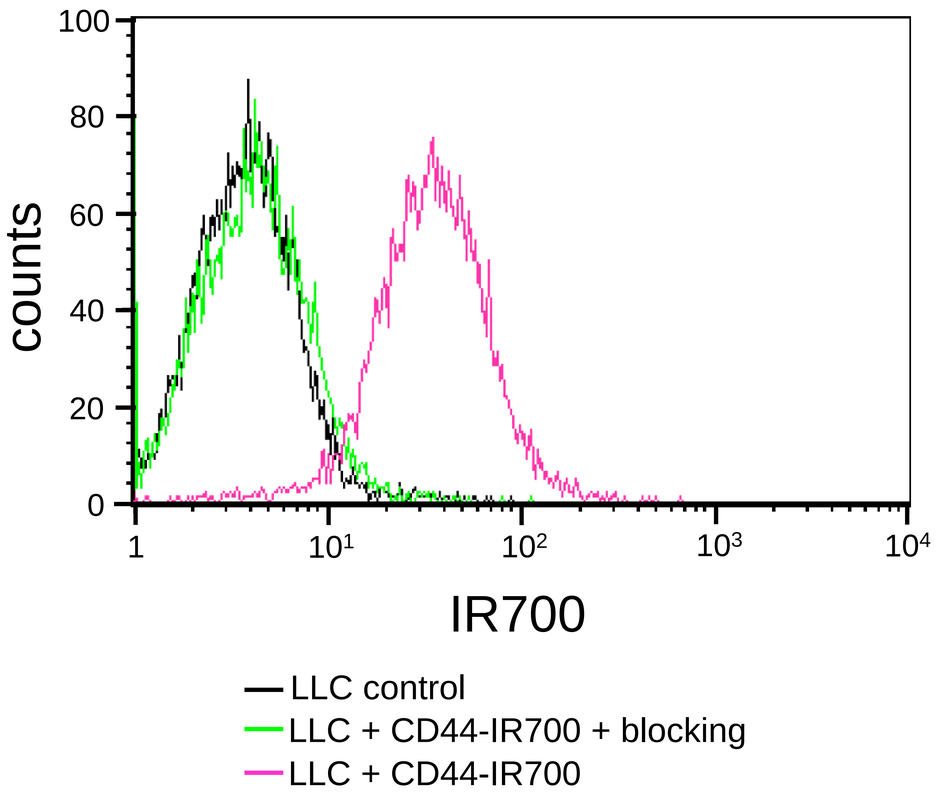
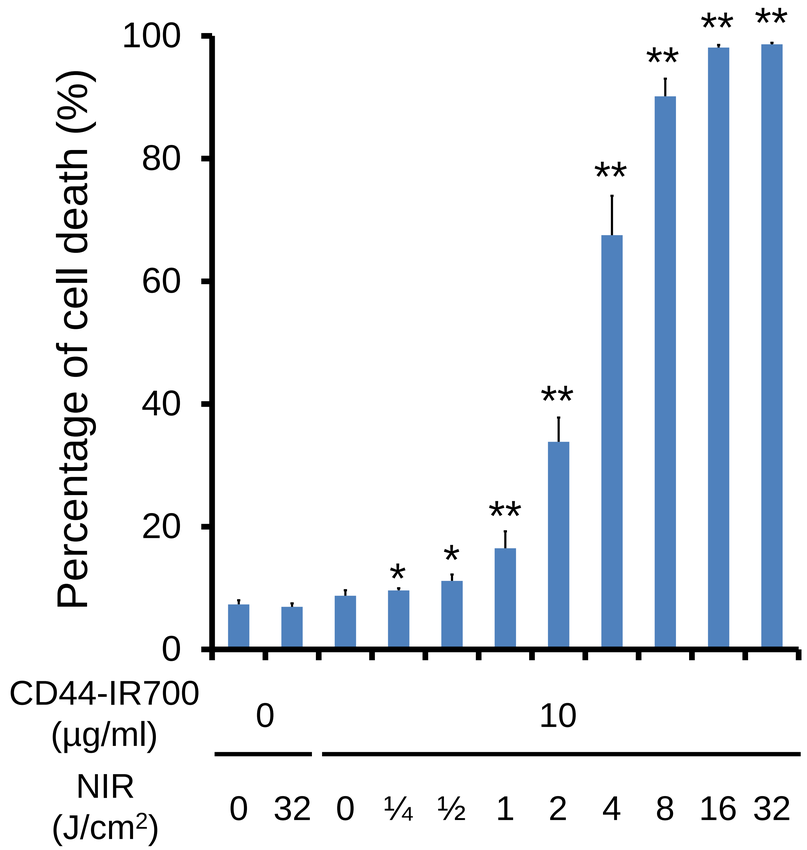
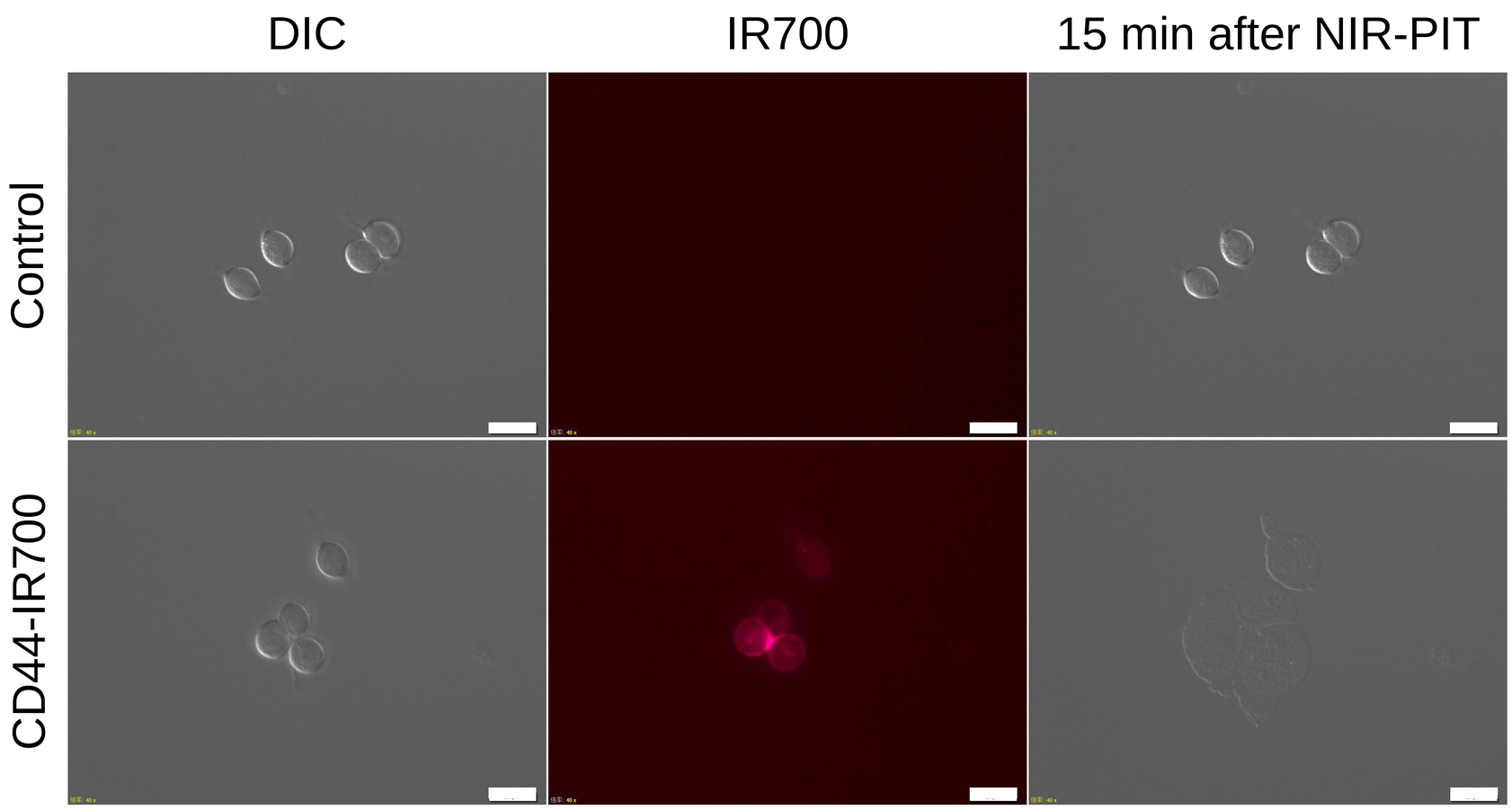
A


100
80
60
40
20
0
counts
1
10¹
10²
10³
10⁴
IR700
LLC control
LLC + CD44-IR700 + blocking
LLC + CD44-IR700

C


Percentage of cell death (%)
100
80
60
40
20
0
**
**
**
**
*
*
**
**
CD44-IR700 (μg/ml)
0
10
NIR (J/cm²)
0 32 0 ¼ ½ 1 2 4 8 16 32

