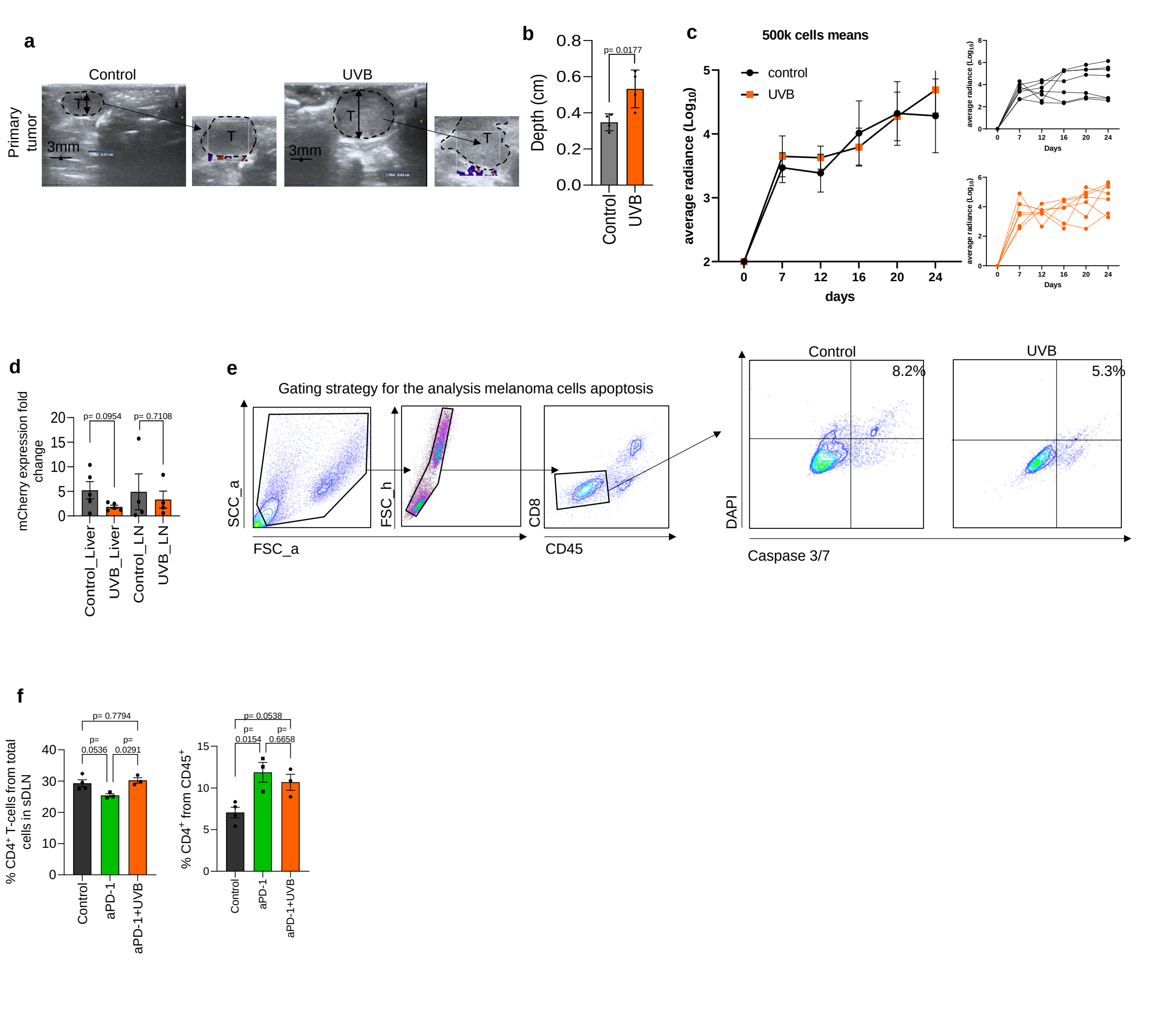


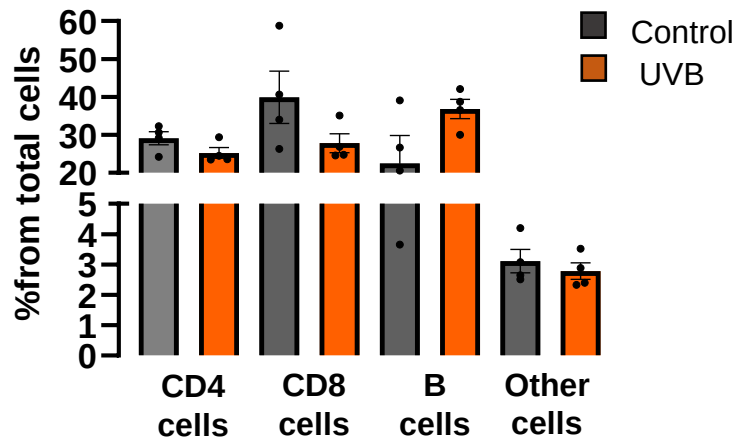
Supplementary Figure 1: Chronic UVB exposure suppresses skin draining lymph node CD8⁺ T cell-mediated killing and induces resistance to melanoma immunotherapy

Supplementary Figure 1, associated with Figure 1:

a. Representative ultrasound images of tumors in UVB- and mock-irradiated mice. The tumors are outlined with dashed lines and locations of depth measurements are marked by arrows. Enlargements show blood flow (in red or blue) in the tumor area. Scale bars, 1 cm. **b,** Tumor depths determined by ultrasound in UVB-treated and mock irradiated (control) mice (n=4 per group). **c,** Average radiance, reflective of lung metastasis development, in UVB-treated or mock irradiated (control) mice injected intravenously with *Ret* melanoma cells ($0.5x10^6$ cells, n=7 per group) on average (left) and in individual mice (right). **d,** Fold change in mRNA levels in $mCherry^+$ melanoma cells from liver (n=5 per condition) and lymph nodes (LN) relative to mice not injected with tumor cells (n=4 per condition). **e,** Flow cytometry gating strategy to evaluate apoptosis of melanoma cells co-cultured with T cells. **f,** Percent $CD4^+$ T cells relative to total cells in sDLN (left) and relative to $CD45^+$ cells in the tumor (right) (n=4 control group and n=3 UVB, UVB PD1 groups). Significance was determined by two-tailed *t* test. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, n.s. not significant. Error bars represent standard errors.

