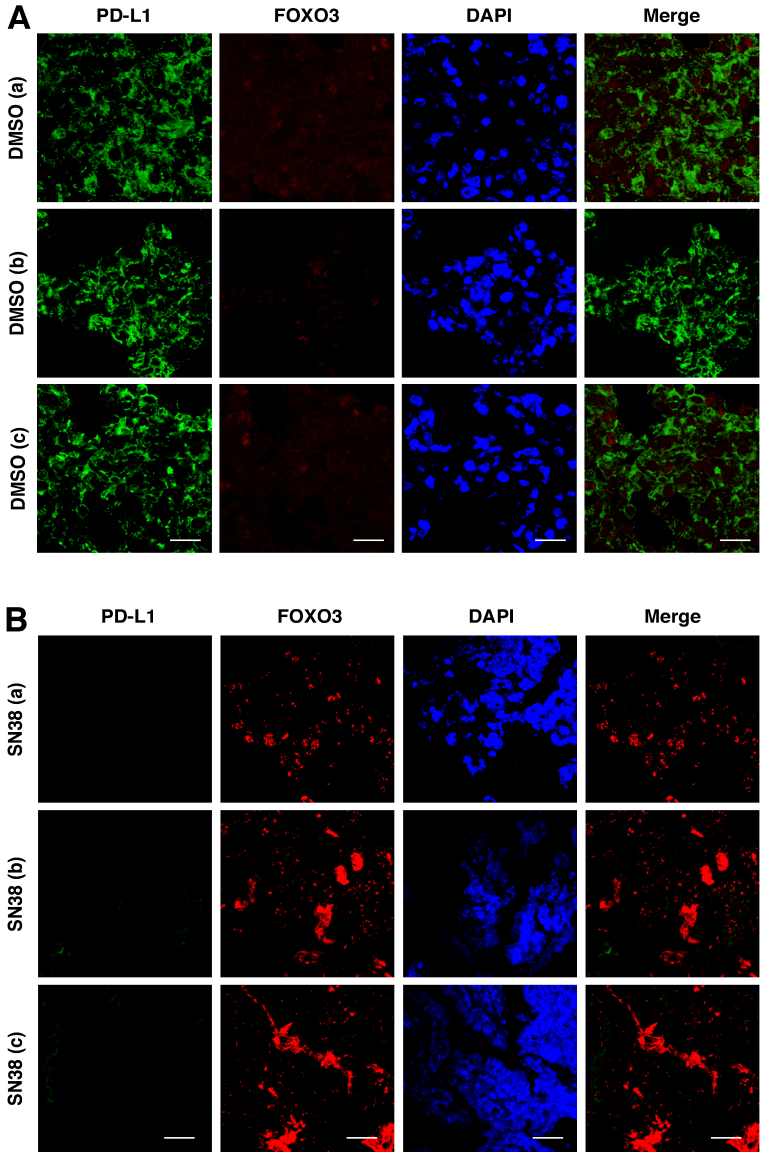
Online supplementary figure S1

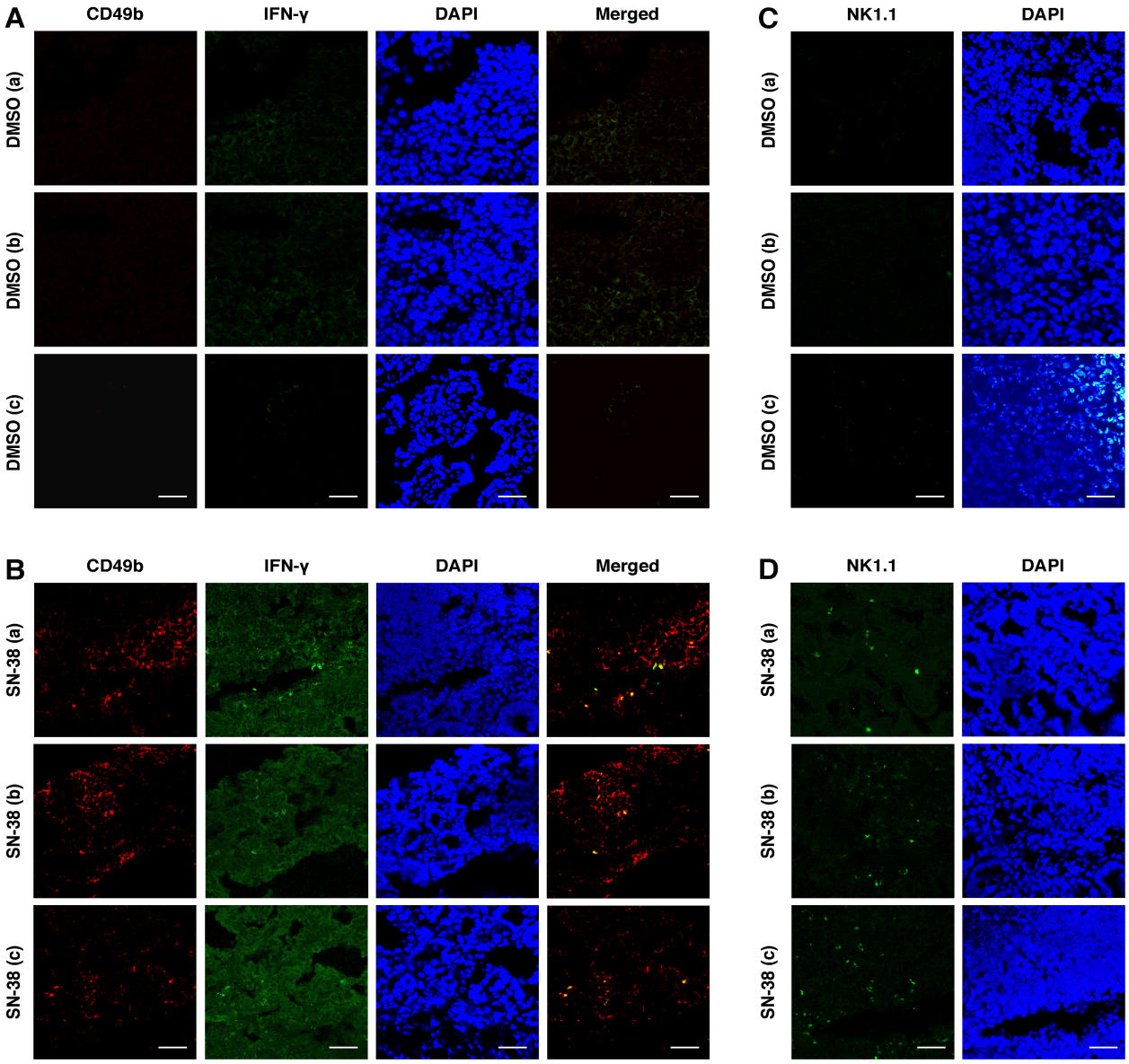
Chung et al.



Online supplementary figure S1. Low-dose SN-38 suppresses PD-L1 and promotes FOXO3 protein expression *in vivo* in mouse ID8 tumor specimens. (A, B) Three slides of samples from ID8 tumors treated with DMSO control (A) or SN-38 (B) were incubated with an antibody (Ab) against mouse PD-L1 or FOXO3 and followed by a corresponding Alexa Fluor 488(green)- or Alexa Fluor 594(red)-conjugated secondary antibody (2nd Ab), and immunofluorescence (IF) analysis using fluorescence confocal microscopy (FCM). DAPI was used to show the nuclei. Scale bar: 20 μm. The relative expressions of PD-L1 and FOXO3 in the TME are shown as histograms in figure 2D, E. Four archetypal IF images displayed in figure 2D are included in this supplementary figure S1.

