Supplementary Materials

TIL-Derived CAR T Cells Improve Immune Cell Infiltration and Survival in the Treatment of CD19-Humanized Mouse Colorectal Cancer

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Supplementary Figure S1 to S6

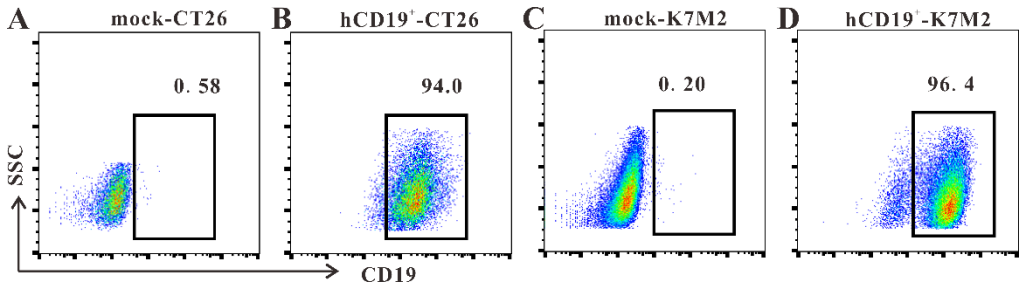


***Figure S1*. Construction of hCD19^{+}-CT26 and hCD19^{+}-K7M2 cell line.**

CD19 expression on the tumor cells were detected by FACS. (A) mock-CT26 cells infected with MSCV-GFP control retrovirus; (B) MSCV-hCD19 retrovirus infected CT26 cells (hCD19^{+}-CT26) by sorting; (C) mock-K7M2 cells infected with MSCV-GFP control retrovirus; (D) MSCV-hCD19 retrovirus infected K7M2 (hCD19^{+}-K7M2) cells by sorting.

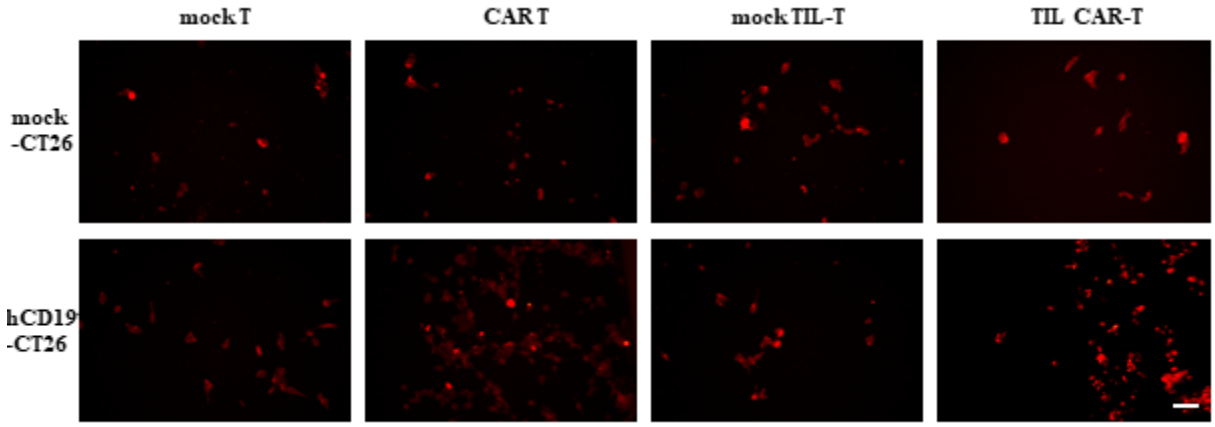


***Figure S2.* TUNEL-staining of CT26 cells after co-cultured with T cells. (scale bar= 200 μm)**

Both CD19-BBζ CAR T and TIL CAR-T could effectively kill the hCD19^{+}-CT26 cells and lead to apoptosis of the tumor cells.

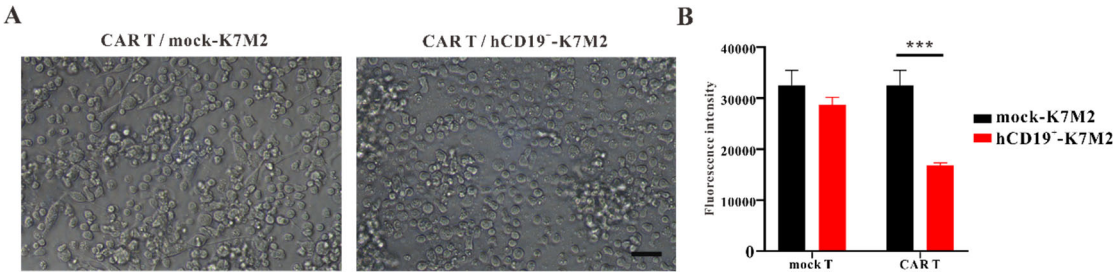


Figure S3. **CAR T killed hCD19-expressed K7M2 cells successfully.**

The CAR T cells were co-cultured with hCD19^{+}-K7M2 cells and mock-K7M2 cells for 12 h.(A) Representative images of CAR T co-cultured with K7M2 cells (scale bar = 200 μm); (B) fluorescence intensity of K7M2 cells detected by Resazurin dye solution (n = 5, $^{***}P < 0.001$).

