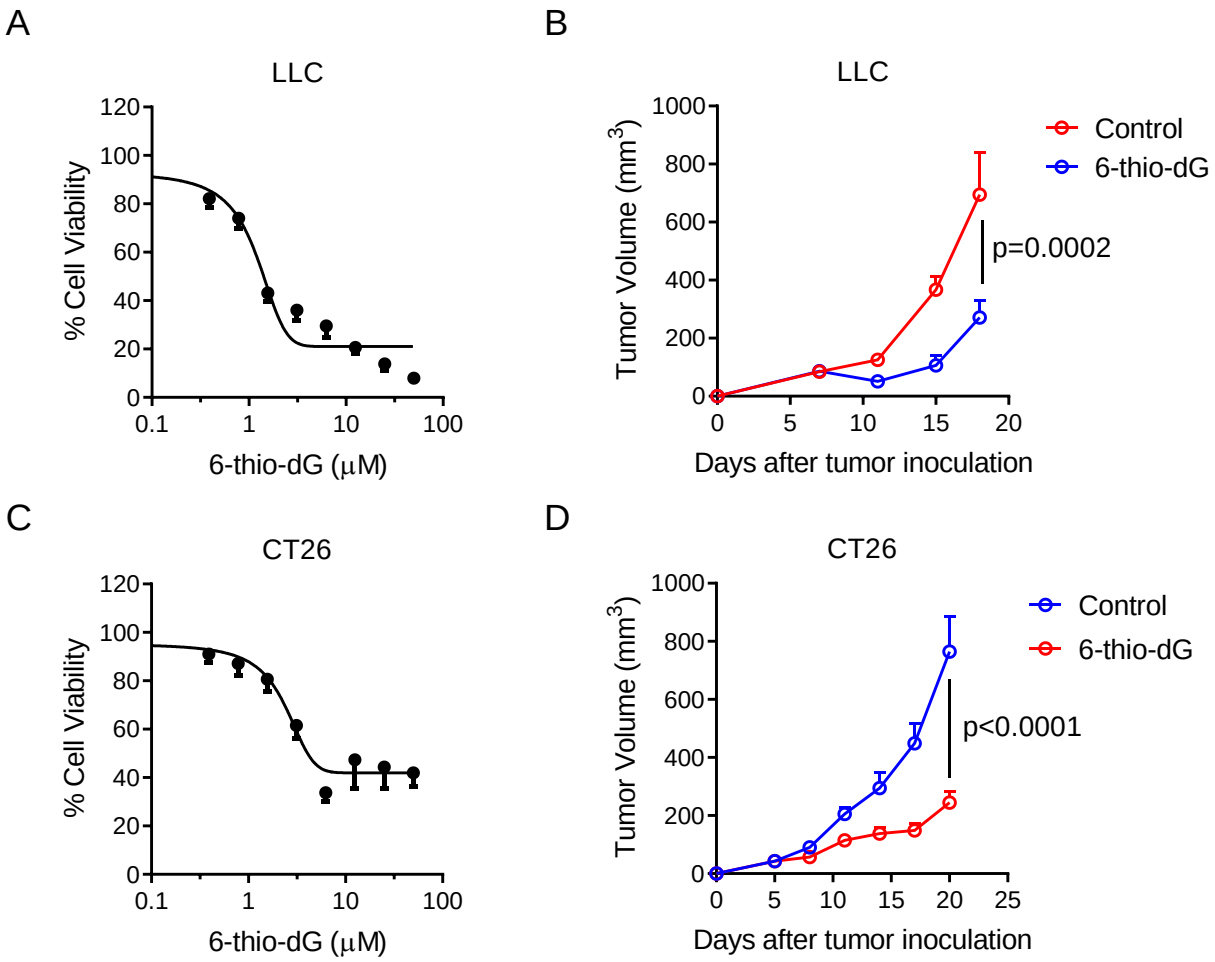

Figure S1. Related to Figure 1

(A) Cell viability (IC50) of 6-thio-dG in LLC murine lung cancer cells. Cells were treated with 6-thio-dG for 4 days.

(B) C57BL/6 mice (n=5) were inoculated with $1x10^6$ LLC tumor cells and treated with 6-thio-dG (3 mg/kg, days 4, 5, 6). Tumor growth was measured every 3 days.

(C) IC50 of 6-thio-dG in CT26 murine colon cancer cells.

