WT with anti-PD-L1 treatment (green), Tet2KO control (CTL) (red), Tetd2KO with anti-PD-L1 treatment (blue). The vertical bars (from left to right) indicate the time points for T cell transfer, and the first and second anti-PD-L1 treatments, respectively.

C. The gating strategy for identifying CD45.2+CD8+ TILs (transferred) and CD45.1+CD8+ (endogenous) TILs.

D. Comparison of the distribution of endogenous CD45.1+CD8+ T cells in tumor (TILs), spleen (SPL) and peripheral blood (PB) in recipient mice transferred with WT (black) and Tet2KO (red) CD45.2+CD8+ T cells. The data were shown as the fold change relative to WT in TILs, spleen and peripheral blood. Data were shown as mean ± S.D; n=6-11, \* *P* < 0.05, by two-tailed Student’s t-test.

E. Percentage of endogenous CD45.1+CD8+ T cells distribution in tumor (TILs), spleen (SPL) and peripheral blood (PB) in recipient mice transferred with WT (left) and Tet2KO (right) CD45.2+CD8+ T cells. Data were shown as mean ± S.D; n=6-11.

**Figure S2. Tet2-deficient TILs exhibit enhanced immune response and cytotoxicity at the early stage of tumor onset.**

A-B. Quantification of flow cytometry analysis results of corresponding markers in endogenous CD45.1+CD8+ T cells in recipient mice transferred with WT (black) and Tet2KO (red) CD45.2+CD8+ T cells for 3 or 8 days. Data were shown as mean ± S.D; n = 7-22, \* *P* < 0.05, \*\*\* *P* < 0.0001, by two-tailed Student’s *t*-test.

C. The gating strategy of flow cytometry analysis to measure the cytotoxicity of WT and Tet2KO CD8+ T cells in vitro. The percentage of caspase 3/7 positive B16-OVA cells were used to indicate the degrees of tumor cell death.

D-E. Cell cycle (Brd-U + 7-AAD staining; D) and cell proliferation analysis (Ki-67 staining; E) in WT and Tet2KO CD8+OTI T cells co-cultured with B16-OVA cells for 4 hrs. Data were shown as mean ± S.D; n=3, \* *P* < 0.05, \*\*\* *P* < 0.0001, by two-tailed Student’s *t*-test.

**Figure S3. scRNA-seq analysis of WT and Tet2KO TILs.**

A. Quality control of scRNA-seq data. Violin plots were used to indicate the total number of genes, UMIs (unique molecular identifier), and the percentage of mitochondrial genes in four experimental groups. nGene: the number of genes; nUMI, the number of unique molecular identifier.

B. List of markers used to identify the cell types using scRNA-seq data in the four indicated experimental groups. CD63: melanoma cells; CD8a: CD8+ T cells; Adgre1: monocytes and macrophages.

**Figure S4. Quality control of** **RNA-seq and ATAC-seq analysis of WT and Tet2KO TILs.**

A. Heatmap representation of Spearman’s correlation coefficients (SCC) between replicates of RNA-seq (left) and ATAC-seq (right) data collected in this study. The scale bar shown from white to red represents the range of SCC from 0.4 to 1.

B. The volcano plots illustrating differentially expressed genes (DEGs; left) between day 0 and day 3 in WT and Tet2KO TILs. (right) The comparison between chromatin accessibility and expression of genes that displayed increased (red) or decreased (blue) expression between WT (top) or Tet2KO (bottom) TILs. TILs were collected from day 0 to day 3 after adoptive transfer.

C. Heatmap representation of WT-specific and Tet2KO-specific DEGs (Day 0 vs Day 3) identified in Figure 4B.

D. GSEA analysis of WT-specific and Tet2KO-specific DEGs identified in Figure 4B.

**Figure S5. ATAC-seq analysis of WT and Tet2KO TILs.**

A. Numbers of differential chromatin accessible regions between Day 0 and Day 3 groups in WT and Tet2KO TILs.

B. Heatmap representation of WT-specific and Tet2KO-specific differential chromatin accessible regions identified in Figure 5A.

C. GREAT analysis of WT-specific and Tet2KO-specific differential chromatin accessible regions identified in Figure 5A.

D. Percentage of genes with ETS1 or BATF binding motifs that displayed positive correlation between expression and chromatin accessibility in WT and Tet2KO TILs.

E. Quantification of caspase 3/7 positive B16-OVA melanoma cells treated with DMSO or ETS inhibitors (100 nM YK-4-279 or 500 nM TK-216) for 24 hrs. Data were shown as mean ± S.D; n=4.

F. Quantification of the percentage of IFN-gamma and TNF-alpha double positive cells in *in vitro* cultured WT and Tet2KO CD8+ T cells treated with DMSO or ETS inhibitors (100 nM YK-4-279 or 500 nM TK-216) for 72 hrs. Data were shown as mean ± S.D; n = 3, \* P < 0.05, by two-tailed Student’s t-test.