Supporting Information

Extending the Scope of ^{1}H NMR Spectroscopy for the Analysis of Cellular Coenzyme A and Acetyl Coenzyme A

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This file contains 15 pages, S1 to S15. The first page is the Title page; page S2 contains Table S1 and Table S2; Pages S3 and S4 contain Table S3 and Table S4, respectively; pages S5 to S15 contain Figure S1 to Figure S11, respectively.

Table S1: Metabolites used for the development of coenzyme A and acetyl coenzyme A analysis method in tissue using ^{1}H NMR.

	Coenzyme/metabolite
1	Coenzyme A (CoA)
2	Acetyl coenzyme A (Acetyl-CoA)
3	Oxidized coenzyme A (CoA-S-S-CoA)
4	Succinyl coenzyme A (Succinyl-CoA)
5	Malonyl coenzyme A (Malonyl –CoA)
6	Glutathione, Reduced (GSH)
7	Glutathione, Oxidized (GSSG)

Table S2: Mice used for developing coenzymes analysis method using ^{1}H NMR

Genotype	**Number**	**Gender**	**Age (months)**
Wild type (WT)	24	Male	5-6 (n=12) 3.5-5.5 (n=12)

Table S3: ^{1}H NMR chemical shifts (in ppm) and *J* couplings (in Hz) for standard coenzyme A (CoA) and acetyl coenzyme A (acetyl-CoA) obtained using a Bruker 800 MHz NMR spectrometer. Characteristic peaks that are isolated in tissue NMR spectra are shown in bold.

Coenzyme A (CoA)			**Acetyl Coenzyme A (Acetyl-CoA)**		
Number*	**Group**	**^{1}H Chemical Shift and *J* couplings**	**Number***	**Group**	**^{1}H Chemical Shift and *J* couplings**
H68	**-CH**	**8.5641**	**H74**	**-CH**	**8.5626**
H67	-CH	8.2796	H73	-CH	8.2729
H73	-CH	6.1795; $^{3}J_{H,H}$ =7.17	H79	-CH	6.1755, $^{3}J_{H,H}$ = 7.16
H71	-CH	4.8517	H77	-CH	4.8519
H70	-CH	4.7648	H76	-CH	4.7632
H69	-CH	4.5771	H75	-CH	4.5758
H63-64	-CH_2	4.2331	H69-70	-CH_2	4.2328
H72	-CH	4.0036	H78	-CH	4.0107
H65-66	-CH_2	3.5352, $^{2}J_{H,H}$=9.62, $^{3}J_{H,31P}$=4.82; 3.8055, $^{3}J_{H,31P}$=4.69	H71-72	-CH_2	3.5408,$^{2}J_{H,H}$=9.77, $^{3}J_{H,31P}$=4.82; 3.8173,$^{3}J_{H,31P}$=4.78
H57-58	-CH_2	3.4658, $^{3}J_{H,H}$ =6.58	H63-64	-CH2$^{\$}$	3.4413,$^{2}J_{H,H}$ =13.42, $^{3}J_{H,H}$ =6.59 3.4589, $^{3}J_{H,H}$ =6.59
H59-60	-CH_2	3.3161,$^{3}J_{H,H}$ =6.65	H65-66	-CH_2	3.3190,$^{3}J_{H,H}$ =6.40
H61-62	**-CH_2**	**2.6089, $^{3}J_{H,H}$ =6.65**	H67-68	-CH_2	2.9642, $^{3}J_{H,H}$ =6.40
H55-56	-CH_2	2.4653, $^{3}J_{H,H}$ =6.58	H61-62	-CH_2	2.4273, $^{3}J_{H,H}$ =6.59
			H52-54	-CH_3	2.3495
H52-54	**-CH_3**	**0.8594**	**H58-60**	**-CH_3**	**0.8674$^{\&}$**
H49-51	**-CH_3**	**0.7315$^{\&}$**	**H55-57**	**-CH_3**	**0.7340**

* Numbers correspond to the hydrogen atom labeling from the recently developed ALATIS, which creates a unique and atom-specific InChI labels (see Figure S3).
$^{\&}$Peaks that are suitable for the determination of the coenzyme concentrations in all tissue types investigated in this study.
$^{\$}$These CH_2 protons are non-equivalent, chemical shifts for these protons were calculated based on the frequencies from the AB part of the ABX_2 multiplet pattern (see Figure S3).

Table S4: Results of recovery experiments performed using mouse tissue extract (n=4; age 5-6 months).		
Coenzyme	Added (μg) to 95 mg tissue extract	Recovered (μg)
Coenzyme-A	6.60	6.36 ± 0.51
Acetyl Coenzyme A	10.68	10.11 ± 1.37
Error is expressed as standard deviation. Endogenous CoA and Acetyl-CoA levels were subtracted to determine the recovered amounts.		

