Expanded View Figures

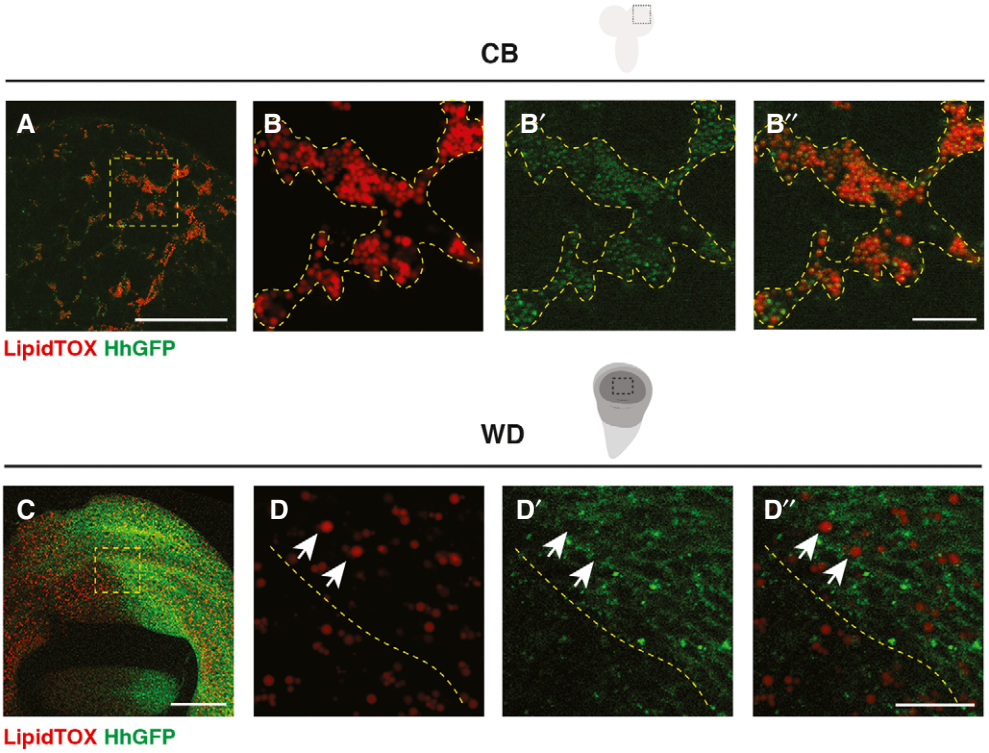

Figure EV1. Hh forms complexes with LDs in the CB but not the wing discs (related Fig 1).

A–B" HhGFP and LDs are associated in the CB glial cells (outlined with yellow dashed lines).
C–D" HhGFP is not associated with LDs in the posterior wing disc (white arrows, the posterior compartment is separated from the anterior with yellow dashed lines).

Data information: (B-B", D-D") are zoomed in images of (A and C), respectively. Scale bar = 50 μm in (A and C). Scale bar = 10 μm in (B-B" and D-D").

