Supplementary Figure

Therapeutic efficacy of anti-CD19 CAR-T cells in a mouse model of systemic lupus erythematosus

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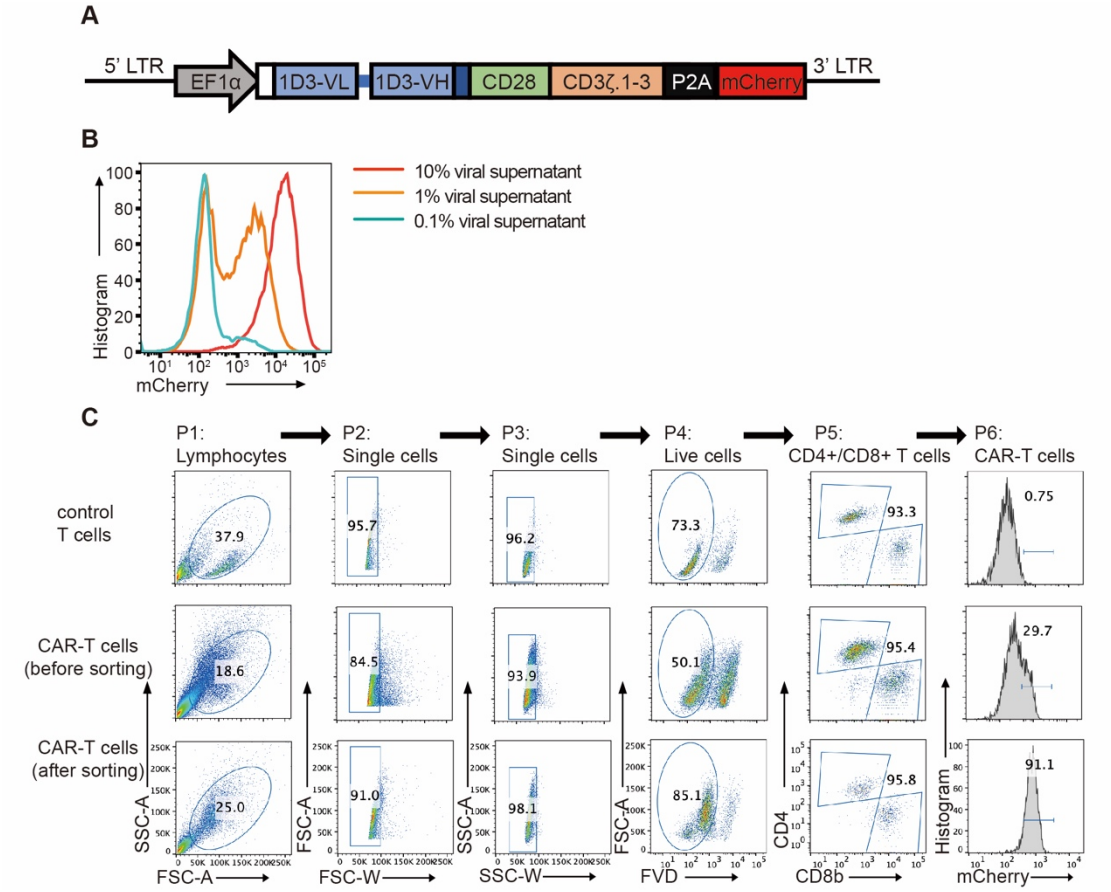
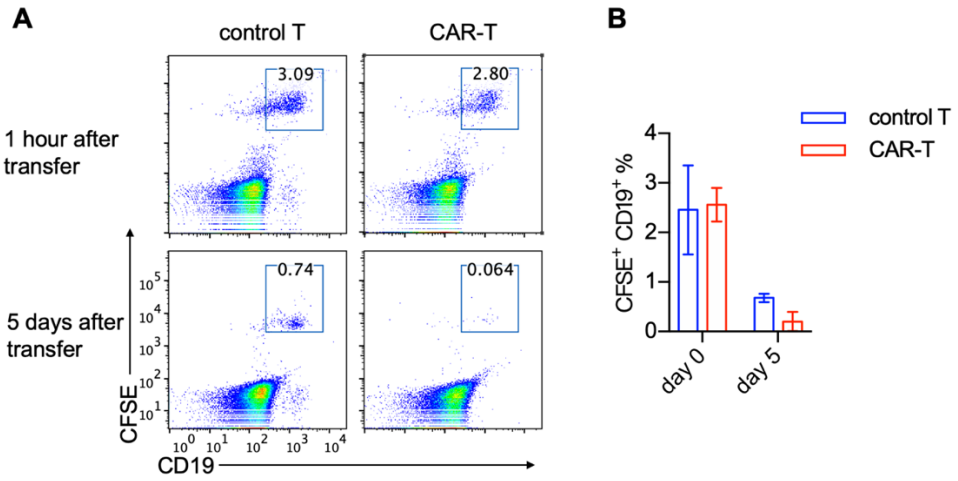


