Genomic and Lipidomic Analyses Differentiate the Compensatory Roles of Two Cyclooxygenase Isoforms during Systemic Inflammation in Mice

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Running title: Differential compensation of COX isoforms in inflammation

Supplemental Table S1: Primers used in quantitative real-time PCR reactions

Gene	Protein	Primer (5’ to 3’)
Rn18s	18S rRNA	F: CTTAGAGGGACAAGTGGCG
		R: ACGCTGAGCCAGTCAGTGTA
Ptgis	PGIS	F: ACAGCATCAAACAATTTGTCGTC
		R: GCATCAGACCGAAGCCATATCT
Ptges	mPGES-1	F:ACGACATGGAGACAATCTATCCT
		R:TGAGGACAACGAGGAAATGT
Hpgds	H-PGDS	F: TGGCCTAAAATCAAGCCAAC
		R: CCACTGCATCAGCTTGACAT
Lpgds	L-PGDS	F: GGTTCCGGGAGAAGAAAGCT
		R: CACTGACACGGAGTGGATGC
Akr1b3	AKR1B3 (PGFS)	F: TCGTGGTGACAGCATATAGTCC
		R: CCAGGAGAGACGGATCTTCA
Tbxas1	TBXAS1	F: TACCATAGTGACTGTGACTCTGC
		R: GGTGCCTGATGCCCAACTT
Il1b	IL-1β	F: TGAAGTTGACGGACCCCAAA
		R: TGATGTGCTGCTGCGAGATT
Il6	IL-6	F: TAGTCCTTCCTACCCCAATTTCC
		R: TTGGTCCTTAGCCACTCCTTC
Ccl2	CCL2 (MCP-1)	F: TTAAAAACCTGGATCGGAACCAA
		R: GCATTAGCTTCAGATTTACGGGT
Ccl3	CCL3 (MIP-1α)	F: CTGCAACCAAGTCTTCTCAGC
		R: CTGCCTCCAAGACTCTCAGG

F, forward; R, reverse.

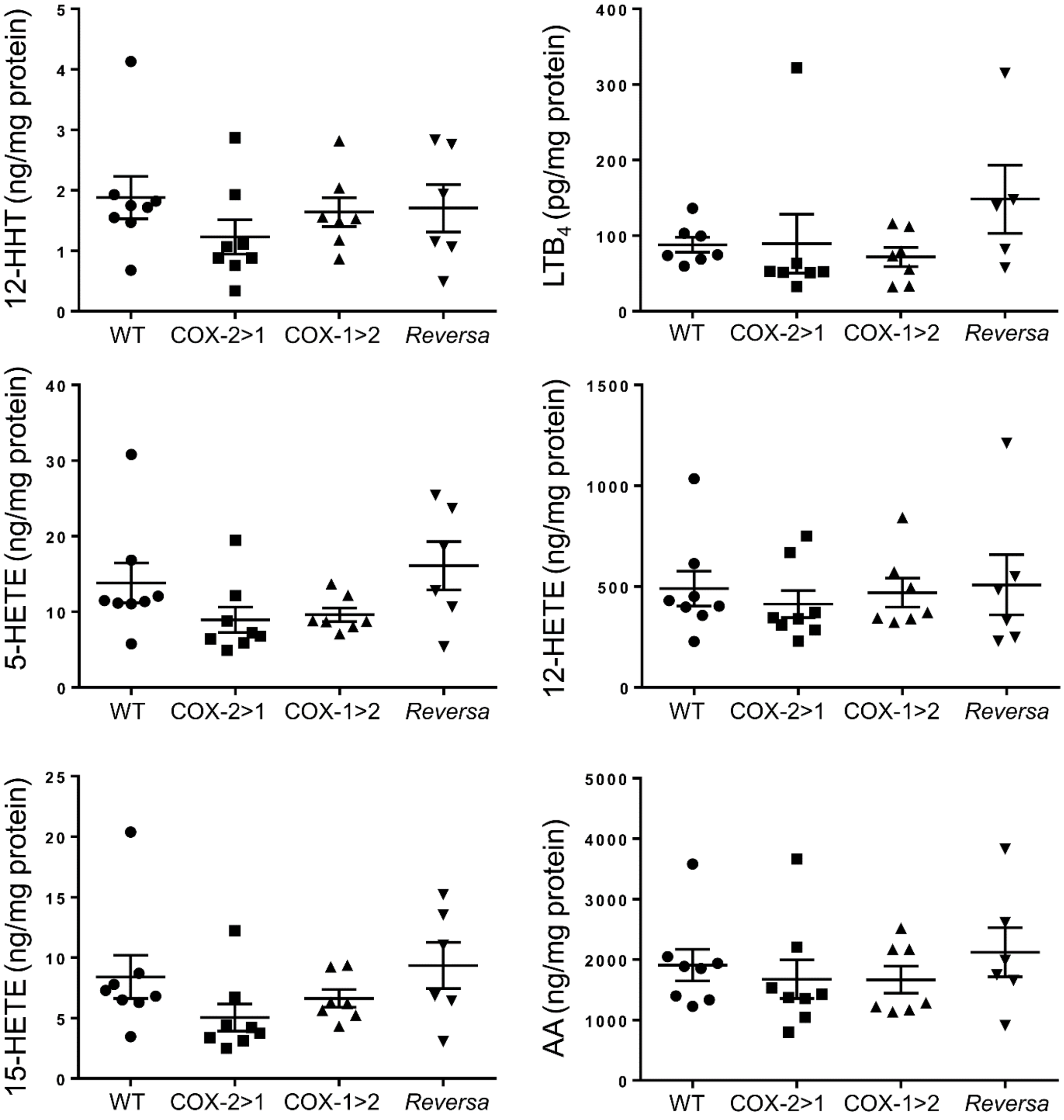
Supplemental Table S2. Fold change of the differentially expressed genes detected via Nanostring analysis in macrophages (n=6)

