Sexual dimorphism in glioma glycolysis underlies sex differences in survival

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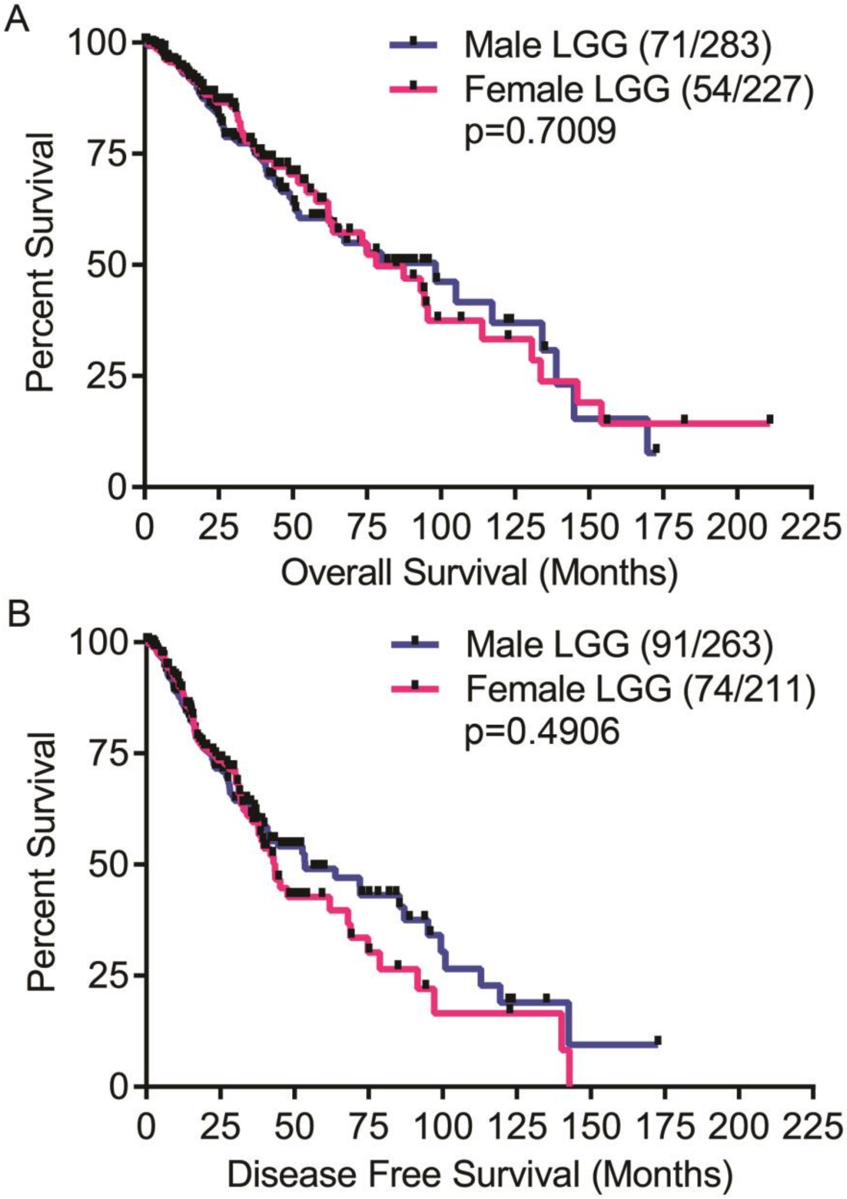
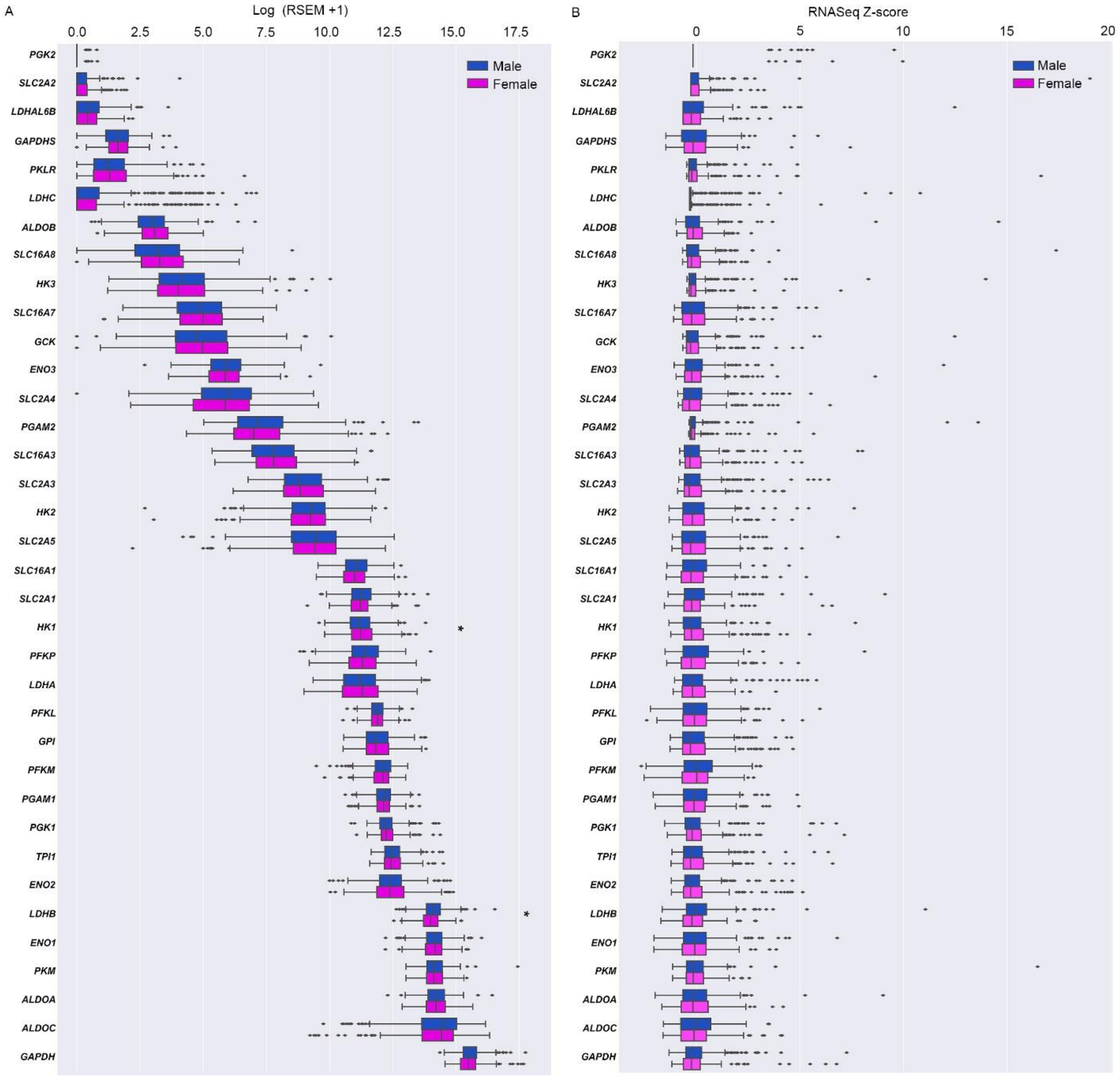
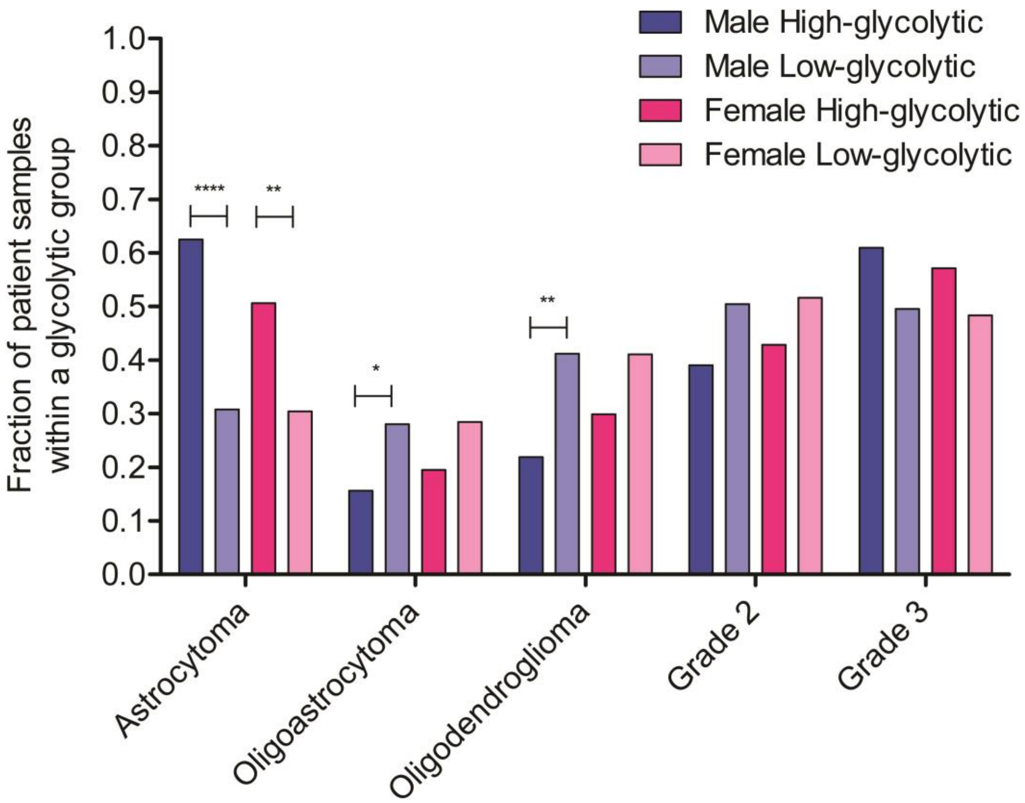


