Supplemental Figure Legends

Supplemental Figure 1. p53 -/- syngeneic TNBC GEM recapitulate human TNBC subtypes and respond to low dose immunostimulatory chemotherapy treatment. **a,** Treatment response using standard of care drugs on T11 mammary tumors. **b,** Cibersort analysis of murine TNBC models. **c**, Single agent CTX treatment led to a reduced tumor burden in Balb/c mice as compared to NSG mice. Data are shown as mean +/-S.E.M, n = 10 per group except NSG PBS, n = 5. Multiple unpaired t tests were used to determine significance. **d,** Co-culture of GFP+ T12 tumor cells and GFP targeting T cells with or without Phosphoramide Mustard (PM). Each treatment was performed in triplicates and repeated twice. **e,** Flow cytometry analysis of CD8+ and CD4+ T cells before and after low-dose CTX treatment, n = 6-7 mice per group. **f**, Antibody depletion of CD4+ T Cells leads to a significant decrease in response to CTX and increased tumor volumes while differences in response due to depletion of CD8+ T Cells neared statistical significance at day 17, p = 0.06 n = 5-7 mice per group. Multiple unpaired t tests were used to determine significance. **g,** Quantification of TAMs upon antibody depletion of CD4+ T cells after treatment and quantification of CD8+ T cells after CD4+ T cell depletion after treatment by single agent CTX**. h,** Quantification of F480+CSF1R+ TAMs after combination treatment with CTX and Pexidartinib using flow cytometry, n= 5, significance was determined by unpaired t-tests. **i,** Quantification of CD8+ T memory cell subsets before and after treatment, n = 4-5 mice per group. Unpaired t-test was used to determine significance. **j,** Reduced tumor volume and improved survival in T12 mammary tumor bearing mice after treatment with CTX and 3.3X lower PXB (75 ppm). Number in parentheses show the specific n values of biologically independent mice per treatment group **k,** Combination of low-dose CTX with SNDX-ms6352 leads to a reduced primary tumor burden of T12 and 2151R p53-/- GEMMs following 4 weekly treatments (arrows). **l,** Immunohistochemistry for F4/80+ TAMs following 1 dose of IgG or SNDX-ms6352 treatment.

Supplemental Figure 2. Combination therapy leads to an expansion of CD8+/CD4+ T cells and B cells in responsive T12 tumors. a, Imaging mass cytometry analysis of the tumor immune microenvironment in T11 tumors before and after treatment. Representative images overlaid with 7 markers (F480, Ly6C, Ly6G, CD11b, CD4, CD8a, B220) for each treatment group. **b**, Neighborhood analysis of T11 tumors in which the color of the squares indicates significant pairwise interactions or avoidance between PhenoGraph defined cellular metaclusters. Highlighted interactions include CD8+/CD4+ T cells (clusters 1-7), B220+ B cells(cluster-15) and CD11C+ CD86+ Dendritic cells (cluster 16). Three Regions of Interest (ROI) were ablated per tumor section. n= 3-5 independent biological replicates per treatment group. **c**, Representative IMC images of T12 and T11 tumors after combination therapy highlighting the spatial localization of CD8+/CD4+ T cells and B cells with respect to proliferating KI67+ cells and F480+ TAMs. **d,** Flow cytometry quantification of F480+ CSF1R+ T12 and T11 tumor associated macrophages after combination therapy, n=3 independent biological replicates per group. **e,** Gene expression heatmap for CD45+ immune cells before and after treatment for all treatment groups. **f** Feature plots showing the expression levels of murine immune cell marker genes-*Cd3e, Cd4, Cd8a, Cd19, Cd68, S100a8, Itgax,* in all 4 treatment groups. **g,** GOBP analysis of *S100a8*+ neutrophils that increase in T12 tumors after single agent PXB treatment.

Supplemental Figure 3. TAMs in responsive (T12) and non-responsive (T11) tumors are phenotypically distinct. a, Heatmap showing top 10 upregulated genes per TAM sub-cluster for T12 and T11. **b,** IHC showing elevated pMAPK signaling in T11 tumors pre-treatment**. c,** Quantification of additional TAM marker genes. **d,** Heatmap representing top

upregulated and downregulated metabolic pathways in T12 TAMs. **e,** qPCR analysis of T12, T11 and 2151R tumors using select genes related to oxidative phosphorylation (n=3 independent biological replicates per tumor model, p value calculated using two-sided T test**.**

Supplemental Figure 4. Combination therapy leads to polyclonal expansion T cells that exhibit memory cell phenotypes. a, Gene expression heatmap top genes for each *Cd8*+ T cell sub-cluster **b,** Feature plots showing the expression of select memory/exhaustion/activation markers in *Cd8*+ T cells. **c.** V(D)J analysis of *Cd8*+ T cells for all 4 treatment groups showing polyclonal expansion of *Cd8*+ T cells after combination treatment. **d,** Treatment schema depicting treatment for mice bearing T12 lung metastasis. **e, f,** Representative images and quantification of surface metastatic lung nodules in all 4 treatment groups-PBS, PXB, CTX, CTX+PXB, n= 5-6 animals per group, $p < 0.05$). **d,** Schema for T12 rechallenge experiments and treatment. **e,f,** Quantification of mice that completely or partially (tumor volume < 50 % of control tumors) rejected T12 mammary tumors injected into the contralateral mammary gland of previously treated T12 tumor bearing mice, n=10. **g,** Images of visible lung metastatic nodules following Veh, PXB, CTX and CTX+PXB. **h,** Immunohistochemistry of T12 macro and micro-metastatic lung lesions for H&E, F480+ TAMs, B220+ B cells and CD4/8+ T cell markers following 30-day treatment of GEMMs**. i,** Quantification of immunohistochemistry showing % positivity of F480, B220, CD4 and CD8 following PBS, PXB, CTX and CTX+PXB.

Supplemental Figure 5. Cd4+ T cell and B cell play an important role in mediating long term tumor regression. a, Heatmap showing top upregulated genes per *Cd4*+ T cell sub-cluster. **b,** Feature plots showing the expression of select memory/exhaustion/activation markers in CD4+ T cells across all 4 treatment groups. **c,** Clonal frequency of Cd4+ T cells in all 4 treatment groups and chord diagrams representing unique V-J region pairings in *Cd4*+ T cells in T12 tumors before and after treatment.