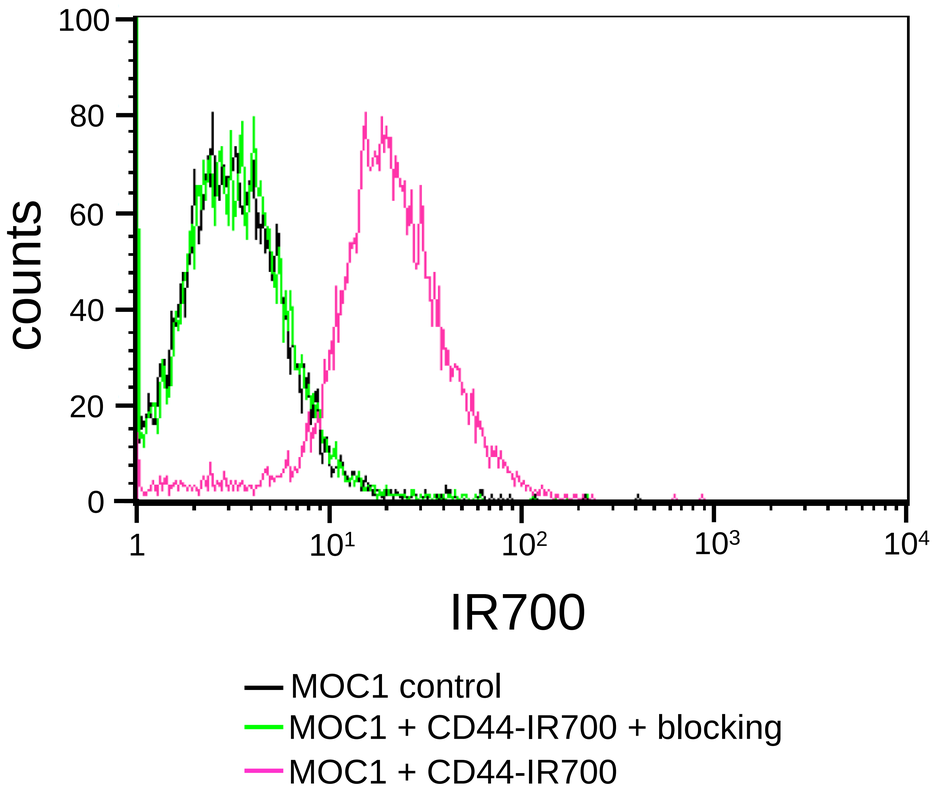
B


DIC
IR700
15 min after NIR-PIT
Control
CD44-IR700

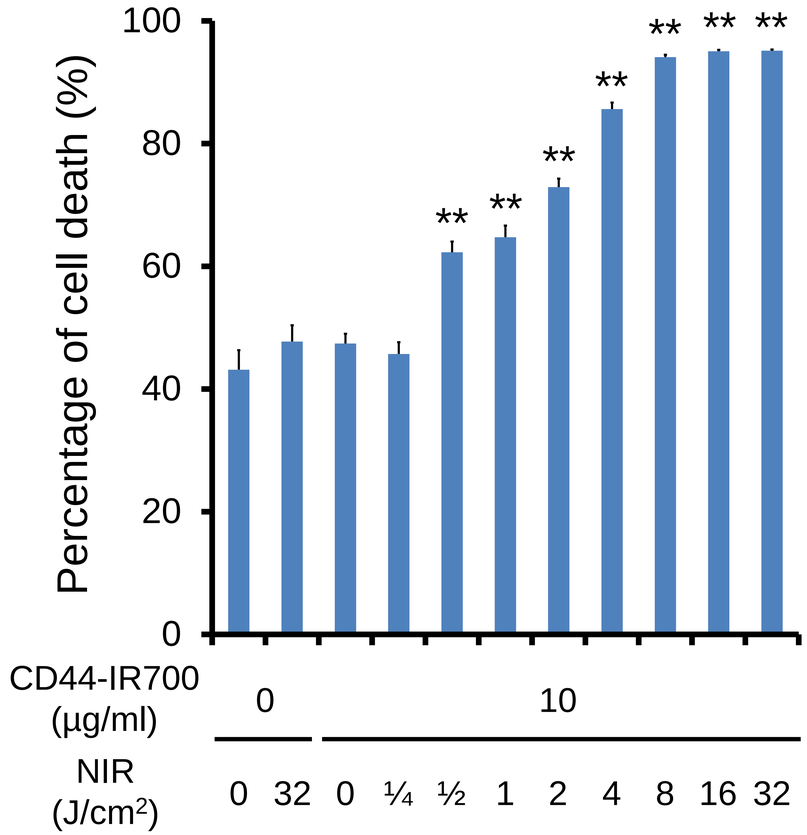
**Supplementary Figure 1. Confirmation of CD44 expression as a target for NIR-PIT in LLC cells and evaluation of *in vitro* NIR-PIT.**

(A) Expression of cell surface CD44 in LLC cells was examined with flow cytometry. CD44 blocking antibody was added to some wells to validate specific staining. Representative histograms shown. (B) Differential interference contrast (DIC) and fluorescence microscopy images of LLC cells. Change in LLC cellular architecture following 15 min of NIR light exposure shown. Scale bars = 20 μm. (C) Membrane permeability of LLC cells, as measured by propidium iodide (PI) staining, after labeling with CD44-IR700 and treatment with NIR-light (n = 5, $^{*}p < 0.05$, $^{**}p < 0.01$, vs untreated control, by Student's t test).

A

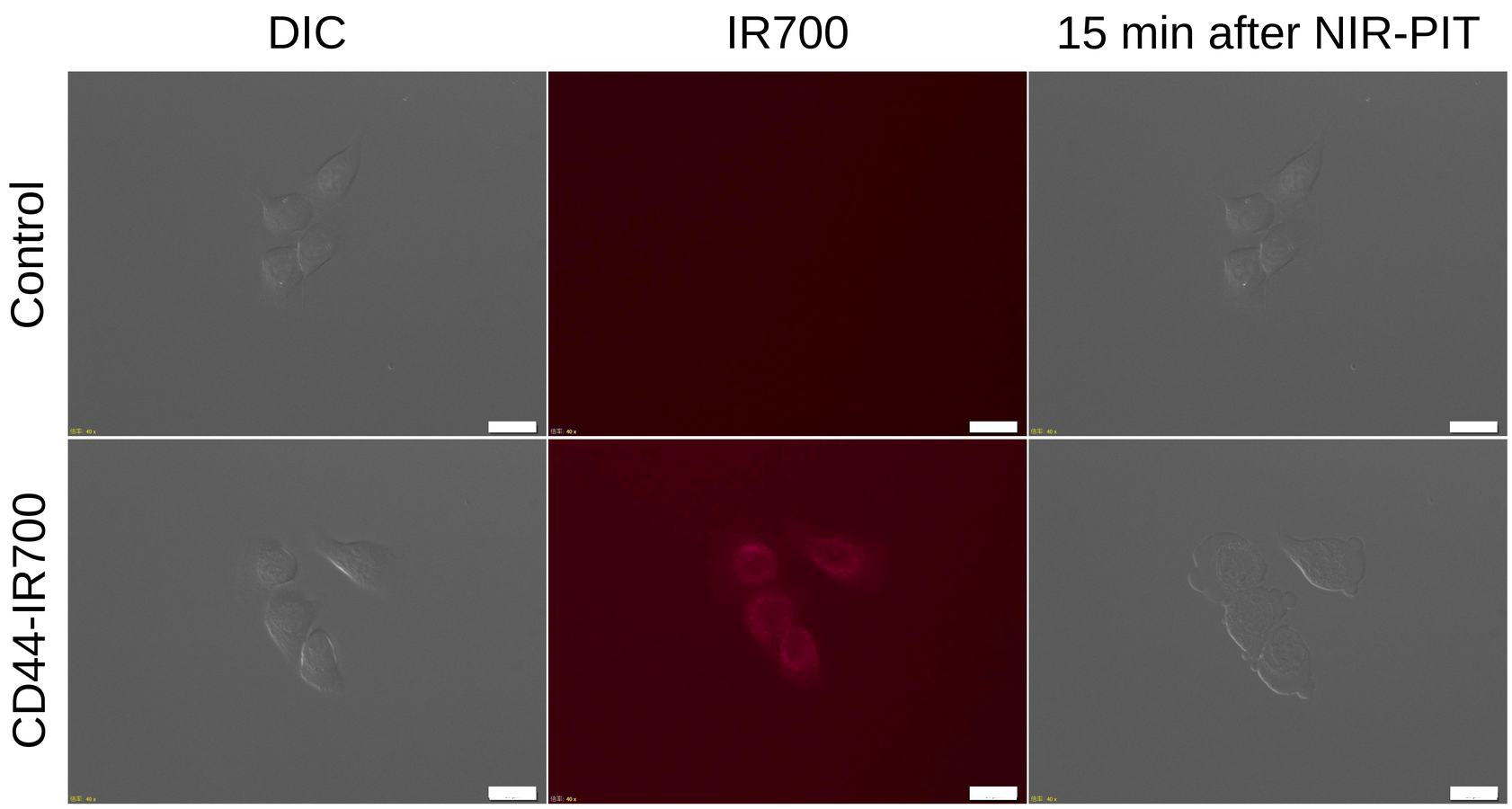

100
80
60
40
20
0
counts
1
10¹
10²
10³
10⁴
IR700
MOC1 control
MOC1 + CD44-IR700 + blocking
MOC1 + CD44-IR700

C


Percentage of cell death (%)
100
80
60
40
20
0
**
CD44-IR700 (μg/ml)
0
10
NIR (J/cm²)
0 32 0 ¼ ½ 1 2 4 8 16 32

sFig2

B

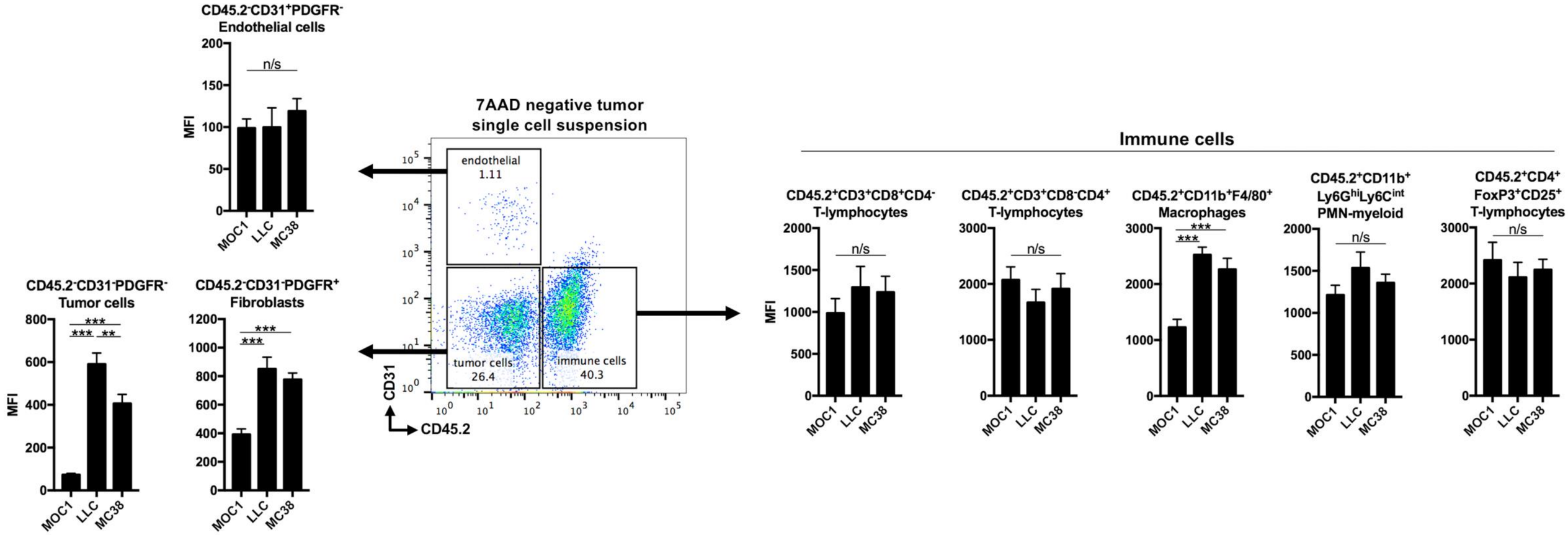

DIC
IR700
15 min after NIR-PIT
Control
CD44-IR700

**Supplementary Figure 2. Confirmation of CD44 expression as a target for NIR-PIT in MOC1 cells and evaluation of *in vitro* NIR-PIT.**

