Supplemental Fig.1

A Carp hepatocyte preparation and in vitro cell culture experiments

Liver slices (0.8-1.0 g per fish) were freshly excised from 5-6 grass carps. After repeated rinsing to remove blood clots, individual slices were diced into 0.5 mm thick fragments with a McIlwain Tissue Chopper (Cavey Lab, Guildford Surrey, UK) and subjected to 30 min digestion with collagenase (type IV , 500 U/ml) and DNase II (0.01 mg/ml) at 28 ^{o}C with gentle shaking. After that, hepatocytes were dispersed by gentle pipetting, filtered through sterilized nylon mesh (~30 µm pore size), and seeded in PEI-precoated 24-well plates at ~0.7x10^{6} cells/ml/well in DMEM/F12 medium (pH 7.6, without serum supplement). The viability of carp hepatocytes was >95% as revealed by trypan blue exclusion test and the average cell yield per cell dispersion was 59.3 ± 7.2 million cells/g liver slices (N = 12). The cells prepared were cultured overnight (~15 hr) at 28 ^{o}C under 5% CO_2 and saturated humidity to allow for recovery from enzyme digestion. On the next day, static incubation experiments with test substances (with/without inhibitors given at the same time) was conducted for the duration as indicated. After drug treatment, total RNA (~4 µg/well) was isolated by TRIzol (Thermo Fisher), reversely transcribed by Superscript II (Invitrogen) using oligo(dT) as the primer, and subjected to real-time PCR for respective gene targets using a Rotor Gene-Q System (Qiagen). Serial dilutions of plasmid DNA with ORF of target genes were used as the standards for data calibration using RotorGene Q-Rex software and parallel measurement of 18S RNA expression was used as internal control. The authenticity of PCR products were routinely confirmed by melting curve analysis after individual real-time PCR assays.

B Time course of carp hepatocyte culture