

## Supplementary Results

### Supplementary Tables

#### Supplementary Table 1: Primer sequences for RT-qPCR assays

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>ACC1</i>	GCCACATCTCATCCAAAC	AGCAGCCCATCACTTCATC
<i>APOB</i>	AAGTGCCACCAGGATCAACT	CAGGGTTGAAGCCATACACC
<i>CD36</i>	GGCTGTGTTTGGAGGTATTC	TGTTGCTGCTGTTTCATCATC
<i>CPT1A</i>	TCCATTGACAGCCTCCAG	AACCTCTTGACATTCCCCC
<i>CYP4A11</i>	GCCATTAGTGACCTGAACAACCTGG	ACTTGGTCTGTGTGCTGATGGGC
<i>DGAT1</i>	CATCCAGGTGGTTTCTCTGTTCCT	GGAATGCAGCCACAGCAAAGACAT
<i>DGAT2</i>	AGTGGCAATGCTATCATCAT	GAGGCCTCGACCATGGAAGAT
<i>FABP1</i>	TCAAGGGGGTGTGCGAAATC	GTGATTATGTCGCCGTTGAGTTC
<i>FASN</i>	AGTGCCAGAGTCGGAGAA	TGTAGCCCACGAGTGTCTCG
<i>FATP2</i>	TCTTGGATGACACAGCAAAAATGT	TCAGAGTTTCAGGGTTTTAGCACTT
<i>GPAT1</i>	GCCGCTTCTGTTTCTACCAG	ACACCGGTTTCTGACTTTGG
<i>HPRT</i>	GGCGTCGTGATTAGTGATG	CAGAGGGCTACAATGTGATG
<i>MTTP</i>	TGAGGCAGTGGCCATAGAAAAT	CTTTGTCTTGATGAGCCTGGTA
<i>PLIN2</i>	GATGGCAGAGAACGGTGTGAAG	CAGGCATAGGTATTGGCAACTGC
<i>RPL13A</i>	AAGGTCGTGCGTCTGAAG	GAGTCCGTGGGTCTTGAG
<i>SCD1</i>	CACCCAGCTGTCAAAGAGAAGG	AGGACGATATCCGAAGAGGTGG
<i>SREBP1C</i>	GCGGAGCCATGGATTGCAC	CTCTTCCTTGATACCAGGCC

#### Supplementary Table 2: Antibodies used for Western blot

- Anti-beta actin rabbit, Abcam, ab 8227, (1/5000)
- Perilipin-2 Rabbit pAb, ABclonal, A6276, (1/1000)
- CPT1A Rabbit pAb, ABclonal, A5307, (1/1000)
- CD36/SR-B3 Rabbit pAb, ABclonal, A14714, (1/1000)
- Secondary antibody, IRDye® 680LT Goat anti-Mouse, LI-COR, 925-68020, (1/20000)
- Secondary antibody, IRDye® 800CW Goat anti-Rabbit, LI-COR, 925-32211, (1/10000)

18 **Supplementary Table 3: Viability of 2D (A) and 3D (B) HepaRG cells after exposure to a**  
 19 **range of concentrations (0.1 – 25 µM) of different EDCs. (A)** Three distinct metabolic  
 20 activities: lysosomal, dehydrogenase and esterase, were measured, respectively, with neutral  
 21 red (displayed in red), resazurin (blue) and CFDA-AM (green) assays after 72 h and 14 days  
 22 of exposure of 2D HepaRG cells. The medium was renewed every 2-3 days. The values are  
 23 the mean (in %) relative to the control of three independent experiments in duplicate. **(B)**  
 24 Viability of Hepoid-HepaRG 3D cells using CellTiter-glo assay after 72 h and 14 days of  
 25 treatment to different EDC families. The values are the mean (in %) relative to the control of  
 26 three independent experiments in triplicate.

**A**

Concentrations	BPA			BPF			BPS			BP			CdCl2			p,p'-DDE			DBP			DEHP			PFOA			PFOS		
	Neutral Red	Resazurin	CFDA-AM	Neutral Red	Resazurin	CFDA-AM	Neutral Red	Resazurin	CFDA-AM	Neutral Red	Resazurin	CFDA-AM	Neutral Red	Resazurin	CFDA-AM	Neutral Red	Resazurin	CFDA-AM	Neutral Red	Resazurin	CFDA-AM	Neutral Red	Resazurin	CFDA-AM	Neutral Red	Resazurin	CFDA-AM	Neutral Red	Resazurin	CFDA-AM
<b>72 h</b>																														
100 nM	101	83	97	105	61	96	105	111	95	86	91	94	94	91	104	95	84	89	98	55	95	91	86	100	104	82	90	106	52	85
1 µM	103	84	96	105	70	96	102	102	95	86	87	94	98	88	101	88	83	93	98	67	103	91	79	102	105	57	85	101	60	84
10 µM	100	84	98	106	74	101	102	102	98	83	98	97	40	96	78	78	88	96	97	69	105	92	90	102	109	69	89	115	70	93
25 µM	104	80	98	105	80	102	105	101	99	88	96	96	20	35	48	73	100	104	96	65	104	89	92	102	101	58	85	107	73	94
<b>14 days</b>																														
100 nM	97	106	96	108	91	90	93	90	86	100	99	98	74	108	92	100	82	90	84	101	87	83	112	91	78	112	96	87	97	92
1 µM	99	86	95	96	86	89	99	84	89	100	92	97	83	100	96	97	83	94	84	102	89	79	98	90	81	101	96	80	102	95
10 µM	98	108	99	117	102	95	98	87	86	98	104	102	4	11	7	92	97	99	77	104	92	85	123	96	77	108	95	83	109	94
25 µM	103	95	99	114	101	100	99	84	86	98	92	99	6	4	6	91	107	105	83	104	104	82	139	96	80	106	99	87	130	102