(D) BALB/C mice (n=5) were inoculated with $5x10^5$ CT26 tumor cells and treated with 6-thio-dG (3 mg/kg, days 5, 6, 7). Tumor growth was measured every 3 days.

Data were shown as mean ± SEM from two independent experiments. P value was determined by two-way ANOVA.

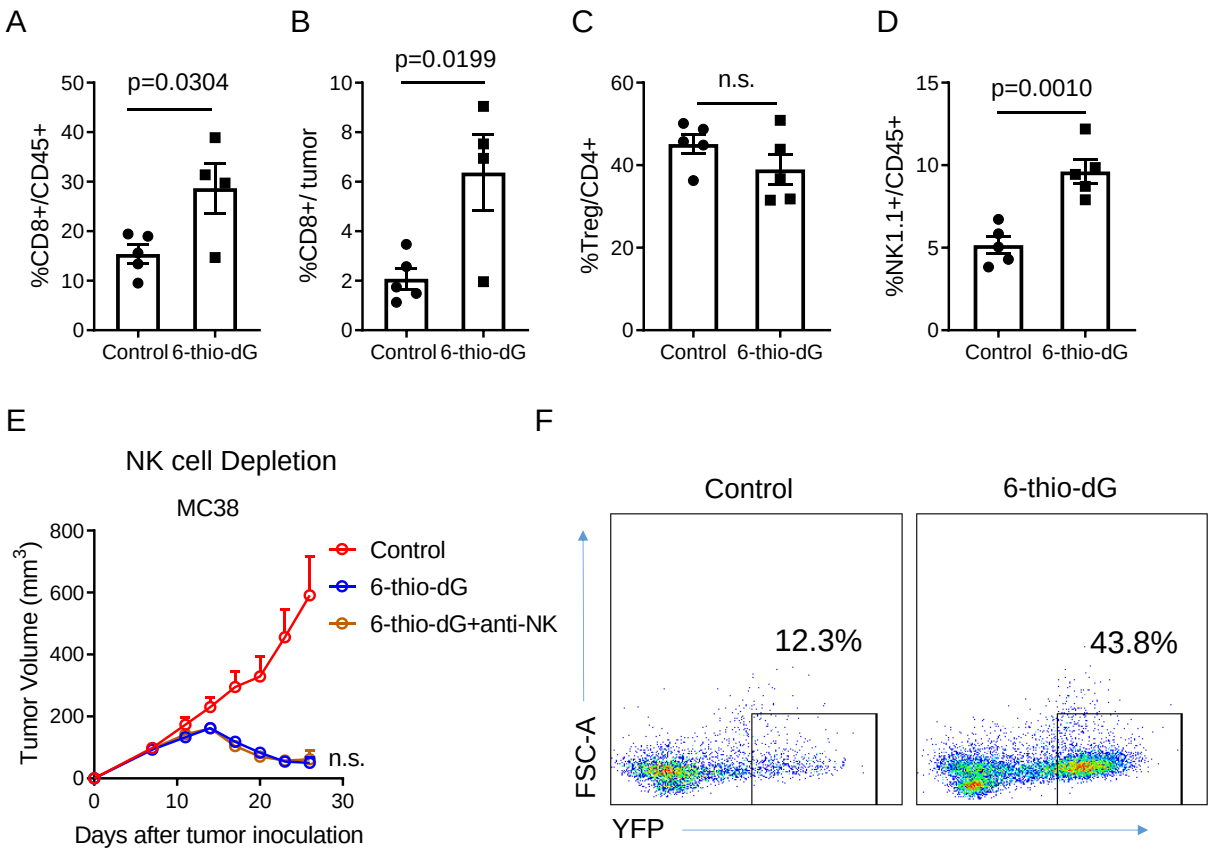

Figure S2. Related to Figure 2

(A-D) C57BL/6 mice (n=4-5) were inoculated with $5x10^5$ MC38 tumor cells and treated with 6-thio-dG (3 mg/kg, days 7, 8, 9). 7 days after first treatment, tumors were analyzed for $CD8^+$ T cells among $CD45^+$ cells (A) and among total tumor cells (B), tumor infiltrating T cells were analyzed for the frequency of $CD4^+Foxp3^+$ Treg cells (C) and NK cells (D).

(E) C57BL/6 mice (n=5) were inoculated with $5x10^5$ MC38 tumor cells and treated with 6-thio-dG (3 mg/kg, days 7, 8, 9). 200 μg anti-NK1.1 was administrated one day before treatment initiation and then twice a week for 3 weeks.

