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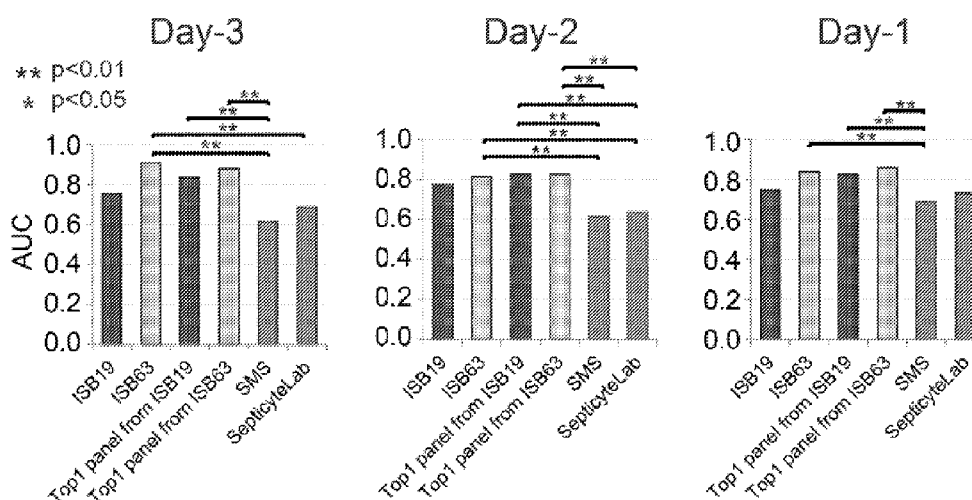
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(54) Title: SEPSIS BIOMARKER PANELS AND METHODS OF USE

FIG. 22A



(57) Abstract: Methods for diagnosing and/or predicting presence of sepsis in a subject using a gene signature of three or more genes are provided. Also provided are sets containing specific binding molecules for each of the three or more genes, and kits containing such sets.



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SEPSIS BIOMARKER PANELS AND METHODS OF USE

CROSS REFERENCE TO RELATED APPLICATIONS

5 This application claims the benefit of U.S. Provisional Application No. 62/755,834, filed November 5, 2018, and U.S. Provisional Application No. 62/911,603, filed October 7, 2019, both of which are incorporated by reference herein in their entirety.

FIELD

10 This disclosure relates to biomarkers for prediction and/or diagnosis of sepsis and methods of their use, including methods for treating sepsis in a subject.

ACKNOWLEDGMENT OF GOVERNMENT SUPPORT

15 This invention was made with government support under Contract number HDTRA1-13-C-0055 awarded by Defense Threat Reduction Agency. The government has certain rights in the invention.

BACKGROUND

 Sepsis occurs when the body's response to infection damages its own tissues and organs, leading to shock, multi-organ failure, and potentially death. In the United States, the incidence of severe sepsis is about 300 cases per 100,000 population with a mortality rate estimated between 28 and 50 percent (Ullah *et al.*, *Pakistan J. Med. Sci.* 32:688-693, 2016). Diagnosis of sepsis is difficult during early stages (*e.g.*, pre-symptomatic phase), which makes it challenging to intervene therapeutically until after the onset of symptoms. Since progression of the condition, once it takes hold, is rapid and often aggressive, effective and early intervention is critical to control the development of sepsis. Among clinical parameters, C-reactive protein (CRP) and procalcitonin (PCT) are established markers to reflect infection or inflammation (Faix, *Crit. Rev. Clin. Lab. Sci.* 50:23-36, 2013). However, due to the lack of specificity, the use of these markers for sepsis diagnosis is limited. For example, both CRP and PCT levels are elevated in sepsis patients as well as in stress, severe trauma, and surgery patients (Matson *et al.*, *Anaesth. Intensive Care* 19:182-186, 1991; Yentis *et al.*, *Intensive Care Med.* 21:602-605, 1995; Aabenhus and Jensen, *Prim. Care Respir. J.* 20:360-367, 2011; Schuetz *et al.*, *BMC Med.* 9:107, 2011).

 The current gold standard for sepsis diagnosis is positive identification of pathogenic bacteria through culture. Unfortunately, such culture-based methods typically take at least 2-5 days to complete, depending on the causative pathogen and often provide negative or inconclusive results (Kim and Weinstein, *Clin. Microbiol. Infect.* 19:513-520, 2013; Ruiz-Giardin *et al.*, *Int. J. Infect. Dis.* 41:6-10, 2015). Patients are often treated with antibiotics prior to definitive identification of the infective agent, which can result in inappropriate or inadequate treatment. The delay of proper intervention likely contributes to the high sepsis mortality rate.

SUMMARY

There is a need to accurately identify patients who have sepsis, are at risk of developing sepsis, or who are in the early or pre-symptomatic phase of sepsis, so that appropriate treatment can be initiated as early as possible.

In some embodiments, disclosed herein are methods of detecting a plurality of markers, such as detecting or measuring expression of a set of at least six genes in a sample from a subject having sepsis, suspected to have sepsis, or at risk of sepsis. The methods include detecting or measuring expression of a set of at least six genes, wherein the set includes CCR1, HLA-DPB1, BATF, C3AR1, ARHGEF18, and C9orf95; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf103; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CCR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; CD177, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, C3AR1, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and SEPHS2; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf103; FES, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FES, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; or C3AR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95. In some examples, the expression of CCR1, C3AR1, C9orf95, BATF, C9orf103, GNA15, CD177, CD63, EMR1, FCER1G, FES, PLAC8, and/or SEPHS2 is increased compared to a control, and/or expression of HLA-DPB1, ARHGEF18, and/or MTCH1 is decreased compared to a control.

Also provided are methods of diagnosing sepsis in a subject including detecting or measuring expression of the set of at least six genes in a sample from a subject, wherein the subject is diagnosed with sepsis when expression of CCR1, C3AR1, C9orf95, BATF, C9orf103, GNA15, CD177, CD63, EMR1, FCER1G, FES, PLAC8, and/or SEPHS2 is increased compared to a control, and/or expression of HLA-DPB1, ARHGEF18, and/or MTCH1 is decreased compared to a control. Further provided are methods of treating a subject with sepsis including detecting or measuring expression of the set of at least six genes in a sample from a subject, wherein expression of CCR1, C3AR1, C9orf95, BATF, C9orf103, GNA15, CD177, CD63, EMR1, FCER1G, FES, PLAC8, and/or SEPHS2 is increased compared to a control, and/or expression of HLA-DPB1, ARHGEF18, and/or MTCH1 is decreased compared to a control and administering one or more antibiotics to the subject.

In other embodiments, disclosed herein are methods of detecting a plurality of markers, such as detecting or measuring expression of a set of at least three genes in a sample from a subject having sepsis

or suspected to have sepsis. In some examples, the methods include detecting or measuring expression of a set of at least three (such as at least 3, 4, 5, or more) genes, wherein the set includes a set of 3, 4, or 5 genes provided in Table 16, for example, LCN2, SLC2A3, and BMX; LCN2, SLC2A3, and GRB10; LCN2, PFKFB3, and GRB10; LCN2, PFKFB3, and BMX; IL1R2, HK3, and BMX; LCN2, HK3, and BMX; LCN2, HK3, and GRB10; GZMA, HK3, and BMX; FCAR, PFKFB2, and BMX; LCN2, PFKFB3, and IL18R1; LCN2, PFKFB3, GRB10, and ST6GALNAC3; LCN2, SLC2A3, BMX, and LGALS2; IL1R2, SLC2A3, BMX, and TCN1; LCN2, SLC2A3, GRB10, and ST6GALNAC3; FCAR, PFKFB2, BMX, and CEACAM1; IL1R2, HK3, BMX, and CD24; IL1R2, PFKFB3, BMX, and CD24; BMX, SLC2A3, GRB10, and CD24; IL1R2, HK3, BMX, and CEACAM1; GZMA, SLC2A3, BMX, and CD24; LCN2, PFKFB3, GRB10, ST6GALNAC3, and RNASE3; LCN2, PFKFB3, GRB10, RNASE2, and ST6GALNAC3; IL1R2, PFKFB3, GRB10, CD24, and ST6GALNAC3; GZMA, PFKFB3, GRB10, ST6GALNAC3, and CD24; HK3, PFKFB3, GRB10, ST6GALNAC3, and CD24; IL1R2, PFKFB3, GRB10, ST6GALNAC3, and TCN1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, and TCN1; LCN2, PFKFB3, GRB10, ST6GALNAC3, and DACH1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, and CD24; or SLC2A3, HK3, BMX, SPOCD1, and LGALS2, wherein expression of the three or more genes is altered compared to a control.

In other examples, the methods include detecting or measuring expression of a set of at least six genes (such as 6, 7, 8, or more), wherein the set includes a set of 6, 7, or 8 genes provided in Table 17, for example, RPS6KA3, BCL6, TPM3, GNG10, STOM, and MPO; RPS6KA3, MAVS, TPM3, BCL6, STOM, and MPO; RPS6KA3, BCL6, TPM3, BMX, STOM, and MPO; RPS6KA3, LTF, TPM3, BCL6, STOM, and PDGFC; RPS6KA3, BCL6, TPM3, CD55, STOM, and MPO; RPS6KA3, BCL6, TPM3, GNG10, STOM, and PDGFC; RPS6KA3, LTF, TPM3, BCL6, STOM, and CYP11B1; LTF, BCL6, TPM3, CD55, STOM, and PDGFC; RPS6KA3, BCL6, TPM3, TLR8, STOM, and MPO; RPS6KA3, BCL6, TPM3, HLA-DRA, GNG10, and MPO; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, CYP27A1, and YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, and YOD1; RPS6KA3, GAS7, TPM3, BCL6, RRBPI, MPO, and YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, and LGALS2; RPS6KA3, GAS7, TPM3, BCL6, GNG10, MPO, and YOD1; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, and YOD1; RPS6KA3, BCL6, TPM3, GNG10, LILRA2, CYP27A1, and YOD1; RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, and LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, and YOD1; RPS6KA3, BCL6, TPM3, CD55, STOM, MPO, and IL17RA; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and LILRA4; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and IL17RA; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and TCN1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and RNASE3; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and NOV; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and RNASE2; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and FAM105A; RPS6KA3, BCL6, TPM3, HLA-DRA, RRBPI, MPO, ERO1L, and YOD1; or LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1,

and C14orf101, wherein expression of the six or more genes is altered compared to a control. An additional set of six or more genes that can be used in the disclosed methods includes LCN2, BMX, MPO, RPS6KA3, BCL6, and MAPK14, wherein expression of the six or more genes is altered compared to a control.

5 Also provided are methods of diagnosing sepsis in a subject, including detecting or measuring expression of the set of at least three or at least six genes (such as the sets of genes in Tables 15 and 16) or the set of at least six genes including LCN2, BMX, MPO, RPS6KA3, BCL6, and MAPK14 in a sample from a subject, wherein the subject is diagnosed with sepsis when expression of the set of at least three genes or at least six genes is altered compared to a control. Further provided are methods of
10 treating a subject with sepsis including detecting or measuring expression of the set of at least three genes or at least six genes (such as the sets of genes in Tables 15 and 16) or the set of at least six genes including LCN2, BMX, MPO, RPS6KA3, BCL6, and MAPK14 in a sample from a subject, wherein expression is altered compared to a control, and administering one or more antibiotics to the subject. In some examples, the subject does not exhibit symptoms of sepsis (for example, is pre-symptomatic or pre-
15 clinical for sepsis). Thus, in some examples, the methods including predicting risk of developing sepsis in a subject.

Also disclosed herein are solid supports including a solid support (such as an array or bead) including binding agents specific for a set of markers disclosed herein. In some embodiments, the solid support includes at least one probe, primer, and/or antibody specific for a set including each of CCR1,
20 HLA-DPB1, BATF, C3AR1, ARHGEF18, and C9orf95; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf103; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CCR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; CD177, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, C3AR1, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and SEPHS2;
30 FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf103; FES, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FES, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; or C3AR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95. In other embodiments, the solid support includes at least one probe, primer, and/or antibody specific for each gene in a set including
35 3, 4, or 5 genes provided in Table 16, for example, LCN2, SLC2A3, and BMX; LCN2, SLC2A3, and GRB10; LCN2, PFKFB3, and GRB10; LCN2, PFKFB3, and BMX; IL1R2, HK3, and BMX; LCN2, HK3, and BMX; LCN2, HK3, and GRB10; GZMA, HK3, and BMX; FCAR, PFKFB2, and BMX;

LCN2, PFKFB3, and IL18R1; LCN2, PFKFB3, GRB10, and ST6GALNAC3; LCN2, SLC2A3, BMX, and LGALS2; IL1R2, SLC2A3, BMX, and TCN1; LCN2, SLC2A3, GRB10, and ST6GALNAC3; FCAR, PFKFB2, BMX, and CEACAM1; IL1R2, HK3, BMX, and CD24; IL1R2, PFKFB3, BMX, and CD24; BMX, SLC2A3, GRB10, and CD24; IL1R2, HK3, BMX, and CEACAM1; GZMA, SLC2A3, BMX, and CD24; LCN2, PFKFB3, GRB10, ST6GALNAC3, and RNASE3; LCN2, PFKFB3, GRB10, RNASE2, and ST6GALNAC3; IL1R2, PFKFB3, GRB10, CD24, and ST6GALNAC3; GZMA, PFKFB3, GRB10, ST6GALNAC3, and CD24; HK3, PFKFB3, GRB10, ST6GALNAC3, and CD24; IL1R2, PFKFB3, GRB10, ST6GALNAC3, and TCN1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, and TCN1; LCN2, PFKFB3, GRB10, ST6GALNAC3, and DACH1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, and CD24; or SLC2A3, HK3, BMX, SPOCD1, and LGALS2. In other embodiments, the solid support includes at least one probe, primer, and/or antibody specific for a set including each gene in a set including 6, 7, or 8 genes provided in Table 17, for example, RPS6KA3, BCL6, TPM3, GNG10, STOM, and MPO; RPS6KA3, MAVS, TPM3, BCL6, STOM, and MPO; RPS6KA3, BCL6, TPM3, BMX, STOM, and MPO; RPS6KA3, LTF, TPM3, BCL6, STOM, and PDGFC; RPS6KA3, BCL6, TPM3, CD55, STOM, and MPO; RPS6KA3, BCL6, TPM3, GNG10, STOM, and PDGFC; RPS6KA3, LTF, TPM3, BCL6, STOM, and CYP1B1; LTF, BCL6, TPM3, CD55, STOM, and PDGFC; RPS6KA3, BCL6, TPM3, TLR8, STOM, and MPO; RPS6KA3, BCL6, TPM3, HLA-DRA, GNG10, and MPO; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, CYP27A1, and YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, and YOD1; RPS6KA3, GAS7, TPM3, BCL6, RRBP1, MPO, and YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, and LGALS2; RPS6KA3, GAS7, TPM3, BCL6, GNG10, MPO, and YOD1; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, and YOD1; RPS6KA3, BCL6, TPM3, GNG10, LILRA2, CYP27A1, and YOD1; RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, and LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, and YOD1; RPS6KA3, BCL6, TPM3, CD55, STOM, MPO, and IL17RA; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and LILRA4; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and IL17RA; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and TCN1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and RNASE3; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and NOV; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and RNASE2; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and FAM105A; RPS6KA3, BCL6, TPM3, HLA-DRA, RRBP1, MPO, ERO1L, and YOD1; or LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and C14orf101. In other embodiments, the solid support includes at least one probe, primer, and/or antibody specific for a set of genes including each LCN2, BMX, MPO, RPS6KA3, BCL6, and MAPK14

Kits that include a disclosed set of specific binding agents (such as a set of primers or probes) are also provided. Such a kit can further include other reagents, such as a buffer, such as a hybridization buffer. Such sets of specific binding agents and kits can be used to perform steps of the disclosed methods.

The foregoing and other features of the disclosure will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

5 FIGS. 1A and 1B are schematic diagrams showing identification of substitutes for the Stanford11 panel. FIG. 1A is a schematic of the overall procedure for identification of substitutions for the Stanford11 panel. FIG. 1B is a schematic diagram showing the six key biological processes represented by the Stanford11 panel.

10 FIGS. 2A-2C are diagrams showing the role of biological processes in classification performance. FIG. 2A is a plot showing the distribution of classification performance of 100,000 random gene sets sorted based on performance. FIG. 2B is a plot showing the number of genes of the top and bottom 250 gene sets in 97 gene ontology biological processes (GOBPs) represented by the Stanford11 panel. FIG. 2C provides clusters of GOBPs in the top 250 and bottom 250 gene sets. Count and percent indicate the average number and percentage of genes in each GOBP cluster.

15 FIGS. 3A-3F are a series of plots showing the performance of panels with one gene substitution. The plots show the distribution of area under the curve (AUC) in the 12 microarray datasets when BATF (FIG. 3A), C3AR1 (FIG. 3B), C9orf95 (FIG. 3C), CEACAM1 (FIG. 3D), GNA15 (FIG. 3E), or MTCH1 (FIG. 3F) was replaced with a substitute gene. * indicates P-value less than 0.05 from DeLong test comparing a substituted panel (box plots) with the median AUC and the original Stanford11 (dots).

20 FIG. 4 is a plot showing the AUCs in the 12 microarray datasets when genes representing all five functional categories were replaced with substitute genes. * and ** indicate P-value less than 0.05 and 0.01, respectively, from DeLong test comparing a substituted panel with the median AUC and the original Stanford11 (filled dots).

25 FIGS. 5A-5C are a series of plots showing AUCs in the 12 microarray datasets when only one gene in PLC, phosphorylation, platelet activation function (FIG. 5A), chemotaxis, angiogenesis, adhesion, migration function (FIG. 5B), or in both processes (FIG. 5C) was retained. * and ** indicate P-value less than 0.05 and 0.01, respectively, from DeLong test comparing a substituted panel with the median AUC and the original Stanford11 (filled dots).

30 FIG. 6 is a plot showing performance of 1,482 six-gene combinations. * and ** indicate P-value less than 0.05 and 0.01, respectively, from DeLong test comparing a substituted panel with the median AUC and the original Stanford11 (filled dots).

35 FIGS. 7A-7F are a series of panels showing the impact of biological function information. Biological function information was evaluated by three different approaches. FIG. 7A shows the 11 highest correlated genes with Stanford11 and the Pearson's correlation coefficient (panel-HC). O in Stanford82 column indicates the gene of Stanford82. FIG. 7B is a plot showing the AUCs of panel-HC (front bars). Back bars show the AUCs of Stanford11. FIG. 7C shows the 14 genes involved in adhesion/migration processes (panel-AM). FIG. 7D is a plot showing the AUCs of panel-AM (front

bars). Back bars show the AUCs of Stanford11. FIG. 7E shows the top-scoring pairs identified by *k*-TSP algorithm (panel-kTSP). FIG. 7F is a plot showing the AUCs of panel-kTSP. * and ** indicate P-value less than 0.05 and 0.01, respectively, from DeLong test.

FIG. 8 is a heat map showing overall expression profile of genes in ISB58 panel. Each column represents samples from patients and each row represent different gene in the set. The sepsis (left) and control (right) patients are indicated on top with the time prior to sepsis diagnosis (Day-3 to Day-1). The red color represents higher expression level than mean while the green represents lower expression level.

FIG. 9 is a heat map showing overall expression profile of genes in ISB355 panel. Each column represents samples from patients and each row represent different gene in the set. The sepsis (left) and control (right) patients are indicated on top with the time prior to sepsis diagnosis (Day-3 to Day-1). The red color represents higher expression level than mean while the green represents lower expression level.

FIG. 10 is a Venn diagram showing overlap of genes between ISB58 and ISB355.

FIGS. 11A and 11B are heat maps showing overall expression profile of genes in predictive panels ISB19 (FIG. 11A) (derived from ISB58 gene set) and ISB63 (FIG. 11B) (derived from ISB355 gene set).

FIG. 12 is a graph summarizing classification performance (Area Under the Curve – AUC) for ISB19 and ISB63 panels. The left bar in each bar represents the performance for the ISB19 gene panel and the right bar in each pair are the results of ISB63. The dataset ID and pathology group are indicated below the X-axis.

FIG. 13 is a graph showing performance comparison between ISB panels with known sepsis diagnosis panels – Stanford11 and Septicyte4. The Y-axis represents the AUC and the X-axis represents the date in relation to sepsis diagnosis (Day 0).

FIG. 14 is a graph showing average AUCs of ISB19 and ISB19-derived 3-, 4-, and 5-gene panels. The Y axis represents the average performance (AUC) of top 3-gene, 4-gene and 5-gene panels, and the X axis indicates the time before sepsis was diagnosed.

FIG. 15 is a graph showing average AUCs of ISB63 and ISB63-derived 6-, 7-, and 8-gene panels. The Y axis represents the average performance (AUC) of top 6-gene, 7-gene and 8-gene panels, and the X axis indicates the time before sepsis was diagnosed.

FIG. 16 is a graph showing average diagnostic performance of sepsis predictive panels in 19 public domain datasets. The AUC is indicated on the bars and the biomarker panels are listed on the X-axis.

FIG. 17 is a graph showing averaged diagnostic performance of 30 smaller panels (top performing 3 genes, 4 genes and 5 genes) derived from ISB19 and (top performing 6 genes, 7 genes and 8 genes) ISB63 (dark color); and with integrated clinical information (bright color) between mild and severe sepsis patients.

FIG. 18 is a summary of analysis approaches, DEGs identified, and performance of the classification panels.

FIGS. 19A-19I show selection of core biological processes that have higher discriminatory power. The procedure for selecting core biological processes is shown in FIG. 19A. Distribution of classification performances of 100,000 random 19 (FIG. 19B) or 63 gene sets (FIG. 19C). Selection of core biological functions associated with ISB19 (FIG. 19D) or ISB63 panels (FIG. 19E). Color scale indicates the number of genes involved in each GOBP among the top 500 and bottom 500 panels (FIGS. 19F-19G). Three and six representative functional terms determined by EnrichmentMap (FIGS. 19H-19I).

FIGS. 20A-20G show study design and patient information. Number of sepsis patients and matched controls (Y-axis) from each recruitment site (X-axis; Heartlands: Heartlands Hospital, LTH: The Leeds Teaching Hospitals Trust, NBNT: North Bristol NHS Trust, Frankfurt: University Hospital Frankfurt, RLU: The Royal Liverpool University Hospital, UCH: University College Hospital, QEH: Queen Elizabeth Hospital, GST: Guys' and St Thomas' Hospital) (FIG. 20A). Number of patients diagnosed with sepsis each day after surgery (FIG. 20B). Organization of samples based on the date sepsis was diagnosed (FIG. 20C). The timeline based on surgery date is indicated on top blue line and the number indicates day after surgery. The sepsis patients and matched controls are indicated in yellow and gray, respectively. The date sepsis was diagnosed is indicated in red as Day0. The distribution of SOFA score (FIG. 20D), CRP concentration (FIG. 20E), and lactate level (FIG. 20F) (Y-axis) across different time points among different sample groups (top) before sepsis was diagnosed (X-axis). The yellow lines represent the mean values for sepsis patients and the black lines represent the mean values for matched controls. The gray areas are the standard distribution. The yellow (Sepsis) and black (Control) dots are levels for individual samples. Overview of the study design to discover, optimize, and validate pre-symptomatic biomarker for sepsis (FIG. 20G).

FIGS. 21A-21C show differentially expressed genes and performance of biomarker panels. Number of DEGs identified in Approach 2 (yellow) and Approach 6 (blue) (FIG. 21A). Number of genes in the biomarker panels (FIG. 21B). The overall expression level changes of genes included in the classification panels: ISB19 and ISB63 (FIG. 21C).

FIGS. 22A-22C show performance of optimized top performing 3-gene and 6-gene panels. Performance comparison of the optimized panels with the original panels; ISB19 and ISB63, and published panels; SMS, SeptiCytel Lab at different time points (indicated on the bottom of the panel) based on Validation sample set (FIG. 22A), or with integration of SOFA or CRP (FIG. 22B), or in patients who developed different severity of sepsis (FIG. 22C). The Y-axis is the AUC and different biomarker panel is indicated on the X-axis.

FIGS. 23A-23E show assessing the performance of 3- and 6-gene panels in diverse immune-related public datasets. Performance of the panels at Day 0 (FIG. 23A). List of public microarray data from sepsis related studies (adult sepsis, pediatric sepsis and neonatal sepsis (FIG. 23B). Classification performances of 3- and 6-gene panels in individual datasets in FIG. 23B (FIG. 23C). List of public

microarray data from bacterial/viral infection and auto immune disease studies (FIG. 23D).

Classification performances of 3- and 6-gene panels in individual datasets in FIG. 23D (FIG. 23E).

FIGS. 24A-24I show qPCR validation. List of 3-gene and 6-gene panels representing each biological function (FIG. 24A). Correlation between microarray and qPCR for 3-gene panel (FIG. 24B).

- 5 Log2 fold change in sepsis when compared to control samples for 3-gene panel in three time points, Day-3, Day-2 and Day-1 (FIG. 24C). Correlation between microarray and qPCR for 6-gene panel and MAPK14 which is a replaceable gene with TPM3. Red arrows indicate the replaceable genes with the original genes in ISB6 (FIG. 24D). Log2 fold change in sepsis when compared to control samples for 6-gene panel in three time points, Day-3, Day-2 and Day-1 (FIG. 24E). AUC of 3-gene and 6-gene panels
- 10 in the Validation set using qPCR of all sepsis patients (FIGS. 24F-24G). AUC of 3-gene and 6-gene panels in Septic shock and Sepsis patients (FIGS. 24H-24I).

DETAILED DESCRIPTION

- It is a challenge to develop large multi-feature diagnostic panels for clinical use. Most of the
- 15 clinical gene panels in current use are relatively small. For example, recently developed influenza and tuberculosis panels each utilize six analytes. Developing large multi-feature panels is expensive and time consuming, including the need to optimize measurements for individual features. In addition, some of the features may show different results due to measurement platform differences. The inventors therefore in some embodiments have reduced the number of features in sepsis diagnosis and/or prognosis
- 20 panels without sacrificing performance in order to further develop panels for clinical use.

I. Terms

- Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in *Lewin's Genes X*, ed. Krebs *et al.*, Jones and Bartlett Publishers, 2009 (ISBN 0763766321); Kendrew *et al.* (eds.), *The Encyclopedia of Molecular*
- 25 *Biology*, published by Blackwell Publishers, 1994 (ISBN 0632021829); Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by Wiley, John & Sons, Inc., 1995 (ISBN 0471186341); and George P. Rédei, *Encyclopedic Dictionary of Genetics, Genomics, Proteomics and Informatics*, 3rd Edition, Springer, 2008 (ISBN: 1402067534), and other similar references.

- 30 Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The singular terms "a," "an," and "the" include plural referents unless the context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. Hence "comprising A or B" means including A, or B, or A and B. It is further to be understood that all
- 35 base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description.

Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety, as are the GenBank Accession numbers (for the sequence present on November 5, 2018). In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

In order to facilitate review of the various embodiments of the disclosure, the following explanations of specific terms are provided:

Altered expression: A difference, such as an increase or decrease, in the conversion of the information encoded in a gene (for example, one or more of those in Tables 5, 6, 11, 15, and/or 16) into messenger RNA, the conversion of mRNA to a protein, or both. In some examples, the difference is relative to a control or reference value (or range of values), such as the average expression value of a group of healthy subjects, such as subjects who do not have sepsis. Detecting altered expression can include measuring a change in gene or protein expression, such as an increase of at least 20%, at least 50%, at least 75%, at least 90%, at least 100%, at least 200%, at least 300% at least 400%, or at least 500%, or a decrease of at least 20%, at least 50%, at least 75%, at least 90%, or at least 95%.

Array: An arrangement of nucleic acids (such as DNA or RNA) or proteins (such as antibodies) in assigned locations on a matrix or substrate. In some examples, the nucleic acid molecules or proteins are attached covalently to the matrix or substrate.

Control: A sample or standard used for comparison with an experimental sample, such as a sample from a healthy subject, for example, a subject who does not have sepsis. In some embodiments, the control is a historical control or standard reference value or range of values (*e.g.*, a previously tested control sample, such as a group of healthy subjects, or group of samples that represent baseline or normal values, such as the level of expression of one or more genes listed in Tables 5, 6, 11, 15, and/or 16). Laboratory standards and values can be set based on a known or determined population value and can be supplied in the format of a graph or table that permits comparison of measured, experimentally determined values.

Detecting or measuring expression: Determining the level expression in either a qualitative, semi-quantitative, or quantitative manner by detection of nucleic acid molecules (*e.g.*, DNA, RNA, and/or mRNA) or proteins. Exemplary methods include microarray analysis, PCR (such as RT-PCR, real-time PCR, or qRT-PCR), Northern blot, Western blot, ELISA, and mass spectrometry.

Sample (or biological sample): A biological specimen containing nucleic acids (for example, DNA, RNA, and/or mRNA), proteins, or combinations thereof, obtained from a subject. Examples include, but are not limited to, peripheral blood, serum, plasma, urine, saliva, tissue biopsy, fine needle aspirate, surgical specimen, and autopsy material. In some examples, a sample includes blood, serum, or plasma.

Sepsis: A condition where a subject has an infection, and the subject's immune response to the infection damages the subject's own tissue(s). As used herein, "sepsis" can refer to sepsis, severe sepsis, or septic shock. Sepsis is typically diagnosed by presence of infection in combination with one or more of altered body temperature (*e.g.*, above 101°F or below 98.6°F), increased respiratory rate (*e.g.*, >20 breaths/minute), and increased heart rate (*e.g.*, >90 beats per minute). Severe sepsis includes sepsis and one or more of altered mental state, low blood pressure (*e.g.*, ≤100 mm Hg systolic pressure), decreased platelet count, respiratory distress, abnormal heart function, and abdominal pain. Septic shock is sepsis or severe sepsis with low blood pressure due to sepsis that does not improve after treatment (such as fluid support). Sepsis, severe sepsis, and septic shock are life-threatening conditions that are usually treated with antibiotics, intravenous fluids, and other supportive measures, such as oxygen, mechanical ventilation and/or dialysis.

Subject: A living multi-cellular vertebrate organism, a category that includes human and non-human mammals. In one example, a subject has or is suspected to have sepsis or is at risk for sepsis. In other example, a subject does not exhibit symptoms of sepsis.

II. Sepsis Biomarkers and Methods

Provided herein are biomarker panels and methods of their use. In some embodiments, the biomarker panels are used in methods of predicting development of sepsis in a subject and/or diagnosing sepsis in a subject. In embodiments, the methods include measuring the expression of three or more (such as 3, 4, 5, 6, 7, 8, 9, 10, or more) genes in a sample from a subject. The methods also include determining whether expression of the three or more genes is altered (such as increased or decreased) compared to a control. In some examples, altered expression of the three or more genes indicates that the subject is predicted to develop or has developed sepsis.

In some embodiments, the methods disclosed herein can predict that a subject will develop sepsis, for example, can diagnose a subject with sepsis when they are pre-symptomatic or pre-clinical (*e.g.*, do not exhibit symptoms of sepsis). In some examples, the methods can diagnose a subject as having sepsis when they do not exhibit symptoms of sepsis, such as at least 1 day (for example, at least 1, 2, 3, 4, 5, or more days) prior to diagnosis of sepsis using current clinical criteria. Thus, in some embodiments, the methods utilize a sample that is from a subject who does not exhibit symptoms of sepsis. Subjects and samples are discussed in greater detail below.

A. ISB6 Panels

Disclosed herein are methods that include measuring expression of six or more (for example, 6, 7, 8, 9, 10, or more) genes in a sample from a subject having or suspected to have sepsis and determining whether expression of the six or more genes is altered (for example, increased or decreased) compared to a control. In some embodiments, the set of six or more genes includes or consists of a set of genes provided in Table 5. In other embodiments, the set of six or more genes includes or consists of a set of genes provided in Table 6, for example, CCR1, HLA-DPB1, BATF, C3AR1, ARHGEF18, and C9orf95; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf103; CCR1, HLA-DPB1, BATF, C3AR1,

MTCH1, and C9orf95; CCR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; CD177, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, C3AR1, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and SEPHS2; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf103; FES, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FES, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; or C3AR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95.

In some examples, expression of one or more of the genes in the set is increased compared to a control and/or expression of one or more of the genes in the set is decreased compared to a control. In particular examples, expression of CCR1, C3AR1, C9orf95, BATF, C9orf103, GNA15, CD177, CD63, EMR1, FCER1G, FES, PLAC8, and/or SEPHS2 is increased compared to the control. In other particular examples, expression of HLA-DPB1, ARHGEF18, and/or MTCH1 is decreased compared to the control.

Also disclosed herein are methods of diagnosing sepsis in a subject that include measuring expression of six or more (for example, 6, 7, 8, 9, 10, or more) genes in a sample from a subject and diagnosing the subject with sepsis when the expression of the six or more genes is altered (for example, increased or decreased) compared to a control. In some embodiments, the set of six or more genes includes or consists of a set of genes provided in Table 5. In other embodiments, the set of six or more genes includes or consists of a set of genes provided in Table 6, for example, CCR1, HLA-DPB1, BATF, C3AR1, ARHGEF18, and C9orf95; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf103; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CCR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; CD177, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, C3AR1, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and SEPHS2; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf103; FES, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FES, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; or C3AR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95.

In some examples, the subject is diagnosed with sepsis when expression of one or more of the genes in the set is increased compared to a control and/or expression of one or more of the genes in the set is decreased compared to a control. In particular examples, expression of CCR1, C3AR1, C9orf95, BATF, C9orf103, GNA15, CD177, CD63, EMR1, FCER1G, FES, PLAC8, and/or SPEHS2 is increased compared to the control. In other particular examples, expression of HLA-DPB1, ARHGEF18, and/or MTCH1 is decreased compared to the control.

Also disclosed herein are methods of treating sepsis in a subject that include measuring expression of six or more (for example, 6, 7, 8, 9, 10, or more) genes in a sample from a subject and administering one or more treatments for sepsis (such as administering one or more antibiotics) when the expression of the six or more genes is altered (for example, increased or decreased) compared to a control. In some embodiments, the set of six or more genes includes or consists of a set of genes provided in Table 5. In other embodiments, the set of six or more genes includes or consists of a set of genes provided in Table 6, for example, CCR1, HLA-DPB1, BATF, C3AR1, ARHGEF18, and C9orf95; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf103; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CCR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; CD177, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, C3AR1, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and SEPHS2; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf103; FES, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FES, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; or C3AR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95. In particular examples, expression of CCR1, C3AR1, C9orf95, BATF, C9orf103, GNA15, CD177, CD63, EMR1, FCER1G, FES, PLAC8, and/or SPEHS2 is increased compared to the control. In other particular examples, expression of HLA-DPB1, ARHGEF18, and/or MTCH1 is decreased compared to the control.

In some embodiments of the methods (such as methods of measuring expression of the six or more genes, methods of diagnosing a subject with sepsis, and/or methods of treating sepsis) further include measuring expression of RPGRIP1 in the sample and determining whether expression of RPGRIP1 is altered compared to a control. In some examples, expression of RPGRIP1 is decreased compared to a control. In other examples, the subject is diagnosed with sepsis when expression of RPGRIP1 is decreased compared to a control.

In other embodiments, the methods further include measuring expression of one or more control genes (such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 control genes), such as one or more housekeeping or internal

control genes. In some examples, a control gene is expressed at a constant level among different tissues and/or is unaffected by sepsis or an experimental treatment and can be used to normalize patterns of gene expression. Exemplary control or housekeeping genes include GAPDH, β -action, 18S ribosomal RNA, tubulin, BRK1, RNF181, GPR155, SUPT4H1, and FAM74A4.

5 **B. ISB 19-Derived Panels**

Disclosed herein are methods that include measuring expression of three or more (for example, 3, 4, 5, 6, or more) genes in a sample from a subject having or suspected to have sepsis or at risk of sepsis and determining whether expression of the three or more genes is altered (for example, increased or decreased) compared to a control. In some embodiments, the set of three or more genes includes or
 10 consists of a three or more genes selected from the set provided in Table 12 as the “ISB 19” panel. In other embodiments, the set of three or more genes includes or consists of a set of 3, 4, or 5 genes provided in Table 16, for example, LCN2, SLC2A3, and BMX; LCN2, SLC2A3, and GRB10; LCN2, PFKFB3, and GRB10; LCN2, PFKFB3, and BMX; IL1R2, HK3, and BMX; LCN2, HK3, and BMX; LCN2, HK3, and GRB10; GZMA, HK3, and BMX; FCAR, PFKFB2, and BMX; LCN2, PFKFB3, and
 15 IL18R1; LCN2, PFKFB3, GRB10, and ST6GALNAC3; LCN2, SLC2A3, BMX, and LGALS2; IL1R2, SLC2A3, BMX, and TCN1; LCN2, SLC2A3, GRB10, and ST6GALNAC3; FCAR, PFKFB2, BMX, and CEACAM1; IL1R2, HK3, BMX, and CD24; IL1R2, PFKFB3, BMX, and CD24; BMX, SLC2A3, GRB10, and CD24; IL1R2, HK3, BMX, and CEACAM1; GZMA, SLC2A3, BMX, and CD24; LCN2, PFKFB3, GRB10, ST6GALNAC3, and RNASE3; LCN2, PFKFB3, GRB10, RNASE2, and
 20 ST6GALNAC3; IL1R2, PFKFB3, GRB10, CD24, and ST6GALNAC3; GZMA, PFKFB3, GRB10, ST6GALNAC3, and CD24; HK3, PFKFB3, GRB10, ST6GALNAC3, and CD24; IL1R2, PFKFB3, GRB10, ST6GALNAC3, and TCN1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, and TCN1; LCN2, PFKFB3, GRB10, ST6GALNAC3, and DACH1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, and CD24; or SLC2A3, HK3, BMX, SPOCD1, and LGALS2.

In some examples, expression of one or more of the genes in the set is increased compared to a control and/or expression of one or more of the genes in the set is decreased compared to a control. In particular examples, expression of one or more of LCN2, SLC2A3, BMX, GRB10, PFKFB3, IL1R2, HK3, FCAR, PFKFB2, IL18R1, ST6GALNAC3, TCN1, CEACAM1, CD24, RNASE3, RNASE2, DACH1 and/or SPOCD1 is increased compared to the control. In other particular examples, expression
 30 of GZMA and/or LGALS2 is decreased compared to the control.

Also disclosed herein are methods of diagnosing or predicting sepsis in a subject that include measuring expression of three or more (for example, 3, 4, 5, 6, or more) genes in a sample from a subject and diagnosing the subject with sepsis or predicting risk of sepsis when the expression of the three or more genes is altered (for example, increased or decreased) compared to a control. In some
 35 embodiments, the three or more genes includes or consists of three or more genes selected from the set provided in Table 12 as the “ISB 19” panel. In other embodiments, the three or more genes includes or consists of a set of 3, 4, or 5 genes provided in Table 16, for example, LCN2, SLC2A3, and BMX;

LCN2, SLC2A3, and GRB10; LCN2, PFKFB3, and GRB10; LCN2, PFKFB3, and BMX; IL1R2, HK3, and BMX; LCN2, HK3, and BMX; LCN2, HK3, and GRB10; GZMA, HK3, and BMX; FCAR, PFKFB2, and BMX; LCN2, PFKFB3, and IL18R1; LCN2, PFKFB3, GRB10, and ST6GALNAC3; LCN2, SLC2A3, BMX, and LGALS2; IL1R2, SLC2A3, BMX, and TCN1; LCN2, SLC2A3, GRB10, and ST6GALNAC3; FCAR, PFKFB2, BMX, and CEACAM1; IL1R2, HK3, BMX, and CD24; IL1R2, PFKFB3, BMX, and CD24; BMX, SLC2A3, GRB10, and CD24; IL1R2, HK3, BMX, and CEACAM1; GZMA, SLC2A3, BMX, and CD24; LCN2, PFKFB3, GRB10, ST6GALNAC3, and RNASE3; LCN2, PFKFB3, GRB10, RNASE2, and ST6GALNAC3; IL1R2, PFKFB3, GRB10, CD24, and ST6GALNAC3; GZMA, PFKFB3, GRB10, ST6GALNAC3, and CD24; HK3, PFKFB3, GRB10, ST6GALNAC3, and CD24; IL1R2, PFKFB3, GRB10, ST6GALNAC3, and TCN1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, and TCN1; LCN2, PFKFB3, GRB10, ST6GALNAC3, and DACH1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, and CD24; or SLC2A3, HK3, BMX, SPOCD1, and LGALS2.

In some examples, the subject is diagnosed with sepsis or predicted to develop sepsis when expression of one or more of the genes in the set is increased compared to a control and/or expression of one or more of the genes in the set is decreased compared to a control. In particular examples, expression of one or more of LCN2, SLC2A3, BMX, GRB10, PFKFB3, IL1R2, HK3, FCAR, PFKFB2, IL18R1, ST6GALNAC3, TCN1, CEACAM1, CD24, RNASE3, RNASE2, DACH1 and/or SPOCD1 is increased compared to the control. In other particular examples, expression of GZMA and/or LGALS2 is decreased compared to the control.

Also disclosed herein are methods of treating sepsis in a subject that include measuring expression of three or more (for example, 3, 4, 5, 6, or more) genes in a sample from a subject and administering one or more treatments for sepsis (such as administering one or more antibiotics) when the expression of the three or more genes is altered (for example, increased or decreased) compared to a control. In some embodiments, the three or more genes includes or consists of three or more genes selected from the set provided in Table 12 as the "ISB 19" panel. In other embodiments, the three or more genes includes or consists of a set of 3, 4, or 5 genes provided in Table 16, for example, LCN2, SLC2A3, and BMX; LCN2, SLC2A3, and GRB10; LCN2, PFKFB3, and GRB10; LCN2, PFKFB3, and BMX; IL1R2, HK3, and BMX; LCN2, HK3, and BMX; LCN2, HK3, and GRB10; GZMA, HK3, and BMX; FCAR, PFKFB2, and BMX; LCN2, PFKFB3, and IL18R1; LCN2, PFKFB3, GRB10, and ST6GALNAC3; LCN2, SLC2A3, BMX, and LGALS2; IL1R2, SLC2A3, BMX, and TCN1; LCN2, SLC2A3, GRB10, and ST6GALNAC3; FCAR, PFKFB2, BMX, and CEACAM1; IL1R2, HK3, BMX, and CD24; IL1R2, PFKFB3, BMX, and CD24; BMX, SLC2A3, GRB10, and CD24; IL1R2, HK3, BMX, and CEACAM1; GZMA, SLC2A3, BMX, and CD24; LCN2, PFKFB3, GRB10, ST6GALNAC3, and RNASE3; LCN2, PFKFB3, GRB10, RNASE2, and ST6GALNAC3; IL1R2, PFKFB3, GRB10, CD24, and ST6GALNAC3; GZMA, PFKFB3, GRB10, ST6GALNAC3, and CD24; HK3, PFKFB3, GRB10, ST6GALNAC3, and CD24; IL1R2, PFKFB3, GRB10, ST6GALNAC3, and TCN1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, and TCN1; LCN2, PFKFB3, GRB10, ST6GALNAC3, and DACH1; SLC2A3,

PFKFB3, GRB10, ST6GALNAC3, and CD24; or SLC2A3, HK3, BMX, SPOCD1, and LGALS2. In particular examples, expression of one or more of LCN2, SLC2A3, BMX, GRB10, PFKFB3, IL1R2, HK3, FCAR, PFKFB2, IL18R1, ST6GALNAC3, TCN1, CEACAM1, CD24, RNASE3, RNASE2, DACH1 and/or SPOCD1 is increased compared to the control. In other particular examples, expression of GZMA and/or LGALS2 is decreased compared to the control. In some embodiments, the subject does not exhibit symptoms of sepsis.

In other embodiments, the methods further include measuring expression of one or more control genes (such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 control genes), such as one or more housekeeping or internal control genes. In some examples, a control gene is expressed at a constant level among different tissues and/or is unaffected by sepsis or an experimental treatment and can be used to normalize patterns of gene expression. Exemplary control or housekeeping genes include GAPDH, β -action, 18S ribosomal RNA, tubulin, BRK1, RNF181, GPR155, SUPT4H1, and FAM74A4.

C. ISB63-Derived Panels

Disclosed herein are methods that include measuring expression of six or more (for example, 6, 7, 8, 9, or more) genes in a sample from a subject having or suspected to have sepsis and determining whether expression of the six or more genes is altered (for example, increased or decreased) compared to a control. In some embodiments, the six or more genes includes or consists of six or more genes selected from the set provided in Table 12 as the "ISB 63" panel. In other embodiments, the six or more genes includes or consists of a set of 6, 7, or 8 genes provided in Table 17, for example, RPS6KA3, BCL6, TPM3, GNG10, STOM, and MPO; RPS6KA3, MAVS, TPM3, BCL6, STOM, and MPO; RPS6KA3, BCL6, TPM3, BMX, STOM, and MPO; RPS6KA3, LTF, TPM3, BCL6, STOM, and PDGFC; RPS6KA3, BCL6, TPM3, CD55, STOM, and MPO; RPS6KA3, BCL6, TPM3, GNG10, STOM, and PDGFC; RPS6KA3, LTF, TPM3, BCL6, STOM, and CYP1B1; LTF, BCL6, TPM3, CD55, STOM, and PDGFC; RPS6KA3, BCL6, TPM3, TLR8, STOM, and MPO; RPS6KA3, BCL6, TPM3, HLA-DRA, GNG10, and MPO; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, CYP27A1, and YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, and YOD1; RPS6KA3, GAS7, TPM3, BCL6, RRBPI, MPO, and YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, and LGALS2; RPS6KA3, GAS7, TPM3, BCL6, GNG10, MPO, and YOD1; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, and YOD1; RPS6KA3, BCL6, TPM3, GNG10, LILRA2, CYP27A1, and YOD1; RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, and LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, and YOD1; RPS6KA3, BCL6, TPM3, CD55, STOM, MPO, and IL17RA; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and LILRA4; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and IL17RA; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and TCN1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and RNASE3; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and NOV; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and RNASE2; LTF, MLLT1, TPM3,

BCL6, GNG10, PDGFC, YOD1, and FAM105A; RPS6KA3, BCL6, TPM3, HLA-DRA, RRBPI, MPO, ERO1L, and YOD1; or LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and C14orf101.

In some examples, expression of one or more of the genes in the set is increased compared to a control and/or expression of one or more of the genes in the set is decreased compared to a control. In particular examples, expression of one or more of RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, BMX, LTF, PDGFC, CD55, CYP1B1, TLR8, MLLT1, YOD1, GAS7, RRBPI, LILRA2, IL17RA, LILRA4, TCN1, RNASE3, RNASE2, FAM105A, ERO1L, and/or C14orf101 is increased compared to the control. In other particular examples, expression of one or more of MAVS, HLA-DRA, CYP27A1, LGALS2, and/or NOV is decreased compared to the control.

Also disclosed herein are methods of measuring expression of a modified set of six or more ISB63 genes comprising LCN2, BMX, MPO, RPS6KA3, BCL6, and MAPK14 in a sample from a subject having or suspected to have sepsis and determining whether expression of the six or more genes is altered (for example, increased or decreased) compared to a control. In particular examples, expression of one or more of LCN2, BMX, MPO, RPS6KA3, BCL6, and MAPK14 is increased compared to the control.

Also disclosed herein are methods of diagnosing or predicting sepsis in a subject that include measuring expression of six or more (for example, 6, 7, 8, 9, or more) genes in a sample from a subject and diagnosing the subject with sepsis or predicting risk of sepsis when the expression of the six or more genes is altered (for example, increased or decreased) compared to a control. In some embodiments, the six or more genes includes or consists of six or more genes selected from the set provided in Table 12 as the "ISB 63" panel. In other embodiments, the six or more genes includes or consists of a set of 6, 7, or 8 genes provided in Table 17, for example, RPS6KA3, BCL6, TPM3, GNG10, STOM, and MPO; RPS6KA3, MAVS, TPM3, BCL6, STOM, and MPO; RPS6KA3, BCL6, TPM3, BMX, STOM, and MPO; RPS6KA3, LTF, TPM3, BCL6, STOM, and PDGFC; RPS6KA3, BCL6, TPM3, CD55, STOM, and MPO; RPS6KA3, BCL6, TPM3, GNG10, STOM, and PDGFC; RPS6KA3, LTF, TPM3, BCL6, STOM, and CYP1B1; LTF, BCL6, TPM3, CD55, STOM, and PDGFC; RPS6KA3, BCL6, TPM3, TLR8, STOM, and MPO; RPS6KA3, BCL6, TPM3, HLA-DRA, GNG10, and MPO; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, CYP27A1, and YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, and YOD1; RPS6KA3, GAS7, TPM3, BCL6, RRBPI, MPO, and YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, and LGALS2; RPS6KA3, GAS7, TPM3, BCL6, GNG10, MPO, and YOD1; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, and YOD1; RPS6KA3, BCL6, TPM3, GNG10, LILRA2, CYP27A1, and YOD1; RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, and LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, and YOD1; RPS6KA3, BCL6, TPM3, CD55, STOM, MPO, and IL17RA; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and LILRA4; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and IL17RA; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and TCN1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and RNASE3;

RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and NOV; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and RNASE2; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and FAM105A; RPS6KA3, BCL6, TPM3, HLA-DRA, RRBPI, MPO, ERO1L, and YOD1; or LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and C14orf101.

5 In some examples, the subject is diagnosed with sepsis or predicted to develop sepsis when expression of one or more of the genes in the set is increased compared to a control and/or expression of one or more of the genes in the set is decreased compared to a control. In particular examples, the subject is diagnosed with sepsis when expression of one or more of RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, BMX, LTF, PDGFC, CD55, CYP1B1, TLR8, MLLT1, YOD1, GAS7, RRBPI, LILRA2, 10 IL17RA, LILRA4, TCN1, RNASE3, RNASE2, FAM105A, ERO1L, and/or C14orf101 is increased compared to the control. In other particular examples, expression of one or more of MAVS, HLA-DRA, CYP27A1, LGALS2, and/or NOV is decreased compared to the control.

Also disclosed herein are methods of diagnosing or predicting sepsis in a subject that include measuring expression of a modified set of six or more ISB63 genes comprising LCN2, BMX, MPO, 15 RPS6KA3, BCL6, and MAPK14 in a sample from a subject and diagnosing the subject with sepsis or predicting risk of sepsis when the expression of the six or more genes is altered (for example, increased or decreased) compared to a control. In particular examples, expression of one or more of LCN2, BMX, MPO, RPS6KA3, BCL6, and MAPK14 is increased compared to the control.

Also disclosed herein are methods of treating sepsis in a subject that include measuring 20 expression of six or more (for example, 6, 7, 8, 9, or more) genes in a sample from a subject and administering one or more treatments for sepsis (such as administering one or more antibiotics) when the expression of the six or more genes is altered (for example, increased or decreased) compared to a control. In some embodiments, the six or more genes includes or consists of six or more genes selected from the set provided in Table 12 as the “ISB 63” panel. In other embodiments, the six or more genes 25 includes or consists of a set of 6, 7, or 8 genes provided in Table 17, for example, RPS6KA3, BCL6, TPM3, GNG10, STOM, and MPO; RPS6KA3, MAVS, TPM3, BCL6, STOM, and MPO; RPS6KA3, BCL6, TPM3, BMX, STOM, and MPO; RPS6KA3, LTF, TPM3, BCL6, STOM, and PDGFC; RPS6KA3, BCL6, TPM3, CD55, STOM, and MPO; RPS6KA3, BCL6, TPM3, GNG10, STOM, and PDGFC; RPS6KA3, LTF, TPM3, BCL6, STOM, and CYP1B1; LTF, BCL6, TPM3, CD55, STOM, and 30 PDGFC; RPS6KA3, BCL6, TPM3, TLR8, STOM, and MPO; RPS6KA3, BCL6, TPM3, HLA-DRA, GNG10, and MPO; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, CYP27A1, and YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, and YOD1; RPS6KA3, GAS7, TPM3, BCL6, RRBPI, MPO, and YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, and LGALS2; RPS6KA3, GAS7, TPM3, BCL6, GNG10, MPO, and YOD1; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, and YOD1; 35 RPS6KA3, BCL6, TPM3, GNG10, LILRA2, CYP27A1, and YOD1; RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, and LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, and YOD1; RPS6KA3, BCL6, TPM3, CD55, STOM, MPO, and IL17RA; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC,

YOD1, and LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and LILRA4; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and IL17RA; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and TCN1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and RNASE3; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and NOV;

5 RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and RNASE2; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and FAM105A; RPS6KA3, BCL6, TPM3, HLA-DRA, RRBPI, MPO, ERO1L, and YOD1; or LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, and C14orf101. In particular examples, expression of one or more of RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, BMX, LTF, PDGFC, CD55, CYP1B1, TLR8, MLLT1, YOD1, GAS7, RRBPI, LILRA2, IL17RA,

10 LILRA4, TCN1, RNASE3, RNASE2, FAM105A, ERO1L, and/or C14orf101 is increased compared to the control. In other particular examples, expression of one or more of MAVS, HLA-DRA, CYP27A1, LGALS2, and/or NOV is decreased compared to the control. In some embodiments, the subject does not exhibit symptoms of sepsis.

Also disclosed herein are methods of treating sepsis in a subject that include measuring

15 expression of a modified set of six or more ISB63 genes comprising LCN2, BMX, MPO, RPS6KA3, BCL6, and MAPK14 in a sample from a subject and administering one or more treatments for sepsis (such as administering one or more antibiotics) when the expression of the six or more genes is altered (for example, increased or decreased) compared to a control. In particular examples, expression of one or more of LCN2, BMX, MPO, RPS6KA3, BCL6, and MAPK14 is increased compared to the control.

20 In other embodiments, the methods further include measuring expression of one or more control genes (such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 control genes), such as one or more housekeeping or internal control genes. In some examples, a control gene is expressed at a constant level among different tissues and/or is unaffected by sepsis or an experimental treatment and can be used to normalize patterns of gene expression. Exemplary control or housekeeping genes include GAPDH, β -action, 18S ribosomal RNA,

25 tubulin, BRK1, RNF181, GPR155, SUPT4H1, and FAM74A4.

D. Subjects and Samples

The methods disclosed herein utilize a sample from a subject. In some embodiments, a subject has or is suspected to have sepsis. In other embodiments, a subject does not exhibit symptoms of sepsis (*e.g.*, does not have sepsis and/or has sepsis, but is pre-symptomatic). In some examples, the subject may

30 be at risk for development of sepsis, for example, a subject with an infection (*e.g.*, a bacterial, viral, fungal, or parasitic infection), a subject who has experienced trauma (*e.g.*, wound, injury, or burn), a subject who has undergone a surgical procedure, a subject who has received an organ transplant, or a subject with an invasive device (*e.g.*, a catheter or breathing tube). In some examples, the subject has an infection with *Enterococcus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, or *Enterobacteriaceae*.

35 In additional examples, a subject at risk for sepsis may be younger than 1 year or older than 65, have diabetes, and/or have a compromised immune system (*e.g.*, subjects with AIDS (or HIV positive), subjects with severe combined immunodeficiency, subjects who have had transplants and who are taking

immunosuppressants, subjects who have cancer or who are receiving chemotherapy for cancer, subjects who do not have a spleen, subjects with end stage kidney disease, and subjects who have been taking corticosteroids or other immune suppressing therapy). In particular examples, a subject at risk for sepsis includes a subject with pneumonia, an abdominal infection (*e.g.*, peritonitis), a kidney infection or urinary tract infection, a subject who has undergone surgery, and/or a subject who has experienced trauma.

In some examples, the subject has experienced a risk factor for development of sepsis (including but not limited to infection, surgery, or trauma) within 1-14 days of collecting a sample from the subject. For example, one or more samples is collected from a subject 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days after undergoing surgery or experiencing a trauma. In other examples, one or more samples are collected from a subject prior to a risk factor for developing sepsis (including but not limited to surgery, placement of an invasive device, or immunosuppressant or chemotherapy), such as 1-14 days prior to the risk factor. In some examples, one or more samples are collected from the subject 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days before the risk factor. Samples may be collected from a subject both before (such as 1-14 days before) and after (such as 1-14 days after) the risk factor, in some examples.

Samples that can be used in the disclosed methods include biological specimens containing nucleic acids (for example, DNA, RNA, and/or mRNA), proteins, or combinations thereof, obtained from a subject. Examples include, but are not limited to, peripheral blood, peripheral blood mononuclear cells (PBMCs), serum, plasma, urine, saliva, sputum, wound secretions, pus, tissue biopsy, fine needle aspirate, surgical specimen, and autopsy material. In particular examples, the sample is peripheral blood from a subject. In some examples, samples are used directly in the methods provided herein. In other examples, samples are manipulated prior to analysis using the disclosed methods, such as through concentrating, filtering, centrifuging, diluting, desalting, denaturing, reducing, alkylating, proteolyzing, or combinations thereof. In some examples, components of the samples (for example, nucleic acids and/or proteins) are isolated or purified from the sample prior to analysis using the disclosed methods, such as isolating cells, proteins, and/or nucleic acid molecules from the samples.

In some embodiments, one or more samples (such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more samples) are collected from a subject. In some examples, multiple samples are collected and tested, for example, to detect changes in gene expression prior to development of symptoms of sepsis. The samples may be collected about every 2 hours, about every 4 hours, about every 8 hours, about every 12 hours, about every 18 hours, about once per day, about every other day, or combinations thereof. The samples may be collected prior to diagnosis with sepsis, after diagnosis with sepsis, or a combination thereof. In some examples, samples are collected from a subject who does not exhibit symptoms of sepsis, such as at least 1 day (for example, at least 1, 2, 3, 4, 5, or more days) prior to diagnosis of sepsis using current clinical criteria. In some examples, one or more samples are collected from a subject after an event that increases their risk for developing sepsis, including but not limited to surgery, trauma or other wound, burn(s),

infection (such as pneumonia, meningitis, urinary tract infection, peritonitis, or skin infection), or presence of an invasive device (such as a catheter or ventilator).

E. Detecting Gene Expression

As described herein, expression of the sepsis biomarkers disclosed herein (*e.g.*, one or more panels provided herein, including in any one of Tables 5, 6, 12, 16, and 17) can be measured or detected using any one of a number of methods. Detecting or measuring expression of nucleic acid molecules (*e.g.*, mRNA, cDNA) or protein is contemplated herein. The detection methods can be qualitative, semi-quantitative, or quantitative.

1. Methods for detecting mRNA

Gene expression can be evaluated by detecting RNA (*e.g.*, mRNA or cDNA) encoding the genes of interest. Thus, the disclosed methods can include evaluating mRNA(s) encoding the genes or sets of genes provided herein (including in any one of Tables 5, 6, 12, 16, and 17). In other examples, mRNA encoding the gene(s) of interest is reverse transcribed to cDNA and the cDNA is measured or detected. In some examples, the mRNA (or cDNA) is quantified. The amount of the mRNA (or cDNA) can be assessed in a sample from a subject and optionally in a control sample (such as a sample from a healthy subject). The amounts of mRNA (or cDNA) can be compared to levels of the mRNA (or cDNA) found in sample(s) from healthy subjects or other controls (such as a standard value or reference value). A significant increase or decrease in the amount can be evaluated using statistical methods. An alteration in the amount of the mRNA or cDNA in a sample from the subject relative to a control, such as an increase or decrease in expression, indicates whether the subject has sepsis or is likely to develop sepsis, as described herein.

RNA can be isolated from a sample from a subject, for example using commercially available kits, such as those from QIAGEN®. General methods for mRNA extraction are disclosed in, for example, including Ausubel *et al.*, Current Protocols of Molecular Biology, John Wiley and Sons (1997). In some examples, RNA can also be extracted from paraffin embedded tissues (*e.g.*, see Rupp and Locker, *Lab Invest.* 56:A67, 1987 and De Andres *et al.*, *BioTechniques* 18:42044, 1995). Total RNA from cells (such as those obtained from a subject) can be isolated using QIAGEN® RNeasy mini-columns. Other commercially available RNA isolation kits include MASTERPURE® Complete DNA and RNA Purification Kit (EPICENTRE® Madison, Wis.), and Paraffin Block RNA Isolation Kit (Ambion, Inc.). Total RNA from tissue samples can be isolated using RNA Stat-60 (Tel-Test). RNA prepared from biological samples can also be isolated, for example, by cesium chloride density gradient centrifugation.

Methods of measuring or detecting gene expression include methods based on hybridization analysis of polynucleotides, methods based on sequencing of polynucleotides, and proteomics-based methods. In some examples, mRNA (or cDNA) expression in a sample is quantified using northern blotting or *in situ* hybridization (Parker & Barnes, *Methods in Molecular Biology* 106:247-283, 1999); RNase protection assays (Hod, *Biotechniques* 13:852-4, 1992); and/or PCR-based methods, such as

reverse transcription polymerase chain reaction (RT-PCR) (Weis *et al.*, *Trends in Genetics* 8:263-4, 1992). Alternatively, antibodies can be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. Representative methods for sequencing-based gene expression analysis include Serial Analysis of Gene Expression (SAGE), and gene expression analysis by massively parallel signature sequencing (MPSS).

In one example, RT-PCR can be used. Generally, the first step in gene expression profiling by RT-PCR is the reverse transcription of the RNA template into cDNA, followed by its exponential amplification in a PCR reaction. Two commonly used reverse transcriptases are avian myeloblastosis virus reverse transcriptase (AMV-RT) and Moloney murine leukemia virus reverse transcriptase (MMLV-RT). The reverse transcription step is typically primed using specific primers, random hexamers, or oligo-dT primers, depending on the circumstances and the goal of expression profiling. For example, extracted RNA can be reverse-transcribed using a GeneAmp RNA PCR kit (Perkin Elmer, Calif., USA), or other commercially available kits, following the manufacturer's instructions. The derived cDNA can then be used as a template in the subsequent PCR reaction.

Although the PCR step can use a variety of thermostable DNA-dependent DNA polymerases, it typically employs the Taq DNA polymerase. TaqMan® PCR typically utilizes the 5'-nuclease activity of Taq or Tth polymerase to hydrolyze a hybridization probe bound to its target amplicon, but any enzyme with equivalent 5' nuclease activity can be used. Two oligonucleotide primers are used to generate an amplicon typical of a PCR reaction. A third oligonucleotide, or probe, is designed to detect nucleotide sequence located between the two PCR primers. The probe is not extendable by Taq DNA polymerase enzyme, and is labeled with a reporter fluorescent dye and a quencher fluorescent dye. Any laser-induced emission from the reporter dye is quenched by the quenching dye when the two dyes are located close together as they are on the probe. During the amplification reaction, the Taq DNA polymerase enzyme cleaves the probe in a template-dependent manner. The resultant probe fragments dissociate in solution, and signal from the released reporter dye is free from the quenching effect of the second fluorophore. One molecule of reporter dye is liberated for each new molecule synthesized, and detection of the unquenched reporter dye provides the basis for quantitative interpretation of the data.

To minimize errors and the effect of sample-to-sample variation, RT-PCR can be performed using an internal standard. The ideal internal standard is expressed at a constant level among different tissues, and/or is unaffected by the experimental treatment. RNAs commonly used to normalize patterns of gene expression are mRNAs for the housekeeping genes glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), beta-actin, tubulin, and 18S ribosomal RNA. Additional internal control genes include BRK1, RNF181, GPR155, SUPT4H1, and FAM74A4.

A variation of RT-PCR is real time quantitative RT-PCR (qRT-PCR), which measures PCR product accumulation through a dual-labeled fluorogenic probe (*e.g.*, TAQMAN® probe). Real time PCR is compatible both with quantitative competitive PCR, where internal competitor for each target sequence is used for normalization, and with quantitative comparative PCR using a normalization gene

contained within the sample, or a housekeeping gene for RT-PCR (see Held *et al.*, *Genome Research* 6:986-994, 1996). Quantitative PCR is also described in U.S. Pat. No. 5,538,848. Related probes and quantitative amplification procedures are described in U.S. Pat. No. 5,716,784 and U.S. Pat. No. 5,723,591. Instruments for carrying out quantitative PCR in microtiter plates are available from PE
 5 Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404 under the trademark ABI PRISM® 7700.

An alternative quantitative nucleic acid amplification procedure is described in U.S. Pat. No. 5,219,727. In this procedure, the amount of a target sequence in a sample is determined by simultaneously amplifying the target sequence and an internal standard nucleic acid segment. The
 10 amount of amplified DNA from each segment is determined and compared to a standard curve to determine the amount of the target nucleic acid segment that was present in the sample prior to amplification.

In some embodiments of this method, the expression of a housekeeping gene or internal control can also be evaluated. These terms include any constitutively or globally expressed gene whose presence
 15 enables an assessment of a sepsis-associated mRNA levels. Such an assessment includes a determination of the overall constitutive level of gene transcription and a control for variations in RNA recovery. Internal control (or internal reference) also refers to genes that show little or minimal change between different conditions (such as presence or absence of sepsis). In one example, an internal reference gene is one that shows <1.1-fold change and p-value >0.05 between sepsis and control (no sepsis) samples.
 20 Exemplary housekeeping genes include but are not limited to GAPDH, 18S ribosomal RNA, β -actin and tubulin. Exemplary internal control genes include but are not limited to BRK1, RNF181, GPR155, SUPT4H1, and FAM74A4. In particular examples, BRK1 and RNF181 are used as internal controls.

In other examples, gene expression is identified or confirmed using a microarray technique. Thus, the expression profile can be measured in a sample from a subject using microarray technology. In
 25 this method, nucleic acid sequences from one or more of the sepsis panels disclosed herein, including but not limited to those included in any one of Tables 5, 6, 12, 16, and 17 (including cDNAs and/or oligonucleotides) are plated, or arrayed, on a microchip substrate. The arrayed sequences are then hybridized with specific DNA probes from cells or tissues of interest. Just as in the RT-PCR method, the source of mRNA typically is total RNA isolated from a sample from a subject, and optionally from
 30 corresponding non-sepsis (*e.g.*, healthy) samples.

In a specific embodiment of the microarray technique, PCR amplified inserts of cDNA clones are applied to a substrate in a dense array. At least probes specific for one or more panels disclosed herein, such as those in any one of Tables 5, 6, 12, 16, and 17 (and in some examples one or more housekeeping and/or internal control genes) are applied to the substrate, and the array can include, consist essentially
 35 of, or consist of these nucleic acids. The microarrayed nucleic acids are suitable for hybridization under stringent conditions. Fluorescently labeled cDNA probes may be generated through incorporation of fluorescent nucleotides by reverse transcription of RNA extracted from a sample of interest (such as a

sample from a subject with sepsis, suspected to have sepsis, or at risk of sepsis). Labeled cDNA probes applied to the chip hybridize with specificity to each spot of DNA on the array. After stringent washing to remove non-specifically bound probes, the chip is scanned by confocal laser microscopy or by another detection method. Quantitation of hybridization of each arrayed element allows for assessment of
5 corresponding mRNA abundance. With dual color fluorescence, separately labeled cDNA probes generated from two sources of RNA are hybridized pairwise to the array. The relative abundance of the transcripts from the two sources corresponding to each specified gene is thus determined simultaneously. The miniaturized scale of the hybridization affords a convenient and rapid evaluation of the expression pattern for the panels disclosed herein. Microarray analysis can be performed by commercially available
10 equipment, following manufacturer's protocols.

Serial analysis of gene expression (SAGE) allows the simultaneous and quantitative analysis of a large number of gene transcripts, without the need of providing an individual hybridization probe for each transcript. First, a short sequence tag (about 10-14 base pairs) is generated that contains sufficient information to uniquely identify a transcript, provided that the tag is obtained from a unique position
15 within each transcript. Then, many transcripts are linked together to form long serial molecules, that can be sequenced, revealing the identity of the multiple tags simultaneously. The expression pattern of any population of transcripts can be quantitatively evaluated by determining the abundance of individual tags, and identifying the gene corresponding to each tag (see, for example, Velculescu *et al.*, *Science* 270:484-7, 1995; and Velculescu *et al.*, *Cell* 88:243-51, 1997, herein incorporated by reference).

In situ hybridization (ISH) is another method for detecting and comparing expression of the panels described herein, including but not limited to those disclosed in any one of Tables 5, 6, 12, 16, and 17. ISH applies and extrapolates the technology of nucleic acid hybridization to the single cell level, and, in combination with the art of cytochemistry, immunocytochemistry and immunohistochemistry, permits the maintenance of morphology and the identification of cellular markers to be maintained and identified,
20 and allows the localization of sequences to specific cells within populations, such as tissues and blood samples. ISH is a type of hybridization that uses a complementary nucleic acid to localize one or more specific nucleic acid sequences in a portion or section of tissue (in situ), or, if the tissue is small enough, in the entire tissue (whole mount ISH).

Sample cells or tissues can be treated to increase their permeability to allow one or more gene-specific probes to enter the cells. The one or more probes are added to the treated cells, allowed to hybridize at pertinent temperature, and excess probe is washed away. The probe can be labeled, for example with a radioactive, fluorescent or antigenic tag, so that the probe's location and in some examples quantity, in the tissue can be determined, for example using autoradiography, fluorescence microscopy or immunoassay. Probes can be designed based on the known sequences of the genes (such
30 as the GenBank accession numbers provided herein) such that the probes specifically bind the gene of interest.

In situ PCR is the PCR based amplification of the target nucleic acid sequences prior to ISH. For detection of RNA, an intracellular reverse transcription step is introduced to generate complementary DNA from RNA templates prior to *in situ* PCR. This enables detection of low copy RNA sequences.

Prior to *in situ* PCR, cells or tissue samples can be fixed and permeabilized to preserve morphology and permit access of the PCR reagents to the intracellular sequences to be amplified. PCR amplification of target sequences is next performed either in intact cells held in suspension or directly in cytocentrifuge preparations or tissue sections on glass slides. In the former approach, fixed cells suspended in the PCR reaction mixture are thermally cycled using conventional thermal cyclers. After PCR, the cells are cytocentrifuged onto glass slides with visualization of intracellular PCR products by ISH or immunohistochemistry. In situ PCR on glass slides is performed by overlaying the samples with the PCR mixture under a coverslip which is then sealed to prevent evaporation of the reaction mixture. Thermal cycling is achieved by placing the glass slides either directly on top of the heating block of a conventional or specially designed thermal cycler or by using thermal cycling ovens.

Detection of intracellular PCR products can be achieved by ISH with PCR-product specific probes, or direct *in situ* PCR without ISH through direct detection of labeled nucleotides (such as digoxigenin-11-dUTP, fluorescein-dUTP, 3H-CTP or biotin-16-dUTP), which have been incorporated into the PCR products during thermal cycling.

Gene expression can also be detected and quantitated using the nCounter® technology developed by NanoString (Seattle, WA; see, for example, U.S. Patent Nos. 7,473,767; 7,919,237; and 9,371,563, which are herein incorporated by reference in their entirety). The nCounter® analysis system utilizes a digital color-coded barcode technology that is based on direct multiplexed measurement of gene expression. The technology uses molecular “barcodes” and single molecule imaging to detect and count hundreds of unique transcripts in a single reaction. Each color-coded barcode is attached to a single target-specific probe corresponding to a gene of interest. Mixed together with controls, they form a multiplexed CodeSet.

Each color-coded barcode represents a single target molecule. Barcodes hybridize directly to target molecules and can be individually counted without the need for amplification. The method includes three steps: (1) hybridization; (2) purification and immobilization; and (3) counting. The technology employs two approximately 50 base probes per mRNA that hybridize in solution. The reporter probe carries the signal; the capture probe allows the complex to be immobilized for data collection. After hybridization, the excess probes are removed and the probe/target complexes are aligned and immobilized in the nCounter® cartridge. Sample cartridges are placed in the digital analyzer for data collection. Color codes on the surface of the cartridge are counted and tabulated for each target molecule. This method is described in, for example, U.S. Patent No. 7,919,237; and U.S. Patent Application Publication Nos. 20100015607; 20100112710; 20130017971, which are herein incorporated by reference in their entirety.

