Humanized Monoclonal Antibody Rescues Rhesus macaques from Aerosolized Ricin Toxin Exposure

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Abstract

Ricin toxin ranks at the top of the list of potential bioweapons of concern to civilian and military personnel alike due to the high potential for morbidity and mortality after inhalation. In non-human primates, aerosolized ricin triggers a severe acute respiratory distress characterized by perivascular and alveolar edema, neutrophilic infiltration, and severe necrotizing bronchiolitis and alveolitis. Despite heightened public health and military concerns, there are currently no approved countermeasures for ricin. In this report, we demonstrate the therapeutic potential of huPB10, a toxin-neutralizing humanized monoclonal antibody (MAb) against an immunodominant epitope on ricin's enzymatic subunit. Five rhesus macaques that received intravenous huPB10 (10 mg/kg) four hours after lethal dose ricin aerosol exposure all survived the toxin challenge, as compared to control animals, which succumbed to ricin intoxication within 30 h. Antibody treatment at 12 h post-ricin exposure resulted in the survival of only one of five monkeys, signifying that, in the majority of animals, ricin intoxication and local tissue damage had progressed beyond the point where huPB10 intervention was beneficial. Principle coordinate analysis of fold change in pro-inflammatory cytokine/chemokines levels in bronchial alveolar lavage fluids before and after toxin challenge successfully clustered monkeys based on survival, as well as treatment group. IL-6 was the most apparent marker of ricin intoxication. This study represents the first demonstration in nonhuman primates that the lethal effects of inhalational ricin exposure can be negated by a drug candidate and opens up a path forward for product development.

52
53 **Introduction**

54 Ricin toxin is considered a high priority biothreat agent by the Centers for
55 Disease Control and Prevention (CDC), the US Department of Defense (DOD), and
56 NATO due to it's accessibility, stability, and high toxicity especially by the aerosol route.
57 (1, 2). In nonhuman primates (NHPs), inhalational ricin exposure elicits the clinical
58 equivalent of acute respiratory distress syndrome (ARDS), characterized by widespread
59 apoptosis of alveolar macrophages, intra-alveolar edema, neutrophilic infiltration,
60 accumulation of pro-inflammatory cytokines, and fibrinous exudate (3). Similar effects
61 are observed in experimental models of aerosol or intranasal ricin challenge conducted
62 in mice, rats and swine (4-8). Ricin toxin, derived from castor beans (*Ricinus*
63 *communis*), is a 65 kDa heterodimeric glycoprotein consisting of two subunits, RTA and
64 RTB, joined via a single disulfide bond. RTB binds to glycoproteins and glycolipids on
65 mammalian cells and facilitates ricin's retrograde transport to the endoplasmic reticulum
66 (ER). In the ER, RTA is liberated from RTB and then retrotranslocated into the
67 cytoplasm via the Sec61 complex (9). RTA is an RNA N-glycosidase that catalyzes the
68 hydrolysis of a conserved adenine residue within the sarcin/ricin loop of 28S rRNA,
69 resulting in protein synthesis arrest (10, 11) and activation of programmed cell death
70 (12). Alveolar macrophages are particularly sensitive to the cytotoxic effects and secrete
71 an array of pro-inflammatory cytokines before undergoing programmed cell death (6, 7,
72 13).

73 In this report we investigated the potential of humanized monoclonal antibody
74 (mAb) huPB10 to serve as a therapeutic in an established Rhesus macaques model of
75 inhalational ricin challenge (3, 14). PB10 was first described as a murine mAb with
76 potent-toxin neutralizing activity *in vitro* and *in vivo* (15). PB10 recognizes an
77 immunodominant epitope situated at the apex of RTA, relative to RTB (16, 17).
78 Chimeric (cPB10) and fully humanized (huPB10) versions of PB10 retain *in vitro* toxin-
79 neutralizing activity and have been shown to passively protect mice against lethal dose
80 ricin toxin administered by injection or inhalation (18, 19). It was also demonstrated that
81 huPB10 can rescue mice from ricin intoxication if administered 4-6 h post toxin
82 exposure (19). Based on these preliminary findings, we now sought to assess the

83 therapeutic potential of huPB10 in a well-established nonhuman primate (NHP) model
84 of ricin aerosol challenge (14). The NHP is believed to be the model most
85 representative of human ricin aerosol exposure; as such, it would likely be the primary
86 efficacy model for approval of an anti-ricin drug by the Food and Drug Administration.
87
88 **Results**
89 A total of 12 rhesus macaques (~7 kg; range 3.8-10.2 kg) bred at Tulane National
90 Primate Research Center (TNPRC) were randomly assigned to three experimental
91 groups and then challenged with ricin by small particle aerosol at a target dose of 18
92 µg/kg (**Table 1; Figure 1**). Group 1 (n=2) received intravenous administration of saline
93 4 h post challenge. Animals in groups 2 and 3 received huPB10 at 4 h or 12 h post
94 challenge, respectively (**Table 1**). huPB10 was administered intravenously at a final
95 dose of 10 mg/kg (**Table S1**). The macaques were subjected to whole body
96 plethysmography and radiotelemetry over the course of the study. Animals surviving on
97 day 14 post challenge were euthanized and subjected to complete necropsy and
98 histopathological analysis, as done for the other animals in the study. Serum and BAL
99 fluids were collected from the animals before and 24 h post ricin exposure.
100 Control animals (n=2) succumbed to ricin toxicosis within 36 hours of ricin
101 exposure (**Table 1; Figure 1**). The clinical progression of ricin-induced intoxication of
102 the control animals mimicked previous studies (3). Approximately 12-16 hours after
103 exposure, animals displayed reduced activity and signs consistent with fever (**Figure 2**).
104 Clinical examination conducted 24 h post exposure revealed respiratory complications,
105 including bilateral congestion and crackles, with dyspnea and tachypnea. Arterial
106 oxygen ranged from 75-85%. The clinical state of the animals continued to decline over
107 the subsequent several hours, manifested by a marked drop in normal activity and
108 mucous membranes appearing cyanotic.
109 In contrast, all five of the Rhesus macaques that received huPB10 at 4 h post-
110 ricin challenge time point survived (*P<0.01* compared to controls) and remained
111 otherwise normal for the duration of the three week post-exposure observation period
112 (**Table 1: Figure 1**). Compared to the control animals, the five macaques in Group 2

113 presented with only minimal signs of distress: mild dyspnea, minimal tachypnea and
114 increased lung sounds upon physical examination at 24 h post-exposure.
115 Finally, only one of the five animals in Group 3 that received huPB10 at 12 h
116 survived ricin challenge (**Table 1; Figure 1;** *P<0.01* and *P<0.05* compared to controls
117 and +4 h group, respectively). The remaining four animals succumbed to ricin
118 intoxication between 44 and 72 h post challenge and followed a clinical course similar to
119 the control animals. HuPB10 was detected in the sera and BAL fluids collected at 24 h
120 post ricin challenge, indicating that biodistribution of huPB10 was similar between
121 groups 2 and 3.
122 Gross examination of the sham-treated control animal lungs showed coalescing
123 hemorrhage with frothy exudate marked by fibrin in lung parenchyma (**Figure S1**). The
124 lungs of control animals were grossly described as fibrinosuppurative bronchointerstitial
125 pneumonia with pulmonary edema and bronchial epithelial necrosis, with severe
126 fibrinosuppurative lymphadenitis in the bronchial lymph nodes. The wet weights of
127 control animal lungs were >150 grams in contrast to a normal wet lung weight of ~30-40
128 grams in naïve animals of approximately the same body weight. Histologically, the lungs
129 of sham-treated animals showed hallmark inflammation consistent with ricin-induced
130 injury, characterized by marked edema, corresponding hemorrhage, and numerous
131 infiltrates. The pathological outcome of the four animals in Group 3 that succumbed to
132 ricin intoxication resembled that of the control animals. There was extensive pulmonary
133 congestion, edema, inflammation with infiltrates, and punctate hemorrhage evident.
134 Gross lung weights were similar to ricin-challenged control animals (>140 g) with clear
135 signs of hemorrhage.
136 Pathological analysis of the lung tissues collected at the time of euthanasia (day 21
137 post challenge) from the five survivors in Group 2 that had been treated with huPB10 at
138 4 h and the single animal in Group 3 (DR61) revealed evidence of chronic inflammation
139 and a distinctive fibrosis proximal to the respiratory bronicholes, reminiscent of past
140 studies in which animals had received sub-lethal exposures to ricin toxin (20).
141 Sera and BAL fluids collected before (day -7) and 24 h post ricin challenge were
142 subjected to analysis with a 29-plex cytokine/chemokine/growth factor Luminex array as
143 a means to assess the impact of huPB10 on local and systemic inflammatory