**B**

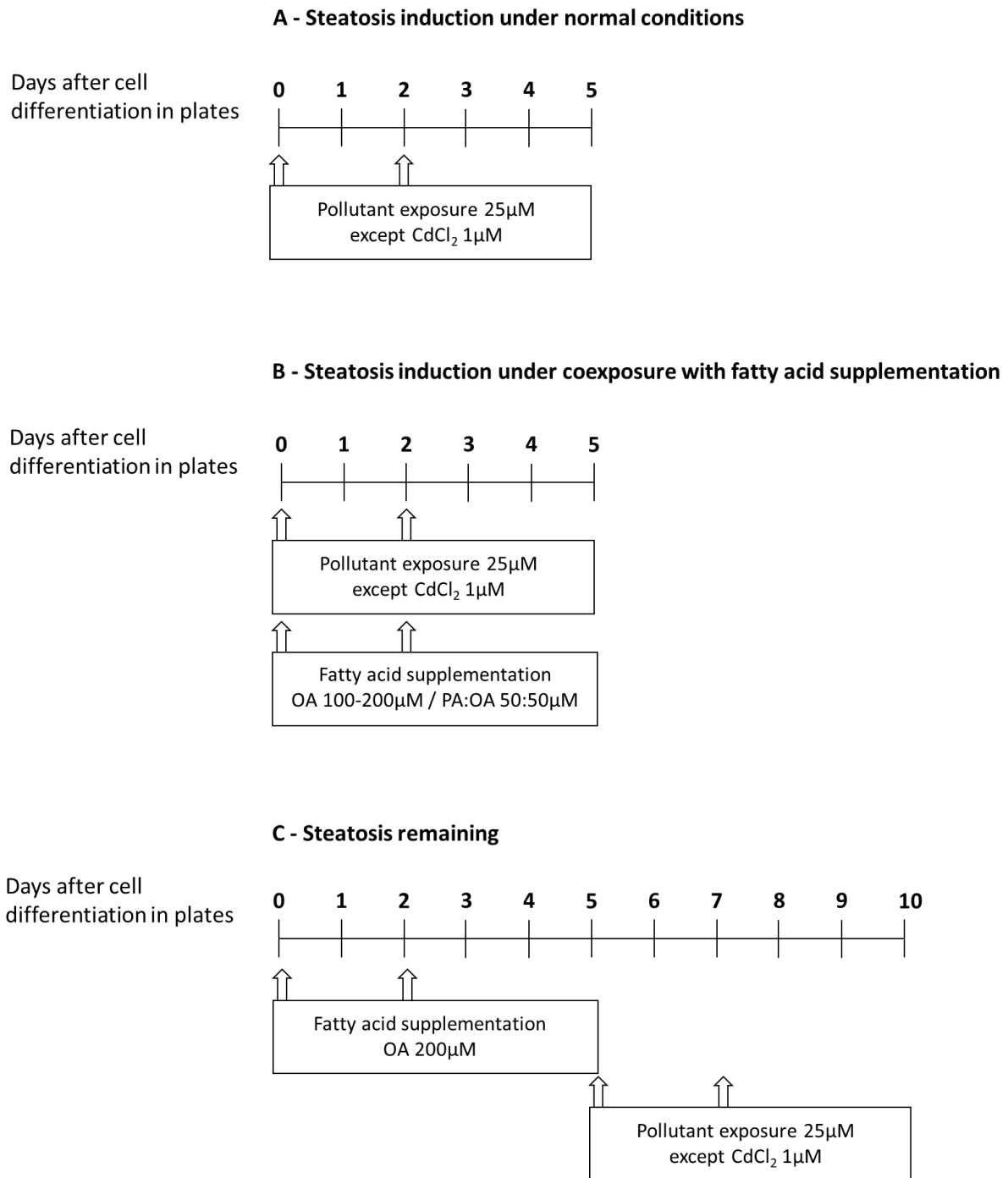
Concentrations	BPA	BPF	BPS	BP	CdCl2	p,p'-DDE	DBP	DEHP	PFOA	PFOS	% of Cell viability / Control
<b>72 h</b>											
0.1 µM	86	96	95	96	93	87	93	89	93	99	175
1 µM	88	87	86	96	78	86	95	90	99	114	150
10 µM	77	70	73	86	41	76	90	81	70	66	125
25 µM	74	68	63	77	29	67	85	74	78	64	100
<b>14 days</b>											
0.1 µM	103	99	100	96	100	98	85	103	95	105	75
1 µM	102	100	100	105	96	98	81	110	92	102	50
10 µM	120	102	92	107	68	85	83	108	93	92	25
25 µM	120	100	90	110	1	93	78	100	90	97	0

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29 **Supplementary Figures**

