Supplemental Table, Figures, and Figure Legends

Table S1. Anti-tumor memory response in previously vaccinated HSCT recipients.

Treatment	No. with tumor[*] / No. injected[†]	% with tumor[‡]
Untreated		
Non-transplanted	9 / 9	100
E.G7-gp96-Ig[§]		
BM + T cells[‖]	0 / 6	0
E.G7-gp96-Ig + IL-2_{S4B6}[§]		
BM + T cells[‖]	2 / 14	14.3

[*]Mice with progressively growing tumors (sacrificed ≥225 mm^2).

[†]Mice were challenged with E.G7 lymphoma cells subcutaneously (1.0 x 10^6).

[‡]Total rejection rate of HSCT recipients was 90% (18/20).

[§]Transplant recipients were ≥100 d post-HSCT (Figure 4-6).

[‖]≥75% $CD4^+$ and $CD8^+$; ≤3% $CD19^+$ (Materials and methods).

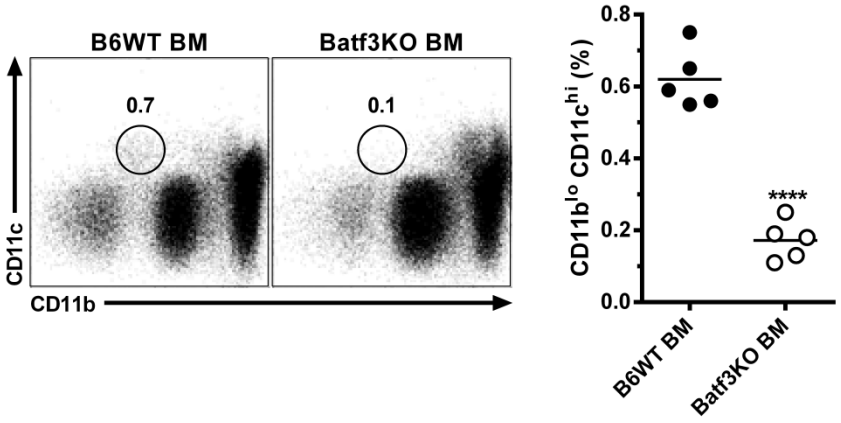

Figure S1. Loss of CD11b^{lo} CD11c^{hi} DC at the site of vaccination in recipients of Batf3 deficient TCD-BM receiving gp96-Ig secreting tumor cells. Conditioned (9.5 Gy) B6 mice were transplanted with 5.0 x 10^6 B6 wild-type (B6WT) or Batf3 deficient (Batf3KO) TCD-BM cells and adoptively transferred with 0.5 x 10^6 CD8$^+$ T cells specific for $OVA_{257-264}$ (OT-I) 5 d later. After a 2 d resting period, recipients were vaccinated intraperitoneally with irradiated (40 Gy) EL-4 lymphoma cells expressing OVA (E.G7) engineered to secrete gp96-Ig (E.G7-gp96-Ig). The peritoneal cavity was analyzed 5 d following vaccination and DC populations were assessed. CD11c^{hi} CD11b^{lo} F4/80^{lo} Gr-1^{lo} frequency in the peritoneal cavity; representative dot plots (left), n = 5 (right).

