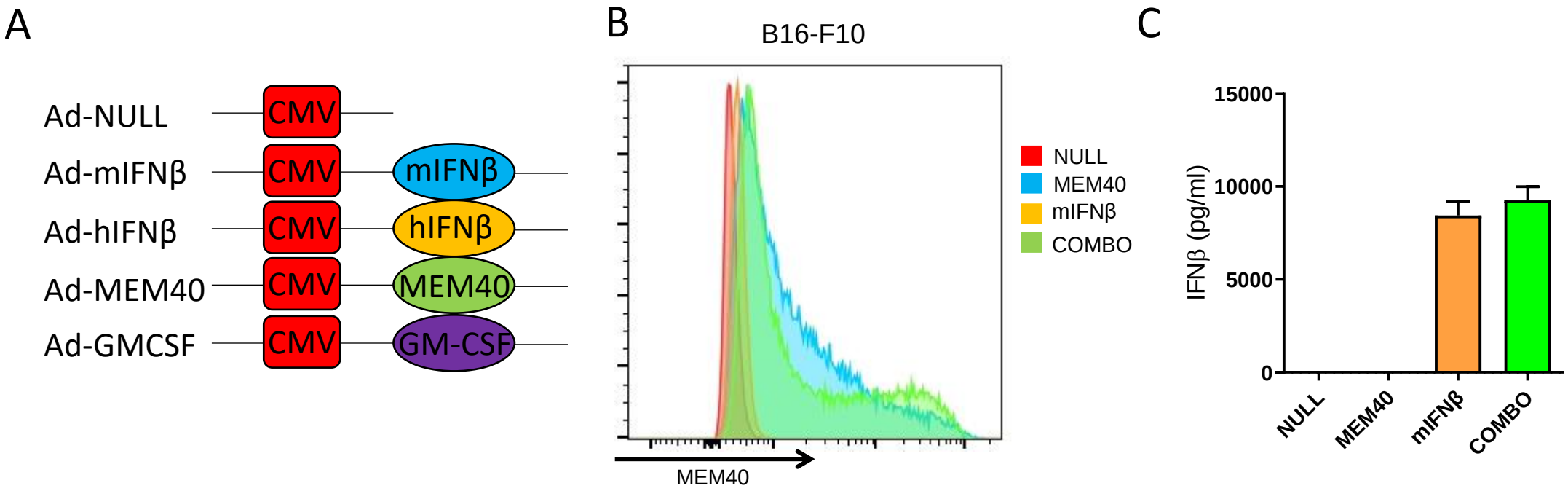
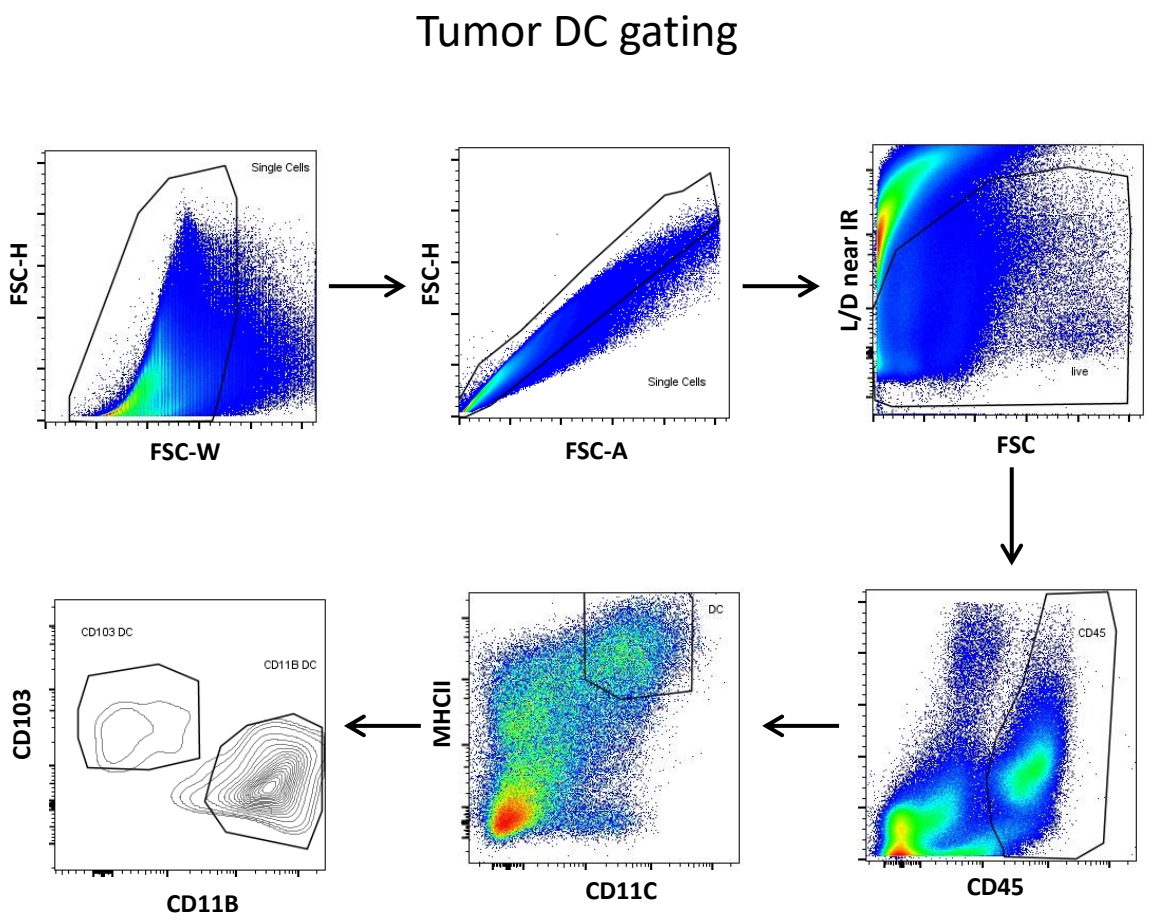
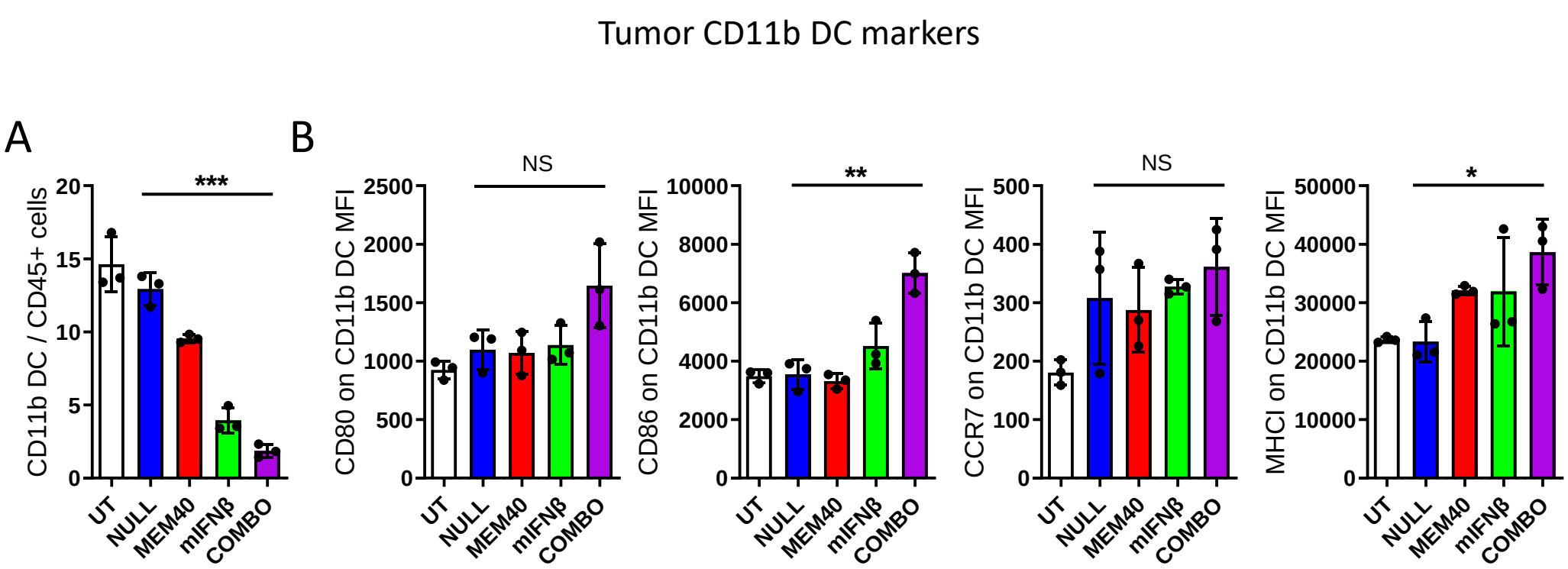


