Supplementary Figure Legends

fig. S1. Analysis of Concanavalin A-induced acute hepatitis model in IPMK$^{f/f}$ and IPMK$^{\Delta CD4}$ mice.

(**A**) Blood ALT levels of sacrificed mice; blood sample was acquired from the retro-orbital vein (with or without ConA injection).

(**B**) Blood AST levels of sacrificed mice; blood sample was acquired from the retro-orbital vein (with or without ConA injection).

(**C**) Flow cytometry analysis of IFNγ produced liver MNCs in IPMK$^{f/f}$ and IPMK$^{\Delta CD4}$ ConA-injected mice.

(**D**) The counts of CD4$^+$ cells in liver MNCs per mouse weight (x10^6/g) in IPMK$^{f/f}$ and IPMK$^{\Delta CD4}$ ConA-injected mice.

(**E**) Flow cytometry analysis of IFNγ in CD4$^+$ cells in liver MNCs in IPMK$^{f/f}$ and IPMK$^{\Delta CD4}$ ConA-injected mice.

(**F**) Flow cytometry analysis of IL-4 in CD4$^+$ cells in liver MNCs in IPMK$^{f/f}$ and IPMK$^{\Delta CD4}$ ConA-injected mice.

(**G**) Flow cytometry analysis of IL-17A in CD4$^+$ cells in liver MNCs in IPMK$^{f/f}$ and IPMK$^{\Delta CD4}$ ConA-injected mice.

All flow cytometric plots presented are representative of experiments. The normality of all data was analyzed using the Shapiro-Wilk test. Data are presented as mean ± SD. ns; $*p < 0.05$, using the Mann-Whitney test (**A** and **B**) or unpaired two-tailed Student's *t*-test (**C-G**) compared to IPMK$^{f/f}$.

fig. S2. Analysis of $CD4^+$ helper T cells and NKT cells in mononuclear cells stimulated by Concanavalin A.

(**A**) Flow cytometry analysis of $CD4^+$ helper T cells ($CD4^+$ $TCR\beta^+$ cells) and NKT cells ($CD4^+$ $TCR\beta^-$ cells) in liver MNCs of $IPMK^{f/f}$ and $IPMK^{\Delta CD4}$ PBS/ConA-injected mice. The numbers of $CD4^+$ helper T cells and NKT cells were calculated based on MNC counts per mouse weight ($x10^6$/g).

(**B**) Flow cytometry analysis of IL-17A in $CD4^+$ $TCR\beta^+$ cells in liver MNCs from $IPMK^{f/f}$ and $IPMK^{\Delta CD4}$ PBS/ConA-injected mice.

All flow cytometric plots presented are representative of experiments. The normality of all data was analyzed using the Shapiro-Wilk test. Data are presented as mean ± SD. ns; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$, using the two-way ANOVA with Tukey's multiple comparisons test.

fig. S3. Decreased PLCγ1 activation and T cell proliferation by the loss of IPMK.

(**A**) Immunoblot analysis of PLCγ1 and its phosphorylation (Y783) in lysates of naïve $CD4^+$ T cells stimulated with Concanavalin A for 15 or 30 minutes.

(**B**) Flow cytometry analysis of $CD4^+$ T cell proliferation polarized into Th0 cells for 72 hours, and the percentage of cells per division measured through the CFSE assay.

(**C**) Flow cytometry analysis of the level of CD25 in naïve $CD4^+$ T cells polarized into Th0 cells for 72 hours by anti-IFNγ and anti-IL-4 antibodies.

(**D**) Flow cytometry analysis of the level of CD69 in naïve $CD4^+$ T cells polarized into Th0 cells for 72 hours by anti-IFNγ and anti-IL-4 antibodies.

All blots and flow cytometric plots presented are representative of at least three independent experiments. Densitometric quantitation results were normalized to control conditions. The normality of all data was analyzed using the Shapiro-Wilk test. Data are presented as mean ± SD. ns; $^{**}p < 0.01$; $^{****}p < 0.0001$, using the two-way ANOVA with Šídák's multiple comparisons test (**A** and **B**) or unpaired two-tailed Student's *t*-test (**C** and **D**) compared to $IPMK^{f/f}$.

fig. S4. Decreased interaction between PLCγ1 and Sam68 by the loss of IPMK.

