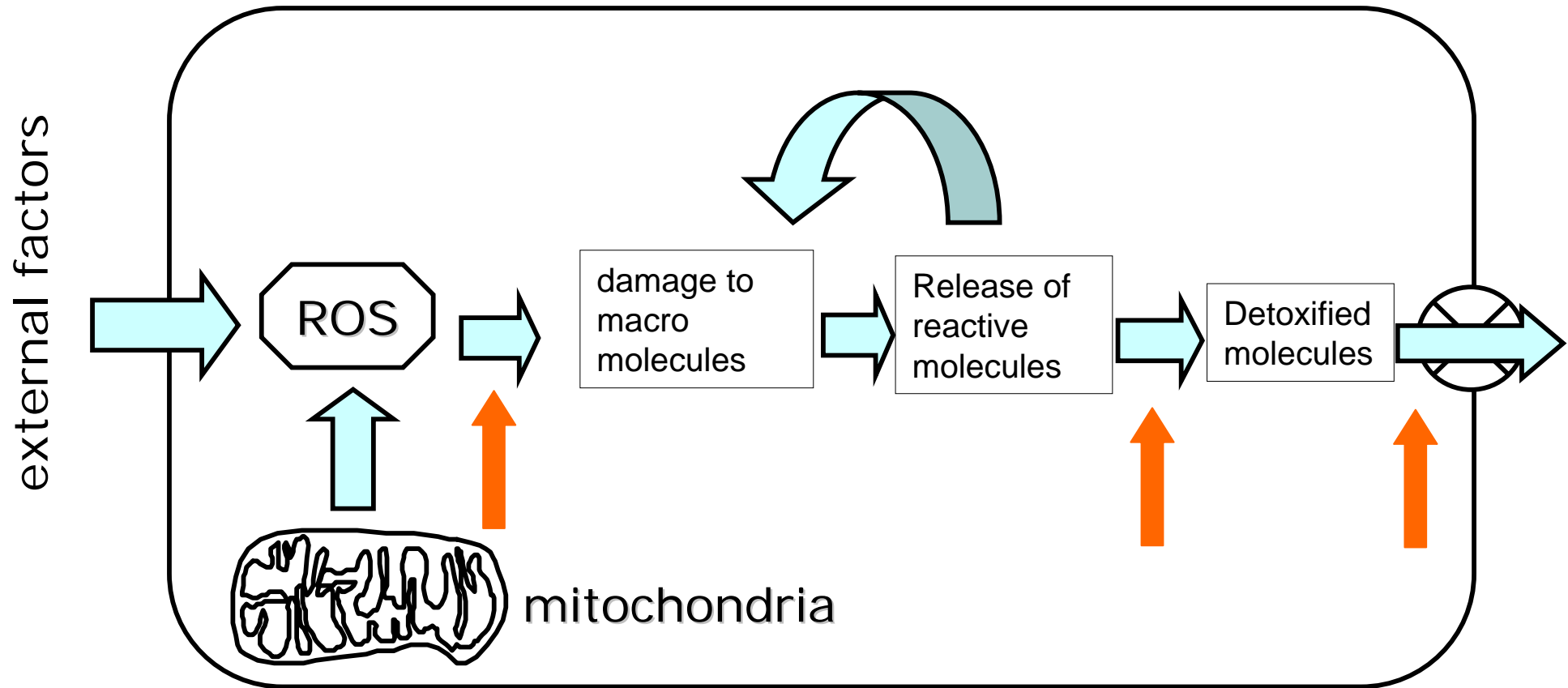


# Analyzing the Relationship Between Oxidative Stress and Aging in *Drosophila*

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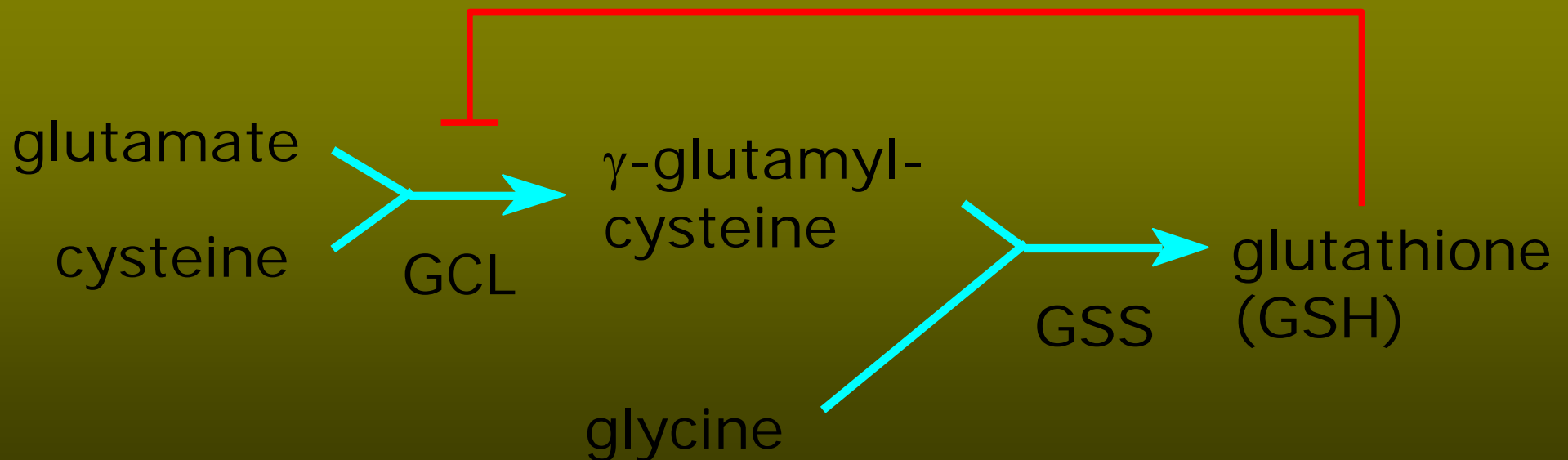
# Glutathione-dependent defence against ROS