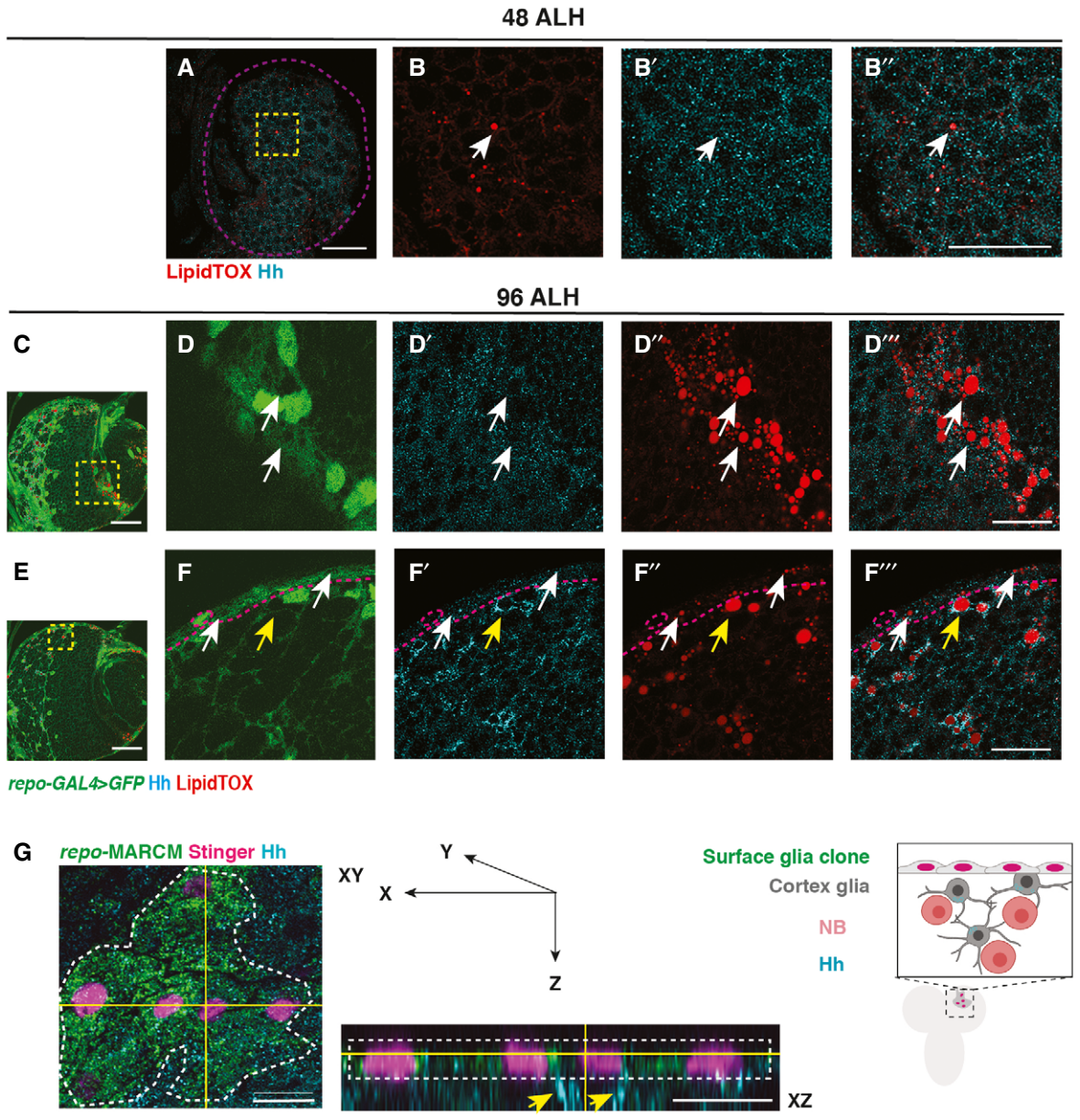

Figure EV2. Hh-LD associations are specifically observed in cortex glial cells in the CB during late larval stages (related to Fig 1).

A–B" Hh and LDs are present at low levels at 48 ALH, and do not form specific association (white arrows, brain lobes are circled with purple dashed lines).

C–D"' Hh and LDs are not associated in the optic lobe glial cells (white arrows).

E–F"' Hh and LDs associate only in the cortex glial cells (yellow arrows) but not the surface glial cells (white arrows, surface glia are separated from cortex with magenta dashed lines).

G Left and middle panel, representative image showing a surface glial clone (circled with white dashed lines, *repo*-MARCM, glial nucleus marked by Stinger in pink). Hh is localised to the cortex glial cells (yellow arrows) underneath the clone marked in green. Right panel, a schematic depicting XZ cross-section of CB glial cells and their relative position.

Data information: Glial cells are visualised with *repo-GAL4 > GFP* in (C-F"'). (B-B", D-D"', F-F"') are zoomed in images of (A, C, E), respectively. (C) is the same image as Fig 1C, with the optic lobe region highlighted in a yellow dashed square. Scale bar = 50 μm in (C, E) and XY section in (G). Scale bar = 20 μm in (A-B", D-D"', F-F"'), Scale bar = 10 μm for XZ section in (G).

Figure EV3. Effects of Hh overexpression and knockdown on Hh level, NB number and EdU index (related to Fig 2).

A–D Representative images showing pan-glial Hh knockdown (*repo-GAL4 > GFP* with *UAS-dcr2*) efficiently reduces Hh staining in the CB (outlined with yellow dashed lines) and brain lobe size, quantified in (C) (n = 6, 6 brain lobes) and (D) (n = 15, 16 brain lobes), respectively.

