ONLINE SUPPLEMENT

Supplemental Results

Supplemental Methods

Supplemental Results

Table S1 Baseline characteristics

.	all	Ph1, n=5	Ph2, n=4	p
Weight (kg)	48 (44-51)	48 (45-52)	47 (43-52)	0.9
Body surface area (per m^2)	1.3 (1.2-1.3)	1.3 (1.2-1.3)	1.2 (1.2-1.3)	0.9
Temperature (°C)	38.7 (37.9-38.9)	38.1 (37.7-38.7)	38.9 (38.0-39.4)	0.2
Hemodynamic parameters				
Mean arterial blood pressure (mmHg)	122 (90-129)	122 (88-127)	121 (83-130)	0.7
Heart rate (bpm)	72 (63-103)	90 (63-105)	72 (55-95)	0.6
Cardiac index	4.4 (3.3-5.3)	4.5 (3.2-6.6)	4.3 (3.4-5.4)	0.7
Mechanical ventilation				
Minute ventilation (L/min)	6.4 (5.5-6.9)	6.1 (5.3-6.9)	6.6 (5.7-6.9)	0.6
Compliance (mL/cmH_2O)	33.4 (26.5-42.3)	33.4 (26.7-53.0)	35.0 (23.8-40.0)	0.7
PEEP (cmH20)	5 (5-5)	5 (5-5)	5 (5-5)	1.0
Plateau pressure (cm H_2O)	15 (13-20)	15 (13-22)	15 (13-22)	1.0
Extravascular lung water index	27 (24-32)	27 (18-37)	28 (25-29)	1.0
Blood gases				
PaO2/FiO2 ratio	494 (412-528)	492 (284-513)	518 (499-533)	0.3
PaCO2 (mmHg)	41 (40-44)	43 (41-47)	41 (39-41)	0.1
Oxyhemoglobin	98 (98-99)	98 (96-99)	98 (98-99)	0.7
Lactate	0.8 (0.4-1.0)	0.8 (0.4-1.1)	0.7 (0.4-0.9)	0.7
Base excess (mmol/L)	2.0 (-0.2-3.9)	2.0 (0.4-4.7)	1.2 (-0.3-3.5)	0.4
Bicarbonate (mmol/L)	27 (24-28)	27 (25-29)	25 (23-27)	0.3
Full blood count				
Hemoglobin (g/L)	113 (105-121)	118 (104-122)	109 (105-119)	0.7
Platelets (10^9/L)	364 (250-413)	278 (204-413)	368 (278-426)	0.6
Neutrophil count (10^9/L)	1.8 (1.6-2.2)	2.1 (1.6-2.8)	1.7 (1.5-1.9)	0.3
Lymphocyte count (10^9/L)	2.9 (2.6-3.6)	2.9 (2.6-4.0)	2.9 (2.5-3.2)	0.7
Biochemistry				
Sodium (mmol/L)	143 (141-145)	143 (141-145)	142 (140-144)	0.4
Potassium (mmol/L)	4.7 (4.3-4.8)	4.3 (4.3-4.7)	4.8 (4.6-5.7)	0.1
Creatinine (mmol/L)	0.06 (0.06-0.08)	0.08 (0.06-0.08)	0.06 (0.05-0.08)	0.3
Bilirubin (umol/L)	4 (3-4)	3 (3-4)	4 (3-4)	0.1
ASAT (IU/L)	93 (85-108)	90 (77-99)	105 (93-134)	0.1
ALP (IU/L)	154 (121-294)	148 (74-213)	247 (140-343)	0.3
CK (IU/L)	192 (119-240)	128 (100-228)	214 (171-250)	0.2
Albumin (g/L)	34.0 (32.5-36.5)	34.0 (32.5-37.0)	34.0 (32.3-36.5)	0.9
Cytokines				
IL-6 plasma (pg/ml)	313 (0.4)	313 (0.4)	313 (0.5)	0.9
IL-8 plasma (pg/ml)	875 (625)	1248 (573)	409 (286)	0.03
IL-10 plasma (pg/ml)	992 (497)	985 (459)	999 (614)	1.0

Parameters displayed as median and IQR, cytokine levels as mean and standard deviation.

Abbreviations: Ph1 and Ph2: phenotype 1 and 2; PEEP: positive end-expiratory pressure; PaCO2 (mmHg): arterial carbondioxide partial pressure; ASAT: aspartate transaminase; ALP: alkaline phosphatase; CK: creatin kinase, IL: interleukin.

