Author contributions
Yanyan Liu: Conceptualization, Methodology, Formal analysis, Investigation, Writing-Original Draft, Visualization. Ping Lu: Investigation, Data Curation. Yifei Ma: Investigation, Resources. Zhucheng Yin: Resources. Hongli Xu: Resources. Lanxin Xiang: Investigation. Wangli Zhang: Investigation. Siwei Li: Conceptualization, Writing-Review & Editing, supervision. Xinjun Liang: Conceptualization, Writing-Review & Editing, supervision, Funding acquisition.

Materials and methods
Patients
This study comprised 35 patients with NSCLC, including 14 with adenocarcinoma and 21 with squamous cell carcinoma, who were diagnosed as clinical stage Ib–IIb from January 2015 to July 2016 at Hubei Cancer Hospital (Hubei, China). The patients’ characteristics are presented in Table S1. Fresh peripheral blood samples (30–40 mL) were obtained two weeks before surgery from patients with early-stage NSCLC. We also enrolled another five patients with advanced NSCLC. They were preparing to receive anti-PD1 blockade treatment at Hubei Cancer Hospital (Hubei, China), from which the peripheral blood specimens were sampled two weeks prior and 90 days after receiving anti-PD1 therapy. The Ethics Committee of Hubei Cancer Hospital approved the protocol and all patients provided written informed consent. Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation using Ficoll-Paque™ Plus (GE Healthcare), washed twice in RPMI-1640 medium (Invitrogen), and immediately stored at −80°C.

Flow cytometry and cell sorting
Flow cytometric procedures were conducted in accordance with the guidelines used for immunological studies [1]. Cryopreserved PBMCs were thawed at 37°C and washed twice with FACS buffer at room temperature. Resuspended single-cell suspensions were incubated for 30 min on ice in the dark with the relevant antibodies: Live/dead-BV510, PD1-PE, CD3-FITC, and CD8-PerCP/Cy5.5 (all Biolegend), and then washed
twice and resuspended in FACS buffer before analysis using a flow cytometer. CD8⁺ T cells with high PD1 expression (PD1^{hi}) and low PD1 expression (PD1^{lo}) were separated based on the mean fluorescence intensity of PD1. Gating strategy was showed in Fig. S1. For the detection of intracellular cytokine release, the cells were stimulated with PMA/ionomycin in the presence of Brefeldin A for 6 h \textit{in vitro}, after which the cells were washed twice and stained with surface marker antibodies, followed by fixation and permeabilization with Cytofix/Cytoperm kit (BD Biosciences). The cells were then stained with the relevant intracellular antibodies: IL-2-BV421, TNF-α-APC/Cy7, and IFN-γ-APC (all Biolegend). All stained samples were measured using a flow cytometer (BD LSR Fortessa) and analyzed using FlowJo software (v. 9.3.2; TreeStar). Gating strategy was showed in Fig. S2. PD1^{hi}CD8⁺ and PD1^{lo}CD8⁺ T cells were sorted based on the PD-1 expression using a BD FACSARia™II cell sorter (BD Bioscience) that employs the live cells CD3⁺-CD8⁻-PD1⁺CD8⁺-PD1^{hi}CD8⁺ or PD1^{lo}CD8⁺ gating strategy. To evaluate Ki-67 expression, PD1^{hi}CD8⁺ and PD1^{lo}CD8⁺ T cells were cultured with CD3/CD28 beads and IL-2 (Thermo Fisher Scientific) for 5 days at 37°C, followed by fixation and permeabilization with Cytofix/Cytoperm kit (BD Biosciences). Subsequently, the cells were stained with anti-Ki-67-APC (Biolegend) and detected using a flow cytometer (BD LSR Fortessa). T cell assays presented in this work were performed following MIATA guidelines.

Preparation of Meso-CAR-T cells based on virus vector
Pure CD3⁺ T cells were obtained from PBMCs using flow cytometry sorting (FACSARia™II, BD), plated with CD3/CD28 antibody (25 µL/mL) before added the cells, and cultured with CD3⁺ T cells using T cell expansion medium. After three days of stimulation, we counted and centrifuged the cells, obtained the virus concentrate, added polybrene (10 µg/mL), resuspended the T cells with the virus solution, seeded the cells into a 48-well plate and centrifuged (2000 rpm, room temperature, 80 min). We changed the fluid 8–12 h after infection and checked the infection efficiency after 48 h. We then incubated the cells for another 10–12 days, changed the medium in time, and gradually moved the cells to a larger dish.
Luciferase *in vitro* killing experiment

A lentivirus expressing luciferase was prepared to infect the MSTO-211 tumor cell line, and puromycin (1 μg/mL, Solabe) was used to screen out a tumor cell line stably expressing luciferase. These cells were then transduced with lentivirus expressing human MSLN protein to obtain the target tumor cell (MSTO-MSLN<sup>+</sup> ffLuc<sup>+</sup>). Tumor cells were plated 24 h in advance. CAR-T cells and control T cells were seeded into the wells according to the different ratios of effector T and tumor cells (effect T cells: tumor cells) at 0 h. The cells were then removed from the well plate after 24 h, and the culture was centrifuged and lysed with substrate D-fluorescein potassium salt (15 mg/mL, YEASEN; protected from light). A microplate reader was used to detect the reaction between luciferase and substrate.