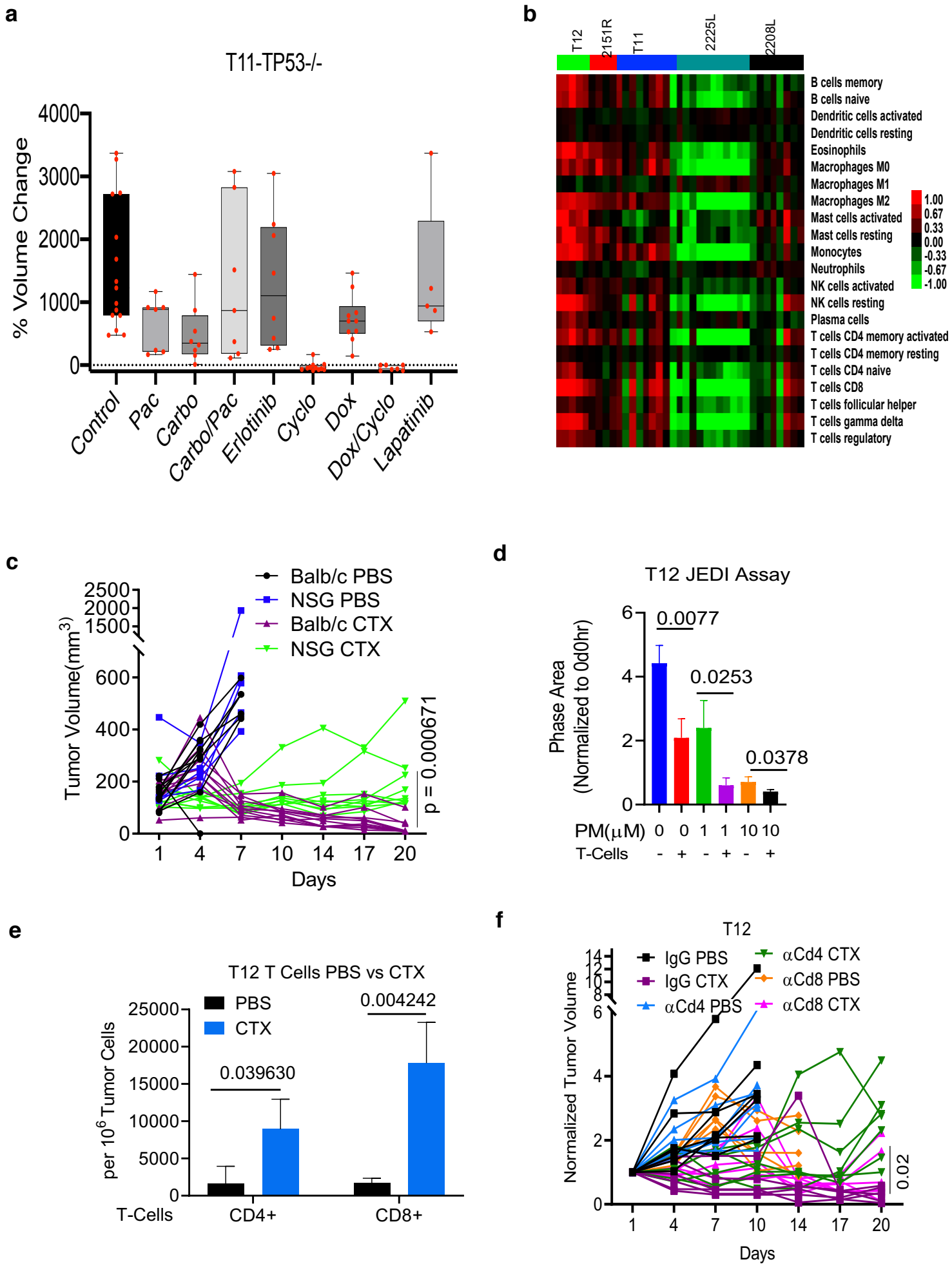
Supplemental Figure 6. Activated B cells expand after combination therapy and may be the main APCs within the tertiary lymphoid structures. a, Heatmap showing highly expressed genes in every B cell sub-cluster**. b,** REACTOME analysis of *Cd86*+ Activated B cells (ABCs) expanded after combination treatment. **c,** H&E and IHC images of TLS using T cell (CD4+ or CD8+) and B cell (CD19+) markers. **d,** Quantification of TLS in all 4 treatment groups in T12 tumors ($p < 0.05$). P value was computed using two-sided T test. **e,** Representative imaging mass cytometry images as well as single channel images depicting the localization of HEV marker Endomucin with B220+ B cells and CD4+ T cells withing TLS.

Supplemental Figure 7. T12 TAM signature is upregulated in patients with Claudin-low breast cancer in all and TNBC only patient samples. a, Downregulation of T11 TAM signature in all claudin-low breast cancer patients in SCANB, TCGA and METABRIC clinical trial datasets. **b,** Upregulation of T12 TAM signature in all claudin-low breast cancer patients in SCANB, TCGA and METABRIC clinical trial datasets.

