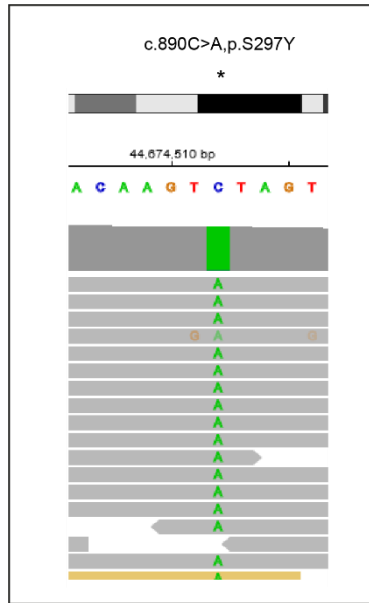


Supplemental Data

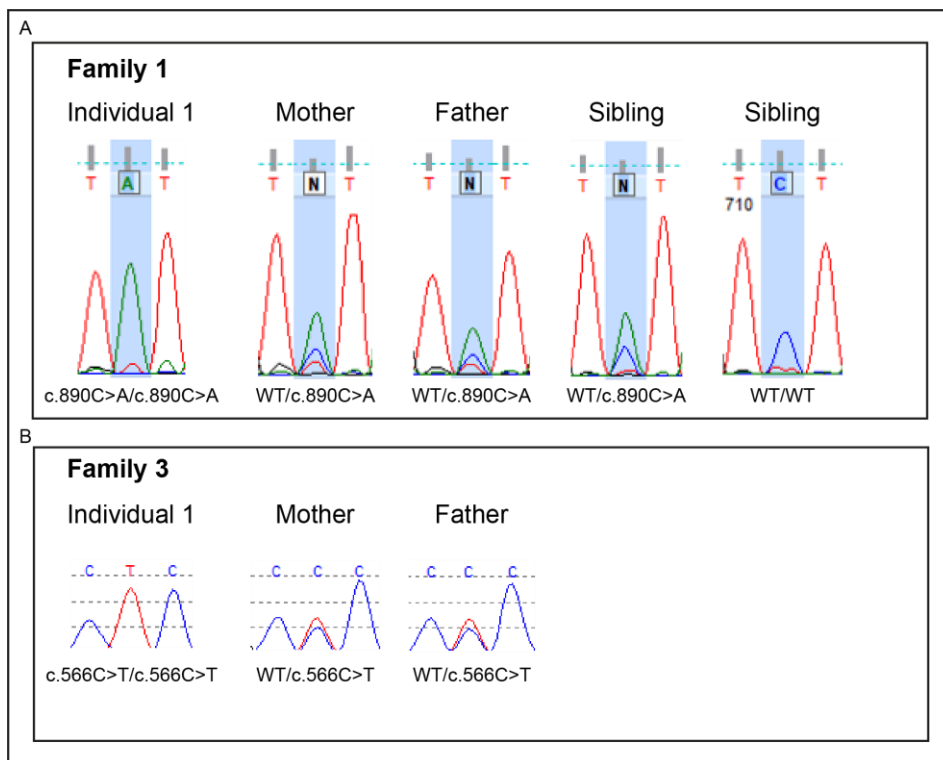
Supplementary Table 1. Additional clinical findings in individuals with homozygous *OGDH* pathogenic variants (separate excel file).

Supplementary Table 2. Variant information for the three *OGDH* variants presented in this study, in addition to the already reported homozygous variant p.Asn320Ser (4).

