N-3 POLYUNSATURATED FATTY ACIDS STIMULATE BILE ACID DETOXIFICATION IN HUMAN CELL MODELS.

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SUPPLEMENTARY MATERIALS

LIST OF ABBREVIATIONS

ASBT,	apical sodium-dependent bile acid transporter
BACAT,	bile acid-Coenzyme A dehydrogenase: amino acid n-acyltransferase
BACL,	bile acid-CoA ligase
BAs,	bile acids
BSEP,	bile salt export pump
CA,	cholic acid
Caco2,	human epithelial colorectal adenocarcinoma cells
CDCA,	chenodeoxycholic acid
CYP,	cytochrome P450
CYP27A1,	sterol 27-hydroxylase
CYP3A4,	cytochrome P450 3A4
CYP7A1,	cholesterol 7α-hydroxylase
CYP8B1,	12α-hydroxylase
DCA,	deoxycholic acid
DHA,	docosahexaenoic acid
DMEM,	Dulbecco’s modified Eagle’s medium
DMEM/HAM-F-12,	Dulbecco's Modified Eagle's Medium and Ham's F-12 Nutrient Mixture
EPA,	eicosapentaenoic acid
FACS,	fluorescence-activated cell sorting
FBS,	fetal bovine serum
FGFR4,	fibroblast growth factor receptor 4
FGF-19,	fibroblast growth factor 19
FXR,	farnesoid X-receptor
GBA,	glyco-conjugated bile acid
HCA,	hyocholic acid

HDCA, hyodeoxycholic acid

HepG2, human hepatocellular liver carcinoma cells

HNF4α, hepatic nuclear factor-4

I-BABP, intestinale bile acid-binding protein

InEpC, Intestinal Epithelial Cells

KCA, ketocholic acid

LCA, lithocholic acid

LCA-S, lithocholic acid sulfate

LC-MS/MS, liquid chromatography-tandem mass spectrometry

LRH-1, Liver receptor homologue-1

LXRα, liver X-receptor alpha

MRP, multi-drug resistance protein

NR, nuclear receptor

NTCP, Na^+ Taurocholate cotransporting polypeptide

N-3 PUFAs, polyunsaturated fatty acids

OATP, organic anion transporting polypeptide

OSTα/β, organic solute transpoter alpha/beta

PBC, primary biliary cholangitis

PI, propidium iodine

PPARα, peroxisome proliferator-activated receptors alpha

PSC, primary sclerosing cholangitis

PUM-1, pumilio RNA-binding family member 1

PXR, pregnane X-receptor

qRT-PCR, quantitative reverse-transcription polymerase chain reaction

RPTEC, renal proximal tubule epithelial cells

RT,	reverse transcription
RXR,	retinoid X receptor
SHP,	small heterodimer partner
SmGM,	smooth Muscle Growth SingleQuot Medium
SULT,	sulfotransferase
TBA,	tauro-conjugated bile acid
TGR5,	G-protein-coupled bile acid receptor Gpbar1
UDCA,	ursodeoxycholic acid

Supplementary Table 1: Primers and conditions used for quantitative real-time PCR experiments.

Gene	Primers	Annealing Temperature (°C)	RT dilution
ASBT	Sense: 5′-TGACCACATGCTCCACACTG Antisense: 5′-CCCAGAGTCGACCCACATTT	62°C	Caco-2 1/50 InEpC 1/25 RPTEC 1/50
BACAT	Sense: 5′-CTGCCAACTTTCTCCTGAGACA Antisense: 5′-CCAATCTGTACTCCTTGACATACA	60°C	HepG2 1/200
BACL	Sense: 5′-GTGGAGGGCGTGTTGTCGCA Antisense: 5′-CCGTCGAAAGTCTGGCCGGG	62°C	HepG2 1/200
BSEP	Sense: 5′-GGGCCATTGTACGAGATCCTAA Antisense: 5′-TGCACCGTCTTTTCACTTTCTG	61°C	HepG2 1/50
CYP3A4	Sense: 5′-CCAAGCTATGCTCTTCACCG Antisense: 5′-TCAGGCTCCACTTACGGTGC	65°C	HepG2 1/50 RPTEC 1/50
CYP27	Sense: 5′-CGGCAACGGAGCTTAGAGG Antisense: 5′-GGCATAGCCTTGAACGAACAG	60°C	HepG2 1/200
CYP7A1	Sense: 5′-AGAAGCATTGACCCGATGGAT Antisense: 5′-AGCGGTCTTTGAGTTAGAGGA	59°C	HepG2 1/50
CYP8B1	Sense: 5′-GAAGCGCATGAGGACCAAG Antisense: 5′-TTGCATATTGCCCAAAGTCTAGT	59°C	HepG2 1/50
FGF19	Sense: 5′-CGGAGGAAGACTGTGCTTTCG Antisense: 5′-CTCGGATCGGTACACATTGTAG	62°C	HepG2 1/50 Caco-2 1/200 InEpC 1/25 RPTEC 1/50
FGFR4	Sense: 5′-GAGGGGCCGCCTAGAGATT Antisense: 5′-CAGGACGATCATGGAGCCT	62°C	HepG2 1/200 Caco-2 1/200 InEpC 1/25 RPTEC 1/50
FXR	Sense: 5′- GGTGTTTTAACAGAACAAGTGGC Antisense: 5′-ACATTGCTGTATTGCGAGTATGG	60°C	HepG2 1/200 Caco-2 1/50 InEpC 1/25 RPTEC 1/500
HNF4α	Sense: 5′-CGACACGTCCCCATCAGAAG Antisense: 5′-CTCGAGGCACCGTAGTGTTT	60°C	HepG2 1/200 Caco-2 1/500 InEpC 1/50 RPTEC 1/100
IBABP	Sense: 5′-ACCGGCAAGTTCGAGATGG Antisense: 5′-CCTTTTCGATTACATCGCTGGA	60°C	Caco-2 1/50 InEpC 1/25
βKLOTHO	Sense: 5′-TTGCCAACGCAAAAGGTCTG Antisense: 5′-GCCAAAGGCAAATCCCAGTG	60°C	HepG2 1/500 Caco-2 1/500 InEpC 1/50 RPTEC 1/100
LRH	Sense: 5′- GAATGCGTGGAGGAAGGAATAA Antisense: 5′-GTCAGAGGGCATAGCTTGGAT	60°C	HepG2 1/200 Caco-2 1/500 InEpC 1/50 RPTEC 1/50
LXRα	Sense: 5′-GCTGCAAGTGGAATTCATCAACC Antisense: 5′-ATATGTGTGCTGCAGCCTCTCCA	64°C	HepG2 1/100 Caco-2 1/500 InEpC 1/50 RPTEC 1/50
Gene	**Primers**	**Annealing Temperature**	**RT dilution**

