Supplemental Data

Figure S1

Phyl mediates down regulation of Dl in the wing imaginal disc and in the mid pupal eye disc.

(**A**) Wild type third instar wing disc showing the expression of Dl (red). In the wing pouch Dl is expressed in two stripes straddling the D/V boundary (arrow).

(**B-C**) *phyl* mutant clones (non-green in **C**) in the wing imaginal disc were generated using *Ubx-flp.*

(**B**) Dl expression (red) in a third instar wing imaginal disc containing *phyl* mutant clones.

(**C**) Merged panel shows an increase in the levels of Dl in cells at the D/V boundary that are mutant for *phyl* (arrow).

(**D**) Wild type mid pupal eye disc showing weak expression of Dl (red) at the apical tips of the ommatidial clusters.

(**E-F**) *phyl* mutant clones (non-green in **F**) in the mid-pupal eye disc.

(**E**) Dl expression (red) in a mid-pupal eye disc containing *phyl* mutant clones.

(**F**) Merged panel shows an increase in the levels of Dl in cells that are mutant for *phyl*.

Figure S2

(**A-I**) Expression of activated Ci, pMAD and activated MAPK in *phyl* mutant cells in third instar eye disc. GFP marks wild–type cells and non-GFP cells are mutant for *phyl*. Arrows mark the morphogenetic furrow.

Wild-type expression of activated Ci **(A)**, pMAD (**D**) and activated MAPK (**G**) in third instar eye discs are shown as controls.

(B-C) Expression of activated Ci (red, **B**) in *phyl* mutant clones (non-green in **C**). The merged panel (**C**) shows no difference in Ci expression in *phyl* mutant cells compared with adjacent wild type cells.

(E-F) Expression of activated pMAD (red **E**) in *phyl* mutant clones. The merged panel (**F**) shows no increase in the levels of PMAD in *phyl* mutant cells.

(H-I) Expression of activated MAPK (red, **H**) in *phyl* mutant clones. The merged panel (**I**) shows no increase in the levels of activated MAPK in *phyl* mutant cells.

(**J-O**) Localization of EGFR and PVR in *phyl* mutant cells in third instar eye disc. GFP marks wild–type cells and non-GFP cells are mutant for *phyl*.

(**J-L**) In an eye disc containing *phyl* mutant clone (non-green **J, L**). The localization of EGFR (red, **K**) in mutant cells is similar to that seen in the adjacent wild-type cells. The merged panel is shown in (**L**).

(**M-O**) In an eye disc containing *phyl* mutant clone (non-green, **M**). The localization of PVR (red, **N**) in mutant cells is similar to that seen in the adjacent wild-type cells. The merged panel is shown in (**O**).

Figure S3

Phyl mediates down regulation of Wg in the third instar wing and antennal discs.

(**A**) Wild-type expression of Wg in the third instar wing pouch showing expression at the D/V boundary (arrow)

(**B**) Expression of Wg in the third instar wing pouch containing *phyl* mutant clones.

Clones mutant for *phyl* were generated using *Ubx-flp*. GFP (green) marks the wild type cells and non-GFP cells are mutant for *phyl* showing increased levels of Wg in *phyl* mutant tissue.

(**C**) Wild type expression of Wg in the third instar antennal disc.

(**D**) Expression of Wg in the third instar antennal disc containing *phyl* mutant clones.

Clones mutant for *phyl* were generated using *hs-flp.* GFP (green) marks the wild-type cells and non-GFP cells are mutant for *phyl* showing increased levels of Wg in *phyl* mutant tissue.

Figure S4

Expression of different endosomal markers in *phyl* mutant tissue in the third instar eye disc**.**

phyl mutant clones were generated using *ey-flp*. Green marks the wild-type tissue and non green tissue is mutant for *phyl*.

(A-C) In *phyl* mutant cells (non-green in **C**), N^{intra} (red, **A**) co-localizes with Avl (blue, **B**) which marks early endosomes. The merged panel is shown in (**C**).

(**D-F**) In *phyl* mutant cells (non-green in **F**), N^{intra} (red, **D**) co-localizes with Dor (blue, **E**) which marks early endosomes. The merged panel is shown in (**F**).

(**G-I**) In *phyl* mutant cells (non-green in I), N^{intra} (red, **G**) does not co-localize with Hook (blue, **H**) which marks late endosomes. The merged panel is shown in (**I**).

