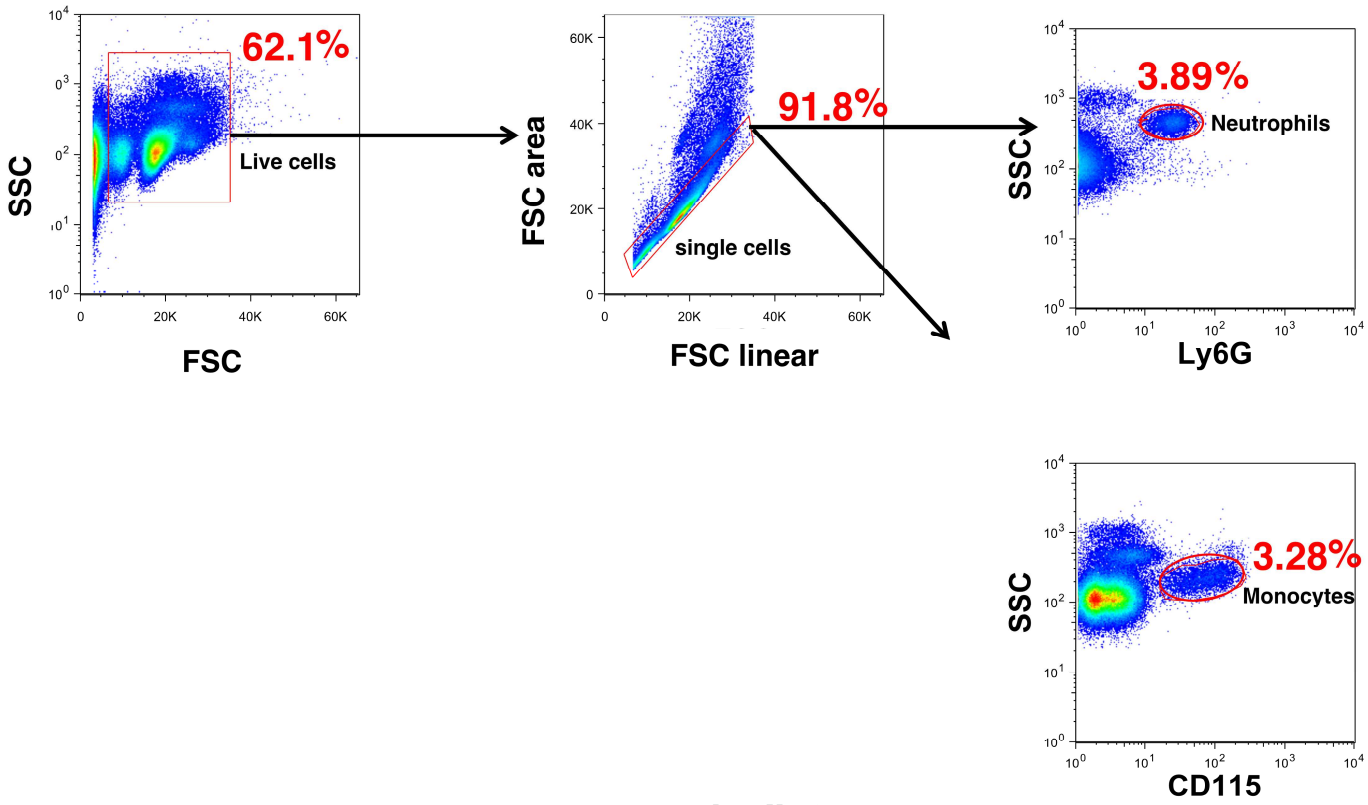
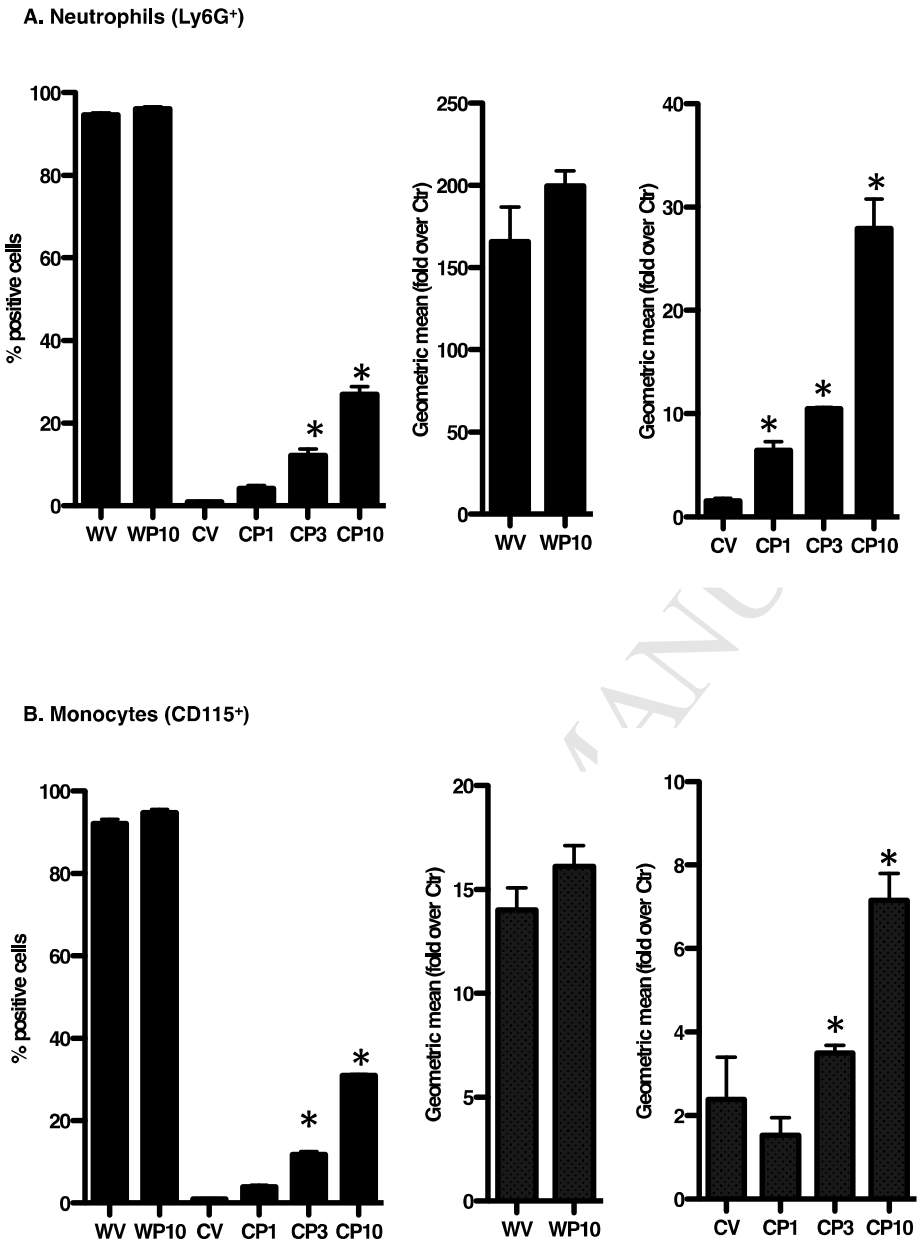


