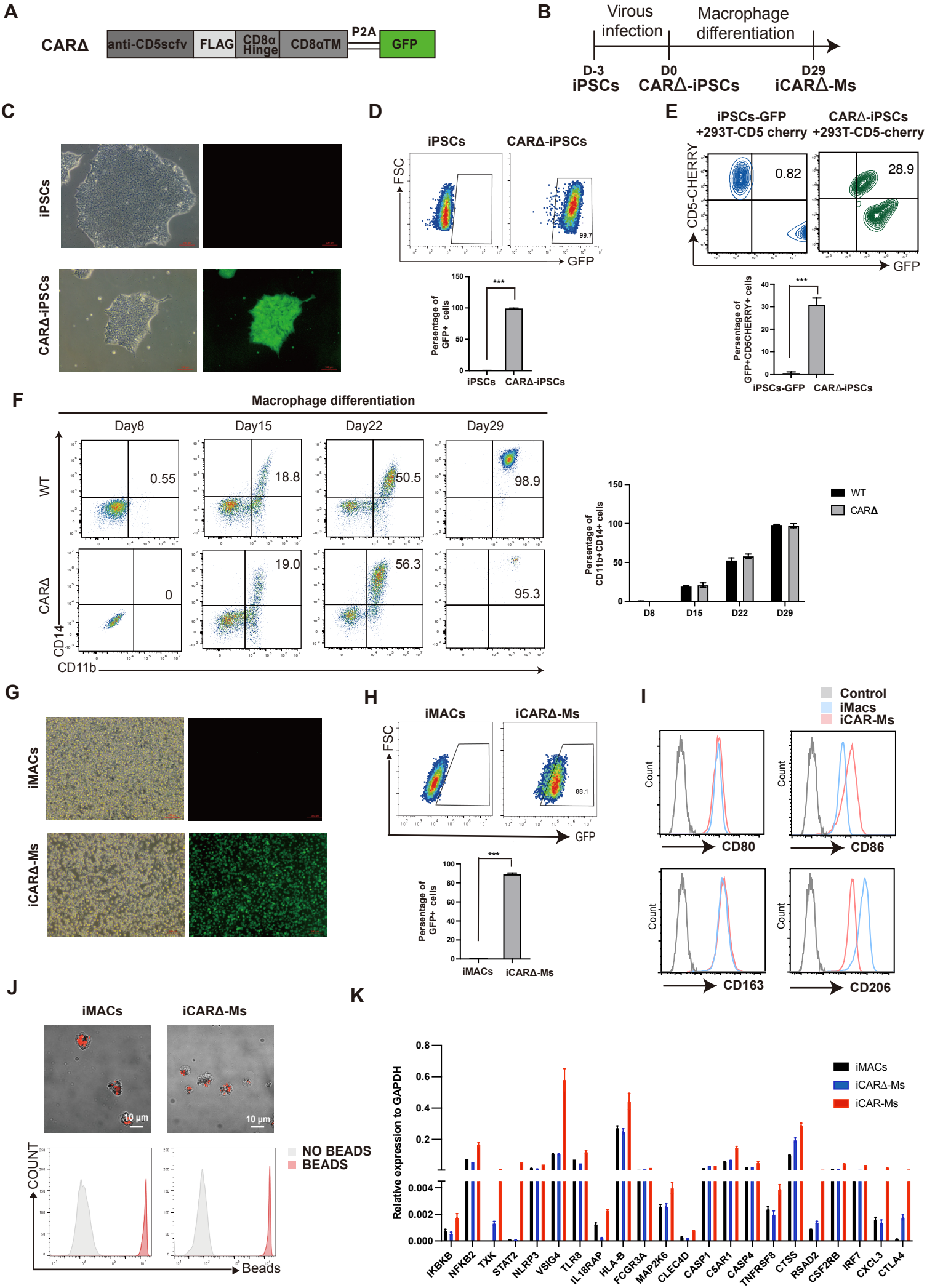

A
CARΔ
anti-CD5scfv
FLAG
CD8α Hinge
CD8αTM
P2A
GFP
B
Virous infection
Macrophage differentiation
D-3 iPSCs
D0 CARΔ-iPSCs
D29 iCARΔ-Ms
C
iPSCs
CARΔ-iPSCs
D
iPSCs
CARΔ-iPSCs
FSC
GFP
99.7
Persentage of GFP+ cells
***
iPSCs
CARΔ-iPSCs
E
iPSCs-GFP +293T-CD5 cherry
CARΔ-iPSCs +293T-CD5-cherry
CD5-CHERRY
GFP
0.82
28.9
Persentage of GFP+CD5CHERRY+ cells
***
iPSCs-GFP
CARΔ-iPSCs
F
Macrophage differentiation
Day8
Day15
Day22
Day29
WT
CARΔ
CD14
CD11b
0.55
18.8
50.5
98.9
0
19.0
56.3
95.3
Persentage of CD11b+CD14+ cells
WT
CARΔ
D8
D15
D22
D29
G
iMACs
iCARΔ-Ms
H
iMACs
iCARΔ-Ms
FSC
GFP
88.1
Persentage of GFP+ cells
***
iMACs
iCARΔ-Ms
I
Control
iMacs
iCAR-Ms
Count
CD80
CD86
CD163
CD206
J
iMACs
iCARΔ-Ms
10 μm
COUNT
Beads
NO BEADS
BEADS
K
Relative expression to GAPDH
iMACs
iCARΔ-Ms
iCAR-Ms
IKBKB
NFKB2
TXK
STAT2
NLRP3
VSIG4
TLR8
IL18RAP
HLA-B
FCGR3A
MAP2K6
CLEC4D
CASP1
C5AR1
CASP4
TNFRSF8
CTSS
RSAD2
CSF2RB
IRF7
CXCL3
CTLA4

Figure S1. Generation of human iCARΔ-Ms targeting CD5. (A)Schematic representation of CARΔ constructs with CD5-targeting. (B) Schematic strategy for generating anti-CD5 CARΔ-Macrophage from human iPSCs. (C) The morphology and green fluorescent protein expression on indicated cells, Scale bar: 100μm. (D) FACS analysis of the iPSCs and GFP $^{+}$CARΔ-iPSCs. Statistics was determined using unpaired two-tailed Student's t-tests, ***$p < 0.001$. These data represent mean ± SD from three independent replicates (n = 3). (E) FACS analysis of the GFP $^{+}$CARΔ-iPSCs and CD5cherry $^{+}$293T-CD5cherry in coculture for 24h. Statistics was determined using unpaired two-tailed Student's t-tests, ***$p < 0.001$. These data represent mean ± SD from three independent replicates (n = 3). (F) FACS analysis of the indicated markers during macrophage differentiation. These data represent mean ± SD from three independent replicates (n=3). (G) The morphology and green fluorescent protein expression of indicated cells, Scale bar: 100μm. (H) FACS analysis the indicated macrophage markers in mature iMACs and iCARΔ-Ms. Undifferentiated human iPSCs serve as control. Statistics was determined using unpaired two-tailed Student's t-tests, ***$p < 0.001$. These data represent mean ± SD from three independent replicates (n = 3). (J) Engulf of red fluorescent latex labeled beads by iMACs and iCARΔ-Ms phagocytosis examined by confocal microscopy (left) and FACS. Scale bar: 10μm. **(K)** RT-qPCR analysis of the indicated gene expression of the iMACs, iCARΔ-Ms and iCAR-Ms cells. These data represent mean ± SD from three independent replicates (n = 3).

