Supplemental Materials

A Proteomics and Redox Proteomics Approach to Understanding ARDS Heterogeneity

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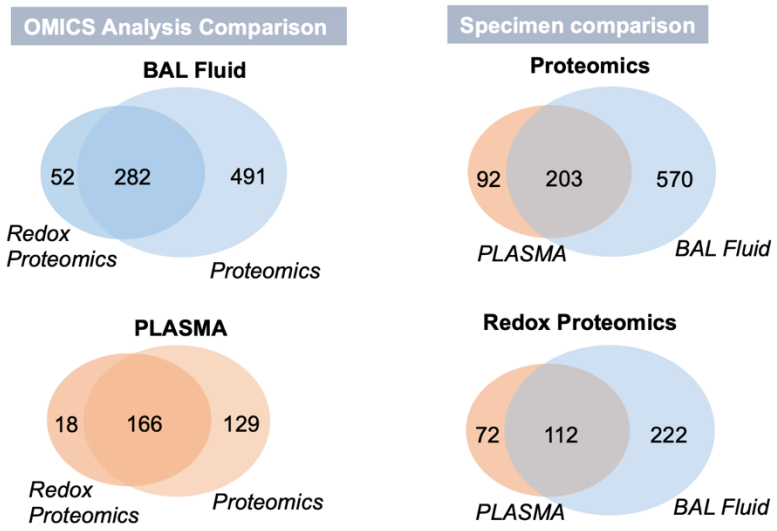
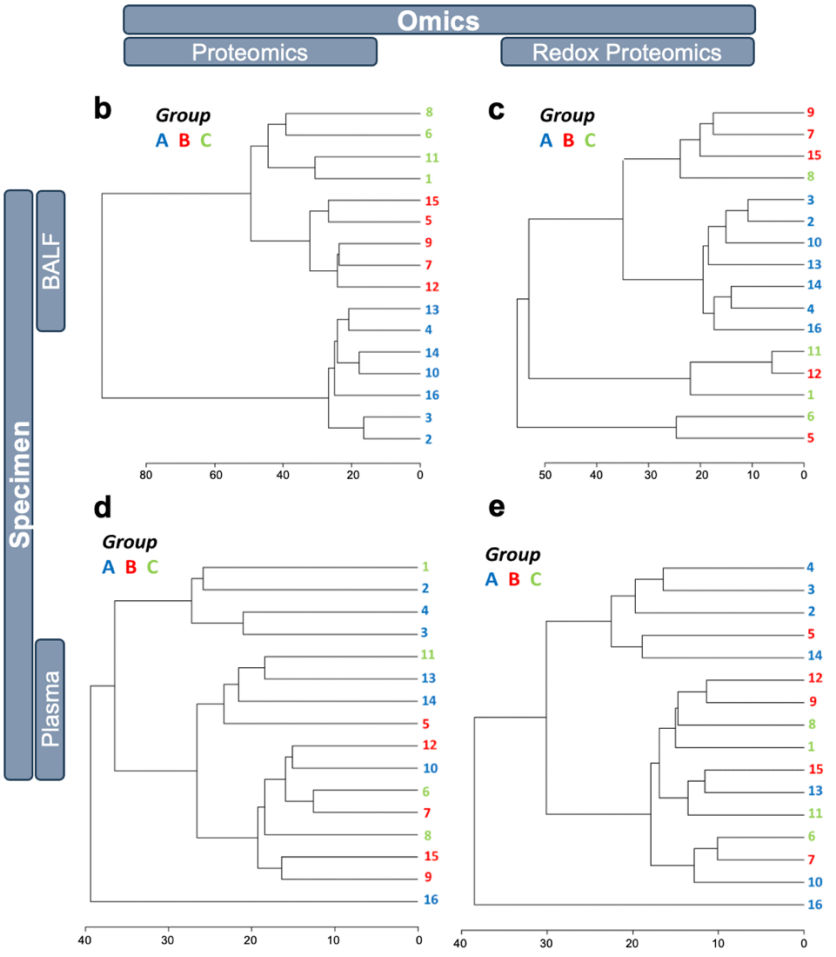
***Supplemental Figure S1*. Venn diagrams summarizing the omics data.** The figure shows the number of common and unique proteins quantified based on omics analysis type (proteomics vs redox proteomics, left panels) and biospecimen type (plasma vs BAL fluid, right panels).



