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# Supplemental Information

# *Rich* Regulates Target Specificity of Photoreceptor Cells and N-Cadherin Trafficking in the *Drosophila* Visual System via Rab6

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## Inventory of Supplemental Information

The Supplemental Information includes detailed Experimental Procedures, 8 figures (S1-S8), and Figure legends. Figure S1 is related to Figure1. Figure S2 is related to Figure 2. Figure S3 is related to Figure 4. Figure S4 is related to Figure 5. Figure S5 is related to Figure 7. Figures S6-S8 are related to Figure 8.

**Supplemental Materials**

**Supplemental Experimental Procedures**

**Genetics**

We generated 4 small deletions using FLPase and FRT-bearing *P*-elements or *PiggyBac* insertions as described before (Parks et al., 2004; Thibault et al., 2004). The following insertions were used: *p[XP]d10386* and *PBac[WH]f01638* (to generate a deletion from 79E3 to 79E6); *p[XP]CG11426 [d05846]* and *PBac[RB]e01384* (to generate a deletion from 79E4 to 80A1'); *p[XP]CG11367 [d05379]* and *PBac[RB] e01384* (to generate a deletion from 80A1 to 80A1'); *p[XP]CG11367 [d05379]* and *PBac[RB] e02858* (to generate a deletion from 80A1 to 80B1).

To generated eye clones, we crossed *y w eyFLP GMR-lacZ; FRT80B ubiGFP, cl / TM3* flies to following stocks 1) *y w; FRT80B iso*; 2) *y w; FRT80B rich$^{1}$ / TM3, Kr-GFP;* or 3) *y w; FRT80B rich$^{2}$ / TM3, Kr-GFP.* We also crossed *y w eyFLP GMR-lacZ; FRT40A ubiGFP* flies to *y w; FRT40 CadN$^{\Delta 14}$ / CyO* flies. *y w eyFLP; FRT40A cl w$^{+}$ / CyO, Kr-GFP* flies was also crossed to the following stocks *y w; FRT40A Rab6$^{D23D}$ / CyO* (Coutelis and Ephrussi, 2007); 2) *y w; FRT40A Liprin α$^{e}$ / CyO* (Choe et al., 2006); or 3) *FRT40A iso*.

To label R7 cells in *rich* mutant eyes, *y w eyFLP GMR-lacZ; Pan-R7 Gal4 / CyO; FRT80B cl / TM6B* flies were crossed to the following stocks 1) *y w*; *UAS-SytGFP; FRT80B rich$^{1}$ / TM6B*; 2) *y w*; UAS-*SytGFP; FRT80B rich$^{2}$ / TM6B*; 3*) y w*; *UAS-SytGFP; FRT80B iso* (control); 4) *y w UAS-SytGFP; FRT40A Rab6$^{D23D}$ / CyO; FRT80B rich$^{1}$ / TM6B*; 5) *y w UAS-SytGFP; FRT40A Rab6$^{D23D}$ / CyO; FRT80B rich$^{2}$ / TM6B*; 6) *y w UAS-SytGFP; FRT40A N-Cad$^{405}$ / CyO; FRT80B rich$^{1}$ / TM6B*; or 7) *y w UAS-SytGFP; FRT40A N-Cad$^{405}$ / CyO; FRT80B rich$^{2}$ / TM6B*. To label R7 cells in *Rab6* mutant eyes, *y w eyFLP GMR-lacZ; FRT40A w$^{+}$ cl / CyO; PanR7 Gal4 / TM6B* flies were crossed to 1) *y w*; *Rab6$^{D23D}$ FRT40A / CyO; UAS-SytGFP* or 2) *y w*; *FRT40A iso; UAS-SytGFP* (control). To label R8 cells in *rich* mutant eyes, *y w eyFLP GMR-lacZ; Rh6 GFP / CyO; FRT80B cl / TM6B* flies were crossed to 1) *y w*; *UAS-SytGFP; FRT80B rich$^{1}$ / TM6B;* 2) *y w*; *UAS-SytGFP; FRT80B iso* (control). To label R4 cells at the 3$^{rd}$ instar larvae stage, we crossed *y w eyFLP GMR-lacZ; FRT80B cl / TM6B* flies to 1) *y w*; *mΔGFP / CyO; FRT80B rich$^{1}$ / TM6B* or 2) *y w*; *mΔGFP / CyO; FRT80B iso* (control) flies. To label R7

cells at the 3rd instar larvae stage; we crossed *y w eyFLP GMR-lacZ; 181Gal4 / CyO; FRT80B cl / TM6B* flies to 1) *y w*; *UAS-SytGFP; FRT80B rich$^{1}$ / TM6B or* 2) *y w*; *UAS-SytGFP; FRT80B iso* (control). To label R2-R5 axons, we crossed *y w eyFLP; Ro-τ- lacZ FRT80B cl / TM6B* flies to 1) *y w*; *FRT80B rich$^{1}$ / TM3, Kr-GFP* or 2) *y w*; *FRT80B iso* (control). To label the R1-R6 cells at the adult stage, we crosses *y w eyFLP; Rh1Gal4 / CyO; FRT80B cl / TM6B* flies to 1) *y w*; *UAS-SytGFP*; *FRT80B rich$^{1}$ / TM6B* or *y w*; *UAS-SytGFP; FRT80B iso* (control).

To test whether the genomic fragment containing only *rich* can rescue *rich* mutants, *y w*; *rich-gRE::VK37 / CyO; FRT80B rich$^{1}$ / TM3* flies were crossed to *y w*; *FRT80B rich$^{1}$ / TM3, Kr-GFP* and the flies without TM3 were scored. The HA tagged genomic fragment (HA-Rich-gRE) rescued flies were kept as a stock: *HA-Rich-gRE::VK37; FRT80B rich$^{1}$*.

