Lysine Glutarylation Is a Protein Posttranslational Modification Regulated by SIRT5

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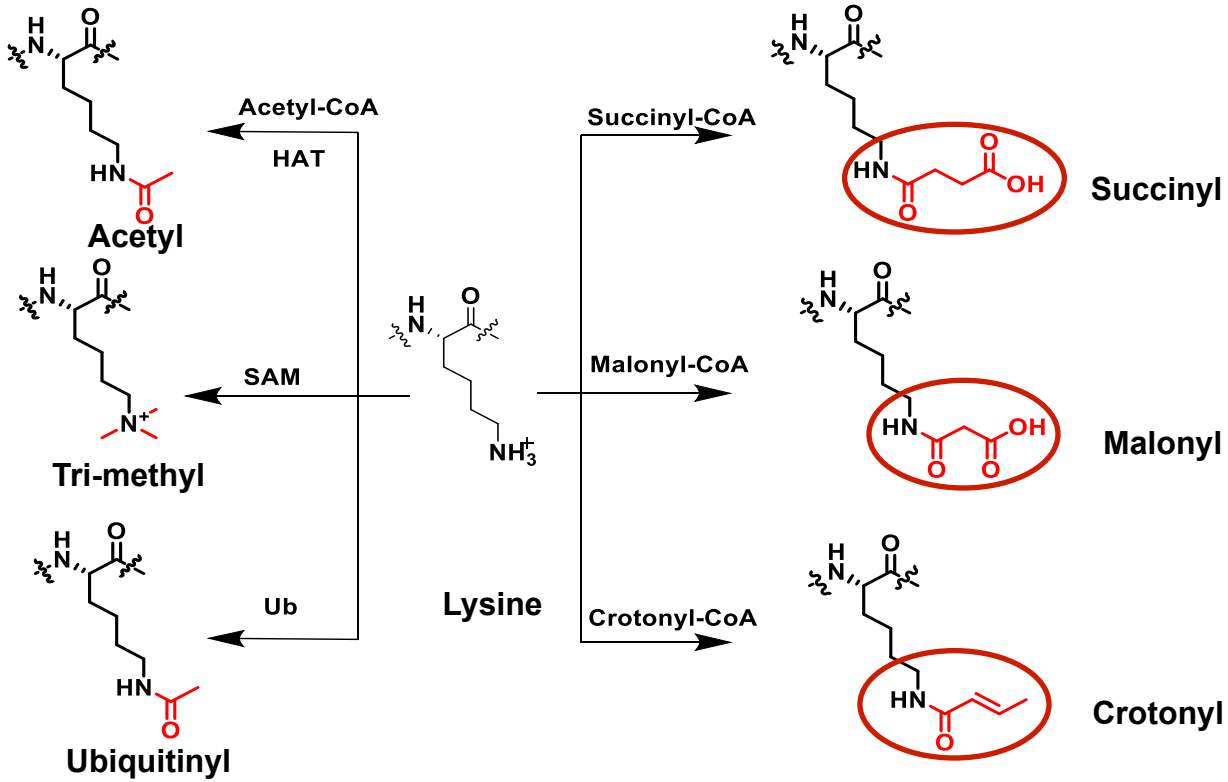
Lysine: the most frequently modified amino acid

PTMs on the lysine residue:

Acetylation, ubiquitylation, methylation, propionylation….

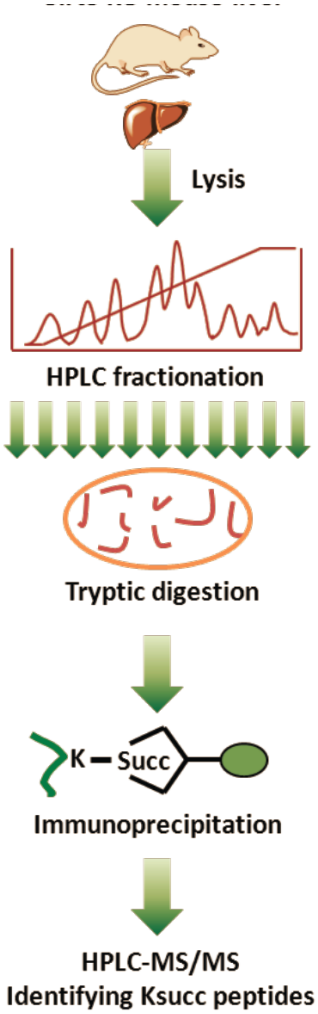


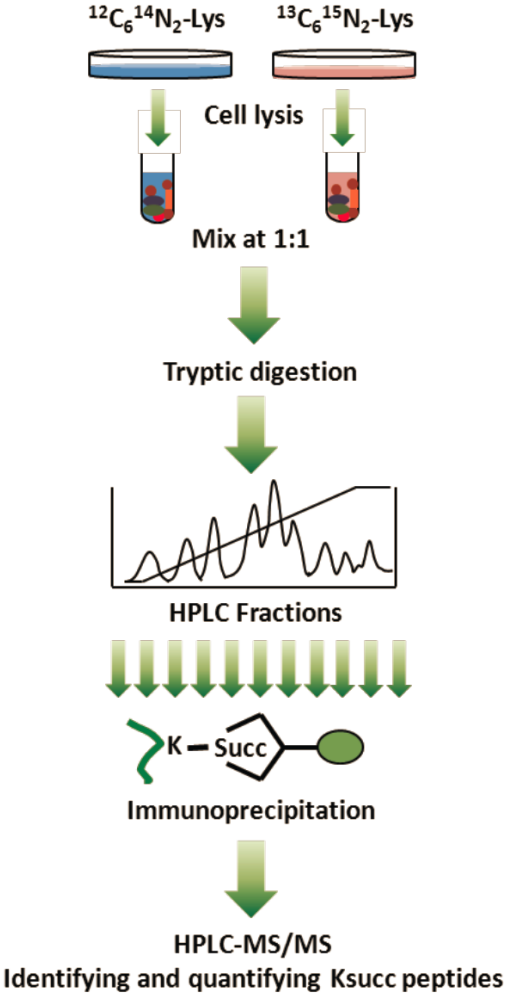
Tan et al, *Cell*. 2011. 146:1016–28.

Zhang and Tan et al *Nature Chem Biol.* 2011.7: 58–63.

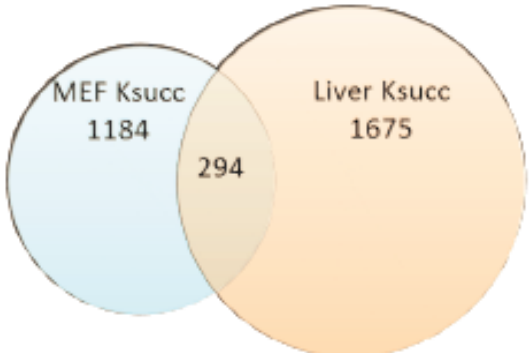
Peng et al, *Mol Cell Proteomics* 2011. M111.012658.

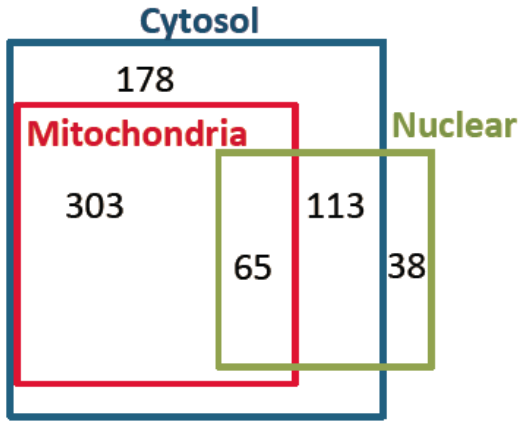
The Ksucc proteome in mammalian cell





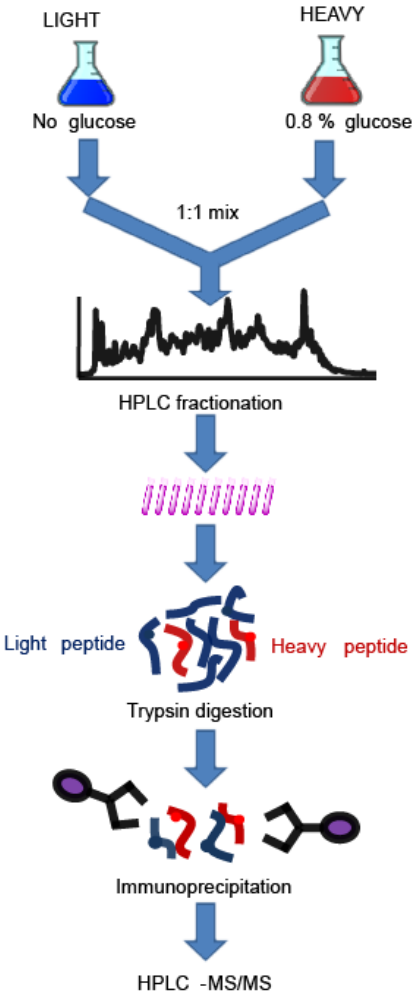
2,565 Ksucc sites on 779 proteins.

