

Statistics and Reproducibility

Figure 1

a-d, n=3 biologically independent samples. The experiments were performed three times with similar results. **a**, ****p < 0.0001 compared with CM by two-tailed unpaired t test, **b**, ***p = 0.001 compared with CM by two-tailed unpaired t test, **c**, ****p < 0.0001 compared with CM by two-tailed unpaired t test, **d**, ****p < 0.0001 by two-tailed unpaired t test.

e, n=4 biologically independent samples. The experiments were performed three times with similar results. **p = 0.0014, ****p < 0.0001 by two-tailed unpaired t test.

f, n=3 biologically independent samples. The experiments were performed once.

g-j, n=3 biologically independent samples. The experiments were performed three times with similar results. **g**, ****p < 0.0001 compared with Sup by two-tailed unpaired t test, **h**, ***p = 0.0002, ****p < 0.0001 compared with Sup by two-tailed unpaired t test, **i**, “ns” p = 0.6597, ***p = 0.0007, and ***p = 0.0002 by two-tailed unpaired t test.

k, l, n=4 biologically independent samples. The experiments were performed three times with similar results.

Figure 2

a, b, n=4 biologically independent samples. CD8⁺ T cells were cultured with fresh media (FM), B16F10 tumor supernatants (Sup), and B16F10 supernatants with methionine supplementation (Sup+Met). GSEA plot showed enriched apoptotic and defective TCR signaling pathways (**a**), as well as defective methionine metabolism signaling (**b**) T cells cultured tumor supernatants.

c-f, n=4 biologically independent samples. The experiments were performed three times with similar results. **d**, ***p = 0.0006 FM vs Sup, and ***p = 0.0005 Sup vs Sup+Met by two-tailed unpaired t test. **e**, ****p < 0.0001 FM vs Sup, and *p = 0.0183 Sup vs Sup+Met by two-tailed unpaired t test. **f**, ****p < 0.0001 FM vs Sup, and **p = 0.0082 Sup vs Sup+Met by two-tailed unpaired t test.

g, h, n=3 biologically independent samples. The experiments were performed three times with similar results. ****p < 0.0001 by two-tailed unpaired t test.

i, j, The experiments were performed three times with similar results.

Figure 3

a, The experiments were performed three times with similar results.

b, c, n=4 biologically independent samples. The experiments were performed three times with similar results. **b**, ****p < 0.0001 by two-tailed unpaired t test. **c**, ****p < 0.0001, *p = 0.0497, **p = 0.0025 by two-tailed unpaired t test.

d, The experiments were performed three times with similar results.

e, n=6 biologically independent samples in fresh CD8⁺, and n=8 biologically independent samples. The experiments were performed twice with similar results. ***p = 0.0001, ****p < 0.0001 by two-tailed unpaired t test.

f, n=4 biologically independent samples. The experiments were performed three times with similar results. *p = 0.0165 by two-tailed unpaired t test.

g-i, n=4 biologically independent samples. The experiments were performed twice with similar results. **g**, *p = 0.0109 by two-tailed unpaired t test. **h**, Day 19 *p = 0.0169, Day 22 and Day 25 ****p < 0.0001 by two-way ANOVA. **i**, *p = 0.014, ***p = 0.0008 by two-tailed unpaired t test.

j, k, n=4 biologically independent samples. The experiments were performed twice with similar results. ****p < 0.0001 by two-tailed unpaired t test.

l, m, n=3 biologically independent samples. The data were from RNA-array of Dot1l^{fl/fl} and Dot1l^{-/-} CD8⁺ T cells.

n, o, The experiments were performed three times with similar results.

p, q, n=3 biologically independent samples. The experiments were performed three times with similar results. **q**, Site1: ***p = 0.0002, ****p < 0.0001 by two-tailed unpaired t test. Site2: ***p = 0.0002 Sup vs FM, ***p = 0.0001 Sup vs Sup+Met.