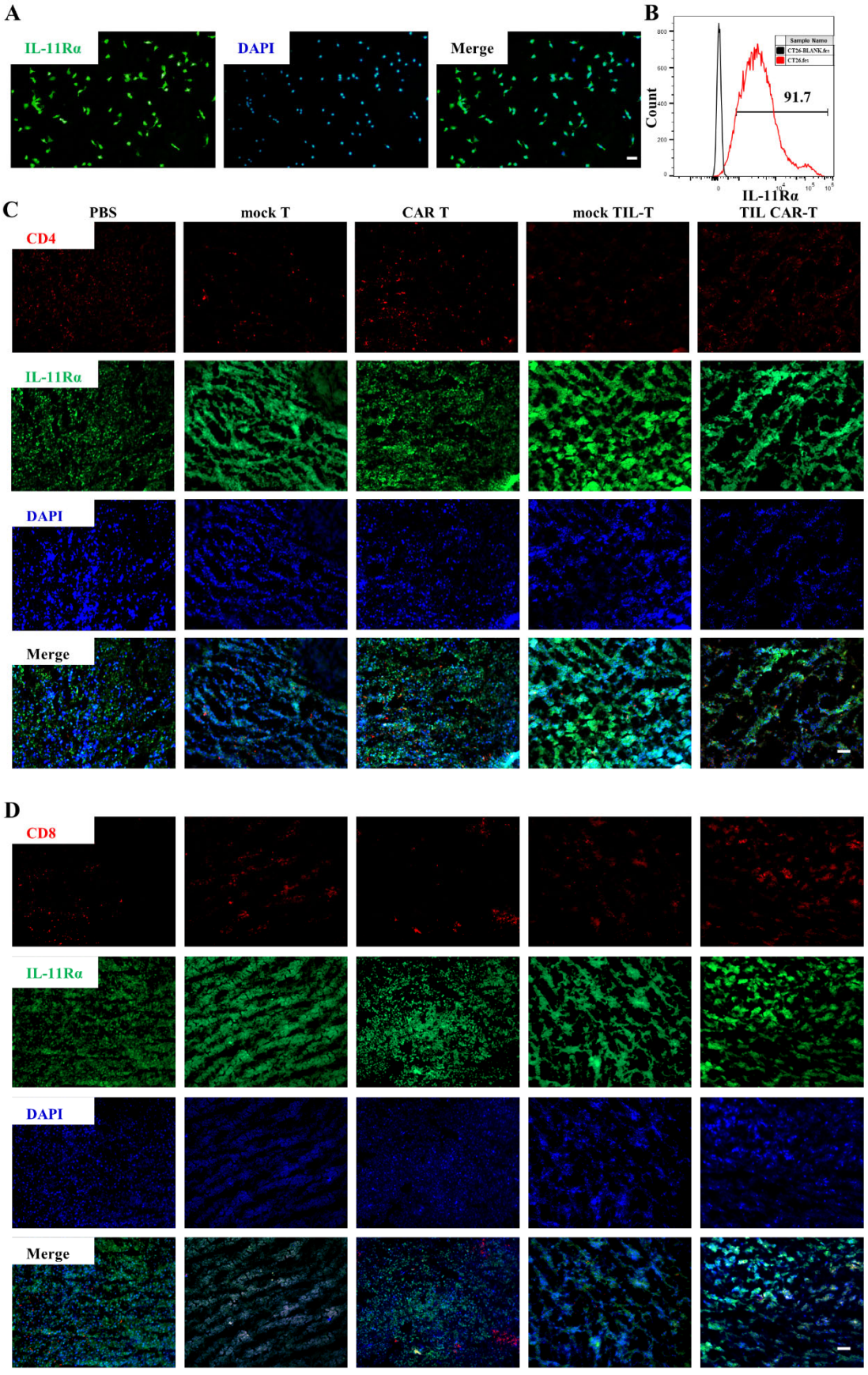


Figure S4. TIL CAR-T promoted immune cell infiltration in the tumor tissues. IL-

11Rα is highly expressed in Colorectal cancer[1,2] and considered to be a tumor-associated antigen (TAA) for a target in cancer therapy[3,4]. (A) Representative images of IL-11Rα (green) and DAPI (blue) fluorescence staining (scale bar = 200 μm) ;(B) The expression of IL-11Rα on CT26 cells by FACS; (C) Representative images of CD4 (red), IL-11Rα(green) and DAPI (blue) fluorescence staining (scale bar =50 μm); (D) Representative images of CD4 (red), IL-11Rα(green) and DAPI (blue) fluorescence staining (scale bar = 50 μm).

