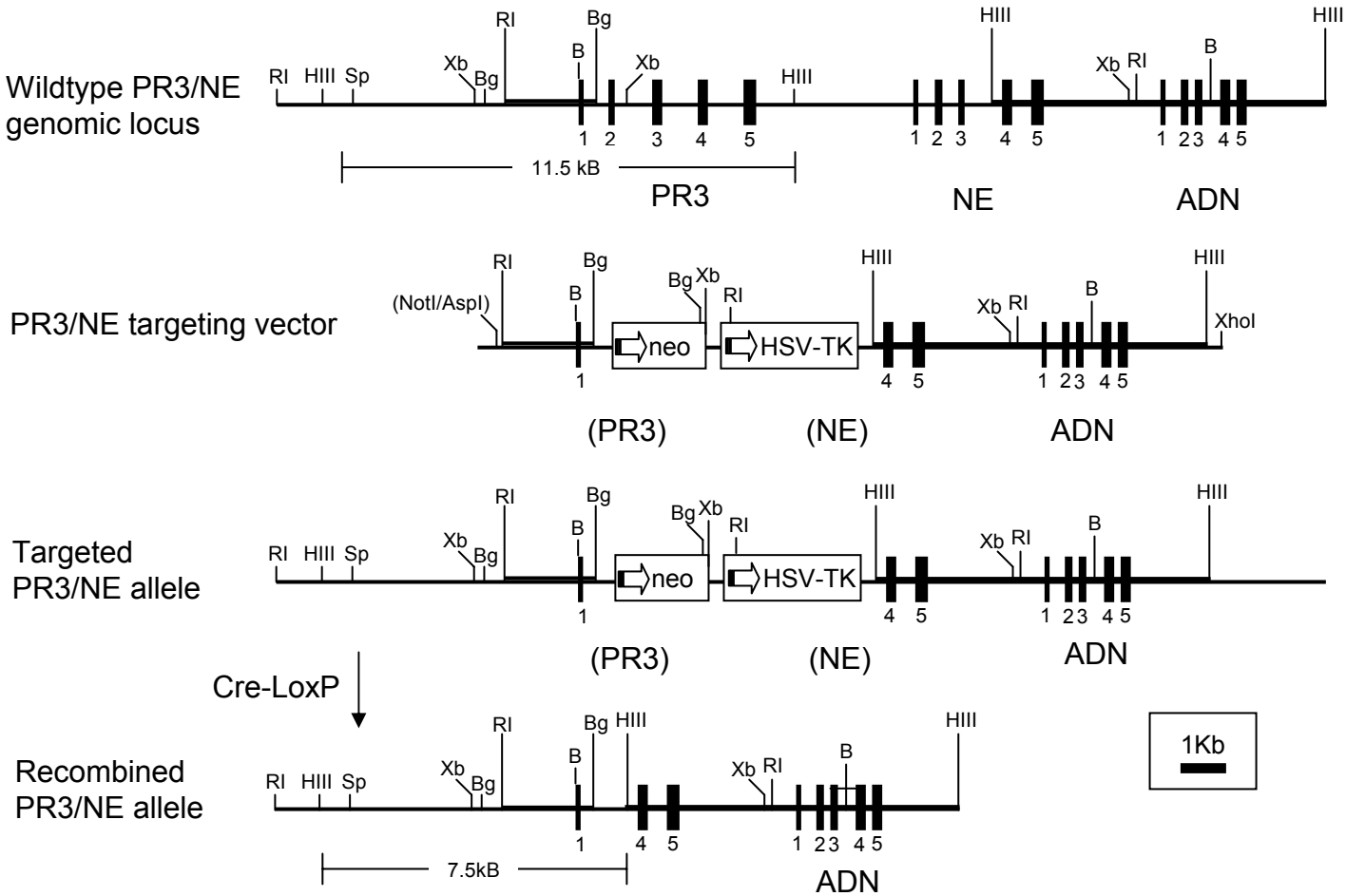
