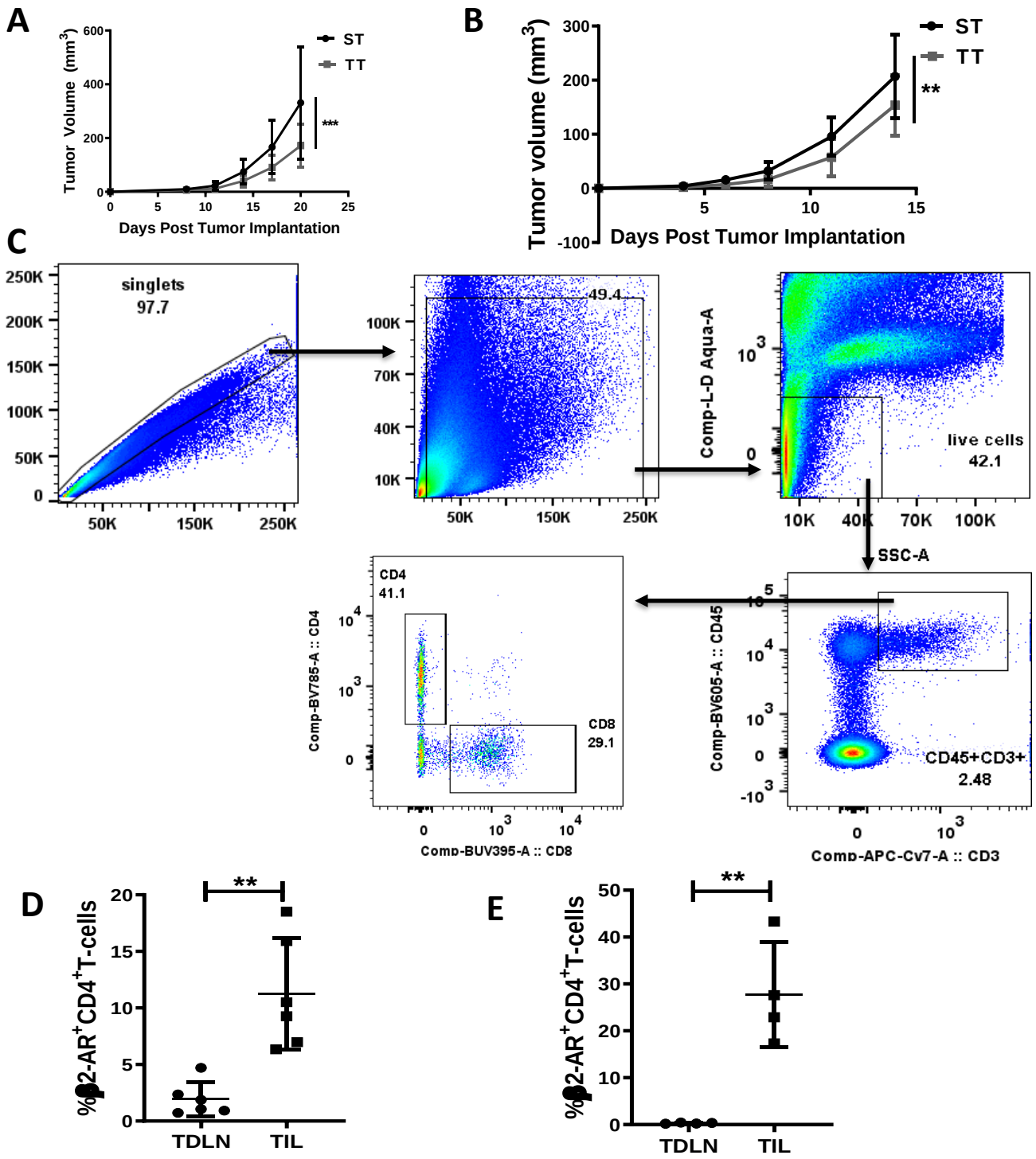
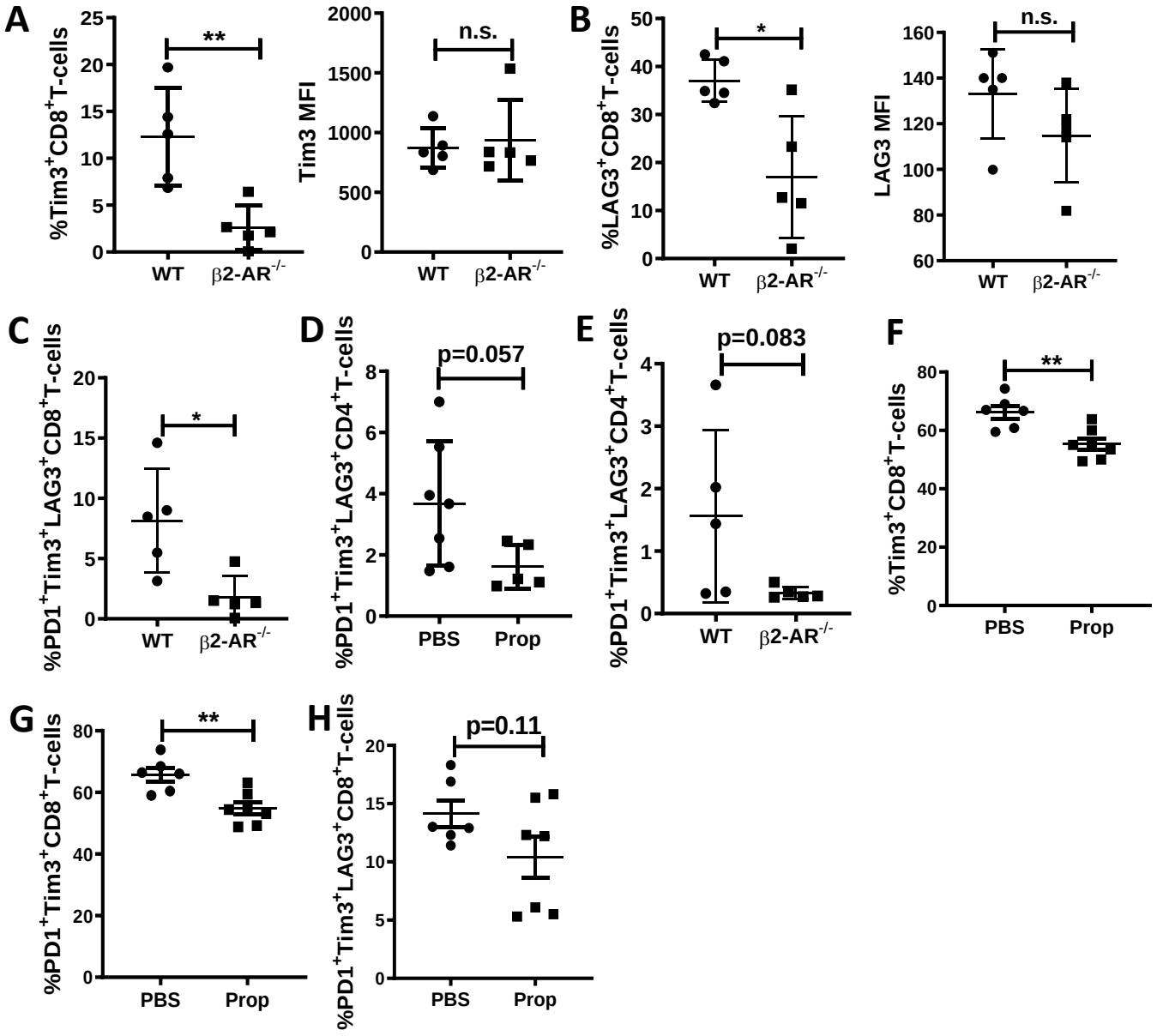


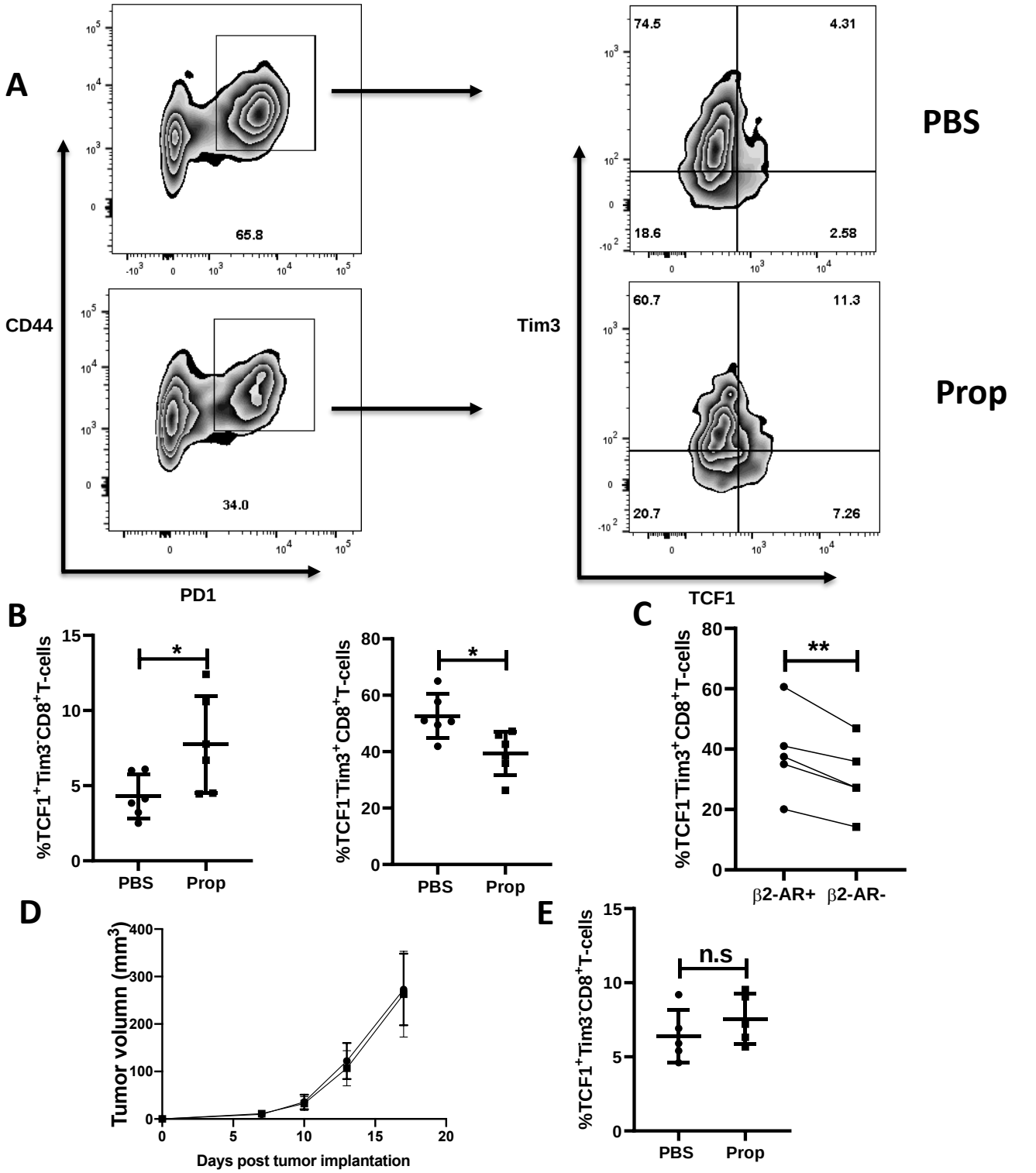
Supplemental Figure 1: TILs express β2-AR, and standard housing temperature induced chronic adrenergic stress in mice accelerates tumor growth

(A-B) Mice were housed at either ST or TT for 3 weeks before tumor implantation. 2×10^5B16-OVA cells (A) or CT26.CL25 (B) were injected into C57BL/6 mice or BALB/c mice, respectively. Tumor growth was monitored every 2-3 days. Data