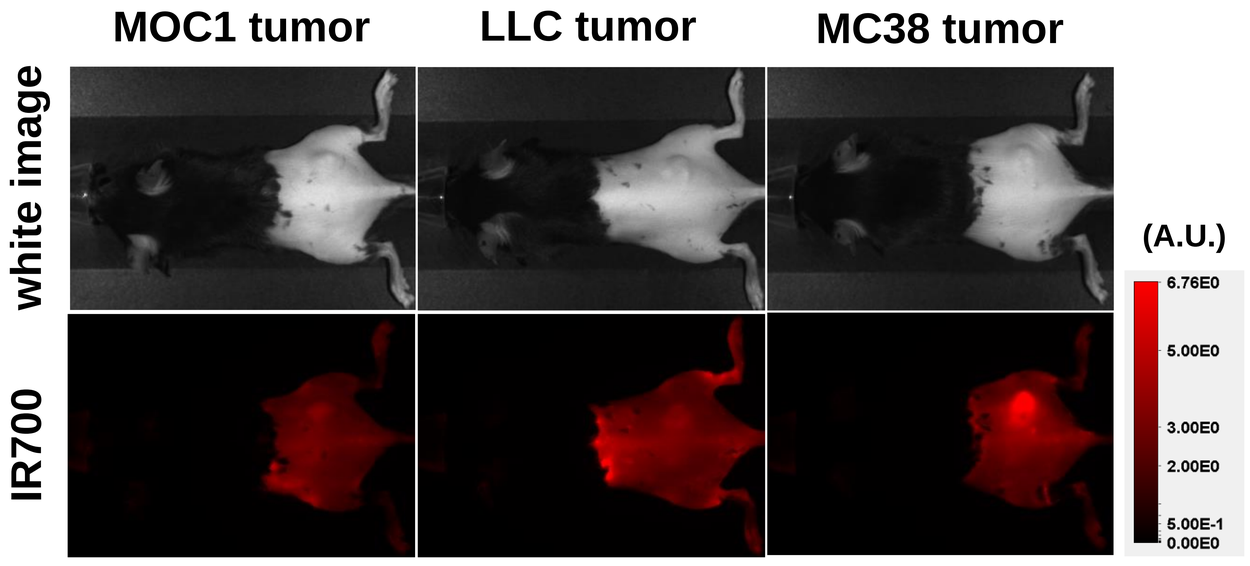
(A) Expression of cell surface CD44 in MOC1 cells was examined with flow cytometry. CD44 blocking antibody was added to some wells to validate specific staining. Representative histograms shown. (B) DIC and fluorescence microscopy images of MOC1 cells. Change in MOC1 cellular architecture following 15 min of NIR light exposure shown. Scale bars = 20 μm. (C) Membrane permeability of MOC1 cells, as measured by PI staining, after labeling with CD44-IR700 and treatment with NIR-light (n = 5, $^{*}p < 0.05$, $^{**}p < 0.01$, vs untreated control, by Student's t test).

A

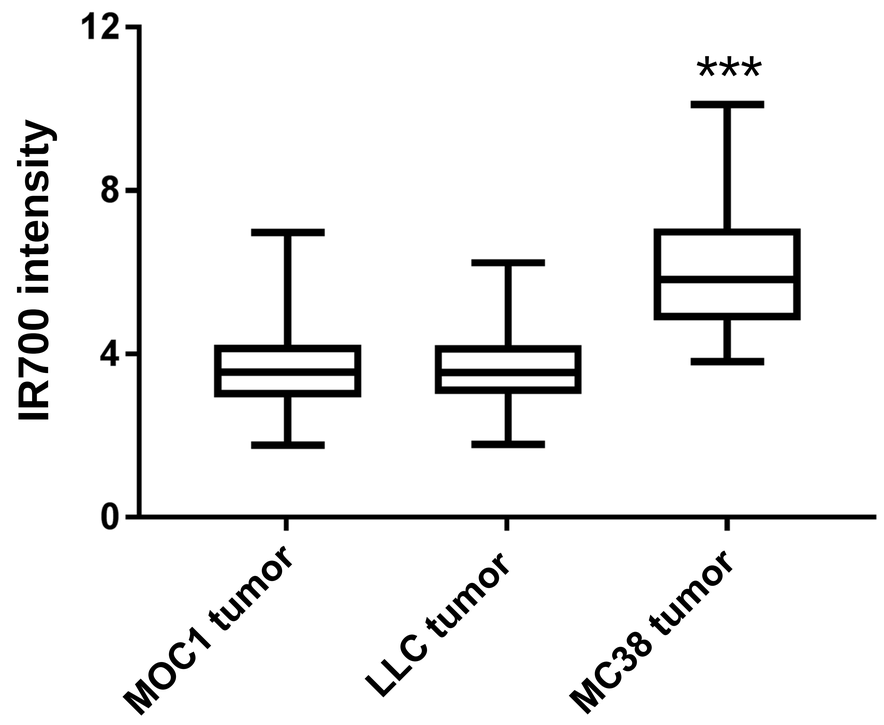
sFig3


CD45.2-CD31+PDGFR-
Endothelial cells
MFI
n/s
MOC1
LLC
MC38
7AAD negative tumor
single cell suspension
endothelial
1.11
tumor cells
26.4
immune cells
40.3
CD31
CD45.2
CD45.2-CD31-PDGFR-
Tumor cells
***
***
**
CD45.2-CD31-PDGFR+
Fibroblasts
***
***
Immune cells
CD45.2+CD3+CD8+CD4-
T-lymphocytes
CD45.2+CD3+CD8-CD4+
T-lymphocytes
CD45.2+CD11b+F4/80+
Macrophages
CD45.2+CD11b+
Ly6GhiLy6Cint
PMN-myeloid
CD45.2+CD4+
FoxP3+CD25+
T-lymphocytes

B


MOC1 tumor
LLC tumor
MC38 tumor
white image
IR700
(A.U.)
6.76E0
5.00E0
3.00E0
2.00E0
5.00E-1
0.00E0

C

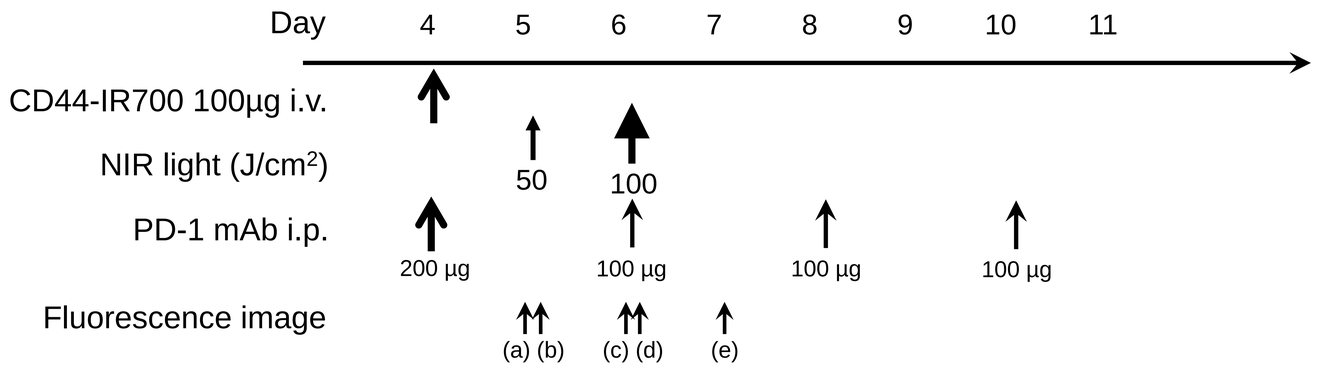

IR700 intensity
***
MOC1 tumor
LLC tumor
MC38 tumor

**Supplementary Figure 3. Baseline CD44 expression within MOC1, LLC and MC38-luc tumor compartments.**

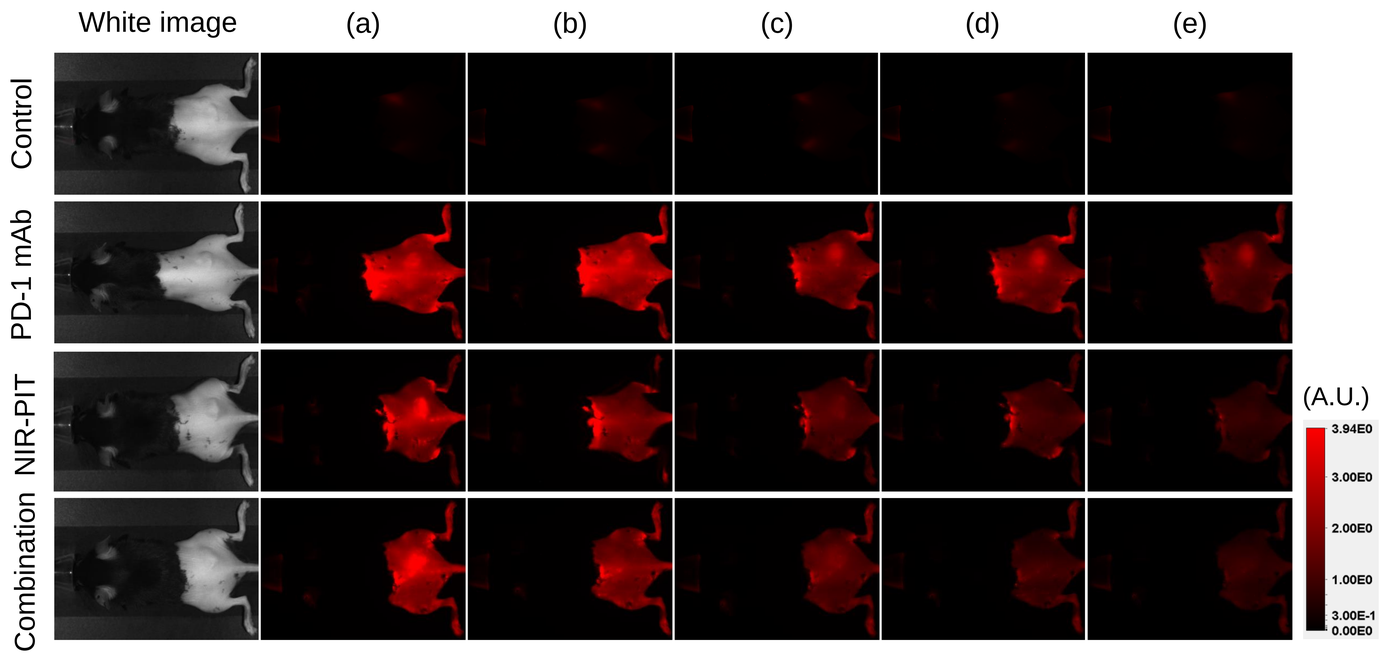
(A) Size matched MOC1 (day 24), LLC (day 10) and MC38-luc (day 10) tumors were harvested digested into a single cell suspension, and assessed for CD44 expression on individual cell types via flow cytometry (n = 3/group). Representative dot plot and gating strategy of a tumor digest shown. Cell surface phenotype of each cell type shown above bar graphs. $^{**}p < 0.01$, $^{***}p < 0.001$, t test with ANOVA. (B) *In vivo* CD44-IR700 fluorescence real-time imaging of tumor bearing mice. Images were obtained of MOC1 (day 18), LLC (day 4) and MC38-luc (day 4) tumors 24 hours after i.v. injection of CD44-IR700. The fluorescence intensity of CD44IR-700 was higher in MC38 tumor compared with the other two tumors. (C) Quantitative analysis of IR700 intensities in MOC1, LLC and MC38-luc tumors. The fluorescence intensities were significantly higher in MC38-luc tumors compared with other tumors ($n \geqq 10$, $^{***}p < 0.001$ vs MOC1 and LLC tumor, Tukey's test with ANOVA).