(F) IFN-γ reporter mice (n=3) were inoculated with $5x10^5$ MC38 tumor cells and treated with 6-thio-dG (3 mg/kg, days 7, 8, 9). Eleven days after the last treatment, tumors were minced and digested for flow cytometric detection of YFP^+ T cells. Representative flow cytometry gating was shown.

Data were shown as mean ± SEM from two independent experiments. P value was determined by two-tailed unpaired t test in (A - D) or two-way ANOVA (E).

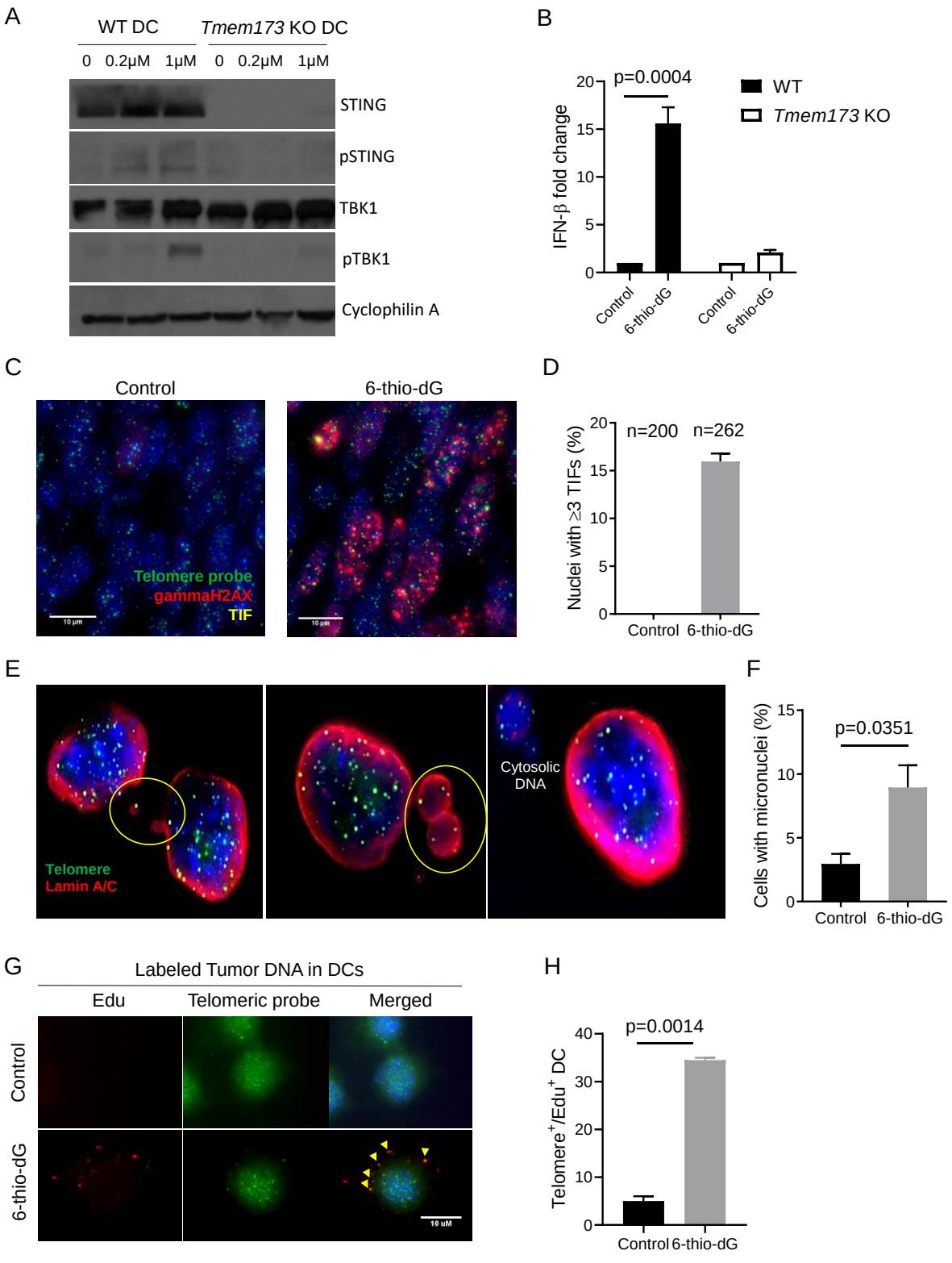

Figure S3. Related to Figure 4

(A) BMDCs were cultured with MC38 tumor cells that were pretreated with 0.2 μM or 1 μM 6-thio-dG for 6 h, and then DCs were purified with magnetic beads and subjected to western blot.
(B) BMDCs from wild WT or *Tmem173* KO mice were cultured with MC38 tumor cells that were pretreated with 200 nM 6-thio-dG for overnight, and then DCs were purified with magnetic beads and qPCR was performed to test the relative abundance of IFN-β.
(C and D) C57BL/6 mice (n=3) were inoculated with $5x10^5$ MC38 tumor cells and treated with 6-thio-dG (3 mg/kg, days 10, 11, 12). 3 days after last injection, mice were sacrificed; tumors were collected and fixed for TIF (Telomere dysfunction Induced Foci) staining. Images were obtained by fluorescence microscope (100X). Red dots show DNA damage (γ-H2AX), green dots show telomeres and yellow dots show TIF (DNA damage on telomeres). Scale bars, 10 μM.