2. Arrays for profiling gene expression

In particular embodiments, arrays (such as a solid support, for example a multi-well plate, a membrane, a bead, or flow cell) are provided that can be used to evaluate gene expression, for example to determine if a subject has or is likely to develop symptoms of sepsis. Such arrays can include a set of specific binding agents (such as nucleic acid probes and/or primers) specific for genes of one or more panels described herein, including but not limited to those in any one of Tables 5, 6, 12, 16, and 17. When describing an array that consists essentially of probes or primers specific for a panel provided herein, including those in any one of Tables 5, 6, 12, 16, and 17, such an array includes probes or primers specific for the genes of the panel, and can further include control probes or primers, such as 1-10 control probes or primers (for example to confirm the incubation conditions are sufficient). In some examples, the array may further comprise additional, such as 1, 2, 3, 4 or 5 additional probes for other genes. In some examples, the array includes 1-10 housekeeping- and/or internal control-specific probes or primers. In one example, an array is a multi-well plate (*e.g.*, 96 or 384 well plate).

In one example, the array includes, consists essentially of, or consists of probes or primers (such as an oligonucleotide or antibody) that can recognize the genes in the panels listed in any one of Tables 5, 6, 12, 16, and 17 (and in some examples also 1-10 housekeeping or control genes). In another example, the array includes, consists essentially of, or consists of probes or primers (such as an oligonucleotide or antibody) that can recognize each of LCN2, BMX, MPO, RPS6KA3, BCL6, and MAPK14 (and in some examples also 1-10 housekeeping or control genes). The oligonucleotide probes or primers can further include one or more detectable labels, to permit detection of hybridization signals between the probe and its target sequence.

3. Methods for detecting protein expression

In some examples, expression of proteins of the panels provided herein, including but not limited to those listed in any one of Tables 5, 6, 12, 16, and 17 is analyzed. Suitable biological samples include samples containing protein obtained from a subject with sepsis, a subject suspected to have sepsis, or a subject at risk for sepsis. An alteration in the amount of the proteins in a sample from the subject relative to a control, such as an increase or decrease in protein expression, indicates whether the subject has sepsis or is likely to develop sepsis, as described herein.

Antibodies specific for the panels of proteins provided herein, including but not limited to those listed in any one of Tables 5, 6, 12, 16, and 17 can be used for protein detection and quantification, for example using an immunoassay method, such as those presented in Harlow and Lane (*Antibodies, A Laboratory Manual*, CSHL, New York, 1988). Exemplary immunoassay formats include ELISA, Western blot, and RIA assays. Thus, protein levels in a sample can be evaluated using these methods. Immunohistochemical techniques can also be utilized protein detection and quantification. General guidance regarding such techniques can be found in Bancroft and Stevens (*Theory and Practice of Histological Techniques*, Churchill Livingstone, 1982) and Ausubel *et al.* (*Current Protocols in Molecular Biology*, John Wiley & Sons, New York, 1998).

To quantify proteins, a biological sample of the subject that includes cellular proteins can be used. Quantification of the proteins of the panels provided herein, including but not limited to those listed in any one of Tables 5, 6, 12, 16, and 17 can be achieved by immunoassay methods. The amount of the proteins can be assessed in a sample from a subject and optionally in a control sample (such as a sample from a healthy subject). The amounts of protein can be compared to levels of the protein found in sample(s) from healthy subjects or other controls (such as a standard value or reference value). A significant increase or decrease in the amount can be evaluated using statistical methods.

Quantitative spectroscopic approaches, such as SELDI, can be used to analyze gene expression in a sample (such as a sample from a subject with sepsis or suspected to have or develop sepsis). In one example, surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) mass spectrometry is used to detect protein expression, for example by using the ProteinChip™ (CIPHERGEN Biosystems, Palo Alto, CA). Such methods are well known in the art (for example see U.S. Pat. No. 5,719,060; U.S. Pat. No. 6,897,072; and U.S. Pat. No. 6,881,586). SELDI is a solid phase method for desorption in which the analyte is presented to the energy stream on a surface that enhances analyte capture or desorption.

The surface chemistry allows the bound analytes to be retained and unbound materials to be washed away. Subsequently, analytes bound to the surface (such as tumor-associated proteins) can be desorbed and analyzed by any of several means, for example using mass spectrometry. When the analyte is ionized in the process of desorption, such as in laser desorption/ionization mass spectrometry, the detector can be an ion detector. Mass spectrometers generally include means for determining the time-of-flight of desorbed ions. This information is converted to mass. However, one need not determine the mass of desorbed ions to resolve and detect them: the fact that ionized analytes strike the detector at different times provides detection and resolution of them. Alternatively, the analyte can be detectably labeled (for example with a fluorophore or radioactive isotope). In these cases, the detector can be a fluorescence or radioactivity detector. A plurality of detection means can be implemented in series to fully interrogate the analyte components and function associated with retained molecules at each location in the array.

Therefore, in a particular example, the chromatographic surface includes antibodies that specifically bind to proteins of the panels listed in any one of Tables 5, 6, 11, 15, or 16. In other examples, the chromatographic surface consists essentially of, or consists of, antibodies that specifically bind to proteins of the panels listed in any one of Tables 5, 6, 11, 15, or 16. In further examples, the chromatographic surface includes antibodies that specifically bind to proteins LCN2, BMX, MPO, RPS6KA3, BCL6, and MAPK14. In some examples, the chromatographic surface includes antibodies that bind other molecules, such as housekeeping proteins (*e.g.*, tubulin, β -actin, GAPDH, or 18S ribosomal RNA) or internal control proteins (*e.g.*, BRK1, RNF181, GPR155, SUPT4H1, or FAM74A4). In another example, antibodies are immobilized onto the surface using a bacterial Fc binding support.

The chromatographic surface is incubated with a sample. The antigens present in the sample can recognize the antibodies on the chromatographic surface. The unbound proteins and mass spectrometric

interfering compounds are washed away and the proteins that are retained on the chromatographic surface are analyzed and detected by SELDI-TOF. The MS profile from the sample can be then compared using differential protein expression mapping, whereby relative expression levels of proteins at specific molecular weights are compared by a variety of statistical techniques and bioinformatic software systems.

F. Controls

The methods disclosed herein include determining expression of one or more genes (such as a panel of genes provided herein, including but not limited to those listed in any one of Tables 5, 6, 11, 15, or 16) is altered compared to a control. In some examples the expression is compared to a control, such as compared to expression in a sample from a subject known not to have sepsis, for example, a healthy subject (or compared to a reference value or range of values representing such).

The control can be any suitable control against which to compare expression of any one of the panels of genes disclosed herein (including in any one of Tables 5, 6, 11, 15, or 16) in a sample from a subject. In some embodiments, the control sample is a sample, or plurality of samples, from a subject(s) known not to have sepsis (e.g., one or more "healthy" subjects). In other examples, the control sample is a sample, or plurality of samples, from a subject(s) known to have sepsis (in which case the increase or decrease in expression correlation to sepsis is reversed). In some embodiments, the control is a reference value. For example, the reference value can be derived from the average expression values obtained from a group of subjects known not to have (or known to have) sepsis.

An increase in expression includes any detectable increase in the production of a gene product, for example, compared to a control. In certain examples, production of a gene product increases by at least 20%, at least 50%, at least 75%, at least 90%, at least 2-fold, at least 3-fold or at least 4-fold, as compared to a control (such as a sample from a subject known not to have sepsis or compared to a reference value or range of values representing such). In one example, a control is a relative amount of gene expression in a biological sample, such as a sample from a subject known not to have sepsis. In some examples, the control is a reference value (or range of values) for an expected amount of expression of each of the panel of genes from a subject known not to have sepsis. In some examples, the control is a sample from a subject known not to have sepsis (which can be analyzed in parallel with a test sample).

A decrease in expression includes any detectable decrease in the production of a gene product, for example, compared to a control. In certain examples, production of a gene product decreases by at least 20%, at least 50%, at least 75%, at least 90%, at least 2-fold, at least 3-fold or at least 4-fold, as compared to a control (such as a sample from a subject known not to have sepsis or compared to a reference value or range of values representing such). In one example, a control is a relative amount of gene expression in a biological sample, such as a sample from a subject known not to have sepsis. In some examples, the control is a reference value (or range of values) for an expected amount of expression of each of the panel of genes from a subject known not to have sepsis. In some examples, the control is a sample from a subject known not to have sepsis (which can be analyzed in parallel with a test sample).

G. Additional Features

In some embodiments, the methods provided herein further include analyzing one or more additional clinical parameters and integrating the information with the panels disclosed herein (such as one or more panels provided herein, including those in any one of Tables 5, 6, 11, 15, or 16). In some examples, the additional clinical parameters alone do not provide good performance for diagnosing or predicting sepsis, however, they can provide improved performance in combination with the panels disclosed herein.

In one example, the methods include determining a Sequential Organ Failure Assessment (SOFA) score for the subject. The SOFA score is a mortality prediction score measurement used to determine the extent of organ failure in a subject based on degree of function/dysfunction of six organ systems. It includes PaO₂, FiO₂, platelet count, Glasgow coma scale, bilirubin level, mean arterial pressure and administration of vasoactive agents, creatinine level, and urine output to generate a score (Vincent *et al.*, *Crit. Care Med.* 26, 1793-1800, 1998; Ferreira *et al.*, *JAMA* 286:1754-1758, 2001). Thus, in some examples, one or more SOFA scores are obtained for a subject having sepsis, suspected to have sepsis, or at risk for sepsis (for example, one or more times per day). The SOFA score is then integrated with the gene panel to determine whether the subject has or is likely to develop sepsis.

In another example, the level of C-reactive protein (CRP) is determined in a sample from the subject. CRP level is commonly used as a non-specific indicator of infection in a subject. Thus, in some examples, a level of CRP is measured in a sample from a subject having sepsis, suspected to have sepsis, or at risk for sepsis (for example, one or more times per day). The CRP level is then integrated with the gene panel to determine whether the subject has or is likely to develop sepsis.

In some examples, both a SOFA score and level of CRP are measured or determined for a subject having sepsis, suspected to have sepsis, or at risk for sepsis (for example, one or more times per day). The SOFA score and CRP level are then integrated with the gene panel to determine whether the subject has or is likely to develop sepsis.

III. Kits

Also provided are kits including sets of specific binding agents, such as sets of nucleic acid probes, nucleic acid primers, and/or antibodies (or antibody fragments) specific for each of the genes in one or more panels provided herein (including those listed in any one of Tables 5, 6, 11, 15, or 16). In some examples, a kit includes a nucleic acid probe specific for each of the genes in one or more panels listed in any one of Tables 5, 6, 11, 15, or 16, one or more nucleic acid primers (*e.g.*, 1, 2, 3, 4, or more primers) specific for each of the genes in one or more panels listed in any one of Tables 5, 6, 11, 15, or 16, an antibody specific for proteins encoded by each of the genes in one or more panels listed in any one of Tables 5, 6, 11, 15, or 16, or combinations thereof. In other examples, a kit includes a nucleic acid probe specific for each of the LCN2, BMX, MPO, RPS6KA3, BCL6, and MAPK14; one or more nucleic acid primers (*e.g.*, 1, 2, 3, 4, or more primers) specific for each of LCN2, BMX, MPO, RPS6KA3, BCL6, and MAPK14; an antibody specific for proteins encoded by each of LCN2, BMX, MPO, RPS6KA3,

BCL6, and MAPK14; or combinations thereof. Such probes, primers, and/or antibodies can be in vials, such as a glass or plastic container, or attached or conjugated to an array (e.g., a solid substrate). In some examples, the probes, primers, and/or antibodies are in a carrier, such as a buffer (e.g., saline). In some examples, such sets further include a nucleic acid probe, one or more nucleic acid primers, or an antibody, specific for at least one housekeeping and/or internal control molecule, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, or about 1 to about 3, or about 1 to about 5, housekeeping and/or internal control molecules (e.g., β -actin, GAPDH, tubulin, BRK1, RNF181, GPR155, SUPT4H1, or FAM74A4). Such kits can include other components, such as a buffer (e.g., hybridization buffer), enzyme(s), and/or one or more detection reagents.

In some embodiments, the kit includes at least one surface with at least one nucleic acid probe, nucleic acid primer, and/or antibody immobilized on the surface (for example on an array, bead, or flow cell). The disclosed kits can include at least one surface with at least one nucleic acid probe, nucleic acid primer, and/or antibody immobilized on the surface in an addressable manner. Some of the surfaces (or substrates) which can be used in the disclosed kits (or methods) are readily available from commercial suppliers. In some embodiments, the surface is a 96-, 384-, or 1536-well microtiter plate, such as modified plates sold by Corning Costar or BD Biosciences (for example, gamma-irradiated plates). In other embodiments, a substrate includes one or more beads (such as a population of beads that can be differentiated by size or color, for example by flow cytometry). In some embodiments, a substrate includes a flow cell (such as a flow cell or a microfluidic chip with a plurality of channels). Alternatively, a surface comprising wells which, in turn, comprise indentations or "dimples" can be formed by micromachining a substance such as aluminum or steel to prepare a mold, then microinjecting plastic or a similar material into the mold to form a structure. Alternatively, a structure comprised of glass, plastic, ceramic, or the like, can be assembled. In some examples, the base is a flat piece of material (for example glass or plastic), in, for example, the shape of the lower portion of a typical microplate used for a biochemical assay. The top surface of the base can be either flat or formed with indentations that will align with a subdivider to provide full subdivisions, or wells, within each sample well. The pieces can be joined by standard procedures, for example the procedures used in the assembly of silicon wafers.

A wide variety of array formats for arrangement of the nucleic acid probes, nucleic acid primers, and/or antibodies can be employed. One suitable format includes a two-dimensional pattern of discrete cells (such as 4096 squares in a 64 by 64 array). Other suitable array formats include but are not limited to slot (rectangular) and circular arrays (see U.S. Patent No. 5,981,185). In some examples, the array is a multi-well plate. In one example, the array is formed on a polymer medium, which is a thread, membrane or film. An example of an organic polymer medium is a polypropylene sheet having a thickness on the order of about 1 mil. (0.001 inch) to about 20 mil., although the thickness of the film is not critical and can be varied over a fairly broad range. The array can include biaxially oriented polypropylene (BOPP) films, which in addition to their durability, exhibit low background fluorescence.

In another example, a surface activated organic polymer is used as the solid support surface. One example of a surface activated organic polymer is a polypropylene material aminated via radio frequency plasma discharge. Other reactive groups can also be used, such as carboxylated, hydroxylated, thiolated, or active ester groups.

5 The solid support of the array can be formed from an organic polymer. Suitable materials for the solid support include, but are not limited to: polypropylene, polyethylene, polybutylene, polyisobutylene, polybutadiene, polyisoprene, polyvinylpyrrolidone, polytetrafluoroethylene, polyvinylidene difluoride, polyfluoroethylene-propylene, polyethylenevinyl alcohol, polymethylpentene, polychlorotrifluoroethylene, polysulfones, hydroxylated biaxially oriented polypropylene, aminated
10 biaxially oriented polypropylene, thiolated biaxially oriented polypropylene, ethyleneacrylic acid, ethylene methacrylic acid, and blends of copolymers thereof (see U.S. Patent No. 5,985,567).

In general, suitable characteristics of the material that can be used to form the solid support surface include: being amenable to surface activation such that upon activation, the surface of the support is capable of covalently attaching a biomolecule such as an oligonucleotide or antibody thereto;
15 amenability to *in situ* synthesis of biomolecules; being chemically inert such that at the areas on the support not occupied by the oligonucleotides or proteins (such as antibodies) are not amenable to non-specific binding, or when non-specific binding occurs, such materials can be readily removed from the surface without removing the oligonucleotides or proteins (such as antibodies).

The array formats of the present disclosure can be included in a variety of different types of
20 formats. A “format” includes any format to which the solid support can be affixed, such as microtiter plates (*e.g.*, multi-well plates), test tubes, inorganic sheets, dipsticks, and the like. For example, when the solid support is a polypropylene thread, one or more polypropylene threads can be affixed to a plastic dipstick-type device; polypropylene membranes can be affixed to glass slides. The particular format is, in and of itself, unimportant. All that is necessary is that the solid support can be affixed thereto without
25 affecting the functional behavior of the solid support or any biopolymer absorbed thereon, and that the format (such as the dipstick or slide) is stable to any materials into which the device is introduced (such as clinical samples and reaction solutions).

In one embodiment, preformed nucleic acid probes, nucleic acid primers, and/or antibodies can be situated on or within the surface of a test region by any of a variety of conventional techniques,
30 including photolithographic or silkscreen chemical attachment, disposition by ink jet technology, capillary, screen or fluid channel chip, electrochemical patterning using electrode arrays, contacting with a pin or quill, or denaturation followed by baking or UV-irradiating onto filters (see, *e.g.*, Rava *et al.* (1996). U.S. Patent No. 5,545,531; Fodor *et al.* (1996). U.S. Patent No. 5,510,270; Zanzucchi *et al.* (1997). U.S. Patent No. 5,643,738; Brennan (1995). U.S. Patent No. 5,474,796; PCT WO 92/10092; PCT
35 WO 90/15070).

In one embodiment, preformed nucleic acid probes or nucleic acid primers are derivatized at the 5' end with a free amino group; dissolved at a concentration routinely determined empirically (*e.g.*, about

1 μM) in a buffer such as 50 mM phosphate buffer, pH 8.5 and 1 mM EDTA; and distributed with a Pixus nanojet dispenser (Cartesian Technologies) in droplets of about 10.4 nanoliters onto specific locations within a test well whose upper surface is that of a fresh, dry DNA Bind plate (Corning Costar). In another embodiment, preformed nucleic acid probes or nucleic acid primers are derivatized at the 3'

5 end with a free amino group and optionally include a carbon spacer. Oligonucleotides are dissolved at 20 μM in 0.5 M Phosphate buffer at pH 8.5 and are contact printed on Falcon 1172 plates, gamma irradiated (BD Biosciences) using capillary pins in a humidified chamber. Depending on the relative rate of attachment and evaporation, it may be required to control the humidity in the wells during preparation.

In another embodiment, nucleic acid probes or nucleic acid primers can be synthesized directly

10 on the surface of a test region, using methods such as, for example, light-activated deprotection of growing oligonucleotide chains (for example, in conjunction with the use of a site directing "mask") or by patterned dispensing of nanoliter droplets of deactivating compound using a nanojet dispenser. Deprotection of all growing oligonucleotides that are to receive a single nucleotide can be done, for example, and the nucleotide then added across the surface. In another embodiment, oligonucleotide

15 anchors are attached to the surface via the 3' ends of the oligonucleotides, using conventional methodology.

In particular examples, the nucleic acid probe(s), nucleic acid primer(s), and/or antibodies are immobilized in an array (such as a microarray) and a label is detected using a microarray imager. Microarray imagers are commercially available and include OMIX, OMIX HD, CAPELLA, or

20 SUPERCAPPELLA imagers (HTG Molecular Diagnostics, Tucson, Arizona), TYPHOON imager (GE Life Sciences, Piscataway, NJ), GENEPIX microarray scanner (Molecular Devices, Sunnyvale, CA), or GENECHIP scanner (Affymetrix, Santa Clara, CA). In other examples, the nucleic acid probes, nucleic acid primers, and/or antibodies can be immobilized on a bead and the label is detected by flow cytometry or related methods (such as utilizing a LUMINEX 200 or FLEXMAP 3D (Luminex Corporation, Austin,

25 TX), or other suitable instrument).

IV. Methods of Treatment

Subjects with sepsis (whether symptomatic or pre-symptomatic) identified using the disclosed methods can be treated for sepsis. Thus, in some embodiments, the disclosed methods include administering one or more treatments for sepsis. The methods disclosed herein can be used to treat a

30 subject who does not exhibit symptoms of sepsis, thereby decreasing the severity and/or duration of sepsis in the subject, or inhibiting development of sepsis. In other embodiments, the methods disclosed herein can be used to treat a subject who exhibits symptoms of sepsis and/or is diagnosed with sepsis by one or more other criteria, such as Sepsis-2 or Sepsis-3 criteria (*e.g.*, Levy *et al.*, *Crit. Care Med.* 31:1250-1256, 2003; Singer *et al.*, *JAMA* 315:801-810, 2016).

In some examples, one or more therapies for sepsis, such as antibiotic therapy (for example, oral, intravenous, and/or intramuscular antibiotics), intravenous fluids, vasopressors (*e.g.*, epinephrine, norepinephrine, and/or vasopressin), or other supportive therapies, can be administered to the subject. In

additional examples, treatment can include surgery, for example to treat or remove infected tissue (including amputation of one or more affected extremities). Additional treatments, including corticosteroids, insulin, blood transfusion, dialysis, and/or mechanical ventilation may be administered in some cases. In other examples, the subject does not exhibit symptoms of sepsis, but is predicted to develop sepsis by the methods disclosed herein, and the subject is monitored for development of symptoms of sepsis. The subject may also be retested one or more times using the methods disclosed herein to diagnose sepsis at a later time point, and may be placed on a treatment protocol if sepsis is diagnosed.

Exemplary antibiotics that can be administered include ceftriaxone, azithromycin, ciprofloxacin, vancomycin, aztreonam, moxifloxacin, nafcillin, daptomycin, meropenem, ceftazidime, cefotaxime, cefepime, piperacillin-tazobactam, ampicillin-sulbactam, imipenem/cilastatin, levofloxacin, clindamycin, and combinations of two or more thereof. Specific antibiotics can be selected if the organism(s) causing the infection are identified. In some examples, the subject is treated with one or more broad-spectrum antibiotics immediately upon diagnosis, for example, prior to identifying a causative agent. The subject can then be administered one or more additional or different antibiotics when a specific causative agent is identified.

In other examples, the subject can be administered antiviral therapy, such as acyclovir, pocapavir or ganciclovir, if the underlying infection is known or suspected to be viral (such as herpes virus or enterovirus).

EXAMPLES

The following examples are provided to illustrate certain particular features and/or embodiments. These examples should not be construed to limit the disclosure to the particular features or embodiments described.

Example 1

Development of a Biological Function-Based Sepsis Panel

Materials and Methods

A previous 11 gene panel for sepsis prediction (CEACAM1, ZDHHC19, C9orf95, GNA15, BATE, C3AR1, KIAA1370, TGFBI, MTCH1, RPGRIP1, and HLA-DPB1; referred to as the “Stanford11” panel) was previously developed from a panel of 82 differentially expressed genes using the greedy forward search method (Sweeney *et al.*, *Sci. Transl. Med.* 7:287ra71, 2015). This set of 82 genes (Stanford82; Table 1) was used as the basis for developing new biological-function based sepsis panels. Exemplary, non-limiting GenBank Accession numbers are provided for each gene in Table 1. Additional sequences for each gene can be identified using publicly available databases.

Table 1. The list of 82 differentially expressed genes (Stanford82)

Gene symbol	GenBank Acc. No.	Summary Effect	Gene symbol	GenBank Acc. No.	Summary Effect*
ADAMTS3	NM_014243	1	PLB1	NM_153021	1
ANKRD22	NM_114590	1	PNPLA1	NM_173676	1
ANXA3	NM_005139	1	PPM1M	NM_144641	1
AP3B2	NM_004644	1	PSTPIP2	NM_024430	1
ARL8A	NM_138795	1	RETN	NM_020415	1
B3GNT8	NM_198540	1	RGL4	NM_153615	1
BATF	NM_006399	1	S100A12	NM_005621	1
BPI	NM_001725	1	SEPHS2	NM_012248	1
BST1	NM_004334	1	SETD8	NM_020382	1
C1orf162	NM_174896	1	SGSH	NM_000199	1
C3AR1	NM_004054	1	SIGLEC9	NM_014441	1
C9orf103	NM_001256915	1	SLC26A8	NM_052961	1
C9orf95	NM_017881	1	SPPL2A	NM_032802	1
CCR1	NM_001295	1	SQRDL	NM_021199	1
CD177	NM_020406	1	TCN1	NM_001062	1
CD63	NM_001780	1	ZDHHC19	NM_001039617	1
CD82	NM_002231	1	ZDHHC3	NM_016598	1
CEACAM1	NM_001712	1	ARHGEF18	NM_015318	-1
CLEC5A	NM_013252	1	CACNA2D3	NM_018398	-1
DHRS9	NM_199204	1	CNNM3	NM_017623	-1
EMR1	NM_001974	1	GLO1	NM_006708	-1
FAM89A	NM_198552	1	GRAMD1C	NM_017577	-1
FCER1G	NM_004106	1	HACL1	NM_012260	-1
FCGR1B	NM_001017986	1	HLA-DPB1	NM_002121	-1
FES	NM_002005	1	KIAA1370	NM_019600	-1
FFAR3	NM_005304	1	KLHDC2	NM_014315	-1
FIG4	NM_014845	1	METAP1	NM_015143	-1
GNA15	NM_002068	1	MRPS35	NM_021821	-1
GPR84	NM_020370	1	MTCH1	NM_014341	-1
HK3	NM_002115	1	NOC3L	NM_022451	-1
HP	NM_005143	1	ODC1	NM_002539	-1
IL10	NM_000572	1	PRKRIR	NM_004705	-1
IL18R1	NM_003855	1	RPGRIP1	NM_020366	-1
KCNE1	NM_000219	1	RPUSD4	NM_032795	-1
LCN2	NM_005564	1	SETD1B	NM_001353345	-1
LIN7A	NM_004664	1	TBC1D4	NM_014832	-1
OSCAR	NM_130771	1	TGFB1	NM_000358	-1
OSTalpha	NM_152672	1	TOMM20	NM_014765	-1
P2RX1	NM_002558	1	UBE2Q2	NM_173469	-1
PADI2	NM_007365	1	WDR75	NM_032468	-1
PECR	NM_018441	1	PLB1	NM_153021	1
PLAC8	NM_016619	1	PNPLA1	NM_173676	1

* Summary Effect indicates the direction of fold changes. '1' and '-1' mean up- and down-regulation in sepsis compared to SIRS/trauma, respectively.

A list of substitutable genes in Stanford11 was generated based on gene ontology biological processes (GOBPs). The biological functions associated with the Stanford82 genes was first analyzed through functional enrichment analyses of GOBPs using DAVID (Database for Annotation Visualization and Integrated Discovery) (Huang *et al.*, *Nature Protocols* 4:44-57, 2009). GO Direct category, which provides GO mappings directly annotated by the source database, was used. The terms with ≥ 1 genes were selected as “Stanford82-associated GOBPs.” The GOBPs that were represented by at least two genes, at least one of which derived from the Stanford11 genes were selected and visualized as a network using Enrichment Map v2.1.0, a Cytoscape plugin (Merico *et al.*, *PLoS One* 5:e13984, 2010). The connected GOBP terms were merged and defined as a single functional category. Genes involved in the merged GOBPs and having the same directional changes were defined as substitutable candidates in the same GOBP.

Twelve microarray datasets that were used in two studies by Sweeney *et al.* (Table 2; Sweeney *et al.*, *Critical Care Medicine* 45:1-10, 2017) were utilized. The first nine datasets are the discovery set that was used to identify the Stanford11 panel and the last three datasets are independent validation sets. The microarray datasets were normalized by the same methods used by Sweeney *et al.* Briefly, Affymetrix datasets were normalized using RMA or gcRMA (R package affy) (Gautier *et al.*, *Bioinformatics* 20:307-315, 2004). Agilent and Illumina datasets were background corrected based on normal-exponential convolution model and then between-arrays quantile normalized using R package limma (Ritchie *et al.*, *Nucleic Acids Research* 43:e47, 2015). The mean of multiple probes for common genes was used as the gene expression level after normalization. In the case of GSE74224, there was no probe for KIAA1370 of Stanford11; therefore, 10 genes were used to compute the performance of Stanford11 for this data.

Table 2. The 9 discovery and 3 independent microarray datasets and the performance of Stanford11.

	Dataset	Platform	Control	Case	# Control	# Case	AUC	Lower bound of 95% CI	Upper bound of 95% CI
Discovery set	GSE28750	GPL570	24 hours post-major surgery	Community-acquired sepsis	11	10	0.96	0.89	1
	GSE32707	GPL10558	Medical ICU $\pm$ SIRS, nonseptic	Sepsis, sepsis + ARDS	55	48	0.8	0.71	0.88
	GSE40012	GPL6947	ICU SIRS (66% trauma)	Sepsis from CAP	24	52	0.71	0.59	0.83
	GSE66099	GPL570	Pediatric ICU SIRS	Sepsis and septic shock	30	199	0.79	0.73	0.86

	Dataset	Platform	Control	Case	# Control	# Case	AUC	Lower bound of 95% CI	Upper bound of 95% CI
	Glue Grant Buffy coat, day [1–3)	GPL570	Trauma patients without infection	Trauma patients $\pm$ 24 hours from diagnosis of infection	65	9	0.91	0.83	1
	Glue Grant Buffy coat, day [3–6)	GPL570	Trauma patients without infection	Trauma patients $\pm$ 24 hours from diagnosis of infection	63	17	0.89	0.8	0.98
	Glue Grant Buffy coat, Day [6–10)	GPL570	Trauma patients without infection	Trauma patients $\pm$ 24 hours from diagnosis of infection	50	15	0.92	0.84	1
	Glue Grant Buffy coat, day [10–18)	GPL570	Trauma patients without infection	Trauma patients $\pm$ 24 hours from diagnosis of infection	22	4	0.85	0.7	1
	Glue Grant Buffy coat, day [18–24)	GPL570	Trauma patients without infection	Trauma patients $\pm$ 24 hours from diagnosis of infection	6	4	0.96	0.84	1
Validation set	GSE65682	GPL13667	ICU noninfected	CAP	33	101	0.78	0.68	0.87
	GSE74224	GPL5175	Postop	Sepsis	31	74	0.88	0.82	0.95
	E-MEXP- 3589	GPL10332	Hospitalized COPD	Infection	14	14	0.74	0.55	0.93

Dataset and platform are NCBI GEO accession numbers. CAP is community acquired pneumonia. COPD is chronic obstructive pulmonary disease. ARDS is acute respiratory distress syndrome.

In order to systematically evaluate the effect of gene substitution and reduction on classification performance, four different procedures were used as follows: 1) substitute one gene at a time; 2) substitute all possible genes; 3) retain only one gene for a GOBP where more than two genes are involved; 4) reduce panel by selecting one gene in each GOBP.

In order to evaluate the impact of biological function information in classification performance, three different approaches were used as follows: 1) use the 11 highest correlated genes based on expression profile with Stanford11 regardless of their biological functions; 2) use 11 randomly selected genes involved in chemotaxis, adhesion and migration biological function – one of GOBP terms associated with Stanford1 1; and 3) *k*-Top Scoring Pairs classifier (*k*TSP) using switchbox R package to identify a small set of paired genes (Afsari *et al.*, *Bioinformatics* 31:273-274, 2015). The AUC of *k*TSP

was calculated by defining the number of votes among k pairs as a diagnostic score (Marchionni *et al.*, *BMC Genomics* 14:336, 2014). The chemotaxis, adhesion and migration GOBP terms were selected as they contain more than 11 genes that can be fully substituted for Stanford11 genes. The classification performance of randomly selected gene sets was also tested by generating 100,000 combinations of 11 genes randomly selected from the Stanford82 genes. Classification performance was analyzed, along with biological processes that the 100,000 gene sets are involved in. The top and bottom 250 gene sets in order of performance were selected. The hierarchical clustering was applied to cluster GOBPs based on the number of genes in each GOBP from the top and bottom 250 gene sets.

Results

The overall procedure and the results of identifying substitutable genes for Stanford11 are summarized in FIG. 1A. There were 503 GOBPs represented by at least one of the Stanford82 genes. The genes in the Stanford11 panel were involved in 97 different GOBPs, with the exception of ZDHHC19 and KIAA1370 which are not associated with known biological function. Among the 97 GOBPs associated with Stanford11, 27 of them had at least one additional gene from the Stanford82 gene pool that is not in the Stanford11 panel and can be used as potential substitution candidates. These 27 GOBPs are represented by six key biological processes; 1) chemotaxis, angiogenesis, adhesion, migration; 2) immune response; 3) transcription by pol II; 4) platelet activation; 5) apoptosis; and 6) metabolism (FIG. 1B). Substitution candidates that are involved in these six key biological processes associated with the Stanford11 panel are summarized in Table 3. Among the six key biological processes, two processes, adhesion/migration and platelet activation, have more than two genes in the panel and the remaining four processes have just one gene. RPGRIP1, a gene associated with cell development and proliferation, was substitutable only with the genes already included in the Stanford11 panel; therefore, it was excluded for gene substitution. Among the substitutable genes, only genes with the same directional changes as genes in the Stanford11 panel were tested, *e.g.*, increased or decreased expression level in sepsis patients compared to controls. Among the 28 substitution candidates, 20 genes were retained for six genes in the Stanford11 panel (CEACAM1, C3AR1, GNA15, BATE, MTCH1, and C9orf95) representing five biological functions. TGFBI for the GOBP chemotaxis, angiogenesis, adhesion, migration was removed since no substitutable gene with the same directional change was retained. There was also no substitutable gene for HLA-DPB1 for immune response function; therefore, HLA-DPB1 was retained to keep all six biological processes during substitution and reduction procedures. Most of the six genes that can be substituted in the Stanford11 panel showed increased expression level in sepsis patients except MTCH1, a gene involved in apoptosis (Table 3).

Table 3. The substitutable genes of Stanford11. ↑ and ↓ indicates up or down regulation in sepsis, respectively. No substitutable gene was retained for TGFBI and HLA-DPB1 after considering consistency in directional changes. Finally, 20 genes were retained for replacing six original Stanford11 genes.

	Chemotaxis, angiogenesis, adhesion, migration	Interferon- gamma, antigen processing, immune response	Transcript ion by RNA pol II	PLC, phosphoryl- ation, platelet activation	Apoptosis	Metabolism
CEACAM1 ↑	ADAMTS3↑, CCR1↑, CD177↑, CD63↑, EMR1↑, FCER1G↑, IL10↑, OSTalpha↑, PSTPIP2↑, SIGLEC9↑, TBC1D4↓					
C3AR1 ↑	ANXA3↑, CCR1↑, CD177↑, EMR1↑, FCER1G↑, FES↑, FFAR3↑, IL10↑, S100A12↑			GPR84↑		
TGFBI ↓	CCR1↑, EMR1↑, FES↑, SIGLEC9↑					
GNA15 ↑	CCR1↑, EMR1↑, FFAR3↑			FCER1G↑, P2RX1↑		
HLA-DPB1 ↓		BPI↑, CCR1↑, FCER1G↑, FCGR1B↑, IL10↑, IL18R1↑				
BATF ↑			IL10↑, PLAC8↑, GLO1↓, PRKRIR↓, WDR75↓			
MTCH1 ↓					LCN2↑, OSTalpha↑, P2RX1↑, ARHGEF1 8↓	
C9orf95 ↑						C9orf103↑, SEPHS2↑

Since three genes (ZDHHC19, KIAA1370, and RPGRIP1) were excluded during the process of identification of substitutable genes, their contribution to the performance of Stanford11 was assessed. Excluding these genes from Stanford11 did not significantly affect the performance in both discovery and validation sets (Table 4). In the case of excluding all three genes, the performance decreased in one discovery and one validation set (Glue grant day [1-3] from 0.9145 to 0.865 and GSE74224 from 0.8814 to 0.8544), while increasing in one discovery set (GSE40012 from 0.7091 to 0.774). Therefore, these three genes contributed marginally to the diagnostic performance of Stanford11.

Table 4. The performances of reduced Stanford11 panels by excluding genes not associated to known biological processes or with no substitutable genes.

	Dataset	AUC				P-value*		
		Stanford 11	Exclude RPGRIP1	Exclude both KIAA1370 and ZDHHC19	Exclude all RPGRIP1, KIAA1370, ZDHHC19	Exclude RPGRIP1	Exclude both KIAA1370 and ZDHHC19	Exclude all RPGRIP1, KIAA1370, ZDHHC19
Discovery set	GSE28750	0.9636	0.9636	0.8273	0.7818	1	0.1	0.0672
	GSE32707	0.7962	0.7894	0.7894	0.7678	0.4702	0.5455	0.0715
	GSE40012	0.7091	0.7147	0.7588 [#]	0.774 [#]	0.7457	0.0174**	0.0123**
	GSE66099	0.7948	0.8028 [#]	0.799	0.8054	0.044**	0.7218	0.3916
	Glue Grant Buffy coat, day [1-3]	0.9145	0.9179	0.8632 [#]	0.865 [#]	0.2997	0.044**	0.0434**
	Glue Grant Buffy coat, day [3-6]	0.8898	0.8693	0.8898	0.8665	0.1139	1	0.2353
	Glue Grant Buffy coat, Day [6-10]	0.9213	0.9107	0.8947	0.8613	0.502	0.2846	0.1136
	Glue Grant Buffy coat, day [10-18]	0.8523	0.8295	0	0.7159	0.3865	0.8076	0.0784
	Glue Grant Buffy coat, day [18-24]	0.9583	0.875	0.9583	0.875	0.398	1	0.398
	GSE65682	0.7792	0.7843	0.7384 [#]	0.7459	0.3798	0.0118**	0.398
Validation set	GSE74224	0.8814	0.8483 [#]	0.8932	0.8544 [#]	0.0018**	0.0838	0.0186**
	E-MEXP-3589	0.7398	0.7449	0.7296	0.7602	0.9025	0.6772	0.6443

* The p-values were calculated by DeLong's test. ** indicates p-value less than 0.05.

[#] indicates significantly higher/lower AUC than Stanford11 (P-value ≤ 0.05).

To determine the impact of biological processes in the performance of a diagnostic panel, 100,000 panels consisting of 11 genes randomly selected from the Stanford82 list were generated. The classification performances of the 100,000 random 11 member gene sets in the 9 discovery datasets were

computed and sorted based on the performance as measured by AUC (FIG. 2A). The GOBP associated with genes in the top 250 and bottom 250 gene sets are summarized in FIG. 2B. The GOBPs represented by the top 250 gene sets were similar to the Stanford11 six key processes (FIG. 2B), such as transcription by pol II (cluster 1 and 2 in FIGS. 2B and 2C), phosphorylation (cluster 4), apoptosis (cluster 5), PLC (cluster 6), chemotaxis (cluster 7), antigen processing and presentation (cluster 11), and metabolic process (cluster 13). The cell development and proliferation process in cluster 3 was also frequently involved in high performing gene sets. RPGRIP1, which has no substitutable gene, was involved in this biological process. However, removing RPGRIP1 from Stanford11 did not significantly decrease diagnostic performance, as shown in Table 4.

A total of 12 microarray datasets from the public domain were used to evaluate the effect of gene substitution/reduction on the diagnostic performances of Stanford11. Nine of the datasets were used in the original discovery of the Stanford11 gene panel. The other three, GSE65682, GSE74224, and E-MTAB-3589, were not used in the development of Stanford11 panel; therefore, they were used as independent validation data in the study.

Based on the substitution candidates listed in Table 3, one gene was changed at a time for the six substitutable genes in the Stanford11 panel. As shown in FIGS. 3A-3F, one gene substitution did not affect the overall diagnostic performance in the nine discovery and three validation datasets. In the discovery datasets, the average AUCs of the substitutions were not significantly lower than the original Stanford11 panel, except using the GSE74224 dataset when replacing GNA15 (FIG. 3E).

The effect of substituting genes representing all functional categories simultaneously was then tested. If a function had more than one substitutable gene, all combinations of genes were enumerated and their classification performances were tested and are summarized in FIG. 4. Except for the GSE40012 and GSE66099 datasets used in the Stanford11 discovery process, there were multiple five gene substitutions that showed similar performance as the original Stanford11. In summary, gene substitution based on the same biological process and direction of concentration changes can provide alternative panels that have similar diagnostic performance.

The possibility of using representative genes from each biological process to reduce the number of features in the diagnostic panel was investigated. Among the six representative biological processes of the Stanford11 panel, two biological functions have more than one gene in the panel. The GOBP “PLC, phosphorylation, platelet activation function” has two genes (C3AR1 and GNA15) and “chemotaxis, angiogenesis, adhesion, migration function” has four genes (CEACAM1, C3AR1, TGFBI, and GNA15) in the panel. We calculated AUCs of panels where only one gene was retained. As shown in Fig 5A, the two panels with only one gene retained from “PLC, phosphorylation, platelet activation function” had similar performance to the Stanford11. The panels with only one gene from the GOBP “chemotaxis, angiogenesis, adhesion, migration function” also have similar performance in the datasets used to identify Stanford11 (Fig 5B). The impact of retaining only one gene from both GOPBs was tested and the average diagnostic performance was not significantly different from the original Stanford11 panel in

all the discovery and validation sets (Fig 5C). In all cases, there were multiple panels with reduced features that delivered better performances in more than half of the independent validation datasets.

To explore the possibility of just using representative genes from key biological processes associated with Stanford11, all possible combinations of 6 gene panels were generated from genes mapped to those six biological processes. Diagnostic performance for all six-gene combinations was
5 computed (FIG. 6). Among the 1,482 six-gene combinations, 73 new panels that have higher performance than the lower bound of 95% confidence intervals in all discovery datasets than the original panel were selected (Table 5). Among the 73 new panels, 22 panels had higher performance even in the two validation sets (GSE65682 and GSE74224, Table 6).

Table 5. 73 new six-gene panels including six genes from the six key biological processes. The 73 panels have higher performance than the lower bound of 95% confidence intervals in all discovery datasets than the original StanfordI1.

* indicates p-value from DeLong's test in comparison with the StanfordI1 less than 0.05.

Chemotaxis, angiogenesis, adhesion, migration	Interferon-gamma, antigen processing, immune response	Transcription by RNA pol II	PLC, phosphorylation, platelet activation	Apoptosis	Metabolism	Discovery set								Validation set			
						GSE 28750	GSE 32707	GSE 40012	GSE 60099	Glue Grant Buffy coat, day 1-3	Glue Grant Buffy coat, day 3-6	Glue Grant Buffy coat, Day [6-10]	Glue Grant Buffy coat, day [10-18]	Glue Grant Buffy coat, day [18-24]	GSE 65682	GSE 74224	E-MEXP-3589
Gene1	Gene2	Gene3	Gene4	Gene5	Gene6												
ADAMTS3	HLA-DPB1	BATF	FCER1G	ARHGEF18	C9orf95	0.8909	0.7648	0.6354	0.7648	0.8359*	0.8711	0.9400	0.7386	0.9583	0.7789	0.7912*	0.5612
ADAMTS3	HLA-DPB1	BATF	FCER1G	MTCH1	C9orf95	0.9182	0.7602	0.6587	0.7665	0.8513*	0.8711	0.9360	0.7386	0.9583	0.8119	0.8156*	0.6378
CCR1	HLA-DPB1	BATF	GPR84	MTCH1	C9orf103	0.8909	0.7348*	0.6338*	0.7372*	0.8462*	0.8936	0.9600	0.8182	0.9167	0.8218	0.8553	0.6378
CCR1	HLA-DPB1	BATF	FCER1G	ARHGEF18	C9orf103	0.9000	0.7117*	0.6042*	0.7504*	0.8735	0.8627	0.9507	0.8750	0.9167	0.7951	0.8535	0.6378
CCR1	HLA-DPB1	BATF	FCER1G	ARHGEF18	C9orf95	0.9364	0.7250*	0.6795	0.7786	0.8650*	0.8655	0.9587	0.8295	0.9167	0.7840	0.8435	0.5969
CCR1	HLA-DPB1	BATF	FCER1G	MTCH1	C9orf95	0.9455	0.7269*	0.7003	0.7826	0.8667*	0.8861	0.9640	0.8523	0.9167	0.8128	0.8588	0.6684
CCR1	HLA-DPB1	BATF	C3AR1	ARHGEF18	C9orf95	0.8909	0.7568	0.6995	0.7896	0.8410*	0.8768	0.9387	0.8750	0.9583	0.7807	0.8854	0.6633
CCR1	HLA-DPB1	BATF	C3AR1	MTCH1	C9orf103	0.9000	0.7227*	0.6675	0.7742	0.8821	0.8814	0.9400	0.8750	0.9167	0.8170	0.9058	0.6990
CCR1	HLA-DPB1	BATF	C3AR1	MTCH1	C9orf95	0.9091	0.7542	0.7204	0.7940	0.8410*	0.8945	0.9440	0.8409	0.9583	0.8095	0.8967	0.6786
CCR1	HLA-DPB1	BATF	GNAI5	MTCH1	C9orf95	0.9545	0.7332*	0.7260	0.8062	0.8342*	0.9094	0.9627	0.8636	0.9167	0.8080	0.8893	0.6378
CD177	HLA-DPB1	BATF	GPR84	MTCH1	C9orf95	0.8909	0.7470*	0.6274*	0.739*	0.8615	0.8534	0.9373	0.7955	1.0000	0.7933	0.8191*	0.6327

Gene1	Gene2	Gene3	Gene4	Gene5	Gene6	Metabo- lism	Discovery set								Validation set			
							GSE 28750	GSE 32707	GSE 40012	GSE 66099	Glue Grant Buffy coat, day [1- 3]	Glue Grant Buffy coat, day [3- 6]	Glue Grant Buffy coat, Day [6-10]	Glue Grant Buffy coat, day [10- 18]	Glue Grant Buffy coat, day [18- 24]	GSE 65682	GSE 74224	E- MLXP- 3589
Chemotaxis, angiogenesis, adhesion, migration	Interferon- gamma, antigen processing, immune response	Transcrip- tion by RNA pol II	PLC, phosphoryl- ation, platelet activation	Apoptosis														
	CD177	HLA-DPB1	BATF	FCER1G	ARHGEF18	C9orf95	0.9182	0.7159*	0.6050*	0.7653	0.8906	0.8142	0.9293	0.8182	1.0000	0.7762	0.8178*	0.6429
	CD177	HLA-DPB1	BATF	FCER1G	MITCH1	C9orf95	0.9273	0.7182*	0.6242*	0.7635	0.8974	0.8161	0.9307	0.8182	1.0000	0.8032	0.8304	0.6378
	CD177	HLA-DPB1	BATF	C3AR1	MITCH1	C9orf95	0.8909	0.7458*	0.6442*	0.7784	0.8838	0.8366	0.9227	0.8295	1.0000	0.8014	0.8827	0.6684
	CD177	HLA-DPB1	BATF	GNA15	ARHGEF18	C9orf95	0.9273	0.7379*	0.6202*	0.7822	0.8718	0.8403	0.9413	0.8409	1.0000	0.7699	0.8413	0.6429
	CD177	HLA-DPB1	BATF	GNA15	MITCH1	C9orf95	0.9455	0.7333*	0.6378*	0.7874	0.8752	0.8450	0.9507	0.8295	1.0000	0.7972	0.8570	0.6531
	CD63	HLA-DPB1	PLAC8	FCER1G	MITCH1	C9orf95	0.9000	0.7231*	0.7244	0.7387*	0.8957	0.8973	0.9067	0.7500	0.9167	0.8158	0.9128	0.6122
	CD63	HLA-DPB1	PLAC8	GNA15	ARHGEF18	C9orf95	0.9273	0.7163*	0.7372	0.7611	0.8427	0.9328*	0.9307	0.7955	1.0000	0.7852	0.9220	0.5663
	CD63	HLA-DPB1	PLAC8	GNA15	MITCH1	C9orf95	0.9636	0.7186*	0.7620	0.7670	0.8444	0.9300	0.9427	0.7727	1.0000	0.8107	0.9333*	0.5918
	CD63	HLA-DPB1	BATF	GPR84	ARHGEF18	C9orf95	0.8909	0.7496*	0.6587	0.7466*	0.8530	0.8758	0.9320	0.7955	1.0000	0.7705	0.8400	0.5969
	CD63	HLA-DPB1	BATF	GPR84	MITCH1	C9orf103	0.8909	0.7223*	0.6386	0.7328*	0.8769	0.8739	0.9453	0.7841	0.8750	0.8116	0.8505	0.6327
	CD63	HLA-DPB1	BATF	GPR84	MITCH1	C9orf95	0.8909	0.7481*	0.6803	0.7497*	0.8547	0.8711	0.9427	0.7841	1.0000	0.7993	0.8553	0.6071
	CD63	HLA-DPB1	BATF	FCER1G	ARHGEF18	C9orf103	0.8909	0.7208*	0.6090*	0.7474*	0.8974	0.8478	0.9387	0.7955	0.8750	0.7942	0.8492	0.6224
	CD63	HLA-DPB1	BATF	FCER1G	ARHGEF18	C9orf95	0.9000	0.7420	0.6771	0.7784	0.8957	0.8347	0.9440	0.7273	1.0000	0.7780	0.8566	0.6122
	CD63	HLA-DPB1	BATF	FCER1G	MITCH1	C9orf103	0.9091	0.7231*	0.6346*	0.7422*	0.9060	0.8609	0.9387	0.7614	0.8750	0.8179	0.8636	0.6684
	CD63	HLA-DPB1	BATF	FCER1G	MITCH1	C9orf95	0.9273	0.7424	0.6979	0.7789	0.9043	0.8478	0.9400	0.7386	0.9583	0.8107	0.8705	0.6276
	CD63	HLA-DPB1	BATF	C3AR1	MITCH1	C9orf95	0.8909	0.7451	0.7147	0.7953	0.8838	0.8609	0.9227	0.7614	1.0000	0.8080	0.9067	0.6633
CD63	HLA-DPB1	BATF	GNA15	ARHGEF18	C9orf95	0.9182	0.7322	0.7188	0.7963	0.8598	0.8702	0.9520	0.7955	0.9583	0.7726	0.8788	0.5969	

Chemotaxis, angiogenesis, adhesion, migration	Interferon- gamma, antigen processing, immune response	Transcrip- tion by RNA pol II	PLC, phosphoryl- ation, platelet activation	Apoptosis	Metabo- lism	Discovery set								Validation set			
						GSE 28750	GSE 32707	GSE 40012	GSE 66099	Glue Grant Buffy coat, day [1- 3]	Glue Grant Buffy coat, day [3- 6]	Glue Grant Buffy coat, day [6-10]	Glue Grant Buffy coat, day [10- 18]	Glue Grant Buffy coat, day [18- 24]	GSE 65682	GSE 74224	E- MLXP- 3589
Gene1	Gene2	Gene3	Gene4	Gene5	Gene6												
CD63	HLA-DPB1	BATF	GNAI5	MITCH1	C9orf95	0.9455	0.7299*	0.7324	0.8005	0.8701	0.8805	0.9547	0.7727	1.0000	0.8029	0.8915	0.6378
EMR1	HLA-DPB1	BATF	FCER1G	ARHGEF18	C9orf95	0.9182	0.7216*	0.6506	0.7658	0.8667	0.8581	0.9440	0.8636	1.0000	0.7768	0.8483	0.6071
EMR1	HLA-DPB1	BATF	FCER1G	MITCH1	C9orf103	0.8909	0.7117*	0.5962*	0.7353*	0.8632	0.8599	0.9453	0.8636	0.8750	0.8161	0.8496	0.6429
EMR1	HLA-DPB1	BATF	FCER1G	MITCH1	C9orf95	0.9182	0.7159*	0.6675	0.7675	0.8701	0.8646	0.9507	0.8636	1.0000	0.8137	0.8596	0.6071
EMR1	HLA-DPB1	BATF	C3AR1	ARHGEF18	C9orf95	0.8909	0.7398*	0.6707	0.7791	0.8462	0.8665	0.9227	0.8750	1.0000	0.7795	0.8867	0.6327
EMR1	HLA-DPB1	BATF	C3AR1	MITCH1	C9orf95	0.8909	0.7341*	0.6931	0.7851	0.8444	0.8702	0.9333	0.8409	1.0000	0.8149	0.8963	0.6582
EMR1	HLA-DPB1	BATF	GNAI5	MITCH1	C9orf95	0.9273	0.7159*	0.6915	0.7844	0.8342	0.8833	0.9467	0.8523	1.0000	0.8044	0.8836	0.6071
FCER1G	HLA-DPB1	PLAC8	C3AR1	MITCH1	C9orf95	0.9000	0.7273*	0.7356	0.7628	0.8615*	0.8599	0.8747	0.8068	1.0000	0.8080	0.9241	0.6531
FCER1G	HLA-DPB1	PLAC8	GNAI5	ARHGEF18	C9orf95	0.9182	0.7330	0.7220	0.7688	0.8496	0.9104	0.9173	0.8182	1.0000	0.7861	0.9098	0.5408
FCER1G	HLA-DPB1	PLAC8	GNAI5	MITCH1	C9orf95	0.9545	0.7322*	0.7460	0.7719	0.8547	0.9188	0.9293	0.7841	1.0000	0.8092	0.9185	0.5816*
FCER1G	HLA-DPB1	BATF	GPR84	ARHGEF18	C9orf95	0.8909	0.7508	0.6587	0.7521*	0.8598	0.8609	0.9307	0.8182	1.0000	0.7747	0.8147*	0.5816
FCER1G	HLA-DPB1	BATF	GPR84	MITCH1	SEPHS2	0.8909	0.7337*	0.6234*	0.7437*	0.8359*	0.8459	0.9360	0.8068	1.0000	0.8038	0.8095*	0.6122
FCER1G	HLA-DPB1	BATF	GPR84	MITCH1	C9orf95	0.8909	0.7424*	0.6755	0.7514*	0.8615	0.8665	0.9387	0.7727	0.9583	0.8041	0.833*	0.6071
FCER1G	HLA-DPB1	BATF	C3AR1	MITCH1	SEPHS2	0.9000	0.7273*	0.6619	0.7995	0.8667	0.8030*	0.9187	0.8409	1.0000	0.8086	0.8867	0.6582
FCER1G	HLA-DPB1	BATF	C3AR1	MITCH1	C9orf95	0.9000	0.7413	0.7083	0.7980	0.8889	0.8413	0.9227	0.8068	1.0000	0.8071	0.8963	0.6480
FCER1G	HLA-DPB1	BATF	GNAI5	ARHGEF18	C9orf95	0.9455	0.7413	0.6947	0.8027	0.8735	0.8533	0.9520	0.7841	0.9583	0.7699	0.8640	0.5867
FCER1G	HLA-DPB1	BATF	GNAI5	MITCH1	SEPHS2	0.9727	0.7242*	0.6747	0.7982	0.8359*	0.8329	0.9480	0.8409	1.0000	0.8077	0.8614	0.6224

Gene1	Gene2	Gene3	Transcription by RNA pol II	PLC, phosphorylation, platelet activation	Apoptosis	Metabolism	Discovery set								Validation set			
							GSE 28750	GSE 32707	GSE 40012	GSE 66099	Glue Grant Buffy coat, day 1-3	Glue Grant Buffy coat, day 3-6	Glue Grant Buffy coat, day 6-10	Glue Grant Buffy coat, day 10-18	Glue Grant Buffy coat, day 18-24	GSE 65682	GSE 74224	E-MEXP-3589
Chemotaxis, angiogenesis, adhesion, migration	Interferon-gamma, antigen processing, immune response	Transcription by RNA pol II																
FCER1G	HLA-DPB1	BATF	Gene4	GNAI5	Gene5	Gene6	0.9818	0.7352	0.7123	0.8040	0.8872	0.8599	0.9547	0.7841	0.9583	0.8026	0.8749	0.6122
OST1alpha	HLA-DPB1	BATF	Gene4	GNAI5	Gene5	Gene6	0.8909	0.7701	0.6338*	0.7496*	0.8940	0.8852	0.9280	0.8409	0.9167	0.7690	0.8147*	0.5918
OST1alpha	HLA-DPB1	BATF	Gene4	GNAI5	Gene5	Gene6	0.8909	0.7295*	0.6242*	0.7367*	0.8855	0.8693	0.9320	0.8523	0.8750	0.8065	0.7960*	0.6276
OST1alpha	HLA-DPB1	BATF	Gene4	GNAI5	Gene5	Gene6	0.9091	0.7640	0.6474	0.7487*	0.8991	0.8805	0.9307	0.8295	0.8750	0.8035	0.8326	0.6224
SIGLEC9	HLA-DPB1	BATF	Gene4	GNAI5	Gene5	Gene6	0.9455	0.7799	0.6907	0.7506*	0.8906	0.8123*	0.9320	0.8182	1.0000	0.8125	0.8745	0.6582
ANXA3	HLA-DPB1	BATF	Gene4	GNAI5	Gene5	Gene6	0.9091	0.7152*	0.6530	0.7930	0.8581	0.8702	0.9373	0.8182	1.0000	0.7993	0.8383	0.6531
FES	HLA-DPB1	PLAC8	Gene4	GPR84	Gene5	Gene6	0.8909	0.7383	0.7027	0.7320*	0.8427	0.8553	0.9160	0.7841	1.0000	0.7939	0.8758	0.6020
FES	HLA-DPB1	PLAC8	Gene4	FCER1G	Gene5	Gene6	0.9091	0.7318*	0.7204	0.7521	0.8906	0.8385	0.8920	0.7841	1.0000	0.8089	0.8945	0.6173
FES	HLA-DPB1	PLAC8	Gene4	GNAI5	Gene5	Gene6	0.8909	0.7292*	0.6643	0.7441*	0.8701	0.8749	0.9187	0.8409	0.8750	0.7870	0.9098	0.6122
FES	HLA-DPB1	PLAC8	Gene4	GNAI5	Gene5	Gene6	0.9000	0.7470	0.7364	0.7735	0.8427	0.8693	0.9147	0.8182	0.9583	0.7768	0.9102	0.5867
FES	HLA-DPB1	PLAC8	Gene4	GNAI5	Gene5	Gene6	0.9545	0.7428	0.7500	0.7760	0.8513	0.8655	0.9120	0.8068	0.9583	0.8005	0.9150	0.6071
FES	HLA-DPB1	BATF	Gene4	GPR84	Gene5	Gene6	0.8909	0.7765	0.6851	0.7553*	0.8479	0.8487*	0.9227	0.7955	1.0000	0.7999	0.8309*	0.6327
FES	HLA-DPB1	BATF	Gene4	FCER1G	Gene5	Gene6	0.8909	0.7348*	0.6050*	0.7513	0.8855	0.8114*	0.9347	0.7841	0.8750	0.7897	0.8069*	0.6633
FES	HLA-DPB1	BATF	Gene4	FCER1G	Gene5	Gene6	0.9000	0.7341*	0.6258*	0.7497	0.8889	0.8226*	0.9333	0.8068	0.8750	0.8158	0.8291	0.6633
FES	HLA-DPB1	BATF	Gene4	C3AR1	Gene5	Gene6	0.9000	0.7799	0.7115	0.7970	0.8667	0.8161*	0.9160	0.7841	1.0000	0.8026	0.8806	0.6786
FES	HLA-DPB1	BATF	Gene4	GNAI5	Gene5	Gene6	0.9273	0.7742	0.7011	0.7968	0.8564	0.8226	0.9320	0.7955	0.9583	0.7672	0.8470	0.6173
FES	HLA-DPB1	BATF	Gene4	GNAI5	Gene5	Gene6	0.9455	0.7670	0.7260	0.8022	0.8598	0.8245	0.9427	0.7841	0.9583	0.7990	0.8627	0.6480

Gene1	Gene2	Gene3	PLC, phosphorylation, platelet activation	Apoptosis	Metabolism	Discovery set								Validation set			
						GSE 28750	GSE 32707	GSE 40012	GSE 66099	Glue Grant Buffy coat, day 1-3)	Glue Grant Buffy coat, day 3-6)	Glue Grant Buffy coat, day 6-10)	Glue Grant Buffy coat, day 10-18)	Glue Grant Buffy coat, day 18-24)	GSE 65682	GSE 74224	E-MEXP-3589
Chemotaxis, angiogenesis, adhesion, migration	Interferon-gamma, antigen processing, immune response	Transcription by RNA pol II															
Gene1	Gene2	Gene3	Gene4	Gene5	Gene6												
S100A12	HLA-DPB1	PLAC8	FCER1G	MITCH1	C9orf95	0.9000	0.7174*	0.6907	0.7367*	0.8855	0.8926	0.9053	0.7955	0.9583	0.8155	0.8718	0.6122
S100A12	HLA-DPB1	BATF	GPR84	ARHGEF18	C9orf95	0.8909	0.7367*	0.6482	0.7476*	0.8479*	0.8768	0.9280	0.7955	1.0000	0.7717	0.7951*	0.6122
S100A12	HLA-DPB1	BATF	GPR84	MITCH1	C9orf95	0.8909	0.7307*	0.6603	0.7504*	0.8513	0.8758	0.9320	0.7841	1.0000	0.8002	0.8112*	0.6122
S100A12	HLA-DPB1	BATF	FCER1G	ARHGEF18	C9orf95	0.9000	0.7280*	0.6482*	0.7782	0.8940	0.8599	0.9507	0.7614	1.0000	0.7786	0.7942*	0.6020
S100A12	HLA-DPB1	BATF	FCER1G	MITCH1	C9orf95	0.9182	0.7242*	0.6611	0.7839	0.8957	0.8693	0.9493	0.7500	1.0000	0.8086	0.8134*	0.6378
S100A12	HLA-DPB1	BATF	C3AR1	MITCH1	C9orf95	0.9000	0.7311*	0.6939	0.7998	0.8752	0.8730	0.9093	0.7955	1.0000	0.8074	0.8670	0.6531
S100A12	HLA-DPB1	BATF	GNAI5	ARHGEF18	C9orf95	0.9182	0.7197*	0.6747	0.7963	0.8564	0.8945	0.9453	0.7841	1.0000	0.7726	0.8352	0.6071
S100A12	HLA-DPB1	BATF	GNAI5	MITCH1	C9orf103	0.9000	0.7106*	0.6202*	0.7693	0.8855	0.9010	0.9547	0.8523	0.8750	0.8128	0.8405	0.6327
S100A12	HLA-DPB1	BATF	GNAI5	MITCH1	C9orf95	0.9455	0.7201*	0.6947	0.8044	0.8684	0.8973	0.9560	0.8068	1.0000	0.8035	0.8461	0.6276
C3AR1	HLA-DPB1	BATF	GNAI5	ARHGEF18	C9orf95	0.9182	0.7583	0.7091	0.8092	0.8513	0.8693	0.9187	0.8182	1.0000	0.7678	0.9050	0.6276
C3AR1	HLA-DPB1	BATF	GNAI5	MITCH1	C9orf95	0.9273	0.7542	0.7228	0.8136	0.8479	0.8711	0.9267	0.8182	1.0000	0.7999	0.9133	0.6480

Table 7. Effect of adding RPGRIP1 to each of the 73 new six-gene panels. * indicates p-value from DeLong's test in comparison with the new 6 gene panels less than 0.05.

