

## LEGENDS FOR SUPPLEMENTAL FIGURES

**Figure 1. RNA binding competition assay.** UV-crosslink assays were performed in the presence of 500ng of recombinant GST-Apobec-1 protein, incubated with constant amount of radiolabeled RNAs (3' UTR A, B, or C) and **A.** with increasing molar excess of the corresponding cold competitor unlabeled RNAs (3'-UTR A, B, or C), and **B.** with increasing molar excess of an unlabeled non-AU-rich RNA.

**Figure 2. Murine Cyp7a1 3'UTR destabilizes mRNA expression but Apobec-1 does not directly restore mRNA stability in HepG2 cells.** **A. Murine Cyp7a1 3' UTR reduces steady-state abundance of a reporter construct.** Wild-type HepG2 cells were transiently transfected with plasmids expressing either the coding region of GFP alone or GFP fused to murine Cyp7a1 full length 3'-UTR. GFP mRNA was analyzed by quantitative PCR and normalized to the neomycin-resistance cassette (G418) mRNA. The expression of GFP mRNA in the absence of the 3'-UTR is 2.5 fold higher than GFP containing the 3'-UTR. Bar graphs represent the mean  $\pm$  SE from 3 independent assays. \*\* indicates  $p < 0.01$ . **B. Murine Cyp7a1 3' UTR reduces mRNA stability of a reporter construct in Tet-Off HepG2 cells.** HepG2 Tet-off cells were transfected with a plasmid expressing  $\beta$ -globin or  $\beta$ -globin fused with the full length murine Cyp7a1 3'-UTR. After 48 hr of transfection, Doxycycline (100 ng/ml, Sigma) was added to the medium. RNA was extracted at indicated times following the addition of Doxycycline.  $\beta$ -globin mRNA levels were determined by quantitative RT-PCR. The  $t_{1/2}$  of  $\beta$ -globin mRNA is 21 hrs versus 4.6h for  $\beta$ -globin mRNA fused to Cyp7a1-3'UTR. Data represent the mean  $\pm$  SE from 6-8 independent experiments. **C. Apobec-1 transfection fails to rescue mRNA stability phenotype in Tet-Off HepG2 cells.** HepG2 Tet-off cells were co-transfected with a plasmid expressing  $\beta$ -globin mRNA fused to full length murine Cyp7a1 3'UTR and either plasmid expressing N-terminal FLAG-tagged murine Apobec-1 or the empty vector pCMV2B. Two days after transfection, Doxycycline (100 ng/ml) was added to the medium and RNA was extracted at indicated times.  $\beta$ -globin mRNA levels were determined by quantitative PCR and normalized to the neomycin-resistance cassette (G418) mRNA. The data are the mean  $\pm$  SE from 6 assays per time point. Apobec-1 protein expression was confirmed by Western blot using rabbit anti-FLAG antibody (insert).

## Supplemental Methods

*Tet-off Plasmid construction and RNA stabilization experiments* - cDNA from C57BL/6 mouse liver was used to amplify the full length Cyp7a1 3'UTR by forward primer (GGTGGTGGTGGATCCGGTTGGAAGAAGAGGACACTGGATGATGTAACG) and reverse primer (GGTGGTGGTAGATCTTCCTGTCTATGTCACTACCCTGCC). The PCR product was digested with BamHI and BglII and cloned into the BglII site downstream from the  $\beta$ -globin coding region in the tet-off plasmid pTet-7B (49). To determine the half-life of  $\beta$ -globin mRNA with or without Cyp7a1 3'UTR, the reporter plasmid pTet7B, with or without the Cyp7a1 3'-UTR was transfected along with pcDNA (used as a transfection control) into Clontech tet-off HepG2 cells (Clontech, Mountain View, CA) using Fugene 6 transfection reagent (Roche, Indianapolis, IN). The cells were grown in serum-containing media for 48 hours. The media was changed, and two hours later 100 ng/ml of doxycycline (Dox) containing media was added. RNA was harvested at 0, 1, 4 and 8 hours after Dox by using TRIzol<sup>®</sup> reagent. cDNA was prepared, and amount of  $\beta$ -globin mRNA measured by quantitative PCR (forward primer TCACCTGGACAACCTCAAAGG, reverse primer CAGGATCCACGTGCAGCTT), normalized to the level of neomycin resistance gene transcript (forward primer GCGCCCGTTCTTT, reverse primer GCCTCGTCCTGCAG). Apobec-1 gain of function experiments were performed by co-transfecting HepG2 Tet-off cells with pTet-7B-Cyp7a1-3'UTR with either pCMV2B-Apobec-1 or pCMV2B for 48 hr. Fresh medium was added for two hours. Doxycycline (100 ng/ml)-containing medium was added and RNA was extracted at 0, 4, 8 and 24 hr using TRIzol as described above and  $\beta$ -globin mRNA was evaluated by quantitative PCR (49).

