Supplementary Information

Self-Limited versus Delayed Resolution of Acute Inflammation: Temporal Regulation of Pro-Resolving Mediators and MicroRNA

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Figure S1

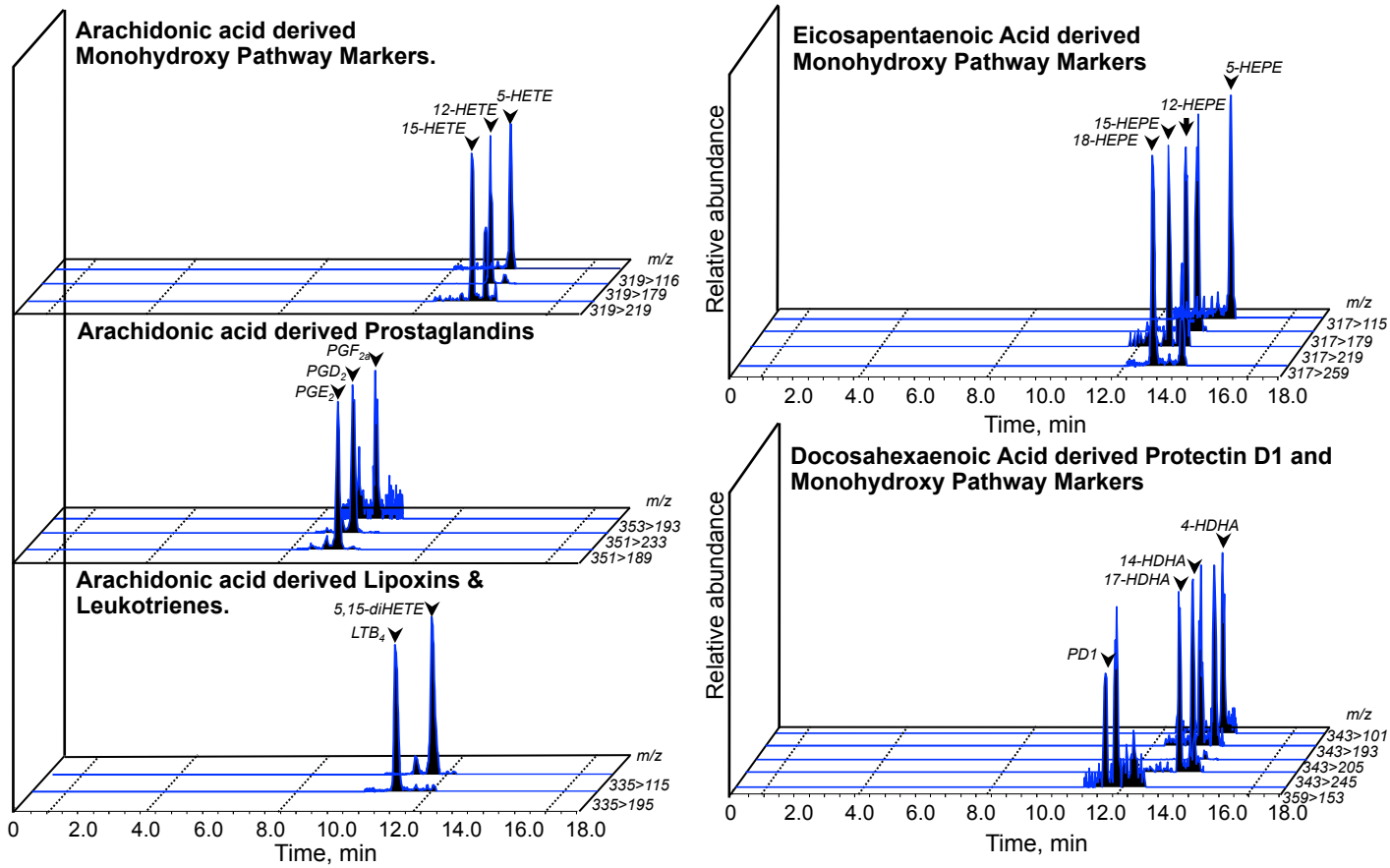
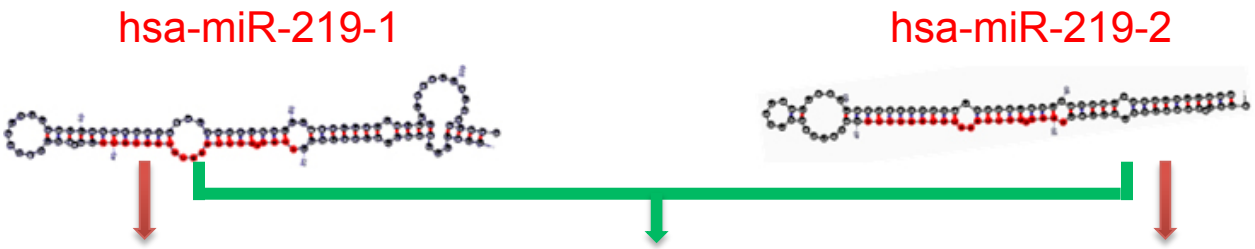


