S1

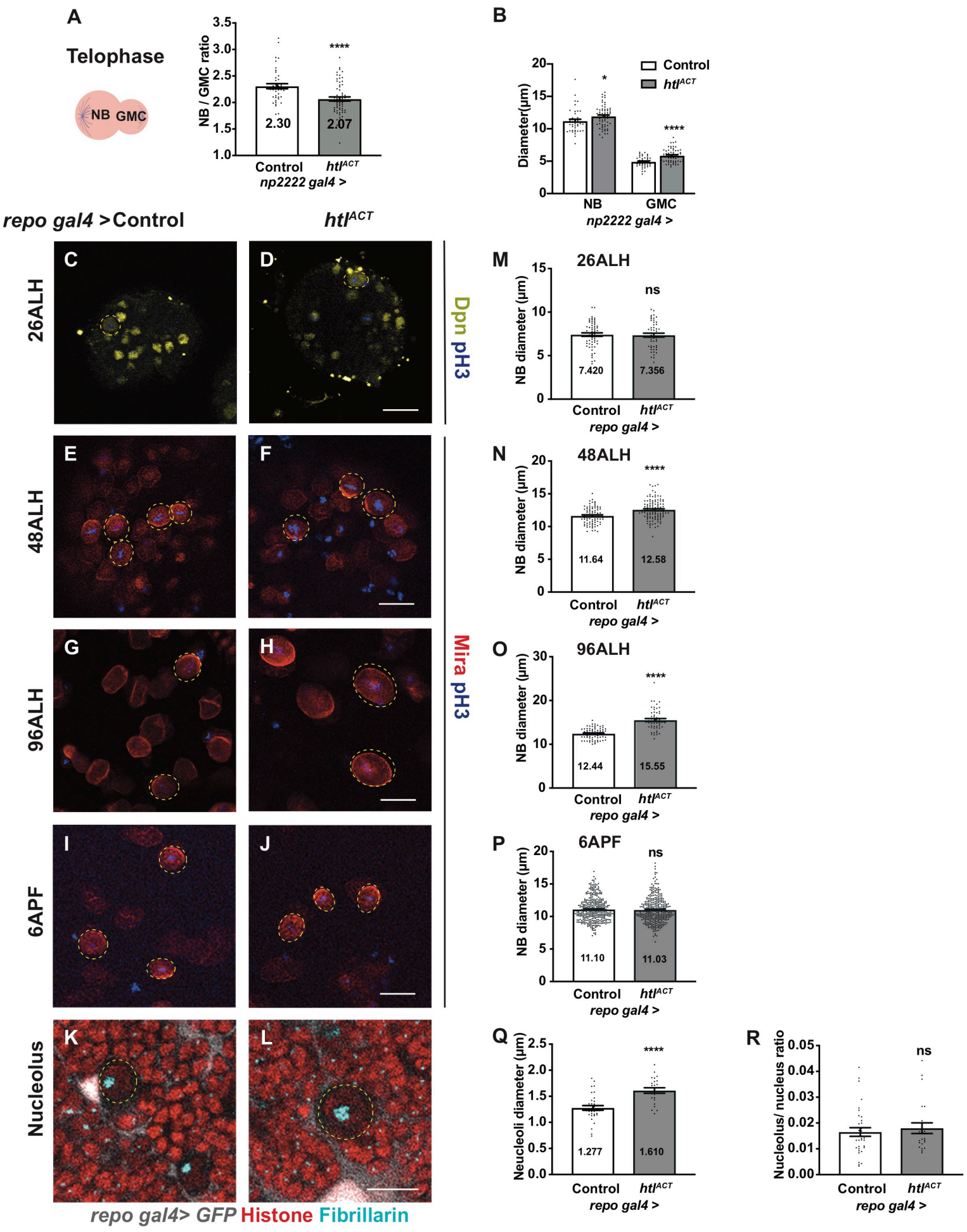

A
Telophase
NB
GMC
NB / GMC ratio
3.5
3.0
2.5
2.0
1.5
1.0
2.30
2.07
****
Control
htlACT
np2222 gal4 >
B
Diameter(µm)
20
15
10
5
0
Control
htlACT
*
****
NB
GMC
np2222 gal4 >
repo gal4 >Control
htlACT
C
D
26ALH
Dpn pH3
E
F
48ALH
G
H
96ALH
Mira pH3
I
J
6APF
K
L
Nucleolus
repo gal4> GFP Histone Fibrillarin
M
26ALH
NB diameter (µm)
15
10
5
0
ns
7.420
7.356
Control
htlACT
repo gal4 >
N
48ALH
NB diameter (µm)
20
15
10
5
0
****
11.64
12.58
Control
htlACT
repo gal4 >
O
96ALH
NB diameter (µm)
30
20
10
0
****
12.44
15.55
Control
htlACT
repo gal4 >
P
6APF
NB diameter (µm)
20
15
10
5
0
ns
11.10
11.03
Control
htlACT
repo gal4 >
Q
Neucleoli diameter (µm)
2.5
2.0
1.5
1.0
0.5
0.0
****
1.277
1.610
Control
htlACT
repo gal4 >
R
Nucleolus/ nucleus ratio
0.05
0.04
0.03
0.02
0.01
0.00
ns
Control
htlACT
repo gal4 >