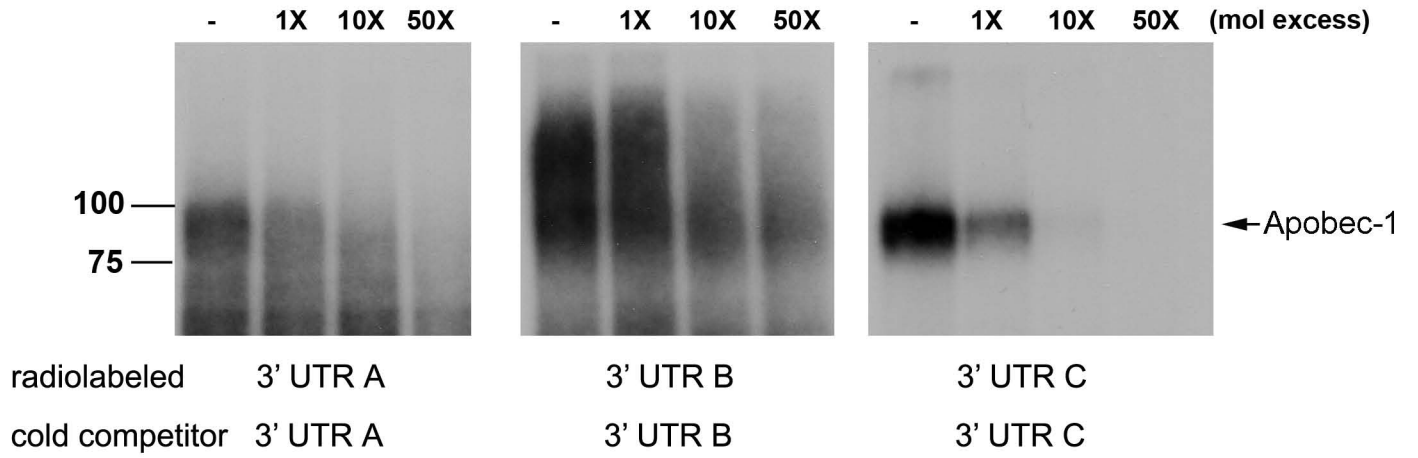
Supplemental Table1. oligodeoxyribonucleotide primer sequences for real-time quantitative PCR

