Supplemental Data

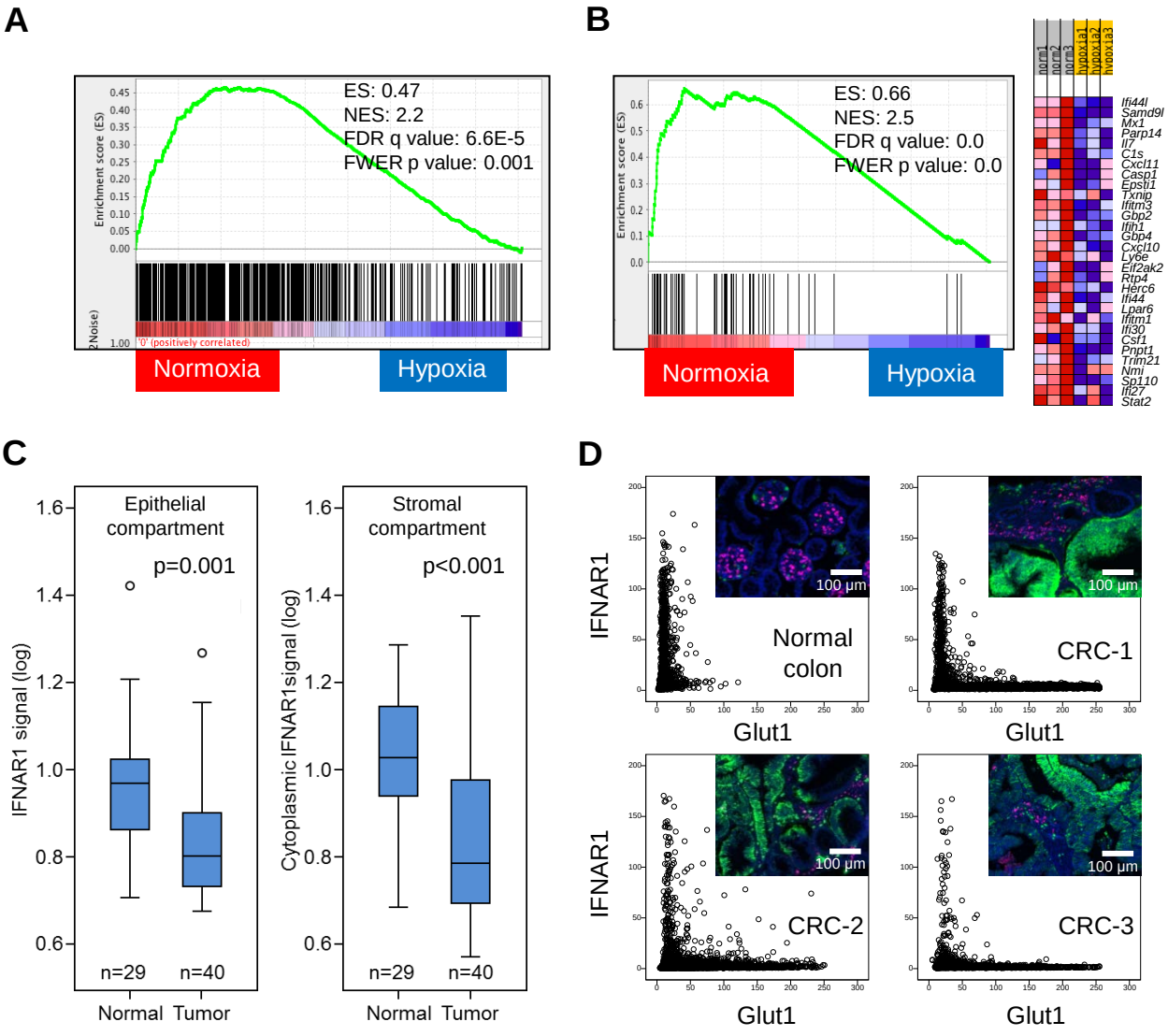

Figure S1 (related to Figure 1). Status of IFN and immune pathways in tumor microenvironment.
(A) Analysis of gene array data on expression of genes downregulated in the hypoxic areas of transplanted rat 9L glioma tumors (reported in (Marotta et al., 2011)). GSEA results for the immune pathway are shown. **(B)** Gene Set Enrichment Analysis (GSEA) (left panel) and heat map (right panel) of IFN signaling pathway genes profiled from the normoxic and hypoxic tumor areas. **(C)** Quantification of immunohistochemical analysis of IFNAR1 protein levels in epithelial and stromal compartments of normal colon and colorectal adenocarcinoma from samples from the University of Pennsylvania's tissue microarray (described in (King et al., 2011). Box plot showing nuclear IFNAR1 levels in representative normal and cancer cases indicates median (dark line), 25-75% range (box), minimum and maximum values (whiskers) and individual scatter plot values (circles) overlaying the box plot. **(D)** Analysis of IFNAR1 and tumor microenvironment stress marker, GLUT1-positive cells in normal colon and colorectal tumors. A panel of 54 colorectal adenocarcinomas and 30 normal colon specimens were co-stained for GLUT1 (green) and IFNAR1 (red). The representative images of normal colon and three CRC samples as well as the scatterplots of quantitative IHC values are shown. Image quantification was performed using Tissue Studio. Scale bars: 100 μm.

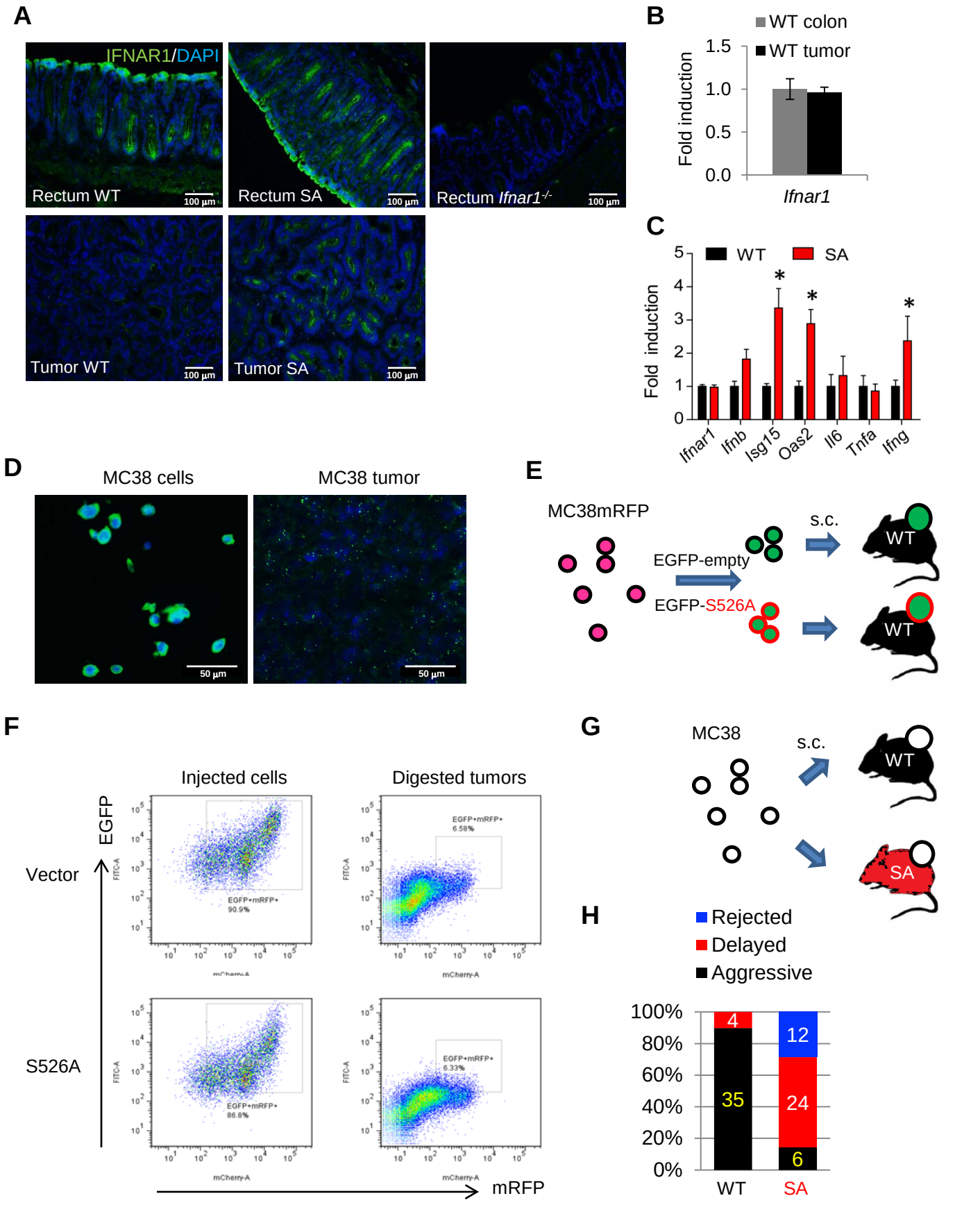
A
IFNAR1/DAPI
Rectum WT
Rectum SA
Rectum Ifnar1-/-
Tumor WT
Tumor SA
100 µm
B
WT colon
WT tumor
Fold induction
1.5
1.0
0.5
0.0
Ifnar1
C
WT
SA
Fold induction
Ifnar1
Ifnb
Isg15
Oas2
Il6
Tnfa
Ifng
D
MC38 cells
MC38 tumor
50 µm
E
MC38mRFP
EGFP-empty
EGFP-S526A
s.c.
WT
WT
F
Injected cells
Digested tumors
EGFP
Vector
S526A
EGFP+mRFP+
90.9%
EGFP+mRFP+
6.58%
EGFP+mRFP+
86.8%
EGFP+mRFP+
6.33%
mCherry-A
mRFP
G
MC38
s.c.
WT
SA
H
Rejected
Delayed
Aggressive
100%
80%
60%
40%
20%
0%
4
35
12
24
6
WT
SA

Figure S2 (related to Figure 2). Role of downregulation of IFNAR1 in the stromal compartment in colorectal carcinogenesis and growth of transplanted adenocarcinomas.

