

Supplementary Figures:

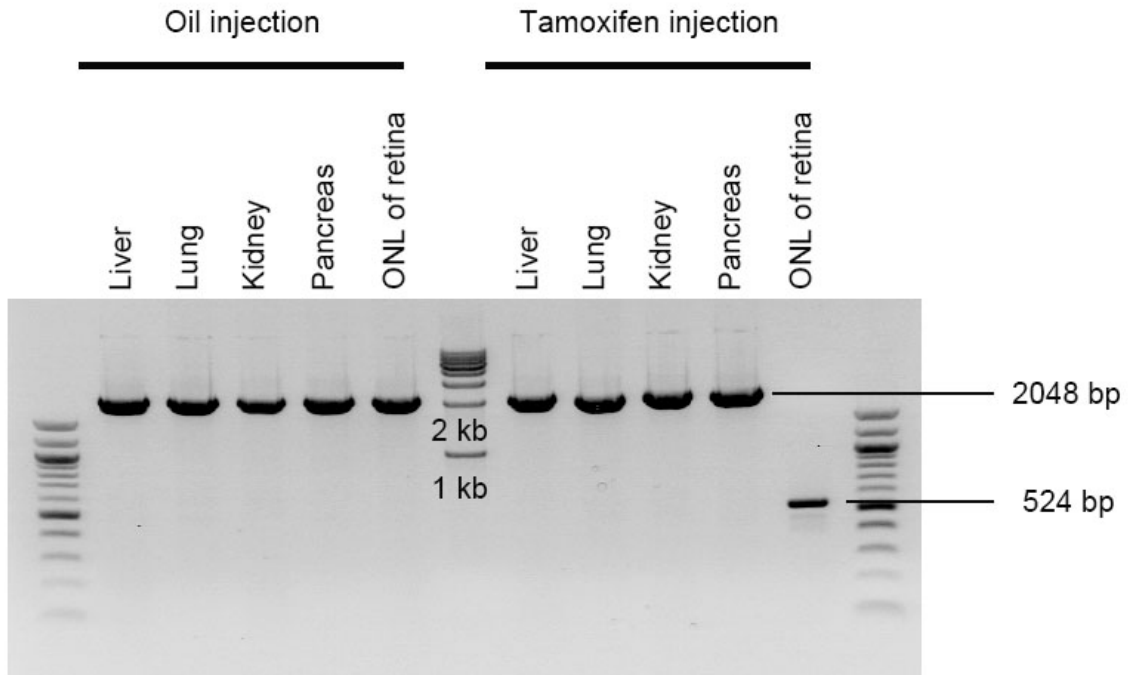


Figure S1. PCR analysis of DNA isolated from the ONL confirms photoreceptor-specific *Sirt6* excision.

The 2,048-bp and 524-bp bands represent nonrecombined and recombined *Sirt6*^{tm1.1Cxd} alleles, respectively. *Pde6g*^{CreERT2} recombinase ablated exons 2 and 3 exclusively in the retinal ONL in *Sirt6*^{-/-} *Pde6b*^{H620Q/H620Q} mice.

