

Supplementary Material

**Antibacterial and Immunosuppressive Effects of a Novel Marine Brown
Algae-Derived Ester in Atopic Dermatitis**

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Supplementary data

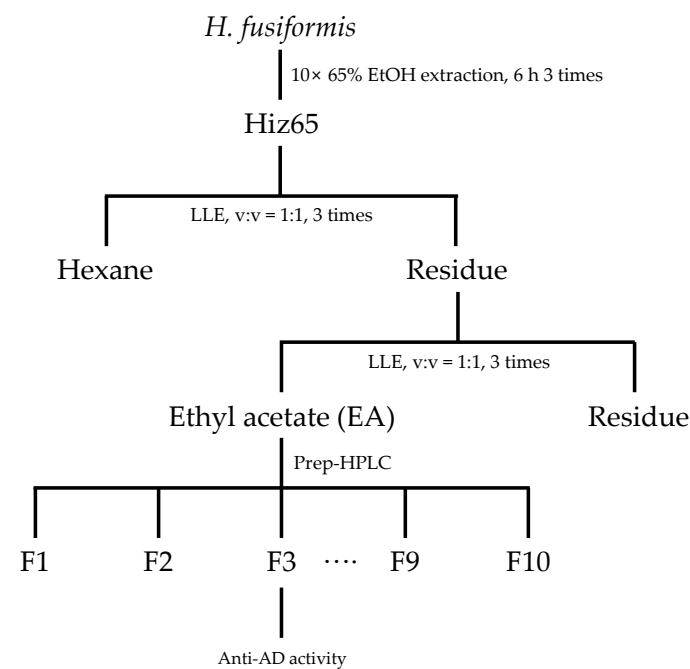


Figure S1: Schematic representation of the extraction of biologically active fractions.

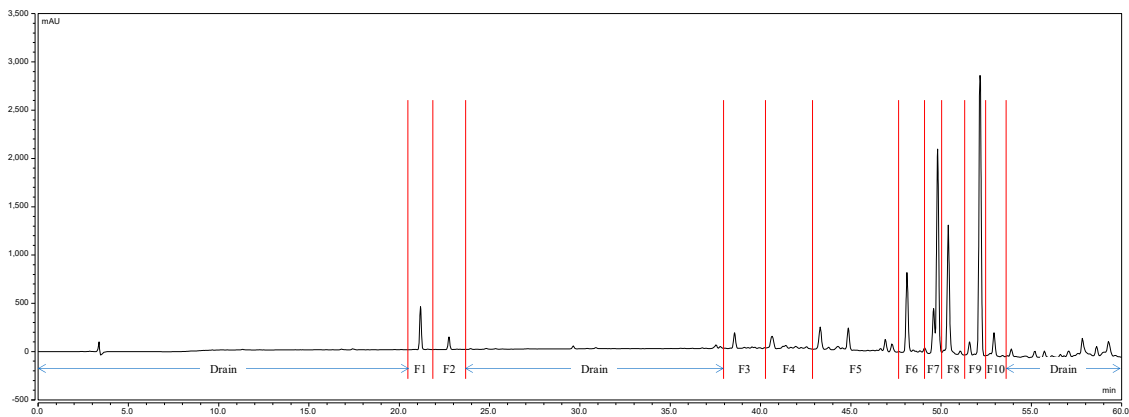


Figure S2: Separation of fractions from *H. fusiformis* EA fraction using Prep-HPLC. "Drain section" refers to areas where no peak is detected during the washing phase.

