Supplementary information:

Gene expression signatures identify paediatric patients with multiple organ dysfunction who require advanced life support in the intensive care unit

Rama Shankar (ramashan@msu.edu)[1,2], Mara L. Leimanis (Mara.Leimanis@spectrumhealth.org)[1,3], Patrick A. Newbury (rnewbury@gmail.com)[1,2], Ke Liu (liuke2@msu.edu)[1,2], Jing Xing (xingjin1@msu.edu)[1,2], Derek Nedveck (dnedveck@gmail.com)[4], Eric J. Kort (eric.kort@helendevoschildrens.org)[1,5,6], Jeremy W Prokop (prokopje@msu.edu)[1,2], Guoli Zhou (zhoug@msu.edu)7, André S Bachmann (bachma26@msu.edu)[1], Bin Chen (chenbi12@msu.edu)[1,2*], Surender Rajasekaran (surender.rajasekaran@spectrumhealth.org)[1,3,4*]

1 Department of Pediatrics and Human Development, College of Human Medicine, Michigan State University, Grand Rapids, MI 49503, USA

2 Department of Pharmacology and Toxicology, College of Human Medicine, Michigan State University, Grand Rapids, MI 49503, USA

3 Pediatric Intensive Care Unit, Helen DeVos Children's Hospital, 100 Michigan Street NE, Grand Rapids, MI, 49503, USA

4 Office of Research, Spectrum Health, 15 Michigan Street NE, Grand Rapids, MI 49503, USA

5 DeVos Cardiovascular Program, Van Andel Research Institute and Fredrik Meijer Heart and Vascular Institute/Spectrum Health, Grand Rapids, MI 49503, USA

6 Pediatric Hospitalist Medicine, Helen DeVos Children's Hospital, 100 Michigan Street NE, Grand Rapids, MI, 49503, USA

7 Biomedical Research Informatics Core (BRIC), Clinical and Translational Sciences Institute (CTSI), Michigan State University, East Lansing, MI 48824, USA

* Correspondence: Dr. Bin Chen (chenbi12@msu.edu) and Dr. Surender Rajasekaran (surender.rajasekaran@spectrumhealth.org)

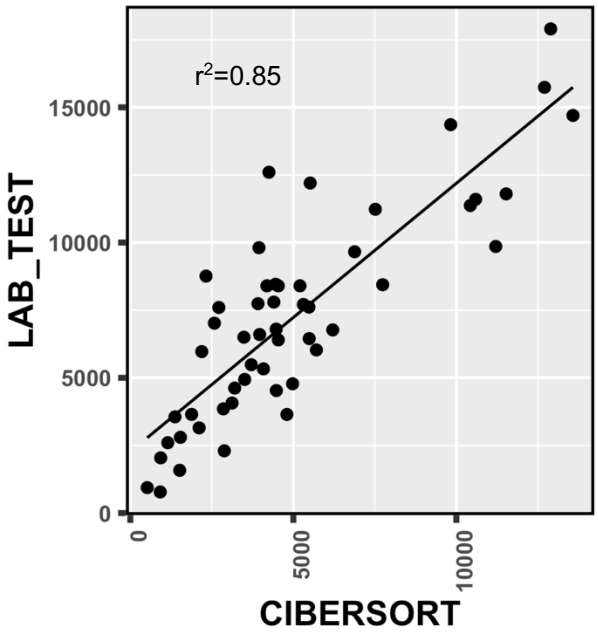

Figure S1. Correlation of neutrophils obtained from lab test data and derived from CIBERSORT. The values are closely correlated with each other (Correlation value= 0.85).

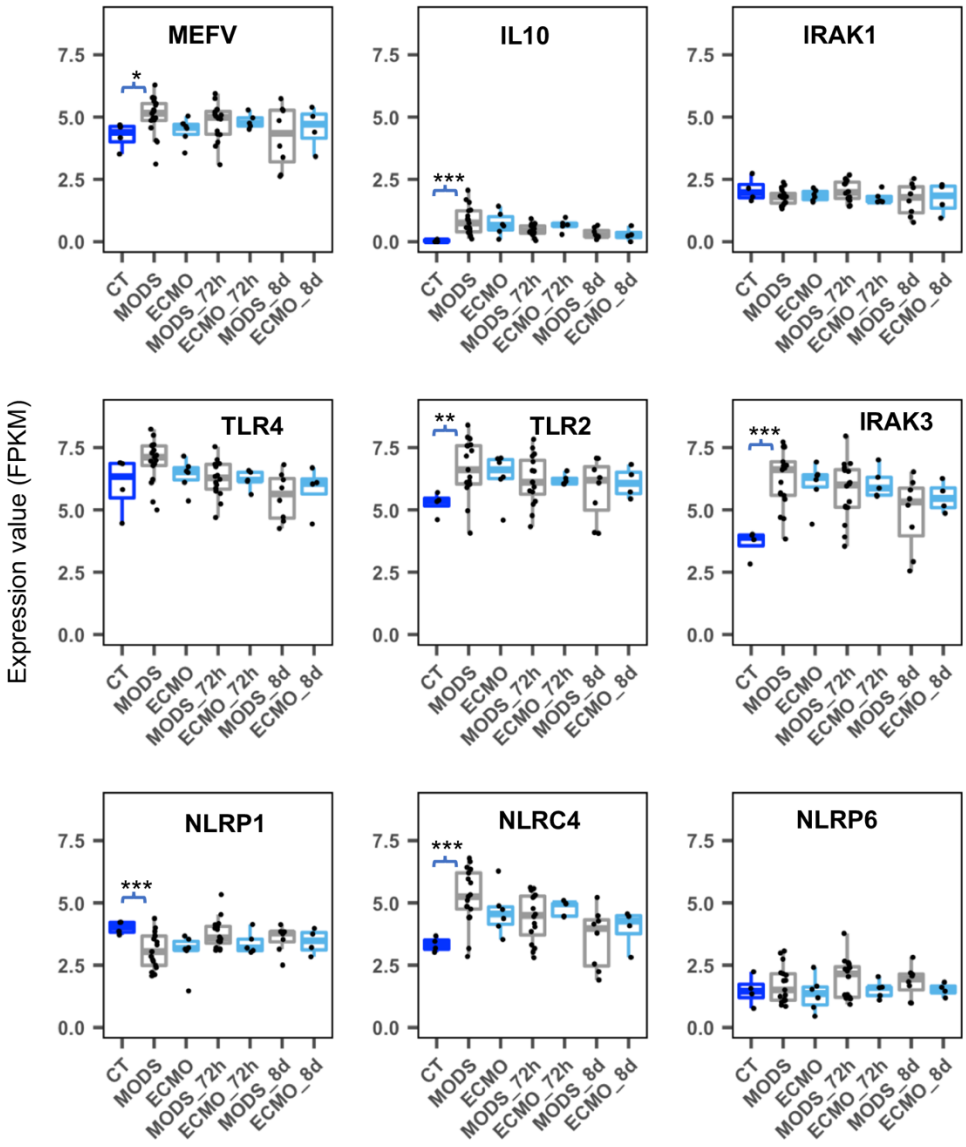

Figure S2. Expression of monocyte genes (based on study of Hall et al., 2007) in control (CT), MODS and ECMO patients at different time points (0h, 72h and 8d). (* 0.01 < P value < 0.05; ** 0.001 < P value < 0.01; *** 7.3e-6 < P value < 0.001). The p-value was estimated by Student's T-test.