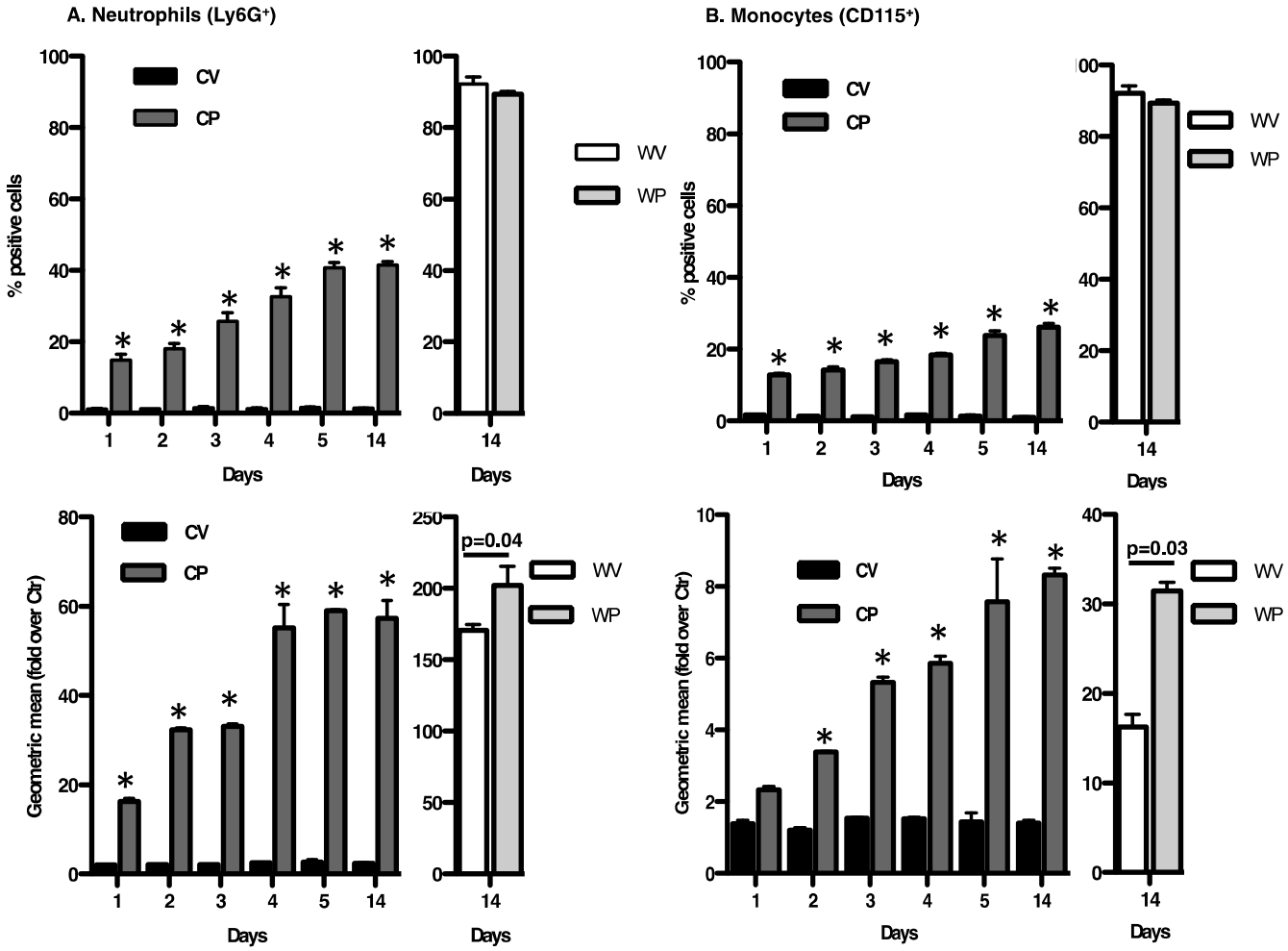
Supplement Figure 1

Gating strategy for identification of neutrophils and monocytes from mouse blood. Following 5 days of treatment with 10 mg/kg/d pioglitazone or vehicle, blood was obtained by cardiac puncture, red cells lysed, remaining leukocytes stained for surface markers and analyzed by flow cytometry. Live cells, with doublets excluded, were plotted as SSC versus Ly6G to identify neutrophils and SSC versus CD115 to identify monocytes.