Figure S1. Representative chromatograms for LMs for peritoneal exudates harvested 4hrs post zymosan initiation. Representative MRM chromatograms for the identified LM. Peak heights represent the relative levels of each mediator in the exudates. Left panel, arachidonic acid derived LMs. Right panel, eicosapentaenoic acid (top) and docosahexaenoic acid (bottom) derived mediators.

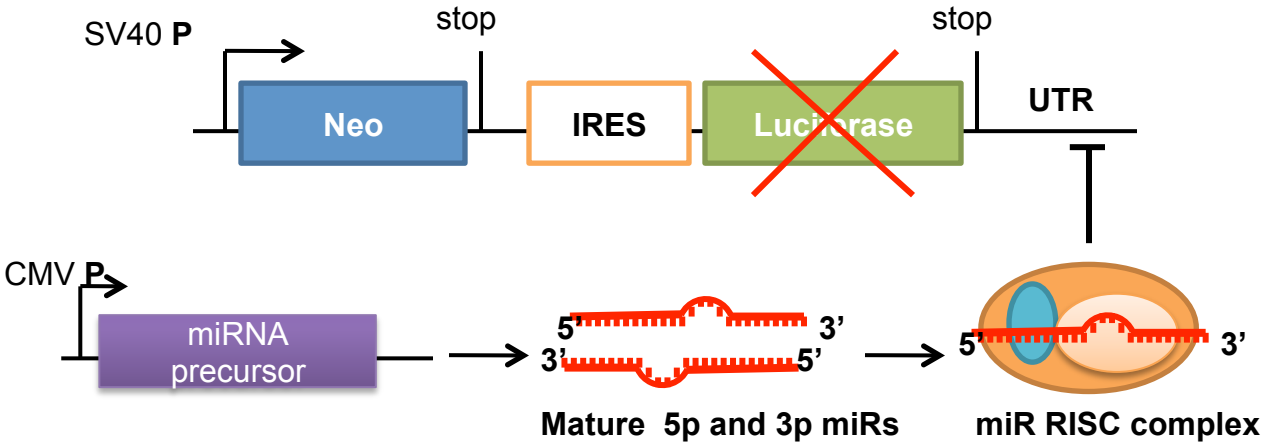
Figure S2

a



Mature miR	**5'-UGAUUGUCCAAACGCAAUUCU-3' miR-219-1-3p**	**5'-AGAGUUGAGUCUGGACGUCCCG-3' miR-219-5p**	**5'-AGAAUUGUGGCUGGACAUCUGU-3' miR-219-2-3p**
Predicte targets	STAM2, TLR4, IL6R, FN1, NOS1AP, ELAVL1, TRAF3	AHR, KLF3, SP4, TGFBR2, NFIB, SMAD4, ITGA2, ESR1, RORA, PPARA, NFIA	5-LOX, P2RY13, C7, TIA1, TIAL1, NFIA, NFIB, BCL2, ESRRG

b



c

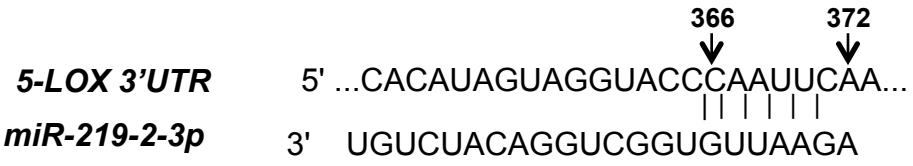


Figure S2. miR-219-1 and miR-219-2 are distinct miRs. (a) Scheme of the difference between miR-219-1 and miR-219-2. Each miR-219 predicted gene targets distinct from each other. (b) Scheme of 3'UTR experiment whereby luminescence will be decreased if direct binding of the miR with the 3'UTR occurs. (c) Predicted sequence binding of miR-219-2-3p with human 5-LOX.

