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SUPPLEMENTARY INFORMATION

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Leukocyte function assessed via serial microlitre sampling of peripheral blood from sepsis patients correlates with disease severity

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Supplementary Information

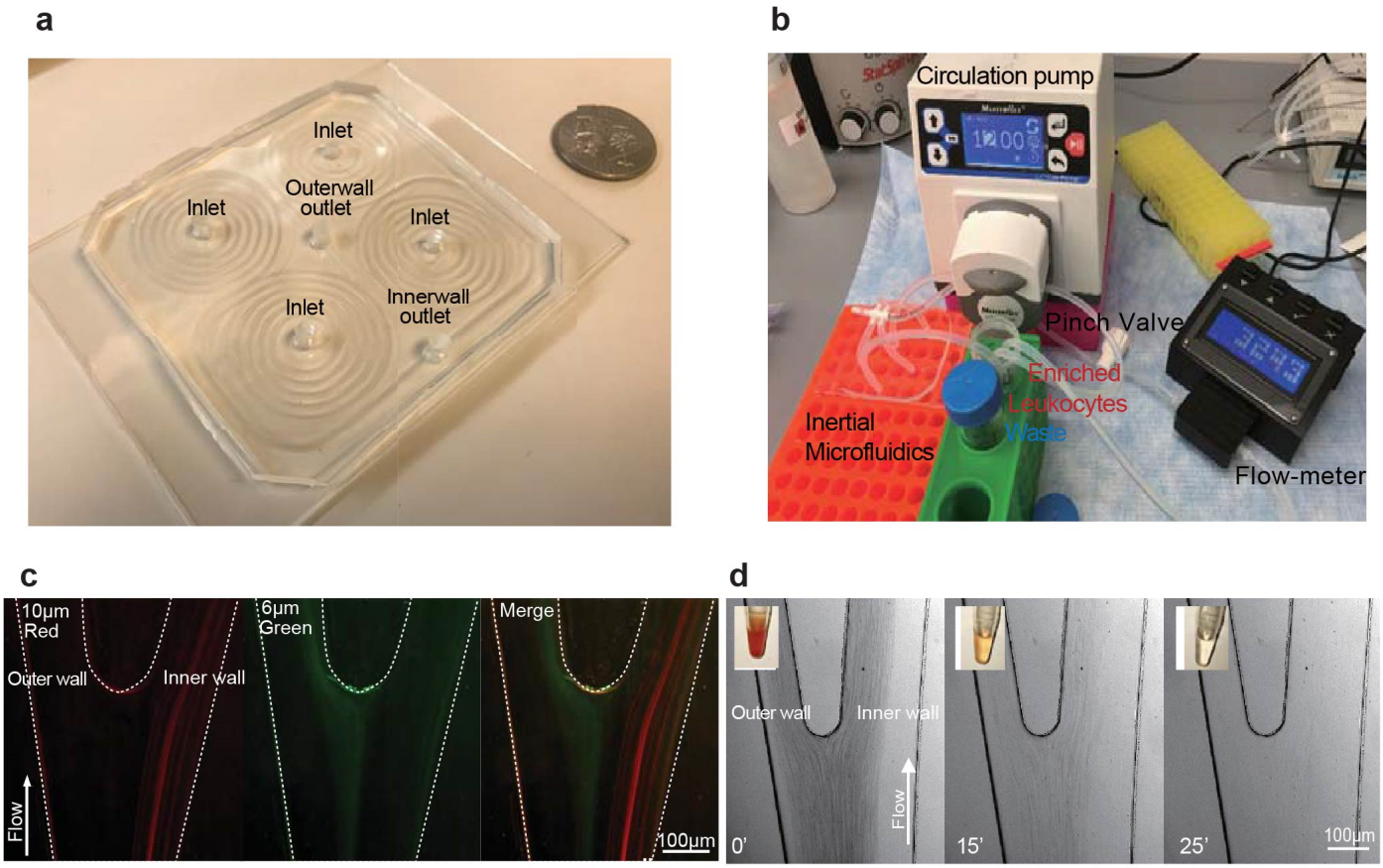

Fig. S1. Closed-loop inertial microfluidic assay. (a) Closed-loop inertial microfluidic used for leukocytes separation from erythrocytes. Two identical layers, each of which has 4-spiral micro-channels with individual inlets and common outlets were vertically stacked. **(b)** Experimental setup for closed-loop inertial microfluidic system. Peristaltic pump was connected in between microfluidics platform and leukocyte/waste tube. Flow meter and adjustable fluidic resistance (pinch valve) was placed between common outlet and waste tube. **(c)** Fluorescent images at outlet of spiral micro-channel showing particle separation during operation. 10 µm red micro-bead and 6 µm green micro-beads were representing PMN and erythrocytes (RBCs), respectively. **(d)** Light microscopy images at outlet of spiral micro-channel (bright-field images) during the separation. RBCs (shown as black

streamlines) were continuously removed from the suspension through outer wall side outlet, while PMN were recirculating via innerwall side outlet.