# Background to GCL



- GCL catalyses the rate-limiting step in glutathione biosynthesis
- Activity is inhibited by glutathione at physiological concentrations



# Why is glutamate cysteine ligase (GCL) potentially important with regard to ageing?



- It is a critical regulator of **Glutathione**, the ‘master antioxidant’.
- Glutathione is an endogenously synthesised antioxidant which is of central importance in regulating levels of reactive oxygen species and environmental toxins in cells.

# GCL in mammals



- Holoenzyme comprises catalytic (GCLC) and modifier (GCLM) subunits
- GCLC has catalytic activity but the holoenzyme is:
  - catalytically more efficient
  - less susceptible to inhibition from GSH
- Non-mammalian GCLM homologues not identified until recently
- Kinetics suggest it may not be required for efficient catalysis in some species

# Do levels of glutathione influence life span?



- We wished to test the hypothesis that an increased capacity to synthesise glutathione would extend life span, using *Drosophila* as a model system.
- Alteration of glutathione levels would be achieved by modulating GCL activity
- This first required the molecular characterisation of GCL in *Drosophila*.....

# Molecular characterisation of *Drosophila* GCL



- DmGCL is a heterodimer comprising a catalytic (DmGCLC) and regulatory (DmGCLM) subunit
- Transcription of DmGCLC is induced by oxidative stress
- DmGCLM modifies activity of DmGCLC in a similar way to the mammalian form
- Disulphide bridges form between DmGCLC and DmGCLM
- The cysteine residues on DmGCLM that could interact with DmGCLC were identified using iodoacetamide modification
- DmGCLM lacking the critical cysteine residues does not form covalent linkages with DmGCLC under non-reducing conditions.

# The cysteine residues in DmGCLM that could interact with DmGCLC identified by iodoacetamide modification



DMGCSL 1 ~~~~~MIPTITKKYQNVVIS**TGN**IIATELGQR**K**...S**NEELYDGLKI**TLHTD**S**TAERV  
 HGCSL 1 MGTDSRAAKALLARARTLHL**QTGN**LLNWGRLR**KK**CPSTH**SEELHDCI**Q**KT**LN**EW**S**S**QINP

DMGCSL 51 VVE**KEI**.**DE**THGRV**QR**ATQELTTRL**TENGRNE**ISIGAKIFL.NRH**STESVNQ**AVEELLHI  
 HGCSL 61 DLV**REFPDV**LECTV**SHAVE**...KINPDER**EEMKVSAK**L**F**IVES**NSSSSTRSA**VD**MACSV**

E

DMGCSL 109 **LSV**THVD**NV**V**L**AYHPNAVATATPVATT**KPP** **S**ED**S**N**V**SRATNWSQRNGKEGVAEL**K**E**LYK**  
 HGCSL 117 **LGVA**Q**LDS**V**IIA**.....**SPPI**EDGV**NLS**.....LEHL**Q**PY**WE**

AB

C

DMGCSL 169 **TLE**QYALK**QQIT**QLGIADLDAA**LEEL**HNSAQVVPTIAQVN**IST** **V**VPPE**IQEF** **T**AHD  
 HGCSL 149 **ELEN**LVQSK**KIVA**IG**TS**DL**DKTQ**LE**QLYQWAQV**K**PNS**NQVN**IAS** **V**MP**PDITAF**AK**QFD**

D

DMGCSL 229 **IQL**N**THSD**PE**LL**PVE**QF**.**DGI**...V**PGYTID**.....**WTL**RYQ**VHVF** **R**GV**LTAKGY**IVG  
 HGCSL 209 **IQL**L**THNDP**K**ELL**SEAS**FQEA**L**QES**IPDIQAHEWVPL**WLL**RY**SVIVK**SRGI**IKSKGY**ILQ