Online supplementary figure S2

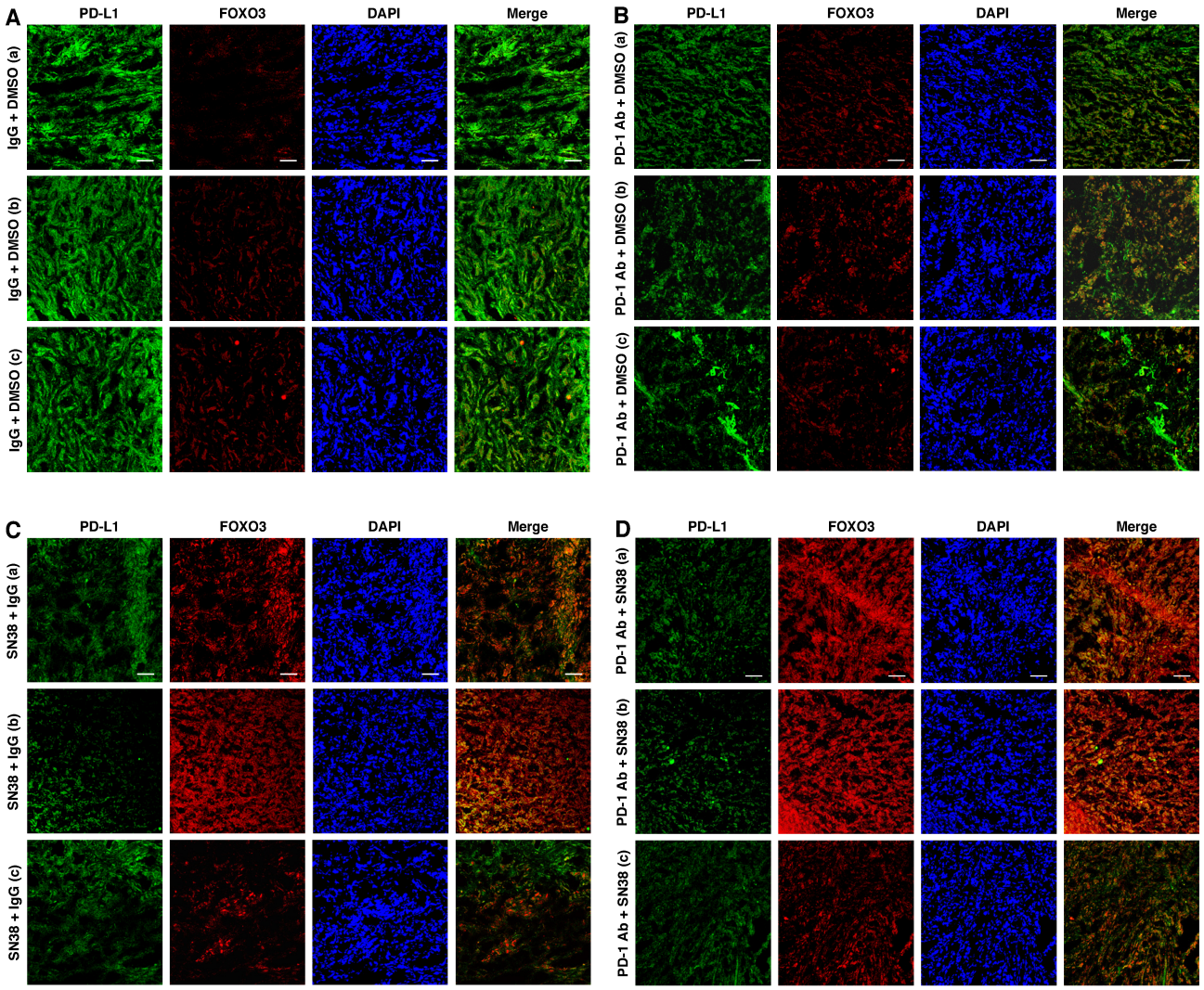
Chung et al.



Online supplementary figure S2. SN-38 engages mouse CD49b/NK1.1-positive NK cells infiltrating the ID8 TME in a syngeneic mouse ID8 tumor model. (A, B) Three slides of samples from ID8 tumors treated with DMSO control (A) or SN-38 (B) were incubated with an antibody against mouse CD49b or IFN-γ or NK1.1 and followed by corresponding fluorescent 2nd Abs and IF analysis using FCM as described above. DAPI was used to show the nuclei. Scale bar: 20 μm. The relative expressions of CD49b, NK1.1, and IFN-γ in the TME are shown as histograms in figure 2F, G, J.

Online supplementary figure S3

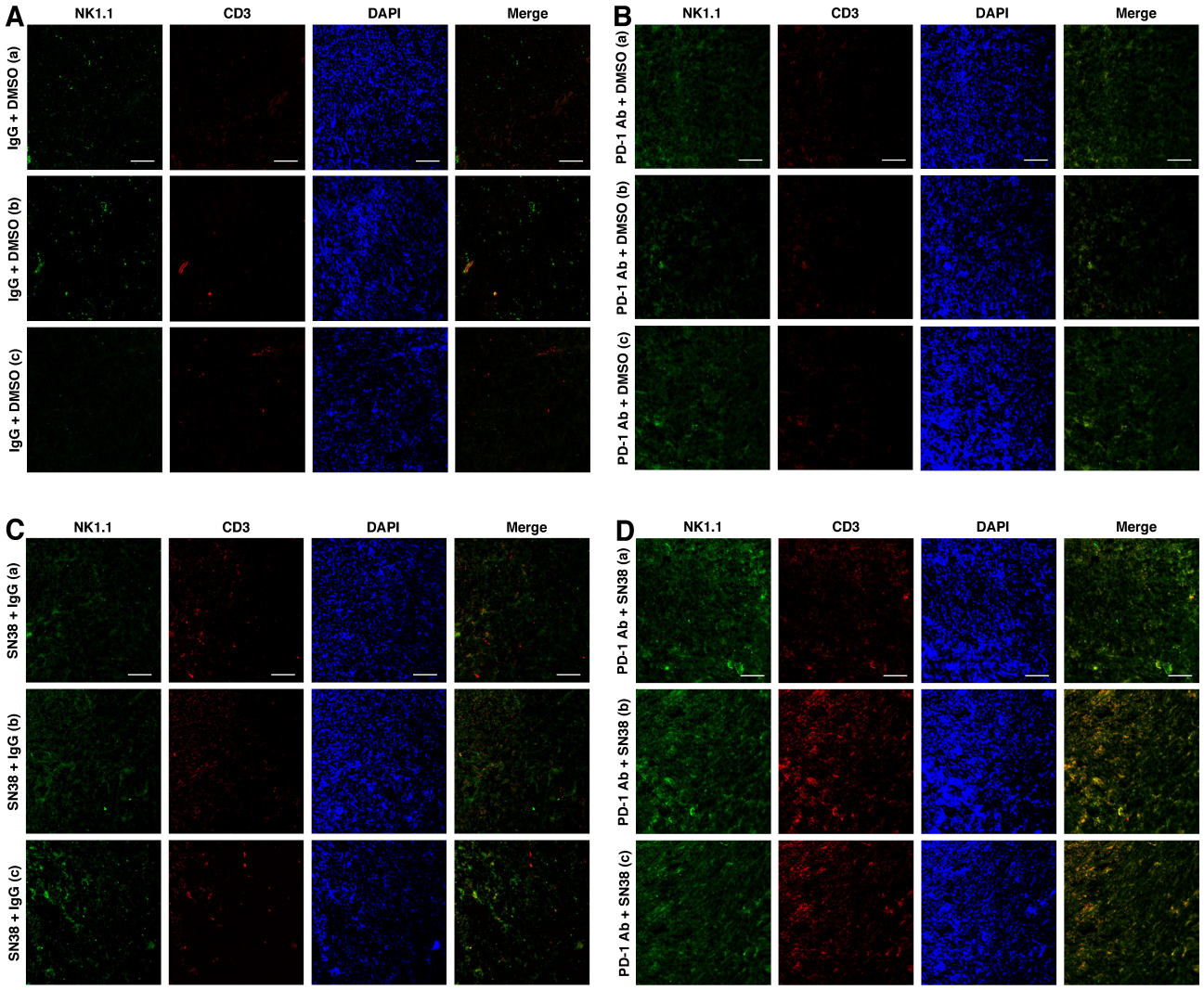
Chung et al.