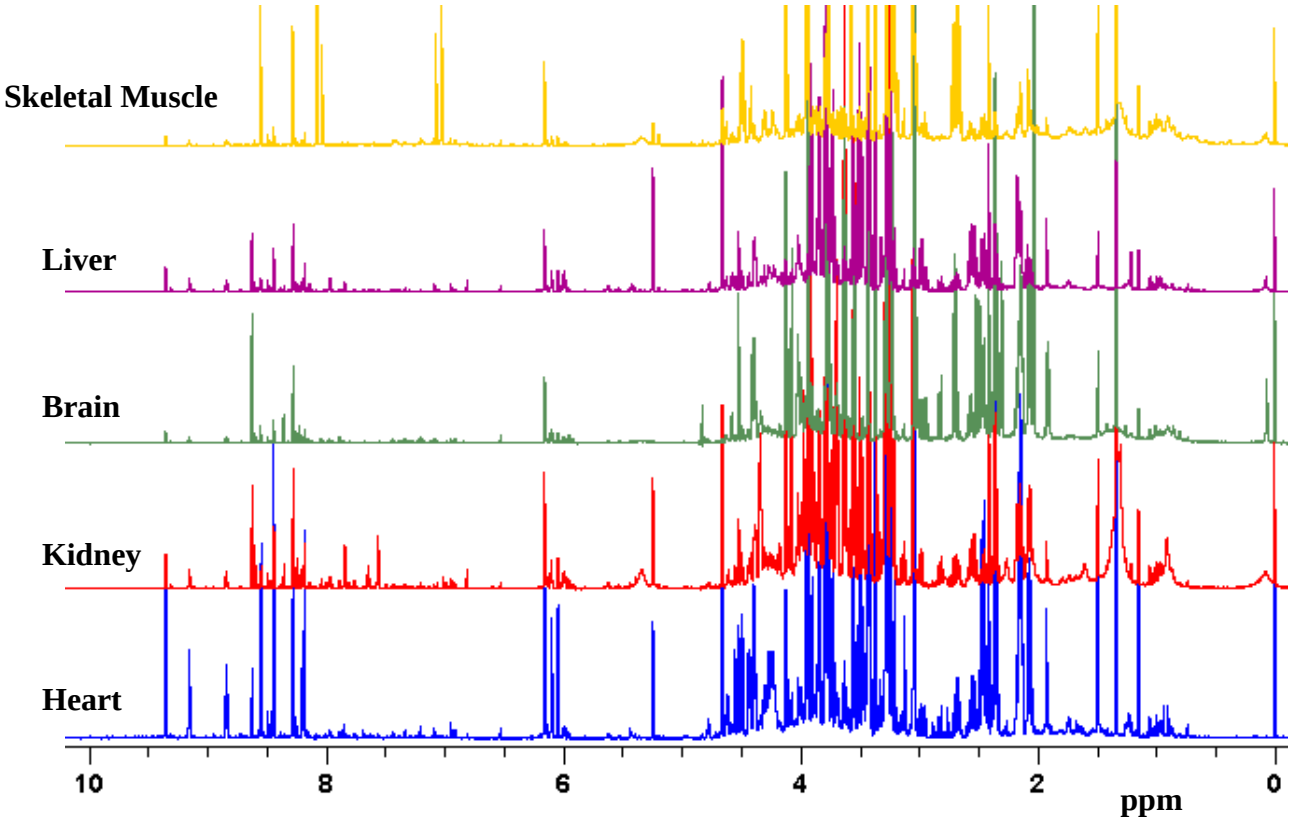

Figure S1. Typical 800 MHz ^{1}H NMR spectra of a mouse heart, kidney, brain, liver and skeletal muscle tissue extracts.