		(°C)	
MRP2	Sense: 5′-CAAACTCTATCTTGCTAAGCAGG Antisense: 5′-TGAGTACAAGGGCCAGCTCTA	59°C	HepG2 1/1000 Caco-2 1/1000 InEpC 1/50 RPTEC 1/50
MRP3	Sense: 5′-CAGAGAAGGTGCAGGTGACA Antisense: 5′-CTAAAGCAGCATAGACGCCC	59°C	HepG2 1/200 Caco-2 1/50 InEpC 1/100 RPTEC 1/50
MRP4	Sense: 5′-GGACAAAGACAACTGGTGTGCC Antisense: 5′-AATGGTTAGCACGGTGCAGTGG	64°C	HepG2 1/200 Caco-2 1/1000 InEpC 1/50 RPTEC 1/500
NTCP	Sense: 5′-TGATATCACTGGTCCTGGTTCTCA Antisense: 5′-GCATGTATTGTGGCCGTTTG	61°C	HepG2 1/50
OATP1B1	Sense: 5′-TGGTCCACCAACAACTGTGGCA Antisense: 5′-AGACAAGCCCAAGTAGACCCTTGAA	60°C	HepG2 1/50
OATP1B3	Sense: 5′-AAGTTGTGCTTTGCGATGCTGAGT Antisense: 5′-GTCAGGCCCTCTAGGAGGTGGG	62°C	HepG2 1/50
OSTα	Sense: 5′-AGATTGCTTGTTCGCCTCC Antisense: 5′-ATTCGTGTCAGCACAGTCATTAG	59°C	HepG2 1/50 Caco-2 1/50 InEpC 1/50 RPTEC 1/50
OSTβ	Sense: 5′-CAGGAGCTGCTGGAAGAGAT Antisense: 5′-GACCATGCTTATAATGACCACCA	59°C	HepG2 1/50 Caco-2 1/50 InEpC 1/50 RPTEC 1/50
PPARα	Sense: 5′- ATATCTCCCTTTTTGTGGCTGCTA Antisense: 5′-TCCGACTCCGTCTTCTTGATGA	60°C	HepG2 1/200 Caco-2 1/100 InEpC 1/50 RPTEC 1/100
PUM1	Sense: 5′-CCGTCGAAAGTCTGGCCGGG Antisense: 5′-CATTAATTACCTGCTGGTCTGAAGGA	62°C	All cells: 1/50
PXR	Sense: 5′-GACAGTGCCAGGCCTGCCGCC Antisense: 5′-CATCTGAGCGTCCATCAGCTCC	62°C	HepG2 1/100 Caco-2 1/100 InEpC 1/50 RPTEC 1/50
RXR	Sense: 5′-ATGGACACCAAACATTTCCTGC Antisense: 5′-GGGAGCTGATGACCGAGAAAG	62°C	HepG2 1/100 Caco-2 1/200 InEpC 1/50 RPTEC 1/200
SHP	Sense: 5′-GTGCCCAGCATACTCAAGAAG Antisense: 5′-TGGGGTCTGTCTGGCAGTT	60°C	HepG2 1/1000 Caco-2 1/50 InEpC 1/25 RPTEC 1/50
SULT2A1	Sense: 5′-ACGGATTCGAGGCCACGTCC Antisense: 5′-TCCGTTTCACTGAGTGCTGTA	62°C	HepG2 1/200 Caco-2 1/500 InEpC 1/50 RPTEC 1/200
TGR5	Sense: 5′-GACTTTGGACCATGAAGACCAG Antisense: 5′-GCCCAGACGGAAGTTTCTTATT	60°C	HepG2 1/50 Caco-2 1/50 InEpC 1/25 RPTEC 1/200

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Dose-dependent and gene-specific modulation of the bile acid-related transcriptome in human hepatoma HepG2 cells treated with EPA and DHA.

HepG2 cells were treated with DMSO (vehicle, 0.1% v/v) or DHA or EPA for 24H at 5, 15, 25 and 50 μM for mRNA measurements. Total RNA was extracted using the TriReagent® protocol to further measure mRNA levels by quantitative real time PCR as detailed in the materials and methods section.

Each data point represents the mean±S.D. of triplicate experiments. The results are representative of two independent experiments. Statistically significant differences were analyzed using one-way analysis of variance (ANOVA) (* $p < 0.05$).

Supplementary Figure 2. Time-dependent and gene-specific modulation of the bile acid-related transcriptome in human HepG2 cells treated with EPA and DHA.

