Supplementary Data

MNAse I sensitivity assay

Heterochromatin content was evaluated in M0, M1 and M2 polarized THP-1 cells (3.6 x 10^6) as described in (1). Briefly, cells were homogenized in RBS buffer (10 mmol/l Tris-HCl (pH 7.4), 10 mM NaCl, 3 mM$MgCl_2$, 0.5% NP-40) for 15 min on ice. Samples were then centrifuged at 4°C for 5 min at 1400 *g*. The medium was discarded and the pellet was washed twice with RBS buffer. Samples were then digested with 2.5 U of micrococcal nuclease in digestion buffer (15 mM Tris-HCl (pH 7.5), 60 mM KCl, 15 mM NaCl, 1 mM $CaCl_2$, 3 mM $MgCl_2$, 20% glycerol, 15 mM β-mercaptoethanol) for 30 seconds, 1, 2 or 5 minutes. Stop solution (50 mM Tris (pH 7.5), 150 mM NaCl, 50 mM EDTA, 0.3% sodium dodecyl sulfate (SDS)) was used to stop digestion in samples. DNA was extracted using phenol/chloroform method and was precipitated with ethanol 100%. DNA was washed once with 70% ethanol, resuspended in H_2O and was separated using 1.2% agarose gel.

Reactive oxygen species measurement

ROS management in M0, M1 and M2 THP-1 cells was evaluated using a fluorescent probe, CM-H_2DCFDA (DCF-DA). THP-1 cells were differentiated and polarized in 6 well plates (8 x 10^5 per well). After polarization, cells were rinsed with PBS. Cells were trypsinized and centrifuged at 1000 rpm for 7 minutes. Cells were resuspended in 2% heat inactivated serum – PBS and were centrifuged at 4°C for 5 minutes at 1200 rpm. Macrophages were incubated with DCFDA dye (10 μM) for 20 minutes at 37°C and centrifuged again at room temperature for 5 minutes at 1200 rpm. Cells were exposed to different concentrations (0.25, 1 and 5 μM) of hydrogen peroxide solution (H_2O_2), for 15 minutes at 37 °C. After rinsing the cells in 2% heat inactivated serum – PBS, cells were resuspended in cold PBS. Fluorescence was measured by flow cytometry using FACS calibur (BD Biosciences).

X-ray irradiation

Four days before irradiation, 800 000 THP-1 monocytes were differentiated in 6-well plates to generate M2 macrophages. To obtain M1 or M0 macrophages, cells were seeded three or two days respectively before irradiation. Just before X-ray irradiation, culture medium was changed by CO_2 independent medium (Gibco #18045054) supplemented with 2 mM L-glutamine and 3.75 g/L of D-glucose. Photon beam (2 Gy/min) was produced with X-RAD 225 kV (Precision X-ray) irradiator and the cells were seeded in six-well plates, positioned at 30 cm from the source. After irradiation, the 6-well plates were replaced in the CO_2 incubator until the samples were recovered.

Gene	Forward	Reverse
CCL22	CACTTCTACTGGACCTCAGAC	AGTAGGCTCTTCATTGGCTCA
EGF	ATGTCCTGCCCTCAACC	GGTTGCATTGACCCATCTGC
IL-6	CCTGAACCTTCCAAAGATGGC	CACCAGGCAAGTCTCCTCATT
IL-8	TCTGTGTGAAGGTGCAGTTTT	GGGGTGGAAAGGTTTGGAGTA
IL-10	TTCCCTGTGAAAACAAGAGCAA	GTAGATGCCTTTCTCTTGGAGCTTA
RPS9	CTGGATGAGGGCAAGATGAAG	GTCTGCAGGCGTCTCTCTAAGAA
TNFα	CTGCACTTTGGAGTGATCGG	TCAGCTTGAGGGTTTGCTAC