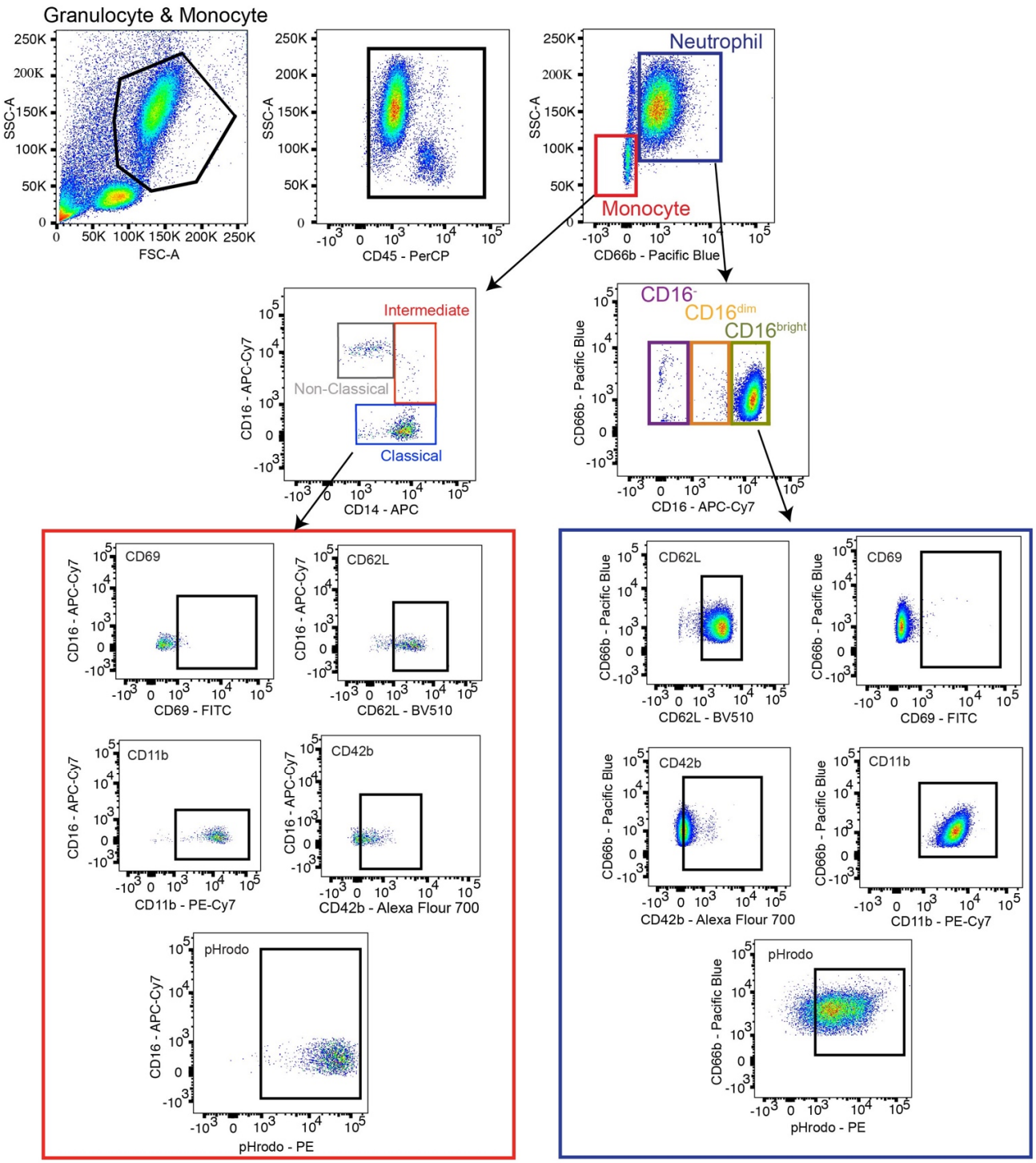

Fig. S2. Gating Strategy for the identification of PMN and monocytes. PMN and monocytes were isolated from 50 µl of peripheral blood using the inertial microfluidic

system. PMNs ($CD45^{+}SSC^{Hi}FSC^{Hi}$) subsets ($CD16^{bright}$, $CD16^{dim}$, $CD16^{-}$) were subsets as determined by CD16 and CD66b surface expression and monocytes ($CD45^{+}FSC^{+}SSC^{-}$) subsets (classical, intermediate, and non-classical monocytes) determined by CD14 and CD16 surface expression. Markers of PMN and monocyte activation were assessed, namely expression of CD69, CD62L, CD11b, CD42b, and pHrodo.

