Supplemental Information

FOXN3 regulates hepatic glucose utilization

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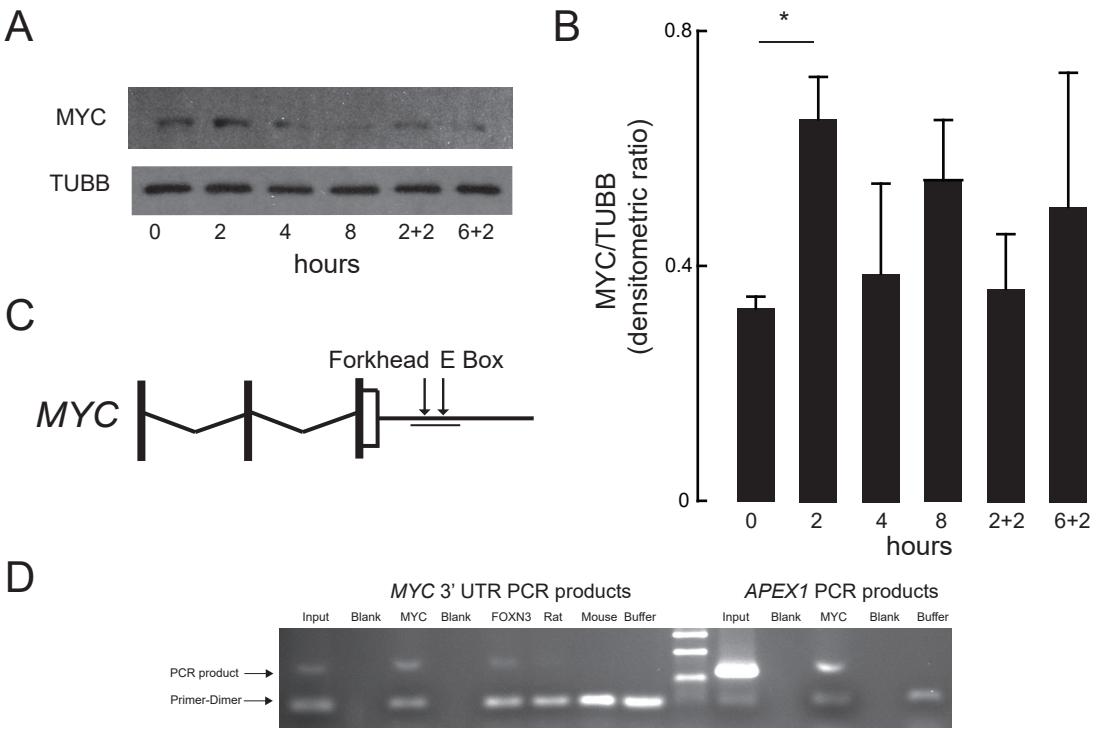
Figure S1. Architecture and population genetics of the rs8004664 SNP in the *FOXN3* gene, Related to Figure 1. Linkage disequilibrium (r^2 values) plots for the 100 kb flanking the rs8004664 are shown for four populations that contributed to the 1000 Genomes project (rendered on the ENSEMBL browser). In none of these populations is this allele in obvious, strong linkage disequilibrium with distant surrounding SNPs, pointing to this intronic region as the causal site.



E

atgataacagccagagttgacagttagaaggaatggcagaaggcaggtgagaaggtgagaggtaggcaaaggagatacaagaggtcaaa
ggtag**cagttaagtacacaaaga**ggcataaggactggggagttgggaggaaggtgaggaagaaactcctgttactttagttaaccagtg
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ttgaacccaggctgtttactcctaacctgtccaagccacctctcagacgacggtag

F

gcttcactacatctttcctaaaagacatcatctttcatattgcttttggtgtacctgctgggcaaaaatatgtctccttcccttcagtg
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tgtcagtcaagaaaaaagttttagtgtcagtcattagtgttgtttacaatcaatattaccaagttaatgaaacttaatcgacaatatgt
gatttatatttatacataagcaccaccagttagtttataagtgttttgtgtaataaaagatgaagttg