Gene Name	Accession #	WT vs. Control	COX-2>COX-1 vs. Control	COX-1>COX-2 vs. Control	*Reversa* vs. Control
Cxcl10	NM_021274.1	16501.61	20069.46	12677.09	12043.35
Csf3	NM_009971.1	2503.34	2550.76	2263.88	1801.37
Cxcl3	NM_203320.2	2796.8	2447.54	1483.52	1677.35
Ccl4	NM_013652.1	1401.6	2014.9	1321.53	2023.95
Nos2	NM_010927.3	1765.59	1557.01	1235.49	1261.36
Ccl7	NM_013654.2	674.78	946.78	683.22	957.95
Ccl3	NM_011337.1	429.08	456.7	408.77	629.49
Ifng	NM_008337.1	342.12	309.33	242.29	167.84
Il1rn	NM_031167.4	279.91	357.64	237.32	195.75
Il22	NM_016971.1	390.44	699.34	237.28	824.19
Cxcl9	NM_008599.2	280.17	226.03	180.79	69.48
Il17a	NM_010552.3	245.89	466.97	156.77	1080.49
Il6	NM_031168.1	146.06	147.64	130.31	169.35
Il1b	NM_008361.3	176.24	184.71	115.15	176.39
Oasl1	NM_145209.2	110.71	149.13	112.28	96.73
Il1a	NM_010554.4	145.93	132.35	104.34	142.97
Il10	NM_010548.1	89.68	190.48	94.48	128.63
Cxcl1	NM_008176.1	129.93	134.49	90.75	81.86
Ccl2	NM_011333.3	88.27	110.15	77.34	125.72
Ifit1	NM_008331.2	81.33	99.23	60.45	77.08
Tlr3	NM_126166.2	61.8	52.68	50.31	35.09
Cxcl2	NM_009140.2	68.87	67.1	50	53.7
Iigp1	NM_021792.3	47.94	44.62	40.09	36.1
Ifi44	NM_133871.2	68.79	79.99	38.46	35.8
Cxcr2	NM_009909.3	32.54	30.93	35.96	48.85
Ccl22	NM_009137.2	42.4	69.8	35.87	72.58
Tnfaip3	NM_009397.2	33.87	34.09	27.78	29.91
Ifit2	NM_008332.2	33.42	47.81	27.59	26.4

