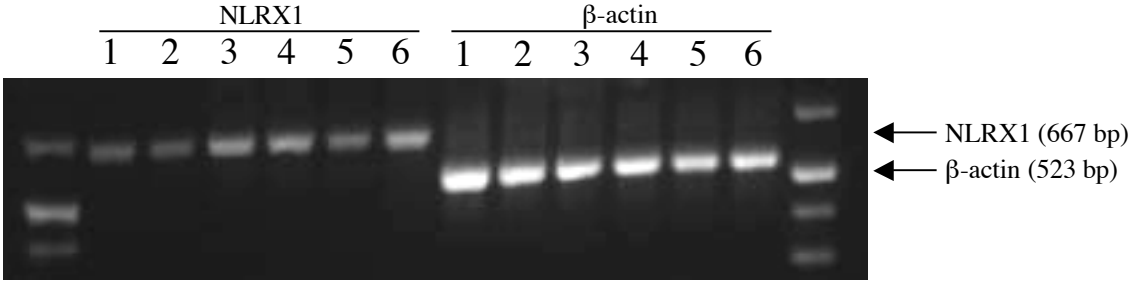
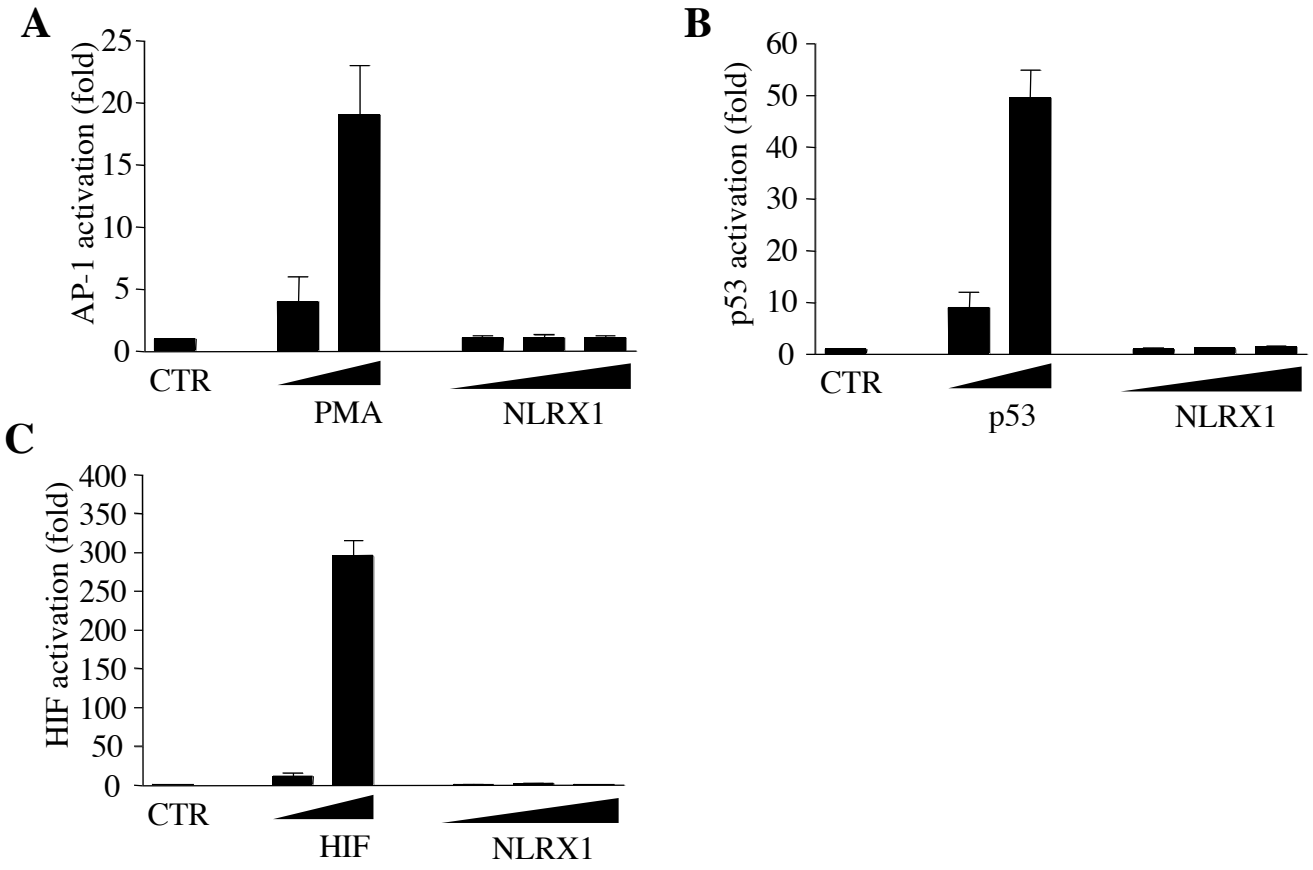
