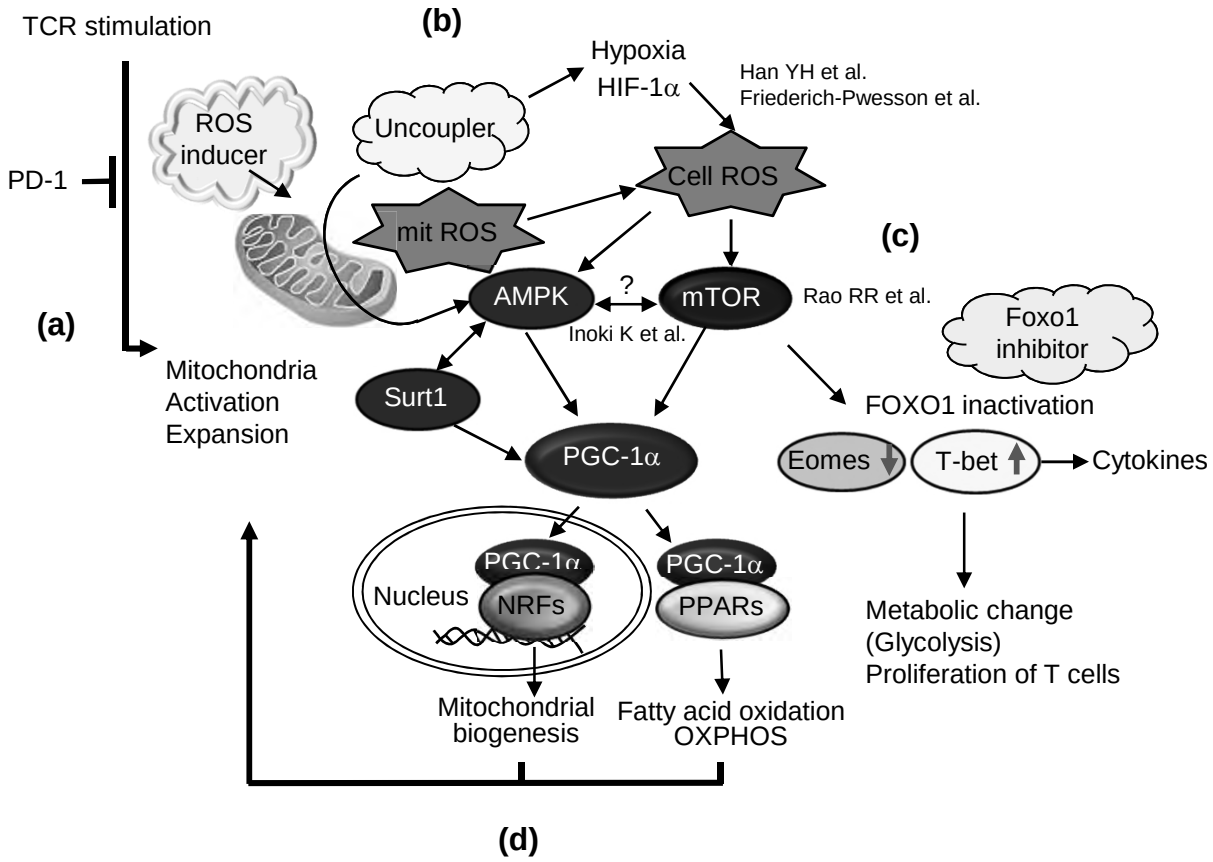
FIGURE 1


TCR stimulation
(b)
Hypoxia
HIF-1α
Han YH et al.
Friederich-Pwesson et al.
ROS inducer
Uncoupler
PD-1
Cell ROS
mit ROS
(c)
AMPK
?
mTOR
Rao RR et al.
Inoki K et al.
Foxo1 inhibitor
(a)
Mitochondria
Activation
Expansion
Surt1
FOXO1 inactivation
PGC-1α
Eomes
T-bet
Cytokines
PGC-1α
PGC-1α
Nucleus
NRFs
PPARs
Metabolic change
(Glycolysis)
Proliferation of T cells
Mitochondrial biogenesis
Fatty acid oxidation
OXPHOS
(d)

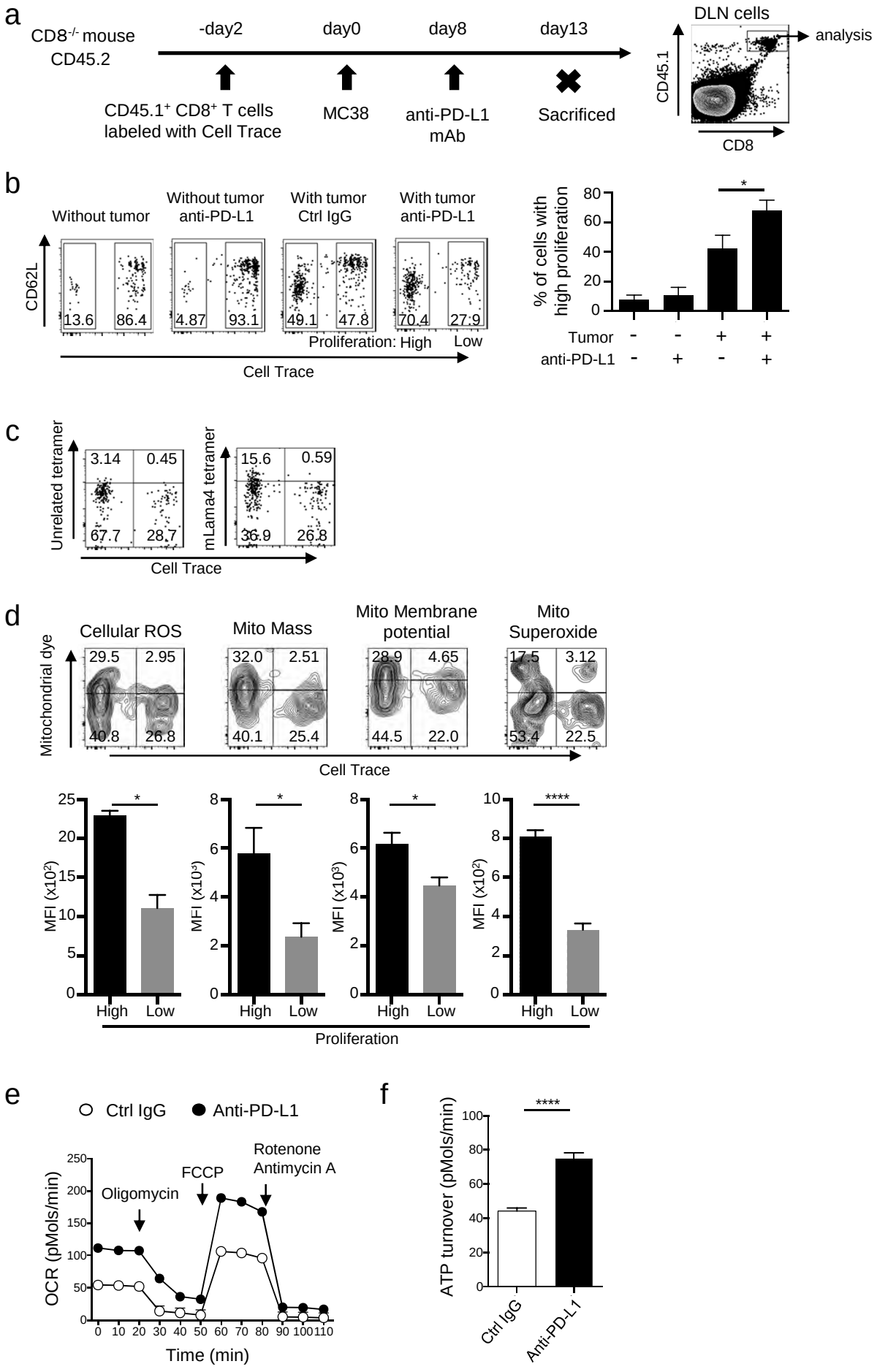
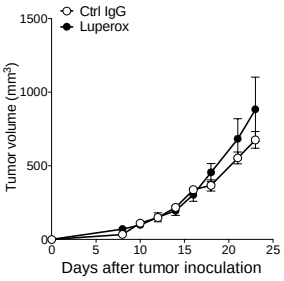
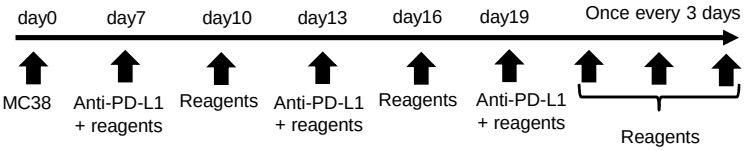