(**A**) Endogenous co-IP test and immunoblot analysis for PLCγ1, Sam68, and IPMK in IPMK KO MEFs. The cell lysates were immunoprecipitated with anti-Sam68 antibodies.

(**B**) Endogenous co-IP test and immunoblot analysis for PLCγ1, Sam68, and IPMK in siIPMK transfected NIH3T3 cells. The cell lysates were immunoprecipitated with anti-Sam68 antibodies.

(**C**) Immunoblot analysis of PLCγ1 and its phosphorylation (Y783) in lysates from CRISPR/Cas9 mediated Sam68 KO Jurkat T cells.

(**D**) Endogenous co-IP test and immunoblot analysis for PLCγ1, LAT, and IPMK in naïve $CD4^+$ T cells. The cell lysate was immunoprecipitated with anti-PLCγ1 antibodies.

(**E**) Endogenous co-IP test and immunoblot analysis for PLCγ1, Sam68, and IPMK in naïve $CD4^+$ T cells stimulated with anti-CD3 and anti-CD28 antibodies for 3 minutes. The cell lysate was immunoprecipitated with anti-PLCγ1 antibodies.

All blots presented are representative of at least three independent experiments. Densitometric quantitation results were normalized to controls. The normality of all data

was analyzed using the Shapiro-Wilk test. Data are presented as mean ± SD. ns; *p < 0.05; **p < 0.01; ***p < 0.001, using the one-sample t-test compared to each control.

fig. S5. Decreased TCR signaling and PLCγ1 Y783 phosphorylation by IPMK-DN.

(**A**) Flow cytometric plots of virus-transduced (GFP^+) $CD4^+$ T cells polarized into Th0 cells for 72 hours.

(**B**) Flow cytometry analysis of the level of CD25 in virus-transduced GFP^+ $CD4^+$ T cells polarized into Th0 cells using anti-IFNγ and anti-IL-4 antibodies.

(**C**) Immunoblot analysis of PLCγ1 and its phosphorylation (Y783) in NIH3T3 cells transfected with GST-tagged IPMK fragments.

All blots and flow cytometric plots presented are representative of at least three independent experiments. Densitometric quantitation results were normalized to controls. The normality of all data was analyzed using the Shapiro-Wilk test. Data are presented as mean ± SD. ns; *p < 0.05; **p < 0.01, using the unpaired two-tailed Student's t-test (**B**) or the one-sample t-test (**C**) compared to each control.