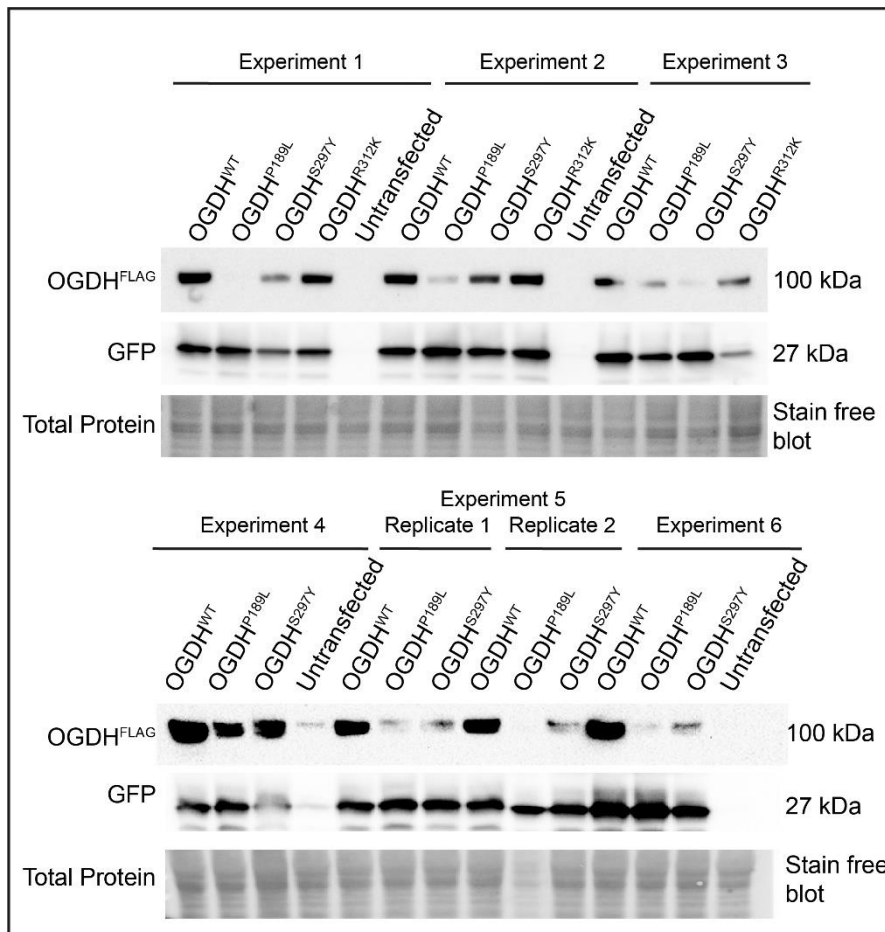
ID	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5 (Yap 2021)	Individual 6 (Yap 2021)	
Variant	c.890C>A p.(Ser297Tyr)		c.566C>T p.(Pro189Leu)	c.935G>A p.(Arg312Lys)/ p.(Phe264_Arg312del)	c.959A>G p.(Asn320Ser)		
Homozygous	Yes		Yes	Yes	Yes		
Transcript	NM_002541		NM_002541	NM_002541	NM_002541		
Ref build	GRCh38		GRCh38	GRCh38	GRCh37		
Location	7:44674512		7:44666784	7:44674557	7:44714800		
Population Frequencies	gnomAD	0	0	0	0		
	Iranome	0	0	0	0		
	GME TP	0	0	0	0		
Predictive Scores	CADD Phred	29.4	24.8	36	24.7		
	SIFT Score	0	0.01	0	0		
	SIFT Prediction	damaging		damaging	damaging	damaging	
	Polyphen	probably damaging (1.00)		probably damaging (0.997)	probably damaging (1.00)	probably damaging (1.00)	
	SpliceAI	Unlikely splice impact		Unlikely splice impact	Donor loss 0.93	Unlikely splice impact	
	REVEL	0.956	0.539	0.899	0.819		
ACMG	Likely pathogenic (PM2 PP3 PS3 PP2)		Likely pathogenic (PM2 PP3 PS3 PP2)	Pathogenic (PVS1 PS3 PM2 PP2)			



Supplementary Figure 1 | Integrative Genomics Viewer (IGV) image of sequencing data for individual 1 p.(Ser297Tyr), aligned to GRCh38 human reference genome.

