

SUPPLEMENTARY DATA

Supplementary Table 1. Diet fatty acid composition

Fatty acid	Control diet (percent of total)	DHA supplemented diet (percent of total)
20:5n3	0.00	9.49
18:3n3	7.16	6.46
18:3n6	0.00	0.00
22:6n3	0.00	8.90
16:1n7	0.00	6.73
20:4n6	0.00	0.00
22:5n3	0.00	0.00
18:2n6	45.68	21.88
20:3n6	0.00	0.00
16:0	18.98	26.19
18:1n9	22.88	19.40
18:0	5.31	0.95
n6/n3 ratio	6.38	0.88

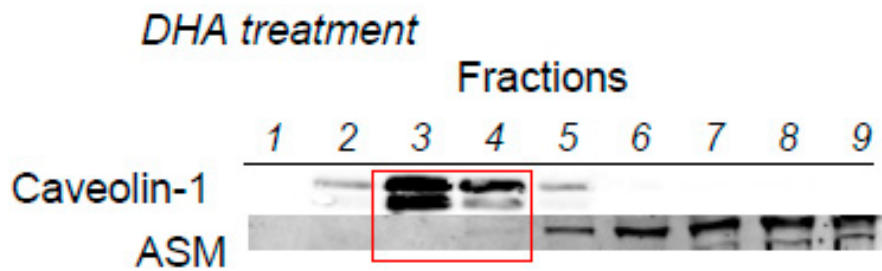
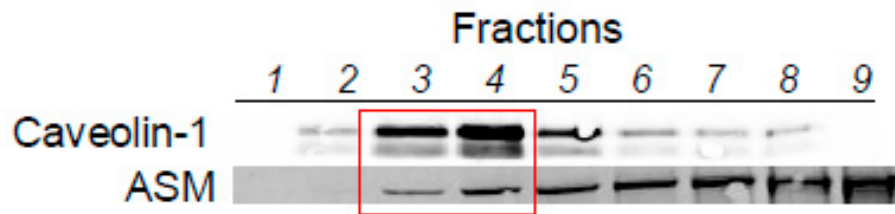
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Supplementary Table 2. RT-PCR primers

Gene name	Sense sequence	Anti-sense sequence
Rat ASM	caactatgggctgaagaagga	acagctgactggcacacatt
Rat ICAM-1	ccaccatcactgtgtattcggt	acggagcagcactactgaga
Rat VEGF A	gctctcttgggtgcactgg	caccacttcatgggctttct
Rat IL-1 β	caaggagagacaagcaacga	gtttgggatccacactctcc
Rat Cyclophilin	cttcttctggtcttgcattct	tggatggcaagcatgtggtctttg
Mouse ASM	aacctggctaccgagttaccaa	tggcctgggtcagattcaagatgt
Mouse ICAM-1	acactatgtggactggcagtgggt	tgaggctcgattgttcagctgcta
Mouse VEGF A	tcaccaaagccagcacataggaga	ttacacgtctcggatcttggaca
Mouse IL-1 β	aagggtgcttccaaaccttgac	atactgctgcctgaagctcttgt
Mouse VCAM-1	cccaggtggaggtctactca	ccagatgggtcaaggataca
Mouse TNF α	tctcatgcaccaccatcaagg	accactctcccttgcagaac
Mouse Cyclophilin	attcatgtgccaggggtgga	ccgtttgtgggtccagca
Human ASM	caacctcgggctgaagaa	tccaccatgtcatcctcaaaa
Human IL-1 β	ggcctcaaggaaaagaatc	ttctgcttgagaggtgctga
Human TNF α	tccttcagacacctcaacc	aggccccagttgaattctt
Human Cyclophilin	caagactgagtgggtgatgg	tggtgatcttctgctggtct