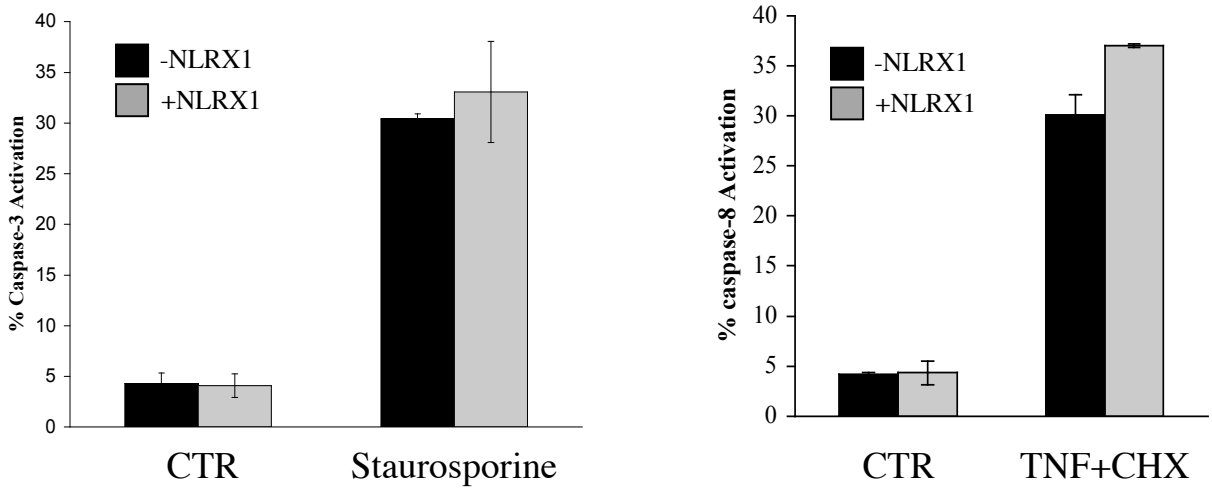