Chemotax, angiogen., adhesion, migration	Immune response	Transcr. by RNA pol II	PLC, phosphoryl- ation, platelet activation	Apoptosis	Metabo- lism	Gene7	Discovery set										Validation set			
							GSE 28750	GSE 32707	GSE 40012	GSE 66099	Gluc Grant Buffy coat, day [1-3]	Gluc Grant Buffy coat, day [3-6]	Gluc Grant Buffy coat, Day [6-10]	Gluc Grant Buffy coat, day [10-18]	Gluc Grant Buffy coat, day [18-24]	GSE 65682	GSE 74224	E- MEXP -3589		
Gene1	Gene2	Gene3	Gene4	Gene5	Gene6															
ADAMTS3	HLA-DPB1	BATF	FCER1G	ARHGEF18	C9orf95	RPGRIP1	0.9182	0.7932	0.6530	0.7554	0.8410	0.8992	0.9440	0.8182	1.0000	0.7525	0.8714*	0.6327		
ADAMTS3	HLA-DPB1	BATF	FCER1G	MTCH1	C9orf95	RPGRIP1	0.9273	0.7886	0.6659	0.7526	0.8462	0.9085	0.9480	0.8295	1.0000	0.7534	0.8945*	0.6582		
CCR1	HLA-DPB1	BATF	GPR84	MTCH1	C9orf103	RPGRIP1	0.9182	0.7496	0.6458	0.7302	0.8444	0.9169	0.9507	0.8636	1.0000	0.7594	0.8836*	0.6531		
CCR1	HLA-DPB1	BATF	FCER1G	ARHGEF18	C9orf103	RPGRIP1	0.9455	0.7261	0.6338	0.7471	0.8735	0.9085*	0.9507	0.8864	0.9583	0.7678	0.8980*	0.6531		
CCR1	HLA-DPB1	BATF	FCER1G	ARHGEF18	C9orf95	RPGRIP1	0.9364	0.7568	0.7019	0.7744	0.8615	0.8926	0.9627	0.8864	1.0000	0.7432	0.8840*	0.6378		
CCR1	HLA-DPB1	BATF	FCER1G	MTCH1	C9orf95	RPGRIP1	0.9545	0.7534	0.7179	0.7764	0.8632	0.9029	0.9680	0.8864	1.0000	0.7390	0.8993*	0.6429		
CCR1	HLA-DPB1	BATF	C3AR1	ARHGEF18	C9orf95	RPGRIP1	0.9091	0.7758	0.7171	0.7814	0.8376	0.9001	0.9453	0.8864	1.0000	0.7405	0.9189*	0.6735		
CCR1	HLA-DPB1	BATF	C3AR1	MTCH1	C9orf103	RPGRIP1	0.9273	0.7508	0.6819	0.7640	0.8667	0.9094	0.9413	0.8864	1.0000	0.7627	0.9381*	0.6735		
CCR1	HLA-DPB1	BATF	C3AR1	MTCH1	C9orf95	RPGRIP1	0.9273	0.7742	0.7308	0.7843	0.8376	0.9085	0.9480	0.8864	1.0000	0.7300	0.9329*	0.6888		
CCR1	HLA-DPB1	BATF	GNAI5	MTCH1	C9orf95	RPGRIP1	0.9455	0.7652	0.7340	0.7941	0.8308	0.9244	0.9667	0.9091	1.0000	0.7348	0.9289*	0.6071		
CD177	HLA-DPB1	BATF	GPR84	MTCH1	C9orf95	RPGRIP1	0.9000	0.7470	0.6546	0.7310	0.8684	0.8786	0.9400	0.8295	1.0000	0.7378	0.8540*	0.6378		
CD177	HLA-DPB1	BATF	FCER1G	ARHGEF18	C9orf95	RPGRIP1	0.9182	0.7424	0.6474	0.7605	0.8957	0.8403	0.9280	0.8182	1.0000	0.7474	0.8614*	0.6786		
CD177	HLA-DPB1	BATF	FCER1G	MTCH1	C9orf95	RPGRIP1	0.9455	0.7436	0.6563	0.7576	0.9009	0.8459*	0.9360	0.8182	1.0000	0.7489	0.8727*	0.6684		
CD177	HLA-DPB1	BATF	C3AR1	MTCH1	C9orf95	RPGRIP1	0.9091	0.7625	0.6675	0.7709	0.8923	0.8590	0.9293	0.8295	1.0000	0.7444	0.9176*	0.6786		
CD177	HLA-DPB1	BATF	GNAI5	ARHGEF18	C9orf95	RPGRIP1	0.9455	0.7564	0.6563	0.7787	0.8752	0.8646	0.9600	0.8409	1.0000	0.7387	0.8862*	0.6684		

Chemotax, angiogen., adhesion, migration	Immune response	Transcr. by RNA pol II	PLC, phosphoryl- ation, platelet activation	Apoptosis	Metabo- lism	Discovery set								Validation set			
						GSE 28750	GSE 32707	GSE 40012	GSE 66099	Glue Grant Buffy coat, day [1-3]	Glue Grant Buffy coat, day [3-6]	Glue Grant Buffy coat, day [6-10]	Glue Grant Buffy coat, day [10-18]	Glue Grant Buffy coat, day [18-24]	GSE 65682	GSE 74224	E- MEXP -3589
Gene1	Gene2	Gene3	Gene4	Gene5	Gene6	Gene7											
CD177	HLA-DPB1	BATF	GNAI5	MTCH1	C9orf95	RPGRIP1	0.9727	0.7538	0.6707	0.7784	0.8821	0.8702	0.9640	1.0000	0.7399	0.8997*	0.6735
CD63	HLA-DPB1	PLAC8	FCER1G	MTCH1	C9orf95	RPGRIP1	0.9182	0.7364	0.7220	0.7266	0.8923	0.9197	0.9200	1.0000	0.7579	0.9394*	0.6173
CD63	HLA-DPB1	PLAC8	GNAI5	ARHGEF18	C9orf95	RPGRIP1	0.9545	0.7201	0.7356	0.7596	0.8427	0.9458	0.9387	1.0000	0.7477	0.9507*	0.6173
CD63	HLA-DPB1	PLAC8	GNAI5	MTCH1	C9orf95	RPGRIP1	0.9636	0.7277	0.7388	0.7611	0.8496	0.9524	0.9440	1.0000	0.7477	0.9568*	0.6173
CD63	HLA-DPB1	BATF	GPR84	ARHGEF18	C9orf95	RPGRIP1	0.8909	0.7595	0.6803	0.7427	0.8547	0.8982	0.9400	1.0000	0.7270	0.8836*	0.6531
CD63	HLA-DPB1	BATF	GPR84	MTCH1	C9orf103	RPGRIP1	0.9000	0.7242	0.6426	0.7233	0.8735	0.9020	0.9467	0.9583	0.7489	0.8849*	0.6224
CD63	HLA-DPB1	BATF	GPR84	MTCH1	C9orf95	RPGRIP1	0.8909	0.7617	0.6907	0.7430	0.8564	0.8982	0.9440	1.0000	0.7279	0.8958*	0.6378
CD63	HLA-DPB1	BATF	FCER1G	ARHGEF18	C9orf103	RPGRIP1	0.9091	0.7258	0.6378	0.7395	0.8957	0.8898	0.9413	0.9583	0.7621	0.9037*	0.6378
CD63	HLA-DPB1	BATF	FCER1G	ARHGEF18	C9orf95	RPGRIP1	0.9273	0.7545	0.6907	0.7704	0.8838	0.8525	0.9493	1.0000	0.7405	0.9067*	0.6786
CD63	HLA-DPB1	BATF	FCER1G	MTCH1	C9orf103	RPGRIP1	0.9364	0.7250	0.6482	0.7327	0.8991	0.8908	0.9453	0.9583	0.7609	0.9102*	0.6480
CD63	HLA-DPB1	BATF	FCER1G	MTCH1	C9orf95	RPGRIP1	0.9364	0.7542	0.6995	0.7717	0.8923	0.8665	0.9560	1.0000	0.7372	0.9167*	0.6633
CD63	HLA-DPB1	BATF	C3AR1	MTCH1	C9orf95	RPGRIP1	0.9091	0.7678	0.7204	0.7841	0.8718	0.8730	0.9413	1.0000	0.7285	0.9446*	0.6990
CD63	HLA-DPB1	BATF	GNAI5	ARHGEF18	C9orf95	RPGRIP1	0.9273	0.7511	0.7083	0.7901	0.8632	0.8758	0.9613	1.0000	0.7303	0.9285*	0.6633
CD63	HLA-DPB1	BATF	GNAI5	MTCH1	C9orf95	RPGRIP1	0.9364	0.7519	0.7196	0.7871	0.8650	0.8861	0.9653	1.0000	0.7300	0.9416*	0.6429
EMR1	HLA-DPB1	BATF	FCER1G	ARHGEF18	C9orf95	RPGRIP1	0.9182	0.7470	0.6691	0.7580	0.8650	0.8908	0.9507	1.0000	0.7378	0.8806*	0.6276
EMR1	HLA-DPB1	BATF	FCER1G	MTCH1	C9orf103	RPGRIP1	0.9545	0.7201	0.3814	0.7256	0.8615	0.8898	0.9467	0.9583	0.7642	0.8840*	0.6531
EMR1	HLA-DPB1	BATF	FCER1G	MTCH1	C9orf95	RPGRIP1	0.9455	0.7420	0.6779	0.7601	0.8667	0.8954	0.9547	1.0000	0.7366	0.8923*	0.6378
EMR1	HLA-DPB1	BATF	C3AR1	ARHGEF18	C9orf95	RPGRIP1	0.9091	0.7648	0.6803	0.7735	0.8530	0.8908	0.9307	1.0000	0.7363	0.9185*	0.6837

Chemotax., adhesion, migration	Immune response	Transcr. by RNA pol II	PLC, phosphoryl- ation, platelet activation	Apoptosis	Metabo- lism	Gene7	Discovery set						Validation set			
							GSE 28750	GSE 32707	GSE 40012	GSE 66099	Glue Grant Buffy coat, day [1-3]	Glue Grant Buffy coat, day [3-6]	Glue Grant Buffy coat, day [6-10]	Glue Grant Buffy coat, day [10-18]	Glue Grant Buffy coat, day [18-24]	E- MEXP -3589
Gene1	Gene2	Gene3	Gene4	Gene5	Gene6	Gene7										
EMR1	HLA-DPB1	BATF	C3AR1	MTCH1	C9orf95	RPGRIP1	0.9182	0.7663	0.6979	0.7760	0.8530	0.8992	0.9320	0.8977	1.0000	0.7330
EMR1	HLA-DPB1	BATF	GNAI5	MTCH1	C9orf95	RPGRIP1	0.9273	0.7489	0.6875	0.7765	0.8393	0.9225	0.9520	0.9318	1.0000	0.7267
FCER1G	HLA-DPB1	PLAC8	C3AR1	MTCH1	C9orf95	RPGRIP1	0.9182	0.7462	0.7412	0.7571	0.8581	0.8936*	0.9013	0.8295	1.0000	0.7465
FCER1G	HLA-DPB1	PLAC8	GNAI5	ARHGEF18	C9orf95	RPGRIP1	0.9455	0.7500	0.7300	0.7675	0.8530	0.9328	0.9293	0.8636	1.0000	0.7495
FCER1G	HLA-DPB1	PLAC8	GNAI5	MTCH1	C9orf95	RPGRIP1	0.9636	0.7481	0.7396	0.7685	0.8462	0.9430	0.9373	0.8523	1.0000	0.7486
FCER1G	HLA-DPB1	BATF	GPR84	ARHGEF18	C9orf95	RPGRIP1	0.8909	0.7708	0.6731	0.7462	0.8547	0.8852	0.9347	0.8068	1.0000	0.7270
FCER1G	HLA-DPB1	BATF	GPR84	MTCH1	SEPHS2	RPGRIP1	0.8909	0.7462	0.6410	0.7410	0.8427	0.8618	0.9400	0.8409	1.0000	0.7245
FCER1G	HLA-DPB1	BATF	GPR84	MTCH1	C9orf95	RPGRIP1	0.9091	0.7693	0.6811	0.7467	0.8598	0.8936	0.9360	0.7841	1.0000	0.7246
FCER1G	HLA-DPB1	BATF	C3AR1	MTCH1	SEPHS2	RPGRIP1	0.9182	0.7428	0.6827	0.7906	0.8615	0.8301	0.9307	0.8750	1.0000	0.7291
FCER1G	HLA-DPB1	BATF	C3AR1	MTCH1	C9orf95	RPGRIP1	0.9000	0.7674	0.7196	0.7898	0.8752	0.8721	0.9293	0.8523	1.0000	0.7276
FCER1G	HLA-DPB1	BATF	GNAI5	ARHGEF18	C9orf95	RPGRIP1	0.9455	0.7659	0.7035	0.7960	0.8718	0.8665	0.9547	0.8523	1.0000	0.7336
FCER1G	HLA-DPB1	BATF	GNAI5	MTCH1	SEPHS2	RPGRIP1	0.9727	0.7383	0.6659	0.7879	0.8427	0.8497	0.9613	0.8750	1.0000	0.7267
FCER1G	HLA-DPB1	BATF	GNAI5	MTCH1	C9orf95	RPGRIP1	0.9636	0.7598	0.7155	0.7925	0.8803	0.8814	0.9627	0.8409	1.0000	0.7324
OSTalpha	HLA-DPB1	BATF	GNAI5	ARHGEF18	C9orf95	RPGRIP1	0.9273	0.7780	0.6522	0.7374	0.8991	0.9076	0.9400	0.8636	0.9583	0.7459
OSTalpha	HLA-DPB1	BATF	GNAI5	MTCH1	SEPHS2	RPGRIP1	0.9364	0.7538	0.6314	0.7231	0.8906	0.9010*	0.9400	0.9091	0.9583	0.7459
OSTalpha	HLA-DPB1	BATF	GNAI5	MTCH1	C9orf95	RPGRIP1	0.9364	0.7788	0.6595	0.7355	0.9026	0.9020	0.9387	0.8750	0.9583	0.7462
SIGLEC9	HLA-DPB1	BATF	GNAI5	MTCH1	C9orf95	RPGRIP1	0.9455	0.8000	0.6931	0.7352	0.8821	0.8263	0.9373	0.8636	1.0000	0.7537
ANXA3	HLA-DPB1	BATF	GNAI5	MTCH1	C9orf95	RPGRIP1	0.9182	0.7470	0.6859	0.7817	0.8564	0.8964	0.9467	0.8750	1.0000	0.7375

Chemotax., adhesion, migration	Immune response	Transcr. by RNA pol II	PLC, phosphoryl- ation, platelet activation	Apoptosis	Metabo- lism	Gene7	Discovery set								Validation set			
							GSE 28750	GSE 32707	GSE 40012	GSE 66099	Glue Grant Buffy coat, day [1-3]	Glue Grant Buffy coat, day [3- 6]	Glue Grant Buffy coat, day [6-10]	Glue Grant Buffy coat, day [10-18]	Glue Grant Buffy coat, day [18- 24]	GSE 65682	GSE 74224	E- MEXP -3589
Gene1	Gene2	Gene3	Gene4	Gene5	Gene6	Gene7												
FES	HLA-DPB1	PLAC8	GPR84	MTCH1	C9orf95	RPGRIP1	0.9000	0.7803	0.7003	0.7286	0.8564	0.8786	0.9107	0.8182	1.0000	0.7510	0.9032*	0.6480
FES	HLA-DPB1	PLAC8	FCER1G	MTCH1	C9orf95	RPGRIP1	0.9273	0.7519	0.7091	0.7466	0.8838	0.8870	0.9173	0.8295	1.0000	0.7615	0.9316*	0.6582
FES	HLA-DPB1	PLAC8	GNAI5	ARHGEF18	C9orf103	RPGRIP1	0.9273	0.7394	0.6635	0.7332	0.8667	0.9160	0.9387	0.8750	0.9583	0.7627	0.9394*	0.6020
FES	HLA-DPB1	PLAC8	GNAI5	ARHGEF18	C9orf95	RPGRIP1	0.9364	0.7727	0.7212	0.7643	0.8530	0.9029*	0.9293	0.8636	1.0000	0.7510	0.9407*	0.6378
FES	HLA-DPB1	PLAC8	GNAI5	MTCH1	C9orf95	RPGRIP1	0.9455	0.7686	0.7356	0.7662	0.8530	0.9076*	0.9400	0.8750	1.0000	0.7492	0.9494*	0.6429
FES	HLA-DPB1	BATF	GPR84	MTCH1	C9orf95	RPGRIP1	0.8909	0.7917	0.6819	0.7484	0.8462	0.8777	0.9387	0.8295	1.0000	0.7300	0.8710*	0.6786
FES	HLA-DPB1	BATF	FCER1G	ARHGEF18	C9orf103	RPGRIP1	0.9000	0.7500	0.6298	0.7462	0.8821	0.8543	0.9413	0.8409	0.9583	0.7606	0.8806*	0.6837
FES	HLA-DPB1	BATF	FCER1G	MTCH1	C9orf103	RPGRIP1	0.9364	0.7436	0.6394	0.7430	0.8889	0.8553	0.9453	0.8523	0.9583	0.7615	0.8945*	0.6837
FES	HLA-DPB1	BATF	C3AR1	MTCH1	C9orf95	RPGRIP1	0.9091	0.7989	0.7188	0.7824	0.8701	0.8385	0.9373	0.8523	1.0000	0.7249	0.9303*	0.7449
FES	HLA-DPB1	BATF	GNAI5	ARHGEF18	C9orf95	RPGRIP1	0.9273	0.7936	0.7035	0.7901	0.8650	0.8497*	0.9480	0.8409	1.0000	0.7342	0.9089*	0.6939
FES	HLA-DPB1	BATF	GNAI5	MTCH1	C9orf95	RPGRIP1	0.9455	0.7924	0.7139	0.7899	0.8718	0.8525*	0.9573	0.8409	1.0000	0.7333	0.9250*	0.6837
S100A12	HLA-DPB1	PLAC8	FCER1G	MTCH1	C9orf95	RPGRIP1	0.9000	0.7299	0.7011	0.7320	0.8752	0.9346*	0.9147	0.8182	1.0000	0.7600	0.8980*	0.6122
S100A12	HLA-DPB1	BATF	GPR84	ARHGEF18	C9orf95	RPGRIP1	0.8909	0.7621	0.6659	0.7420	0.8479	0.9001	0.9293	0.7955	1.0000	0.7261	0.8135*	0.6327
S100A12	HLA-DPB1	BATF	GPR84	MTCH1	C9orf95	RPGRIP1	0.8909	0.7583	0.6731	0.7402	0.8513	0.8982	0.9293	0.7841	1.0000	0.7225	0.8548*	0.6378
S100A12	HLA-DPB1	BATF	FCER1G	ARHGEF18	C9orf95	RPGRIP1	0.9091	0.7451	0.6787	0.7737	0.8872	0.8852	0.9520	0.8295	1.0000	0.7360	0.8514*	0.6378
S100A12	HLA-DPB1	BATF	FCER1G	MTCH1	C9orf95	RPGRIP1	0.9182	0.7390	0.6899	0.7735	0.8957	0.8964	0.9587	0.8068	1.0000	0.7360	0.8710*	0.6327
S100A12	HLA-DPB1	BATF	C3AR1	MTCH1	C9orf95	RPGRIP1	0.9000	0.7602	0.6987	0.7881	0.8667	0.8898	0.9307	0.8636*	1.0000	0.7240	0.9106*	0.6888
S100A12	HLA-DPB1	BATF	GNAI5	ARHGEF18	C9orf95	RPGRIP1	0.9273	0.7527	0.6915	0.7950	0.8581	0.9094	0.9520	0.8523	1.0000	0.7276	0.8888*	0.6327

Chemotax, angiogen., adhesion, migration	Immune response	Transcr. by RNA pol II	PLC, phosphoryl- ation, platelet activation	Apoptosis	Metabo- lism	Gene7	GSE 28750	GSE 32707	GSE 40012	GSE 66099	Discovery set						Validation set		
											Glue Grant Buffy coat, day [1-3]	Glue Grant Buffy coat, day [3-6]	Glue Grant Buffy coat, day [6-10]	Glue Grant Buffy coat, day [10-18]	Glue Grant Buffy coat, day [18-24]	GSE 65682	GSE 74224	E- MEXP -3589	
Gene1	Gene2	Gene3	Gene4	Gene5	Gene6	Gene7	GSE 28750	GSE 32707	GSE 40012	GSE 66099	Glue Grant Buffy coat, day [1-3]	Glue Grant Buffy coat, day [3-6]	Glue Grant Buffy coat, day [6-10]	Glue Grant Buffy coat, day [10-18]	Glue Grant Buffy coat, day [18-24]	GSE 65682	GSE 74224	E- MEXP -3589	
S100A12	HLA-DPB1	BATF	GNAI5	MTCH1	C9orf103	RPGRIP1	0.9545	0.7254	0.6514	0.7625	0.8872	0.9374	0.9547	0.8636	0.9583	0.7513	0.8976*	0.6224	
S100A12	HLA-DPB1	BATF	GNAI5	MTCH1	C9orf95	RPGRIP1	0.9364	0.7481	0.7043	0.7953	0.8718	0.9150	0.9560	0.8409	1.0000	0.7243	0.9010*	0.6327	
C3AR1	HLA-DPB1	BATF	GNAI5	ARHGEF18	C9orf95	RPGRIP1	0.9273	0.7836	0.7220	0.8017	0.8513	0.8749	0.9360	0.8864	1.0000	0.7195	0.9381*	0.7143	
C3AR1	HLA-DPB1	BATF	GNAI5	MTCH1	C9orf95	RPGRIP1	0.9455	0.7830	0.7340	0.8030	0.8427	0.8898	0.9373	0.8750	1.0000	0.7177	0.9459*	0.7041	

Table 6. 22 new six gene panels with higher performance in the validation sets. Among 73 six gene panels that had higher performance than the lower bound of 95% confidence intervals of the original Stanford11 panel in all discovery datasets, 22 panels had even higher performance in two independent datasets.

Chemotaxis angiogenesis, adhesion, migration	Interferon- gamma, antigen processing, immune response	Transcrip tion by RNA pol II	PLC, phosphoryl- ation, platelet activation	Apoptosis	Metabolism	GSE65682	GSE74224	E- MEXP- 3589
CCR1	HLA-DPB1	BATF	C3AR1	ARHGEF18	C9orf95	0.7807	0.8854	0.6633
CCR1	HLA-DPB1	BATF	C3AR1	MTCH1	C9orf103	0.8170	0.9058	0.6990
CCR1	HLA-DPB1	BATF	C3AR1	MTCH1	C9orf95	0.8095	0.8967	0.6786
CCR1	HLA-DPB1	BATF	GNA15	MTCH1	C9orf95	0.8080	0.8893	0.6378
CD177	HLA-DPB1	BATF	C3AR1	MTCH1	C9orf95	0.8014	0.8827	0.6684
CD63	HLA-DPB1	PLAC8	FCER1G	MTCH1	C9orf95	0.8158	0.9128	0.6122
CD63	HLA-DPB1	PLAC8	GNA15	ARHGEF18	C9orf95	0.7852	0.9220	0.5663
CD63	HLA-DPB1	PLAC8	GNA15	MTCH1	C9orf95	0.8107	0.9333	0.5918
CD63	HLA-DPB1	BATF	C3AR1	MTCH1	C9orf95	0.8080	0.9067	0.6633
CD63	HLA-DPB1	BATF	GNA15	MTCH1	C9orf95	0.8029	0.8915	0.6378
EMR1	HLA-DPB1	BATF	C3AR1	MTCH1	C9orf95	0.8149	0.8963	0.6582
EMR1	HLA-DPB1	BATF	GNA15	MTCH1	C9orf95	0.8044	0.8836	0.6071
FCER1G	HLA-DPB1	PLAC8	C3AR1	MTCH1	C9orf95	0.8080	0.9241	0.6531
FCER1G	HLA-DPB1	PLAC8	GNA15	ARHGEF18	C9orf95	0.7861	0.9098	0.5408
FCER1G	HLA-DPB1	PLAC8	GNA15	MTCH1	C9orf95	0.8092	0.9185	0.5816
FCER1G	HLA-DPB1	BATF	C3AR1	MTCH1	SEPHS2	0.8086	0.8867	0.6582
FCER1G	HLA-DPB1	BATF	C3AR1	MTCH1	C9orf95	0.8071	0.8963	0.6480
FES	HLA-DPB1	PLAC8	FCER1G	MTCH1	C9orf95	0.8089	0.8945	0.6173
FES	HLA-DPB1	PLAC8	GNA15	ARHGEF18	C9orf103	0.7870	0.9098	0.6122
FES	HLA-DPB1	PLAC8	GNA15	MTCH1	C9orf95	0.8005	0.9150	0.6071
FES	HLA-DPB1	BATF	C3AR1	MTCH1	C9orf95	0.8026	0.8806	0.6786
C3AR1	HLA-DPB1	BATF	GNA15	MTCH1	C9orf95	0.7999	0.9133	0.6480

5

Though removing RPGRIP1 involved in cell development and proliferation process from the original Stanford11 panel did not decrease the overall diagnostic performance (Table 4), we tested the effect of adding RPGRIP1 to the new 6 gene panels. The results showed that adding RPGRIP1 to the 6-

gene panel only improved the performance in one of the validation datasets, GSE74224, but not in the other two datasets (Table 7).

The performance differences between biological function-based gene substitution and expression correlation-based substitution were tested. Based on expression profiles of all the discovery datasets, the highest correlated genes with features in the Stanford11 panel were selected (referred to as panel-HC), regardless of the biological functions (FIG. 7A). Most of the highest correlated genes with features in the Stanford11 panel were not from Stanford82 nor involved in the same GOBPs represented by Stanford11. For instance, BATF of Stanford11 is involved in transcription by pol II, but the highest correlated gene, DDAH2 is involved in nitric oxide biosynthetic process. The average AUCs of the panel-HC in the discovery and validation sets were 0.8238 and 0.6846, respectively, which are 3.58% and 14.40% lower than the Stanford11 (average AUC of 0.8544 and 0.7997, respectively, in the discovery and validation sets, p-value from DeLong's test of less than 0.01 in two validation datasets, GSE65682 and GSE74224, and less than 0.1 in one validation dataset, E-MEXP-3589, FIG. 7B), which suggests maintaining biological processes associated with disease condition is more critical to generating high performance diagnostic panels than maintaining features with highly correlated expression profiles.

364 panels (referred to as panel-AM) were also generated by randomly selecting 11 genes from 14 genes involved in adhesion/migration process (FIG. 7C). The performance of panel-AM was also lower than Stanford11 (average AUC of 0.7999 and 0.7546 in the discovery and validation sets, respectively, and p-value from DeLong's test of less than 0.01 in GSE74224 in FIG. 7D), which suggests genes from one biological process do not deliver sufficient diagnostic performance.

Lastly, TSP and *k*-TSP algorithms were applied to identify a small set of paired genes. TSP identified two genes that have only 67% classification accuracy in the three independent validation sets. Therefore, we applied *k*-TSP to increase performance by including additional pairs of genes. It resulted in three pairs which include six genes in total (referred to as panel-kTSP, FIG. 7E). The panel-kTSP also showed lower performance in independent datasets (AUC of 0.6408 and p-value from DeLong's test of less than 0.05 in all validation data sets in FIG. 7F).

Example 2

Identification of Gene Panels to Predict Development of Sepsis

Peripheral blood samples were collected from elective surgery patients (n=155) prior to surgery (Pre-Op) and daily up until one or two days post-sepsis diagnosis (Post-Op). The patients had undergone a wide range of surgeries, with the most common being large bowel resection, vascular surgery, and pancreatic surgery. The control group included an equal number of patients matched by age, sex, and surgical procedure who did not develop sepsis. The sepsis patients, as well as matched controls, were divided into three separate groups: Discovery, Test, and Validation sample sets (Table 8). Samples from the Discovery set were used to identify various differentially expressed genes (DEGs) and corresponding biomarker panel candidates. The Test set and the Validation set were used to compare performances of

the candidate panels and to assess the ability of the selected panel based on the test set to predict sepsis, respectively.

Table 8. Groups of surgery patients used for sepsis prognostic biomarker discovery.

	Discovery Set	Test Set	Validation Set	Total
Sepsis	64	31	60	155
Control	63	30	60	153

5 * Numbers indicate patient numbers in each group.

Based on the study design, the longitudinal samples from each patient were organized according to the date when sepsis was diagnosed (Day 0). Using this nomenclature, the day before diagnosis date is Day-1 and one day post-diagnosis is Day+1. There were significantly fewer samples available on Day-4 (almost 50% decrease from Day-3). Since the performance and reliability of biomarkers depends on the number of samples analyzed and the purpose of this study was to find a predictive marker for risk sepsis, the analyses were concentrated on data from samples taken on Day-3 to Day-1. Total RNA from whole blood was isolated with miRNeasy® kit (Qiagen, Germantown, MD) according to the manufacturer's instructions. Fluorescent-labeled (Cy3) probes were prepared with the single color labeling kit from Agilent (Santa Clara, CA). Probes were then loaded on the array hybridization chambers then hybridized at 65°C for 17 hours. The slides were washed and scanned (Agilent, Santa Clara, CA). The microarray data was then normalized by quantile normalization.

To identify genes associated with the development of sepsis for the samples, three factors were considered during statistical analysis: 1) Pre-Op normalization, 2) paired vs unpaired testing, and 3) time points. By considering only probes corresponding to mRNA, eight sets of DEGs were identified (Table 9). The Pre-Op normalization factor was applied to remove transcriptome changes associated with surgery from Post-Op samples to reduce the individual differences and emphasize the gene expression changes associated with infection processes. The second factor was to explore the effect of matched sepsis-control patients individually or as a group. The third factor was to compare the gene expression profile changes between the sepsis and control groups at individual timepoints before sepsis is diagnosed (Day 0). It is reasonable to assume that a sepsis-specific signature would be more prominent as getting closer to time of diagnosis. Grouping all pre-diagnosis sepsis time points together could potentially dilute out the stronger signal present at Day-1. Statistical analysis was therefore performed comparing control and sepsis at each time-point. DEGs identified in at least two individual timepoints were considered.

Support Vector Machine was then combined with Recursive Feature Elimination (SVM-RFE) and applied in conjunction with a repeat cross-validation procedure to select genes that were most relevant for the classification between sepsis patients and controls from the selected DEGs identified from the eight different procedures. The performances of the resultant eight candidate panels were then evaluated in the test sample set (Table 9).

Table 9. Classification performance of gene panels identified using different statistical approaches.

Pre-Op norm.	Statistical Test	Comparison	Approach	# DEGs	# Genes in panel	Avg. AUC	Day-3 AUC	Day-2 AUC	Day-1 AUC
With Pre-Op norm.	Paired Test	All time points together	1	76	37	0.7036	0.6198	0.6266	0.7805
		Each time point	2	58	19	0.7669	0.7355	0.6842	0.8506
	Unpaired Test	All time points together	3	77	39	0.7396	0.6694	0.6692	0.8299
		Each time point	4	62	21	0.7617	0.7273	0.6892	0.8322
Without Pre-Op norm.	Paired Test	All time points together	5	345	50	0.8475	0.8347	0.8421	0.8747
		Each time point	6	355	63	0.8751	0.8347	0.8596	0.9092
	Unpaired Test	All time points together	7	342	85	0.8433	0.8099	0.8321	0.8678
		Each time point	8	338	54	0.8724	0.7934	0.8521	0.9126

Four different approaches using Pre-Op normalization were first performed (Table 9).

- 5 Differentially expressed genes and their optimal feature sets from SVM-RFE were identified for each approach. Among the four approaches, 58 genes (ISB58) were identified from approach 2. A 19 gene panel (ISB19) was selected from ISB58 using SVM-RFE. ISB19 showed the highest performance from panel genes identified from the four approaches utilizing Pre-Op normalization (Approach 2 (bold in Table 9)). ISB58 is summarized in Table 10, and the overall expression profile is shown in FIG. 8.

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Table 10. Gene list for ISB58 (81 probes)

Probe id	Gene Symbol	Day-3		Day-2		Day-1	
		logFC	P.Value	logFC	P.Value	logFC	P.Value
A_33_P3238993	AGFG1	0.86	5.47E-04	0.62	2.01E-03	0.67	3.91E-04
A_23_P146058	ATP6V1C1	0.77	2.27E-03	0.55	9.33E-03	0.69	1.15E-04
A_23_P380614	ATP9A	0.99	5.62E-05	0.85	1.97E-04	0.97	1.49E-06
A_23_P253602	BMX	0.69	4.61E-03	0.65	3.39E-03	0.80	7.55E-05
A_23_P330561	C19orf59	1.26	5.58E-03	0.92	2.34E-02	1.41	2.78E-04
A_21_P0011751	CD177	1.61	1.31E-03	1.33	7.07E-03	1.83	4.38E-05
A_23_P259863	CD177	1.76	1.45E-03	1.30	9.30E-03	1.88	4.39E-05
A_33_P3232080	CD177	0.60	5.29E-03	0.73	1.91E-03	0.90	3.41E-06
A_33_P3369844	CD24	0.61	2.63E-02	0.65	5.29E-03	0.71	5.22E-03
A_33_P3389060	CDK5RAP2	0.73	6.37E-03	0.67	4.01E-03	0.77	1.73E-04
A_23_P83110	CDK5RAP2	0.84	7.75E-03	0.72	9.41E-03	0.84	9.12E-04
A_24_P382319	CEACAM1	0.83	9.78E-04	0.75	6.56E-04	0.97	1.39E-05
A_33_P3352578	CLEC4D	0.82	4.30E-03	0.63	2.14E-02	0.89	2.50E-04
A_33_P3258977	CLEC4D	0.85	4.35E-03	0.68	1.28E-02	0.89	2.41E-04

Probe id	Gene Symbol	Day-3		Day-2		Day-1	
		logFC	P.Value	logFC	P.Value	logFC	P.Value
A 33 P3316786	DACH1	0.65	1.56E-03	0.64	2.08E-03	0.77	6.35E-06
A 23 P32577	DACH1	0.68	2.06E-05	0.58	2.54E-04	0.64	2.29E-06
A 23 P19482	DDAH2	0.62	1.21E-02	0.65	2.11E-03	0.90	8.12E-06
A 23 P56559	DHRS9	0.96	6.34E-04	0.69	5.53E-03	0.90	5.85E-05
A 33 P3301410	EXOSC4	0.81	1.81E-04	0.83	7.14E-05	1.02	3.90E-08
A 23 P208768	FCAR	0.73	3.91E-03	0.55	1.17E-02	0.61	3.04E-03
A 23 P23221	GADD45A	0.88	8.47E-04	0.74	1.45E-03	0.97	1.57E-05
A 23 P67847	GALNT14	0.77	6.03E-03	0.67	1.65E-02	0.81	5.96E-04
A 23 P74290	GBP5	-0.81	1.94E-04	-0.45	6.16E-02	-0.74	1.52E-03
A 23 P25155	GPR84	1.10	9.83E-04	1.02	2.87E-04	1.24	6.20E-07
A 23 P122863	GRB10	0.74	7.28E-03	0.53	2.03E-02	0.89	1.04E-04
A 24 P235266	GRB10	0.67	1.35E-02	0.60	9.13E-03	0.96	2.70E-05
A 23 P29422	GYG1	1.05	3.05E-04	0.84	1.65E-03	0.96	5.70E-05
A 21 P0013518	GYG1	0.96	1.99E-04	0.79	1.50E-03	0.92	2.42E-05
A 23 P384517	GYG1	0.91	5.52E-03	0.78	9.45E-03	1.04	1.39E-04
A 33 P3376821	GZMA	-0.59	8.29E-03	-0.61	3.32E-03	-0.46	2.74E-02
A 23 P128993	GZMH	-0.68	6.67E-03	-0.69	3.37E-03	-0.52	1.10E-02
A 23 P213584	HK3	0.83	3.40E-03	0.67	4.98E-03	0.69	1.30E-03
A 23 P206760	HP	1.08	5.79E-03	0.75	4.17E-02	1.20	4.06E-04
A 33 P3289236	HPR	1.07	4.00E-03	0.73	2.35E-02	1.18	7.87E-05
A 24 P103886	IDI1	0.73	1.50E-03	0.52	3.43E-03	0.61	4.00E-04
A 33 P3251876	IL18R1	0.59	3.99E-03	0.52	2.52E-03	0.82	1.16E-06
A 33 P3211666	IL18R1	0.79	5.69E-03	0.68	7.68E-03	1.19	5.12E-07
A 24 P208567	IL18R1	0.97	1.02E-03	0.78	1.75E-03	1.10	6.53E-06
A 24 P63019	IL1R2	0.93	1.71E-02	0.90	2.93E-03	1.32	7.98E-06
A 23 P169437	LCN2	0.89	4.13E-03	0.74	3.05E-03	0.96	6.47E-04
A 23 P120902	LGALS2	-0.70	1.01E-03	-0.66	2.93E-03	-1.03	3.14E-06
A 23 P50638	LRG1	0.63	3.33E-03	0.44	2.10E-02	0.62	4.66E-04
A 23 P40174	MMP9	1.08	2.05E-03	0.81	8.96E-03	1.21	1.21E-04
A 23 P40174	MMP9	1.03	3.31E-03	0.79	1.22E-02	1.20	1.51E-04
A 23 P40174	MMP9	0.99	5.17E-03	0.78	1.27E-02	1.20	1.62E-04
A 23 P40174	MMP9	1.00	5.35E-03	0.78	1.26E-02	1.23	1.20E-04
A 23 P40174	MMP9	1.04	3.23E-03	0.77	1.30E-02	1.22	1.43E-04
A 23 P40174	MMP9	1.02	3.60E-03	0.79	1.07E-02	1.20	1.26E-04
A 23 P40174	MMP9	1.03	2.84E-03	0.79	8.99E-03	1.20	1.27E-04
A 23 P40174	MMP9	1.06	2.06E-03	0.81	7.56E-03	1.22	1.04E-04
A 23 P40174	MMP9	1.02	3.67E-03	0.79	9.06E-03	1.20	1.39E-04
A 23 P40174	MMP9	0.99	4.43E-03	0.75	1.30E-02	1.16	1.81E-04
A 23 P161458	OLAH	0.66	1.64E-02	0.85	2.18E-03	0.98	2.58E-05
A 24 P181254	OLFM4	1.23	1.31E-03	0.98	4.41E-04	1.42	8.48E-05
A 23 P170186	OPLAH	0.75	4.67E-03	0.72	3.70E-03	0.99	2.04E-05
A 24 P413669	PFKFB2	1.11	1.05E-03	0.97	1.10E-03	1.29	6.05E-06
A 33 P3300635	PFKFB2	0.88	3.82E-03	0.74	1.73E-03	1.11	9.25E-06
A 24 P261259	PFKFB3	0.90	4.65E-03	0.74	6.84E-03	0.99	1.16E-04
A 23 P208747	PGLYRP1	0.62	6.66E-03	0.46	2.03E-02	0.63	3.35E-03
A 24 P183128	PLAC8	0.76	1.76E-03	0.77	2.31E-03	0.90	3.30E-05
A 23 P119222	RETN	1.15	6.90E-04	1.04	1.76E-03	1.24	1.74E-04
A 33 P3350863	RETN	1.09	6.27E-03	0.97	6.58E-03	1.43	1.30E-04
A 23 P306941	RGL4	0.86	5.79E-03	0.76	4.36E-03	0.97	4.49E-04
A 23 P151637	RNASE2	0.66	5.78E-03	0.62	1.99E-03	0.58	2.37E-03
A 23 P163025	RNASE3	0.68	3.84E-04	0.65	2.19E-04	0.64	4.61E-05

Probe id	Gene Symbol	Day-3		Day-2		Day-1	
		logFC	P.Value	logFC	P.Value	logFC	P.Value
A 33 P3385785	S100A12	1.02	5.23E-03	0.82	1.04E-02	1.13	1.54E-04
A 23 P29005	SAMSN1	0.96	9.04E-04	0.62	1.32E-02	0.87	3.11E-04
A 24 P81900	SLC2A3	0.66	2.72E-03	0.57	5.11E-03	0.63	3.87E-04
A 23 P139669	SLC2A3	0.64	4.30E-04	0.64	2.35E-04	0.54	3.19E-04
A 23 P431388	SPOCD1	0.41	1.61E-02	0.59	3.64E-05	0.60	1.19E-04
A 33 P3275055	ST6GALNAC3	0.59	9.78E-04	0.46	4.80E-03	0.64	6.07E-06
A 23 P154605	SULF2	-0.73	7.04E-05	-0.68	8.76E-04	-0.59	1.61E-04
A 33 P3309075	TBC1D8	0.73	1.38E-04	0.62	9.18E-04	0.72	1.66E-05
A 23 P64372	TCN1	0.67	2.59E-04	0.63	2.13E-04	0.72	9.74E-05
A 32 P208350	TDRD9	0.94	1.94E-03	0.88	7.49E-04	1.10	5.86E-06
A 23 P5392	TP53I3	0.69	3.05E-04	0.48	7.18E-03	0.62	9.33E-05
A 33 P3392077	TP53I3	0.67	4.62E-04	0.49	4.64E-03	0.68	1.96E-04
A 24 P27977	TRPM2	0.61	5.61E-04	0.45	1.70E-03	0.59	8.09E-06
A 23 P313389	UGCG	0.92	1.24E-04	0.71	1.68E-03	0.95	1.01E-05
A 23 P351275	UPP1	0.93	3.61E-04	0.71	2.65E-03	0.71	1.87E-03
A 33 P3399571	VNN1	0.84	9.70E-03	0.63	3.76E-02	0.88	8.39E-04

Second, from the comparisons without Pre-Op normalization, gene panels identified from approach 6 showed the best performance (Approach 6 highlighted with bold in Table 9). With this approach, 355 DEGs were identified (ISB355) (Table 11). Among the 355 genes, 63 genes (ISB63) were then identified as an optimal feature set using SVM-RFE. The overall expression profiles for the ISB355 is shown in Figure 9.

Table 11. Gene list for ISB355 (503 probes)

Probe id	Gene Symbol	Day-3		Day-2		Day-1	
		logFC	P.Value	logFC	P.Value	logFC	P.Value
A 33 P3424577	A23747	-0.69	5.95E-04	-0.68	1.29E-04	-0.81	1.59E-05
A 23 P17242	ABHD1	0.08	7.25E-01	-0.67	8.33E-03	-0.71	3.96E-04
A 23 P107336	ACAP1	0.57	1.83E-05	0.59	4.75E-07	0.59	1.85E-08
A 23 P59528	ACN9	0.64	1.24E-05	0.62	3.82E-05	0.72	4.98E-07
A 23 P110212	ACSL1	0.80	8.58E-06	0.75	2.73E-08	0.85	1.52E-08
A 23 P110212	ACSL1	0.79	2.45E-05	0.77	8.12E-09	0.82	3.35E-08
A 23 P110212	ACSL1	0.80	2.34E-05	0.75	2.26E-08	0.85	2.33E-09
A 23 P110212	ACSL1	0.78	2.05E-05	0.74	6.12E-08	0.84	1.01E-08
A 23 P110212	ACSL1	0.81	1.84E-05	0.76	1.15E-08	0.83	3.77E-08
A 23 P110212	ACSL1	0.83	1.06E-05	0.75	1.80E-08	0.82	9.08E-09
A 23 P110212	ACSL1	0.81	2.60E-05	0.75	2.08E-08	0.81	1.52E-08
A 23 P110212	ACSL1	0.81	2.11E-05	0.75	1.04E-08	0.82	4.49E-08
A 23 P110212	ACSL1	0.80	4.98E-05	0.76	1.36E-08	0.83	1.36E-08
A 23 P110212	ACSL1	0.81	1.26E-05	0.76	1.01E-08	0.84	1.64E-08
A 23 P217564	ACSL4	0.73	6.00E-07	0.71	1.70E-09	0.74	5.99E-11
A 33 P3256848	ADAM12	-0.10	7.01E-01	-0.75	3.68E-04	-0.61	1.79E-03
A 33 P3245489	ADAMTSL5	-0.63	1.12E-02	-0.59	3.18E-03	-0.68	5.82E-04
A 33 P3238997	AGFG1	0.62	2.96E-06	0.55	6.53E-08	0.69	3.18E-11
A 33 P3238993	AGFG1	1.00	3.67E-07	0.91	5.08E-08	0.96	5.73E-10
A 33 P3279470	AGRP	0.01	9.77E-01	-0.64	4.28E-03	-0.65	1.93E-03
A 23 P169278	AGTPBP1	0.66	1.82E-05	0.65	1.71E-08	0.63	3.39E-07
A 33 P3215797	AHDC1	0.03	8.87E-01	-0.78	4.00E-04	-0.73	4.55E-04

Probe id	Gene Symbol	Day-3		Day-2		Day-1	
		logFC	P.Value	logFC	P.Value	logFC	P.Value
A 32 P44394	AIM2	0.40	1.54E-02	0.61	1.20E-04	0.67	5.39E-06
A 23 P104464	ALOX5	0.78	1.83E-04	0.72	1.46E-07	0.70	1.27E-06
A 23 P104464	ALOX5	0.75	9.19E-06	0.73	1.36E-07	0.70	3.28E-07
A 23 P104464	ALOX5	0.73	1.81E-05	0.71	1.02E-07	0.66	1.10E-06
A 23 P104464	ALOX5	0.73	2.15E-05	0.69	2.26E-07	0.67	2.38E-06
A 23 P104464	ALOX5	0.72	1.87E-05	0.72	2.48E-08	0.67	3.10E-07
A 23 P104464	ALOX5	0.68	4.07E-05	0.72	2.33E-08	0.66	9.83E-07
A 23 P104464	ALOX5	0.77	5.64E-06	0.69	1.02E-07	0.68	3.93E-07
A 23 P104464	ALOX5	0.69	1.18E-04	0.71	3.68E-07	0.69	2.36E-07
A 23 P104464	ALOX5	0.75	2.22E-05	0.72	7.63E-08	0.69	2.40E-07
A 23 P104464	ALOX5	0.74	2.63E-05	0.74	2.37E-08	0.69	1.88E-07
A 24 P347378	ALOX5AP	0.61	9.85E-04	0.47	1.76E-03	0.69	2.80E-06
A 24 P353619	ALPL	0.81	3.00E-05	0.85	1.80E-06	0.77	3.34E-05
A 23 P156748	ANKS1A	0.66	1.84E-05	0.56	1.37E-08	0.67	5.49E-11
A 23 P94501	ANXA1	0.63	5.27E-05	0.71	3.99E-07	0.55	1.81E-05
A 23 P121716	ANXA3	1.06	2.36E-05	1.03	8.35E-07	1.14	1.58E-07
A 23 P121716	ANXA3	1.08	1.52E-05	1.04	1.21E-06	1.14	1.60E-07
A 23 P121716	ANXA3	1.08	1.68E-05	1.03	8.55E-07	1.11	3.31E-07
A 23 P121716	ANXA3	1.07	2.39E-05	1.05	8.57E-07	1.12	2.42E-07
A 23 P121716	ANXA3	1.10	1.87E-05	1.03	3.96E-07	1.11	1.70E-07
A 23 P121716	ANXA3	1.12	6.39E-06	1.04	6.47E-07	1.12	1.62E-07
A 23 P121716	ANXA3	1.10	2.04E-05	1.02	7.71E-07	1.13	2.06E-07
A 23 P121716	ANXA3	1.11	2.07E-05	1.03	5.49E-07	1.12	2.29E-07
A 23 P121716	ANXA3	1.09	1.57E-05	1.04	6.23E-07	1.12	2.16E-07
A 23 P121716	ANXA3	1.11	1.71E-05	1.04	6.80E-07	1.11	3.40E-07
A 33 P3352382	ARG1	1.11	3.03E-04	1.21	1.56E-06	1.46	1.47E-07
A 33 P3319967	ARG1	1.03	1.39E-03	1.19	8.05E-06	1.51	1.45E-07
A 23 P143016	ARID5A	0.60	5.05E-05	0.58	2.47E-06	0.73	7.00E-10
A 23 P217712	ARSD	-0.01	9.65E-01	-0.68	4.76E-03	-0.62	5.37E-03
A 23 P216094	ASPH	0.58	3.37E-04	0.69	2.77E-08	0.81	6.30E-11
A 24 P295245	ASPH	0.71	1.65E-04	0.79	5.17E-08	0.98	4.23E-11
A 24 P161973	ATP11A	0.59	6.42E-06	0.50	1.23E-07	0.66	2.56E-09
A 23 P212522	ATP11B	0.64	3.33E-06	0.70	5.00E-08	0.74	7.05E-11
A 24 P405205	ATP2B4	0.58	1.10E-04	0.60	1.24E-07	0.66	7.41E-11
A 23 P146058	ATP6V1C1	0.98	2.85E-06	0.86	1.26E-06	1.01	1.41E-09
A 33 P3380897	ATP6V1C1	0.76	3.49E-05	0.75	1.21E-06	0.92	1.75E-10
A 23 P380614	ATP9A	0.92	4.51E-05	0.93	8.45E-06	1.10	4.78E-09
A 23 P18372	B3GNT5	0.64	8.59E-06	0.58	1.17E-05	0.73	2.72E-09
A 24 P239731	B4GALT5	0.86	1.61E-07	0.92	3.63E-10	1.05	4.34E-13
A 23 P213385	BASP1	0.68	3.73E-05	0.65	2.37E-07	0.66	2.07E-06
A 23 P128974	BATF	0.48	1.14E-03	0.60	2.79E-06	0.73	3.42E-09
A 23 P152002	BCL2A1	0.82	2.13E-05	0.76	2.65E-06	0.84	3.74E-07
A 23 P57856	BCL6	0.81	9.14E-05	0.92	2.38E-11	0.89	8.26E-08
A 23 P310911	BLMH	0.83	6.56E-05	0.78	4.50E-06	0.89	3.57E-08
A 23 P253602	BMX	0.73	1.51E-04	0.79	1.66E-06	0.91	3.84E-08
A 23 P131785	BPI	0.71	2.32E-04	0.65	5.72E-04	0.85	2.74E-05
A 33 P3245389	C14orf101	0.59	2.90E-04	0.59	5.06E-06	0.68	1.77E-09
A 23 P26557	C16orf59	-0.12	6.21E-01	-0.62	5.07E-03	-0.75	2.90E-04
A 23 P330561	C19orf59	1.38	4.51E-05	1.35	2.20E-06	1.78	1.56E-09
A 24 P297078	C20orf3	0.74	8.60E-05	0.62	7.13E-06	0.61	3.11E-05
A 21 P0000149	C2orf3	-0.07	7.89E-01	-0.71	9.00E-03	-0.71	1.88E-03
A 33 P3347869	C3	0.02	9.50E-01	-0.73	7.90E-03	-0.88	7.80E-04
A 23 P259506	C5orf32	0.96	2.79E-05	1.07	7.74E-08	1.13	5.48E-08
A 33 P3431595	C8orf31	0.02	9.38E-01	-0.73	9.11E-03	-0.74	6.72E-03
A 23 P123732	C9orf103	-0.15	3.38E-01	0.59	2.38E-03	0.63	1.65E-04
A 23 P123732	C9orf103	0.10	5.78E-01	0.64	4.66E-04	0.70	2.68E-05
A 23 P123732	C9orf103	0.32	5.26E-02	0.67	1.01E-04	0.69	5.77E-06

Probe id	Gene Symbol	Day-3		Day-2		Day-1	
		logFC	P.Value	logFC	P.Value	logFC	P.Value
A 23 P123732	C9orf103	0.12	4.53E-01	0.65	1.91E-04	0.63	3.00E-05
A 33 P3359223	C9orf173	-0.54	3.28E-02	-0.75	3.71E-04	-0.94	1.07E-06
A 33 P3282614	C9orf173	-0.34	1.12E-01	-0.66	4.79E-04	-0.80	4.71E-06
A 23 P20804	C9orf25	-0.63	3.06E-03	-0.37	4.90E-02	-0.66	9.36E-04
A 23 P4096	CA4	0.70	4.63E-04	0.66	1.12E-04	0.79	1.97E-06
A 23 P79426	CAB39	0.68	1.08E-06	0.65	2.26E-08	0.71	5.46E-10
A 33 P3228612	CACNA1E	0.59	3.17E-04	0.75	3.45E-07	0.71	3.78E-09
A 23 P408830	CAMKK2	0.60	8.95E-05	0.60	2.46E-05	0.57	2.19E-05
A 33 P3281816	CAP1	0.59	8.98E-05	0.66	9.74E-08	0.69	4.95E-08
A 33 P3340847	CARD6	0.51	1.60E-03	0.62	6.84E-06	0.89	1.02E-10
A 23 P41854	CARD6	0.67	5.31E-05	0.64	1.90E-05	0.87	1.65E-10
A 23 P344884	CARNS1	-0.34	6.24E-02	-0.69	2.29E-04	-0.70	4.89E-06
A 23 P166306	CBS	0.44	1.51E-02	0.70	9.15E-06	0.76	1.60E-05
A 33 P3246613	CCDC78	-0.66	1.99E-02	-0.67	5.17E-03	-0.98	5.21E-05
A 24 P148717	CCR1	0.63	1.13E-03	0.60	3.26E-04	0.58	2.35E-04
A 23 P250302	CCR3	-0.38	9.50E-02	-0.59	1.82E-03	-0.98	2.85E-06
A 23 P33723	CD163	0.77	1.26E-03	0.45	2.68E-02	0.77	5.22E-06
A 24 P380536	CD164	0.49	1.99E-05	0.48	8.75E-06	0.63	3.14E-12
A 23 P254756	CD164	0.59	1.10E-05	0.56	1.18E-07	0.51	8.99E-08
A 21 P0011751	CD177	1.91	9.92E-06	1.98	5.61E-07	2.38	1.43E-08
A 23 P259863	CD177	1.87	5.48E-05	1.77	5.98E-06	2.21	1.43E-07
A 33 P3232080	CD177	0.65	1.48E-04	0.80	3.24E-05	0.92	2.24E-07
A 33 P3375541	CD3D	-0.63	1.90E-03	-0.84	1.89E-06	-0.95	8.63E-08
A 33 P3294509	CD44	0.65	5.42E-04	0.63	5.40E-06	0.58	6.31E-07
A 24 P188377	CD55	0.64	1.78E-04	0.81	3.54E-08	0.91	7.33E-10
A 24 P270144	CD63	0.59	7.45E-05	0.64	4.70E-06	0.76	1.31E-08
A 23 P107735	CD79A	-0.79	3.79E-03	-0.54	4.90E-03	-0.79	7.88E-05
A 23 P1782	CD82	0.52	1.53E-05	0.59	9.87E-08	0.66	4.35E-10
A 33 P3389060	CDK5RAP2	0.88	7.32E-05	0.97	3.11E-07	1.09	9.85E-10
A 23 P83110	CDK5RAP2	0.98	1.22E-04	1.03	1.92E-06	1.21	8.99E-09
A 33 P3268507	CEACAM1	0.79	1.41E-06	0.64	2.18E-05	0.78	9.58E-09
A 24 P382319	CEACAM1	1.12	5.80E-06	1.05	3.03E-06	1.25	2.12E-09
A 24 P120115	CFLAR	0.66	8.29E-07	0.60	3.88E-09	0.63	2.44E-08
A 23 P137665	CHI3L1	-0.83	3.49E-03	-0.96	2.47E-04	-0.75	1.66E-03
A 24 P945293	CHMP3	-0.35	1.60E-01	-0.79	2.31E-04	-0.77	3.17E-04
A 23 P48056	CKAP4	0.71	7.08E-05	0.81	2.26E-07	0.92	5.54E-08
A 23 P128470	CLEC12A	0.83	7.92E-04	0.70	4.78E-03	0.55	4.81E-03
A 33 P3352578	CLEC4D	1.07	1.36E-05	1.08	1.92E-06	1.29	5.58E-10
A 33 P3258977	CLEC4D	1.00	2.43E-05	0.99	1.12E-05	1.15	4.45E-08
A 23 P411113	CNTNAP1	-0.80	3.31E-03	-0.63	5.82E-03	-0.55	2.77E-03
A 33 P3216448	COL11A2	-0.35	1.64E-01	-0.81	4.30E-04	-0.80	3.93E-04
A 23 P258164	CORT	-0.25	2.97E-01	-0.65	8.71E-03	-0.94	1.74E-05
A 23 P251937	CPEB4	0.61	2.69E-05	0.54	1.28E-06	0.72	5.06E-09
A 23 P256821	CR1	0.77	5.81E-06	0.93	2.06E-09	0.99	3.02E-11
A 33 P3319126	CR1L	0.75	6.38E-07	0.87	1.07E-09	0.86	5.73E-12
A 23 P149892	CSGALNACT2	0.89	3.62E-07	0.91	9.16E-10	0.94	2.28E-10
A 33 P3402526	CSGALNACT2	0.79	2.18E-06	0.80	8.75E-09	0.99	4.05E-13
A 23 P68601	CST7	0.69	8.01E-03	0.76	9.89E-05	0.88	3.11E-06
A 23 P94533	CTSL1	0.03	8.89E-01	-0.60	6.58E-03	-0.59	2.85E-03
A 23 P209625	CYP1B1	0.77	2.49E-04	0.77	1.45E-04	0.92	9.86E-08
A 33 P3290343	CYP1B1	0.90	6.13E-06	0.83	6.07E-05	0.91	1.01E-07
A 33 P3361422	CYP27A1	-0.74	2.07E-04	-0.80	5.48E-06	-0.58	1.29E-04
A 33 P3316786	DACH1	0.81	3.38E-06	0.88	1.54E-07	1.00	5.35E-11
A 23 P32577	DACH1	0.76	7.46E-08	0.77	3.93E-07	0.80	2.00E-09
A 23 P19482	DDAH2	1.04	7.45E-06	1.20	9.46E-11	1.32	9.34E-12
A 23 P66719	DHRS13	0.56	1.27E-03	0.59	1.31E-05	0.65	3.50E-07
A 23 P56559	DHRS9	1.34	3.99E-06	1.04	1.40E-05	1.31	6.05E-09

Probe id	Gene Symbol	Day-3		Day-2		Day-1	
		logFC	P.Value	logFC	P.Value	logFC	P.Value
A 21 P0011611	DNAH17	-0.65	2.31E-02	-1.04	1.11E-04	-0.95	3.79E-05
A 33 P3253144	DOK3	0.56	1.10E-03	0.62	4.21E-06	0.73	1.53E-08
A 23 P99163	DRAM1	0.63	1.58E-05	0.64	1.29E-06	0.76	1.17E-10
A 23 P39931	DYSF	0.86	2.28E-06	0.80	2.30E-08	0.91	9.25E-10
A 23 P401606	EDIL3	-0.37	1.71E-01	-0.90	3.24E-03	-0.99	1.03E-04
A 23 P126241	EIF4G3	0.63	7.25E-07	0.60	7.25E-09	0.74	4.61E-12
A 24 P322635	ELMO2	0.55	2.96E-05	0.60	5.84E-09	0.59	1.05E-09
A 33 P3359900	EMB	0.74	9.32E-06	0.86	4.39E-09	1.00	1.49E-13
A 24 P684186	EMB	0.61	2.26E-05	0.61	8.87E-08	0.69	1.91E-09
A 23 P27315	EMILIN2	0.61	3.81E-04	0.54	2.42E-04	0.67	3.70E-08
A 23 P106145	EOIL	0.60	3.10E-05	0.44	1.71E-04	0.59	2.27E-07
A 24 P314179	ETS2	0.84	4.91E-08	0.70	1.78E-07	0.75	5.06E-09
A 33 P3301410	EXOSC4	0.78	5.97E-05	0.84	1.78E-06	1.04	5.36E-11
A 23 P133438	FAM105A	0.64	5.20E-06	0.55	4.34E-06	0.62	5.56E-10
A 23 P334864	FAM126B	0.68	1.21E-05	0.71	4.04E-08	0.77	3.51E-08
A 32 P108254	FAM20A	0.65	5.55E-04	0.66	5.94E-04	0.86	7.22E-07
A 23 P214026	FBN2	0.18	5.49E-01	-0.79	3.85E-03	-0.77	2.29E-03
A 33 P3329549	FBR5	-0.46	2.59E-02	-0.67	1.06E-03	-0.72	3.17E-04
A 23 P208768	FCAR	0.90	6.42E-06	0.84	7.12E-07	0.92	4.16E-08
A 24 P348265	FCAR	0.75	4.88E-06	0.74	6.18E-08	0.94	2.40E-10
A 23 P103765	FCER1A	-0.69	1.29E-04	-0.65	7.66E-06	-0.92	2.24E-07
A 23 P103765	FCER1A	-0.59	5.06E-03	-0.59	1.69E-04	-0.95	1.51E-06
A 23 P103765	FCER1A	-0.49	1.11E-02	-0.62	1.56E-05	-0.94	1.14E-07
A 23 P103765	FCER1A	-0.49	7.16E-03	-0.66	9.88E-06	-0.89	2.22E-07
A 21 P0010561	FCGR1B	0.95	4.43E-06	0.85	2.38E-05	0.72	3.23E-05
A 21 P0010728	FCGR1B	0.91	9.34E-06	0.83	1.13E-05	0.72	1.88E-05
A 23 P63390	FCGR1B	0.83	1.56E-04	0.87	7.95E-06	0.77	1.81E-05
A 33 P3298810	FFAR3	0.63	2.70E-03	0.68	3.39E-04	0.84	7.53E-07
A 23 P217319	FGF13	0.55	2.84E-02	0.60	4.46E-03	0.82	6.09E-05
A 24 P38081	FKBP5	1.08	3.82E-06	1.08	1.23E-08	1.19	3.31E-09
A 23 P214603	FLOT1	0.59	1.34E-04	0.54	8.09E-07	0.60	7.96E-08
A 24 P253818	FLOT2	0.80	7.79E-08	0.71	1.61E-07	0.64	4.19E-08
A 24 P223124	FNDC3B	0.59	6.27E-06	0.51	7.47E-07	0.62	2.31E-09
A 23 P23221	GADD45A	1.06	1.08E-06	1.16	8.65E-10	1.32	1.86E-11
A 23 P67847	GALNT14	1.07	1.22E-04	1.20	8.53E-08	1.26	2.31E-09
A 24 P353794	GALNT2	0.60	7.90E-06	0.65	6.78E-07	0.63	2.26E-08
A 24 P82466	GAS7	0.61	2.82E-06	0.63	1.13E-07	0.62	3.50E-11
A 23 P28485	GCA	0.74	5.27E-05	0.64	4.19E-07	0.73	3.33E-07
A 33 P3343155	GNAQ	0.60	2.77E-05	0.61	6.24E-09	0.71	1.73E-12
A 23 P112260	GNG10	0.62	9.66E-05	0.60	3.04E-07	0.64	1.37E-06
A 23 P8640	GPER	0.59	3.45E-03	0.46	3.80E-03	0.71	4.73E-06
A 23 P6943	GPR15	0.09	7.55E-01	-0.89	3.21E-03	-0.85	2.48E-03
A 23 P167005	GPR160	0.64	2.72E-04	0.63	3.97E-06	0.70	2.52E-06
A 23 P25155	GPR84	1.25	3.72E-05	1.28	1.64E-07	1.50	3.40E-11
A 23 P140760	GPR97	0.59	8.37E-06	0.69	2.97E-06	0.76	4.30E-08
A 33 P3331687	GPSM1	-0.38	1.00E-01	-0.68	1.40E-03	-0.76	1.30E-04
A 23 P122863	GRB10	0.79	3.54E-04	0.86	2.04E-06	1.23	5.13E-11
A 23 P122863	GRB10	0.77	4.92E-04	0.90	8.44E-07	1.21	1.65E-10
A 23 P122863	GRB10	0.89	1.71E-04	0.88	1.58E-06	1.28	4.32E-10
A 23 P122863	GRB10	0.91	1.62E-04	0.90	1.15E-06	1.23	1.23E-10
A 23 P122863	GRB10	0.79	3.57E-04	0.93	4.22E-07	1.24	3.57E-11
A 23 P122863	GRB10	0.79	6.06E-04	0.92	7.58E-07	1.26	5.91E-11
A 23 P122863	GRB10	0.80	3.16E-04	0.90	1.19E-06	1.23	8.09E-11
A 24 P235266	GRB10	1.00	3.49E-05	1.04	1.03E-07	1.32	4.39E-11
A 23 P122863	GRB10	0.81	2.53E-04	0.90	3.05E-07	1.21	9.48E-11
A 23 P122863	GRB10	0.82	2.17E-04	0.91	7.43E-08	1.21	5.06E-11
A 23 P122863	GRB10	0.82	2.38E-04	0.93	2.31E-07	1.23	7.15E-11

Probe id	Gene Symbol	Day-3		Day-2		Day-1	
		logFC	P.Value	logFC	P.Value	logFC	P.Value
A 23 P153945	GTDC1	0.61	1.67E-05	0.61	5.57E-06	0.71	7.76E-09
A 23 P29422	GYG1	1.28	4.10E-07	1.22	9.68E-08	1.35	2.78E-11
A 21 P0013518	GYG1	1.18	1.42E-07	1.14	3.43E-08	1.28	2.09E-11
A 23 P384517	GYG1	1.12	1.46E-05	1.15	7.06E-07	1.36	5.73E-10
A 33 P3376821	GZMA	-0.62	1.31E-03	-0.76	2.39E-05	-0.60	1.87E-03
A 23 P156218	GZMK	-0.64	6.94E-04	-0.65	2.23E-04	-0.63	3.96E-04
A 33 P3306624	HCRT	-0.09	7.88E-01	-0.88	3.36E-03	-0.95	3.50E-04
A 23 P47034	HHEX	0.57	7.86E-05	0.60	1.84E-05	0.61	8.46E-06
A 24 P363548	HIP1	0.64	1.22E-06	0.53	1.76E-07	0.71	9.41E-11
A 23 P213584	HK3	1.22	6.82E-08	1.11	2.91E-08	1.22	7.15E-13
A 24 P50245	HLA-DMA	-0.28	2.01E-02	-0.60	8.98E-08	-0.68	2.11E-09
A 32 P51968	HLA-DMB	-0.66	4.17E-04	-0.84	4.52E-07	-0.94	9.74E-08
A 23 P30913	HLA-DPA1	-0.42	9.92E-03	-0.70	5.24E-07	-0.74	4.10E-06
A 33 P3234277	HLA-DPA1	-0.32	2.49E-02	-0.64	4.28E-06	-0.84	1.34E-07
A 24 P166443	HLA-DPB1	-0.43	6.24E-04	-0.67	5.96E-08	-0.66	4.63E-06
A 33 P3271651	HLA-DPB1	-0.36	1.51E-02	-0.61	2.05E-05	-0.68	2.10E-05
A 23 P8108	HLA-DQB1	-0.26	5.05E-02	-0.62	5.67E-06	-0.81	2.45E-08
A 32 P87697	HLA-DRA	-0.29	8.75E-02	-0.72	6.77E-06	-0.86	2.97E-07
A 24 P343233	HLA-DRB1	-0.41	3.93E-04	-0.60	7.09E-08	-0.71	5.22E-08
A 24 P343233	HLA-DRB1	-0.37	1.21E-03	-0.59	1.29E-07	-0.73	4.69E-08
A 24 P343233	HLA-DRB1	-0.31	9.20E-03	-0.68	1.18E-07	-0.80	2.81E-09
A 24 P343233	HLA-DRB1	-0.32	1.02E-02	-0.71	1.76E-08	-0.86	7.40E-10
A 24 P343233	HLA-DRB1	-0.31	8.66E-03	-0.71	4.37E-08	-0.82	1.14E-09
A 24 P343233	HLA-DRB1	-0.26	1.20E-02	-0.59	6.36E-07	-0.74	6.06E-08
A 24 P343233	HLA-DRB1	-0.27	2.61E-02	-0.74	2.70E-09	-0.85	6.07E-10
A 33 P3383912	HLA-DRB3	-0.18	2.21E-01	-0.61	2.43E-06	-0.68	1.57E-06
A 33 P3394605	HMG20B	-0.37	1.35E-01	-0.62	2.72E-04	-0.59	7.82E-04
A 23 P155765	HMGB2	0.52	3.74E-03	0.65	3.78E-06	0.77	9.47E-08
A 23 P206760	HP	1.39	1.21E-04	1.33	6.27E-05	1.77	4.23E-09
A 33 P3289236	HPR	1.31	9.79E-05	1.15	1.01E-04	1.54	1.32E-09
A 23 P142125	HRC	-0.18	4.65E-01	-0.72	8.73E-04	-0.75	2.35E-04
A 24 P103886	ID11	0.82	1.01E-05	0.73	6.78E-07	0.70	2.01E-06
A 23 P52266	IFIT1	-0.60	4.99E-02	-0.80	1.18E-03	-1.07	9.52E-05
A 33 P3224809	IL17RA	0.60	3.03E-06	0.47	6.85E-07	0.59	1.31E-08
A 23 P17706	IL17RA	0.65	1.88E-06	0.58	2.38E-09	0.67	1.01E-10
A 33 P3540143	IL17RA	0.61	4.77E-05	0.57	3.65E-07	0.73	3.86E-10
A 33 P3251876	IL18R1	0.75	1.72E-04	0.85	2.07E-07	1.05	1.93E-12
A 33 P3211666	IL18R1	0.97	4.81E-04	1.09	4.00E-06	1.44	8.00E-12
A 24 P208567	IL18R1	1.20	2.07E-05	1.25	1.63E-07	1.47	3.69E-12
A 23 P28334	IL18RAP	1.02	6.51E-05	1.11	5.60E-08	1.28	2.88E-09
A 33 P3221960	IL18RAP	1.06	3.41E-05	1.09	3.61E-08	1.28	2.04E-09
A 24 P63019	IL1R2	1.56	1.24E-06	1.54	1.51E-08	1.81	2.94E-10
A 23 P170857	IL1RAP	0.85	2.91E-07	0.64	1.59E-05	0.61	4.27E-05
A 23 P129556	IL4R	0.67	8.61E-04	0.78	1.20E-07	0.83	2.40E-07
A 33 P3349045	IL4R	0.64	2.40E-06	0.60	1.15E-07	0.63	3.03E-09
A 23 P109907	ILDR1	0.32	2.75E-01	0.62	1.92E-03	0.61	9.38E-04
A 23 P109907	ILDR1	0.27	3.46E-01	0.60	2.28E-03	0.61	8.51E-04
A 23 P18119	IMPG2	-0.23	3.61E-01	-0.69	1.75E-03	-0.79	1.80E-04
A 23 P73780	IRAK1	-0.39	7.29E-02	-0.61	1.11E-03	-0.71	1.17E-04
A 23 P162300	IRAK3	0.86	9.29E-06	0.88	5.97E-09	1.15	4.94E-12
A 23 P128084	ITGA7	0.64	2.46E-03	0.68	6.30E-04	0.86	2.54E-05
A 23 P124108	ITGAM	0.71	1.00E-05	0.58	2.22E-06	0.69	8.34E-08
A 23 P124108	ITGAM	0.69	4.16E-05	0.57	2.94E-06	0.70	3.94E-08
A 23 P124108	ITGAM	0.70	2.00E-05	0.57	3.09E-06	0.67	1.12E-07
A 23 P124108	ITGAM	0.87	6.18E-04	0.58	3.11E-06	0.72	6.37E-08
A 23 P124108	ITGAM	0.69	2.76E-05	0.62	4.64E-07	0.71	2.89E-08
A 23 P124108	ITGAM	0.69	1.70E-05	0.59	1.34E-06	0.74	1.48E-08

Probe id	Gene Symbol	Day-3		Day-2		Day-1	
		logFC	P.Value	logFC	P.Value	logFC	P.Value
A 23 P124108	ITGAM	0.71	1.14E-05	0.58	2.60E-06	0.72	3.98E-08
A 23 P124108	ITGAM	0.69	1.28E-05	0.59	1.71E-06	0.72	2.40E-08
A 23 P124108	ITGAM	0.70	1.13E-05	0.60	1.71E-06	0.71	4.26E-08
A 23 P124108	ITGAM	0.73	1.05E-05	0.59	1.33E-06	0.72	2.11E-08
A 24 P59667	JAK3	0.62	4.52E-04	0.69	1.47E-07	0.85	1.64E-11
A 33 P3338793	KCNC3	-0.16	4.60E-01	-0.68	8.58E-04	-0.73	9.52E-05
A 23 P109026	KCNK15	-0.45	2.55E-02	-0.71	6.23E-05	-0.71	8.15E-05
A 23 P109026	KCNK15	-0.46	1.62E-02	-0.64	3.56E-04	-0.72	1.14E-04
A 23 P109026	KCNK15	-0.26	2.90E-01	-0.69	2.38E-04	-0.69	2.11E-04
A 23 P123393	KCNQ3	-0.60	2.30E-02	-0.75	5.04E-03	-0.74	7.23E-04
A 23 P123393	KCNQ3	-0.95	1.29E-03	-0.27	2.95E-01	-0.68	6.81E-03
A 23 P123393	KCNQ3	-0.79	4.91E-03	-0.25	3.30E-01	-0.63	6.95E-03
A 23 P123393	KCNQ3	-0.79	5.05E-03	-0.31	2.12E-01	-0.64	7.98E-03
A 23 P123393	KCNQ3	-0.40	8.32E-02	-0.70	3.34E-03	-0.76	6.79E-04
A 23 P123393	KCNQ3	-0.27	3.35E-01	-0.90	1.10E-04	-0.92	1.72E-04
A 23 P123393	KCNQ3	-0.57	5.90E-02	-0.95	9.64E-05	-1.12	1.29E-05
A 23 P201287	KIF1B	0.85	1.29E-06	0.81	6.98E-09	0.89	2.71E-11
A 24 P145066	KIF1B	0.71	6.77E-10	0.54	9.37E-08	0.68	2.63E-13
A 24 P649624	KIF1B	0.76	6.79E-07	0.66	3.54E-06	0.80	9.10E-11
A 23 P104741	KIRREL3	-0.07	7.91E-01	-0.80	9.75E-04	-0.84	1.13E-04
A 23 P104741	KIRREL3	-0.19	4.81E-01	-0.69	5.05E-03	-0.76	6.15E-04
A 23 P104741	KIRREL3	-0.54	4.95E-02	-0.96	7.95E-05	-0.95	5.36E-05
A 23 P104741	KIRREL3	-0.37	1.85E-01	-0.70	4.96E-03	-0.95	1.85E-04
A 23 P72503	KLHL2	0.90	1.22E-06	0.88	1.85E-08	0.97	4.57E-09
A 23 P68851	KREMEN1	0.70	2.51E-04	0.66	2.31E-06	0.81	1.33E-06
A 23 P211401	KREMEN1	0.63	1.88E-06	0.60	2.47E-07	0.57	3.86E-07
A 23 P107465	KRT31	-0.12	5.89E-01	-0.63	6.33E-03	-0.64	7.12E-04
A 33 P3315303	KRT73	-0.31	3.45E-01	-0.91	5.82E-04	-0.97	3.82E-05
A 33 P3357651	KRTAP10-12	-0.58	2.30E-02	-0.93	4.50E-05	-0.86	2.74E-05
A 33 P3247473	KRTAP23-1	-0.24	3.15E-01	-0.71	7.98E-04	-0.59	3.02E-03
A 23 P116765	LALBA	-0.06	8.43E-01	-0.88	8.47E-04	-0.94	3.87E-05
A 23 P116765	LALBA	-0.24	4.47E-01	-0.95	1.89E-04	-0.90	3.77E-04
A 23 P116765	LALBA	-0.57	4.61E-02	-1.03	5.99E-05	-1.03	3.54E-05
A 23 P103361	LCK	-0.57	1.41E-03	-0.59	2.10E-04	-0.64	1.20E-04
A 23 P169437	LCN2	0.90	2.10E-03	0.66	3.30E-03	0.94	9.15E-04
A 23 P47565	LDHA	0.85	1.34E-05	0.77	1.10E-06	0.84	1.06E-08
A 24 P117029	LDLR	0.67	2.23E-05	0.58	2.77E-06	0.70	6.63E-08
A 23 P120902	LGALS2	-0.81	2.94E-02	-1.18	9.96E-04	-1.30	2.79E-05
A 23 P142205	LILRA2	0.76	1.32E-05	0.62	4.74E-06	0.57	3.03E-06
A 23 P79094	LILRA3	0.80	1.95E-03	0.61	5.84E-03	0.84	5.65E-05
A 23 P90497	LILRA4	0.70	5.83E-06	0.60	1.57E-06	0.53	5.69E-07
A 24 P370172	LILRA5	0.96	3.14E-06	0.95	2.70E-08	1.01	1.16E-09
A 32 P70158	LILRB3	0.70	2.15E-04	0.64	2.00E-07	0.73	7.20E-08
A 23 P376088	LIME1	-0.31	1.78E-01	-0.71	8.11E-05	-0.73	5.36E-05
A 24 P353300	LIMK2	0.74	1.54E-06	0.76	1.40E-11	0.73	1.51E-09
A 33 P3311285	LMNA	-0.36	1.25E-01	-0.62	2.70E-03	-0.72	7.25E-05
A 23 P50638	LRG1	0.64	2.17E-04	0.50	3.11E-04	0.68	4.11E-07
A 33 P3306948	LRP6	-0.22	3.44E-01	-0.63	2.51E-03	-0.66	1.04E-03
A 23 P29851	LRPAP1	0.64	3.45E-05	0.58	9.76E-06	0.62	4.30E-08
A 23 P41664	LRRC70	0.61	2.69E-04	0.76	2.61E-07	0.69	1.67E-07
A 23 P166848	LTF	1.18	1.62E-03	0.86	4.21E-03	1.07	2.53E-03
A 23 P136870	MAGEA6	-0.16	4.91E-01	-0.64	1.31E-03	-0.64	8.54E-04
A 23 P162211	MANSC1	0.66	1.84E-05	0.58	1.14E-04	0.65	1.33E-06
A 33 P3300308	MAP1LC3A	-0.23	2.37E-01	-0.63	3.62E-03	-0.72	2.21E-04
A 23 P207445	MAP2K6	0.62	1.04E-04	0.53	5.64E-05	0.63	8.42E-07
A 24 P283288	MAPK14	0.96	5.48E-07	0.95	3.20E-10	0.95	4.09E-10
A 33 P3272527	MAVS	-0.19	4.39E-01	-0.81	4.21E-03	-0.71	3.18E-03

Probe id	Gene Symbol	Day-3		Day-2		Day-1	
		logFC	P.Value	logFC	P.Value	logFC	P.Value
A 24 P244944	MCTP2	0.73	5.32E-06	0.76	2.25E-09	0.88	1.65E-12
A 23 P65789	MCTP2	0.76	8.23E-06	0.84	6.55E-10	0.87	6.21E-12
A 33 P3341676	MEF2A	0.67	1.30E-04	0.63	4.40E-06	0.82	8.08E-10
A 23 P103104	MFNG	-0.33	2.44E-01	-0.81	6.46E-03	-0.90	1.21E-04
A 23 P42897	MGAM	0.80	2.38E-05	0.83	2.38E-08	0.90	1.73E-08
A 33 P3324884	MICAL1	0.69	4.18E-06	0.72	5.77E-09	0.69	2.65E-10
A 33 P3289541	MLLT1	0.56	6.80E-06	0.60	1.39E-06	0.64	1.12E-09
A 33 P3282394	MLLT1	0.79	1.20E-05	0.75	1.98E-07	0.85	3.50E-11
A 23 P9823	MLXIP	0.68	6.16E-08	0.62	1.98E-08	0.72	1.74E-11
A 23 P40174	MMP9	1.20	5.43E-05	1.11	6.58E-06	1.46	1.85E-09
A 23 P40174	MMP9	1.15	8.33E-05	1.09	7.06E-06	1.45	1.56E-09
A 23 P40174	MMP9	1.17	1.15E-04	1.13	4.55E-06	1.48	1.46E-09
A 23 P40174	MMP9	1.16	1.91E-04	1.12	8.16E-06	1.48	1.34E-09
A 23 P40174	MMP9	1.21	6.16E-05	1.11	4.48E-06	1.49	1.17E-09
A 23 P40174	MMP9	1.18	7.30E-05	1.13	3.03E-06	1.45	1.43E-09
A 23 P40174	MMP9	1.19	7.41E-05	1.12	2.17E-06	1.44	2.15E-09
A 23 P40174	MMP9	1.20	5.89E-05	1.13	2.02E-06	1.46	1.44E-09
A 23 P40174	MMP9	1.21	6.39E-05	1.15	1.77E-06	1.46	1.32E-09
A 23 P40174	MMP9	1.18	7.54E-05	1.12	2.34E-06	1.45	1.50E-09
A 23 P141173	MPO	0.55	2.93E-03	0.61	3.10E-03	0.72	2.38E-03
A 23 P75769	MS4A4A	0.95	4.78E-05	0.92	3.31E-05	1.00	3.08E-07
A 23 P217778	MSL3	0.61	2.74E-04	0.53	4.98E-05	0.69	3.49E-08
A 23 P61426	MSRA	0.63	3.96E-05	0.63	4.85E-05	0.71	1.46E-07
A 33 P3417281	MUC4	-0.41	3.81E-02	-0.76	1.08E-04	-0.68	3.69E-04
A 24 P417706	MXD3	0.66	1.69E-06	0.60	1.20E-09	0.83	7.70E-05
A 23 P360240	MYEOV	-0.23	3.19E-01	-0.64	6.62E-03	-0.60	8.30E-04
A 23 P110473	NAIP	0.93	2.30E-05	0.86	1.80E-06	1.00	4.08E-08
A 23 P110473	NAIP	0.93	1.87E-05	0.83	3.82E-06	1.02	3.62E-08
A 23 P110473	NAIP	0.87	5.38E-05	0.82	1.35E-05	1.08	3.33E-08
A 23 P110473	NAIP	0.91	2.12E-05	0.83	3.19E-06	1.01	1.69E-08
A 21 P0012992	NAIP	0.90	6.87E-07	0.91	9.83E-08	0.94	2.93E-10
A 23 P110473	NAIP	0.92	5.40E-05	0.83	1.03E-05	1.01	2.29E-08
A 23 P110473	NAIP	0.95	1.51E-05	0.84	2.98E-06	1.02	3.11E-08
A 23 P110473	NAIP	0.94	2.45E-05	0.87	1.62E-06	1.00	3.30E-08
A 23 P110473	NAIP	0.95	1.51E-05	0.84	4.99E-06	1.01	2.48E-08
A 23 P110473	NAIP	1.06	1.54E-05	0.85	2.84E-06	0.99	3.65E-08
A 23 P110473	NAIP	0.94	2.26E-05	0.88	1.62E-06	1.00	2.85E-08
A 21 P0013998	NAIP	1.14	8.49E-07	1.02	1.85E-08	1.06	3.59E-09
A 33 P3364864	NAMPT	0.57	1.36E-03	0.67	6.35E-06	0.64	2.43E-05
A 23 P87329	NAT10	-0.63	2.97E-03	-0.33	9.38E-02	-0.63	1.89E-03
A 33 P3341970	NEGR1	-0.28	2.74E-01	-0.73	4.87E-03	-0.83	1.12E-04
A 23 P119835	NLRC4	0.86	2.82E-05	0.84	8.46E-08	1.05	2.49E-10
A 23 P47579	NLRP14	-0.05	8.41E-01	-0.63	5.94E-03	-0.61	3.93E-03
A 23 P82929	NOV	-0.60	2.73E-04	-0.68	2.74E-07	-0.75	1.82E-07
A 23 P58953	NQO2	0.73	2.92E-04	0.87	4.27E-07	0.76	3.43E-06
A 24 P7121	NSUN7	0.64	1.04E-03	0.74	8.66E-06	0.90	7.03E-09
A 23 P423331	NTNG2	0.53	1.03E-03	0.68	3.74E-08	0.81	7.30E-10
A 23 P161458	OLAH	0.78	1.08E-03	1.05	1.51E-05	1.15	9.99E-09
A 24 P181254	OLFM4	1.47	2.04E-04	1.20	2.07E-04	1.72	9.61E-06
A 23 P170186	OPLAH	0.98	7.45E-06	1.10	2.64E-08	1.34	2.50E-12
A 33 P3376090	OR1J4	0.03	9.02E-01	-0.61	4.53E-03	-0.65	2.28E-03
A 23 P94647	OR1L3	0.03	9.26E-01	-0.97	1.66E-03	-0.91	1.19E-03
A 23 P166408	OSM	0.98	4.63E-06	0.77	7.87E-06	0.89	1.66E-07
A 23 P124003	P2RX2	-0.29	1.45E-01	-0.73	6.63E-04	-0.60	3.11E-03
A 23 P124003	P2RX2	0.08	7.74E-01	-0.73	2.34E-03	-0.72	1.14E-03
A 24 P187970	PADI2	0.61	1.01E-04	0.39	2.22E-02	0.64	5.21E-08
A 33 P3216890	PAG1	0.78	9.41E-08	0.70	6.46E-10	0.84	9.56E-13

