

Appendix

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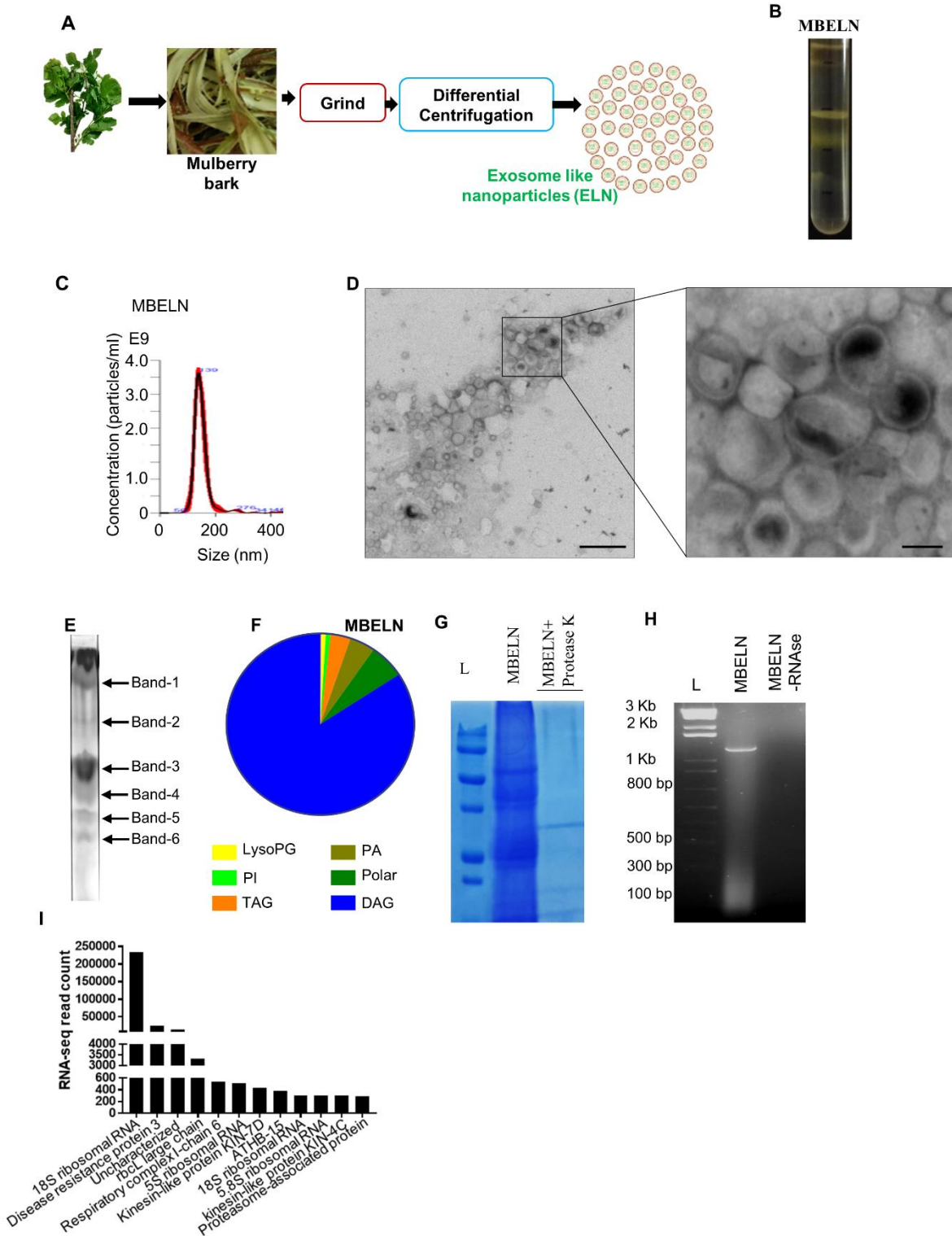
Exosome-like nanoparticles from Mulberry bark prevent DSS-induced colitis by AhR/COPS8 pathway

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Table of contents:

