Figure 1S

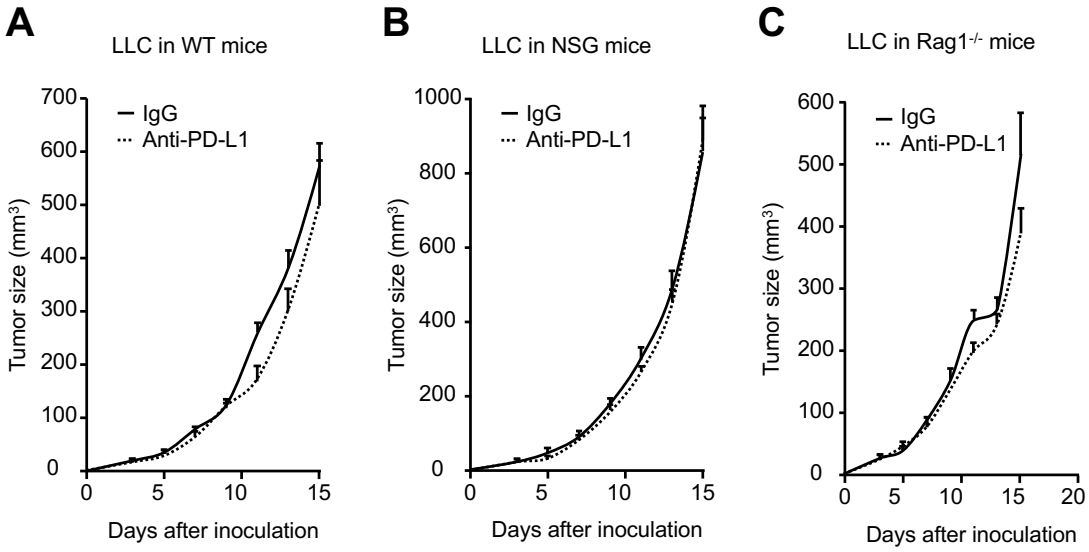

A
LLC in WT mice
IgG
Anti-PD-L1
Tumor size (mm3)
Days after inoculation
B
LLC in NSG mice
IgG
Anti-PD-L1
Tumor size (mm3)
Days after inoculation
C
LLC in Rag1-/- mice
IgG
Anti-PD-L1
Tumor size (mm3)
Days after inoculation

