Figure S1. Deletion of AGK in macrophages leads to enhanced anti-tumor activity

(A, B) qRT-PCR and immunoblotting analysis of the expression of AGK in $Agk^{fl/fl}$ or Agk^{cKO} macrophages (n = 3 per group). (**C**) H&E and TUNEL, Ki67 staining of tumor tissues from LLC-bearing $Agk^{fl/fl}$ and Agk^{cKO} mice. (**D**) CD31 (Red) /α-SMA (Green) double immunostaining of tumor tissues from LLC-bearing $Agk^{fl/fl}$ and Agk^{cKO} mice. (**E-F**) The percentages of M1 ($CD11C^{+}CD206^{-}$) or M2 ($CD11C^{-}CD206^{+}$) among gated $F4/80^{+}CD11b^{+}$ macrophages in tumor tissues from LLC-bearing $Agk^{fl/fl}$ and Agk^{cKO} mice were determined and quantified by flow cytometry (n = 5). Data were shown as mean ±SEM and were analyzed by unpaired two-tailed t-test (A, D, F). Data in (A-F) are representative of three independent experiments; ns, no significance; *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure S2. Deficiency of AGK in macrophages enhances T cell anti-tumor response

(**A**) Flow gating strategy for cytokines and cell surface staining in $CD8^{+}$ T cells in $Agk^{fl/fl}$ or Agk^{cKO} B16-OVA melanoma-bearing mice. (**B, C**) Representative percentages and immunofluorescence staining of $CD4^{+}$ T cell and $CD8^{+}$ T cell in LLC tumor tissues from $Agk^{fl/fl}$ and Agk^{cKO} tumor mice (n ≥ 5 per group). (**D**) Representative plots and percentages of $PD1^{+}Tim3^{+}CD8^{+}$ T cells in the tumor tissues from $Agk^{fl/fl}$ and Agk^{cKO} LLC-bearing mice (n ≥ 4 per group). (**E**) Representative plots and percentages of $CD4^{+}$ $Foxp3^{+}$ T cells population. (**F**) Representative plots of $CD4^{+}$ T cells and $CD8^{+}$ T cells isolated from LLC tissues of $Agk^{fl/fl}$ and Agk^{cKO} mice treated with PBS or anti-CD4/8 antibody (n ≥ 3 per group). Data were means ± SEM and were analyzed by two-tailed, unpaired Student's *t*-test (B-E). Data were representative of one (E, F) or three (B, D) experiments. ns, no significance; * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$.

Figure S3. AGK deficiency in macrophage enhances $CD8^{+}$ T cell stemness

(**A, B**) Representative plots and percentages of $PD1^{+}Tcf1^{+}Tim3^{-}CD8^{+}$ T cells population determined by flow cytometry in LLC-bearing $Agk^{fl/fl}$ and Agk^{cKO} mice (n = 6 per group). Data were shown as means ± SEM and were analyzed by two-tailed, unpaired Student's *t*-test (B). Data were representative of two (A, B) experiments. ns, no significance; * $p < 0.05$; ** $p < 0.01$,*** $p < 0.001$.

Figure S4. Macrophage AGK deficiency promotes cGAS-STING signaling pathway and type I IFN response

(A-F) The expressions of Type I IFN related genes were determined by RT-PCR in $Agk^{fl/fl}$ and Agk^{cKO} BMDMs upon treatment with B16-TCM (B16 CM:DMEM = 0：1; 1：5; 1：3) for 6 h. (**G**) Immunoblot analysis of the amounts of (AGK, cGAS, p-Tbk1, Tbk1, p-IRF3, IRF3, p-P65, P65 and STING) in $Agk^{fl/fl}$ and Agk^{cKO} BMDMs that were treated with B16F10-TCM (LLC CM:DMEM = 1：3) for 0, 30, 60, 120 and 180 min. Data were means ± SEM and were analyzed by and two-way ANOVA (A-F). Data were representative of two (G) or three (A-F) experiments. ns, no significance; * $p < 0.05$;** $p < 0.01$; *** $p < 0.001$.

Figure S5. AGK-deficient macrophages promote the anti-tumor effects by inducing mitochondrial ROS and enhancing cGAS-STING-type I IFN response

(**A**) 5×10^5 LLC cells were subcutaneously injected into $Agk^{fl/fl}$ and Agk^{cKO} mice that were treated with PBS or anti-IFNAR antibody i.p 250 μg at day 2, 4, 7 and 11 and the tumor volumes were measured at the indicated times (n = 5 per group). Flow cytometric analysis and the percentages of $CD107a^{+}CD8^{+}$ T cells in four groups were quantified.