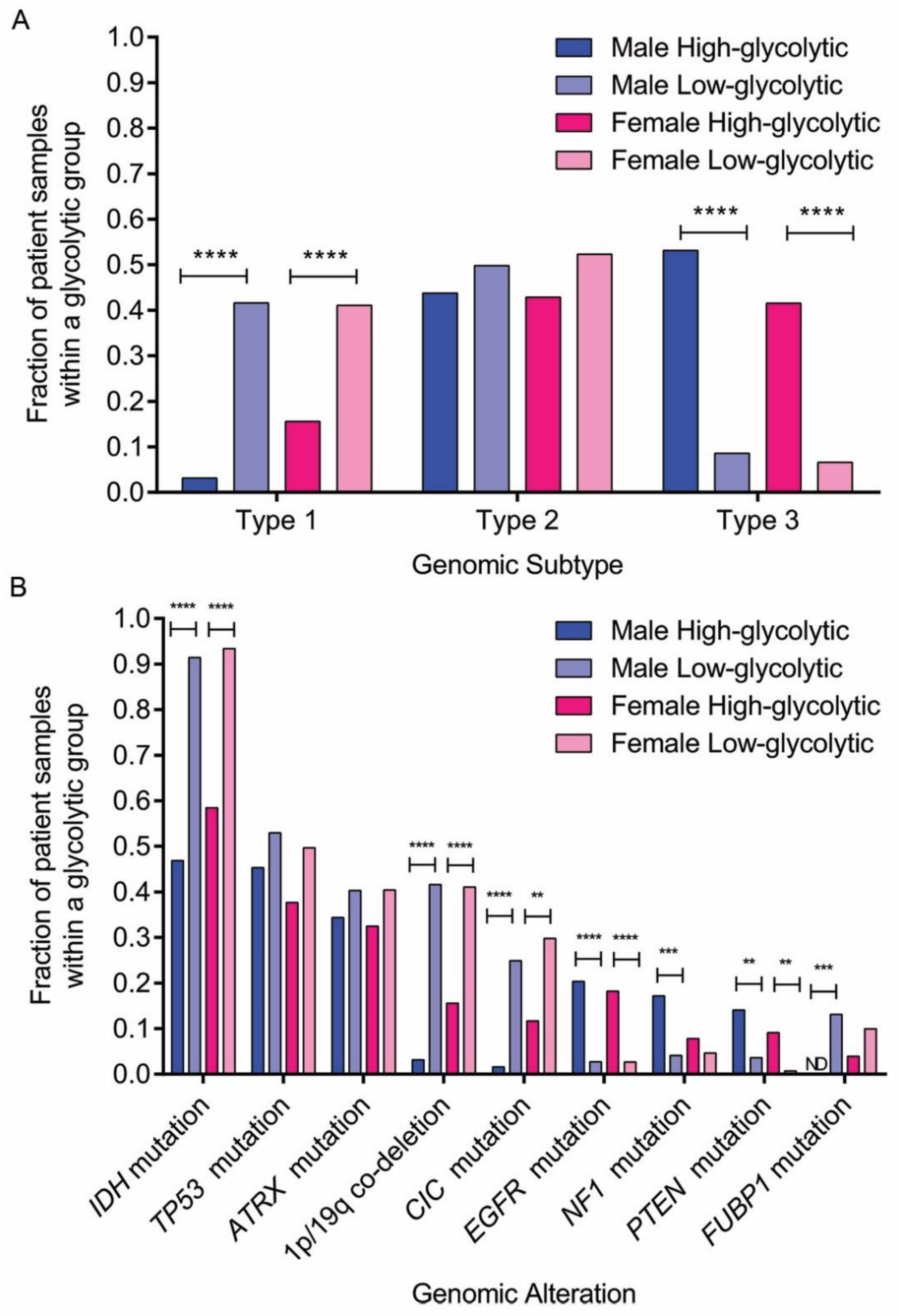
Supplemental Figure 1. Sex-based overall survival and disease free survival analyses of TCGA LGG Datasets. There are no sex-differences in **A.** overall survival (OS) or **B.** disease free survival (DFS). Significance calculated using the log-rank test.