Figure 2S

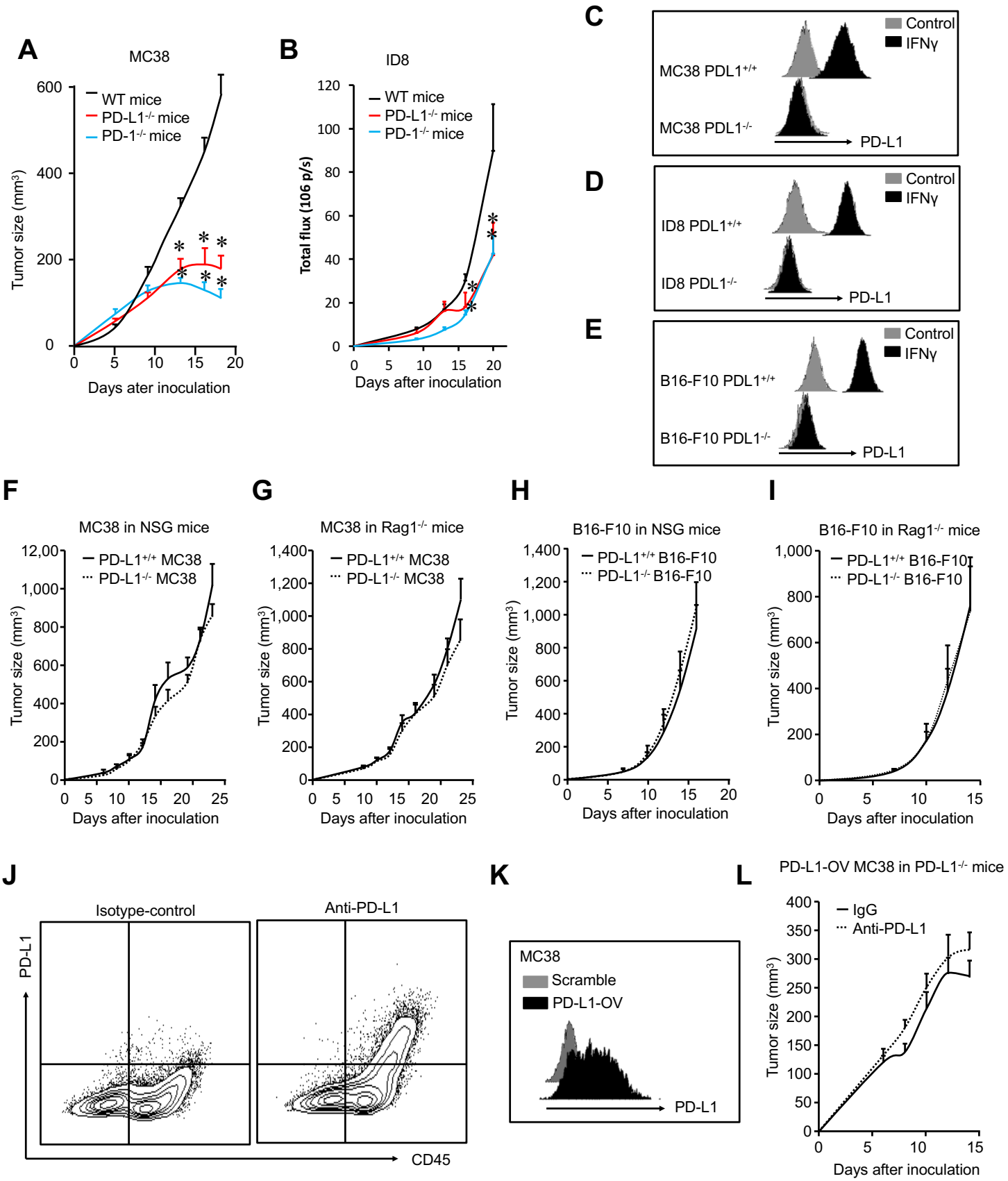

A
MC38
WT mice
PD-L1-/- mice
PD-1-/- mice
Tumor size (mm3)
Days ater inoculation
B
ID8
Total flux (106 p/s)
Days after inoculation
C
Control
IFNγ
MC38 PDL1+/+
MC38 PDL1-/-
PD-L1
D
ID8 PDL1+/+
ID8 PDL1-/-
E
B16-F10 PDL1+/+
B16-F10 PDL1-/-
F
MC38 in NSG mice
PD-L1+/+ MC38
PD-L1-/- MC38
G
MC38 in Rag1-/- mice
H
B16-F10 in NSG mice
PD-L1+/+ B16-F10
PD-L1-/- B16-F10
I
B16-F10 in Rag1-/- mice
J
Isotype-control
Anti-PD-L1
CD45
K
MC38
Scramble
PD-L1-OV
L
PD-L1-OV MC38 in PD-L1-/- mice
IgG
Anti-PD-L1