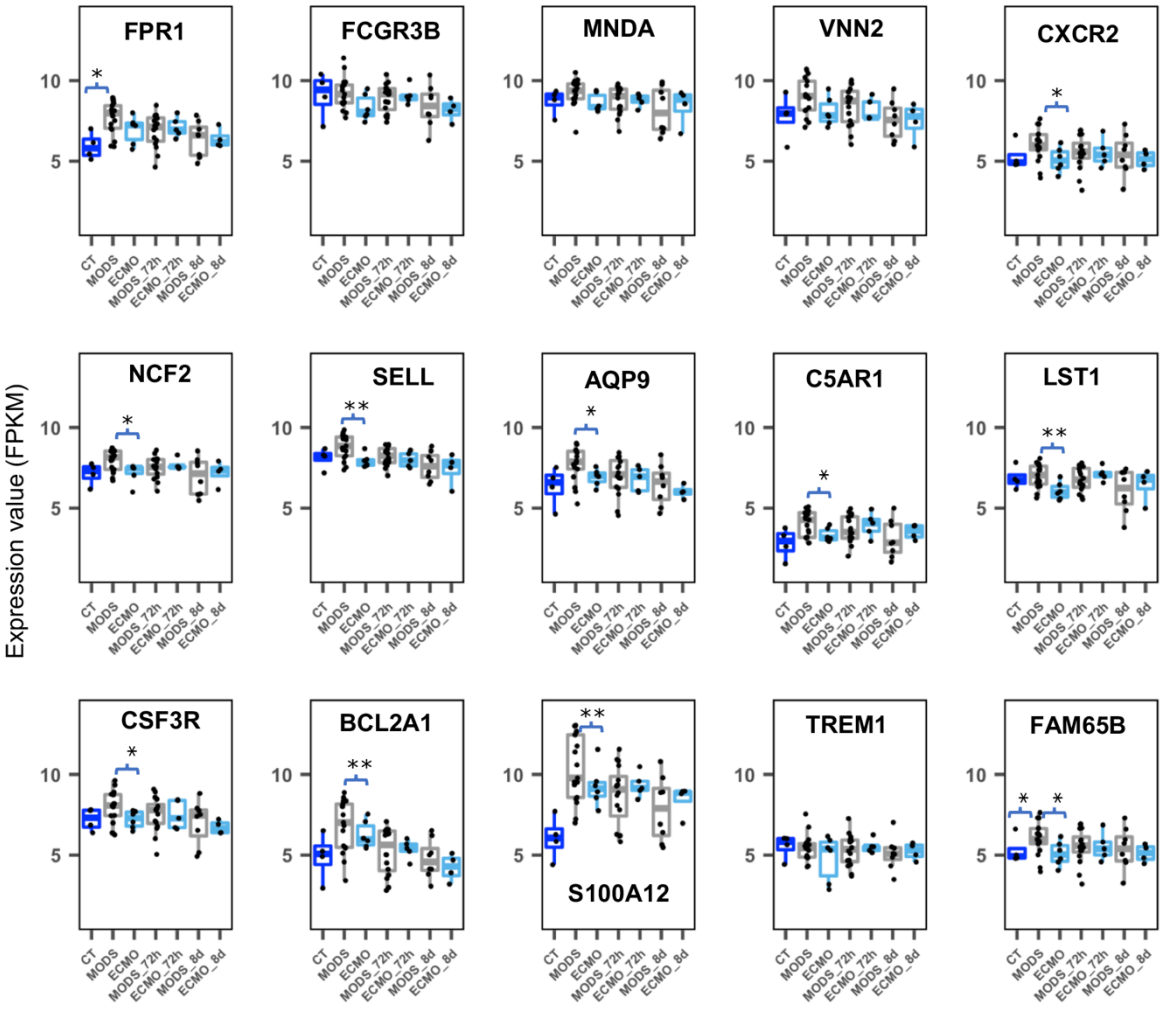

Figure S3. Expression of neutrophils genes (based on CIBERSORT cell marker) in control (CT), MODS and ECMO patients at different time points(0h, 72h and 8d). (* 0.05 > p <= 0.01 and ** 0.01 > p < 0.001). The p-value was estimated by Student's T-test.