Figure S1. Glial FGF activation increases NB size and cellular growth

A) Schematic representing neuroblast division during telophase (left panel, A). The ratio of the NB to GMC cell diameter was significantly reduced upon cortex glial (*np2222-gal4*) htl^{ACT} overexpression (2.07 ± 0.04, n = 63), compared to control (*w1118*) (2.31 ± 0.05, n= 43), quantified in (right panel, A).

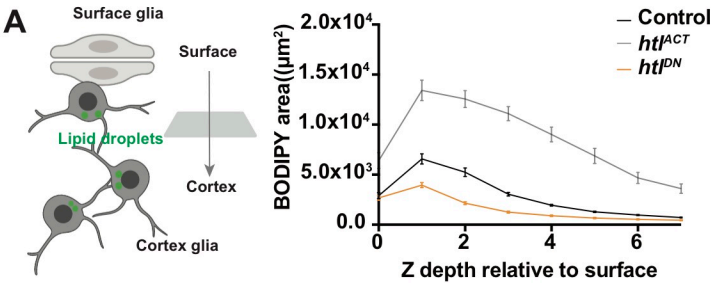
B) The diameter of both the NB and the GMC was significantly increased by cortex glial htl^{ACT} overexpression (NB: 11.92 ± 0.2 μm, n = 63; GMC: 5.86 ± 0.13 μm, n = 63), compared to the control (*w1118*) (NB: 11.19 ± 0.28 μm, n = 43; GMC: 4.90 ± 0.12 μm, n = 43).

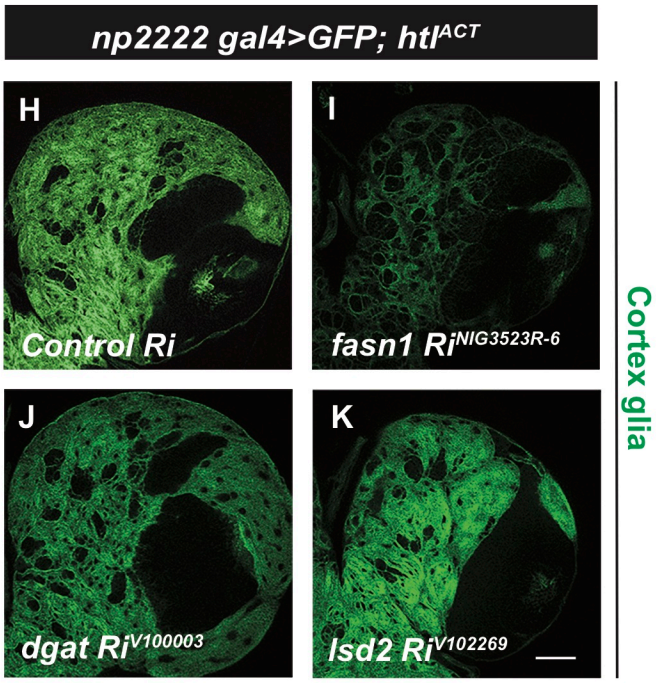
C-D, M) Representative images showing that the diameter of mitotic NBs marked with Dpn (yellow, outlined in yellow) and pH3 (blue), was not significantly altered by glial (*repo-gal4*) htl^{ACT} overexpression at 26 ALH, quantified in (M) (Control (*w1118*): 7.42 ± 0.20 μm, n = 58; htl^{ACT}: 7.36 ± 0.22 μm, n = 50). Scale bar = 20 μm.