DMGCSL 280 **AS**RSSV  
 HGCSL 269 **AK**R**R**GS

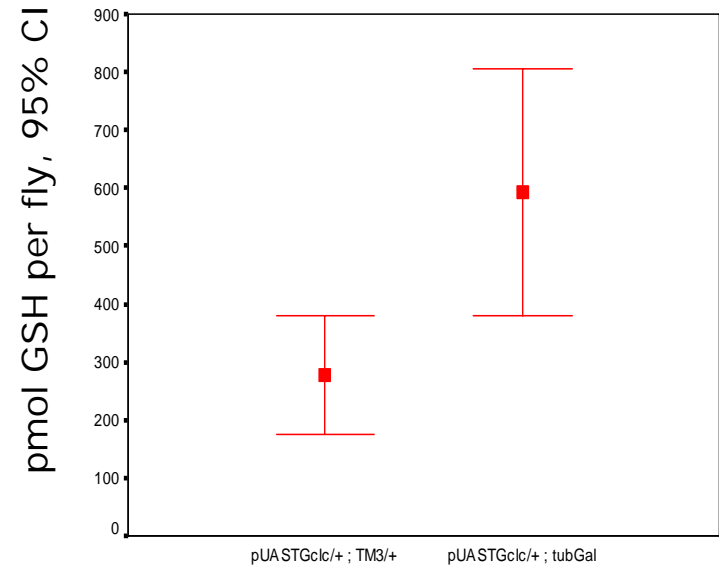
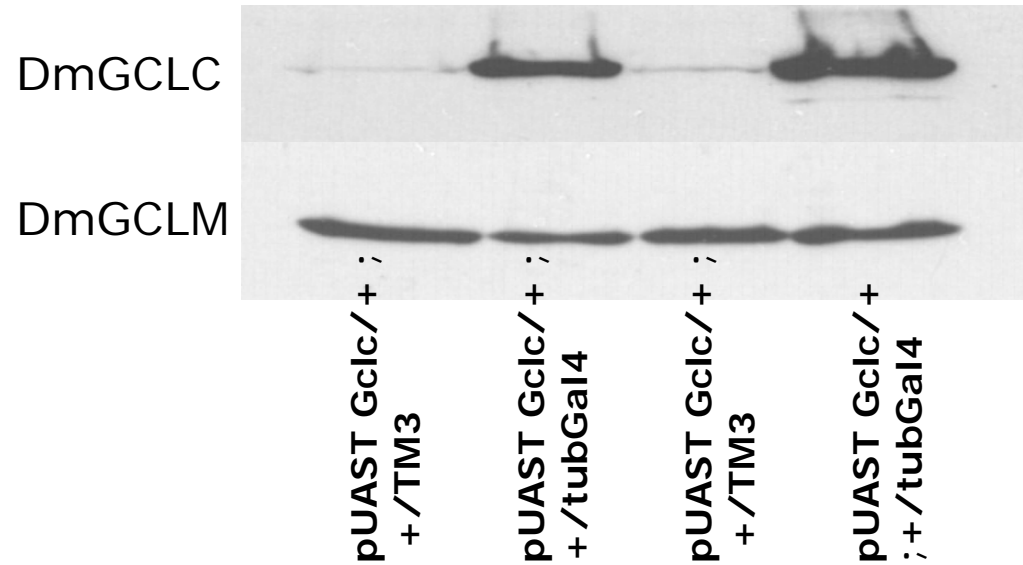
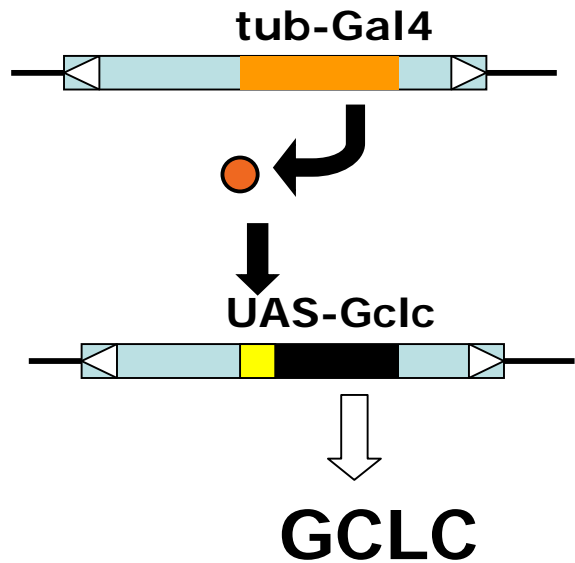
DmGCLM lacking the critical cysteine residues A B and D fails to establish covalent linkage with DmGCLC