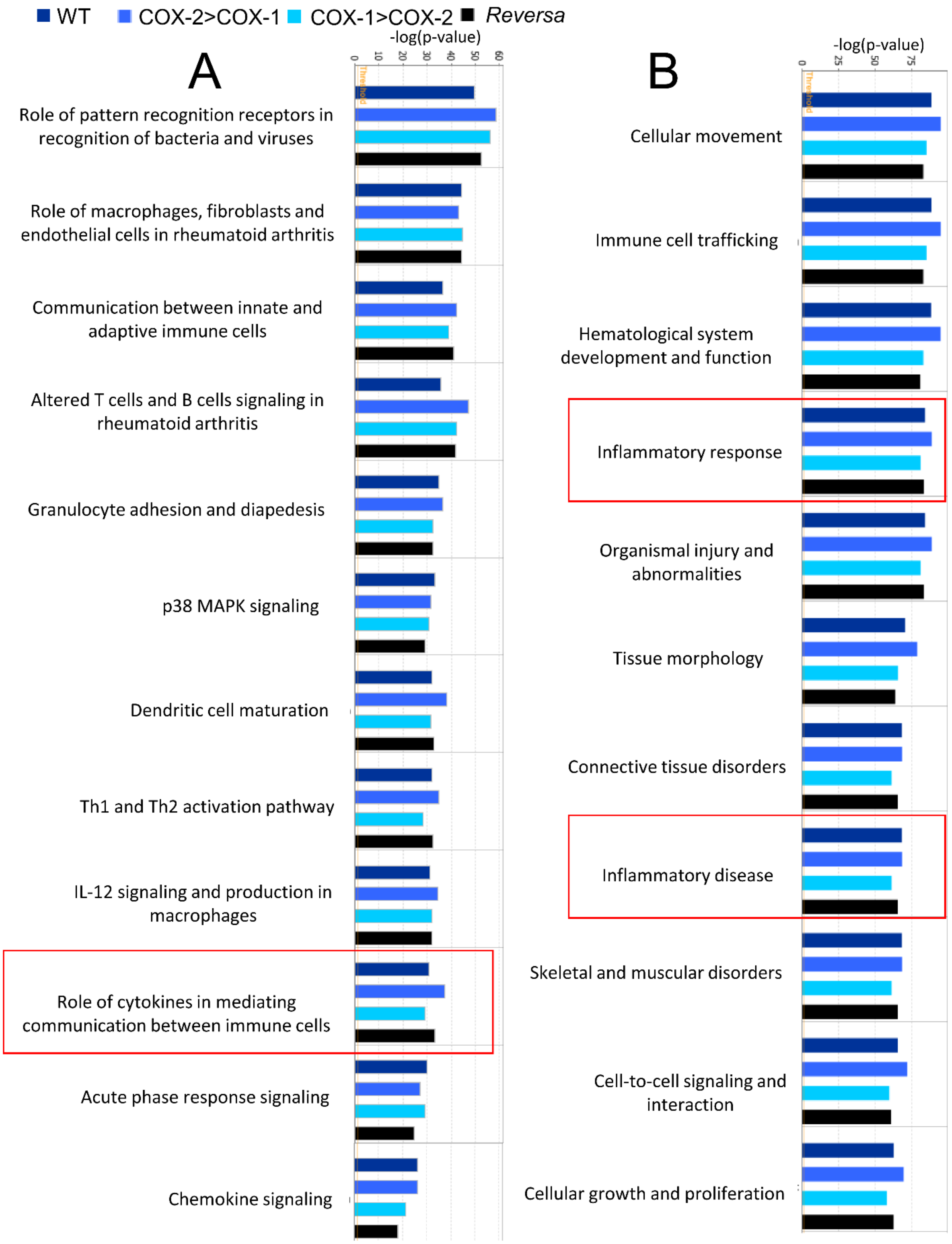
Ifit3	NM_010501.1	27.85	41.59	27.49	25.71
Cxcl5	NM_009141.2	45.07	42.22	24.45	21.65
Flt1	NM_010228.3	15.55	11.91	23.77	26.17
Ccl8	NM_021443.2	33.03	30.13	21.64	40.1
Maff	NM_010755.3	17.33	20.54	20.09	18.69
Irf7	NM_016850.2	18.78	19.73	18.7	16.79
Mef2b	NM_001045484.1	14.01	20.13	18.62	12.5
Ccl5	NM_013653.1	27.06	26.62	18.4	12.43
Tnf	NM_013693.1	10.2	10.24	14.76	16.57
Mx2	NM_013606.1	15.06	22.54	13.57	14.58
Il13	NM_008355.2	5.01	9.61	13.39	26.2
C1s	NM_144938.2	11.78	7.15	9.57	7.49
Areg	NM_009704.3	9.8	13.59	9.28	21.64
Mx1	NM_010846.1	12.66	11.57	8.63	9.3
Nlrp3	NM_145827.3	10.31	7.66	7.68	6.51
Il5	NM_010558.1	7.6	15.58	7.46	40.51
Csf2	NM_009969.4	16.8	16.99	7.38	34.97
Cfb	NM_008198.2	7.99	6.36	6.97	5.23
Ccl17	NM_011332.2	8.13	9.65	6.62	6.5
Oas1a	NM_145211.2	6.06	6.15	6.11	4.97
Daxx	NM_007829.3	6.21	6.13	5.74	5.48
Hif1a	NM_010431.2	6.53	6.81	5.64	5.48
Irf1	NM_008390.1	6.19	5.61	5.54	5.41
Jun	NM_010591.2	4.58	5.12	4.82	5.85
Tgfb3	NM_009368.2	8.13	11.24	4.39	6.75
Il15	NM_008357.1	4.16	4.13	4.31	3.24
Csf1	NM_001113530.1	3.95	3.38	3.8	4.02
Il23r	NM_144548.1	5.74	4.41	3.73	5.97
Cd40	NM_011611.2	3.79	4.02	3.67	3.54
Pla2g4a	NM_008869.2	4.41	4.18	3.64	3.33
Il4	NM_021283.1	2.29	2.98	3.6	5.84
Nod2	NM_145857.2	3.24	2.27	3.57	3.16

