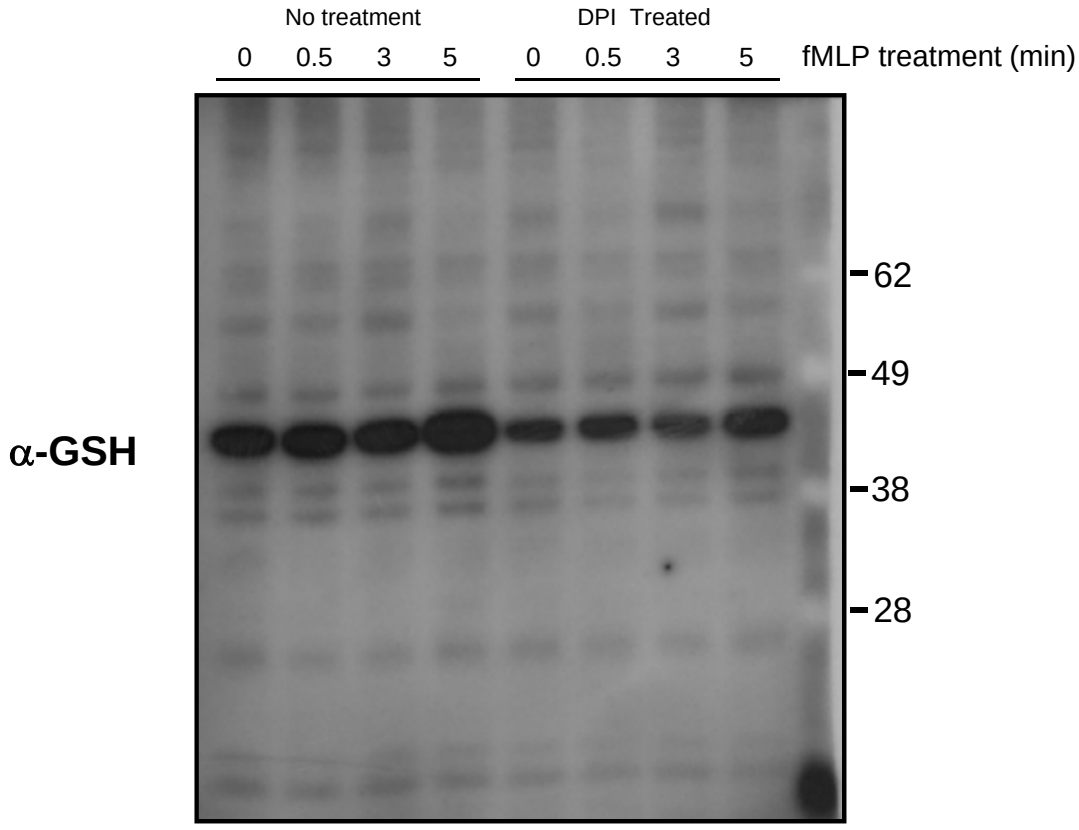

Figure S1. Actin is the major glutathionylated protein in neutrophils. Image is a longer exposure of the α-GSH western blot shown in Fig 1E. Human neutrophils (10^6) pretreated with (or without) 50 μM DPI for 30 min were stimulated (or not unstimulated) with 100 nM fMLP. At indicated time points, neutrophils were lysed and probed using the α-GSH antibody (1:1000).