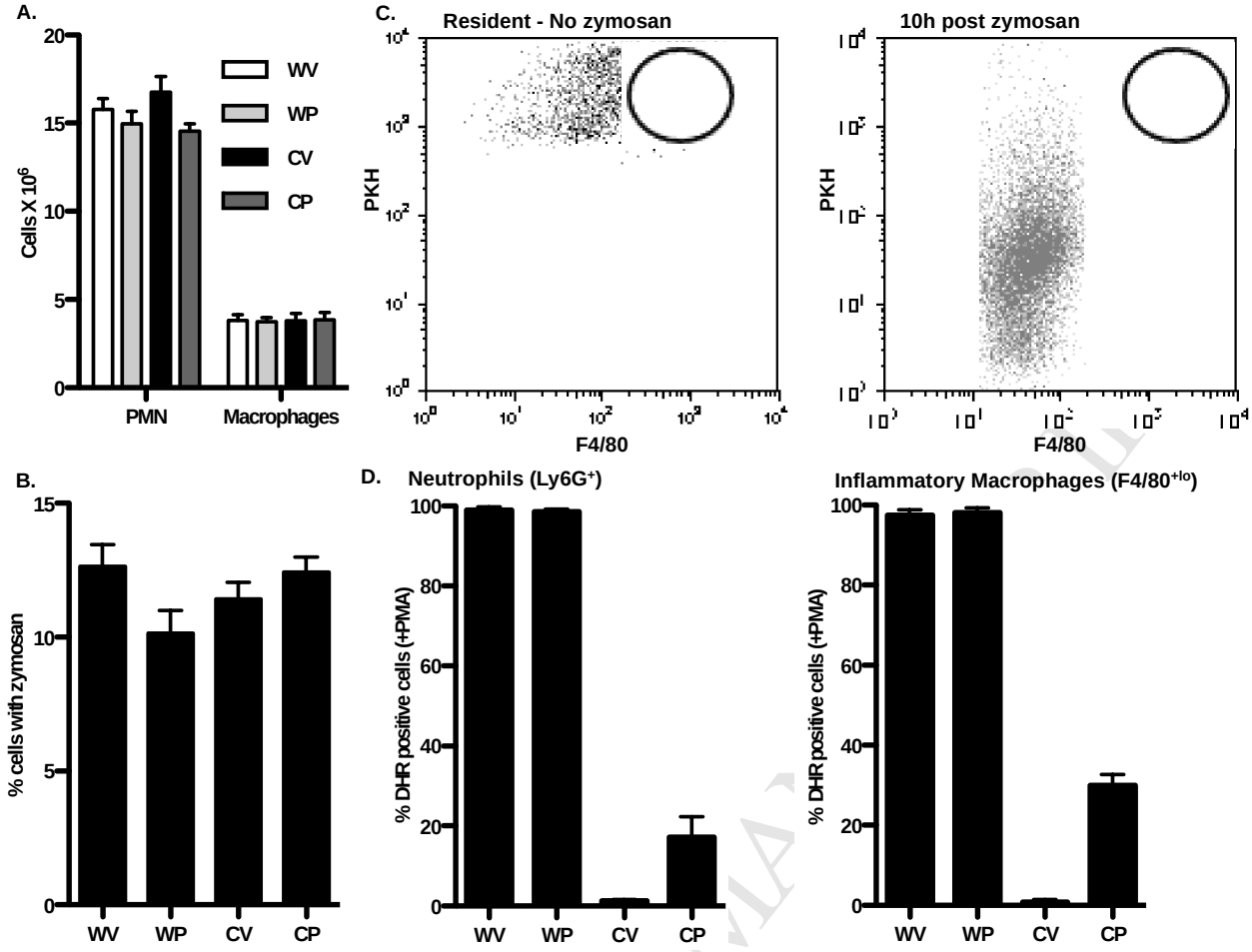
Supplement Figure 2

Pioglitazone dose-response for production of stimulated ROS by blood phagocytes. Following 5 days of treatment with 1, 3, or 10 mg/kg/d pioglitazone or vehicle, blood neutrophil ($Ly6G^+$) (A) and monocytes ($CD115^+$) (B) from wild type (WP, WV) and $gp91^{phox-/-}$ (CP, CV) mice were harvested and tested for ROS production in response to PMA as in Fig. 1. The percentage of ROS positive cells and the geometric mean for the population, expressed as fold over controls without PMA stimulation are shown. N = 3 mice each treatment group. $p \leq 0.05$ for *compared to CV.