Supplementary Fig. 1. Adenoviruses scheme and MEM40&IFNβ expression in B16-F10. (A) Schematic representation of all replication-deficient adenoviruses used in paper. B16-F10 cells were infected with adenoviruses Ad-Null, Ad-MEM40, Ad-mIFNβ or Ad-MEM40+Ad-mIFNβ(Ad COMBO) at MOI=10. MEM40 expression was detected by flow cytometry (B) and secretion of IFNβwas detected by ELISA (C).

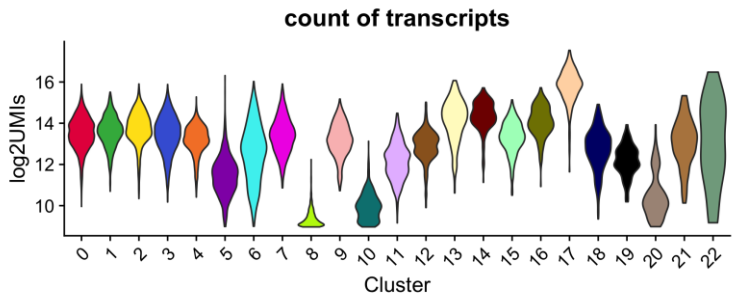


Supplementary Fig. 2. Flow cytometry analysis scheme of B16-F10 to detect populations of DCs in tumor.

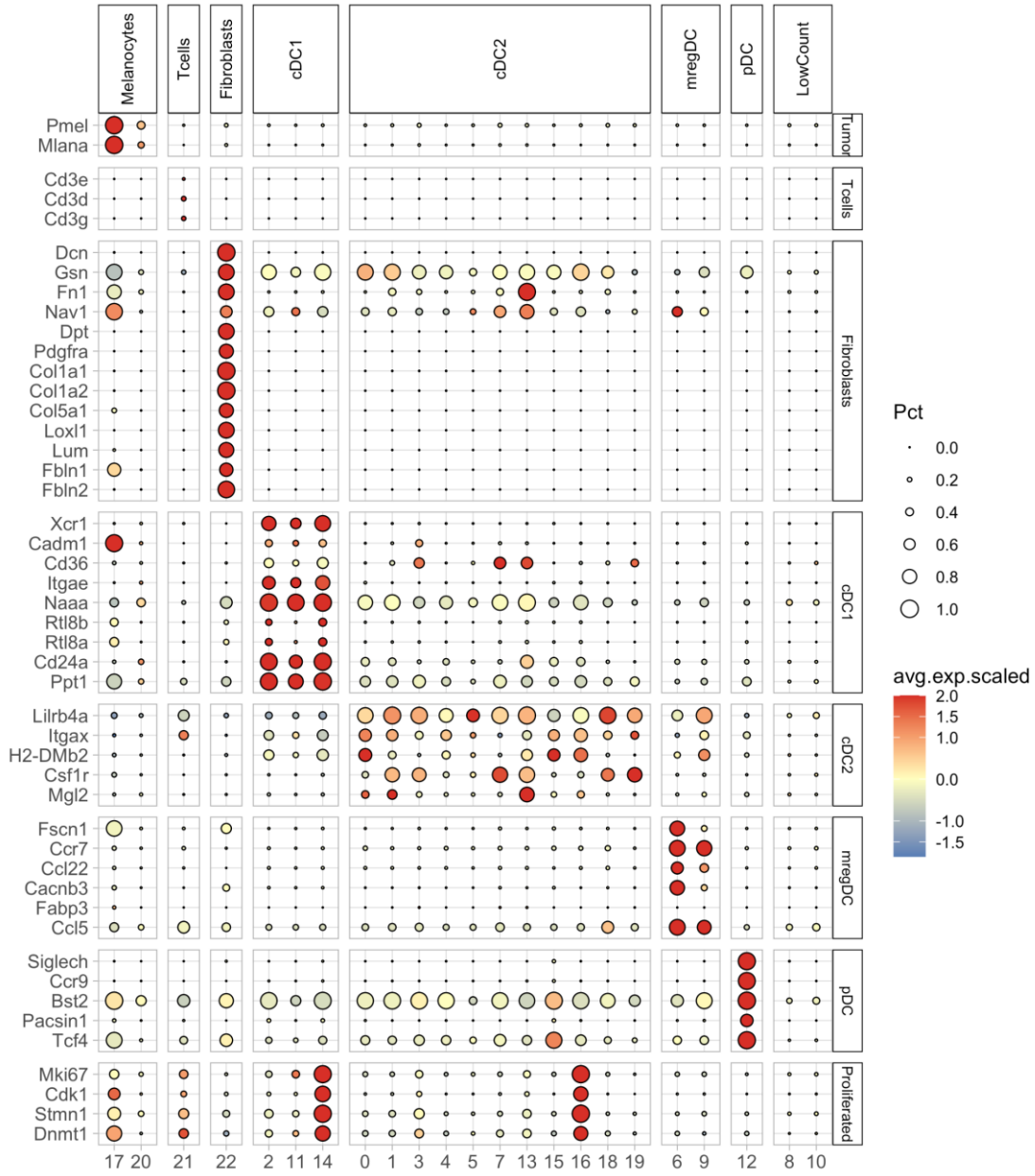


Supplementary Fig. 3. Tumor levels and activation of cDC2. Presence of CD11b^{+}cDC2 (A) and indicated activation markers (B) on cDC2 are indicated.

