

**Supplementary Table 1. Genotype frequencies for *TLR4* Asp299Gly and Thr399Ile SNPs in previous studies of infants.**

Manuscript (ref.)	Country	<i>TLR4</i> SNP	n	% Mutations		OR	p
				Cases	Controls		
Tal G. et. al. JID 2004 (14).	Israel	Both pooled	181	20	4	4.9 (1.6 - 15.3)	0.003
Paulus S.C. et. al. Clin Immunol 2007 (17).	Canada	Asp299Gly	342	5	10	NA	NS
Puthothu B. Dis Markers 2006 (16).	Germany	Asp299Gly	401	4	8	NA	0.05
Kresfelder T.L. et. al. J Med Virol 2011 (18).	South Africa	Asp299Gly	409	6	12	0.48 (0.23-0.99)	NA
Inoue Y. et. al. JID 2007 (15).	Japan	Both	104	0	0	NA	NA

NA: not available

NS: p>0.05

**Supplementary Table 2. Overall socioeconomic status in West, South and Central communities in Buenos Aires (2003 - 2006)**

	<b>low SES‡</b>	<b>high SES</b>
<b>Under poverty line* (%)</b>	60	0
<b>Crowding** (%)</b>	45	3
<b>Well water (%)</b>	38	0
<b>No sewage (%)</b>	62	0
<b>Incomplete elementary school (%)</b>	47	1

\* income less than US \$161/month

\*\* more than three per room

‡ West + South regions

**From**

National Institute of Statistics and Census, Ministry of Economy, Argentina. Censo nacional de población, hogares y viviendas. 2001  
Hospital Posadas and Hospital Evita Pueblo epidemiological records.

**Supplementary Table 3. Demographic characteristics of infants in the first population.**

Region	RSV positives (n = 418)					RSV negatives (n = 350)					p***
	West	South	p*	Center	p**	West	South	p*	Center	p**	
<i>n</i>	122	171		125		152	131		67		
<b>Males (%)</b>	66(54)	87(51)	0.58	75(62)	0.17	86(57)	75(57)	0.9	42(63)	0.46	0.34
<b>Breastfed (%)</b>	98(80)	138(80)	0.93	109(87)	0.13	123(81)	101(77)	0.43	50(75)	0.52	0.14
<b>Smokers at home (%)</b>	73(60)	116(68)	0.16	55(44)	<b>&lt;0.001</b>	87(57)	82(62)	0.36	21(31)	<b>0.001</b>	0.25
<b>Daycare (%)</b>	4(3)	2(1)	0.24	9(7)	<b>0.02</b>	7(5)	3(2)	0.28	10(15)	0.05	0.16
<b>Sib &lt;14 y (med)</b>	1	1	0.53	1	0.31	1	2	0.16	1	0.19	0.60
<b>Age (mo, mean)</b>	4.3 ± 2.8	3.6 ± 2.2	0.1	4.2 ± 2.5	0.41	5.2 ± 2.1	4.0 ± 2.3	<b>0.001</b>	5.3 ± 2.1	0.06	<b>0.03</b>

