Supplementary Materials

Supplementary Figure 1

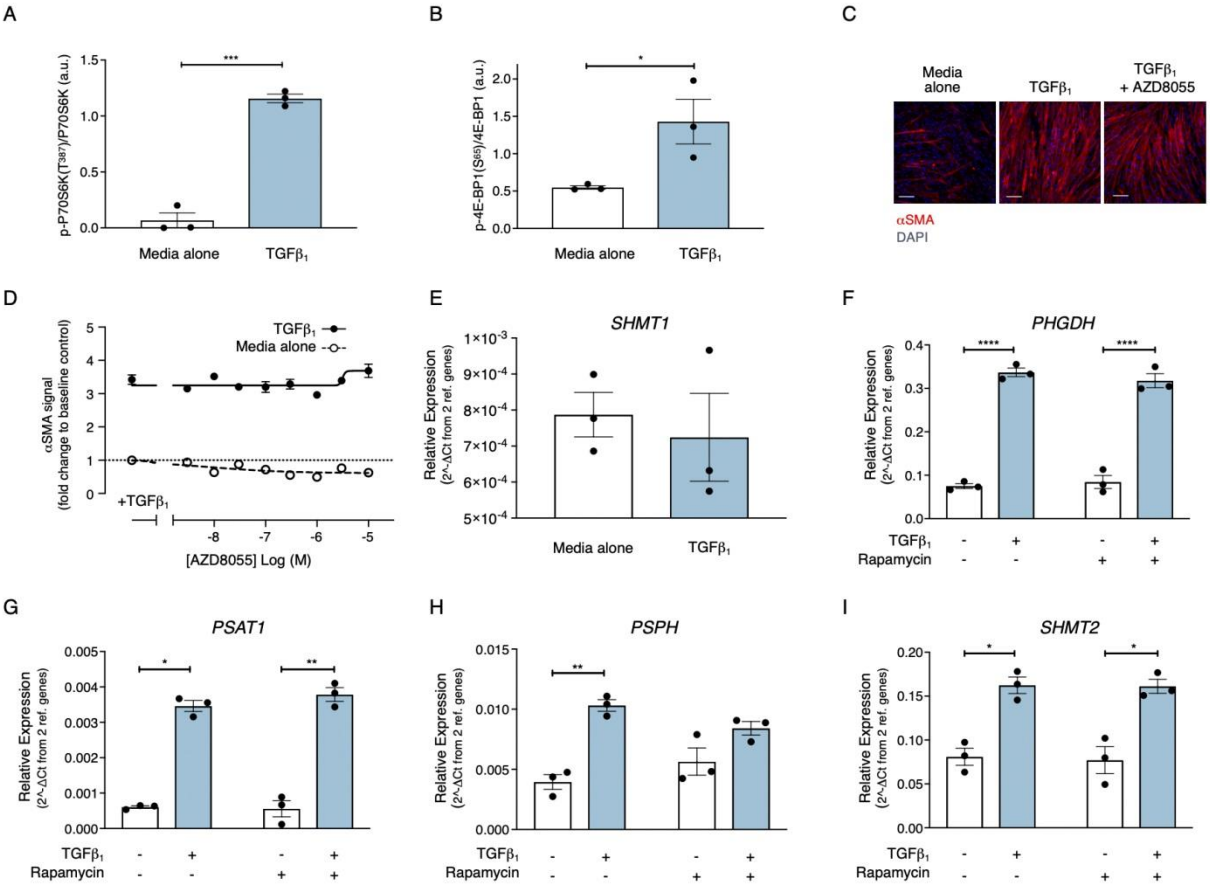

fig. S1. TGF-β_1 induced α-SMA expression is insensitive to AZD8055 treatment and the TGF-β_1 induced serine-glycine biosynthetic pathway is insensitive to rapamycin treatment

