Supplementary Information

Figure legends

Figure S1. **A.** Confirmation of HEK293T permanently overexpressing CYP2D6^{*1} and CYP2D6^{*4} cell model. Expression levels of Flag tagged functional wild-type CYP2D6 and non-functional mutant CYP2D6 were checked by immunoblotting analysis using actin as loading control. **B.** Confirmation of HEK293T cells permanently overexpressing CYP2D6^{*1} and CYP2D6^{*4}, adenovirus overexpressing POR and CYB5A were checked by immunoblotting analysis using actin as loading control. **C.** Detection of CYP2D6 catalytic activity in HEK293T cells stalely overexpressing wild-type (*CYP2D6*1*) or LoF CYP2D6 alleles, and transiently overexpressing POR and CYB5A. The formation of dextrorphan was measured at indicated time points after incubation with 10μM CYP2D6 specific substrate dextromethorphan by LC-MS/MS. **D.** Cell growth rate was measured in HEK293T parental, CYP2D6$^{*1\text{-POR-B5A}}$ and CYP2D6$^{*4\text{-POR-B5A}}$ cells. No significantly different growth rates were observed.

Figure S2. **A.** Dose response to 13 identified hit compounds in the HEK293T cell model. Name of 13 compounds: AZD-3463, CYC-16, EHT-1864, Etoposide, Everolimus, GDC-0349, Lenvatinib, MK-8776, PHA-680632, Talazoparib, Tyrphostin 9, VX-702 and WZ-3146. **B.** IC_{50} of 13 identified hit compounds in HEK293T CYP2D6$^{*1\text{-POR-B5A}}$ and CYP2D6$^{*4\text{-POR-B5A}}$ cells. One representative experiment with three technical replicates is shown (mean $\pm$ SD). To calculate the half-maximal inhibitory concentration (IC_{50}), at least 5 different concentrations were made. The half-maximal inhibitory concentration (IC_{50}) was calculated using Dose-Response inhibition (inhibition vs normalized response) analysis in GraphPad prism 8.

Figure S3. **A.** Dose response to 13 identified hit compounds in the HepG2 parental and CYP2D6 KO clones. Name of 13 compounds: AZD-3463, CYC-16, EHT-1864, Etoposide, Everolimus, GDC-0349, Lenvatinib, MK-8776, PHA-680632, Talazoparib, Tyrphostin 9, VX-702 and WZ-3146. **B.** IC_{50} of 13 identified hit compounds in the HepG2 parental and CYP2D6 KO clones. One representative experiment with three technical replicates is shown (mean $\pm$ SD). To calculate the half-maximal inhibitory concentration (IC_{50}), at least 5 different concentrations were made. The half-maximal inhibitory concentration (IC_{50}) was calculated using Dose-Response inhibition (inhibition vs normalized response) analysis in GraphPad prism 8.

Figure S4. **A.** Protein expression of HepG2 CYP2D6^{Wt} and CYP2D6^{Mut} variants by immunoblotting. A Flag-tag antibody was used to detect recombinant CYP2D6 expression with β-actin as loading control. **B.** Left: Detection of CYP1A2 and CYP2C9 catalytic activity in HepG2 parental or overexpressing cells. CYP1A2 inhibitor α-naphthoflavone (5 μM), CYP2C9 inhibitor sulfaphenazole (5 μM). Right: Dose response to the identified 3 hit compounds Talazoparib, GDC-0349 and MK-8776 in HepG2 CYP1A2 and CYP2C9 overexpressing cells absent or present inhibitors. One representative experiment with three technical replicates is shown (mean ± SD, * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001, unpaired t-test, Two-stage step-up (Benjamini, Krieger, and Yekutieli).

Figure S5. **A.** Representative images for spheroids formed by HEK293T parental and HepG2 parental cells. **B.** Dose response to GDC-0349 in spheroids formed by HEK293 CYP2D6*1 and CYP2D6*4 cells, and HepG2 Parental and *CYP2D6* KO cells. One representative experiment with three technical replicates is shown (mean ± SD, * P<0.05, ** P<0.01, unpaired t-test, Two-stage step-up (Benjamini, Krieger, and Yekutieli)). Cell viability was measured by the resazurin assay after 72 h of incubation with the indicated drugs. **C.** Representative images for HEK293T *CYP2D6*$^{*1\text{-}POR\text{-}B5A}$ and *CYP2D6*$^{*4\text{-}POR\text{-}B5A}$ cells were taken by Incucyte after 72 h of incubation with GDC-0349. Diameters were measured and spheroids volumes were calculated by equation Volume=4/3*3.14*R3(R=radius). One representative experiment with three technical replicates is shown (mean ± SD, * P<0.05, unpaired t-test, Two-stage step-up (Benjamini, Krieger, and Yekutieli)). **D.** Dose response of human HCCOs to MK-8776 and GDC-0349. Sample number marked as HCCO-C975, HCCO-D045, HCCO-D046, HCCO-D324, HCCO-D386, HCCO-D415, and HCCO-D455 were determined as normal metabolizer; sample number marked as HCCO-C798, HCCO-C948, HCCO-C949, and HCCO-D359 were determined as intermediate metabolizer.