

**Cell Metabolism, Volume 29**

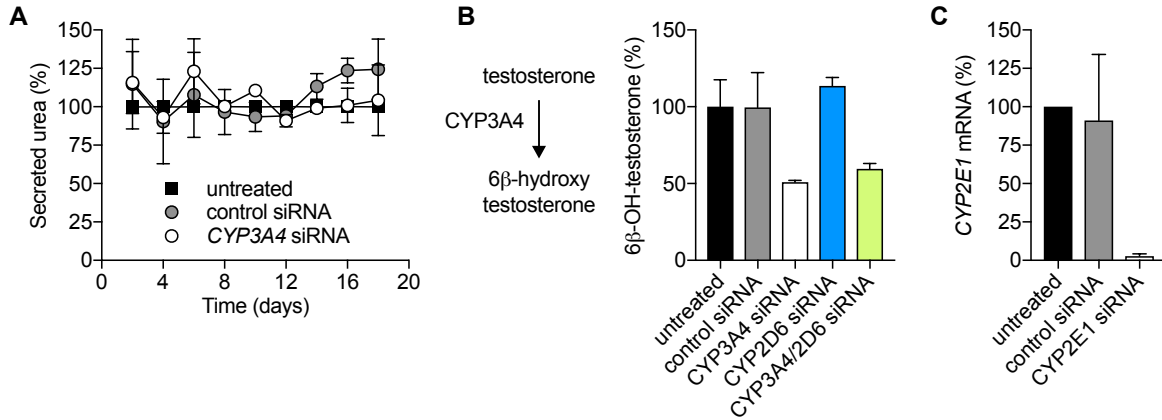
**Supplemental Information**

**Improving Drug Discovery by Nucleic Acid**

**Delivery in Engineered Human Microlivers**

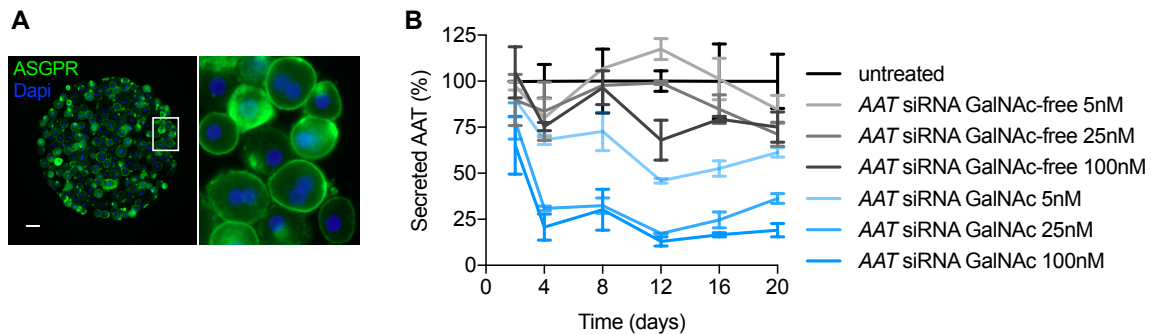
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SUPPLEMENTAL DATA



**Figure S1. CYP3A4 silencing in MPCCs. Related to Figures 2 and 3.**

(A) Relative urea levels in the culture supernatant of CYP3A4-silenced MPCCs, as determined by a colorimetric assay. Values are mean  $\pm$  SEM (n=2). (B) Relative 6 $\beta$ -hydroxytestosterone levels in the supernatant measured by mass spectrometry after incubation with testosterone on day 4 post-transfection (schematic of the reaction shown on the left). 6 $\beta$ -hydroxytestosterone peak area was normalized to untreated cells. Values are mean  $\pm$  SEM (n=2). (C) *CYP2E1* mRNA levels at day 3 post-transfection. Values (mean  $\pm$ SEM, n=2) were normalized to untreated MPCCs.



**Figure S2. GalNAc-mediated silencing in MPCCs. Related to Figure 4.**

(A) ASGPR expression in one of the 3 assayed donors at the time of siRNA addition. A representative hepatocyte island is shown, with a zoomed inset on the right. Scale bar, 50  $\mu$ m. (B) GalNAc- and dose-dependent AAT silencing in free-uptake mode. Values were normalized to untreated cells (mean  $\pm$  SD). A representative of 3 tested hepatocyte donors is shown.

**Table S1.** List of GalNAc-conjugated and unconjugated siRNA sequences. **Related to STAR Methods.**

siRNA	Sense (5'-3')	Antisense (3'-5')
<i>CD81</i>	UGCUCUUCGUCUUCAAUUUCA	UGAAAUUGAAGACGAAGAGCAGG
<i>SERPINA1 (AAT)</i>	CACCUGGAAAAUGAACUCACA	UGUGAGUUCAUTUCCAGGUGCU

**Table S2.** List of primer sequences. **Related to STAR Methods.**

Primer	Forward	Reverse
<i>CYP3A4</i>	CCGAGTGGATTCCTTCAGCTG	TGCTCGTGGTTTCATAGCCAGC
<i>CYP2D6</i>	GCAAGAAGTCGCTGGAGCAGTG	CTCACGGCTTTGTCCAAGAGAC
<i>CYP2E1</i>	GAGCACCATCAATCTCTGGACC	CACGGTGATACCGTCCATTGTG
<i>gapdh</i>	GTCTCCTCTGACTTCAACAGCG	ACCACCTGTTGCTGTAGCCAA