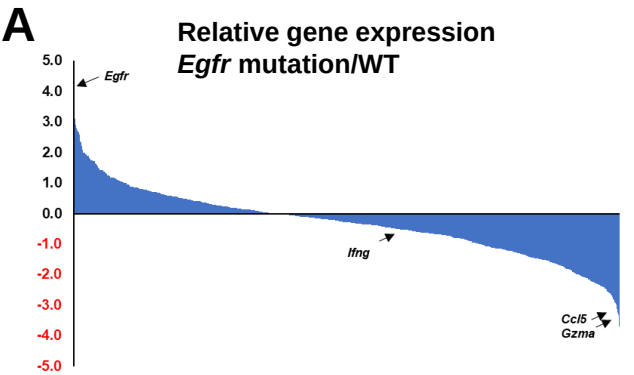
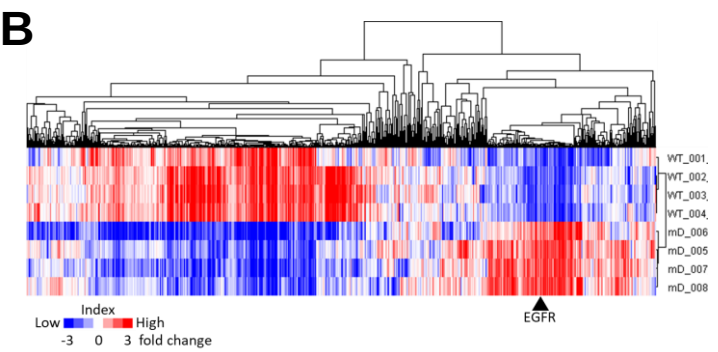
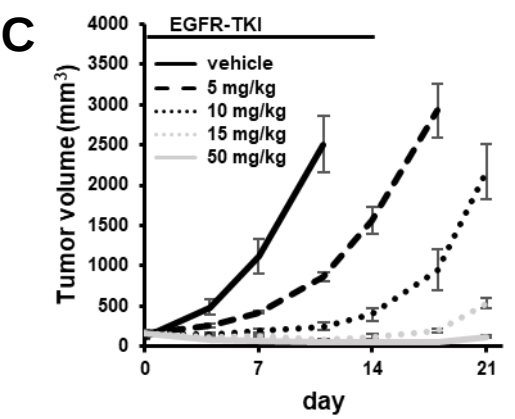
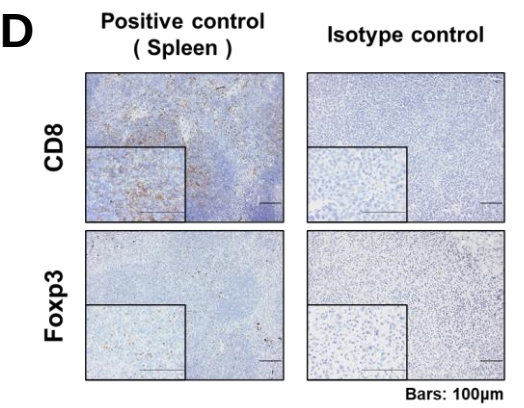
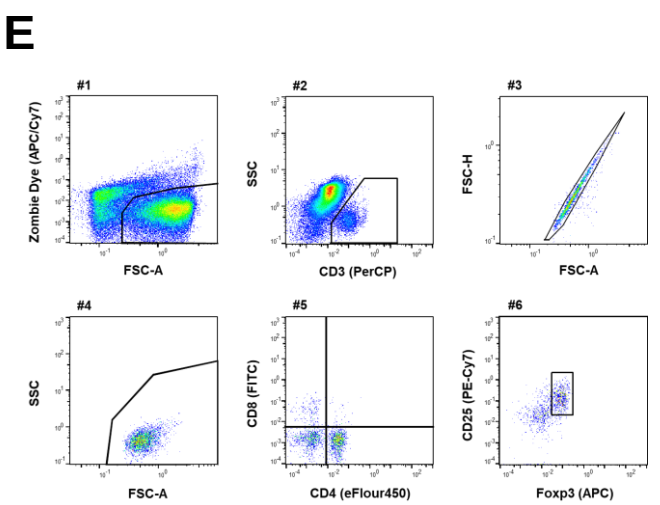
Suppl. Fig. S1


A
Relative gene expression
Egfr mutation/WT
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
-4.0
-5.0
Egfr
Ifng
Ccl5
Gzma

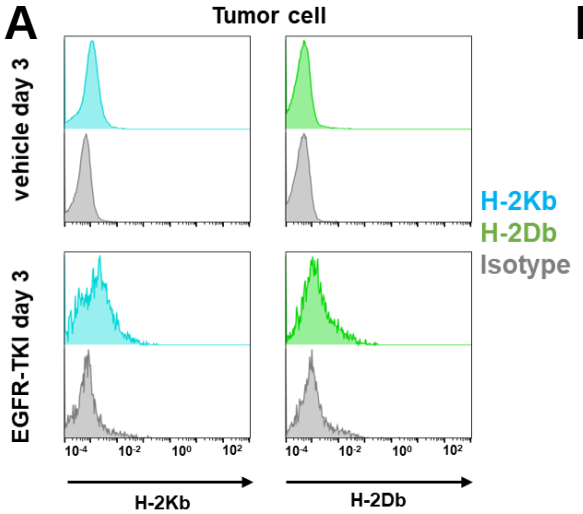

B
WT_001
WT_002
WT_003
WT_004
mD_006
mD_005
mD_007
mD_008
EGFR
Index
Low
High
-3
0
3 fold change