Figure S3

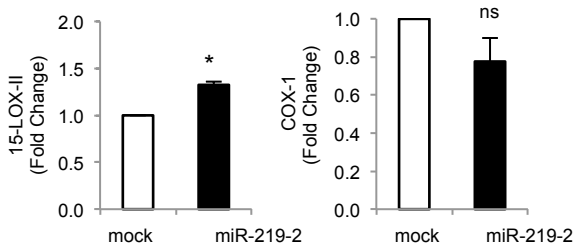


Figure S3 miR-219-2 modulation of 15-LOX-II.

Human macrophages were transfected with either mock or miR-219-2 (72 hrs, 37° C). mRNAs were isolated and analyzed using qPCR. (a) 15-LOX-II, (b) COX-1. Results are mean ± SEM of n=6 donors. *P<0.05, mock vs. miR-219-2.

Table S1. Lipid mediator profiles in self-limited versus delayed resolution exudates*

Compound	**Q1**	**Q3**	*1mg Zym 4h (pg/exudate)*	*10 mg Zym 4h (pg/exudate)*
5-HETE	**319**	**116**	2,711 ± 365	14,068 ± 1,773
12-HETE	**319**	**179**	4,844 ± 1576	2,833 ± 542
15-HETE	**319**	**219**	1,097 ± 101	3,129 ± 232
4-HDHA	**343**	**101**	16,491 ± 5,222	40,818 ± 2,649
7-HDHA	**343**	**141**	245 ± 46	2,614 ± 147
14-HDHA	**343**	**205**	3,471 ± 1,880	2,082 ± 662
17-HDHA	**343**	**245**	1,524 ± 494	1,493 ± 200
5-HEPE	**317**	**115**	6,036 ± 620	22,799 ± 3,531
12-HEPE	**317**	**179**	468 ± 177	210 ± 40
15-HEPE	**317**	**219**	354 ± 115	142 ± 36
18-HEPE	**317**	**259**	441 ± 121	490 ± 149
LTB_4	**335**	**195**	741 ± 168	12,070 ± 1,904
LXA_4	**351**	**115**	70 ± 25	289 ± 67
5,15-diHETE	**335**	**115**	225 ± 67	981 ± 136
PGF_{2a}	**353**	**193**	461 ± 43	1,358 ± 190
PGD_2	**351**	**233**	1,416 ± 125	3,319 ± 466
PGE_2	**351**	**189**	2,073 ± 316	6,739 ± 1,089
RvD1	**375**	**215**	23 ± 10	8 ± 1
RvD2	**375**	**175**	*	*
PD1	**359**	**153**	1,140 ± 330	324 ± 108
RvD5	**359**	**199**	177 ± 93	86 ± 25
RvD6	**359**	**101**	63 ± 30	45 ± 21

*LC-MS-MS-based LM metabololipidomics were carried out with self-limited or delayed resolution lavage exudates collected at 4 hours after zymosan injection. The detection limits were ~10 pg. (*) denotes below limits of detection. Lipid mediators were profiled using multiple reaction monitoring (MRM) and identified by direct comparison with synthetic and authentic standards using matching criteria including retention times and 6 diagnostic ions. Q1: M-H (parent ion), Q3: A diagnostic ion in the MS-MS (daughter ion). Q1-Q3 ion pairs were used for quantification. Specific bioactive lipid mediator and precursor/pathway markers are expressed as mean ± SEM, n=4 separate murine lavage exudates for each of the mediators.

