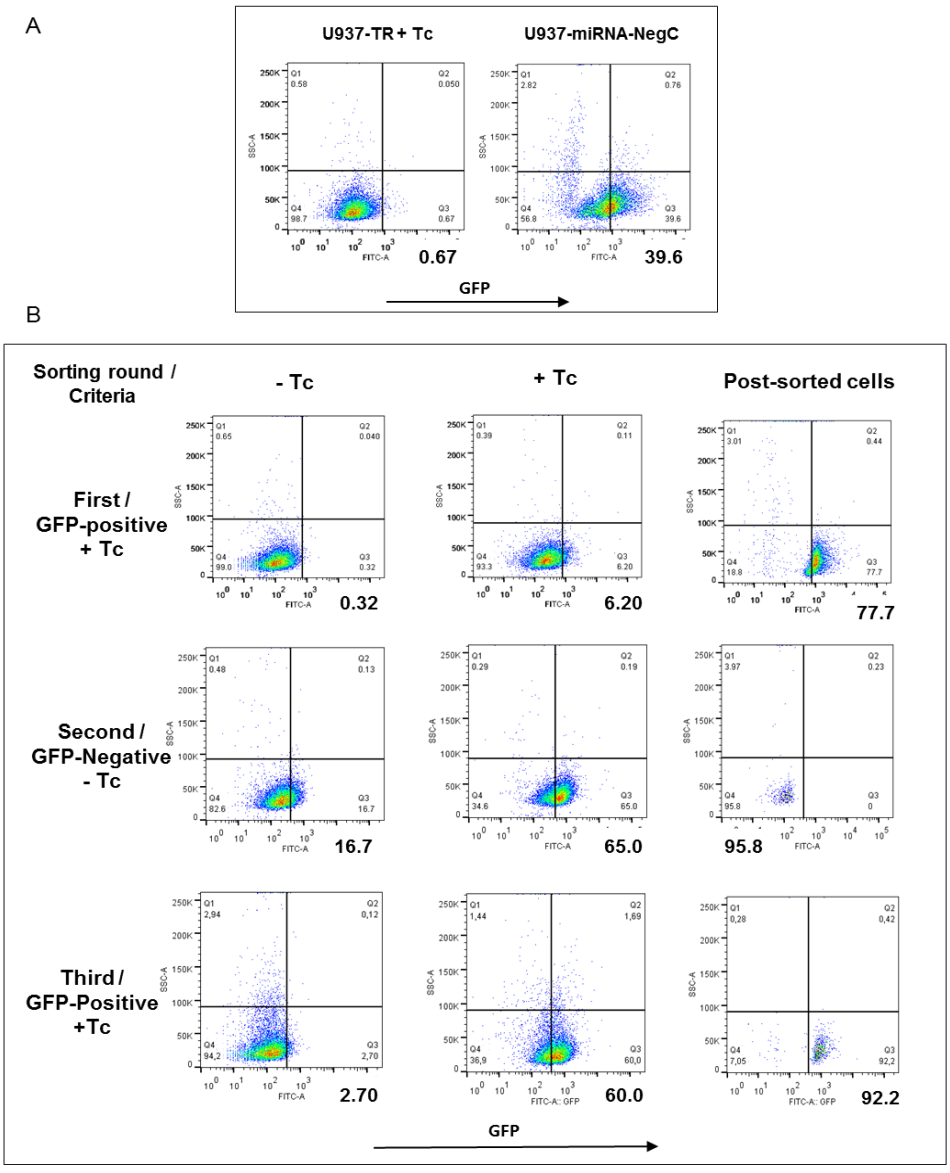
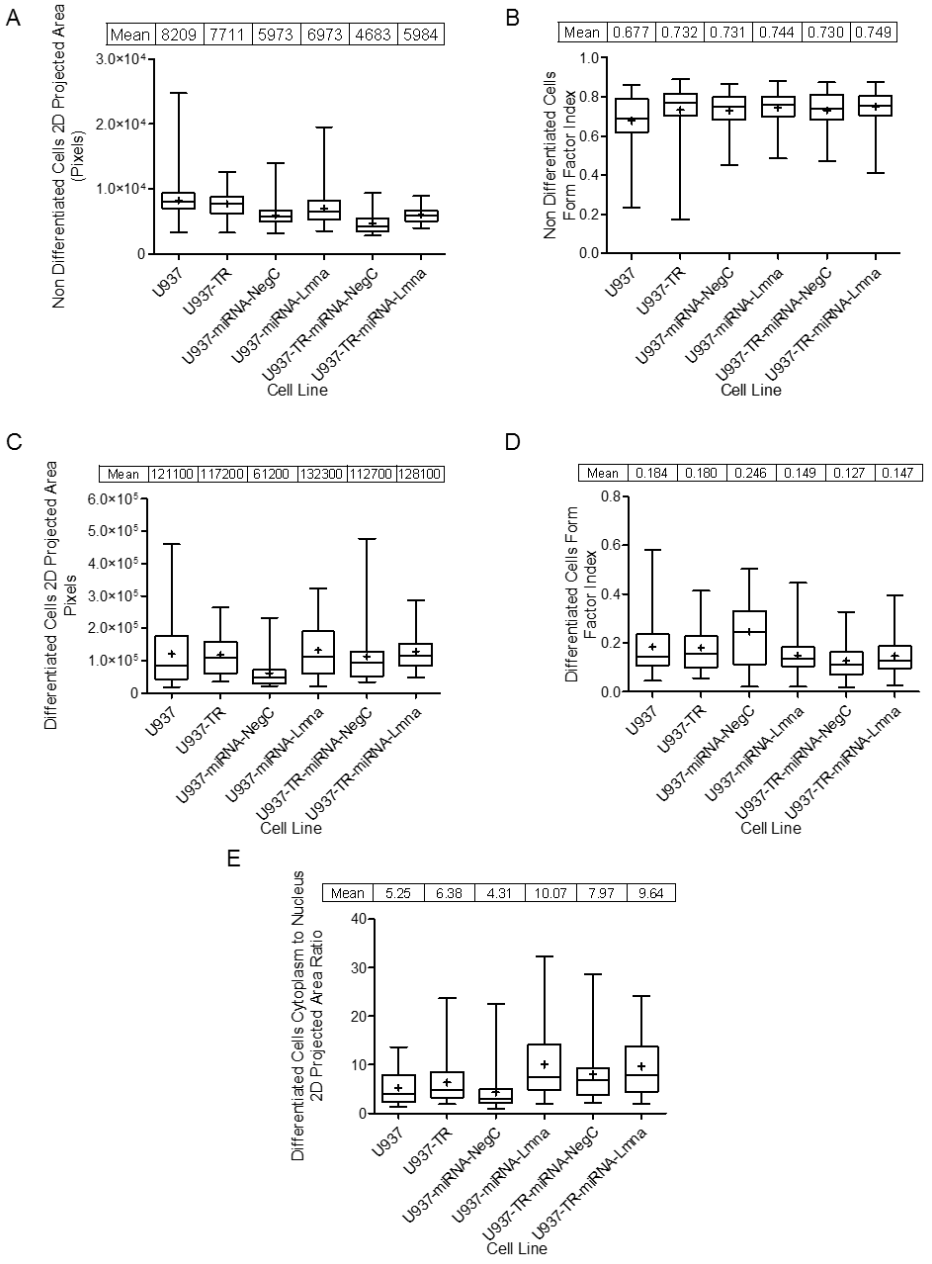
Generation and characterization of U937-TR: a platform cell line for inducible gene expression in human macrophages

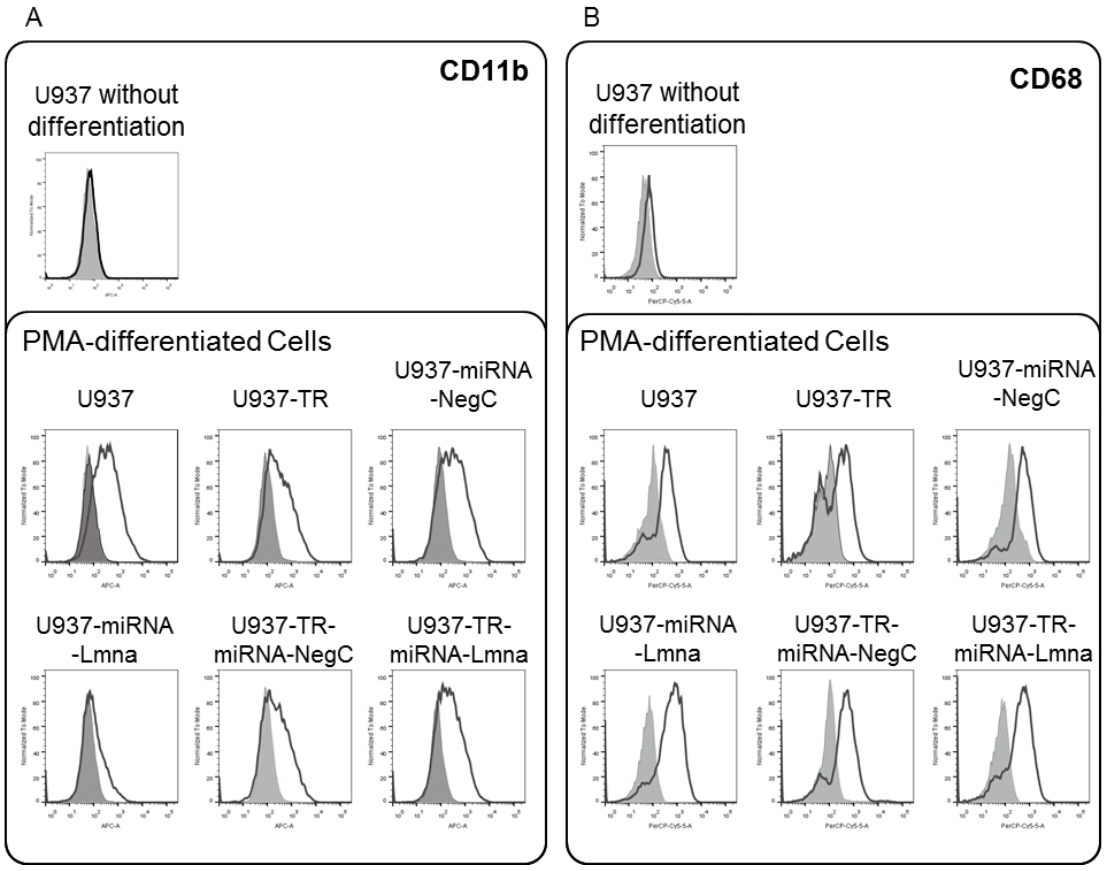
Supplementary Figures



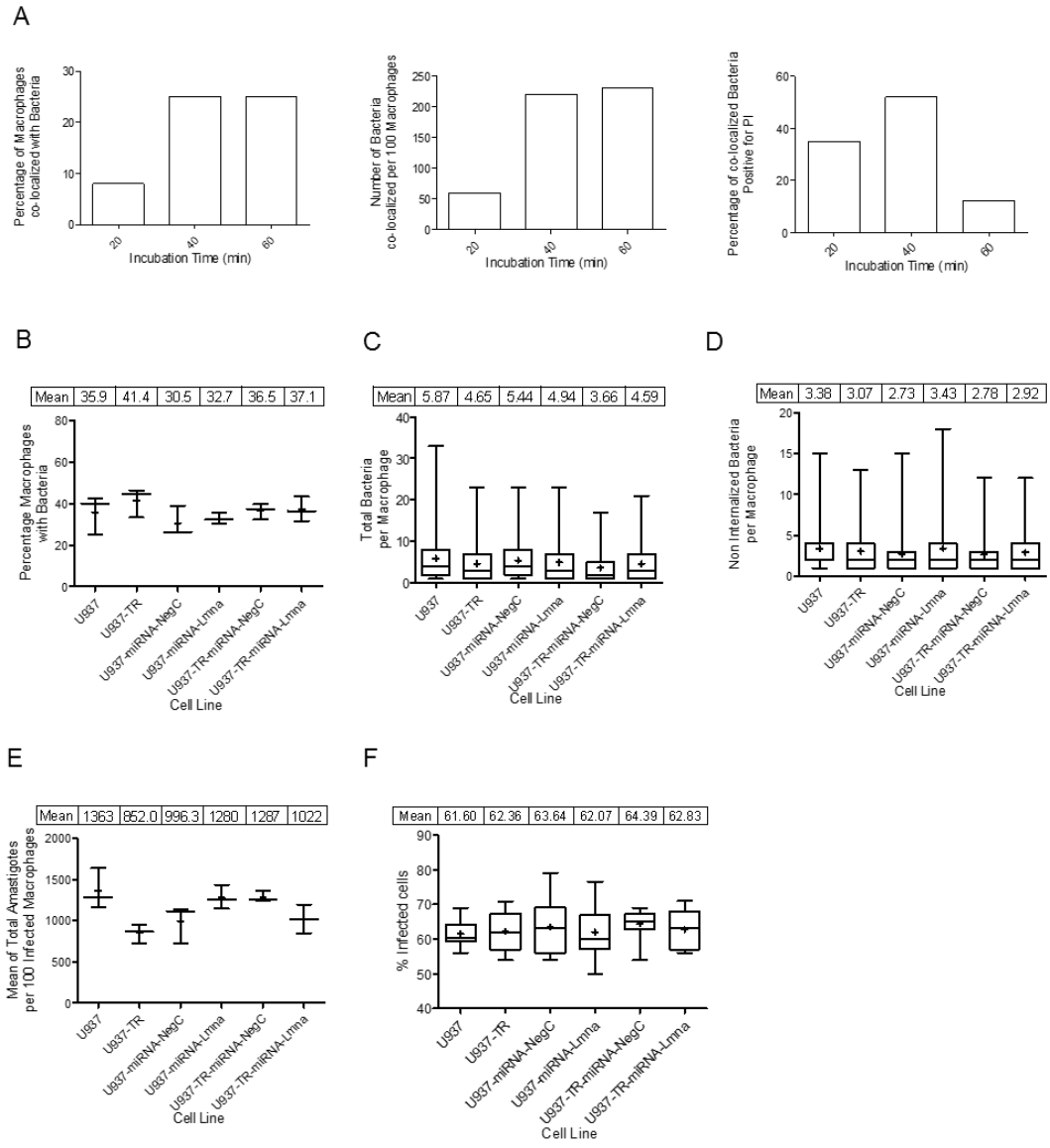
Supplementary Figure 1. Tetracycline-induced GFP expression in U937-TR-miRNA-Lmna cells subjected to three rounds of fluorescence-activated cell sorting (FACS). U937-TR-miRNA-NegC and U937-TR-miRNA-Lmna, cells were treated with tetracycline (100ng/ml) for 3 days. Then cells were sorted based on their GFP expression. (A) Dot plots showing the autofluorescence levels of U937-TR cells used as negative control (left) and U937-miRNA-NegC, constitutively expressing GFP cells, used as positive control (right). (B) Representative dot plots from the three rounds of GFP-based cell sorting. Positive (first and third sorting) and negative selection (second sorting) were performed. The shown plots belong to U937-TR-miRNA-Lmna cells but similar results were obtained with U937-TR-miRNA-NegC cell line. Tc: Tetracycline. Percentage values are shown for some quadrants for comparison effects.