Online supplementary figure S3. SN-38 alone and combined regimens, PD1 antibody (Ab) plus (+) SN38, significantly downregulate PD-L1 and upregulate FOXO3 protein expressions in ID8 tumor specimens. (A-D) Three slides of samples from ID8 tumors treated with various regimens containing IgG plus DMSO (A) or PD-1 Ab plus DMSO (B) or SN-38 plus IgG (C) or PD-1 Ab plus SN-38 (D) were incubated with an Ab against mouse PD-L1 or FOXO3 and followed by corresponding fluorescent 2nd Abs and IF analysis using FCM as described above. DAPI showed the nuclei. Scale bar: 50 μm. DMSO and IgG are negative controls. The relative expressions of PD-L1 and FOXO3 in the TME are shown as histograms in figure 4C. Eight archetypal IF images displayed in figure 3B are included in this supplementary figure S3.

Online supplementary figure S4

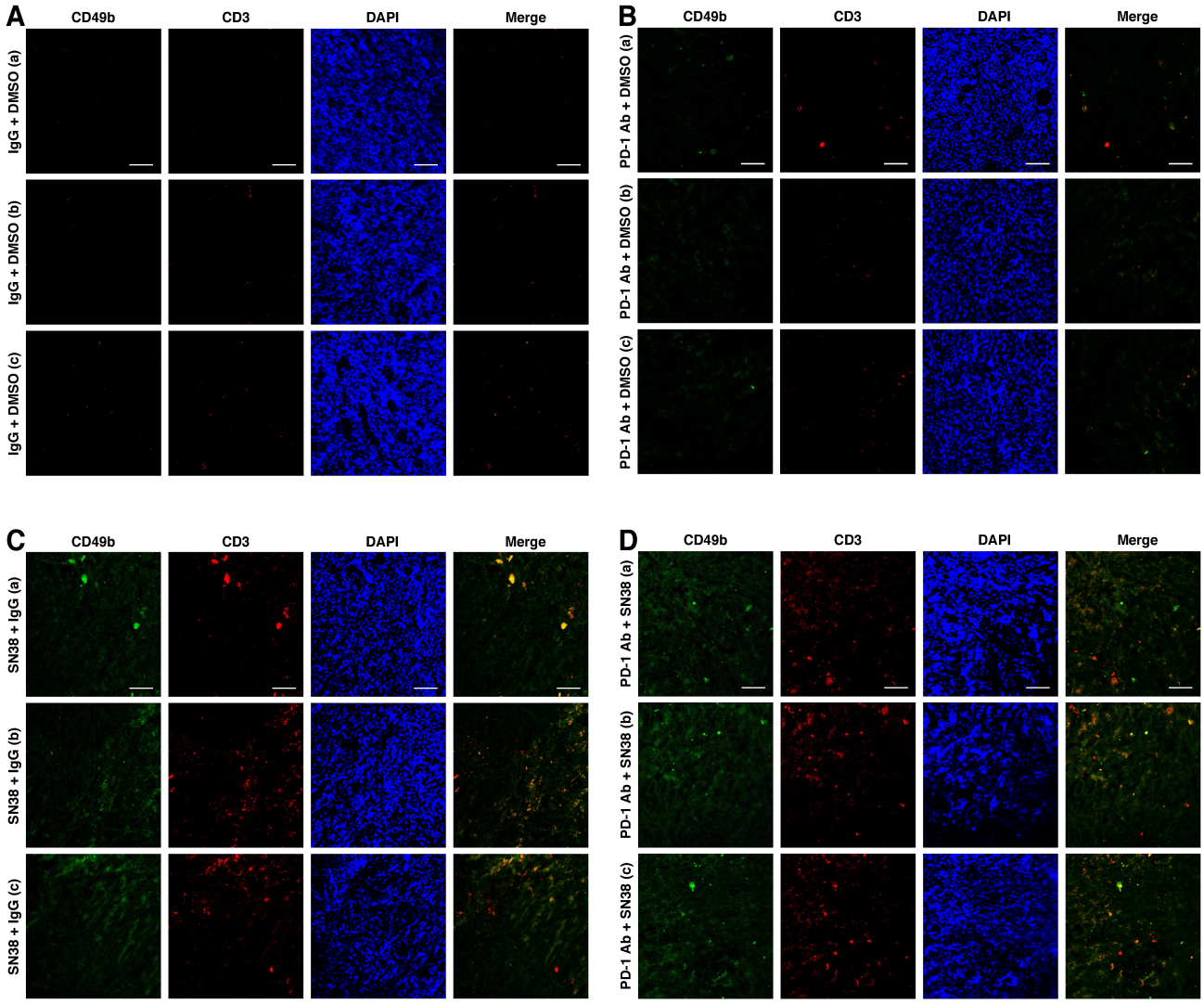
Chung et al.



Online supplementary figure S4. SN-38 alone or combined anti-PD-1 antibody (Ab) and SN-38 significantly engage mouse NK1.1-positive NK cells and, to a lesser extent, CD3-positive T cells to infiltrate the ID8 TME in a syngeneic tumor model. (A-D) Three slides of samples from ID8 tumors treated with various regimens containing IgG plus DMSO (A) or PD-1 Ab plus DMSO (B) or SN-38 plus IgG (C) or PD-1 Ab plus SN-38 (D) were incubated with an Ab against mouse NK1.1 or CD3 and followed by corresponding fluorescent 2nd Abs and IF analysis using FCM as described above. DAPI showed the nuclei. Scale bar: 20 μm. DMSO and IgG are negative controls. The relative expressions of NK1.1 and CD3 in the TME are shown as histograms in figure 4E. Eight paradigmatic IF images displayed in figure 4D are included in this supplementary figure S4.