(**A-B**) Confluent pHLFs were incubated with or without TGF-β_1 for 1 hour followed by immunoblotting and densitometric quantification of p-p70S6K relative to p70S6k (Thr^{389})(**A**) and p-4E-BP1 relative to 4E-BP1(Ser^{65})(**B**), N=3 biological replicates and data are representative of 3 independent experiments. (**C-D**) Confluent pHLFs were pre-incubated with vehicle (DMSO) or increasing concentrations of AZD8055 and stimulated for 48 hours with TGF-β_1. α-SMA stress fiber formation was assessed by high content imaging. Immunofluorescence images are shown as indicated (Scale bar=100μm) (**C**) and (**D**) N=3 biological replicates and data are representative of 3 independent experiments. (**E**) *SHMT1* relative mRNA abundance was measured by RT-qPCR at 24 hours following incubation with media plus TGF-β_1 or media alone (N=3 biological replicates). (**F–I**) Confluent pHLFs were

pre-incubated with rapamycin or vehicle (DMSO) prior to TGF-β_1 incubation or media alone. After 24 hours, the relative mRNA abundance of *PHGDH, PSAT1*, *PSPH* and *SHMT2* were measured by RT-qPCR (N=3 biological replicates and data are representative of 3 independent experiments). Differences between groups were evaluated by unpaired t-test (A,B,E) or two-way ANOVA (F-I) with Tukey post-hoc testing. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p< 0.0001$.