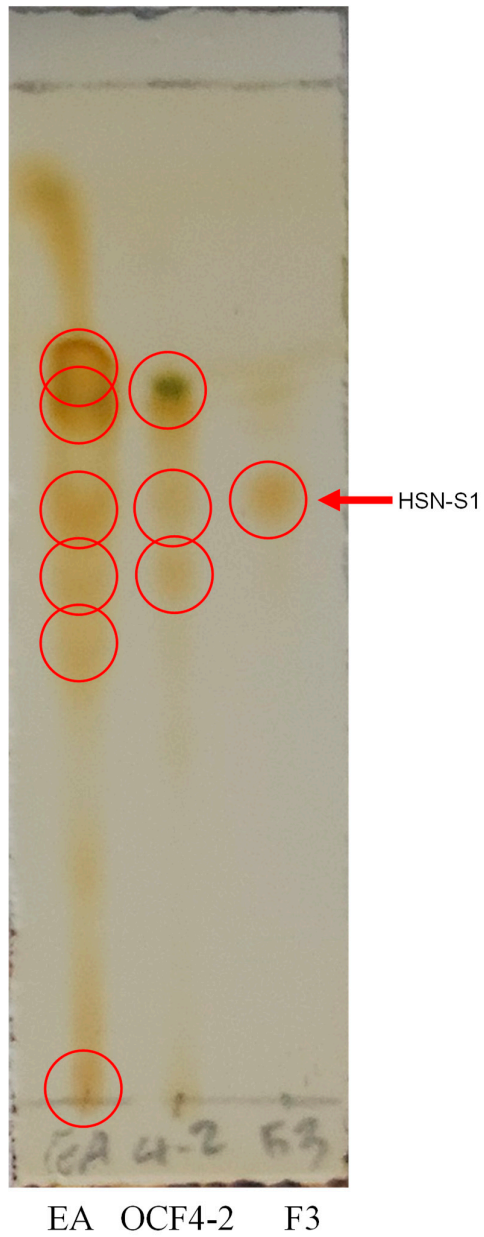
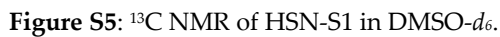
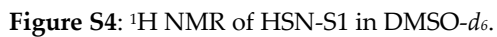


Figure S3: TLC analysis results of *Hizikia* EA fraction using silica gel chromatography fractions.



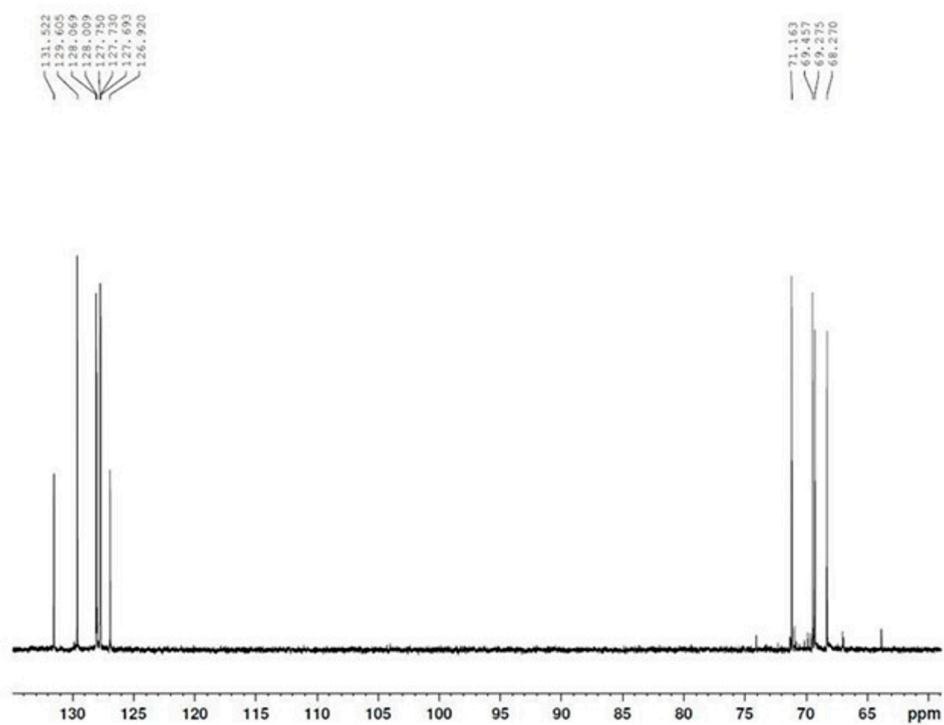


Figure S6: DEPT-90 of HSN-S1 in DMSO- d_6 .

