Table S1 Target sites and sequences

Name	Sequence(5'→3')
Section1: sgRNAs targeting *LAG-3*	
sgRNA1	atgtgggaggctcagttcct
sgRNA2	gctgcagaaacagcaagccc
sgRNA3	tgctgtttctgcagccgctt
sgRNA4	gctgtttctgcagccgcttt
sgRNA5	gtttctgcagccgctttggg
Section2: Primers for generating in vitro transcription template	
Forward	taatacgactcactatagNNNNNNNNNNNNNNNNNN(20bp target sequence) gtttaagagctatgctggaaac
Reverse	aaaagcaccgactcggtgcc
Section3: Genotyping primers for TIDE analysis and single clone sequencing primer	
LAG-3 TIDE seq F	attccggcctctggtcatcc
LAG-3 TIDE seq R	ggagaggcttcactaggtgag
M13F	gtaaaacgacggccagt
Section4: Genotyping primers for off-target analysis	
PCR primers of the off-taget sites	
Offsite1- PCR F	gacaccaaacttccaagactc
Offsite1- PCR R	ctgcctgatcctgtgctcca
Offsite2- PCR F	gggaggaaatgaaaggatatg
Offsite2- PCR R	gagacgtaatgtcaaagatg
Offsite3- PCR F	gttgaggcaaagatgagtgg
Offsite3- PCR R	gcagcagtgataagtcagga
Offsite4- PCR F	cctcagaagtaggcattctc
Offsite4- PCR R	gtaagatgaggaagtggaggc
Offsite5- PCR F	agcatcactgcatccacagc
Offsite5- PCR R	gaataattgcacggctgagct
Oligo nucleotides for TIDE sequencing	
Offsite1- seq F	cttccaagactcaagaatg
Offsite2- seq F	aggatatgtgcattggacat
Offsite3- seq F	atgagtggggtgggagttcc
Offsite4- seq F	gcattctcctctccacctacagc
Offsite5- seq F	atgcactcccttcttagcccttc

Table S2 Off-target candidates genotyping

Locus name	In exon	In coding exon	Gene	Position chromosome	Mismatch sequence	TIDE analysis
LAG-3 gRNA5 on target	Yes	Yes	*LAG-3*	chr12:+6772899	GTTTCTGCAGCCGCTTTGGG	
offsite 1	No	No	CDC42BPB	chr14:+102966770	GCTTCTGCAGCCGCTTTGGG	0
offsite 2	No	No	AKIP1	chr11:+8914657	TTCTCTGCAGCCACTTTGGG	0
offsite 3	No	No	CTIF	chr18:-48742415	GGGTCTGCAGCCCCTTTGGG	0
offsite 4	No	No	CTB-41I6.1	chr17:-8967817	GTTTTTCCAGCAGCTTTGGG	0
offsite 5	No	No	LOC105370369	chr13:+111626415	ATGTCTGCCCCCGCTTTGGG	0

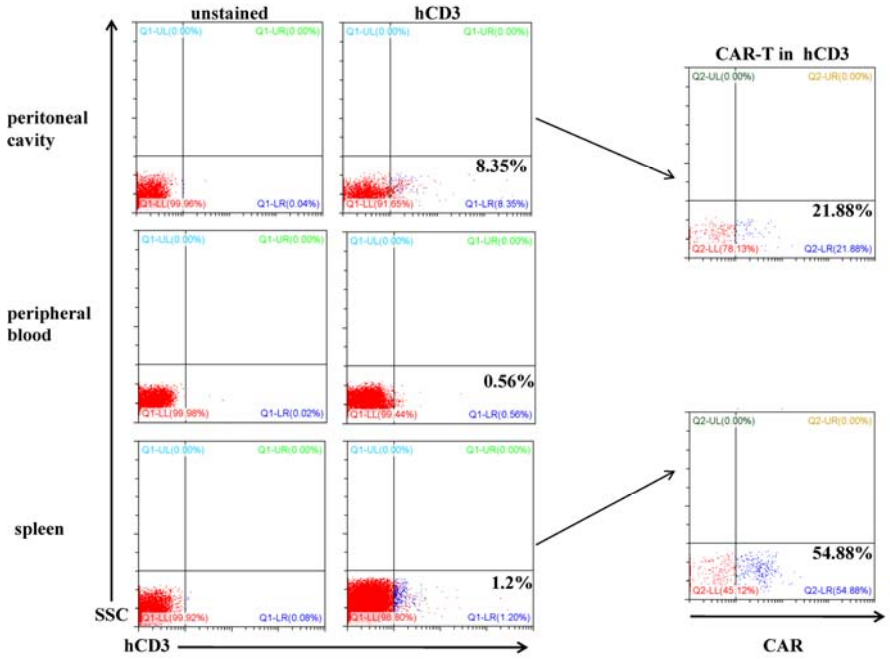

Fig. S1 The persistence of the *LAG-3* knock-out CAR-T cells *in vivo*. One of the *LAG-3* knock-out CAR-T cells treated mice was sacrificed and the peripheral blood, peritoneal wash fluid and spleen cells were collected for human T cells and CAR-T cells detect using flow cytometry. The left panel showed the unstained control and human CD3 (hCD3) expression in peripheral blood, peritoneal wash fluid and spleen cells respectively. The right panel showed the CAR expression ratio in the human CD3 cells of peritoneal wash fluid and spleen cells.