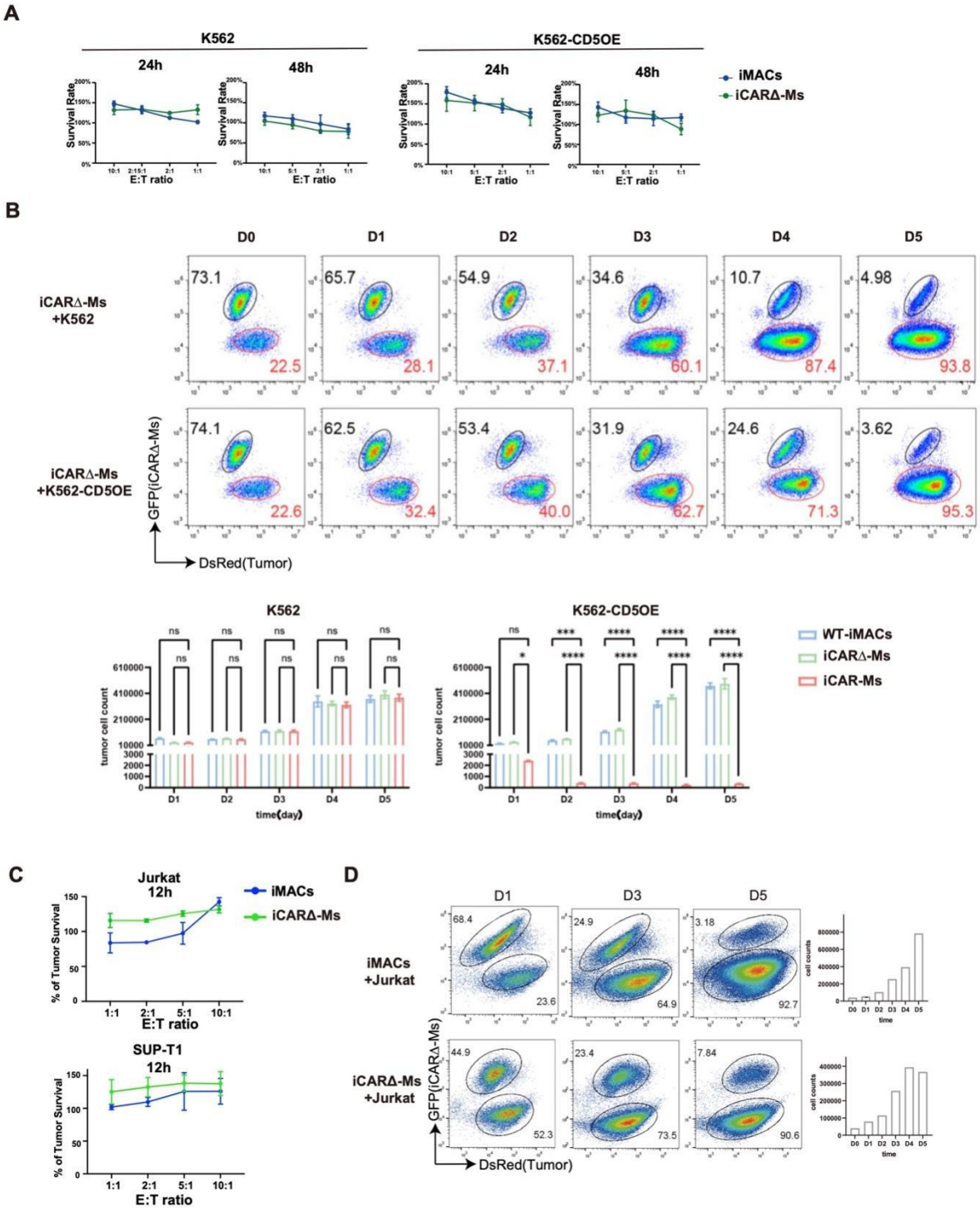



Figure S2. (A) Killing assay of indicated tumor by iCARΔ-Ms or iMACs at indicated E:T ratios. These quantitative data represent mean ± SD from three independent replicates (n=3). (B) FACS analysis of DsRed+ WT K562 or K562-CD5OE cells with iCARΔ-Ms(top). The remaining tumor cells were counted at indicated time point during co-culture(bottom). Statistics was determined using unpaired two-tailed Student's t-tests. These data represent mean ± SD from three independent replicates (n = 3). (C) Killing assay of indicated tumor by iCARΔ-Ms or iMACs after 12h of co-culture at indicated E:T ratios (bottom). These quantitative data represent mean ± SD from three independent

replicates (n=3). **(D)** FACS analysis of DsRed$^{+}$ WT K562 or K562-CD5OE cells with iCARΔ-Ms. The remaining tumor cells were counted at indicated time point during co-culture.

