Supporting Information

Ward et al. 10.1073/pnas.1412608111

SI Materials and Methods

Patient Parameters. A total of 49 patients, including 7 with ischemic hepatitis (Dataset S1, Tab S1), were evaluated using serum or plasma samples obtained from Banner Good Samaritan Medical Center, Phoenix [provided by S.C.C.; institutional review board (IRB) no. 0004299], University of Kansas Medical Center, Kansas City (provided by H.J. and M.R.M.; IRB no. 11962), and University of Massachusetts Medical School (provided by J.W.; IRB no. 13198) according to IRB approval from each institution. The etiology of ischemic hepatitis for these patients was primarily due to drug overdose by narcotics and sedative hypnotics and was not due to underlying cardiac illness. Patients represented a range of age, sex, acetaminophen (APAP) ingestion, and alanine aminotransferase (ALT) elevations. Blood draws available during hospitalization and treatment with *N*-acetyl cysteine (NAC) were assayed for changes in the microRNA (miRNA) profile. Protein adduct levels were used to help identify APAP overdose. Multiple time points were available for most of the patients, resulting in 156 total samples assayed. The control group included 12 healthy individuals consisting of 50% females, aged 20–60 y who had not taken any APAP in the prior week. Healthy liver tissue was obtained from the University of Massachusetts Medical School Tissue and Tumor Bank.

Sample Preparation and Quantitative Real-Time PCR. Blood plasma was isolated by spinning EDTA-treated blood at 1,500 × *g* for 20 min at 4 °C and was stored at −80 °C. After thawing, the plasma was spun in at 16,000 × *g* for 10 min to remove debris and deplete platelets. Then, 200 μL of plasma was mixed with 100 μL of lysis buffer [6.4 M guanidine HCl, 5% (vol/vol) Triton, 5% (vol/vol) Tween, 120 mM Na_2EDTA, 120 mM Tris (pH 8.0)] and digested with ∼6 mAu Proteinase K at 65 °C with shaking for 15 min. After cooling to room temperature, 250 μL of phenol: chloroform 5:1 (pH 8.0) and 250 μL of nuclease free water were added. The mixture was shaken for 5 min at room temperature and spun at 16,000 × *g* for 5 min at room temperature. The aqueous layer (∼450–500 μL) was removed to a clean 2-mL tube. Three volumes of cold 100% ethanol were added and loaded onto an Enzymax Tini spin column. The column was washed twice with 400 μL of cold buffer [80% (vol/vol) ethanol, 2 mM Tris (pH 7.5), 20 mM NaCl] and eluted twice with 6 μL of heated nuclease free water. QIAGEN reagents were used in the reverse transcription of RNA, preamplification, and qRT-PCR, according to the manufacturer's instructions. Preamplified cDNA prepared from the RNA equivalent of 7.5 μL of plasma was assayed using the QIAGEN miScript miRNA PCR Array for Human Serum and Plasma. The 372 miRNA assays were chosen by QIAGEN after detection in plasma from healthy and lung, breast, and ovarian cancer patients. Reverse transcription control (miRTC) primer assay and positive PCR control (PPC) were used to evaluate the reverse transcription efficiency (as a measure of RNA purity) and the performance of the quantitative real-time PCR (qRT-PCR), respectively. qRT-PCR reactions were run on the Viia 7 (Life Technologies).