Figure 3S

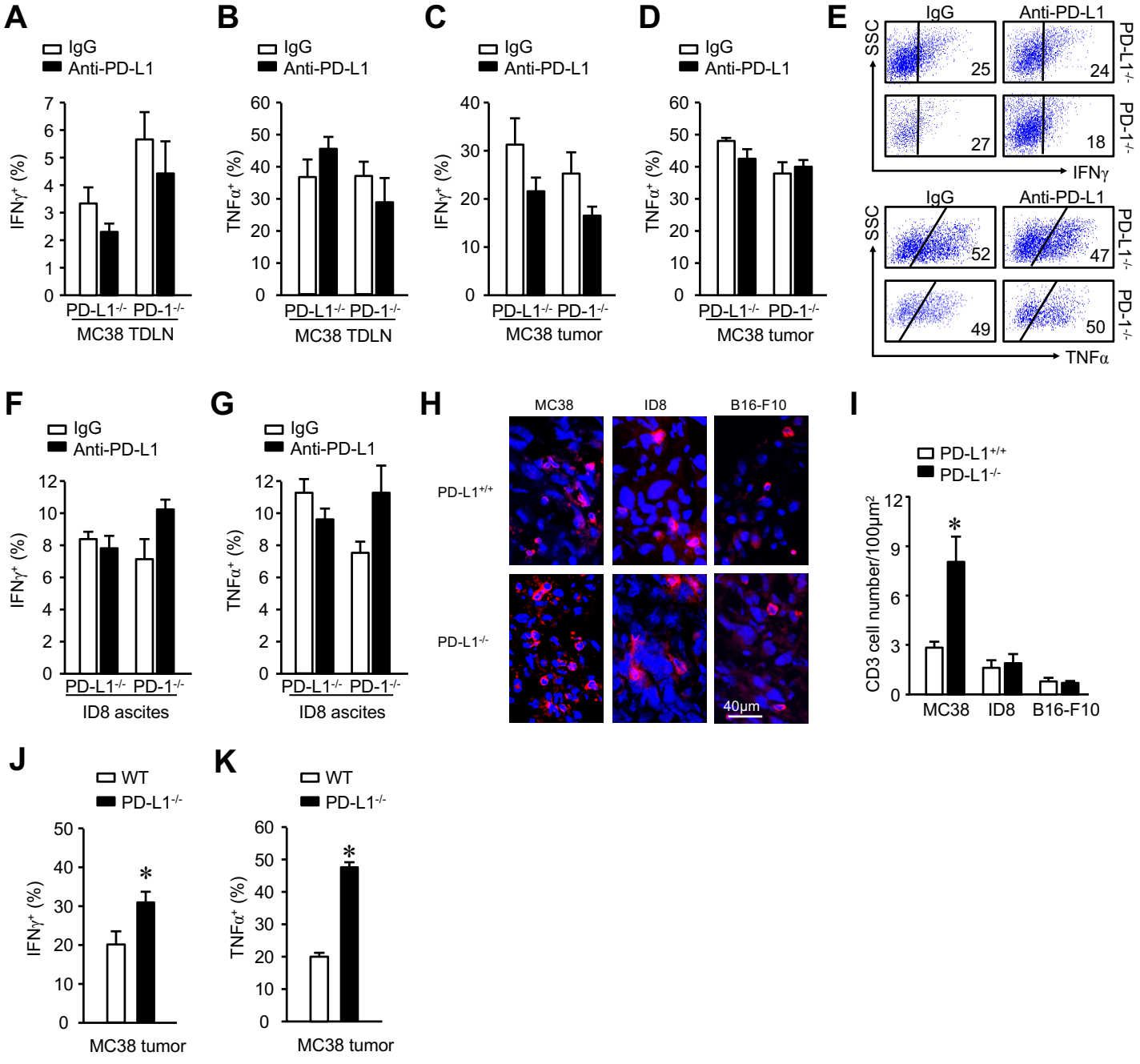

A
IgG
Anti-PD-L1
IFNγ+ (%)
PD-L1-/- PD-1-/-
MC38 TDLN
B
TNFα+ (%)
C
MC38 tumor
D
E
SSC
IFNγ
TNFα
F
ID8 ascites
G
H
MC38
ID8
B16-F10
PD-L1+/+
PD-L1-/-
40μm
I
PD-L1+/+
CD3 cell number/100μm2
J
WT
K

