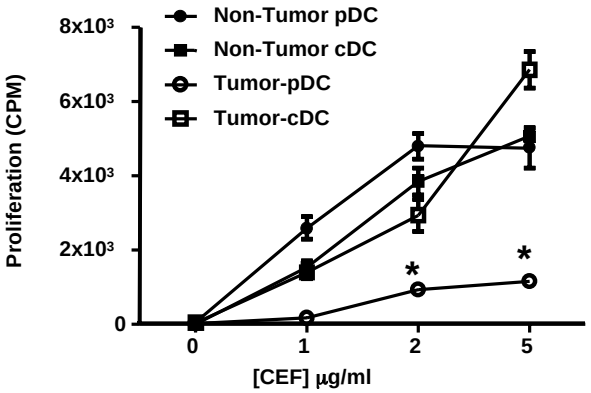
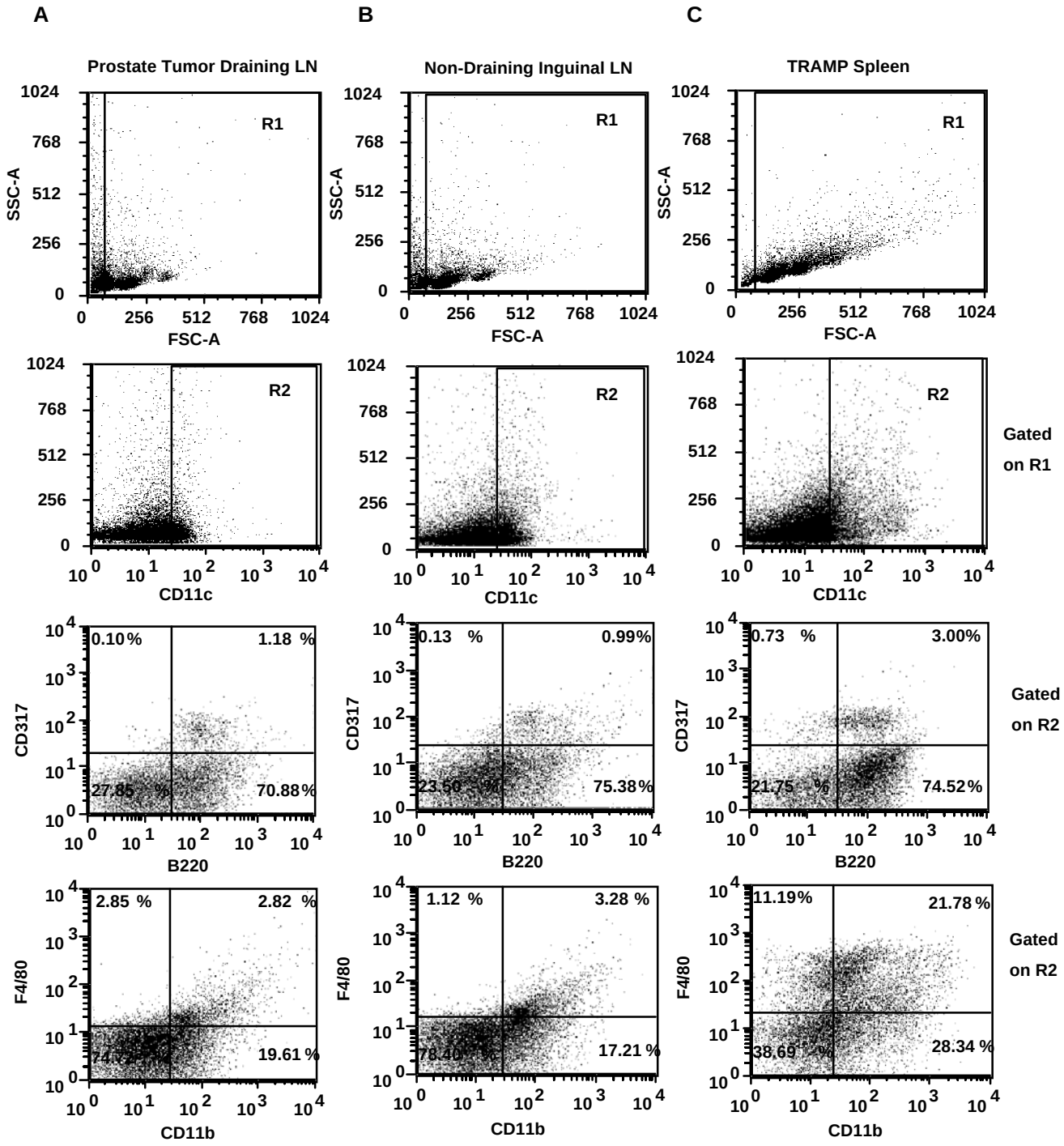
A