# Elevating GSH titre by transgenic overexpression of DmGCL

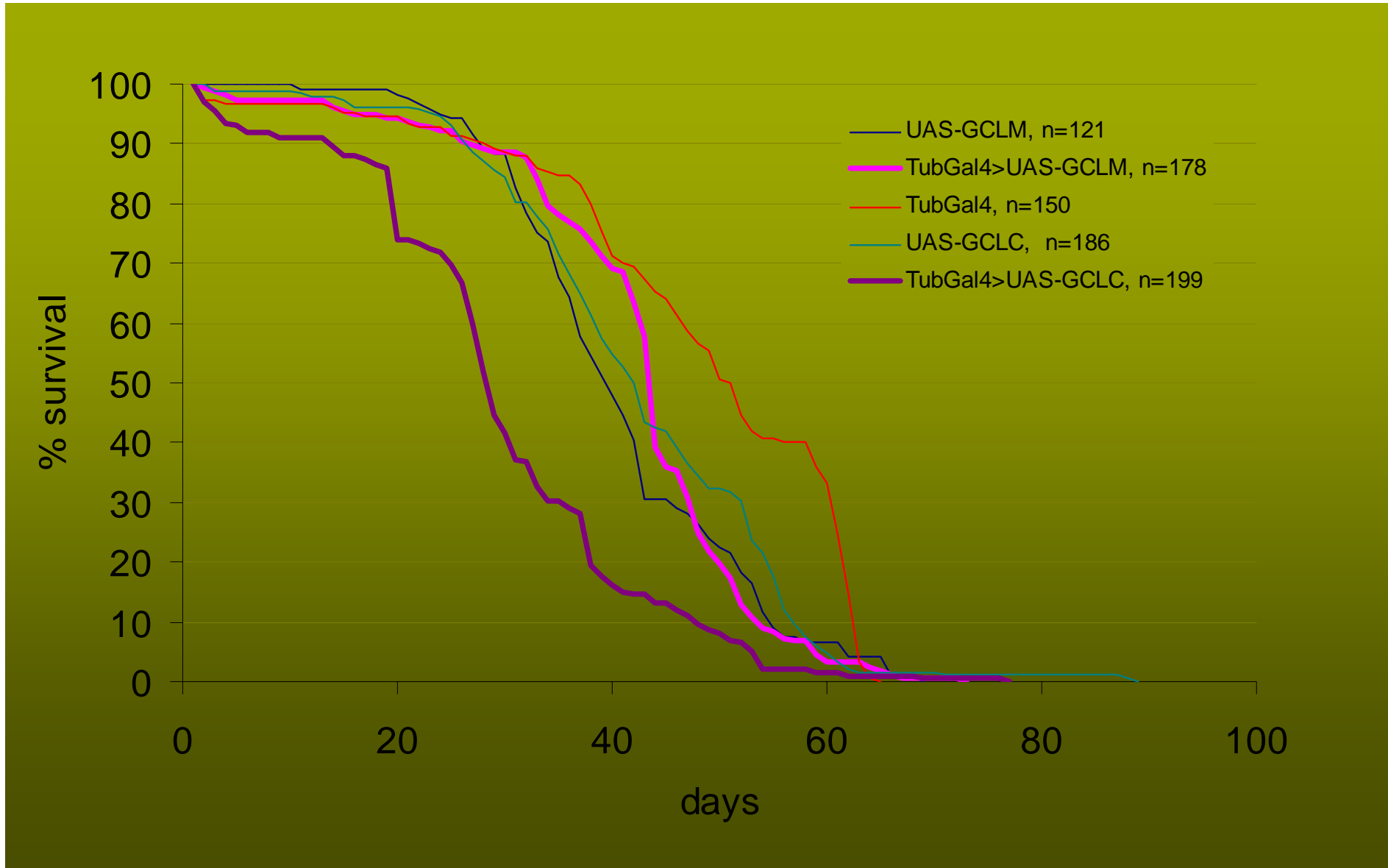


- Technology for transgenic expression well established in *Drosophila*
- We have used the Gal4-UAS system
  - Wide selection of P elements expressing the transcription factor Gal4 in specific spatiotemporal patterns
  - Gal4 drives expression of transgene via the UAS
- Can mix and match Gal4 drivers with UAS responders
- **Cautionary notes:** the expression patterns of drivers may be unexpected; effects on complex traits may be masked by genetic background

