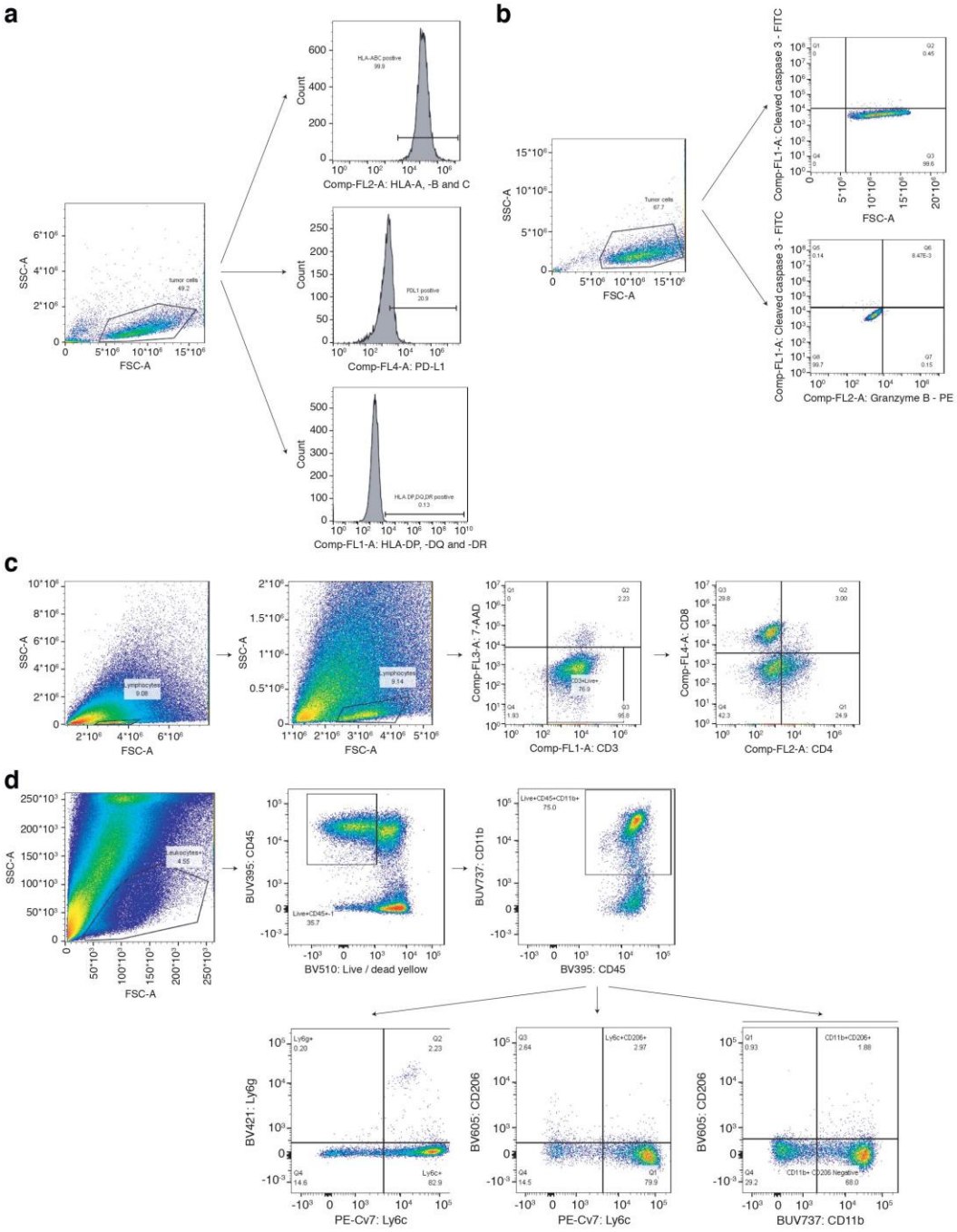
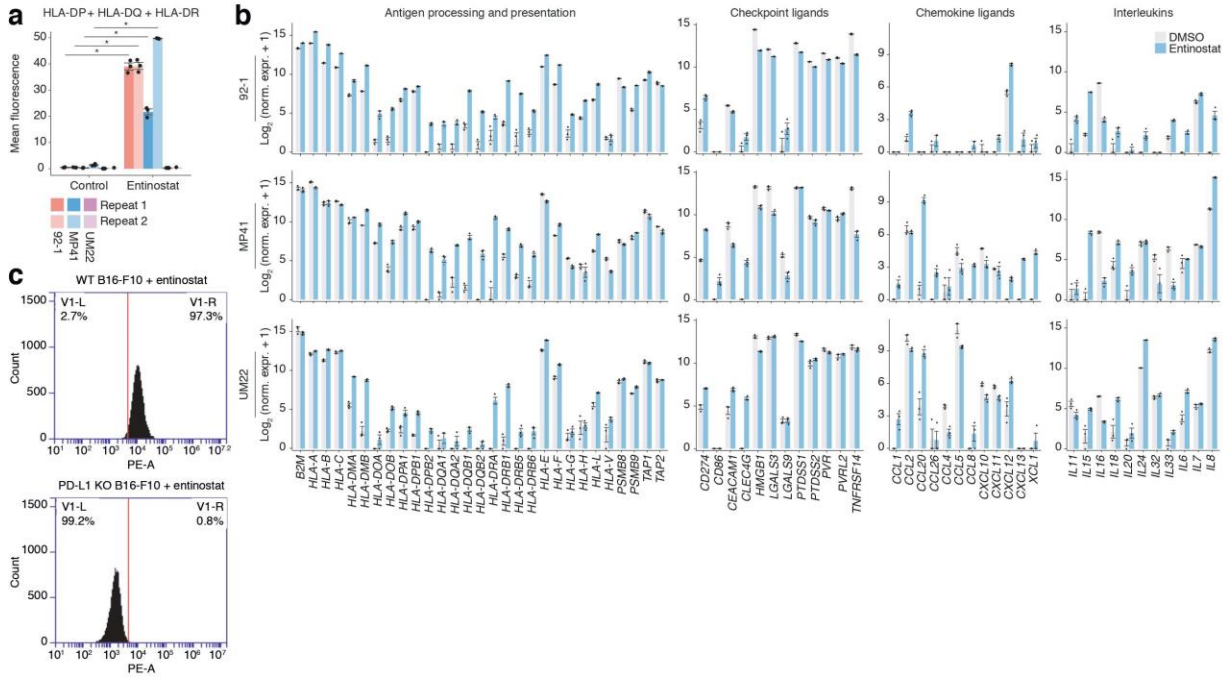
Supplementary Fig. 1. Gating strategy for flow cytometry analyses. (a) A gating strategy for excluding debris and choosing tumor cells based on high forward scatter (FSC) was employed and used for estimating levels of HLA-A, -B, and -C, PD-L1 and HLA-DP, -DQ and -DR in different cell lines. Experiments from entinostat-treated UM22 cells are shown as representative examples. (b) Granzyme B and cleaved caspase 3 measurements in cell lines co-cultured with MART-1-reactive TILs. Experiments from MP41 are shown as representative examples. (c) Within the in vivo B16-F10 tumor suspension, leukocytes were identified by a low side scatter (SSC) and low forward scatter (FSC) with gates for estimating levels of live CD3+ cells for CD4+ and CD8+ TILs, as well as (d) gates for estimating levels of live CD45 cells for CD11b+, Ly6c+, Ly6g+, CD206+ myeloid infiltrating cells.


