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Supplementary information to:

Original article:

OKADAIC ACID INFLUENCES XENOBIOTIC METABOLISM IN HEPARG CELLS

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Supplementary Table 1: Temperature profile used for the qPCR

Reaction	Temperature	Time	Repeats
Initialization	50°C 95°C	2 min 10 min	1
Denaturation, primer binding and elongation	95°C 60°C 72°C	10 s 15 s 20 s	40
Dissociation curve	95°C 60°C 95°C	15s 15s 15s	1

Supplementary Table 2: Used primer sequences for the qPCR

Protein name	Forward primer	Reverse primer
CYP1A1	ACCCTGAAGGTGACAGTTCC	TCTTGGAGGTGGCTGAGGTA
CYP1A2	AATCAGGTGGTGGTGTCAGT	TGAGTGTTCTTCACGAGGCT
CYP2B6	TTCGGCGATTCTCTGTGACC	ATGAGGGCCCCCTTGGAT
CYP2C8	CCTCTCATCAAATCTTCCCATT	GCAGTGACCTGAACAACTCTCC
CYP2C9	AAATGGAGAAGGAAAAGCACAACC	TCAACTGCAGTGTTTTCCAAGC
CYP2C19	CCTGGAACGCATGGTGGT	TCCATTGCTGAAAACGATTCCAAAT
CYP2E1	CATGAGATTCAGCGGTTCATC	GGTGTCTCGGGTTGCTTCA
CYP3A4	TCACAAACCGGAGGCCTTTT	TGGTGAAGGTTGGAGACAGC
CYP7A1	GACACACCTCGTGGTCCTCT	TTTCATTGCTTCTGGGTTCC
GUSB	CACCAGGGACCATCCAATAC	ATGTAGGTGGTGGGTGTCGT
GAPDH	TTAAAAGCAGCCCTGGTGAC	CTCTGCTCCTCCTGTTCGAC

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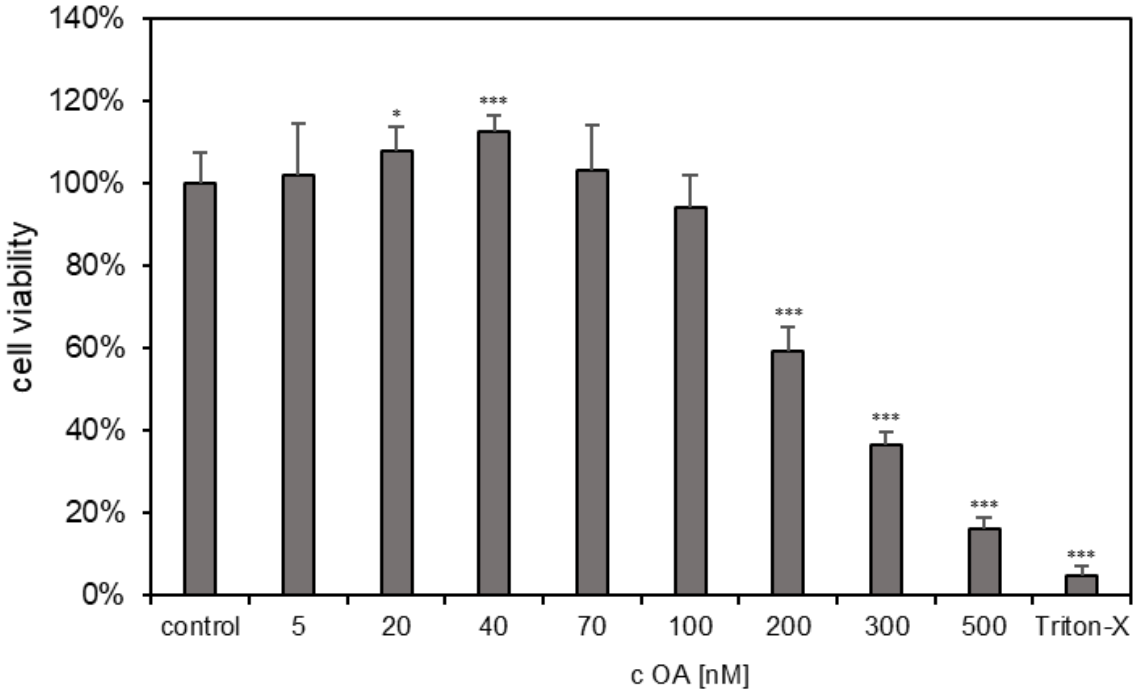
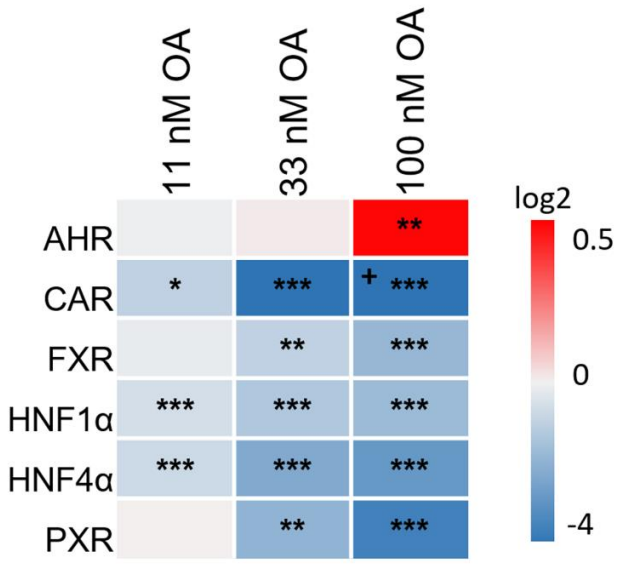