Probe id	Gene Symbol	Day-3		Day-2		Day-1	
		logFC	P.Value	logFC	P.Value	logFC	P.Value
A 32 P61684	PAG1	0.82	1.85E-07	0.75	2.45E-09	0.84	2.88E-09
A 23 P252681	PCYT1A	0.59	4.03E-06	0.66	5.26E-09	0.69	4.42E-11
A 23 P58396	PDGFC	0.41	1.54E-02	0.62	1.71E-04	0.78	2.02E-07
A 23 P161152	PDSS1	0.66	8.22E-06	0.55	8.82E-05	0.65	1.26E-08
A 23 P91140	PECR	0.59	1.24E-05	0.64	6.82E-08	0.74	5.11E-12
A 24 P413669	PFKFB2	1.40	5.56E-06	1.44	2.86E-08	1.59	6.10E-11
A 33 P3300635	PFKFB2	1.19	2.32E-05	1.09	2.80E-07	1.36	2.02E-09
A 24 P261259	PFKFB3	1.24	9.92E-06	1.30	2.77E-09	1.47	4.16E-12
A 24 P206604	PFKFB3	0.54	1.02E-05	0.60	2.89E-07	0.78	1.10E-09
A 23 P126623	PGD	0.80	7.62E-06	0.67	7.38E-06	0.72	7.32E-08
A 23 P34510	PHC2	0.61	4.51E-07	0.61	1.51E-07	0.69	3.45E-08
A 24 P393864	PHTF1	0.61	1.17E-06	0.52	1.05E-06	0.64	9.48E-10
A 33 P3376234	PHTF1	0.63	7.36E-06	0.67	1.27E-06	0.77	1.86E-10
A 24 P183128	PLAC8	0.93	3.77E-05	1.00	1.20E-05	1.12	9.92E-10
A 23 P56356	PLB1	0.88	1.41E-04	0.87	3.13E-07	0.93	2.65E-08
A 33 P3400763	PLIN4	0.83	2.09E-05	0.84	4.91E-08	1.04	2.43E-11
A 23 P39251	PLIN5	0.72	8.03E-05	0.68	2.59E-07	0.86	1.81E-08
A 24 P74932	PLP2	0.67	1.74E-04	0.62	8.40E-07	0.51	7.50E-06
A 23 P69109	PLSCR1	0.94	7.63E-06	0.81	4.76E-06	0.86	2.16E-06
A 33 P3220422	POM121L12	0.41	1.93E-01	-0.74	7.00E-03	-0.79	8.87E-04
A 24 P29723	POR	0.84	4.89E-06	0.72	4.03E-06	0.76	1.07E-08
A 33 P3835524	POU2F2	-0.28	1.85E-01	-0.63	6.26E-04	-0.78	1.59E-05
A 23 P362759	PRDM5	0.55	1.93E-04	0.60	1.44E-05	0.82	7.46E-12
A 24 P97342	PROK2	0.70	4.24E-04	0.71	1.85E-05	0.66	1.13E-04
A 24 P322353	PSTPIP2	0.62	2.71E-07	0.56	1.22E-06	0.61	6.45E-11
A 23 P48676	PSGL	0.63	1.69E-03	0.59	1.57E-06	0.68	1.60E-06
A 23 P12463	QSOX1	0.55	2.54E-04	0.63	1.51E-07	0.83	5.34E-11
A 24 P373174	RAB27A	0.67	5.70E-07	0.61	3.42E-08	0.62	2.76E-08
A 24 P303480	RAB32	0.61	3.56E-05	0.52	1.11E-04	0.61	5.59E-08
A 24 P337746	RABGEF1	0.72	2.96E-07	0.68	1.10E-07	0.80	3.27E-12
A 33 P3421571	RAPH1	-0.69	1.64E-02	-0.70	3.75E-03	-0.87	5.23E-05
A 23 P1962	RARRES3	-0.55	1.73E-03	-0.65	2.21E-05	-0.62	1.21E-04
A 24 P384397	RAVER1	-0.67	2.26E-02	-0.76	1.66E-03	-0.98	8.52E-05
A 23 P132910	RBM47	0.70	2.93E-06	0.61	4.95E-08	0.69	8.01E-10
A 33 P3271490	RBMS1	0.62	1.42E-05	0.67	1.58E-07	0.76	3.76E-10
A 23 P119222	RETN	1.37	2.08E-05	1.34	1.82E-05	1.52	6.33E-08
A 33 P3350863	RETN	1.50	5.17E-06	1.52	8.78E-07	1.83	3.97E-09
A 23 P306941	RGL4	1.05	3.39E-05	1.10	3.06E-09	1.20	1.35E-08
A 23 P151637	RNASE2	0.75	1.16E-05	0.59	6.44E-04	0.61	2.95E-04
A 23 P163025	RNASE3	0.69	2.28E-06	0.59	1.55E-04	0.62	1.73E-05
A 23 P257201	RNF146	0.61	2.80E-04	0.59	1.79E-06	0.61	1.06E-06
A 33 P3271316	RPP25	-0.02	9.65E-01	-0.91	3.63E-03	-1.01	8.51E-04
A 24 P808522	RPS14	-0.55	1.92E-07	-0.60	6.43E-08	-0.65	5.46E-10
A 23 P417331	RPS6KA3	0.48	1.90E-04	0.63	2.17E-09	0.69	1.06E-12
A 23 P120566	RRBP1	0.65	7.80E-07	0.55	3.54E-07	0.73	1.15E-12
A 33 P3211804	RUNX1	0.49	3.42E-04	0.60	3.36E-07	0.78	5.49E-10
A 23 P74001	S100A12	0.84	5.71E-04	0.93	2.01E-06	1.09	2.11E-08
A 33 P3385785	S100A12	1.04	1.04E-04	1.08	7.82E-07	1.30	2.57E-09
A 23 P23048	S100A9	0.87	3.07E-05	0.81	2.10E-06	0.80	1.36E-06
A 23 P29005	SAMSN1	1.25	3.17E-07	1.13	1.20E-08	1.28	3.26E-10
A 23 P145006	SCGB3A2	-0.19	3.73E-01	-0.63	1.71E-03	-0.69	3.72E-04
A 23 P152548	SCPEP1	0.60	6.32E-04	0.52	4.18E-04	0.59	7.46E-06
A 21 P0012051	SEPT14	0.64	4.85E-06	0.73	4.13E-09	0.80	8.70E-09
A 21 P0010748	SEPT14	0.68	7.47E-06	0.82	1.20E-09	0.89	4.88E-09
A 21 P0011898	SEPT14	0.57	1.03E-04	0.67	2.20E-08	0.72	2.32E-07
A 21 P0011897	SEPT14	0.75	5.28E-06	0.80	5.63E-11	0.86	2.75E-11
A 21 P0013195	SEPT14	0.70	1.32E-06	0.83	4.15E-11	0.84	1.07E-12

Probe id	Gene Symbol	Day-3		Day-2		Day-1	
		logFC	P.Value	logFC	P.Value	logFC	P.Value
A 23 P214330	SERPINB1	0.81	2.05E-05	0.82	1.12E-07	1.00	3.98E-11
A 24 P148750	SH3BP5	0.77	1.40E-06	0.67	1.05E-07	0.68	2.34E-09
A 33 P3298356	SH3GLB1	0.54	4.74E-04	0.63	1.90E-06	0.77	1.34E-07
A 23 P137470	SIPAIL2	0.89	5.35E-07	0.89	7.93E-10	0.97	3.57E-10
A 33 P3764802	SIRT5	0.59	1.04E-04	0.61	6.77E-06	0.71	1.21E-08
A 23 P363313	SLC16A11	0.13	5.60E-01	-0.67	8.35E-03	-0.62	6.39E-03
A 24 P286114	SLC1A3	0.69	3.10E-05	0.46	6.47E-04	0.69	1.52E-07
A 23 P156180	SLC22A4	0.58	6.61E-05	0.62	1.99E-07	0.61	5.01E-08
A 23 P106258	SLC25A47	-0.15	5.23E-01	-0.63	6.21E-04	-0.65	1.39E-04
A 23 P30950	SLC26A8	0.60	1.45E-05	0.57	5.41E-07	0.76	2.21E-12
A 24 P81900	SLC2A3	0.98	2.08E-07	1.00	3.57E-10	1.07	1.09E-11
A 23 P139669	SLC2A3	0.87	9.03E-08	0.92	1.79E-09	0.87	5.37E-10
A 33 P3251093	SLC36A1	0.51	3.58E-03	0.63	9.44E-07	0.89	1.97E-10
A 23 P95130	SLC37A3	0.71	4.51E-04	0.80	1.94E-07	0.94	9.40E-09
A 24 P295963	SLC38A2	0.62	6.77E-07	0.51	1.27E-06	0.64	1.70E-07
A 33 P3242458	SLC41A3	-0.37	1.21E-01	-0.74	2.69E-04	-0.78	1.41E-04
A 24 P58054	SLC9A8	0.53	3.41E-05	0.59	5.41E-08	0.67	5.92E-10
A 32 P154342	SLC04C1	0.59	5.90E-05	0.57	2.30E-05	0.71	2.47E-08
A 24 P277807	SNX3	0.67	3.39E-07	0.64	2.93E-07	0.51	8.39E-05
A 23 P207058	SOCS3	0.97	1.79E-04	0.94	9.87E-07	1.06	2.45E-07
A 33 P3409625	SORBS3	-0.59	4.11E-02	-0.70	6.50E-03	-0.96	1.46E-04
A 24 P325520	SORT1	0.88	1.96E-06	0.86	8.42E-07	0.94	8.01E-11
A 23 P117546	SOS2	0.53	5.88E-05	0.57	1.12E-08	0.65	7.28E-10
A 23 P117546	SOS2	0.53	5.46E-05	0.58	5.68E-09	0.68	8.69E-10
A 23 P117546	SOS2	0.58	5.34E-05	0.58	6.11E-09	0.64	1.08E-09
A 23 P117546	SOS2	0.51	1.02E-04	0.55	3.05E-08	0.69	2.16E-09
A 23 P117546	SOS2	0.54	2.70E-05	0.57	5.48E-09	0.69	6.91E-10
A 23 P117546	SOS2	0.47	4.44E-04	0.57	1.41E-07	0.68	7.44E-10
A 23 P117546	SOS2	0.52	6.85E-05	0.58	6.22E-09	0.66	9.95E-10
A 23 P117546	SOS2	0.51	1.29E-04	0.57	9.94E-09	0.67	3.73E-10
A 23 P117546	SOS2	0.53	5.01E-05	0.59	3.48E-09	0.54	3.97E-04
A 23 P117546	SOS2	0.53	6.92E-05	0.58	8.96E-09	0.69	2.13E-10
A 24 P385611	SP100	0.61	3.59E-07	0.63	1.25E-10	0.72	2.31E-14
A 23 P431388	SPOCD1	0.53	1.07E-02	0.64	4.14E-05	0.64	2.86E-04
A 33 P3214943	SPOCK2	-0.50	3.14E-02	-0.90	9.28E-06	-0.94	6.17E-06
A 33 P3222139	SREBF1	-0.76	3.63E-03	-0.61	3.18E-03	-0.79	2.46E-04
A 23 P19543	SRPK1	0.57	5.04E-04	0.64	2.01E-07	0.67	1.50E-07
A 23 P429560	SSH1	0.61	4.65E-06	0.72	1.68E-09	0.85	1.64E-13
A 24 P181055	ST3GAL4	0.62	3.58E-04	0.59	1.97E-05	0.71	3.23E-05
A 33 P3275055	ST6GALNAC3	0.59	1.52E-04	0.60	1.24E-05	0.75	5.91E-10
A 33 P3356220	STARD3	-0.47	3.72E-02	-0.59	2.62E-03	-0.74	4.31E-05
A 24 P141214	STOM	0.95	5.85E-08	0.87	7.22E-07	0.88	5.79E-08
A 23 P154605	SULF2	-0.70	5.25E-05	-0.81	3.72E-06	-0.72	1.14E-05
A 33 P3335920	SYNE1	-0.40	5.80E-02	-0.73	1.45E-04	-0.74	2.03E-04
A 23 P70733	TAA2	-0.39	1.64E-01	-0.79	1.50E-03	-0.75	2.82E-03
A 24 P148590	TACR1	0.13	5.57E-01	-0.61	9.51E-03	-0.83	4.65E-05
A 33 P3378659	TARP	-0.40	3.61E-02	-0.64	1.46E-03	-0.68	2.63E-04
A 33 P3309075	TBC1D8	0.98	1.45E-07	0.98	2.53E-08	1.08	2.42E-13
A 23 P64372	TCN1	0.75	3.10E-04	0.68	2.17E-04	0.79	3.43E-05
A 32 P208350	TDRD9	1.19	1.30E-05	1.28	1.59E-08	1.43	7.32E-11
A 23 P143845	TIPARP	0.78	3.84E-06	0.74	4.07E-07	0.72	5.53E-10
A 23 P85903	TLR5	0.83	1.26E-05	0.84	3.24E-09	1.12	1.63E-10
A 23 P73837	TLR8	0.60	1.49E-04	0.55	1.20E-06	0.61	8.30E-08
A 33 P3257279	TMEM145	-0.29	2.02E-01	-0.67	3.85E-03	-0.75	6.91E-04
A 33 P3411612	TMEM221	0.06	8.12E-01	-0.61	5.83E-03	-0.60	2.48E-03
A 23 P126844	TNFRSF25	-0.59	5.43E-04	-0.58	1.37E-04	-0.64	8.61E-05
A 33 P3286157	TNFRSF4	-0.31	1.10E-01	-0.64	5.32E-05	-0.67	7.97E-06

Probe id	Gene Symbol	Day-3		Day-2		Day-1	
		logFC	P.Value	logFC	P.Value	logFC	P.Value
A 33 P3364582	TNXB	-0.04	8.73E-01	-0.71	3.45E-03	-0.72	8.17E-04
A 23 P5392	TP53I3	0.83	2.67E-06	0.66	6.22E-05	0.78	2.14E-08
A 33 P3392077	TP53I3	0.83	4.55E-06	0.69	2.54E-05	0.83	9.84E-08
A 33 P3223980	TPM3	0.58	2.17E-07	0.59	1.57E-08	0.61	1.97E-10
A 24 P27977	TRPM2	0.77	1.44E-06	0.63	3.13E-06	0.77	2.06E-10
A 33 P3413216	TSPAN4	-0.38	8.06E-02	-0.84	6.95E-05	-0.79	1.97E-05
A 33 P3251148	TSPO	0.76	1.03E-04	0.70	5.88E-06	0.78	1.72E-08
A 24 P11436	TTC22	-0.22	2.93E-01	-0.62	6.57E-04	-0.63	2.95E-04
A 32 P148796	UBXN2B	0.66	1.00E-05	0.55	2.73E-07	0.63	1.33E-09
A 23 P17330	UCKL1	-0.22	4.23E-01	-0.71	3.86E-03	-1.06	6.10E-06
A 23 P313389	UGCG	1.17	1.07E-07	1.12	3.26E-09	1.29	7.12E-13
A 24 P112160	UPK3B	-0.13	6.18E-01	-0.66	2.87E-03	-0.62	4.86E-03
A 23 P351275	UPP1	1.03	1.38E-06	0.92	6.89E-07	1.02	1.02E-08
A 33 P3399571	VNN1	1.27	3.39E-05	1.20	5.27E-06	1.39	3.69E-09
A 32 P10396	WDFY3	0.73	2.67E-06	0.63	5.64E-09	0.67	1.88E-08
A 33 P3411925	WDR18	-0.45	4.73E-02	-0.82	2.57E-05	-0.84	1.98E-05
A 23 P4353	WSB1	0.68	2.15E-05	0.67	5.55E-08	0.85	2.48E-11
A 32 P178945	YOD1	0.69	1.63E-03	0.63	2.62E-03	0.59	3.10E-03
A 23 P99397	ZDHHC20	0.80	2.74E-07	0.77	2.22E-07	0.96	3.41E-14
A 33 P3376449	ZDHHC23	-0.47	3.24E-02	-0.81	2.06E-05	-0.80	4.65E-06
A 24 P351420	ZDHHC3	0.61	9.15E-06	0.49	1.18E-05	0.60	1.49E-08
A 23 P27424	ZNF418	-0.26	3.19E-01	-0.63	6.88E-03	-0.77	4.62E-04
A 23 P161156	ZNF438	0.70	4.37E-05	0.72	5.15E-08	0.81	8.60E-10
A 33 P3263756	ZNF446	-0.10	6.04E-01	-0.60	1.49E-03	-0.66	1.27E-04
A 33 P3345132	ZNF578	-0.05	8.43E-01	-0.72	7.57E-03	-0.74	3.74E-04

There is a significant overlap (54 genes) between the genes in ISB 58 and ISB 355 (FIG. 10).

The genes included in the ISB19 and ISB63 panels are listed in Table 12 and the overall expression profiles for those genes are shown in FIGS. 11A and 11B. Exemplary, non-limiting GenBank Accession numbers are provided for each gene in Table 1. Additional sequences for each gene can be identified using publicly available databases.

Table 12. List of genes included in ISB19 and ISB63

Gene Symbol	GenBank Acc. No.	ISB19	ISB63	Gene Symbol	GenBank Acc. No.	ISB19	ISB63
ADAMTSL5	NM_213604		X	NQO2	NM_000904		X
ARID5A	NM_212481		X	NSUN7	NM_024677		X
ATP11B	NM_014616		X	OLAH	NM_018324		X
ATP6V1C1	NM_001695	X	X	P2RX2	NM_016318		X
ATP9A	NM_006045	X		PDGFC	NM_016205		X
B4GALT5	NM_004776		X	PDSS1	NM_014317		X
BMX	NM_001721	X		PFKFB3	NM_004566	X	X
CA4	NM_000717		X	PGLYRP1	NM_005091	X	
CARNS1	NM_020811		X	PLIN4	NM_001080400		X
CD164	NM_006016		X	POM121L12	NM_182595		X
CD55	NM_000574		X	POU2F2	NM_002698		X
CD63	NM_001780		X	PYGL	NM_002863		X
CD82	NM_002231		X	RBM47	NM_019027		X
CFLAR	NM_003879		X	RGL4	NM_153615	X	
CLEC12A	NM_138337		X	RNASE3	NM_002935		X

Gene Symbol	GenBank Acc. No.	ISB19	ISB63	Gene Symbol	GenBank Acc. No.	ISB19	ISB63
CST7	NM_003650		X	RNF146	NM_030963		X
CYP27A1	NM_000784		X	RRBP1	NM_004587		X
DOK3	NM_024872		X	RUNX1	NM_001754		X
ETS2	NM_005239		X	SCGB3A2	NM_054023		X
FCAR	NM_002000	X		SEPT14	NM_207366		X
FFAR3	NM_005304		X	SH3BP5	NM_004844		X
GALNT14	NM_024572	X		SLC2A3	NM_006931	X	X
GBP5	NM_052942	X		SPOCK2	NM_014767		X
GPER	NM_001505		X	ST6GALNAC3	NM_152996		X
GPR15	NM_005290		X	STOM	NM_004099		X
HK3	NM_002115	X		SYNE1	NM_033071		X
HLA-DRA	NM_019111		X	TAAR2	NM_014626		X
IL18R1	NM_003855	X		TARP	NM_001003799		X
IL1R2	NM_004633	X		TCN1	NM_001062	X	X
IL1RAP	NM_002182		X	TP53I3	NM_004881	X	
KREMEN1	NM_032045		X	TPM3	NM_153649		X
LDLR	NM_000527		X	UCKL1	NM_017859		X
LGALS2	NM_006498	X	X	UGCG	NM_003358	X	
LRG1	NM_052972	X	X	UPP1	NM_003364	X	
LTF	NM_002343		X	WSB1	NM_015626		X
MFNG	NM_002405		X	YOD1	NM_018566		X
MICAL1	NM_022765		X	ZDHHC23	NM_173570		X
MSL3	NM_078628		X	ZNF446	NM_017908		X

Example 3

Validation of Gene Panels for Predicting Development of Sepsis

An SVM model for each panel that was trained using the Discovery sample set (Example 2) was generated and subsequently tested in the Validation sample set. Classification performance was summarized and listed with four metrics: AUC, Accuracy, Sensitivity, and Specificity in Table 13. Both panels provided high predictive performance. For example, the AUC values of ISB19 are > 0.7 and the AUC values of ISB63 were even higher, at ≥ 0.8 at all time points. ISB63 showed greater than 77% of predictive accuracy for the development of sepsis three days prior to sepsis diagnosis (Day-3). The two panels ISB19 and ISB63, showed very little overlap of genes in the panel (Table 11).

Table 13. Classification performances of ISB19 and ISB63 in Validation sample set.

	ISB19			ISB63		
	Day-3	Day-2	Day-1	Day-3	Day-2	Day-1
AUC	0.7538	0.7690	0.8005	0.8897	0.8263	0.8558
Accuracy	0.6622	0.6866	0.7457	0.7703	0.7313	0.7341
Sensitivity	0.6486	0.7391	0.7701	0.8649	0.7681	0.8161
Specificity	0.6757	0.6308	0.7209	0.6757	0.6923	0.6512

Example 4

Assessing Ability of ISB Panels to Diagnose Sepsis Using Diverse Datasets

Even though the ISB panels were developed using pre-sepsis diagnosis gene expression data to predict the development of sepsis, the ability of the ISB19 and ISB63 gene panels (Example 2) were assessed to diagnose patients with sepsis using 19 publicly available sepsis related datasets derived from 1,636 patients (Table 14). The public datasets can be grouped based on patient information, such as bacterial or viral infection, or adult or pediatric sepsis. With this information, the datasets were grouped into 6 groups based on clinical parameters: 1) Sepsis/severe sepsis, 2) Pediatric sepsis, 3) Neonatal sepsis, 4) Sepsis associated with bacterial infection, 5) Sepsis associated with bacterial/viral infection, and 6) Sepsis associated with viral infection.

Table 14. Public datasets used to assess the diagnostic performance of ISB19 and ISB63 panels

Subcategory	Accession	Platform	Clinical Comparison	# Control	# Cases
Sepsis or severe sepsis vs Control	GSE69528	Illumina HumanHT-12 V4.0 expression beadchip	Adults with sepsis, many from burkholderia		83
	GSE57065	Affymetrix Human Genome U133 Plus 2.0 Array	Septic shock at admission, 24 hr, 48 hr	25	82
	GSE28750	Affymetrix Human Genome U133 Plus 2.0 Array	Community-acquired sepsis at admission to ICU	20	10
Pediatric sepsis vs Control	GSE66099	Affymetrix Human Genome U133 Plus 2.0 Array	Pediatric sepsis	47	199
	E-MEXP-3567	Affymetrix Human Genome U133A Array	Children with meningococcal sepsis ± HIV coinfection	3	12
	GSE11755	Affymetrix Human Genome U133 Plus 2.0 Array	Children with meningococcal sepsis	3	6
Neonatal sepsis vs Control	GSE25504	Illumina HumanHT-12 V3.0 expression beadchip	Neonatal sepsis	35	28
	GSE25504	Affymetrix Human Genome U219 Array	Neonatal sepsis	6	14
	GSE25504	Affymetrix Human Genome U133 Plus 2.0 Array	Neonatal sepsis	3	2
Bacterial infection with sepsis vs Control	GSE65682	Affymetrix Human Genome U219 Array	Adults in ICU with CAP	42	101
	GSE33341	Affymetrix Human Genome U133A 2.0 Array	Bloodstream infection Staphylococcus aureus or Escherichia coli	43	51
Bacterial or viral infection with sepsis vs Control	GSE40396	Illumina HumanHT-12 V4.0 expression beadchip	Children with infection + fever	22	30
	E-MEXP-3589	Agilent-026652 Whole Human Genome Microarray 4x44K v2	Hospitalized chronic obstructive pulmonary disease patients with infection	4	14
Viral infection with sepsis	GSE68310	Illumina HumanHT-12 V4.0 expression beadchip	Outpatients with acute viral illness at days 0 and 2	243	258

Subcategory vs Control	Accession	Platform	Clinical Comparison	# Control	# Cases
	GSE17156	Affymetrix Human Genome U133A 2.0 Array	Viral challenge peak symptoms	56	27
	GSE21802	Illumina human-6 v2.0 expression beadchip	Severe H1N1 influenza with mechanical ventilation	4	12
	GSE27131	Illumina human-6 v2.0 expression beadchip	Severe H1N1 influenza with mechanical ventilation	4	12
	E-MTAB-3162	Affymetrix Human Genome U133 Plus 2.0 Array	Dengue fever ($\pm$ severe) within 48 hr of fever	15	30
	GSE51808	Affymetrix HT HG-U133+ PM Array Plate	Dengue fever ($\pm$ severe) at admission	9	28

Even though the ISB19 and ISB63 panels were developed for predicting the development of sepsis, these panels still showed good performance in diagnosing patients with sepsis in unrelated and independent datasets obtained from the public domain (FIG. 12). Importantly, these datasets were generated with different sample preparation methods, measurement platforms, and diverse patient cohorts. The results shown in FIG. 12 suggest that the measurement platform, disease condition and sample preparation method have very little effect on the performance of the ISB19 and ISB63 panels. Also, both panels showed high classification performance in most sepsis datasets and bacterial or bacterial/viral infection datasets (AUC > 0.8), except for ISB63 in neonatal sepsis and ISB19 in one of the bacterial/viral infection datasets). However, both panels showed lower performance in two of the viral infection datasets, GSE68310 and GSE17156. Importantly, viral infection is not a common cause of sepsis post-surgery.

Example 5

ISB19 and ISB63 Panels are Both Prognostic and Diagnostic for Sepsis

To determine the utility of ISB19 and ISB63 panels, the ability of ISB panels to predict the development and diagnose sepsis was assessed and compared with panels reported in literature (Stanford11) and approved by FDA (Septicyte4). The results show that the previously identified sepsis panels, Stanford11 and Septicyte4, were able to accurately diagnose but not predict sepsis development (FIG. 13). In contrast, the ISB panels performed well to predict the development of sepsis in the pre-symptomatic period (from Day-3 to Day-1) (FIG. 13). In addition, the panels, in particular ISB63, showed better or similar ability as the Stanford11 and Septicyte4 panels to diagnose (Day 0) and monitor (Day 1 and Day 2) sepsis. These findings suggest that the ISB panels described herein can not only predict the development of sepsis at the pre-symptomatic period, but also diagnose and monitor the sepsis condition in patients.

Example 6

Biological Function-Based Approach to Optimize Panels

The methods described in Example 1 to optimize gene panels were applied to the ISB19 and ISB63 panels. The biological processes associated with ISB19 and ISB 63 panels were first determined.

5 The Gene Ontology (GO) enrichment test showed a total of 89 GOBPs associated with genes in the ISB19 panel and 326 GOBPs associated with the ISB63. The EnrichmentMap tool summarized the 89 GOBPs associated with ISB19 into three representative functional terms: immune response, signal transduction, and metabolism. Similarly, the 326 GOBPs associated with ISB63 were summarized into six representative terms: immune response, signal transduction, metabolism, apoptosis, transcription, and

10 adhesion/migration (Table 15).

Genes from the original ISB58 and ISB355 sets that shared the same functional terms and similar direction of concentration changes with genes already included in the ISB19 and ISB63 were then used. From ISB58 gene set, 13, 3, and 4 genes were identified that have the same direction of concentration changes with genes in ISB19 and are associated with immune response, signal transduction, and

15 metabolism, respectively. From ISB355 gene set, 67, 55, 41, 53, 7 and 40 genes were identified that have the same direction of concentration changes with genes in ISB63 and are associated with immune response, signal transduction, metabolism, apoptosis, transcription and adhesion/migration, respectively (Table 15).

20 Table 15. List of functional terms associated with ISB19 and ISB63 and available alternative candidates from ISB58 and ISB355 for genes in the ISB19 and ISB63 panels

Functional term	ISB19	ISB63
Immune response	13	67
Signal transduction	3	55
Metabolism	4	40
Apoptosis		41
Transcription		53
Adhesion/Migration		7

The genes identified from ISB58 and ISB355 (number of available genes for individual functional terms are listed in Table 15) were then used to compute classification performance of three-

25 gene combinations that represent three functional terms associated with ISB19 and six gene combinations for ISB63. In total, the performance of 156 three-gene combinations and randomly selected 100,000 (out of 2.24×10^9 possibilities) six-gene combinations, respectively, were assessed. The top 10 performing three (from ISB19) and six (from ISB63) gene panels are listed in Tables 16 and 17, respectively. The results showed slightly higher or similar average performance for the ISB19 derived three gene panels

30 than the original ISB19 at all three time points (FIG. 14). For ISB63 derived six gene panels, slightly lower performances at all time points were observed compared to the original ISB63 (FIG. 15). Since some of the genes in the ISB58 or ISB355 that were not in the biological processes associated with ISB19 or ISB63 can still contribute to the classification performance, additional genes were added (one

gene at a time) to the top 10 ISB19 derived 3-gene or ISB63 derived 6-gene panels. The results showed a significant improvement of overall performance when adding one or two genes for both ISB19 and ISB63 derived panels (Tables 16 and 17, and FIGS. 14 and 15). The slight decrease in performance of the panels with smaller number of features than the original 19 or 63 gene panels is a trade-off that provides advantages with respect to future development and application in a clinical setting.

Table 16. The top 10 performance of 3, 4 and 5 gene panels derived from ISB19 based on biological function.

Panels	Composition	Day-3	Day-2	Day-1
ISB19		0.7929	0.8021	0.7786
ISB19 derived 3 gene panels	LCN2, SLC2A3, BMX	0.8284	0.8279	0.8233
	LCN2, SLC2A3, GRB10	0.7959	0.7885	0.8002
	LCN2, PFKFB3, GRB10	0.8033	0.7355	0.7759
	LCN2, PFKFB3, BMX	0.8003	0.7351	0.7599
	IL1R2, HK3, BMX	0.7722	0.7690	0.7284
	LCN2, HK3, BMX	0.8358	0.7944	0.7645
	LCN2, HK3, GRB10	0.8225	0.7794	0.7722
	GZMA, HK3, BMX	0.8343	0.7654	0.7577
	FCAR, PFKFB2, BMX	0.7737	0.7178	0.7737
	LCN2, PFKFB3, IL18R1	0.7870	0.7396	0.7716
ISB19 derived 4 gene panels	LCN2, PFKFB3, GRB10, ST6GALNAC3	0.8047	0.7255	0.7845
	LCN2, SLC2A3, BMX, LGALS2	0.8136	0.8374	0.8291
	IL1R2, SLC2A3, BMX, TCN1	0.8018	0.8478	0.8143
	LCN2, SLC2A3, GRB10, ST6GALNAC3	0.7737	0.7758	0.8245
	FCAR, PFKFB2, BMX, CEACAM1	0.7840	0.7088	0.7746
	IL1R2, HK3, BMX, CD24	0.7870	0.7763	0.7457
	IL1R2, PFKFB3, BMX, CD24	0.8121	0.7332	0.7703
	BMX, SLC2A3, GRB10, CD24	0.8136	0.7939	0.8143
	IL1R2, HK3, BMX, CEACAM1	0.7959	0.7722	0.7445
	GZMA, SLC2A3, BMX, CD24	0.8388	0.8143	0.8381
ISB19 derived 5 gene panels	LCN2, PFKFB3, GRB10, ST6GALNAC3, RNASE3	0.8018	0.7373	0.7993
	LCN2, PFKFB3, GRB10, RNASE2, ST6GALNAC3	0.8077	0.7355	0.7996
	IL1R2, PFKFB3, GRB10, CD24, ST6GALNAC3	0.8240	0.7400	0.8014
	GZMA, PFKFB3, GRB10, ST6GALNAC3, CD24	0.8402	0.7450	0.8159
	HK3, PFKFB3, GRB10, ST6GALNAC3, CD24	0.8402	0.7554	0.8107
	IL1R2, PFKFB3, GRB10, ST6GALNAC3, TCN1	0.8047	0.7767	0.8033
	SLC2A3, PFKFB3, GRB10, ST6GALNAC3, TCN1	0.8077	0.7966	0.8384
	LCN2, PFKFB3, GRB10, ST6GALNAC3, DACH1	0.8047	0.7364	0.7922
	SLC2A3, PFKFB3, GRB10, ST6GALNAC3, CD24	0.8299	0.7831	0.8381
	SLC2A3, HK3, BMX, SPOCD1, LGALS2	0.7811	0.7853	0.7925

- Exemplary GenBank Accession Nos. are provided for the genes in Table 16 either in Table 12, or as follows: GRB10 – GenBank Accession No. NM_001350814; GZMA - GenBank Accession No. NM_006144; PFKFB2 - GenBank Accession No. NM_006212; CD24 - GenBank Accession No. NM_037362; RNASE2 - GenBank Accession No. NM_002934; DACH1 - GenBank Accession No. NM_004392; SPOCD1 - GenBank Accession No. NM_144569.

Table 17. The top 10 performance of 6, 7 and 8 gene panels derived from ISB63 based on biological function

Panels	Composition	Day-3	Day-2	Day-1
ISB63		0.9157	0.8071	0.8347
ISB63 derived 6 gene panels	RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO	0.8772	0.8216	0.8590
	RPS6KA3, MAVS, TPM3, BCL6, STOM, MPO	0.8772	0.8243	0.8575
	RPS6KA3, BCL6, TPM3, BMX, STOM, MPO	0.8861	0.8170	0.8571
	RPS6KA3, LTF, TPM3, BCL6, STOM, PDGFC	0.8891	0.8333	0.8655
	RPS6KA3, BCL6, TPM3, CD55, STOM, MPO	0.8802	0.8184	0.8575
	RPS6KA3, BCL6, TPM3, GNG10, STOM, PDGFC	0.8654	0.8211	0.8565
	RPS6KA3, LTF, TPM3, BCL6, STOM, CYP1B1	0.8905	0.8342	0.8685
	LTF, BCL6, TPM3, CD55, STOM, PDGFC	0.8905	0.8270	0.8633
	RPS6KA3, BCL6, TPM3, TLR8, STOM, MPO	0.8817	0.8197	0.8584
	RPS6KA3, BCL6, TPM3, HLA-DRA, GNG10, MPO	0.8476	0.7649	0.8350
ISB63 derived 7 gene panels	RPS6KA3, MLLT1, TPM3, BCL6, GNG10, CYP27A1, YOD1	0.8728	0.8143	0.8402
	RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1	0.8891	0.8066	0.8285
	RPS6KA3, GAS7, TPM3, BCL6, RRBPI, MPO, YOD1	0.9009	0.8202	0.8313
	RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, LGALS2	0.9157	0.8125	0.8399
	RPS6KA3, GAS7, TPM3, BCL6, GNG10, MPO, YOD1	0.8891	0.8179	0.8307
	LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1	0.9157	0.8179	0.8334
	RPS6KA3, BCL6, TPM3, GNG10, LILRA2, CYP27A1, YOD1	0.8654	0.8225	0.8347
	RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, LGALS2	0.8876	0.8279	0.8534
	RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, YOD1	0.8935	0.8134	0.8414
	RPS6KA3, BCL6, TPM3, CD55, STOM, MPO, IL17RA	0.8964	0.8220	0.8605
ISB63 derived 8 gene panels	RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, LGALS2	0.8876	0.8098	0.8282
	RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, LILRA4	0.8920	0.8030	0.8300
	RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, IL17RA	0.8979	0.8111	0.8285

Panels	Composition	Day-3	Day-2	Day-1
	RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, TCN1	0.9172	0.8252	0.8374
	RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE3	0.8950	0.8084	0.8307
	RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, NOV	0.8876	0.7971	0.8264
	RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE2	0.8920	0.8066	0.8304
	LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, FAM105A	0.9112	0.8116	0.8331
	RPS6KA3, BCL6, TPM3, HLA-DRA, RRBPI, MPO, ERO1L, YOD1	0.8950	0.7799	0.8177
	LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, C14orf101	0.9112	0.8211	0.8353

Exemplary GenBank Accession Nos. are provided for the genes in Table 17 either in Table 12, with respect to Table 16, or as follows: RPS6KA3 – GenBank Accession No. NM_004586; BCL6 - GenBank Accession No. NM_001706; GNG10 - GenBank Accession No. NM_001017998; MPO - GenBank Accession No. NM_000250; MAVS - GenBank Accession No. NM_020746; CYP1B1 - GenBank Accession No. NM_000104; TLR8 - GenBank Accession No. NM_016610; MLLT1 - GenBank Accession No. NM_005934; GAS7 - GenBank Accession No. NM_003644; LILRA2 - GenBank Accession No. NM_006866; IL17RA - GenBank Accession No. NM_014339; LILRA4 - GenBank Accession No. NM_012276; NOV - GenBank Accession No. NM_002514; FAM105A - GenBank Accession No. NM_019018; ERO1L - GenBank Accession No. GenBank Accession No. NM_014584; C14orf101 - GenBank Accession No. NM_017799.

Example 7

Diagnostic Performance of Reduced Size Panels

Since the ISB predictive panels ISB19 and ISB63 also showed good diagnostic performance, public domain data were used to assess the ability of smaller gene panels to accurately diagnose sepsis. The average AUCs of the 19 datasets are summarized in FIG. 16. Even though the public datasets consist of post-symptomatic samples, the ISB biomarker panels still showed good performance compared to other sepsis diagnostic panels including Stanford11 and the recently FDA approved Septicyte 4. This finding suggests that like the ISB19 and ISB63 panels, the performance of smaller panels were not significantly impacted by sample preparation methods, measurement platforms and patient cohort demographics.

Example 8

Integrating Clinical Information Enhances Performance of Biomarker Panels

The Sequential Organ Failure Assessment (SOFA) score is used to determine the extent of a patient's organ failure. C-reactive protein (CRP) levels are commonly used clinically as a non-specific

indicator of infection. These two clinical parameters are often utilized as part of the sepsis diagnosis, and for most patients in this study this clinical information was available. SOFA score or CRP levels alone did not provide good performance in either diagnosing or predicting the development of sepsis (Table 18). However, integrating these two clinical parameters with the ISB gene panels slightly increased sensitivity, but not specificity (Table 18).

Table 18. Performance summaries of ISB gene panels integrated with SOFA and CRP levels

Panel and combination	AUC			ACCURACY			Sensitivity			Specificity		
	Day-3	Day-2	Day-1	Day-3	Day-2	Day-1	Day-3	Day-2	Day-1	Day-3	Day-2	Day-1
SOFA	0.65	0.65	0.64	0.59	0.61	0.61	0.58	0.63	0.53	0.60	0.59	0.68
CRP	0.47	0.41	0.50	0.54	0.61	0.46	1.00	1.00	1.00	0.00	0.00	0.00
SOFA+CRP	0.47	0.67	0.78	0.41	0.60	0.66	0.56	0.64	0.79	0.25	0.53	0.55
ISB19 derived 3 gene panels	0.81	0.77	0.77	0.73	0.69	0.72	0.74	0.70	0.75	0.73	0.69	0.69
ISB19 derived 3 gene panels +SOFA	0.79	0.74	0.76	0.63	0.60	0.66	0.77	0.72	0.78	0.50	0.48	0.52
ISB19 derived 3 gene panels +CRP	0.83	0.75	0.85	0.62	0.58	0.70	0.87	0.80	0.91	0.38	0.35	0.48
ISB19 derived 3 gene panels +SOFA+CRP	0.82	0.73	0.81	0.57	0.56	0.64	0.90	0.78	0.91	0.24	0.33	0.35
ISB19 derived 4 gene panels	0.80	0.78	0.79	0.72	0.72	0.73	0.70	0.71	0.74	0.73	0.73	0.72
ISB19 derived 4 gene panels +SOFA	0.79	0.76	0.78	0.63	0.62	0.66	0.73	0.72	0.78	0.52	0.52	0.53
ISB19 derived 4 gene panels +CRP	0.79	0.76	0.86	0.61	0.58	0.71	0.87	0.77	0.91	0.35	0.38	0.50
ISB19 derived 4 gene panels +SOFA+CRP	0.81	0.74	0.83	0.55	0.56	0.65	0.87	0.77	0.91	0.23	0.35	0.37
ISB19 derived 5 gene panels	0.81	0.76	0.81	0.74	0.71	0.74	0.75	0.70	0.79	0.73	0.72	0.69
ISB19 derived 5 gene panels +SOFA	0.80	0.74	0.80	0.63	0.60	0.68	0.77	0.70	0.81	0.50	0.50	0.54
ISB19 derived 5 gene panels +CRP	0.82	0.77	0.88	0.62	0.60	0.71	0.87	0.80	0.92	0.38	0.39	0.49
ISB19 derived 5 gene panels +SOFA+CRP	0.83	0.74	0.85	0.58	0.56	0.66	0.89	0.77	0.91	0.26	0.35	0.39
ISB63 derived 6 gene panels	0.88	0.82	0.86	0.77	0.77	0.78	0.80	0.78	0.81	0.74	0.76	0.76
ISB63 derived 6 gene panels +SOFA	0.87	0.78	0.83	0.68	0.63	0.68	0.83	0.75	0.83	0.52	0.51	0.53
ISB63 derived 6 gene panels +CRP	0.90	0.79	0.89	0.65	0.63	0.71	0.92	0.83	0.93	0.37	0.43	0.48
ISB63 derived 6 gene panels +SOFA+CRP	0.88	0.77	0.85	0.58	0.60	0.64	0.90	0.83	0.91	0.26	0.36	0.35
ISB63 derived 7 gene panels	0.89	0.82	0.84	0.81	0.75	0.78	0.81	0.73	0.78	0.81	0.77	0.78
ISB63 derived 7 gene panels +SOFA	0.87	0.79	0.81	0.68	0.65	0.68	0.80	0.78	0.81	0.55	0.52	0.54

Panel and combination	AUC			ACCURACY			Sensitivity			Specificity		
	Day-3	Day-2	Day-1	Day-3	Day-2	Day-1	Day-3	Day-2	Day-1	Day-3	Day-2	Day-1
ISB63 derived 7 gene panels +CRP	0.92	0.80	0.88	0.68	0.62	0.71	0.93	0.82	0.89	0.43	0.41	0.53
ISB63 derived 7 gene panels +SOFA+CRP	0.88	0.78	0.84	0.58	0.59	0.65	0.91	0.82	0.89	0.26	0.36	0.39
ISB63 derived 8 gene panels	0.90	0.81	0.83	0.80	0.74	0.76	0.81	0.73	0.76	0.80	0.74	0.76
ISB63 derived 8 gene panels +SOFA	0.87	0.79	0.80	0.65	0.65	0.68	0.81	0.79	0.80	0.49	0.51	0.56
ISB63 derived 8 gene panels +CRP	0.92	0.80	0.87	0.68	0.61	0.72	0.92	0.82	0.88	0.43	0.40	0.55
ISB63 derived 8 gene panels +SOFA+CRP	0.87	0.78	0.83	0.57	0.59	0.65	0.89	0.83	0.89	0.24	0.35	0.39

Example 9

Prognostic Performance of Reduced Panels in Patients with Different Levels of Severity With and Without Integrated Clinical Performance

5 To determine if there were differences in prediction performance based on the severity of sepsis, patients who required vasopressor support and who met septic shock criteria in all iterations of the sepsis guidelines as severe sepsis were identified. All remaining patients were classified as mild sepsis. Based on this stratification, the classification performance of 60 small gene panels derived from ISB19 and ISB63 (Tables 16 and 17) were evaluated for all Sepsis samples, Mild Sepsis and Severe Sepsis compared to Controls. The ISB panels showed higher classification performance between Severe Sepsis and Control (FIG. 17). This result suggested that the prognostic performance of the panels is particularly accurate in identifying patients who are at risk of developing severe sepsis. The classification performance of each panel integrated with clinical information (SOFA score and CRP level) was then computed. Similar to what was observed earlier, integrating with clinical parameters in the panel enhanced classification performance especially in Severe Sepsis compared to Control (AUC: 0.9522 at Day-1) (FIG. 17).

Example 10

Pre-Symptomatic Blood Biomarker Panels for Sepsis

20 The current study utilized longitudinal blood samples collected from patients undergoing elective surgery to identify a blood mRNA-based panel that could diagnose sepsis prior to onset of detectable clinical symptoms, allowing for much earlier therapeutic intervention. Using a combination of differential gene expression analysis, machine learning tools, and a biological function-based biomarker panel optimization process, we have identified and validated biomarker panels consisting of only three or six genes that can identify patients developing sepsis three days prior to the onset of symptoms.

Methods

Patient and sample description. The patients were recruited across eight hospitals in England and Germany: Guys' and St Thomas' Hospital (London, U.K.), Heartlands Hospital (Birmingham, U.K.), North Bristol NHS Trust (Bristol, U.K.), Queen Elizabeth Hospital (Birmingham, U.K.), The Leeds Teaching Hospitals Trust (Leeds, U.K.), The Royal Liverpool University Hospital (Liverpool, U.K.), University College Hospital (London, U.K.), and University Hospital Frankfurt (Frankfurt DE). All subjects gave written informed consent to participate and the study was approved by Southampton & South West Hampshire Research Ethics Committee with reference number 06/Q1702/152.

The patients underwent a wide range of major elective surgeries, with the most common being large bowel resection, vascular surgery, and pancreatic surgery (detailed patient information is shown in Table 19). Peripheral blood samples were collected from patients prior to surgery (Pre-Op) and daily up to five days post-sepsis diagnosis (Post-Op). Each patient's demographic parameters, vital signs, hematology, clinical chemistry, and pathogen detection results were recorded. The control group included age, sex, and surgical procedure matched patients who did not develop sepsis. Consensus evaluations were made by nine physicians for the study. This multi-year project was initiated prior to the release of the Sepsis-3 definition; therefore, all patients were diagnosed based on the Sepsis-2 criteria.

Whole blood RNA isolation and microarray analysis. Total RNA from RNAlater-preserved whole blood was isolated with miRNeasy kit (Qiagen, Germantown, MD) according to the manufacturer's instructions. The quantity and quality of RNA were assessed with the Agilent 2100 Bioanalyzer (Santa Clara, CA) and NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE). Whole blood gene expression profiling experiments were performed using Agilent Human Whole Genome 8x60 microarrays and fluorescent probes were prepared using Agilent QuickAmp Labeling Kit according to the manufacturer's instructions (Santa Clara, CA). Gene expression information was obtained with Agilent's Feature Extractor and processed with in-house SLIM pipeline (Marzolf *et al.*, *Source Code Biol. Med.* 1:5, 2006).

Differential expression analysis. The microarray data were normalized using a quantile method. Probes having signals lower than the global mean were removed from further analysis as it is known that low abundance transcripts have higher detection variabilities. To identify genes associated with the development of sepsis, three factors were considered during analysis: 1) the use of Pre-Op data, 2) paired or unpaired analysis between controls and sepsis samples, and 3) combining time point data prior to Day0 or not. Combinations of these 3 different factors resulted in 8 different analysis approaches (Approach 1 to 8, FIG. 18). DEGs from each analysis approach were identified based on the criteria of $p\text{-value} < 0.01$ and absolute $\log_2\text{-fold-change} > 0.585$ (*i.e.*, more than 1.5 fold concentration change). When comparisons were made using individual time points, only genes showing differential expression in at least two time points were selected.

Feature selection. To identify a biomarker panel consisting of a subset of genes among DEGs, support vector machine with recursive feature elimination (SVM-RFE) (Guyon *et al.*, *Machine Learning*

46:389-422, 2002) was applied with 5-fold cross-validation using pathClass R package (Johannes *et al.*, *Bioinformatics* 27:1442-1443, 2011). From each loop of the cross-validation procedure, an optimal set of features was selected by SVM-RFE, therefore, five different sets of features (genes) could be identified. Considering the randomness of shuffling and partitioning of samples in a cross-validation procedure, the cross-validation procedure was repeated 100 times, resulting in a total of 500 sets of features. The importance of each feature to the classification was determined as the frequency of how often each feature was selected among the 500 sets. The features were sorted in order of their importance. SVM models with different numbers of features were constructed by adding features from the most important to the least and average AUCs were computed by repeating the 5-fold cross-validation procedure 100 times. The final optimal feature set was determined at the highest average AUC.

Panel optimization. To determine the biological processes associated with the classification, the Gene Ontology (GO) terms associated with the genes (features) in the top performing diagnostic panels were determined using the database for annotation visualization and integrated discovery (DAVID) (Huang *et al.*, *Nat. Protoc.* 4:44-57, 2009). Then we tried to select core biological processes that have higher discriminatory power (FIG. 19A). We generated 100,000 panels that consist of the same number of genes with the panels identified by SVM-RFE. The genes were randomly selected from DEGs. The Discovery set was divided into two sets of equal size. One set was used to train random 19- or 63-gene panels and the other set was used to compute their classification performance. Among the random 100,000 panels, the top 500 high performing and bottom 500 low performing panels (1% of the random 100,000 panels) were selected. For each panel, the number of genes involved in the biological processes associated with ISB19 and ISB63 were counted. For each GOBP, the ratio of panels that have more than one gene in the corresponding GOBP were computed and sorted in decreasing order. The number of core biological processes were selected based on the Elbow method. Then the selected core biological processes were summarized to representative functional terms with EnrichmentMap tool (Merico *et al.*, *PLoS One* 5:e13984, 2010). Genes in each of the DEG sets that shared the same functional terms and had the same directional changes between sepsis and control were identified as substitutable genes representing each functional term. To optimize the panel, one gene from each functional term was assembled and trained using all the samples in the Discovery sample set, and the classification performances were calculated using the Test sample set.

Throughout the analyses, classification performance of a biomarker panel was evaluated by the area under the curve (AUC) of receiver operating characteristic (ROC) curve. Statistical significance of the difference between AUCs from different panels was computed with DeLong's method (Robin *et al.*, *BMC Bioinformatics* 12:77, 2011).

qPCR validation. The Fluidigm 96.96 integrated fluidic circuit (IFC) plates were used for qPCR validation of the 3- and 6-gene panels. The analysis included 665 blood RNA samples from 155 sepsis and 153 controls from the Discovery, Test, and Validation sets. The Discovery and Test sets were used to train classifiers using the panels and the Validation set was used to calculate their performances.

The samples were randomized across 8 IFC chips and each chip included four wells of a pooled sample to measure inter-chip variability and two wells of no template controls. The whole blood cDNA was synthesized from 100 ng of RNA using the Reverse Transcription Master mix (Fluidigm PN 100-6297). Pre-amplification was performed using a pool of all assays using the Preamp Master Mix (Fluidigm PN 100-5744). The preamp reaction was then cleaned up using Exonuclease 1 (New England Biolabs PN M0293S). The amplified cDNA was diluted 1:10 using DNA suspension buffer, and then the Fluidigm chips were run on the Biomark HD machine according to the manufacturer's instructions using the GE 96.96 Standard v1 protocol. Ct values from all eight chips were combined using the Biomark Data Analysis software. BRK1 and RNF181 were used as reference genes. The two reference genes were selected based on the following procedure: 1) a set of invariant genes was selected in which each gene had less than 5% of coefficient of variation in each of sepsis and control datasets, below bottom 5% of fold-change distribution between sepsis and control, and within top 5% of an intensity distribution of all genes. Genes that were not defined well or were suspected to be saturated in intensity were not considered in further steps. 2) A total of 41 genes were selected from the first step and used as an input for RefFinder that provides a comprehensive ranking of genes by comparing four computational programs (delta-CT, BestKeeper, NormFinder, and geNorm) for a reference gene identification. In RefFinder, sepsis and control datasets were analyzed separately and gene rankings from each dataset were averaged. Top two ranked genes, BRK1 and RNF181, were finally selected and used as reference genes.

Table 19. Characteristics of Discovery, Test, and Validation cohorts.

Sample set	Discovery		Test		Validation	
Characteristics Mean (Standard Deviation)	Control (n=63)	Sepsis (n=64)	Control (n=30)	Sepsis (n=31)	Control (n=60)	Sepsis (n=60)
Age	65.9 (8.0)	64.9 (9.6)	62.6 (14.8)	64.2 (13.0)	61.1 (13.5)	61.1 (13.8)
Gender						
Female	23	23	6	6	11	11
Male	40	41	24	25	49	49
Race						
Black or Black British		1	2		2	3
Asian or Asian British			1	1		1
Chinese or other ethnic group				2		2
White	62	63	27	27	56	52
Not recorded	1			1	2	2
Surgery type, N						
Abdomen						
APR	2	3	2	2	4	4
Biliary surgery	3	2	1	1	1	
Bowel resection/anastomosis	15	14	4	4	11	10
Cystectomy	2	2			1	1
Cystoprostatectomy						1
Esophagectomy/Gastrectomy	12	12	8	9	22	22
Hernia/abdominal wall reconstruction						1
Liver surgery	3	3	4	4	4	4
Nephrectomy			1	1	1	

Sample set		Discovery		Test		Validation	
		12	13	5	5	8	9
	Pancreaticoduodenectomy						
	Vascular surgery	10	9	2	2	3	2
Neck	Mitrofanoff/Max fax	2	2	1	1		
Thorax	Lung resection/vascular surgery	2	4	2	2	5	6
Patient Outcome, N							
	Death		1		1		5
	caused by sepsis						1
	not caused by sepsis						
	Discharged	25	19	10	6	27	16
	Inpatient	8	18	12	19	16	31
	Not recorded	30	26	8	5	17	7
Microbiology, N (%)							
Blood	Positive		10 (16%)		5 (16%)		8 (13%)
	Negative	1 (2%)	16 (25%)		14 (45%)	2 (3%)	27 (45%)
Sputum	Positive	1 (2%)	25 (6%)		12 (39%)		11 (18%)
	Negative	3 (5%)	4 (6%)		1 (3%)	3 (5%)	9 (15%)
Urine	Positive		3 (5%)				8 (13%)
	Negative	6 (10%)	7 (11%)	1 (3%)	12 (39%)	1 (2%)	9 (15%)
Throat	Positive		2 (3%)				1 (2%)
	Negative	1 (2%)	3 (5%)	3 (10%)	1 (3%)	1 (2%)	2 (3%)
Wound Site	Positive	2 (3%)	27 (42%)		9 (29%)		21 (35%)
	Negative	1 (2%)	2 (3%)	1 (3%)	1 (3%)		4 (7%)

APR indicates abdominoperineal excision of rectum & end colostomy.

Results

Patient cohort and study design. Surgery patients were recruited across eight hospitals located in England and Germany (FIG. 20A). Detailed patient information is shown in Table 19. Peripheral blood samples were collected from elective surgery patients prior to surgery (Pre-Op) and then daily up to four or five days post-sepsis diagnosis (Post-Op). About 4,000 patients consented and participated in the study, and approximately 4% of the enrolled patients developed sepsis (n = 155) with most developing the disease two to seven days after surgery (FIG. 20B). Sepsis patients were diagnosed by a panel of nine clinicians using the Sepsis-2 criteria. The sepsis-related mortality rate in this cohort was approximately 5% (7/155 sepsis patients). The control group included age, sex, and surgical procedure matched patients who did not develop sepsis.

The sepsis patients and matched controls were each split into three groups: Discovery (64 sepsis and 63 control), Test (31 sepsis and 30 control), and Validation (60 sepsis and 60 control) sets. Samples from the Discovery set were used to identify biomarker panel(s) based on differentially expressed genes (DEGs) from whole blood between sepsis patients and controls. The Test samples were used to optimize the biomarker panel(s) and the Validation set was used to verify the panels' classification performance.

To identify pre-symptomatic biomarkers, the longitudinal samples from each patient were organized such that the day sepsis was diagnosed was labeled as Day0 (FIG. 20C). One day before the sepsis diagnosis date was set as Day-1 while one day post-diagnosis was designated as Day+1. Analysis for pre-symptomatic biomarker detection was focused on Day-3 to Day-1 time points (three to one days prior to the day of sepsis diagnosis, respectively). We could not examine earlier time points due to an insufficient number of available samples earlier than Day-3 (Table 20). There were no significant differences in clinical parameters commonly used for sepsis including Sequential Organ Failure Assessment (SOFA) Score, blood C reactive protein (CRP), and lactate levels among the three sample group populations (Discovery, Test, Validation) (FIG. 20D-20F).

Table 20. Number of samples in time points rearranged by sepsis diagnosis day

Time point	Discovery Set		Test Set		Validation Set	
	sepsis	control	sepsis	control	sepsis	control
Pre-Operation	64	63	31	30	60	60
Sepsis Day -7	1	2				
Sepsis Day -6	8	9			1	1
Sepsis Day -5	16	17			1	1
Sepsis Day -4	20	21	1	1	1	1
Sepsis Day -3	39	38	11	11	26	26
Sepsis Day -2	48	46	21	19	48	46
Sepsis Day -1	62	57	29	30	58	56
Sepsis Day 0	55	49	27	5	54	6
Sepsis Day +1	44	40	23	4	46	5

Time point	Discovery Set		Test Set		Validation Set	
	sepsis	control	sepsis	control	sepsis	control
Sepsis Day +2	31	26	4	5	4	5
Sepsis Day +3	21	17	4	4	4	4
Sepsis Day +4	9	10	4	3	3	2
Sepsis Day +5	3	6	3	2		1
Total	421	401	158	114	306	214

Identification of pre-symptomatic sepsis diagnostic panels. Because of the study design, different approaches were used to identify gene expression changes associated with sepsis development (FIG. 20G). Specifically, variables included 1) adjusting the Post-Op whole blood transcriptome data with Pre-Op data, 2) individual patient-control pairs comparison vs. unpaired all patients vs. all controls analysis, and 3) individual time points vs. combining all pre-sepsis diagnosis time points. The use of Pre-Op data to adjust Post-Op array data reduces the variation among individuals by controlling potential confounding variables from an individual and emphasizing the gene expression changes associated with surgery and infection. The paired and unpaired t-test was used to determine whether to analyze individual patient-control pairs or group the patient and control samples together. The third factor was to compare the gene expression profile changes between the sepsis and control groups either at individual time points (e.g., Day-3 sepsis vs. Day-3 control) or by grouping all three time points (Day-3+Day-2+Day-1) from each condition together. In total, eight different approaches were used (FIG. 18). As expected, there were fewer DEGs when using Pre-Op adjusted data (Approaches 1 to 4, FIG. 18) and most of the DEGs identified with Pre-Op adjusted data were also included in data without Pre-Op adjustment (Approaches 5 to 8, FIG. 18). However, a few DEGs were observed only in Pre-Op adjusted data. For example GBP5 (Guanylate Binding Protein 5) and GZMH (Granzyme H) were identified as DEGs with Approaches 1 to 4 but not with Approaches 5 to 8.

Using the DEGs identified from the Discovery sample set, a classifier capable of separating pre-symptomatic sepsis patients from control patients was identified using support vector machine combined with recursive feature elimination (SVM-RFE) in conjunction with a repeat cross-validation procedure for each of the DEG sets from different analysis approaches. The performances (indicated as area under the receiver operating characteristic curve (AUC) for each day prior to the day of sepsis diagnosis) of the panels were then computed (summarized in FIG. 18). In general, panels identified from the datasets without Pre-Op adjustment (from Approaches 5 - 8) showed higher performance than the panels from the Pre-Op adjusted datasets. Among the four different Pre-Op adjusted datasets, the 19-gene panel (ISB19) derived from 58 DEGs (ISB58) that were identified by Approach 2 showed the highest performance based on the average AUC of three time points (Day-3 to Day-1) (average AUC = 0.77). The 63-gene panel (ISB63) derived from 355 DEGs (ISB355) that were identified by Approach 6 gave the highest performance based on the average AUC of three time points among datasets without Pre-Op adjustment (average AUC = 0.88). Apart from the difference with or without Pre-Op adjustment, Approaches 2 and

6 both tested individual patient-control pairs and individual time points. We observed significant overlap
(54 genes) in DEGs between the ISB58 and ISB355 sets (FIG. 21A and Table 21); however, the panels
(ISB19 and ISB63) derived from these DEGs contained only 6 genes in common (FIG. 21B and Table
21). The genes included in the two panels are listed in Table 21 and the overall expression profiles of
5 those genes among samples in the Discovery set are shown in FIG. 21C

Table 21. Differentially expressed genes (DEGs) identified from approach 2 (ISB58) and approach 6 (ISB355)

Probe ID	Gene Symbol	PreO P norm, Day-3 logFC	P Value	PreO P norm, Day-2 logFC	P Value	PreOP norm, Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach 6 (ISB58)	Approach 6 (ISB55)	ISB19	ISB63
A 23 P13765	<i>FCER1A</i>	-0.54	0.19	-0.33	0.14	-0.69	0.36	-0.70	0.13	-0.65	0.00	-0.92	0.00		X		
A 24 P11729	<i>LDLR</i>	0.49	0.22	0.38	0.62	0.47	0.72	0.67	0.22	0.58	0.28	0.70	0.00		X		X
A 23 P117546	<i>SOS2</i>	0.31	0.62	0.24	0.75	0.38	0.17	0.53	0.59	0.57	0.00	0.65	0.73		X		
A 23 P14464	<i>ALOX5</i>	0.47	0.58	0.39	0.19	0.43	0.15	0.78	0.18	0.73	0.00	0.72	0.00		X		
A 24 P82466	<i>GLS7</i>	0.75	0.29	0.71	0.15	0.46	0.26	0.61	0.00	0.63	0.00	0.62	0.00		X		
A 23 P122863	<i>GRB1</i>	0.60	0.28	0.55	0.26	0.97	0.47	0.79	0.35	0.86	0.24	1.23	0.00		X		
A 23 P411113	<i>CNTNAP1</i>	-0.33	0.27	-0.12	0.59	-0.66	0.65	-0.83	0.34	-0.64	0.58	-0.55	0.28		X		
A 33 P3352382	<i>ARG1</i>	0.97	0.16	0.73	0.23	1.87	0.85	1.11	0.33	1.21	0.00	1.46	0.00		X		
A 23 P61426	<i>MSR4</i>	0.55	0.19	0.54	0.37	0.62	0.26	0.63	0.40	0.63	0.48	0.77	0.00		X		
A 23 P2676	<i>HP</i>	1.85	0.58	0.75	0.42	1.21	0.46	1.39	0.12	1.33	0.63	1.77	0.00	X	X		
A 23 P121716	<i>ANXA3</i>	0.75	0.32	0.55	0.77	0.69	0.12	1.61	0.24	1.33	0.00	1.14	0.00		X		
A 23 P17735	<i>CD79A</i>	-0.18	0.54	0.83	0.67	-0.40	0.28	-0.79	0.38	-0.54	0.49	-0.80	0.79		X		
A 23 P21287	<i>KIF1B</i>	0.58	0.62	0.47	0.68	0.57	0.46	0.85	0.00	0.89	0.00	0.89	0.00		X		
A 23 P12418	<i>ITGAM</i>	0.52	0.15	0.37	0.36	0.45	0.80	0.71	0.13	0.58	0.00	0.69	0.00		X		
A 23 P47565	<i>LDHA</i>	0.61	0.62	0.47	0.97	0.55	0.18	0.85	0.13	0.77	0.00	0.84	0.00		X		
A 23 P12847	<i>CLEC12A</i>	0.38	0.62	0.35	0.16	0.15	0.17	0.83	0.79	0.70	0.48	0.55	0.49		X		X
A 32 P18254	<i>FAM2A</i>	0.45	0.42	0.32	0.17	0.44	0.66	0.66	0.55	0.66	0.59	0.86	0.00		X		
A 23 P82929	<i>NOV</i>	-0.56	0.62	-0.49	0.12	-0.53	0.79	-0.62	0.27	-0.68	0.00	-0.75	0.00		X		
A 23 P129556	<i>IL4R</i>	0.42	0.76	0.37	0.55	0.45	0.22	0.67	0.87	0.78	0.00	0.83	0.00		X		
A 23 P14464	<i>ALOX5</i>	0.49	0.21	0.42	0.12	0.44	0.57	0.75	0.00	0.73	0.00	0.70	0.00		X		
A 24 P385611	<i>SP1</i>	0.24	0.46	0.16	0.17	0.27	0.23	0.61	0.00	0.63	0.13	0.72	0.00		X		
A 33 P321689	<i>PAG1</i>	0.37	0.18	0.23	0.54	0.42	0.39	0.79	0.00	0.74	0.65	0.84	0.00		X		
A 23 P121716	<i>ANXA3</i>	0.77	0.27	0.56	0.70	0.68	0.13	1.85	0.15	1.42	0.00	1.14	0.00		X		

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISB58)	Approach6 (ISB55)	ISB19	ISB63
A_23_P161458	<i>OLAH</i>	0.66	0.16	0.85	0.22	0.99	0.26	0.78	0.18	1.54	0.16	1.15	0.00	X	X		X
A_23_P152548	<i>SCPEP1</i>	0.42	0.51	0.32	0.54	0.40	0.44	0.65	0.63	0.52	0.42	0.59	0.00		X		
A_23_P123732	<i>C9orf13</i>	-0.14	0.43	0.44	0.47	0.44	0.83	-0.15	0.34	0.59	0.24	0.63	0.16		X		
A_23_P122863	<i>GRB1</i>	0.55	0.39	0.50	0.32	0.86	0.21	0.77	0.49	0.90	0.00	1.29	0.16		X		
A_33_P3361422	<i>CYP27A1</i>	-0.51	0.50	-0.45	0.78	-0.32	0.35	-0.74	0.27	-0.83	0.00	-0.58	0.13		X		X
A_23_P9451	<i>ANXA1</i>	0.37	0.49	0.36	0.23	0.28	0.39	0.63	0.53	0.79	0.00	0.55	0.19		X		
A_33_P3352578	<i>CLEC4D</i>	0.82	0.43	0.63	0.21	0.89	0.25	1.72	0.14	1.82	0.00	1.29	0.56	X	X		
A_23_P79426	<i>CAB39</i>	0.43	0.34	0.27	0.28	0.38	0.64	0.68	0.00	0.65	0.00	0.71	0.55		X		
A_33_P3421571	<i>RAPH1</i>	-0.14	0.64	-0.13	0.59	-0.52	0.12	-0.69	0.16	-0.70	0.37	-0.87	0.52		X		
A_23_P119222	<i>RETN</i>	1.15	0.69	1.43	0.18	1.24	0.17	1.37	0.28	1.34	0.18	1.52	0.00	X	X		
A_24_P343233	<i>HILA-DRB1</i>	-0.27	0.56	-0.24	0.26	-0.53	0.17	-0.41	0.39	-0.62	0.00	-0.71	0.00		X		
A_23_P121716	<i>ANXA3</i>	0.79	0.26	0.56	0.78	0.67	0.16	1.88	0.17	1.32	0.00	1.11	0.00		X		
A_23_P122863	<i>GRB1</i>	0.72	0.18	0.51	0.26	0.97	0.50	0.89	0.17	0.88	0.00	1.28	0.43		X		
A_23_P123393	<i>KCNO3</i>	-0.20	0.47	-0.26	0.27	-0.22	0.22	-0.61	0.23	-0.75	0.54	-0.74	0.72		X		
A_24_P363548	<i>HIP1</i>	0.41	0.48	0.28	0.14	0.42	0.13	0.64	0.00	0.53	0.00	0.71	0.00		X		
A_23_P12418	<i>ITGAM</i>	0.55	0.15	0.37	0.38	0.48	0.41	0.69	0.42	0.57	0.00	0.73	0.00		X		
A_24_P819	<i>SLC2A3</i>	0.66	0.27	0.57	0.52	0.63	0.39	0.98	0.00	1.00	0.36	1.67	0.00	X	X	X	X
A_24_P59667	<i>JAK3</i>	0.19	0.34	0.19	0.18	0.45	0.83	0.62	0.45	0.69	0.00	0.85	0.00		X		
A_24_P314179	<i>ETS2</i>	0.56	0.16	0.50	0.84	0.45	0.16	0.84	0.00	0.73	0.00	0.75	0.00		X		X
A_33_P3316786	<i>DACH1</i>	0.65	0.16	0.64	0.28	0.77	0.00	0.81	0.00	0.89	0.00	1.42	0.00	X	X		
A_33_P3376821	<i>GZMA</i>	-0.59	0.83	-0.61	0.33	-0.46	0.27	-0.62	0.13	-0.76	0.24	-0.65	0.19	X	X		
A_23_P12566	<i>RRBP1</i>	0.28	0.36	0.18	0.11	0.37	0.13	0.65	0.00	0.55	0.00	0.73	0.00		X		X
A_23_P123393	<i>KCNO3</i>	-0.37	0.21	0.23	0.32	-0.42	0.57	-0.95	0.13	-0.27	0.30	-0.68	0.69		X		
A_23_P7253	<i>KLHL2</i>	0.62	0.15	0.43	0.39	0.55	0.55	0.92	0.00	0.88	0.00	0.97	0.00		X		

