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Supplemental information

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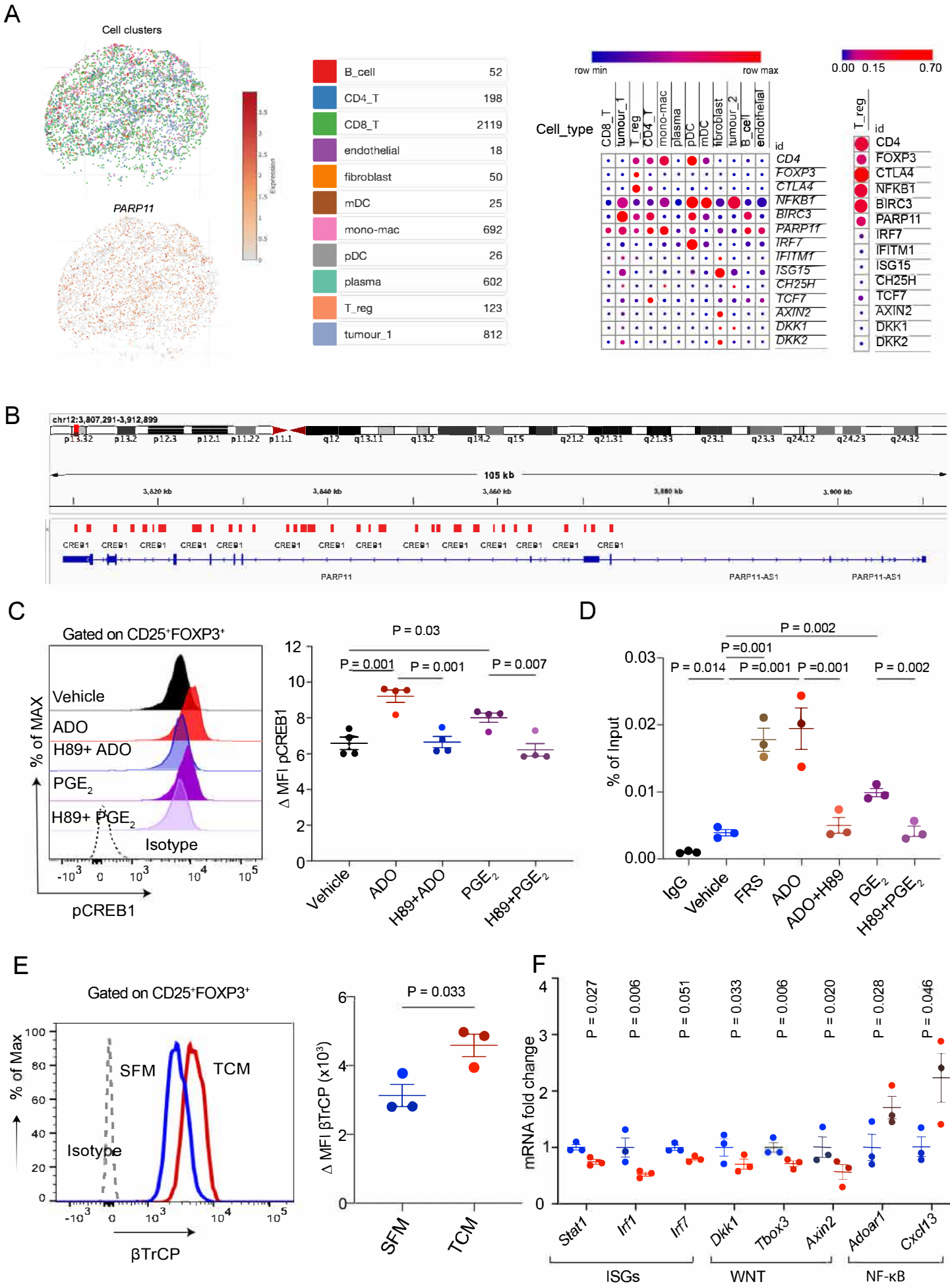
A
Cell clusters
PARP11
B_cell 52
CD4_T 198
CD8_T 2119
endothelial 18
fibroblast 50
mDC 25
mono-mac 692
pDC 26
plasma 602
T_reg 123
tumour_1 812
row min
row max
0.00 0.15 0.70
Cell_type
CD8_T
tumour_1
T_reg
CD4_T
mono-mac
plasma
pDC
mDC
fibroblast
tumour_2
B_cell
endothelial
id
CD4
FOXP3
CTLA4
NFKB1
BIRC3
PARP11
IRF7
IFITM1
ISG15
CH25H
TCF7
AXIN2
DKK1
DKK2
B
chr12:3,807,291-3,912,899
105 kb
PARP11
PARP11-AS1
C
Gated on CD25+FOXP3+
Vehicle
ADO
H89+ ADO
PGE2
H89+ PGE2
Isotype
% of MAX
pCREB1
Δ MFI pCREB1
P = 0.03
P = 0.001
P = 0.001
P = 0.007
Vehicle
ADO
H89+ADO
PGE2
H89+PGE2
D
P = 0.002
P =0.001
P = 0.014
P =0.001
P =0.001
P = 0.002
% of Input
IgG
Vehicle
FRS
ADO
ADO+H89
PGE2
H89+PGE2
E
Gated on CD25+FOXP3+
% of Max
SFM
TCM
Isotype
βTrCP
Δ MFI βTrCP (x10³)
P = 0.033
SFM
TCM
F
P = 0.027
P = 0.006
P = 0.051
P = 0.033
P = 0.006
P = 0.020
P = 0.028
P = 0.046
mRNA fold change
Stat1
Irf1
Irf7
Dkk1
Tbox3
Axin2
Adoar1
Cxcl13
ISGs
WNT
NF-κB

Figure S1. ***Expression of PARP11 is upregulated in TI-Tregs and associated with failure of ICB therapies related to Figure 1.***

A. Color-coded representation of snRNA-seq profiles across various cell types with differential *PARP11* expression. Intensity of color corresponds to *PARP11* expression levels. Accompanying dot plots detail the expression of key genes in NF-κB, type I interferon, and Wnt pathways. Dot size reflects gene expression level, while color intensity indicates the proportion of cells expressing each gene within the clusters.