sFig4

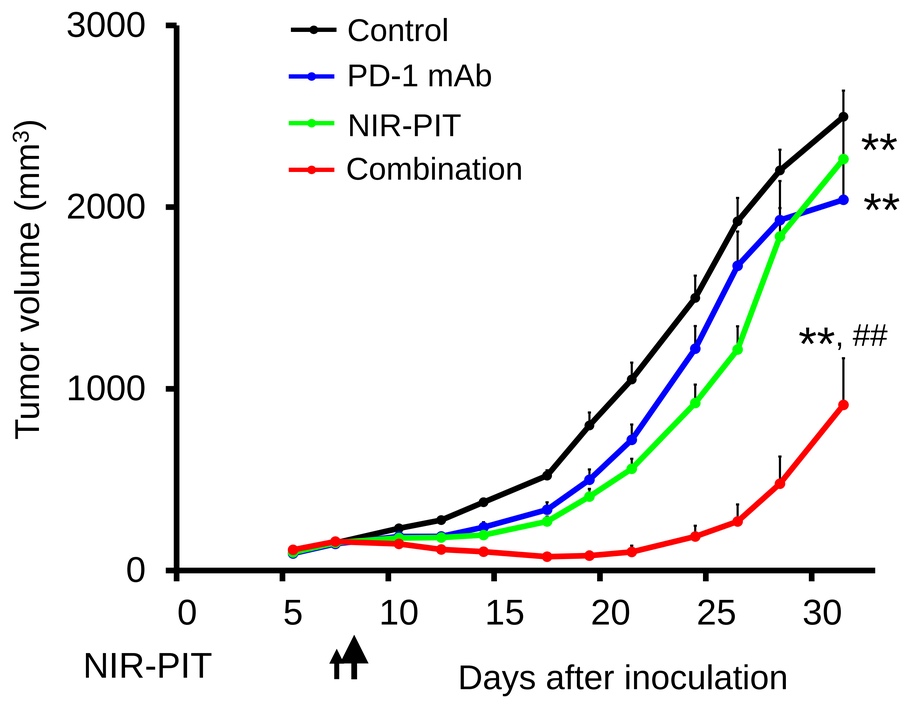
A


Day
4
5
6
7
8
9
10
11
CD44-IR700 100μg i.v.
NIR light (J/cm2)
50
100
PD-1 mAb i.p.
200 μg
100 μg
100 μg
100 μg
Fluorescence image
(a) (b)
(c) (d)
(e)

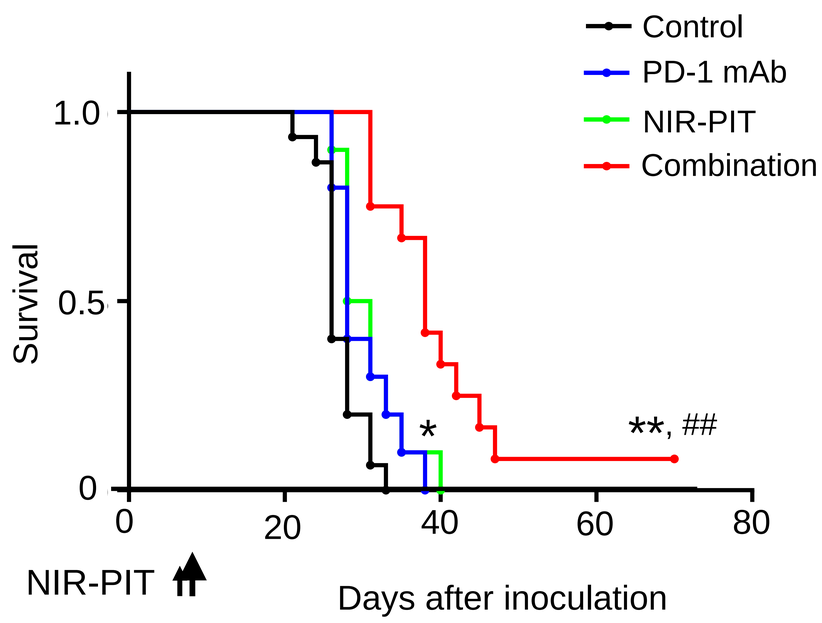
B


White image
(a)
(b)
(c)
(d)
(e)
Control
PD-1 mAb
NIR-PIT
Combination
(A.U.)
3.94E0
3.00E0
2.00E0
1.00E0
3.00E-1
0.00E0

C


Control
PD-1 mAb
NIR-PIT
Combination
Tumor volume (mm3)
3000
2000
1000
0
**
**
**, ##
0
5
10
15
20
25
30
NIR-PIT
Days after inoculation

D

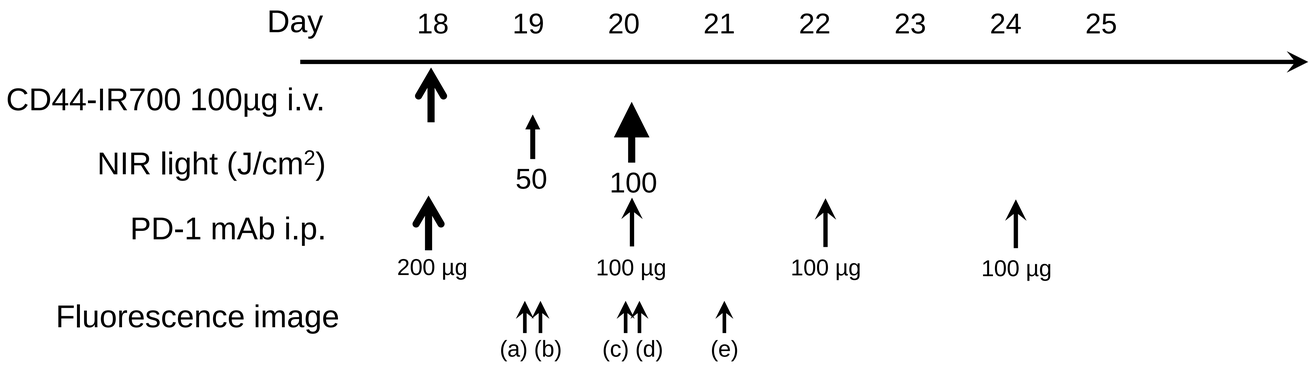

Control
PD-1 mAb
NIR-PIT
Combination
Survival
1.0
0.5
0
*
**, ##
0
20
40
60
80
NIR-PIT
Days after inoculation

**Supplementary Figure 4. *In vivo* effect of NIR-PIT and PD-1 mAb in mice bearing a unilateral LLC tumor.**

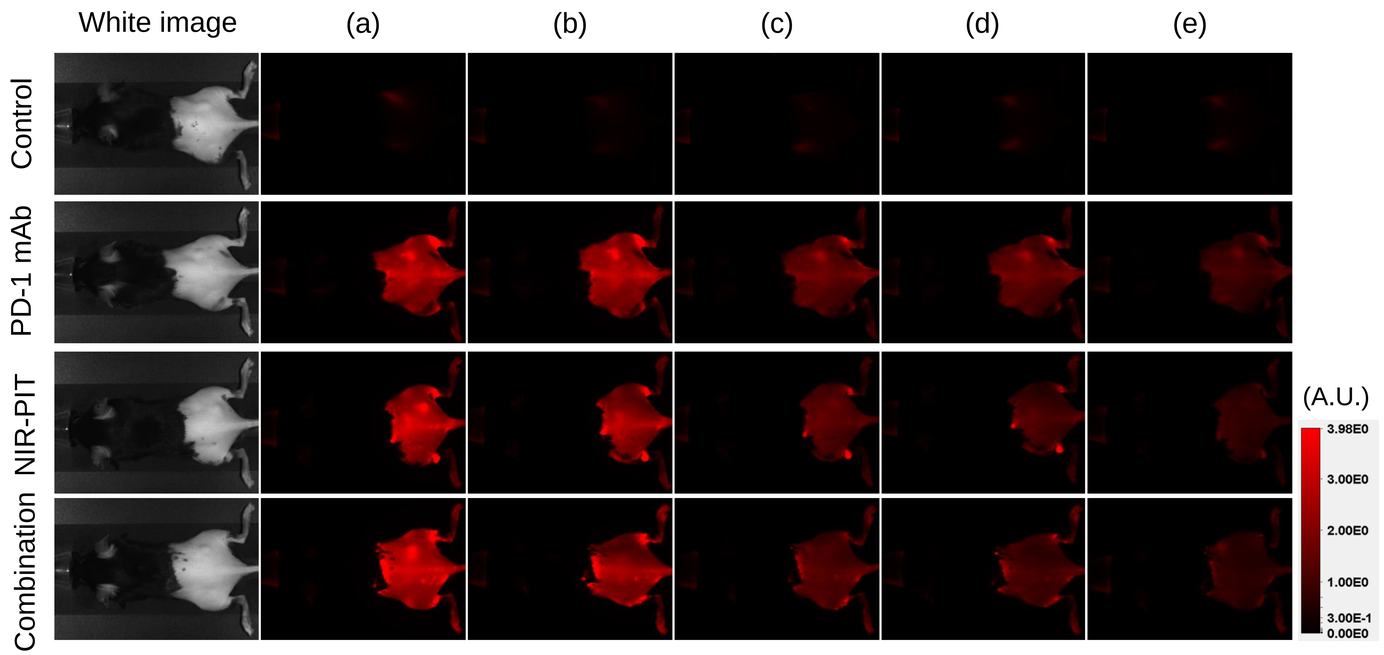
(A) NIR-PIT regimen. Bioluminescence and fluorescence images were obtained at each time point as indicated. (B) *In vivo* IR700 fluorescence real-time imaging of tumor-bearing mice in response to NIR-PIT alone or in combination with PD-1 mAb. Mice in the PD-1 mAb group also received CD44-IR700 but were not treated with NIR. (C) LLC tumor growth curves following NIR-PIT treatment with and without PD-1 mAb (n $\geqq$ 10, $^{**}p < 0.01$ vs control, $^{\#\#}p < 0.01$ vs PD-1 mAb and NIR-PIT groups, Tukey's t test with ANOVA). (D) Kaplan-Meier survival analysis (n $\geqq$ 10, $^{*}p < 0.05$, $^{**}p < 0.01$ vs control, $^{\#\#}p < 0.01$ vs PD-1 mAb and NIR-PIT groups, Log rank test).

