Hilden, Germany) for 2 h at 4 °C with gentle shaking. The gel was packed into a column and washed successively with 10 mM Tris-buffered saline (TBS) (pH 8.0) containing 10 mM imidazole and then 10 mM Tris-buffer (TB) (pH 8.0) containing 1 M NaCl. Human HRG was eluted by 0.5 M imidazole in 10 mM TBS (pH 8.0). The protein extract was further purified by a Mono Q column (GE Healthcare, Little Chalfont, UK) with NaCl gradient. Purified human HRG was identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting with a human HRG-specific antibody.

Cecal ligation puncture model. All animal experiments were approved by the University's committee on animal experimentation and performed according to the guidelines of Okayama University on animal experiments. Male C57BL/6N mice (22-25 g, 7-8 wk) were obtained from Japan SLC (Shizuoka, Japan). Sepsis was induced in mice by cecal ligation and puncture (CLP). Animals were anesthetized and a ligature was placed below the ileocecal valve. The cecum was punctured once (mild sepsis) or twice (severe sepsis) with an 18-gauge needle and then returned to the peritoneal cavity. Animals were treated with vehicle (PBS) or with 4 or 20 mg/kg of human HRG or protein control (HSA; 20 mg/kg) immediately or 6 h and at 24 h and 48

h after CLP induction. Sham mice underwent the same surgical procedures without ligation and puncture. Sickness behaviors were evaluated at 24 h after CLP. Details of the assessments used and the scoring are as follows: 1= nomal (locomotion, appearance); 2= slight signs of illness (lethargy, ptosis or piloerection); 3= obvious illness (very little movement, ptosis and piloerection, curled body posture); 4= very sick animal (virtually no movement, impaired breathing, unresponsiveness, plus the above symptoms)[48]. Whole blood from mouse heart at 24 h after CLP was plated on LB agar dishes and incubated at 37°C; colony-forming units (CFUs) were counted after 24 h. The results were expressed as CFU/mL. The number of RBC, WBC, and platelet were counted in whole blood from mouse at 24 h after CLP. (Subcontract animal clinical examination: Oriental east, Tokyo, Japan). The blood were mixed with 3.2 % sodium citrate solution (blood: sodium citrate solution = 9:1) and separated the plasma from the blood. The plasma samples were used for coagulation and fibrinolysis tests (Subcontract animal clinical examination: Monoris, Tokyo, Japan). The cells present in the peritoneal cavity were collected by injecting 5 mL of saline. The cells were counted using a Burker-Turk counting chamber.

Lung wet-to-dry weight ratio. The whole lung from mouse at 24 h after CLP treated

with PBS, HSA or HRG was weighed, dried in an oven at 65 °C for 48 h, and weighed again. The lung wet/dry weight ratio was then calculated.

Western blot analysis of HRG levels in plasma. Mouse plasma was electrophoresed on polyacrylamide gel (12.5%) and transferred onto a polyvinylidene diflouride (PVDF) membrane (Bio-Rad, Hercules, CA). After the membrane was stained with SYPRO Ruby (Life Technologies), it was blocked with 10% skim milk for 1 h and incubated overnight at 4 °C with rabbit anti-hHRG polyclonal Ab followed by anti-rabbit IgG goat polyclonal IgG-HRP (MBL, Nagoya, Japan) for 2 h at room temperature. The signals were finally visualized using an enhanced chemiluminescence system (Pierce Biotechnology, Rockford, IL).

***In vivo* RNAi experiments.** Invivofectamine 2.0 reagent and HRG siRNA or negative control siRNA (Life Technologies, Carlsbad, CA) were mixed and incubated for 30 min at 50 °C. The mixtures were dialyzed by PBS and injected via tail veins of male C57BL/6N mice (7 weeks old). One week after injection, mouse blood was obtained by cutting the tail, and the plasma levels of HRG were determined using Western blotting. Then, a mild CLP sepsis (with one puncture) was induced in the

RNAi-treated mice.