(A) Representative immunofluorescent analysis of IFNAR1 levels (green) in rectums from WT (upper left), SA (upper middle), and *Ifnar1*$^{-/-}$ (upper right – serves as a negative control) mice and rectal tumors from AOM-DSS-treated WT (lower left) and SA (lower right) mice. Here and thereafter, the sections were counterstained with DAPI (blue). Scale bar: 100 μm. **(B)** qPCR analysis of *Ifnar1* mRNA expression in tumors and benign tissues from AOM-DSS-treated mice. Mean values (the group of 3 mice)±SEM are shown. **(C)** qPCR analysis of mRNA for indicated genes in AOM-DSS-induced tumors from WT and SA mice. Mean values (8 tumors from the group of 5 mice)±SEM are shown. Here and thereafter: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

(D) Immunofluorescent analysis of IFNAR1 levels (green) on MC38-derived tumors compared to cultured MC38 cancer cells. Samples were contrasted with DAPI (blue). Scale bar: 50 μm; **(E)** Design of experiments restoring IFNAR1 levels in cancer cells. MC38mRFP cells were transduced with pCIG-EGFP or pCIG-IFNAR1^{S526A}-EGFP constructs and GFP-positive tumor cells were injected into the flank of syngeneic WT mice ($1x10^6$ per mouse). **(F)** FACS analysis of MC38mRFP cells transduced with pCIG-EGFP or pCIG-IFNAR1^{S526A}-EGFP constructs was carried out either in cells immediately prior to injection into animals ("Injected cells") or in cells obtained from resulting tumors upon their digestion with collagenase ("Digested tumors"). MC38mRFP and MC38 parental cells were used as controls for gating on GFP/mRFP positive cells. **(G)** Design of experiments restoring IFNAR1 levels in the stroma. **(H)** Combined data depicting the outcome of MC38 tumor growth in WT and SA are shown. Tumors smaller than median tumor size in WT minus 2σ (SD) at day 32 were considered as delayed.

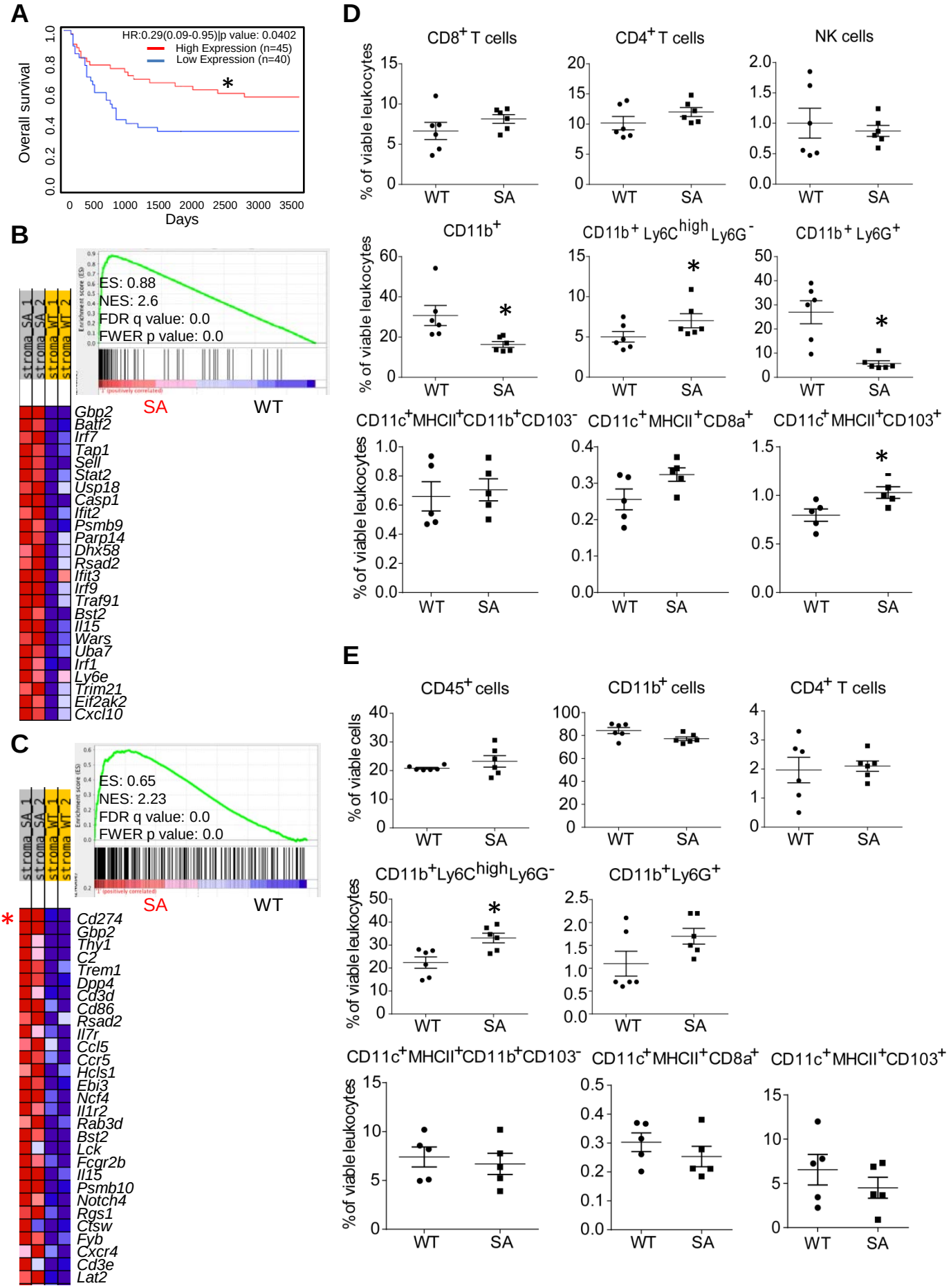
A
HR:0.29(0.09-0.95)|p value: 0.0402
High Expression (n=45)
Low Expression (n=40)
Overall survival
Days
B
ES: 0.88
NES: 2.6
FDR q value: 0.0
FWER p value: 0.0
SA
WT
Gbp2
Batf2
Irf7
Tap1
Sell
Stat2
Usp18
Casp1
Ifit2
Psmb9
Parp14
Dhx58
Rsad2
Ifit3
Irf9
Traf91
Bst2
Il15
Wars
Uba7
Irf1
Ly6e
Trim21
Eif2ak2
Cxcl10
C
ES: 0.65
NES: 2.23
FDR q value: 0.0
FWER p value: 0.0
SA
WT
Cd274
Gbp2
Thy1
C2
Trem1
Dpp4
Cd3d
Cd86
Rsad2
Il7r
Ccl5
Ccr5
Hcls1
Ebi3
Ncf4
Il1r2
Rab3d
Bst2
Lck
Fcgr2b
Il15
Psmb10
Notch4
Rgs1
Ctsw
Fyb
Cxcr4
Cd3e
Lat2
D
CD8+ T cells
CD4+ T cells
NK cells
% of viable leukocytes
CD11b+
CD11b+ Ly6Chigh Ly6G-
CD11b+ Ly6G+
CD11c+MHCII+CD11b+CD103-
CD11c+MHCII+CD8a+
CD11c+MHCII+CD103+
WT
SA
E
CD45+ cells
CD11b+ cells
CD4+ T cells
% of viable cells
CD11b+Ly6Chigh Ly6G-
CD11b+Ly6G+
CD11c+MHCII+CD11b+CD103-
CD11c+MHCII+CD8a+
CD11c+MHCII+CD103+
% of viable leukocytes
WT
SA

Figure S3 (related to Figure 3). Gene expression associated with IFNAR1 downregulation and prognosis in CRC patients.

(A) Survival of adjusted for the stage CRC patients (GSE30378) harboring expression pattern of 30 selected genes (Table S1) similar to MC38 that grew either in WT (blue) or in SA (red) mice **(B)** GSEA results and heat map of IFNα/β signaling pathway genes of the transcriptome profiles of enriched stromal (mRFP negative) cells sorted from MC38mRFP tumors harvested at day 21 (time point A1) and used for RNA isolation and microarray analysis. **(C)** GSEA results and heat map of immune system genes described in panel B. Asterisk denotes the most dramatically altered *Cd274* gene expression. **(D)** Percentage of splenic leukocyte populations in MC38 tumor bearing animals. Cell populations were defined as follows: $CD4^+$T cells ($CD11b^-CD11c^-NK1.1^-CD3^+CD4^+$), $CD8^+$ T cells ($CD11b^-CD11c^-NK1.1^-CD3^+CD8^+$), NK cells ($CD3^-NK1.1^+$). **(E)** Percentage of infiltrating leukocytes in MC38 tumors grown in WT and SA mice after s.c. injection of $1x10^6$ MC38 cells (as in panel D). Tumors were excised at the size of 200-300 mm^2 and digested with collagenase to prepare single cell suspensions.

Data shown as Mean±SD (n = 5-6).

Table S1 (related to Figure 3). The list of 30 genes upregulated in the enriched stromal compartment (mRFP-negative cells) from SA MC38mRFP tumors compared to WT MC38mRFP tumors (harvested at the A0 time point in experiment as described in Figure 3A).

Nup210	nucleoporin 210kDa
Irf7	interferon regulatory factor 1
Cd69	CD69 molecule
Ifit2	interferon-induced protein with tetratricopeptide repeats 2
Daxx	Death domain-associated protein 6
Il10ra	IL10RA interleukin 10 receptor, alpha
Mx2	MX dynamin-like GTPase 2
Usp18	ubiquitin specific peptidase 18
Sh2d2a	SH2 domain containing 2A
Ass1	argininosuccinate synthase 1
Hpx	hemopexin
Clec7a	C-type lectin domain family 7, member A
Lcn2	lipocalin 2
Cd40	TNF receptor superfamily member 5
Csf2ra	colony stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage)
Cxcl9	chemokine (C-X-C motif) ligand 9
Ccl17	chemokine (C-C motif) ligand 17
Slc7a8	solute carrier family 7 (amino acid transporter light chain, L system), member 8
Cd86	CD86 molecule
Tlr4	toll-like receptor 2
Sema4a	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4A
Sdc3	syndecan 3
Cd83	CD83 molecule
Cytip	cytohesin 1 interacting protein
Oas3	2'-5' oligoadenylate synthetase 3
Tiam1	T-cell lymphoma invasion and metastasis 1
Il1rn	interleukin 1 receptor antagonist
Cxcl10	chemokine (C-X-C motif) ligand 10
Aim1	absent in melanoma 1
Stat4	signal transducer and activator of transcription 4