sFig5

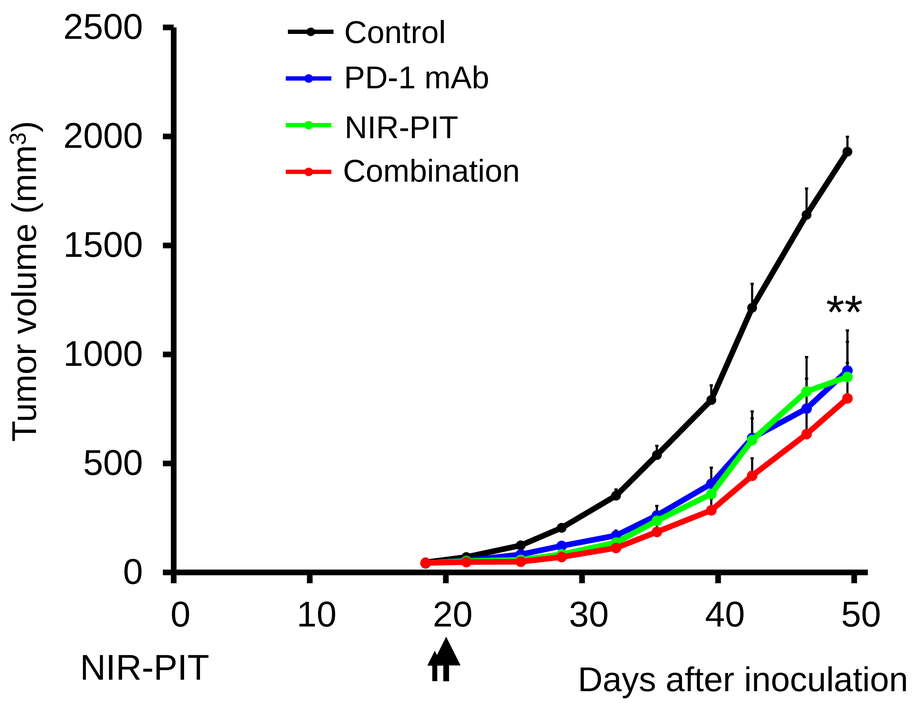
A


Day
18
19
20
21
22
23
24
25
CD44-IR700 100μg i.v.
NIR light (J/cm2)
50
100
PD-1 mAb i.p.
200 μg
100 μg
100 μg
100 μg
Fluorescence image
(a) (b)
(c) (d)
(e)

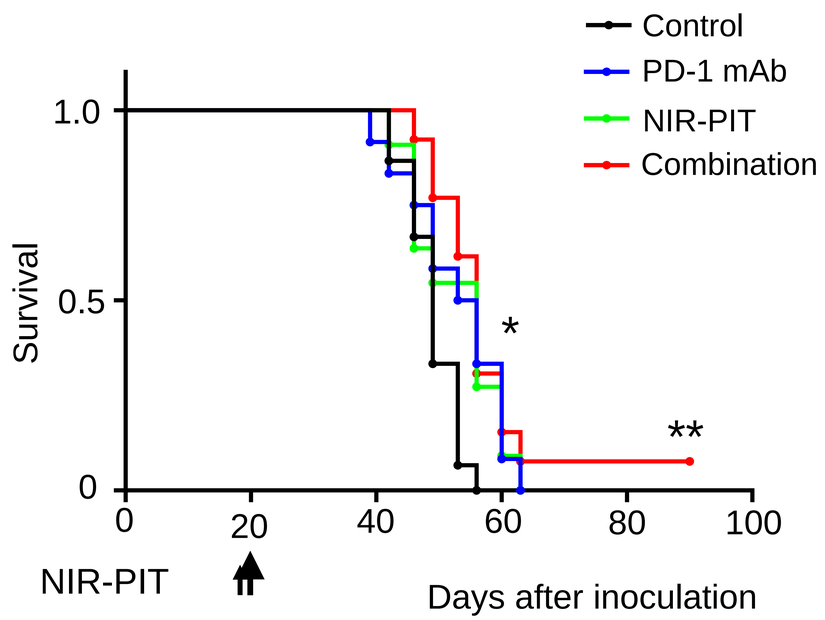
B


White image
(a)
(b)
(c)
(d)
(e)
Control
PD-1 mAb
NIR-PIT
Combination
(A.U.)
3.98E0
3.00E0
2.00E0
1.00E0
3.00E-1
0.00E0

C


Control
PD-1 mAb
NIR-PIT
Combination
Tumor volume (mm3)
2500
2000
1500
1000
500
0
**
0
10
20
30
40
50
NIR-PIT
Days after inoculation

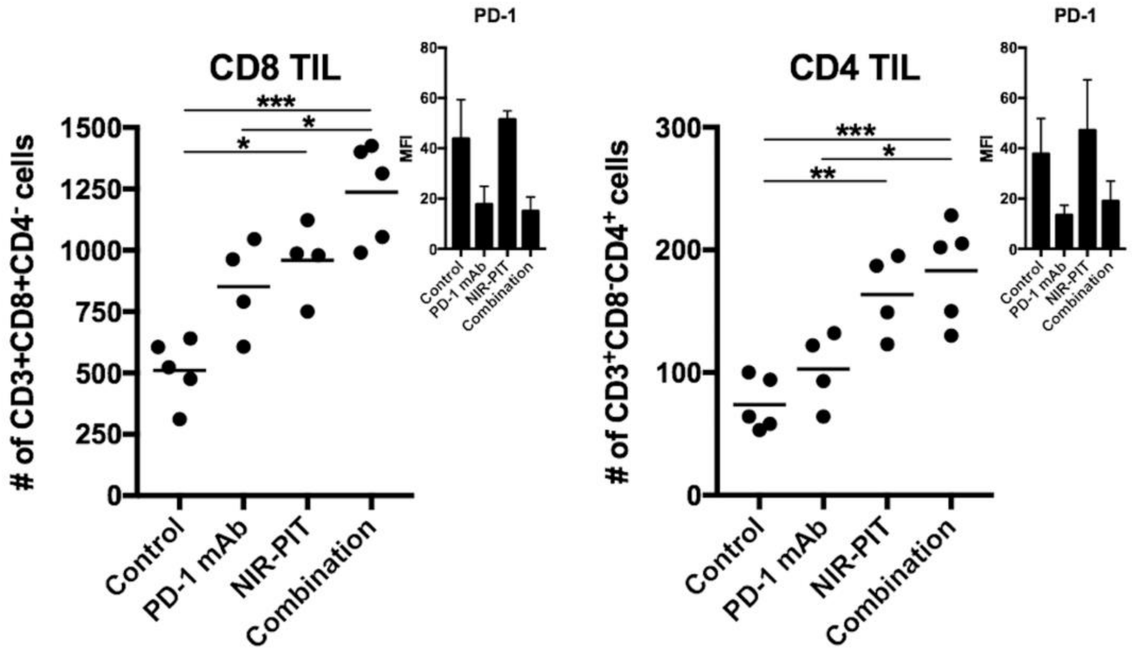
D


Control
PD-1 mAb
NIR-PIT
Combination
Survival
1.0
0.5
0
*
**
0
20
40
60
80
100
NIR-PIT
Days after inoculation

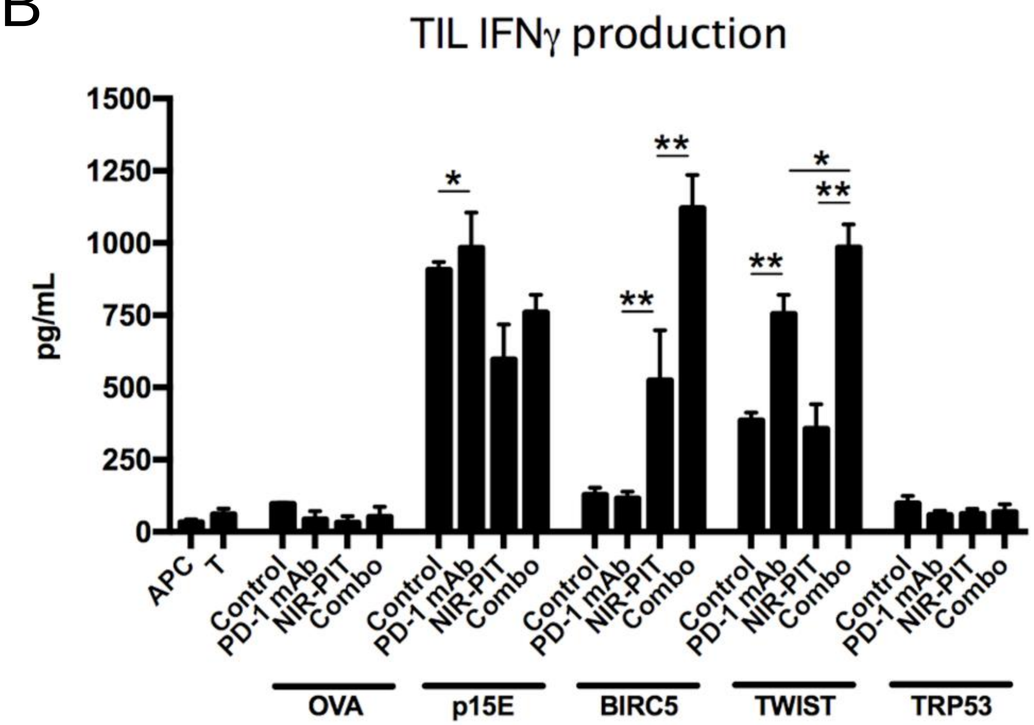
**Supplementary Figure 5. *In vivo* effect of NIR-PIT and PD-1 mAb in mice bearing a unilateral MOC1 tumor.**

(A) NIR-PIT regimen. Bioluminescence and fluorescence images were obtained at each time point as indicated. (B) *In vivo* IR700 fluorescence real-time imaging of tumor-bearing mice in response to NIR-PIT alone or in combination with PD-1 mAb. Mice in the PD-1 mAb group also received CD44-IR700 but were not treated with NIR. (C) MOC1 tumor growth curves following NIR-PIT treatment with and without PD-1 ($n \geqq 10$, $^{**}p < 0.01$ vs control, Tukey's test with ANOVA). (D) Kaplan-Meier survival analysis ($n \geqq 10$, $^{*}p < 0.05$, $^{**}p < 0.01$ vs control, Log rank test).

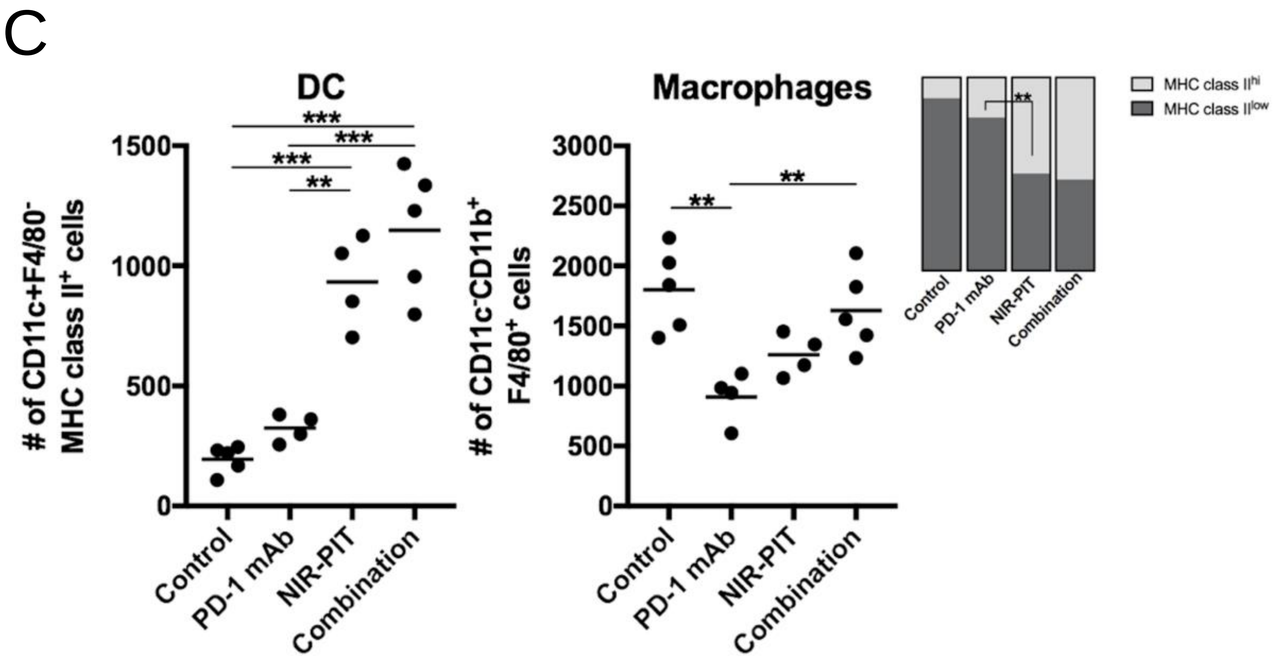
A


CD8 TIL
# of CD3+CD8+CD4- cells
PD-1
MFI
Control
PD-1 mAb
NIR-PIT
Combination
CD4 TIL
# of CD3+CD8-CD4+ cells