a
CD8-/- mouse
CD45.2
-day2
day0
day8
day13
CD45.1+ CD8+ T cells labeled with Cell Trace
MC38
anti-PD-L1 mAb
Sacrificed
DLN cells
analysis
CD45.1
CD8
b
Without tumor
Without tumor anti-PD-L1
With tumor Ctrl IgG
With tumor anti-PD-L1
CD62L
13.6
86.4
4.87
93.1
49.1
47.8
70.4
27.9
Proliferation: High Low
Cell Trace
% of cells with high proliferation
Tumor
anti-PD-L1
c
Unrelated tetramer
3.14
0.45
67.7
28.7
mLama4 tetramer
15.6
0.59
36.9
26.8
Cell Trace
d
Cellular ROS
Mito Mass
Mito Membrane potential
Mito Superoxide
Mitochondrial dye
29.5
2.95
40.8
26.8
32.0
2.51
40.1
25.4
28.9
4.65
44.5
22.0
17.5
3.12
53.4
22.5
Cell Trace
MFI (x10²)
MFI (x10⁴)
MFI (x10³)
MFI (x10²)
High
Low
Proliferation
e
Ctrl IgG
Anti-PD-L1
OCR (pMols/min)
Oligomycin
FCCP
Rotenone
Antimycin A
Time (min)
f
ATP turnover (pMols/min)
Ctrl IgG
Anti-PD-L1

FIGURE 3

a


Ctrl IgG
Luperox
Tumor volume (mm3)
1500
1000
500
0
0
5
10
15
20
25
Days after tumor inoculation

b


C57BL/6N mice
day0
day7
day10
day13
day16
day19
Once every 3 days
MC38
Anti-PD-L1 + reagents
Reagents
Anti-PD-L1 + reagents
Reagents
Anti-PD-L1 + reagents
Reagents

c

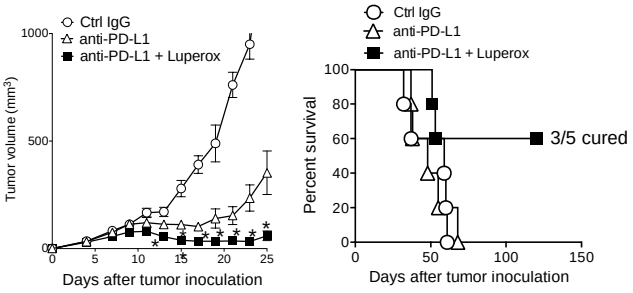

Ctrl IgG
anti-PD-L1
anti-PD-L1 + Luperox
Tumor volume (mm3)
1000
500
0
0
5
10
15
20
25
Days after tumor inoculation
Ctrl IgG
anti-PD-L1
anti-PD-L1 + Luperox
Percent survival
100
80
60
40
20
0
3/5 cured
0
50
100
150
Days after tumor inoculation

