Mineral particles stimulate innate immunity through neutrophil extracellular traps containing HMGB1

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Supplementary Information

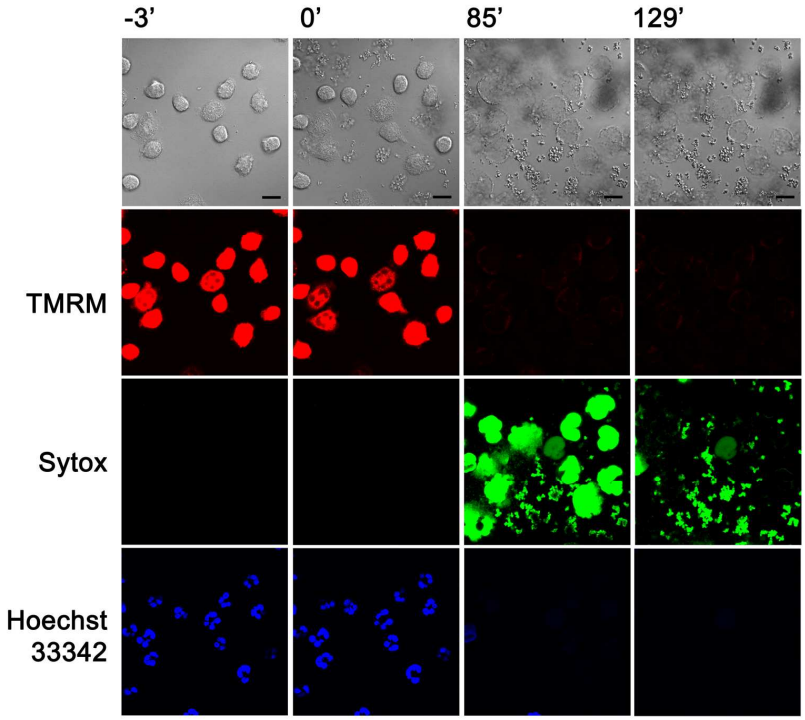

Figure S1. Mineral particles induce loss of mitochondrial potential in neutrophils. Neutrophils were exposed to mineral particles and an indicator of mitochondrial membrane potential (tetramethylrhodamine methyl ester, TMRM; red). Nuclear DNA was stained blue (Hoechst 33342), while NET DNA was stained green (Sytox). Data are representative of three independent experiments. Scale bar, 10 μm.

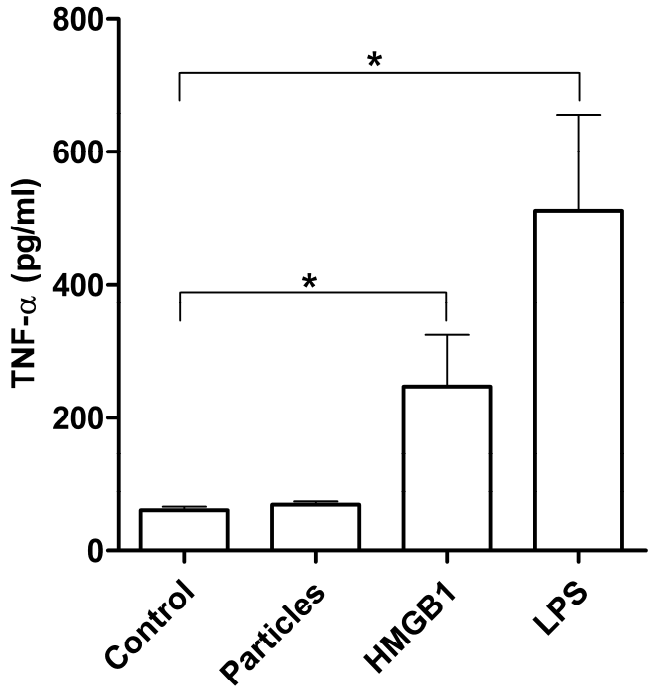

Figure S2. Priming THP-1 macrophages with PMA does not facilitate TNF-α secretion upon stimulation with mineral particles. THP-1 cells previously primed with PMA were cultured with mineralo-organic particles ("Particles"), or recombinant HMGB1 ("HMGB1") or LPS ("LPS") as positive control. Data are shown as means ± SEM and the results of at least three independent experiments. *$p < 0.05$, vs. untreated neutrophils.