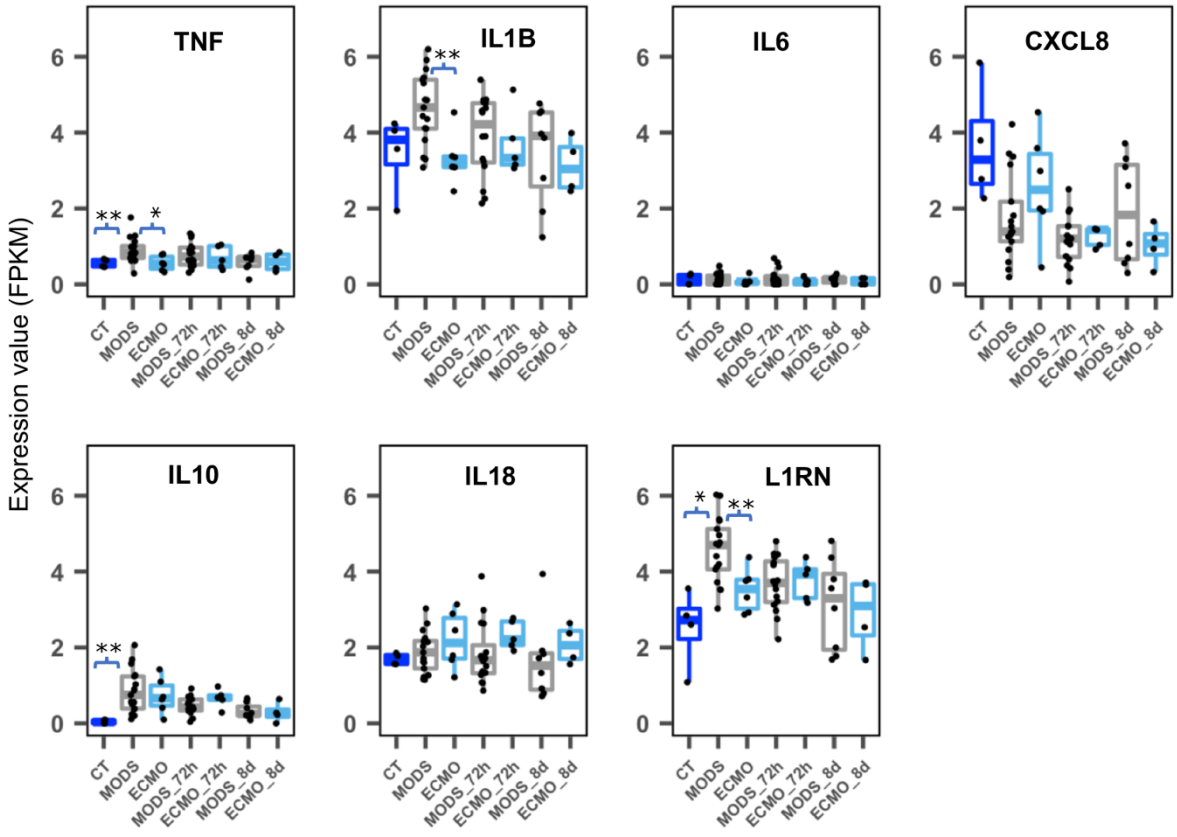

Figure S4. Expression of cytokines genes (based on study of Hall et al., 2007) in control (CT), MODS and ECMO patients at different time points(0h, 72h and 8d). (* 0.05 > p <= 0.01 and ** 0.01 > p < 2.5e-05). The p-value was estimated by Student's T-test.

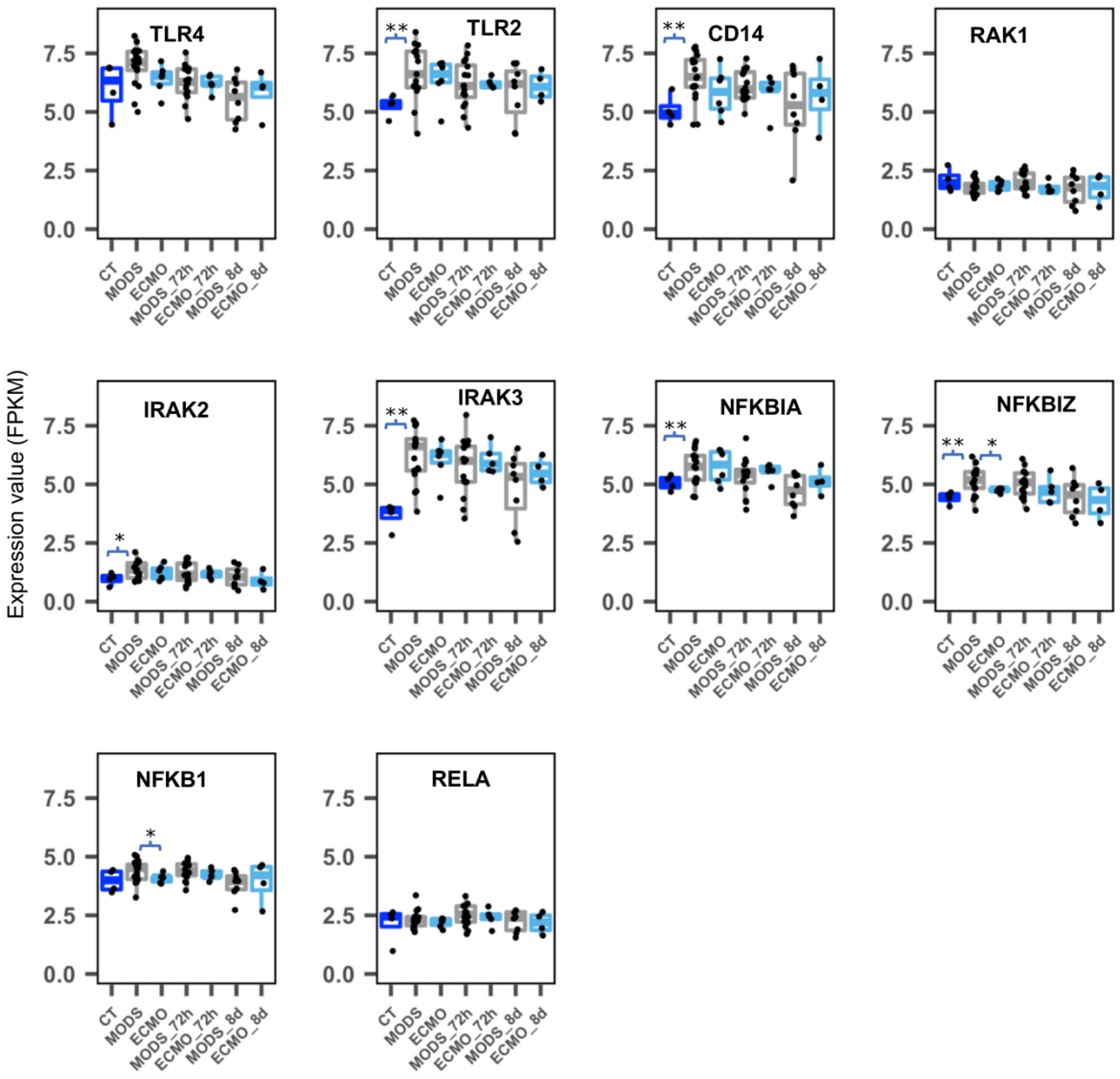

Figure S5. Expression of NF-kB signaling pathway (based on study of Hall et al., 2007) in control (CT), MODS and ECMO patients at different time points(0h, 72h and 8d). (* 0.05 > p <= 0.01 and ** 0.01 > p < 7.3e-05). The p-value was estimated by Student's T-test.

