Expanded View Figures

**Figure EV1.** Depletion of Mettl3 or Mettl14 enhanced the response to immunotherapy. (Related to Fig 1).

A  CT26 tumor volume was measured from tumor-bearing mice treated with IgG (IgG control), P (anti-PD-1 antibody), and P plus C (anti-CTLA-4 antibody) therapeutic modalities. n, the numbers of mice. Data are mean ± SEM of the indicated number of mice. *P < 0.05; **P < 0.001 by Student’s t-tests.

B  Survival analysis after various treatments of mice bearing CT26 tumors. n, the numbers of mice. Data are mean ± SEM of the indicated number of mice. ***P < 0.001 by Student’s t-tests.

C, D  C57BL/6 or BALB/c mice bearing control and Mettl3- or Mettl14-depleted tumors (C, CT26; D, B16) were treated with various therapeutic modalities as indicated. Tumor volume was recorded over time as indicated. Each line represents one mouse.

E, F  Immunoblots of Mettl3 and Mettl14 were carried out in the indicated CT26 and B16 mouse tumors in triplicates with Gapdh as a loading control.

G  Representative images of Ki-67 were stained by IHC analysis. Tissue sections from BALB/c mice bearing the indicated knockout of genes with treatment of PD1 antibody. Scale bars, 50 μm.

Source data are available online for this figure.
Figure EV1.
Figure EV2. Loss of Mettl3 or Mettl14 has no effect to cell proliferation and tumor growth.

A  Cell proliferation was assessed in knockout of Mettl3, Mettl14, and non-targeting control (NTC) CT26 and B16 cells using MTS assay in vitro. Mean ± SD of n = 3.
B, C  Tumor growth of xenografts from CT26 and B16 cells with Mettl3- or Mettl14-depleted genes and control as indicated. n, the numbers of mice. Data are mean ± SEM of the indicated number of mice.
D, E  Tumor growth from C57BL/6 or BALB/c mice with control and Mettl3 or Mettl14-depleted tumors (D, CT26; E, B16). Tumor volume was recorded over time as indicated. Each line represents one mouse.
Figure EV3.
Figure EV3  Tumor-infiltrating CD8⁺ T cells and chemokines concentration were altered in Mettl3 or Mettl14 null tumors. (Related to Fig 2).

A Representative examples for CD8⁺ T cells from FACS analyses in CT26 tumors.

B Percentage of tumor-infiltrating CD8⁺ T cells and NK cells were analyzed from control and Mettl3- or Mettl14-deficient B16 tumors using flow cytometry. Each spot represents one mouse. Data are mean ± SEM of the indicated number of mice. *P < 0.05; **P < 0.01 by Student's t-tests.

C Percentage of granzyme B-expressing CD8⁺ T cells from B16 tumors as indicated. Each spot represents one mouse. *P < 0.05; **P < 0.01 by Student's t-tests.

D Secretion of Cxcl9 in serum from the indicated BALB/c mice by ELISA. Each spot represents one mouse. *P < 0.05 by Student's t-tests.

E Intratumoral Cxcl9 concentration were determined by ELISA in the indicated CT26 tumor extracts and then calculated by the total protein concentration. Each spot represents one mouse. n, the numbers of mice. *P < 0.05 by Student's t-tests.

F Secretion of Cxcl10 in serum from the indicated BALB/c mice by ELISA. Each spot represents one mouse.

G Intratumoral Cxcl10 concentration were determined by ELISA in the indicated CT26 tumor extracts and then calculated by the total protein concentration. Each spot represents one mouse. n, the numbers of mice. *P < 0.05; **P < 0.01 by Student's t-tests.
Figure EV4.
Figure EV4. Gene expression changes and analysis of m^6^A modification in Mettl3- or Mettl14-depleted tumors (Related to Fig 3).

A Transcriptional analysis of the indicated genes identified from the RNA-seq data using quantitative RT–PCR. mRNA levels in Mettl3- or Mettl14-deficient tumors are presented as the relative fold change compared to control sgRNA tumor. The mean ± SD of five replicates is shown. *P < 0.05; **P < 0.01; ***P < 0.001 by Student's t-tests.

B Dot blot of the total m^6^A levels in mRNA extracted from Mettl3- or Mettl14-depleted and control tumors.

C Number of consensus m^6^A peaks identified from two biological replicates in the indicated tumors.

D Venn diagram of upregulated m^6^A containing genes, downregulated m^6^A containing genes, and common m^6^A genes without expression level changes as indicated. Orange shade represents upregulated m^6^A containing genes from Mettl3-depleted tumors compared to control. Blue shade represents downregulated m^6^A containing genes from Mettl3-depleted tumors compared to control. Green shade represents upregulated m^6^A containing genes from Mettl14-depleted tumors compared to control. Yellow shade represents downregulated m^6^A containing genes from Mettl14-depleted tumors compared to control. Gray shade represents common m^6^A genes without any changes from Mettl3- and Mettl14-depleted tumors compared to control.

E GO analysis was performed on 64 co-upregulated m^6^A containing genes from D as indicated.

F Distribution of m^6^A peaks in the indicated tumors. Pie charts show the proportion of m^6^A peaks in the 5'-UTR (orange), CDS (gray), 3'-UTR (yellow), and promoter-TSS (blue).

G Representative genes with m^6^A sites generated by integrative genomics viewer. Blue represents reads coverage of input sample and red represents reads coverage of IP sample. Rectangular cyan shade represents the m^6^A peaks located on transcripts.

Source data are available online for this figure.
**Figure EV5.** Stat1 and Irf1 are targets regulated by Mettl3 and Mettl14 (Related to Fig 3).

A, B Immunoblot analysis of the protein levels of Mettl3, Mettl14, Irf1, and Stat1 in CT26 cells as indicated. Gapdh served as a control.

C–E Tumor growth in BALB/c mice bearing the lacking indicated genes treated with PD-1 antibody. Each line represents one mouse.

F Survival analysis of tumors with the lacking indicated genes and control were observed in CT26 colon cancer. n, the numbers of mice. Data are mean ± SEM of the indicated number of mice in each group. **P < 0.01 by Student's t-tests.

Source data are available online for this figure.