144 responses. In the sera of the two control animals, there were 12 cytokines/chemokines
145 that were significantly different in serum post- versus pre-challenge; 11 cytokines were
146 increased, while one (IL-8) was decreased (**Figure 3A; S2**). Most notable were IL-6
147 (~500-fold increase) and IL-1RA (~256-fold increase). VEGF was also elevated,
148 possibly reflecting response to pulmonary insult. Analysis of sera from animals that
149 received huPB10 at 4 h post challenge indicated that only four cytokine/chemokines
150 were significantly different from pre-challenge levels (3 up;1 down), including a ~4-fold
151 increase in IL-6. IL-1RA and VEGF levels were unchanged. In animals that received
152 huPB10 at 12 h post challenge, a total of six cytokines/chemokines changed relative to
153 pre-challenge levels, although the magnitude of these changes was less compared to
154 the control animals, possibly reflecting a dampening of the inflammatory response as a
155 consequence of huPB10 intervention. Principle component analysis (PCA) of fold-
156 change in cytokine/chemokine levels from serum samples from all 12 monkeys did not
157 reveal any clustering by experimental group or survival (**Figure S3A-C**), signifying that
158 serum inflammatory responses are not indicative of experimental outcome.
159 The impact of huPB10 intervention was much more apparent in BAL fluids than in
160 sera (**Figure 3B**). Within group 2, a total of 21 cytokines/chemokines were significantly
161 elevated following ricin challenge (as compared to pre-challenge levels), with IL-6 being
162 the most pronounced. In group 3, there were 25 cytokines/chemokines that were
163 significantly elevated compared to pre-challenge levels. Seven cytokines/chemokines
164 differed between the groups 2 and 3 with the most notable being IL-6, which was 32-fold
165 elevated in group 2 and 181-fold elevated in group 3. PCA successfully clustered
166 animals by both group and survival status, demonstrating that the localized cytokine
167 responses in the BAL are more closely tied to survival than the systemic responses
168 (**Figure S3D-E**). The relative contributions of the different cytokines/chemokines
169 responsible for segregating the animals into clusters are shown in **Figure S3F**.
170

171 **Discussion**

172 The results of this study constitute a major advance in longstanding efforts to
173 develop effective medical countermeasures against ricin toxin (2, 21). Foremost, it is the
174 first demonstration in NHPs that a drug candidate can rescue animals from the lethal

175 effects of aerosolized ricin toxin exposure. This is significant considering that licensure
176 of anti-ricin products for human will need to adhere to the Food and Drug
177 Administration's Animal Rule; human challenge studies with ricin are obviously unethical
178 (22). The well-established aerosolized ricin challenge model in Rhesus macaques was
179 a prerequisite for the therapeutic study conducted herein (3, 14, 23). Comparative
180 models in mice (4, 6, 24) and swine (8) may also be important for therapeutic
181 development under Animal Rule guidelines.

182 The fact that all five animals in group 2 (+4 h huPB10 intervention), and one animal
183 in group 3 (+12 h huPB10 intervention), survived toxin exposure indicates that a
184 significant proportion of ricin remains accessible to huPB0 in the alveolar space and/or
185 interstitial fluids for hours after inhalational exposure. This finding is remarkably
186 consistent with what has been reported in mice with huPB10 and other anti-ricin mAbs
187 (4, 5, 19, 25), but surprising all the same considering the rate that ricin can gain entry
188 into mammalian cells (26) and the extraordinary sensitivity of the lung mucosa to the
189 effects of toxin exposure. We postulate high dose intravenous delivery of huPB10
190 results in the accumulation of huPB10 within the lung mucosa and alveolar space where
191 it can engage free (soluble) or receptor-bound ricin. Whether transudation or active
192 transport of antibodies from serum into the lung mucosa is triggered by ricin exposure
193 itself has not been evaluated (27). Nonetheless, once in the lung mucosa, huPB10
194 presumably limits toxin uptake into target cells (e.g., macrophages and airway epithelial
195 cells) and/or interferes with ricin intracellular transport in the event that endocytosis of
196 ricin-antibody complexes should occur (28, 29). Work by Magun and colleagues made
197 it clear more than a decade ago that protecting alveolar macrophages is paramount in
198 limiting toxin-induced lung damage (7, 30).

199 It is worth underscoring the value of the 29-plex monkey cytokine/chemokine/growth
200 factor array in not only identifying local inflammatory markers like IL-6 that arise
201 following ricin exposure but also enabling through PCA the unbiased clustering of
202 specific animals based on survival or experimental group. Identifying an inflammatory
203 “fingerprint” associated with ricin exposure has obvious applications in diagnostics,
204 especially in the context of biodefense where early clinical symptoms following
205 exposure to different toxins and pathogens may in fact be indistinguishable (31). As

206 noted above, IL-6 levels were particularly elevated following ricin exposure, which is
207 consistent with what has been observed in mice (32). IL-6 is a particularly potent driver
208 of pulmonary inflammation and could very well be a major contributor to ricin-induced
209 pathology (33). In fact, we have observed that human lung epithelial cell lines
210 preferentially secrete IL-6 following ricin exposure, particularly in conjunction with co-
211 stimulatory molecules like TRAIL (Y. Rong, J. Westfall, D. Ehrbar, T. LaRocca, and N.
212 Mantis, *manuscript in revision*). The possibility that the huPB10 therapeutic window
213 might be extended with anti-inflammatory agents is worth investigating (25).
214
215 **Methods**
216 **Ricin toxin and huPB10.** Purified ricin toxin derived from castor beans (*Ricinus*
217 *communis*) was produced at the University of Texas Southwestern and was procured
218 from BEI resources (14). huPB10 was expressed using a *Nicotiana benthamiana*-based
219 manufacturing platform. The properties of huPB10 used in this study are shown in
220 **Table S1.**
221
222 **Animal care and use.** Rhesus macaques were born and housed at the Tulane
223 National Primate Research Center (Covington, LA), which is US Department of
224 Agriculture-licensed and fully accredited by the Association for Assessment and
225 Accreditation of Laboratory Animal Care (AAALAC). Aerosolization, dosing and delivery
226 of ricin were performed as described (14). The LD_{50} of ricin is 5.8 µg/kg body weight
227 and the target dose for this experiment was set at the equivalent of three LD_{50}s (≈18
228 µg/kg). The mean inhaled dose of ricin across all animals was 4.4 ± 1.4 LD_{50}s.
229 At 4 h or 12 hours post-exposure, designated animal groups received a single
230 intravenous administration of huPB10 by slow infusion at an individualized unit dose of
231 10 mg/kg. Sham-treated animals were administered saline for injection at the 4h time
232 point. Treated animals were observed for signs of adverse reactions to the antibody
233 during administration and throughout anesthesia recovery period. Animals were bled
234 just before and 24 h following aerosol challenge. Blood was also collected when the
235 animals either succumbed to intoxication or 21 d after challenge, when the experiment
236 was terminated. Animals determined to be in respiratory distress and those that

survived for 21 d after exposure to ricin were euthanized by an overdose of sodium pentobarbital, consistent with the recommendation of the American Veterinary Medical Association's Panel on Euthanasia, and submitted for necropsy. All methods were approved by the Tulane Institutional Animal Care and Use Committee (IACUC). After gross necropsy, tissues were collected in neutral buffered zinc-formalin solution (Z-Fix Concentrate, Anatach). Tissues were processed, sectioned, and stained as previously described (3).