Table S2 Clinical and laboratory parameters at T12 and T24

	at T12			at T24		
	all, n=9	**Ph1, n=5**	**Ph2, n=4**	**all, n=9**	**Ph1, n=5**	**Ph2, n=4**
Temperature (°C)	38.9 (37.8-39.4)	39.8 (37.3-39.2)	39.3 (38.1-39.9)	38.7 (38.2-39.4)	38.6 (38.2-39.1)	39.2 (38.1-39.9)
Hemodynamic parameters						
Mean arterial blood pressure (mmHg)	71 (61-74)	71 (54-74)	71 (66-80)	76 (63-78)	64 (58-78)	77 (72-81)
Use of noradrenaline (mcg/kg/min)	0.17 (0.10-0.27)	0.17 (0.03-0.27)	0.19 (0.15-0.33)	0.25 (0.17-0.34)	0.25 (0.21-0.29)	0.28 (0.15-0.39)
Heart rate (bpm)	107 (61-74)	105 (96-130)	124 (96-142)	122 (101-128)	116 (101-126)	122 (99-133)
Cardiac index	4.9 (3.7-6.2)	3.9 (3.5-6.2)	5.6 (5.0-6.4)	6.5 (5.8-7.4)	6.2 (5.4-6.9)	7.1 (6.0-9.0)
Mechanical ventilation						
Minute ventilation (L/min)	10.8 (9.1-11.9)	10.7 (8.4-11.0)	11.9 (9.2-14.9)	14.7 (10.8-15.8)	13.8 (10.8-15.2)	15.4 (11.4-17.0)
Compliance (mL/cmH_2O)	17.4 (14.7-21.0)	15.6 (13.3-23.7)	17.7 (15.7-19.5)	19.0 (15.5-22.4)	18.5 (13.5-20.4)	21.0 (16.6-25.0)
Plateau pressure (cm H_2O)	22 (21-26)	22 (19-27)	22.5 (21.8-27.4)	26 (21-28)	26 (21-28)	24 (20-28)
Extravascular lung water index	49 (42-55)	55 (39-55)	49 (39-54)	50 (43-55)	50 (32-55)	48 (42-54)
Blood gases						
PaO2/FiO2 ratio	272 (209-324)	220 (174-290)	295 (273-329)	268 (216-309)	258 (136-278)	299 (265-339)
PaCO2 (mmHg)	47 (42-58)	47 (42-60)	48 (39-56)	52 (41-59)	52 (41-57)	53 (40-63)
Lactate	2.4 (2.0-4.3)	2.3 (2.0-3.3)	3.6 (1.7-6.5)	2.6 (1.6-4.3)	2.3 (1.6-2.6)	3.8 (1.3-8.7)
Base excess (mmol/L)	-0.8 (-3.4-0.1)	0 (-1.9-0.5)	-2.4 (-4.3—0.1)	-3.2 (-4.3—1.8)	-3.2 (-3.9-0.4)	-3.7 (-6.6—2.4)
Full blood count						
Hemoglobin (g/L)	113 (97-123)	113 (96-118)	113 (96-126)	108 (98-115)	108 (103-117)	103 (92-113)
Platelets (10^9/L)	237 (142-267)	250 (189-267)	176 (82-271)	188 (91-223)	217 (123-234)	140 (51-217)
Neutrophil count (10^9/L)	1.2 (0.8-1.5)	0.8 (0.7-1.2)	1.5 (1.2-2.0)	0.7 (0.3-1.3)	0.4 (0.2-0.7)	1.3 (0.6-1.5)
Lymphocyte count (10^9/L)	0.9 (0.8-1.3)	1.0 (0.6-1.4)	0.9 (0.9-1.2)	1.4 (0.9-1.8)	1.2 (0.9-1.8)	1.4 (0.9-1.9)
Biochemistry						
Sodium (mmol/L)	144 (143-147)	144 (142-148)	145 (143-147)	146 (144-150)	146 (144-151)	147 (144-151)
Potassium (mmol/L)	5.3 (4.8-6.5)	4.9 (4.4-6.3)	5.8 (5.0-6.6)	5.3 (4.9-6.3)	5.3 (4.8-6.2)	5.4 (4.9-6.8)
Creatinine (mmol/L)	0.12 (0.08-0.15)	0.12 (0.08-0.15)	0.14 (0.09-0.16)	0.14 (0.11-0.25)	0.14 (0.12-0.26)	0.15 (0.11-0.24)
Bilirubin (umol/L)	6 (2-13)	4 (0.5-15)	8 (4-14)	8 (6-19)	6 (4-20)	9 (7-19)
ASAT (IU/L)	196 (160-242)	180 (139-233)	215 (192-314)	353 (295-501)	353 (261-477)	383 (317-544)
ALP (IU/L)	107 (62-184)	80 (35-122)	184 (100-249)	89 (52-195)	75 (31-123)	195 (96-224)
CK (IU/L)	8119 (4597-12030)	6709 (2150-9514)	11157 (8557-14990)	21462 (15409-25673)	21462 (12105-31112)	21610 (18656-24386)
Albumin (g/L)	21.0 (19.5-22.0)	22.0 (21.0-22.5)	19.5 (16.5-21.0)	20.0 (17.0-21.5)	21.0 (18.5-22.0)	18.5 (14.8-20.8)
Cytokines						
IL-6 plasma (pg/ml)	217133 (183445)	1105456 (81890)	350480 (195261)	198673 (151525)	127902 (59632)	287137 (194173)
IL-8 plasma (pg/ml)	815 (658)	918 (865)	685 (341)	1524 (1430)	1916 (1562)	1034 (1275)
IL-10 plasma (pg/ml)	2458 (925)	2482 (865)	2427 (1106)	2789 (892)	2922 (940)	2625 (938)

