**Supplementary Figures and Legends for**

# Lipid metabolic perturbation is an early-onset phenotype in adult *spin* mutants: a *Drosophila* model for lysosomal storage disorders

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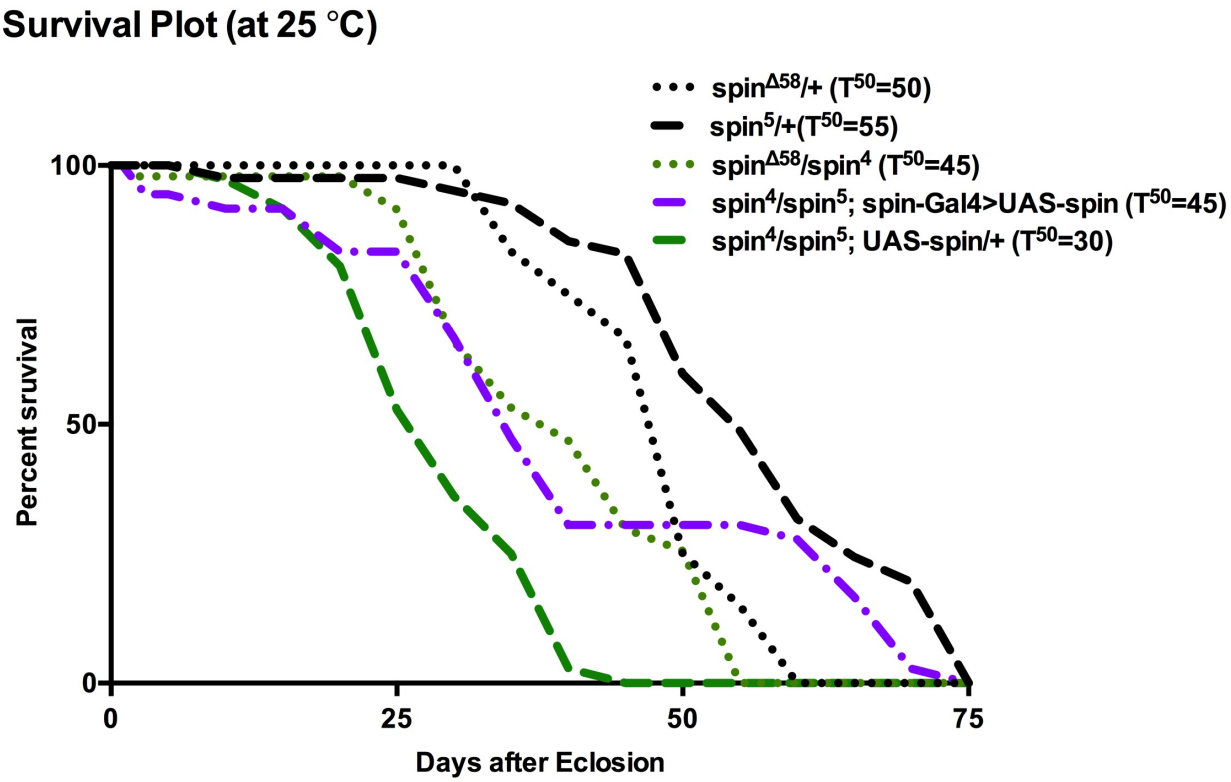
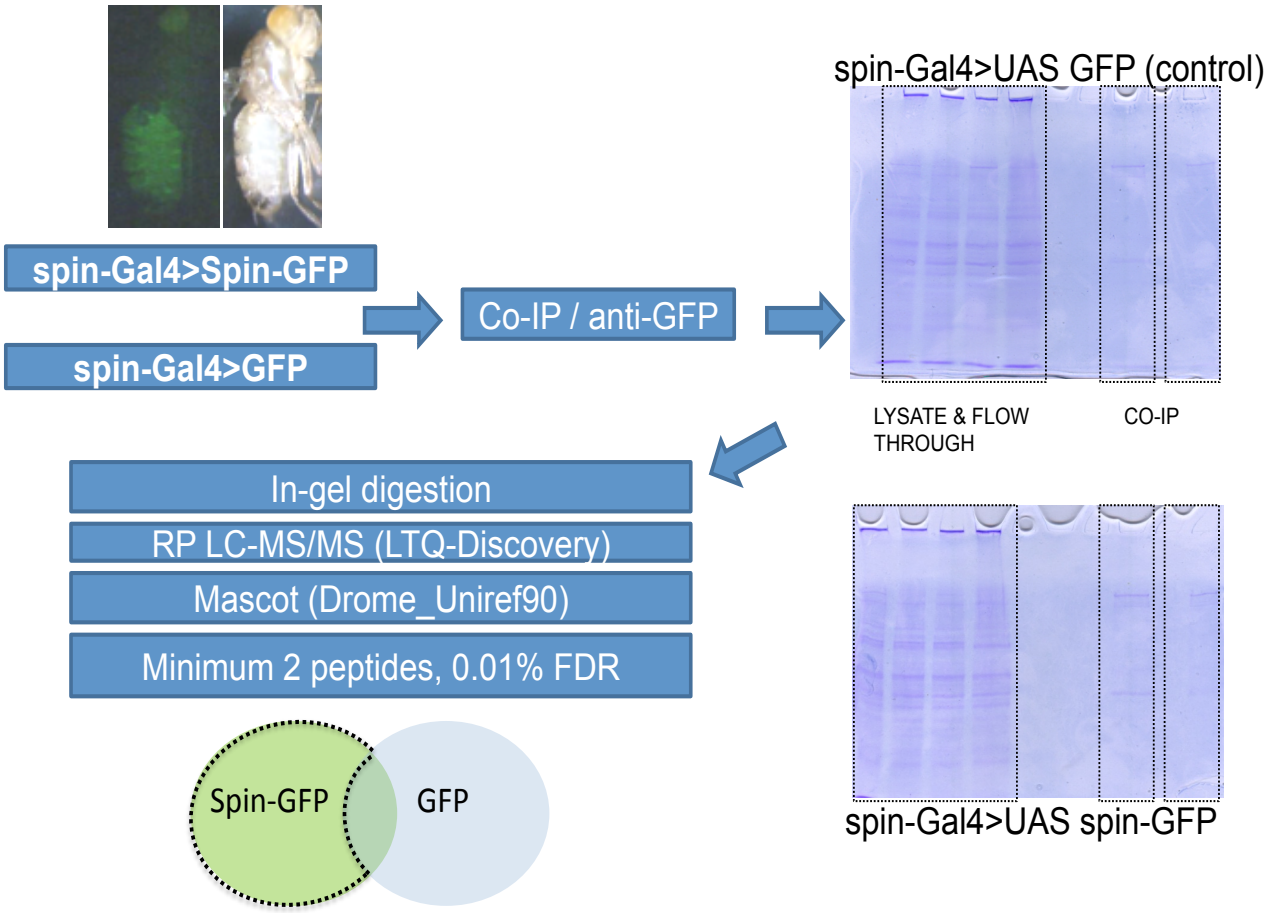
**Supplemental Figure S1**:

Decreased adult lifespan in *spin* mutants
Survival plot comprises line graphs generated using percentage of surviving adults over time at 25°C for *spin* mutants (green lines) and in a rescue condition (purple) in relation to their genetic controls (black). This data comes from multiple sets of 15 flies constituting a sample size of at least 50-100 flies. For $spin^4/spin^5$ the population size is below 50 because of the inability to obtain large number of escaper adults. Legend displays the genotypes and $T^{50}$ values in parenthesis



**Supplemental Figure S2: Strategy for characterization of interactors of spin using LC-MS.**

Adult flies overexpressing Spin-GFP (*spin-Gal4>spin-GFP*; fluorescent and corresponding bright-field image) were used for protein extraction. As a control we also used protein extracts from flies overexpressing only GFP (*spin-Gal4>GFP*). A pull-down was achieved using a GFP antibody. The initial lysate, flow-through following the binding, and final eluent (Co-IP) were run on a SDS_PAGE gel. Images from two independent experiments for each (*spin-Gal4>spin-GFP and spin-Gal4>GFP*) are presented here. These were subjected to routine in gel-digestion followed by LC-MS workflow as described in methods. Schematic Venn Diagram highlights subset of proteins that are specific interactors of Spin-GFP (black dotted

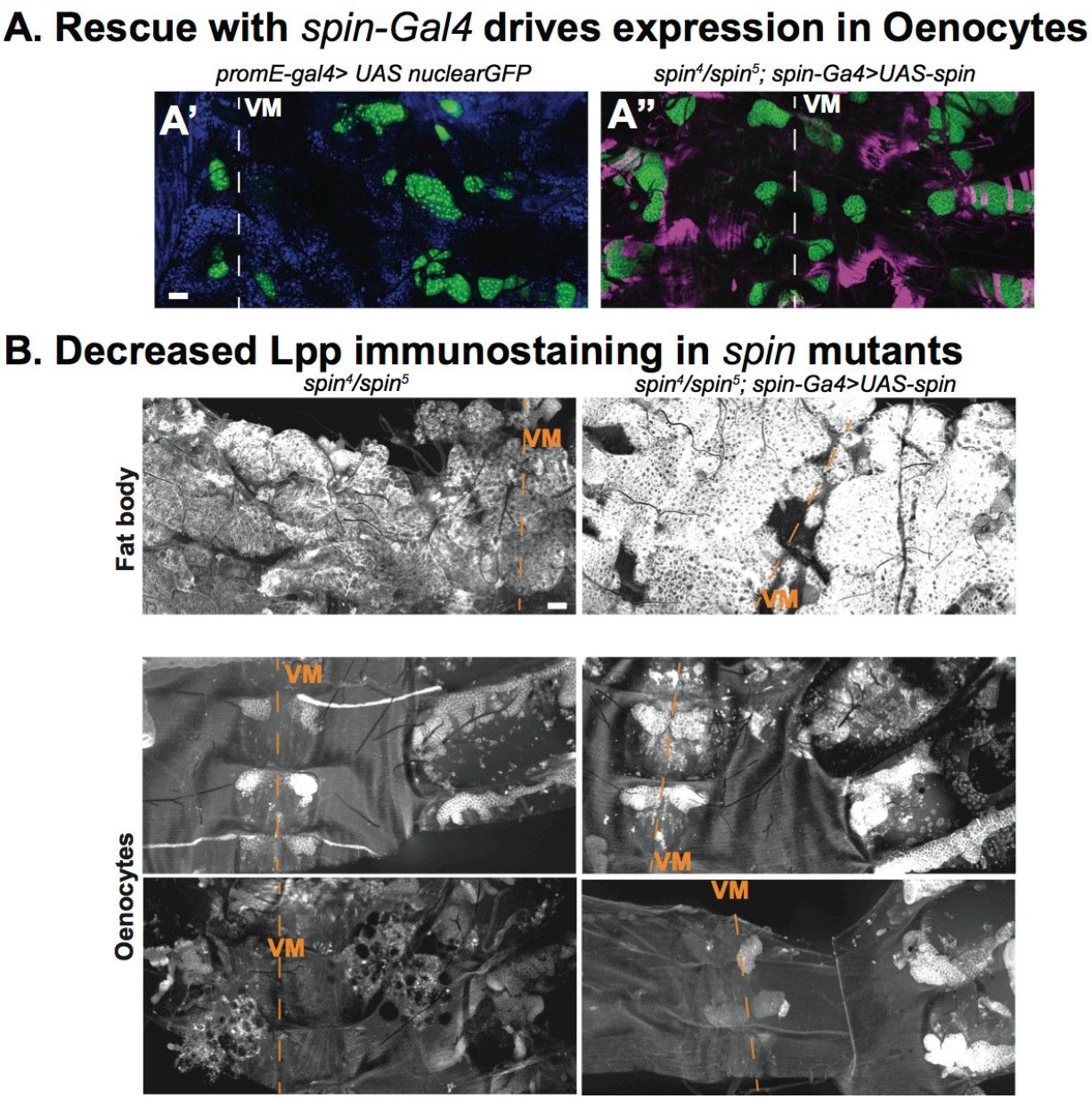



outline), i.e. not common to the GFP control pull-down are presented in Table 1.