fig. S1

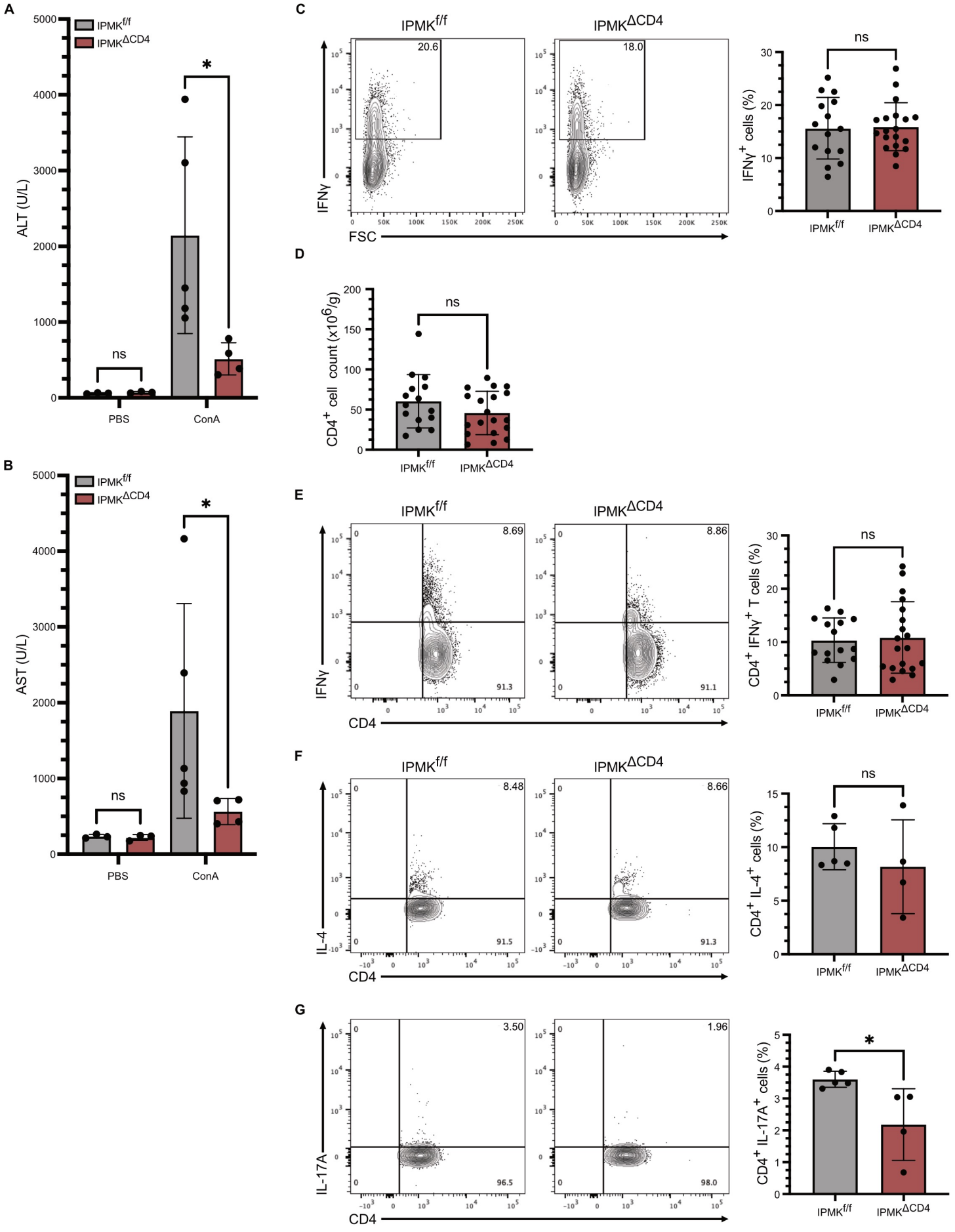

A
IPMK f/f
IPMK ΔCD4
ALT (U/L)
ns
*
PBS
ConA
B
AST (U/L)
C
IFNγ
FSC
20.6
18.0
IFNγ+ cells (%)
D
CD4+ cell count (x10^6/g)
E
CD4
8.69
8.86
91.3
91.1
CD4+ IFNγ+ T cells (%)
F
IL-4
8.48
8.66
91.5
91.3
CD4+ IL-4+ cells (%)
G
IL-17A
3.50
1.96
96.5
98.0
CD4+ IL-17A+ cells (%)

