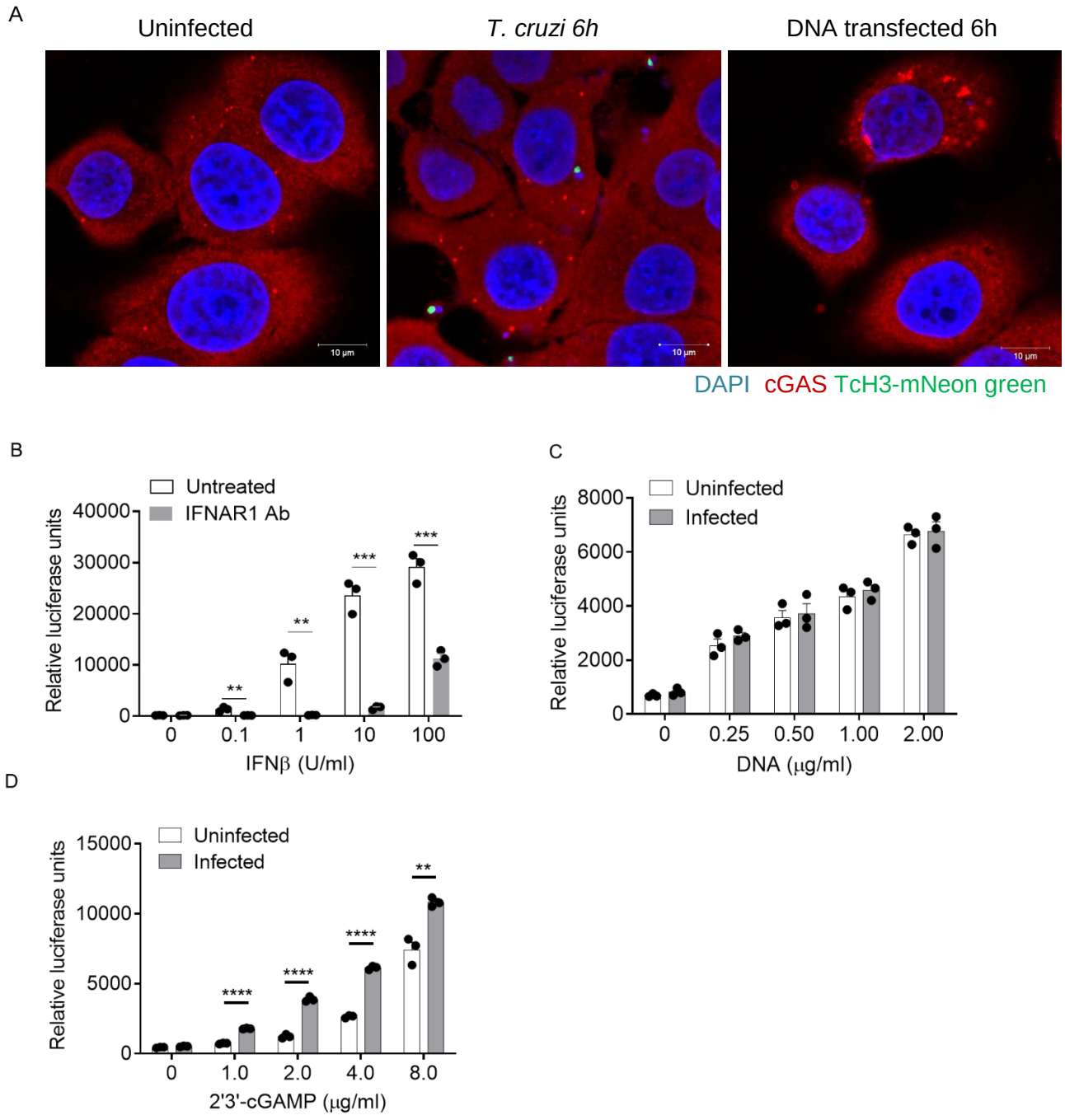
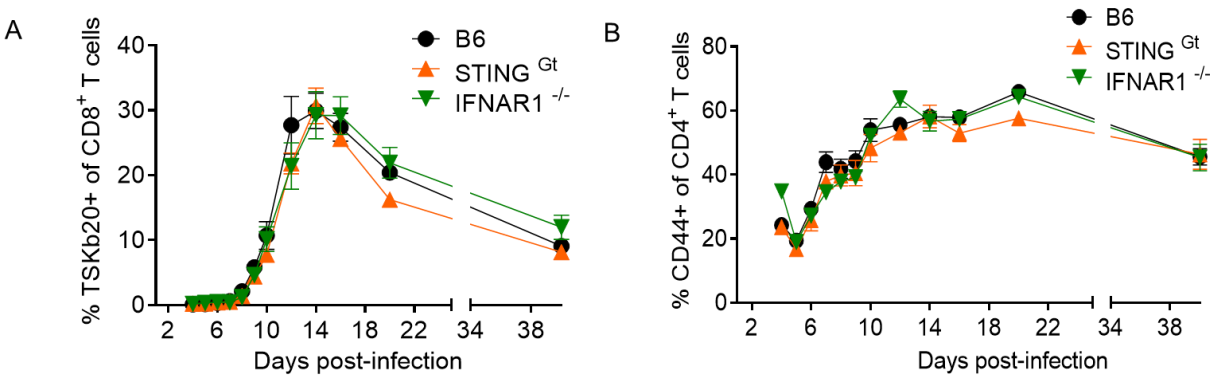
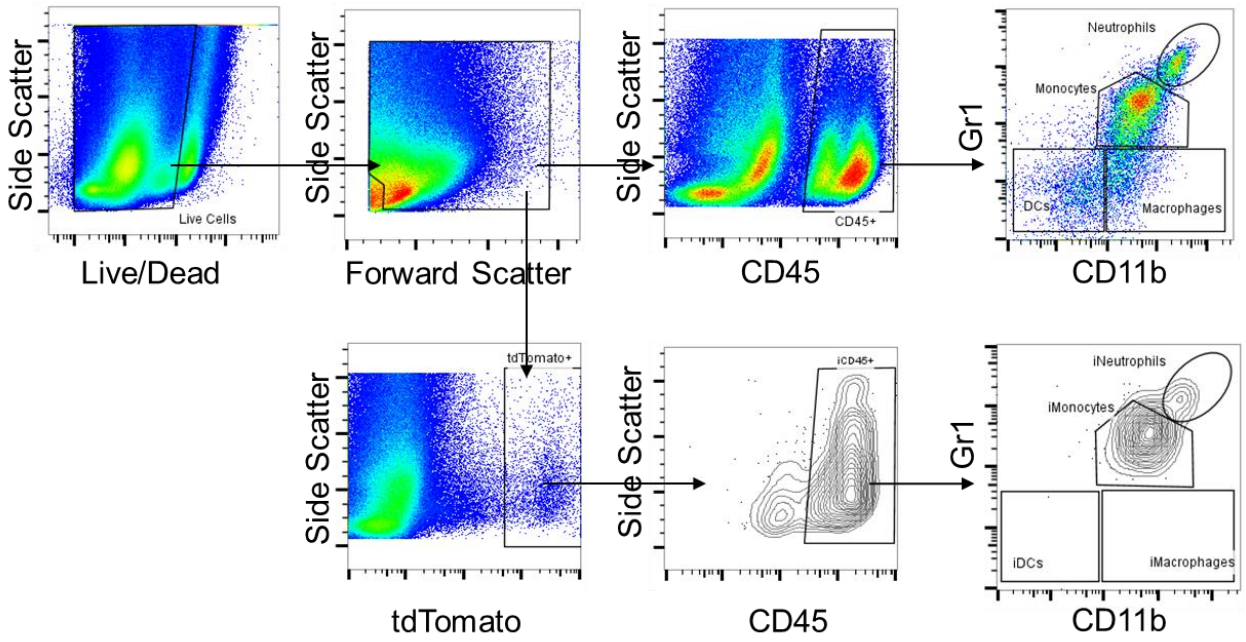


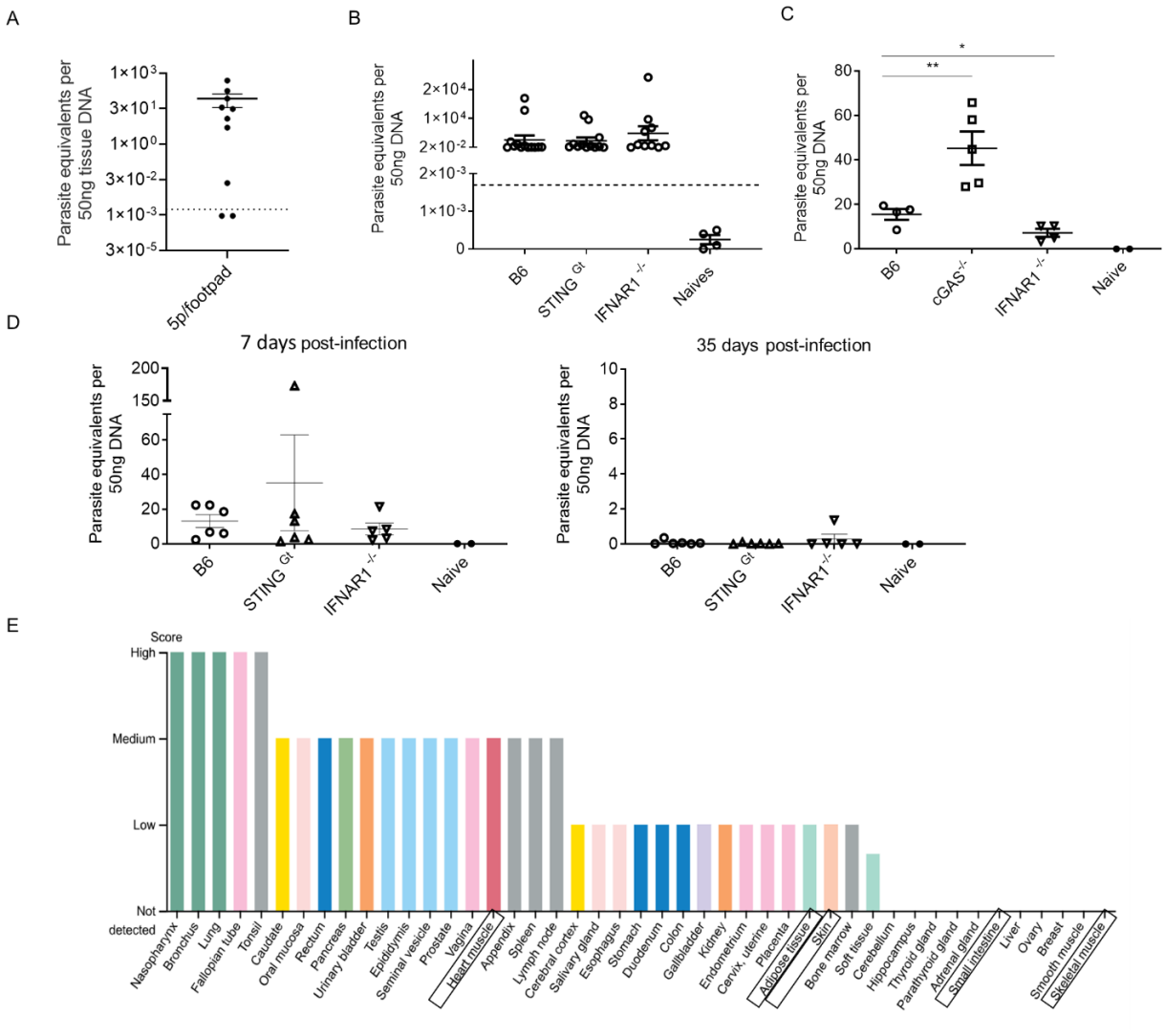
Supplemental Figure 1: *T. cruzi* activates the cGAS-STING pathway in primary cells to induce a modest IFN-I response without active suppression. A) Infrequent occurrence of HeLa host cells showing small puncta at 6h post *T. cruzi* infection compared to larger puncta following DNA transfection. B) Raw-Lucia-ISG macrophage cells were treated with IFNAR mAb MAR1-5A3 (7.5ug/ml), stimulated with increasing concentrations of IFNβ, and quantified Lucia-luciferase activity in the supernatant 24h post-stimulation to assess the effective blocking of IFNAR1. C-D) IRF reporter expression in uninfected or infected macrophages costimulated or not with DNA (C) or 2'3'-cGAMP (D) were assessed by measuring Lucia-luciferase activity in the supernatant 24h post-stimulation. Data are representative of 2 or more independent experiments with n=3 infection replicates.