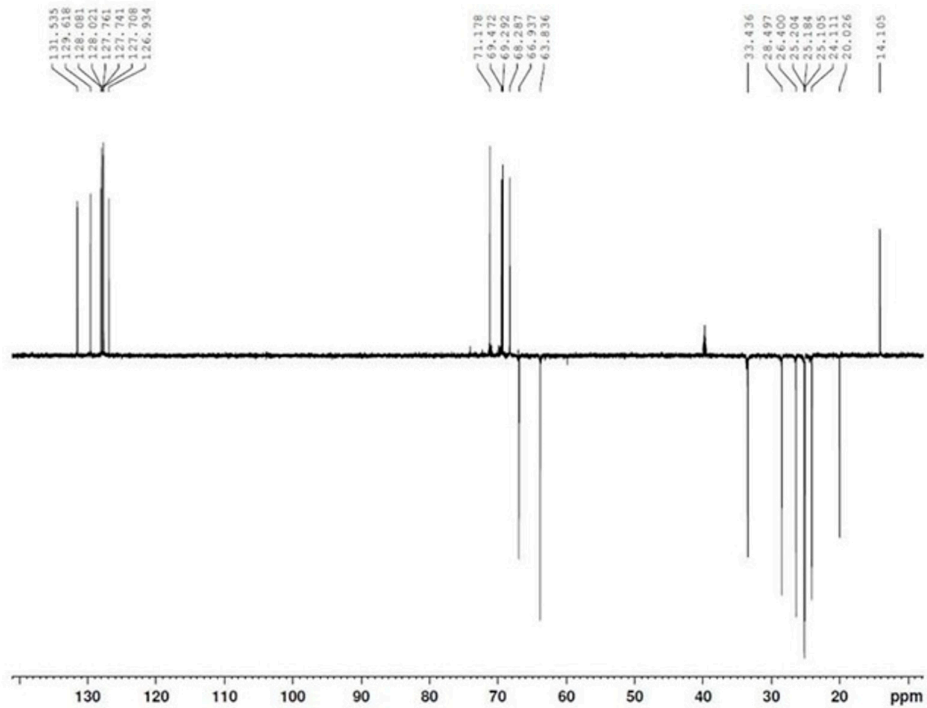


Figure S7: DEPT-135 of HSN-S1 in DMSO- d_6 .

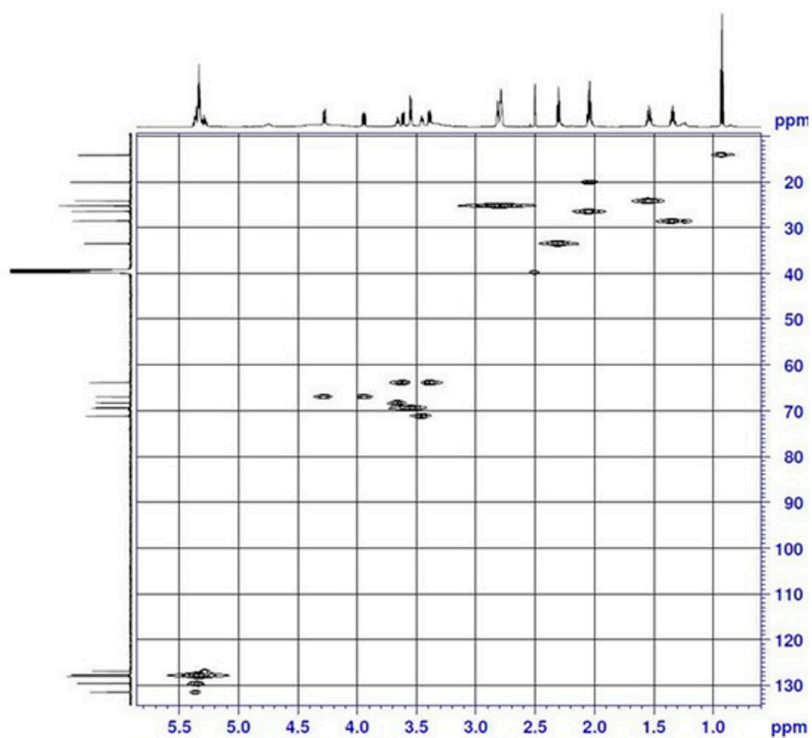


Figure S8: HSQC of HSN-S1 in DMSO- d_6 .