HepG2 cells were treated with DMSO (vehicle, 0.1% v/v) or DHA or EPA at 50 μM for 6, 12, 18, 24 and 48H for mRNA measurements. Total RNA was extracted using the TriReagent® protocol to further measure mRNA levels by quantitative real time PCR as detailed in the materials and methods section.

Each data point represents the mean±S.D. of triplicate experiments. The results are representative of two independent experiments. Statistically significant differences were analyzed using one-way analysis of variance (ANOVA) (* $p < 0.05$).

Supplementary Figure 3. Additive and/or synergistic effects of the EPA+DHA combination on the bile acid-related transcriptome in human hepatoma HepG2 cells.

HepG2 cells were treated with DMSO (vehicle, 0.1% v/v) or DHA and/or EPA for 24H at 25 and/or 50 μM for mRNA measurements. Total RNA was extracted using the TriReagent® protocol to further measure mRNA levels by quantitative real time PCR as detailed in the materials and methods section.

Each data point represents the mean±S.D. of triplicate experiments. The results are representative of two independent experiments. Statistically significant differences were analyzed using one-way analysis of variance (ANOVA) (* $p < 0.05$).

Supplementary Figure 4. Preparation of the PUFA solution has minimum impact on the response of BA-related genes in HepG2 cells.

HepG2 cells were exposed to EPA/DHA (25/25 or 50/50µM) prepared either in DMSO or in culture medium containing 125µM BSA for 24H. Total RNA was extracted using the TriReagent® protocol and CYP7A1, CYP27 and MRP3 mRNA levels were determined by quantitative real time PCR as detailed in the materials and methods section.

Statistically significant differences were analyzed using one-way analysis of variance (ANOVA) (* $p < 0.05$).

Supplementary Figure 5. Time-dependent effects of n-3 PUFAs on the bile acid-induced activation of the pro-apoptotic Caspase 3 pathway in human hepatoma HepG2 cells.

HepG2 cells pretreated with DMSO (vehicle, 0.1% v/v) and DHA/EPA (50/50µM) **(A)**: for 24H and exposed to 100 µM BAs (CA, CDCA, LCA, CDA) for 0,5, 1, 2, 3, 6 and 24H, **(B)**: for 3, 6, 16 and 24H and exposed to 100 µM BA (CA, CDCA, LCA, CDA) for 2H. The caspase-3 activity was determined as indicated in the materials and methods section.

The results (mean±S.D.) are representative of two independent experiments. Statistical differences between two groups were analyzed using unpaired two-side *t*-test *versus* vehicle (*) or BAs (ǂ) ($p < 0.05$).

Supplementary Figure 6. Dose-dependent and additive/synergistic effects of EPA and/or DHA on the bile acid-related transcriptome in human colon carcinoma Caco-2 cells.

Human colon carcinoma Caco-2 were treated with DMSO (vehicle, 0.1% v/v) or DHA and/or EPA for 24H at 10, 25 and/or 50µM for mRNA measurements. Total RNA was extracted using the TriReagent® protocol to further measure mRNA levels by quantitative real time PCR as detailed in the materials and methods section.

Each data point represents the mean±S.D. of triplicate experiments. The results are representative of two independent experiments. Statistically significant differences were analyzed using one-way analysis of variance (ANOVA) (* $p < 0.05$).

Supplementary Figure 7. Time-dependent and gene-specific modulation of the bile acid-related transcriptome in colon carcinoma Caco-2 cells treated with EPA and DHA.

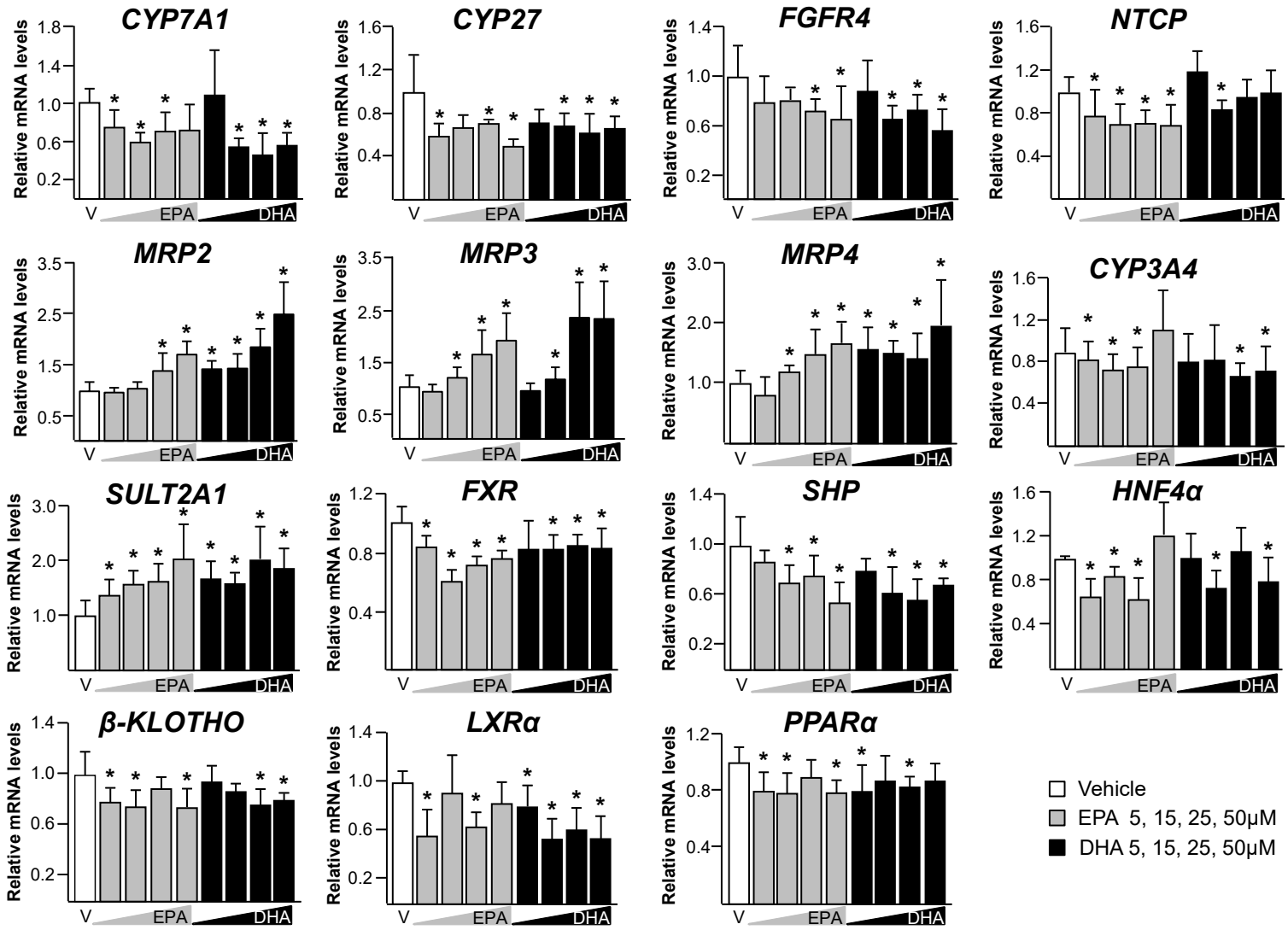
Human colon carcinoma Caco-2 were treated with DMSO (vehicle, 0.1% v/v) or DHA and EPA at 50 and 25μM respectively for 6, 12, 18, 24 and 48H for mRNA measurements. Total RNA was extracted using the TriReagent® protocol to further measure mRNA levels by quantitative real time PCR as detailed in the materials and methods section.