Supplementary Figure 1: Expression of NLRX1 in human cell lines. 1: HeLa, 2: HEK293T, 3: MCF-7, 4:Ramos, 5:Jurkat, 6: THP1. The following oligonucleotides were used to amplify NLRX1 and β-actin:

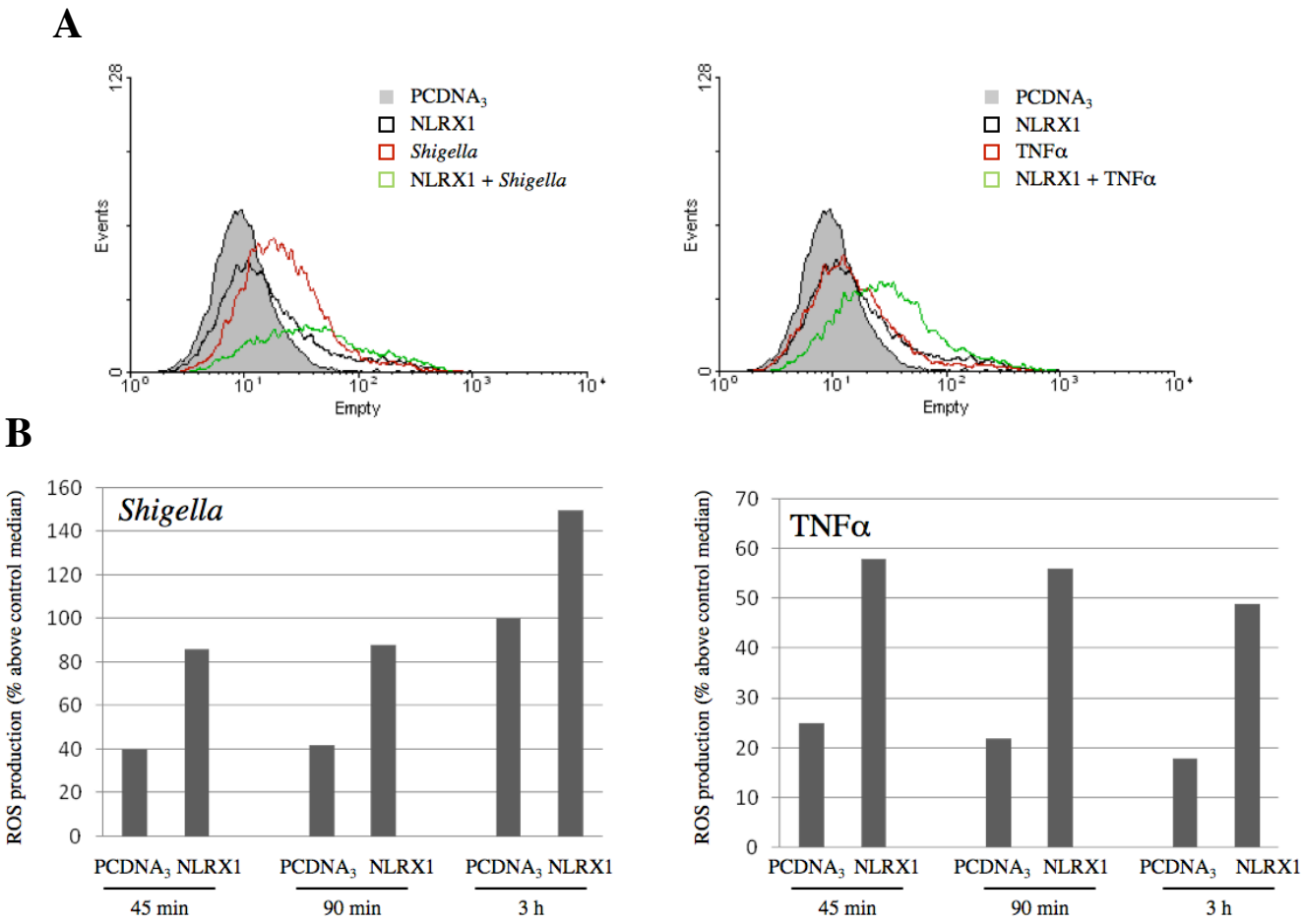
NLRX1:
5'-GCTCCATGGCTTAGAGCATC-3' (forward)
5'-AACTCCTCCTCCGTCCTGAT-3' (reverse)
β-actin
5'-GTGGGGCGCCCCAGGCACCA-3' (forward)
5'-CTCCTTAATGTCACGCACGATTTC-3' (reverse)



Supplementary Figure 2: NLRX1 overexpression does not trigger AP-1-, p53- or HIF-dependent pathways. HEK293T epithelial cells were transfected with increasing amounts (10, 100 and 250 ng) of NLRX1 expression vector together with AP-1- (A), p53- (B) and hypoxia inducible factor- (HIF) (C) luciferase-reporter constructs. Different positive controls were used, depending of the luciferase-reporter construct used: PMA (A; 10 and 100ng/ml) or p53 (B) and HIF-1α (C) expression vectors (transfection of 20 or 200 ng). Data show the mean ± s.e.m of duplicates and is representative of three independent experiments. CTR, control.

