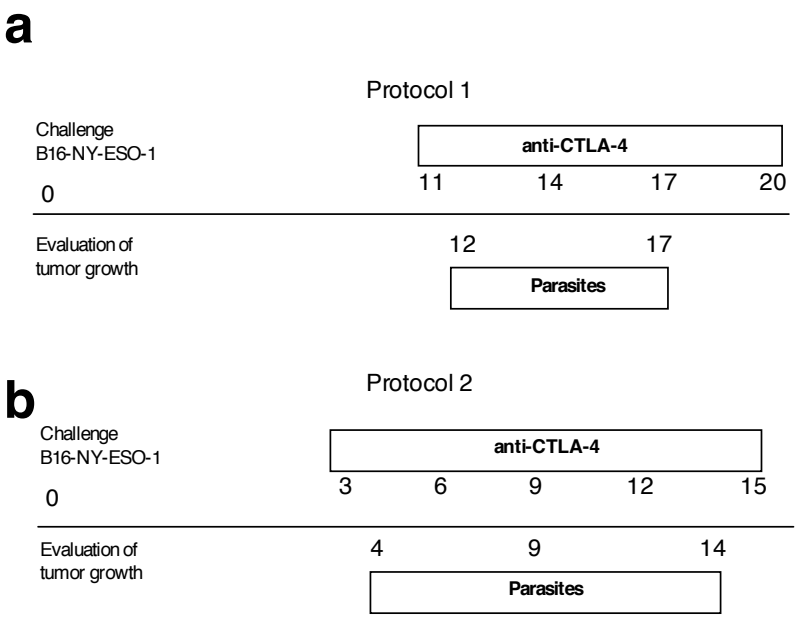
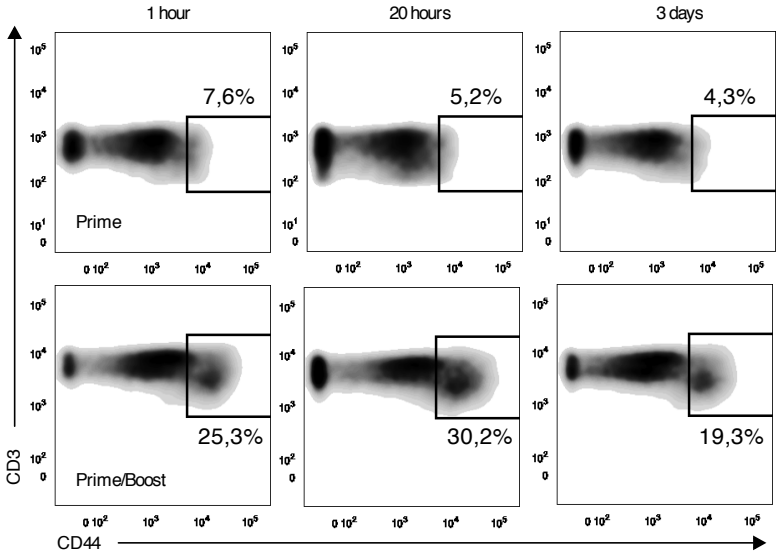
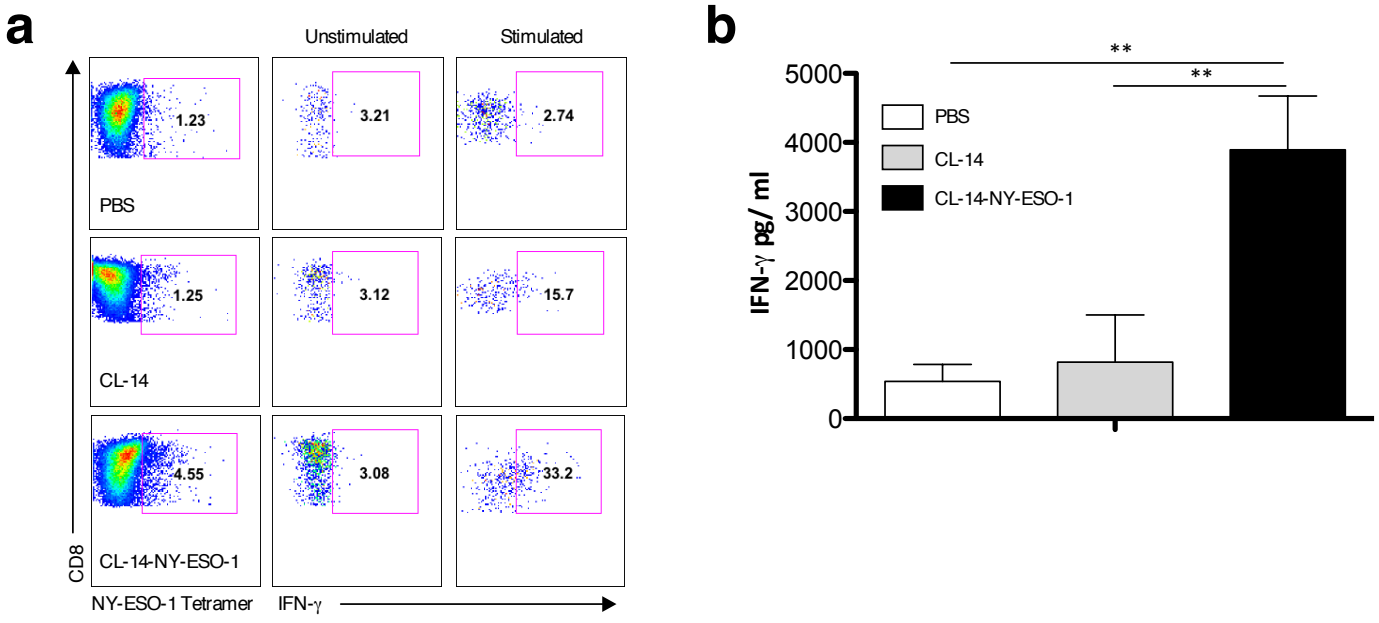


