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/6J 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.

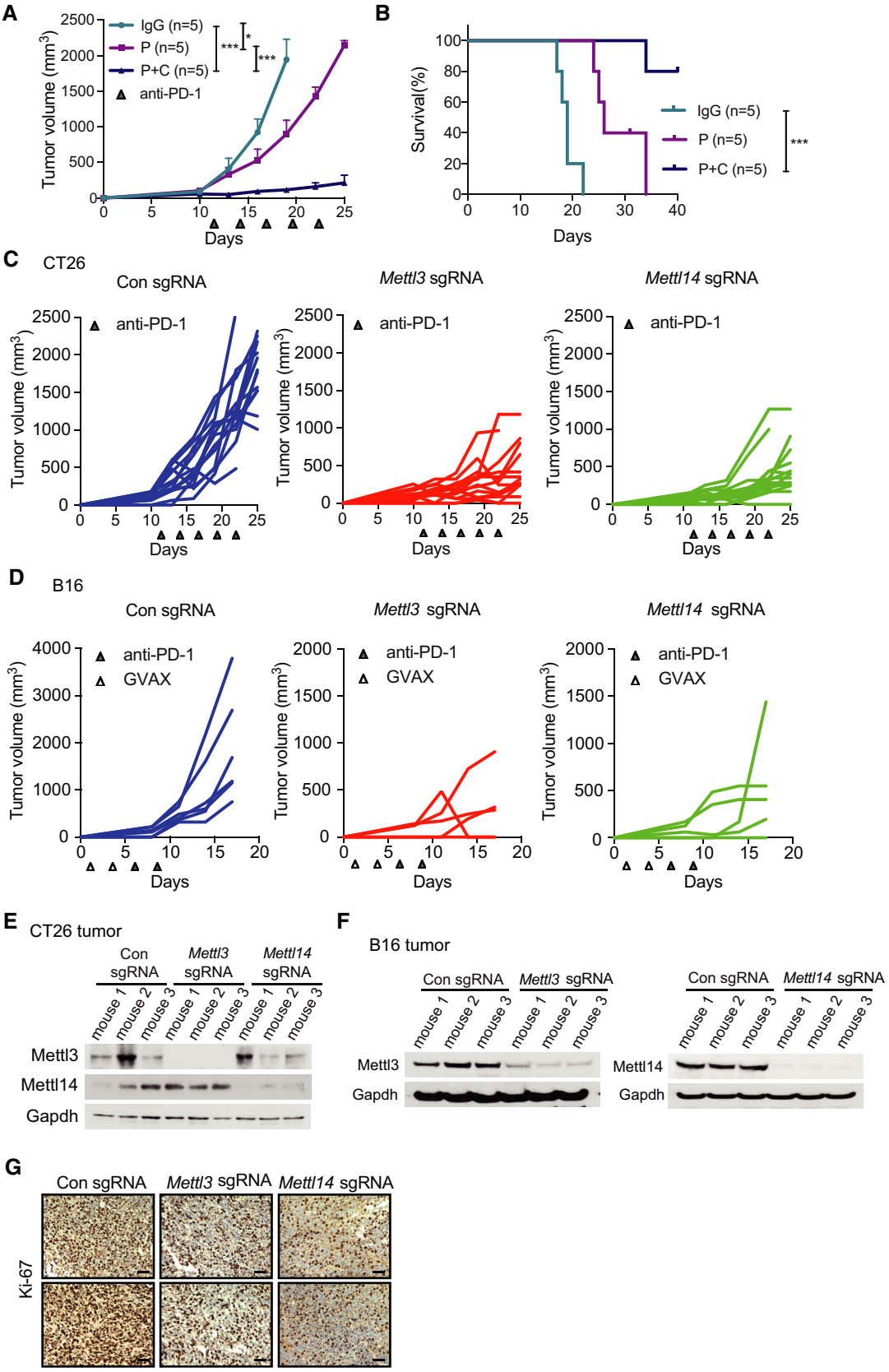
A
IgG (n=5)
P (n=5)
P+C (n=5)
anti-PD-1
Tumor volume (mm3)
Days
B
Survival(%)
IgG (n=5)
P (n=5)
P+C (n=5)
Days
C
CT26
Con sgRNA
Mettl3 sgRNA
Mettl14 sgRNA
anti-PD-1
Tumor volume (mm3)
Days
D
B16
Con sgRNA
Mettl3 sgRNA
Mettl14 sgRNA
anti-PD-1
GVAX
Tumor volume (mm3)
Days
E
CT26 tumor
Con sgRNA
Mettl3 sgRNA
Mettl14 sgRNA
mouse 1
mouse 2
mouse 3
Mettl3
Mettl14
Gapdh
F
B16 tumor
Con sgRNA
Mettl3 sgRNA
Mettl14 sgRNA
mouse 1
mouse 2
mouse 3
Mettl3
Gapdh
Mettl14
Gapdh
G
Con sgRNA
Mettl3 sgRNA
Mettl14 sgRNA
Ki-67