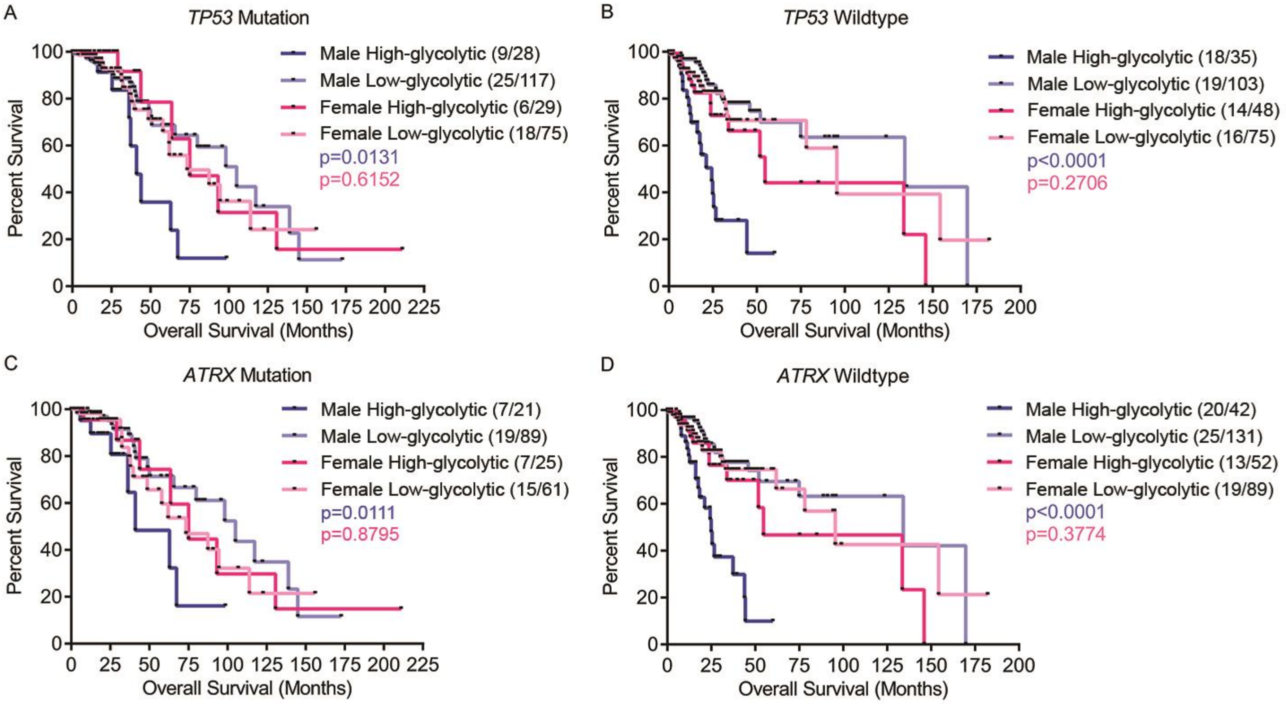
Supplemental Figure 2. There are few sex differences in expression of 36 glucose metabolism genes involved in hexose transport, glycolysis, and monocarboxylate transport. A. Male (blue) and female (pink) RSEM values are plotted together for each gene. Only 2 genes (*HK1* and *LDHB*) display subtle but statistically significant differences in expression between males and females. Genes arranged from lowest to highest median RSEM expression. **B.** Sex-specific Z-score transformation of RSEM values reveals no sex differences in gene expression. Significance calculated using two-tailed t-test, * $p<0.05$. Data are represented as the mean ± 95% C.I.