(**B**) *Agk*$^{fl/fl}$ or *Agk*^{cKO} BMDMs were cultured with or without Mito-TEMPO (500μM) for 24 h and MitoSOX levels were determined by flow cytometry. Data were means ± SEM and were analyzed by two-tailed, unpaired Student's *t*-test (A) or two-way ANOVA (B). Data were representative of one (A) or three (B) experiments. ns, no significance; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure S1

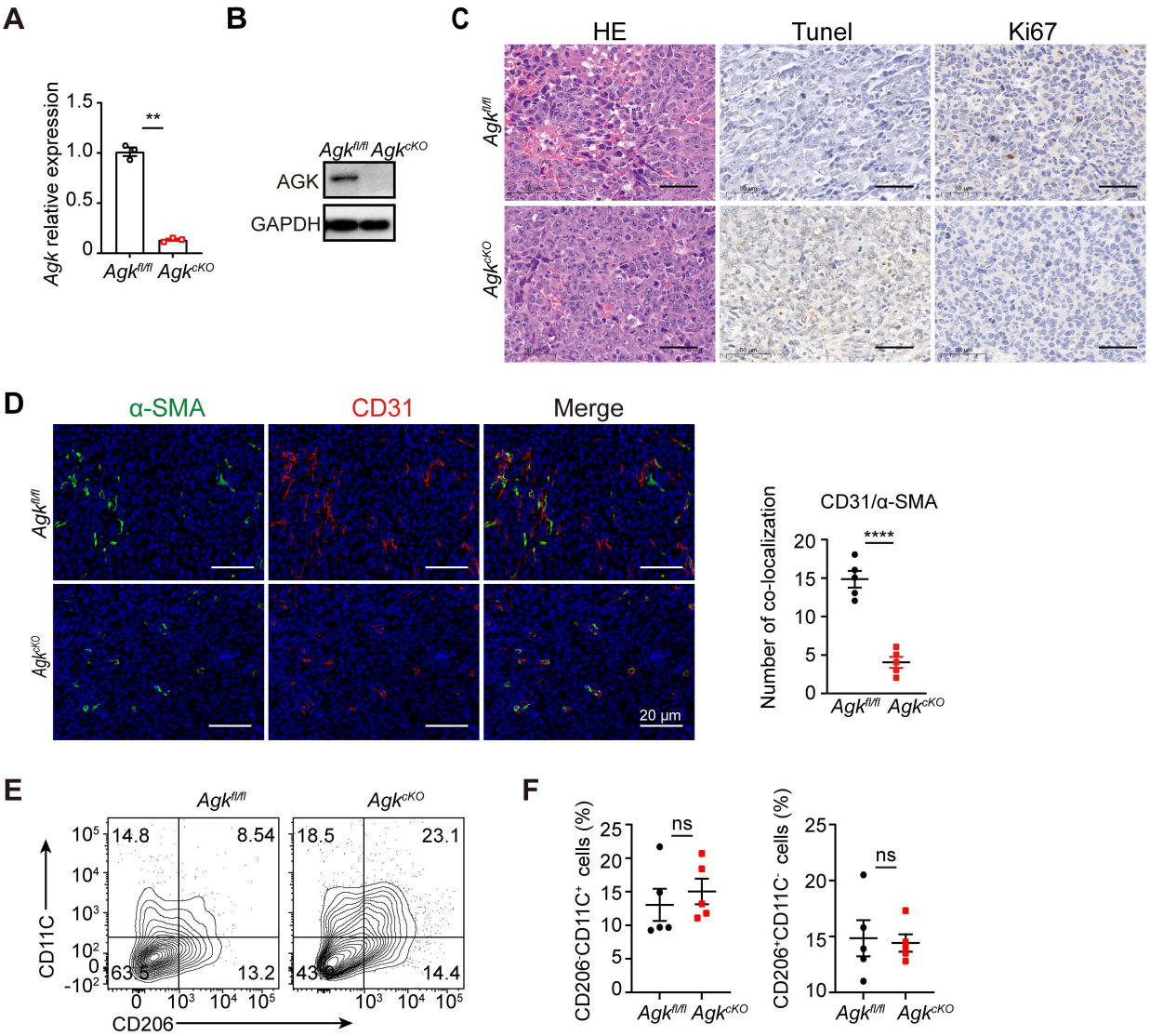
A
Agk relative expression
1.5
1.0
0.5
0
**
Agkfl/fl
AgkcKO
B
Agkfl/fl AgkcKO
AGK
GAPDH
C
HE
Tunel
Ki67
Agkfl/fl
AgkcKO
D
α-SMA
CD31
Merge
Agkfl/fl
AgkcKO
20 μm
CD31/α-SMA
Number of co-localization
****
20
15
10
5
0
Agkfl/fl
AgkcKO
E
Agkfl/fl
AgkcKO
CD11C
CD206
14.8
8.54
63.5
13.2
18.5
23.1
43.1
14.4
F
CD206-CD11C+ cells (%)
ns
25
20
15
10
5
0
Agkfl/fl
AgkcKO
CD206+CD11C- cells (%)
ns
25
20
15
10
Agkfl/fl
AgkcKO