Supplementary Figure 2

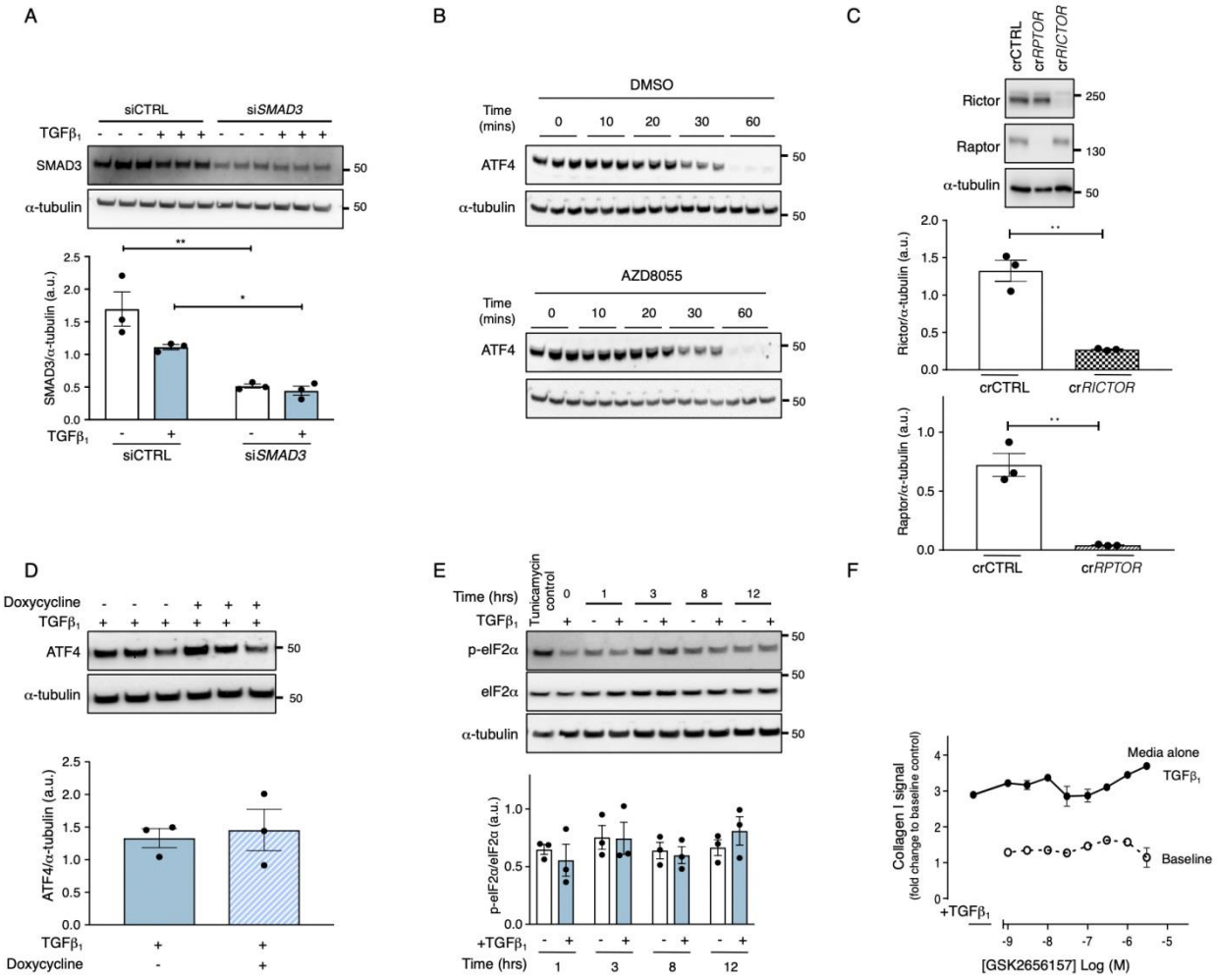

fig. S2. Demonstration of successful gene and protein manipulation and evidence that TGF-β_1 stimulation in fibroblasts is not associated with PERK activation

(**A**) Confluent pHLFs were transfected with scrambled control siRNA (siCTRL) or Smad3 siRNA (si*SMAD3*) before incubation with or without TGF-β_1 for 24 hours. Representative immunoblot and densitometric quantification for Smad3 are shown (N=3 biological replicates). (**B**) Confluent pHLFs were treated with TGF-β_1 for 13 hours and lactimidomycin was added followed by vehicle (DMSO) or AZD8055. Immunoblotting for ATF4 and α-tubulin was performed at indicated time points over the subsequent hour. (**C**) pHLFs were modified by CRISPR-Cas9 gene editing of *RPTOR* or *RICTOR* before being incubated in media alone for 24 hours. Immunoblot and densitometric quantification of Raptor and Rictor are shown (N=3 biological replicates). (**D**) Confluent pHLFs were treated with doxycycline or media alone for 24 hours prior to TGF-β_1 stimulation. Immunoblot for ATF4 and densitometric quantification were performed at 24 hours (N=3 biological replicates). (**E**)

Confluent pHLFs were incubated with or without TGF-β_1 and immunoblotting and densitometric quantification for phosphorylated p-eIF2α and total-eIF2α were performed at indicated time points. Tunicamycin was added as a positive control and lysates collected at 12 hours post TGF-β_1 stimulation (N=3 biological replicates). (**F**) Confluent pHLFs were pre-incubated with vehicle (DMSO) or the PERK inhibitor GSK2656157 and stimulated for 48 hours with or without TGF-β_1 and collagen deposition assessed by high content imaging. N=3 biological replicates per condition and data are representative of 3 independent experiments. Difference between groups were assessed by two-way ANOVA (A,E) with Tukey post-hoc test or unpaired T-test (C,D). *p<0.05, **p<0.01, ***p< 0.001, ****p< 0.0001.