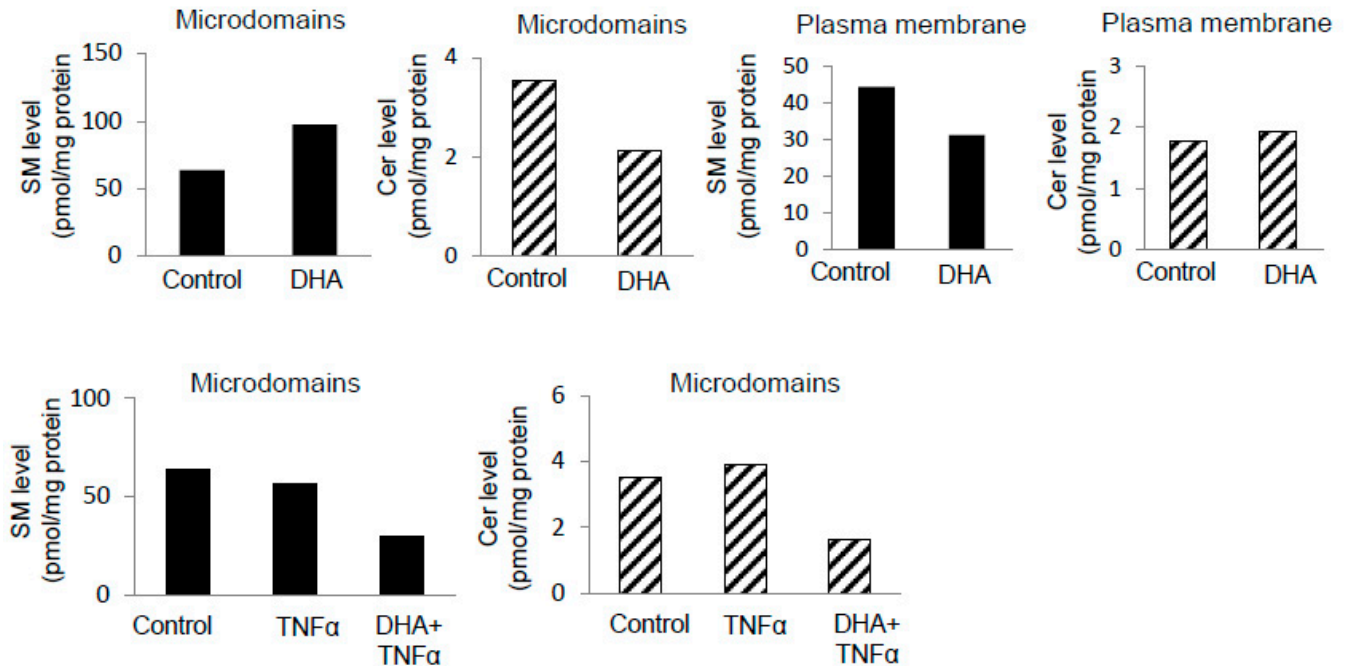
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Supplementary Figure 1. ASM protein expression in caveolae and non-caveolae fractions isolated from control and DHA-treated HREC. Caveolae fractions were isolated by sucrose discontinuous gradient ultracentrifugation based on their insolubility in Triton-X-100 at 4°C and identified by western blotting as fractions 3-4 based on the caveolin-1 (a marker for caveolae) distribution



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Supplementary Figure 2. Absolute values of Sphingomyelin (SM) and ceramide (Cer) species in caveolae and plasma membrane fractions. Sphingomyelin and ceramide levels were determined by tandem mass spectrometry precursor ion mode scanning for the characteristic ceramide product ion at m/z 264.4 and sphingomyelin species by positive ion mode PI m/z 184 after alkaline hydrolysis of glycerophospholipids. The data is presented from HREC isolated from one representative donor.