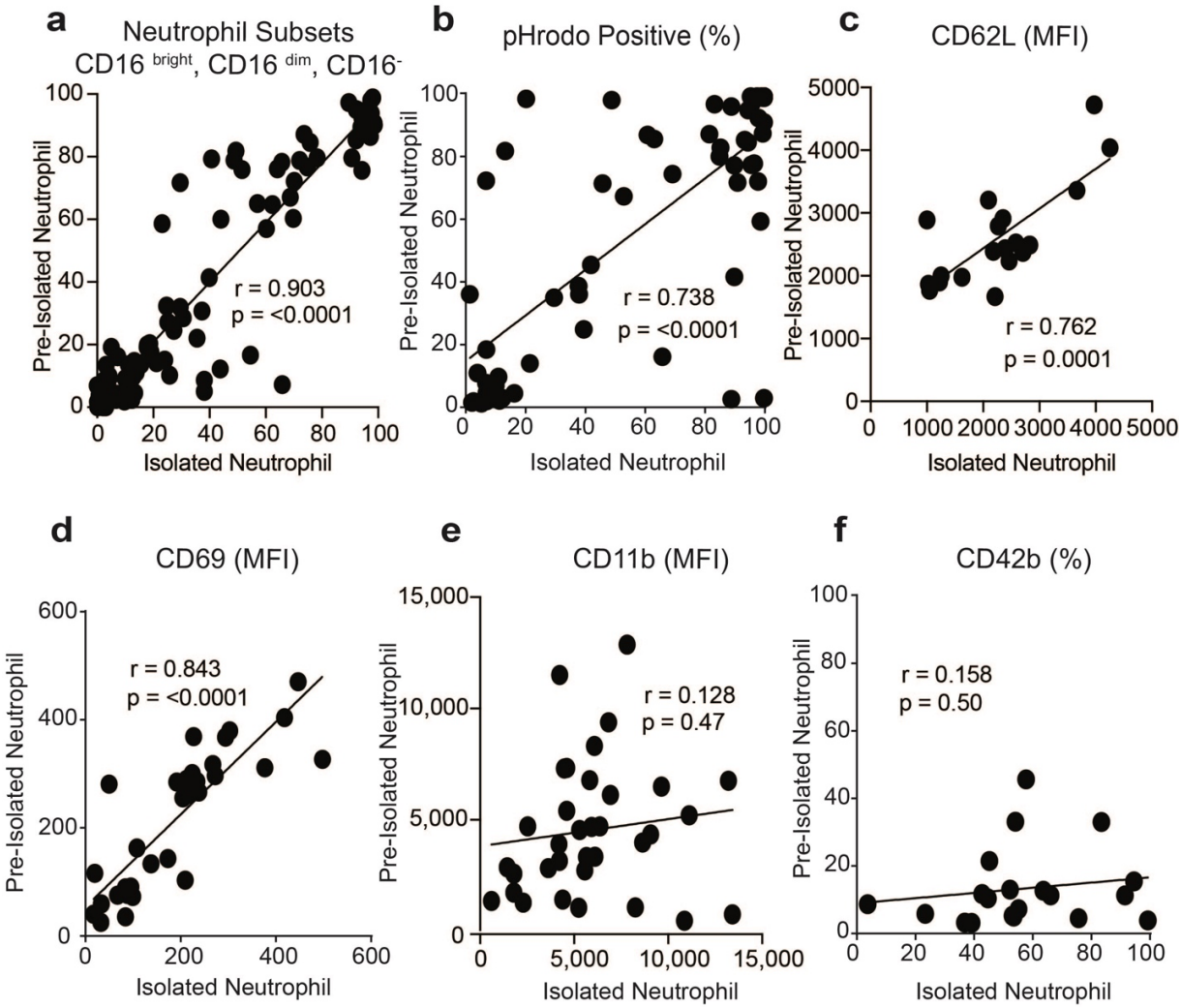

Fig. S3. Effect of inertial microfluidics isolation on markers of PMN activation in sepsis and health. PMN were isolated from 50 µl of freshly obtained peripheral blood from healthy subjects or sepsis patients (Day 0, 3, and 7 of hospitalization) using the inertial microfluidic system (see methods). The correlation of **(a)** PMN subsets ($CD16^{bright}$, $CD16^{dim}$, and $CD16^{-}$) (n=117 biospecimens from 23 donors), **(b)** pHrodo (%) positive ($CD16^{bright}$, $CD16^{dim}$, and $CD16^{-}$) (n=62 from 20 donors)**,** $CD16^{bright}$ of **(c)** CD62L MFI (n=19 from 13 donors), **(d)** CD69 MFI (n=34 from 21 donors), **(e)** CD11b MFI (n=31 from 20 donors), and **(f)** CD42b (%) (n=20 from 16 donors) with pre-isolation and after inertial

microfluidics were identified. Pearson correlation r value and significance are noted, and regression lines are shown. MFI, Median Fluoresce Intensity.

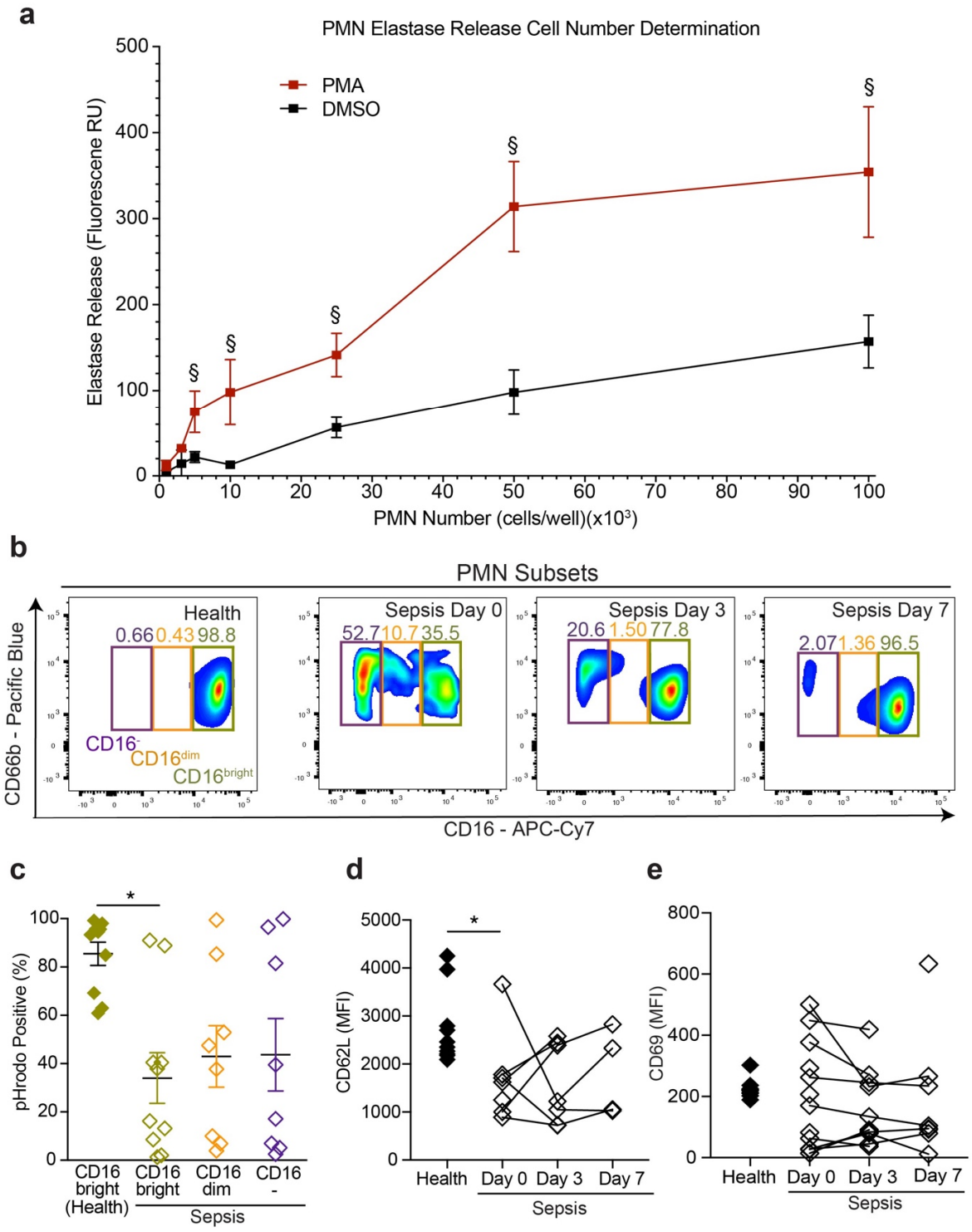

Fig. S4. PMN subsets and activation in sepsis and health. PMNs were isolated from 50 µl of freshly obtained peripheral blood using the inertial microfluidic system (see

methods). **(a)** Effect of PMN number per well on elastase release after exposure to PMA (20nM) and vehicle (DMSO 0.002% v/v) (n=32). **(b)** Representative flow cytometry zebra plot of PMN ($CD45^+FSC^+SSC^+$) subsets as determined by CD16 and CD66b surface expression. Numbers represent percentage of PMN subsets. **(c)** Frequency of PMN subsets expressing pHrodo-labelled *E. coli* particles after incubation for 15 minutes (37^oC) in sepsis (day 0) (n=10) and health (n=10). Markers of PMN activation were assessed, namely expression of **(d)** CD62L MFI (n=10 healthy and n=18 sequential samples from 7 sepsis patients) **(e)** CD69 MFI (n=10 healthy and n=31 sequential samples from 13 sepsis patients) in $CD16^{bright}$ PMNs of healthy donors and patients with sepsis at days 0, 3, and 7 of hospitalization. Values are expressed as the mean +/- s.e.m. § P< 0.05 for PMA vs. Vehicle (DMSO) by paired, two-tailed t-test, *P<0.05 sepsis vs. healthy by unpaired, two-tailed student's t-test.

