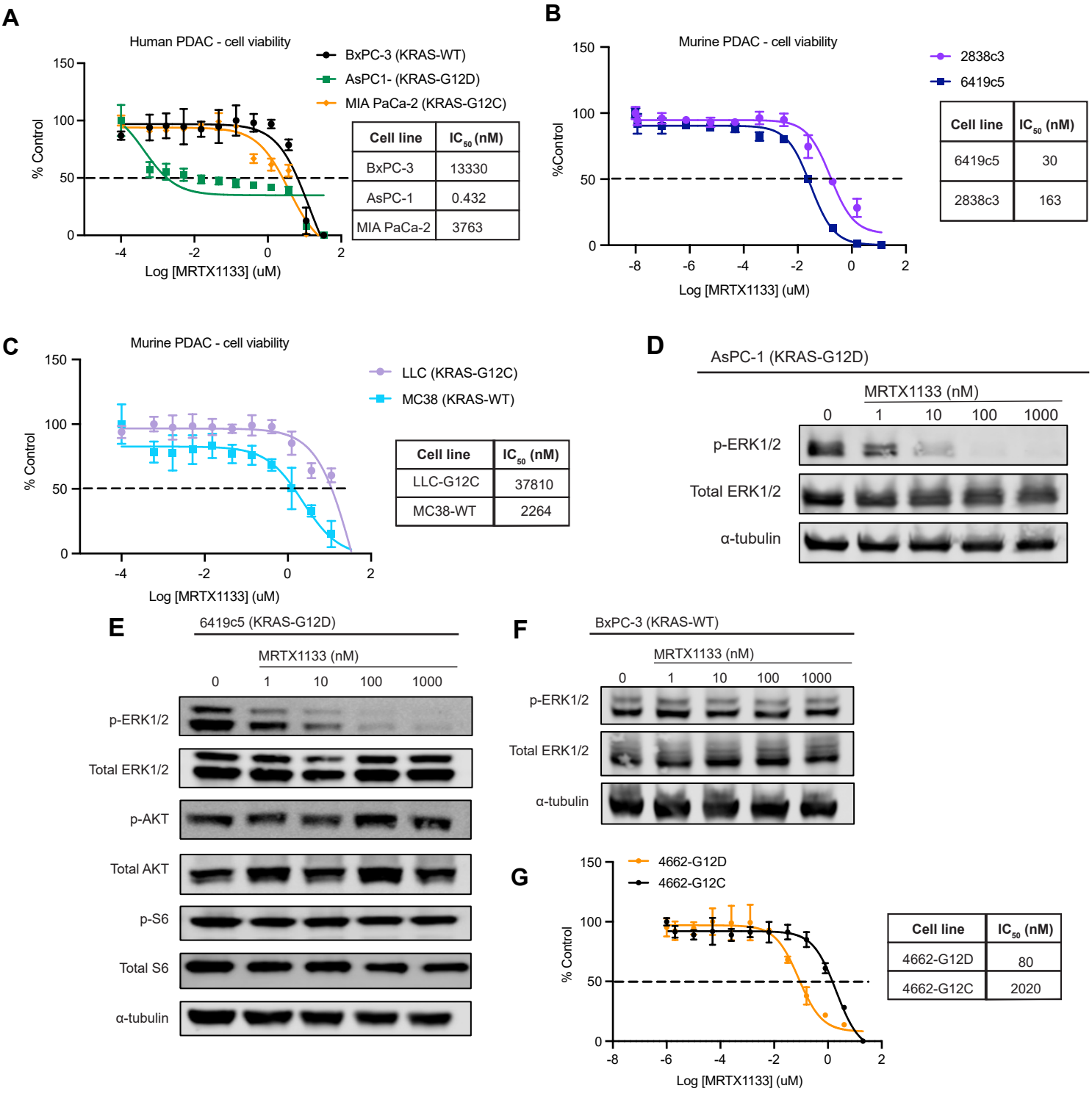
Supplementary Materials

Supplementary Figures S1-7

Supplementary Table

Figure S1


A
Human PDAC - cell viability
BxPC-3 (KRAS-WT)
AsPC1- (KRAS-G12D)
MIA PaCa-2 (KRAS-G12C)
% Control
Log [MRTX1133] (uM)
Cell line
IC50 (nM)
BxPC-3
13330
AsPC-1
0.432
MIA PaCa-2
3763
B
Murine PDAC - cell viability
2838c3
6419c5
%Control
Log [MRTX1133] (uM)
Cell line
IC50 (nM)
6419c5
30
2838c3
163
C
Murine PDAC - cell viability
LLC (KRAS-G12C)
MC38 (KRAS-WT)
% Control
Log [MRTX1133] (uM)
Cell line
IC50 (nM)
LLC-G12C
37810
MC38-WT
2264
D
AsPC-1 (KRAS-G12D)
MRTX1133 (nM)
0
1
10
100
1000
p-ERK1/2
Total ERK1/2
α-tubulin
E
6419c5 (KRAS-G12D)
MRTX1133 (nM)
0
1
10
100
1000
p-ERK1/2
Total ERK1/2
p-AKT
Total AKT
p-S6
Total S6
α-tubulin
F
BxPC-3 (KRAS-WT)
MRTX1133 (nM)
0
1
10
100
1000
p-ERK1/2
Total ERK1/2
α-tubulin
G
4662-G12D
4662-G12C
% Control
Log [MRTX1133] (uM)
Cell line
IC50 (nM)
4662-G12D
80
4662-G12C
2020

Supplementary Figure 1. MRTX1133 selectively inhibits $KRAS^{G12D}$ and downstream MAPK signaling *in vitro*.

(A) MRTX1133 dose response of BxPC-3 ($KRAS^{WT}$), AsPC-1 ($KRAS^{G12D}$), and MIA PaCa-2 ($KRAS^{G12C}$) cells after 72h of treatment. 3-fold serial dilutions were used, and cell viability was measured with CellTiter-Glo. IC_{50} values are listed.