Table S1. Primers used for RT-qPCR

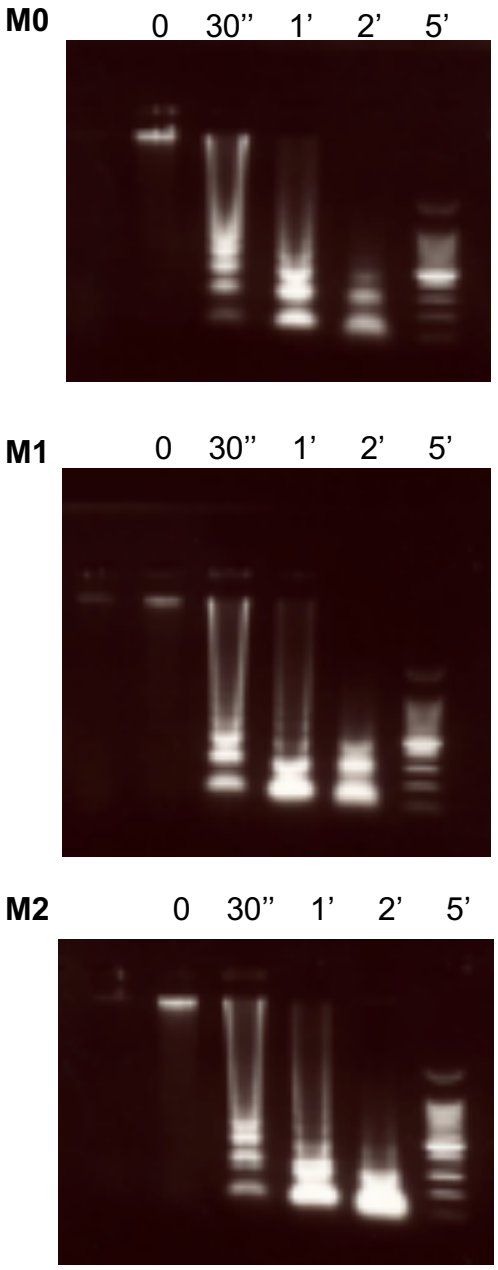


Figure S1. Heterochromatin content in M0, M1 and M2 macrophages
Heterochromatin content was evaluated by MNAse assay. DNA was extracted from M0, M1 and M2 macrophages and then exposed to MNAse enzyme for 30 seconds, 1, 2 or 5 minutes. Resulting fragments were separated by electrophoresis. Representative images show the migration profile of fragmented DNA in M0, M1 and M2 macrophages after MNAse treatment (N=5).