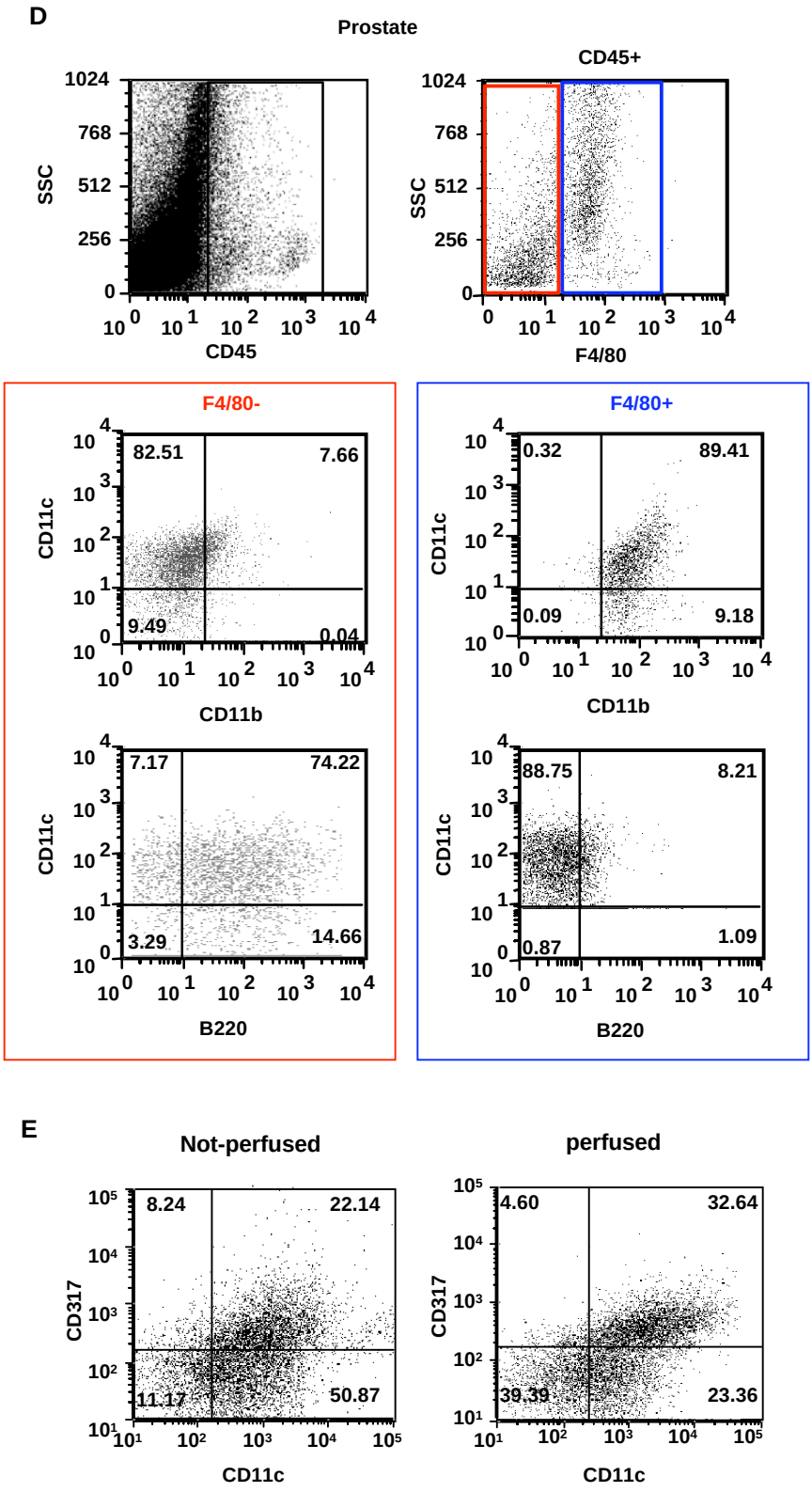
CD45+	3.4%
CD14+/CD16+	63%
CD11c+	21%
CD123+/ILT7+	14%