(B) MRTX1133 dose response of two mouse $KRAS^{G12D}$ lines (2838c3, 6419c5) after 72h of treatment. 8-fold serial dilutions were used. IC_{50} values are listed.

(C) MRTX1133 dose response of LLC ($KRAS^{G12C}$) and MC38 (KRAS-WT) cells after 72h of treatment. 3-fold serial dilutions were used. IC_{50} values are listed.

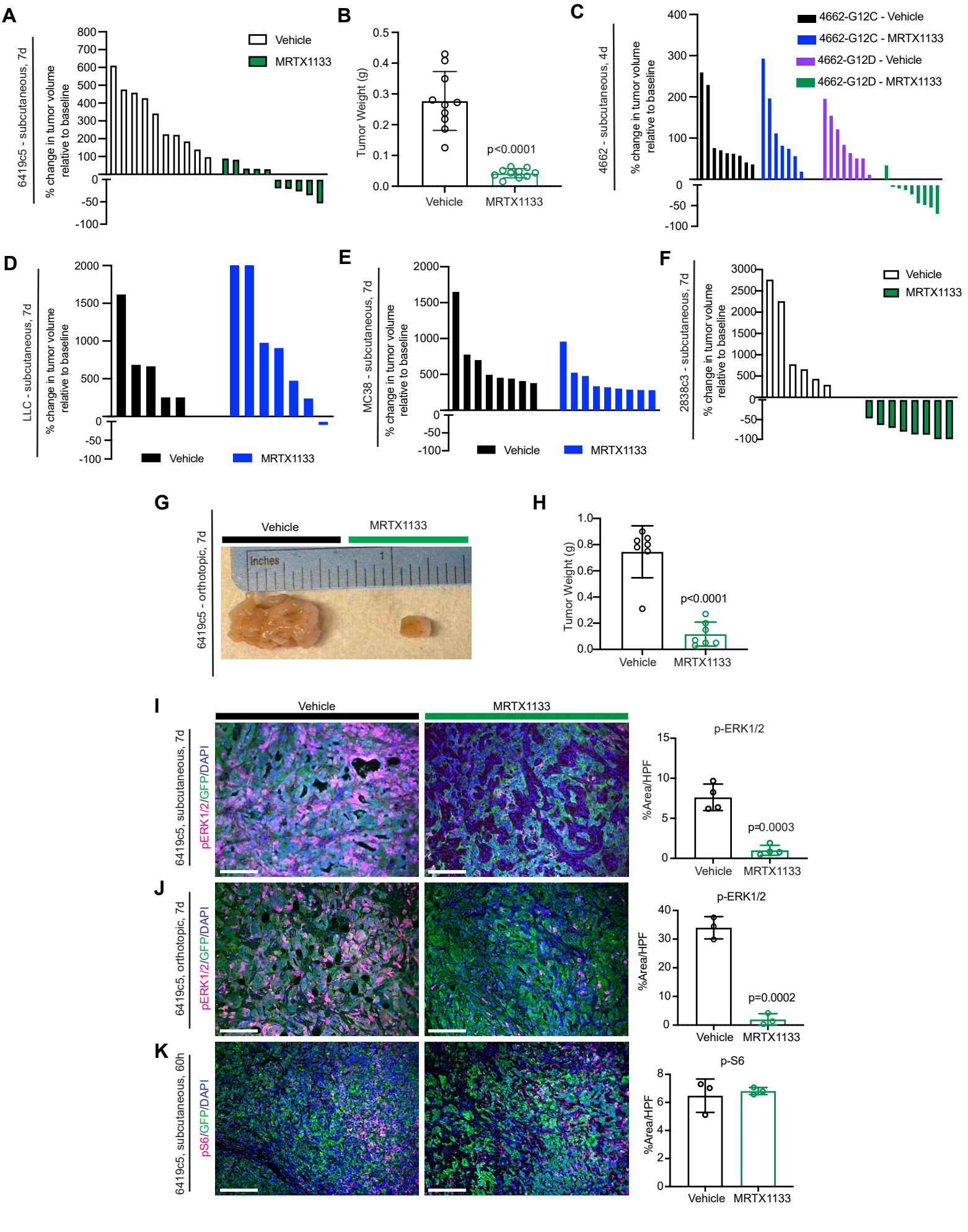
(D) Representative western blot of cell lysates from AsPC-1 ($KRAS^{G12D}$) cells treated with MRTX1133 or DMSO control for two hours.

(E) Representative western blot of cell lysates from 6419c5 ($KRAS^{G12D}$) cells treated with MRTX1133 or DMSO control for two hours.

(F) Representative western blot of cell lysates from BxPC-3 (KRAS-WT) cells treated with MRTX1133 or DMSO control for two hours.

(G) MRTX1133 dose response of 4662-$KRAS^{G12D}$ and 4662-$KRAS^{G12C}$ after 72h of treatment. 5-fold serial dilutions were used. IC_{50} values are listed.

Figure S2


A
6419c5 - subcutaneous, 7d
% change in tumor volume relative to baseline
Vehicle
MRTX1133
B
Tumor Weight (g)
p<0.0001
Vehicle
MRTX1133
C
4662 - subcutaneous, 4d
% change in tumor volume relative to baseline
4662-G12C - Vehicle
4662-G12C - MRTX1133
4662-G12D - Vehicle
4662-G12D - MRTX1133
D
LLC - subcutaneous, 7d
% change in tumor volume relative to baseline
Vehicle
MRTX1133
E
MC38 - subcutaneous, 7d
% change in tumor volume relative to baseline
Vehicle
MRTX1133
F
2838c3 - subcutaneous, 7d
% change in tumor volume relative to baseline
Vehicle
MRTX1133
G
6419c5 - orthotopic, 7d
Vehicle
MRTX1133
inches
H
Tumor Weight (g)
p<0.0001
Vehicle
MRTX1133
I
6419c5, subcutaneous, 7d
pERK1/2/GFP/DAPI
Vehicle
MRTX1133
p-ERK1/2
%Area/HPF
p=0.0003
Vehicle
MRTX1133
J
6419c5, orthotopic, 7d
pERK1/2/GFP/DAPI
p-ERK1/2
%Area/HPF
p=0.0002
Vehicle
MRTX1133
K
6419c5, subcutaneous, 60h
pS6/GFP/DAPI
p-S6
%Area/HPF
Vehicle
MRTX1133

Supplementary Figure 2. MRTX1133 is selective for KRAS^{G12D} in immunocompetent implantation tumor models.