Il12b	NM_008352.1	3.4	5.79	3.49	3.16
Bcl2l1	NM_009743.4	4.3	4.08	3.24	3.39
Cd86	NM_019388.3	3.6	4.84	3.16	3.54
Il11	NM_008350.2	3.45	4	3.07	7.15
Stat2	NM_019963.1	2.98	2.59	3.07	2.78
Myd88	NM_010851.2	3.66	2.62	3.04	2.96
Myc	NM_010849.4	2.92	3.85	3	3.33
Il18rap	NM_010553.2	5.16	3.03	2.67	2.36
Stat3	NM_213659.2	2.91	3.39	2.64	2.68
Mafk	NM_010757.2	2.65	2.8	2.61	2.58
Stat1	NM_009283.3	2.85	2.62	2.58	2.17
Il1r1	NM_001123382.1	4.41	2.95	2.53	2.41
Relb	NM_009046.2	2.64	2.77	2.24	2.42
Chi3l3	NM_009892.1	2.43	4.71	2.13	9.11
Mknk1	NM_021461.4	-2.61	-2.72	-2.21	-2.48
Ccr4	NM_009916.2	-2.58	-2.22	-2.24	-2.56
C1qb	NM_009777.2	-2.19	-2.68	-2.39	-3.48
Hmgb1	NM_010439.3	-2.47	-2.47	-2.49	-2.31
Ppp1r12b	NM_001081307.1	-2.8	-5.87	-2.52	-2.64
Nfatc3	NM_010901.2	-2.49	-2.75	-2.55	-2.39
Itgb2	NM_008404.4	-2.45	-2.63	-2.61	-2.77
Prkcb	NM_008855.2	-2.74	-2.84	-2.61	-2.61
Ccr1	NM_009912.4	-2.18	-2.17	-2.77	-2.64
Mmp9	NM_013599.2	-2.69	-2.57	-2.82	-2.56
Il6ra	NM_010559.2	-3.46	-4.63	-2.9	-3.64
Map3k5	NM_008580.4	-2.32	-2.1	-3.23	-2.79
Map3k1	NM_011945.2	-3.92	-3.35	-3.24	-3.36
Tyrobp	NM_011662.2	-3.03	-2.88	-3.27	-3.8
Mef2c_Mm	NM_025282.2	-2.98	-2.92	-3.38	-3.83
Prkca	NM_011101.3	-2.75	-2.7	-3.43	-2.75
Twist2	NM_007855.2	-3.18	-5.6	-3.65	-3.29
Fxyd2	NM_052823.2	-2.06	-2.08	-3.69	-4.16