Supplementary Figure 1: MTT-Assay of selected OA concentrations on HepaRG cells. Cells were seeded in 96-well plates and grown for 2 weeks. They were then differentiated for 2 weeks prior to 24 h incubation. Cell viability is proportional to absorption at 570 nm and was compared to an untreated control. Based on the MTT assay, 100 nM OA was picked as the highest concentration for incubation. 11 and 33 nM were picked as non-toxic concentrations based on the 100 nM. Statistical analysis was performed using t-test (*p < 0.05; **p < 0.01; ***p < 0.001).



Supplementary Figure 2: Effect of OA on relevant transcription factors. HepaRG cells were treated with 11 nM, 33 nM and 100 nM OA, and mRNA levels were determined by qPCR and normalized to the housekeeping genes GUSB and GAPDH. The heatmap shows the log2 values of the resulting fold changes as mean of three independent replicates, relative to the solvent control. Marking with "+" indicates a fold change value outside of the color scheme (i.e. value <-4). Statistical analysis (=3) was performed using one-way ANOVA followed by Dunnett's post-hoc test (*p < 0.05; **p < 0.01; ***p < 0.001).

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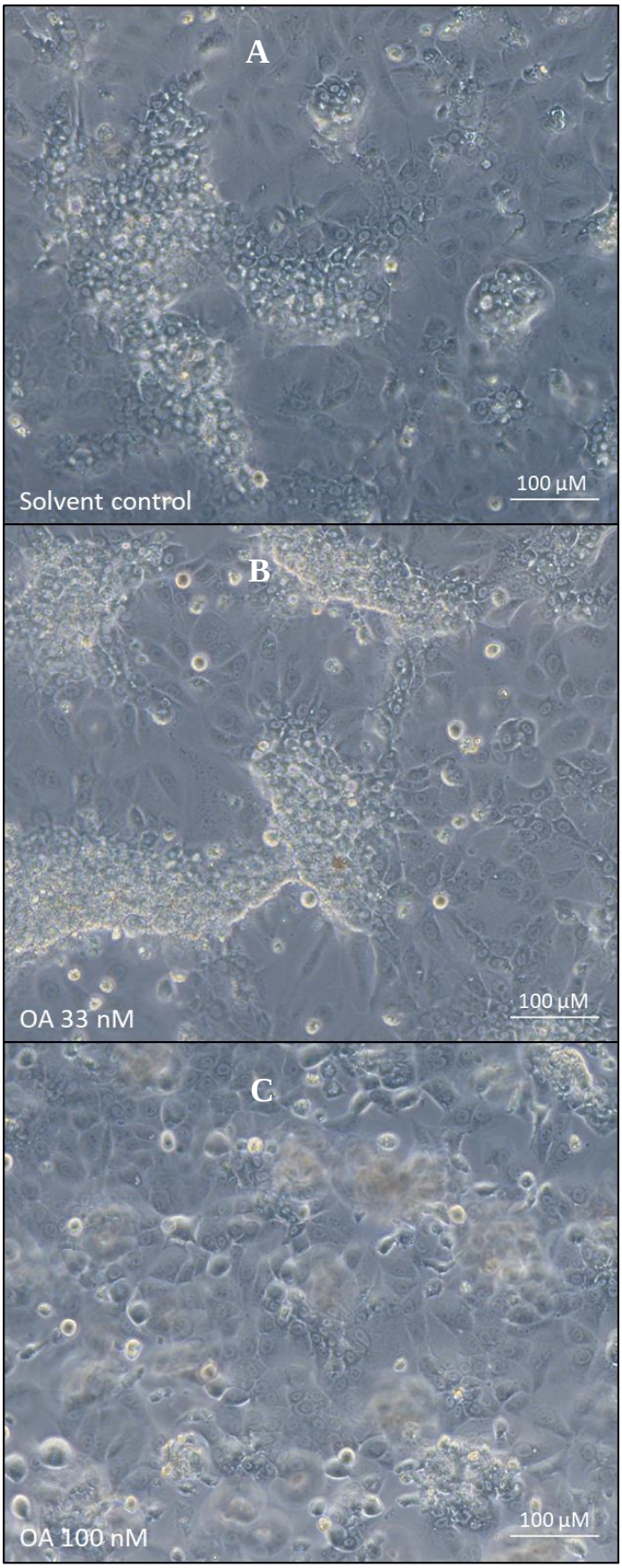

Supplementary Figure 3: Series of micrographs of the used HepaRG cells. Cells were were seeded in 12-well plates and grown for 2 weeks. They were then differentiated for 2 weeks prior to 24 h incubation. Cells were visualized using the Zeiss Axio Observer (Zeiss, Göttingen) and the Software ZEN 2.3 Pro for editing. **A** shows differentiated, untreated HepaRG cells after 2 weeks differentiation. **B** shows differentiated HepaRG cells incubated with 33 nM OA for 24 h. **C** shows differentiated HepaRG cells incubated with 100 nM OA for 24 h.