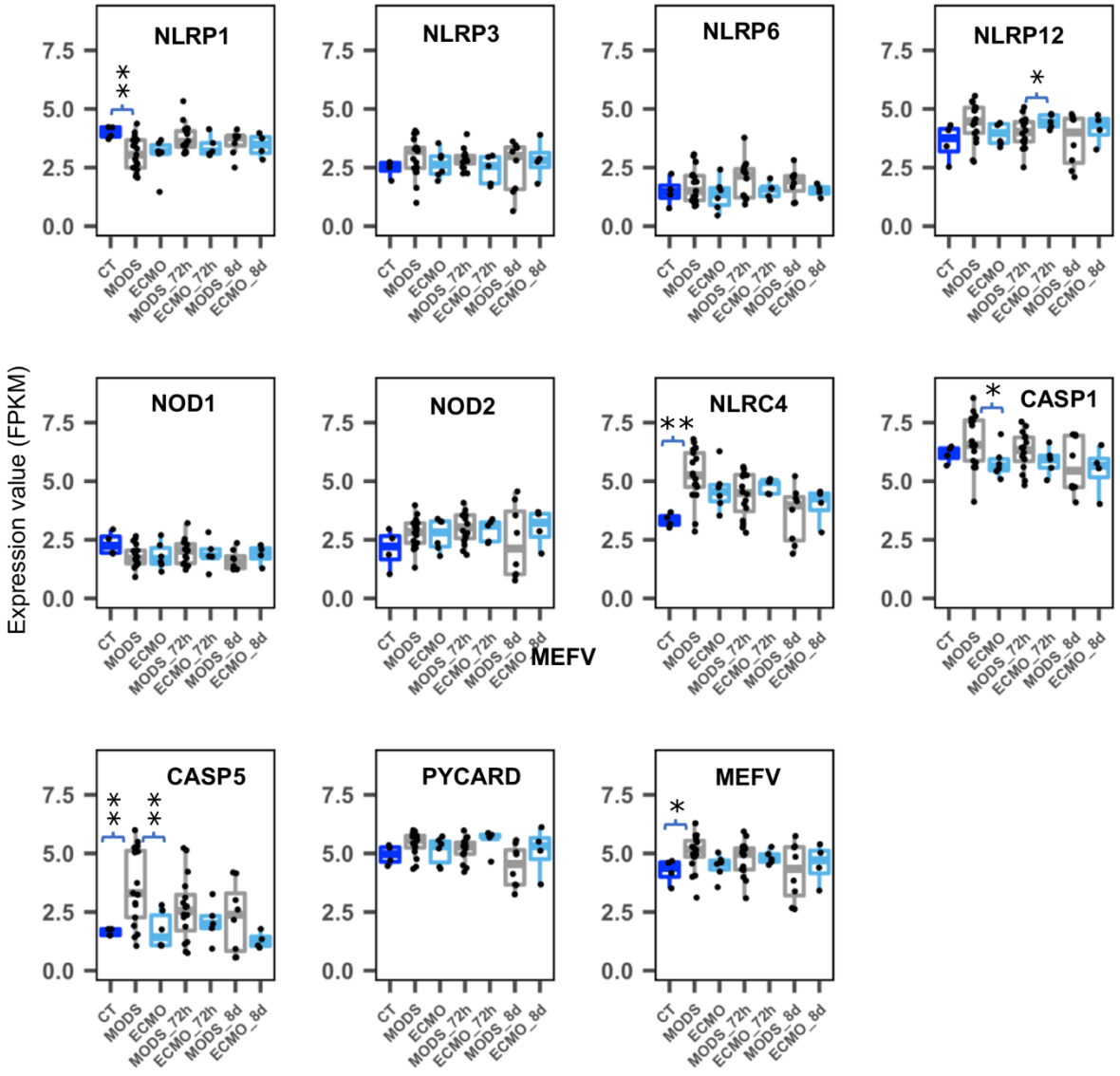

Figure S6. Expression of genes involved in inflammasome elements (based on study of Hall et al., 2007) in control (CT), MODS and ECMO patients at different time points(0h, 72h and 8d). (* 0.05 > p <= 0.01 and ** 0.01 > p < 7.8e-06). The p-value was estimated by Student's T-test.