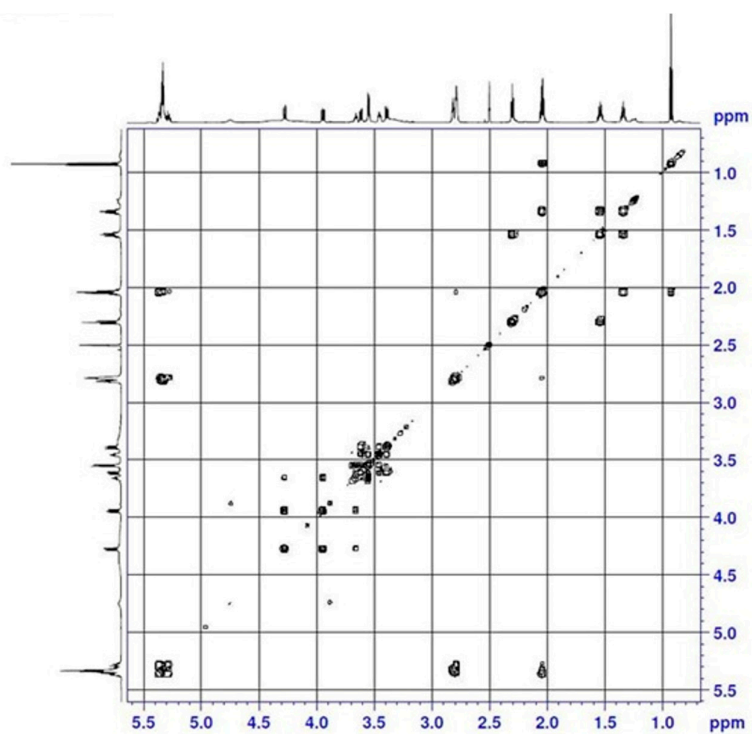


Figure S9: COSY of HSN-S1 in DMSO- d_6 .

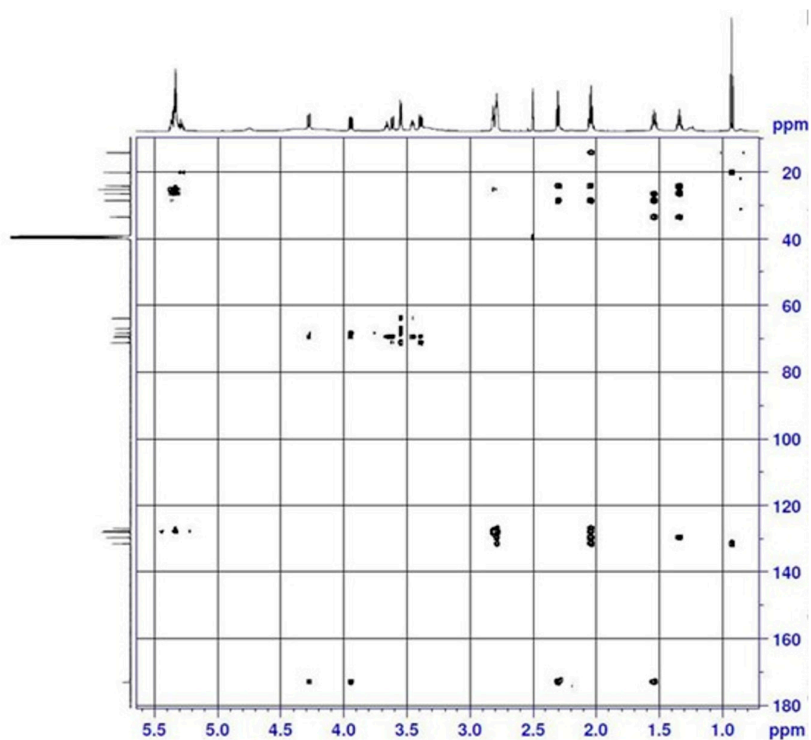


Figure S10: HMBC of HSN-S1 in DMSO-*d*₆.