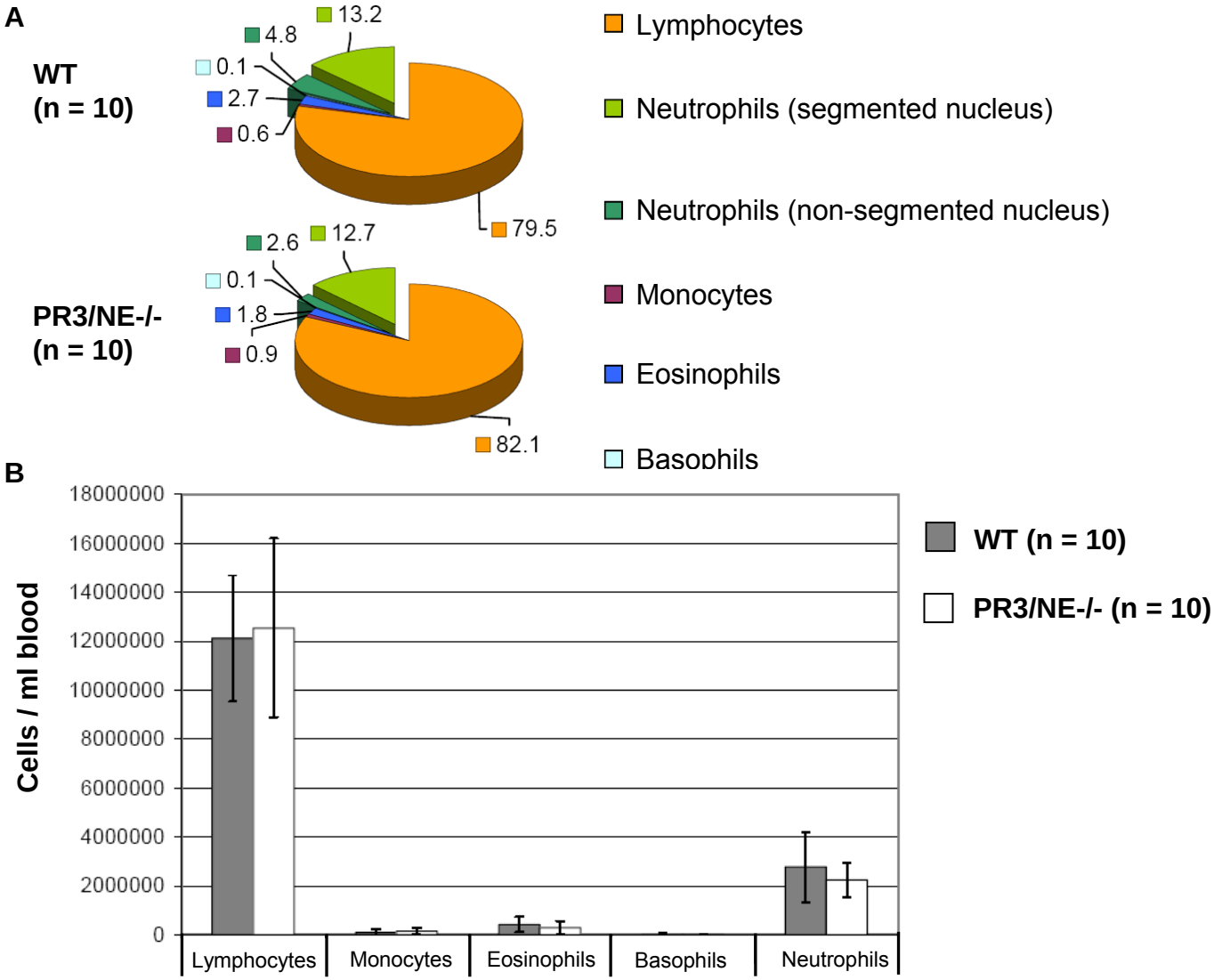


