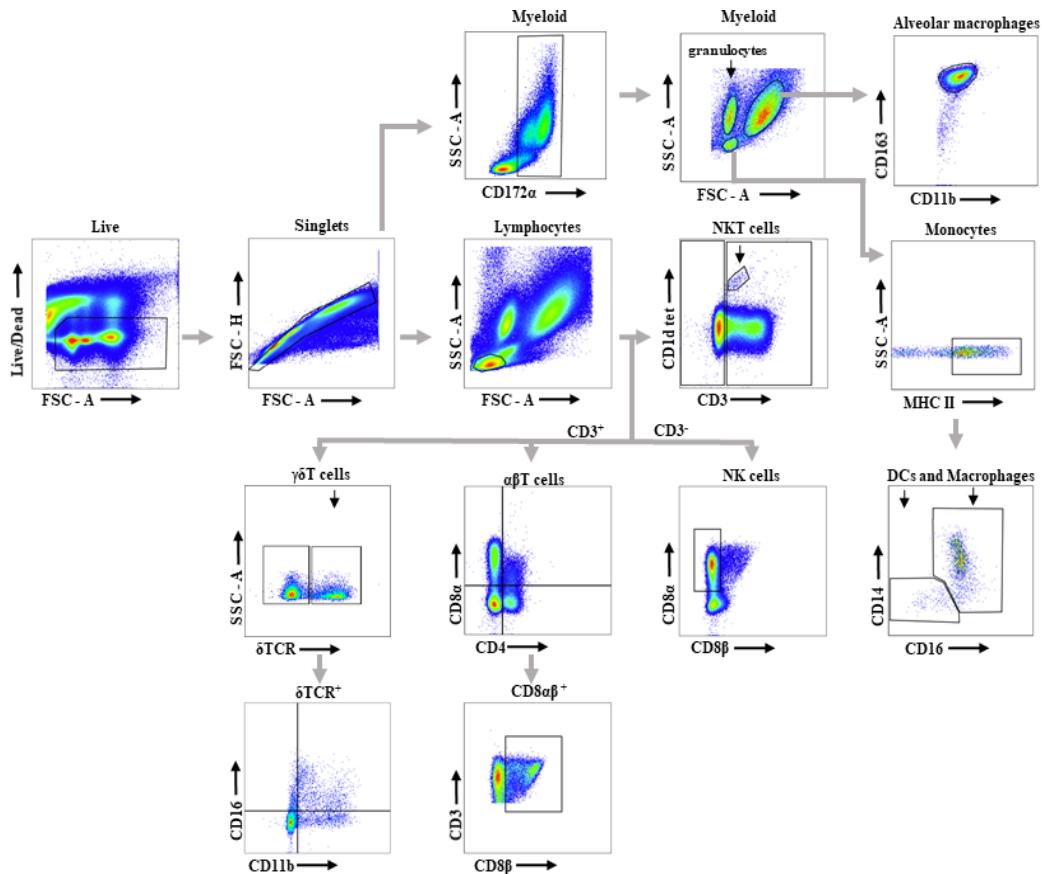


Supplementary Material

Supplementary Figure 1



Supplemental Figure 1. Gating strategy to distinguish immune cell populations in tissues. $\alpha\beta$ and $\gamma\delta$ T cells were distinguished by sequentially gating live single lymphocytes, $CD3^+$ cells, and then $CD4^+$, $CD8\alpha^+$, $CD8\beta^+$, $TCR-\delta^+$ cells. iNKT-cells were distinguished from conventional T cells according to $CD1d$ -tetramer staining. NK cells were identified as $CD8\alpha^+$ $CD3^-$ cells after first gating on single live lymphocytes. T cell and NK cell subsets were distinguished according to their surface expression of $CD11b$ and $CD16$. Monocytes, macrophages, and granulocytes were identified after initially gating on live single $CD172\alpha^+$ cells. Thereafter, monocytes were identified as $MHCII^+$ $CD14^+$ cells and alveolar macrophages were identified as SSC^{high} $CD163^+$ $CD11b^+$ cells. Granulocytes were identified according to FSC and SSC. FSC, forward scatter; SSC, side scatter; A, signal area; H, signal height. One representative lung sample is shown.