Gene	Forward primer	Reverse primer
<i>Abca1</i>	5'-AGGGTTTCTTTGCTCAGATTGTC -3'	5'-TGCCAAAGGGTGGCACA -3'
<i>Abcg5</i>	5'-TTGCGATACACAGCGATGCT-3'	5'-TGACTGCCTCTACCT TCTTGTGT -3'
<i>Abcg8</i>	5'-CCGTCGTGATTTCCAATGA-3'	5'-GGCTTCCGACCCATGAATG-3'
<i>ApoAIV</i>	5'-CCCGGGCTGAGGTCACCT-3'	5'-GCATTGTTGCTTAGCTGGGTAA-3'
<i>ApoB</i>	5'-TGAATGCACGGGCAATGA-3'	5'-GGCATTACTTGTTCCATGGTTCT-3'
<i>ApoC2</i>	5'-CCCCCTGTGGCTTTGTTG-3'	5'-GCCCCCTCCTCCATATCAG-3'
<i>ApoC3</i>	5'-TGAAAGGCTACTGGAGCAAGTTT-3'	5'-TGGTTGGTCCTCAGGGTTAGA-3'
<i>Asbt</i>	5'-TGTCTGTCCCCCAAATGCA-3	5'-TGCATTGAAGTTGCTCTCAGGTA-3'
<i>Bsep/Abcb11</i>	5'-GTTCTCCGTTCAAACATTGG-3'	5'-TCCCCATACTTGATGTTGTCCAT-3'
<i>Cav1</i>	5'-CCGTGCATCAAGAGCTTCCT-3'	5'-TCGCAGAAGGTATGGACGTAGA-3'
<i>Cyp2b10</i>	5'-GGGAAAGCGCATTGTCTTG-3'	5'-ATGGACGTGAAGAAAAGGAACAAT-3'
<i>Cyp2c38</i>	5'-CCTTATCAGGGATAAGGCATCTTC-3'	5'-AACCGCATGTGTTTGCTAGGT-3'
<i>Cyp3a11</i>	5'-ACT CAC CCT GAT ATC CAG AAA AAA T-3'	5'-CGT GGG AGG TGC CTT GTT-3'
<i>Cyp4a14</i>	5'-ATAGCCATGCTTATCTGCCATTC-3'	5'-TCAGCTCGTTCATAGCAAACCTGTT-3'
<i>Cyp7a1</i>	5'-CCATGATGCAAAACCTCCAAT-3'	5'-ACCCAGACAGCGCTCTTTGA-3'
<i>Cyp8b1</i>	5'-ATCCATGCAGGACAAATTTAACTT T-3'	5'-ACAGGTGGGTCCCGTGTTTC-3'
<i>Cyp27a1</i>	5'-GGATTGGCTGCCTGAAACC-3'	5'-GGAACATGATTGCAACAGATCTG-3'
<i>Fas</i>	5'-GGCATCATTGGGCACTCCTT-3'	5'-GCTGCAAGCACAGCCTCTCT-3'
<i>Fxr</i>	5'-CACGAAGATCAGATTGCTTTGC-3'	5'-CCGCCGAACGAAGAAACA-3'
<i>Hmgcr</i>	5'-ATTCTGGCAGTCAGTGGGAACT-3'	5'-CCTCGTCCTTCGATCCAATTTA-3'
<i>Ldlr</i>	5'-GCTCCATAGGCTATCTGCTCTTCA-3'	5'-CTGCGGTCCAGGGTCATC-3'
<i>Lfabp</i>	5'CCAGGAGAACTTTGAGCCATTC-3'	5'-TGTCCTTCCCTTTCTGGATGA-3'
<i>Lxra</i>	5'-GCTCTGCTCATTGCCATCAG-3'	5'-TGTTGCAGCCTCTCTACTTGA-3'
<i>Mdr2/Abcb4</i>	5'-CTTGAGGCAGCGAGAAACG-3'	5'-GGTTGCTGATGCTGCCTAGTT-3'
<i>Ntcp</i>	5'-TCTGGTAGTTATGTTGCTGCTCATC-3'	5'-AGTGAGCCTTGATCTTGCTGAAC-3'
<i>Oatp2</i>	5'-CTAAAGGAAGGATTACAGGATAATGGA-3'	5'-CCTCGGTTTTCTCCTTGATT-3'
<i>Oatp4</i>	5'-TTTATAGAGCAGCAGTTTGGTCAGA-3'	5'-CTTGCCATAGTGGGTATGGTTATAAC-3'
<i>Ppara</i>	5'-TATTCGGCTGAAGCTGGTGTAC-3'	5'-CTGGCATTGTCCGGTTCT-3'
<i>PXR</i>	5'-TCCAGCGCAGCGTGGTA-3'	5'-GATATGGCCGACTACACTCAATGT-3'