Ezyme-linked immunosorbant assay (ELISA). In accordance with the ethics approval and guidelines of Okayama University, written informed consent was obtained from 10 healthy subjects and 8 septic patients. The septic patients were evaluated by clinical criteria of infections with SIRS in intensive-care unit (ICU). The plasma sample of septic patients was obtained when they were wheeled into the ICU. Human plasma HRG levels were assessed by ELISA using solid-phase-immobilized anti-HRG monoclonal antibodies (#75-14) and Ni-NTA labeled with HRP (Qiagen, Hilden, Germany)

High-speed scan spinning-disk confocal microscopy system. Images were acquired with an inverted microscope IX 73 (Olympus, Tokyo, Japan) using a x20/0.75 objective lens. The microscope was equipped with a CSU-X1 confocal scanner (Yokokawa, Tokyo, Japan) and an iXON3 EMCCD camera (Andor Technology, Belfast, Northern Ireland). Image acquisition software iQ2 (Andor Technology) was used to drive the confocal microscope.

***In vivo* neutrophil imaging.** Neutrophils were stained with FITC-labeled anti-Gr-1 antibody (20 μg/mouse) via i.v. injection before the *in vivo* imaging experiments. Sham-operated or CLP-treated mice were anesthetized with isoflurane, the abdomen was opened, and the exposed mesentery vessels were observed by a high-speed scan spinning-disk confocal microscopy system. Heating pads were used to keep the body temperature at 37 °C.

Micro Channel Array Flow Analyzer (MC-FAN). The blood or neutrophil samples were prepared as follows. Mice were sacrificed 24 h after CLP. Blood was obtained from the heart and mixed with heparin for the hematocrit test or with ACD solution for the microcapillary passage test. Human whole blood was withdrawn from the cubital vein and mixed with ACD solution. The blood was incubated with rat anti-human HRG monoclonal antibody (clone # 75- 14) or rat IgG for 30 min at 37°C. The human neutrophils isolated were treated with one of the reagents (BSA, HSA, HRG, fMLP) for 1 h at 37°C. The whole blood or neutrophil samples were forced to flow through artificial micro-channels made of silicon (width 7 μm, depth 4.5 μm, length 30 μm) under a constant suction of -20 cm H_2O (Micro Channel Array Flow Analyzer; MC-FAN, MC Lab, Tokyo, Japan). The passage time of the 100 μl samples through

microcapillaries was determined.

Immunohistochemistry. Immunohistochemical staining of nuclei, Gr-1, CD42d, and fibrinogen/fibrin was performed on 5 μm lung sections with DAPI, FITC-labeled rat anti-mouse Gr-1 Ab at 5μg/ml, anti-mouse CD42d Ab at 5μg/ml followed by Alexa Fluor 568-labeled anti-sheep IgG, and anti-mouse fibrinogen/fibrin Ab at 5μg/ml followed by Alexa Fluor 647-labeled anti-rabbit IgG, nuclei, Gr-1, HRG, and fibrinogen/fibrin was performed on 5 μm lung sections with DAPI, FITC-labeled rat anti-mouse Gr-1 Ab at 5μg/ml, anti-HRG Ab at 5μg/ml followed by Alexa Fluor 568-labeled anti-rabbit IgG, and anti-mouse fibrinogen/fibrin Ab at 5μg/ml followed by Alexa Fluor 647-labeled anti-sheep IgG, or nuclei (NETs) and Gr-1 was performed on 5 μm lung sections with SYTOX Blue and Alexa Fluor 594-labeled rat anti-mouse Gr-1 Ab at 5μg/ml after antigen retrieval; incubation of the lung section with 0.3% pepsin (Sigma) for 30 min at 37°C. Gr-1-positive neutrophils and NETs were counted in three fields of a lung section at x 200 magnification under a confocal laser scanning microscopy. The number of Gr-1, CD42d, and fibrin/fibrinogen triple positive sites was counted in three fields of a lung section at x 400 magnification under a confocal laser scanning microscopy. The results were expressed as the number of Gr-1-positive

neutrophils, Gr-1, CD42d, and fibrin/fibrinogen triple positive sites and NETs per square millimeter. The percentages of CD42d and fibrin/fibrinogen positive sites among Gr-1 positive neutrophils in each group were also determined.