(A) Waterfall plot of vehicle and MRTX1133 treated tumors (6419c5, subcutaneous) showing change in tumor volume after 7d of treatment compared to baseline at day 0. Each bar represents a single tumor. n=10 mice/group.

(B) Final tumor weights (g) from **(A)** after 7d of treatment. n=10/group. p-values were determined using Student's unpaired t-test. Error bars indicate SD.

(C) Waterfall plot of 4662^{G12C} and 4662^{G12D} subcutaneous tumors treated with vehicle or MRTX1133. Plot shows change in tumor volume after 4d of treatment. Each bar represents a single tumor. n=7-9/group.

(D) Waterfall plot of vehicle and MRTX1133 treated tumors (LLC, subcutaneous) showing change in tumor volume after 7d of treatment. Each bar represents a single tumor. n=5-7/group.

(E) Waterfall plot of vehicle and MRTX1133 treated tumors (MC38, subcutaneous) showing change in tumor volume after 7d of treatment. Each bar represents a single tumor. n=5-7/group.

(F) Waterfall plot of vehicle and MRTX1133 treated tumors (2838c3, subcutaneous) showing change in tumor volume after 7d of treatment. Each bar represents a single tumor. n=6-8/group.

(G) Representative gross images of orthotopic tumors 7d after treatment with vehicle or MRTX1133.

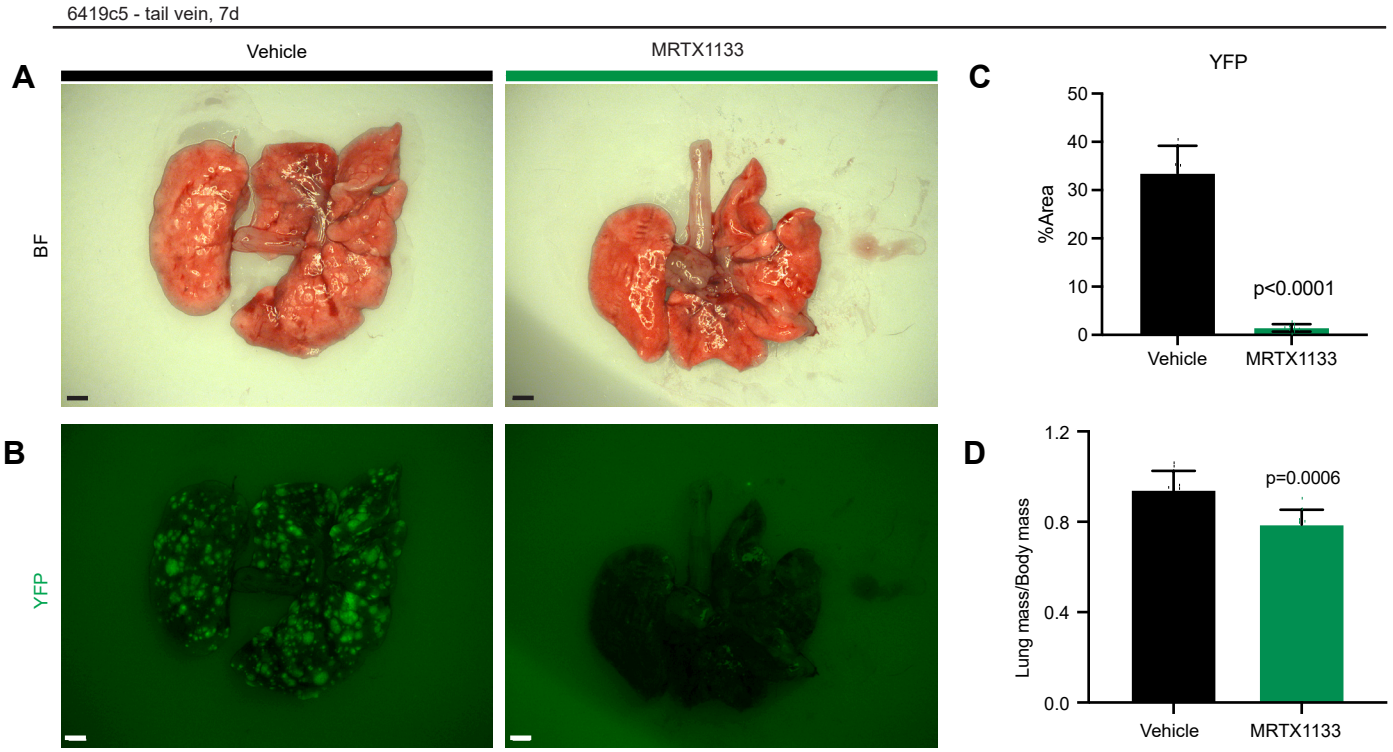
(H) Final tumor weights (g) from **(G)** after 7d of treatment. n=10/group. p-values were determined using Student's unpaired t-test. Error bars indicate SD.

(I) Left: Representative co-immunofluorescence images of p-ERK1/2, GFP, and DAPI in vehicle and MRTX1133 treated tumors (6419c5, subcutaneous, 7d). Scale bars, 100 μm. Objective, 20x. Right: Quantitation of p-ERK1/2 expression as percent area per high power field (HPF) in vehicle (n= 4) and MRTX1133 treated tumors (n=4). At least 3 fields of view were averaged per tumor. p-value was determined by Student's unpaired t-test. Error bars indicate SD.