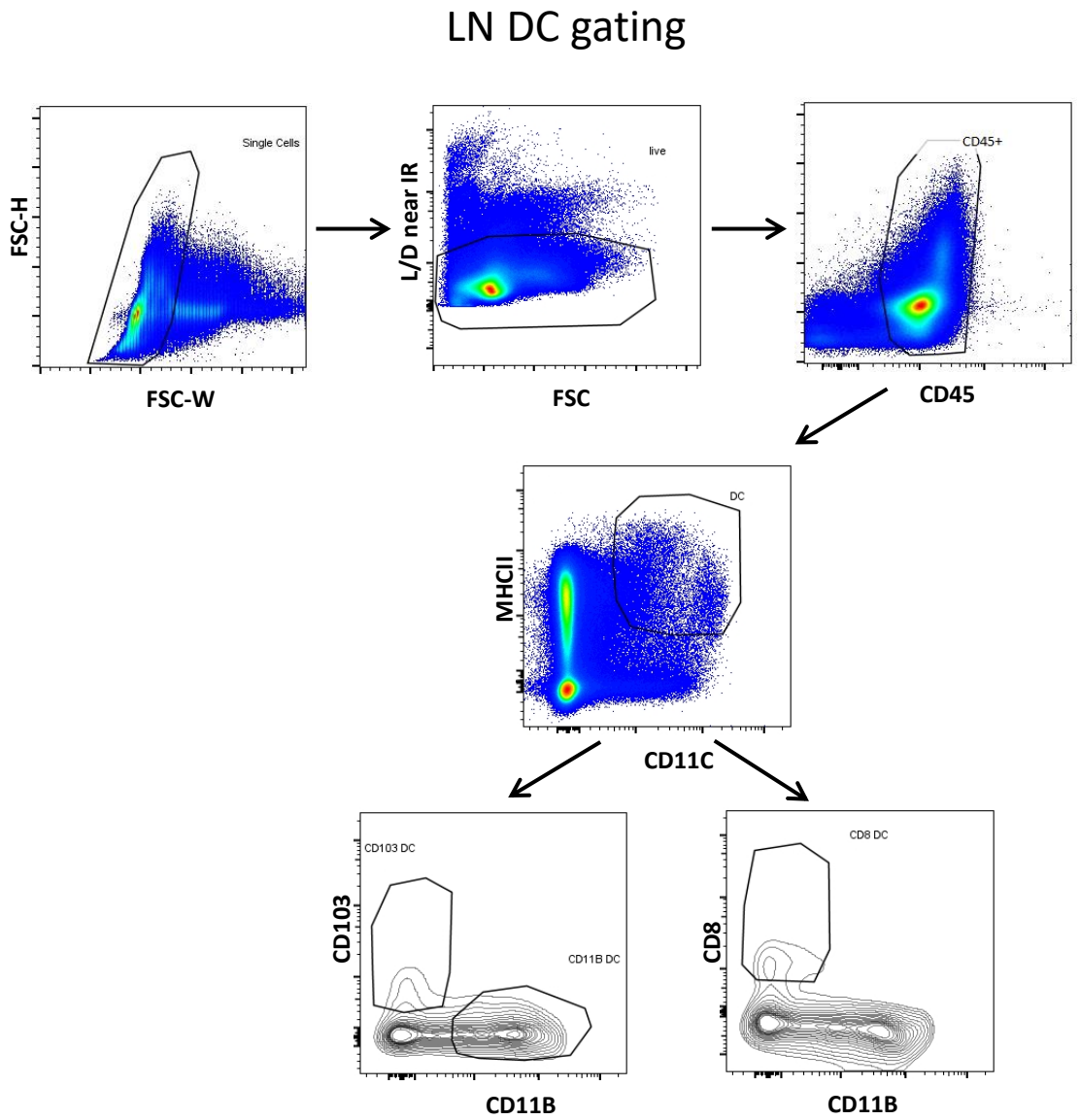
A



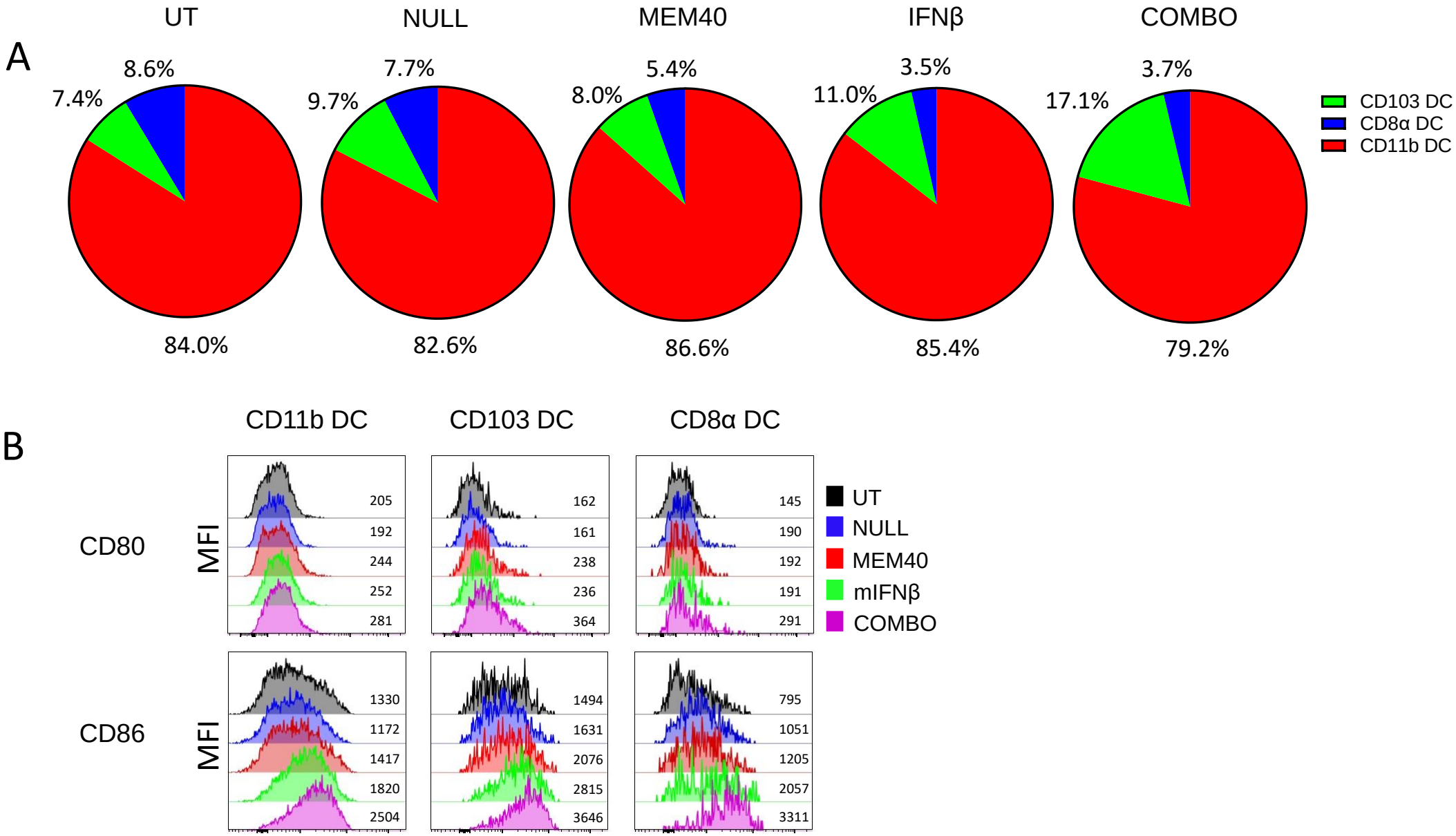
B



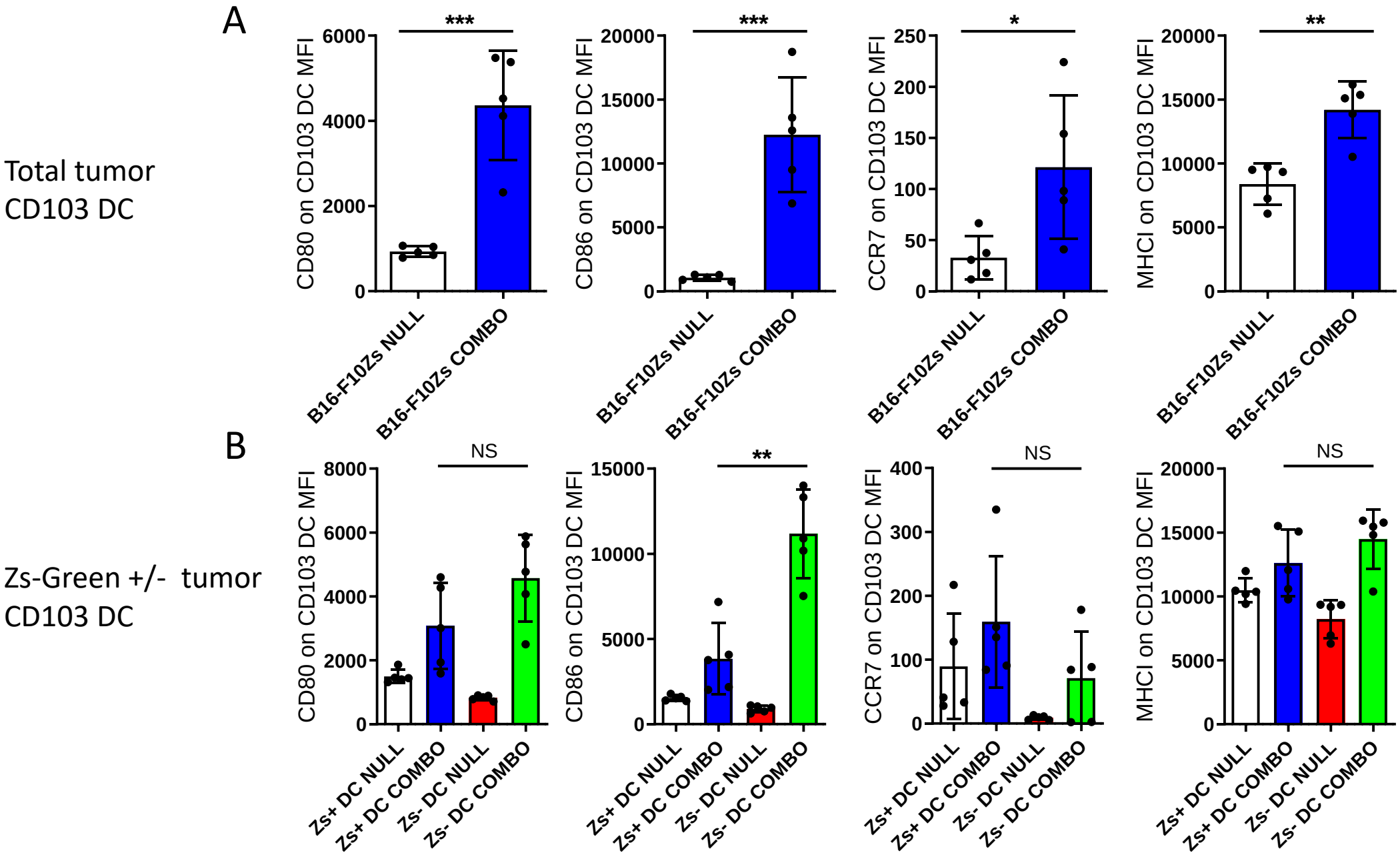
Supplementary Fig. 4. Single cell RNA sequencing of tumor DCs. (A) Transcript counts in indicated clusters. (B) Marker gene bubble plot showing gene-level average expression calculated for each cluster and then Z-score normalized.