To test the genetic interaction between *rich*, *DLAR*, *liprin α*, and *jeb*, the flies with following genotypes were generated: 1): *y w eyFLP; FRT80B rich$^{1}$ / FRT80B cl UbiGFP*; 2): *y w eyFLP; FRT80B rich$^{2}$ / FRT80B cl UbiGFP*; 3): *y w eyFLP; FRT40A DLAR / +; FRT80B rich$^{1}$ / FRT80B cl UbiGFP*; 4): *y w eyFLP; FRT40A DLAR / +; FRT80B rich$^{2}$ / FRT80B cl UbiGFP*; 5): *y w eyFLP; FRT40A Liprin α$^{e}$ / +; FRT80B rich$^{1}$ / FRT80B cl UbiGFP*; 6): *y w eyFLP; FRT40A Liprin α$^{e}$ / +; FRT80B rich$^{2}$ / FRT80B cl UbiGFP*; 7): *y w eyFLP; FRT40A Jeb / +; FRT80B rich$^{1}$ / FRT80B cl UbiGFP*; 8): *y w eyFLP; FRT40A Jeb / +; FRT80B rich$^{2}$ / FRT80B cl UbiGFP*.

To generate *CadN, rich* double mutant animals, the genomic rescue allele *rich-gRE* was recombined on the *FRT40A* chromosome. *y w*; *FRT40A rich-gRE::VK37 / CyO; 3L6 deletion / TM3* flies were crossed to *y w eyFLP*; *FRT40A CadN$^{\Delta 14}$ / CyO; FRT80B rich$^{1}$ / TM6B*.

Eye MARCM analysis: We used the MARCM technique (Lee and Luo, 1999) to study *rich*'s role in the R cells. To label mutant clones only in R cells, the flies with the following genotypes were generated: 1) *y w ey3.5FLP; Act>Gal4 UAS-SytGFP / UAS-SytGFP; FRT80B rich$^{1}$ / FRT80B tub-Gal80, M(3)*; 2) *y w ey3.5FLP; Act>Gal4 UAS-SytGFP / UAS-SytGFP; FRT80b iso / FRT80B tub-Gal80, M(3)* (control). To label mutant clones only in R7 cells, we generated the flies with following genotypes: 1) *y w GMRFLP; GMR-Gal4 / UAS-SytGFP; FRT80B rich$^{1}$ / FRT80B tub-Gal80, M(3)*; 2) *y w GMRFLP; GMR-Gal4 / UAS-SytGFP; FRT80B iso / FRT80B tub-Gal80, M(3)*. To reduce

nonspecific labeling of R7 cells, the flies were kept at 18°C before dissection. To express the mutant Rab6 proteins in *rich* mutant cells, we generated the flies with the following genotypes: 1). *y w eyFLP / UAS-YFPRab6 Q71L; Act>Gal4 UAS-SytGFP / CyO; FRT80B rich$^{1}$ / FRT80B tub-Gal80, M(3)*; 2) *y w eyFLP; Act>Gal4 UAS-SytGFP / UAS-YFPRab6 T26N; FRT80B rich$^{1}$ / FRT80B tub-Gal80, M(3)*; 3) *y w eyFLP; Act>Gal4 UAS-SytGFP / UAS-SytGFP; FRT80B rich$^{1}$ / FRT80B tub-Gal80, M(3)*; 4) *y w eyFLP; Act>Gal4 UAS-SytGFP / UAS-SytGFP; FRT80B iso / FRT80B tub-Gal80, M(3)* (control). To overexpress CadN in the *rich* mutant cells, we generated flies with the following genotype : *y w eyFLP; Act>Gal4 UAS-SytGFP / UAS-CadN; FRT80B M (3), tubGal80 / FRT 80B rich$^{1}$*. To generated mutant clones in ORNs, We generated flies with following genotypes: 1) *y w eyFLP; UAS-SytGFP / Or22a Gal4; FRT80B iso / FRT80B tub-Gal80, M(3)* (control); 2) *y w eyFLP; UAS-SytGFP / Or22a Gal4; FRT80B rich$^{1}$ / FRT80b tub-Gal80, M(3)*; 3) *y w eyFLP; FRT40A Rab6$^{D23D}$ / FRT40A tub-Gal80; UAS-SytGFP / Or22a Gal4;* 4) *y w eyFLP; UAS-SytGFP / Or46a Gal4; FRT80B iso / FRT80B tub-Gal80, M(3)* (control); 5) *y w eyFLP; UAS-SytGFP / Or46a Gal4; FRT80B rich$^{1}$ / FRT80B tub-Gal80, M(3)*; 6) *y w eyFLP; FRT40A Rab6$^{D23D}$ / FRT40A tub-Gal80; UAS-SytGFP / Or46a Gal4;* 7) *y w eyFLP; UAS-SytGFP / Or47b Gal4; FRT80B iso / FRT80B tub-Gal80, M(3)* (control); 8) *y w eyFLP; UAS-SytGFP / Or47b Gal4; FRT80B rich$^{1}$ / FRT80B tub-Gal80, M(3)*; 9) *y w eyFLP; FRT40A Rab6$^{D23D}$ / FRT40A tub-Gal80; UAS-SytGFP / Or47b Gal4*; 10) *y w eyFLP; FRT40A CadN $^{M19}$ / FRT40A tub-Gal80; Or22a Gal4, UAS-SytGFP*; 11) *y w eyFLP; FRT40A CadN $^{M19}$ / FRT40A tub-Gal80; Or46a Gal4, UAS-SytGFP*; 12) *y w eyFLP; FRT40A CadN $^{M19}$ / FRT40A tub-Gal80; Or47b Gal4, UAS-SytGFP*

**Immunohistochemistry**

Antibody dilution used: mAb24B10 1:50 (DHSB) (Zipursky et al., 1984); guinea pig anti-Senseless (1:1000) (Nolo et al., 2000); rat anti-Elav (1:25, DHSB)(O'Neill et al., 1994); chicken anti-GFP (Abcam) (1:1000); mouse anti-HA.11 (16B12) (1:500) (Covance); rabbit anti-Hrp (1:1000) (Jackson ImmunoResearch Laboratories); mouse anti-Nc82 (1:10) (DHSB) (Wagh et al., 2006); mouse anti-V5 (1:1000) (Invitrogen); rabbit anti-beta-GAL (1:500) (Cappel); rabbit anti-dGM130 (1:200) (Yano et al., 2005);

mouse anti-120KD protein (1:200) (Calbiochem); rabbit anti-dSYX16 (1:500) (Xu et al., 2002); rat anti-N-Cadherin 1:50 (DHSB) (Iwai et al., 1997); mouse anti-DLAR 1:10 (DHSB) (Sun et al., 2000); mouse anti-PTP69D (1:10) (DHSB) (Desai et al., 1994); rabbit anti-Caps (1:200) (Shinza-Kameda et al., 2006); Guinea pig anti-Jeb (1:5000) (Englund et al., 2003) Pre-absorbed secondary antibodies conjugated to FITC, Cy3, Cy5 (Jackson ImmunoResearch Laboratories) were used at 1:500.