Supplementary Figure 3

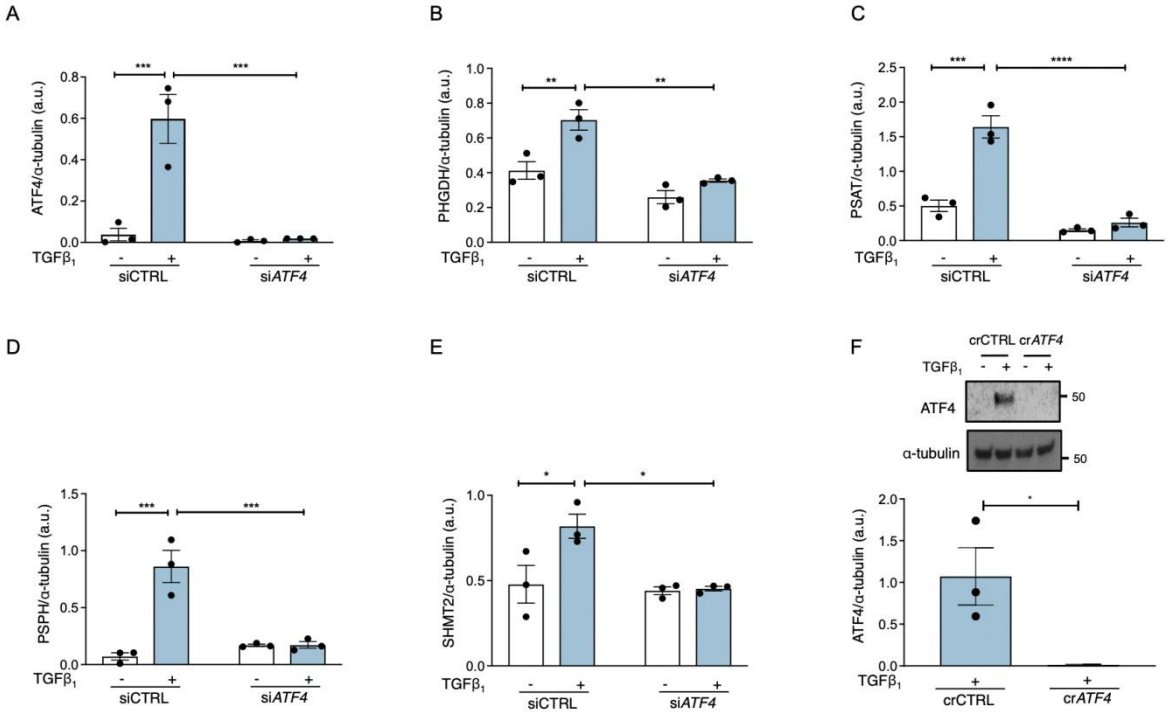

fig. S3 ATF4 knockdown abrogates the TGF-β_1–induced increase in glycine biosynthesis enzyme protein production.

Confluent pHLFs were transfected with scrambled control siRNA (siCTRL) or ATF4 siRNA (si*ATF4)* were incubated with or without TGF-β_1 for 24 hours. Immunoblotting performed and densitometric quantification for ATF4(**A**), PHGDH(**B**), PSAT1(**C**), PSPH(**D**) and SHMT2(**E**) are presented (N=3 biological replicates). (**F**) Confluent wildtype and *ATF4*$^{-/-}$ crispred pHLFs were incubated with or without TGF-β_1. Representative immunoblotting and densitometric quantification for ATF4 are shown at 24 hours (N=3 biological replicates). Difference between groups were assessed by two-way ANOVA (A-E) with Tukey post-hoc test or unpaired T-test (F). *p<0.05, **p<0.01, ***p< 0.001, ****p< 0.0001.