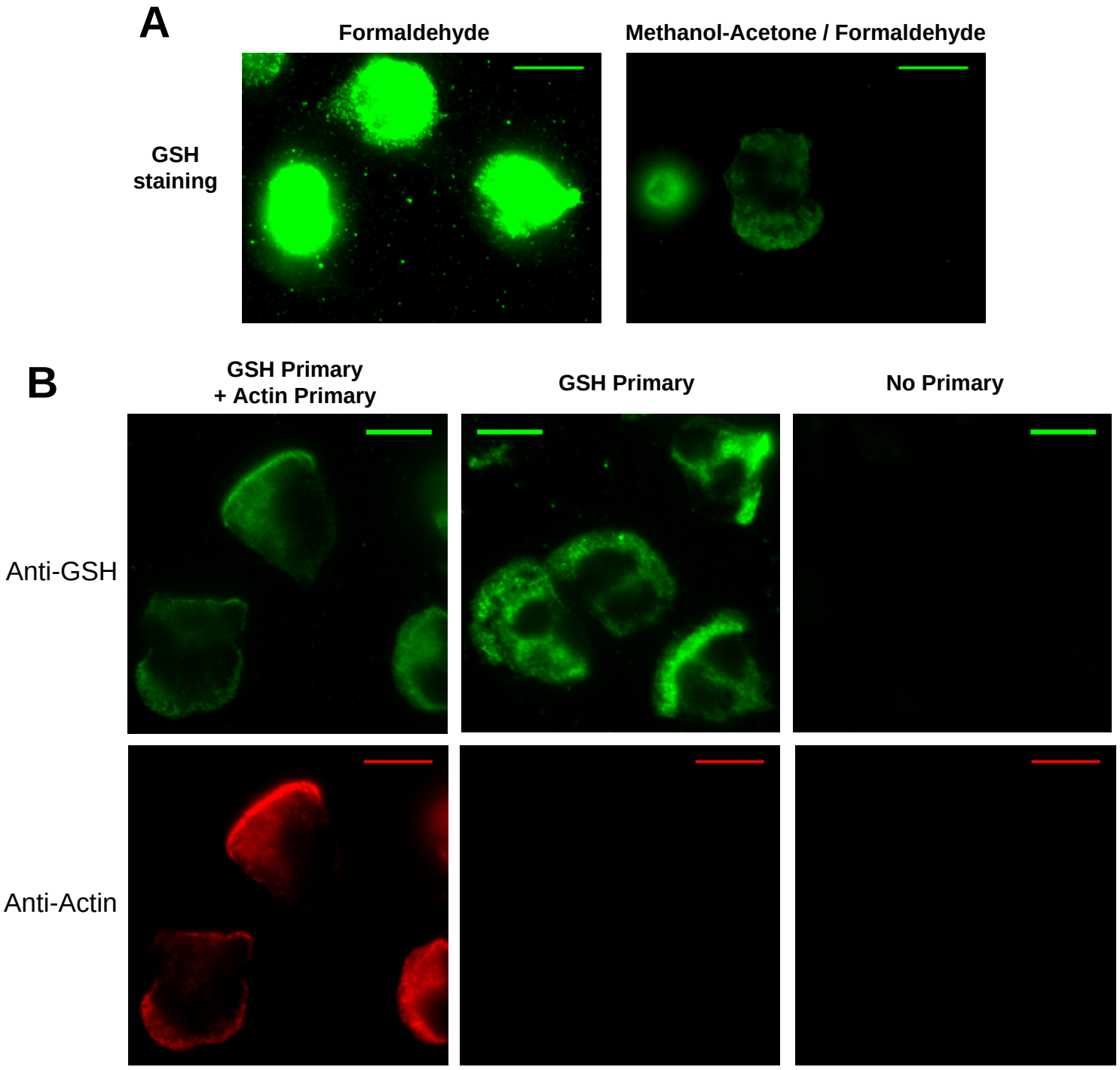

Figure S2. Controls for glutathionylation immunostaining. (A) Sequential fixation using Methanol-Acetone and formaldehyde removes free glutathione from neutrophils. Neutrophils were uniformly stimulated with 25nM fMLP on coverslips and fixed either with formaldehyde only (left, stains both glutathionylated proteins and free GSH) or fixed with Methanol-Acetone (only stains glutathionylated proteins), washed twice with PBS and then fixed again with formaldehyde (right). Fixed neutrophils were then permeablized, preblocked and stained using an anti-GSH antibody. Representative fluorescence images of stained cells are shown. Scale bar, 10 µm. **(B)** Negative controls for actin and protein glutathionylation co-staining. Representative images of fMLP stimulated neutrophils that were fixed sequentially as described above, permeabilized, preblocked and stained simultaneously with anti-GSH and anti-actin antibodies (left) or stained with anti-GSH antibody only (middle) or with no primary antibodies (right). All three samples were then incubated with mouse IgG (green) and rabbit IgG (red), and imaged in the green (top panel) or red (bottom panel) fluorescence channel. Scale bar,10 µm.

