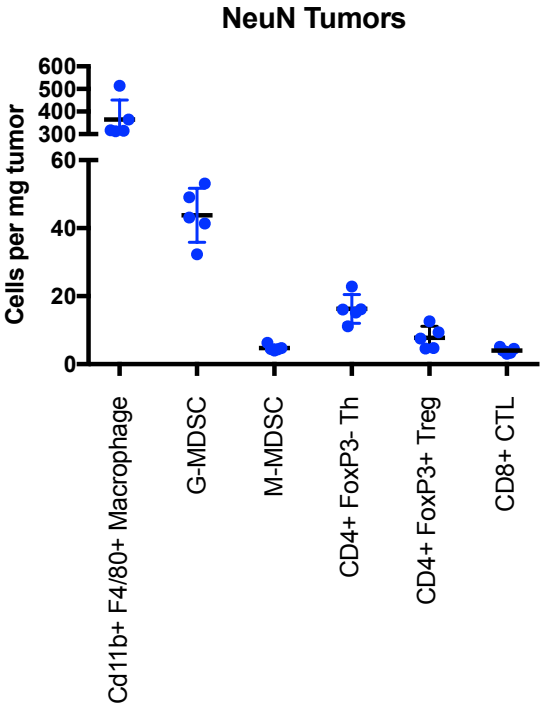
SUPPLEMENTARY MATERIAL

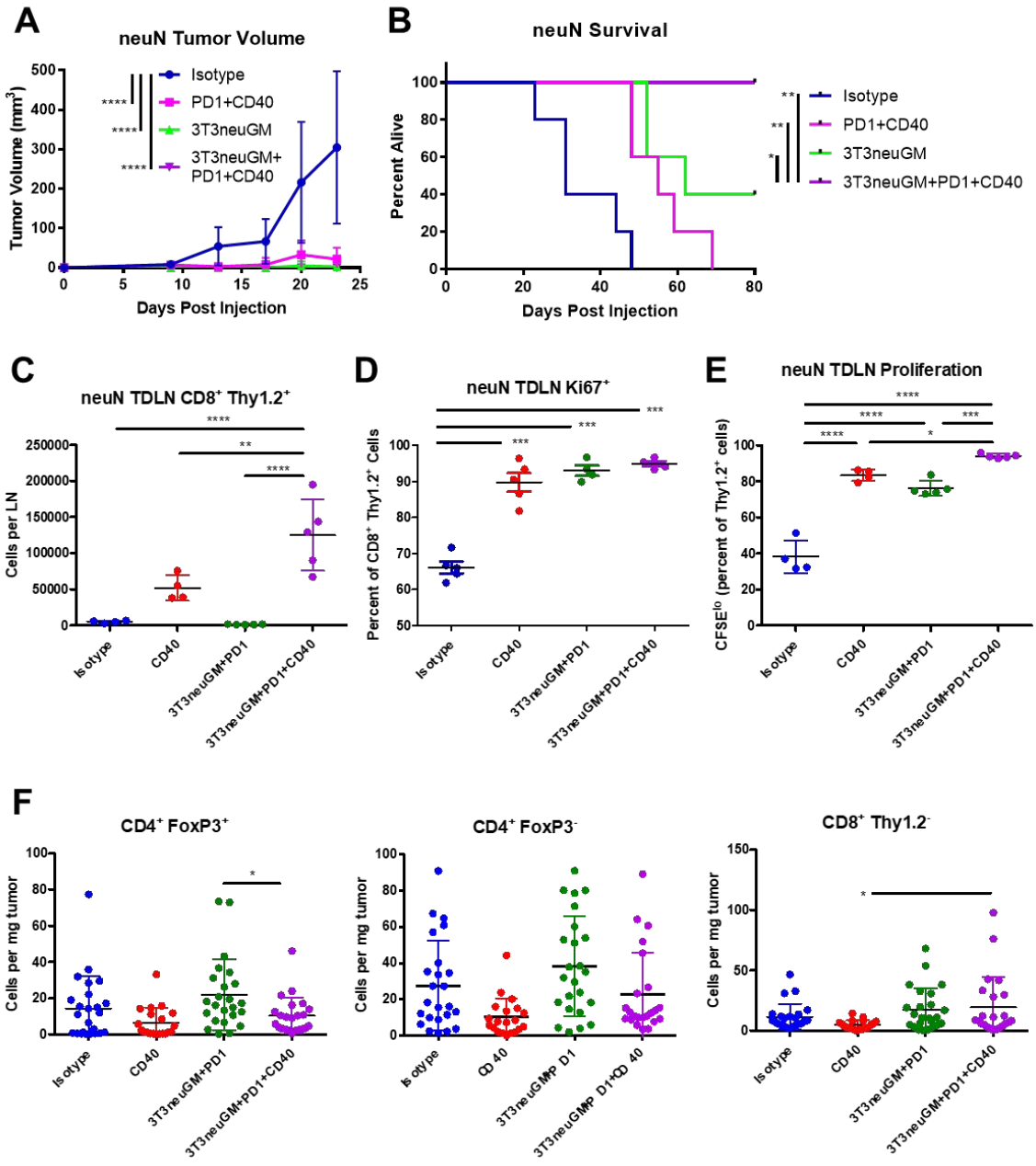
Supplementary Table S1. Fluorescent conjugated antibodies for flow cytometry