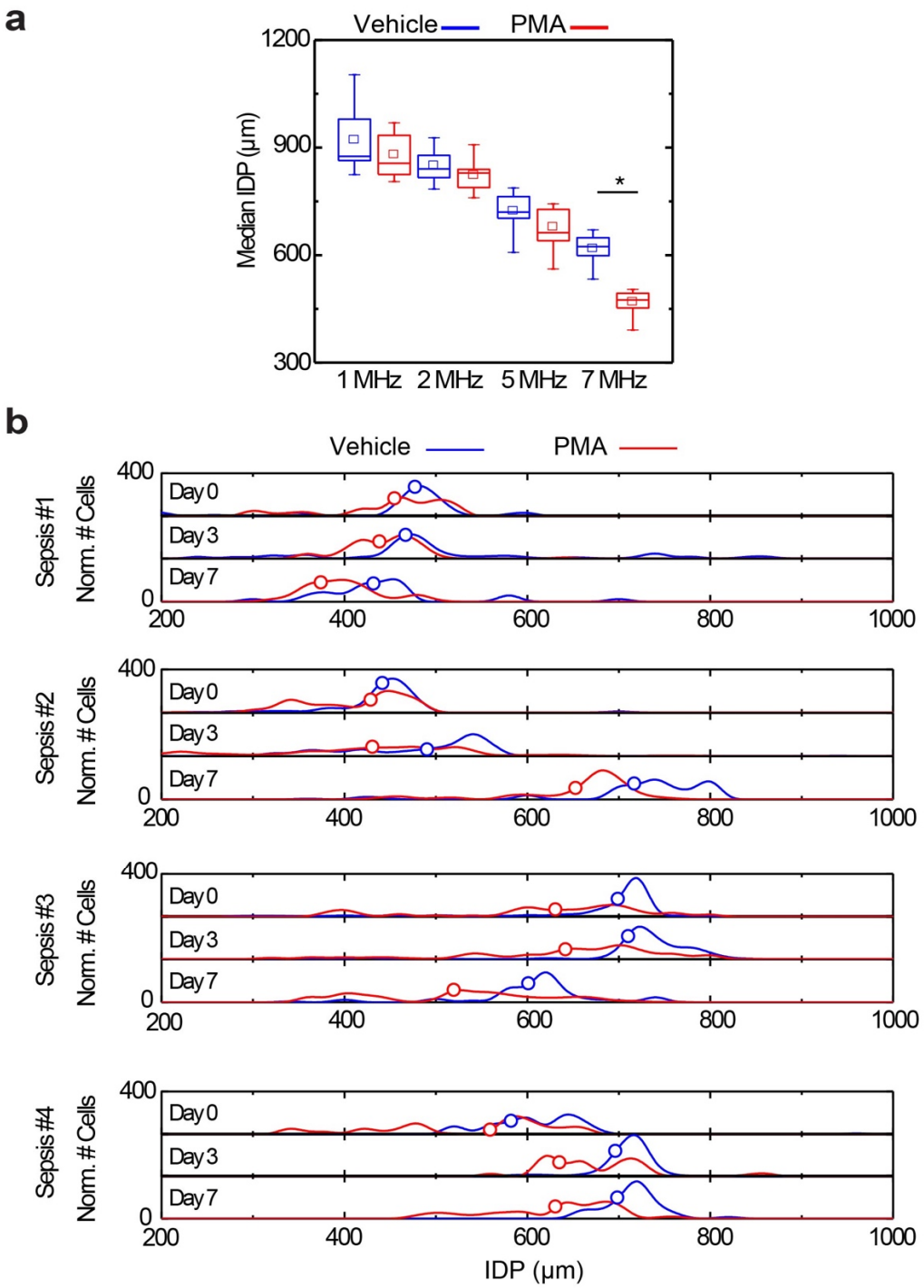

Fig. S5. IDP distributions of PMA-treated and non-treated PMN from sepsis patients and healthy donors. (a) Box-and-whisker plots (median, 25th and 75th percentile) of median IDP of PMA-stimulated and non-stimulated PMNs from healthy donors (n=10).

(b) After exposure to PMA (20nM, 30 min, RT) or vehicle, the IDPs of PMNs from sepsis patients were distributed at 7 MHz electrical application. The median IDP values were determined for PMA-activated cells (red circle) relative to vehicle (blue circle) at days 0, 3, and 7 of hospitalization. *P<0.05 PMA stimuli vs. vehicle by paired, two-tailed student's t-test.

