Supplementary file: **Anti-inflammatory activities of *Zophobas morio* larvae lipids on lipopolysaccharide-induced activated macrophages: Reprogramming macrophage polarization and attenuating oxidative stress**

Table S1. Primary antibodies used in the Western blot assay

Gene name	Dilution factor	Company	Cat No.
iNOS	1:1000	Cell Signaling Technology	13120S
COX-2	1:1000	Cell Signaling Technology	12282S
β-actin	1:500	Santa Cruz Biotechnology	sc-8432
IκB	1:1000	Cell Signaling Technology	4812S
p-IκB	1:1000	Cell Signaling Technology	2859S
NF-κB	1:1000	Cell Signaling Technology	8242S
p-NF-κB	1:1000	Cell Signaling Technology	3033S
β-tubulin	1:1000	abcam	ab108342
Lamin B1	1:1000	abcam	ab16048
p38	1:1000	Cell Signaling Technology	9212S
p-p38	1:1000	Cell Signaling Technology	9211S
ERK	1:500	Santa Cruz Biotechnology	sc-514302
p-ERK	1:1000	Cell Signaling Technology	4370S
JNK	1:1000	Cell Signaling Technology	9252S
p-JNK	1:1000	Cell Signaling Technology	4668S
HO-1	1:1000	Cell Signaling Technology	43966S
Keap1	1:1000	Cell Signaling Technology	8047S
Nrf2	1:1000	Cell Signaling Technology	12721S

Table S2. Primer sequences used in RT-qPCR analysis

Gene name		Sequence
TNFα	Forward	5′-TGGAACTGGCAGAAGAGGCACT-3′
	Reverse	5′-AGAGGCTGAGACATAGGCACCG-3′
IL6	Forward	5′-CACGATTTCCCAGAGAACATGTG-3′
	Reverse	5′-ACAACCACGGCCTTCCCTACTT-3′
Arg1	Forward	5′-TGCGCCACATGAAAACCATC -3′
	Reverse	5′- TTGGGAGGAGAAGGCGTTTG -3′
IL10	Forward	5′-GCTCTTACTGACTGGCATGAG -3′
	Reverse	5′-CGCAGCTCTAGGAGCATGTG -3′
β-actin	Forward	5′-CGTGCGTGACATCAAAGAGAA-3′
	Reverse	5′-GCTCGTTGCCAATAGTGATGA-3′

Table S3. FT-IR spectra of ZML.

No.	Wavenumber (cm^{-1})	Structure	Assignment
1	3006	C=C	Unsaturated carbon atoms
2	2924 and 2854	$-CH_2$ and $-CH_3$	Long-chain alkyl group (Stretching bands)
3	1744	-C-O-C	Typical ester carbonyl
4	1463 and 1378	$-CH_2$ and $-CH_3$	Symmetric vibrations (Bending bands)
5	1260 and 1162	-C-O- and C-C-O	Organic
6	722	$-(CH_2)_n-(CH_3)$	All fatty acids

Table S4. ^{1}H-NMR spectra of ZML

Peak notation	δ (ppm) (Multiplicity)	Proton	Assignment
1	0.80-0.82 (m)	$CH_2CH_2CH_2C\underline{H}_3$	All fatty acids (acyl group except for n-3)
2	0.89-0.92 (t)	$=CH_2\text{-}CH_2\text{-}C\underline{H}_3$	Linolenic acid
3	1.18-1.31 (m)	$\text{-}(C\underline{H}_2)_n\text{-}$	All fatty acids (acyl group)
4	1.92-2.00 (m)	$C\underline{H}_2\text{-}CH=CH$	Oleic acid
5	2.69-2.71 (t)	$=CH\text{-}C\underline{H}_2\text{-}CH=$	Diunsaturated acyl group (Linoleic acid)
6	4.06-4.09 (dd)	$C\underline{H}H$-OCOR sn- 1,3	Triglyceride
7	4.21-4.24 (dd)	$CH\underline{H}$-OCOR sn- 1,3	Triglyceride
8	5.23-5.32 (m)	$CH_2\text{-}C\underline{H}=C\underline{H}$	All Unsaturated fatty acids

Figure S1. Effect of ZML on the cell viability of macrophages. RAW264.7 cells were treated with ZML for 24 h, and then the cell viability was analyzed using the CCK-8 assay. $^{***}p < 0.001$ compared to the ZML treatment group with the non-treatment group.

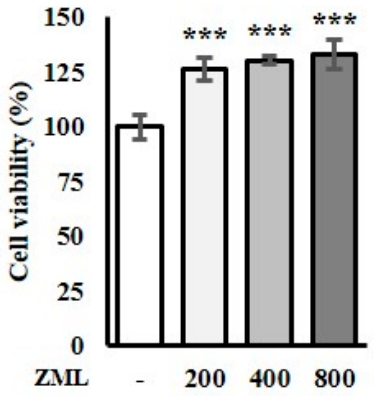


Figure S2. Effect of ZML on the secretion of pro-inflammatory cytokines in LPS-induced activated macrophage. RAW264.7 cells were pretreated with 1 h of ZML, then cotreated with LPS (1 μg/mL) for 24 h, and then the pro-inflammatory cytokine secretion was analyzed using ELISA. $^{###}p < 0.001$ compared to the non-treatment group with the LPS-only treatment group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to the LPS+ZML cotreatment group with LPS-only treatment group