Retnla	NM_020509.3	-2.43	-3.17	-3.71	-6.24
C3ar1	NM_009779.2	-3.84	-4.18	-3.75	-3.82
C1qa	NM_007572.2	-3.61	-3.98	-4	-6.36
Ltb4r1	NM_008519.2	-3.28	-4.14	-4.04	-2.51
Ptger2	NM_008964.4	-5.19	-4.15	-4.12	-5.39
Mapk14	NM_011951.2	-4.83	-5.63	-4.16	-4.42
Rps6ka5	NM_153587.2	-4.68	-4.01	-4.58	-5.2
Cd40lg	NM_011616.2	-3.7	-2.83	-4.65	-5.37
Tslp	NM_021367.1	-4.36	-4.55	-4.72	-6.32
Mapk3	NM_011952.2	-5.34	-5.72	-4.87	-5.44
Trem2	NM_031254.2	-4.54	-7.54	-4.9	-13.57
Cd4	NM_013488.2	-5.85	-3.85	-7.26	-9.25
Cysltr1	NM_021476.4	-5.93	-6.3	-7.49	-7.26
Cd55	NM_010016.2	-7.49	-8.71	-7.73	-4.98
Alox5	NM_009662.2	-6.45	-7.57	-7.79	-7.37
Alox15	NM_009660.3	-8.28	-6.85	-10.13	-9.71
Ltb	NM_008518.2	-13.15	-9.54	-16.2	-17.32
Ccr2	NM_009915.2	-25.7	-27.33	-29.53	-17.11
Tbxa2r	NM_001277265.1	-42.99	-43.15	-42.21	-38.66
Map2k6	NM_011943.2	-52.09	-32.98	-52.17	-34.25
Tgfb2	NM_009367.1	-226.65	-150.14	-118.03	-129.45



Supplemental Fig. S1. AA and downstream eicosanoid profiles in the renal medulla. Renal medulla was dissected after 6 h of LPS peritoneal administration, and then subjected to LC-MS/MS assays. Data are presented as mean ± SEM, n =6-8.



■ WT ■ COX-2>COX-1 ■ COX-1>COX-2 ■ *Reversa*
A
-log(p-value)
0
10
20
30
40
50
60
Threshold
Role of pattern recognition receptors in recognition of bacteria and viruses
Role of macrophages, fibroblasts and endothelial cells in rheumatoid arthritis
Communication between innate and adaptive immune cells
Altered T cells and B cells signaling in rheumatoid arthritis
Granulocyte adhesion and diapedesis
p38 MAPK signaling
Dendritic cell maturation
Th1 and Th2 activation pathway
IL-12 signaling and production in macrophages
Role of cytokines in mediating communication between immune cells
Acute phase response signaling
Chemokine signaling
B
-log(p-value)
0
25
50
75
Threshold
Cellular movement
Immune cell trafficking
Hematological system development and function
Inflammatory response
Organismal injury and abnormalities
Tissue morphology
Connective tissue disorders
Inflammatory disease
Skeletal and muscular disorders
Cell-to-cell signaling and interaction
Cellular growth and proliferation

Supplemental Fig. S2. Comparison of the canonical pathways (A) and biological function (B) among the genes affected by the *Ptgs* gene exchange. Significant regulated pathways were determined by Ingenuity Pathway Analysis (IPA). Core analysis between the Control group and 4 LPS-challenged genotypes of mice was performed prior to the comparison analysis between 4 LPS-challenged groups (WT, COX-2>COX-1, COX-1>COX-2, and *Reversa*).