Primer sequence for real-time PCR. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (NM_008084) F-5' TGA CGT GCC GCC TGG AGA AA 3', R-5' AGT GTA GCC CAA GAT GCC CTT CAG 3'; TNF-α (NM_013693) F-5' GAC CCT CAC ACT CAG ATC ATC CTT CT 3', R-5' GCG CTG GCT CAG CCA CTC 3'; iNOS (NM_010927) F-5' GAT TTT GCA TGA CAC TCT TCA 3', R-5' GGA GCC ATA ATA CTG GTT GAT 3'; IL-6 (NM_031168) F-5' GAC CTG TCT ATA CCA CTT CAC A 3', R-5' CTC TGG AAG TTT CAG ATT GTT 3'; PAI-1 (NM_008871) F-5' CTA TGG CGT GTC CTC GGT GCT 3', R-5' CAT TCT TGT TCC ACG GCC CCA T 3'; Neutrophil elastase (NM_015779) F-5' CTA CTG GCA TTG TTC CTG GGT G 3', R-5' GCT GAC ATG ACG AAG TTC CTG G 3'; RAGE (NM_007425) F-5'CTA GAG CCT GGG TGC TGG TTC 3'; R-5' GTT TCC ATT CTA GCT GCT GGG GC 3' ; HRG (NM_053176) F-5' TGC TCA CCA CAG CAT TGC TT 3'; R-5' CAC TCC TCC GCC CTT TAT TGA 3'

Real-time quantitative PCR. Total RNA was isolated from the mouse lung or liver using an RNeasy mini kit (Qiagen, Hilden). Complementary DNA was synthesized with a Takara RNA PCR kit Ver. 3.0 (Takara Bio, Nagahama, Japan) according to the manufacturer's instructions. Real-time PCR was performed with a Light Cycler (Roche, Basel, Switzerland) according to the manufacturer's instructions. Reaction mixtures contained cDNA template, SYBR premix Ex Taq (Takara Bio), and sequence-specific primers (Supplemental Methods). GAPDH expression was used to normalize cDNA levels. The PCR products were analyzed by a melting curve to ascertain the specificity of amplification.

Cytometric Bead Array (CBA). IL-6, TNF-α, and IL-10 concentration in the serum from mouse at 24 h after CLP treated with PBS, HSA and HRG were measured by CBA kits according to the manufacturer's instructions (BD Biosciences, San Diego, USA).

Neutrophil shape and adhesion assay. Purified human neutrophils pre-stained with Hoechst33342 (Nuclei) and Calcein-AM (Cytosol) were aliquoted in a volume of 100 μl ($5x10^4$ cells /well) to polystyrene wells or confluent wells of EA.hy926 cells (ATCC CRL-2922), a hybridoma of HUVEC and the human epithelial cell line A549. The

incubation started with one of the reagents (BSA, HSA, HRG, fMLP: each at a final concentration 1 μM) and continued for indicated periods at 37 °C. The cell shape and fluorescence intensity were analyzed by using an In Cell Analyzer 2000 (GE Healthcare, Waukesha, WI) and In Cell Analyzer Workstation software (GE Healthcare). Neutrophil adhesion was evaluated by measuring the fluorescence intensity of the wells by a Flexstation3 (Molecular Devices, Sunnyvale, CA) before and after the wells were gently washed twice to remove any nonadherent cells. When HRG's effects on intracellular calcium were examined, BAPTA or Fluo-4-AM was preloaded for 20 min.