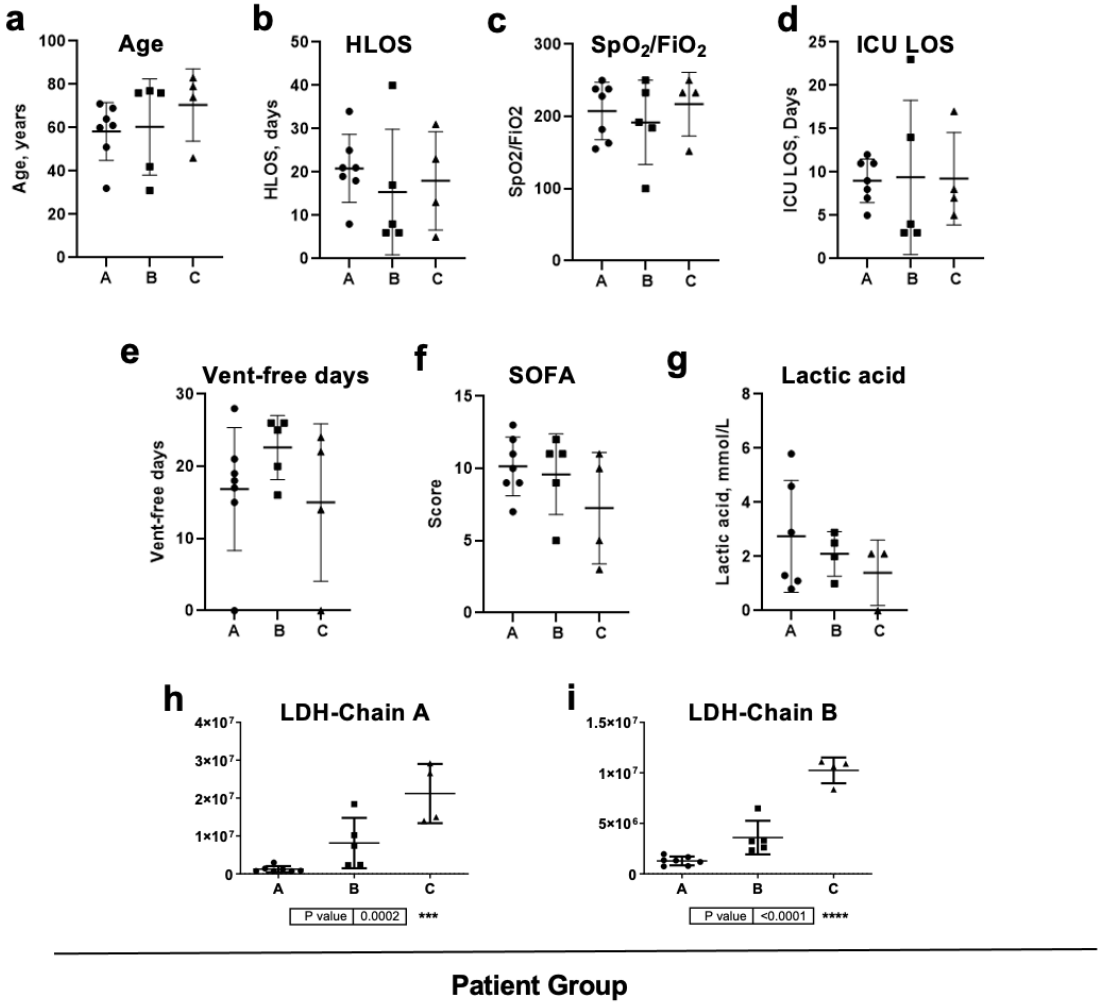
Supplemental Figure S2. **Day 0 (Admission) dendrograms of BAL fluid and plasma Data.** Datasets were analyzed in R-Studio (version 2025.09.2, Build 418) for silhouette-based clustering assessment, and MetaboAnalyst (v6.0) by Ward clustering and Euclidean distancing. Silhouette analysis (**a**) showed average silhouette widths for k=2-4 across BAL fluid and plasmid proteomic and redox proteomic datasets. Given the abundance of proteins (n=773) identified in the global BAL fluid (**b**) compared to the other datasets, this was anticipated to be the most informative for defining the proteomics-informed patient Groups (A, blue; B, red; C, green). This assumption held true for the Group alignments in the BAL fluid redox proteomics (**c**): Group A, 7 of 7 patients clustering together; Group B, 3 of 5 patients clustering together; and Group C, 3 of 4 patients clustering together. The fidelity of the plasma (**d, e**) clustering tended to be lower.

a

Dataset	k=2	k=3	k=4
BAL fluid proteomics	0.335	0.299	0.211
BAL fluid redox proteomics	0.417	0.321	0.323
Plasma proteomics	0.283	0.138	0.109
Plasma redox proteomics	0.422	0.152	0.15



Supplemental Figure S3. **Clinical data across patient groups and other supporting data.** Routine clinical parameters (**a-g**) were stratified by the proteomic-informed patient Groups for comparison by ANOVA or pairwise T-tests. No statistically significant differences were observed for the indicated clinical parameters. The LDH abundance data (**h, i**) were derived from the BAL fluid proteomic data and differed significantly by patient Group, as indicated by the stated ANOVA P-values. The data shown are the mean ± SD.