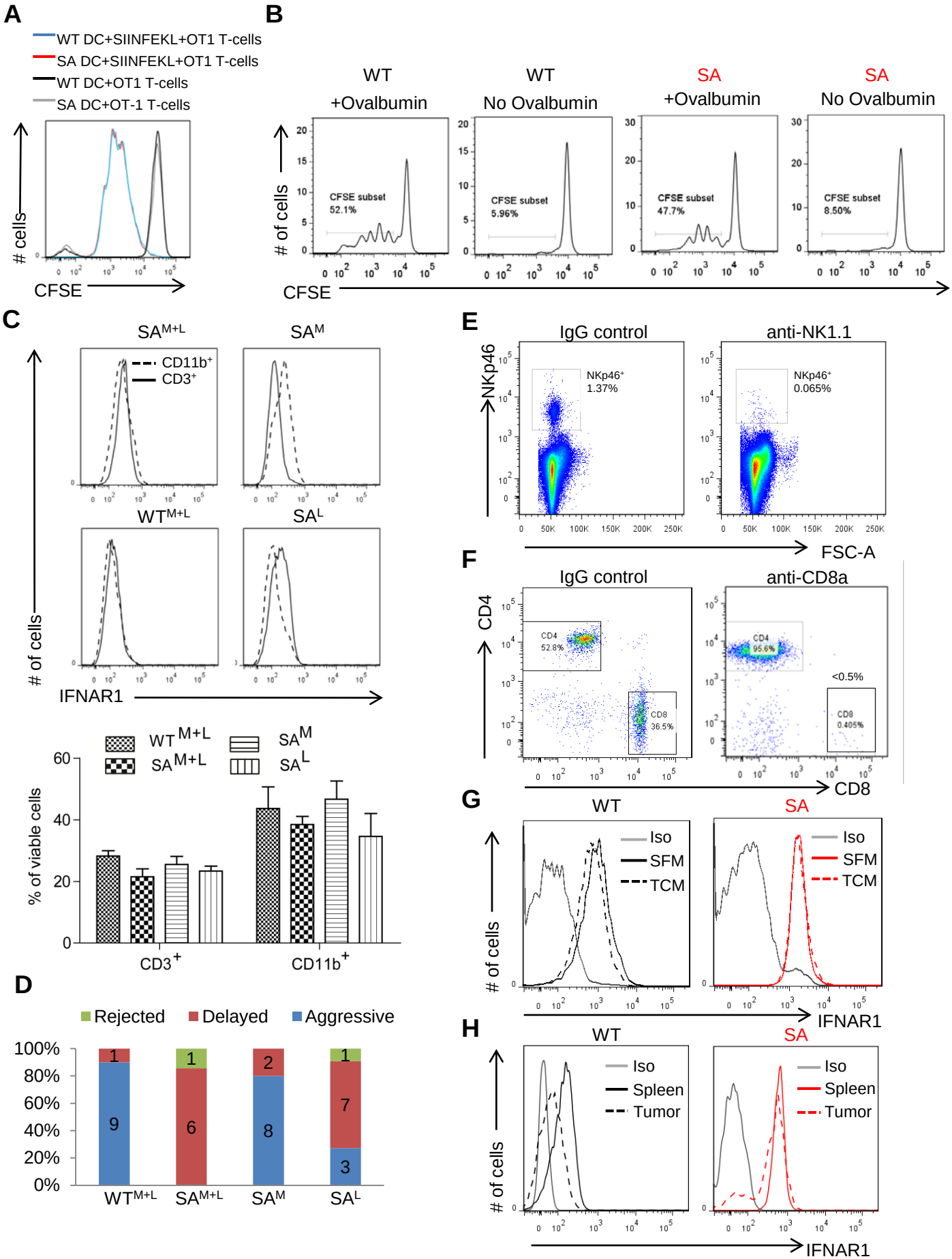
A
WT DC+SIINFEKL+OT1 T-cells
SA DC+SIINFEKL+OT1 T-cells
WT DC+OT1 T-cells
SA DC+OT-1 T-cells
cells
CFSE
B
WT
+Ovalbumin
WT
No Ovalbumin
SA
+Ovalbumin
SA
No Ovalbumin
# of cells
CFSE subset
52.1%
CFSE subset
5.96%
CFSE subset
47.7%
CFSE subset
8.50%
CFSE
C
SA^M+L
SA^M
CD11b+
CD3+
WT^M+L
SA^L
# of cells
IFNAR1
WT^M+L
SA^M+L
SA^M
SA^L
% of viable cells
CD3+
CD11b+
D
Rejected
Delayed
Aggressive
100%
80%
60%
40%
20%
0%
WT^M+L
SA^M+L
SA^M
SA^L
E
IgG control
anti-NK1.1
NKp46
NKp46+
1.37%
NKp46+
0.065%
FSC-A
F
IgG control
anti-CD8a
CD4
CD4
52.8%
CD8
36.5%
CD4
95.6%
<0.5%
CD8
0.405%
CD8
G
WT
SA
Iso
SFM
TCM
# of cells
IFNAR1
H
WT
SA
Iso
Spleen
Tumor
# of cells
IFNAR1

Figure S4 (related to Figure 4). Downregulation of IFNAR1 in cytotoxic T lymphocytes and immunosuppressive tumor microenvironment in CRC.

(A) Representative flow cytometry analysis of proliferation of CFSE-labeled OT-1 $CD3^+CD8^+$ T cells stimulated with $CD11c^+$ splenic WT and SA dendritic cells loaded with SIINFEKL peptide. Dilution of the cell proliferation dye (CFSE) was measured 60 hr later. Data are representative of two independent experiments. **(B)** Representative flow cytometry analysis of proliferation of CFSE-labeled $CD3^+CD8^+$ OT-1 WT T cells in response to ovalbumin cross-presented by WT or SA $CD11c^+$ dendritic cells. $CD11c^+$ cells were isolated from the spleens of WT or SA mice and co-cultured with irradiated, ovalbumin-loaded (left panels) or unloaded (right panels) MHC class I mismatched splenocytes and CFSE-labeled OT-1 T cells. After 72 hr incubation, proliferation of OT-1 T cells was determined by CSFE dilution. Histograms represent CFSE levels in the $CD8^+$ T cells. Data are representative of at least two independent experiments that yielded similar results. **(C)** Representative histograms of cell surface IFNAR1 levels on myeloid and lymphoid cells (upper panels) and the percentage of these cells in the spleens from indicated bone marrow chimeras (lower panel) at the end of experiment shown in Figure 4D. Data are shown as Mean±SEM (n=4-6). **(D)** Outcome of MC38 tumor growth in mixed bone marrow chimeras. Tumors smaller than median tumor size in WT^{M+L} minus 2σ (SD) at day 32 were considered as delayed. **(E)** Percentage of $NKp46^+$ NK cells in the spleens of tumor bearing mice treated with IgG control or anti-NK.1.1 antibodies at the end of experiment. **(F)** Percentage of $CD3^+CD8^+$ cells in the spleens of tumor bearing mice treated with anti-CD8a antibodies at the end of the experiment. **(G)** Representative histograms of cell surface IFNAR1 levels on $CD8^+$ T cells isolated from spleens of WT or SA mice and incubated for 2 hr in the presence of serum-free media (SFM) or media conditioned by MC38 tumor cells (TCM). **(H)** Representative histograms of cell surface IFNAR1 levels on $CD3^+CD8^+$ cells isolated from spleens or MC38 tumors from WT (left panel) or SA (right panel) tumor-bearing mice.

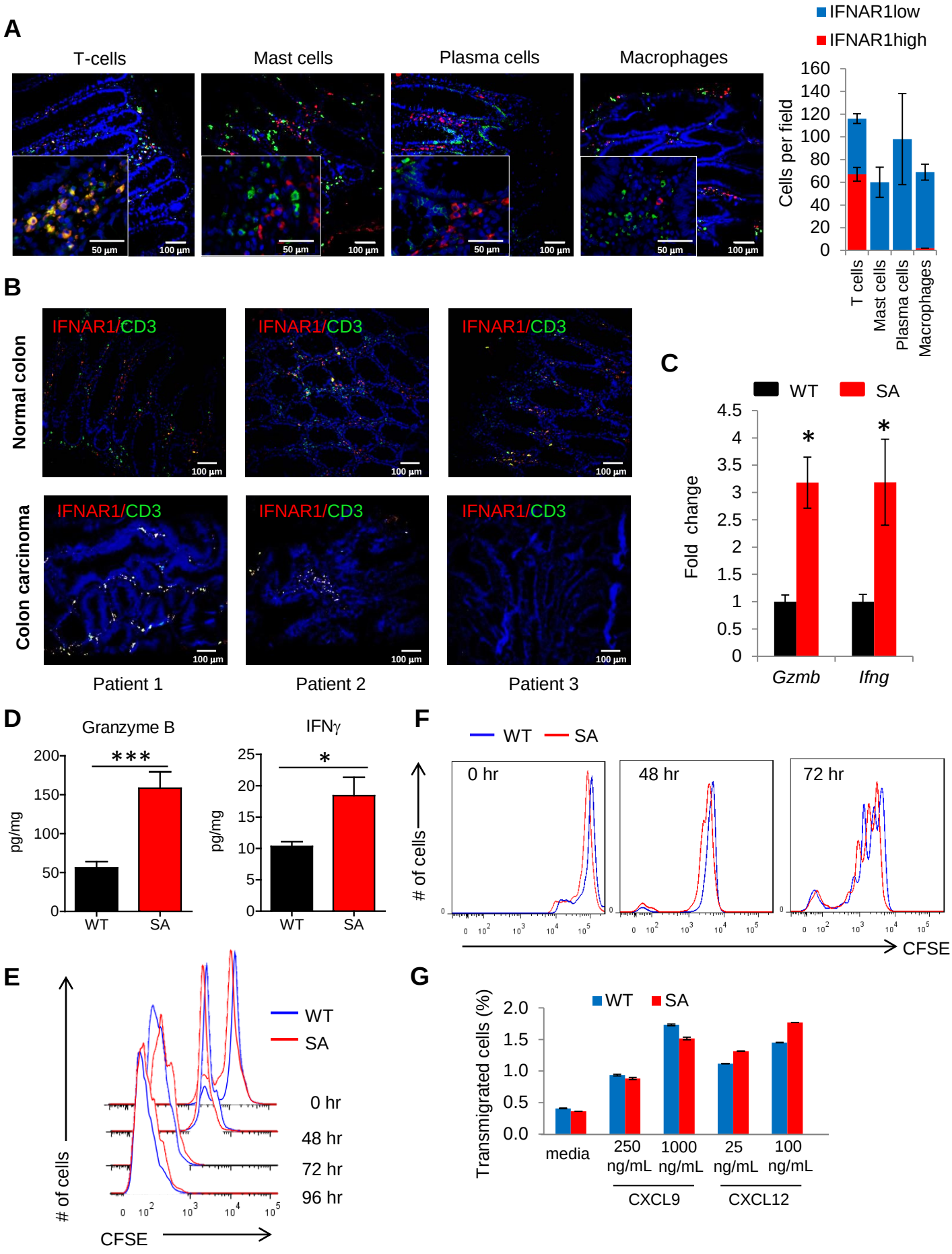
A
T-cells
Mast cells
Plasma cells
Macrophages
50 µm
100 µm
IFNAR1low
IFNAR1high
Cells per field
160
140
120
100
80
60
40
20
0
T cells
Mast cells
Plasma cells
Macrophages
B
Normal colon
Colon carcinoma
IFNAR1/CD3
100 µm
Patient 1
Patient 2
Patient 3
C
WT
SA
Fold change
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
*
*
Gzmb
Ifng
D
Granzyme B
IFNγ
pg/mg
200
150
100
50
0
***
WT
SA
25
20
15
10
5
0
*
E
WT
SA
0 hr
48 hr
72 hr
96 hr
# of cells
CFSE
F
WT
SA
0 hr
48 hr
72 hr
# of cells
CFSE
G
WT
SA
Transmigrated cells (%)
2.0
1.5
1.0
0.5
0.0
media
250 ng/mL
1000 ng/mL
25 ng/mL
100 ng/mL
CXCL9
CXCL12

Figure S5 (related to Figure 5). Role of IFNAR1 downregulation in survival/intra-tumoral accumulation of CTL.