S.No.	Description	Page number
1.	Appendix figure S1	2
2.	Appendix figure S2	4
3.	Appendix figure S3	6
4.	Appendix figure S4	8
5.	Appendix Table S1: List of primers	9
6.	Appendix Table S2: List of antibodies	11

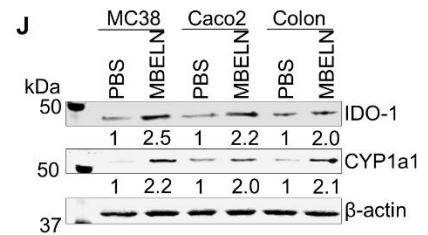
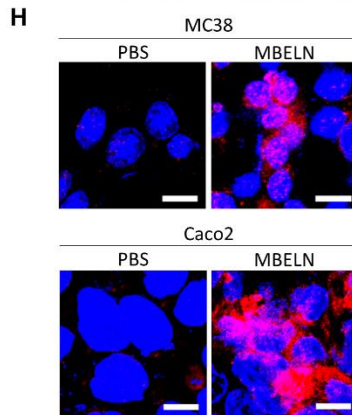
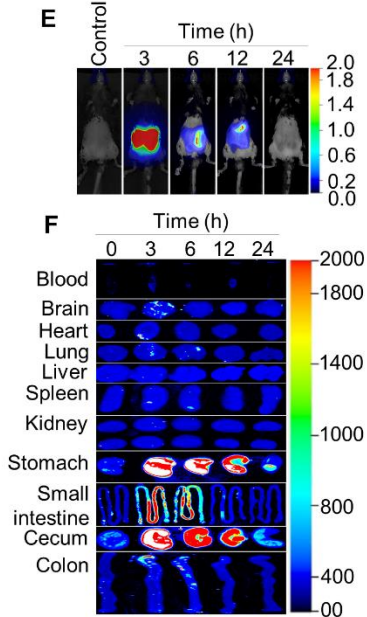
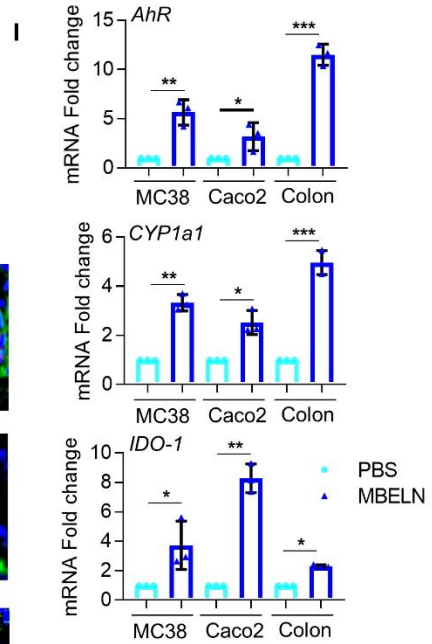
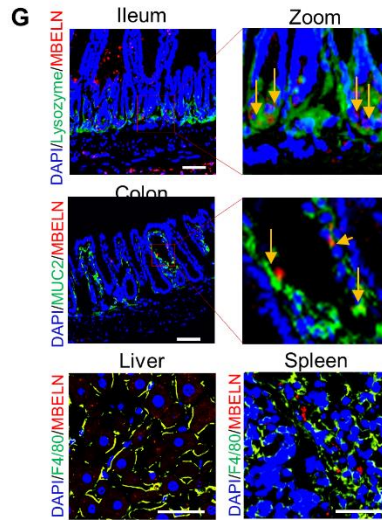
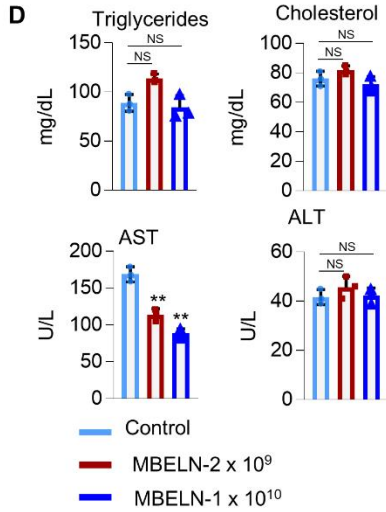
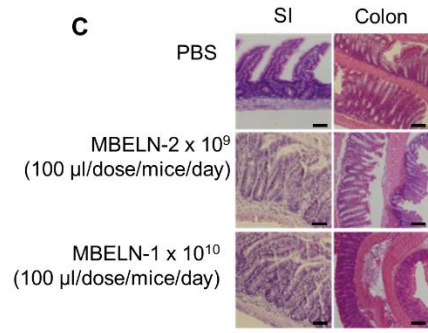
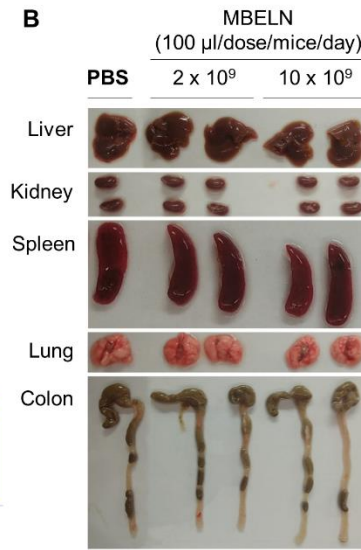
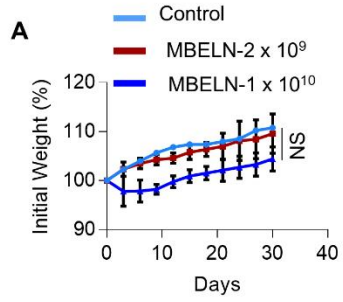
Appendix figures