Target	Clone	Fluorophore	Supplier
Arg-1	HepG2	PE	R&D Systems
CD11b	M1/70	PE Dazzle	BioLegend
CD11b	M1/70	AF700	BD Pharmingen
CD11c	HL3	APC	BD Pharmingen
CD169	3D6.112	PE	BioLegend
CD19	1D3	BV510	BD Pharmingen
CD3	SK7	V450	BD Pharmingen
CD3	17A2	BV510	BioLegend
CD3	145-2C11	PE CF594	BD Pharmingen
CD4	RM4-5	Pac Blue, PerCPCy5.5	BioLegend, eBioscience
CD4	RM4-5	PerCPCy5.5	eBioscience
CD44	IM7	PE	BD Pharmingen
CD45	30-F11	AF700	BD Pharmingen
CD62L	MEL-14	APC	BioLegend
CD8	53-6.7	APC, APC Fire, PECy7	BD Pharmingen, BioLegend
CD8	RA3-6B2	Pac Blue	BD Pharmingen
CD80	16-10A1	PE Dazzle	BioLegend
CD86	GL-1	PE	BioLegend
CTLA4	UC10-4F10-11	PE	BD Pharmingen
F480	BM8	PECy7	eBioscience
FoxP3	FJK-16s	FITC	eBioscience
Gal3	M3/38	AF647	BioLegend
Granzyme B	NGZB	PECy7	eBioscience
INFg	XMG1.2	PerCPCy5.5	eBioscience
Ly6C	HK1.4	PerCPCy5.5, eFluor450	BioLegend, eBioscience
Ly6G	AL-21	BV421	BD Pharmingen
Ly6G	1A8	FITC	BD Pharmingen
MHCII	2G9	FITC	BD Pharmingen
PD-1	29F.1A12	PECy7	BioLegend
PD-L1	MIH5	PE	eBioscience
Thy1.2	53-2.1	APC	BD Pharmingen
Thy1.2	30-H12	AF700	BioLegend
TIM3	8B.2C12	eFluor450	eBioscience
TNFa	MP6-XT22	PE Dazzle	BioLegend