B

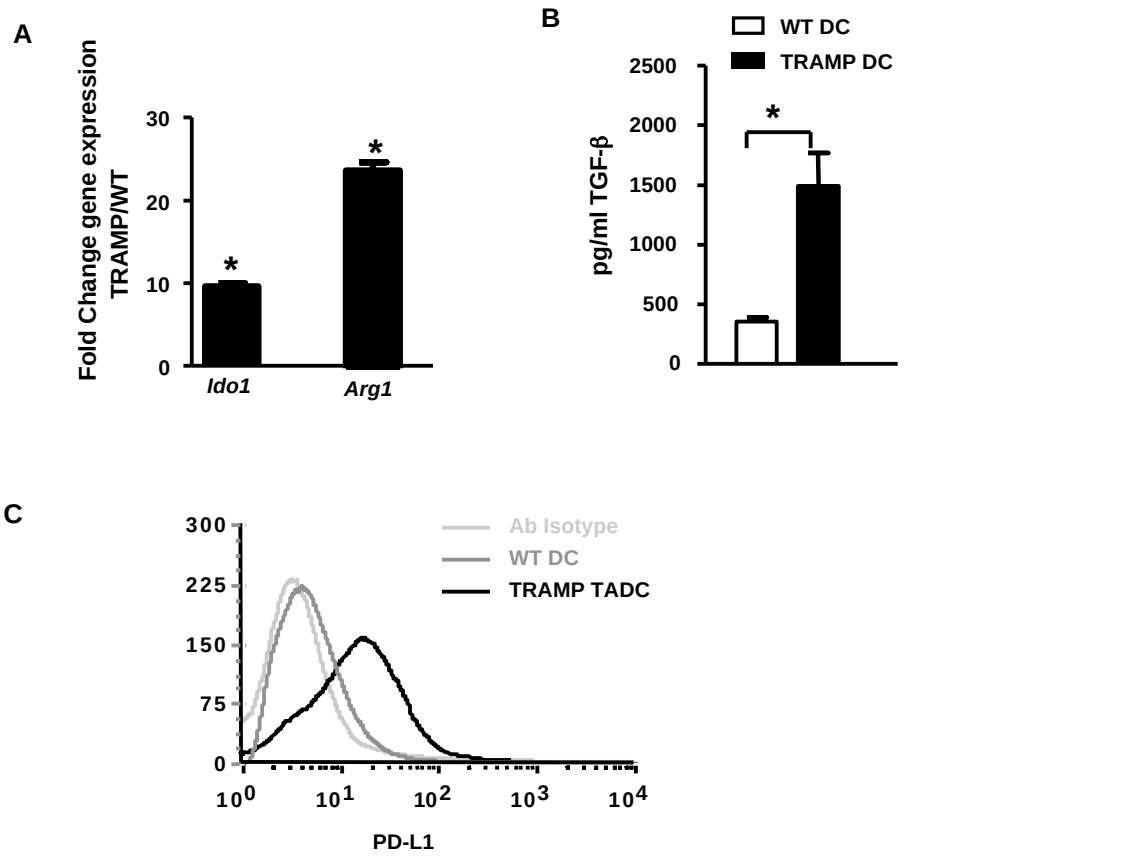


Supplemental Figure 1: pDCs isolated from human prostate tumors induce T cell tolerance. (A) Myeloid cell populations were quantified in human prostate tumor samples by first gating on $CD45^+$ cells. (B) DC subsets were tested for their ability to induce T cell tolerance. *$p<0.01$ for Tumor pDC vs. Non-Tumor pDC. Data are presented as the combined average of triplicates for two patients $\pm$ SEM.

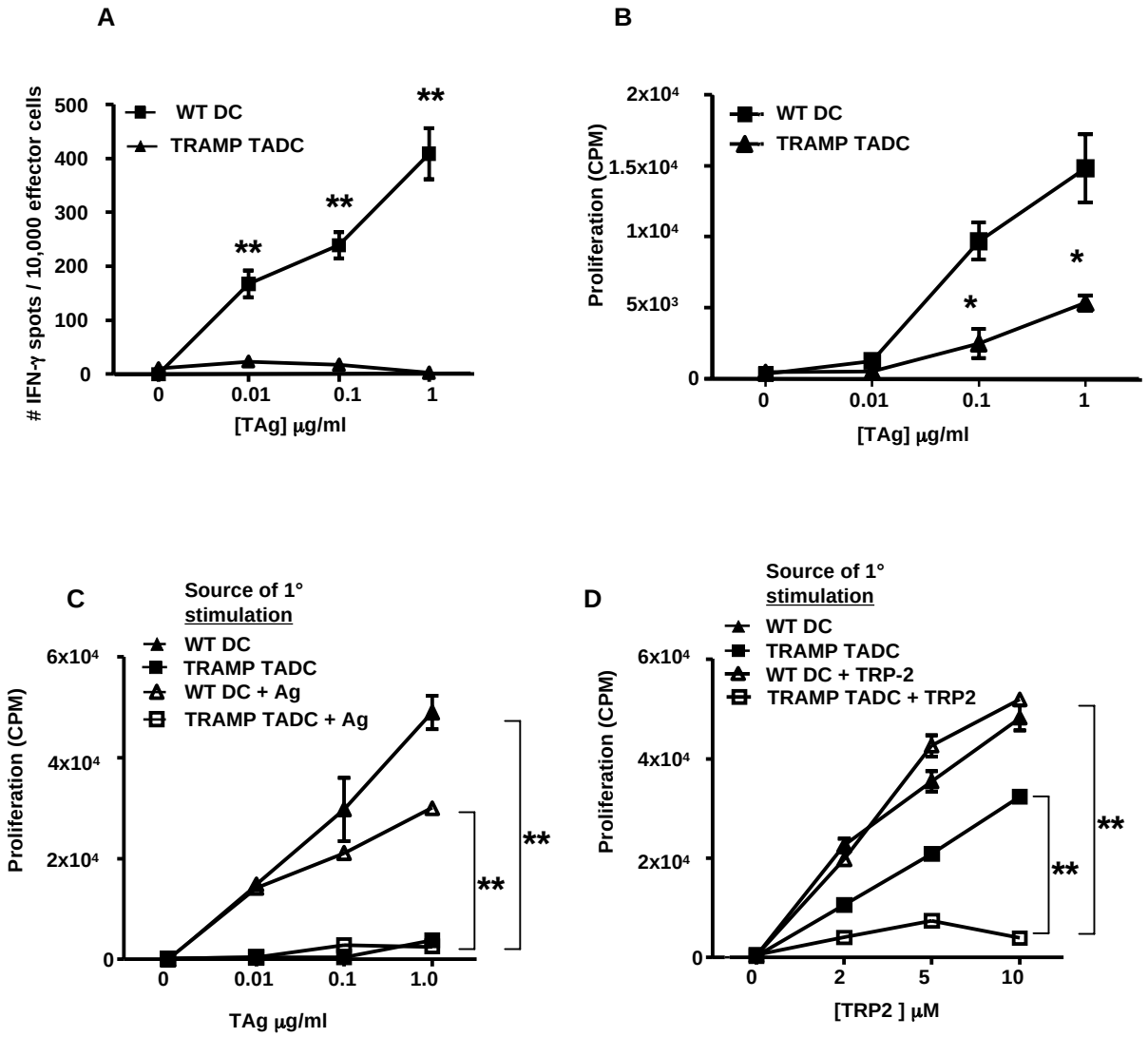

A
B
C
Prostate Tumor Draining LN
Non-Draining Inguinal LN
TRAMP Spleen
SSC-A
FSC-A
R1
R2
CD11c
CD317
B220
F4/80
CD11b
Gated on R1
Gated on R2
Gated on R2
0.10%
1.18 %
27.85 %
70.88%
0.13 %
0.99%
23.50 %
75.38%
0.73 %
3.00%
21.75 %
74.52%
2.85 %
2.82 %
19.61 %
1.12 %
3.28 %
78.40 %
17.21 %
11.19%
21.78 %
38.69 %
28.34 %



