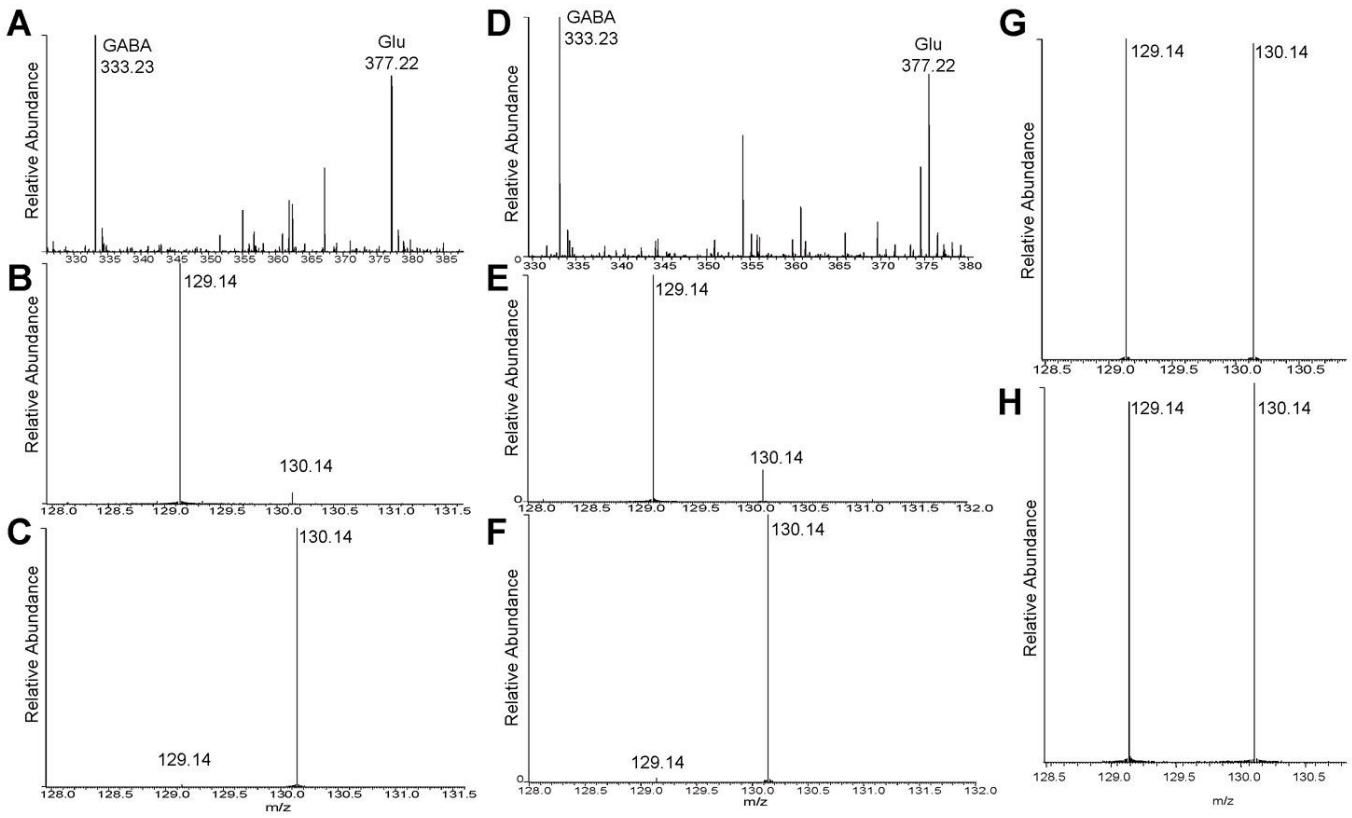


**Supplementary information, Figure S2** Neither GadA nor GadB is able to use Gln as the substrate for α-decarboxylation.

Panels **A**, **B**, and **C** illustrate the fact that GadB efficiently decarboxylates Glu to GABA. (**A**) The mass spectrum of a mixture of TMT-129-labeled Glu alone (as the control) and TMT-130-labeled reaction products (of an equal amount of Glu) with GadB. The TMT-labeled Glu was observed at m/z of 377.22 and its TMT-labeled reaction product GABA was observed at 333.23. (**B**) The MS/MS spectrum of TMT-labeled Glu at m/z 377.22, in which the dominant fragment was observed at 129.14, indicating that most Glu in the mixture was derived from the control sample (Glu alone) and most Glu in the presence of GadB had been converted to GABA. (**C**) The MS/MS spectrum of TMT-labeled GABA at m/z 333.234, in which the dominant fragment was observed at 130.14, indicating that GABA was exclusively derived from

Glu by GadB. Panels **D**, **E**, and **F**, which correspond to panels **A**, **B**, and **C**, respectively, illustrates the fact that GadA efficiently decarboxylates Glu to GABA. (**G**) GadB is unable to decarboxylate Gln. Free Gln was labeled with TMT-129 as the control, and the reaction products of an equal amount of Gln with GadB were labeled with TMT-130. Then the mixture was analyzed by mass spectrometry. The MS/MS spectrum of TMT-labeled Gln at m/z 376.24 contains two fragments, at m/z 129.14 and 130.14, of roughly equal abundance. In addition, no labeled 4-aminobutanamide (decarboxylation product of Gln) was detected in the mass spectra of the reaction products. These results show that Gln was not decarboxylated by GadB. (**H**) GadA is unable to decarboxylate Gln. This panel is similar to panel **G**.