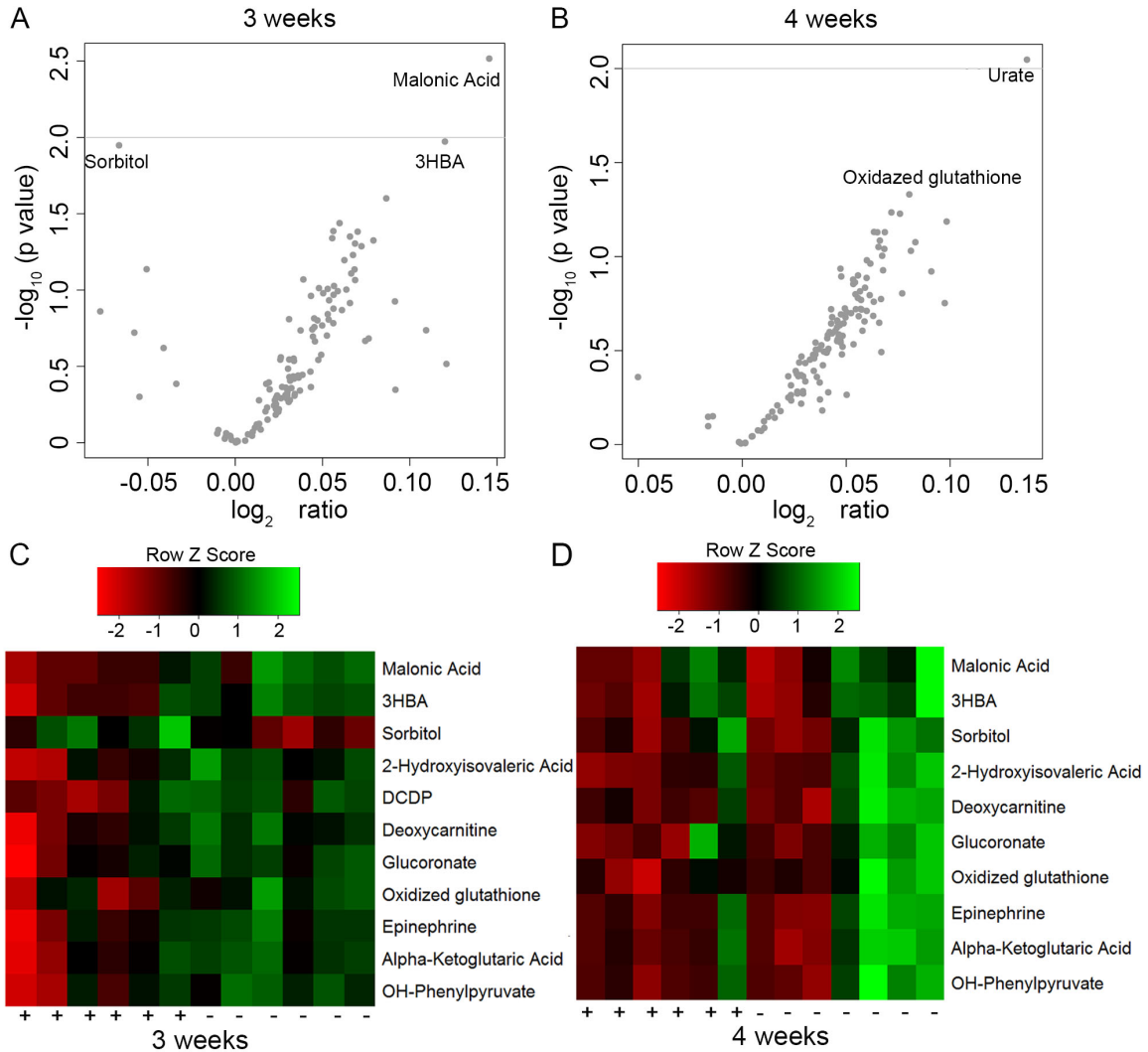


Figure S2. *Sirt6* deficiency enhances glucose metabolism

(A-B) Double log-transformed graph of LC-MS data identified more than 100 downstream targets of *Sirt6*. Each circle represents the ratio of $Sirt6^{-/-}Pde6b^{H620Q/H620Q} : Sirt6^{loxP/loxP}Pde6b^{H620Q/H620Q}$ metabolite levels; values that fall in $x \leq 0$ indicate down-regulation, whereas values at $y \geq 1$ indicate $P < 0.01$.