(J) Left: Representative co-immunofluorescence images of p-ERK1/2, GFP and DAPI in vehicle and MRTX1133 treated tumors (6419c5, orthotopic, 7d). Scale bars, 100 μm. Objective, 20x. Right: Quantitation of p-ERK1/2 expression as percent area per high power field (HPF) in vehicle (n= 4) and MRTX1133 treated tumors (n=4). At least 3 fields of view were averaged per tumor. p-value was determined by Student's unpaired t-test. Error bars indicate SD.

(K) Left: Representative co-immunofluorescence images of p-S6, GFP and DAPI in vehicle and MRTX1133 treated tumors (6419c5, subcutaneous, 60h). Scale bars, 100 μm. Objective, 20x. Right: Quantitation of p-S6 expression as percent area per high power field (HPF) in vehicle (n= 3) and MRTX1133 treated tumors (n=3). At least 3 fields of view were averaged per tumor. p-value was determined by Student's unpaired t-test. Error bars indicate SD.

Figure S3


6419c5 - tail vein, 7d
Vehicle
MRTX1133
A
BF
B
YFP
C
YFP
%Area
50
40
30
20
10
0
p<0.0001
Vehicle
MRTX1133
D
Lung mass/Body mass
1.2
0.8
0.4
0.0
p=0.0006
Vehicle
MRTX1133

Supplementary Figure 3. MRTX1133 reduces metastatic burden in a tail vein model.

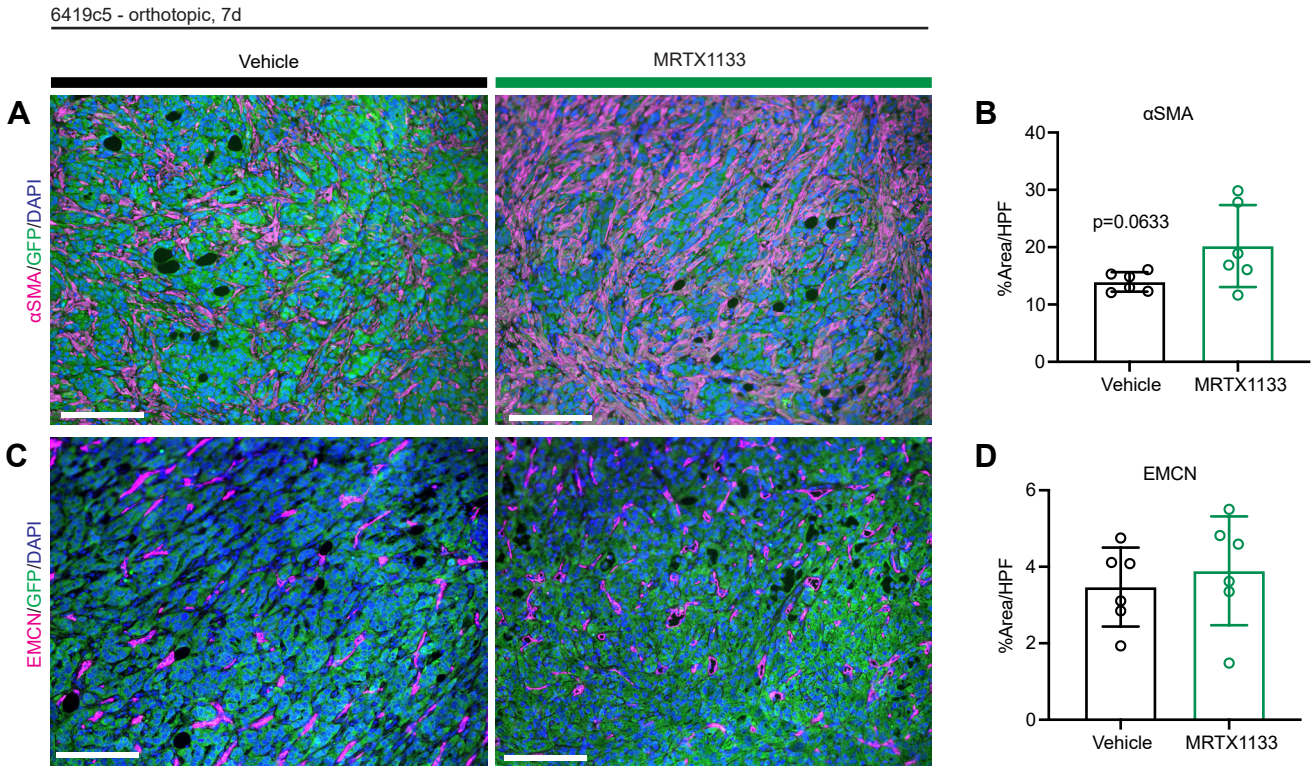
(A) Representative brightfield images of lungs from vehicle and MRTX1133 (6419c5, tail vein, 7d) treated mice. Scale bars, 1 mm.

(B) Representative fluorescent images of YFP signal in lungs from vehicle and MRTX1133 (6419c5, tail vein, 7d) treated mice. Scale bars, 1 mm.

(C) Quantitation of YFP expression as percent area in vehicle (n= 7) and MRTX1133 treated (n=9) mice. p-value was determined by Student's unpaired t-test. Error bars indicate SD.

(D) Ratio of lung mass to body mass in vehicle (n=9) and MRTX1133 treated (n=9) mice. p-value was determined by Student's unpaired t-test. Error bars indicate SD.

Figure S4


6419c5 - orthotopic, 7d
Vehicle
MRTX1133
A
αSMA/GFP/DAPI
B
αSMA
%Area/HPF
p=0.0633
Vehicle
MRTX1133
C
EMCN/GFP/DAPI
D
EMCN
%Area/HPF
Vehicle
MRTX1133

Supplementary Figure 4. MRTX1133 elicits changes in the tumor stroma and vasculature.