B. Prediction of CREB1 binding sites on *PARP11* DNA sequence using Gene Transcription Regulation Database (GTRD).

C. Levels of phosphorylated CREB1 in induced T regulatory cells (iTregs) following treatment with adenosine (ADO; 1mM), prostaglandin E2 (PGE_2; 1μM), and their combinations with or without the PKA inhibitor H89 (10μM) for 3h. (n=4).

D. ChIP-qPCR analysis of phosphor-CREB1 binding to *Parp11* promoter in iTreg cells following treatment with forskolin (10μM), adenosine (ADO; 1mM), prostaglandin E2 (PGE_2; 1μM), and their combinations with or without the PKA inhibitor H89 (10μM) for 1h (n=3).

E. Levels of βTrCP in iTregs treated with either B16F10 tumor conditioned media (TCM, red dots) or with serum free media (SFM, blue dots) for 6h (n=3).

F. qPCR analysis of the type I interferon pathway (*Stat1*, *Irf1* and *Irf7*), Wnt pathway (*Dkk1*, *TBox3* and *Axin2*) and NF-κB pathway (*Adoar1* and *Cxcl13*) genes in iTregs exposed with TCM (red dots) or SFM (blue dots) for 24h (n=3).

Data are presented as mean ± SEM. Statistical analysis was performed using 1-way ANOVA with Tukey's multiple-comparison test (**C, D**) or 2-tailed unpaired Students' t test (**E, F**).

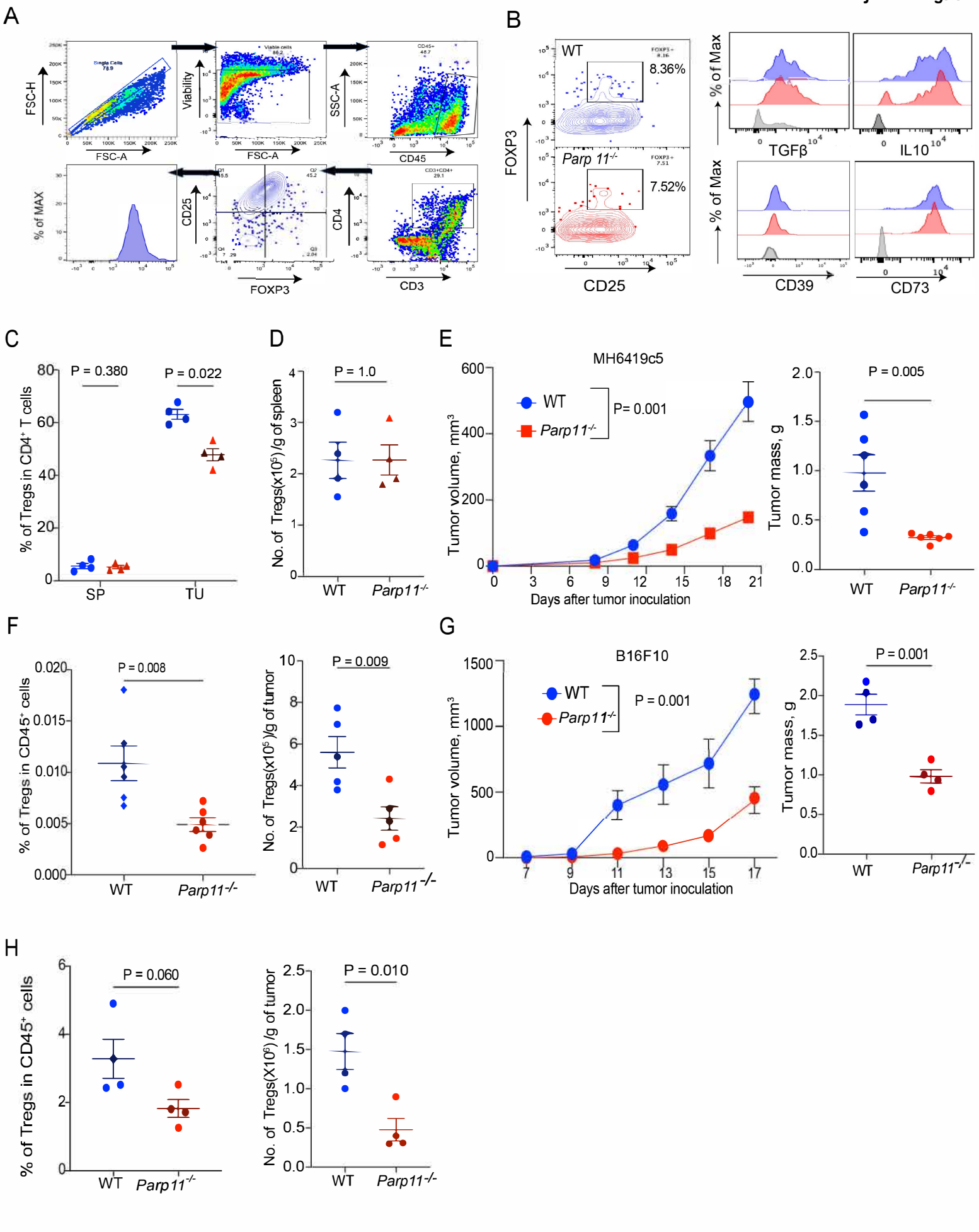
A
FSC-H
FSC-A
Viability
SSC-A
CD45
% of MAX
CD25
FOXP3
CD4
CD3
B
WT
8.36%
Parp 11-/-
7.52%
FOXP3
CD25
% of Max
TGFβ
IL10
CD39
CD73
C
P = 0.380
P = 0.022
% of Tregs in CD4+ T cells
SP
TU
D
P = 1.0
No. of Tregs(x10^5)/g of spleen
WT
Parp11-/-
E
MH6419c5
WT
Parp11-/-
P= 0.001
Tumor volume, mm3
Days after tumor inoculation
P = 0.005
Tumor mass, g
F
P = 0.008
% of Tregs in CD45+ cells
P = 0.009
No. of Tregs(x10^5)/g of tumor
G
B16F10
P = 0.001
P = 0.001
H
P = 0.060
% of Tregs in CD45+ cells
P = 0.010
No. of Tregs(X10^6)/g of tumor

