Appendix

Infiltrative and drug-resistant slow-cycling cells support metabolic heterogeneity in glioblastoma

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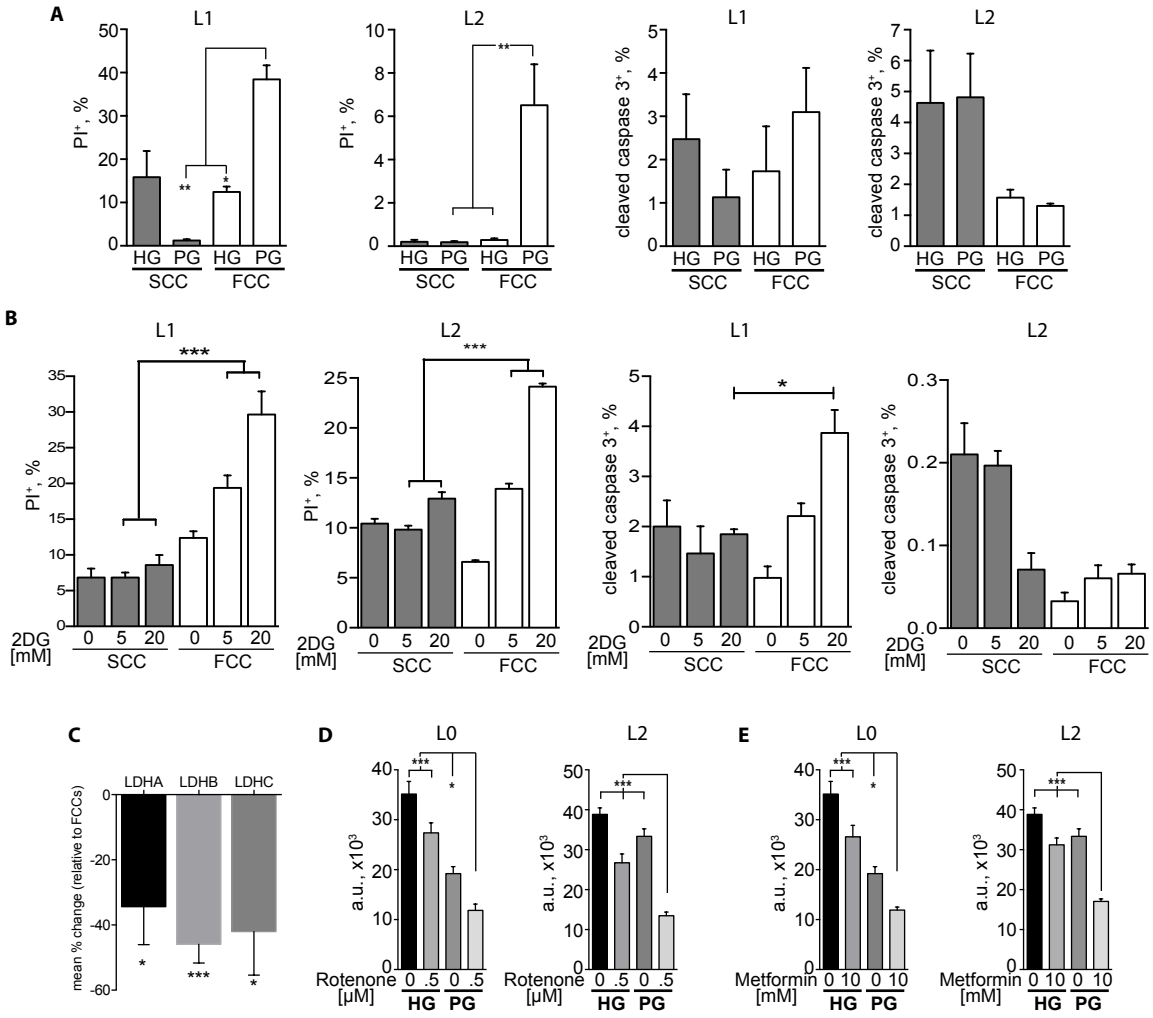
Appendix Figure Legends

Appendix Figure S1: (**A**) SCCs and FCCs were cultured in high glucose (HG, > 500 mg/dL) or physiological glucose (PG, 90-110 mg/dL) conditions for 24 hours. Cell death was quantified by flow cytometry through propidium iodide (PI) incorporation (L1, n=2; L2, n=4) (**A**) and cleaved caspase 3 expression (L1, n=2; L2, n=3) (**B**) SCCs and FCCs were cultured in 0, 5, or 20 mM 2DG for 24 hours and cell death quantified by flow cytometry through propidium iodide (PI) incorporation (L1, n=2; L2, n=4) and cleaved caspase 3 expression (L1, n=2; L2, n=3). (**C**) Using RT-qPCR, expression levels of the isoforms A, B, and C of lactate dehydrogenase were compared between SCCs and FCCs (n=3 technical replicates for each line) of the three hGBM cell lines. Changes in transcript levels are given as the mean percent change relative to FCCs. * $p < 0.05$, *** $p < 0.001$, one sample t-test. The combinatorial effect of administering glucose restriction and mitochondrial targeting with rotenone (n=16, **D**) or metformin (n=16, **E**) was measured using the CyQUANT assay after 24 hours of treatment for L0 and L2 hGBM cell lines (* $p < 0.05$, *** $p < 0.001$, one-way ANOVA with Tukey post-test, $^{\#\#}$ $p < 0.01$, t-test).