Supplementary Figure 2. Morphometric changes induced by PMA treatment in U937 and derived cell lines. PMA-treated and non-treated cells were stained with Giemsa stain, photographed and analyzed using a CellProfiler Software pipeline (Carpenter et al., 2006), designed to measure the Area and the Form Factor Index of the bi-dimensional projection of the non-differentiated (A and B) and differentiated (C and D) cells of each cell line. Area values (in pixels) of cytoplasm and nucleus were determined to calculate the Cytoplasm to nucleus area ratio of differentiated cells (E). The entire population distribution of recorded values for each cell line is shown (n ranges from 93 to 122 records for A and B; 31 to 55 records for C and D; 27 to 55 records for E). Box and whiskers graphs show maximum value, minimum value, median and mean (+ sign and table), 25th, 50th and 75th percentiles of each registered population. Kruskal-Wallis test was performed followed by Dunn's Multiple Comparison Test ($P < 0.05$).

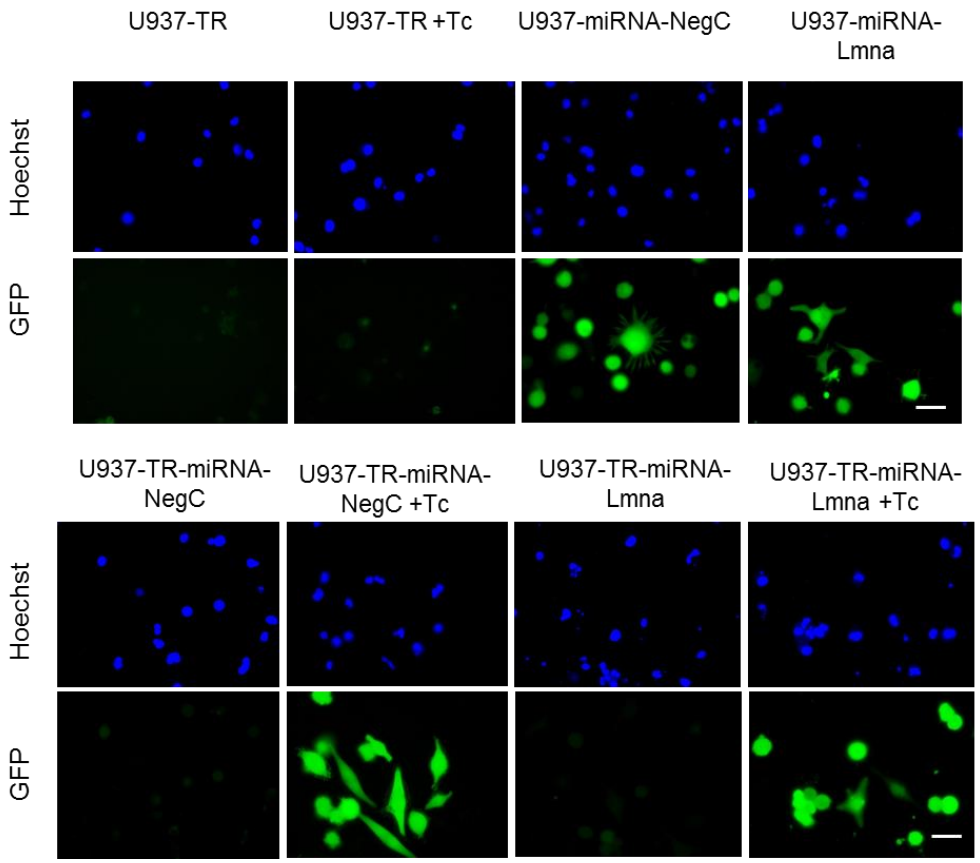


Supplementary Figure 3. **CD11b and CD68 expression changes induced by PMA treatment on U937 and derived cell lines**. Macrophage-like cells were obtained in suspension by incubation with StemPro® Accutase® (Life Technologies) during 15 minutes at 37°C. Fc-receptors were blocked with 1µg human IgG (Invitrogen 02-7102) during 15 minutes. Cells were incubated for 30 minutes at RT in the dark, with 4µl of anti-CD11b (301409, Biolegend) conjugated to APC or the corresponding isotype control (400119, Biolegend). Cells were fixed with 4% paraformaldehyde (PFA) 15 minutes at RT. Cells were kept at 4°C until flow cytometry analysis. PBS washes were used between each step. For CD68 detection, cells were fixed with 4% PFA in 15 min. PFA traces were blocked with 50nM ammonium chloride for 5 min. Cells were made permeable with 0.2% Saponin in PBS for 20 minutes. Then non-specific protein binding was reduce with 1% BSA for 20 min. Cells were incubated for 30 min in the dark, with 4µl of anti-CD68 (333813, Biolegend) monoclonal antibody conjugated to PerCP/Cy5.5 or the corresponding isotype control IgG2b (400337, Biolegend). Then, cells were kept at 4°C until flow cytometry analysis. PBS washes were used between each step in the protocol. Flow cytometry assay showing changes in CD11b (A) and CD68 (B) marker expression from undifferentiated to differentiated U937 and derived cell lines. Filled histograms illustrate isotype control signal whereas continuous lines depict the signal of anti-CD11b- APC conjugated monoclonal antibody (Panel A) or the signal of anti-CD68-PerCP/Cy5.5 conjugated monoclonal antibody (Panel B). Readings were performed in a FACS Aria II Flow Cytometer (BD Biosciences) using the FACSDiva TM Software (BD Biosciences). 10,000 events were acquired. Each flow cytometry result is presented as a frequency histogram (numbers of events (cells) Normalized to the Mode vs fluorescence intensity signal (log scale)). Histograms are representative from 2 independent experiments.