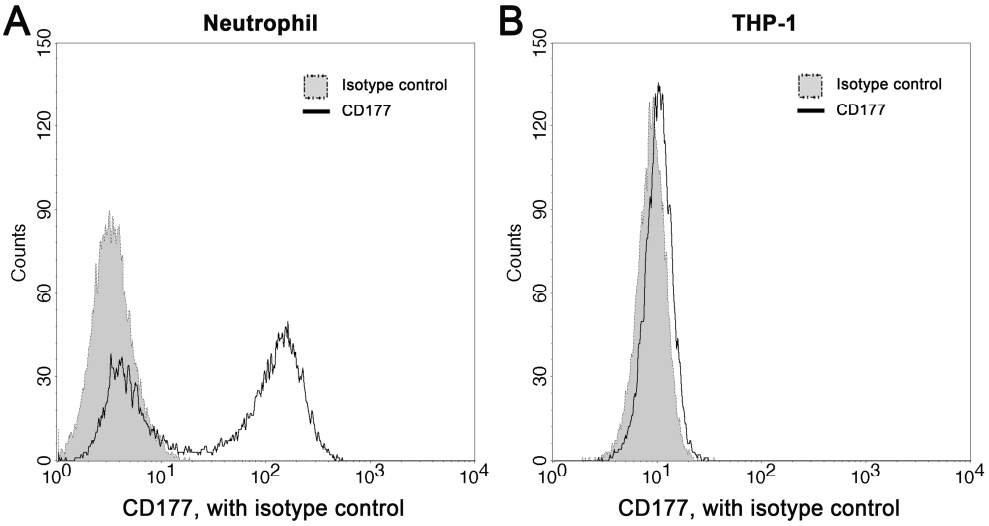

Figure S3. Flow cytometry analysis of isolated neutrophils and cultured macrophages. The isolated neutrophil population (A) stained positive for CD177, whereas cultured THP-1 cells (B) did not. A portion of neutrophils remained CD177 negative; the CD177-negative population is considered part of the neutrophil profile of CD77 expression.

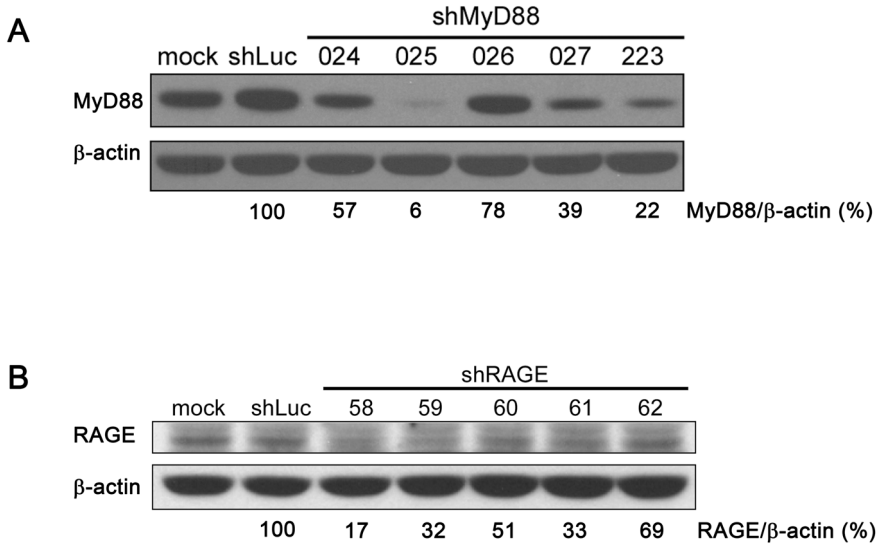

Figure S4. Downregulation of MyD88 and RAGE by stable shRNA expression in THP-1 cells. THP-1 cell clones stably expressing shRNA targeting either MyD88 (A) or RAGE (B) were generated, and expression of the target proteins was assessed using Western blotting. Mock: mock-transduced cells; shLuc: THP-1 cells expressing shRNA directed against luciferase as a control; 024, 025, 026, 027, and 233: MyD88-targeting shRNA clone TRCN0000008024, TRCN0000008025, TRCN0000008026, TRCN0000008027 and TRCN0000011223, respectively; 58, 59, 60, 61, and 62: RAGE-targeting shRNA clone TRCN0000062658, TRCN0000062659, TRCN0000062660, TRCN0000062661 and TRCN0000062662, respectively.

Video S1. Live cell imaging of neutrophil response to mineral particles
Microscopy images were taken every 3 min. The playback speed is 5 frames/sec. Blue, DAPI; green, Sytox.

Video S2. Depiction of 3D-reconstruction of NET-macrophage interactions
The movie was generated by Z-stacking with section thickness = 1 μm. Blue, DAPI; green, Sytox; red, THP-1.