

Supplementary information and material:

Comparative *Burkholderia pseudomallei* natural history virulence studies using an aerosol murine model of infection.

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SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Gross pathological progression of melioidosis in mice.

Representative images of organs during each of the three stages of melioidosis infection.

Supplemental Figure 2. Chemokine/cytokine analysis from homogenized lung tissue.

Levels of G-CSF, KC, IL-6, MCP-1, IL-17 and TNF- α in homogenized lung tissue (pg/ml) of animals infected with HB PUB10303a (closed circles) or K96243 (closed triangles) compared to the uninfected control animals (dashed line). The symbols represent values of individual animals. The bars indicate the mean for each group and the dashed line represents the mean for the uninfected control animals. The asterisk (*) indicates significantly different values when comparing HB PUB10303a to the uninfected control animals, $p < 0.05$. The single cross (†) represents significantly different values when comparing K96243 to the uninfected controls, $p < 0.05$. The double cross (‡) indicates significantly different values when comparing HB PUB10303a to K96243, $p < 0.05$.

Stage

Lung

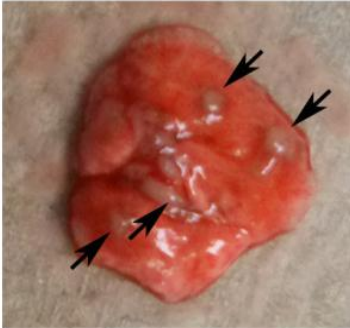
Spleen

Liver

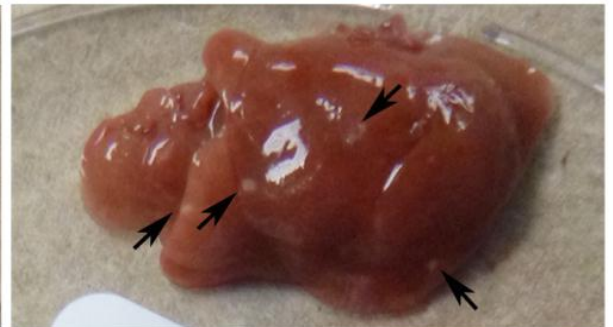
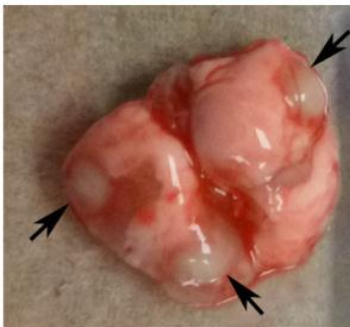
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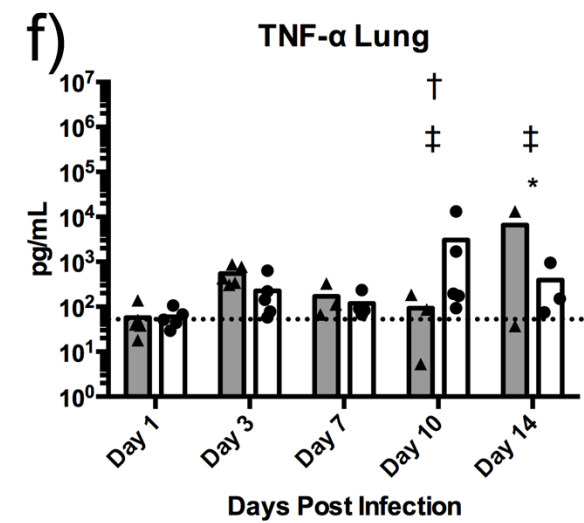
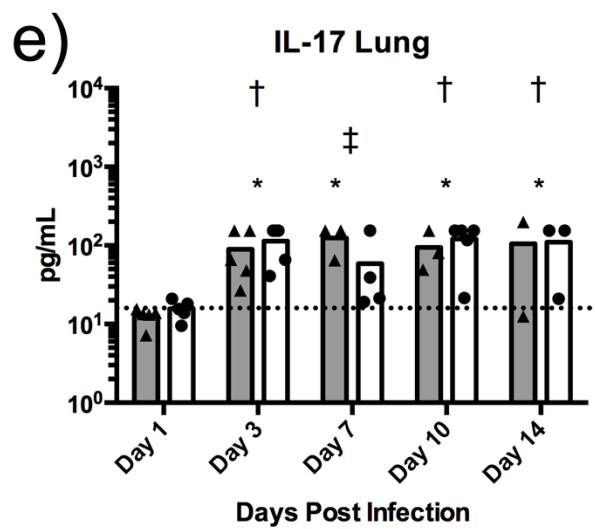
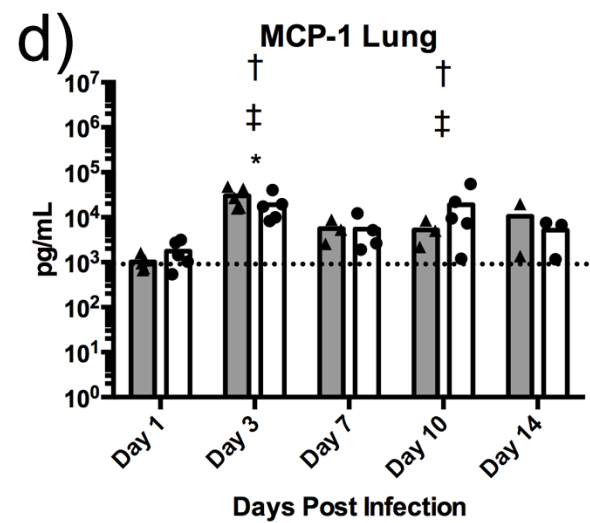
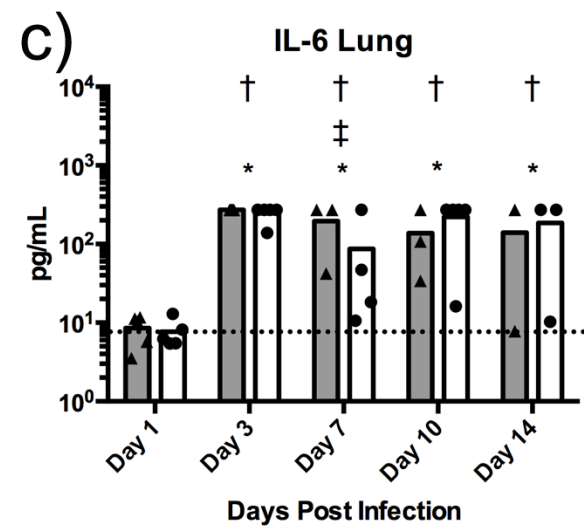
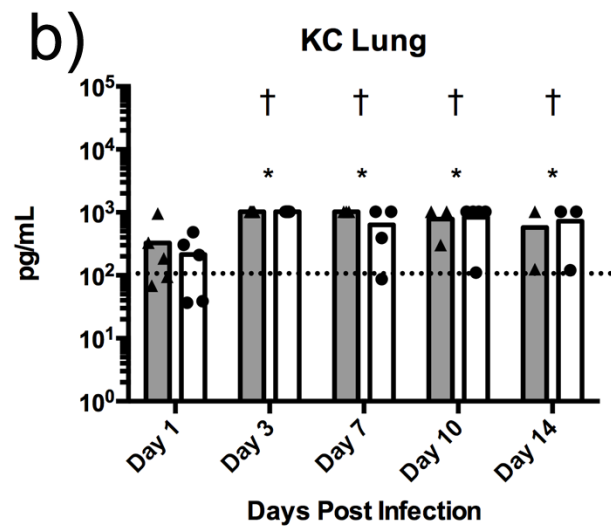
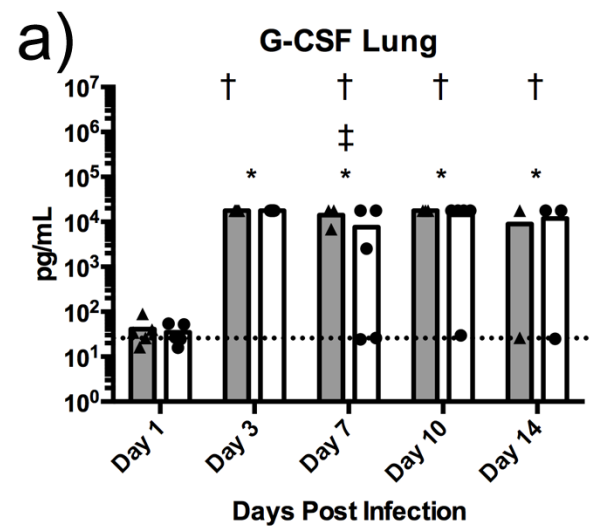