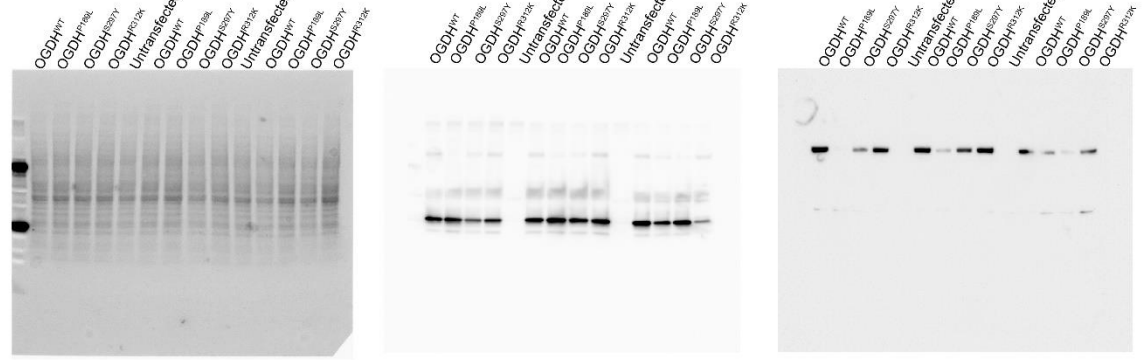


Supplementary Figure 2 | Segregation analysis. (A) Segregation data for family 1 harbouring the p.(Ser297Tyr) variant. (B) Segregation data from family 3 harbouring the p.(Pro189Leu) variant.



Supplementary Figure 3 | Replicates of HEK293 transfection with pcDNA3.1 containing a flag tagged OGDH variant of interest. Transfection of either pcDNA3.1-OGDH^{WT}-FLAG, pcDNA3.1-OGDH^{p.(Pro189Leu)}-FLAG, pcDNA3.1-OGDH^{p.(Ser297Tyr)}-FLAG, pcDNA3.1-OGDH^{p.(Arg312Lys)}-FLAG or transfected with vector only was performed, with GFP and total protein staining for normalisation. The data shown (excluding experiment 5 replicate 2) was used for statistical analysis presented in Figure 2D.

