Supplementary supporting data

Table 1. Differential expression of cytokine and chemokine related genes in the lungs of *nrf2* -deficient and wild-type mice following treatment with LPS.

Gene title	Gene symbol	30 min (LPS / Vehicle)		1 h (LPS / Vehicle)		6 h (LPS / Vehicle)		12 h (LPS / Vehicle)		24 h (LPS / Vehicle)	
		Nrf2 -/-	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*
Chemokine (C-C motif) ligand 12 (Monocyte chemotactic protein 5)	CCL12 (MCP5)	19.7 ± 0.6	7.5 ± 0.4	27.7 ± 0.6	12.5 ± 0.4	19.3 ± 0.6	8.6 ± 0.4	19.6 ± 0.7	15.0 ± 0.4	29.2 ± 0.7	14.9 ± 0.4
Chemokine (C-C motif) ligand 17 (Thymus- and activation-regulated chemokine)	CCL17 (TARC)	4.5 ± 0.4	1.8 ± 0.4	6.1 ± 0.4	4.2 ± 0.4	9.1 ± 0.4	7.0 ± 0.4	7.1 ± 0.4	7.1 ± 0.4	- - -	- - -
Chemokine (C-C motif) ligand 2 (Monocyte chemoattractant protein-1)	CCL2 (MCP1)	6.3 ± 0.5	- - -	24.8 ± 0.4	20.5 ± 0.6	20.4 ± 0.5	11.9 ± 0.6	6.0 ± 0.6	8.8 ± 0.6	4.7 ± 0.6	5.7 ± 0.5
Chemokine (C-C motif) ligand 20 (Macrophage inflammatory protein 3 alpha)	CCL20 (MIP3α)	- - -	- - -	21.4 ± 0.5	32.0 ± 0.7	- - -	- - -	- - -	- - -	- - -	- - -
Chemokine (C-C motif) ligand 3 (Macrophage inflammatory protein 1-alpha)	CCL3 (MIP1α)	40.5 ± 0.9	25.3 ± 0.5	321.8 ± 0.8	501.5 ± 0.5	120.3 ± 0.8	170.1 ± 0.4	39.1 ± 0.8	73.5 ± 0.5	- - -	- - -
Chemokine (C-C motif) ligand 4 (Macrophage inflammatory protein 1-beta)	CCL4 (MIP1β)	3.3 ± 0.4	1.7 ± 0.4	12.8 ± 0.4	11.4 ± 0.5	8.1 ± 0.5	8.2 ± 0.4	1.9 ± 0.4	2.3 ± 0.4	- - -	1.6 ± 0.4
Chemokine (C-C motif) ligand 6	CCL6	2.5 ± 0.4	- - -	1.4 ± 0.4	1.7 ± 0.4	1.6 ± 0.5	1.7 ± 0.4	- - -	- - -	- - -	- - -
Chemokine (C-C motif) ligand 8 (Monocyte chemoattractant protein 2)	CCL8 (MCP2)	2.1 ± 0.5	- - -	- - -	- - -	- - -	- - -	1.6 ± 0.4	- - -	- - -	- - -
Chemokine (C-C motif) receptor 7	CCR7	- - -	- - -	- - -	- - -	3.5 ± 0.4	2.4 ± 0.5	3.1 ± 0.4	2.3 ± 0.5	1.5 ± 0.4	- - -
Chemokine (C-C motif) receptor-like 2	CCRL2	5.3 ± 0.4	3.3 ± 0.4	8.7 ± 0.4	11.6 ± 0.4	3.9 ± 0.4	3.7 ± 0.4	1.7 ± 0.4	1.8 ± 0.4	- - -	- - -
Chemokine (C-X3-C motif) ligand 1	CX3CL1	- - -	- - -	2.8 ± 0.4	5.0 ± 0.7	- - -	- - -	- - -	- - -	- - -	- - -
Chemokine (C-X-C motif) ligand 1 (Platelet-derived growth factor-inducible protein)	CXCL1 (KC)	16.0 ± 0.4	6.8 ± 0.5	34.1 ± 0.4	26.0 ± 0.4	12.9 ± 0.5	9.7 ± 0.4	5.3 ± 0.4	5.7 ± 0.4	1.7 ± 0.5	2.0 ± 0.4
Chemokine (C-X-C motif) ligand 10 (Gamma-IP10)	CXCL10 (IP-10)	14.7 ± .6	4.3 ± 0.5	40.5 ± 0.5	25.8 ± 0.4	187.4 ± 0.6	112.2 ± 0.4	40.2 ± 0.6	34.3 ± 0.4	5.0 ± 0.7	5.6 ± 0.4
Chemokine (C-X-C motif) ligand 11(Interferon-inducible T-cell alpha chemoattractant)	CXCL11 (ITAC)	- - -	- - -	3.9 ± 0.5	- - -	177.3 ± 0.5	198.1 ± 0.8	24.8 ± 0.5	41.6 ± 0.9	- - -	- - -
Chemokine (C-X-C motif) ligand 13 (B lymphocyte chemoattractant)	CXCL13 (BLC)	2.6 ± 0.5	- - -	- - -	1.9 ± 0.5	8.6 ± 0.5	4.9 ± 0.4	9.2 ± 0.4	8.0 ± 0.5	10.6 ± 0.4	8.3 ± 0.4
Chemokine (C-X-C motif) ligand 14	CXCL14	- - -	- - -	- - -	- - -	1.5 ± 0.4	- - -	2.3 ± 0.5	- - -	- - -	- - -
Chemokine (C-X-C motif) ligand 2 (Macrophage inflammatory protein 2)	CXCL2 (MIP2)	123.6 ± 0.4	56.9 ± 0.4	250.7 ± 0.4	215.3 ± 0.4	76.6 ± 0.5	66.7 ± 0.4	35.8 ± 0.5	28.2 ± 0.5	3.9 ± 0.4	5.1 ± 0.4
Chemokine (C-X-C motif) ligand 5 (lipopoly-saccharide induced C-X-C chemokine)	CXCL5 (LIX)	- - -	- - -	- - -	3.2 ± 0.7	4.1 ± 0.4	2.4 ± 0.5	- - -	- - -	- - -	- - -

