SUPPLEMENTAL DATA

Najjar, Menk et al. 2019

Figure S1. GEMM-derived Clone 24 murine melanoma has poor immune infiltration and is resistant to PD-1 blockade therapy. (A) Schematic of Clone 24 murine melanoma cell line generation. (B) Representative OCR vs ECAR of MelanA (B6 melanocyte cell line) and Clone 24 (left) and tabulated baseline OCR and ECAR (right) from multiple experiments. (C) Representative flow cytogram (left) and tabulated data from multiple experiments (right) of CD8 and $CD4^+$ T cells from Clone 24 and B16-bearing mice. LN, lymph node, TIL, tumor infiltrating lymphocytes. (n=3 per group) (D) Tumor growth curves of C57/BL6 inoculated with 250,000 Clone 24 melanoma cells intradermally. Mice received 200 μg anti-PD1 or isotype controls thrice weekly when palpable tumors were detected (typically day 5 or 6) (n=4 per group). Each line represents one animal. Data represent 3 independent experiments. * $p < 0.05$, ** $p < 0.01$, ns not significant by unpaired t-test. Error bars indicate s.e.m.

Figure S2. RNA interference to metabolic genes represses target proteins and does not impair tumor cell proliferation. (A) Representative immunoblot (left) and tabulated densitometry (right) of Glut1 and Ndufs4 from Clone 24 melanoma cells transfected with either scrambled control, *Slc2a1* (Glut1), or *Ndufs4* (complex I) shRNA . (B) Tabulated OCR and ECAR from multiple experiments of Clone 24 melanoma cells transfected with either scrambled control, *Slc2a1* (Glut1), or *Ndufs4* (complex I) shRNA. (C) *In vitro* proliferation of Clone 24 cells as in A. Data represent 3 independent experiments. * $p < 0.05$, ** $p < 0.01$, ns not significant by unpaired t-test. Error bars indicate s.e.m.

Figure S3. (A) Representative flow cytogram of IFNγ and TNFα from patients as in Figure 4B-4D. (B) Representative flow cytogram of MitotrackerFM and 2NBDG of $CD8^+$ T cells from patients as in Figure 4E.