Supplementary Figure 4

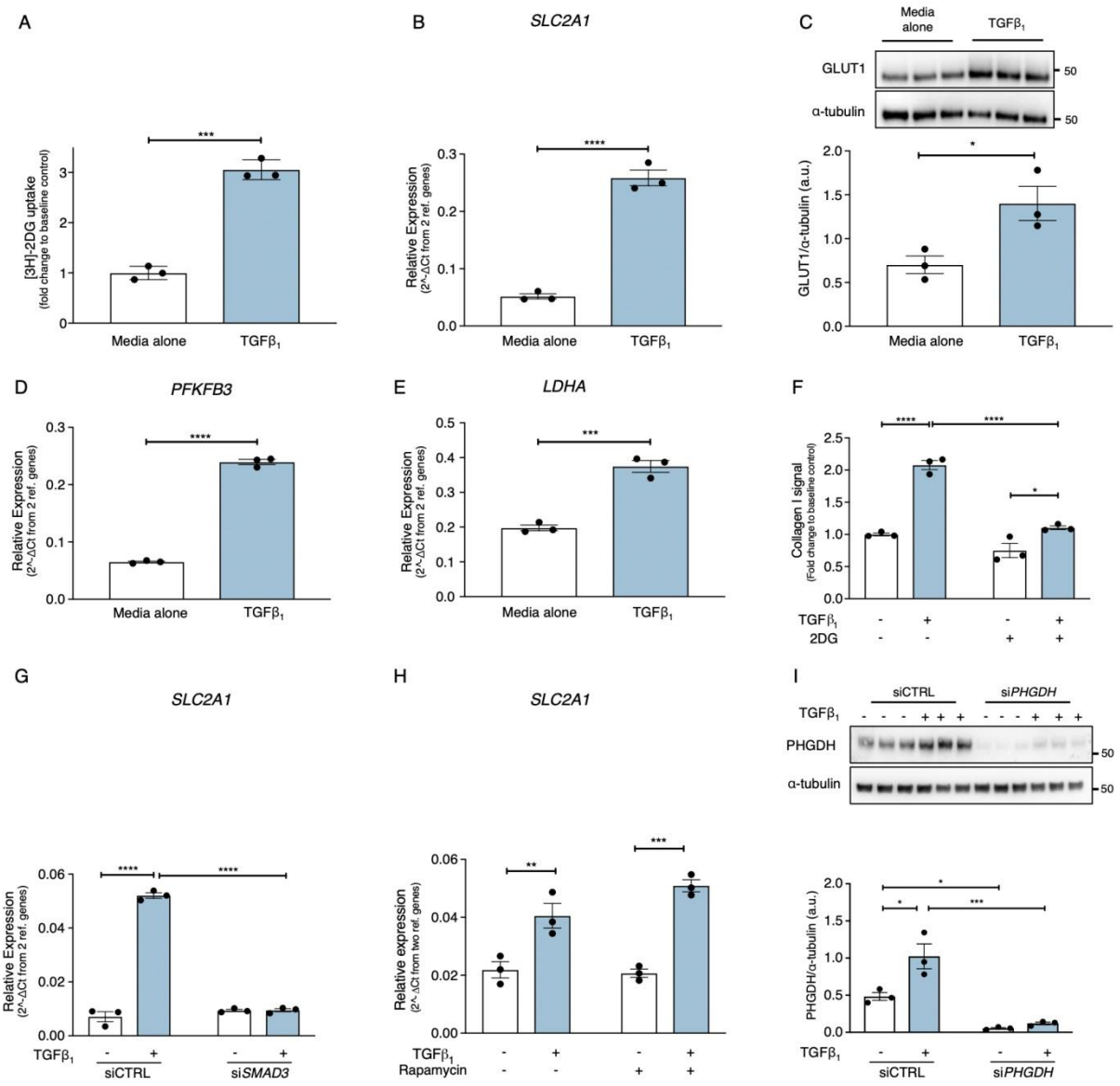

fig. S4. TGF-β_1 increases glucose uptake, *SLC2A1*/GLUT1 and glycolytic enzyme expression

(**A**) Confluent pHLFs were incubated with or without TGF-β_1 for 24 hours and ^{3}H-2DG uptake was measured (N=3 biological replicates and data are representative of 3 independent experiments). (**B-E**) Confluent pHLFs were incubated with or without TGF-β_1 and (**B**) *SLC2A1* relative mRNA abundance(24 hours), (**C**) GLUT1 protein abundance (24 hours), (**D**) *PFKFB3* (3 hours) and (**E**) *LDHA* (24 hours) relative mRNA abundance were measured (N=3 biological replicates). **(F)** Confluent pHLFs were incubated in the presence of 2-deoxyglucose and incubated for 48 hours with or without TGF-β_1. Collagen deposition at 48 hours was assessed by high content imaging. N=3 biological replicates per condition and data are representative of 3 independent experiments. **(G)** Confluent pHLFs were transfected with

scrambled control siRNA (siCTRL) or Smad3 siRNA (si*SMAD3*), before incubation with or without TGF-β_1; *SLC2A1* relative mRNA abundance was measured at 24 hours (N=3 biological replicates). (**H**) Confluent pHLFs were pre-incubated with rapamycin (or DMSO control) and exposed to media plus TGF-β_1 or media alone; *SLC2A1* relative mRNA abundance was measured at 24 hours. (**I**) Confluent pHLFs were transfected with scrambled control siRNA (siCTRL) or PHGDH siRNA (si*PHGDH*) before incubation with or without TGF-β_1; immunoblotting and densitometric quantification of PHGDH were performed at 24 hours (N=3 biological replicates). Differences between groups were evaluated by unpaired t-test (A-E) or two-way (F-I) ANOVA test with Tukey post-hoc test. *p<0.05, **p<0.01, ***p< 0.001, ****p< 0.0001.