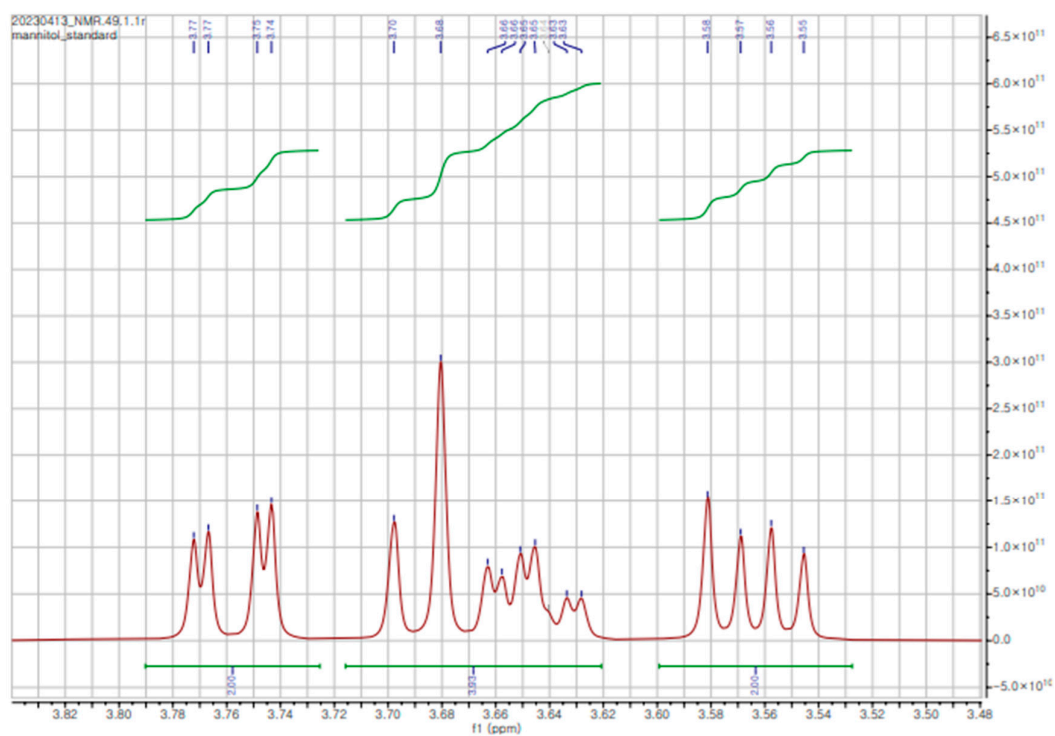


Figure S11: ¹H NMR of mannitol standard in D₂O.