Statistics. Statistical analysis was carried out with GraphPad Prism 6 (GraphPad Software, La Jolla California USA). The difference in outcomes between groups was determined by Fisher's exact test (two-tailed) and the mean survival times after exposure to ricin were compared by log-rank analysis of Kaplan–Meier survival curves. The statistical significance of the effects of ricin challenge and huPB10 intervention on cytokine levels were analyzed with two-way repeated measures ANOVAs in both serum and BAL, with the repeated measures being pre- and post-exposure status, and treatment group as the independent measure. Resulting p-values were corrected with the Benjamini, Krieger, and Yekutieli method to control false discovery rate. All analyses were performed on $\log^2$ transformed raw fluorescent intensity values to avoid the need to censor values. PCA analysis was performed using singular value decomposition with the R package FactoMineR (34). Heatmap construction using raw fold change values was done using GraphPad Prism 6. Hierarchical clustering and scaled heatmap construction was completed with the R package pheatmap (35).

Author contributions. NB, OB, DK, MP, KW and ESV generated reagents; PJD, LDM, YR, and CJR conducted animal studies and animal tissue/sample analysis; DJE performed statistical analysis; CJR, NJM and LZ are responsible for experimental design and manuscript preparation.

Conflicts of interest. NB, OB, DK, MP are Mapp Biopharmaceutical employees and shareholders. KW and LZ are employees, shareholders, and co-owners of Mapp Biopharmaceutical. The remaining authors declare no conflicts of interest.

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380
381

382 **Figure Legends**

383

384 **Figure 1. Ricin aerosol challenge and effect of treatment and macaque**
385 **survival.** A. Animals were aerosol challenged with ricin aerosols and the individual ricin
386 doses, expressed in µg/kg inhaled, and clustered by treatment group, show minimal
387 variation in dosing, bars represent group mean ± standard deviation; the aerosol target
388 dose of 18 µg/kg represented by segmented line. B. Macaque survival is represented by
389 survival curve. The two control animals succumbed to intoxication by day 2; treatment
390 with huPB10 at 4 h post ricin challenge resulted in 5/5 survival, while treatment with
391 huPB10 at 12 h resulted in only 1/5 survivors.

392

393 **Figure 2. Physiological response to ricin.** A. Radiotelemetry of core temperature of
394 rhesus macaques treated with huPB10 either at 4 h or 12 h post-exposure to ricin
395 aerosol. Continuous monitoring showed significant differences ($P<0.005$) between the
396 initiation and tempo of pyrexia that was dependent on the time to treat. B. Fever
397 intensity showed differences between the relative change (increase) based upon time to
398 treat. Respiratory function measured by whole-body plethysmography. Respiratory
399 function was measured via head-out conductance plethysmography prior to and at
400 timed intervals post challenge. C. Significant changes ($P<0.05$) in post-exposure group
401 tidal volume was observed among animals rescued from ricin intoxication, (D) although
402 compensatory changes in frequency resulted in minimal observable changes in minute
403 volume, with some animals producing increased minute volumes +24 h post-exposure
404 to ricin.

405

406 **Figure 3. Cytokine profiles of serum and BAL in ricin challenged Rhesus**
407 **macaques.** Heatmaps present $\log_2$-fold change of cytokines in (A) serum and (B) BAL,
408 comparing samples collected 24 hours post-challenge to those collected 7 days pre-
409 challenge. Animals are separated into groups for controls, 4-hour, and 12-hour huPB10
410 interventions. Increases in cytokine levels are shown in red, while decreases are shown
411 in blue. Specific values for the fold changes are shown within each cell.

412

413

414 **Tables**

415

Table 1. Experimental groups and outcome of ricin challenge

					huPB10 (μg/ml)[c]			
Group	**ID**	**kg**	**M/F[a]**	**RT[b]**	**serum**	**BAL**	**TTD[d]**	**#/total**
Control	DH27	9.7	M	14	-	-	28 h	
	JP56	8.4	M	26	-	-	30 h	0/2
+4 h huPB10	LJ80	4.4	M	31	157	2.8	+	
	LB98	4.9	M	29	177	2.7	+	
	EM61	9.5	M	31	207	2.5	+	
	IC70	5.9	F	26	225	3.6	+	
	IL40	6.1	F	33	147	3.2	+	5/5
+12 h huPB10	JM39	9.4	M	22	205	42.8	72 h	
	DR61	10.2	F	24	265	3.9	+	
	KP48	5.8	M	30	203	25.7	48 h	
	KR49	5.7	M	29	151	3.1	49 h	
	LB26	3.8	M	29	191	11.3	44 h	1/5

[a], sex of animal; [b], ricin toxin (RT) dose (μg/kg) received per animal; [c], huPB10 in serum and BAL from samples collected at 24 h post challenge. The average in serum was 182.6 μg/ml and 203 μg/ml for the +4 and +12 h groups, respectively. In the BAL, the was 3.04 μg/ml and 17.3 μg/ml, respectively. Times of huPB10 administration are approximate (~ 30 minutes of point estimate); the logistics of performing slow intravenous infusion of huPB10 in monkeys initiated and was times at 4 hours from each of the individual ricin aerosol challenge events and was performed in sequence; [d], time to death (TTD); +, indicates survival for 21 day duration of study.

416

417

418 **Supplemental Material**

419

420 Supplemental Methods

421

422 **Animal Husbandry and Telemetry.** Rhesus macaques were born and housed at the
423 Tulane National Primate Research Center (Covington, LA), which is US Department of
424 Agriculture-licensed and fully accredited by the Association for Assessment and
425 Accreditation of Laboratory Animal Care. Subcutaneous radiotelemetry transmitters
426 combined with sensors capable of detecting biopotential signals of an electrocardiogram
427 as well as thermistor-type sensors capable of detecting temperature signals (T34G-8;
428 Konigsberg Instruments) were surgically implanted under aseptic conditions in 8 of the
429 12 vaccinated macaques and both control macaques before the start of the study.
430 Animals determined to be in respiratory distress and those that survived for 21 d after
431 exposure to ricin were euthanized by an overdose of sodium pentobarbital, consistent
432 with the recommendation of the American Veterinary Medical Association's Panel on
433 Euthanasia, and submitted for necropsy. All methods were approved by the Tulane
434 Institutional Animal Care and Use Committee (IACUC).