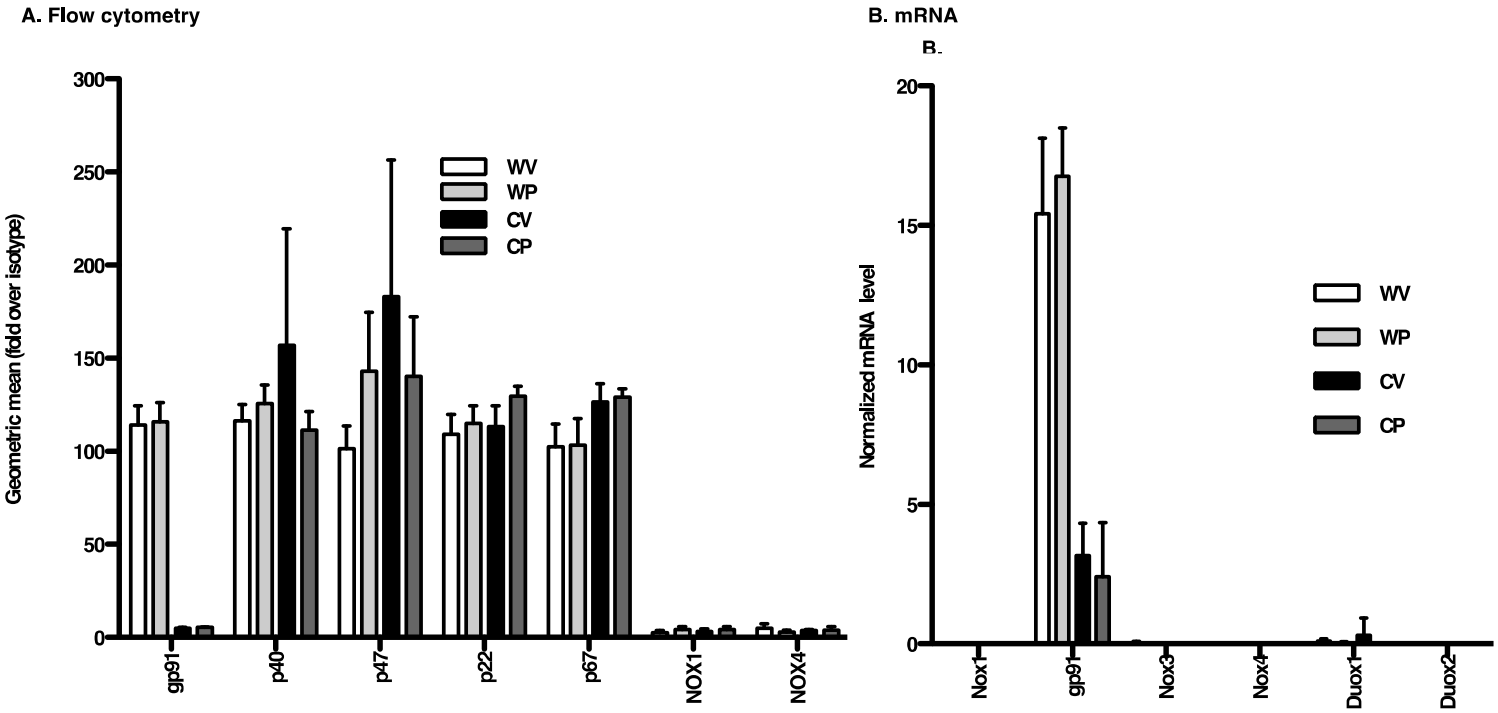
Supplement Figure 3

ROS response following prolonged treatment with pioglitazone. Blood neutrophil ($Ly6G^+$) (A) and monocytes ($CD115^+$) (B) from wild type (WV, WP) and $gp91^{phox-/-}$ (CV, CP) mice were tested for ROS production in response to PMA as in Fig. 1 following 1 to 14 days of treatment with 10 mg/kg/d pioglitazone or vehicle. For simplicity, ROS production for leukocytes from wild type mice is shown only for Day 14. N = 3 mice each treatment group.



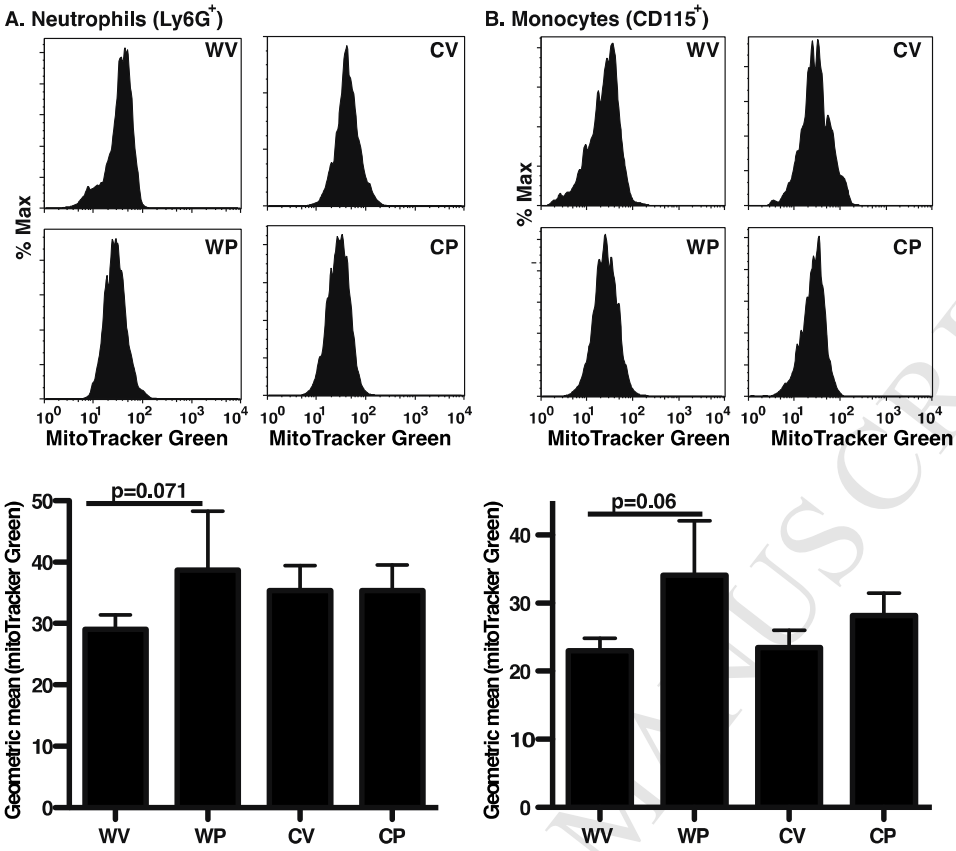
Supplement Figure 4

Characterization of recruited phagocytes in zymosan-induced peritonitis. Following 5 days of treatment with pioglitazone or vehicle, wild type (WP, WV) and gp91$^{phox-/-}$ (CP, CV) mice were injected intraperitoneally with zymosan. A) Phagocyte numbers and differentials on cytospins were determined at 10 hours post zymosan. Similar numbers and types of inflammatory leukocytes were recruited to the peritoneum for both genotypes and treatment groups. B) The percentage of phagocytes from each genotype and treatment group positive for zymosan ingestion was not different[29], N = 10. C) Inflammatory macrophages (F4/80^{lo+} PKH^{lo}) derived from recruited monocytes, but not resident peritoneal macrophages (F4/80^{hi} PKH^{hi}) are recovered in 10-hour harvests from inflamed peritonea of mice injected with i.p. zymosan. Mice were injected with PKH 24 hours prior to zymosan or PBS and then lavaged 10 hours later. Resident peritoneal macrophages F4/80^{hi} PKH^{hi} were lavaged from mice receiving PBS, while only inflammatory macrophages, F4/80^{lo+} PKH^{lo}, are lavaged from mice receiving zymosan. F480^{hi+} resident peritoneal macrophages are not recovered by lavage at this early stage of peritonitis. D) Vehicle or pioglitazone-treated WT (WV, WP) and gp91$^{phox-/-}$ (CV, CP) mice were injected intraperitoneally with zymosan and phagocytes harvested at 10 hours, loaded with DHR, stimulated with PMA (200 ng/ml for 15 min), and analyzed for DHR fluorescence by flow cytometry. Aggregate data with PMA stimulation are expressed as the percentage of cells exhibiting a shift in DHR fluorescence.



