Supplemental Figure Legends:

Figure S1. Duration of protective effect seen in immunized mice.

Eight week old male C57BL/6 mice were immunized by infection with $5x10^3$ GFP-DDDHA. At 4, 6, 7, 9, 12 and 15 months post immunization, mice were challenged with a lethal dose ($5x10^5$) of WT Tulahuen *T. cruzi.* Age match non-immunized mice were infected with the same dose as control animals. As shown, immunized mice had essentially no mortality when challenged even at 15 months post immunization, while non-immunized mice all died rapidly from the same WT infection.

Figure S2. In the absence of TMP-lactate GFP-DDDHA immunization provides cross protection and protection against challenge infection.

A. Eight week old male C3H mice were immunized with $5x10^3$ GFP-DDDHA Tulahuen strain for 42 days without TMP-lactate treatment. When challenged with $5x10^5$ Brazil strain (WT), these immunized mice did not develop parasitemia. In contrast, mice that had not been immunized mice had a significant parasitemia (top panel). All of the immunized mice survived the challenge infection, while all of the non-immunized mice died during the challenge infection (bottom panel) (n=5).

B. Eight week old male C57BL/6 mice were immunized with $5x10^3$ GFP-DDDHA Tulahuen strain for 42 days without TMP-lactate treatment and then challenged with

$5x10^5$ Brazil strain (WT). Immunized mice had almost no parasitemia, while non-immunized mice had a significant higher parasitemia (top panel). All of the immunized mice survived the challenge infection, while 40% of the non-immunized mice died from the challenge infection (bottom panel) (n=5).

C. Eight week old male C57BL/6 were immunized with $5x10^3$ GFP-DDDHA Tulahuen strain for 42 days without TMP-lactate treatment and then challenged with $5x10^5$ Tulahuen strain (WT). Immunized mice did not develop parasitemia while non-immunized mice had a high parasitemia (top panel). All of the immunized mice survived the challenge infection, while all of the non-immunized mice died during the acute phase of the challenge infection (bottom panel) (n=5).

Data in A, B and C were analyzed by using an unpaired two-tailed *t* test.

**P*<0.05, ***P*<0.01, ****P*<0.001

Figure S3. Immunization with the GFP-DDDHA strain induces early and strong recall responses in monocytes and neutrophils.

Immunized C57BL/6 mice were re-infected with half million of WT Tulahuen parasites at 12 and 24 hours of post infection. Splenocytes were analyzed by FACS and gated on monocytes and neutrophils. Monocytes from immunized mice have early strong responses by expressing CD86, CD40, MHCII, iNOS, TNF-α and IL-12 as compared to that of non-immunized mice. Neutrophils from immunized mice expressed higher INF-α than that of non-immunized mice.

Data are representative of three separate experiments. All three experiments produced similar results.

Figure S4. Immunization with the GFP-DDDHA strain induces early and strong recall responses in dendritic cells.

Immunized C57BL/6 mice were re-infected with half million of WT Tulahuen parasites at 12 and 24 hours of post infection. Splenocytes were analyzed by FACS and gated on dendritic cells. Dendritic cells from immunized mice have early strong responses by expressing CD86, CD40, MHCII, iNOS, TNF-α as compared to that of non-immunized mice.

Data are representative of three separate experiments. All three experiments produced similar results.

Figure S5. Immunization with the GFP-DDDHA strain induces early and strong recall responses in $CD4^+$ and $CD8^+$ T cells.

Immunized C57BL/6 mice were re-infected with half million of WT Tulahuen parasites at 12 and 24 hours of post infection. Splenocytes were analyzed by FACS and gated on $CD4^+$ and $CD8^+$ T cells. A subset of effector memory T cells ($CD44^+$ $CD62L^-$ $CD4^+$ and $CD44^+$ $CD62L^-$ $CD8^+$) from immunized mice quickly and strongly expressed granzyme B compared to that of non-immunized mice. $CD4^+$ and $CD8^+$ T cells from immunized mice also had early expression of IFN- γ.

Data are representative of three separate experiments. All three experiments produced similar results.