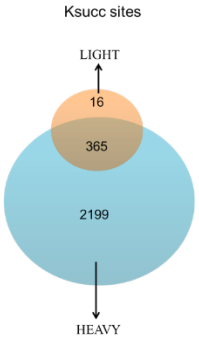


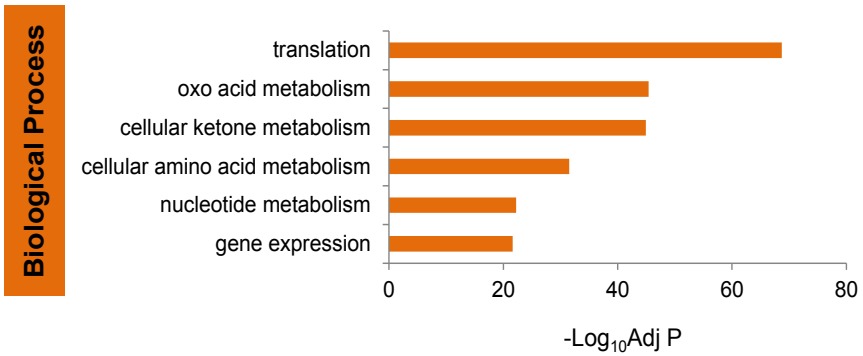


Kim et al, *Mol Cell.* 2013 50:919-30

Proteome-wide *E. Coli* Ksucc Substrates

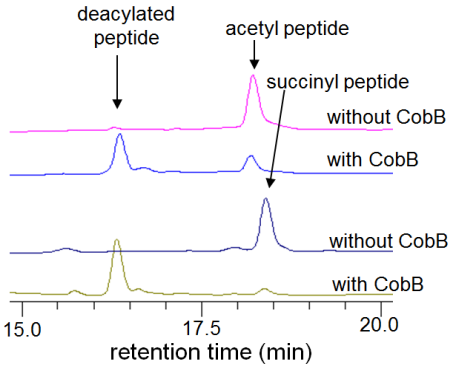






2487 Ksucc sites on 658 proteins

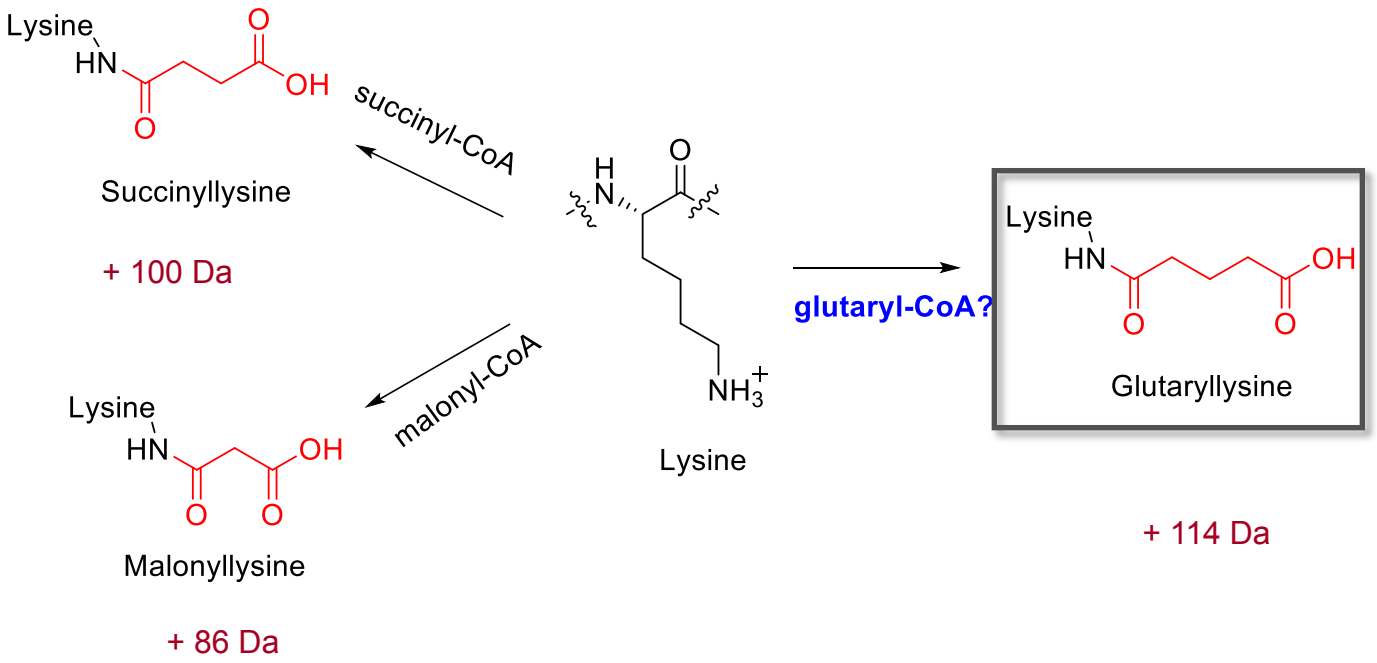
~16% *E. coli* proteome



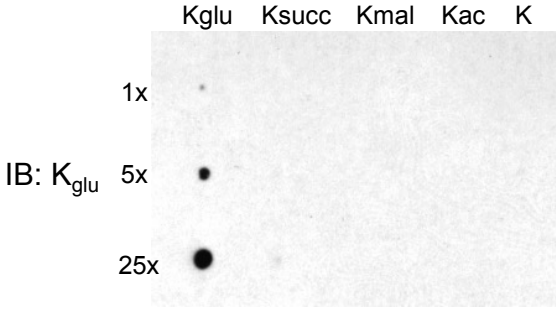
CobB is a desuccinylase and deacetylase

Gozde, Tan et al. ***Mol Cell Proteomics***. 2013, 3509-20

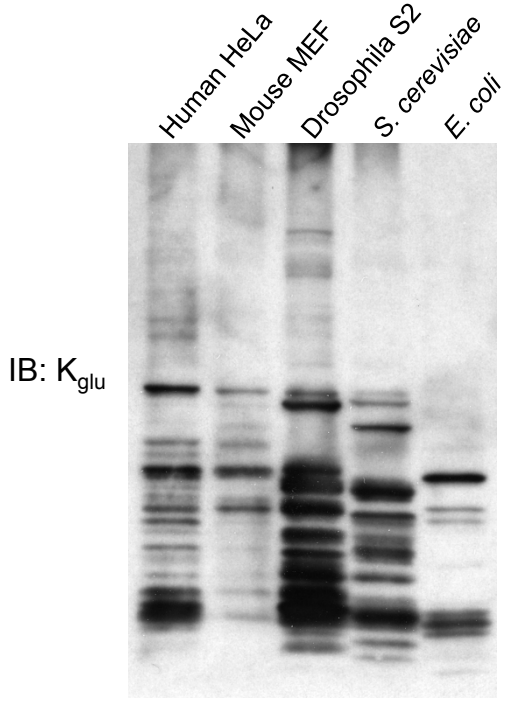
Lysine glutarylation (Kglu)



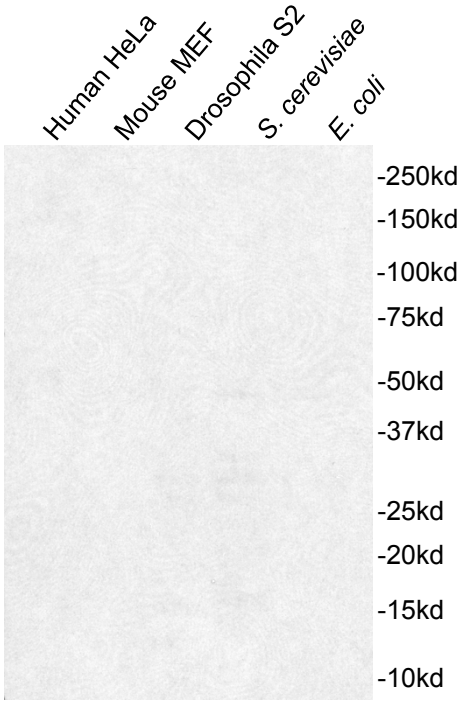
Detection of Kglu signals by anti-Kglu antibody



Dot spot assay

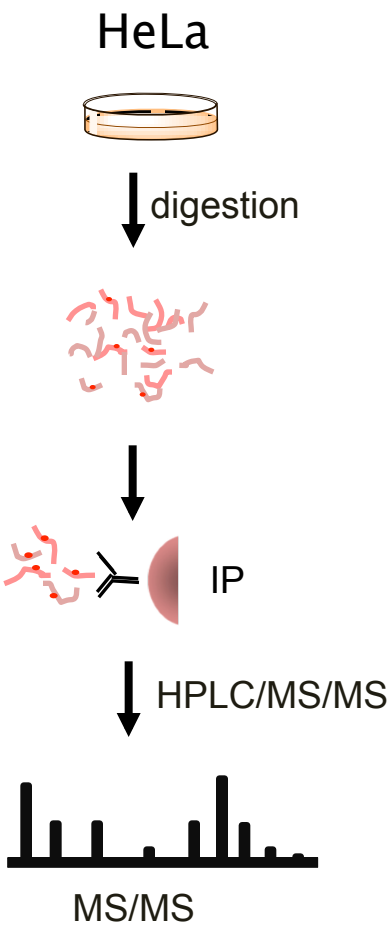


Competed with
unmodified BSA tryptic peptides



Competed with
glutarylated-BSA tryptic peptides

Identification of Kglu peptides by MS

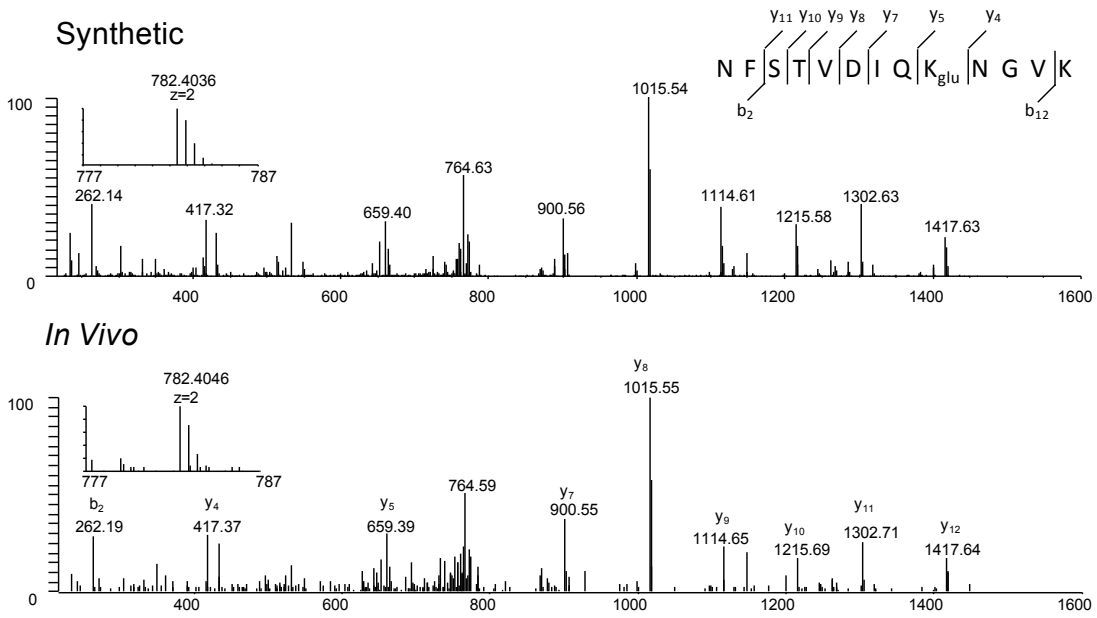


Kglu (+114 Da) peptides identified by MS from HeLa cells.

