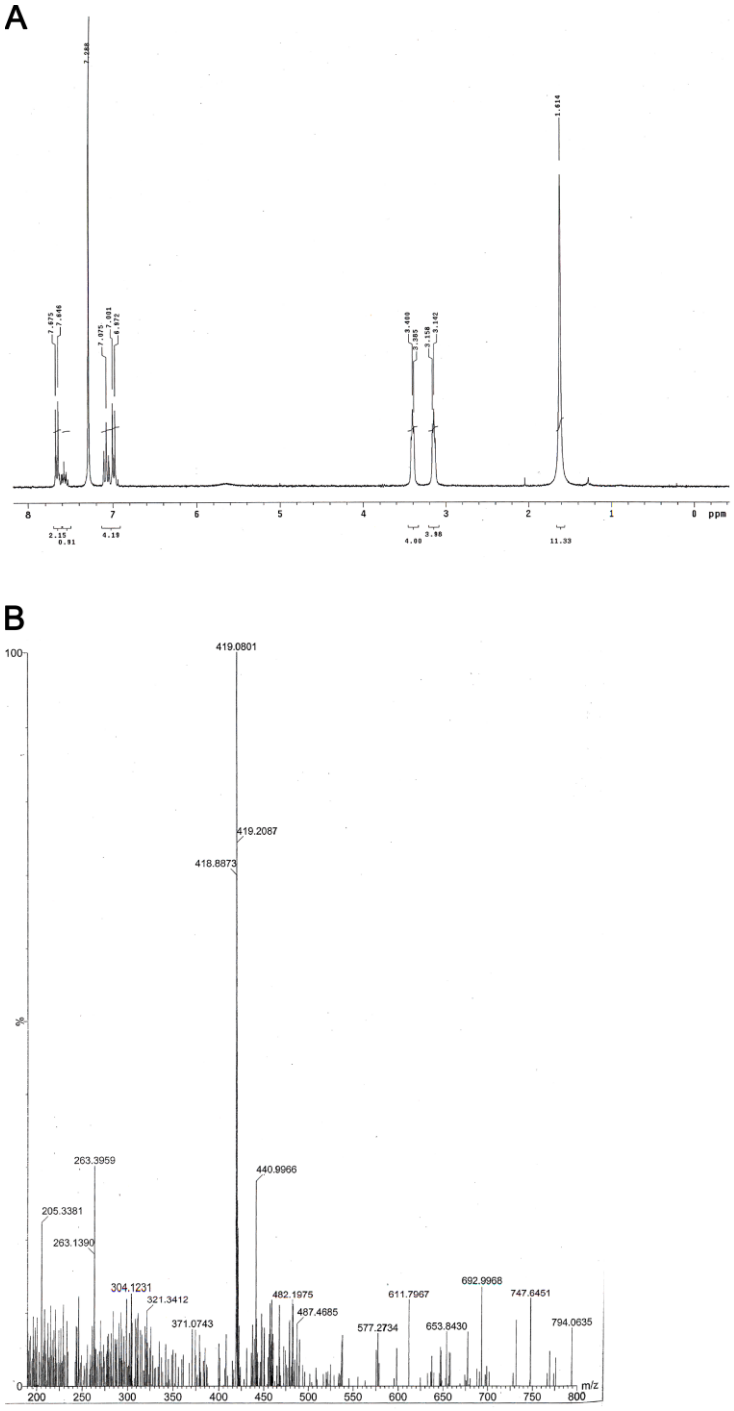
SUPPLEMENTARY INFORMATION

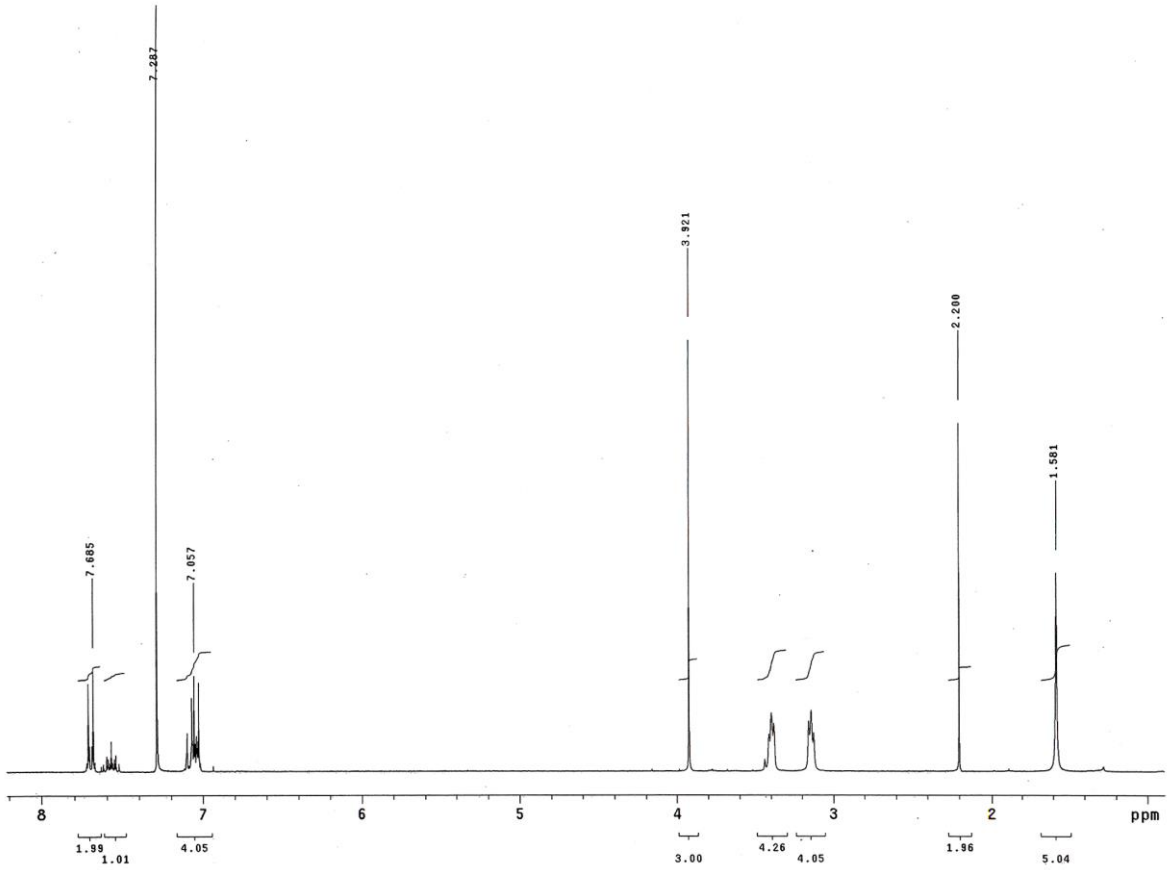
PET imaging of tumor glycolysis downstream of hexokinase through non-invasive measurement of pyruvate kinase M2

Timothy H Witney[1], Michelle L James[1], Bin Shen[1], Christoph Pohling[1], Edwin Chang[1], Natasha Arksey[1], Deepika Bodapati[1], Judith Weber[1], Aileen Hoehne [1], Adam Shuhendler[1], Jun-Hyung Park[1], Gayatri Gowrishankar[1], Jianghong Rao[1], Frederick T Chin[1] & Sanjiv Sam Gambhir[1]