Online supplementary figure S5

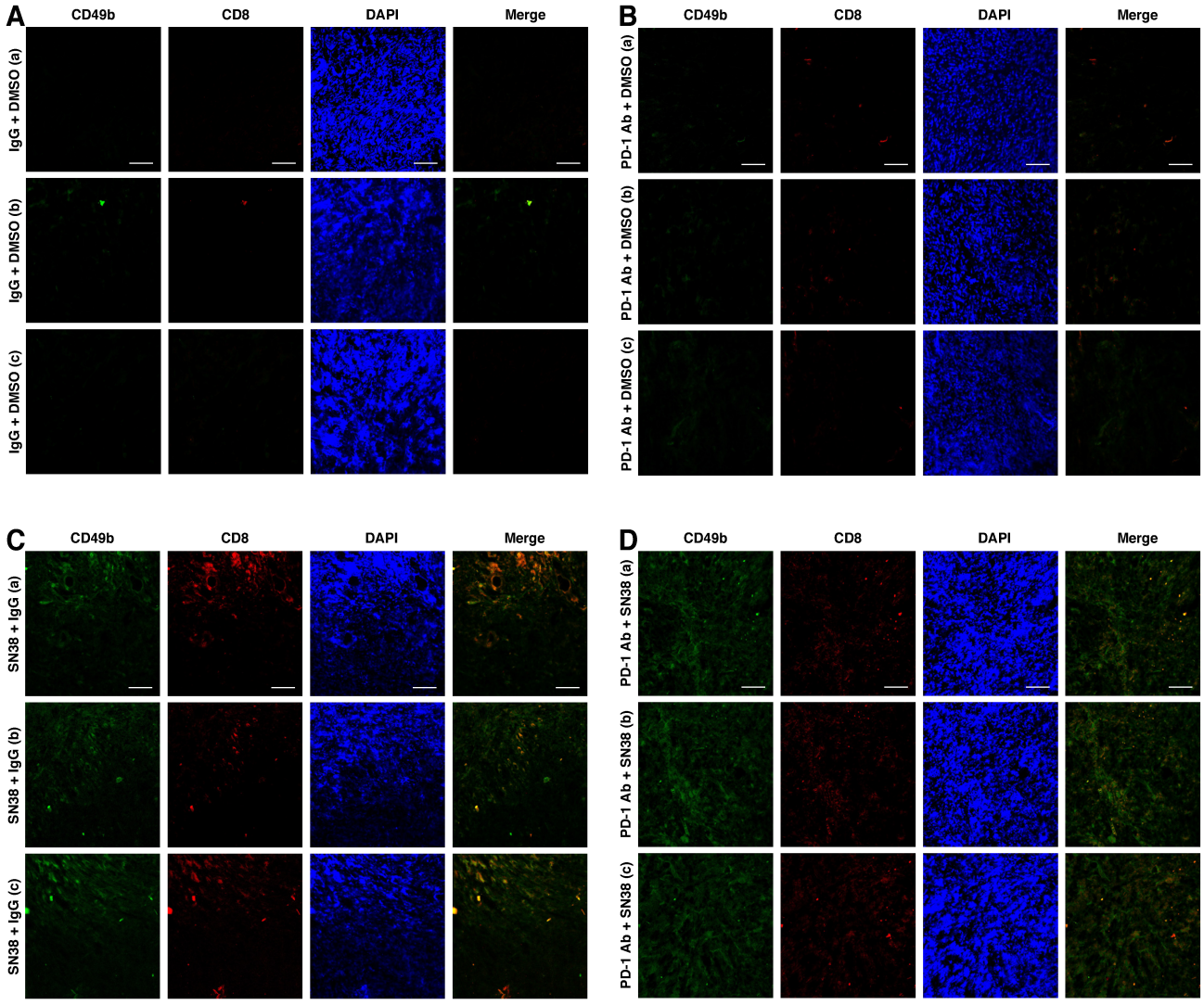
Chung et al.



Online supplementary figure S5. SN-38 alone or combined anti-PD-1 antibody (Ab) and SN-38 significantly engage mouse CD49b-positive NK cells and, to a lesser extent, CD3-positive T cells to infiltrate the ID8 TME in a syngeneic tumor model. (A-D) Three slides of samples from ID8 tumors treated with various regimens containing IgG plus DMSO (A) or PD-1 Ab plus DMSO (B) or SN-38 plus IgG (C) or PD-1 Ab plus SN-38 (D) were incubated with an Ab against mouse CD49b Ab or CD3 Ab and followed by corresponding fluorescent 2nd Abs and IF analysis using FCM as described above. DAPI showed the nuclei. Scale bar: 20 μm. DMSO and IgG are negative controls. The relative expressions of CD49b and CD3 in the TME are shown as histograms in figure 4E. Eight paradigmatic IF images displayed in figure 4F are included in this supplementary figure S5.

Online supplementary figure S6

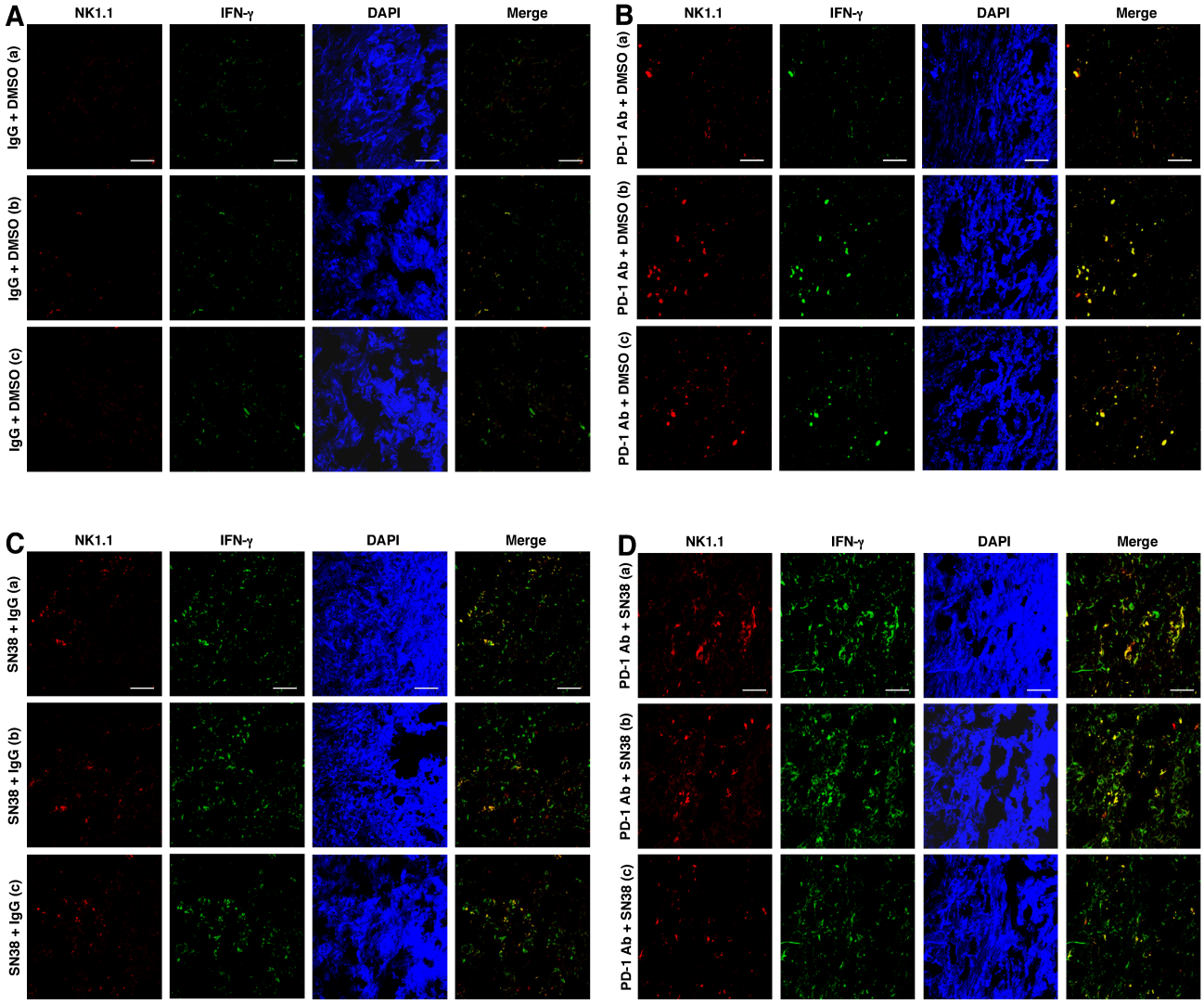
Chung et al.



