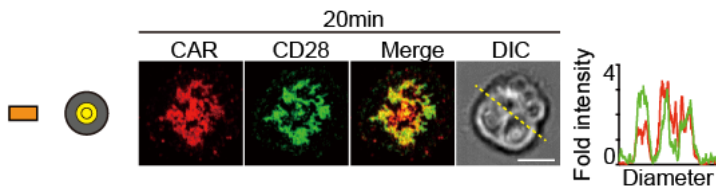


1

2 **Supplementary Fig. 1**

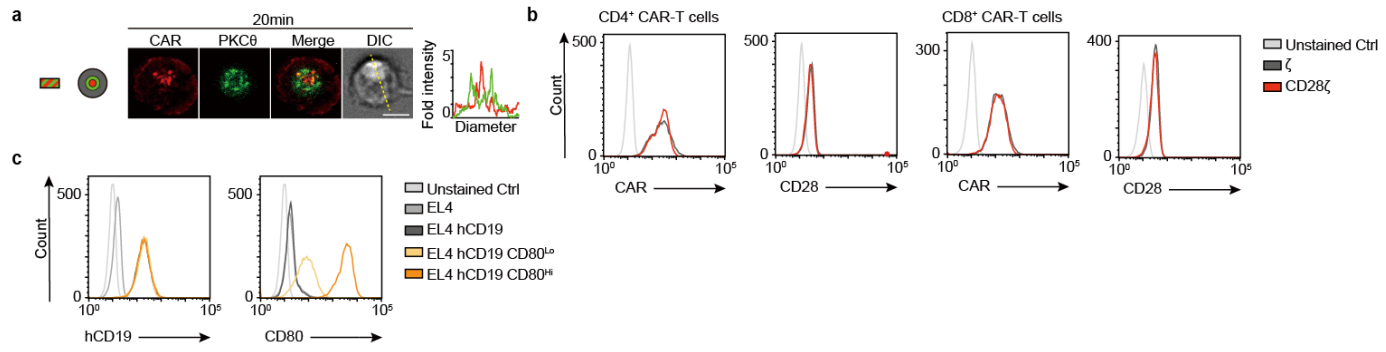
3 Primary CD8⁺ T cells were transduced with ζ . and CD28 ζ . and 4-1BB ζ .CAR. Cell surface
4 expression of CAR was analyzed by flow cytometry.



5

6 **Supplementary Fig. 2**

7 The other distribution pattern of CAR and CD28 at the T cell–SLB interface imaged in
8 the same condition as in **Fig. 3c** (see also **Fig. 3e**). The histogram shows fold fluorescent
9 intensities of CAR and CD28 on the diagonal yellow lines in the DIC images. Bars, 5 μm .

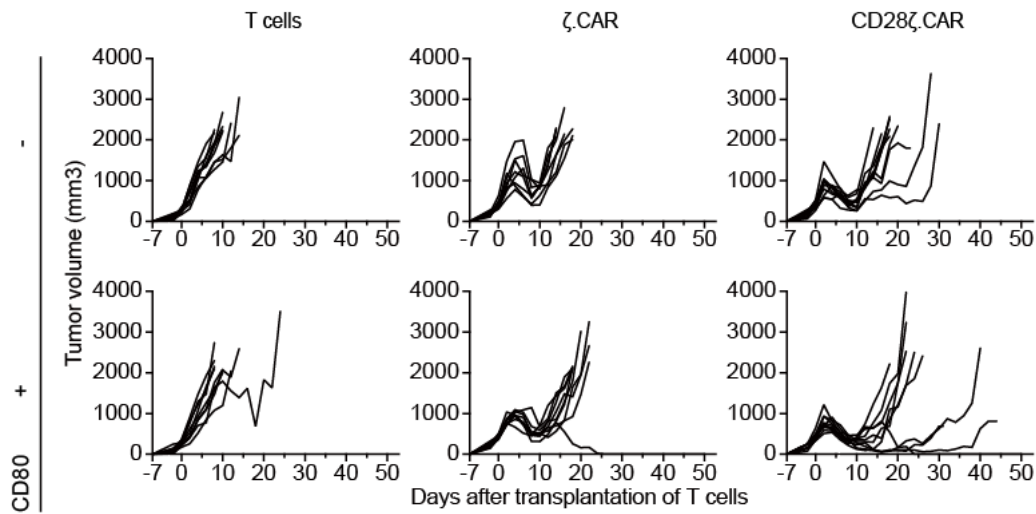


Supplementary Fig. 3

a The other distribution pattern of CAR and PKCθ at the T cell-SLB interface imaged in the same condition as in **Fig. 4c** (see also **Fig. 4e**). The histogram shows fold fluorescent intensities of CAR and PKCθ on the diagonal yellow lines in the DIC images. Bars, 5 μm.

b Splenic CD4⁺ or CD8⁺ T cells were transduced with ζ. or CD28ζ.CAR and analyzed by flow cytometry for cell surface expression of each CAR and CD28.

c EL-4 cells were transduced with hCD19 and/or CD80 and sorted by different expression level of CD80: low (CD80^{Lo}) and high (CD80^{Hi}). The cell surface expression of hCD19 and CD80 were analyzed by flow cytometry.



Supplementary Fig. 4

The graphs show the individual growth curves of EL-4 cells inoculated into the mice in Fig. 5b.