\* West vs. South

\*\* West + South vs. Center

\*\*\* RSV positives vs. RSV negatives

**Supplementary Table 4. Epidemiological risk factors for severe RSV bronchiolitis.**

	RSV positives				RSV negatives			
	low LPS	high LPS	All infants		low LPS	high LPS	All infants	
			univariate	multivariate			univariate	multivariate
<b>n</b>	125	293	418	418	67	283	305	350
<b>Males OR (95% CI)</b>	0.66 (0.29-1.49)	0.89 (0.54-1.46)	0.83 (0.55-1.26)	0.84 (0.55-1.26)	1.14 (0.39-3.33)	0.92 (0.56-1.52)	0.91 (0.59-1.42)	0.91 (0.58-2.98)
<b>Breastfeeding OR (95% CI)</b>	3.22 (0.97-10.69)	0.68 (0.36-1.29)	0.96 (0.56-1.64)	0.96 (0.56-1.65)	0.70 (0.19-2.65)	0.68 (0.37-1.27)	0.69 (0.4-1.19)	0.71 (0.41-1.22)
<b>Smokers OR (95% CI)</b>	0.72 (0.33-1.59)	1.32 (0.79-2.19)	1.10 (0.73-1.67)	1.10 (0.72-1.67)	2.66 (0.72-9.89)	1.47 (0.89-2.44)	1.34 (0.86-2.08)	1.46 (0.93-2.30)
<b>Daycare OR (95% CI)</b>	0.5 (0.11-2.27)	0.23 (0.04-1.43)	0.35 (0.11-1.1)	0.35 (0.11-1.13)	1.17 (0.25-5.4)	0.38 (0.09-1.66)	1.10 (0.42-2.89)	0.94 (0.35-2.51)
<b>Siblings &lt;14 y OR (95% CI)</b>	<b>1.5* (1.01-2.23)</b>	1.14 (0.97-1.34)	<b>1.2* (1.04-1.39)</b>	<b>1.2* (1.03-1.39)</b>	1.19 (0.84-1.69)	<b>1.36* (1.13-1.63)</b>	<b>1.30* (1.11-1.52)</b>	<b>1.32* (1.12-1.55)</b>
<b>Age (months) OR (95% CI)</b>	<b>0.81* (0.69-0.96)</b>	<b>0.82* (0.74-0.90)</b>	<b>0.82* (0.75-0.89)</b>	<b>0.82* (0.75-0.89)</b>	1.12 (0.93-1.34)	<b>0.85* (0.77-0.94)</b>	<b>0.91* (0.84-0.99)</b>	<b>0.91* (0.84-0.99)</b>

\* p<0.05

**Supplementary Table 5. Additional indicators of severity in infants with RSV bronchiolitis (2003-2006).**

<b>Region</b>	<b>West</b>	<b>South</b>	<b>Center</b>
Number of infants	78	99	69
Oxygen supplementation (d; mean $\pm$ SD)	5 $\pm$ 3	6 $\pm$ 2	6 $\pm$ 3
Hospitalization (d; mean $\pm$ SD)	6 $\pm$ 2	7 $\pm$ 2	7 $\pm$ 3
Need for pediatric intensive care (n)	1	2	4
Deaths (n)	0	0	0

Note: No significant differences ( $p < 0.05$ ) observed between groups.

**Supplementary Table 6. Genotype frequencies for *TLR4* Asp299Gly and Thr399Ile SNPs in infants with bronchiolitis.**

SNP	<i>TLR4</i> Asp299Gly*		Total
	RSV positives n (%)	RSV negatives n (%)	n
CC	369 (92.95)	310 (93.66)	679
CT	27 (6.80)	21 (6.34)	48
TT	1 (0.25)	0	1
Total	397 (100)	331 (100)	728

SNP	<i>TLR4</i> Thr399Ile**		Total
	RSV positives n (%)	RSV negatives n (%)	n
AA	372 (92.77)	317 (94.07)	689
AG	28 (6.98)	20 (5.93)	48
GG	1 (0.25)	0	1
Total	401 (100)	337 (100)	738

\* sequence failed in 40 for *TLR4* Asp299Gly.

\*\* sequence failed in 30 for *TLR4* Thr399Ile.

**Supplementary Table 7. Ethnic specific genomic markers\* to evaluate population admixture and reported relative frequencies in different ethnicities.**