Supplementary Figures

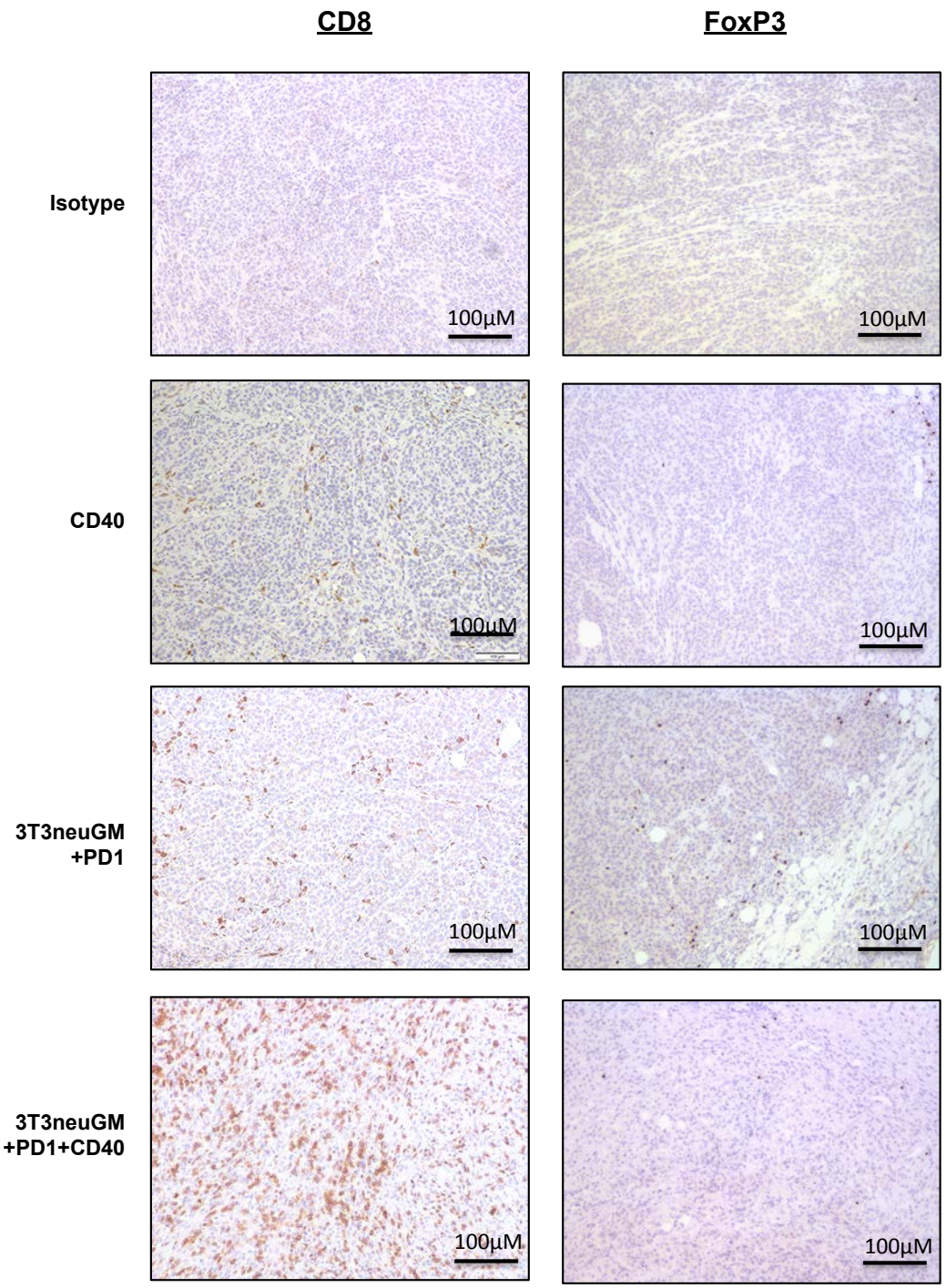


Supplementary Figure S1. *Neu*-N mammary tumor immune infiltrate at baseline. *Neu*-N mice were inoculated with 1×10^6 NT2.5 cells on Day 0. Tumors were subsequently harvested on Day 14 and various immune cell populations were quantified by flow cytometry. Cells were defined as follows: macrophages ($CD11b^+$ $F4/80^+$), G-MDSCs ($CD11b^+$ $F4/80^-$ $Ly6G^+$), M-MDSCs ($CD11b^+$ $F4/80^-$ $Ly6C^+$), Th cells ($CD4^+$ $FoxP3^-$), Tregs ($CD4^+$ $FoxP3^-$), and CTLs ($CD8^+$). All populations were represented as the number of cells per mg of tumor.