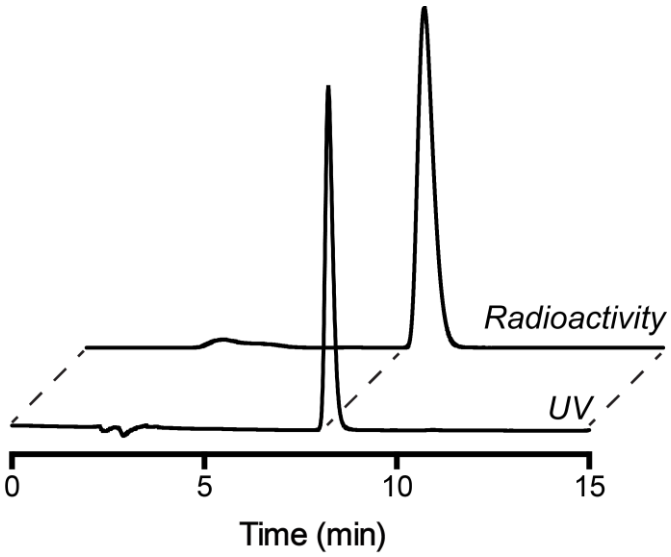
SUPPLEMENTARY FIGURES



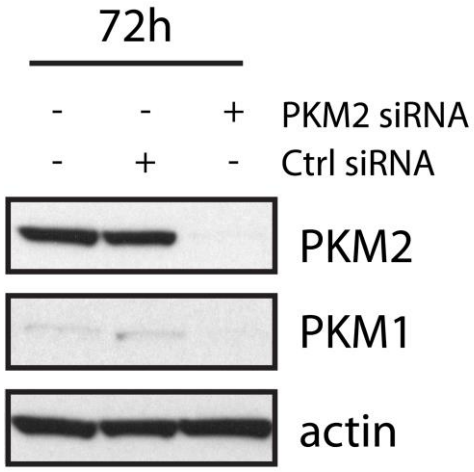
Supplementary Figure 1. Precursor structural determination. Identification was performed by ^{1}H-NMR spectrum (**A**) and mass spectrometry (**B**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) = 7.661 (d, $^{3}J_{H,H}$ = 8.7 Hz, 2H), 7.574 (m, 1H), 7.075 (m, 2H), 6.987 (d, $^{3}J_{H,H}$ = 8.7 Hz, 2H), 5.648 (bs, 1H), 3.393 (m, 4H), 3.150 (m, 4H). MS (CI) *m/z:* $[M+H]^+$ calculated for $C_{16}H_{16}F_2N_2O_5S_2$ 419.05; found 419.08.



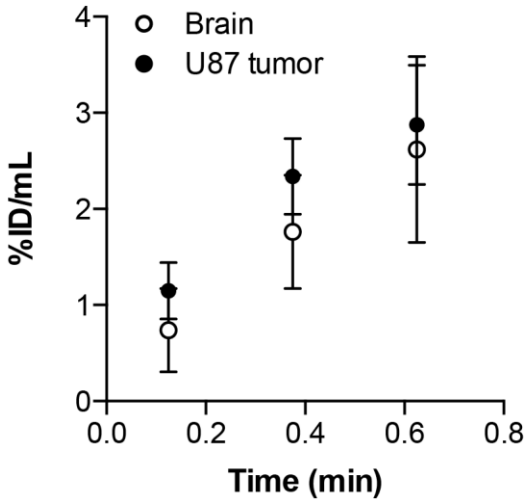
Supplementary Figure 2. Confirmation of DASA-23 identity by ^{1}H-NMR spectrum. ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) = 7.685 (d, $^{3}J_{H,H}$ = 9.4 Hz, 2H), 7.563 (m, 1H), 7.057 (m, 4H), 3.921 (s, 3H), 3.392 (m,4H), 2.200 (s, C_3H_6O impurity in $CDCl_3$), 3.151 (m,4H).