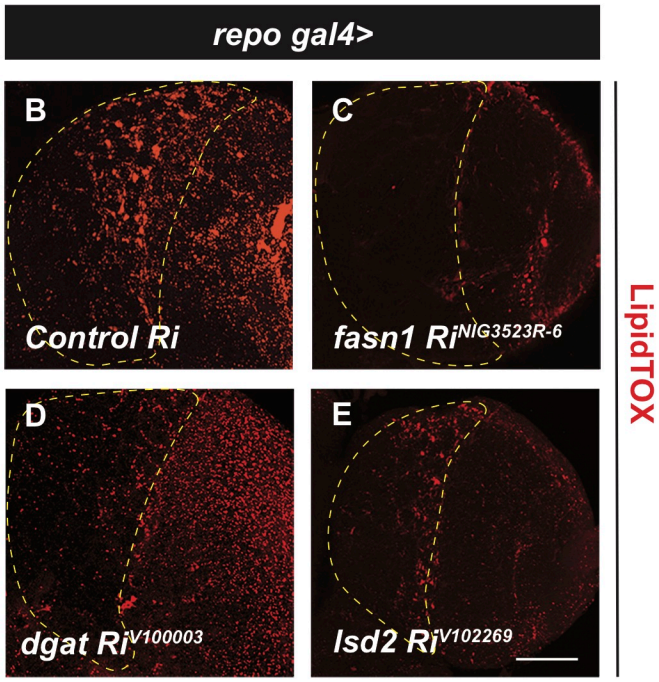
E-J, N-P) Representative images showing M phase NBs (outlined in yellow) marked with Mira (red) and pH3 (blue) at 48ALH, 96ALH, and 6APF (E-J). Upon glial (*repo*-gal4) htl^{ACT} overexpression, the diameter of mitotic NBs was significantly increased by 1 μm at 48ALH, quantified in (N). Control (*w1118*): 11.64 ± 0.15 μm, n = 78; htl^{ACT}: 12.58 ± 0.14 μm, n = 115. The difference in NB diameter was further increased to around 3 μm, at 96 ALH, quantified in (O). Control (*w1118*): 12.44 ± 0.16 μm, n = 70; htl^{ACT}: 15.55 ± 0.34 μm, n = 53. However, the difference in NB diameter was no longer observed at 6APF, quantified in (P). Control (*w1118*): 11.10 ± 0.09 μm, n = 364; htl^{ACT}: 11.03 ± 0.11 μm, n = 331. Scale bar = 20 μm.

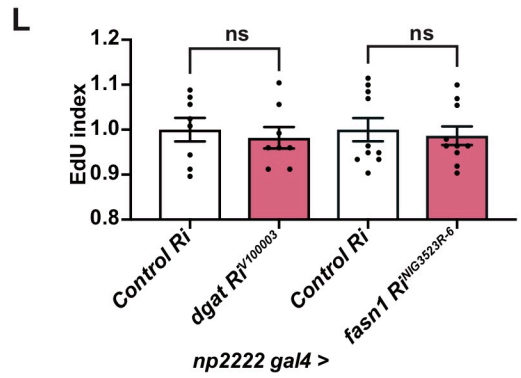
K-L, Q-R) Representative images showing NBs marked by Histone (red), surrounded by glial cells (grey, *repo-gal4 > GFP*) with nucleolus marked by Fib (Cyan). The diameter of the NB nucleolus was significantly enlarged in glial htl^{ACT} compared to control, quantified in (Q). Control (*w1118*): 1.28 ± 0.05 μm, n = 33; htl^{ACT}: 1.61 ± 0.05 μm, n = 23. However, the ratio of nucleolar to nuclear volume was not altered, quantified in (R). Control (*w1118*): 0.02 ± 0.002 μm, n = 33; htl^{ACT}: 0.02 ± 0.002 μm, n = 23. Scale bar = 10 μm.

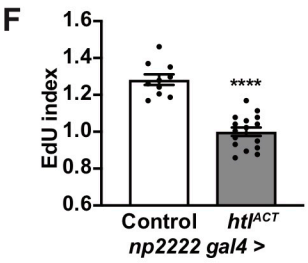
S2

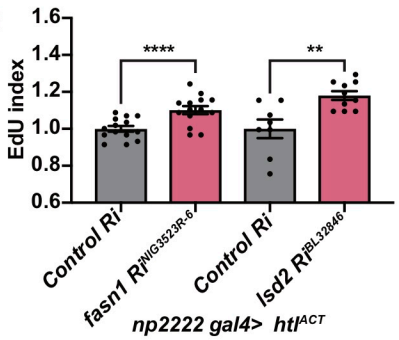

A
Surface glia
Surface
Lipid droplets
Cortex
Cortex glia
BODIPY area((µm²)
2.0x10⁴
1.5x10⁴
1.0x10⁴
5.0x10³
0.0
0
2
4
6
Z depth relative to surface
Control
htl^ACT
htl^DN

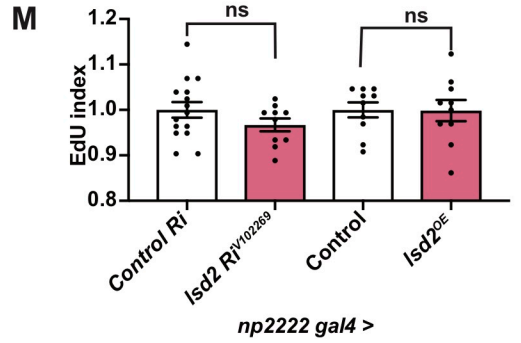

np2222 gal4>GFP; htl^ACT
H
Control Ri
I
fasn1 Ri^NIG3523R-6
J
dgat Ri^V100003
K
lsd2 Ri^V102269
Cortex glia


repo gal4>
B
Control Ri
C
fasn1 Ri^NIG3523R-6
D
dgat Ri^V100003
E
lsd2 Ri^V102269
LipidTOX