are presented as mean ± SD and from one of two independent experiments; Data was analyzed using 2-way ANOVA. (C) TILs were gated by following strategy: singlets>cells>live cells>$CD45^+CD3^+$T-cells>$CD4/CD8^+$T-cells (D-E) Frequency of β2-AR on $CD4^+$T-cells from TDLN and TME of untreated WT mice in B16-OVA (D) and CT26 (E) model were tested by flow cytometry; n=4-6/group; Data are presented as mean ± SD, and from one of two independent experiments; Data was analyzed using unpaired Student's t-test. **p<0.01, ***p<0.001



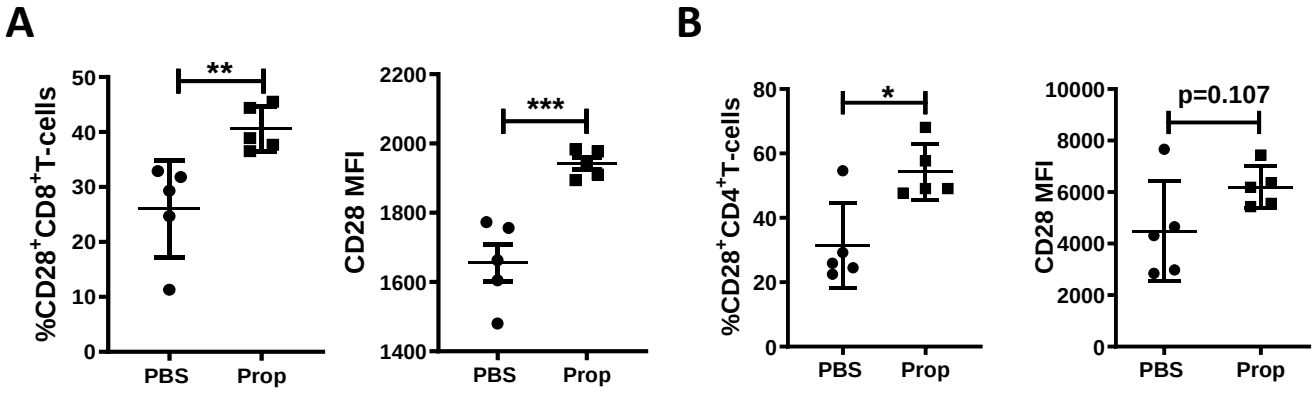
Supplemental Figure 2: Blockade of adrenergic signaling in stressed mice reduces immune checkpoint receptors expression on TILs

Single-cell suspensions were made from B16-OVA or CT26 tumors from mice treated with PBS or propranolol or from WT or β2-AR$^{-/-}$ mice (A-C & E). (A-B) Frequency and MFI of Tim3^{+} (A) and LAG3^{+} (B) CD8^{+}TILs from B16-OVA tumor. (C) Frequency of PD1^{+}Tim3^{+}LAG3^{+}CD8^{+}TILs from