Figure EV1.

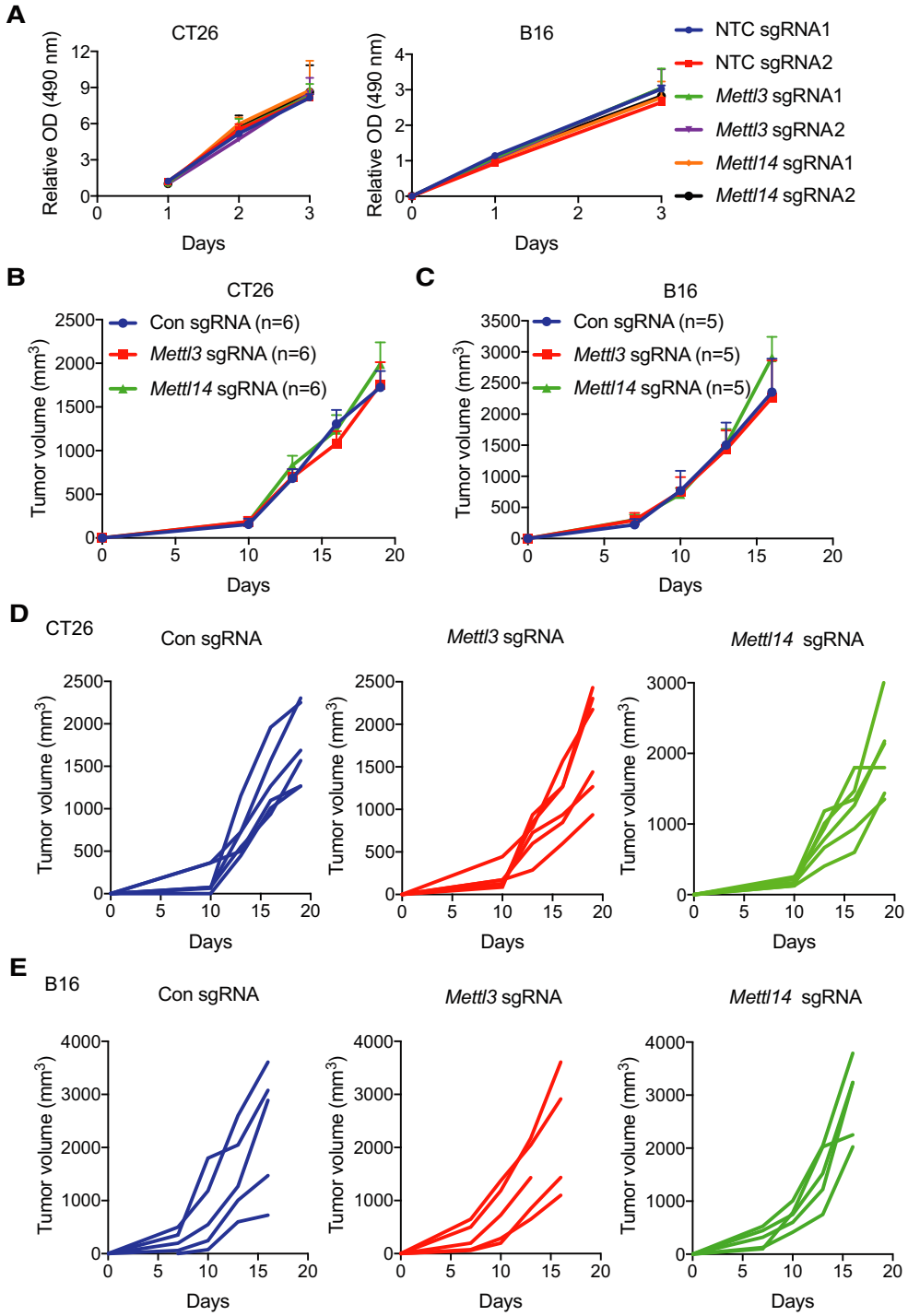

Figure EV2. Loss of Mettl3 or Mettl14 has no effect to cell proliferation and tumor growth.

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/6J 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.

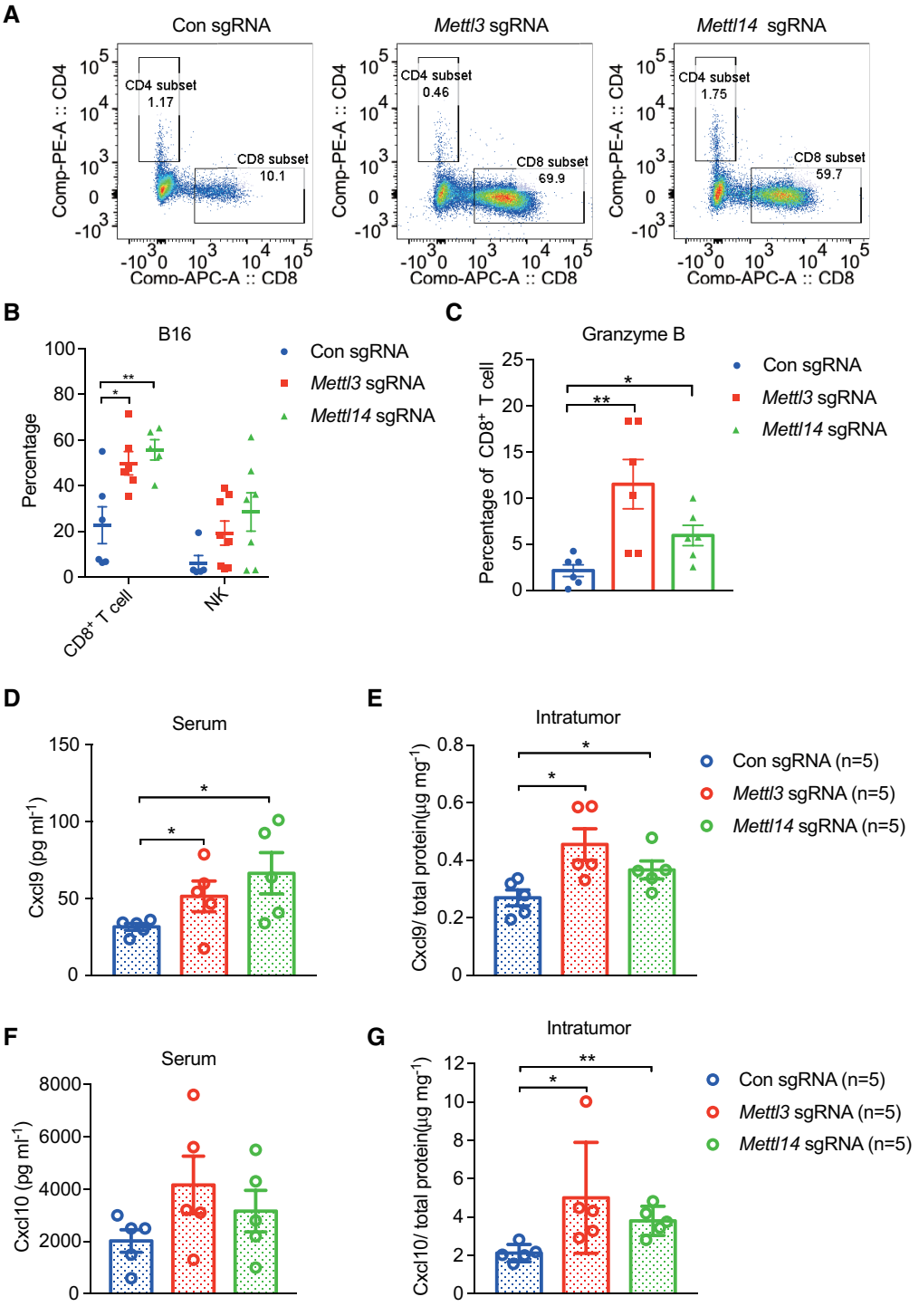
A
Con sgRNA
Mettl3 sgRNA
Mettl14 sgRNA
Comp-PE-A :: CD4
Comp-APC-A :: CD8
CD4 subset
1.17
CD8 subset
10.1
CD4 subset
0.46
CD8 subset
69.9
CD4 subset
1.75
CD8 subset
59.7
B
B16
Percentage
CD8+ T cell
NK
Con sgRNA
Mettl3 sgRNA
Mettl14 sgRNA
C
Granzyme B
Percentage of CD8+ T cell
D
Serum
Cxcl9 (pg ml-1)
E
Intratumor
Cxcl9/ total protein(μg mg-1)
Con sgRNA (n=5)
Mettl3 sgRNA (n=5)
Mettl14 sgRNA (n=5)
F
Serum
Cxcl10 (pg ml-1)
G
Intratumor
Cxcl10/ total protein(μg mg-1)