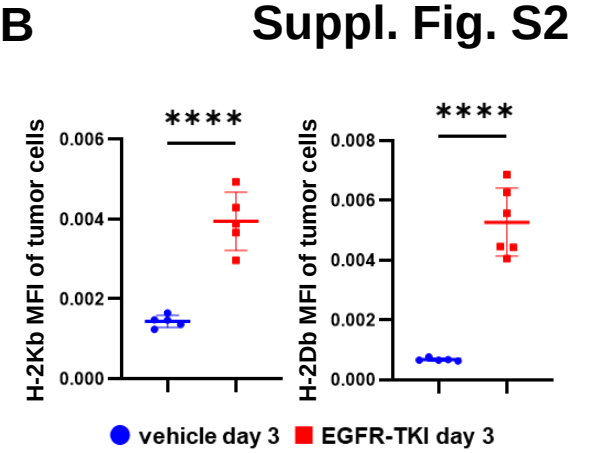

C
EGFR-TKI
vehicle
5 mg/kg
10 mg/kg
15 mg/kg
50 mg/kg
Tumor volume (mm3)
4000
3500
3000
2500
2000
1500
1000
500
0
0
7
14
21
day

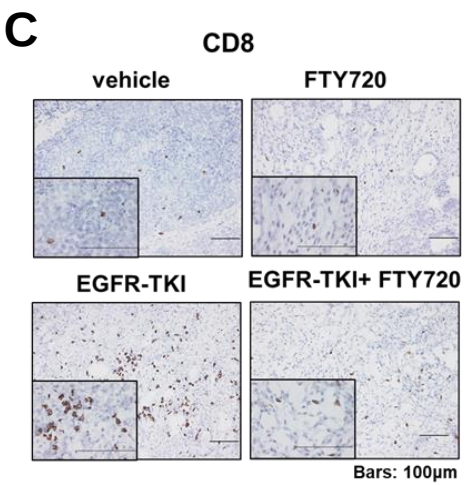

D
Positive control
(Spleen)
Isotype control
CD8
Foxp3
Bars: 100μm

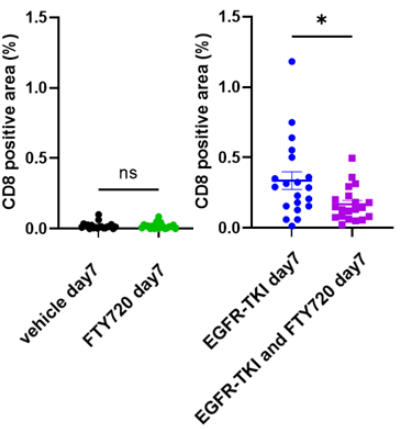

E
#1
Zombie Dye (APC/Cy7)
FSC-A
#2
SSC
CD3 (PerCP)
#3
FSC-H
FSC-A
#4
SSC
FSC-A
#5
CD8 (FITC)
CD4 (eFlour450)
#6
CD25 (PE-Cy7)
Foxp3 (APC)

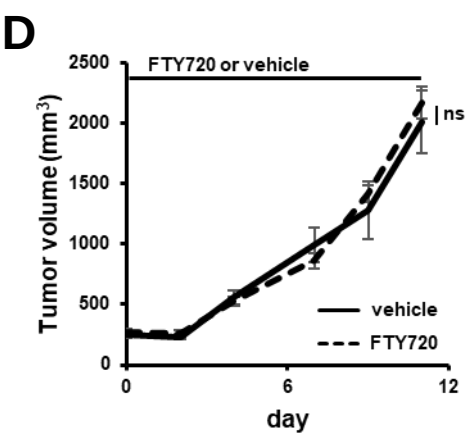
Suppl. Fig. S2

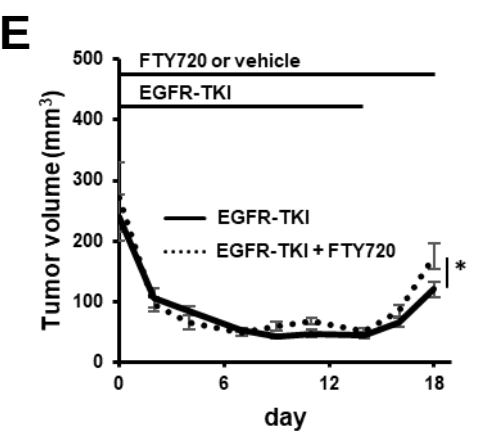

A
Tumor cell
vehicle day 3
EGFR-TKI day 3
H-2Kb
H-2Db
Isotype
H-2Kb
H-2Db


B
H-2Kb MFI of tumor cells
H-2Db MFI of tumor cells
****
****
vehicle day 3
EGFR-TKI day 3