E Representative image from EdU incorporation assays used throughout the manuscript. During a 15 min EdU pulse, type I NB (yellow arrow) and its GMC (white arrow) both incorporate EdU. EdU index quantifications include only EdU^+ type I NBs.

F Hh knockdown (*repo-GAL4 > GFP* with *UAS-dcr2*) significantly reduces NB EdU index (n = 12, 9 brain lobes).

G Hh knockdown or overexpression in glial cells (*repo-Gal4>*) does not significantly alter the number of CB NBs (n = 12, 10; 12, 12 brain lobes).

H Schematic depicting EdU pulse-chase experiment. Larvae are fed with EdU-containing food for 3 h and then chased with EdU-free food for 3 h before CNS dissection at wandering stages. NBs and newly generated GMCs are marked with Mira; GMCs and newly generated neurons are marked with ProsGFP.

I–K Representative images showing that Hh knockdown in cortex glial cells (*NP2222-GAL4*) significantly reduced the number of EdU^+ cells that are marked by $ProsGFP^+$, quantified in (K) (Box plot, the boxes extend from the 25^{th} to 75^{th} percentiles; the median is marked by a central band inside the box; and the whiskers go down to the minimum value and up to the maximum value. n = 94, 104 NB lineages imaged from 8, 8 brain lobes, respectively).

L–O Representative images showing that Hh overexpression in cortex glial cells (*NP2222-GAL4 > mGFP*) does not alter cortex glial membrane size and total $Repo^+$ glial cell numbers, quantified in (N) (n = 15, 14 brain lobes) and (O) (n = 15, 14 brain lobes), respectively.

P–Q" Representative images showing Ptc:mcherry is expressed in NBs (yellow arrows). (Q-Q") are zoomed in images of (P).

Data information: Scale bar = 50 μm in (A, B, E, L, M). Scale bar = 20 μm in (I, J, P, Q-Q"). Error bar represents SEM. In (C): unpaired t-test, (****) $P < 0.0001$. In (D): Mann–Whitney test, (**) $P = 0.0017$. In (F): Welch's t-test, (*) $P = 0.0101$. In (G): unpaired t-test, (ns) $P = 0.3645$; Mann–Whitney test, (ns) $P = 0.7621$. In (K): Welch's t-test, (****) $P < 0.0001$. In (N): unpaired t-test, (ns) $P = 0.5151$. In (O): unpaired test, (ns) $P = 0.9690$.