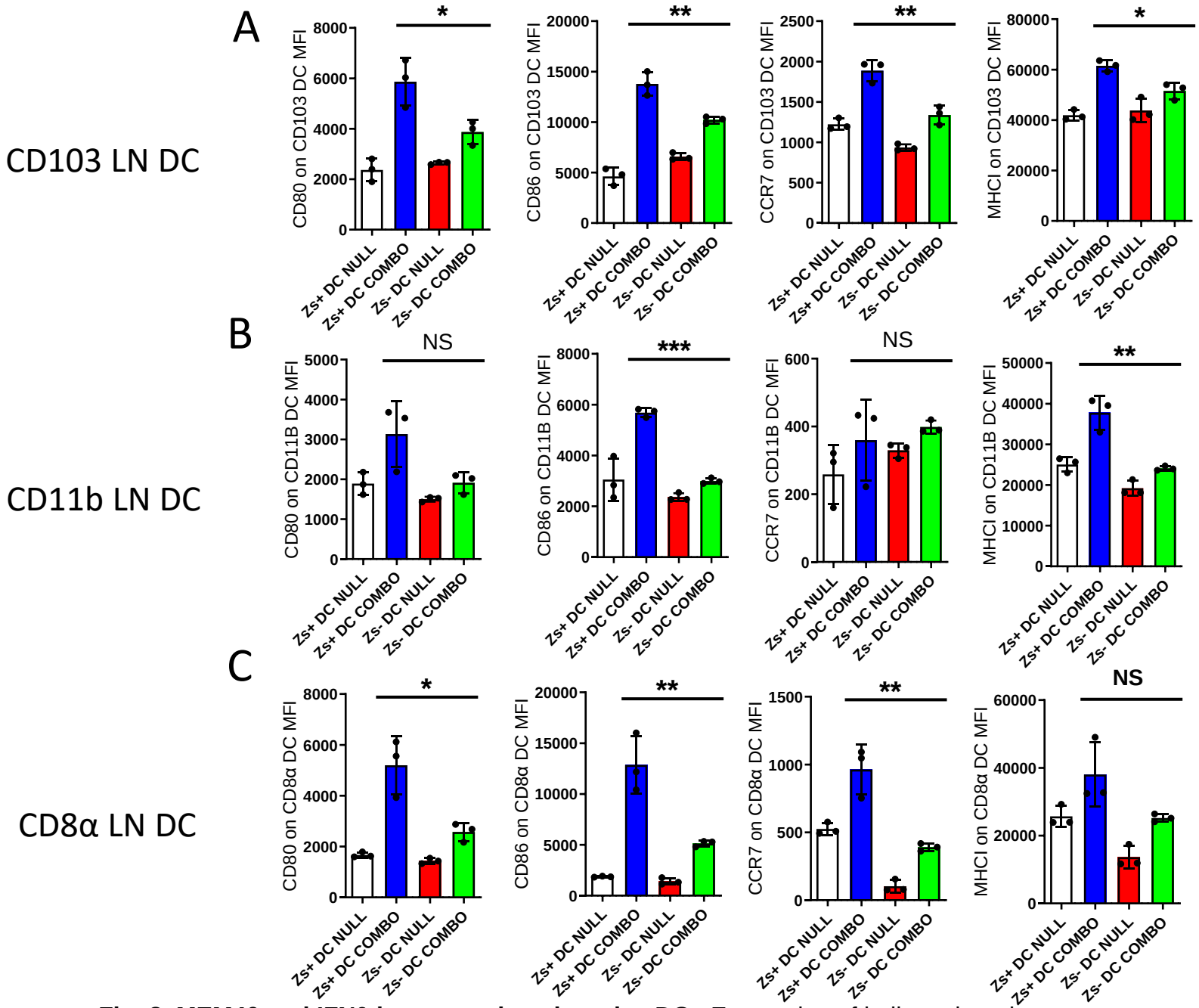
Supplementary Fig. 5. Flow cytometry analysis scheme of B16-F10 to detect populations of DCs in draining lymph nodes.



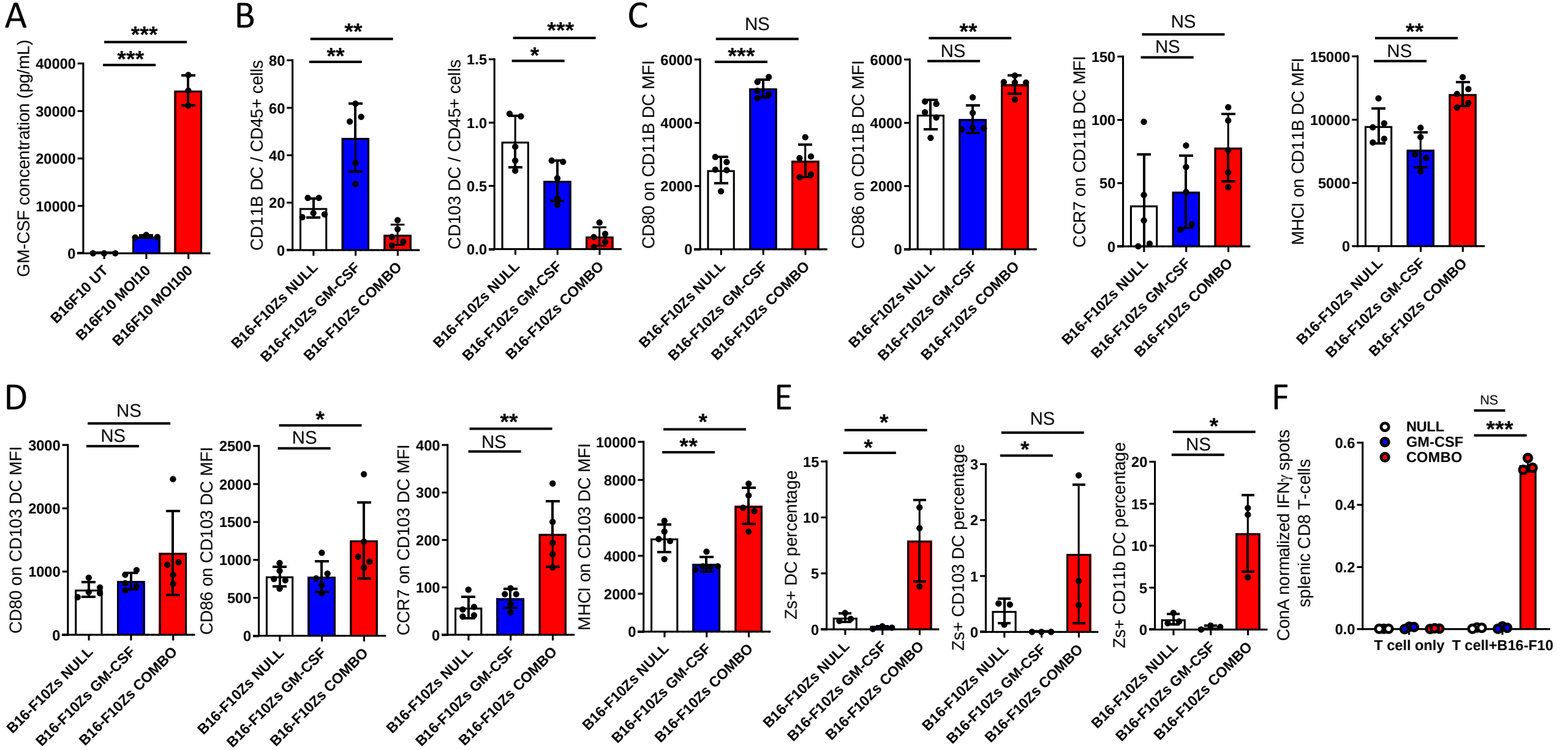
Supplementary Fig. 6. MEM40 and IFNβ impact on lymph nodes cDCs. (A) LNs of mice injected with indicated viruses were pooled and used to determine levels of indicated DC subsets. (B) Levels of CD80 and CD86 expression in CD11b^{+}cDC2, CD103^{+}cDC1and CD8α^{+}cDC1 along with MFI are indicated.



