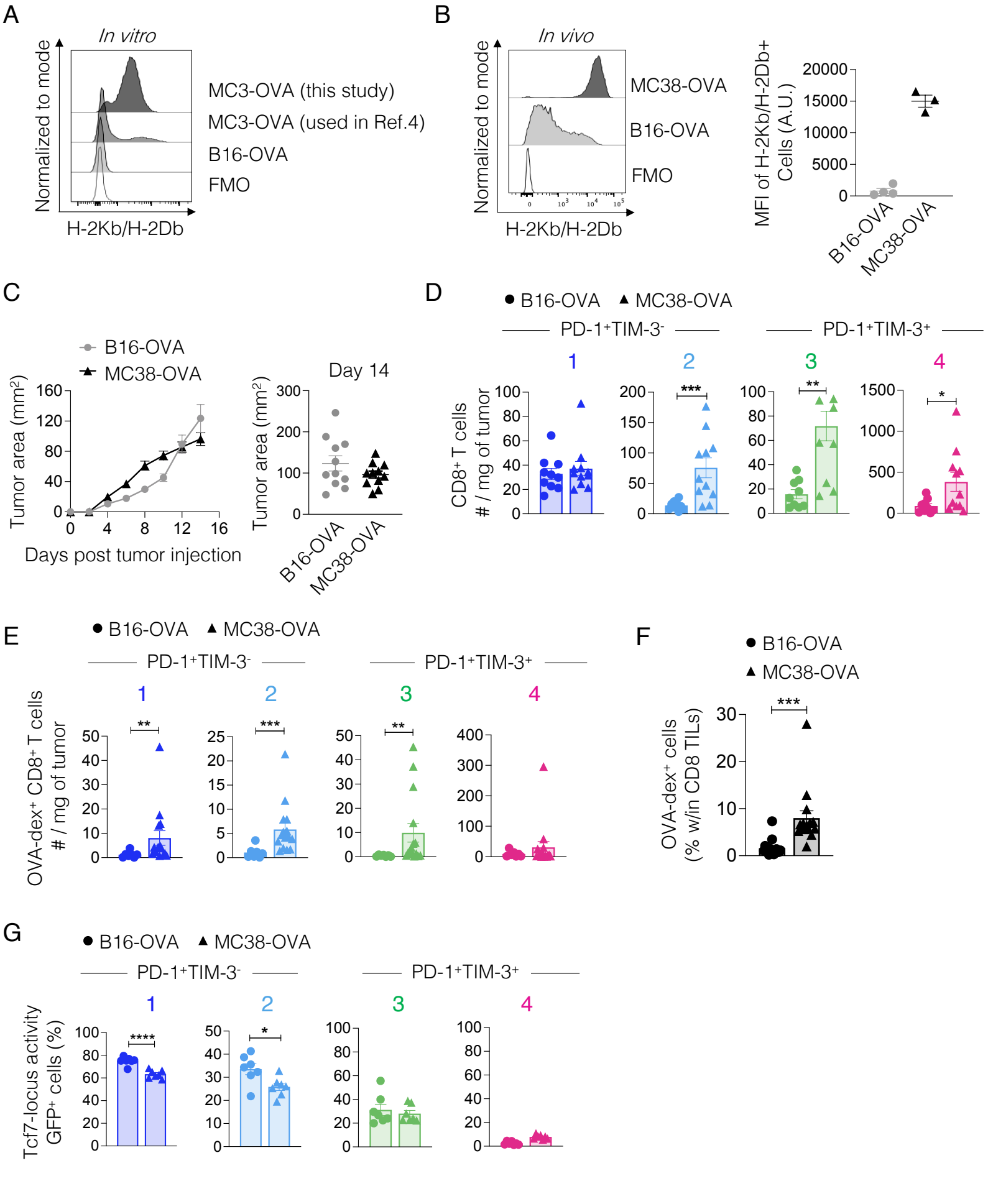
# SUPPLEMENTARY FIGURE 1

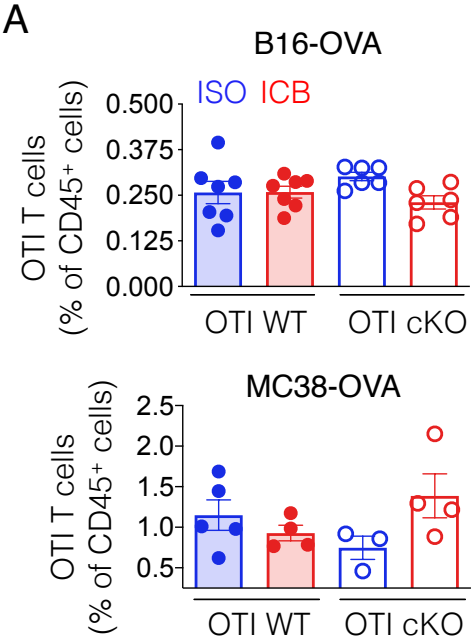


**Supplementary Figure 1. Characterization of B16-OVA and MC38-OVA tumors. Related to Figure 1**

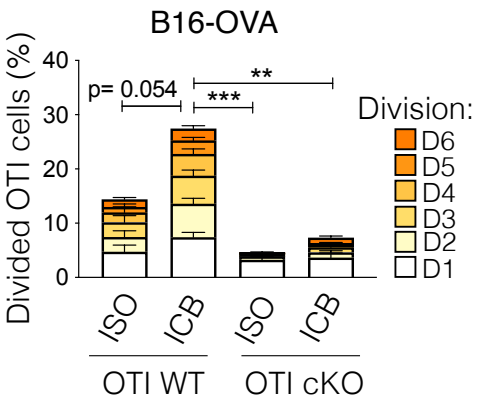
(A) Representative histograms of MHCI expression in B16-OVA, MC38-OVA (used in this study) and MC38-OVA tumor cells (used in Kurtulus et al., Ref 4) *in vitro*. (B) Representative histograms and quantification of MHC-I expression level (MFI) in B16-OVA or MC38-OVA tumors, 14 days post tumor implant in WT mice. (C) B16-OVA and MC38-OVA tumor area overtime in WT mice. For each mouse (dots) the tumor area at time of analysis (day 14) is shown. n=11, two experiments combined. (D) Number of cells per mg of tumor tissue. PD-$1^{+}$TIM-$3^{-}$SLAMF$6^{+}$CX3CR$1^{-}$$CD8^{+}$ population 1 (blue), PD-$1^{+}$TIM-$3^{-}$SLAMF$6^{lo/neg}$CX3CR$1^{+}$$CD8^{+}$ population 2 (light blue), PD-$1^{+}$TIM-$3^{+}$$CD39^{lo/neg}$SLAMF$6^{+}$$CD8^{+}$ population 3 (green), and PD-$1^{+}$TIM-$3^{+}$$CD39^{+}$SLAMF$6^{-}$ $CD8^{+}$ population 4 (pink) are gated as defined in Figure 1F within total $CD8^{+}$ TILs from MC38-OVA or B16-OVA-bearing WT mice. Analysis is done 14 days post tumor implant. Mann-Whitney t test, *p<0.05, **p<0.01, ***p<0.001. n=10-11, two