Supplemental Table 1. Reagents used for flow cytometry analysis of surface markers

Antigen	Clone	Isotype	Conjugation	Source
CD1d tetramer	N/A	N/A	PE	NIH Tetramer Core
Live/Dead	N/A	N/A	IR	Invitrogen
Anti-CD3ε	BB23-8E6-8C8	Mouse IgG2a κ	PE-Cy7	BD Biosciences
Anti-CD4	74-12-4	Mouse IgG2b κ	PE & Alexa647	Southern Biotech
Anti-CD8α	76-2-11	Mouse IgG2a κ	Alexa488 & Biotin	Southern Biotech
Anti-CD8β	PPT23	Mouse IgG1	A488	Bio-Rad
Anti-TCRδ	PGBL22A	Mouse IgG1	Alexa647	WSU mAB Center
Anti-CD14	MIL2	Mouse IgG2b	Alexa488	Bio-Rad
Anti-CD16	G7	Mouse IgG1	Biotin	BD Biosciences
Anti-CD11b	M1/70	Rat IgG2b κ	Alexa647	Biolegend
Anti-MHC class II	H42A	Mouse IgG2a κ	Alexa647	WSU mAb Center
Anti-CD163	2A10/11	Mouse IgG1	PE	Bio-Rad
Anti-CD172α	74-22-15A	Mouse IgG2b κ	PerCP	BD Biosciences

Supplemental Table 2. Flow cytometric analysis of immune cell populations in lung

	MM (n=6)	MC (n=10)	OS (n=8)	αGC (n=10)	αGCOS (n=8)
Lymphocytes (% of live cells)	26.6 ± 4.5 ^a	28.5 ± 3.8 ^a	29.3 ± 5.0 ^a	26.6 ± 2.7 ^a	25.8 ± 2.3 ^a
Myeloid cells (% of live cells)	70.1 ± 3.3 ^a	65.8 ± 2.9 ^{ab}	57.1 ± 4.7 ^{ab}	63.0 ± 1.7^b	67.5 ± 3.2 ^{ab}
iNKT-cells (% of CD3 ⁺)	0.31±0.13 ^{ab}	0.13±0.03 ^a	0.08±0.02 ^a	0.40±0.19 ^{ab}	0.31±0.16 ^a
CD8α ⁺ CD8β ⁺ CD4 ⁻ (% of CD3 ⁺)	16.7 ± 5.8 ^a	17.6 ± 4.7 ^a	26.4 ± 10.8 ^a	15.0 ± 3.2 ^a	20.3 ± 6.2 ^a
CD8α ⁻ CD4 ⁺ (% of CD3 ⁺)	8.15 ± 0.9 ^a	7.76 ± 1.0 ^a	6.09 ± 2.1 ^a	9.39 ± 1.4 ^a	7.69 ± 0.9 ^a
δTCR ⁺ (% of CD3 ⁺)	53.0 ± 8.7 ^a	50.1 ± 7.0 ^a	48.6 ± 6.4 ^a	48.3 ± 5.3 ^a	30.1 ± 3.3^b
NK cells (% of live cells)	7.8 ± 1.8 ^a	7.2 ± 1.1 ^a	8.7 ± 1.9^b	7.9 ± 1.2 ^a	6.8 ± 0.9 ^a
Alveolar macrophages (% of live cells)	36.2 ± 2.3 ^a	38.5 ± 3.2 ^a	32.8 ± 1.9 ^a	38.1 ± 1.4 ^a	40.6 ± 1.7 ^a
Granulocytes (% of live cells)	18.6 ± 2.8 ^a	14.3 ± 1.8 ^a	20.7 ± 4.4 ^a	16.7 ± 2.1 ^a	19.0 ± 2.0 ^a

Values expressed in means and ± SEM, P<0.05 calculated using R function glm followed by Tukey's test. Values within rows with no common superscript letters differ significantly. Values with the same superscript are not statistically different. Values in bold are statistically different from non-infected controls.