Table S2. Lipid mediator profiles in self-limited versus delayed resolution exudates with or without apoptotic PMN *

			Self-limited		Delayed Resolution	
Compound	**Q1**	**Q3**	*Zym*	*Zym + AP*	*Zym*	*Zym + AP*
5-HETE	**319**	**116**	233 ± 57	165 ± 47	498 ± 53	319 ± 66
12-HETE	**319**	**179**	1,870 ± 867	1,347 ± 84	1,310 ± 250	2,183 ± 302
15-HETE	**319**	**219**	389 ± 87	815 ± 284	3,512 ± 400	4,860 ± 831
4-HDHA	**343**	**101**	192 ± 47	200 ± 43	302 ± 45	173 ± 11
7-HDHA	**343**	**141**	64 ±12	60 ±14	108 ± 24	89 ± 23
14-HDHA	**343**	**205**	1,392 ± 803	750 ± 252	928 ± 174	1,673 ± 132
17-HDHA	**343**	**245**	185 ± 26	186 ± 38	490 ± 61	466 ± 71
5-HEPE	**317**	**115**	2,425 ± 568	2,077 ± 572	3,414 ± 432	2,062 ± 308
12-HEPE	**317**	**179**	330 ± 139	163 ± 50	222 ± 22	98 ± 18
15-HEPE	**317**	**219**	72 ± 4	75 ± 15	161 ± 13	142 ± 16
18-HEPE	**317**	**259**	214 ± 22	165 ± 48	312 ± 24	184 ± 39
LTB_4	**335**	**195**	10 ± 0.8	2.5 ±1.3 §	25 ± 2	14 ± 3 #
LXA4	**351**	**115**	*	*	*	*
5,15-diHETE	**335**	**115**	1.8 ± 1.0	4.0 ± 1.4 *	56.9 ± 11.0	98.2 ± 4.3 #
PGF_{2a}	**353**	**193**	73 ± 8	216 ± 33	790 ± 108	1,274 ± 472
PGD_2	**351**	**233**	5 ± 1	498 ± 95 §§	151 ± 43	1,279 ± 558 #
PGE_2	**351**	**189**	308 ± 47	536 ± 196	2,801 ± 174	2,929 ± 240
RvD1	**375**	**215**	*	*	*	*
RvD2	**375**	**175**	*	*	*	*
PD1	**359**	**153**	22.4 ± 4.4	40.6 ± 6.9 §	15.5 ± 1.3	78.5 ± 18.0 #
RvD5	**359**	**199**	*	*	*	*
RvD6	**359**	**101**	18 ± 12	*	37 ± 4	31 ± 10

*LC-MS-MS-based LM metabololipidomics were carried out with self-limited or delayed resolution lavage exudates collected at 24 hours after zymosan injection. The detection limits were ~10 pg. (*) denotes below limits of detection. Lipid mediators were profiled using multiple reaction monitoring (MRM) and identified by direct comparison with synthetic and authentic standards using matching criteria including retention times and 6 diagnostic ions. Q1: M-H (parent ion), Q3: A diagnostic ion in the MS-MS (daughter ion). Q1-Q3 ion pairs were used for quantification. Specific bioactive lipid mediator and precursor/pathway markers are expressed as mean ± SEM, n=4 separate murine lavage exudates for each of the mediators identified. §$p<0.05$, §§$p<0.01$ Self-limited zymosan (Zym) versus self-limited zymosan plus apoptotic PMN (Apop PMN). #$p<0.05$ Delayed resolution zymosan versus delayed resolution zymosan plus apoptotic PMN.

Table S3A. Primer designed and used for *in vitro* and *in vivo* experiments

Genes	**Forward (5'→3')**	**Reverse (5'→3')**
Human 5-LOX	GGAAACACGGCAAAAACA	ATCGATGCTCAAGGGGAAG
Human LTA4H	CCACCATCCTTCCCTTAT	AAACAATCGTCCGCAAAT
Human 12-LOX	GATGATCTACCTCCAAATATG	CTGGCCCCAGAAGATCTGATC
Human 15-LOX	GACCGAGGGTTTCCTGTCTC	TGTCTCCAGCGTTGCATCC
Human 15-LOX-2	GATCTTCAACTTCCGGAGGAC	ACTGGGAGGCGAAGAAGG
Human GAPDH	AGCCACATCGCTCAGACAC	GCCCAATACGACCAAATCC
Mouse 5-LOX	GGCACGGGAAAAACAGTATC	TGGCATTTGGCATCAATACTC
Mouse PTGS2	AAGGAACTCAGCACTGCATCC	ACAGGGATTGGAACAGCAAGGA
	Qiagen Catalog No.	
RNAU1A	MS00013986	
miR-219-5p	MS00006776	
miR-219-2-3p	MS00009135	
miR-21	MS00009079	
miR-146b-5p	MS00003542	
miR-208a	MS00003794	
Human PTGS1	QT00210280	
Human PTGS2	QT00040586	
Human HPGD	QT00013454	