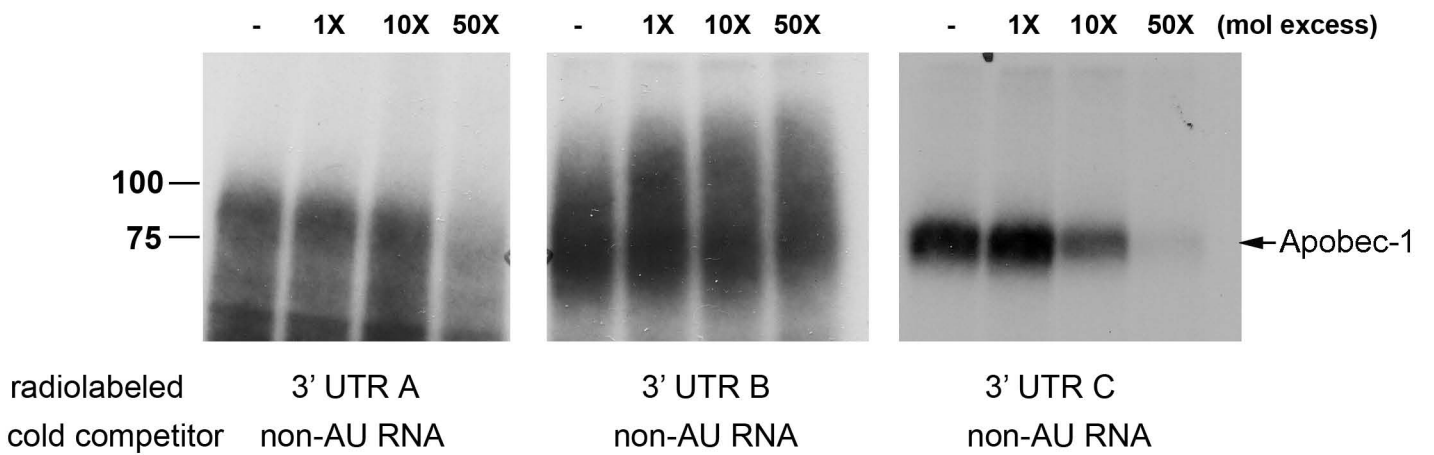
<i>Scd1</i>	5'-TCGAAGGACCCGAGGTGTT-3'	5'-CACCTCTTAGCAGCTACTTACAGACACT-3'
<i>Scd2</i>	5'-GTACCGCTGGCACATCAACTT-3'	5'-ACACTCTCT TCCGGTCGTAAG C-3'
<i>Scp2</i>	5'-GGCTTTGATGACTGGAAA	5'- CCCATGTTACCAGCAATCTTCA-3'
<i>Shp</i>	5'-CAGGCACCCTTCTGGTAGATCT-3'	5'-GTCTTCAAGGAGTTCAGTGATGTCA-'
<i>Srb1</i>	5'-TCAGAAGCTGTTCTTGGTCTG AAC-3'	5'-GTTTCATGGGGATCCCAGAGA-3'
<i>Srebp1c</i>	5'-GGAGCCATGGATTGCACATT-3'	5'-CCTGTCTCACCCCCAGCATA-3'
<i>Srebp2</i>	5'-ACAGCCGCCCTTCAAGTG-3'	5'-TCACAGGCATTGTGGTCAGAA-3'
<i>Ucp2</i>	5'-TCACTGTGCCCTTACCATGCT-3'	5'-AGGCAT GAACCCCTTGTAGAAG-3'
<i>18S</i>	5'-CGGCTACCACATCCAAGGAA-3'	5'-GCTGGAATTACCGCGGCT-3'
* <i>Cyp7a1</i>	5'-TCTGTCATGAGACCTCCGGG-3'	5'-CCCTCATGTACAGGTTCTTGTGC-3'
* <i>Apobec-1</i>	5'-ATTAGCAGCGGTGTGACTATCC-3'	5'-GCCCAACTCCCAGAAGTCATT-3'
* <i>β-gal</i>	5'- GTCGAAAACCCGAAACTGTGG -3'	5'- GGTTCCGATAATGCGAACAGC-3'

\* Primers for RT-PCR.