Parameters displayed as median and IQR, cytokine levels as mean and standard deviation.

Abbreviations: Ph1 and Ph2: phenotype 1 and 2; PaCO2 (mmHg): arterial carbondioxide partial pressure; ASAT: aspartate transaminase; ALP: alkaline phosphatase; CK: creatine kinase, IL: interleukin; IFNy: interferon gamma.

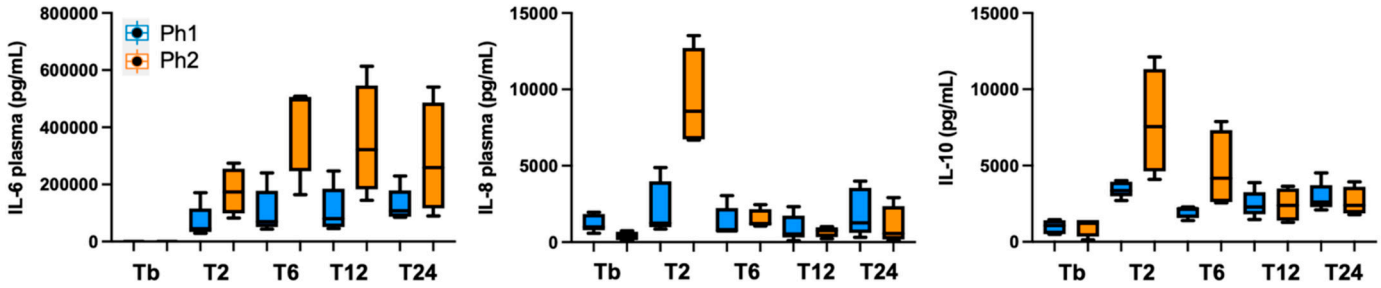

Figure S1 Cytokine levels in plasma among phenotypes at different time points during observation time. Abbreviations: Ph1 and Ph2: phenotype 1 and 2; IL: interleukin; Tb: baseline; T: time point.

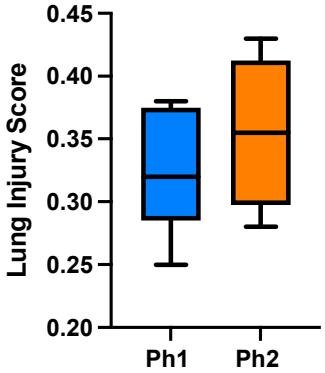

Figure S2 Histopathological assessment of the lungs at study end. Abbreviations: Ph1 and Ph2: phenotype 1 and 2

