Supplementary Data

Supplementary Tables

Table 1S: qRT-PCR Primers

Mouse		Accession#	Forward Primer	Reverse Primer
Collagen1A1	Col1A1	NM_007742.3	TAGGCCATTGTGTATGCAGC	ACATGTTCAGCTTTGTGGACC
Collagen1A2	Col1A2	NM_007743.2	GTCCTAGTCGATGGCTGCTC	AGCACCACCAATGTCCAGAG
Collagen 4A1	Col4A1	NM_009931.2	TTTGGCTCGCCACCATAGAG	GCAGAGGCGAGCATCATAGT
Collagen7A1	Col7A1	NM_007738.3	ACCGCATCTTCTGTTGAGCA	TTCCAAGCCACTCTCACGAC
Sm. muscle Actin	Acta2	NM_007392.2	AAGCCCAGCCAGTCGCTGTCA	AGCCCAGAGCCATTGTCGCA
Lysyl Oxidase	Lox	NM_010728.3	GGGCCAGAACGGCTTGTGTA	TCTGGGAAAGCGCACAGAGT
Lox-Like-1	LoxL1	NM_010729.3	TGTGGGGCTGGCCATGTAAG	AGGACAGGATGGCTCTTCGC
Lox-Like-2	LoxL2	NM_033325.2	AAGTGCAGGAGCCGCTATGA	TGCCAAAGCTCGGATGGGAT
Lox-Like-3	LoxL3	NM_013586.4	CAGTGTGTGGTATTGCTGCCC	TCTGAGAAACCGGGAAGGCG
Cyclophilin		NM_008907	CTTCTTGCTGGTCTTGCCATTCCT	GATGGCAAGCATGTGGTCTTTG
Human				
Collagen1A1	Col1A1	NM_000088.3	AATGGTGCTCCTGGTATTGC	ACCAGGTTCACCGCTGTTAC
Hydroxy methylbilane Synthase	HMBS	NM_000190.3	TCTGCGGAGACCAGGAGTCA	GTAGGCTGTGTGTGGGTGC