Appendix fig S1. Preparation, characterization and phenotypic effect of mulberry bark derived exosome-like nanoparticles (MBELN).

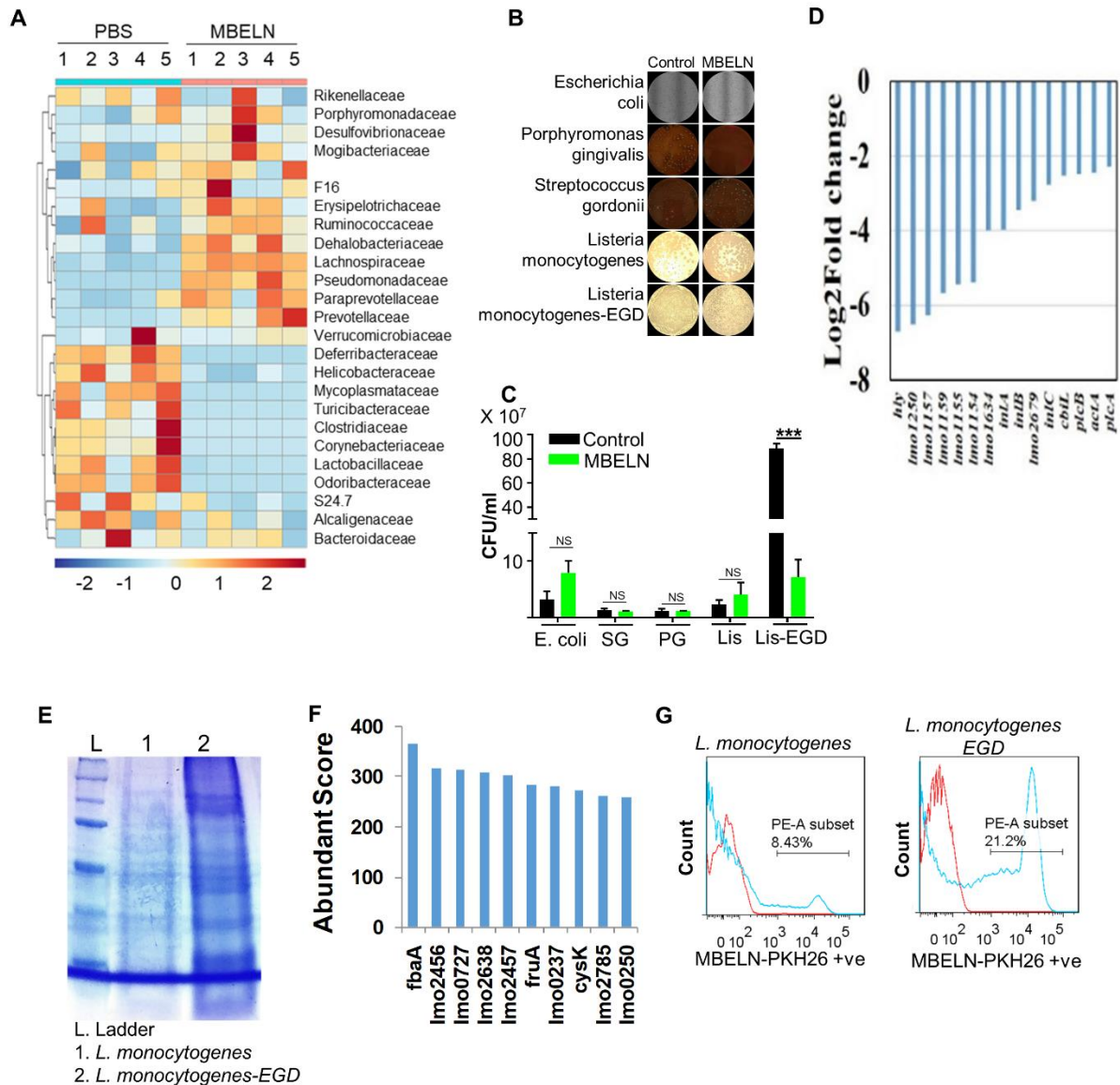
(A) Schematic diagrams of the MBELN preparation process.