(**J-L**) endocytic N^{intra} in *phyl* mutant tissue is ubiquitinated.

phyl mutant clones in the eye disc (non- green in **L**) were co-stained for N^{intra} (red, **J**) and antibody directed against poly-ubiquitin epitope (blue, **K**). The merged panel (**L**) shows co-localization of N^{intra} and poly-Ubi.

(**M-O**). endocytic vesicles in *phyl* mutant tissue are positive for Lysotracker Red staining. In the third instar eye disc, *phyl* mutant tissue (non-green, **M**) exhibit positive Lysotracker Red staining for endocytic vesicles (red, **N**). The merged panel is shown in (**O**).

(**P-Q**) In *phyl* mutant clones (non-green in **R**), Dl (red, **P**) does not co-localize with Rab11 (blue, **Q**) which marks the recycling endosomes. The merged panel is show non-overlapping vesicles (arrows) (**R**).

Figure S5

Elevated levels of Dl and N^{intra} in *phyl* mutant cells is independent of Tamtrack function in third instar eye discs. Arrows mark the morphogenetic furrow.

(A-C) Expression of Tamtrack in *wild-type* **(A),** *GMR-ttk* **(B)** and *sev-ttk* **(C)**.

(D-F) Expression of Dl in *wild-type* (**D**), *GMR-ttk* (**E**) and *sev-ttk* (**F**) showing that over-expression of TTK in cells behind the furrow does not cause an increase in the levels of Dl or mislocalization of Dl.

(G-I) Expression of N^{intra} in *wild-type* (**G**), *GMR-ttk* (**H**) and *sev-ttk* (**I**) showing over-expression of TTK in cells behind the furrow does not cause an increase in the levels of N^{intra}.

(**J-O**) Expression of Dl (**J-L**) and N^{intra} (**M-O**) in *sina* mutant cells in third instar eye discs. Wild-type cells are marked with GFP (green) and non-GFP cells are mutant for

sina. The merged panels **L** and **O** show normal expression and localization of Dl and N^{intra} in *sina* mutant cells.

(P-S) Localization of N^{intra} (red) in cells doubly mutant for *phyl* and *ttk***.** Cells negative for GFP (green) and β-Gal (blue) are doubly mutant for *phyl* and *ttk* (outlined by white dotted line). The merged panel (**S**) shows vesicular N^{intra} in cells that are mutant for both *phyl* and *ttk* (arrows).

(**T-V**) Expression of Wg in $sina^{A16}$ mutant clones in the third instar eye disc. Wild type cells are marked with GFP (green, **T**) and non-GFP cells are mutant for *sina.* The merged panel (**V**) shows normal expression and levels of Wg in *sina* mutant tissue.

Figure S6

(**A**). Dl expression (green) in wild type third instar eye disc showing down regulation of Dl protein by column 8 behind the furrow. Arrow marks the position of the morphogenetic furrow.

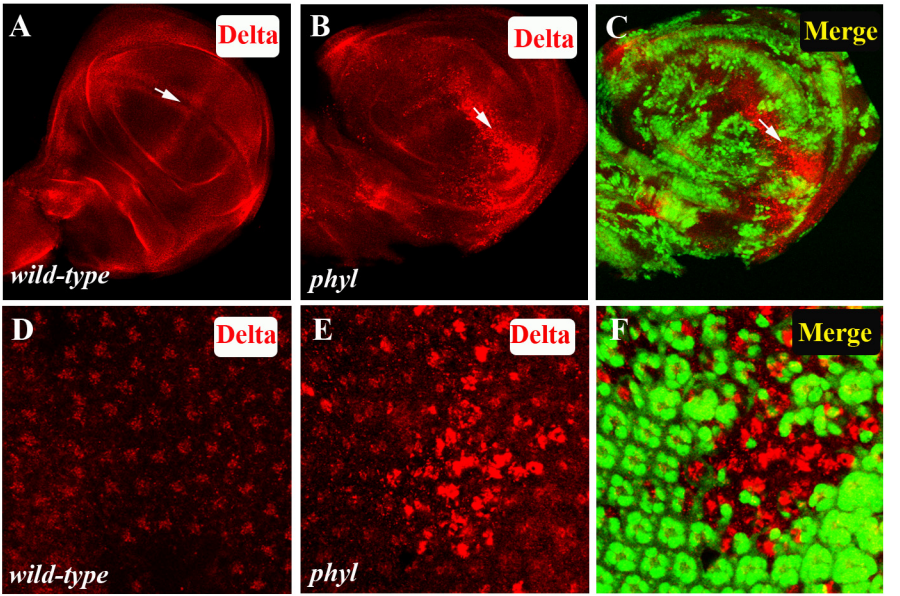
(B) *elav-gal4 UAS-Dl* third instar eye disc stained for Dl. In this background, Dl continues to be expressed beyond column 8 behind the furrow. Arrow marks the position of the morphogenetic furrow.

