Regulation of drug metabolism by the interplay of inflammatory signaling, steatosis, and xeno-sensing receptors in HepaRG cells

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Supplementary figures and legends
Supplementary Figure 1. Cytotoxicity testing of HepaRG cells. Cells were incubated in the presence of increasing concentrations of an equimolar O/P (1:1) mixture (A, C) or in the presence of increasing concentrations of IL-6 (B, D). Incubation was performed for either 24h (A, B) or for 14 days with medium change and repeated treatment every 2\textsuperscript{nd} day (C, D). Data from the MTT and CTB tests are depicted as means ± SD (two independent biological replicates, each assayed in quintuple technical replicates) and given relative to solvent-treated control cells (NC). PC (positive control): cells treated with 0.1% Triton X-100. The non-toxic concentrations of 10 ng/mL IL-6 and 125 µM O/P were selected for further analyses.
Supplementary Figure 2. Verification of cellular steatosis by Oil Red O staining. HepaRG cells were treated for 24h with control medium (A) or with 125 µM of O/P (125 µM of each of the fatty acids) (B) and images were taken under a light microscope. The scale bars in the images denote a distance of 200 µm. (C) For quantitative analysis of the dose-dependency of fat incorporation Oil Red O staining was measured on a multi-well plate reader following incubation of differentiated HepaRG cells with the indicated concentrations of O/P for 24h. Means + SD (assayed in quintuple determination) are given relative to untreated cells (set to 1)." A non-toxic and steatosis-inducing concentration of 125 µM O/P was selected for further analyses.
Supplementary Figure 3. Heat-map visualization of the expression of inflammation-related genes in HepaRG cells and their regulation by nuclear receptor agonists, O/P, and IL-6. Cells were treated according to the scheme presented in Figure 1 and mRNA expression levels were analyzed using a Fluidigm PCR system. For more details, please refer to the legend to Figure 2 and the raw data in Supplemental Table 1. Mean values (n=3 independent biological replicates) are given relative; asterisks indicate statistical significance in comparison to solvent control (p<0.05); hashmarks indicate statistical significance compared to solvent control of cells not treated with IL-6 and/or O/P.
**Supplementary Figure 4.** Heat-map visualization of the expression of fat metabolism-related genes in HepaRG cells and their regulation by nuclear receptor agonists, O/P, and IL-6. For more details, please refer to the legend to Figure 2 and the raw data in Supplemental Table 1. Mean values (n=3 independent biological replicates) are given; asterisks indicate statistical significance in comparison to solvent control (p<0.05); hashmarks indicate statistical significance compared to solvent control of cells not treated with IL-6 and/or O/P.
**Supplementary Figure 5.** Heat-map visualization of the expression of non-CYP genes related to phase I of drug metabolism in HepaRG cells and their regulation by nuclear receptor agonists, O/P, and IL-6. For more details, please refer to the legend to Figure 2 and the raw data in Supplemental Table 1. Mean values (n=3 independent biological replicates) are given; asterisks indicate statistical significance in comparison to solvent control (p<0.05); hashmarks indicate statistical significance compared to solvent control of cells not treated with IL-6 and/or O/P.
**Supplementary Figure 6.** Heat-map visualization of the expression of genes related to phase II or phase III of drug metabolism in HepaRG cells and their regulation by nuclear receptor agonists, O/P, and IL-6. For more details, please refer to the legend to Figure 2 and the raw data in Supplemental Table 1. Mean values (n=3 independent biological replicates) are given; asterisks indicate statistical significance in comparison to solvent control (p<0.05); hashmarks indicate statistical significance compared to solvent control of cells not treated with IL-6 and/or O/P.