(A) Representative immunofluorescent staining of normal human colon sections with antibodies against IFNAR1 (red, all panels) and indicated cell specific markers (green): anti-CD3 (pan T cells), anti-mast cell tryptase (mast cells), anti-CD138 (plasma cells), anti-CD68 (macrophages). Sections were contrasted with DAPI (blue). Scale bars: 100 μm. Inserts with a higher magnification are shown in the bottom left corner of each image (scale bars 50 μm). Quantification of $IFNAR1^{high}$ and $IFNAR1^{low}$ cells (from at least 20 random fields) is shown on the right. **(B)** Representative immunofluorescent analysis of $CD3^+$ T cells infiltration of human CRC. Tumor sections stained with antibodies against IFNAR1 (red) and CD3 (green) and contrasted with DAPI (blue). **(C)** Real time PCR analysis of mRNA of indicated genes in MC38 tumors grown in WT and SA mice. Tumors were harvested at the size of 200-300 mm^2 and used for RNA isolation. Mean values ±SEM (n= 6 tumors for each group) are shown. **(D)** Results of ELISA analysis of IFNγ and granzyme B in the lysates from indicated tumors described in panel C. Mean values ±SEM (n= 6 tumors for each group) are shown. **(E)** Proliferation of CFSE labeled OT-1 $CD3^+CD8^+$ WT and OT-1 $CD3^+CD8^+$ SA upon stimulation of splenocytes with SIINFEKL peptide. Dilution of CFSE was measured after indicated time periods. Data are representative of 2 independent experiments (each in triplicates). **(F)** Proliferation of CFSE labeled OT-1 $CD3^+CD8^+$ WT and OT-1 $CD3^+CD8^+$ SA T cells from spleens of MC38-OVA tumor bearing *Rag1*$^{-/-}$ mice. *Rag1*$^{-/-}$ mice were s.c. injected with MC38-OVA (bright, $2x10^6$/mouse). When the tumors reached ~100 mm^2 (around 20 days), each animal received $5x10^6$ CFSE labeled WT or SA OT-1 T cells (i.v.). Levels of CFSE in spleen-derived $CD3^+CD8^+$ cells were assessed at 0, 48 hr and 72 hr after adoptive transfer. Data are representative of 3 independent experiments (each in triplicates). **(G)** The percentage of transmigrated CD3/CD28-activated T cells from WT and SA mice in response to CXCL12 or CXCL9 in trans-well migration assay. Data represent Mean±SD of two independent experiments (each in triplicates).

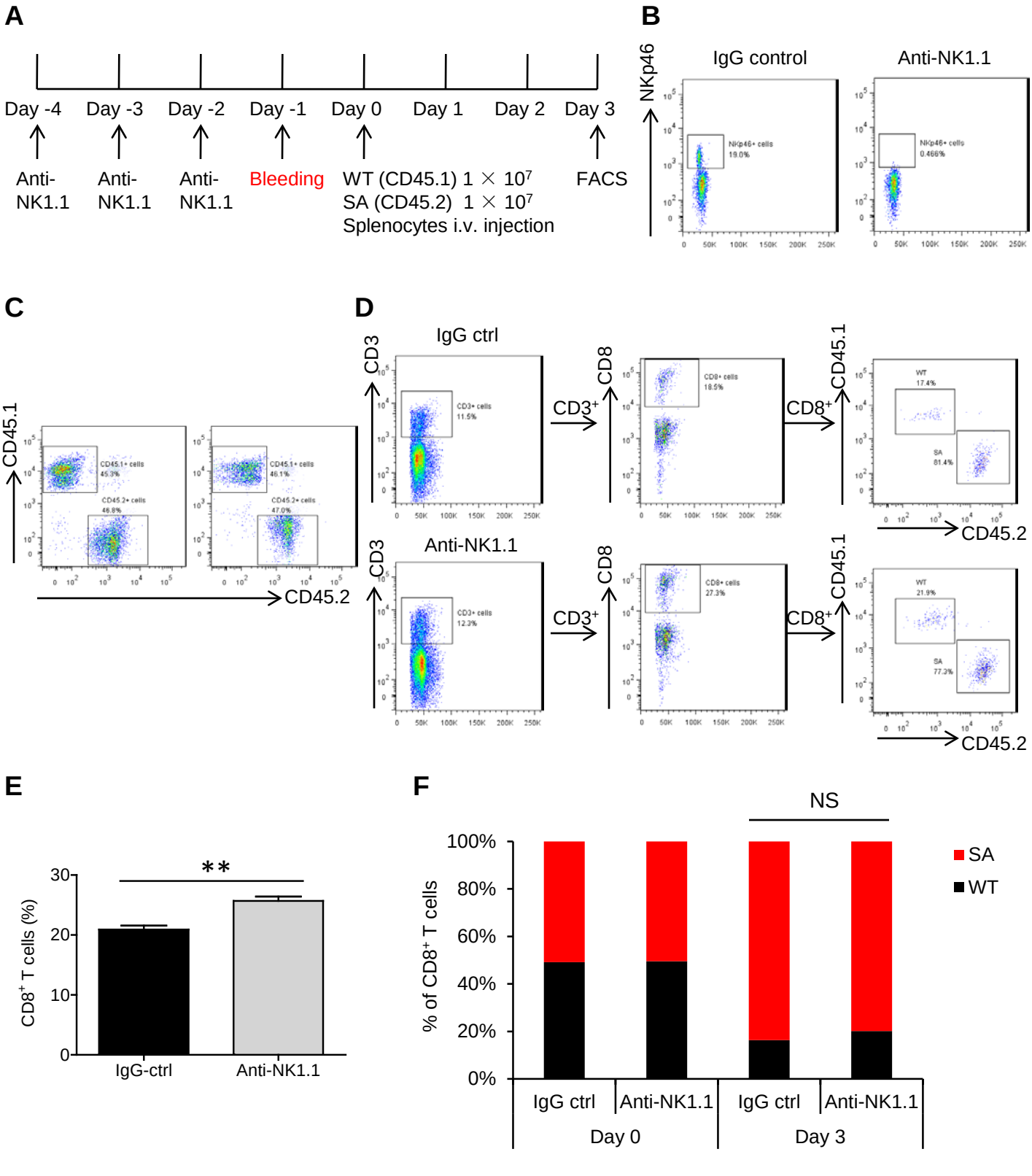
A
Day -4
Day -3
Day -2
Day -1
Day 0
Day 1
Day 2
Day 3
Anti-NK1.1
Anti-NK1.1
Anti-NK1.1
Bleeding
WT (CD45.1) 1 × 10^7
SA (CD45.2) 1 × 10^7
Splenocytes i.v. injection
FACS
B
IgG control
Anti-NK1.1
NKp46
NKp46+ cells 19.0%
NKp46+ cells 0.466%
C
CD45.1
CD45.2
CD45.1+ cells 45.3%
CD45.2+ cells 46.8%
CD45.1+ cells 46.1%
CD45.2+ cells 47.0%
D
IgG ctrl
Anti-NK1.1
CD3
CD8
CD45.1
CD45.2
CD3+ cells 11.5%
CD8+ cells 18.5%
WT 17.4%
SA 81.4%
CD3+ cells 12.3%
CD8+ cells 27.3%
WT 21.9%
SA 77.3%
CD3+
CD8+
E
CD8+ T cells (%)
30
20
10
0
**
IgG-ctrl
Anti-NK1.1
F
NS
100%
80%
60%
40%
20%
0%
% of CD8+ T cells
SA
WT
IgG ctrl
Anti-NK1.1
IgG ctrl
Anti-NK1.1
Day 0
Day 3

Figure S6 (related to Figure 6). Role of NK cells in the survival of WT or SA T cells.

Rag1 knockout mice were depleted (or not) of NK cells and viability of $CD8^+$ T cells derived from the spleens from WT (CD45.1) or SA (CD45.2) co-injected into these mice in 1:1 ratio was assessed. **(A)** Schematic illustration for the experiment to determine the extent of NK-mediated killing of WT and SA T cells *in vivo*. **(B)** FACS analysis of the percentage of $NKp46^+$ NK cells in the peripheral blood of *Rag1*$^{-/-}$ mice treated with IgG control (left) or anti-NK.1.1 (right) antibodies before cells injection. **(C)** FACS analysis of the composition of splenocytes mix (10^7 cells from each WT (CD45.1) and SA (CD45.2) mice prior to injection into *Rag1*$^{-/-}$ mice. **(D)** Representative of FACS analysis of composition of WT and SA $CD8^+$ T cells in *Rag1*$^{-/-}$ mice (treated with anti-NK1.1 or control antibody) three days after adoptive transfer. **(E)** Quantification of total $CD8^+$T cells found in *Rag1*$^{-/-}$ mice (as % of $CD3^+$ cells) three days after adoptive transfer. **(F)** Quantification of the fraction of WT (CD45.1) and SA (CD45.2) $CD8^+$ T cells in *Rag1*$^{-/-}$ mice three days after adoptive transfer. NS, not significant.

Data are shown as Mean±SEM (n=5-6).