B16-OVA tumor. (D-E) Frequency of PD1^{+}Tim3^{+}LAG3^{+}CD4^{+}TILs from B16-OVA tumor bearing mice treated with PBS or propranolol (D) or from WT or β2-AR$^{-/-}$ mice (E). (F-H) Frequency of Tim3^{+}(F), PD1^{+}Tim3^{+} (G) PD1^{+}Tim3^{+}LAG3^{+} (H) on CD8^{+}TILs from CT26 tumor. n=5-7/group; Data are presented as mean ± SD, and from one of two independent experiments. Data was analyzed using unpaired Student's t-test, *p<0.05, **p<0.01



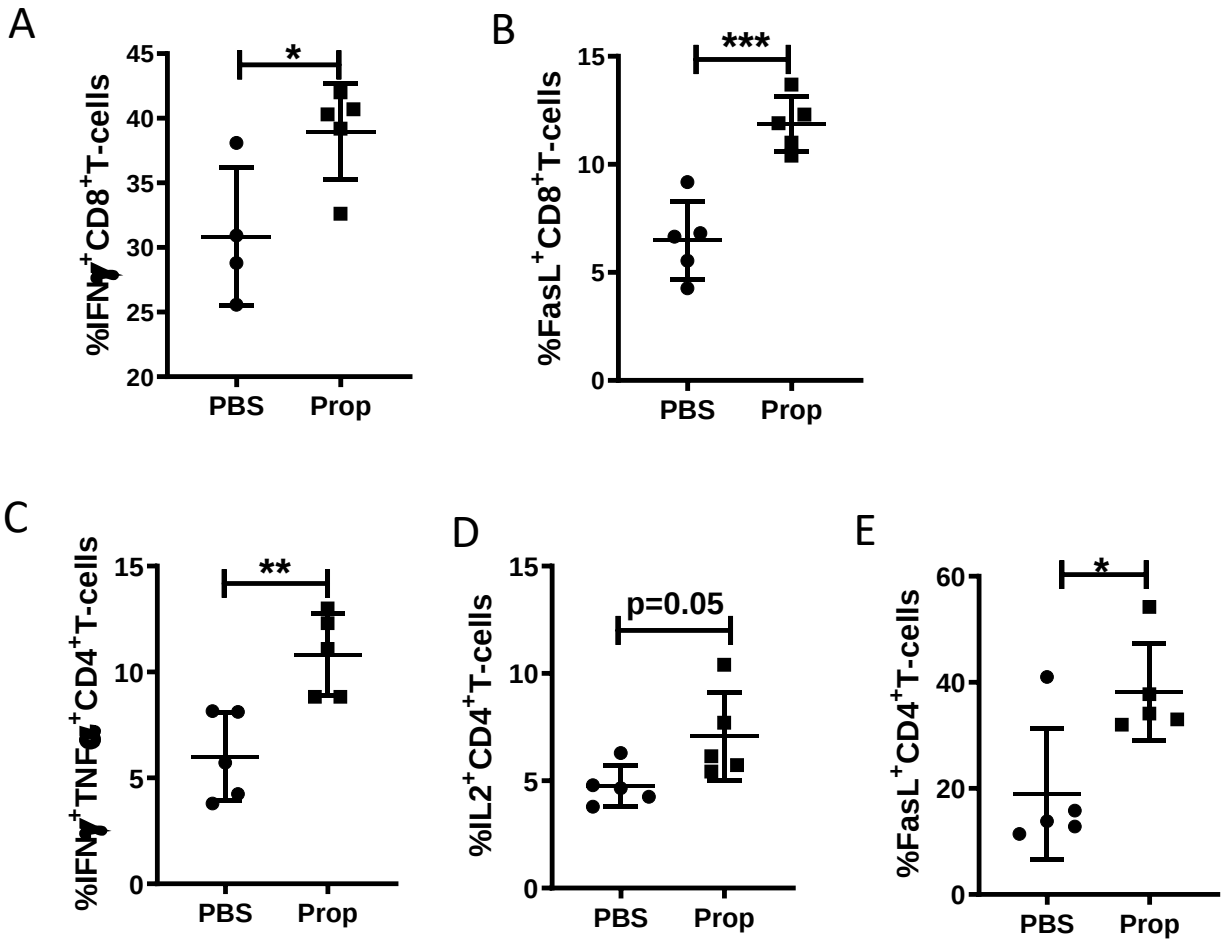
