**Inflammatory and tissue injury marker dynamics in pediatric acute respiratory distress syndrome**

**DATA SUPPLEMENT**

**Supplementary Table 1:** Biomarkers

| Marker | Assay | Comments |
|---|---|---|
| **Inflammatory cytokines** | | |
| IL-1α | Luminex | • Non-specific pro-inflammatory cytokine |
| IL-6, sTNFR1 | ELLA | • Non-specific pro-inflammatory cytokines<br>• Discriminates ARDS subphenotypes |
| **Proteases** | | |
| MMP8 | Luminex | • Neutrophil collagenase<br>• Also released by some tissues |
| Granzyme B | Luminex | • Pro-apoptotic serine protease released by NK cells and cytotoxic T lymphocytes<br>• Also released by some tissues, including type-II alveolar epithelial cells |
| **Chemokines** | | |
| IL-8 | Luminex | • Neutrophil attractant |
| CCL7 | ELLA | • Chemoattractant for multiple leukocytes, including monocytes, NK cells, and activated T lymphocytes |
| CCL22 | ELLA | • Chemoattractant for multiple leukocytes, including monocytes, NK cells, and Th2 lymphocytes<br>• Macrophage-derived |
| MIP-1α, MIP-1β | Luminex | • Chemoattractant for multiple leukocytes, including monocytes, NK cells, and Th1 lymphocytes |
| **Tissue injury markers** | | |
| Angiopoietin-2 | ELISA | • Endothelial injury marker |
| P3NP | ELISA | • Marker of collagen turnover and fibrosis |
| Soluble RAGE | ELISA | • Pro-inflammatory scavenger receptor for AGEs<br>• Expressed in high levels in type-I alveolar epithelia<br>• Pleiotropic expression in endothelium and leukocytes |
| Surfactant protein D | ELISA | • Type-II alveolar epithelial cell marker |
| **DAMPs** | | |
| Heat shock protein 70 | Luminex | • Released during cell death and can propagate inflammation via TLR2 and TLR4 |
| Nucleosomes | ELISA | • DNA/histone complexes released during cell death with histones acting as DAMPs |
| COX4 (nuclear DNA) | RT-PCR | • Unclear if directly a DAMP or whether a bystander released alongside histones and nucleosomes |
| COX1, ND1 (mtDNA) | RT-PCR | • Released during cell death and can propagate inflammation via TLR9 |

**Supplementary Table 2:** Cohort stratified by immunocompromised status.

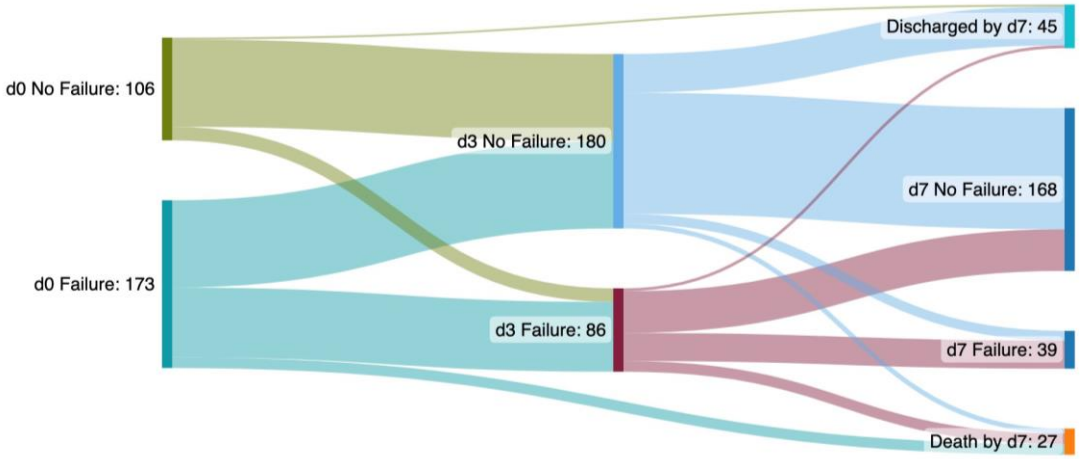
| Variables | All Patients (n = 279) | Immuno-competent (n = 206) | Immuno-compromised (n = 73) | P value |
|---|---|---|---|---|
| Age (years) | 6.8 [2, 13.5] | 6.3 [1.6, 13.1] | 7.1 [3.3, 14.4] | 0.078 |
| Female (%) | 124 (44) | 93 (45) | 31 (42) | 0.784 |
| Severity of illness | | | | |
| PRISM III at 12h | 11 [6, 18] | 10 [5, 17] | 14 [8, 19] | 0.072 |
| Non-pulmonary organ failures | 2 [1, 3] | 1 [1, 2] | 2 [2, 3] | < 0.001 |
| Vasopressor score | 8 [0, 20] | 8 [0, 17] | 10 [0, 40] | 0.127 |
| Stem cell transplant (%) | 35 (13) | - | 35 (48) | - |
| Etiology of ARDS (%) | | | | |
| Infectious pneumonia | 132 (47) | 104 (50) | 28 (38) | |
| Non-pulmonary sepsis | 70 (25) | 40 (19) | 30 (41) | 0.002 |
| Aspiration | 44 (16) | 38 (18) | 6 (8) | |
| Other | 33 (12) | 24 (12) | 9 (12) | |
| Day 0 parameters | | | | |
| $PaO_2/FiO_2$ | 150 [94, 217] | 146 [88, 213] | 156 [118, 220] | 0.247 |
| OI | 11.3 [7.8, 22.6] | 12.1 [7.6, 23.9] | 10.1 [8.2, 15.9] | 0.372 |
| PIP ($cmH_2O$) | 31 [27, 36] | 31 [26, 36] | 32 [27, 37] | 0.624 |
| PEEP ($cmH_2O$) | 10 [8, 12] | 10 [8, 12] | 10 [8, 12] | 0.927 |
| ΔP ($cmH_2O$) | 21 [17, 25] | 21 [16, 25] | 21 [17, 24] | 0.578 |
| Ancillary therapies (%) | | | | |
| Inhaled nitric oxide | 110 (39) | 79 (38) | 31 (42) | 0.578 |
| Corticosteroids | 142 (51) | 92 (45) | 50 (68) | 0.001 |
| Neuromuscular blockade | 149 (53) | 108 (52) | 41 (56) | 0.682 |
| Prone positioning | 16 (6) | 11 (5) | 5 (7) | 0.573 |
| Alternative ventilator modes | 69 (25) | 53 (26) | 16 (22) | 0.636 |
| ECMO | 17 (6) | 9 (4) | 8 (11) | 0.082 |
| PICU mortality (%) | 64 (23) | 30 (15) | 34 (47) | < 0.001 |