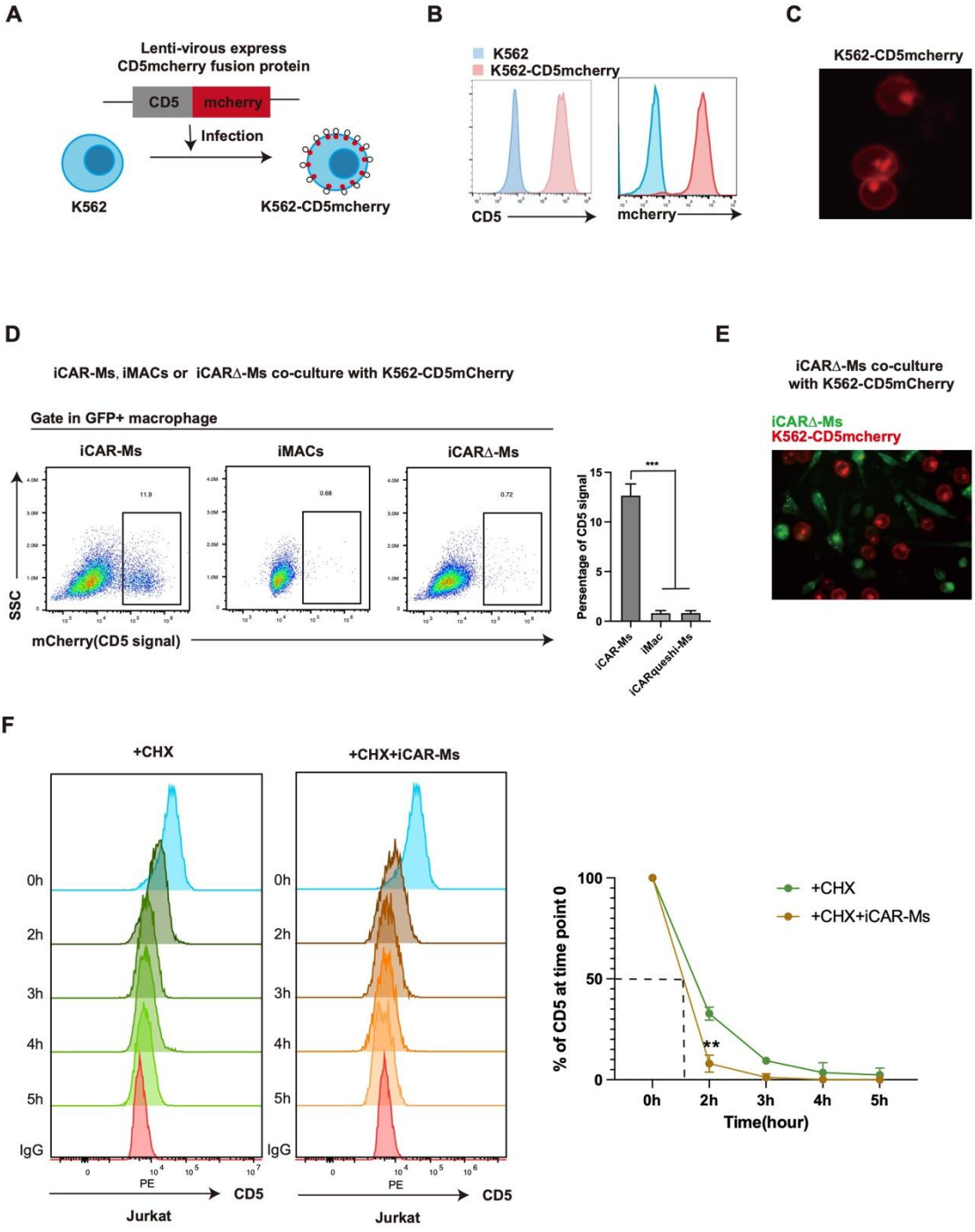