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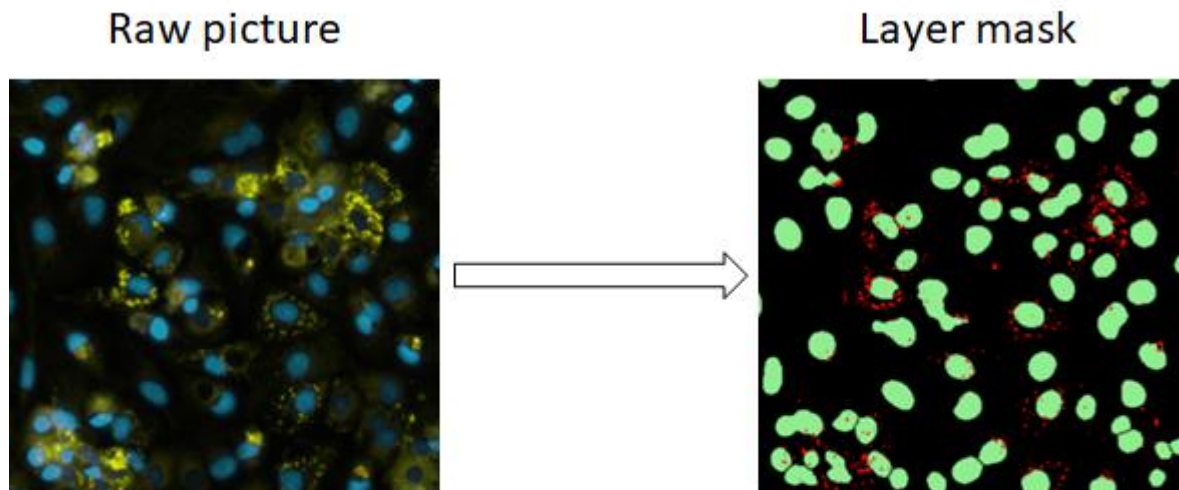
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33 **Supplementary Figure 1: *In vitro* exposure protocols of 2D HepaRG cells to assess**  
34 **impact of EDCs on lipid accumulation.** Time 0 corresponds, in all cases, to 5 days after  
35 seeding into 6-, 12- or 96-well plates (see Materials and Methods, 2.1 Compounds and cell  
36 culture). **(A)** Steatosis induction of pollutants, alone, after 5 days of exposure under normal  
37 culture conditions of HepaRG. **(B)** Steatosis induction of pollutants in combination with fatty

38 acids. Different fatty acid supplementations were used: 100  $\mu\text{M}$  or 200  $\mu\text{M}$  oleic acid (OA), a  
39 mixture of palmitic and oleic acid (PA:OA, 50:50  $\mu\text{M}$ ). (C) Steatosis remaining; to assess the  
40 capacity of EDCs to maintain lipid overload. Cells were exposed for 5 days to 200  $\mu\text{M}$  OA to  
41 induce steatosis. The fatty acid supplementation was then interrupted and cells were exposed  
42 to pollutants alone for 5 more days. A steatosis maintenance percentage was specifically  
43 defined: a maintenance of 100 % corresponds to a steatosis score identical to that of cells  
44 treated for 5 days with 200  $\mu\text{M}$  OA, while 0 % corresponds to the score of cells not exposed  
45 to any supplementation. The arrows indicate the days when the medium was renewed.

46



**Steatosis index =**

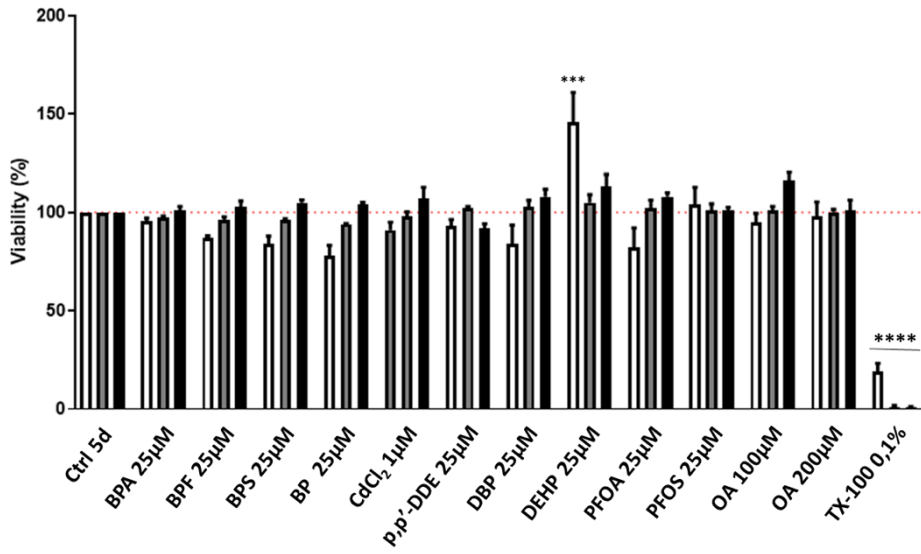
$$\text{mean surface of lipid droplets} \times \text{density of lipid droplets by cell}$$

47

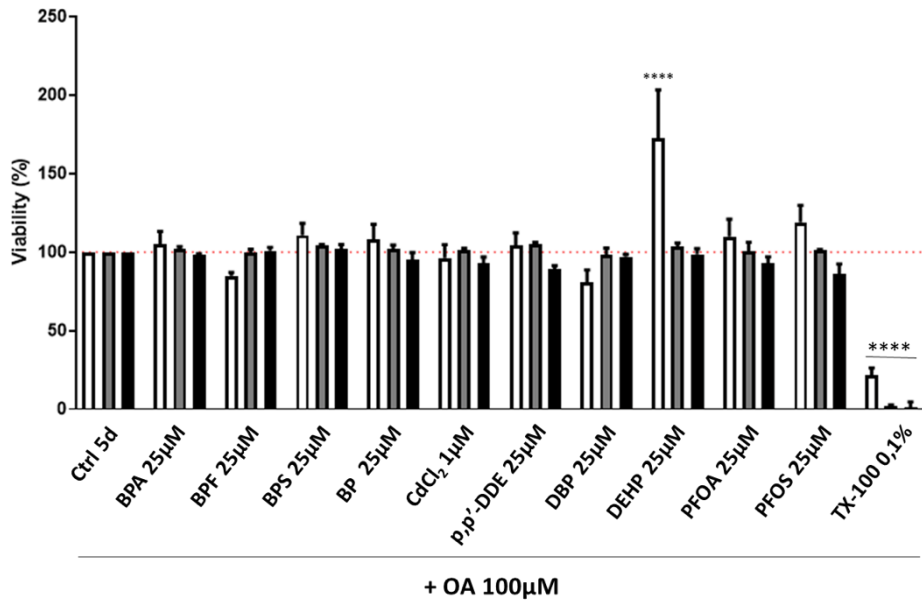
48 **Supplementary Figure 2: Fluorescence microscopy image acquisition with data analysis**  
49 **and steatosis scoring for 2D HepaRG.** After exposure following the different protocols  
50 (described in **Supplementary Figure 1**), cells were stained with Hoechst for nuclei (blue) and  
51 Nile Red for neutral lipids (yellow). A layer mask was then created to select nuclei (green)  
52 and lipid droplets (red) in the cells by using the size and the intensity (exceeding the  
53 background noise). To compare the impact of different treatments, a steatosis index,  
54 consisting of the mean surface area of the lipid droplets multiplied by the mean number of  
55 lipid droplets per cell, was defined.