Figure S2

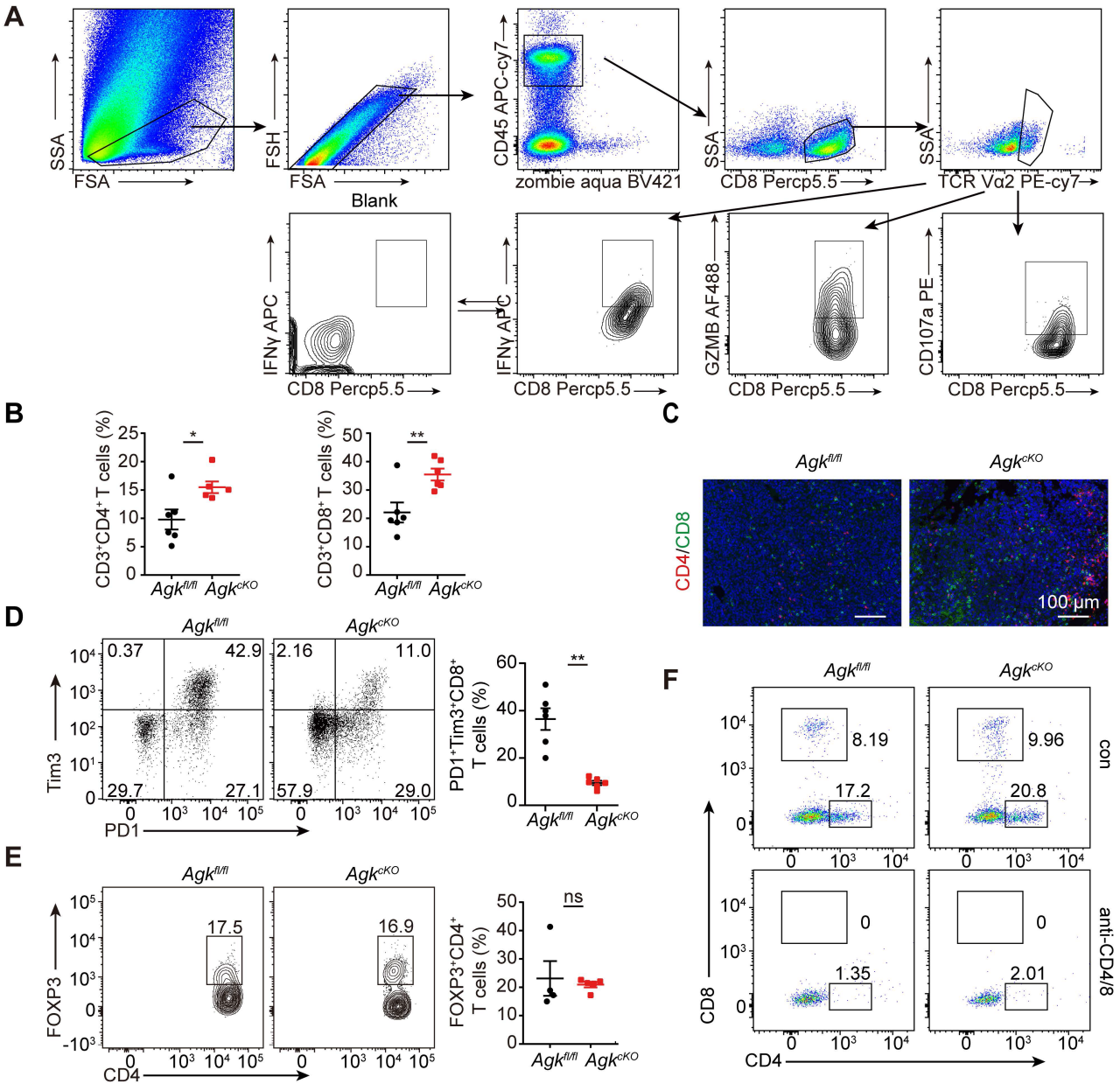
A
SSA
FSA
FSH
FSA
Blank
CD45 APC-cy7
zombie aqua BV421
SSA
CD8 Percp5.5
SSA
TCR Vα2 PE-cy7
IFNγ APC
CD8 Percp5.5
IFNγ APC
CD8 Percp5.5
GZMB AF488
CD8 Percp5.5
CD107a PE
CD8 Percp5.5
B
CD3+CD4+ T cells (%)
*
Agkfl/fl
AgkcKO
CD3+CD8+ T cells (%)
**
Agkfl/fl
AgkcKO
C
Agkfl/fl
AgkcKO
CD4/CD8
100 μm
D
Agkfl/fl
AgkcKO
0.37
42.9
2.16
11.0
29.7
27.1
57.9
29.0
Tim3
PD1
PD1+Tim3+CD8+ T cells (%)
**
Agkfl/fl
AgkcKO
E
Agkfl/fl
AgkcKO
17.5
16.9
FOXP3
CD4
FOXP3+CD4+ T cells (%)
ns
Agkfl/fl
AgkcKO
F
Agkfl/fl
AgkcKO
8.19
9.96
con
17.2
20.8
0
0
anti-CD4/8
1.35
2.01
CD8
CD4