Table 2S: MOUSE FIBROSIS ARRAY GENE TABLE

Unigene	GeneBank	Symbol	Description	Gene Name
Mm.213025	NM_007392	**Acta2**	Actin, alpha 2, smooth muscle, aorta	0610041G09Rik, Actvs, SMalphaA, a-SMA, alphaSMA
Mm.301626	NM_007428	**Agt**	Angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	AI265500, AngI, AngII, Aogen, Serpina8
Mm.6645	NM_009652	**Akt1**	Thymoma viral proto-oncogene 1	Akt, PKB, PKB, Akt, PKBalpha, Rac
Mm.257460	NM_009741	**Bcl2**	B-cell leukemia/lymphoma 2	AW986256, Bcl-2, C430015F12Rik, D630044D05Rik, D830018M01Rik
Mm.392150	NM_007557	**Bmp7**	Bone morphogenetic protein 7	OP1
Mm.28278	NM_007616	**Cav1**	Caveolin 1, caveolae protein	Cav, Cav-1
Mm.4686	NM_011330	**Ccl11**	Chemokine (C-C motif) ligand 11	Scya11, eotaxin
Mm.867	NM_011331	**Ccl12**	Chemokine (C-C motif) ligand 12	MCP-5, Scya12
Mm.1282	NM_011337	**Ccl3**	Chemokine (C-C motif) ligand 3	AI323804, G0S19-1, LD78alpha, MIP-1alpha, MIP1-(a), MIP1-alpha, Mip1a, Scya3
Mm.6272	NM_009915	**Ccr2**	Chemokine (C-C motif) receptor 2	Cc-ckr-2, Ccr2a, Ccr2b, Ckr2, Ckr2a, Ckr2b, Cmkbr2, mJe-r
Mm.439656	NM_009883	**Cebpb**	CCAAT/enhancer binding protein (C/EBP), beta	C, EBPbeta, CRP2, IL-6DBP, LAP, LIP, NF-IL6, NF-M, Nfil6
Mm.277792	NM_007743	**Col1a2**	Collagen, type I, alpha 2	AA960264, AI325291, Col1a-2, Cola-2, Cola2, oim
Mm.249555	NM_009930	**Col3a1**	Collagen, type III, alpha 1	AW550625, Col3a-1, MMS10-W, Ms10w, mKIAA4231
Mm.390287	NM_010217	**Ctgf**	Connective tissue growth factor	Ccn2, Fisp12, Hcs24, fisp-12
Mm.1401	NM_009911	**Cxcr4**	Chemokine (C-X-C motif) receptor 4	CD184, Cmkar4, LESTR, PB-CKR, PBSF, SDF-1, Sdf1r
Mm.56769	NM_007833	**Dcn**	Decorin	DC, DSPG2, PG40, PGII, PGS2, SLRR1B, mDcn
Mm.14543	NM_010104	**Edn1**	Endothelin 1	ET-1, preproET
Mm.252481	NM_010113	**Egf**	Epidermal growth factor	AI790464
Mm.225297	NM_007932	**Eng**	Endoglin	AI528660, AI662476, CD105, Endo, S-endoglin
Mm.3355	NM_010177	**Fasl**	Fas ligand (TNF superfamily, member 6)	APT1LG1, CD178, CD95-L, CD95L, Fas-L, Faslg, Tnfsf6, gld
Mm.166318	NM_011824	**Grem1**	Gremlin 1	Cktsf1b1, Drm, Grem, ld
Mm.267078	NM_010427	**Hgf**	Hepatocyte growth factor	C230052L06Rik, HGF, SF, NK1, NK2, SF, HGF
Mm.240327	NM_008337	**Ifng**	Interferon gamma	IFN-g, Ifg
Mm.874	NM_010548	**Il10**	Interleukin 10	CSIF, Il-10
Mm.1284	NM_008355	**Il13**	Interleukin 13	Il-13
Mm.368330	NM_008356	**Il13ra2**	Interleukin 13 receptor, alpha 2	CD213a2
Mm.15534	NM_010554	**Il1a**	Interleukin 1 alpha	Il-1a
Mm.222830	NM_008361	**Il1b**	Interleukin 1 beta	IL-1beta, Il-1b
Mm.276360	NM_021283	**Il4**	Interleukin 4	BSF-1, Il-4
Mm.4461	NM_010558	**Il5**	Interleukin 5	Il-5
Mm.274846	NM_010562	**Ilk**	Integrin linked kinase	AA511515, ESTM24
Mm.3510	NM_008382	**Inhbe**	Inhibin beta E	-
Mm.317280	NM_001033228	**Itga1**	Integrin alpha 1	CD49A, E130012M19Rik, Vla1
Mm.5007	NM_008396	**Itga2**	Integrin alpha 2	CD49B, DX5
Mm.57035	NM_013565	**Itga3**	Integrin alpha 3	AA407068, CD49C, GAPB3
Mm.227	NM_008402	**Itgav**	Integrin alpha V	1110004F14Rik, 2610028E01Rik, CD51, D430040G12Rik
Mm.263396	NM_010578	**Itgb1**	Integrin beta 1 (fibronectin receptor beta)	4633401G24Rik, AA409975, AA960159, CD29, ENSMUSG00000051907, Fnrb, Gm9863, gpIIa
Mm.87150	NM_016780	**Itgb3**	Integrin beta 3	CD61, GP3A, INGRB3
Mm.6424	NM_010580	**Itgb5**	Integrin beta 5	AA475909, AI874634, ESTM23, [b]-5, [b]5, [b]5A, [b]5B, beta-5, beta5
Mm.98193	NM_021359	**Itgb6**	Integrin beta 6	2210409C20Rik, 4831415H04Rik
Mm.217000	NM_177290	**Itgb8**	Integrin beta 8	4832412O06Rik
Mm.275071	NM_010591	**Jun**	Jun oncogene	AP-1, Junc, c-jun
Mm.488403	NM_010728	**Lox**	Lysyl oxidase	AI893619, TSC-160, rrg
Mm.269747	NM_019919	**Ltbp1**	Latent transforming growth factor beta binding protein 1	9430031G15Rik, 9830146M04, Ltbp-1, Tgfb, b2b1000Clo
Mm.5022	NM_008607	**Mmp13**	Matrix metallopeptidase 13	Clg, MMP-13, Mmp1
Mm.486486	NM_008608	**Mmp14**	Matrix metallopeptidase 14 (membrane-inserted)	AI325305, MT-MMP-1, MT1-MMP, sabe