Each data point represents the mean±S.D. of triplicate experiments. The results are representative of two independent experiments. Statistical differences between two groups were analyzed using unpaired two-side *t*-test ($p < 0.05$).

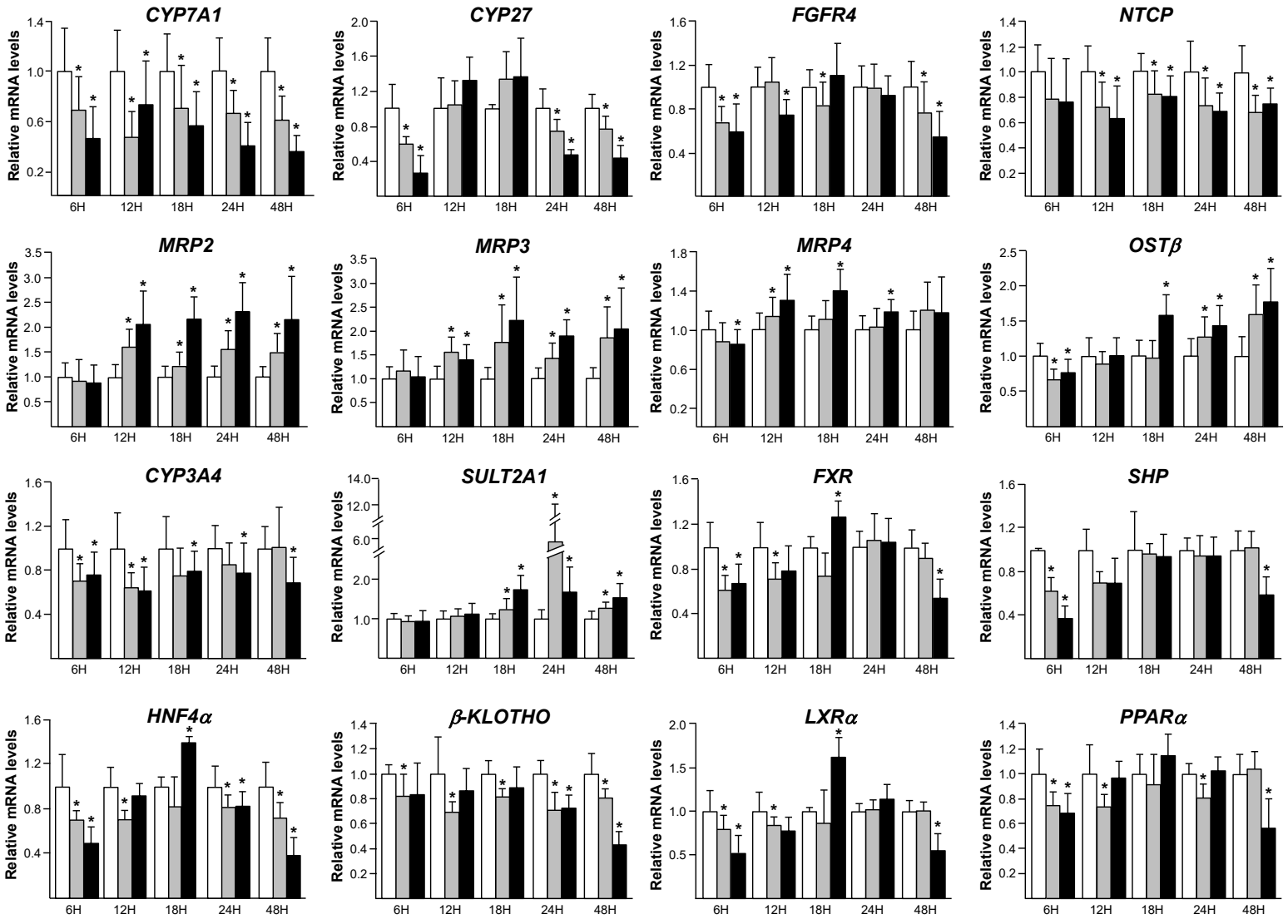
Supplementary Figure 8. Time-dependent and gene-specific modulation of the bile acid-related transcriptome in RPTEC treated with EPA and DHA.