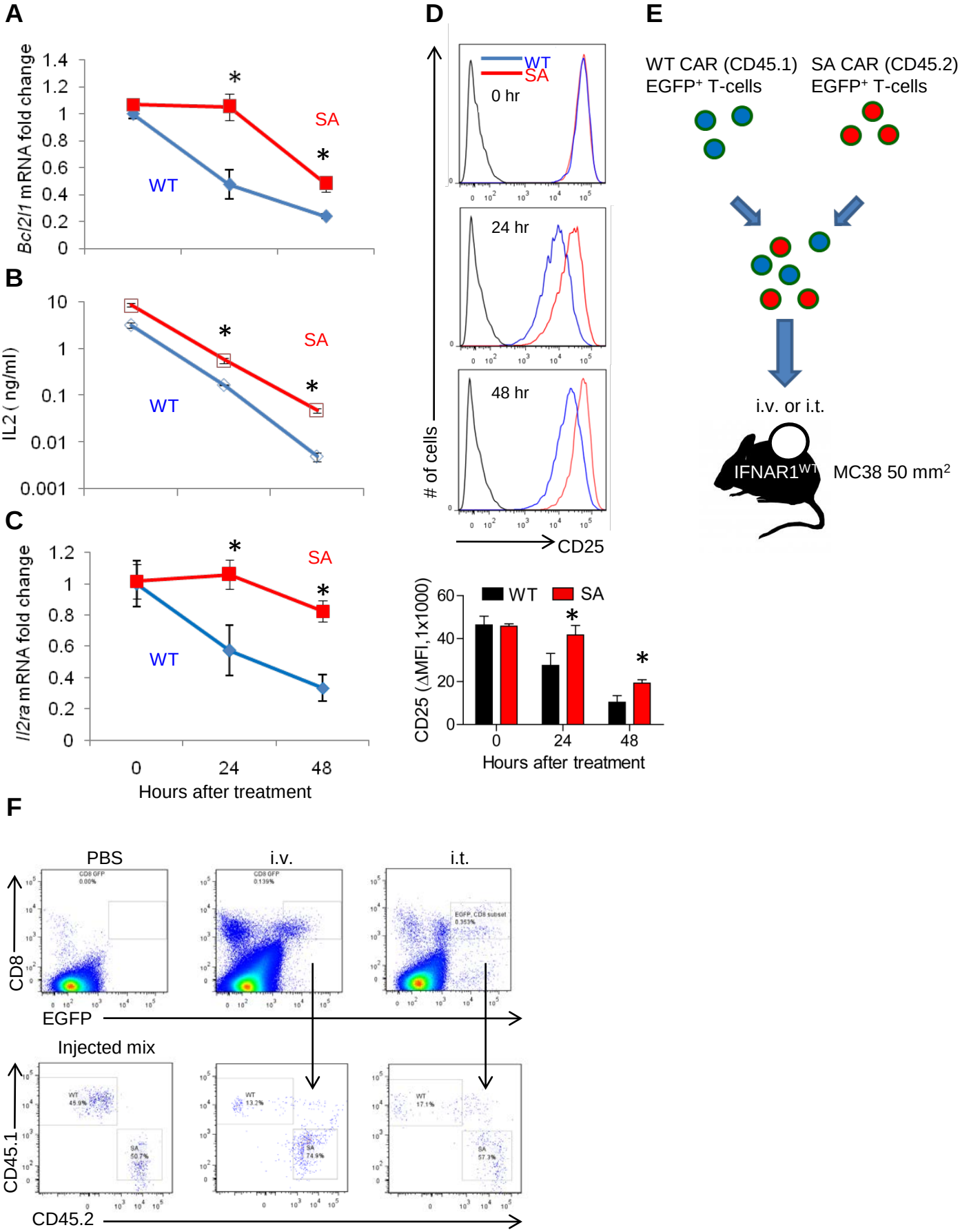
A
Bcl2l1 mRNA fold change
1.4
1.2
1
0.8
0.6
0.4
0.2
0
SA
WT
*
*
B
IL2 (ng/ml)
10
1
0.1
0.01
0.001
SA
WT
*
*
C
Il2ra mRNA fold change
1.2
1
0.8
0.6
0.4
0.2
0
SA
WT
*
*
0
24
48
Hours after treatment
D
WT
SA
0 hr
24 hr
48 hr
# of cells
CD25
CD25 (ΔMFI, 1x1000)
60
40
20
0
WT
SA
*
*
0
24
48
Hours after treatment
E
WT CAR (CD45.1)
EGFP+ T-cells
SA CAR (CD45.2)
EGFP+ T-cells
i.v. or i.t.
IFNAR1WT
MC38 50 mm2
F
PBS
i.v.
i.t.
CD8
EGFP
Injected mix
CD45.1
CD45.2

Figure S7 (related to Figure 6). Role of downregulation of IFNAR1 on cytotoxic T lymphocytes in their survival within tumor microenvironment.

(A) Splenocytes from WT OT1 or SA OT1 mice were exposed to SIINFEKL peptide (0.5 μg/mL for 48 hr) and then cultured for indicated times and evaluated for viability and expression of apoptotic regulators. RT-PCR analysis of *Bcl2l1* mRNA expression levels in activated splenocytes from WT and SA OT-1 mice. Data are shown as Mean±SD (n=3 for each of 3 independent experiments). **(B)** ELISA analysis of IL2 levels in the supernatants of activated splenocytes cultured as in panel A. Data are shown as Mean±SD (n=3 for each of 3 independent experiments). **(C)** RT-PCR analysis of mRNA levels of *Il2α* chain expression in the culture of splenocytes from WT and SA OT-1 mice activated as in panel A. Data are shown as Mean±SD (n=3 for each of 3 independent experiments). **(D)** Representative histograms of CD25 expression on $CD3^+CD8^+$ cells at indicated time points from the culture of activated splenocytes from WT and SA OT-1 mice as described in panel A. Data are shown as Mean±SD (n=3 for each of 3 independent experiments, bottom panel). **(E)** Schematic representation of experiment described in Panel F. **(F)** Representative flow cytometry analysis of the fraction of viable FAP-CAR $EGFP^+$ WT (CD45.1) or FAP-CAR $EGFP^+$ SA (CD45.2) CTLs in the MC38 tumors 72 hr after injection (1:1 ratio) intravenously (i.v.) or directly into the tumors (i.t.) of WT MC38 tumor bearing mice.

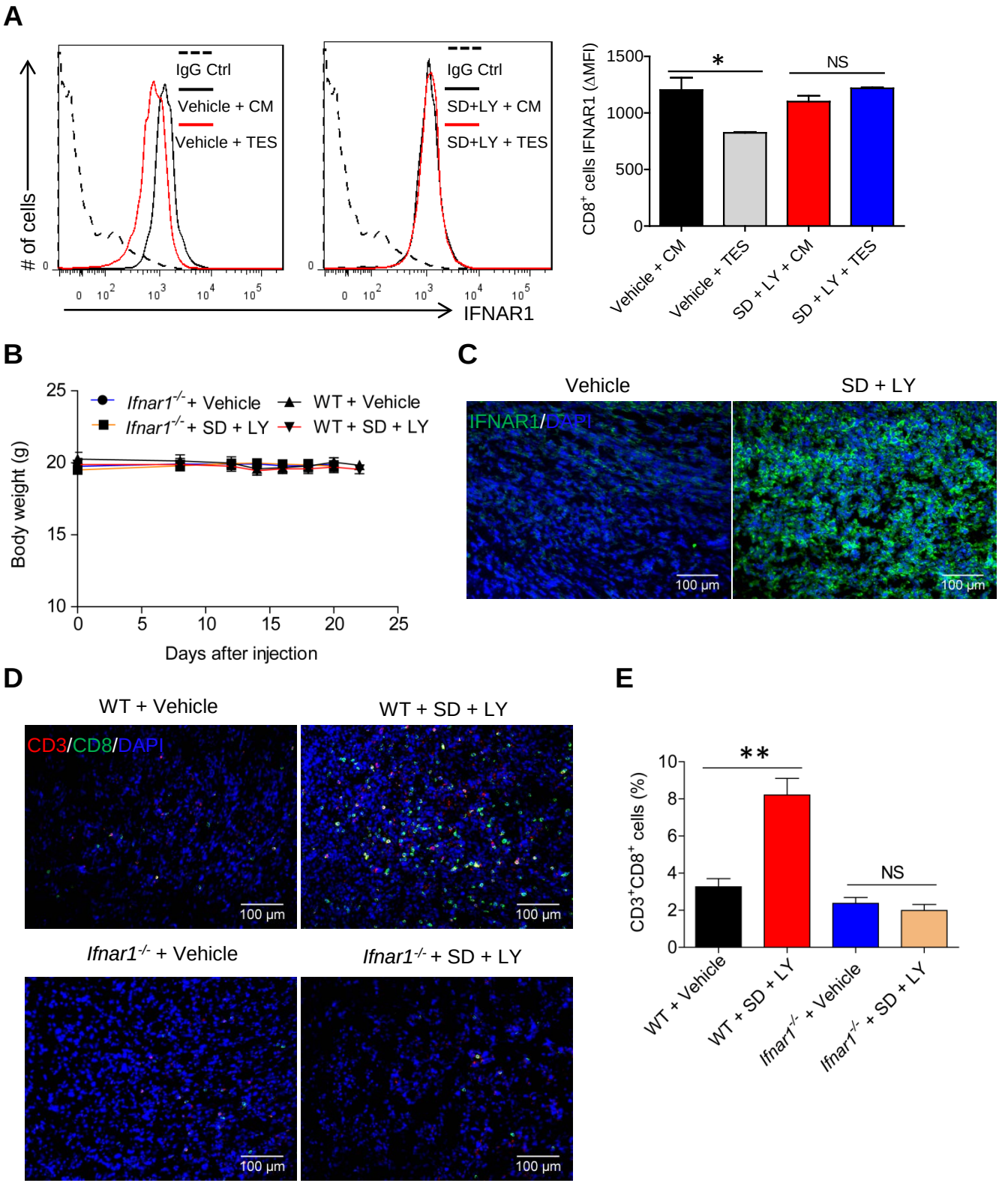
A
IgG Ctrl
Vehicle + CM
Vehicle + TES
SD+LY + CM
SD+LY + TES
# of cells
IFNAR1
CD8+ cells IFNAR1 (ΔMFI)
1500
1000
500
0
*
NS
Vehicle + CM
Vehicle + TES
SD + LY + CM
SD + LY + TES
B
Ifnar1-/- + Vehicle
Ifnar1-/- + SD + LY
WT + Vehicle
WT + SD + LY
Body weight (g)
Days after injection
C
Vehicle
SD + LY
IFNAR1/DAPI
100 μm
D
WT + Vehicle
WT + SD + LY
CD3/CD8/DAPI
Ifnar1-/- + Vehicle
Ifnar1-/- + SD + LY
100 μm
E
CD3+CD8+ cells (%)
**
NS
WT + Vehicle
WT + SD + LY
Ifnar1-/- + Vehicle
Ifnar1-/- + SD + LY

Figure S8 (related to Figure 8). Effects of agents stabilzing IFNAR1 on the immune privileged niche.