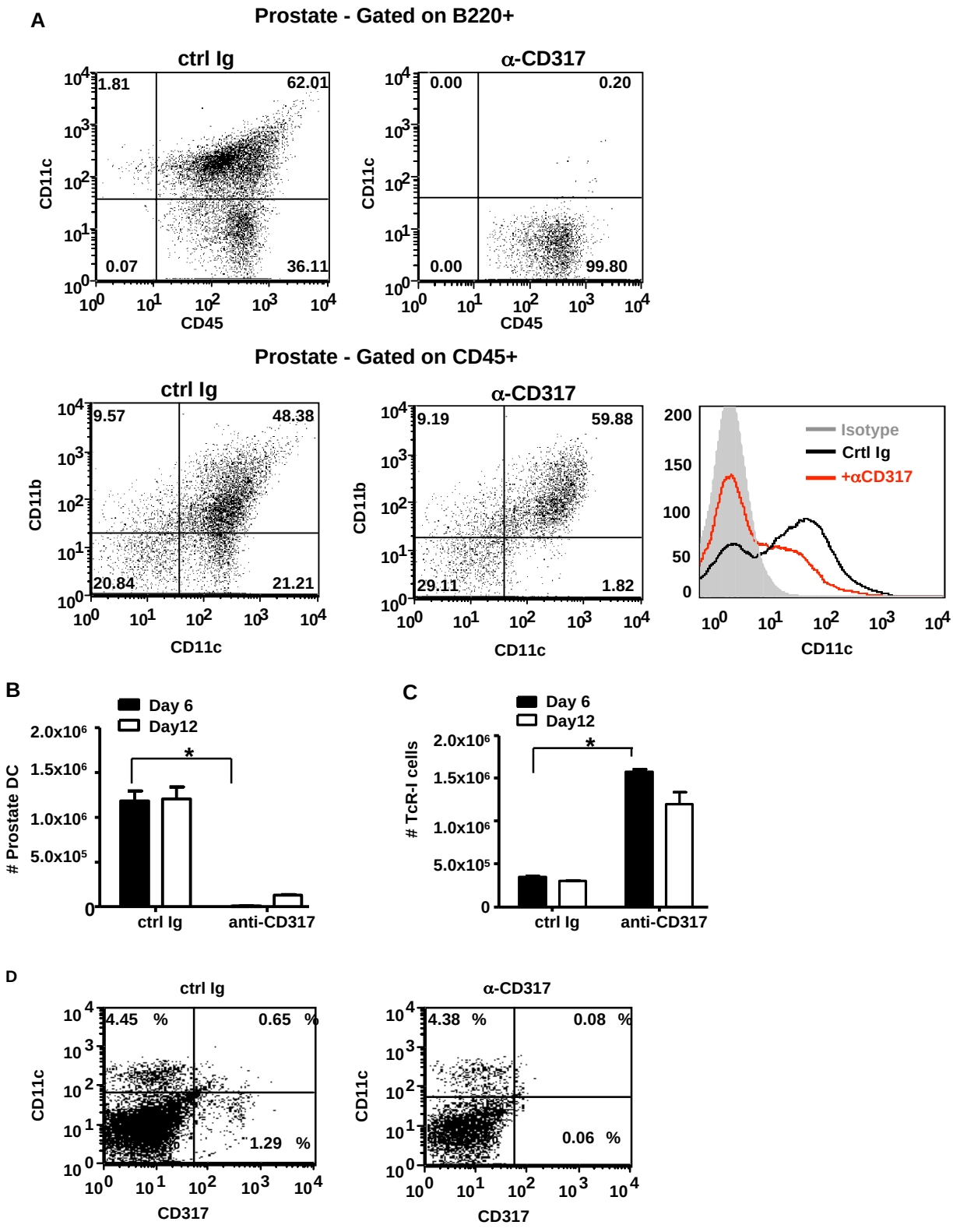
Supplemental Figure 2: Phenotypic analyses of myeloid cells in TRAMP mice. Dendritic cell subsets were identified in (A) tumor draining lymph nodes, (B) inguinal, non-draining lymph nodes, (C) spleen and (D) prostates (gated on CD45+). B220+/CD317+/F4/80- cells were classified as pDC, CD11c+/CD11b+/F4/80-cells were classified as cDC, and CD11c+/CD11b+/F4/80+cells were classified as TAMs. (E) DC frequency, number and phenotype were consistent in perfused and non-perfused prostates (CD45+ gating applied).