**Supplementary Table 3:** Cohort stratified by corticosteroid use.

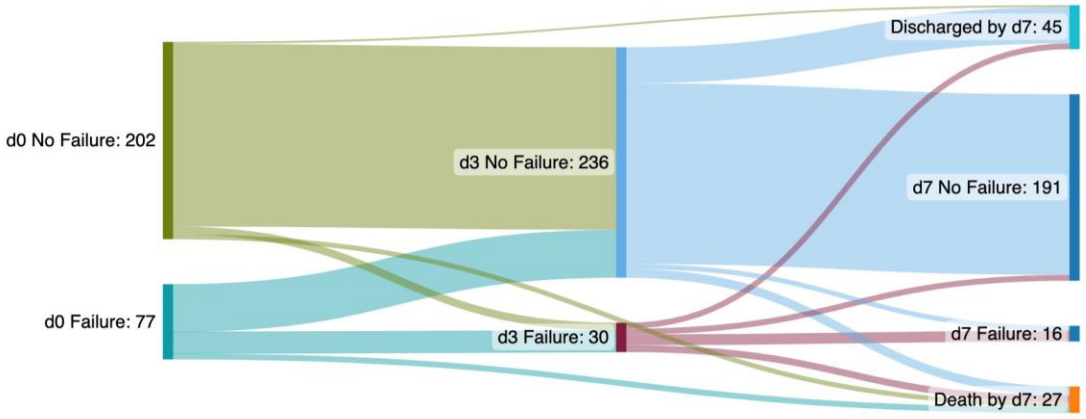
| Variables | All Patients (n = 279) | No corticosteroids (n = 137) | Corticosteroids (n = 142) | P value |
|---|---|---|---|---|
| Age (years) | 6.8 [2, 13.5] | 6.9 [2, 13.1] | 6.3 [2, 14] | 0.986 |
| Female (%) | 124 (44) | 53 (39) | 71 (50) | 0.071 |
| Severity of illness | | | | |
| PRISM III at 12h | 11 [6, 18] | 11 [6, 18] | 12 [6, 18] | 0.597 |
| Non-pulmonary organ failures | 2 [1, 3] | 2 [1, 2] | 2 [1, 3] | 0.854 |
| Vasopressor score | 8 [0, 20] | 8 [0, 15] | 10 [0, 25] | 0.229 |
| Immunocompromised (%) | 73 (26) | 23 (17) | 50 (35) | 0.001 |
| Stem cell transplant (%) | 35 (13) | 11 (8) | 24 (17) | 0.030 |
| Etiology of ARDS (%) | | | | |
| Infectious pneumonia | 132 (47) | 55 (40) | 77 (54) | |
| Non-pulmonary sepsis | 70 (25) | 33 (24) | 37 (26) | 0.015 |
| Aspiration | 44 (16) | 26 (19) | 18 (13) | |
| Other | 33 (12) | 23 (17) | 10 (7) | |
| Day 0 parameters | | | | |
| $PaO_2/FiO_2$ | 150 [94, 217] | 155 [101, 230] | 146 [83, 197] | 0.067 |
| OI | 11.3 [7.8, 22.6] | 10.1 [6.8, 17.6] | 12.9 [8.7, 25.6] | 0.002 |
| PIP ($cmH_2O$) | 31 [27, 36] | 29 [26, 34] | 34 [30, 38] | < 0.001 |
| PEEP ($cmH_2O$) | 10 [8, 12] | 10 [8, 12] | 10 [10, 12] | 0.029 |
| ΔP ($cmH_2O$) | 21 [17, 25] | 19 [16, 22] | 22 [18, 26] | < 0.001 |
| Ancillary therapies (%) | | | | |
| Inhaled nitric oxide | 110 (39) | 40 (29) | 70 (49) | 0.001 |
| Neuromuscular blockade | 149 (53) | 60 (44) | 89 (63) | 0.002 |
| Prone positioning | 16 (6) | 5 (4) | 11 (8) | 0.198 |
| Alternative ventilator modes | 69 (25) | 30 (22) | 39 (27) | 0.332 |
| ECMO | 17 (6) | 5 (4) | 12 (8) | 0.273 |
| PICU mortality (%) | 64 (23) | 25 (18) | 39 (27) | 0.087 |

**Supplementary Figure 1:** Specific organ failure trajectories (Goldstein definitions). Organ failures status from day 0 to day 3 are stratified according to whether the organ was failing (aqua) or not (olive). Similarly, trajectories between days 3 and 7 are stratified according to whether organs were failing on day 3 (red) or not (blue). By day 7, 45 subjects had been discharged alive from the PICU, and 27 had died. Note that these 27 non-survivors within 7 days of ARDS onset represent a subset of the total (n = 64) that died in the PICU.