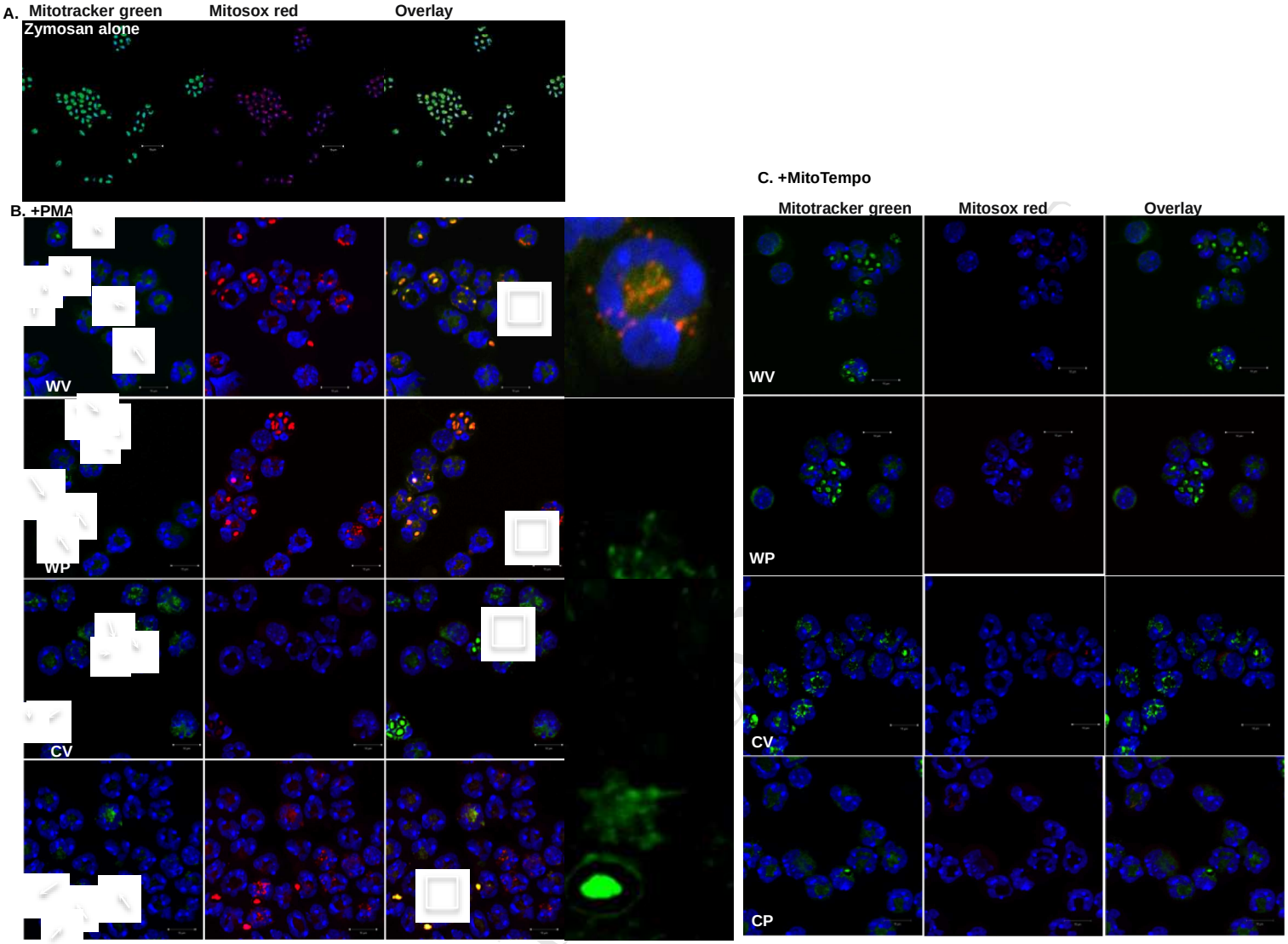
Supplement Figure 5

NOX and DUOX proteins in peritoneal phagocytes from gp91$^{phox-/-}$ and wild type mice following pioglitazone or vehicle treatment. Following 5 days of treatment with pioglitazone or vehicle, wild type (WP, WV) and gp91$^{phox-/-}$ (CP, CV) mice were injected intraperitoneally with zymosan. A) Ten hours post zymosan, cells were harvested, permeaballized and stained with antibodies as described in Methods and analyzed by flow cytometry. B) Trizol reagent (Life technologies) was used to prepare total RNA (2µg) that was treated with DNase (1U/ml) and reverse transcribed using a first-strand complementary DNA synthesis kit (Life technologies). PCR amplification was performed using fast universal PCR buffer and predesigned primers from Life technologies on a 7900H platform. Primer pairs targeting at least 2 exons were chosen, and for gp91^{phox} primer pairs targeting exon 2 and 3 were chosen (exon 3 is deleted in these knock out mice[27]). Relative expression levels for mRNAs were calculated using the comparative C_T method normalized to GUSb RNA. N=7 mice.