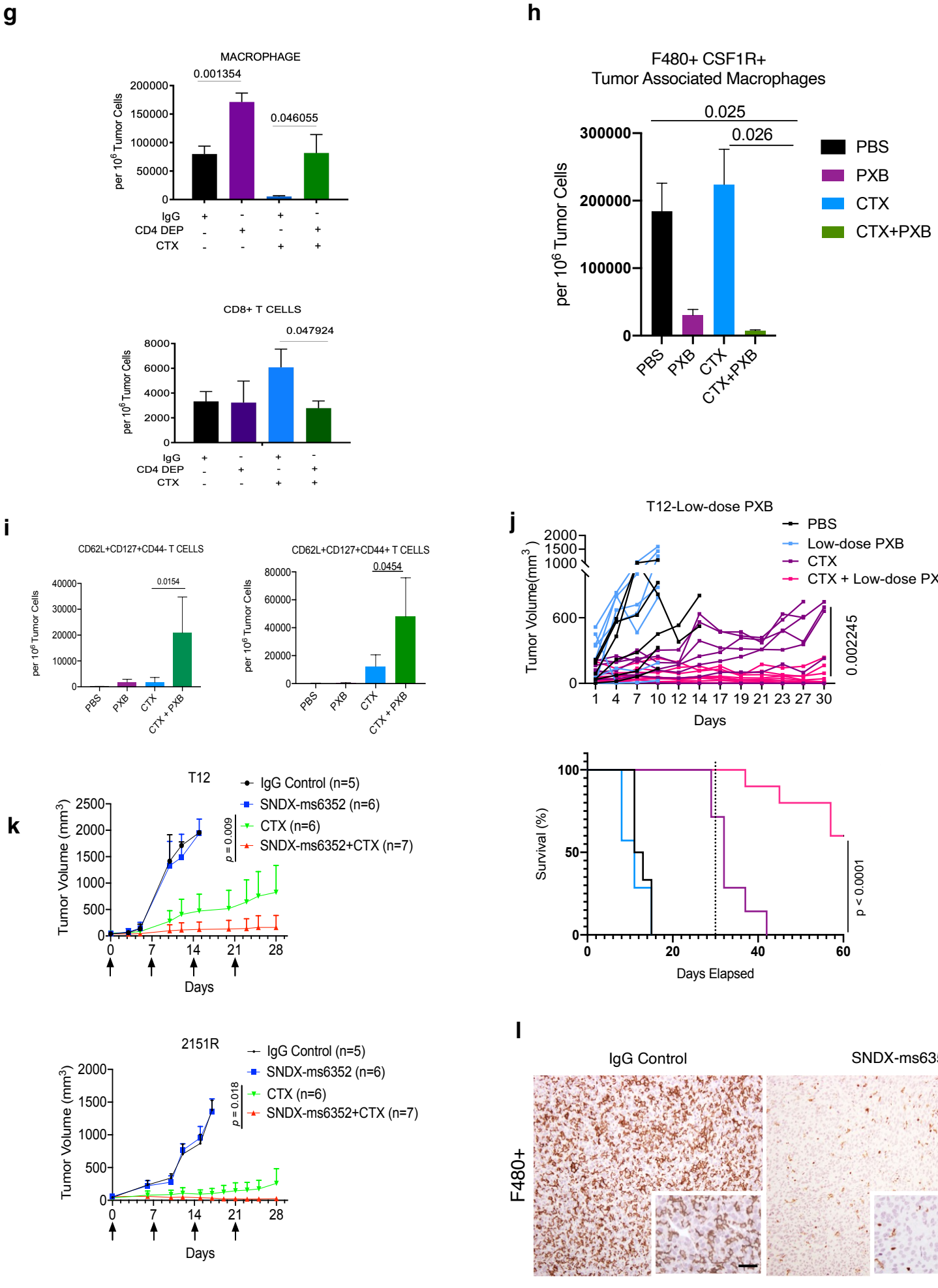
Supplementary Table 1. List of primer sequences used in qPCR analysis.

Supplementary Table 2. a, List of T11 vs T12 Signature Genes derived from single cell RNA

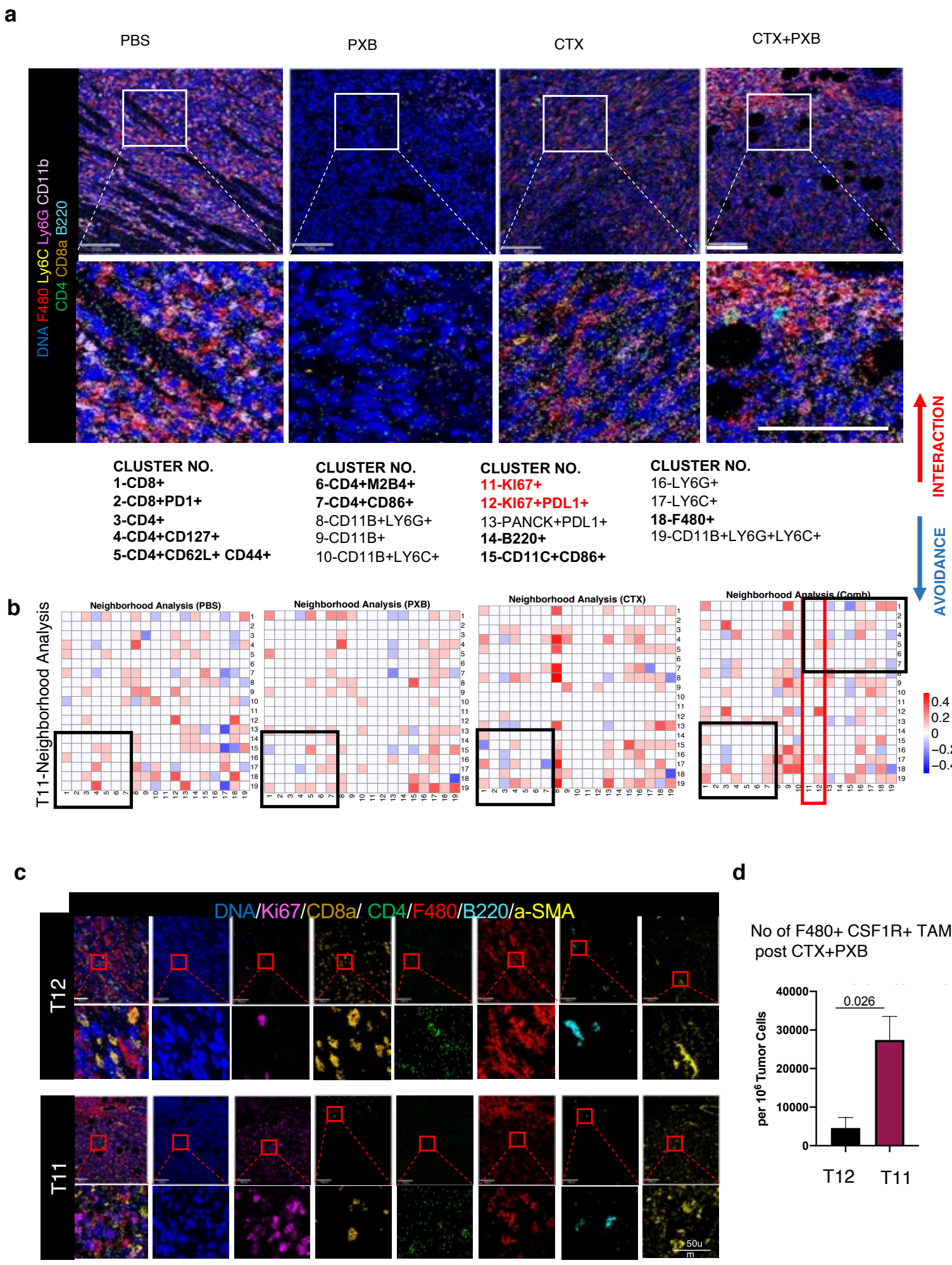
sequencing data for CD68+ Myeloid Cells**. b,** List of T12 vs T11 Signature Genes derived from single cell RNA sequencing data for CD68+ Myeloid Cells**.**



Supplementary Figure 1 for Figure 1, Singh et al.