Mm.156952	NM_032006	**Mmp1a**	Matrix metallopeptidase 1a (interstitial collagenase)	Mcol-A, Mcola
Mm.29564	NM_008610	**Mmp2**	Matrix metallopeptidase 2	Clg4a, GelA, MMP-2
Mm.4993	NM_010809	**Mmp3**	Matrix metallopeptidase 3	SLN-1, SLN1, STR-1, Stmy1, Str1
Mm.16415	NM_008611	**Mmp8**	Matrix metallopeptidase 8	BB138268
Mm.4406	NM_013599	**Mmp9**	Matrix metallopeptidase 9	AW743869, B, MMP9, Clg4b, MMP-9, pro-MMP-9
Mm.2444	NM_010849	**Myc**	Myelocytomatosis oncogene	AU016757, Myc2, Niard, Nird, bHLHe39
Mm.256765	NM_008689	**Nfkb1**	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, p105	NF-KB1, NF-kappaB, NF-kappaB1, p105, p50, p50, p105
Mm.2675	NM_008808	**Pdgfa**	Platelet derived growth factor, alpha	-
Mm.144089	NM_011057	**Pdgfb**	Platelet derived growth factor, B polypeptide	PDGF-B, Sis
Mm.154660	NM_008872	**Plat**	Plasminogen activator, tissue	AU020998, AW212668, D8Ertd2e, tPA
Mm.4183	NM_008873	**Plau**	Plasminogen activator, urokinase	u-PA, uPA
Mm.971	NM_008877	**Plg**	Plasminogen	AI649309, Pg
Mm.439692	NM_009243	**Serpina1a**	Serine (or cysteine) peptidase inhibitor, clade A, member 1a	Aat-2, Aat2, Dom1, PI1, Spi1-1, Spi1-3
Mm.250422	NM_008871	**Serpine1**	Serine (or cysteine) peptidase inhibitor, clade E, member 1	PAI-1, PAI1, Planh1
Mm.22708	NM_009825	**Serpinh1**	Serine (or cysteine) peptidase inhibitor, clade H, member 1	BERF-1, Cbp1, Cbp2, Hsp47, J6, Serpinh2, gp46
Mm.490934	NM_010754	**Smad2**	MAD homolog 2 (Drosophila)	7120426M23Rik, Madh2, Madr2, Smad-2, mMad2
Mm.7320	NM_016769	**Smad3**	MAD homolog 3 (Drosophila)	AU022421, Madh3
Mm.100399	NM_008540	**Smad4**	MAD homolog 4 (Drosophila)	AW743858, D18Wsu70e, DPC4, Madh4
Mm.325757	NM_008542	**Smad6**	MAD homolog 6 (Drosophila)	Madh6, b2b390Clo
Mm.34407	NM_001042660	**Smad7**	MAD homolog 7 (Drosophila)	Madh7
Mm.2093	NM_011427	**Snai1**	Snail homolog 1 (Drosophila)	AI194338, Sna, Sna1, Snail, Snail1
Mm.4618	NM_013672	**Sp1**	Trans-acting transcription factor 1	1110003E12Rik, AA450830, AI845540, Sp1-1
Mm.487336	NM_009283	**Stat1**	Signal transducer and activator of transcription 1	2010005J02Rik, AA408197
Mm.121721	NM_009284	**Stat6**	Signal transducer and activator of transcription 6	-
Mm.248380	NM_011577	**Tgfb1**	Transforming growth factor, beta 1	TGF-beta1, TGFbeta1, Tgfb, Tgfb-1
Mm.18213	NM_009367	**Tgfb2**	Transforming growth factor, beta 2	BB105277, Tgf-beta2, Tgfb-2
Mm.3992	NM_009368	**Tgfb3**	Transforming growth factor, beta 3	Tgfb-3
Mm.197552	NM_009370	**Tgfbr1**	Transforming growth factor, beta receptor I	ALK5, AU017191, Alk-5, TbetaR-I, TbetaRI
Mm.172346	NM_009371	**Tgfbr2**	Transforming growth factor, beta receptor II	1110020H15Rik, AU042018, DNIIR, RIIDN, TBR-II, TbetaR-II, TbetaRII
Mm.101034	NM_009372	**Tgif1**	TGFB-induced factor homeobox 1	AA959811, AI462167, Tgif
Mm.4159	NM_011580	**Thbs1**	Thrombospondin 1	TSP-1, TSP1, Thbs-1, tbsp1
Mm.26688	NM_011581	**Thbs2**	Thrombospondin 2	TSP2, Thbs-2
Mm.8245	NM_011593	**Timp1**	Tissue inhibitor of metalloproteinase 1	Clgi, TIMP-1, Timp
Mm.206505	NM_011594	**Timp2**	Tissue inhibitor of metalloproteinase 2	D11Bwg1104e, Timp-2
Mm.4871	NM_011595	**Timp3**	Tissue inhibitor of metalloproteinase 3	Timp-3
Mm.255607	NM_080639	**Timp4**	Tissue inhibitor of metalloproteinase 4	TIMP-4
Mm.1293	NM_013693	**Tnf**	Tumor necrosis factor	DIF, TNF-a, TNF-alpha, TNFSF2, TNFalpha, Tnfa, Tnfsf1a
Mm.282184	NM_009505	**Vegfa**	Vascular endothelial growth factor A	Vegf, Vpf
Mm.391967	NM_007393	**Actb**	Actin, beta	Actx, E430023M04Rik, beta-actin
Mm.163	NM_009735	**B2m**	Beta-2 microglobulin	Ly-m11, beta2-m, beta2m
Mm.304088	NM_008084	**Gapdh**	Glyceraldehyde-3-phosphate dehydrogenase	Gapd
Mm.3317	NM_010368	**Gusb**	Glucuronidase, beta	AI747421, Gur, Gus, Gus-r, Gus-s, Gus-t, Gus-u, Gut, asd, g
Mm.2180	NM_008302	**Hsp90ab1**	Heat shock protein 90 alpha (cytosolic), class B member 1	90kDa, AL022974, C81438, Hsp84, Hsp84-1, Hsp90, Hspcb
N/A	SA_00106	**MGDC**	Mouse Genomic DNA Contamination	MIGX1B

