

## ONLINE DATA SUPPLEMENT

### IL1F9 STIMULATES CHEMOKINE PRODUCTION AND NEUTROPHIL INFLUX IN MOUSE LUNGS

**Ravisankar A Ramadas\***, **Susan L Ewart<sup>†</sup>**, **Benjamin D Medoff<sup>‡</sup>**, **Ann Marie LeVine\***

\*Department of Pediatrics, University of Florida, Gainesville, FL. <sup>†</sup>Department of Large Animal Clinical Sciences, Michigan State University, Lansing, MI. <sup>‡</sup> Pulmonary and Critical Care Unit, Center for Immunology and Inflammatory Diseases, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA.

#### **Corresponding author:**

Ann Marie LeVine, MD

Pediatrics & Communicable Diseases

Pediatric Critical Care Medicine

University of Michigan Medical School

C.S. Mott Children's Hospital

Mott F-6882/Box 0243

1500 East Medical Center Drive

Ann Arbor, MI 48109

Telephone: (734) 764-5302

Fax: (734) 647-5624

E-mail address: [anlevine@med.umich.edu](mailto:anlevine@med.umich.edu)

**Running title:** IL1F9 is proinflammatory in lung diseases

**Keywords:** IL1F9, chemokines, lung diseases, inflammation, neutrophil

Table S1. Primers used in qRT-PCR mini-arrays.

No	Gene	Primer name	Primer sequence 5'-3'
1	CCL1	SYBR PF - CCL1	ACAGGAGGAGCCCATCTTTC
	CCL1	SYBR PR - CCL1	CTGCCGTGTGGATACAGGAT
2	CCL2	SYBR PF - CCL2	TCTCCAGCCTACTCATTGGG
	CCL2	SYBR PR - CCL2	AGGTCCCTGTCATGCTTCTG
3	CCL3	SYBR PF - CCL3	TAGGAGAAGCAGCAGGCAGT
	CCL3	SYBR PR - CCL3	CCTCTGTCACCTGCTCAACA
4	CCL4	SYBR PF - CCL4	AGAAACAGCAGGAAGTGGGA
	CCL4	SYBR PR - CCL4	GCTCTGTGCAAACCTAACCC
5	CCL5	SYBR PF - CCL5	CACTTCTTCTCTGGGTTGGC
	CCL5	SYBR PR - CCL5	CTGCTGCTTTGCCTACCTCT
6	CCL6	SYBR PF - CCL6	TGGGATCTGTGTGGCATAAG
	CCL6	SYBR PR - CCL6	TTATCCTTGTGGCTGTCCTTG
7	CCL7	SYBR PF - CCL7	TCCTCTTGGGGATCTTTTGTT
	CCL7	SYBR PR - CCL7	ACCATGAGGATCTCTGCCAC
8	CCL8	SYBR PF - CCL8	CTCGTAGCTTTTCAGCACCC
	CCL8	SYBR PR - CCL8	TTCTTTGCCTGCTGCTCATA
9	CCL9	SYBR PF - CCL9	TTGAAAGCCCATGTGAAACA

	CCL9	SYBR PR - CCL9	ACTGCCCTCTCCTTCCTCAT
10	CCL11	SYBR PF - CCL11	CTATGGCTTTCAGGGTGCAT
	CCL11	SYBR PR - CCL11	TCACTTCCTTCACCTCCCAG
11	CCL12	SYBR PF - CCL12	CCTGAAGATCACAGCTTCCC
	CCL12	SYBR PR - CCL12	GTCCTCAGGTATTGGCTGGA
12	CCL17	SYBR PF - CCL17	ATCCCTGGAACACTCCACTG
	CCL17	SYBR PR - CCL17	TGCTTCTGGGGACTTTTCTG
13	CCL19	SYBR PF - CCL19	CACAGACAGGCAGCAGTCTT
	CCL19	SYBR PR - CCL19	CACTCACTCTCTGTGGCCTG
14	CCL20	SYBR PF - CCL20	GGAAGGAAGAGGCGTCTGTA
	CCL20	SYBR PR - CCL20	ACTCCTGGAGCTGAGAATGG
15	CCL22	SYBR PF - CCL22	CAGGTCCTCCTCCCTAGGAC
	CCL22	SYBR PR - CCL22	TCTGGACCTCAAATCCTGC
16	CCL24	SYBR PF - CCL24	CTTATGGCCCTTCTTGGTGA
	CCL24	SYBR PR - CCL24	CTGTGACCATCCCCTCATCT
17	CCL25	SYBR PF - CCL25	CTAGCATGCCGGAGAACATT
	CCL25	SYBR PR - CCL25	GAAACTGTGGCTTTTTGCCT
18	CCL27	SYBR PF - CCL27	GGTTCTGGGGATGAACACAG
	CCL27	SYBR PR - CCL27	ATAGACAGCCACTCCCAAGC
19	CCL28	SYBR PF - CCL28	AGACACCTCAGTGCAACAGC
	CCL28	SYBR PR - CCL28	TCACCTGAGTCATTGCCAGA
20	CXCL1	SYBR PF - CXCL1	GTGCCATCAGAGCAGTCTGT
	CXCL1	SYBR PR - CXCL1	GCACCCAAACCGAAGTCATA
21	CXCL2	SYBR PF - CXCL2	CATCAGGTACGATCCAGGCT