Human renal proximal tubule epithelial cells (RPTEC) were treated with DMSO (vehicle, 0.1% v/v) or DHA and EPA at 25 and 50μM, respectively for 6, 12, 18, 24 and 48H for mRNA measurements. Total RNA was extracted using the TriReagent® protocol to further measure mRNA levels by quantitative real time PCR as detailed in the materials and methods section.

Each data point represents the mean±S.D. of triplicate experiments. The results are representative of two independent experiments. Statistical differences between two groups were analyzed using unpaired two-side *t*-test ($p < 0.05$).



Supplementary Figure 1



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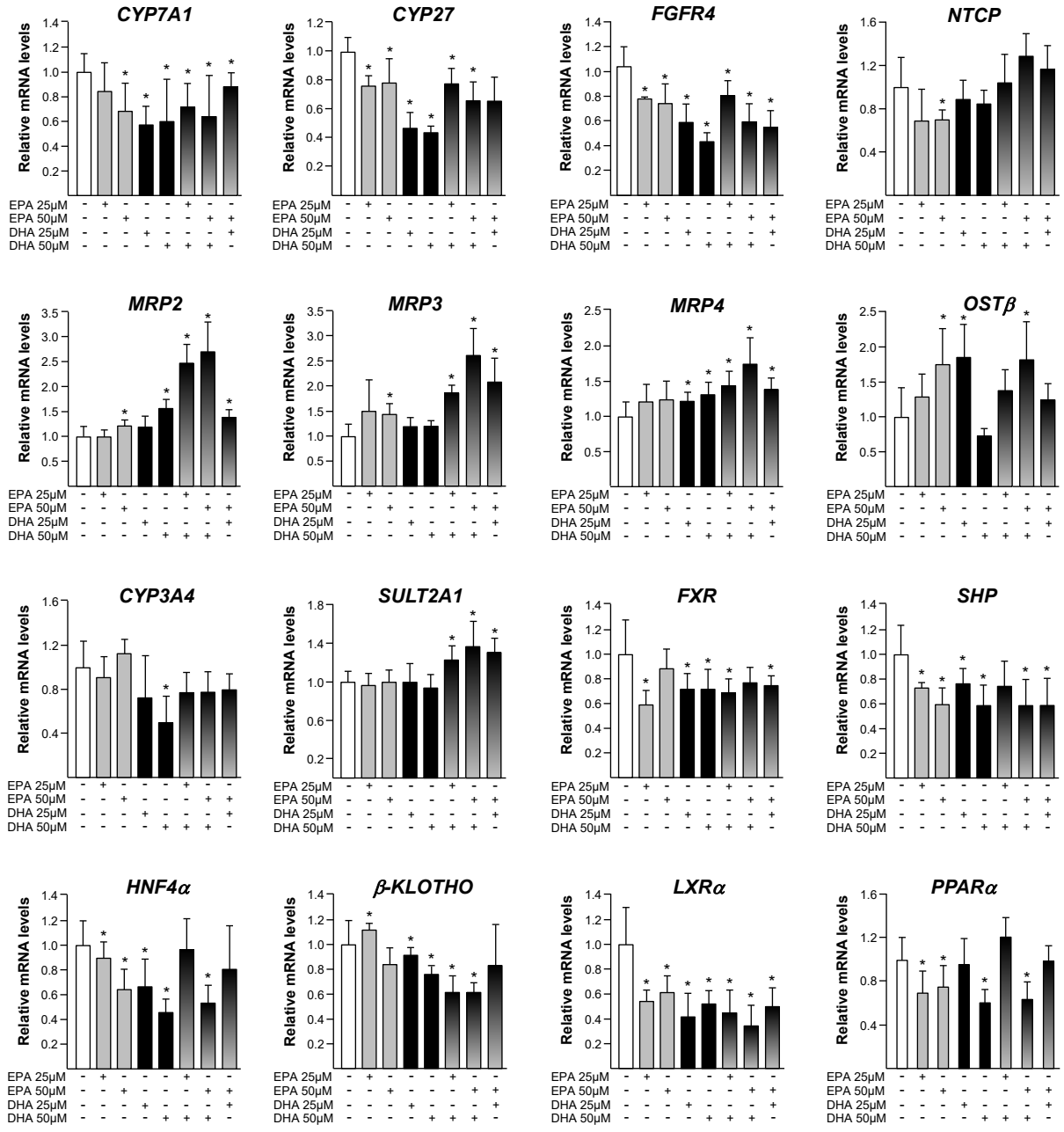