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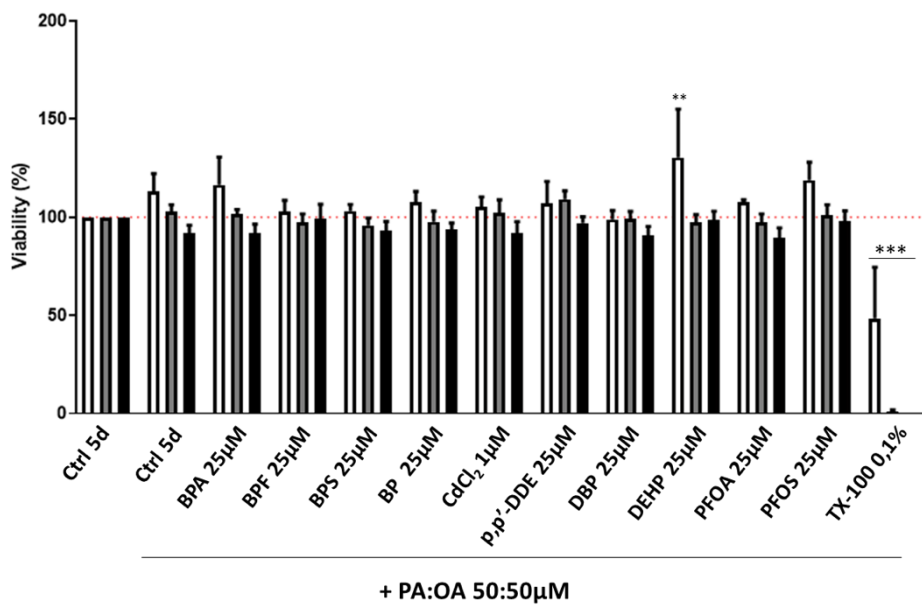
**A**