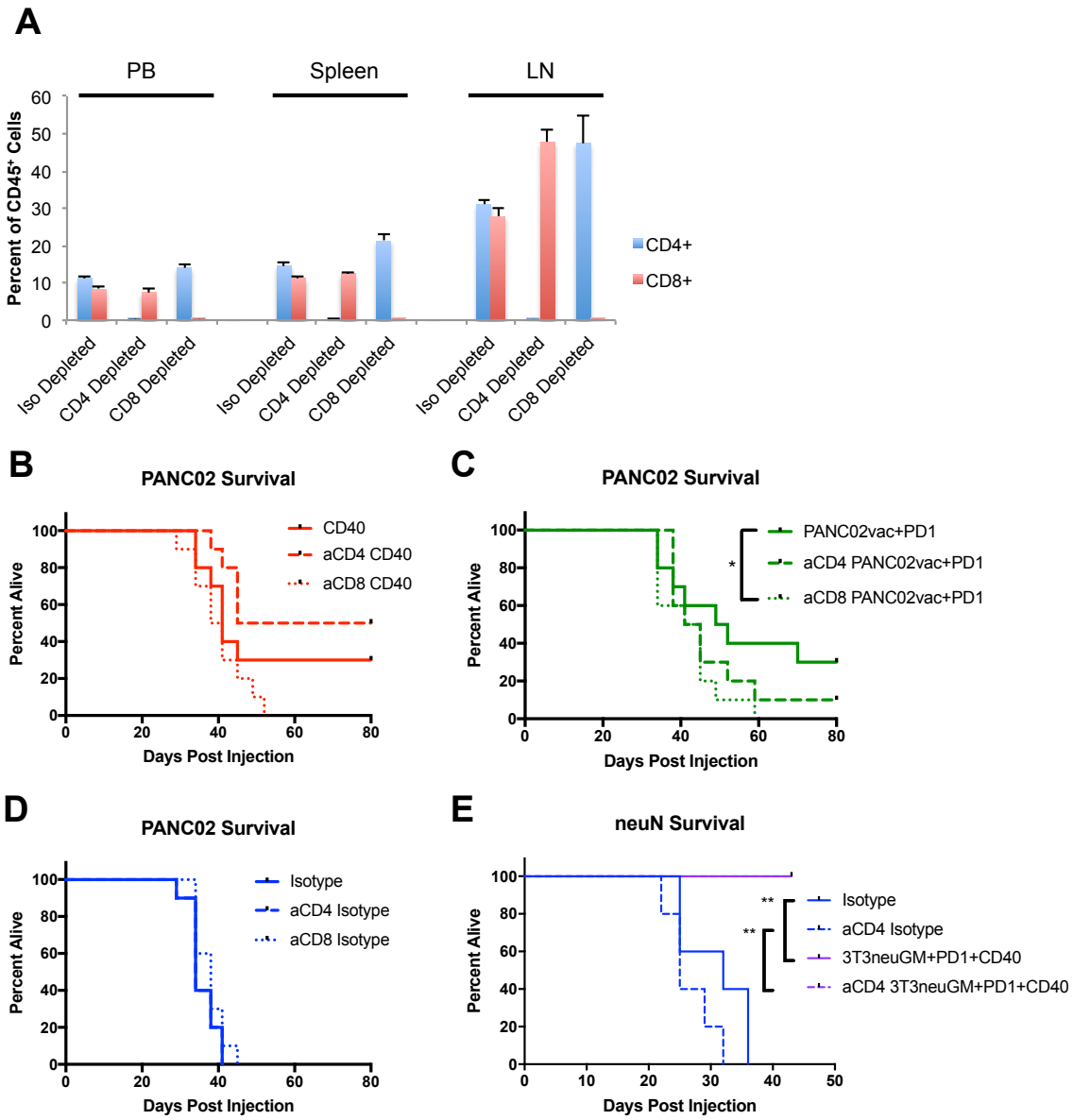


Supplementary Figure S2. Response of murine mammary tumors to a CD40 agonist alone or in combination with PD-1 antagonist and/or vaccine.

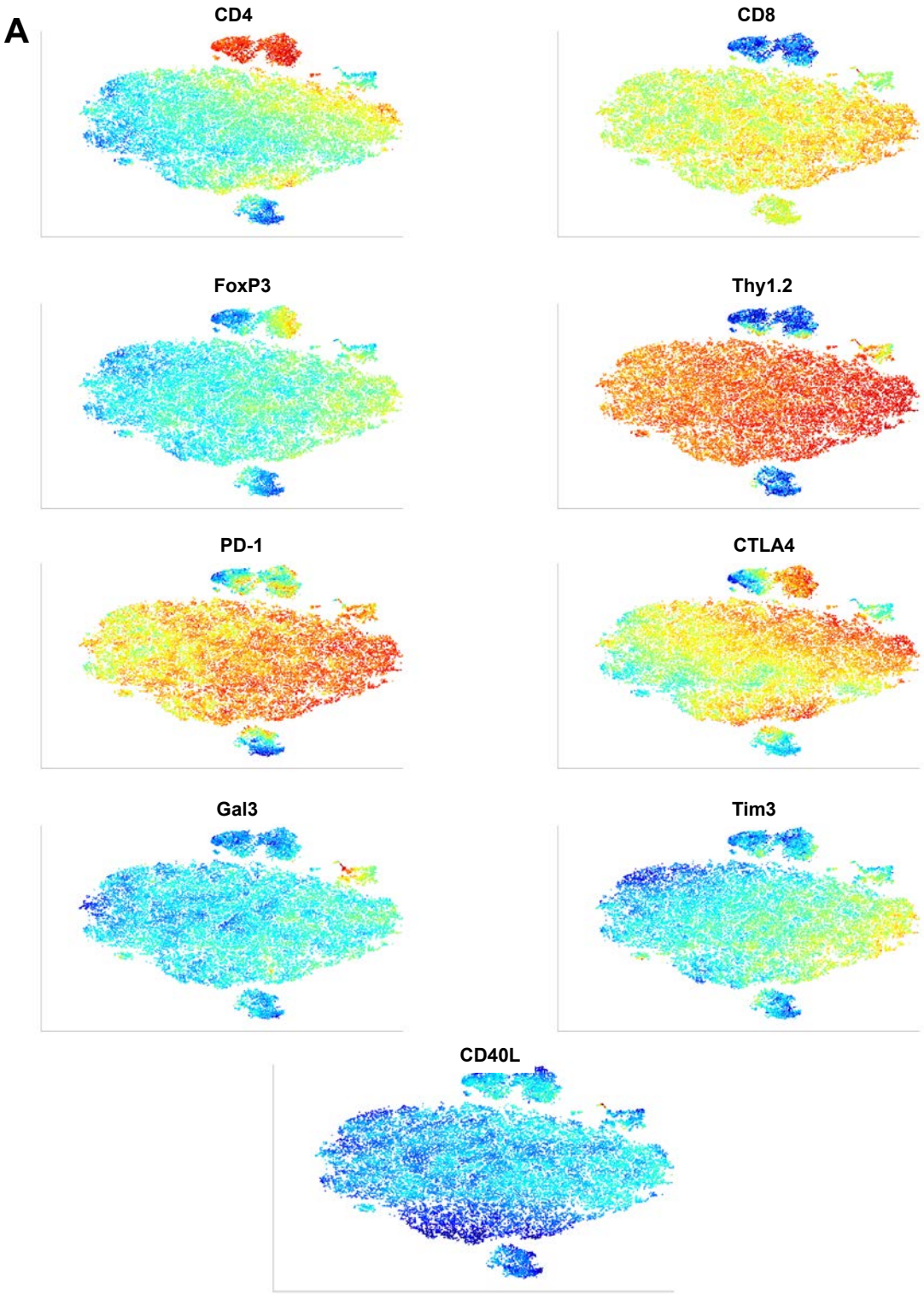
Supplementary Figure S2. Response of murine mammary tumors to a CD40 agonist alone or in combination with PD-1 antagonist and/or vaccine. A) Tumor volume and B) overall survival of *neu*-N mice treated with 3T3neuGM alone or anti-PD-1 + CD40 agonist combination (n = 5 mice per group). All mice received 2×10^6 *neu*-specific T cells on Day 1. C-E) *Neu*-N mice received 6×10^6 CFSE labeled TCR transgenic T cells ($Thy1.2^+$) and were treated with 3T3neuGM, anti-PD-1 mAb and/or CD40 mAb. C) Absolute number of $CD8^+$ $Thy1.2^+$ T cells per TDLN. D) T cell proliferation rate as measured by percentage of $CD8^+$ $Thy1.2^+$ cells which are $Ki67^+$ in the TDLN. E) T cell proliferation rate as measured by percentage of $CD8^+$ $Thy1.2^+$ cells that are $CFSE^{lo}$ in the tdLN. F) Tumor infiltration of $CD4^+$ $FoxP3^+$, $CD4^+$ $FoxP3^-$ and $CD8^+$ $Thy1.2^-$ cells in *neu*-N mice (n = 20-24 mice per group). All cell populations were represented as the number of cells per mg of tumor. *, $p<0.05$, **, $p<0.01$.