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.

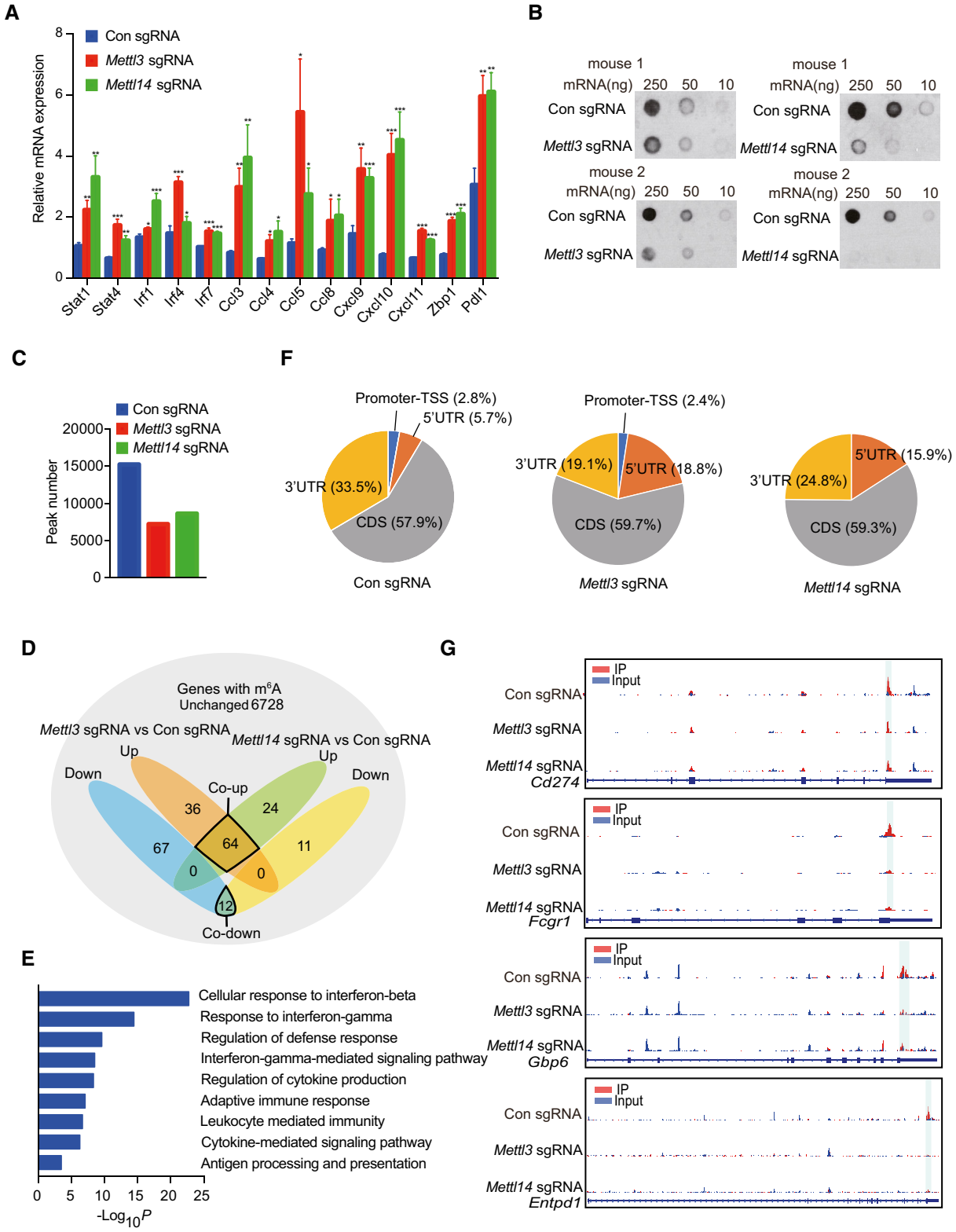
A
Con sgRNA
Mettl3 sgRNA
Mettl14 sgRNA
Relative mRNA expression
Stat1
Stat4
Irf1
Irf4
Irf7
Ccl3
Ccl4
Ccl5
Ccl8
Cxcl9
Cxcl10
Cxcl11
Zbp1
Pdl1
B
mouse 1
mRNA(ng) 250 50 10
Con sgRNA
Mettl3 sgRNA
mouse 1
mRNA(ng) 250 50 10
Con sgRNA
Mettl14 sgRNA
mouse 2
mRNA(ng) 250 50 10
Con sgRNA
Mettl3 sgRNA
mouse 2
mRNA(ng) 250 50 10
Con sgRNA
Mettl14 sgRNA
C
Con sgRNA
Mettl3 sgRNA
Mettl14 sgRNA
Peak number
F
Promoter-TSS (2.8%)
5'UTR (5.7%)
3'UTR (33.5%)
CDS (57.9%)
Con sgRNA
Promoter-TSS (2.4%)
3'UTR (19.1%)
5'UTR (18.8%)
CDS (59.7%)
Mettl3 sgRNA
5'UTR (15.9%)
3'UTR (24.8%)
CDS (59.3%)
Mettl14 sgRNA
D
Genes with m6A
Unchanged 6728
Mettl3 sgRNA vs Con sgRNA
Mettl14 sgRNA vs Con sgRNA
Up
Down
Co-up
36
24
67
64
11
0
0
12
Co-down
E
Cellular response to interferon-beta
Response to interferon-gamma
Regulation of defense response
Interferon-gamma-mediated signaling pathway
Regulation of cytokine production
Adaptive immune response
Leukocyte mediated immunity
Cytokine-mediated signaling pathway
Antigen processing and presentation
-Log10P
G
IP
Input
Con sgRNA
Mettl3 sgRNA
Mettl14 sgRNA
Cd274
Fcgr1
Gbp6
Entpd1

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.