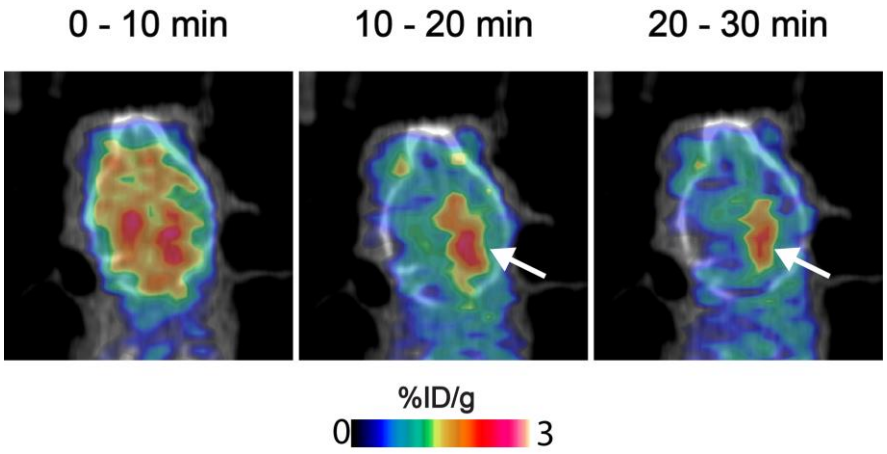
Supplementary Figure 3. Analytical HPLC chromatogram of purified [^{11}C]DASA-23. Cold [^{12}C]DASA-23 was co-injected as a standard to confirm the identity of the radiotracer.



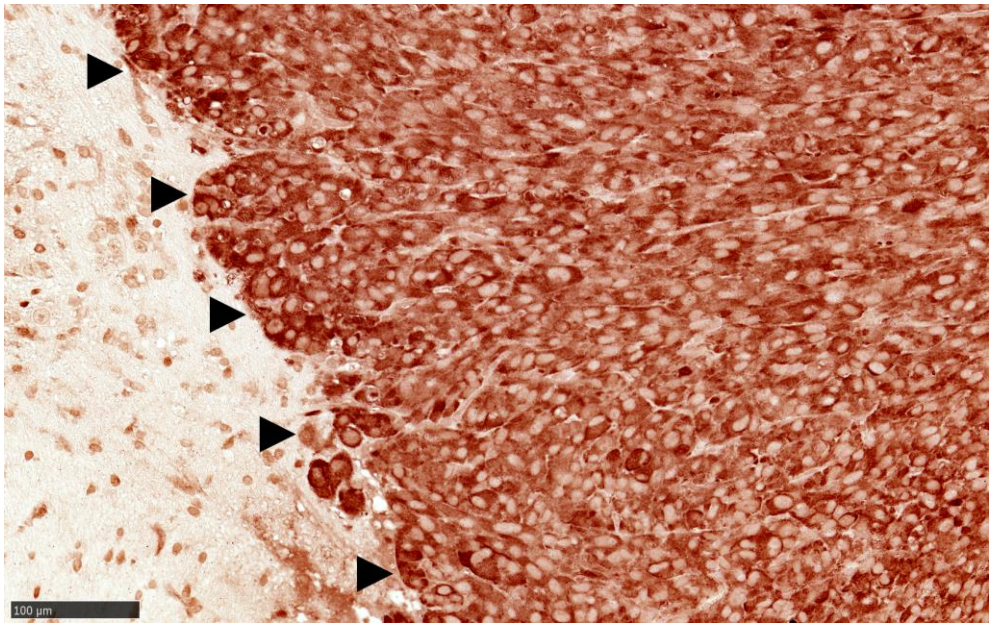
Supplementary Figure 4. Pyruvate kinase protein expression in HeLa cells. Expression was measured 72 hours after transfection with siPKM2 or siCtrl. Untreated cells were used for comparison. Representative western blot from whole cell lysate is shown, with actin used as a loading control. Blots probed for PKM2 and actin were exposed for 1 minute, with blots probed for PKM1 exposed for 30 min.



