

Legends to Supplementary Figures:

Supplementary Figure 1: (a). Episomal expression of centrin1 protein in the *LdCen1*^{-/-} parasites restores positive growth in cultured macrophages. Survival of parasites of *LdCen1*^{-/-} and *LdCen1*^{-/-} episomally expressing LdCen1 protein (add back; *LdCen1*^{-/-} AB) and wild type (Wt) in macrophages is shown. Newly differentiated macrophages *in vitro* from freshly elutriated human monocytes in the presence of Macrophage colony-stimulating factor (M-CSF) in culture for 7 days were infected with metacyclic parasites and the percent of infected macrophages at different time periods post infection up to day 10 were measured. The data presented are the mean and S.E.M. of three independent experiments. At each time point in an experiment at least 300 total macrophages were counted. (b) Heat-killed (HK) *LdCen1*^{-/-} parasites do not protect mice against virulent challenge. BALB/c mice vaccinated with either *LdCen1*^{-/-} parasites, heat killed *LdCen1*^{-/-} or PBS alone were challenged 5 wk post immunization with virulent *L. donovani*. Graph shows the number of challenge parasites in spleen and liver measured 8 wk post challenge. The data presented are representative of two experiments with similar results. Mean and S.E.M. of four animals in each group are shown. *, p<0.01.

Supplementary Figure 2: *Leishmania* antigen specific stimulation of nitric oxide synthase (NOS₂) in the *in vitro* cultured peritoneal macrophages from naïve mice, mice immunized with *LdCen1*^{-/-} for 5 wk followed by 10 wk challenge or naïve challenged mice. The activity of NOS₂, indicated by the amount of released nitrite (NO) in the splenocyte supernatant, was measured by the Griess reaction. The data presented are representative of two experiments with similar results. Mean and S.E.M. of three or more mice in each group are shown. Cha': challenged; FTAg: Freeze-thaw antigen; Imm': immunized; WI: wk after immunization; WC: wk after challenge *, p<0.01.

Fig S1

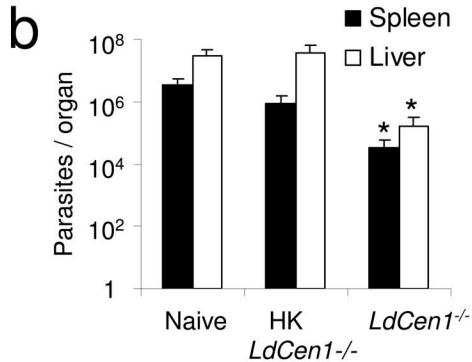
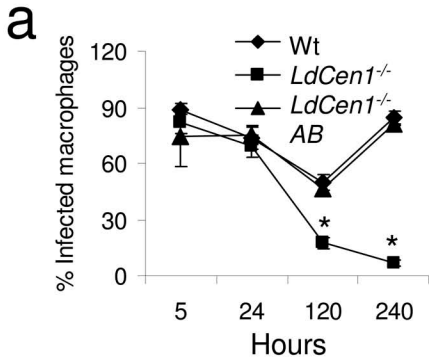
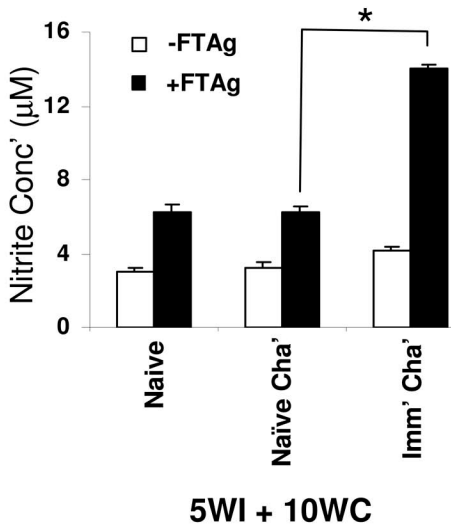


Fig S2



Supplemental Table 1. Real-time PCR confirmation of parasite presence in spleens of mice infected with either the virulent or *LdCen1*^{-/-} parasites

Groups	Ct Values ^a
Naïve	39.6 ± 0.6
6 wk infection with wild type parasites	31.2 ± 4.9
5 wk infection with <i>LdCen1</i> ^{-/-} parasites	31.9 ± 1.8
12 wk infection with <i>LdCen1</i> ^{-/-} parasites	37.7 ± 2.3
Intracellular Amastigotes ^b (cell count per reaction)	
10	28.5 ± 0.4
1	30.1 ± 0.4
0.1	33.5 ± 0.3

^a Mean and S.E.M. of three mice in each group are shown.

^b Standard curve of ex vivo infected macrophages, 4 replicates at each concentration (see Materials and Methods for details)