Figure S2. ***PARP11 supports immune suppressive activities of TI-Tregs, related to Figure 2.***

A. Flow cytometry gating strategy in analysis of cellular components of splenic or tumor tissues.
B. Frequencies of Tregs (% of CD4+ T cells) and levels of TGFβ, IL10, CD39, CD73 in Tregs isolated from spleens of naïve WT or *Parp11* knockout mice.
C. Frequencies of Tregs (% of CD4+ T cells) isolated from spleens and MC38 tumors of mice described in **Figure 2A** (n=4).
D. Numbers (per gram of tissue) of SP-Tregs from MC38 tumor-bearing mice described in **Figure 2A** (n=4).
E. Volume and mass (on Day 21) of s.c MH6419c5 tumors growing in WT or *Parp11* knockout mice (n=6).
F. Frequencies (% of CD45+ cells) and numbers (per gram of tissue) of Tregs isolated from s.c MH6419c5 tumors described in panel **E**.
G. Volume and mass (Day 17) of s.c B16F10 tumors growing in WT or *Parp11* knockout mice (n=4).
H. Frequencies (% of CD45+ cells) and numbers (per gram of tissue) of Tregs isolated from s.c B16F10 tumors described in panel **G.**

Data are presented as mean ± SEM. Statistical analysis was performed using 2-tailed unpaired Students' t test (C-H) or 1-way ANOVA with Tukey's multiple-comparison test (E and G).

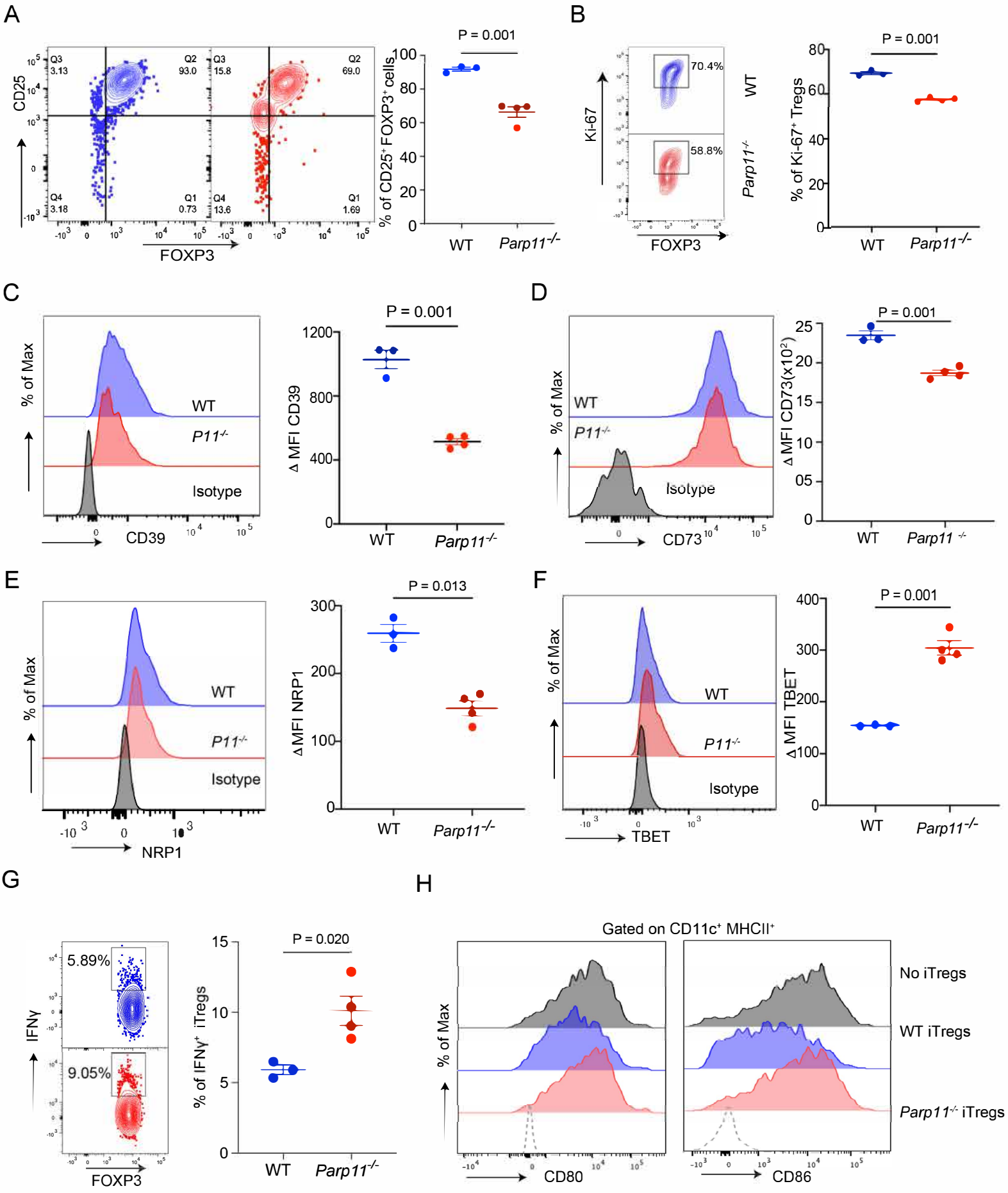
A
CD25
FOXP3
Q3 3.13
Q2 93.0
Q4 3.18
Q1 0.73
Q3 15.8
Q2 69.0
Q4 13.6
Q1 1.69
% of CD25+ FOXP3+ cells
P = 0.001
WT
Parp11-/-
B
Ki-67
FOXP3
70.4%
58.8%
WT
Parp11-/-
% of Ki-67+ Tregs
P = 0.001
C
% of Max
WT
P11-/-
Isotype
CD39
Δ MFI CD39
P = 0.001
D
% of Max
CD73
Δ MFI CD73(x10²)
P = 0.001
E
% of Max
NRP1
ΔMFI NRP1
P = 0.013
F
% of Max
TBET
ΔMFI TBET
P = 0.001
G
IFNγ
5.89%
9.05%
FOXP3
% of IFNγ+ iTregs
P = 0.020
H
Gated on CD11c+ MHCII+
% of Max
CD80
CD86
No iTregs
WT iTregs
Parp11-/- iTregs