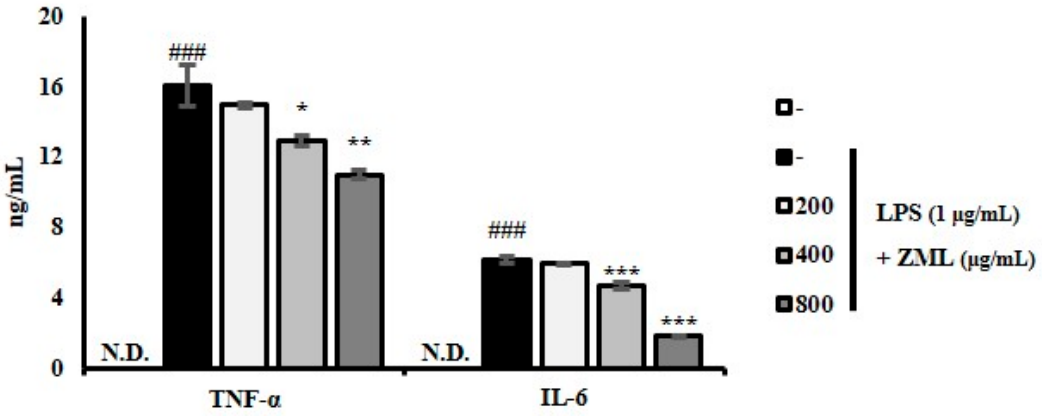


Figure S3. Successful cytosolic/nuclear fractionation was performed in the experiment. (a) NFκB translocation assay performed in Figure 4B. (b) Nrf2 translocation assay performed in Figure 5B.

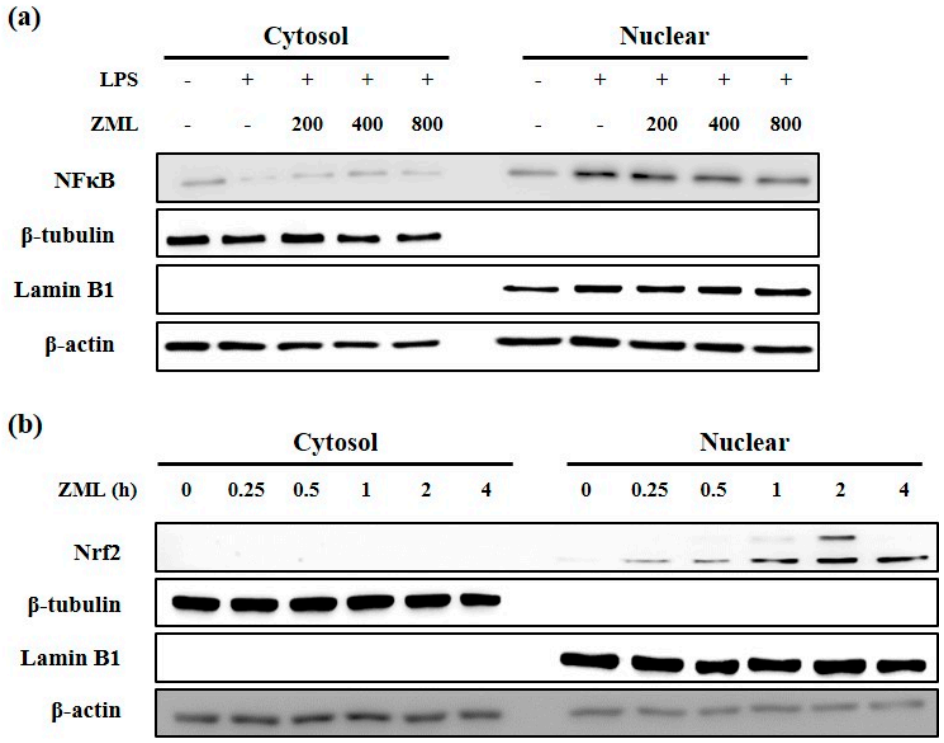


Figure S4. ZML increased Nrf2 expression in macrophages. RAW264.7 cells were treated with 800 μg/mL of ZML for 0.25-4 h. The expression of Nrf2 was analyzed using Western blot analysis. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to the ZML treatment group with the non-treatment group.

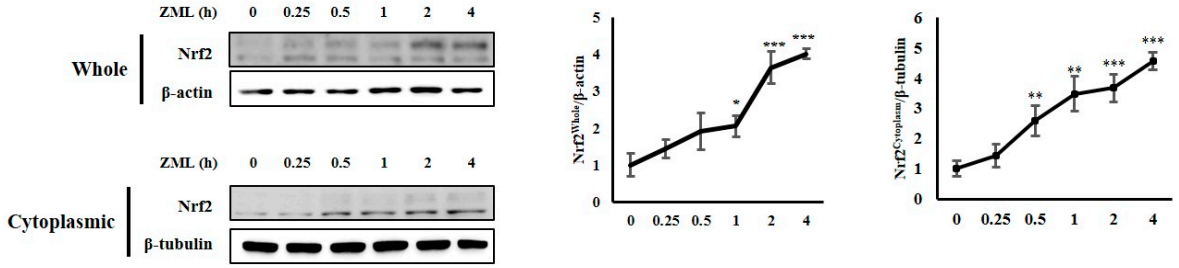


Figure S5. Comparison of the effects of ZML and LPS on the phosphorylation levels of MAPK signaling factors. RAW264.7 cells were treated with ZML (800 μg/mL) or LPS (1 μg/mL) for 30 min for the MAPK signaling pathway factors. The phosphorylation of MAPK signaling factors was analyzed using Western blot analysis.

