

Supplementary Materials for

PGE₂ production at sites of tissue injury promotes an anti-inflammatory neutrophil phenotype and determines the outcome of inflammation resolution in vivo

Catherine A. Loynes, Jou A. Lee, Anne L. Robertson, Michael JG. Steel, Felix Ellett, Yi Feng, Bruce D. Levy, Moira K.B. Whyte, Stephen A. Renshaw*

*Corresponding author. Email: s.a.renshaw@sheffield.ac.uk

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Fig. S1. Reverse transcription PCR demonstrates successful exon deletion.

Fig. S2. PGE₂ drives resolution of inflammation in the absence of macrophages by 12 hpi and does not alter neutrophil apoptosis.

Fig. S3. Amino acid positions 353 and 418 determine LOX functionality.

Fig. S4. Genotyping method for *ptges* crispant.

Supplementary Materials

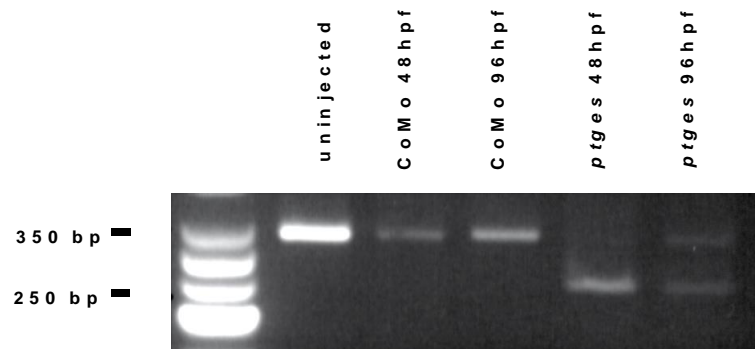


Fig. S1. Reverse transcription PCR demonstrates successful exon deletion. Gel electrophoresis showing almost complete knockdown of *ptges* at 48 and partial knockdown remaining at 96 hpf. A splice variant missing exon 2 in *ptges* morphants of 250bp compared to a WT product of 350bp is visible.

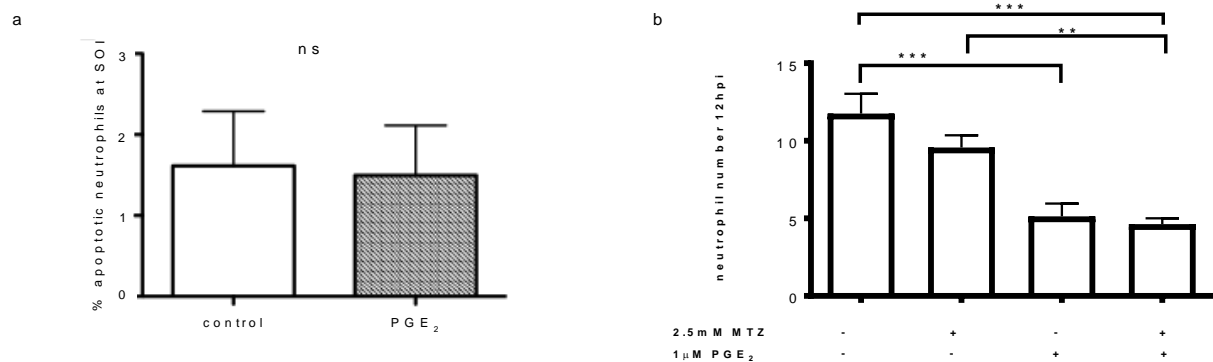


Fig. S2. PGE₂ drives resolution of inflammation in the absence of macrophages by 12 hpi and does not alter neutrophil apoptosis. (A) Graph showing PGE₂ does not affect neutrophil apoptosis during inflammation resolution. Larvae were injured, treated with PGE₂ and fixed at 12 hpi for TSA and TUNEL staining. The percentage of apoptotic neutrophils at the site of injury was not altered by the addition of PGE₂, n=36, from 2 experimental repeats. (B) Neutrophil counts 12 hpi in 3 dpf *Tg(mpx:EGFP)i114;Tg(cfms:Gal4)i186;Tg(UAS:nfsB-mCherry)i149* larvae treated with and without 2.5mM metronidazole and 1μM PGE₂. PGE₂, added at 8 hpi, can significantly drive inflammation resolution by 12 hpi, even when

macrophages are still being recruited to the site of injury in control larvae or in their complete absence when treated with metronidazole.

