Stojkov et al., https://doi.org/10.1083/jcb.201611168

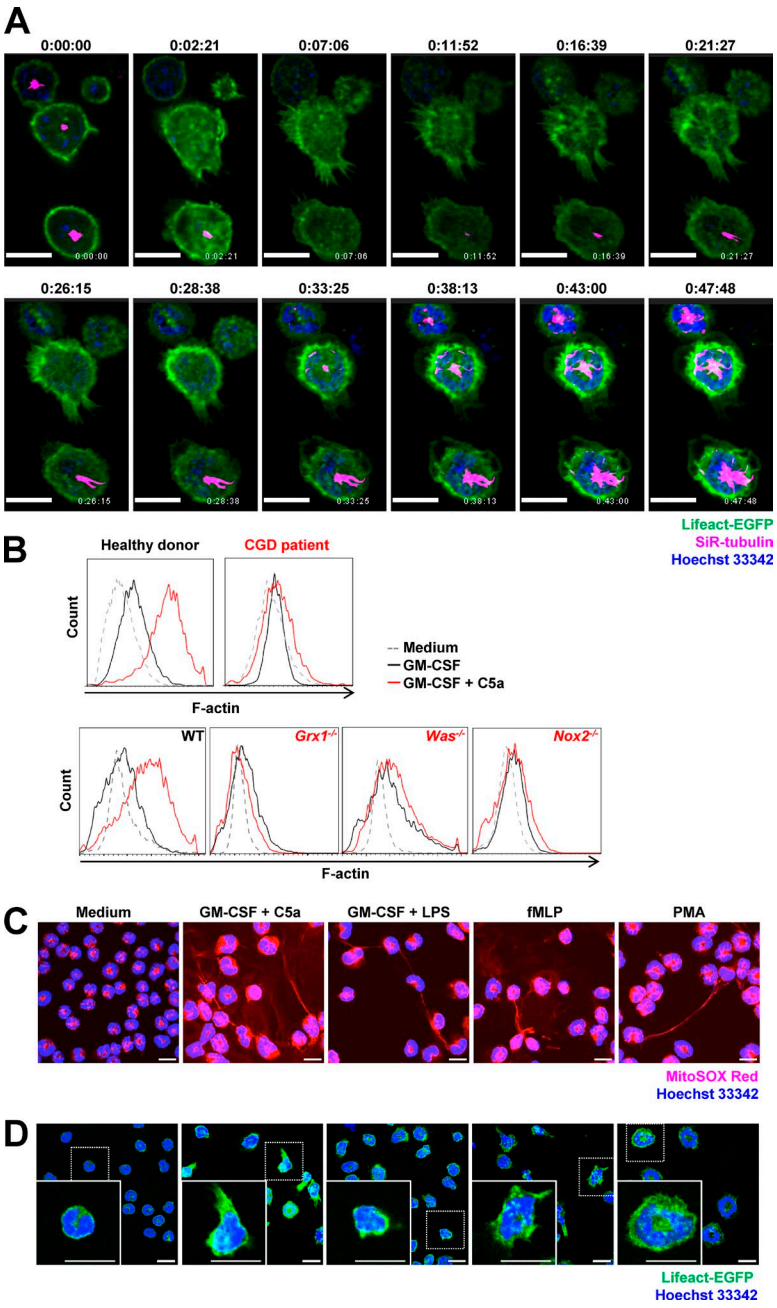


Figure S1. **Actin and tubulin rearrangements on neutrophil activation and NET formation.** Neutrophils (2 × 10^6 cells/ml) were primed with 25 ng/ml GM-CSF for 20 min and subsequently stimulated with 10^{-8} M C5a for 15 min. **(A)** Confocal microscopy. Snapshots of live cell analysis by using *Lifeact-EGFP* mouse neutrophils labeled with SiR-tubulin and Hoechst 33342 (corresponding to Video 1). **(B)** Flow cytometry. Analysis of F-actin polymerization on activation by using phalloidin (green). $n = 5$. **(C)** Confocal microscopy. DNA release was analyzed after short-term stimulation (total 35 min) of mouse neutrophils with the indicated triggers. $n = 3$. **(D)** Confocal microscopy. F-actin distribution and morphological changes were analyzed after short-term stimulation (total 35 min) of mouse neutrophils with the indicated triggers. $n = 3$. Bars, 10 µm.