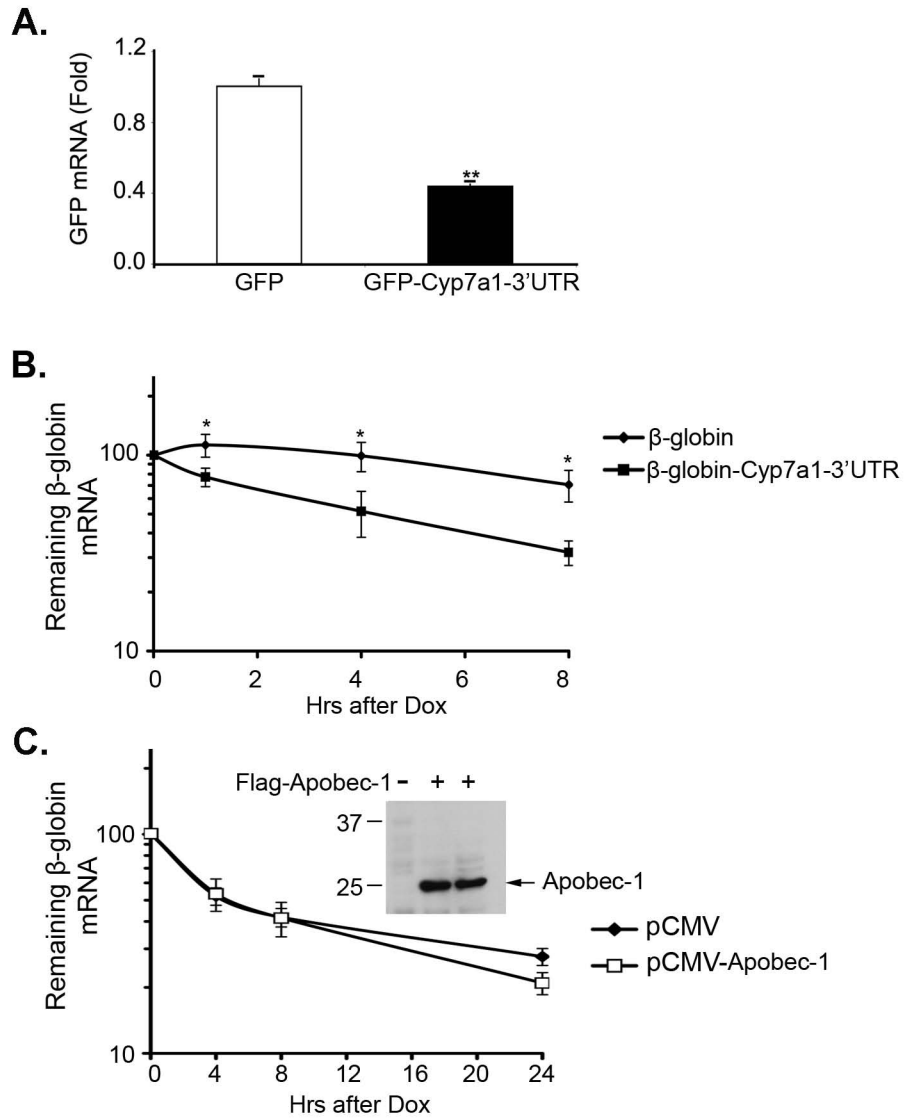
**A.**



**B.**



**Supplemental figure 1**



**Supplemental figure 2**