**B**

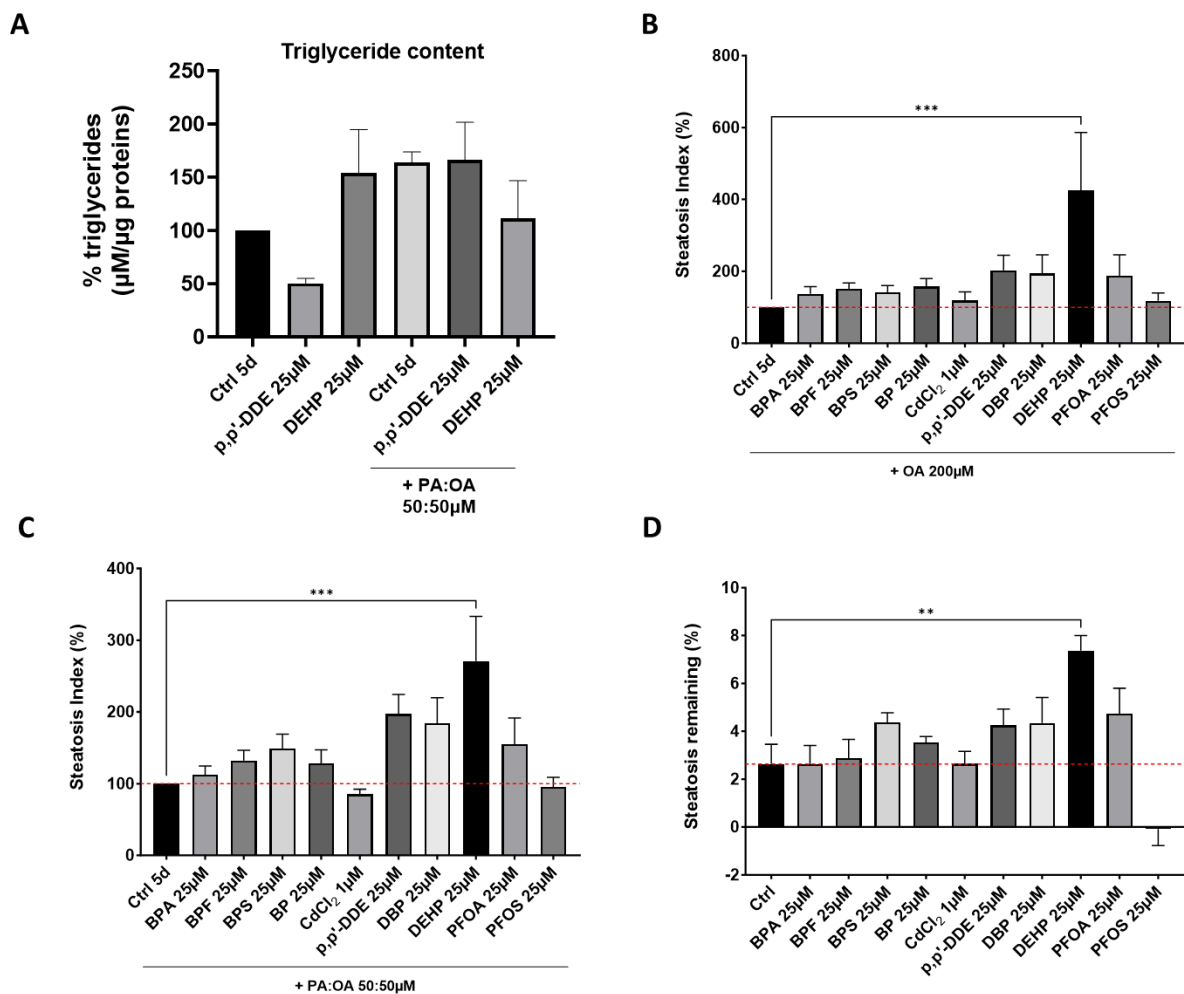


**C**



58 **Supplementary Figure 3:** Viability of 2D HepaRG cells following 5 days of exposure to (A)  
59 EDCs under normal culture conditions, (B) EDCs in combination with 100  $\mu$ M oleic acid  
60 (OA) and (C) EDCs in combination with a mixture of oleic and palmitic acid (PA:OA, 50:50  
61  $\mu$ M). Three distinct metabolic activities: dehydrogenase (white), esterase (grey) and  
62 lysosomal (black) were measured, respectively, with neutral red, resazurin and CFDA-AM  
63 assays. The medium was renewed every 2 days after the first treatment. The values are the  
64 mean (in %) relative to the control of three to four independent experiments in triplicate.  
65 Statistical analysis was performed using two-way ANOVA followed by a Tukey post-hoc test.  
66 Stars represent the significance of the fold change as compared to the control (\*\*,  $p < 0.01$ ;  
67 \*\*\*,  $p < 0.001$ ; \*\*\*\*,  $p < 0.0001$ ).