- (B) Size of MBELNs on the sucrose gradient. (n=3 biological replicates)
- (C) Graph showing size of MBELNs using NS300 NanoSight. (n=3 biological replicates)
- (D) Electron microscopy picture showing bilayer structure and size of MBELNs. Scale bar, 500 μ m (zoom scale bar 100 μ m). (n=3 biological replicates)
- (E) Thin layer liquid chromatography for lipid derived from MBELNs. (n=3 biological replicates)
- (F) Lipid profile showing differential lipid composition in MBELNs. (n=3 biological replicates)
- (G) Polyacrylamide gel electrophoresis showing presence of protein in MBELNs. (n=3 biological replicates)
- (H) Visualization of total RNA on 1% agarose gels. (n=3 biological replicates)
- (I) mRNA Seq analysis of MBELN derived RNA. (n=3 biological replicates)



Appendix fig S2.

- (A) Graph showing changes in initial weight of mice while treated with mulberry bark derived exosome-like nanoparticles (MBELN) at two different doses. Data are mean \pm SEM of five biological replicates, NS- non-significant using Mann–Whitney test.
- (B) Pictorial representation of morphological changes in internal organs while mice are treated with MBELN. (n=5 biological replicates)
- (C) Hematoxylin and eosin (HE) staining to show histological changes due to MBELN administration. Scale bar 100 μ m, n=5 biological replicates.
- (D) Graph showing plasma level of triglycerides, total cholesterol, aspartate transaminase (AST) and alanine aminotransferase (ALT) in mice treated with MBELN. Data are mean \pm SEM of three biological replicates **P<0.01, NS- non-significant using one-way ANOVA.
- (E) *In vivo* imaging showing trafficking of DiR labeled MBELN. (n=5 biological replicates)
- (F) *Ex vivo* imaging showing localization of DiR labeled MBELNs in different organs. (n=5 biological replicates)
- (G) Distribution of PKH26 labelled MBELNs *in vivo* in C57BL/6 mice ileum (Paneth cells- yellow arrow), colon, liver and spleen tissues following oral administration of MBELNs. Scale bar, 100 μ m (n=3 biological replicates).
- (H) Confocal microscopy showing expression of total AhR in MC38 cells and Caco2 cells while being treated with MBELNs. Scale bar, 10 μ m (n=3 biological replicates).
- (I) *mRNA* expression of aryl hydrocarbon receptor (*AhR*), *Cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1a1)* and Indoleamine 2,3-dioxygenase (*IDO-1*) in MC38, Caco2 cells and colon epithelial cells. Data are mean \pm SEM. (n=3 biological replicates) *P<0.05, **P<0.01, ***P<0.001 using Student's t-test.
- (J) Western blot analysis of CYP1a1 and IDO-1 in MC38 cells, Caco2 cells and mice colon epithelial cells following administration of MBELN. Data are mean \pm SEM of three biological replicates, Student's t-test.