Chromosome location	rs Number	SNP	Major allele frequency			
			Nat Am	European	African	Hispanics
1q23.2	rs2814778	A/G	0.99	0.99	0	0.84
1q32.1	rs10800899	A/G		0.067	0.017	
1q32.1	rs2065160	C/T	0.83	0.08	0.5	0.63
1q32.3	rs723632	G/C	0.33	0.08	0.9	0.27
1q42	rs2752	G/T	0.33	0.46	0.09	
2p16.1	rs3287	A/G	0.79	0.8	0.27	0.68
3q22.3	rs584059	A/C	0.53	0.86	0.51	0.73
5p13.2	rs930072	C/T	0.45	0.1	0.96	0.34
5q23.1	rs3317	A/G	0.73	0.59	0.05	0.54
5q33.2	rs3340	A/G	0.35	0.81	0.94	0.6
7p14.3	rs13226868	C/G		0.1	0.75	
7p14.3	rs1985080	A/G	0.03	0.36	0.9	0.3
7p22.3	rs2763	C/G	0.48	0.84	0.86	0.72
7q22.1	rs776745	A/C		0.867	0.083	
7q22.1	rs2161	A/G	0.62	0.3	0.44	0.38
8p21.3	rs285	C/T	0.55	0.48	0.03	0.49
9q21.31	rs2695	A/G	0.78	0.14	0.19	0.44
9q33.3	rs518116	A/G	0.42	0.33	0.87	0.5
10q23.1	rs235936	C/T	0.37	0.49	0.18	0.42
11q11	rs1042602	A/C	0.05	0.47	0	0.28
11q23.1	rs1079598	C/T	0.63	0.14	0.06	0.26
11q23.1	rs1800498	C/T	0.91	0.35	0.86	0.6
11q23.1	rs1800404	A/G	0.48	0.72	0.14	0.54
14q32.12	rs736394	C/T	0.99	0.74	0.52	0.79
15q14	rs2862	C/T	0.69	0.17	0.38	0.4
15q21.2	rs4646	A/C	0.72	0.29	0.32	0.42
16q24.3	rs2228478	A/G	0.96	0.86	0.49	0.88
17p12	rs2816	C/T	0.92	0.51	1	0.67
17p13.2	rs2891	A/G	0.57	0.49	0.98	0.59
17q21.33	rs203096	G/T	0.28	0.72	0.65	0.59
19q13.42	rs1989486	C/T	0.4	0.58	0.04	0.51
20q11.22	rs16434	Del/Ins	0.66	0.65	0.17	0.61
21q21.1	rs1035451	A/G		0.448	0.881	

\* Adapted from Hoggart CJ et al. *Am J Hum Genet* 72:1492-1504, 2003.

**Supplementary Table 8. Genotype frequencies for *CD14*-159 and -550 SNPs in infants with bronchiolitis (2003-2006).**

SNP	<i>CD14</i> -159*		Total
	RSV positives n (%)	RSV negatives n (%)	n
CC	107 (26.62)	111 (32.65)	679
CT	216 (53.73)	161 (47.35)	48
TT	79 (19.65)	68 (20.00)	1
Total	402 (100)	340 (100)	742

SNP	<i>CD14</i> -550**		Total
	RSV positives n (%)	RSV negatives n (%)	n
CC	216 (54.82)	199 (61.04)	415
CT	143 (36.29)	107 (32.83)	250
TT	35 (8.88)	20 (6.13)	55
Total	394 (100)	326 (100)	720

\* sequence failed in 26 for *CD14*-159.

\*\* sequence failed in 48 for *CD14*-550.



**Supplementary Table 9. Genotype frequencies for *IL-13* A-144G and *IL-4* C-590T in infants with bronchiolitis (2003-2006).**

SNP	<i>IL-13</i> -144*		Total
	RSV positives n (%)	RSV negatives n (%)	n
GG	167 (41.44)	135 (39.94)	302
AG	171 (42.43)	153 (45.27)	324
AA	65 (16.13)	50 (14.79)	115
Total	403 (100)	338 (100)	741

SNP	<i>IL-4</i> -590**		Total
	RSV positives n (%)	RSV negatives n (%)	n
CC	183 (44.53)	148 (43.27)	331
CT	168 (40.88)	157 (45.91)	325
TT	59 (14.36)	36 (10.53)	95
Total	410 (100)	341 (100)	751

\* sequence failed in 9 for *IL-13*-144.