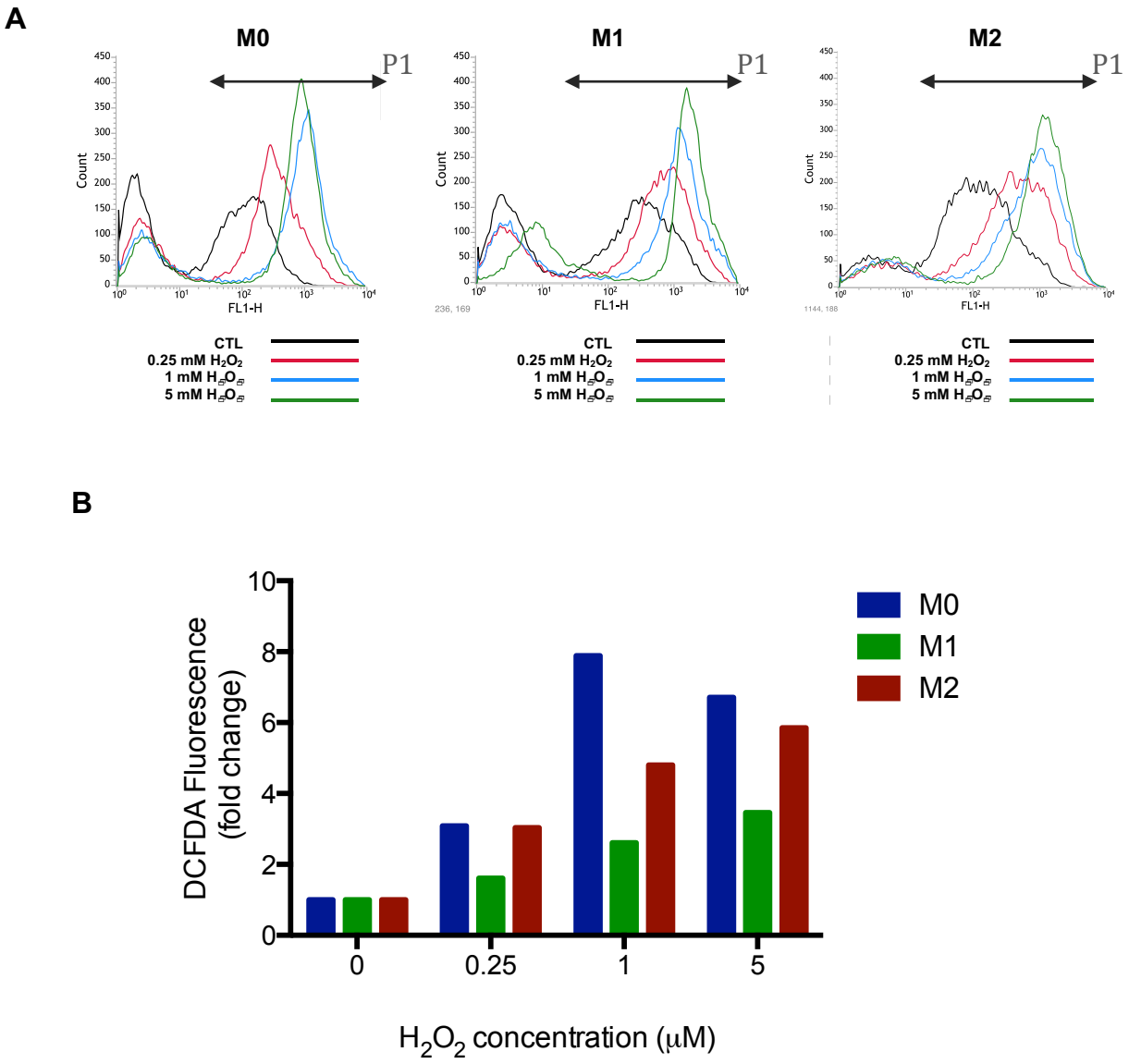