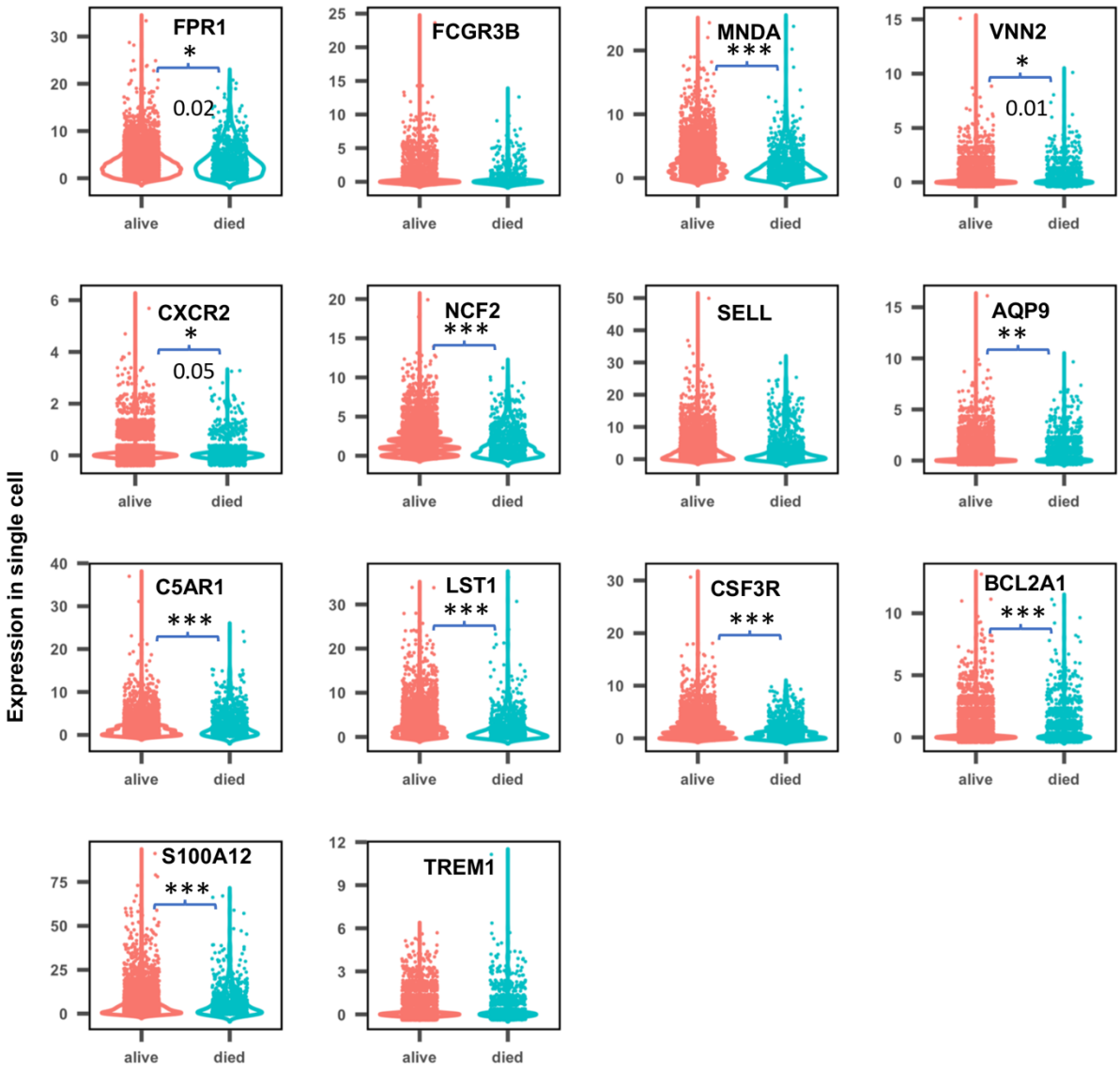

Figure S7. Expression of marker genes for neutrophils cells in single cell data of ECMO adult patients (Kort et al., 2019). Red- Surviving ECMO patients and Green- Died ECMO patients. (* 0.01 < P value < 0.05; ** 0.001 < P value < 0.01; *** 2e-16 < P value < 0.001). The p-value was estimated by Student's T-test.

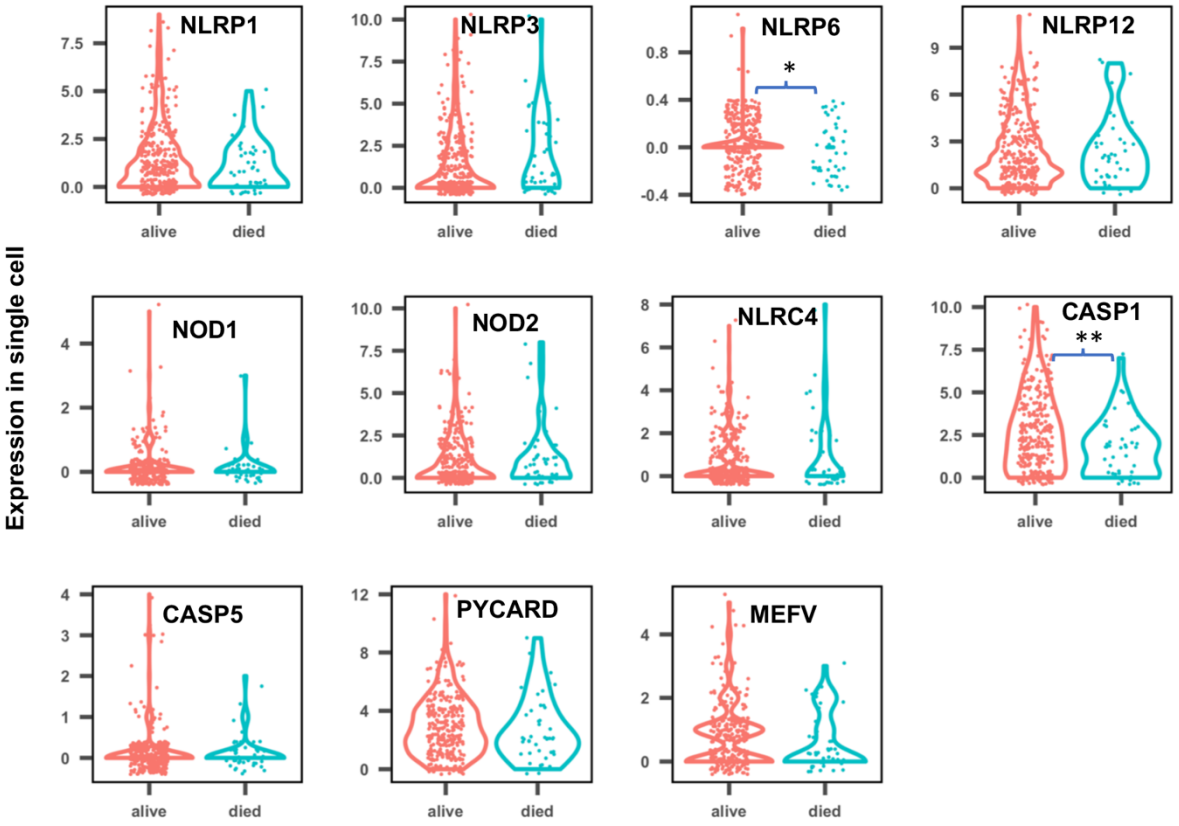

Figure S8. Expression of genes involved in inflammatory response in each cell from the single cell data of ECMO adult patients (Kort et al., 2019). Red- Surviving ECMO patients and Green- Died ECMO patients. (* 0.01 < P value < 0.05 and ** 0.0008 < P value < 0.01. The p-value was estimated by Student's T-test.

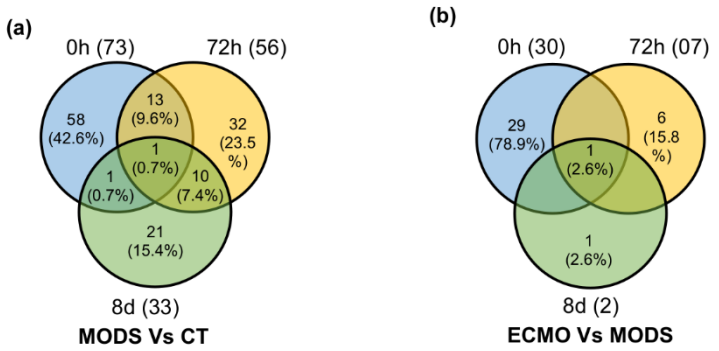

Figure S9. Comparisons of differential gene expression. Venn diagram showing the comparisons of differentially expressed genes in between (a) MODS and control (CT) and (b) in between ECMO and MODS patients at different time points; baseline (0h), 72h and 8d.