Figure S3. ***Loss of PARP11 is associated with reduced immunoregulatory functions of Tregs, related to Figure 3.***

A. Frequencies of $CD25^{+}FOXP3^{+}$ cells in $CD4^{+}$ iTreg cells within the WT or *Parp11*$^{-/-}$ mice (n=3-4).
B. Flow cytometry analysis of percentage of Ki-67^{+} Tregs from experiment indicated in panel **A**.
C-F. Levels of CD39, CD73, NRP1, and TBET in WT or PARP11-null iTregs described in panel **A**.
G. Flow cytometry analysis of percentage of IFNγ+ iTregs from experiment described in panel **A**.
H. Analysis of cell surface levels of CD80 and CD86 on dendritic cells described in **Figure 3F** (n=3).

Data are presented as mean ± SEM. Statistical analysis was performed using 2-tailed unpaired Students' t test (**A-G**).

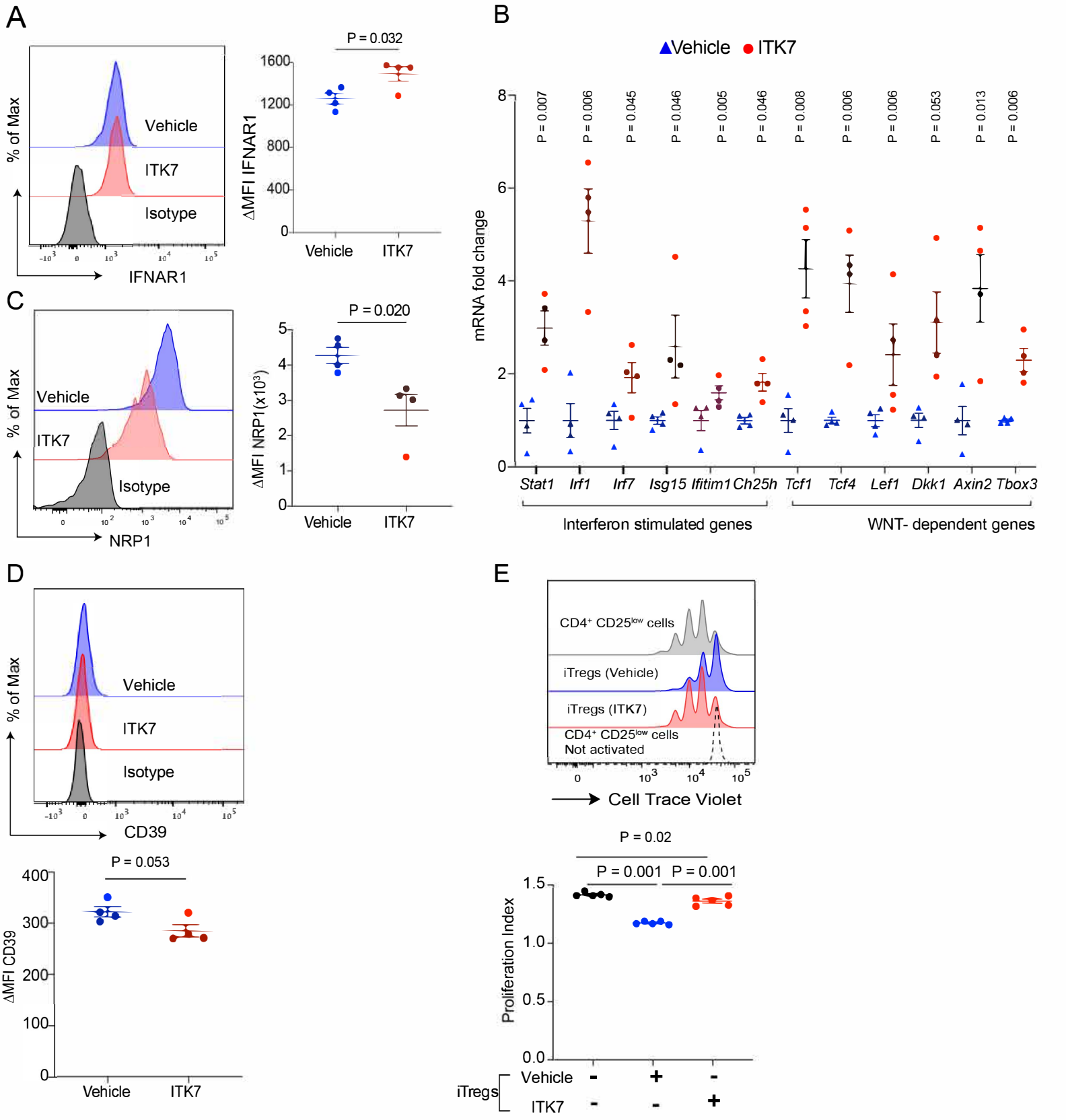
A
% of Max
Vehicle
ITK7
Isotype
IFNAR1
P = 0.032
ΔMFI IFNAR1
1600
1200
800
400
0
Vehicle
ITK7
B
Vehicle
ITK7
mRNA fold change
P = 0.007
P = 0.006
P = 0.045
P = 0.046
P = 0.005
P = 0.046
P = 0.008
P = 0.006
P = 0.006
P = 0.053
P = 0.013
P = 0.006
Stat1
Irf1
Irf7
Isg15
Ifitim1
Ch25h
Tcf1
Tcf4
Lef1
Dkk1
Axin2
Tbox3
Interferon stimulated genes
WNT- dependent genes
C
% of Max
Vehicle
ITK7
Isotype
NRP1
P = 0.020
ΔMFI NRP1(x10³)
Vehicle
ITK7
D
% of Max
Vehicle
ITK7
Isotype
CD39
P = 0.053
ΔMFI CD39
Vehicle
ITK7
E
CD4⁺ CD25ˡᵒʷ cells
iTregs (Vehicle)
iTregs (ITK7)
CD4⁺ CD25ˡᵒʷ cells
Not activated
Cell Trace Violet
P = 0.02
P = 0.001
P = 0.001
Proliferation Index
iTregs
Vehicle
ITK7

