Supporting Information

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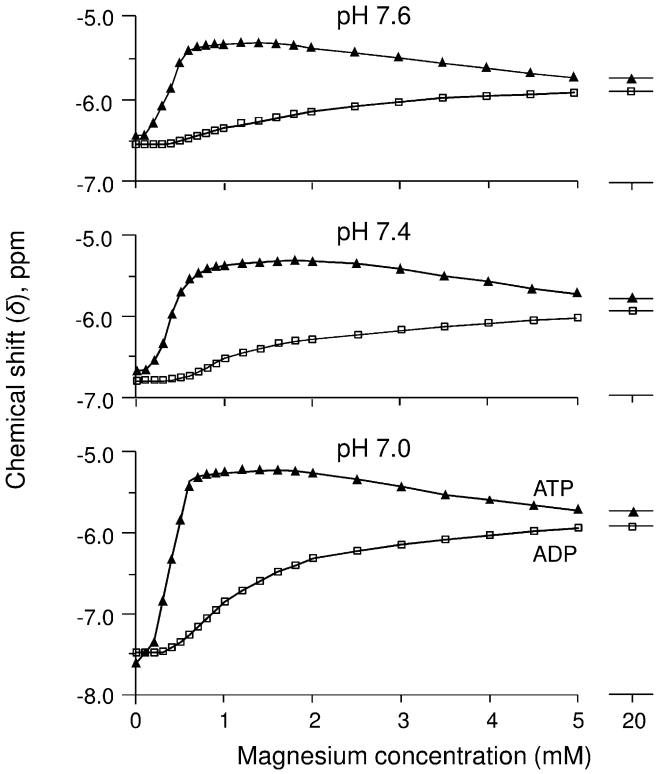

Fig. S1. Calibration curves linking γ-ATP and β-ADP chemical shifts to [$MgSO_4$] at different pH values. Solutions containing 0.6 mM ATP, 0.15 mM ADP, 150 mM KNO_3, and 20 mM Hepes buffer at pH 7.0, 7.4, and 7.6 were analyzed in a 10-mm NMR tube by ^{31}P-NMR. Acquisition time, 15 min (1,500 scans). The total magnesium concentrations corresponding to the midpoints used to calculate K_d values were 335 ± 3 μM for ATP and 1,345 ± 50 μM for ADP, at the three pH values tested. A detailed description of how [ATP], [MgATP], [ADP], [MgADP], and [Mg^{2+}] were calculated from the chemical shift data is provided given in *Materials and Methods*.

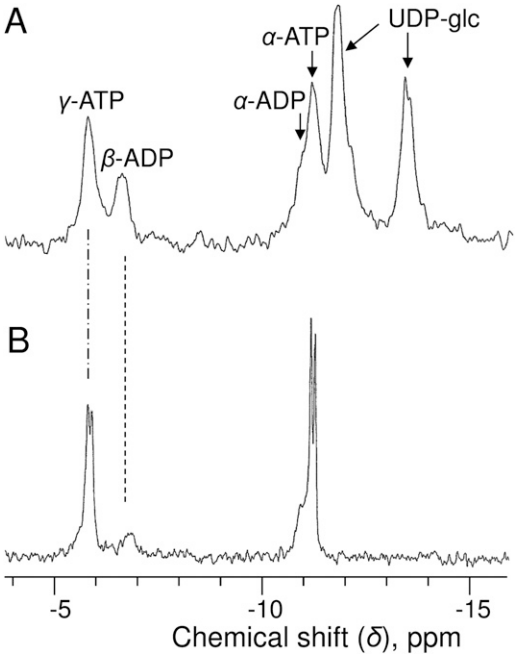

Fig. S2. Enlarged portions of in vivo/in vitro proton-decoupled ^{31}P-NMR spectra of hypoxic sycamore cells (*A*) and a solution of ATP + ADP (*B*). Experimental conditions were as follows. (*A*) Cells were harvested at 5 d after subculture in standard medium and perfused in a 25-mm NMR tube at 20 °C, with NM containing 50 μM Pi at pH 6.0. The O_2 bubbling in perfusing NM was replaced by N_2 bubbling 5 min before acquisition. Acquisition time, 20 min (2,000 scans). (*B*) A solution of 0.6 mM ATP + 0.15 mM ADP containing 1.1 mM $MgSO_4$, 150 mM KNO_3, and 20 mM Hepes buffer (pH 6.8) was perfused in a 10-mm NMR tube. Acquisition time, 15 min (1,500 scans). This in vitro simulation fit the in vivo spectrum.

