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).
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* (nM) | Average AUC | Standard Deviation | SEMb | % 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