FIGURE 4

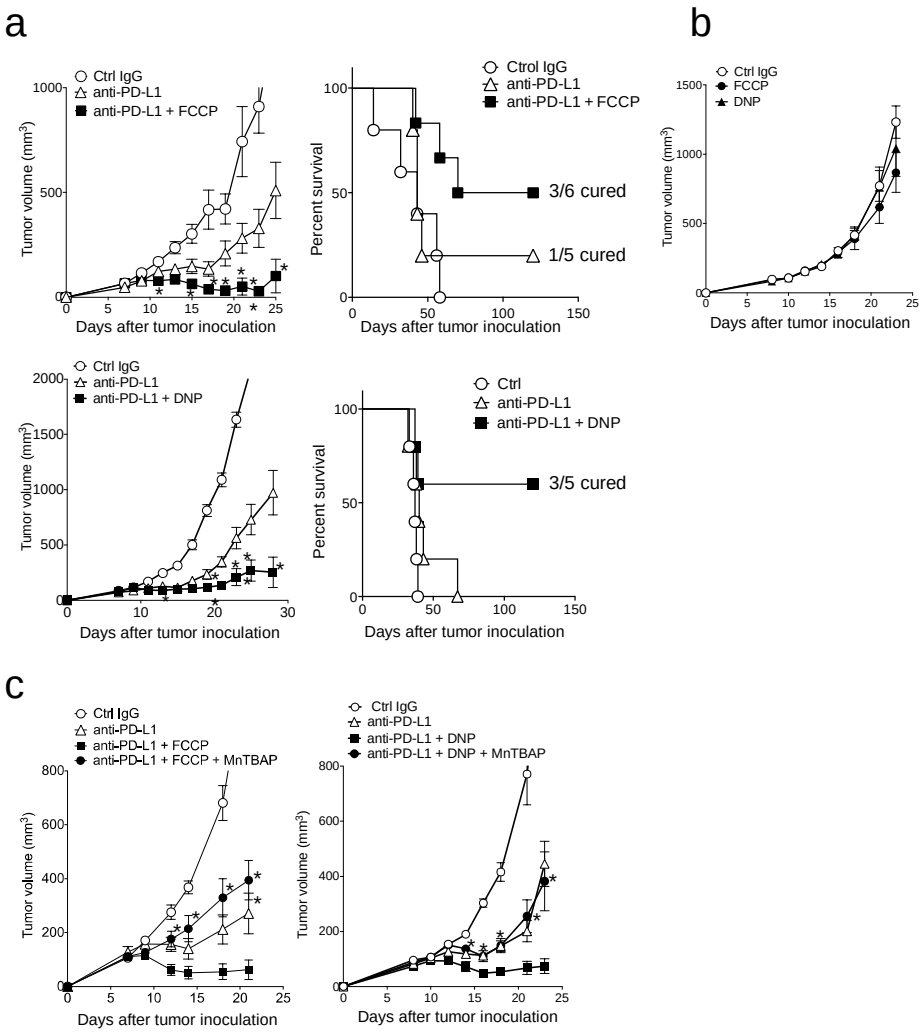
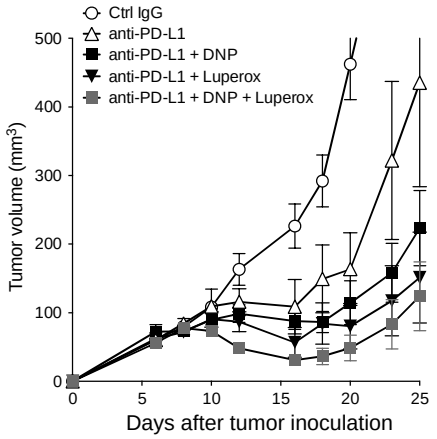


FIGURE 5



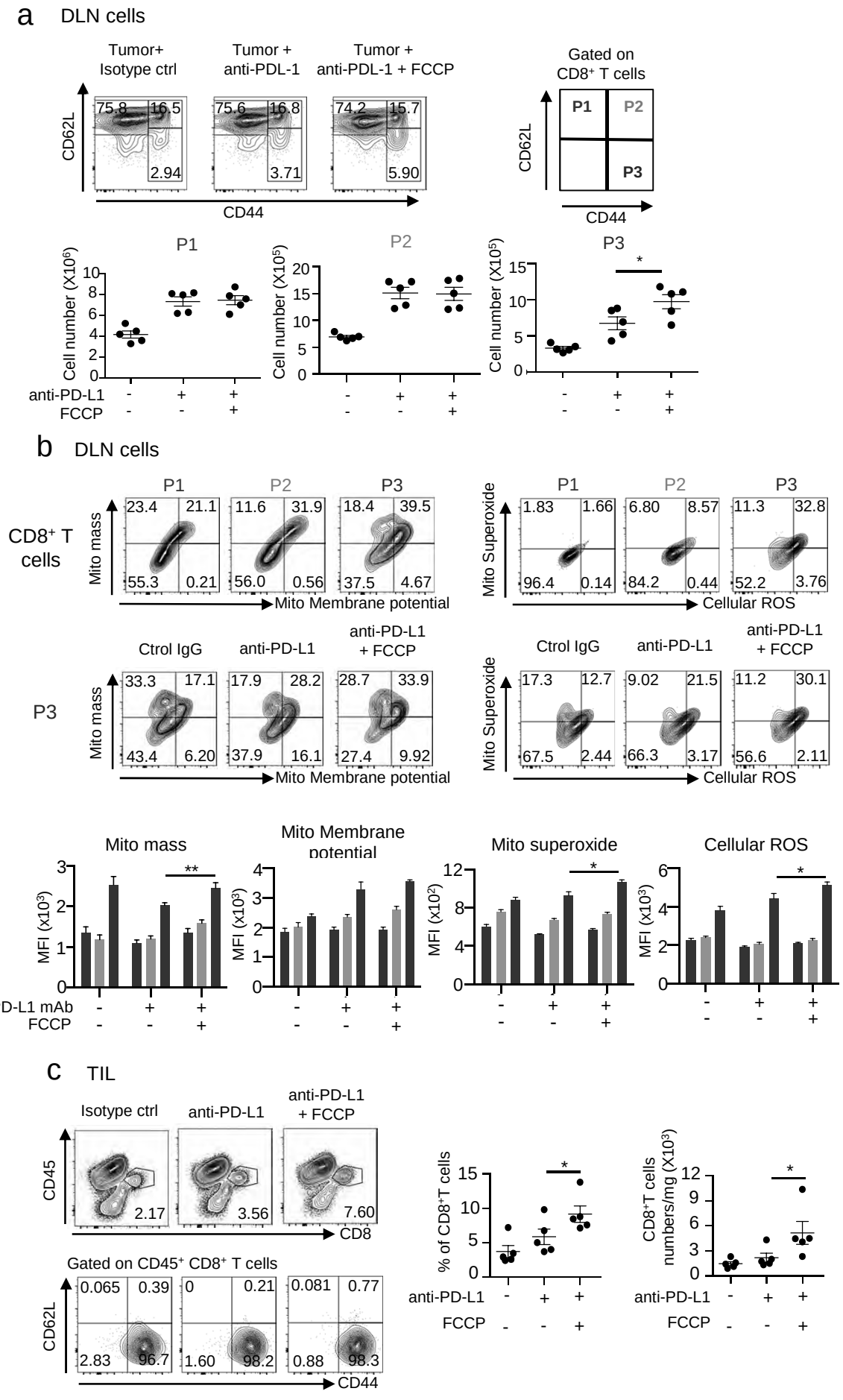
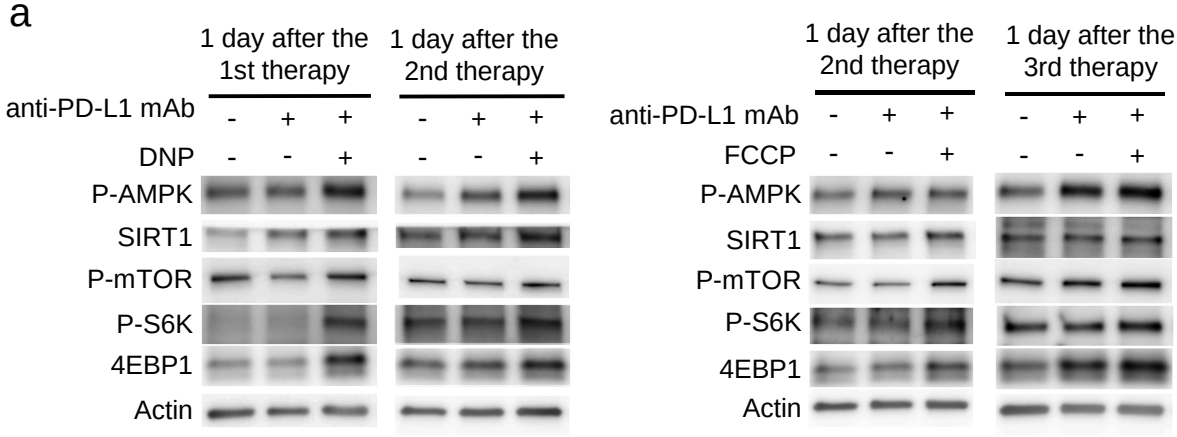
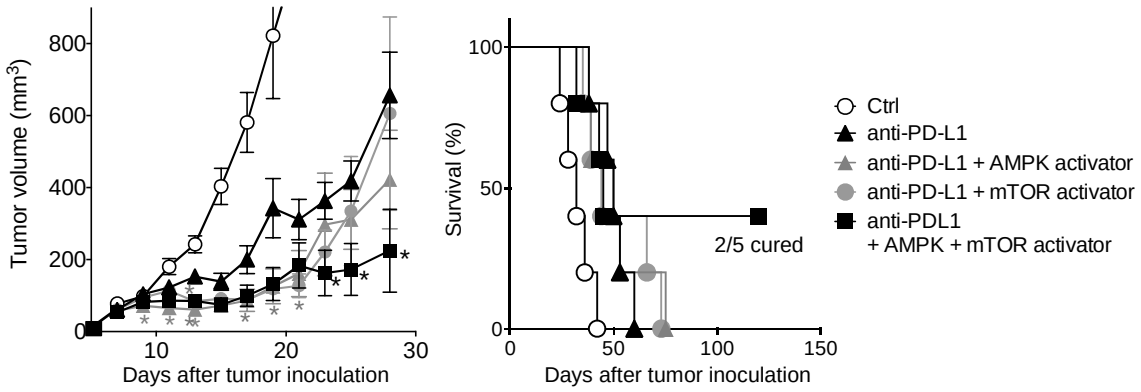