experiments combined. (E) Number of cells per mg of tumor tissue. Shown are the populations defined in Figure 1F and gated within OVA-dextramer$^{+}$ $CD8^{+}$ TILs. Mann-Whitney t test, **p<0.01, ***p<0.001. n=5-15, two experiments combined. (F) Frequency of OVA-dextramer$^{+}$ $CD8^{+}$ TILs in MC38-OVA or B16-OVA tumors. Student's t test. ***P<0.001. n=15-16, two experiments combined. (G) Frequency of $GFP^{+}$ cells in $CD8^{+}$ TIL subsets identified in Figure 1F from MC38-OVA or B16-OVA tumors implanted in *Tcf7*-GFP reporter mice. Analysis is done 14 days post tumor implant. Student's t test, *p<0.05, ****p<0.0001. n=7, one experiment. All values are reported as mean±SEM.

# SUPPLEMENTARY FIGURE 2

A

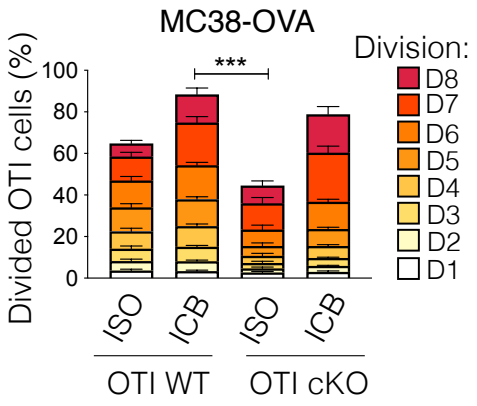



B



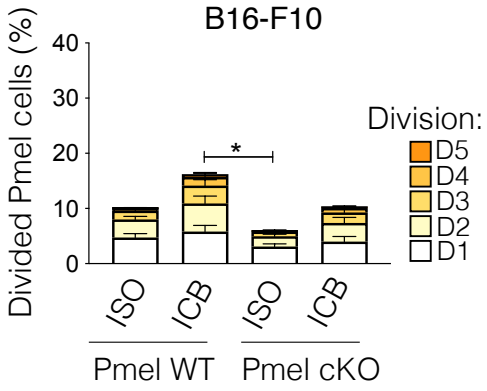

| Divisions | WT ISO | KO ISO | KO ICB |
|---|---|---|---|
| D1 | n.s. | ** | * |
| D2 | * | **** | **** |
| D3 | n.s. | *** | ** |
| D4 | n.s. | ** | ** |
| D5 | * | *** | *** |
| D6 | n.s. | *** | n.s. |

C




| Divisions | WT ISO | KO ISO | KO ICB |
|---|---|---|---|
| D1 | n.s. | n.s. | n.s. |
| D2 | n.s. | ** | n.s. |
| D3 | n.s. | ** | * |
| D4 | n.s. | *** | * |
| D5 | n.s. | *** | * |
| D6 | n.s. | ** | n.s. |
| D7 | n.s. | n.s. | n.s. |
| D8 | n.s. | n.s. | n.s. |