Appendix figure S3.

(A) Effect of Mulberry bark derived exosome-like nanoparticles (MBELN) on microbiome profile of fecal bacteria at family level. (n=5 biological replicates)

(B-C) Bacteriostatic effect of MBELNs evaluated on *Escherichia coli* (*E. coli*), *Porphyromonas gingivalis* (PG), *Streptococcus gordonii* (SG), *Listeria monocytogenes* (Lis) and *Listeria monocytogenes-EGD* (Lis-EGD). Data are mean \pm SEM. ***p < 0.001, NS- non-significant using Student's t test.

(D) Change in mRNA level in *Listeria (L.) monocytogenes-EDG* compared to control (no treatment). (n=3 biological replicates)

(E) Virulent vs non-virulent *Listeria* proteins that bind with biotin labelled MBELNs and shown on an agarose gel. (n=3 biological replicates)

(F) Mass spectrometry (MS) analysis of protein samples: *Listeria (L.) monocytogenes* and *L. monocytogenes-EGD* total protein binding with biotin labelled MBELN protein. Top 10 most

abundant proteins from the virulent strain of *L. monocytogenes* shown interacting with MBELNs.
(n=3 technical replicates)

(g) Uptake of PKH26 labelled MBELNs in the virulent and non-virulent strains of *L. monocytogenes*. (n=3 biological replicates)

A

>>HSP90_Human 853 bp
 Waterman-Eggert score: 3289; 682.9 bits; E(1) < 1.6e-200 (853 aa)
 71.2% identity (87.1% similar) in 720 aa overlap (8-703:137-853)