Figure S7

Expression of Wg in the third instar eye disc containing $EGFR^{ts}$ mutant clones.

$EGFR^{ts}$ clones in the third instar eye disc generated using *eye-flp* (**A**, non-green) was stained for Wg (**B**). The merged panel (**C**) shows the accumulation of Wg in endocytic

vesicles only in the mutant tissue. A high magnification view of the panels is shown in **D**, **E** and **F**.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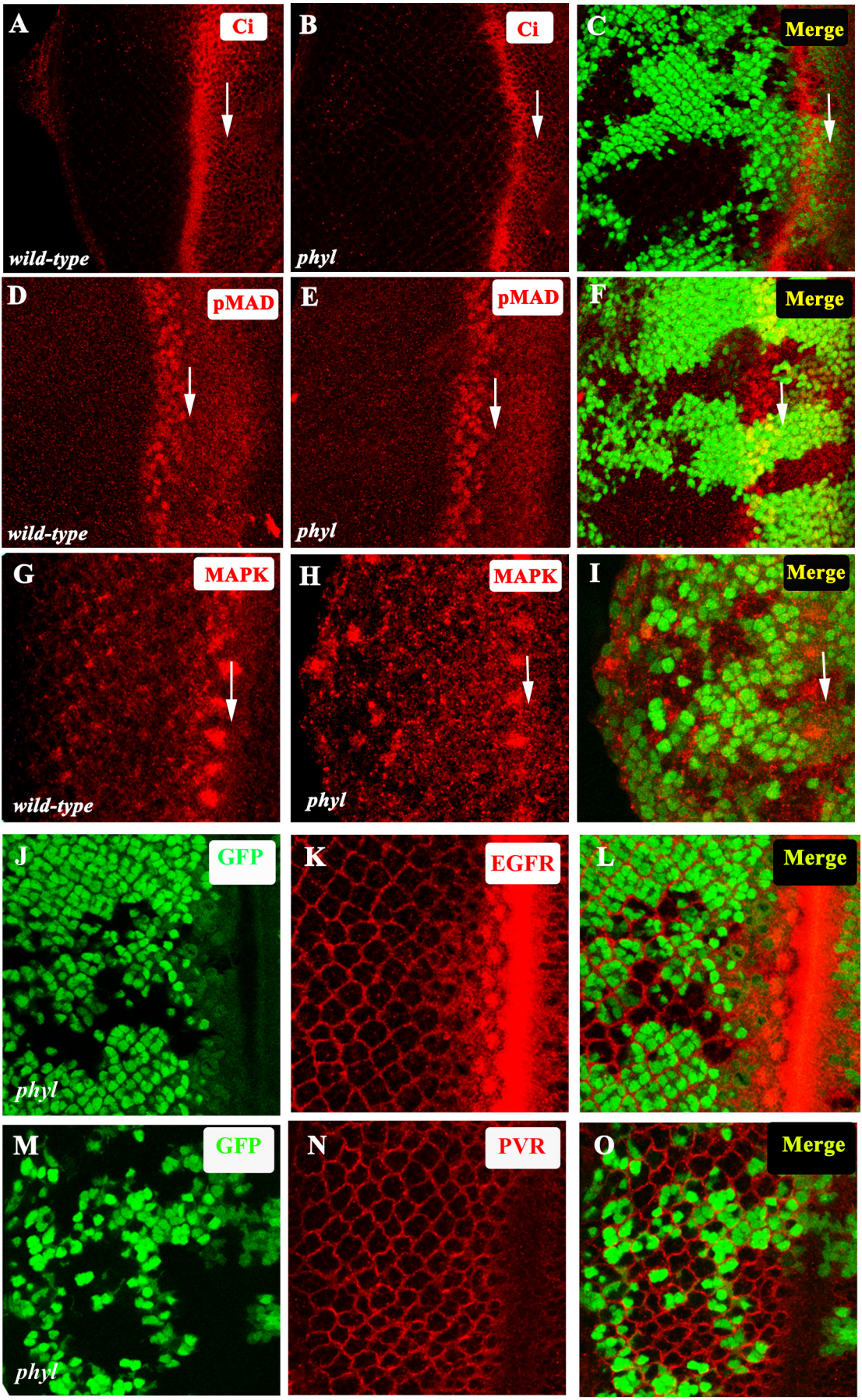
wild-type
B
Delta
phyl
C
Merge
D
Delta
wild-type
E
Delta
phyl
F
Merge

