



Supplementary Figure 1. Selection of CAR-expressing T cells

FACS analysis of one representative experiment. After transduction at day 6, the transduced cells were stained with the anti-LNGFR mAb C40-1457, and analysed by FACS (upper panels). Then CAR-T cells endowed with the NWL spacer were immuno-selected by the use of a single step protocol with the mAb ME20.4 coupled to magnetic Microbeads (CliniMACS CD271-LS MicroBeads, Miltenyi), whereas CD44v6-NWN2.CAR T and CD44v6-NMS.CAR T cells were purified with the C40-PE mAb followed by anti-PE coupled Microbeads. As shown in the lower panels, the immunoselection processes allowed the isolation of highly enriched (>90%) populations of CAR T cells, which were then used for all the *in vitro* and *in vivo* functional studies.