**GEF assay**

GST fusion proteins dRab6 and Rac1 (a positive control) were made and purified from *E coli* as described in the manufacturer's manual. The GST tag was removed by digesting with biotin labeled Thrombin followed by strepavidin beads and Glutathione beads absorption. To load GDP to the small GTPases, 1 uM dRab6 or Rac1 in 110 mM NaCl, 50 mM Tris-HCl pH8.0, 1 mM EDTA, 0.8 mM DTT, 0.005% Triton X-100 was incubated with 50 uM BODIPY-GDP for 60 min at 30°C. The reactions were stopped by adding $MgCl_2$. S2 cells were transfected with pAcRich alone or together with pAc CG1116-PB, pAcyRgp1p. HEK293T cells were transfected with pCMV Tiam1. Cell lysates were made by sonicating transfected cells in 110 mM NaCl, 50 mM Tris-HCl pH8.0, 12 mM $MgCl_2$, 0.8 mM DTT. 0.1 uM BODIPY-GDP loaded dRab6 was incubated with cell lysates with or without Rich, Rich+CG1116-PB, Rich+yRgp1p overexpression in presence of 2 mM GDP. As a positive control, BODIPY-GDP loaded Rac1 was incubated with cell lysates with or without Tiam1 overexpression. The fluorescence intensity was read automatically by plate reader every 2 mins for 50 mins.

**RNA isolation and real-time PCR**

Total RNA was isolated from 40 adult heads with largest mutant clones (Based on white patches) using Absolutely RNA miniprep Kit (Stratagene). Reverse transcription was done using the SuperScript One-Step RT-PCR system with Platinum Taq kit (Invitrogen). The real-time PCR was performed as described before (Fan et al., 2009). Relative levels of mRNAs were calculated using Rotor-Gene 6.0 software and normalized to the levels of endogenous tubulin in the same samples. The relative transcript level of the control was set as 1. The relative transcript levels of other samples were compared to the control,

and the fold-changes shown in the graph. For each experiment, qPCR reactions were done in triplicate. The primers used are: CadN Forward: AGGAGCGACTTTCAATCGTGGG; CadN Reverse: GACGTGGGTCCGATGTTGAAGG; Tubulin Forward: GTGAATTTTCCTTGTCGCGTG; Tubulin Reverse: CTCCAGTCTCGCTGAAGAAG.

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**Figure legends:**

**Figure S1: R1-R6 axons terminate in the lamina at both larval and adult stages (related to Figure 1)**. The photoreceptor terminals in 3rd instar larval brains (A, B) and adult brains (C, D) were labeled with 24B10 (red). The R2-R5 axons at 3rd instar larval optic lobe were labeled with Ro-τ-LacZ (blue). The R1-R6 axons in the adult brains were labeled with Rh1>GFP (green). The animal genotypes for each image are (A) *y w eyFLP; Ro-τ- lacZ FRT80B cl / FRT80B iso*; (B) *y w eyFLP; Ro-τ- lacZ FRT80B cl / FRT80B 3L61*; (C) *y w eyFLP; Rh1Gal4 / UAS-sytGFP; FRT80B cl / FRT80B iso*; (D) *y w eyFLP; Rh1Gal4 / UAS-SytGFP; FRT80B cl / FRT80B 3L61*.

**Figure S2: The early differentiation of the photoreceptor cells in *3L61* mutants is intact (related to Figure 2).** (A, B) 3rd instar larvae eye imaginal discs were stained with anti-Senseless (red), anti-Elav (blue); anti-GFP (green: mutant clones are non-GFP region). The genotypes are (A) *eyFLP; FRT80B ubiGFP cl / FRT80B iso*; (B) *eyFLP; FRT80B ubiGFP cl / FRT80B 3L61*. (C, D) R7 cells in 3rd instar larval eye imaginal discs were labeled with GFP (green). 24B10 (red) labels the R cells. The animal genotypes are (C) *y w eyFLP; 181 Gal4 / UAS-SytGFP; FRT80B cl / FRT80B iso*; (D) *y w eyFLP;181Gal4 / UAS-SytGFP ; FRT80B cl / FRT80B 3L61*. (E, F) R4 cells in 3rd instar larvae eye imaginal discs were labeled with GFP (green). The animal genotypes are (E) *y w eyFLP; m ΔGal4 UASGFP / +; FRT80B cl / FRT80B iso*; (F) *y w eyFLP; m ΔGal4 UASGFP / +; FRT80B cl / FRT80B 3L61*. (G, H) The retina thick sections of (G) control animal (*y w eyFLP; FRT80B cl / FRT80B iso*) and (H) *3L61* mutants (*y w eyFLP; FRT80B cl / FRT80B 3L61*).

**Figure S3: The expression pattern of Rich substantially overlaps with that of CadN in the optic lobe (related to Figure 4).** Optic lobes of a $rich^1$ mutant animal carry a HA-Rich-gRE rescue construct (rescue animal: *y w*; *HA-Rich-gRE::VK37; FRT80B* $rich^1$) at the 3rd instar larval stage (A) or 50h APF (B) were stained with anti-HA (red) and anti-CadN (blue) antibodies. Rich protein is enriched in the lamina (LA) plexus and medulla (ME) where CadN is expressed. (C-E) Rab6 is broadly distributed in the developing eyes.

Eye discs (50hrs APF) (E) or optic lobes of 3rd instars (C) or 50hrs APF (D) animals (*y w;* Act-Gal4 / UAS-Rab6YFP) were stained with anti-GFP (red) and anti-Hrp (green) antibodies. Rab6 YFP is present in both cell body and neuropil.