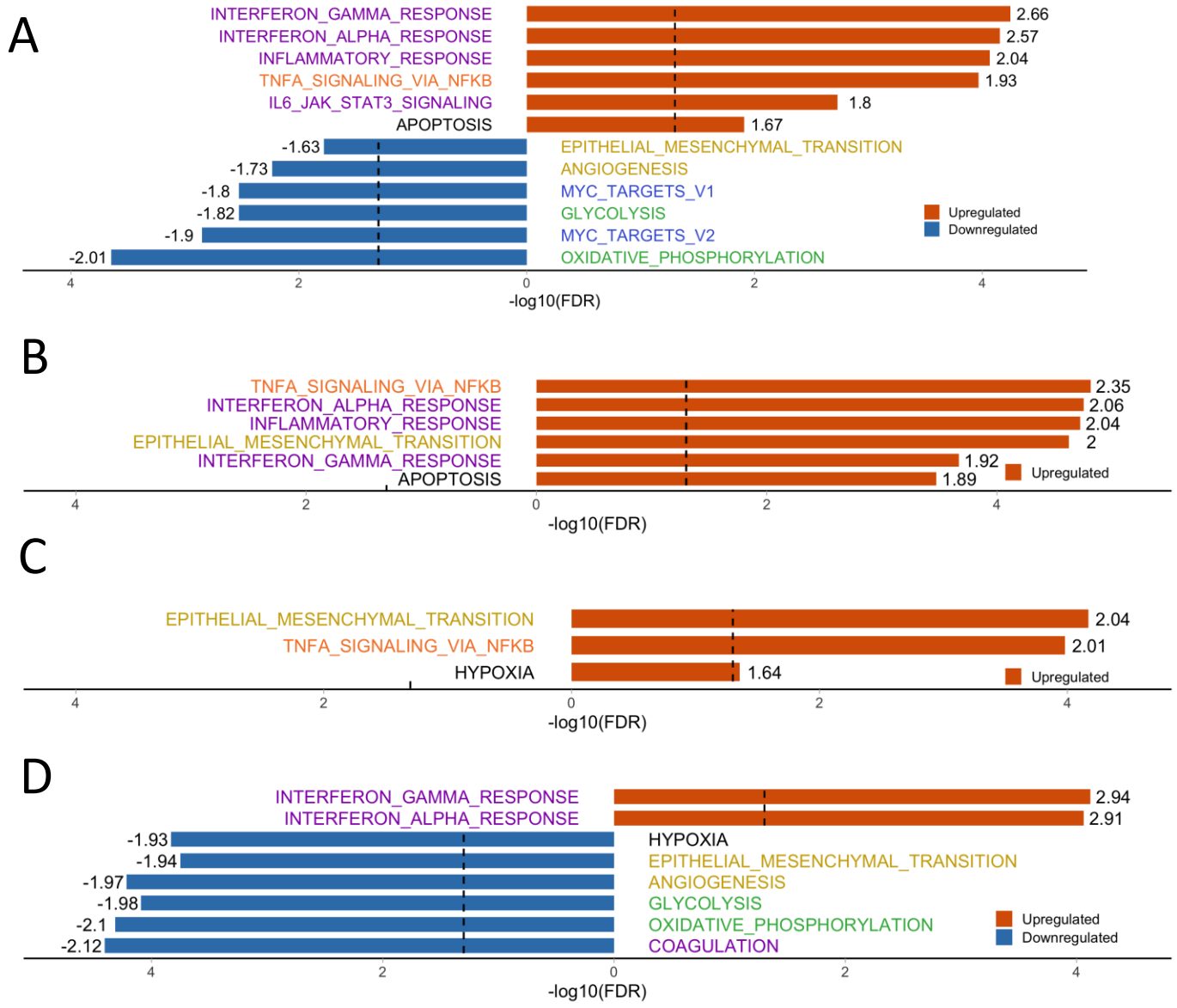
Supplementary Fig. 7. MEM40 and IFNβ impact on tumor cDC1 in Zs-Green expressing B16-F10. (A) Expression of indicated markers on tumor CD103^{+}cDC1 after Ad-Null and Ad COMBO virus treatments. (B) Expression of markers were separately assessed on Zs-Green^{+} and Zs-Green^{-}CD103 tumor DC. T-test was used to determine significance of differences and indicated by p-values *p<0.05, **p<0.01, ***p<0.001. NS: not significant.



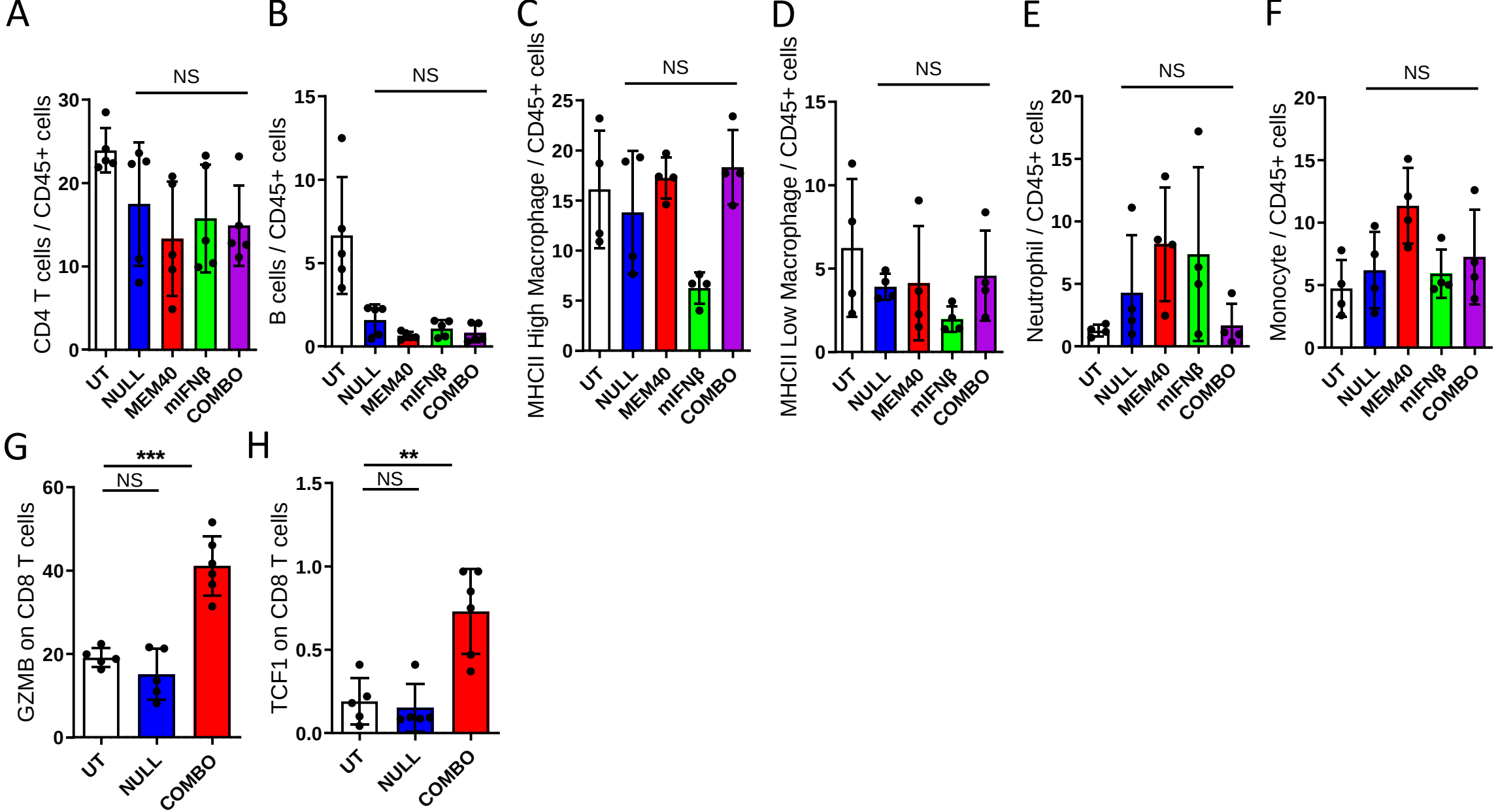
Supplementary Fig. 8. MEM40 and IFNβ impact on lymph node cDC. Expression of indicated markers were separately assessed on Zs-Green^{+} and Zs-Green^{-}in (A) CD103^{+}LN cDC1, (B) CD11b^{+}LN cDC2, and (C) CD8α^{+}LN cDC1. T-test was used to determine significance of differences and indicated by p-values *p<0.05, **p<0.01, ***p<0.001. NS: not significant.