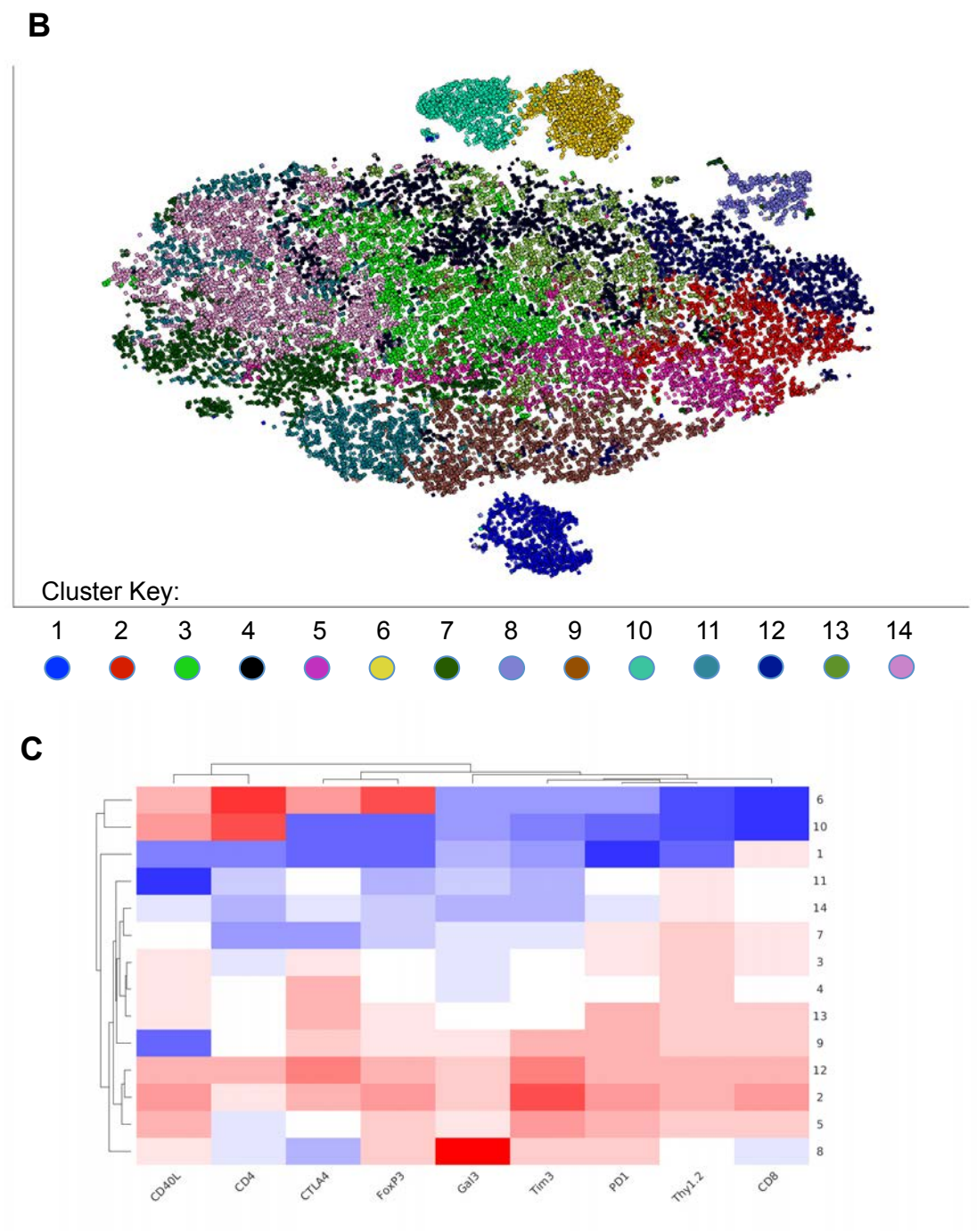
Supplementary Figure S3. 3T3neuGM + anti-PD-1 + CD40 agonist triple therapy results in increased CD8$^+$ T lymphocyte infiltration. *Neu*-N mice were inoculated with 1×10^6 NT2.5 cells on Day -8 and treated with 3T3neuGM vaccine on Day 0. All mice received 6×10^6 *neu*-specific T cells on Day 1, followed by anti-PD-1 and/or CD40 agonist antibody, or rat IgG isotype control, on Day 2. Tumors were harvested on Day 6 and characterized via immunohistochemistry for CD8 and FoxP3 expression. Slides were imaged at 10X magnification. Black scale bar represents 100 μM.