Figure S1

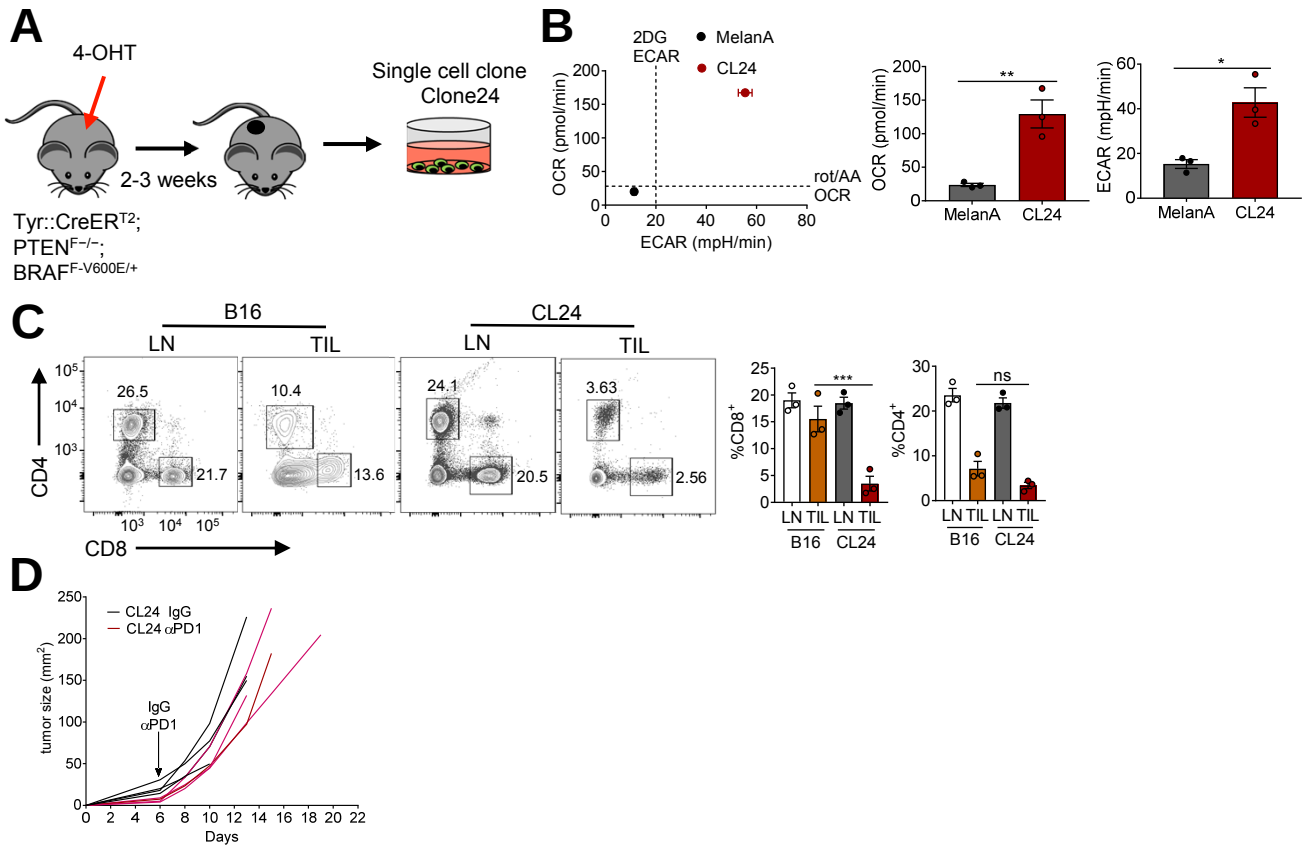

A
4-OHT
2-3 weeks
Single cell clone
Clone24
Tyr::CreER^{T2};
PTEN$^{F-/-}$;
BRAF$^{F-V600E/+}$
B
2DG
ECAR
MelanA
CL24
OCR (pmol/min)
ECAR (mpH/min)
rot/AA
OCR
MelanA
CL24
ECAR (mpH/min)
C
B16
CL24
LN
TIL
CD4
CD8
26.5
21.7
10.4
13.6
24.1
20.5
3.63
2.56
%CD8+
%CD4+
ns
B16
CL24
D
CL24 IgG
CL24 αPD1
IgG
αPD1
tumor size (mm2)
Days