Appendix Figure S2: List of all the pathways up-regulated in the SCCs, based on metabolites that are over-represented in SCCs compared to FCCs (fold change ≥ 2).

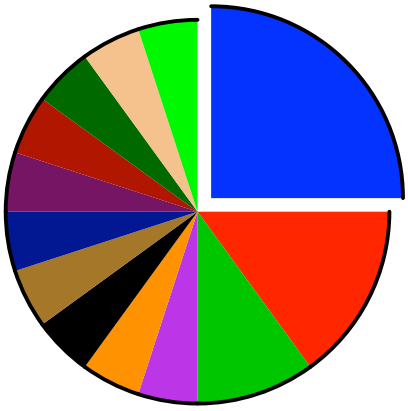
Appendix Figure S3: (**A**) Raw values of mean fluorescence intensity (MFI) in SCCs and FCCs following LipidTox staining. GSEA of SCC and FCC RNA-seq data sets for enrichment of the autophagosome-lysosome signature, as defined by Perera *et. al.* (Perera et al., 2015) (**B**) and Jegga *et. al.* (Jegga et al., 2011) (**C**). FDR, false discovery rate; NES, normalized enrichment score; Nom., nominal. Raw values of mean fluorescence intensity (MFI) in SCCs and FCCs following immunostaining for LC3B (**D**) and LAMP2 (**E**).

Appendix Figure S1



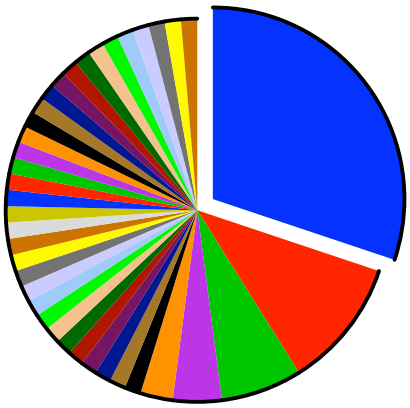
Appendix Figure S2

hGBM L0



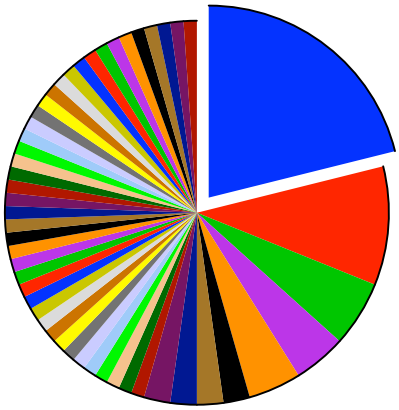
- Lipid pathway
- Pyrimidine metabolism
- purine metabolism
- Aerobic glycolysis
- Glutathione metabolism
- Oxocarboxylic acid metabolism
- Pentose phosphate metabolism
- Phenylpropanoid biosynthesis
- protein biosynthesis
- ribose metabolism
- tryptophan metabolism
- Other 1
- Other 2

hGBM L1



- Lipid metabolism
- Purine metabolism
- Pyrimidine metabolism
- Pentose metabolism
- Galactose metabolism
- Acetyl-CoA metabolism
- Aerobic glycolysis
- Oxocarboxylic acid metabolism
- Alanine, aspartate and glutamate metabolism
- Amino sugar and nucleotide sugar metabolism
- Ascorbate and aldarate metabolism
- Caffeine metabolism
- Carbohydrate metabolism
- Glucose metabolism
- Glycine, serine and threonine metabolism
- Indole diterpene alkaloid biosynthesis
- Nicotinate and nicotinamide metabolism
- Phenylalanine metabolism
- ribose metabolism
- Carcinogens
- Other 1
- Other 2
- Other 3
- Other 4
- Other 5
- Other 6
- Other 7
- Other 8
- Other 9
- Other 10
- Other 11
- Other 12
- Other 13
- Other 14
- Other 15
- Other 16
- Other 17
- Other 18

hGBM L2



- Lipid metabolism
- Purine metabolism
- Pentose metabolism
- Galactose metabolism
- Pyrimidine metabolism
- Oxidative phosphorilation
- Glutathione metabolism
- Carbohydrates metabolism
- Carcinogen
- Acetyl-CoA metabolism
- Aerobic glycolysis
- Glutamine metabolism
- CoA biosynthesis
- Alanine, aspartate and glutamate metabolism
- Amino sugar and nucleotide sugar metabolism
- Arginine biosynthesis
- Caffeine metabolism
- Caprolactam degradation
- Folate biosynthesis
- Fructose and mannose metabolism
- Glycine, serine and threonine metabolism
- Histidine metabolism
- Indole diterpene alkaloid biosynthesis
- Metabolism of xenobiotics by cytochrome P450
- Nicotinate and nicotinamide metabolism
- Oxocarboxylic acid metabolism
- Phenylalanine metabolism
- Phosphonate and phosphinate metabolism
- Propanoate metabolism
- Starch and sucrose metabolism
- Other 1
- Other 2
- Other 3
- Other 4
- Other 5
- Other 6
- Other 7
- Other 8
- Other 9
- Other 10
- Other 11
- Other 12
- Other 13
- Other 14
- Other 15
- Other 16
- Other 17
- Other 18
- Other 19
- Other 20

Appendix Figure S3