**Functional evaluation of gene silencing on macrophages derived from U937 cells using interference RNA (shRNA) in a model of macrophages infected with *Leishmania (Viannia) braziliensis***

Supplementary material



Sup. Fig.1.CellProfiler® standardized pipeline for image analysis: module 1 loads images of membranes labeled with DiD stain and images of nucleic acids labeled with Hoechst stain; modules 2 and 3 change color images to gray scale; module 4 identifies macrophages according to their area (100 to 400 pixels) and their signal intensity (0,1 to 1) using Otsu per object as fragmentation algorithm; module 5 enhances contrast between objects and subtracts background to facilitate amastigotes identification; module 6 overlaps images of membranes labeled with DiD and images of nucleic acids labeled with Hoechst in order to discard artefacts outside the macrophages area and to confirm identified amastigotes are inside the macrophages area; module 7 allows amastigotes identification according to their sizes of both nucleus and kinetoplast (7,3 to 15,9 pixels) and their signal intensity (0,052 to 1) using Robust background per object as fragmentation algorithm; step 8 associates each parasite identified with its corresponding host cell; step 9 counts the number of amastigotes per cell and finally step 10 exports these data to a spread sheet. These data were used to determine percentage of infection, number of parasites per sampled macrophage and number of parasites per infected macrophage.



Sup. Fig. 2. Frequency distributions of amastigotes per infected macrophage in the parental cell line (U937) and the cell lines generated: (A) cell lines with expression (U937-GFP) and silencing (U937-GFP/shRNA-GFP) of gfp and the corresponding negative control (U937/shRNA-NR-MOI: 10); (B) cell lines with lmna silencing (U937/shRNA-LMNA) and the corresponding negative control (U937/shRNA-NR, MOI 400); and (C) cell lines with gro-β silencing (U937/shRNA-Gro-β) and the corresponding negative control (U937/shRNA-NR, MOI 0.2). Data illustrated corresponds to the entire set of infection experiments performed in triplicate and in three independent occasions.