**Supplemental Figure S3: Abdominal body wall staining in *spin* mutants**

A: Oenocytes Identification: Representative images from dissected adult abdominal fillets of females of
A': Oenocyte driver (*promE (800)- Gal4*; Billeter et al., 2009) driving nuclear GFP (Green). Oenocytes that are located dorsally and ventrally located are clearly evident. Ventral mid-line is indicated with a dashed line. The fat bodies (blue) are stained with Lipid droplet dye, LD450 (Spandl et al, 2009).
A": *spin-Gal4* rescue in *spin* mutants clearly shows the rescue construct (UAS *spin-GFP*; green)is expressed abundantly in oenocytes (green). The musculature is labeled with Phalloidin tagged Alexa-Fluor 568 (shown in magenta)

B: Examples of different adult abdominal fillets for mutants ($spin^4/spin^5$) and rescue ($spin^4/spin^5$; *spin-Gal4>UAS spin*) stained with LPP antibody showing fat-body and oenocyte staining.



**Suppl Table 1: Genotypes used in this study**

| Genotype | Achieved genetic perturbation |
|---|---|
| +/+;*spin*$^{4}$/*spin*$^{5}$;+ | *spin* mutant heteroallelic combination /transheterozygote (Sweeney and Davis, 2002) constituting a genetic null. |
| +/+;*spin*$^{4}$/*spin*$^{\Delta 58}$;+ | *spin* mutant heteroallelic combination /transheterozygote (Milton et al., 2011; Sweeney and Davis, 2002) constituting a genetic hypomorph |
| +/+;*spin*$^{5}$/*spin*$^{\Delta 58}$;+ | spin mutant heteroallelic combination /transheterozygote (Milton et al., 2011; Sweeney and Davis, 2002) constituting a genetic hypomorph |
| +/+;*spin*$^{4}$/*spin*$^{5}$; *spin-Gal4>UAS spin-GFP;*+ | Rescue of spin levels in a transheterozygote in endogenous spin producing cells (Sweeney and Davis, 2002) |
| +/+; UAS spin-GFP/+; spin-Gal4/+ | Expression of spin-tagged GFP in endogenous spin producing cells (Sweeney and Davis, 2002) |
| +/+;*spin*$^{4}$/*spin*$^{5}$; *UAS spin-GFP/*+ | *spin* mutant background for overexpression experiments |
| +/+;*spin*$^{4}$/*spin*$^{5}$; *spin-Gal4>UAS slab* | *spin* mutant and an overexpression of *slab* (Rohrbough et al., 2004) |
| +/+;*spin*$^{4}$/*spin*$^{5}$; *slab*$^{2}$/+ | *spin* mutant and *slab*$^{2}$ heterozygosity (Rohrbough et al., 2004) |

**Supplemental Table 2: Details for Internal Standard (IS) mix used:**

I. IS composition (for single brain analyses; corresponding to Fig. 2)

| Lipid Standard | Amount (picomoles) |
|---|---|
| Cer_35:1 | 11.62 |
| PC_31:1 | 9.88 |
| PE_31:1 | 9.56 |
| PE-OO | 11.98 |
| PI_31:1 | 8.32 |
| TAG_51:0 | 13.88 |
| CerPE_29:1 | 11.12 |
| TOTAL | 76.36 |

II. IS composition (for pooled brain extracts; corresponding to Fig. 3/4)

| Lipid Standard | Amount (picomoles) |
|---|---|
| CerPE-C12 Sphingosyl PE [d17:1] | 7.97 |
| PC [17:0-14:1] | 4.43 |
| PE [17:1-14:0] | 4.29 |
| C17-Cer [d18:1/17:0] | 10.41 |
| PE-OO [40:00] | 7.16 |
| Sphingosine [d17:1] | 7.1 |
| PI [17:0-14:1] | 3.72 |
| GluCer[d18:1/12:0] | 7.89 |
| PS [17:0-14:1] | 2.95 |
| TOTAL | 55.92 |