Table 1 Cont'd,

Gene title	Gene symbol	30 min (LPS / Vehicle)		1 h (LPS / Vehicle)		6 h (LPS / Vehicle)		12 h (LPS / Vehicle)		24 h (LPS / Vehicle)	
		Nrf2 -/-	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*
Chemokine (C-X-C motif) ligand 9 (Gamma interferon induced monokine)	CXCL9 (MIG)	14.7 ± 0.5	- - -	11.7 ± 0.5	- - -	820.3 ± 0.5	576.0 ± 0.5	837.5 ± 0.5	739.3 ± 0.6	116.2 ± 0.7	68.6 ± 0.7
Colony stimulating factor 1 (macrophage)	CSF1	3.0 ± 0.4	2.2 ± 0.4	8.2 ± 0.4	7.0 ± 0.4	4.9 ± 0.4	4.9 ± 0.4	3.4 ± 0.4	3.9 ± 0.4	1.7 ± 0.4	2.0 ± 0.4
Colony stimulating factor 2 (granulocyte-macrophage)	CSF2	6.3 ± 0.8	- - -	70.5 ± 1.0	49.9 ± 0.5	65.8 ± 0.9	106.9 ± 0.4	12.5 ± 1.0	24.3 ± 0.5	- - -	- - -
Colony stimulating factor 3 (granulocyte)	CSF3	- - -	- - -	40.2 ± 0.5	27.5 ± 0.5	39.9 ± 0.6	20.1 ± 0.5	13.2 ± 0.6	10.8 ± 0.5	- - -	- - -
Interferon gamma	IFNG	- - -	- - -	- - -	- - -	7.5 ± 0.8	5.3 ± 0.9	- - -	- - -	- - -	- - -
Interleukin 1 alpha	IL1α	4.9 ± 0.6	2.2 ± 0.4	11.2 ± 0.6	6.2 ± 0.5	- - -	- - -	- - -	- - -	- - -	- - -
Interleukin 1 beta	IL1β	21.0 ± 0.4	17.6 ± 0.4	27.7 ± 0.4	40.8 ± 0.5	13.8 ± 0.4	14.3 ± 0.4	10.6 ± 0.4	11.8 ± 0.4	4.9 ± 0.4	6.7 ± 0.4
Interleukin 1 family, member 9	IL1F9	3.6 ± 0.6	1.8 ± 0.4	25.6 ± 0.4	19.0 ± 0.5	3.8 ± 0.4	3.7 ± 0.5	6.1 ± 0.4	5.9 ± 0.5	1.8 ± 0.4	2.1 ± 0.5
Interleukin 1 receptor antagonist	IL1RN	9.8 ± 0.6	5.0 ± 0.5	34.1 ± 0.4	36.3 ± 0.4	42.8 ± 0.4	38.9 ± 0.4	22.6 ± 0.4	23.3 ± 0.4	5.4 ± 0.5	6.2 ± 0.4
Interleukin 10	IL10	2.2 ± 0.4	- - -	2.2 ± 0.5	1.8 ± 0.4	2.7 ± 0.4	2.0 ± 0.4	4.3 ± 0.6	2.6 ± 0.4	- - -	- - -
Interleukin 12b	IL12β	1.8 ± 0.4	- - -	4.4 ± 0.4	3.1 ± 0.4	- - -	- - -	- - -	- - -	- - -	- - -
Interleukin 15 receptor, alpha chain	IL15Rα	- - -	- - -	- - -	- - -	4.3 ± 0.4	- - -	2.5 ± 0.5	1.9 ± 0.4	- - -	- - -
Interleukin 22	IL22	- - -	- - -	- - -	- - -	3.4 ± 0.8	- - -	- - -	- - -	- - -	- - -
Interleukin 23, alpha subunit p19	IL23p19	6.0 ± 0.5	- - -	8.1 ± 0.5	14.5 ± 0.5	- - -	- - -	- - -	- - -	- - -	- - -
Interleukin 6	IL6	171.3 ± 0.7	36.3 ± 0.9	362.0 ± 0.7	176.1 ± 0.9	97.7 ± 0.8	38.6 ± 0.9	25.5 ± 0.8	14.5 ± 0.9	5.2 ± 0.7	5.2 ± 0.8
Suppressor of cytokine signaling 1	SOCS1	- - -	- - -	1.9 ± 0.5	- - -	7.9 ± 0.6	7.9 ± 0.6	3.1 ± 0.6	2.2 ± 0.5	- - -	- - -
Suppressor of cytokine signaling 3	SOCS3	3.5 ± 0.4	2.5 ± 0.4	8.7 ± 0.4	7.0 ± 0.4	6.5 ± 0.4	5.3 ± 0.4	3.4 ± 0.4	3.1 ± 0.4	1.8 ± 0.4	2.0 ± 0.4
Tumor necrosis factor	TNF	39.4 ± 0.4	21.9 ± 0.5	24.3 ± 0.6	28.6 ± 0.4	29.4 ± 0.4	23.9 ± 0.4	18.3 ± 0.4	19.6 ± 0.4	7.8 ± 0.5	- - -
Tumor necrosis factor (ligand) superfamily, member 14	TNFSF14	- - -	- - -	- - -	- - -	3.4 ± 0.6	- - -	- - -	- - -	- - -	- - -
Tumor necrosis factor (ligand) superfamily, member 9	TNFSF9	10.8 ± 0.4	5.8 ± 0.5	16.1 ± 0.4	14.4 ± 0.4	2.4 ± 0.4	- - -	- - -	- - -	- - -	- - -

Values are mean fold change ± SE; ---, No change or less than 1.5 fold.

Table 2. Differential expression of transcripts for cell surface adhesion molecules and receptors associated with inflammation in the lungs of *nrf2* -deficient and wild-type mice following treatment with LPS.