**Figure S4: The R7 cells target to the correct layer in *rich*$^{1}$ mutant clones generated by *GMRFLP* (related to Figure 5).** The mutant R7 cells were labeled with GFP (green). The PR cell terminals are labeled with 24B10 (red). (A', B') are enlarged image for (A, B). The animal genotypes are (A, A') *y w GMRFLP; GMR-Gal4 / UAS-SytGFP; tub-Gal80 M(3)FRT80B cl / FRT80B iso.* (B, B') *y w GMRFLP; GMR-Gal4 / UAS-SytGFP; tub-Gal80 M(3)FRT80B cl / FRT80B rich*$^{1}$.

**Figure S5: Rich does not exhibit Rab6 GEF activity (related to Figure 7).** (A) *Drosophila* Rab6 (dRab6) was purified from *E.coli* and loaded with BODIPY-GDP followed by incubating with the cell lysates containing overexpressed Rich, Rich+CG1116-PB, or Rich+yRgp1. The bound BODIPY-GDP percentage was determined by the BODIPY fluorescence intensity at 2 mins intervals divided by the BODIPY florescence intensity at 0 min. Rac1 and Tiam were used as a positive control for the GEF assay. The lysates of cells without transfection were used as negative control. (B) Rich does not interact with Rgp1p-like protein CG1116-PB or yeast Rgp1p. V5-tagged Rich or yRic1p was expressed in S2 cells alone or together with either HA-tagged CG1116-PB or HA-tagged Rgp1p. IP yRic1p could pull down yRgp1p. However, IP Rich could not detect any co-precipitation of either CG1116-PB or yRgp1p. The expression of Rgp1p and CG116-PB was detect by blotting the whole cell lysate with anti-HA antibody. (*) is a possible dimer of the yRic1p protein.

**Figure S6: Distribution of the cell surface proteins DLAR, Jeb, PTP69D, and endosomal protein Sec15 in the lamina of *rich*$^{1}$ or *Rab6* mutants at 24APF are not altered (Related to Figure 8).** (A) The animal genotypes are (control) *y w eyFLP; Act>Gal4 UAS-SytGFP / UAS-SytGFP; FRT80B iso / FRT80B tub-Gal80, M (3)* ; (*rich*$^{1}$):. *y w eyFLP; Act>Gal4 UAS-SytGFP / UAS-SytGFP; FRT80B rich*$^{1}$*/ FRT80B tub-Gal80, M (3)*; (*Rab6*): *y w eyFLP; FRT40A tub-Gal80 / FRT40A Rab6*$^{D23D}$*; tubGal4/*

*UAS-SytGFP*. The side view of the lamina was shown. GFP marks the mutant cells. (B) Removal of one copy of *DLAR*, *liprin α*, or *Jeb* does not enhance the R7 targeting phenotype of *rich*$^{2}$. The adult brains of the indicated genotypes were stained with 24B10 antibody (red). The genotypes are (*eyFLP; rich*$^{1}$): *y w eyFLP; FRT80B rich*$^{1}$ */ FRT80B cl UbiGFP*; (*eyFLP; rich*$^{2}$): *y w eyFLP; FRT80B rich*$^{2}$ */ FRT80B cl UbiGFP*; (*eyFLP; Lar / +; rich*$^{1}$): *y w eyFLP; FRT40A LAR +; FRT80B rich*$^{1}$ */ FRT80B cl UbiGFP*; (*eyFLP; Lar / +; rich*$^{2}$): *y w eyFLP; FRT40A LAR / +; FRT80B rich*$^{2}$ */ FRT80B cl UbiGFP*; (*eyFLP; Liprin-α*$^{e}$ */ +; rich*$^{1}$): *y w eyFLP; FRT40A Liprin-α*$^{e}$ */ +; FRT80B rich*$^{1}$ */ FRT80B cl UbiGFP*; (*eyFLP; Liprin-α*$^{e}$ */ +; rich*$^{2}$): *y w eyFLP; FRT40A Liprin-α*$^{e}$ */ +; FRT80B rich*$^{2}$ */ FRT80B cl UbiGFP*; (*eyFLP; Jeb / +; rich*$^{1}$): *y w eyFLP; FRT40A Jeb / +; FRT80B rich*$^{1}$ */ FRT80B cl UbiGFP*; (*eyFLP; Jeb / +; rich*$^{2}$): *y w eyFLP; FRT40A Jeb / +; FRT80B rich*$^{2}$ */ FRT80B cl UbiGFP*. (C) The quantification of mistargeted R7 cells (mean ± SD). *eyFLP; rich*$^{1}$: (18.9 ± 3.4 %, n=450) ; *eyFLP; rich*$^{2}$: (8.15 ± 1.5%, n=517) ; *eyFLP; Lar / +; rich*$^{1}$: (19.7 ± 2.4%, n=656) ; *eyFLP; Lar / +; rich*$^{2}$: (7.3 ± 1.5%, n=438); *eyFLP; Liprin-α*$^{e}$ */ +; rich*$^{1}$: (17.7 ± 2.1%, n=362); *eyFLP; Liprin-α*$^{e}$ */ +; rich*$^{2}$: (8.6 ± 1.9%, n=345); *eyFLP; Jeb / +; rich*$^{1}$: ( 18.5± 2.2%, n=514); *eyFLP; Jeb / +; rich*$^{2}$: (8.9 ± 1.5%, n=363).

**Figure S7: CadN has a normal distribution in medulla of both *rich*$^{1}$ and *Rab6* mutants. (Related to Figure 8).** Adult brains were stained with anti-CadN (red) and 24B10 (green) antibody. The animal genotypes are CTL (*y w eyFLP; FRT80B iso / FRT80B cl UbiGFP*); *rich*$^{1}$ (*y w eyFLP; FRT80B rich*$^{1}$ */ FRT80B cl UbiGFP*); *Rab6* (*y w eyFLP; FRT40A Rab6 / FRT40A cl*); *CadN (PR)* (*y w GMRFLP; FRT40A CadN*$^{\Delta 14}$ */ FRT40A cl*). (B) The CadN RNA level in *rich* mutant does not change when assessed with the real-time PCR assay. Relative levels of CadN mRNAs were calculated using Rotor-Gene 6.0 software and normalized to the levels of endogenous tubulin in the same samples. The relative transcript level of the control was set as 1. The relative transcript levels of other samples were compared to the control, and the fold-changes shown in the graph. The animal genotypes are CTL: (*y w eyFLP; FRT80B iso / FRT80B cl UbiGFP*) ; *CadN*: (*y w eyFLP; FRT40A CadN*$^{M19}$ */ FRT40A cl*); *rich*$^{1}$ (*y w eyFLP; FRT80B rich*$^{1}$ */ FRT80B cl UbiGFP*).