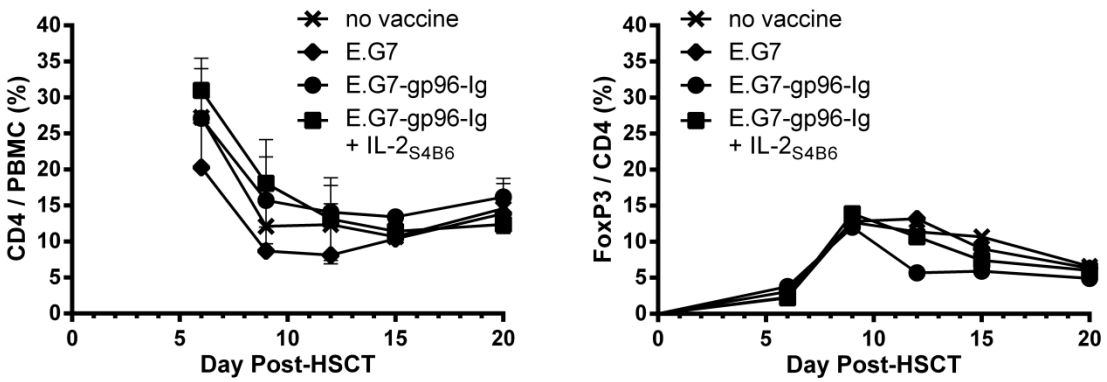

Figure S2. Systemic $CD4^+$ T cell and Treg levels were marginally changed following $IL\text{-}2_{S4B6}$ treatment in syngeneic HSCT recipients. Conditioned (9.5 Gy) B6 recipients received B6-$CD45.1^+$ TCD-BM cells supplemented with 2.0 x 10^6 B6-$CD90.1^+$ $CD4^+$ and $CD8^+$ T lymphocytes obtained from E.G7 lymphoma bearing donors, containing ~1000 tumor-reactive $CD8^+$ T cells (OT-I). Recipients were inoculated intraperitoneally with 1.0 x 10^5 E.G7 lymphoma cells 1 d post-HSCT to simulate relapse post-transplant. Mice were vaccinated intraperitoneally with irradiated (40 Gy) E.G7 cells secreting gp96-Ig 2 d post-HSCT (repeated every 3 d for a total of 5 vaccinations) and received IL-2 pre-bound to anti-IL-2 mAb clone S4B6 ($IL\text{-}2_{S4B6}$) 1 d following each vaccination as indicated. **(Left)** Systemic $CD4^+$ T cell levels were unchanged following $IL\text{-}2_{S4B6}$ treatment in syngeneic HSCT recipients. $CD4^+$ T cell frequency in the peripheral blood; n = 10 from pool of 2 experiments; **x**: no vaccine, ♦: E.G7, ●: E.G7-gp96-Ig, ■: E.G7-gp96-Ig + $IL\text{-}2_{S4B6}$. **(Right)** Systemic $CD4^+$ $FoxP3^+$ Treg levels were marginally changed following $IL\text{-}2_{S4B6}$ treatment in syngeneic HSCT recipients. $FoxP3^+$ frequency within $CD4^+$ T cells in the peripheral blood utilizing mice expressing red fluorescent protein under control of the FoxP3 promoter as T cell donors; n = pool of 5; **x**: no vaccine, ♦: E.G7, ●: E.G7-gp96-Ig, ■: E.G7-gp96-Ig + $IL\text{-}2_{S4B6}$.

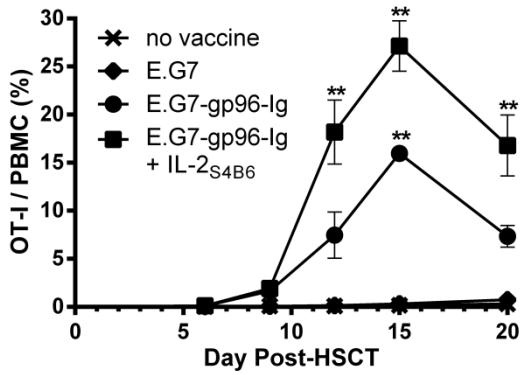

Figure S3. Tumor-reactive CD8$^+$ T cells reached maximal levels in the peripheral blood 2 wk post-HSCT following vaccination and IL-2 therapy. Transplants and tumor inoculation were performed as in Figure S2 and mice were treated as indicated; n = 20 from pool of 4 experiments; **x**: no vaccine, ♦: E.G7, ●: E.G7-gp96-Ig, ■: E.G7-gp96-Ig + IL-2$_{S4B6}$. The 'no vaccine,' 'E.G7,' and 'E.G7-gp9-Ig' groups from Figure 4B were included as a reference to illustrate the kinetics of expansion with vaccination and IL-2$_{S4B6}$.