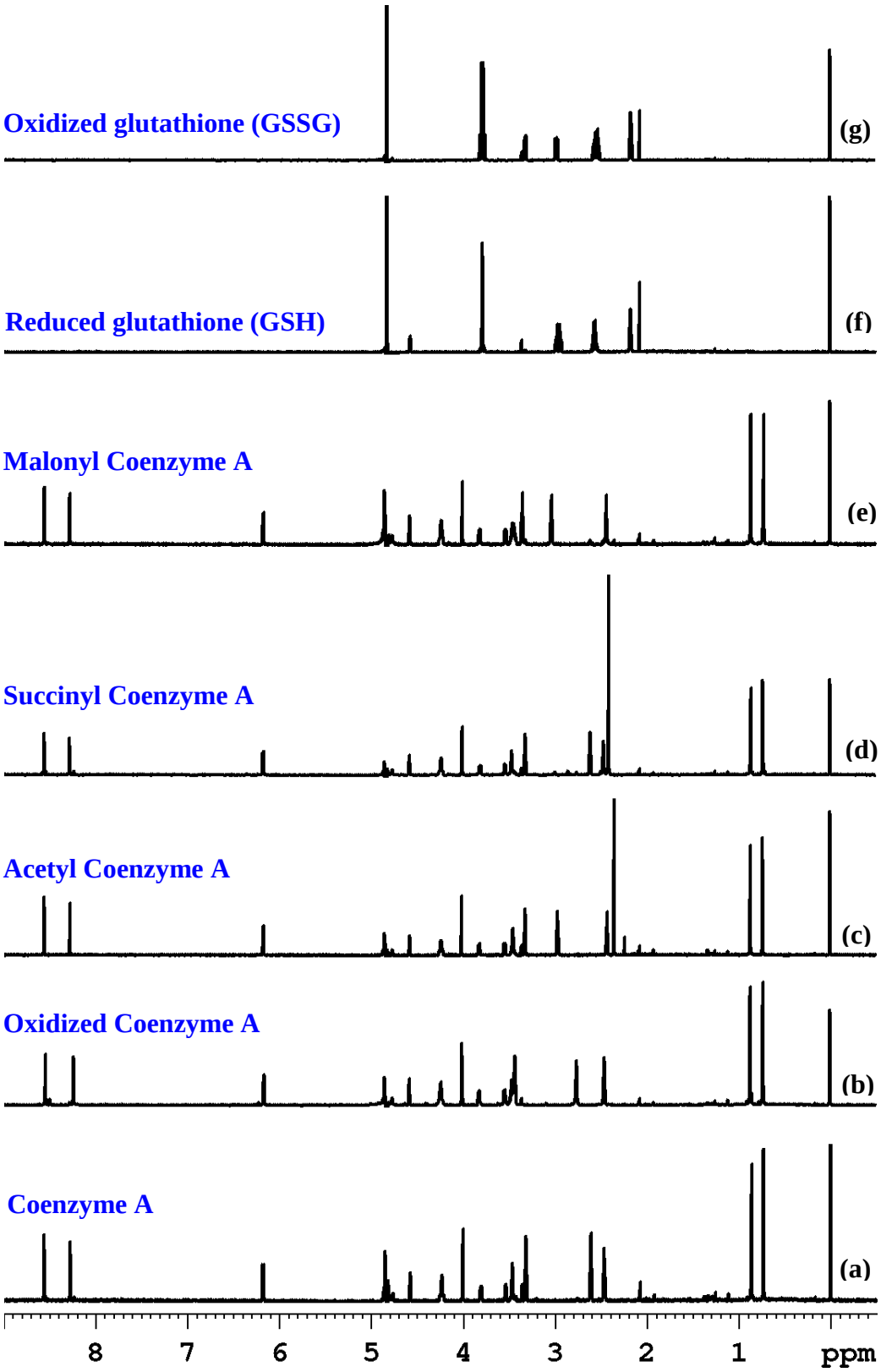

Figure S2. Typical 800 MHz ^{1}H NMR spectra of standard solutions (~100 μM) of (a) coenzyme A (CoA); (b) oxidized coenzyme A (CoA-S-S-CoA); (c) acetyl coenzyme A (acetyl-CoA); (d) succinyl coenzyme A; (e) malonyl coenzyme A; (f) reduced glutathione (GSH); and (g) oxidized glutathione (GSSG). The spectra were obtained using similar conditions as used for tissue extracts to aid CoA and acetyl-CoA identification in tissue NMR spectra.

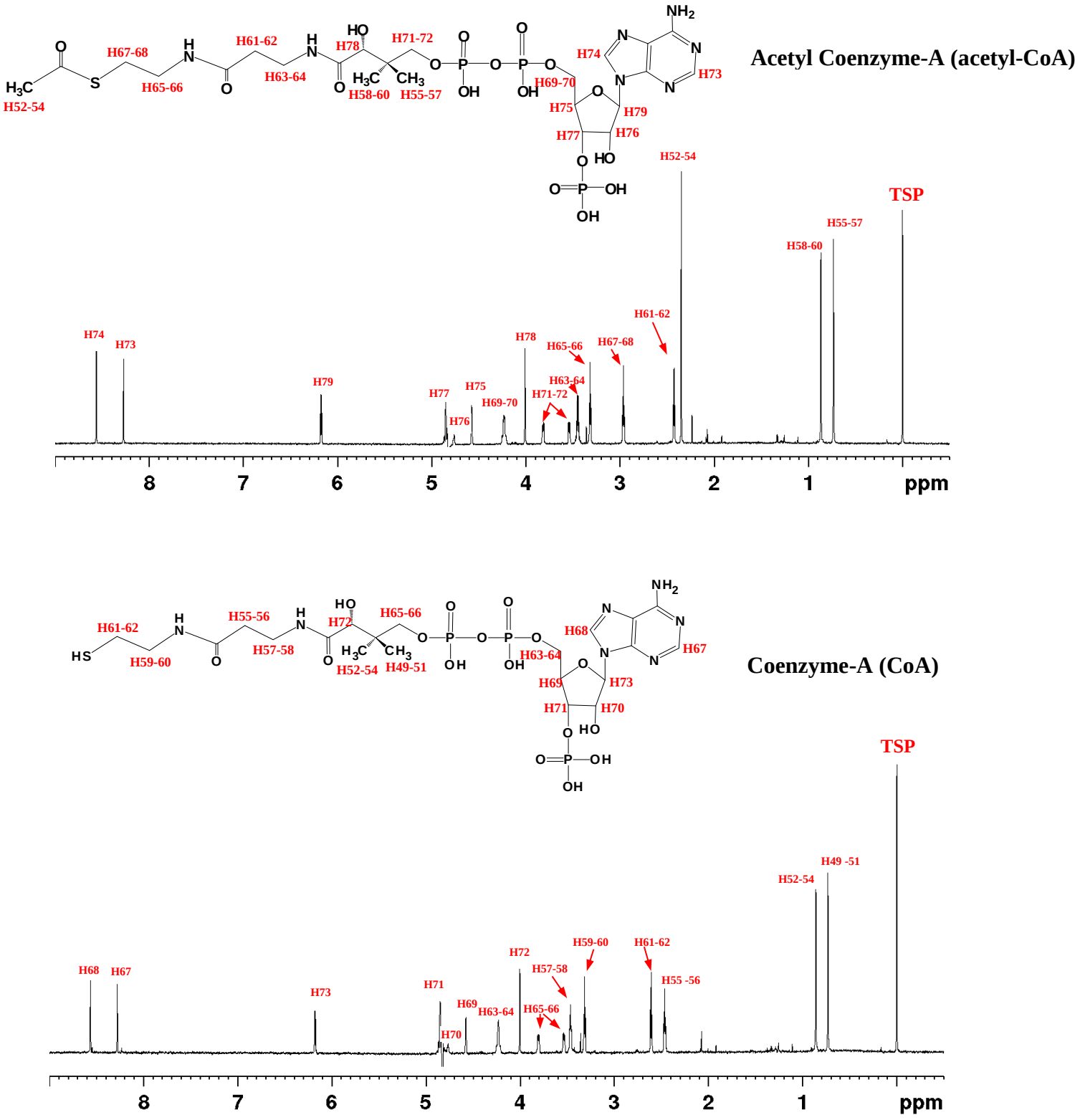

Figure S3: 800 MHz ^{1}H NMR spectra of coenzyme A (CoA) and acetyl coenzyme A (acetyl-CoA) along with their molecular structures. The spectra were obtained using similar conditions as used for tissue extracts to aid CoA and acetyl-CoA identification in tissue NMR spectra. Peak labels correspond to the number for proton(s) as shown in their molecular structure. Note, peaks for protons from NH, NH_2, OH and SH groups are not detected as they are labile and exchange with D_2O. Peak labels correspond to the hydrogen atom labeling based on the recently developed ALATIS, which creates a unique and atom-specific InChI labels.[38]

