Supplementary Figures

Fig. S1 A. Full scan MS/MS profile of carnosine. The MS/MS spectrum depicting fragment ion of carnosine at medium collision energy (25 V). **S1B.** The MRM chromatogram of three precursor ions (m/z 210.1, 227.2, 249.1)

A

B

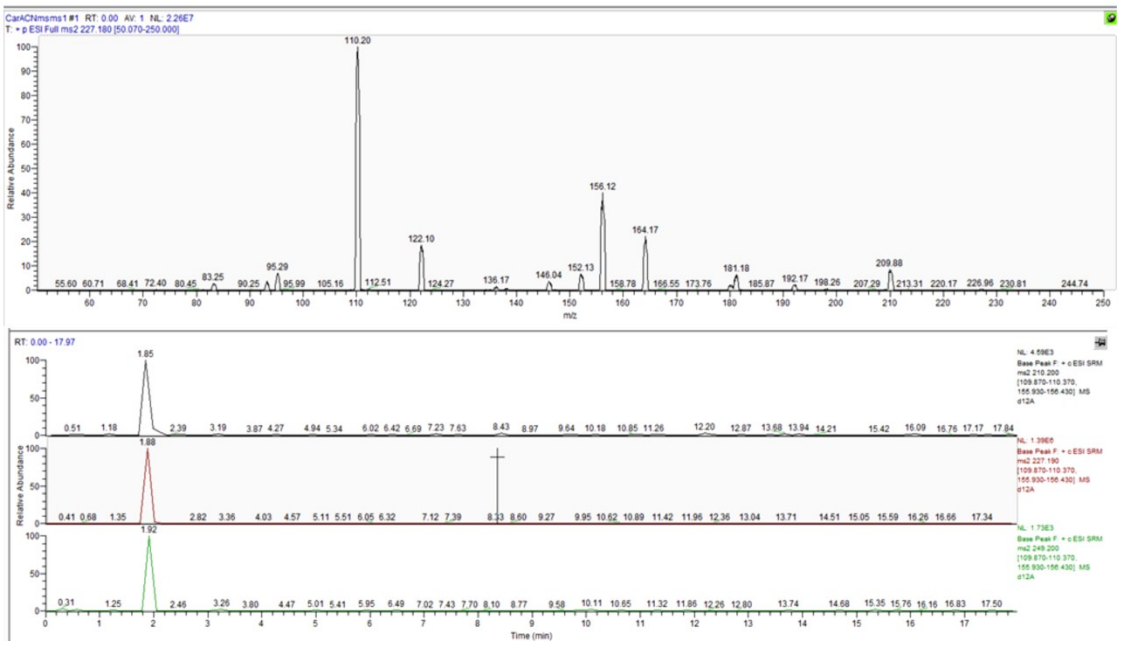


Fig. S2 A-F. MRM chromatogram of carnosine at different concentrations. Out of 15 different concentrations of carnosine analyzed for the standard curve, chromatograms for six concentrations were depicted with peak areas in panel A to F.