Figure S4. ***PARP11 specific inhibitor ITK7 mimics the loss of PARP11 phenotype on Tregs suppressive function, related to Figure 4.***

A. Flow cytometry analysis of cell surface IFNAR1 on iTregs treated with vehicle or ITK7 (5nM, 48h; n=4).
B. qPCR analysis of IFN1 pathway (*Stat1, Irf1* and *Irf7*) and WNT pathway (*Dkk1, TBox3* and *Axin2*) genes in WT iTregs treated with vehicle or ITK7 (5nM, 24h; n=4).
C. Flow cytometry analysis of cell surface NRP1 in iTregs described in panel **A**.
D. Flow cytometry analysis of cell surface CD39 in iTregs described in panel **A**.
E. Flow cytometry analysis and quantification of $CD4^{+}CD25^{low}$ T cell proliferation index in vitro. Activated WT CD8+ T cells stained with CellTrace Violet were co-cultured for 72h with or without WT iTreg cells (Treg:CD4=1:2) pre-treated or not with ITK7 (5nM, 48h; n=5).

Data are presented as mean ± SEM. Statistical analysis was performed using 2-tailed unpaired Students' t test (**A-D**) or 1-way ANOVA with Tukey's multiple-comparison test (**E**).

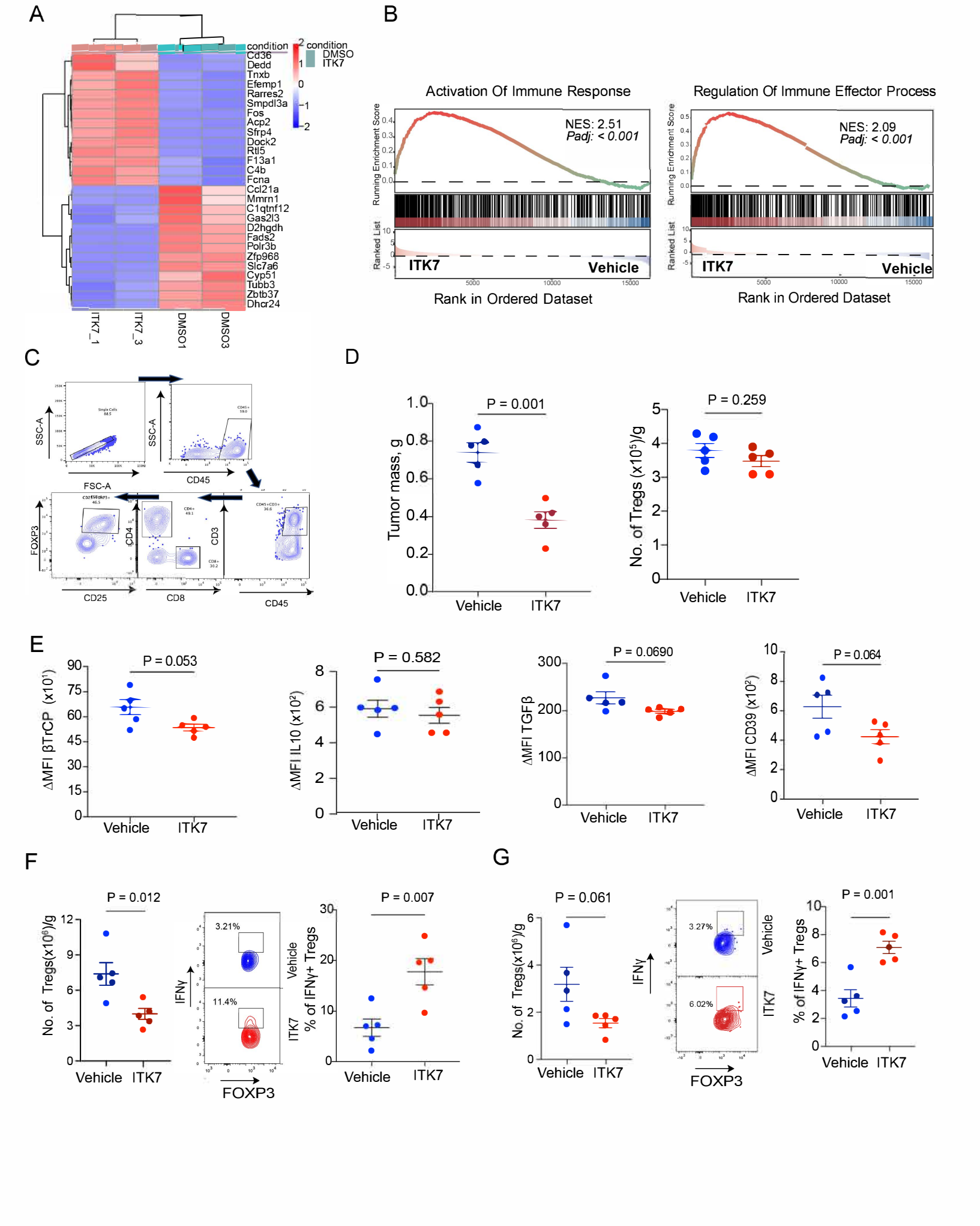
A
condition
Cd36
Dedd
Tnxb
Efemp1
Rarres2
Smpdl3a
Fos
Acp2
Sfrp4
Dock2
Rtl5
F13a1
C4b
Fcna
Ccl21a
Mmrn1
C1qtnf12
Gas2l3
D2hgdh
Fads2
Polr3b
Zfp968
Slc7a6
Cyp51
Tubb3
Zbtb37
Dhcr24
ITK7_1
ITK7_3
DMSO1
DMSO3
condition
DMSO
ITK7
B
Activation Of Immune Response
NES: 2.51
Padj: < 0.001
Running Enrichment Score
Ranked List
ITK7
Vehicle
Rank in Ordered Dataset
Regulation Of Immune Effector Process
NES: 2.09
Padj: < 0.001
Running Enrichment Score
Ranked List
ITK7
Vehicle
Rank in Ordered Dataset
C
SSC-A
FSC-A
CD45
FOXP3
CD4
CD3
CD25
CD8
CD45
D
Tumor mass, g
P = 0.001
Vehicle
ITK7