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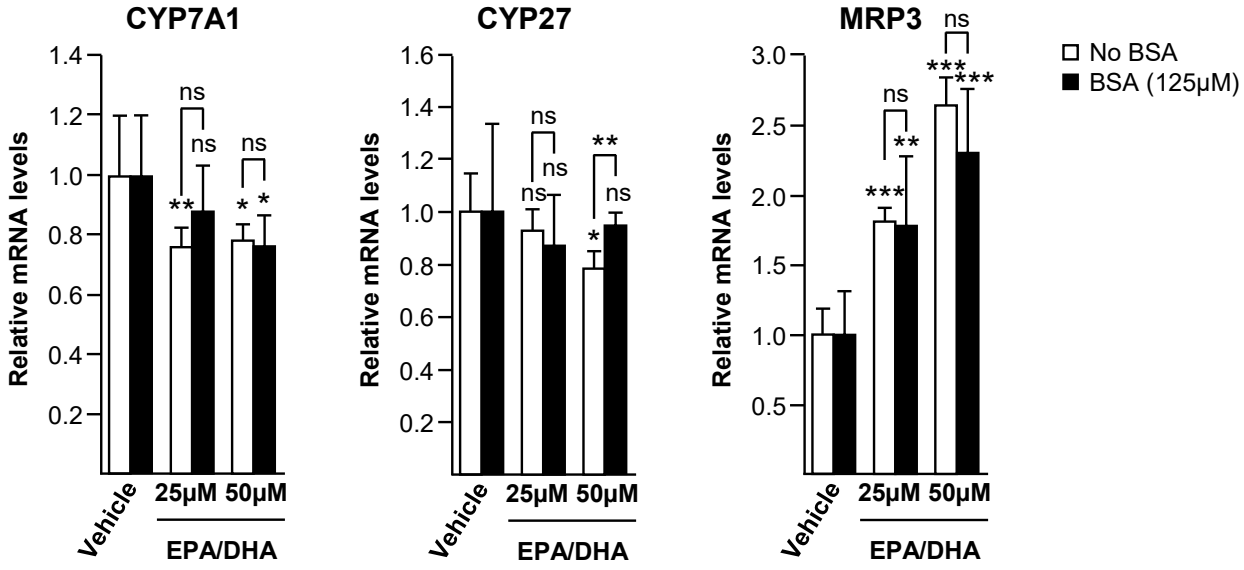

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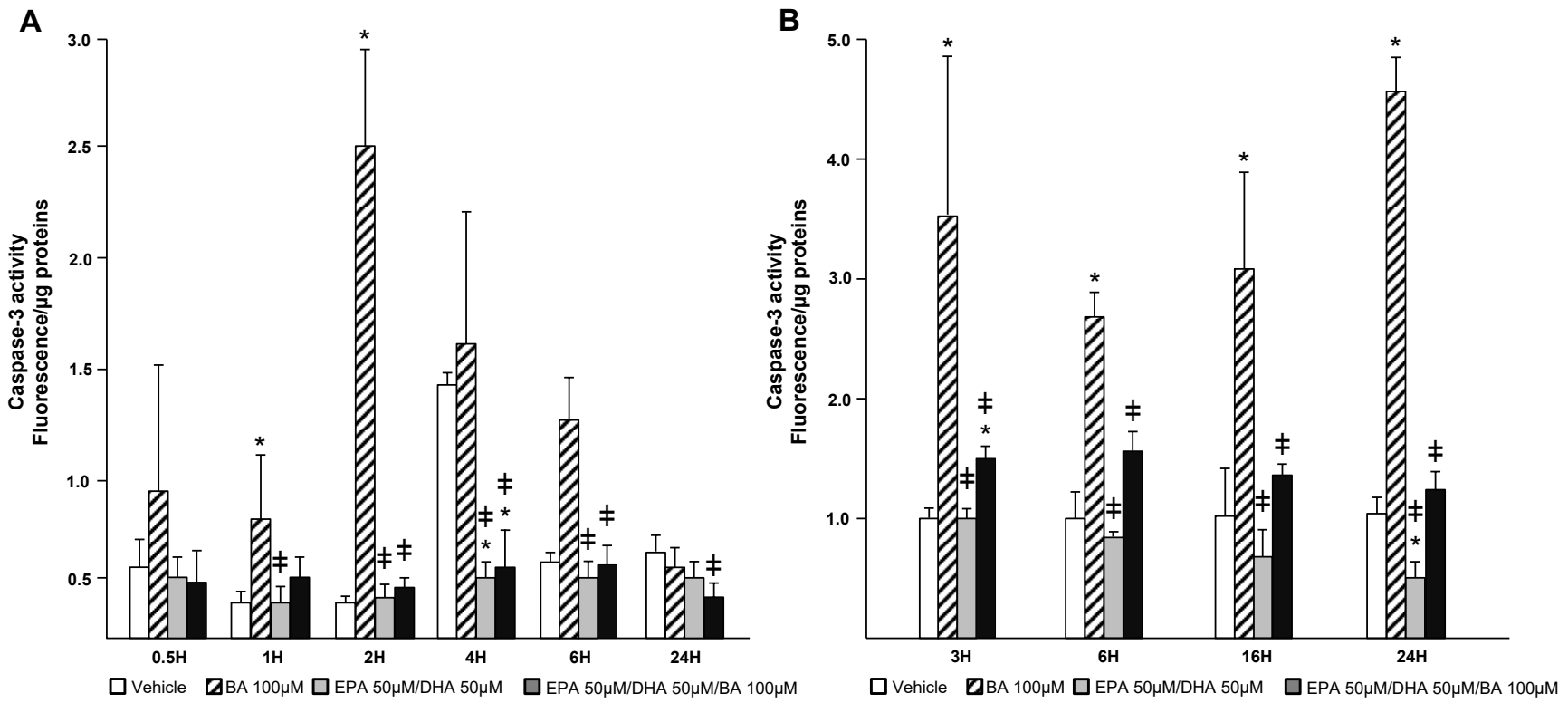

