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Supplemental Information

Elevated Levels of the Reactive Metabolite Methylglyoxal Recapitulate Progression of Type 2 Diabetes

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Supplemental Information

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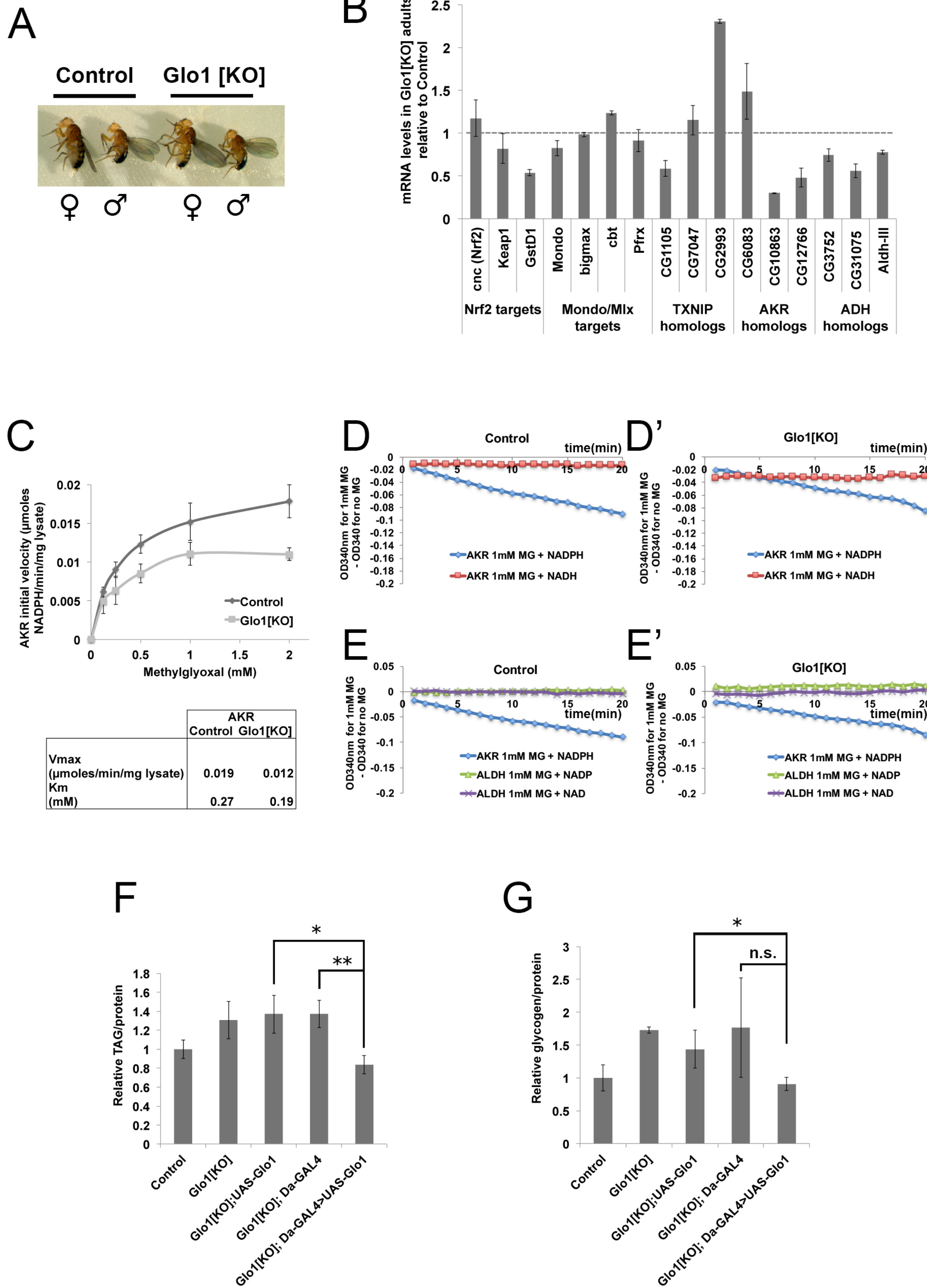
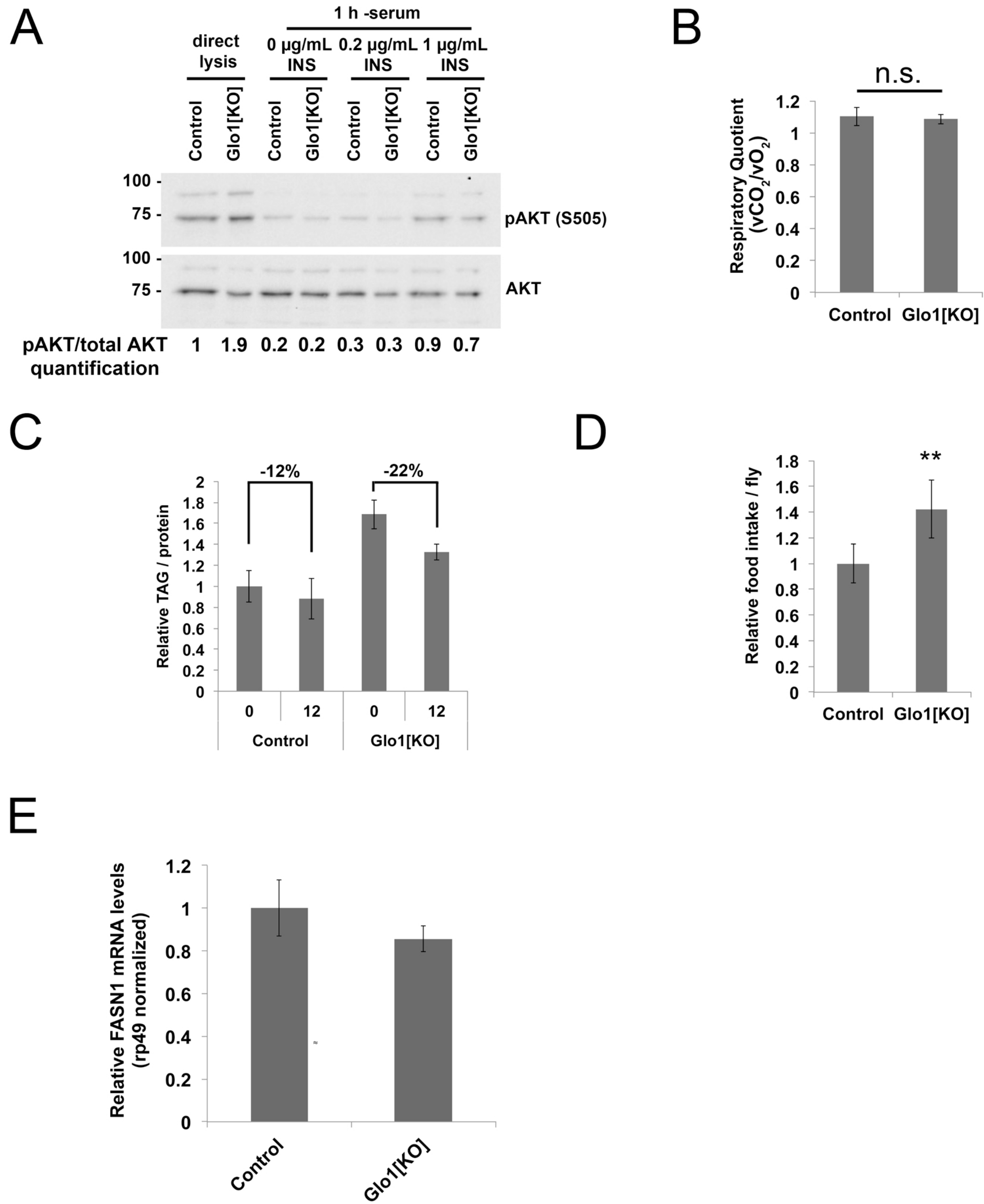
Supplemental Figure S1