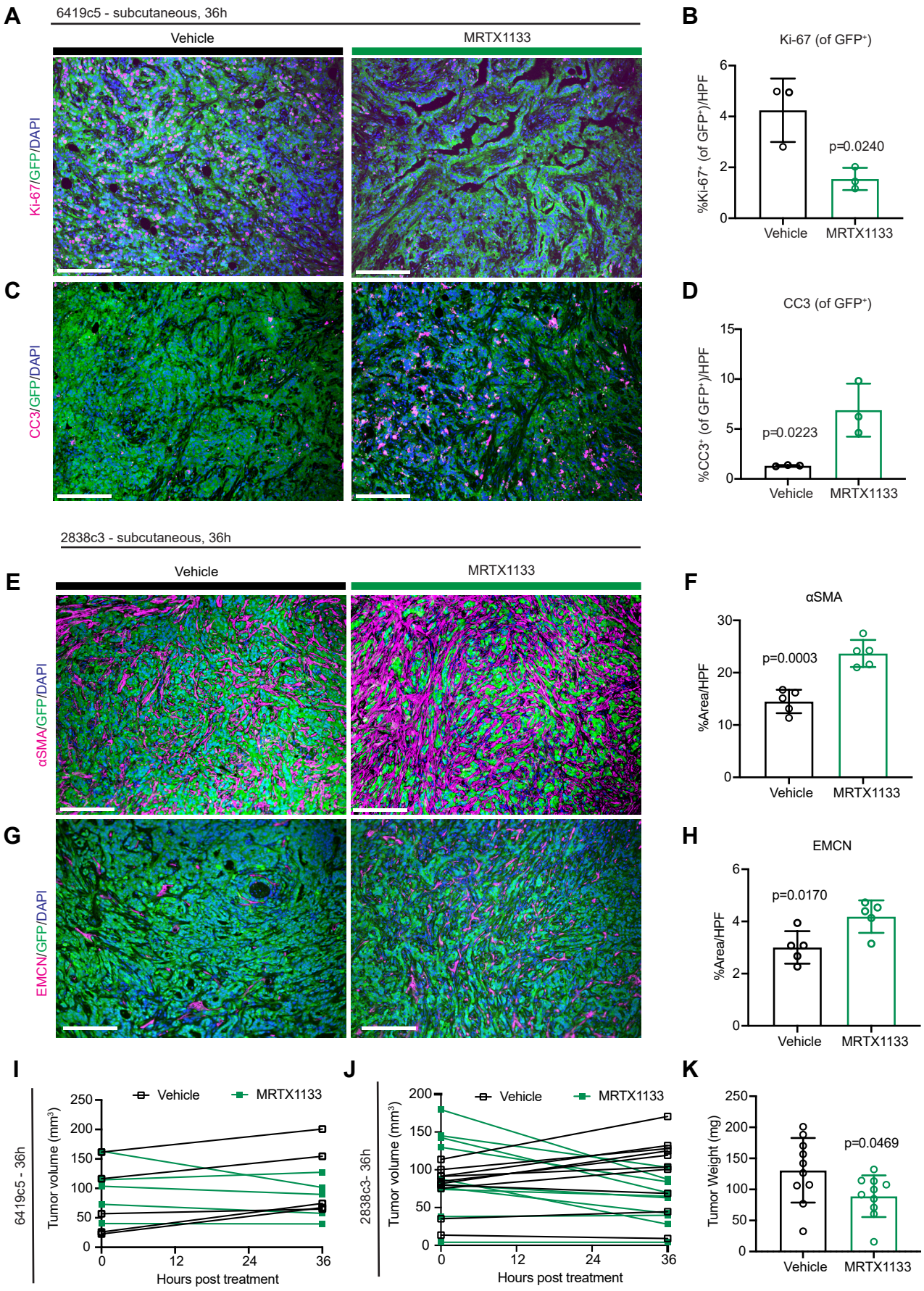
(A) Representative co-immunofluorescence images of αSMA, GFP, and DAPI in vehicle and MRTX1133 treated tumors (6419c5, orthotopic, 7d). Scale bars, 100 μm. Objective, 20x.

(B) Quantitation of fibroblasts (αSMA^+) as percent area per high power field (HPF) in vehicle (n= 6) and MRTX1133 treated tumors (n=6). At least 3 fields of view were averaged per tumor. p-value was determined by Student's unpaired t-test. Error bars indicate SD.

(C) Representative co-immunofluorescence images of EMCN, GFP, and DAPI in vehicle and MRTX1133 treated tumors (6419c5, orthotopic, 7d). Scale bars, 100 μm. Objective, 20x.

(D) Quantitation of blood vessels ($EMCN^+$) as percent area per high power field (HPF) in vehicle (n= 6) and MRTX1133 treated tumors (n=6). At least 3 fields of view were averaged per tumor. p-value was determined by Student's unpaired t-test. Error bars indicate SD.

Figure S5


A
6419c5 - subcutaneous, 36h
Vehicle
MRTX1133
Ki-67/GFP/DAPI
B
Ki-67 (of GFP+)
%Ki-67+ (of GFP+)/HPF
p=0.0240
Vehicle
MRTX1133
C
CC3/GFP/DAPI
D
CC3 (of GFP+)
%CC3+ (of GFP+)/HPF
p=0.0223
Vehicle
MRTX1133
2838c3 - subcutaneous, 36h
Vehicle
MRTX1133
E
αSMA/GFP/DAPI
F
αSMA
%Area/HPF
p=0.0003
Vehicle
MRTX1133
G
EMCN/GFP/DAPI
H
EMCN
%Area/HPF
p=0.0170
Vehicle
MRTX1133
I
6419c5 - 36h
Tumor volume (mm3)
Vehicle
MRTX1133
Hours post treatment
J
2838c3- 36h
Tumor volume (mm3)
Vehicle
MRTX1133
Hours post treatment
K
Tumor Weight (mg)
p=0.0469
Vehicle
MRTX1133

Supplementary Figure 5. MRTX1133 exerts *in vivo* effects early in treatment.

(A) Representative co-immunofluorescence images of Ki-67, GFP, and DAPI in vehicle and MRTX1133 treated tumors (6419c5, subcutaneous, 36h). Scale bars, 100 μm. Objective, 20x.