a DLN cells
Tumor+ Isotype ctrl
Tumor + anti-PDL-1
Tumor + anti-PDL-1 + FCCP
CD62L
CD44
75.8 16.5 2.94
75.6 16.8 3.71
74.2 15.7 5.90
Gated on CD8+ T cells
P1 P2 P3
P1 P2 P3
Cell number (X10^6)
Cell number (X10^5)
anti-PD-L1
FCCP
b DLN cells
CD8+ T cells
Mito mass
Mito Membrane potential
Mito Superoxide
Cellular ROS
23.4 21.1 55.3 0.21
11.6 31.9 56.0 0.56
18.4 39.5 37.5 4.67
1.83 1.66 96.4 0.14
6.80 8.57 84.2 0.44
11.3 32.8 52.2 3.76
P3
Ctrol IgG
anti-PD-L1
anti-PD-L1 + FCCP
33.3 17.1 43.4 6.20
17.9 28.2 37.9 16.1
28.7 33.9 27.4 9.92
17.3 12.7 67.5 2.44
9.02 21.5 66.3 3.17
11.2 30.1 56.6 2.11
Mito mass
Mito Membrane potential
Mito superoxide
Cellular ROS
MFI (x10^3)
MFI (x10^2)
PD-L1 mAb
FCCP
c TIL
Isotype ctrl
anti-PD-L1
anti-PD-L1 + FCCP
CD45
CD8
2.17 3.56 7.60
Gated on CD45+ CD8+ T cells
CD62L
CD44
0.065 0.39 2.83 96.7
0 0.21 1.60 98.2
0.081 0.77 0.88 98.3
% of CD8+T cells
CD8+T cells numbers/mg (X10^3)
anti-PD-L1
FCCP

FIGURE 7

a


1 day after the 1st therapy
1 day after the 2nd therapy
anti-PD-L1 mAb
DNP
P-AMPK
SIRT1
P-mTOR
P-S6K
4EBP1
Actin
1 day after the 2nd therapy
1 day after the 3rd therapy
anti-PD-L1 mAb
FCCP
P-AMPK
SIRT1
P-mTOR
P-S6K
4EBP1
Actin

b


Tumor volume (mm3)
Days after tumor inoculation
Survival (%)
2/5 cured
Days after tumor inoculation
Ctrl
anti-PD-L1
anti-PD-L1 + AMPK activator
anti-PD-L1 + mTOR activator
anti-PDL1
+ AMPK + mTOR activator

c

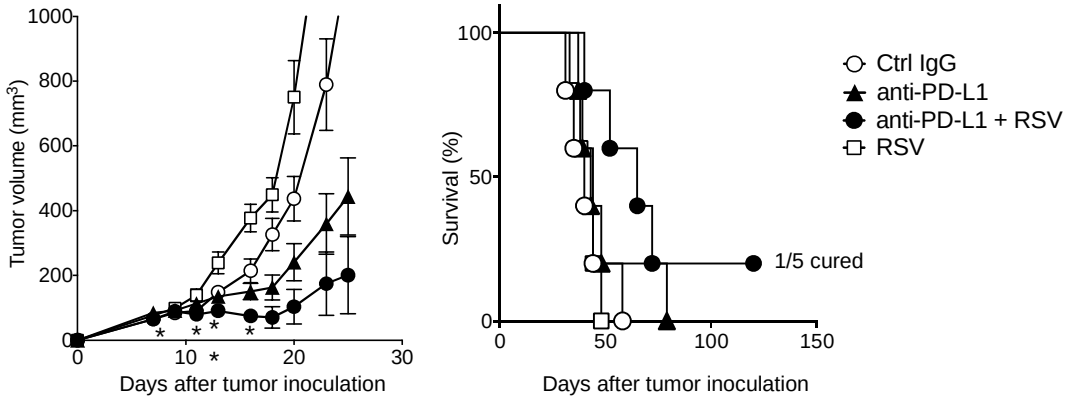

Tumor volume (mm3)
Days after tumor inoculation
Survival (%)
1/5 cured
Days after tumor inoculation
Ctrl IgG
anti-PD-L1
anti-PD-L1 + RSV
RSV