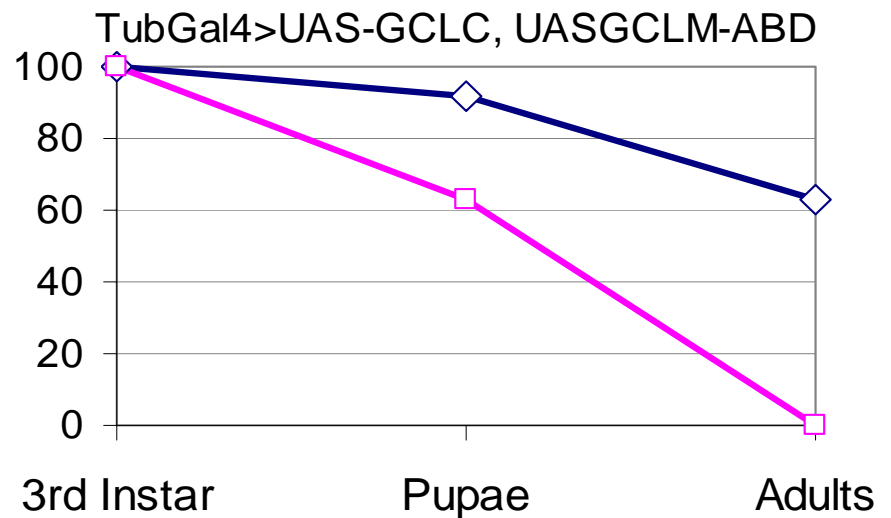
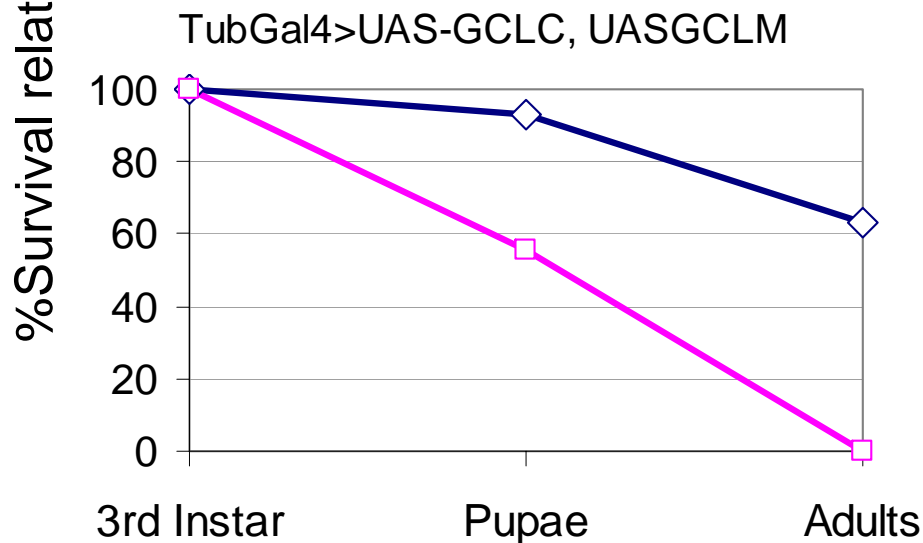
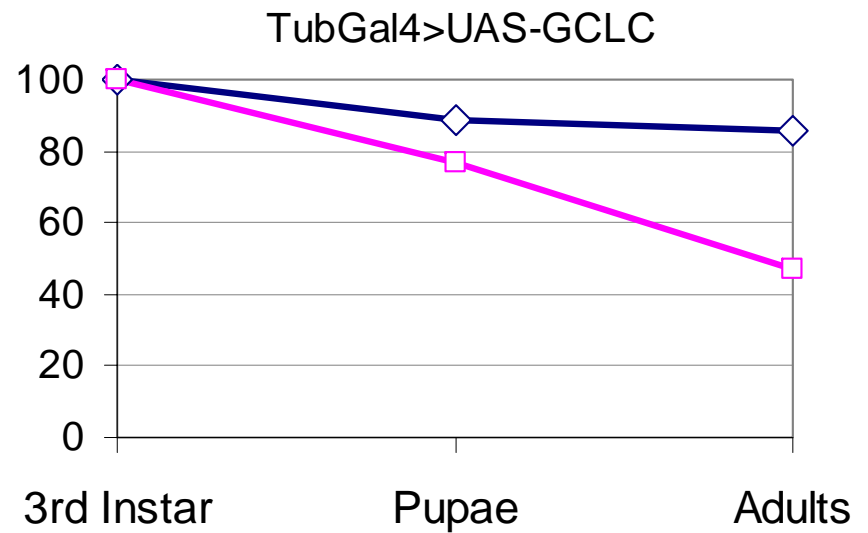
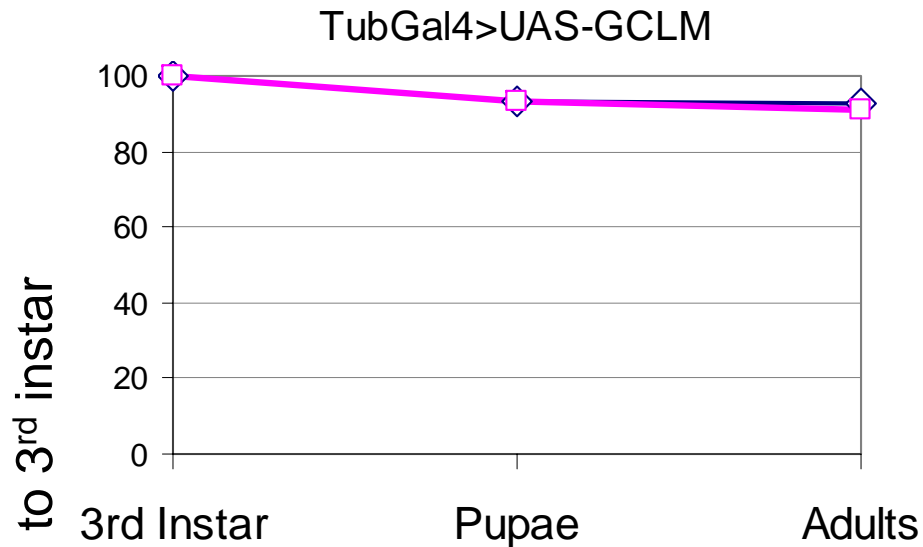
# *tubGal4* drives overexpression of *pUASTGclc*