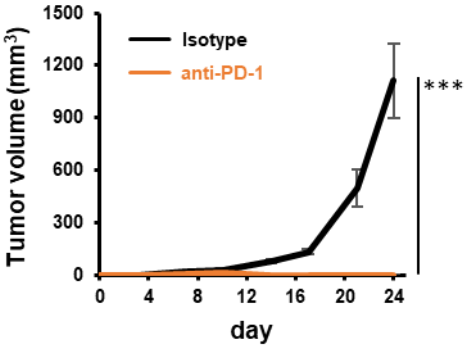

C
CD8
vehicle
FTY720
EGFR-TKI
EGFR-TKI+ FTY720
Bars: 100μm

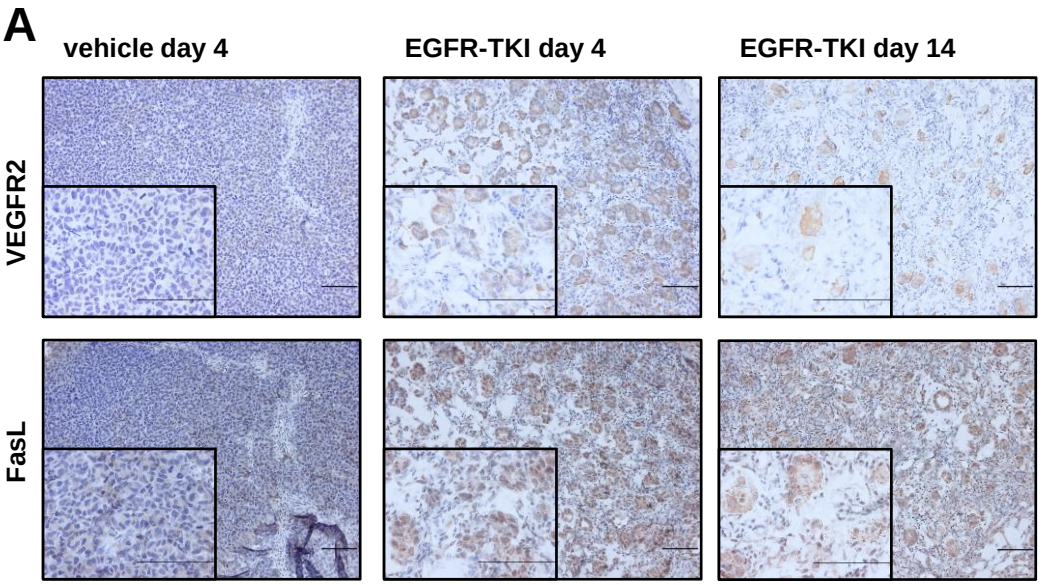

CD8 positive area (%)
ns
*
vehicle day7
FTY720 day7
EGFR-TKI day7
EGFR-TKI and FTY720 day7