L
EdU index
1.2
1.1
1.0
0.9
0.8
ns
ns
Control Ri
dgat Ri^V100003
Control Ri
fasn1 Ri^NIG3523R-6
np2222 gal4 >


F
EdU index
1.6
1.4
1.2
1.0
0.8
0.6
****
Control
htl^ACT
np2222 gal4 >


G
EdU index
1.6
1.4
1.2
1.0
0.8
0.6
****
**
Control Ri
fasn1 Ri^NIG3523R-6
Control Ri
lsd2 Ri^BL32846
np2222 gal4> htl^ACT


M
EdU index
1.2
1.1
1.0
0.9
0.8
ns
ns
Control Ri
lsd2 Ri^V102269
Control
lsd2^OE
np2222 gal4 >

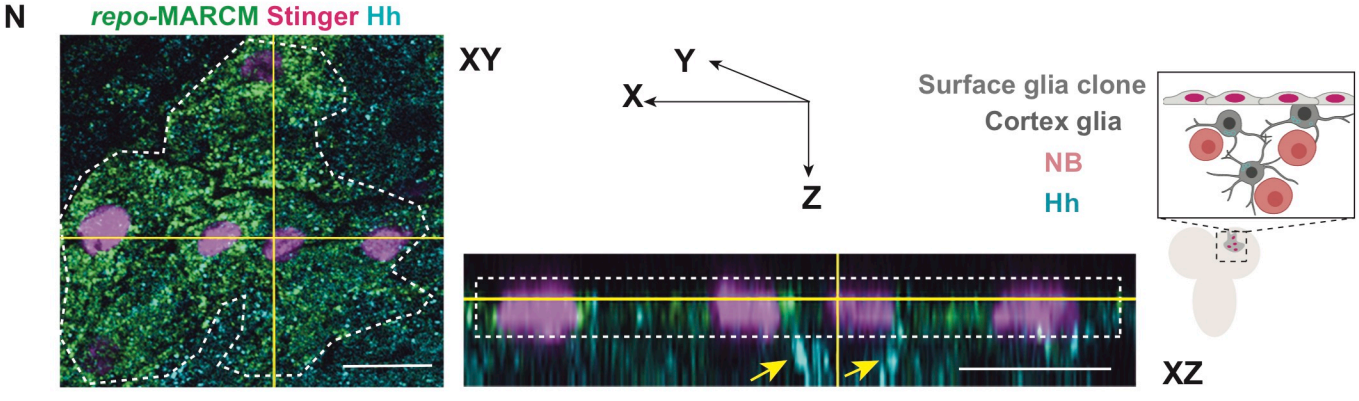

N
repo-MARCM Stinger Hh
XY
Y
X
Z
Surface glia clone
Cortex glia
NB
Hh
XZ

Figure S2. Glial lipid droplets are not required to maintain NB proliferation during development. (Related to **Figure 4**)

A) left panel: schematic depicting lipid droplets are localised to cortex glial cells. right panel: The amount of lipid droplets (BODIPY) was significantly altered by pan-glial (*repo-gal4*) FGF signalling (htl^{ACT} and htl^{DN} compared to control (*w1118*), quantified at each sampling depth relative to the surface.

B-E) Representative images showing that the amount of lipid droplets (LipidTOX, red, region of interest outlined by yellow dashed lines) was effectively reduced upon pan-glial (*repo-gal4*) knockdown of *fasn1, dgat* or *lsd2*, with RNAis used in Figure 4H and Figure S2G-M.

F) Cortex glial (*np2222-gal4*) htl^{ACT} overexpression reduced NB EdU incorporation, normalised to htl^{ACT}. Control: 1.28 ± 0.03, n = 10; htl^{ACT}: 1.00 ± 0.02, n = 16.

G) The slowdown of NB EdU incorporation due to cortex glial (*np2222-gal4*) overexpression of htl^{ACT} (F), was partially rescued by overexpression of RNAi lines against *fasn1 and lsd2*. *These* are additional RNAi lines to the ones used in Figure 3G. G, *Control Ri (mcherry Ri*): 1.00 ± 0.02, n = 14; *fasn1Ri*: 1.10 ± 0.02, n = 14. *Control Ri* (*mcherry Ri*): 1.00 ± 0.02, n = 8; *lsd2 Ri*: 1.18 ± 0.02, n=10.

H-K) Representative images showing glial overgrowth due to cortex glial (*np2222-gal4*) specific htl^{ACT} expression, was significantly reduced by overexpression of *fasn1RNAi*, but not RNAis against *dgat and lsd2. Control Ri = mcherry Ri*.