	CXCL2	SYBR PR - CXCL2	CCTGGTTCAGAAAATCATCCA
22	CXCL4	SYBR PF - CXCL4	GATCTCCATCGCTTTCTTCG
	CXCL4	SYBR PR - CXCL4	GGTCTTGACATGAGCGTCG
23	CXCL5	SYBR PF - CXCL5	TGCATTCCGCTTAGCTTTCT
	CXCL5	SYBR PR - CXCL5	GCCCTACGGTGGAAGTCATA
24	CXCL7	SYBR PF - CXCL7	GAATGGGATTCCAGAGATGG
	CXCL7	SYBR PR - CXCL7	CTGCCCACTTCATAACCTCC
25	CXCL9	SYBR PF - CXCL9	TTTCCCCCTCTTTTGCTTTT
	CXCL9	SYBR PR - CXCL9	CCCAATTGCAACAAAACCTGA
26	CXCL10	SYBR PF - CXCL10	CTTCCCTATGGCCCTCATTC
	CXCL10	SYBR PR - CXCL10	AAGTGCTGCCGTCATTTTCT
27	CXCL11	SYBR PF - CXCL11	GCTTTCTCGATCTCTGCCAT
	CXCL11	SYBR PR - CXCL11	AACAGGAAGGTCACAGCCAT
28	CXCL12	SYBR PF - CXCL12	GGCGTCTGACTCACACCTCT
	CXCL12	SYBR PR - CXCL12	ACACTCCAAACTGTGCCCTT
29	CXCL13	SYBR PF - CXCL13	CCGACAACAGTTGAAATCACTC
	CXCL13	SYBR PR - CXCL13	CAGAATGAGGCTCAGCACAG
30	CXCL14	SYBR PF - CXCL14	GATGAAGCGTTTGGTGCTCT
	CXCL14	SYBR PR - CXCL14	AAGCTGGAAATGAAGCCAAA
31	CXCL15	SYBR PF - CXCL15	TCTCAGGTCTCCCAAATGAAA
	CXCL15	SYBR PR - CXCL15	CCATGGGTGAAGGCTACTGT
32	CXCL16	SYBR PF - CXCL16	ACTGGCTTGAGGCAAATGTT
	CXCL16	SYBR PR - CXCL16	GGTTCCAGTTGCAGTCCAAA
33	XCL1	SYBR PF - XCL1	CCCCTCCCAGATGATATAGGT

	XCL1	SYBR PR - XCL1	TGACTTTCCTGGGAGTCTGC
34	CX3CL1	SYBR PF - CX3CL1	ACTCCTGGTTTAGCTGATAGCG
	CX3CL1	SYBR PR - CX3CL1	CGCGTTCTTCCATTTGTGTA
35	CCR1	SYBR PF - CCR1	GCTGAGGAACTGGTCAGGAA
	CCR1	SYBR PR - CCR1	AGGTTGGGACCTTGAACCTT
36	CCR2	SYBR PF - CCR2	TCCTTTGATTTGTTTTGCAGAT
	CCR2	SYBR PR - CCR2	TGCCATCATAAAGGAGCCAT
37	CCR3	SYBR PF - CCR3	CCCCAGCTCTTTGATTCTGA
	CCR3	SYBR PR - CCR3	AAAGGACTTAGCAAAATTCACCA
38	CCR4	SYBR PF - CCR4	GTTTCATCCTGGGTGGTGTC
	CCR4	SYBR PR - CCR4	CGGCATTGCTTCATAGACTG
39	CCR5	SYBR PF - CCR5	AGCCGCAATTTGTTTCACAT
	CCR5	SYBR PR - CCR5	GAGACATCCGTTCCCCCTAC
40	CCR6	SYBR PF - CCR6	CTGGAACTCTGCAGAACGCT
	CCR6	SYBR PR - CCR6	TGGCCAGTCTACTTTGGAGC
41	CCR7	SYBR PF - CCR7	ACACAGGAAGGCTGTGCTTT
	CCR7	SYBR PR - CCR7	CATGGACTGCTATCTGCGTC
42	CCR8	SYBR PF - CCR8	GAGGAGGAACTCTGCGTCAC
	CCR8	SYBR PR - CCR8	AAACCTCAGAAGAAAGGCTCG
43	CCR9	SYBR PF - CCR9	CATCTGTGGAAGCAGTGGAG
	CCR9	SYBR PR - CCR9	ACTCACCTCCGCCGTACAT
44	CXCR3	SYBR PF - CXCR3	CAGAAGAAAGGCAAAGTCCG
	CXCR3	SYBR PR - CXCR3	AAGTTCCCAACCACAAGTGC
45	CXCR4	SYBR PF - CXCR4	CTCCAGACCCCACTTCTTCA