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Supplemental Table 1: Blood Chemistry and Hematology

		Control	HB PUB10303a					K96243				
			Day 1	Day 3	Day 7	Day 10	Day 14	Day 1	Day 3	Day 7	Day 10	Day 14
		(n=22)	(n=8-10)	(n=6-10)	(n=6-9)	(n=5-8)	(n=2-7)	(n=9-10)	(n=7-10)	(n=4-9)	(n=3-8)	(n=2-7)
Leukocytes												
WBC	(K/ μ L)	3.90±0.37	3.48±0.88	6.35±0.76	7.32±1.00 ^a	8.43±0.86 ^b	6.28±2.09	6.93±0.91 ^b	5.39±1.09	6.95±1.02 ^a	8.32±0.83 ^d	4.46±0.93
LY	(K/ μ L)	2.69±0.28	2.62±0.64	2.52±0.27	3.14±0.43	2.04±0.23	4.02±2.13	4.62±0.60 ^b	2.71±0.76	3.49±0.54	3.19±0.64	2.00±0.45
MO	(K/ μ L)	0.19±0.02	0.13±0.03	0.31±0.05	0.45±0.06 ^c	0.50±0.2 ^d	0.49±0.10 ^c	0.31±0.03	0.22±0.06	0.27±0.04	0.45±0.07 ^d	0.24±0.09
EO	(K/ μ L)	0.10±0.03	0.08±0.03	0.30±0.06 ^b	0.22±0.07	0.26±0.13	0.11±0.02	0.21±0.04	0.13±0.06	0.17±0.07	0.12±0.03	0.08±0.03
BA	(K/ μ L)	0.04±0.01	0.03±0.01	0.09±0.02	0.06±0.02	0.10±0.06	0.01±0.01	0.06±0.01	0.04±0.02	0.04±0.02	0.03±0.01	0.02±0.01
NE	(%)	22.60±2.23	14.72±2.01	47.20±3.55 ^d	46.13±4.04 ^c	65.65±4.75 ^d	34.65±7.52	24.00±1.67	44.26±5.26 ^d	41.24±5.00 ^c	53.11±6.45 ^d	41.62±8.17 ^b
LY	(%)	68.93±2.50	78.38±2.08	41.83±3.17 ^d	43.23±3.69 ^c	24.78±3.05 ^d	51.94±9.80	67.52±1.78	48.35±4.01 ^c	51.84±4.96 ^a	39.15±6.57 ^d	51.02±8.46 ^a
MO	(%)	4.91±0.50	4.47±0.53	5.34±0.93	7.06±1.52	5.61±2.16	9.98±1.71 ^b	4.91±0.62	4.55±0.81	4.40±0.95	5.48±0.77	5.41±1.28
EO	(%)	2.59±0.74	1.74±0.38	4.43±0.63	2.84±0.71	2.90±1.48	3.11±1.36	2.81±0.36	2.10±0.53	2.01±0.60	1.79±0.65	1.57±0.43
BA	(%)	0.98±0.28	0.69±0.11	1.21±0.21	0.74±0.18	1.05±0.61	0.33±0.11	0.76±0.13	0.74±0.25	0.51±0.19	1.48±0.21	0.27±0.13
Erythrocytes												
RBC	(M/ μ L)	7.16±0.40	6.30±0.75	8.28±0.74	7.83±0.17	7.95±0.24	5.33±1.22	8.52±0.31	8.76±0.88	8.38±0.42	8.20±0.33	6.78±0.85
HGB	(g/dL)	10.30±0.82	9.61±0.94	12.27±1.10	11.58±0.24	11.24±0.41	13.04±2.53	12.69±0.41	13.84±0.29	12.36±0.56	12.23±0.50	9.89±1.22
HCT	(%)	37.32±2.14	33.09±3.95	42.30±3.80	39.02±0.42	38.50±1.43	24.90±5.68	45.12±1.69	45.69±4.63	42.27±2.02	40.95±1.81	33.87±4.33
MCV	(fL)	51.99±0.25	52.36±0.77	50.90±0.38	49.90±0.59 ^a	48.40±0.40 ^d	46.68±1.24 ^d	52.93±0.19	52.06±0.28	50.58±1.78	49.88±0.51 ^a	49.97±0.47 ^a