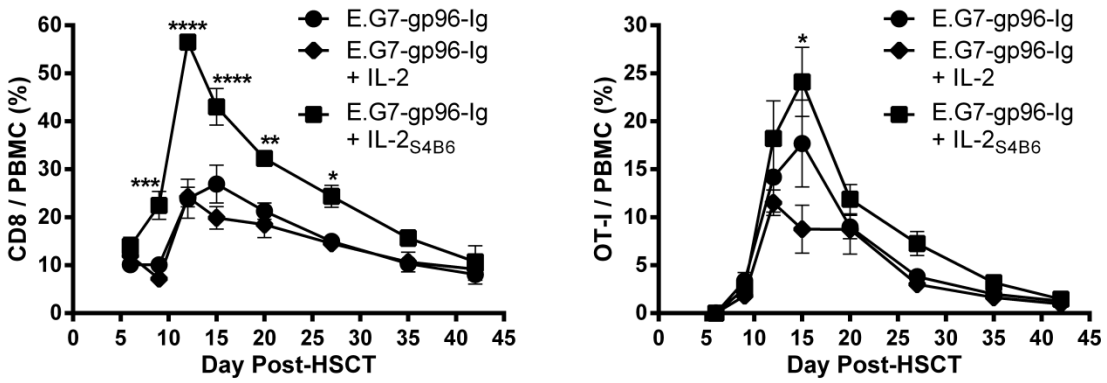

Figure S4. Non-complexed, unbound IL-2 in combination with vaccination failed to expand CD8$^+$ T cells or enhance tumor-reactive CD8$^+$ T lymphocyte levels. Transplants and tumor inoculation were performed as in Figure S2 and mice were treated as indicated, including IL-2 not pre-bound to anti-IL-2. **(Left)** Non-complexed, unbound IL-2 in combination with vaccination failed to expand CD8$^+$ T lymphocytes. CD8$^+$ T cell frequency in the peripheral blood; n = 5; ●: E.G7-gp96-Ig, ♦: E.G7-gp96-Ig + IL-2, ■: E.G7-gp96-Ig + IL-2$_{S4B6}$. **(Right)** Non-complexed, unbound IL-2 in combination with vaccination failed to expand tumor-reactive CD8$^+$ T lymphocytes. Tumor-reactive CD8$^+$ T cell frequency in the peripheral blood; n = 5. ●: E.G7-gp96-Ig, ♦: E.G7-gp96-Ig + IL-2, ■: E.G7-gp96-Ig + IL-2$_{S4B6}$. Extended analyses of these CD8$^+$ T lymphocyte frequencies in mice treated with vaccination and IL-2$_{S4B6}$ suggested that slightly increased levels were maintained for at least 6 wk post-HSCT, or 1 mo following cessation of treatment.

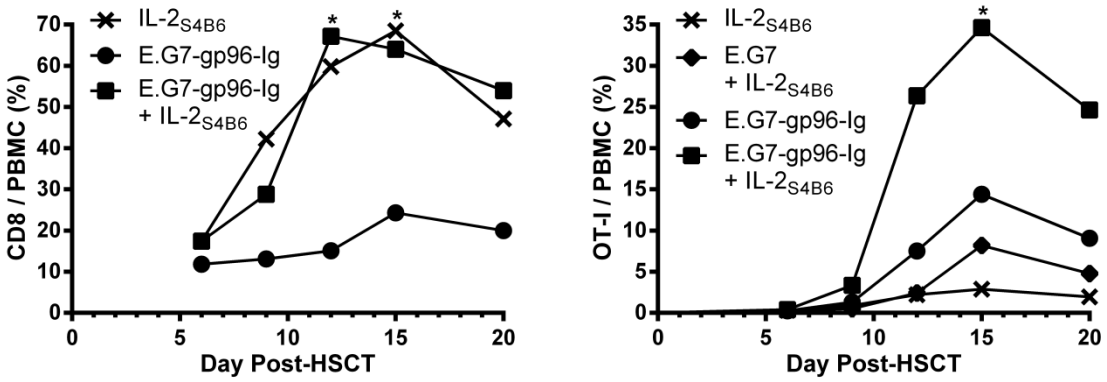

Figure S5. IL-2_{S4B6} in the absence of vaccination markedly expanded $CD8^+$ T cells, but only elicited a small response by tumor-reactive $CD8^+$ T lymphocytes. Transplants and tumor inoculation were performed as in Figure S2 and recipients were treated as indicated, including IL-2_{S4B6} in the absence of vaccination. **(Left)** IL-2_{S4B6} in the absence of vaccination markedly expanded $CD8^+$ T cells. $CD8^+$ T cell frequency in the peripheral blood; n = pool of 5; **x**: IL-2_{S4B6}, ●: E.G7-gp96-Ig, ■: E.G7-gp96-Ig + IL-2_{S4B6}. **(Right)** IL-2_{S4B6} in the absence of vaccination only elicited a small response by tumor-reactive $CD8^+$ T lymphocytes. Tumor-reactive $CD8^+$ T cell frequency in the peripheral blood; n = pool of 5; **x**: IL-2_{S4B6}, ♦: E.G7 + IL-2_{S4B6}, ●: E.G7-gp96-Ig, ■: E.G7-gp96-Ig + IL-2_{S4B6}.