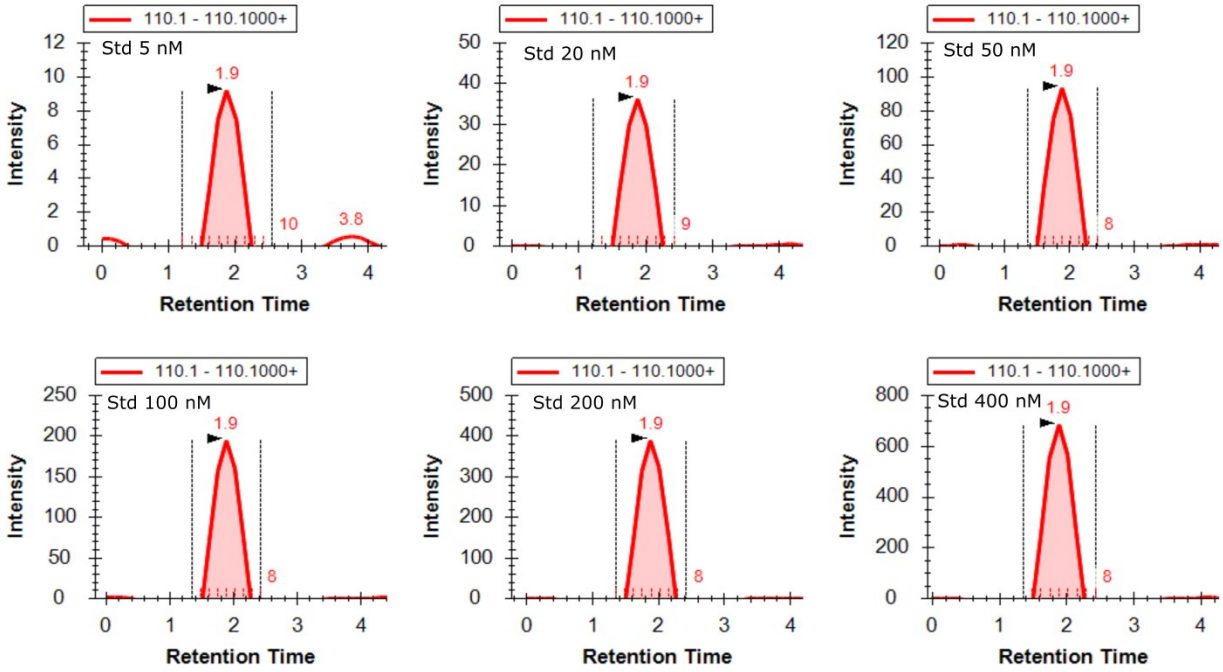


Fig. S3 A-F. Elution of standard carnosine fragments and their retention time in MRM analysis. Fig. A, C, and E shows intensity of precursors with m/z 227.2, 210.2 and 249.2 respectively. Fig. B, D, and F shows retention time of the fragments for these three precursors.

