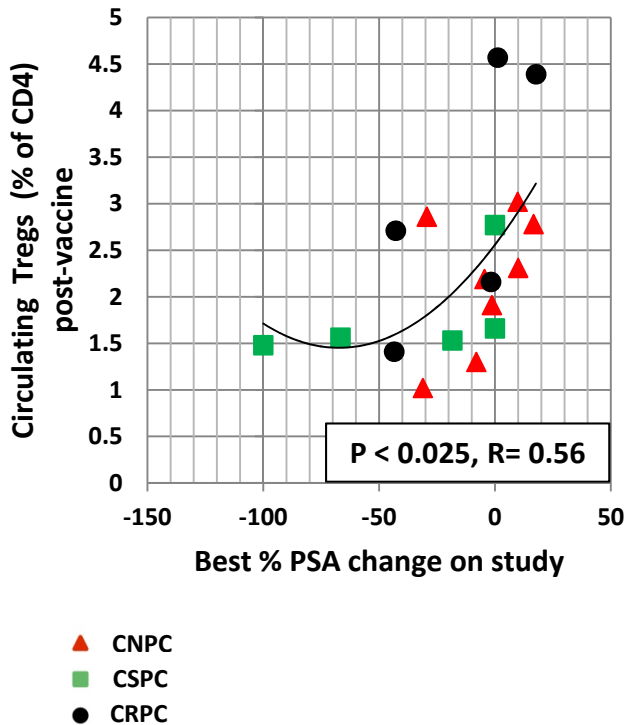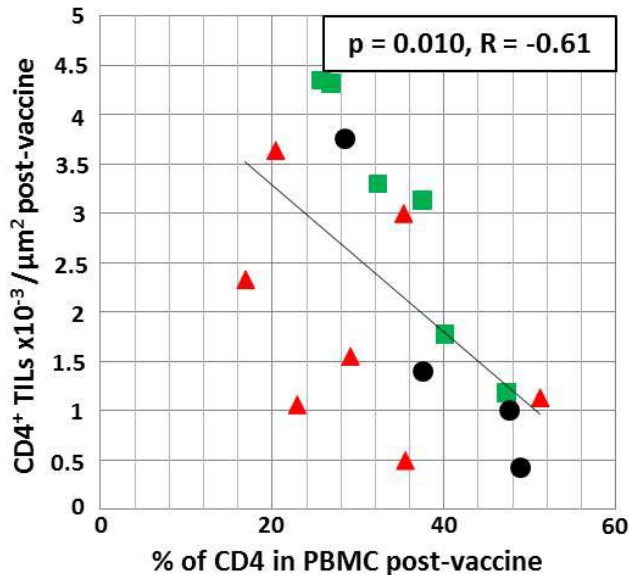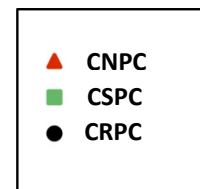# Supplemental Figure 1

Supplemental Figure 1. Low frequency of Tregs in PBMC post-vaccine correlated with decreased serum PSA values. Nonlinear regression analysis by centered second order polynomial (quadratic) equation comparing best percentage PSA change on study vs. circulating Tregs (% of CD4) post-vaccine. Red triangles: castration-naïve prostate cancer patients (CNPC). Green squares: castration-sensitive prostate cancer patients (CSPC). Black circles: castration-resistant prostate cancer patients (CRPC).

Supplemental Figure 2

a

b
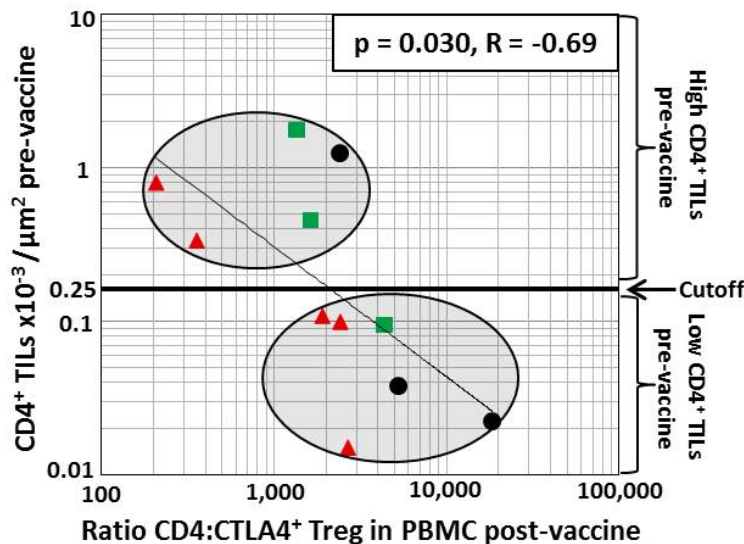
Supplemental Figure 2. Low infiltration of CD4$^+$ TILs correlated with high CD4$^+$ circulating lymphocytes. (*a*) Regression analysis between CD4$^+$ TILs post-vaccine and frequency of CD4$^+$ T lymphocytes in PBMCs post-vaccine. Linear regression line is shown. (*b*) Regression analysis between CD4$^+$ TILs pre-vaccine and ratio CD4$^+$ lymphocytes vs. highly suppressive CTLA4$^+$ Tregs in PBMCs post-vaccine. Patients were divided based on low or high CD4$^+$ TILs pre-vaccine, having as a cutoff 0.25 x10^{-3} CD4$^+$ TILs /µm^2. Power regression line and cutoff for CD4$^+$ TILs high vs. low are shown. Red triangles: castration-naïve prostate cancer patients (CNPC). Green squares: castration-sensitive prostate cancer patients (CSPC). Black circles: castration-resistant prostate cancer patients (CRPC).