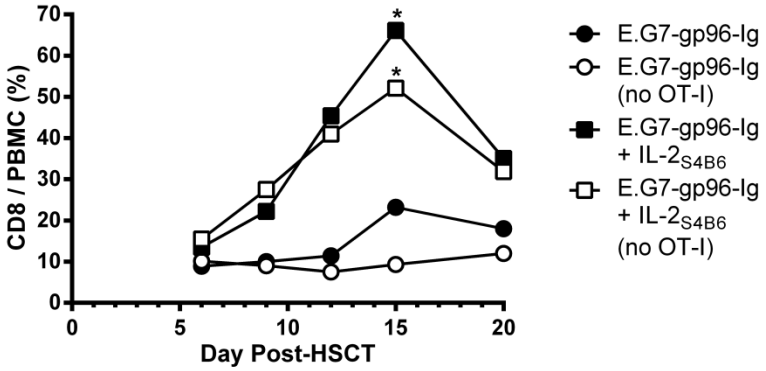

Figure S6. CD8$^+$ T cells markedly expanded following vaccination and IL-2$_{S4B6}$ in the absence of transgenic CD8$^+$ OT-I T lymphocytes. Transplants and tumor inoculation were performed as in Figure S2 utilizing T cell donors with or without transgenic CD8$^+$ OT-I T cells and recipients were treated as indicated. CD8$^+$ T cell frequency in the peripheral blood; n = pool of 5; ●: E.G7-gp96-Ig, ○: E.G7-gp96-Ig (no OT-I), ■: E.G7-gp96-Ig + IL-2$_{S4B6}$, □: E.G7-gp96-Ig + IL-2$_{S4B6}$ (no OT-I). The small increase observed in CD8$^+$ T lymphocyte frequency in both groups containing CD8$^+$ OT-I T cells likely represents the presence of the transgenic CD8$^+$ T cell population.

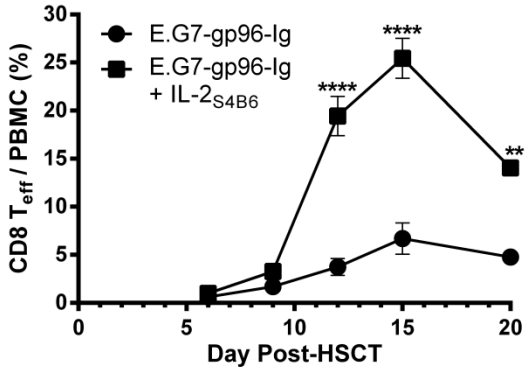

Figure S7. IL-2_{S4B6} in combination with vaccination elicits potent effector $CD8^+$ T cell response. Transplants and tumor inoculation were performed as in Figure S2, but without the addition of $CD8^+$ OT-I T cells, and recipients were treated as indicated. $CD8^+$ T_{eff} ($CD62L^-$ $CD44^+$) cell frequency in the peripheral blood; n = 21 from pool of 4 experiments; ●: E.G7-gp96-Ig, ■: E.G7-gp96-Ig + IL-2_{S4B6}.