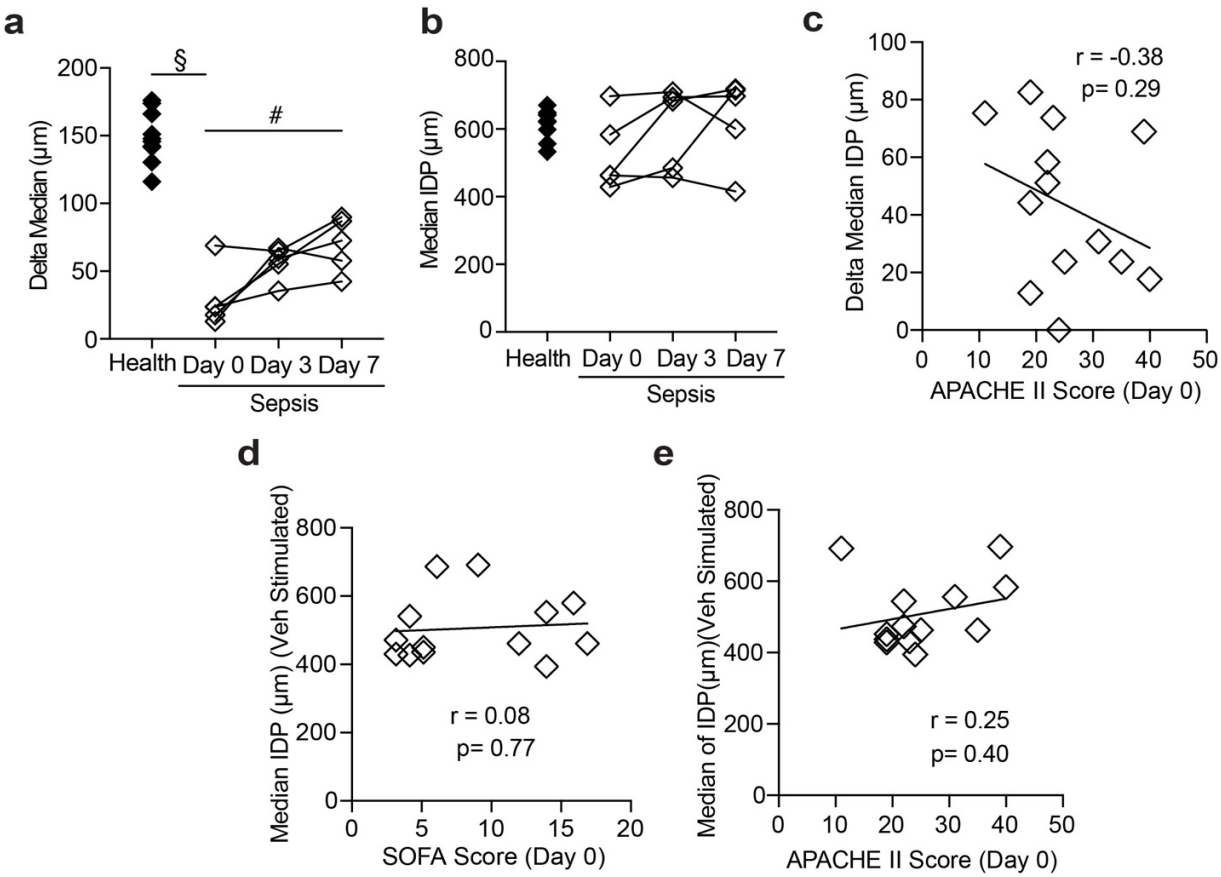

Fig. S6. Correlation of measures of clinical severity during sepsis and isodielectric separation of activated from non-activated human PMNs. (a) Delta median (Δ_{med}) IDP and **(b)** Median IDP in PMNs from healthy subjects (n = 10) or patients presenting with sepsis (n=15 sequential samples from 5 sepsis patients) at day 0, 3, and 7 of hospitalization. The relationship between the severity indicator scores with **(c)** delta median (Δ_{med}) and **(d, e)** median IDP (µm) was determined in sepsis patients (n=13) upon presentation (Day 0). Pearson correlation r value is noted, and a regression line is shown. §P<0.05 health vs. sepsis by unpaired, two-tailed student's t-test, #P<0.05 one-way ANOVA-test for n=5 sepsis patients at day 0, 3, and 7 of hospitalization.

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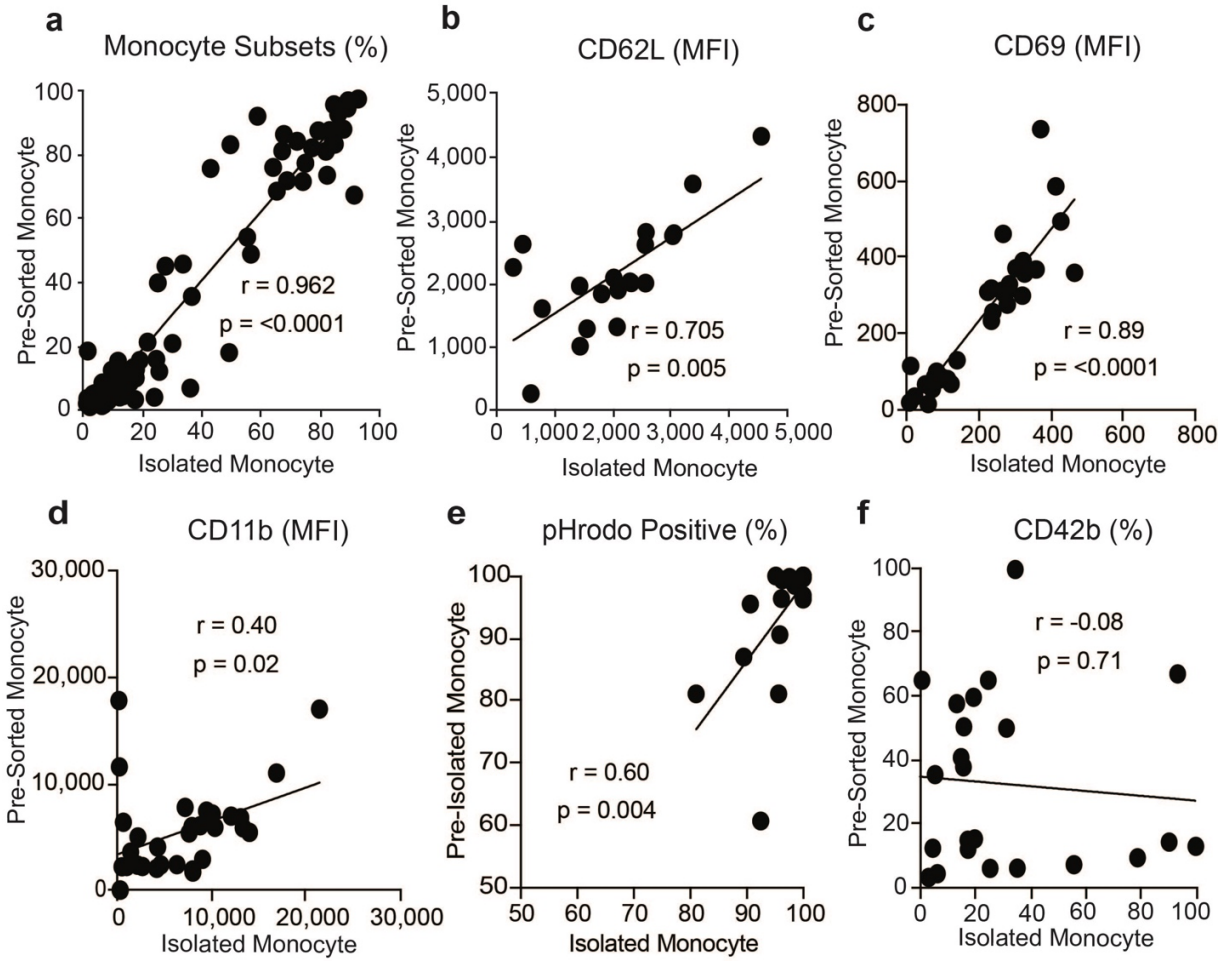

Fig. S7. Effect of inertial microfluidics isolation on markers of monocyte activation in sepsis and health. Monocytes were isolated from 50 µl of freshly obtained peripheral blood from healthy subjects or sepsis patients (Day 0, 3, and 7 of hospitalization) using the inertial microfluidic system (see methods). The correlation of **(a)** Monocyte subsets (n=99 from 21 donors) (non-classical ($CD16^{high}CD14^{low}$), intermediate ($CD16^{high}CD14^{high}$), and classical ($CD16^{low}CD14^{high}$) monocytes), classical monocytes expression of **(b)** CD62L MFI (n=20 from 14 donors), **(c)** CD69 MFI (n=34 from 21 donors), **(d)** CD11b MFI (n=31 from 20 donors), **(e)** pHrodo Positive (%) (n=21 from 14 donors), and **(f)** CD42b (%) (n=23 from 16 donors) with pre-isolation and after inertial

microfluidics. Pearson correlation r value and significance are noted, and regression lines are shown. MFI, Median Fluoresce Intensity.