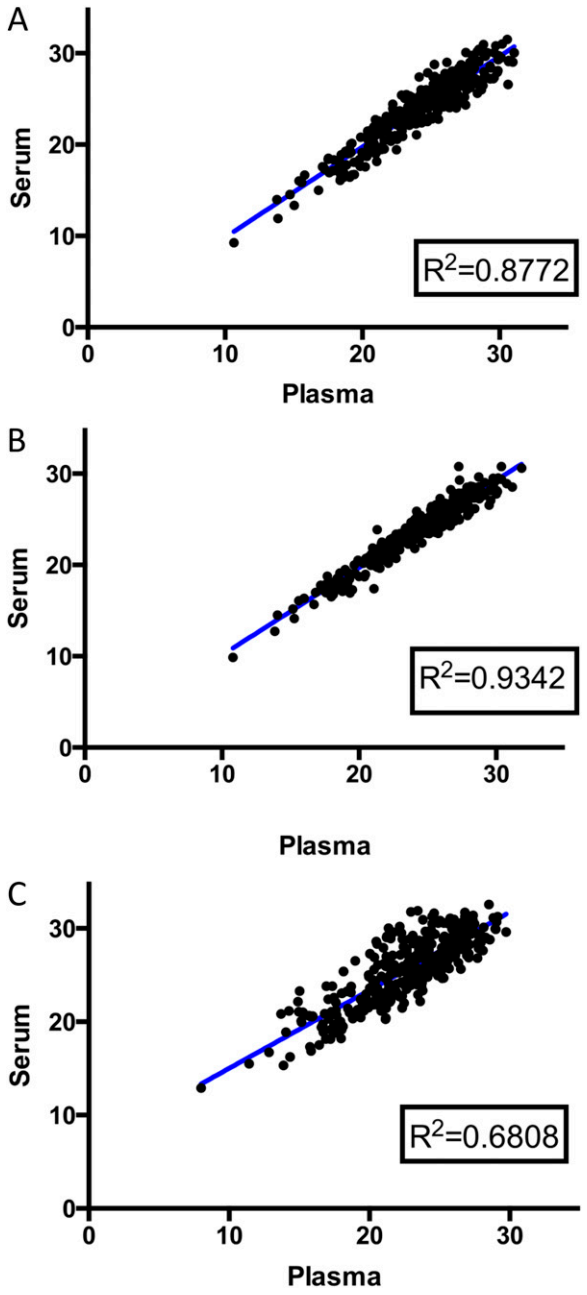

Fig. S1. Comparison of serum and plasma miRNA profiles. Scatter plots comparing CT values of 347 miRNAs (with a CT $\leq$ 32) profiled from serum and plasma for three different non–APAP-exposed healthy controls (*A*–*C*).

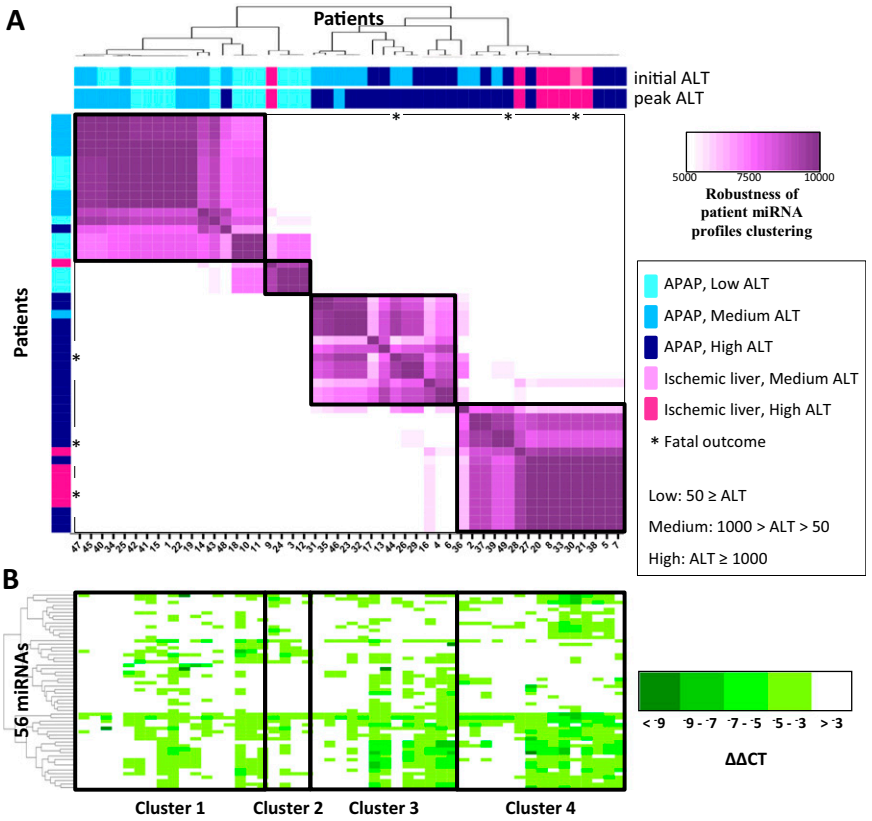

Fig. S2. Heat map of miRNAs that are decreased in the earliest miRNA profile. (*A*) The clusters as shown in Fig. 1, reproduced here for reference. (*B*) A heat map of 56 miRNAs that were decreased by $\Delta\Delta CT \geq 3$ in the earliest available sample (within 2 d of hospital admittance) in at least five patients.