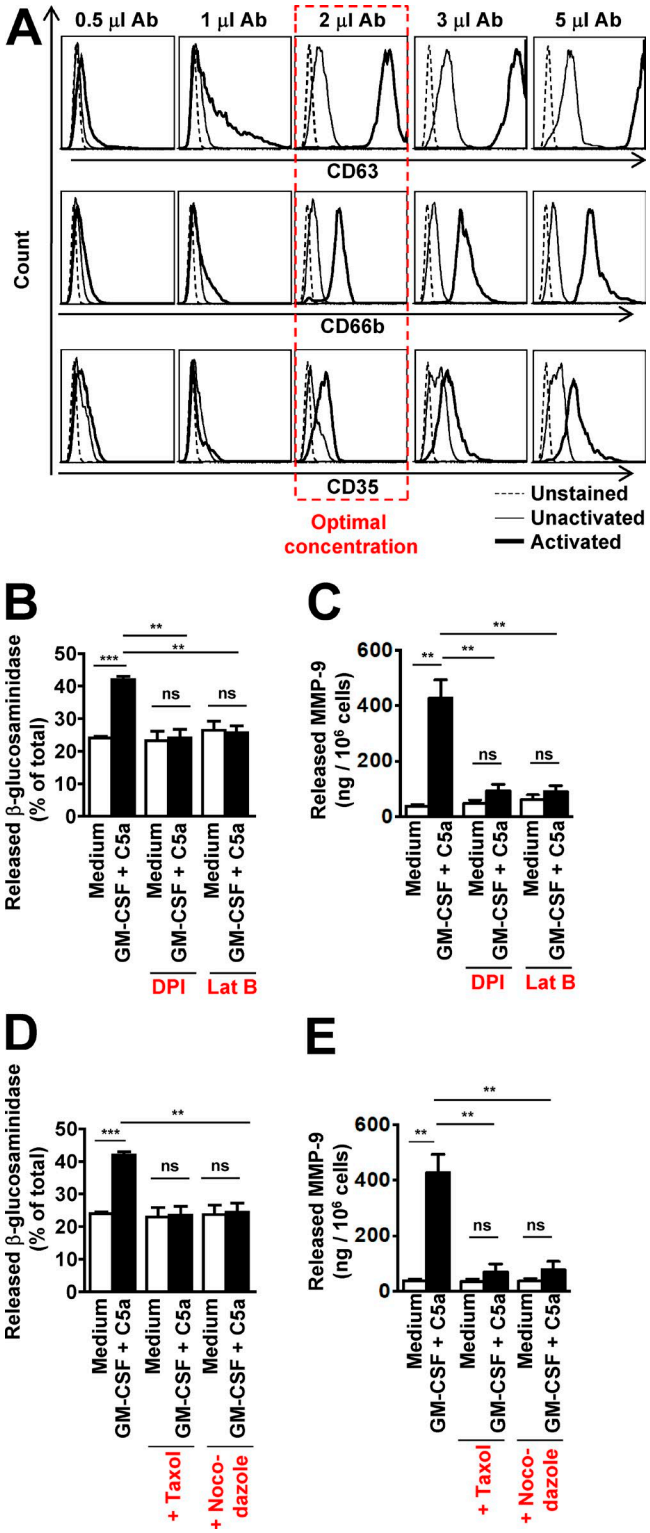


Figure S2. **Degranulation in the activated human neutrophils: surrogate markers and the release of azurophilic (N-acetyl-β-glucosaminidase) and tertiary (MMP-9) granules.** Human neutrophils (4 × 10^6 cells/ml) were primed with 25 ng/ml GM-CSF for 20 min followed by 15 min stimulation with 10^{-8} M C5a. Pretreatment of neutrophils with 5 µM DPI, 10 µM Lat B, 1 µM taxol, or 5 µM nocodazole was done 30 min before activation. **(A)** Flow cytometry. Optimal antibody titer for cell surface expression of CD63, CD66b, and CD35. **(B and D)** β-glucosaminidase release. **(C and E)** MMP-9 release. Data are means ± SEM. **, P < 0.0078; ***, P < 0.001.