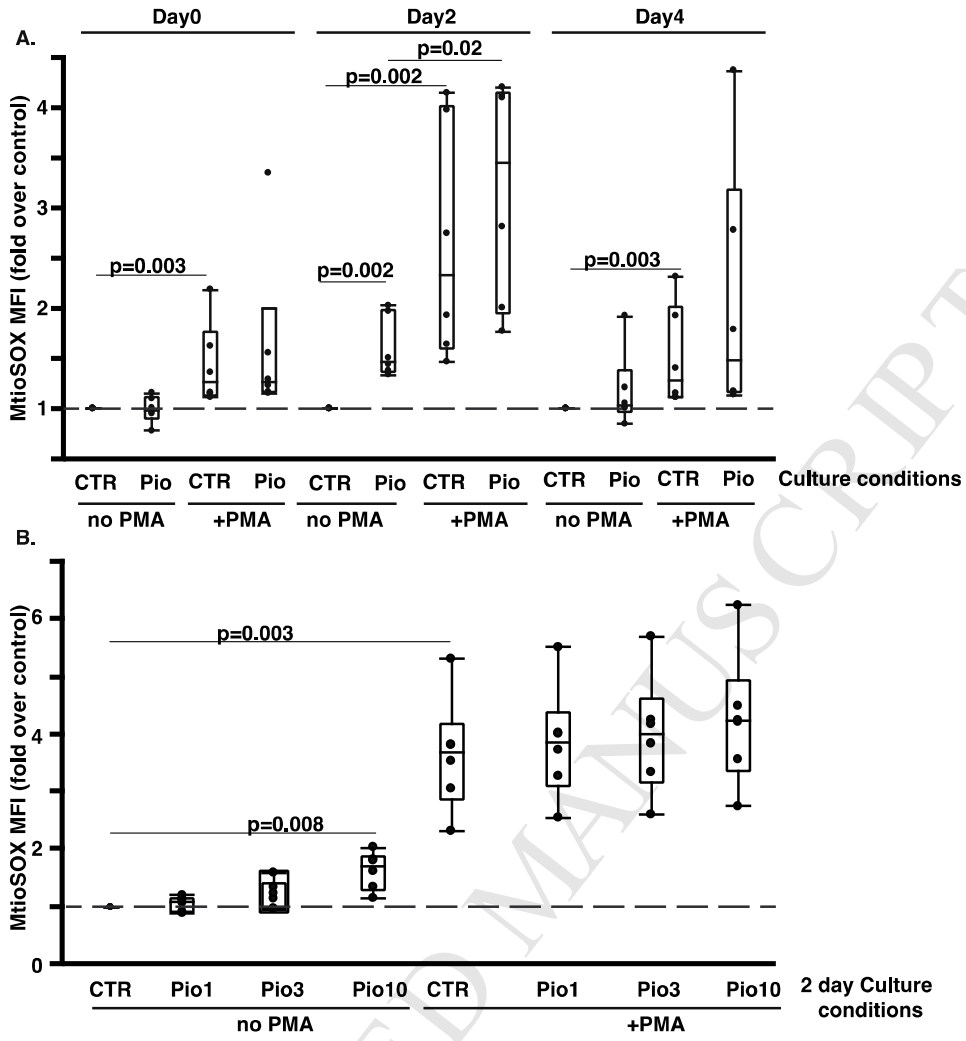
Supplement Figure 6

Pioglitazone treatment has little effect on mitochondrial content in blood neutrophils (A) and monocytes (B). Blood leukocytes from vehicle- or pioglitazone-treated WT (WV, WP) and gp91$^{phox-/-}$ (CV, CP) mice were stained with surface markers, followed by MitoTracker Green and then analyzed by flow cytometry. Representative histograms (top) and aggregate data (bottom) are shown. N = 6 mice/group.