Online supplementary figure S6. SN-38 alone and combined anti-PD-1 antibody (Ab) and SN-38 significantly engage mouse CD49b-positive NK cells and, to a lesser extent, CD3-positive T cells to infiltrate the ID8 TME in a syngeneic tumor model. (A-D) Three slides of samples from ID8 tumors treated with various regimens containing IgG plus DMSO (A) or PD-1 Ab plus DMSO (B) or SN-38 plus IgG (C) or PD-1 Ab plus SN-38 (D) were incubated with an Ab against mouse CD49b Ab or CD3 Ab and followed by corresponding fluorescent 2nd Abs and IF analysis using FCM as described above. DAPI showed the nuclei. Scale bar: 20 μm. DMSO and IgG are negative controls. The relative expressions of CD49b and CD3 in the TME are shown as histograms in figure 4E. Eight paradigmatic IF images displayed in figure 4H are included in this supplementary figure S6.

Online supplementary figure S7

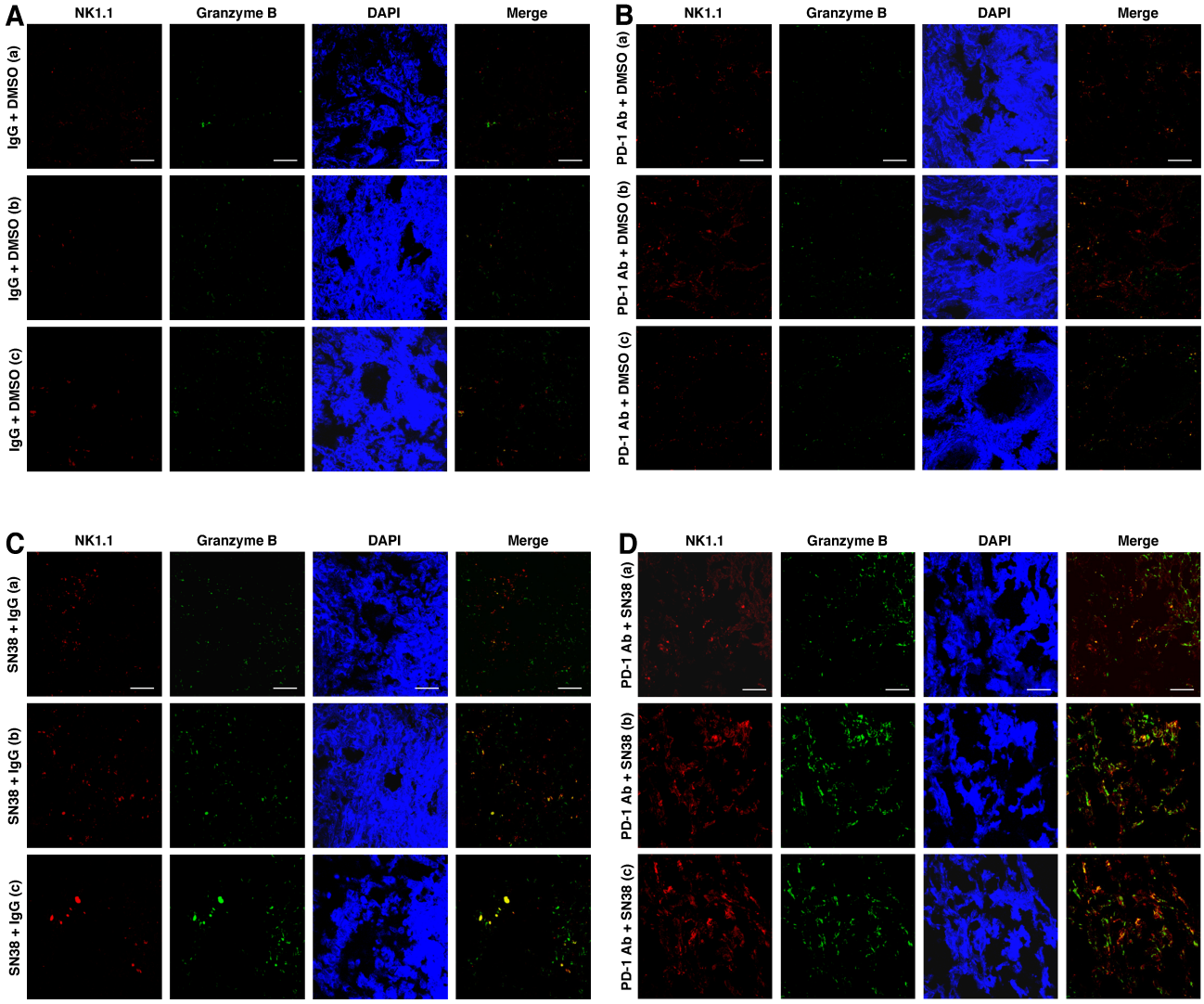
Chung et al.



Online supplementary figure S7. SN-38 alone and combined anti-PD-1 antibody (Ab) and SN-38 significantly increased IFN-γ protein levels in the ID8 TME in a syngeneic tumor model. (A-D) Three slides of samples from ID8 tumors treated with various regimens containing IgG plus DMSO (A) or PD-1 Ab plus DMSO (B) or SN-38 plus IgG (C) or PD-1 Ab plus SN-38 (D) were incubated with an Ab against mouse NK1.1 or IFN-γ and followed by corresponding fluorescent 2nd Abs and IF analysis using FCM as described above. DAPI showed the nuclei. Scale bar: 20 μm. The relative expressions of NK1.1 and IFN-γ in the TME are shown as histograms in figure 6A. DMSO and IgG are negative controls.

