Supplemental Figure 1. Relates to Figure 2.

A) Pigment glial (*54C-GAL4)* or neuronal (*Elav-GAL4)* knockdown of *Sln* **(b, f)**, *out* **(c, g)** and *Bsg* **(d, h)** does not affect cellular integrity or alter LD accumulation in 1-day-old animals. **B) a-b**. Whole eye clones of $sicily^{E}$ and $Marf^{B}$ created with *GMRhid FRT19A; Eyeless-GAL4, UAS-Flp* show high levels of glial LD when stained with Nile Red. **c-f.** Glial LD accumulation is reduced with whole eye (*Eyeless-GAL4*) knockdown of *Sln* and *out* using RNAi. **C)** Quantification of B. **D) a-b.** Nile Red stained whole-mount retina show LD accumulation in the $sicily^{E}$ and $Marf^{B}$ mutant clones. **c-f.** One copy loss of Sln^{D1} or Bsg^{1217} in the mutant background reduces LD accumulation. **E) a-b.** $sicily^{E}$ and $Marf^{B}$ mutant clones exhibit photoreceptor degeneration after 5 days as stained by phalloidin (F-actin) which is ameliorated with whole eye (*Eyeless-GAL4*) knockdown of *Sln* or *out* **(c-f)** or one copy loss of Sln^{D1} or Bsg^{1217}**(g-j)**. All data points represent mean +/- SEM Student's t-tests were used to calculate significance (**P<0.05, **P<0.005, ***P<0.0005,* n=10 animals each)

Supplemental Figure 2. Relates to Figure 3.

A) Primary murine olfactory bulb co-cultured cells show astrocytes, neurons and olfactory bulb ensheathing glia. **B) a-b,** OB co-culture (C57BL/6J) treated with 2µM rotenone did not accumulate significant glial LD accumulation with the addition of 1.5mM AD4. c-d. Blocking MCTs using 50 µM MCTi2 (SP13800) lead to less LD accumulation**. e-f.** 2µM rotenone combined with 200nM MCTi and 1.5mM AD4 did not lead to high levels of glial LD accumulation. **C)** Single culture of olfactory bulb neurons or astrocytes do not accumulate LD in response to elevated ROS. **D)** Quantification of the percentage of total neuron/glia with LD accumulation. **E)** TUNEL staining measures cell death. 24 hrs treatment with 2 µM rotenone increased cell death to ~20%. Inhibiting lactate transport immediately or 12 hrs after rotenone using MCTi reduced cell death to a level comparable to vehicle control (n > 200 cells). **F)** Addition of 11mM lactate to co-cultured cells at 5DIV did not alter number of cleaved caspase 3 positive cells at 11 DIV (n > 200 cells). **G)** Wire hang assay. Mice treated with 3mg/kg/day rotenone for 8 days exhibit motor deficits (Student's t-test. n = 5 per treatment.) All data points represent mean +/- SEM. Student's t-tests were used to calculate significance, (**P<0.05, **P<0.005, ***P<0.0005*). Scale bar: 50 µm.

Supplemental Figure 3. Relates to Figure 4.

A) Nile Red staining of 1-day-old whole mount retina shows neuronal (*Elav-GAL4)* or glial (*54C-GAL4)* knockdown of *Ldh* or *Pdha* does not affect cellular integrity or LD accumulation at day 1. **B)** Glial knockdown of *Ldh* **(a)** ameliorated LD accumulation while knockdown of *Pdha* **(b)** in the *Rh-ND42 IR* background did not. Neuronal knockdown of *Pdha* **(e)** or *Ldh* **(f)** lead to a reduction of glial LD accumulation in the *Rh-ND42 IR* background. **C)** Removing 1 copy of *Pdha* or citrate synthase (*Kdn*) reduces LD accumulation in *Rh-ND42 IR* **(a-c)**, *Rh-Marf IR* **(d-f)** and *Rh-Aats-met IR* **(g-i)** retinas. **D)** *N-Syb-GAL4* overexpression of *UAS-SREBP* and *UAS-JNK* leads to glial LD accumulation **(a, b)**. Neuronal knockdown of MCTs (*Sln* [**c, d**] and *out* [**e, f**]) and metabolic enzymes (*Ldh* [**g, h**] and *Pdha* [**i, j**]) ameliorates glial LD accumulation. Data are represented as mean ± SEM. Student's t-tests were used to calculate significance (**P<0.05, **P<0.005, ***P<0.0005.* n = 10).

Supplemental Figure 4. Relates to Figure 5

A) Nile Red stain of whole-mount retina reveal that Neuronal (*Elav-GAL4)* or glial (*54C-GAL4)* knockdown of *Fatp* does not affect cellular integrity at day 1. **B)** Neuronal or glial knockdown of *Fatp* in the *Rh-ND42 IR* background reveal a reduced number of LD accumulated in glial cells **C)** *Eyeless-GAL4* knockdown of *Fatp* (b, d) in the $sicily^{E}$ and $Marf^{B}$ mutant clones reduces glial LD accumulation. **D)** Quantification of C. **E)** Immunohistological staining for FATP1 and FATP4 in wildtype (BL6) cells. (blue: DAPI, red: Tuj1, gray: GFAP). **F) a-d**. Cells transduced with non-targeting sgRNA have intact FATP4 protein localization can to accumulate glial LD after being treated with 2µM rotenone. **e-h.** sgRNA knockout of FATP4 leads to a disruption of protein localization and cells were unable to accumulate LD after 2µM rotenone treatment. Data are represented as mean ± SEM. Student's t-tests were used to calculate significance (**P<0.05, **P<0.005, ***P<0.0005.* n = 10). Scale bar: 50 µm.

Supplemental Figure 5. Relates to Figure 6

A) Neuronal (*Elav-GAL4)* or glial (*54C-GAL4)* knockdown of *Glaz* or *Nlaz* does not affect cellular integrity at day 1. **B)** One-copy-loss of Glaz or Nlaz in the sicily mutant background delays photoreceptor degeneration. **C) a.** Control flies have dark red eyes. **b.** *54C-GAL4* (pigment cell driver) knockdown of *white* leads to yellow colored eyes. **c.** $Glaz^{T2A\text{-}GAL4}$ (glial apolipoprotein driver) knockdown of *white* results in a subtle loss of red pigment. **D)** $Glaz^{T2A\text{-}GAL4}$ expressing *UAS- mCD8GFP* represents one copy loss of *Glaz* with expression of *UAS-mCD8::GFP*.

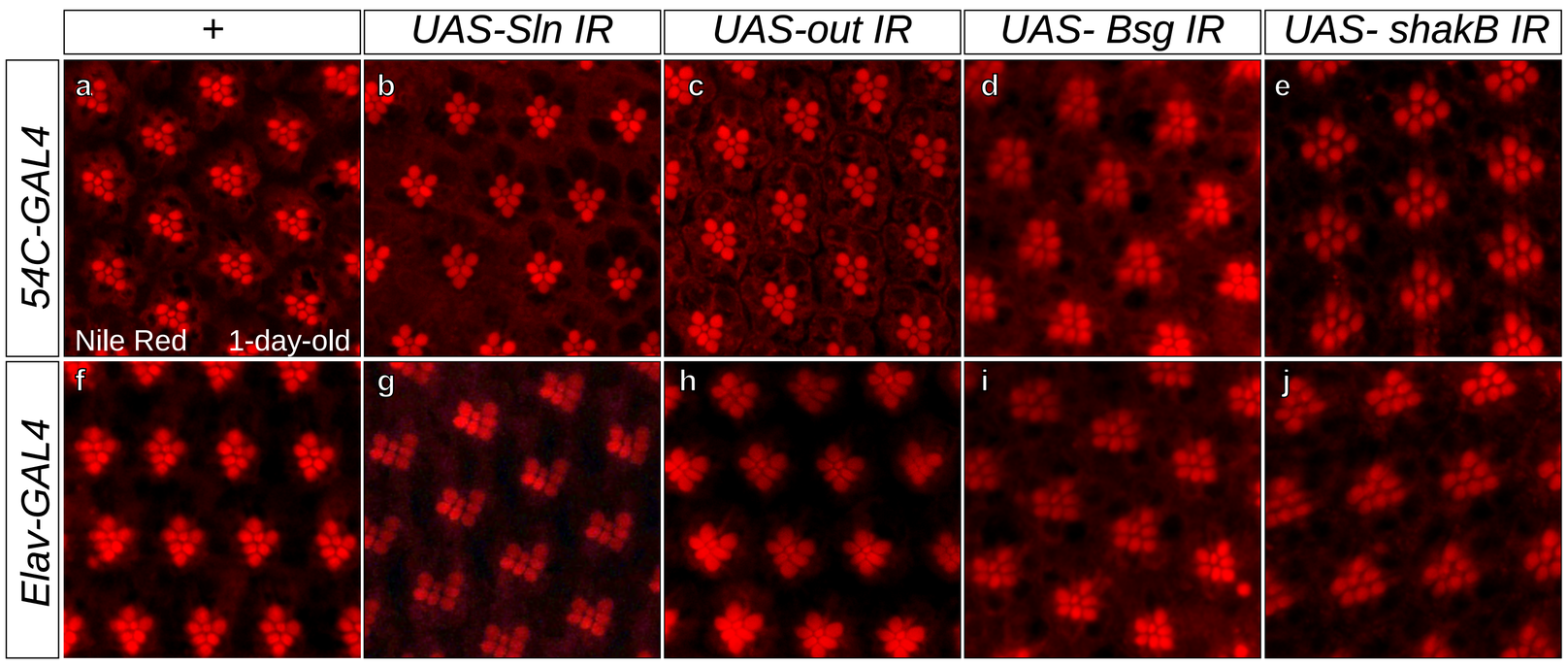
Glaz$^{T2A\text{-}GAL4}$ expression of *Glaz* and the *APOE* alleles does not lead to glial LD accumulation. **E)** *Daughterless-GAL4* ubiquitous overexpression of *UAS-APOE* alleles reveal similar levels of protein expression in third instar larvae. Actin used as loading control. Data are represented as mean ± SEM. Student's t-tests were used to calculate significance (**P<0.05, **P<0.005, ***P<0.0005.* n = 10).