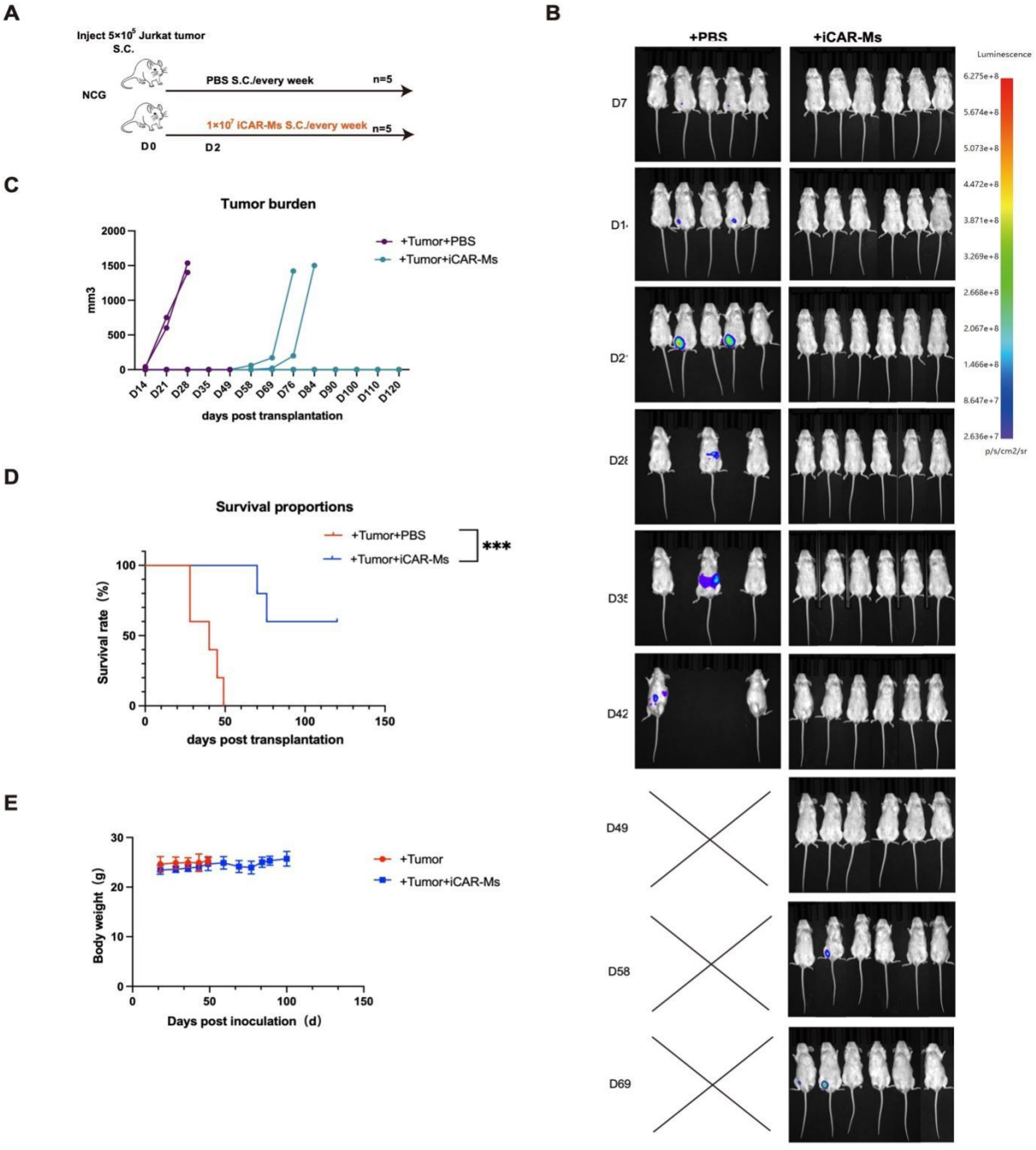