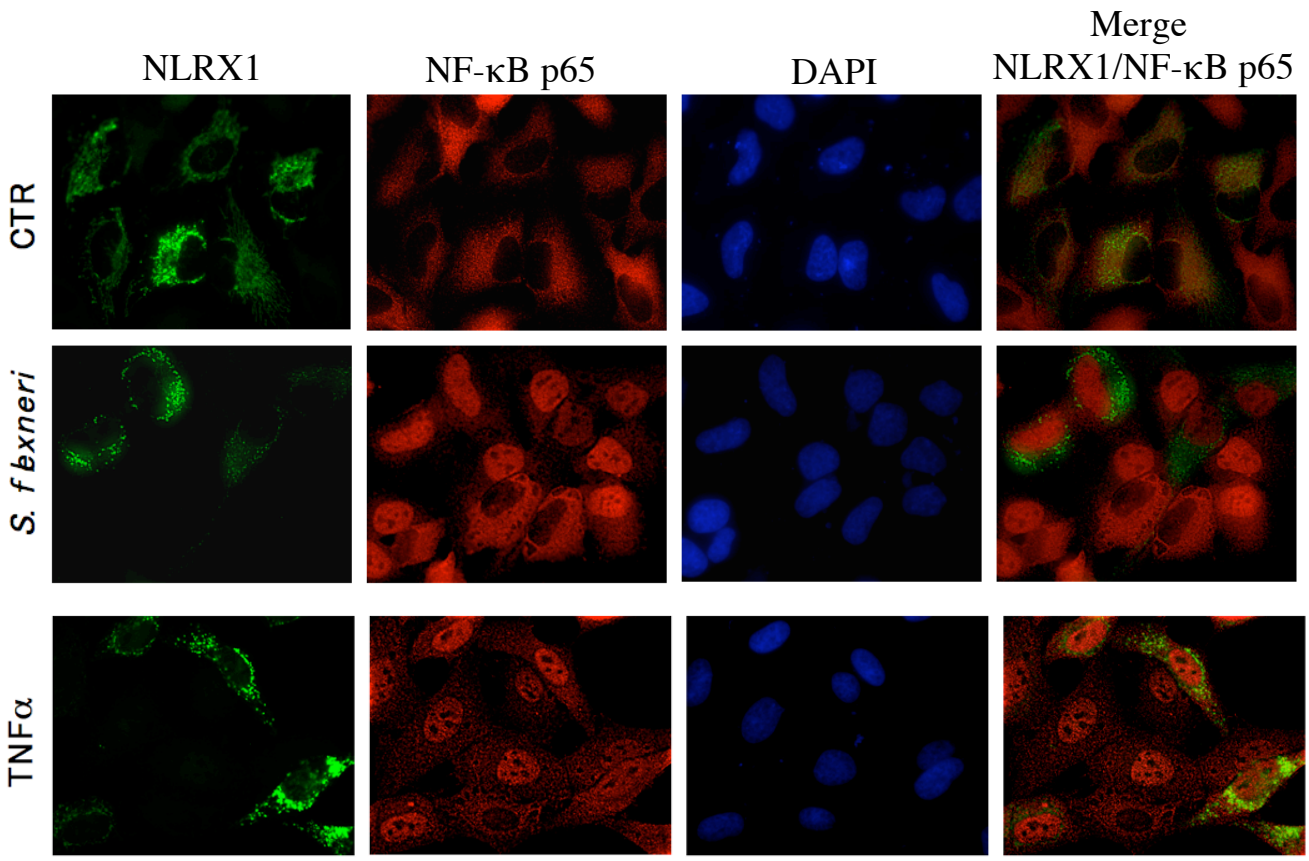


Supplementary Figure 3: NLRX1 overexpression does not significantly alter apoptosis. HeLa cells were transfected overnight with NLRX1 and stimulated for 2 hours with either 2 μM staurosporine (left panel) or TNF (10 ng/ml) plus cycloheximide (CHX; 0,5 μg/ml) (right panel). Activation of caspase-3 and caspase-8 were quantified by immunofluorescence using antibodies that detect the cleaved caspase-3 and cleaved caspase-8 in cells over-expressing or not NLRX1. The expression of NLRX1 was determined using an antibody against the FLAG epitope. For each experiment, a minimum of 200 cells were quantified, and the results presented are representative of three independent experiments.