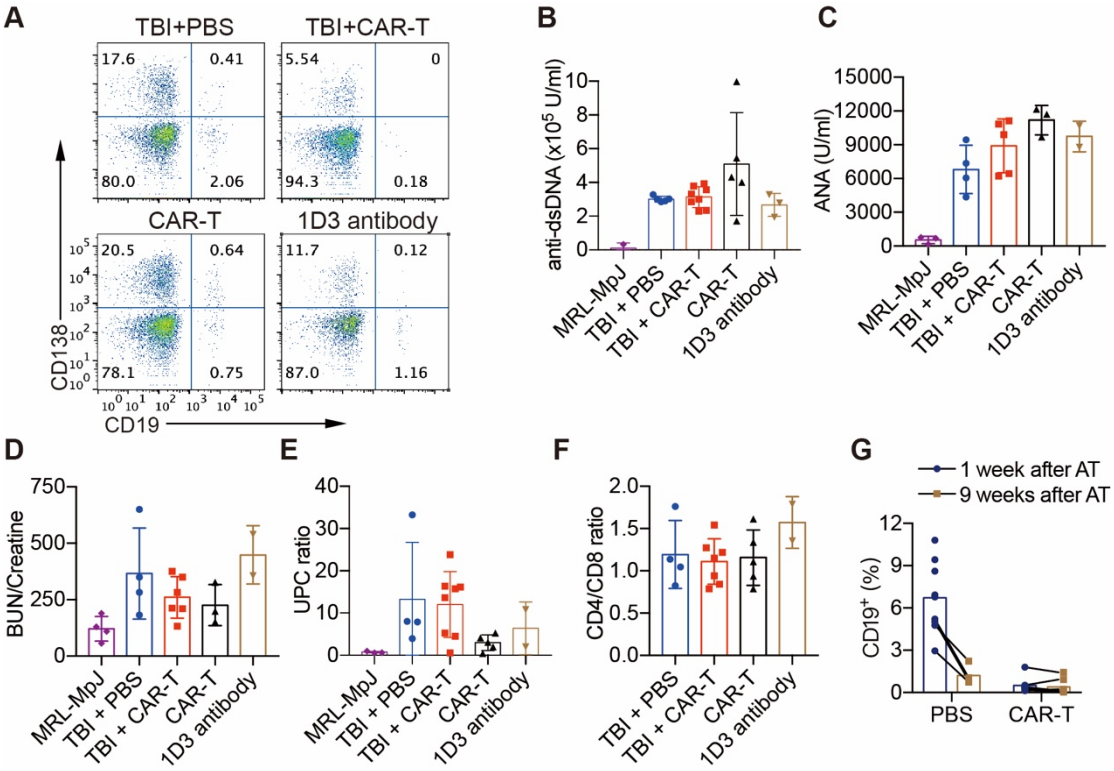
Supplementary Figure 1. Generation of CAR-T cells.

(A) Diagram of the DNA encoding 1D3-28Z.1-3 CAR. (B) After 24h of transduction with indicated concentration of lentiviral supernatant containing 8 μg/ml polybrene, mCherry expression by 293FT cells was analyzed by flow cytometry. (C) Gating strategy and sorting efficiency of CAR-T cells; control T cells are mock-virus transduced T cells; CAR-T cells are CAR-expressing lentivirus transduced primary T cells. Control T cells in P5 and CAR-T cells in P6 were sorted. The data are representative of at least two independent experiments with similar results.