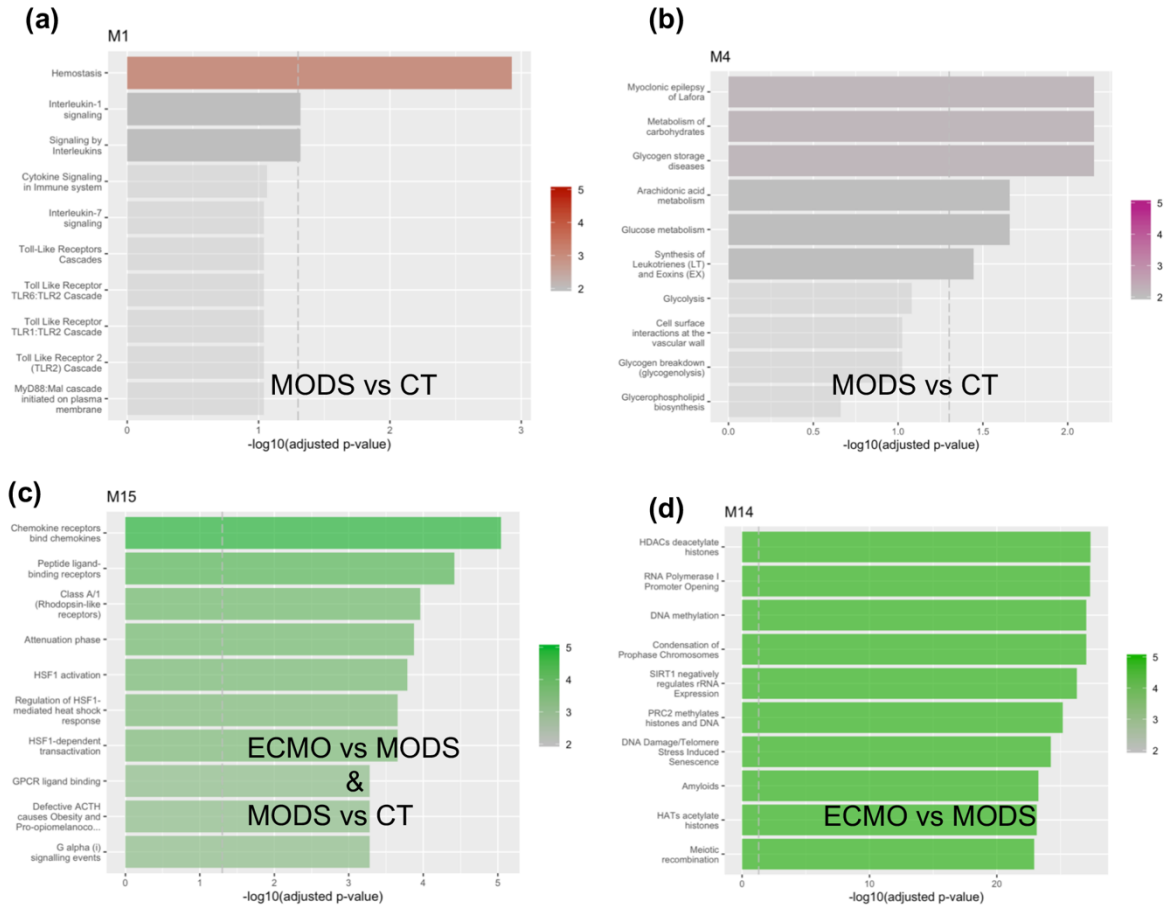

Figure S10. Pathways enriched by the genes present in different co-expressed networks module. (a, b) Enriched pathways are shown by the DE genes from MODS vs CT and mapped on two co-expressed networks. (c) Enriched pathways shared by the DE genes in MODS vs CT and in ECMO vs MODS are shown. This showed the transition from MODS to ECMO. (d) In addition, epigenetic modifications related processes were activated in the severe MODS patients, who require ECMO support.

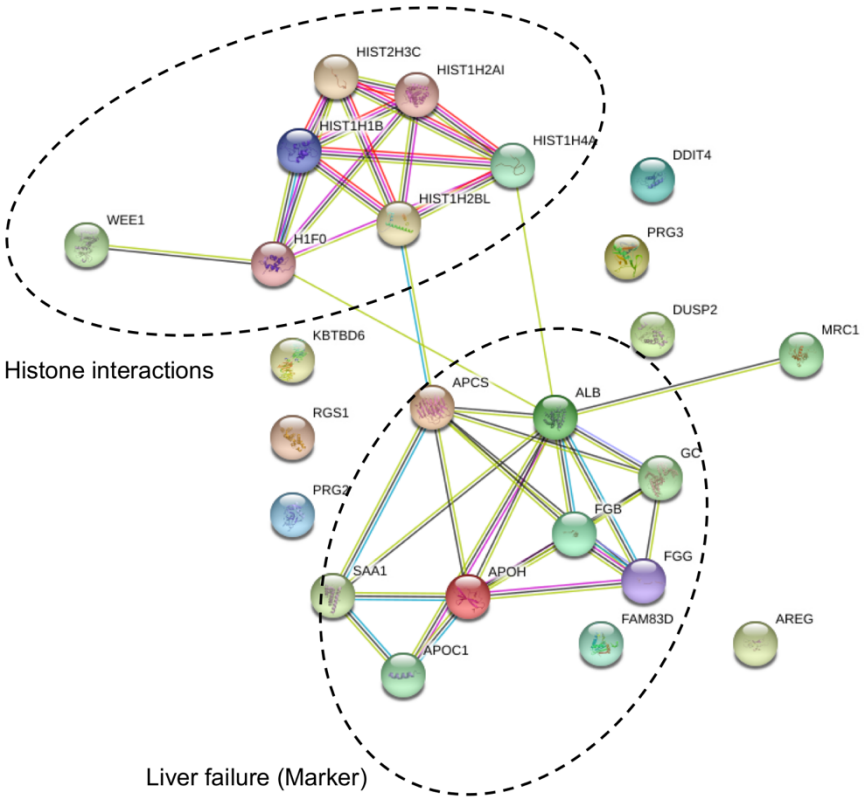

Figure S11. Interaction of genes associated with ECMO at baseline (0h). Two main networks (Histone interactions and gene markers for liver failure) were enriched.