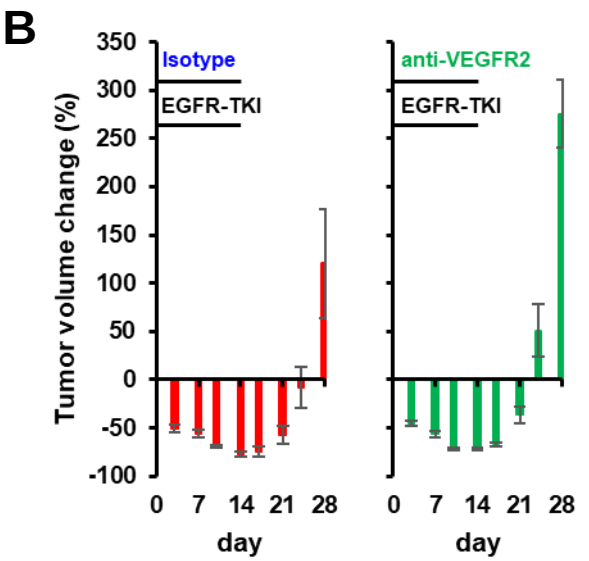

D
FTY720 or vehicle
Tumor volume (mm3)
ns
vehicle
FTY720
day


E
FTY720 or vehicle
EGFR-TKI
EGFR-TKI
EGFR-TKI + FTY720
Tumor volume (mm3)
*
day

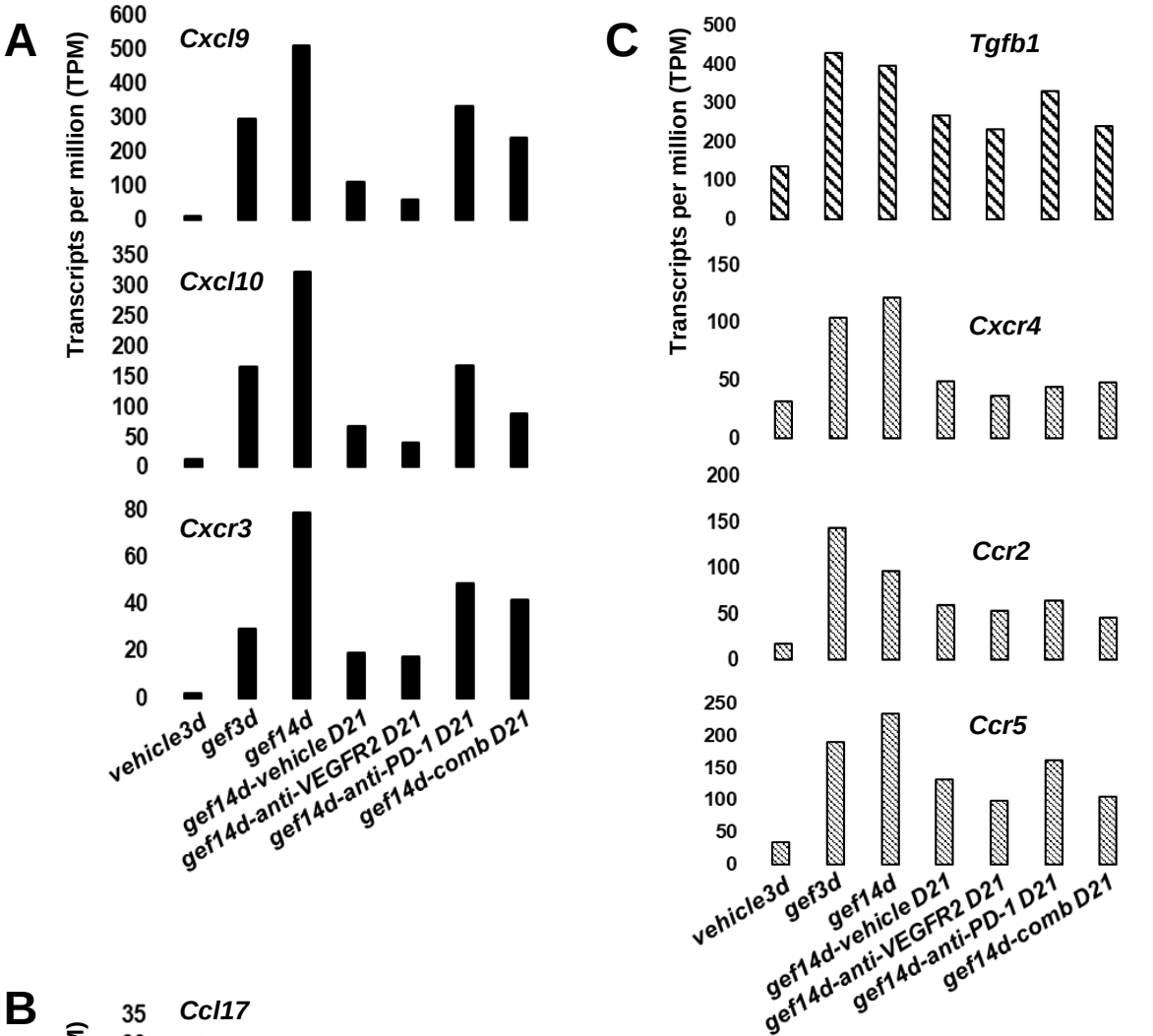
Suppl. Fig. S3


1500
1200
900
600
300
0
Tumor volume (mm3)
Isotype
anti-PD-1
***
0
4
8
12
16
20
24
day

