**Supplementary Methods**

**Lentivirus production**

Human embryonic kidney 293T cells were seeded at 5 × 106 per 10-cm dish prior to 24 h of transduction. All plasmid DNA was purified using the QIAGEN Endo-Free Maxi prep kit (Qiagen, Valencia, CA). The 293T cells were transfected with 20 μg of the empty vector (mock) or the recombinant expression vector in addition to the 15 μg of packaging plasmid psPAX2 and 5 μg of the vesicular stomatitis virus (VSV-G) envelope plasmid pMD2.G (generous gifts from Dr. T. Didier) using a calcium phosphate transfection system (1).The viral supernatant was harvested at 48 or 72 h after transfection.

**Western blot analysis**

To confirm the levels of GPC3 expression in various HCC cell lines, 5 × 106 HCC cells were lysed in 100 µl lysis buffer for 30 min on ice. Cell lysate was then removed by centrifugation at 12,000 × g for 10 min. Each sample was denatured under reducing conditions (in the presence of DTT) and electrophoresed by 12% SDS–PAGE. The samples were then transferred to a nitrocellulose membrane (Bio-Rad, Hercules, CA) and immunoblotted with the mAb 9C2 against GPC3 (Abcam, Cambridge, MA). To confirm whether the expression of Bcl-XL in αGPC3-28BBZ CAR T cells was increased in the presence of GPC3 protein, we cocultured the GPC3-targeted CAR or 2D3-28BBZ CAR CD8+ T cells with GPC3-positive Huh-7 or GPC3-negativeSK-HEP-1 cells for 24 h at an effector : target ratio of 3 : 1. The T cells were then isolated, lysed and immunoblotted using anti-Bcl-XL antibody (Cell Signaling Technology, USA), β-actin was as a reliable loading control. The blots were incubated with horseradish peroxidase-conjugated anti-mouse or rabbit IgG (Kangchen Biotech, Shanghai, China) and detected using the ECL western blot analysis system (Pierce, Thermo Scientific, Rockford, IL) in accordance with the manufacturer’s instructions.

**Flow cytometric analysis**

Surface expression of GPC3 on HCC cells was detected by the mAb 9C2 (Abcam, Cambridge, MA), using normal mouse IgG as a negative control, followed by FITC-conjugated goat anti-mouse IgG (Kangchen Biotech, Shanghai, China). The transduction efficiencies of the various genetically modified T cells were detected using the autofluorescence of eGFP. Untreated T cells were used as negative control. The following conjugated antibodies were purchased from BD Biosciences: mouse anti-human CD3 (FITC or PerCP), anti-human CD4 (FITC), anti-human CD8 (PE), anti-human CD64 (PE), anti-human CD86 (PE), isotype-matched control antibodies The expression of CD64 or CD86 on K562 cells transduced with lentiviral vectors encoding the membrane-binding extra-domains of CD64 or CD86 was detected by PE-labeled CD64 or PE labeled CD86. PE labeled isotype control antibody was used in all analyses. In T cells transfer experiments, peripheral blood was collected from retro-orbital bleeding, and the absolute number of human CD3+, CD4+, and CD8+ T cells was determined. After gating on the human CD3+ population, the CD4+ and CD8+ T cells were quantified using TruCount tubes (BD Biosciences, San Jose, CA) as described in the manufacturer’s instructions. GFP-positive T cells were directly analyzed using TruCount tubes. Flow cytometric data were analyzed by WinMDI 2.9 software.

**Cytokine release assay**

Cytokine measurements were performed through the coculture of 4 × 104 transduced T cells with 4 × 104 target cells per well, in triplicate wells of 96-well plates in a final volume of 200 μl of T cell medium. After inoculation for 24 h, the IFN-γ and IL-2 cytokines secreted by the genetically modified T cells that were stimulated by the targeted HCC cells were measured using an ELISA kit according to the manufacturer’s instructions (MultiSciences Biotechnology, Hangzhou, China).

**Immunohistochemistry**

The HLiv-HCC150CS-01 microarray (Outdo Biotech, Shanghai, China) containing 75 primary HCC samples and the sections of the normal kidney (n=10) and gastric glands (n=10) were immunostained using an anti-GPC3 antibody (mAb 1G12, BioMosaics Inc, Burlington, VT) to determine the expression of GPC3. To assess the persistence of human T cells in tumors, formalin-ﬁxed, parafﬁn-embedded tumor tissues were immunostained using an anti-CD3 antibody (Thermo Scientific RM-9107-S0). A normal rabbit IgG served as an isotype control. The procedures were performed as previously described (5). Briefly, after deparafﬁnization and rehydration, the sections were exposed to 3% H2O2 in methanol to eliminate endogenous peroxidase activity. Bovine serum albumin (1%) was used to block for 30 min at room temperature (RT). The primary rabbit anti-human CD3 monoclonal antibody was incubated overnight at 4°C. Sections were then washed with PBS and incubated with an HRP-conjugated goat anti-rat secondary antibody (Kangchen Biotech, Shanghai, China) for 45 min at RT. The sections were visualized using a diaminobenzidine staining kit (Tiangen Biotech, Beijing, China) and then counterstained with hematoxylin, dehydrated, cleared, mounted and photographed. In evaluating CD3 expression, brown membrane staining was considered positive.

**References**

1. Wang H, Zhou M, Shi BZ, et al. Identification of an exon 4-deletion variant of epidermal growth factor receptor with increased metastasis-promoting capacity. Neoplasia 2011;13:461-471.
2. Zhang PN, Zhang PF, Shi BZ, et al. Galectin-1 overexpression promotes progression and chemoresistance to cisplatin in epithelial ovarian cancer. Cell Death Dis 2014; 5:e991.