\*\* sequence failed in 17 for *IL-4*-590.

**Supplementary Table 10. Socioeconomic status in low and high income groups in Buenos Aires (2010-2013).**

	<b>low SES‡</b>	<b>high SES</b>
<b>House material* (%)</b>	25	0
<b>Crowding** (%)</b>	59	5
<b>Well water (%)</b>	30	0
<b>No sewage (%)</b>	62	0
<b>Incomplete elementary school (%)</b>	12	0

\* house material % of tin/mud

\*\* more than three people per room

‡ West + South regions

**From**

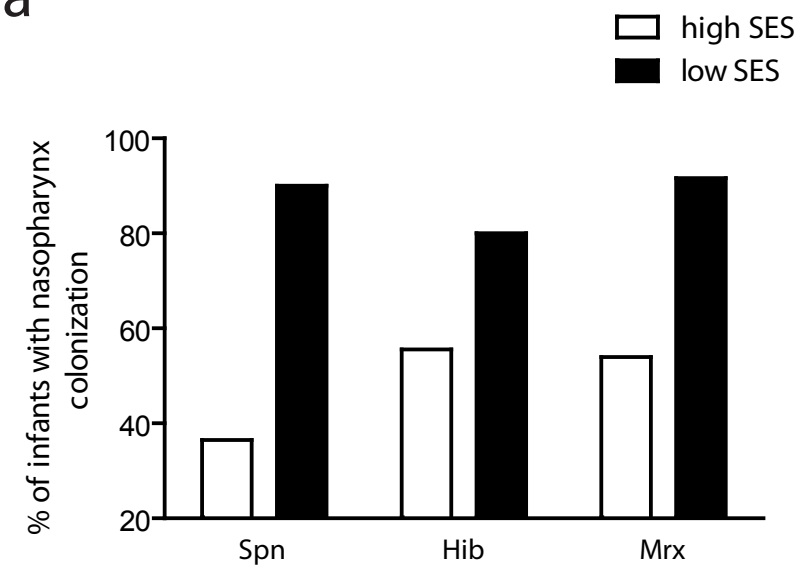
Latinoamerican Foundation of Economics Research. Valorización de la canasta básica alimentaria y canasta básica total 2010

**Supplementary Table 11.** Failsafe buffers, primer sequences and restriction enzymes used for the RFLP assays.

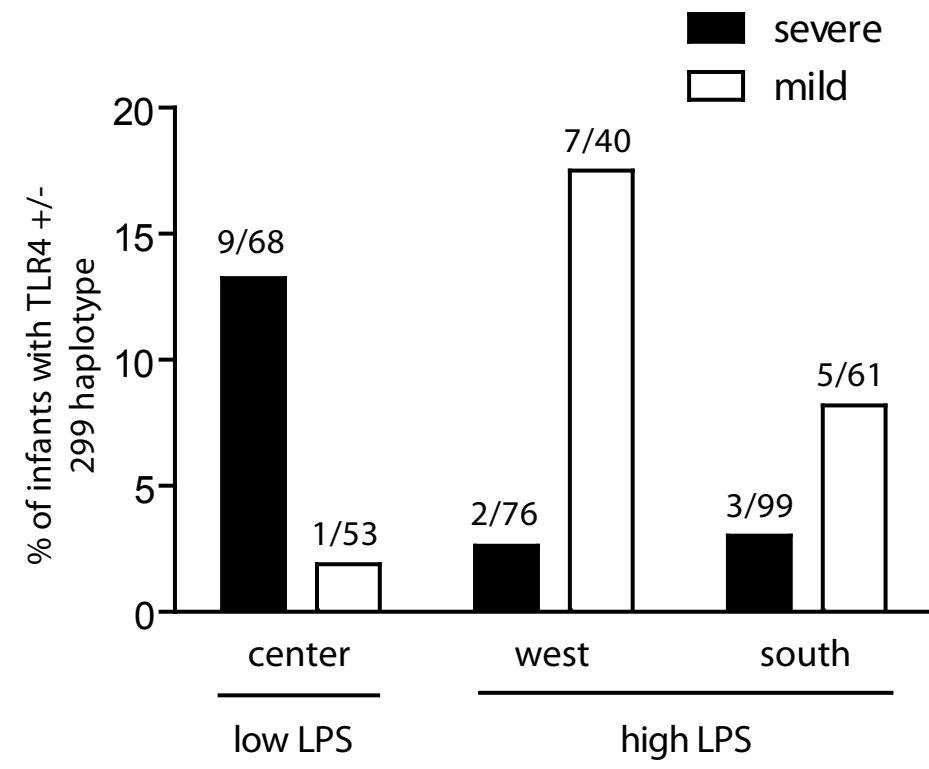
<b>Polymorphic site</b>	<b>Failsafe buffer</b>	<b>Primer sequence</b>	<b>Restriction enzyme</b>
CD14 C-159T rs2569190	G	Forward 5'-ATC ATC CTT TTC CCA CAC C-3' Reverse 5'-AAC TCT TCG GCT GCC TCT-3'	Hae III
CD14 C-550T rs5744455	H	Forward 5'-GGA AGG GGG AAT TTT TCT TTA GGC-3' Reverse 5'-GGC AGT GTC CTG ATG ACT CAG G-3'	Hae III
IL4 C-590T rs2243250	F	Forward 5'-TAA ACT TGG GAG AAC ATG GT-3' Reverse 5'-TGG GGA AAG ATA GAG TAA TA-3'	Ava II