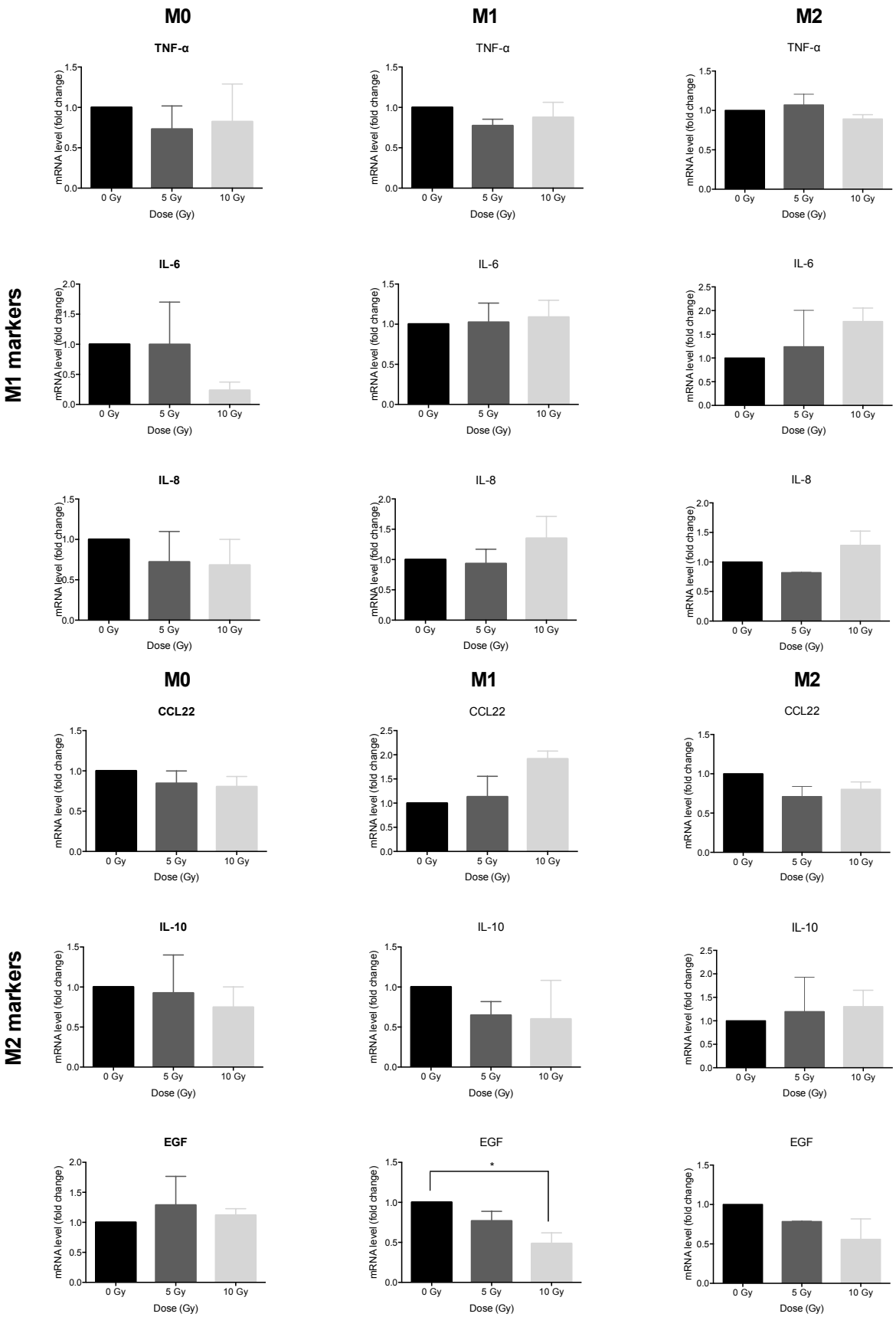