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISB58)	Approach6 (ISB55)	ISB19	ISB63
A_23_P68851	KREMEN1	0.24	0.29	0.25	0.29	0.34	0.95	0.75	0.26	0.66	0.00	0.87	0.13		X		X
A_33_P329459	CD44	0.49	0.67	0.45	0.14	0.35	0.66	0.65	0.54	0.63	0.00	0.58	0.00		X		
A_23_P3451	PHC2	0.26	0.82	0.29	0.85	0.25	0.56	0.67	0.00	0.61	0.00	0.69	0.00		X		
A_23_P32577	DACH1	0.68	0.36	0.58	0.25	0.64	0.00	0.76	0.00	0.77	0.00	0.83	0.00	X	X		
A_23_P64372	TCN1	0.67	0.26	0.63	0.21	0.72	0.97	0.75	0.31	0.68	0.22	0.79	0.34	X	X	X	X
A_33_P322489	IL17R4	0.28	0.54	0.18	0.18	0.24	0.38	0.60	0.00	0.47	0.00	0.59	0.00		X		
A_23_P1476	GPR97	0.57	0.13	0.58	0.20	0.54	0.46	0.59	0.00	0.69	0.00	0.76	0.00		X		
A_23_P14464	ALOX5	0.47	0.22	0.49	0.12	0.48	0.87	0.73	0.19	0.79	0.00	0.66	0.00		X		
A_33_P3424577	A23747	-0.49	0.23	-0.38	0.57	-0.58	0.23	-0.69	0.59	-0.68	0.13	-0.81	0.16		X		
A_23_P123732	C9orf13	-0.83	0.73	0.35	0.75	0.48	0.15	0.14	0.58	0.64	0.47	0.70	0.27		X		
A_23_P251937	CPEB4	0.35	0.59	0.19	0.14	0.42	0.99	0.66	0.27	0.54	0.00	0.72	0.00		X		
A_33_P3258977	CLEC4D	0.86	0.43	0.68	0.13	0.90	0.24	1.43	0.24	0.99	0.11	1.15	0.00	X	X		
A_23_P14658	ATP6V1C1	0.77	0.23	0.55	0.93	0.69	0.11	0.98	0.00	0.86	0.13	1.62	0.00	X	X	X	X
A_23_P117546	SOS2	0.42	0.34	0.33	0.37	0.47	0.26	0.53	0.55	0.58	0.00	0.69	0.87		X		
A_21_P1251	SEPT14	0.15	0.29	0.13	0.22	0.36	0.93	0.64	0.00	0.73	0.00	0.80	0.00		X		X
A_23_P9513	SLC37A3	0.52	0.34	0.46	0.60	0.68	0.26	0.79	0.45	0.83	0.00	0.94	0.00		X		
A_32_P7158	LILRB3	0.27	0.13	0.17	0.23	0.35	0.18	0.72	0.22	0.64	0.00	0.74	0.00		X		
A_23_P28334	IL18RAP	0.62	0.58	0.59	0.26	0.76	0.29	1.23	0.65	1.11	0.00	1.28	0.00		X		
A_23_P14464	ALOX5	0.47	0.18	0.44	0.13	0.43	0.76	0.73	0.22	0.69	0.00	0.67	0.00		X		
A_24_P74932	PLP2	0.52	0.13	0.48	0.15	0.38	0.89	0.67	0.17	0.62	0.00	0.51	0.00		X		
A_23_P11473	NAP	0.46	0.98	0.23	0.29	0.45	0.28	0.93	0.23	0.86	0.00	1.19	0.00		X		
A_23_P4174	NMIP9	1.79	0.25	0.81	0.90	1.21	0.13	1.22	0.54	1.11	0.00	1.46	0.00	X	X		
A_23_P29635	CYP11B1	0.30	0.19	0.24	0.23	0.51	0.56	0.77	0.25	0.77	0.14	0.93	0.00		X		
A_24_P223124	FNDC3B	0.36	0.19	0.15	0.21	0.24	0.30	0.59	0.00	0.51	0.00	0.62	0.00		X		

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISB58)	Approach6 (ISB55)	ISB19	ISB63
A_21_P1748	SEPT14	0.13	0.41	0.15	0.25	0.30	0.25	0.68	0.00	0.82	0.00	0.89	0.00		X		X
A_33_P33896	CDK3RAP2	0.73	0.64	0.67	0.49	0.77	0.17	0.88	0.73	0.97	0.00	1.86	0.98	X	X		
A_21_P1561	FCGR1B	0.32	0.17	0.35	0.12	0.46	0.86	0.95	0.00	0.85	0.24	0.72	0.32		X		
A_23_P25362	BMX	0.69	0.47	0.66	0.34	0.86	0.76	0.73	0.16	0.79	0.00	0.91	0.00	X	X	X	
A_21_P1728	FCGR1B	0.23	0.29	0.25	0.23	0.25	0.92	0.91	0.00	0.83	0.11	0.72	0.19		X		
A_33_P322196	IL18RAP	0.67	0.39	0.58	0.23	0.77	0.23	1.62	0.35	1.91	0.00	1.28	0.00		X		
A_23_P11212	ACSL1	0.49	0.38	0.29	0.13	0.39	0.29	0.82	0.00	0.75	0.00	0.85	0.00		X		
A_24_P27787	SNX3	0.54	0.32	0.31	0.37	0.64	0.64	0.67	0.00	0.64	0.00	0.55	0.84		X		
A_23_P117546	SOS2	0.37	0.34	0.27	0.46	0.37	0.17	0.58	0.53	0.58	0.00	0.64	0.00		X		
A_23_P119835	NLR4	0.53	0.39	0.36	0.93	0.65	0.16	0.86	0.28	0.84	0.00	1.55	0.25		X		
A_23_P14464	ALOX5	0.41	0.43	0.37	0.23	0.38	0.17	0.72	0.19	0.72	0.00	0.67	0.00		X		
A_24_P14566	KIF1B	0.56	0.43	0.26	0.28	0.39	0.36	0.78	0.68	0.54	0.00	0.68	0.00		X		
A_23_P3913	HLA-DPA1	-0.29	0.13	-0.37	0.15	-0.58	0.26	-0.42	0.99	-0.70	0.00	-0.74	0.00		X		
A_33_P3253144	DOK3	0.41	0.44	0.39	0.20	0.53	0.66	0.56	0.11	0.62	0.00	0.73	0.00		X		X
A_33_P3251148	TSP0	0.57	0.83	0.50	0.74	0.62	0.79	0.76	0.13	0.72	0.00	0.78	0.00		X		
A_24_P37172	IL18A5	0.63	0.12	0.52	0.22	0.65	0.15	0.96	0.00	0.95	0.00	1.95	0.00		X		
A_23_P133438	FAM15A	0.54	0.89	0.28	0.46	0.34	0.26	0.64	0.00	0.55	0.00	0.62	0.56		X		
A_23_P11212	ACSL1	0.42	0.90	0.24	0.25	0.34	0.54	0.79	0.25	0.77	0.00	0.82	0.00		X		
A_23_P4856	CKAP4	0.43	0.46	0.43	0.29	0.65	0.57	0.77	0.78	0.81	0.00	0.92	0.00		X		
A_24_P353619	ALPL	0.60	0.16	0.56	0.23	0.43	0.82	0.86	0.33	0.85	0.00	0.77	0.33		X		
A_24_P41776	MXD3	0.33	0.34	0.23	0.50	0.45	0.42	0.66	0.00	0.60	0.00	0.83	0.77		X		
A_33_P34763	PLIN4	0.43	0.39	0.39	0.99	0.53	0.26	0.83	0.29	0.85	0.00	1.37	0.00		X		X
A_33_P326857	CEACAM1	0.56	0.72	0.45	0.29	0.60	0.85	0.79	0.00	0.64	0.22	0.78	0.00		X		
A_23_P121716	ANXA3	0.77	0.30	0.57	0.67	0.67	0.16	1.73	0.24	1.53	0.00	1.12	0.00		X		

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISB58)	Approach6 (ISB55)	ISB19	ISB63
A_23_P169778	AGTPBP1	0.42	0.37	0.31	0.40	0.33	0.29	0.66	0.18	0.65	0.00	0.63	0.00		X		
A_23_P5392	TP53f3	0.69	0.35	0.48	0.72	0.62	0.93	0.83	0.00	0.66	0.62	0.78	0.00	X	X	X	
A_33_P3298356	SH3GLB1	0.27	0.17	0.29	0.78	0.44	0.33	0.54	0.47	0.63	0.00	0.77	0.00		X		
A_24_P393864	PHTF1	0.46	0.75	0.27	0.24	0.39	0.46	0.68	0.00	0.52	0.00	0.64	0.95		X		
A_24_P384397	RAVER1	0.16	0.62	0.11	0.66	-0.31	0.19	-0.67	0.23	-0.76	0.17	-0.99	0.85		X		
A_23_P14464	ALOX5	0.41	0.48	0.40	0.17	0.44	0.14	0.68	0.47	0.72	0.00	0.66	0.00		X		
A_32_P1396	WDFY3	0.43	0.35	0.23	0.17	0.30	0.36	0.73	0.00	0.63	0.00	0.67	0.00		X		
A_23_P23221	GADD45A	0.88	0.85	0.74	0.15	0.97	0.16	1.63	0.00	1.16	0.87	1.32	0.00	X	X		
A_23_P169437	LCN2	0.89	0.41	0.74	0.35	0.96	0.65	0.99	0.21	0.66	0.33	0.94	0.92	X	X		
A_24_P166443	HLA-DPB1	-0.24	0.12	-0.30	0.27	-0.46	0.19	-0.43	0.62	-0.67	0.00	-0.66	0.00		X		
A_23_P36559	DHRS9	0.96	0.63	0.69	0.55	0.90	0.59	1.34	0.00	1.45	0.14	1.31	0.00	X	X		
A_23_P11212	ACSL1	0.48	0.55	0.28	0.15	0.40	0.24	0.80	0.23	0.75	0.00	0.85	0.00		X		
A_23_P16145	ERO1L	0.36	0.24	0.21	0.35	0.33	0.97	0.60	0.40	0.44	0.17	0.59	0.00		X		
A_23_P25956	C5orf32	0.70	0.15	0.57	0.22	0.76	0.22	0.97	0.28	1.70	0.00	1.13	0.00		X		
A_23_P11226	GNG1	0.28	0.14	0.15	0.32	0.26	0.75	0.62	0.97	0.64	0.00	0.64	0.00		X		
A_23_P1675	GPR16	0.55	0.13	0.38	0.35	0.53	0.33	0.64	0.27	0.63	0.00	0.70	0.00		X		
A_33_P325193	SLC36A1	0.27	0.14	0.33	0.29	0.59	0.32	0.58	0.36	0.63	0.00	0.89	0.20		X		
A_23_P73837	TLR8	0.35	0.51	0.25	0.78	0.32	0.19	0.63	0.15	0.55	0.00	0.66	0.00		X		
A_23_P11212	ACSL1	0.46	0.52	0.27	0.16	0.38	0.31	0.79	0.25	0.74	0.00	0.84	0.00		X		
A_33_P338997	ATP6V1C1	0.56	0.69	0.43	0.18	0.61	0.36	0.76	0.35	0.75	0.00	0.92	0.17		X	X	
A_23_P11212	ACSL1	0.45	0.64	0.23	0.22	0.34	0.59	0.87	0.18	0.76	0.00	0.83	0.00		X		
A_23_P213584	HK3	0.83	0.34	0.67	0.50	0.69	0.13	1.22	0.00	1.16	0.00	1.22	0.00	X	X	X	
A_23_P28485	GCA	0.45	0.44	0.25	0.16	0.46	0.62	0.74	0.53	0.64	0.00	0.73	0.00		X		
A_24_P141214	STOM	0.69	0.91	0.48	0.14	0.34	0.79	0.95	0.00	0.87	0.00	0.88	0.00		X		X

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISB58)	Approach6 (ISB55)	ISB19	ISB63
A_23_P11473	<i>NAIP</i>	0.47	0.86	0.24	0.28	0.47	0.26	0.93	0.19	0.83	0.38	1.15	0.00		X		
A_23_P15618	<i>SLC22A4</i>	0.38	0.41	0.38	0.38	0.26	0.52	0.58	0.67	0.62	0.00	0.68	0.00		X		
A_33_P3238997	<i>AGFG1</i>	0.40	0.19	0.23	0.38	0.45	0.54	0.62	0.00	0.55	0.00	0.69	0.00		X		
A_32_P178045	<i>YOD1</i>	0.53	0.11	0.32	0.85	0.19	0.57	0.69	0.16	0.63	0.36	0.59	0.40		X		X
A_24_P181254	<i>OLEM4</i>	1.23	0.14	0.98	0.45	1.42	0.85	1.47	0.24	1.22	0.27	1.72	0.00	X	X		
A_33_P3251876	<i>IL18R1</i>	0.59	0.40	0.52	0.25	0.82	0.00	0.75	0.17	0.85	0.00	1.48	0.00	X	X	X	
A_23_P59528	<i>ACN9</i>	0.52	0.44	0.46	0.70	0.56	0.26	0.64	0.12	0.62	0.38	0.72	0.00		X		
A_24_P253818	<i>FLOT2</i>	0.58	0.17	0.49	0.17	0.36	0.94	0.80	0.00	0.71	0.00	0.65	0.00		X		
A_24_P413669	<i>PFKFB2</i>	1.16	0.15	0.97	0.11	1.29	0.00	1.40	0.00	1.44	0.00	1.59	0.00	X	X		
A_23_P864	<i>GPER</i>	0.43	0.85	0.29	0.13	0.52	0.16	0.59	0.34	0.46	0.38	0.79	0.00		X		X
A_24_P3854	<i>SLC9A8</i>	0.23	0.96	0.27	0.23	0.32	0.49	0.53	0.34	0.59	0.00	0.67	0.59		X		
A_23_P217319	<i>FGF13</i>	0.42	0.87	0.48	0.23	0.70	0.19	0.55	0.28	0.63	0.45	0.82	0.69		X		
A_24_P188377	<i>CD55</i>	0.47	0.16	0.49	0.16	0.54	0.82	0.65	0.18	0.90	0.00	0.91	0.73		X	X	
A_23_P58396	<i>PDGFC</i>	0.39	0.17	0.56	0.55	0.68	0.00	0.47	0.15	0.62	0.18	0.78	0.00		X		X
A_23_P149892	<i>CSGALNA CT2</i>	0.51	0.87	0.38	0.90	0.46	0.58	0.89	0.00	0.98	0.92	0.94	0.23		X		
A_23_P123732	<i>C9orf13</i>	0.13	0.58	0.47	0.37	0.52	0.58	0.32	0.53	0.67	0.17	0.69	0.00		X		
A_24_P6319	<i>ILIR2</i>	0.93	0.17	0.90	0.29	1.32	0.00	1.56	0.00	1.54	0.00	1.81	0.29	X	X	X	
A_33_P33599	<i>EMB</i>	0.45	0.86	0.33	0.28	0.58	0.31	0.74	0.00	0.86	0.00	1.41	0.00		X		
A_23_P42897	<i>MGAM</i>	0.52	0.42	0.37	0.66	0.48	0.12	0.84	0.24	0.83	0.00	0.94	0.00		X		
A_23_P11212	<i>ACSL1</i>	0.59	0.39	0.28	0.15	0.37	0.35	0.83	0.16	0.76	0.00	0.82	0.00		X		
A_23_P1997	<i>ILDR1</i>	-0.44	0.13	-0.33	0.17	-0.17	0.45	0.32	0.28	0.62	0.19	0.69	0.94		X		
A_23_P11212	<i>ACSL1</i>	0.46	0.58	0.26	0.18	0.36	0.43	0.85	0.27	0.75	0.00	0.81	0.00		X		
A_23_P423331	<i>NTNG2</i>	0.16	0.29	0.22	0.95	0.42	0.65	0.53	0.13	0.68	0.00	0.88	0.73		X		
A_33_P3376234	<i>PHIFI</i>	0.43	0.85	0.38	0.17	0.50	0.69	0.63	0.00	0.67	0.00	0.77	0.19		X		

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISB58)	Approach6 (ISB55)	ISB19	ISB63
A 24 P684186	EMB	0.48	0.16	0.35	0.21	0.45	0.39	0.65	0.23	0.61	0.00	0.69	0.00		X		
A 23 P121716	ANX43	0.78	0.27	0.55	0.77	0.66	0.16	1.96	0.19	1.34	0.00	1.18	0.00		X		
A 23 P29422	GYGI	1.46	0.35	0.84	0.16	0.96	0.57	1.28	0.00	1.22	0.00	1.36	0.00	X	X		
A 23 P117546	SOS2	0.31	0.56	0.24	0.89	0.43	0.82	0.59	0.12	0.55	0.00	0.69	0.00		X		
A 23 P139669	SLC243	0.65	0.44	0.64	0.24	0.54	0.32	0.87	0.00	0.92	0.00	0.87	0.54	X	X	X	X
A 21 P11751	CD177	1.68	0.14	1.33	0.78	1.83	0.44	1.98	0.00	1.98	0.00	2.38	0.00	X	X		
A 33 P339571	FN1	0.84	0.97	0.63	0.38	0.88	0.84	1.27	0.34	1.23	0.00	1.39	0.00	X	X		
A 33 P322139	SREBF1	-0.11	0.70	0.73	0.97	-0.39	0.48	-0.76	0.36	-0.61	0.32	-0.79	0.25		X		
A 21 P13518	GYGI	0.96	0.20	0.79	0.15	0.92	0.24	1.18	0.00	1.14	0.00	1.28	0.00	X	X		
A 23 P31911	BLMH	0.56	0.15	0.44	0.83	0.66	0.14	0.83	0.66	0.78	0.00	0.89	0.00		X		
A 33 P3341676	MEF2A	0.41	0.50	0.31	0.69	0.48	0.19	0.68	0.13	0.63	0.44	0.82	0.88		X		
A 24 P322353	PSTPIP2	0.40	0.21	0.37	0.19	0.45	0.83	0.62	0.00	0.56	0.00	0.67	0.00		X		
A 23 P11473	NAIP	0.47	0.13	0.23	0.32	0.58	0.13	0.87	0.54	0.82	0.14	1.88	0.00		X		
A 24 P343233	HLA-DRB1	-0.21	0.13	-0.22	0.47	-0.52	0.96	-0.37	0.13	-0.59	0.00	-0.73	0.00		X		
A 21 P11898	SEPT14	0.99	0.53	0.62	0.60	0.19	0.14	0.57	0.13	0.68	0.00	0.72	0.00		X		X
A 33 P33141	EXOSC4	0.81	0.18	0.83	0.71	1.22	0.00	0.78	0.60	0.84	0.00	1.41	0.00	X	X		
A 23 P13765	FCER1A	-0.38	0.14	-0.26	0.34	-0.70	0.73	-0.59	0.56	-0.59	0.17	-0.95	0.00		X		
A 23 P217564	ACSL4	0.40	0.93	0.33	0.12	0.35	0.59	0.74	0.00	0.71	0.00	0.74	0.00		X		
A 24 P261259	PFKEB3	0.96	0.46	0.74	0.68	0.99	0.12	1.24	0.99	1.30	0.00	1.48	0.00	X	X	X	
A 23 P1997	ILDR1	-0.53	0.78	-0.39	0.54	-0.17	0.44	0.27	0.35	0.60	0.23	0.66	0.85		X		
A 23 P166848	LTF	0.95	0.18	0.75	0.19	0.87	0.13	1.18	0.16	0.86	0.42	1.74	0.25		X		X
A 24 P343233	HLA-DRB1	-0.26	0.96	-0.43	0.26	-0.57	0.11	-0.38	0.92	-0.68	0.00	-0.83	0.00		X		
A 23 P1962	RAREES3	-0.47	0.63	-0.25	0.19	-0.34	0.48	-0.55	0.17	-0.65	0.22	-0.62	0.13		X		
A 33 P3286157	TNFRSF4	0.97	0.64	-0.16	0.28	-0.20	0.16	-0.39	0.11	-0.64	0.53	-0.67	0.00		X		

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISR58)	Approach6 (ISR355)	ISB19	ISB63
A_24_P27977	TRPM2	0.69	0.57	0.45	0.17	0.60	0.00	0.77	0.00	0.63	0.00	0.78	0.26	X	X		
A_33_P3280236	HPR	1.73	0.43	0.73	0.23	1.18	0.79	1.36	0.98	1.15	0.11	1.54	0.00	X	X		
A_23_P14464	ALOX5	0.50	0.17	0.38	0.22	0.46	0.12	0.77	0.00	0.69	0.00	0.68	0.00		X		
A_23_P131785	BPI	0.49	0.14	0.53	0.42	0.74	0.19	0.77	0.23	0.65	0.57	0.85	0.27		X		
A_21_P11897	SEPT14	0.36	0.67	0.37	0.38	0.41	0.28	0.75	0.00	0.80	0.00	0.87	0.00		X		X
A_23_P42956	SSH1	0.43	0.24	0.44	0.72	0.57	0.00	0.61	0.00	0.72	0.00	0.85	0.00		X		
A_23_P4174	MMIP9	1.28	0.33	0.79	0.12	1.20	0.15	1.15	0.83	1.88	0.00	1.45	0.00	X	X		
A_23_P122863	GRB1	0.74	0.73	0.53	0.23	0.89	0.14	0.96	0.16	0.90	0.00	1.23	0.12	X	X		
A_23_P126241	EIF4G3	0.28	0.69	0.16	0.64	0.32	0.13	0.63	0.00	0.61	0.00	0.74	0.00		X		
A_23_P122863	GRB1	0.58	0.29	0.52	0.27	0.91	0.67	0.79	0.36	0.93	0.00	1.24	0.00		X		
A_33_P3369844	CD24	0.66	0.26	0.65	0.53	0.71	0.52	0.58	0.19	0.53	0.30	0.55	0.17	X			
A_33_P334945	IL4R	0.42	0.12	0.27	0.35	0.33	0.22	0.64	0.00	0.67	0.00	0.63	0.00		X		
A_32_P61684	PAG1	0.48	0.12	0.39	0.39	0.46	0.12	0.82	0.00	0.75	0.00	0.84	0.00		X		
A_23_P161152	PDSSI	0.62	0.14	0.50	0.13	0.57	0.00	0.66	0.00	0.55	0.88	0.65	0.00		X		X
A_23_P4174	MMIP9	0.99	0.52	0.78	0.13	1.21	0.16	1.17	0.12	1.14	0.00	1.48	0.00	X	X		
A_23_P212522	ATP11B	0.53	0.56	0.44	0.19	0.48	0.48	0.64	0.00	0.70	0.00	0.74	0.00		X		X
A_23_P11473	NAP	0.49	0.85	0.26	0.25	0.49	0.22	0.91	0.21	0.83	0.00	1.13	0.00		X		
A_23_P14464	ALOX5	0.44	0.37	0.42	0.15	0.47	0.40	0.69	0.12	0.76	0.00	0.69	0.00		X		
A_23_P17336	ACAP1	0.45	0.24	0.47	0.23	0.35	0.22	0.57	0.18	0.59	0.00	0.59	0.00		X		
A_23_P334864	FAM126B	0.22	0.19	0.96	0.51	0.19	0.17	0.68	0.12	0.71	0.00	0.77	0.00		X		
A_21_P12992	NAP	0.60	0.41	0.45	0.23	0.52	0.13	0.91	0.00	0.91	0.00	0.94	0.29		X		
A_23_P17465	KRT31	-0.35	0.90	-0.39	0.64	-0.28	0.27	-0.12	0.59	-0.63	0.63	-0.64	0.71		X		
A_23_P33561	C19orf59	1.26	0.56	0.92	0.23	1.47	0.28	1.38	0.45	1.35	0.00	1.78	0.00	X	X		
A_23_P213385	BASP1	0.45	0.32	0.33	0.74	0.40	0.16	0.68	0.37	0.65	0.00	0.66	0.00		X		

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISB58)	Approach6 (ISB55)	ISB19	ISB63
A_23_P123393	KCNQ3	-0.13	0.74	0.38	0.90	-0.11	0.52	-0.79	0.49	-0.25	0.33	-0.63	0.70		X		
A_23_P17186	OPLAH	0.75	0.47	0.72	0.37	1.00	0.24	0.98	0.00	1.15	0.00	1.34	0.00	X	X		
A_23_P12418	ITGAM	0.52	0.14	0.36	0.39	0.45	0.66	0.74	0.20	0.57	0.00	0.67	0.00		X		
A_23_P18372	B3GNT5	0.49	0.13	0.29	0.16	0.39	0.36	0.64	0.00	0.58	0.12	0.73	0.00		X		
A_23_P123732	C9orf13	-0.15	0.94	0.46	0.15	0.47	0.67	0.12	0.45	0.65	0.19	0.63	0.32		X		
A_23_P4883	CAMKK2	0.40	0.46	0.33	0.78	0.33	0.54	0.60	0.90	0.60	0.25	0.57	0.22		X		
A_33_P329343	CYP11B1	0.52	0.15	0.40	0.56	0.52	0.80	0.90	0.00	0.83	0.67	0.97	0.00		X		
A_33_P3311285	LMNA	0.15	0.59	-0.93	0.65	-0.29	0.94	-0.36	0.13	-0.62	0.27	-0.72	0.73		X		
A_23_P13361	LCK	-0.49	0.48	-0.42	0.43	-0.44	0.23	-0.57	0.14	-0.59	0.21	-0.64	0.12		X		
A_23_P29851	LRPA1	0.49	0.25	0.46	0.17	0.41	0.84	0.64	0.34	0.58	0.00	0.62	0.00		X		
A_23_P38953	NOO2	0.44	0.26	0.48	0.14	0.40	0.75	0.73	0.29	0.87	0.43	0.76	0.00		X		X
A_23_P4174	MMP9	1.00	0.53	0.78	0.13	1.23	0.12	1.16	0.19	1.12	0.00	1.48	0.00	X	X		
A_32_P87697	HLA-DRA	-0.23	0.20	-0.37	0.20	-0.62	0.22	-0.29	0.88	-0.72	0.00	-0.86	0.00		X		X
A_33_P335622	STAR3	0.12	0.66	0.28	0.89	-0.27	0.12	-0.48	0.37	-0.59	0.26	-0.74	0.44		X		
A_23_P117546	SOS2	0.34	0.33	0.25	0.64	0.45	0.83	0.54	0.27	0.57	0.00	0.69	0.69		X		
A_23_P123393	KCNQ3	-0.24	0.40	0.18	0.40	-0.26	0.16	-0.79	0.55	-0.31	0.21	-0.64	0.80		X		
A_23_P123393	KCNQ3	-0.76	0.78	-0.24	0.23	-0.23	0.23	-0.40	0.83	-0.74	0.33	-0.76	0.68		X		
A_23_P4174	MMP9	1.38	0.32	0.78	0.13	1.22	0.14	1.28	0.62	1.11	0.00	1.50	0.00	X	X		
A_24_P29723	POR	0.68	0.12	0.52	0.79	0.55	0.66	0.84	0.49	0.72	0.00	0.76	0.00		X		
A_23_P117546	SOS2	0.29	0.20	0.25	0.13	0.43	0.72	0.47	0.44	0.57	0.00	0.68	0.74		X		
A_23_P11473	NAP	0.54	0.83	0.27	0.23	0.55	0.13	0.92	0.54	0.83	0.13	1.12	0.00		X		
A_33_P335863	RETN	1.89	0.63	0.97	0.66	1.44	0.13	1.53	0.00	1.52	0.00	1.83	0.00		X		
A_33_P334197	NEGR1	-0.19	0.45	-0.48	0.19	-0.48	0.79	-0.28	0.27	-0.73	0.49	-0.83	0.11		X		
A_33_P3281816	CAP1	0.21	0.90	0.23	0.48	0.30	0.66	0.60	0.90	0.66	0.00	0.69	0.00		X		

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISB58)	Approach6 (ISB55)	ISB19	ISB63
A_33_P3285541	<i>MLLT1</i>	0.46	0.66	0.44	0.54	0.39	0.15	0.56	0.68	0.60	0.00	0.64	0.00		X		
A_23_P295	<i>S4MSN1</i>	0.96	0.94	0.62	0.13	0.87	0.31	1.25	0.00	1.13	0.00	1.28	0.33	X	X		
A_24_P343733	<i>HLA-DRB1</i>	-0.21	0.19	-0.40	0.47	-0.62	0.00	-0.32	0.12	-0.76	0.00	-0.86	0.74		X		
A_23_P155765	<i>HMGCB2</i>	0.35	0.12	0.30	0.18	0.62	0.16	0.53	0.37	0.65	0.00	0.77	0.00		X		
A_33_P334847	<i>CARD6</i>	0.32	0.80	0.30	0.18	0.67	0.00	0.51	0.16	0.62	0.00	0.90	0.12		X		
A_23_P417331	<i>RPS6K43</i>	0.33	0.14	0.38	0.77	0.46	0.00	0.48	0.19	0.63	0.00	0.69	0.00		X		
A_23_P67847	<i>GALNT14</i>	0.77	0.63	0.67	0.16	0.87	0.60	1.69	0.12	1.23	0.00	1.26	0.00	X	X	X	
A_33_P3338	<i>MAP1LC3</i>	0.12	0.65	-0.16	0.43	-0.17	0.37	-0.23	0.24	-0.64	0.36	-0.72	0.22		X		
A_24_P244944	<i>MC1P2</i>	0.60	0.24	0.42	0.73	0.57	0.19	0.73	0.00	0.76	0.00	0.88	0.00		X		
A_33_P3211666	<i>IL18R1</i>	0.79	0.57	0.68	0.77	1.19	0.00	0.97	0.48	1.88	0.00	1.44	0.00	X	X	X	
A_33_P3343155	<i>GNAQ</i>	0.27	0.33	0.27	0.48	0.36	0.25	0.60	0.28	0.61	0.00	0.71	0.00		X		
A_32_P351968	<i>HLA-DMB</i>	-0.56	0.62	-0.53	0.95	-0.67	0.53	-0.66	0.42	-0.84	0.00	-0.94	0.00		X		
A_23_P9823	<i>MLXIP</i>	0.25	0.17	0.23	0.14	0.23	0.91	0.68	0.00	0.62	0.00	0.72	0.00		X		
A_23_P3624	<i>MYEOV</i>	0.92	0.69	-0.28	0.28	-0.15	0.33	-0.23	0.32	-0.64	0.66	-0.60	0.83		X		
A_24_P38536	<i>CD164</i>	0.35	0.50	0.29	0.18	0.42	0.14	0.49	0.20	0.48	0.00	0.63	0.00		X		X
A_23_P13747	<i>SIPA1L2</i>	0.44	0.13	0.25	0.15	0.47	0.63	0.89	0.00	0.89	0.79	0.97	0.36		X		
A_23_P25155	<i>GPR84</i>	1.97	0.98	1.19	0.29	1.24	0.00	1.25	0.37	1.28	0.00	1.54	0.00	X	X		
A_24_P18155	<i>ST3GAL4</i>	0.50	0.14	0.39	0.28	0.47	0.17	0.62	0.36	0.59	0.20	0.71	0.32		X		
A_23_P2758	<i>SOC3</i>	0.66	0.20	0.45	0.74	0.65	0.11	0.97	0.18	0.94	0.00	1.57	0.00		X		
A_23_P12463	<i>QSOX1</i>	0.34	0.59	0.32	0.27	0.59	0.47	0.55	0.25	0.63	0.00	0.83	0.00		X		
A_23_P121716	<i>ANXA3</i>	0.84	0.16	0.58	0.67	0.67	0.13	1.12	0.00	1.40	0.00	1.12	0.00		X		
A_23_P25721	<i>RNF146</i>	0.37	0.54	0.28	0.57	0.26	0.69	0.67	0.28	0.59	0.00	0.66	0.00		X		X
A_33_P3345132	<i>ZNF578</i>	0.20	0.45	-0.34	0.22	-0.13	0.55	-0.54	0.84	-0.72	0.76	-0.74	0.37		X		
A_33_P3245389	<i>C14orf11</i>	0.47	0.11	0.36	0.19	0.50	0.14	0.59	0.29	0.59	0.00	0.68	0.00		X		

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISB58)	Approach6 (ISB55)	ISB19	ISB63
A_23_P13765	FCER1A	-0.30	0.16	-0.26	0.18	-0.67	0.34	-0.49	0.11	-0.62	0.16	-0.94	0.00		X		
A_23_P21433	SERPINB1	0.52	0.17	0.42	0.33	0.62	0.42	0.89	0.25	0.82	0.00	1.00	0.00		X		
A_24_P4525	ATP2B4	0.24	0.17	0.17	0.17	0.23	0.36	0.59	0.20	0.64	0.00	0.66	0.00		X		
A_33_P3375541	CD3D	-0.38	0.65	-0.48	0.98	-0.56	0.58	-0.63	0.19	-0.84	0.00	-0.95	0.00		X		
A_23_P2532	CCR3	-0.28	0.25	-0.24	0.24	-0.72	0.17	-0.38	0.95	-0.59	0.18	-0.98	0.00		X		
A_33_P3376659	TARP	-0.13	0.51	-0.28	0.12	-0.34	0.66	-0.42	0.37	-0.64	0.15	-0.68	0.26		X		X
A_33_P3234277	HLA-DPA1	-0.27	0.26	-0.32	0.52	-0.63	0.22	-0.32	0.25	-0.64	0.00	-0.84	0.00		X		
A_33_P342526	CSGALNA CT2	0.54	0.42	0.34	0.33	0.59	0.60	0.79	0.22	0.85	0.00	0.99	0.00		X		
A_23_P818	HLA-DQB1	-0.17	0.37	-0.47	0.24	-0.62	0.12	-0.26	0.55	-0.62	0.00	-0.88	0.00		X		
A_23_P15465	SULF2	-0.73	0.74	-0.68	0.88	-0.59	0.16	-0.70	0.53	-0.81	0.00	-0.72	0.11	X	X		
A_23_P28768	FCAR	0.73	0.40	0.55	0.12	0.61	0.34	0.94	0.00	0.84	0.00	0.92	0.00	X	X	X	
A_23_P48676	PYGL	0.44	0.92	0.29	0.13	0.47	0.74	0.63	0.17	0.59	0.00	0.68	0.00		X		X
A_33_P322398	TPM3	0.23	0.88	0.16	0.54	0.18	0.18	0.58	0.00	0.59	0.00	0.67	0.20		X		X
A_23_P12418	ITGAM	0.70	0.19	0.37	0.34	0.50	0.47	0.87	0.62	0.58	0.00	0.72	0.00		X		
A_23_P496	C44	0.68	0.14	0.55	0.19	0.74	0.42	0.74	0.46	0.66	0.11	0.80	0.00		X		X
A_24_P27144	CD63	0.39	0.22	0.43	0.80	0.58	0.18	0.59	0.74	0.64	0.00	0.76	0.00		X		X
A_23_P8593	TLR5	0.57	0.24	0.45	0.68	0.68	0.28	0.83	0.13	0.84	0.00	1.12	0.16		X		
A_23_P4353	HSBI	0.33	0.12	0.19	0.27	0.49	0.14	0.68	0.22	0.67	0.00	0.85	0.00		X		X
A_33_P321184	RUNX1	0.33	0.45	0.40	0.15	0.60	0.00	0.49	0.34	0.60	0.00	0.78	0.55		X		X
A_33_P3359223	C9orf173	0.67	0.80	-0.89	0.66	-0.43	0.15	-0.54	0.33	-0.75	0.38	-0.94	0.00		X		
A_23_P6339	FCGR1B	0.89	0.67	0.19	0.37	0.85	0.96	0.84	0.16	0.87	0.00	0.77	0.18		X		
A_23_P21694	ASPH	0.28	0.14	0.24	0.14	0.52	0.35	0.58	0.34	0.70	0.00	0.87	0.00		X		
A_23_P122863	GRB1	0.59	0.36	0.50	0.38	0.91	0.15	0.79	0.66	0.92	0.00	1.26	0.00		X		
A_23_P258164	CORT	-0.23	0.92	-0.22	0.34	-0.48	0.11	-0.26	0.30	-0.65	0.87	-0.94	0.17		X		

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISB58)	Approach6 (ISB55)	ISB19	ISB63
A_23_P128974	BATF	0.30	0.68	0.34	0.47	0.44	0.48	0.11	0.60	0.00	0.73	0.00			X		
A_33_P3287614	C9orf173	0.21	0.36	-0.88	0.62	-0.36	-0.34	0.11	-0.66	0.48	-0.82	0.00			X		
A_23_P6861	CST7	0.60	0.37	0.56	0.24	0.63	0.62	0.89	0.76	0.99	0.89	0.32			X		X
A_23_P117546	SOS2	0.29	0.75	0.23	0.86	0.40	0.82	0.68	0.58	0.00	0.66	0.99			X		
A_23_P121716	ANXA3	0.78	0.27	0.54	0.80	0.67	0.15	0.24	1.19	0.00	1.13	0.00			X		
A_24_P3348	RAB32	0.55	0.69	0.45	0.43	0.44	0.56	0.69	0.52	0.11	0.70	0.00			X		
A_33_P3364864	NAMPT	0.45	0.80	0.58	0.97	0.85	0.52	0.58	0.14	0.67	0.00	0.64	0.24		X		
A_24_P14859	TACR1	0.43	0.97	-0.18	0.44	-0.29	0.16	0.13	0.56	-0.61	0.95	-0.84	0.46		X		
A_33_P3835524	POU2F2	-0.30	0.92	-0.28	0.77	-0.46	0.28	0.19	-0.63	0.63	-0.79	0.16			X		X
A_24_P2664	PFKFB3	0.29	0.36	0.21	0.99	0.43	0.29	0.54	0.12	0.60	0.00	0.78	0.00		X		X
A_24_P343233	HLA-DRA1	-0.20	0.15	-0.40	0.37	-0.57	0.00	-0.31	0.87	-0.71	0.00	-0.82	0.00		X		
A_23_P11212	ACSL1	0.47	0.57	0.24	0.21	0.35	0.52	0.81	0.22	0.75	0.00	0.82	0.00		X		
A_23_P33723	CD163	0.45	0.12	0.12	0.66	0.35	0.93	0.77	0.13	0.46	0.27	0.77	0.00		X		
A_23_P384517	GYGI	0.95	0.55	0.79	0.95	1.37	0.14	1.12	0.15	1.15	0.00	1.36	0.57	X	X		
A_24_P12115	CFLAR	0.25	0.20	0.13	0.28	0.11	0.32	0.66	0.00	0.64	0.00	0.63	0.00		X		X
A_33_P3324884	MICAL1	0.37	0.19	0.41	0.22	0.33	0.20	0.69	0.00	0.72	0.00	0.69	0.27		X		X
A_33_P376482	SIRT5	0.53	0.73	0.47	0.12	0.60	0.13	0.59	0.14	0.62	0.00	0.77	0.00		X		
A_23_P256821	CR1	0.49	0.15	0.50	0.72	0.66	0.60	0.77	0.00	0.93	0.00	0.99	0.00		X		
A_23_P116765	LALBA	0.29	0.26	-0.35	0.12	-0.39	0.38	-0.55	0.84	-0.88	0.85	-0.94	0.39		X		
A_33_P3329549	FBR5	-0.83	0.64	-0.25	0.12	-0.36	0.44	-0.46	0.26	-0.67	0.16	-0.72	0.32		X		
A_23_P66719	DHRS13	0.45	0.18	0.37	0.34	0.46	0.39	0.56	0.13	0.59	0.13	0.65	0.00		X		
A_32_P154342	SLCO4C1	0.59	0.48	0.39	0.13	0.55	0.88	0.59	0.59	0.57	0.23	0.72	0.00		X		
A_23_P7429	GBP5	-0.81	0.19	-0.45	0.62	-0.74	0.15	-0.37	0.49	-0.31	0.14	-0.38	0.58	X		X	
A_23_P36356	PLB1	0.49	0.45	0.36	0.76	0.54	0.26	0.89	0.15	0.88	0.00	0.93	0.00		X		

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISB58)	Approach6 (ISB55)	ISB19	ISB63
A_23_P57856	BCL6	0.25	0.30	0.21	0.28	0.29	0.11	0.89	0.91	0.92	0.00	0.89	0.00		X		
A_32_P148796	UBXN2B	0.34	0.69	0.18	0.18	0.23	0.32	0.66	0.14	0.55	0.00	0.63	0.00		X		
A_23_P14741	KIRREL3	0.36	0.30	-0.29	0.22	-0.22	0.24	-0.66	0.79	-0.80	0.98	-0.84	0.11		X		
A_33_P3257279	TMEM145	0.64	0.79	-0.16	0.39	-0.28	0.16	-0.29	0.22	-0.67	0.39	-0.75	0.69		X		
A_23_P259863	CD177	1.76	0.14	1.30	0.93	1.88	0.44	1.87	0.55	1.77	0.60	2.29	0.00	X	X		
A_23_P75769	MS4A4A	0.66	0.13	0.53	0.35	0.63	0.12	0.95	0.48	0.92	0.34	1.13	0.00		X		
A_23_P8311	CDK3RAP2	0.84	0.78	0.72	0.94	0.84	0.91	0.98	0.12	1.27	0.00	1.25	0.00	X	X		
A_23_P121716	ANXA3	0.82	0.22	0.55	0.72	0.67	0.16	1.18	0.27	1.39	0.00	1.12	0.00		X		
A_23_P17857	ILIRAP	0.49	0.25	0.18	0.23	0.22	0.15	0.85	0.00	0.64	0.16	0.70	0.43		X		X
A_23_P741	SLA12	0.88	0.28	0.67	0.23	0.93	0.89	0.85	0.57	0.93	0.00	1.89	0.00		X		
A_24_P295245	ASPH	0.58	0.29	0.47	0.30	0.74	0.17	0.78	0.17	0.79	0.00	0.98	0.00		X		
A_23_P252681	PCYT1A	0.38	0.26	0.35	0.15	0.45	0.00	0.59	0.00	0.66	0.00	0.69	0.00		X		
A_23_P351275	UPP1	0.93	0.37	0.71	0.27	0.77	0.19	1.28	0.00	0.93	0.00	1.24	0.00	X	X	X	
A_23_P13291	RBM47	0.31	0.76	0.13	0.34	0.23	0.64	0.70	0.00	0.69	0.00	0.69	0.81		X		X
A_23_P12418	ITGAM	0.53	0.12	0.49	0.17	0.48	0.44	0.69	0.28	0.62	0.00	0.71	0.00		X		
A_33_P33975	TBC1D8	0.73	0.14	0.62	0.92	0.72	0.17	0.98	0.00	0.98	0.00	1.82	0.00	X	X		
A_23_P1292	LGALS2	-0.70	0.11	-0.66	0.29	-1.33	0.00	-0.88	0.29	-1.18	1.00	-1.36	0.28	X	X	X	
A_24_P3881	FKBP5	0.76	0.15	0.57	0.28	0.73	0.30	1.78	0.00	1.76	0.00	1.19	0.00		X		
A_23_P11212	ACSL1	0.47	0.60	0.26	0.17	0.38	0.36	0.82	0.50	0.76	0.00	0.83	0.00		X		
A_33_P3338793	KCNK3	0.37	0.11	-0.85	0.64	-0.28	0.44	-0.16	0.46	-0.68	0.86	-0.73	0.95		X		
A_23_P4734	HHEX	0.50	0.62	0.35	0.25	0.33	0.16	0.57	0.79	0.62	0.18	0.62	0.00		X		
A_23_P217778	MSL3	0.46	0.33	0.29	0.85	0.45	0.27	0.69	0.27	0.53	0.50	0.69	0.00		X		X
A_33_P333592	SYNE1	-0.47	0.81	-0.36	0.59	-0.46	0.22	-0.45	0.58	-0.73	0.14	-0.74	0.23		X		X
A_33_P32338	CD177	0.60	0.53	0.73	0.19	0.93	0.00	0.65	0.15	0.84	0.32	0.92	0.00	X	X		

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISB58)	Approach6 (ISB55)	ISB19	ISB63
A_23_P27424	ZNF418	0.88	0.73	-0.18	0.38	-0.39	0.98	-0.26	0.32	-0.63	0.69	-0.77	0.46		X		
A_23_P14741	KIRREL3	0.24	0.31	-0.18	0.43	-0.19	0.32	-0.19	0.49	-0.70	0.55	-0.76	0.61		X		
A_33_P327555	STGALNA C3	0.59	0.98	0.46	0.48	0.64	0.00	0.59	0.15	0.60	0.12	0.75	0.59	X	X		X
A_23_P41854	CARD6	0.45	0.60	0.37	0.64	0.58	0.29	0.67	0.54	0.64	0.19	0.87	0.17		X		
A_23_P9497	ILIR44	0.36	0.19	0.40	0.16	0.23	0.49	0.74	0.58	0.60	0.00	0.53	0.00		X		
A_33_P3228612	CACNA1E	0.28	0.58	0.44	0.14	0.42	0.81	0.59	0.32	0.76	0.00	0.71	0.00		X		
A_23_P156218	GZMK	-0.33	0.54	-0.35	0.36	-0.47	0.57	-0.65	0.69	-0.65	0.22	-0.63	0.40		X		
A_23_P1776	IL17R4	0.17	0.25	0.13	0.36	0.19	0.97	0.65	0.00	0.58	0.00	0.67	0.17		X		
A_33_P3331687	GPSM1	0.21	0.37	-0.82	0.64	-0.23	0.27	-0.38	0.15	-0.68	0.14	-0.76	0.14		X		
A_23_P39931	DYSF	0.55	0.13	0.44	0.19	0.53	0.25	0.86	0.00	0.82	0.00	0.91	0.93		X		
A_24_P286114	SLC1A3	0.56	0.25	0.28	0.64	0.55	0.49	0.69	0.40	0.46	0.65	0.69	0.00		X		
A_23_P6919	PLSCR1	0.59	0.12	0.32	0.11	0.34	0.85	0.94	0.00	0.81	0.00	0.86	0.00		X		
A_33_P3385785	SLA12	1.17	0.52	0.82	0.15	1.13	0.15	1.36	0.14	1.89	0.00	1.30	0.00	X	X		
A_23_P4174	MMIP9	1.21	0.37	0.79	0.17	1.25	0.13	1.18	0.73	1.13	0.00	1.45	0.00	X	X		
A_23_P13765	FCER1A	-0.35	0.15	-0.35	0.12	-0.64	0.46	-0.49	0.72	-0.66	0.00	-0.89	0.00		X		
A_23_P12418	ITGAM	0.51	0.12	0.38	0.28	0.49	0.45	0.69	0.17	0.59	0.00	0.74	0.00		X		
A_23_P1782	CD82	0.35	0.13	0.34	0.95	0.43	0.65	0.52	0.15	0.59	0.00	0.66	0.44		X		X
A_24_P649624	KIF1B	0.63	0.12	0.45	0.96	0.54	0.19	0.76	0.00	0.66	0.35	0.83	0.00		X		
A_23_P117546	SOS2	0.34	0.62	0.25	0.62	0.42	0.42	0.58	0.13	0.57	0.00	0.67	0.37		X		
A_23_P431388	SPOCD1	0.41	0.17	0.59	0.36	0.65	0.12	0.53	0.17	0.64	0.41	0.64	0.29	X	X		
A_33_P3364582	TNXB	0.16	0.49	-0.38	0.78	-0.29	0.12	-0.39	0.87	-0.75	0.34	-0.72	0.82		X		
A_24_P18797	PADI2	0.37	0.24	0.13	0.38	0.32	0.82	0.61	0.11	0.39	0.22	0.64	0.00		X		
A_21_P13195	SEPT14	0.40	0.12	0.41	0.23	0.41	0.14	0.70	0.00	0.83	0.00	0.84	0.00		X		X
A_23_P4174	MMIP9	1.32	0.28	0.79	0.90	1.20	0.13	1.20	0.75	1.13	0.00	1.45	0.00	X	X		

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISB58)	Approach6 (ISB55)	ISB19	ISB63
A_23_P16648	OSM	0.67	0.61	0.33	0.14	0.53	0.11	0.99	0.00	0.77	0.00	0.89	0.00		X		
A_33_P3263756	ZNF446	0.73	0.67	-0.30	0.72	-0.30	0.24	-1.00	0.64	-0.60	0.15	-0.66	0.13		X		X
A_23_P99397	ZDHC2	0.54	0.42	0.41	0.78	0.63	0.00	0.82	0.00	0.77	0.00	0.96	0.00		X		
A_23_P143845	TIPARP	0.64	0.56	0.48	0.18	0.49	0.26	0.78	0.38	0.74	0.00	0.72	0.55		X		
A_23_P122863	GRB1	0.62	0.19	0.52	0.25	0.92	0.72	0.80	0.32	0.96	0.00	1.23	0.00		X		
A_33_P3282394	MLLT1	0.40	0.19	0.35	0.12	0.49	0.60	0.79	0.12	0.75	0.00	0.85	0.00		X		
A_23_P1243	P2RX2	0.86	1.00	-0.48	0.17	-0.34	0.98	-0.29	0.15	-0.73	0.66	-0.60	0.32		X		X
A_23_P14316	ARID5A	0.45	0.29	0.36	0.84	0.51	0.89	0.64	0.55	0.58	0.00	0.73	0.70		X		X
A_24_P28567	IL18R1	0.97	0.12	0.78	0.17	1.12	0.00	1.22	0.27	1.26	0.00	1.47	0.00	X	X	X	
A_24_P353794	GALNT2	0.49	0.12	0.54	0.65	0.48	0.32	0.60	0.00	0.65	0.00	0.63	0.00		X		
A_23_P39251	PLIN5	0.34	0.80	0.26	0.99	0.49	0.46	0.72	0.83	0.68	0.00	0.86	0.00		X		
A_23_P99163	DR4MI	0.43	0.11	0.40	0.51	0.50	0.71	0.63	0.16	0.64	0.00	0.76	0.12		X		
A_23_P11473	NAIP	0.52	0.58	0.28	0.21	0.54	0.15	0.95	0.16	0.84	0.00	1.16	0.00		X		
A_23_P38614	ATP9A	0.99	0.56	0.85	0.20	0.98	0.00	0.92	0.46	0.93	0.00	1.14	0.00	X	X	X	
A_23_P363313	SLC16A11	0.23	0.38	-0.44	0.71	-0.23	0.27	0.13	0.56	-0.67	0.83	-0.62	0.64		X		
A_23_P121716	ANXA3	0.79	0.22	0.56	0.64	0.69	0.13	1.93	0.16	1.45	0.00	1.12	0.00		X		
A_23_P52266	IFT1	-0.72	0.27	-0.79	0.27	-1.58	0.13	-0.60	0.50	-0.80	0.12	-1.72	0.95		X		
A_33_P3319967	ARG1	0.88	0.28	0.75	0.30	1.15	0.59	1.28	0.14	1.19	0.00	1.51	0.00		X		
A_33_P354143	IL17R4	0.25	0.16	0.17	0.22	0.39	0.22	0.62	0.48	0.57	0.00	0.73	0.39		X		
A_33_P33635	PFKEB2	0.88	0.38	0.74	0.17	1.17	0.00	1.19	0.23	1.95	0.00	1.36	0.00	X	X		
A_23_P4174	MDIP9	1.62	0.26	0.85	0.76	1.22	0.14	1.20	0.59	1.14	0.00	1.46	0.00	X	X		
A_23_P161156	ZNF438	0.41	0.38	0.30	0.70	0.45	0.17	0.70	0.44	0.72	0.00	0.81	0.86		X		
A_23_P87329	NAT1	-0.14	0.57	0.63	0.74	-0.32	0.82	-0.63	0.30	-0.33	0.94	-0.63	0.19		X		
A_21_P149	C2orf3	0.13	0.59	-0.39	0.13	-0.30	0.16	-0.70	0.79	-0.78	0.90	-0.71	0.19		X		

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISB58)	Approach6 (ISB55)	ISB19	ISB63
A_33_P339277	TP53I3	0.67	0.46	0.49	0.46	0.68	0.20	0.83	0.00	0.69	0.25	0.84	0.00	X	X	X	
A_23_P2348	SLA9	0.44	0.76	0.37	0.95	0.53	0.50	0.87	0.37	0.90	0.00	0.80	0.00		X		
A_23_P6943	GPR15	0.15	0.61	-0.66	0.26	-0.42	0.18	0.95	0.76	-0.89	0.32	-0.85	0.25		X		X
A_23_P123393	KCNQ3	0.18	0.43	-0.39	0.58	-0.47	0.51	-0.27	0.34	-0.92	0.20	-0.92	0.17		X		
A_23_P116765	LALBA	0.32	0.23	-0.32	0.15	-0.31	0.16	-0.24	0.45	-0.95	0.19	-0.95	0.38		X		
A_33_P3216448	COL11A2	0.13	0.57	-0.23	0.25	-0.38	0.36	-0.35	0.16	-0.81	0.43	-0.80	0.39		X		
A_24_P161973	ATP11A	0.26	0.66	0.13	0.22	0.34	0.31	0.59	0.00	0.52	0.00	0.66	0.00		X		
A_23_P12418	ITGAM	0.56	0.66	0.48	0.20	0.57	0.29	0.75	0.11	0.58	0.26	0.72	0.00		X		
A_23_P11473	NAP	0.49	0.89	0.25	0.27	0.46	0.27	0.94	0.24	0.87	0.00	1.00	0.00		X		
A_24_P337746	RABGEF1	0.57	0.36	0.46	0.21	0.62	0.00	0.72	0.00	0.68	0.00	0.83	0.00		X		
A_23_P128993	GZMH	-0.68	0.67	-0.69	0.34	-0.52	0.20	-0.42	0.18	-0.52	0.39	-0.33	0.19	X			
A_24_P5245	HLA-DMA	-0.11	0.39	-0.28	0.12	-0.44	0.16	-0.28	0.27	-0.64	0.00	-0.68	0.00		X		
A_33_P3215797	AHDC1	0.39	0.12	-0.34	0.12	-0.24	0.24	0.35	0.89	-0.78	0.40	-0.73	0.45		X		
A_24_P295963	SLC38A2	0.34	0.17	0.17	0.13	0.32	0.91	0.62	0.00	0.51	0.00	0.64	0.00		X		
A_23_P153945	GTDC1	0.44	0.33	0.37	0.78	0.51	0.11	0.66	0.17	0.61	0.00	0.71	0.00		X		
A_24_P32552	SORT1	0.58	0.17	0.54	0.12	0.53	0.34	0.88	0.00	0.86	0.00	0.94	0.00		X		
A_33_P3247473	KRTAP23-1	0.34	0.29	-0.22	0.29	-0.12	0.53	-0.24	0.31	-0.78	0.80	-0.59	0.32		X		
A_23_P41664	LRRC7	0.50	0.77	0.50	0.68	0.47	0.18	0.61	0.27	0.76	0.00	0.69	0.00		X		
A_24_P183128	PLAC8	0.76	0.18	0.77	0.24	0.93	0.33	0.93	0.38	1.00	0.12	1.12	0.99	X			
A_23_P217712	ARSD	0.13	0.59	-0.46	0.26	-0.35	0.76	-0.19	0.96	-0.68	0.48	-0.62	0.54		X		
A_23_P65789	MCTP2	0.44	0.19	0.41	0.12	0.48	0.97	0.77	0.00	0.84	0.65	0.87	0.00		X		
A_24_P3533	LMK2	0.38	0.17	0.37	0.93	0.29	0.19	0.74	0.00	0.76	0.00	0.73	0.00		X		
A_23_P117546	SOS2	0.31	0.60	0.25	0.72	0.26	0.12	0.53	0.51	0.60	0.00	0.54	0.40		X		
A_33_P3272527	M4VS	0.14	0.64	-0.52	0.33	-0.20	0.43	-0.19	0.44	-0.81	0.43	-0.71	0.32		X		

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISB58)	Approach6 (ISB55)	ISB19	ISB63
A_23_P313389	UGCG	0.92	0.12	0.77	0.17	0.95	0.11	1.17	0.00	1.12	0.00	1.29	0.00	X	X	X	
A_23_P17242	ABHD1	0.15	0.46	-0.46	0.32	-0.40	0.38	0.79	0.73	-0.67	0.83	-0.71	0.40		X		
A_23_P156748	ANKS1A	0.37	0.27	0.21	0.59	0.39	0.92	0.66	0.18	0.56	0.00	0.67	0.00		X		
A_23_P254756	CD164	0.32	0.53	0.25	0.32	0.17	0.15	0.60	0.11	0.57	0.00	0.58	0.00		X		X
A_23_P162211	MANSC1	0.44	0.58	0.31	0.19	0.45	0.18	0.66	0.18	0.58	0.11	0.65	0.00		X		
A_23_P21141	KREMENT	0.37	0.84	0.37	0.46	0.26	0.26	0.63	0.00	0.65	0.00	0.58	0.00		X		X
A_33_P3242458	SLC11A3	0.15	0.70	-0.25	0.22	-0.27	0.20	-0.37	0.13	-0.74	0.27	-0.78	0.14		X		
A_33_P3431595	C8orf31	0.23	0.47	-0.42	0.16	-0.19	0.45	0.28	0.94	-0.73	0.92	-0.74	0.67		X		
A_23_P11473	NAP	0.51	0.62	0.26	0.26	0.54	0.16	0.95	0.16	0.84	0.00	1.13	0.00		X		
A_33_P3411925	WDR18	0.14	0.58	-0.25	0.17	-0.34	0.71	-0.45	0.47	-0.82	0.26	-0.84	0.20		X		
A_33_P339465	FMG2B	0.43	0.15	0.44	0.82	-0.84	0.69	-0.37	0.14	-0.62	0.27	-0.59	0.78		X		
A_24_P945293	CHMP3	0.42	0.87	-0.39	0.49	-0.38	0.64	-0.35	0.16	-0.79	0.23	-0.77	0.32		X		
A_23_P116765	LALBA	0.84	0.98	-0.53	0.25	-0.45	0.72	-0.57	0.46	-1.32	0.60	-1.33	0.35		X		
A_23_P94647	ORIL3	0.22	0.42	-0.67	0.14	-0.42	0.11	0.26	0.93	-0.97	0.17	-0.95	0.12		X		
A_23_P126623	PGD	0.68	0.48	0.58	0.52	0.57	0.14	0.80	0.00	0.67	0.00	0.72	0.00		X		
A_23_P137665	CH13L1	-0.65	0.27	-0.69	0.18	-0.62	0.78	-0.83	0.35	-0.96	0.25	-0.75	0.17		X		
A_24_P14875	SH3BP5	0.57	0.11	0.39	0.34	0.42	0.68	0.77	0.14	0.67	0.00	0.68	0.00		X		X
A_23_P12418	ITGA4	0.49	0.14	0.36	0.39	0.47	0.52	0.69	0.13	0.59	0.00	0.72	0.00		X		
A_23_P151637	RNASE2	0.66	0.58	0.62	0.20	0.58	0.24	0.75	0.12	0.59	0.64	0.62	0.29	X	X		
A_33_P327149	RBMS1	0.27	0.81	0.23	0.85	0.40	0.93	0.62	0.14	0.67	0.00	0.76	0.38		X		
A_23_P126844	TNFRSF25	-0.53	0.17	-0.41	0.24	-0.51	0.54	-0.59	0.54	-0.58	0.14	-0.64	0.86		X		
A_23_P14741	KIRREL3	0.44	0.88	-0.48	0.43	-0.33	0.17	-0.54	0.49	-0.96	0.79	-0.95	0.54		X		
A_33_P3411612	TMEM221	0.23	0.37	-0.38	0.64	-0.24	0.20	0.55	0.81	-0.61	0.58	-0.63	0.25		X		
A_23_P1926	KCNK15	-0.14	0.96	-0.36	0.39	-0.26	0.16	-0.45	0.26	-0.76	0.62	-0.76	0.82		X		

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISB58)	Approach6 (ISB55)	ISB19	ISB63
A_23_P19543	SRPK1	0.26	0.25	0.18	0.29	0.30	0.47	0.57	0.54	0.64	0.00	0.67	0.00		X		
A_23_P27445	M4P2K6	0.48	0.16	0.30	0.88	0.64	0.20	0.62	0.14	0.53	0.56	0.63	0.00		X		
A_23_P1623	IRAK3	0.54	0.23	0.36	0.85	0.71	0.44	0.86	0.93	0.88	0.00	1.15	0.00		X		
A_23_P1733	UCKL1	0.27	0.92	-0.25	0.26	-0.57	0.27	-0.22	0.42	-0.78	0.39	-1.63	0.00		X		X
A_23_P14464	ALOX5	0.46	0.31	0.38	0.22	0.43	0.14	0.75	0.22	0.73	0.00	0.69	0.00		X		
A_23_P1243	P2RX2	0.22	0.42	-0.50	0.11	-0.37	0.78	0.79	0.77	-0.73	0.23	-0.72	0.11		X		X
A_23_P21463	FLOT1	0.44	0.12	0.37	0.20	0.44	0.12	0.59	0.13	0.54	0.00	0.60	0.00		X		
A_24_P322635	ELMO2	0.33	0.26	0.35	0.99	0.30	0.63	0.55	0.30	0.60	0.00	0.59	0.00		X		
A_33_P336948	LRP6	0.12	0.64	-0.25	0.21	-0.19	0.27	-0.22	0.34	-0.63	0.26	-0.66	0.14		X		
A_23_P5638	LRG1	0.63	0.33	0.44	0.30	0.62	0.47	0.64	0.22	0.54	0.32	0.68	0.00	X	X	X	
A_33_P3417281	MUC4	0.35	0.99	-0.38	0.38	-0.19	0.35	-0.48	0.38	-0.76	0.18	-0.68	0.37		X		
A_23_P16325	RNASE3	0.68	0.38	0.65	0.22	0.64	0.46	0.69	0.23	0.59	0.16	0.62	0.17	X	X		X
A_23_P344884	CARNS1	-0.94	0.96	-0.38	0.34	-0.28	0.72	-0.34	0.62	-0.69	0.23	-0.75	0.00		X		X
A_33_P3319126	CRIL	0.54	0.16	0.50	0.74	0.51	0.85	0.75	0.00	0.87	0.00	0.87	0.00		X		
A_23_P94533	CTSL1	0.31	0.26	-0.18	0.52	-0.15	0.47	0.33	0.89	-0.63	0.66	-0.59	0.28		X		
A_23_P36941	RGL4	0.86	0.38	0.76	0.44	0.97	0.45	1.48	0.34	1.12	0.00	1.20	0.00	X	X	X	
A_23_P12884	ITGA7	0.55	0.29	0.54	0.16	0.88	0.24	0.64	0.25	0.68	0.63	0.86	0.25		X		
A_33_P322422	POM121L1	0.67	0.35	-0.48	0.52	-0.42	0.75	0.48	0.19	-0.75	0.74	-0.79	0.89		X		X
A_23_P21426	FBN2	0.53	0.19	-0.34	0.24	-0.17	0.49	0.18	0.55	-0.79	0.39	-0.77	0.23		X		
A_23_P14741	KIRREL3	0.49	0.87	-0.17	0.55	-0.30	0.24	-0.37	0.19	-0.71	0.50	-0.95	0.19		X		
A_33_P3413216	TSPAN4	-0.37	0.88	-0.50	0.48	-0.39	0.39	-0.38	0.86	-0.84	0.70	-0.79	0.20		X		
A_24_P7121	NSUN7	0.44	0.59	0.43	0.25	0.66	0.80	0.64	0.14	0.74	0.00	0.90	0.00		X		X
A_24_P35142	ZDHHC3	0.54	0.32	0.34	0.63	0.47	0.96	0.70	0.00	0.49	0.12	0.63	0.00		X		
A_23_P16636	CBS	0.32	0.12	0.29	0.99	0.32	0.57	0.44	0.16	0.71	0.00	0.76	0.16		X		

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISB58)	Approach6 (ISB55)	ISB19	ISB63
A_24_P11436	<i>ITIC22</i>	0.65	0.76	-0.32	0.82	-0.24	0.18	-0.22	0.29	-0.62	0.66	-0.63	0.30		X		
A_23_P28747	<i>PGLYRP1</i>	0.62	0.67	0.46	0.23	0.63	0.34	0.45	0.53	0.39	0.38	0.49	0.84	X		X	
A_24_P343733	<i>HLA-DRA1</i>	-0.18	0.18	-0.32	0.24	-0.54	0.68	-0.26	0.12	-0.59	0.00	-0.74	0.00		X		
A_24_P239731	<i>B4GALT5</i>	0.28	0.54	0.28	0.26	0.38	0.39	0.86	0.00	0.92	0.36	1.48	0.00		X		X
A_33_P3245489	<i>ADAMTS1</i>	0.11	0.70	0.12	0.60	-0.18	0.46	-0.63	0.11	-0.59	0.32	-0.68	0.58		X		X
A_33_P3238993	<i>AGFG1</i>	0.86	0.55	0.62	0.21	0.67	0.40	1.00	0.00	0.99	0.00	0.96	0.57	X	X		
A_24_P29778	<i>C2orf3</i>	0.58	0.84	0.43	0.96	0.34	0.48	0.74	0.86	0.62	0.00	0.61	0.31		X		
A_23_P4166	<i>EDIL3</i>	0.15	0.65	-0.43	0.94	-0.34	0.18	-0.37	0.17	-0.94	0.32	-0.99	0.13		X		
A_23_P7378	<i>IRAK1</i>	-0.22	0.91	-0.19	0.28	-0.21	0.19	-0.39	0.73	-0.61	0.12	-0.78	0.12		X		
A_23_P14464	<i>ALOX5</i>	0.47	0.76	0.44	0.74	0.43	0.64	0.75	0.26	0.74	0.00	0.69	0.00		X		
A_24_P235266	<i>GRB1</i>	0.67	0.13	0.60	0.91	0.96	0.27	1.00	0.35	1.37	0.00	1.32	0.00	X	X		
A_23_P9114	<i>PECR</i>	0.59	0.21	0.48	0.27	0.59	0.00	0.59	0.12	0.64	0.00	0.74	0.00		X		
A_23_P122863	<i>GRB1</i>	0.63	0.15	0.56	0.12	0.88	0.82	0.85	0.25	0.92	0.00	1.26	0.00		X		
A_23_P123393	<i>KCNQ3</i>	-0.17	0.95	-0.38	0.96	-0.49	0.35	-0.57	0.59	-0.95	0.96	-1.12	0.13		X		
A_24_P347378	<i>ALOX5AP</i>	0.61	0.15	0.45	0.19	0.68	0.13	0.61	0.99	0.47	0.18	0.69	0.00		X		
A_33_P3347869	<i>C3</i>	0.32	0.34	-0.44	0.11	-0.27	0.28	0.18	0.95	-0.73	0.79	-0.88	0.78		X		
A_23_P14225	<i>LILRA2</i>	0.44	0.42	0.31	0.41	0.25	0.14	0.76	0.13	0.62	0.00	0.57	0.00		X		
A_24_P348765	<i>FCAR</i>	0.47	0.20	0.27	0.12	0.53	0.28	0.75	0.00	0.74	0.00	0.94	0.24		X		
A_23_P1522	<i>BCL2A1</i>	0.63	0.13	0.39	0.55	0.55	0.35	0.82	0.21	0.76	0.00	0.84	0.00		X		
A_23_P26557	<i>C16orf59</i>	0.22	0.43	-0.35	0.15	-0.27	0.19	-0.12	0.63	-0.62	0.57	-0.75	0.29		X		
A_23_P141173	<i>MPO</i>	0.50	0.26	0.57	0.15	0.59	0.15	0.55	0.29	0.61	0.40	0.72	0.24		X		
A_23_P395	<i>SLC26A8</i>	0.39	0.79	0.33	0.20	0.46	0.12	0.61	0.14	0.57	0.00	0.76	0.00		X		
A_23_P122863	<i>GRB1</i>	0.64	0.18	0.56	0.11	0.92	0.49	0.82	0.22	0.91	0.00	1.22	0.00		X		
A_23_P1926	<i>KCNK15</i>	0.22	0.92	-0.18	0.33	-0.27	0.15	-0.47	0.16	-0.64	0.36	-0.72	0.11		X		

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISB58)	Approach6 (ISB55)	ISB19	ISB63
A_33_P33769	<i>ORL14</i>	0.27	0.31	-0.39	0.59	-0.23	0.27	0.31	0.92	-0.65	0.45	-0.65	0.23		X		
A_23_P4174	<i>AMMP9</i>	1.20	0.37	0.79	0.96	1.20	0.14	1.25	0.64	1.15	0.00	1.46	0.00	X	X		
A_23_P122863	<i>GRB1</i>	0.64	0.17	0.54	0.17	0.91	0.89	0.82	0.24	0.93	0.00	1.73	0.00		X		
A_24_P373174	<i>RAB27A</i>	0.45	0.55	0.33	0.18	0.37	0.43	0.67	0.00	0.66	0.00	0.62	0.00		X		
A_33_P3271316	<i>RPP25</i>	0.52	0.14	-0.39	0.18	-0.25	0.35	-0.16	0.96	-0.97	0.36	-1.12	0.86		X		
A_24_P343233	<i>HILA-DRB1</i>	-0.15	0.33	-0.41	0.40	-0.59	0.82	-0.27	0.27	-0.74	0.00	-0.85	0.67		X		
A_23_P12418	<i>ITGAM</i>	0.52	0.13	0.38	0.28	0.47	0.59	0.74	0.11	0.60	0.00	0.72	0.00		X		
A_33_P3357651	<i>KRTAP1-12</i>	0.37	0.90	-0.24	0.29	-0.32	0.12	-0.58	0.23	-0.93	0.45	-0.86	0.27		X		
A_23_P142125	<i>HRC</i>	0.13	0.62	-0.39	0.15	-0.26	0.22	-0.18	0.47	-0.72	0.87	-0.75	0.23		X		
A_23_P11473	<i>NAP</i>	0.62	0.43	0.25	0.27	0.46	0.27	1.63	0.15	0.85	0.00	0.99	0.00		X		
A_23_P27315	<i>EMILIN2</i>	0.26	0.17	0.25	0.16	0.33	0.17	0.67	0.39	0.54	0.24	0.67	0.00		X		
A_33_P336624	<i>HCRT</i>	0.38	0.29	-0.27	0.40	-0.89	0.73	-0.94	0.79	-0.88	0.34	-0.95	0.36		X		
A_23_P7994	<i>LILRA3</i>	0.37	0.38	0.22	0.13	0.36	0.74	0.82	0.20	0.61	0.58	0.84	0.57		X		
A_32_P2835	<i>TDRD9</i>	0.94	0.19	0.88	0.75	1.14	0.00	1.19	0.13	1.28	0.00	1.43	0.00	X	X		
A_24_P13886	<i>IDH1</i>	0.73	0.15	0.52	0.34	0.62	0.40	0.82	0.15	0.74	0.00	0.70	0.00	X	X		
A_23_P37688	<i>LIMB1</i>	0.14	0.56	-0.28	0.11	-0.26	0.16	-0.32	0.18	-0.80	0.81	-0.73	0.54		X		
A_23_P1314	<i>MFNG</i>	-0.85	0.74	-0.52	0.57	-0.42	0.67	-0.33	0.24	-0.81	0.65	-0.94	0.12		X		X
A_23_P7733	<i>TADR2</i>	0.19	0.53	-0.23	0.29	-0.18	0.67	-0.39	0.16	-0.79	0.15	-0.75	0.28		X		
A_23_P12418	<i>ITGAM</i>	0.55	0.96	0.38	0.30	0.48	0.45	0.73	0.15	0.59	0.00	0.72	0.00		X		
A_24_P382319	<i>CEACAM1</i>	0.83	0.98	0.75	0.66	0.97	0.14	1.12	0.58	1.54	0.00	1.25	0.00	X	X		
A_23_P1926	<i>KCNK15</i>	0.23	0.39	-0.25	0.17	-0.25	0.28	-0.26	0.29	-0.69	0.24	-0.70	0.21		X		
A_23_P362759	<i>PRDM5</i>	0.40	0.23	0.36	0.49	0.57	0.00	0.55	0.19	0.60	0.14	0.82	0.00		X		
A_33_P349625	<i>SORBS3</i>	0.33	0.95	-0.41	0.87	-0.52	0.16	-0.59	0.42	-0.70	0.65	-0.96	0.15		X		
A_23_P47579	<i>NLRP14</i>	0.54	0.85	-0.32	0.24	-0.12	0.66	-0.49	0.85	-0.63	0.59	-0.67	0.39		X		

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISB58)	Approach6 (ISB55)	ISB19	ISB63
A_33_P3271651	HLA-DPB1	-0.24	0.16	-0.28	0.67	-0.53	0.12	-0.36	0.15	-0.61	0.25	-0.68	0.30		X		
A_23_P19482	DDA4H2	0.62	0.13	0.65	0.22	0.94	0.00	1.47	0.00	1.20	0.00	1.32	0.00	X	X		
A_24_P283788	MADPK14	0.62	0.45	0.47	0.14	0.46	0.79	0.96	0.00	0.95	0.32	0.95	0.49		X		
A_23_P16258	SLC25A47	0.42	0.95	-0.34	0.86	-0.24	0.89	-0.15	0.52	-0.64	0.63	-0.65	0.14		X		
A_23_P1456	SCGB3A2	0.16	0.58	-0.11	0.64	-0.69	0.75	-0.19	0.37	-0.63	0.17	-0.69	0.37		X		X
A_23_P117546	SOS2	0.32	0.62	0.24	0.77	0.41	0.59	0.53	0.69	0.58	0.00	0.69	0.21		X		
A_23_P11212	ACSL1	0.45	0.58	0.25	0.19	0.37	0.43	0.81	0.13	0.76	0.00	0.84	0.00		X		
A_23_P121716	ANXA3	0.77	0.39	0.54	0.75	0.63	0.23	1.17	0.17	1.37	0.00	1.16	0.00		X		
A_33_P3256848	ADAM12	0.22	0.37	-0.39	0.53	-0.23	0.33	-0.98	0.71	-0.75	0.37	-0.69	0.18		X		
A_33_P331533	KRT73	0.13	0.68	-0.34	0.15	-0.32	0.18	-0.37	0.34	-0.91	0.58	-0.97	0.38		X		
A_24_P11216	UPK3B	-0.32	0.93	-0.42	0.58	-0.34	0.20	-0.13	0.62	-0.66	0.29	-0.62	0.49		X		
A_21_P11611	DNAH17	-0.18	0.57	-0.49	0.82	-0.50	0.76	-0.65	0.24	-1.38	0.12	-0.95	0.38		X		
A_33_P327947	AGRP	0.17	0.54	-0.28	0.27	-0.68	0.78	0.73	0.98	-0.64	0.43	-0.65	0.19		X		
A_33_P3246613	CCDC78	-0.36	0.99	-0.12	0.96	-0.54	0.17	-0.66	0.20	-0.67	0.52	-0.98	0.52		X		
A_24_P148717	CERI	0.14	0.33	0.13	0.32	0.14	0.24	0.63	0.11	0.63	0.33	0.58	0.24		X		
A_32_P443594	AIM2	0.11	0.58	0.32	0.61	0.33	0.32	0.42	0.15	0.61	0.12	0.68	0.00		X		
A_33_P3214943	SPOCK2	0.25	0.94	-0.33	0.17	-0.41	0.49	-0.50	0.31	-0.95	0.00	-0.95	0.00		X		X
A_24_P88522	RPS14	-0.27	0.20	-0.19	0.57	-0.21	0.32	-0.55	0.00	-0.62	0.00	-0.65	0.55		X		
A_23_P18119	IMPG2	0.23	0.46	-0.28	0.17	-0.27	0.18	-0.23	0.37	-0.69	0.18	-0.79	0.18		X		
A_24_P97342	PROK2	0.41	0.14	0.20	0.46	0.27	0.18	0.70	0.42	0.77	0.19	0.66	0.11		X		
A_33_P3376449	ZDHHC23	-0.69	0.77	-0.38	0.62	-0.39	0.27	-0.47	0.32	-0.82	0.26	-0.81	0.00		X		X
A_23_P11473	NAIP	0.44	0.13	0.22	0.31	0.44	0.32	0.95	0.23	0.88	0.00	1.00	0.00		X		
A_21_P13998	NAIP	0.76	0.92	0.46	0.38	0.56	0.15	1.14	0.00	1.20	0.00	1.64	0.00		X		
A_33_P3383912	HLA-DRB3	-0.62	0.71	-0.30	0.32	-0.55	0.11	-0.18	0.22	-0.70	0.00	-0.68	0.00		X		

Probe ID	Gene Symbol	PreO P norm. Day-3 logFC	P Value	PreO P norm. Day-2 logFC	P Value	PreOP norm. Day-1 logFC	P Value	Day-3 logFC	P Value	Day-2 logFC	P Value	Day-1 logFC	P Value	Approach2 (ISB58)	Approach6 (ISB55)	ISB19	ISB63
A_23_P284	<i>C9orf25</i>	0.87	0.97	0.15	0.44	-0.23	0.24	-0.63	0.36	-0.37	0.50	-0.66	0.94		X		
A_33_P329881	<i>FFAR3</i>	0.52	0.13	0.57	0.58	0.74	0.58	0.63	0.27	0.68	0.34	0.84	0.00		X		X
A_23_P13687	<i>MAGEA6</i>	0.22	0.43	-0.32	0.11	-0.24	0.32	-0.16	0.50	-0.64	0.14	-0.64	0.85		X		
A_23_P4174	<i>MMP9</i>	0.99	0.44	0.75	0.13	1.16	0.19	1.19	0.75	1.12	0.23	1.45	0.00	X	X		

Optimization of the classification panels. We used a biological function-based process to reduce the number of features in the two top performing classification panels (Lee *et al.*, *Sci Rep.* 9:7365, 2019; incorporated herein by reference in its entirety). There were 89 and 326 biological processes associated with at least one of the genes in ISB19 and ISB63, respectively. To determine the biological processes with higher discriminatory power, 100,000 randomly selected 19-gene or 63-gene panels were generated from ISB58 or ISB355, respectively (FIG. 19A). Among the random 100,000 panels, the biological processes associated with the top 500 high performing and bottom 500 low performing panels (1% of the random 100,000 panels) based on the Discovery sample set were determined (FIG. 19B-19C). There were 19 and 46 biological functions from the top 500 performing panels that overlapped with the ISB19 and ISB63 panels, respectively (FIG. 19D-19E). The association of ISB19 and ISB63 genes from the bottom 500 panels with the 19 and 46 biological functions were lower compared to the top 500 panels (FIG. 19F-19G).