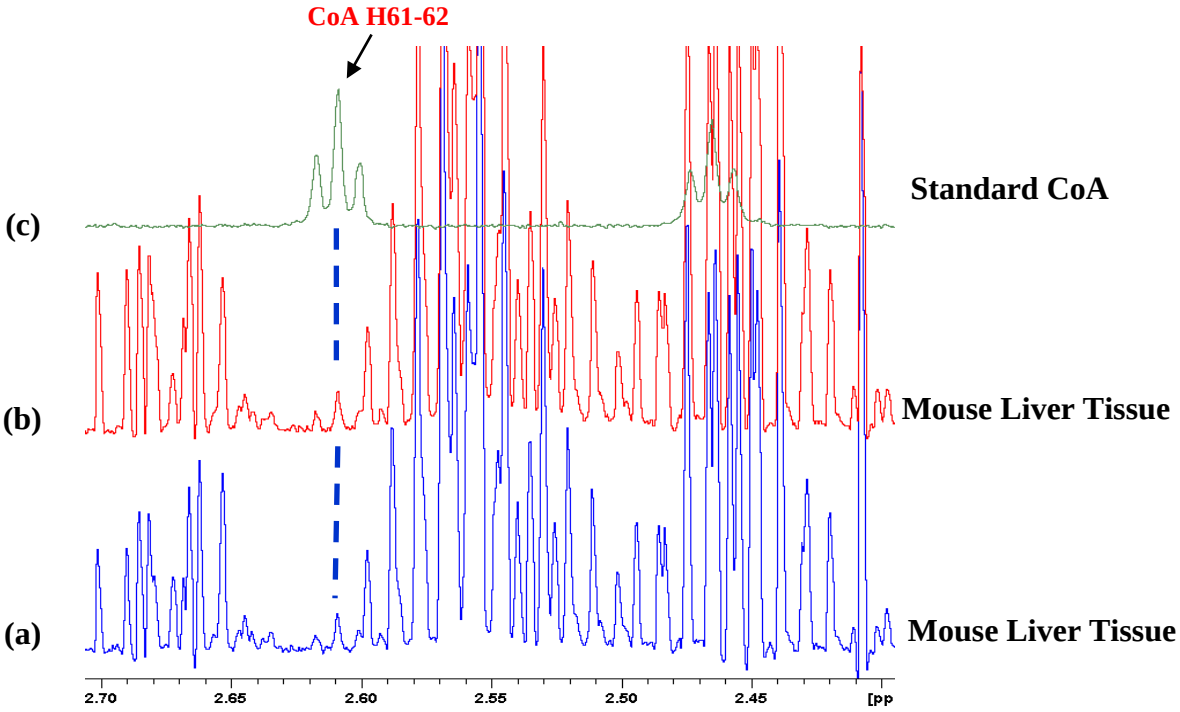

Figure S4. Portions of typical 800 MHz ^{1}H NMR spectra of mouse liver tissue extracts (a and b) with highlighting signals from the Coenzyme A. Portion of the spectrum in (c) is for the standard CoA, shown here for comparison (also see Table S3 and Figure S3). Peak labels correspond to the hydrogen atom labeling based on the recently developed ALATIS, which creates a unique and atom-specific InChI labels.[38]

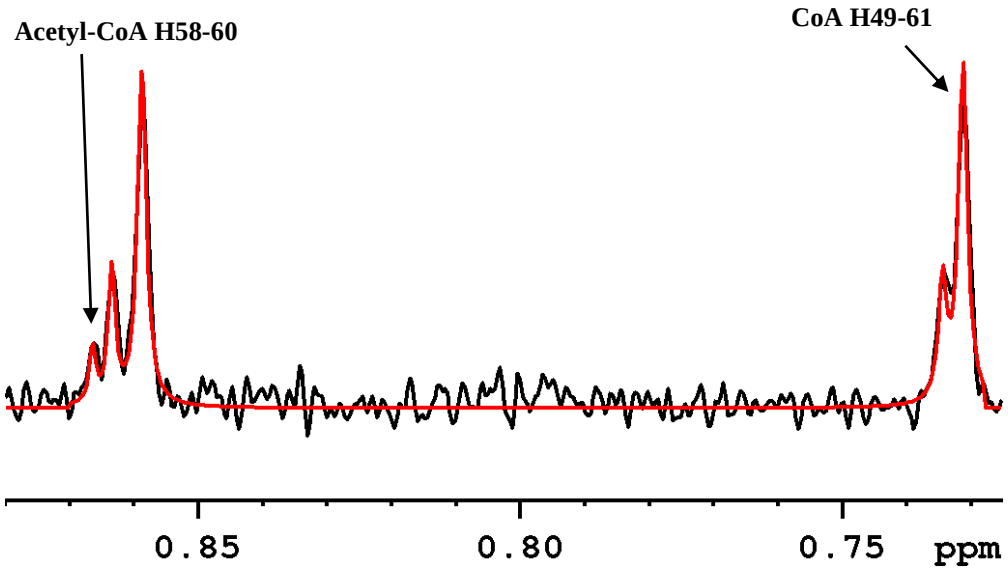

Figure S5. A portion of 800 MHz ^{1}H NMR spectrum of a typical mouse heart tissue extract highlighting deconvolution of the characteristic peaks of CoA and Acetyl-CoA for peak integration: Black: experimental spectrum; and Red: Deconvoluted spectrum using Bruker TopSpin 3.5pl7 software. Peak labels correspond to the hydrogen atom labeling based on the recently developed ALATIS, which creates a unique and atom-specific InChI labels.[38]

