**Supplemental Fig.1.** Phenotypic similarity between isolated CD45RO+CD62L+ human TCM expanded following lentiviral transduction with spacer variant 2G-CARs.

**(A)** Timeline of CD8+ TCM isolation, transduction and expansion prior to experimental use. **(B)** Immunomagnetic isolation method and purity of CD8+ TCM cells (CD45RO+CD62L+). **(C)** Phenotype of CD8+ TE(CM) transduced with different spacer variants of 2G-CAR at time of experimental use.

**Supplemental Fig.2.** Similar CD171 expression levels by xenografted NB cell lines and patient NB specimens.

**(A)** IF detection of CD171+ in human NB biopsies imaged at 20x with the Nuance Multispectral imaging system. Pictured are examples of low, medium, and high expression. Mean optical density (OD) of tumor area was measured using inForm analysis software. The threshold for positivity was set at 0.3 OD, based on controls. **(B)** IF detection of CD171 by intracranially injected Be2 and SK-N-DZ xenografts as in (A).

**Supplemental Fig.3.** *In vitro* and *in vivo* antitumor activity of CD171-specific 2G-CAR spacer variant CD8+ TE(CM) against CD171lowSK-N-DZ human NB xenografts.

**(A)** Lytic potency of 2G-CAR spacer variants in CRA against SK-N-DZ. **(B)** SK-N-DZ stimulation of IFNγ secretion by spacer variant 2G-CAR-CTLs. (**C**) Biophotonic SK-N-DZ tumor signal response to intratumorally infused 2G-CAR(SS, MS or LS)-CD8+ TE(CM) (n=5 per group). **(D)** Kaplan-Meier survival of treated cohorts.

**Supplemental Fig. 4.** Expression of Fas and FasL after siRNA knockdown.

**(A)** Frequency of Fas+ LS 2G-CAR-CTLs after siRNA knockdown of Fas relative to LS 2G-CAR-CTLs treated with scr siRNA (%Fas+ values derived from an average of 4 independent experiments). **(B)** Frequency of FasL+ LS 2G-CAR-CTLs after siRNA knockdown of FasL as described in (A).