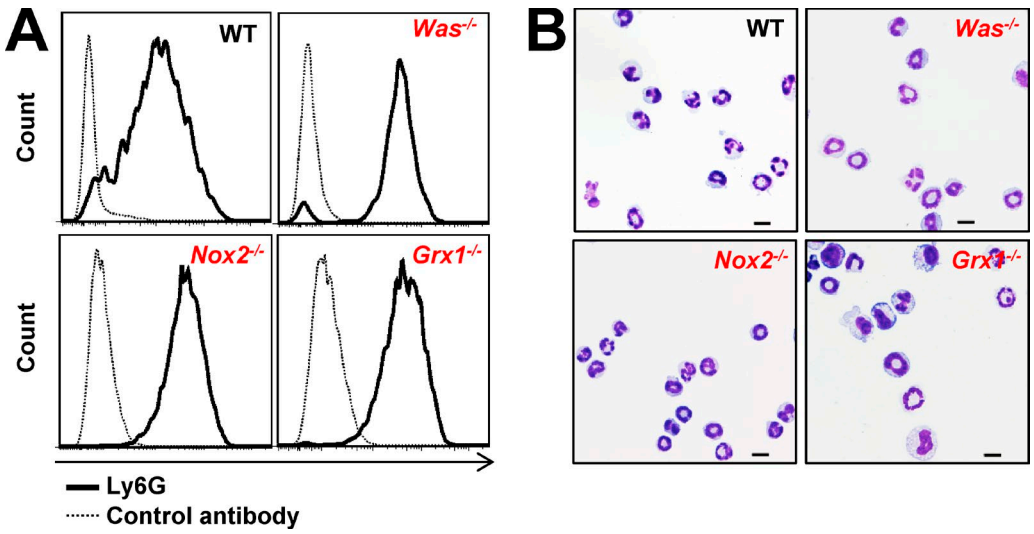


Figure S3. **Immunophenotypic and morphological characterization of mature Hoxb8 mouse neutrophils. (A)** Flow cytometry. Cell surface expression of Ly6G in WT, *Nox2*$^{-/-}$, *Grx1*$^{-/-}$, and *Was*$^{-/-}$ Hoxb8 mouse neutrophils after differentiation. **(B)** Cytology. Nuclear morphology of Hoxb8 mouse neutrophils was analyzed by light microscopy. Representative data are shown.