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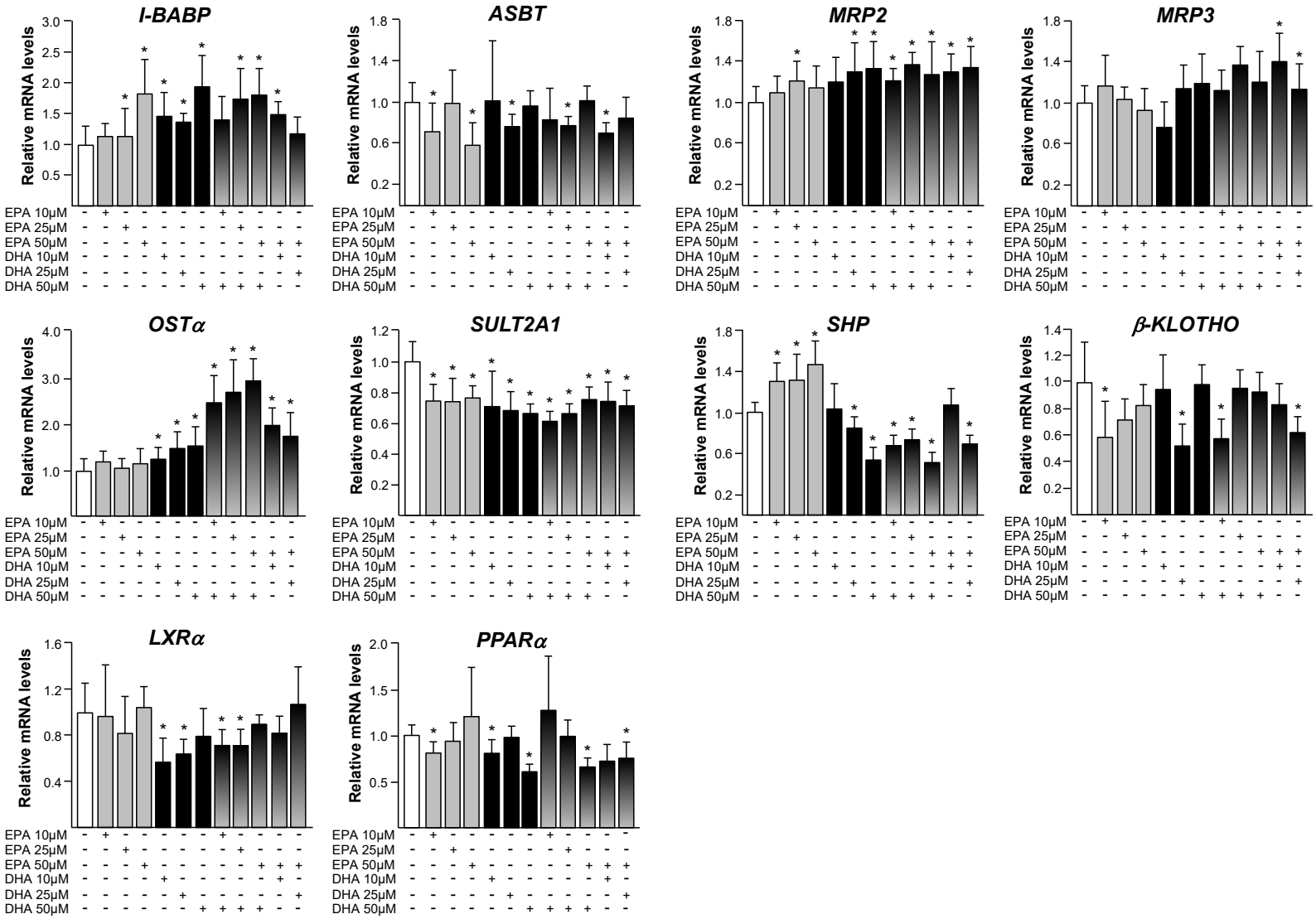