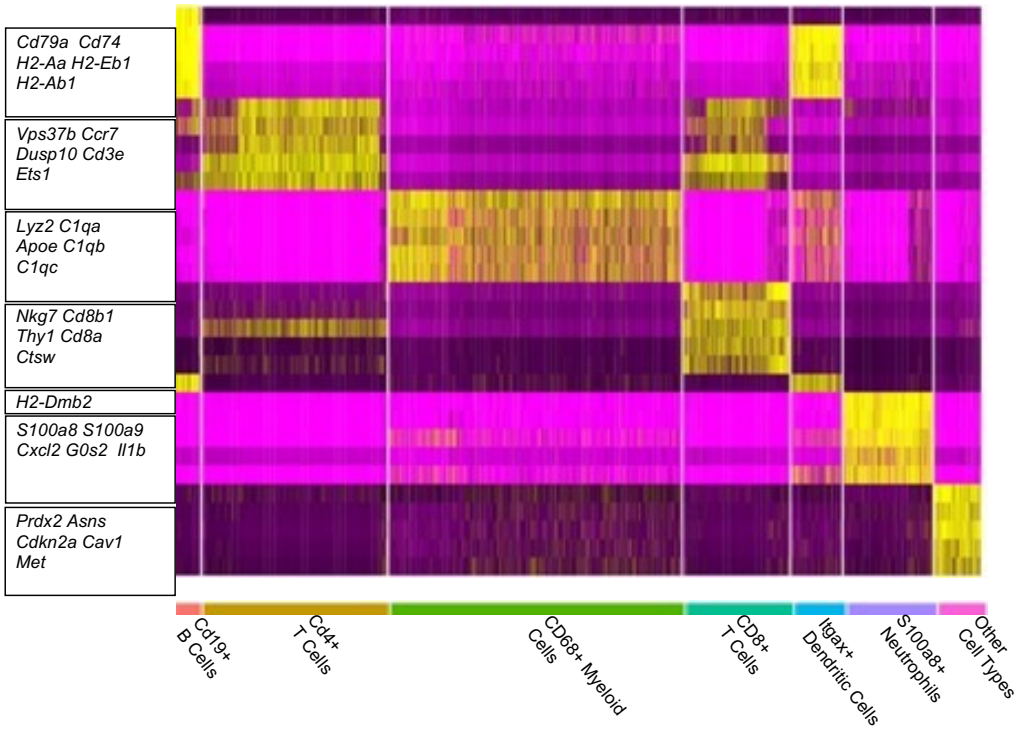


Supplementary Figure 1-2 for Figure 1, Singh et al.



Supplementary Figure 2-1 for Figure 2, Singh et al.

e



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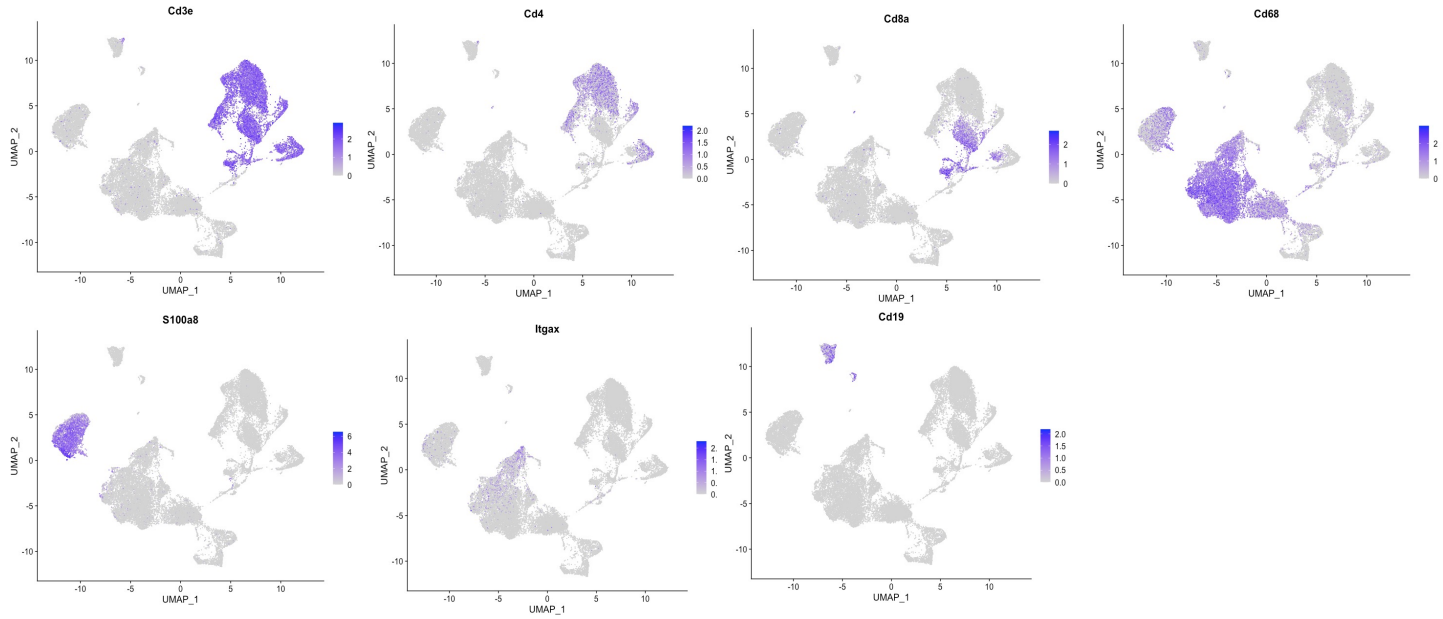
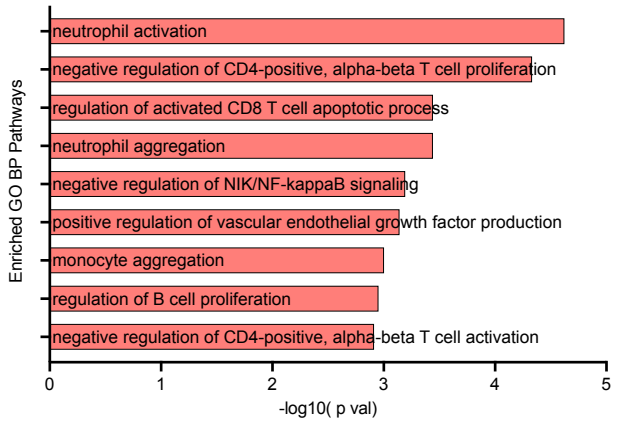


