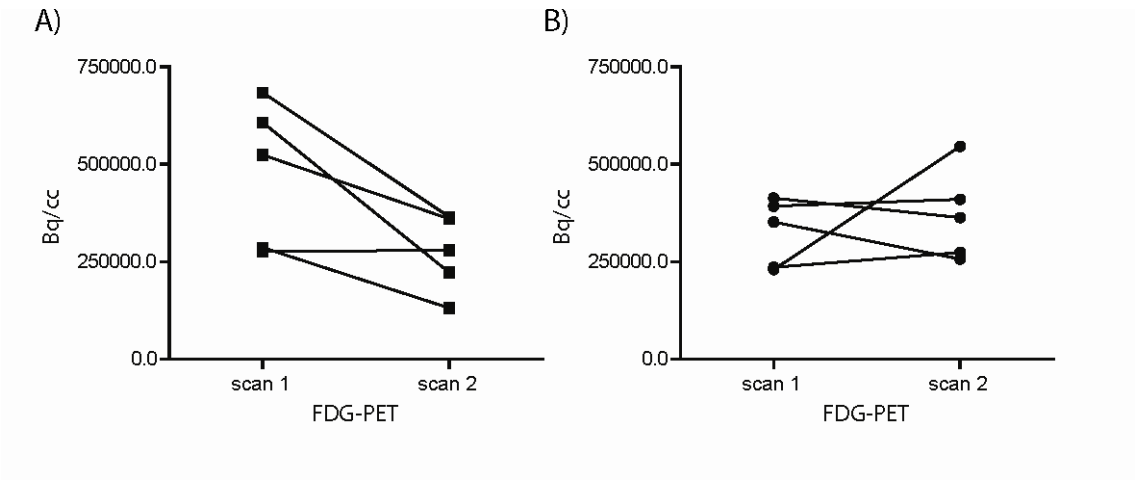
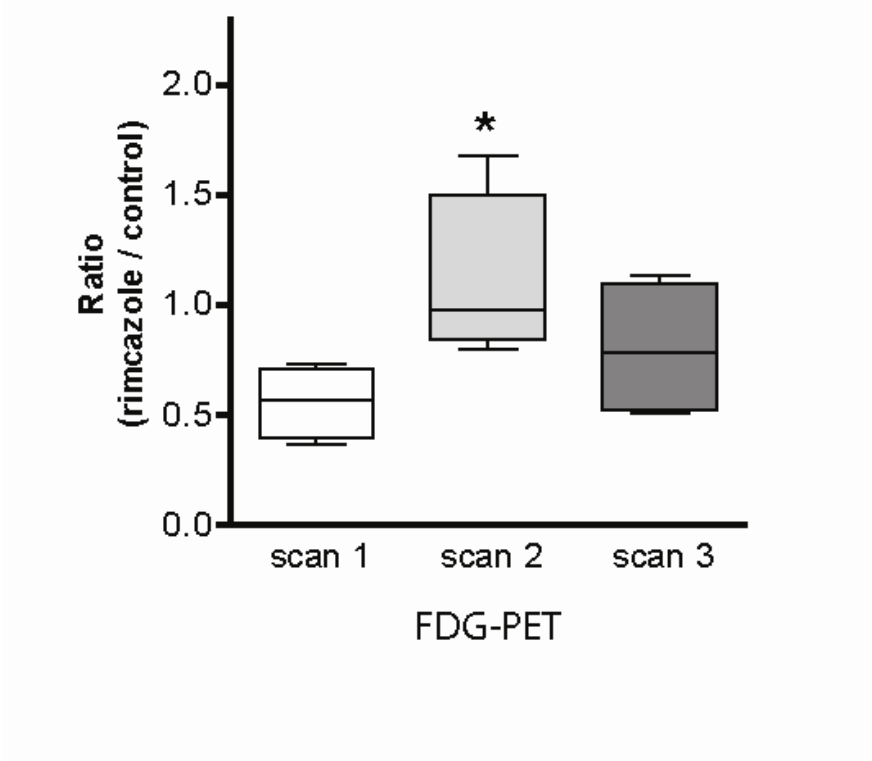
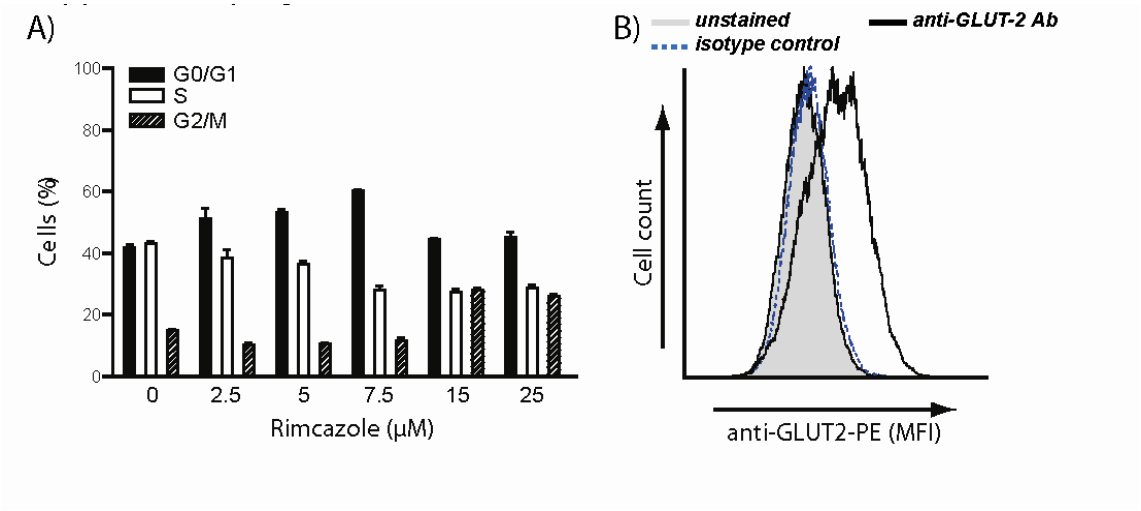