L-M) Knockdown of lipogenesis enzymes *fasn1, dgat* and *lsd2* or overexpression of *lsd2* using a cortex glial driver ((*np2222-gal4*) does not significantly affect NB EdU incorporation during development. L: *Control Ri (mcherry Ri*): 1.00 ± 0.03, n = 8; *dgat Ri*: 0.98 ± 0.02, n = 8. *Control Ri*; 1.00 ± 0.03, n = 10; *fasn1 Ri*: 0.99 ± 0.02, n = 10. M: *Control Ri*; 1.00 ± 0.02, n = 15; *lsd2Ri*: 0.97 ± 0.01, n = 10. Control (*luc*): 1.00 ± 0.02, n = 10; *lsd2 overexpression*: 1.00 ± 0.02, n = 10.

N) Left and middle panel, representative image showing a surface glial clone (*repo*-MARCM clone, green and outlined in white dashed lines, nucleus marked by Stinger, pink). In XY plane (left) and XZ plane (middle), Hh (yellow arrows, Hh antibody staining, cyan) are localised to cortex glial cells (yellow arrows) underneath the surface. Right panel, a schematic depicting XZ cross-section of CB glial cells and their relative position.
Scale bar = 10 μm

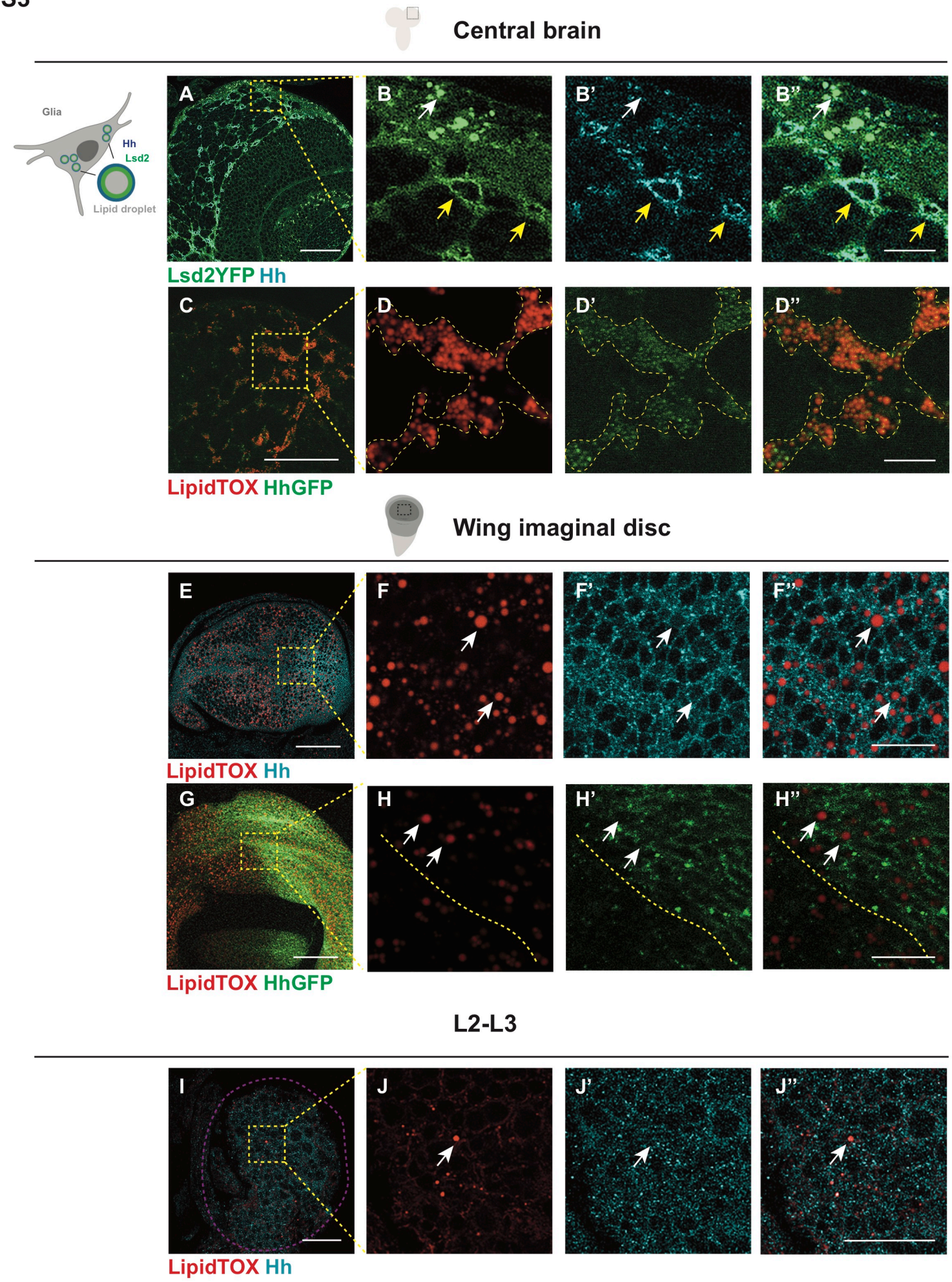

Central brain
Glia
Hh
Lsd2
Lipid droplet
A
B
B'
B''
Lsd2YFP Hh
C
D
D'
D''
LipidTOX HhGFP
Wing imaginal disc
E
F
F'
F''
LipidTOX Hh
G
H
H'
H''
LipidTOX HhGFP
L2-L3
I
J
J'
J''
LipidTOX Hh

Figure S3 Hh localizes to the surface of lipid droplets in larval CB, but not wing imaginal disc. (Related to **Figure 4**)

(B-B''), (D-D''), (F-F''), (H-H''), (J-J'') are enlarged view of (A), (C), (E), (G) and I, respectively (outlined with yellow dashed square).

A-H'') Representative images showing that Hh (Hh antibody staining, cyan in A-B'' and E-F'; Hh(BAC)GFP, green in C-D'', G-H') localized to the surface of lipid droplets (surrounded by Lsd2YFP, green in A-B''; lipidTOX, red in C-D'') in late larval CB. The Hh and lipid droplet association was observed in cortex glia (yellow arrows in B-B'', circled by yellow dashed lines in D-D''), but not surface glia (white arrows), or posterior compartments of wing imaginal discs (region of interest lies right of the dashed line (H), white arrows, F-F'', H-H'').

I-J'') Representative images showing that at L2-L3, Hh and lipid droplets were present at low levels and were weakly associated (white arrows).

Scale bar = 50 μm for (A, C, E, G). Scale bar = 10 μm for (B-B'', D-D'', F-F'' and H-H''). Scale bar = 20 μm for (I-J'').

