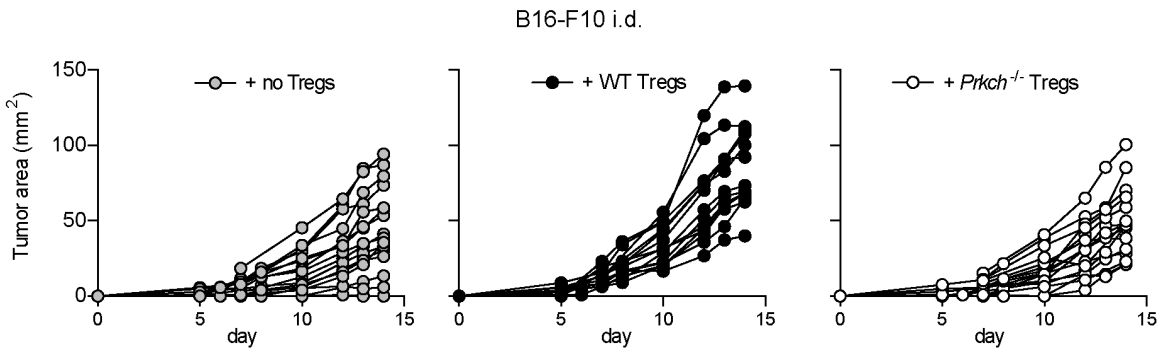
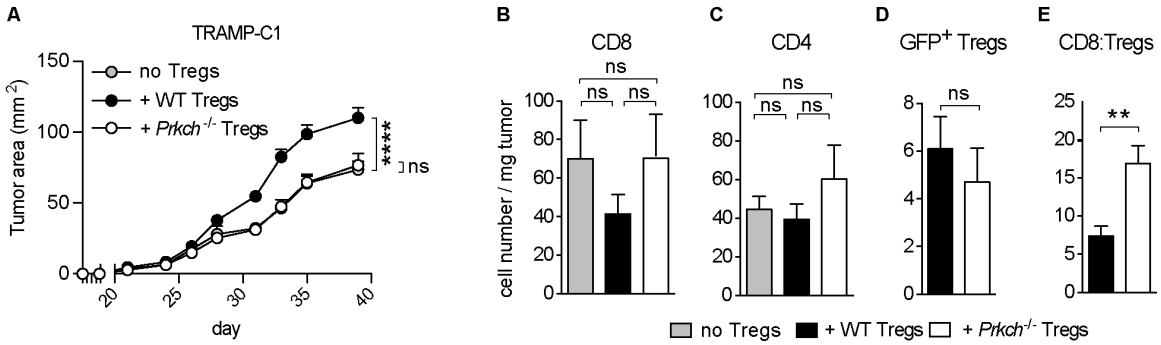
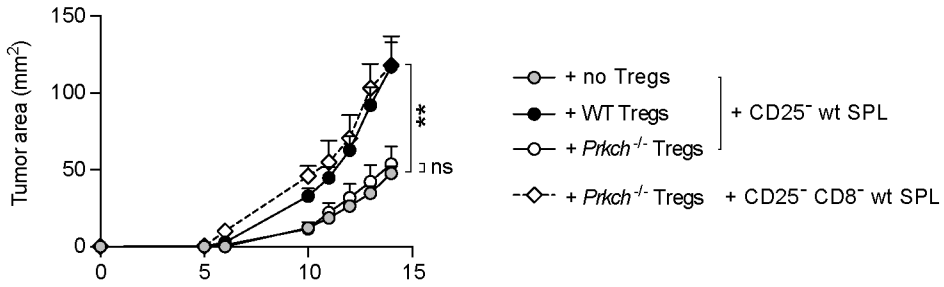


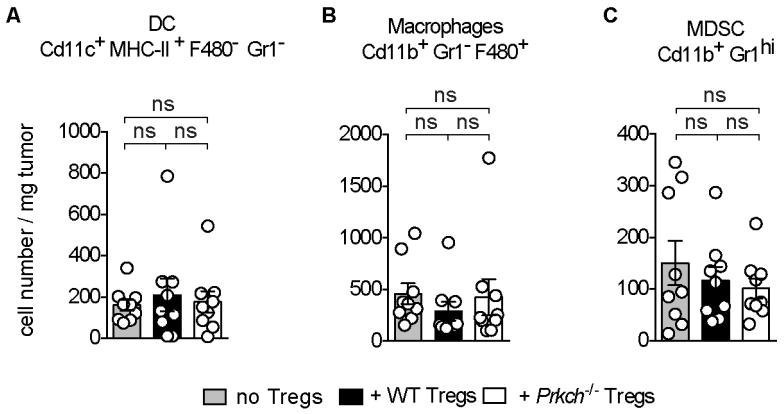
Supplementary Figure 1: Impact of *Prkch*$^{-/-}$ Tregs on tumor growth. *Rag1*$^{-/-}$ mice received CD25-depleted C57Bl/6 spleen cells as a source of Teff cells either alone (+ no Tregs, grey) or together with CD4^{+}GFP^{+} Tregs from WT (black) or *Prkch*$^{-/-}$ FIG mice (white). B16-F10 melanoma cells were inoculated 1 day later (0.5 x 10^{6} i.d.). Tumors sizes were measured 3 times/week, and tumor areas (mm^{2}) are shown. Each curve represents a single mouse, and cumulative data of 4 experiments are shown. no Tregs, n=16; + WT Tregs, n=14; + *Prkch*$^{-/-}$ Tregs, n=16.