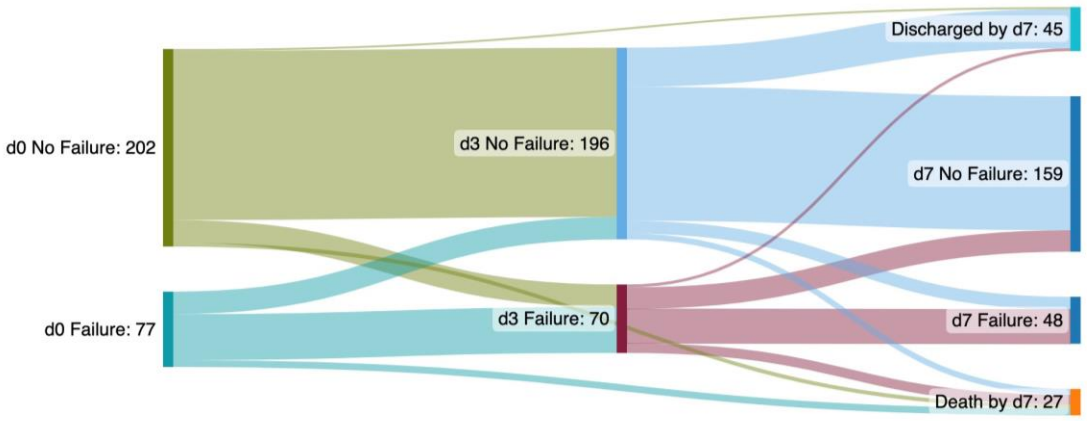
**Cardiovascular**



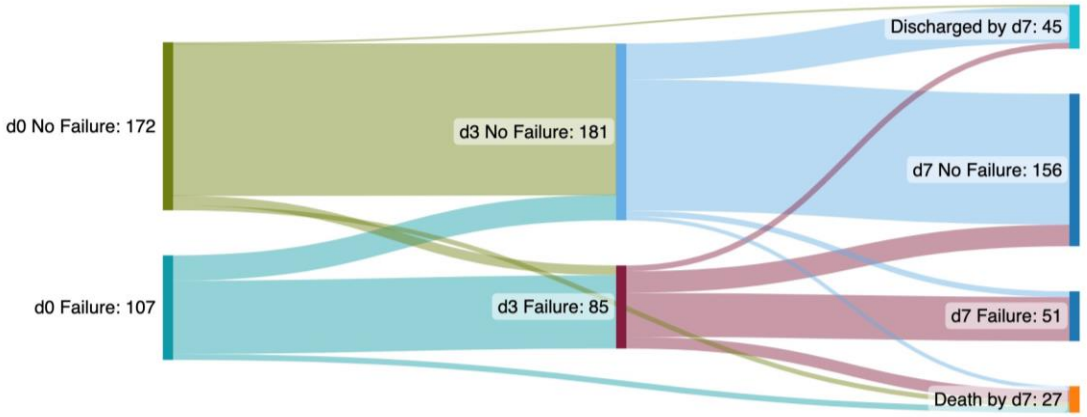

**Renal**



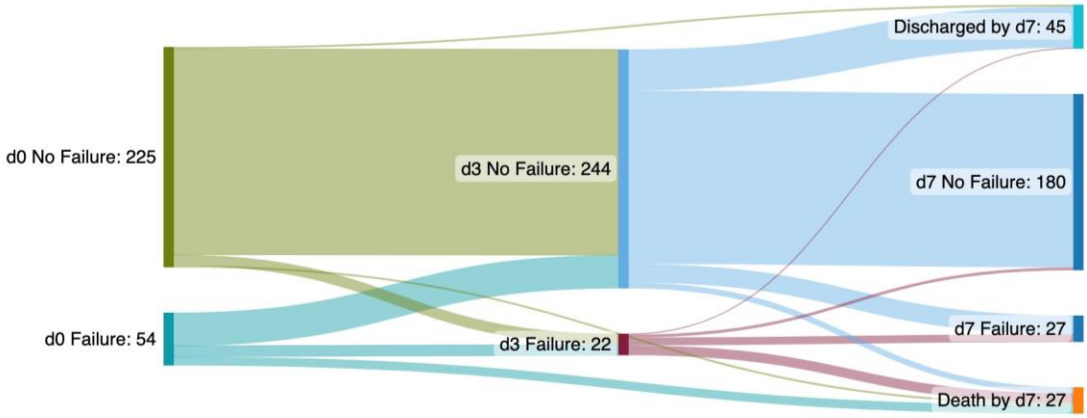
**Liver**



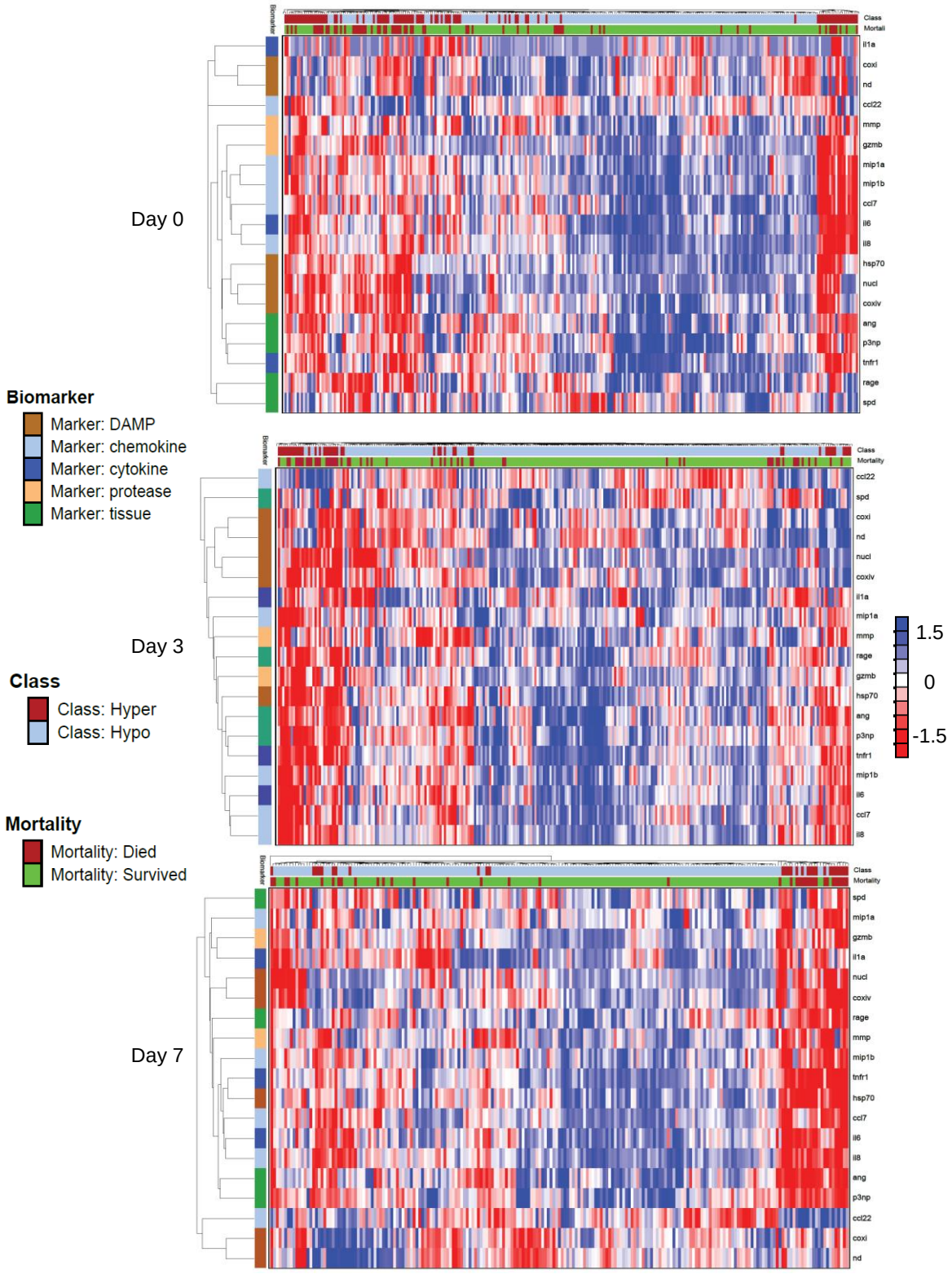

**Hematologic**



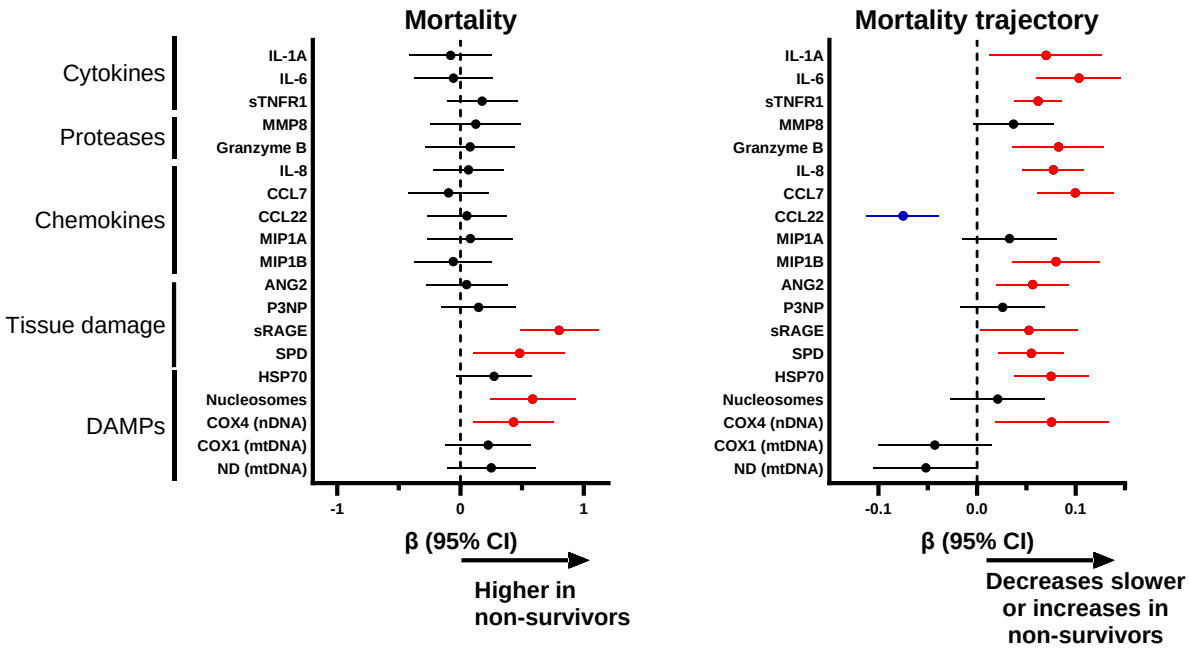

**Neurologic**



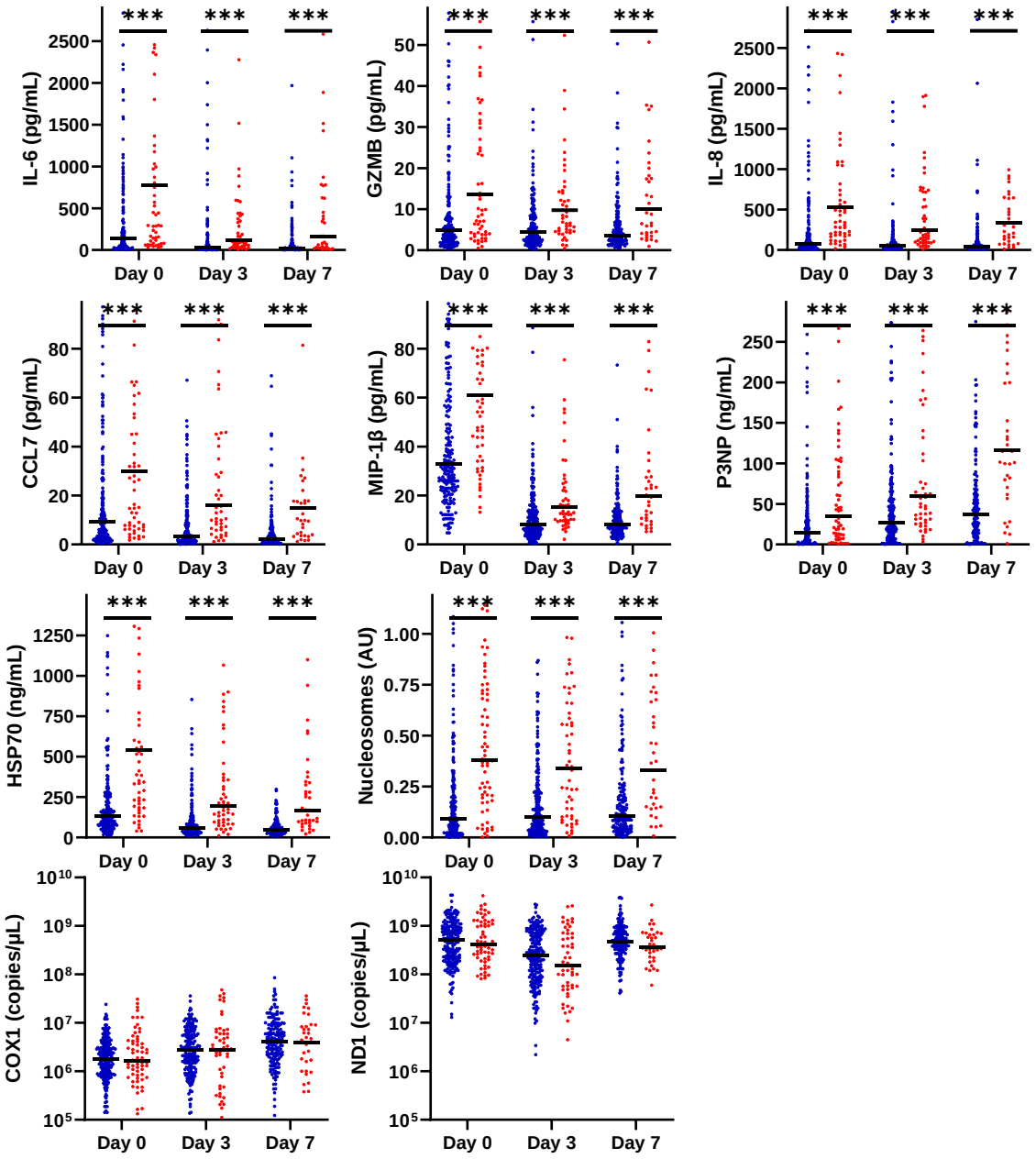
**Supplementary Figure 2:** Hierarchical clustering of biomarkers and subjects on days 0, 3, and 7 after ARDS onset. DAMPs, tissue injury markers, and cytokines