Figure S2. FOXN3 binds a Forkhead sequence in the 3'-UTR of *MYC*, Related to Figure 4. (A,B) Immunoblot and densitometric analysis was performed for human MYC for the samples shown in Figure 2B (the TUBB immunoblot is reproduced here for clarity). Where indicated, samples were returned to complete medium for the indicated time ('+'). All times are in hours. Mean ± s.e.m. values are shown. **P* = 0.05 in a 2-sided Student's *t*-test. (C) Structure of the human *MYC* gene (~5 kb shown). A Forkhead binding site and a MYC binding site (E box) are located distal to the 3'-untranslated region (3'UTR) were identified using the ENCODE database. The target PCR product for the ChIP assay is underlined. (D) The gel shows a PCR products from DNA templates precipitated from chromatin extracted from HepG2 cells with the indicated antibodies (MYC or FOXN3), antisera (Rat and Mouse) or controls (input and buffer). As a positive control, ChIP was performed on an additional MYC target gene *APEX1*. (E) Human *MYC* sequences distal to the 3'-UTR where the Forkhead binding site (blue) and E-box (red) were identified in the ENCODE database. (F) Zebrafish *mycb* sequences distal to the 3'-UTR where the Forkhead binding site (blue) and E-box (red) were identified with JASPAR modeling and then validated in Figure 4.

