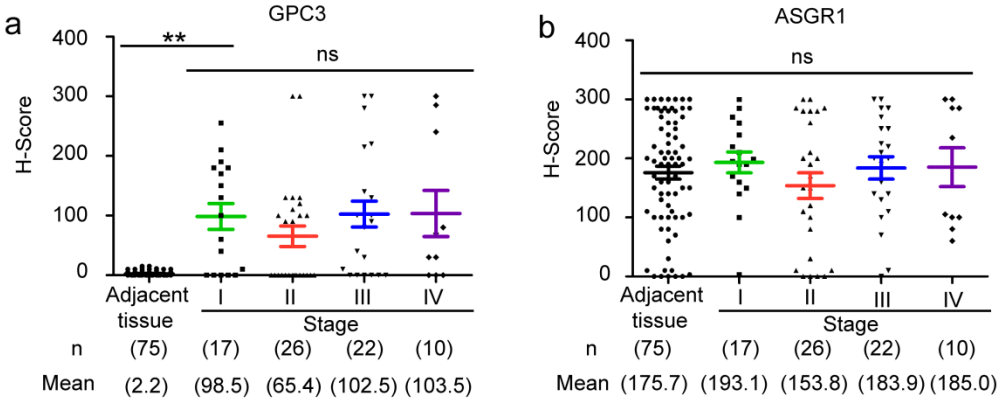
Supplementary Table 1. Primers used for the construction of the vectors in this study.

Splice site	Primer	Sequence (5'→3') [a]
mCherry	mCherry-F	5'-acgcgtcctagcgctaccggtcgccaccatggtgagcaagggcgaggaggataac-3' (*Mlu*I)
	mCherry-R	5'-caaaattcaaagtctgtttcactccggacttgtacagctcgtccatgccgccggtg-3'
F2A	F2A-F	5'-tccggagtgaaacagactttgaattttgaccttctgaagttggcaggagacgttgag-3'
	F2A-R	5'-caaggcggtcactggtaaggccatgggcccagggttggactcaacgtctcctgcca-3'
CD8α signaling peptide	SP-F	5'-atggccttaccagtgaccgccttgctcctgccgctggccttgctgctccacgccg-3'
	SP-R	5'-cagactccaacagctgcacctccggcctggcggcgtggag-3'
anti-ASGR1 dAb(H)	dASGR1-F1	5'-gaggtgcagctgttggagtctgggggaggcttggtacagcctgggggtccctgcgtc-3'
	dASGR1-R1	5'-gccatcgcatacttctcaaaggtgaatccggaggctgcacaggagagacgcagggaccc-3'
	dASGR1-F2	5'-gaagtatgcgatggcgtgggtccgccaggccccagggaagggtctggagtgggtctcac-3'
	dASGR1-R2	5'-cggagtctgcgtagtatgtcgtcacacccctcgccgaaatccgtgagacccactccag-3'
	dASGR1-F3	5'-ctacgcagactccgtgaagggccggttcaccatctcccgcgacaattccaagaacac-3'
	dASGR1-R3	5'-cggtgtcctcagcacgcaggctgttcatttgcagatacagcgtgttcttggaattg-3'
	dASGR1-F4	5'-gtgctgaggacaccgcggtatattactgtgcgaaacataagcggcacgagcatac-3'
	dASGR1-R4	5'-gctcgagacggtgaccagggttccctgaccccaggagtcaaaacgagtatgctcgtgcc-3'
CD8α hinge*	CD8-HF	5'-gtcaccgtctcgagcaccacgacgccagcgccgcgaccaccaacac-3'
	CD8-HR	5'-caaccaccaccagcacccaaaaatcacaggcgaagtccagc-3'
CD28 TM & CD28/4-1BB endo-domian [b]	CD28-TF	5'-ttttgggtgctggtggtggttggtggagtcctg-3'
	BB-endo-R	5'-gtcgacctacagttcacatcctccttcttcttc-3'(*Sal*I)
GPC3	GPC3-F	5'- acgcgtcctagcgctaccggtcgccaccatggccgggaccgtgcgcaccgcg -3' (*Mlu*I)
	GPC3-R	5'-gtcgacctatcagtgcaccaggaagaagaagcacaccaccgagatggccatg-3' (*Sal*I)
ASGR1	ASGR1-F	5'-acgcgtcctagcgctaccggtcgccaccatgaccaaggagtatcaagaccttc-3' (*Mlu*I)
	ASGR1-R	5'-gtcgacctattaaaggagag gtggctcctggctggc-3' (*Sal*I)