Figure S2. Differential ROS management in M0, M1 and M2 macrophages
M0, M1 and M2 macrophages were treated with different H_2O_2 concentrations (0, 0.25, 1 and 5 μM) and ROS content was detected by flow cytometry (DCFDA fluorescence). **A**, Evaluation of ROS content in M0, M1 and M2 macrophages after exposure to different H_2O_2 concentrations. **B**, Quantification of the mean fluorescence (ROS level) in macrophages in gated population (P1). Results are normalized to the untreated condition (N=1).


M0
M1
M2
M1 markers
M2 markers
TNF-α
IL-6
IL-8
CCL22
IL-10
EGF
mRNA level (fold change)
0 Gy
5 Gy
10 Gy
Dose (Gy)
*

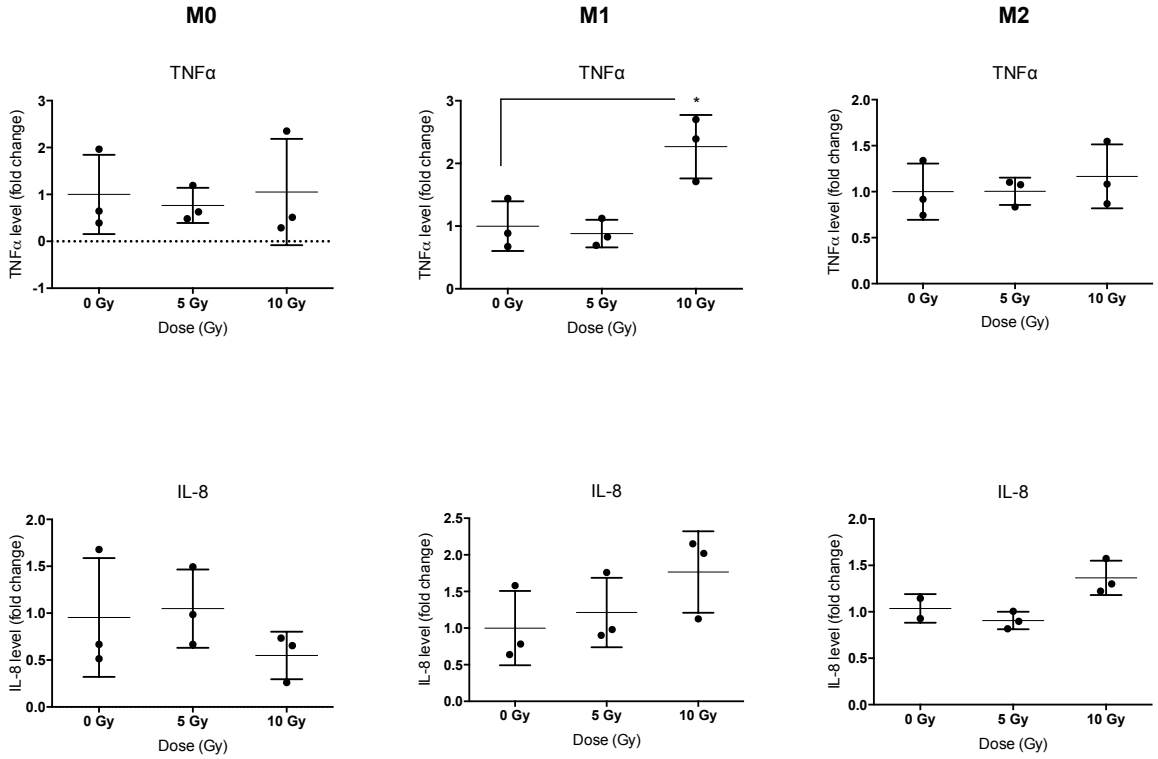


Figure S3. **X-ray irradiation does not induce macrophage reprogramming**
M0, M1 and M2 macrophages were irradiated with different doses of X-rays (0, 5 and 10 Gy) **A,** 24h after X-ray irradiation, mRNA levels of M1 (TNFα, IL-6, IL-8) and M2 (CCL22, IL-10, EGF) markers were assessed by RT-qPCR (N=3, mean ± SD). One-way ANOVA analyses followed by Dunnett's multiple comparison tests were performed to evaluate the significance (* $p \leq 0.05$; ** $p < 0.01$; *** $p < 0.001$). **B,** 24h after X-ray irradiation, TNFα and IL-8 secretion was evaluated by ELISA. Results are expressed in pg/ng of proteins and are normalized to the non-irradiated condition (fold change) for each macrophage phenotype (N=3, mean ± SD). One-way ANOVA analyses followed by Kruskal Wallis multiple comparison tests were performed on data (* $p \leq 0.05$; ** $p < 0.01$; *** $p < 0.001$).