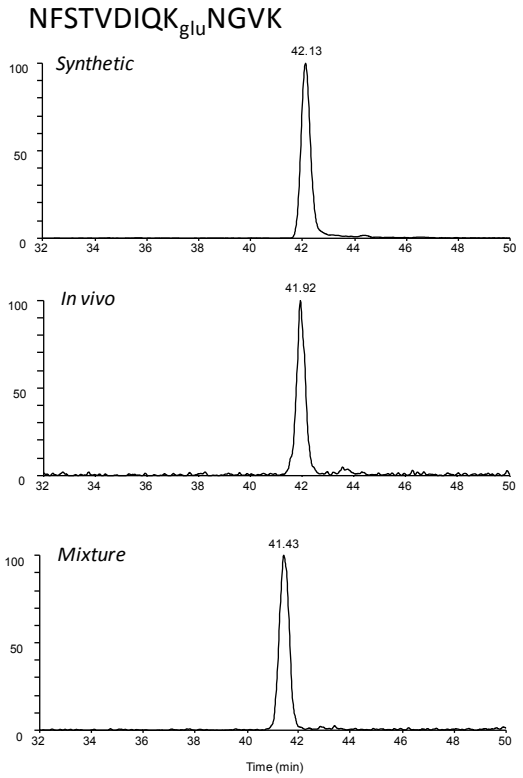
Peptide Sequence	Protein Name	Site
NFSTVDIQKNGVK	DNA mismatch repair protein Msh2	K546
TVDGPSGKLWR	Glyceraldehyde-3-phosphate dehydrogenase	K194
VGMGGMEAKVK	Delta-1-pyrroline-5-carboxylate synthase	K311
SDVYYFSPSGKK	Methyl-CpG-binding domain protein 2	K185
ADGKISEQSDAK	ATP synthase subunit alpha	K531
TPVTDPATGAVKEK	Very-long-chain specific acyl-CoAdehydrogenase	K322
VIQGAGKLPR	Zinc finger/BTB domain-containing protein 7A	K396
NFGTKISAR	6-phosphofructokinase type C	K688
GKGGEIQPVSVK	10 kDa heat shock protein	K56
FASDPIIKGSGTAEVELK	Pyruvate kinase	K105

Validation of Kglu

1. MS/MS analysis

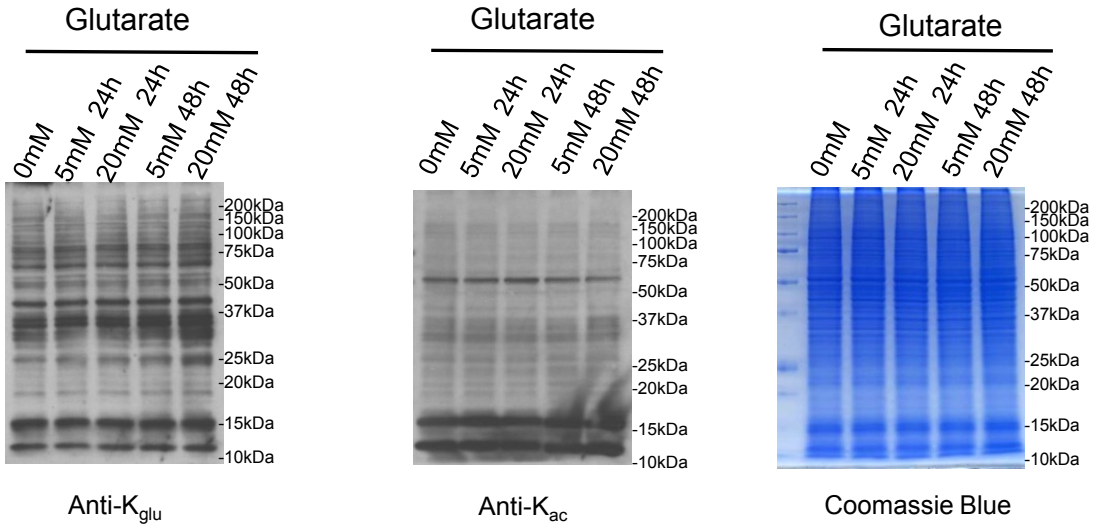


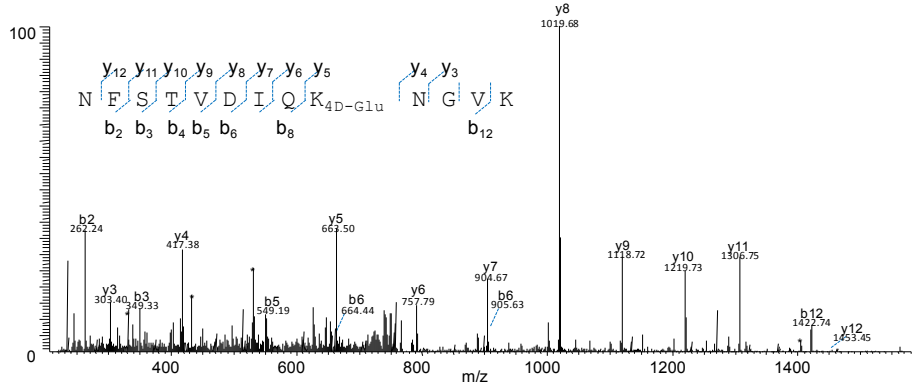
2. HPLC Co-elution



Validation of Kglu

3. In vivo D4-glutrate labeling

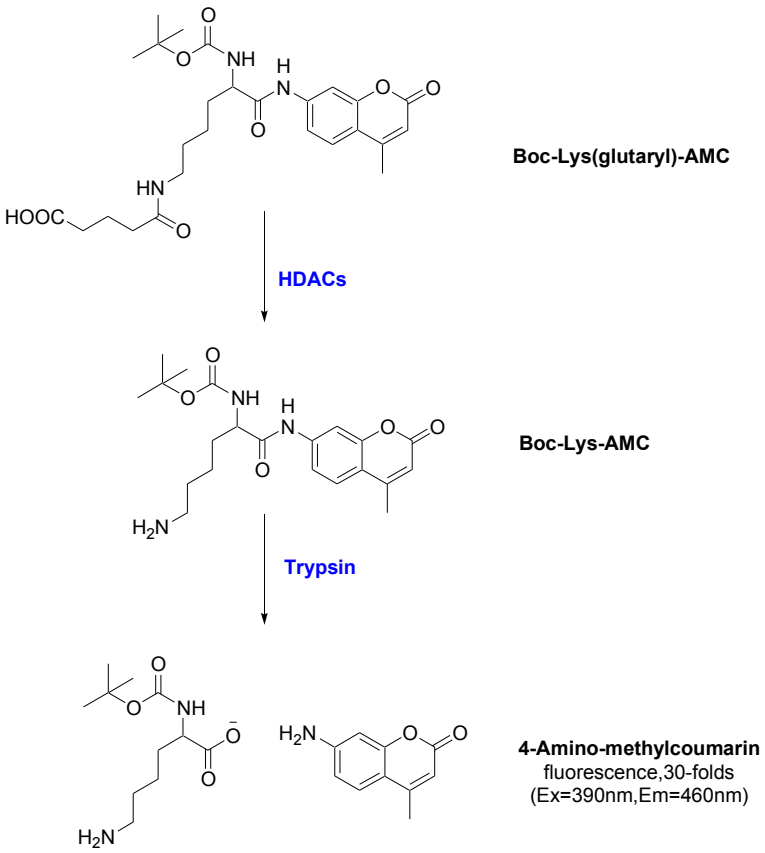


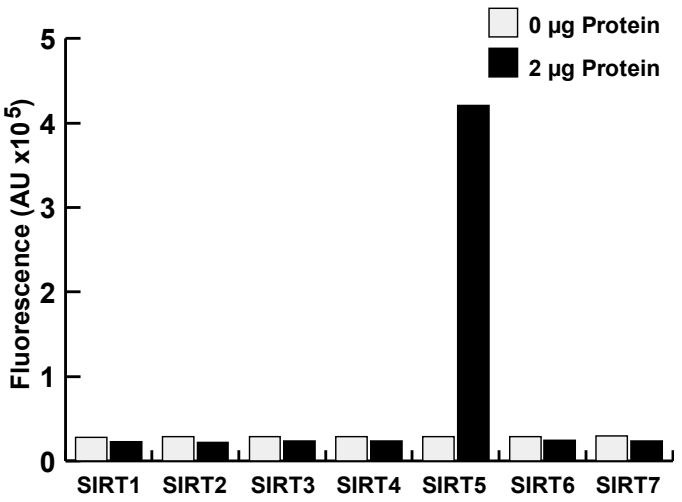


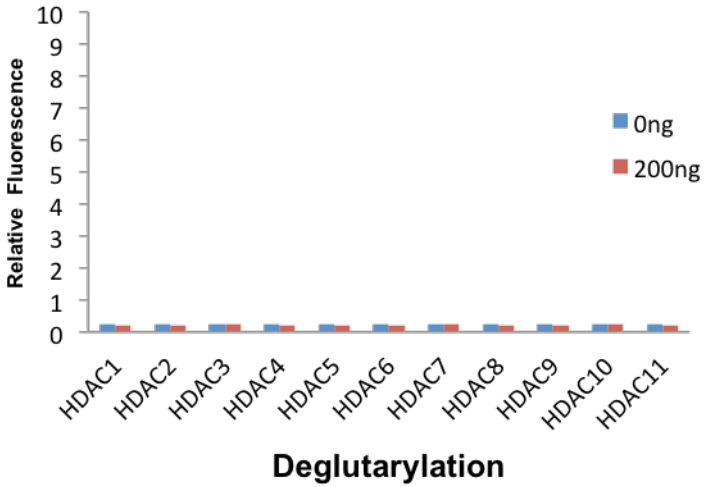
What is the regulatory enzyme?