**Figure S3. (A)** Generation of K562 cells expressing CD5-mcherry fusion protein. Schematic representation of experimental design. **(B)** FACS analysis of CD5 and mcherry expression on K562-CD5mcherry, WT K562 as control. **(C)** Confocal analysis of mcherry expression on K562-CD5mcherry, Red, mcherry. **(D)** FACS analysis of $mCherry^{+}$ CD5 of iCAR-Ms, iMACs and iCARΔ-Ms after co-culture with K562-CD5mCherry. **(E)**

Fluorescence microscopy images of iCARΔ-Ms and K562-CD5mcherry after co-culture 3h. Green: iCARΔ-Ms, Red: K562-CD5mcherry. Scale bar: 20μm. **(F)** Representative FACS analysis of CD5 and quantification of CD5 signal in Jurkat cells treated with 100 mg/mL cycloheximide (CHX) in the presence or absence of iCAR-Ms for the indicated times.




## Figure S4.

**(A)**Experimental design: NCG mice were s.c. injected with luciferase_SUP-T1 and treated with s.c. PBS or iCAR-Ms as shown. **(B)** Imaging of luciferase-expressing SUP-T1 tumor-bearing mice. Tumor burden was measured by bioluminescence (total flux) at

indicated time. **(C)**Tumor burden monitored by measuring tumor volumes over 120 days. Each line represents 1 mouse. **(D)** Kaplan–Meier survival curve for animals injected with Jurkat tumor cells and indicated treatments. Statistical significance was calculated using the log-rank Mantel–Cox test. **(E)** Mouse weight monitoring. Data are represented as mean ± SD.

**Movie S1**. iCAR-M mediate trogocytosis on target tumor cells. iCAR-Ms stripped and internalized the cell-surface CD5-mCherry protein in co-culture. Green, iCAR-Ms; Red, K562-CD5mcherry. Scale bar: 10μm

**Movie S2.** iCAR-M mediate phagocytosis on target tumor cells. Green, iCAR-Ms; Red, K562-CD5mcherry. Scale bar: 10μm

**Movie S2**. iMACs has no interaction with tumor cells. iMACs(GFP overexpressing) co-culture with K562-CD5mcherry. Green, iMACs; Red, K562-CD5mcherry. Scale bar: 10μm

Supplemental Table 1 List of Chemicals and Antibodies

| REAGENT | SOURCE | IDENTIFIER |
|---|---|---|
| Antibodies | | |
| anti-CD3ζ antibody | Abcam | Cat# ab243874 |
| Goat anti rabbit IgG HRP | Abcam | Cat# ab6721 |
| HRP-conjugated GAPDH Monoclonal antibody | Proteintech | Cat# HRP-60004 |
| Mouse Anti-Human CD5 APC | Elabscience | Cat# E-AB-F1041E |
| Mouse Anti-Human CD5 PE | BD Biosciences | Cat# 561897 |
| Mouse Anti-Human CD11b APC-Cy7 | BD Biosciences | Cat# 560914 |
| Mouse Anti-Human CD14 PE | BD Biosciences | Cat# 561707 |
| Mouse Anti-Human CD80 APC | BD Biosciences | Cat# 565157 |
| Mouse Anti-HumanCD163 PE | BD Biosciences | Cat# 560933 |
| Mouse Anti-HumanCD206 BV421 | BD Biosciences | Cat# 566281 |
| Mouse Anti-HumanCD86 APC | BD Biosciences | Cat# 560956 |
| Chemicals and Recombinant Proteins | | |
| DMEM/F12 | Thermo Fisher Scientific | Cat# 11330-032 |
| mTeSR1 | Stem Cell Technologies | Cat# 85850 |
| StemPro34 | Thermo Fisher Scientific | Cat# 10639011 |
| Matrigel | Corning | Cat# 354230 |
| Penicillin/Streptomycin | Hyclone | Cat# SV30010 |
| Y-27632 | Selleck | Cat# S1049 |
| TRIzol | MRC | Cat# TR118-500 |
| DPBS | Thermo Fisher Scientific | Cat# 14190-144 |
| FBS | ExCell Bio | Cat# FSD500 |
| EDTA | Thermo Fisher Scientific | Cat# AM9262 |
| Accutase | Sigma | Cat# A6964 |
| RPMI-1640 | Thermo Fisher Scientific | Cat# C11875500BT |
| thiazovivin | Selleck | Cat# S1459 |
| human BMP4 | Peprotech | Cat# 120-05ET |
| human ACTIVIN A | Peprotech | Cat# 120-14P-250 |
| human bFGF | Sino Biological | Cat# 10014-HNAE |
| human VEGF | Sino Biological | Cat# 10008-HNAB-100 |
| human SCF | Peprotech | Cat# 300-07-100 |
| human thrombopoietin | Sino Biological | Cat# 13194-H08B-100 |
| human IL-3 | Sino Biological | Cat# 11858-HNAE-100 |
| human IL-6 | Sino Biological | Cat# 10395-HNAE-100 |
| human FLT3L | Peprotech | Cat# 300-19-100 |
| human M-CSF | Sino Biological | Cat# 11792-HNAH-20 |
| human GM-CSF | Sino Biological | Cat# 10015-HNAH-20 |
| LPS | InvivoGen | Cat# tlrl-peklps |
| human IFN-γ | Sino Biological | Cat# 11725-HNAS-20 |
| human IL-4 | Sino Biological | Cat# 11846-HNAE-5 |