Supplementary Data

Figure Legends:

Figure 1S. Activation of TLR2 and TLR4 using authentic ligands.

Hek-Blue^{TM}mTLR2 and Hek-blue^{TM}mTLR4 cells (Invivogen) were treated with PAM3CSK4 and LPS-B5, TLR2 and TLR4 agonist, respectively, overnight as described in Materials and Methods and Fig. 1. NFκB activation was quantified by absorbance at 620 nm.

Figure 2S. Assessment of fatty acid synthesis in LX2 human stellate cells.

Panels A and B: The relative abundance of mRNAs encoding proteins involved in *de novo* lipogenesis [DNL] (acetyl CoA carboxylase-1 [ACC1], fatty acid synthase [FASN]), fatty acid elongation (Elovl2, Elovl5) and desaturation (FADS1, FADS2); and two transcription factors (SREBP1a, SREBP1c) controlling expression of these enzymes was quantified by qRTPCR and RNA from human hepatoma (HepG2) and stellate (LX2) cells as described previously (1). The results are reported as Relative mRNA abundance, mean $\pm$ SD, N=3. **Panel C:** Confluent LX2 wells were treated with ^{14}C-acetate, ^{14}C-linoleate or ^{14}C-α-linoleate for 6 hours as described previously (1, 2). The labeling of cells was terminated by treatment with 0.4 M KOH in 80% methanol to saponify lipid. The recovered ^{14}C-labeled fatty acids were separated by RP-HPLC and radioactivity was quantified using an in-line β-scintillation counter. Results are represented as % Distribution of ^{14}C-fatty acids. Results are representative of 2 separate studies.

This analysis compared the level of expression of enzymes involved in DNL (ACC1, FASN), elongation and desaturation (Elovl2, Elovl5, FADS1, FADS2) and 2 transcription factors (SREBP1 & 2) involved in fatty acid synthesis in HepG2 (human hepatoma cells) and LX2 cells. While both HepG2 and LX2 cells express key enzymes involved in DNL, elongation and desaturation, levels of expression of these enzymes is different in the two cell types. HepG2 cells appear to have the high capacity for DNL, while LX2 cells may have higher capacity for long chain fatty acid elongation and desaturation. The capacity of HepG2 cells to carry out DNL, elongate and de-saturate fatty acids was previously reported