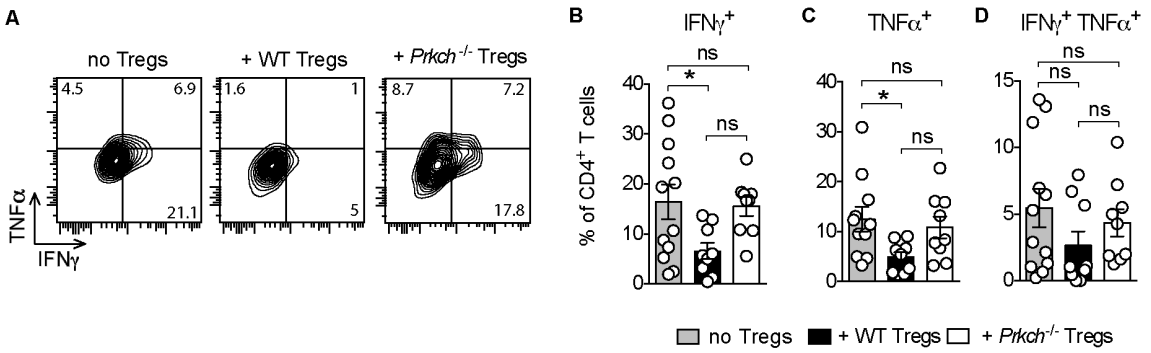
Supplementary Figure 2: Impact of *Prkch*$^{-/-}$ Tregs on TRAMP-C1 tumor growth and intratumoral T cell infiltrates. *Rag1*$^{-/-}$ mice received an adoptive transfer of spleen cells with or without Tregs as in Fig. S1, and TRAMP-C1 prostate adenocarcinoma cells (5 x 10^6) were inoculated s.c. 1 day later. (**A)** Tumors sizes were measured as in Fig. S1, and cumulative data of 4 experiments are shown as mean +/- sem. no Tregs, n=12; + WT Tregs, n=14; + *Prkch*$^{-/-}$Tregs, n=12. Statistical significance was determined against the no Tregs group. (**B-E**) Numbers of tumor-infiltrating CD8$^+$ Teff cells (**B**), CD4$^+$ Teff cells (**C**) and GFP$^+$ Tregs (**D**) per mg of tumor and CD8:Treg ratios (**E**) were analyzed on day 40. Cumulative data of 3 experiments are shown as mean +/- sem. no Tregs, n=11; + wt Tregs, n=10; + *Prkch*$^{-/-}$ Tregs n=9. Statistical significance of differences between groups was determined by repeated mesaures two-way ANOVA (**A**) or one-way ANOVA (**B-E**) followed by Tukey's multiple comparisons test. ns $P > 0.05$, **, $P \leq 0.01$, ****, $P \leq 0.0001$.