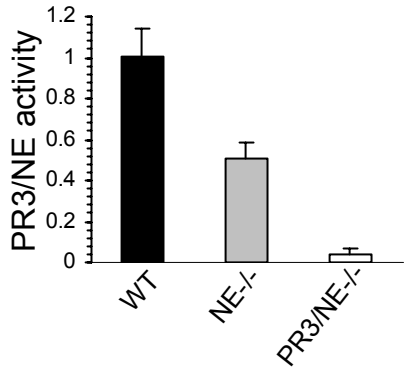
Supplementary Figure 1

Generation and characterization of PR3/NE knockout mice. Schematic representation of the wildtype PR3/NE locus, gene-targeting vector, targeted allele and the correctly recombined PR3/NE knockout allele. Gene-targeting results in deletion of exons 2-5 of the PR3 gene as well as exons 1-3 of the NE gene leaving the neighbored ADN gene unchanged.

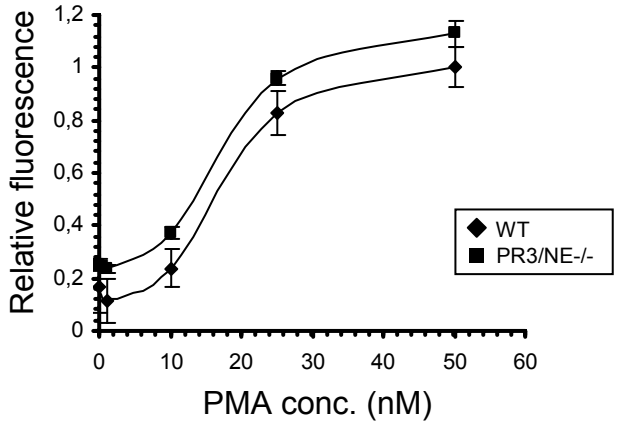


Supplementary Figure 2

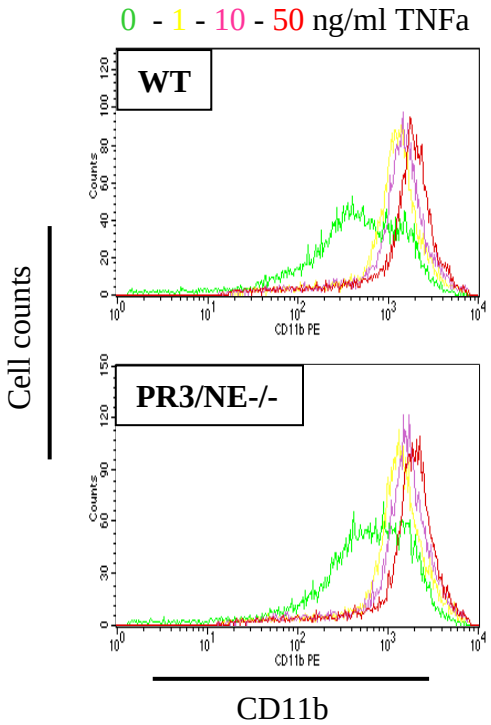
Normal leukocyte populations in PR3/NE-depleted animals. (**A**) Blood smears of peripheral blood were stained by "Diff-Quick" and leukocytes were morphologically determined in 10 random microscopic fields. Cell counts are shown as percentage. Normal leukocyte populations were observed in PR3/NE-deficient animals. **(B)** The cell number of each leukocyte population per mililiter (ml) blood was determined using a hemocytometer and the mean values of blood samples from 10 mice are shown and the standard deviation is indicated. No significant difference of neutrophil counts was observed between WT and PR3/NE-deficient mice ($p = 0.288$).



Supplementary Figure 3
PR3/NE enzyme activity of the neutrophil lysates was measured in triplicates using a PR3/NE specific chromogenic substrate after 15 min incubation. Data is shown as mean +/- SD of triplicates. Compared to WT lysates, NE-/- neutrophils show ~50% residual activity. No activity was observed in PR3/NE-negative neutrophils



Supplementary Figure 4
Neutrophils were activated by PMA at concentrations ranging from 0.1 to 50 nM. After one hour of stimulation, no difference of ROS production was detected between WT and PR3/NE-deficient neutrophils. Data is representative of 3 independent experiments and SD of triplicates is shown.



Supplementary Figure 5
Neutrophils were incubated with increasing concentrations of TNFα and the “priming” response was measured via the surface upregulation of CD11b using FACS. Both WT and PR3/NE-depleted neutrophils displayed a comparable of CD11b on the cell surface after 30 min indicating a normal “priming” response in the absence of PR3 and NE.

