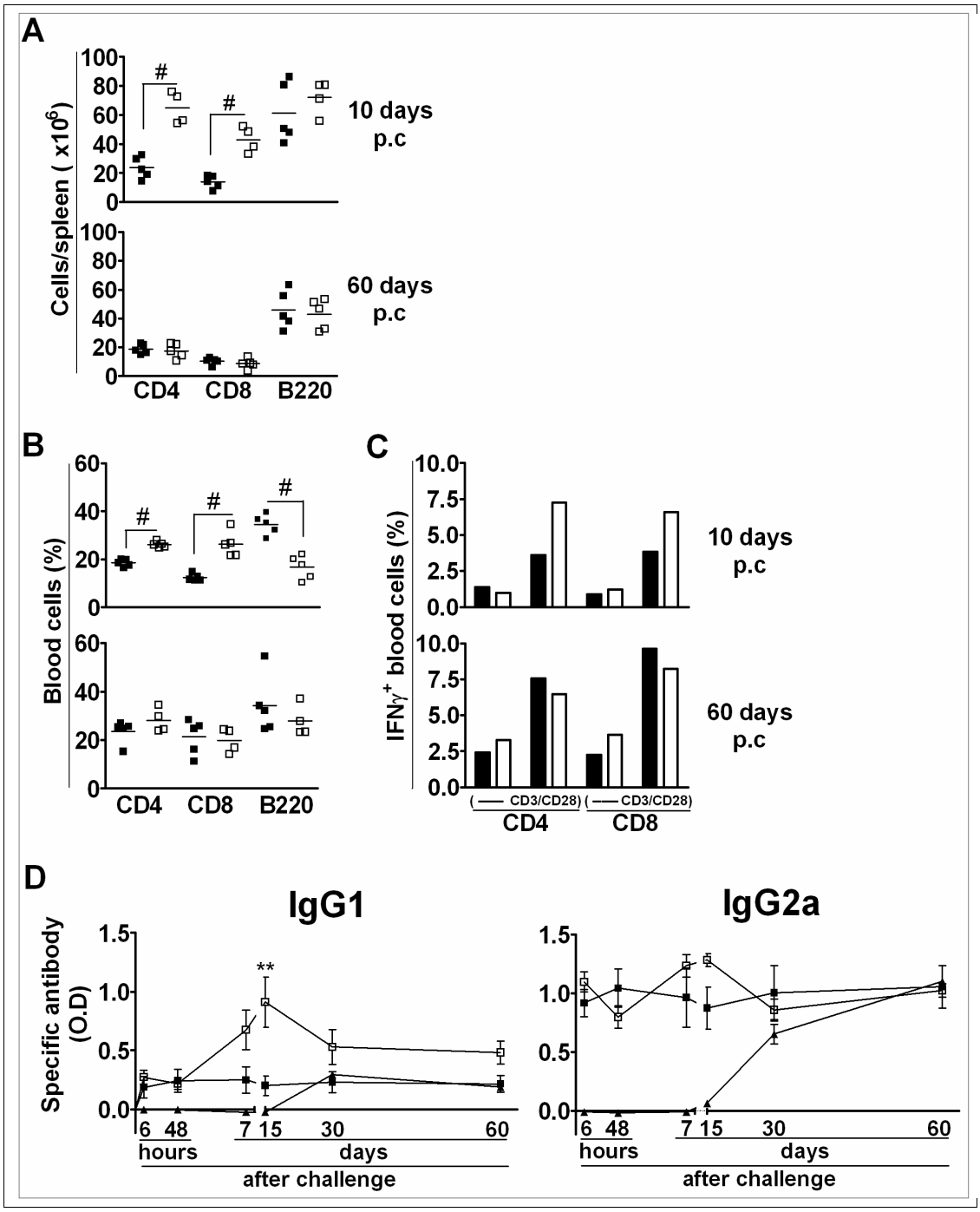
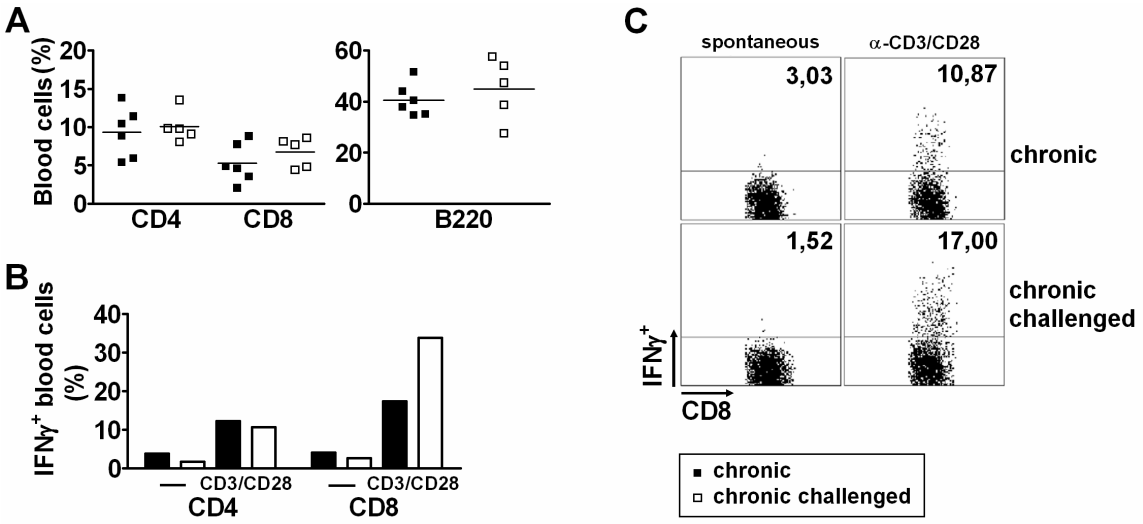