PBMC shape and adhesion assay. Purified human PBMC (including monocytes, lymphocytes and platelets) pre-stained with Hoechst33342 (Nuclei) and Calcein-AM (Cytosol) were aliquoted in a volume of 100 μl ($5x10^4$ cells /well) to confluent wells of EA.hy926 cells. The incubation started with one of the reagents (BSA, HSA, HRG, fMLP: each at a final concentration 1 μM) and continued for indicated periods at 37 °C. The cell shape and fluorescence intensity were analyzed by using an In Cell Analyzer 2000 (GE Healthcare, Waukesha, WI) and In Cell Analyzer Workstation software (GE Healthcare). PBMC adhesions were evaluated by counting Calsein-positive cell number before and after the wells were gently washed twice to remove any

non-adherent cells.

F-actin distribution and cell surface structure in neutrophils. Neutrophils (5 x 10^6 cells/ml) were seeded onto poly L-lysine-coated cover glass (Matsunami, Tokyo, Japan) and incubated with one of the reagents (BSA, HSA, HRG, or fMLP: each at a final concentration of 1 μM) at 37 °C for 1 h. For actin staining, the neutrophils were fixed with 4% PFA and treated with 0.1% TritonX-100 followed by staining with Phalloidin-Alexa568 (F-actin), DNaseI-Alexa488 (G-actin), and DAPI (Nuclei). The samples were observed using a confocal microscope (LSM 510, Carl Zeiss, Oberkochen, Germany). For electron microscopy, the samples were fixed in 4% paraformaldehyde and 2.5% glutaraldehyde for 24 h at 4 °C, then post-fixed using 1% osmium tetroxide for 1 h at 4 °C. OsO_4 coating with an osmium coater HPC-1S (Vacuum Device, Mito, Japan) was used. Each sample was examined using an s-4800 scanning laser microscope (Hitachi, Tokyo, Japan).

Determination of HRG uptake into neutrophils. HRG and HSA were labeled by a fluorescein using a fluorescein-labeling kit-NH_2 (Dojindo, Kumamoto, Japan). The neutrophils or EA.hy926 cells were aliquoted to a 96-well plate and incubated with

fluorescein-labeled HRG or HSA (each at a final concentration of 1 μM) at 37 °C for 1 h. The samples were assayed using In Cell Analyzer 2000, and the data on granule count were analyzed by In Cell Analyzer Workstation software.

Determination of ROS production. The neutrophil suspension with isoluminol (final concentration, 50 mM) and horse radish peroxidase type IV (final concentration 4U/ml) were aliquoted to a 96-well plate (BD, Franklin Lakes, NJ) in a volume of 100 μl (5 x 10^4 cells) with one of the reagents (BSA, HSA, HRG, each at a final concentration of 1 μM). Neutrophil extracellular ROS production was evaluated at 15 min after the start of incubation at 37 °C by the measurement of chemiluminescence intensity using Flexstation3. Intracellular ROS was determined by pre-loading of CM-H_2DCFDA into neutrophils for 20 min.

Chemotaxis assay. The transfer of neutrophils to the vertical direction was examined using a 24-well Chemotaxicell chamber (Kurabo, Kurashiki, Japan). Neutrophils (5 x 10^5 cells in 100 μl) were added to the upper wells of the chamber and were separated by a polycarbonate membrane 5 μm in diameter pores from lower wells containing one of the reagents (BSA, HSA, HRG, or fMLP). Following 1 h incubation, the cells that had

migrated to lower wells were counted under a light microscope. For the horizontal chemotaxis assay, neutrophils (5 x 10^5 cells/ 10 μl) were added to one well in agarose gel (5 mm thick) and were allowed to migrate toward the other well (2 mm from the former) containing a possible chemoattractant (1 μM). Two hours after the start of migration, the neutrophils were fixed and stained with Wright's stain, and the distance from the origin to the leading edge was measured.

Detection of ICAM-1, P-selectin and phosphatidylserine on EA. hy926 cell.