Online supplementary figure S8

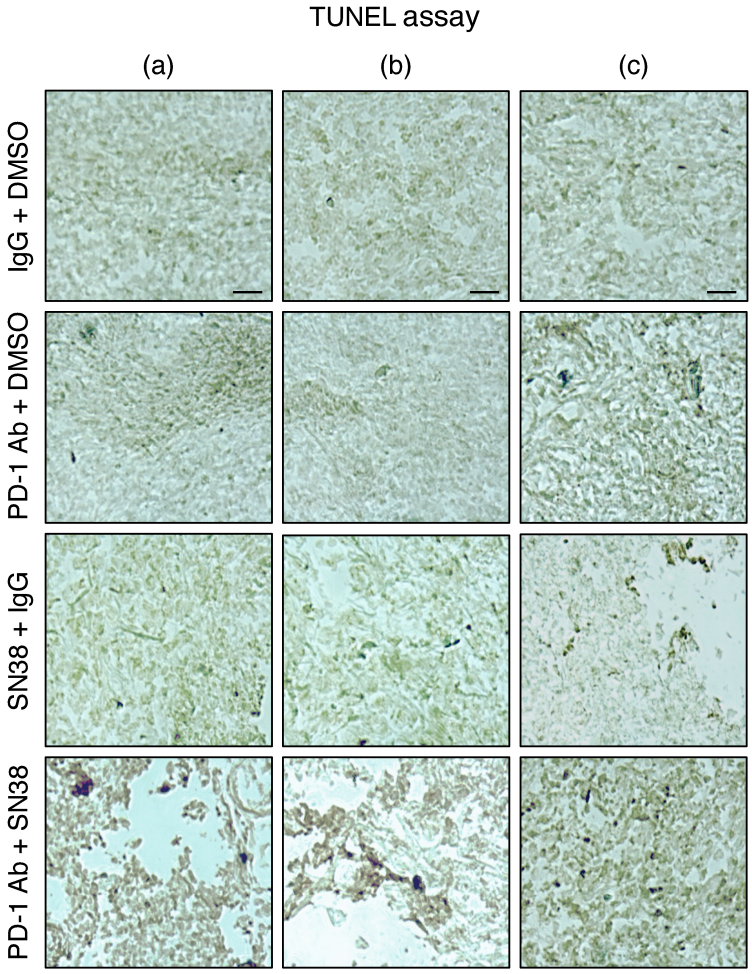
Chung et al.



Online supplementary figure S8. SN-38 alone and combined anti-PD-1 antibody (Ab) and SN-38 significantly increased granzyme B protein levels in the ID8 TME in a syngeneic tumor model. (A-D) Three slides of samples from ID8 tumors treated with various regimens containing IgG plus DMSO (A) or PD-1 Ab plus DMSO (B) or SN-38 plus IgG (C) or PD-1 Ab plus SN-38 (D) were incubated with an Ab against mouse NK1.1 or granzyme B and followed by corresponding fluorescent 2nd Abs and IF analysis using FCM as described above. DAPI showed the nuclei. Scale bar: 20 μm. DMSO and IgG are negative controls. The relative expressions of NK1.1 and granzyme B in the TME are shown as histograms in figure 6B.

Online supplementary figure S9

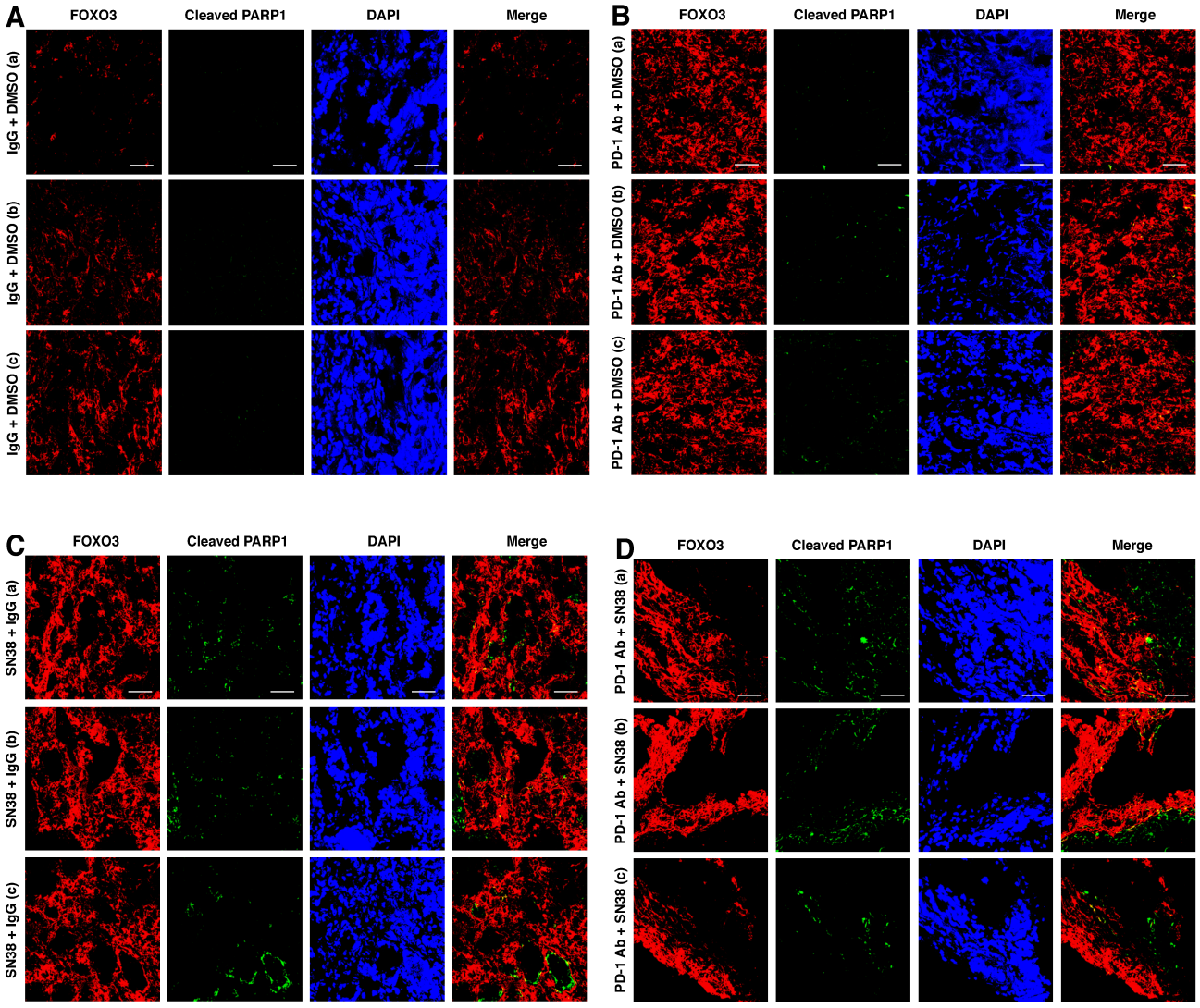
Chung et al.



Online supplementary figure S9. NK cells promote apoptosis in ID8 tumor cells in the TME in a syngeneic tumor model. Three slides of samples from ID8 tumors treated with various regimens containing IgG plus DMSO or PD-1 antibody (Ab) plus DMSO or SN-38 plus IgG or PD-1 Ab plus SN-38 were subjected to TUNEL assays (Promega) for determining cellular apoptosis. Scale bar: 20 μm. IgG plus DMSO are negative controls. The relative TUNEL-positive apoptotic tumors cells in the TME are shown as histograms in figure 6C. Four archetypal TUNEL images displayed in figure 6C are included in this supplementary figure S6.