Supplemental figure 1. **Changes in ^{18}F-FDG uptake before (scan 1) and after (scan 2) treatment:** A) in each control animal; B) in each rimcazole-treated animal.



Supplemental figure 2. **Maximal ^{18}F-FDG uptake at different time points after rimcazole treatment.**



Supplemental figure 3. **FACS analysis of:** A) cell cycle distribution; and B) membrane GLUT-2 expression in rimcazole-treated A375M cells.

SUPPLEMENTAL METHODS

Culture Media and Drugs

DMEM (high-glucose) medium was purchased from Sigma. Fetal calf serum (FCS) was obtained from Bodinco (Alkmaar, The Netherlands), trypsin was a product of Invitrogen (Breda, The Netherlands), and matrigel was from Becton Dickinson (Breda, The Netherlands). Water-soluble form of rimcazole (BW234U; 9-[3-(*cis*-3,5-Dimethyl-1-piperazinyl)propyl]-9*H*-carbazole dihydrochloride) was purchased from Tocris Bioscience (Bristol, United Kingdom). Isoflurane was from Pharmachemie BV (Haarlem, The Netherlands).

Image reconstruction

Reconstructions were performed using microPET Manager 2.3.3.6 (Siemens, USA). List-mode data were processed by a static reconstruction (94 slices with a slice thickness of 0.796 mm, and an in-plane image matrix of 128 x 128 pixels of size 0.47 x 0.47 mm^3) from the period 8-52 min after tracer injection. Emission sinograms were iteratively reconstructed (OSEM2D) after being normalized, corrected for attenuation, and corrected for decay of radioactivity. A separate transmission scan (515 sec) with a Co-57 point source was acquired for attenuation correction of 511 keV photons by tissue.

Histology and Immunohistochemistry

Liver samples were formalin-fixed for 24 h, embedded in paraffin, cut into 4 μm sections and stained with hematoxylin-eosin. Liver histopathology (hepatic tissue injury and inflammation) was evaluated by an independent researcher who was blinded to all data (Dr. Arjan Diepstra, Department of Pathology, University Medical Center Groningen, The Netherlands).

Tumor samples were rapidly frozen and stored at -80˚C until further analyses. For staining, tumors were cut into 5.5 μm sections and fixed in acetone. A two-step immunoperoxidase technique was used. Sections were stained with primary antibodies: anti-Ki-67 (ab9260, Chemicon/Milipore, Amsterdam, The Netherlands) and rabbit polyclonal anti-OPRS1 (ab89655, Abcam, Cambridge, UK) at dilution 1:50 and polyclonal anti-PGRMC1 (ARP46752-P050, Aviva Systems Biology, Amsterdam, The Netherlands) at dilution 1:500, 45 min, RT. Subsequently, endogenous peroxidase was blocked with H_2O_2, followed by staining with the secondary antibody: goat-anti-rabbit-peroxidase (GaR^{PO}, P0448 dilution