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10      20      30      40      50      60
HSPAB_ ETETFAFAQAEIQLLSLIINFYSNKEIFRLRELISNASDALDKIRFESLTKSKLDAQPE
HSP90_ EVETFAFAQAEIQLLSLIINFYSNKEIFRLRELISNASDALDKIRYRESLTPSKLDSGKE
140     150     160     170     180     190
HSPAB_ LFIKLVDPKASKTSLIISDGIKADLVNNGITARSGETKFMALQAGADVSMIGQFG
HSP90_ LHIHLIPNKQORTLTIVDTGIGMTKADLNNLGTIAKSGTKAFMEALQAGADISMIQFG
200     210     220     230     240     250
HSPAB_ VGFYSALVAEKVIVTKHNDDQVIMESQAGGSFVTRDTEGEQLGRGTITLFLKADQ
HSP90_ VGFYSALVAEKVIVTKHNDDQVIMESQAGGSFVTRDTEGEQLGRGTITLFLKADQ
260     270     280     290     300     310
HSPAB_ LEYLEERRKDLVKKHSEFISYIPLWTKTEKEISDDEEIEKKKEE-----
HSP90_ TEYLEERRKDLVKKHSEFISYIPLWTKTEKEISDDEEIEKKKEE-----
320     330     340     350     360     370
HSPAB_ -GDVEDV--DEEKESK---KRRKVKVSEHQLVKNQKPIWLRKPEITKDEYAFYK
HSP90_ KPEIEDVSGDEEKKDKGDKKKKIKKIKYIQEELNKKPIWLRKPEITKDEYAFYK
380     390     400     410     420     430
HSPAB_ SLTNDWEDHLAVKHFVSEVQLEFKAILFVPRAPFDLFDTRKKTNNIKLYRVRVIMDNC
HSP90_ SLTNDWEDHLAVKHFVSEVQLEFKAILFVPRAPFDLFDTRKKTNNIKLYRVRVIMDNC
440     450     460     470     480     490
HSPAB_ EELIPEYLGFKGVVSDPLNISREMLQNKILKIRKLVKCKIEMFNEIAENKEDY
HSP90_ EELIPEYLGFKGVVSDPLNISREMLQNKILKIRKLVKCKIEMFNEIAENKEDY
500     510     520     530     540     550
HSPAB_ AKFYDAFSKNLKGIHEDSQNRKALADLLRHYSTKSGDEMSTLKDVTVMKEGQKDIYYI
HSP90_ KKFYEQFSKNLKGIHEDSQNRKALADLLRHYSTKSGDEMSTLKDVTVMKEGQKDIYYI
560     570     580     590     600     610
HSPAB_ TGESKKAVENTSPFLERLKKGGYELVYMDAIDEYAVAGLKEYDGKLVSATKEGLKLDDE
HSP90_ TGETKQVANSFAVRLRKHGLEVIYMIPEIDYCVQQLKEFEGKLVSVTKEGLELP-E
620     630     640     650     660     670
HSPAB_ TEEEEKKKEKKSFENLCKTIKIDLGDKVEKVVSDRIVDSCCLVTGEYGTANMERI
HSP90_ DEEEKKKEKKSFENLCKTIKIDLGDKVEKVVSDRIVDSCCLVTGEYGTANMERI
680     690     700     710     720     730
HSPAB_ MKAQALRDSMSYSSKKTMEINPDNGIMEELRKRKRAVDKNDKSVKDLVLLFETALLT
HSP90_ MKAQALRDSMSYSSKKTMEINPDNGIMEELRKRKRAVDKNDKSVKDLVLLFETALLS
740     750     760     770     780     790
HSPAB_ SGFSLDPPNTFASRIHRMLKGLSIDDEAGADDT-----DMPPLEEAGNEESKMEVD
HSP90_ SGFSLDPPQTHANRIYRMKIKLGLSIDDEDDPTADDTSAAVTEEMPLE-GDDDTSRMEVD
800     810     820     830     840     850

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B

Score	Expect	Method	Identities	Positives	Gaps
287 bits(735)	4e-93	Compositional matrix adjust.	174/499(35%)	272/499(54%)	18/499(3%)
Query 28	AIGIDIGTSQCSVAVWNGSQVELLKNTRNQKMMRSVYTKDDIPSGGVSNQLSNEHEMLS				87
Sbjct 6	A+GID+GT+ V V+ +VE++ N + + SYV + D + + N+ M				63
Query 88	GAAIFNMKRLVGRVDTDPVVAHAKCSLPLVQTLDIGVRFIAALVNNHRSITPEEVMMA				146
Sbjct 64	+F+ KRL+GR D VV + K PF+V D G RP + +S PEEV +				121
Query 147	IFLVELRAMAELQKLRPIRHVLTIPVSGFRFQLTRIERACAMAGHLVRLMPEPTAVAL				206
Sbjct 122	+ L +++ +AE L + + + V+T+P F Q + + A +AGL+VLR++ EPTA A+				181
Query 207	LYAQQQQTTTHDNMGSGSEKIALIFNMGAGYTDVAVTATAGGVSQIRALAGSA-IGGEDL				265
Sbjct 262	Y ++ G+E+ LIF++G G DV++ G+ ++++ AG +GGED				233
Query 186	LQNMWHYLLPNADTLFSSHSINEIKAMGLRVAQTQDAIHKLSQTQTSVPIINLD-LGMGSKI				324
Sbjct 234	M+++ + +A+ LR A + A LS+ T I +D L G				293
Query 325	NKVLHRQEFEEVNRKVFKECETLVQCLHDAKVEIEDVNDVIVVGGCSYIPKVDLVTSA				384
Sbjct 294	+ R FEE+N +F V + L DAK++ ++D+++VGG + IPK++ L+				353
Query 385	CKRKELYKGMNPLEAAVCGAALLEGAVASISDPFGNL-DLLTIQIATLAIGIRADGGNFI				443
Sbjct 354	KEL K +NP EA GAA++ A+ SG D N+ DLL + + L++GI GG				411
Query 444	PIIPRNTTMAPKEMTFTTADHNTQEAIVVYEGEGQKVENHLLGYFKLVGIPPAKGA				503
Sbjct 412	+I RNTT+P ++ TFTT DNQ LI VYEGE ++N+LLG F++ GIPPAP+G				471
Query 504	PEISVCMVDVDSANLVRVFA 522		Query= Mulberry HPA8		
Sbjct 472	P+I V D+DA+ +L V A 490		Subject= Human HSPA8		