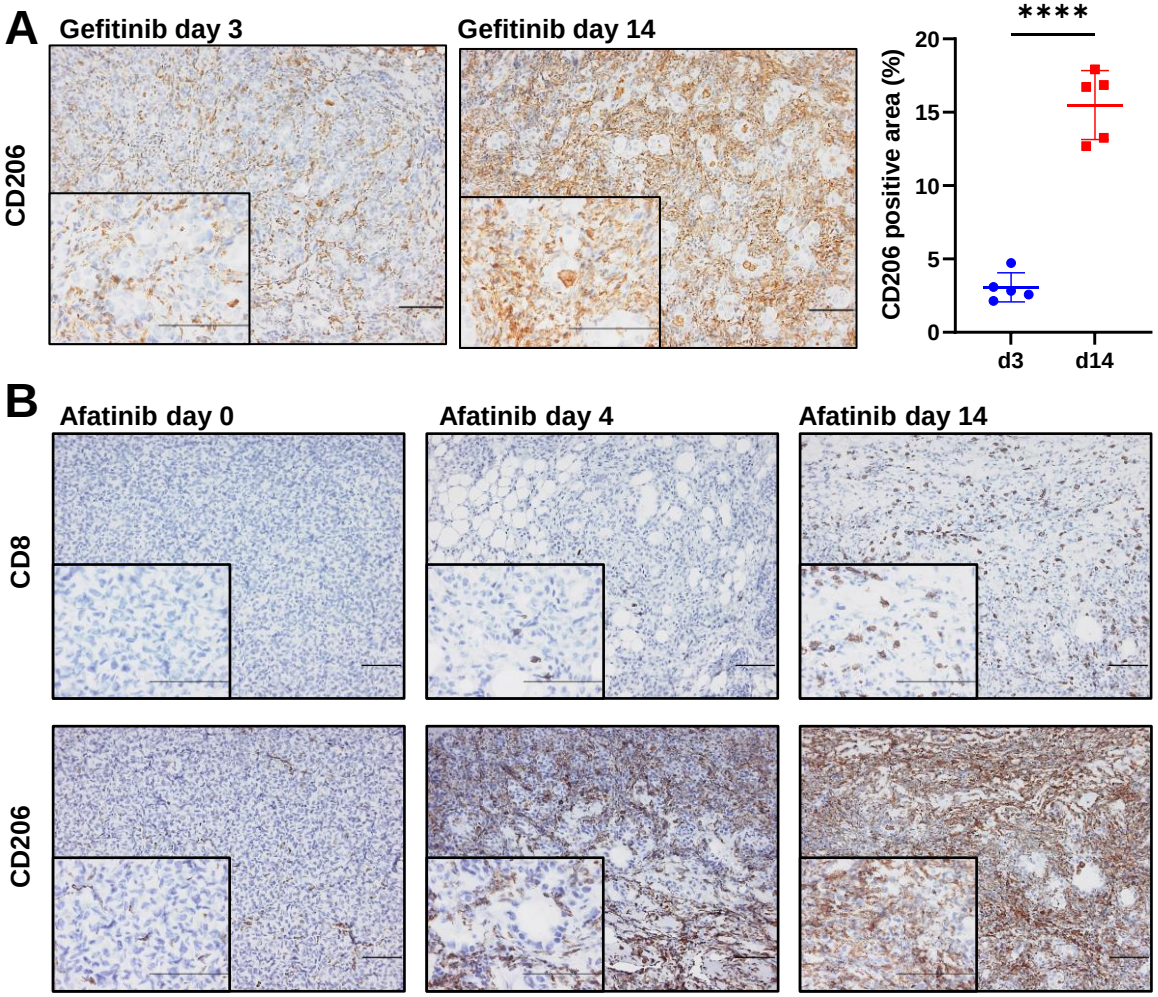

A
vehicle day 4
EGFR-TKI day 4
EGFR-TKI day 14
VEGFR2
FasL

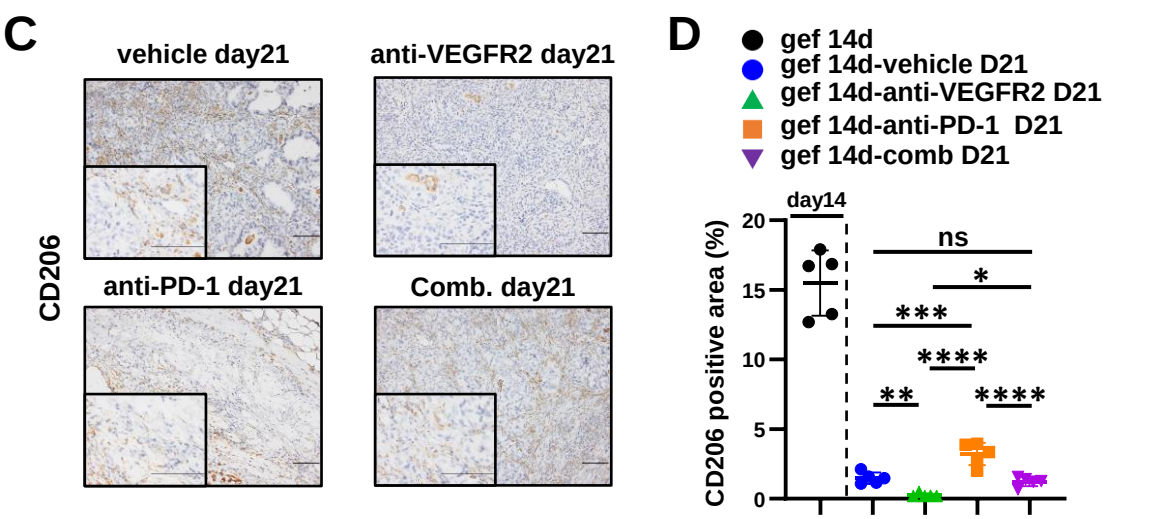

B
Isotype
EGFR-TKI
anti-VEGFR2
EGFR-TKI
Tumor volume change (%)
350
300
250
200
150
100
50
0
-50
-100
0 7 14 21 28
day
0 7 14 21 28
day