D

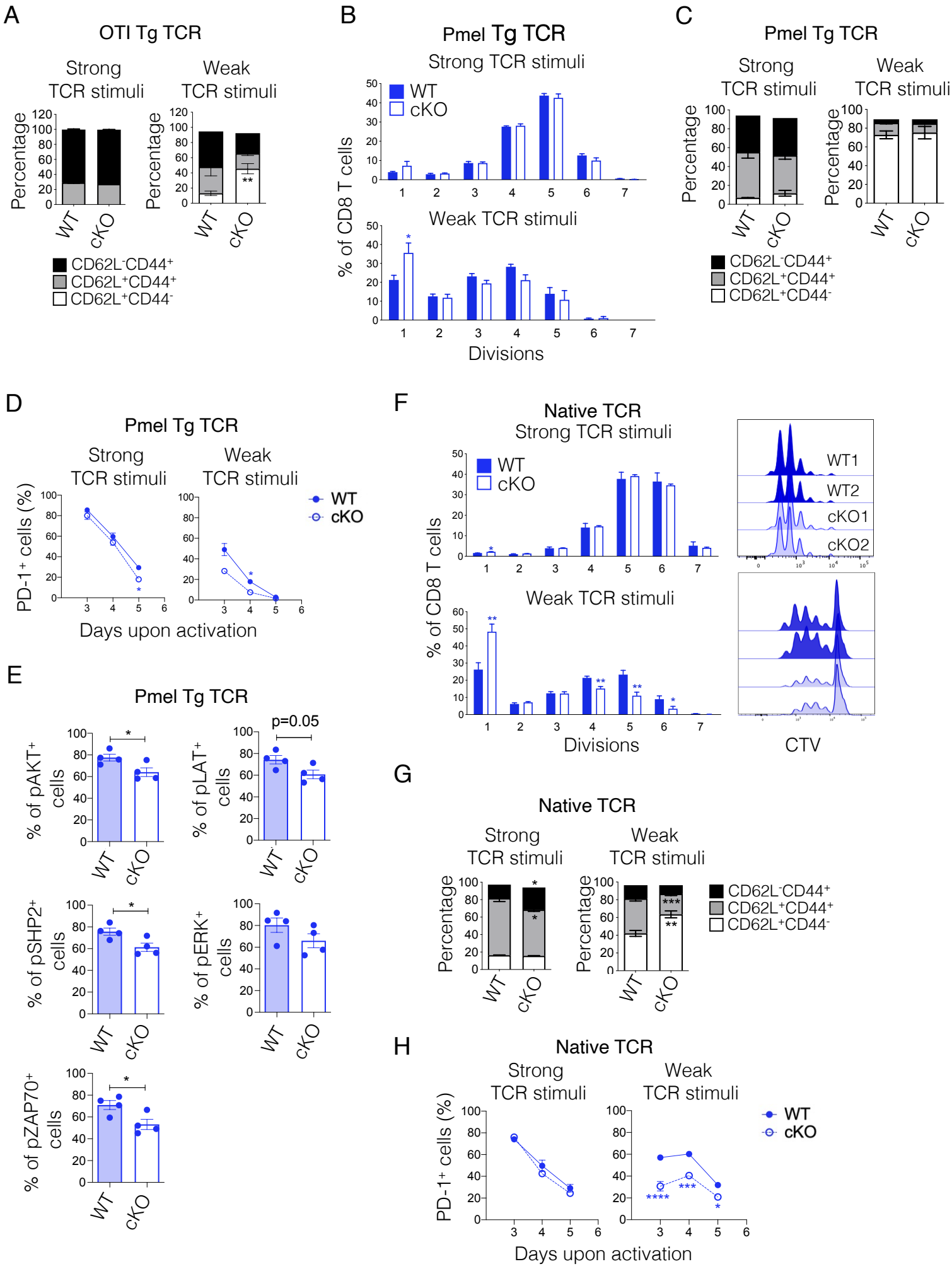



| Divisions | WT ISO | KO ISO | KO ICB |
|---|---|---|---|
| D1 | n.s. | n.s. | n.s. |
| D2 | n.s. | * | n.s |
| D3 | n.s. | * | n.s. |
| D4 | n.s. | * | n.s |
| D6 | n.s. | n.s. | n.s. |

**Supplementary Figure 2. Defective proliferation of TCF1 cKO T cells in the TDLN of poorly immunogenic tumors. Related to Figure 2**