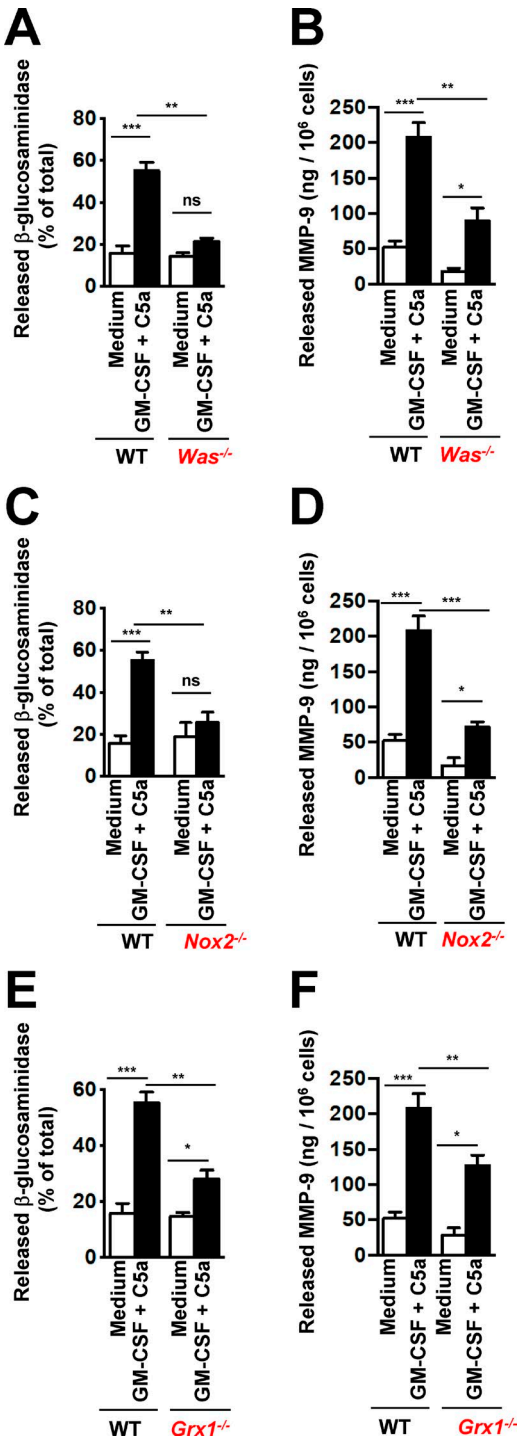


Figure S4. **Degranulation of activated *Was*$^{-/-}$, *Nox2*$^{-/-}$, and *Grx1*$^{-/-}$neutrophils was compromised compared with that of WT neutrophils.** Mouse neutrophils (4 × 10^6 cells/ml) were primed with 25 ng/ml GM-CSF for 20 min followed by 15 min stimulation with 10^{-8} M C5a. MMP-9 release was measured in the supernatants of activated cells by ELISA. The amount of β-glucosaminidase activity released in supernatants of neutrophils was expressed as a percentage of the total β-glucosaminidase. **(A and B)** Comparison between WT and *Was*$^{-/-}$ neutrophils. **(C and D)** Comparison between WT and *Nox2*$^{-/-}$ neutrophils. **(E and F)** Comparison between WT and *Grx1*$^{-/-}$ neutrophils. Data are means ± SEM. ***, P < 0.001; **, P < 0.01; *, P < 0.05. *n* = 4.