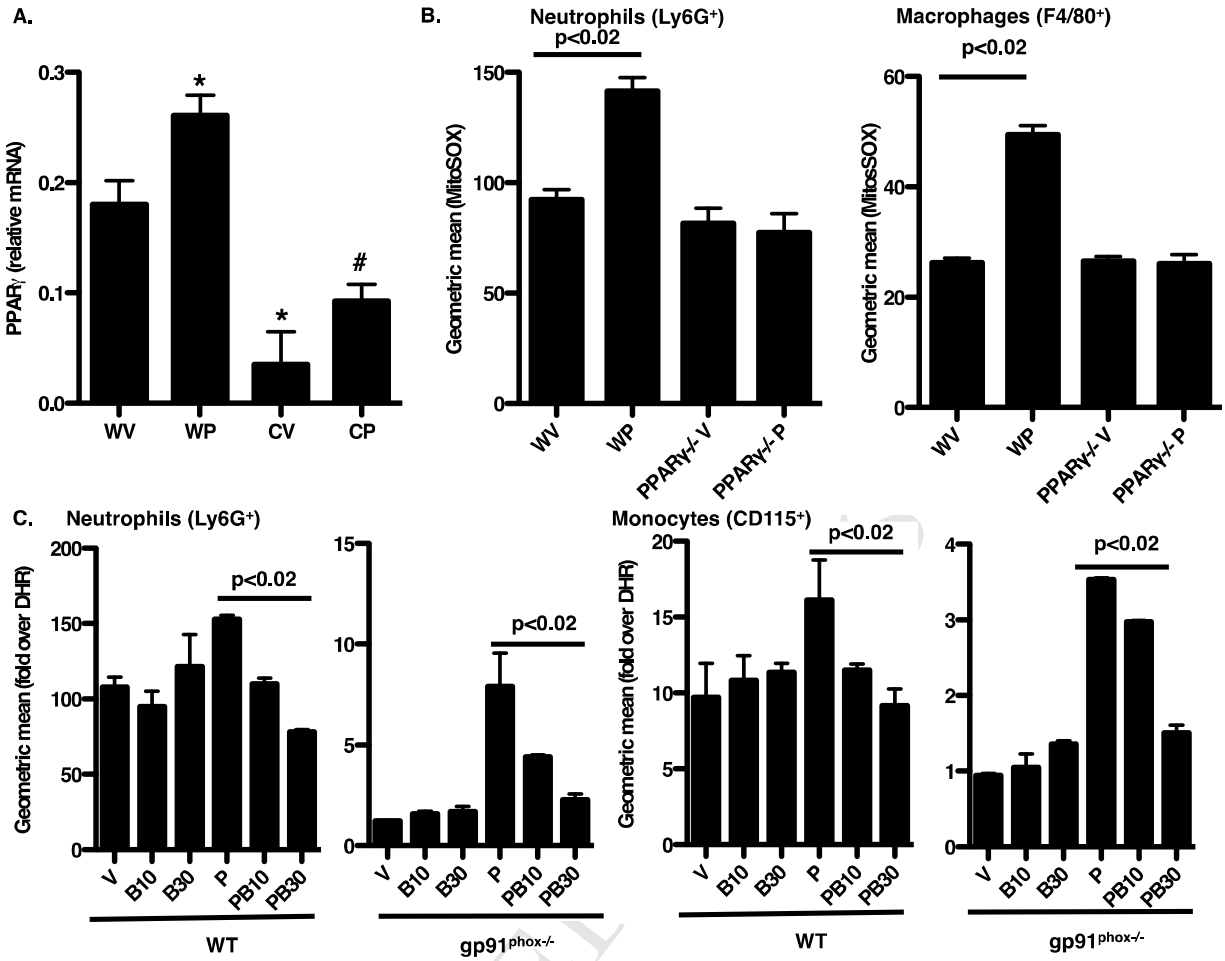
Supplement Figure 7

Pioglitazone enhancement of gp91$^{phox-/-}$ mice ROS production in recruited phagocytes is of mitochondrial origin. A) Zymosan particles (no cells) were stained with DAPI, MitoTracker Green (25 nM for 15 min), and MitoSOX Red (4 μM for 15 min), washed and then analyzed by confocal microscopy using a 63X oil immersion lens to determine their absorption of the stains. B) Recruited phagocytes were harvested 10h following intraperitoneal zymosan injection from vehicle- (WV) or pioglitazone-treated (WP) wild type mice, and vehicle- (CV) or pioglitazone-treated (CP) gp91$^{phox-/-}$ mice. Harvested cells were then stained with MitoTracker Green (25nM for 15 min), and MitoSOX Red (4μM for 15 min) with PMA, washed, stained with DAPI and analyzed by confocal microscopy using a 63X oil immersion lens (Methods). High-resolution images of a single cell (white box) are shown in the last panel. Arrows denote zymosan in the first column. C) Harvested cells were pretreated with MitoTEMPO (100 μM) for 30 min prior to staining and PMA stimulation as in (B). Representative images are shown for each genotype and treatment group (N=3).



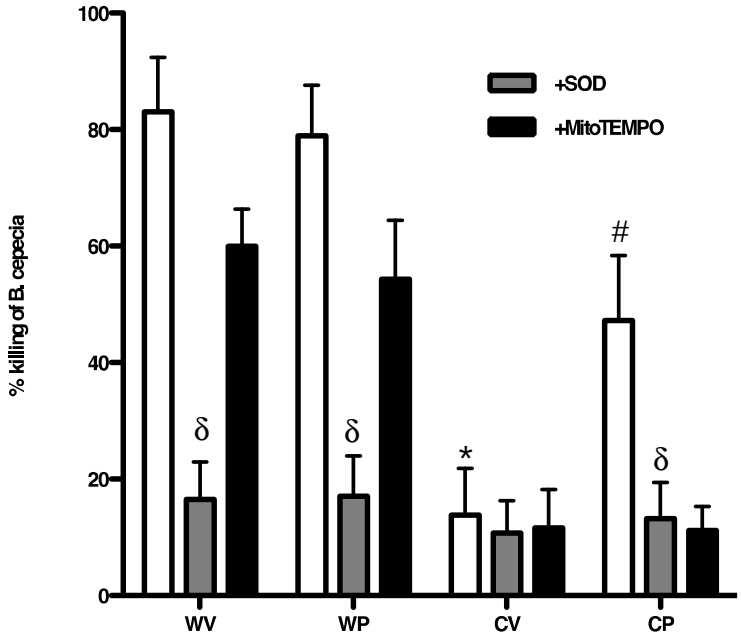
Supplement Figure 8

Pioglitazone enhances production of mtROS by stimulated human monocytes in a time and dose dependent manner. Human monocytes were isolated from blood, plated, and treated as indicated. A) 10 μM pioglitazone was added to monocyte cultures for 1 hr (Day0 cultures), 2 days or 4 days, and then the monocytes were stained with MitoSOX Red, stimulated with or without PMA, and analyzed by flow cytometry. B) Monocytes were cultured for 2 days in different concentrations of pioglitazone (0, 1, 3, 10 μM) before MitoSOX staining, stimulation with PMA, and analyses as in (A). N=6 monocytes from normal subjects. Data are normalized to each individual untreated control.