Supplemental Figure 2: The timing and frequency of anti- *T. cruzi* T cell responses do not vary significantly in the STING and IFNAR -deficient mice relative to WT mice.
A) Kinetics of *T. cruzi* specific $CD8^+$ T cells in B6, $STING^{Gt}$ and IFNAR1 $^{-/-}$ infected in the feet and determined by staining of peripheral blood for specific binding of MHC-I tetramers containing the *T. cruzi* trans-sialidase TSKB20 peptide. B) Kinetics of activated $CD4^+$ T cells in B6, $STING^{Gt}$ and $IFNAR1^{-/-}$ infected in the feet and determined by staining of peripheral blood for activation marker CD44. Data represent n>3 mice; 2 independent experiments.



Supplemental Figure 3. Gating strategy for phenotyping the various leukocyte populations recruited to the ear. Live cells (Live/Dead Aqua-) that were gated or not on infected (tdTomato+) cells, were gated on CD45+ cells, followed by CD11b and Gr-1 to distinguish neutrophils, monocytes, macrophages and dendritic cells (DCs).



Supplemental Figure 4: STING activation does not impact the initial establishment of infection but constrains acute parasite load in the heart independently of IFN-I. A) The establishment of infection in the skeletal muscle as determined by detection of parasite loads followed by immunosuppression after 30 days post infection with a low dose of 5 CL in the hind footpad, with 2 mice showing parasite loads below the limit of detection (represented by the dotted line). B) Parasite loads in the skeletal muscle following the similar protocol to (A) in B6, STING^{Gt} and IFNAR1$^{-/-}$ mice. n=10 mice per group. C) *T. cruzi* DNA in the hearts of cGAS$^{-/-}$ mice in comparison to control B6 and IFNAR1$^{-/-}$ mice infected intraperitoneally with Colombiana strain parasites as determined by quantitative real-time PCR at 14 days post-infection. n=6 mice per group. D) *T. cruzi* DNA in the hearts of STING^{Gt} mice in comparison to control B6 and IFNAR1$^{-/-}$ mice infected intraperitoneally with Colombiana strain parasites as determined by quantitative real-time PCR at 7 days post-infection (left) and 35 days post-infection (right). Data are representative of 2 independent experiments. E) Relative basal STING protein expression in human tissues based on analyses of data from the Human Protein Atlas database[1] (Image available from[2]).

[1] M. Uhlen, L. Fagerberg, B.M. Hallstrom, C. Lindskog, P. Oksvold, A. Mardinoglu, A. Sivertsson, C. Kampf, E. Sjostedt, A. Asplund, I. Olsson, K. Edlund, E. Lundberg, S. Navani, C.A. Szigyarto, J. Odeberg, D. Djureinovic, J.O. Takanen, S. Hober, T. Alm, P.H. Edqvist, H. Berling, H. Tegel, J. Mulder, J. Rockberg, P. Nilsson, J.M. Schwenk, M. Hamsten, K. von Feilitzen, M. Forsberg, L. Persson, F. Johansson, M. Zwahlen, G. von Heijne, J. Nielsen, F. Ponten, Proteomics. Tissue-based map of the human proteome, Science 347(6220) (2015) 1260419.
[2] Human Protein Atlas, v22. https://www.proteinatlas.org/ENSG00000184584-STING1/tissue.