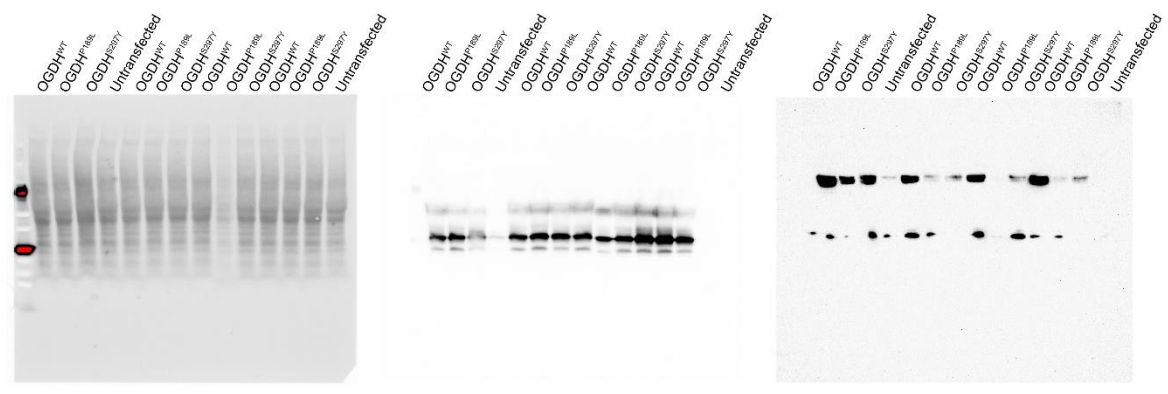
A



Stain Free Total Protein Blot

anti-GFP

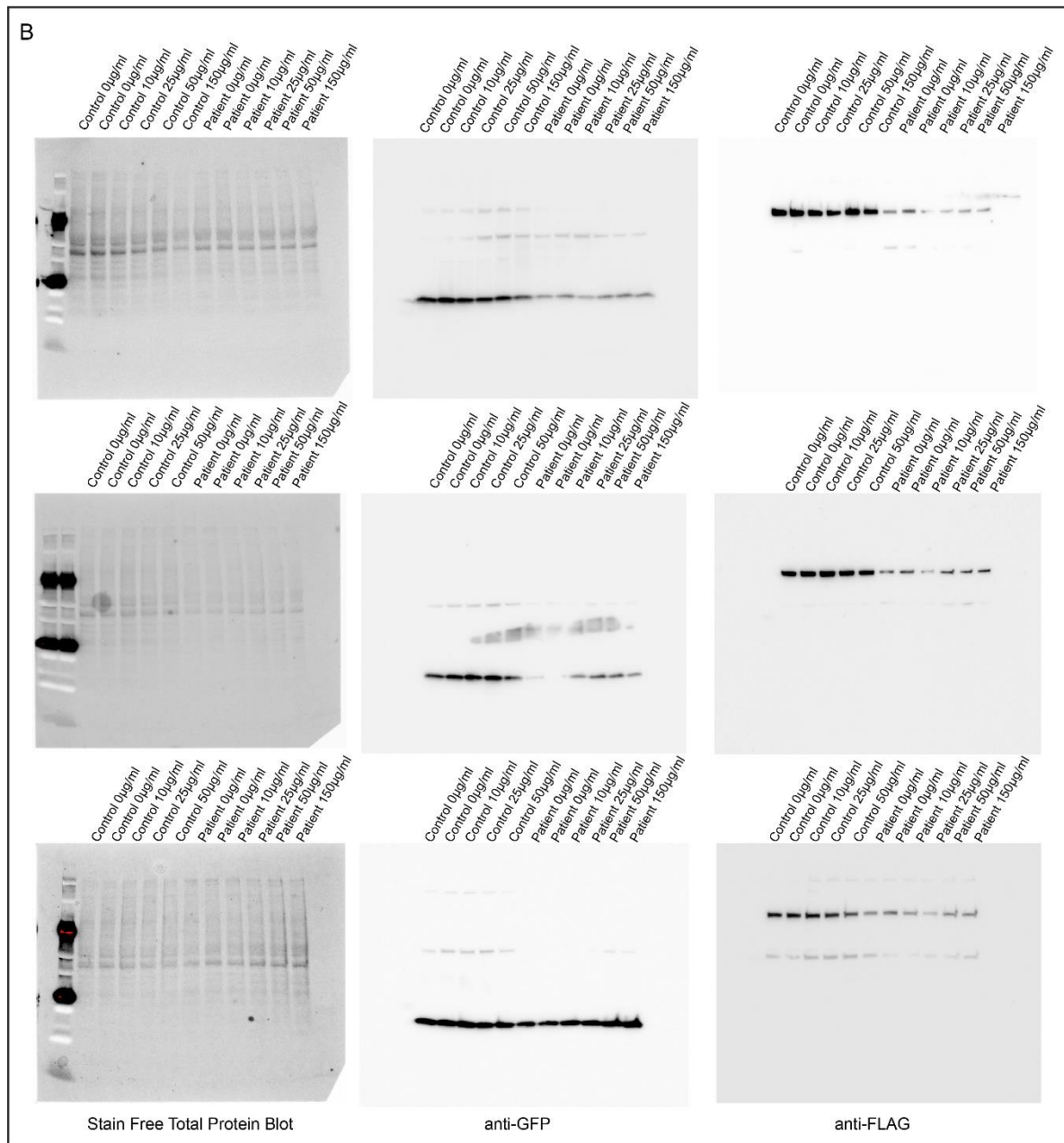
anti-FLAG



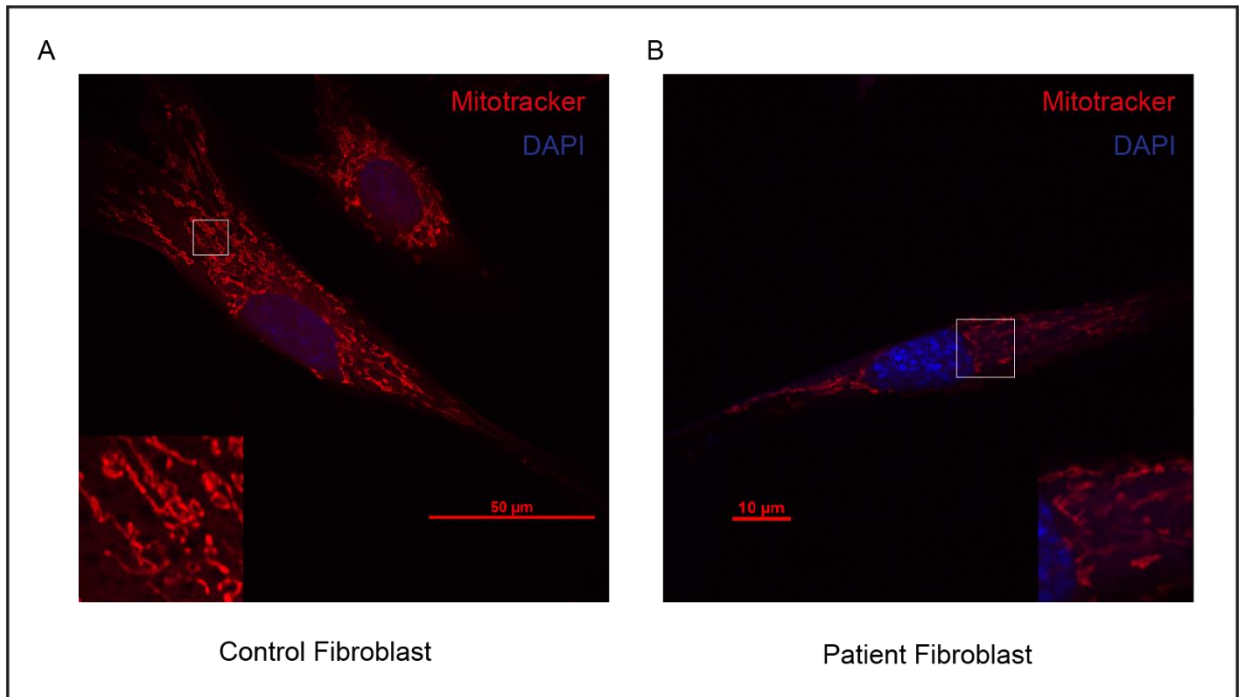
Stain Free Total Protein Blot

anti-GFP

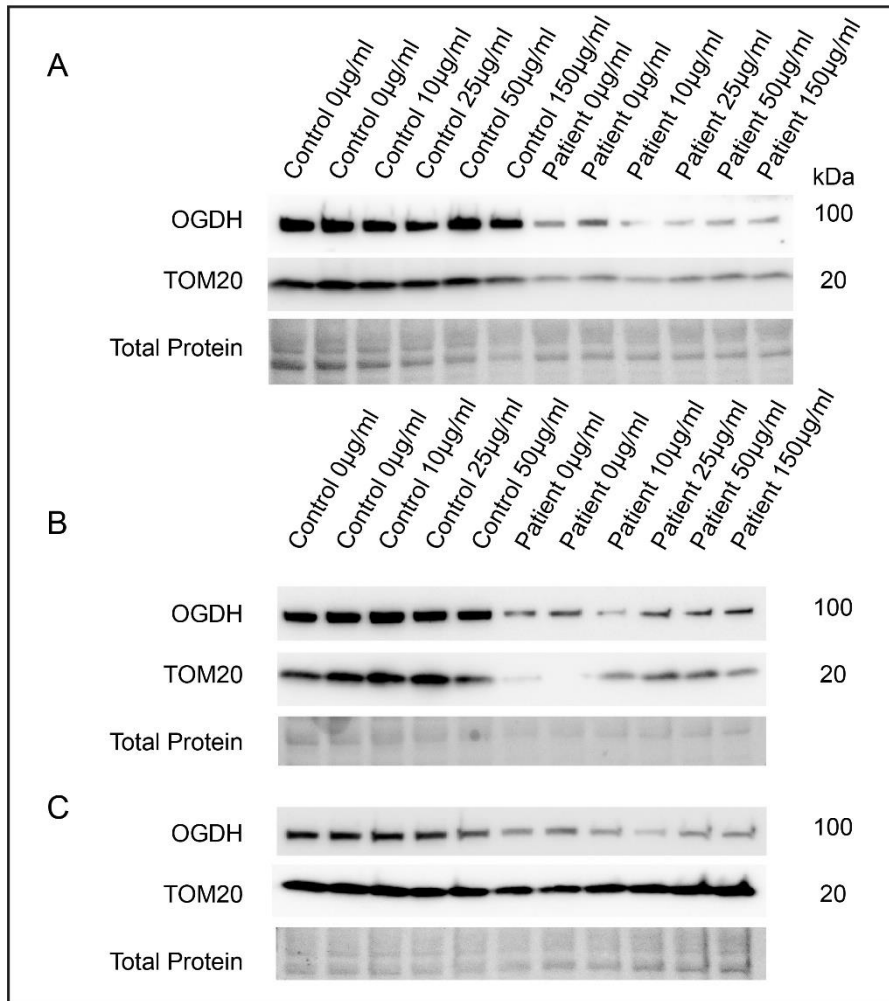
anti-FLAG



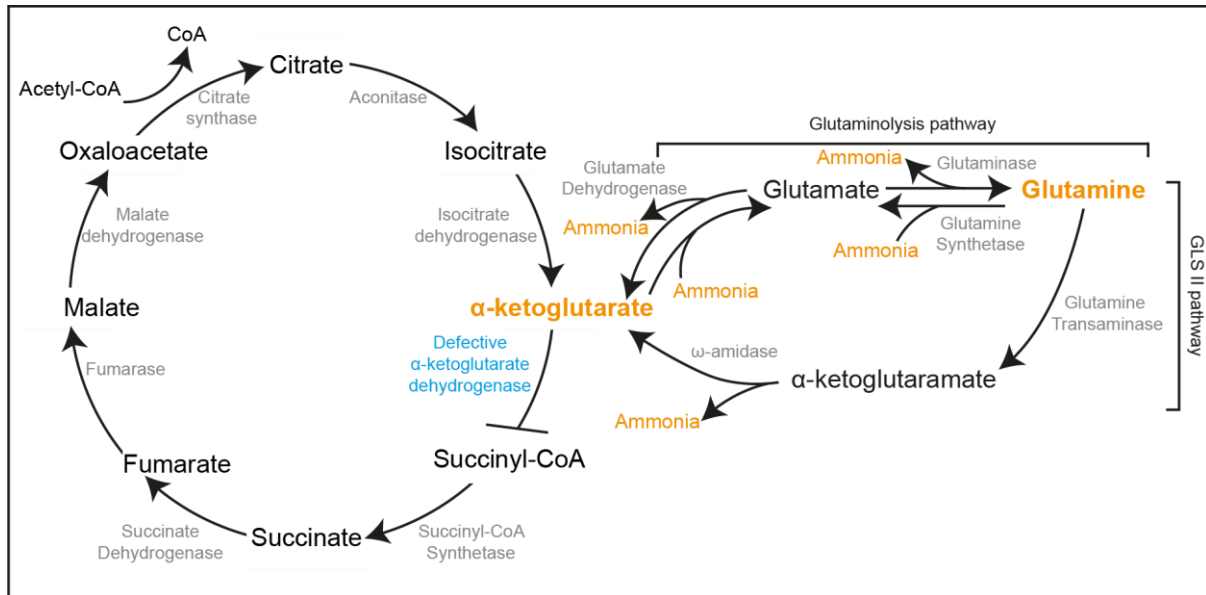
Supplementary Figure 4 | Full western blot images. (A) Western blot data corresponding to Figure 2D and Supplementary Figure 3 HEK293 pcDNA3.1 transfections. (B) Western blot data corresponding to Figure 2E and Supplementary Figure 6.



Supplementary Figure 5 | Visualisation of mitochondria in individual 1 p.(Ser297Tyr) and control fibroblasts using Mitotracker (red) and DAPI (blue) to stain the nucleus. (A) Healthy control fibroblasts. (B) Individual 1 fibroblasts harbouring the p.(Ser297Tyr) variant.



Supplementary Figure 6 | Replicates of individual 1 p.(Ser297Tyr) and control fibroblast OGDH protein detection following a 24 hour treatment with cycloheximide. A, B, C showing three separate experiment results. (C) Figure included in the main text body (Figure 2E). 150µg/ml cycloheximide treatment not included in all experiments due to toxicity in control cells.



Supplementary Figure 7 | The TCA cycle and glutamine synthesis pathways. The TCA cycle with a block at the conversion of α -ketoglutarate to succinyl-coA due to a defective α -ketoglutarate dehydrogenase complex, shown in blue. Next to the TCA cycle, the pathways for the conversion between glutamine and α -ketoglutarate are shown. Metabolites increased in patients are shown in orange.