Supplemental Figure 2. Biochemical assays of the HK1 E847K mutation. (a) HK1-WT and HK1-E847K were cloned into pcDNA3 with N-terminal Flag-tag, and over-expressed in 293T cells. Proteins were purified by anti-flag-M2 magnetic beads and analyzed by SDS-PAGE and coomassie staining. (b) Hexokinase activities were measured using Hexokinase colorimetric assay kit with purified HK1-WT and HK1-E847K proteins (0.1 μg each) (n=3). (c) The crystal structure of recombinant human hexokinase type I with 1,5-anhydroglucitol 6-phosphate (PDB ID: 4FPB). Yellow arrow indicates the site of mutation E847 (d) HK1 mRNA levels were analyzed using RT-qPCR two days after transfection in 293 cells (n=3). The Ct values of HK1 were normalized by β-actin. (e) HK1 protein levels were measured by Western blot analysis using 20 μg of total proteins from each 293T cell lysate sample. n.s.: p-value >0.05 (Student’s t-test). All the values are shown as mean±SD.