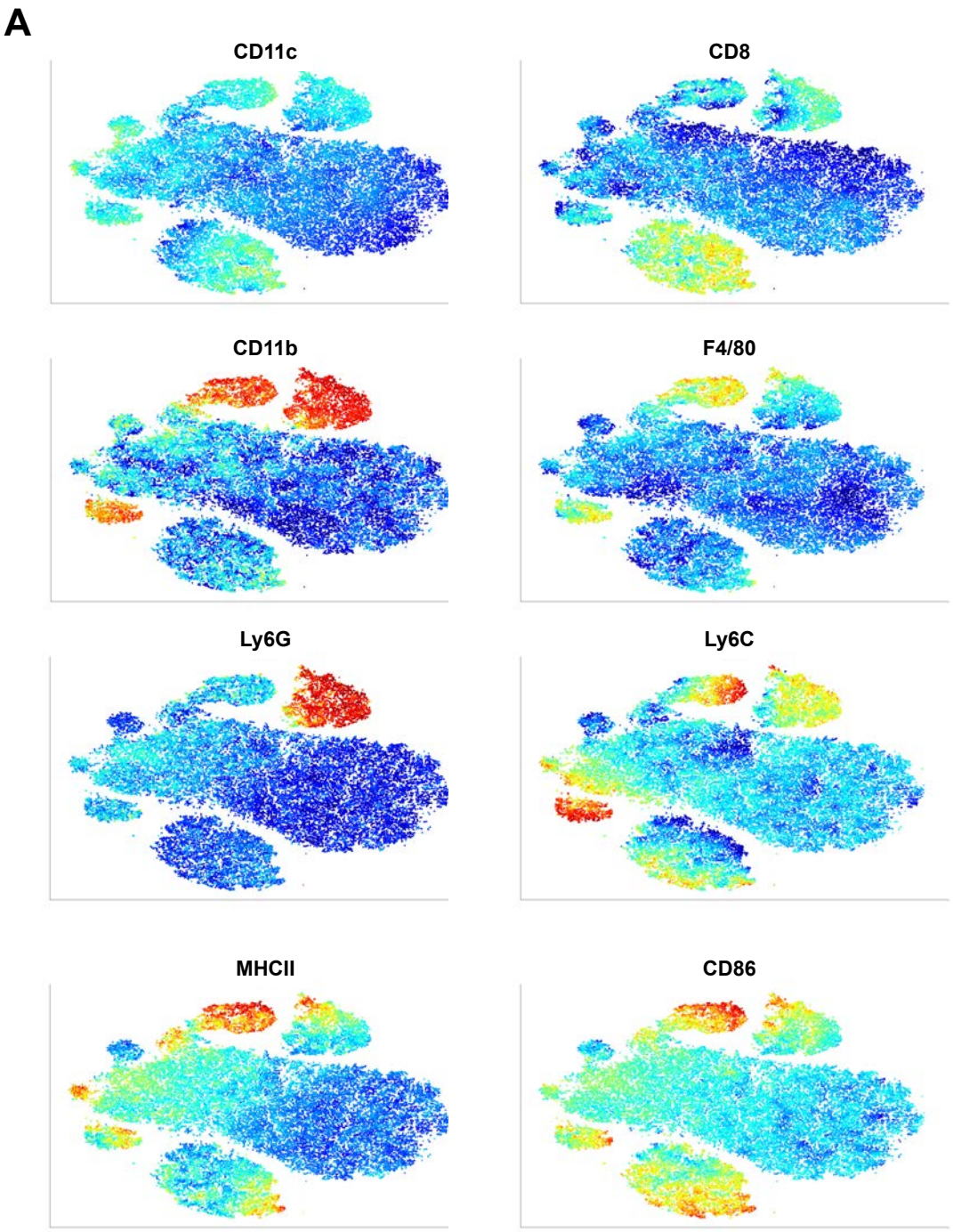
Supplementary Figure S4. Depletion of CD8$^+$ T cells results in a significant decrease in anti-tumor efficacy with PANC02vac + anti-PD-1 therapy. A) C57BL/6 mice were treated with depletion mAbs against CD8α, CD4, or isotype control mAb on Day -2. Spleens, peripheral blood (PB), and axillary lymph nodes (LN) were harvested on Day 0 (n = 3 per group) to confirm T cell depletion. B) Overall survival of PANC02 sc tumor injected mice treated with CD40 mAb, C) PANC02vac+PD1 or D) isotype control mAb and depletion mAbs against CD8α, CD4, or isotype control. E) Overall survival of *neu*-N mice treated with depleting mAb against CD4 or isotype control mAb. All mice received 2×10^6 *neu*-specific T cells on Day 1. *, $p<0.05$, **, $p<0.01$.