No. of Tregs (x10⁵)/g
P = 0.259
Vehicle
ITK7
E
ΔMFI βTrCP (x10¹)
P = 0.053
Vehicle
ITK7
ΔMFI IL10 (x10²)
P = 0.582
Vehicle
ITK7
ΔMFI TGFβ
P = 0.0690
Vehicle
ITK7
ΔMFI CD39 (x10²)
P = 0.064
Vehicle
ITK7
F
No. of Tregs(x10⁶)/g
P = 0.012
Vehicle
ITK7
3.21%
11.4%
IFNγ
FOXP3
Vehicle
ITK7
% of IFNγ+ Tregs
P = 0.007
Vehicle
ITK7
G
No. of Tregs(x10⁶)/g
P = 0.061
Vehicle
ITK7
3.27%
6.02%
IFNγ
FOXP3
Vehicle
ITK7
% of IFNγ+ Tregs
P = 0.001
Vehicle
ITK7

Figure S5. ***Selective PARP11 inhibitor ITK7 disrupts the immune suppressive activities of TI-Tregs and activates the immune pathways in the TME, related to Figure 5.***

A. Heatmap for differentially expressed genes in MC38 s.c. tumors from mice administered with ITK7 described in panel Figure **5A** (n=2).

B. GSEA and KEGG plots of indicated signatures detected in MC38 tumors described in panel **5A**.

C. The gating strategy for flow cytometry analysis of the effects of ITK7 treatment (as described in **Figure 5D**) on TME immune infiltration and status of Tregs and $CD8^+$ T cells**.**

D. Mass of MC38 tumors and absolute numbers (per gram of spleen) Tregs isolated from spleens from MC38 tumor- bearing mice described in **Figure 5D**.

E. Levels of β-TrCP, IL10, TGFβ and CD39 in Tregs isolated from spleens from MC38 tumor- bearing mice described in **Figure 5D**.

F. Absolute numbers and IFN-γ expression of TI-Treg isolated form subcutaneous MH6499c4 tumors (n=5)**.**

G. Absolute numbers and IFN-γ expression of TI-Treg isolated form mouse orthotopic pancreatic MH6499c4 tumors (n=5)**.**

Data are presented as mean ± SEM. Statistical analysis was performed using 2-tailed unpaired Students' t test (**D-G**).