Medalox12	-HPDDGLIPIAIIQLEQT--PGLDTPIFLPSDPPLAWLLAKMWRHAEFQVFPQLLSHLLRT	396
Zfalox12	-HPEKGLIPIAIIQLEQK--PDKDTPVFLPSDPPLAWLLAKMWRHAEFQVFPQLLSHLLRT	371
Cfalox12	-HPESGLIPIAIIQLEQN--PGKDTPIFLPNDPPLAWLLAKMWRHAEFQVFPQLLSHLLRT	370
Halox12	-EPNGKLPQPMVIQIQPPNPSSPTTFLFLPSDPPLAWLLAKSWVRNSDFOLHEIQYHLLNT	364
Malox12	-DPGGKLLPMAIQIQPPNPSSPAPTFLFLPSDPPLAWLLAKIWRVNSDFOLQELQFHLLNT	364
Malox15	-QPDGQLLPPIAIIQLELPKGTGTPPPIFTPLDPPMDWLLAKCWVRSSDLOLHELQAHLLRG	365
Ralox12	-QPDGKLLPMPVIQLHLPKIGSSPPPLFLPTDPPMVWLLAKCWVRSSDLOVHELNSHLLRG	365
Ralox15	QQPSTVGLVICLELHLPKIGSSPPPLFLPTDPPMVWLLAKCWVRSSDFOVHELNSHLLRG	495
Halox15	-QPDGKLLPMPVIQLQLPRTGSPPLFLPTDPPMAWLLAKCWVRSSDFOLHELQSHLLRG	364
Palox15	-QPDGKLLPMPVIQLQLPHEGSPLPPLFLPTDPPMVWLLAKCWVRSSDFOLHELHSHLLRG	365
Malox8	SSGSGPLLPPIAIIQLKQT--PGPDNPIFLPSDDTDWLLAKTWRVNSDFYIHEAVTHLLHA	378
Medalox15	MNPEGKLMPLAIQLNQK--ASAENPIFLPTDPEKDWLLAKLFFKSADLLECEVVHLLIT	388
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Medalox12	HLVVEVFCVATLRQLPAVHPIYKLLAPHQRYTLEINCRGRTQLLSETGIFKRVVSTGGEG	456
Zfalox12	HLMVEVICVATLRQLPAVHPIYKLLTPHLRYTLEINCRGRTQLLSEPIGIFKRVVSTGGEG	431
Cfalox12	HLVVEVICVSTLRHLPAVHPIYKLLTPHLRYTLEINCRGRTQLISPEGIFKRVVSTGGTG	430
Halox12	HLVAEVI AVATMRCLPGLHPIFKFLIPHIRYTMETRARTQLISDGGIFDQAVSTGGGG	424
Malox12	HLVAEVI AVATMRCLPGLHPIFKLLVPHIRYTMETRARTQLISDGGIFDQAVSTGGGG	424
Malox15	HLVAEVFAVATMRCLPSVHPVFKLLVPHLLYTMETRARTQLISERGFDFKVMSTGGGG	425
Ralox12	HLMAEVFTVATMRCLPSIHPVFKLIVPHRYTLEINVRARNGLVSDFGIFDQIMSTGGGG	425
Ralox15	HLMAEVFTVATMRCLPSIHPVFKLIVPHRYTLEINVRARNGLVSDFGIFDQIMSTGGGG	555
Halox15	HLMAEVI VVATMRCLPSIHPFKLIPHLRYTLEINVRARTGLVSDMGIFDQIMSTGGGG	424
Palox15	HLMAEVI AVATMRCLPSIHPFKLIPHPRYTLEINVRARNGLVSDLGIFDQAVSTGGGG	425
Malox8	HLIPEVFALATLRQLPCHPLFKLLIPHIRYTLHINTLARELLVAPGKLIDKSTGLGTGG	438
Medalox15	HFLSEVFAVATLRCPFTIHPHLKLLIPHRFTLHINIMGREALLGPDGALC-ASSFGLGEG	447
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Fig. S3. Amino acid positions 353 and 418 determine LOX functionality. Alignments of mammalian and fish 12- and 15-LO proteins. A Phenylalanine (F) at position 353 (magenta box) in reference to rabbit 12-LO, and a bulky amino acid Valine (V) at position 418 (green box) dictates preferential oxygenation at carbon 15, indicating zebrafish 12-LO functions as a 15-LO lipoxygenase.

