Supporting Information

Vlashi et al. 10.1073/pnas.1106704108

SI Materials and Methods

Western Blotting. Cells were lysed on ice using Mammalian Protein Extraction Reagent (Thermo Scientific). The lysate was centrifuged at 10,000 × *g* for 10 min, and the protein concentration in the supernatant was determined using the MicroBCA protocol (Pierce, Thermo Scientific). The protein lysate was separated by SDS/PAGE and then transferred to PVDF membranes (BioRad). The membranes were blocked with 5% BSA/Tris buffered saline with Tween 20 (TBS-T) and then probed with rabbit anti-PK-M1/PK-M2 primary antibodies (1:2,000) (15) for 1 h at room temperature. After washing, the blots were incubated with an anti-rabbit HRP-conjugated secondary antibody (1:5,000) (GE Healthcare) and visualized by ECL Plus Membrane Blotting detection System (GE Healthcare).

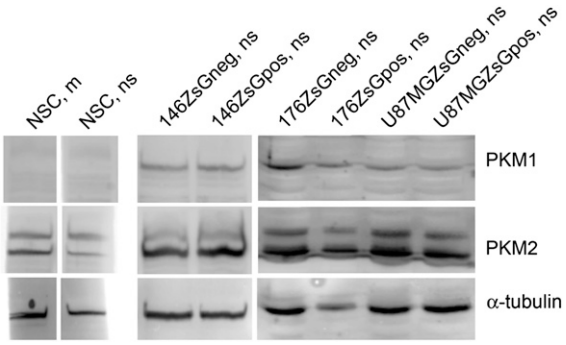

Fig. S1. Expression of PK-M1 and PK-M2 in subpopulations of glioma cells in neurospheres. GSCs and progenitor cells from GBM-146, GBM-176, and U87MG neurospheres expressed both isoforms of pyruvate kinase, PK-M1 and PK-M2. In contrast, normal neuronal stem cells (NSCs) lacked expression of PK-M1.

Table S1. Cell populations

Parameter	Monolayer cultures	Neurospheres	Differentiated cells	Progenitor cells	Stem cells
Proteasome activity	High	Low	High	Low	Very low
ZsGreen-positive cells	Mainly ZsGreen-negative, <1% positive	Mainly ZsGreen-negative, <10% positive	ZsGreen-negative	Mainly ZsGreen-negative or weakly positive	Highly positive for ZsGreen
Source	Unsorted	Unsorted	ZsGreen-negative sorted from monolayer	ZsGreen-negative sorted from neurospheres	ZsGreen-positive sorted from neurospheres