Figure S3

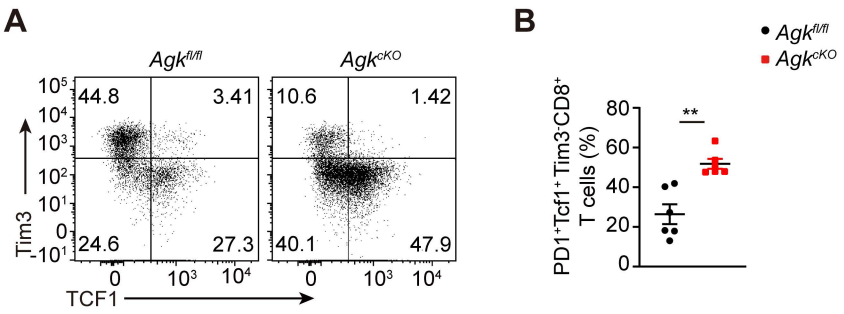
A
Agkfl/fl
AgkcKO
44.8
3.41
24.6
27.3
10.6
1.42
40.1
47.9
Tim3
TCF1
B
Agkfl/fl
AgkcKO
**
PD1+Tcf1+ Tim3-CD8+ T cells (%)

Figure S4

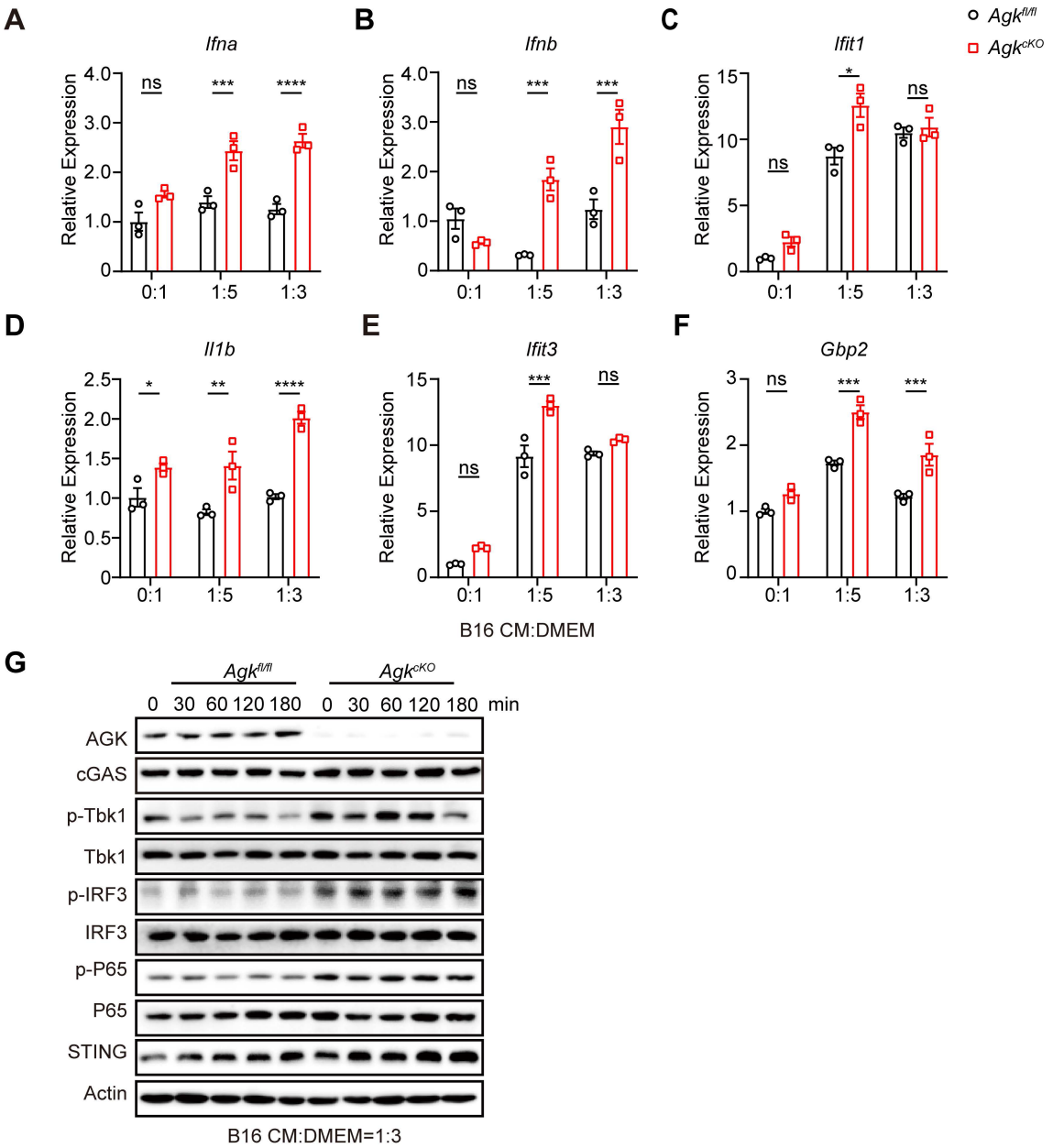
A
Ifna
B
Ifnb
C
Ifit1
Agkfl/fl
AgkcKO
Relative Expression
ns
***
****
*
0:1
1:5
1:3
D
Il1b
**
E
Ifit3
F
Gbp2
B16 CM:DMEM
G
Agkfl/fl
AgkcKO
0 30 60 120 180 0 30 60 120 180 min
AGK
cGAS
p-Tbk1
Tbk1
p-IRF3
IRF3
p-P65
P65
STING
Actin
B16 CM:DMEM=1:3