fig. S2

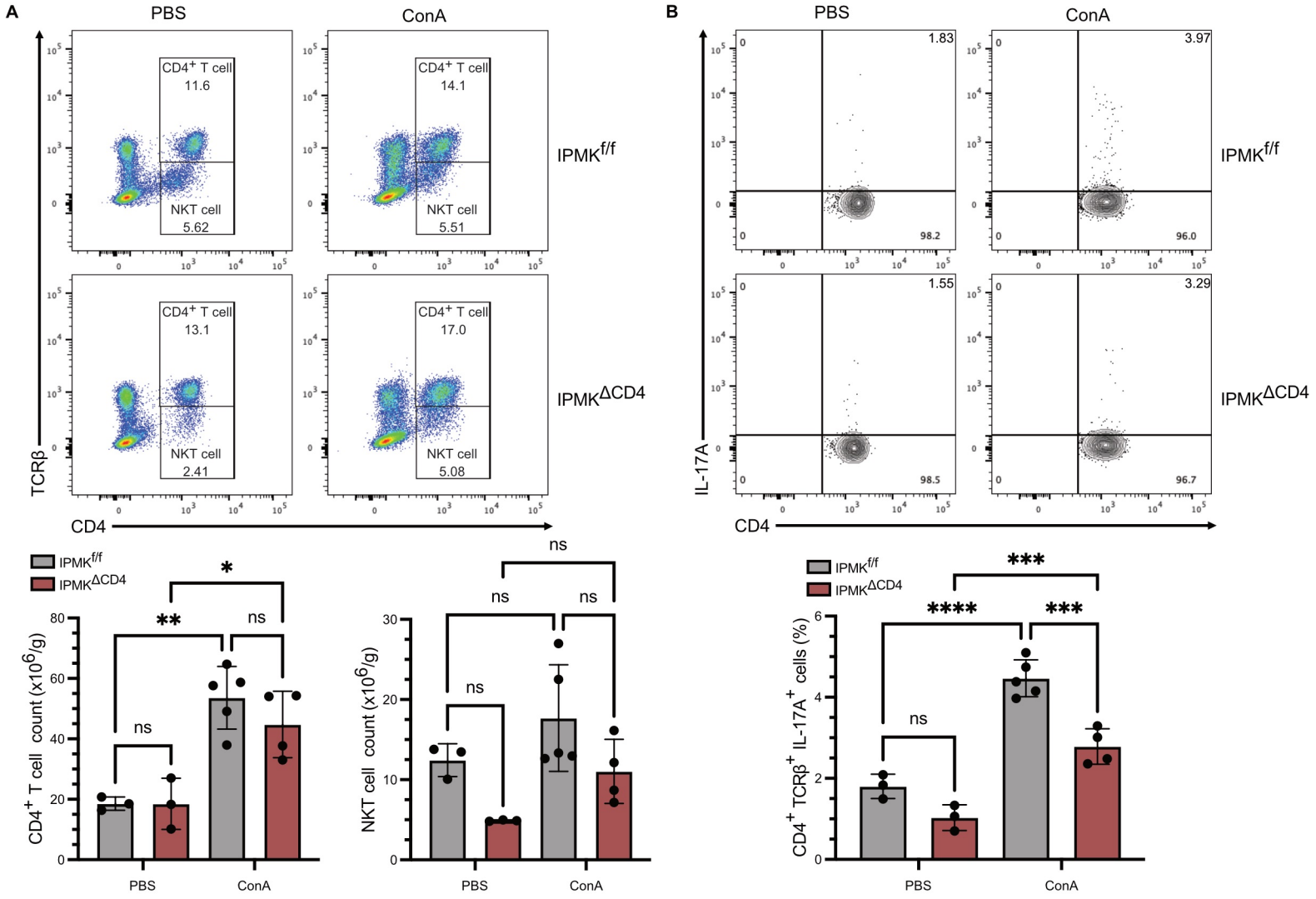

A
PBS
ConA
CD4+ T cell
11.6
NKT cell
5.62
CD4+ T cell
14.1
NKT cell
5.51
IPMKf/f
CD4+ T cell
13.1
NKT cell
2.41
CD4+ T cell
17.0
NKT cell
5.08
IPMKΔCD4
TCRβ
CD4
B
PBS
ConA
1.83
98.2
3.97
96.0
IPMKf/f
1.55
98.5
3.29
96.7
IPMKΔCD4
IL-17A
CD4
IPMKf/f
IPMKΔCD4
CD4+ T cell count (x10^6/g)
NKT cell count (x10^6/g)
CD4+ TCRβ+ IL-17A+ cells (%)
PBS
ConA
ns
*
**
***
****