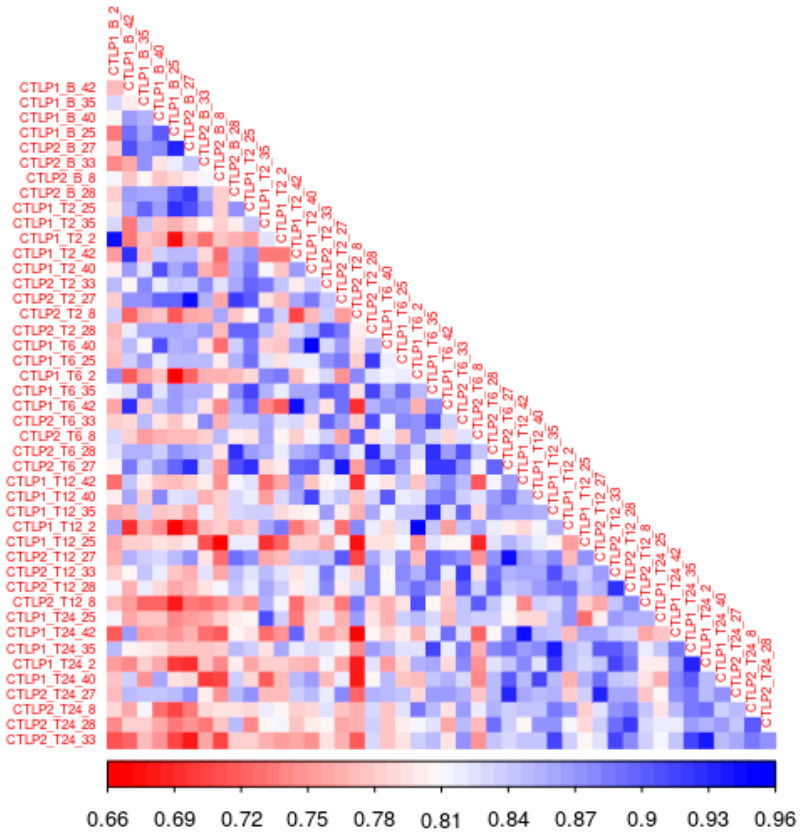

Figure S3 Correlation plot of all samples**.** Abbreviations: P1 and P2 = phenotype 1 and 2; CTL = control; T = time point

Table S3 Differentially expressed proteins among Ph1 and Ph2 at every time point

Baseline	protein	p (wilc)
	apolipoprotein A-II [Ovis aries]	0.015873016
	CD5 antigen-like [Ovis aries]	0.015873016
	complement factor I [Ovis aries]	0.015873016
	hypothetical protein JEQ12_008015 [Ovis aries]	0.015873016
	hypothetical protein JEQ12_008126 [Ovis aries]	0.015873016
	hypothetical protein JEQ12_010483 [Ovis aries]	0.015873016
	hypothetical protein JEQ12_014972 [Ovis aries]	0.015873016
	immunoglobulin J chain [Ovis aries]	0.015873016
	complement component C8 gamma chain [Ovis aries]	0.028571429
	lumican [Ovis aries]	0.028571429
	clusterin [Ovis aries]	0.031746032
	heparin cofactor 2 [Ovis aries]	0.031746032
	hypothetical protein JEQ12_001510 [Ovis aries]	0.031746032
	hypothetical protein JEQ12_002713 [Ovis aries]	0.031746032
	inter-alpha-trypsin inhibitor heavy chain H2 isoform X2 [Ovis aries]	0.031746032
	plasma protease C1 inhibitor [Ovis aries]	0.031746032
T2	**Protein**	**p (wilc)**
	synaptotagmin-like protein 4 isoform X3 [Ovis aries]	0.031746032
T6	**Protein**	**p (wilc)**
	hypothetical protein JEQ12_002713 [Ovis aries]	0.015873016
	hypothetical protein JEQ12_008129, partial [Ovis aries]	0.028571429
T12	**Protein**	**p (wilc)**
	hypothetical protein JEQ12_008126 [Ovis aries]	0.031746032
T24	**Protein**	**p (wilc)**
	adiponectin isoform X1 [Ovis aries]	0.015873016
	hypothetical protein JEQ12_008022 [Ovis aries]	0.015873016
	hypothetical protein JEQ12_010483 [Ovis aries]	0.015873016
	immunoglobulin J chain [Ovis aries]	0.015873016
	lumican [Ovis aries]	0.015873016
	plasma protease C1 inhibitor [Ovis aries]	0.015873016
	apolipoprotein E [Ovis aries]	0.028571429
	C4b-binding protein alpha chain isoform X8 [Ovis aries]	0.028571429
	alpha-2-HS-glycoprotein precursor [Ovis aries]	0.031746032
	apolipoprotein A-I isoform X1 [Ovis aries]	0.031746032
	apolipoprotein A-IV [Ovis aries]	0.031746032
	complement factor I [Ovis aries]	0.031746032
	gelsolin isoform X4 [Ovis aries]	0.031746032
	hypothetical protein JEQ12_002713 [Ovis aries]	0.031746032
	hypothetical protein JEQ12_003887 [Ovis aries]	0.031746032
	hypothetical protein JEQ12_008126 [Ovis aries]	0.031746032
	hypothetical protein JEQ12_013893 [Ovis aries]	0.031746032