cluster together on days 0 and 3. Hyperinflammatory ARDS and non-survivors are enriched within these signatures.



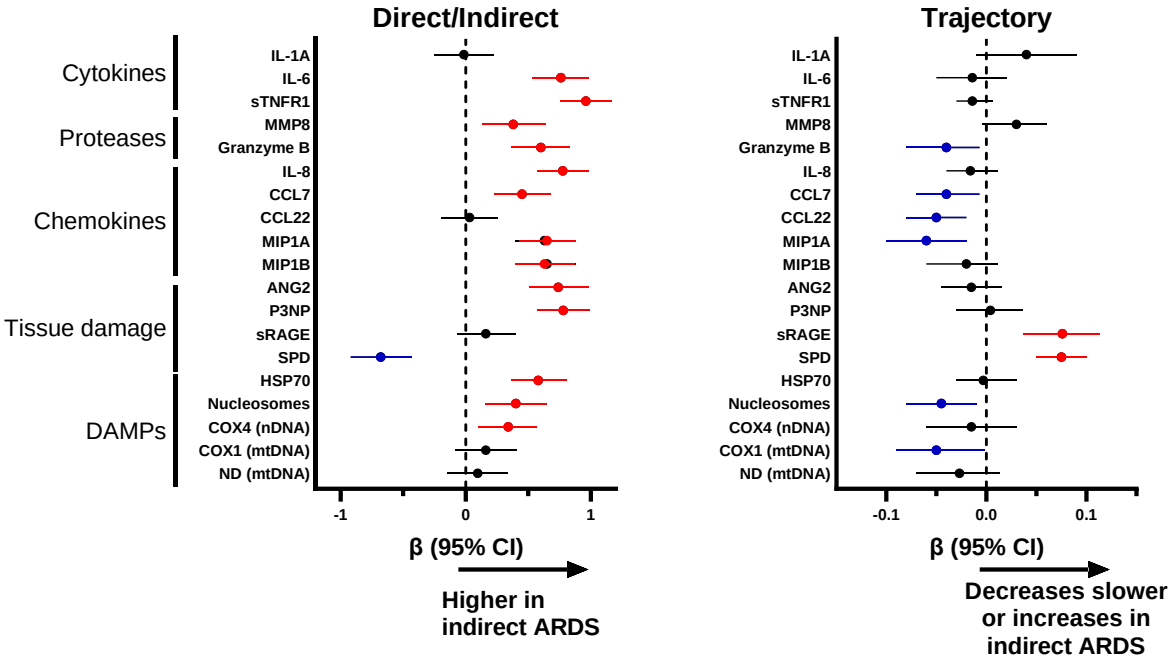
**Supplementary Figure 3:** Association between biomarker levels and trajectory over the first 7 days of ARDS with PICU mortality restricted to subjects alive and in the PICU for all three timepoints (n = 207). Beta coefficients (and 95% confidence intervals) are plotted for the association between the overall biomarker level in the first 7 days of ARDS and the trajectory with PICU mortality. Biomarker levels are log-transformed and standardized (set to mean = 0, SD = 1), and then adjusted for age, ARDS etiology, immunocompromised status, and initial PaO2/FIO2 in a multivariable mixed effects model. Red dots represent biomarkers with adjusted $p < 0.05$ with higher levels in non-survivors, blue dots represent biomarkers with adjusted $p < 0.05$ with lower levels in non-survivors, and black dots represent those with $p > 0.05$.