(A) Frequency of adoptively transferred WT or TCF1 cKO OTI T cells in the TDLN of MC38-OVA (n=3-5, one experiment) or B16-OVA-bearing (n=6-7, two experiments combined) mice three days post adoptive transfer. (B-C) OTI WT and TCF1 cKO T cells are co-transferred at day 5 post tumor inoculation. Mice are treated at day 6 with one dose of ISO (IgG2a + IgG2b) or ICB (anti-TIM-3 + anti-PD-L1). T cells are analyzed three days post transfer (day 8). Frequency of proliferating WT ($CD45.1^+CD45.2^+$) or TCF1 cKO ($CD45.2^+$) OTI T cells in each cell division in the TDLN of B16-OVA- (B, n=10-11, two experiments combined) or MC38-OVA-bearing (C, n=12-13, two experiments combined) WT mice. (D) WT ($Thy1.2^+Thy1.1^+$) or TCF1 cKO ($Thy1.2^+$) Pmel T cells were co-transferred into B16-F10-bearing WT mice at day 5 post tumor injection. Mice are treated at day 6 with one dose of ISO (IgG2a + IgG2b) or ICB (anti-TIM-3 + anti-PD-L1). T cells are analyzed three days post transfer (day 8). Frequency of proliferating WT and TCF1 cKO Pmel T cells in the TDLN. n=13, two experiments combined. In Figure B-D one-way ANOVA is used to calculate the statistics related to the total frequency of proliferating cells (all comparisons are done versus the WT + ICB group). Statistics within each cell division is calculated using one-way ANOVA and is reported in the tables (all comparisons are done versus the WT + ICB group). *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$. All values are reported as mean±SEM.

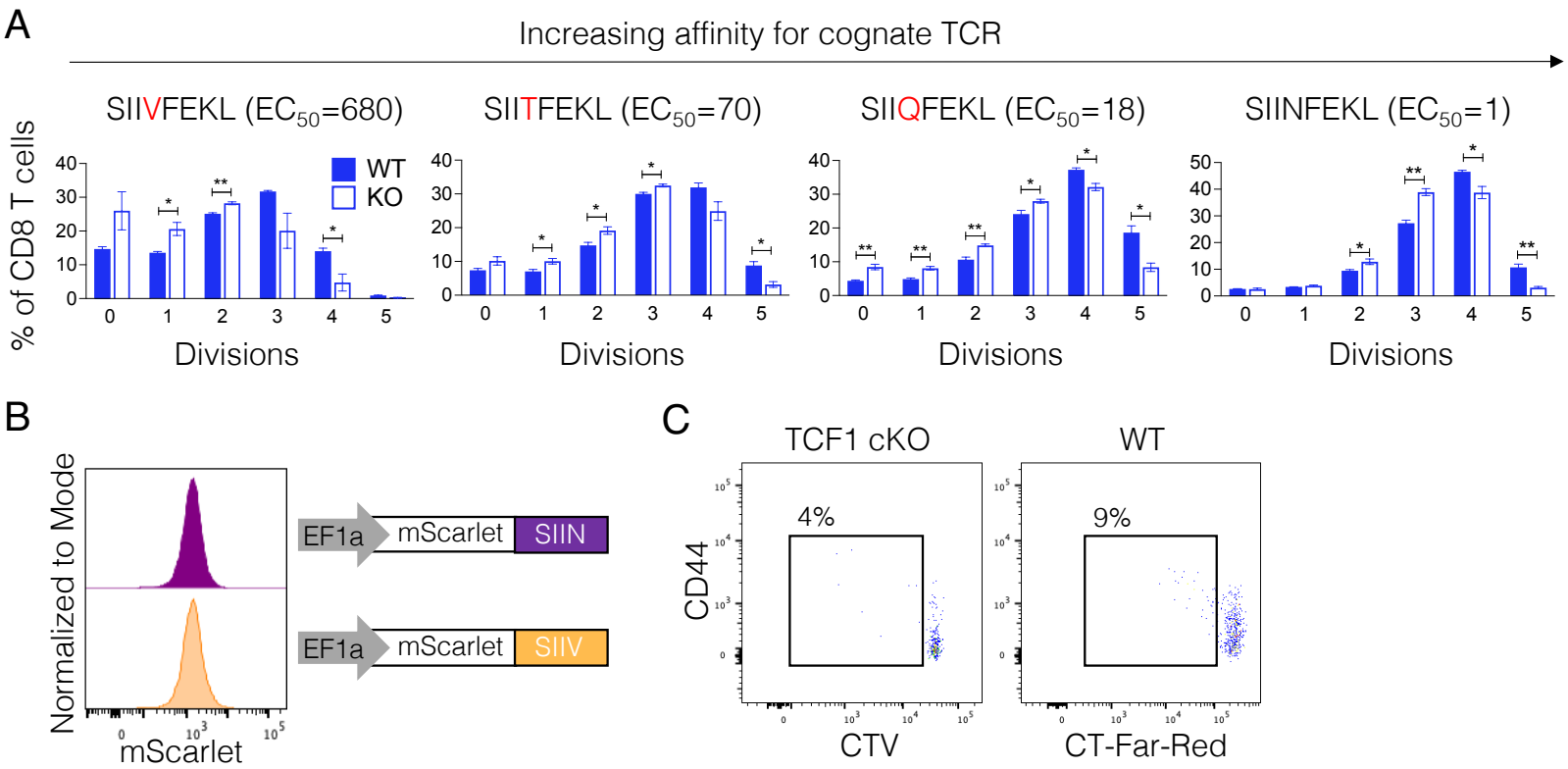
# SUPPLEMENTARY FIGURE 3



**Supplementary Figure 3. Defective proliferation and reduced TCR signaling in TCF1 cKO T cells activated *in vitro* with low TCR stimuli. Related to Figure 3.**