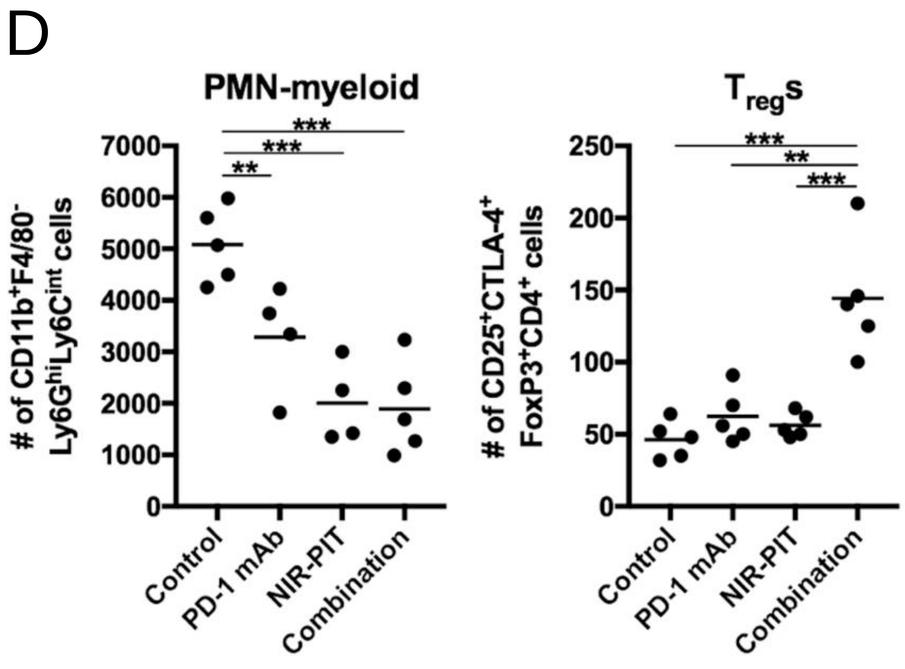
B


TIL IFNγ production
pg/mL
APC
T
Control
PD-1 mAb
NIR-PIT
Combo
OVA
p15E
BIRC5
TWIST
TRP53

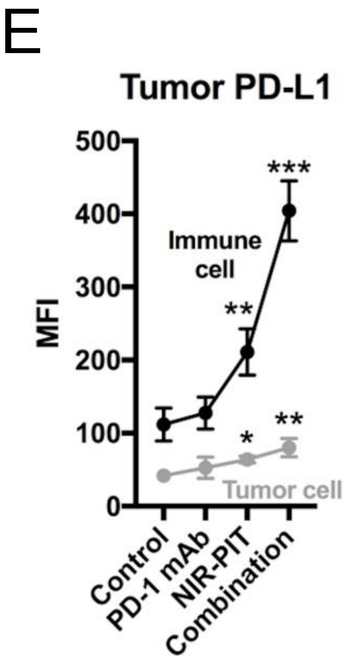
C


DC
# of CD11c+F4/80- MHC class II+ cells
Macrophages
# of CD11c-CD11b+ F4/80+ cells
MHC class IIhi
MHC class IIlow
Control
PD-1 mAb
NIR-PIT
Combination

D


PMN-myeloid
# of CD11b+F4/80- Ly6GhiLy6Cint cells
Treg s
# of CD25+CTLA-4+ FoxP3+CD4+ cells
Control
PD-1 mAb
NIR-PIT
Combination

E

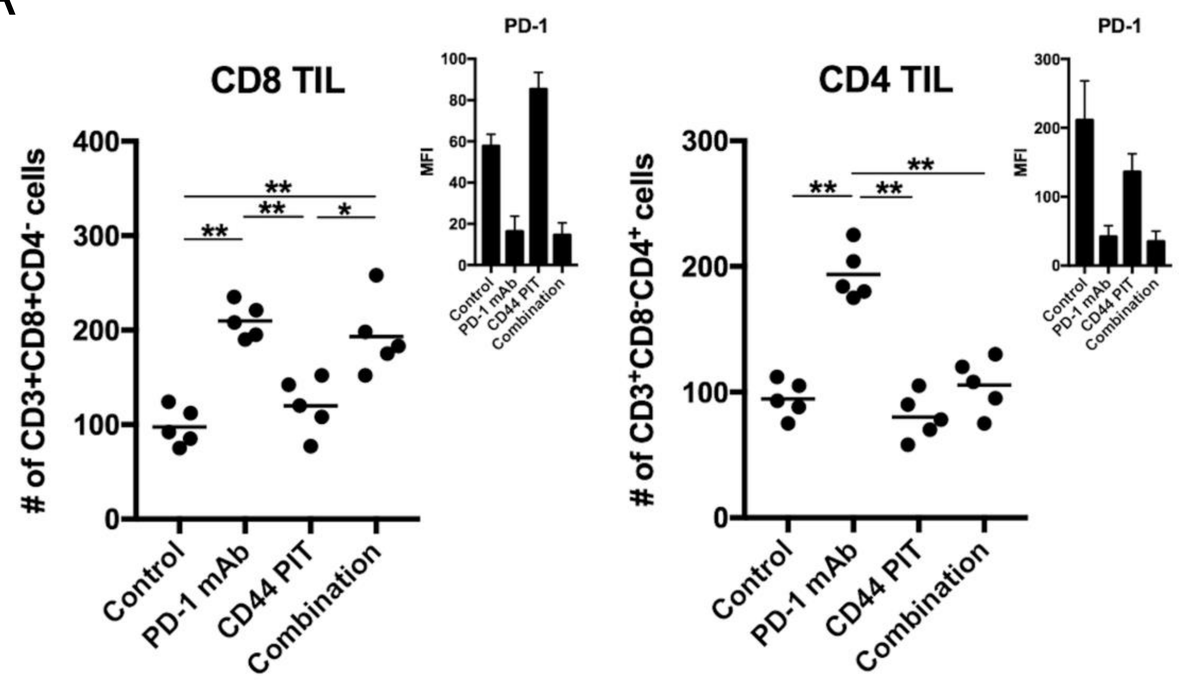

Tumor PD-L1
MFI
Immune cell
Tumor cell
Control
PD-1 mAb
NIR-PIT
Combination

**Supplementary Figure 6. Immune correlative and functional effects of NIR-PIT and PD-1 mAb in mice bearing a unilateral LLC tumor.**

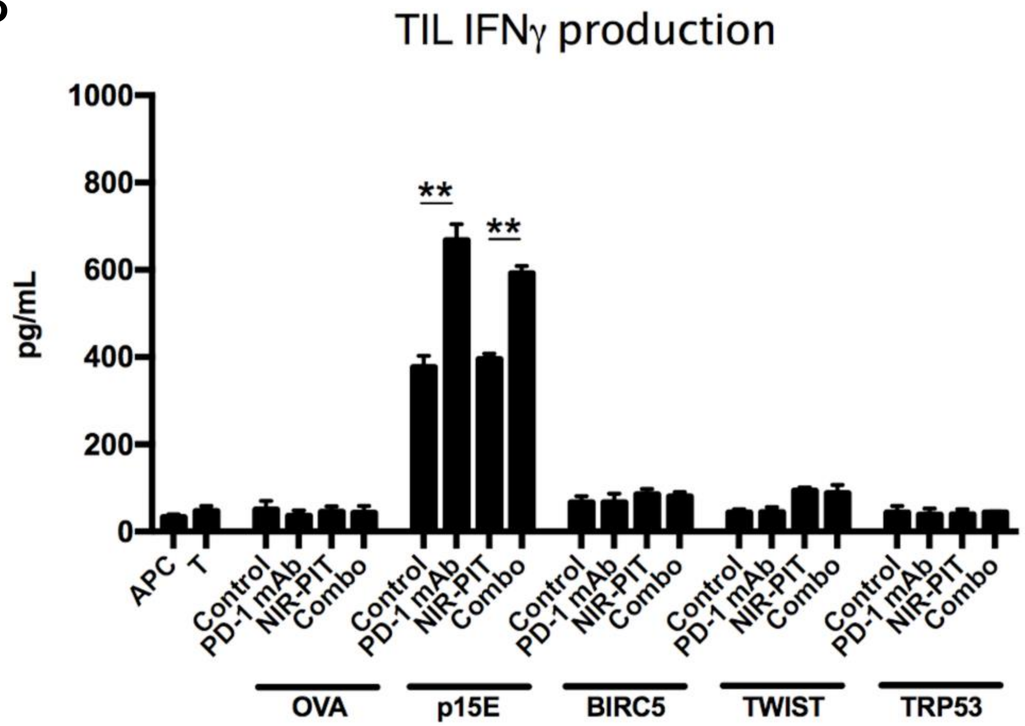
(A) LLC tumors (day 10, n = 5/group) treated with NIR-PIT with and without systemic PD-1 mAb and controls were harvested, digested into single-cell suspensions, and analyzed for tumor infiltrating lymphocytes (TIL) infiltration via flow cytometry. Presented as absolute number of infiltrating cells per $1.5 \times 10^4$ live cells analyzed. PD-1 expression shown as inset (MFI, mean fluorescence intensity). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, t test with ANOVA. (B) TIL were extracted from tumors treated as above (n = 5/group) via an IL-2 gradient, enriched via negative magnetic selection, and stimulated with irradiated splenocytes pulsed with peptides representing known MHC class I-restricted epitopes from selected tumor-associated antigens. IFNγ levels determined by ELISA from supernatants collected 24 hours after stimulation. Supernatants from splenocytes (APC) alone, TIL (T) alone, and a MHC-class I-restricted epitope from ovalbumin (OVA, SIINFEKL) used as controls. *$p < 0.05$, **$p < 0.01$, t test with ANOVA. (C) Flow cytometric analysis of tumor infiltrating dendritic cells (DC) and macrophages, with quantification of macrophage polarization

based on MHC class II expression. $^{**}p < 0.01$, $^{***}p < 0.001$, t test with ANOVA. (E) Flow cytometric analysis of tumor infiltrating granulocytic myeloid derived suppressor cells PMN-myeloid and $T_{reg}$s. $^{**}p < 0.01$, $^{***}p < 0.001$, t test with ANOVA. (F) Flow cytometric analysis of PD-L1 expression on $CD45.2^{-}CD31^{-}PDGFR^{-}$ tumor cells and $CD45.2^{+}CD31^{-}$ immune cells. N = 5/group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, t test with ANOVA.