Supplemental Table 3. Flow cytometric analysis of immune cell populations in BALF

	MM (n=6)	MC (n=10)	OS (n=8)	α GC (n=10)	α GCOS (n=8)
Lymphocytes (% of live cells)	4.2 ± 0.9 ^a	6.3 ± 1.6 ^a	7.0 ± 1.1 ^a	6.1 ± 0.9 ^a	7.1 ± 1.5 ^a
Myeloid cells (% of live cells)	91.7 ± 0.8 ^a	88.7 ± 1.5 ^a	88.9 ± 1.7 ^a	89.2 ± 1.2 ^a	90.2 ± 1.7 ^a
iNKT-cells (% of CD3 ⁺)	0.57 ± 0.26 ^a	0.37 ± 0.12 ^a	0.13 ± 0.05 ^a	0.36 ± 0.12 ^a	0.57 ± 0.25 ^a
CD8 α^+ CD8 β^+ CD4 $^-$ (% of CD3 ⁺)	14.2 ± 3.3 ^{ab}	14.6 ± 2.6 ^a	14.4 ± 2.6 ^{ab}	20.5 ± 4.9 ^{ab}	19.5 ± 3.5^b
CD8 α^- CD4 $^+$ (% of CD3 ⁺)	4.7 ± 1.5 ^a	7.9 ± 2.0 ^a	3.7 ± 1.2 ^a	7.9 ± 1.7 ^a	5.8 ± 2.0 ^a
δ TCR $^+$ (% of CD3 ⁺)	40.8 ± 3.4 ^a	37.5 ± 4.0 ^a	38.6 ± 4.3 ^a	44.0 ± 5.7 ^a	34.4 ± 3.3 ^a
NK cells (% of live cells)	5.0 ± 1.7 ^a	3.9 ± 0.7 ^a	4.4 ± 1.1 ^a	5.3 ± 1.3 ^a	8.0 ± 1.9^b
Alveolar macrophages (% of live cells)	60.9 ± 4.4 ^a	65.8 ± 5.8 ^a	64.4 ± 2.4 ^a	66.7 ± 2.4 ^a	62.8 ± 3.7 ^a
Granulocytes (% of live cells)	15.9 ± 3.3 ^a	11.2 ± 3.1 ^a	10.4 ± 1.6 ^a	11.1 ± 1.2 ^a	12.7 ± 1.8 ^a

Values expressed in means and ± SEM, P<0.05 calculated using R function glm followed by Tukey's test. Values within rows with no common superscript letters differ significantly. Values with the same superscript are not statistically different. Values in bold are statistically different from non-infected controls.

Supplemental Table 4. Flow cytometric analysis of immune cell populations in spleen