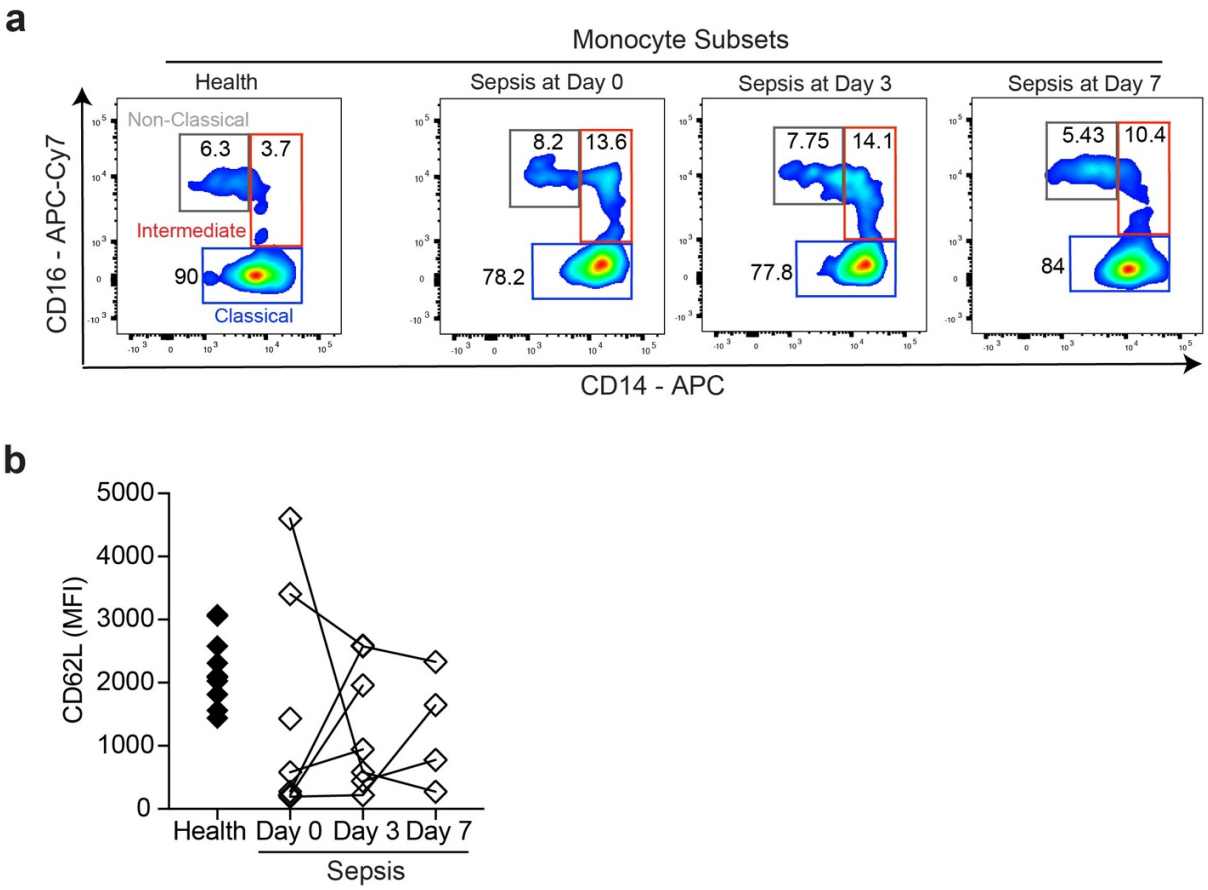

Fig. S8. Monocyte subsets and activation in sepsis and health. Monocytes were isolated from 50 µl of whole blood using the inertial microfluidic system. **(a)** Representative flow cytometry zebra plot of monocyte ($CD45^{+}SSC^{Low}FSC^{High}$) subsets as determined by CD14 and CD16 surface expression. Frequency of non-classical ($CD16^{high}CD14^{low}$, grey), intermediate ($CD16^{high}CD14^{high}$, red), and classical ($CD16^{low}CD14^{high}$, blue) monocytes in healthy subjects or sepsis patients. Numbers represent percentage of monocyte subsets. Markers of monocyte activation were assessed, namely expression **(b)** CD62L MFI in classical monocytes of healthy donors (n = 10) and patients with sepsis (n = 18 sequential samples from 7 sepsis patients) at days 0, 3, and 7 of hospitalization.