# Supplementary Figure 1

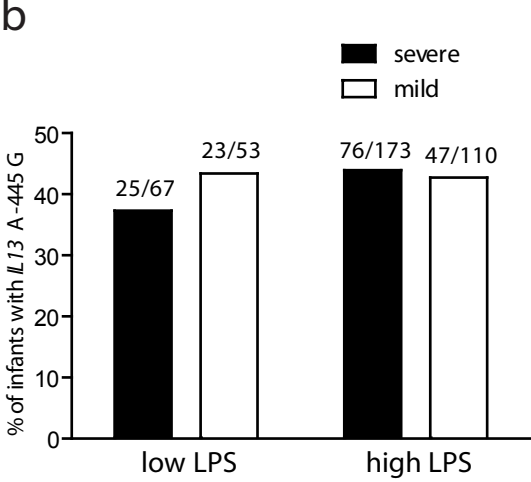
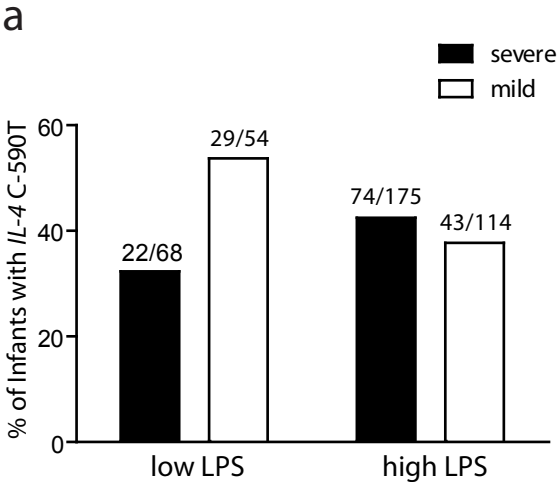
a