Go Analysis Biological Processes-
S100a8+ Neutrophils

g



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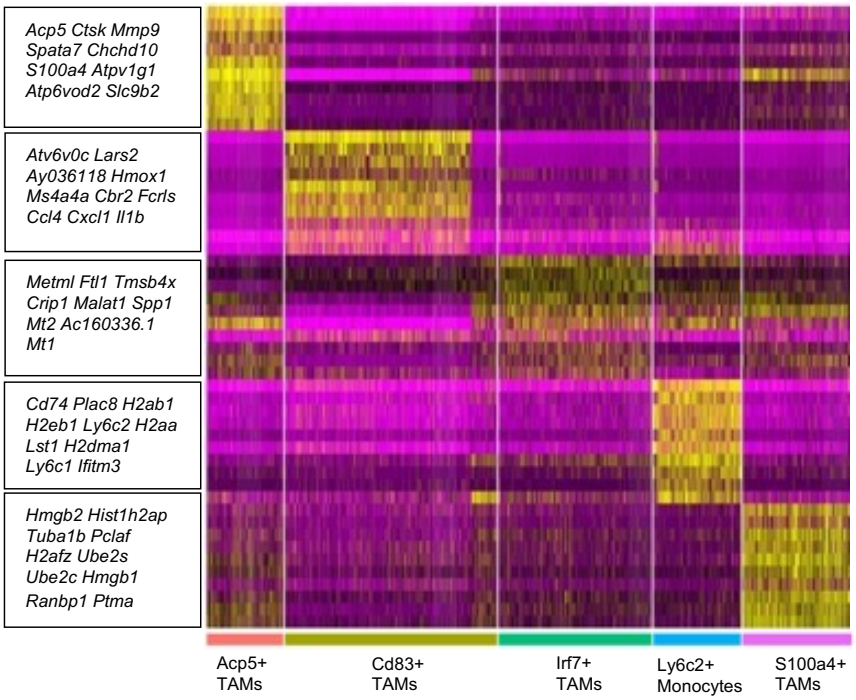
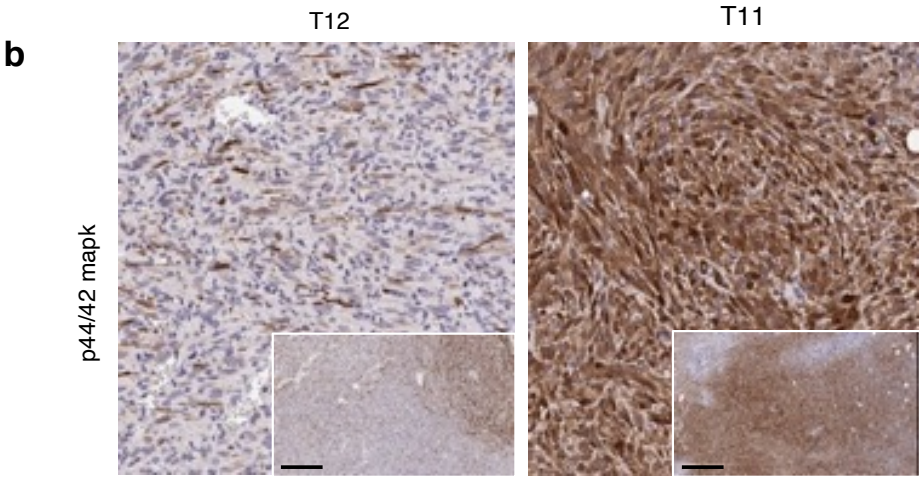
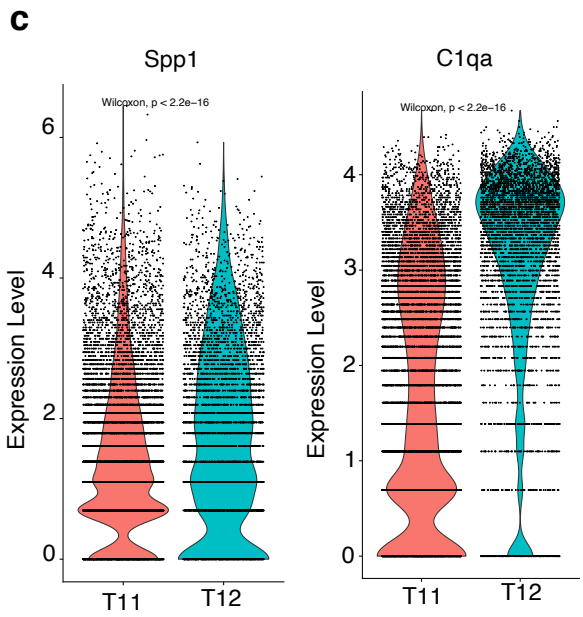
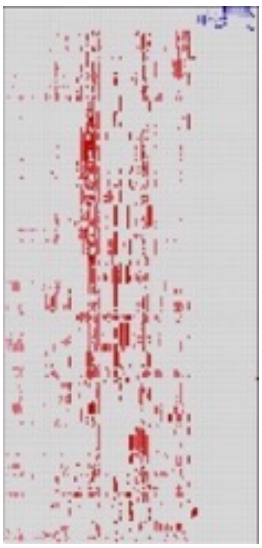