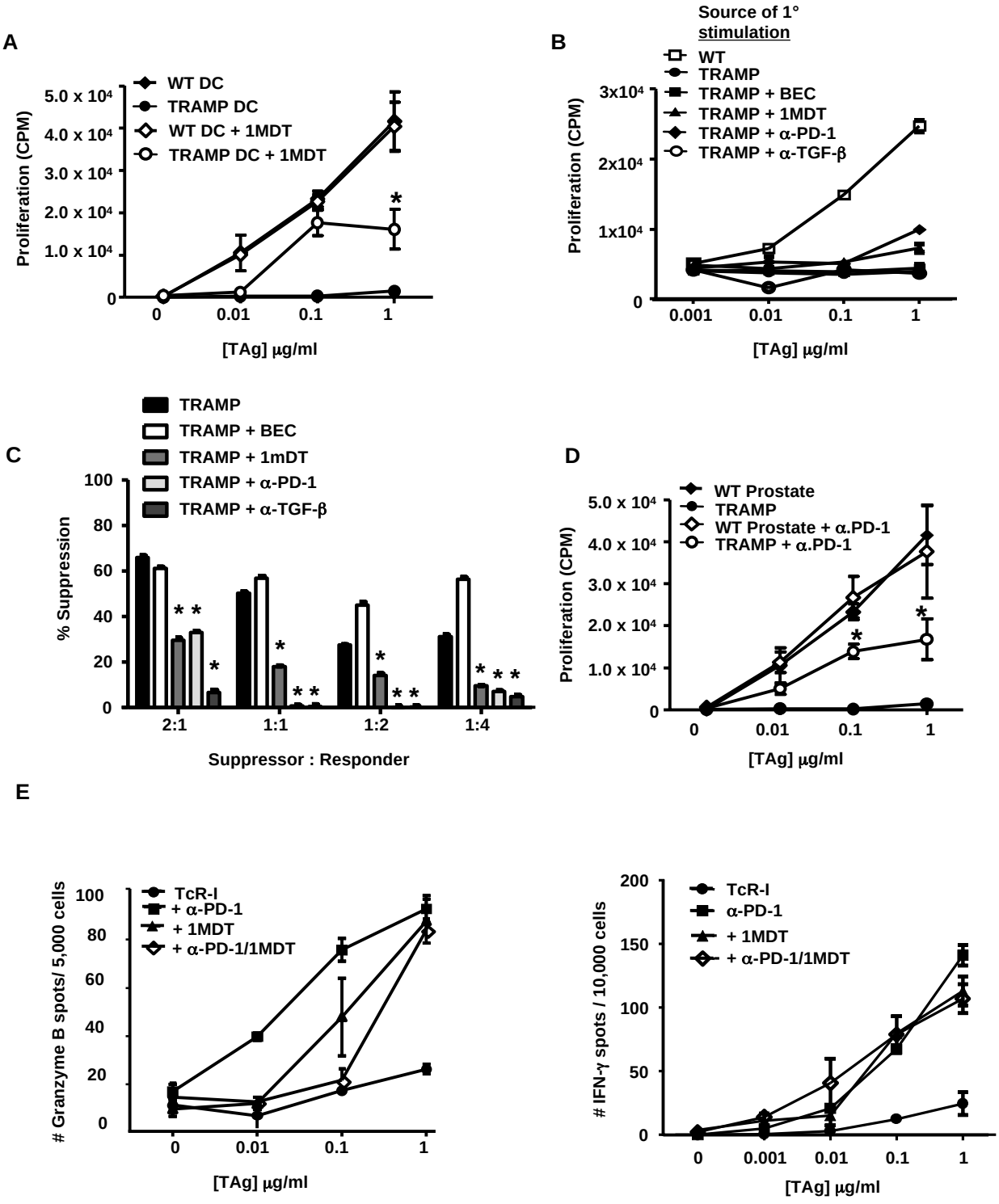
Supplemental Figure 3: TRAMP DC express elevated IDO, Arginase, TGF-β, and PD-L1. For detection of suppressive mediators, microarray findings presented in Table I were confirmed by (A) real-time qPCR for IDO and ARG, (B) ELISA for TGF-β production or (C) flow cytometric analysis for PD-L1. *p<0.001 (Student's *t*-test) TRAMP vs. WT. Data representative of 4 independent trials, 3-5 mice per group, mean +S.D. (A,B)