	MM (n=6)	MC (n=10)	OS (n=8)	αGC (n=10)	αGCOS (n=8)
Lymphocytes (% of live cells)	65.9 ± 3.6 ^a	63.9 ± 1.2 ^a	60.2 ± 1.5 ^a	61.5 ± 1.7 ^a	59.7 ± 1.5 ^a
Myeloid cells (% of live cells)	25.5 ± 2.5 ^a	22.0 ± 1.6 ^a	27.7 ± 1.6 ^a	25.0 ± 1.8 ^a	26.7 ± 2.7 ^a
iNKT-cells (% of CD3 ⁺)	0.06 ± 0.03 ^a	0.08 ± 0.01 ^a	0.10 ± 0.02 ^a	0.36 ± 0.23 ^a	0.29 ± 0.21 ^a
CD8α ⁺ CD8β ⁺ CD4 ⁻ (% of CD3 ⁺)	6.8 ± 0.7 ^a	7.7 ± 0.8 ^a	10.8 ± 1.4 ^a	7.6 ± 0.8 ^a	12.5 ± 4.0^b
CD8α ⁻ CD4 ⁺ (% of CD3 ⁺)	22.3 ± 1.2 ^a	19.5 ± 1.4 ^a	21.2 ± 2.0 ^a	17.30 ± 2.3 ^a	14.0 ± 2.2 ^b
δTCR ⁺ (% of CD3 ⁺)	38.0 ± 3.9 ^a	34.5 ± 2.2 ^a	35.2 ± 3.9 ^a	30.8 ± 2.1 ^a	28.1 ± 2.9 ^a
NK cells (% of live cells)	3.7 ± 0.6 ^a	4.3 ± 1.0 ^a	4.9 ± 2.0 ^{ab}	3.7 ± 1.0 ^a	5.9 ± 2.5^b
Granulocytes (% of live cells)	10.2 ± 1.0 ^a	7.5 ± 0.9 ^a	9.4 ± 1.7 ^a	9.1 ± 0.9 ^a	10.2 ± 1.5 ^a
Macrophages (% of live cells)	3.3 ± 0.6 ^a	2.7 ± 0.3 ^a	2.6 ± 0.3 ^a	2.0 ± 0.2 ^a	3.1 ± 0.6 ^a
Dendritic cells (% of live cells)	1.3 ± 0.2 ^a	1.9 ± 0.6 ^a	2.1 ± 0.7 ^a	2.0 ± 0.5 ^a	1.1 ± 0.4 ^a

Values expressed in means and ± SEM, P<0.05 calculated using R function glm followed by Tukey's test. Values within rows with no common superscript letters differ significantly. Values with the same superscript are not statistically different. Values in bold are statistically different from non-infected controls.

Supplemental Table 5. List of 84 transcripts queried through Pig Inflammatory Cytokines and Receptors PCR arrays in lung samples

Gene	Normalized to MM		Normalized to MC					
	MC		OS		αGC		αGCOS	
	Fold change	P value	Fold change	P value	Fold change	P value	Fold change	P value
<i>AIMP1</i>	-1.16	0.4503	1.09	0.590	1.05	0.714	1.09	0.641
<i>BMP2</i>	1.04	0.7640	1.32	0.261	1.09	0.357	1.49	0.087
<i>C5</i>	-1.27	0.7127	1.25	0.674	1.21	0.648	1.19	0.721
<i>CCL1</i>	1.62	0.0057	1.20	0.361	-1.08	0.911	1.88	0.086
