SUPPORTING INFORMATION

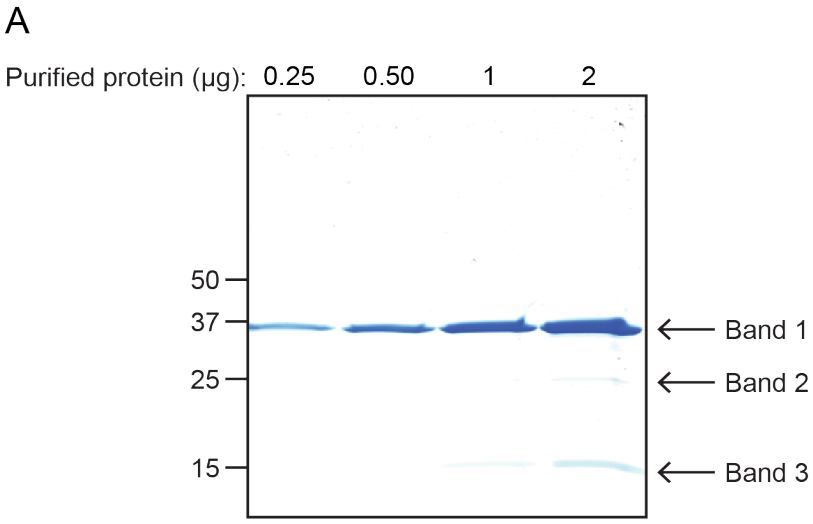


Figure S1. Assessment of the purity of AKR1A1. Coomassie-stained SDS-PAGE gel illustrating serial dilution of the purified protein. The identity of the highlighted bands is shown in Table S1.

Table S1. Results of protein identification from three bands in Fig. S1

Protein name	Database accession ID	Molecular weight (Da)
AKR1A1 protein.- Bos taurus (Bovine) Band 1; major band	Q3ZCJ2_BOVIN	36594
AKR1A1 protein.- Bos taurus (Bovine) Band 2	Q3ZCJ2_BOVIN	36594
hemoglobin beta chain [validated] – Bos Taurus (Bovine) Band 3	HBBOB	15944.3

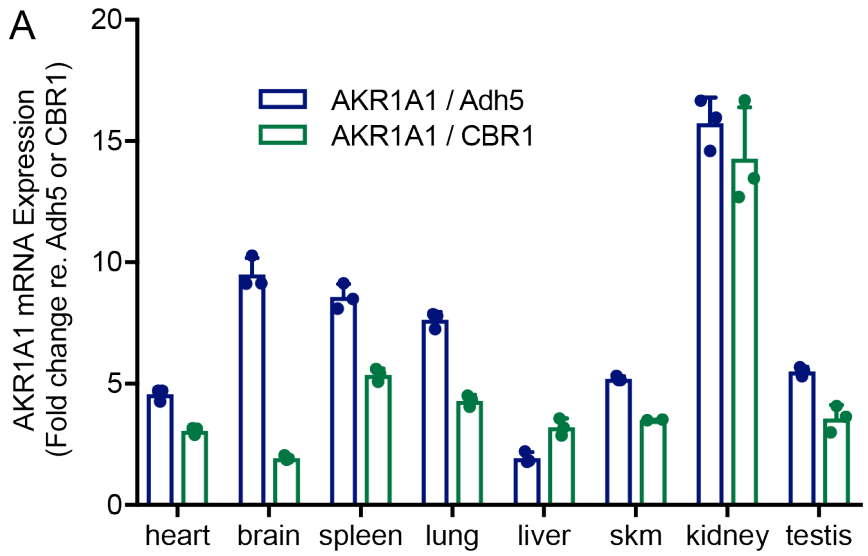
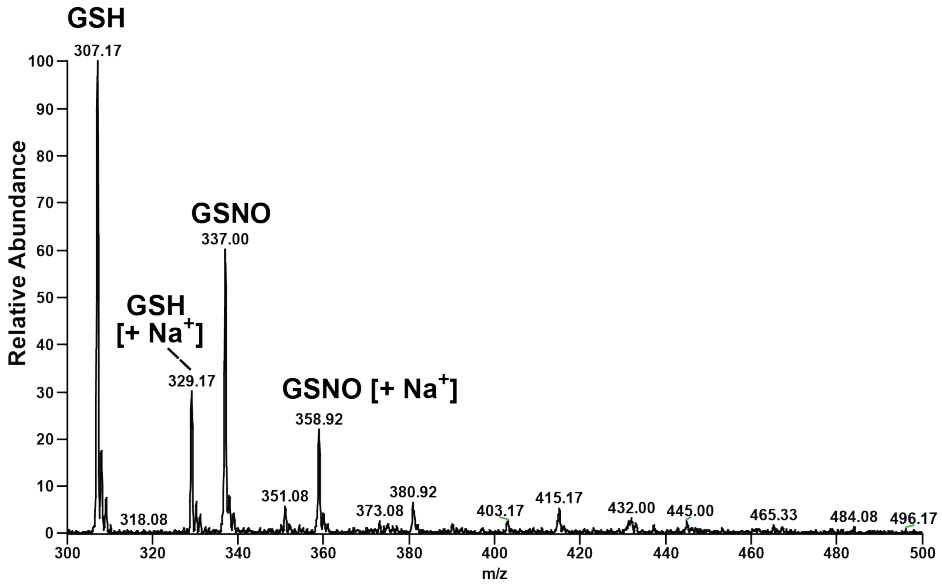
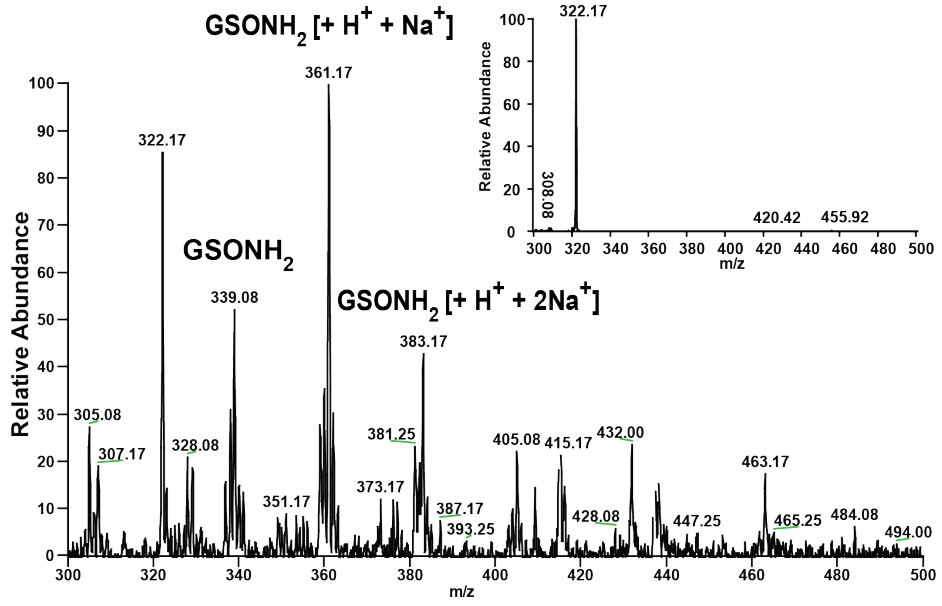