**Figure S8: Rich, Rab6, and CadN function in a common pathway, but overexpression CadN cannot rescue *rich* targeting phenotypes (related to Figure 8).** (A). Axon terminals of Or46a, OR47b ORNs in *rich*, *Rab6*, and *CadN* mutants fail to converge on their appropriate glomeruli and spread out over the surface of the antenna lobe. Or22a ORN axon terminals in CadN mutants spread out over the surface of the lobe, but there is no obvious defect in both *rich* and *Rab6* mutants. Different ORN classes (Or46a, Or47b, Or22a) are shown in wild type (*y w eyFLP; UAS-SytGFP / OrGal4; FRT80B iso / FRT80B tub-Gal80, M(3)*), *rich* mutant (*y w eyFLP; UAS-SytGFP / OrGal4; FRT80B rich$^{1}$ / FRT80B tub-Gal80, M(3)*), *Rab6* mutants (*y w eyFLP; FRT40A Rab6$^{D23D}$ / FRT40A tub-Gal80; UAS-SytGFP / OrGal4*), and CadN mutants (*y w eyFLP; FRT40A CadN $^{M19}$ FRT40A tub-Gal80; OrGal4>UAS-SytGFP*). The glomeruli are labeled with anti-nc82 (red). The ORN axons are labeled with anti-GFP antibody (green). (B) The adult brains of the animals with indicated genotypes were stained with anti-GFP (green), 24B10 (red), and anti-CadN (blue) antibody. The mutant cells were marked with GFP and the targeting patterns of photoreceptor cells were shown by 24B10 staining. (C) Quantification of mistargeted R7 cells in the animals with indicated genotypes. The mistargeted R7 terminals were counted and divided by the total number of R7 cells in the same image. CTL (*y w eyFLP; Act>Gal4 UAS-SytGFP / UAS-SytGFP; FRT80B M (3), tub-Gal80 / FRT80B iso*) *; rich$^{1}$* (*y w eyFLP; Act>Gal4 UAS-SytGFP / UAS-SytGFP; FRT80B M (3), tub-Gal80 / FRT80B rich$^{1}$*) (20.24±2% (n=252)); rich$^{1}$, Act CadN (*y w eyFLP; Act>Gal4 UAS-SytGFP / UAS-CadN; FRT80B M (3), tubGal80 / FRT 80B rich$^{1}$*)( 20.12 ±2% (n= 348)).