Figure 4

a, n=10 biologically independent animals. The experiments were performed once. Day 17 *p = 0.0291 by two-tailed unpaired two-way ANOVA.

b, n=7 biologically independent animals. *p = 0.0114 by two-tailed unpaired t test.

c, n=6 biologically independent animals. *p = 0.0292 by two-tailed unpaired t test.

d, n=6 biologically independent animals. *p = 0.0395 by two-tailed unpaired t test.

e, n=8 biologically independent animals. IL-2: *p = 0.0106 by two-tailed unpaired t test. TNFα: *p = 0.0131 by two-tailed unpaired t test. IFNγ: **p = 0.0027 by two-tailed unpaired t test.

f, n=20 biologically independent animals. The experiments were performed once. Day 41 *p = 0.0046 by two-tailed unpaired two-way ANOVA.

g, n=14 biologically independent animals. IL-2: *p = 0.0161 by two-tailed unpaired t test. TNFα: **p = 0.0046 by two-tailed unpaired t test. IFNγ: **p = 0.0024 by two-tailed unpaired t test.

h, n=6 biologically independent samples. IL-2: ***p = 0.0004 by two-tailed unpaired t test. TNFα: ****p < 0.0001 by two-tailed unpaired t test. IFNγ: *p = 0.0328 by two-tailed unpaired t test.

i-l, n=7 Colorectal cancer patients received methionine supplementation. **i, j**, Representative results were shown from 4 patients. **k, l**, TNFα: *p = 0.0181 by two-tailed paired t test. IFNγ: *p = 0.0404 by two-tailed paired t test. Annexin V: *p = 0.0497 by two-tailed paired t test.

Figure 5

a-d, n=3 biologically independent animals. The experiments were performed three times with similar results. **a**, ***p = 0.0001, ****p < 0.0001 by two-tailed unpaired t test. **b-d**, ****p < 0.0001 by two-tailed unpaired t test.

e, The experiments were performed three times with similar results.

f, n=8 biologically independent animals. The experiments were performed twice with similar results. Day 27 ***p = 0.0006 by two-tailed unpaired two-way ANOVA.

g, n=6 biologically independent animals. The experiments were performed twice with similar results. TNFα: *p = 0.0494 by two-tailed unpaired t test. IFNγ: **p = 0.0496 by two-tailed unpaired t test. Granzyme B: *p = 0.0434 by two-tailed unpaired t test.

h, n=18 biologically independent animals. The experiments were performed twice with similar results. Day 22 **p = 0.0023 anti-PD-L1+BCH vs anti-PD-L1, ****p < 0.0001 anti-PD-L1+BCH vs PBS+IgG or BCH by two-tailed unpaired two-way ANOVA.

i, n=5 in PBS+IgG, n=9 in other groups biologically independent animals. The experiments were performed twice with similar results. TNFα: *p = 0.0263 anti-PD-L1+BCH vs anti-PD-L1, **p = 0.0082 anti-PD-L1+BCH vs PBS+IgG by two-tailed unpaired t test. IFNγ: **p = 0.0046 anti-PD-L1+BCH vs PBS+IgG, **p = 0.0013 anti-PD-L1+BCH vs BCH, **p = 0.0086 anti-PD-L1+BCH vs anti-PDL1 by two-tailed unpaired t test. Granzyme B: *p = 0.0235 anti-PD-L1+BCH vs PBS+IgG, **p = 0.0426 anti-PD-L1+BCH vs BCH, **p = 0.0484 anti-PD-L1+BCH vs anti-PDL1 by two-tailed unpaired t test.

j, k, n=9 biologically independent animals. The experiments were performed twice with similar results. Day 33 *p = 0.0167 anti-PD-L1+BCH vs anti-PD-L1, **p = 0.0038 anti-PD-L1+BCH vs BCH, ****p < 0.0001 anti-PD-L1+BCH vs PBS+IgG by two-tailed unpaired two-way ANOVA.

l, n=5 in PBS+IgG, n=7 in other groups biologically independent animals. The experiments were performed twice with similar results. TNF α : *p = 0.0478 anti-PD-L1+BCH vs PBS+IgG, *p = 0.0498 anti-PD-L1+BCH vs BCH, *p = 0.0425 anti-PD-L1+BCH vs anti-PD-L1 by two-tailed unpaired t test. IFN γ : *p = 0.0269 anti-PD-L1+BCH vs PBS+IgG, **p = 0.0013 anti-PD-L1+BCH vs BCH, **p = 0.0331 anti-PD-L1+BCH vs anti-PDL1 by two-tailed unpaired t test.

m, RNA-seq analysis showed expression of SLC43A2 transcripts in tumors and paired adjacent normal tissues in several types of tumor. CHOL: Cholangiocarcinoma, n=9, **p = 0.0013; ESCA: Esophageal Carcinoma, n=11, **p = 0.0070; HNSC: Head-Neck Squamous Cell Carcinoma, n=43, **p = 0.0016; KICH: Kidney Chromophobe, n=24, ****p < 0.0001; LIHC: Liver Hepatocellular Carcinoma, n=50, *p=0.0273 by two-tailed paired t test.