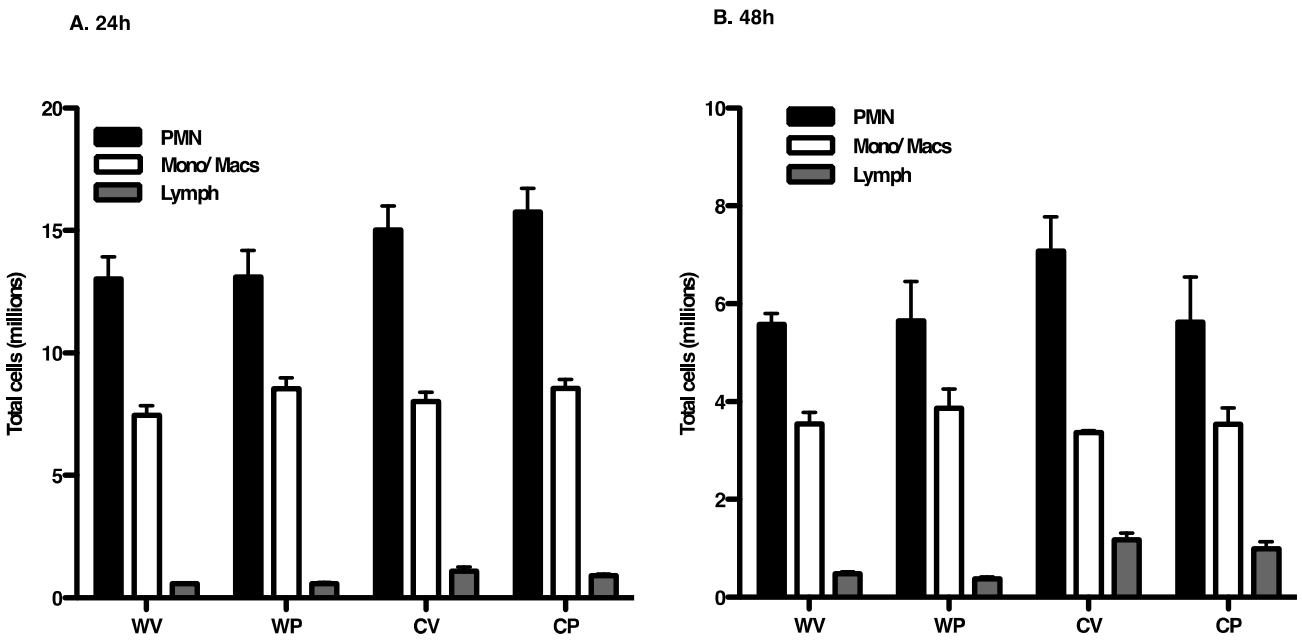
Supplement Figure 9

Pioglitazone enhancement of mitochondrial ROS production is PPARγ dependent. A) Vehicle or pioglitazone-treated WT (WV, WP), gp91$^{phox-/-}$ (CP, CV) mice were injected intraperitoneally with zymosan and phagocytes harvested at 10 h. PPARγ expression (mRNA) was assessed by q-PCR and normalized to GUSb. N=6; *p <0.02 compared to WV and $^{\#}$p<0.02 compared to CV. B) Recruited neutrophils and inflammatory macrophages were harvested 10 hours after zymosan from WV and WP mice as in (A) and vehicle and pioglitazone treated mice with genetic deletion of PPARγ in myeloid cells. Phagocytes were stained for MitoSOX Red and analyzed by flow cytometry without further stimulation as in Fig. 5. Geometric mean fluorescence is shown, N=3. C) Following vehicle (V), pioglitazone (P), BADGE (10 or 30 mg/kg/d) (B10, B30) or the combination of pioglitazone and BADGE (PB10, PB30) treatment for 5 days, blood leukocytes were obtained from WT (WV, WP, WB, WPB) and gp91$^{phox-/-}$ (CV, CP, CB, CPB) mice, loaded with DHR, stimulated with PMA (200 ng/ml for 15 min), and analyzed for DHR fluorescence by flow cytometry. The change in geometric mean fluorescence (fold over controls without PMA) for the whole population of either neutrophils or monocytes is shown. N = 4 mice in each treatment group.



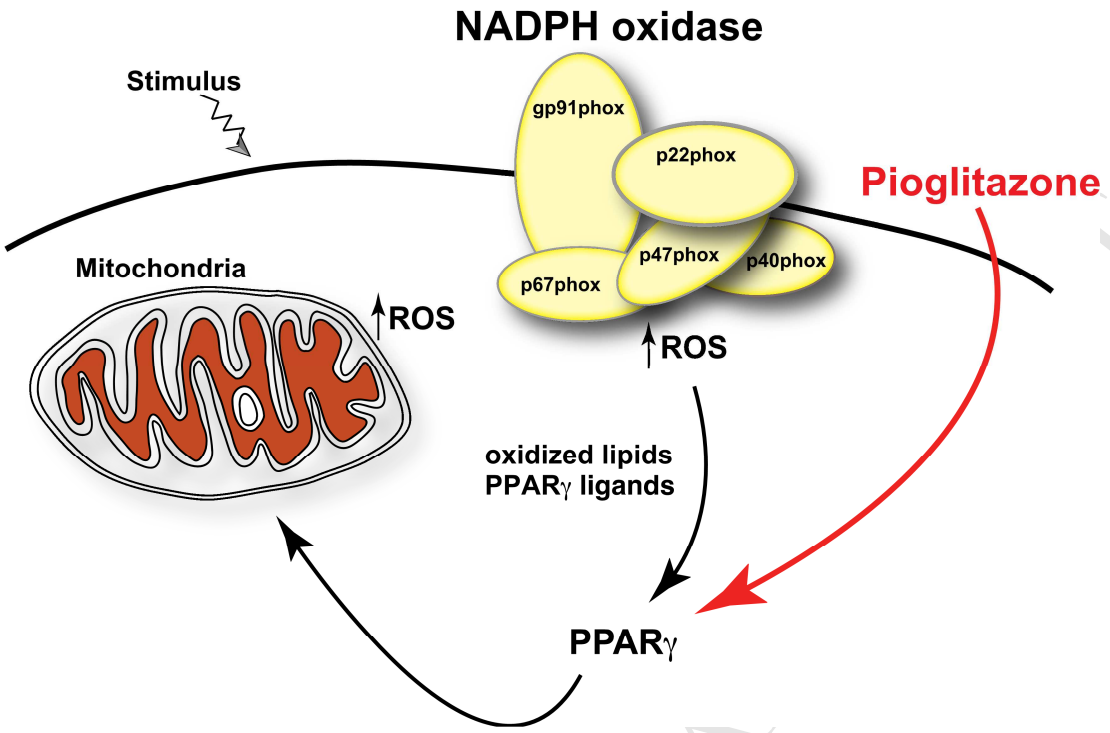
Supplement Figure 10

Recruited phagocytes from pioglitazone-treated gp91$^{phox-/-}$ mice show enhanced killing of *B. cepecia ex vivo.* Recruited, peritoneal phagocytes (1x10^6) from WV, WP, CV, CP mice as in Fig. 2 were treated with or without SOD or MitoTEMPO *ex vivo* for 30 min, co-incubated with *B.cepecia* (1x10^7 CFU, grown overnight in Lauryl Broth and washed twice with saline) at 37ºC for 2h in 100 µl RPMI (phenol red free, 1% mouse serum). The phagocytes were then lysed with 0.1% trition X for 10 min, the remaining viable bacteria enumerated using the Alamar blue assay and the percent of bacteria killed determined. p≤0.02 *compared to phagocytes from WV, $^{\#}$ compared to phagocytes from CV, and $^{\delta}$compared to phagocytes from respective treatment group in the absence of inhibitors. N=7 mice/group.



Supplement Figure 11

Cell numbers and differentials following intraperitoneal injection of *S. aureus.* Vehicle or pioglitazone-treated WT (WV, WP) and gp91$^{phox-/-}$ (CV, CP) mice were injected intraperitoneally with *S.aureus*. Phagocyte numbers and differentials at 24h (A) and 48h (B) were determined on cytospins. N=16 mice at 24h and 11 mice at 48h.



Supplement Figure 12

Cross talk between NADPH oxidase and mitochondria. There is crosstalk between the NADPH oxidase and mitochondria under normal circumstances that leads to enhanced mitochondrial ROS and optimal antimicrobial responses by phagocytes. In the absence of a functional NADPH oxidase (CGD), this signaling is lost, but importantly, can be largely restored at the level of mitochondrial ROS production by pioglitazone treatment.