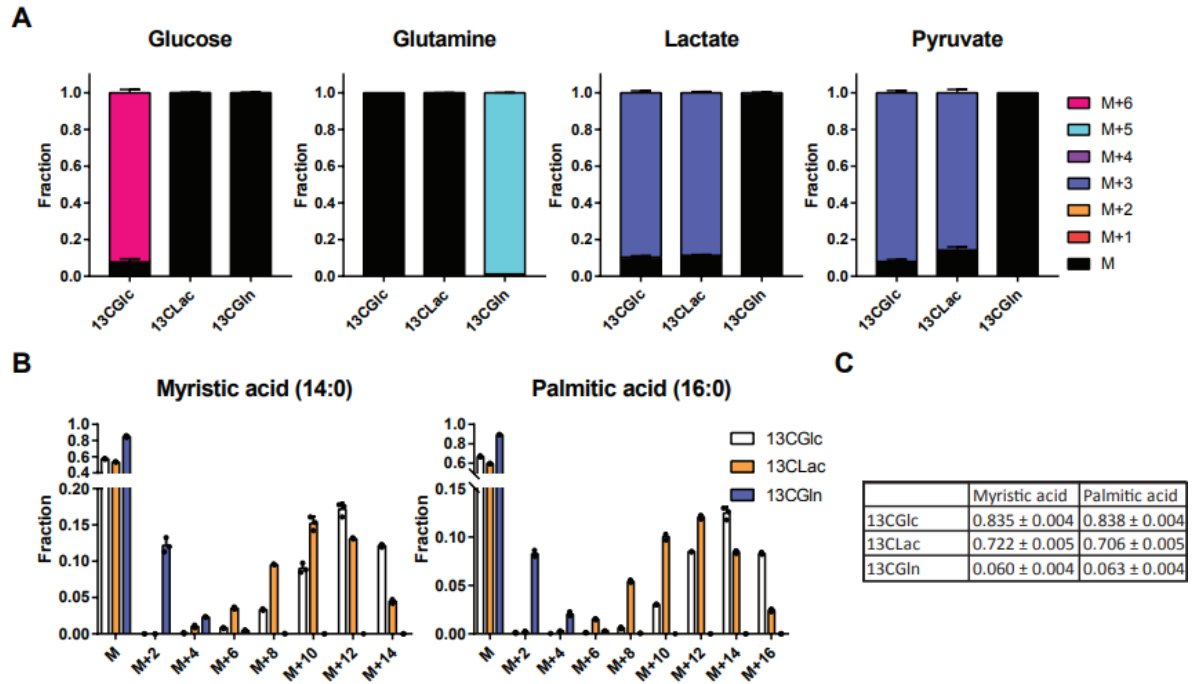
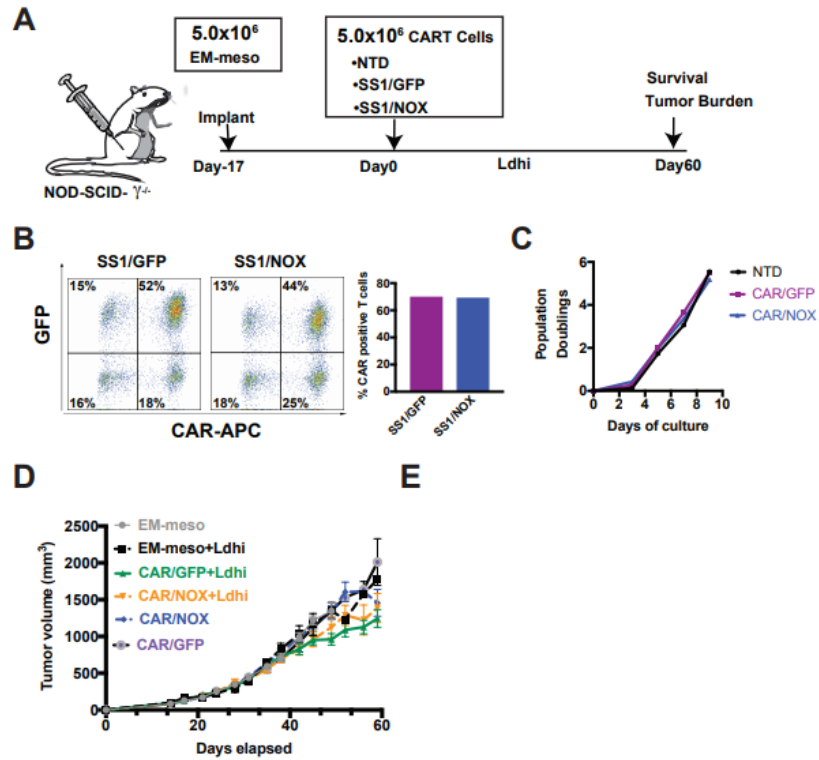


**Figure S1. CAR design.** (A) Schematic representation of the mesothelin-specific 28ζ lentiviral CAR construct containing an SS1 single chain variable fragment (scFv) which is linked via a CD8α hinge as well as a CD28 TM to the CD28 and CD3ζ intracellular signaling domains. TM, transmembrane. (B) The 4-1BBζ CAR construct has a CD8α hinge, CD8α TM and 4-1BB intracellular signaling domain.



**Figure S2. Lactate metabolism supports long chain fatty acid synthesis in murine T-cells.** (A) Metabolite labeling patterns in activated CD8<sup>+</sup> T-cells (day after a 24 h incubation with U-13-C glucose, U-13-C-lactate, or U-13C-glutamine (mean ± SD, *n* = 3). (B) Myristate and palmitate labeling from U-13-C glucose, U-13-C-lactate, or U-13C-glutamine following a 24 h incubation time (mean ± SD, *n* = 3). (C) Fractional contribution to myristate and palmitate labeling from U-13-C glucose, U-13-C-lactate, or U-13C-glutamine (mean ± SD, *n* = 3). 13CGlc, U-13-C glucose; 13CLac, U-13-C-lactate; 13CGln, U-13C-glutamine.



**Figure S3.** Non-additive effects of Ldhi with CAR T-cell anti-tumor function in vivo.

Experimental layout including a schematic of the EM-meso xenograft model and combinatorial treatment approach. (A)  $5 \times 10^6$  EM-meso cells were injected subcutaneously into adult NSG mice.  $5 \times 10^6$  28 $\zeta$  CAR+ T-cells were I.V. injected 17 days after tumor implantation ( $n = 6-9$  per group). 75mg/kg Ldhi or vehicle (PBS) were administered twice weekly (days 21–49). Tumor volumes were measured by caliper at the indicated time-points as described in the Materials and Methods section. Values represent mean  $\pm$  S.E.M. for each group. (B&C) Tumor size, independent of CAR cytolytic activity, was compared following Ldhi inhibition. (C&D) The anti-tumor function of CAR T-cells following Ldhi inhibition was compared by Two-way ANOVA using a Holm-Sidak multiple comparison post-hoc test. Tumor size was significantly less ( $*p < 0.05$  CAR [ $n = 7$ ] relative to Tumor alone [ $n = 7$ ]; CAR +Ldhi [ $n = 7$ ] relative to Tumor alone) during the terminal phase of the experiment (day 49). However, no differences were observed between CAR vs CAR+Ldhi.