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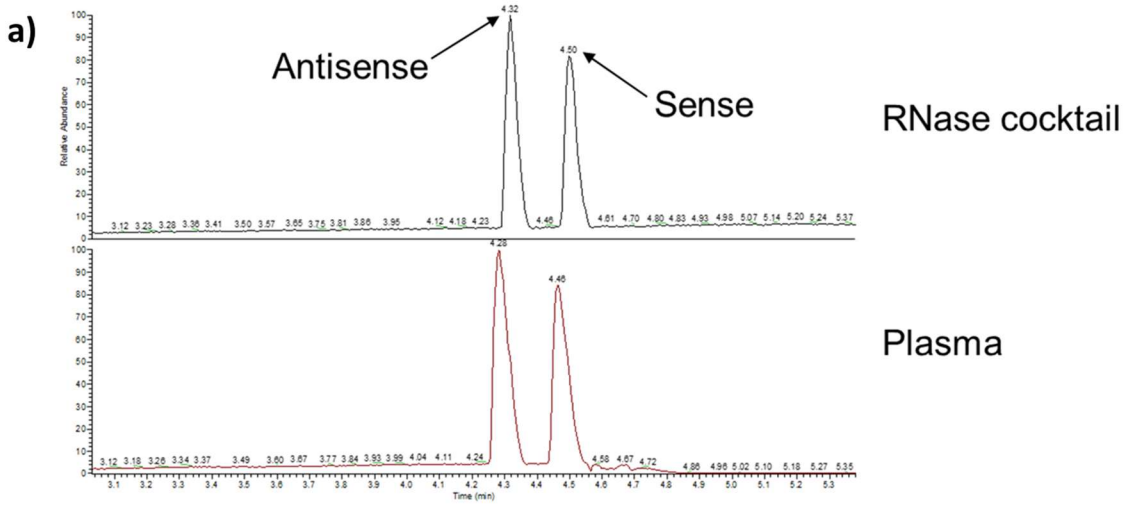