Mouse model and *in vivo* experiment

Female NSG mice (6–8 weeks old) were obtained from Charles River Laboratories (Beijing, China) and maintained at Tongji Medical College Animal Care Unit. All animal protocols were pre-approved by the Institutional Animal Care and Use Committee of Tongji Medical College. The mice were anesthetized by intraperitoneal injection of 1.5% pentobarbital sodium. Anesthesia was given for approximately 10 min, and the hair on the dorsal side of the right lower limb was shaved. There were four mice per group. MSTO-MSLN<sup>+</sup> ffLuc<sup>+</sup> tumor cells were injected subcutaneously, at a total of 6 × 10<sup>5</sup> cells. We then observed the tumor formation status in all mice. After the tumor was visible, we measured the size using a vernier caliper and calculated the tumor size using the following formula: tumor volume (mm<sup>3</sup>) = 1/2 (length × width × width). On the 7<sup>th</sup> day (D7) and 21<sup>st</sup> day (D21), 1 × 10<sup>6</sup> CAR-T cells (200 μL in volume) were infused into the mice via tail vein injection, and the control group was infused with an equal amount of mock T cells. We continued to monitor tumor size and mouse survival.

Statistical analysis

We used GraphPad Prism 6.0 (GraphPad Soft Inc., USA) for statistical analysis.
Differences between two groups and among multiple groups were assessed using Student’s t-test and analysis of variance, respectively. A p-value of < 0.05 was considered statistically significant.

Reference
1. Cossarizza A, Chang HD, Radbruch A, Abrignani S, Addo R, Akdis M, Andrä I, et al. Guidelines for the use of flow cytometry and cell sorting in immunological studies (third edition). Eur J Immunol. 2021;51(12):2708-3145.
Figure S1. Gating strategy for the identification of PD1\textsuperscript{hi}CD8\textsuperscript{+} and PD1\textsuperscript{lo}CD8\textsuperscript{+} T cells. Peripheral blood samples were stained with relevant antibodies described in Materials and methods, then PD1\textsuperscript{hi}CD8\textsuperscript{+} and PD1\textsuperscript{lo}CD8\textsuperscript{+} T cells were gated according to the gating strategy (lymphocytes → singlets → live cells → CD3\textsuperscript{+} → CD3\textsuperscript{+}CD8\textsuperscript{+} → PD1\textsuperscript{hi}CD8\textsuperscript{+} or PD1\textsuperscript{lo}CD8\textsuperscript{+}).
Figure S2. Gating strategy for intracellular cytokine analysis. PBMC were stimulated with PMA/ionomycin for 6h in the presence of Brefeldin A, followed by fixation and permeabilization, then cells were stained with relevant antibodies and detected using flow cytometry. Cytokine producing PD1^+CD8^+ T cells was obtained according to the gating strategy (lymphocytes \rightarrow \text{singlets} \rightarrow \text{live cells} \rightarrow CD3^+ \rightarrow CD3^+CD8^+ \rightarrow PD1^+CD8^+ \rightarrow CD8^+INF-\gamma^+ or CD8^+INF-\gamma^- \rightarrow IL-2^+TNF-\alpha^+).
Table S1. Patient characteristics (n=35)

|                          | PFS > 2 years | PFS < 2 years |
|--------------------------|---------------|---------------|
|                          | (n=19)        | (n=16)        |
| Age, years               |               |               |
| Median (range)           | 68 (56-76)    | 65 (54-75)    |
| Gender, n (%)            |               |               |
| Male                     | 13 (68.4)     | 10 (62.5)     |
| Female                   | 6 (31.6)      | 6 (37.5)      |
| Smoking history, n (%)   |               |               |
| Former                   | 4 (21.1)      | 5 (31.3)      |
| Current                  | 10 (52.6)     | 7 (43.8)      |
| Never                    | 5 (26.3)      | 4 (25.0)      |
| Histology, n (%)         |               |               |
| Adenocarcinoma           | 8 (42.1)      | 6 (37.5)      |
| Squamous cell carcinoma  | 11 (57.9)     | 10 (62.5)     |
| Stage at diagnosis, n (%)|               |               |
| I                        | 5 (26.3)      | 5 (31.3)      |
| II                       | 14 (73.7)     | 11 (68.8)     |