	AKR Control	AKR Glo1[KO]
Vmax (μmoles/min/mg lysate)	0.019	0.012
Km (mM)	0.27	0.19

Supplemental Figure S2



Supplemental Figure S3

A

Intracellular MG

Methylglyoxal (pmol/10^6 cells)

1200 1000 800 600 400 200 0

0 2 4 6 8 10 12 14 16 18 20 22 24

Time after MG addition (hrs)

Extracellular MG

Methylglyoxal (µM)

1200 1000 800 600 400 200 0

0 2 4 6 8 10 12 14 16 18 20 22 24

Time after MG addition (hrs)

B

ELISA with anti MG-H1 antibody

OD 490nm

1.8 1.6 1.4 1.2 1 0.8 0.6 0.4 0.2 0

1:500 1:1000 1:2000 1:4000 1:8000 1:16000 1:32000 1:64000

Serum dilution

BSA

MG-H1 conjugate HSA

CEL conjugate HSA

CML conjugate HSA

C

MG - +

MG-H1

Tubulin

D

MG(24h): 0 10 µM 100 µM 500 µM 1 mM 2 mM

MGH1

Tubulin

E

Relative FASN1 mRNA levels (rp49 normalized)

1.2 1 0.8 0.6 0.4 0.2 0

***

MG(1mM,24h) - +

F

mmol MG-H1/mol Arg

6.00 5.00 4.00 3.00 2.00 1.00 0.00

0 0.5 1 2 4 8

Methylglyoxal (mM)