(1). To verify the capacity of LX2 cells to carry out fatty acid metabolism, cells were treated with ^{14}C-precursors to long chain fatty acids, i.e., acetate, linoleate and α-linolenate. LX2 cells convert ^{14}C-acetate to $C_{16\text{-}18}$ saturated (18:0) and monounsaturated fatty acids (16:1,ω7). Note that 16:0, 18:1,ω7 and 18:1,ω9 co-migrate in the RP-HPLC. ^{14}C-linoleate was converted to $C_{18\text{-}22}$ ω6 PUFA and ^{14}C-α-linolenate was converted to $C_{18\text{-}22}$ ω3 PUFA. Thus, LX2 cells have the capacity to synthesize fatty acids *de novo,* elongate and de-saturate essential fatty acids and modify fatty acids by peroxisomal β-oxidation, i.e., converted exogenous DHA to EPA, see **Fig. 8.**

Figure 3S: Diet effects on expression of the hepatic chemokines & cytokines, growth factors and transcription factors.

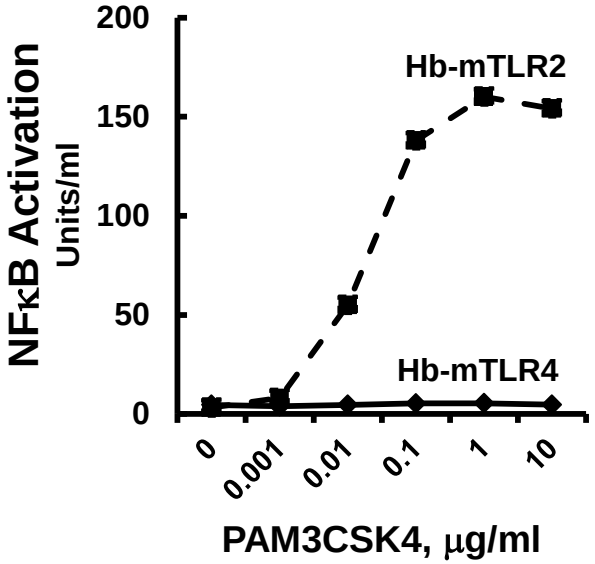
Transcript abundance of proteins encoding chemokines and cytokines (**Panel A**), growth factors (**Panel B**) and transcription factors (**Panel C**) was quantified using data from the qRTPCR fibrosis array data described in **Fig. 2**. Results are represented as mRNA Abundance-Fold Change, mean $\pm$ SD, n=8; *, *$p \leq 0.05$ versus RD; #, $p \leq 0.05$ versus WD + O.*

Literature cited.

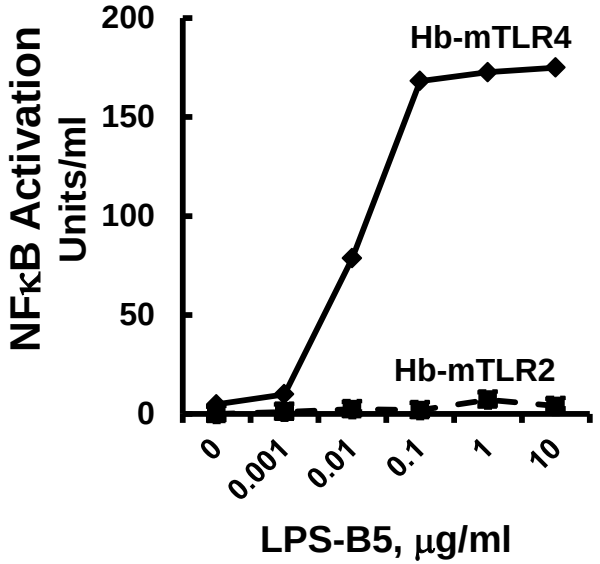
1. Jump, D. B., M. Torres-Gonzalez, and L. K. Olson. 2011. Soraphen A, an inhibitor of acetyl CoA carboxylase activity, interferes with fatty acid elongation. *Biochem Pharmacol* **81**: 649-660.
2. Tripathy, S., Lytle, K.A., Stevens, R.D., Bain, J.R., Newgard, C.B., Greenberg, A.S., Huang, L-S., and Jump, D.B 2014. Fatty acid elongase-5 (Elovl5) regulates hepatic triglyceride catabolism in obese C57BL/6J mice *J Lipid Res* **55**: 1448-1464.