Gene title	**Gene symbol**	**30 min (LPS / Vehicle)**		**1 h (LPS / Vehicle)**		**6 h (LPS / Vehicle)**		**12 h (LPS / Vehicle)**		**24 h (LPS / Vehicle)**	
		Nrf2 -/-	***Nrf2 +/+***	***Nrf2 -/-***	***Nrf2 +/+***	***Nrf2 -/-***	***Nrf2 +/+***	***Nrf2 -/-***	***Nrf2 +/+***	***Nrf2 -/-***	***Nrf2 +/+***
CD14 antigen	CD14	9.6 ± 0.4	3.7 ± 0.5	20.3 ± 0.4	14.6 ± 0.4	10.9 ± 0.4	7.7 ± 0.4	8.6 ± 0.4	5.9 ± 0.4	3.4 ± 0.4	3.4 ± 0.4
C-type lectin domain family 4, member d	CLEC4D	8.9 ± 0.5	3.6 ± 0.5	33.6 ± 0.4	28.2 ± 0.4	6.6 ± 0.4	5.9 ± 0.4	7 ± 0.4	5.7 ± 0.4	2.9 ± 0.4	3.5 ± 0.4
C-type lectin domain family 4, member e	CLEC4E	34.8 ± 0.5	15.9 ± 0.5	111.4 ± 0.4	93.1 ± 0.5	11.2 ± 0.4	9.3 ± 0.5	13.9 ± 0.4	11.2 ± 0.5	6.2 ± 0.4	8.5 ± 0.5
Complement component 5, receptor 1	C5R1	3.4 ± 0.5	- - -	7.8 ± 0.4	9.1 ± 0.4	5.4 ± 0.4	4.1 ± 0.4	5.4 ± 0.4	4.8 ± 0.4	3.2 ± 0.4	2.8 ± 0.4
Peptidoglycan recognition protein 1	PGLYRP1	2.1 ± 0.4	- - -	7.9 ± 0.4	4.0 ±0.5	4.8 ± 0.4	2.4 ± 0.5	6.6 ± 0.4	3.9 ± 0.5	4.2 ± 0.4	2.5 ± 0.5
Selectin, endothelial cell	SELE	37.8 ± 0.5	15.2 ± 0.5	69.6 ± 0.5	67.2 ± 0.5	4.7 ± 0.5	5.4 ± 0.5	3.8 ± 0.6	6.2 ± 0.5	- - -	- - -
Selectin, platelet	SELP	- - -	- - -	44.6 ± 0.7	17.4 ± 0.5	49.5 ± 0.7	26.2 ± 0.4	15.1 ± 0.9	10.6 ± 0.4	- - -	3.2 ± 0.5
Toll-like receptor 2	TLR2	4.2 ± 0.5	2.4 ± 0.4	11.6 ± 0.4	12.3 ± 0.4	7.0 ± 0.4	6.0 ± 0.4	3.3 ± 0.5	3.6 ± 0.4	2.0 ± 0.4	1.9 ± 0.4
Triggering receptor expressed on myeloid cells 1	TREM1	18.0 ± 0.6	4.7 ± 0.7	151.2 ± 0.4	121.9 ± 0.7	51.3 ± 0.4	45.6 ± 0.6	42.5 ± 0.4	19.7 ± 0.6	8.5 ± 0.5	2.9 ± 0.7
Triggering receptor expressed on myeloid cells 3	TREM3	3.9 ± 0.7	- - -	44.3 ± 0.6	52.7 ± 0.8	17.4 ± 0.7	27.1 ± 0.8	13.1 ± 0.7	17.9 ± 0.8	13.3 ± 0.6	17.8 ± 0.8
Urokinase plasminogen activator receptor	PLAUR	6.1 ± 0.4	3.2 ± 0.4	7.2 ± 0.4	6.0 ± 0.4	4.8 ± 0.4	4.3 ± 0.4	3.1 ± 0.4	2.7 ± 0.4	1.8 ± 0.4	1.6 ± 0.4
Vascular cell adhesion molecule 1	VCAM1	3.0 ± 0.4	1.9 ± 0.4	5.0 ± 0.4	4.9 ± 0.4	3.8 ± 0.4	3.2 ± 0.4	1.5 ± 0.4	1.9 ± 0.4	- - -	- - -

Values are mean fold change ± SE; ---, No change or less than 1.5 fold.

Table 3. Differential expression of genes associated with transcriptional regulation of inflammatory molecules in the lungs of *nrf2*-deficient and wild-type mice following treatment with LPS.