435

436 **Treatment of Rhesus macaques**. At 4 h or 12 hours postexposure, designated animal
437 groups received a single intravenous administration of huPB10 by slow infusion at an
438 individualized unit dose of 10 mg/kg. Sham-treated animals were administered saline for
439 injection at the four-hour time point. Treated animals were observed for signs of
440 adverse reactions to the antibody during administration and throughout anesthesia
441 recovery period. Animals were bled just before and 24 h following aerosol challenge.
442 Blood was also collected when the animals either succumbed to intoxication or 21 d
443 after challenge, when the experiment was terminated.

444

445 **Ricin aerosolization, dosing, and calculation**. Aerosolization, dosing and delivery of
446 ricin were performed as described (14). Inductive plethysmography that measures
447 volume of air breathed by each individual animal per minute was performed just before
448 the ricin exposure. Ricin was dissolved in 10 mL sterile phosphate buffer saline to the

449 desired concentration for each animal based on plethysmography data obtained 2 d
450 before the exposure. Aerosols were generated directly into a head-only chamber using
451 a Collision three jet-nebulizer (BGI) with fully automated management control system
452 (Biaera Technologies, Hagerstown, MD) all within a Class III biological safety cabinet
453 housed within the TNPRC high-containment (BSL-3) laboratories. The nebulizer
454 operated at 18 lb/inch^2 equating to a flow of 7.5 L/min and produced 3.0E+04 particles
455 per cc with a mass median aerodynamic diameter of ~1.4 μm. Each discrete aerosol
456 exposure lasted 10 minutes (per animal). Air samples were continuously obtained
457 during the exposure and the protein concentrations of these samples were determined
458 using a micro-BSA protein assay kit (Thermo Scientific). The aerosol concentrations
459 were determined and the inhaled dose of RT for each animal was calculated by
460 multiplying the empirically determined aerosol exposure concentration (μg/liter of air) in
461 the chamber by volume of air estimated to have been breathed by the animal (via
462 results of plethysmography just before exposure). The LD_{50} of ricin is 5.8 μg/kg body
463 weight and the target dose for this experiment was set at the equivalent of three LD_{50}s
464 (≈18 μg/kg). The mean inhaled dose of ricin across all animals was 4.4 ± 1.4 LD_{50}s.
465
466 **Statistics**. Statistical analysis was carried out with GraphPad Prism 6 (GraphPad
467 Software, La Jolla California USA). The difference in outcomes between groups was
468 determined by Fisher's exact test (two-tailed) and the mean survival times after
469 exposure to ricin were compared by log-rank analysis of Kaplan–Meier survival curves.
470 The statistical significance of the effects of ricin challenge and huPB10 intervention on
471 cytokine levels were analyzed with two-way repeated measures ANOVAs in both serum
472 and BAL, with the repeated measures being pre- and post-exposure status, and
473 treatment group as the independent measure. Resulting p-values were corrected with
474 the Benjamini, Krieger, and Yekutieli method to control false discovery rate. All
475 analyses were performed on log^2 transformed raw fluorescent intensity values to avoid
476 the need to censor values. PCA analysis was performed using singular value
477 decomposition with the R package FactoMineR (34). Heatmap construction using raw
478 fold change values was done using GraphPad Prism 6. Hierarchical clustering and
479 scaled heatmap construction was completed with the R package pheatmap (35).

480

481 **Tissue Collection, Histological Analysis, and Special Stains**. After gross necropsy,
482 tissues were collected in neutral buffered zinc-formalin solution (Z-Fix Concentrate,
483 Anatach). Tissues were processed, sectioned, and stained as previously described (3)
484

485

486 **Supplemental Table**

487

Table S1. Characteristics of huPB10 used in study		
Test Parameters	**Test Method**	**Result**
Concentration	UV Absorbance	19.7 mg/mL
Appearance	Visible	clear, liquid
Physical/chemical	pH Determination	5.6
Purity	SDS-PAGE	> 99%
Osmolality	Micro-Osmometer	412 mOS/kg
Bioburden	Bioburden Testing	0 CFU/mL
Aggregates	Size Exclusion HPLC	1.90%
Potency	ELISA	20.0 mg/mL
Safety	Endotoxin	0.2 EU/mg

488

489

490

491

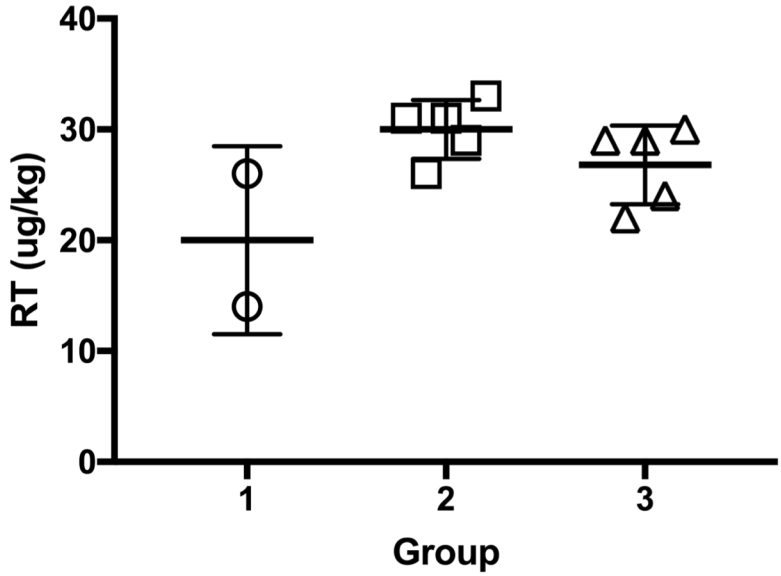
492 **Figure S1. Gross pathology associated with ricin exposure and huPB10**
493 **intervention**. The lungs of sham-treated animals (Figure 3a) show hallmark
494 inflammation, marked edema, corresponding hemorrhage, and numerous infiltrates;
495 grossly, wet weight at 3x normal with coalescing hemorrhage. Animals treated with
496 huPB10 at 4 h (Figure 3b & 3c) show remarkably little pulmonary damage, with
497 occasional infiltrates; lung wet weights were essentially normal. Animals treated with
498 huPB10 at 12 hours (Figure 3d) demonstrated mild to moderate inflammation with
499 infiltrates, edema, and punctate hemorrhage evident; gross lung weight were
500 approximately 2x with clear signs of hemorrhage. Original magnification at 10x
501

502 **Fig. S2 Scaled heatmap and cluster analysis of log2-fold changes in cytokine**
503 **levels in individual macaques following ricin challenge.** Heatmaps visualizing log2-
504 fold changes in cytokines in serum (A) and BAL (B). Both individual animals and
505 cytokines are arranged by hierarchical clustering, represented by dendrograms on top
506 and to the right of the heatmaps, respectively. Surviving animals are marked in white in
507 the top bar above the heatmap, while dead animals are in black. In the bar below this,
508 the 4-hour group of animals are marked in red, the 12-hour group in blue, and the
509 control group in green. Fold change values are centered and scaled for each cytokine
510 by first subtracting the mean fold change from each value and then dividing by the
511 standard deviation of that cytokine.
512

513 **Fig. S3 Principal component analysis of cytokine fold changes following ricin**
514 **challenge.** Scatter plots of first 2 principal components of log2-transformed cytokine
515 fold changes in A-C) BAL and (D-F) serum from all animals included in the study,
516 calculated used singular value decomposition. Each dot represents an individual
517 monkey; red for those in the 4-hour group, black for the 12-hour group, and green for
518 the control group. 95% confidence ellipses of the group means are represented in the
519 color of the group they correspond to. Eigenvectors for (C) BAL and (F) and analyses
520 are colored to show the percent contribution of each variable to the principal
521 components.