Suppl. Fig. S4

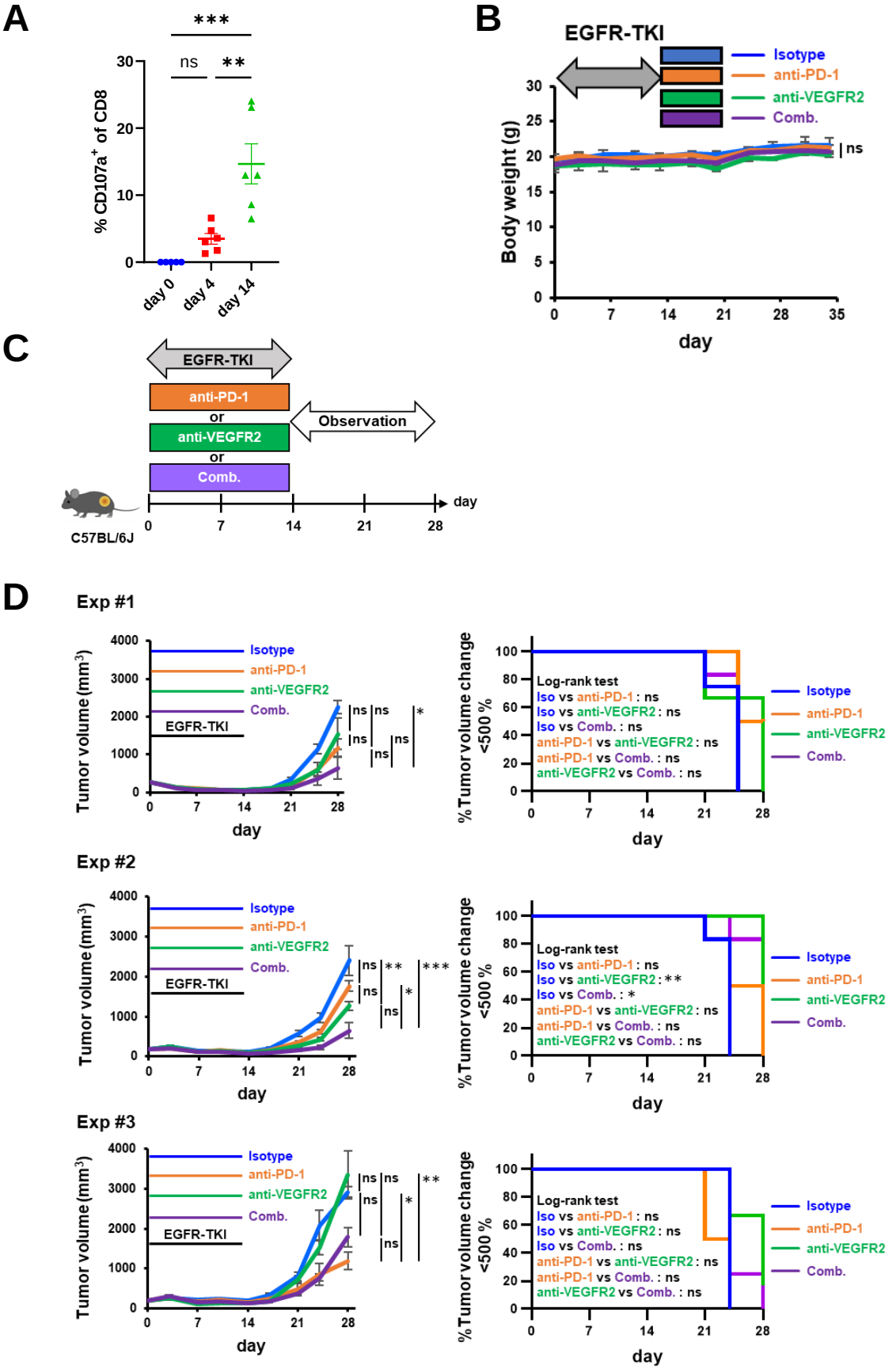

A
Cxcl9
Cxcl10
Cxcr3
Transcripts per million (TPM)
vehicle3d
gef3d
gef14d
gef14d-vehicle D21
gef14d-anti-VEGFR2 D21
gef14d-anti-PD-1 D21
gef14d-comb D21
B
Ccl17
C
Tgfb1
Cxcr4
Ccr2
Ccr5

Suppl. Fig. S5


A
Gefitinib day 3
Gefitinib day 14
CD206
CD206 positive area (%)
****
20
15
10
5
0
d3
d14
B
Afatinib day 0
Afatinib day 4
Afatinib day 14
CD8
CD206


C
vehicle day21
anti-VEGFR2 day21
anti-PD-1 day21
Comb. day21
CD206
D
gef 14d
gef 14d-vehicle D21
gef 14d-anti-VEGFR2 D21
gef 14d-anti-PD-1 D21
gef 14d-comb D21
day14
CD206 positive area (%)
20
15
10
5
0
ns
*
***
****
**
****

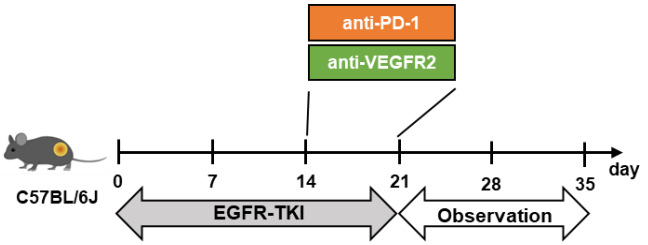
Suppl. Fig. S6


A
***
ns
**
% CD107a+ of CD8
day 0
day 4
day 14
B
EGFR-TKI
Isotype
anti-PD-1
anti-VEGFR2
Comb.
Body weight (g)
ns
day
C
EGFR-TKI
anti-PD-1
or
anti-VEGFR2
or
Comb.
Observation
C57BL/6J
day
D
Exp #1
Exp #2
Exp #3
Tumor volume (mm3)
EGFR-TKI
% Tumor volume change <500 %
Log-rank test
Iso vs anti-PD-1 : ns
Iso vs anti-VEGFR2 : ns
Iso vs Comb. : ns
anti-PD-1 vs anti-VEGFR2 : ns
anti-PD-1 vs Comb. : ns
anti-VEGFR2 vs Comb. : ns
Iso vs anti-VEGFR2 : **
Iso vs Comb. : *

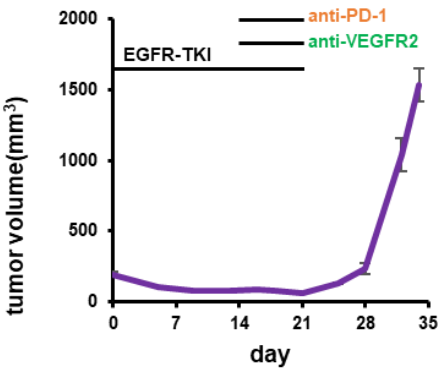
Suppl. Fig. S7

Suppl. Fig. S8

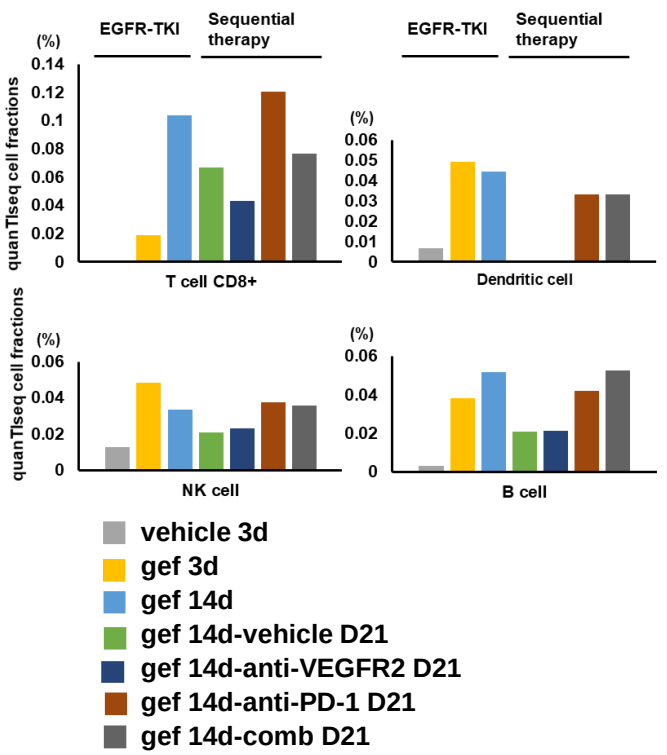
A



B



C



Suppl. Fig. S1.