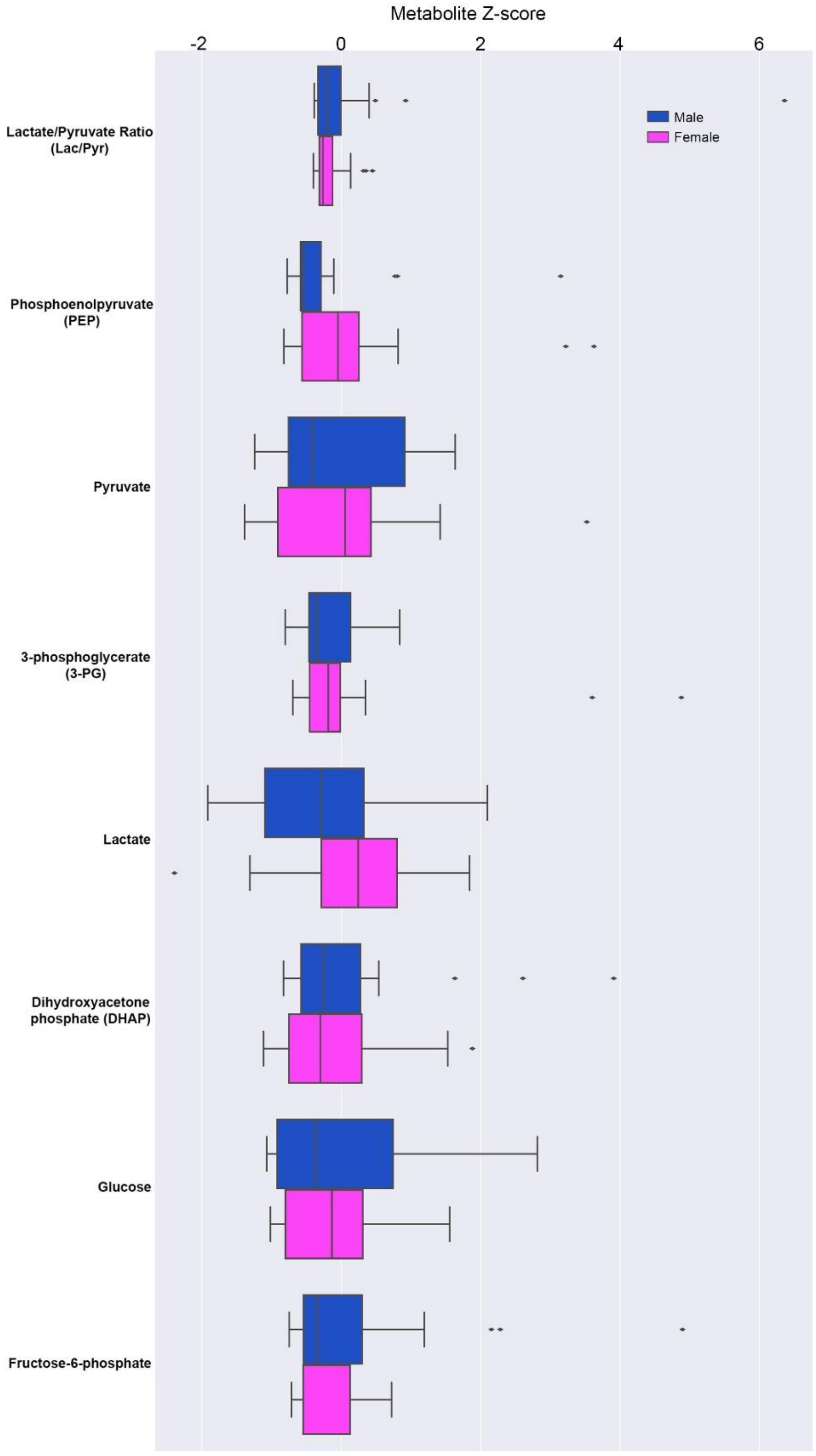
Supplemental Figure 3. Glycolytic tumor samples are enriched for astrocytomas. Enrichment of histopathologic and WHO grade classifiers were assessed in the glycolytic and non-glycolytic groups. Oligoastrocytomas and oligodendrogliomas are less abundant in the glycolytic group where astrocytomas are enriched in the glycolytic group. Significance calculated using the Fisher exact test. *p<0.05, **p<0.01, ****p<0.0001.