Gene title	Gene symbol	30 min (LPS / Vehicle)		1 h (LPS / Vehicle)		6 h (LPS / Vehicle)		12 h (LPS / Vehicle)		24 h (LPS / Vehicle)	
		Nrf2 -/-	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*
Stat											
Signal transducer and activator of transcription 4	STAT4	6.8 ± 1.0	- - -	5.1 ± 0.9	- - -	- - -	- - -	- - -	- - -	- - -	- - -
NF-κB related											
Ankyrin repeat domain 22	ANKRD22	- - -	- - -	34.1 ± 0.7	11.6 ± 0.4	- - -	- - -	- - -	- - -	- - -	- - -
Avian reticulo-endotheliosis viral (v-rel) oncogene related B	RELB	2.5 ± 0.4	1.5 ± 0.4	6.6 ± 0.4	4.3 ± 0.4	4.3 ± 0.4	3.2 ± 0.4	2.9 ± 0.4	2.6 ± 0.4	2.0 ± 0.4	1.8 ± 0.4
Reticuloendotheliosis oncogene	C-REL	3.5 ± 0.4	2.2 ± 0.4	7.3 ± 0.4	7.1 ± 0.4	- - -	- - -	- - -	- - -	- - -	- - -
B-cell leukemia/lymphoma 3	BCL3	3.0 ± 0.4	1.8 ± 0.4	8.5 ± 0.4	6.5 ± 0.4	9.1 ± 0.4	8.4 ± 0.4	3.5 ± 0.4	3.4 ± 0.4	1.6 ± 0.5	2.0 ± 0.4
CAMP responsive ele - ment binding protein 5	CREB5	2.5 ± 0.4	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -
CCAAT/enhancer binding protein (C/EBP), beta	CEBPB	4.9 ± 0.4	3.1 ± 0.4	6.4 ± 0.4	5.8 ± 0.4	5.6 ± 0.4	4.6 ± 0.4	4.4 ± 0.4	3.4 ± 0.4	2.4 ± 0.4	2.2 ± 0.4
Inhibitor of kappa b kinase epsilon	IKBKE	- - -	- - -	11.0 ± 0.5	4.5 ± 0.6	17.1 ± 0.5	11.0 ± 0.4	21.9 ± 0.5	17.1 ± 0.4	6.9 ± 0.5	6.8 ± 0.4
Interleukin-1 receptor-associated kinase 3	IRAK3	- - -	- - -	7.2 ± 0.4	4.0 ± 0.4	8.3 ± 0.4	5.9 ± 0.4	6.9 ± 0.4	6.0 ± 0.4	3.6 ± 0.4	3.6 ± 0.4
Max dimerization protein	MAD	5.5 ± 0.6	3.5 ± 0.4	17.3 ± 0.4	18.6 ± 0.4	13.1 ± 0.4	12.9 ± 0.4	7.2 ± 0.4	6.7 ± 0.5	1.8 ± 0.4	2.3 ± 0.4
Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta	NFKBIZ	20.5 ± 0.4	16.7 ± 0.4	22.5 ± 0.4	32.7 ± 0.3	6.0 ± 0.4	7.7 ± 0.4	4.2 ± 0.4	5.2 ± 0.4	1.9 ± 0.4	2.3 ± 0.4
Nuclear factor of kappa light polypeptide gene enhancer in B-cells 2, p49/p100	NFKB2	2.5 ± 0.4	2.2 ± 0.4	7.7 ± 0.4	4.9 ± 0.4	3.5 ± 0.4	2.8 ± 0.4	2.5 ± 0.4	2.3 ± 0.4	1.7 ± 0.4	1.8 ± 0.4
Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon	NFKBIE	3.2 ± 0.4	1.8 ± 0.4	5.9 ± 0.4	5.7 ± 0.4	3.7 ± 0.4	3.2 ± 0.4	2.8 ± 0.4	2.5 ± 0.4	1.7 ± 0.4	1.8 ± 0.4
TRAF family member-associated NF-kappa B activator	TANK	2.6 ± 0.4	1.9 ± 0.4	4.3 ± 0.4	5.7 ± 0.4	- - -	- - -	- - -	- - -	- - -	- - -
Interferon related											
Interferon activated gene 202B	IFI202B	2.5 ± 0.4	- - -	3.5 ± 0.5	1.9 ± 0.5	39.4 ± 0.4	21.0 ± 0.4	14.9 ± 0.4	8.7 ± 0.4	6.5 ± 0.4	4.8 ± 0.4
Interferon activated gene 204	IFI204	4.3 ± 0.4	- - -	4.8 ± 0.7	1.9 ± 0.5	31.8 ± 0.4	29.9 ± 0.4	12 ± 0.5	9.4 ± 0.4	7.1 ± 0.5	3.7 ± 0.4

Table 3 Cont'd

Gene title	Gene symbol	30 min (LPS / Vehicle)		1 h (LPS / Vehicle)		6 h (LPS / Vehicle)		12 h (LPS / Vehicle)		24 h LPS / Vehicle	
		Nrf2 -/-	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*
Interferon regulatory factor 1	IRF1	5.7 ± 0.4	4.2 ± 0.4	4.5 ± 0.4	3.7 ± 0.4	4.9 ± 0.4	4.5 ± 0.4	2.5 ± 0.4	2.4 ± 0.4	- - -	- - -
Interferon regulatory factor 5	IRF5	1.7 ± 0.4	- - -	2.4 ± 0.4	1.7 ± 0.4	3.8 ± 0.4	3.1 ± 0.4	2.5 ± 0.4	2.2 ± 0.4	2.2 ± 0.4	2.1 ± 0.4
Interferon regulatory factor 7	IRF7	- - -	- - -	1.9 ± 0.4	- - -	22.6 ± 0.4	15.6 ± 0.4	16.3 ± 0.4	13.1 ± 0.4	7.7 ± 0.5	6.0 ± 0.4
Interferon-induced protein 44	IFI44	- - -	- - -	- - -	- - -	17.9 ± 0.4	10.6 ± 0.4	6.6 ± 0.4	5.5 ± 0.4	3.1 ± 0.4	1.8 ± 0.4
Interferon-induced protein with tetra-tricopeptide repeats 2 (ISG54)	IFIT2	- - -	- - -	- - -	- - -	39.9 ± 0.4	23.1 ± 0.4	11.8 ± 0.6	8.2 ± 0.5	2.5 ± 0.5	2.1 ± 0.4
Interferon-induced protein with tetra-tricopeptide repeats 3 (GARG-49)	IFIT3	- - -	- - -	- - -	- - -	18.4 ± 0.4	9.9 ± 0.4	6.3 ± 0.4	5.8 ± 0.4	2.9 ± 0.5	2.4 ± 0.4
Myxovirus (influenza virus) resistance 1	Mx1	---	- - -	- - -	2.1 ± 0.5	49.9 ± 0.4	23.8 ± 0.4	6.9 ± 0.7	4.7 ± 0.4	2.1 ± 0.4	1.9 ± 0.5
Stat											
Signal transducer and activator of transcription 4	STAT4	6.8 ± 1.0	- - -	5.1 ± 0.9	- - -	- - -	- - -	- - -	- - -	- - -	- - -
Other transcription factors											
Early growth response 2	EGR2	8.5 ± 0.4	6.5 ± 0.4	6.1 ± 0.4	5.6 ± 0.4	- - -	- - -	- - -	- - -	- - -	- - -
Early growth response 3	EGR3	84.4 ± 0.4	71.0 ± 0.4	44 ± 0.4	67.6 ± 0.4	- - -	- - -	- - -	- - -	- - -	- - -
Spi-C transcription factor (Spi-1/PU.1 related)	SPIC	- - -	- - -	- - -	- - -	31.8 ± 1.0	19.2 ± 0.6	20.0 ± 0.8	21.4 ± 0.5	35.0 ± 0.8	35.0 ± 0.5
TGFB-induced factor 2	TGIF2	8.1 ± 0.4	4.1 ± 0.8	7.0 ± 0.5	10.9 ± 0.5	- - -	- - -	- - -	- - -	- - -	- - -
Transcription factor E3	TCFE3	1.4 ± 0.4	- - -	2.1 ± 0.3	- - -	- - -	- - -	- - -	- - -	- - -	- - -
Transforming growth factor, beta induced	TGFBI	1.5 ± 0.4	- - -	1.5 ± 0.4	1.5 ± 0.4	2.1 ± 0.4	2.4 ± 0.4	2.8 ± 0.4	2.5 ± 0.4	3.1 ± 0.4	3.3 ± 0.4
V-maf musculo-aponeurotic fibro-sarcoma oncogene family, protein F (avian)	MAFF	5.5 ± 0.4	3.5 ± 0.4	8.5 ± 0.4	7.0 ± 0.4	6.1 ± 0.4	5.4 ± 0.4	5.1 ± 0.4	4.0 ± 0.4	- - -	- - -