Screening regulatory enzymes for deKglu

Acyllysine compound based assay

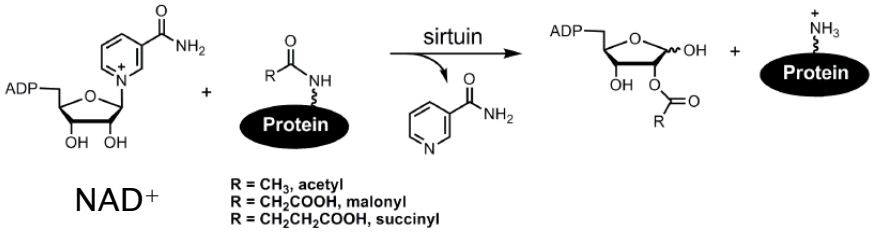


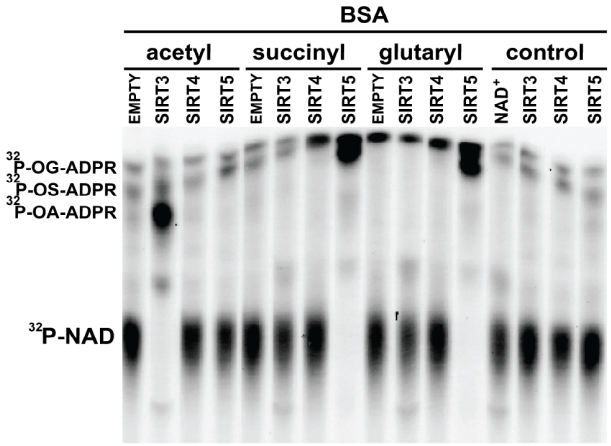




SIRT5 as a deKglu enzyme in vitro

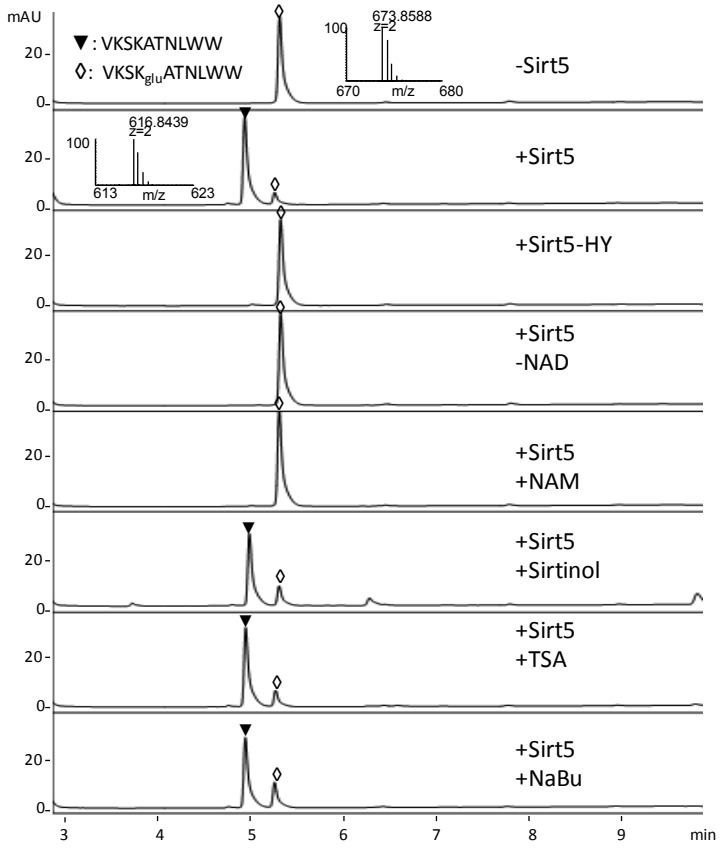
^{32}P-NAD^{+} consumption assay



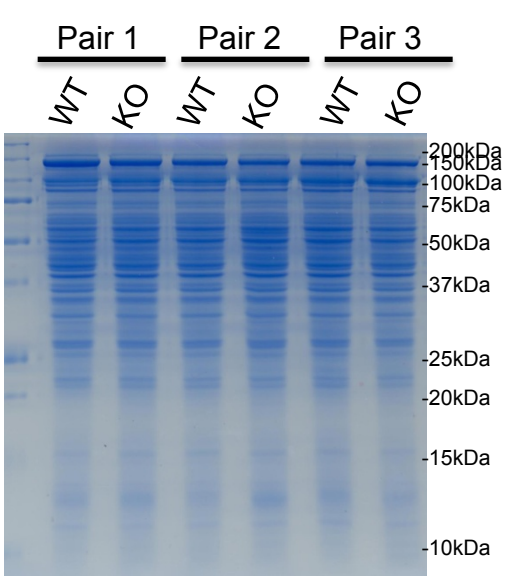


o-glutaryl-ADP ribose, OG-ADPR;
o-succinyl-ADP ribose, OS-ADPR;
o-acetyl-ADP ribose, OA-ADPR.

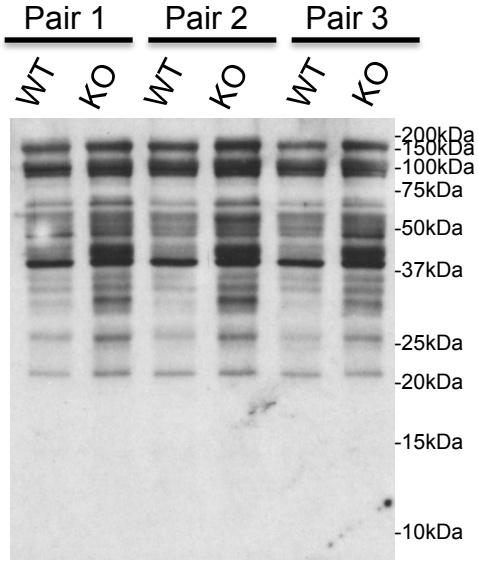
Kglu peptide assay



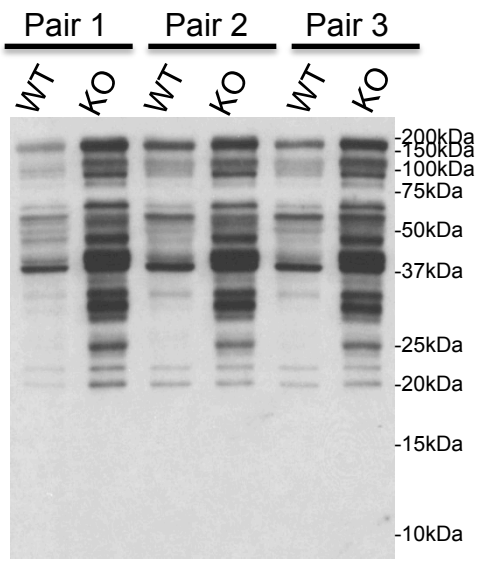
SIRT5 catalyzes deKglu in vivo



Coomassie Blue



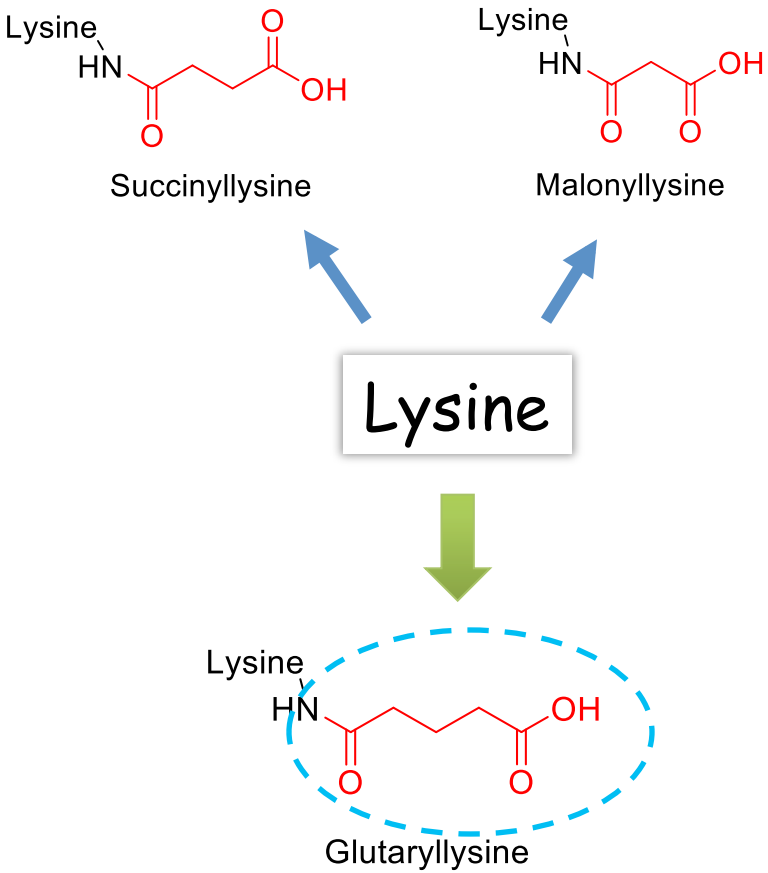
Anti-K_{glu}

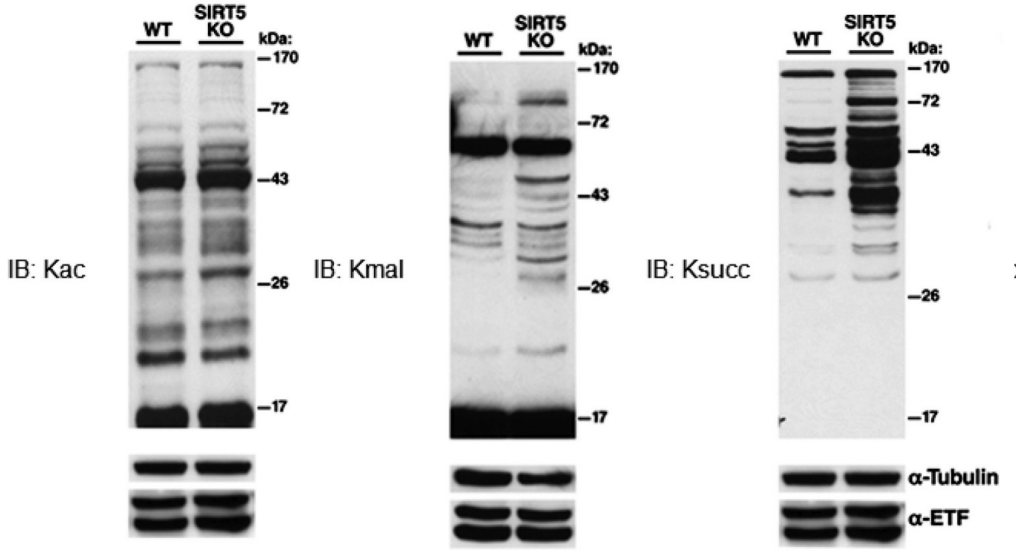


Anti-K_{succ}

Mitochondrial lysates from WT and Sirt5 KO mouse livers

Sirt5 is the regulatory enzyme for Kmal/Ksucc

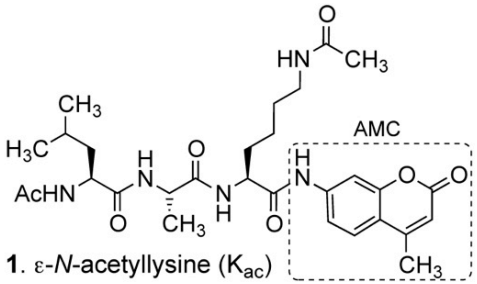




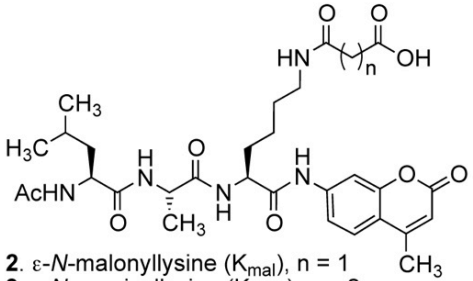
Peng et al, Mol Cell Proteomics. 2011, ,M111.012658.