Supplementary Fig. 9. MEM40 and IFNβ versus GM-CSF in tumor and lymph node cDC. (A) B16-F10 cells were infected with adenoviruses Ad-Null or Ad-GM-CSF at MOI=10 and 100. Secretion of GM-CSF was detected by ELISA. (B) Presence of CD11b$^+$cDC2 and CD103$^+$cDC1 in B16-F10-ZsGreen tumors 3 days after Ad-Null, Ad-GM-CSF and Ad-MEM40 + Ad-IFNβ (Ad-COMBO) virus treatments. (C) Expression of indicated markers on tumor CD11b$^+$cDC2. (D) Expression of indicated markers on tumor CD103$^+$cDC1. (E) Zs-Green$^+$ presence on total, CD103$^+$ and CD11b$^+$ dLN cDCs. (F) Comparison of T cell activation of Ad-Null, Ad-GM-CSF or Ad-COMBO by ELISPOT. T-test was used to determine significance of differences and indicated by p-values *p<0.05, **p<0.01, ***p<0.001. NS: not significant.

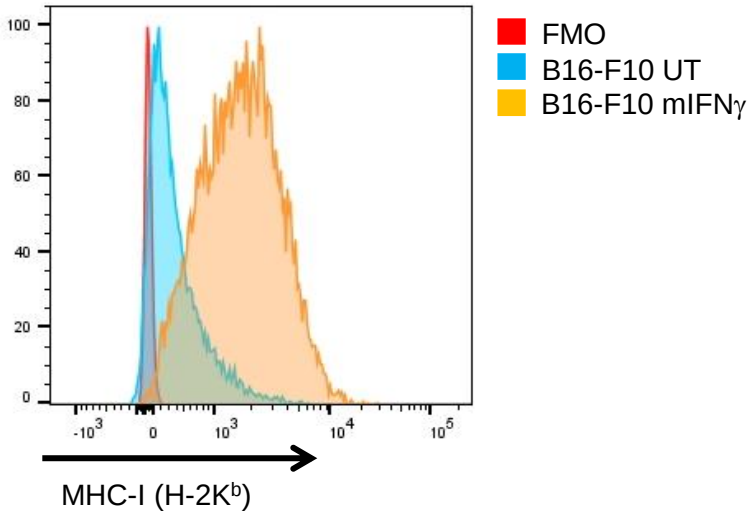


Supplementary Fig. 10. Pathway analysis in human DCs. RNA-seq was used to determine changes in gene expression in HLA-DR^{+}CD11c^{+} sorted DCs derived from monocytes of 3 healthy donors. Hallmark pathway analysis was used to determine differences in pathway activation in (A) IFNβ vs NULL , (B) MEM40 vs NULL, (C) COMBO vs IFNβ, (D) COMBO vs MEM40.



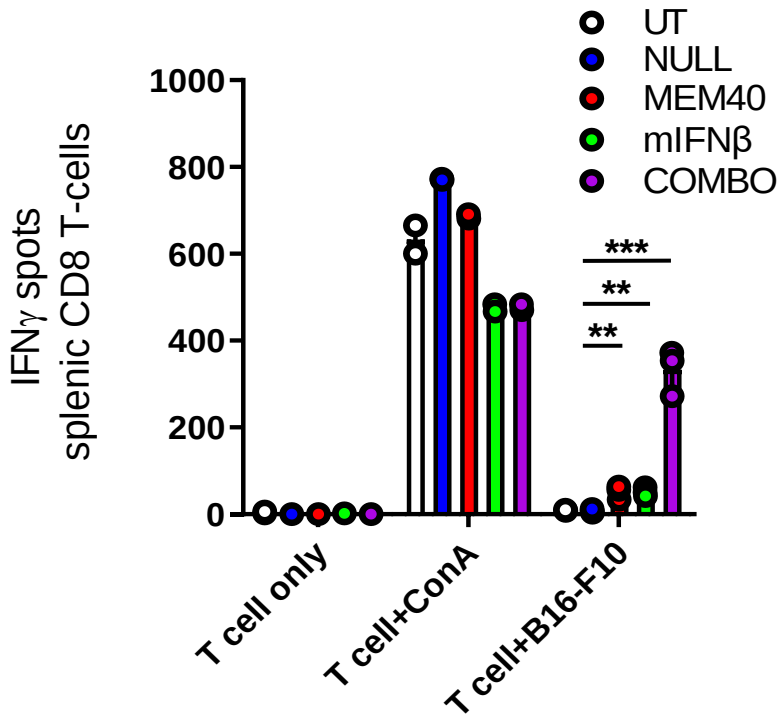
Supplementary Fig. 11. MEM40 and IFNβ impact on CD4⁺ T cells, B cells and myeloid cells. Results from experiment shown in Fig. 4 indicating levels of (A) CD4⁺T cells, (B) CD19⁺ B cells, (C) MHC-II^{high} macrophages (F4/80⁺Ly6C⁺Ly6G⁻MHCII^{high}), (D) MHC-II^{low} macrophages (F4/80⁺Ly6C⁺Ly6G⁻MHCII^{low}), (E) neutrophils (F4/80⁻CD11c⁻CD11b⁺Ly6C⁺Ly6G⁺) and (F) monocytes (F4/80⁻CD11c⁻CD11b⁺Ly6C⁺Ly6G⁻). Mice were injected with B16 tumors and treated with Ad-Null or Ad-MEM40 + Ad-IFNβ (COMBO). Tumors were collected 10 days after the second virus infection and analyzed by flow cytometry. The intracellular markers (G) GZMB and (H) TCF1 on tumor CD8 T cells are indicated. T-test was used to determine significance of differences and indicated by p-values *p<0.05, **p<0.01, ***p<0.001 NS: not significant.