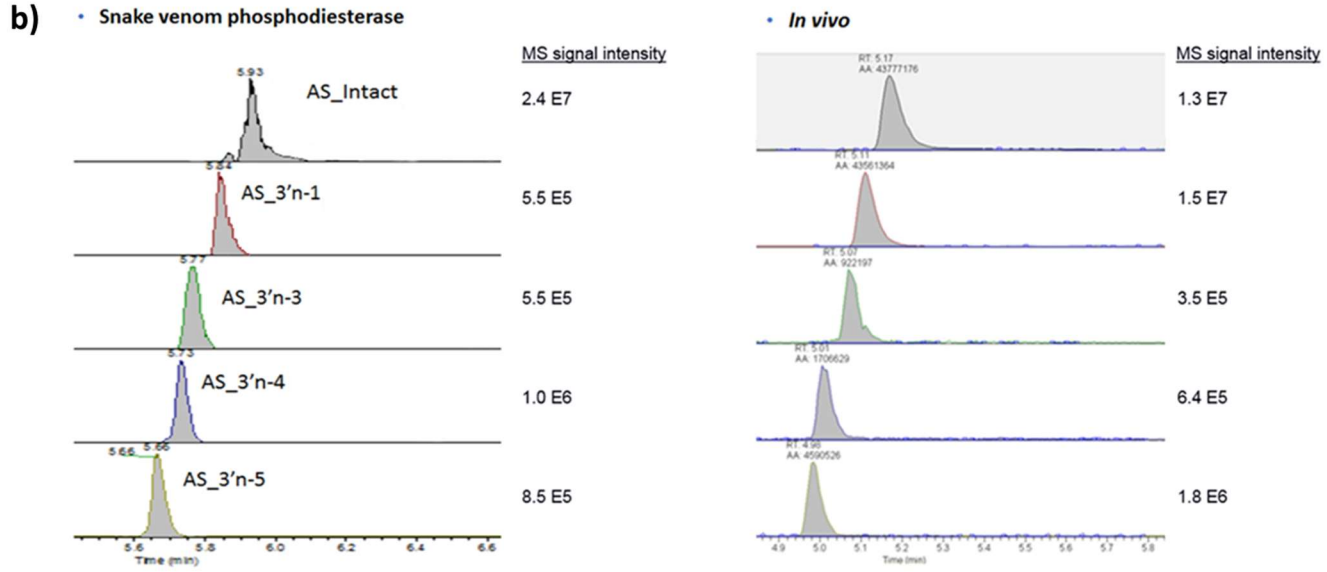
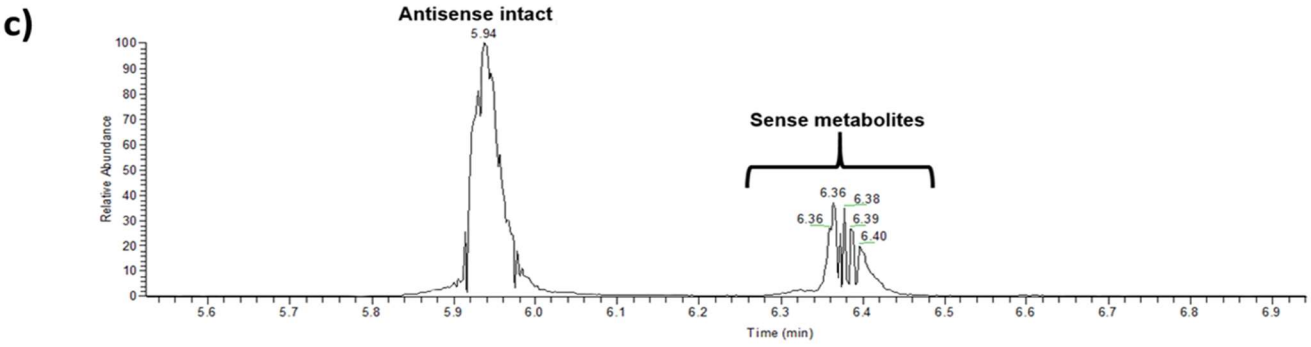
Supplemental Information