Supplement: Fig. 1S

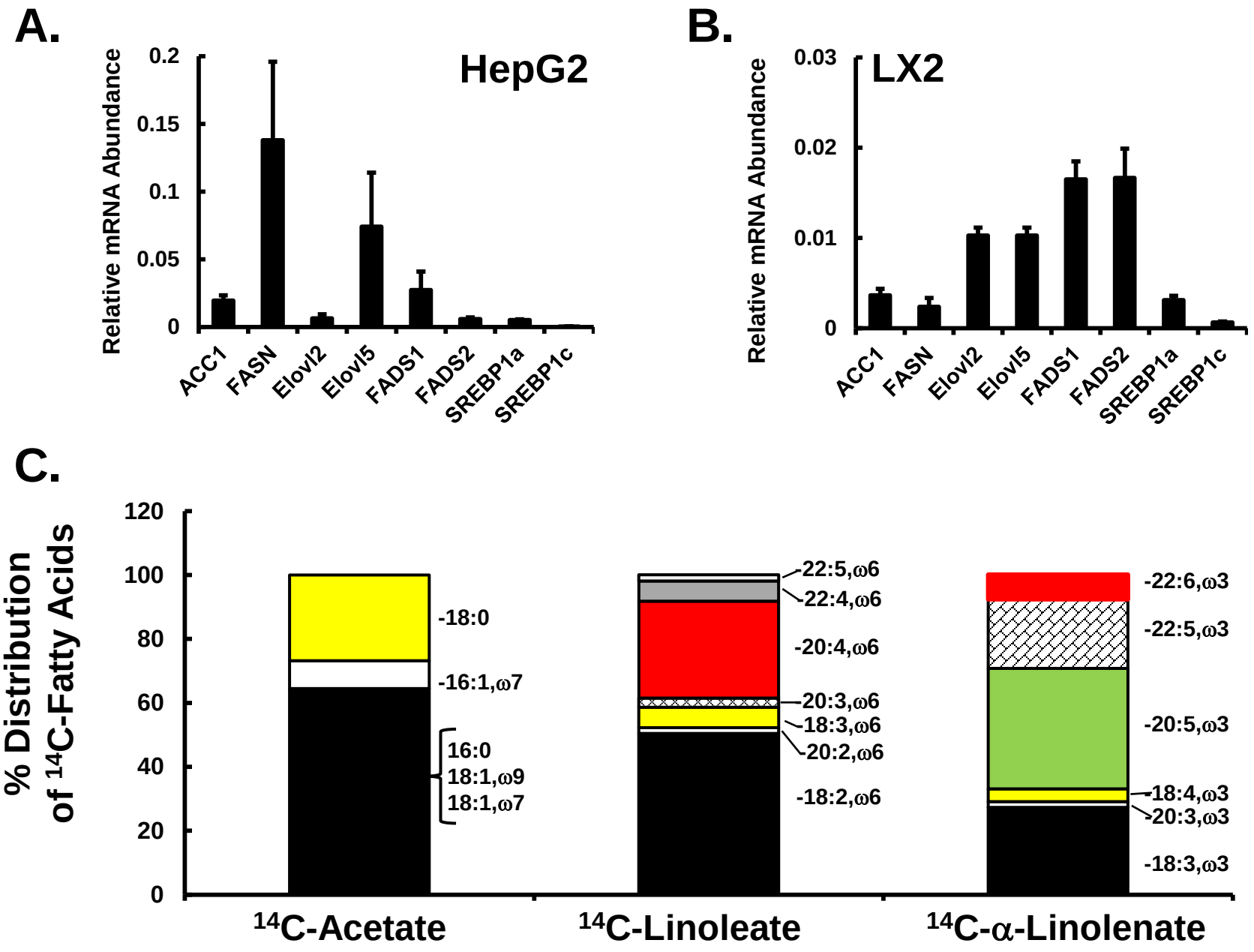
A.