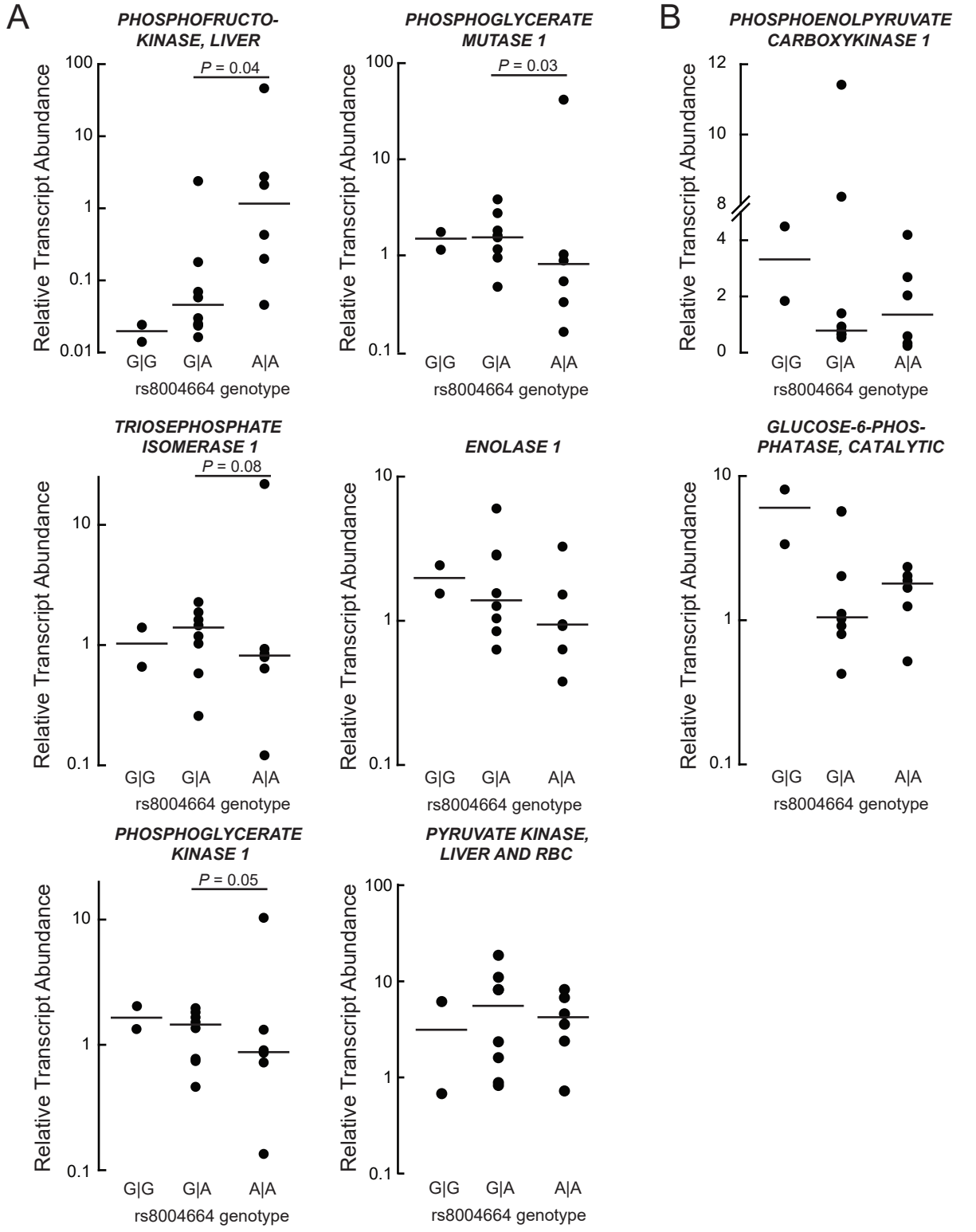

Figure S3. Glycolytic and gluconeogenic gene transcript abundance in the primary human hepatocytes samples, Related to Figure 5. Steady-state transcript abundance of the indicated glycolytic (A) and gluconeogenic (B) transcripts in cryopreserved human hepatocytes from donors with the indicated rs8004664 genotypes, G|G (n=2), A|G (n=8), and A|A (n=6). Horizontal lines indicate the median value. For *PHOSPHOGLYCERATE KINASE 1*, a 1-tailed Student's *t*-test and an outlier test were performed. For *PHOSPHOGLYCERATE MUTASE 1*, a 2-tailed Student's *t*-test and an outlier test were performed. For *TRIOSEPHOSPHATE ISOMERASE*, a 2-tailed Student's *t*-test and an outlier test were performed. FOR *PHOSPHOFRUCTOKINASE, LIVER,* a 2-tailed Student's *t*-test and an outlier test were performed.

Table S1. Phenotypic characterization of the 16 human hepatocyte samples studied, Related to Figures 1 and 4.

Lot Number	Age (years)	Sex	Body mass index (kg/m^2)	rs8004664 genotype	Transcript abundance (normalized to *GAPDH*)		
					FOXN3-T003	***FOXN3-T004***	***MYC***
ZBH1665	15	Male	17.6	G|G	0.015	0.518	8.697
ZBH2347-P10	1.4	Female	19.2	G|G	0.046	0.787	4.654
ZBH0614	54	Male	25.8	G|A	0.246	0.250	2.485
ZBH0129	51	Male	36.6	G|A	0.046	0.421	3.055
HUM4058	67	Female	28	G|A	1.271	0.808	2.101
ZBH0486	14	Female	20.7	G|A	0.046	0.617	7.987
HUM4035	51	Female	33	G|A	1.278	0.247	2.271
ZBH1835 U10	17	Female	23.5	G|A	0.068	0.318	12.831
ZBH012513	51	Female	33.5	G|A	0.267	0.901	15.504
HUM4060B	23	Male	18	G|A	0.978	0.434	94.252
HUM4024	77	Male	26	A|A	0.715	0.901	1.003
HUM4069C	30	Male	25	A|A	0.652	0.455	0.668
ZBH1989-P	25	Female	26.1	A|A	1.041	0.371	4.660
HUM4031	53	Female	27	A|A	0.842	0.742	1.377
HUM4048	43	Female	23	A|A	1.729	23.637	0.369
HUM4050	42	Female	29.1	A|A	1.358	0.362	0.622

Table S2. Primer sequences, Related to Figures 1, 2, and 4.