(E and F) 6-thio-dG treatment induced micronuclei in MC38 cells. (E) Representative picture of two daughter cells in late telophase contain telomere signals and coated and uncoated micronuclei in MC38 cells. Green dots represent telomeric signals and red color represents lamin A/C (nuclear envelop biomarker). (F) Quantification of 1 μM 6-thio-dG treatment induced micronuclei after 48 h.
(G and H) $1x\ 10^5$ MC38 cells were seeded in 6-well plate and cells were labeled with 25 μM EdU. 2 days later, cells were washed out and incubated with 1 μM 6-thio-dG in fresh media O/N. Cells were then washed out and co-cultured with DCs O/N. The next day, DCs were purified with magnetic beads. Purified DCs were then fixed and cytospun for immuno-FISH. Telomeric probe: green, EdU: red, DAPI: blue. Images were captured at 63X magnification with an Axio Imager Z2 equipped with an automatic capture system and analyzed with ISIS software (camera: coolcube 1-metasystems). Representative imaging (G) and quantification data (H) were shown, n=100. Scale bars, 10 μM.
Data were shown as mean ± SEM from two to three independent experiments. P value was determined by two-tailed unpaired t test (B, F and H).

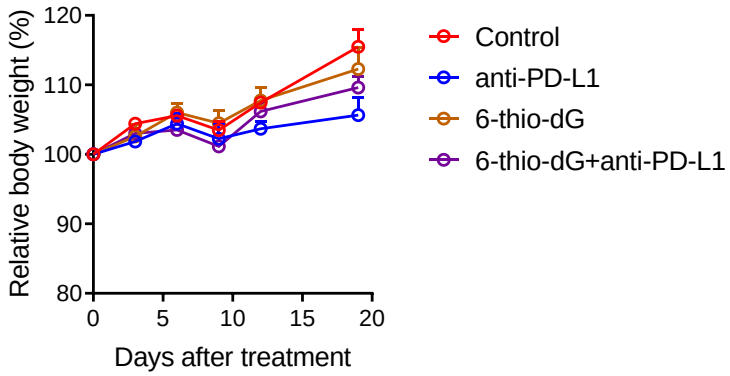

Figure S4. Related to Figure 5

(A) C57BL/6 mice (n=5) were inoculated with $5x10^5$ MC38 tumor cells and treated with 6-thio-dG (3 mg/kg, days 10 and 11). 50 μg anti-PD-L1 antibody was administrated on days 13 and 17. Mice body weight were measured.

Data were shown as mean ± SEM.