fig. S3

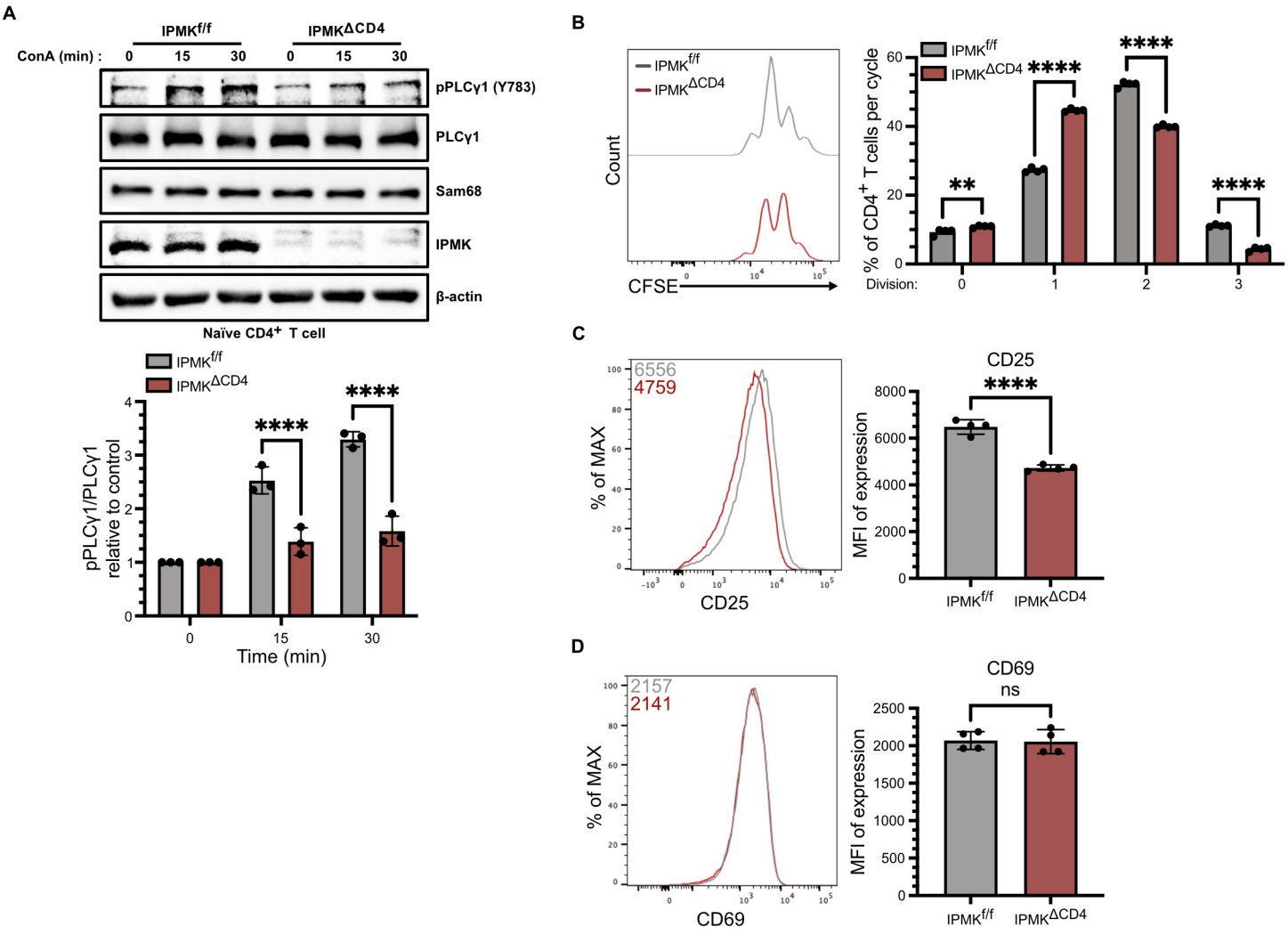

A
IPMKf/f
IPMKΔCD4
ConA (min) : 0 15 30 0 15 30
pPLCγ1 (Y783)
PLCγ1
Sam68
IPMK
β-actin
Naïve CD4+ T cell
IPMKf/f
IPMKΔCD4
pPLCγ1/PLCγ1 relative to control
****
****
Time (min)
B
IPMKf/f
IPMKΔCD4
Count
CFSE
% of CD4+ T cells per cycle
**
****
****
****
Division: 0 1 2 3
C
6556
4759
% of MAX
CD25
CD25
****
MFI of expression
IPMKf/f
IPMKΔCD4
D
2157
2141
% of MAX
CD69
CD69
ns
MFI of expression
IPMKf/f
IPMKΔCD4