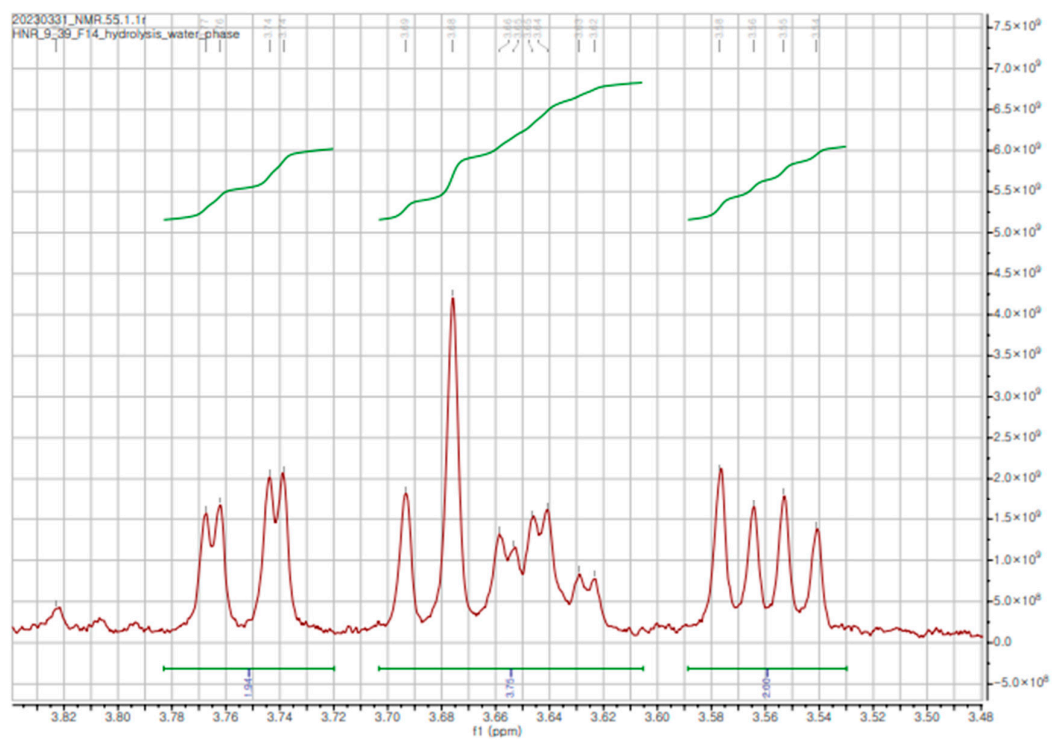


Figure S12: ^1H NMR of the HSN-S1 sugar alcohol in D_2O .

Table S1: Results of cytotoxicity analysis on HSN-S1.

Cell	Dose range for cell treatment ($\mu\text{g/mL}$)	CC ₅₀ ($\mu\text{g/mL}$)	Remarks
HaCaT	1.0 to 100.0	24.2 \pm 0.1	
RAW264.7	1.0 to 100.0	54.4 \pm 1.8	
Splenocytes	1.0 to 100.0	62.8 \pm 0.2	
Naïve CD4 ⁺ Th	1.0 to 100.0	> 100	
Jurkat	1.0 to 100.0	50.3 \pm 0.1	
RBL-2H3	0.3 to 100	14.6 \pm 0.4	
Jurkat Lucia-NFAT	1.0 to 100.0	57.9 \pm 2.8	Luciferase activity of transfected cells

Table S2: HPLC gradient elution conditions for *H. fusiformis* EA fraction.

Time (min)	Flow (mL/min)	% D.W.	% ACN
0.0	1.0	100.0	0.0
53.0	1.0	0.0	100.0
58.0	1.0	0.0	100.0
60.0	1.0	100.0	0.0

D.W: distilled water, ACN: acetonitrile.

Table S3: Effect of different subfractions separated from *H. fusiformis* on the production of IL-2 in anti-CD3 ϵ -induced splenocytes and expression of NF- κ B in LPS-induced THP-1 Lucia™ NF- κ B cells.

Fraction No	Splenocytes (% of Anti-CD3 ϵ group)	THP-1 NF κ B (% of LPS group)
F1	48.13 \pm 0.01	36.03 \pm 0.63
F2	39.05 \pm 0.66	71.34 \pm 0.19
F3	30.53 \pm 0.15	20.48 \pm 0.38
F4	39.34 \pm 2.04	80.77 \pm 0.48
F5	65.69 \pm 4.09	31.24 \pm 0.10
F6	45.24 \pm 3.76	84.10 \pm 0.30
F7	54.63 \pm 6.29	84.07 \pm 0.32
F8	75.06 \pm 8.15	76.69 \pm 0.94
F9	106.55 \pm 4.11	61.46 \pm 0.57
F10	61.22 \pm 2.63	80.26 \pm 0.87

Table S4: Elution conditions for silica gel open column chromatography (A) and Prep-HPLC gradient elution (B) for purification of anti-AD compounds.