Supplemental Figure 6. Relates to Figure 7

A) Nile Red stained whole mount retina of neuronal and glial overexpression of *UAS-mCD8::GFP* shows no LD accumulation. Neuronal and glial overexpression of *Glaz*, *APOE2* and *APO3* variants leads to more glial LD accumulation compared to overexpression of *APOE4* variant. **B)** F-actin (phalloidin) staining of photoreceptors from 1-day-old adult flies. All flies were raised on 25 µM rotenone and APOE4 expressing flies exhibit "comet" tails on a subset of rhabdomeres. Dotted outlines point out the comet structures. **C)** Flies raised on 25 µM rotenone were aged for 10 days. F actin staining reveal photoreceptor loss. Dotted outline denotes missing rhabdomeres.

Figure S1

A



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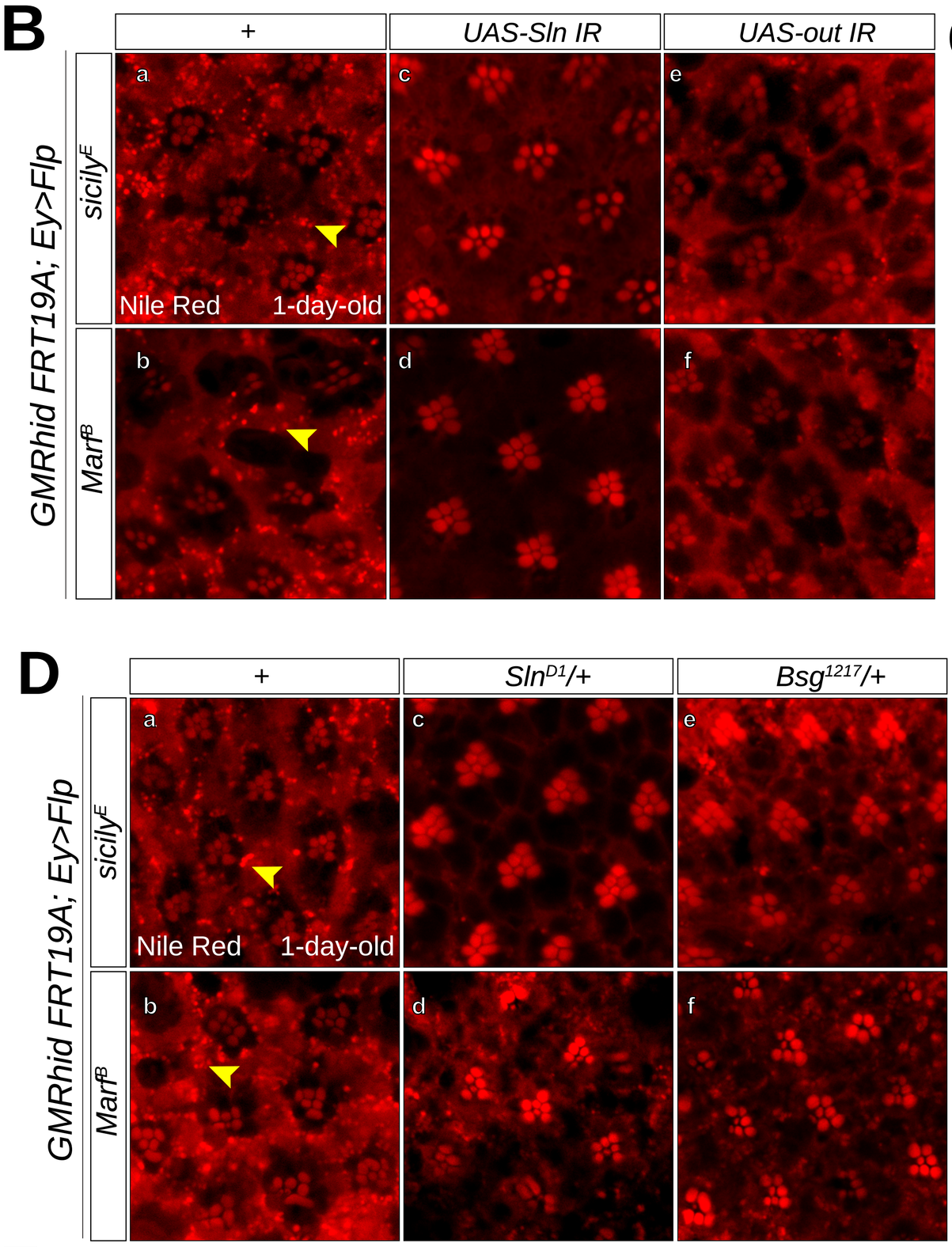

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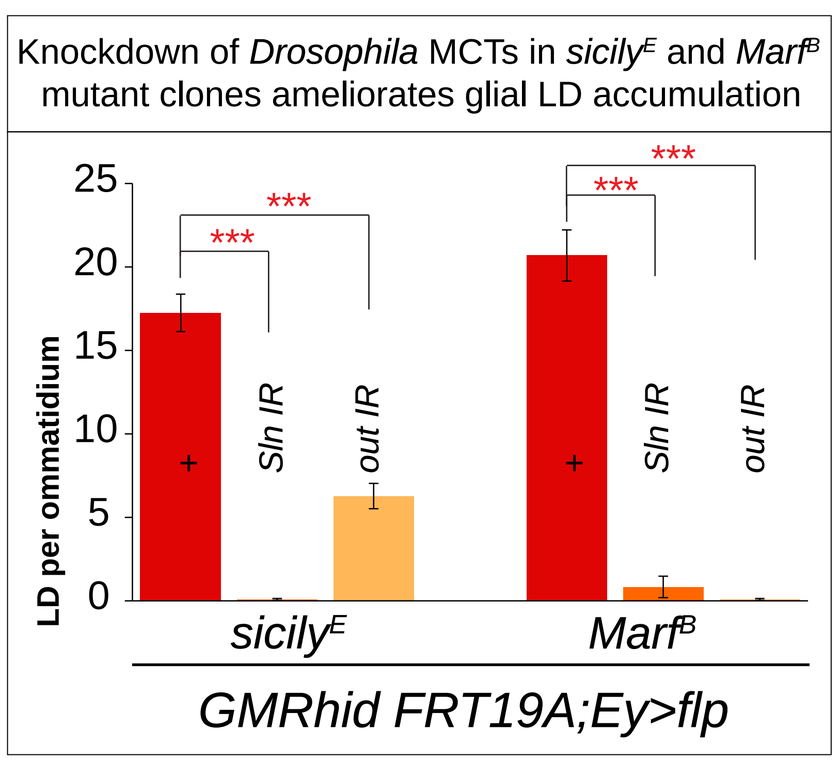