fig. S4

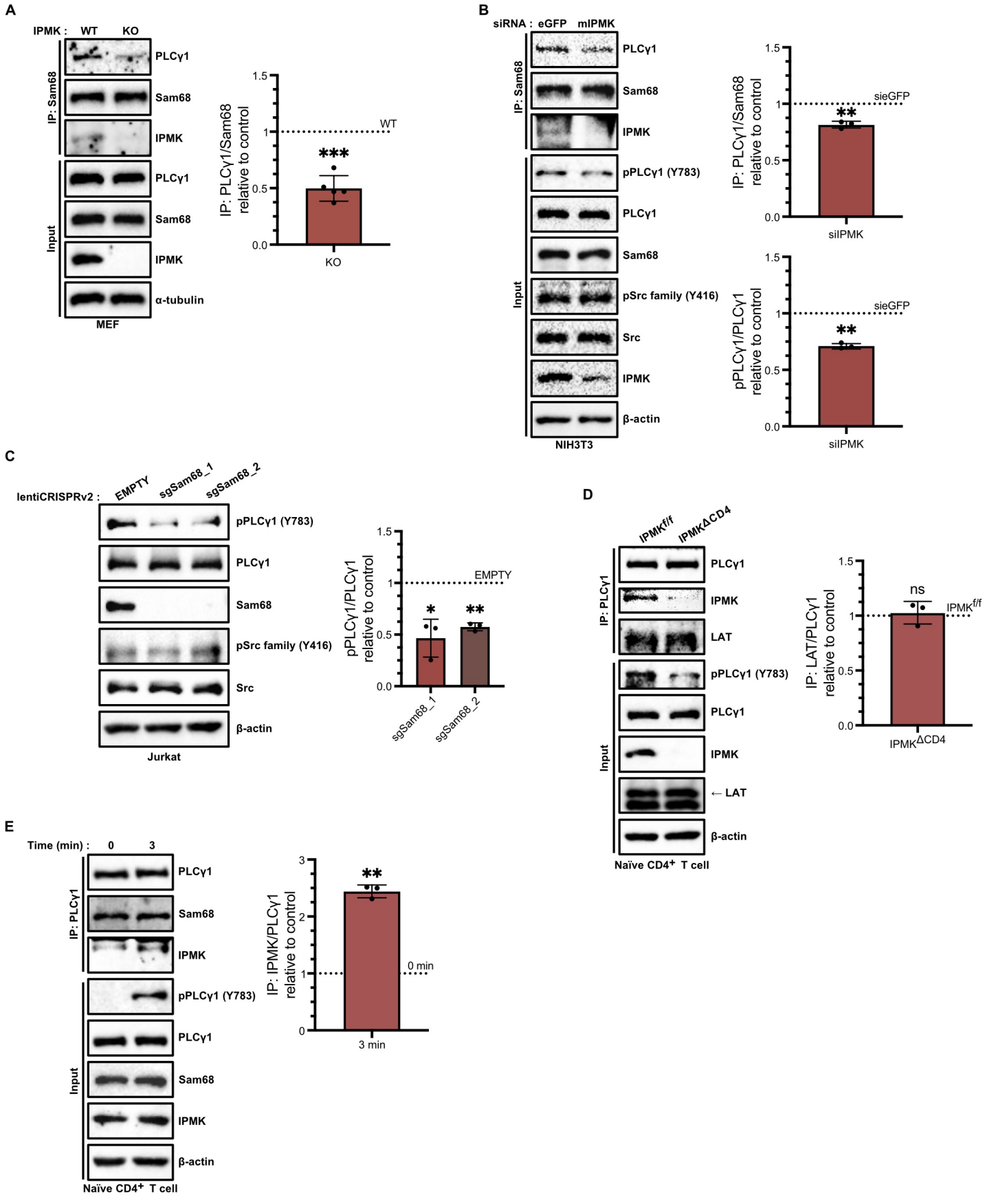
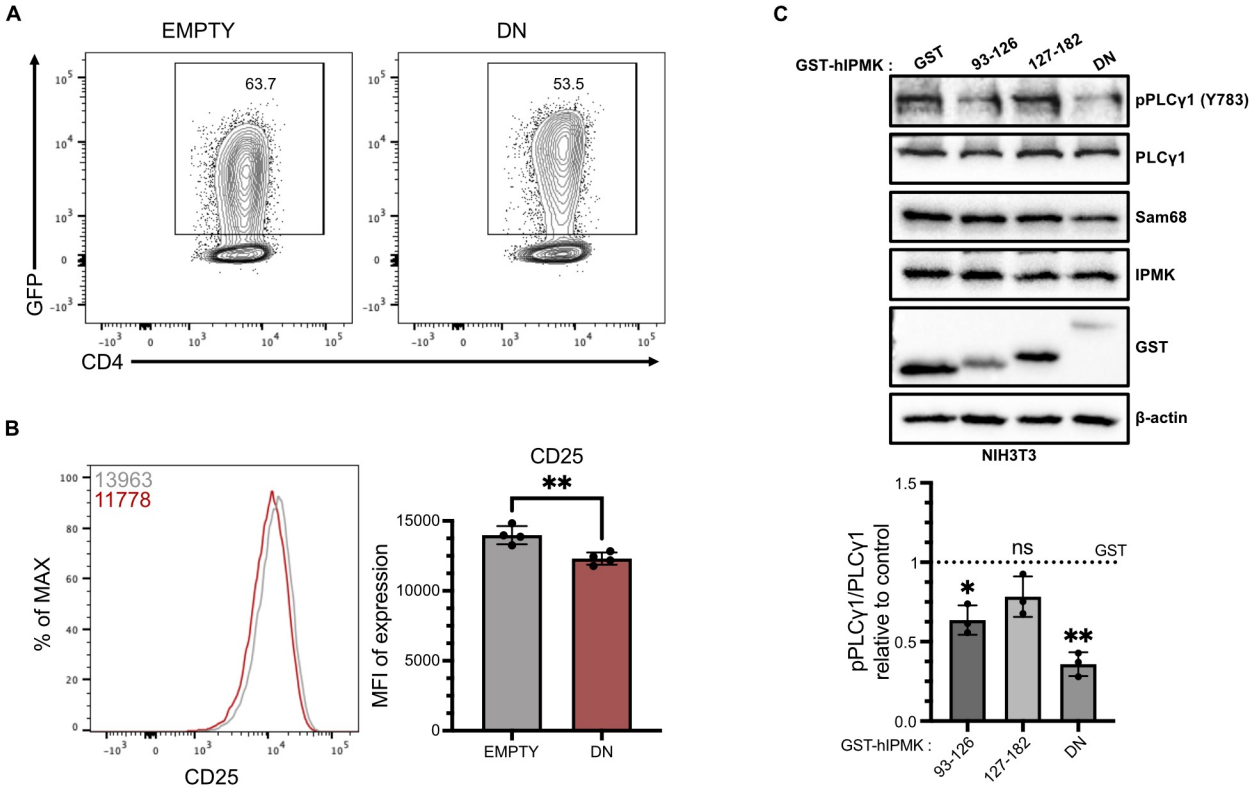