EA.hy926 cell monolayer was stimulated by the mixture of HBSS, BSA, HSA or HRG (1μM each) and LPS (10ng/ml) or TNF-α (10ng/ml) for 30min at 37 °C in 5% CO_2 atmosphere. The cells were stained by Alexa Fluor 488-labeled anti-human CD54 Ab or DyLight 488-labeled anti-human CD62P Ab for 2hrs at RT, or anti-phosphatidylserine Ab followed by Alexa Fluor 488-labeled anti-rabbit IgG for 1hr at RT. The number of phosphatidylserine positive-cells was counted in six fields at x 100 magnification under a confocal laser scanning microscopy. The number of CD54 or CD62P positive-cells was counted in four fields at x 200 magnification under a confocal laser scanning microscopy.

FACS analysis. C5a, IL-8 or fMLP were preincubated with HSA or HRG for 60 min at 37 °C. Purified human neutrophils were stimulated with the mixture for 60 min at 37 °C in 5% CO_2 atmosphere. The stimulation was stopped by ice-cold cooling for 10 min. The neutrophils were stained by FITC-labeled anti-human CD11b Ab, FITC-labeled anti-human activated CD11b Ab, FITC-labeled anti-human CD62L Ab or PE-labeled anti-human CD162 Ab for 25 min at 4 °C. The fluorescence intensity of the cells was detected by FACS calibur (Becton Dickinson, Franklin Lakes, NJ). These data were analyzed by Flow Jo software (TOMY Digital Biology, Tokyo, Japan).

Statistical analysis. The statistical analysis across multiple treatment groups was determined with ANOVA, followed by Dunnett test. The statistical difference between paired groups were determined using Mann-Whitney test. All data are presented as the means ± SEM. P values < 0.05 were considered statistically significant. The Kaplan-Meier method was used for the survival experiments, and the differences were analyzed using the log-rank test.

References

1. Aziz, M., Jacob, A., Yang, W.L., Matsuda, A. & Wang, P. Current trends in inflammatory and immunomodulatory mediators in sepsis. *J. Leuc. Biol.* **93**, 329-342 (2013).

2. Dellinger, R.P. *et al.* Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. *Crit. Care Med.* **41**, 580-637 (2013).

3. Hotchkiss, R.S., Monneret, G. & Payen, D. Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach. *Lancet Infect. Dis.* **13**, 260-268 (2013).

4. Semeraro, N., Ammollo, C.T., Semeraro, F. & Colucci, M. Sepsis, thrombosis and organ dysfunction. *Thromb. Res.* **129**, 290-295 (2012).

5. Piccinini, A.M. & Midwood, K.S. DAMPening inflammation by modulating TLR signalling. *Mediators Inflamm.* **2010**, 1-21 (2010).

6. Ward, P.A. New approaches to the study of sepsis. *EMBO Mol. Med.* **4**, 1234-1243 (2012).

7. Salomão, R. *et al.* TLR signaling pathway in patients with sepsis. *Shock* **30**, 73-77 (2008).

8. Williams, M.R., Azcutia, V., Newton, G., Alcaide, P. & Luscinskas, F.W. Emerging mechanisms of neutrophil recruitment across endothelium. *Trends Immunol.* **32**, 461-469 (2011).

9. Grommes, J. & Soehnlein, O. Contribution of neutrophils to acute lung injury. *Mol. Med.* **17**, 293-307 (2011).

10. Matthay, M.A. & Zemans, R.L. The acute respiratory distress syndrome: pathogenesis and treatment. *Annu. Rev. Pathol.* **6**, 147-163 (2011).

11. Moreland, J.G., Bailey, G., Nauseef, W.M. & Weiss, J.P. Organism-specific neutrophil-endothelial cell interactions in response to Escherichia coli, Streptococcus pneumoniae, and Staphylococcus aureus. *J. Immunol.* **172**, 426-432 (2004).