Supplementary Figure 4. Experimental assessment of phagocytic capacity of U937 and derived cell lines. (A) Heat-inactivated *E. coli* were labeled with DAPI, washed and added to PMA-differentiated U937 cells. After incubation for 20, 40 or 60 minutes at 37°C (phagocytosis), cells were washed and exposed to Propidium Iodide (PI) 5µM and incubated at room temperature for 5 additional minutes. After washing excess PI, labeled bacteria presence (or lack thereof) on cells was registered by observation using a fluorescence microscope. 100 differentiated cells (macrophages) per experimental unit were registered as either co-localized or not with bacteria and the number of positive labeled bacteria for DAPI, PI or both. Percentage of U937 differentiated cells co-localized with at least one bacterium is displayed in left chart. Number of bacteria per 100 U937 differentiated cells is shown in the chart at the center of the panel. (B) From the experiment described in Figure 2A-B, the percentage of differentiated cells (macrophages) co-localized with at least one bacterium is shown. (C) Individual cell records from three replicates (Figure 2A-B) were combined to show the entire population distribution of values for each cell line (n ranges from 91 to 123 records) for total bacteria per macrophage. (D). Number of non-internalized

bacteria per macrophage (See figure 2A-B). From the experiment described in Figure 2C-D, the mean of total amastigotes per 100 infected macrophages per cell line is shown in (E) and the percentage of infected cell is depicted in (F). Box and whiskers graphs show maximum value, minimum value, median and mean (+ sign and table), 25th, 50th and 75th percentiles of each register population. For results shown in B-D, Kruskal-Wallis test was performed followed by Dunn's Multiple Comparison Test ($P < 0.05$).



Supplementary Figure 5. Constitutive or Tetracycline-induced GFP expression in U937-derived cell lines. 2x10^5 viable cells were plated over glass coverslips for each cell line. Differentiation was induced by treatment with PMA 100ng/ml for 5 days and cultured at 37°C and 5% CO_2 atmosphere. For the indicated experimental units (+Tc), the culture medium was supplemented with Tetracycline at a final concentration of 100ng/ml, since day 2 of incubation. At day 4 of incubation, nuclei were labeled with Hoechst 1.2µg/ml and incubated for 16 hours before harvest for image acquisition with a fluorescence microscope. The panel shows representative images captured using the blue channel (Hoechst) and green channel (GFP) (Scale bar=50µm).