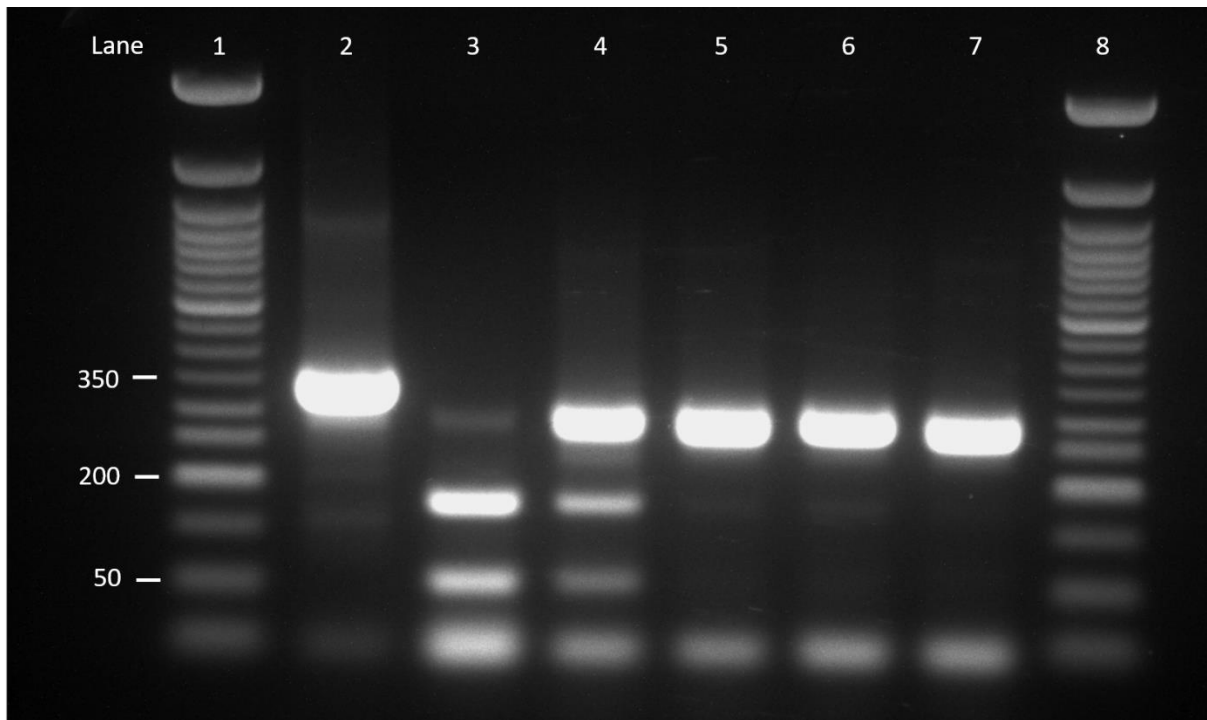


Fig. S4. Genotyping method for *ptges* crisprant. Electrophoresis gel shows different genotypes from *ptges* CRISPR injection. Lane 1: NEB 50bp ladder. Lane 2: undigested PCR product 345bp. Lane 3: fully cut WT 184bp, 109bp and 52bp. Lane 4: Heterozygous CRISPRant with 293bp band, 184bp, 109bp and 52bp. Lane 5-7: Homozygous CRISPRants with 293bp and 52bp bands. Very weak 184bp and 109bp bands can be seen, indicating a small amount of WT *ptges* is still present, allowing for normal development in these almost complete knockdown CRISPRant larvae. Lane 8: NEB 50bp ladder.