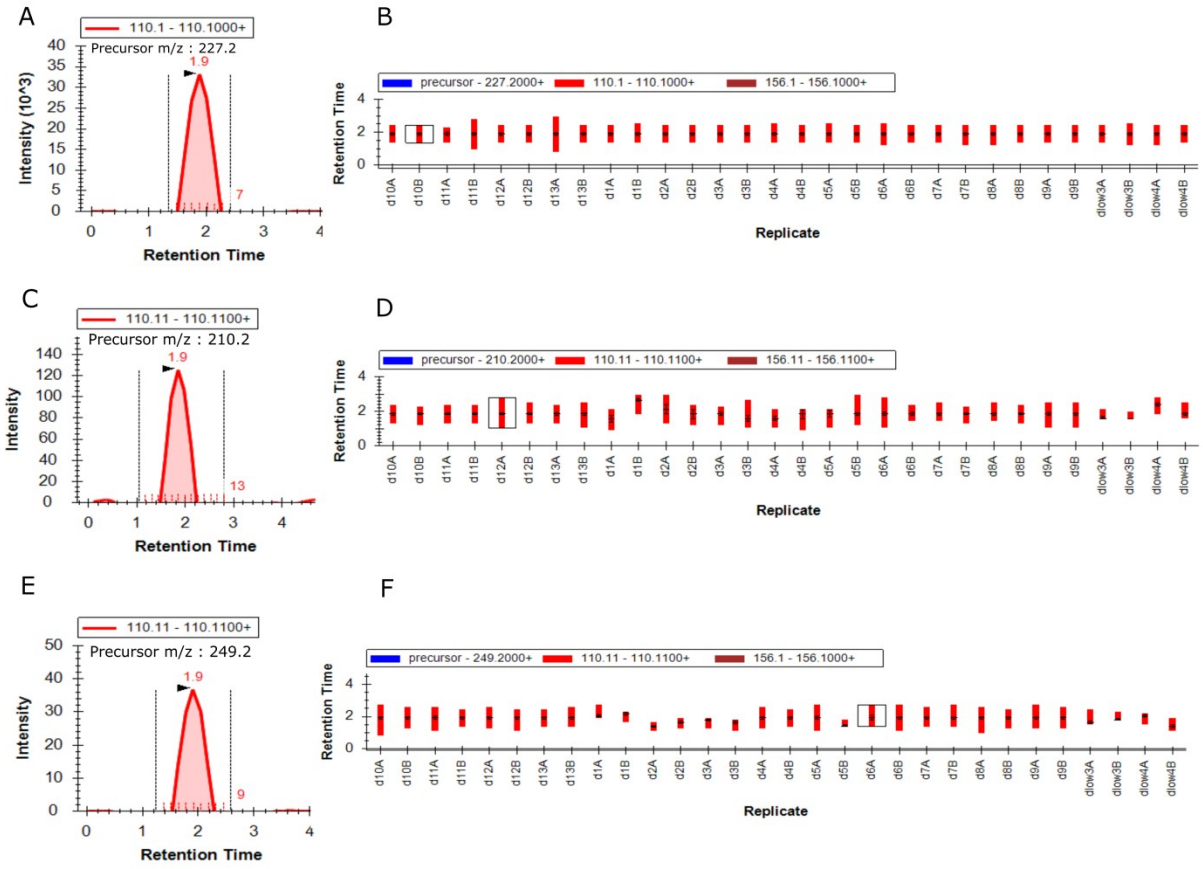


Fig. S4 A-F. Elution of carnosine fragments in plasma samples and their retention time in MRM analysis. Fig. A, C, and E shows intensity of precursors with m/z 227.2, 210.2 and 249.2 respectively. Fig. B, D, and F shows retention time of the fragments for these three precursors.

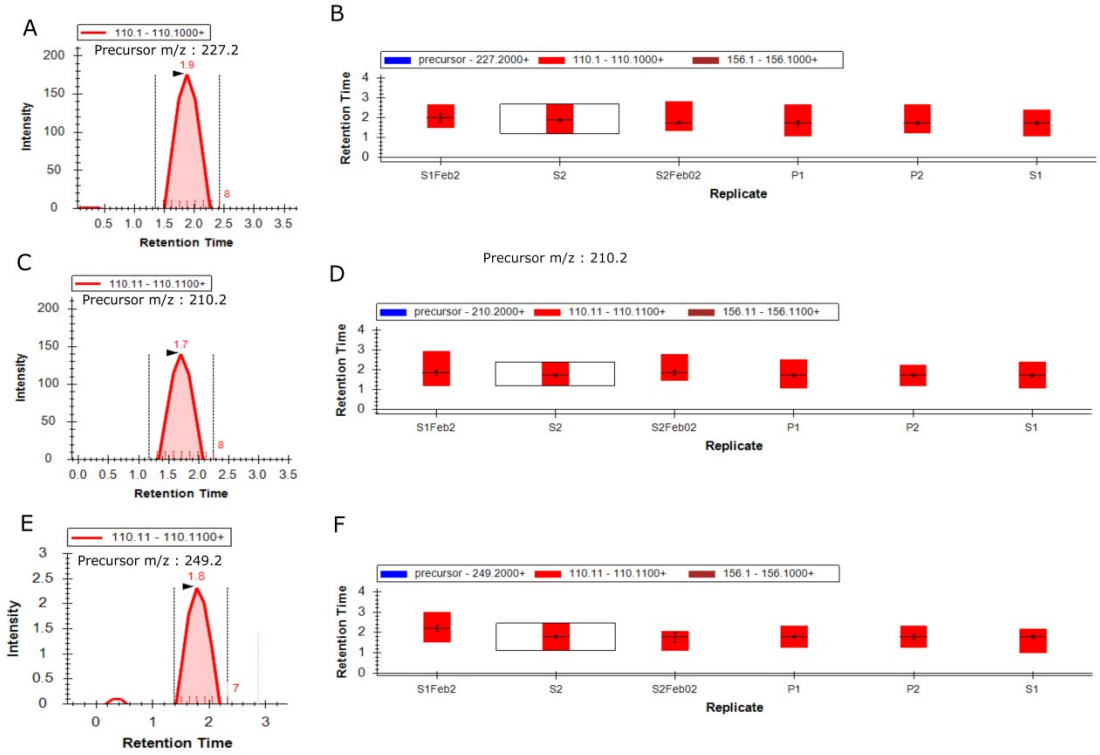


Fig S5. SDS-PAGE image of CN1 and CN2. The affinity purified proteins were separated on 12 % SDS-PAGE and stained with coomassie brilliant blue (CBB).