# Ubiquitous overexpression of GCL does not extend lifespan

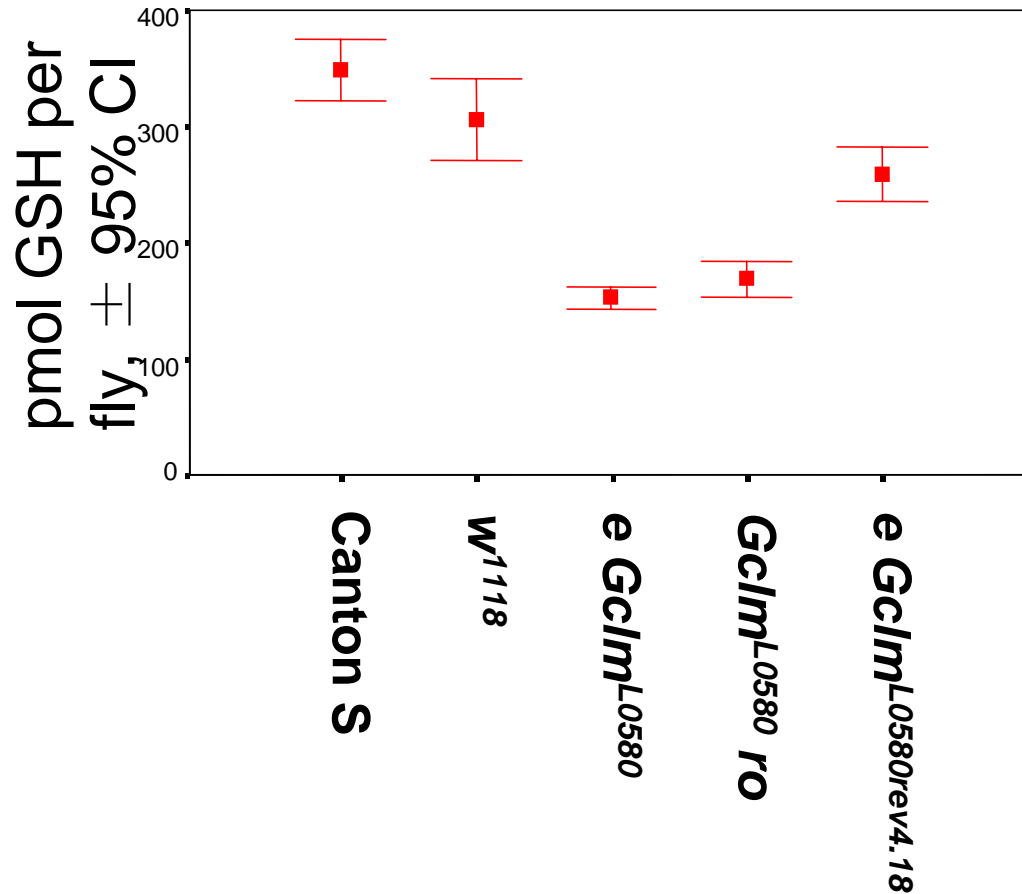


# Ubiquitous overexpression of