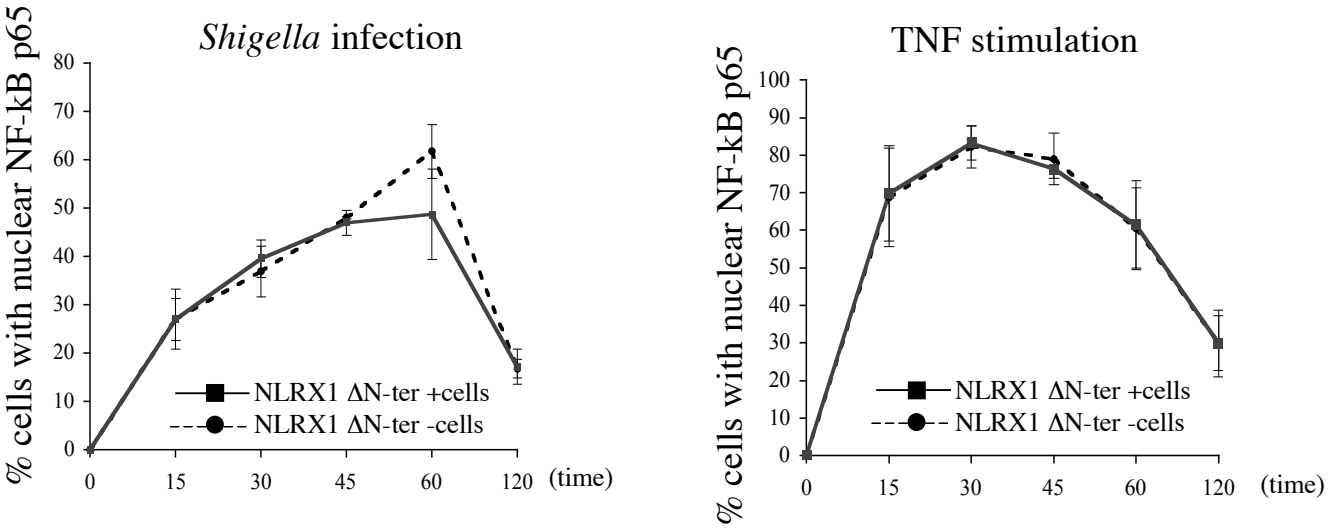


Supplementary Figure 4: Kinetics of reactive oxygen species production following *Shigella* infection or TNFα stimulation for up to 3h in cells over-expressing NLRX1. HeLa cells were transfected overnight with pcDNA3 or NLRX1 vectors and infected for 3 hours with *Shigella* (left panel) or stimulated for 3 hours with 10 ng/ml TNFα (right panel). ROS production was measured using a redox-sensitive dye (CM-H_2DCFDA) on live cells, followed by flow cytometry analysis. (B) Time-course analysis of ROS production induced by *Shigella* (left panel) and TNFα (right panel) in cells transfected with pcDNA3 or NLRX1, as in (A). The results presented are representative of three independent experiments.

Tattoli et al. Figure S4



Supplementary Figure 5: HeLa cells grown on glass coverslips were transfected overnight with NLRX1 expression vector and infected with *Shigella* or stimulated with 10 ng/ml TNFα for 1 hour. NLRX1 over-expressing cells were stained using a monoclonal anti-Flag antibody, and a polyclonal anti-NF-κB p65 antibody was used to assess the cellular localization of this NF-κB subunit. Cells in which the NF-κB is activated display nuclear localization of NF-κB p65. DAPI was used to stain nuclei. Such experimental set-up was used to perform quantification analyses presented in Figure 4C-D. Note that for quantification purposes, fields were picked randomly and > 500 cells (from 3 independent experiments) per condition/time point were analyzed for NLRX1 over-expression and NF-κB p65 nuclear translocation.



Supplementary Figure 6: NLRX1 localization to the mitochondria is critical for the modulation of *Shigella*- and TNF-dependent NF-κB activation. HeLa cells grown on glass coverslips were transfected overnight with NLRX1 ΔN-ter expression vector and infected with *Shigella* (left) or stimulated with 10 ng/ml TNFα (right) for various times, as indicated. The nuclear translocation of NF-κB p65 subunit (as an indicator of the activation of the NF-κB pathway) was evaluated by immunofluorescence in cells over-expressing NLRX1 ΔN-ter (NLRX1 ΔN-ter +cells) or not (NLRX1 ΔN-ter -cells) . Data show the mean ± s.e.m of three independent experiments, and for each condition and time point, a minimum of 500 cells were counted.