Figure 4S

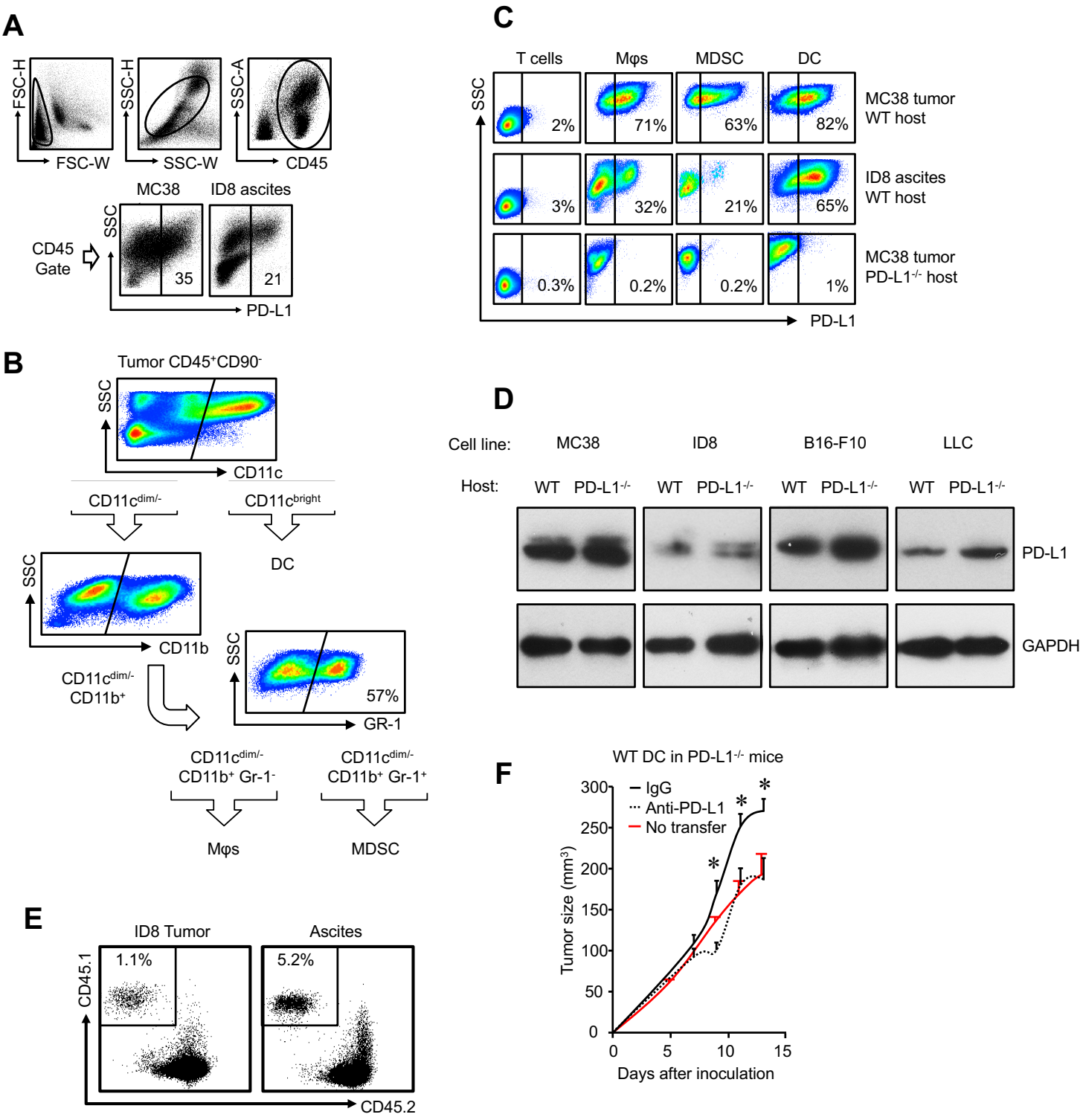
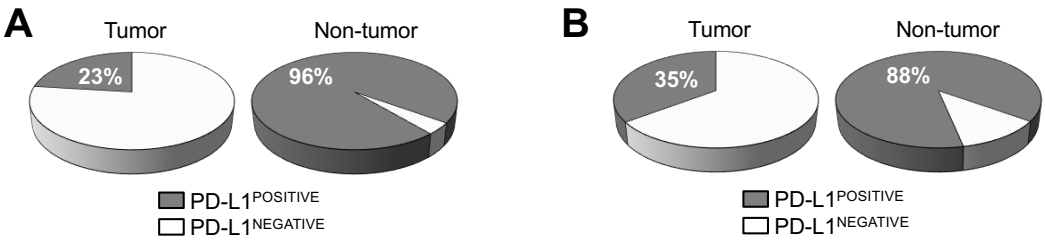

A
FSC-H
FSC-W
SSC-H
SSC-W
SSC-A
CD45
MC38
ID8 ascites
SSC
CD45 Gate
35
21
PD-L1
B
Tumor CD45+CD90-
SSC
CD11c
CD11cdim/-
CD11cbright
DC
CD11b
CD11cdim/- CD11b+
57%
GR-1
CD11cdim/- CD11b+ Gr-1-
CD11cdim/- CD11b+ Gr-1+
Mφs
MDSC
C
T cells
Mφs
MDSC
DC
2%
71%
63%
82%
MC38 tumor WT host
3%
32%
21%
65%
ID8 ascites WT host
0.3%
0.2%
0.2%
1%
MC38 tumor PD-L1-/- host
PD-L1
D
Cell line:
MC38
ID8
B16-F10
LLC
Host:
WT
PD-L1-/-
PD-L1
GAPDH
E
ID8 Tumor
Ascites
1.1%
5.2%
CD45.1
CD45.2
F
WT DC in PD-L1-/- mice
IgG
Anti-PD-L1
No transfer
Tumor size (mm3)
Days after inoculation
300
250
200
150
100
50
0
0
5
10
15