Extended Data Figure 1

a-c, n=4 biologically independent samples. The experiments were performed three times with similar results. **a**, ****p < 0.0001 by two-tailed unpaired t test. **b**, ***p = 0.0002 100μM vs FM, ***p = 0.0005 50μM vs FM, ***p = 0.0019 30μM vs FM by two-tailed unpaired t test. **c**, *p = 0.0372, ****p < 0.0001 by two-tailed unpaired t test.

d, e, n=4 biologically independent samples. The experiments were performed twice with similar results. **d**, *p = 0.038 Sup vs FM, *p = 0.0139 Sup vs Sup+Met by two-tailed unpaired t test. **e**, TNFα: ***p = 0.0003 Sup vs FM, ****p < 0.0001 Sup vs Sup+Met by two-tailed unpaired t test. IFNγ: ****p < 0.0001 Sup vs FM, ***p = 0.0003 Sup vs Sup+Met by two-tailed unpaired t test.

f, g, n=11 biologically independent donors in healthy control, and n=14 biologically independent donors in ovarian cancer patient. The experiments were performed once. **g**, *p = 0.0201 by two-tailed unpaired t test.

h, n=3 biologically independent samples. The experiments were performed once. *p = 0.0531 by two-tailed unpaired t test.

i, n=3 biologically independent samples. The experiments were performed twice with similar results. *p = 0.0306, **p = 0.0090 by two-tailed unpaired t test.

k-m, n=5 biologically independent samples. The experiments were performed twice with similar results. ****p < 0.0001 by two-tailed unpaired t test.

o, p, n=4 biologically independent animals. The experiments were performed twice with similar results. EC50 was determined by nonlinear regression (log (agonist) vs. response).

Extended Data Figure 2

a-e, n=4 biologically independent samples. RNA-sequence in CD8⁺ T cells.

f, n=4 biologically independent samples. The experiments were performed twice with similar results.

h, i, n=4 biologically independent samples. The experiments were performed twice with similar results. **h**, ****p Sup vs FM, **p = 0.0097 Sup vs Sup+Met by two-tailed unpaired t test. **i**, **p = 0.0040 Sup vs FM, **p = 0.0075 Sup vs Sup+Met by two-tailed unpaired t test.

j, k, The experiments were performed three with similar results.

Extended Data Figure 3

a, b, The experiments were performed three times with similar results.

c-e, n=4 biologically independent samples. Gene signature comparison between Dot1l^{-/-} and Dot1l^{ff/ff} CD8⁺ T cells through RNA-array.

f, n=4 biologically independent samples. The experiments were performed twice with similar results. TNFα: *p = 0.0254 Sup by two-tailed unpaired t test. IFNγ: **p = 0.0032 by two-tailed unpaired t test. Granzyme B: ***p = 0.0006 by two-tailed unpaired t test.

g, n=5 biologically independent animals. The experiments were performed once. Day 16 *p = 0.0246 Dot1l^{ff/ff} vs Dot1l^{-/-}, ****p < 0.0001 Dot1l^{ff/ff} vs Dot1l^{-/-}+anti-PDL1, “ns”, p = 0.9402 Dot1l^{-/-} vs Dot1l^{-/-}+anti-PDL1 by two-tailed unpaired two-way ANOVA.

h, n=5 biologically independent samples. The experiments were performed twice with similar results. Day 14 **p = 0.003 by two-tailed unpaired two-way ANOVA.

i, n=4 biologically independent samples. The experiments were performed twice with similar results. *p = 0.0199 in DLN CD8, and *p = 0.0142 in tumor CD8 by two-tailed unpaired t test.

j, n=4 biologically independent samples. The experiments were performed twice with similar results. stat5a: *p = 0.0111 in fresh Dot1l^{ff/ff} vs Dot1l^{-/-}, *p = 0.0144 in activated Dot1l^{ff/ff} vs Dot1l^{-/-}. stat5b: *p = 0.0116 in fresh Dot1l^{ff/ff} vs Dot1l^{-/-}, ****p < 0.0001 in activated Dot1l^{ff/ff} vs Dot1l^{-/-}.

k, n=3 biologically independent samples. The experiments were performed twice with similar results. stat5b: *p = 0.0140 Sup vs FM, **p = 0.0087 Sup vs Sup+Met.

l-n, n=3 biologically independent samples. RNA-seq showed the effect of DOT1L inhibitor (SGC0946) on human CD8⁺ T cells (Database: GSE108694).