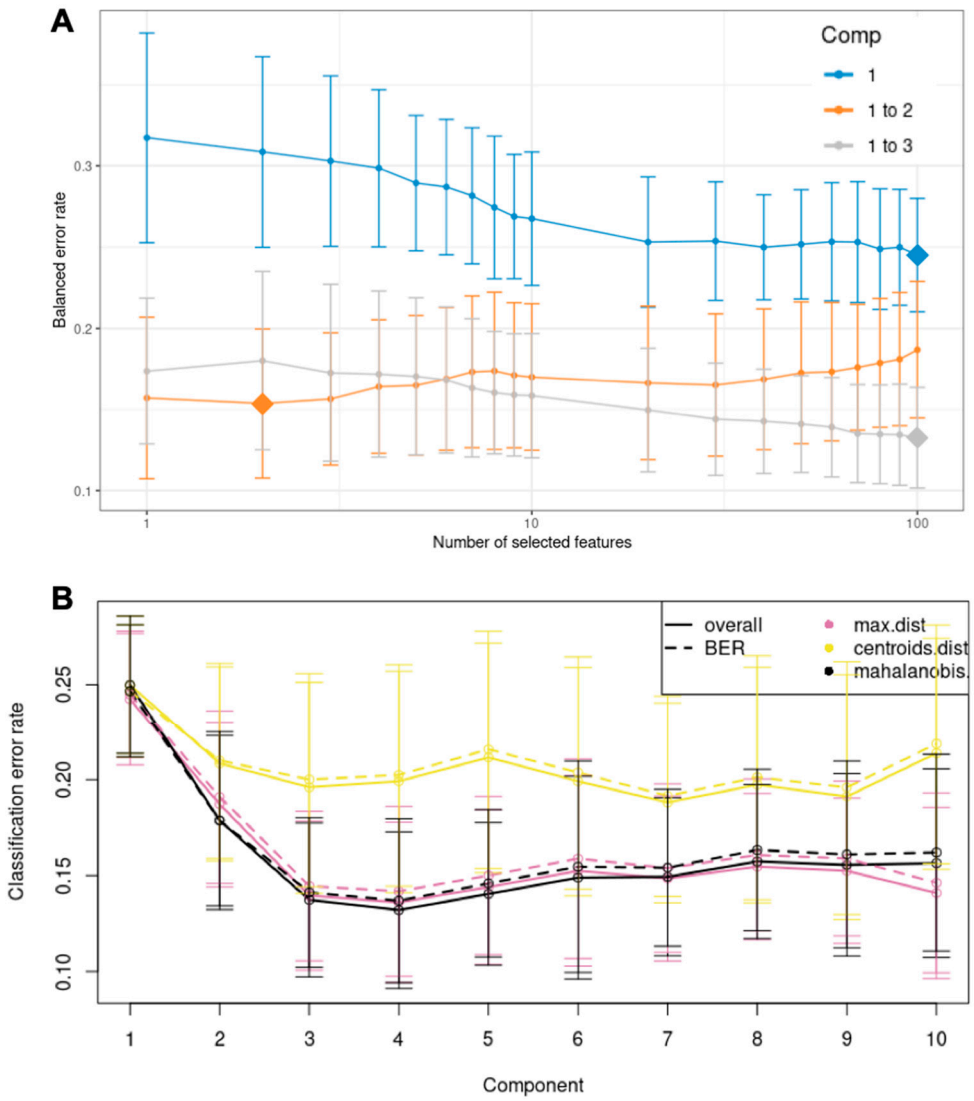

Figure S4 PLS-DA: features per component and initial error rate**.** Abbreviations: Comp: component, BER: balanced error rate; max. dist.: maximal distance; centroids dist.: centroids distance