A. NanoString analysis on lungs from wild-type mice (n=4) and genetically engineered mice bearing tumors with the *Egfr* exon 19 deletion (n=4) were assessed. Relative gene expression of 750 genes related to cancer immunity between the lungs from wild-type mice and those from the genetically engineered mice harboring *Egfr* exon 19 deletion. **B.** Cluster analysis of the expression profiles of 750 genes related to cancer immunity between the lungs from wild-type mice (n=4 lungs. 4 mice per group) and genetically engineered mice harboring *Egfr* exon 19 deletion (n=4 lungs. 4 mice per group).

C. Tumor growth in the syngeneic *Egfr*-mutant lung cancer model treated with gefitinib of indicated dosage for 14 days and subsequently observed for 7 days. Gefitinib (5, 10, 15 or 50 mg/kg, p.o., 5 days/week). n=4 tumors per group. 2 mice per group. Bars, mean±standard error.

D. IHC analysis of CD8 and Foxp3 expression in the spleen tissue as a positive control from mice treated with saline with 0.5% polyoxyethylene sorbitan monooleate for 3 days. Magnification: ×200 or ×800. Bars: 100 μm.

E. FCM analysis of $CD8^+$ T cells ($CD3^+CD8^+$) and Tregs ($CD4^+/CD25^+/FOXP3^+$) within the dissociated *Egfr*-mutant lung tumor cells from mice treated with saline with 0.5% polyoxyethylene sorbitan monooleate for 3 days and representative gating setting data.

Suppl. Fig. S2.

A. B. FCM analysis of MHC class I proteins H-2Kb and H-2Db within the dissociated *Egfr*-mutant tumor cells from mice treated with saline with 0.5% polyoxyethylene sorbitan monooleate as vehicle for 3 days or EGFR-TKI, gefitinib (50mg/kg, p.o.) and representative FCM data. n=5-6 tumors per group. 3 mice per group. **C.** Left: A representative image of CD8 staining in the *Egfr*-mutated lung cancer treated with the indicated therapy for 7 days. EGFR-TKI: gefitinib (50 mg/kg, p.o., 7 days/week), FTY720 (300 μg/kg/day, p.o., 7 days/week), Magnification: ×200 or ×800. Bars: 100 μm. Right: CD8-positive area are quantified using ImageJ software. (n=20 field-of-view per group, Magnification: ×200)

D. Tumor growth in the syngeneic *Egfr*-mutated lung cancer model treated with saline with 0.5% polyoxyethylene sorbitan monooleate as vehicle or FTY720 (300 μg/kg/day, p.o., 7 days/week) for 12 days (n=6 tumors per group. 3 mice per group).

E. Tumor growth in the syngeneic *Egfr*-mutated lung cancer model treated with gefitinib (50 mg/kg, p.o., 7 days/week) for 14 days and saline with 0.5% polyoxyethylene sorbitan monooleate as vehicle or FTY720 (300 μg/kg/day, p.o., 7 days/week) for 18 days (n=8 tumors per group. 4 mice per group.).

Bars, mean±standard error. ns, not significant. *p<0.05, ****p<0.0001, t-test.

Suppl. Fig. S3.

Tumor growth in the syngeneic graft tumor derived from MC-38 cells treated with anti-PD-1 for 24 days. Isotype antibody group (n=5 tumors per group. 5 mice per group), anti-PD-1 group (10 mg/kg/day, i.p., every 5 days) (n=5 tumors per group. 5 mice per group). Bars, mean±standard error. ***p<0.001, t-test.

Suppl. Fig. S4.

A. A representative image of VEGFR2 and FasL staining in the *Egfr*-mutant lung tumors treated with the indicted therapy. EGFR-TKI: gefitinib (50 mg/kg, p.o., 7 days/week), Magnification: ×200 or ×800. Bars: 100 μm.

B. Tumor volume change rate in the *Egfr*-mutant lung cancer model treated with gefitinib (50 mg/kg, p.o., 5 days/week) and/or anti-VEGFR2 (10 mg/kg/day, i.p., every 3 days) for 14 days and subsequently observed for 14 days, n=6 tumors per group. 3 mice per group. Bars, mean±standard error.

Suppl. Fig. S5.

A, B, C. RNA expression in the tumors treated with vehicle as saline with 0.5% polyoxyethylene sorbitan monooleate for 3 days or indicated drug at 3, 14 and 21 days. Comb., combination anti-PD-1/anti-VEGFR2; gef, geftinib (50 mg/kg 7 days/week); d, days; gef14d-vehicle D21, gefitinib for 14 days followed by isotype antibody for 7 days; gef14d-anti-VEGFR2 D21, gefitinib for 14 days followed by anti-VEGFR2 (10 mg/kg/day, i.p., every 3 days) for 7 days; gef14d-anti-PD-1 D21, gefitinib for 14 days followed by anti-PD-1 (10 mg/kg/day, i.p., per 5 days) for 7 days; gef14d-comb D21, gefitinib for 14 days followed by combination anti-PD-1/anti-VEGFR2 for 7 days.

Suppl. Fig. S6.

A. Left: A representative image of CD206 staining in the *Egfr*-mutant lung cancer treated with gefitinib (50 mg/kg, p.o., 7 days/week) for 3 or 14 days. Magnification: ×200 or ×800. Bars: 100 μm. Right: The CD206-positive area is quantified using ImageJ software. Bars, mean±standard error. (n=5 field-of-view per group, Magnification: ×200). ****p<0.0001, t-test.