Values are mean fold change ± SE; ---, No change or less than 1.5 fold.

Table 4. Differential expression of mebers of immunoglobulin and MHC class II family in the lungs of *nrf2* -deficient and wild-type mice 30 min after LPS challenge.

Gene name	Gene symbol	*Nrf2 -/-*, LPS / Vehicle	*Nrf2 +/+*, LPS / Vehicle
Histocompatibility 2, class II antigen A, alpha	H2-Aα	1.6 ± 0.4	- - -
Histocompatibility 2, class II antigen A, beta 1	H2-Aβ1	2.0 ± 0.4	- - -
Histocompatibility 2, class II antigen E alpha	H2-Eα	5.1 ± 0.7	- - -
Histocompatibility 2, class II, locus dma	H2-DMA	2.3 ± 0.4	- - -
Histocompatibility 2, class II, locus Mb1	H2-DMB1	2.3 ± 0.4	- - -
Histocompatibility 2, class II, locus Mb2	H2-DMB2	1.6 ± 0.4	- - -
Immunoglobulin heavy chain (gamma polypeptide)	IGHγ	12.9 ± 0.7	- - -
Immunoglobulin heavy chain (J558 family)	IGH-VJ558	4.7 ± 0.4	- - -
Immunoglobulin heavy chain 4 (serum igg1)	IGH-4	38.9 ± 1.0	- - -
Immunoglobulin heavy chain 6 (heavy chain of igm)	IGH-6	29.7 ± 0.8	2.1 ± 0.4
Immunoglobulin joining chain	IGJ	7.5 ± 0.5	- - -
Immunoglobulin kappa chain variable 21 (V21)	IGK-V21	9.9 ± 0.6	- - -
Immunoglobulin kappa chain variable 32 (V32)	IGK-V32	13.9 ± 0.9	- - -
Immunoglobulin kappa chain variable 8 (V8)	IGK-V8	4.1 ± 0.4	- - -
Immunoglobulin lambda chain, variable 1	IGL-V1	3.7 ± 0.7	- - -
Immunoglobulin superfamily, member 6	IGSF6	10.3 ± 0.5	4.3 ± 0.5
Ig kappa chain	IGM	6.7 ± 0.5	- - -

Values are mean fold change ± SE; ---, No change or less than 1.5 fold.

Table 5. Differential expression of genes encoding acute phase proteins in the lungs of *nrf2*-deficient and wild-type mice following treatment with LPS.