Supplementary Figure 5

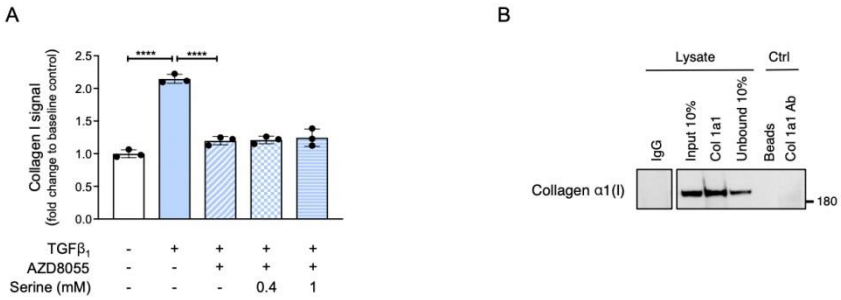

fig. S5. Exogenous serine does not rescue the inhibitory effects of ATP-competitive mTOR inhibition of TGF-β_1 induced collagen deposition

(**A**) Confluent pHLFs were incubated with AZD8055 and supplemented with or without increasing concentration of serine as described, then incubated with or without TGF-β_1; collagen deposition was assessed by high content imaging at 48 hours. N=3 biological replicates per condition and data are representative of 3 independent experiments. (**B**) Confluent pHLFs were stimulated with TGF-β_1 prior to cell lysis and immunoprecipitated with antibodies targeting IgG or collagen α1(I), followed by immunoblotting for collagen α1(I) abundance in whole lysate (input), antibody-bound fraction (collagen α1(I) IP) and unbound fraction. Protein G agarose beads and collagen type α1(I) antibody alone were immunoblotted as controls. Differences between groups were evaluated by one-way ANOVA test with Tukey post-hoc test (A). *=p<0.05, **=p<0.01, ***p< 0.001, ****p< 0.0001.