D

E

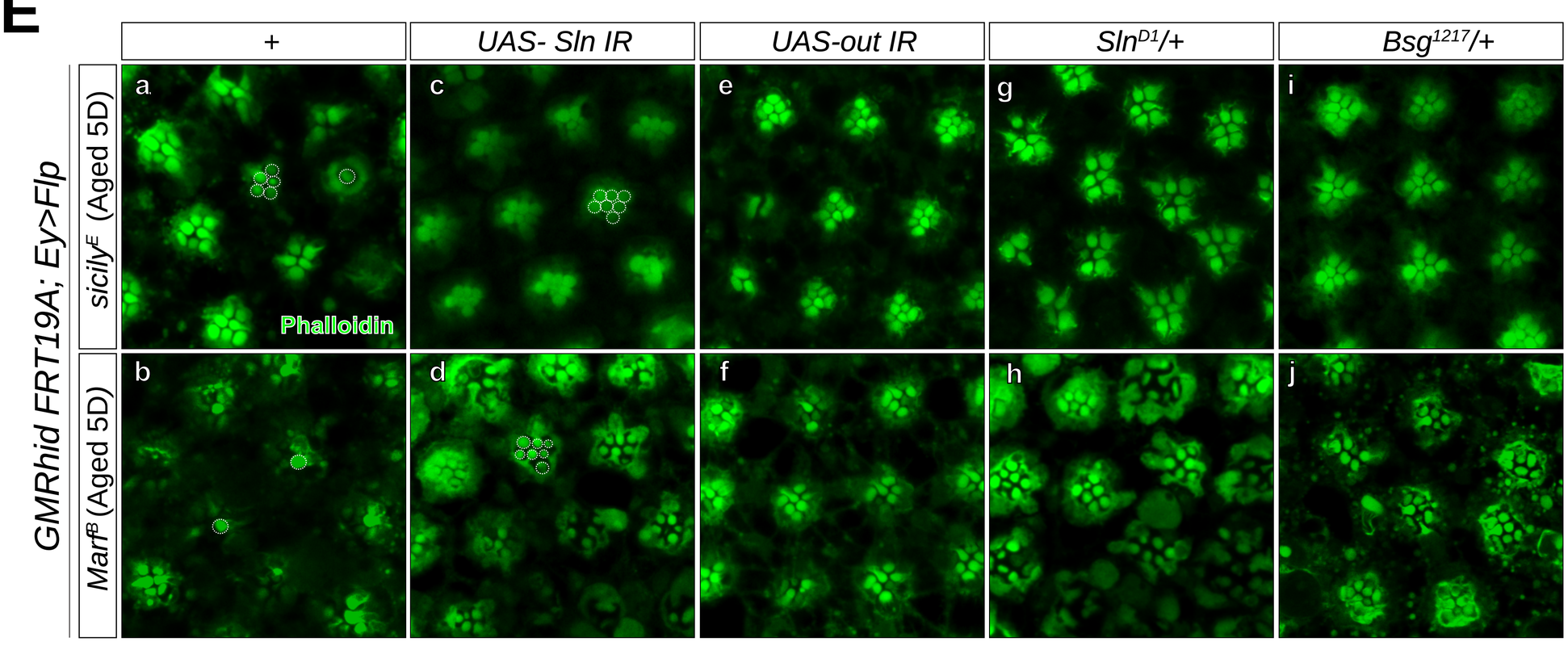
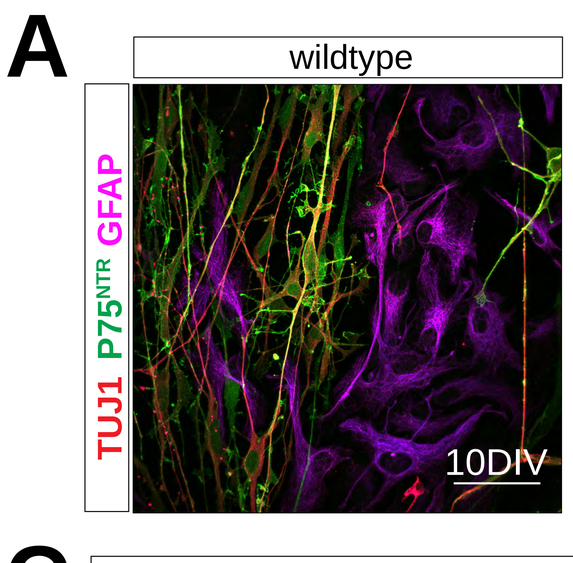
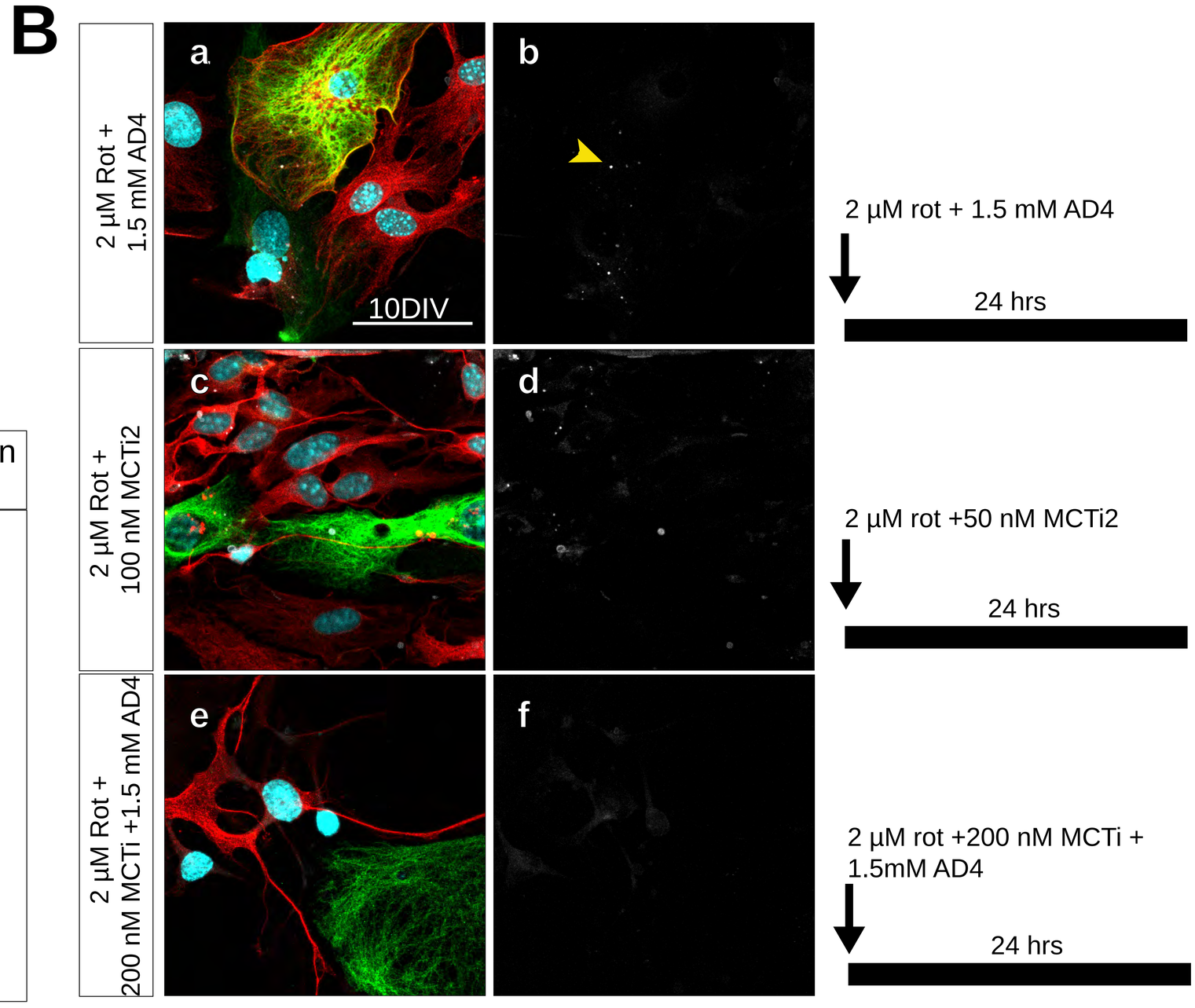
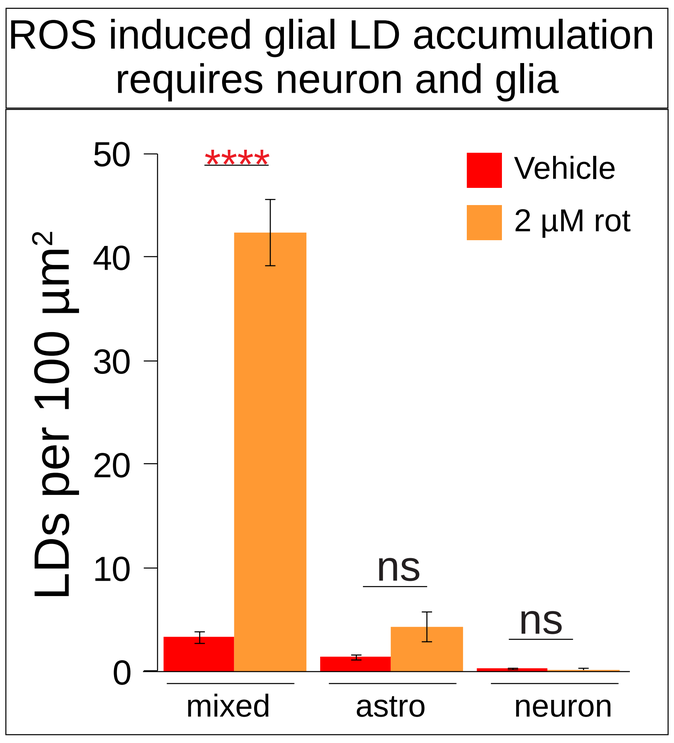
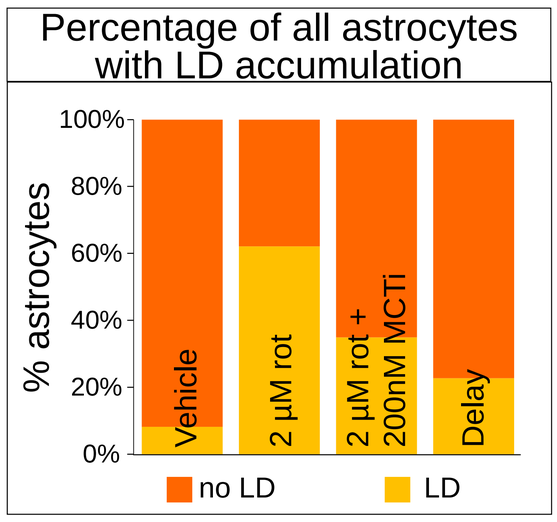


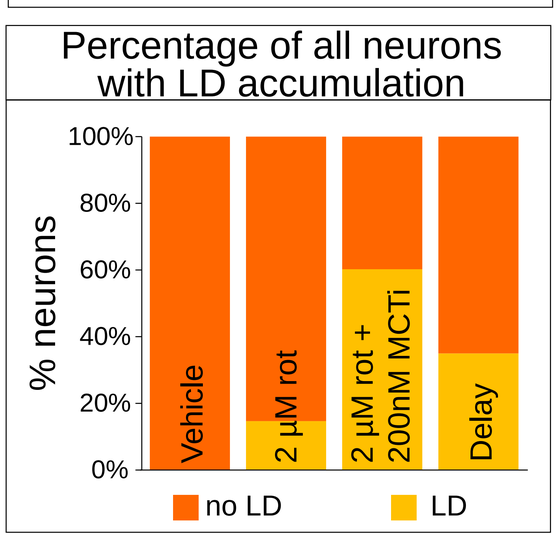
Figure S2.