G

mmol MG-H1/mol Arg

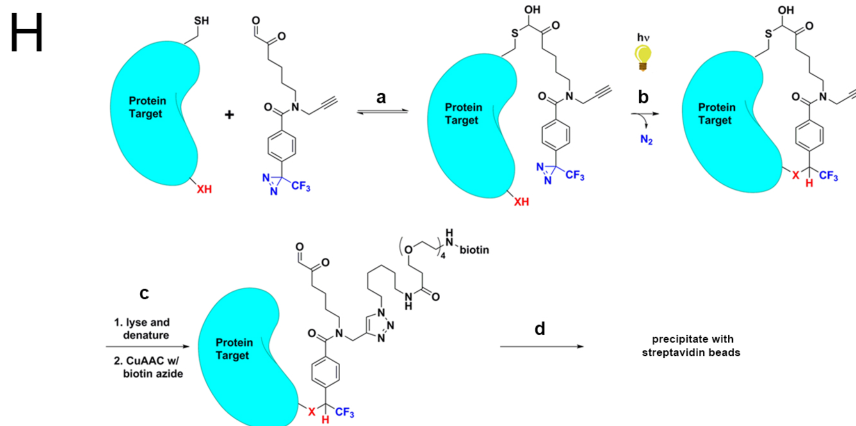
12 10 8 6 4 2 0

*

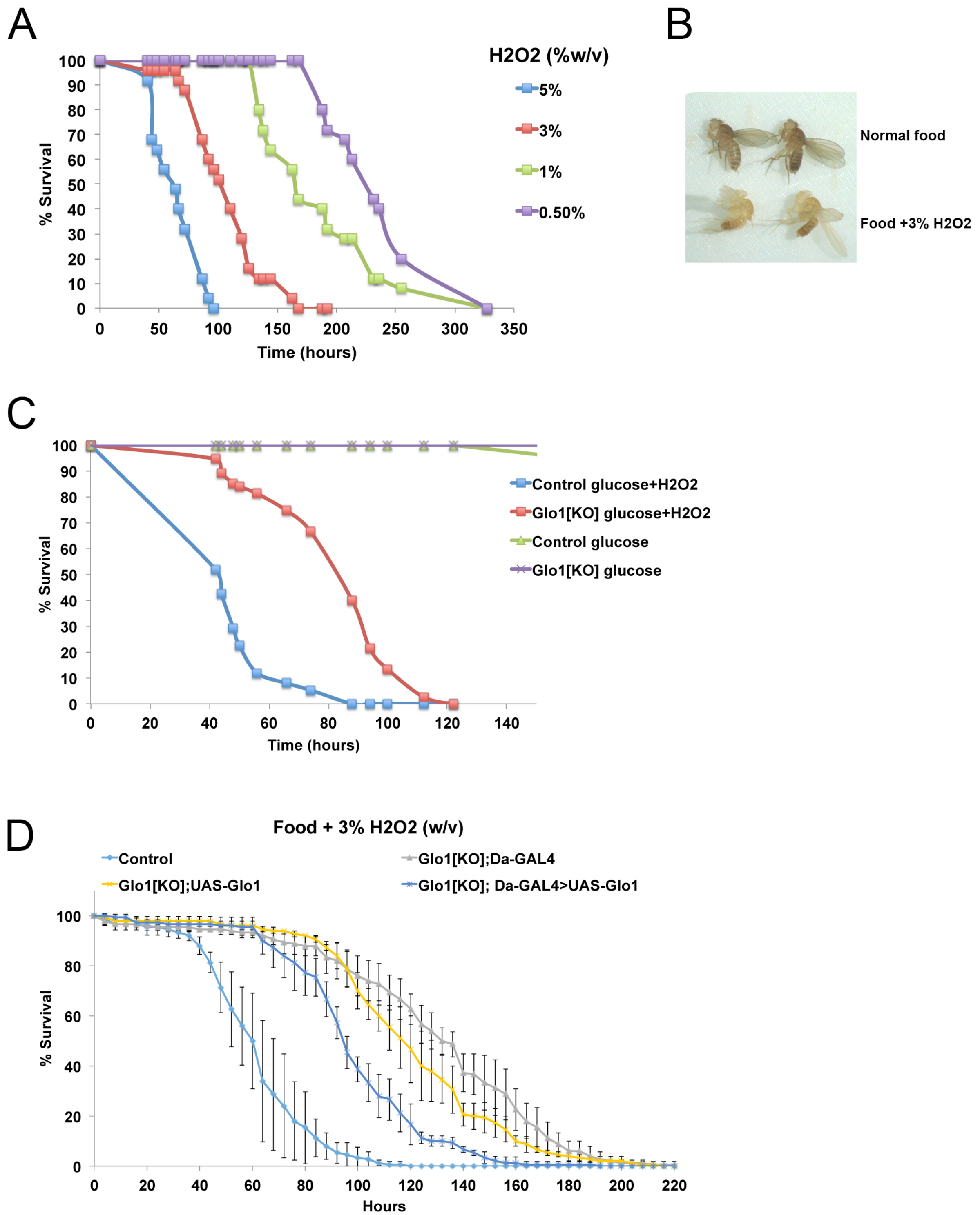
-MG +MG

FASN1

H



Supplemental Figure S4



Legends to Supplemental Figures

Supplemental Figure S1. Related to Figure 1.

(A) Glo1^{KO} flies are developmentally normal, with normal size and morphology.

(B) Quantification of mRNA levels in 1-week old female Glo1^{KO} adults relative to controls, for genes described to be involved in MG detoxification (AKR and ALD), oxidative stress response (keap1/Nrf2 pathway), or sugar metabolism (Mondo/Mlx and TXNIP). mRNA levels were determined by RT-Q-PCR, normalized to rp49.

(C) Glo1^{KO} mutant larvae do not show a compensatory increase in aldo-keto reductase (AKR) activity. Lower panel: Michaelis–Menten kinetic constants for AKR in control and Glo1^{KO} mutants, calculated from the graph above. (n=3x10 animals)

(D-D') Unlike AKR activity using NADPH as a co-factor (blue lines), which was detectable in both control (D) and Glo1^{KO} animals (D'), AKR activity using NADH as a co-factor was not detectable above background in lysates of either control or Glo1^{KO} animals (red lines, D and D' respectively). Net OD340 values are shown, by subtracting OD340 values for the reaction without MG from those of the reaction with 1mM MG. (n=3 for each curve).

(E-E') MG dehydrogenase activity was not detectable above background in lysates of either control or Glo1^{KO} animals (green and purple lines using NADP and NAD respectively, panels E-E'). AKR activity using NADPH as a co-factor (blue lines), which was detectable in both control (E) and Glo1^{KO} animals (E'), is shown as a positive control.