Introducing an *In Vitro* Liver Stability Assay Capable of Predicting the *In Vivo* Pharmacodynamic Efficacy of siRNAs for IVIVC

Babak Basiri, Fang Xie, Bin Wu, Sara C. Humphreys, Julie M. Lade, Mai B. Thayer, Pam Yamaguchi, Monica Florio, and Brooke M. Rock


a)
Antisense
Sense
RNase cocktail
Plasma
Relative Abundance
Time (min)


b)
Snake venom phosphodiesterase
MS signal intensity
AS_Intact
2.4 E7
AS_3′n-1
5.5 E5
AS_3′n-3
5.5 E5
AS_3′n-4
1.0 E6
AS_3′n-5
8.5 E5
Time (min)
In vivo
MS signal intensity
1.3 E7
1.5 E7
3.5 E5
6.4 E5
1.8 E6


c)
Antisense intact
5.94
Sense metabolites
6.36
6.38
6.36
6.39
6.40
Relative Abundance
Time (min)

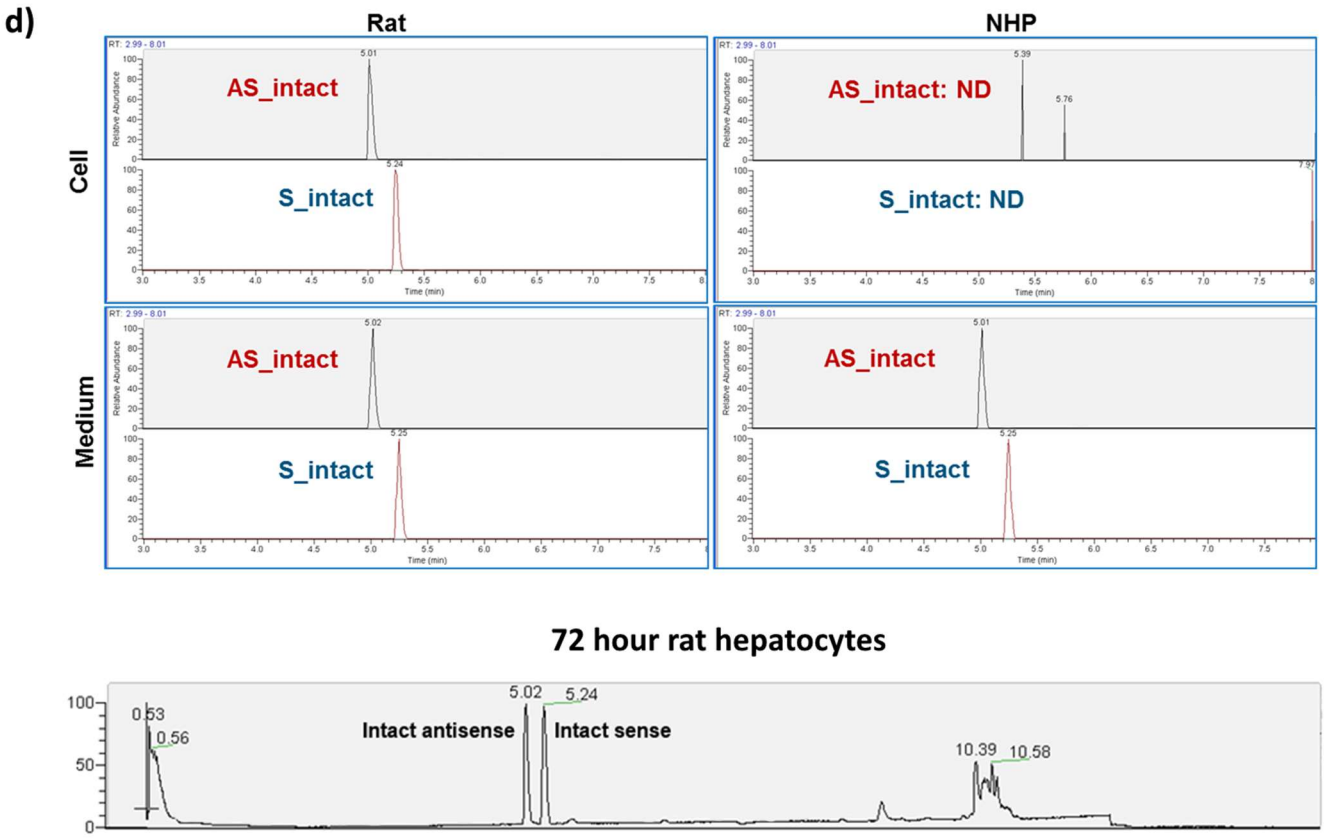


Figure S1: Several biological matrices were evaluated in search of an *in vitro* system that can recapitulate *in vivo* siRNA biotransformation.

a) No siRNA metabolism was observed after 72 hours of incubation in either plasma or RNase A/T1 cocktail. Sense and antisense strands remained intact and no metabolite peaks were observed.

b) Snake venom phosphodiesterase I successfully catalyzed the cleavage of the antisense strand. Nevertheless, it generated all different shortmers at an approximately equal rate, whereas some shortmers are produced at much higher rates than the others *in vivo*. For example, it can be observed that the *in vivo* production of 3'n-1 metabolite of the antisense strand is much higher, while *in vivo* generation of 3'n-3 is much lower than what happens in the presence of phosphodiesterase. Meanwhile, the sense strand was not metabolized in this system.

c) In contrast to snake venom phosphodiesterase, rat tritosome and human lysosome only metabolized the sense strand and the antisense strand remained intact.

d) While siRNA uptake was successfully observed in rat hepatocytes, no uptake happened for cynomolgus monkey hepatocytes despite siRNA presence in the medium. Moreover, as shown in the bottom panel, even in rat hepatocytes no metabolism of either strand was observed.