Supplemental Table 1: Study Schematic

Days   1   28   57   85   113

Prime (s.c.)   Boost (i.t.)   Boost (i.t.)   Boost (i.t.)   Biopsy

Cohort	Prime (Day 1)	Boosts (Days 28, 57, 88)	
	s.c.	i.t.	s.c.
1 (n=3)	rV-PSA-TRICOM 2×10^8 pfu rF-GM-CSF 2×10^7 pfu	rF-PSA-TRICOM 4×10^7 pfu	
2 (n=3)	rV-PSA-TRICOM 2×10^8 pfu rF-GM-CSF 2×10^7 pfu	rF-PSA-TRICOM 4×10^8 pfu	
3 (n=3)	rV-PSA-TRICOM 2×10^8 pfu rF-GM-CSF 2×10^7 pfu	rF-PSA-TRICOM 4×10^8 pfu rF-GM-CSF 1×10^7 pfu	
4 (n=6)	rV-PSA-TRICOM 2×10^8 pfu rF-GM-CSF 2×10^7 pfu	rF-PSA-TRICOM 4×10^8 pfu rF-GM-CSF 1×10^8 pfu	
5 (n=6)	rV-PSA-TRICOM 2×10^8 pfu rF-GM-CSF 2×10^7 pfu	rF-PSA-TRICOM 4×10^8 pfu rF-GM-CSF 1×10^8 pfu	rF-PSA-TRICOM 4×10^8 pfu rF-GM-CSF 1×10^7 pfu

s.c.: subcutaneous; **i.t.:** intratumoral; **pfu:** plaque-forming units. Number of patients enrolled per cohort is shown.

Supplemental Table 2:
Immune Subsets of PBMC Analyzed by Flow Cytometry

#	Subset
1	CD4$^+$
2	CD4$^+$ CTLA-4$^+$
3	Central Memory CD4$^+$= CD45RA$^-$ CCR7$^+$
4	Effector Memory CD4$^+$= CD45RA$^-$ CCR7$^-$
5	Effector Memory CD4$^+$ PD-1$^+$
6	Terminal (EMRA) CD4$^+$= CD45RA$^+$ CCR7$^-$
7	Naïve CD4$^+$= CD45RA$^+$ CCR7$^+$
8	Ratio CD4$^+$ vs. CD8$^+$
9	CD8$^+$
10	CD8$^+$ CTLA-4$^+$
11	CD8$^+$ PD-1$^+$
12	CD8$^+$ Tim-3$^+$
13	Central Memory CD8$^+$= CD45RA$^-$ CCR7$^+$
14	Effector Memory CD8$^+$= CD45RA$^-$ CCR7$^-$
15	Terminal (EMRA) CD8$^+$= CD45RA$^+$ CCR7$^-$
16	Naïve CD8$^+$= CD45RA$^+$ CCR7$^+$
17	Tregs (% of CD4$^+$)= CD4$^+$ CD25$^+$ FoxP3$^+$ CD127$^-$
18	Treg CTLA-4$^+$
19	Ratio CD4$^+$ vs. Treg
20	Ratio CD4$^+$ vs. Treg CTLA-4$^+$
21	Ratio CD8$^+$ vs. Treg
22	Ratio CD8$^+$ vs. TregCTLA-4$^+$
23	NK cells= CD56$^+$ CD3$^-$
24	NK Tim-3$^+$
25	NK CD16$^+$ CD56^{br}
26	NK CD16$^+$ CD56^{br} Tim-3$^+$
27	NK CD16$^+$ CD56^{dim}
28	NK CD16$^+$ CD56^{dim} Tim-3$^+$
29	NK CD16$^-$ CD56^{br}
30	NK CD16$^+$ CD56^{br} Tim-3$^+$
31	MDSC= CD33$^+$ HLA-DR$^-$ CD11b$^+$

Supplemental Table 3:
TIL Counts by Digital Immunohistochemistry

Patient	CD4 x10^{-3}/μm²			CD8x10^{-3}/μm²		
	pre	Post	Fold change	pre	post	Fold change
1	0.015	0.494	32.72	0.169	0.719	4.25
2	0.100	1.130	11.32	0.115	0.266	2.31
3	1.770	3.130	1.77	1.360	2.140	1.57
4	-	3.640	-	-	1.720	-
5	0.109	2.330	21.38	0.121	1.860	15.37
6	0.807	3.000	3.72	0.331	1.980	5.98
7	0.458	1.770	3.86	0.238	1.360	5.71
8	-	1.550	-	-	0.870	-
9	-	4.320	-	0.530	4.880	9.21
10	0.338	1.060	3.14	0.630	0.181	2.87
11	-	-	-	-	1.400	-
12	-	3.300	-	-	3.370	-
13	0.095	4.350	45.98	0.256	3.760	14.69
14	-	1.180	-	-	0.776	-
15	-	-	-	-	-	-
16	1.250	3.76	3.01	0.770	1.280	1.66
17	0.038	0.424	11.22	0.079	0.727	9.20
18	-	1.400	-	-	1.760	-
19	-	-	-	-	-	-
20	0.023	1.000	44.22	0.081	0.858	10.55
21	0.258	2.460	9.53	0.161	1.950	12.11
No.	12[a]	18[a]	12[b]	13[a]	19[a]	13[b]

a: number of prostate biopsies analyzed by digital IHC.
b: number of prostate biopsies for the analysis of pre- vs. post-vaccine comparison.