A
IPMK : WT KO
IP: Sam68
PLCγ1
Sam68
IPMK
Input
PLCγ1
Sam68
IPMK
α-tubulin
MEF
IP: PLCγ1/Sam68 relative to control
WT
KO
***
B
siRNA : eGFP mIPMK
IP: Sam68
PLCγ1
Sam68
IPMK
Input
pPLCγ1 (Y783)
PLCγ1
Sam68
pSrc family (Y416)
Src
IPMK
β-actin
NIH3T3
IP: PLCγ1/Sam68 relative to control
sieGFP
siIPMK
**
pPLCγ1/PLCγ1 relative to control
C
lentiCRISPRv2 : EMPTY sgSam68_1 sgSam68_2
pPLCγ1 (Y783)
PLCγ1
Sam68
pSrc family (Y416)
Src
β-actin
Jurkat
pPLCγ1/PLCγ1 relative to control
EMPTY
*
**
sgSam68_1
sgSam68_2
D
IPMKf/f IPMKΔCD4
IP: PLCγ1
PLCγ1
IPMK
LAT
Input
pPLCγ1 (Y783)
PLCγ1
IPMK
← LAT
β-actin
Naïve CD4+ T cell
IP: LAT/PLCγ1 relative to control
ns
IPMKf/f
IPMKΔCD4
E
Time (min) : 0 3
IP: PLCγ1
PLCγ1
Sam68
IPMK
Input
pPLCγ1 (Y783)
PLCγ1
Sam68
IPMK
β-actin
Naïve CD4+ T cell
IP: IPMK/PLCγ1 relative to control
0 min
3 min
**

fig. S5


A
EMPTY
DN
63.7
53.5
GFP
CD4
B
13963
11778
% of MAX
CD25
CD25
**
MFI of expression
EMPTY
DN
C
GST-hIPMK :
GST
93-126
127-182
DN
pPLCγ1 (Y783)
PLCγ1
Sam68
IPMK
GST
β-actin
NIH3T3
pPLCγ1/PLCγ1 relative to control
ns
GST
*
**
GST-hIPMK :
93-126
127-182
DN