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Supplementary Figure 3. Retinal gene expression in ASM^{-/-} and ASM^{+/+} mice. Extensive quantitative PCR analysis of retinas isolated from ASM^{+/+} and ASM^{-/-} for angiogenic and pro-inflammatory molecules. Ang1= Angiopoietin 1; Ang2 = Angiopoietin 2; Anp =Alanyl (membrane) aminopeptidase; Bai1= Brain-specific angiogenesis inhibitor 1; Ccl2= Chemokine (C-C motif) ligand 2; Cdh5= Cadherin 5; Col18= collagen type VIII, alpha 1; Col4= collagen type IV, alpha 3; Csf = Colony stimulating factor 3; Cxcl1 = Chemokine ligand 1; Cxcl2 = Chemokine ligand 2; Cxcl5 = Chemokine ligand 5; Tymp = Thymidine phosphorylase; S1pr1 = Sphingosine 1 phosphate receptor1; Efna1= Ephrin A1; Efnb2= Ephrin B2; Egf= Epidermal growth factor; Eng= Endoglin; Epas1= Endothelial PAS domain protein 1; Ephb4= Eph receptor B4; Ereg = Epiregulin; F2= Coagulation factor II; Fgf2= Fibroblast growth factor 1; Fgf6 = Fibroblast growth factor 6; Fgfr3= Fibroblast growth factor 3; Figf= C-fos induced growth factor; Flt1= FMS-like tyrosine kinase; Hand2= Heart and neural crest derivatives expressed transcript 2; Hgf= Hepatocyte growth factor; Ifgn= Interferon gamma; Igf1 = Insulin-like growth factor 1; IL-1b = Interleukin 1 beta; IL-6 = Interleukin 6; Itgav = Integrin alpha V; Itgb3= Integrin beta 3; Jag1 = Jagged 1; Kdr = Kinase insert domain protein receptor; Lama5= Laminin alpha 5; Lect1= Leukocyte cell derived chemotaxin 1; Lep = Leptin; Mapk14 = Mitogen-activated protein kinase 14; Mdk = Midkine; Mmp19 = Matrix metalloproteinase 19; Mmp2 = Matrix metalloproteinase 2; Mmp9 = Matrix metalloproteinase 9; Npr1 = Natriuretic peptide receptor 1; Nrp2 = Neuropilin 2; Pdgfa = Platelet derived growth factor alpha; Pecam1 = Platelet/endothelial cell adhesion molecule 1; Pgf= placental growth factor; Plau= Plasminogen activator urokinase; Plg= Plasminogen; Plxdc1= Plexin domain containing 1; Ptgs1= Prostaglandin-endoperoxide synthase 1; Serpin= Serine (or cysteine) peptidase inhibitor; Sphk1 = Sphingosine kinase 1; Stab = Stabilin 1; Tbx1= T-box 1; Tbx4= T-box 4; Tek = Endothelial specific receptor tyrosine kinase; Tgfa = Transforming growth factor alpha; Tgfb1= Transforming growth factor, beta 1; Tgfb2= Transforming growth factor, beta 2; Tgfb3= Transforming growth factor, beta 3; Tgfb1r= Transforming growth factor, beta receptor 1; Thbs1= Thrombospondin 1; Thbs2= Thrombospondin2; Timp2= Tissue inhibitor of metalloproteinase 2; Tsp6= Transmembrane serine protease 6; TNF = Tumor necrosis factor; Tnfai2 = Tumor necrosis factor alpha-induced protein 2; VegfA= Vascular endothelial growth factor A; VegfB= Vascular endothelial growth factor B; VegfC= Vascular endothelial growth factor C; Ccl11= Chemokine (C-C motif) ligand 11; Ctgf = Connective tissue growth factor; Gna13= Guanine nucleotide binding protein, alpha 13; Smad5= MAD homolog 5 (Drosophilla); TNFsf12 = Tumor necrosis factor (ligand) superfamily, member 12; Fgf1= Fibroblast growth factor 1; Fzd5= Frizzled homolog 5 (Drosophilla); Nrp1= Neuropilin 1; Hif1a =Hypoxia inducible factor 1, alpha subunit.

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