MCH	(pg)	13.76±0.82	15.91±0.80	14.75±0.12	14.82±.20	14.10±0.17	19.73±6.13 ^a	14.91±0.16	14.51±0.11	14.78±0.27	14.94±0.16	14.70±0.41
MCHC	(K/ μ L)	26.40±1.57	30.49±1.70	29.01±0.18	29.67±0.38	29.22±0.32	40.98±11.10 ^b	28.19±0.26	27.80±0.28	29.27±0.31	29.99±0.25	29.46±0.69
RDW	(K/ μ L)	16.93±0.16	1.99±1.29	17.62±0.25	18.32±0.58	19.36±0.27	20.30±1.06	17.43±0.18	17.63±0.34	18.00±0.44	18.81±0.48	19.17±0.77
Thrombocytes												
PLT	(K/ μ L)	247.65±49.86	200.50±51.58	225.00±35.54	604.67±130.48 ^a	760±89.67 ^c	269.40±128.66	270.50±59.89	449.00±68.910	743.56±122.84 ^d	576.88±146.73 ^a	384.29±91.64
MPV	(fL)	5.05±0.10	5.610±0.18 ^a	5.34±0.08	5.13±0.10	5.04±0.18	5.62±0.31	5.07±0.10	5.13±0.14	5.16±0.17	5.09±0.18	5.56±0.20
Blood Chemistry												
ALT	(u/L)	79.14±14.61	156.67±76.97	139.00±54.61	45.86±17.63	54.00±16.86	252.00±224.00	43.44±7.48	33.11±3.098	35.00±3.59	42.88±11.90	50.00±21.48
AMY	(u/L)	1298.64±155.10	912.22±37.09	937.88±66.98	671.14±46.32	688.80±126.42	1028.50±369.50	972.45±24.22	812.22±45.11	805.00±92.07	771.50±73.94	855.83±74.39
TBIL	(mg/dL)	0.33±0.01	0.30±0.02	0.29±0.01	0.30±0.00	0.30±0.00	0.30±0.00	0.31±0.01	0.31±0.01	0.29±0.01	0.30±0.00	0.30±0.00
BUN	(mg/dL)	16.55±0.62	18.33±1.30	14.70±1.04	13.43±1.40	13.60±0.68	16.00±2.00	17.33±0.80	16.44±1.10	14.75±0.92	18.25±3.42	17.50±1.61
CA	(mg/dL)	8.95±0.33	8.29±0.74	9.21±0.65	10.29±0.54	7.66±0.87	7.00±3.00	9.97±0.22	10.43±0.18	10.03±0.21	10.36±0.61	10.65±0.67
PHOS	(mg/dL)	7.68±0.48	7.90±0.33	7.34±0.50	7.54±0.55	6.64±0.64	8.95±1.45	8.09±0.45	7.38±0.28	6.30±0.44	7.35±0.44	7.13±0.43
CRE	(mg/dL)	0.21±0.02	0.21±0.01	0.23±0.03	0.26±0.04	0.20±0.00	0.20±0.00	0.23±0.02	0.20±0.00	0.20±0.00	0.24±0.02	0.23±0.02
GLU	(mg/dL)	217.46±6.25	235.33±9.67	149.40±12.50 ^d	138.29±21.39 ^d	128.60±14.72 ^d	159.00±61.00	234.00±10.30	208.33±13.25	174.88±19.25	156.25±14.35 ^c	156.50±20.45 ^b
NA+	(mMol/L)	146.55±0.67	149.33±2.05	146.14±0.97	144.43±1.70	147.40±1.25	153.50±5.50	144.44±0.69	144.44±0.73	145.86±0.77	150.13±4.45	148.67±6.86
K+	(mMol/L)	6.04±0.28	6.64±0.24	6.35±0.21	5.75±0.50	7.26±0.51	6.85±1.15	5.62±0.16	5.68±0.18	5.66±0.24	5.50±0.26	6.28±0.46
TP	(g/dL)	4.77±0.07	4.58±0.06	5.90±0.28 ^d	5.73±0.20 ^d	5.82±0.27 ^c	5.25±0.05	4.87±0.05	5.72±0.16 ^d	5.16±0.14	5.69±0.30 ^d	5.80±0.50 ^d