Redundant and mutually overlapping terms from the 19 and 46 core biological functions were clustered and summarized (FIG. 19H-19I). Three functional terms were associated with features (transcripts) in the ISB19 panel: immune response, metabolism, and signal transduction, whereas 6 terms were associated with the ISB63 panel: immune response, metabolism, signal transduction, apoptosis, transcription, and adhesion/migration (FIG. 19H-19I). The terms immune response, metabolism, and signal transduction were shared between the two panels. From the 58 DEGs from which ISB19 panel was derived, there were 13, 3, and 4 genes that are associated with immune response, signal transduction, and metabolism, respectively. Among the 355 DEGs from which ISB63 was derived, there were 67, 55, 41, 53, 7 and 40 genes associated with immune response, signal transduction, apoptosis, transcription, adhesion/migration, and metabolism, respectively.

Next, it was determined whether using a transcript representing each biological function could reduce the overall number of features while preserving the diagnostic performance of the panel(s). All 3-gene combinations that represent functional terms associated with ISB19 and 6-gene combinations representing ISB63 were generated by selecting one gene from each biological process, respectively. The performances of all possible 3-gene combinations ($156 = 13 \times 3 \times 4$, Table 22) were assessed using the Test sample set. However, it is prohibitory to do all 6-gene panels as there are more than 2 billion combinations ($2,242,101,400 = 67 \times 55 \times 41 \times 53 \times 7 \times 40$). To reduce the number of possible 6-gene combinations for the ISB63 derived panels, the genes that had better classification capability were first determined. The performances of randomly selected 100,000 6-gene combinations were assessed with the Test sample set and then sorted based on classification performance. For each biological process, the most frequently appearing genes among the top performing 1,000 6-gene panels (top 1% of the 100,000 combinations tested) were identified. This analysis yielded 16, 12, 8, 10, 2, and 10 genes for immune response, signal transduction, apoptosis, transcription, adhesion/migration and metabolism, respectively (Table 22). From these top performing genes, 307,200 ($16 \times 12 \times 8 \times 10 \times 2 \times 10$) 6-gene panels were generated and their performances were also assessed with the Test sample set. The performances of the

ISB19 and ISB63 panels were also determined with the Test sample set. Seventy-eight of the 3-gene panels and 32,540 of the 6-gene panels showed performances similar or better than the original ISB19 and ISB63 panels based on DeLong's test (p -value > 0.05). Therefore, these panels could be considered alternative optimized panels for the original ISB19 and ISB63.

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Table 22. Substitutable genes in each biological process

Functional term	ISB19	ISB63
Immune response	<i>IL1R2, IL18R1, FCAR, GZMA, PGLYRP1, GZMH, LCN2, BMX, VNN1, CLEC4D, S100A12, SLC2A3, HK3</i>	<i>BCL6, HLA-DRA, HLA-DMA, BPI, LCN2, MAVS, HLA-DPB1, GNG10, PDGFC, CD55, HLA-DPA1, TLR8, GZMA, BMX, HLA-DRB1, OR1J4</i>
Signal transduction	<i>IL18R1, GRB10, BMX</i>	<i>STOM, RRBP1, MAVS, MICAL1, LILRA2, SH3BP5, GNG10, WSB1, MAPK14, RUNX1, BMX, RAPH</i>
Metabolism	<i>PFKFB3, PFKFB2, HK3, SLC2A3</i>	<i>MPO, CYP27A1, MICAL1, CAP1, PDGFC, ALOX5, NQO2, CD163, CYP1B1, B3GNT5</i>
Apoptosis		<i>MPO, LTF, LCN2, HLA-DRA, MAVS, RPS6KA3, CFLAR, GCA</i>
Transcription		<i>BCL6, MLLT1, LTF, GAS7, MXD3, SPOCD1, ZNF446, MAVS, BASP1, RUNX1</i>
Adhesion/Migration		<i>TPM3, MAPK14</i>

Evaluation of the biomarker panels using the Validation cohort. The diagnostic performances of the ISB19 and ISB63 panels were further assessed with the Validation cohort.

- 10 Compared to their diagnostic performances in the Test cohort (FIG. 18), the panels performed similarly in the Validation cohort or produced even higher scores, except at Day-1 for both the ISB19 and ISB63 panels (FIG. 22A). Among the alternative panels, the top-performing 3-gene panel (LCN2, BMX, and SLC2A3) from ISB19 and 6-gene panel (GNG10, STOM, MPO, RPS6KA3, BCL3, and TPM3) from ISB63 were also assessed with the Validation cohort. The overall performances of the ISB3 and ISB6
- 15 panels were not significantly different from those of the original ISB19 and ISB63 panels across all time points (Day-3 to Day-1) in the Validation cohort (FIG. 22A) and ranged from AUC = 0.82 – 0.83 and AUC = 0.83 – 0.88 between Day-3 and Day-1, respectively. Importantly, the performances of both ISB3 and ISB6, and the original ISB19 and ISB63 were significantly better at all pre-diagnosis (Day-3, Day-2

and Day-1) time points than the SMS (AUC = 0.61 – 0.69) or SeptiCyte™ LAB (AUC = 0.63 – 0.73) panels (FIG. 22A).

Integration of top panel with clinical parameters does not significantly increase performance of the biomarker panels. A patient's clinical parameters such as the SOFA score and blood CRP level have been used to identify individuals having or suspected to have sepsis. Therefore, the diagnostic performances of SOFA and CRP were evaluated in the pre-symptomatic phase. In addition, the impact of integrating clinical information with the gene panels was evaluated to determine if this would increase the panels' performance. Neither the SOFA score nor the CRP level alone or combined performed well in diagnosing sepsis in the pre-symptomatic phase in the Validation cohort (FIG. 22B). Integration of either SOFA score, CRP level or both SOFA and CRP level with the gene panels did not significantly increase the performance of either the ISB3 (average AUC of 0.80 and 0.83 with and without SOFA and CRP, respectively) or ISB6 (average AUC of 0.84 and 0.85 with and without SOFA and CRP, respectively) (FIG. 22B).

The impact of disease severity on the performance of panels. To determine whether the panels' performances were affected by disease severity, patients who required vasopressors and met septic shock criteria were identified and labeled as "Septic Shock." All remaining sepsis patients were grouped as "Sepsis" (this includes sepsis and severe sepsis categories according to Sepsis-2 criteria). The classification performances of the smaller 3- and 6-gene panels were then assessed in these groups. The panels had higher overall performances in identifying "Septic Shock" patients (average AUC of 0.91 and 0.94 from ISB3 and ISB6, respectively) prior to the onset of clinical symptoms compared to "Sepsis" patients (average AUC of 0.80 and 0.84 from ISB3 and ISB6, respectively) (FIG. 22C) and were significantly higher on Day-3 (p-value < 0.05), suggesting the classifiers might be even more accurate in identifying patients at risk of developing a more severe condition.

Assessing the accuracy and specificity of detecting sepsis in the symptomatic phase. Although the biomarker panels identified were aimed at detecting sepsis at the pre-symptomatic stage, the ISB19 and ISB63 panels also showed good diagnostic performance for patients with clinical symptoms of sepsis in the Test sample set (FIG. 18). Therefore, the diagnosis performance was further assessed in Validation samples where the AUCs were higher than 0.9 at Day0 (FIG. 23A). Data from nine publicly available sepsis related studies were used to further assess the panels' ability to specifically diagnose sepsis during the symptomatic phase. These datasets were obtained with different measurement platforms on different cohorts with a total of 633 individuals, including 197 controls and 436 sepsis patients (FIG. 23B). Based on the patient characteristics, we split the data into three groups: 1) Adult sepsis/severe sepsis, 2) Pediatric sepsis, 3) Neonatal sepsis. For the 3-gene panel, the average AUC = 0.95 for all the samples, 0.95 for Adult sepsis, 0.98 for Pediatric sepsis and 0.92 for Neonatal sepsis. For the 6-gene panel, the average AUC = 0.81 for all the samples, 0.86 for Adult sepsis, 0.86 for Pediatric sepsis, and 0.70 for Neonatal sepsis (FIG. 23C). These results are comparable to SMS (AUC = 0.98) but significantly better than SeptiCyte™ LAB (average AUC = 0.67).

We also assessed the specificity of the panels for sepsis diagnosis using eleven different public domain datasets including four sets representing bacterial infections without sepsis, four sets representing viral infection, and three sets representing autoimmune diseases (FIG. 23D). The panels performed significantly less well in all non-sepsis datasets. Specifically, the 3-gene panel had an average AUC = 0.70 for all the samples and average AUC = 0.76 for bacterial infection without sepsis, 0.62 for viral infection and 0.72 for autoimmune diseases. The 6-gene panel had an overall average AUC of 0.62, and 0.63 for bacterial infection without sepsis, 0.56 for viral infection, and 0.69 for autoimmune diseases (FIG. 23E). Collectively, these results suggest that both ISB3 and ISB6 have a much higher ability to detect bacterial-associated sepsis compared to non-sepsis bacterial infection, viral or non-infection related immune disorders.

Validation of ISB3 and ISB6 using an alternative measurement platform. The performances of the 3-gene and 6-gene panels were further verified using qPCR (FIG. 24A). In the case of the 3-gene panel (ISB3), the qPCR measurements correlated well with the microarray measurements (FIG. 24B-24C). However, the qPCR measurements for GNG10 (immune response), STOM (signal transduction), and TPM3 (adhesion/migration) from the 6-gene panel (ISB6) correlated poorly with microarray measurements (FIG. 24D-24E). Since the panel optimization process also provides a list of substitutable genes (Table 22), we used this list to replace GNG10 with LCN2 (immune response gene), STOM with BMX (signal transduction), and TPM3 with MAPK14 (adhesion/migration), a process that resulted in improved correlation between the qPCR measurements and the microarray data.

Although the performances were slightly decreased compared to the microarray results (average ISB3 AUC = 0.83 and average ISB6 AUC = 0.85), the AUCs from qPCR results were still greater than 0.7 at all time points for both ISB3 (average AUC = 0.77) and ISB6 (average AUC = 0.74) (FIG. 24F). The panels also showed higher performance in patients with septic shock (average AUC of 0.86 and 0.81 for ISB3 and ISB6, respectively) compared to sepsis (average AUC of 0.72 and 0.71 for ISB3 and ISB6, respectively) (FIG. 24G).

In view of the many possible embodiments to which the principles of the disclosure may be applied, it should be recognized that the illustrated embodiments are only examples and should not be taken as limiting the scope of the invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

We claim:

1. A method, comprising measuring expression of a set of at least six genes in a sample from a subject having or suspected to have sepsis, wherein the set of at least six genes comprises or consists of:
 - 5 LCN2, BMX, MPO, RPS6KA3, BCL6, MAPK14; RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO; RPS6KA3, MAVS, TPM3, BCL6, STOM, MPO; RPS6KA3, BCL6, TPM3, BMX, STOM, MPO; RPS6KA3, LTF, TPM3, BCL6, STOM, PDGFC; RPS6KA3, BCL6, TPM3, CD55, STOM, MPO; RPS6KA3, BCL6, TPM3, GNG10, STOM, PDGFC; RPS6KA3, LTF, TPM3, BCL6, STOM, CYP1B1; LTF, BCL6, TPM3, CD55, STOM, PDGFC; RPS6KA3, BCL6, TPM3, TLR8, STOM, MPO; or
 - 10 RPS6KA3, BCL6, TPM3, HLA-DRA, GNG10, MPO;
 - RPS6KA3, MLLT1, TPM3, BCL6, GNG10, CYP27A1, YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1; RPS6KA3, GAS7, TPM3, BCL6, RRBP1, MPO, YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, LGALS2; RPS6KA3, GAS7, TPM3, BCL6, GNG10, MPO, YOD1; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1; RPS6KA3, BCL6, TPM3, GNG10,
 - 15 LILRA2, CYP27A1, YOD1; RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, YOD1; or RPS6KA3, BCL6, TPM3, CD55, STOM, MPO, IL17RA; or
 - RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, LILRA4; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC,
 - 20 YOD1, IL17RA; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, TCN1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE3; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, NOV; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE2; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, FAM105A; RPS6KA3, BCL6, TPM3, HLA-DRA, RRBP1, MPO, ERO1L, YOD1; or LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1,
 - 25 C14orf101;

wherein the expression of the set of genes is altered compared to a control.
2. A method of diagnosing and/or predicting sepsis in a subject comprising measuring expression of a set of at least six genes in a sample from a subject, wherein the set of at least six genes comprises or
 - 30 consists of:
 - LCN2, BMX, MPO, RPS6KA3, BCL6, MAPK14; RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO; RPS6KA3, MAVS, TPM3, BCL6, STOM, MPO; RPS6KA3, BCL6, TPM3, BMX, STOM, MPO; RPS6KA3, LTF, TPM3, BCL6, STOM, PDGFC; RPS6KA3, BCL6, TPM3, CD55, STOM, MPO; RPS6KA3, BCL6, TPM3, GNG10, STOM, PDGFC; RPS6KA3, LTF, TPM3, BCL6, STOM, CYP1B1;
 - 35 LTF, BCL6, TPM3, CD55, STOM, PDGFC; RPS6KA3, BCL6, TPM3, TLR8, STOM, MPO; or RPS6KA3, BCL6, TPM3, HLA-DRA, GNG10, MPO;

- RPS6KA3, MLLT1, TPM3, BCL6, GNG10, CYP27A1, YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1; RPS6KA3, GAS7, TPM3, BCL6, RRBP1, MPO, YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, LGALS2; RPS6KA3, GAS7, TPM3, BCL6, GNG10, MPO, YOD1; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1; RPS6KA3, BCL6, TPM3, GNG10, LILRA2, CYP27A1, YOD1; RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, YOD1; or RPS6KA3, BCL6, TPM3, CD55, STOM, MPO, IL17RA; or
- RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, LILRA4; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, IL17RA; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, TCN1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE3; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, NOV; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE2; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, FAM105A; RPS6KA3, BCL6, TPM3, HLA-DRA, RRBP1, MPO, ERO1L, YOD1; or LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, C14orf101; and

diagnosing the subject with sepsis and/or predicting development of sepsis in the subject when the expression of the set of genes is altered compared to a control.

3. A method, comprising measuring expression of a set of at least six genes in a sample from a subject having or suspected to have sepsis, wherein the set of at least six genes is selected from the set listed as ISB63 in Table 12, wherein the expression of the set of genes is altered compared to a control.
4. A method of diagnosing and/or predicting sepsis in a subject comprising:
 - measuring expression of a set of at least six genes in a sample from a subject, wherein the set of at least six genes is selected from the set listed as ISB63 in Table 12; and
 - diagnosing the subject with sepsis and/or predicting development of sepsis in the subject when the expression of the set of genes is altered compared to a control.
5. The method of any one of claims 1 to 4, wherein expression of one or more of LCN2, RPS6KA3, BCL6, MAPK14, TPM3, GNG10, STOM, MPO, BMX, LTF, PDGFC, CD55, CYP1B1, TLR8, MLLT1, YOD1, GAS7, RRBP1, LILRA2, IL17RA, LILRA4, TCN1, RNASE3, RNASE2, FAM105A, ERO1L, and/or C14orf101 is increased compared to the control and/or expression of one or more of MAVS, HLA-DRA, CYP27A1, LGALS2, and/or NOV is decreased compared to the control.
6. A method, comprising measuring expression of a set of at least three genes in a sample from a subject having or suspected to have sepsis, wherein the set of at least three genes comprises or consists of:

LCN2, SLC2A3, BMX; LCN2, SLC2A3, GRB10; LCN2, PFKFB3, GRB10; LCN2, PFKFB3, BMX; IL1R2, HK3, BMX; LCN2, HK3, BMX; LCN2, HK3, GRB10; GZMA, HK3, BMX; FCAR, PFKFB2, BMX; or LCN2, PFKFB3, IL18R1;

LCN2, PFKFB3, GRB10, ST6GALNAC3; LCN2, SLC2A3, BMX, LGALS2; IL1R2, SLC2A3, BMX, TCN1; LCN2, SLC2A3, GRB10, ST6GALNAC3; FCAR, PFKFB2, BMX, CEACAM1; IL1R2, HK3, BMX, CD24; IL1R2, PFKFB3, BMX, CD24; BMX, SLC2A3, GRB10, CD24; IL1R2, HK3, BMX, CEACAM1; or GZMA, SLC2A3, BMX, CD24; or

LCN2, PFKFB3, GRB10, ST6GALNAC3, RNASE3; LCN2, PFKFB3, GRB10, RNASE2, ST6GALNAC3; IL1R2, PFKFB3, GRB10, CD24, ST6GALNAC3; GZMA, PFKFB3, GRB10, ST6GALNAC3, CD24; HK3, PFKFB3, GRB10, ST6GALNAC3, CD24; IL1R2, PFKFB3, GRB10, ST6GALNAC3, TCN1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, TCN1; LCN2, PFKFB3, GRB10, ST6GALNAC3, DACH1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, CD24; or SLC2A3, HK3, BMX, SPOCD1, LGALS2;

wherein the expression of the set of genes is altered compared to a control.

15

7. A method of diagnosing and/or predicting sepsis in a subject comprising measuring expression of a set of at least three genes in a sample from a subject, wherein the set of at least three genes comprises or consists of:

LCN2, SLC2A3, BMX; LCN2, SLC2A3, GRB10; LCN2, PFKFB3, GRB10; LCN2, PFKFB3, BMX; IL1R2, HK3, BMX; LCN2, HK3, BMX; LCN2, HK3, GRB10; GZMA, HK3, BMX; FCAR, PFKFB2, BMX; or LCN2, PFKFB3, IL18R1;

LCN2, PFKFB3, GRB10, ST6GALNAC3; LCN2, SLC2A3, BMX, LGALS2; IL1R2, SLC2A3, BMX, TCN1; LCN2, SLC2A3, GRB10, ST6GALNAC3; FCAR, PFKFB2, BMX, CEACAM1; IL1R2, HK3, BMX, CD24; IL1R2, PFKFB3, BMX, CD24; BMX, SLC2A3, GRB10, CD24; IL1R2, HK3, BMX, CEACAM1; or GZMA, SLC2A3, BMX, CD24; or

LCN2, PFKFB3, GRB10, ST6GALNAC3, RNASE3; LCN2, PFKFB3, GRB10, RNASE2, ST6GALNAC3; IL1R2, PFKFB3, GRB10, CD24, ST6GALNAC3; GZMA, PFKFB3, GRB10, ST6GALNAC3, CD24; HK3, PFKFB3, GRB10, ST6GALNAC3, CD24; IL1R2, PFKFB3, GRB10, ST6GALNAC3, TCN1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, TCN1; LCN2, PFKFB3, GRB10, ST6GALNAC3, DACH1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, CD24; or SLC2A3, HK3, BMX, SPOCD1, LGALS2; and

diagnosing the subject with sepsis and/or predicting development of sepsis in the subject when the expression of the set of genes is altered compared to a control.

8. A method, comprising measuring expression of a set of at least three genes in a sample from a subject having or suspected to have sepsis, wherein the set of at least three genes is selected from the set listed as ISB19 in Table 12, wherein the expression of the set of genes is altered compared to a control.

- 9 A method of diagnosing and/or predicting sepsis in a subject comprising:
 measuring expression of a set of at least three genes in a sample from a subject, wherein the set
 of at least three genes is selected from the set listed as ISB19 in Table 12; and
 5 diagnosing the subject with sepsis and/or predicting development of sepsis in the subject when
 the expression of the set of genes is altered compared to a control.
10. The method of any one of claims 6 to 9, wherein expression of one or more of LCN2, SLC2A3,
 BMX, GRB10, PFKFB3, IL1R2, HK3, FCAR, PFKFB2, IL18R1, ST6GALNAC3, TCN1, CEACAM1,
 10 CD24, RNASE3, RNASE2, DACH1 and/or SPOCD1 is increased compared to the control and/or
 expression of GZMA and/or LGALS2 is decreased compared to the control.
11. The method of any one of claims 1 to 10, further comprising determining a Sequential Organ
 15 Failure Assessment (SOFA) score for the subject and/or measuring C-reactive protein (CRP) level in a
 sample from the subject.
12. A method of treating sepsis comprising,
 i) measuring expression of a set of three or more genes in a sample from a subject, wherein the
 20 three or more genes comprise or consist of:
 LCN2, SLC2A3, BMX; LCN2, SLC2A3, GRB10; LCN2, PFKFB3, GRB10; LCN2, PFKFB3,
 BMX; IL1R2, HK3, BMX; LCN2, HK3, BMX; LCN2, HK3, GRB10; GZMA, HK3, BMX; FCAR,
 PFKFB2, BMX; or LCN2, PFKFB3, IL18R1;
 LCN2, PFKFB3, GRB10, ST6GALNAC3; LCN2, SLC2A3, BMX, LGALS2; IL1R2, SLC2A3,
 25 BMX, TCN1; LCN2, SLC2A3, GRB10, ST6GALNAC3; FCAR, PFKFB2, BMX, CEACAM1; IL1R2,
 HK3, BMX, CD24; IL1R2, PFKFB3, BMX, CD24; BMX, SLC2A3, GRB10, CD24; IL1R2, HK3,
 BMX, CEACAM1; or GZMA, SLC2A3, BMX, CD24; or
 LCN2, PFKFB3, GRB10, ST6GALNAC3, RNASE3; LCN2, PFKFB3, GRB10, RNASE2,
 ST6GALNAC3; IL1R2, PFKFB3, GRB10, CD24, ST6GALNAC3; GZMA, PFKFB3, GRB10,
 30 ST6GALNAC3, CD24; HK3, PFKFB3, GRB10, ST6GALNAC3, CD24; IL1R2, PFKFB3, GRB10,
 ST6GALNAC3, TCN1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, TCN1; LCN2, PFKFB3, GRB10,
 ST6GALNAC3, DACH1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, CD24; or SLC2A3, HK3, BMX,
 SPOCD1, LGALS2,
 wherein expression of one or more of LCN2, SLC2A3, BMX, GRB10, PFKFB3, IL1R2, HK3,
 35 FCAR, PFKFB2, IL18R1, ST6GALNAC3, TCN1, CEACAM1, CD24, RNASE3, RNASE2, DACH1
 and/or SPOCD1 is increased compared to the control and/or expression of GZMA and/or LGALS2 is
 decreased compared to the control; and

ii) administering one or more treatments for sepsis to the subject.

13. A method of treating sepsis comprising,

i) measuring expression of a set of six or more genes in a sample from a subject, wherein the six
5 or more genes comprise or consist of:

LCN2, BMX, MPO, RPS6KA3, BCL6, MAPK14; RPS6KA3, BCL6, TPM3, GNG10, STOM,
MPO; RPS6KA3, MAVS, TPM3, BCL6, STOM, MPO; RPS6KA3, BCL6, TPM3, BMX, STOM, MPO;
RPS6KA3, LTF, TPM3, BCL6, STOM, PDGFC; RPS6KA3, BCL6, TPM3, CD55, STOM, MPO;
RPS6KA3, BCL6, TPM3, GNG10, STOM, PDGFC; RPS6KA3, LTF, TPM3, BCL6, STOM, CYP1B1;
10 LTF, BCL6, TPM3, CD55, STOM, PDGFC; RPS6KA3, BCL6, TPM3, TLR8, STOM, MPO; or
RPS6KA3, BCL6, TPM3, HLA-DRA, GNG10, MPO;

RPS6KA3, MLLT1, TPM3, BCL6, GNG10, CYP27A1, YOD1; RPS6KA3, MLLT1, TPM3,
BCL6, GNG10, PDGFC, YOD1; RPS6KA3, GAS7, TPM3, BCL6, RRB1, MPO, YOD1; RPS6KA3,
MLLT1, TPM3, BCL6, GNG10, MPO, LGALS2; RPS6KA3, GAS7, TPM3, BCL6, GNG10, MPO,
15 YOD1; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1; RPS6KA3, BCL6, TPM3, GNG10,
LILRA2, CYP27A1, YOD1; RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, LGALS2; RPS6KA3,
MLLT1, TPM3, BCL6, GNG10, MPO, YOD1; or RPS6KA3, BCL6, TPM3, CD55, STOM, MPO,
IL17RA; or

RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, LGALS2; RPS6KA3, MLLT1,
20 TPM3, BCL6, GNG10, PDGFC, YOD1, LILRA4; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC,
YOD1, IL17RA; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, TCN1; RPS6KA3,
MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE3; RPS6KA3, MLLT1, TPM3, BCL6,
GNG10, PDGFC, YOD1, NOV; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE2;
LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, FAM105A; RPS6KA3, BCL6, TPM3, HLA-
25 DRA, RRB1, MPO, ERO1L, YOD1; or LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1,
C14orf101,

wherein expression of one or more of RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, BMX,
LTF, PDGFC, CD55, CYP1B1, TLR8, MLLT1, YOD1, GAS7, RRB1, LILRA2, IL17RA, LILRA4,
TCN1, RNASE3, RNASE2, FAM105A, ERO1L, and/or C14orf101 is increased compared to the control
30 and/or expression of one or more of MAVS, HLA-DRA, CYP27A1, LGALS2, and/or NOV is decreased
compared to the control; and

ii) administering one or more treatments for sepsis to the subject.

14. The method of claim 12 or claim 13, wherein the treatment for sepsis comprises one or more of
35 antibiotic treatment, vasopressors, intravenous fluids, oxygen, dialysis, and monitoring for sepsis.

15. The method of any one of claims 1 to 14, wherein the subject does not exhibit symptoms of sepsis.
16. A method, comprising measuring expression of a set of at least six genes in a sample from a
 5 subject having or suspected to have sepsis, wherein the set of at least six genes comprises or consists of:
 CCR1, HLA-DPB1, BATF, C3AR1, ARHGEF18, and C9orf95; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf103; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CCR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; CD177, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; CD63, HLA-DPB1,
 10 PLAC8, GNA15, ARHGEF18, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, C3AR1, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and
 15 SEPHS2; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf103; FES, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FES, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; or C3AR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95;
 20 wherein the expression of CCR1, C3AR1, C9orf95, BATF, C9orf103, GNA15, CD177, CD63, EMR1, FCER1G, FES, PLAC8, and/or SEPHS2 is increased compared to a control and the expression of HLA-DPB1, ARHGEF18, and/or MTCH1 is decreased compared to a control.
17. A method of diagnosing sepsis in a subject comprising measuring expression of a set of at least
 25 six genes in a sample from a subject, wherein the set of at least six genes comprises or consists of:
 CCR1, HLA-DPB1, BATF, C3AR1, ARHGEF18, and C9orf95; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf103; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CCR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; CD177, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; CD63, HLA-DPB1,
 30 PLAC8, GNA15, ARHGEF18, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, C3AR1, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and
 35 SEPHS2; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf103; FES,

HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FES, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; or C3AR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; and

diagnosing the subject with sepsis when the expression of CCR1, C3AR1, C9orf95, BATF, C9orf103, GNA15, CD177, CD63, EMR1, FCER1G, FES, PLAC8, and/or SEPHS2 is increased compared to a control and the expression of HLA-DPB1, ARHGEF18, and/or MTCH1 is decreased compared to a control.

18. A method, comprising measuring expression of a set of at least six genes in a sample from a subject having or suspected to have sepsis, wherein the set of at least six genes comprises or consists of a set of six genes listed in Table 5, wherein the expression of the set of genes is altered compared to a control.

19. A method of diagnosing sepsis in a subject comprising:
measuring expression of a set of at least six genes in a sample from a subject, wherein the set of at least six genes comprises or consists of a set of six genes listed in Table 5; and
diagnosing the subject with sepsis when the expression of the set of genes is altered compared to a control.

20. The method of any one of claims 16 to 19, further comprising measuring expression of RPGRIP1, wherein the expression of RPGRIP1 is decreased compared to a control.

21. A method of treating sepsis comprising,
i) measuring expression of a set of six or more genes in a sample from a subject, wherein the six or more genes comprise or consist of:

CCR1, HLA-DPB1, BATF, C3AR1, ARHGEF18, and C9orf95; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf103; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CCR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; CD177, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, C3AR1, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and SEPHS2; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf103; FES,

- HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FES, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; or C3AR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95,
 wherein the expression of CCR1, C3AR1, C9orf95, BATF, C9orf103, GNA15, CD177, CD63, EMR1, FCER1G, FES, PLAC8, and/or SEPHS2 is increased compared to a control and the expression of
 5 HLA-DPB1, ARHGEF18, and/or MTCH1 is decreased compared to a control; and
 ii) administering one or more treatments for sepsis to the subject.
22. The method of claim 21, wherein the treatment for sepsis comprises one or more of antibiotic treatment, vasopressors, intravenous fluids, oxygen, and dialysis.
- 10 23. The method of any one of claims 1 to 22, wherein the sample is whole blood, peripheral blood mononuclear cells, serum, or plasma.
24. The method of any one of claims 1 to 23, wherein measuring expression comprises measuring
 15 mRNA expression, protein expression, or both.
25. The method of any one of claims 1 to 24, further comprising measuring expression of at least one housekeeping or internal control molecule.
- 20 26. The method of any one of claims 1 to 25, wherein the control is a reference value or a sample from a healthy subject.
27. The method of any one of claims 1 to 26, wherein measuring expression comprises real-time PCR, quantitative real-time reverse transcriptase PCR, reverse transcriptase PCR, and/or microarray
 25 analysis.
28. A solid support comprising binding agents specific for a set of genes selected from:
 CCR1, HLA-DPB1, BATF, C3AR1, ARHGEF18, and C9orf 95; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf 103; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CCR1,
 30 HLA-DPB1, BATF, GNA15, MTCH1, and C9orf 95; CD177, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; EMR1,
 35 HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, C3AR1, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and

SEPHS2; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf103; FES, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FES, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; or C3AR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95,

5 wherein the specific binding agents are covalently attached to the solid substrate.

29. A solid support comprising binding agents specific for a set of genes selected from:

LCN2, SLC2A3, BMX; LCN2, SLC2A3, GRB10; LCN2, PFKFB3, GRB10; LCN2, PFKFB3, BMX; IL1R2, HK3, BMX; LCN2, HK3, BMX; LCN2, HK3, GRB10; GZMA, HK3, BMX; FCAR, PFKFB2, BMX; or LCN2, PFKFB3, IL18R1;
 LCN2, PFKFB3, GRB10, ST6GALNAC3; LCN2, SLC2A3, BMX, LGALS2; IL1R2, SLC2A3, BMX, TCN1; LCN2, SLC2A3, GRB10, ST6GALNAC3; FCAR, PFKFB2, BMX, CEACAM1; IL1R2, HK3, BMX, CD24; IL1R2, PFKFB3, BMX, CD24; BMX, SLC2A3, GRB10, CD24; IL1R2, HK3, BMX, CEACAM1; or GZMA, SLC2A3, BMX, CD24; or
 15 LCN2, PFKFB3, GRB10, ST6GALNAC3, RNASE3; LCN2, PFKFB3, GRB10, RNASE2, ST6GALNAC3; IL1R2, PFKFB3, GRB10, CD24, ST6GALNAC3; GZMA, PFKFB3, GRB10, ST6GALNAC3, CD24; HK3, PFKFB3, GRB10, ST6GALNAC3, CD24; IL1R2, PFKFB3, GRB10, ST6GALNAC3, TCN1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, TCN1; LCN2, PFKFB3, GRB10, ST6GALNAC3, DACH1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, CD24; or SLC2A3, HK3, BMX,
 20 SPOCD1, LGALS2,

wherein the specific binding agents are covalently attached to the solid substrate.

30. A solid support comprising binding agents specific for a set of genes selected from:

RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO; RPS6KA3, MAVS, TPM3, BCL6, STOM, MPO; RPS6KA3, BCL6, TPM3, BMX, STOM, MPO; RPS6KA3, LTF, TPM3, BCL6, STOM, PDGFC; RPS6KA3, BCL6, TPM3, CD55, STOM, MPO; RPS6KA3, BCL6, TPM3, GNG10, STOM, PDGFC; RPS6KA3, LTF, TPM3, BCL6, STOM, CYP1B1; LTF, BCL6, TPM3, CD55, STOM, PDGFC; RPS6KA3, BCL6, TPM3, TLR8, STOM, MPO; RPS6KA3, BCL6, TPM3, HLA-DRA, GNG10, MPO; or LCN2, BMX, MPO, RPS6KA3, BCL6, MAPK14;
 30 RPS6KA3, MLLT1, TPM3, BCL6, GNG10, CYP27A1, YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1; RPS6KA3, GAS7, TPM3, BCL6, RRBP1, MPO, YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, LGALS2; RPS6KA3, GAS7, TPM3, BCL6, GNG10, MPO, YOD1; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1; RPS6KA3, BCL6, TPM3, GNG10, LILRA2, CYP27A1, YOD1; RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, LGALS2; RPS6KA3,
 35 MLLT1, TPM3, BCL6, GNG10, MPO, YOD1; or RPS6KA3, BCL6, TPM3, CD55, STOM, MPO, IL17RA; or

RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, LILRA4; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, IL17RA; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, TCN1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE3; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, NOV; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE2; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, FAM105A; RPS6KA3, BCL6, TPM3, HLA-DRA, RRBPI, MPO, ERO1L, YOD1; or LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, C14orf101,

wherein the specific binding agents are covalently attached to the solid substrate.

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31. The solid support of any one of claims 28 to 30, further comprising one or more binding agents specific for one or more housekeeping or internal control genes.

32. The solid support of any one of claims 28 to 31, wherein the solid support is an array.

15

33. A kit comprising a set of primers and/or probes capable of specifically binding to a set of genes selected from:

CCR1, HLA-DPB1, BATF, C3AR1, ARHGEF18, and C9orf95; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf103; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CCR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; CD177, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, C3AR1, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and SEPHS2; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf103; FES, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FES, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; or C3AR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95.

34. A kit comprising a set of primers and/or probes capable of specifically binding to a set of genes selected from:

LCN2, SLC2A3, BMX; LCN2, SLC2A3, GRB10; LCN2, PFKFB3, GRB10; LCN2, PFKFB3, BMX; IL1R2, HK3, BMX; LCN2, HK3, BMX; LCN2, HK3, GRB10; GZMA, HK3, BMX; FCAR, PFKFB2, BMX; or LCN2, PFKFB3, IL18R1;

- LCN2, PFKFB3, GRB10, ST6GALNAC3; LCN2, SLC2A3, BMX, LGALS2; IL1R2, SLC2A3, BMX, TCN1; LCN2, SLC2A3, GRB10, ST6GALNAC3; FCAR, PFKFB2, BMX, CEACAM1; IL1R2, HK3, BMX, CD24; IL1R2, PFKFB3, BMX, CD24; BMX, SLC2A3, GRB10, CD24; IL1R2, HK3, BMX, CEACAM1; or GZMA, SLC2A3, BMX, CD24; or
- 5 LCN2, PFKFB3, GRB10, ST6GALNAC3, RNASE3; LCN2, PFKFB3, GRB10, RNASE2, ST6GALNAC3; IL1R2, PFKFB3, GRB10, CD24, ST6GALNAC3; GZMA, PFKFB3, GRB10, ST6GALNAC3, CD24; HK3, PFKFB3, GRB10, ST6GALNAC3, CD24; IL1R2, PFKFB3, GRB10, ST6GALNAC3, TCN1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, TCN1; LCN2, PFKFB3, GRB10, ST6GALNAC3, DACH1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, CD24; or SLC2A3, HK3, BMX,
- 10 SPOCD1, LGALS2.
35. A kit comprising a set of primers and/or probes capable of specifically binding to a set of genes selected from:
- RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO; RPS6KA3, MAVS, TPM3, BCL6, STOM,
- 15 MPO; RPS6KA3, BCL6, TPM3, BMX, STOM, MPO; RPS6KA3, LTF, TPM3, BCL6, STOM, PDGFC; RPS6KA3, BCL6, TPM3, CD55, STOM, MPO; RPS6KA3, BCL6, TPM3, GNG10, STOM, PDGFC; RPS6KA3, LTF, TPM3, BCL6, STOM, CYP1B1; LTF, BCL6, TPM3, CD55, STOM, PDGFC; RPS6KA3, BCL6, TPM3, TLR8, STOM, MPO; RPS6KA3, BCL6, TPM3, HLA-DRA, GNG10, MPO; or LCN2, BMX, MPO, RPS6KA3, BCL6, MAPK14;
- 20 RPS6KA3, MLLT1, TPM3, BCL6, GNG10, CYP27A1, YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1; RPS6KA3, GAS7, TPM3, BCL6, RRBPI, MPO, YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, LGALS2; RPS6KA3, GAS7, TPM3, BCL6, GNG10, MPO, YOD1; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1; RPS6KA3, BCL6, TPM3, GNG10, LILRA2, CYP27A1, YOD1; RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, LGALS2; RPS6KA3,
- 25 MLLT1, TPM3, BCL6, GNG10, MPO, YOD1; or RPS6KA3, BCL6, TPM3, CD55, STOM, MPO, IL17RA; or
- RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, LILRA4; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, IL17RA; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, TCN1; RPS6KA3,
- 30 MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE3; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, NOV; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE2; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, FAM105A; RPS6KA3, BCL6, TPM3, HLA-DRA, RRBPI, MPO, ERO1L, YOD1; or LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, C14orf101.
- 35
36. The kit of any one of claims 33 to 35, further comprising one or more primers and/or probes specific for one or more housekeeping or internal control genes.

37. The kit of any one of claims 33 to 36, further comprising one or more buffers, enzymes, and/or detection reagents.

AMENDED CLAIMS

received by the International Bureau on 28 April 2020 (28.04.2020)

1. A method, comprising measuring expression of a set of at least six genes in a sample from a subject having or suspected to have sepsis, wherein the set of at least six genes comprises or consists of:

5 LCN2, BMX, MPO, RPS6KA3, BCL6, MAPK14; RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO; RPS6KA3, MAVS, TPM3, BCL6, STOM, MPO; RPS6KA3, BCL6, TPM3, BMX, STOM, MPO; RPS6KA3, LTF, TPM3, BCL6, STOM, PDGFC; RPS6KA3, BCL6, TPM3, CD55, STOM, MPO; RPS6KA3, BCL6, TPM3, GNG10, STOM, PDGFC; RPS6KA3, LTF, TPM3, BCL6, STOM, CYP1B1; LTF, BCL6, TPM3, CD55, STOM, PDGFC; RPS6KA3, BCL6, TPM3, TLR8, STOM, MPO; or
10 RPS6KA3, BCL6, TPM3, HLA-DRA, GNG10, MPO;

RPS6KA3, MLLT1, TPM3, BCL6, GNG10, CYP27A1, YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1; RPS6KA3, GAS7, TPM3, BCL6, RRBPI, MPO, YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, LGALS2; RPS6KA3, GAS7, TPM3, BCL6, GNG10, MPO, YOD1; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1; RPS6KA3, BCL6, TPM3, GNG10,
15 LILRA2, CYP27A1, YOD1; RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, YOD1; or RPS6KA3, BCL6, TPM3, CD55, STOM, MPO, IL17RA; or

RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, LILRA4; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC,
20 YOD1, IL17RA; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, TCN1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE3; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, NOV; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE2; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, FAM105A; RPS6KA3, BCL6, TPM3, HLA-DRA, RRBPI, MPO, ERO1L, YOD1; or LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1,
25 C14orf101;

wherein the expression of the set of genes is altered compared to a control.

2. A method of diagnosing and/or predicting sepsis in a subject comprising measuring expression of a set of at least six genes in a sample from a subject, wherein the set of at least six genes comprises or
30 consists of:

LCN2, BMX, MPO, RPS6KA3, BCL6, MAPK14; RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO; RPS6KA3, MAVS, TPM3, BCL6, STOM, MPO; RPS6KA3, BCL6, TPM3, BMX, STOM, MPO; RPS6KA3, LTF, TPM3, BCL6, STOM, PDGFC; RPS6KA3, BCL6, TPM3, CD55, STOM, MPO; RPS6KA3, BCL6, TPM3, GNG10, STOM, PDGFC; RPS6KA3, LTF, TPM3, BCL6, STOM, CYP1B1;
35 LTF, BCL6, TPM3, CD55, STOM, PDGFC; RPS6KA3, BCL6, TPM3, TLR8, STOM, MPO; or RPS6KA3, BCL6, TPM3, HLA-DRA, GNG10, MPO;

RPS6KA3, MLLT1, TPM3, BCL6, GNG10, CYP27A1, YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1; RPS6KA3, GAS7, TPM3, BCL6, RRBPI, MPO, YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, LGALS2; RPS6KA3, GAS7, TPM3, BCL6, GNG10, MPO, YOD1; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1; RPS6KA3, BCL6, TPM3, GNG10, LILRA2, CYP27A1, YOD1; RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, YOD1; or RPS6KA3, BCL6, TPM3, CD55, STOM, MPO, IL17RA; or

RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, LILRA4; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, IL17RA; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, TCN1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE3; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, NOV; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE2; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, FAM105A; RPS6KA3, BCL6, TPM3, HLA-DRA, RRBPI, MPO, ERO1L, YOD1; or LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, C14orf101; and

diagnosing the subject with sepsis and/or predicting development of sepsis in the subject when the expression of the set of genes is altered compared to a control.

3. A method, comprising measuring expression of a set of at least six genes in a sample from a subject having or suspected to have sepsis, wherein the set of at least six genes is selected from the set listed as ISB63 in Table 12, wherein the expression of the set of genes is altered compared to a control.

4. A method of diagnosing and/or predicting sepsis in a subject comprising:
measuring expression of a set of at least six genes in a sample from a subject, wherein the set of at least six genes is selected from the set listed as ISB63 in Table 12; and

diagnosing the subject with sepsis and/or predicting development of sepsis in the subject when the expression of the set of genes is altered compared to a control.

5. The method of any one of claims 1 to 4, wherein expression of one or more of LCN2, RPS6KA3, BCL6, MAPK14, TPM3, GNG10, STOM, MPO, BMX, LTF, PDGFC, CD55, CYP1B1, TLR8, MLLT1, YOD1, GAS7, RRBPI, LILRA2, IL17RA, LILRA4, TCN1, RNASE3, RNASE2, FAM105A, ERO1L, and/or C14orf101 is increased compared to the control and/or expression of one or more of MAVS, HLA-DRA, CYP27A1, LGALS2, and/or NOV is decreased compared to the control.

6. A method, comprising measuring expression of a set of at least three genes in a sample from a subject having or suspected to have sepsis, wherein the set of at least three genes comprises or consists of:

LCN2, SLC2A3, BMX; LCN2, SLC2A3, GRB10; LCN2, PFKFB3, GRB10; LCN2, PFKFB3, BMX; IL1R2, HK3, BMX; LCN2, HK3, BMX; LCN2, HK3, GRB10; GZMA, HK3, BMX; FCAR, PFKFB2, BMX; or LCN2, PFKFB3, IL18R1;

LCN2, PFKFB3, GRB10, ST6GALNAC3; LCN2, SLC2A3, BMX, LGALS2; IL1R2, SLC2A3, BMX, TCN1; LCN2, SLC2A3, GRB10, ST6GALNAC3; FCAR, PFKFB2, BMX, CEACAM1; IL1R2, HK3, BMX, CD24; IL1R2, PFKFB3, BMX, CD24; BMX, SLC2A3, GRB10, CD24; IL1R2, HK3, BMX, CEACAM1; or GZMA, SLC2A3, BMX, CD24; or

LCN2, PFKFB3, GRB10, ST6GALNAC3, RNASE3; LCN2, PFKFB3, GRB10, RNASE2, ST6GALNAC3; IL1R2, PFKFB3, GRB10, CD24, ST6GALNAC3; GZMA, PFKFB3, GRB10, ST6GALNAC3, CD24; HK3, PFKFB3, GRB10, ST6GALNAC3, CD24; IL1R2, PFKFB3, GRB10, ST6GALNAC3, TCN1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, TCN1; LCN2, PFKFB3, GRB10, ST6GALNAC3, DACH1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, CD24; or SLC2A3, HK3, BMX, SPOCD1, LGALS2;

wherein the expression of the set of genes is altered compared to a control.

7. A method of diagnosing and/or predicting sepsis in a subject comprising measuring expression of a set of at least three genes in a sample from a subject, wherein the set of at least three genes comprises or consists of:

LCN2, SLC2A3, BMX; LCN2, SLC2A3, GRB10; LCN2, PFKFB3, GRB10; LCN2, PFKFB3, BMX; IL1R2, HK3, BMX; LCN2, HK3, BMX; LCN2, HK3, GRB10; GZMA, HK3, BMX; FCAR, PFKFB2, BMX; or LCN2, PFKFB3, IL18R1;

LCN2, PFKFB3, GRB10, ST6GALNAC3; LCN2, SLC2A3, BMX, LGALS2; IL1R2, SLC2A3, BMX, TCN1; LCN2, SLC2A3, GRB10, ST6GALNAC3; FCAR, PFKFB2, BMX, CEACAM1; IL1R2, HK3, BMX, CD24; IL1R2, PFKFB3, BMX, CD24; BMX, SLC2A3, GRB10, CD24; IL1R2, HK3, BMX, CEACAM1; or GZMA, SLC2A3, BMX, CD24; or

LCN2, PFKFB3, GRB10, ST6GALNAC3, RNASE3; LCN2, PFKFB3, GRB10, RNASE2, ST6GALNAC3; IL1R2, PFKFB3, GRB10, CD24, ST6GALNAC3; GZMA, PFKFB3, GRB10, ST6GALNAC3, CD24; HK3, PFKFB3, GRB10, ST6GALNAC3, CD24; IL1R2, PFKFB3, GRB10, ST6GALNAC3, TCN1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, TCN1; LCN2, PFKFB3, GRB10, ST6GALNAC3, DACH1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, CD24; or SLC2A3, HK3, BMX, SPOCD1, LGALS2; and

diagnosing the subject with sepsis and/or predicting development of sepsis in the subject when the expression of the set of genes is altered compared to a control.

8. A method, comprising measuring expression of a set of at least three genes in a sample from a subject having or suspected to have sepsis, wherein the set of at least three genes is selected from the set listed as ISB19 in Table 12, wherein the expression of the set of genes is altered compared to a control.

9 A method of diagnosing and/or predicting sepsis in a subject comprising:
measuring expression of a set of at least three genes in a sample from a subject, wherein the set
of at least three genes is selected from the set listed as ISB19 in Table 12; and

5 diagnosing the subject with sepsis and/or predicting development of sepsis in the subject when
the expression of the set of genes is altered compared to a control.

10. The method of any one of claims 6 to 9, wherein expression of one or more of LCN2, SLC2A3,
BMX, GRB10, PFKFB3, IL1R2, HK3, FCAR, PFKFB2, IL18R1, ST6GALNAC3, TCN1, CEACAM1,
10 CD24, RNASE3, RNASE2, DACH1 and/or SPOCD1 is increased compared to the control and/or
expression of GZMA and/or LGALS2 is decreased compared to the control.

11. The method of any one of claims 1 to 10, further comprising determining a Sequential Organ
15 Failure Assessment (SOFA) score for the subject and/or measuring C-reactive protein (CRP) level in a
sample from the subject.

12. A method of treating sepsis comprising,

i) measuring expression of a set of three or more genes in a sample from a subject, wherein the
20 three or more genes comprise or consist of:

LCN2, SLC2A3, BMX; LCN2, SLC2A3, GRB10; LCN2, PFKFB3, GRB10; LCN2, PFKFB3,
BMX; IL1R2, HK3, BMX; LCN2, HK3, BMX; LCN2, HK3, GRB10; GZMA, HK3, BMX; FCAR,
PFKFB2, BMX; or LCN2, PFKFB3, IL18R1;

LCN2, PFKFB3, GRB10, ST6GALNAC3; LCN2, SLC2A3, BMX, LGALS2; IL1R2, SLC2A3,
25 BMX, TCN1; LCN2, SLC2A3, GRB10, ST6GALNAC3; FCAR, PFKFB2, BMX, CEACAM1; IL1R2,
HK3, BMX, CD24; IL1R2, PFKFB3, BMX, CD24; BMX, SLC2A3, GRB10, CD24; IL1R2, HK3,
BMX, CEACAM1; or GZMA, SLC2A3, BMX, CD24; or

LCN2, PFKFB3, GRB10, ST6GALNAC3, RNASE3; LCN2, PFKFB3, GRB10, RNASE2,
ST6GALNAC3; IL1R2, PFKFB3, GRB10, CD24, ST6GALNAC3; GZMA, PFKFB3, GRB10,
30 ST6GALNAC3, CD24; HK3, PFKFB3, GRB10, ST6GALNAC3, CD24; IL1R2, PFKFB3, GRB10,
ST6GALNAC3, TCN1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, TCN1; LCN2, PFKFB3, GRB10,
ST6GALNAC3, DACH1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, CD24; or SLC2A3, HK3, BMX,
SPOCD1, LGALS2,

wherein expression of one or more of LCN2, SLC2A3, BMX, GRB10, PFKFB3, IL1R2, HK3,
35 FCAR, PFKFB2, IL18R1, ST6GALNAC3, TCN1, CEACAM1, CD24, RNASE3, RNASE2, DACH1
and/or SPOCD1 is increased compared to the control and/or expression of GZMA and/or LGALS2 is
decreased compared to the control; and

ii) administering one or more treatments for sepsis to the subject.

13. A method of treating sepsis comprising,

i) measuring expression of a set of six or more genes in a sample from a subject. wherein the six
5 or more genes comprise or consist of:

LCN2, BMX, MPO, RPS6KA3, BCL6, MAPK14; RPS6KA3, BCL6, TPM3, GNG10, STOM,
MPO; RPS6KA3, MAVS, TPM3, BCL6, STOM, MPO; RPS6KA3, BCL6, TPM3, BMX, STOM, MPO;
RPS6KA3, LTF, TPM3, BCL6, STOM, PDGFC; RPS6KA3, BCL6, TPM3, CD55, STOM, MPO;
RPS6KA3, BCL6, TPM3, GNG10, STOM, PDGFC; RPS6KA3, LTF, TPM3, BCL6, STOM, CYP1B1;
10 LTF, BCL6, TPM3, CD55, STOM, PDGFC; RPS6KA3, BCL6, TPM3, TLR8, STOM, MPO; or
RPS6KA3, BCL6, TPM3, HLA-DRA, GNG10, MPO;

RPS6KA3, MLLT1, TPM3, BCL6, GNG10, CYP27A1, YOD1; RPS6KA3, MLLT1, TPM3,
BCL6, GNG10, PDGFC, YOD1; RPS6KA3, GAS7, TPM3, BCL6, RRBPI, MPO, YOD1; RPS6KA3,
MLLT1, TPM3, BCL6, GNG10, MPO, LGALS2; RPS6KA3, GAS7, TPM3, BCL6, GNG10, MPO,
15 YOD1; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1; RPS6KA3, BCL6, TPM3, GNG10,
LILRA2, CYP27A1, YOD1; RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, LGALS2; RPS6KA3,
MLLT1, TPM3, BCL6, GNG10, MPO, YOD1; or RPS6KA3, BCL6, TPM3, CD55, STOM, MPO,
IL17RA; or

RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, LGALS2; RPS6KA3, MLLT1,
20 TPM3, BCL6, GNG10, PDGFC, YOD1, LILRA4; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC,
YOD1, IL17RA; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, TCN1; RPS6KA3,
MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE3; RPS6KA3, MLLT1, TPM3, BCL6,
GNG10, PDGFC, YOD1, NOV; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE2;
LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, FAM105A; RPS6KA3, BCL6, TPM3, HLA-
25 DRA, RRBPI, MPO, ERO1L, YOD1; or LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1,
C14orf101,

wherein expression of one or more of RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, BMX,
LTF, PDGFC, CD55, CYP1B1, TLR8, MLLT1, YOD1, GAS7, RRBPI, LILRA2, IL17RA, LILRA4,
TCN1, RNASE3, RNASE2, FAM105A, ERO1L, and/or C14orf101 is increased compared to the control
30 and/or expression of one or more of MAVS, HLA-DRA, CYP27A1, LGALS2, and/or NOV is decreased
compared to the control; and

ii) administering one or more treatments for sepsis to the subject.

14. The method of claim 12 or claim 13, wherein the treatment for sepsis comprises one or more of
35 antibiotic treatment, vasopressors, intravenous fluids, oxygen, dialysis, and monitoring for sepsis.

15. The method of any one of claims 1 to 14, wherein the subject does not exhibit symptoms of sepsis.

16. A method, comprising measuring expression of a set of at least six genes in a sample from a subject having or suspected to have sepsis, wherein the set of at least six genes comprises or consists of:

CCR1, HLA-DPB1, BATF, C3AR1, ARHGEF18, and C9orf 95; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf 103; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CCR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf 95; CD177, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, C3AR1, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf103; FES, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FES, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; or C3AR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95;

wherein the expression of CCR1, C3AR1, C9orf95, BATF, C9orf103, GNA15, CD177, CD63, EMR1, FCER1G, FES, PLAC8, and/or SEPHS2 is increased compared to a control and the expression of HLA-DPB1, ARHGEF18, and/or MTCH1 is decreased compared to a control.

17. A method of diagnosing sepsis in a subject comprising measuring expression of a set of at least six genes in a sample from a subject, wherein the set of at least six genes comprises or consists of:

CCR1, HLA-DPB1, BATF, C3AR1, ARHGEF18, and C9orf 95; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf 103; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CCR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf 95; CD177, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, C3AR1, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf103; FES,

HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FES, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; or C3AR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; and

diagnosing the subject with sepsis when the expression of CCR1, C3AR1, C9orf95, BATF, C9orf103, GNA15, CD177, CD63, EMR1, FCER1G, FES, PLAC8, and/or SEPHS2 is increased compared to a control and the expression of HLA-DPB1, ARHGEF18, and/or MTCH1 is decreased compared to a control.

18. A method, comprising measuring expression of a set of at least six genes in a sample from a subject having or suspected to have sepsis, wherein the set of at least six genes comprises or consists of a set of six genes listed in Table 5, wherein the expression of the set of genes is altered compared to a control.

19. A method of diagnosing sepsis in a subject comprising:
measuring expression of a set of at least six genes in a sample from a subject, wherein the set of at least six genes comprises or consists of a set of six genes listed in Table 5; and
diagnosing the subject with sepsis when the expression of the set of genes is altered compared to a control.

20. The method of any one of claims 16 to 19, further comprising measuring expression of RPGRIP1, wherein the expression of RPGRIP1 is decreased compared to a control.

21. A method of treating sepsis comprising,
i) measuring expression of a set of six or more genes in a sample from a subject, wherein the six or more genes comprise or consist of:

CCR1, HLA-DPB1, BATF, C3AR1, ARHGEF18, and C9orf 95; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf 103; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CCR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf 95; CD177, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, C3AR1, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and SEPHS2; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf103; FES,

HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FES, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; or C3AR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95,

wherein the expression of CCR1, C3AR1, C9orf95, BATF, C9orf103, GNA15, CD177, CD63, EMR1, FCER1G, FES, PLAC8, and/or SEPHS2 is increased compared to a control and the expression of HLA-DPB1, ARHGEF18, and/or MTCH1 is decreased compared to a control; and

ii) administering one or more treatments for sepsis to the subject.

22. The method of claim 21, wherein the treatment for sepsis comprises one or more of antibiotic treatment, vasopressors, intravenous fluids, oxygen, and dialysis.

23. The method of any one of claims 1 to 22, wherein the sample is whole blood, peripheral blood mononuclear cells, serum, or plasma.

24. The method of any one of claims 1 to 23, wherein measuring expression comprises measuring mRNA expression, protein expression, or both.

25. The method of any one of claims 1 to 24, further comprising measuring expression of at least one housekeeping or internal control molecule.

26. The method of any one of claims 1 to 25, wherein the control is a reference value or a sample from a healthy subject.

27. The method of any one of claims 1 to 26, wherein measuring expression comprises real-time PCR, quantitative real-time reverse transcriptase PCR, reverse transcriptase PCR, and/or microarray analysis.

28. A solid support comprising binding agents specific for a set of genes selected from:

CCR1, HLA-DPB1, BATF, C3AR1, ARHGEF18, and C9orf 95; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf 103; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CCR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf 95; CD177, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, C3AR1, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and

SEPHS2; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf103; FES, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FES, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; or C3AR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95,

5 wherein the specific binding agents are covalently attached to the solid substrate.

29. A solid support comprising binding agents specific for a set of genes selected from:

LCN2, SLC2A3, BMX; LCN2, SLC2A3, GRB10; LCN2, PFKFB3, GRB10; LCN2, PFKFB3, BMX; IL1R2, HK3, BMX; LCN2, HK3, BMX; LCN2, HK3, GRB10; GZMA, HK3, BMX; FCAR, PFKFB2, BMX; or LCN2, PFKFB3, IL18R1;

LCN2, PFKFB3, GRB10, ST6GALNAC3; LCN2, SLC2A3, BMX, LGALS2; IL1R2, SLC2A3, BMX, TCN1; LCN2, SLC2A3, GRB10, ST6GALNAC3; FCAR, PFKFB2, BMX, CEACAM1; IL1R2, HK3, BMX, CD24; IL1R2, PFKFB3, BMX, CD24; BMX, SLC2A3, GRB10, CD24; IL1R2, HK3, BMX, CEACAM1; or GZMA, SLC2A3, BMX, CD24; or

LCN2, PFKFB3, GRB10, ST6GALNAC3, RNASE3; LCN2, PFKFB3, GRB10, RNASE2, ST6GALNAC3; IL1R2, PFKFB3, GRB10, CD24, ST6GALNAC3; GZMA, PFKFB3, GRB10, ST6GALNAC3, CD24; HK3, PFKFB3, GRB10, ST6GALNAC3, CD24; IL1R2, PFKFB3, GRB10, ST6GALNAC3, TCN1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, TCN1; LCN2, PFKFB3, GRB10, ST6GALNAC3, DACH1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, CD24; or SLC2A3, HK3, BMX, SPOCD1, LGALS2,

wherein the specific binding agents are covalently attached to the solid substrate.

30. A solid support comprising binding agents specific for a set of genes selected from:

RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO; RPS6KA3, MAVS, TPM3, BCL6, STOM, MPO; RPS6KA3, BCL6, TPM3, BMX, STOM, MPO; RPS6KA3, LTF, TPM3, BCL6, STOM, PDGFC; RPS6KA3, BCL6, TPM3, CD55, STOM, MPO; RPS6KA3, BCL6, TPM3, GNG10, STOM, PDGFC; RPS6KA3, LTF, TPM3, BCL6, STOM, CYP1B1; LTF, BCL6, TPM3, CD55, STOM, PDGFC; RPS6KA3, BCL6, TPM3, TLR8, STOM, MPO; RPS6KA3, BCL6, TPM3, HLA-DRA, GNG10, MPO; or LCN2, BMX, MPO, RPS6KA3, BCL6, MAPK14;

RPS6KA3, MLLT1, TPM3, BCL6, GNG10, CYP27A1, YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1; RPS6KA3, GAS7, TPM3, BCL6, RRBP1, MPO, YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, LGALS2; RPS6KA3, GAS7, TPM3, BCL6, GNG10, MPO, YOD1; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1; RPS6KA3, BCL6, TPM3, GNG10, LILRA2, CYP27A1, YOD1; RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, YOD1; or RPS6KA3, BCL6, TPM3, CD55, STOM, MPO, IL17RA; or

RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, LILRA4; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, IL17RA; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, TCN1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE3; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, NOV; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE2; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, FAM105A; RPS6KA3, BCL6, TPM3, HLA-DRA, RRBPI, MPO, ERO1L, YOD1; or LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, C14orf101,

wherein the specific binding agents are covalently attached to the solid substrate.

31. The solid support of any one of claims 28 to 30, further comprising one or more binding agents specific for one or more housekeeping or internal control genes.

32. The solid support of any one of claims 28 to 31, wherein the solid support is an array.

33. A kit comprising a set of primers and/or probes capable of specifically binding to a set of genes selected from:

CCR1, HLA-DPB1, BATF, C3AR1, ARHGEF18, and C9orf95; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf103; CCR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CCR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; CD177, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; CD63, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; CD63, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; EMR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, C3AR1, MTCH1, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf95; FCER1G, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and SEPHS2; FCER1G, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, FCER1G, MTCH1, and C9orf95; FES, HLA-DPB1, PLAC8, GNA15, ARHGEF18, and C9orf103; FES, HLA-DPB1, PLAC8, GNA15, MTCH1, and C9orf95; FES, HLA-DPB1, BATF, C3AR1, MTCH1, and C9orf95; or C3AR1, HLA-DPB1, BATF, GNA15, MTCH1, and C9orf95.

34. A kit comprising a set of primers and/or probes capable of specifically binding to a set of genes selected from:

LCN2, SLC2A3, BMX; LCN2, SLC2A3, GRB10; LCN2, PFKFB3, GRB10; LCN2, PFKFB3, BMX; IL1R2, HK3, BMX; LCN2, HK3, BMX; LCN2, HK3, GRB10; GZMA, HK3, BMX; FCAR, PFKFB2, BMX; or LCN2, PFKFB3, IL18R1;

LCN2, PFKFB3, GRB10, ST6GALNAC3; LCN2, SLC2A3, BMX, LGALS2; IL1R2, SLC2A3, BMX, TCN1; LCN2, SLC2A3, GRB10, ST6GALNAC3; FCAR, PFKFB2, BMX, CEACAM1; IL1R2, HK3, BMX, CD24; IL1R2, PFKFB3, BMX, CD24; BMX, SLC2A3, GRB10, CD24; IL1R2, HK3, BMX, CEACAM1; or GZMA, SLC2A3, BMX, CD24; or

LCN2, PFKFB3, GRB10, ST6GALNAC3, RNASE3; LCN2, PFKFB3, GRB10, RNASE2, ST6GALNAC3; IL1R2, PFKFB3, GRB10, CD24, ST6GALNAC3; GZMA, PFKFB3, GRB10, ST6GALNAC3, CD24; HK3, PFKFB3, GRB10, ST6GALNAC3, CD24; IL1R2, PFKFB3, GRB10, ST6GALNAC3, TCN1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, TCN1; LCN2, PFKFB3, GRB10, ST6GALNAC3, DACH1; SLC2A3, PFKFB3, GRB10, ST6GALNAC3, CD24; or SLC2A3, HK3, BMX, SPOCD1, LGALS2.

35. A kit comprising a set of primers and/or probes capable of specifically binding to a set of genes selected from:

RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO; RPS6KA3, MAVS, TPM3, BCL6, STOM, MPO; RPS6KA3, BCL6, TPM3, BMX, STOM, MPO; RPS6KA3, LTF, TPM3, BCL6, STOM, PDGFC; RPS6KA3, BCL6, TPM3, CD55, STOM, MPO; RPS6KA3, BCL6, TPM3, GNG10, STOM, PDGFC; RPS6KA3, LTF, TPM3, BCL6, STOM, CYP1B1; LTF, BCL6, TPM3, CD55, STOM, PDGFC; RPS6KA3, BCL6, TPM3, TLR8, STOM, MPO; RPS6KA3, BCL6, TPM3, HLA-DRA, GNG10, MPO; or LCN2, BMX, MPO, RPS6KA3, BCL6, MAPK14;

RPS6KA3, MLLT1, TPM3, BCL6, GNG10, CYP27A1, YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1; RPS6KA3, GAS7, TPM3, BCL6, RRBPI, MPO, YOD1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, LGALS2; RPS6KA3, GAS7, TPM3, BCL6, GNG10, MPO, YOD1; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1; RPS6KA3, BCL6, TPM3, GNG10, LILRA2, CYP27A1, YOD1; RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO, LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, MPO, YOD1; or RPS6KA3, BCL6, TPM3, CD55, STOM, MPO, IL17RA; or

RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, LGALS2; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, LILRA4; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, IL17RA; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, TCN1; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE3; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, NOV; RPS6KA3, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, RNASE2; LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, FAM105A; RPS6KA3, BCL6, TPM3, HLA-DRA, RRBPI, MPO, ERO1L, YOD1; or LTF, MLLT1, TPM3, BCL6, GNG10, PDGFC, YOD1, C14orf101.

36. The kit of any one of claims 33 to 35, further comprising one or more primers and/or probes specific for one or more housekeeping or internal control genes.

37. The kit of any one of claims 33 to 36, further comprising one or more buffers, enzymes, and/or detection reagents.

5 38. A method of identifying a mRNA biomarker panel for diagnosing pre-symptomatic sepsis, comprising:

collecting blood samples from patients prior to a surgery and daily until five days post-sepsis diagnosis;

collecting blood samples from age, gender and procedure matched control surgical subjects
10 who did not develop sepsis;

preparing RNA from the blood samples;

performing whole blood differential gene expression analysis using clinical information on the samples with approaches comprising:

with or without using pre-surgery data;

15 paired or unpaired analysis between controls and sepsis samples; and

with or without combining time point data prior to Day 0 or not;

selecting mRNAs with differential expression in at least two times points; and

identifying a biomarker panel comprising utilizing support vector machine with recursive feature elimination.