12. Engelmann, B. & Massberg, S. Thrombosis as an intravascular effector of innate immunity. *Nat. Rev. Immunol.* **13**, 34-45 (2013).

13. Brinkmann, V. *et al.* Neutrophil extracellular traps kill bacteria. *Science* **303**, 1532-1535 (2004).

14. Yipp, B.G. & Kubes, P. NETosis: how vital is it? *Blood* **122**, 2784-2794 (2013).

15. Alves-Filho, J.C., de Freitas, A., Spiller, F., Souto, F.O. & Cunha, F.Q. The role of neutrophils in severe sepsis. *Shock* **1**, 3-9 (2008).

16. Koide, T., Foster, D., Yoshitake, S. & Davie, E.W. Amino acid sequence of human histidine-rich glycoprotein derived from the nucleotide sequence of its cDNA. *Biochemistry* **25**, 2220-2225 (1986).

17. Poon, I.K., Patel, K.K., Davis, D.S., Parish, C.R. & Hulett, M.D. Histidine-rich glycoprotein: the Swiss Army knife of mammalian plasma. *Blood* **117**, 2093-2101 (2011).

18. Borza, D.B., Tatum, F.M. & Morgan, W.T. Domain structure and conformation of histidine-proline-rich glycoprotein. *Biochemistry* **35**, 1925-1934 (1996).

19. Lijnen, H.R., Hoylaerts, M. & Collen, D. Heparin binding properties of human histidine-rich glycoprotein. Mechanism and role in the neutralization of heparin in plasma. *J. Biol. Chem.* **258**, 3803-3808 (1983).

20. Leung, L.L. Interaction of histidine-rich glycoprotein with fibrinogen and fibrin. *J. Clin. Invest.* **77**, 1305-1311 (1986).

21. Peterson, C.B., Morgan, W.T. & Blackburn, M.N. Histidine-rich glycoprotein modulation of the anticoagulant activity of heparin. Evidence for a mechanism involving competition with both antithrombin and thrombin for heparin binding. *J. Biol. Chem.* **262**, 7567-7574 (1987).

22. Silverstein, R.L., Leung, L.L., Harpel, P.C. & Nachman, R.L. Platelet

thrombospondin forms a trimolecular complex with plasminogen and histidine-rich glycoprotein. *J. Clin. Invest.* **75**, 2065-2073 (1985).

23. Gorgani, N.N., Parish, C.R., Easterbrook, Smith, S.B. & Altin, J.G. Histidine-rich glycoprotein binds to human IgG and C1q and inhibits the formation of insoluble immune complexes. *Biochemistry* **36**, 6653-6662 (1997).

24. Morgan, W.T. The histidine-rich glycoprotein of serum has a domain rich in histidine, proline, and glycine that binds heme and metals. *Biochemistry* **24**, 1496-1501 (1985).

25. Poon, I.K., Hulett, M.D. & Parish, C.R. Histidine-rich glycoprotein is a novel plasma pattern recognition molecule that recruits IgG to facilitate necrotic cell clearance via FcgammaRI on phagocytes. *Blood* **115**, 2473-2482 (2010).

26. Doñate, F. *et al.* Peptides derived from the histidine-proline domain of the histidine-proline-rich glycoprotein bind to tropomyosin and have antiangiogenic and antitumor activities. *Cancer Res.* **64**, 5812-5817 (2004).

27. Juarez, J.C. *et al.* Histidine-proline-rich glycoprotein has potent antiangiogenic activity mediated through the histidine-proline-rich domain. *Cancer Res.* **62**, 5344-5350 (2002).

28. Wake, H., Mori, S., Liu, K., Takahashi, H.K. & Nishibori, M. Histidine-rich

glycoprotein inhibited high mobility group box 1 in complex with heparin-induced angiogenesis in matrigel plug assay. *Eur. J. Pharmacol.* **623**, 89-95 (2009).