(A) Cell surface IFNAR1 levels on WT $CD3^+CD8^+$splenocytes pre-treated with SD-208 PKD inhibitor (SD, 1 μM) and LY2228820 p38 inhibitor (LY, 1 μM) or vehicle (DMSO) for 2 hr and then treated with tumor explant supernatant (TES) or control media (CM) for additional 2 hr. A representative experiment and overall quantification (Mean±SEM from 3 independent experiments, each in triplicates) are shown. **(B)** Body weight of *Ifnar1*$^{-/-}$ and WT mice treated with SD-208 PKD inhibitor (SD) and LY2228820 p38 inhibitor (LY) as described in Figure 8E and Materials and Methods. Data are shown as Mean±SEM (n=5 from each of 3 independent experiments). **(C)** Immunofluorescent analysis of IFNAR1 levels (green) in MC38 tumors grown in WT mice treated with vehicle (left panel) or PKD2 (SD) and p38 (LY) inhibitors (right panel) as described in Figure 8B-E. Samples were contrasted with DAPI (blue). Scale bars: 100 μm. **(D)** Representative immunofluorescent analysis of $CD3^+CD8^+$ cells infiltration of MC38 tumors from Panel C. Tumor sections stained with indicated antibodies and contrasted with DAPI (blue). **(E)** Quantification of experiment described in Panel D. Scale bars: 100 μm. Percent of $CD3^+CD8^+$cells among total DAPI-positive cells calculated as Mean±SEM from at least 20 random fields of 5 tumors for each treatment group is shown.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Experiments in animals and statistical analysis.

All experiments with animals were carried out under the protocol 803995 approved by the IACUC of The University of Pennsylvania. All mice were on the C57Bl/6 background and had water ad libitum and were fed regular chow. *Rag1*$^{-/-}$, OT-1, *Ubc9-Cre*^{ERT2} and CD45.1 congenic mice were obtained from the Jackson Laboratory. Littermate C57BL/6 ("WT") and C57BL/6 *Ifnar1*^{S526A} mice ("SA") were described previously (Bhattacharya et al., 2014). *Mapk14*$^{f/f}$ and *Ifnar1*$^{-/-}$ mice were generously provided by Yibin Wang (UCLA) and Susan Weiss (University of Pennsylvania), respectively. Other strains were generated by intercrossing and the littermates of 6-8 weeks of age were used in the experiments. Mice were maintained in a specific pathogen-free facility in accordance with American Association for Laboratory Animal Science guidelines. Littermate animals from different cages were randomly assigned into the experimental groups, which were either co-housed or systematically exposed to other groups' bedding to ensure equal exposure to all group's microbiota.

All described results are representative of at least three independent experiments (n=5 mice per group in each experiment unless specified otherwise). All in vitro analyses using cells or tissues from each of these animals were done at least in biological triplicates (e.g. samples from 3 tumors, 3 spleens, etc). Data are presented as average ± S.E.M. Statistical analysis was performed using Microsoft Excel (Microsoft) or GraphPad Prism 7 software (GraphPad Prism Software Inc). Unpaired Student t test was used for the comparison between two groups. One-way ANOVA or two-way ANOVA analysis followed by the Bonferroni post-hoc test were used for the multiple comparisons. Repeated-measure two-way ANOVA (mixed-model) followed by the Bonferroni post-hoc test was used for the analysis of tumor growth curve. The Kaplan-Meier survival analysis is based on reaching the endpoint of tumor volume ~200 mm^2. A value of $p < 0.05$ was considered significant.

Analysis of human cancers and statistical analysis

Use of pre-existing previously collected under informed consent human de-identified CRC tissue arrays and samples that could not be directly or indirectly linked to individual human subjects was exempt from the institutional review. Archived de-identified and de-coded, formalin-fixed, paraffin-embedded colorectal carcinomas and normal colon tissues in standard or CEMA tissue array format (LeBaron et al., 2005) were obtained from US Biomax, Inc (Derwood, MD; 242 adenocarcinomas with clinical outcome and matched adjacent normal tissue), and Thomas Jefferson University (54 adenocarcinomas and 30 normal epithelia (Wilson et al., 2014). Tissue array containing samples (40 adenocarcinomas and 20 normal epithelia) from an additional de-identified and coded cohort obtained from the University of Pennsylvania was previously described (King et al., 2011).

IFNAR1, phospho-tyrosine Stat2 (pTyr-Stat2) or GLUT1 were detected by chromogen or immunofluorescence IHC as previously described (Peck et al., 2011) with the following specifications: antigen retrieval with citric acid buffer (pH 6.0), rabbit polyclonal IFNAR1 antibody (Sigma, HPA018015; 1:200), rabbit polyclonal pTyr-Stat2 antibody (Sigma, SAB4503836; 1:400), mouse monoclonal GLUT1 (Thermo-Fisher, SPM498; 1:200), mouse monoclonal anti-pancytokeratin (clone AE1/AE3, DAKO, 1:100). Quantitative analysis was performed as previously described (Peck et al., 2016) using the ScanScope FL line scanner (Leica Biosystems) to capture high-resolution digital images followed by quantification of biomarker levels using Tissue Studio image analysis software (Definiens). Briefly, user-guided machine learning was performed to identify DAPI-stained cell nuclei and cytokeratin-stained cells within the epithelial or stromal regions of each tissue. Mean nuclear or cytoplasmic biomarker signal intensity then was calculated for epithelial and stromal regions. Differences in normal versus tumor specimens were compared by boxplot of log-transformed values and two-tailed t-test. Elements of the boxplot included median (dark line), 25-75 percentile (box), minimum and maximum values (whiskers) and scatter plot overlayed on the boxplot (each circle representing an individual data point).

For survival analysis, data-driven optimal cutpoint was determined by X-tile software (Camp et al., 2004) which dichotomized cases into high and low IFNAR1 categories. IBM SPSS Statistics 24 software was used for the survival analyses. Survival was analyzed using Kaplan-Meier survival curve estimator, log-rank test and Cox proportional hazards model.

Cell lines, culture conditions and viral infection. 293T and Phoenix cells (kindly provided by Z. Ronai, The Burnham Institute, San Diego, CA), MC38 colon adenocarcinoma cells (provided by S. Ostrand-Rosenberg, University of Maryland, Baltimore, MD), MC38OVAbright (Gilfillan et al., 2008), a gift from M. Smyth, Peter MacCallum Cancer Center), MC38mRFP cells (Powell et al., 2011), a gift from M. Wong, Oregon Health & Science University) were maintained at 37°C with 5% CO_2 in DMEM supplemented with 10% heat-inactivated FBS, penicillin, streptomycin and L-glutamine.

For lentiviral transduction, 293T cells were transfected with pCIG plasmids encoding enhanced green fluorescent protein (EGFP) or mouse ubiquitination deficient mutant IFNAR1^{S526A} together with EGFP (described in (Huangfu et al., 2012) using Lipofectamine Plus (Invitrogen). After 24-48 hr supernatants were passed through 0.45 µm nylon filter, mixed with Polybrene (8 µg/ml, Santa Cruz Biotech) and transferred to plated cancer cells. Transduction efficiency was verified by FACS analysis and usually was more than 80%. Flow-sorted GFP-positive cells were used for injections and tumor growth experiments described in Figures 2C and S2B-C.

Immunoprecipitation, immunoblotting and ELISA. Tumor samples or normal tissues were flash frozen and lysed by sonication. IFNAR1 was immunoprecipitated with anti-IFNAR1 antibodies (Leinco Technologies). Western blots were stained with rabbit anti-IFNAR1 antibodies (MyBioSource), anti-PKD2 (Bethyl Laboratories), anti-phospho-PKD2 (Abcam), anti-p38 (Santa Cruz Biotech), anti-phospho-p38 (Cell signaling) or anti-β-actin antibodies followed by anti-rabbit-HRP conjugated secondary antibodies (both from Sigma-Aldrich). ELISA-based assessment of levels of IFNγ, Granzyme B and IL2 in tumor lysates or CTLs supernatants was carried out using appropriate kits (Cat# BMS609, eBioscience for IFNγ; Cat# BMS6029, eBioscience for Granzyme B and Cat#431004, Biolegend for IL2).

Cancer cell transplantation and organ harvest. Cancer cells were inoculated subcutaneously in the right flank at 1x10^6 cells (unless specified otherwise) in 100 µl Dulbecco-modified phosphate buffered saline (DPBS). Tumors were measured by caliper and size was calculated as (length × width). Mice were euthanized when the tumor reached 200-300 mm^2, organs were dissected, and single cell suspension was prepared from tumor tissue by digestion of 1- to 2-mm pieces with 1 mg/ml Collagenase D (Roche) and 100 µg/ml DNase I (Roche) in RPMI medium with 2% FBS for 1 hours with continuous agitation. Digestion mixture was passed through 70 µm cell strainer and washed with PBS supplemented with 2mM EDTA and 1% FBS. Single cells suspension from spleen was obtained by mechanical disruption. After erythrocyte lysis using red blood cell lysis buffer (RBC lysis buffer (155 mM NH_4Cl, 12 mM $KHCO_3$, and 0.1 mM EDTA) cells were used for immune staining. Intestinal epithelial cells (IECs) were isolated from the distal part of the colon by incubation of colon pieces in Hank's balanced salt solution containing 5 mM EDTA at 4 °C for 30 min.

Tumor explant supernatants (TES) were prepared from excised non-ulcerated tumors ~1.5 cm in diameter as described elsewhere (Ramakrishnan et al., 2014). Briefly, tumor tissues were bathed in 70% isopropanol for 30 seconds and then transferred to a Petri dish. Tumors were minced into pieces < 3 mm in diameter and digested in 2 mg/ml collagenase Type IV at 37˚C for 1 hr. The digested tissue pieces were then pressed through a 70 µm cell strainer to create a single-cell suspension. Cells were washed with PBS and re-suspended in RPMI 1640 supplemented with 20 mM HEPES, 2 mM L-glutamine, 200 U/ml penicillin plus 50 mg/ml streptomycin, and 10% FBS. Cells were cultured for 24 hr at 10^7 cells/ml, and the cell free supernatants were collected and kept at -80˚C. The complete RPMI 1640 medium was control medium (CM).

Tumor conditioned media were prepared from cultured MC38 cells. Briefly, MC38 cells were cultured with DMEM supplemented with 10% FBS, 1% Penicillin/Streptomycin. When the cells were

80% confluent, the medium was replaced with serum-free medium. 48 hr later, the medium were collected and filtered with a 0.2 μm filter, stored at -80^{o}C until use.