a
b
c
d
SSC-A
FSC-A
Count
Comp-FL2-A: HLA-A, -B and C
Comp-FL4-A: PD-L1
Comp-FL1-A: HLA-DP, -DQ and -DR
Comp-FL1-A: Cleaved caspase 3 - FITC
Comp-FL2-A: Granzyme B - PE
Comp-FL3-A: 7-AAD
Comp-FL1-A: CD3
Comp-FL4-A: CD8
Comp-FL2-A: CD4
BV510: Live / dead yellow
BV395: CD45
BV737: CD11b
BV421: Ly6g
PE-Cy7: Ly6c
BV605: CD206

Supplementary Fig. 2. Entinostat increases HLA expression in human UM cell lines and mouse B16-F10 melanomas. (a) HLA class 2 expression as assessed by flow cytometry in human UM cell lines 92-1, MP41 and UM22 treated with DMSO or entinostat. The experiment was repeated twice with n = 3 biological replicates per cell line and condition, except in the case of UM22, where n = 5 and n = 1 replicates were used for the first and second experiments, respectively (excluded from statistical tests due to nearly absent expression in all cases). Significance was assessed by t-tests and adjusted p-values <0.05 (Benjamini-Hochberg correction) were considered statistically significant, as indicated by asterisks. (b) Immune-associated gene expression levels inferred from RNA sequencing data after entinostat treatment, relative to DMSO controls, as shown in Figure 1c. (c) Flow cytometry analysis of parental B16-F10 cells and CRISPR/Cas9-generated PD-L1 knockout B16-F10 cells after treatment with entinostat for 24 h.


a
HLA-DP+ HLA-DQ + HLA-DR
Mean fluorescence
Control
Entinostat
Repeat 1
Repeat 2
92-1
MP41
UM22
b
Antigen processing and presentation
Checkpoint ligands
Chemokine ligands
Interleukins
DMSO
Entinostat
Log2 (norm. expr. + 1)
B2M
HLA-A
HLA-B
HLA-C
HLA-DMA
HLA-DMB
HLA-DOA
HLA-DOB
HLA-DPA1
HLA-DPB1
HLA-DPB2
HLA-DQA1
HLA-DQA2
HLA-DQB1
HLA-DQB2
HLA-DRA
HLA-DRB1
HLA-DRB5
HLA-DRB6
HLA-E
HLA-F
HLA-G
HLA-H
HLA-L
HLA-V
PSMB8
PSMB9
TAP1
TAP2
CD274
CD86
CEACAM1
CLEC4G
HMGB1
LGALS3
LGALS9
PTDSS1
PTDSS2
PVR
PVRL2
TNFRSF14
CCL1
CCL2
CCL20
CCL26
CCL4
CCL5
CCL8
CXCL10
CXCL11
CXCL12
CXCL13
XCL1
IL11
IL15
IL16
IL18
IL20
IL24
IL32
IL33
IL6
IL7
IL8
c
WT B16-F10 + entinostat
V1-L
2.7%
V1-R
97.3%
Count
PE-A
PD-L1 KO B16-F10 + entinostat
V1-L
99.2%
V1-R
0.8%
Count
PE-A