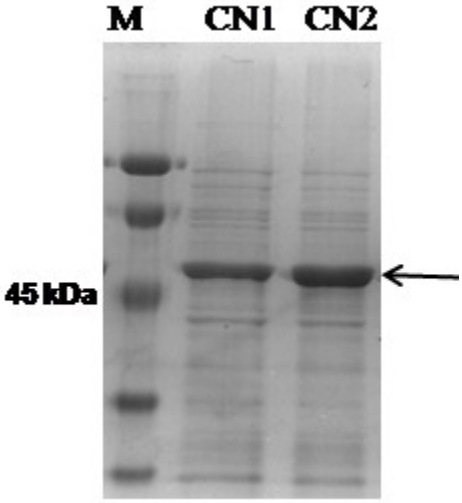
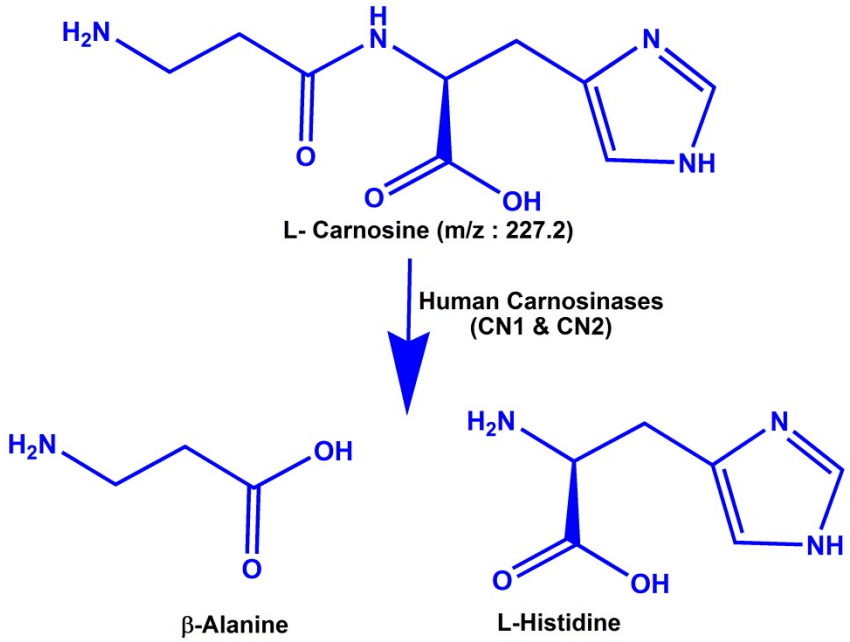


Fig. S6. Scheme of carnosine hydrolysis by human carnosinases (CN1 and CN2).



Supplementary Table: S1. Details of standard curve of carnosine.

C[a] (nM)	Average AUC	Standard Deviation	SEM[b]	% SEM
1.00	1205.67	95.00	54.85	4.55
5.00	3018.77	392.63	226.69	7.51
20.00	13852.95	647.05	373.58	2.70
50.00	44156.26	8646.89	4992.28	11.31
100.00	71905.76	6176.29	3565.88	4.96
200.00	155853.13	10223.73	5902.67	3.79
400.00	300122.35	12102.79	6987.55	2.33
700.00	546154.74	5706.82	3294.84	0.60
1000.00	760074.88	24177.45	13958.85	1.84
1500.00	1200083.86	9007.88	5200.70	0.43
2000.00	1566594.83	6238.18	3601.62	0.23
3000.00	2482731.83	52444.80	30279.02	1.22
4000.00	3301119.86	29776.46	17191.44	0.52
8000.00	6466348.67	98206.09	56699.31	0.88
15000.00	13794366.53	282271.43	162969.49	1.18

[a]: working concentration of standard carnosine; [b]: standard error of mean