Figure 1

A.


40
30
20
10
0
RT (ug/kg)
1
2
3
Group

B.

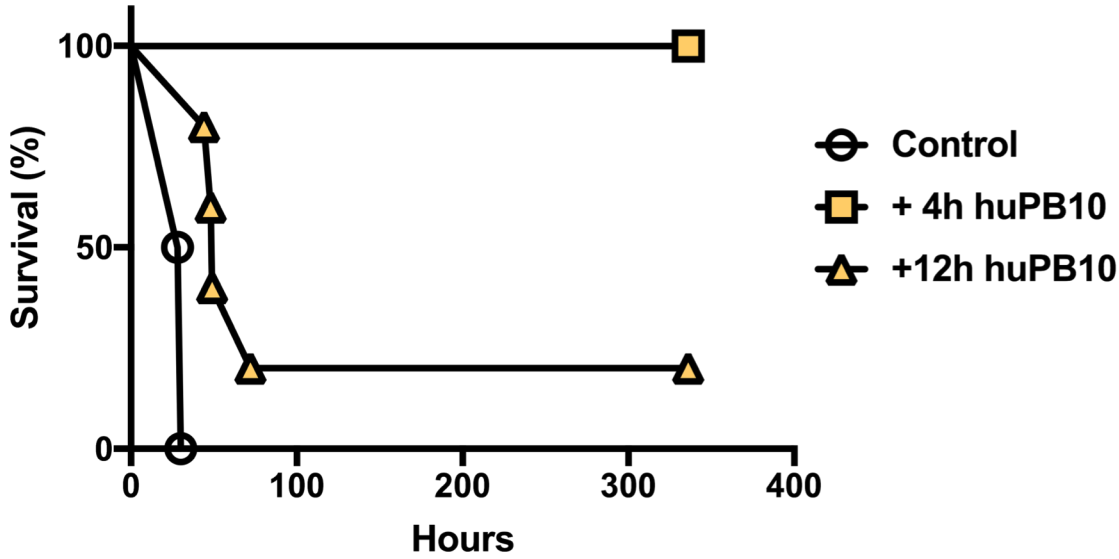

100
50
0
Survival (%)
0
100
200
300
400
Hours
Control
+ 4h huPB10
+12h huPB10

Figure 2

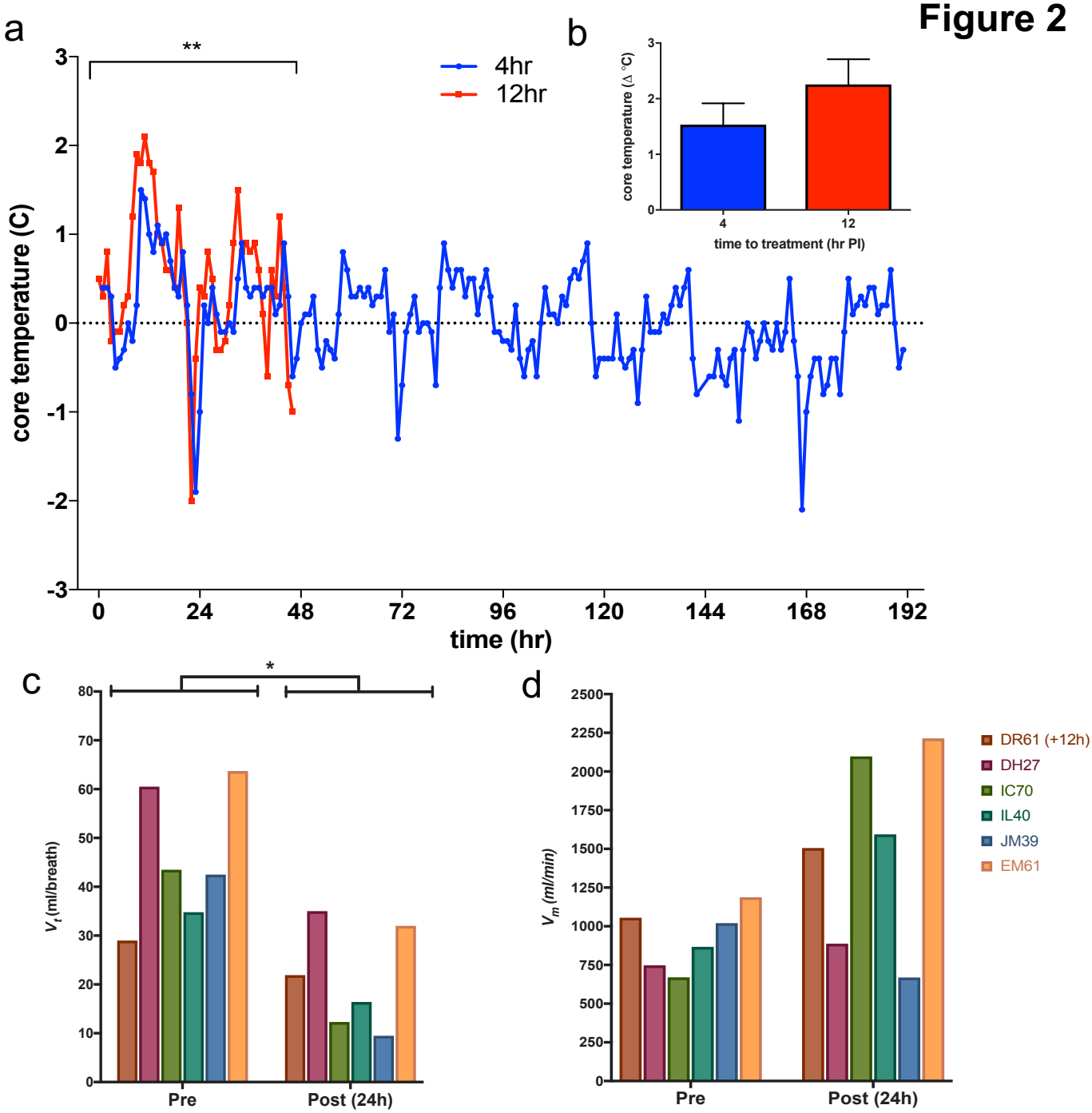
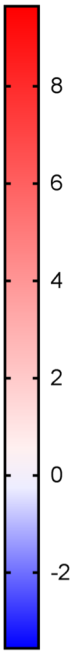

a
**
4hr
12hr
core temperature (C)
time (hr)
b
core temperature (Δ °C)
time to treatment (hr PI)
c
*
V_t (ml/breath)
Pre
Post (24h)
d
V_m (ml/min)
Pre
Post (24h)
DR61 (+12h)
DH27
IC70
IL40
JM39
EM61