Fig S1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wild-type
B
Ci
phyl
C
Merge
D
pMAD
wild-type
E
pMAD
phyl
F
Merge
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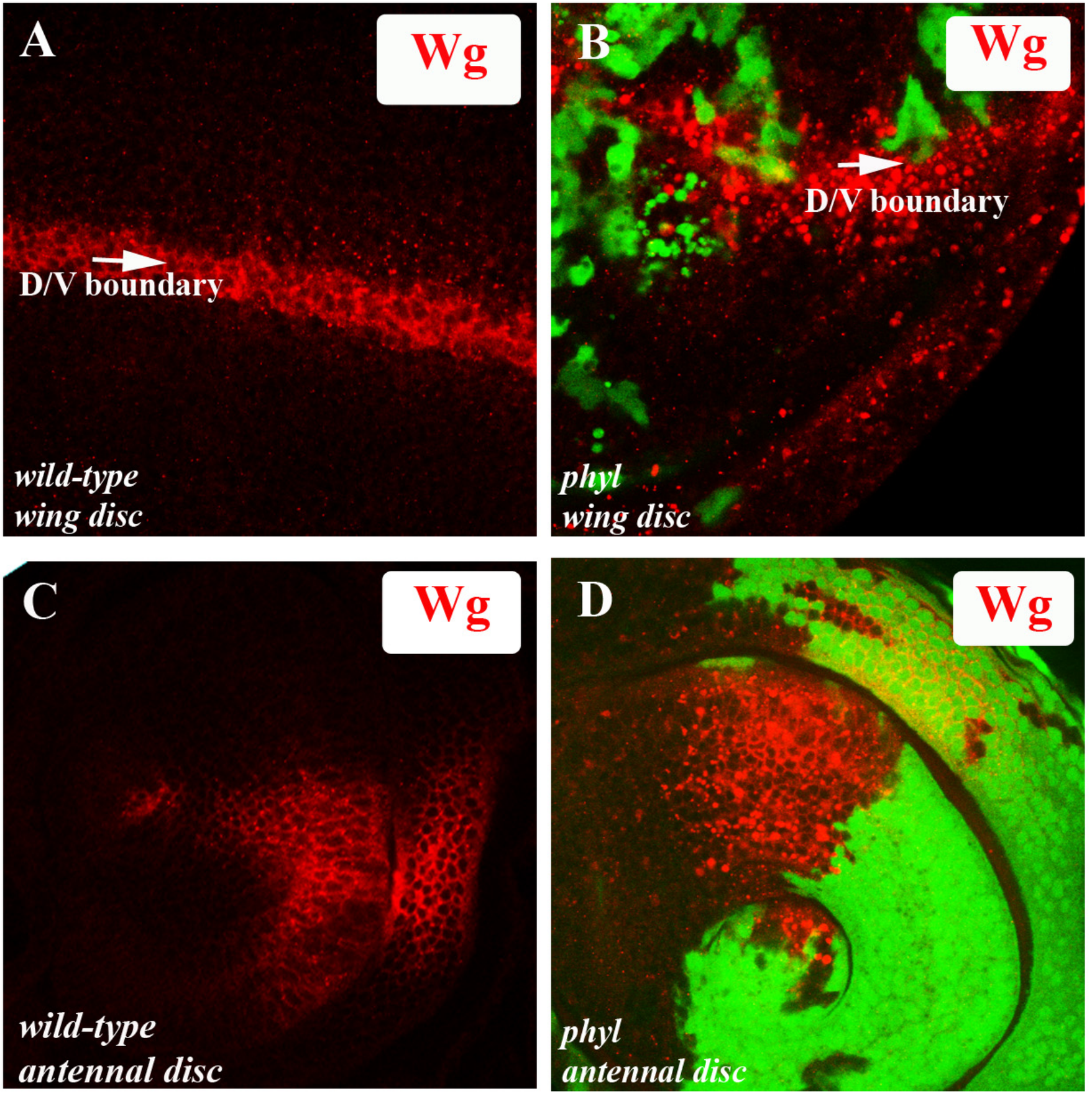
MAPK
wild-type
H
MAPK
phyl
I
Merge
J
GFP
phyl
K
EGFR
L
Merge
M
GFP
phyl
N
PVR
O
Merge