Supplemental Table S1a. **BAL Fluid proteomics Top 25 by ANOVA.** Data are derived from analyses in MetaboAnalyst (v6.0). UniProt protein names are included to facilitate reference to heatmap designations. The table lists proteins in the decreasing f.value.

Uniprot ID	Protein name	f.value	p.value	-log$_{10}$(p)	FDR	Fisher's LSD
UBA1	Ubiquitin-like modifier-activating enzyme 1	79.232	5.23E-08	7.2815	1.54E-05	B - A; C - A; C - B
LDHB	L-lactate dehydrogenase B chain	78.469	5.54E-08	7.2563	1.54E-05	B - A; C - A; C - B
FLNA	Filamin-A	73.84	7.98E-08	7.0981	1.54E-05	B - A; C - A; C - B
VPS35	Vacuolar protein sorting-associated protein 35	69.745	1.12E-07	6.9504	1.62E-05	B - A; C - A; C - B
FKB1A	Peptidyl-prolyl cis-trans isomerase A	56.059	4.06E-07	6.3919	4.50E-05	B - A; C - A; C - B
GBB1	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	54.739	4.66E-07	6.3317	4.50E-05	B - A; C - A; C - B
MVP	Major vault protein	50.216	7.67E-07	6.1152	6.34E-05	B - A; C - A; C - B
PSB4	Proteasome subunit alpha type-4	47.801	1.02E-06	5.9923	7.37E-05	C - A; C - B
HSP74	Heat shock 70 kDa protein 4	45.471	1.35E-06	5.8685	8.71E-05	B - A; C - A; C - B
CLIC1	Chloride intracellular channel protein 1	39.892	2.83E-06	5.5479	0.000164	B - A; C - A; C - B
PSA3	Proteasome subunit alpha type-3	38.72	3.34E-06	5.4757	0.000176	C - A; C - B
RAN	GTP-binding nuclear protein Ran	35.925	5.06E-06	5.2956	0.000244	B - A; C - A; C - B
ARP2	Actin-related protein 2	35.112	5.74E-06	5.241	0.000256	B - A; C - A; C - B
1433Z	14-3-3 protein zeta/delta	34.051	6.79E-06	5.168	0.000281	B - A; C - A; C - B
ACTBL	Beta-actin-like protein 2	32.941	8.13E-06	5.0897	0.00031	B - A; C - A; C - B
IDHC	Isocitrate dehydrogenase [NADP] cytoplasmic	32.129	9.31E-06	5.031	0.00031	B - A; C - A; C - B
PDC6I	Programmed cell death 6-interacting protein	32	9.52E-06	5.0216	0.00031	B - A; C - A; C - B
PPIB	Peptidyl-prolyl cis-trans isomerase B	31.703	1.00E-05	4.9997	0.00031	C - A; C - B
V9HW88	Calreticulin	31.601	1.02E-05	4.9922	0.00031	C - A; C - B
ARPC4	Actin-related protein 2/3 complex subunit 4	30.676	1.19E-05	4.9228	0.000346	B - A; C - A; C - B
KPYM	Pyruvate kinase PKM	29.957	1.36E-05	4.8677	0.000374	B - A; C - A
ENOA	Alpha-enolase	29.564	1.46E-05	4.837	0.000376	B - A; C - A; C - B
IQGA1	Ras GTPase-activating-like protein IQGAP1	29.423	1.49E-05	4.8259	0.000376	B - A; C - A; C - B
AATC	Aspartate aminotransferase, cytoplasmic	28.39	1.80E-05	4.7436	0.000427	C - A; C - B
GDIR1	Rho GDP-dissociation inhibitor 1	28.277	1.84E-05	4.7344	0.000427	B - A; C - A

Supplemental Table S1b. ***BAL fluid* redox proteomics Top 25 by ANOVA.** Data are derived from analyses in MetaboAnalyst (v6.0). UniProt protein names are included to facilitate reference to heatmap designations. The table lists proteins in the decreasing f.value.