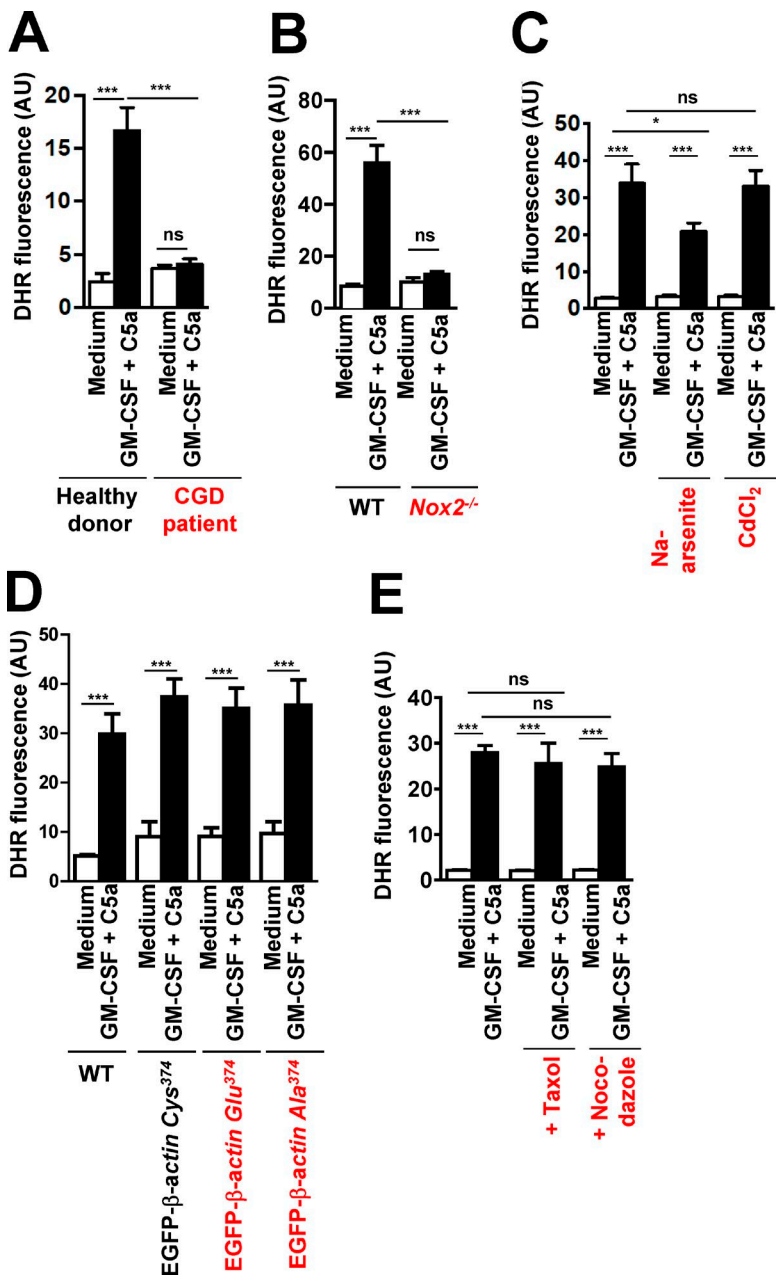


Figure S5. **NADPH oxidase but not actin glutathionylation or MT formation regulates ROS production in mouse and human neutrophils.** Flow cytometry. Total ROS activity of human and mouse neutrophils after short-term stimulation (total 35 min) with the indicated triggers was assessed by using DHR123 fluorescence. **(A)** Comparison between control and CGD human neutrophils. $n = 3$. **(B)** Comparison between WT and *Nox2*$^{-/-}$ mouse neutrophils. $n = 4$. **(C)** Comparison between human neutrophils in the presence and absence of the indicated inhibitors. $n = 5$. **(D)** Comparison between differentiated Hoxb8 mouse neutrophils and neutrophils transfected with EGFP-β-actin-Cys^{374} (WT) or point-mutated constructs having Cys^{374} changed to Ala^{374} or Glu^{374}. $n = 5$. **(E)** Comparison between human neutrophils in the presence and absence of the indicated inhibitors. $n = 5$. Data are means ± SEM. ***, $P < 0.001$; *, $P < 0.05$.