Figure 3

A. Serum

	Control		4 hr intervention					12 hr intervention				
	DH27	JP56	LB98	IC70	IL40	LJ80	EM61	KP48	KR49	LB26	DR61	JM39
EGF	1.53	0.83	-0.17	0.73	0.28	-0.83	0.64	0.27	0.64	-0.67	0.79	1.42
Eotaxin	0.62	0.91	-0.01	0.48	0.31	0.28	0.40	-0.29	0.01	-0.05	-0.17	0.61
FGF-basic	0.38	1.49	0	0.33	-0.27	-0.32	0.40	0.25	0.28	-0.35	0.29	0.73
G-CSF	2.58	4.92	-0.07	0.05	-0.93	-0.22	-0.33	-0.14	-0.21	-0.33	-0.10	0.21
GM-CSF	1.58	2.16	-0.21	-0.05	0.08	-0.07	0.07	-0.03	0.04	-0.18	-0.07	0.26
HGF	0.33	0.68	-0.24	-0.10	-1.07	-0.22	0.04	-0.17	-0.16	-0.04	-0.15	0.23
IFN-g	0.53	0.74	-0.20	0.05	-1.15	-0.22	0	-0.13	-0.21	0	-0.24	0.57
IL-1b	0.99	1.56	-0.15	-0.01	-0.33	-0.06	0.15	-0.06	-0.03	-0.07	-0.38	0.44
IL-1RA	7.17	8.47	0.09	0.04	0.12	-0.47	1.73	1.68	0.84	1.55	1.35	3.74
IL-2	1.04	1.64	-0.08	-0.10	0.10	0	0.07	-0.07	0.07	-0.03	-0.21	0.13
IL-4	0.23	0.35	-0.15	-0.08	-0.58	-0.12	-0.11	0	-0.07	0.08	-0.14	-0.07
IL-5	1.04	1.95	-0.21	0.26	-0.97	-0.39	0.18	-0.06	0.15	-0.20	-0.38	0.06
IL-6	8.65	9.64	1.41	1.94	2.23	2.14	2.61	2.30	0.64	2.49	2.24	4.18
IL-8	-0.85	-1.52	-1.74	-1.87	-1.83	-2.01	-0.84	-2.28	-2.80	-1.78	-3.56	-1.90
IL-10	0.60	0.85	-0.06	-0.03	-0.77	-0.03	0.06	-0.01	0.10	0.05	0	0.12
IL-12	-0.44	0.05	-0.13	-0.27	-0.82	-0.43	0.07	-0.32	-0.25	-0.10	-0.22	-0.55
IL-15	0.57	0.96	-0.18	0.05	-0.61	-0.16	0.27	-0.08	0.02	-0.45	-0.11	0.21
IL-17	0.05	-0.08	-0.10	-0.12	-0.23	-0.40	0.04	-0.21	-0.18	-0.42	-0.18	0.14
I-TAC	-0.32	-0.17	-0.57	-0.74	0.50	-0.56	0.50	-0.19	-0.81	-0.71	-0.36	0.18
MCP-1	3.05	4.02	-0.16	0.73	0.72	0.28	1.68	-0.22	0.30	0.26	-0.31	1.41
MDC	-0.25	0.19	-0.16	-0.28	-0.04	0.17	0.33	0	0.23	0.08	-0.42	-0.36
MIF	2.00	1.74	0.27	1.09	0.22	-0.67	0.78	0.62	0.67	-0.48	1.39	0.86
MIG	0.31	0.38	-0.26	-0.18	-0.94	-0.07	-0.04	0.10	-0.18	-0.17	-0.20	0.25
MIP-1a	0.71	0.77	-0.16	0	-1.04	0.13	0.10	-0.15	0.17	0	-0.18	0.24
MIP-1b	0.44	0.82	-0.17	-0.04	-0.77	-0.20	0.08	-0.10	-0.10	0.14	-0.06	0.42
RANTES	0.04	0.08	-0.02	-0.03	-0.02	0.01	0.03	-0.04	-0.01	-0.06	-0.07	-0.01
TNF-a	0.30	0.58	-0.19	-0.02	-1.34	-0.11	0	-0.32	0.11	-0.21	-0.12	0.15
VEGF	3.18	3.71	0.32	0.31	1.16	0.14	0.67	0.73	0.20	0.67	0.44	1.57
IP-10	0.33	0.71	-0.10	-0.23	-1.12	-0.07	-0.10	-0.15	0	-0.17	-0.03	0.13



B. BAL

	Control	4 hr intervention					12 hr intervention				
	DH27	LB98	IC70	IL40	LJ80	EM61	KP48	KR49	LB26	DR61	JM39
EGF	0.10	0.07	0.12	0.26	-0.10	0.05	-0.02	0.19	0.22	0.22	0.29
Eotaxin	2.25	0.98	2.84	1.97	1.73	1.09	3.78	2.00	3.69	2.74	4.23
FGF-basic	0.62	0.41	0.13	0.14	-0.08	0.03	0.62	0.49	0.45	0.56	0.62
G-CSF	1.21	0.17	0.20	0.29	0.21	0.20	0.19	0.25	1.21	0.38	3.74
GM-CSF	1.02	0.26	0.50	0.64	0.80	0.57	1.00	1.23	1.29	1.11	1.65
HGF	0.21	0.09	0	0.29	0.13	0.05	0.42	0.37	0.52	0.23	1.02
IFN-g	-0.17	0.09	0.13	0	0.11	0	0.47	0.28	0.35	0.28	0.47
IL-1b	1.76	0.84	0.57	2.16	0.82	1.29	1.04	1.32	1.52	2.76	2.36
IL-1RA	2.28	0.10	0.98	2.09	1.10	1.52	2.25	2.81	1.75	3.72	3.41
IL-2	0.92	0.03	0.21	0.40	0.16	0.16	0.90	0.90	1.04	0.88	1.39
IL-4	0.22	0.15	0.08	0.03	0.12	0	0.35	0.14	0.22	0.29	0.51
IL-5	1.07	0.25	0.58	0.63	0.28	0.29	1.12	1.56	1.46	1.43	1.90
IL-6	6.25	3.79	5.20	5.40	4.16	4.53	6.79	5.27	7.74	7.96	7.79
IL-8	0.85	0.62	0.58	0.77	1.01	0.50	0.55	1.03	1.04	1.08	1.03
IL-10	0.08	0.04	0.08	0.06	0.03	0.01	0.27	0.73	0.34	0.23	0.53
IL-12	0.04	0.04	0.11	0.15	0.17	0.12	0.28	0.24	0.26	0.25	0.55
IL-15	-0.21	-0.02	0.09	0.16	-0.02	0.03	0.54	0.11	0.26	0.20	0.32
IL-17	0.20	0.07	0.17	0.58	0.16	0.03	0.74	0.69	1.52	0.22	0.69
I-TAC	-0.81	1.84	0.93	1.65	0.64	1.63	0.33	1.04	0.75	1.78	1.42
MCP-1	-0.89	2.69	2.02	3.02	3.78	3.51	3.83	3.71	5.19	1.97	4.65
MDC	-0.06	1.73	1.62	2.01	1.34	2.70	1.30	1.51	0.56	2.72	3.62
MIF	1.47	1.10	0.73	0.51	0.73	0.46	-0.48	1.11	0.07	-0.48	-0.86
MIG	0	0	-0.13	0.06	0.06	0.13	0.14	-0.21	0.16	0.29	0.20
MIP-1a	0.51	0.47	0.45	1.78	1.09	0.45	0.42	0.48	2.45	0.78	0.91
MIP-1b	0.64	0.06	0.13	0.27	0.13	0.07	0.72	0.49	1.42	0.46	0.81
RANTES	0.37	-0.22	-0.31	-0.39	-0.18	-0.09	-0.76	-0.08	-0.63	-0.58	-0.40
TNF-a	0.49	0.28	0.36	0.77	0.51	0.47	0.65	0.75	0.74	0.77	1.37
VEGF	-1.36	-0.24	0.50	-0.29	0.27	0.14	0.62	-0.30	1.34	2.02	0.39
IP-10	0.10	0.08	0.14	0.17	0.08	0.15	0.74	0.24	0.68	0.43	0.76