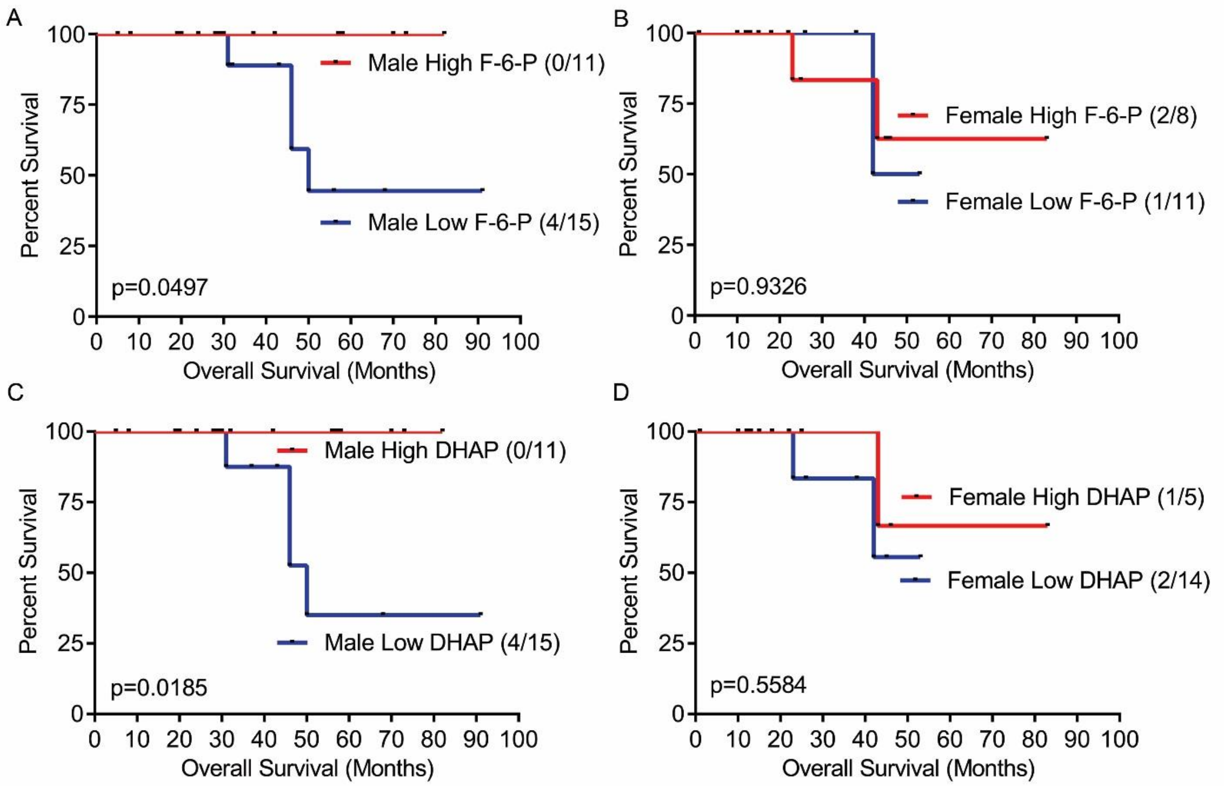
Supplemental Figure 4. Glycolysis-based stratification correlates with genomic alterations in gliomas. A. Progression from the genomic type 1 (1p/19q co-deletion/ *IDH* mutant) LGG to the more aggressive genomic type 3 (*IDH* wildtype) LGG enriches for the glycolytic tumor classifier. **B.** Genomic alterations significantly depleted in the glycolytic group include *FUBP1*, *CIC*, *IDH* mutations and 1p/19q co-deletion). Alterations significantly enriched in the glycolytic group include *PTEN*, *EGFR*, and *NF1* mutations. *TP53* and *ATRX* were not enriched in either group. Significance calculated using the two-tailed Fisher exact test. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.



Supplemental Figure 5. Glycolysis stratifies males independently of *TP53* and *ATRX* status in gliomas. A. *TP53* mutant samples. **B.** *TP53* wildtype samples. **C.** *ATRX* mutant samples. **D.** *ATRX* wildtype samples. p-values were calculated using the log-rank test. Numbers in parentheses refer to number of deaths / total patients in that group.