(B) Quantitation of proliferating tumor cells (Ki-67 of GFP^+) as percent area per high power field (HPF) in vehicle (n= 3) and MRTX1133 treated tumors (n=3). At least 3 fields of view were averaged per tumor. p-value was determined by Student's unpaired t-test. Error bars indicate SD.

(C) Representative co-immunofluorescence images of CC3, GFP, and DAPI in vehicle and MRTX1133 treated tumors (6419c5, subcutaneous, 36h). Scale bars, 100 μm. Objective, 20x.

(D) Quantitation of apoptotic tumor cells (CC3 of GFP^+) as percent area per high power field (HPF) in vehicle (n= 3) and MRTX1133 treated tumors (n=3). At least 3 fields of view were averaged per tumor. p-value was determined by Student's unpaired t-test.

(E) Representative co-immunofluorescence images of αSMA, GFP, and DAPI in vehicle and MRTX1133 treated tumors (2838c3, subcutaneous, 36h). Scale bars, 100 μm. Objective, 20x.

(F) Quantitation of fibroblasts (αSMA^+) as percent area per high power field (HPF) in vehicle (n= 5) and MRTX1133 treated tumors (n=5). At least 3 fields of view were averaged per tumor. p-value was determined by Student's unpaired t-test. Error bars indicate SD.

(G) Representative co-immunofluorescence images of EMCN, GFP, and DAPI in vehicle and MRTX1133 treated tumors (2838c3, subcutaneous, 36h). Scale bars, 100 μm. Objective, 20x.

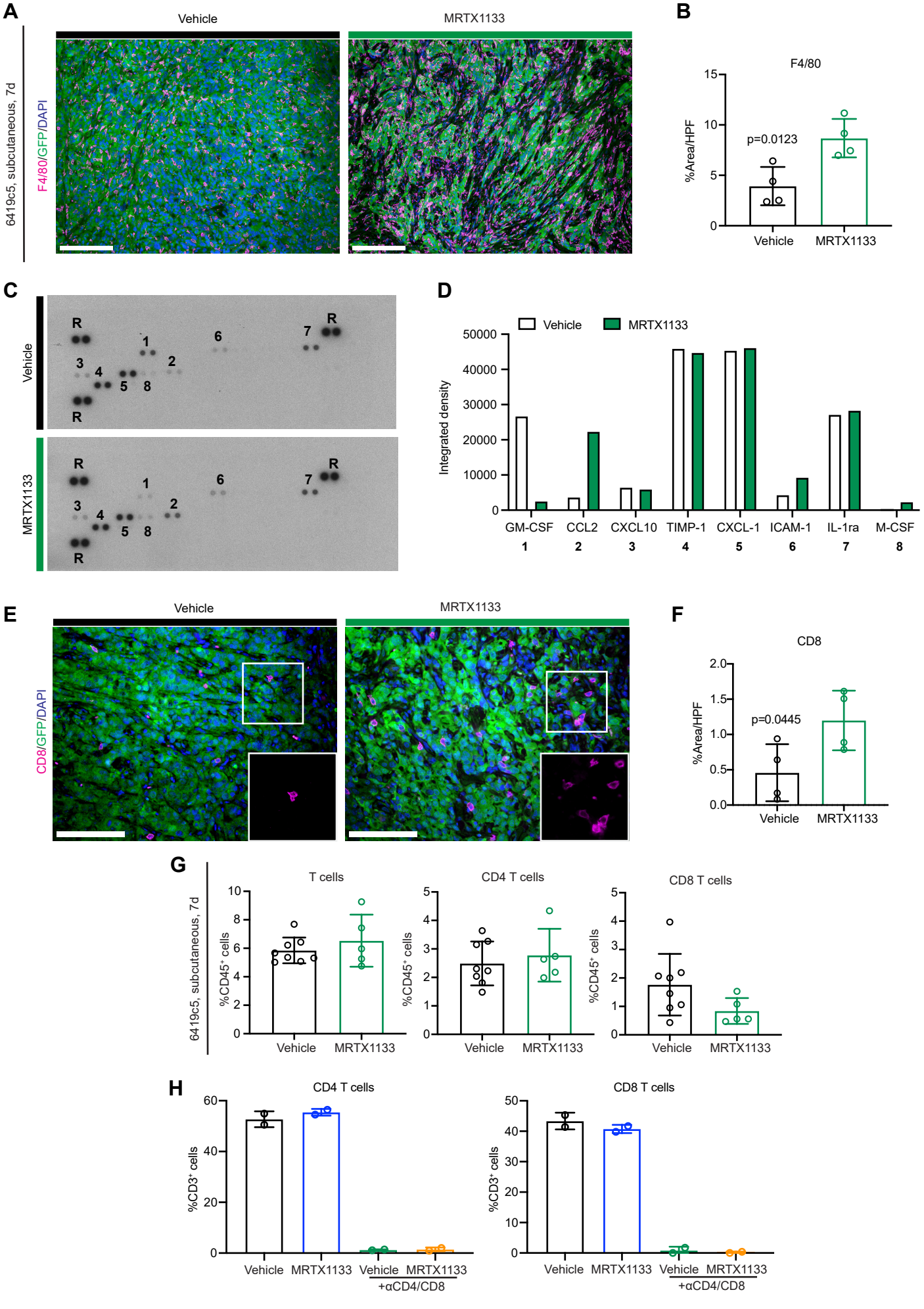
(H) Quantitation of blood vessels ($EMCN^+$) as percent area per high power field (HPF) in vehicle (n= 5) and MRTX1133 treated tumors (n=5). At least 3 fields of view were averaged per tumor. p-value was determined by Student's unpaired t-test. Error bars indicate SD.

(I) Tumor volumes (mm^3) of 6419c5 (subcutaneous) tumors treated with vehicle or MRTX1133 for 36h. n=5/group. Each line represents a single tumor.