	CXCR4	SYBR PR - CXCR4	AGGTGCAGGTAGCAGTGACC
46	CXCR5	SYBR PF - CXCR5	CGTTTGTTCCTCCTGGCTGTT
	CXCR5	SYBR PR - CXCR5	CTGGAACAAAGCTACTGGGC
47	XCR1	SYBR PF - XCR1	AGATGGTGGAAAAGAAGATGACA
	XCR1	SYBR PR - XCR1	GACTTGAAACCCTGACATGGA

Table S2. Fold change in mRNA expression following HDM or IL1F9 challenges.

Gene	Fold change in expression			
	1h	48h	6h	24h
	HDM	HDM	IL1F9	IL1F9
<b>CCL1</b>	NS	NS	2.31	NS
<b>CCL2</b>	9.78	54.42	227.54	NS
<b>CCL3</b>	24.00	NS	594.69	NS
<b>CCL4</b>	53.45	NS	1274.75	NS
<b>CCL5</b>	2.29	NS	6.62	NS
<b>CCL6</b>	2.34	9.96	1.80	1.04
<b>CCL7</b>	15.32	73.01	132.51	NS
<b>CCL8</b>	28.25	1406.60	14.10	30.32
<b>CCL9</b>	15.71	NS	13.10	NS
<b>CCL11</b>	6.36	61.39	2.18	NS
<b>CCL12</b>	27.05	49.66	44.51	20.51
<b>CCL17</b>	NS	7.30	4.72	NS
<b>CCL19</b>	NS	-1.18	2.27	NS

<b>CCL20</b>	NS	NS	124.16	NS
<b>CCL22</b>	-1.16	3.48	5.59	NS
<b>CCL24</b>	4.18	142.22	NS	-1.07
<b>CCL25</b>	1.48	0.00	1.66	NS
<b>CCL27</b>	0.00	1.47	-1.36	-1.84
<b>CXCL1</b>	12.15	43.90	15.69	NS
<b>CXCL2</b>	44.86	NS	156.72	NS
<b>CXCL4</b>	1.23	2.91	3.07	NS
<b>CXCL5</b>	NS	45.76	43.71	NS
<b>CXCL7</b>	-1.02	1.48	1.04	1.12
<b>CXCL9</b>	7.86	NS	599.66	45.95
<b>CXCL10</b>	NS	NS	533.00	NS
<b>CXCL11</b>	NS	NS	262.83	NS
<b>CXCL12</b>	1.09	2.73	94.75	17.38
<b>CXCL13</b>	NS	13.79	25.71	3.24
<b>CXCL14</b>	-1.36	-1.31	3.07	NS
<b>CXCL15</b>	1.20	6.16	1.80	3.34
<b>CXCL16</b>	1.01	2.95	4.48	3.20
<b>XCL1</b>	NS	NS	6.01	17.53
<b>CCR1</b>	8.65	NS	19.89	NS
<b>CCR2</b>	2.98	NS	7.68	NS
<b>CCR5</b>	NS	4.77	24.08	NS
<b>CCR6</b>	NS	NS	-1.58	NS
<b>CCR8</b>	NS	NS	NS	-1.30

<b>CCR9</b>	NS	NS	2.87	NS
<b>CXCR3</b>	NS	NS	NS	3.83
<b>CXCR4</b>	1.23	NS	2.60	1.94
<b>CXCR5</b>	NS	NS	6.18	5.58
<b>XCR1</b>	NS	NS	1.15	NS

**TABLE S2.** Overlapping as well as distinct lung chemokine and chemokine receptor expression patterns in lungs of mice challenged with HDM or IL1F9 compared to PBS controls. Lungs were collected 1 and 48 following the last HDM challenge or 6 and 24h following the last of four IL1F9 challenges, RNA isolated and qPCR mini-array analyses performed. Data reported include genes that are significantly upregulated ( $p < 0.05$ ) in HDM challenged mouse lungs compared to PBS challenged controls 1 and 48h following the last challenge. Data reported also include genes that are significantly upregulated ( $p < 0.05$ ) in IL1F9 challenged mouse lungs compared to PBS challenged controls 6h and 24h following the last of four intratracheal challenges. Data are presented as fold change in mRNA expression relative to 18S rRNA expression ( $n = 4-5$  mice/group/timepoint). NS = not significant.