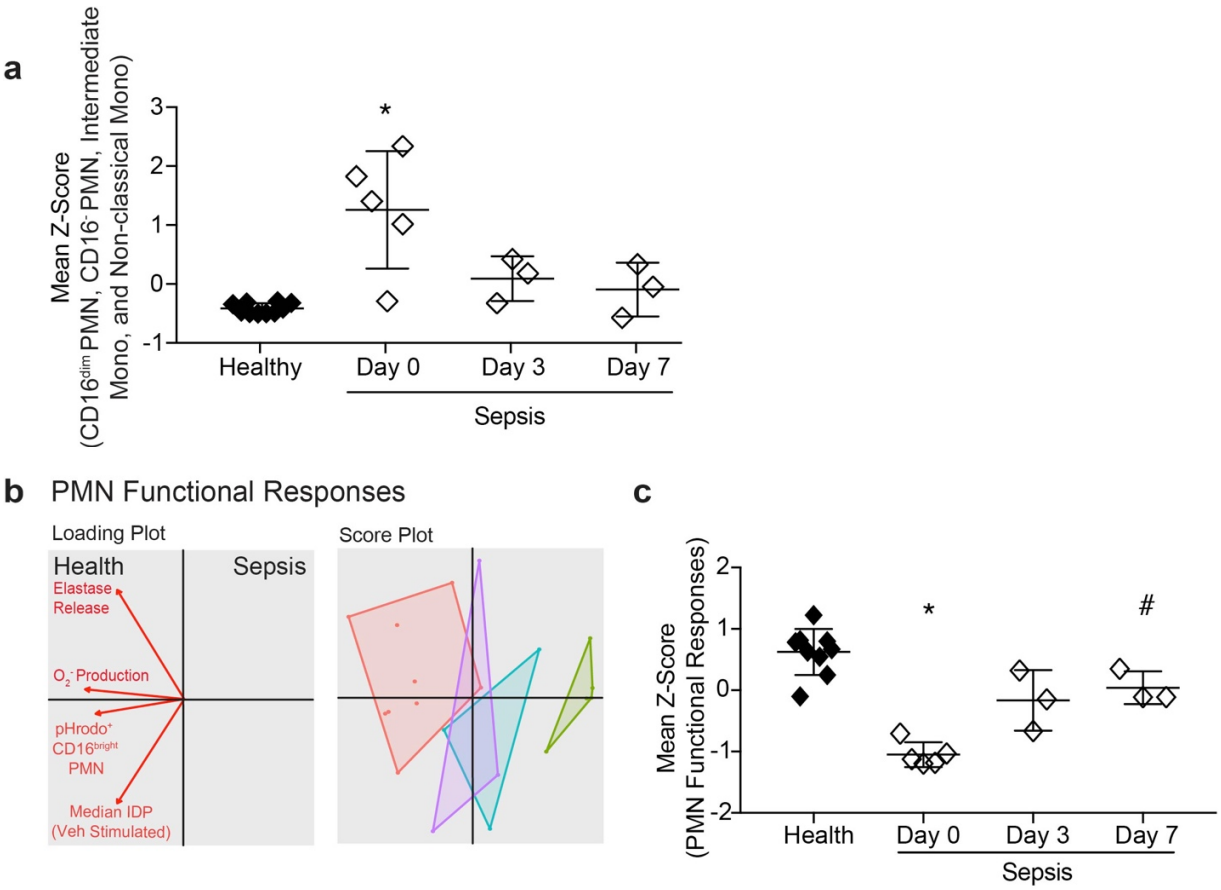

Fig. S9. Mean z-score for leukocyte phenotype and functional responses. (a) Mean z-score was determined for leukocyte phenotype markers ($CD16^{high}$ $CD14^{low}$, $CD16^{high}$ $CD14^{high}$, $CD16^{dim}$ and $CD16^{-}$). Two-dimensional score plots and loading plots from multivariate principal component analyses and mean Z-score was determined for **(b, c)** PMN functional responses. Mean z-scores were derived from individual variable z-scores in a given subject. *P< 0.05 sepsis day 0 vs. health, #P< 0.05 sepsis day 7 vs. day 0 by unpaired, student's two-tailed t-test for n=9 healthy and 5 patients with sepsis (n=11).

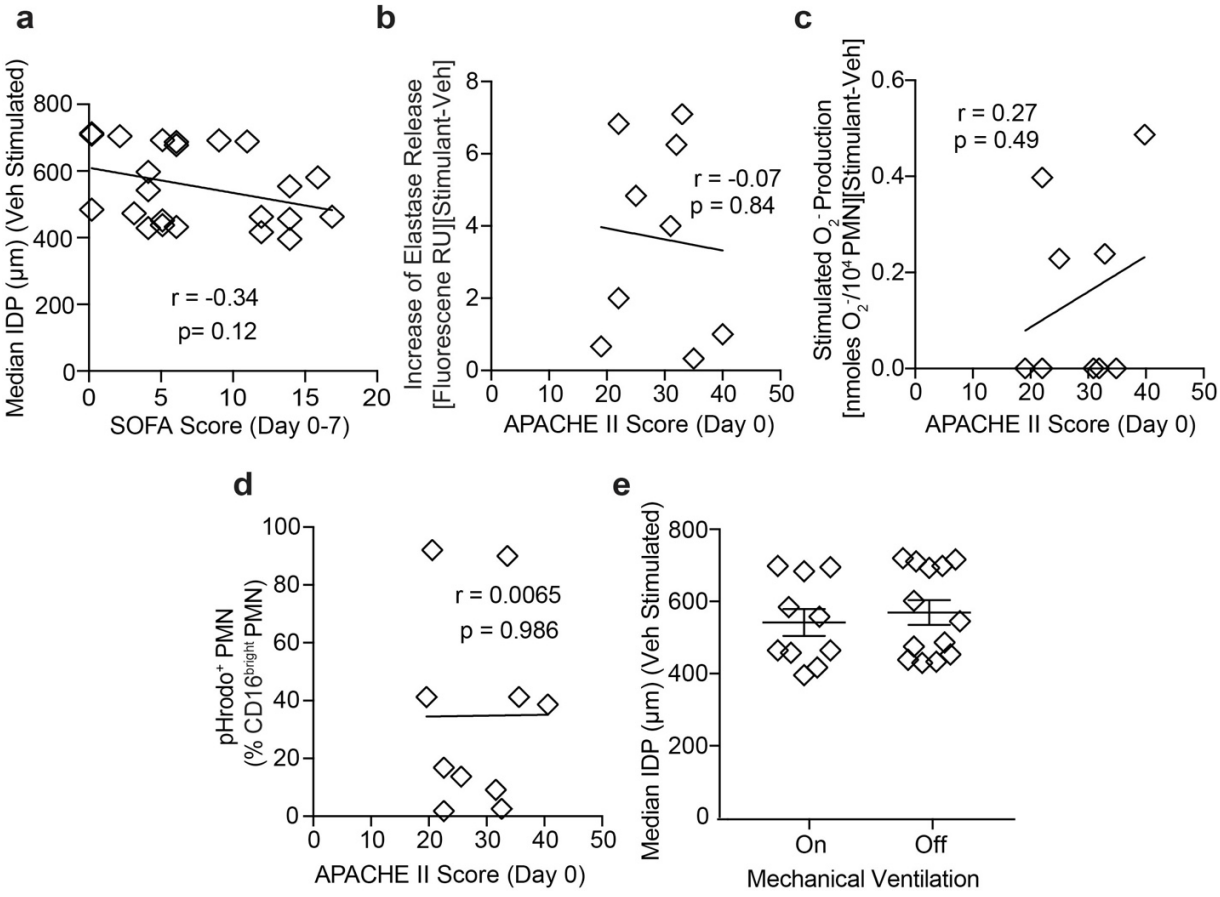

Fig. S10. Correlation of measures of clinical severity during sepsis and PMNs functional responses in sepsis. The relationship between clinical severity indicators scores and PMN functional responses, namely **(a)** Median IDP (veh stimulated) in PMNs (n=23 from 13 sepsis patients) from sepsis patients at day 0, 3, and 7 **(b)** PMN elastase release (LTB_4 stimulated, day 0) (n=9), **(c)** PMN O_2^- production (LTB_4 stimulated, day 0) (n=9), **(d)** $CD16^{bright}$ PMN expressing pHrodo positive in sepsis (day 0) (n=10), and **(e)** Median IDP (µm) (n=23) with mechanical ventilation in sepsis patients at day 0, 3 and 7. Pearson correlation r value and significance are noted, and regression lines are shown.