(A)

Fraction No	Solvent	BV
OCF1	DCM	2.5
OCF2	DCM:EA=1:1	2.5
OCF3	EA	2.5
OCF4-1	DCM:MeOH=7:1	2.5
OCF4-2	DCM:MeOH=7:1	2.5
OCF5 (wash)	MeOH	2.5

BV: bed volume, DCM: dichloromethane, EA: ethyl acetate, and MeOH: methanol.

(B)

Time (min)	Flow (mL/min)	% D.W	% ACN
0.0	1.0	50.0	50.0
53.0	1.0	0.0	100.0
58.0	1.0	0.0	100.0
60.0	1.0	50.0	50.0

D.W: distilled water and ACN: acetonitrile.

Table S5: Primer sequences for quantitative reverse transcription polymerase chain reaction.

Species	Gene	Direction	Sequence (5' to 3')	Accession
Human	TNF- α	Forward	5'- CTC TTC TCC TTC CTG ATC GT	NM_000594.4
		Reverse	5'- CTG GTT ATC TCT CAG CTC CA	
	MCP-1	Forward	5'- TAG AAG AAT CAC CAG CAG CA	NM_002982.4
		Reverse	5'- GTC TTC GGA GTT TGG GTT TG	
	CXCL10	Forward	5'- CTA AGT GGC ATT CAA GGA GT	NM_001565.4
		Reverse	5'- AGA CCT TTC CTT GCT AAC TG	
	β -actin	Forward	5'- ACC TGA CTG ACT ACC TCA TG	NM_001101.5
		Reverse	5'- CTC ATT GCC AAT GGT GAT GA	
Mouse	IL-1 β	Forward	5'- CTG TGT AAT GAA AGA CGG CA	NM_008361.4
		Reverse	5'- TAT GTC CTG ACC ACT GTT GT	
	IL-2	Forward	5'- CAC TTC AAG CTC CAC TTC AA	NM_008366.3
		Reverse	5'- AGT CAA ATC CAG AAC ATG CC	
	IL-4	Forward	5'- TGA GAG AGA TCA TCG GCA TT	NM_021283.2
		Reverse	5'- TGA TGT GGA CTT GGA CTC AT	
	IL-9	Forward	5'- TGA CAT ACA TCC TTG CCT CT	NM_008373.2
		Reverse	5'- AGT CTT GAT TTC TGT GTG GC	
	IL-10	Forward	5'- ACA ATA ACT GCA CCC ACT TC	NM_010548.2
		Reverse	5'- CCA CTG CCT TGC TCT TAT TT	
	IL-13	Forward	5'- TCT GAC CCT TAA GGA GCT TA	NM_008355.3
		Reverse	5'- TAC AGA GGC CAT GCA ATA TC	
	IL-17A	Forward	5'- CTC CAG AAT GTG AAG GTC AA	NM_010552.3
		Reverse	5'- AAC AGA ATT CAT GTG GTG GT	
	IL-21	Forward	5'- CCT GAA CTT CTA TCA GCT CC	NM_021782.3
		Reverse	5'- ATC ACA GGA AGG GCA TTT AG	
	IL-22	Forward	5'- CTC CCC CAG TCA GAC AGG TT	NM_016971.2
		Reverse	5'- AAA CAG CAG GTC CAG TTC CC	
	IL-33	Forward	5'- CTG TTG ACA CAT TGA GCA TC	NM_001360725.1
		Reverse	5'- CCA GAT GTC TGT GTC TTT GA	
	COX-2	Forward	5'- CGA TGT CAT GGA ACT GTA CC	NM_011198.5
		Reverse	5'- ATC TTA AAA CCC ACT TCG CC	
	GATA3	Forward	5'- TCG TAC ATG GAA GCT CAG TA	XM_030247533.2
		Reverse	5'- AAG AGA TGA GGA CTG GAG TG	
	β -actin	Forward	5'-CAT CAA AGA GAA GCT GTG CT	NM_007393.5
		Reverse	5'-GAA GGA AGG CTG GAA AAG AG	