**Figure S1**

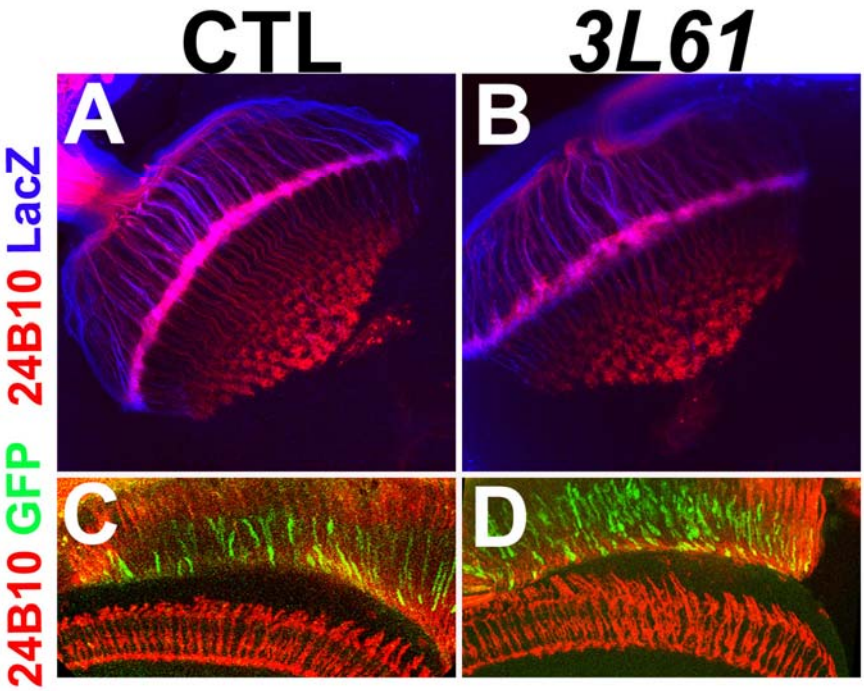
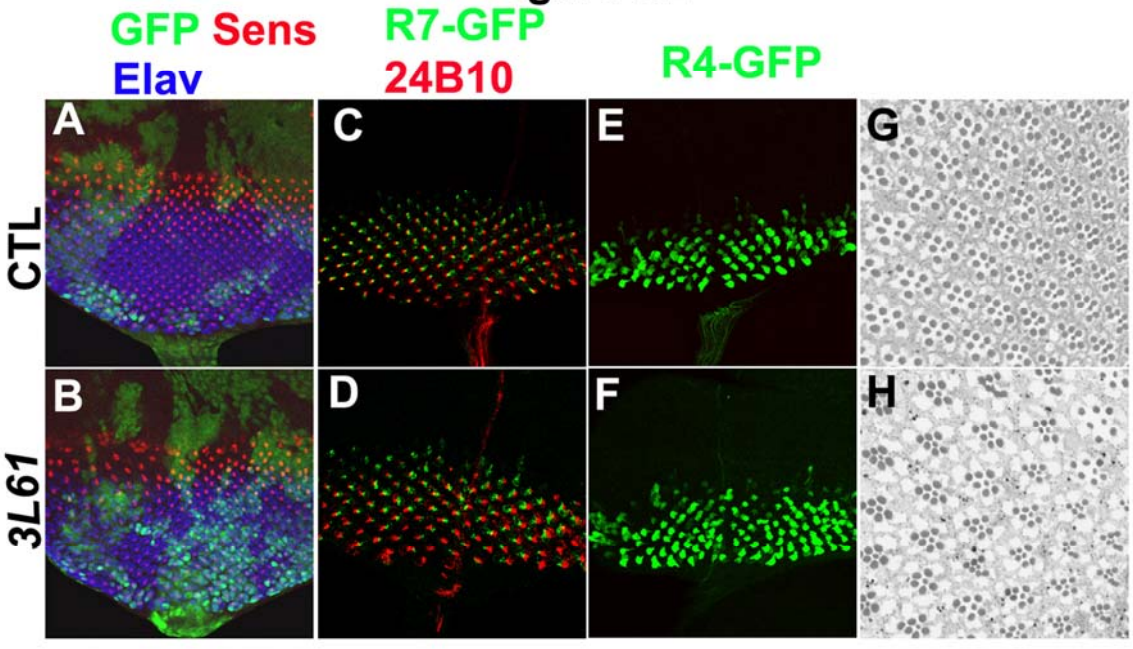
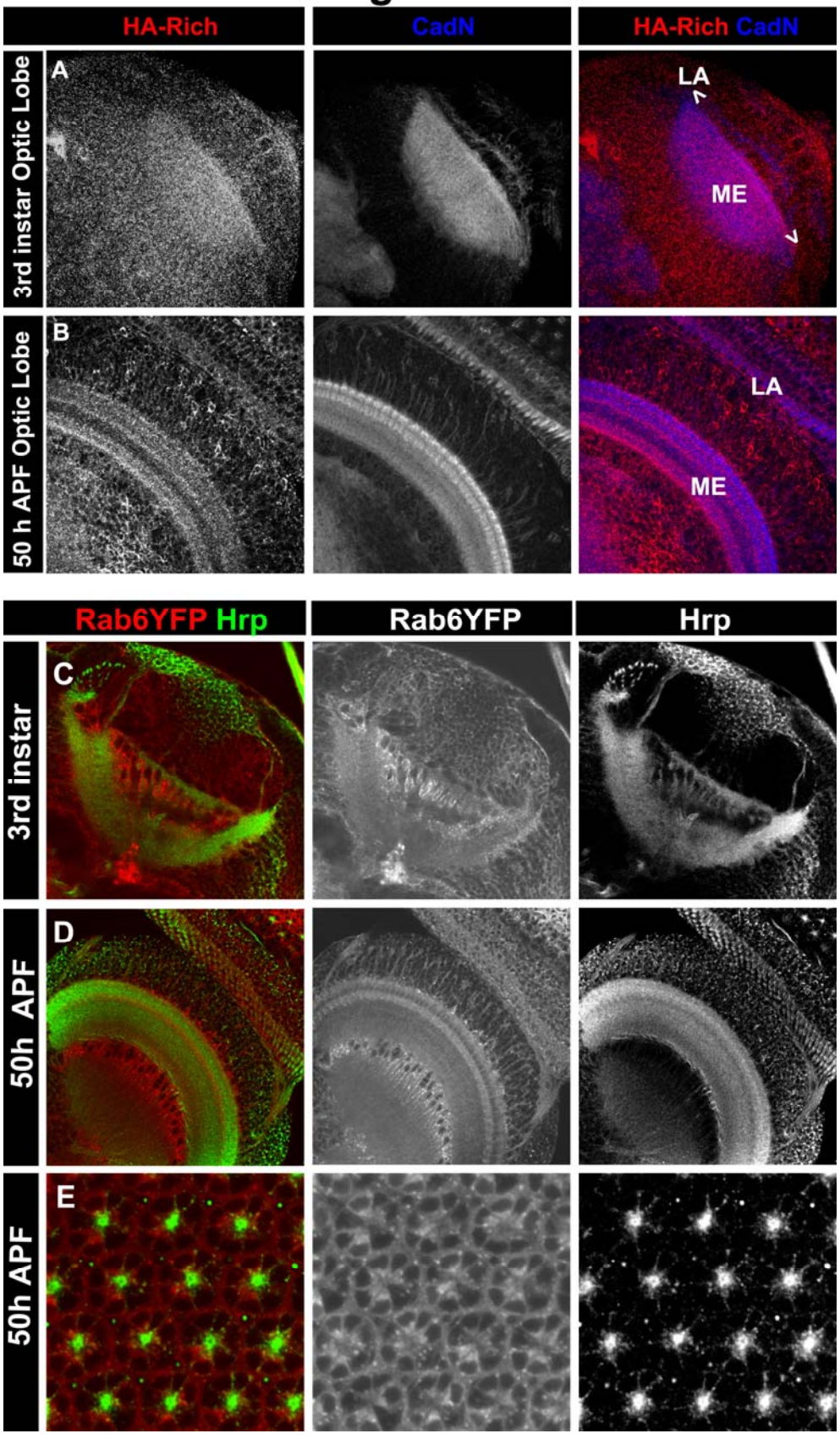


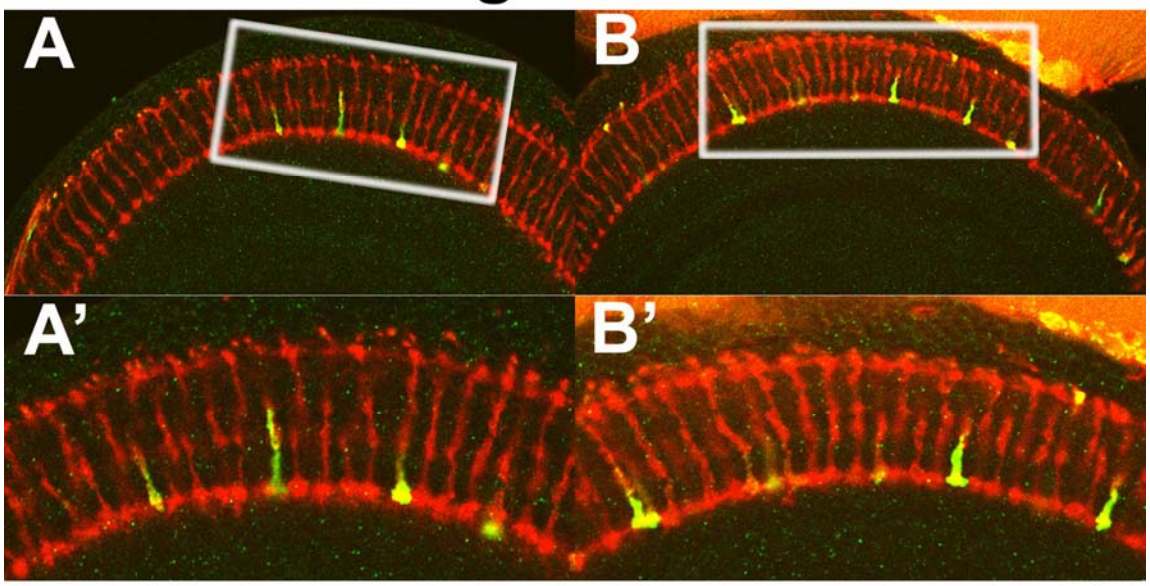

**Figure S2**



# Figure S3


HA-Rich
CadN
HA-Rich CadN
3rd instar Optic Lobe
A
LA
ME
50 h APF Optic Lobe
B
LA
ME
Rab6YFP Hrp
Rab6YFP
Hrp
3rd instar
C
50h APF
D
50h APF
E