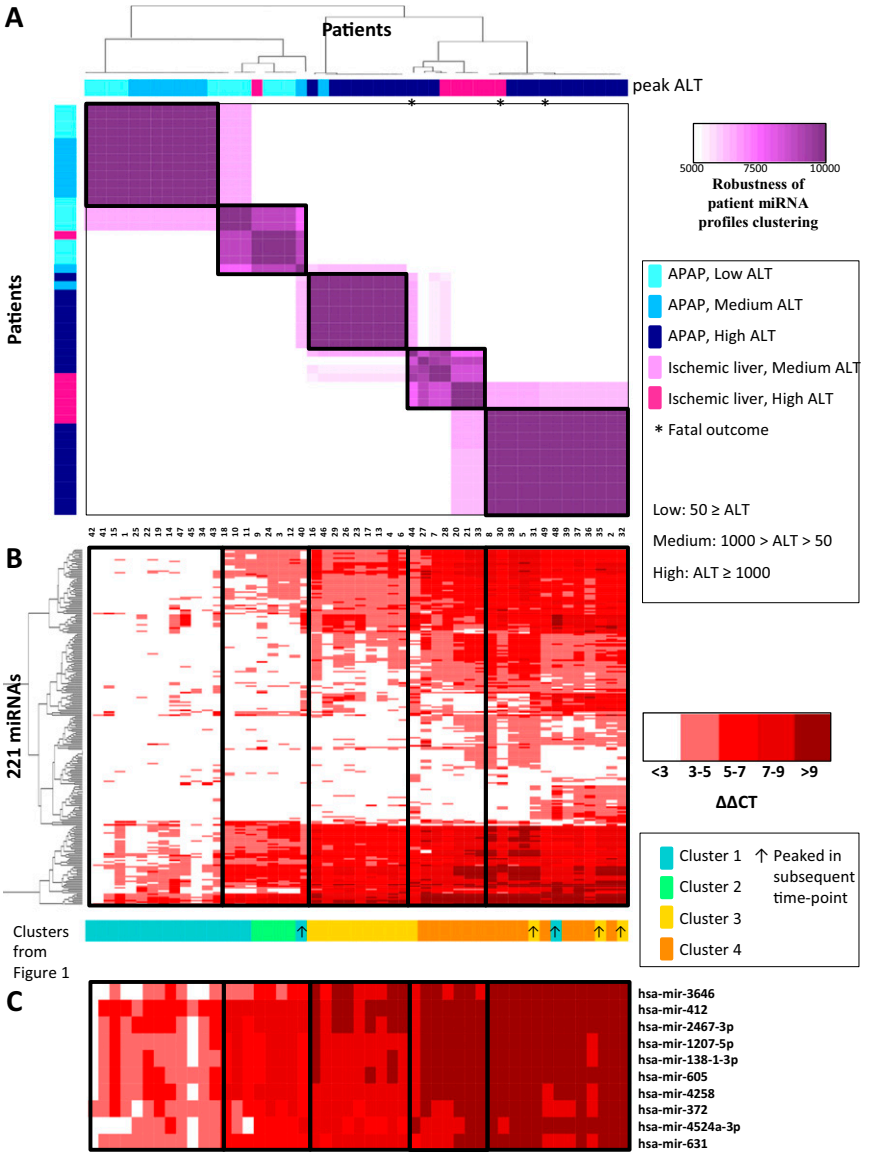

Fig. S3. Patient clustering based on peak miRNA profiles. A clustering of 49 patients based on peak miRNA profiles. For 44 patients, the earliest available sample was the peak as in Fig. 1; miRNA profiles peaked for patients no. 40, no. 31, no. 48, no. 35, and no. 32 at a subsequent time point as indicated in Fig. 3. Each patient is designated according to APAP overdose or ischemic hepatitis, initial ALT, peak ALT during hospital stay, and outcome. (*A*) Each heat map cell corresponds to the number of times that the patient's miRNA profiles were coclustered out of 10,000 runs of the random *k*-means algorithm (see details in *Materials and Methods*). (*B*) The heat map of 221 miRNAs displaying the $\Delta\Delta CT = CT_{Sample} - \text{Mean } CT_{Healthy}$ in the peak miRNA profile. Columns correspond to patients, and rows correspond to unique miRNAs. The patients are also color-coded with their initial classification to clusters in Fig. 1. (*C*) A magnification of the heat map in *B*, showing the top 10 elevated miRNAs (Dataset S1, Tab S7).

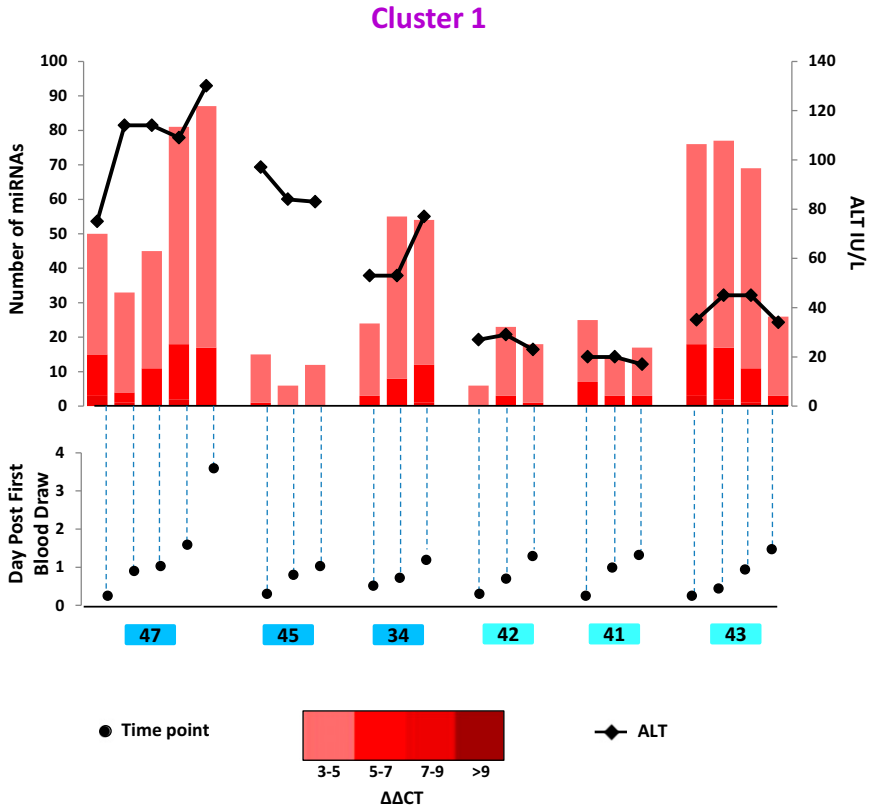

Fig. S4. Dynamics of miRNA profiles during NAC treatment. Profiles of ALT (black line), miRNA changes (red bars), and sample time point (triangle or oval) are shown for patients with at least two available samples within 4 d of hospital admittance, that displayed less than 100 miRNAs elevated ($\Delta\Delta CT \geq 3$) during this time window. Patients with a higher number of elevated miRNAs are shown in Fig. 3. Patients are labeled with patient ID and color-coded according to their peak ALT as in Fig. 1.

Dataset S1.

Dataset S1

Tab S1, patients' clinical data; tab S2, raw CT values from qRT-PCR (49 patients, 146 samples, and 12 healthy controls); tab S3, miRNAs in the normalization set; tab S4, normalized CT values of tab S2; tab S5, ΔΔCTs for individual miRNAs in each of the four clusters (all patients); tab S6, average ΔΔCT for individual miRNAs in each of the four clusters; tab S7, partial list of miRNAs that show the highest elevation in high ALT patients (clusters 3 and 4); tab S8, partial list of miRNAs that are elevated only in high ALT patients in clusters 3 and 4; and tab S9, raw CT values from qRT-PCR (healthy liver tissue, three samples).