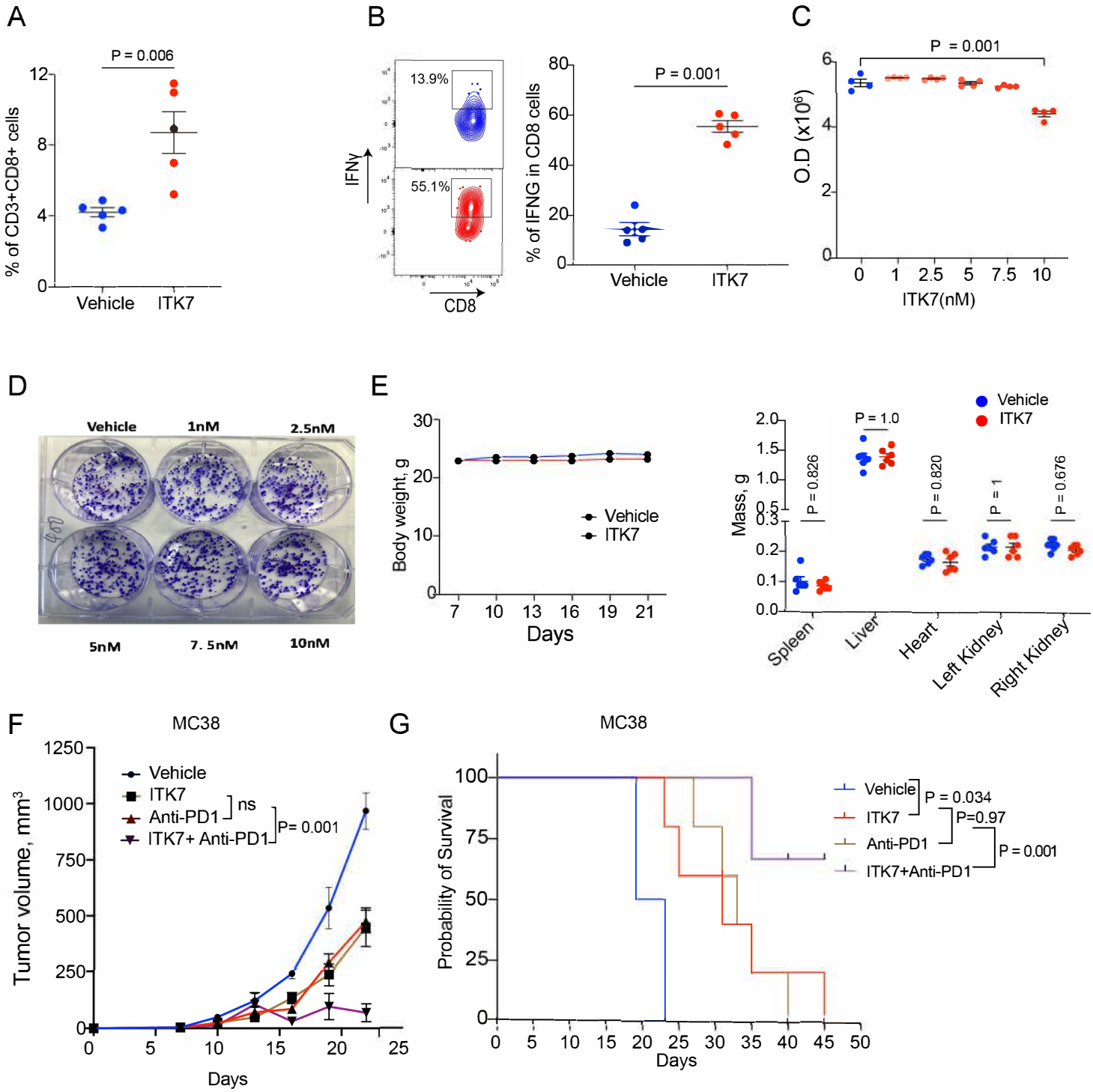
A
P = 0.006
% of CD3+CD8+ cells
Vehicle
ITK7
B
13.9%
55.1%
IFNγ
CD8
P = 0.001
% of IFNG in CD8 cells
Vehicle
ITK7
C
P = 0.001
O.D (x10^6)
ITK7(nM)
D
Vehicle
1nM
2.5nM
5nM
7. 5nM
10nM
E
Body weight, g
Vehicle
ITK7
Days
Vehicle
ITK7
P = 1.0
P = 0.826
P = 0.820
P = 1
P = 0.676
Mass, g
Spleen
Liver
Heart
Left Kidney
Right Kidney
F
MC38
Tumor volume, mm3
Vehicle
ITK7
Anti-PD1
ITK7+ Anti-PD1
ns
P= 0.001
Days
G
MC38
Probability of Survival
Vehicle
ITK7
Anti-PD1
ITK7+Anti-PD1
P = 0.034
P=0.97
P = 0.001
Days

Figure S6. ***ITK7 reactivates CTLs and elicits anti-tumor effects alone and in combination with immunotherapies, related to Figure 6.***

A. Percentage of $CD3^+CD8^+$ cells in $CD45^+$ cells isolated from subcutaneous MH6499c4 tumors grown in mice treated or not with ITK7 (100μg/mouse) as in **Figure 6E** (n=5)**.**
B. Flow cytometry analysis of percentage of $IFN\gamma^+$ $CD3^+CD8^+$ cells described in panel **A**.
C. Proliferation of MC38 cells in the presence of indicated ITK concentrations *in vitro* as assessed by the CellTiter-Glo® luminescence assay (n=4).
D. Colony formation by MC38 cells treated either with vehicle or indicated of ITK7 *in vitro* (n=2).
E. Body weight and the weight of indicated internal organs from s.c. MC38 tumor-bearing mice administered with ITK7 (100 μg/mouse i.p) on days 7, 10, 13, 16 and 19 after tumor inoculation (n=6).
F. Volume of s. c MC38 tumors growing in syngeneic WT mice treated with anti-PD1 (200μg/mouse) and ITK7 (100μg/mouse) as indicated (n=5).
G. The Kaplan-Meier survival analysis of MC38 tumor-bearing described in panel **F.** Mice were sacrificed when the tumor volume reached $1000mm^3$ (n=5).