Table S1. MetaCore pathways enriched in the rapamycin-insensitive, mTOR module

Pathway	P value	Genes
Glycine, serine, cysteine and threonine metabolism	5.45E-05	CBS,GARS,PHGDH,PISD,PSAT1,PSPH,SHMT2
Signal transduction_mTORC1 downstream signaling	0.000743439	CCND2,EIF4A2,EIF4EBP1,GRB10,MTHFD2,POLR3K, SLC2A1,SREBF2
L-Alanine and L-cysteine metabolism	0.000942113	CBS,GOT1,GPT2,PC
Translation_Insulin regulation of translation	0.002325036	EIF2B3,EIF2S2,EIF2S3,EIF4A2,EIF4EBP1,TSC1
Translation_Translation regulation by Alpha-1 adrenergic receptors	0.004874917	ADRA1B,EIF4A2,EIF4EBP1,GNB1,GNG11,RHOA
Tau pathology in Alzheimer disease	0.007691877	GPC1,GRIN2A,MARK1,SDC1,SDC2,TUBA1B,TUBB4B
Hypertrophy of asthmatic airway smooth muscle cells	0.009638728	EIF2B3,EIF2S2,EIF2S3,EIF4EBP1,IFNAR2,MAX
Immune response_Distinct metabolic pathways in naive and effector CD8+ T cells	0.011569384	EIF4EBP1,SLC1A5,SLC2A1,SLC7A5,TSC1
Translation_Opioid receptors in regulation of translation	0.013771852	EIF4EBP1,GNB1,GNG11,PENK
Apoptosis and survival_Endoplasmic reticulum stress response pathway	0.014249639	BAK1,BCL2L11,EIF2S2,EIF2S3,HERPUD1
Folic acid metabolism	0.016973265	ALDH1L2,MTHFD2,SHMT2
Stem cells_Excitotoxicity of Glutamate in glioblastoma	0.017731496	GNB1,GNG11,GOT1,GRIN2A
Regulation of lipid metabolism_Alpha-1 adrenergic receptors signaling via arachidonic acid	0.018431849	ADRA1B,GNB1,GNG11,PTGS1,RHOA
Fenofibrate in treatment of type 2 diabetes and metabolic syndrome X	0.01906206	ABCA1,ACSL4,RBP4
DeltaF508-CFTR traffic / Sorting endosome formation in CF	0.021286737	NSF,RAB7A,VPS25
Apoptosis and survival_Role of nuclear PI3K in NGF/ TrkA signaling	0.023647542	ACIN1,BCL2L11,NPM1
Role of ER stress in obesity and type 2 diabetes	0.024016297	ATF3,PCK2,SREBF2,TRIB3
Transcription_HIF-1 targets	0.025269769	ADRA1B,CCNG2,LOXL2,LOXL4,NPM1,SLC2A1
Cysteine-glutamate metabolism	0.027393787	CBS,GOT1
Muscle contraction_S1P2 receptor-mediated smooth muscle contraction	0.031496271	GNB1,GNG11,MYL7,RHOA
Proteolysis_Role of Parkin in the Ubiquitin-Proteasomal Pathway	0.032207644	PSMD11,SIAH2,TUBA1B,TUBB4B,UBE2G2
Sulfur metabolism	0.032360777	CBS,GOT1
Apoptosis and survival_Granzyme A signaling	0.033557078	ANP32A,APEX1,HIST1H2BD,HIST2H2BE

Neurophysiological process_Dynein-dynactin motor complex in axonal transport in neurons	0.037276388	DYNLRB2,IPO5,MAPRE3,RAB7A,TUBA1B,TUBB4B
Development_PIP3 signaling in cardiac myocytes	0.042916214	CCND2,EIF4EBP1,GNB1,GNG11,TSC1
Stem cells_Role of GSK3 beta in cardioprotection against myocardial infarction	0.043951681	ADM2,BDKRB2,PENK
Transcription_CoREST complex-mediated epigenetic gene silencing	0.045019326	ELAC2,GRIN2A,SIN3A,SMARCC1
Signal transduction_AKT signaling	0.04754385	BCL2L11,CCND2,EIF4EBP1,TSC1

Table S2. Primer sequence

ATF4 (F)	5'-GCTAAGGCGGGCTCCTCCGA-3'
ATF4 (R)	5'-ACCCAACAGGGCATCCAAGTCG-3'
LDHA (F)	5’-GGAGATTCCAGTGTGCCTGT-3’
LDHA (R)	5’-GTCCAATAGCCCAGGATGTG-3’
PFKFB3 (F)	5’-AAAAGTGTTCAACGTCGGGG-3’
PFKFB3 (R)	5’-CATGGCTTCCTCATTGTCGG-3’
PHGDH (F)	5’-GGAGGAGATCTGGCCTCTCT-3’
PHGDH (R)	5’-GTCATTCAGCAAGCCTGTCG-3’
PSAT1 (F)	5’-GCGGCCATGGAGAAGCTTAG-3’
PSAT1 (R)	5’-ATGCCTCCCACAGACACGTA-3’
PSPH (F)	5’-GAGGACGCGGTGTCAGAAAT-3’
PSPH (R)	5’-GGTTGCTCTGCTATGAGTCTCT-3’
SHMT1 (F)	5’-GTGACCACCACCACTCACAA-3’
SHMT1 (R)	5’-ACAGCAACCCCTTTCCTGTAG-3’
SHMT2 (F)	5’-GCTGCCCTAGACCAGAGTTG-3’
SHMT2 (R)	5’-GCAGAGGCCGAGCCG-3’
SLC2A1 (F)	5’-ACTGTCGTGTCGCTGTTTGT-3’
SLC2A1 (R)	5’-GATGGCCACGATGCTCAGAT-3’