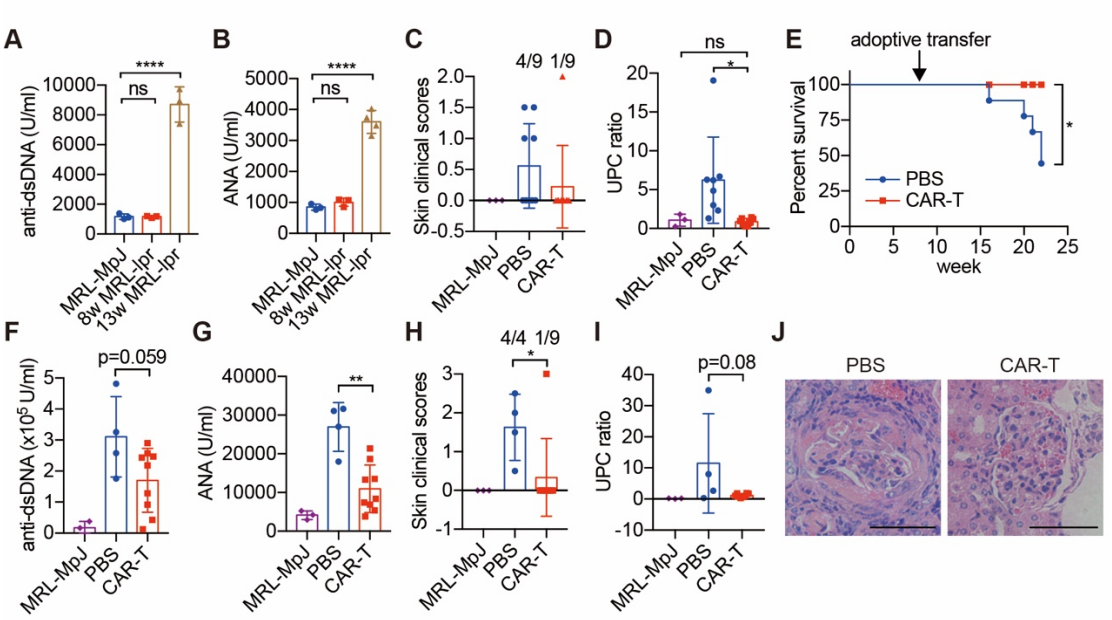
Supplementary Figure 2. *In vivo* killing test of CAR-T cells.

(A and B) MRL-lpr mice were treated with control or CAR-T cell, both with low-dose TBI as preconditioning. 8 weeks later, these MRL-lpr mice received $3x10^7$ CFSE labeled $CD19^+$ B cells *i.v.*. 1 hour or 5 days after B cell infusion, blood B cell percentages were analyzed by FACS. The representative flow cytometry (A) and quantification (B) data of the transferred B cells.



Supplementary Figure 3. Plasma cell percentage, antibody levels and nephritis biochemical analysis of mice received different treatments.

MRL-lpr mice received different treatments at 13 weeks of age (Fig. 2A), and were sacrificed at 22-weeks of age for following analysis. (A) Percentage of blood $CD138^+$ cells 9 weeks after transfer were analyzed by flow cytometry; live $CD45^+$ cells were gated. (B, C) Sera were isolated; anti-dsDNA antibody (B) and anti-nuclear antibody (ANA) (C) levels were detected by ELISA. (D) Blood urea nitrogen to creatinine ratios in serum were analyzed by chemistry analyzer. (E) Urine protein to creatinine ratios from urine were analyzed by chemistry analyzer. (F) Blood cells were analyzed by flow cytometry. (G) The percentages of blood $CD19^+$ cells 1 week and 9 weeks after transfer were analyzed by flow cytometry, and the percentages of $CD19^+$ cells of the same mice were paired. The data are representative of at least two independent experiments with similar results.



Supplementary Figure 4. CAR-T cell transfer at 8 weeks of age delayed SLE pathogenesis.

Sera anti-dsDNA antibody (A) and anti-nuclear antibody (B) level of non-treated MRL-lpr mice of different age were measured. (C) Skin lesion of 13-week-old mice were scored. (D) Urine protein to creatinine ratios from urine of 13-week-old mice were analyzed. (E) The survival rate of mice; the difference between the 2 groups was compared by Logrank (Mantel-Cox test) calculation. Sera anti-dsDNA antibody (F) and anti-nuclear antibody (G) level of MRL-lpr mice at 22 weeks of age. (H) Skin lesion of 22-week-old mice were scored. (I) Urine protein to creatinine ratios from urine of 22-week-old mice were analyzed. (J) The representative hematoxylin and eosin (H&E) stained sections of glomerular areas from kidneys of 22-week-old MRL-lpr mice. Original magnification × 400. Bars represent 50um. (A, B, D, F, G, H, I) The data were analyzed by Student's *t* test and significance is indicated by *P < 0.05.

	TBI + PBS (N=11)	TBI + 4-1BB CAR-T (N=10)
Survival rate	27.3% (3/11)	60% (6/10)
Blood $CD19^+$ %	1.31±0.62	0.46±0.29
Spleen/body weight %	1.87±1.02	1.66±0.35
Anti-dsDNA ($x10^5$ U/mL)	4.32±2.31	4.21±0.71
ANA ($x10^4$ U/ml)	4.92±2.35	3.87±1.82
UPC ratio	3.25±1.10	3.42±1.92

Table 1. Long-term therapeutic effect of 4-1BB CAR-T cells.

MRL-lpr mice received TBI + PBS or TBI + 4-1BB CAR-T cells at 13 weeks of age, and the parameters of disease severity were analyzed at 30 weeks of age. CD19+ B cell percentage of the blood were analyzed by FACS. Sera anti-dsDNA antibody and ANA levels were detected by ELISA. Urine protein to creatinine ratios were analyzed.