Gene title	Gene symbol	30 min (LPS / Vehicle)		1 h (LPS / Vehicle)		6 h (LPS / Vehicle)		12 h (LPS / Vehicle)		24 h (LPS / Vehicle)	
		Nrf2 -/-	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*
Heat shock protein 1A	HSPA1A	30.1 ± 0.4	23.3 ± 0.5	2.8 ± 0.5	1.5 ± 0.4	- - -	- - -	- - -	- - -	- - -	1.7 ± 0.4
Heat shock protein 8	HSPA8	2.1 ± 0.4	4.3 ± 0.5	1.5 ± 0.4	- - -	- - -	- - -	- - -	- - -	1.7 ± 0.4	2.4 ± 0.4
Metallothionein 2	MT2	1.8 ± 0.5	- - -	5.6 ± 0.5	3.6 ± 0.4	8.5 ± 0.5	6.2 ± 0.4	7.5 ± 0.5	5.2 ± 0.4	2.0 ± 0.6	1.6 ± 0.4
Orosomucoid 1	ORM1	- - -	- - -	1.6 ± 0.5	- - -	22.9 ± 0.4	14.8 ± 0.7	21.1 ± 0.5	12.0 ± 0.7	3.1 ± 0.6	5.1 ± 0.7
Orosomucoid 2	ORM2	- - -	- - -	- - -	- - -	6.0 ± 0.4	3.8 ± 0.6	7.2 ± 0.5	3.8 ± 0.5	3.5 ± 0.5	3.3 ± 0.5
Serine (or cysteine) proteinase inhibitor, clade A, member 1a	SERPINA1A	- - -	- - -	- - -	- - -	- - -	- - -	- - -	43.1 ± 0.5	- - -	- - -
Serine (or cysteine) proteinase inhibitor, clade A, member 3C	SERPINA3C	- - -	- - -	1.8 ± 0.5	- - -	6.7 ± 0.4	8.2 ± 0.5	3.6 ± 0.7	3.3 ± 0.5	- - -	1.6 ± 0.4
Serine (or cysteine) proteinase inhibitor, clade A, member 3G	SERPINA3G	1.9 ± 0.5	- - -	3.2 ± 0.5	1.5 ± 0.4	14.7 ± 0.4	9.4 ± 0.4	10.1 ± 0.4	7.0 ± 0.4	2.6 ± 0.5	- - -
Serine (or cysteine) proteinase inhibitor, clade A, member 3M	SERPINA3M	- - -	- - -	- - -	- - -	8.0 ± 0.4	5.7 ± 0.4	10.9 ± 0.5	3.5 ± 0.4	3.2 ± 0.5	2.0 ± 0.4
Serine (or cysteine) proteinase inhibitor, clade A, member 3N	SERPINA3N	- - -	- - -	4.2 ± 0.6	3.7 ± 0.6	11.2 ± 0.5	31.3 ± 0.4	12.5 ± 0.5	30.7 ± 0.4	6.7 ± 0.5	16.3 ± 0.4
Serine (or cysteine) proteinase inhibitor, clade B, member 2	SERPINB2	14.3 ± 0.6	- - -	18.5 ± 0.5	10.1 ± 0.6	5.0 ± 0.6	2.1 ± 0.5	3.9 ± 0.7	- - -	2.9 ± 0.6	- - -
Serine (or cysteine) proteinase inhibitor, clade E, member 1	SERPINE1	10.9 ± 0.4	8.1 ± 0.4	32.4 ± 0.4	24.3 ± 0.4	23.8 ± 0.4	23.8 ± 0.4	9.3 ± 0.5	15.7 ± 0.4	2.3 ± 0.5	3.8 ± 0.5
Serum amyloid A 1	SAA1	- - -	- - -	3.1 ± 0.5	- - -	93.1 ± 0.4	95.7 ± 0.5	66.3 ± 0.4	76.6 ± 0.5	23.4 ± 0.4	32.7 ± 0.5
Serum amyloid A 2	SAA2	- - -	- - -	- - -	- - -	28.1 ± 0.4	19.8 ± 0.4	16.2 ± 0.4	12.5 ± 0.4	5.1 ± 0.5	- - -
Serum amyloid A 3	SAA3	3.0 ± 0.5	- - -	18.0 ± 0.4	4.0 ± 0.9	85.6 ± 0.4	25.5 ± 0.8	90.5 ± 0.5	24.9 ± 0.8	61.0 ± 0.4	22 ± 0.8

Values are mean fold change ± SE; ---, No change or less than 1.5 fold.

Table 6. Differential expression of selected genes that modulate inflammation in the lungs of *nrf2* -deficient and wild-type mice following treatment with LPS.

Gene title	Gene symbol	30 min (LPS / Vehicle)		1 h (LPS / Vehicle)		6 h (LPS / Vehicle)		12 h (LPS / Vehicle)		24 h (LPS / Vehicle)	
		Nrf2 -/-	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*
Arginase II	ARG2	4.1 ± 0.4	1.8 ± 0.4	7.0 ± 0.4	7.5 ± 0.4	7.0 ± 0.4	5.2 ± 0.4	4.6 ± 0.4	2.9 ± 0.4	1.8 ± 0.4	1.5 ± 0.4
Immune-responsive gene 1	IRG1	286.0 ± 0.6	29.0 ± 0.8	1858.0 ± 0.4	1082.0 ± 0.4	552.0 ± 0.4	304.0 ± 0.5	313.0 ± 0.4	183.5 ± 0.7	53.0 ± 0.4	64.1 ± 0.5
Indoleamine-pyrrole 2,3 dioxygenase	INDO	2.2 ± 0.5	- - -	- - -	- - -	25.6 ± 0.6	19.8 ± 0.5	9.3 ± 0.5	8.5 ± 0.6	- - -	- - -
Neutrophil cytosolic factor 1	NCF1	4.9 ± 0.5	2.0 ± 0.4	16.3 ± 0.4	13.5 ± 0.4	5.8 ± 0.4	4.3 ± 0.4	6.6 ± 0.4	4.7 ± 0.4	2.8 ± 0.4	2.4 ± 0.4
Neutrophil cytosolic factor 4	NCF4	2.7 ± 0.4	- - -	5.7 ± 0.4	4.7 ± 0.4	5 ± 0.3	4.1 ± 0.4	6.2 ± 0.3	4.8 ± 0.4	4.0 ± 0.4	3.9 ± 0.4
Nitric oxide synthase 2, inducible, macrophage	NOS2	- - -	- - -	- - -	- - -	14.7 ± 0.5	7.9 ± 0.6	- - -	- - -	- - -	- - -
Pleckstrin	PLEK	4.3 ± 0.4	2.5 ± 0.4	9.6 ± 0.4	10.3 ± 0.4	3.3 ± 0.4	3.1 ± 0.4	2.2 ± 0.4	2.4 ± 0.4	2.0 ± 0.4	2.1 ± 0.4
Properdin factor, complement	PFC	2.6 ± 0.5	- - -	2.6 ± 0.5	2.4 ± 0.4	3.0 ± 0.5	2.3 ± 0.4	3.6 ± 0.5	2.5 ± 0.4	5.5 ± 0.5	3.8 ± 0.4

Values are mean fold change ± SE; ---, No change or less than 1.5 fold.

Table 7. Antioxidative genes that are constitutively elevated in the lungs of wild-type compared to *nrf2* -deficient mice.