<i>CCL17</i>	1.53	0.0129	1.42	0.282	-1.01	0.893	1.90	0.094
<i>CCL2</i>	1.75	0.3182	-1.63	0.411	-1.93	0.128	-1.49	0.319
<i>CCL20</i>	2.43	0.0007	1.17	0.305	2.27	0.011	-1.39	0.386
<i>CCL21</i>	1.28	0.1543	1.29	0.334	-1.32	0.130	-1.28	0.104
<i>CCL22</i>	1.16	0.5154	-1.38	0.455	-1.04	0.877	1.90	0.094
<i>CCL3L1</i>	1.16	0.7266	-1.42	0.172	-1.07	0.454	1.20	0.214
<i>CCL4</i>	1.10	0.7576	-1.06	0.776	-1.16	0.421	1.38	0.178
<i>CCL5</i>	1.45	0.0270	1.78	0.143	1.01	0.724	2.23	0.005
<i>CCL8</i>	1.24	0.4638	1.01	0.952	-1.37	0.238	1.11	0.949
<i>CCR1</i>	1.23	0.2664	1.37	0.292	-1.03	0.848	1.63	0.106
<i>CCR10</i>	1.12	0.5358	1.21	0.363	1.03	0.684	1.92	0.081
<i>CCR2</i>	1.45	0.0738	1.06	0.734	-1.17	0.149	1.04	0.901
<i>CCR3</i>	1.22	0.2986	1.63	0.217	-1.09	0.289	2.28	0.029
<i>CCR4</i>	1.32	0.0177	1.36	0.262	-1.12	0.278	1.40	0.179
<i>CCR5</i>	1.63	0.1202	1.12	0.783	-1.14	0.464	1.27	0.393
<i>CCR7</i>	1.21	0.2722	-1.10	0.534	-1.01	0.877	1.46	0.137
<i>CD40LG</i>	1.28	0.2005	1.43	0.135	-1.19	0.265	1.19	0.371
<i>CD70</i>	1.51	0.0382	1.24	0.279	1.02	0.981	1.35	0.156
<i>CSF1</i>	1.09	0.4686	1.02	0.810	-1.19	0.344	1.15	0.244
<i>CSF2</i>	-1.09	0.9730	1.08	0.979	1.16	0.674	1.28	0.512
<i>CSF3</i>	1.18	0.3153	1.35	0.301	1.03	0.660	1.83	0.084
<i>CXCL10</i>	4.00	0.4869	-1.39	0.492	-1.67	0.351	-1.06	0.499
<i>CXCL11</i>	1.85	0.3866	1.26	0.597	-1.37	0.355	1.53	0.524
<i>CXCL12</i>	1.17	0.3228	-1.18	0.432	-1.14	0.303	-1.21	0.196
<i>LOC396594</i>	1.24	0.2751	-1.08	0.833	1.70	0.030	-1.32	0.079
<i>CXCL9</i>	3.10	0.3803	1.00	0.813	-1.80	0.339	1.48	0.956
<i>CXCR2</i>	-1.34	0.0713	1.34	0.048	1.24	0.060	1.40	0.088
<i>CXCR4</i>	-1.49	0.0267	1.13	0.465	1.08	0.598	1.32	0.076
<i>FASLG</i>	1.36	0.0436	1.39	0.221	1.03	0.775	1.73	0.033
<i>FLT3LG</i>	1.12	0.4384	1.14	0.400	-1.00	0.947	1.68	0.106
<i>IFNG</i>	1.82	0.1129	1.08	0.727	-1.26	0.284	1.36	0.295