# Figure S4




*GMRFLP; GMRGal4; 3LMARCM CTL* *GMRFLP; GMRGal4; 3LMARCM rich$^{1}$*

# Figure S5

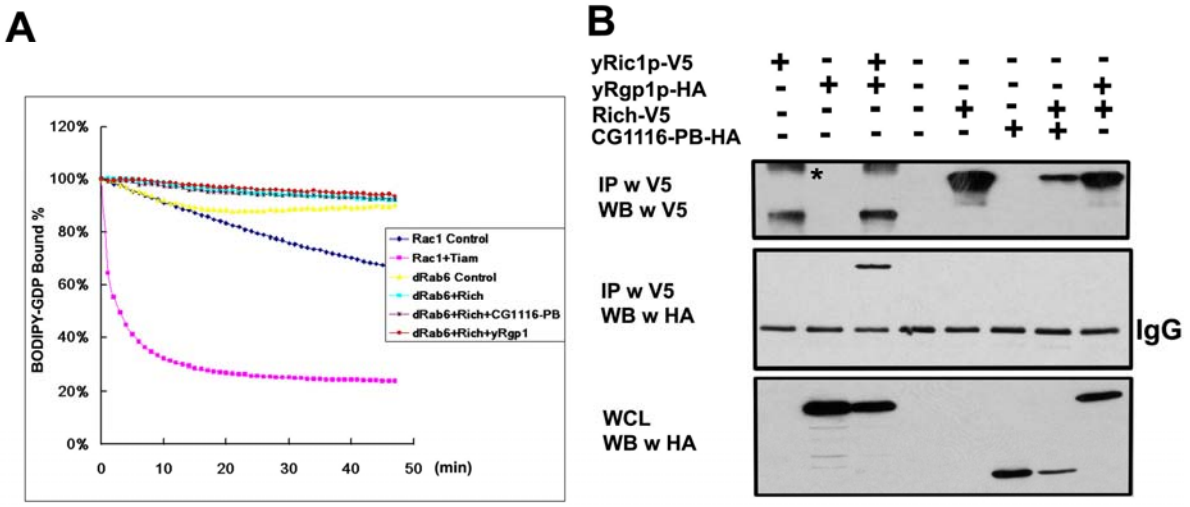
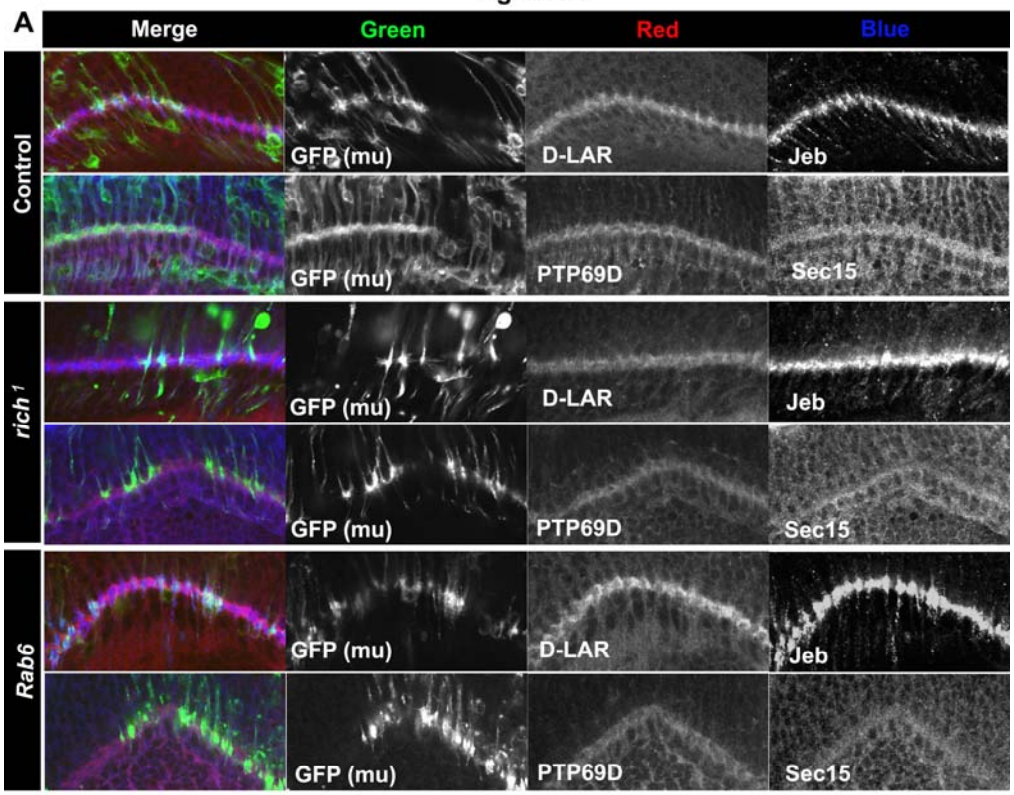
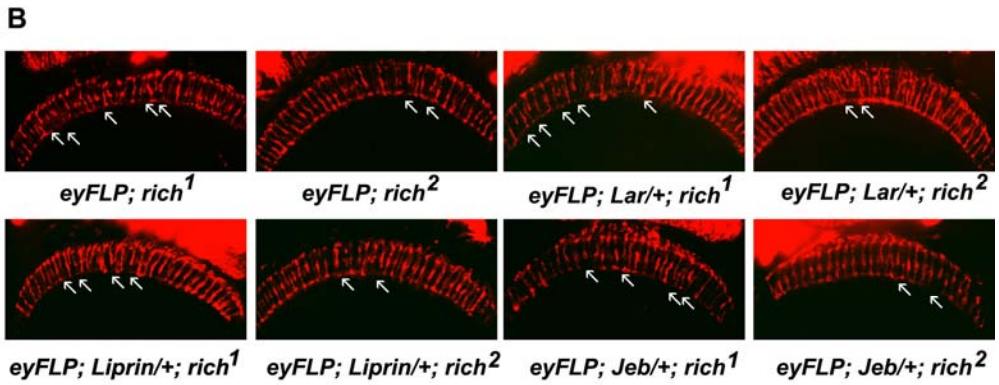