a

Immune cell types	markers
CD4 T cells	$CD3^+$ $CD4^+$; $CD8^-$ $CD11b^-$ IgD^- $TCRgd^-$ $NK1.1^-$
CD8 T cells	$CD3^+$ $CD8^+$; $CD4^-$ $CD11b^-$ IgD^- $TCRgd^-$ $NK1.1^-$
B cells	$B220^+$ or IgD^+ ; $CD11b^-$ $TCRgd^-$ $NK1.1^-$
Other cells- innate immune cells	$CD11C^+$ or $CD11B^+$ or $TCRgd^+$ or $NK1.1^+$



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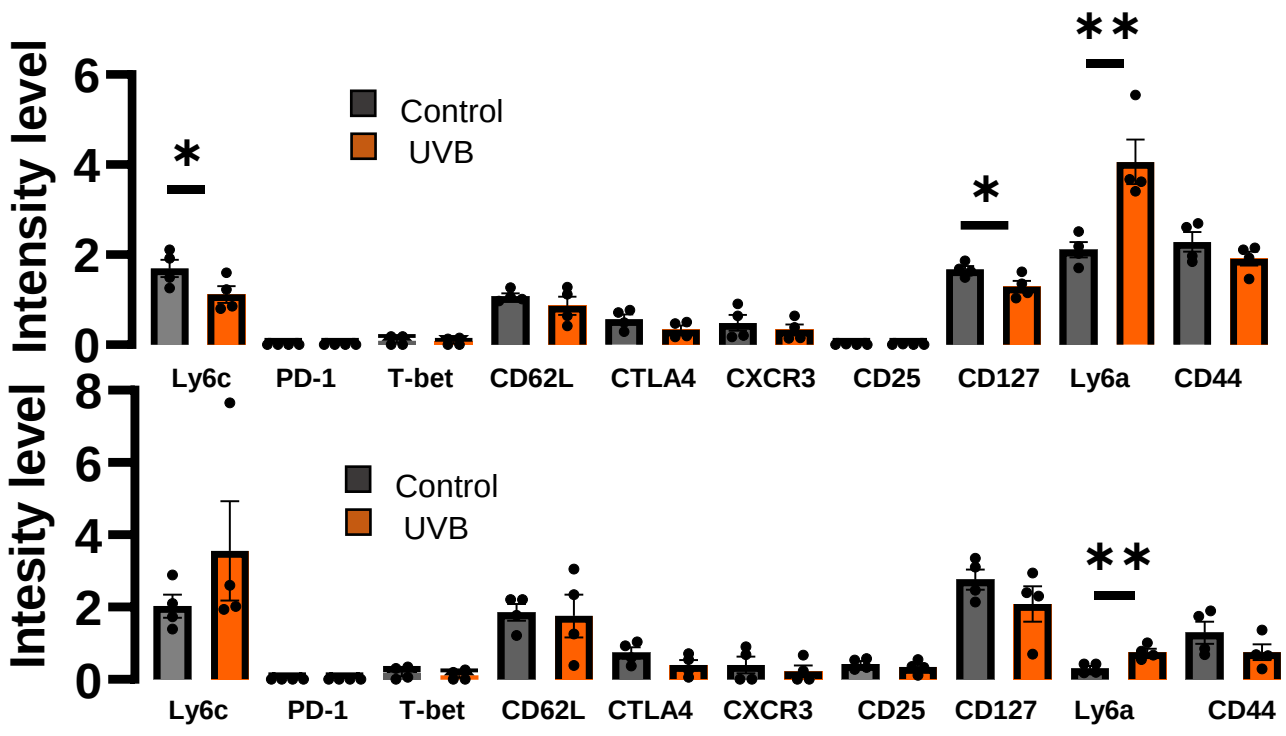

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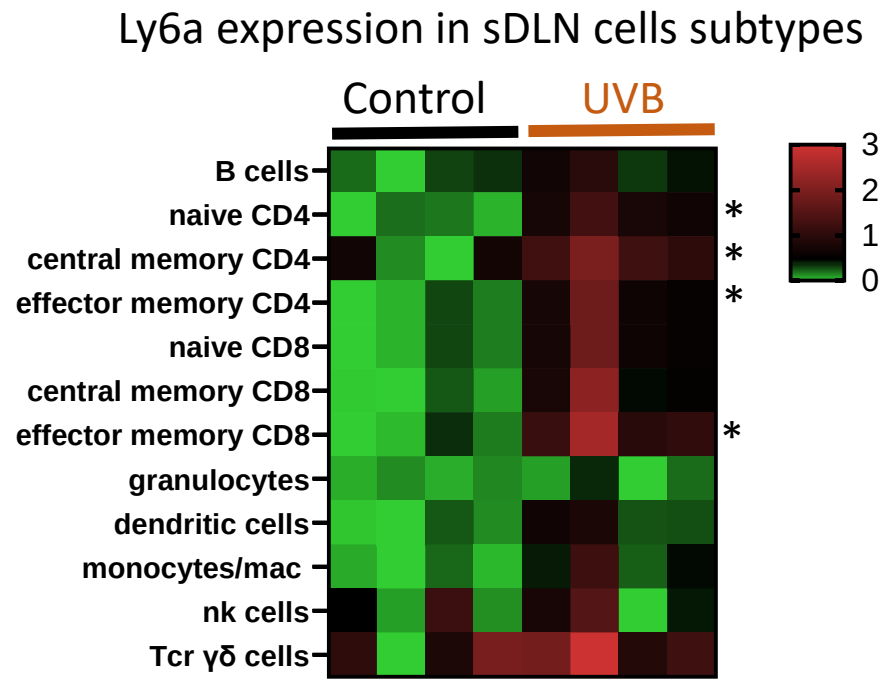

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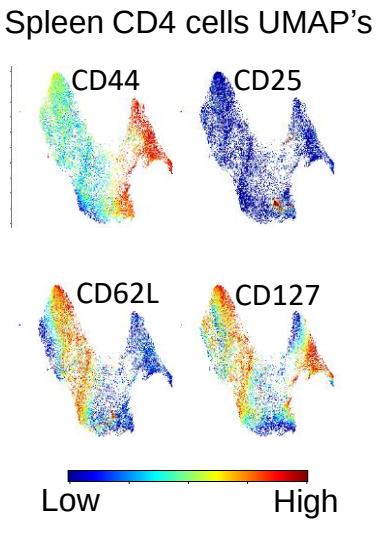

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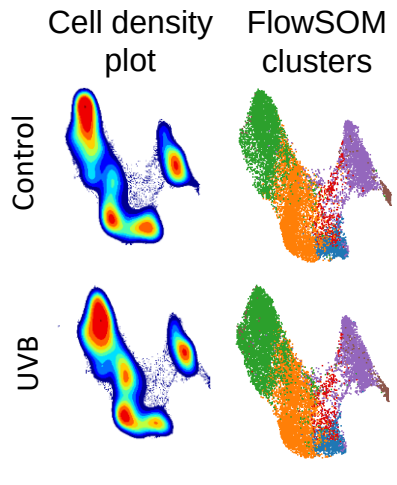