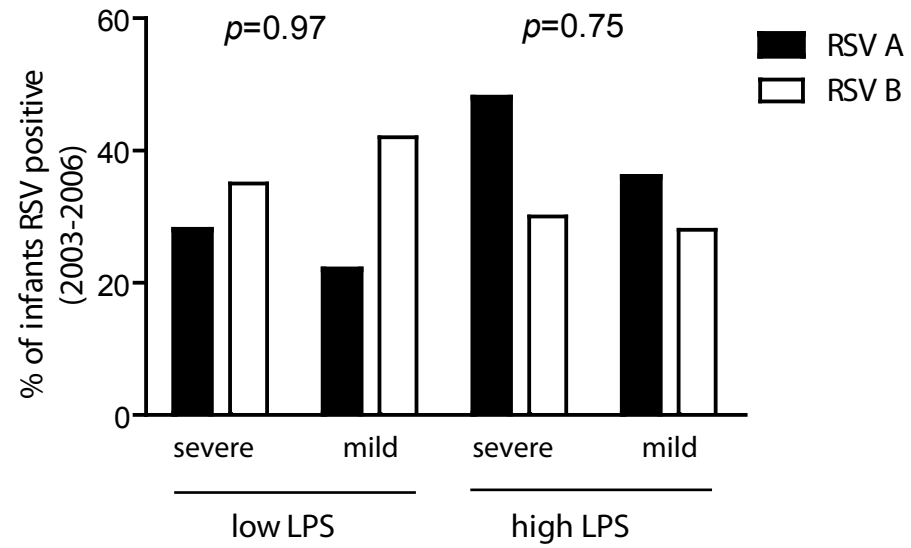
Supplementary Figure 2.



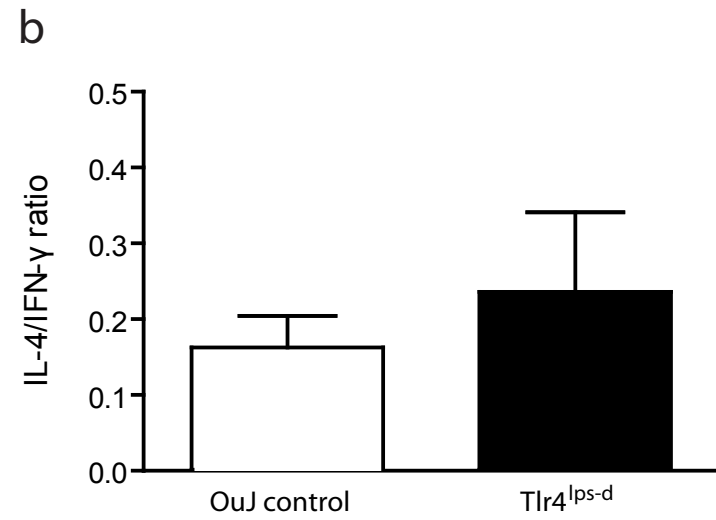
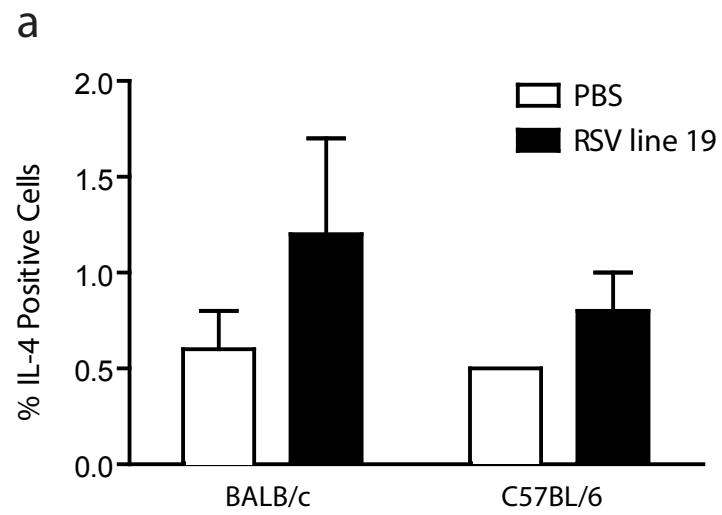
# Supplementary Figure 3.