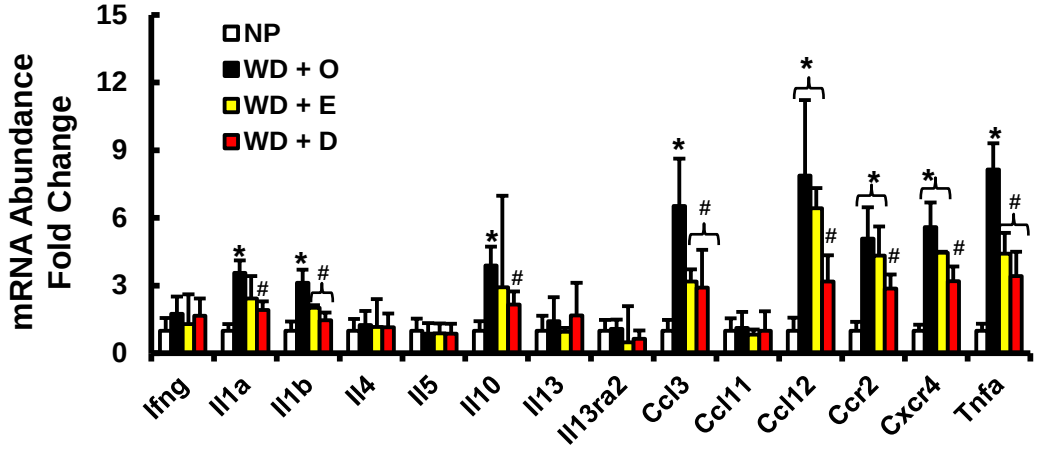
B.



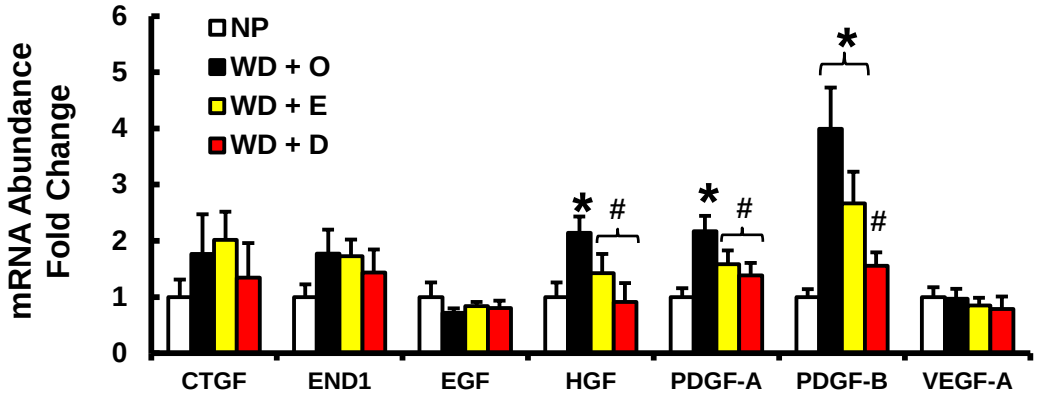
Supplement: Fig. 2S



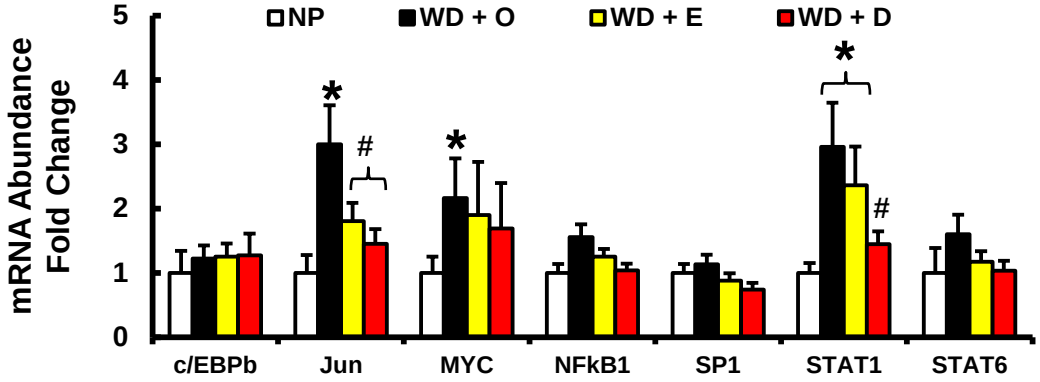
A. Chemokines & Cytokines



B. Growth Factors



C. Transcription Factors



Supplement: Fig. 3S