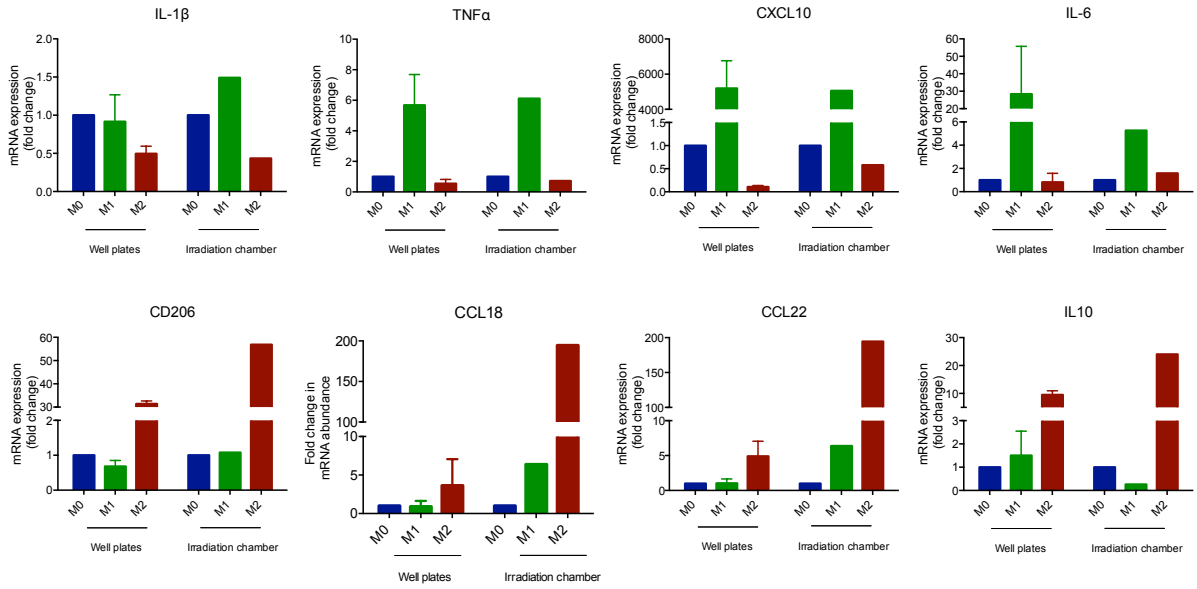


Figure S4. Evaluation of macrophage phenotype after differentiation and polarization in the irradiation chambers

THP-1 cells were differentiated and polarized into M0, M1 and M2 macrophages in conventional well plates and irradiation chambers. mRNA levels of M1 (IL-1β, TNFα, CXCL10 and IL-6) and M2 (CD206, CL18, CCL22 and IL-10) markers were assessed by RT-qPCR in M0 (blue), M1 (green) and M2 (red) macrophages (N=2 for well plate; N=1 for irradiation chamber).

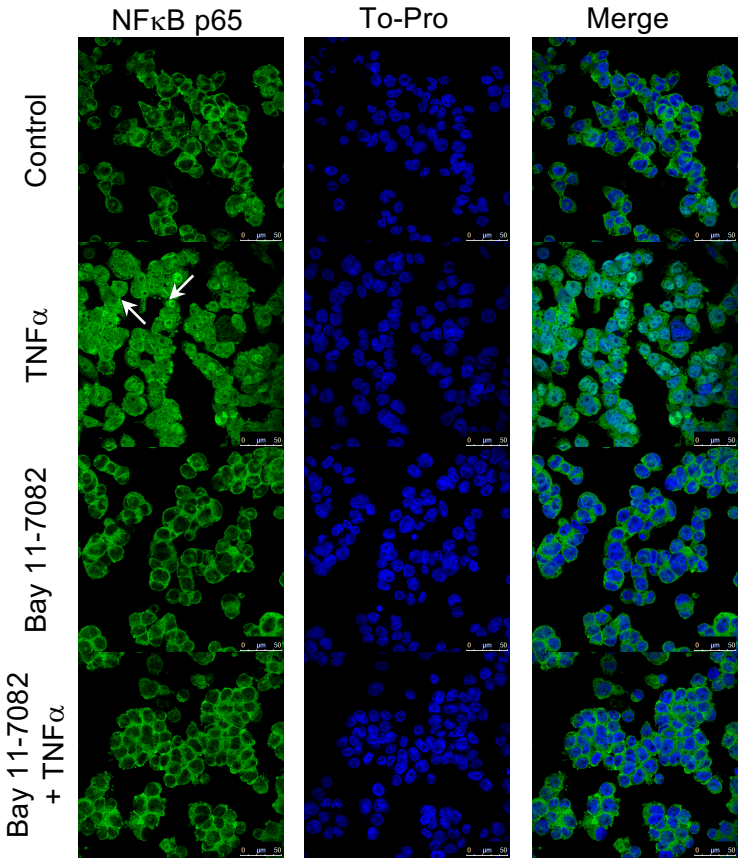


Figure S5. Inhibition of nuclear NFκB p65 translocation after Bay 11-7082 exposure
M0 macrophages were exposed to TNFα (20 ng/ml) or to Bay 11-7082 inhibitor (5 μM) as well as to the combination of both TNFα and Bay 11-7082 for 12h. The nuclear translocation of NFκB p65 was evaluated by immunofluorescence staining. Representative immunofluorescence staining images display NFκB p65 in green and nuclei in blue (N=1).

1. Peixoto P, Castronovo V, Matheus N, Polese C, Peulen O, Gonzalez A, et al. HDAC5 is required for maintenance of pericentric heterochromatin, and controls cell-cycle progression and survival of human cancer cells. Cell Death Differ. 2012;19(7):1239-52.