Figure 5S


A
Tumor
Non-tumor
23%
96%
PD-L1POSITIVE
PD-L1NEGATIVE
B
Tumor
Non-tumor
35%
88%
PD-L1POSITIVE
PD-L1NEGATIVE

Supplementary figure legends

Figure S1. Effect of anti-PD-L1 treatment on LLC volume in mice.

(**A-C**) WT, NSG, and Rag1$^{-/-}$ mice were inoculated with LLC tumor cells. The mice were treated from day 3 every 3 days with anti-PD-L1 or isotype control (rIgG1). Tumor volume was monitored. n = 5-7. Wilcoxon test was used for two-way comparisons.

Figure S2. Expression and effect of tumor PD-L1 on tumor growth.

(**A-B**) WT, PD-L1$^{-/-}$, and PD-1$^{-/-}$ mice were inoculated with MC38 (**A**), and ID8 (**B**) tumor cells. Tumor volume was monitored. n = 5-7. T-test was used for two-way comparisons (*P < 0.05). (**C-E**) CRISPR PD-L1 homozygous PD-L1 knockout (PD-L1$^{-/-}$) tumor cell clones were made for MC38 (**C**), ID8 (**D**), and B16-F10 (**E**). PD-L1$^{-/-}$ and control tumor cells were stimulated for 48 hours with or without IFNγ (10ng/ml). PD-L1 expression was analyzed by FACS. One of 4 experiments is shown. (**F-I**) PD-L1$^{-/-}$ MC38 (**F, G**), PD-L1$^{-/-}$ B16-F10 (**H, I**) and wild type control tumor cells were inoculated into NSG and Rag1$^{-/-}$ mice. Tumor load was monitored. n=5 mice per group. (**J**) PD-L1$^{-/-}$ MC38 cells were inoculated into wild type mice. Single cells were prepared from tumor tissues on day 15. PD-L1 expression was analyzed by FACS on CD45^{+} immune cells and CD45^{-} tumor cells. One of 5 is shown. (**K-L**) PD-L1 overexpressed (PD-L1-OV) or scrambled MC38 cells were analyzed for PD-L1 expression by FACS (**K**). PD-L1$^{-/-}$ mice were inoculated with PD-L1-OV MC38 cells (**L**, n = 8) and treated with anti-PD-L1 or isotype control (rIgG1). Tumor volume was monitored. Wilcoxon test was used for two-way comparisons. (*P < 0.05).

Figure S3. Effect of anti-PD-L1 on T cell effector cytokine expression.

(**A-G**) T cell effector cytokines were analyzed with intracellular staining in MC38 tumor draining lymph nodes (TDLN) (**A, B**), MC38 tumor tissues (**C-E**), and ID8 tumor ascites (**F, G**) in PD-L1$^{-/-}$ and PD-1$^{-/-}$ mice. Data are expressed as the mean ± SEM (n = 3-5 per group). Representative original flow cytometry data are shown (**E**). (**H, I**) T cell infiltration was analyzed with immunofluorescence staining for CD3 in tumors from PD-L1$^{+/+}$ and PD-L1$^{-/-}$ mice. Data are expressed as the mean ± SEM (**I**, n = 10 per group). Representative staining images are shown (**H**). T-test was used for two-way comparisons (*P < 0.05). (**J, K**) T cell effector cytokines were analyzed with intracellular staining in MC38 tumor in WT and PD-L1$^{-/-}$ mice, n = 5. T-test was used for two-way comparisons (*P < 0.05).