Table S3B. Commercial human antibodies used for flow cytometry

Antibodies	**Company**	**Catlog No.**
5-LOX	Cell Signaling Technology	3289
COX-2	Cayman Chemical	160113
12-LOX	Novus Biologicals	H00000239-M01
15-LOX-I	Origene	TA504358
LTA4H	Origene	TA500663
PGDH	Novus Biologicals	NB200-179G
Alexa Fluor 647-conjugated anti-Rabbit IgG	Jackson ImmunoResearch Laboratories	111-605-003

Table S4. Plasmids used with human macrophages

Plasmids	ACCN	Sequences	Cat No.
miR-219-2	MI0000740	ACUCAGGGGCUUCGCCACUGAUUGUCCAAACGCAAUUCUUGUACGAGUCUGCGGCCAACCGAGAAUUGUGGCUGGACAUCUGUGGCUGAGCUCCGGG	SC400285
miR-219-1	MI0000296	CCGCCCCGGGCCGCGGCUCCUGAUUGUCCAAACGCAAUUCUCGAGUCUAUGGCUCCGGCCGAGAGUUGAGUCUGGACGUCCCGAGCCGCCGCCCCCAAACCUCGAGCGGG	SC400284
miR-146b	MI0003129	CCUGGCACUGAGAACUGAAUUCCAUAGGCUGUGAGCUCUAGCAAUGCCCUGUGGACUCAGUUCUGGUGCCCGG	SC400179
miR-208a	MI0000555	UUCCUUUGACGGGUGAGCUUUUGGCCCGGGUUAUACCUGACACUCACGUAUAAGACGAGCAAAAAGCUUGUUGGUCAGAGGAG	SC400911
mock	empty	Vector for miRNA expression clone with GFP as reporter	PCMVMIR
5-LOX 3'UTR	NM_000698	CAATTGGCAGAGCTCAGAATTCAAGCGATCGCCGGATTCCGAACAGTGTGGCCATC**TGA**GCACACTGCCAGTCTCACTGTGGGAAGGCCAGCTGCCCCAGCCAGATGGACTCCAGCCTGCCTGGCAGGCTGTCTGGCCAGGCCTCTTGGCAGTCACATCTCTTCCTCCGAGGCCAGTACCTTTCCATTTATTCTTTGATCTTCAGGGAACTGCATAGATTGATCAAAGTGTAAACACCATAGGGACCCATTCTACACAGAGCAGGACTGCACAGCGTCCTGTCCACACCCAGCTCAGCATTTCCACACCAAGCAGCAACAGCAAATCACGACCACTGATAGATGTCTATTCTTGTTGGAGACATGGGATGATTATTTTCTGTTCTATTTGTGCTTAGTCCAATTCCTTGCACATAGTAGGTACCCAATTCAATTACTATTGAATGAATTAAGAATTGGTTGCCATAAAAATAACGCGTAAGCGGCCGCGGCATCTAGATTCGAAGAAAATGACCG	SC205988
COX-1 3'UTR	NM_080591	CTGCTGTGGAGCGACCATCCACAGAGCTCT**TGA**GGGGCAGGAAAGCAGCATTCTGGAGGGGAGAGCTTTGTGCTTGTCATTCCAGAGTGCTGAGGCCAGGGCTGATGGTCTTAAATGCTCATTTTCTGGTTTGGCATGGTGAGTGTTGGGGTTGACATTTAGAACTTTAAGTCTCACCCATTATCTGGAATATTGTGATTCTGTTTATTCTTCCAGAATGCTGAACTCCTTGTTAGCCCTTCAGATTGTTAGGAGTGGTTCTCATTTGGTCTGCCAGAATACTGGGTTCTTAGTTGACAACCTAGAATGTCAGATTTCTGGTTGATTTGTAACACAGTCATTCTAGGATGTGGAGCTACTGATGAAATCTGCTAGAAAGTTAGGGGGTTCTTATTTTGCATTCCAGAATCTTGACTTTCTGATTGGTGATTCAAAGTGTTGTGTTCCTGGCTGATGATCCAGAACAGTGGCTCGTATCCCAAATCTGTCAGCATCTGGCTGTCTAGAATGTGGATTTGATTCATTTTCCTGTTCAGTGAGATATCATAGAGACGGAGATCCTAAGGTCCAACAAGAATGCATTCCCTGAATCTGTGCCTGCACTGAGAGGGCAAGGAAGTGGGGTGTTCTTCTTGGGACCCCCACTAAGACCCTGGTCTGAGGATGTAGAGAGAAGAAGCAGGTGGGCTGTATTCACGCCATTGGTTGGAAGCTACCAGAGCTCTATCCCCATCCAGGTCTTGACTCATGGCAGCTGTTTCTCATGAAGCTAATAAAATTCGCTTTCTAAAGTTACCTGTTATATATCTCTTTTGGTCCCATCCTCTAAAGCAGAGGCAACACTGGAACATGGCTAGCCTTTCTTGTAGCCATGGCTGGGCGTGCTAGAGGTTGCAGCATGAGACTTTCTGCTGGGATCCTTGGGCCCATCACTGTATAGACATGCTACCACTGGTACTTCCTTTCTCCCTGCGGGCCAGGCACTGCCCTTTTCAGGAAGCTCTCTTAAAATACCCATTTGCCCCAGACCTGGAAGATATAACATTCAGTTCCCACCATCTGATTAAAACAACTTCCTCCCTTACAGAGCATACAACAGAGGGGGCACCCGGGGAGGAGAGCACATACTGTGTTCCAATTTCACGCTTTTAATTCTCATTTGTTCTCACACCAACAGTGTGAAGTGCGTGGTATAATCTCCATTTCAAAACCAAGGAAGCAGCCTCAGAGTGGTCGAGTGACACACCTCACGCAGGCTGAGTCCAGAGCTTGTGCTCCTCTTGATTCCTGGTTTGACTCAGTTCCAGGCCTGATCTTGCCTGTCTGGCTCAGGGGTCAAAGACAGAATGGTGGAGTGTAGCCTCCACCTGATATTCAGGCTACTCATTCAGTCCCAAATATGTATTTTCCTAAGTGTTTACTATGGCCAGTTCCTGTAACAGGTGTGGGGACACAGCAGTGAGTAATCAATACAGACAAGGTTCTGCCCTTATGGAGCTCACACTCCAGTGGCAGACAAACAGACCATAAATAAGGAAACGATGAAATAAGATATATACAAGGTGAGTGTGACTTCCCTTCTAACCCCCTCTGCTCTGTCCTCCCCTATTGCGCTCTCAAGACCAGAGACCCAACAGCAGTGATCTCAGGGCAGACAGCCCTCCACTCCAGCTCTGAGCACCTTTTCTCAGGACCTCTGTAGGCAGCAGAGAGAGGACAGAGGGGTAAGATGAGGGGTTGAGGGAAGGTTCTTCATGATCCACACTTTGGGCTTAGTATTTCTCAGGAAGAGCTATGGCCCAGAAACAACAGGGGAAACTAGAGTTCGGTCTGACAGTCCTTGGGGTTAAGTCTCCTGTCTTATGGTCCAGAAACTCCTGTTTCTCCTTAGTTGGCTGGAAACTGCTCCCATCATTCCTTCTGGCCTCTGCTGAATGCAGGGAATGCAATCCTTCCCTGCTCTTGCAGTTGCTCTGACGTAGAAAGATCCTTCGGGTGCTGGAAGTCTCCATGAAGAGCTTGTGTCCTGTCCTTTCTTGCAGATTCTATTTCCCCTCTTCTGCTAATACCTCTTACTTTGCTTGAGAATCCTCTCCTTTCTTATTAATTTCAGTCTTGGTGGTTCTATCAGGGGTGCATTCTGGCCAAGGGGTGGGCCTGTGAATCAATCCTGGGCAATCAGACACCCTCTCCTTAAAAACTGGCCCGTGGAGACTGAGATCACTGACTCTGACTCATCCCCACAGCTGGCTCTGACAAGATGGTCCATTTGTTCCTGCTTCCGAGATCCCCAGGGCAGCCTGGATCCCTGCCCTTCTCAAGACTTTAGCTTTCCTTCCATCCGGTGGCCTATTCCAGGAATTCCTCTTTTGCTTAAATCAGTTGGAGTTTGTGTCTGTTGCTTGTAATCAAGCCTTTATGGCTGCTGGGCTGAGTGACACAAGCACTTTAATGGCCTGGAGGGACTTTTAATCAGTGAAGATGCAATCAGACAAGTGTTTTGGAAAGAGCACCCTCGAGAAGGGTGGATGACAGGGCAGAGCAGGAAGGACAGGAAGCTGGCAGAACGGAGGAGGCTGCAGCCGTGGTCCAACCAGGAGCTGATGGCAGCTGGGGCTAGGGGAAGGGCTTTGAGGGTGGAAGGATGGGATGGGTTCCAGAGGTATTCCTCTCTTAAATGCAAGTGCCTAGATTAGGTAGACTTTGCTTAGTATTGACAACTGCACATGAAAGTTTTGCAAAGGGAAACAGGCTAAATGCACCAAGAAAGCTTCTTCAGAGTGAAGAATCTTAATGCTTGTAATTTAAACATTTGTTCCTGGAGTTTTGATTTGGTGGATGTGATGGTTGGTTTATTTGTCAGTTTGGTTGGGCTATAGCACACAGTTATTTAATCAAACAGTAATCTAGGTGTGGCTGTGAAGGTATTTTGTAGATGTGATTAACATCTACAATCAGTTGACTTTAAGTGAAAGAGATTACTTAAATAATTTGGGTGAGCTGCACCTGATTAGTTGAAAGGCCTCAAGAACAAACACTGCAGTTTCCTGGAAAAGAAGAAACTTTGCCTCAAGACTATAGCCATCGACTCCTGCCTGAGTTTCCAGCCTGCTAGTCTGCCCTATGGATTTGAAGTTTGCCAACCCCAACAATTGTGTGAATTA	SC220383
COX-2 3'UTR	NM_000963	ACGTTCGACTGAACTG**TAG**AAGTCTAATGATCATATTTATTTATTTATATGAACCATGTCTATTAATTTAATTATTTAATAATATTTATATTAAACTCCTTTATGTTACTTAACATCTTCTGTAACAGAAGTCAGTACTCCTGTTGCGGAGAAAGGAGTCATACTTGTGAAGACTTTTATGTCACTACTCTAAAGATTTTGCTGTTGCTGTTAAGTTTGGAAAACAGTTTTTATTCTGTTTTATAAACCAGAGAGAAATGAGTTTTGACGTCTTTTTACTTGAATTTCAACTTATATTATAAGAACGAAAGTAAAGATGTTTGAATACTTAAACACTGTCACAAGATGGCAAAATGCTGAAAGTTTTTACACTGTCGATGTTTCCAATGCATCTTCCATGATGCATTAGAAGTAACTAATGTTTGAAATTTTAAAGTACTTTTGGTTATTTTTCTGTCATCAAACAAAAACAGGTATCAGTGCATTATTAAATGAATATTTAAATTAGACATTACCAGTAATTTCATGTCTACTTTTTAAAATCAGCAATGAAACAATAATTTGAAATTTCTAAATTCATAGGGTAGAATCACCTGTAAAAGCTTGTTTGATTTCTTAAAGTTATTAAACTTGTACATATACCAAAAAGAAGCTGTCTTGGATTTAAATCTGTAAAATCAGTAGAAATTTTACTACAATTGCTTGTTAAAATATTTTATAAGTGATGTTCCTTTTTCACCAAGAGTATAAACCTTTTTAGTGTGACTGTTAAAACTTCCTTTTAAATCAAAATGCCAAATTTATTAAGGTGGTGGAGCCACTGCAGTGTTATCTTAAAATAAGAATATTTTGTTGAGATATTCCAGAATTTGTTTATATGGCTGGTAACATGTAAAATCTATATCAGCAAAAGGGTCTACCTTTAAAATAAGCAATAACAAAGAAGAAAACCAAATTATTGTTCAAATTTAGGTTTAAACTTTTGAAGCAAACTTTTTTTTATCCTTGTGCACTGCAGGCCTGGTACTCAGATTTTGCTATGAGGTTAATGAAGTACCAAGCTGTGCTTGAATAATGATATGTTTTCTCAGATTTTCTGTTGTACAGTTTAATTTAGCAGTCCATATCACATTGCAAAAGTAGCAATGACCTCATAAAATACCTCTTCAAAATGCTTAAATTCATTTCACACATTAATTTTATCTCAGTCTTGAAGCCAATTCAGTAGGTGCATTGGAATCAAGCCTGGCTACCTGCATGCTGTTCCTTTTCTTTCTTCTTTTAGCCATTTTGCTAAGAGACACAGTCTTCTCATCACTTCGTTTCTCCTATTTTGTTTTACTAGTTTTAAGATCAGAGTTCACTTTCTTTGGACTCTGCCTATATTTTCTTACCTGAACTTTTGCAAGTTTTCAGGTAAACCTCAGCTCAGGACTGCTATTTAGCTCCTCTTAAGAAGATTAAAAGAGAAAAAAAAAGGCCCTTTTAAAAATAGTATACACTTATTTTAAGTGAAAAGCAGAGAATTTTATTTATAGCTAATTTTAGCTATCTGTAACCAAGATGGATGCAAAGAGGCTAGTGCCTCAGAGAGAACTGTACGGGGTTTGTGACTGGAAAAAGTTACGTTCCCATTCTAATTAATGCCCTTTCTTATTTAAAAACAAAACCAAATGATATCTAAGTAGTTCTCAGCAATAATAATAATGACGATAATACTTCTTTTCCACATCTCATTGTCACTGACATTAATGGTACTGTATATTACTTAATTTATTGAAGATTATTATTTATGTCTTATTAGGACACTATGGTTATAAACTGTGTTTAAGCCTACAATCATTGATTTTTTTTTGTTATGTCACAATCAGTATATCTTCTTTGGGGTTACCTCTCTGAATATTATGTAAACAATCCAAAGAAATGATTGTATTAAGATTTGTGAATAAATTTTTAGAAATCTGATTGGCATATTGAGATATTTAAGGTTGAATGTTTGTCCTTAGGATAGGCCTATGTGCTAGCCCACAAAGAATATTGTCTCATTAGCCTGAATGTGCCATAAGACTGACCTTTTAAAATGTTTTGAGGGATCTGTGGATGCTTCGTTAATTTGTTCAGCCACAATTTATTGAGAAAATATTCTGTGTCAAGCACTGTGGGTTTTAATATTTTTAAATCAAACGCTGATTACAGATAATAGTATTTATATAAATAATTGAAAAAAATTTTCTTTTGGGAAGAGGGAGAAAATGAAATAAATATCATTAAAGATAACTCAGGAGAATCTTCTTTACAATTTTACGTTTAGAATGTTTAAGGTTAAGAAAGAAATAGTCAATATGCTTGTATAAAACACTGTTCACTGTTTTTTTTAAAAAAAAAACTTGATTTGTTATTAACATTGATCTGCTGACAAAACCTGGGAATTTGGGTTGTGTATGCGAATGTTTCAGTGCCTCAGACAAATGTGTATTTAACTTATGTAAAAGATAAGTCTGGAAATAAATGTCTGTTTATTTTTTGTACTATTTTAAAAATTGACAGATCTTTTCTGAAG	SC219115
Vector control	pMirTarget	AAGGGCGGAAAGATCGCCGTGTAACAATTGGCAGAGCTCAGAATTCAAGCGATCGCTTGGCGCGCCCGGACCGTTACGCGTAAGCGGCCGCGGCATCTAGATTCGAAGAAAATGACCG	PS100062