(J) Tumor volumes (mm^3) of 2838c3 (subcutaneous) tumors treated with vehicle or MRTX1133 for 36h. n=10/group. Each line represents a single tumor.

(K) Final tumor weight (mg) after 36h of treatment. n=10/group. p-value was determined by Student's unpaired t-test. Error bars indicate SD.

Figure S6


A
Vehicle
MRTX1133
6419c5, subcutaneous, 7d
F4/80/GFP/DAPI
B
F4/80
%Area/HPF
p=0.0123
Vehicle
MRTX1133
C
Vehicle
MRTX1133
D
Vehicle
MRTX1133
Integrated density
GM-CSF
CCL2
CXCL10
TIMP-1
CXCL-1
ICAM-1
IL-1ra
M-CSF
E
CD8/GFP/DAPI
F
CD8
%Area/HPF
p=0.0445
G
6419c5, subcutaneous, 7d
T cells
CD4 T cells
CD8 T cells
%CD45+ cells
H
CD4 T cells
CD8 T cells
%CD3+ cells
+αCD4/CD8

Supplementary Figure 6. $KRAS^{G12D}$ inhibition alters the tumor immune microenvironment.

(A) Representative co-immunofluorescent images of F4/80, GFP, and DAPI in vehicle and MRTX1133 treated tumors (6419c5, subcutaneous, 7d). Scale bars, 100 μm. Objective, 20x.

(B) Quantitation of macrophages ($F4/80^+$) as percent area per high power field (HPF) in vehicle (n= 4) and MRTX1133 treated tumors (n=4). At least 3 fields of view were averaged per tumor. p-values were determined by Student's unpaired t-test. Error bars indicate SD.

(C) Representative proteome profiler cytokine array of conditioned media from 6419c5 tumor cells treated with vehicle (top) or MRTX1133 (bottom) for 48h. R denotes reference spots. Detectable cytokines are numbered 1-8. 1, GM-CSF; 2, CCL2; 3, CXCL10; 4, TIMP-1; 5, CXCL1; 6, ICAM-1; 7, IL-1ra; 8, M-CSF.

(D) Quantitation of cytokine arrays in (C). Integrated density was measured using the protein array analyzer macro in FIJI. Results representative of two independent experiments.

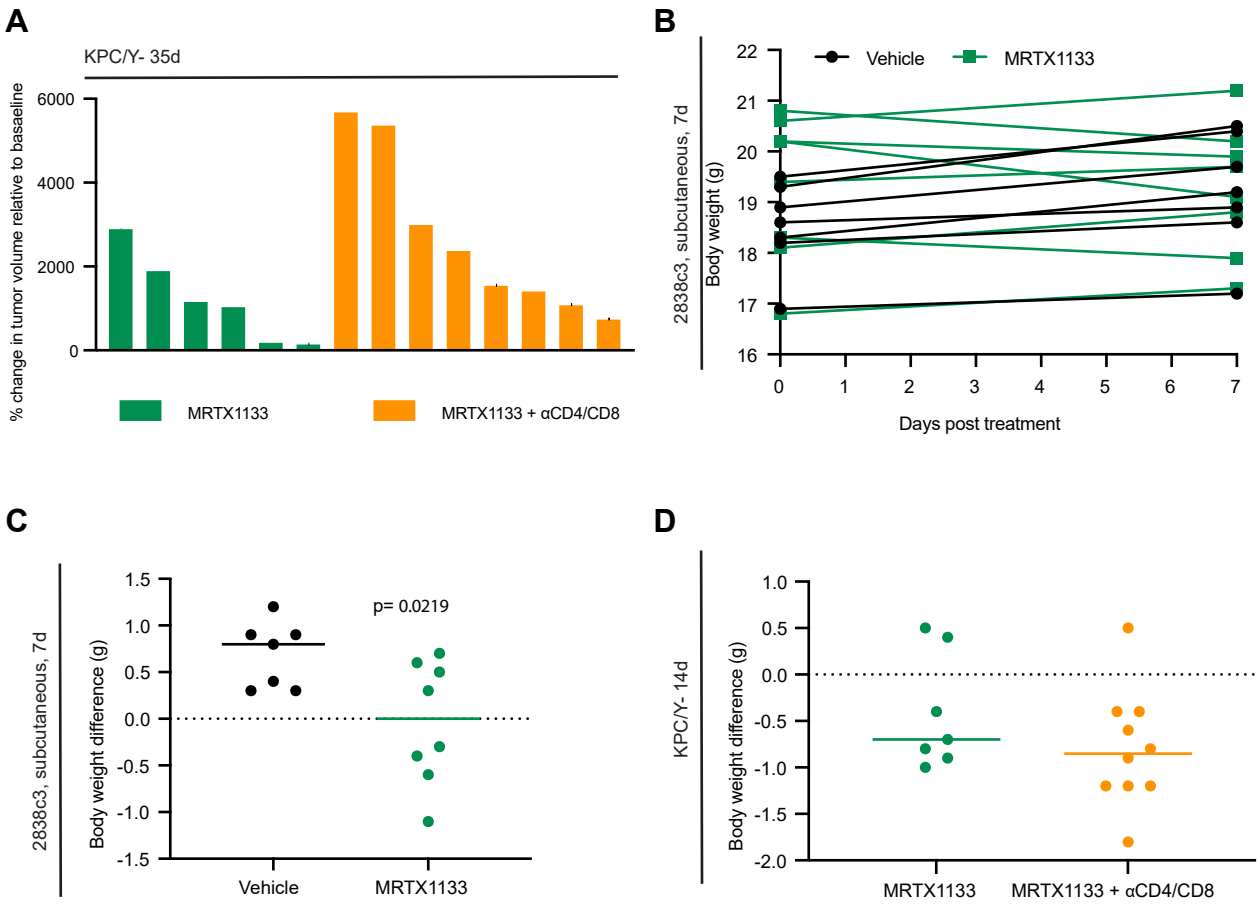
(E) Representative co-immunofluorescent images of CD8, GFP, and DAPI in vehicle and MRTX1133 treated tumors (6419c5, subcutaneous, 60h). Inset represents higher magnification of $CD8^+$ T cells in boxed area. Scale bars, 100 μm. Objective, 40x.