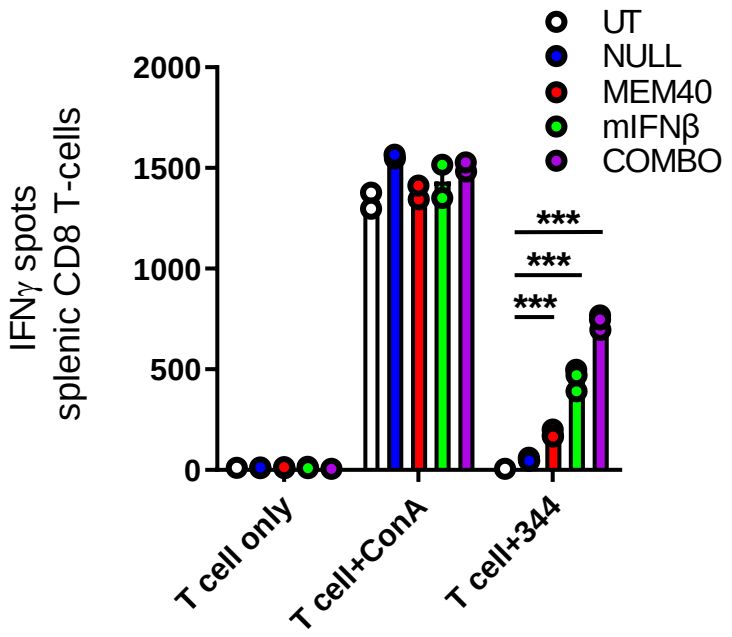
A



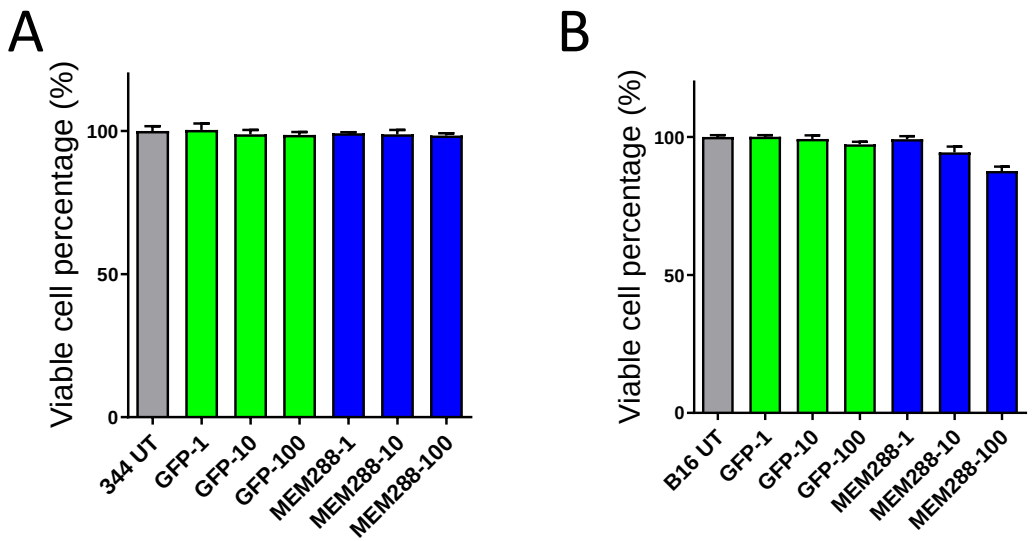
B

Non-normalized:

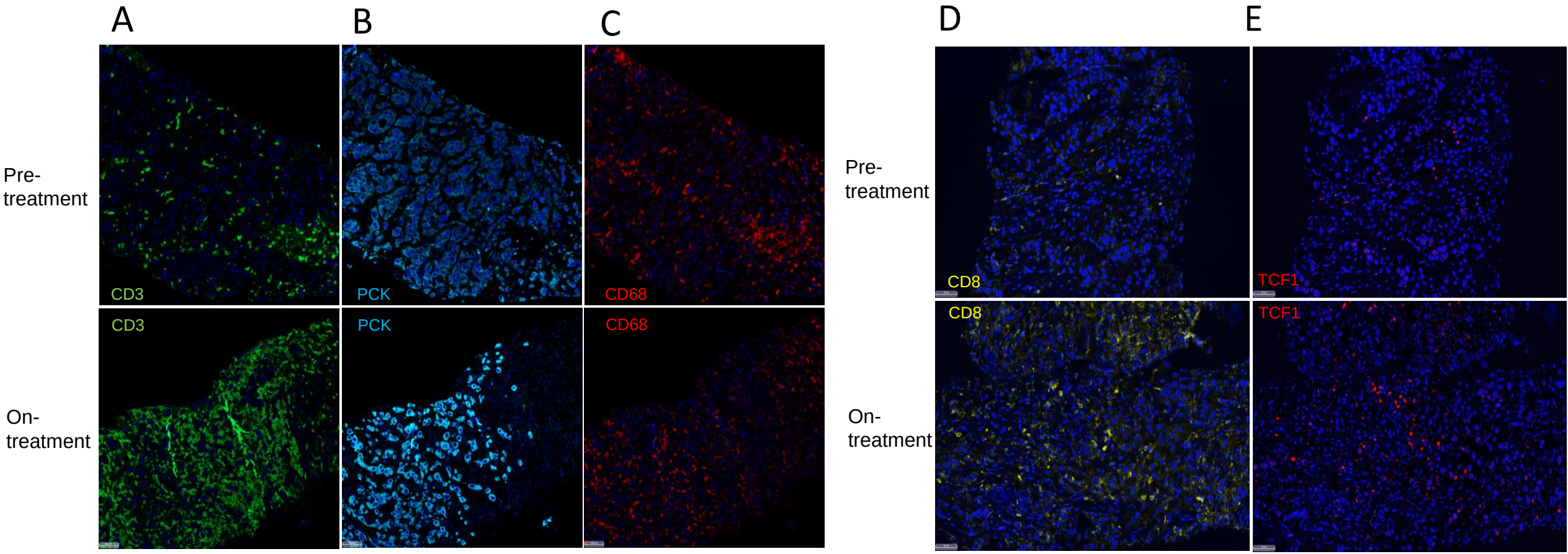




Supplementary Fig. 12. ELISPOT assay. (A) ELISPOT studies used IFNγ treatment of tumor cells to increase MHC-I expression. Shown here is the impact of 6 hr IFNγ treatment on MHC-I expression in B16-F10. (B) Non-normalized ELISPOT results showing spot numbers in data from Fig. 4E, I.



Supplementary Fig. 13. MEM-288 oncolytic effects. (A) 344 (A) and (B) B16-F10 mouse tumor cell lines were infected with indicated oncolytic viruses Ad-GFP (GFP) or MEM-288 at different indicated MOIs (1, 10, 100) for 2 days. Cell viability was determined by trypan blue staining assay.



Supplementary Fig. 14. MEM-288 modulation of the TME and systemic T cell immunity in NSCLC. Single-color images of pre- and on-treatment biopsies with indicated markers and DAPI. A, B and C top-panel correspond to the **Figure 7A** image. A, B and C bottom-panel correspond to the **Figure 7B** image. D and E top-panel correspond to the **Figure 7C** image. D and E bottom-panel correspond to the **Figure 7D** image.