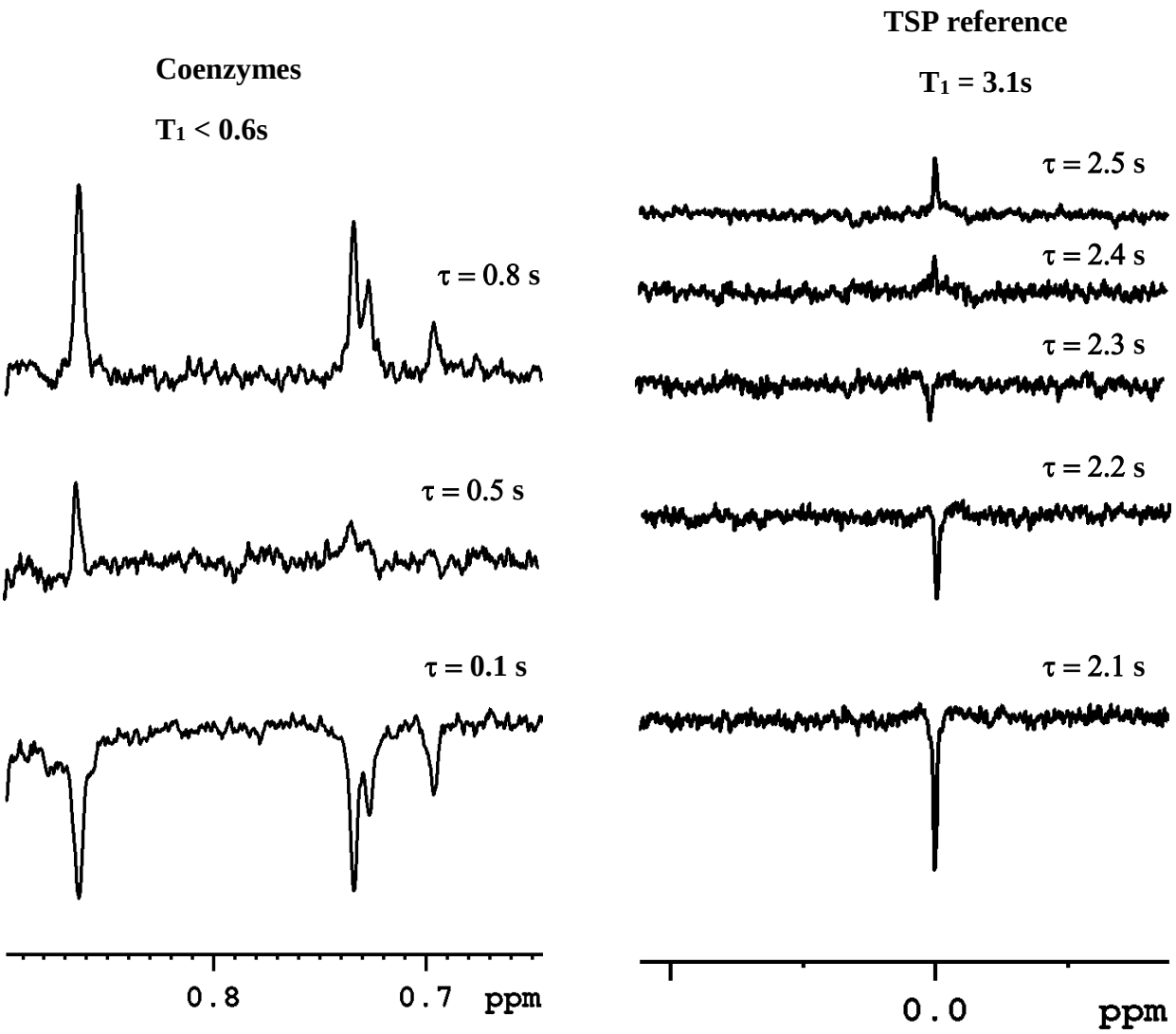

Figure S6: Portions of typical 1D ^{1}H NMR spectra from a mouse heart tissue obtained using the inversion recovery pulse sequence. The recovery time at which the inverted signal becomes zero ($\tau = \tau_{null}$) was used to measure longitudinal relaxation time (T_1) for TSP reference peak as well as the characteristic coenzyme peaks. Note, the peak with the longest T_1 (among the peaks used for quantitation) is from the TSP reference.