68

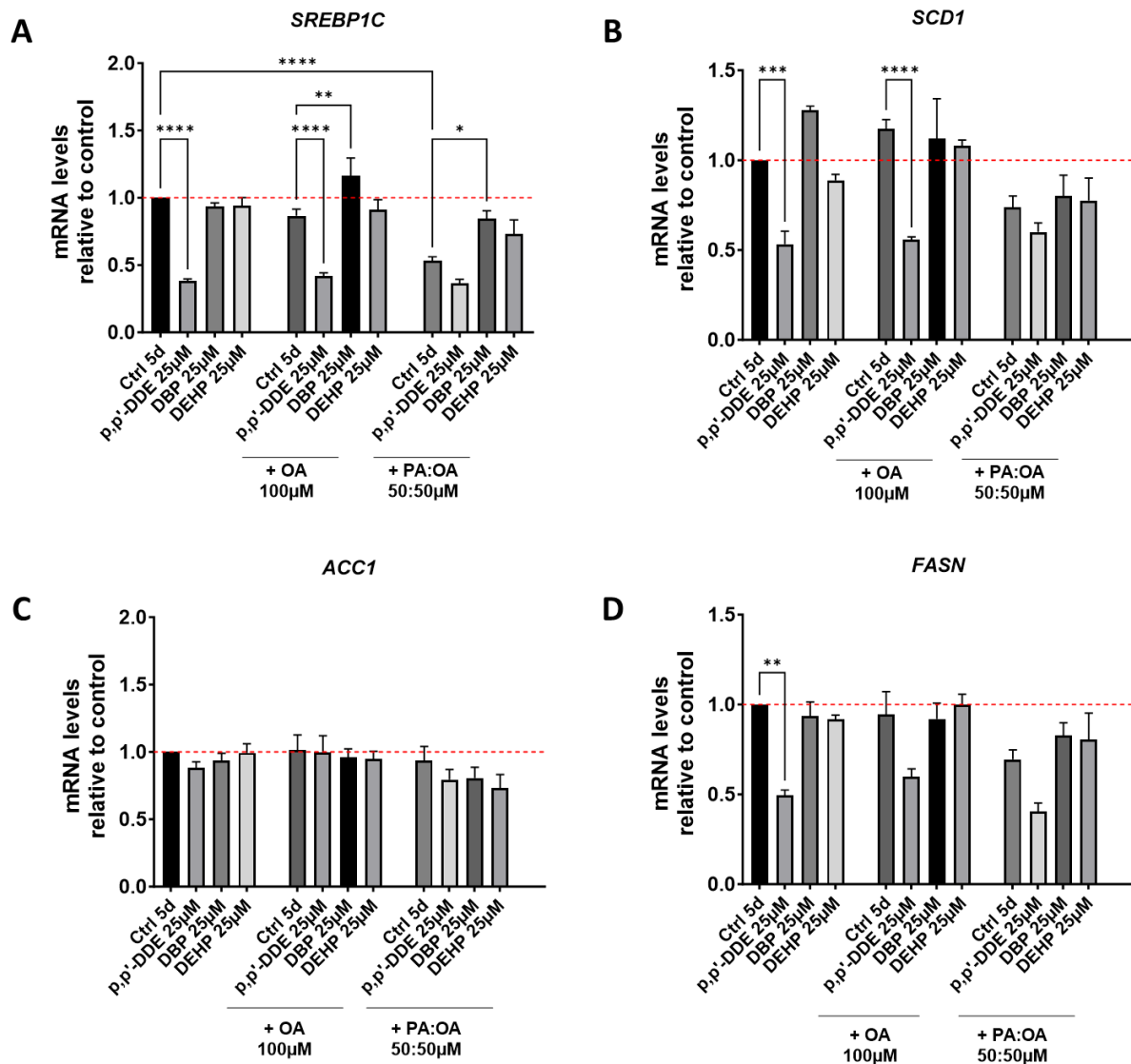


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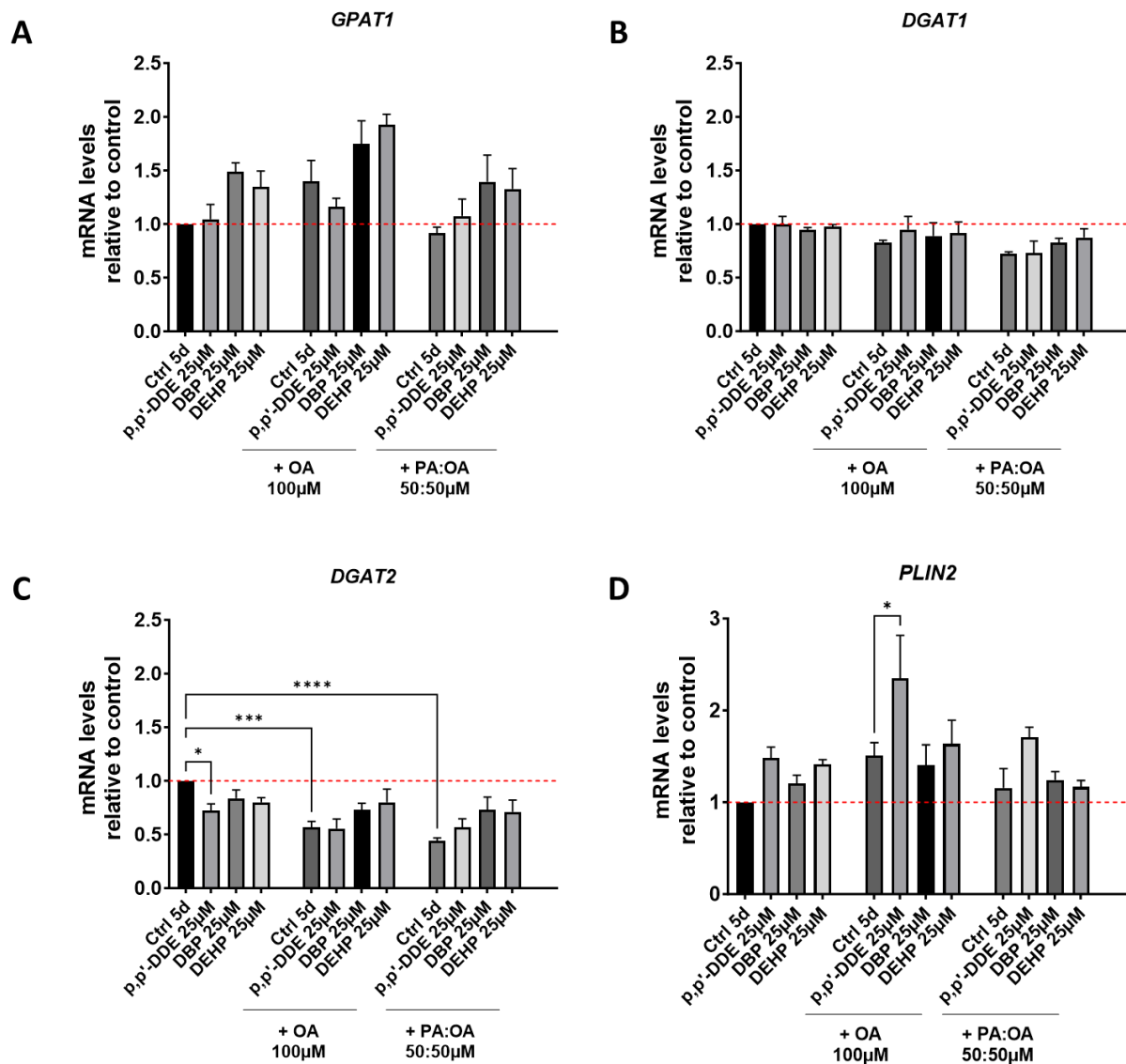
71 **Supplementary Figure 4:** (A) Assessment of triglyceride accumulation in HepaRG cells  
 72 after 5 days of exposure to EDCs without or with supplementation with FA (PA:OA, 50:50  
 73 μM). (B, C, D) Assessment of steatosis index by fluorescence microscopy of HepaRG cells  
 74 following exposure to EDCs. Lipid accumulation was evaluated after 5 days of exposure to  
 75 EDCs (B) in combination with 200 μM oleic acid (OA) or (C) under co-exposure with a  
 76 mixture of palmitic and oleic acid (PA:OA, 50:50 μM). (D) The steatosis remaining was  
 77 measured in HepaRG cells by triglyceride assessment with fluorescence microscopy. Cells  
 78 were exposed for 5 days with 200 μM OA to induce a marked steatotic state and then exposed  
 79 for 5 days, without OA supplementation, to 25 μM EDCs. A steatosis maintenance percentage  
 80 was specifically defined: a maintenance of 100 % corresponds to a steatosis score identical to  
 81 that of cells treated for 5 days with 200 μM OA, while 0 % corresponds to the score of cells  
 82 not exposed to any supplementation. To assess triglyceride accumulation, a steatosis index  
 83 (cf. **Supplementary Figure 2**) was used based on Nile Red staining and analysis with an  
 84 automated fluorescence microscope. Values are the mean ± SEM relative to the control (in %)



85 from at least 5 (**B**), 4 (**C**) or 3 (**A, D**) independent experiments in triplicate. Statistical analysis  
86 was performed using Kruskal-Wallis test with Dunn's correction (**B**), or using one-way  
87 ANOVA followed by a Dunnett post-hoc correction (**A, C, D**). \*\*,  $p < 0,01$ ; \*\*\*,  $p < 0,001$ .  
88