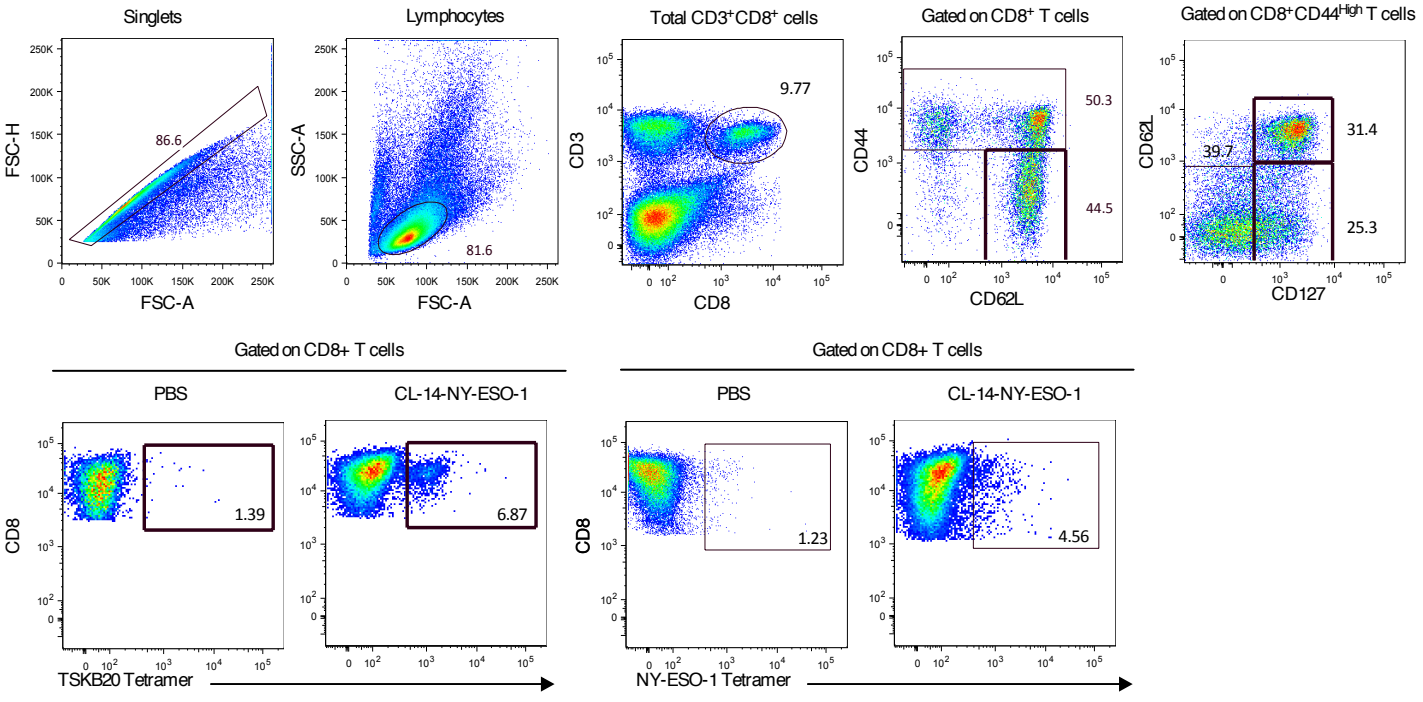
Supplementary Fig.1. Protocols used to immunotherapy. To establish subcutaneous tumors, mice received 5×10^4 B16-NY-ESO-1 cells (day 0) and the treatment was initiated on day 11 (**a**) or 3 (**b**) depending on the adopted protocol. The anti-CTLA-4 was inoculated every 3 days (100µg, i.p.) and the parasite CL-14-NY-ESO-1 with the interval of 5 days (10^7 trypomastigotes, i.p.).