d

Pathways Analysis
T12 Cd68+ Myeloid Cells



NAME	NES	NOM p-val
Oxidative phosphorylation	-2.3721688	0
Generation of precursor metabolites and energy	-2.0811594	0
Mitochondrial organization	-1.7881126	0.00763359
GOBP_EPITHELIUM_DEVELOPMENT	-1.2340722	0.1827957
ATP metabolic processes	-1.2040108	0.16666667
Protein containing complex subunit organization	-0.9362941	0.5684931
Intracellular transport signaling	-0.9282206	0.60227275
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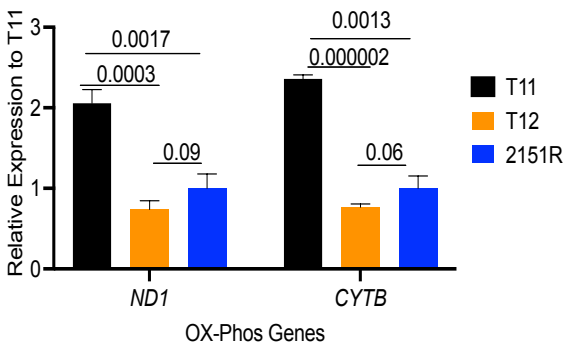
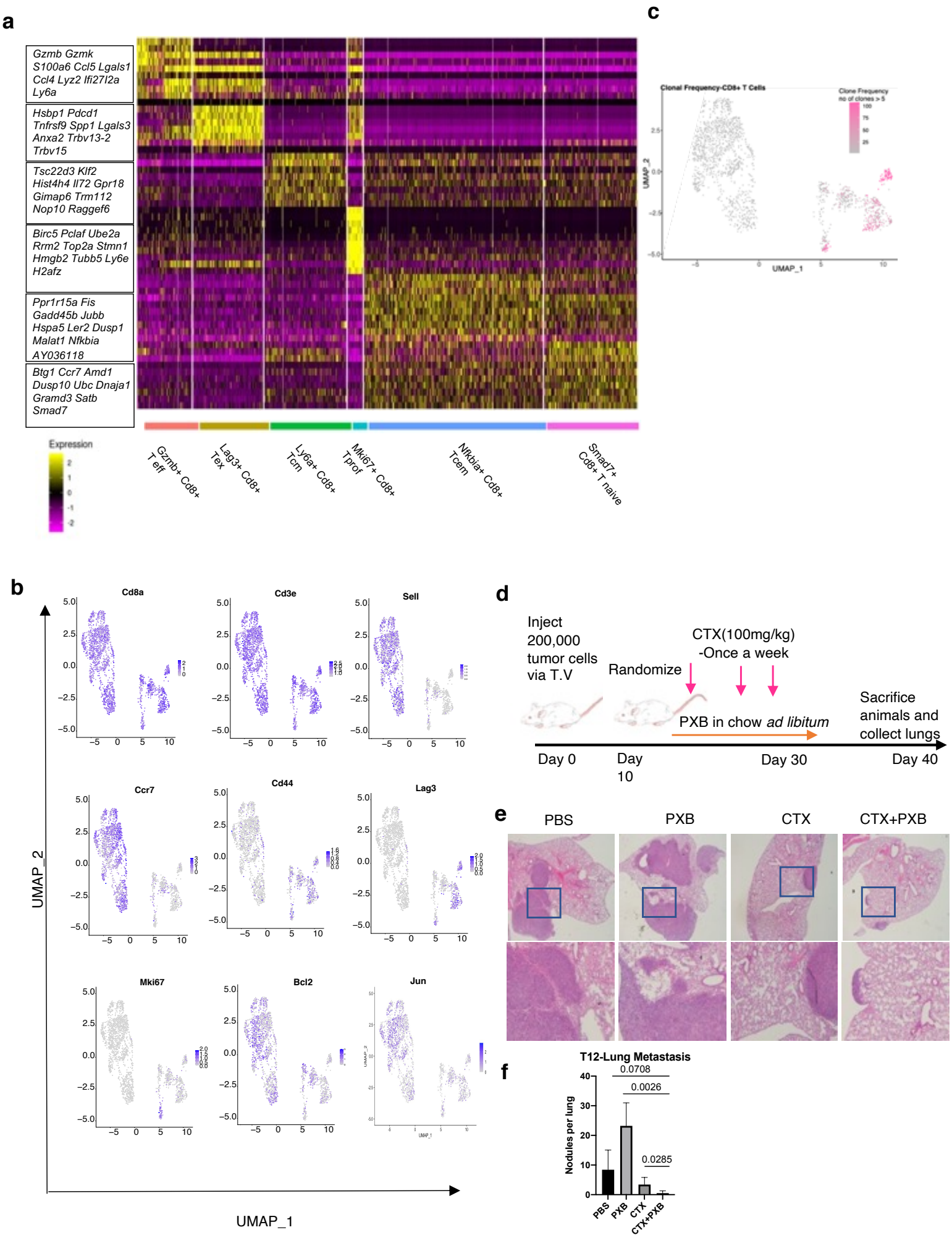
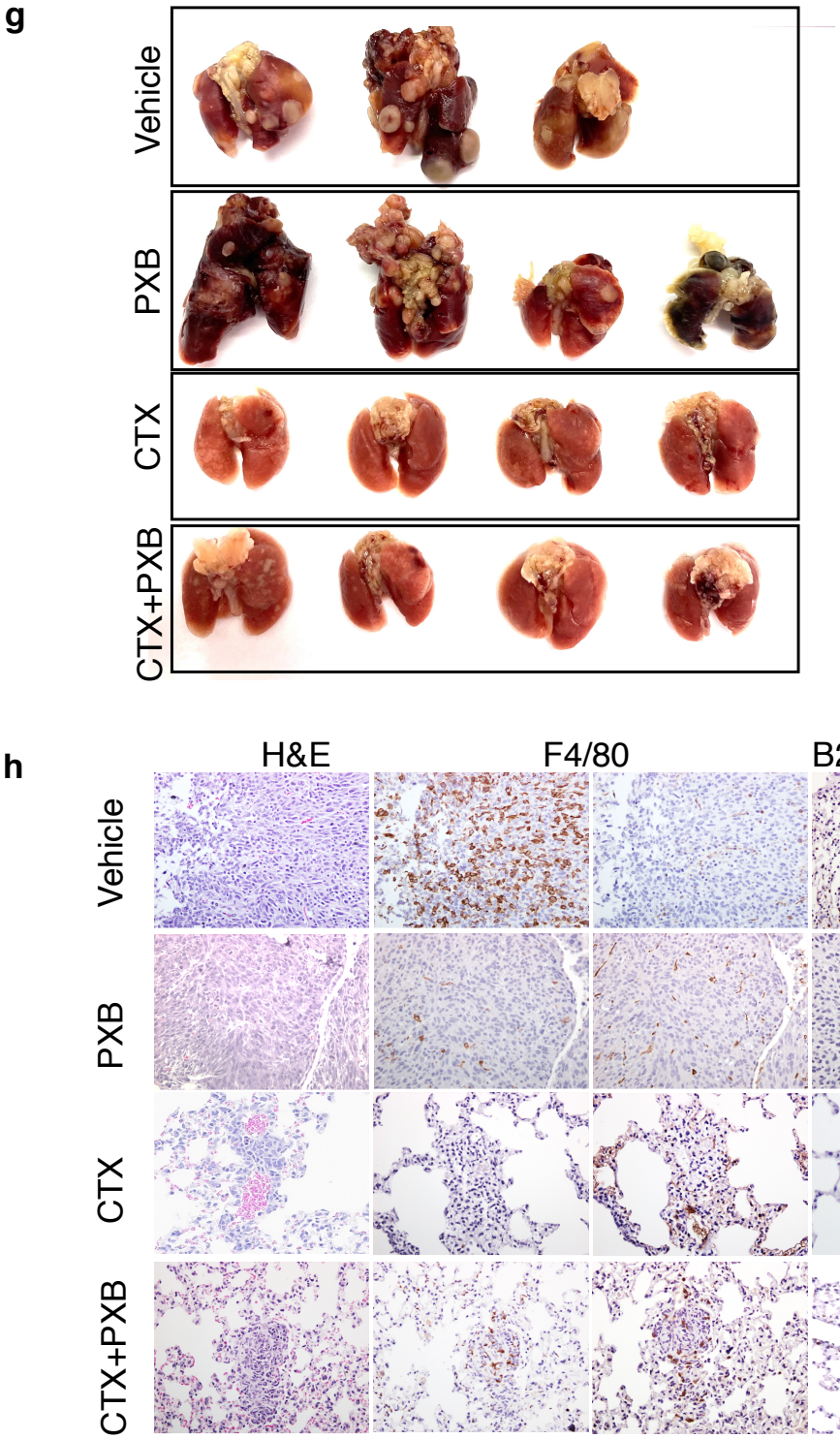
Protein localization to organelles	-0.6916076	0.9444444