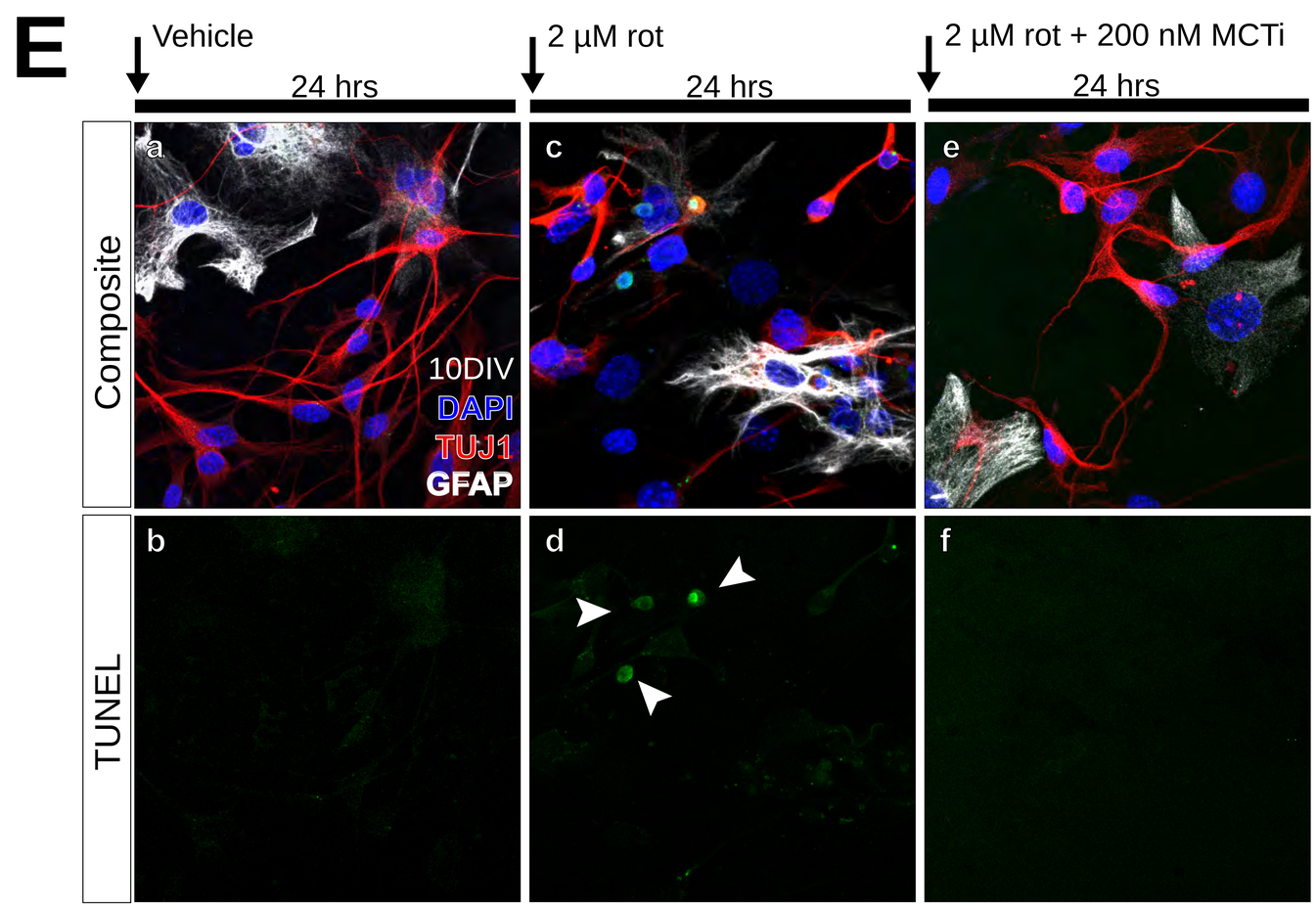


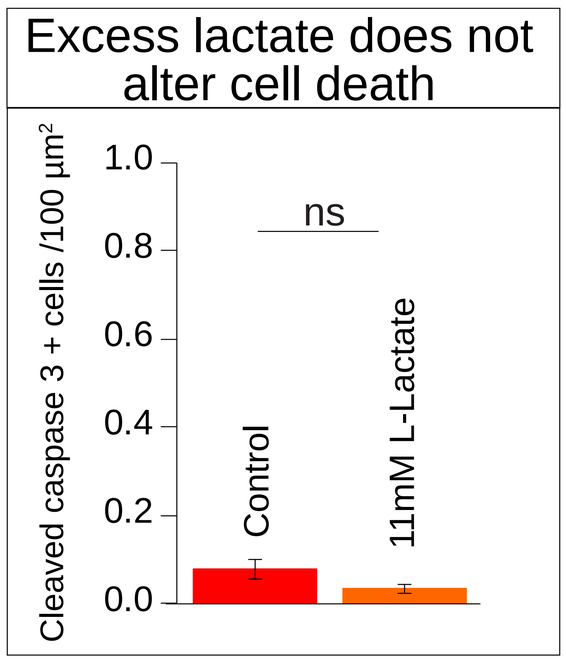


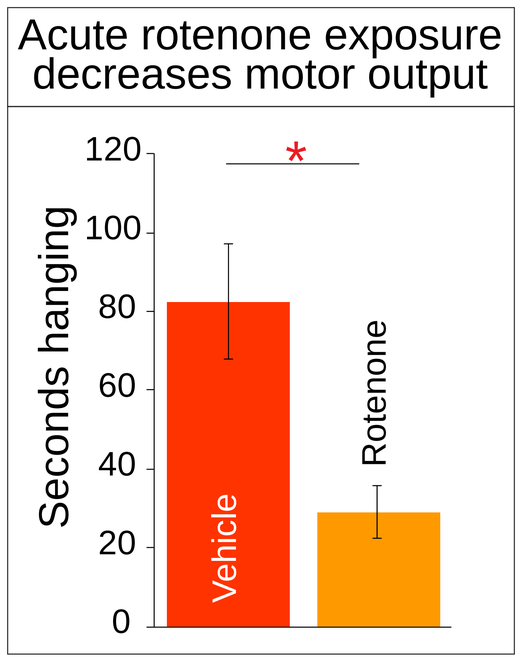












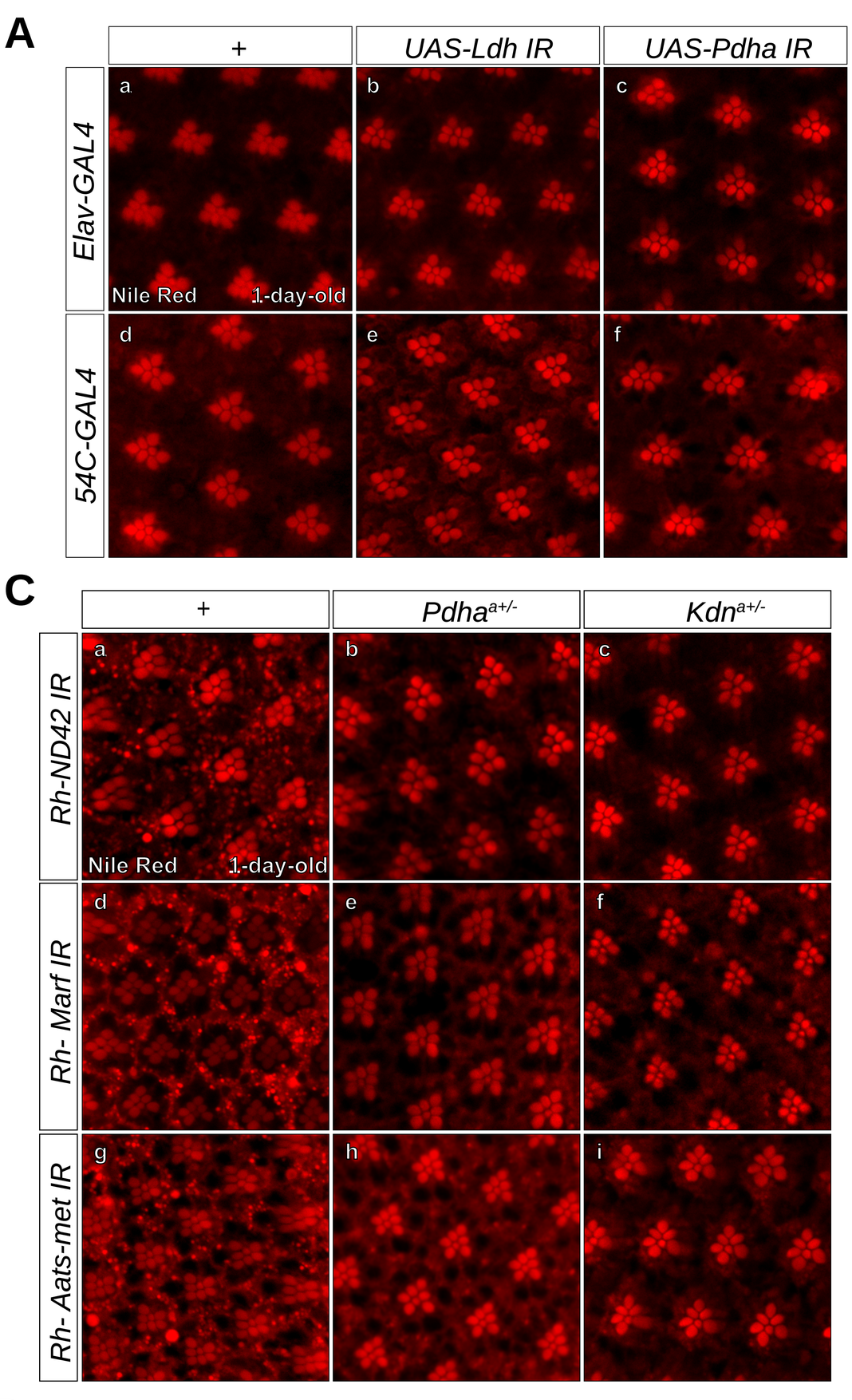
H

% ^{13}C measurements from LC-MS ion counts

media additions	biological replicates (± std deviation*)				average
	1	2	3		± std deviation
^{13}C-glucose	36.0 ± 0.2 %	32.4 ± 0.5 %	33.1 ± 0.5%		33.8 ±1.9 %
^{13}C-glucose + lactate	20.9 ± 0.3 %	20.1 ± 0.1 %	20.4 ± 0.2 %		20.5 ± 0.4 %

QTOF, 10ppm windows
*standard deviation for three LC-MS technical replicates of a single culture extract

Figure S3.


A
+
UAS-Ldh IR
UAS-Pdha IR
Elav-GAL4
54C-GAL4
a
b
c
d
e
f
Nile Red
1-day-old
B
+
UAS- Pdha IR
UAS- Ldh IR
54C-GAL4; Rh-ND42 IR
Elav-GAL4; Rh-ND42 IR
C
+
Pdha a+/-
Kdn a+/-
Rh-ND42 IR
Rh- Marf IR
Rh- Aats-met IR
g
h
i