Extended Data Figure 4

a, n=6 biologically independent samples. **p = 0.0036 by two-tailed unpaired t test.

b, n=6 biologically independent samples. **p = 0.0016 by two-tailed unpaired t test.

c, n=5 biologically independent samples. ****p < 0.0001 by two-tailed unpaired t test.

d, n=5 biologically independent samples. p = 0.0666 by two-tailed unpaired t test.

e, n=6 biological independent clinical samples. The experiment was performed once.

f, n=6 biologically independent samples. *p = 0.0483 by two-tailed unpaired t test.

g, n=6 biologically independent samples. **p = 0.0446 by two-tailed unpaired t test.

h, n=5 biologically independent samples. **p = 0.0055 by two-tailed unpaired t test.

i, n=5 biologically independent samples. p = 0.0596 by two-tailed unpaired t test.

j, n=4 biologically independent samples. ****p < 0.0001 by two-tailed unpaired t test.

k, n=4 biologically independent samples. ****p < 0.0001 by two-tailed unpaired t test.

l, n=4 biologically independent samples. *p = 0.0155 by two-tailed unpaired t test.

m, n=4 biologically independent samples. ****p < 0.0001 by two-tailed unpaired t test.

n, n=5 biologically independent samples. “ns”, p = 0.7891 in CD45- Tumor, *p = 0.0217 in T cell by two-tailed unpaired t test.

o, n=10 biologically independent samples. **p = 0.0045 by two-tailed unpaired t test.

p, n=10 biologically independent animals. ****p < 0.0001 by two-tailed unpaired two-way ANOVA.

q, n=10 biologically independent samples. **p = 0.0011 Met+anti-PDL1 vs PBS+IgG, *p = 0.0219 Met+anti-PDL1 vs Met+IgG, *p = 0.0492 Met+anti-PDL1 vs PBS+anti-PDL1 by two-tailed unpaired t test.

r, n=10 biologically independent samples. *p = 0.0220 Met+anti-PDL1 vs PBS+IgG, *p = 0.0444 Met+anti-PDL1 vs PBS+anti-PDL1 by two-tailed unpaired t test.

Extended Data Figure 5

a, n=3 biologically independent samples. The experiments were performed three times with similar results. **p = 0.0067, ****p < 0.0001 by two-tailed unpaired t test.

b, n=3 biologically independent samples. The experiments were performed three times with similar results. ***p = 0.0002, ****p < 0.0001 by two-tailed unpaired t test.

c, n=3 biologically independent samples. The experiments were performed twice with similar results. ****p < 0.0001 by two-tailed unpaired t test.

d, The experiments were performed twice with similar results.

e, The experiments were performed three times with similar results.

f, The experiments were performed twice with similar results.

g, n=3 biologically independent samples. The experiments were performed once. *p = 0.0277, **p = 0.0065 by two-tailed unpaired t test.

h, n=5 biologically independent animals. The experiments were performed once.

i, n=7 biologically independent animals. The experiments were performed once.

j, n=5 biologically independent samples. The experiments were performed twice with similar results. *p = 0.0263 by two-tailed unpaired t test.

k, n=8 biologically independent animals. The experiments were performed once. ****p < 0.0001 by two-tailed unpaired two-way ANOVA.

l, The experiments were performed twice with similar results.

m, n=9 biologically independent animals. The experiments were performed once.

n, n=5 biologically independent animals. The experiments were performed twice with similar results. Day 35 *p = 0.0102 by two-tailed unpaired two-way ANOVA.

o, n=4 biologically independent samples. The experiments were performed twice with similar results. *p = 0.0415 in ID8 ascites, **p = 0.0389 in ID8 tumor by two-tailed unpaired t test.

p, n=5 biologically independent samples. The experiments were performed twice with similar results. T cells: **p = 0.0029 BCH+anti-PDL1 vs PBS+IgG, **p = 0.0094 BCH+anti-PDL1 vs BCH, *p = 0.0461 BCH+anti-PDL1 vs anti-PDL1 by two-tailed unpaired t test. CD8 cells: **p = 0.0049 BCH+anti-PDL1 vs PBS+IgG, *p = 0.0136 BCH+anti-PDL1 vs BCH, *p = 0.0480 BCH+anti-PDL1 vs anti-PDL1 by two-tailed unpaired t test.

q-s, Kaplan-Meier survival curves showed the relationship between levels of SLC43A2 and patient survival in different types of tumor: Cholangiocarcinoma (CHOL, **p**), low grade glioma (LGG, **q**), and lung squamous cell carcinoma (LUSC, **r**). The raw data was from TCGA.

t-y, n=12 independent patients. Single cell RNA-seq analyses were based on GSE72056. **t**, *p = 0.0485 by two-tailed paired t test. **v**, Correlation was analyzed using Pearson correlation analysis.