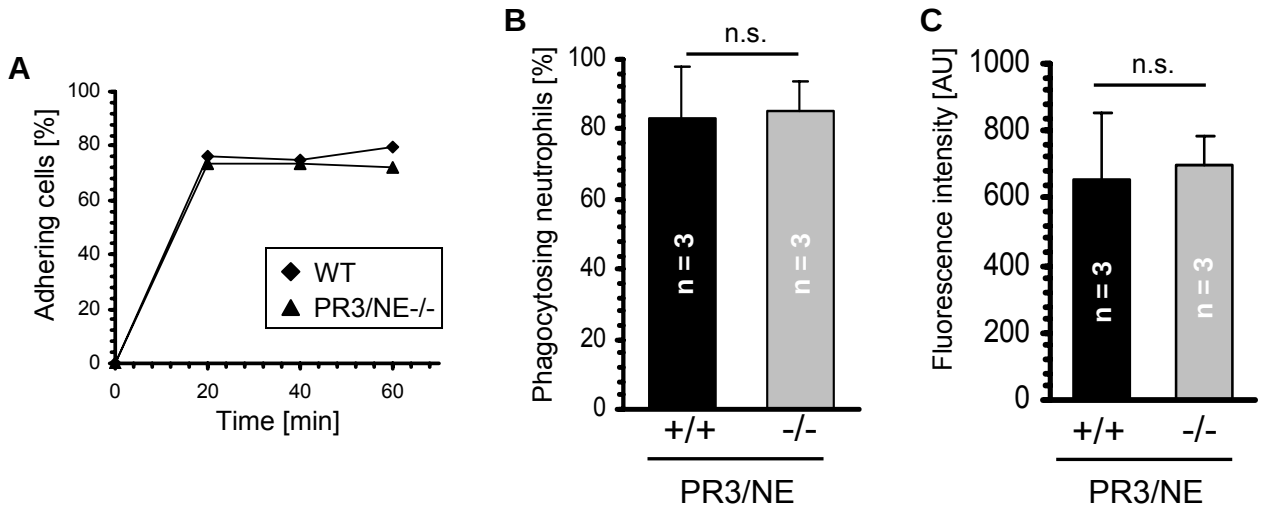


Figure 6

Normal neutrophil endocytosis and adhesion to ICs in the absence of PR3 and NE. **(A)** Calcein AM-labeled neutrophils were incubated on ICs in the presence of TNFα and the percentage of adherent cells was measured after washing at indicated time points. PR3/NE-/- neutrophils adhered normally to ICs. Data is representative of three independent experiments. No difference in phagocytosis of opsonized E.coli was found between WT and PR3/NE-/- neutrophils after 30 min incubation regarding percentage (**B**) and fluorescence intensity (**C**) of phagocytosing neutrophils as analyzed using Phagotest®. Data is shown as mean + SD and is representative of three independent experiments. Mann-Witney U-test revealed that the differences are statistically not significant (n.s.).

Supplementary Movie 1
Isolated neutrophils from WT and PR3/NE-/- mice embedded into a three dimensional collagen gel migrated towards C5a gradient; time-lapse over 17 min (20 sec/frame; 51 frames).

SUPPLEMENTARY METHODS

Generation of PR3/NE-deficient mice

To generate the PR3/NE gene targeting vector (pLF3), initially the second HindIII site of the vector pBSloxP-neo/tk-loxP upstream of the selection cassette was removed by partial endonuclease digestion of the vector followed by blunt-ending with the Klenow fragment and religation. The vector contained a 1.6 kb large neomycin-resistance gene for positive selection and a thymidine kinase gene of 3 kb for negative selection in embryonic stem (ES) cells, which were both driven by phosphoglycerokinase promoters and provided with phosphoglycerokinase polyadenylation signals, respectively. The selection cassette of the vector was flanked by two loxP sites for excision with the help of Cre recombinase. For the construction of the 5´ homologous arm of the targeting vector, a 3 kb from cosmid K13 subcloned EcoRI-BglII fragment, extending from -2.5 kb to intron 1 of mPR3, was excised with Asp718I and BglII restriction endonucleases, blunt-ended at the Asp718I site and inserted into the blunt-ended NotI and BglII sites of the vector. A 9.5-kb HindIIII fragment obtained from cosmid K79 was ligated into the single HindIII site of the modified targeting vector downstream of the selection cassette and served as the long arm for homologous recombination. The HindIII fragment emanates from intron 3 of the mNE gene and ends 4.2 kb downstream of the adipsin gene which is therefore in its entire structure covered by the 3´arm of the construct. Cosmids K13, 14 and K79 originate from a 129/SvJ mouse genomic library.