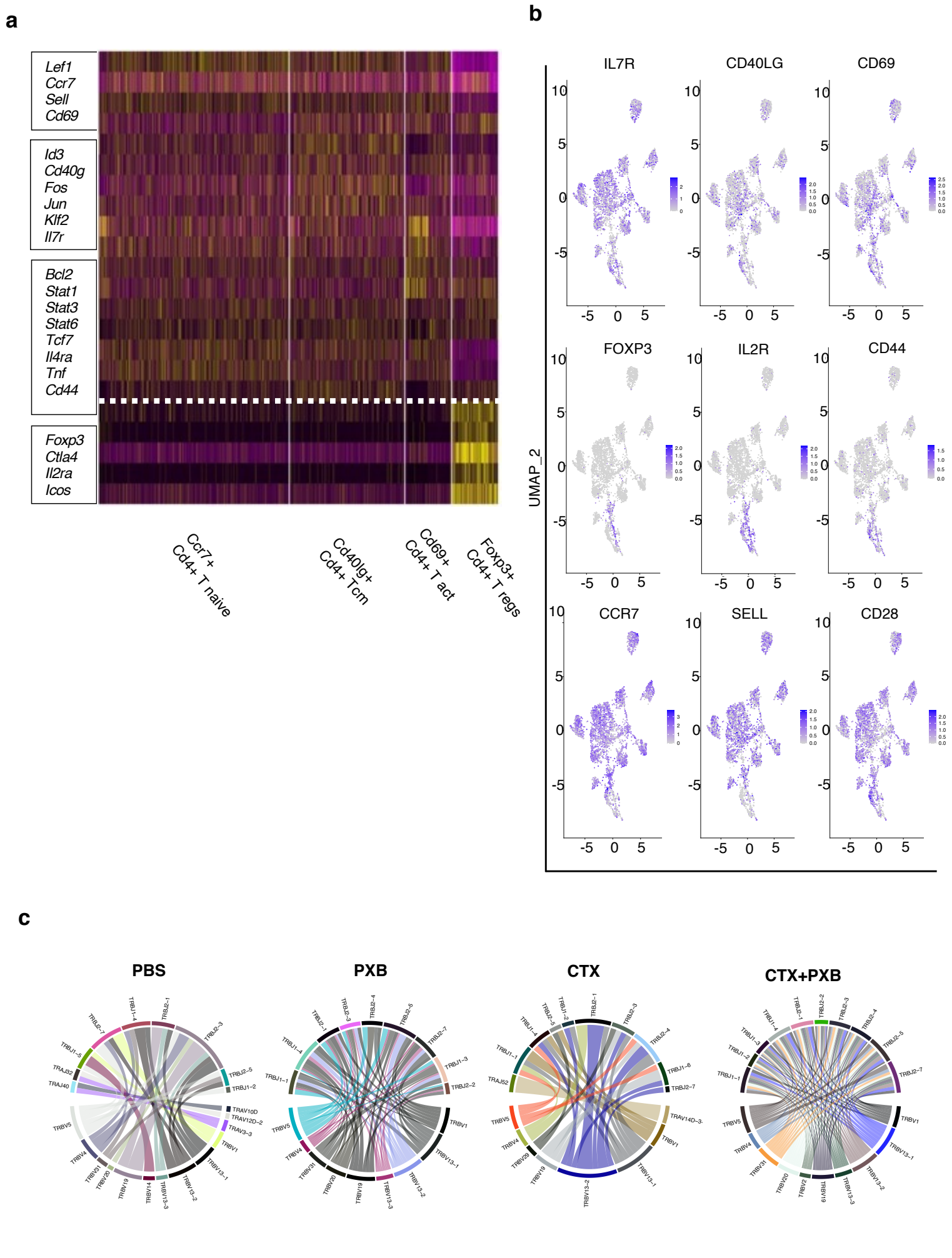
Supplementary Figure 3 for Figure 3, Singh et al.



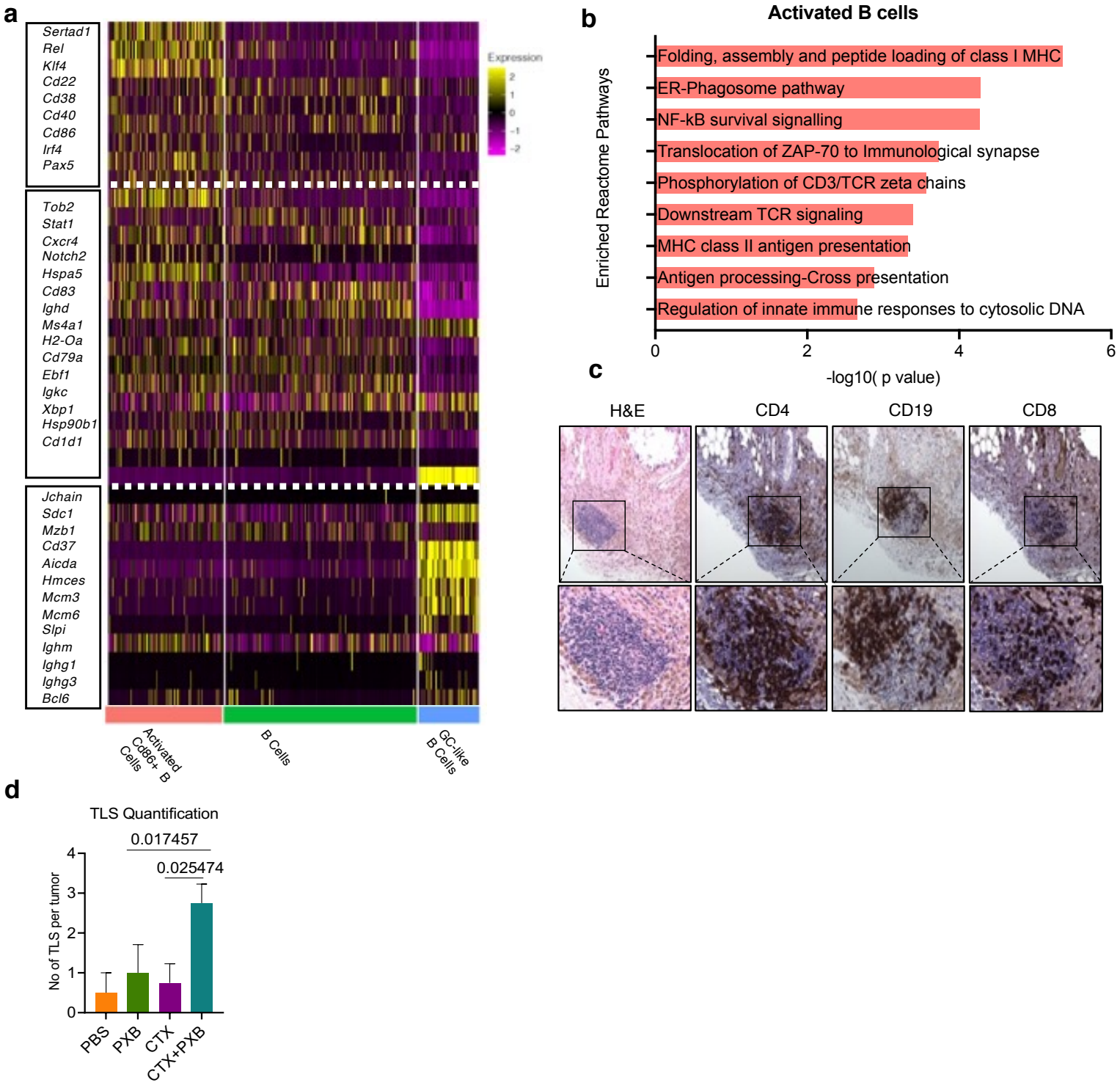
Supplementary Figure 4 for Figure 4, Singh et al.