a. The underlined nucleotides are restriction sites of the enzymes indicated in the brackets at the ends.

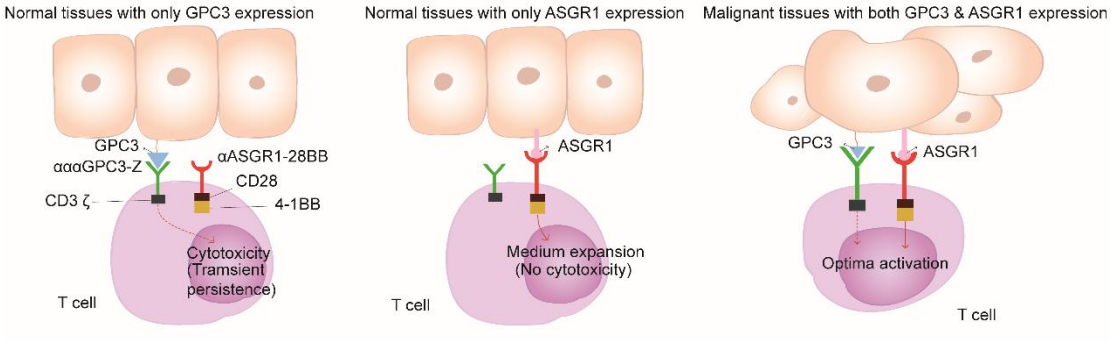
b. The PCR template is the previously constructed vector αGPC3-28BBZ CAR [1].

Supplementary Figure 1.



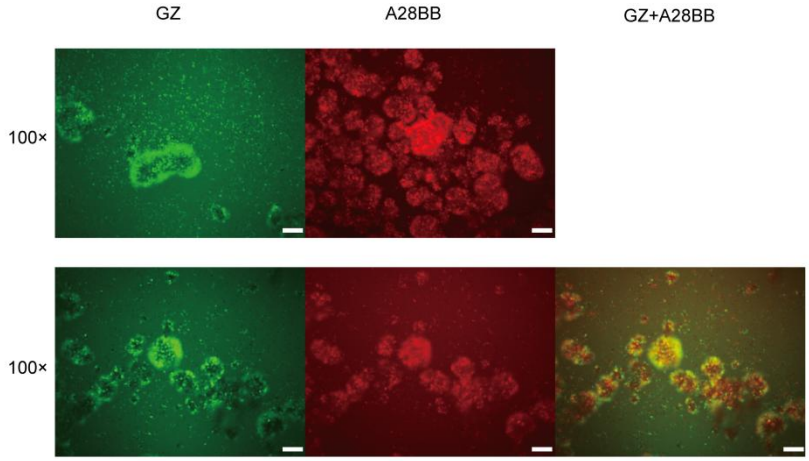
The H-score of GPC3 and ASGR1 staining in scatter plots. The scattering of H-scores for GPC3 and ASGR1 expression in hepatocellular carcinoma (HCC) and adjacent normal liver is illustrated in scatter plots. The mean and standard errors are indicated. **a** The overexpression of GPC3 was detected in tumor samples, and there was no differential expression among the HCC samples at different stages. **b** The H-scores [2] for ASGR1 of each category were not significantly different.

Supplementary Figure 2.