# Supplementary Figure 4



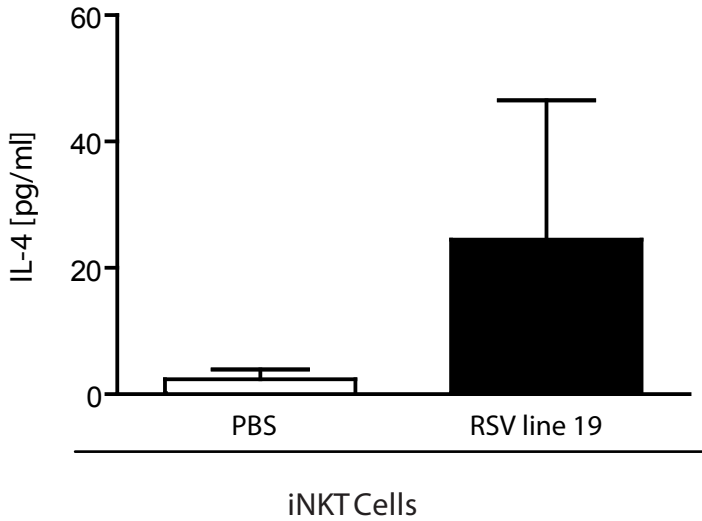
# Supplementary Figure 5



CD4+ T Cells



# Supplementary Figure 6



**Supplementary Figure 1.** Nasopharyngeal colonization with *S. pneumoniae*, non-typable *H. influenzae*, and *M. catarrhalis* in a convenience sample of 100 infants of low vs. high SES; % of infants with detectable bacterial DNA; Chi-Square;  $P < 0.001$  for all three comparisons.

**Supplementary Figure 2.** Association of the *TLR4* Asp299Gly heterozygous genotype in RSV-infected infants with mild (white bars) and severe (black bars) bronchiolitis in two groups from rural homes exposed to high environmental levels of LPS (high LPS West and South), and a group from urban homes with low LPS exposure (low LPS) in 2003-2006.

**Supplementary Figure 3.** (A) Distribution of a gain-of-function *IL4* SNP (C-590T) in infants with RSV bronchiolitis. Consistent with studies in industrialized countries ([9](#), [10](#)), C-590T associated with severity in low LPS environments;  $p = 0.04$ . No effect was observed in the rural population exposed to high levels of LPS.  $p = \text{NS}$  (B) Conversely, *IL13* SNP (A-445G) analysis did not associate with differences in severity;  $p = \text{NS}$  for both comparisons.

**Supplementary Figure 4.** RSV isolates were analyzed for RSV subgroups A and B by RT-PCR in both low and high LPS groups.

**Supplementary Figure 5.** Mice were inoculated with PBS or  $10^6$  pfu of RSV line 19 IN. Five days post infection lung mononuclear cells were harvested from lung homogenates. Cells were stimulated *ex vivo* with PMA/ionomycin (50 ng/ml and 2  $\mu\text{g/ml}$ , respectively) 4 hours at  $37^\circ\text{C}$ . Cells were stained for intracellular cytokines and analyzed by flow cytometry. A) Percentage of IL-4 positive CD4+T cells in RSV and PBS inoculated mice ( $p < 0.05$  for both comparisons). B) OuJ and *Tlr4*<sup>lps-d</sup> (HeJ) mice infected with  $10^6$  pfu of RSV line 19. IL-4/IFN- $\gamma$  ratio was compared between groups ( $p < 0.05$ ).

**Supplementary Figure 6.** C57BL/6 mice were inoculated with PBS or  $10^6$  pfu of RSV line 19 IN. Five days post inoculation mononuclear cells from lung homogenates were harvested and iNKT cells were isolated using magnetic beads (Miltenyi Biotec). Cells were stimulated *ex vivo* with  $\alpha$ -Galactosylceramide and IL-2 (100 ng and 1 ng/ml, respectively) for 24 hours at  $37^\circ\text{C}$ . IL-4 was analyzed in supernatants fluids by immunoassays ( $p < 0.05$ ). A second experiment assaying iNKT cells for IL-4 by flow cytometry after 4 hs of *ex vivo* stimulation with  $\alpha$ -Galactosylceramide and IL-2 did not detect excess IL-4 production in RSV infected animals (not shown).