Primers for quantitative PCR			
Gene	**Forward primer**	**Reverse Primer**	**Reference**
		Zebrafish	
aldoaa	CTGCCAGGAGGAGTTCATAAAG	CCTTATCTCCCGAGGACACATA	
ef1a	TTGAGAAGAAAATCGGTGGTGCTG	GGAACGGTGTGATTGAGGGAAATTC	(Karanth et al., 2009)
eno1a	CTTCATCGCTGACCTTGTAGTC	TCAGTGCTTCAGATGGGTTTC	
gck	TGCCAGGAGGAGCTAAAGA	CTGCCTTCTTCTGACTGGATAAA	
gludb1	CAACGGAGAATGGGAGGTTATC	AACCTCATCCACAGACACATC	
*foxn3*_T002	GTGACGACGAGGAGATGAAAG	CGTCCGGTTGGTGATATTGT	
*foxn3*_T201	GCTACCTGTCTCTGTCTGATTC	CTCCTTCGCCTTTGTTTGTTT	
myca	TCCTGGACACTCCACCTAAC	CTCTTCTTCCTCCTCCTCTTCT	
mycb	CCACAACACTTCCACTGGATAC	CCACGTCAATTTCTTCCTCCTC	
pck1	GCTGCTGAACACAAAGGTAAAG	CTGACCGAAGTTATAGCCGAAG	
pfklb	CACAGACTTTGAGCACAGGA	TCGTCGAAACTGGTCTGATATTT	
pgam1b	AAACACCTGGAGGGAATGTC	CATAGGCTTCACTGGCTTCA	
pgk1	AAATGGCTGCATCACCATTATC	CTCTGGCAGAAGAGCCTTAAA	
slc2a2	CCACGTCCAGCAGCTATT	GCTTCCACAGAGACTCACTAAA	
tpi1a	ACTCTGTGCGCATCATCTAC	TTTCAGAGCGTCTGGTTGAG	
		Human	
ALDOA	GTTGTGGGCATCAAGGTAGA	GCTCCGTCCTTCTTGTACTG	
APEX1	ACGAACAACCCAGAACCAAG	CTAAGCCAGAGACCCTCACG	(Barrilleaux et al., 2013)
ENO1	TGCCCTGGTTAGCAAGAAAC	GCGTTCGCACCAAACTTAGA	
*FOXN3*_T003	CAGGCATATCAAAGCACATCAG	AATGTCAGGATCTTGGAGAAG	
*FOXN3*_T004	CCTTCTCCAAGTTCCTCCAGGA	ACAAGATGGCTCACTCTCAGTC	
GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG	(Cicinnati et al., 2008)
GCK1 M1	GCGGAGAAGCCTTGGATATT	ATCTGCTCTACCTTCTCCTTCT	
G6PC	GGCTCAACCTCGTCTTTAAGTG	CTCCCTGGTCCAGTCTCACA	(Zhang et al., 2014)
HMBS	TGCAACGGCGGAAGAAAA	ACGAGGCTTTCAATGTTGCC	(Cicinnati et al., 2008)
PCK1	ACGGATTCACCCTACGTGGT	CCCCACAGAATGGAGGCATTT	(Zhang et al., 2014)
PFKL	AAGGCATGAACGCTGCT	CCCTCATAGCCCTCGTAGAT	
PGAM1	CATCTGGAGGGTCTCTCTGAA	AACTGCATGGGCTTGATAGG	
PGK1	CTGGACAAGCTGGACGTTAAA	TTGGGACAGCAGCCTTAATC	
PKLR	GGCATTGAAAGTGGAAAGCTC	CCCGCATGATGTTGGTGTA	
TPI	AAGATGAACGGGCGGAAG	GCGAAGTCGATATAGGCAGTAG	

Primers for genotyping SNP rs8004664	
Forward primer	**Reverse Primer**
GGTCTTTGCACAGGCTCT	ACATTATGCTAGTTGTTGGGTTATG

Primers for Transgenesis		
Gateway multisite cloning - middle Entry clone (pME)	**Primers**	
zebrafish *foxn3*	*att*B1	GGGGACAAGTTTGTACAAAAAAGCAGGCTACTGTGGTCACTGCTGAA
	*att*B2	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTTTTGCTCTTTCTGCCCCTT
Human *FOXN3*	*att*B1	GGGGACAAGTTTGTACAAAAAAGCAGGATGGGTCCAGTCATGCCTCCCAGT
	*att*B2	GGGGACCACTTTGTACAAGAAAGCTGGGTATTTTTTGTGGTTTCCTTTTGC

Chromatin Immunoprecipitation-DNA primers		
	Forward primer	**Reverse Primer**
Forkhead binding sites near *MYC* 3'UTR	CAGCCAGAGTTGACAGTTAG	TTGTGAAGGCAGCAGAAG
Forkhead binding sites near *mycb* 3'UTR	AGGTCCTGTTAACTTGGTATCAC	TGACAGCAGACCAGTTTACTTAT

Supplementary References

Cicinnati, V.R., Shen, Q., Sotiropoulos, G.C., Radtke, A., Gerken, G., and Beckebaum, S. (2008). Validation of putative reference genes for gene expression studies in human hepatocellular carcinoma using real-time quantitative RT-PCR. BMC Cancer *8*, 350.

Karanth, S., Lall, S., Denovan-Wright, E., and Wright, J. (2009). Differential transcriptional modulation of duplicated fatty acid-binding protein genes by dietary fatty acids in zebrafish (Danio rerio): evidence for subfunctionalization or neofunctionalization of duplicated genes. BMC Evol Biol *9*, 219.

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