FIGURE 8

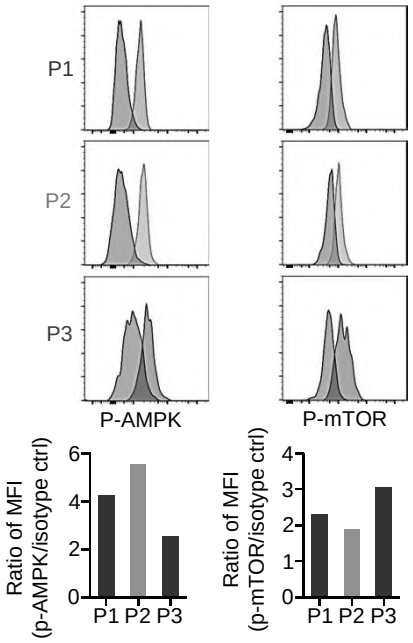


FIGURE 9

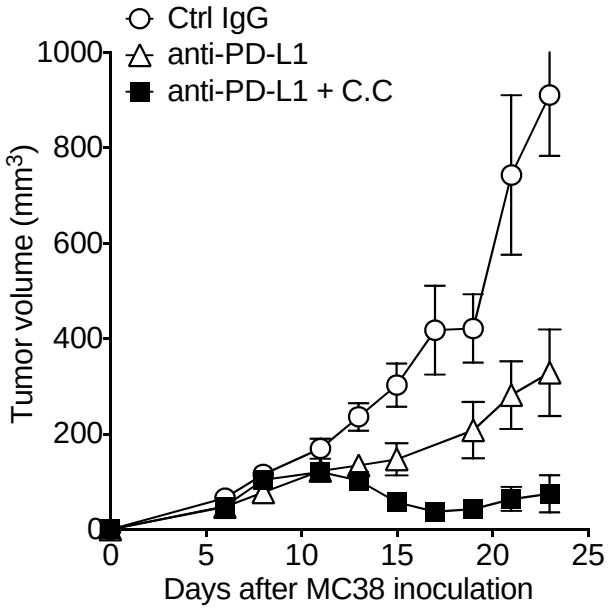


FIGURE 10

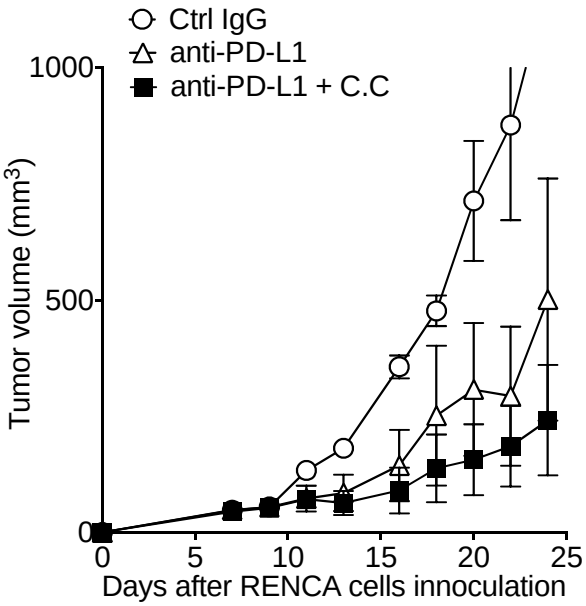


FIGURE 11

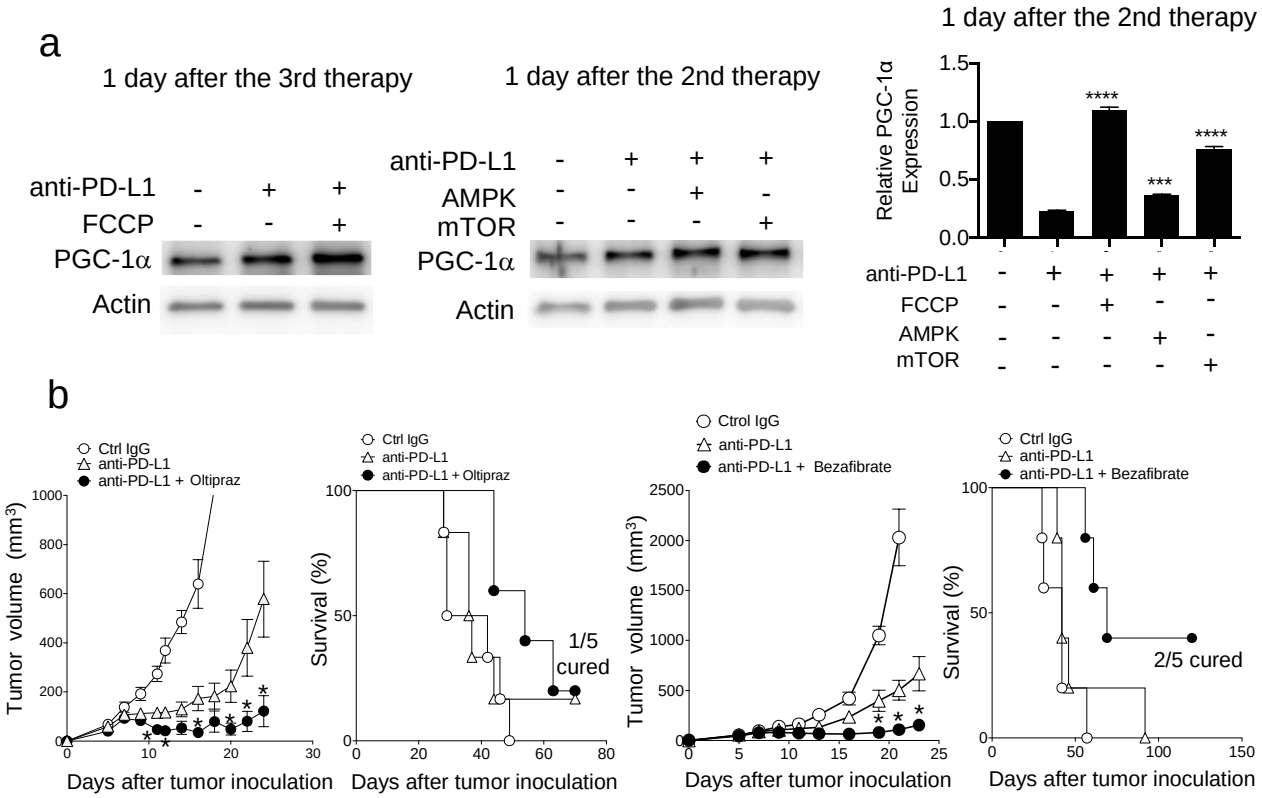


FIGURE 12

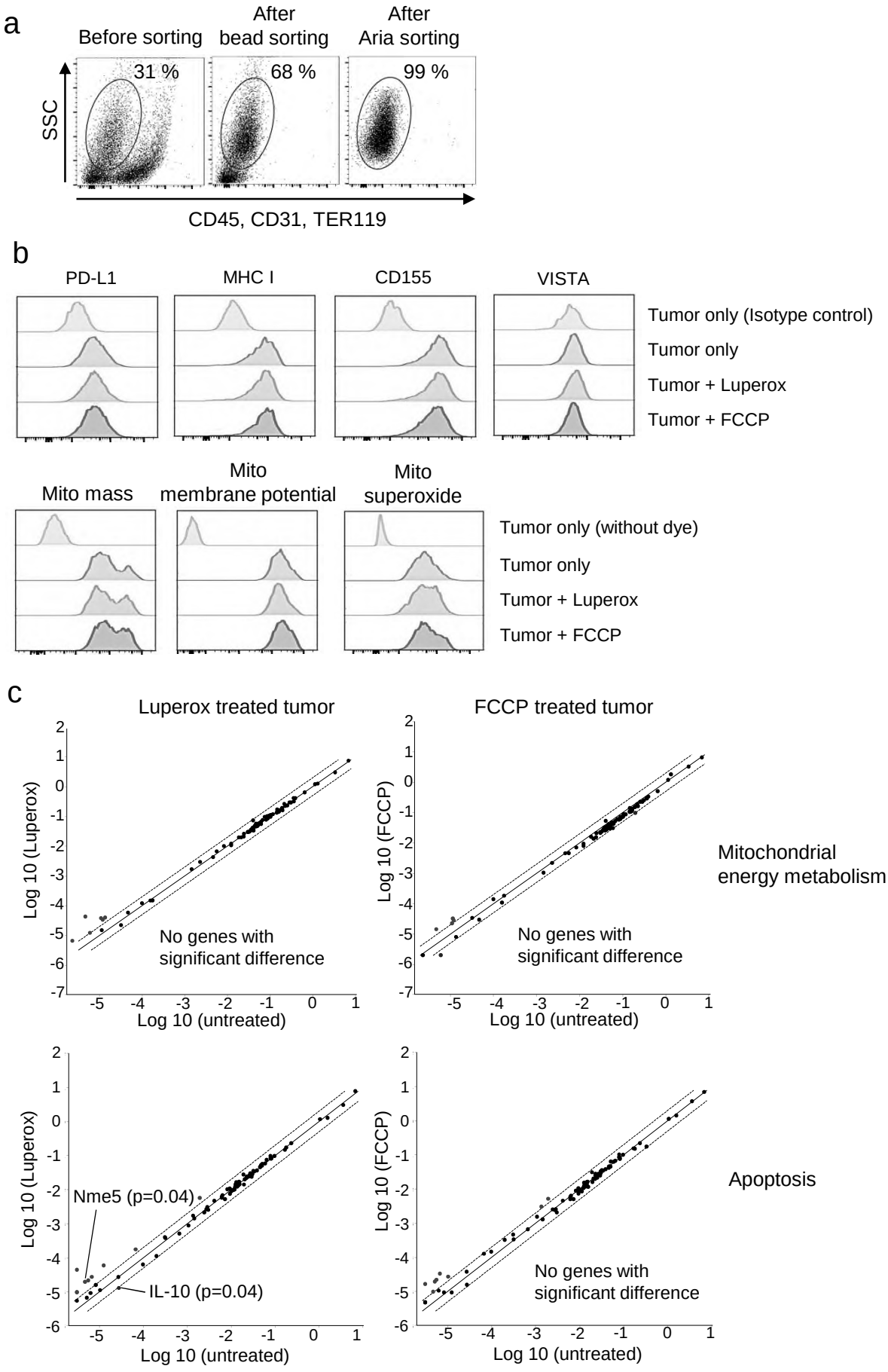

a
Before sorting
After bead sorting
After Aria sorting
31 %
68 %
99 %
SSC
CD45, CD31, TER119
b
PD-L1
MHC I
CD155
VISTA
Tumor only (Isotype control)
Tumor only
Tumor + Luperox
Tumor + FCCP
Mito mass
Mito membrane potential
Mito superoxide
Tumor only (without dye)
Tumor only
Tumor + Luperox
Tumor + FCCP
c
Luperox treated tumor
FCCP treated tumor
Log 10 (Luperox)
Log 10 (FCCP)
Log 10 (untreated)
No genes with significant difference
Mitochondrial energy metabolism
Nme5 (p=0.04)
IL-10 (p=0.04)
Apoptosis