What are the preferred deacylation susbtrates of SIRT5?

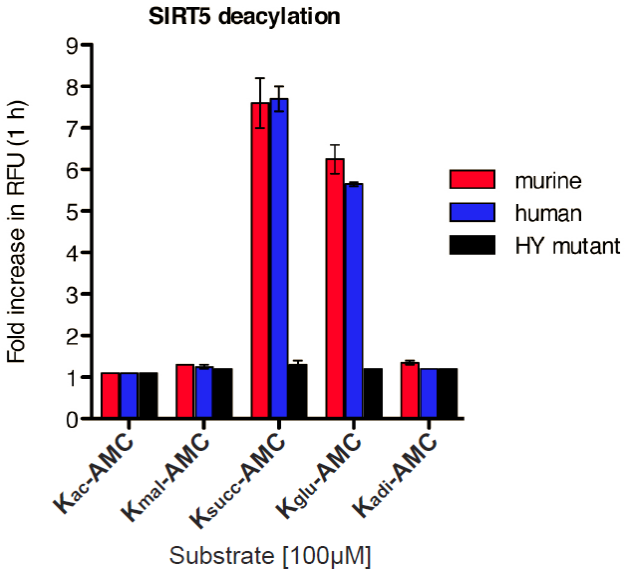
Kinetic studies for SIRT5 deacylation activity

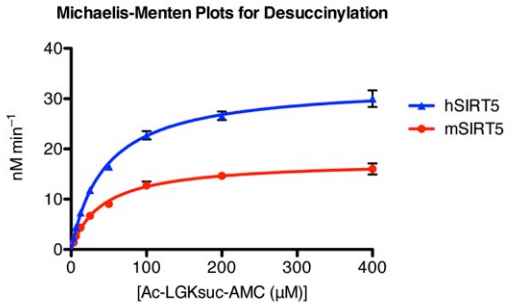


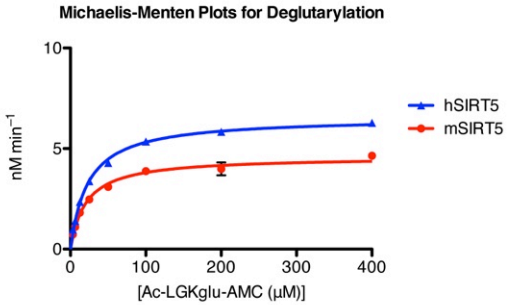
1. ε-*N*-acetyllysine (K_{ac})



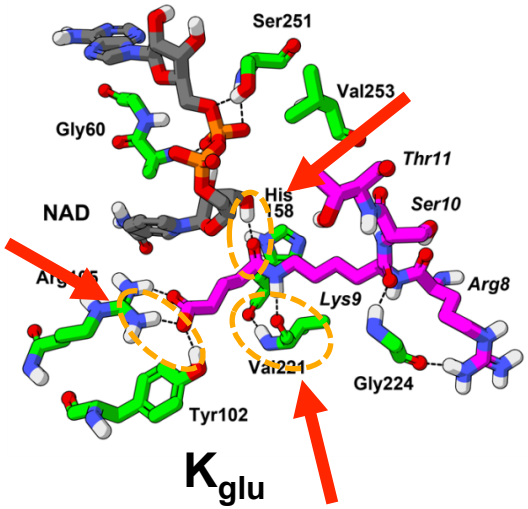
2. ε-*N*-malonyllysine (K_{mal}), n = 1
3. ε-*N*-succinyllysine (K_{succ}), n = 2
4. ε-*N*-glutaryllysine (K_{glu}), n = 3
5. ε-*N*-adipoyllysine (K_{adi}), n = 4