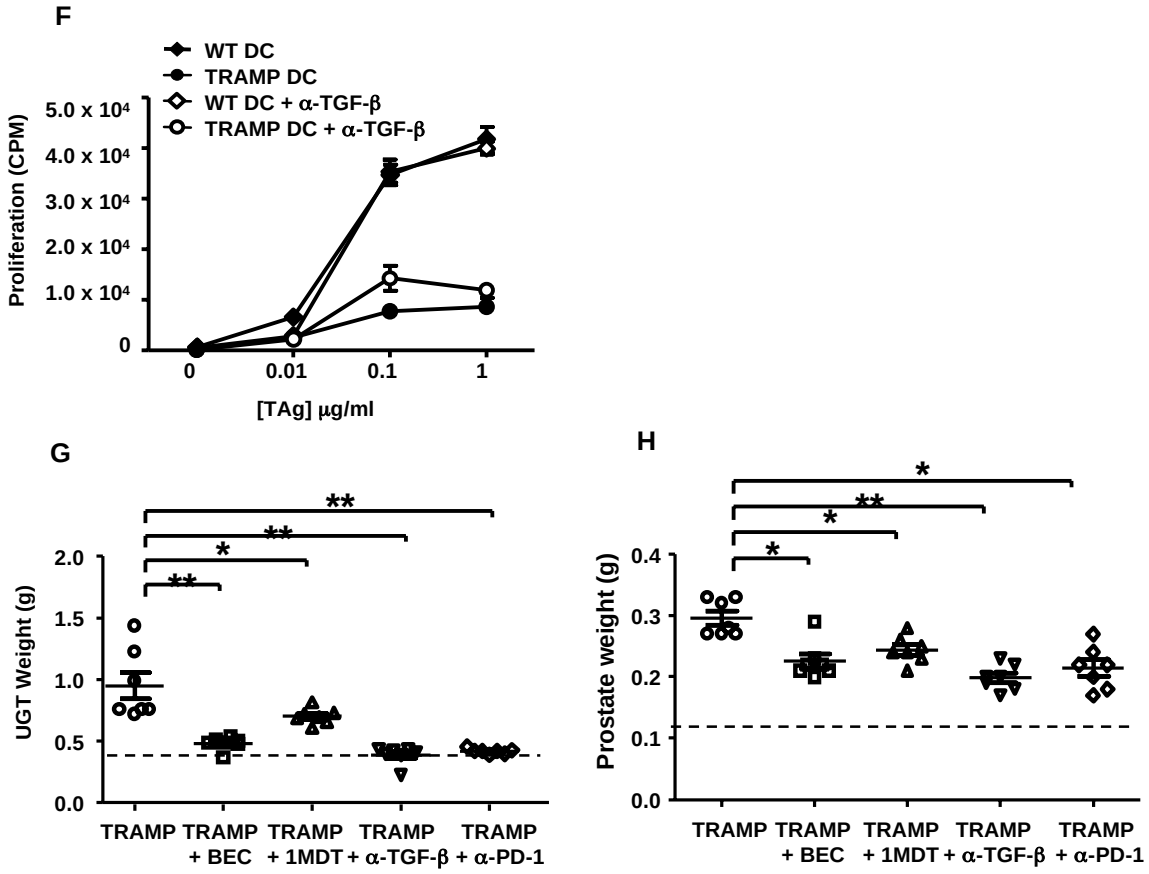


Supplemental Figure 4: TRAMP TADC tolerize naïve and effector TcR-I T cells *in vitro*. Naïve (A) or effector (B) TcR-I T cells were co-cultured *in vitro* with TAg peptide and prostatic DCs from TRAMP or WT mice for 4 days prior to secondary stimulation with TAg peptide and splenic APCs. DCs were isolated from 3-5 mice per group. (C,D) TADC were co-cultured *in vitro* with (C) TcR-I or (D) TcR-Mel T cells +/- antigen for 4 days prior to secondary stimulation with splenocytes and TAg or TRP2, respectively. Data representative of 2 independent trials, of 3 mice per group, mean ± S.D. **p<0.0001, *p<0.001 (Student's *t*-test) TRAMP vs. WT.