Supplementary Figure 1. Effects of challenge with a single dose of homologous *T. cruzi* parasites in the spleen and blood lymphocyte subpopulation frequencies, IFN-γ production by blood lymphocytes and in the kinetics of *T. cruzi* –specific IgG1 and IgG2a serum antibodies of chronically infected mice. (A-C)

Spleen and blood lymphomononuclear cells from unchallenged chronically infected mice and chronically infected mice that were submitted to i.v. challenge with a single dose of homologous parasites were euthanized 10 and 60 days p.c to analyze the frequency and number of $CD4^+$, $CD8^+$ and B ($B220^+$) cells in the spleen (A); the frequency of $CD4^+$, $CD8^+$ and B ($B220^+$) cells in the blood (B) and the frequency of spontaneous and anti-CD3/CD28-induced IFN-γ-producing $CD4^+$ and $CD8^+$ cells in the blood. D) Kinetics of anti-*T. cruzi* IgG1 and IgG2a antibody serum levels (the 1/12.000 dilution is shown) after single challenge with homologous parasites. Unchallenged chronic mice (■), challenged chronic mice (□).* $p<0.05$, ** $p<0.01$, # $p<0.001$, compared to unchallenged chronically infected mice. Representative experiments are shown.



Supplementary Figure 2. Subpopulation frequencies and IFN-γ production among blood lymphocytes from chronically infected mice that were subjected to sustained challenge with homologous *T. cruzi* parasites. Peripheral blood lymphomononuclear cells from unchallenged chronically infected mice and chronically infected mice that were submitted to sustained challenge with homologous *T. cruzi* parasites were analyzed 1-2 months after the last challenge dose for (A) the frequency of $CD4^+$, $CD8^+$ and B ($B220^+$) cells and (B) the frequency of $CD4^+$ and $CD8^+$ cells producing IFN-γ, either spontaneously or after *in vitro* stimulation for 12 h with anti-CD3/anti-CD28 mAbs. Unchallenged (■) and challenged (□) chronic mice. (C) Representative dot blots of anti-CD3/anti-CD28 mAbs-stimulated production of IFN-γ by $CD8^+$ blood cells from chronically infected, unchallenged and chronically infected mice that were subjected to sustained challenge with homologous parasites. Numbers in dot-blots indicate the frequency of IFN-γ-producing cells. Representative experiment out of two experiments.