Figure S4. APC subsets in tumor tissues and tumor PD-L1 expression *in vivo.*

(**A-C**) PD-L1 expression in tumor infiltrating immune cells. **(A)** PD-L1 expression on $CD45^+$ immune cells in MC38 tumor tissues. (**B**) FACS gating on different immune cell subsets in MC38 tumor tissues. Representative flow cytometry data showed gates on $CD45^+CD90^-$ cells, DCs, macrophages, and MDSCs. (**C**) The percentages of PD-L1 expression in each immune cell subset in MC38 and ID8 ascites. One of five replicates is shown. (**D**) PD-L1 expression on tumor cells *in vivo*. Tumor cells were isolated from wild type and PD-$L1^{-/-}$ mice. PD-L1 expression was detected by Western blots in tumor cells. Experiments were performed in triplicates; representative replicate is shown. (**E**) $CD45.1^+$ peritoneal APCs were injected into ID8 tumor bearing $CD45.2^+$ mice. 48 hours after APC injection, $CD45.1^+$ APCs were examined in ID8 tumor tissues and ascites by flow cytometry. n = 5. (**F**) MC38 tumor bearing PD-$L1^{-/-}$ mice were adoptively transferred with (black) or without (red) WT DCs. Mice were treated with anti-PD-L1 or isotype IgG1. Tumor volume was monitored. Wilcoxon test was used for two-way comparisons (n = 7, $*P < 0.05$).

Figure S5. PD-$L1^+$ APCs and tumor cells in patients with melanoma and ovarian cancer.

(**A-B**) Percentages of patients with PD-$L1^+$ tumor cells or PD-$L1^+$ non-tumor cells were shown in melanoma (**A**) and ovarian cancer (**B**) tissues.

Supplementary Table 1. Characteristics of melanoma patients and PD-L1 expression pattern

Patient	Gender	Response	tissue type	Tumor	Non-tumor		(%)	
					positivity	score	mDC	MQ
1	M	CR	sinus primary	negative	Positive	4	50	30
2	M	CR	lymph node metastasis	single cells	Positive	3	20	0
3	M	CR	skin primary	negative	Positive	3	5	10
4	M	CR	subcutaneous metastasis	negative	Positive	3	20	5
5	M	CR	lymph node metastasis	negative	Positive	3	50	5
6	F	CR	lymph node metastasis	negative	Positive	4	20	50
7	F	CR	skin primary	positive	Positive	3	30	20
8	M	CR	lymph node metastasis	negative	Positive	4	50	50
9	M	CR	lymph node metastasis	single cells	Positive	3	20	40
10	M	CR	skin primary	single cells	Positive	4	50	40
11	M	PR	sinus metastasis	negative	Positive	2	20	0
12	M	PR	brain metastasis	negative	Positive	1	1	5
13	M	PR	subcutaneous metastasis	negative	Positive	3	30	20
14	M	PR	soft tissue metastasis	negative	Positive	1	5	1
15	M	PR	lymph node metastasis	negative	Positive	2	20	10
16	F	PR	skin primary	negative	Positive	1	1	10
17	F	SD	uvea primary	negative	Positive	3	50	10
18	M	SD	skin primary	positive	Positive	1	5	1
19	M	SD	lymph node metastasis	negative	Positive	3	30	30
20	M	SD	soft tissue metastasis	positive	Positive	1	1	1
21	F	SD	lymph node metastasis	negative	Positive	3	20	50
22	M	PD	lymph node metastasis	negative	Positive	2	5	0
23	F	PD	lymph node metastasis	negative	Positive	2	30	20
24	F	PD	skin/vulvar primary	negative	negative	0	0	0
25	M	PD	lung metastasis	negative	Positive	1	1	1
26	F	PD	dermal/subcutaneous metastasis	negative	Positive	1	1	1

Supplementary Table 2. Characteristics of ovarian cancer patients and PD-L1 expression pattern

Patient	Gender	Response	Tumor	Non-tumor		(%)	
				positivity	score	mDC	MQ
1	F	CR	Negative	Positive	4	20	50
2	F	CR	Negative	Positive	3	10	20
3	F	PR	Negative	Positive	4	40	40
4	F	SD	Positive	Positive	4	10	20
5	F	SD	Negative	Positive	2	1	10
6	F	SD	Negative	Positive	4	40	50
7	F	SD	Negative	Positive	2	1	10
8	F	SD	Negative	Positive	2	10	5
9	F	PD	Positive	Positive	3	20	40
10	F	PD	Negative	Positive	1	1	1
11	F	PD	Negative	Positive	2	5	5
12	F	PD	Positive	Negative	0	0	0
13	F	PD	Positive	Negative	0	0	0
14	F	PD	Negative	Positive	2	5	5
15	F	PD	Negative	Positive	2	5	5
16	F	PD	Positive	Positive	2	5	10
17	F	PD	Negative	Positive	2	5	1

