Supporting Information

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SI Materials and Methods

DNAS

Localized in Vivo ¹H MRS. Anatomic imaging and *in vivo* ¹H spectroscopy were obtained by a butterfly-shaped ¹H surface coil with a long axis of 2 cm and a shot axis of 1.2 cm. Scout images were acquired by using a turbo fast low-angle shot (TurboFlash) sequence (1). The *in vivo* ¹H spectra were acquired by using the point-resolved spectroscopy (PRESS) localization approach (2) with a $4 \times 4 \times 4$ mm³ voxel size mainly covering the rat cortical region. Out volume suppression (OVS) was performed by B₁-insensitive selective train to obliterate signal (BISTRO) method (3). The spectral acquisition parameters were

as follows: a 5,000-Hz spectral width, 128 scans, 1,500 data points, a 3-s repetition time, and a 13-ms echo time.

In Vivo ³¹P Chemical Shift Imaging (CSI). The one-dimensional ³¹P chemical shift image was performed on 9.4T, using the same ${}^{1}H/{}^{31}P$ RF surface coil and the Fourier series window imaging technique (4) with a 6,000-Hz spectral width; a 2.5-cm field of view, and a spatial resolution of 0.5 cm (nominal resolution of 0.36 cm); 512 data points; 3-s repetition time and total 250 scans per CSI with acquisition time of 12.5 min.

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Fig. S1. Localized *in vivo* ¹H spectra obtained from a rat brain under IsoF (*A*), low-Pen (*B*), and high-Pen (*C*) anesthesia conditions. An increase in the lactate was not evident under the iso-electric (high-Pen) condition. Assignments of spectral peaks: *N*-acetyl aspartate (NAA), lactate (Lac), glutamate (Glu), glutamine (Gln), creatine (Cr), phosphocreatine (PCr), and choline (Cho).



Fig. S2. Localization of in vivo ³¹P MT measurements. To evaluate the source of the Pi signal detected by the ³¹P RF coil in this study, we performed a concurrent measurement of ¹H anatomical imaging (A) and one-dimensional (1D) ³¹P chemical shift imaging (CSI) (B). In this experiment, the RF coil was intentionally set toward the lateral side of the rat head for detecting the ³¹P signals from both the brain tissue and surrounding muscles. The results show that Pi peak was almost invisible in the ³¹P spectrum obtained from the region (Voxel 2) dominated by muscles; and, in contrast, a well resolved and intensive Pi resonance peak was observed in the region (Voxel 5) dominated by brain tissue. Therefore, the Pi signal detected in our ³¹P MT study can be predominantly attributed to the brain tissue. Moreover, for all in vivo ³¹P MT measurements as reported in this article, the ³¹P coil was positioned at the center of the rat head; thus, the possible Pi contribution from surrounding muscles is further suppressed and becomes negligible, and the measured forward ATP reaction flux should present only the ATP metabolic activity of the brain tissue. It is well known that the cellular PCr content of the skeletal muscle is significantly higher than that of brain tissue, which is also evident in B (e.g., voxel 2 of muscle versus voxel 5 of brain tissue). However, the possible contribution of the PCr signal from muscle was relatively small and it was estimated to be <20% with the RF coil set-up in our in vivo ³¹P MT study. The conclusion from this rat study was consistent with the human brain studies (1-3). The white dashed lines in A show the voxel locations of the 1D³¹P CSI (B), and the white double line indicates the RF coil position (cross section) in this measurement and the white solid curve defines the coil sensitive region (with >5% of the maximal signal detected by the ³¹P RF coil) indicating a signal penetration of ~1 cm in depth. It is well documented that the metabolic rate is not uniform across different types of tissues and is much higher in the cortical or subcortical gray matter than the white matter. Thus, the ratios of baseline to functional metabolic activity could vary among these tissue compartments. Nevertheless, the fraction of the white matter volume is small in the rat brain owing to a very small volume ratio of white mater to gray matter of <0.14 (4), and this is also evident in the rat anatomic image as shown in A. Moreover, the ³¹P RF surface coil used in this study provides the favorable detection sensitivity in the superficial brain region that is mainly occupied by the cortical gray matter. Thereby, the measured ³¹P signals and the reaction fluxes should be mainly attributed to the cortical gray matter tissue in this study.

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