B. A representative image of CD8 and CD206 staining in the *Egfr*-mutated lung cancer treated with afatinib (15mg/kg, p.o., 7 days/week) for 4 or 14 days. Magnification: ×200 or ×800. Bar: 100 μm.

C. A representative image of CD206 staining in the *Egfr*-mutated lung cancer treated with EGFR-TKI (gefitinib, 50 mg/kg, p.o., 7 days/week) for 14 days, followed by the indicated treatment with vehicle, anti-PD-1, anti-VEGFR2, or combination anti-PD-1/anti-VEGFR2 for 7 days. Isotype control as vehicle (n=6 tumors per group. 3 mice per group), anti-VEGFR2 (10 mg/kg/day, i.p., every 3 days; n=6 tumors per group. 3 mice per group), anti-PD-1 (10 mg/kg/day, i.p. every 5 days; n=6 tumors pe r group. 3 mice per group), combination anti-PD-1/anti-VEGFR2 (n=6 tumors per group. 3 mice per group). Magnification: ×200 or ×800. Bars: 100 μm.

D. CD206-positive area is quantified using ImageJ software. (n=5 field-of-view per group, Magnification: ×200) Bars, mean±standard error. ns, not significant. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, one-way ANOVA with post-hoc Tukey's test.

Comb., combination anti-PD-1/anti-VEGFR2; gef, geftinib (50 mg/kg, p.o., 7 days/week); d, days; gef14d-vehicle D21, gefitinib for 14 days followed by isotype antibody for 7 days; gef14d-anti-VEGFR2 D21, gefitinib for 14 days followed by anti-VEGFR2 (10 mg/kg/day, i.p., every 3 days) for 7 days; gef14d-anti-PD-1 D21, gefitinib for 14 days followed by anti-PD-1 (10 mg/kg/day, i.p., per 5 days) for 7 days; gef14d-comb D21, gefitinib for 14 days followed by combination anti-PD-1/anti-VEGFR2 for 7 days.

Suppl. Fig. S7.

A. FCM analysis of CD107a on $CD8^+$ T cells within the dissociated *Egfr*-mutated lung tumor from mice treated with gefitinib (50 mg/kg, p.o., 7 days/week). n=5-6 tumors per group. 3 mice per group. Bars, mean±standard error. **p<0.01, ***p<0.001, one-way ANOVA with post-hoc Tukey's test.

B. The mice are treated with gefitinib group (50 mg/kg, p.o., 7 days/week) for 14 days followed by the indicated therapy for 7 days and subsequently observed for 14 days. Isotype control, anti-VEGFR2 (10 mg/kg/day, i.p., every 3 days), anti-PD-1 (10 mg/kg/day, i.p. every 5 days), combination anti-PD-1 (10 mg/kg/day, i.p. every 5 days)/anti-VEGFR2 (10 mg/kg/day, i.p., every 3 days). Body weight loss is not observed in the mice. n=6 tumors per group. 3 mice per group. Bars, mean±standard error. ns, not significant. one-way ANOVA with post-hoc Tukey's test.

C. Schematic image of the treatment schedule.

D. Left: Tumors in the *Egfr* -mutant lung cancer model were treated with the indicated therapy for 14 days and subsequently observed for 14 days. The gefitinib with isotype antibody, gefitinib with anti-VEGFR2 (10 mg/kg/day, i.p., every 3 days), gefitinib with anti-PD-1 (10 mg/kg/day, i.p., every 5 days), and gefitinib with the combination anti-PD-

1/anti-VEGFR2. Gefitinib (50 mg/kg, p.o., 5 days/week). n =6 tumors per group. 3 mice per group. one-way ANOVA with post-hoc Tukey's test. Right: The survival probability is calculated using the Kaplan–Meier method, and differences in survival are evaluated using the Log-rank test with Bonferroni correction. Kaplan–Meier plot showing percentage of animals with tumor burden below 500% compared to those at Day 14 for the duration of this study. n =6 tumors per group. 3 mice per group. Bars, mean±standard error. ns, not significant. *p<0.05, **p<0.01, ***p<0.001.

Comb., combination anti-PD-1/anti-VEGFR2

Suppl. Fig. S8.

A. Schematic image of the treatment schedule.

B. Tumors in the *Egfr*-mutated lung cancer model are treated with the gefitinib for 14 days and subsequently treated with gefitinib, anti-PD-1and anti-VEGFR2 for 7 days (n=6). Gefitinib (50 mg/kg, p.o., 7 days/week), anti-VEGFR2 (10 mg/kg/day, i.p., every 3 days), anti-PD-1 (10 mg/kg/day, i.p., every 5 days). n=6 tumors per group. 3 mice per group. Bars, mean±standard error.

C. quanTIseq analysis to estimate the fraction of immune cells in the *Egfr*-mutated lung tumor from mice treated with the indicated therapy. vehicle 3d, saline with 0.5%

polyoxyethylene sorbitan monooleate for 3 days, gef, gefitinib (50 mg/kg 7 days a week); d, days; gef14d-vehicle D21, gefitinib for 14 days followed by isotype antibody for 7 days; gef14d-anti-VEGFR2 D21, gefitinib for 14 days followed by anti-VEGFR2 (10 mg/kg/day, i.p., every 3 days) for 7 days; gef14d-anti-PD-1 D21, gefitinib for 14 days followed by anti-PD- (10 mg/kg/day, i.p., per 5 days) for 7 days; gef14d-comb D21, gefitinib for 14 days followed by a combination anti-PD-1/anti-VEGFR2 for 7 days.