Data are presented as mean ± SEM. Statistical analysis was using 2-tailed Students' t test (**A-E**) or 1-way ANOVA with Tukey's multiple-comparison Test (**F**) or log rank test (**G**).

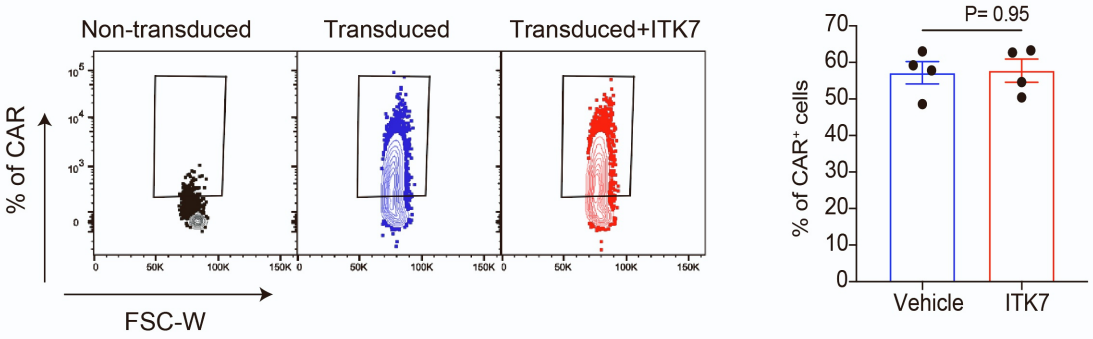

Figure S7. ITK7 improves the efficacy of CAR T therapy, related to Figure 7.
Percentage of CAR$^+$ T cells treated with vehicle or ITK7 (10nM) for 72h (n=4). Data are presented as mean ± SEM. Statistical analysis was using 2-tailed Students' t test.

Supplementary Table 2. List of oligonucleotides for qPCR, ChIP- qPCR and Genotyping, related to STAR METHODS.

Primers (5'- 3')	**Forward**	**Reverse**
Mouse β-Actin	TTCCAGCCTTCCTTCTTGGG	TGTTGGCATAGAGGTCTTTACGG
Mouse *Parp11*	GCAGATGAATCTTGTCACTGGG	TGGCCTCATTCTCACAGATGTA
Mouse *Btrc*		TCTCTTGGTTTATGCAAAGCCTG
Mouse *Stat1*	CGCGCATGCAACTGGCATATAACT	AAGCTCGAACCACTGTGACATCCT
Mouse *Irf3*	GAGAGCCGAACGAGGTTCAG	CTTCCAGGTTGACACGTCCG
Mouse *Irf7*	GAGACTGGCTATTGGGGGAG	GACCGAAATGCTTCCAGGG
Mouse *Isg15*	GGTGTCCGTGACTAACTCCAT	TGGAAAGGGTAAGACCGTCCT
Mouse *Ch25h*	TGCTACAACGGTTCGGAGC	AGAAGCCCACGTAAGTGATGAT
Mouse *Axin2*	TGACTCTCCTTCCAGATCCCA	TGCCCACACTAGGCTGACA
Mouse *Lef1*	TGTTTATCCCATCACGGGTGG	CATGGAAGTGTCGCCTGACAG
Mouse *Dickkopf*	GACCTGCTACGAGACCTGGA	CTGGAGAGGGTATGGTTGCC
Mouse *Tcf4*	CGAAAAGTTCCTCCGGGTTTG	CGTAGCCGGGCTGATTCAT
Mouse *Tbax3*	ACTCGGGGTCGGAACTGAA	GGAGGGGGCGATTTTGTTTTT
Mouse *Tcf1*	TGAATCACCACCCGGAATGG	CTGGGCCAACTTCACATCCC
Mouse *Nfkb1*	ATGGCAGACGATGATCCCTAC	TGTTGACAGTGGTATTTCTGGTG
Mouse *Il1b*	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
Mouse *Adoar1*	TGTGCCCGGAAATGTACTGG	TCTGTGGCCCAATGTTGATAAG
Mouse *Cxcl10*	CCAAGTGCTGCCGTCATTTTC	GGCTCGCAGGGATGATTTCAA
Mouse *Cxcl13*	GGCCACGGTATTCTGGAAGC	GGGCGTAACTTGAATCCGATCTA
ChIP- qPCR		
Mouse *Parp11*	CGTAGGTTCTGCATGGAGGA	AAACTCGCTCCGCCTCTATG
Genotyping		
***Parp11*$^{-/-}$**		
*Parp11*_TU	CAT GCT CAT GGA AAC CTG GAA AG	CCC TGT CTA AGC TCA CCA CTG
*Parp11*_NeoIn	TTC GGC TAT GAC TGG GCA CAA CAG	TAC TTT CTC GGC AGG AGC AAG GTG
***Foxp3*$^{YFP\text{-}Cre}$**		
Foxp3$^{YFP\text{-}Cre}$ *WT*	CAG TGT GGA CCG TAG ATG AA	AGT GCT GTT GCT GTG TAA GG
Foxp3$^{YFP\text{-}Cre}$ Mut	AGGATGTGAGGGACTACCTCCTGTA	TCCTTCACTCTGATCTGGCAATTT