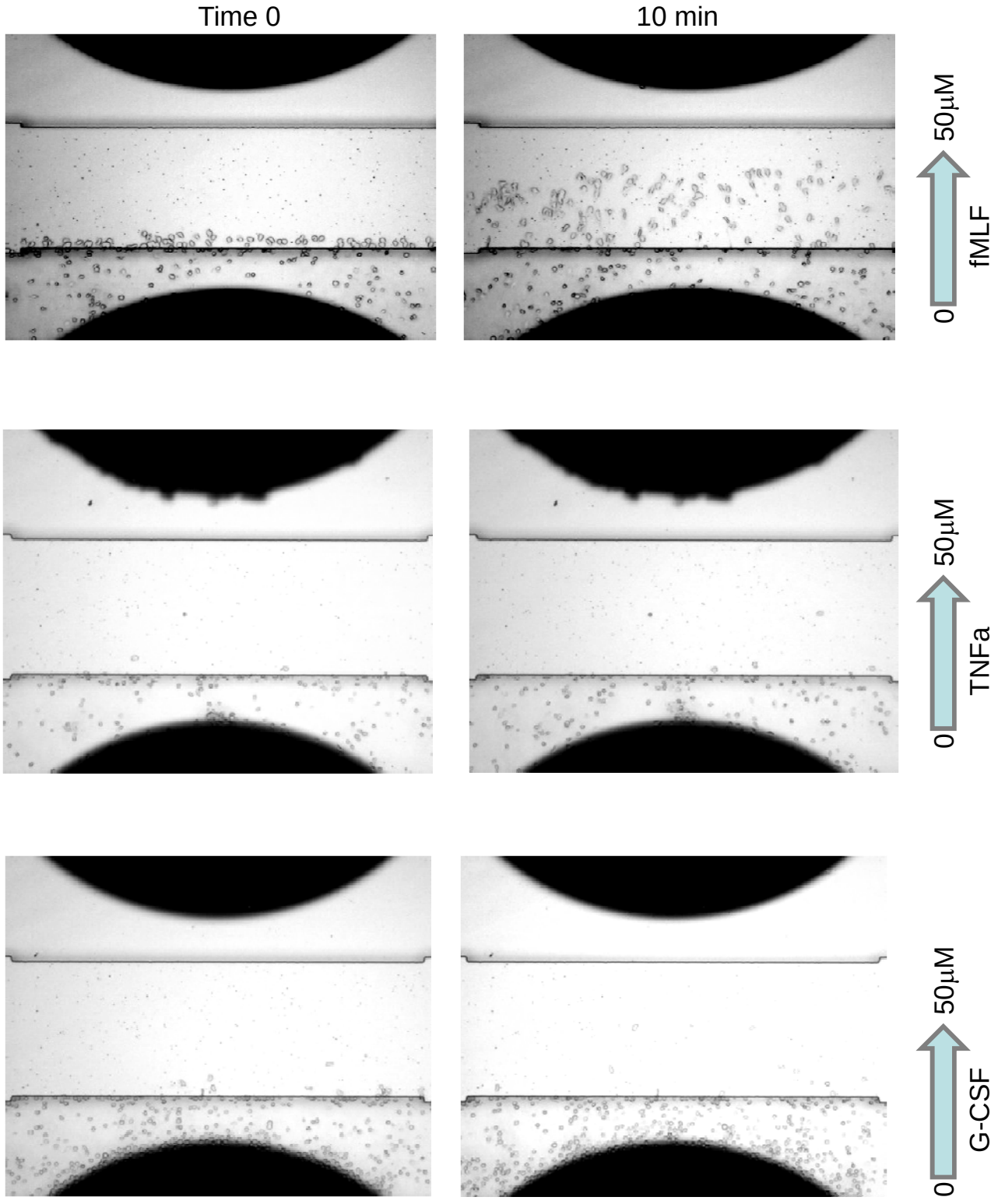

Figure S3. Neutrophils do not chemotax efficiently in the gradient of G-CSF or TNFa. Neutrophils chemotaxis was analyzed using EZ-taxiscan device in the indicated chemoattractant gradients as described in Figure 6D.