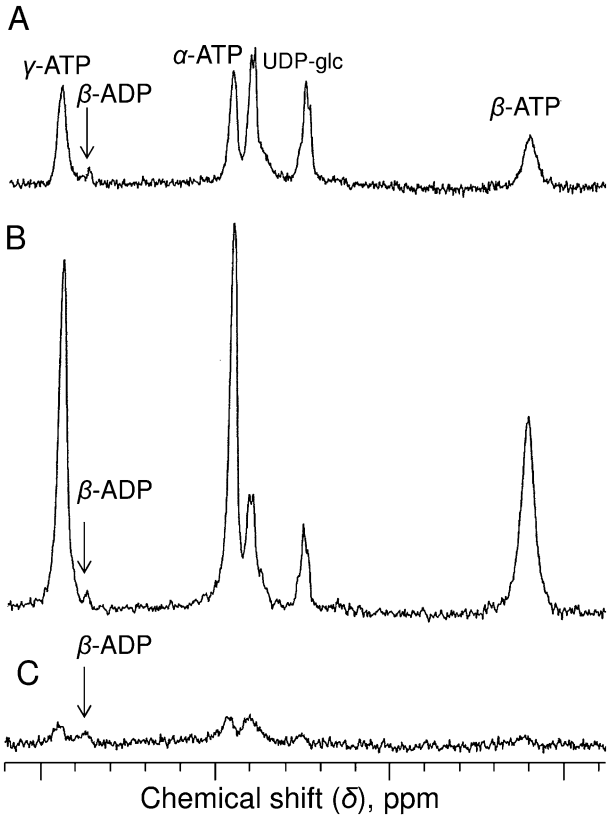

Fig. S3. Enlarged portions of in vivo proton-decoupled ^{31}P-NMR spectra of sycamore cells. (*A*) Cells harvested in standard NM at 5 d after subculture and perfused in a 25-mm NMR tube with a well-oxygenated diluted NM. (*B*) Cells preincubated in adenine-supplied NM for 12 h and perfused in the presence of 1 mM adenine. (*C*) Cells preincubated in a Pi-free NM for 5 d and perfused with a Pi-free NM. Acquisition time, 16 h (96,000 scans). The 16-h spectrum is the sum of four successive 4-h blocks showing no change across the 16-h time course of the experiment.

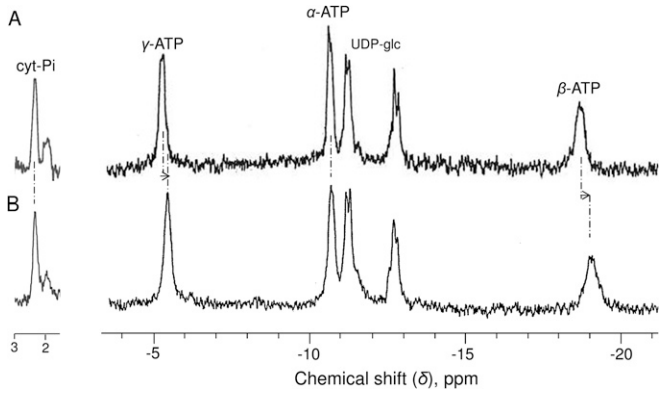

Fig. S4. Enlarged portions of in vivo proton-decoupled ^{31}P-NMR spectra of sycamore cells. (*A*) Spectrum accumulated 1 h after the addition of 1 mM $MgSO_4$ to the nutrient medium of 2-wk Mg-deprived cells. Acquisition time, 1 h (6,000 scans). (*B*) Spectrum accumulated 2 h later. Acquisition time, 2 h (12,000 scans). Horizontal arrows indicate the upfield shift (toward the right of the spectra) of the γ- and β-ATP resonance peaks after the first hour of recovery. Note that just before the addition of Mg^{2+}, the chemical shifts of β-ATP and γ-ATP were −-19.5 and −5.9 ppm, respectively (Fig. 6*A*), which are even more upfield than those shown in *B*. This suggests that the massive influx of Mg^{2+} transiently saturated ATP in the cytosol to equilibrate within the storage compartments (vacuole).

Table S1. Phosphorylation rate of 1 mM adenine, 0.1 mM choline, and 50 mM glycerol by standard and 2-wk Mg-deprived sycamore cells

	ATP	P-choline	Glycerol 3-P
+ Mg^{2+} (standard cells)	28 ± 3	600 ± 50	950 ± 90
− Mg^{2+} (Mg-deprived cells)	2.0 ± 0.5	20 ± 5	25 ± 5

Measurements were performed from 1-h in vivo proton-decoupled ^{31}P-NMR spectra. Values correspond to nmol of phosphorylated compound h^{-1} g^{-1} cell wet weight, and are reported as mean ± SD (n = 5).

Table S2. Cytosolic concentration of free-magnesium in sycamore cells measured from the chemical shifts of β-ATP, γ-ATP, and β-ADP signals after 1 h and 16 h of data accumulation

	From $\delta_{\gamma\text{-ATP}}$	From $\delta_{\beta\text{-ATP}}$	From $\delta_{\beta\text{-ADP}}$
After 1 h, μM	250 ± 30	270 ± 60	—
After 16 h, μM	250 ± 20	260 ± 30	250 ± 30

Concentrations were measured as indicated in *Materials and Methods*. The data accumulation times of in vivo ^{31}P-NMR spectra were 1 h (6,000 scans) and 16 h (96,000 scans). Values are mean ± SD (n = 5).