AOM/DSS colorectal carcinogenesis. Co-housed experimental mice were intraperitoneally injected with 10 mg/kg azoxymethane (AOM; Sigma). A week later, they were supplied with tap water containing 2.5% dextran sodium sulfate (DSS, TdB Consultancy) for 7 days, followed by 14 days of regular water. This cycle was repeated three times and mice were sacrificed 2 weeks after the end of the last DSS cycle or at the end of 10 weeks. Colons were harvested, washed of feces with DPBS, and slit open longitudinally to count tumors. Tumors were flash frozen in liquid nitrogen or embedded into OCT media.

Flow cytometric analysis. Single-cell suspension was re-suspended in FACS buffer (PBS, 1% BSA) and blocked with anti-mouse CD16/32 antibodies and rat IgG for 10 min prior to staining with specific antibodies. Antibodies against cell surface markers: anti-CD3-FITC (APC, 145-2C11), anti-CD4-APC-Cy7 (RMA4-5), anti–CD8a-AF700 (PE, APC-Cy7, 53-6.7), anti-CD11b-PerCP-Cy5.5 (FITC, M1/70), anti-DX5-PE (DX5), anti–Gr-1–PE (RB6-8C5), anti-CD45-APC (FITC, 30-F11), anti-F4/80-APC (BM8), anti-CD11c-PE (PE-Cy7, FITC, N418), anti-NKp46-FITC (29A1.4), anti-NK1.1 PE-Cy7 (APC, PK136), anti-CD103 (BV 605^{TM}, 2E7), anti-I-A/I-E-PE/Cy7 (MHC-II, M5/114.15.2), anti-H-2K^{b}-SIINFEKL (PE, 25-D1.16), anti-Ly6C-FITC (APC-Cy7, HK1.4), anti-Ly6G APC-Cy7 (PE, 1A8), anti-IFNAR1-PE (MAR1-5A3) were purchased from Biolegend. Samples were mixed with DAPI (1 ug/ml) and acquired on LSRFortessa flow cytometer (BD Biosciences), and data were analyzed with FlowJo software (Tree Star).

Immunofluorescence and immunohistochemistry in mouse tissues. Cancer cells, tumors and organs harvested from mice were frozen in Tissue-Tek O.C.T. compound and cryosectioned in Leica CM3050 S Cryostats, fixed in acetone, washed and blocked with PBS containing 5% goat serum. Antibodies against cell surface markers: anti-CD3-biotin (145-2C11), anti-CD8a-AF488 (53-6.7) and streptavidin-dylight594 were purchased from Biolegend, antibody against IFNAR1 was from Sino Biological, Alexa Fluor 488 labeled goat antibodies against rabbit or rat IgG were from Life technologies. The sections were incubated with primary antibodies for 1 hr, washed and incubated with secondary Alexa Fluor 488 labeled goat antibodies for 1 hr. For double immune-staining sections were simultaneously stained with different primary Alexa Fluor 488-labeled and biotinylated antibodies followed by the incubation with streptavidin-dylight594. The sections were then washed and mounted with cover slip in ProLong gold antifade reagent containing 4′,6-diamidino-2-phenylindole (DAPI). Immunofluorescent staining of human samples was performed on a Dako Autostainer. Antigen retrieval was performed using the DAKO PT-module with Tris/EDTA buffer (pH 9.0). Slides were coincubated with rabbit anti-pYSTAT2 (SAB4503836; Sigma-Aldrich) or anti-IFNAR1 (HPA018015; Sigma-Aldrich) and mouse anti-CD3 (F7.2.38; MDR), anti-CD138 (MI15; Biolegend), anti-CD68 (PGM1; Abcam), anti-Glut1 (SPM498; Abcam), anti-CD4 (1F6; Thermo), or anti-pancytokeratin (AE1/AE3; Dako). Slides were incubated with horseradish peroxidase–conjugated anti-mouse immunoglobulin G or Alexa-555-conjugated anti-rabbit immunoglobulin G secondary antibodies, followed by incubation with Cy5-tyramide (Perkin Elmer, Waltham, MA). Slides were coverslipped using DAPI-containing mounting media.

T cell proliferation analysis in vivo

Rag1$^{-/-}$ mice were s.c. injected with MC38-OVA (bright) (2x10^{6}/mouse). When the tumor size was reached to approximately 100 mm^{2}, mice received an intravenous transfer CFSE-labeled WT OT-1 T cells or SA OT-1 T cells (5x10^{6} per mouse). At 48 hr and 72 hr after cells transfer, the splenocytes were isolated and CFSE level in CD3^{+}CD8^{+} cells were analyzed by FACS.

NK killing assay in vivo

8 weeks *Rag1*$^{-/-}$ mice were intraperitoneally injected daily with 100 μg anti-NK1.1 antibody (Cat# 108712, Biolegend) or its IgG2a control antibody (Cat# 400281, Biolegend) for three days. After three days of injections, mice were bled to check the efficacy of NK cell depletion. Then WT (CD45.1) and SA (CD45.2) splenocytes were isolated and mixed in 1:1 ratio. The mixed splenocytes (total cell

number $2x10^7$ in 200µl PBS for each mouse) were intravenously injected into the *Rag1*$^{-/-}$ mice. Three days after cell transfer, splenocytes were isolated and the fraction of WT and SA $CD3^+CD8^+$ cells were analyzed by FACS.

Antibody and small molecules treatments. To deplete NK cells mice were intraperitoneally injected with 100 µg of anti-NK1.1 antibodies (PK136, Biolegend) in 200 µl of DPBS at day -2, 0, 7, 14, 21, 27 (relative to tumor injection). For $CD8^+$ T cell depletion experiments, 200 µg anti-CD8 (2.43; Bio-XCell) or control Ab (LTF-2; Bio-XCell) per mouse was delivered by i.p. injection at day -1, 0, 4, 8, 12, 16, 18, 22. For the PD-1 blockade experiment, 100 µg anti–PD-1 (RMP1-14; Bio-XCell) or control Ab (2A3; Bio-XCell) was administered i.p. to mice every 4 days starting at day 8 for a total of six times. For IFNAR1 neutralizing experiments, the mice were injected with neutralizing IFNAR1 antibody (I-401, Leinco Tech) or IgG control antibody (I-536, Leinco Tech) at 1 mg/mouse (i.p. once a day; one injection for every five days starting on the next day after tumor cells inoculation).

To inhibit PKD and p38 kinases in vivo, MC38 tumor-bearing mice were treated by gavage with PKD inhibitor SD-208 (Selleckchem, 3mg/kg) and p38 inhibitor LY2228820 (Selleckchem, 1mg/kg) prepared in methylcellulose at the day 8, 9, 10, 12, 14, 16, 18, 20, and 22 after MC38 cells ($1x10^6$) inoculation. Gavage with analogous volumes of pure methylcellulose was used as a vehicle control procedure. For in vitro experiments, inhibitors were administered in DMSO at 1µM.

Bone marrow chimeras. Mixed bone marrow chimeric mice were obtained as described elsewhere (Diamond et al., 2011; Fuertes et al., 2011). Briefly, pooled tibial and femoral bone marrow cells from donor mice were lysed with RBC lysis buffer. To generate the "SA^L" mixed bone marrow chimeras, four parts of bone marrow from *Rag1*$^{-/-}$ mice (WT *Ifnar1*) were mixed with one part of bone marrow from SA mice (*Ifnar1*^{SA}). To prepare the "SA^M" chimeras, 4 parts of bone marrow from SA Rag1$^{-/-}$ mice were mixed with one part of bone marrow from WT mice. Control "SA^{M+L}" and "WT^{M+L}" chimeras were prepared by mixing four parts of bone marrow from SA *Rag1*$^{-/-}$ or WT *Rag1*$^{-/-}$ mice with 1 part of bone marrow from SA or WT mice, respectively. Resulting mixtures of indicated bone marrow cells injected retro-orbitally into recipient WT mice (10^7 cells per each recipient mouse) irradiated with a single dose of 9.5 Gy as described previously elsewhere (Diamond et al., 2011; Fuertes et al., 2011). Animals were maintained on trimethoprim-sulfamethoxazole (Hi-Tech Pharmacal) antibiotic water for 1 day prior and 2 weeks after irradiation, and tumor transplantation of chimeric mice was performed at least 8 weeks after reconstitution. Hematopoietic reconstitution of all animals was verified by flow cytometry of splenocytes at the end of the experiment. The levels of IFNAR1 on $CD11b^+$ and $CD3^+$ cells analyzed in tumor bearing mice at the end of experiments were used as a control for the expression of the *Ifnar1*^{SA} allele in specifically myeloid or lymphoid cell compartments (as shown in Supplemental Figure 4C).

Microarray analyses with Illumina whole-genome arrays. WT or SA mice were inoculated with MC38mRFP tumor cells and, upon tumor growth to volumes indicated in Figure 3A, tumors were excised and digested to produce the single-cell suspensions. Enriched total stromal (mRFP-negative) cells were obtained from flow sorting and used for gene expression profiling. Total RNA was isolated with miRNeasy mini kit (QIAGEN). Biotin-labeled cRNA preparations were obtained using TargetAmp™-Nano Labeling Kit (Epicentre) as recommended by the manufacturer. Thereafter 0.75 µg cRNA was hybridized to Illumina Sentrix Mouse-6 v.1 BeadChips, which were scanned with an Illumina BeadStation 500 (both from Applied Biosystems-Life Technologies Inc.). Data were collected with Illumina BeadStudio 3.1.1.0 software, and statistical analyses were conducted on the IlluminaGUI R-package. Gene sets from microarray data were analyzed for overlap with curated data sets (C5, H) in MSigDB using the web interface available at http://www.broadinstitute.org/gsea/msigdb/index.jsp. The raw data have been deposited to NCBI (**accession number GSE76889**). Prognostic value of the 30 gene signature that separates early SA tumor from early WT tumors was tested using the PROGgene2 software (Goswami and Nakshatri, 2013) and published gene expression data (adjusted for stage) from two separate datasets GSE41258 and GSE30378 (Agesen et al., 2012; Sheffer et al., 2009).