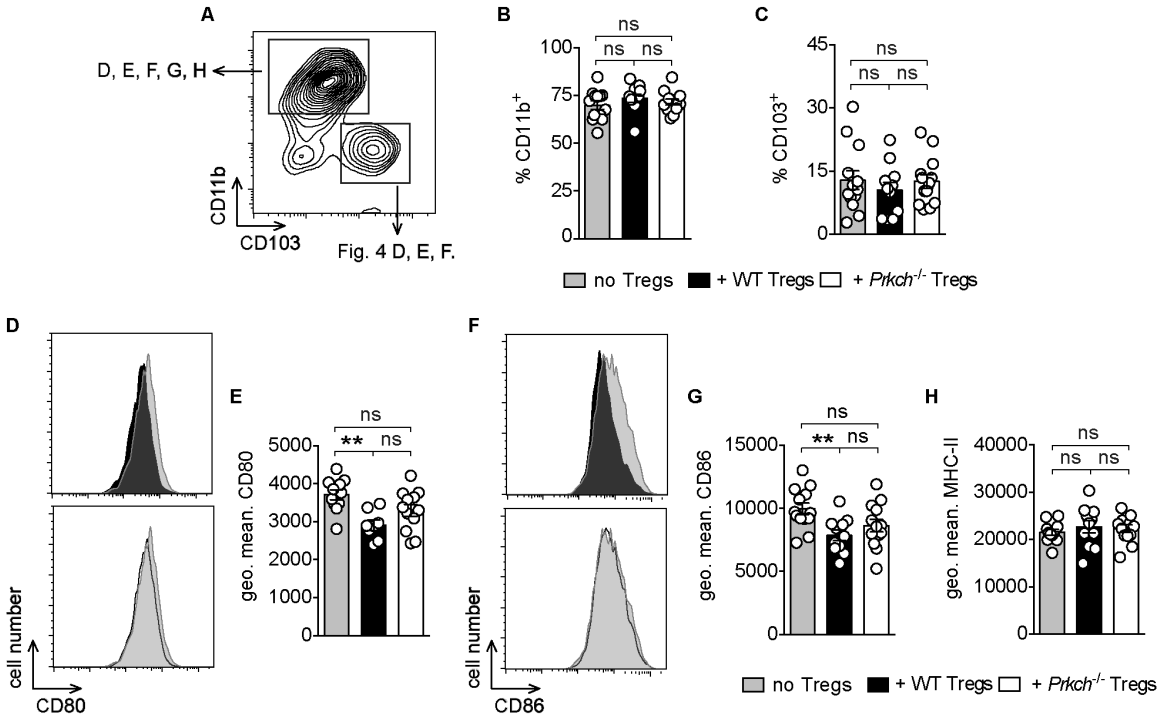
Supplementary Figure 3: Reduced tumor growth in presence of *Prkch*$^{-/-}$ Tregs depends on CD8^{+} T cells. *Rag1*$^{-/-}$ mice received an adoptive transfer of spleen cells with or without Tregs as in Fig. S1. In one group of mice receiving *Prkch*$^{-/-}$ Tregs from FIG mice, the transferred spleen cells were also depleted of CD8^{+} T cells (broken line). B16-F10 melanoma cells were inoculated i.d. 1 day later. Tumor sizes were measured to calculate tumor area (mm^2), and are displayed as mean +/- sem. CD25^{-} spleen cells w/o Tregs, n=6; CD25^{-} spleen cells + WT Tregs, n=6; CD25^{-} spleen cells + *Prkch*$^{-/-}$ Tregs, n=6; CD25^{-}CD8^{-} spleen + *Prkch*$^{-/-}$ Tregs, n=4. Statistical significance between groups was determined as in Fig. S2. ns, $P > 0.05$; **, $P \leq 0.01$. Statistical significance was determined against the no Tregs group.



Supplementary Figure 4: The presence of *Prkch*$^{-/-}$ Tregs does not affect tumor infiltration by myeloid cells. *Rag1*$^{-/-}$ mice received an adoptive transfer of spleen cells with or without Tregs as in Fig. S1, and B16-F10 melanoma cells were inoculated 1 day later. Numbers of tumor-infiltrating DCs (**A**), macrophages (**B**), and myeloid derived suppressor cells (MDSCs; **C**) on day 14 are shown. n=9 mice/group. ns, $P > 0.05$. Statistical significance of differences between groups was determined by a 1-way ANOVA and Tukey's multiple comparisons test.



Supplementary Figure 5: The presence of *Prkch*$^{-/-}$ Tregs favors intratumoral CD4^{+} TILs functionality. *Rag1*$^{-/-}$ mice received an adoptive transfer of spleen cells -/+ Tregs as in Fig. S1, and B16-F10 melanoma cells were inoculated i.d. 1 day later. TILs were harvested on day 14 and briefly stimulated ex vivo with PMA plus ionomycin to assess IFNγ and TNFα expression in CD4^{+} T cells by intracellular staining. Representative dot plots (**A**), and percentage of IFNγ^{+} (**B**), TNFα^{+} (**C**) or IFNγ^{+}TNFα^{+} (**D**) cells among CD8^{+} TILs are shown. Cumulative data of 3 experiments (no Tregs, n=12; + WT Tregs, n=9; + *Prkch*$^{-/-}$ Tregs, n=9). ns, $P > 0.05$; *, $P \leq 0.05$. Statistical significance of differences between groups was determined by 1-way ANOVA and Tukey's multiple comparisons test.



Supplementary Figure 6: Impact of *Prkch*$^{-/-}$ Tregs on intratumoral DC subsets and CD11b^{+} DC activation. *Rag1*$^{-/-}$ recipient mice received an adoptive transfer of spleen cells -/+ Tregs as in Fig. S1. OVA-expressing B16-F10 melanoma cells (B16-OVA) were inoculated i.d. 1 day later. CTV-labeled naive OT-I CD8^{+} CD45.1^{+} T cells were transferred i.v. into tumor-bearing mice on day 10. Data are derived from the same experiments as in Fig. 4. (**A-C**) DC subsets boxed in (**A**) were analyzed to determine the % of CD11b^{+} (**B**) and CD103^{+} (**C**) subsets on day 13. (**D**-**H)** The expression of CD80 (**D, E**), CD86 (**F, G**) and MHC-II (**H**) by CD11b^{+} DCs was analyzed. Representative FACS plots (**D, F**) and cumulative data of 3 experiments (**E, G, H**) are shown (no Tregs, n=10; + WT Tregs, n=8; + *Prkch*$^{-/-}$ Tregs, n=10). *ns, P* > 0.05; **, *P* ≤ 0.01. Statistical significance of differences between groups was determined as in Fig. S5.