(F-G) Ubiquitous expression of Glo1 from a UAS-Glo1 transgene rescues both the elevated triglycerides (F) and elevated glycogen (G) levels of Glo1^{KO} flies. Ubiquitous expression was achieved with the daughterless-GAL4 driver. Glo1^{KO}; UAS-Glo1 and Glo1^{KO}; Da-GAL4 are parental genotypes that do not express Glo1 from a transgene, unless combined to yield the experimental Glo1^{KO}; Da-GAL4>UAS-Glo1 animals which do.

Error Bars: Std. Dev. * ttest<0.05, ** ttest<0.01

Supplemental Figure S2. Related to Figure 2.

(A) Glo1^{KO} animals display insulin resistance starting early in development. Control or Glo1^{KO} 3rd instar female larvae were either lysed directly or first explanted into Schneider's medium lacking serum for 1h, and then stimulated for 15 min with increasing amounts of insulin as indicated. Activation of the insulin/PI3K/TORC1 pathway detected via phosphorylation of AKT (n=6 animals per sample. Representative blot for three biological replicates.)

(B) Glo1^{KO} adults do not have an elevated respiratory quotient, suggesting they have unaltered lipid catabolism compared to controls. (n=3x10 animals)

(C) Glo1^{KO} adults have no obvious defect in lipid mobilization. Control and Glo1^{KO} females were fasted for 12 hours, and total body triglycerides, normalized to total body protein, was quantified before and after fasting (n=3x6 females).

(D) Food intake is mildly elevated in Glo1^{KO} adults as compared to control animals. (n=5x8 animals per genotype)

(E) FASN1 mRNA levels, determined by RT-Q-PCR, are not increased in Glo1^{KO} mutant flies.