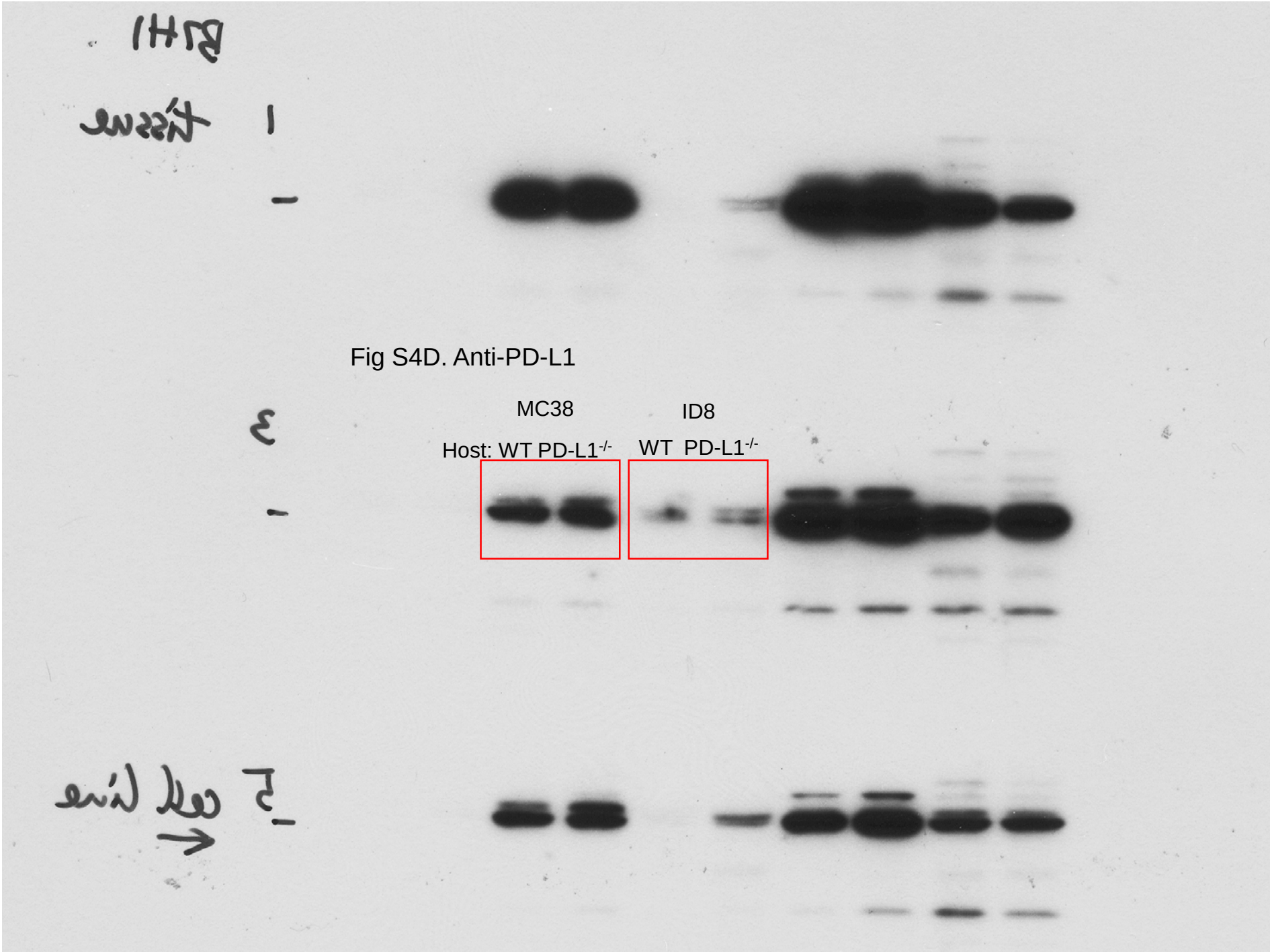


Fig S4D. Anti-PD-L1

Fig S4D. Anti-PD-L1 B16-F10

Host: WT PD-L1$^{-/-}$