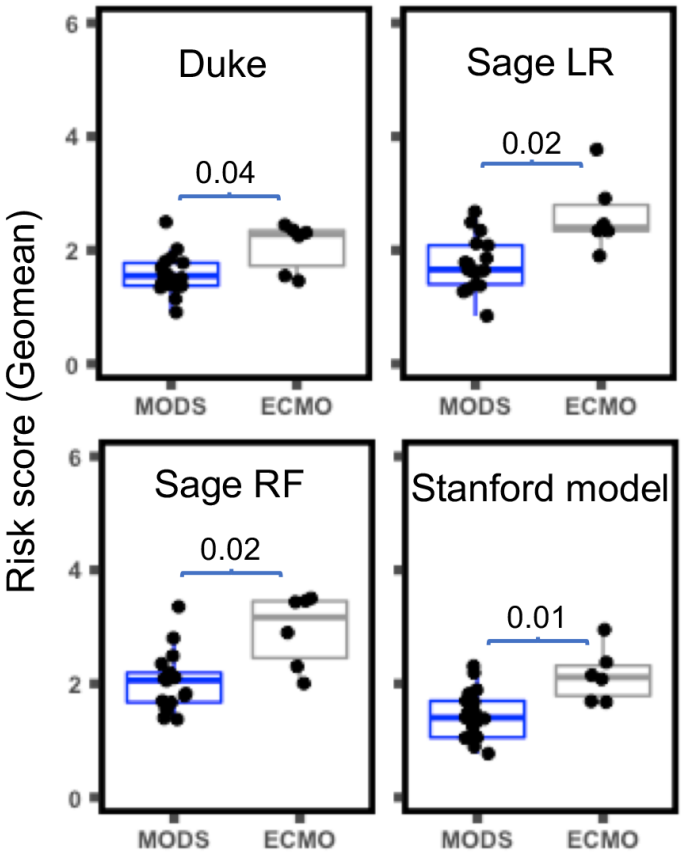

Figure S12. Risk scores derived from the putative signatures predicted for sepsis patients (Sweeney et al., 2018). The signatures have been derived from different models namely, Duke, Sage LR, Sage RF and Stanford. These signatures are composed of two categories, i.e, positively and negatively associated with patients mortality. However, only the signature which are positively associated with patients mortality showed the difference in MODS and ECMO.

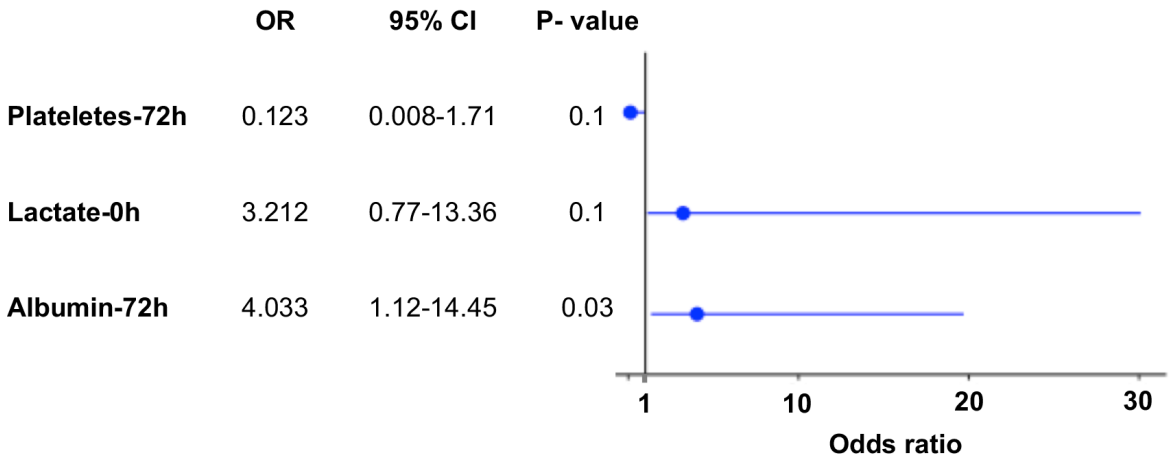

Figure S13. Odds ratio for clinical data. Clinical data available for all the ECMO and MODS patients at different time points were used to compute the odds ratio. Significantly (P value ≤ 0.03, Student's T-test) higher odds ratio for Albumin was observed in ECMO as compared to MODS patients.

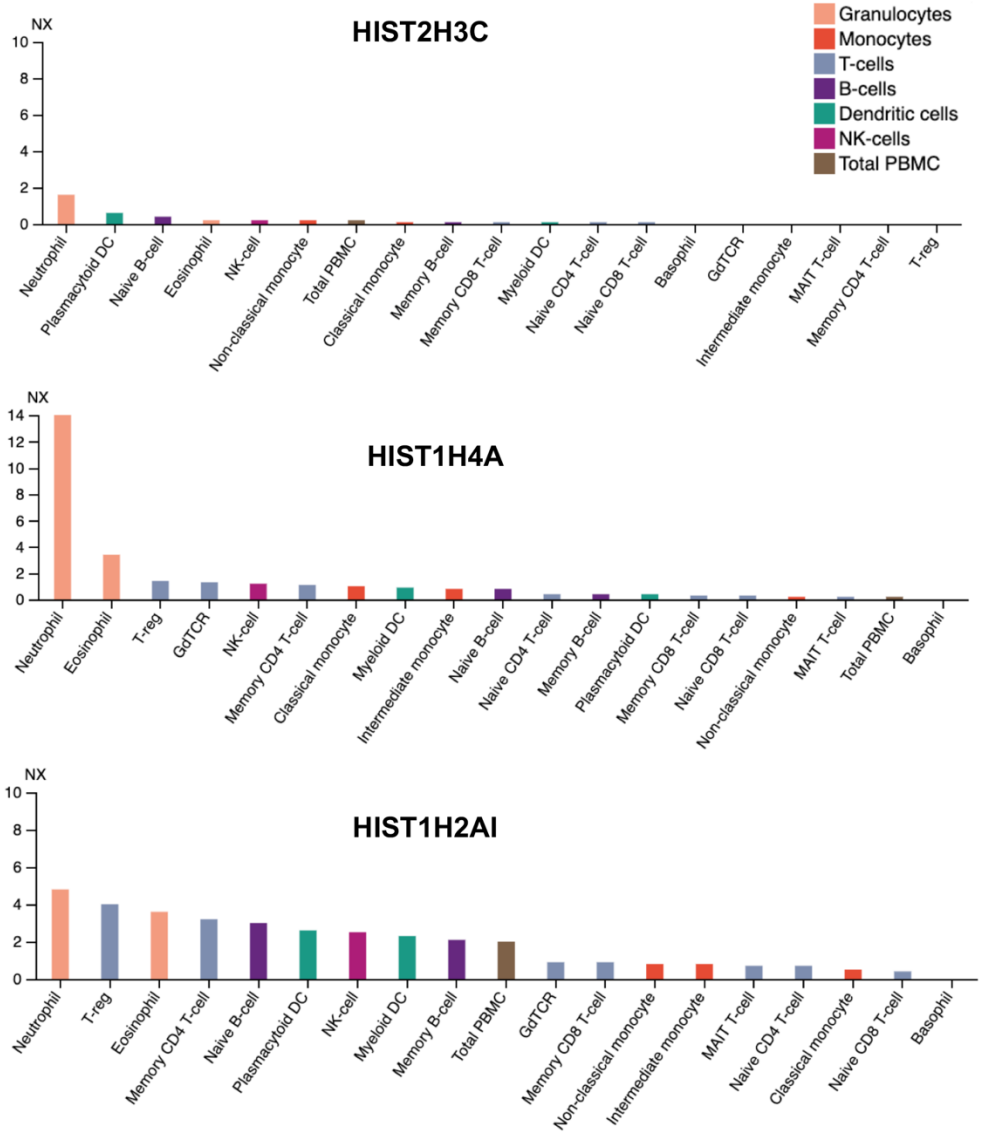

Figure S14. Expression pattern of some of histone genes in blood cells obtained from Human protein atlas. HIST2H3C, HIST1H4A, HIST1H2AI are highly expressed in neutrophils.