(F) Quantitation of CD8 T cells ($CD8^+$) as percent area per high power field (HPF) in vehicle (n= 4) and MRTX1133 treated tumors (n=4). At least 3 fields of view were averaged per tumor. p-values were determined by Student's unpaired t-test. Error bars indicate SD.

(G) Flow cytometry of indicated T cell subsets from 6419c5 (subcutaneous) tumors after 7d of treatment with vehicle (n=8) or MRTX1133 (n=5). p-values were determined by Student's unpaired t-test. Error bars indicate SD.

(H) Flow cytometry of T cell subsets (as %CD3) from the blood of tumor-bearing (2838c3) animals after 8 days of treatment of vehicle (n=2), MRTX1133 (n=2), vehicle + αCD4/CD8 (n=2), and MRTX1133 + αCD4/CD8 (n=2).

Figure S7


A
KPC/Y- 35d
% change in tumor volume relative to basaeline
6000
4000
2000
0
MRTX1133
MRTX1133 + αCD4/CD8
B
Vehicle
MRTX1133
2838c3, subcutaneous, 7d
Body weight (g)
22
21
20
19
18
17
16
0
1
2
3
4
5
6
7
Days post treatment
C
2838c3, subcutaneous, 7d
Body weight difference (g)
p= 0.0219
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
Vehicle
MRTX1133
D
KPC/Y- 14d
Body weight difference (g)
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
MRTX1133
MRTX1133 + αCD4/CD8

Supplementary Figure 7. Effects of MRTX1133 in KPC/Y and subcutaneous models.

(A) Waterfall plot of MRTX1133 and MRTX1133+ αCD4/CD8 treated KPC/Y tumors showing change in tumor volume after removal of MRTX1133. Each bar represents a single tumor. n=6-8/group.

(B) Body weight (g) over 7d of treatment (2838c3, subcutaneous) with either vehicle or MRTX1133 (30mg/kg). Each line represents a single mouse. n=7-8/group.

(C) Body weight difference (g) after 7d of treatment (2838c3, subcutaneous) with either vehicle or MRTX1133 (30mg/kg) compared to starting weight. Each dot represents a single mouse. n=7-8/group. p-value was determined by Student's unpaired t-test. Error bars indicate SD. Results representative of 2 independent experiments.

(D) Body weight difference (g) of KPC/Y mice after 14d of treatment with either MRTX1133 or MRTX1133+ αCD4/CD8 compared to starting weight. Each dot represents a single mouse. n=7-19/group. Error bars indicate SD.

Supplementary Table

Flow cytometry antibodies

Flow Antibody	Supplier	Clone	Dilution	Catalog Number
CD4 BV650	Biolegend	RM4-5	1:100	100546
Ly-6C BV570	Biolegend	HK1.4	1:100	128030
CD11b PerCP/Cy5.5	BD	M1/70	1:100	550993
CD11c BV605	Biolegend	N418	1:100	117334
F4/80 APC/Cy7	Biolegend	BM8	1:100	123118
CD45 AF700	Biolegend	30-F11	1:100	103128
Ly-6G V450	BD	1A8	1:100	560603
CD8a PE/Cy7	Biolegend	53-6.7	1:100	100722
CD3e PE/Cy5	Biolegend	145-2C11	1:100	100310
CD103 PE/Dazzle 594	Biolegend	2E7	1:100	121430
CD206 (MMR) PE/Cy7	Biolegend	C068C2	1:100	141719
I-A/I-E (MHC II) APC/Cy7	Biolegend	M5/114.15.2	1:100	107628
H-2K^{b}/H-2D^{b} (MHC I) PerCP/Cy5.5	Biolegend	28-8-6	1:100	114619
CD4 BUV395	BD	GK1.5	1:100	563790
CD8a BUV805	BD	53.-6.7	1:100	564920
CD3 BV711	Biolegend	17A2	1:100	100241
Ki-67 PerCP/Cy5.5	Biolegend	16A8	1:100	652424
CD45 APC	Biolegend	30-F11	1:100	103112
CD3 FITC	Biolegend	145-2C11	1:100	100306
CD4 PE/Cy7	Biolegend	RM4-5	1:100	100528
CD8 APC/Cy7	Biolegend	53-6.7	1:100	100714
GZMB PE	Biolegend	QA16A02	1:20	372208
IFNγ APC	BD	XMG1.2	1:100	554413
CD3 BUV395	BD	17A2	1:100	740268
CD4 BUV805	BD	GK1.5	1:100	612900
CD8 BV785	Biolegend	53-6.7	1:100	100750
TNFα BV421	Biolegend	MP6-XT22	1:50	506327

Immunostaining and immunoblotting antibodies

Antibody	Supplier	Catalog Number	IF dilution	WB dilution
Endomucin (EMCN)	Santa Cruz	sc-65495	1:200	-
Alpha-smooth muscle actin (αSMA)	Sigma-Aldrich	A2547	1:1000	-
Cleaved caspase 3 (CC3)	Cell Signaling	9661	1:100	-
Ki-67	eBioscience	14-5698-82	1:100	-
GFP	Abcam	Ab13970	1:200	
F4/80	Cell Signaling	70076	1:250	-
CD8	Cell Signaling	98941	1:400	-
CK19 (TROMA-III)	Iowa Developmental Hybridoma Bank	AB-2133570	1:200	
ERK1/2	Cell Signaling	4695	-	1:1000
p-ERK1/2	Cell Signaling	4370	1:100	1:1000
AKT	Cell Signaling	4691	-	1:1000
p-AKT	Cell Signaling	4060	-	1:1000
S6	Cell Signaling	2217	-	1:1000
p-S6	Cell Signaling	2211	1:100	1:1000
α-tubulin	Cell Signaling	3873	-	1:2000