S4

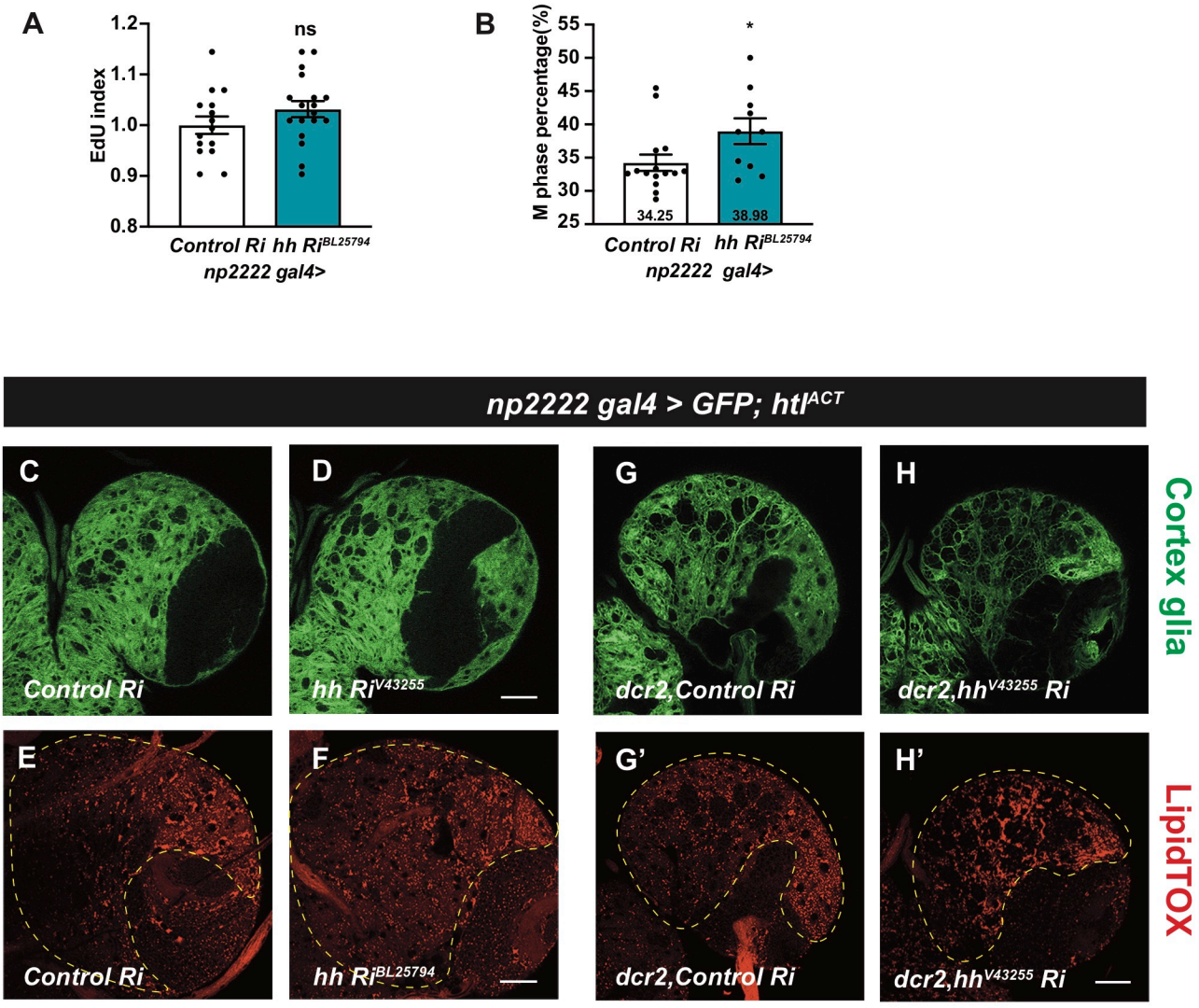

A
EdU index
1.2
1.1
1.0
0.9
0.8
ns
Control Ri
hh Ri BL25794
np2222 gal4>
B
M phase percentage(%)
55
50
45
40
35
30
25
*
34.25
38.98
Control Ri
hh Ri BL25794
np2222 gal4>
np2222 gal4 > GFP; htl ACT
C
Control Ri
D
hh Ri V43255
G
dcr2,Control Ri
H
dcr2,hh V43255 Ri
Cortex glia
E
Control Ri
F
hh Ri BL25794
G'
dcr2,Control Ri
H'
dcr2,hh V43255 Ri
LipidTOX

Figure S4. Hedgehog signalling regulates gliogenesis (related to Figure 5**).**

A-B) Cortex glial (*np2222-gal4*) overexpression of *hh RNAi* significantly increased the percentage of NBs in mitosis, quantified in (B) (from 34.25% ± 1.24%, n = 15 to 38.98% ± 1.93%, n = 10), but not EdU index, quantified in (A). *Control Ri* (*mcherry Ri*): 1.00 ± 0.02, n = 15; *hh Ri*: 1.03 ± 0.02, n = 18.

C-F) Expression of htl^{ACT} and *hh RNAi* in cortex glial cells (*np2222-gal4*) did not significantly change glial membrane morphology or LD content (LipidTOX, region of interest outlined in yellow dashed line) compared to Control (expression of htl^{ACT} and *mcherry Ri*).

G-H') Expression of htl^{ACT} and *hh RNAi* together with *dcr2* in cortex glial cells (*np2222-gal4*) significantly reduced glial membrane volume but not LD content (LipidTOX, region of interest outlined in yellow dashed line) compared to Control (expression of htl^{ACT}, *mcherry Ri* and *drc2*).