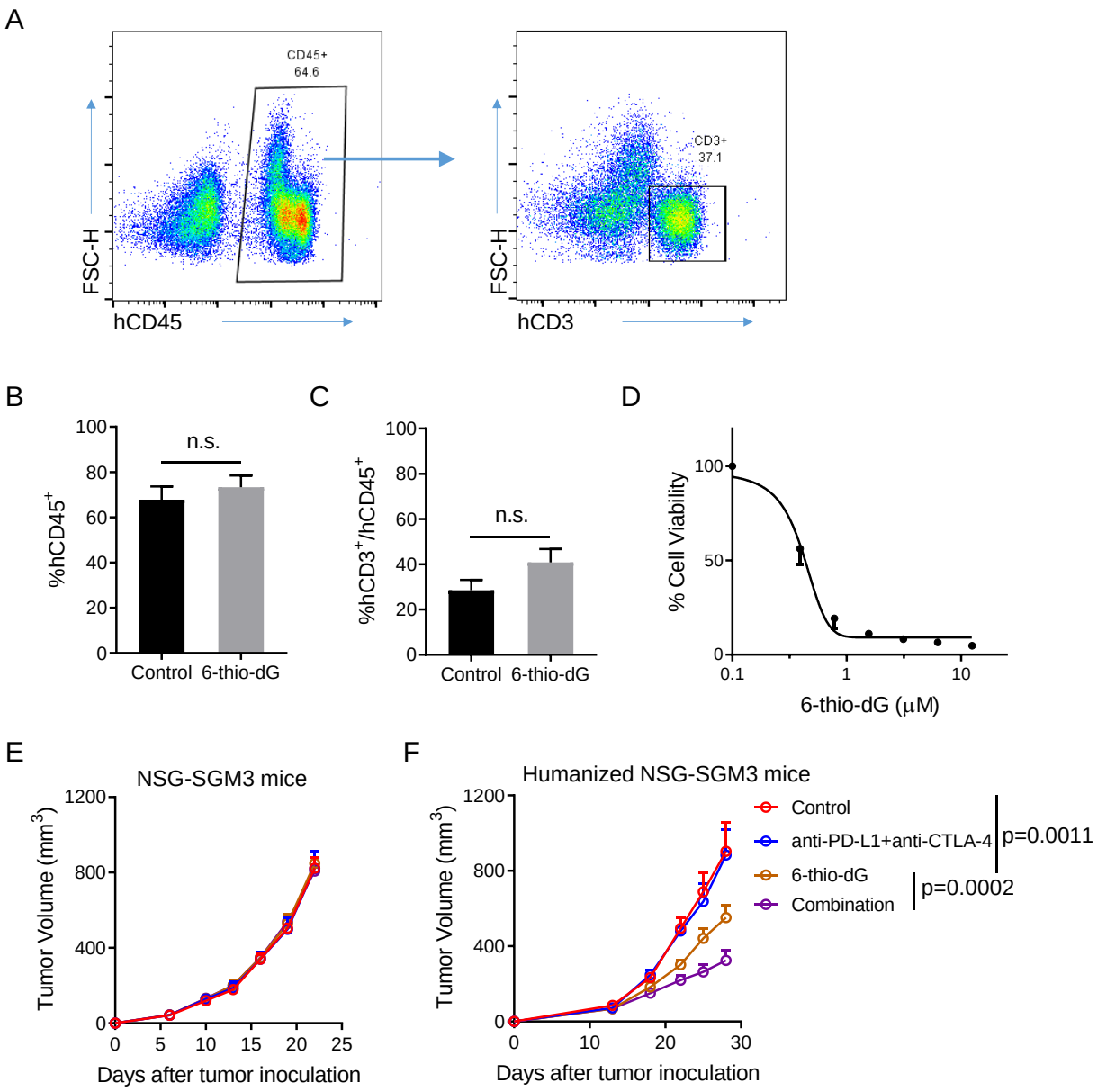

Figure S5. Related to Figure 6

(A-C) 12 weeks after humanized mouse reconstitution, human $CD45^+$ cells and $CD3^+$ T cells in mouse peripheral blood were tested by flow cytometry. Representative flow cytometric plot was shown in (A). CD45 and CD3 frequency in control and 6-thio-dG groups before treatment were shown in (B) and (C), n=5.

(D) IC50 of 6-thio-dG in A375 human melanoma cancer cells. Cells were treated with 6-thio-dG for 4 days.

(E) NSG-SGM3 mice (n=5) were inoculated with $2x10^6$ A375 tumor cells and treated with 6-thio-dG (3mg/kg, days 7 and 8) or anti-PD-L1 plus anti-CTLA-4 (200 μg i.p., days 10 and 13) or the combination of 6-thio-dG plus anti-PD-L1 and anti-CTLA-4. Tumor growth was measured every 3 days.

(F) Humanized NSG-SGM3 mice (n=5-7) were inoculated with $2x10^6$ A375 tumor cells and treated with 6-thio-dG (3 mg/kg, days 13 and 14) or anti-PD-L1 plus anti-CTLA-4 (200 μg i.p., days 16 and 19) or the combination of 6-thio-dG plus anti-PD-L1 and anti-CTLA-4. Tumor growth was measured every 3 days.

Data were shown as mean ± SEM. P value was determined by two-tailed unpaired t test (B and C, n.s. $p > 0.05$) or two-way ANOVA (F).