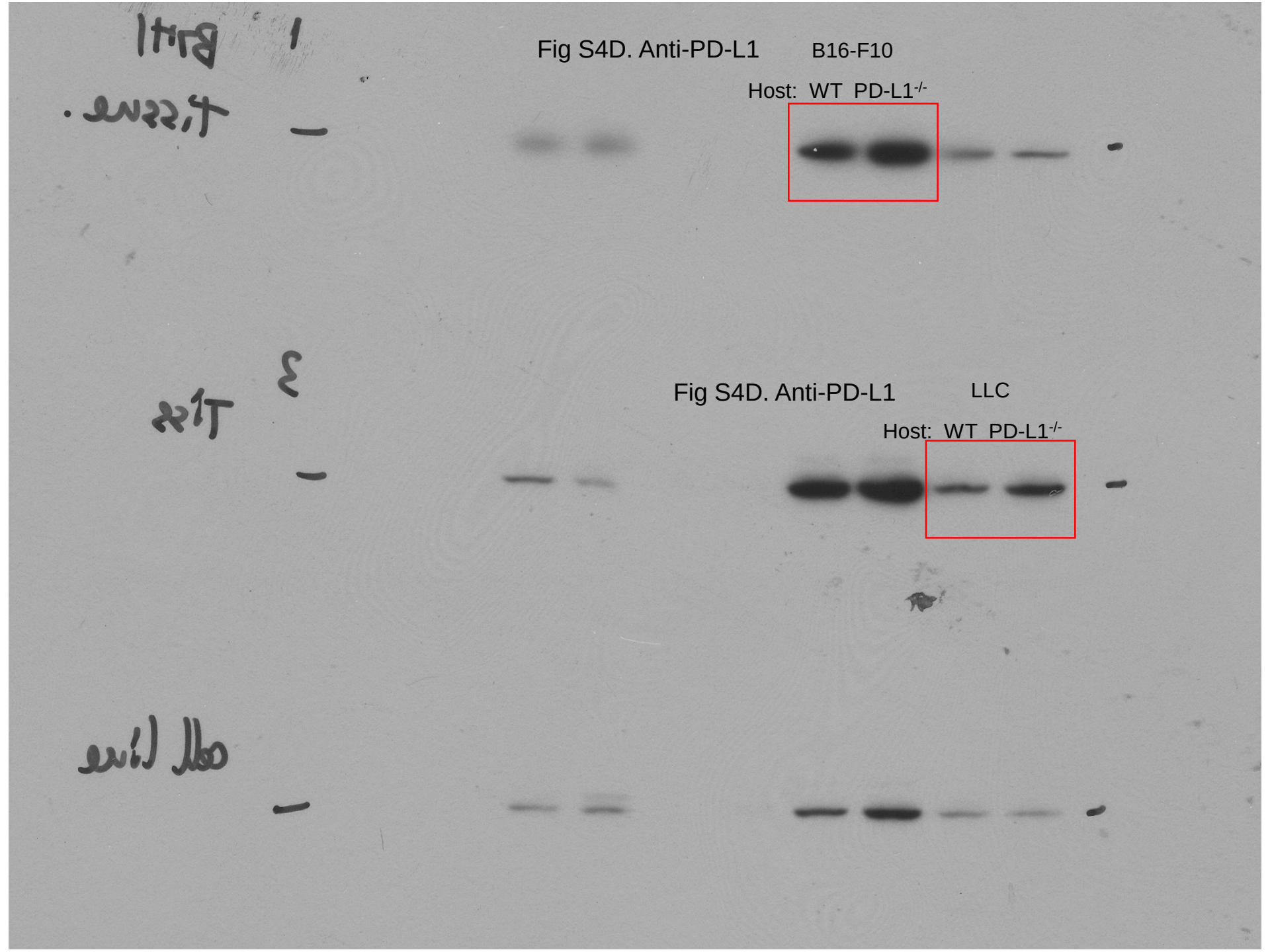


Fig S4D. Anti-PD-L1 LLC

Host: WT PD-L1$^{-/-}$

Fig S4D. Anti-GAPDH