both subunits of GCL is lethal





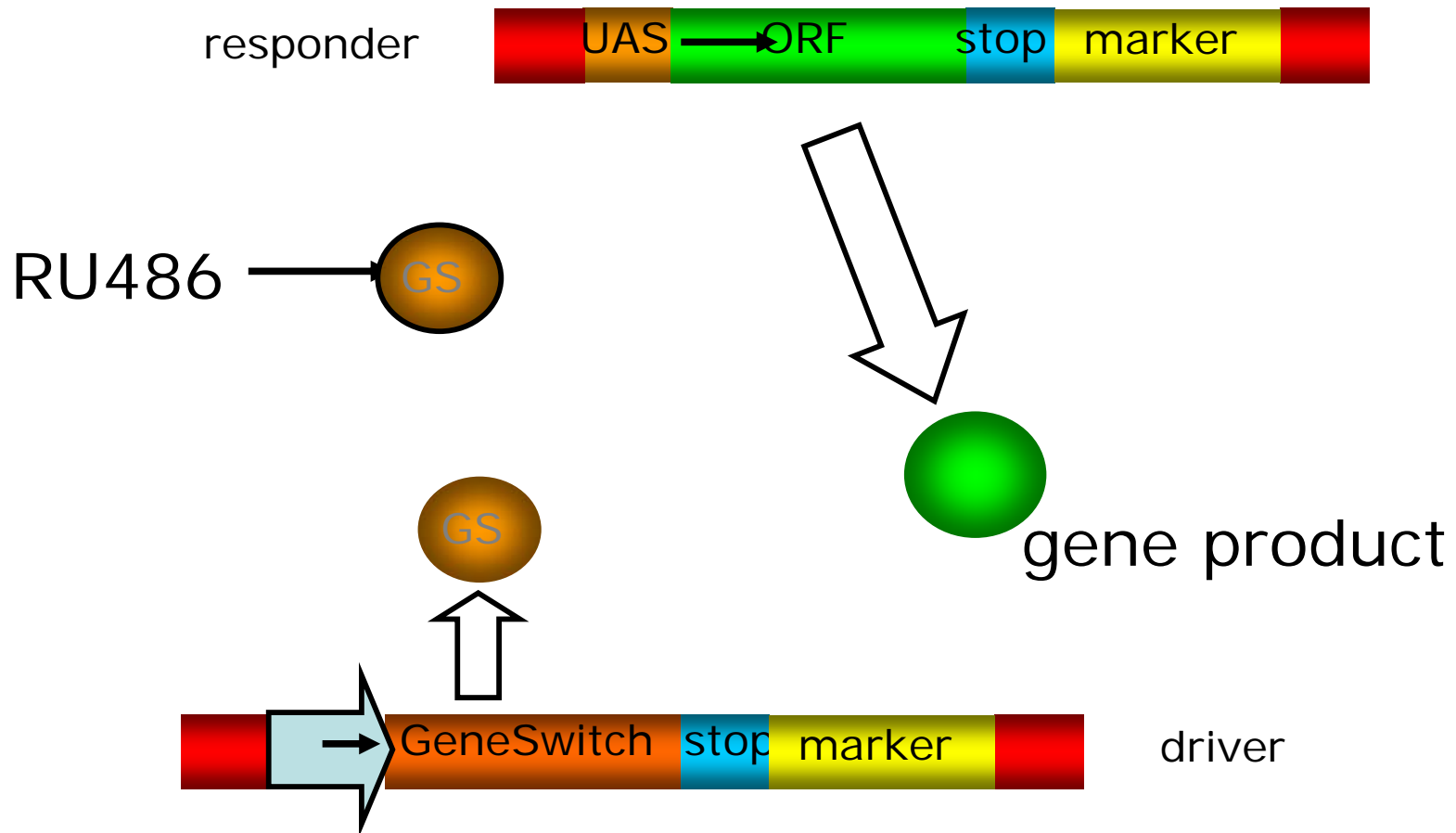
# *Gclm*<sup>L0580</sup> is a strongly hypomorphic allele