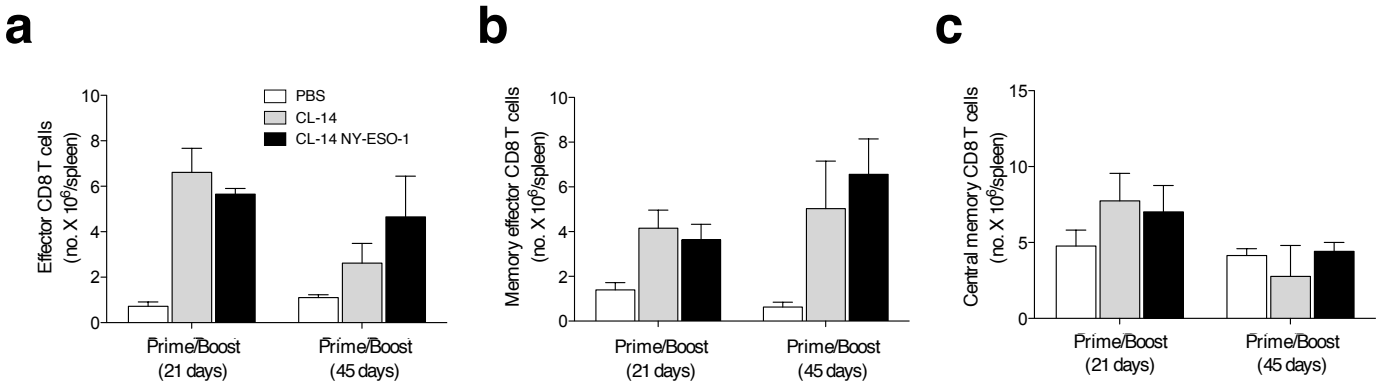
Supplementary Fig.2. Two doses of CL-14-NY-ESO-1 were sufficient to ensure stimulation of T cells. The lymph nodes were collected 1 hour or 1 or 3 days after infection with one (upper panels) or two doses (lower panels) of the transgenic parasite and the frequency of stimulated T cells ($CD3^+CD44^{High}$) was analyzed by flow cytometry.



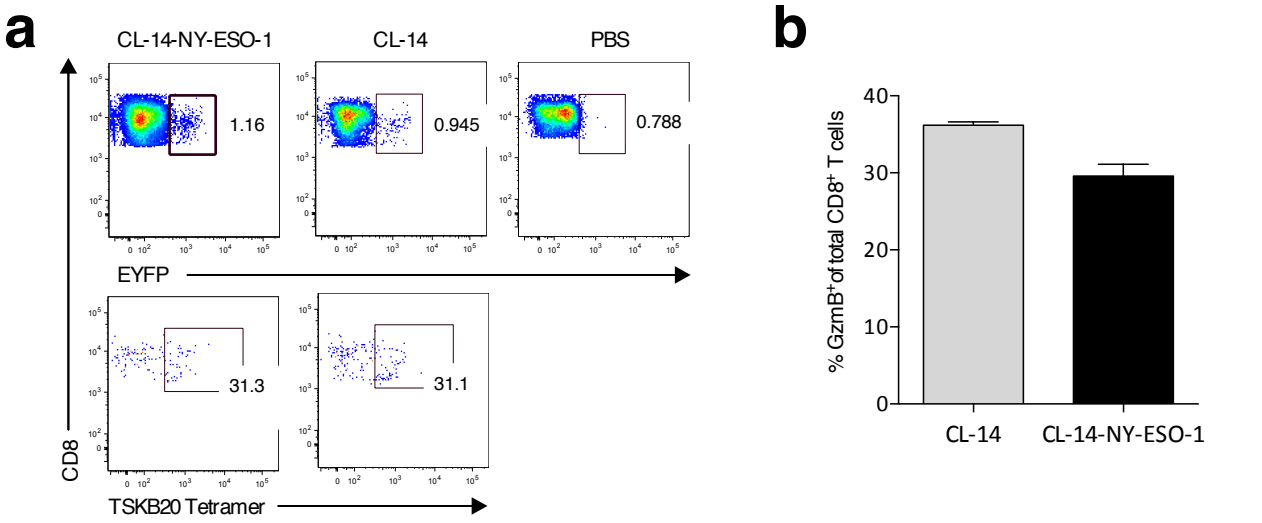
Supplementary Fig.3. Vaccinations with transgenic parasite induce antitumor-specific immune response. (**a**) Representative pseudo-color plots demonstrate NY-ESO-1-specific CD8+ T cells collected 21 days after the boost, re-stimulated with rNY-ESO-1 and evaluated for their ability to produce IFN-γ. (**b**) Production of IFN-γ were assessed in the supernatant after 48h of culture B16-NY-ESO-1 melanoma cells and splenocytes from mice that received two doses of transgenic parasite and had the spleen harvested at day 21 after boost. Control groups received PBS or CL-14 parasites.