Gene name (Gene symbol)	**Vehicle, *Nrf2* +/+ // *Nrf2* -/-**	**LPS, *Nrf2* +/+ // *Nrf2* -/-**				
		30 min	**1 h**	**6 h**	**12 h**	**24 h**
Glutamate-cysteine ligase, catalytic subunit (GCLC)	2.1 ± 0.4	- - -	1.9 ± 0.4	1.7 ± 0.5	1.6 ± 0.4	2.1 ± 0.4
Glutathione peroxidase 2 (GPX2)	5.3 ± 0.5	4.8 ± 0.5	4.4 ± 0.5	3.4 ± 0.6	2.3 ± 0.5	4.0 ± 0.7
Glutathione S-transferase, alpha 3 (GSTA3)	2.6 ± 0.4	3.3 ± 0.4	2.5 ± 0.4	2.7 ± 0.5	4.0 ± 0.5	2.4 ± 0.4
Glutathione S-transferase, alpha 4 (GSTA4)	1.7 ± 0.4	- - -	1.5 ± 0.4	- - -	- - -	- - -
Glutathione S-transferase, mu 1 (GSTM1)	2.4 ± 0.4	2.6 ± 0.4	2.4 ± 0.3	1.9 ± 0.4	1.7 ± 0.4	1.5 ± 0.4
Glutathione S-transferase, mu 2 (GSTM2)	1.6 ± 0.4	1.9 ± 0.3	1.6 ± 0.3	- - -	1.5 ± 0.4	- - -
Malic enzyme, supernatant (MOD1)	1.9 ± 0.8	1.9 ± 0.3	1.8 ± 0.4	1.5 ± 0.4	1.5 ± 0.4	1.6 ± 0.4
Catalase (CAT)	- - -	- - -	- - -	- - -	- - -	3.3 ± 0.5
Thioredoxin reductase 1 (TXNRD1)	1.8 ± 0.4	- - -	- - -	- - -	- - -	- - -

Values are mean fold change ± SE; ---, No change or less than 1.5 fold.

Table 8. Validation by real time-PCR of selected LPS inducible genes identified by microarray analysis in the lungs of mice of both genotypes challenged with LPS.

Gene symbol	*Nrf2* -/-, LPS / Vehicle										*Nrf2* +/+, LPS / Vehicle									
	30 min		1 h		6 h		12 h		24 h		30 min		1 h		6 h		12 h		24 h	
	Real Time PCR	Micro-array	Real Time PCR	Micro-array	Real Time PCR	Micro-array	Real Time PCR	Micro-array	Real Time PCR	Micro-array	Real Time PCR	Micro-array	Real Time PCR	Micro-array	Real Time PCR	Micro-array	Real Time PCR	Micro-array	Real Time PCR	Micro-array
CCL12/MCP5	18.1	19.7	27.8	27.7	15.3	19.3	18.3	19.6	25.3	29.2	6.5	7.5	11.3	12.5	7.2	8.6	6.5	15.0	15.2	14.9
CCL2/MCP1	7.2	6.3	23.1	24.8	15.6	20.4	7.6	6.0	1.3	4.7	1.8	1.8	4.8	4.2	6.3	7.0	6.8	7.1	1.3	1.2
CD14	8.1	9.6	22.3	20.3	12.3	10.9	7.5	8.6	2.6	3.4	4.3	3.7	11.3	14.6	7.8	7.7	4.5	5.9	2.4	3.4
CSF2	7.2	6.3	58.6	70.5	44.6	65.8	12.8	12.5	1.4	1.3	1.3	1.2	38.2		75.8	49.9	20.4	106.9	1.5	24.3
CXCL2/ MIP2	75.2	123.6	210.2	250.0	48.3	76.6	25.6	35.8	4.1	3.9	32.1	56.9	175.2	215.3	36.2	66.7	26.3	28.2	4.7	5.1
H2-Eα	3.9	5.1	1.2	---	0.7	---	0.5	---	0.4	---	3.0	---	1.0	---	0.5	---	0.7	---	0.4	---
IGH-4	12.9	38.9	0.5	---	0.3	---	0.2	---	0.4	---	3.0	---	0.9	---	0.6	---	0.7	---	1.1	---
IHSF6	10.5	10.3	15.2		3.2		4.1		3.2		3.1		10.6		2.8		4.0		2.6	
IL-1α	5.1	4.9	9.8	11.2	1.6	---	1.6	---	1.3	---	1.3	---	1.9	---	1.32	---	0.8	---	0.9	---
IL-6	99.1	171.3	202.1	362.0	70.0	97.7	7.94	25.5	2.28	5.2	30.2	36.3	140.6	176.1	20.9	38.6	20.5	14.5	2.9	5.2
IRG1	486.3	286.0	2548.4	1858.4	370.5	552.0	208.9	313.0	63.2	53.0	64.6	29	2100.0	1082.0	332.4	304	170.8	183.5	73.3	64.1
STAT4	3.6	6.8	3.0	5.1	0.8	---	0.7	---	0.8	---	1.5	---	1.8	---	0.7	---	0.6	---	0.7	---
TNFα	35.2	39.4	21.1	24.3	25.3	29.6	16.5	18.3	6.4	7.8	21.3	21.9	19.5	28.6	23.1	23.9	17.2	19.6	1.3	---

Values are the ratio of mean fold change of LPS treatment to vehicle control (*n*=3).

Table 9. Differential expression of IRF3 regulated genes in lungs of *nrf2*-deficient and wild-type mice after LPS stimulus.