(A) Frequency of $CD62L^{+}CD44^{-}$, $CD44^{+}CD62L^{+}$ and $CD62L^{-}CD44^{+}$ cells within WT or TCF1 cKO OTI T cells, three days post activation with a high (1ug/ml; n=3, shown one out of 3 experiments) or low (0.3ug/ml; n=5-6, two experiments combined) dose of anti-CD3 + anti-CD28 *in vitro*. **p<0.01, Student's t test. (B) Frequency of WT or TCF1 cKO Pmel T cells in each cell division. Analyses is done three days post activation with a high (2ug/ml; n=3, one experiment) or low dose (0.3ug/ml; n=4, two experiments combined) of anti-CD3 + anti-CD28 *in vitro*. Student's t test, *p<0.05. (C) Frequency of $CD62L^{+}CD44^{-}$, $CD44^{+}CD62L^{+}$ and $CD62L^{-}CD44^{+}$ cells within WT or TCF1 cKO Pmel T cells from B. (D) Frequency of PD-$1^{+}$ cells in WT or TCF1 cKO Pmel T cells from B. Two-way ANOVA and Sidak's multiple comparison test, *p<0.05 (E) Frequency of phospho-AKT, phospho-LAT, phospho-SHP2, phospho-ERK and phospho-ZAP70 in WT or TCF1 cKO Pmel T cells 48 hours post activation with anti-CD3 + anti-CD28 (1ug/ml) *in vitro*. Student's t test, *p<0.05. n=4, two experiments combined. (F) Representative histograms and frequency of WT or TCF1 cKO T cells (expressing a native TCR repertoire) in each cell division. Analyses is done three days post activation with a high (4ug/ml; n=3) or low (0.3ug/ml; n=6, two experiments combined) dose of anti-CD3 + anti-CD28 antibodies *in vitro*. Student's t test, *p<0.05, **p<0.01. (G) Frequency of $CD62L^{+}CD44^{-}$, $CD44^{+}CD62L^{+}$ and $CD62L^{-}CD44^{+}$ cells within WT or TCF1 cKO T cells from F. (H) Frequency of PD-$1^{+}$ cells in WT or TCF1 cKO T cells from F. Student's t test, *p<0.05, **p<0.01, ***p<0.001. All values are reported as mean±SEM.