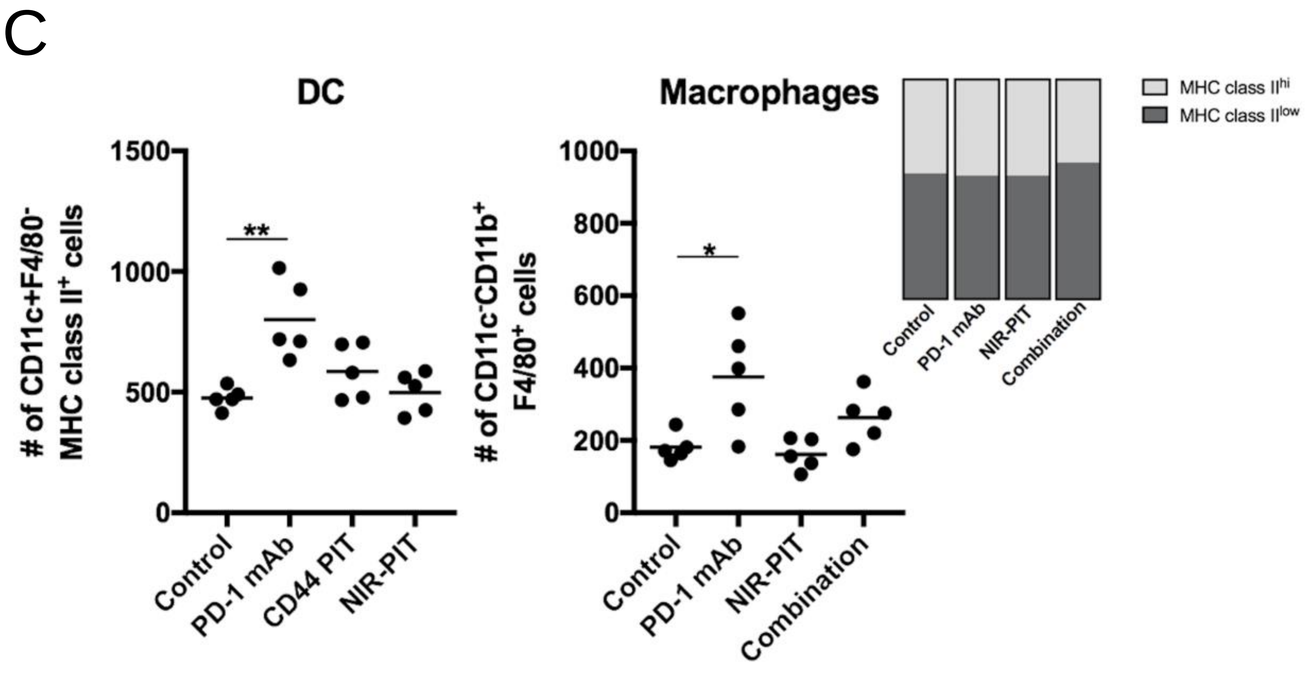
A


CD8 TIL
# of CD3+CD8+CD4- cells
400
300
200
100
0
**
**
*
Control
PD-1 mAb
CD44 PIT
Combination
PD-1
MFI
100
80
60
40
20
0
Control
PD-1 mAb
CD44 PIT
Combination
CD4 TIL
# of CD3+CD8-CD4+ cells
300
200
100
0
**
**
**
Control
PD-1 mAb
CD44 PIT
Combination
PD-1
MFI
300
200
100
0
Control
PD-1 mAb
CD44 PIT
Combination

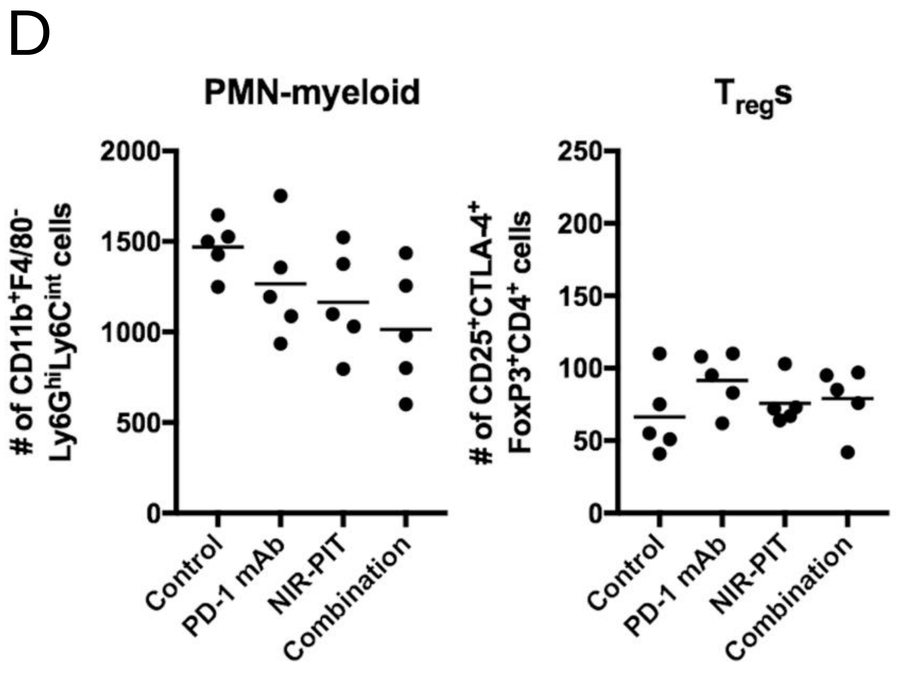
B


TIL IFNγ production
pg/mL
1000
800
600
400
200
0
**
**
APC
T
Control
PD-1 mAb
NIR-PIT
Combo
OVA
p15E
BIRC5
TWIST
TRP53

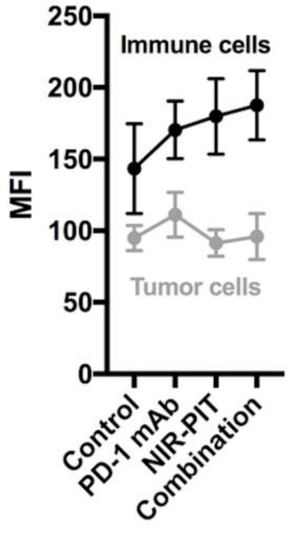
C


DC
# of CD11c+F4/80- MHC class II+ cells
1500
1000
500
0
**
Control
PD-1 mAb
CD44 PIT
NIR-PIT
Macrophages
# of CD11c-CD11b+ F4/80+ cells
1000
800
600
400
200
0
*
Control
PD-1 mAb
NIR-PIT
Combination
MHC class IIhi
MHC class IIlow
Control
PD-1 mAb
NIR-PIT
Combination

D


PMN-myeloid
# of CD11b+F4/80- Ly6GhiLy6Cint cells
2000
1500
1000
500
0
Control
PD-1 mAb
NIR-PIT
Combination
Treg s
# of CD25+CTLA-4+ FoxP3+CD4+ cells
250
200
150
100
50
0
Control
PD-1 mAb
NIR-PIT
Combination

E

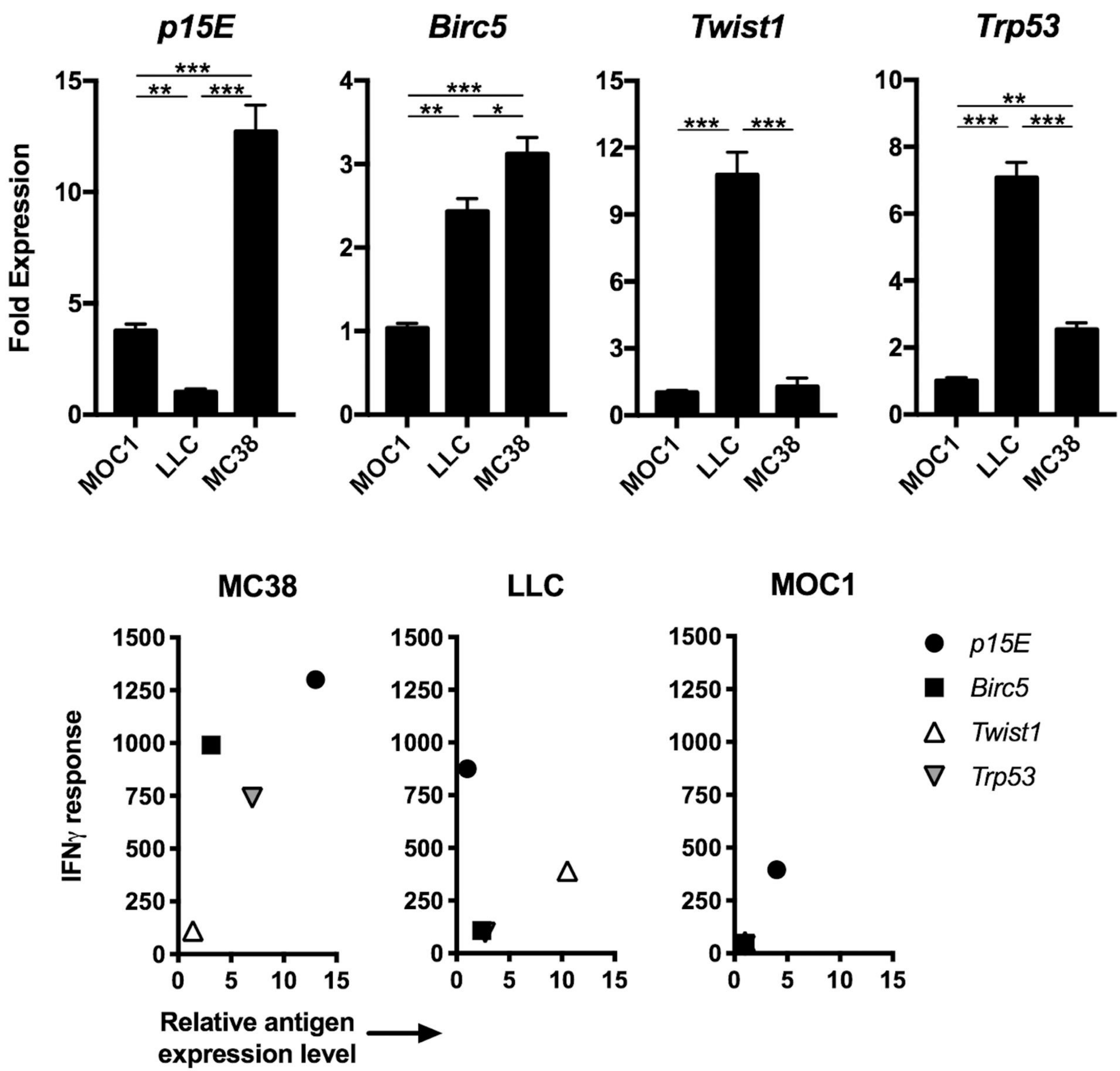

Tumor PD-L1
MFI
250
200
150
100
50
0
Immune cells
Tumor cells
Control
PD-1 mAb
NIR-PIT
Combination