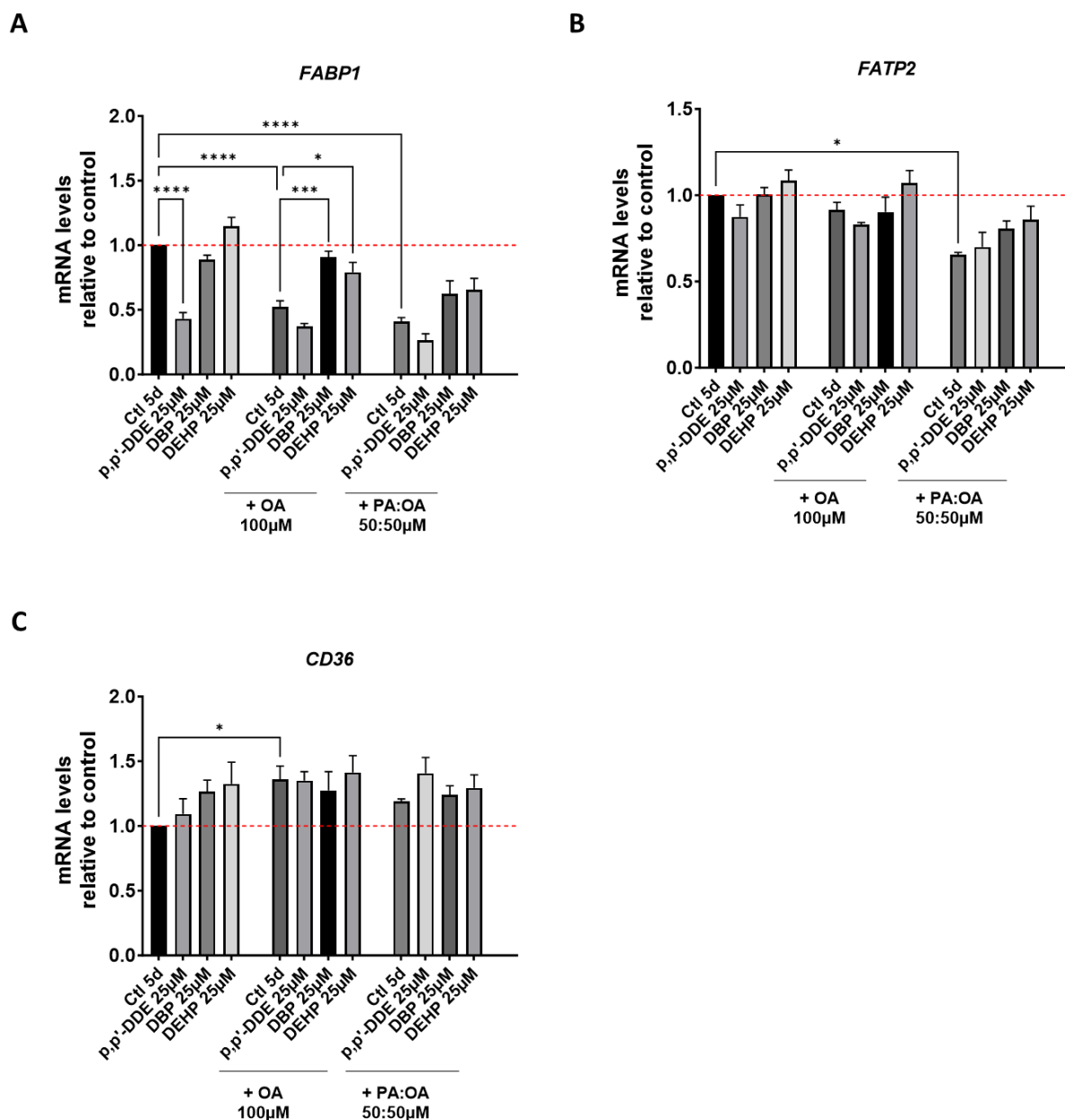
89  
 90 **Supplementary Figure 5: Impact of p,p'-DDE, DBP and DEHP exposure on *de novo***  
 91 **lipogenesis gene expression in 2D HepaRG cells.** The mRNA levels of (A) *SREBP1C*, (B)  
 92 *SCD1*, (C) *ACC1* and (D) *FASN* were measured after 5 days of exposure to 25 μM EDCs  
 93 alone or in combination with 100 μM oleic acid (OA) or a mixture of palmitic and oleic acid  
 94 (PA:OA, 50:50 μM). Values are the mean ± SEM relative to the control from at least 3  
 95 independent experiments. Statistical analysis was performed on treatments having the same  
 96 supplementation condition using one-way ANOVA followed by a Bonferroni correction (A,  
 97 B, C) or Kruskal-Wallis test followed by a Dunn's correction (D). \*, p < 0.05; \*\*, p < 0.01;  
 98 \*\*\* p < 0.001; \*\*\*\*, p < 0.0001.



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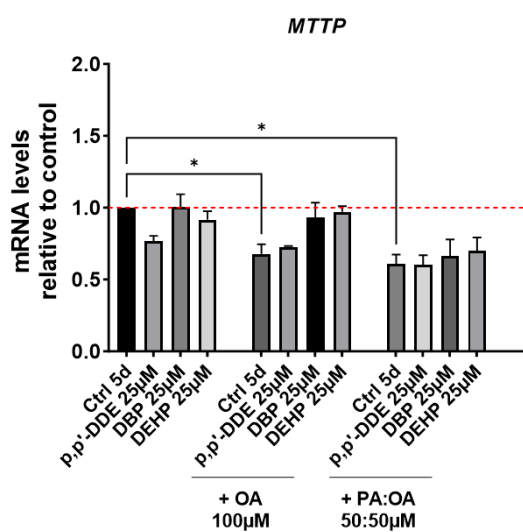
100 **Supplementary Figure 6: Impact of p,p'-DDE, DBP and DEHP exposure on the**  
 101 **expression of genes related to triglyceride synthesis and storage in 2D HepaRG cells.** The  
 102 mRNA levels of (A) *GPAT1*, (B) *DGAT1*, (C) *DGAT2* and (D) *PLIN2* were measured after 5  
 103 days of exposure to 25 μM EDCs alone or in combination with 100 μM oleic acid (OA) or a  
 104 mixture of palmitic and oleic acid (PA:OA, 50:50 μM). Values are the mean ± SEM relative  
 105 to the control from at least 3 independent experiments. Statistical analysis was performed on  
 106 treatments having the same supplementation condition using one-way ANOVA followed by a  
 107 Bonferroni correction (A, C, D) or Kruskal-Wallis test followed by a Dunn's correction (B).  
 108 \*,  $p < 0.05$ ; \*\*\*,  $p < 0.001$ ; \*\*\*\*,  $p < 0.0001$ .