Figure S2

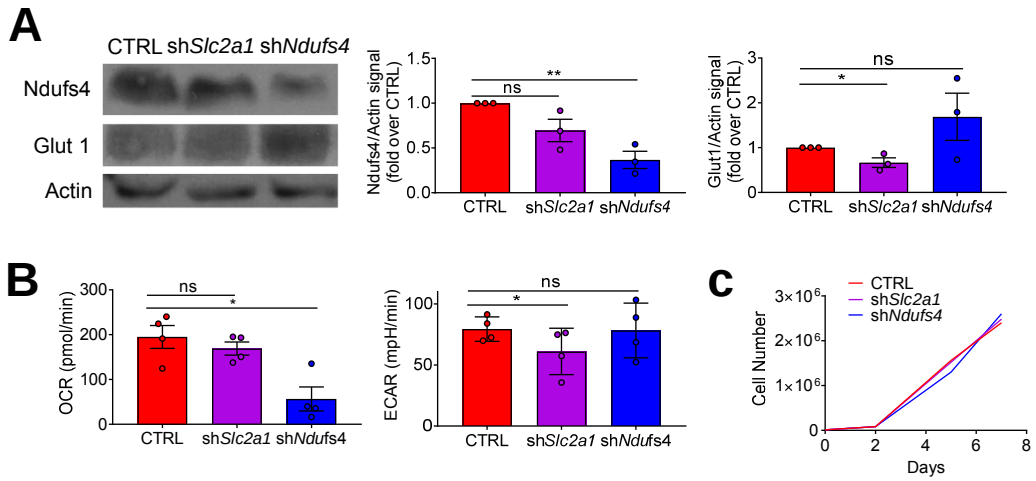
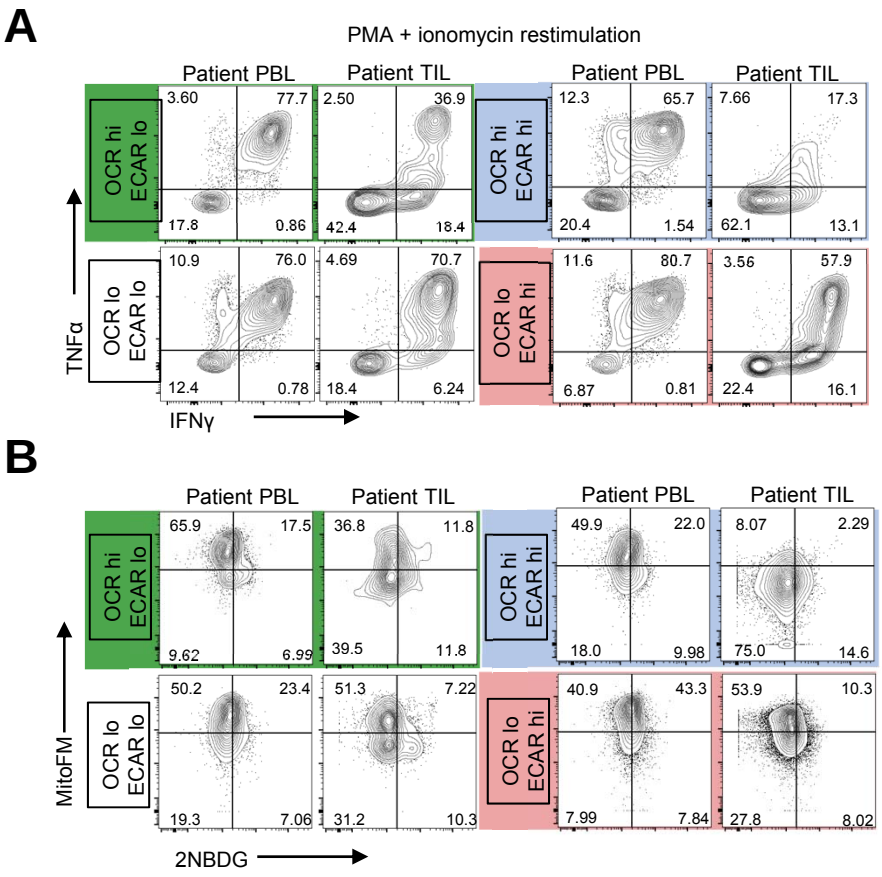

A
CTRL shSlc2a1 shNdufs4
Ndufs4
Glut 1
Actin
Ndufs4/Actin signal (fold over CTRL)
1.5
1.0
0.5
0.0
**
ns
CTRL
shSlc2a1 shNdufs4
Glut1/Actin signal (fold over CTRL)
3
2
1
0
ns
*
CTRL
shSlc2a1 shNdufs4
B
OCR (pmol/min)
300
200
100
0
ns
*
CTRL
shSlc2a1 shNdufs4
ECAR (mpH/min)
100
50
0
ns
*
CTRL
shSlc2a1 shNdufs4
C
CTRL
shSlc2a1
shNdufs4
Cell Number
3×10^6
2×10^6
1×10^6
0
0
2
4
6
8
Days

Figure S3


A
PMA + ionomycin restimulation
Patient PBL
Patient TIL
Patient PBL
Patient TIL
OCR hi ECAR lo
OCR hi ECAR hi
OCR lo ECAR lo
OCR lo ECAR hi
3.60
77.7
17.8
0.86
2.50
36.9
42.4
18.4
12.3
65.7
20.4
1.54
7.66
17.3
62.1
13.1
10.9
76.0
12.4
0.78
4.69
70.7
18.4
6.24
11.6
80.7
6.87
0.81
3.56
57.9
22.4
16.1
TNFα
IFNγ
B
Patient PBL
Patient TIL
Patient PBL
Patient TIL
OCR hi ECAR lo
OCR hi ECAR hi
OCR lo ECAR lo
OCR lo ECAR hi
65.9
17.5
9.62
6.99
36.8
11.8
39.5
11.8
49.9
22.0
18.0
9.98
8.07
2.29
75.0
14.6
50.2
23.4
19.3
7.06
51.3
7.22
31.2
10.3
40.9
43.3
7.99
7.84
53.9
10.3
27.8
8.02
MitoFM
2NBDG