**Supplementary Figure 7. Immune correlative and functional effects of NIR-PIT and PD-1 mAb in MOC1 tumor-bearing mice.**

(A) MOC1 tumors (day 10, n = 5/group) treated with NIR-PIT with and without systemic PD-1 mAb and controls were harvested, digested into single-cell suspensions, and analyzed for tumor infiltrating lymphocytes (TIL) infiltration via flow cytometry. Presented as absolute number of infiltrating cells per $1.5 \times 10^4$ live cells analyzed. PD-1 expression shown as inset (MFI, mean fluorescence intensity). *$p < 0.05$, **$p < 0.01$, t test with ANOVA. (B) TIL were extracted from tumors treated as above (n = 5/group) via an IL-2 gradient, enriched via negative magnetic selection, and stimulated with irradiated splenocytes pulsed with peptides representing known MHC class I-restricted epitopes from selected tumor-associated antigens. IFNγ levels determined by ELISA from supernatants collected 24 hours after stimulation. Supernatants from splenocytes (APC) alone, TIL (T) alone, and a MHC-class I-restricted epitope from ovalbumin (OVA, SIINFEKL) used as controls. **$p < 0.01$, t test with ANOVA. (C) Flow cytometric analysis of tumor infiltrating dendritic cells (DC) and macrophages, with quantification of macrophage polarization based on

MHC class II expression. *$p < 0.05$, **$p < 0.01$, t test with ANOVA. (D) Flow cytometric analysis of tumor infiltrating PMN-myeloid and $T_{reg}$s. (E) Flow cytometric analysis of PD-L1 expression on $CD45.2^{-}CD31^{-}PDGFR^{-}$ tumor cells and $CD45.2^{+}CD31^{-}$ immune cells. N = 5/group.

sFig8

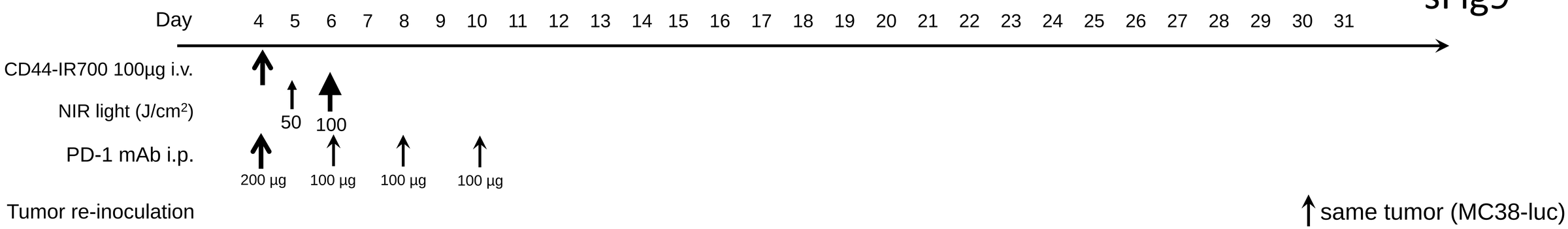

p15E
Birc5
Twist1
Trp53
Fold Expression
MOC1
LLC
MC38
***
**
*
MC38
LLC
MOC1
IFNγ response
Relative antigen expression level
p15E
Birc5
Twist1
Trp53

**Supplementary Figure 8. Relative tumor associated antigen gene expression.**

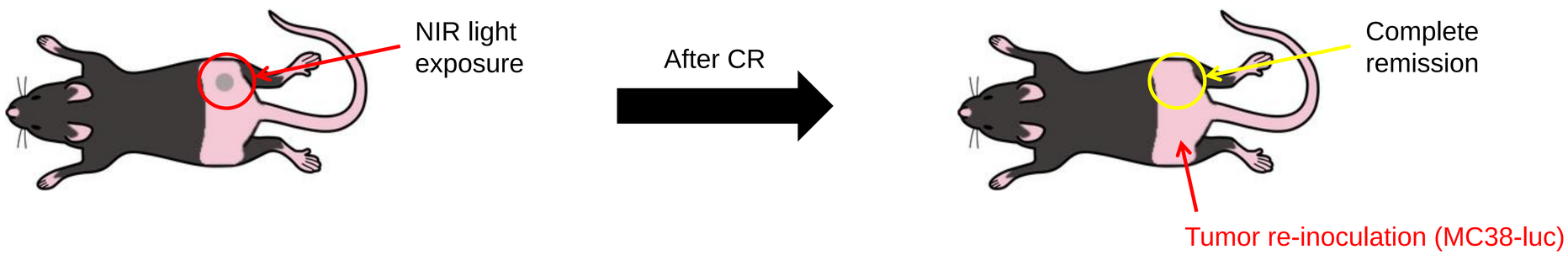
MC38-luc, LLC and MOC1 cells were processed and assessed for gene expression of *p15E*, *Birb5*, *Twist1* and *Trp53* by qRT-PCR using custom primers designed to flank the region encoding the MHC class I-restricted epitope (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, t test with ANOVA.). Two-dimensional plot of relative antigen expression level vs baseline antigen-specific IFNγ responses in TIL for each model shown on bottom.

sFig9

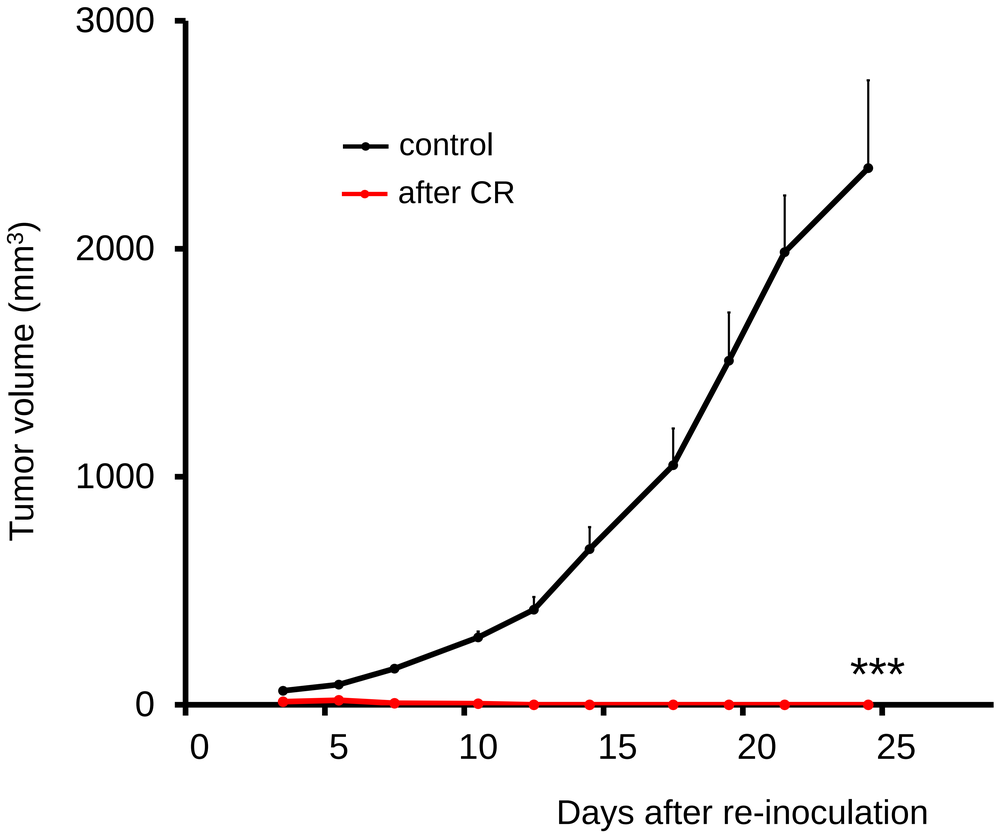
A


Day
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
CD44-IR700 100μg i.v.
NIR light (J/cm2)
50
100
PD-1 mAb i.p.
200 μg
100 μg
100 μg
100 μg
Tumor re-inoculation
same tumor (MC38-luc)

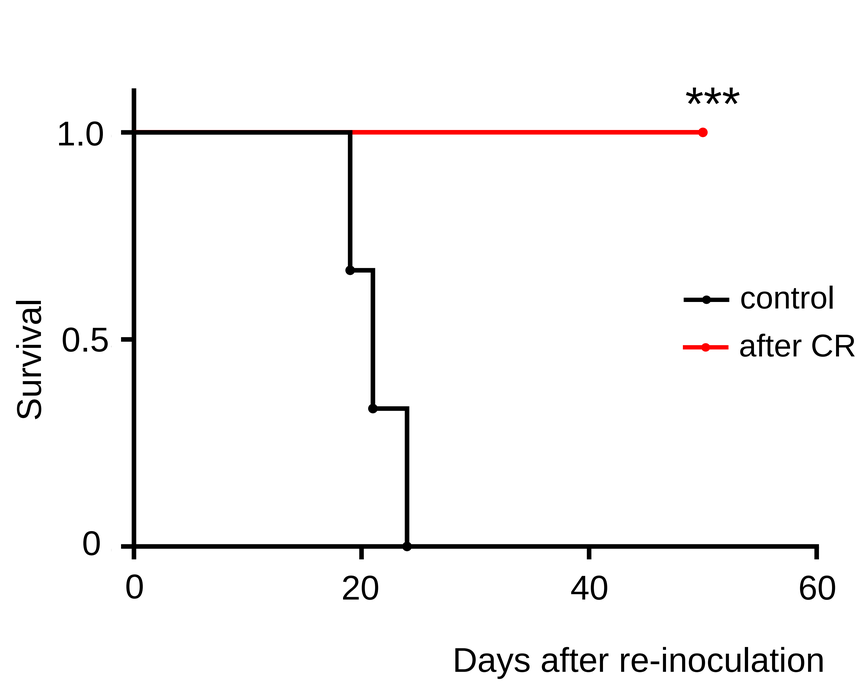
B


NIR light exposure
After CR
Complete remission
Tumor re-inoculation (MC38-luc)

C


3000
2000
1000
0
Tumor volume (mm3)
control
after CR
***
0 5 10 15 20 25
Days after re-inoculation

D


1.0
0.5
0
Survival
***
control
after CR
0 20 40 60
Days after re-inoculation

**Supplementary Figure 9. Resistance to re-challenge with MC38-luc cells following complete tumor rejection with combination NIR-PIT and PD-1 mAb treatment**

(A) The regimen of tumor re-challenge in mice that completely rejected (CR) tumors with combination treatment. Tumor was inoculated on the contralateral side 30 days after first inoculation. (B) Mice receiving re-inoculation of MC38-luc cells. (C) Growth curves of control and CR mice challenged with MC38-luc cells in the contralateral flank. (D) Kaplan-Meier survival analysis (n = 9, ***$p < 0.001$, by Tukey's test with ANOVA for growth curves, ***$p < 0.001$, by Log-rank test for survival).

# Supplementary Videos
# 1 - 3

Supplementary Video 1:
Microscopic image of *in vitro* NIR-PIT for MC38 cell

Supplementary Video 2:
Microscopic image of *in vitro* NIR-PIT for LLC cell

Supplementary Video 3:
Microscopic image of *in vitro* NIR-PIT for MOC1 cell