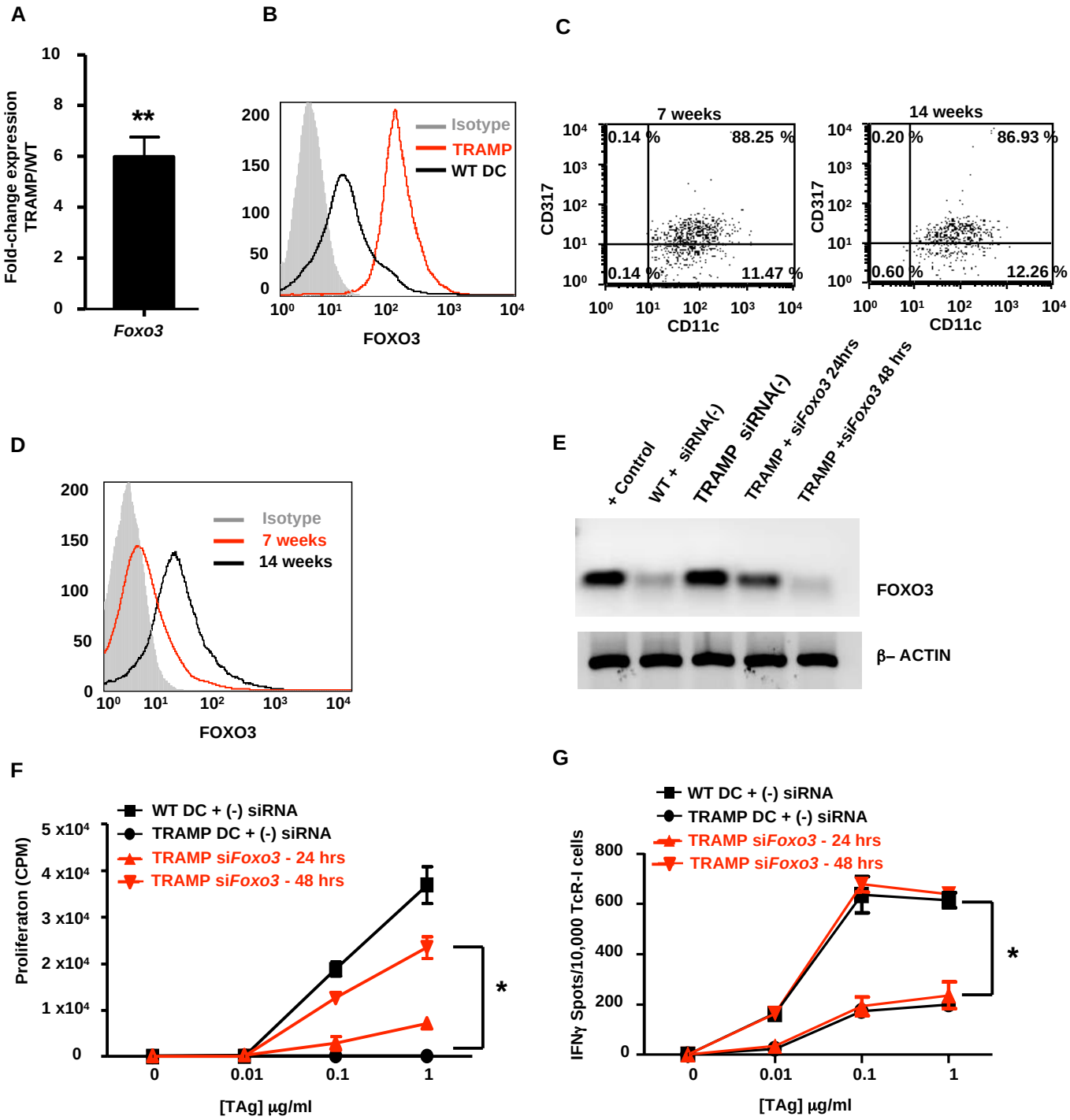


Supplemental Figure 5: Depleting TADC with anti-CD317 Ab results in increased TcR-I cell infiltration into the prostate. Anti-CD317 was injected i.p. on days -1 and 0 relative to T cell transfer. Prostatic tissues were harvested 6 or 12 days post-T cell transfer. Prostate digests were assayed for the presence of (A,B) $B220^{+}/CD11c^{+}$ TADC and (C) TcR-I T cells. Data representative of 4 independent trials of 5 mice per group, mean ± S.D. *$p<0.001$ (D) CD317 depletion was also assessed in the spleen using a non-cross-reacting anti-CD317 Ab. Similar pDC depletion efficiency was observed in WT mice.

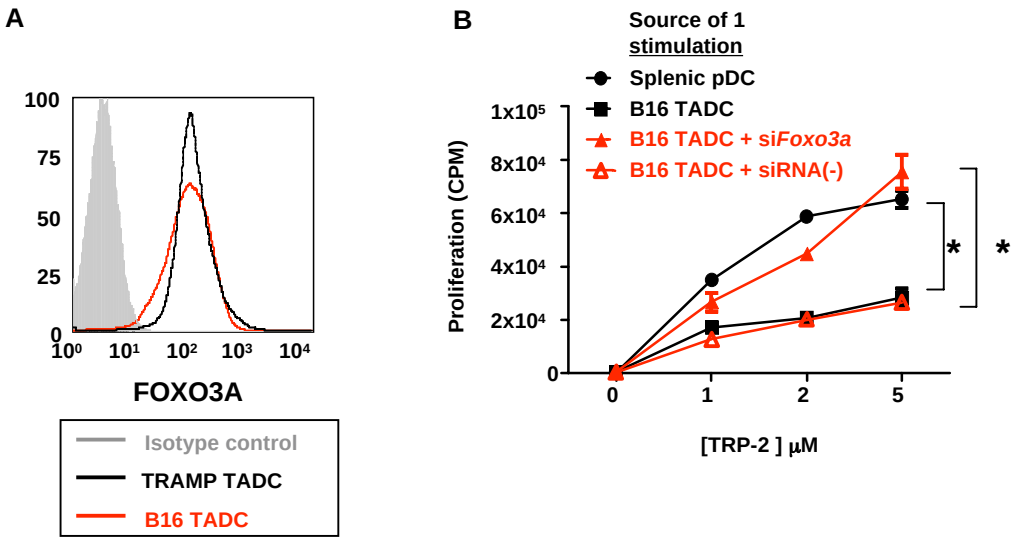

A
WT DC
TRAMP DC
WT DC + 1MDT
TRAMP DC + 1MDT
Proliferation (CPM)
5.0 x 10⁴
4.0 x 10⁴
3.0 x 10⁴
2.0 x 10⁴
1.0 x 10⁴
0
0
0.01
0.1
1
[TAg] μg/ml
*
B
Source of 1° stimulation
WT
TRAMP
TRAMP + BEC
TRAMP + 1MDT
TRAMP + α-PD-1
TRAMP + α-TGF-β
Proliferation (CPM)
3x10⁴
2x10⁴
1x10⁴
0
0.001
0.01
0.1
1
[TAg] μg/ml
C
TRAMP
TRAMP + BEC
TRAMP + 1mDT
TRAMP + α-PD-1
TRAMP + α-TGF-β
% Suppression
100
80
60
40
20
0
2:1
1:1
1:2
1:4
Suppressor : Responder
D
WT Prostate
TRAMP
WT Prostate + α.PD-1
TRAMP + α.PD-1
Proliferation (CPM)
5.0 x 10⁴
4.0 x 10⁴
3.0 x 10⁴
2.0 x 10⁴
1.0 x 10⁴
0
0
0.01
0.1
1
[TAg] μg/ml
E
TcR-I
+ α-PD-1
+ 1MDT
+ α-PD-1/1MDT
# Granzyme B spots/ 5,000 cells
100
80
60
40
20
0
0
0.01
0.1
1
[TAg] μg/ml
TcR-I
α-PD-1
+ 1MDT
+ α-PD-1/1MDT
# IFN-γ spots / 10,000 cells
200
150
100
50
0
0
0.001
0.01
0.1
1
[TAg] μg/ml