1:100, 30 min, RT), and the tertiary antibody: rabbit-anti-goat-peroxidase (RaG^{PO}, P0160, dilution 1:100, 30 min, RT), both from Dako (Glostrup, Denmark). The reaction was developed using brown/red 3-Amino-9-Ethyl-Carbazole/H_2O_2. Sections were counterstained using Mayer's Hematoxylin Solution (Merck, Darmstadt, Germany). A negative control was obtained by omission of the primary antibody. The percentages of Ki67-positive tumor cells were quantified by calculating total and Ki67-positive cell number per randomly chosen microscope field in tissue section of each animal at 20x magnification. Mitotic figures in liver slices were quantified in three randomly chosen microscope fields per tissue section per animal at 20x magnification. Histological parameters were quantified using ImageJ.

Western Blotting

A375M cells were plated in 6 cm petri dishes ($1x10^6$ cells in 3 ml medium) and left to adhere overnight at 37 °C. The next day, cells were treated with rimcazole (15 and 25 μM) for the indicated times, or remained untreated. For preparation of whole cell lysates, A375M cells were washed twice in ice-cold phosphate-buffered saline (PBS), harvested and centrifuged (4 min, 1900 rpm, 4 °C). The pellet was suspended in 4× Laemmli sample buffer (8% SDS, 10% β-mercaptoethanol, 40% glycerol, 0.2 M Tris, pH 8.0) supplemented with phosphatase inhibitor cocktail II (Sigma-Aldrich, Munich, Germany) and protease inhibitor cocktail (Roche), and sonicated for 15 seconds. Protein samples were boiled for 5 min at 96 °C. Samples were separated by SDS-PAGE and transferred to nitrocellulose membranes by semi-dry blotting. Next, nonspecific binding sites were blocked (30 min) by incubation with Tris-buffered saline containing 0.05% Tween 20 and 5% dry milk. To detect the proteins of interest, membranes were incubated overnight with corresponding primary antibodies: rabbit polyclonal anti-HKII (1:1000, ab37593, Abcam, Cambridge, UK), anti-Akt and anti-phospho-Akt (Ser473) (1:3000, 9272 and 9271, respectively, Cell Signaling, Frankfurt am Main, Germany), anti-GLUT2 (1:500, 9117, Santa Cruz, Heidelberg, Germany), mouse monoclonal anti-PARP (1:1000, 556494, BD Pharmingen, Heidelberg, Germany) and α-tubulin (1:4000, MS-581P, NeoMarkers, Fremont, USA). In experiments dedicated to quantitative analysis of relative amounts of proteins, we used fluorescent secondary antibodies (1:10000), IRDye 800CW-conjugated goat-anti-rabbit (926-32211) and anti-mouse (926-32210) IgG (LI-COR, Lincoln, NE) and quantified corresponding protein bands using the LI-COR Odyssey infrared imaging system.

Flow Cytometry

A375M cells were plated at 3.0×10^4 cells/well in a 48-well plate containing 0.25 ml of culture medium and allowed to adhere overnight. Subsequently, cell medium was exchanged and the cells were concurrently treated with rimcazole in a total volume of 0.25 ml. After 20h of incubation, the cells were washed with 0.25 ml of warm PBS, incubated in 0.1 ml trypsin and re-suspended in 0.2 ml of culture medium. Supernatants and trypsinized cells were collected into a 2 ml tube and centrifuged (1800 rpm, 3 min.). The cell pellet was subsequently re-suspended in 1 ml PBS. For GLUT2 staining, cells were incubated for 1h at 4°C with rabbit polyclonal anti-GLUT2 antibody (1:100, 9117, Santa Cruz, Heidelberg, Germany) recognizing the extracellular epitope of GLUT2. This was followed by staining for 1h at 4°C with R-phycoerythrin(PE)-labeled polyclonal goat anti-rabbit IgG (1:100, sc-3739, Santa Cruz, Heidelberg, Germany). For PI staining, cells were fixed for 15 min on ice in 2.5 ml ethanol (final concentration approx. 70% v/v). Fixed cells were washed three times in PBS, where each washing step was followed by centrifugation (1500 rpm, 5 min), and stained with PI solution containing: 50 μg/ml PI, 0.01 mg/ml RNase A and 0.05% Triton X-100 in PBS (40 min, 37ºC). Samples were analyzed with FACSCalibur (Becton Dickinson). Data were plotted using CellQuest software (Becton Dickinson); at least 5,000 events were analyzed for each sample.