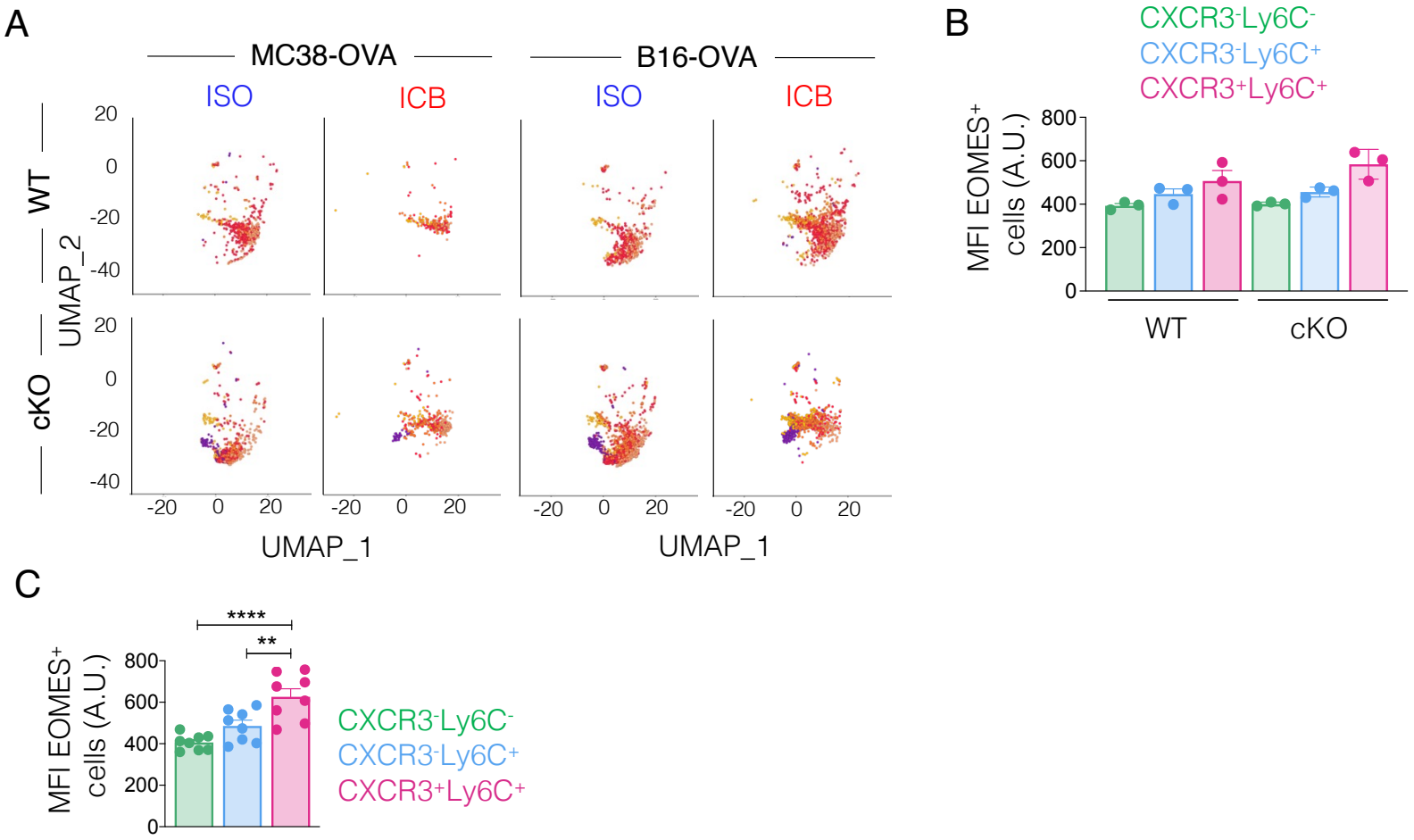
# SUPPLEMENTARY FIGURE 4



**Supplementary Figure 4. Defective proliferation of TCF1 cKO T cells stimulated with low affinity OVA peptide variants. Related to Figure 3.**

(A) Frequency (mean±SEM) of WT and TCF1 cKO OTI T cells in each cell division, three days post *in vitro* co-culture with BM-DC pulsed with SII**V**FEKL (17.78nM), SII**T**FEKL (0.219nM), SII**Q**FEKL (0.024nM), and SII**N**FEKL (0.024nM) peptides. For each peptide, the highest dose tested that resulted in overt defective proliferation in TCF1 cKO T cells is shown. Student's t test. *p,0.05, **p<0.01. n=3, one experiment. (B) Representative plot of mScarlet expression in MC38 tumor cells engineered with the indicated lentiviral vectors. (C) Representative plots of proliferating (cell trace dye-diluted) WT or TCF1 cKO OTI T cells adoptively co-transferred into mice implanted with MC38-SII**V**FEKL tumors and harvested from the TDLN three days post T cell transfer (i.e. day 9 post tumor injection).