Error Bars: Std. Dev. * ttest<0.05, ** ttest<0.01, ***ttest<0.001

Supplemental Figure S3. Related to Figure 3.

(A) Treatment of cells in culture with MG does not lead to sustained elevated levels of MG. *Drosophila* S2 cells (1 million cells in 1mL of medium) were treated with 1mM MG for the indicated amount of time, and both intracellular and extracellular free MG levels were determined by LC-MS/MS.

(B) Specificity of the anti MG-H1 antibody was tested by indirect ELISA against the indicated MG adducts.

(C) MG-H1 antibody detects increased modification of proteins by immunoblotting of lysates from S2 cells treated +/- MG (1mM) for 24 hours.

(D) 1mM MG is the lowest concentration of MG that causes elevated MG-H1 adducts of *Drosophila* S2 cells in culture as detected by anti-MG-H1 antibody. Cells were treated with the indicated concentrations of MG for 24 hours and lysates were immunobloted with anti-MG-H1 antibody or tubulin.

(E) FASN1 mRNA levels, determined by RT-Q-PCR, are decreased in S2 cells treated with MG (1mM, 24h). ***p<0.001 by t-test.

(F) Titration of MG added to lysates of S2 cells (4 million cells/mL, circa 0.5mg protein/mL) for 6h to test the effect on MG-H1 modification of the proteins, quantified by mass spectrometry on fully hydrolyzed proteins, normalized to arginine levels. Treatment with 1mM MG doubles the MG-H1 content relative

to untreated lysates, which is less than the 10-fold increase observed in diabetic patients (Ahmed et al., 2005).

(G) Fatty Acid Synthase (FASN) is highly modified with MG-H1 adducts compared to the average proteome (compare to panel F), and becomes more modified when lysates are treated with 500 μM MG for 6 hours. S2 cell lysates were treated +/- MG, then FASN protein was immunoprecipitated, gel extracted, and hydrolyzed to completion prior to MG-H1 and Arg quantification. n=3 biological replicates. Error bars: std. dev. $^{*}p<0.05$ by t-test.

(H) Enrichment of MG target proteins with the trifunctional probe Click-MG. (a) Proteins form reversible hemi-thioacetal bonds with Click-MG. (b) Upon irradiation with UV light, the diazirine functionality in Click-MG extrudes nitrogen gas (N2) to make a highly reactive intermediate that will form an irreversible chemical bond with proximally located amino acid side chains or back bones, leading to preferential labeling of proteins which make hemi-thioacetal bonds with Click-MG. (c) Proteins covalently bound to Click-MG are then labeled with biotin via a copper catalyzed azide alkyne cycloaddition (CuAAC) between the alkyne handle in Click-MG and biotin azide. (d) Biotin labeled proteins are enriched with streptavidin beads.

Supplemental Figure S4. Related to Figure 4.

(A) Increasing levels of H_2O_2 in food causes a dose-dependent decrease in fly viability. *Drosophila* w^{1118} flies were exposed to indicated levels of H_2O_2 added into normal food and the surviving animals were quantified over time (n=25 flies for each concentration)

(B) Upon exposure to H_2O_2 flies lose their pigmentation, showing penetrance of the H_2O_2 into their tissues.

(C) Glo1^{KO} flies have increased survival to oxidative stress when exposed to 3% H_2O_2 in 5% glucose/1%agarose/PBS (n=3x20 1-week old adult females)

(D) Ubiquitous expression of Glo1 from a UAS-Glo1 transgene rescues the increased resistance to oxidative stress of Glo1^{KO} mutants. Ubiquitous expression was achieved with the daughterless-GAL4 driver. Glo1^{KO}; UAS-Glo1 and Glo1^{KO}; Da-GAL4 are parental genotypes that do not express Glo1 from a transgene, unless combined to yield the experimental Glo1^{KO}; Da-GAL4>UAS-Glo1 animals which do.

Error Bars: Std. Dev. n=3x50 animals.

(Supplemental Tables continue on next page)

Supplemental Tables

Table S1: Oligos, related to STAR Methods.