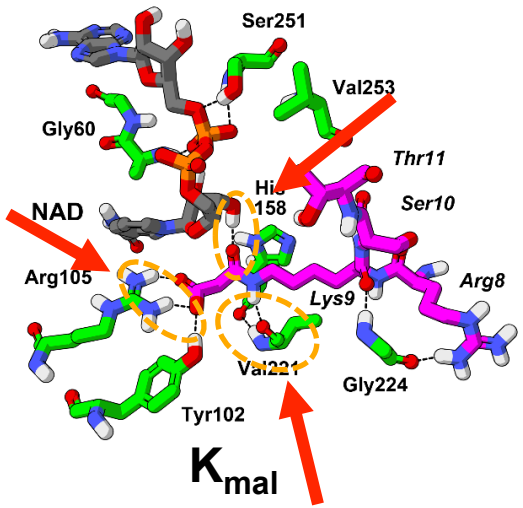


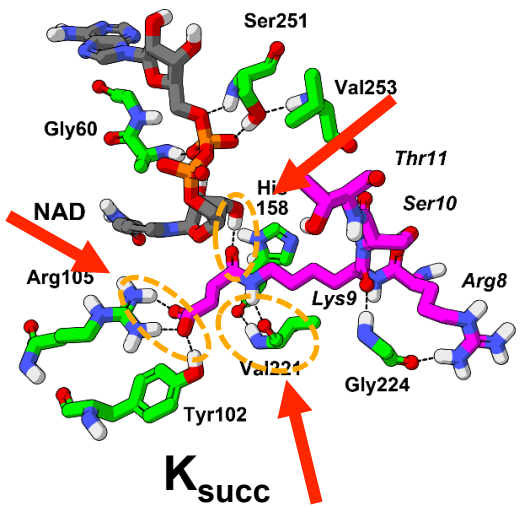


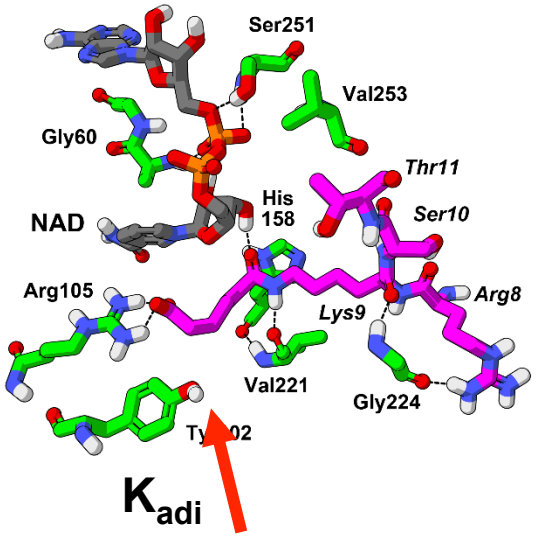


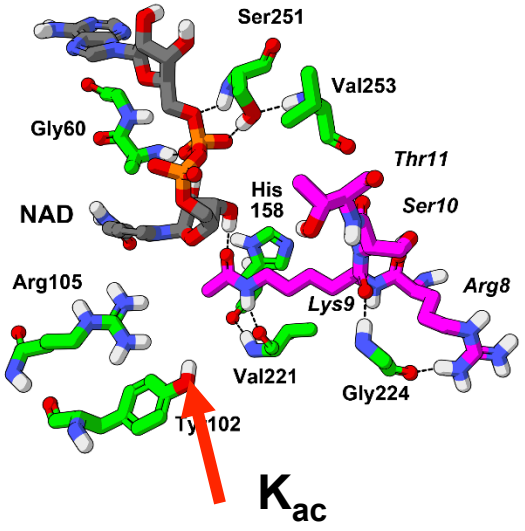
In silico molecular modeling



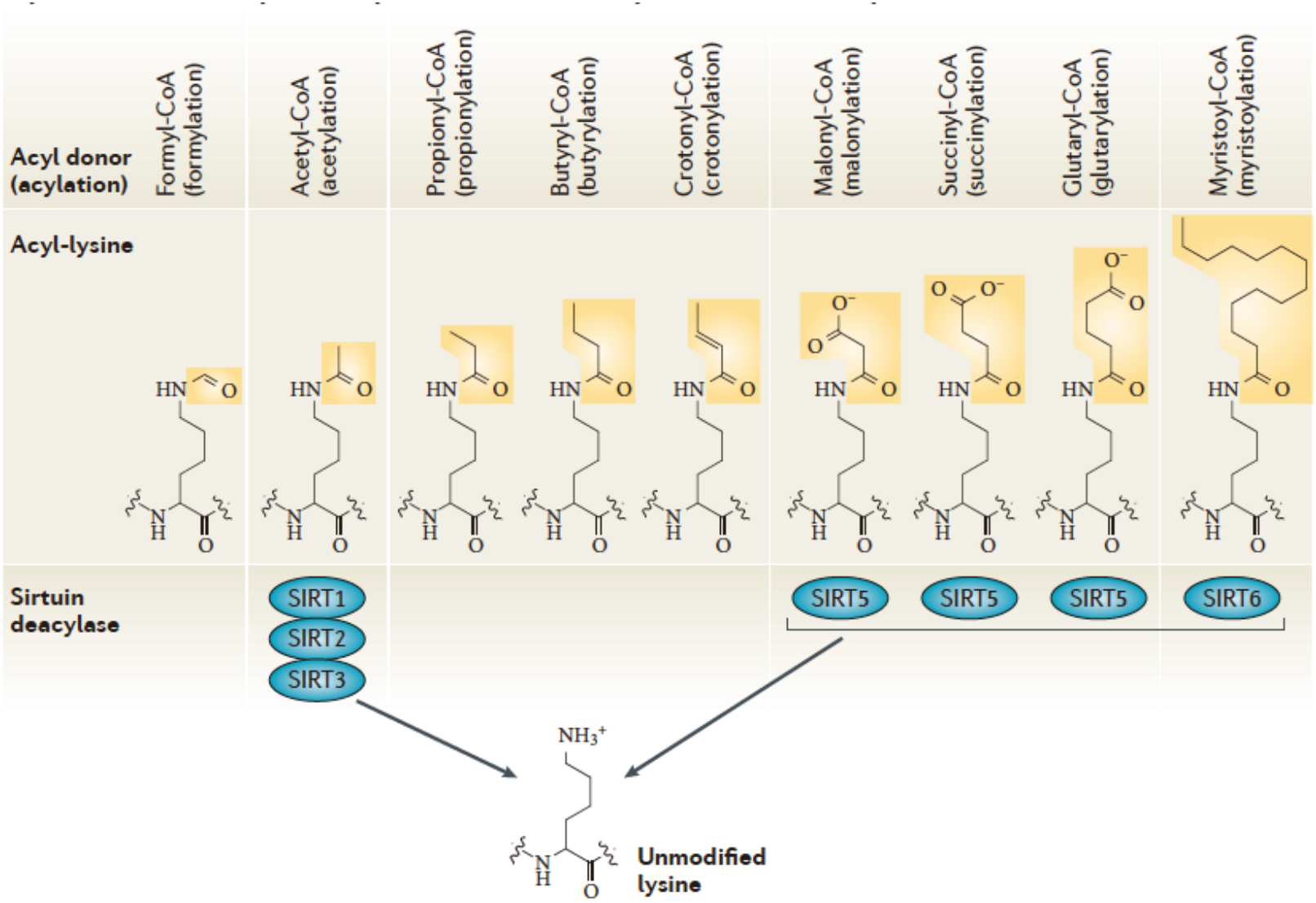








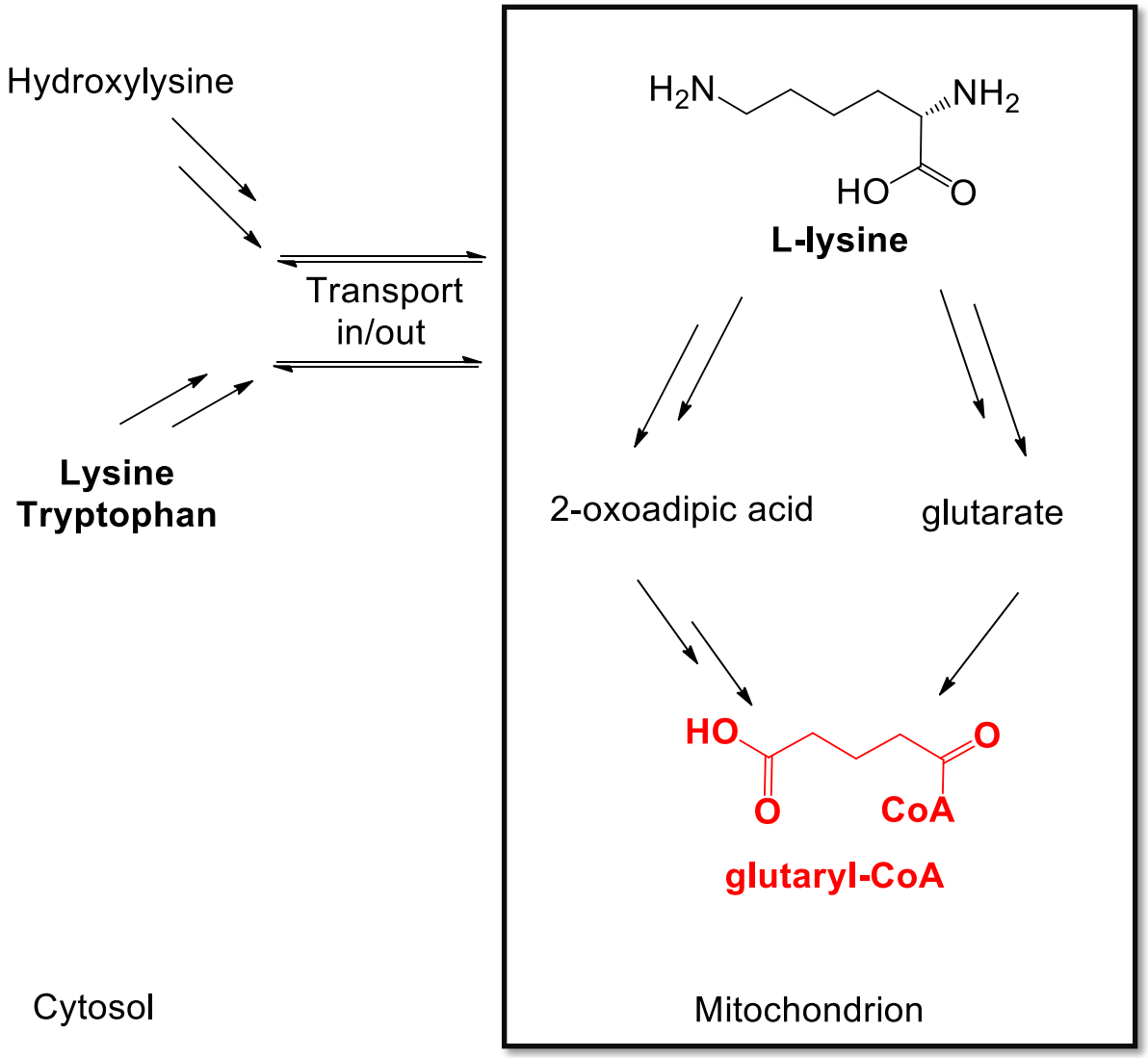
Lys Acylation and Sirtuins



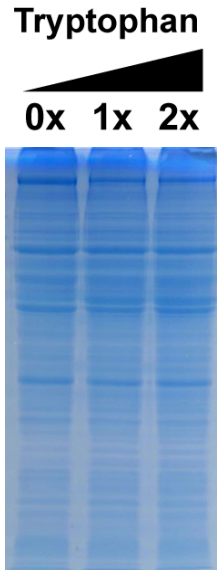
Mann M., et al, Nat Rev Mol Cell Biol. 2014 Aug;15:536-50.

Physiological significane of Kglu?

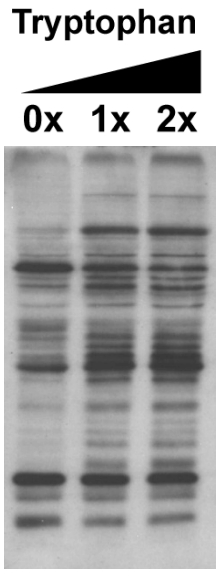
Biosynthetic Pathway of Glutaryl-CoA



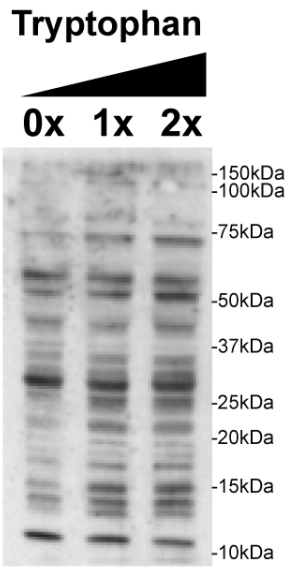
Protein hyperglutarylation is sensitive to dietary changes



Coomassie Blue



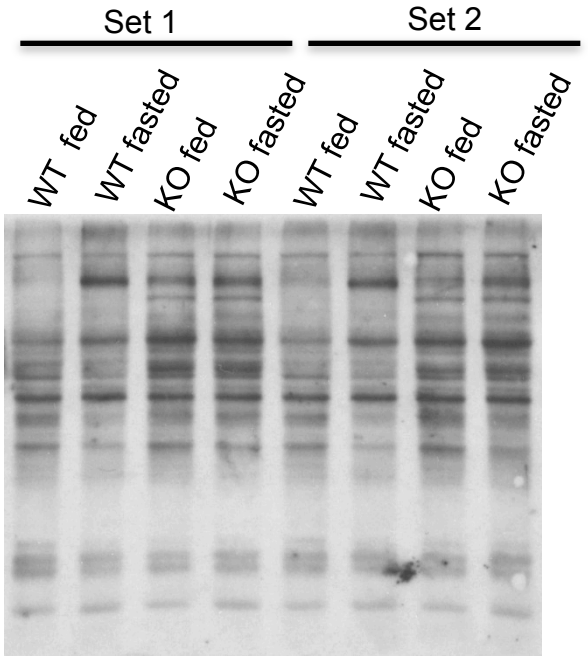
Anti-K_{ac}



Anti-K_{glu}

The effect of tryptophan on the Kglu levels of Drosophila

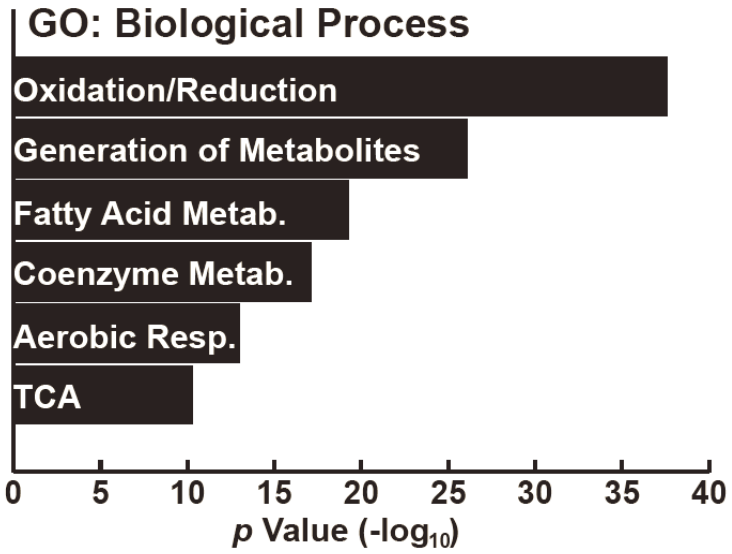
Protein hyperglutarylation is sensitive to dietary changes

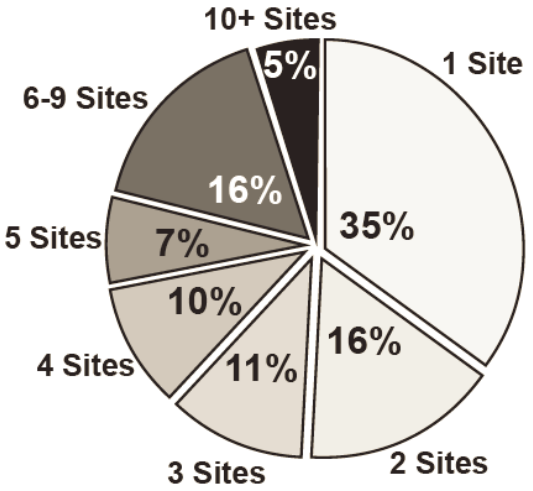


Lysate from WT and Sirt5 KO 48h-fed and fasted mice livers.