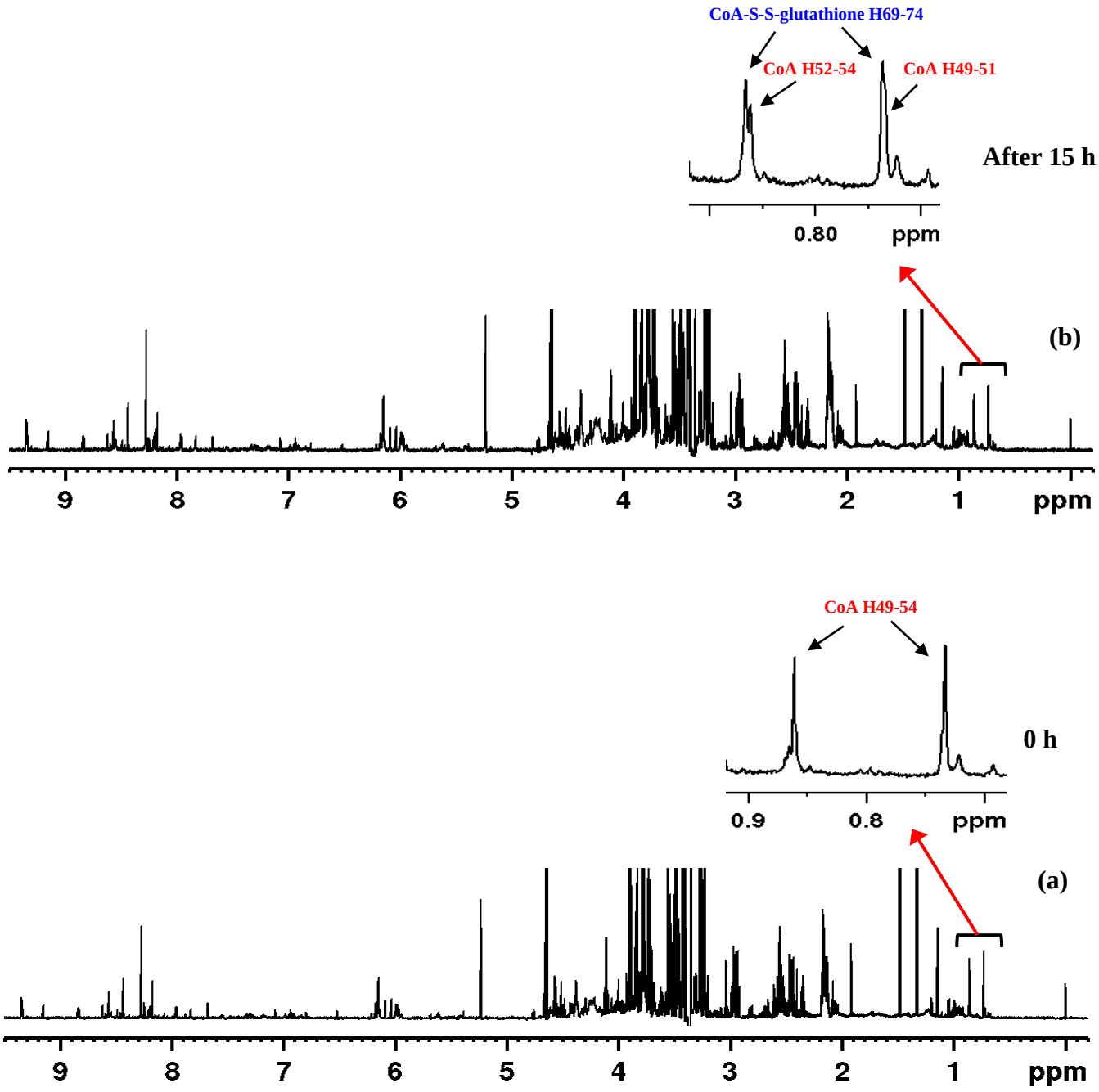

Figure S7. 800 MHz ^{1}H NMR spectra of typical mouse kidney tissue extract obtained after spiking with 60uL (1 mM) standard coenzyme A (CoA) solution (a) obtained as soon as spiking with CoA; and (b) obtained 15 hr after spiking with CoA. Note, after 15 h more than half of CoA is oxidized in solution to form CoA-S-S-glutathione. Peak labels correspond to the hydrogen atom labeling based on the recently developed ALATIS, which creates a unique and atom-specific InChI labels (Figure S3).[38]