Supplemental Figure 6. Glycolytic metabolites are not significantly different between males and females with grade 2 gliomas. p-values were calculated using the two-tailed Student's, t-test. Data are represented as the mean ± 95% C.I.



Supplemental Figure 7. Glycolytic metabolites stratify males but not females with grade 2 gliomas. Fructose-6-phosphate (F-6-P) stratifies **A.** male but not **B.** female gliomas. Dihydroxyacetone phosphate (DHAP) stratifies **C.** male but not **D.** female gliomas. p-values were calculated using the log-rank test. Numbers in parentheses refer to number of deaths / total patients in that group.

Supplemental Table 1. Mean gene expression Z-score values for 36 glycolytic genes in male and female K-means clustering groups.

Gene	Mean Male Cluster 1 (N=253)	Mean Male Cluster 2 (N=32)	Mean Female Cluster 1 (N=201)	Mean Female Cluster 2 (N=27)
PGK1	-0.251090882	1.985187288	-0.214404549	1.601263323
GPI	-0.224738361	1.776837669	-0.257712031	1.941684189
LDHA	-0.224635066	1.776020994	-0.129156407	0.997340487
TPI1	-0.217632393	1.720656108	-0.180050541	1.357309715
SLC2A3	-0.20728892	1.638878024	-0.229728886	1.732651284
HK1	-0.196008634	1.549693261	-0.274679296	2.059720466
ENO2	-0.184465253	1.458428409	-0.283740943	2.105656386
ENO1	-0.180196439	1.424678093	-0.099304355	0.78629729
GAPDH	-0.174307468	1.378118418	-0.11417666	0.858473082
ALDOA	-0.161913515	1.280128726	-0.203041727	1.541344557
PKM2	-0.160385865	1.268050746	-0.17233714	1.276187244
PGAM1	-0.132677239	1.048979423	-0.221259066	1.650484798
PKLR	-0.128093139	1.012736379	-0.087614677	0.659159434
SLC16A3	-0.127423129	1.007439117	0.039009844	-0.261043457
PFKP	-0.126394921	0.999309842	-0.260120558	1.972898623
HK3	-0.110588762	0.874342398	0.028824933	-0.195899608
PFKL	-0.095587628	0.755739686	-0.069100829	0.53573822
SLC2A1	-0.073610802	0.581985403	-0.015020757	0.073874619
SLC16A8	-0.072578737	0.573825636	-0.104359164	0.784722618
GCK	-0.055825886	0.441373408	-0.015307403	0.137992407
HK2	-0.054148047	0.428107994	0.074621381	-0.516890009
SLC2A2	-0.051389924	0.40630159	0.010698048	-0.062200703
SLC16A1	-0.027820355	0.219954681	0.083839343	-0.605444771
PGK2	-0.019876106	0.157145468	-0.001724718	0.01899839
SLC16A7	-0.015353848	0.121391357	-0.076115078	0.505629845
ALDOB	-0.000796865	0.006300211	-0.068786178	0.533986296
LDHAL6B	-5.22E-06	4.13E-05	-0.008959949	0.096456034
LDHC	0.000532518	-0.004210218	0.006328065	-0.033848191
PFKM	0.000552602	-0.004369011	-0.104045365	0.748761714
SLC2A5	0.005684456	-0.044942731	0.073780488	-0.517104992
SLC2A4	0.010205535	-0.080687513	0.035009697	-0.234868712
LDHB	0.01382981	-0.109341938	0.061729914	-0.467884066
PGAM2	0.014342112	-0.113392322	0.045690772	-0.327183814
ALDOC	0.021763087	-0.172064409	-0.01686641	0.15647868
GAPDHS	0.030218655	-0.238916244	-0.002435995	0.055912094
ENO3	0.050185012	-0.396775247	0.068148665	-0.472910228

Supplemental Table 2. Optimization of Z-score sliding threshold for sex differences in glycolysis. NA: Could not be defined due to insufficient sample size or lack of definable median OS. Significant p-values are bolded with asterisks.