Gene title	Gene symbol	30 min (LPS / Vehicle)		1 h (LPS / Vehicle)		6 h (LPS / Vehicle)		12 h (LPS / Vehicle)		24 h LPS / Vehicle	
		Nrf2 -/-	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*	*Nrf2 -/-*	*Nrf2 +/+*
Chemokine (C-X-C motif) ligand 10 (Gamma-IP10)	CXCL10 (IP-10)	14.7 ± .6	4.3 ± 0.5	40.5 ± 0.5	25.8 ± 0.4	187.4 ± 0.6	112.2 ± 0.4	40.2 ± 0.6	34.3 ± 0.4	5.0 ± 0.7	5.6 ± 0.4
Chemokine (C-X-C motif) ligand 11(Interferon-inducible T-cell alpha chemoattractant)	CXCL11 (ITAC)	- - -	- - -	3.9 ± 0.5	- - -	177.3 ± 0.5	198.1 ± 0.8	24.8 ± 0.5	41.6 ± 0.9	- - -	- - -
Chemokine (C-X-C motif) ligand 9 (Gamma inter- feron induced monokine)	CXCL9 (MIG)	14.7 ± 0.5	- - -	11.7 ± 0.5	- - -	820.3 ± 0.5	576.0 ± 0.5	837.5 ± 0.5	739.3 ± 0.6	116.2 ± 0.7	68.6 ± 0.7
Epstein-Barr virus induced gene 3	Ebi3	- - -	- - -	9.6 ± 0.4	12.2 ± 0.4	8.8 ± 0.4	6.2 ± 0.4	8.2 ± 0.4	6.7 ± 0.4	4.2 ± 0.5	4.0 ± 0.4
Immune-responsive gene 1	IRG1	286.0 ± 0.6	1858 ± 0.4	552 ± 0.4	313 ± 0.4	53 ± 0.4	29 ± 0.8	1082 ± 0.4	304 ± 0.5	183.5 ± 0.7	64.1 ± 0.5
Interferon activated gene 202B	IFI202B	2.5 ± 0.4	- - -	3.5 ± 0.5	1.9 ± 0.5	39.4 ± 0.4	21.0 ± 0.4	14.9 ± 0.4	8.7 ± 0.4	6.5 ± 0.4	4.8 ± 0.4
Interferon activated gene 204	IFI204	4.3 ± 0.4	- - -	4.8 ± 0.7	1.9 ± 0.5	31.8 ± 0.4	29.9 ± 0.4	12 ± 0.5	9.4 ± 0.4	7.1 ± 0.5	3.7 ± 0.4
Interferon regulatory factor 1	IRF1	5.7 ± 0.4	4.2 ± 0.4	4.5 ± 0.4	3.7 ± 0.4	4.9 ± 0.4	4.5 ± 0.4	2.5 ± 0.4	2.4 ± 0.4	- - -	- - -
Interferon regulatory factor 5	IRF5	1.7 ± 0.4	- - -	2.4 ± 0.4	1.7 ± 0.4	3.8 ± 0.4	3.1 ± 0.4	2.5 ± 0.4	2.2 ± 0.4	2.2 ± 0.4	2.1 ± 0.4
Interferon regulatory factor 7	IRF7	- - -	- - -	1.9 ± 0.4	- - -	22.6 ± 0.4	15.6 ± 0.4	16.3 ± 0.4	13.1 ± 0.4	7.7 ± 0.5	6.0 ± 0.4
Interferon-induced protein 44	IFI44	- - -	- - -	- - -	- - -	17.9 ± 0.4	10.6 ± 0.4	6.6 ± 0.4	5.5 ± 0.4	3.1 ± 0.4	1.8 ± 0.4
Interferon-induced protein with tetra-tricopeptide repeats 2 (ISG54)	IFIT2	- - -	- - -	- - -	- - -	39.9 ± 0.4	23.1 ± 0.4	11.8 ± 0.6	8.2 ± 0.5	2.5 ± 0.5	2.1 ± 0.4
Interferon-induced protein with tetra-tricopeptide repeats 3 (GARG-49)	IFIT3	- - -	- - -	- - -	- - -	18.4 ± 0.4	9.9 ± 0.4	6.3 ± 0.4	5.8 ± 0.4	2.9 ± 0.5	2.4 ± 0.4
Myxovirus (influenza virus) resistance 1	Mx1	---	- - -	- - -	2.1 ± 0.5	49.9 ± 0.4	23.8 ± 0.4	6.9 ± 0.7	4.7 ± 0.4	2.1 ± 0.4	1.9 ± 0.5

Values are mean fold change ± SE; ---, No change or less than 1.5 fold.

Figure 1

Serum levels of p55 and p75 analyzed by ELISA (R & D Systems) in *nrf2*-deficient and wild-type mice after 6h of treatment with either vehicle and or LPS (1.5 mg/mouse). * Differs from vehicle control of the same genotype; $P<0.05$. ND, Not detected.

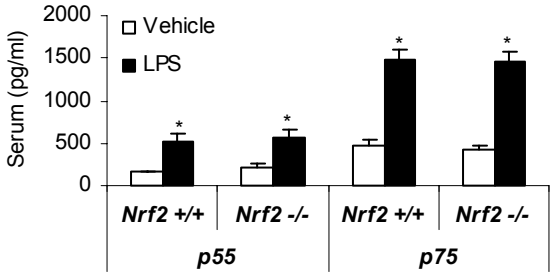


Figure 2

Constitutive protein levels of TLR4 **(A)** and CD14 **(B)** in whole cell extracts obtained from peritoneal macrophages of *nrf2*-/- and *nrf2* +/+ mice by immunoblot analysis. Immunoblot analysis was performed as described in the methods section using antibodies specific for the TLR4 and CD14.

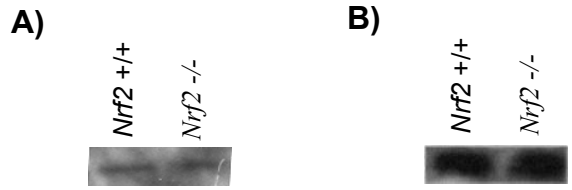
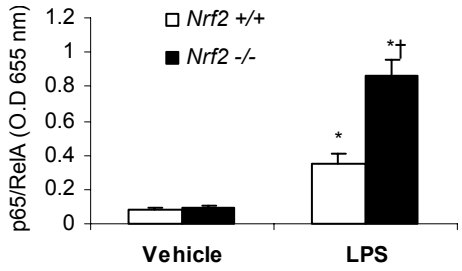


Figure 3 **(A)** DNA binding activity assay showed the increased binding of p65/Rel A subunit from the lung nuclear extracts obtained from LPS treated nrf2 -/- mice to an NF-κB binding sequence compared with its wild-type counterpart. (**B)** In response to LPS or TNF-α treatment, nuclear extracts from *nrf2 -/-* MEFs showed increased binding of p65/Rel A subunit to NF-κB binding sequence when compared to wild-type MEFs.

A



B