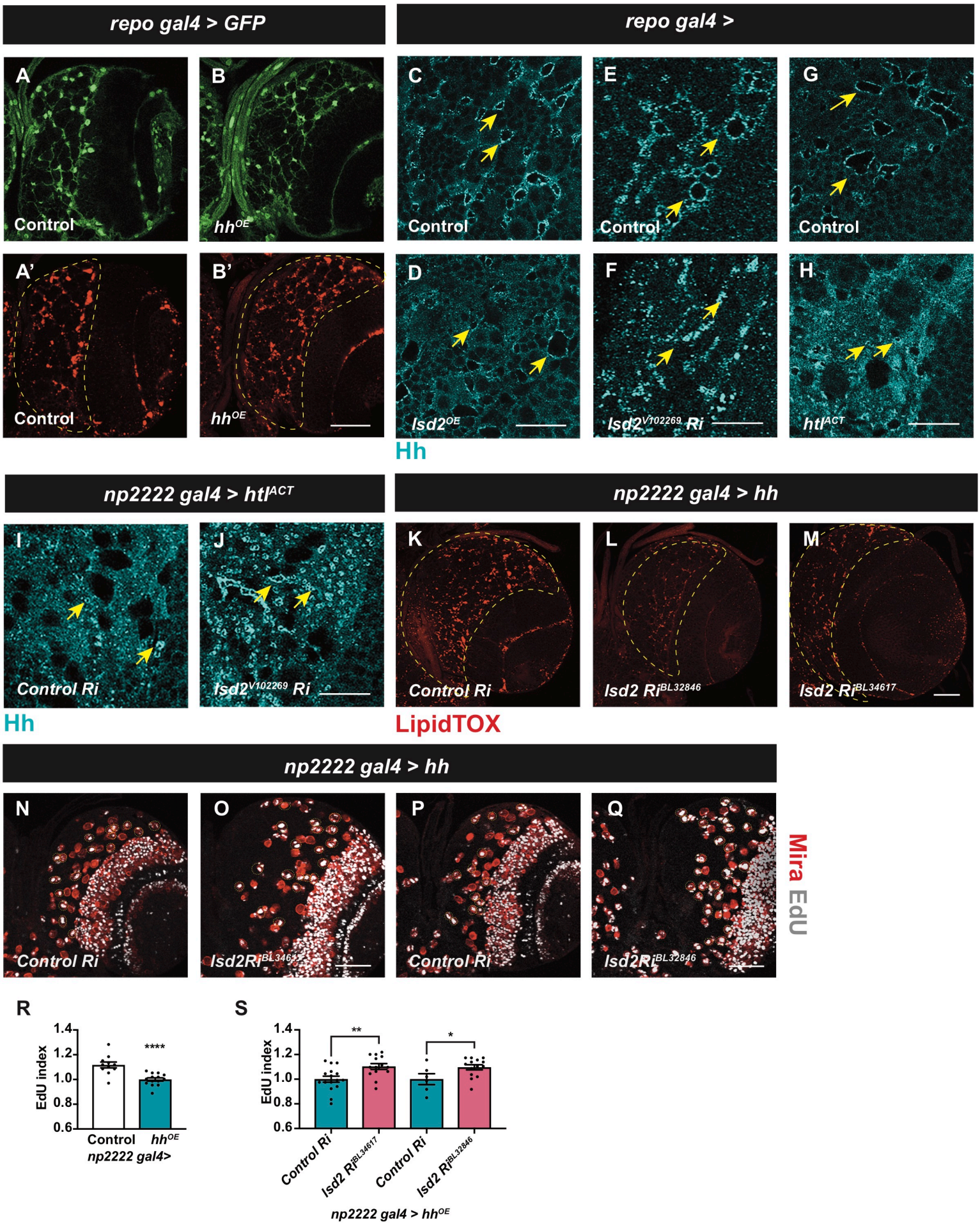

repo gal4 > GFP
repo gal4 >
Glia
LipidTOX
A
Control
B
hh^OE
C
Control
E
Control
G
Control
A'
Control
B'
hh^OE
D
lsd2^OE
F
lsd2^V102269 Ri
H
htl^ACT
Hh
np2222 gal4 > htl^ACT
np2222 gal4 > hh
I
Control Ri
J
lsd2^V102269 Ri
K
Control Ri
L
lsd2 Ri^BL32846
M
lsd2 Ri^BL34617
Hh
LipidTOX
np2222 gal4 > hh
N
Control Ri
O
lsd2Ri^BL34617
P
Control Ri
Q
lsd2Ri^BL32846
Mira EdU
R
EdU index
1.4
1.2
1.0
0.8
0.6
****
Control
hh^OE
np2222 gal4>
S
EdU index
1.4
1.2
1.0
0.8
0.6
**
*
Control Ri
lsd2 Ri^BL34617
Control Ri
lsd2 Ri^BL32846
np2222 gal4 > hh^OE

Figure S5. Lipid droplet surface protein, Lsd2, regulates Hh localization and activity (related to Figure 5).

A-B') Representative images showing that ectopic overexpression of *hh* in glia (*repo-gal4*) was not sufficient to alter neither the glial cell number, nor the amount of lipid droplets. The region of interest is outlined with yellow dashed line in A' and B'. Control = *w1118*.

C-H) Representative images showing that Hh (cyan, yellow arrows) accumulates in a typical ring-like pattern in Control (*luc* in C, *mcherryRi* in E, and *w1118* in G), indicative of Hh-Lipid droplet association (as seen in Figure 4). Pan-glial (*repo-gal4*) overexpression of *lsd2* (D), RNAi against *lsd2* (F) as well as htl^{ACT} caused disruption to Hh localisation.

I-J) Representative images showing that disruption to Hh localisation (cyan, yellow arrows) by cortex glial-specific htl^{ACT} overexpression, were partially restored by overexpression of *lsd2 RNAi* compared to control*: mcherry Ri.* Scale bar = 20 μm.

K-M) Representative images showing that overexpression of two independent *lsd2 RNAis* in cortex glia (*np2222-gal4*), where *hh* was also overexpressed, was sufficient to reduce the amount of lipid droplets compared to Control (*mcherryRi*). The region of interest is outlined with yellow dashed line in K-M.

R) Overexpression of *hh* in cortex glia (*np2222-gal4*) reduced NB EdU incorporation compared to control. Control (*w1118*): 1.12 ± 0.02, n = 11; *hh* overexpression: 1.00 ± 0.01, n = 14 (data normalised to hh overexpression).

N-Q, S) The glial-*hh*- induced NB cell cycle slow down was significantly rescued by glial *lsd2* knockdown (via two independent RNAis), quantified in (S). Control: *mcherryRi*
EdU+ NBs (Mira, red; EdU, grey) are circled with yellow dashed lines.