Cont. Supplemental Table 5. List of 84 transcripts queried through Pig Inflammatory Cytokines and Receptors PCR arrays in lung samples

Gene	Normalized to MM		Normalized to MC					
	MC		OS		αGC		αGCOS	
	Fold change	P value	Fold change	P value	Fold change	P value	Fold change	P value
<i>IL10</i>	1.03	0.8229	-1.1	0.578	-1.04	0.710	1.06	0.885
<i>IL10RA</i>	1.26	0.0826	1.31	0.315	-1.04	0.795	1.65	0.097
<i>IL10RB</i>	1.06	0.5386	1.19	0.206	1.03	0.697	1.14	0.287
<i>IL12B</i>	1.29	0.1875	-1.50	0.434	1.06	0.549	2.06	0.074
<i>IL13</i>	1.52	0.4106	1.62	0.287	-1.22	0.378	1.44	0.298
<i>IL15</i>	1.24	0.1116	1.13	0.435	1.10	0.359	1.35	0.087
<i>IL16</i>	1.25	0.0056	1.25	0.288	1.02	0.686	1.36	0.112
<i>IL17A</i>	-1.05	0.9814	1.51	0.310	1.09	0.967	2.63	0.045
<i>IL17F</i>	1.03	0.8871	1.58	0.256	1.19	0.257	1.98	0.080
<i>IL18</i>	-1.15	0.2879	1.23	0.096	-1.11	0.334	1.15	0.276
<i>IL18RI</i>	1.28	0.1945	1.05	0.623	-1.02	0.935	-1.09	0.322
<i>IL1A</i>	1.08	0.6679	-1.61	0.084	-1.13	0.336	-1.21	0.321
<i>IL1B</i>	1.12	0.5335	1.39	0.301	1.01	0.912	2.14	0.097
<i>IL1RN</i>	1.15	0.6687	1.10	0.732	-1.23	0.316	1.31	0.538
<i>IL21</i>	1.45	0.3383	1.57	0.262	-1.18	0.427	2.17	0.063
<i>IL23A</i>	1.12	0.5668	1.56	0.248	-1.05	0.750	1.85	0.070
<i>IL27</i>	-1.00	0.7592	1.63	0.271	1.21	0.614	2.37	0.078
<i>IL2RG</i>	1.23	0.0091	1.45	0.247	-1.02	0.642	1.28	0.067
<i>IL4</i>	1.29	0.0579	-1.13	0.441	1.04	0.660	1.74	0.093
<i>IL4R</i>	1.33	0.0851	1.20	0.329	-1.07	0.581	1.37	0.090
<i>IL5</i>	1.17	0.2299	1.27	0.323	-1.03	0.741	1.53	0.128
<i>IL5RA</i>	1.30	0.0147	1.02	0.584	1.05	0.476	1.05	0.441
<i>IL6</i>	1.41	0.3889	1.24	0.426	-1.04	0.643	1.47	0.311
<i>IL6R</i>	1.05	0.5318	-1.04	0.831	-1.01	0.983	1.11	0.240
<i>IL6ST</i>	1.03	0.7342	1.05	0.667	1.05	0.624	-1.03	0.699
<i>IL7</i>	1.11	0.2073	1.06	0.580	-1.00	0.981	1.09	0.389
<i>IL7R</i>	1.21	0.4104	1.13	0.642	1.01	0.993	-1.12	0.711
<i>CXCL8</i>	1.16	0.4022	1.36	0.291	1.23	0.104	1.54	0.140
<i>IL9</i>	1.24	0.4571	1.39	0.303	-1.06	0.583	2.05	0.091
<i>LIF</i>	1.58	0.1535	-1.07	0.747	-1.16	0.651	1.28	0.339
<i>OSM</i>	1.09	0.5958	1.54	0.248	1.07	0.452	2.01	0.069
<i>IL17B</i>	1.47	0.0768	1.38	0.276	-1.13	0.855	1.68	0.107
<i>IL33</i>	-1.04	0.9174	-1.05	0.891	-1.03	0.929	-1.09	0.528
<i>TNFSF14</i>	1.28	0.2285	1.61	0.222	-1.06	0.940	1.83	0.123
<i>IL9R</i>	1.24	0.2142	1.29	0.323	1.04	0.712	1.62	0.131

Cont. Supplemental Table 5. List of 84 transcripts queried through Pig Inflammatory Cytokines and Receptors PCR arrays in lung samples

Gene	Normalized to MM		Normalized to MC					
	MC		OS		αGC		αGCOS	
	Fold change	P value	Fold change	P value	Fold change	P value	Fold change	P value
<i>LOC100621682</i>	1.14	0.5615	1.41	0.290	-1.04	0.870	2.05	0.097
<i>IL2RB</i>	1.3	0.0443	1.68	0.156	1.16	0.124	1.88	0.042
<i>LTA</i>	1.44	0.1861	1.30	0.326	-1.04	0.886	1.83	0.126
<i>LTB</i>	1.15	0.2934	1.28	0.322	-1.09	0.701	1.20	0.323
<i>MIF</i>	-1.09	0.2081	1.09	0.365	-1.10	0.096	-1.04	0.559
<i>NAMPT</i>	-1.00	0.9252	1.08	0.698	1.07	0.634	-1.14	0.380
<i>SPP1</i>	1.41	0.5498	-1.09	0.892	-1.76	0.160	-1.16	0.497
<i>TGFB2</i>	-1.06	0.3733	-1.08	0.344	-1.11	0.326	-1.09	0.178
<i>TNF</i>	1.34	0.1863	-1.29	0.399	-1.34	0.336	-1.13	0.778
<i>TNFRSF11B</i>	-1.05	0.7505	1.01	0.892	-1.02	0.943	-1.07	0.576
<i>TNFSF10</i>	1.36	0.3374	-1.37	0.344	-1.06	0.569	-1.01	0.762
<i>TNFSF13B</i>	1.19	0.0975	1.08	0.419	1.01	0.890	1.09	0.391
<i>TNFSF4</i>	1.21	0.4030	1.55	0.258	1.15	0.250	1.75	0.116