Gene targeting was performed in R1 ES cells (129/SvJ; kindly provided by Dr. A. Nagy, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada). The targeting vector pLF3 was linearized at the unique XhoI site in the remaining linker sequence downstream of the 3´homologous arm and 60 µg of phenol-chloroform extracted DNA was electroporated into approximately 4 x 10^7 cells. After 24 hrs, selection was initiated with 400µg/ml G418. Resistant ES cell colonies were isolated after 5 to 7 days and genomic DNA was screened upon digestion with BglII, XbaI, or SpeI by Southern blot analysis using a 5' external probe. For the hybridization with the internal neo probe, the ES cell DNA was restricted with EcoRI, BamHI, or XbaI. The 5' probe was generated by subcloning a 320 bp BglII-EcoRI fragment directly connecting upstream to the position of the 5´homologous arm from cosmid K14. Two correctly

targeted ES cell clones were expanded and 5 x 10^6 cells of each clone were electroporated in a second round with 10 µg of the Cre expression plasmid pIC-Cre. 72 hrs after transfection, the medium was supplemented with 2µm FIAU in order to select for those cells which excised the selection cassette from the targeted allele. After 7 days, resistant ES cell colonies were isolated of each clone and HindIII restricted genomic DNA was analyzed again with the 5´and neo probe in Southern blot analysis.

Correctly recombined ES cell clones were injected into 3.5-d postcoitus C57BL/6J blastocysts, which were then transferred into the uteri of 2.5-d postcoitus pseudopregnant CD1 mice. Germ line-transmitting male chimeras were obtained from two independent cell lines. PR3/NE heterozygous offspring from chimeras were crossed into two different genetic backgrounds (C57BL/6J and 129S6/SvEv) to generate an inbred and outcross line. Genotyping of offspring was performed by PCR using primers DJ186 (5'-CTCGGCCTTATGTGGCATCC; exon 2 PR3) and DJ187 (5'-CCGGCATAGGAAGGTGACCA-3'; exon 4 PR3 reverse) for the wild-type allele and the primer pair DJ218 (5'-AGCTCCCATGCTGTGTTTC-3'; Intron 1 PR3) and DJ104 (5'-TCGGTCTTTGGGATGGGTAAG-3', exon 5 NE reverse) for the recombinant allele. PCR mixtures contained 1 µM of each primer, 1.5 mM $MgCl_2$, 250 µM of each deoxynucleoside triphosphate, 1 x Taq polymerase buffer, and 1.5 U of Taq DNA polymerase (Perkin Elmer). The PCR amplification program consisted of an initial 5-min denaturation step at 95 C, followed by 35 cycles of annealing (65 C, 60 sec), extension (72 C, 60 sec for the recombinant allele or 90 sec for the wildtype allele), and denaturation (93 C, 60 sec), with a 5-min extension after the final cycle. The mutant-specific amplification product was cloned and sequenced to confirm correct loxP-mediated Cre recombination.

Measurement of PR3/NE enzyme activity

Enzymatic activity in lysates from WT, NE-/- and PR3/NE-/- neutrophils was determined using Boc-Ala-Pro-Nva p-chlorothiobenzylester (BAPN-TBE; Sigma), a chromogenic substrate widely used to monitor NE and PR3 activity. Lysates of neutrophils purified from the casein-inflamed peritoneum were prepared and PR3/NE activity in the lysate of 8 x 10^4 neutrophils was measured in 50 mM Hepes, 0.75 M NaCl, 0.05% Nonidet

containing 1 mM BAPN-TBE and 0.5 mM DTNB. The OD_{405} was measured at 37°C over time using FLUOstar Optima (BMG Labtech).

Isolation of human neutrophils

Human peripheral blood neutrophils were isolated by density centrifugation using a Pancoll® gradient. Briefly, 10 ml blood containing EDTA was diluted in 10 ml phosphate buffered saline (PBS) and layered on 10 ml Pancoll (PAN Biotech GmbH, Cat.No: P04-60500). After 30 min centrifugation at 500 g without break, granulocytes were further purified from the cellular pellet by dextran sedimentation (Dextran T500, Pharmacia Biotech, Cat.No: 17-0320-01). Residual erythrocytes were removed by hypertonic lysis and, after washing, neutrophil granulocytes were resuspended in RPMI (Gibco, Cat.No: 61870). Neutrophil purity as assessed by forward and side scatter with flow cytometry was routinely ~95%. Human granulocytes were subjected to IC- and PMA activation as mentioned before and the effect of 100 nM PGRN on the oxidative burst was evaluated.