(C-D) At three weeks, malonic acid, 3-hydroxybenzoic acid (3HBA), 2-hydroxyisovaleric acid, deoxycytidine diphosphate, deoxycarnitine, glucocoronate, oxidized glutathione, epinephrine/normetanephrine, α -ketoglutaric acid, and OH-phenylpyruvate were significantly up-regulated in the $Sirt6^{-/-}Pde6b^{H620Q/H620Q}$ mice ($P < 0.05$), whereas sorbitol was significantly down-regulated ($P < 0.05$). At four weeks, the most dramatically changed metabolites at the three-week time point all maintained upregulated values, although all lost significance except for oxidized glutathione ($P < 0.05$). (n = 6 for $Sirt6^{-/-}Pde6b^{H620Q/H620Q}$ and $Sirt6^{loxP/loxP}Pde6b^{H620Q/H620Q}$ mice at 3

weeks. n = 7 for *Sirt6*^{-/-}*Pde6b*^{H620Q/H620Q} and n = 6 for *Sirt6*^{loxP/loxP}*Pde6b*^{H620Q/H620Q} mice at 4 weeks.

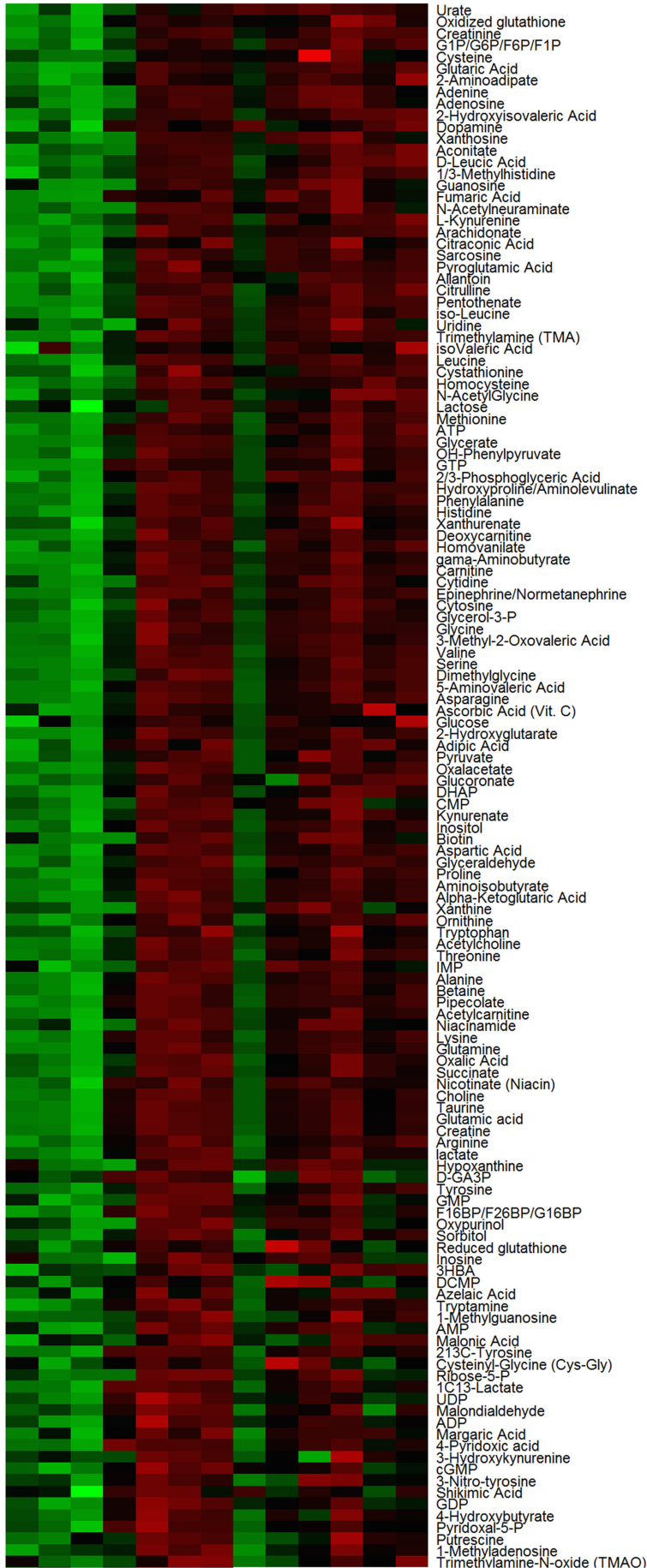


- Malonic Acid
- 3-HBA
- Sorbitol
- 2-Hydroxyisovaleric Acid
- DCDP
- Deoxycarnitine
- Glucuronate
- Oxidized glutathione
- Epinephrine
- Alpha-ketoglutaric Acid
- Phenylpyruvic acid
- Salicylurate
- Glycerate
- Pentothenate
- Tyrosine
- Tryptamine
- 3-Methyl-2-Oxovaleric Acid
- Leucine
- Ascorbic Acid (Vit. C)
- Trimethylamine (TMA)
- CMP
- Acetylcholine
- N-Acetylneuraminic acid
- Aminoisobutyrate
- Betaine
- Asparagine
- Pipecolate
- gamma-Aminobutyrate
- Aconitate
- Maleic Acid
- Inositol
- Acetylcarnitine
- GTP
- Hydroxyproline/Aminolevulinic acid
- Lysine
- Xanthurenate
- Serine
- Glycine
- Glutamine
- Carnitine
- Alanine
- Allantoin
- Nicotinate (Niacin)
- Succinate
- Cysteinyl-Glycine (Cys-Gly)
- D-Leucic Acid
- 2-Hydroxyglutarate
- Guanidinoacetate
- Urate
- Glucose
- Tyramine
- N-AcetylGlycine
- iso-Leucine
- Oxalic Acid
- Arginine
- 1/3-Methylhistidine
- Niacinamide