FIGURE 13

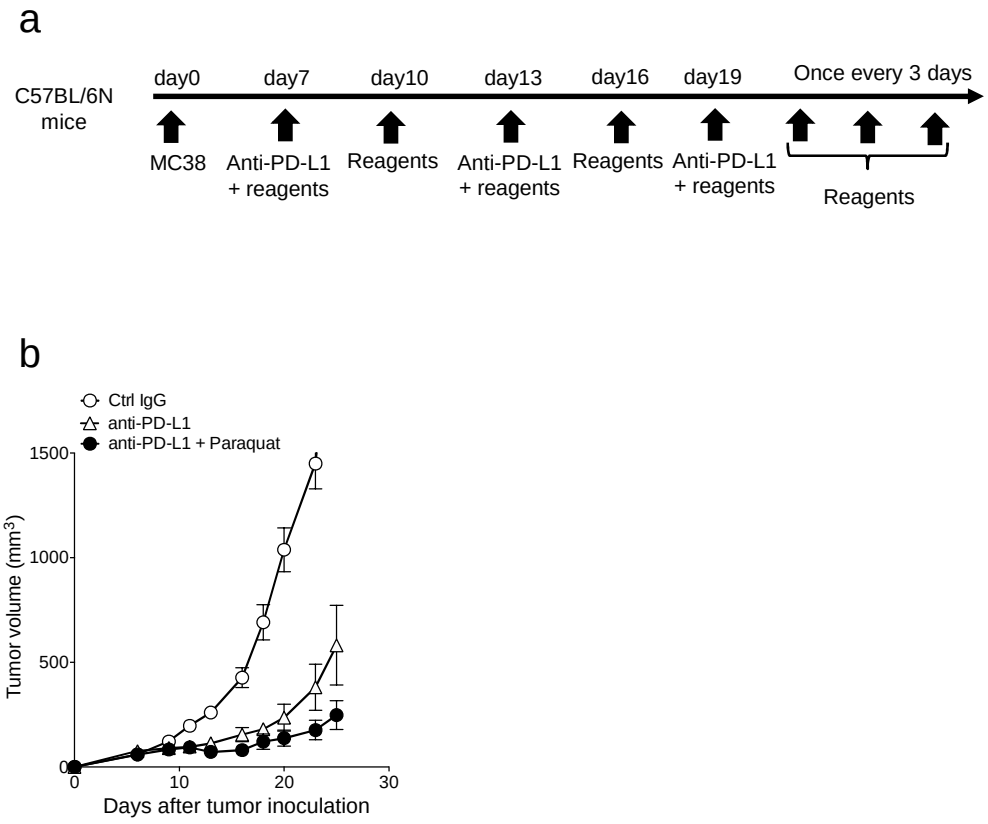


FIGURE 14

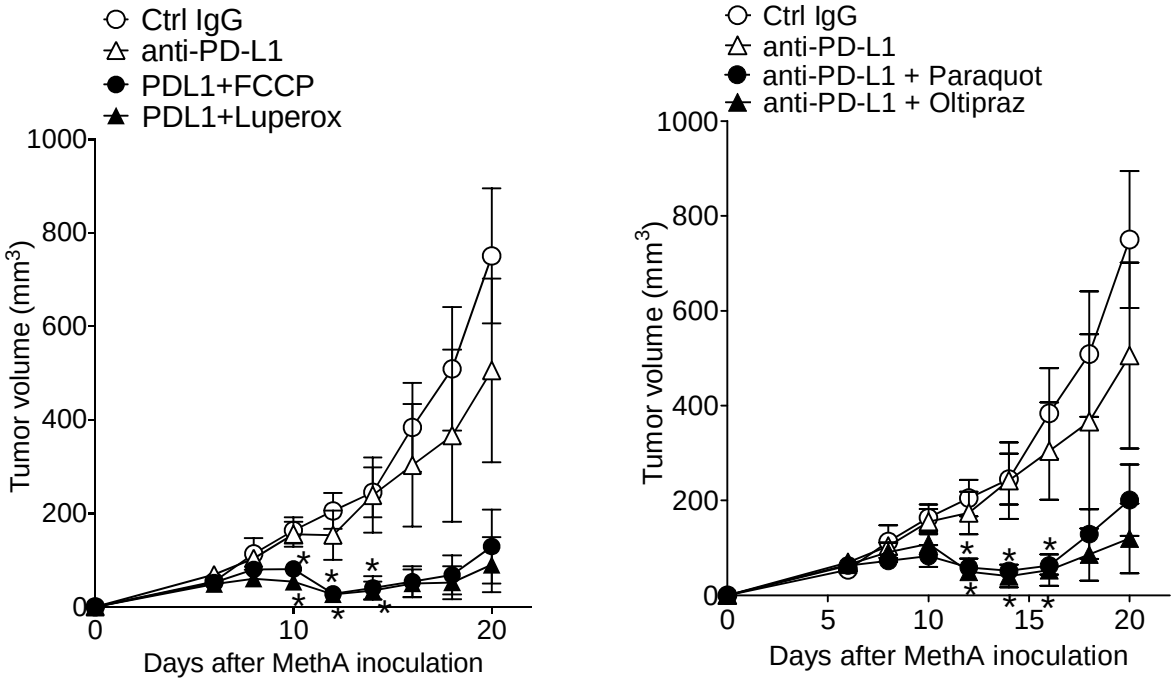


FIGURE 15

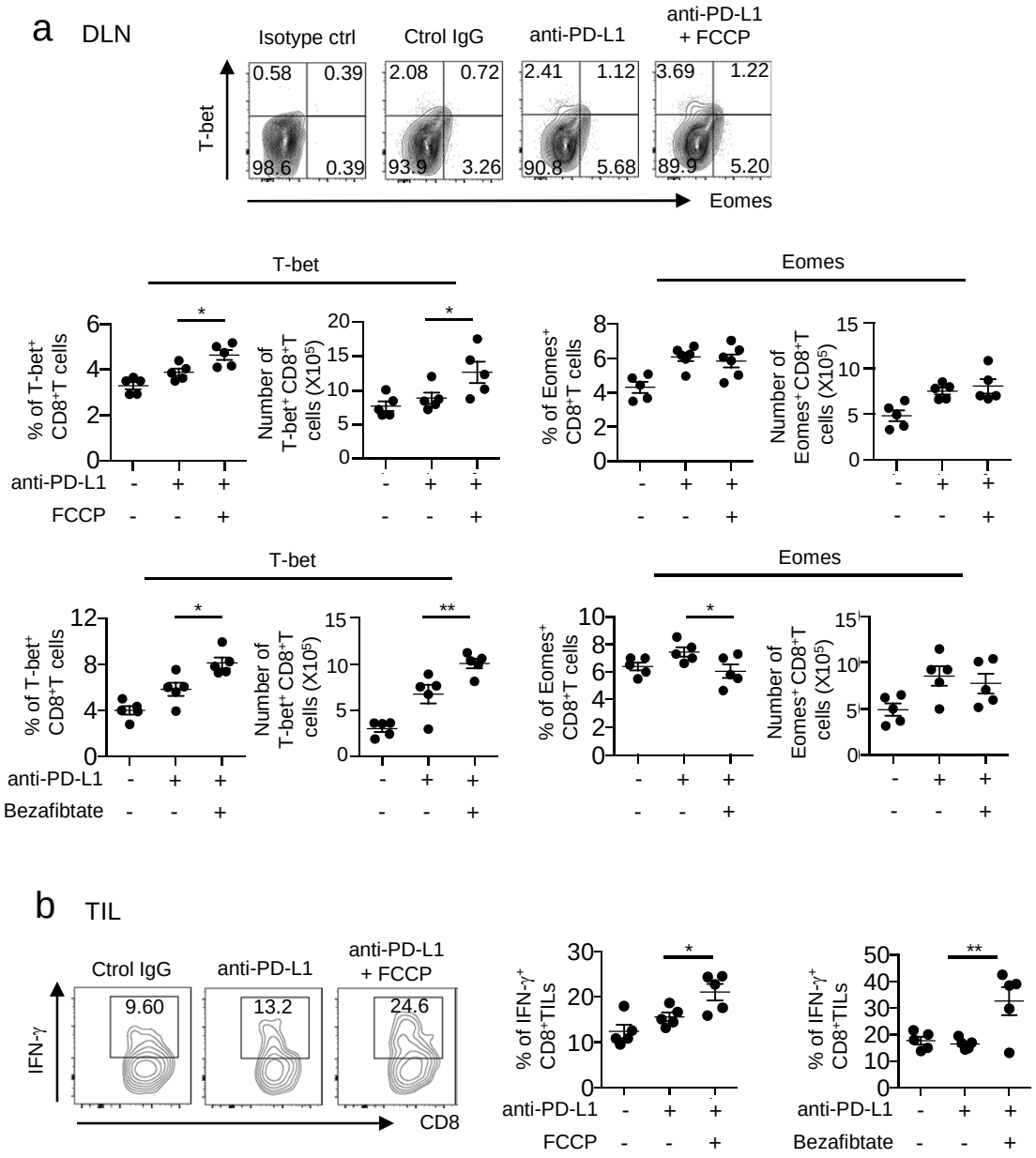


FIGURE 16

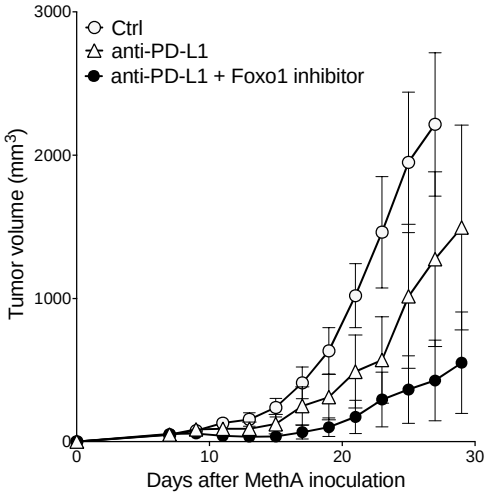


FIGURE 17

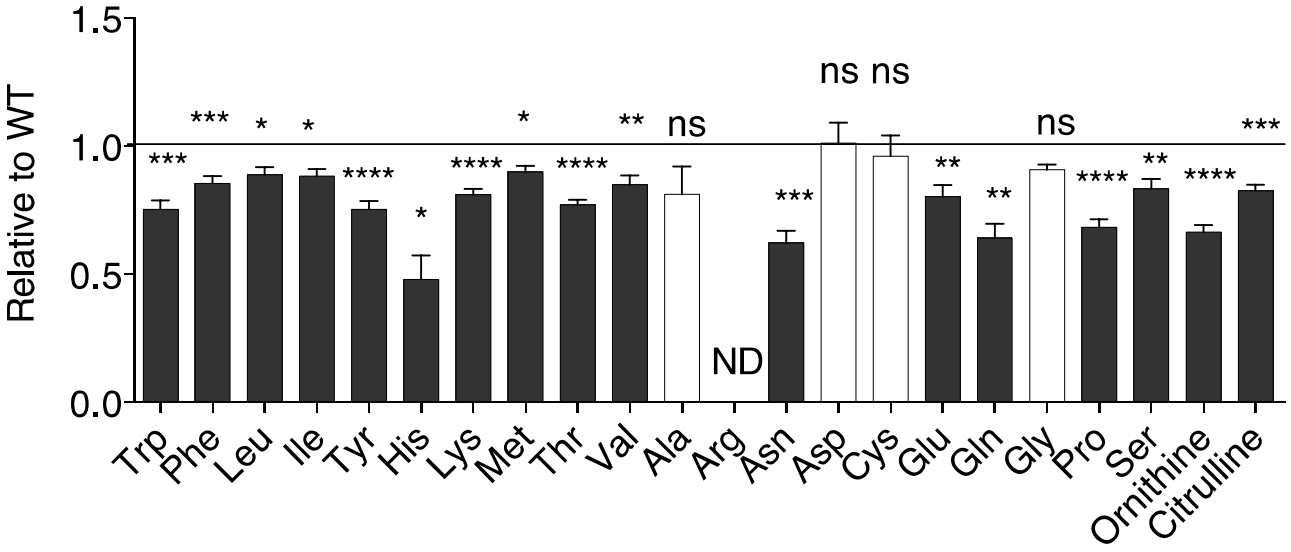


FIGURE 18

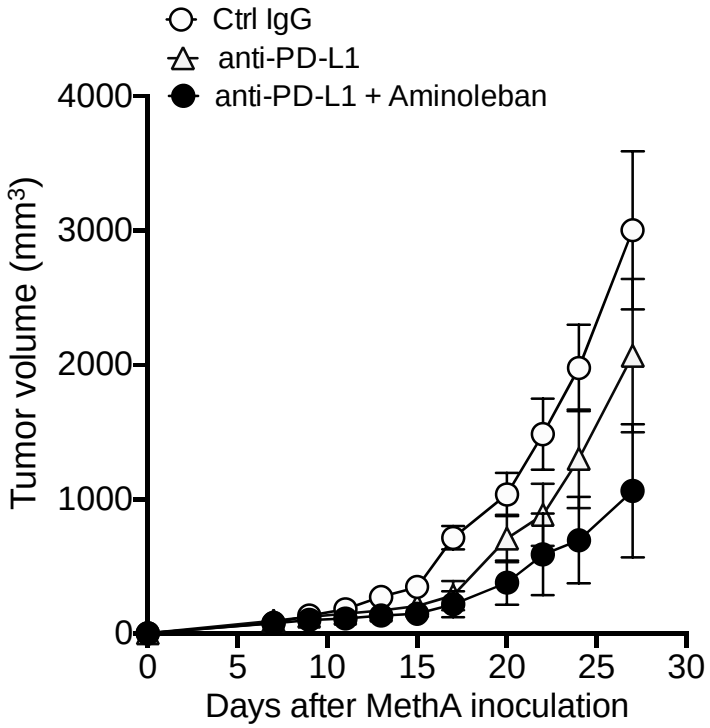
COMPOSITION

Each 1000ml of **AMINOLEBAN** contains:

	Aminoacetic acid	9.0 g
	L-alanine	7.5 g
	L-arginine HCl	7.3 g
	(L-arginine equivalent)	(6.0 g)
	L-cysteine HCl monohydrate	0.4 g
	(L-cysteine equivalent)	(0.3 g)
Essential	L-histidine HCl monohydrate	3.2 g
	(L-histidine equivalent)	(2.4 g)
Essential	L-isoleucine	9.0 g
Essential	L-leucine	11.0 g
Essential	L-Lysine HCl	7.6 g
	(L-lysine equivalent)	(6.1 g)
Essential	L-methionine	1.0 g
Essential	L-phenylalanine	1.0 g
	L-proline	8.0 g
	L-serine	5.0 g
Essential	L-threonine	4.5 g
Essential	L-tryptophan	0.7 g
Essential	L-valine	8.4 g
	Water for injection q.s	1000 ml

Amino Acids	7.99% (w/v)
Branched-chain amino acids	35.5% (w/w) of total amino acids

FIGURE 19


Ctrl IgG
anti-PD-L1
anti-PD-L1 + Aminoleban
Tumor volume (mm3)
4000
3000
2000
1000
0
0
5
10
15
20
25
30
Days after MethA inoculation