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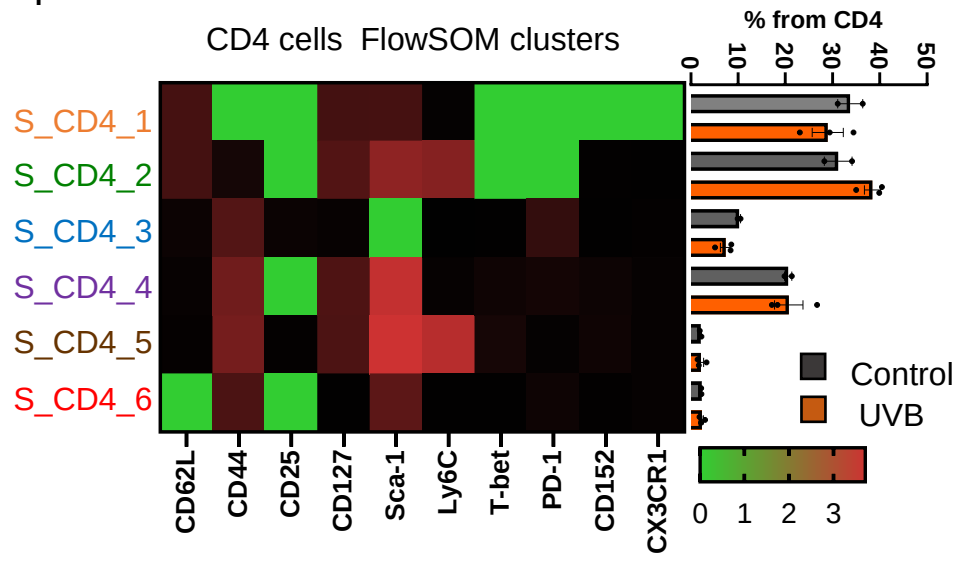

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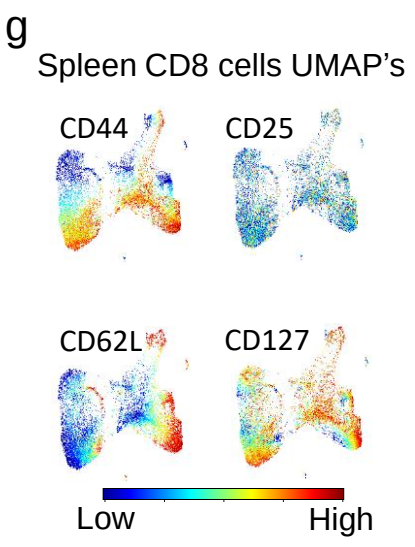

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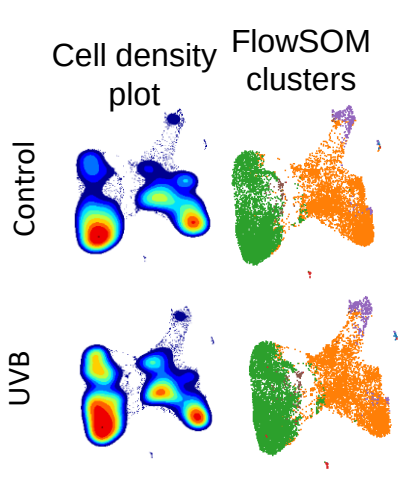

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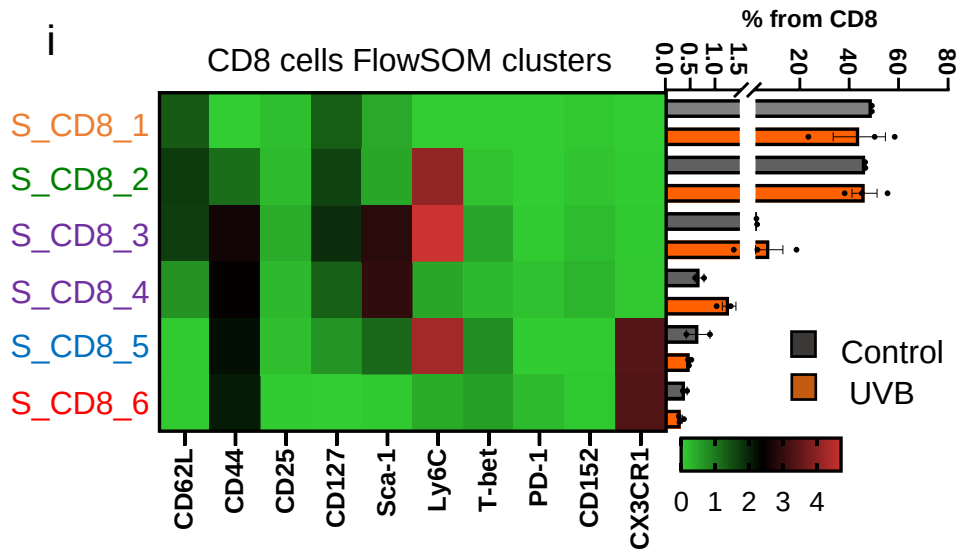

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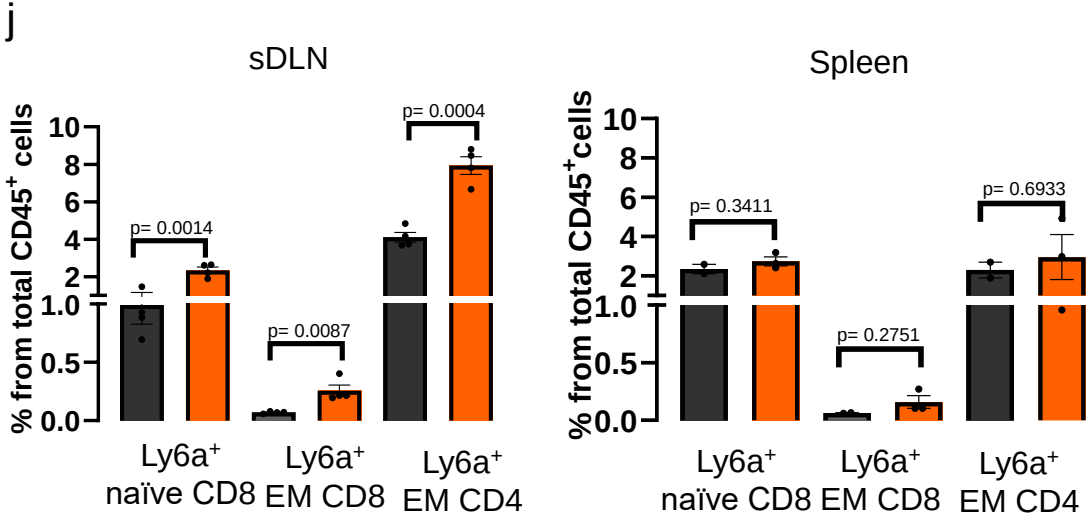