Supplementary Figure 4-2 for Figure 4, Singh et al.



Supplementary Figure 5 for Figure 5, Singh et al.



Supplementary Figure 6 for Figure 6, Singh et al.

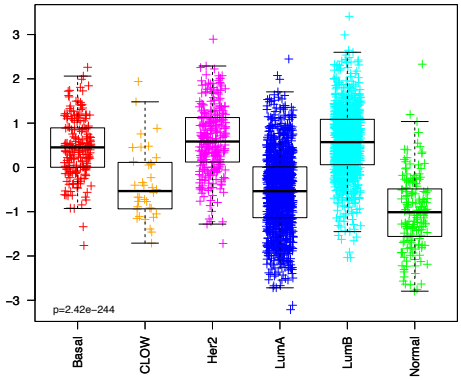
a

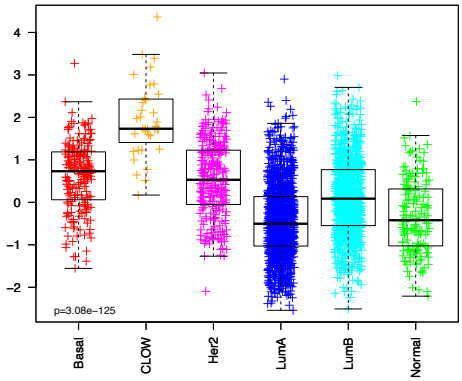
b

T11 TAM Signature

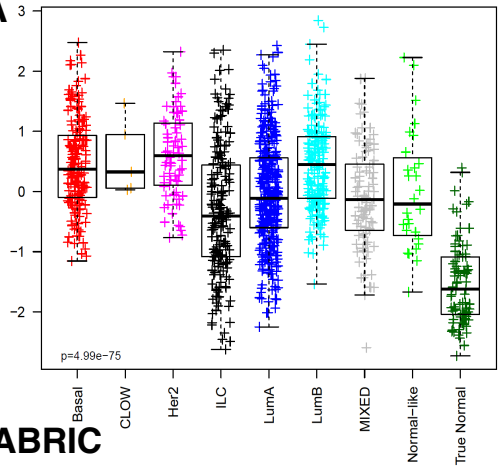
T12 TAM Signature

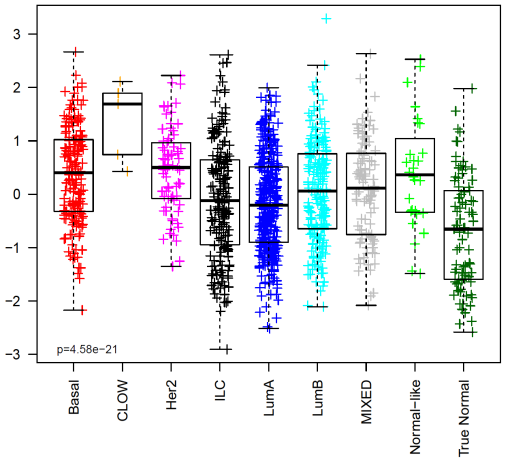
SCANB



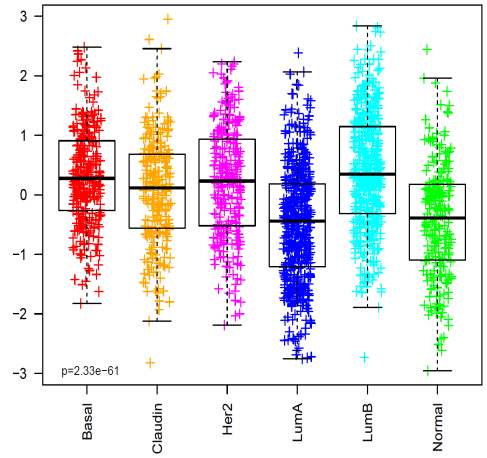


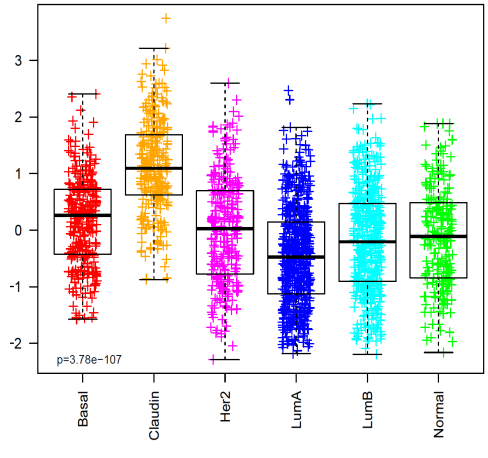
TCGA





METABRIC





Supplementary Figure 7 for Figure 7, Singh et al.