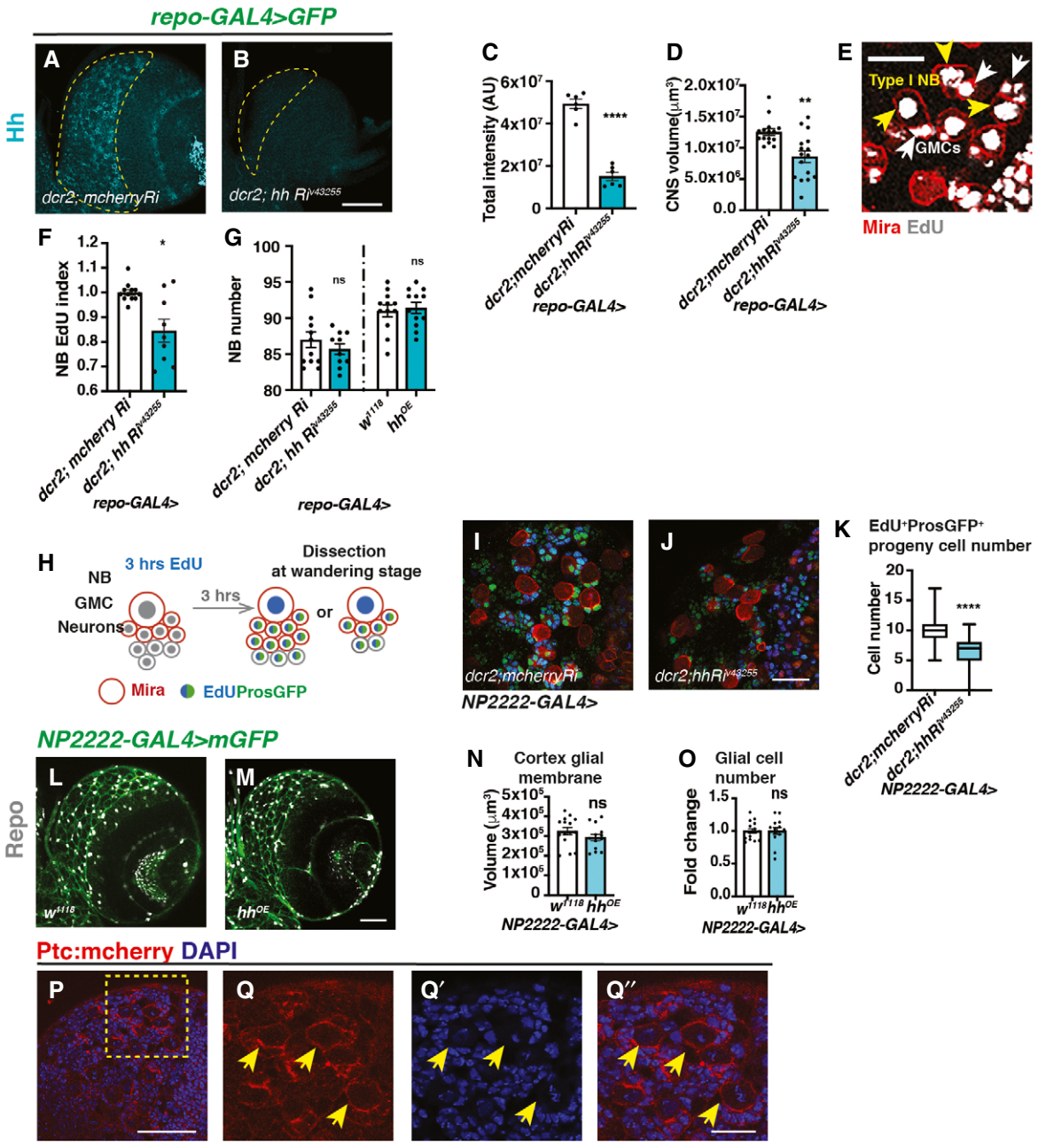

Figure EV3.