Proteome-wide survey of Kglu sites in SIRT5KO mouse liver

683 Kglu sites in 191 proteins

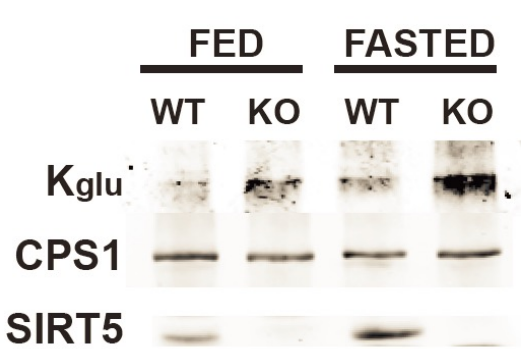


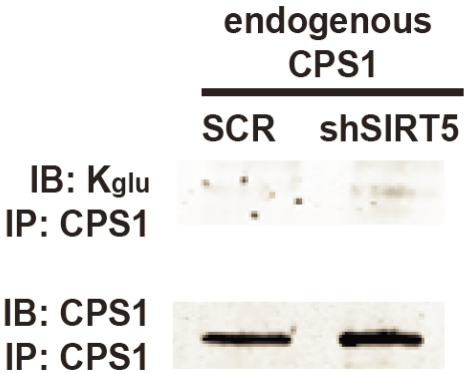


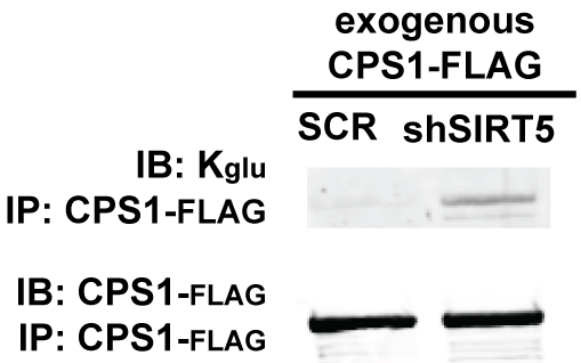
Carbamoyl-phosphate synthetase 1 氨甲酰磷酸合成酶 (CPS1): 33 Kglu sites