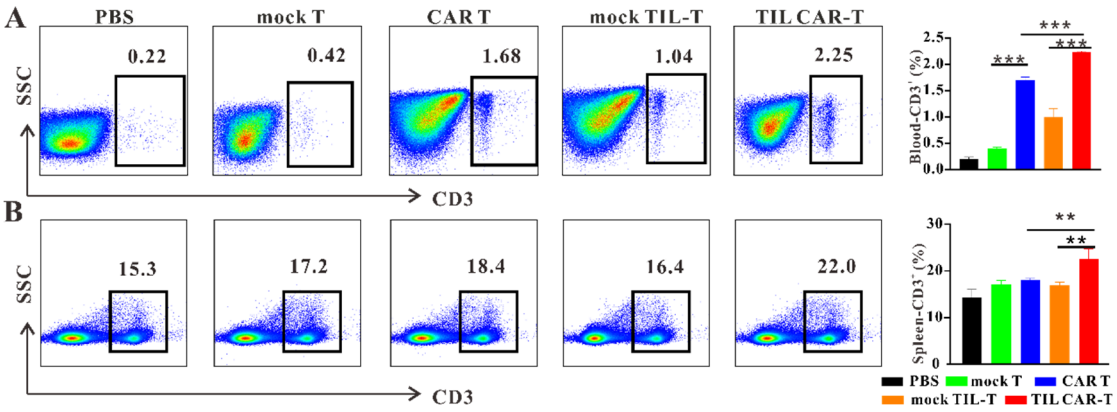


Figure S5. **T cell proportion in the peripheral blood and spleen of tumor-bearing mice.**

The frequency of T cell in the blood and spleen were detected by FACS. (A) The percentage of T cell in peripheral blood; (B) The percentage of T cells in spleen of tumor-bearing mice after cell transfusion ($n = 3$, $^{**}P < 0.01$; $^{***}P < 0.001$).

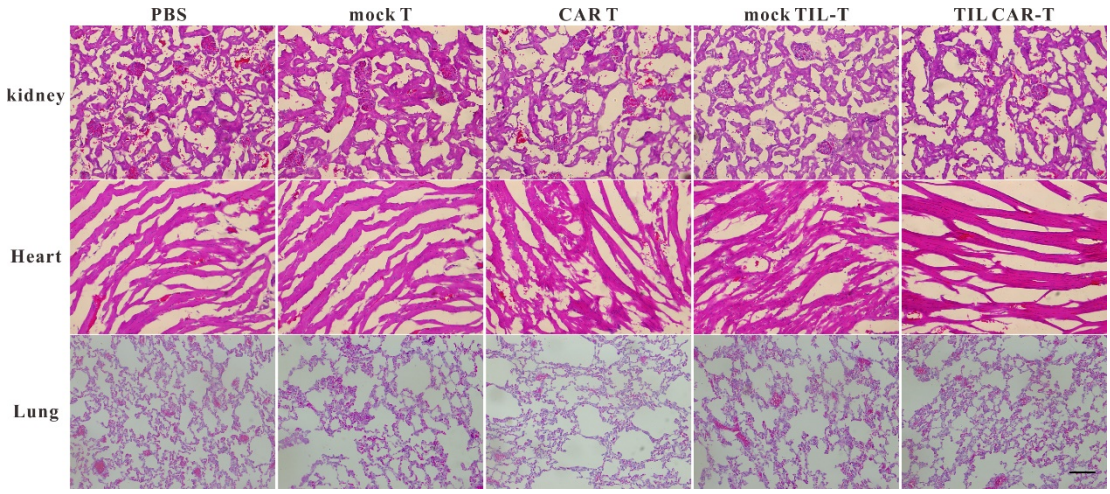


Figure S6. **H&E staining of kidney, heart, and lung of tumor-bearing mice.**

H&E staining was performed on the kidney, heart, and lung sections of different groups of tumor-bearing mice, and no obvious pathological injury was observed (scale bar = 200 μm).

References

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