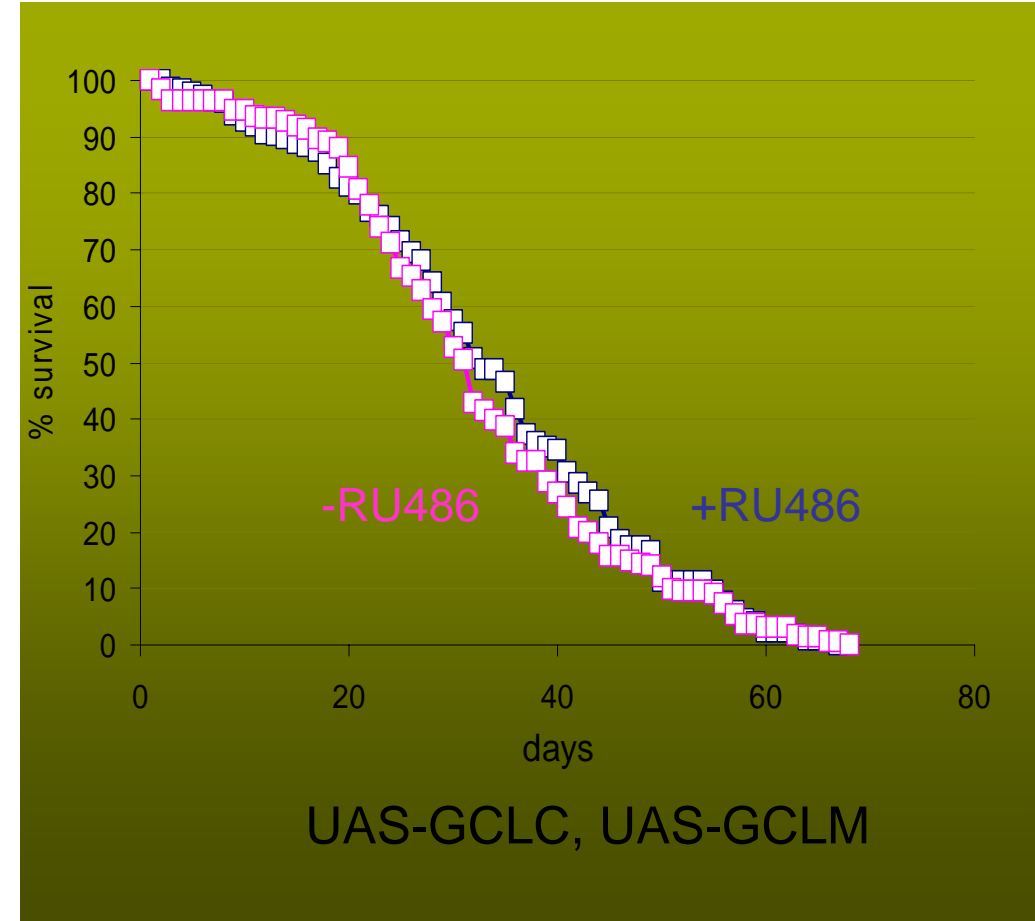
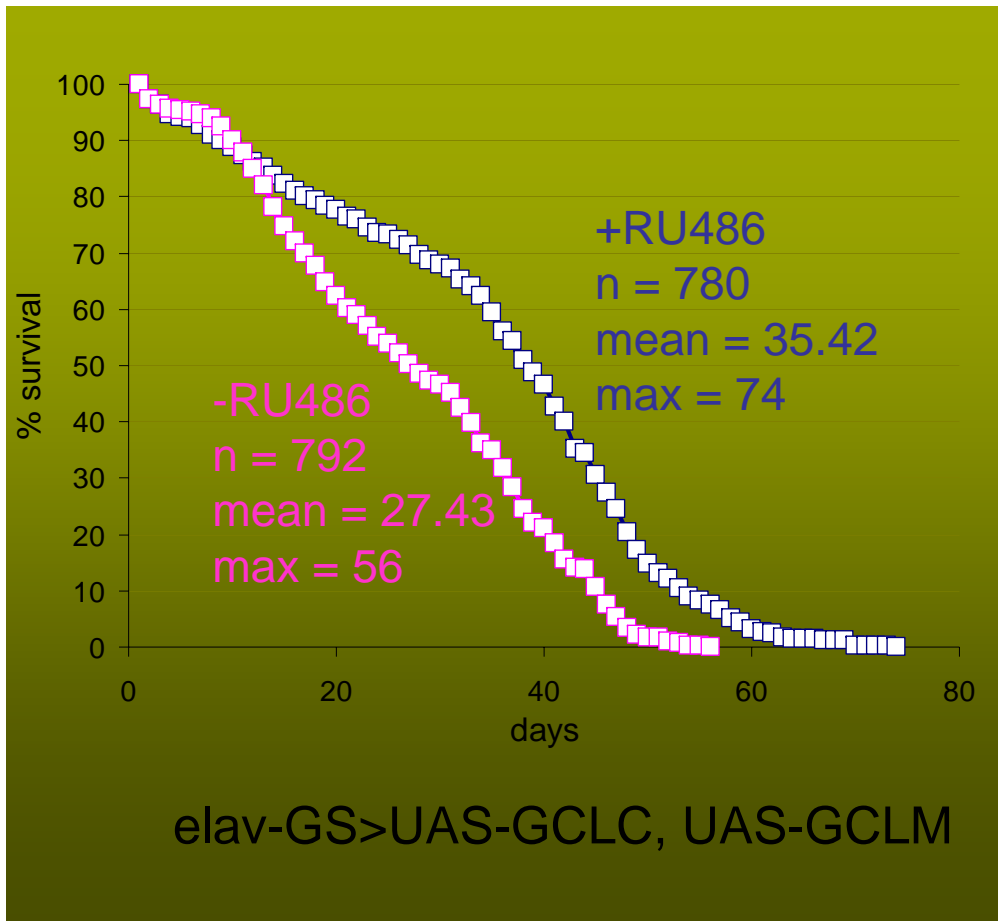
*DmGclm*<sup>L0580</sup> is an insertional allele, and is strongly hypomorphic.

- Loss of *DmGCLM* did not substantially increase sensitivity to oxidative stress caused by diethylmaleate, nor did it appear to significantly shorten lifespan under the conditions used.

# The GeneSwitch-UAS system



# Overexpression of GCL in a pan-neural pattern can extend lifespan



Statistically significant extension of lifespan in males, but not in females

# Summary



- DmGCL appears to be regulated in a similar manner to mammalian GCL
- Identified cysteine residues as candidates for interaction with DmGCLC
- Overexpression of DmGCL leads to elevation of GSH titres
- Overexpression of DmGCLC reduces viability
- Overexpression of DmGCL in neural tissues extends lifespan in males
- Loss of DmGCLM reduces GSH titre but does not affect OS resistance or lifespan

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