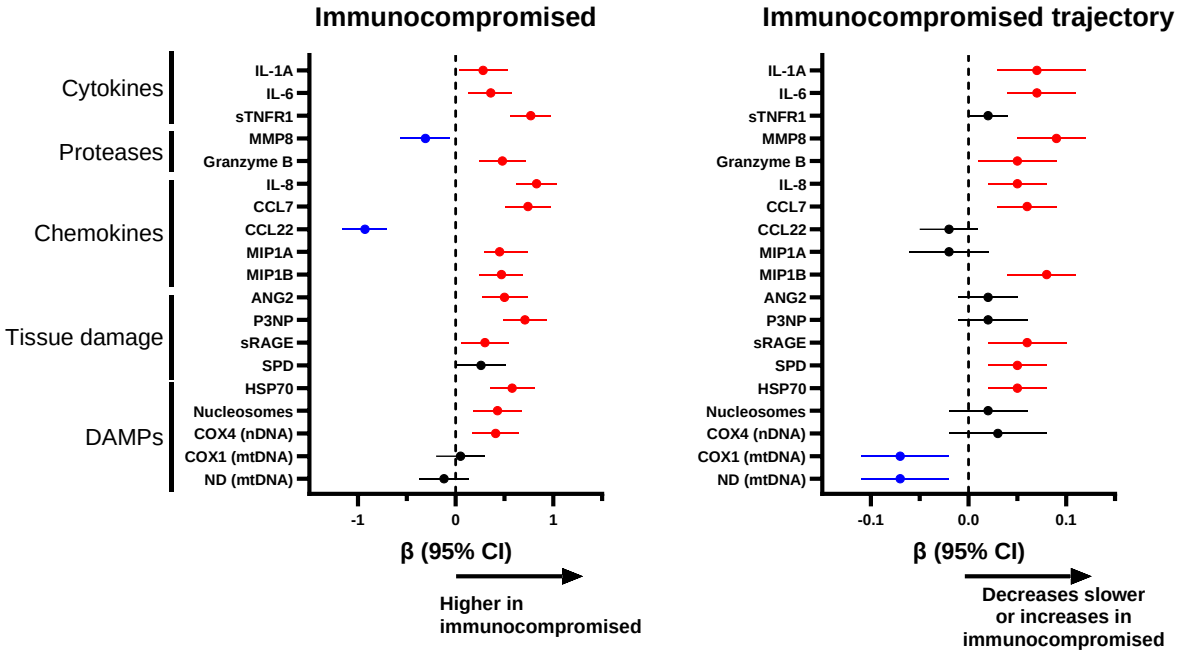
**Supplementary Figure 4:** Unadjusted plasma biomarker levels between survivors (blue) and non-survivors (red) on days 0, 3, and 7 of ARDS. Black bars are median values. Unadjusted Wilcoxon rank sum tests compare survivors and non-survivors on days 0, 3, and 7 (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$). Select biomarkers are shown, with the remainder in main Figure 6.



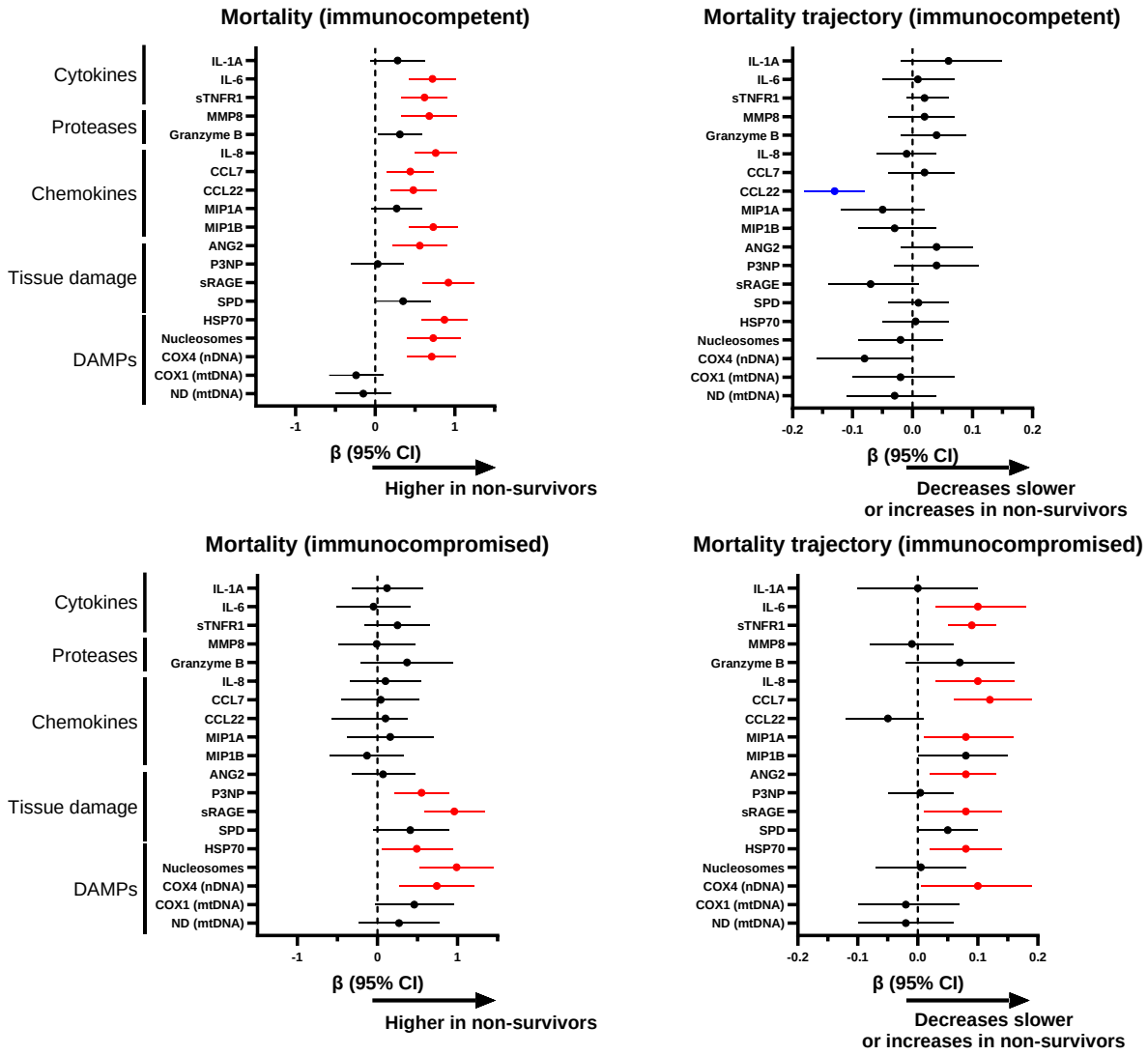
**Supplementary Figure 5:** Association between biomarker levels and trajectory over the first 7 days of ARDS with direct versus indirect ARDS. Beta coefficients (and 95% confidence intervals) are plotted for the association between the overall biomarker level in the first 7 days of ARDS and the trajectory with indirect ARDS (baseline = direct ARDS). Biomarker levels are log-transformed and standardized (set to mean = 0, SD = 1), and then adjusted for age, immunocompromised status, and initial PaO2/FIO2 in a multivariable mixed effects model. Red dots represent biomarkers with adjusted $p < 0.05$ with higher levels in indirect ARDS, blue dots represent biomarkers with adjusted $p < 0.05$ with lower levels in indirect ARDS, and black dots represent those with $p > 0.05$.