Supplemental Figure 3: Blockade of adrenergic signaling in stressed mice reduces terminally exhausted CD8⁺ TILs

(A) $CD8^+$ T-cells were first gated by PD-1^+CD44^+ then progenitor exhausted T-cells were gated by $TCF1^+Tim3^-$; terminally exhausted T-cells were gated by $TCF1^-Tim3^+$ (B) Frequency of progenitor and terminally exhausted $CD8^+$ TILs from CT26; (C) Frequency of terminally exhausted T-cells in β2-AR^+ and β2-AR^- $CD8^+$ TILs; Data was analyzed using paired Student's t-test (D) Tumor growth rate when mice were treated with PBS or propranolol 7 days after tumor implantation; (E) Frequency of progenitor exhausted T-cells in TME from mice treated with PBS or propranolol 7 days after tumor implantation. n=5-6/group; Data are presented as mean ± SD. Data was analyzed using unpaired Student's t-test except (C), *$p<0.05$, **$p<0.01$



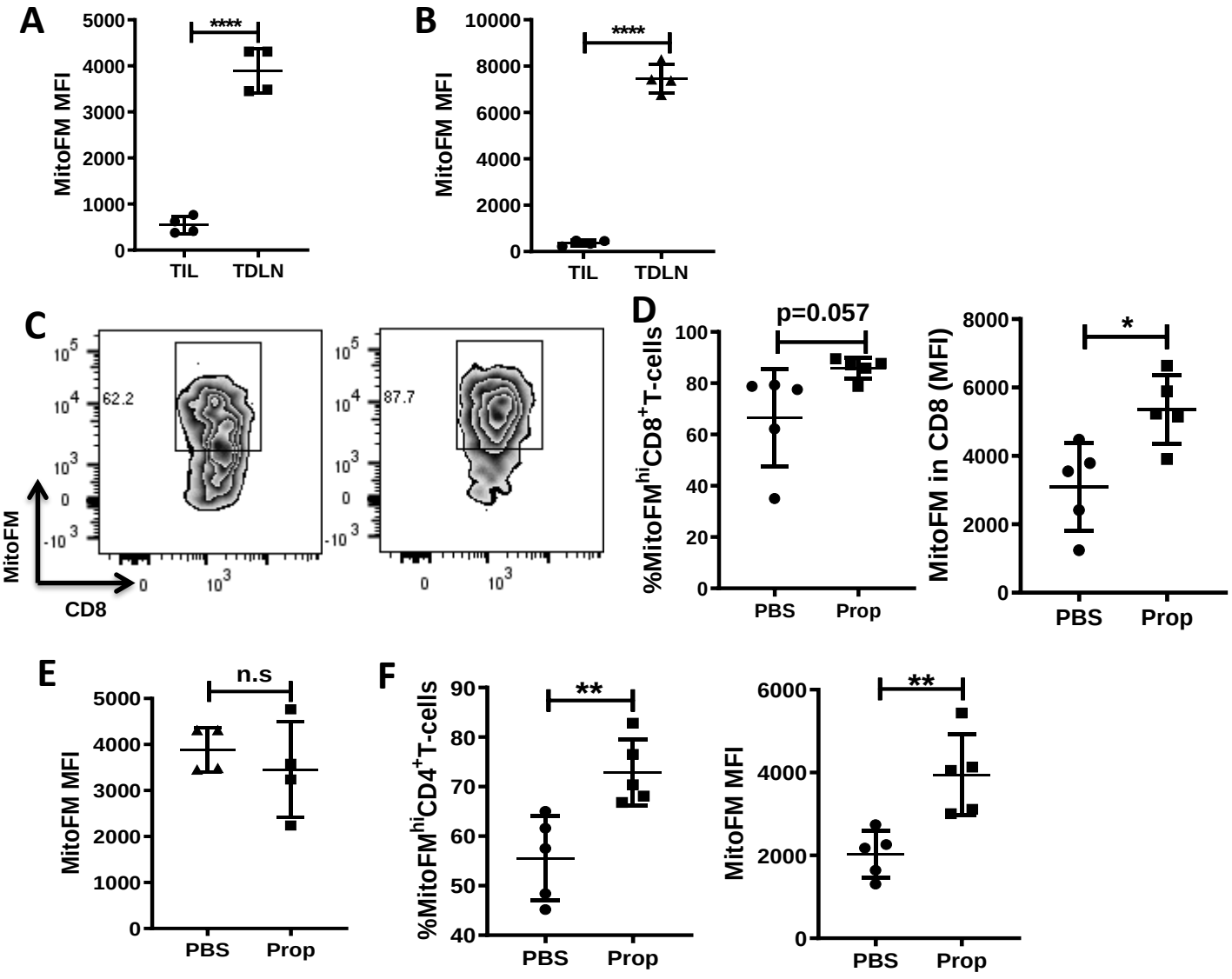
Supplemental Figure 4: Blockade of adrenergic signaling in stressed mice increases CD28 expression on TILs.