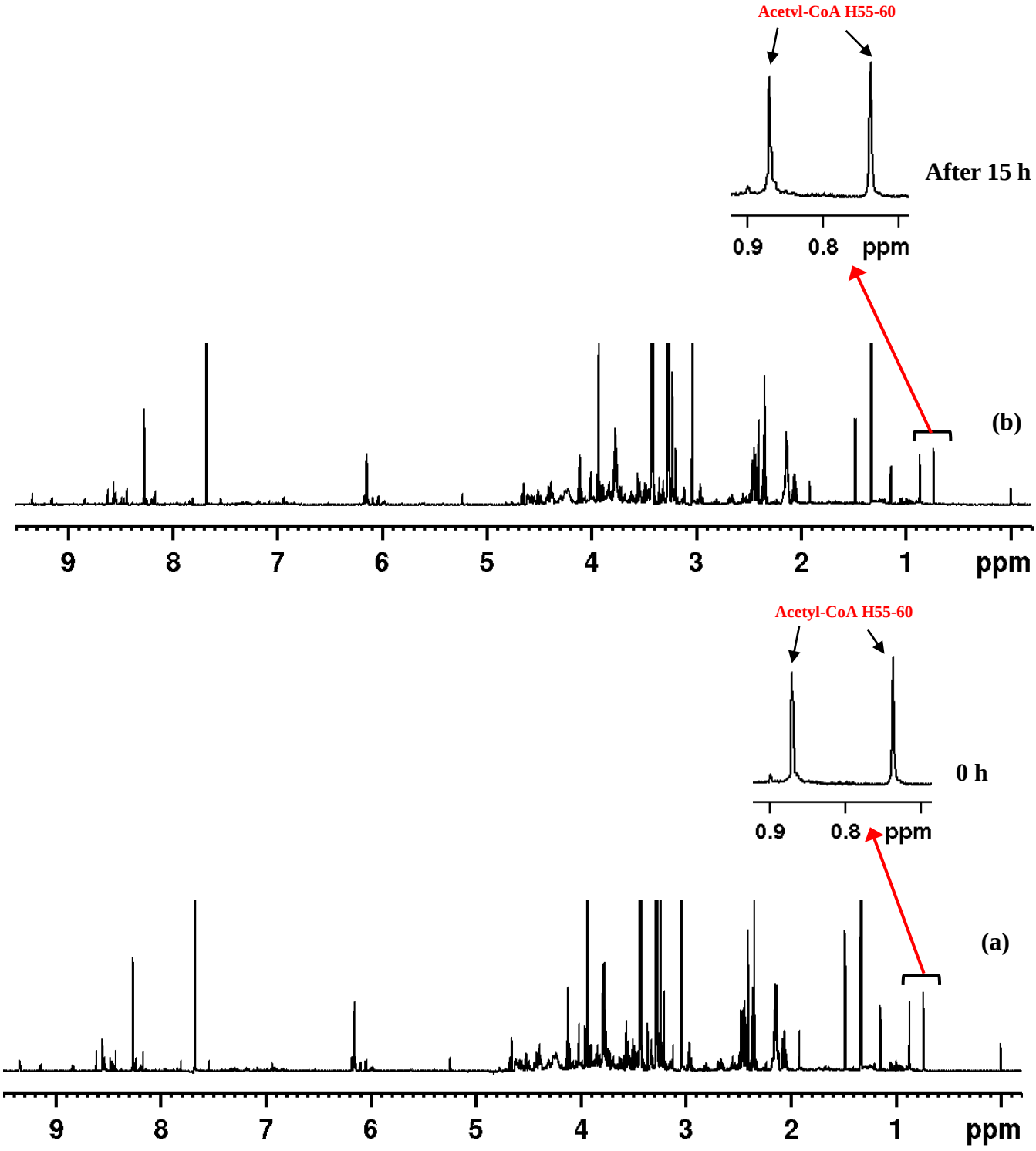

Figure S8. 800 MHz ^{1}H NMR spectra of typical mouse liver tissue extract obtained after spiking with 60uL (1 mM) standard acetyl coenzyme A (acetyl-CoA) solution (a) obtained as soon as spiking with acetyl-CoA; and (b) obtained 15 h after spiking with acetyl-CoA. Note, unlike Co-A, acetyl-CoA peaks are unaltered even after 15 h of spiking. Peak labels correspond to the hydrogen atom labeling based on the recently developed ALATIS, which creates a unique and atom-specific InChI labels (Figure S3).[38]

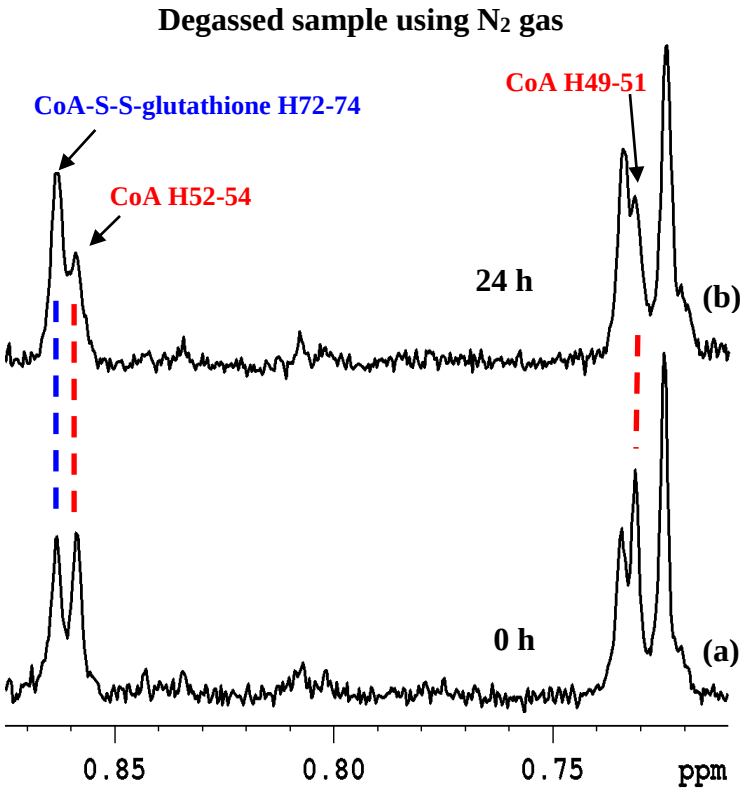

Figure S9. Portions of 800 MHz ^{1}H NMR spectra of a typical mouse liver tissue extract with degassing of both the NMR tube and solvent using nitrogen gas: (a) obtained immediately after sample preparation; and (b) obtained 24 h after preparation. Note, the CoA level reduced with a concomitant increase of the CoA-S-S-glutathione level 24 h after sample preparation even with degassing using nitrogen. The reduction in the CoA levels, however, was not as drastic as observed in the sample without any degassing (see Figure 5). Although reason for the reduction in the CoA level in the sample degassed with nitrogen is unknown, it is likely that the nitrogen gas was contaminated with oxygen. Peak labels correspond to the hydrogen atom labeling based on the recently developed ALATIS, which creates a unique and atom-specific InChI labels (Figure S3).[38]