20

FIG. 1A

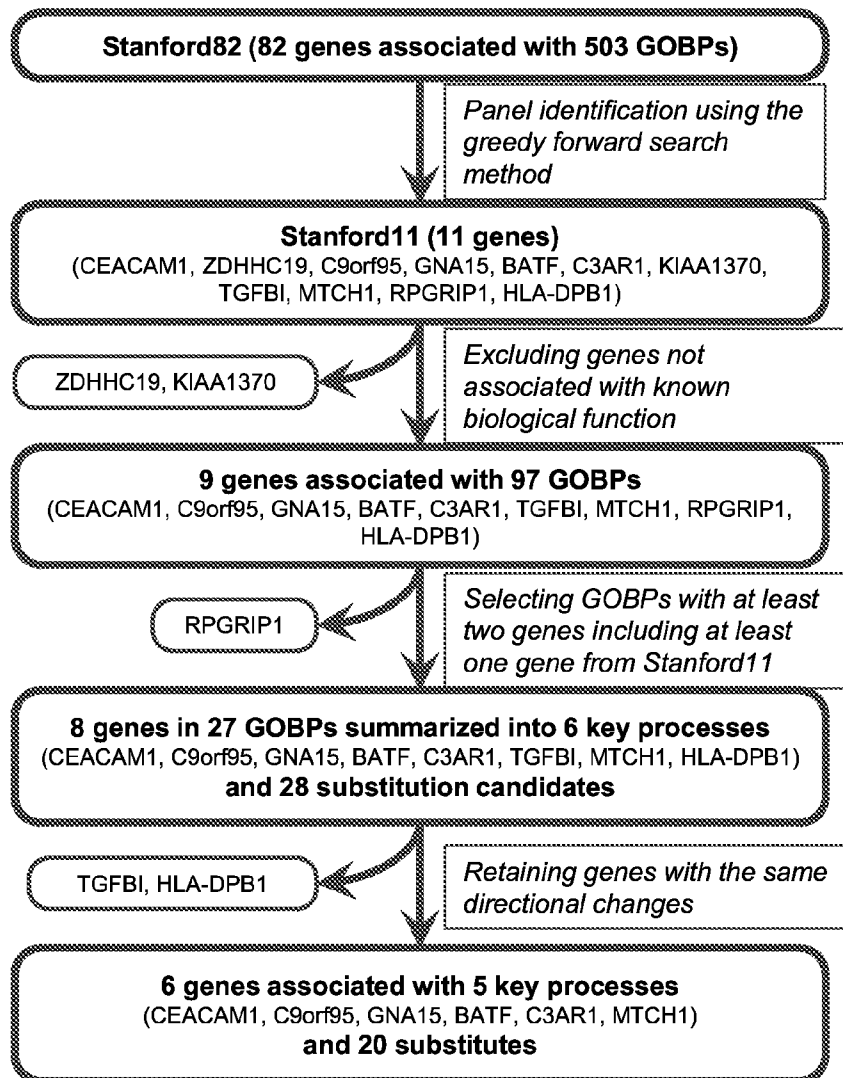


FIG. 1B

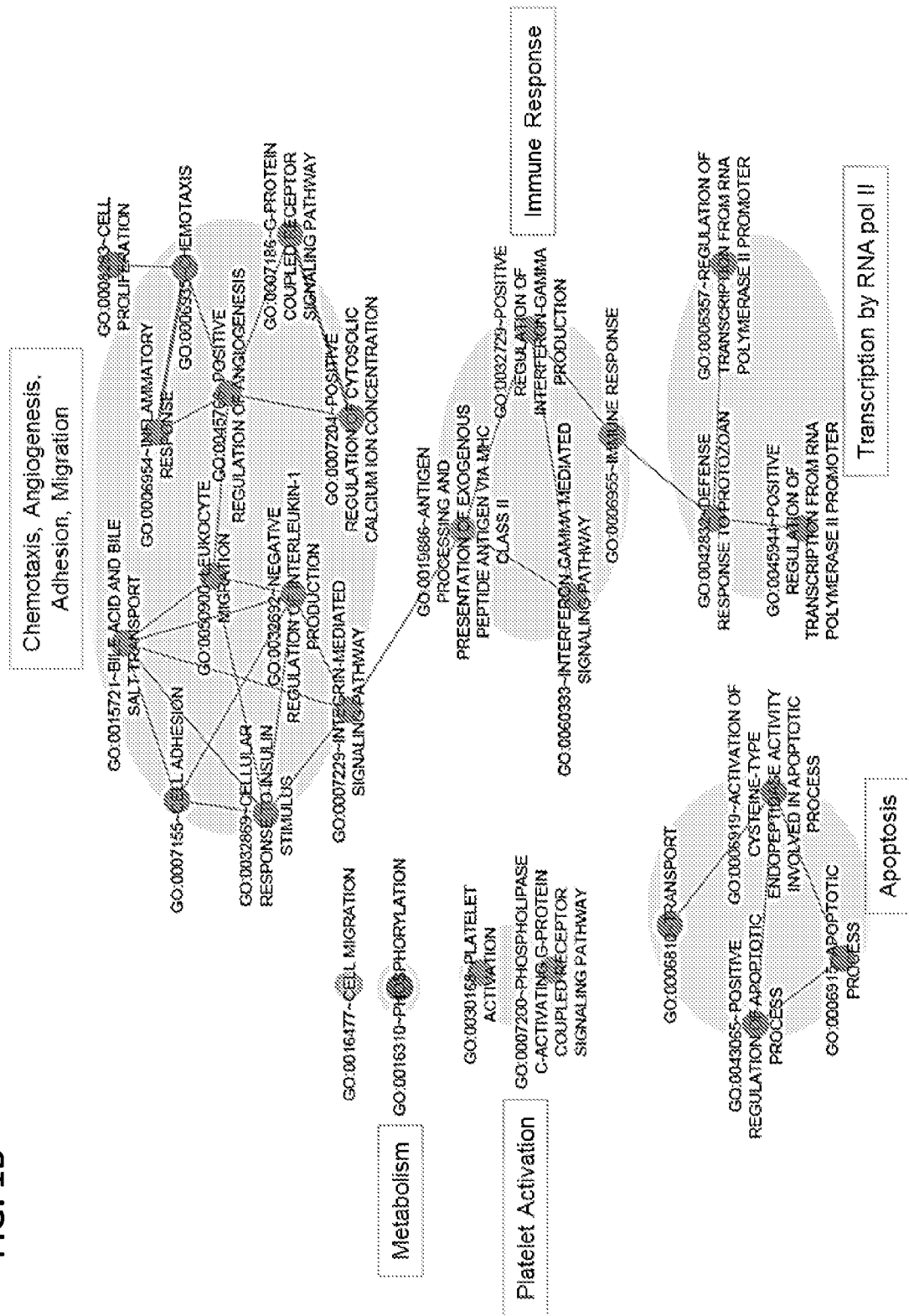


FIG. 2A

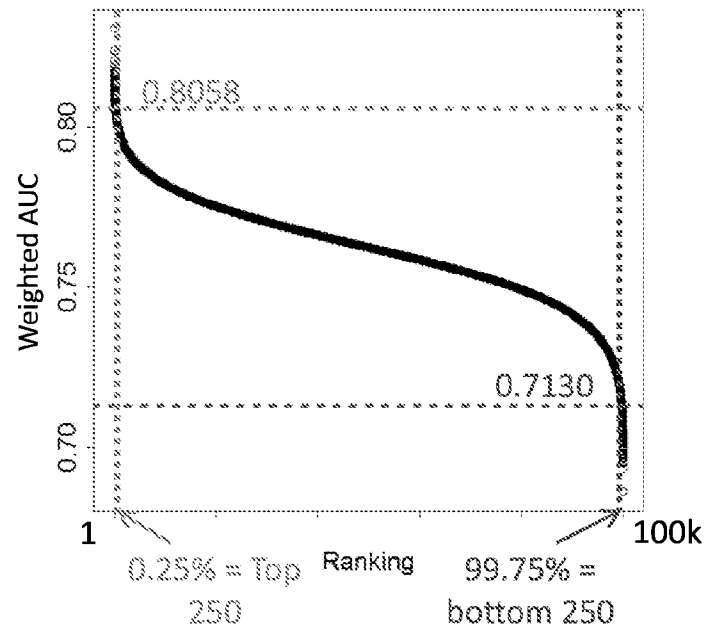


FIG. 2B

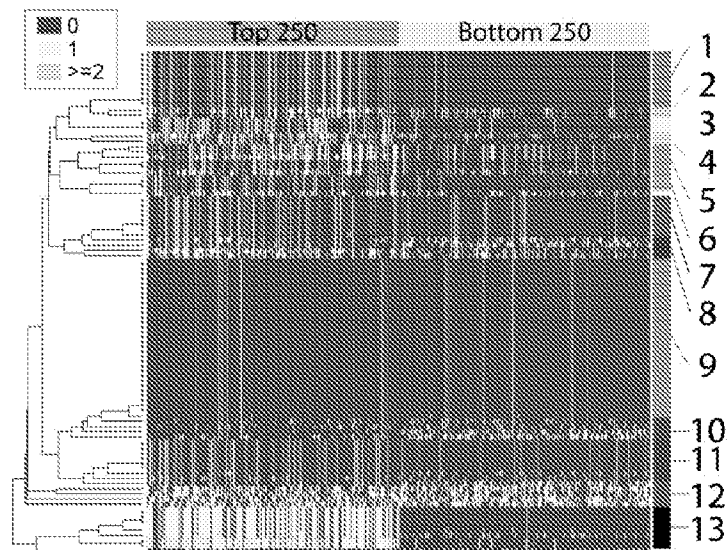


FIG. 2C

Cluster	GO Biological Process	Top 250		Bottom 250	
		Count	Percent	Count	Percent
1	Cell differentiation, DNA damage, transcription from RNA polymerase II promoter	69	27.60%	4	1.60%
2	positive regulation of transcription from RNA polymerase II promoter, defense response to protozoan	116	46.40%	46	18.40%
3	eye photoreceptor cell development, neural precursor cell proliferation, retina development	103	41.20%	7	2.80%
4	phosphorylation, NAD biosynthetic process	135	54.00%	61	24.40%
5	apoptotic process, signaling transduction	160	64.00%	62	24.80%
6	phospholipase C activity	68	27.20%	7	2.80%
7	blood circulation, cell chemotaxis, complement activation	66	26.40%	16	6.40%
8	Leukocyte migration, angiogenesis, chemotaxis	158	63.20%	172	68.80%
9	Cell-cell adhesion, blood vessel development, common myeloid progenitor cell proliferation, negative regulation of T cell mediated signaling pathway, wound healing, spreading of cells	11	4.40%	10	4.00%
10	Cell migration	88	35.20%	191	76.40%
11	T cell receptor signaling pathway, antigen processing and presentation, positive regulation of T cell activation/proliferation	79	31.60%	67	26.80%
12	Inflammatory response, immune response	231	92.40%	234	93.60%
13	angiogenesis, response to stimulus, visual perception, cellular protein metabolic process, chondrocyte differentiation, extracellular matrix organization, negative regulation of cell adhesion	215	86.00%	54	21.60%

FIG. 3A

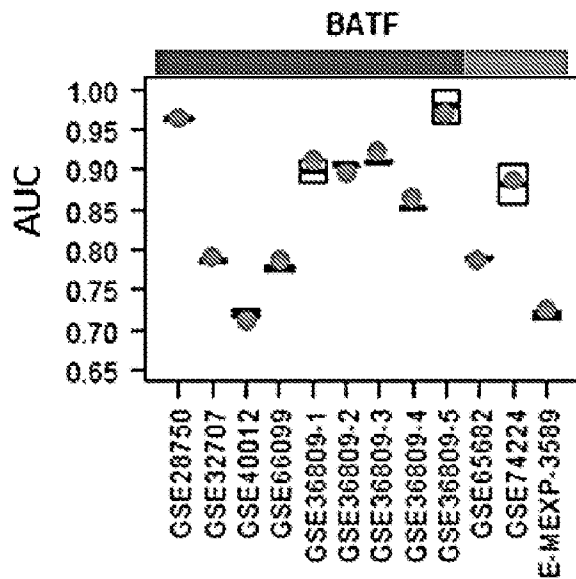


FIG. 3B

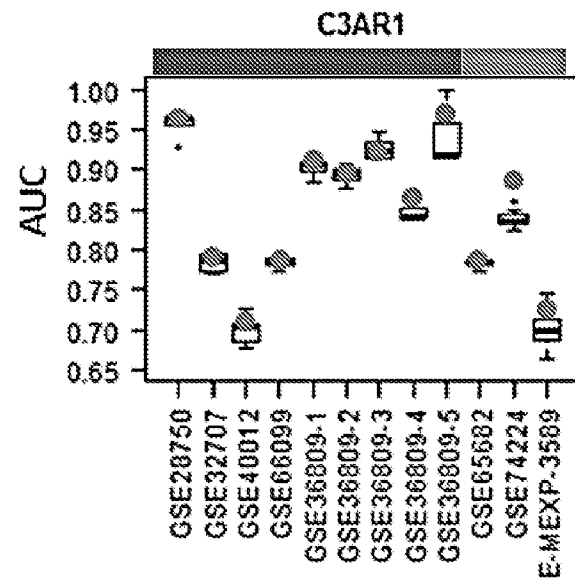
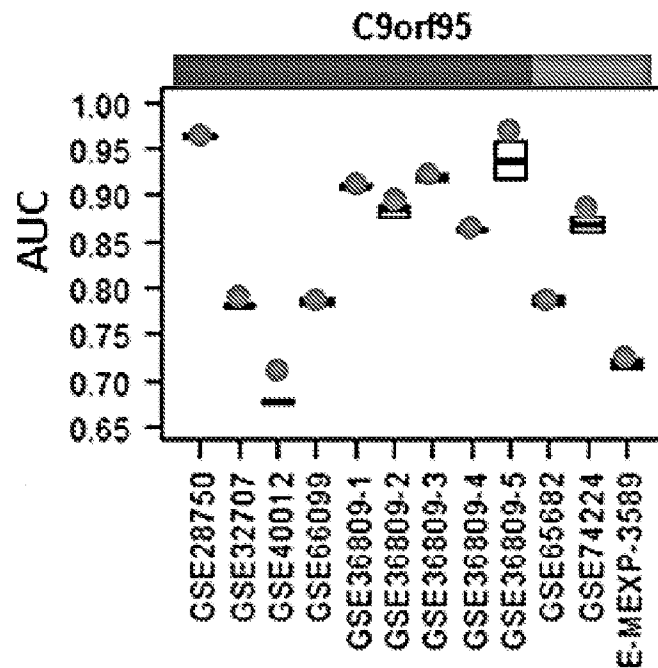


FIG. 3C

* $p < 0.05$

Discovery
Validation

FIG. 3D

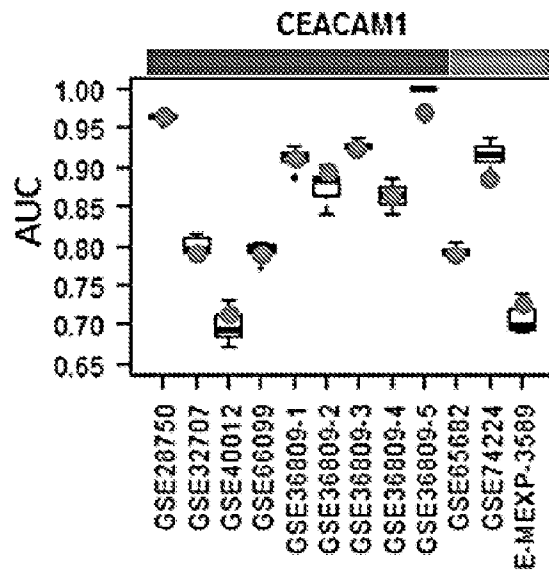


FIG. 3E

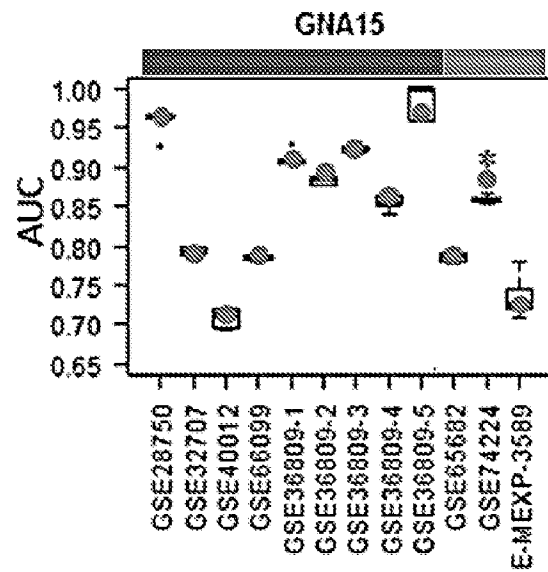


FIG. 3F

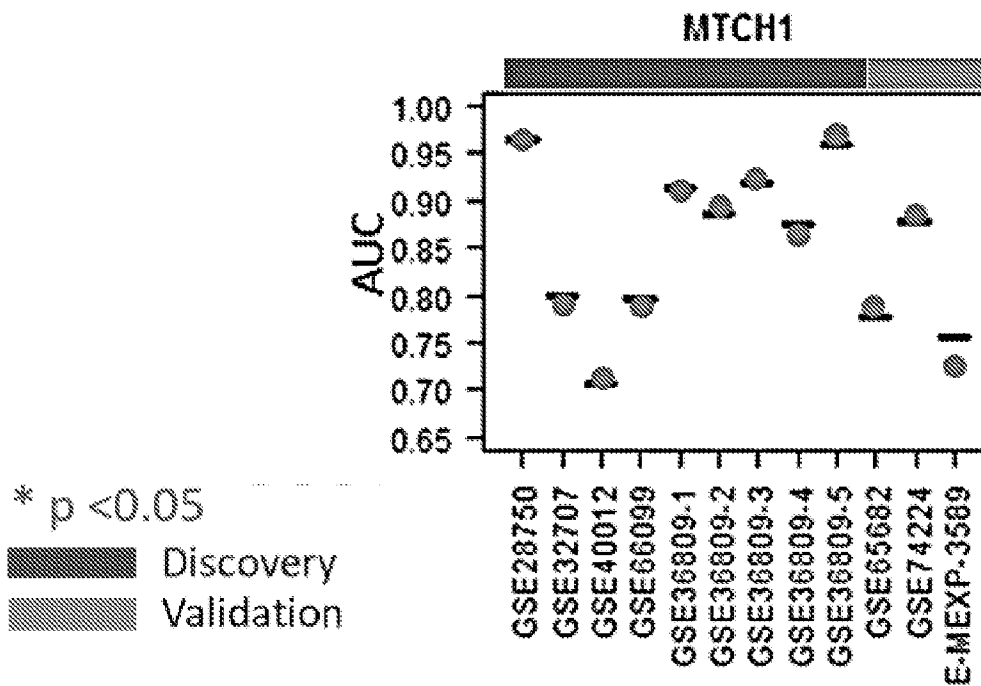
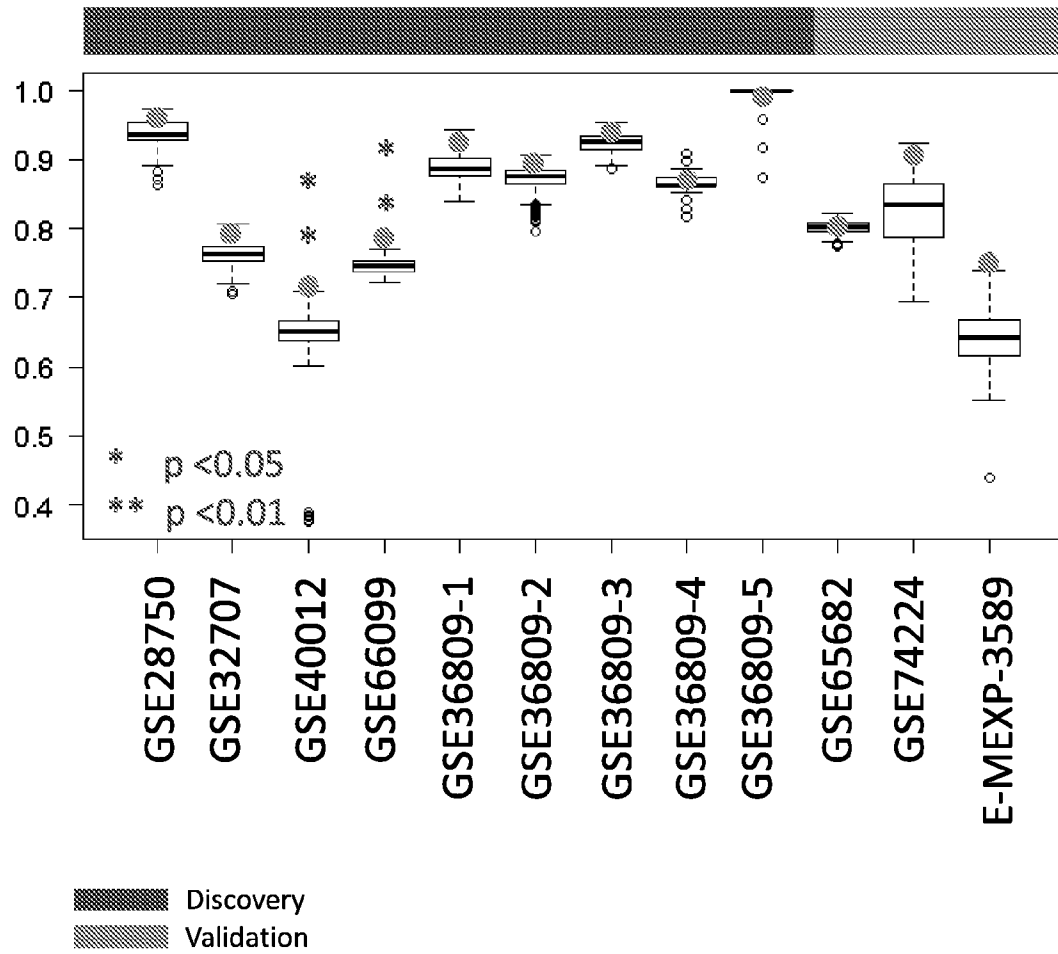


FIG. 4



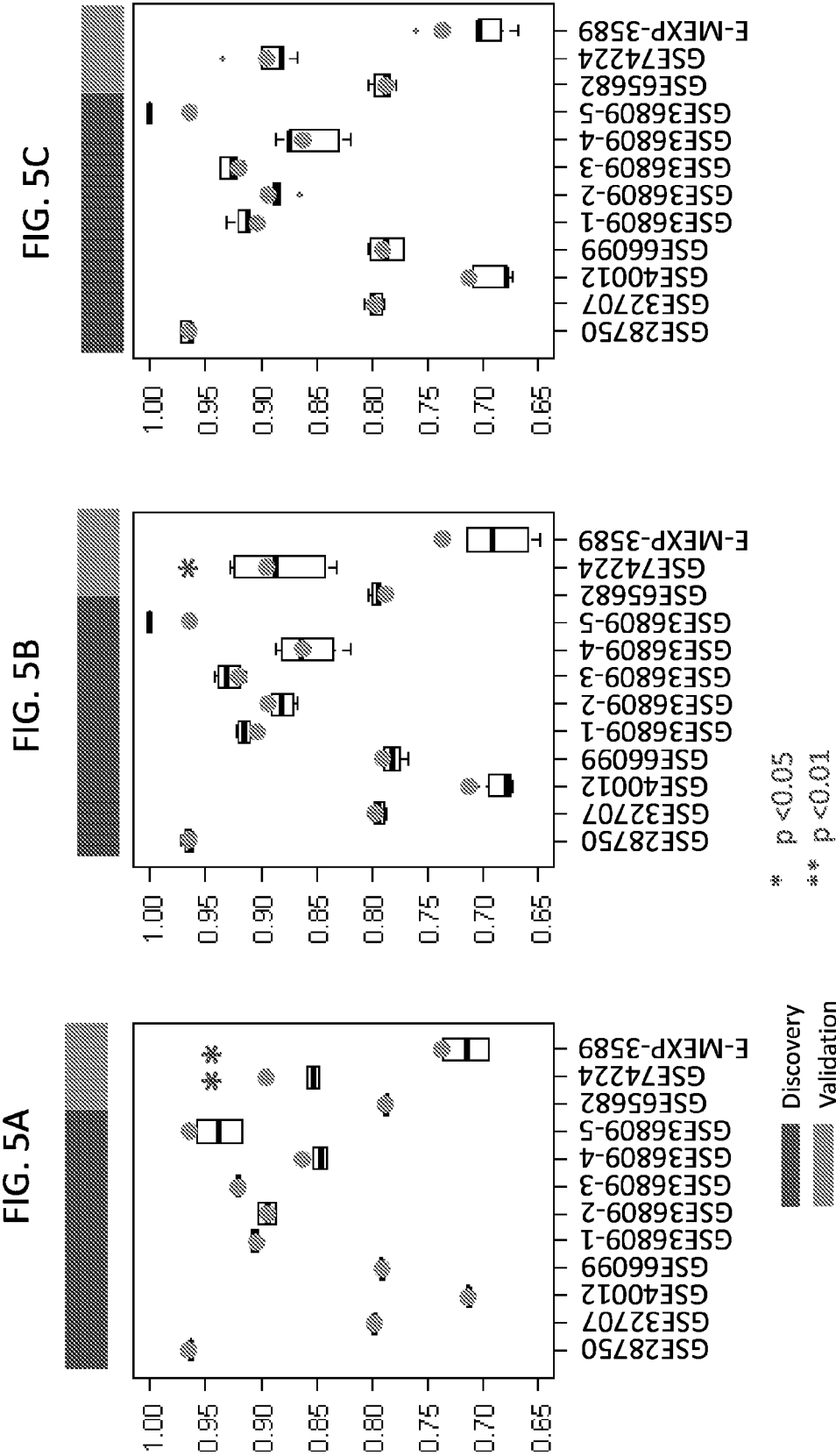


FIG. 6

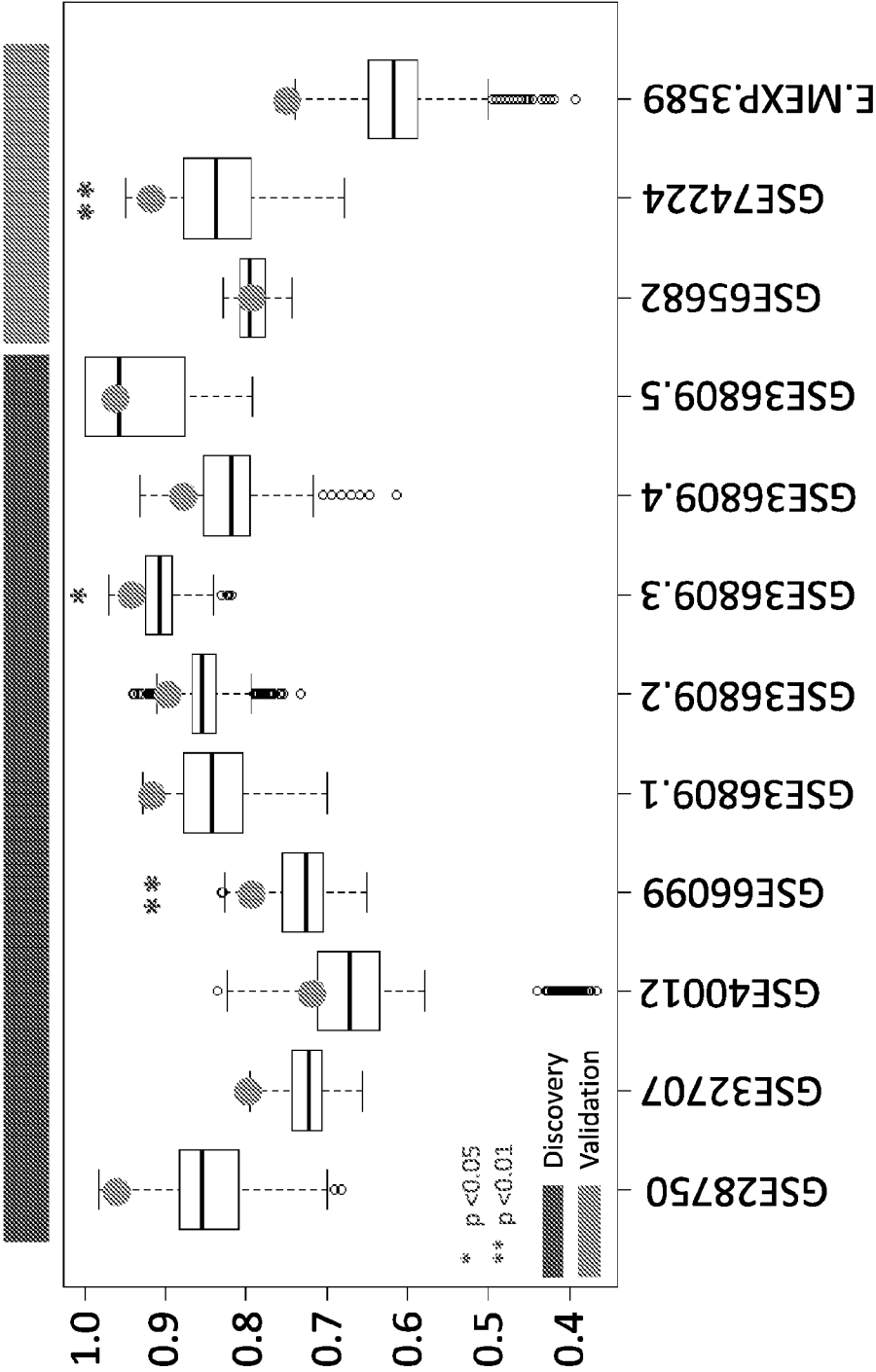


FIG. 7A

Stanford11	Top 1 highly correlated gene panel	Correlation coefficient	Stanford 82
BATF	DDAH2	0.7642	
C3AR1	SQRDL	0.5393	O
C9orf95	PTPN22	0.4870	
CEACAM1	GPR84	0.8169	O
GNA15	FERMT3	0.5874	
ZDHHC19	GPR84	0.7408	O
HLA-DPB1	HLA-DMB	0.7329	
KIAA1370	KIAA1468	0.6517	
MTCH1	CDK5RAP3	0.6607	
RPGRIP1	NOV	0.5266	
TGFBI	CPVL	0.8078	

FIG. 7B

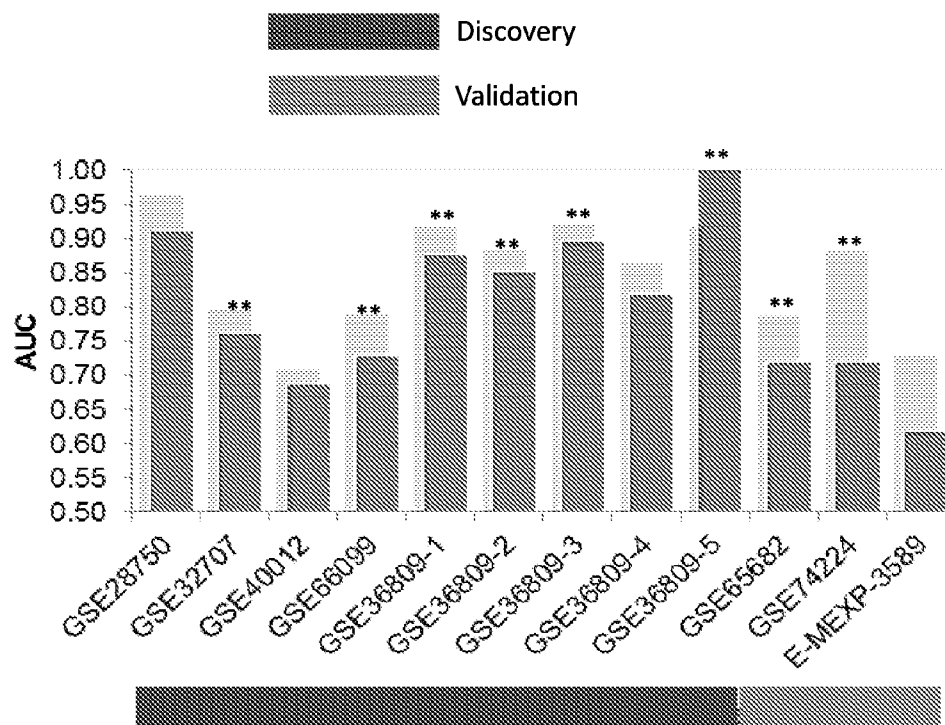


FIG. 7C

14 adhesion/migration related genes
C3AR1, CD177, FCER1G, CEACAM1, ADGRE1, CCR1, TGFBI, SIGLEC9, CD63, PSTPIP2, FES, ANXA3, IL10, RETN

FIG. 7D

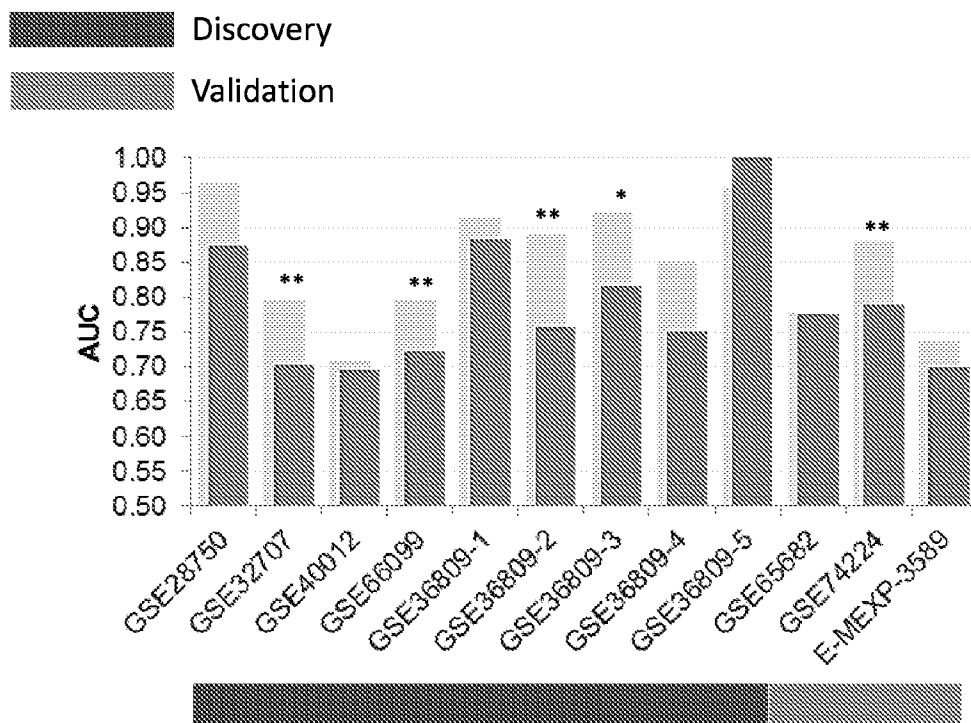


FIG. 7E

3 Top scoring pairs		
1	CACNA2D3	FAM89A
2	RPGRIP1	AP3B2
3	TGFBI	RETN

FIG. 7F

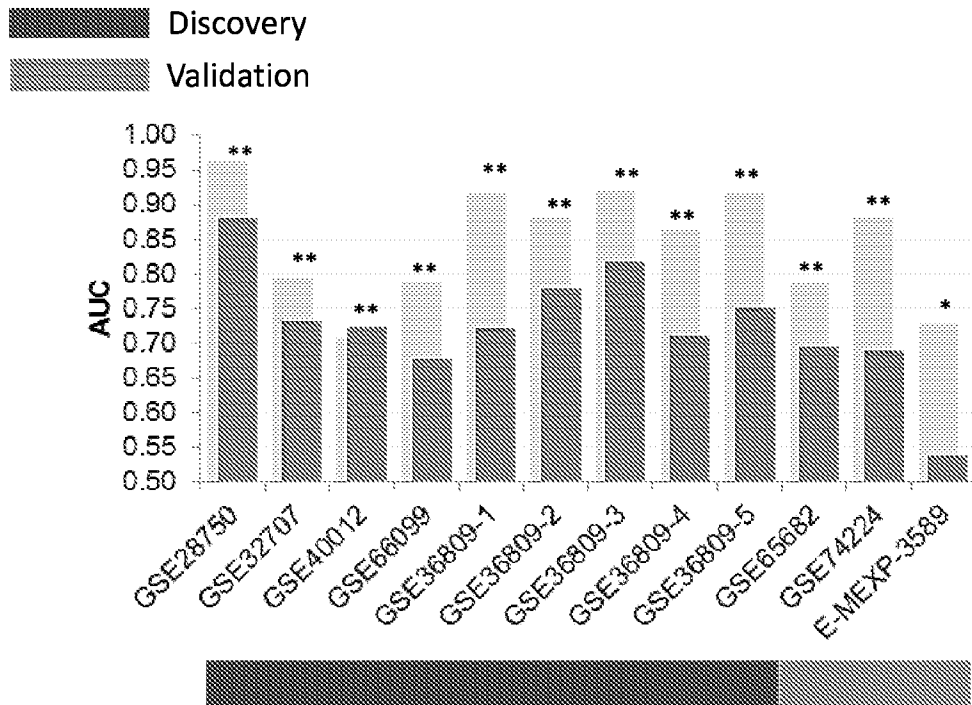


FIG. 8

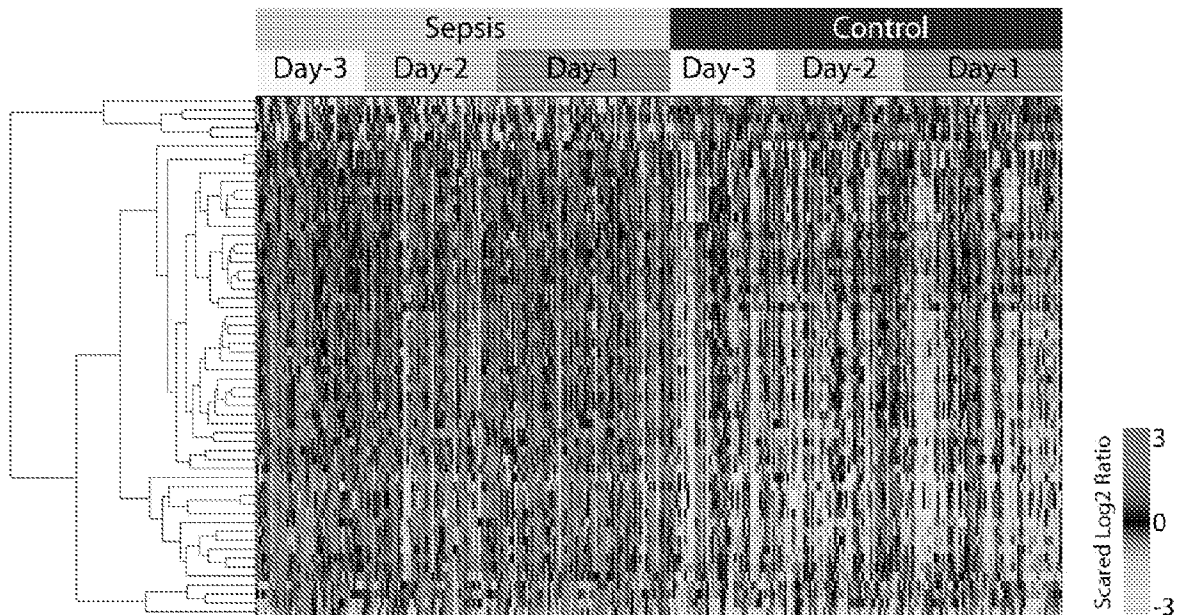


FIG. 9

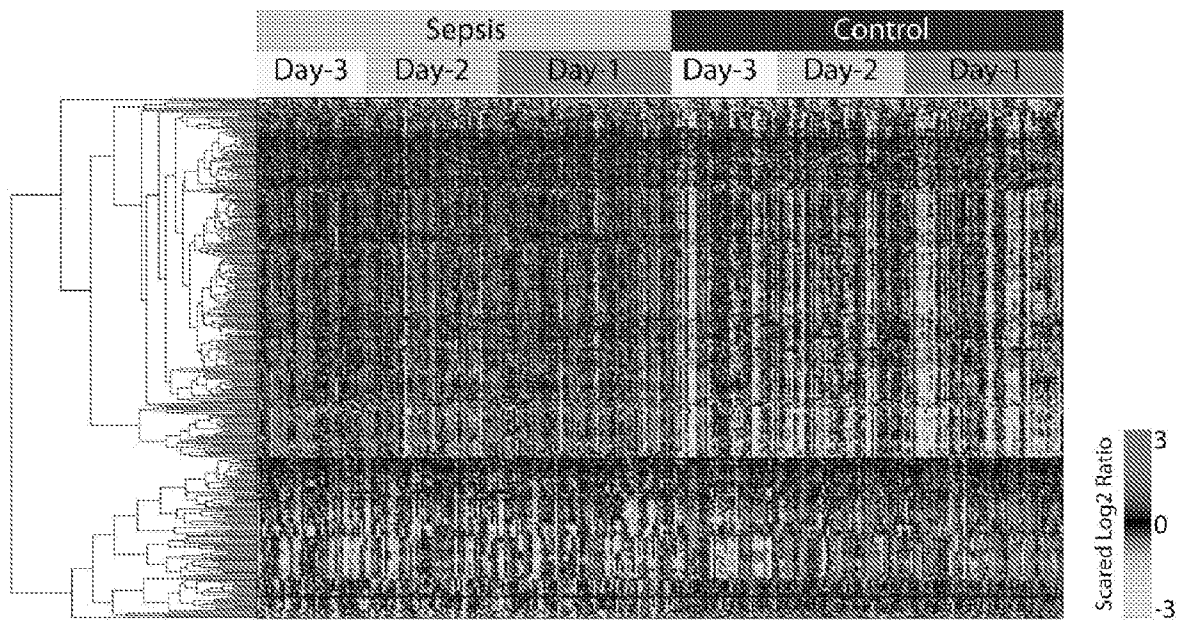


FIG. 10

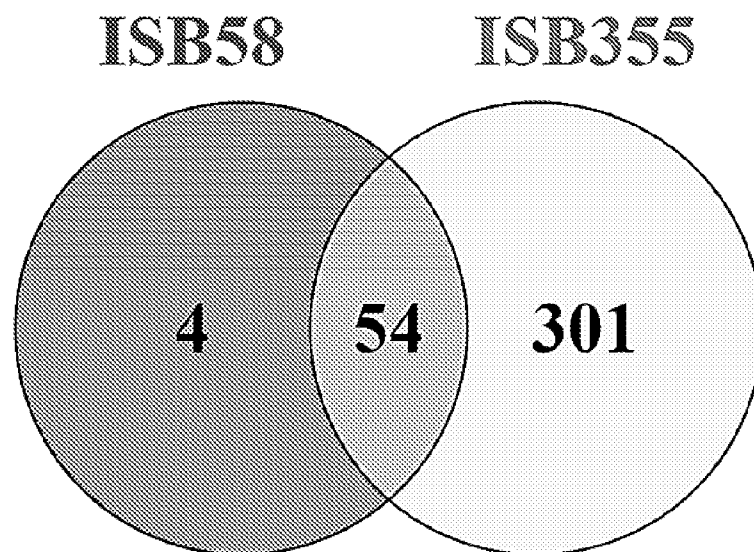


FIG. 11A

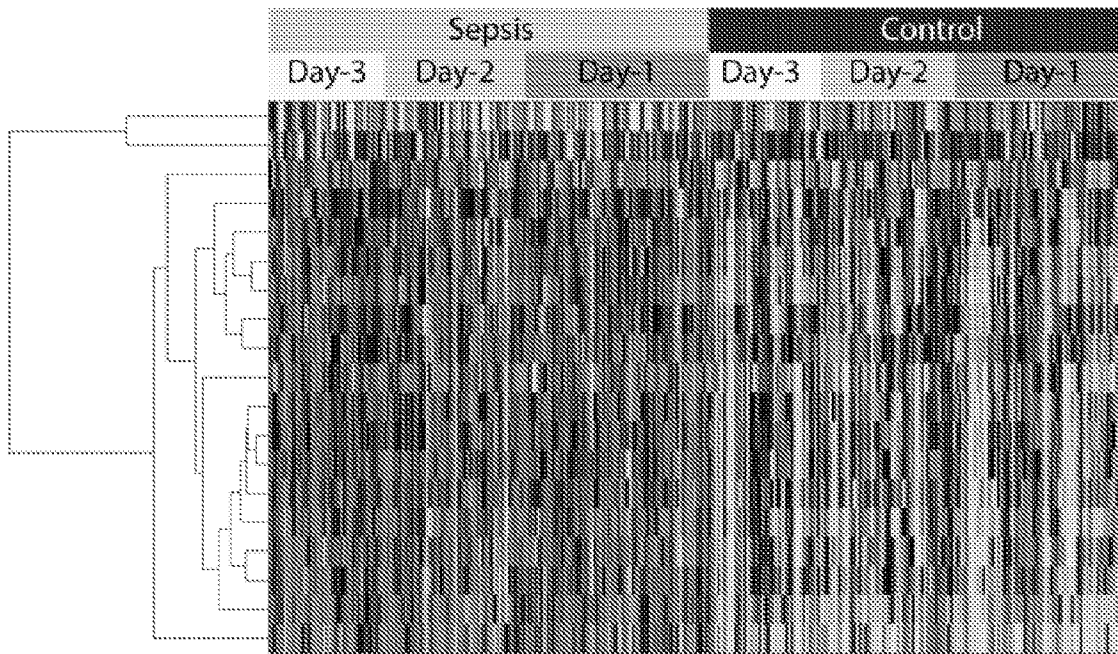


FIG. 11B

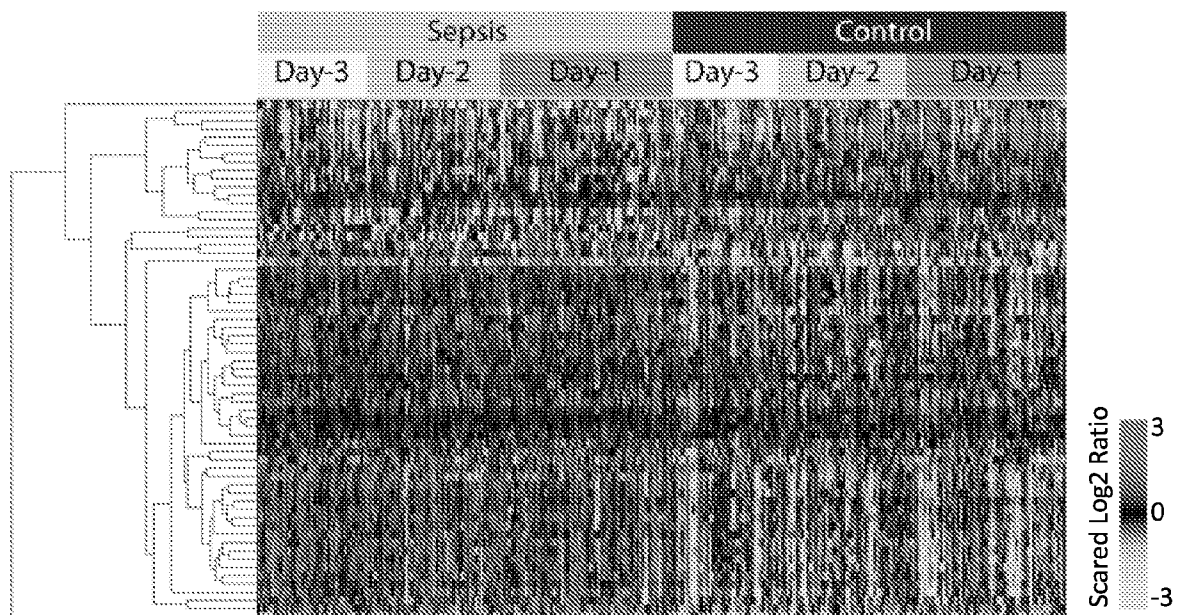


FIG. 12

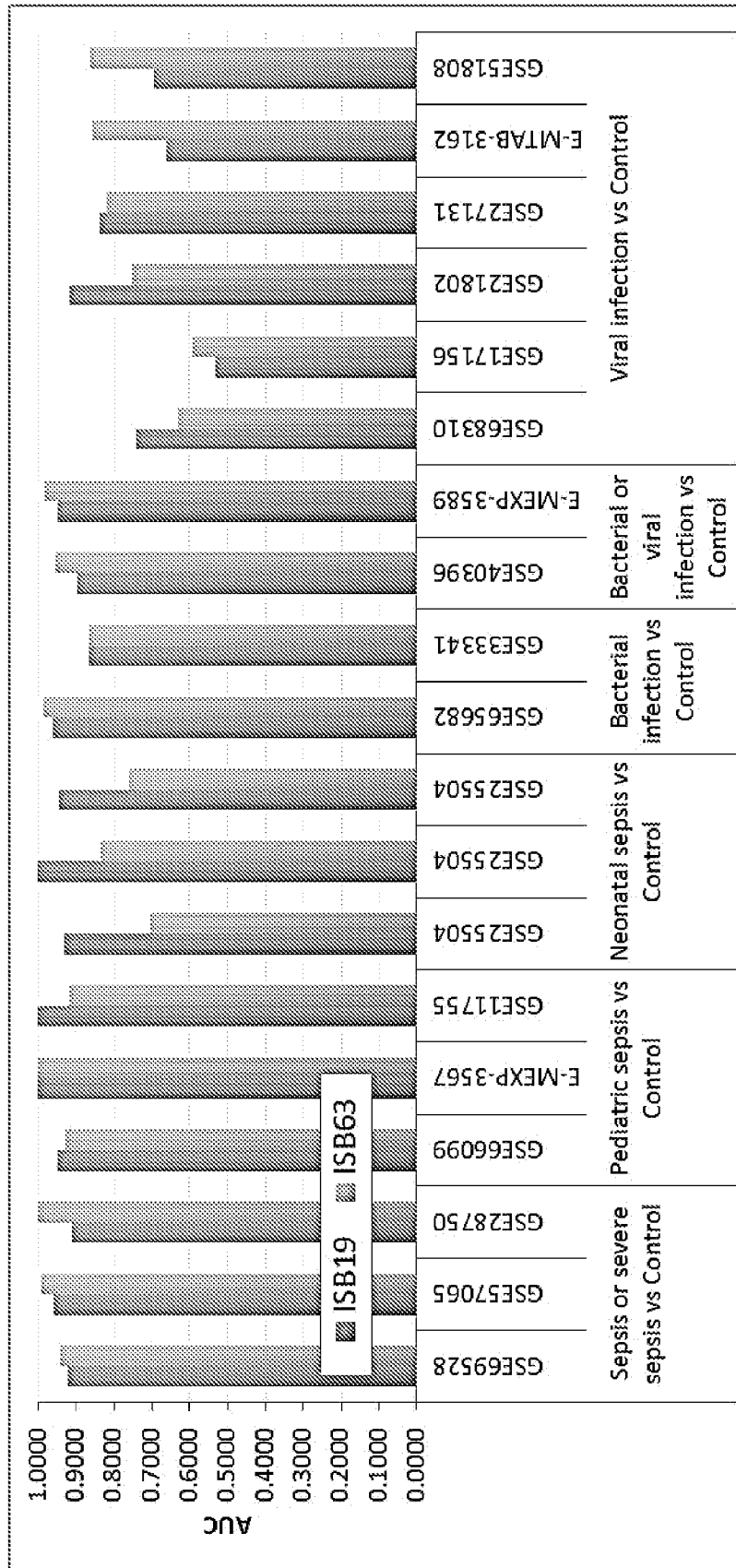


FIG. 13

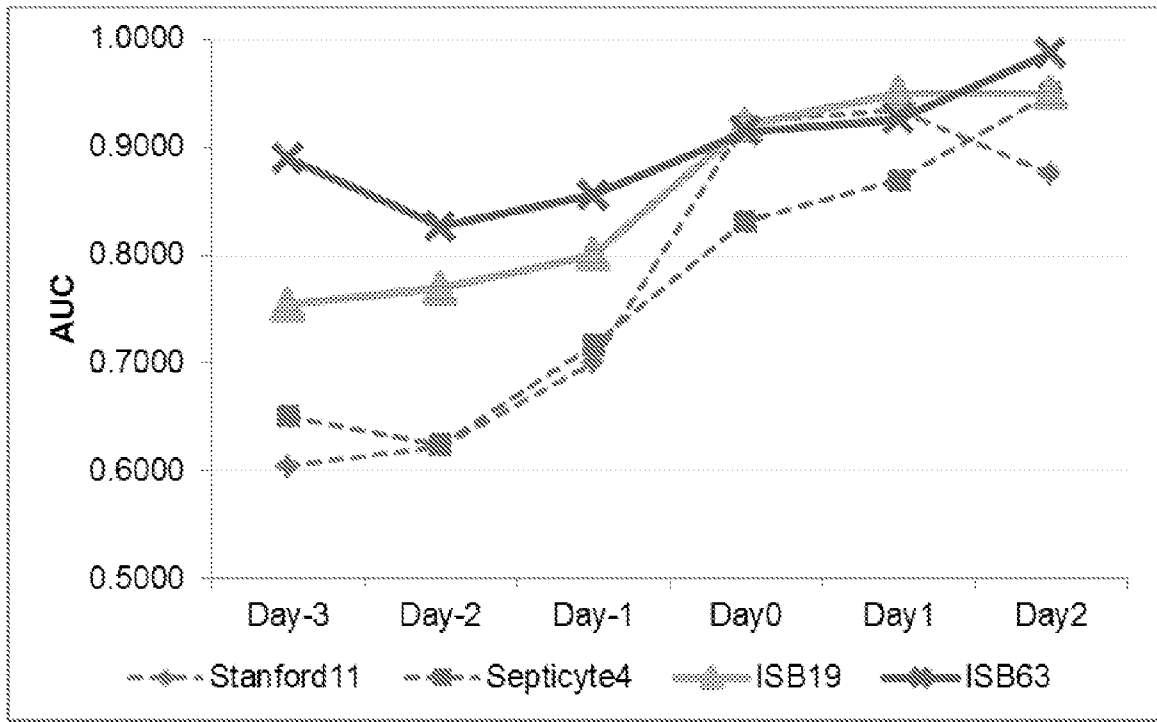


FIG. 14

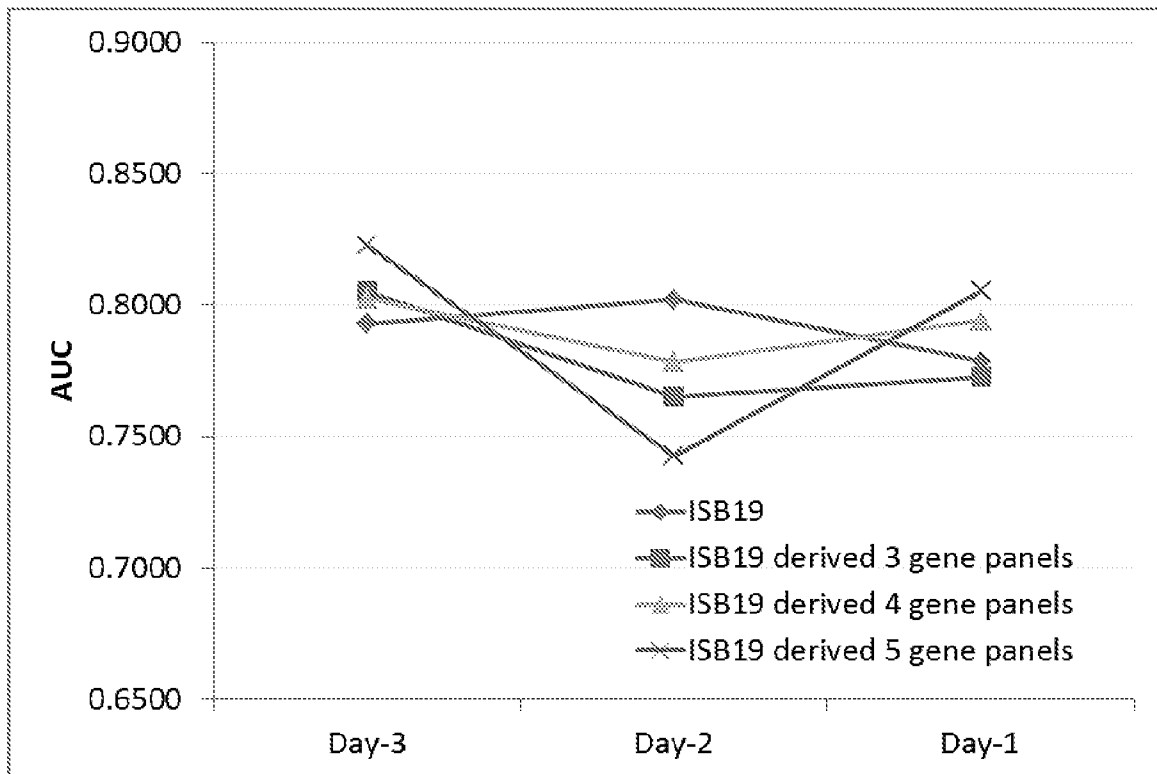


FIG. 15

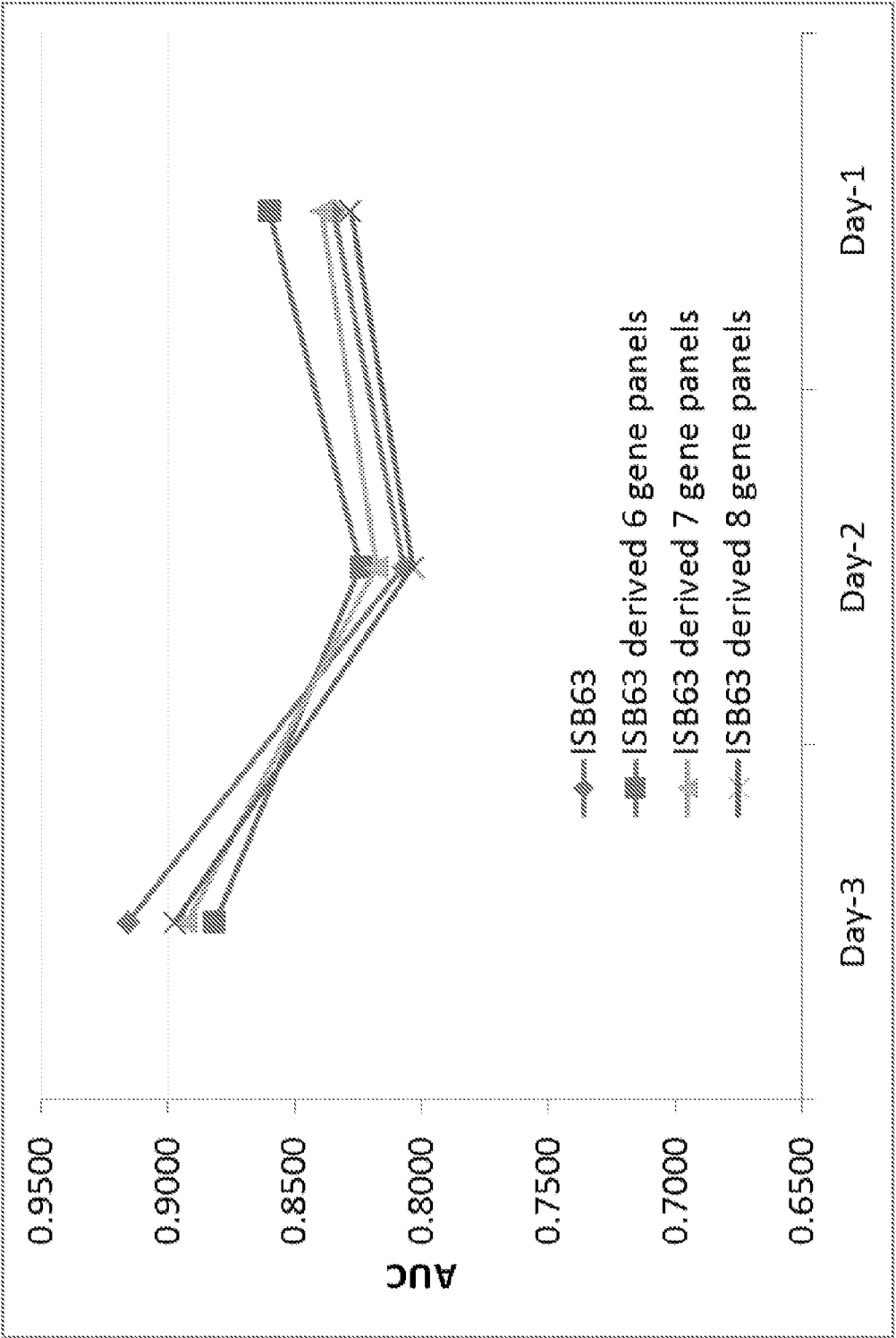


FIG. 16

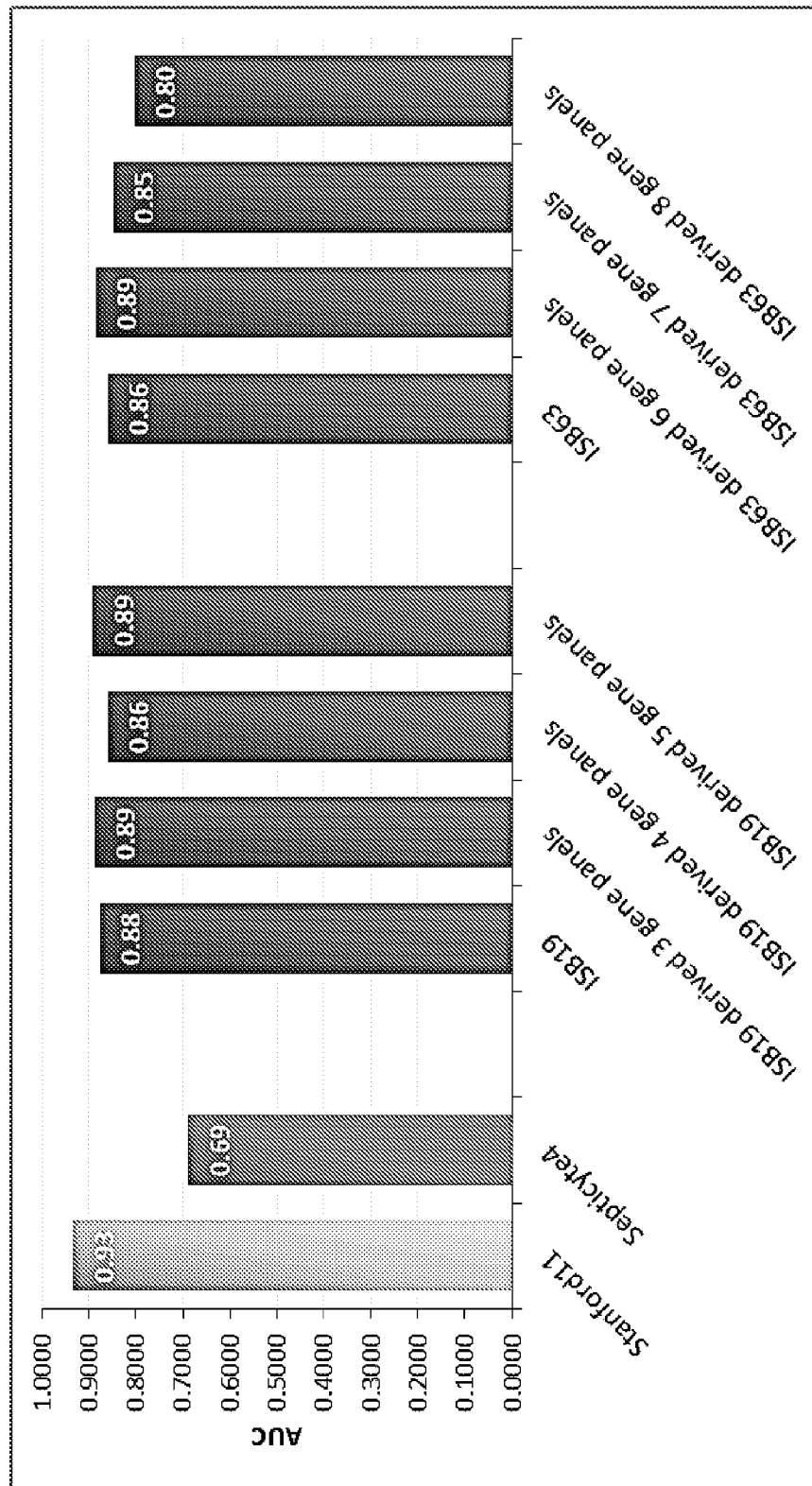


FIG. 17

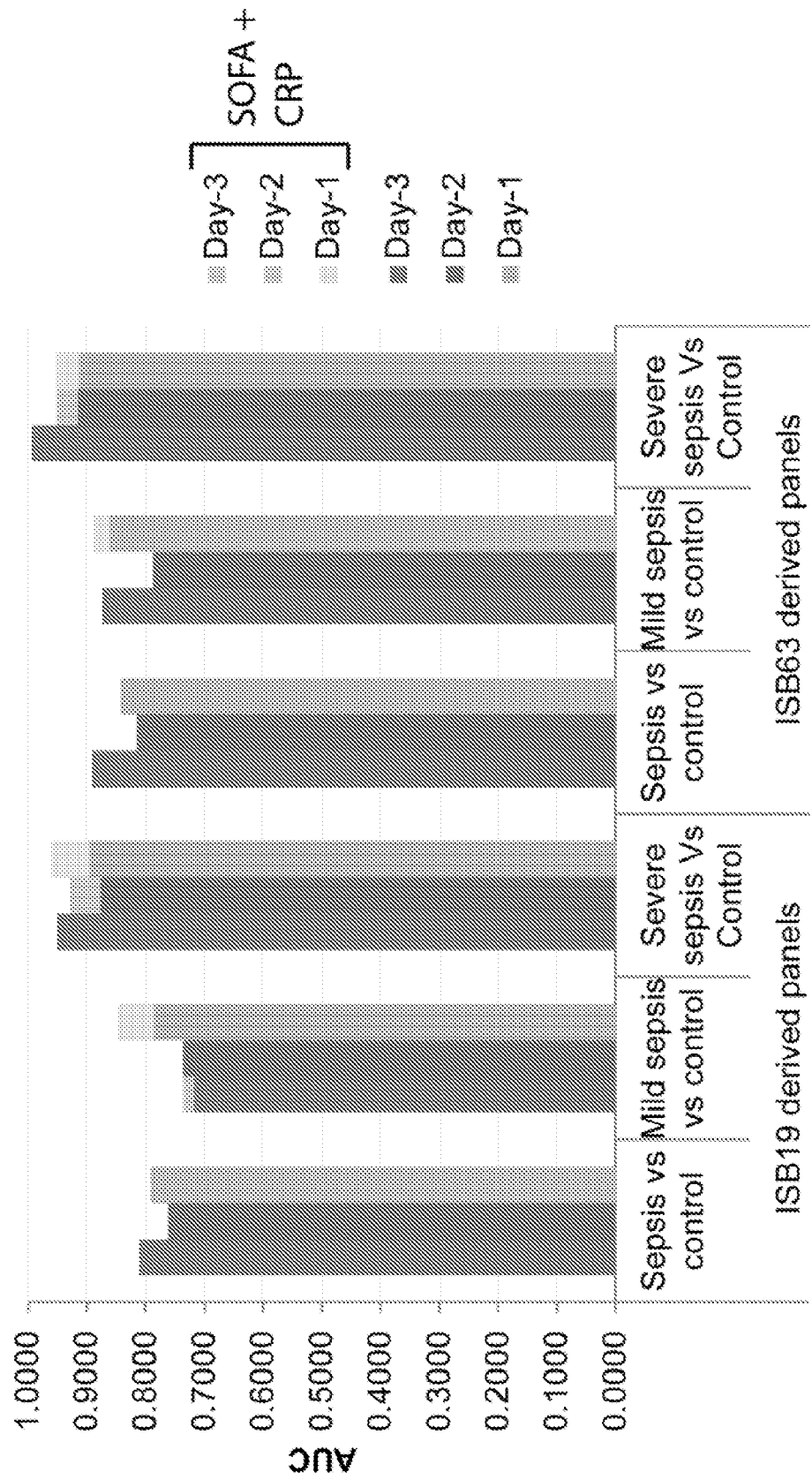


FIG. 18

Pre-Op normalization	Statistical Test	Comparison	Approach	# DEGs	# Genes in panel	Three time points before sepsis	Day-3	Day-2	Day-1	Day0
With Pre-Op normalization	Paired Test	All time points together	Approach1	76	37	0.70	0.62	0.63	0.78	0.84
		Each time point	Approach2	58	19	0.77	0.74	0.68	0.85	0.92
	Unpaired Test	All time points together	Approach3	77	39	0.74	0.67	0.67	0.83	0.84
		Each time point	Approach4	62	21	0.76	0.73	0.69	0.83	0.94
Without Pre-Op normalization	Paired Test	All time points together	Approach5	345	50	0.85	0.83	0.84	0.87	0.93
		Each time point	Approach6	355	63	0.88	0.83	0.86	0.91	0.93
	Unpaired Test	All time points together	Approach7	342	85	0.84	0.81	0.83	0.87	0.92
		Each time point	Approach8	338	54	0.87	0.79	0.85	0.91	0.95

FIG. 19A

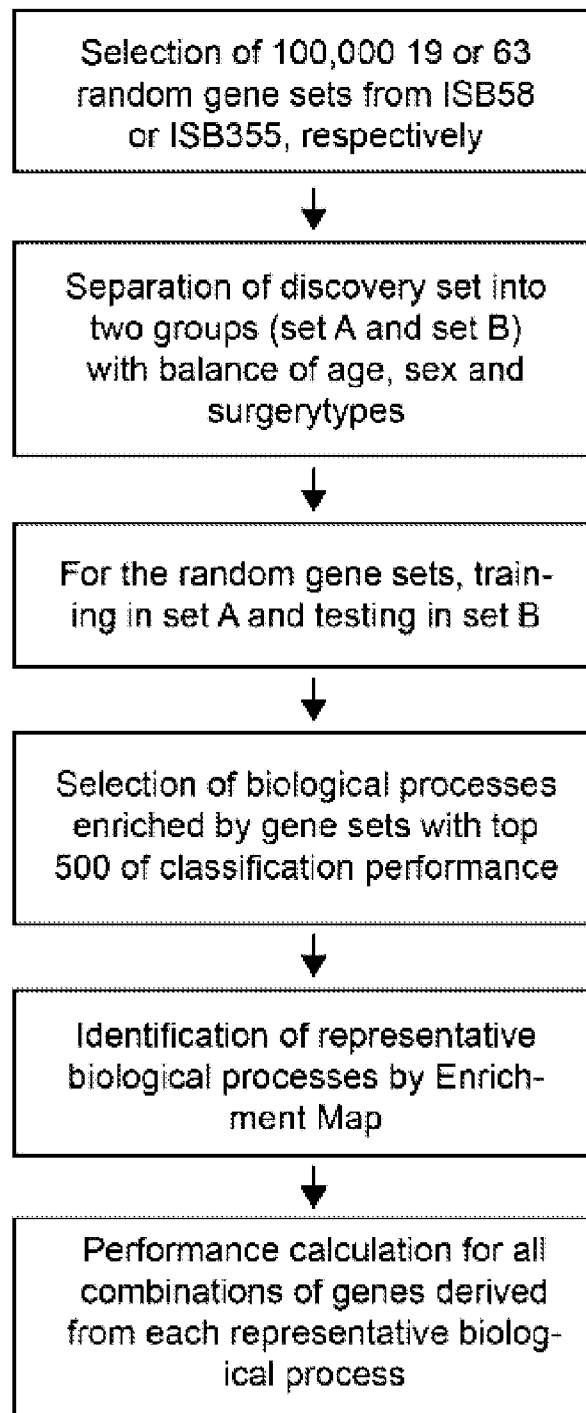


FIG. 19B

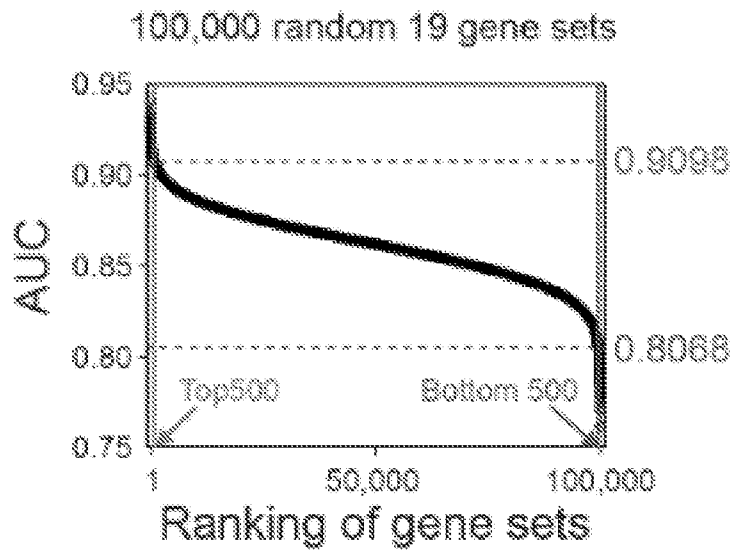


FIG. 19C

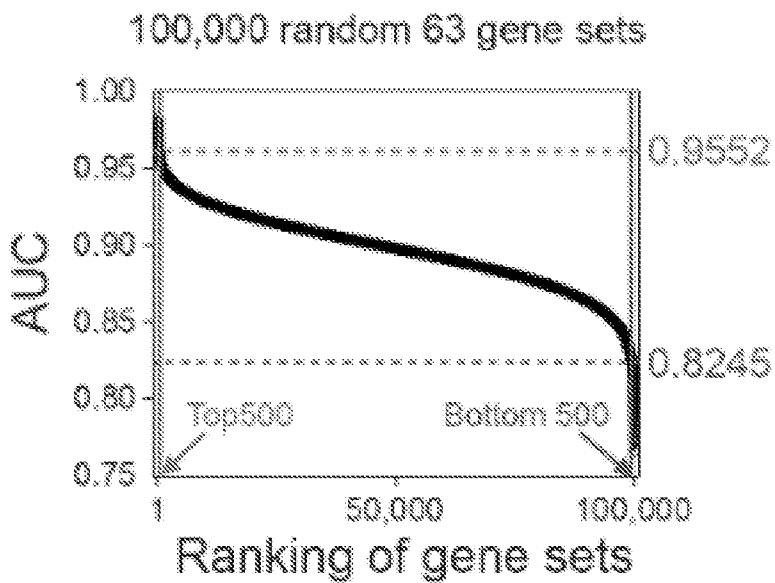


FIG. 19D

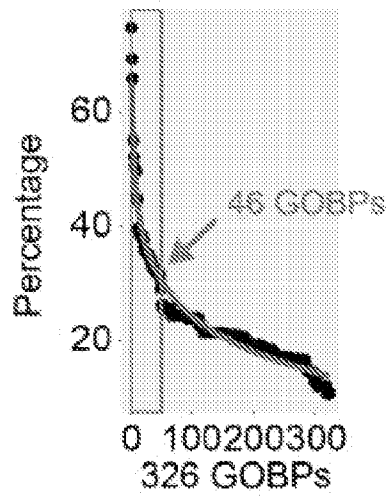
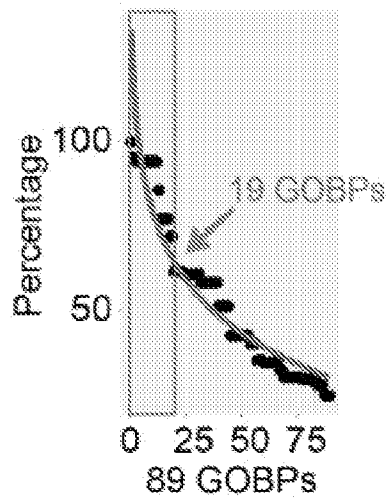