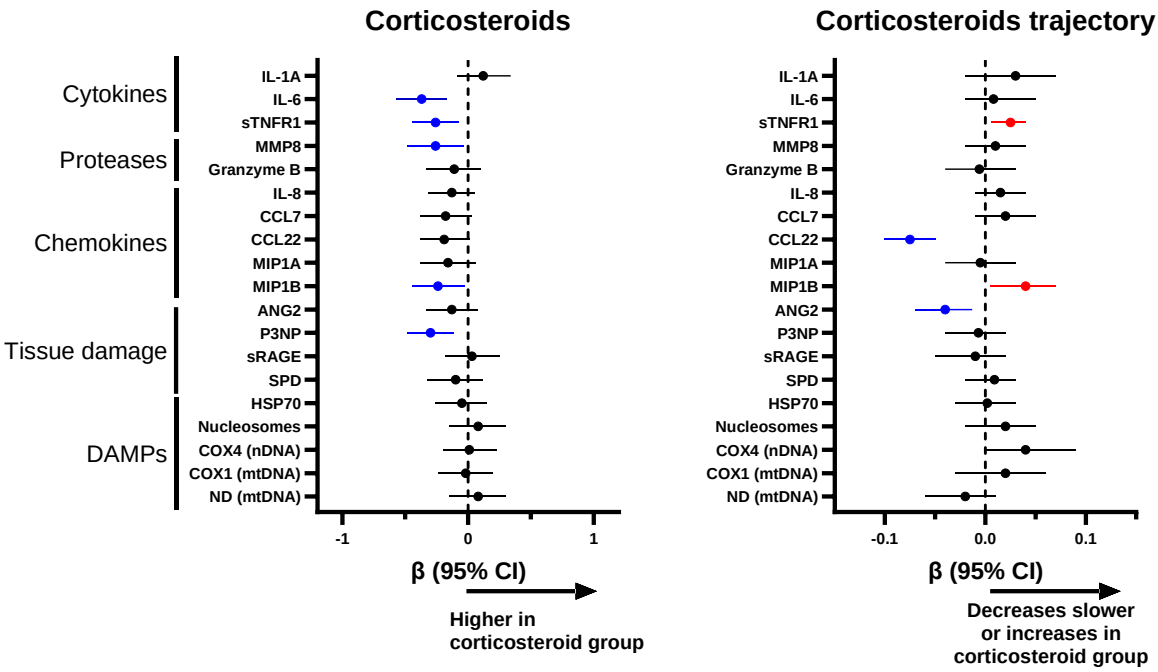
**Supplementary Figure 6:** Association between biomarker levels and trajectory over the first 7 days of ARDS with immunocompromised status. Beta coefficients (and 95% confidence intervals) are plotted for the association between the overall biomarker level in the first 7 days of ARDS and the trajectory with immunocompromised status (baseline = immunocompetent). Biomarker levels are log-transformed and standardized (set to mean = 0, SD = 1), and then adjusted for age, ARDS etiology, and initial PaO2/FIO2 in a multivariable mixed effects model. Red dots represent biomarkers with adjusted $p < 0.05$ with higher levels in immunocompromised subjects, blue dots represent biomarkers with adjusted $p < 0.05$ with lower levels in immunocompromised subjects, and black dots represent those with $p > 0.05$.