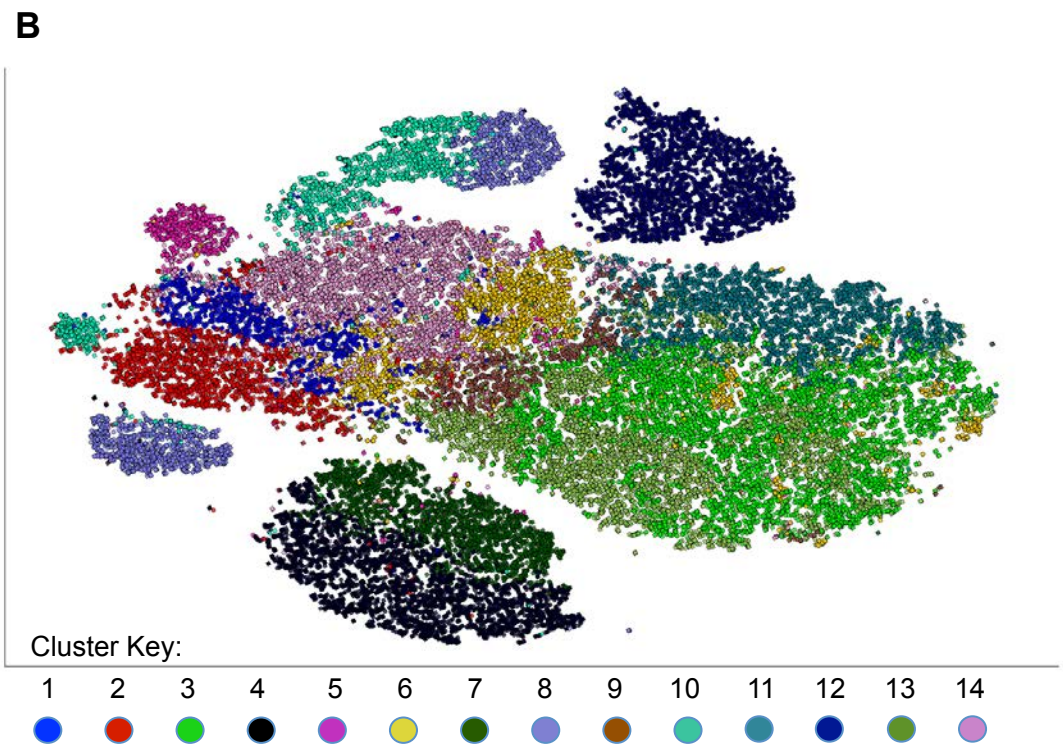
Supplementary Figure S5. Lymphoid panel t-SNE heat maps.