Supplemental Figures

Figure S1

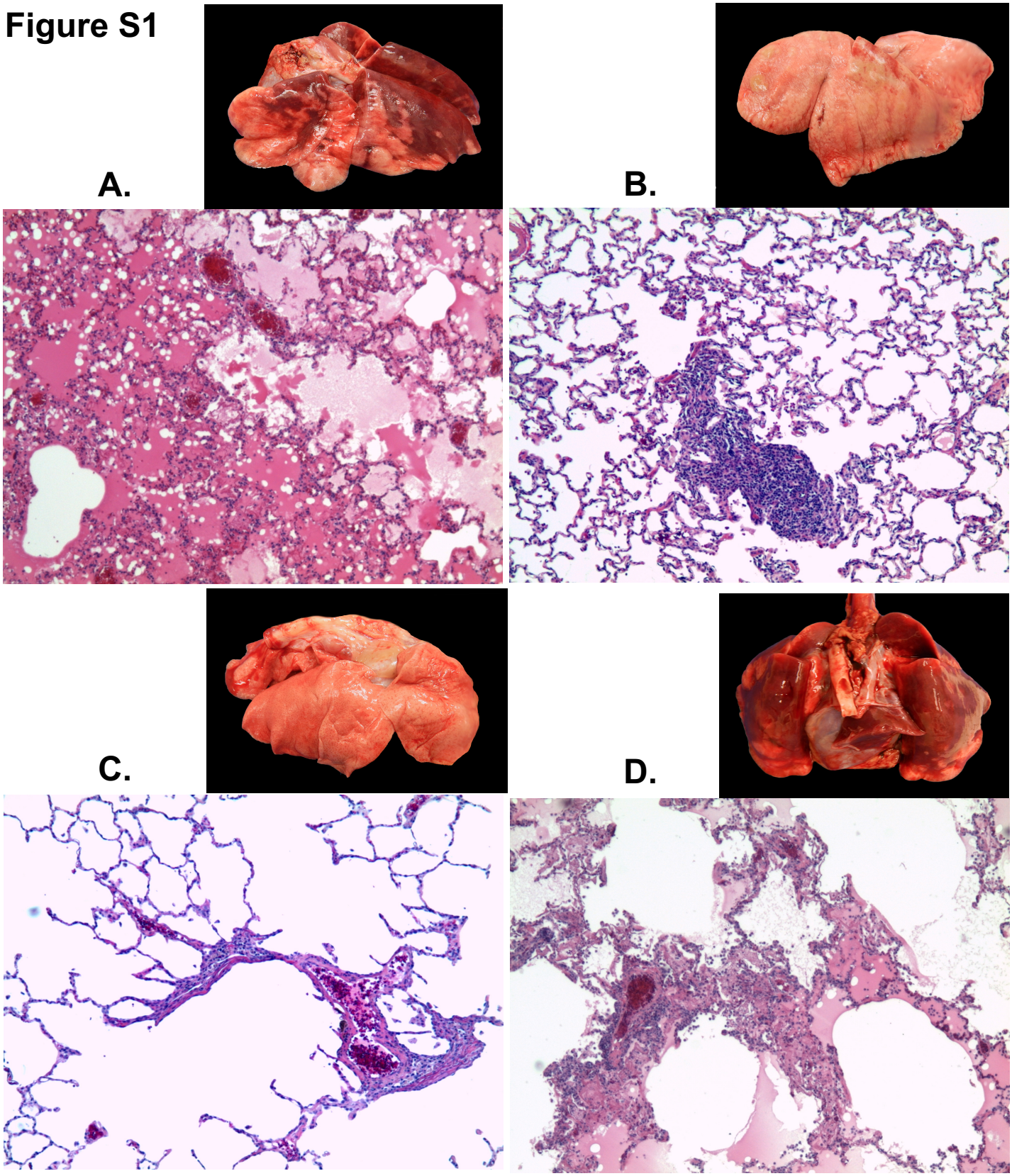


Figure S2

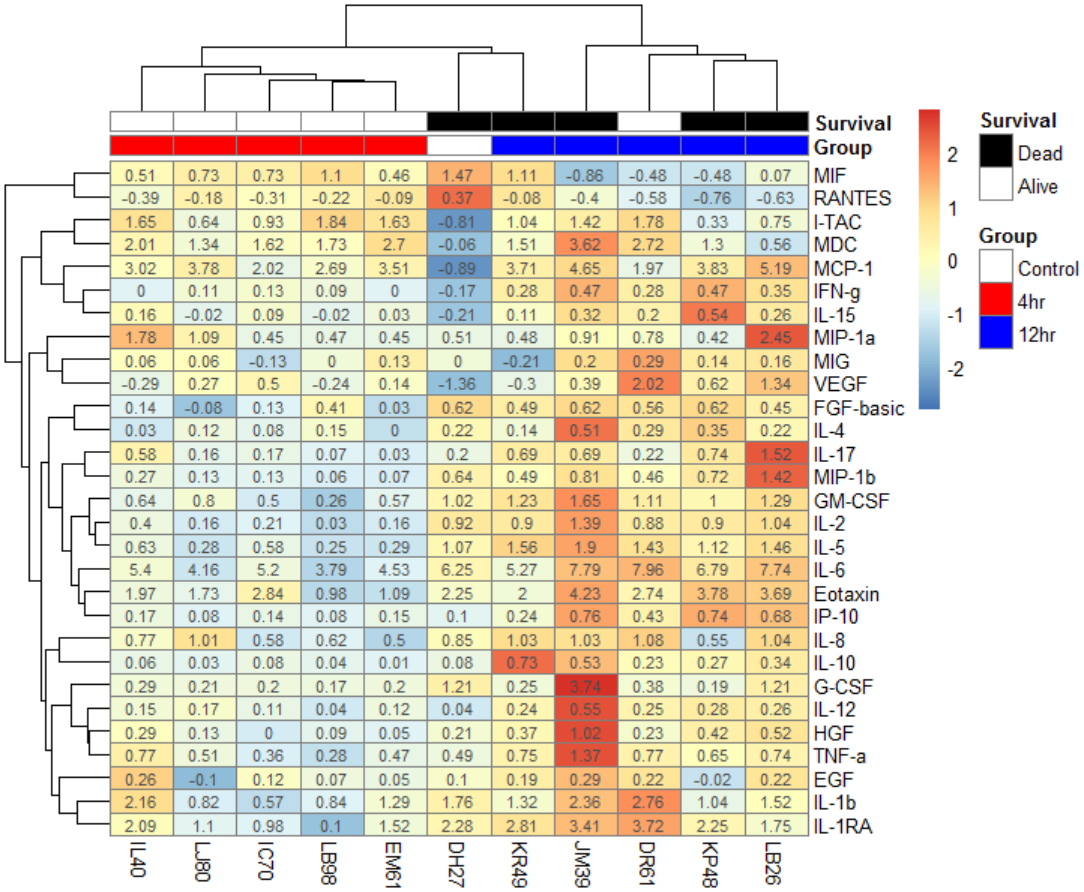
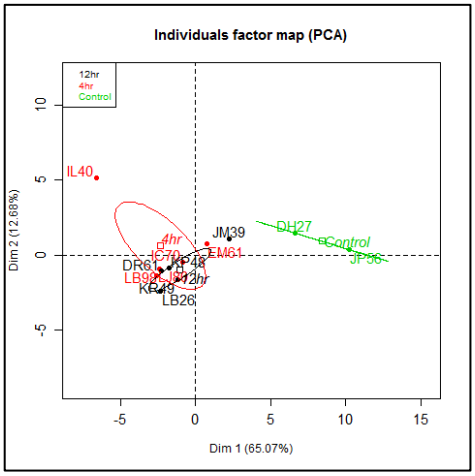