Fig S2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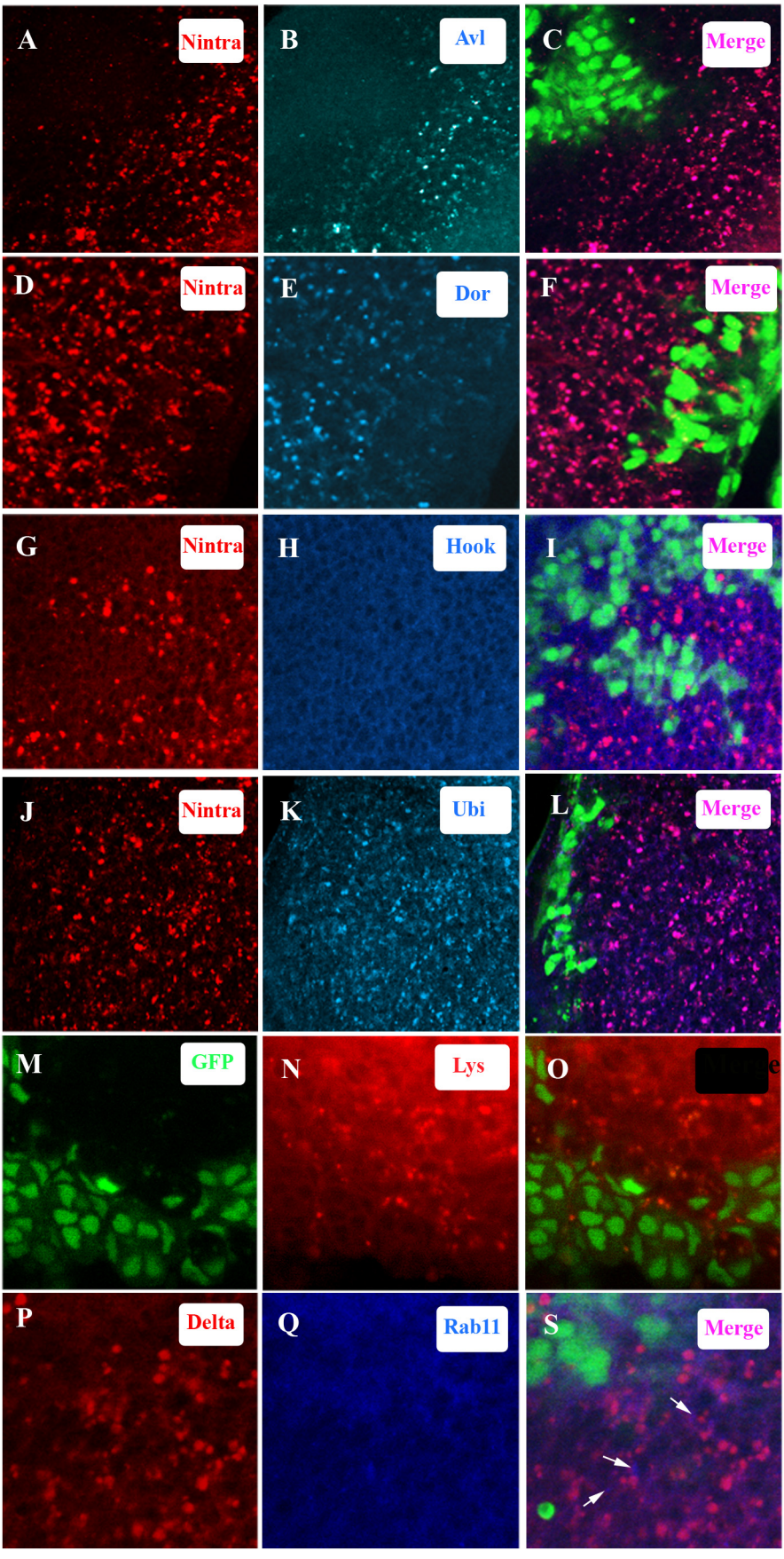
Wg
D/V boundary
wild-type
wing disc
B
Wg
D/V boundary
phyl
wing disc
C
Wg
wild-type
antennal disc
D
Wg
phyl
antennal disc