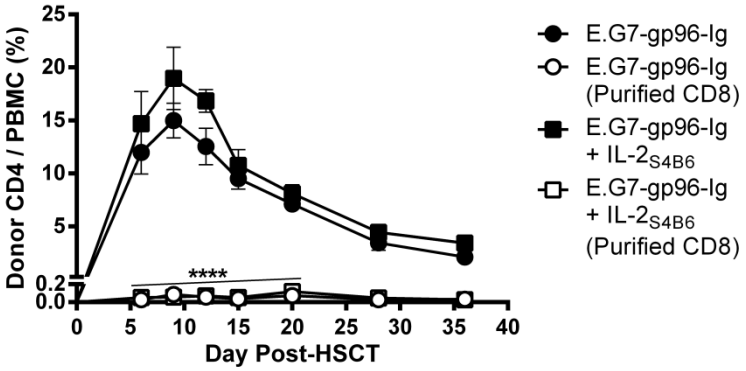

Figure S8. Donor CD4$^+$ T cells were minimally detectable in recipients of purified CD8$^+$ T cells. Transplants and tumor inoculation were performed as in Figure S2, but without the addition of CD8$^+$ OT-I T cells, including groups with TCD-BM supplemented with purified CD8$^+$ T cells, and mice were treated as indicated. CD4$^+$ T cell frequency in the peripheral blood; n = 5; ●: E.G7-gp96-Ig, ○: E.G7-gp96-Ig (Purified CD8), ■: E.G7-gp96-Ig + IL-2$_{S4B6}$, □: E.G7-gp96-Ig + IL-2$_{S4B6}$ (Purified CD8). The results for 'E.G7-gp96-Ig + IL-2$_{S4B6}$' and 'E.G7-gp96-Ig + IL-2$_{S4B6}$ (Purified CD8)' were repeated in an independent experiment.

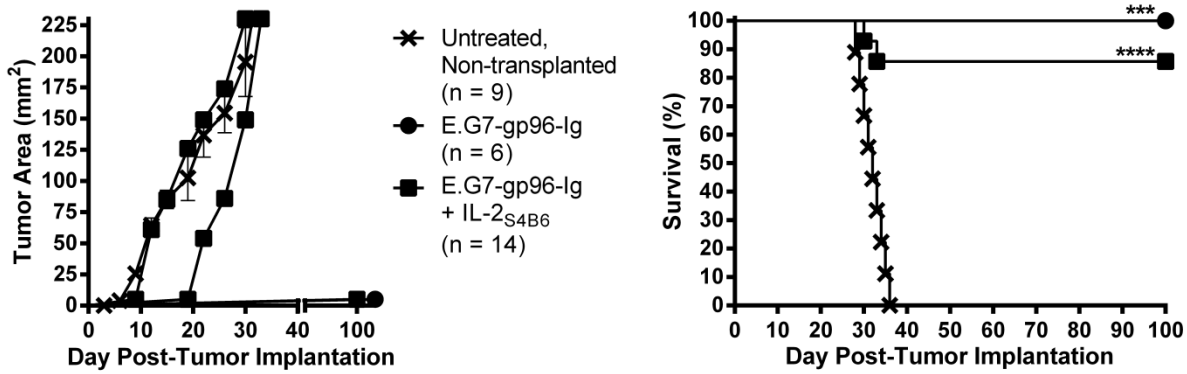

Figure S9. Kinetics of tumor growth and survival in previously vaccinated HSCT recipients challenged with a lethal number of tumor cells. Previously vaccinated transplant recipients (>100 d post-HSCT; Figures 4-6) were challenged with a lethal number (1.0 x 10^6) of tumor cells subcutaneously to determine if anti-tumor memory had been generated by either vaccine strategy. **(Left)** Kinetics of tumor growth and **(right)** survival post-challenge; n = 6-14 from pool of 2 subcutaneous challenge experiments; **x**: Untreated and Non-transplanted (pooled), ●: E.G7-gp96-Ig (individual), ■: E.G7-gp96-Ig + IL-2_{S4B6} (individual).

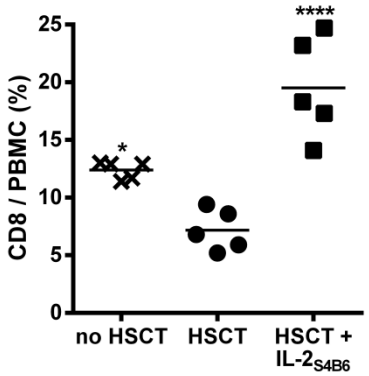

Figure S10. IL-2_{S4B6} infusion induced expansion of $CD8^+$ T cells in HSCT recipients immediately prior to *Listeria monocytogenes* challenge. Transplants were performed as in Figure S2 and HSCT recipients were infused with IL-2_{S4B6} 3 d post-HSCT and every 3 d for a total of 4 infusions as indicated. $CD8^+$ T cell frequency in the peripheral blood 2 wk post-HSCT; n = 5 from representative of 2 experiments; **x**: no HSCT, ●: HSCT, ■: HSCT + IL-2_{S4B6}.