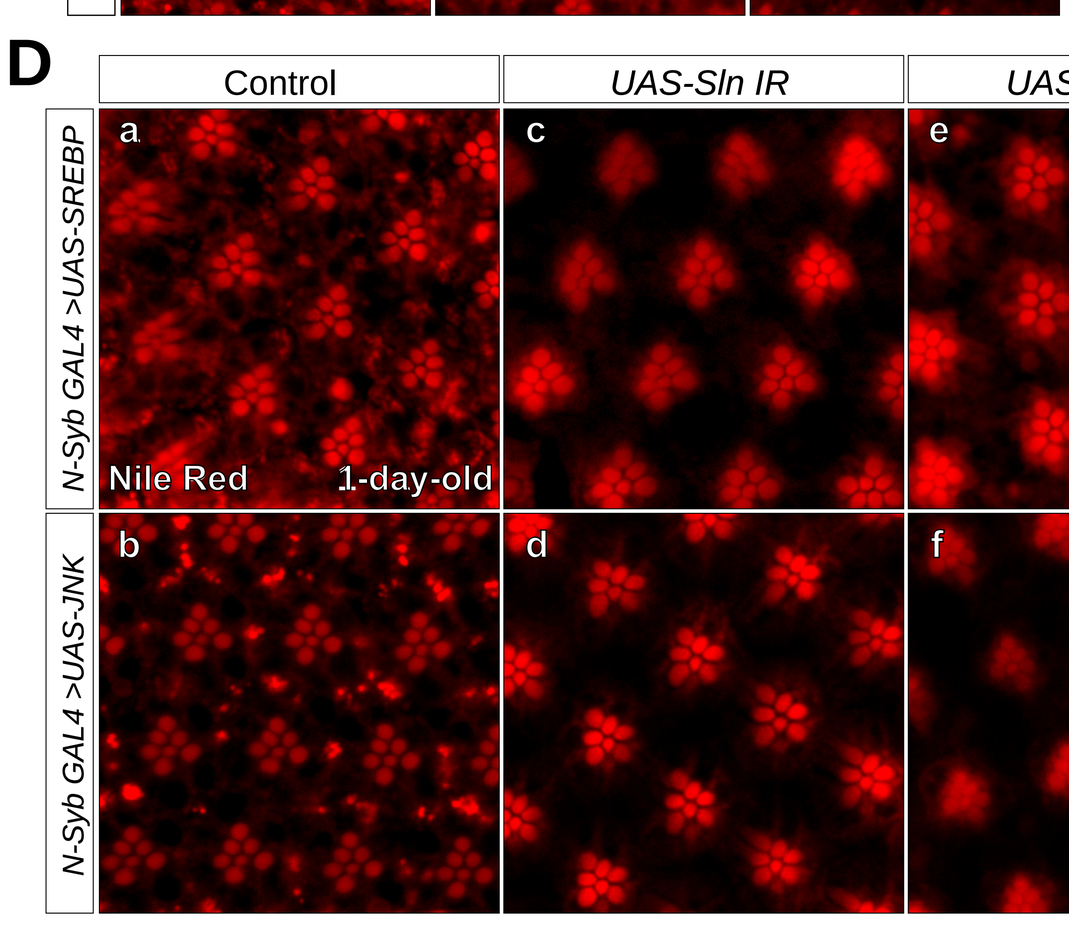
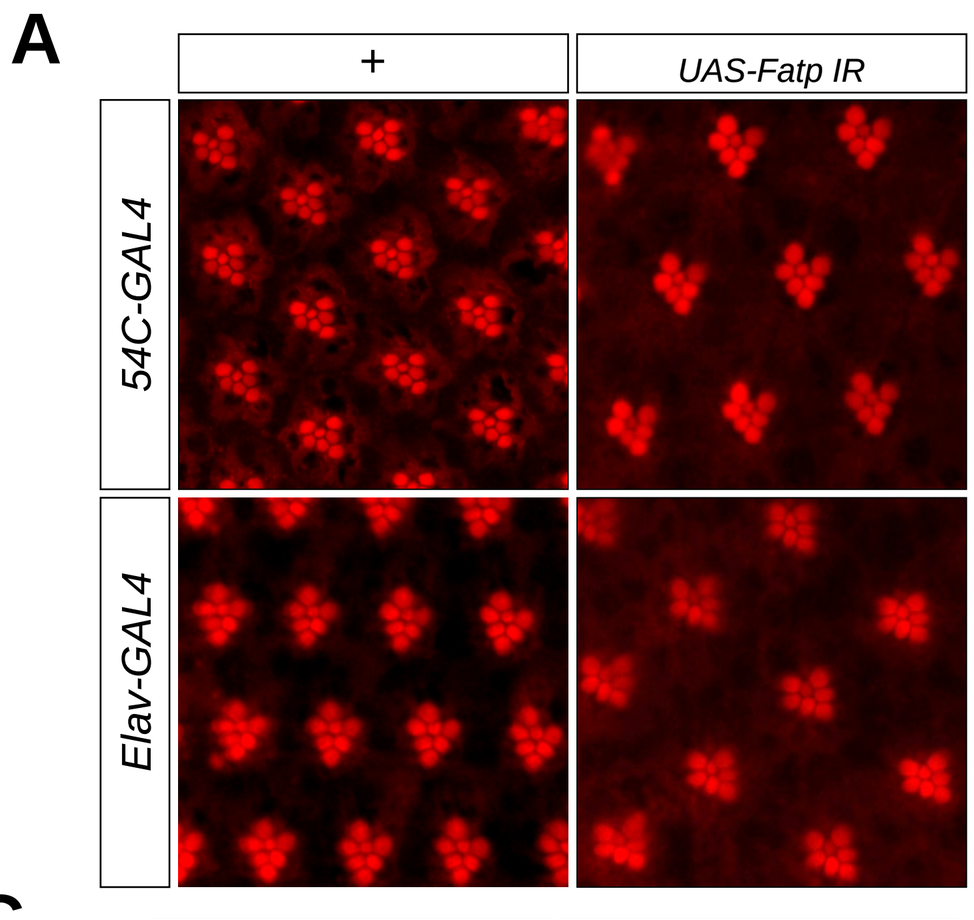
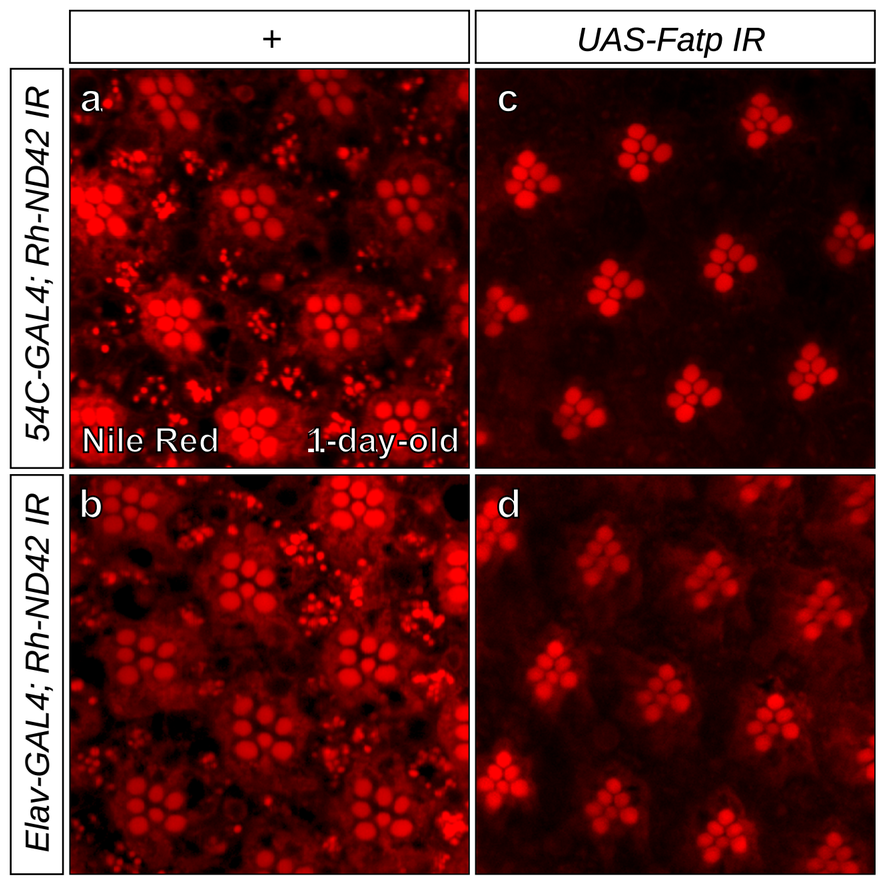
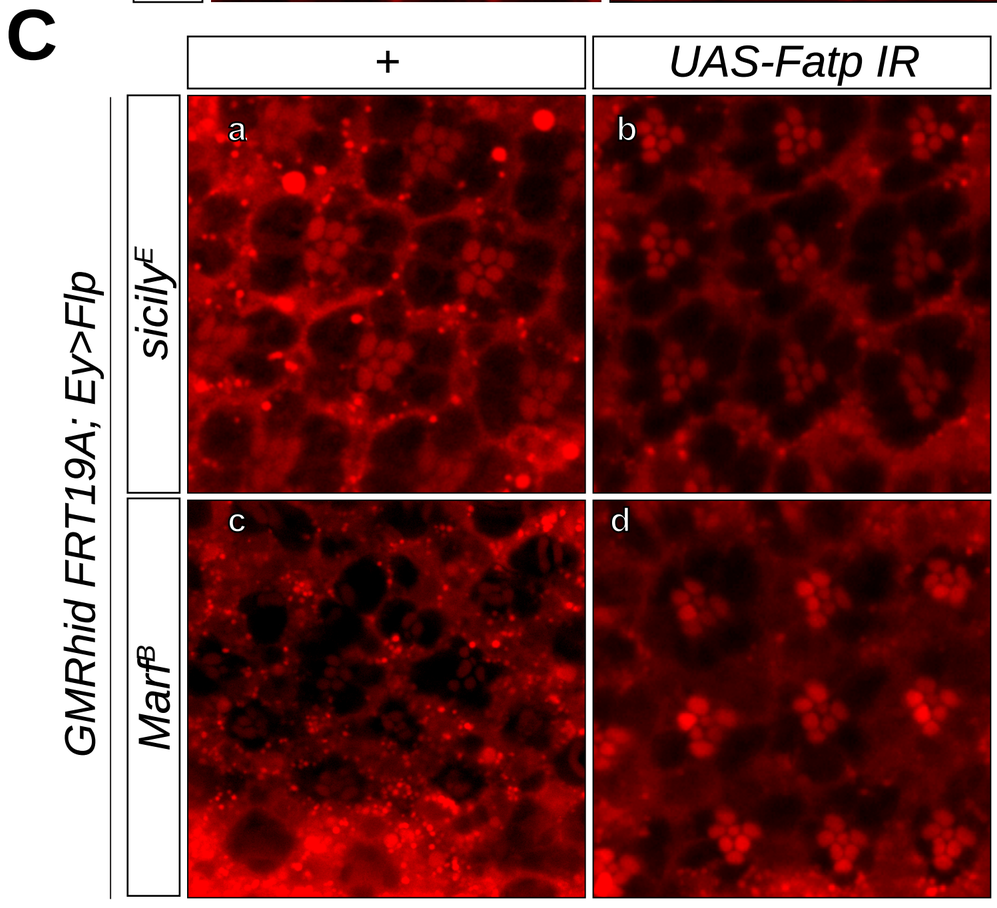