Uniprot ID	Protein name	f.value	p.value	$-\log_{10}(p)$	FDR	Fisher's LSD
STIP1	Stress-induced-phosphoprotein 1	45.562	1.34E-06	5.8734	0.000401	C - A; C - B
IQGA1	Ras GTPase-activating-like protein IQGAP1	23.077	5.28E-05	4.2772	0.007652	B - A; C - A; C - B
MYL6	Myosin light polypeptide 6	21.437	7.65E-05	4.1162	0.007652	B - A; C - A; C - B
S10A9	Protein S100-A9	19.791	0.000114	3.9448	0.008516	B - A; C - A; C - B
ITB2	Integrin beta-2	15.7	0.000341	3.4673	0.019008	B - A; C - A
EFHD2	EF-hand domain-containing protein D2	15.233	0.000391	3.4073	0.019008	B - A; C - A; C - B
HS71B	Heat shock 70 kDa protein 1B	14.819	0.000444	3.3531	0.019008	B - A; C - A
ACTN1	Alpha-actinin-1	13.455	0.000682	3.1664	0.025563	B - A; C - A; C - B
TPPP3	Tubulin polymerization-promoting protein family member 3	12.139	0.001062	2.9739	0.0354	C - A; C - B
KNG1	Kininogen-1	11.547	0.00131	2.8827	0.036014	A - B; A - C
RINI	Ribonuclease inhibitor	11.525	0.001321	2.8793	0.036014	B - A; C - A; C - B
CPNS1	Calpain small subunit 1	11.135	0.001523	2.8174	0.036242	B - A; C - A; C - B
PGK1	Phosphoglycerate kinase 1	11.051	0.001571	2.804	0.036242	B - A; C - A
TPIS	Triosephosphate isomerase	10.602	0.001858	2.7309	0.039817	B - A; C - A
ALBU	Albumin	9.5504	0.002807	2.5517	0.056146	A - B; A - C
CPN2	Carboxypeptidase N subunit 2	8.9592	0.003583	2.4458	0.065091	A - B; A - C
PGAM1	Phosphoglycerate mutase 1	8.8902	0.003689	2.4331	0.065091	B - A; C - A
IGHG4	Immunoglobulin heavy constant gamma 4	8.5096	0.004341	2.3625	0.069451	A - B; A - C
MYH9	Myosin-9	8.4789	0.004399	2.3567	0.069451	C - A
TSN1	Tetraspanin-1	8.1737	0.005029	2.2986	0.072068	B - A; C - A
1433F	14-3-3 protein eta	8.0711	0.005263	2.2788	0.072068	C - A; C - B
FHR2	Complement factor H-related protein 2	8.0618	0.005285	2.277	0.072068	A - B; A - C
CO8G	Complement component C8 gamma chain	7.8195	0.005894	2.2296	0.076878	A - B; A - C
TLN1	Talin-1	7.4121	0.00711	2.1481	0.087584	B - A; C - A
A1BG	Alpha-1B-glycoprotein	7.2758	0.00758	2.1203	0.087584	A - B; A - C

Supplemental Table S1c. ***Oxidized BAL fluid proteins significantly different by patient groups that potentially contribute to ARDS.*** BAL fluid redox proteomics that were significantly enriched Group C or Group A were explored to evaluate relevance to ARDS. Data are derived from analyses in MetaboAnalyst (v6.0). The FDR-adjusted p values are given.

		BALF, P_{FDR}	**Effect when Oxidized**	**Reference**
Enriched Oxidation in Group C				
RAS GTPase activating-like protein	IQGAP1	7.65E-03	regulates endothelial migration	(1)
phosphoglycerate mutase 1	PGM1	0.065	unknown in ARDS	
			upregulation in the neutrophils of patients with sepsis	(2)
Stress-induced-phosphoprotein 1	STIP1	4.01E-04	Anti-inflammatory in mouse spinal injury model; modulates NFκB activity	(2, 3)
Protein S100-A9	S100-A9	0.0085	anti-inflammatory	(4)
phosphoglycerate kinase 1	PGK1	0.036	decreased enzyme activity, which inhibits cellular production of IL1β and IL6	(5)
			(when reduced, activation improved survival in mouse model of sepsis)	(6)
Enriched Oxidation in Group A				
kininogen 1		0.036	oxidation decreases activity	(7, 8)
			decreased activity contributes to impaired coagulation and vasodilation	(9)
albumin		0.056	associated with a poorer prognosis	(10)
complement component C8		0.076	unknown in ARDS	