Appendix fig S4.

(A) Comparison and alignment of amino acid sequence of mulberry heat shock protein family A (Hsp70) member 8 (HSPA8) protein and human heat shock protein HSP90aa1.
 (B) Comparison and alignment of amino acid sequence of mulberry and human heat shock protein family A (Hsp70) member 8 (HSPA8) protein.

Appendix Table S1: List of primers

Gene name	Forward sequence (5'-3')	Reverse sequence (5'-3')
Real-time PCR primers sequence for Human		
AhR	CAAATCCTTCCAAGCGGC ATA	CGCTGAGCCTAAGAAGCTGAA AG
CYP1a1	ATCCTGGAGACCTTCCGAC A	ACAAAGACACAACGCCCTT A
IDO1	TTCAGTGCTTTGACGTCCT G	TGGAGGAACTGAGCAGCAT G
Real-time PCR primers sequence for Mouse		
AhR	GGCTTTCAGCAGTCTGATG TC	CATGAAAGAAGCGTTCTCTGG TC
CYP1a1	CCTCATGTACCTGGTAACC A	AAGGATGAATGCCGGAAGGT A
IDO1	TGAGCATTGCAAGGAAAG TG	TATAGGCCATCAGGCAGTCC TG
Defa-rs1	CACCACCCAAGCTCCAAA TACACAG	ATCGTGAGGACCAAAAAGCAA ATGG
Defa1	TGCCTGCTCATCCTAATCC	GCTCCTCAGTTTTAGTCTCTTC
Defa22	TCCAAAACACAGATGAAG AGAC	GGCAGATCAGATCTCTCGAC AGAC
Defa21	CCAGGGGAAGATGACCAG GCTG	TGCAGCGACGATTTCTACAAA GGC
CRS1C	CACCACCCAAGCTCCAAA TACACAG	ATCGTGAGGACCAAAAAGCAA ATGG
CRS4C	TCGCAGCCATGAAGAAAC	CAAAAGAGACAGACACAGCC
Reg3b	TTCCTGTCCTCCATGATCA AAA	CATCCACCTCTGTTGGGTTCA AAA

REG3g	ATGCTGCTCTCCTGCCTGA TG	CTAATGCGTGCGGAGGGTATA TTC
Ang4	ACTCTGGCTCAGAATGAA AGGT	TCACAGTATCTGTTCGTCCTCCG
LYZ2	TCAGCACGAGAGCAATTA TAAC	TTGCCATCATTACACCAGTAT C
COPS8	AAGGAGACGCGCCTTTGC C	GTGGAAGAGGCTGTGAAAGG C
TNF- α	TCTATGGCCCAGACCCTCA C	GACGGCAGAGAGGAGGTTGA C
IL-17A	TTTAACTCCCTTGGCGCAA AA	CTTTCCCTCCGCATTGACAC
IFN- γ	TCAGCAACAGCAAGGCGA AAAAGG	CCACCCCGAATCAGCAGCGA