Male					
Z-score	**Median OS (months) Z-score > threshold**	**Median OS (months) Z-score < threshold**	**Difference in median OS**	**Log-rank p value**	**Log-rank Hazard Ratio (HR)**
0	98.16	NA	NA	NA	NA
0.25	98.16	NA	NA	NA	NA
0.5	79.93	NA	NA	0.3294	NA
0.75	79.93	138.93	-59	0.0625	0.2953
1	74.97	138.9	-63.93	**0.0307***	2.366
1.25	74.97	138.9	-63.93	**0.0481***	1.968
1.5	74.97	138.9	-63.93	**0.0053****	2.255
1.75	62.91	138.9	-75.99	**0.0018****	2.46
2	62.91	105.1	-42.19	**0.001****	2.044
2.25	50.1	105.1	-55	**0.0031****	1.984
2.5	62.91	105.1	-42.19	**0.0357***	1.633
2.75	62.91	98.16	-35.25	0.0821	1.422
3	62.91	98.16	-35.25	0.0703	1.423
Female					
Z-score	**Median OS (months) Z-score > threshold**	**Median OS (months) Z-score < threshold**	**Difference in median OS**	**Log-rank p value**	**Log-rank Hazard Ratio (HR)**
0	78.15	NA	NA	NA	NA
0.25	78.15	NA	NA	0.7421	NA
0.5	78.15	NA	NA	0.5285	NA
0.75	78.15	NA	NA	0.288	2.737
1	78.15	NA	NA	0.1333	2.801
1.25	75.1	114	-38.9	0.4169	1.418
1.5	75.1	114	-38.9	0.4953	1.269
1.75	75.1	94.45	-19.35	0.9541	0.9828
2	78.15	94.45	-16.3	0.9538	1.017
2.25	78.15	94.45	-16.3	0.3769	0.7872
2.5	87.39	78.15	9.24	0.4323	0.8079
2.75	93.13	73.42	19.71	0.491	0.8262
3	93.13	73.42	19.71	0.507	0.8459

Supplemental Table 3. Survival analysis of significant glycolytic Genes using the Z = 1.75 threshold. Undefined: lack of definable median OS. Significant p-values are bolded with asterisks.

Male				
Gene	**Median OS (Z>1.75)**	**Median OS (Z<1.75)**	**Log-rank p value**	**HR (95% CI)**
GAPDH	36.33	105.1	__< 0.0001****__	4.078 (4.661 to 59.90)
LDHA	18.43	98.16	__< 0.0001****__	6.321 (25.33 to 720.8)
PGK1	24.38	98.16	__0.0002***__	4.199 (4.277 to 105.6)
SLC16A3	24.38	98.16	__< 0.0001****__	4.803 (6.639 to 203.0)
HK3	26.74	98.16	__0.0004***__	6.248 (10.13 to 2672)
PFKL	24.38	98.16	__0.0018**__	3.789 (2.781 to 79.71)
SLC2A5	37.35	98.16	__0.0485*__	2.261 (1.013 to 11.12)
GCK	22.84	98.16	__< 0.0001****__	6.12 (15.92 to 1097)
GPI	24.9	98.16	__0.0005***__	3.645 (3.007 to 47.82)
PGAM2	28.94	98.16	__0.043*__	3.088 (1.076 to 55.77)
SLC16A8	21.29	98.16	__< 0.0001****__	7.287 (22.03 to 9022)
Female				
Gene	**Median OS (Z>1.75)**	**Median OS (Z<1.75)**	**Log-rank p value**	**HR (95% CI)**
GAPDH	Undefined	78.15	0.2576	undefined
LDHA	133.6	78.15	0.4288	0.7023 (0.3199 to 1.589)
PGK1	Undefined	78.15	0.8706	0.8506 (0.1374 to 5.345)
SLC16A3	51.84	93.13	0.1017	1.913 (0.8468 to 6.650)
HK3	33.94	93.13	__0.0073**__	3.264 (1.796 to 40.33)
PFKL	Undefined	78.15	0.3386	undefined
SLC2A5	63.5	93.13	0.6428	1.22 (0.4967 to 3.120)
GCK	29.11	87.39	0.181	1.834 (0.6954 to 7.571)
GPI	75.1	146	0.2405	0.441 (0.2075 to 1.479)
PGAM2	54.73	87.39	0.4424	1.723 (0.3308 to 12.73)
SLC16A8	75.1	93.13	0.1508	0.2626 (0.1565 to 1.321)

Supplemental Table 4. Co-expression of the 11 significant glycolytic transcripts in male and female samples

Transcript Number co-expressed in the same sample	Number of Male Samples	Number of Female Samples
0	220	150
1	38	38
2	10	23
3	3	8
4	7	7
5	1	0
6	2	1
7	1	0
8	1	0
9	0	0
10	0	0
11	0	0