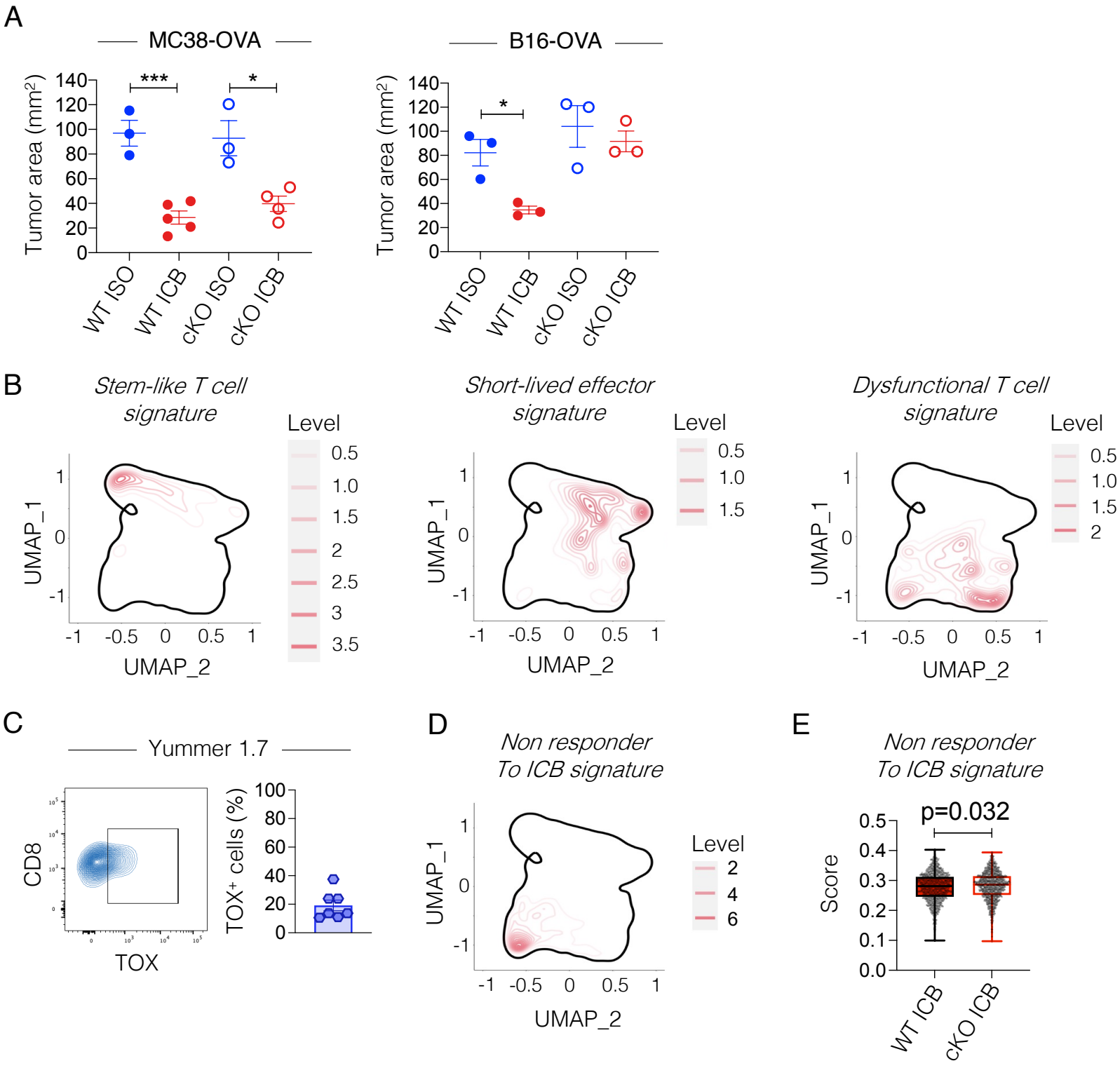
# SUPPLEMENTARY FIGURE 5



**Supplementary Figure 5. Composition of naïve cells within WT and TCF1 cKO OTI T cells present in the TDLN of MC38-OVA- or B16-OVA-bearing mice**. **Related to Figure 5.**

(A) Distribution of single-cell events from the naïve cluster (from Figure 5A) in each experimental sample and treatment condition. Single-cells are colored and grouped based on the 5 naïve clusters from Figure 5A. (B) EOMES expression level (MFI, mean±SEM) in the three naïve subsets defined in Figure 5E and identified in tumor-free WT and TCF1 cKO mice. n=3, shown one out of two experiments. (C) EOMES expression level (MFI, mean±SEM) of in the indicated naïve subsets found in adoptively transferred WT or TCF1 cKO OTI T cells harvested from the TDLN of MC38-OVA-bearing mice (n=8, shown one out of two experiments). Analysis is done three days post adoptive T cell transfer. One-way ANOVA, **p<0.01, ****p<0.0001.