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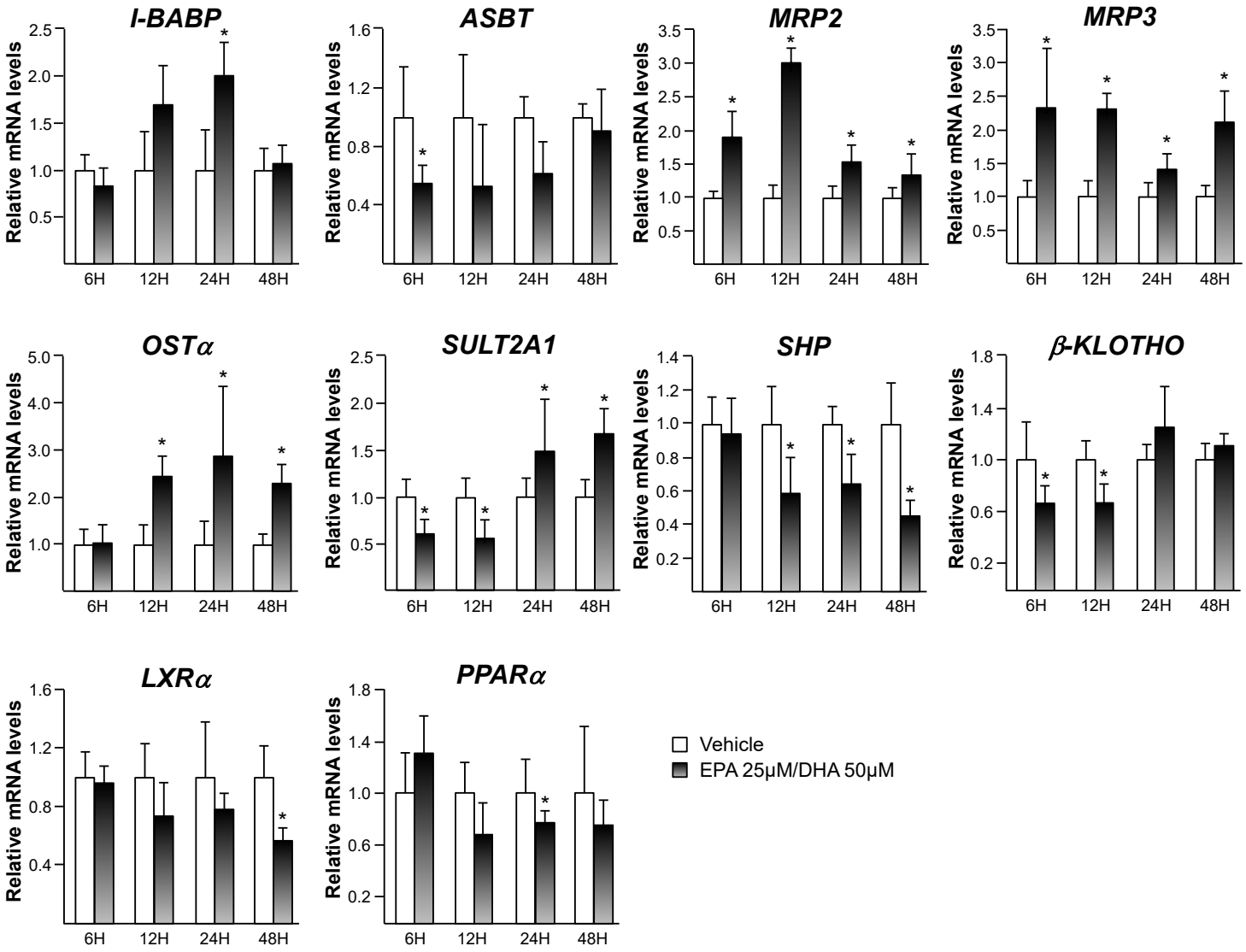
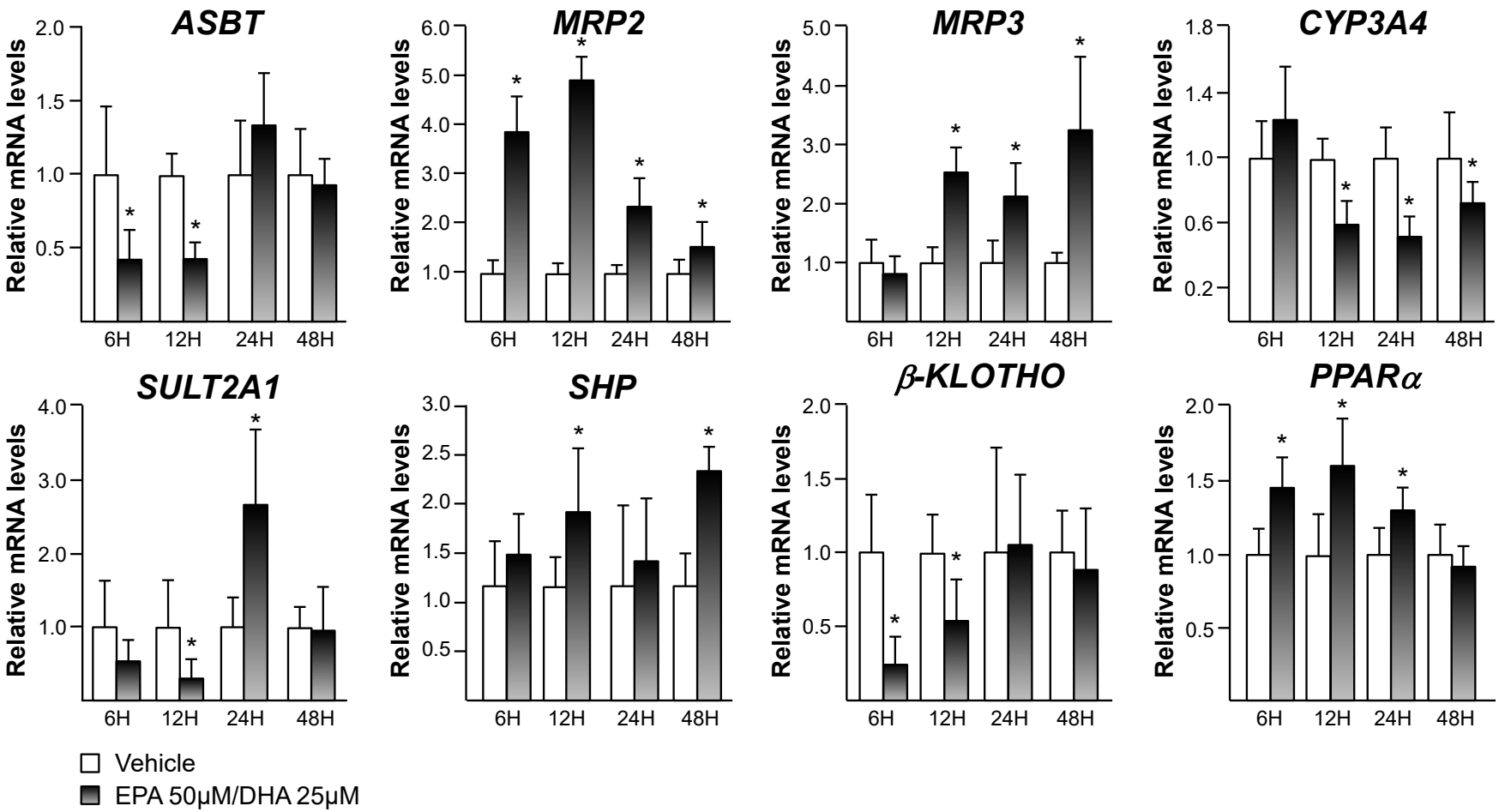


Supplementary Figure 7



Supplementary Figure 8