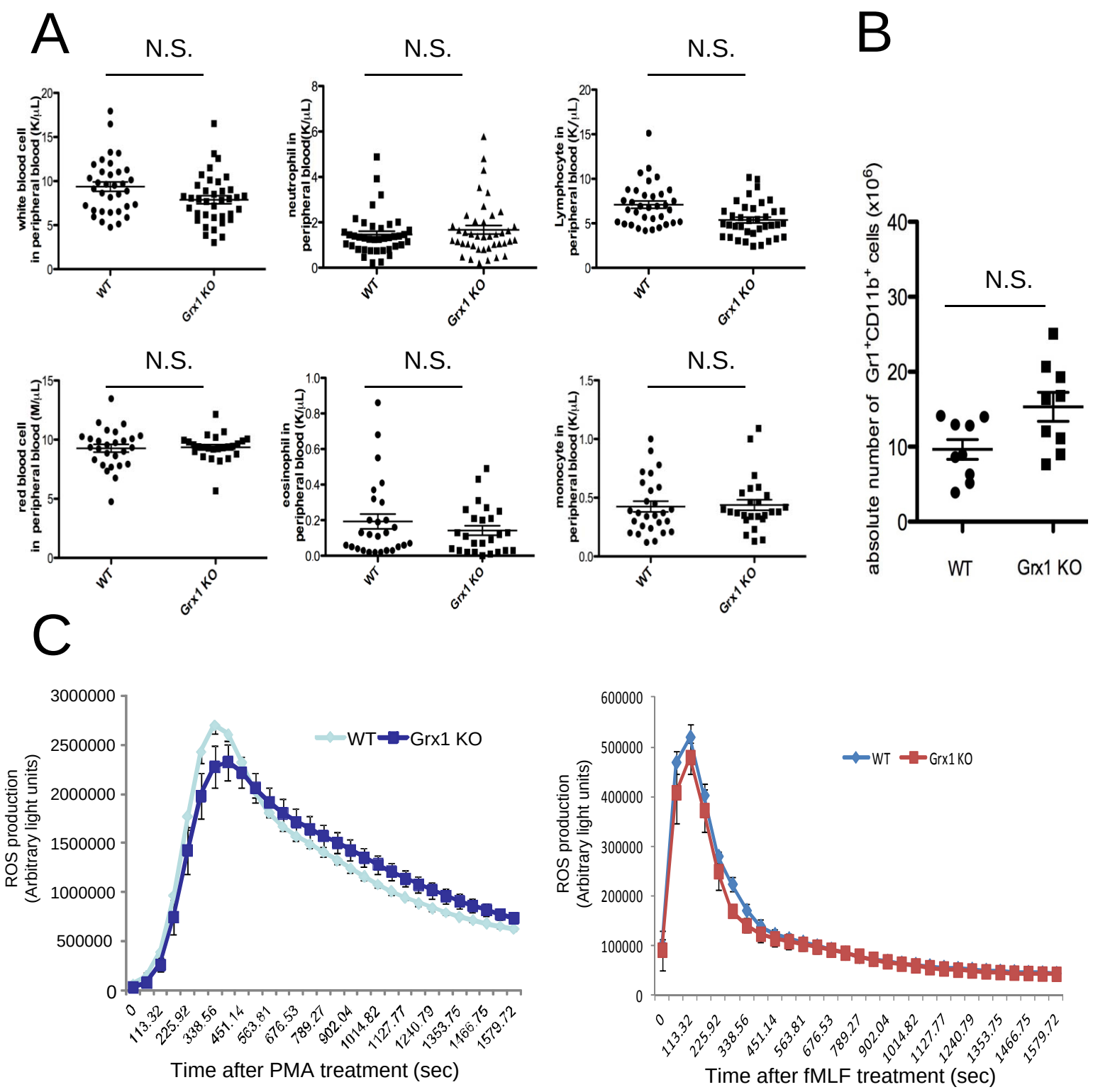

Figure S4. (A) Grx1-deficient mice are similar to their WT littermates in peripheral blood differential leukocyte counts**.** The total cellularity and the differential leukocyte count were measured using a Hemavet950FS Hematology system. Data shown are mean ± SD of n>20 mice. **(B)** Grx1 disruption does not affect bone marrow neutrophil (Gr1+CD11b+ cells) count. The number of Gr1+CD11b+ cells in the BM was measured by flow-cytometry. The absolute cell numbers per tibia and femur are shown. Data shown are mean ± SD of n=9 mice. N.S., p>0.05. **(C).** Grx1-deficient neutrophils generated the same amount of superoxide as wild-type neutrophils. Bone marrow derived neutrophils ($4x10^5$) from WT and $Grx1^{-/-}$ mice were stimulated with 100 nM fMLF or 122 nM phorbol-12-myristate-13-acetate (PMA), a PKC activator. ROS production was monitored in the presence of 50 μM isoluminol and 0.8U of HRP in a luminometer at 37°C. Chemiluminescence (Arbitrary Light Units) was recorded (for 2 sec) at indicated time points. WT and $Grx1^{-/-}$ neutrophils were assayed in parallel. Data are mean ± SD of three experiments.