Single-cell suspensions were made from CT26 tumors from mice treated with PBS or propranolol. (A) Frequency and MFI of CD28 expression on $CD8^+$TILs. (B) Frequency and MFI of CD28 expression on $CD4^+$ TILs. $n = 5$/group; Data are presented as mean ± SD, and from one of two independent experiments; Data was analyzed using unpaired Student's t-test, *$p<0.05$.



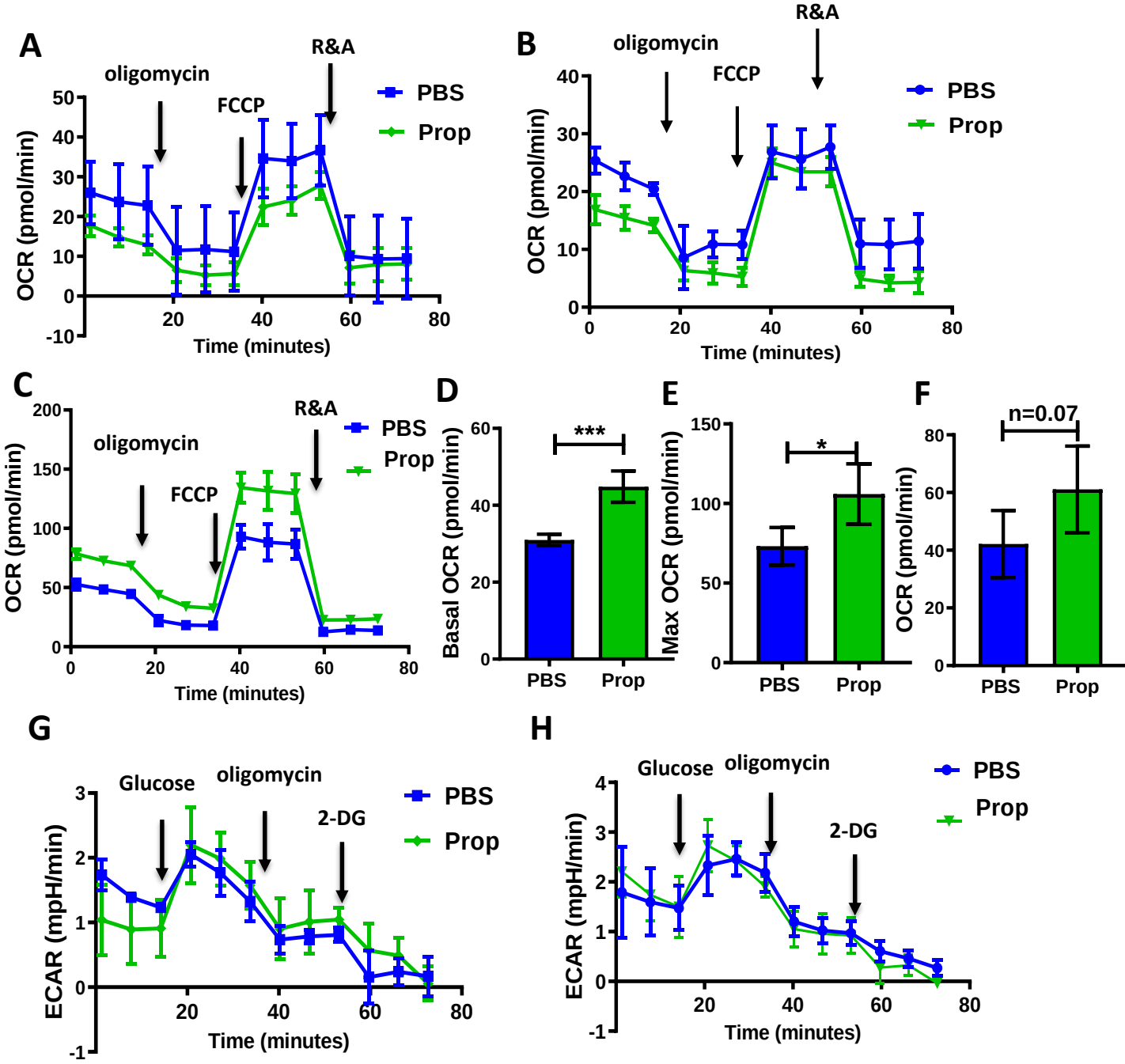
Supplemental Figure 5: Blocking β-AR signaling in stressed mice increases expression of anti-tumor cytokines and cytotoxic proteins in TILs.