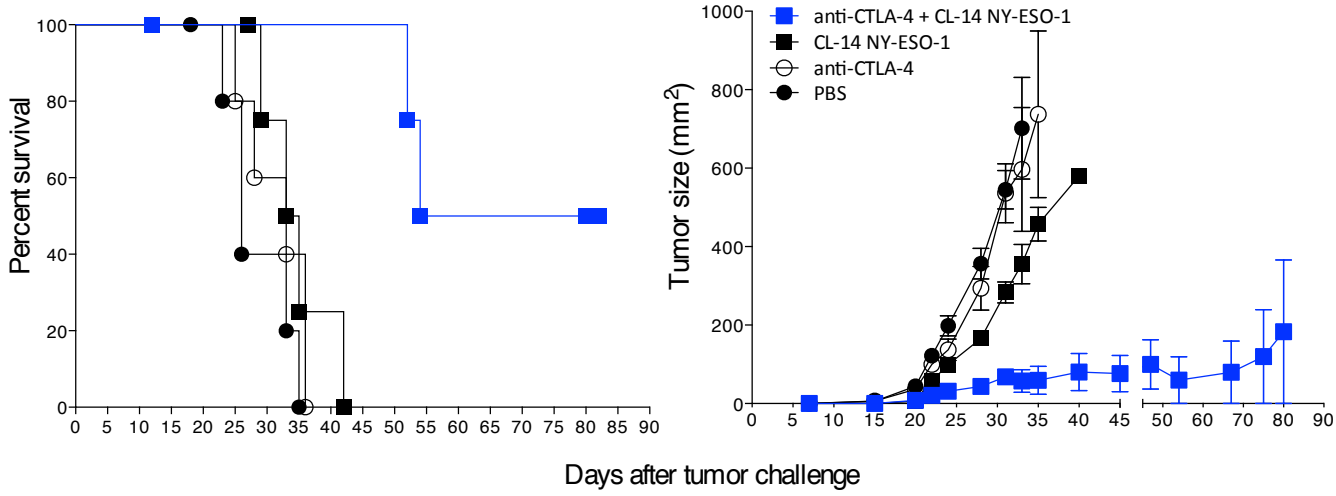
Supplementary Fig.4. **Gating strategy for flow cytometry analysis.** Cells were first gated for singlets (FSC-H vs. FSC-A) and lymphocytes (SSC-A vs. FSC-A). The lymphocyte gate is further analyzed to determine total $CD8^{+}$T cells (CD3 vs. CD8). The profile of activation ($CD44^{High}$) and naïve cells ($CD44^{Low}CD62L^{High}$) were defined from total CD8+ T cells. The subpopulations of CD8+ T cells were depicted from activated cells: effector ($CD62L^{Low}CD127^{Low}$), memory effector ($CD62L^{Low}CD127^{High}$) and central memory ($CD62L^{High}CD127^{High}$). MHC-tetramers-PE exhibiting the specific epitopes for parasite (TSKB20) or cancer testis (NY-ESO-1) were evaluated from total lymphocytes T CD8.