109

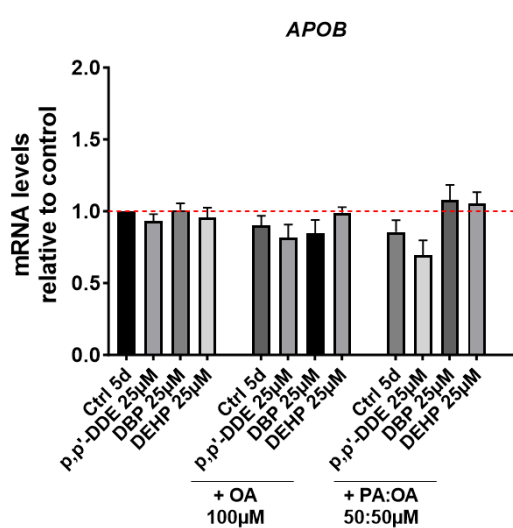


110  
 111 **Supplementary Figure 7: Impact of p,p'-DDE, DBP and DEHP exposure on the**  
 112 **expression of genes related to fatty acid uptake in 2D HepaRG cells.** The mRNA levels of  
 113 (A) *FABP1*, (B) *FATP2* and (C) *CD36* were measured after 5 days of exposure to 25 μM  
 114 EDCs alone or in combination with 100 μM oleic acid (OA) and a mixture of palmitic and  
 115 oleic acid (PA:OA, 50:50 μM). Values are the mean ± SEM relative to the control from at  
 116 least 3 independent experiments. Statistical analysis was performed on treatments having the  
 117 same supplementation condition using one-way ANOVA followed by a Bonferroni correction  
 118 (A) or Kruskal-Wallis test followed by a Dunn's correction (B, C). \*, p < 0.05; \*\*\*, p <  
 119 0.001; \*\*\*\*, p < 0.0001.

A



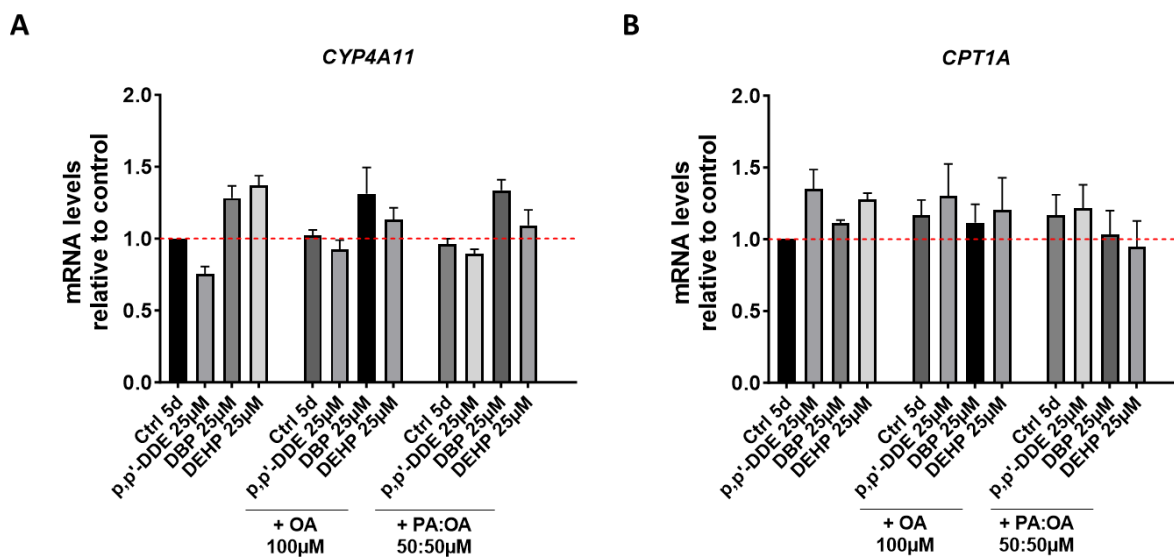
B



120

121 **Supplementary Figure 8: Impact of p,p'-DDE, DBP and DEHP exposure on the**  
 122 **expression of genes related to VLDL export in 2D HepaRG cells.** The mRNA levels of  
 123 *MTP* (A) and *APOB* (B) were measured after 5 days of exposure to 25 µM EDCs alone or in  
 124 combination with 100 µM oleic acid (OA) or a mixture of palmitic and oleic acid (PA:OA,  
 125 50:50 µM). Values are the mean ± SEM relative to the control from at least 3 (A, B)  
 126 independent experiments. Statistical analysis was performed on treatments having the same  
 127 supplementation condition using one-way ANOVA followed by a Bonferroni correction (B)  
 128 or Kruskal-Wallis test followed by a Dunn's correction (A) \*,  $p < 0.05$ .

129



130

131 **Supplementary Figure 9: Impact of p,p'-DDE, DBP and DEHP on fatty acid oxidation**  
 132 **in 2D HepaRG cells.** The mRNA levels of (A) *CYP4A11* and (B) *CPT1A* were measured  
 133 after 5 days of exposure to 25 μM EDCs alone or in combination with 100 μM oleic acid  
 134 (OA) and a mixture of palmitic and oleic acid (PA:OA, 50:50 μM). Values are the mean ±  
 135 SEM relative to the control from at least 3 independent experiments (A, B). Statistical  
 136 analysis was performed on treatments having the same supplementation condition using a  
 137 Kruskal-Wallis test with a Dunn's correction (A, B).

138