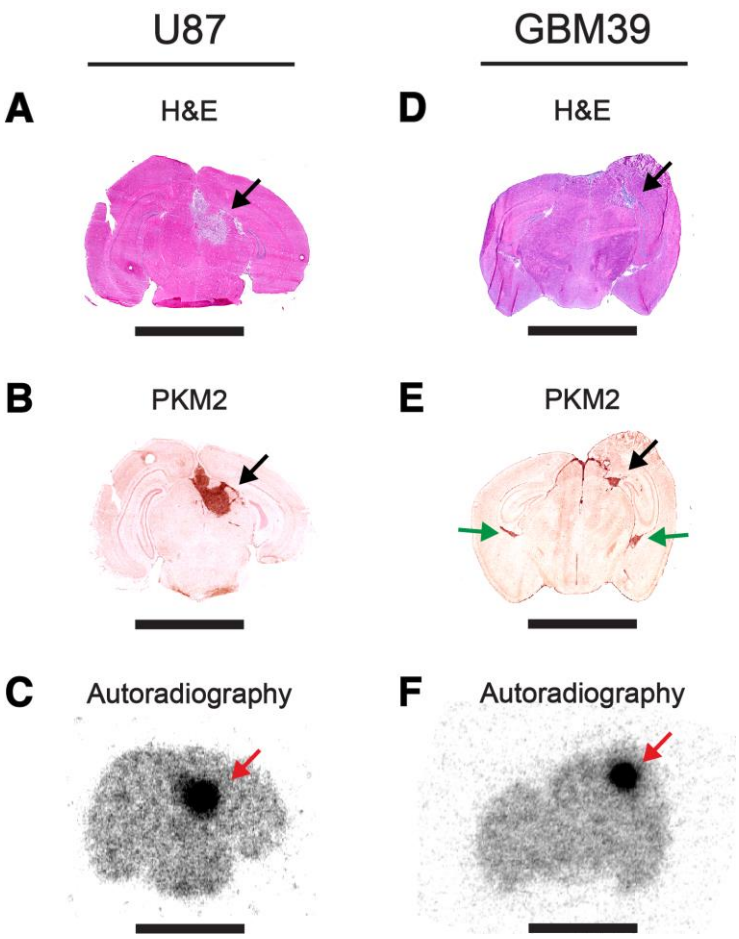
Supplementary Figure 5. Initial delivery of [^{11}C]DASA-23 to orthotopic U87 tumors and corresponding contralateral normal brain. The TAC was taken from dynamic [^{11}C]DASA-23-PET/CT images. Data shown as mean ± SD (*n* = 6 animals).



Supplementary Figure 6. Time course images of [^{11}C]DASA-23 uptake in the brain of an orthotopic U87 tumor-bearing mouse. [^{11}C]DASA-23 PET/CT was characterized by rapid uptake and subsequent efflux in healthy brain tissue, with radioactivity retained in the tumor, as identified by the white arrow.



Supplementary Figure 7. Cytosolic immunostaining of tumor-specific PKM2 at the tumor margin. PKM2 staining is denoted by black arrow heads (20×; scale bar = 100 µm).



Supplementary Figure 8. *Ex vivo* histopathological and autoradiographic analysis of orthotopic GBM tumors. [^{11}C]DASA-23 uptake and correlation with tumor-associated PKM2 expression was assessed in U87 (**A-C**) and GBM39 tumors (**D-F**). Whole brain sections were either stained with H&E (**A, D**) or an antibody against PKM2 (**B, E**), and compared to adjacent [^{11}C]DASA-23 *ex vivo* autoradiography sections taken 20 min after radiotracer injection (**C, F**). Scale bar = 5 mm. Black and red arrows identify the tumor. Green arrows indicate PKM2 staining of suspected small tumor lesions not present in adjacent sections, as shown by H&E.

SUPPLEMENTARY VIDEO LEGENDS

Supplemental movie 1. Overlaid PET/CT 3D VRT movie of a mouse with an orthotopic U87 xenograft. The PET image represents radioactivity 10-30 min after radiotracer injection.

Supplemental movie 2. Overlaid PET/CT 3D VRT movie of a non tumor-bearing mouse. The PET image represents radioactivity 10-30 min after radiotracer injection.

Supplemental movie 3. Overlaid PET/CT 3D VRT movie of a mouse containing an orthotopic GBM39 PDX. The PET image represents radioactivity 10-30 min after radiotracer injection.

Supplemental movie 4. Overlaid PET/CT 3D VRT movie of the same mouse as in movie S3, after TEPP-46 treatment. The movie was obtained 1 hour after blocking with TEPP-46 (50 mg/kg). The PET image represents radioactivity 10-30 min after radiotracer injection.