Figure S2. Fold difference in basal *AKR1A1* mRNA expression compared relative to *ADH5* or *CBR1*. qRT-PCR analysis was performed using indicated mouse tissues and expression of *AKR1A1*, *CBR1*, and *ADH5* was analyzed with normalization to expression of 18S rRNA. Data are represented as a ratio of normalized expression.

A


Before AKR1A1 addition
GSH
307.17
GSNO
337.00
GSH
[+ Na+]
329.17
GSNO [+ Na+]
358.92
Relative Abundance
m/z

B


After AKR1A1 addition
GSONH2 [+ H+ + Na+]
361.17
322.17
GSONH2
339.08
GSONH2 [+ H+ + 2Na+]
383.17
Relative Abundance
m/z

C

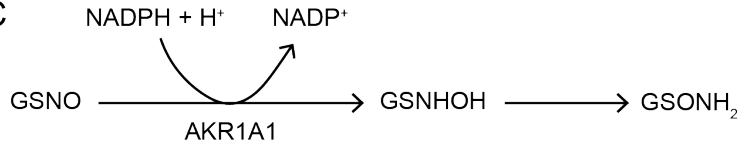

NADPH + H+
NADP+
GSNO
GSNHOH
GSONH2
AKR1A1

Figure S3. Product analysis of GSNO reduction by AKR1A1. (A and B) Electron-spray ionization mass spectra of the reactants (A) and products (B) of assay of AKR1A1 purified from bovine kidney (for clarity, the range of m/z spectra shown is from 300 to 500). In A and B, clusters of peaks at 22 atomic mass unit intervals represent sodiated species. In B, peaks at m/z 339 and 361 correspond to glutathione-sulfinamide and its Na^+ adduct, respectively. Inset shows the MS/MS spectra of the peak at m/z 339. Presence of a peak at m/z 322 indicates a loss of $NH3^+$, confirming glutathione-sulfinamide as the major product. (C) Enzymatic reaction carried out by AKR1A1.

Table S2

Table S2. Enzyme Kinetics for SCoR and mutants.

Substrate	Enzyme	K_m[a] (μM)	kcat[a] (min^{-1})
GSNO	WT	184 ± 8	959
	W22A	169 ± 19	493
	K23A	122 ± 28	1161
	K127A	336 ± 24	953
	W220A	143 ± 17	824
	R312	943 ± 104	383

[a]K_m and V_{max} were determined from Michaelis-Menten curves using GraphPad Prism 7. kcat was calculated by dividing V_{max} by the enzyme concentration in each assay. Enzyme assays were performed in triplicate.