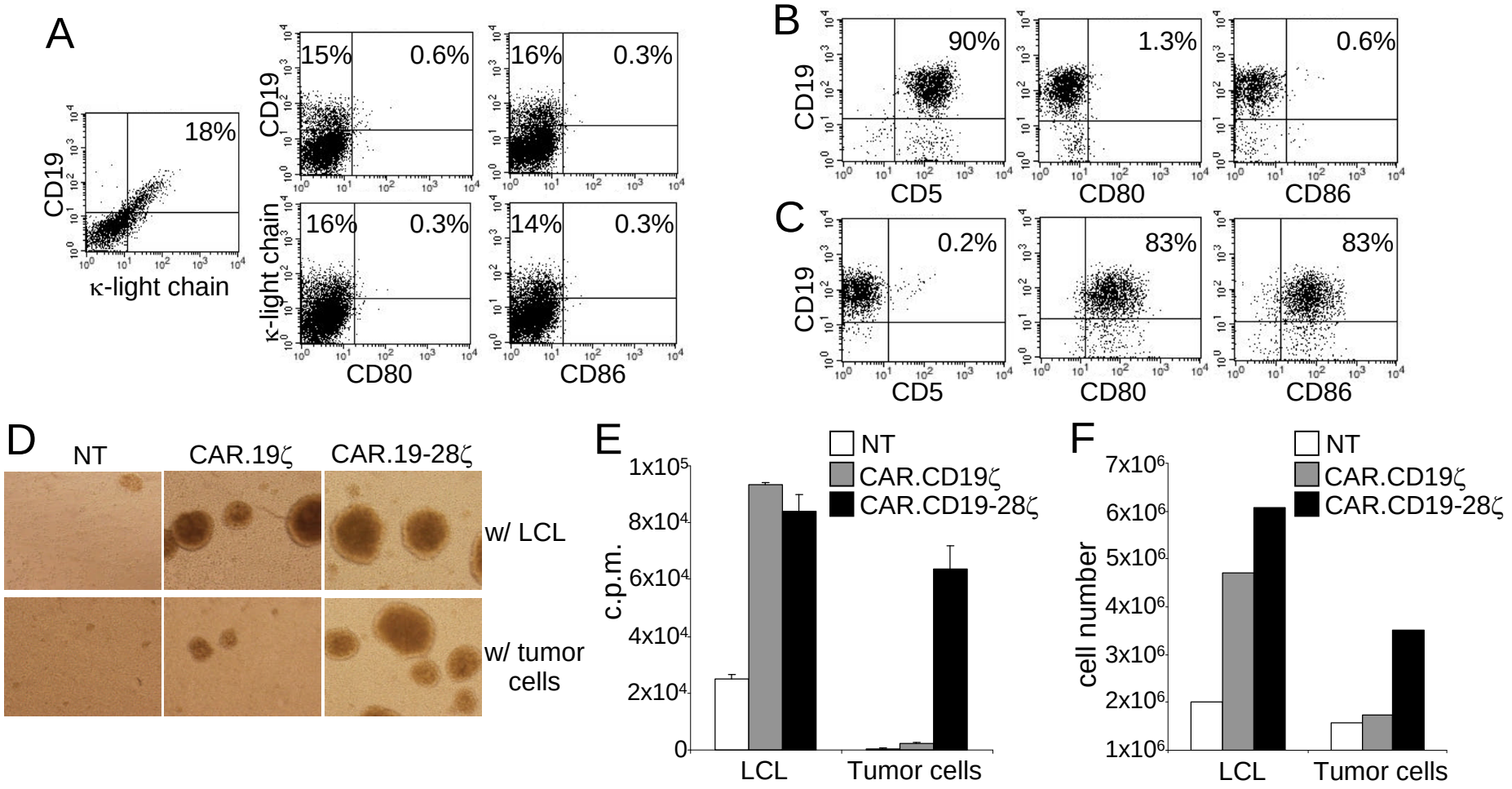
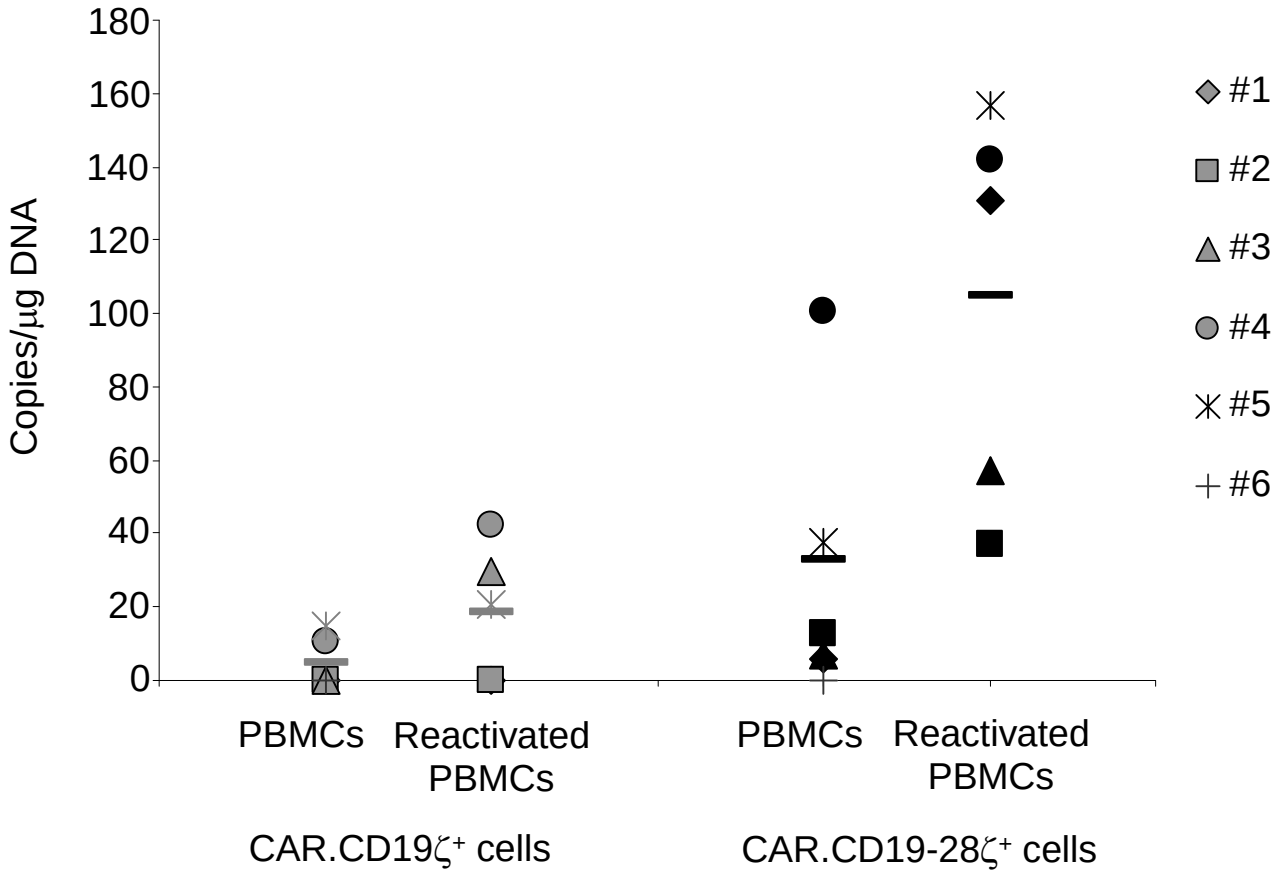
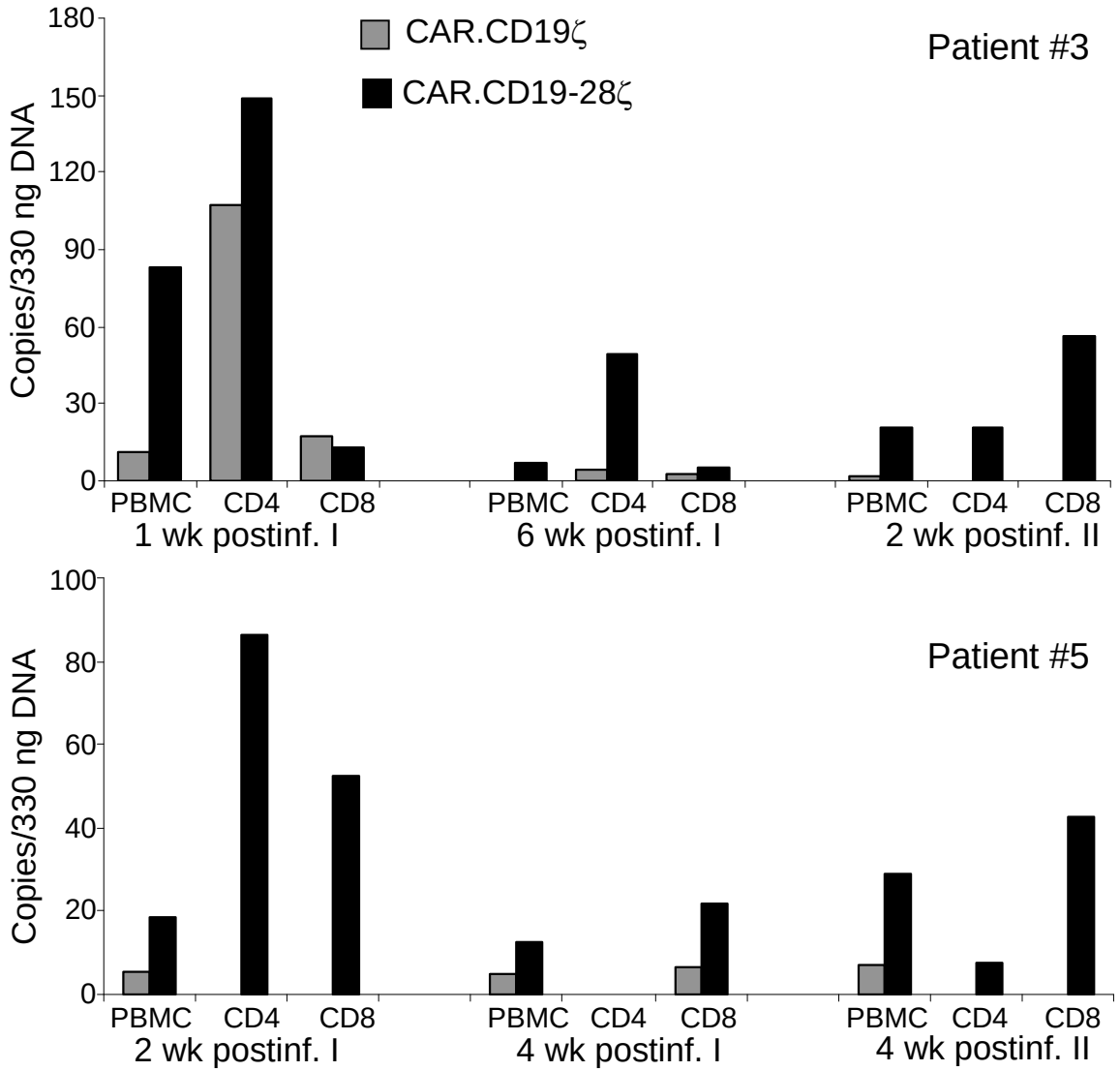


Supplementary Figure 1. Role of CD28 costimulation in vitro. The expression of CD80 and CD86 molecules was assessed by FACS analysis in two fresh preinfusion tissues available from Pt #2 (panel A) and Pt #1(panel B). In both cases monoclonal CD19$^+$ tumor cells lacked CD80 and CD86 expression. Panel C illustrates that Epstein Barr Virus infected cells (LCLs) obtained from Pt # 1 expressed CD80 and CD86 molecules. Panel D shows NT, CAR.CD19ζ$^+$ and CAR.CD19-28ζ$^+$ T cells from Pt #1 co-cultured with autologous LCLs or autologous tumor cells (ratio 1 to 1). Both CAR.CD19ζ$^+$ and CAR.CD19-28ζ$^+$ T cells (expressing the native CD28 as shown in Fig. 1) proliferated in the presence of LCLs, while only CAR.CD19-28ζ$^+$ T cells proliferated in response to tumor cells (magnification 10X). Thymidine incorporation at 72 h (panel E) and cell counts 1 week (panel F) post stimulation with LCLs or primary tumor cells confirmed that only CAR.CD19-28ζ$^+$ T cells proliferated in response to tumor cells while both CAR.CD19ζ$^+$ and CAR.CD19-28ζ$^+$ T cells proliferated in response to LCLs. These data confirm that CD28 endodomain incorporated within the CAR is essential to provide co-stimulation when the tumor cells lack the expression of co-stimulatory molecules.