Real-time RT-PCR. RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) and cDNA was prepared using the High Capacity cDNA Reverse Transcription Kit (Life Technologies).Real-time PCR was performed using SYBR reagent (Applied Biosystems, Carlsbad, CA, USA). The expression of each gene was calculated based on the cycle threshold (CT), set within the linear range of DNA amplification. The relative expression was calculated by Ct method, with normalization of raw data to a housekeeping gene (β-actin). The following primer sequences were used:

Ifnar1 FW 5′-CGACCAAGTGTGAATTCTCTTTAC , RV 5′- ATCAACCTCATTCCACGAAGAT; ; *Ifnb* FW 5'-AGCTCCAAGAAAGGACGAACAT-3', RV 5'-GCCCTGTAGGTGAGGTTGATCT-3'; *Ifng* FW 5'-ATGAACGCTACACACTGCATC-3', RV 5'-CCATCCTTTTGCCAGTTCCTC-3'; *Isg15* FW 5'-GGAACGAAAGGGGCCACAGCA-3', RV 5'-CCTCCATGGGCCTTCCCTCGA-3'; *Oas2* FW 5'-CCCTGTGAAGGAAGTGGCTA -3', RV 5'- CTGTTGGAAGCAGTCCATGA -3'; *Irf7* FW 5'-CCACACCCCCATCTTCGA-3', RV 5'-CCTCCGAGCCCGAAACTC-3'; *Stat1* FW 5'-CGCGCATGCAACTGGCATATAACT-3', RV 5'-AAGCTCGAACCACTGTGACATCCT-3'; *Tnfa* FW 5'-CATCTTCTCAAAATTCGAGTGACAA, RV 5'-TGGGAGTAGACAAGGTACAACCC-3'; *Il6* FW 5'-GAGGATACCACTCCCAACAGACC-3', RV 5'-AAGTGCATCATCGTTGTTCATACA-3' ; *Gzmb* FW 5'-CCCCGATGATCTCCCCTGCCTTTG-3', RV 5'-TCTTGACGCTGGGACCTAGGCG-3'; *Bcl2l1* FW 5'-TGCATTGTTCCCGTAGAGATCCA-3', RV 5'-TCTGAATGACCACCTAGAGCCTT-3'; *Il2ra* FW 5'-AACACCACCGATTTCTGGCT-3', RV 5'-GTGGGTTGTGGGAAGTCTGT-3'; *Ido1* FW 5′-CCCACACTGAGCACGGACGG-3', RV 5′-TTGCGGGGCAGCACCTTTCG-3'; *Ly6c1* FW 5′-TTGTCTGAGAGGAACCCTTC-3', RV 5′-GCACTCCATAGCACTCGTAG-3'; *Cxcl9* FW 5′-ATTGTGTCTCAGAGATGGTGCTAATG-3', RV 5′-TGAAATCCCATGGTCTCGAAAG-3'; *Gbp2* FW 5′-GATCCACATGTCGGAACCCA-3', RV 5′-GGAAAAGCCTGTCCTCTTCCC-3', *Pdcd1lg* FW 5′-GACCAGCTTTTGAAGGGAAATG-3', RV 5′-CTGGTTGATTTTGCGGTATGG-3', *Actb* FW 5′-AGAGGGAAATCGTGCGTGAC-3', RV 5′-CAATAGTGATGACCTGGCCGT-3'.

T-cell cells isolation, CFSE staining, activation and adoptive transfer. Pan T cells and $CD8^+$ T cells were purified from mouse spleens with naive T cell isolation kit from Stem Cell and naive $CD8^+$ cell isolation kit from Miltenyi Biotec respectively. $1x10^7$ T cells were reconstituted in RPMI media, stained with 2.5 mM CFSE at room temperature for 5 min and washed with DPBS supplemented with 10% FBS. For antigen specific activation of $CD3^+CD8^+$ cells, splenocytes from OT-1 WT and SA were stimulated with SIINFEKL peptide (0.5 μg/ml). For adoptive transfer of activated T-cells, splenocytes from OT-1 WT and SA were stimulated with SIINFEKL (0.5 μg/ml) peptide in presence of human IL2 (50 IU/ml). After 2 days, cells were washed and split into new medium supplemented with IL2. Following 3 days of activation $2x10^7$ T cells were used for 4 consecutive daily i.v. injections.

Trans-well migration assay. Naive T cells from CD45.1 WT animals and CD45.2 SA animals were activated with plate bound anti-CD3 antibodies and soluble anti-CD28 antibodies for 2 days. Then cells were transferred to new plates and rested for additional 2 days. For trans-well migration assay cells were reconstituted in RPMI 1640/0.5% BSA (assay media) and transferred to upper chambers (100 μl, $0.5x10^6$ cells) (insert). Inserts were then placed into 24 well plate filled with 600 μl of assay media with different concentration of CXCL12 and CXCL9. Plate was placed in CO_2 incubator at 37^oC. After 2 hr inserts were removed and transmigrated cells from the bottom chamber were spin down, manually count to determine the absolute number of migrated cells and stain with anti-CD3-APC/CD8-AF700/CD4-APC-Cy7/CD45.1-PE/CD45.1-FITC antibody cocktail and DAPI.

Analysis of antigen presentation and cross-presentation. $CD11c^+$ dendritic cells were isolated from mouse spleens with CD11c MicroBeads (Miltenyi Biotec) and incubated with the OVA257–264 peptide (SIINFEKL; AnaSpec), or control human papillomavirus E7 protein (49-57) peptide (RAHYNIVTF; AnaSpec) for 1 hr at 37°C in (RPMI supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 10% FBS, 50 μM 2-βME, and 2 mM L-glutamine (complete medium). The cells were further washed three times with DPBS, re-suspended in complete medium and plated at $2x10^4$ cells/well in 96-well round-bottom plates (Corning) containing $2x10^5$ CFSE-labeled OT-1 cells. Proliferation of T cells was analyzed by flow cytometry after 2.5 days of culture. To evaluate antigen cross-presentation $2.5×10^7$ splenocytes from C3H/Hej mice were incubated in 170 μl of hypertonic

medium (0.5 M sucrose, 10% wt/vol polyethylene glycol 1000, and 10 mM Hepes in RPMI 1640, pH 7.2) alone or in the presence of 10 mg/ml ovalbumin (Calbiochem) for 10 min at 37°C. 2.2 ml of pre-warmed hypotonic medium (40% H_2O, 60% RPMI 1640) was added followed by an additional 2 min incubation at 37°C. The cells were centrifuged, washed twice with cold PBS and irradiated (1,350 rads). For all cultures, $5x10^4$ irradiated ovalbumin loaded $CD45.2^+$ C3H/Hej splenocytes were incubated in a round bottom 96-well plate (final volume: 200µl) with $5x10^4$ CFSE labeled $CD45.1^+$ OT-1 transgenic T cells and $5x10^4$ splenic $CD45.2^+CD11c^+$ DCs from SA and WT mice in the presence of GM-CSF (150 IU/ml). As a positive control, $5x10^4$ CFSE labeled $CD45.1^+$ OT-1 transgenic T cells and $5x10^4$ splenic $CD45.2^+CD11c^+$ cells from WT or SA mice were cultured in the continuous presence of 1 µM SIINFEKL (Ova 257-264) peptide. After 72 hr, cells were stained for CD45.1-APC and CD8-APC-Cy-7 and percentages of $CD45.1^+CD8^+CFSE^{low}$ cells were assessed by flow.

To estimate antigen presentation in vivo naive $IFNAR1^{WT}$ T cells were isolated from the spleens of OT-1 CD45.1 mice, labeled with CFSE and injected into SA and WT mice through the retro-orbital sinus (each mouse received $2.5x10^6$ cells). Next day mice were s.c. injected with MC38OVA cells ($1x10^6$ cells) into the right flank. After 6 days spleens were isolated and the percentage and proliferation of $CD45.1^+CD45.2^-CD8^+$ cells were quantified.

To analyze the antigen cross presentation by intra-tumoral DCs, *Rag1*$^{-/-}$ WT and *Rag1*$^{-/-}$ SA mice were subcutaneously injected with $2x10^6$ MC38-OVA^{bright} cells (100 µl). Tumors were harvested (at size ~100 mm^2) and digested. The levels of OVA peptide (SIINFEKL)-H2Kb complex on the surface of $CD45^+CD11c^+MHCII^+CD103^+$ cells were assessed by flow cytometry using the 25-D1.16 monoclonal antibody (as described in (Baghdadi et al., 2013)).

Generation of mouse anti-FAP-CARs, retrovirus production and T cell transduction. The CAR migR1-Mu-FAP-CAR-GFP-JS construct used in this study was previously described (Wang et al., 2014). The mouse FAP-specific scFv used for generating the anti-FAP-CAR was derived from the FAP antibody (Wang et al., 2014). Retroviral supernatants were generated by co-transfecting Phoenix cells ($5×10^6$ cells plated on 100 mm plate 1 day before transfection) with the migR1-Mu-FAP-CAR-GFP-JS plasmid (18 µg/plate) and pCI-Eco packaging plasmid (9 µg/plate) the appropriate using Lipofectamine reagent 2000 (Invitrogen). Retroviral supernatants were collected at 48 hours after transfection, passed through 0.45 µm nylon filter, mixed with Polybrene (5 µg/ml, Santa Cruz Biotech) and transferred to 24-well plates. Activated T cells ($2×10^6$ per well, at $2×10^6$ cells/ml in complete media supplemented with human IL2 (100 U/ml, eBioscience)) were then spun onto the retrovirus plates for 60 min at 1,300 g. Activated T cells were transduced overnight, then a half of the supernatant was removed from the plates and fresh media (1.5 ml/well) were added to each well. Cells were expanded for 3-4 days and adoptively transferred by retro-orbital injection into venous plexus of anesthetized tumor-bearing mice when tumors were typically 30-50 mm^2. The adoptively transferred T cells demonstrated >50% transduction efficiency determined by GFP expression.

To generate activated mouse T cells for transduction, purified T-cells ($5×10^6$ per well, at $2.5×10^6$ cells/ml in IL2 containing T cell media) were activated for 48 hours in 12-well plates pre-coated with anti-CD3e (1 µg/well) and CD28 (2 µg/well) antibodies. To ablate p38α expression FAP-CAR T cells were generated from mice of the following genotypes: *Ifnar1*$^{+/+}$ *Mapk14*$^{f/f}$ (*Mapk14*$^{f/f}$, WT); Ubc-Cre^{ERT2} *Ifnar1*$^{+/+}$ *Mapk14*$^{f/f}$ (*Mapk14*$^{Δ/Δ}$, WT); *Ifnar1*$^{-/-}$ *Mapk14*$^{f/f}$ (*Mapk14*$^{f/f}$, Null); and Ubc-Cre^{ERT2} *Ifnar1*$^{-/-}$ *Mapk14*$^{f/f}$ (*Mapk14*$^{Δ/Δ}$, Null). These FAP-CAR T cells were treated for 24h with 1 µg/ml of 4-hydroxytamoxifen (Sigma) one day before adoptive transfer.

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