^aP<0.05^bP<0.01^cP<0.0005^dP<0.0001

Supplemental Table 2: Blood Serum Chemokines/Cytokines

Cytokine (pg/mL)	Control	HB PUB10303a					K96243				
	(n=18)	Day 1 (n=8)	Day 3 (n=9)	Day 7 (n=5)	Day 10 (n=4)	Day 14 (n=4)	Day 1 (n=9)	Day 3 (n=8)	Day 7 (n=8)	Day 10 (n=6)	Day 14 (n=6)
IL-1 β	124.10 \pm 9.96	125.26 \pm 12.68	167.57 \pm 21.26	248.76 \pm 17.36	449.99 \pm 271.01 ^d	287.79 \pm 225.79 ^a	159.53 \pm 7.02	195.55 \pm 25.22	182.13 \pm 27.64	170.15 \pm 11.49	134.39 \pm 20.51
IL-2	18.95 \pm 3.50	20.43 \pm 3.31	11.43 \pm 3.95	36.42 \pm 7.77 ^a	23.82 \pm 9.00	15.99 \pm 8.55	29.75 \pm 2.39	25.39 \pm 3.11	27.23 \pm 4.00	31.95 \pm 4.89	30.18 \pm 8.64
IL-3	6.79 \pm 1.85	2.15 \pm 0.98	2.24 \pm 1.41	12.02 \pm 3.56	0.50 \pm 0.40	2.76 \pm 2.31	9.76 \pm 3.02	2.01 \pm 0.49	7.61 \pm 1.53	3.60 \pm 1.59	4.28 \pm 1.36
IL-5	17.61 \pm 3.18	11.11 \pm 1.85	12.04 \pm 3.65	39.08 \pm 7.65 ^b	19.36 \pm 3.78	11.21 \pm 7.12	17.40 \pm 3.25	10.98 \pm 2.91	28.79 \pm 6.84	18.65 \pm 4.53	20.36 \pm 6.62
IL-9	181.12 \pm 52.89	56.98 \pm 56.89	13.78 \pm 13.68	375.09 \pm 62.99	75.47 \pm 75.37	167.59 \pm 129.75	261.13 \pm 54.68	193.24 \pm 73.89	259.41 \pm 90.50	253.51 \pm 69.85	382.58 \pm 90.13
IL-10	24.71 \pm 3.81	17.87 \pm 3.34	49.37 \pm 6.18 ^b	76.70 \pm 11.03 ^c	55.75 \pm 16.67 ^b	39.84 \pm 21.56	30.42 \pm 5.27	34.56 \pm 7.61	48.64 \pm 12.80 ^b	30.50 \pm 6.09	25.05 \pm 6.37
IL-12(p40)	1042.72 \pm 122.98	1050.44 \pm 180.39	986.10 \pm 143.77	2674.69 \pm 1126.46 ^d	1250.51 \pm 351.74	1130.83 \pm 638.77	690.74 \pm 113.47	648.35 \pm 158.53	1138.56 \pm 194.34	870.58 \pm 243.61	938.54 \pm 247.94
IL-12(p70)	92.07 \pm 13.99	73.47 \pm 9.09	135.24 \pm 48.69	138.35 \pm 24.35	103.85 \pm 30.77	81.61 \pm 23.67	127.52 \pm 20.67	92.29 \pm 11.06	119.62 \pm 19.69	84.90 \pm 7.50	91.86 \pm 22.36
IL-13	2459.29 \pm 215.21	2275.28 \pm 127.67	2003.43 \pm 169.05	3308.34 \pm 358.74	2345.99 \pm 248.05	1468.36 \pm 746.05	2699.23 \pm 168.83	2154.79 \pm 65.53	2751.02 \pm 196.95	2543.45 \pm 224.53	3394.54 \pm 1134.18
Eotaxin	554.50 \pm 96.18	212.69 \pm 73.75	659.61 \pm 224.93	1122.03 \pm 170.47 ^a	595.13 \pm 241.24	315.34 \pm 183.09	930.20 \pm 143.98	736.59 \pm 126.14	772.79 \pm 147.92	520.80 \pm 96.66	231.94 \pm 128.63
GM-CSF	121.42 \pm 32.64	61.80 \pm 25.21	133.22 \pm 35.12	380.95 \pm 38.98 ^c	280.67 \pm 108.61	204.91 \pm 105.35	209.50 \pm 40.68	155.99 \pm 41.31	254.20 \pm 62.82 ^a	153.55 \pm 39.84	151.27 \pm 63.44
IFN- γ	27.01 \pm 5.21	11.02 \pm 3.00	121.36 \pm 23.83 ^d	49.28 \pm 7.33	107.36 \pm 59.99 ^c	99.69 \pm 69.18 ^b	31.58 \pm 8.20	43.94 \pm 9.75	34.10 \pm 6.01	21.88 \pm 5.64	36.92 \pm 9.12
MIP-1 α	57.52 \pm 9.91	43.73 \pm 7.08	50.82 \pm 10.67	76.53 \pm 12.84	56.91 \pm 17.38	53.08 \pm 248.47	55.38 \pm 10.10	31.84 \pm 5.49	47.22 \pm 5.33	31.75 \pm 8.73	32.88 \pm 10.98
MIP-1 β	24.61 \pm 3.96	17.15 \pm 3.04	49.92 \pm 4.88	57.84 \pm 6.13	213.48 \pm 183.93 ^d	82.48 \pm 68.45	32.62 \pm 5.00	34.49 \pm 5.60	39.37 \pm 7.86	31.20 \pm 5.09	32.74 \pm 8.38
RANTES	48.51 \pm 5.60	44.73 \pm 6.44	61.56 \pm 12.03	52.21 \pm 10.97	39.97 \pm 17.86	23.96 \pm 6.23	32.97 \pm 6.08	25.74 \pm 8.89	49.25 \pm 11.66	18.29 \pm 6.33 ^a	26.06 \pm 8.99