Single-cell suspensions were made from CT26 tumors from mice treated with PBS or propranolol. (A-B) Frequency of IFN-γ (A) and IL2 (B) expression in $CD8^{+}$TILs. (C-E) Frequency of IFN-γ (C), IL2 (D) and FasL (E) expression in $CD4^{+}$T-cells from CT26 tumor. $n = 5$; Data are presented as mean ± SD, and from one of two independent experiments. Data were analyzed using unpaired Student's *t* -test *p<0.05, ***p<0.001



Supplemental Figure 6: Blocking β-AR signaling in stressed mice alleviates mitochondrial dysfunction of CD8$^+$T-cells in the TME

Single-cell suspensions were made from B16-OVA or CT26 from mice treated with PBS or propranolol. (A) MFI of mitochondrial mass of CD8$^+$T-cells from B16-OVA tumor or TDLN. (B) MFI of mitochondrial mass of CD4$^+$T-cells from B16-OVA tumor or TDLN. (C) Representative flow plots of mitochondrial mass of CD8$^+$TILs from CT26; (D) Frequency and MFI of mitochondrial mass of CD8$^+$; (E) MFI of mitochondrial mass of TDLN CD8$^+$T-cells from B16-OVA tumor-bearing mice treated with either PBS or propranolol. (F) Frequency and MFI of mitochondrial mass of CD4$^+$ TILs from CT26. $n = 4$-5/group; Data are presented as mean ± SD, and from one of two independent experiments. Data were analyzed using unpaired Student's *t*-test, *p<0.05, **p<0.01, ****p<0.0001



Supplemental Figure 7: Blocking β-AR signaling does not change metabolism of CD8⁺T-cells from TDLN or spleen

CD8⁺T-cells of spleen or TDLN or tumor from tumor bearing mice treated with either PBS or propranolol (pooled out from 5 mice/group) were isolated through CD8⁺T-cell isolation kit. OCR or ECAR were measured by adding 1×10^5CD8⁺T-cells to Seahorse Extracellular Flux Analyzer. (A) OCR of CD8⁺T-cells from TDLN; (B) OCR of CD8⁺T-cells from spleen; (C-F) CD8⁺TILs from CT26 (pooled from 5 mice per group) (C) plots of OCR of CD8⁺TILs. (D) Basal OCR of CD8⁺ TILs; (E) maximum OCR of CD8⁺ TILs; (F) spare respiratory capacity (SRC) of CD8⁺ TILs [addition of reagents indicated by arrows: (1) oligomycin; (2) FCCP; and (3) antimycin A and rotenone] (G) ECAR of CD8⁺T-cells from TDLN. (H) ECAR of CD8⁺T-cells from spleen. [addition of reagents indicated by arrows: (1) glucose; (2) oligomycin; and (3) 2-DG] $n = 4$-5; Data are presented as mean ± SD, and from one of two independent experiments; Data was analyzed using Student's t-test.