Gene cloning primers

Human AhR	ATCATCATCACAGCAGCG GCGCTCTGAATGGCTTTGT ATTAGTTG	AAGGTTCTTTAAGTAATCTGT CACGTATTTTCGTTTTTCGTA TGC
HSPA8 (Mulberry)	AAAATCTATACTTCCAAGG AGACTACAAAGACGATGA CGACAAGATGGCTGAACA AGCATAAC	TAGCAGCCTGTACTGAGGGAC TATATTTTCTTCTGAACACTG ACC

AhR: aryl hydrocarbon receptor, CYP1a1: cytochrome P450 family 1 subfamily A member 1; Ahrr: aryl-hydrocarbon receptor repressor; Arnt: aryl hydrocarbon receptor nuclear translocator; IDO1: indoleamine 2,3-dioxygenase 1; Defa-rs1: defensin, alpha, related sequence 1; Defa: defensin, alpha; CRS: cryptdin-related sequence; Reg: regenerating islet-derived; Ang: angiogenin; LYZ: lysozymes; COPS8: COP9 constitutive photomorphogenic homolog subunit 8; HSPA8: heat shock protein family A (Hsp70) member 8; ZO-1: Zonula occludens-1; TNF- α : Tumor necrosis factor- α ; IL-17A: Interleukin 17A; IFN- γ : Interferon gamma;.

Appendix table S2: List of Antibodies and their details

S. No.	Antibody-	Application;	Company	(Catalog)
1.	COPS8-	WB, IF, IP;	Abcam	ab77300
2.	COPS5-	WB;	BIOMOL	PW8365
3.	COPS6-	WB;	Santa Cruz	Sc-393023
4.	COPS7-	WB;	Santa Cruz	sc-398882
5.	Cullin 1-	WB;	EPITOMICS	2436-1
6.	Cullin 3-	WB;	EPITOMICS	2506-1
7.	CYP1a1-	WB;	Invitrogen	PA5-15213
8.	IDO-1-	WB;	Proteintech	66528-1-IG
9.	AhR-	WB, IF, IP;	Invitrogen	MA1-514
10.	Nf-kB-	WB;	BD Biosciences	610869
11.	β -actin-	WB;	Santa Cruz	sc-47778
12.	α -tubulin-	WB;	Santa Cruz	sc-5286
13.	Zonula occludens-1 (ZO-1)-	WB;	Invitrogen	33-9100
14.	IL-17a-	ELISA;	eBioscience™	14-7175-81
15.	IL-6-	ELISA;	eBioscience™	14-7069-81
16.	TNF- α -	ELISA;	eBioscience™	14-7325-81
17.	IL-10-	ELISA;	eBioscience™	14-7101-81
18.	IL-1b-	ELISA;	eBioscience™	14-7012-81
19.	IFN- γ -	ELISA;	eBioscience™	14-7313-81
20.	PE anti-mouse IL-17A-	FC;	BioLegend	506904
21.	APC anti-mouse IL-17A-	FC;	BioLegend	506916
22.	FITC anti-mouse IL-17A-	FC;	BioLegend	506908
23.	FITC anti-human IFN- γ -	FC;	BioLegend	502507
24.	APC anti-human IFN- γ -	FC;	BioLegend	506510
25.	PE anti-mouse FOXP3-	FC;	BioLegend	126404
26.	APC anti-CD11b-	FC;	BioLegend	101212
27.	FITC anti-CD11b-	FC;	BioLegend	101206
28.	PE anti-CD11b-	FC;	BioLegend	101208
29.	APC anti-CD4-	FC;	BioLegend	100412
30.	FITC anti-CD4-	FC;	BioLegend	100406
31.	PE anti-CD4-	FC;	BioLegend	100512
32.	PE/Cyanine7 anti-CD3-	FC;	BioLegend	100220
33.	APC/Cyanine7 anti-CD3-	FC;	BioLegend	100222
34.	PE/Cyanine7 anti-Gr-1-	FC;	BioLegend	108416
35.	FITC anti-Ly-6G-	FC;	BioLegend	127606
36.	PE anti-Ly-6G-	FC;	BioLegend	127608
Abbreviation: WB-Western blot; IF-Immunofluorescence; IP-Immuno-precipitation; FC- Flow cytometry				