- G1P/G6P/F6P/F1P
- Agmatine
- Histidine
- Cadaverine
- Creatine
- Glutamic acid
- Creatinine
- Arachidonate
- D-GA3P
- Homocysteine
- Citrulline
- Phenylalanine
- Reduced glutathione
- Valine
- DHAP
- Cytosine
- Aspartic Acid
- Choline
- Glycerol-3-P
- Quinolinate
- 1-Methyladenosine
- 2/3-Phosphoglyceric Acid
- Biotin
- Taurine
- Glyceraldehyde
- Sarcosine
- Putrescine
- Histamine
- Proline
- Inosine
- PEP
- 4-Hydroxybutyrate
- lactate
- 5-Aminovaleric Acid
- Xanthine
- Dimethylglycine
- IMP
- Ribose-5-P
- Ornithine
- Uridine
- Shikimic Acid
- Glutaric Acid
- Cytidine
- 4-Pyridoxic acid
- 3C13-Lactate
- 213C-Tyrosine
- Oxaloacetate
- Methylmalonate
- Hypoxanthine
- AMP
- Margaric Acid
- Guanosine
- Citraconic Acid
- Trimethylamine-N-oxide (TMAO)
- Tryptophan
- Fumaric Acid
- Threonine
- Sucrose
- Methionine
- Geranyl Pyrophosphate
- Azelaic Acid
- Pyruvate
- Adenosine
- Kynurenate
- Adipic Acid
- Adenine

- - - - - + + + + +

Figure S3. Heat mapping at three weeks reveals overall changes in metabolic intermediate concentration in *Sirt6*-deficient mice

Heat mapping corroborated the findings in **Fig. S2** and reflects variations in metabolite levels between mice. Black boxes indicate no difference between levels in control and treated mice. The brightest hues of red indicate that higher levels of that metabolite were observed in the *Sirt6*^{loxP/loxP}*Pde6b*^{H620Q/H620Q} mice, while the brightest green boxes indicate that higher levels were observed in the *Sirt6*^{-/-}*Pde6b*^{H620Q/H620Q} mice. The majority of metabolites were upregulated in the *Sirt6*^{-/-}*Pde6b*^{H620Q/H620Q} mice compared to controls. ((+) represents *Sirt6*^{loxP/loxP}*Pde6b*^{H620Q/H620Q} and (-) represents *Sirt6*^{-/-}*Pde6b*^{H620Q/H620Q}. n = 6 per group)