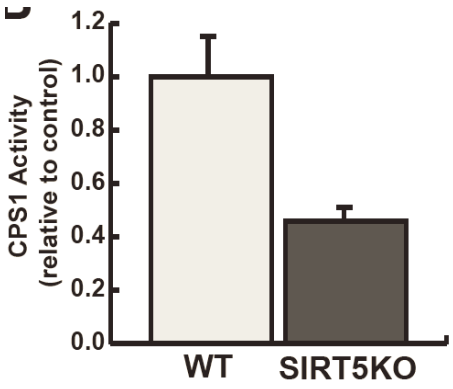
Key enzyme for ammonia detoxification

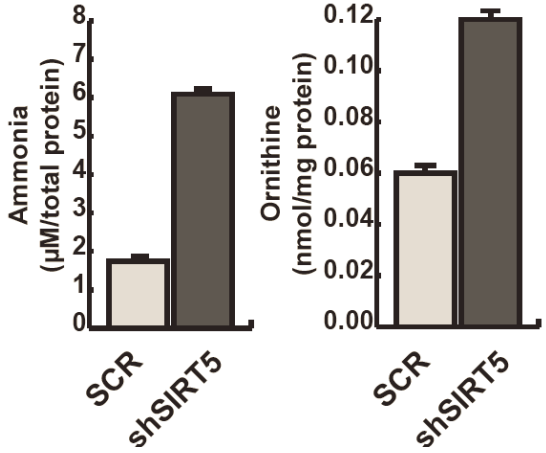
CPS1 is targeted for deKglu by SIRT5





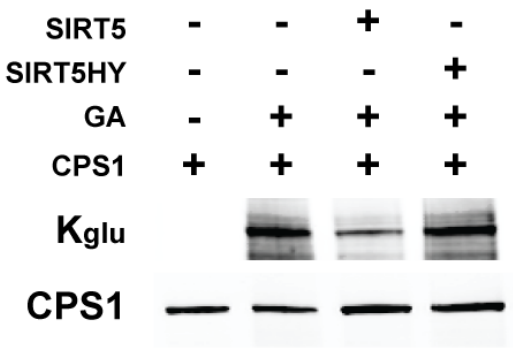






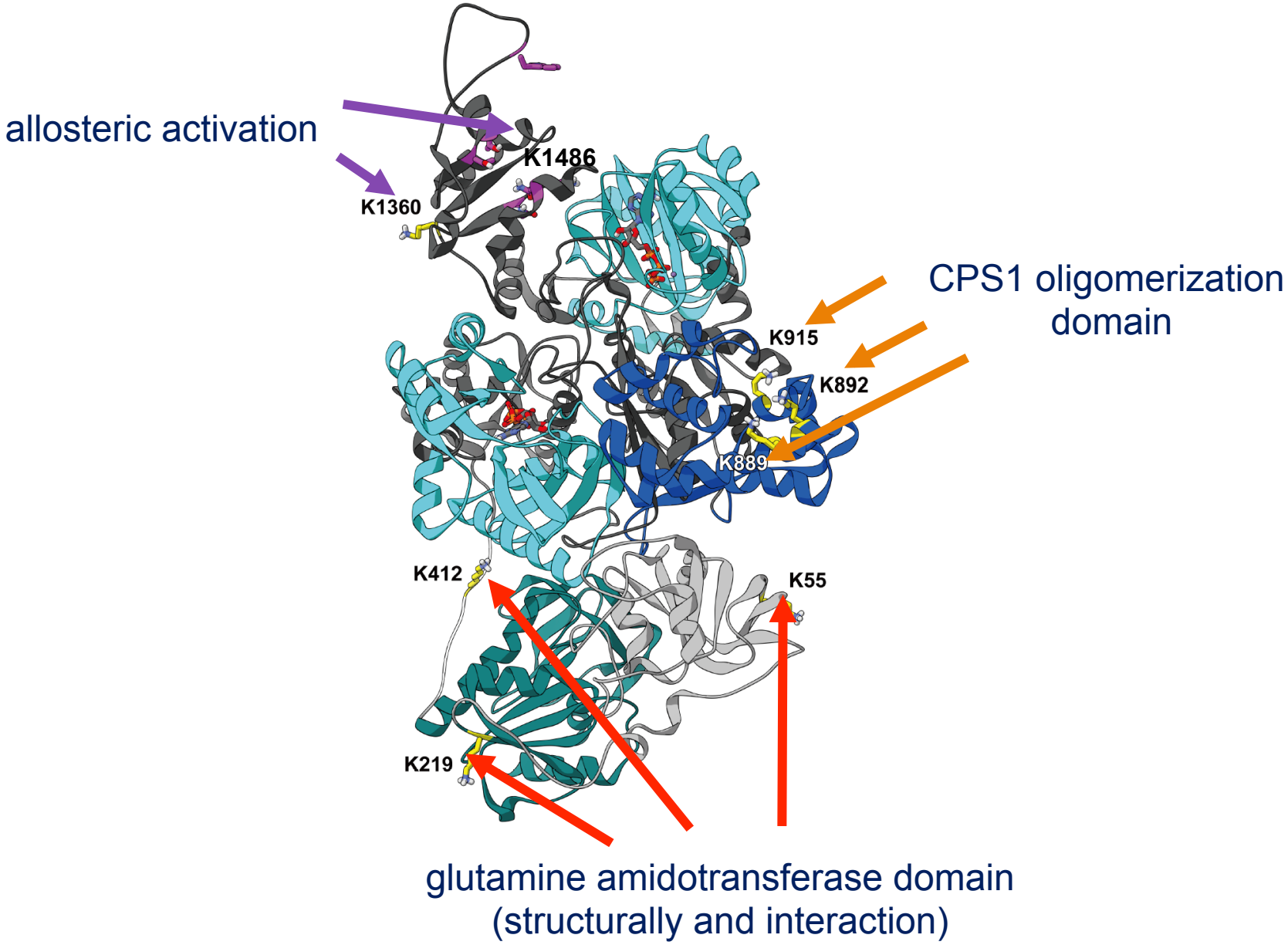
Candidate Kglu sites regulated by SIRT5

Chemical glutarylated CPS1



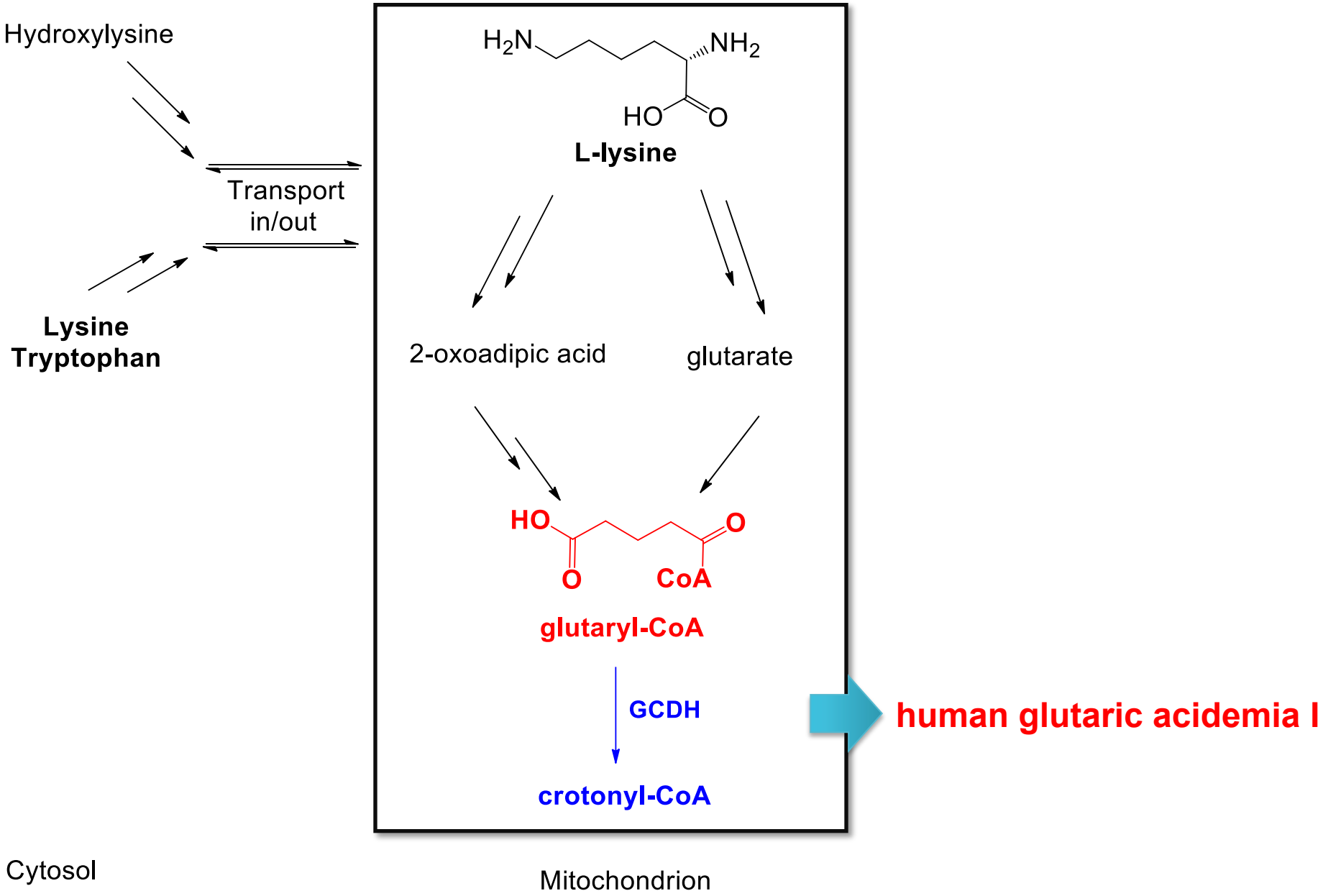
Kglu site	Peptide Sequence	SIRT5 -	SIRT5 +	In vivo
K55	AQTAHIVLEDGTKMK	Yes	No	Yes
K219	GNPTKVVAVDCGIK	Yes	No	Yes
K412	ATTITSVLPKPALVASR	Yes	No	Yes
K889	DILNMEKTLK	Yes	No	Yes
K892	TLKGLNSESMTEETLK	Yes	No	Yes
K915	AKEIGFSDKQISK	Yes	No	Yes
K1360	IPQKGILIGIQQSFRPR	Yes	No	Yes
K1486	KVDSKSLFHYR	Yes	No	Yes

Structural analysis on glutarylated CPS1 homology model

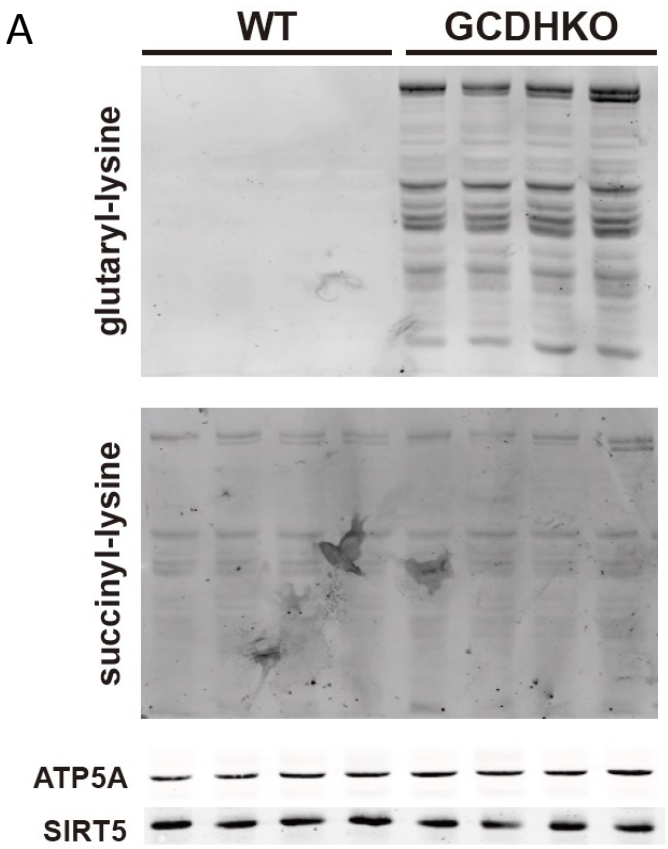


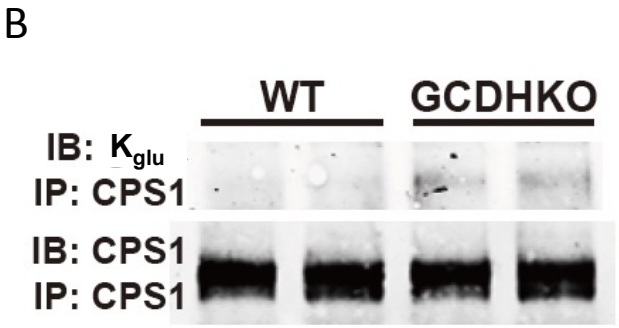
Pathological singnificance of Kglu?

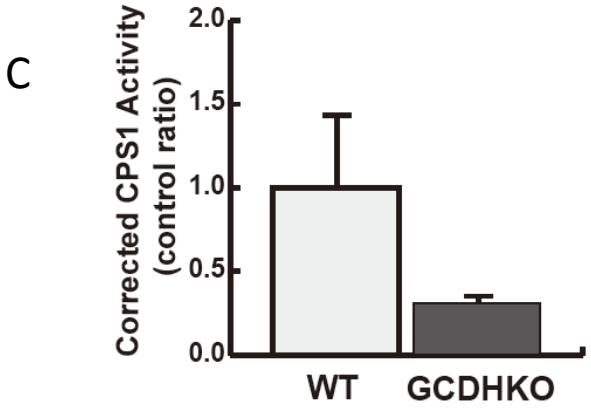
Biosynthetic Pathway of Glutaryl-CoA



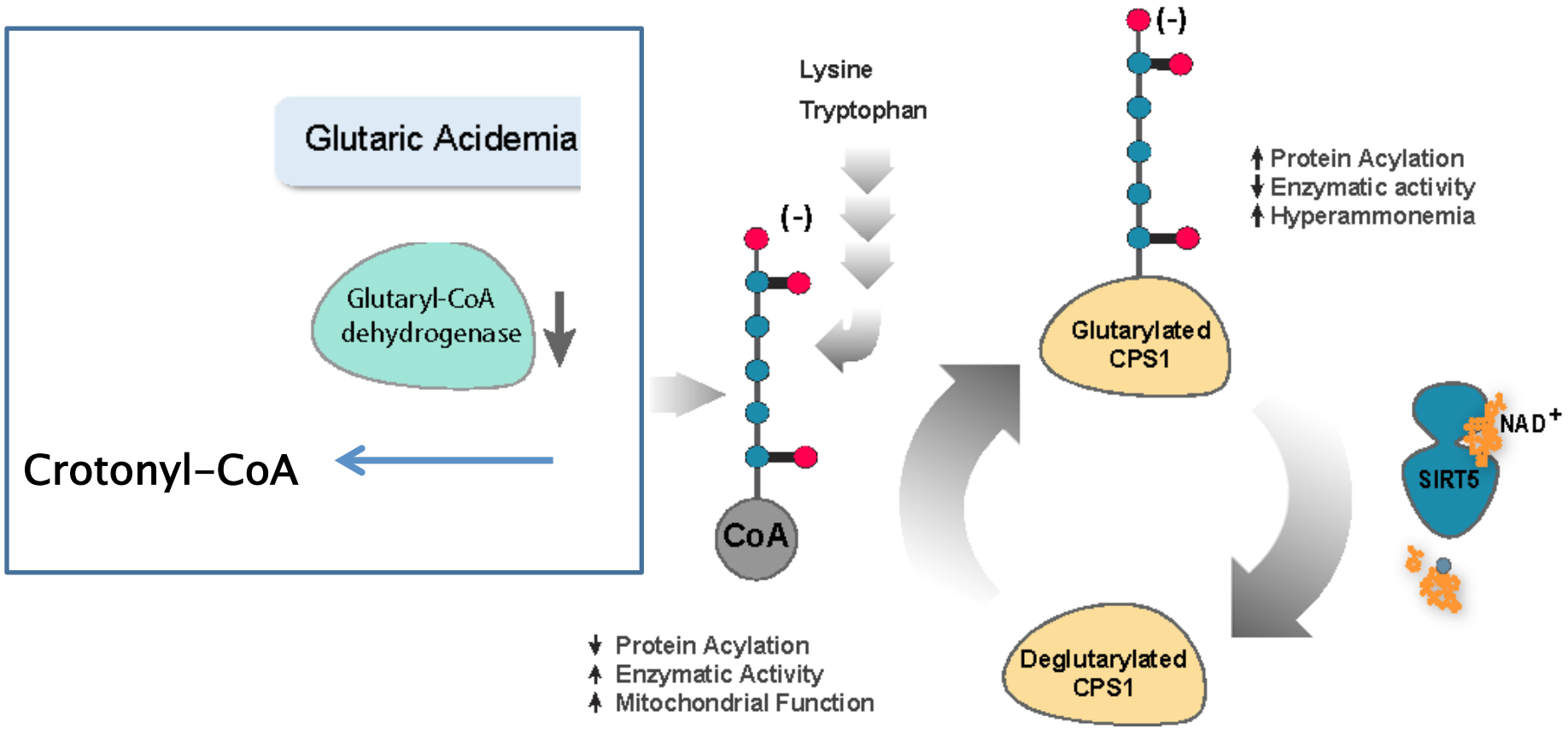
Protein hyperglutarylation in GCDH deficient mouse







Kglu and Glutaric Acidemia I (GA)



Tan et al, *Cell Metabolism*, 2014, 19, 605-17.

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