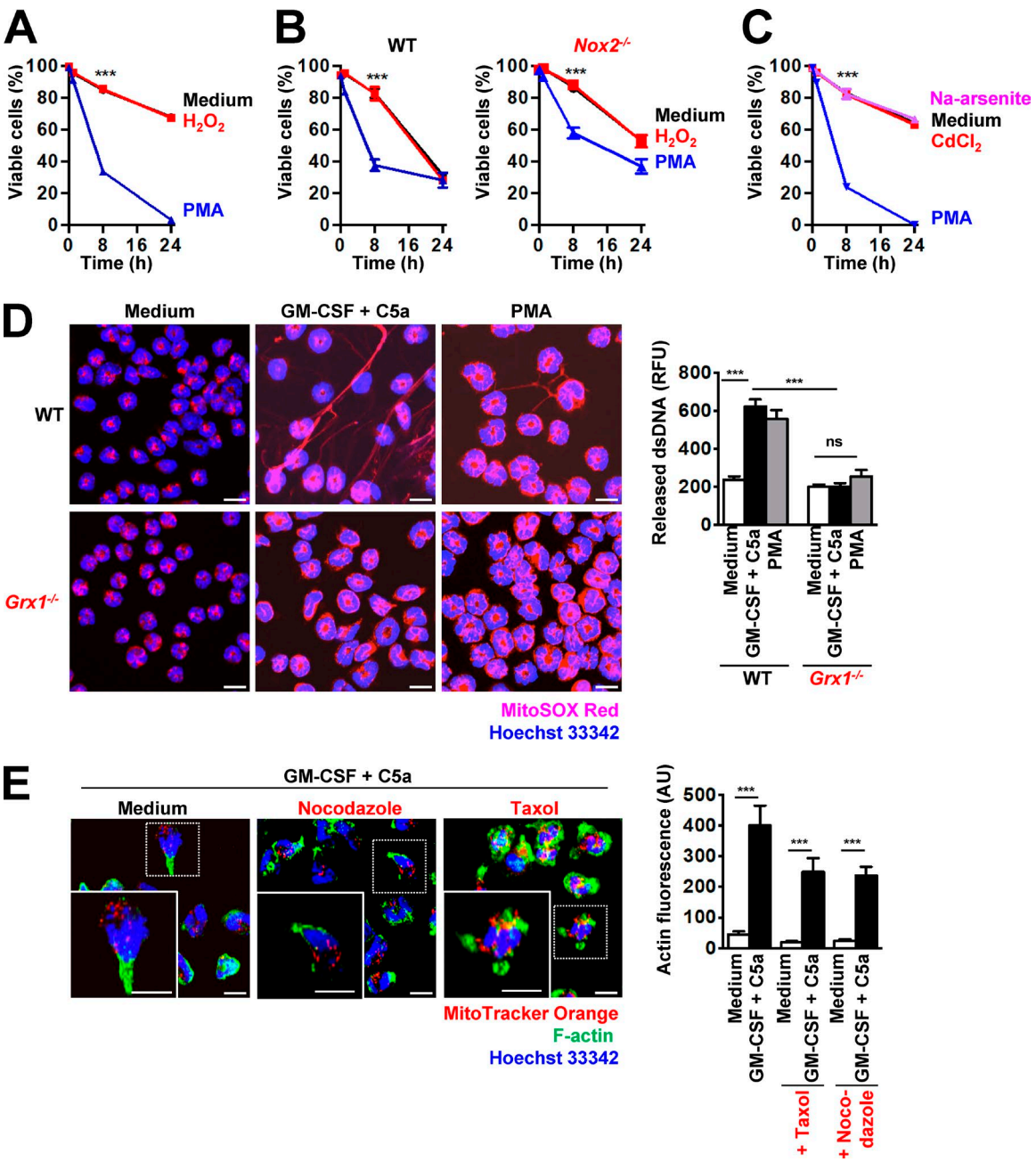
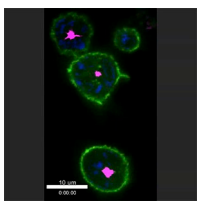


Figure S6. **Sodium-arsenite (50 µM), $CdCl_2$ (2 µM), and H_2O_2 (50 µM) do not induce death in human and mouse neutrophils. (A–C)** Flow cytometry. Viability of mouse and human neutrophils was analyzed in a time-dependent manner by using an ethidium bromide exclusion assay with flow cytometry. **(A and C)** Human neutrophils. **(B)** WT and *Nox2*$^{-/-}$ mouse neutrophils. **(D)** *Grx1*$^{-/-}$ mouse neutrophils are unable to release DNA. Confocal microscopy. DNA release was analyzed after short-term stimulation (total 35 min) of WT and *Grx1*$^{-/-}$ mouse neutrophils with the indicated triggers. Right: Quantification of released dsDNA in supernatants of activated neutrophils. **(E)** Disruption of MT network formation does not affect actin polymerization. Confocal microscopy. F-actin distribution and morphological changes were analyzed after pretreatment and short-term stimulation (total 35 min) of human neutrophils with the indicated inhibitors and triggers. Right: Quantification of F-actin was performed by automated analysis of microscopic images by using Imaris software. Data are means ± SEM. ***, $P < 0.001$. $n = 5$. Bars, 10 µM.



Video 1. **Time-lapse confocal microscopy.** Cytoplasmic actin and tubulin rearrangements were observed on mouse neutrophil activation over a period of 47 min. Please see also Fig. S1 A. *Lifeact-EGFP* mouse neutrophils were primed briefly with GM-CSF and activated with C5a. Neutrophils expressed EGFP-labeled actin and were further stained for 30 min with SiR-tubulin to visualize the tubulin. Nuclear DNA was stained with Hoechst 33342. At the earlier time points, cortical F-actin was seen in a ring-like manner close to the cell membrane. GM-CSF–primed and C5a-activated neutrophils demonstrated F-actin accumulation at the leading edge of the cells and increasing MT network formation. Bar, 10 µm.