Supplemental Figures

Application of Mice Humanised for Cytochrome P450 CYP2D6 to the Study of Tamoxifen Metabolism and Drug-Drug Interaction with Antidepressants

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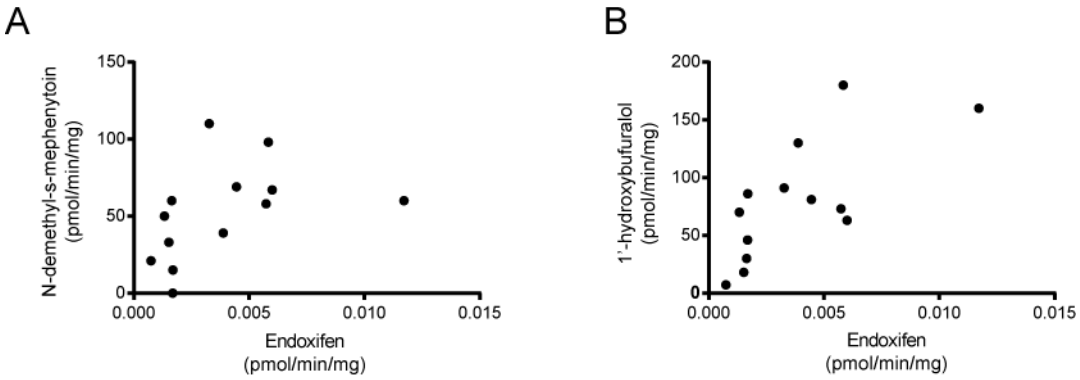
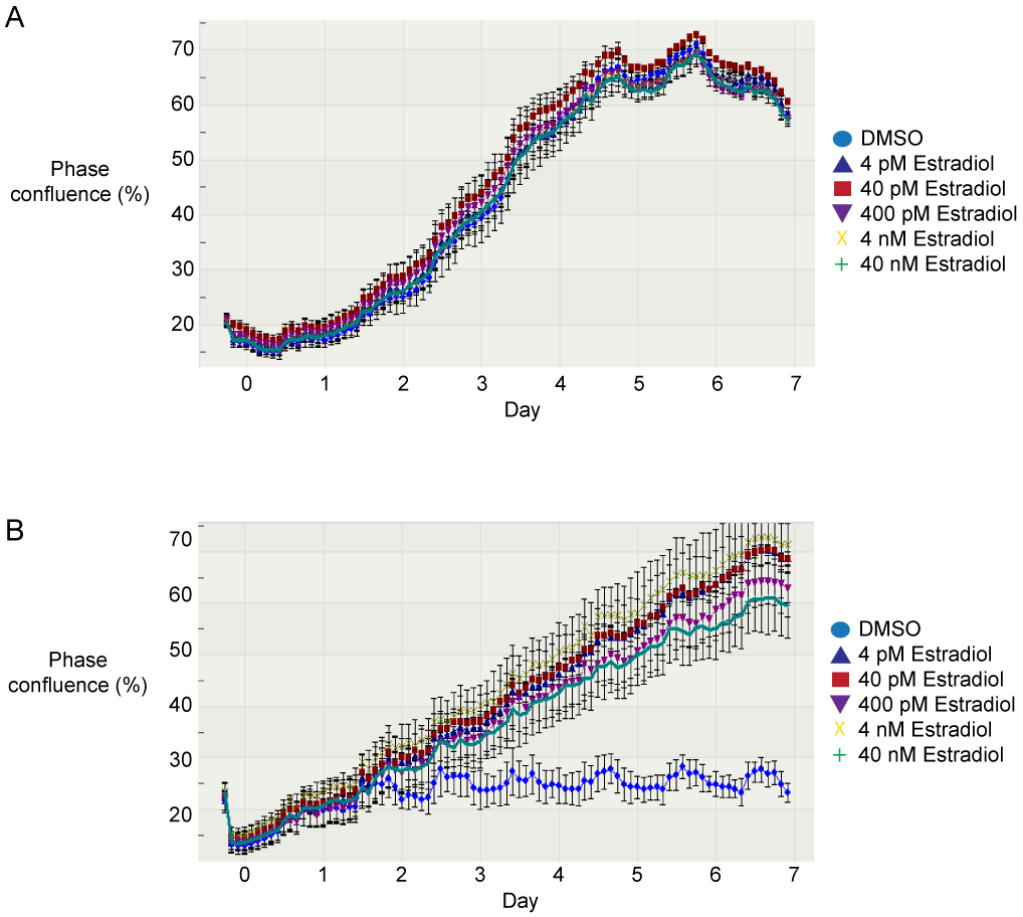
Drug Metabolism and Disposition



Supplemental Figure 1. Spearman's rank correlation of NDT hydroxylation with CYP2B6 and and CYP2D6 probe activities. As detailed in Table 1, conversion of NDT to endoxifen correlated with **(A)** S-mephenytoin N-demethylation (CYP2B6 activity, SRCC = 0.572, p = 0.045) and **(B)** bufuralol 1'-hydroxylation (CYP2D6 activity, SRCC = 0.698, p = 0.010).



Supplemental Figure 2. E0771 cell proliferation is independent of oestradiol *in vitro*. (A) E0771 and **(B)** MCF7 (positive control) cells were cultured in the presence of a range of oestradiol concentrations, or with the vehicle (DMSO) only, for seven days. The experimental protocol was adapted from those of Johnson (Johnson et al., 2004) and Aakvaag (Aakvaag et al., 1990). Briefly, cells were routinely cultured in DMEM containing 10% foetal bovine serum (FBS, both Thermo Fisher Scientific). On the day before seeding, cell monolayers (70% confluency) were washed once every hour for five hours with experimental medium (phenol red-free DMEM containing 10% charcoal-stripped FBS, Thermo Fisher Scientific) then incubated in this medium overnight. The following morning, cells were trypsinised (phenol red-free trypsin-EDTA, Thermo Fisher Scientific) and seeded into 96-well plates at a density of 1.6 x 10^4 cells per well. Five hours later, medium was replaced with experimental medium containing a range of concentrations of oestradiol (Sigma) and growth monitored by measurement of phase confluency on the InCucyte ZOOM platform (Essen BioScience, Ann Arbor, MI, USA) every two hours.

Supplemental References

Aakvaag A, Utaaker E, Thorsen T, Lea OA and Lahooti H (1990) Growth control of human mammary cancer cells (MCF-7 cells) in culture: effect of estradiol and growth factors in serum-containing medium. *Cancer Res* **50:**7806-7810.

Johnson MD, Zuo H, Lee KH, Trebley JP, Rae JM, Weatherman RV, Desta Z, Flockhart DA and Skaar TC (2004) Pharmacological characterization of 4-hydroxy-N-desmethyl tamoxifen, a novel active metabolite of tamoxifen. *Breast Cancer Res Treat* **85:**151-159.