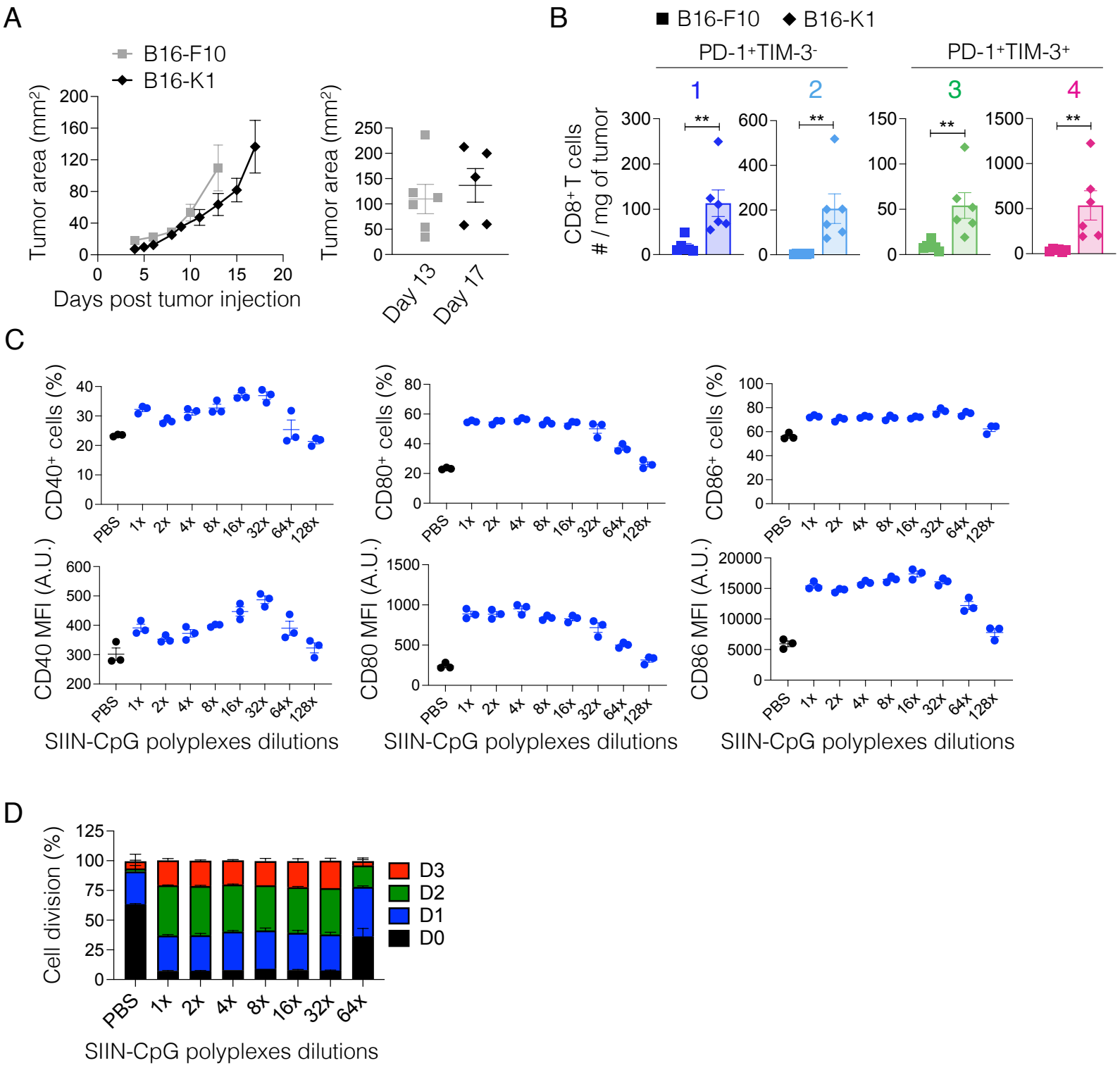
# SUPPLEMENTARY FIGURE 6



**Supplementary Figure 6. Transcriptional analysis of WT and TCF1 cKO CD8$^+$ TILs from MC38-OVA or B16-OVA tumors. Related to Figures 6 and 7.**

(A) MC38-OVA or B16-OVA area (mean±SEM, day 11 or 12 post tumor injection, respectively) from WT (n=3-5) or TCF1 cKO (n=3-4) mice used to perform scRNA-seq on CD8$^+$ TILs. Mice received either control or anti-PD-L1 + anti-TIM-3 antibodies on day 6 and 9 prior to harvesting CD8$^+$ TILs. (B) Projection of stem-like, short-lived effector and dysfunctional T cell signatures displayed as single-cell density over the global UMAP from Figure 6A. (C) Representative plot and quantification (frequency, mean±SEM) of TOX expression in PD-1$^+$TIM-3$^+$CD8$^+$ TILs present in Yummer1.7 melanomas. n=7, one experiment. (D) Projection of non-responder to ICB signature displayed as single-cell density over the global UMAP from Figure 6A. (E) Expression score of the non-responder to ICB signature on the proliferating TOX$^+$TIM-3$^+$PD-1$^+$CD8$^+$ TILs (cluster 8 from figure 6A) from WT or TCF1 cKO B16-OVA-bearing mice treated with ICB. Mann-Whitney test, *$p<0.05$. Bottom, middle, and upper lines denote lower quartile (Q1), median and upper quartile (Q3), respectively. Whiskers denote minimum (Q1 − 1.5 x interquartile range) and maximum (Q3 + 1.5 x interquartile range).

# SUPPLEMENTARY FIGURE 7



**Supplementary Figure 7. Characterization of B16-F10 and B16-K1 tumors and of the SIIN-CpG polyplex vaccine. Related to Figure 8.**

(A) B16-F10 and B6-K1 tumor growth (area, mean±SEM) in WT mice. For each mouse (dots) the tumor area at time of analysis (day 13 for B16-F10 or day 17 for B16-K1) is shown. n=6, one experiment. (B) Number of cells (mean±SEM) per mg of tumor tissue. PD-$1^{+}$TIM-$3^{-}$ SLAMF$6^{+}$CX3CR$1^{-}$CD$8^{+}$ population 1 (blue), PD-$1^{+}$TIM-$3^{-}$SLAMF$6^{lo/neg}$CX3CR$1^{+}$CD$8^{+}$ population 2 (light blue), PD-$1^{+}$TIM-$3^{+}$CD$39^{lo/neg}$SLAMF$6^{+}$CD$8^{+}$ population 3 (green), and PD-$1^{+}$TIM-$3^{+}$CD$39^{+}$SLAMF$6^{-}$ CD$8^{+}$ population 4 (pink) are gated as defined in Figure 1F within total CD$8^{+}$ TILs present in B16-F10- or B16-K1-tumor-bearing WT mice. Analysis is done at 13 (B16-F10) or 17 (B16-K1) days post tumor implant. n=6, one experiment. Mann-Whitney t test, **$p<0.01$. (C) Frequency (mean±SEM) and levels (MFI, mean±SEM) of the indicated co-stimulatory molecules expressed on splenic dendritic cells 18 hours after pulsing with increasing dilutions of the SIIN-CpG polyplexes or with PBS as control. n=3, one experiment. (D) Frequency (mean±SEM) of CD$8^{+}$ T cells in the indicated cell divisions three days after co-culture with splenic DCs pulsed with the indicated dilutions of SIIN-CpG polyplexes. n=3, one experiment.