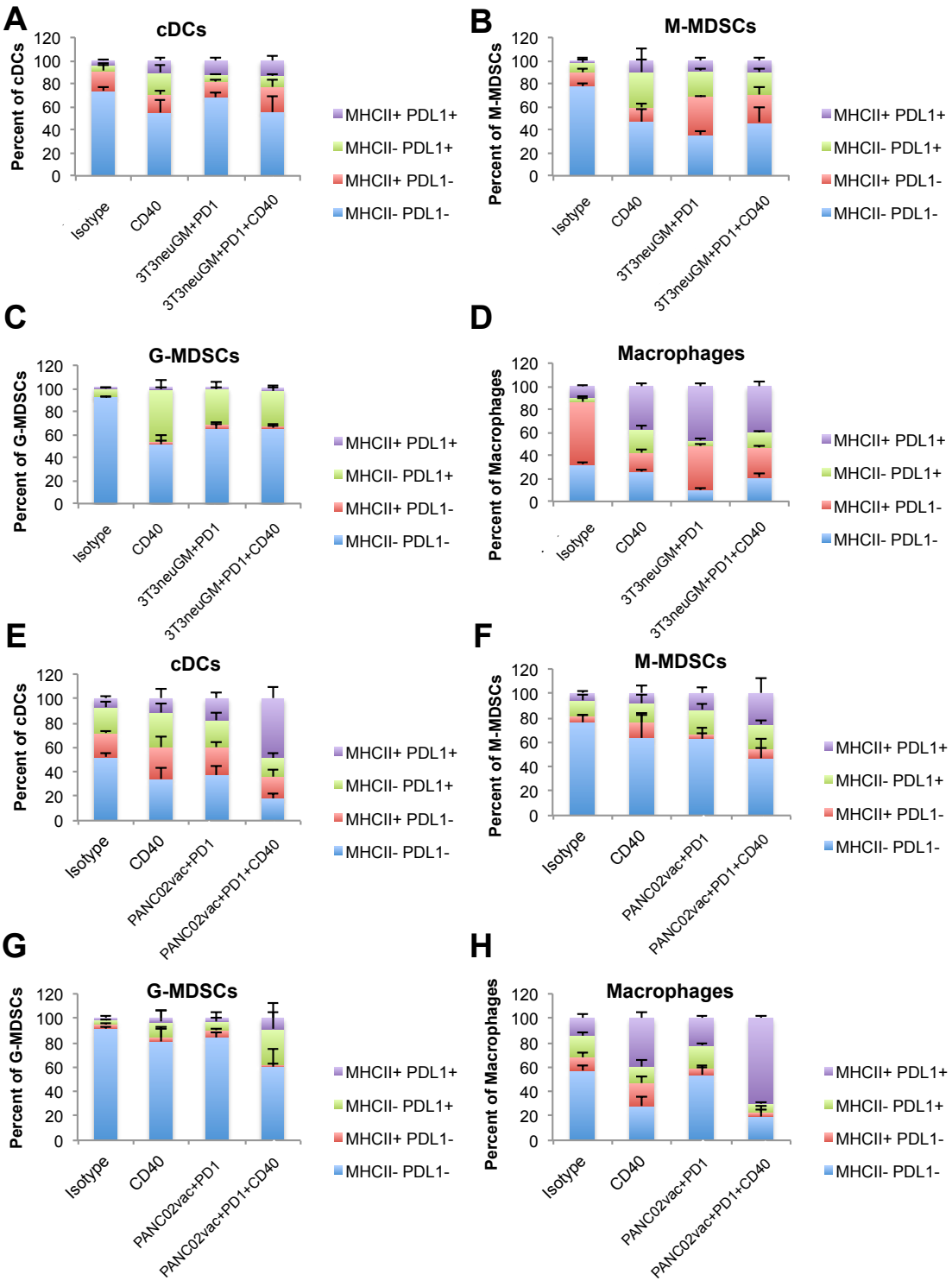
Supplementary Figure S5 (continued). Lymphoid panel t-SNE heat maps. Tumors were harvested from treated *neu*-N mice and stained with a panel of lymphoid markers. A fixed number of Live $CD4^+$ or $CD8^+$ T cells (3000 events per sample, if available) were clustered via a network-graph based algorithm and visualized by t-SNE. A) Expression of T cell markers overlaid on t-SNE plot to visualize co-localization of T cell markers within clusters. B) Clustering solution overlaid on t-SNE map with all treatment cohorts combined (n=4 mice per group). C) Heat map showing expression of each T cell marker relative to Cluster number. t-SNE = t-distributed stochastic neighbor embedding.



Supplementary Figure S6. Myeloid panel t-SNE heat maps.



Supplementary Figure S6 (continued). Myeloid panel t-SNE heat maps. Tumors were harvested from treated *neu*-N mice and stained with a panel of myeloid markers. A fixed number of Live $CD3^-$ $CD19^-$ cells (3000 events per sample, if available) were clustered via a network-graph based algorithm and visualized by t-SNE. A) Expression of myeloid cell markers overlaid on t-SNE plot to visualize co-localization of myeloid markers within clusters. B) Clustering solution overlaid on t-SNE map with all treatment cohorts combined (n=4 mice per group). C) Heat map showing expression of each myeloid marker relative to Cluster number. t-SNE = t-distributed stochastic neighbor embedding.



Supplementary Figure S7. 3T3neuGM + anti-PD-1 + CD40 agonist therapy results in APC maturation in the TME. MHCII and PD-L1 expression of A) $CD11c^+$ cDCs, B) $CD11b^+$ $F4/80^-$ $Ly6C^+$ M-MDSCS, C) $CD11b^+$ $F4/80^-$ $Ly6G^+$ G-MDSCs, and D) $CD11b^+$

F4/80^{+} macrophages in tumors of *neu*-N mice, represented as percent of parent population. MHCII and PD-L1 expression of E) CD11c^{+} cDCs, F) CD11b^{+} F4/80^{-} Ly6C^{+} M-MDSCS, G) CD11b^{+} F4/80^{-} Ly6G^{+} G-MDSCs, and H) CD11b^{+} F4/80^{+} macrophages in the livers of PANC02 metastatic tumor-bearing mice, represented as percent of parent population. APC=antigen presenting cells; TME=tumor microenvironment.