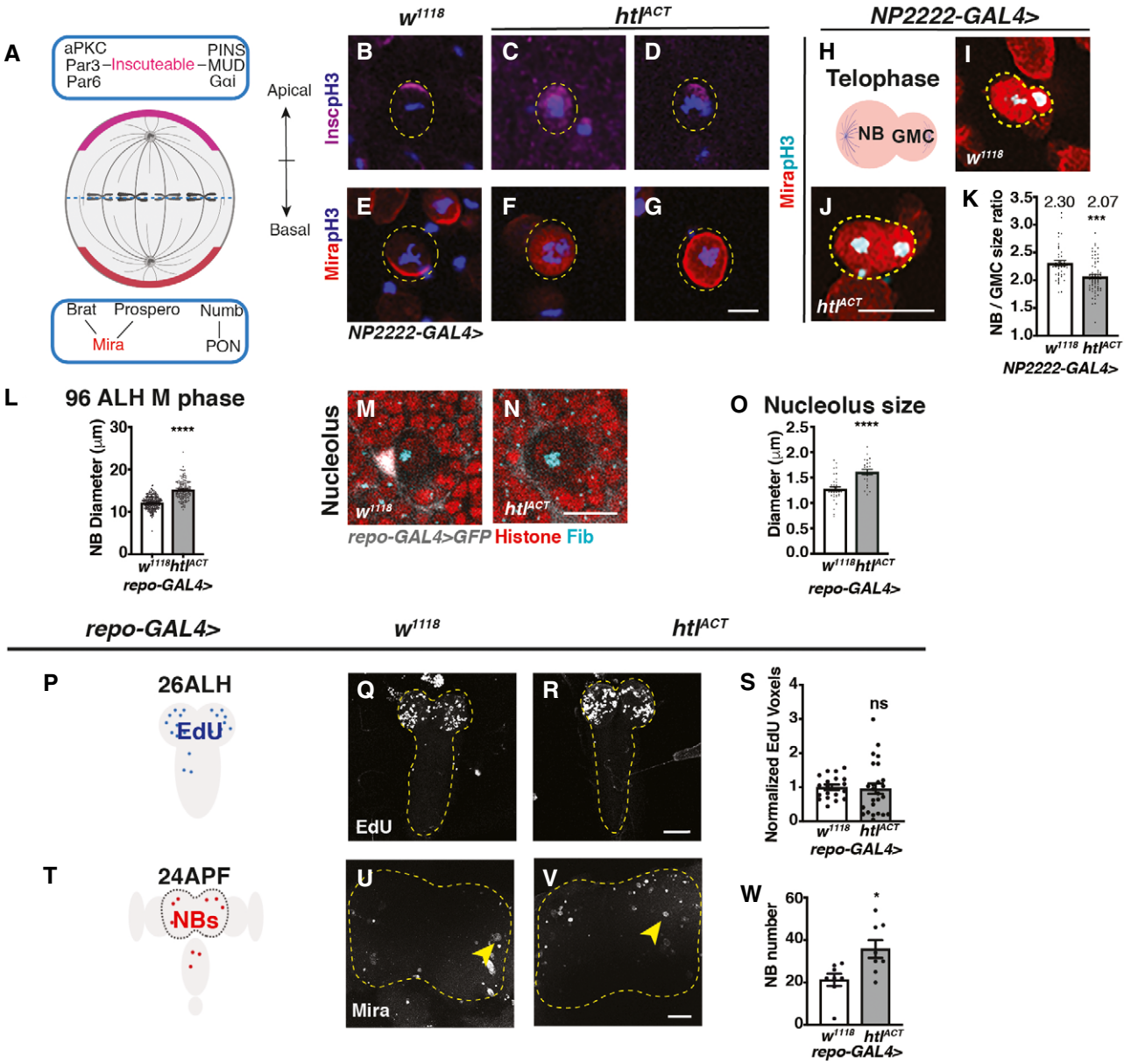

Figure EV4. Glial *htl*^{ACT} overexpression affects NB asymmetric division, size and cell cycle exit (related to Fig 5).

A Schematic depicting the distribution of polarity proteins in M phase NBs. Apical polarity proteins include the Par complex (aPKC/Par3/Par6), the PINS/MUD/Gαi complex and the adaptor protein, inscuteable (magenta); basal polarity complex comprises the cell fate determinants Brat/ Pros/ Numb and their adaptor proteins Mira (red) and PON.

B–G Representative images showing that in pH3^{+} NBs, Insc and Mira mislocalise to the cytoplasm or cortex upon FGF activation in cortex glia (*NP2222-GAL4 > htl*^{ACT}).

H Schematic depicting a NB undergoing telophase.

I–K Representative images showing that NBs in telophase (Mira^{+}; pH3^{+}) give rise to more size-symmetric daughter cells upon cortex glial (*NP2222-GAL4>*) *htl*^{ACT} overexpression, quantified in (K) (n = 43, 63 NBs from 10, 9 brain lobes, respectively).

L Glial (*repo-GAL4>*) *htl*^{ACT} overexpression causes an increase in M phase NB diameter (n = 70, 53 NBs from 12, 7 brain lobes, respectively).

M–O Representative images showing that NB nucleoli are significantly enlarged upon glial (*repo-GAL4>*) *htl*^{ACT} overexpression, quantified in (O) (n = 33, 23 NBs from 9, 7 brain lobes). NBs are marked by Histone (red), surrounded by glial cells (grey, *repo-GAL4 > GFP*), nucleoli are marked by Fib (Cyan).

P–S Representative images showing that the timing of NB cell cycle entry (visualised by EdU incorporation at 26ALH) is not significantly altered by pan-glial (*repo-GAL4>*) *htl*^{ACT} overexpression, quantified in (S), where EdU voxels are normalised to control (n = 19, 25 brains). The region of interest is outlined in yellow.

T–W Representative images showing that the number of CB NBs (Mira^{+}) at 24APF is significantly increased with pan-glial (*repo-GAL4>*) *htl*^{ACT} overexpression, quantified in (W) (n = 8, 8 brains). The region of interest is outlined by yellow dashed lines and NBs are marked with yellow arrows.

Data information: NBs are outlined with yellow dashed lines in (B-G, I and J). Scale bar = 50 μm in (Q, R, U, V). Scale bar = 10 μm in (B-G); Scale bar = 20 μm in (I, J, M and N). Error bar represents SEM. In (K): Mann–Whitney test, (***) P = 0.0002. In (L): Welch's t-test, (****) P < 0.0001. In (O): unpaired t-test, (****) P < 0.0001. In (S): Welch's t-test, (ns) P = 0.8152. In (W): unpaired t-test, (*) P = 0.0134.