Supplementary Figure 2. Reactivation of CAR-modified T lymphocytes ex vivo. PBMCs were collected at 4-6 weeks after the first T-cell infusion and were stimulated ex vivo with immobilized OKT3/CD28 antibodies. Each symbol represents a single patient and the horizontal bars denote mean group values. Gray symbols indicate CAR.CD19ζ$^+$ T-cell lines. Black symbols indicate CAR.CD19-28ζ$^+$ T-cell lines. A significant increase in molecular signals after ex vivo activation was apparent only among CAR.CD19-28ζ$^+$ cells.



Supplementary Figure 3. Relative contribution of CAR-transduced CD4 and CD8 subsets to expand T-cell populations at different postinfusion intervals. $CD4^+$ and $CD8^+$ T cells were FACS sorted (TCH Core Facility, Houston, TX) from freshly isolated PBMCs at 1, 2, 4 and 6 weeks postinfusion and DNA extracted for Q-PCR amplification. The relative percentage of $CD4^+$ and $CD8^+$ cells for patient #3 were 55% and 43% among CAR.CD19ζ^+ T cells vs. 57% and 42% among CAR.CD19-28ζ^+ T cells. For patient #5 the corresponding percentages were 7% and 90% for the CAR.CD19ζ^+ T cells vs. 10% and 85% for CAR.CD19-28ζ^+ T cells.

Supplementary Table 1. Clinical characteristics of treated patients						
Patient no.	**Diagnosis**	**Age/Sex**	**Previous therapy**	**Disease status at T-cell infusion**	**T-cell dose**	**Clinical outcome**
1	Stage IVA SLL with history of EBV+ NHL	53/M	Fludarabine, cyclophosphamide and rituximab	Active disease (blood and cervical, axillary, retroperitoneal and inguinal lymph nodes)	2×10^7 cells/m^2	Stable disease 10 mo; progressive disease 6 mo post 2nd infusion
2	Relapsed stage IVB follicular lymphoma with transformation to DLBCL	56/M	Multiagent chemotherapy with rituximab	Active disease (cervical lymph nodes)	2×10^7 cells/m^2	Progressive disease 5 wk post 1st infusion
3	Relapsed stage IIIB DLBCL	46/M	Multiagent chemotherapy with rituximab	Active disease (retroperitoneal lymph nodes)	1×10^8 cells/m^2	Stable disease 3 mo; progressive disease 4 wk post 2nd infusion

4	Relapsed/refractory stage IIA DLBCL	57/M	Multiagent chemotherapy with rituximab, ASCT	Active disease (cervical and retroperitoneal lymph nodes)	1×10^8 cells/m^2	Progressive disease 6 wk post 1st infusion
5	Relapsed stage IVB follicular lymphoma with transformation to DBLCL	59/F	Multiagent chemotherapy with rituximab, ASCT	Active disease (muscle and skin)	2×10^8 cells/m^2	Progressive disease 6 wk after 1st infusion
6	Relapsed primary central nervous system DLBCL with systemic relapse	49/M	Multiagent chemotherapy with rituximab, ASCT	Active disease (brain and retroperitoneum)	2×10^8 cells/m^2	Progressive disease 2 wk after 1st infusion

SLL – Small lymphocytic lymphoma; DLBCL – Diffuse large B cell lymphoma; ASCT – autologous stem cell transplant.