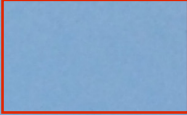


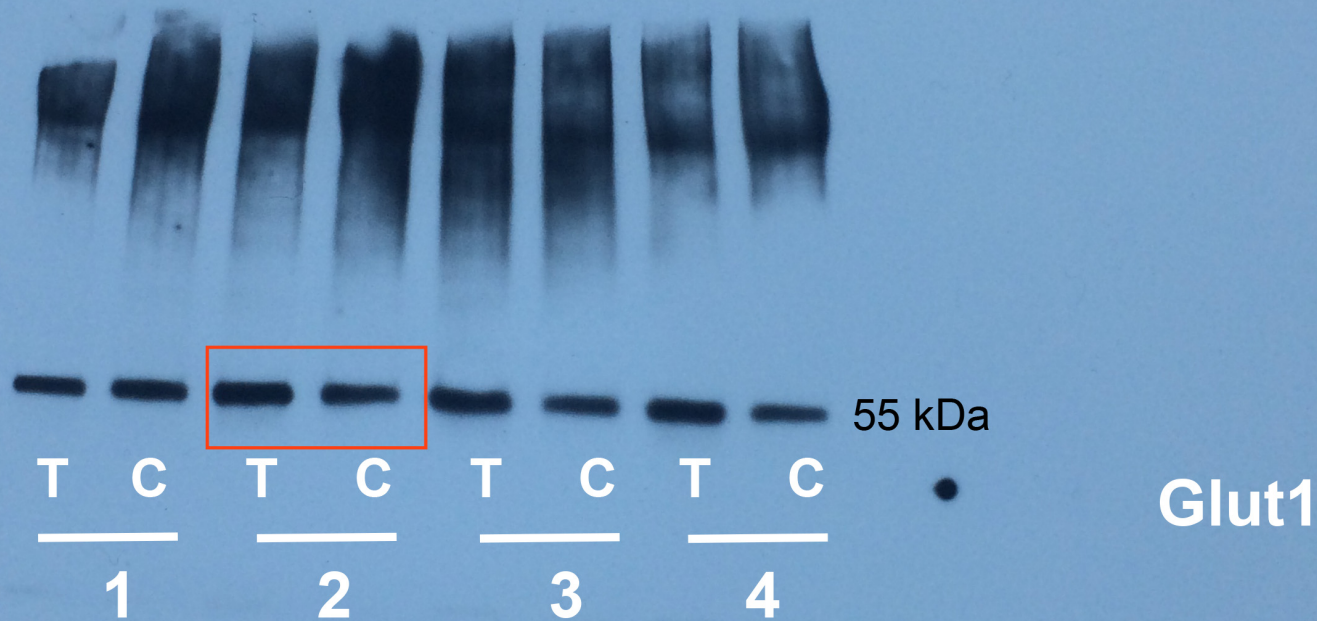
+ + + + + - - - - -

Figure S4. Heat mapping at 4 weeks reveals overall changes in metabolic intermediate concentration in *Sirt6*-deficient mice