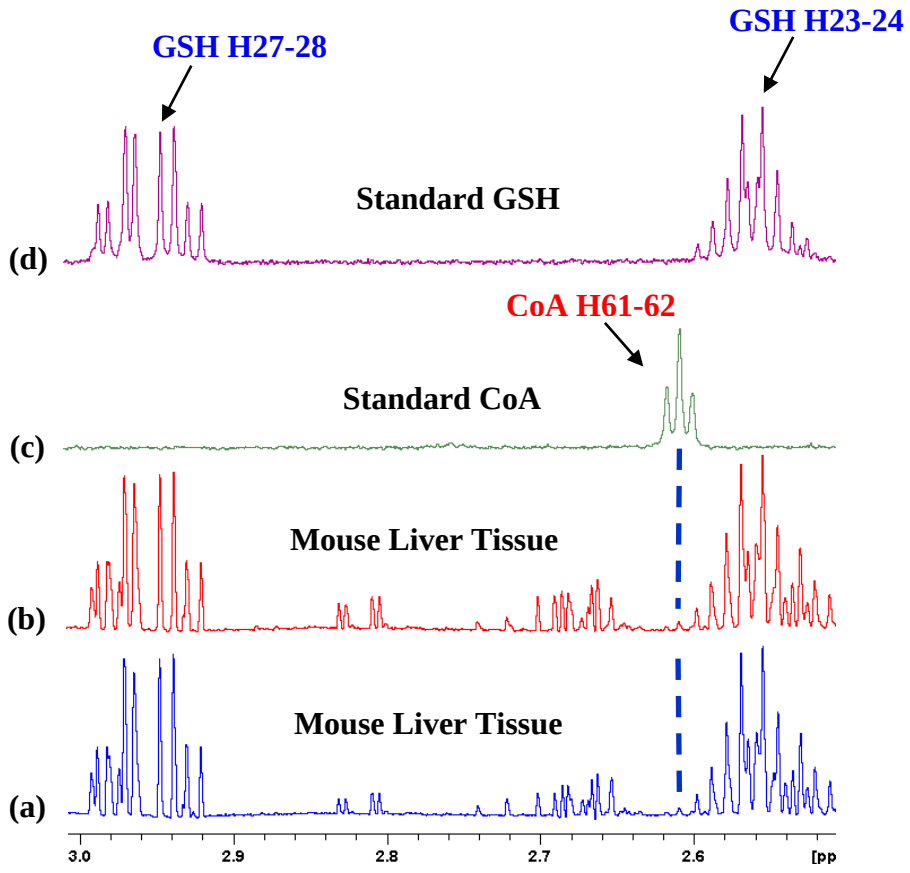

Figure S10. Portions of typical 800 MHz ^{1}H NMR spectra of mouse liver tissue extracts (a and b) with highlighting peaks for coenzyme A (CoA) and reduced glutathione (GSH). In the tissue samples (a and b), GSH concentration is higher than CoA by a factor of > 55. Portions of spectra (c) and (d) are for standard CoA and GSH, respectively, shown here for comparison. Peak labels correspond to the hydrogen atom labeling based on the recently developed ALATIS, which creates a unique and atom-specific InChI labels (Figure S3).[38]

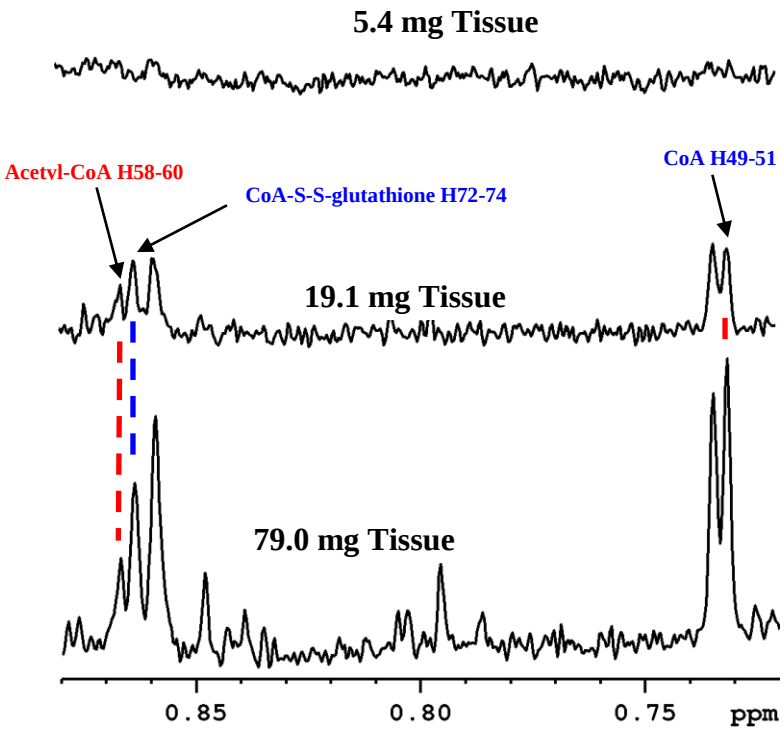

Figure S11. Portions of 800 MHz ^{1}H NMR spectra of a heart tissue extract obtained using different amounts of tissue with highlighting peak intensity as a function of tissue weight for CoA, acetyl-CoA and CoA-S-S-glutathione. All three compounds were detected in 79.0 and 19.1 mg tissue; none of them was detected in 5.4 mg tissue. Each spectrum was obtained with a 15 min data acquisition. Peak labels correspond to the hydrogen atom labeling based on the recently developed ALATIS, which creates a unique and atom-specific InChI labels (Figure S3).[38]