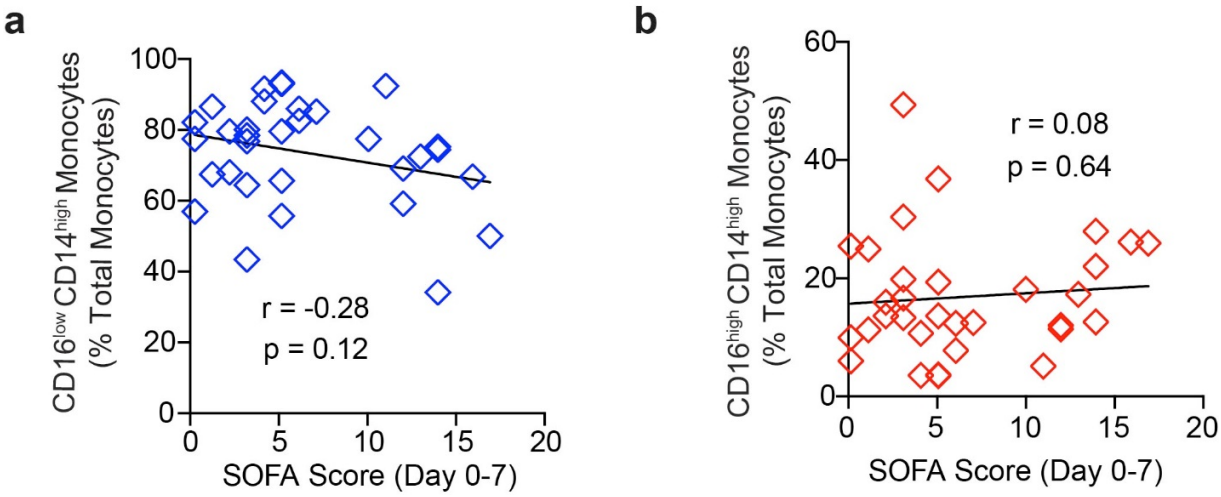

Fig. S11. Correlation of SOFA score and monocyte subsets in sepsis. The relationship between the SOFA score and monocyte subsets, namely **(a)** classical monocytes ($CD16^{high}CD14^{high}$, blue) **(b)** intermediate monocytes ($CD16^{high}$ $CD14^{high}$, red) in sepsis patients (n=32 from 13 sepsis patients) at day 0, 3, and 7 of hospitalization. Pearson correlation r value and significance are noted, and regression lines are shown.

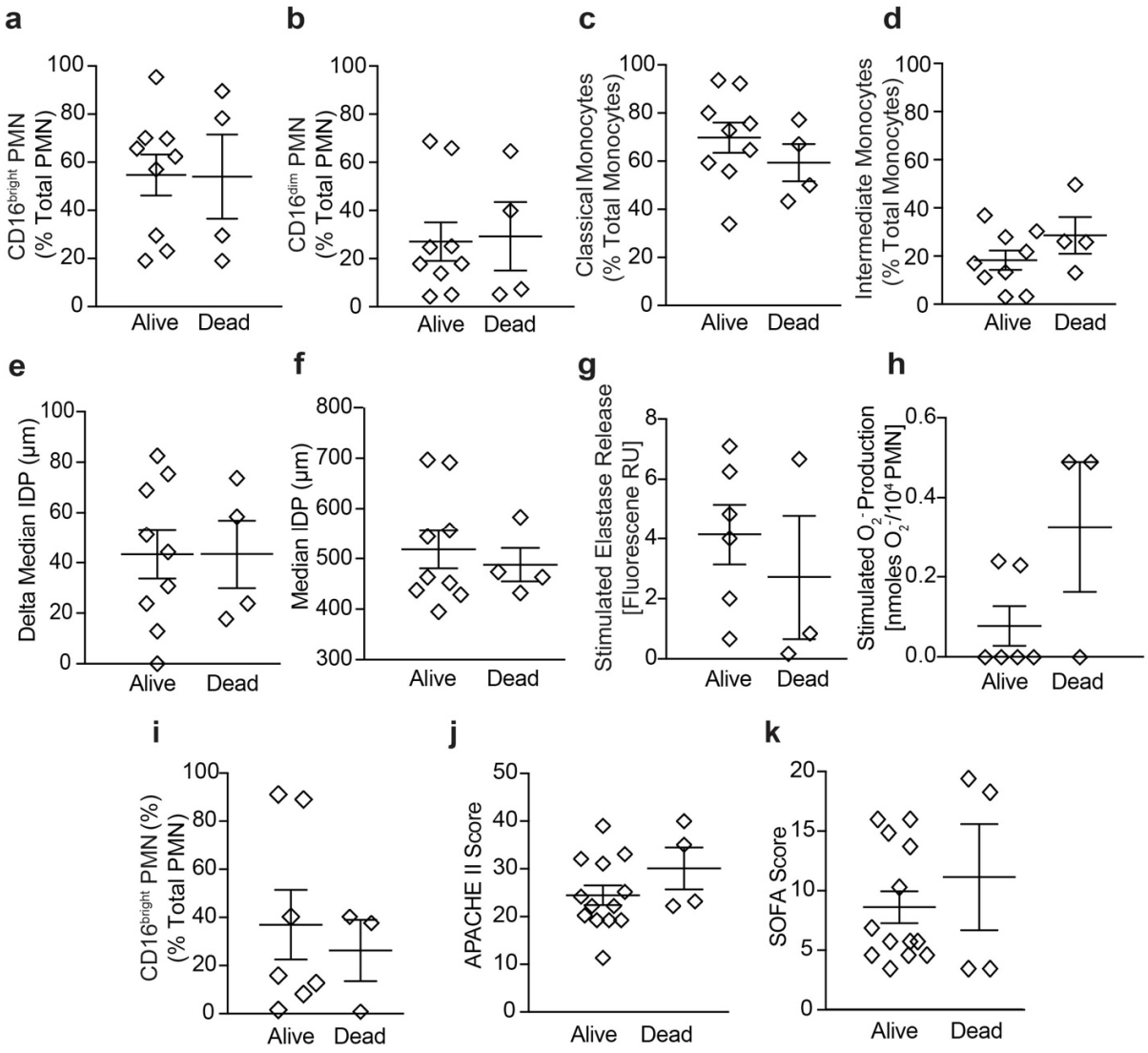

Fig. S12. Correlation between leukocyte responses and mortality outcome in sepsis. Relationship between mortality outcome and **(a)** $CD16^{bright}$ (n=13), **(b)** $CD16^{dim}$ (n=13), **(c)** Classical monocyte (n=13), **(d)** Intermediate Monocyte (n=13), **(e)** Delta Median IDP (n=13), **(f)** Median IDP (n=13), **(g)** elastase release (LTB_4-stimulated) (n=9), **(h)** O_2^- production (LTB_4-stimulated) (n=9), **(i)** Frequency of $CD16^{bright}$ PMN expressing pHrodo-labelled (n=10), **(j)** APACHE II score (n=17), **(k)** SOFA score (n=17).