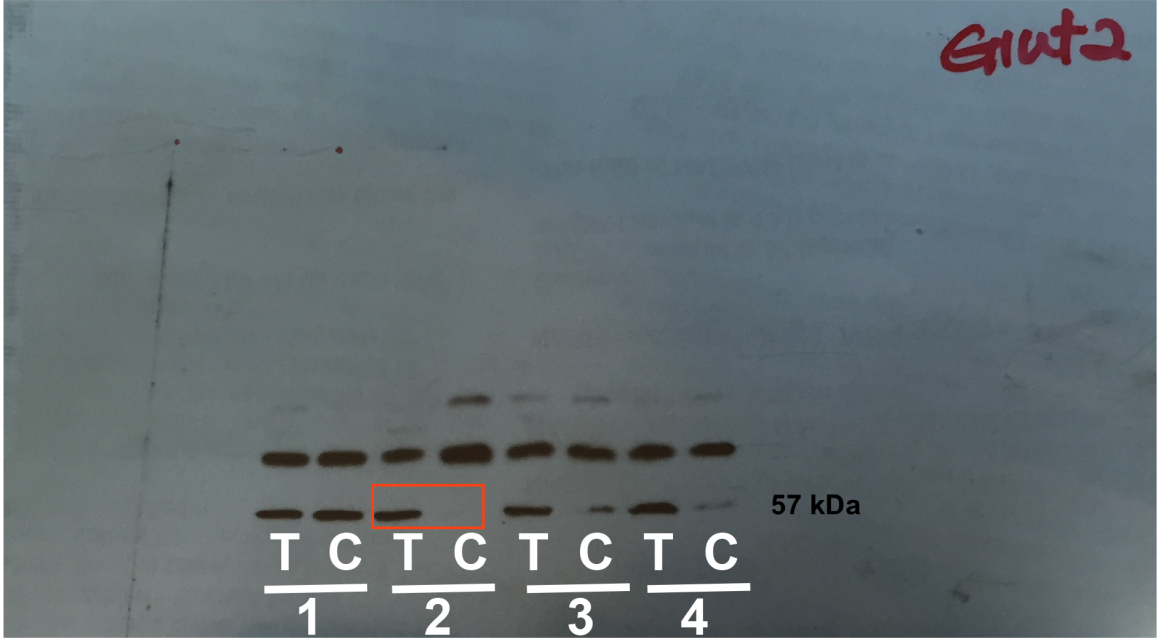
Heat mapping at 4 weeks corroborated the findings in **Fig. S2B,D** and reflects variations in metabolite levels between mice. Black boxes indicate no difference between levels in control and treated mice. The brightest hues of red indicate that higher levels of that metabolite were observed in the *Sirt6*^{loxP/loxP}*Pde6b*^{H620Q/H620Q} mice, while the brightest green boxes indicate that higher levels were observed in the *Sirt6*^{-/-}*Pde6b*^{H620Q/H620Q} mice. The majority of metabolites were upregulated in the *Sirt6*^{-/-}*Pde6b*^{H620Q/H620Q} mice compared to controls, although some metabolites lost statistical significance at this later time point. ((+) represents *Sirt6*^{loxP/loxP}*Pde6b*^{H620Q/H620Q} and (-) represents *Sirt6*^{-/-}*Pde6b*^{H620Q/H620Q}. n = 6 for *Sirt6*^{loxP/loxP}*Pde6b*^{H620Q/H620Q} and n = 7 for *Sirt6*^{-/-}*Pde6b*^{H620Q/H620Q})

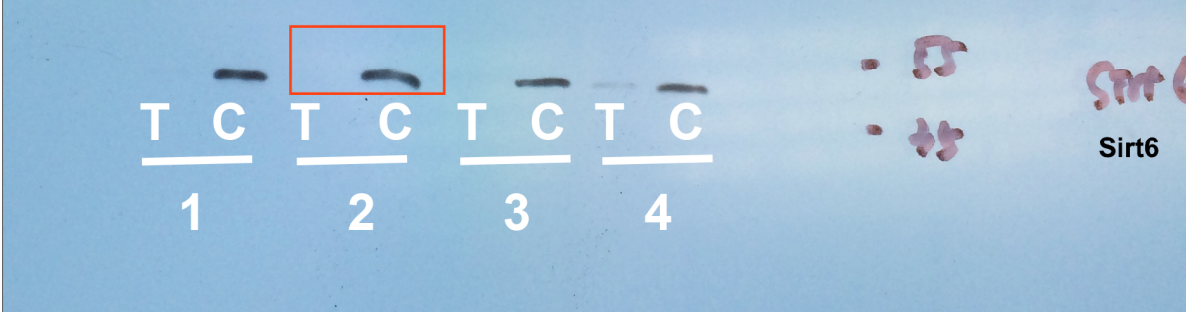
Key: T = treated (Sirt6^{-/-})
C = control (Sirt6^{loxP/loxP})
1 = Sample 1 (2 retina, 1 mouse)
2 = Sample 2
etc...