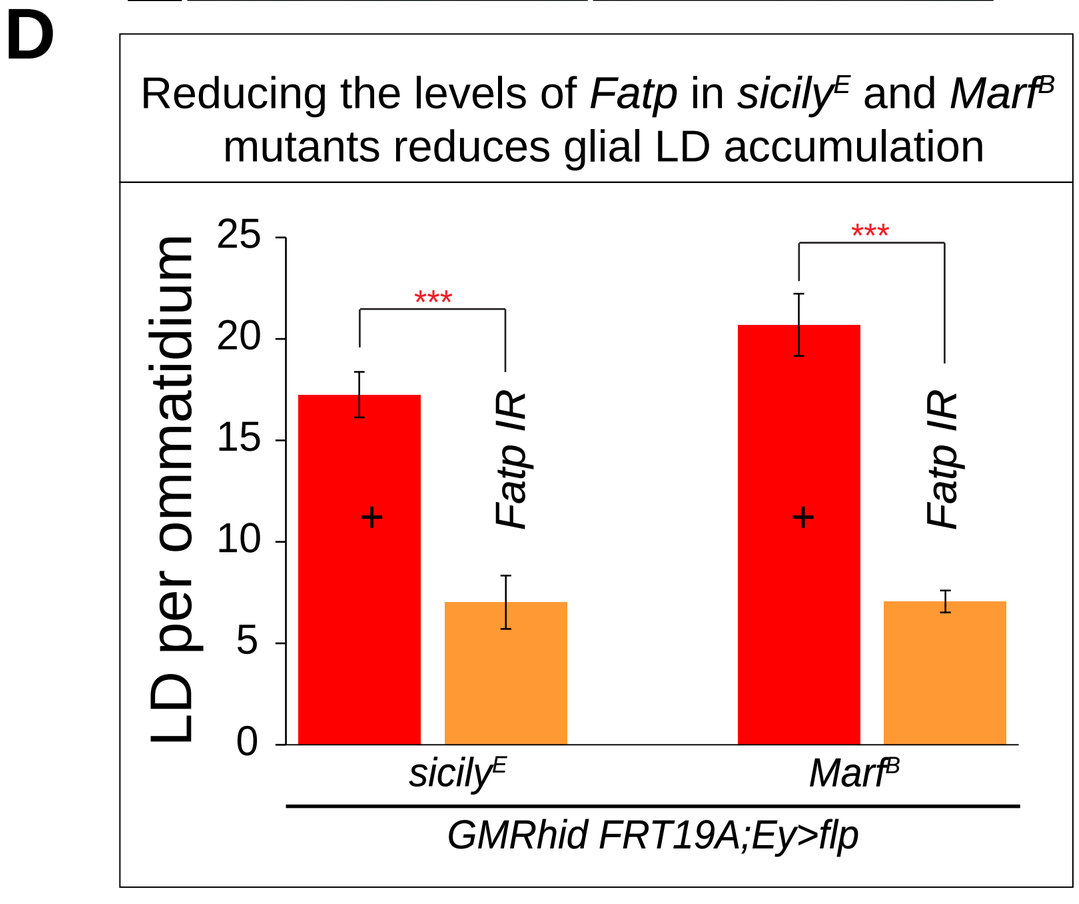

D
Control
UAS-Sln IR
UAS-out IR
UAS-Ldh IR
UAS-Pdha IR
N-Syb GAL4 >UAS-SREBP
N-Syb GAL4 >UAS-JNK
a
b
c
d
e
f
g
h
i
j
Nile Red
1-day-old

