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 ± SEM.
A) Prostate Tumor Draining LN  
B) Non-Draining Inguinal LN  
C) TRAMP Spleen

Gated on R1

Gated on R2

Gated on R2

SSC-A

FSC-A

CD11c

CD317

B220

F4/80

CD11b

R1

R2

R2

R1
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.
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. (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. 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.