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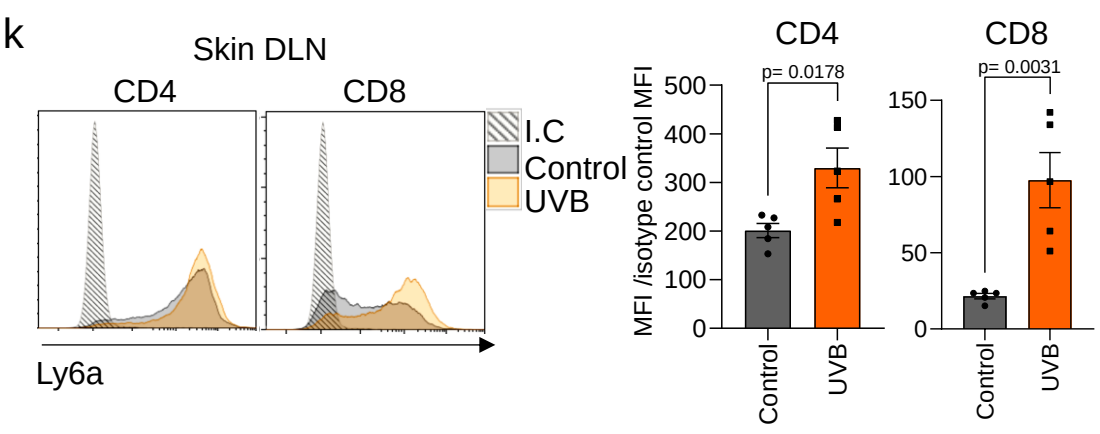

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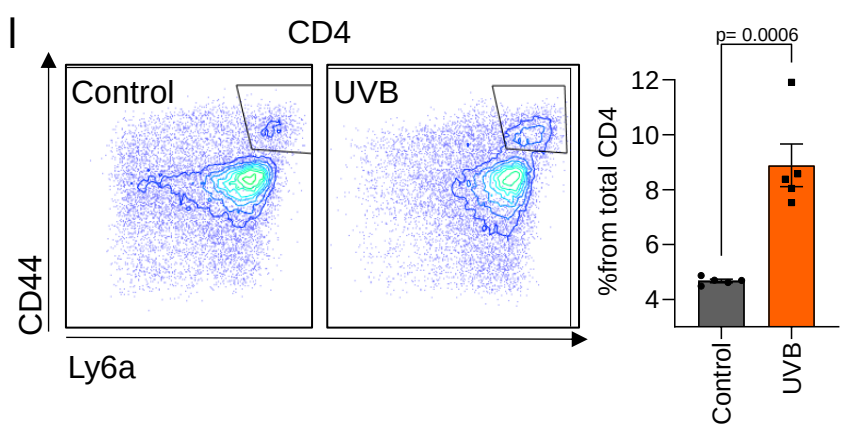

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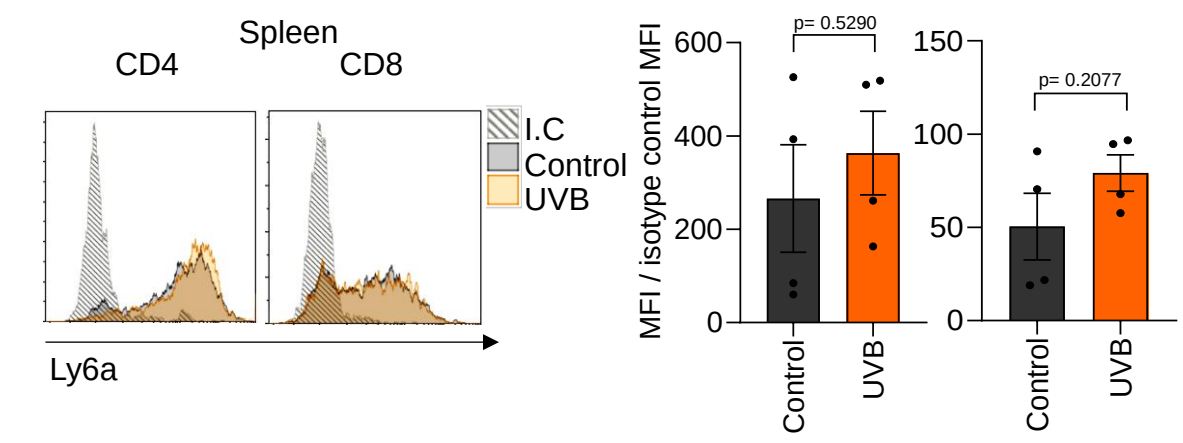