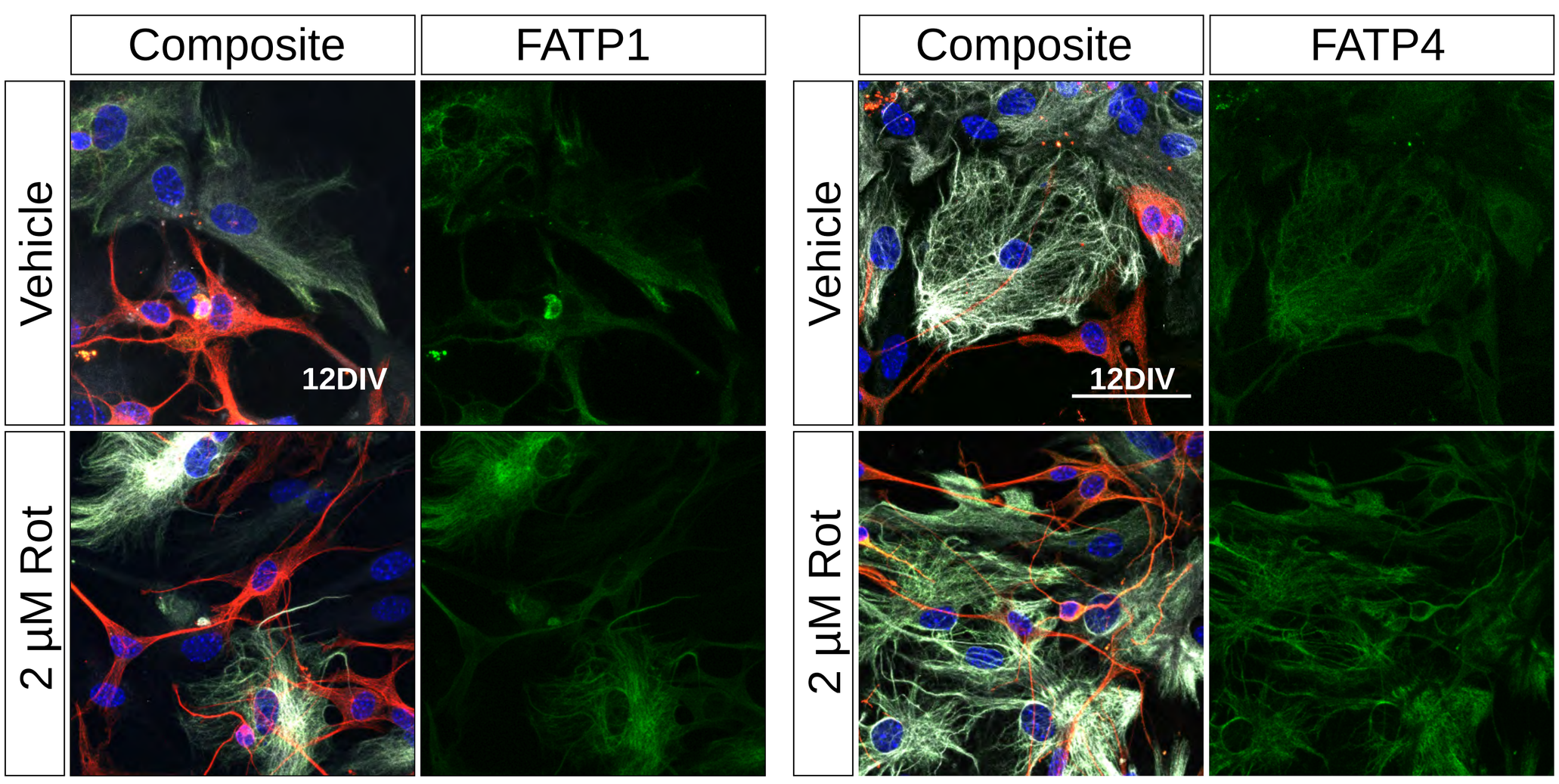
Figure S4.

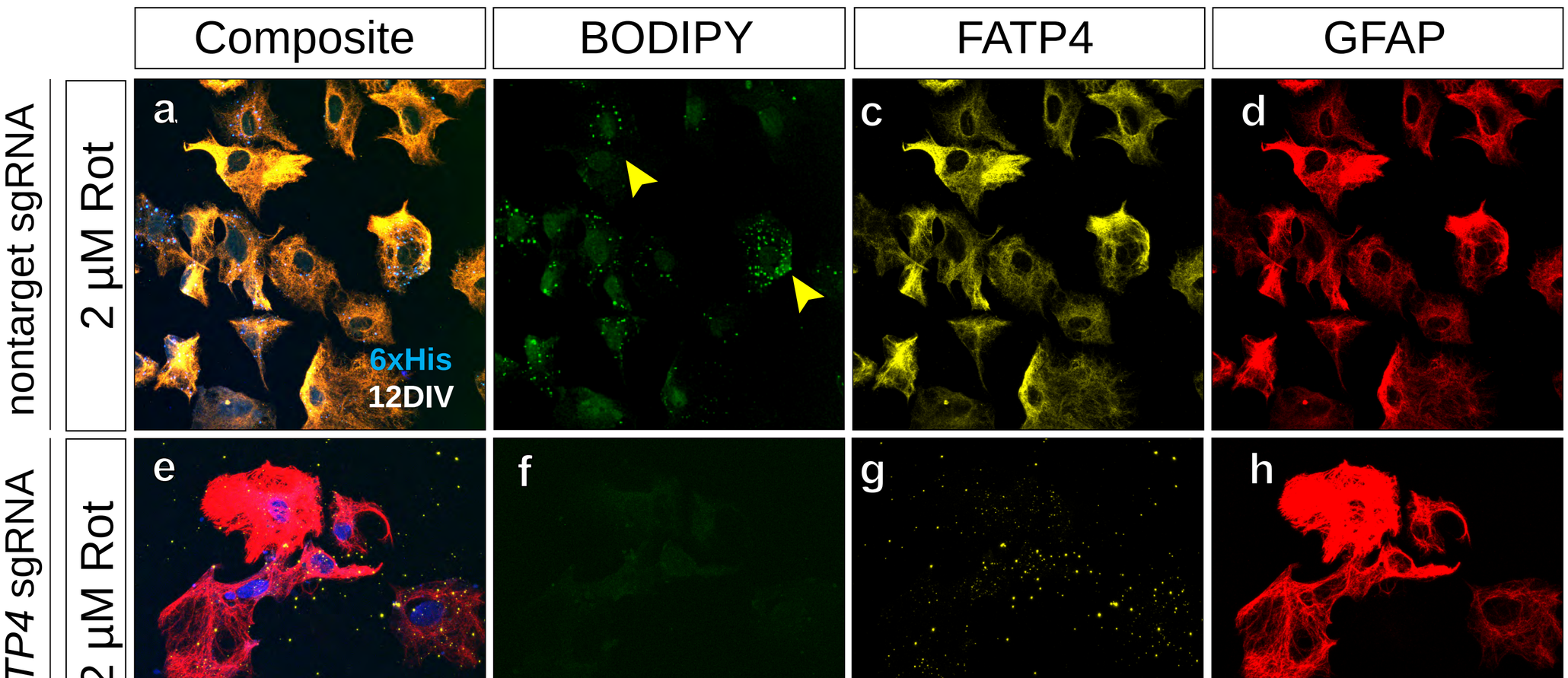

A
+
UAS-Fatp IR
54C-GAL4
Elav-GAL4


B
+
UAS-Fatp IR
54C-GAL4; Rh-ND42 IR
Elav-GAL4; Rh-ND42 IR
a
c
b
d
Nile Red
1-day-old