References for Supplemental Table S1c

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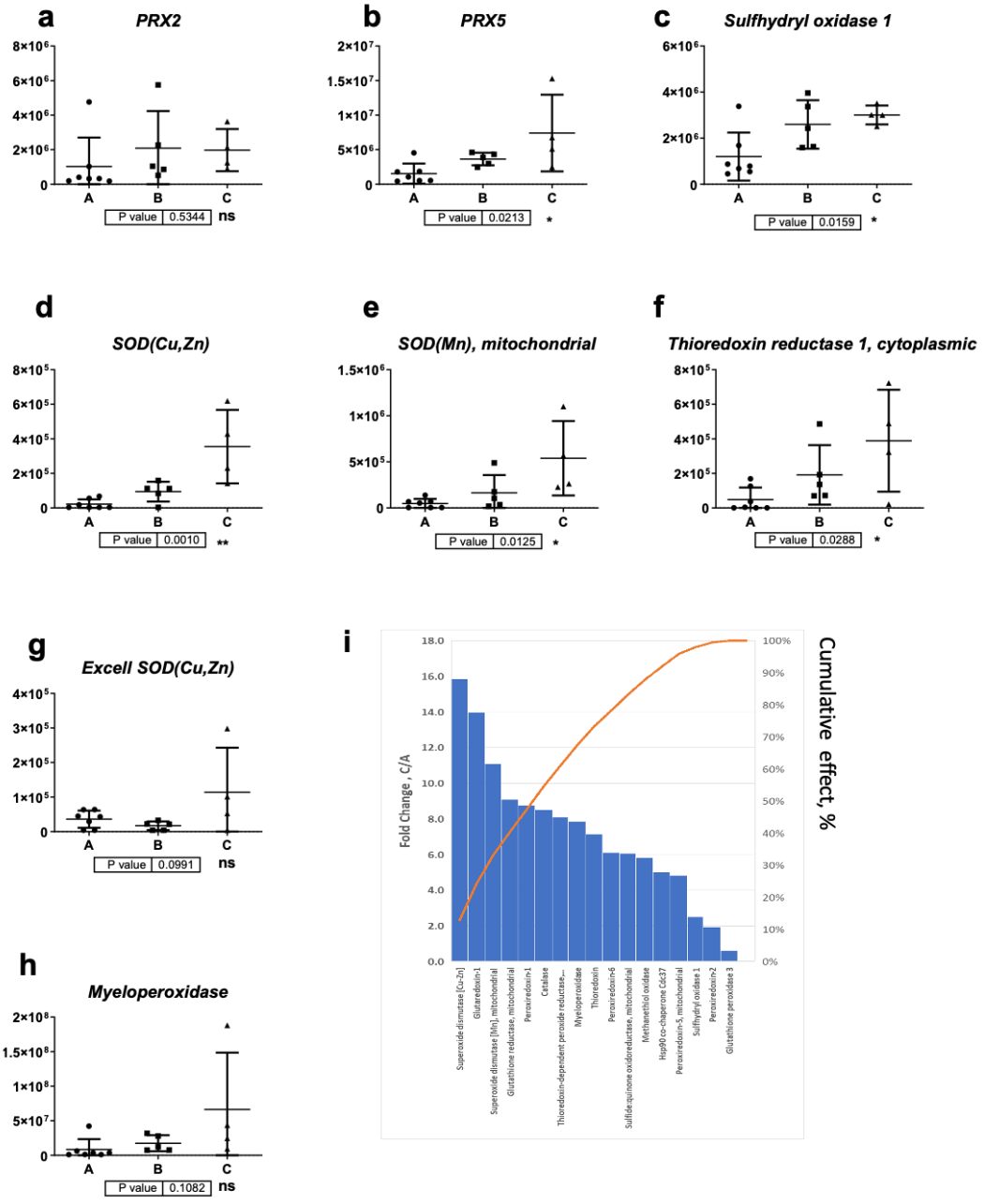
Supplemental Figure S4. **Comparison analysis of additional antioxidant proteins across groups.** BAL fluid antioxidant proteins (**a-h**) were evaluated (one-way ANOVA) across the patient Groups and found to be significantly different as indicated: * p=<0.05, ** p=<0. 002. The data are the mean ± SD. The Pareto plot (**i**) shows the relative contribution of the respective antioxidant protein based on fold change (FC) for the proteomic-informed Group C vs Group A (C/A; based on mean values).



Supplemental Figure S5. **Plasma proteomics PLS-DA analysis.** Plasma proteomic data (study admission/Day 0) was subjected to PLS-DA analysis using MetaboAnalyst, v6.0. The resultant Scores plot showed considerable overlap among the patient Groups (ovals represent the 95% confidence interval). Pairwise analysis by PERMANOVA (in MetaboAnalyst) yielded p values of Groups A vs B (0.655), Groups A vs C (0.45) and Groups B vs C (0.214).