Online supplementary figure S10

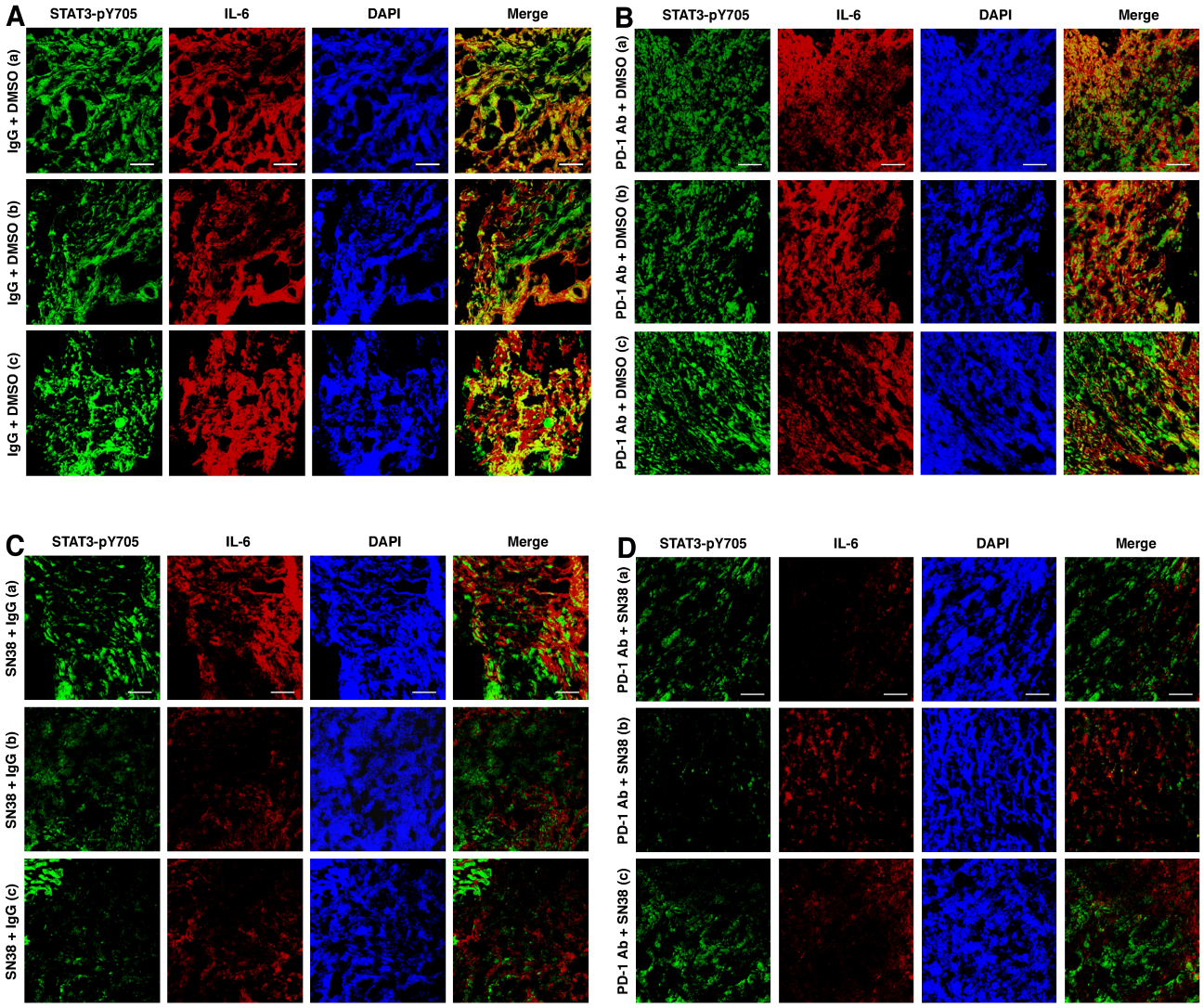
Chung et al.



Online supplementary figure S10. SN-38 alone and combined anti-PD-1 antibody (Ab) and SN-38 significantly increased cleaved-PARP1 protein levels in the ID8 TME in a syngeneic tumor model. (A-D) Three slides of samples from ID8 tumors treated with various regimens containing IgG plus DMSO (A) or PD-1 Ab plus DMSO (B) or SN-38 plus IgG (C) or PD-1 Ab plus SN-38 (D) were incubated with an Ab against mouse FOXO3 or cleaved-PARP1 peptide and followed by corresponding fluorescent 2nd Abs and IF analysis using FCM as described above. DAPI showed the nuclei. Scale bar: 20 μm. DMSO and IgG are negative controls. The relative expressions of FOXO3 and cleaved-PARP1 in the TME are shown as histograms in figure 6D.

Online supplementary figure S11

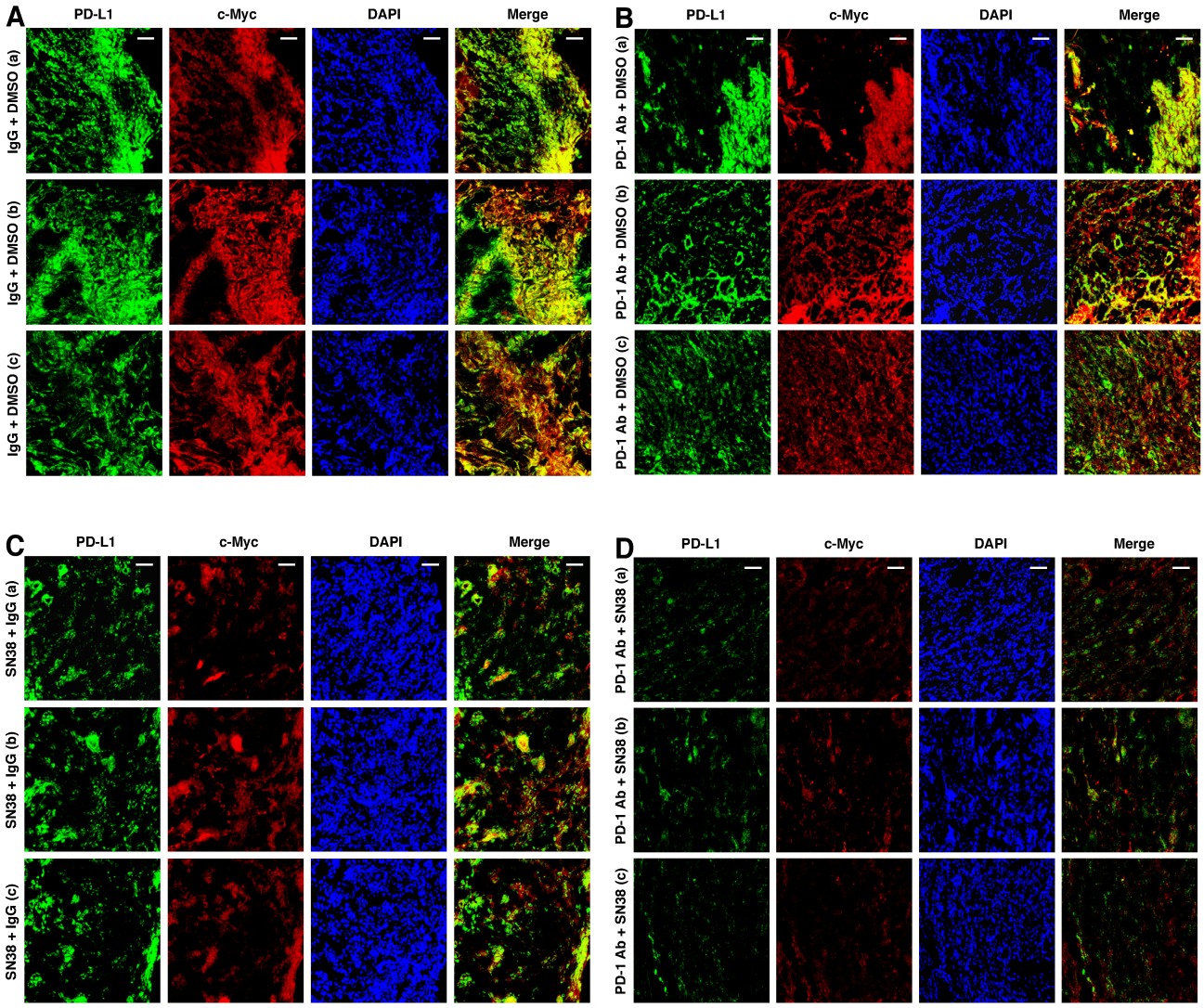
Chung et al.