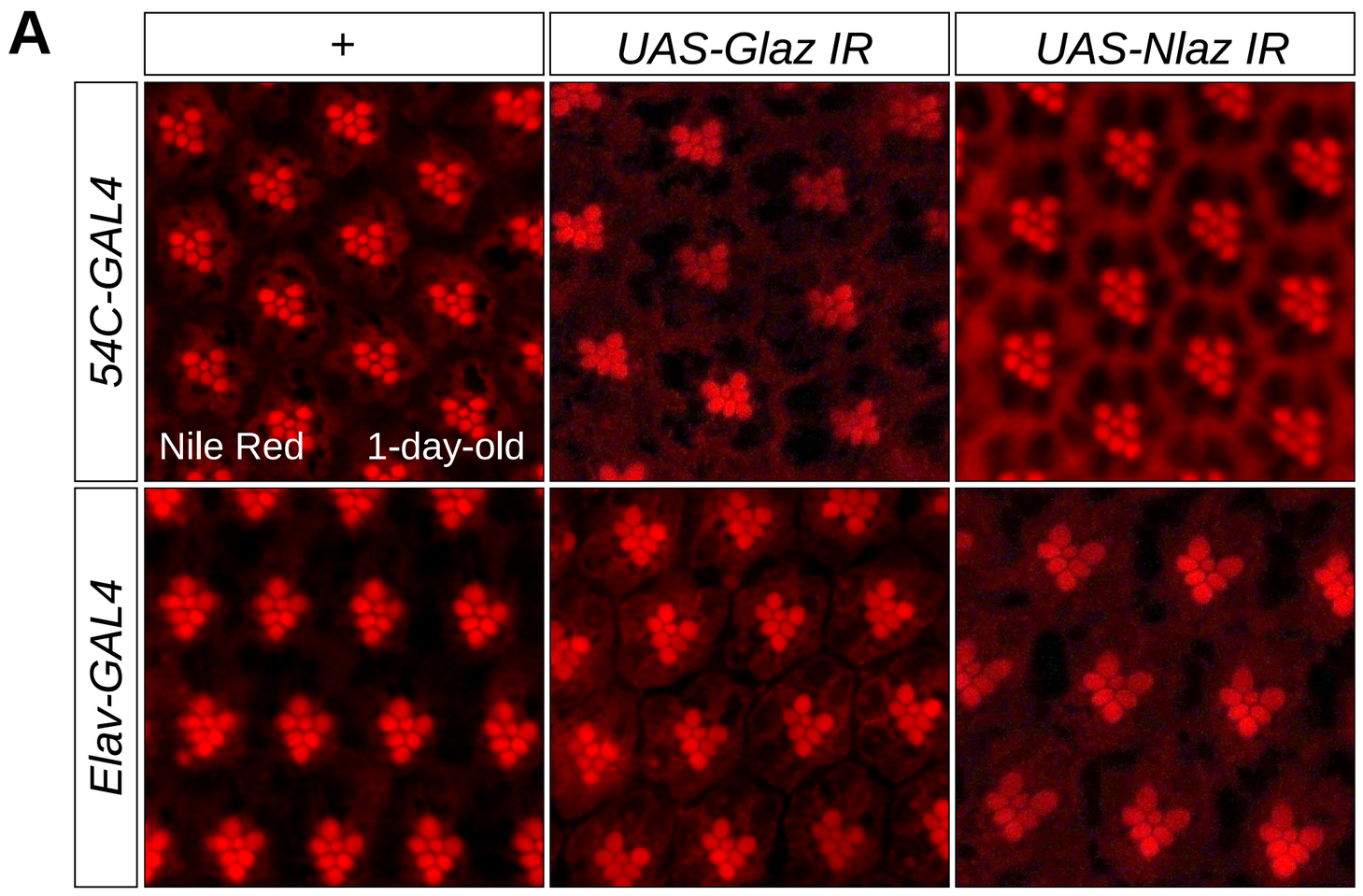

C
+
UAS-Fatp IR
GMRhid FRT19A; Ey>Flp
sicily^E
Marf^B
a
b
c
d

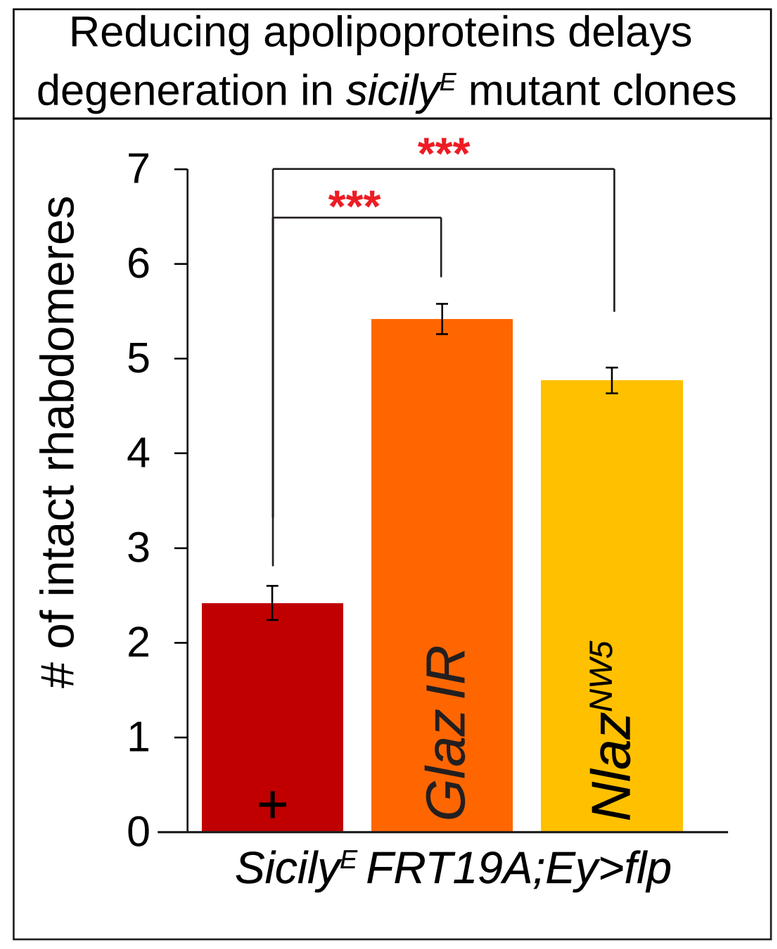

D
Reducing the levels of Fatp in sicily^E and Marf^B mutants reduces glial LD accumulation
LD per ommatidium
25
20
15
10
5
0
***
***
+
Fatp IR
sicily^E
Marf^B
GMRhid FRT19A;Ey>flp

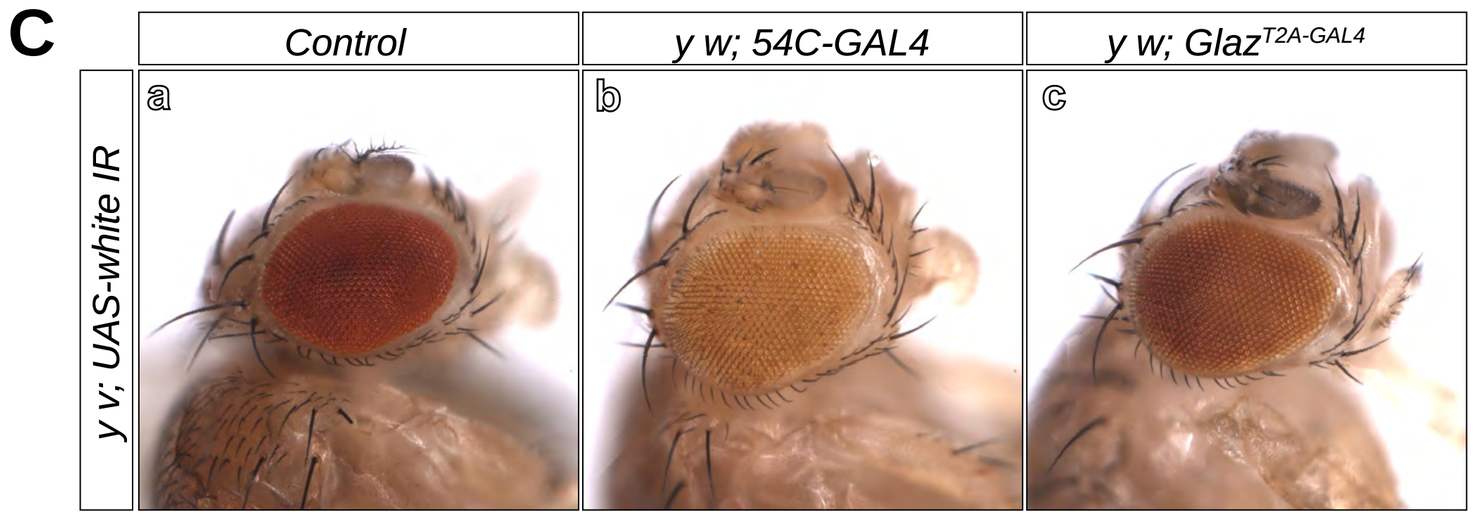

E
Composite
FATP1
FATP4
Vehicle
2 μM Rot
12DIV

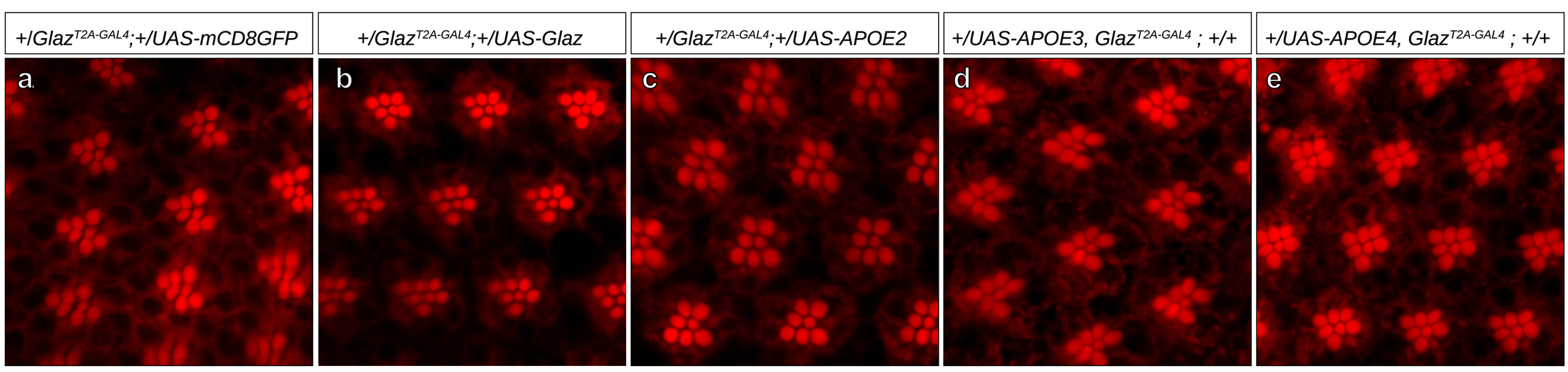

F
Composite
BODIPY
FATP4
GFAP
nontarget sgRNA
FATP4 sgRNA
2 μM Rot
a
c
d
e
f
g
h
6xHis
12DIV

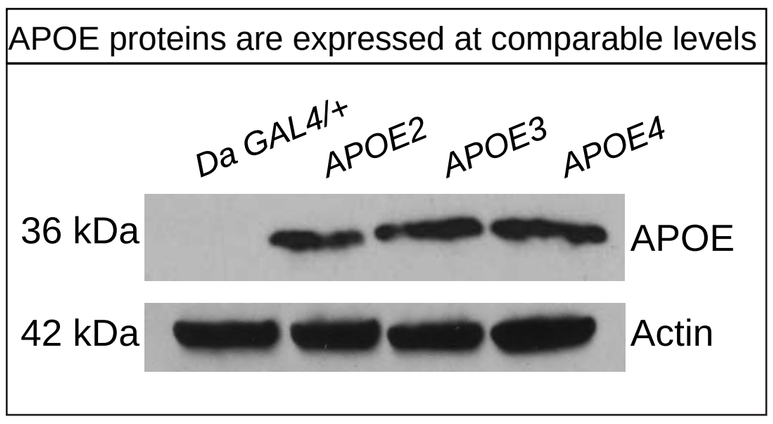
Supplemental Figure 5











Supplemental Figure 6