Table S4 Biological processes and associated proteins in pathway analysis

biological process and involved proteins	strength	FDR
regulation of cholesterol and lipid metabolism	2.98	0.0115
APOC3		
APOA2		
phospholipid efflux	2.32	0.0301
APOC3		
APOA2		
Oxygen carrier and binding activity	2.24	0.027
HBB		
ENSOARP00000011736		
Complement and coagulation cascades	1.88	<0.0001
KNG1		
SERPINC1		
F2		
ENSOARP00000002890		
ENSOARP00000000771		
Negative regulation of endopeptidase activity	1.35	0.0097
ENSOARP00000016410		
ITIH2		
KNG1		
SERPINC1		
Inflammatory response	1.1	0.023
ENSOARP00000002890		
KNG1		
F2		
SAA1		
ENSOARP00000000771		
Negative regulation of catalytic activity	1.05	0.0015
ENSOARP00000000771		
ENSOARP00000016410		
SERPINC2		
KNG1		
ITIH2		
APOC3		
APOA2		
Defense responses	0.81	0.0314
ENSOARP00000000771		
ENSOARP00000002890		
APOA2		
F2		
KNG1		
SAA1		
IGJ		

Abbreviations: FDR: false discovery rate; APOA2: apolipoprotein A-II; ENSOARP00000000771: uncharacterized protein; ITIH2: inter-alpha-trypsin inhibitor heavy chain H2; HBB: hemoglobin subunit beta; IGJ: immunoglobulin J chain; ENSOARP00000011736: hemoglobin subunit alpha; SAA1: serum amyloid A protein; ENSOARP00000016410: uncharacterized protein, belongs to serpin family; APOC3: apolipoprotein CIII; F2: thrombin; ENSOARP00000002890: complement C4-like isoform X1; SERPINC1: antithrombin-III precursor; KNG1: kininogen-1 isoform X2.

Supplemental Methods

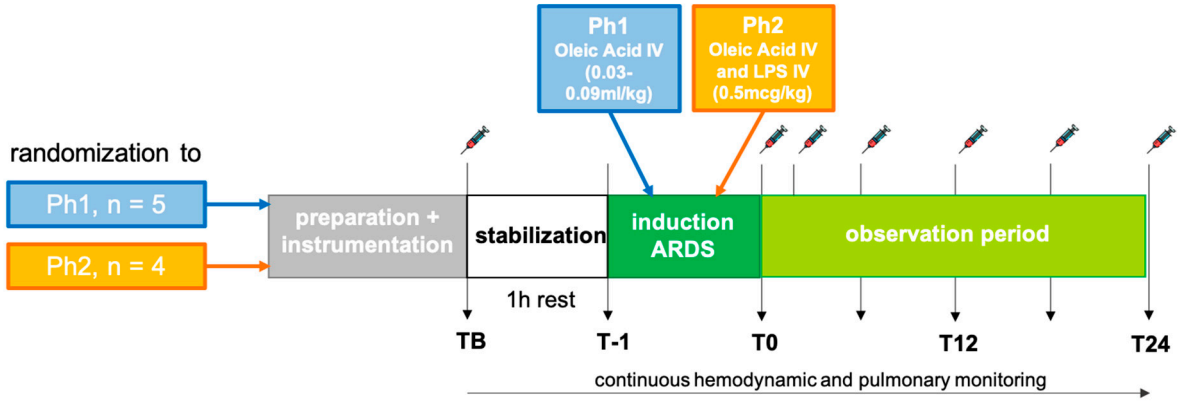

Figure S5 Study design and time line. Abbreviations: ARDS: Acute Respiratory Distress Syndrome; Ph1 and Ph2: phenotype 1 and 2; IV: intravenously; LPS: lipopolysaccharides; T: time point; TB: baseline time point

Adherence to main features of experimental ALI according to the ATS definition

The main features of experimental animal models of ALI are:

a) *Histological evidence of tissue injury* is reported in the LIS score that consists of the components alveolar neutrophils, interstitial neutrophils, hyaline membranes and septal thickening (Figure S2).

b) *Alteration of the Alveolar Capillary Barrier* is reported as the extent of extravascular lung water (Table S1 and S2).

c) *Inflammatory Response* to the lung injury is demonstrated in Figure S1 as cytokine levels at different time points.

d) *Physiological Dysfunction* is described as the extent of decrease in oxygenation and alteration in pulmonary mechanics (Table S1 as compared to Table S2).