 = indicates which blot was presented in the figure (Fig 6)

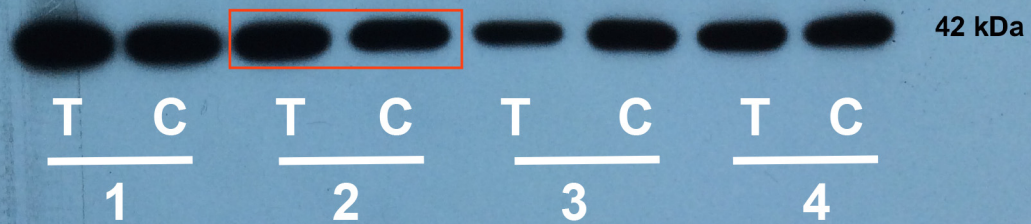
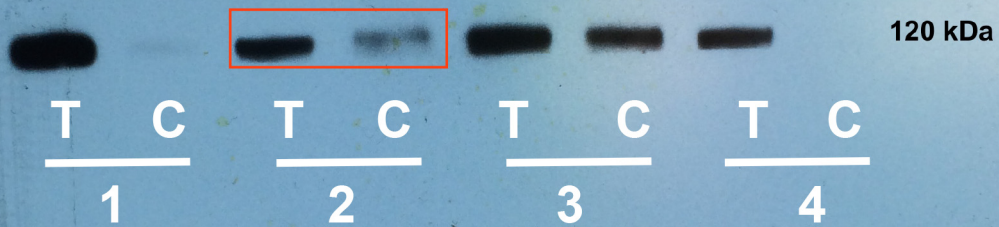