Fig S3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B
Avl
C
Merge
D
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E
Dor
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Hook
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Merge
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Nintra
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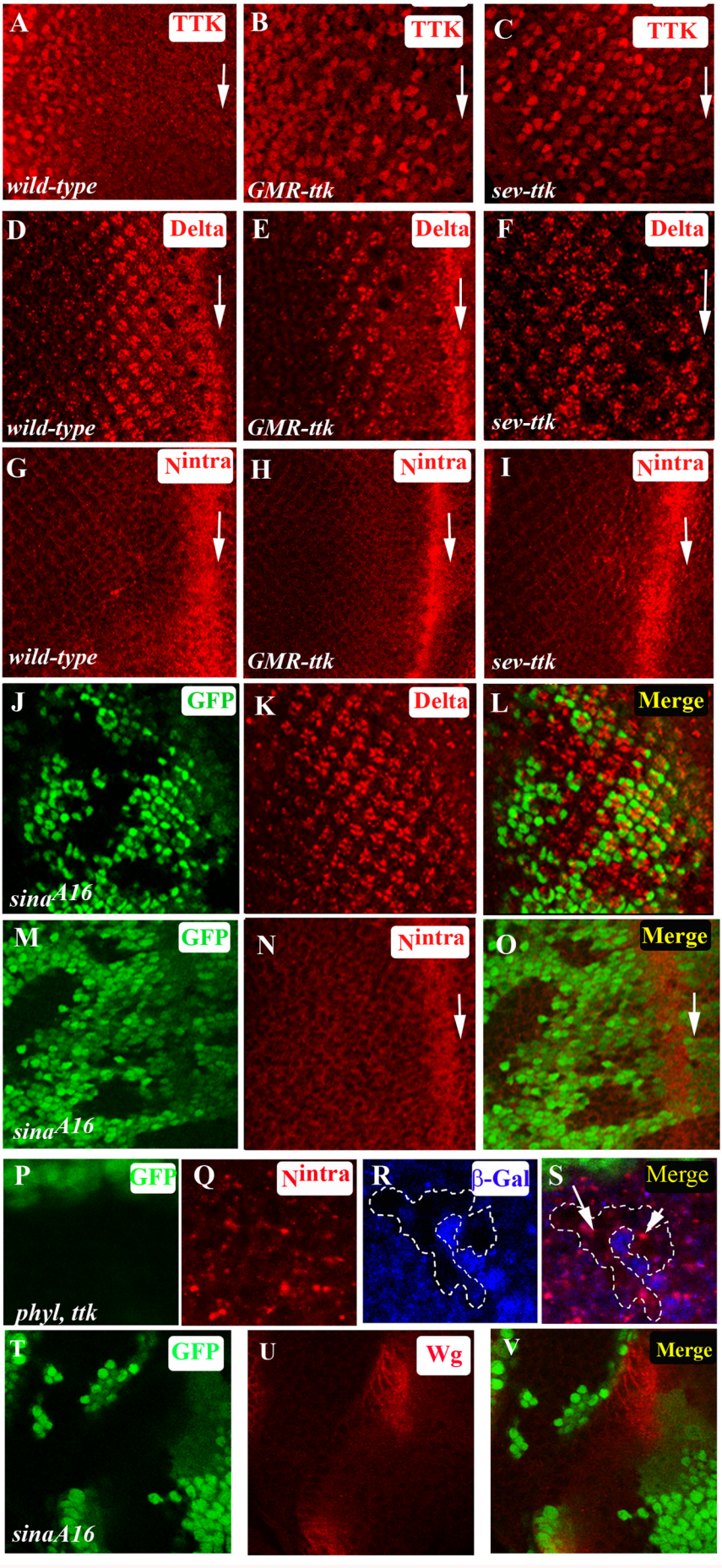
Lys
O
P
Delta
Q
Rab11
S
Merge