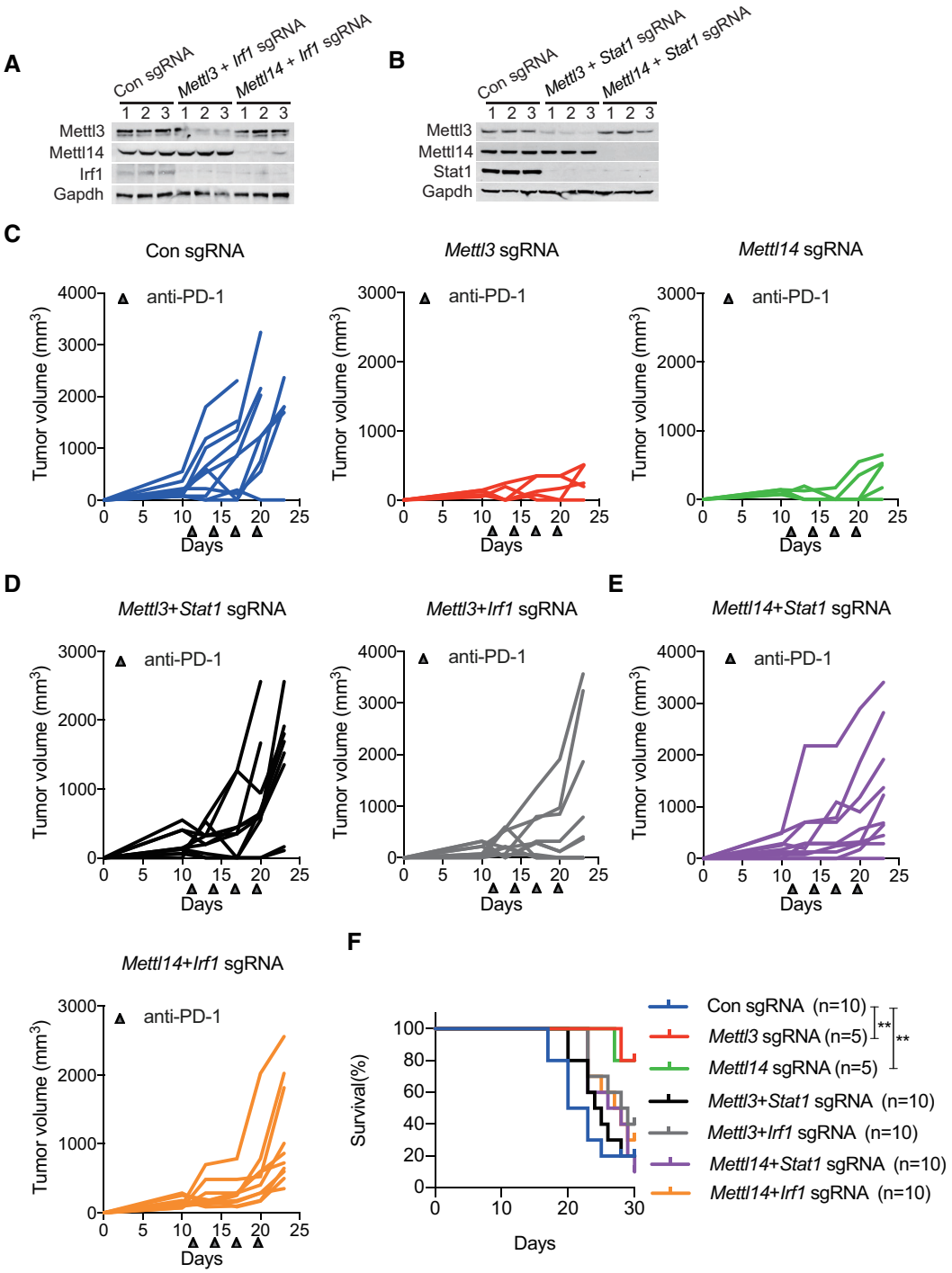

Figure EV5. Stat1 and Irf1 are targets regulated by Mettl3 and Mettl14 (Related to Fig 3).

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.