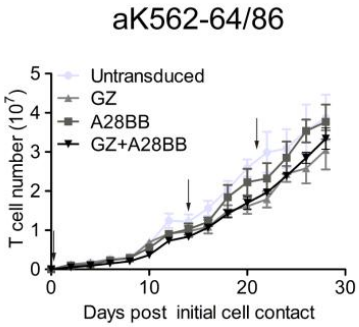
A schematic diagram depicting the mechanism of dual-targeted T cell activation. This dual-targeted strategy mimics the natural activation process of T cells. T cells only completely activate when they recognize GPC3 and ASGR1 simultaneously. If either GPC3 or ASGR1 is absent, as occurs in normal tissue, the T cells will not undergo optimal activation.

Supplementary Figure 3.



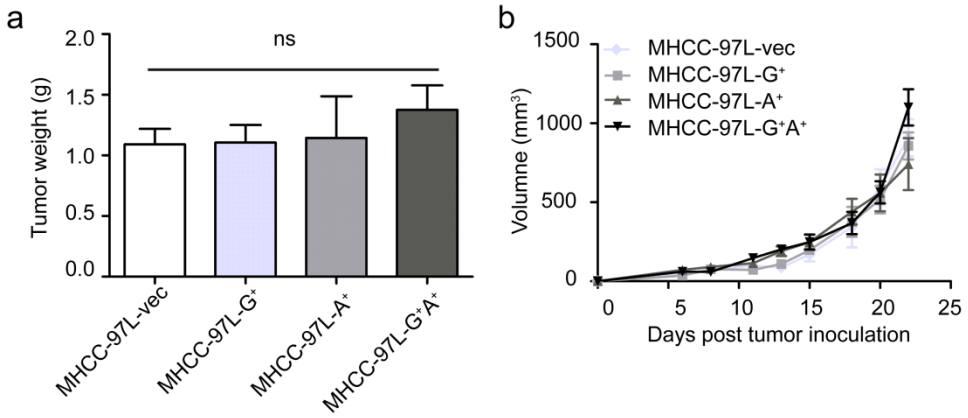
T cells were effectively transduced with the αASGR1-28BB- or/and αGPC3-Z-encoding vectors. Representative green fluorescence (eGFP) and red fluorescence (mCherry) photomicrographs (scale bar, 50 μm) of GZ, A28BB and GZ+A28BB T cells after gene transfer.

Supplementary Figure 4.



The *in vitro* expansion of T cells after re-stimulation with irradiated αK562-64/86. On day 14, at the end of one cycle of activation, T cells were then re-stimulated by irradiated αK562-64/86 cells. The arrows indicate re-stimulation of T cells using freshly irradiated αK562-64/86 cells every week.

Supplementary Figure 5.



The *in vivo* growth of MHCC-97L cells was not significantly affected by the introduction of GPC3 and/or ASGR1. 2 × 10^6 MHCC-97L-vec, MHCC-97L-G^+, MHCC-97L-A^+, or MHCC-97L-G^+A^+ cells were injected subcutaneously into the right flank of NOD/SCID mice (n=5). **a** The tumor weight of xenografts treated with saline. The adoptively transferred human T cells in subsequent animal experiments were re-suspended in saline. **b** Growth curve of the vector- and gene-transduced MHCC-97L cells (P> 0.05).

References

1. Gao H, Li K, Tu H, Pan X, Jiang H, Shi B, Kong J, Wang H, Yang S, Gu J, Li Z (2014) Development of T cells redirected to glypican-3 for the treatment of hepatocellular carcinoma. Clin Cancer Res 20 (24):6418-6428. doi:10.1158/1078-0432.CCR-14-1170

2. Shi B, Abrams M, Sepp-Lorenzino L (2013) Expression of asialoglycoprotein receptor 1 in human hepatocellular carcinoma. J Histochem Cytochem 61 (12):901-909. doi:10.1369/0022155413503662