^aP<0.05

^bP<0.01

^cP<0.0005

^dP<0.0001

Supplemental Table 3: Lung Tissue Chemokines/Cytokines

Cytokine (pg/mL)	Control	HB PUB10303a					K96243				
	(n=18)	Day 1 (n=5)	Day 3 (n=5)	Day 7 (n=4)	Day 10 (n=5)	Day 14 (n=3)	Day 1 (n=5)	Day 3 (n=5)	Day 7 (n=3)	Day 10 (n=3)	Day 14 (n=2)
IL-1 α	26.15 \pm 2.22	22.37 \pm 3.68	386.73 \pm 0.00 ^d	386.730 \pm 0.00 ^d	258.38 \pm 128.33 ^d	204.155 \pm 185.58 ^b	33.52 \pm 11.22	353.17 \pm 33.56 ^d	129.11 \pm 86.03 ^a	317.38 \pm 69.35 ^d	270.55 \pm 116.18 ^d
IL-1 β	86.33 \pm 24.58	114.55 \pm 25.82	61473.51 \pm 17454.14	52288.89 \pm 5880.65	34731.27 \pm 17812.29	140075.10 \pm 139974.6 ^b	160.63 \pm 91.96	47461.14 \pm 17128.21	8347.76 \pm 17128.21	279872.60 \pm 96953.02 ^d	53832.40 \pm 27282.33
IL-3	0.92 \pm 0.42	0.61 \pm 0.42	0.87 \pm 0.27	3.73 \pm 1.04 ^a	1.16 \pm 0.56	2.59 \pm 1.80	1.47 \pm 0.79	1.00 \pm 0.59	2.90 \pm 1.13	3.20 \pm 1.11 ^a	1.37 \pm 0.34
IL-4	10.74 \pm 3.25	9.62 \pm 4.06	17.47 \pm 1.75	16.09 \pm 1.86	13.17 \pm 3.87	22.74 \pm 15.05	13.11 \pm 5.09	15.33 \pm 3.27	20.83 \pm 3.76	30.31 \pm 4.73 ^b	22.65 \pm 1.52
IL-5	40.57 \pm 24.34	27.69 \pm 17.36	10.71 \pm 1.28	8.83 \pm 1.26	5.99 \pm 1.55	28.01 \pm 4.81	65.85 \pm 37.08	10.65 \pm 1.29	52.22 \pm 17.64	49.80 \pm 17.33	59.15 \pm 22.47
IL-9	301.72 \pm 87.43	207.25 \pm 113.68	71.04 \pm 18.79	52.25 \pm 0.00	52.25 \pm 0.00	139.47 \pm 27.27	236.38 \pm 62.12	281.89 \pm 69.37	369.95 \pm 118.75	216.55 \pm 94.90	126.45 \pm 74.20
IL-10	67.90 \pm 26.77	46.26 \pm 17.03	694.70 \pm 414.01	72.99 \pm 8.05	29.35 \pm 19.51	8505.93 \pm 8494.29	28.76 \pm 9.51	120.62 \pm 49.43	139.61 \pm 39.54	122558.50 \pm 122276.80 ^c	103.60 \pm 24.77
IL-12(p40)	4805.54 \pm 3209.67	28067.31 \pm 27740.67	6542.86 \pm 1852.98	11694.87 \pm 3940.94	4433.38 \pm 1871.29	5149.04 \pm 3122.36	20761.11 \pm 13305.66	7308.06 \pm 1921.53	27524.80 \pm 16205.50	65060.79 \pm 40451.08 ^c	39916.49 \pm 15748.40