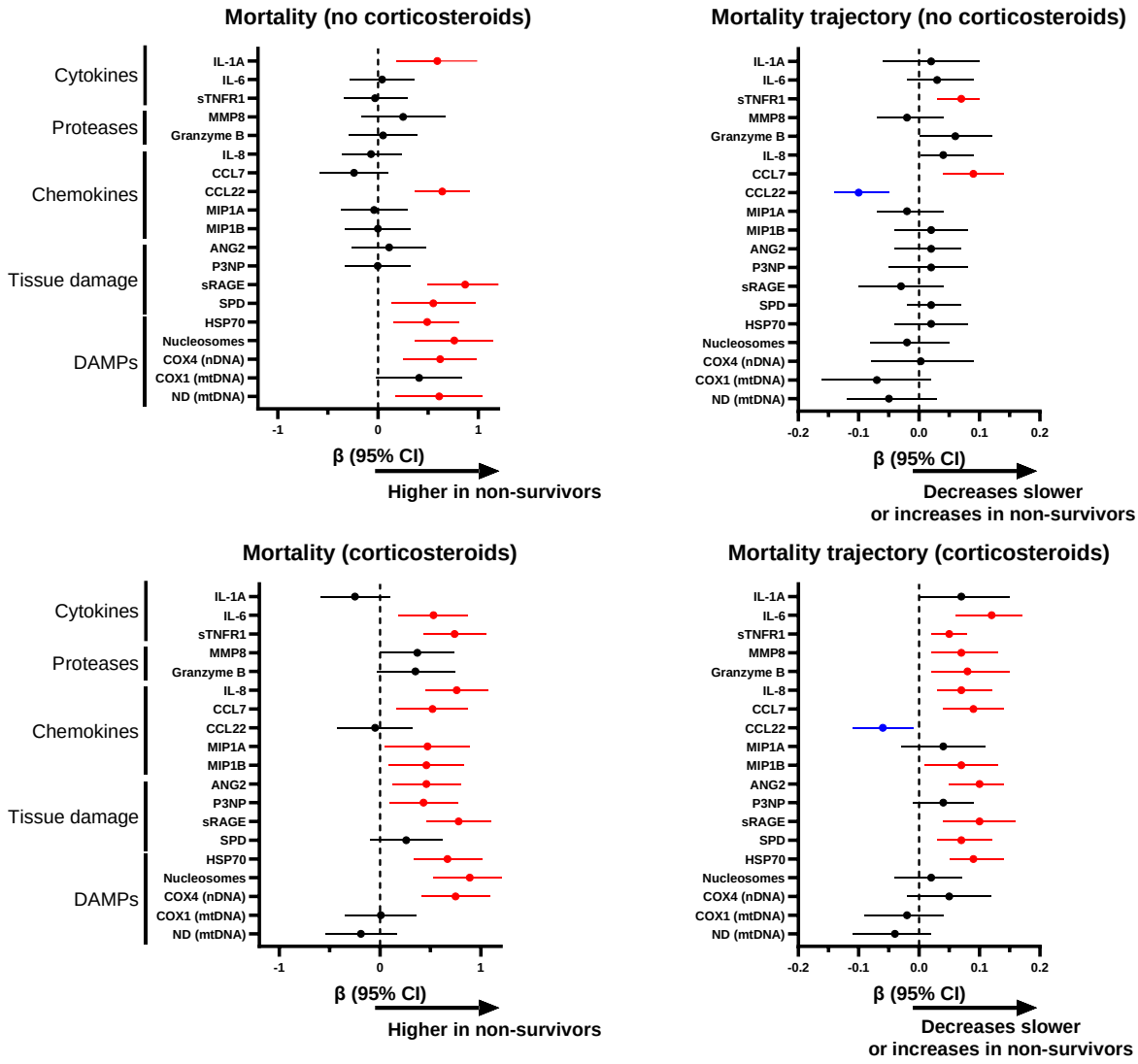
**Supplementary Figure 7:** Association between biomarker levels and trajectory over the first 7 days of ARDS with PICU mortality, stratified by baseline immune status (immunocompetent or immunocompromised). Beta coefficients (and 95% confidence intervals) are plotted for the association between the overall biomarker level in the first 7 days of ARDS with PICU mortality. Biomarker levels are log-transformed and standardized (set to mean = 0, SD = 1), and then adjusted for age, ARDS etiology, immunocompromised status, and initial PaO2/FIO2 in a multivariable mixed effects model. Red dots represent biomarkers with adjusted $p < 0.05$ with higher levels in non-survivors, blue dots represent biomarkers with adjusted $p < 0.05$ with lower levels in non-survivors, and black dots represent those with $p > 0.05$.



**Supplementary Figure 8:** Association between biomarker levels and trajectory over the first 7 days of ARDS with exposure to corticosteroids within the first 3 days of ARDS. Beta coefficients (and 95% confidence intervals) are plotted for the association between the overall biomarker level in the first 7 days of ARDS and the trajectory with corticosteroid use (baseline = no corticosteroid use). Biomarker levels are log-transformed and standardized (set to mean = 0, SD = 1), and then adjusted for age, ARDS etiology, immunocompromised status, and initial PaO2/FIO2 in a multivariable mixed effects model. Red dots represent biomarkers with adjusted $p < 0.05$ with higher levels in the corticosteroid group, blue dots represent biomarkers with adjusted $p < 0.05$ with lower levels in the corticosteroid group, and black dots represent those with $p > 0.05$.



**Supplementary Figure 9:** Association between biomarker levels and trajectory over the first 7 days of ARDS with PICU mortality, stratified by corticosteroid use within the first 3 days of ARDS. Beta coefficients (and 95% confidence intervals) are plotted for the association between the overall biomarker level in the first 7 days of ARDS with PICU mortality. Biomarker levels are log-transformed and standardized (set to mean = 0, SD = 1), and then adjusted for age, ARDS etiology, immunocompromised status, and initial PaO2/FIO2 in a multivariable mixed effects model. Red dots represent biomarkers with adjusted $p < 0.05$ with higher levels in non-survivors, blue dots represent biomarkers with adjusted $p < 0.05$ with lower levels in non-survivors, and black dots represent those with $p > 0.05$.



**Supplementary Figure 10:** Directed acyclic graph (DAG) depicting the relationship between the exposures (biomarkers) and the outcome (PICU mortality, persistent ARDS, or persistent MODS). Note that the confounders (red) that were adjusted for in regression models were chosen for plausible upstream associations with both the exposure (biomarker) and the eventual outcomes (e.g., mortality). Mediators (blue) were not adjusted for as they were thought to mediate the association between the biomarker and outcomes along a causal pathway.