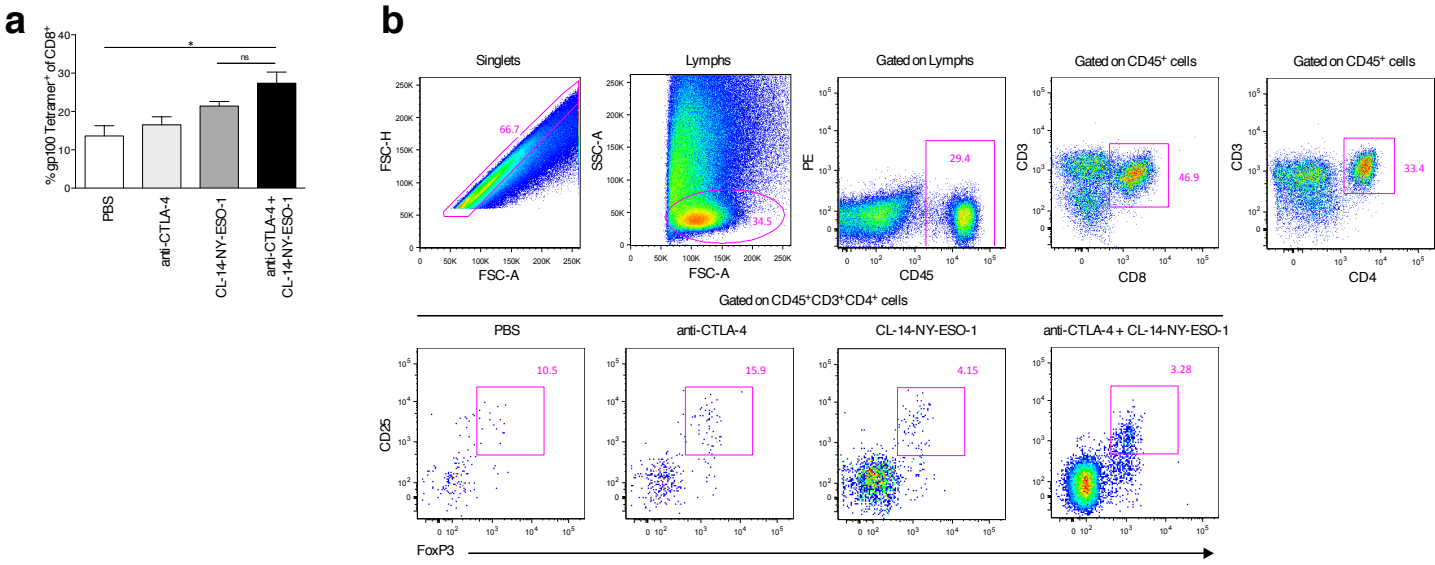
Supplementary Fig.5. **Numbers of effector and memory effector CD8+ T cells generated with immunization.** Splenocytes were harvested 21 and 45 days after boost vaccination. The markers CD44, CD62L and CD127 were used to define subpopulations within $CD3^{+}CD8^{+}$ T cells. To evaluate the profile of the activated cells $CD44^{high}$ was defined. The subpopulations were depicted: (**a**) effector ($CD62L^{Low}CD127^{Low}$), (**b**) memory effector ($CD62L^{Low}CD127^{High}$) and (**c**) central memory ($CD62L^{High}CD127^{High}$). Similar results were found in three independent experiments with four animals in each group.



Supplementary Fig.6. Transgenic parasites were able to induce granzyme B tumor-specific $CD8^{+}$ T cells. gzmBCreERT2/ROSA26EYFP mice were vaccinated with two doses of PBS or the *T. cruzi* strain CL-14 expressing or not expressing NY-ESO-1. Treatment with tamoxifen was performed during 5 days as described in the methods section, and the splenocytes were collected 21 days after the boost vaccination. (**a**) The expression of EYFP, which is restricted to cells expressing granzyme B (gzmB), was used for gating (upper panels). Parasite-specific cells stained with the TSKB20 tetramer were assessment on the gate of $CD8^{+}EYFP^{+}$ cells. (**b**) To confirm the expression of gzmB induced by transgenic parasite, C57BL/6 were vaccinated with the same protocol and the expression of gzmB were evaluated using a monoclonal antibody for this protein.



Supplementary Fig.7. Immunotherapy with anti-CTLA-4/CL14-NY-ESO-1 controls tumor growth. For the therapeutic vaccination, the mice were subjected to the treatment starting on day 3 after challenge with B16-NY-ESO-1 melanoma cells and the tumor growth and survival were monitored for 84 days.



Supplementary Fig.8. **Treatment with anti-CTLA-4/CL14-NY-ESO-1 enhanced antitumor immune response.** C57BL/6 mice were challenged with B16-NY-ESO-1 cells. (**a**) After 28 days, splenocytes were harvested and specificity to melanocytic cells was determined using gp100. (**b**) The gate strategy used to the analysis in the tumor infiltrate was demonstrated on the dotplots.

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