Figure S5

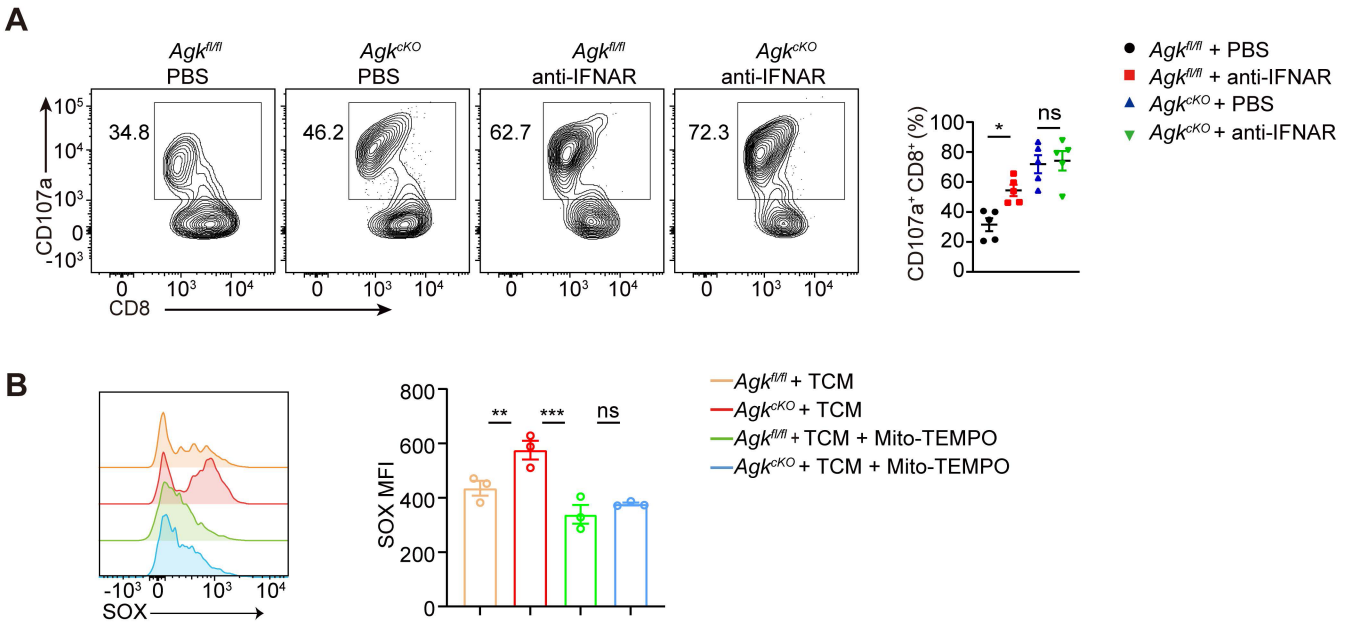
A
Agkfl/fl PBS
AgkcKO PBS
Agkfl/fl anti-IFNAR
AgkcKO anti-IFNAR
34.8
46.2
62.7
72.3
CD107a
CD8
Agkfl/fl + PBS
Agkfl/fl + anti-IFNAR
AgkcKO + PBS
AgkcKO + anti-IFNAR
CD107a+ CD8+ (%)
*
ns
B
SOX
SOX MFI
**
***
ns
Agkfl/fl + TCM
AgkcKO + TCM
Agkfl/fl + TCM + Mito-TEMPO
AgkcKO + TCM + Mito-TEMPO

Table S1. Oligonucleotide primers for qPCR

Target	Sequence
Mouse-Agk (F)	TTTGAGTCATACCCTCTTTGCC
Mouse-Agk (R)	TCAAGTGGAACTGTCTCTCCTT
Mouse-Ifna (F)	GCTTTCCTGATGGTTTTGGTG
Mouse-Ifna (R)	AGGCTTTCTTGTTCCTGAGG
Mouse-Ifnb (F)	CCAGCTCCAAGAAAGGACGA
Mouse-Ifnb (R)	TGGATGGCAAAGGCAGTGTA
Mouse-Ifit1 (F)	AGAGTCAAGGCAGGTTTCTG
Mouse-Ifit1 (R)	TGTGAAGTGACATCTCAGCTG
Mouse-Il1b (F)	GCAACTGTTCCTGAACTCAACT
Mouse-Il1b (R)	ATCTTTTGGGGTCCGTCAACT
Mouse-Ifit3 (F)	CTGAAGGGGAGCGATTGATT
Mouse-Ifit3 (R)	AACGGCACATGACCAAAGAGTAGA
Mouse-Gbp2 (F)	CTGCACTATGTGACGGAGCTA
Mouse-Gbp2 (R)	GAGTCCACACAAAGGTTGGAAA
Mouse-mtDNA Dloop1 (F)	AATCTACCATCCTCCGTGAAACC
Mouse-mtDNA Dloop1 (R)	TCAGTTTAGCTACCCCCAAGTTTAA
Mouse-mtDNA Dloop2 (F)	CCCTTCCCCATTTGGTCT
Mouse-mtDNA Dloop2 (R)	TGGTTTCACGGAGGATGG
Mouse-mtDNA 16S (F)	CACTGCCTGCCCAGTGA
Mouse-mtDNA 16S (R)	ATACCGCGGCCGTTAAA
Mouse-mtDNA ND4 (F)	AACGGATCCACAGCCGTA
Mouse-mtDNA ND4 (R)	AGTCCTCGGGCCATGATT