Enut2





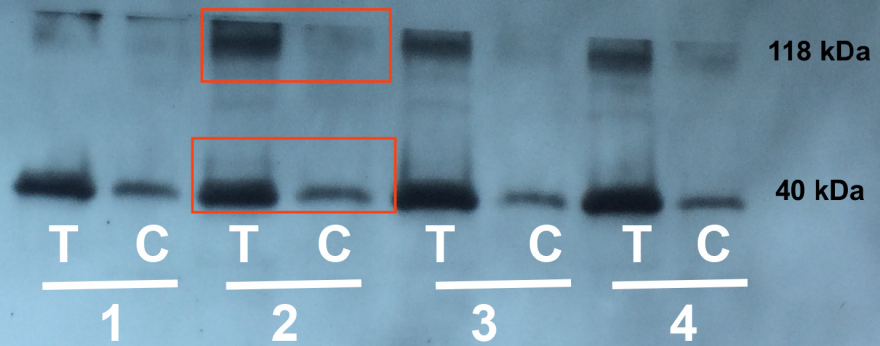
H7f1a



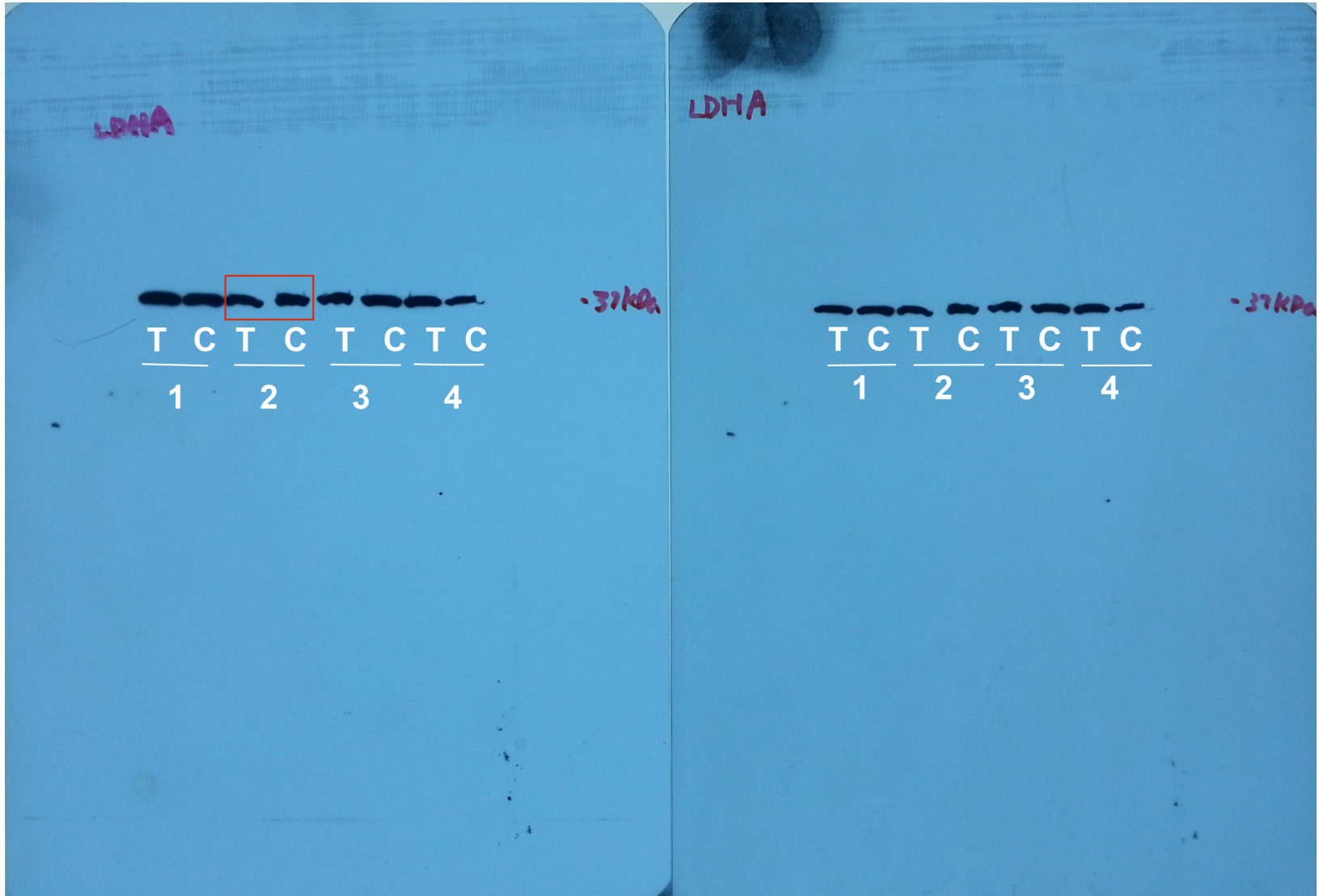
B-actin

442a

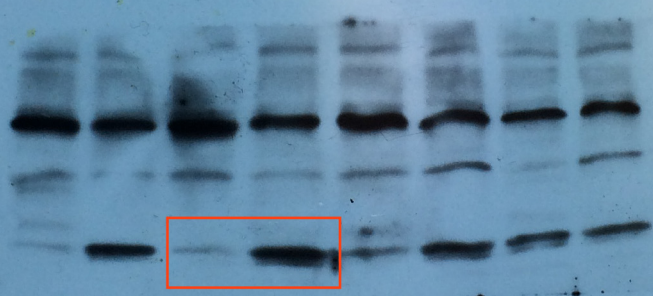
myc



LDHA



VHL



->5

T C T C T C T C
1 2 3 4

T C T C T C T C