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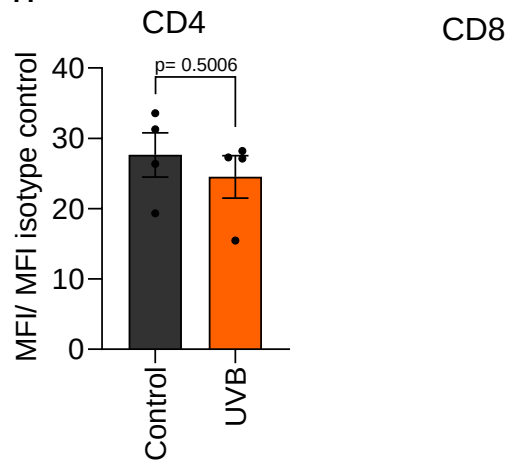
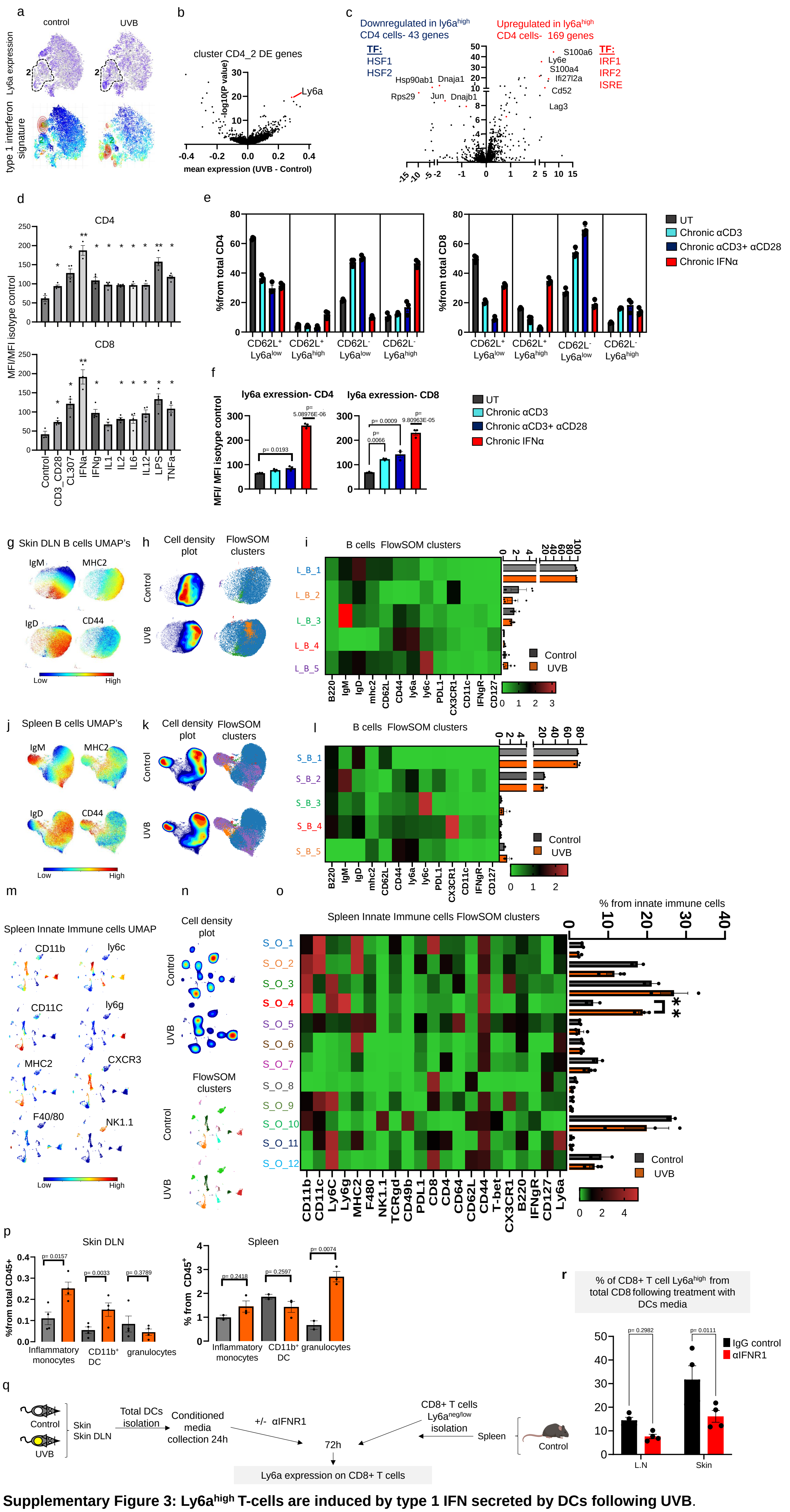


o

Supplementary Figure 2: UVB induces a Ly6a^{high} T cell subpopulation in the skin drain lymph nodes.

Supplementary Figure 2, associated with Figure 2:

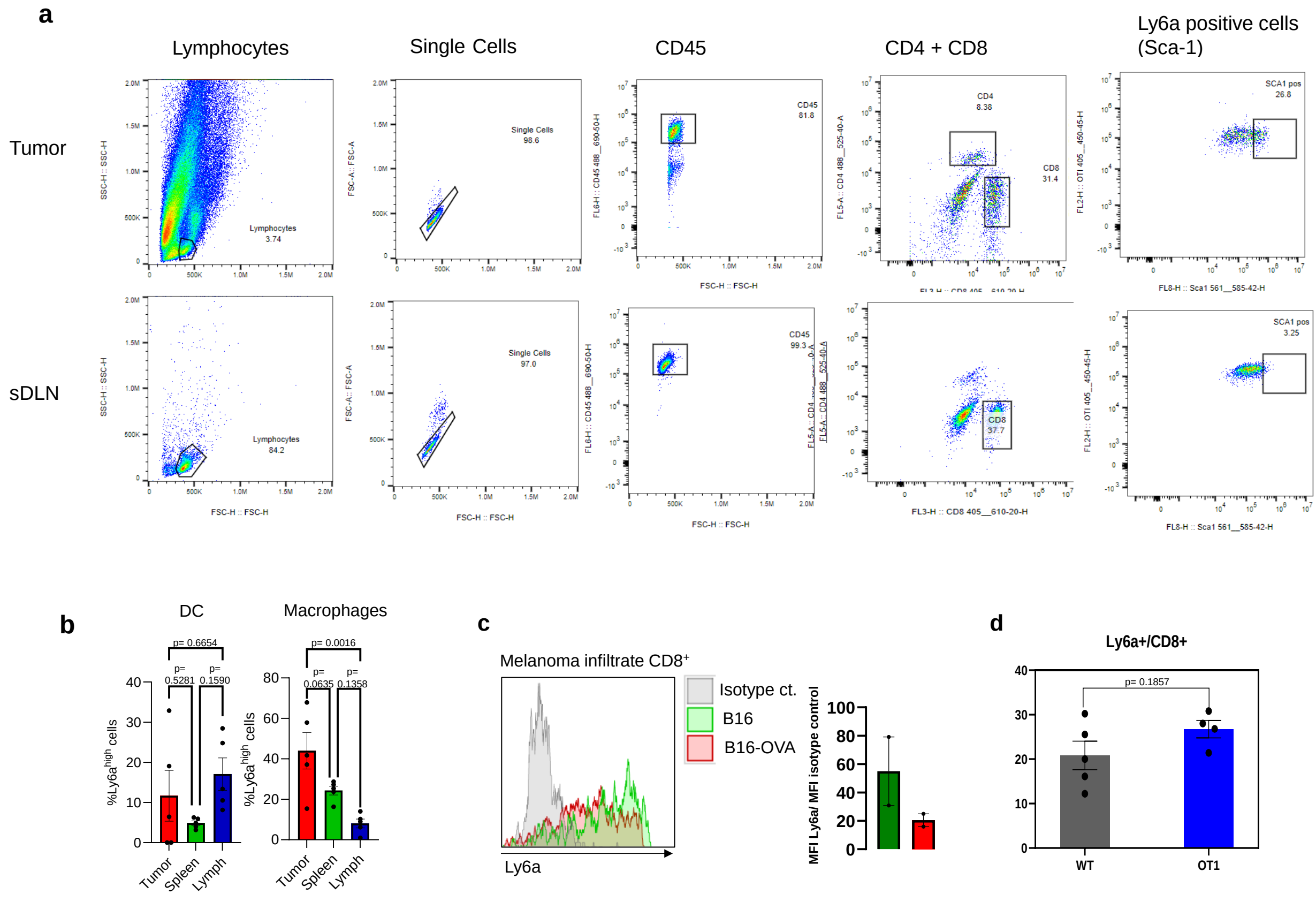
a, Left: The markers for each cell type analyzed. Right: Percentages of the indicated cell types from total live $CD45^+$ cells based on supervised analysis of mass cytometry data (n=4 mice per group). **b,** Median intensities of indicated markers in $CD4^+$ (upper) and $CD8^+$ (lower) cells (n=4 mice per group). **c,** Heatmap of median expression levels of Ly6a in the indicated cell subtypes in sDLNs. Colors reflect the transformed ratio relative to the minimum expression of Ly6a in the indicated cells. (n=4 per condition). **d-f**, Mass cytometry analysis of mouse spleen following UVB exposure as in Figure 2 (n=2 control and n=3 UVB). **d,** Representative UMAPs for $CD4^+$ subset marker levels (red, high; blue, low) in cells from spleens. **e**, Cell density plots (left) and UMAPs of $CD4^+$ clusters (indicated by different colors) generated by FlowSOM (right) from spleen $CD4^+$ cells. **f,** Left: Heatmap of $CD4^+$ FlowSOM clusters with colors indicative of the transformed ratio relative to the minimum expression of the indicated marker in $CD4^+$ cells. Right: Percentage of each cluster from the total population of $CD4^+$ cells in UVB-treated and mock-irradiated mice. **g-i,** Analysis of spleen $CD8^+$ cells as in d to f. **j,** Supervised mass cytometry analysis of T cell subtypes that significantly increased upon UVB treatment in spleen (left) and sDLN (right) according to the unsupervised analysis: $CD62l^+/CD44^-/Ly6a^+/CD8^+$ ($Ly6a^+$ naïve CD8), $CD62l^-/CD44^+/Ly6a^+/CD8^+$ ($Ly6a^+$ EM CD8), and $CD62l^-/CD44^+/Ly6a^+/Ly6c^-/CD4^+$ ($Ly6a^+$ EM CD4). **k,** Left: Representative trace of Ly6a median fluorescence intensity (MFI) in $CD4^+$ and $CD8^+$ cells from sDLN relative to isotype control MFI (diagonal stripes) in UVB-treated (UVB, orange) and mock-irradiated (control, gray) mice as determined by flow cytometry. Right: Averages of Ly6a versus isotope MFIs (n=5 per group). **l,** Left: Representative flow cytometry analyses of $CD44^+/Ly6a^+/CD4^+$ cells from sDLN of UVB-treated and mock-treated (control) mice. Right: Means of percentages of $CD44^+/Ly6a^+$ cells from total $CD4^+$ cells (n=5 per group). **m-n,** Flow cytometry analysis of Ly6a levels in T cells from **m)** spleens (n=5 per group) and **n)** Mesenteric lymph nodes (n=4 per group) of UVB-treated and mock-treated mice. **o,** Left: Percent of $Ly6a^+/PD\text{-}1^-$ and $Ly6a^+/PD\text{-}1^+$ cells from total $CD8^+$ cells from skin, sDLN, and spleen following UVB or mock irradiation (control) treatment (n=4 per group). Right: Representative image. Significance was determined by two-tailed *t* test. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, n.s. not significant. Error bars represent standard errors.



Supplementary Figure 3: Ly6a^{high} T-cells are induced by type 1 IFN secreted by DCs following UVB.

Supplementary Figure 3, associated with Figure 2:

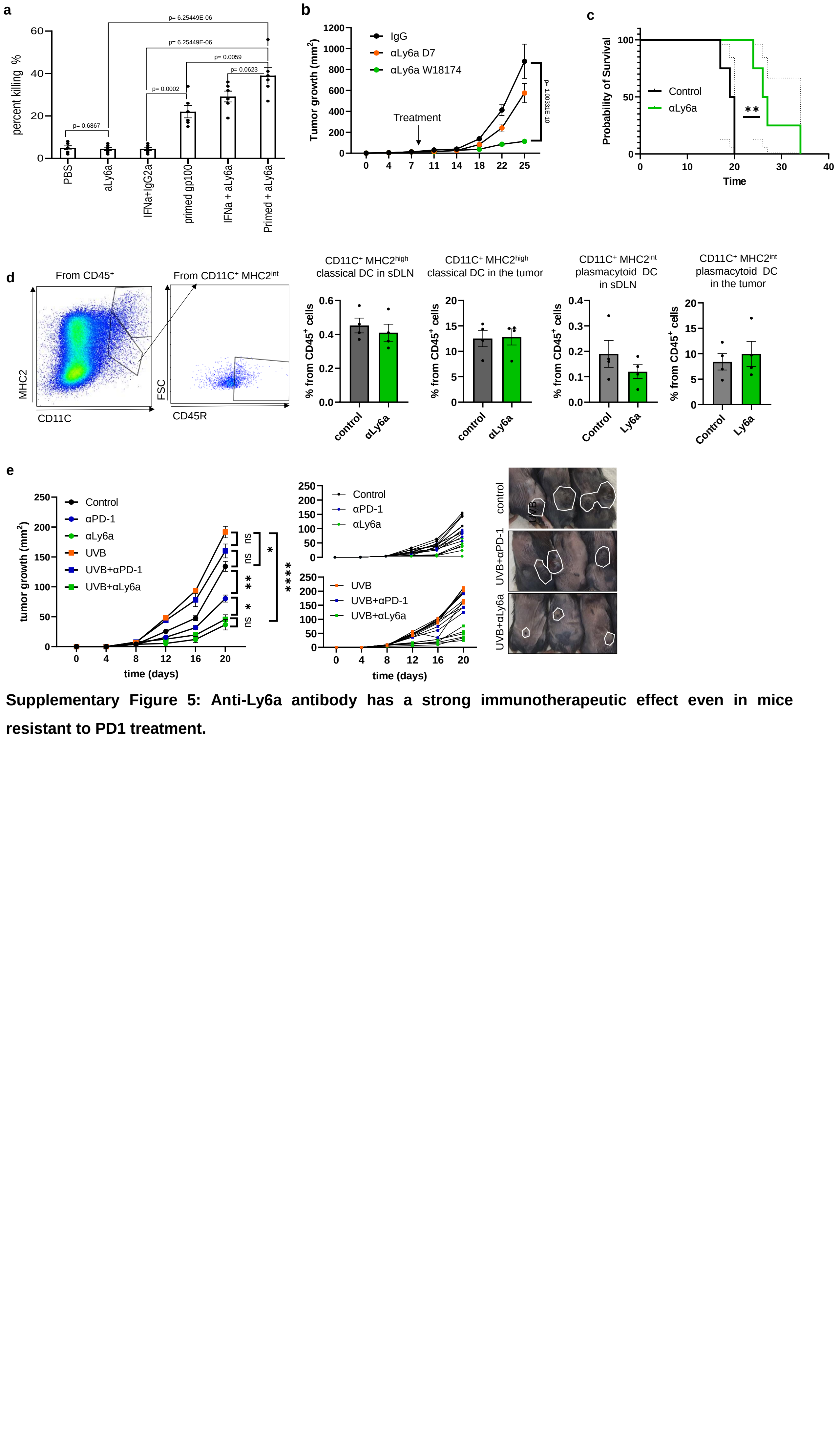
a, t-SNE projections colored by the expression levels of Ly6a (upper) and type 1 IFN signature genes (lower) for UVB- and mock-irradiated (control) *CD4*$^{+}$ cells (cluster 2 of clusters defined in Figure 3a) from single-cell RNA-seq data of sDLN T cells from UVB- and mock-irradiated mice. **b,** Volcano plot of the differently expressed genes in *CD4*$^{+}$ cells (cluster 2) between UVB-treated and control mice. *Ly6a* is marked in red. **c,** Volcano plots of genes upregulated or downregulated in Ly6a^{high} effector memory and exhausted cells (analysis of published data[1]). The x axis represents fold change; y axis represents $\log_{10}$ of adjusted p value. Representative transcription factors associated with gene groups are indicated. **d,** MFI of Ly6a on CD4^{+} (upper) and CD8^{+} (lower) T cells at 24 hours after treatment with the indicated cytokine or factor (n=3 per condition). **e,** Percentages of CD62L^{+}/Ly6a^{low}, CD62L^{+}/Ly6a^{high}, CD62L^{-}/Ly6a^{low}, and CD62L^{-}/Ly6a^{high} cells from CD4^{+} (left) and CD8^{+} (right) compartments after the indicated treatments (n=3 per group). **f,** MFI versus that of isotype control of Ly6a on CD4^{+} (left) and CD8^{+} (right) T cells after the indicated treatments (n=3 per group). **g,** UMAP of mass cytometry data analysis of B cells from sDLN of mice treated with UVB or mock irradiated (n=4 per group). Marker levels are indicated by color (red, high; blue, low). **h,** Cell density plots (left) and UMAPs of B cell clusters (indicated by different colors) generated by FlowSOM (right). **i,** Left: Heatmap of transformed ratio relative to the minimum expression of the indicated marker in B cells. Right: Percentage of each cluster from the total population of B cells in UVB- and mock-treated mice (n=4 per group). **j-o,** Mass cytometry analyses of **j-i)** spleen B cells and **m-o,** spleen innate immune cells (or non-B, non-T cells) of mice treated with UVB (n=3) or mock irradiated (n=2) as in **g-i**, **p,** Supervised mass cytometry analysis of the myeloid cell subtypes that significantly increase upon UVB in the sDLN (left) and the spleen (right): CD11b^{+}/Ly6g^{-}/Ly6c^{high}/CXCR3^{+} cells (inflammatory monocytes), CD11b^{+}/Ly6g^{-}/MHC2^{high}/CD11c^{+} cells (CD11b^{+} DCs), and CD11b^{+}/Ly6g^{+}/Ly6c^{low} cells (granulocytes) (n=4 per group). **q**, Experimental flowchart: *C57BL/6* mice were irradiated with UVB or mock irradiated for 8 weeks (n=4 per group). CD11b^{+} cells were isolated from sDLN and the skin and cultured for 24 hours. Conditioned media was collected and added to Ly6a$^{neg/low}$/CD8^{+} cells from untreated mice, with or without anti-IFNR1 blocking antibody. After 72 hours of culturing with the conditioned media, the percentage of Ly6a^{high} T cells was determined. **r**, Mean percentages of Ly6a^{high}/CD8^{+} cells in indicated samples (n=4 per group). Significance was determined by two-tailed *t* test. * p<0.05, ** p<0.01, *** p<0.001, n.s. not significant. Error bars represent standard errors.



Supplementary Figure 4: Ly6a^{high} T-cells are enriched in the tumor microenvironment independently to UVB exposure.

Supplementary Figure 4, associated with Figure 4:

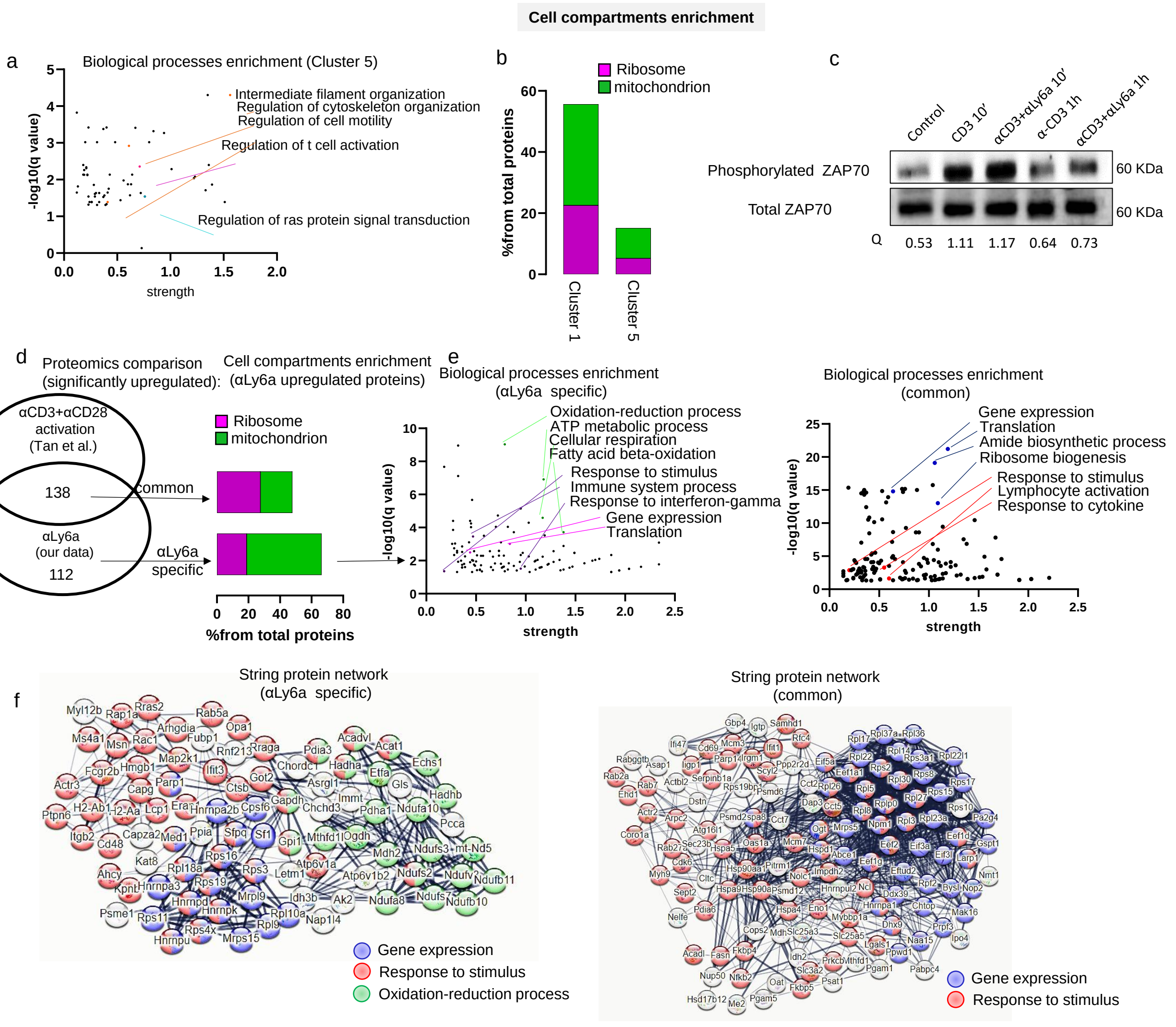
a, Gating strategy for Figure 4a-b. **b,** Percent of DCs and macrophages that express Ly6a in the tumor microenvironment, spleen, and sDLN from Figure 4a (n=4) **c,** *B16* or *B16-OVA* tumor cells were subcutaneously injected into *C57BL/6 OT-1* mice (n=2 per group). After 16 days, the expression of Ly6a on TILs was measured by flow cytometry. Left: Representative trace of Ly6a median fluorescence intensity (MFI). Right: Averages of Ly6a versus isotope MFIs. **d**, *C57Bl/6* (n=5) and OT-I mice (n=4) were injected subcutaneously with *B16-OVA* melanoma cells. Once tumors were palpable, the percentage of $Ly6a^{high}$ in infiltrating $CD8^{+}$ T cells was analyzed by staining with H-2Kb:OVA (SIINFEKL) tetramer (MBL, cat# TB-5001-4). Significance was determined by two-tailed *t* test (panels c, d) or by one-way ANOVA test with Tukey correction (panel b). * $p<0.05$, ** $p<0.01$, *** $p<0.001$, n.s. not significant. Error bars represent standard errors.



Supplementary Figure 5: Anti-Ly6a antibody has a strong immunotherapeutic effect even in mice resistant to PD1 treatment.

Supplementary Figure 5, associated with Figure 5:

a, *B16F10* melanoma cell killing assay following co-culture with splenic $CD8^+$ T cells that were either primed with gp100 or exhausted with IFNα, in the presence of anti-Ly6a antibody or IgG2 control (n=6 per condition). **b,** Tumor mean diameters in mice subcutaneously injected with MC38 tumor cells and treated with anti-Ly6a antibody clone W18174, anti-Ly6a clone D7, or IgG2a isotype control on day indicated by arrows (n=3 per group). **c,** Survival curves of mice following treatment with anti-Ly6a. According to the ethical guidelines, mice with tumors of 1.5 cm^3 were sacrificed (n=4 per group). **d**, Analyses of DC subtypes from the lymph nodes and tumor microenvironment following treatment with anti-Ly6a as in Figure 5e. Left: Gating strategy. Right: Percent of the DC subtype from total immune cells. n=4 per group. **e,** Mean tumor diameters (left) individual mouse data (middle) and representative images (right) for mice treated with UVB or mock-irradiated (control) and injected subcutaneously with *Ret* melanoma cells then treated with anti-Ly6a antibody, anti-PD-1 or IgG control (n=6 per group). Arrows indicate days of treatment. Significance was determined by two-way ANOVA test with Tukey correction (panels b and e) or by one-way ANOVA test with Tukey correction (panel a) or by two-tailed t test (panel d) or by log-rank test (panel c). * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, n.s. not significant. Error bars represent standard errors.



Supplementary Figure 6: Anti-Ly6a antibody crosslinking enhances CD8⁺ T-cells activity and prevent loss of mitochondrial function that is associated with T-cells exhaustion via cMYC signaling

Supplementary Figure 6, associated with Figure 6:

a, GO terms of biological processes significantly associated with proteins of cluster 5, which are downregulated in the presence of melanoma cells and anti-Ly6a and upregulated in cells treated with IgG in the absence of melanoma cells as described in Figure 5c. **b,** Percentage of proteins associated with ribosome or mitochondria based on cellular compartment GO terms for the proteins of cluster 1 and cluster 5 from Figure 4d. **c,** Western blot analysis of phosphorylated and total Zap70 protein at 10 minutes and 1 hour after activation of $CD8^+$ T cells with anti-CD3 or anti-Ly6a antibody plus anti-CD3 antibody (n=1). Numbers under lanes are the intensity of phosphorylated Zap70 divided by the intensity of total Zap70. **d,** Venn diagram of overlap between proteins significantly upregulated by anti-Ly6a antibody treatment with those significantly upregulated upon anti-CD3 antibody plus anti-CD28 antibody treatment ("common"), and the genes that upregulated by anti-Ly6a antibody treatment but not significantly upregulated upon anti-CD3 antibody plus anti-CD28 antibody treatment ("unique"). Data from[43]. Right: Percentage of proteins associated with ribosome or mitochondria based on cellular compartment GO terms for the “common” and “unique” proteins. **e,** GO terms for biological processes significantly associated with the "common" (left) and "unique" (right) proteins. **f,** String protein networks of the “common" (left) and "unique" (right) proteins.