Survival
Group
MIF 0.51 0.73 0.73 1.1 0.46 1.47 1.11 -0.86 -0.48 -0.48 0.07
RANTES -0.39 -0.18 -0.31 -0.22 -0.09 0.37 -0.08 -0.4 -0.58 -0.76 -0.63
I-TAC 1.65 0.64 0.93 1.84 1.63 -0.81 1.04 1.42 1.78 0.33 0.75
MDC 2.01 1.34 1.62 1.73 2.7 -0.06 1.51 3.62 2.72 1.3 0.56
MCP-1 3.02 3.78 2.02 2.69 3.51 -0.89 3.71 4.65 1.97 3.83 5.19
IFN-g 0 0.11 0.13 0.09 0 -0.17 0.28 0.47 0.28 0.47 0.35
IL-15 0.16 -0.02 0.09 -0.02 0.03 -0.21 0.11 0.32 0.2 0.54 0.26
MIP-1a 1.78 1.09 0.45 0.47 0.45 0.51 0.48 0.91 0.78 0.42 2.45
MIG 0.06 0.06 -0.13 0 0.13 0 -0.21 0.2 0.29 0.14 0.16
VEGF -0.29 0.27 0.5 -0.24 0.14 -1.36 -0.3 0.39 2.02 0.62 1.34
FGF-basic 0.14 -0.08 0.13 0.41 0.03 0.62 0.49 0.62 0.56 0.62 0.45
IL-4 0.03 0.12 0.08 0.15 0 0.22 0.14 0.51 0.29 0.35 0.22
IL-17 0.58 0.16 0.17 0.07 0.03 0.2 0.69 0.69 0.22 0.74 1.52
MIP-1b 0.27 0.13 0.13 0.06 0.07 0.64 0.49 0.81 0.46 0.72 1.42
GM-CSF 0.64 0.8 0.5 0.26 0.57 1.02 1.23 1.65 1.11 1 1.29
IL-2 0.4 0.16 0.21 0.03 0.16 0.92 0.9 1.39 0.88 0.9 1.04
IL-5 0.63 0.28 0.58 0.25 0.29 1.07 1.56 1.9 1.43 1.12 1.46
IL-6 5.4 4.16 5.2 3.79 4.53 6.25 5.27 7.79 7.96 6.79 7.74
Eotaxin 1.97 1.73 2.84 0.98 1.09 2.25 2 4.23 2.74 3.78 3.69
IP-10 0.17 0.08 0.14 0.08 0.15 0.1 0.24 0.76 0.43 0.74 0.68
IL-8 0.77 1.01 0.58 0.62 0.5 0.85 1.03 1.03 1.08 0.55 1.04
IL-10 0.06 0.03 0.08 0.04 0.01 0.08 0.73 0.53 0.23 0.27 0.34
G-CSF 0.29 0.21 0.2 0.17 0.2 1.21 0.25 3.74 0.38 0.19 1.21
IL-12 0.15 0.17 0.11 0.04 0.12 0.04 0.24 0.55 0.25 0.28 0.26
HGF 0.29 0.13 0 0.09 0.05 0.21 0.37 1.02 0.23 0.42 0.52
TNF-a 0.77 0.51 0.36 0.28 0.47 0.49 0.75 1.37 0.77 0.65 0.74
EGF 0.26 -0.1 0.12 0.07 0.05 0.1 0.19 0.29 0.22 -0.02 0.22
IL-1b 2.16 0.82 0.57 0.84 1.29 1.76 1.32 2.36 2.76 1.04 1.52
IL-1RA 2.09 1.1 0.98 0.1 1.52 2.28 2.81 3.41 3.72 2.25 1.75
IL40 LJ80 IC70 LB98 EM61 DH27 KR49 JM39 DR61 KP48 LB26
2
1
0
-1
-2
Survival
Dead
Alive
Group
Control
4hr
12hr

Figure S3

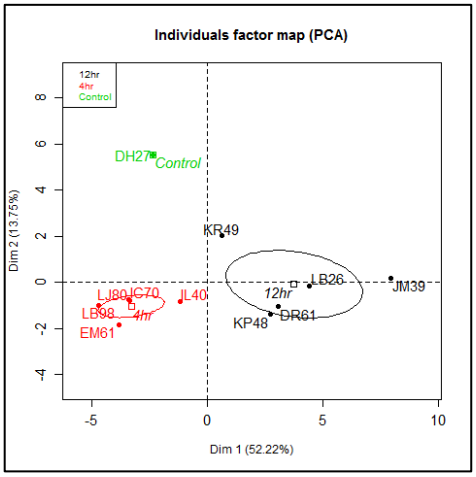
Serum

BAL

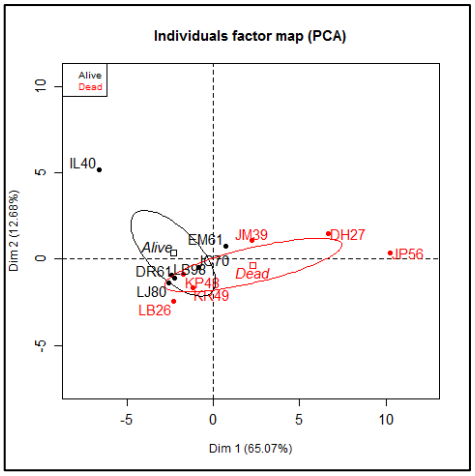
A.



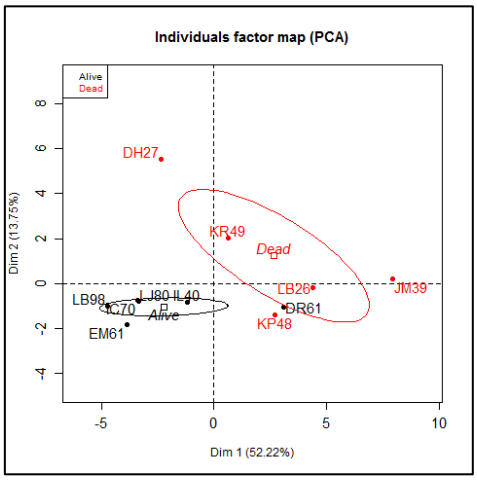
D.



B.



E.



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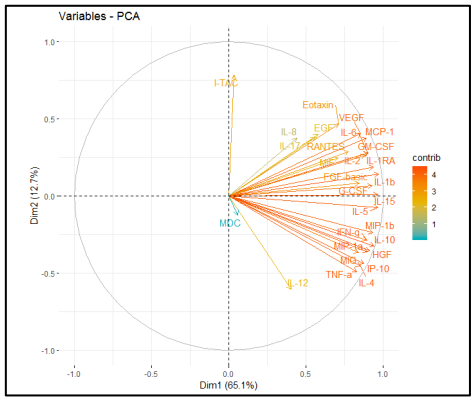
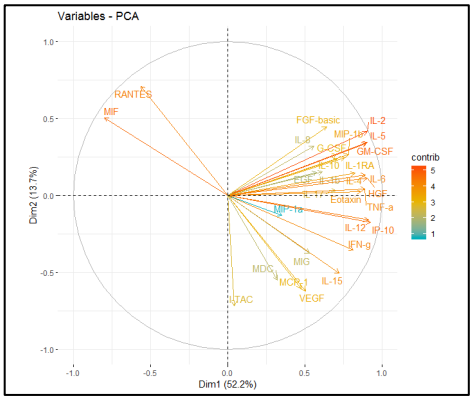
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