FIG. 19E

FIG. 19F

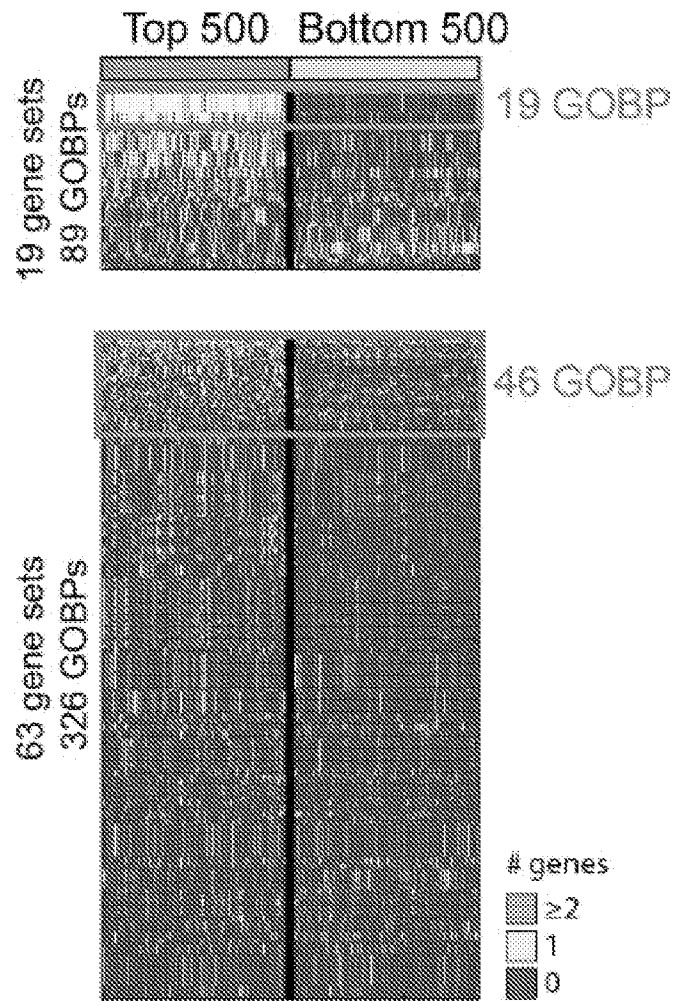


FIG. 19G

FIG. 19H

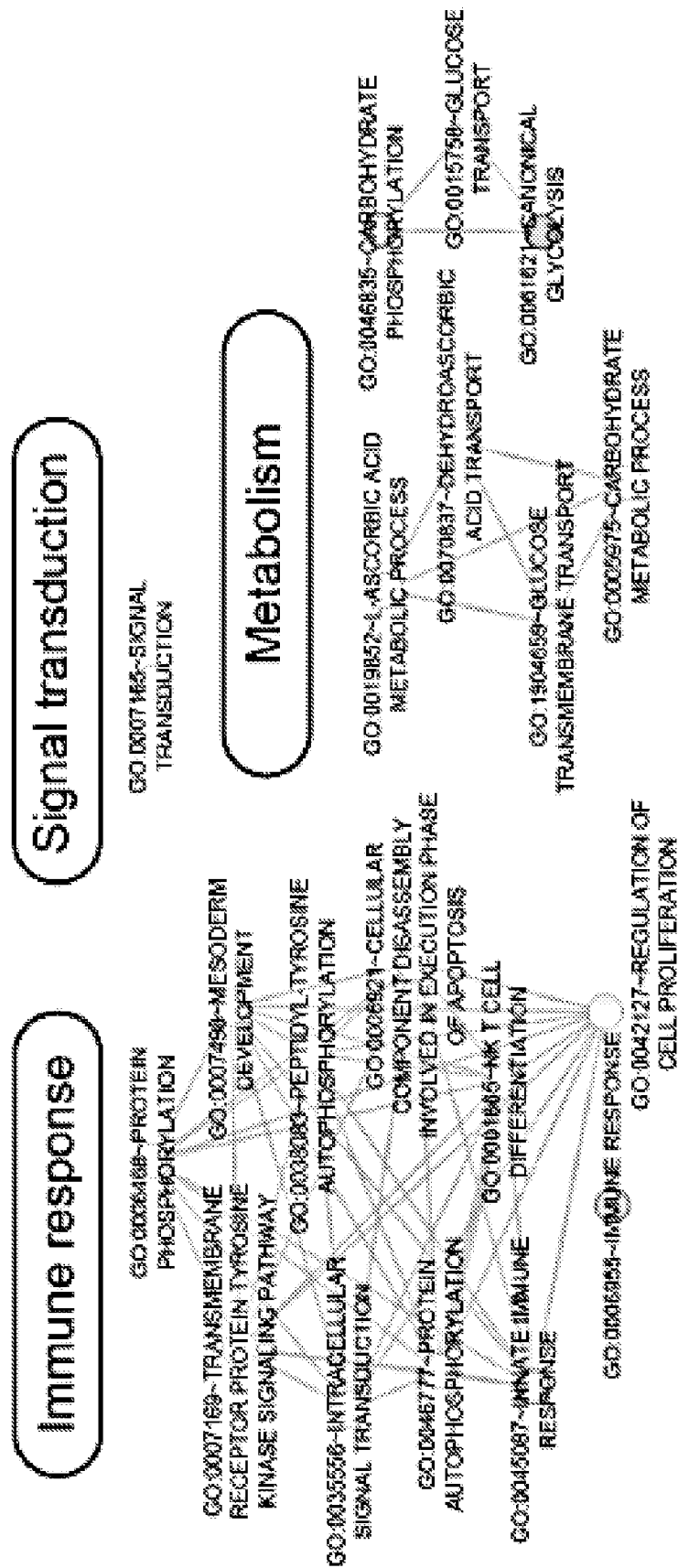


FIG. 19I

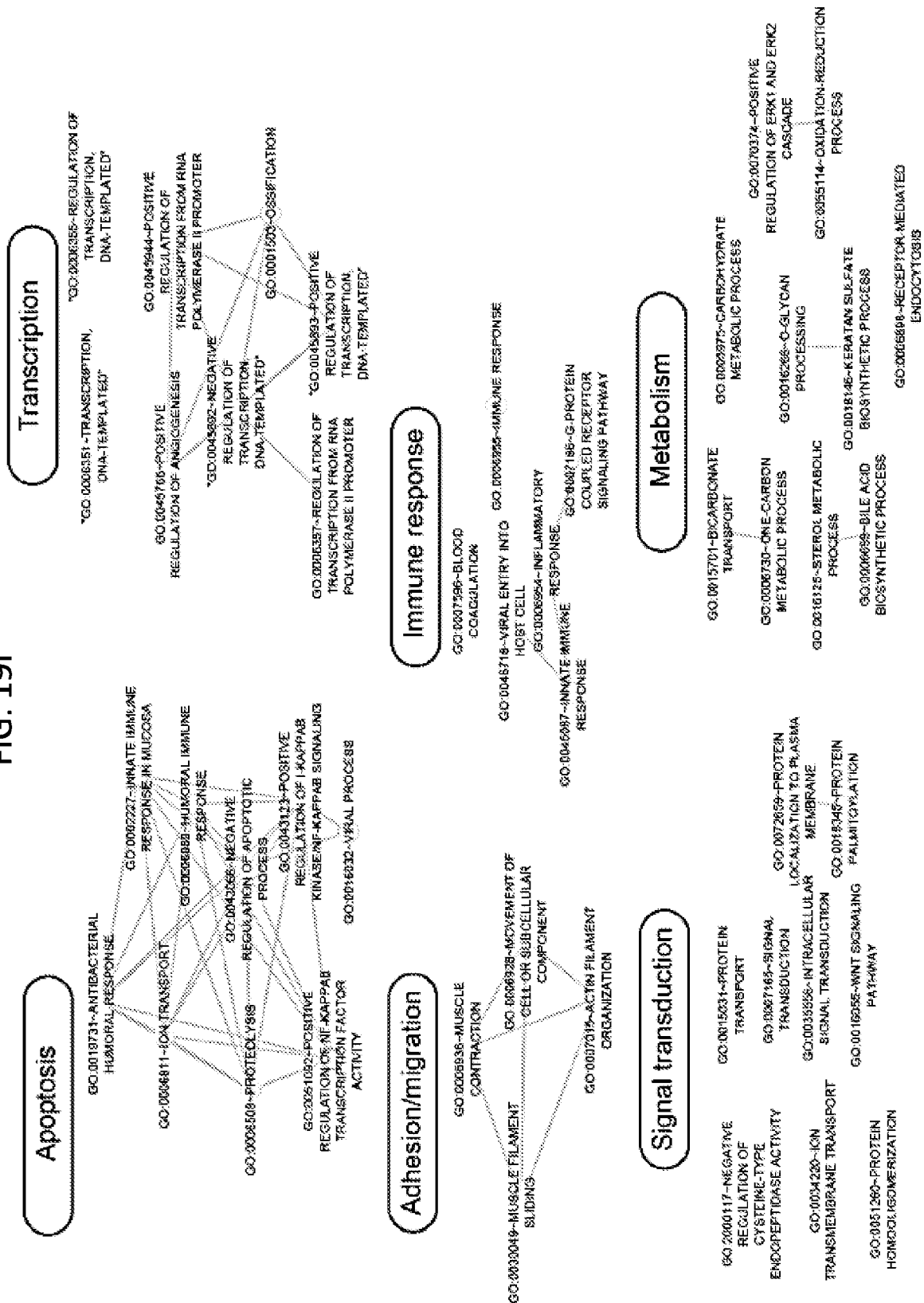


FIG. 20B

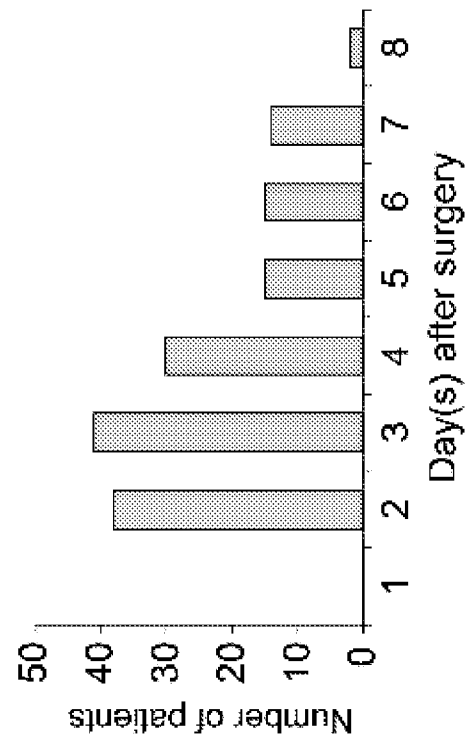


FIG. 20A

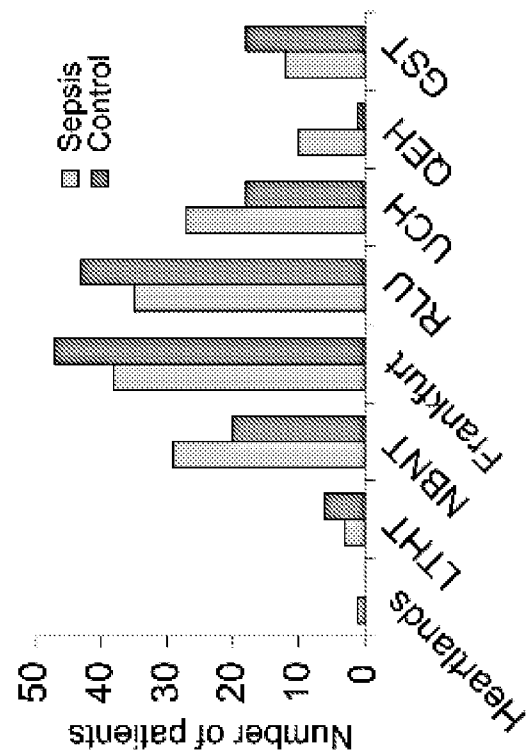


FIG. 20C

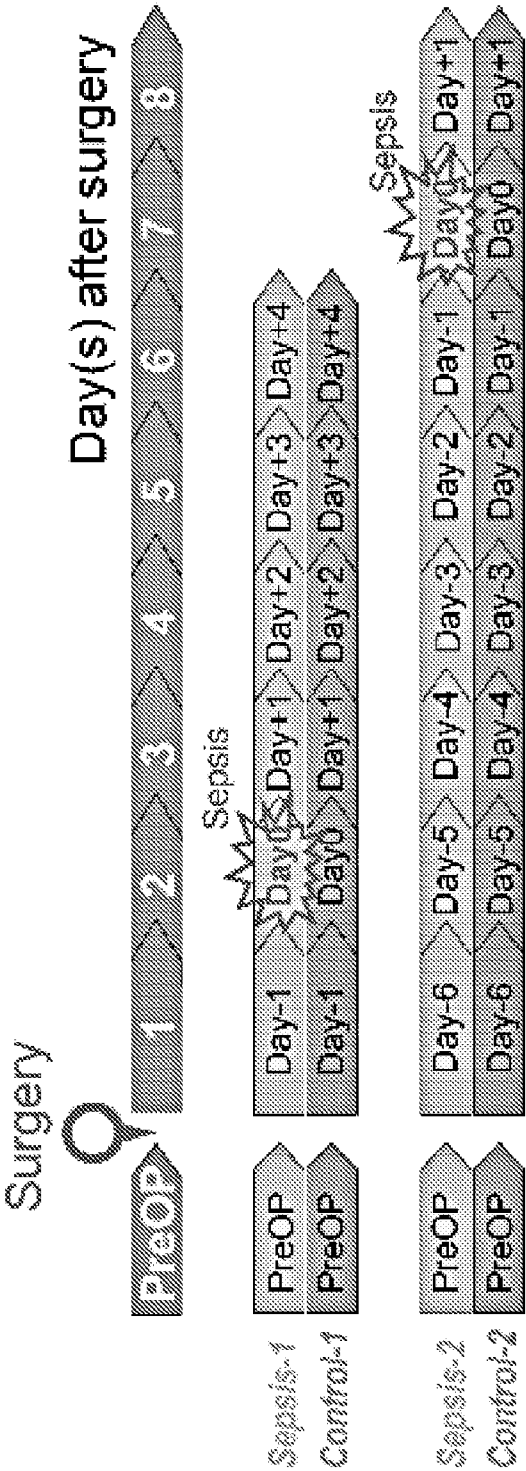


FIG. 20D

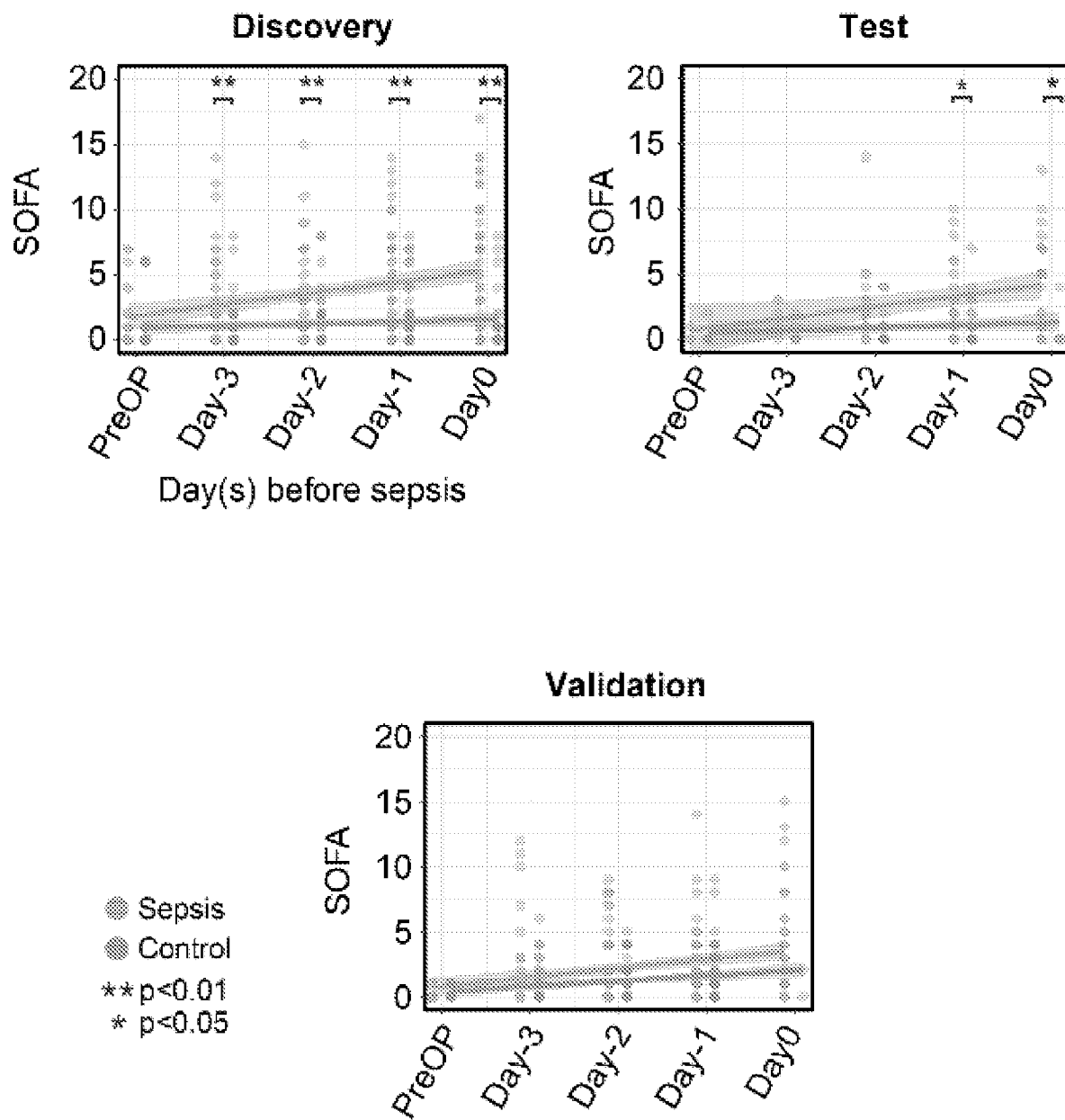


FIG. 20E

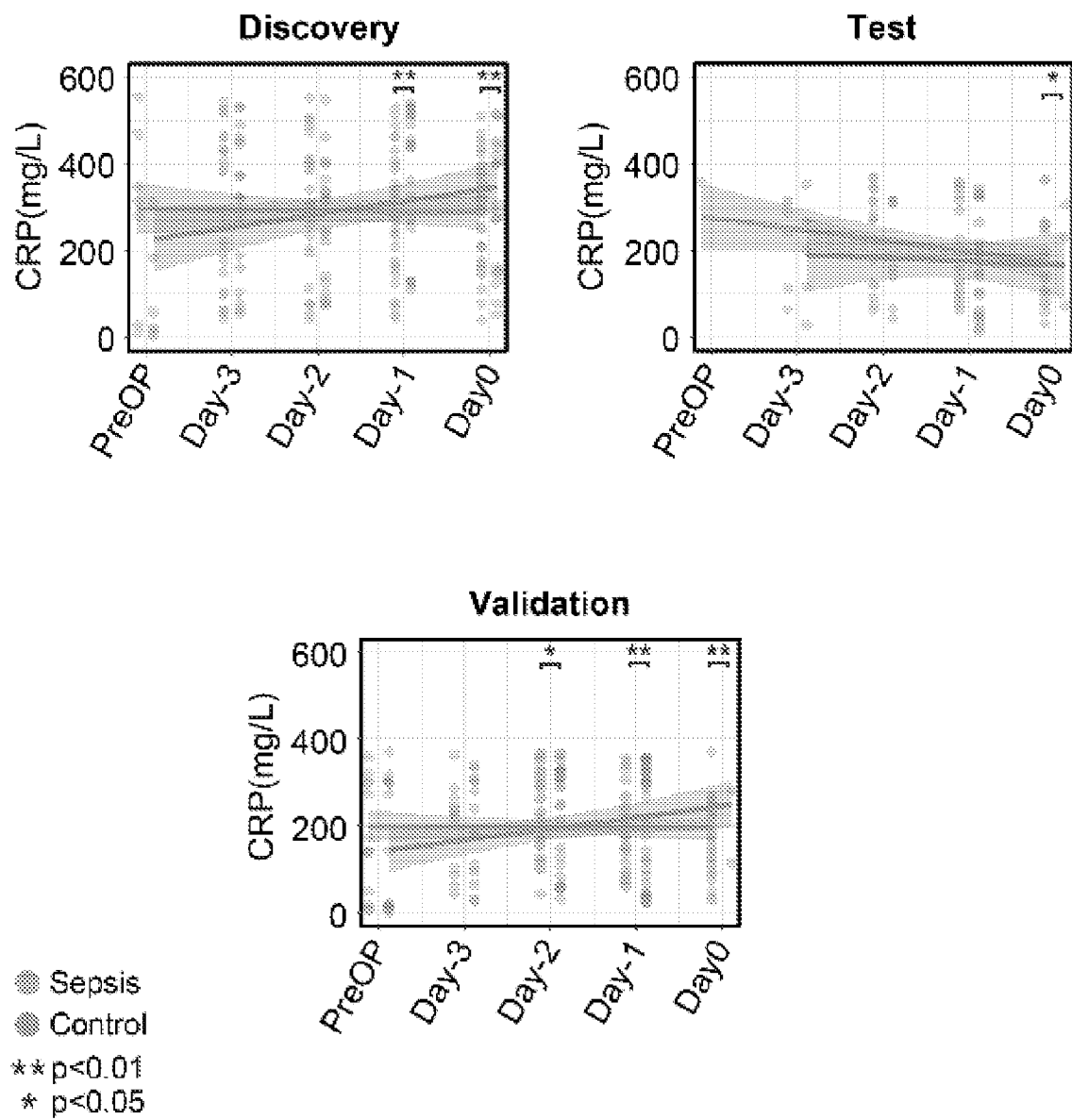


FIG. 20F

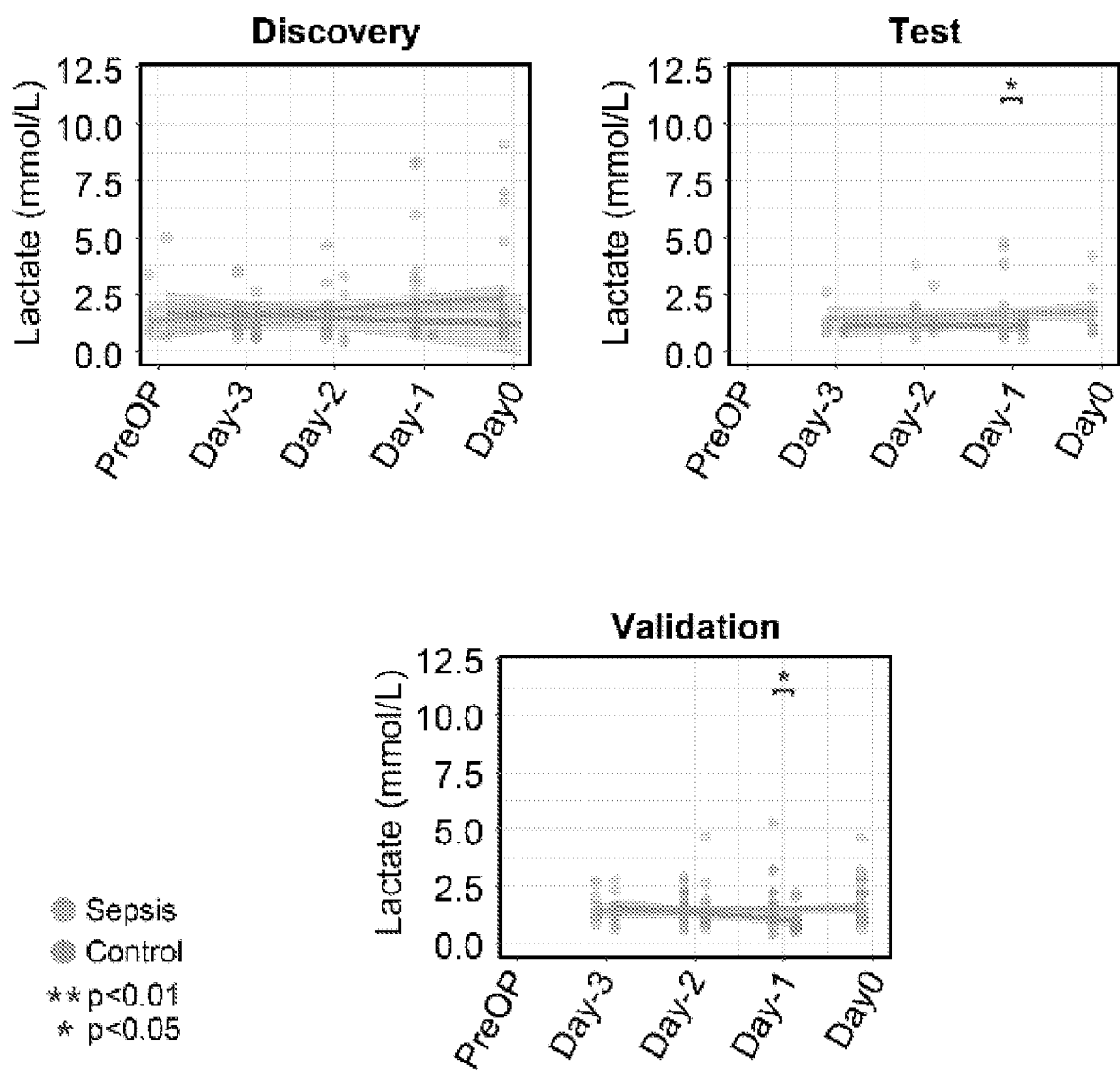


FIG. 20G

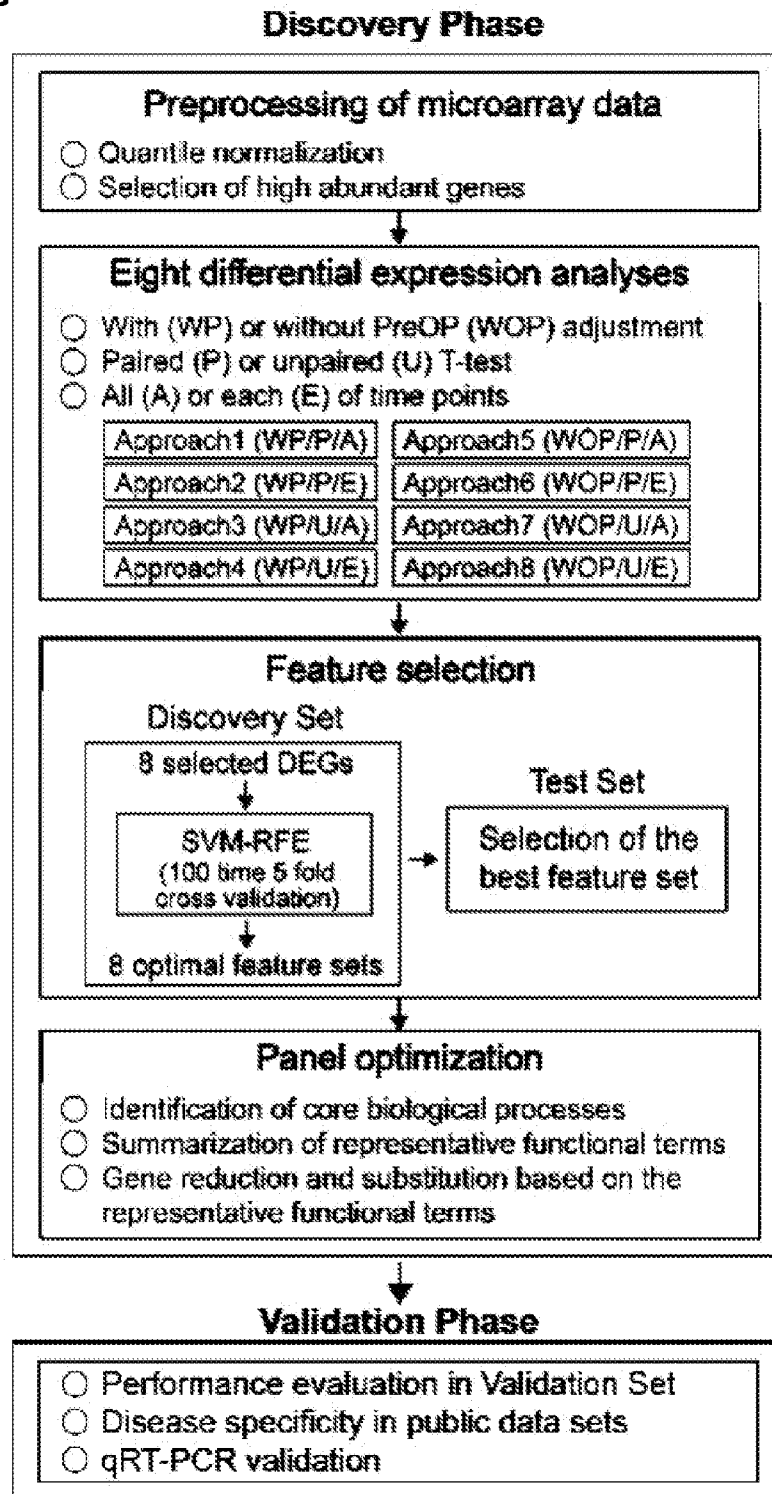


FIG. 21A

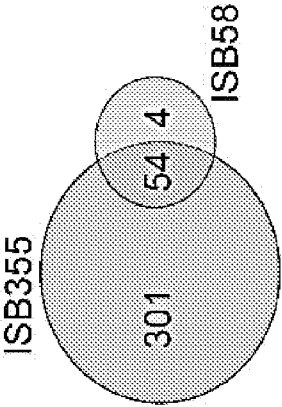


FIG. 21B

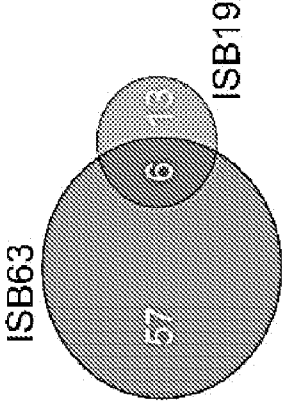


FIG. 21C

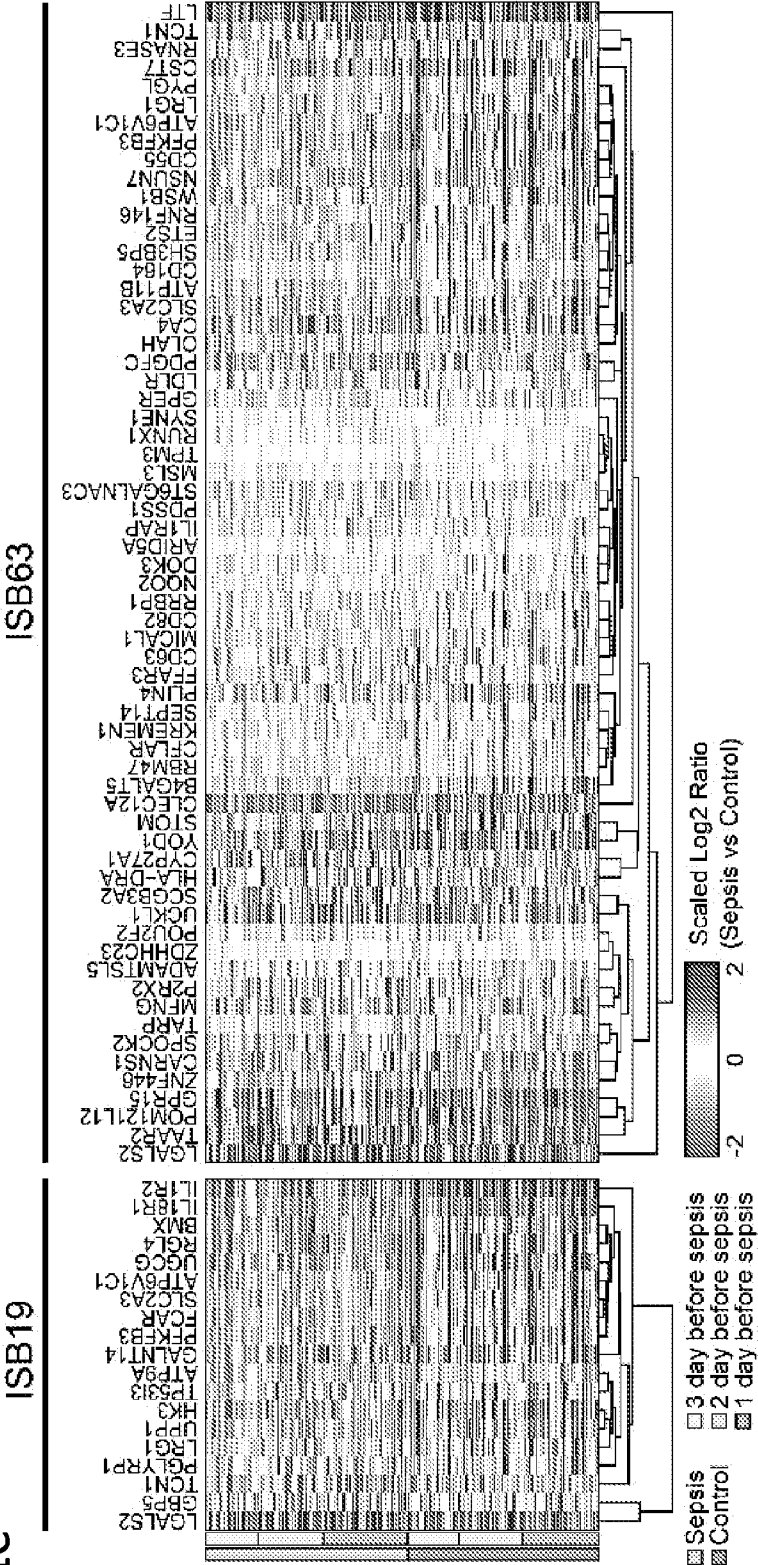


FIG. 22A

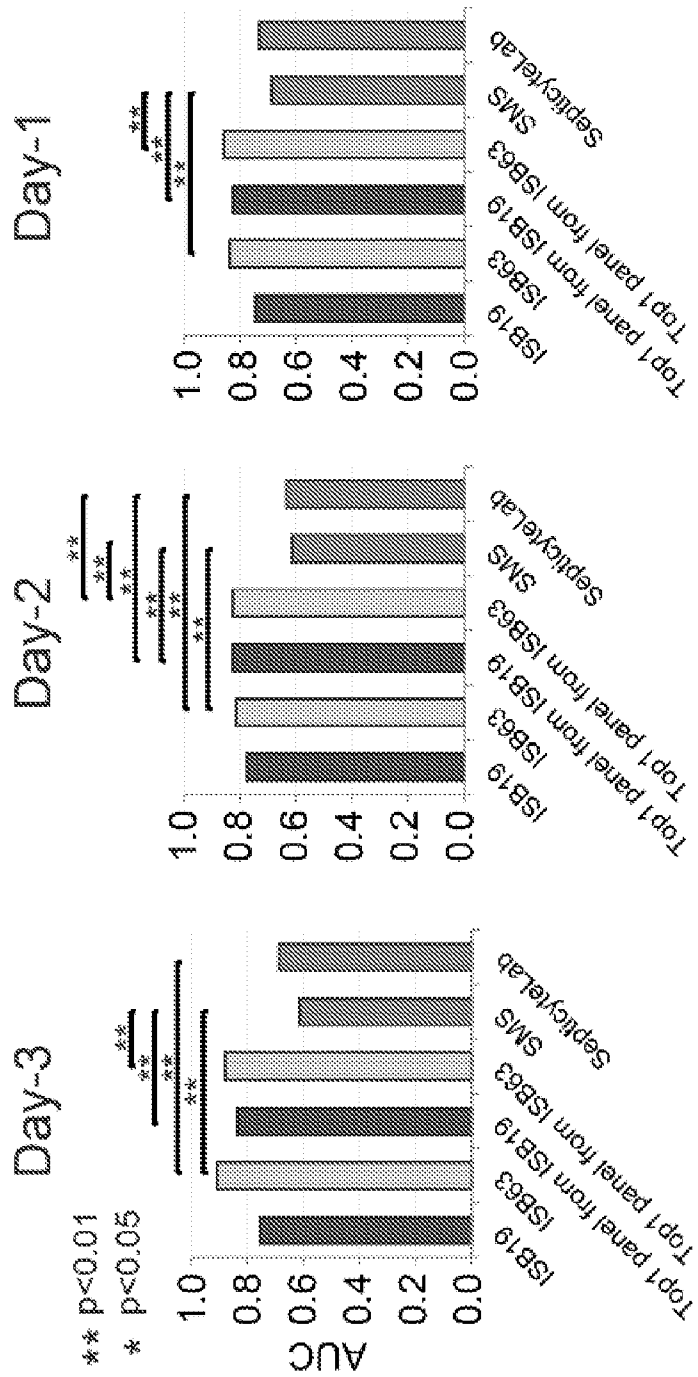


FIG. 22C

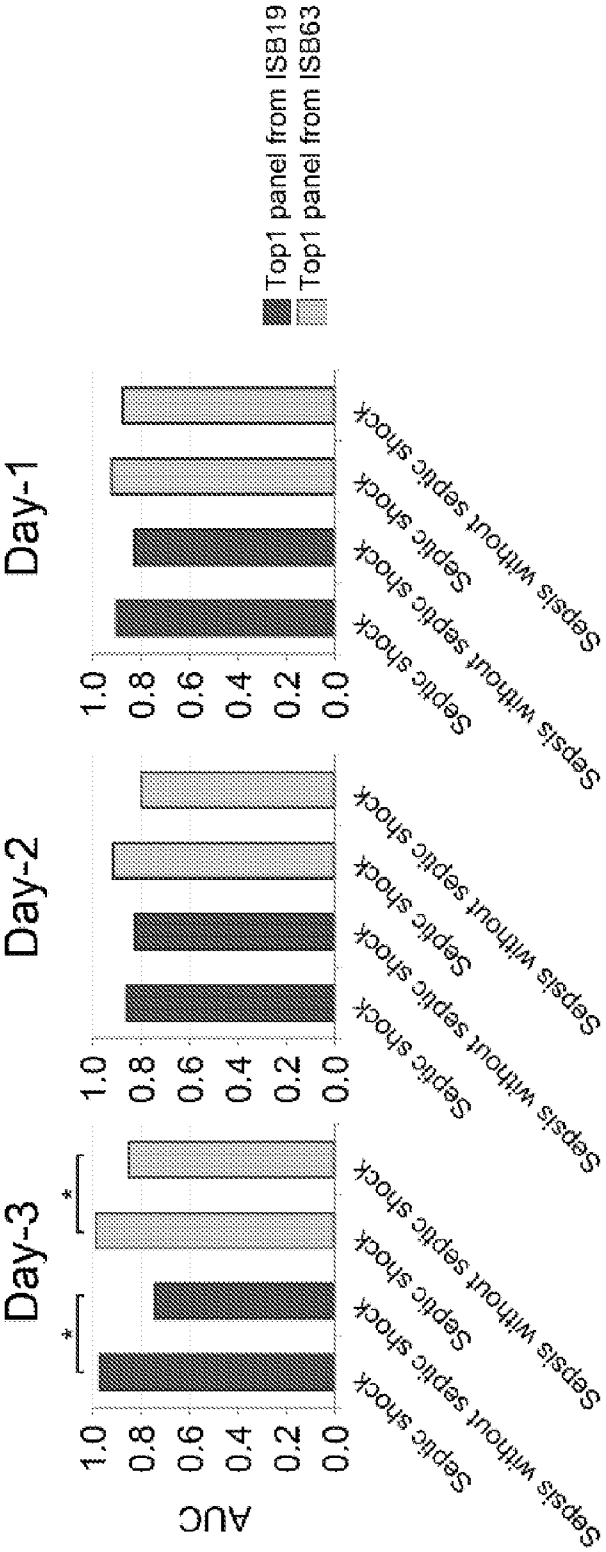


FIG. 22B

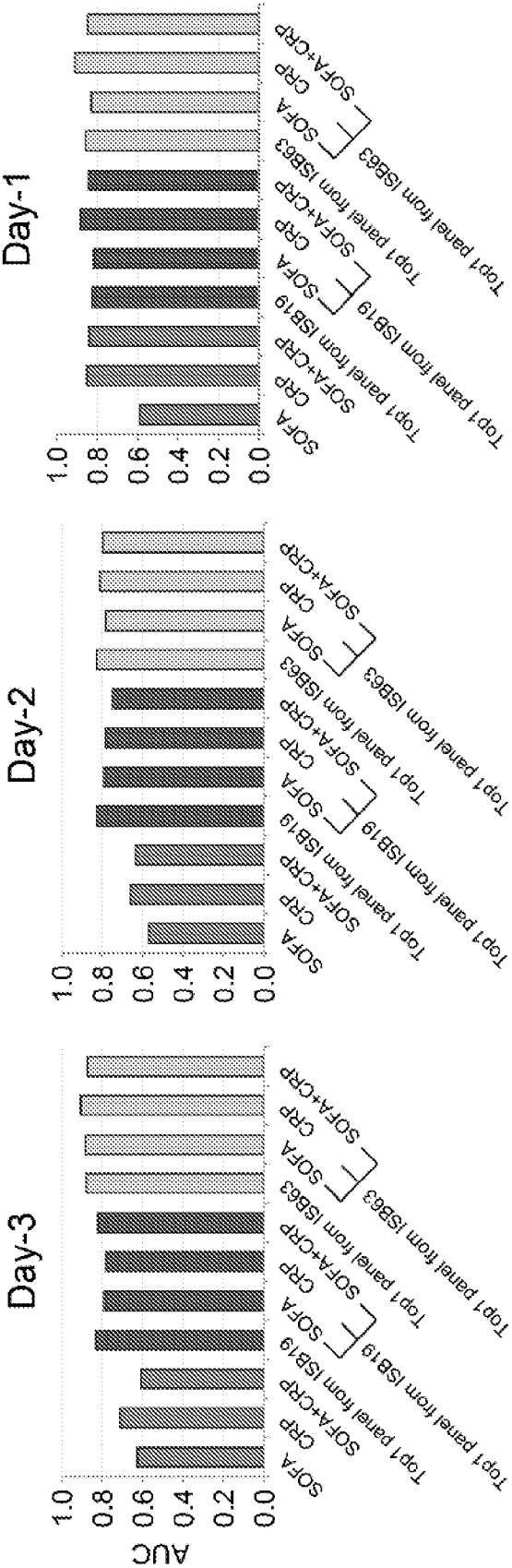


FIG. 23A

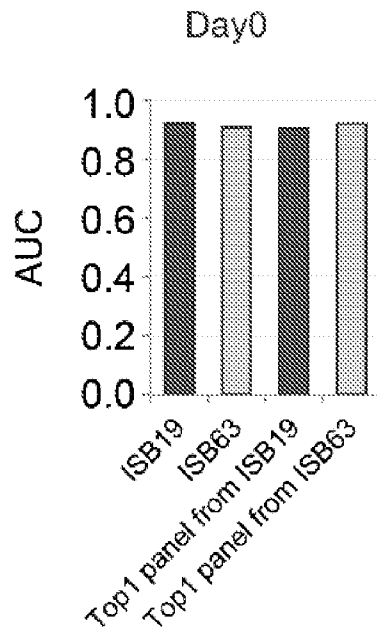


FIG. 23C

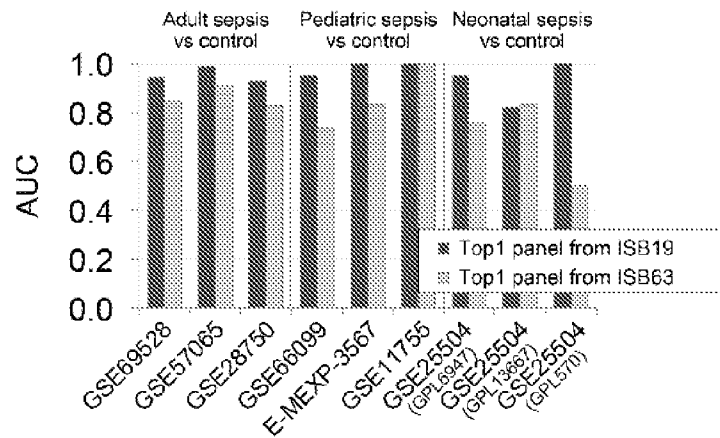


FIG. 23B

Subcategory	Accession	Platform	Clinical Comparison	# Control	# Case
Adult sepsis vs control	GSE69528	GPL10558	Adults with sepsis, many from burkholderia	55	83
	GSE57065	GPL570	Septic shock at admission, 24 hr, 48 hr	25	82
	GSE28750	GPL570	Community-acquired sepsis at admission to ICU	20	10
Pediatric sepsis vs control	GSE66099	GPL570	Pediatric sepsis	47	199
	E-MEXP-3567	GPL571	Children with meningococcal sepsis ± HIV coinfection	3	12
	GSE11755	GPL570	Children with meningococcal sepsis	3	6
Neonatal sepsis vs control	GSE25504	GPL6947	Neonatal sepsis	35	28
	GSE25504	GPL13667	Neonatal sepsis	6	14
	GSE25504	GPL570	Neonatal sepsis	3	2

FIG. 23D

Bacterial infection vs control	GSE64456	GPL10558	Bacterial infection vs Control	19	89
	GSE42834	GPL10558	TB vs Control	113	35
	GSE42830	GPL10558	TB vs Control	38	16
	GSE22098	GPL6947	Staphylococcus and Streptococcus vs Control	81	52
Viral infection vs control	GSE38900	GPL6884	RSV/Rhino/Influenza vs Control	31	153
	GSE68310	GPL10558	Outpatients with acute viral illness at days 0 and 2	243	258
	GSE17156	GPL571	Viral challenge peak symptoms	56	27
	E-MTAB-3162	GPL570	Dengue fever ($\pm$ severe) within 48 hr of fever	15	30
Autoimmune disease	GSE65391	GPL10558	SLE vs Healthy Control	72	924
	GSE29536	GPL6102	SLE vs Control	116	96
	GSE17590	GPL6106	SoJIA vs Healthy Control	21	64

FIG. 23E

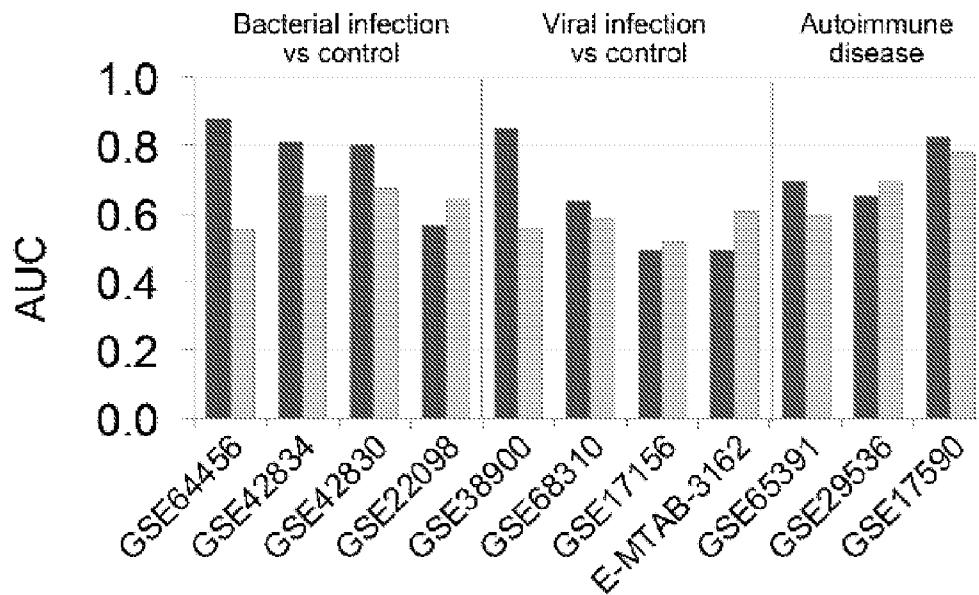


FIG. 24A

	Top1 panel from ISB19	Top1 panel from ISB63	
Immune response	<i>LCN2</i>	<i>GNG10</i>	Replaceable genes
Signal transduction	<i>BMX</i>	<i>STOM</i>	Replaceable genes
Metabolism	<i>SLC2A3</i>	<i>MPO</i>	
Apoptosis		<i>RPS6KA3</i>	
Transcription		<i>BCL6</i>	
Adhesion/Migration		<i>TPM3</i>	Replaceable genes

FIG. 24B

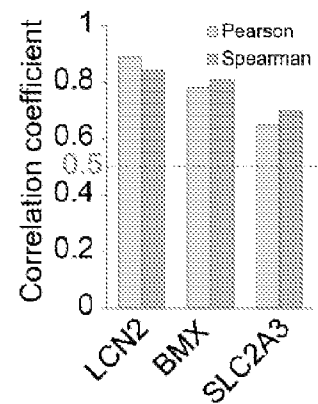
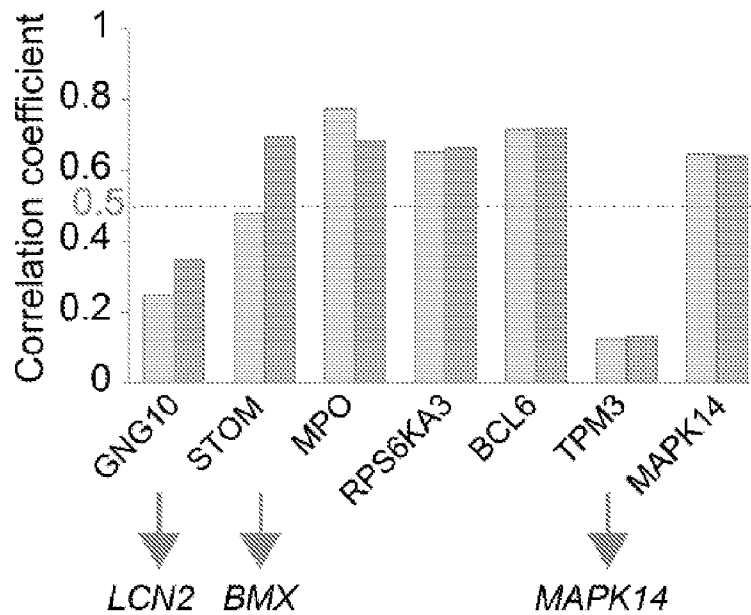


FIG. 24D



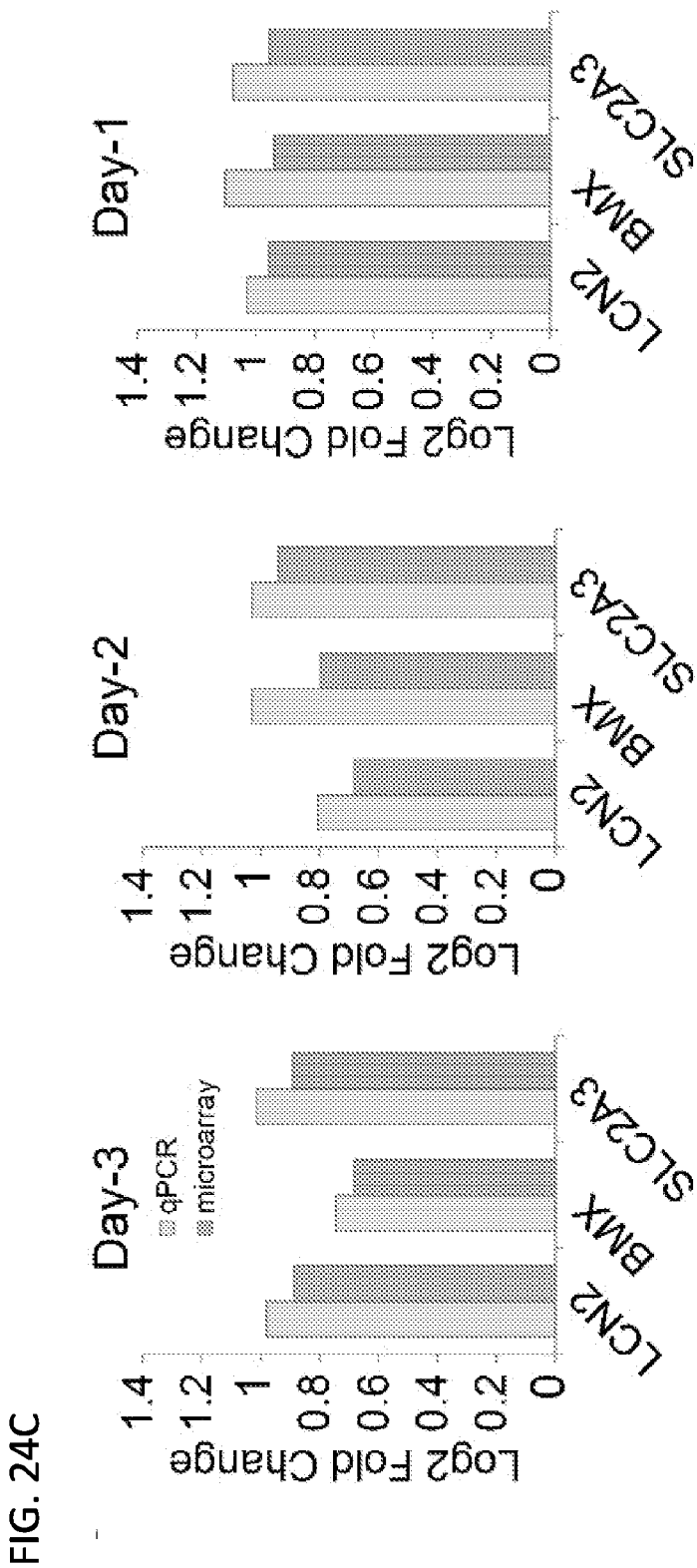


FIG. 24E

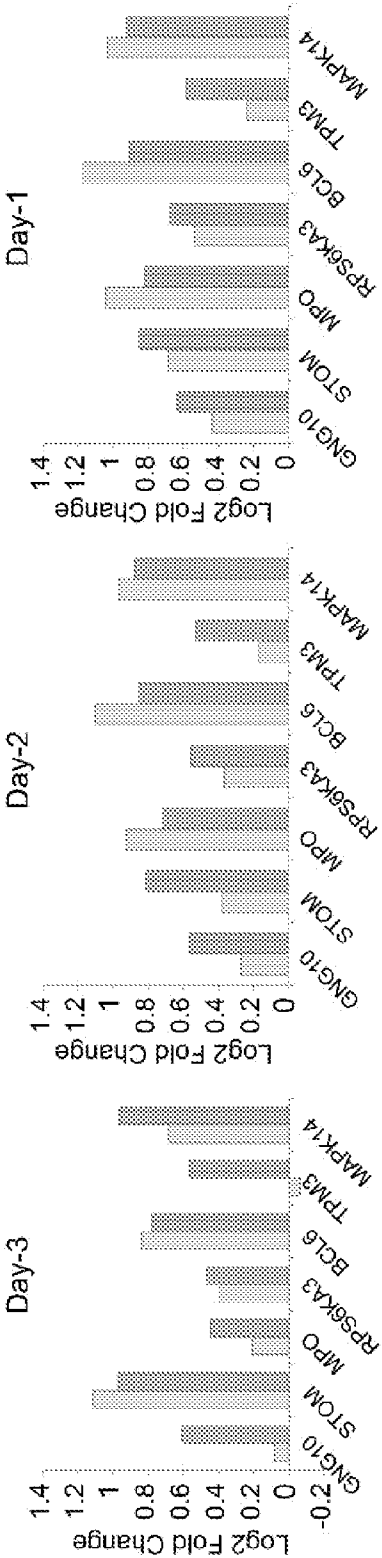


FIG. 24G

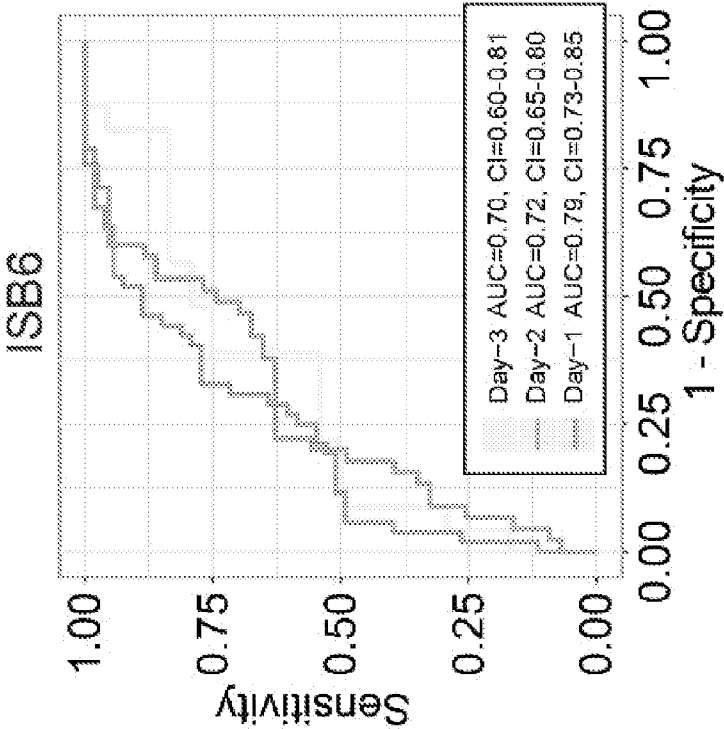


FIG. 24F

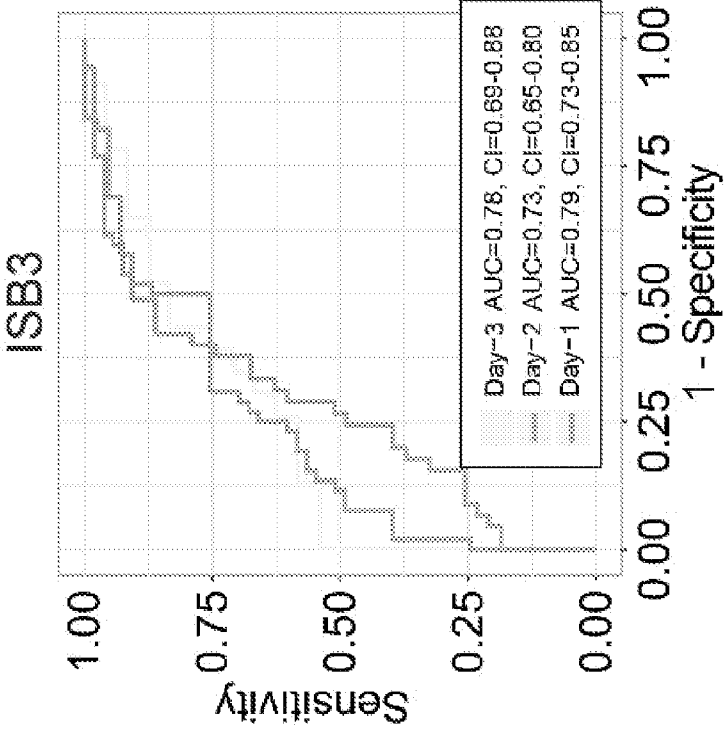


FIG. 24H

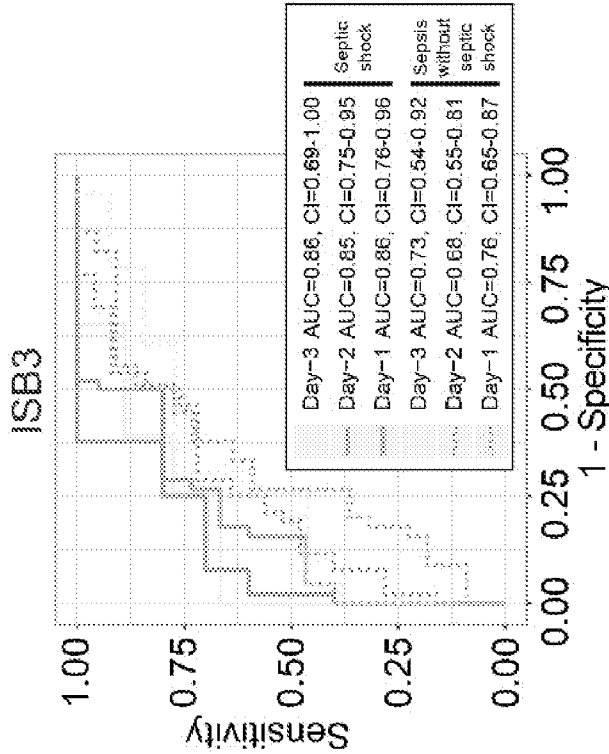
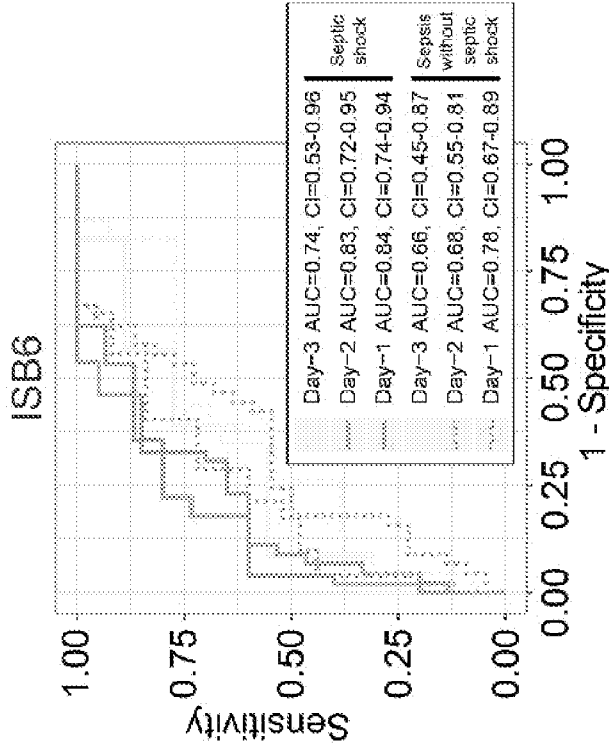


FIG. 24I



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US19/59707

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12Q 1/68; G01N 33/53; C40B 40/08 (2020.01)

CPC - C12Q 1/68; G01N 33/53; C40B 40/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2016/145426 A1 (THE BOARD OF TRUSTEES OF THE LELAND) 15 September 2016; claims 1, 18	1-2, 5/1-2, 13, 14/13, 30, 35
A	WO 2014/209238 A1 (ACUMEN RESEARCH LABORATORIES PTE. LTD.) 31 December 2014; claim 1	1-2, 5/1-2, 13, 14/13, 30, 35
A	WO 2008/143890 A2 (CHILDREN'S HOSPITAL MEDICAL CENTER) 27 November 2008; abstract; paragraph [0077]	1-2, 5/1-2, 13, 14/13, 30, 35

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

17 March 2020 (17.03.2020)

Date of mailing of the international search report

01 APR 2020

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US19/59707

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 11, 15, 23-27, 32, 37
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- Please See within the Next Supplemental Page-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Group 1+ Claims 1-2 (each in-part), 5 (in-part), 13-14 (each in-part), 30 (in-part), and 35 (in-part); LCN2, BMX, MPO, RPS6KA3, BCL6, MAPK14 (gene signature set)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

-***-Continued from Box III Observations where unity of invention is lacking -***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+, Claims 1-10, 12-14, 16-22, 28-31, 33-36; LCN2, BMX, MPO, RPS6KA3, BCL6, MAPK14 (gene signature set) are directed toward methods for diagnosing and/or predicting presence of sepsis in a subject using a gene signature set as well as binding molecules and kits containing such sets.

The methods will be searched to the extent that they encompass a gene signature set comprising LCN2, BMX, MPO, RPS6KA3, BCL6, and MAPK14 (first exemplary gene signature set). Applicant is invited to elect additional gene signature set(s), and available as an option within at least one searchable claim, to be searched. Additional gene signature set(s) will be searched upon the payment of additional fees. It is believed that claims 1-2 (each in-part), 5 (in-part), 13-14 (each in-part), 30 (in-part), and 35 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass LCN2, BMX, MPO, RPS6KA3, BCL6, and MAPK14 (gene signature set). Applicants must specify the searchable claims that encompass any additionally elected gene signature set(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be RPS6KA3, BCL6, TPM3, GNG10, STOM, MPO (gene signature set).

No technical features are shared between the gene signature sets of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features including: a method, comprising measuring expression of a set of at least six genes in a sample from a subject having or suspected to have sepsis, wherein the expression of the set of genes is altered compared to a control; a method of diagnosing and/or predicting sepsis in a subject comprising measuring expression of a set of at least six genes in a sample from a subject, diagnosing the subject with sepsis and/or predicting development of sepsis in the subject when the expression of the set of genes is altered compared to a control; a method, comprising measuring expression of a set of at least three genes in a sample from a subject having or suspected to have sepsis, wherein the expression of the set of genes is altered compared to a control; a method, comprising measuring expression of a set of at least three genes in a sample from a subject having or suspected to have sepsis, wherein the expression of the set of genes is altered compared to a control; a method of diagnosing and/or predicting sepsis in a subject comprising measuring expression of a set of at least three genes in a sample from a subject, diagnosing the subject with sepsis and/or predicting development of sepsis in the subject when the expression of the set of genes is altered compared to a control; a method of treating sepsis comprising, i) measuring expression of a set of three or more genes in a sample from a subject; wherein expression of one or more genes is increased compared to the control; and ii) administering one or more treatments for sepsis to the subject; a method of treating sepsis comprising, i) measuring expression of a set of six or more genes in a sample from a subject; wherein expression of one or more genes is increased compared to the control; and ii) administering one or more treatments for sepsis to the subject; a method, comprising measuring expression of a set of at least six genes in a sample from a subject having or suspected to have sepsis, wherein the expression of genes is increased compared to a control and the expression of genes is decreased compared to a control; a method of diagnosing sepsis in a subject comprising measuring expression of a set of at least six genes in a sample from a subject, diagnosing the subject with sepsis wherein the expression of genes is increased compared to a control and the expression of genes is decreased compared to a control; a method of treating sepsis comprising, i) measuring expression of a set of six or more genes in a sample from a subject; wherein the expression of genes is increased compared to a control and the expression of genes is decreased compared to a control; and ii) administering one or more treatments for sepsis to the subject; a solid support comprising binding agents specific for a set of genes, wherein the specific binding agents are covalently attached to the solid substrate; a kit comprising a set of primers and/or probes capable of specifically binding to a set of genes; these shared technical features are previously disclosed by WO 2016/145426 A1 (THE BOARD OF TRUSTEES OF THE LELAND) (hereinafter 'Leland').

-***-Continued on Next Supplemental Page-***-

-Continued from Previous Supplemental Page-:

Leland discloses a method (claim 1), comprising measuring expression of a set of at least six genes in a sample from a subject having or suspected to have sepsis (comprising measuring expression of a set of at least six genes in a sample from a subject having or suspected to have sepsis; claims 1, 18), wherein the expression of the set of genes is altered compared to a control (wherein the expression of the set of genes is altered compared to a control; claim 1); a method of diagnosing and/or predicting sepsis in a subject comprising measuring expression of a set of at least six genes in a sample from a subject (method of diagnosing and/or predicting sepsis in a subject comprising measuring expression of a set of at least six genes in a sample from a subject; claim 1), diagnosing the subject with sepsis (claim 1) and/or predicting development of sepsis in the subject when the expression of the set of genes is altered compared to a control (when the expression of the set of genes is altered compared to a control; claim 1); a method (claim 1), comprising measuring expression of a set of at least three genes in a sample from a subject having or suspected to have sepsis (comprising measuring expression of a set of at least three genes in a sample from a subject having or suspected to have sepsis; claims 1, 18), wherein the expression of the set of genes is altered compared to a control (claim 1); a method of diagnosing and/or predicting sepsis in a subject comprising measuring expression of a set of at least three genes in a sample from a subject (a method of diagnosing and/or predicting sepsis in a subject comprising measuring expression of a set of at least three genes in a sample from a subject; claim 1), diagnosing the subject with sepsis and/or predicting development of sepsis in the subject when the expression of the set of genes is altered compared to a control (diagnosing the subject with sepsis and/or predicting development of sepsis in the subject when the expression of the set of genes is altered compared to a control; claim 1); a method of treating sepsis (a method of treating sepsis; claim 9) comprising, i) measuring expression of a set of three or more genes in a sample from a subject (measuring expression of a set of three or more genes in a sample from a subject; claims 1, 9); wherein expression of one or more genes is increased compared to the control (wherein expression of one or more genes is increased compared to the control; claim 1); and ii) administering one or more treatments for sepsis to the subject (administering one or more treatments for sepsis to the subject; claim 9); a method of treating sepsis (claim 9) comprising, i) measuring expression of a set of six or more genes in a sample from a subject (measuring expression of a set of six or more genes in a sample from a subject; claims 1, 9); wherein expression of one or more genes is increased compared to the control (wherein expression of one or more genes is increased compared to the control; claim 1); and ii) administering one or more treatments for sepsis to the subject (administering one or more treatments for sepsis to the subject; claim 9); a method, comprising measuring expression of a set of at least six genes in a sample from a subject having or suspected to have sepsis (a method, comprising measuring expression of a set of at least six genes in a sample from a subject having or suspected to have sepsis; claims 1, 18), wherein the expression of genes is increased compared to a control and the expression of genes is decreased compared to a control (wherein the expression of genes is increased compared to a control and the expression of genes is decreased compared to a control; claim 1); a method of diagnosing sepsis in a subject comprising measuring expression of a set of at least six genes in a sample from a subject (a method of diagnosing sepsis in a subject comprising measuring expression of a set of at least six genes in a sample from a subject; claim 1), diagnosing the subject with sepsis wherein the expression of genes is increased compared to a control and the expression of genes is decreased compared to a control (diagnosing the subject with sepsis wherein the expression of genes is increased compared to a control and the expression of genes is decreased compared to a control; claim 1); a method of treating sepsis comprising, i) measuring expression of a set of six or more genes in a sample from a subject (a method of treating sepsis comprising, i) measuring expression of a set of six or more genes in a sample from a subject; claims 1, 9); wherein the expression of genes is increased compared to a control and the expression of genes is decreased compared to a control (wherein the expression of genes is increased compared to a control and the expression of genes is decreased compared to a control; claim 1); and ii) administering one or more treatments for sepsis to the subject (claim 1); a solid support comprising binding agents specific for a set of genes (a solid support comprising binding agents specific for a set of genes; page 26, lines 6-12), wherein the specific binding agents are covalently attached to the solid substrate (wherein the specific binding agents are covalently attached to the solid substrate; page 26, lines 21-24); a kit comprising a set of primers and/or probes capable of specifically binding to a set of genes (a kit comprising a set of primers and/or probes capable of specifically binding to a set of genes; page 26, lines 11-12; claims 14, 16).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Leland reference, unity of invention is lacking.