SFig 4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wild-type
B
TTK
GMR-ttk
C
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sev-ttk
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F
Delta
sev-ttk
G
Nintra
wild-type
H
Nintra
GMR-ttk
I
Nintra
sev-ttk
J
GFP
sinaA16
K
Delta
L
Merge
M
GFP
sinaA16
N
Nintra
O
Merge
P
GFP
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β-Gal
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Merge
T
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Wg
V
Merge

Fig S5

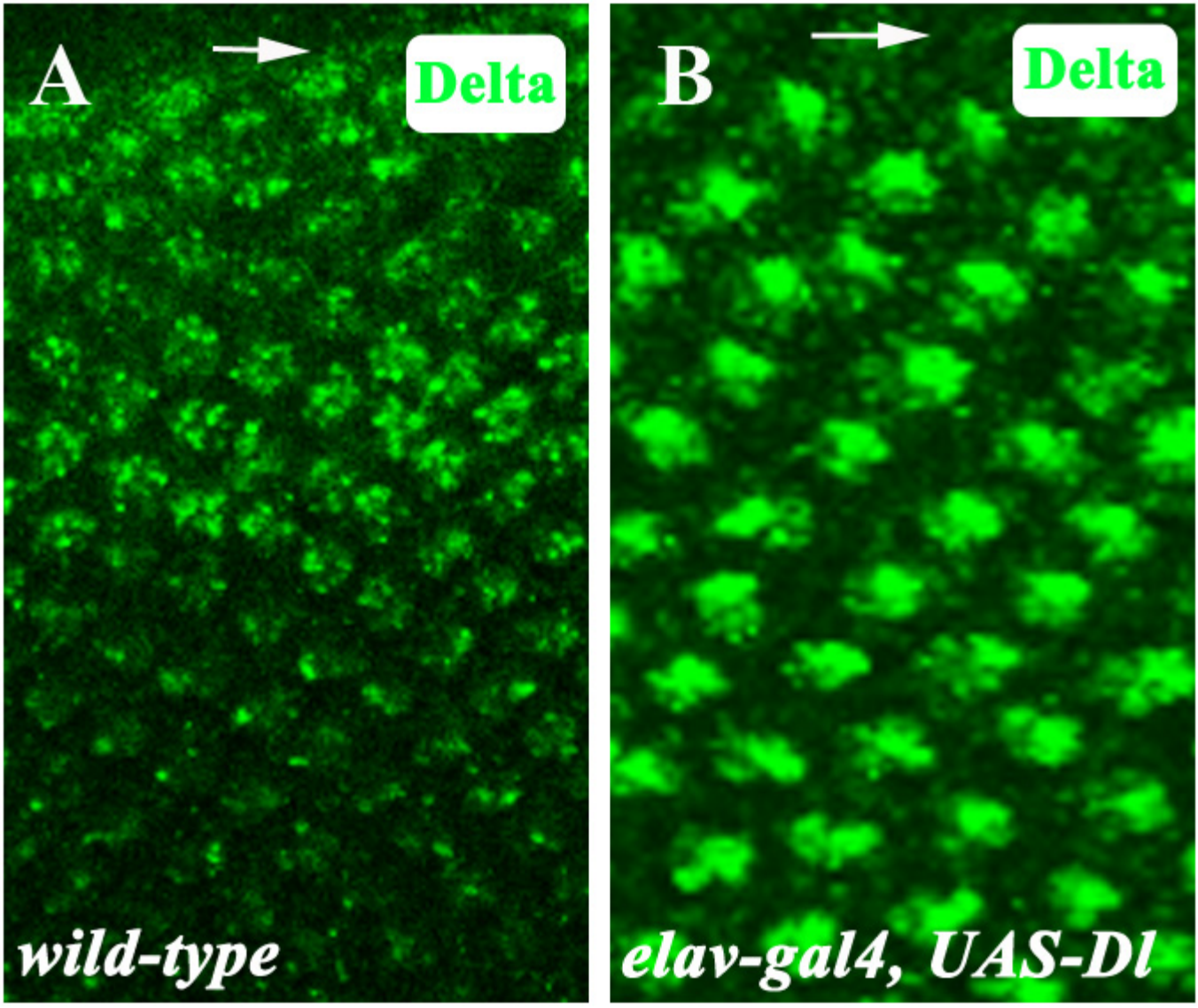

Fig. S6

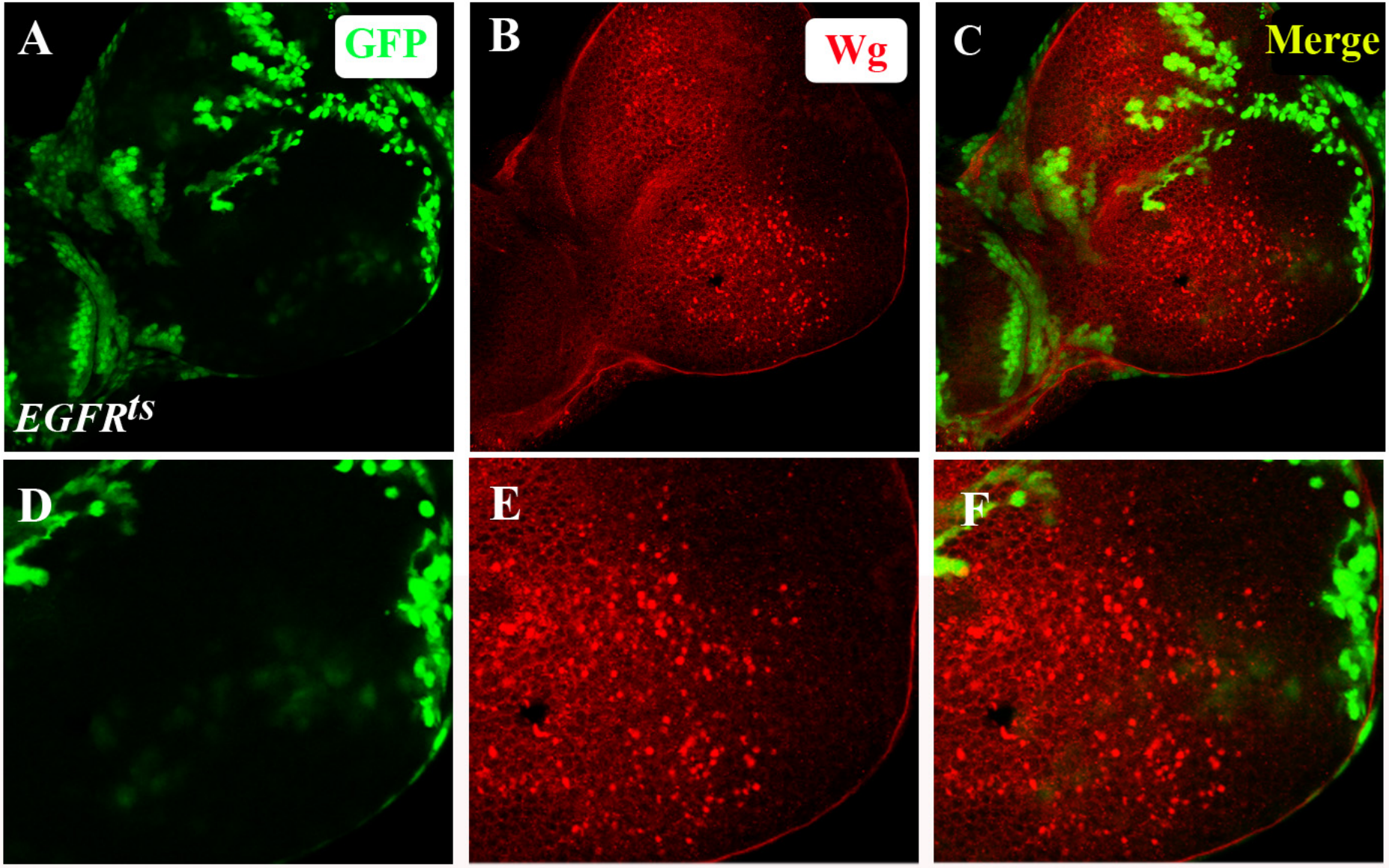

Fig S7