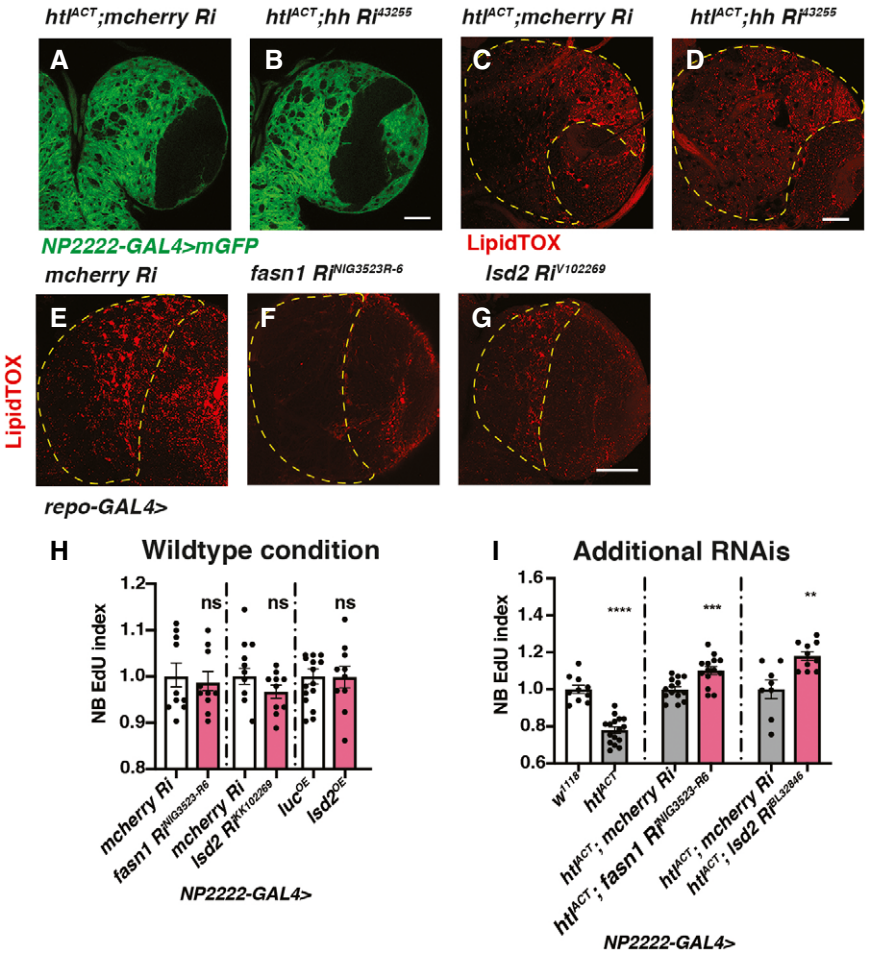

Figure EV5. Characterisation of the effects of glial *hh*, *fasn1* and *lsd2* RNAis on glial size, LDs and NB proliferation (related to Fig 6).

A–D Representative images showing that induction of *hh RNAi* in cortex glial cells with htl^{ACT} overexpression do not alter the size of cortex glial membrane (*NP2222-GAL4 > mGFP*) nor the number of LDs in CB (outlined by yellow dashed lines).

E–G Representative images showing that glial (*repo-GAL4>*) induction of RNAis against *fasn1* and *lsd2* efficiently reduce the number of LDs in CB (outlined by yellow dashed lines).

H Knockdown of lipogenesis genes *fasn1* and *lsd2* or overexpression of *lsd2* using a cortex glial driver (*NP2222-GAL4>*) do not significantly affect NB EdU index (n = 10, 10; 15, 10; 10, 10 brain lobes).

I The NB EdU incorporation defects due to cortex glial (*NP2222-GAL4*) overexpression of htl^{ACT} is rescued by overexpression of additional RNAis lines against *fasn1* and *lsd2* (related to Fig 6J; n = 10, 16; 14, 14; 8, 10 brain lobes). The *NP2222-GAL4 > w^{1118}* versus htl^{ACT} columns depict the same data as those in Fig 5E.

Data information: Scale bar = 50 μm in (A–G). Error bar represents SEM. In (H): Mann–Whitney test, (ns) P = 0.9555; unpaired t-test, (ns) P = 0.1799; unpaired t-test, (ns) P = 0.9574. In (I): unpaired t-test, (****) P < 0.0001; unpaired t-test, (***) P = 0.0008; unpaired t-test, (**) P = 0.0032.