IL-12(p70)	4.94 \pm 3.05	6.90 \pm 6.43	1113.71 \pm 267.74	873.92 \pm 309.01	879.47 \pm 470.75	3606.20 \pm 3605.73 ^c	3.872 \pm 3.402	889.45 \pm 456.74	563.24 \pm 556.24	3430.47 \pm 1745.29 ^d	669.85 \pm 338.54
IL-13	127.201 \pm 28.03	105.08 \pm 45.63	63.10 \pm 7.47	28.19 \pm 5.05	23.73 \pm 5.22	98.49 \pm 26.50	156.50 \pm 50.94	99.52 \pm 18.47	212.66 \pm 63.72	153.55 \pm 47.46	99.88 \pm 39.77
Eotaxin	3631.74 \pm 158.27	2902.33 \pm 244.23	3072.81 \pm 255.08	2658.10 \pm 0.00	2658.10 \pm 0.00	3680.69 \pm 1022.59	3329.27 \pm 205.97	3689.60 \pm 230.82	4051.78 \pm 173.95	4437.49 \pm 621.30	3399.41 \pm 371.55
GM-CSF	47.75 \pm 16.90	21.57 \pm 17.33	474.17 \pm 54.75 ^a	209.97 \pm 63.82	121.23 \pm 82.84	574.99 \pm 570.75	86.51 \pm 33.08	372.97 \pm 109.70	305.21 \pm 153.15	984.34 \pm 544.00 ^d	270.20 \pm 81.92
IFN- γ	8.65.00 \pm 0.00	8.65.00 \pm 0.00	874.98 \pm 397.53 ^b	902.19 \pm 480.56 ^b	636.33 \pm 423.16	735.89 \pm 727.21	8.65.00 \pm 0.00	315.91 \pm 118.23	812.34 \pm 803.69 ^b	586.88 \pm 251.38 ^a	791.97 \pm 393.78 ^a
MIP-1 α	48.75 \pm 33.39	32.04 \pm 22.81	217174.30 \pm 105749.00	1244.82 \pm 247.34	1038.84 \pm 906.67	7.95e+015 \pm 7.95e+015 ^d	145.69 \pm 64.92	302203.10 \pm 262776.30	2086.09 \pm 1241.10	6.36e+015 \pm 3.90e+015 ^d	90681.70 \pm 46014.99
MIP-1 β	25.14 \pm 3.15	29.61 \pm 11.16	947.93 \pm 466.46	192.14 \pm 48.58	82.05 \pm 45.94	1390.34 \pm 1368.81 ^c	31.31 \pm 9.10	345.93 \pm 127.39	167.34 \pm 59.10	1216.75 \pm 631.24 ^d	200.02 \pm 74.52
RANTES	5514.35 \pm 1025.96	4046.91 \pm 1653.94	7879.59 \pm 0.00	7879.59 \pm 0.00	5253.11 \pm 2626.48	7879.59 \pm 0.00	5898.32 \pm 1511.68	4990.89 \pm 1770.67	7879.59 \pm 0.00	7879.59 \pm 0.00	7879.59 \pm 0.00

^aP<0.05

^bP<0.01

^cP<0.0005

^dP<0.0001