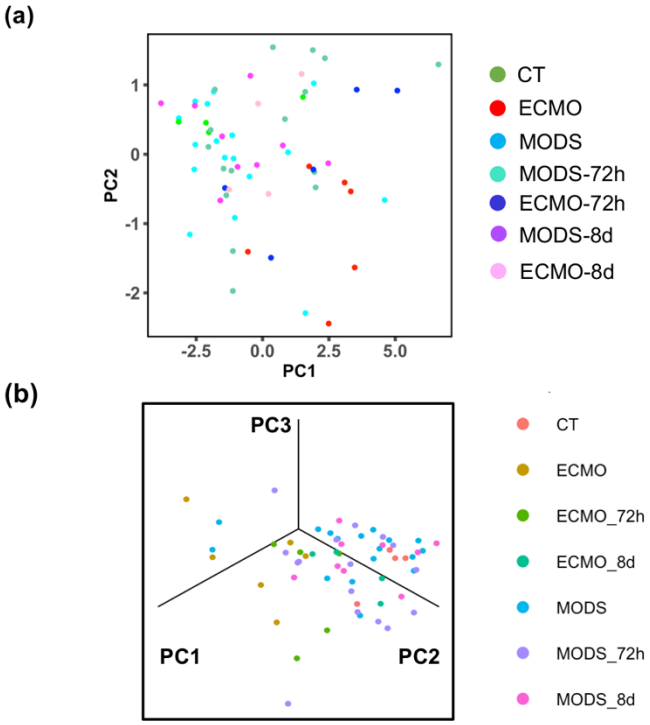

Figure S15. Labelled PCA comparing CT, MODS and ECMO at different time points. **(a)** Labelled PCA based on gene signature separated all the patients of different time points into CT, MODS and ECMO group. **(b)** PC1 and PC2 with PC3 provide more clear differentiation of patients.

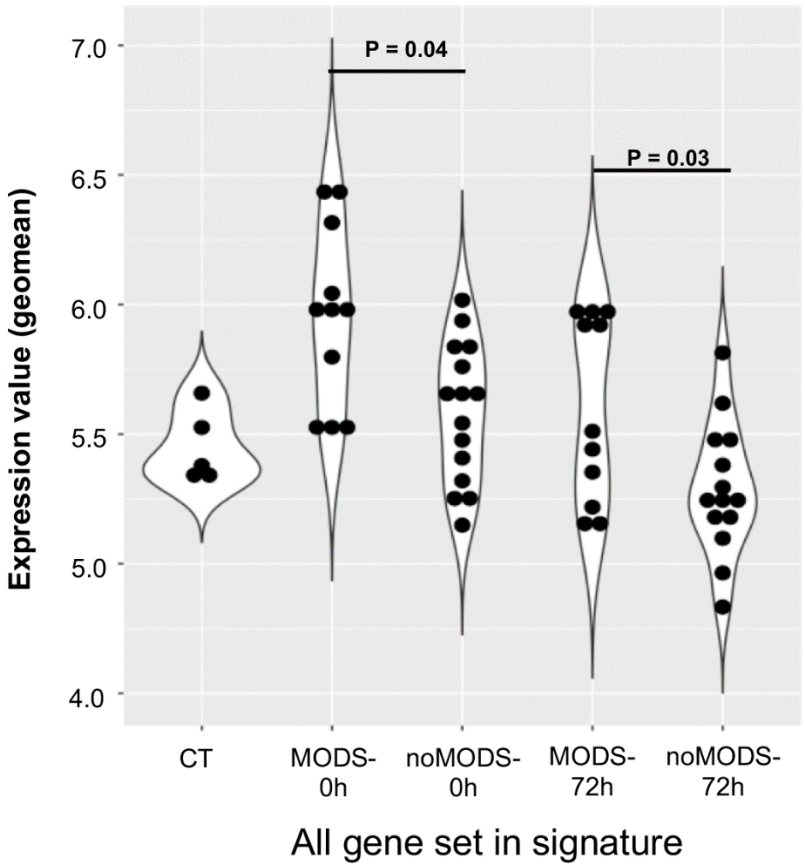

Figure S16. Comparison of all genes in validation data (Cabrera et at., 2017). The violin plot displayed differences in the expression values in MODS and noMODS patients at (P = 0.04) 0h and (P = 0.03) 72h time point. The p-value was estimated by Student's T-test.

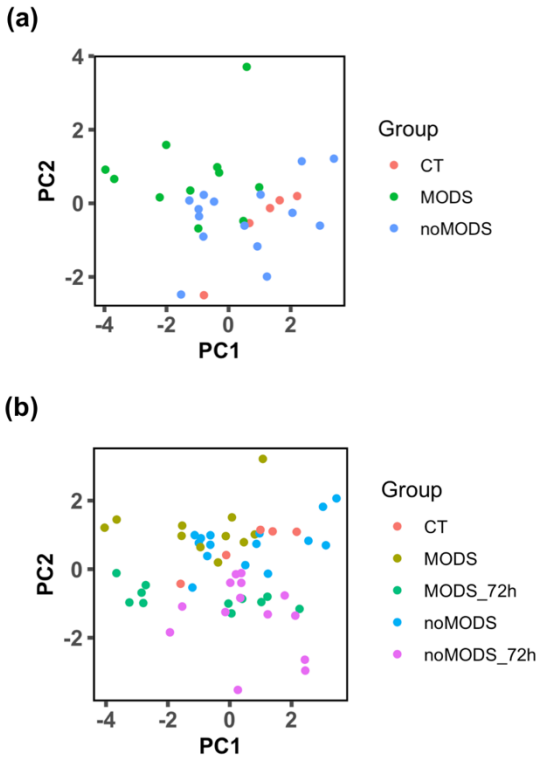

Figure S17. Labelled PCA separated the control (CT), noMODS (patients doesn't develop MODS) and MODS (develop MODS) patients in the validation cohort (Cabrera et at., 2017). (a) The labelled PCA using the signature genes associated with ECMO separated the MODS and noMODS. (b)The labelled PCA from different time points also separated MODS and noMODS patients with minimal overlap of patients at 72h. Although patients in the validation cohort data were adult, the remarkable separation indirectly validated the signature genes associated with paediatrics ECMO.