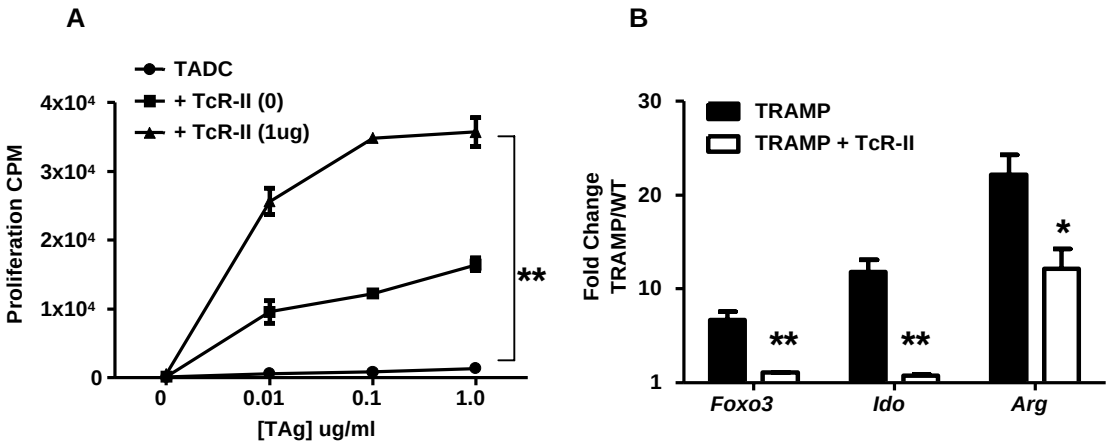
Supplemental Figure 6: Blocking suppressive factors enhances T cell responses and reduces tumor burden(A) 1MDT was added to purified cultures to inhibit IDO activity during DC stimulation of TcR-I proliferation. (B) Tolerance was assessed by testing secondary stimulation with splenocytes and Ag 4 days after primary culture with blocking agents to suppressive mediators. (C) T cell suppressive activity was measured after a 4 day co-culture with the indicated blocking agents. p(D) PD-1 ligation was blocked by anti-PD-1 Ab during DC stimulation (E) Mice were treated with both anti-PD-1 and 1MDT and tested for Granzyme B and IFN-γ secretion on day 6 after transfer. Data is representative of 3 independent trials of 3-5 mice per group, mean ± S.D. (F) anti-TGF-β Ab was added to cultures of TcR-I T cells and TRAMP or WT prostate DCs during primary stimulation. (G) UGT weights and (H) prostate weights were assessed on day 12 after TcR-I transfer. Dashed lines represent the average WT tissue weight. Data are presented for two combined studies with 7 mice total per treatment group, mean ±S.E.M. *p<0.05, **p<0.001 (Student's *t*-test). Data representative of 2 independent trials of 3-5 mice per group, mean ±S.D. Similar results were obtained for anti-PD-1 and BEC treatments.



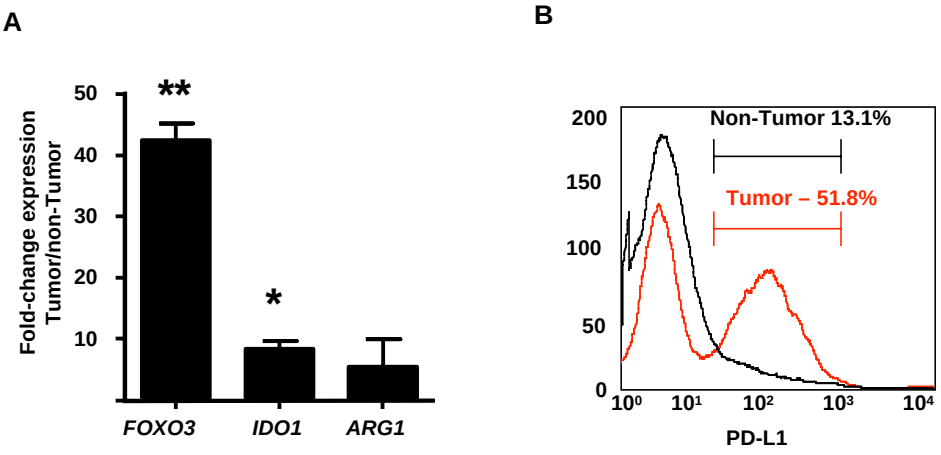
Supplemental Figure 7: Silencing *Foxo3* expression by TADCs.
(A) TRAMP TADCs express a 6-fold increase in *Foxo3* mRNA levels as compared to WT prostate DC. *Foxo3* mRNA was measured in prostatic DC isolated from 5 individual mice from each group by real-time qPCR. Mean ± S.D., **p<0.0001 (student's *t*-test). (B) Increased FOXO3 protein levels were detected in TADC (red) compared to WT prostate DC (black) by flow cytometric analysis. Grey: isotype control. (C,D) TRAMP DC from tumor-bearing. but not non tumor-bearing mice have increased *Foxo3* expression. (E) Protein lysates were assayed by Western blot to confirm gene silencing lysates were pooled from DC isolated from 3 mice per group. TRAMP DCs were added to naïve TcR-I cells and proliferation (F) or cytokine secretion (G) were tested after 60 hours.



Supplemental Figure 8: FOXO3^{+} pDC from B16 tumors induce T cell tolerance. TADC were isolated via magnetic bead coupled to anti-CD317 and (A) assessed for FOXO3 expression by flow cytometry or (B) tested for their ability to induce tolerance in TcR-Mel T cells. Data represented as the average of 4 total experiments. *p<0.01 Tumor pDC vs. Spleen, or siFoxo3 vs. siRNA(-) Control.



Supplemental Figure 9: TcR-II T cells reverse TADC tolerogenicity following *in vitro* co-culture. TcR-II cells were cultured with TADC for 24 hours with the indicated Ag dose prior to testing TADC tolerogenicity (A) or measuring gene expression of *Foxo3, Ido,* and *Arg* (B). Data representative of 2 individual experiments. **p<0.0001, *p<0.001.



Supplemental Figure 10. Human TADC express elevated levels of tolerogenic mediators. Microarray data was confirmed by (A) real-time qPCR for relative gene expression of *FOXO3*, *IDO1* and *ARG1* and (B) flow cytometry for PD-L1 expression.