Online supplementary figure S11. SN-38 alone and combined anti-PD-1 antibody (Ab) and SN-38 significantly reduce phospho-STAT3 (Tyr-705) (STAT3-pY705) and interleukin-6 (IL-6) protein levels in the ID8 TME in a syngeneic tumor model. (A-D) Three slides of samples from ID8 tumors treated with various regimens containing IgG plus DMSO (A) or PD-1 Ab plus DMSO (B) or SN-38 plus IgG (C) or PD-1 Ab plus SN-38 (D) were incubated with an Ab against mouse STAT3-pY705 or IL-6 and followed by corresponding fluorescent 2nd Abs and IF analysis using FCM as described above. DAPI showed the nuclei. Scale bar: 20 μm. DMSO and IgG are negative controls. The relative expressions of STAT3-pY705 and IL-6 in the TME are shown as histograms in figure 8A, B.

Online supplementary figure S12

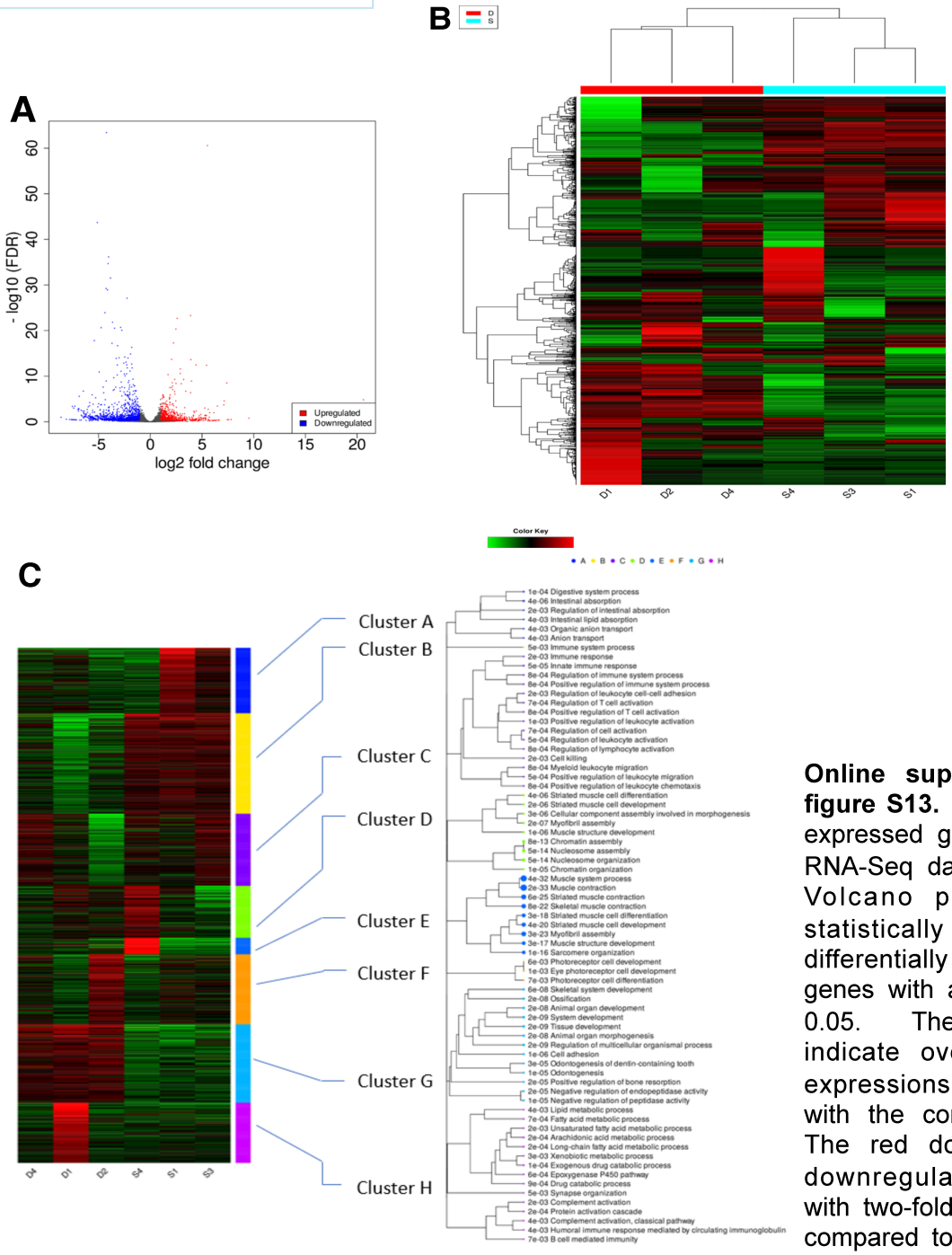
Chung et al.



Online supplementary figure S12. SN-38 alone and combined anti-PD-1 antibody (Ab) and SN-38 significantly decrease c-Myc and PD-L1 protein levels concurrently in the ID8 TME in a syngeneic tumor model. (A-D) Three slides of samples from ID8 tumors treated with various regimens containing IgG plus DMSO (A) or PD-1 Ab plus DMSO (B) or SN-38 plus IgG (C) or PD-1 Ab plus SN-38 (D) were incubated with an Ab against mouse c-Myc or PD-L1 and followed by corresponding fluorescent 2nd Abs and IF analysis using FCM as described above. DAPI showed the nuclei. Scale bar: 20 μm. DMSO and IgG are negative controls. The relative expressions of c-Myc and PD-L1 in the TME are shown as histograms in figure 8C.

Online supplementary figure S13

Chung et al.



Online supplementary figure S13. Differentially expressed genes in the RNA-Seq data. (A) The Volcano plot shows statistically significant differentially expressed genes with a P-value < 0.05. The blue dots indicate over two-fold expressions compared with the control group. The red dots indicate downregulated genes with two-fold expression compared to the control group. (B) RNA-seq data analysis of control group (DMSO + IgG) vs drug-treated group (SN-38 + PD-1 ab). Heatmap of hierarchical clustering indicates differentially expressed genes (rows) between two groups (n=3/group). The red color bar indicates up-regulation, and the green color bar indicates down-regulation. (C) The enriched gene ontology classification by k-means clustering was performed via the iDEP93 web-based tool. Eight classes were generated and defined using highly variable 3,600 genes.