Supplemental Table 2. Sequence of primers used for quantitative PCR

| Gene | Forward primer | Reverse primer |
|---|---|---|
| *IKBKB* | GGAAGTACCTGAACCAGTTTGAG | GCAGGACGATGTTTTCTGGCT |
| *NFKB2* | ATGGAGAGTTGCTACAACCCA | CTGTTCCACGATCACCAGGTA |
| *TXK* | CATCCAGTCGGTTTTCTGTTGC | TGCGACGCTGGGTGTATTTT |
| *STAT2* | CCAGCTTTACTCGCACAGC | AGCCTTGGAATCATCACTCCC |
| *NLRP3* | GATCTTCGCTGCGATCAACAG | CGTGCATTATCTGAACCCCAC |
| *VSIG4* | GGGGCACCTAACAGTGGAC | GTCTGAGCCACGTTGTACCAG |
| *TLR8* | ATGTTCCTTCAGTCGTCAATGC | TTGCTGCACTCTGCAATAACT |
| *IL18RAP* | ATGCTCTGTTTGGGCTGGATA | GTGAGAGTCGATTTCTGTGGC |
| *HLA-B* | CAGTTCGTGAGGTTCGACAG | CAGCCGTACATGCTCTGGA |
| *FCGR3A* | CCTCCTGTCTAGTCGGTTTGG | TCGAGCACCCTGTACCATTGA |
| *MAP2K6* | GAAGCATTTGAACAACCTCAGAC | CCTGGCTATTTACTGTGGCTC |
| *CLEC4D* | CTGATACCTTCGGTTATTGCTGT | GCACTCCTGTGCCTCTCTTAC |
| *CASP1* | TTTCCGCAAGGTTCGATTTTCA | GGCATCTGCGCTCTACCATC |
| *C5AR1* | TCCTTCAATTATACCACCCCTGA | ACGCAGCGTGTTAGAAGTTTTAT |
| *CASP4* | CAAGAGAAGCAACGTATGGCA | AGGCAGATGGTCAAACTCTGTA |
| *TNFRSF8* | TCCACGGAGCACACCAATAAC | ACTGAGAGCATGACATCGCTG |
| *CTSS* | AAACGGCTGGTTTGTGTGC | CAGTGGTGATCCAGGGTAGG |
| *RSAD2* | TGGGTGCTTACACCTGCTG | GAAGTGATAGTTGACGCTGGTT |
| *CSF2RB* | AGCGGCTTCAGGACTCTTG | CTGGGCATGAGGTGCTCTG |
| *IRF7* | GCTGGACGTGACCATCATGTA | GGGCCGTATAGGAACGTGC |
| *CXCL3* | CGCCCAAACCGAAGTCATAG | GCTCCCCTTGTTCAGTATCTTTT |
| *CTLA4* | GCCCTGCACTCTCCTGTTTTT | GGTTGCCGCACAGACTTCA |