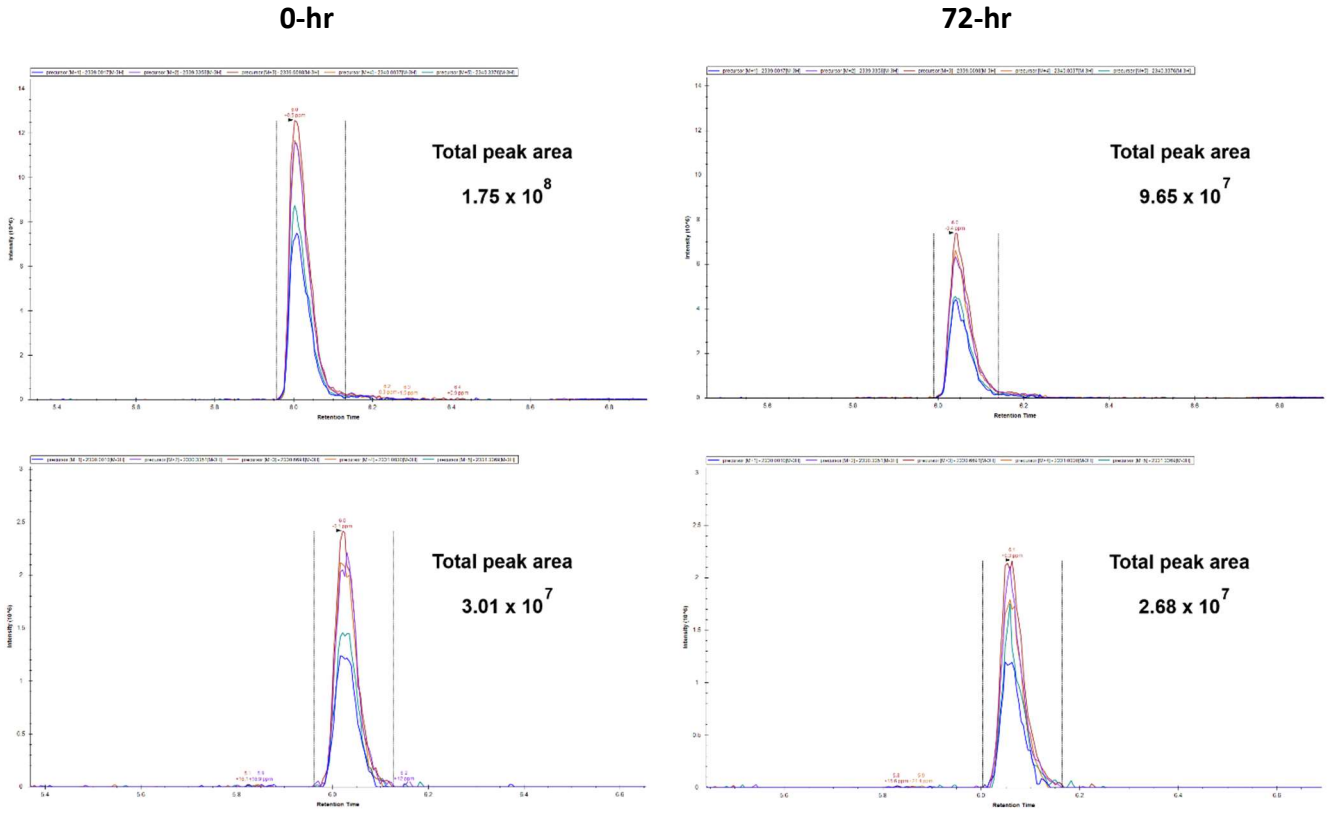


Figure S2: An example of siRNA stability determination using LC-MS results.

The panels on top show the extracted ion chromatograms (5 top isotopic peaks) for the antisense strand of a test siRNA and the bottom panels are extracted ion chromatograms for the internal standard (IS). It can be seen that while the IS response remains largely the same for 0-hr and 72-hr samples, the MS response for the antisense strand of the test siRNA decreases in 72-hr samples. This indicates that a portion of siRNA has been degraded in 72-hr samples. In this particular example, siRNA stability was calculated to be 61.97%.