Oligo	Sequence	Use
OAM137	ccatgggcATGCCCGCCCGATTCGCCGAG	to clone FASN1 N-terminal fragment into pETM11 for anti-FASN1 antibody production
OAM138	GATATGCGCGAAGGAAAGtaagcggccgc	to clone FASN1 N-terminal fragment into pETM11 for anti-FASN1 antibody production
OAM003	ggcctaatacgactcactatagggTGGGCGACGTGACAGGACT	dsRNA for knockdown of Glo I – upper
OAM004	ggcctaatacgactcactatagggTCGGTGGCGTTCTCGTACC	dsRNA for knockdown of Glo I – lower
OJA368	GGCCTAATACGACTCACTATAGGGAGGCCCGAAGGCTACGTCCAG	dsRNA for knockdown of GFP – upper
OJA377	GGCCTAATACGACTCACTATAGGGAGGGATGGGGGTGTTCTGCTG	dsRNA for knockdown of GFP – lower
OAT1996	CCAGCCCGTGGAAACGATC	qpcr CG3523 (FASN1) – upper
OAT1997	CCTCGTCCAGCTTGGCAGA	qpcr CG3523 (FASN1) –lower
OAT243	GCTAAGCTGTCGCACAAA	qpcr rp49 – upper
OAT244	TCCGGTGGGCAGCATGTG	qpcr rp49 – lower
OAM217	GCGGCGTTACAACATAAAGA	qpcr mondo - upper
OAM218	CTCCATGCGCAAAGCTTCAA	qpcr mondo - lower
OAM219	GCCAAGTTTCAAGTGTTCCAG	qpcr mlx - upper
OAM220	CTCCAGCCAGGGGATAATG	qpcr mlx - lower
OAM221	ATGCCTTCTCGCTCTCATGT	qpcr cabut - upper
OAM222	TCCTGGAAAGAAGTGGCATC	qpcr cabut - lower
OAM223	GATCAAGCCCAGCGAGATT	qpcr Aldh-III - upper
OAM224	CGCAGACAACTGGATAGCAA	qpcr Aldh-III - lower
OAM225	AGAGCGAGTACAACCTGAGC	qpcr PFK2 -

		upper
OAM226	TAGCGCATTGGCATACTGGT	qpcr PFK2- lower
OAM227	GAATGACCGCCGATCTCTTGG	qpcr cnc (cap-n-collar Drosophila Nrf2 homologue) - upper
OAM228	GGAGCCCATCGAACTGACA	qpcr cnc (cap-n-collar Drosophila Nrf2 homologue) - lower
OAM233	CCTATACGTCGTTGGGAGGC	qpcr CG10863 - upper
OAM234	TAACGCCCTCGGCAATCTTT	qpcr CG10863 - lower
OAM235	CCGATATGTGATTGAGCTGGGA	qpcr CG12766 - upper
OAM236	ACTCTTGATGGCTTGTCGGG	qpcr CG12766 - lower
OAM237	ACAACCCGTGGAACACCTAC	qpcr CG1105 - upper
OAM238	GGAACCGAATGATAATACCTCGGA	qpcr CG1105 - lower
OAM239	ATCAGTTGCCAAACAAACGCT	qpcr dvdup1 (CG7047) - upper
OAM240	CGTACGCCTGTACACACAAAG	qpcr dvdup1 (CG7047) - lower
OAM241	ACTATATACCCATGATGCCGCC	qpcr CG2993 - upper
OAM242	ACCGGATAACGCGGCTTAAA	qpcr CG2993 - lower
OAM249	CAGGTGCTCCTACGCTTTCA	qpcr CG6083 - upper
OAM250	ATAGGCCGCCTTCATGGTCA	qpcr CG6083 - lower
OAM251	AAACCATCAACCCGACCACA	qpcr CG3752 - upper
OAM252	GCCATGGAGATCCCAACTTGA	qpcr CG3752- lower
OAM253	TCGCTCAATATGGCCGATCC	qpcr CG31075 - upper
OAM254	TATCAGCCTTATCTCCCTCGG	qpcr CG31075 - lower
OAM277	gaattcATGCCCGCCCGATTCGC	to clone FASN1 CDNA into pMT-Flag C terminal
OAM280	ggatccGTTGAACAGACGCTTCAG	to clone FASN1 CDNA into pMT-Flag C terminal
OAM283	ACTCCGGAACGAGAGGTC	to clone FASN1 CDNA into pMT-Flag C terminal

OAM284	AGGTCTTGGCGGATTCGG	to clone FASN1 CDNA into pMT-Flag C terminal
OAM285	AAACTAATCCAGTTGTC	to clone FASN1 CDNA into pMT-Flag C terminal
OAM286	ATTGTTCGTTCTGACACC	to clone FASN1 CDNA into pMT-Flag C terminal