29. Rydengård, V., Olsson, A.K., Mörgelin, M. & Schmidtchen, A. Histidine-rich glycoprotein exerts antibacterial activity. *FEBS J.* **274**, 377-389 (2007).
30. Kacprzyk, L. *et al.* Antimicrobial activity of histidine-rich peptides is dependent on acidic conditions. *Biochim. Biophys. Acta.* **1768**, 2667-80 (2007).
31. Rydengård, V. *et al.* Histidine-rich glycoprotein protects from systemic Candida infection. *PLoS Pathog.* **4**, e1000116 (2008).
32. Bosshart, H. & Heinzelmann, M. Endotoxin-neutralizing effects of histidine-rich peptides. *FEBS Lett.* **553**, 135-140 (2003).
33. Shannon, O. *et al.* Histidine-rich glycoprotein promotes bacterial entrapment in clots and decreases mortality in a mouse model of sepsis. *Blood* **116**, 2365-2372 (2010).
34. Saigo, K., Yoshida, A., Ryo, R., Yamaguchi, N. & Leung, L.L. Histidine-rich glycoprotein as a negative acute phase reactant. *Am. J. Hematol.* **34**, 149-150 (1990).
35. Amulic, B., Cazalet, C., Hayes, G.L., Metzler, K.D. & Zychlinsky, A. Neutrophil function: from mechanisms to disease. *Annu. Rev. Immunol.* **30**, 459-489 (2012).

36. DiStasi, M.R. & Ley, K. Opening the flood-gates: how neutrophil-endothelial interactions regulate permeability. *Trends. Immunol.* **30**, 547-556 (2009).

37. Phillipson, M. & Kubes, P. The neutrophil in vascular inflammation. *Nat. Med.* **17**, 1381-1390 (2011).

38. Cain, R.J. & Ridley, A.J. Phosphoinositide 3-kinases in cell migration. *Biol. Cell.* **101**, 13-29 (2009).

39. Gambardella, L. & Vermeren, S. Molecular players in neutrophil chemotaxis - focus on PI3K and small GTPases. *J. Leukoc. Biol.* **94**, 603-612 (2013).

40. Lehmann, K. *et al.* PI3Kγ controls oxidative burst in neutrophils via interaction with PKCα and p47phox. *Biochem. J.* **419**, 603-610 (2009).

41. Yamamori, T., Inanami, O., Nagahata, H. & Kuwabara, M. Phosphoinositide 3-kinase regulates the phosphorylation of NADPH oxidase component p47(phox) by controlling cPKC/PKCdelta but not Akt. *Biochem. Biophys. Res. Commun.* **316**, 720-730 (2004).

42. Opal, S.M. & van der Poll, T. Endothelial barrier dysfunction in septic shock. *J. Intern. Med.* **277**, 277-293 (2015).

43. Opal, S.M. *et al.* Effect of Eritoran, an antagonist of MD2-TLR4, on mortality in patients with severe sepsis. *JAMA* **309**, 1154-1162 (2013).

44. Ranieri, V. M. *et al.* Drotrecogin alfa (activated) in adults with septic shock. *N. Engl. J. Med.* **366**, 2055-2064 (2012).

45. Rice, T.W. *et al.* A randomized, double-blind, placebo-controlled trial of TAK-242 for the treatment of severe sepsis. *Crit. Care Med.* **38**, 1685-1694 (2010).

46. Russell, J.A. Management of sepsis. *N. Engl. J. Med.* **355**, 1699-1713 (2006).

47. Mori, S., Takahashi, H.K., Yamaoka, K., Okamoto, M. & Nishibori, M. High affinity binding of serum histidine-rich glycoprotein to nickel-nitrilotriacetic acid: the application to microquantification. *Life Sci.* **73**, 93-102 (2003).

48. Sudom, K., Turrin, N.P., Hayley, S. & Anisman, H. Influence of chronic interleukin-2 infusion and stressors on sickness behaviors and neurochemical change in mice. *Neuroimmunomodulation.* **11**, 341-350 (2004).