Figure S6


A
Merge
Green
Red
Blue
Control
GFP (mu)
D-LAR
Jeb
GFP (mu)
PTP69D
Sec15
rich 1
GFP (mu)
D-LAR
Jeb
GFP (mu)
PTP69D
Sec15
Rab6
GFP (mu)
D-LAR
Jeb
GFP (mu)
PTP69D
Sec15


B
eyFLP; rich1
eyFLP; rich2
eyFLP; Lar/+; rich1
eyFLP; Lar/+; rich2
eyFLP; Liprin/+; rich1
eyFLP; Liprin/+; rich2
eyFLP; Jeb/+; rich1
eyFLP; Jeb/+; rich2

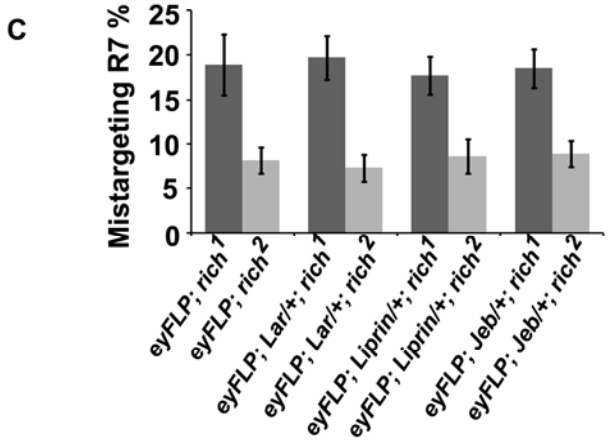

C
Mistargeting R7 %
25
20
15
10
5
0
eyFLP; rich 1
eyFLP; rich2
eyFLP; Lar/+; rich 1
eyFLP; Lar/+; rich2
eyFLP; Liprin/+; rich1
eyFLP; Liprin/+; rich2
eyFLP; Jeb/+; rich1
eyFLP; Jeb/+; rich2

Figure S7

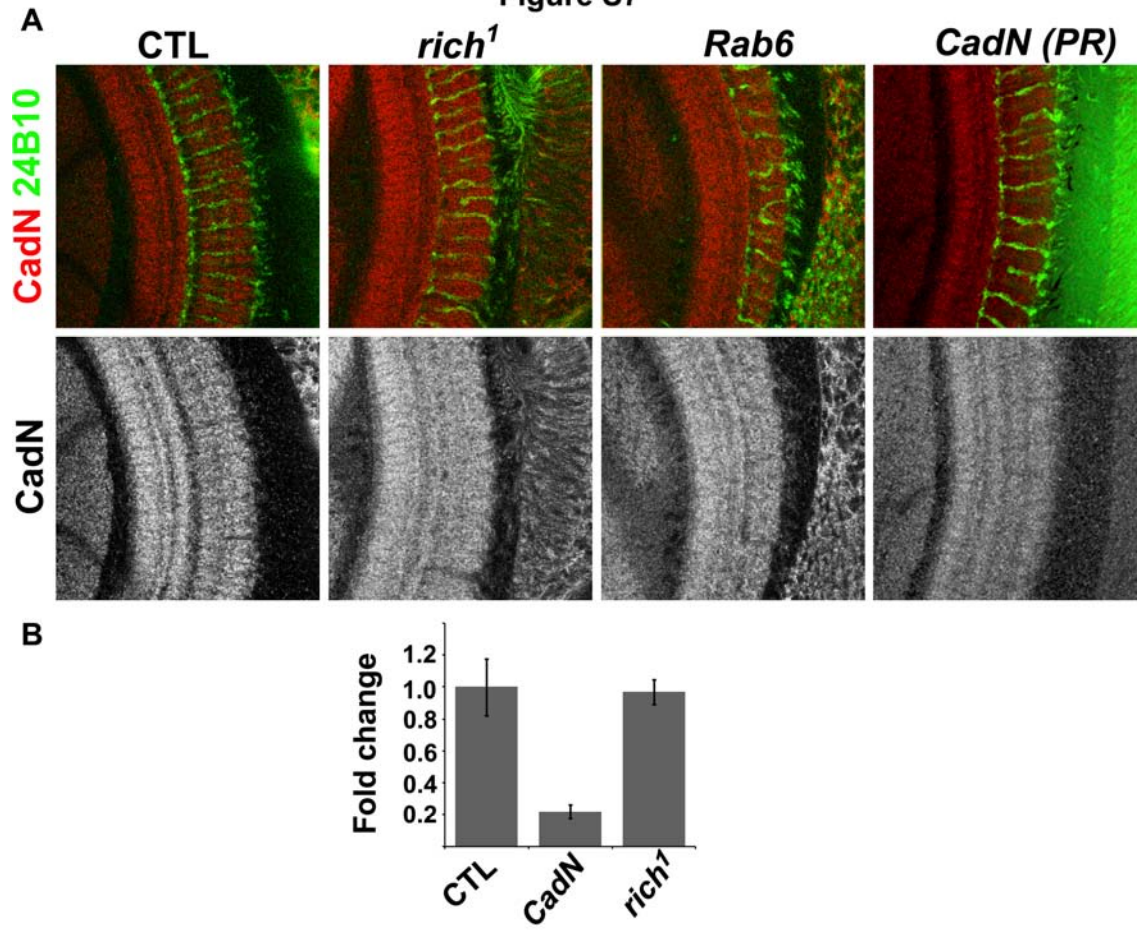
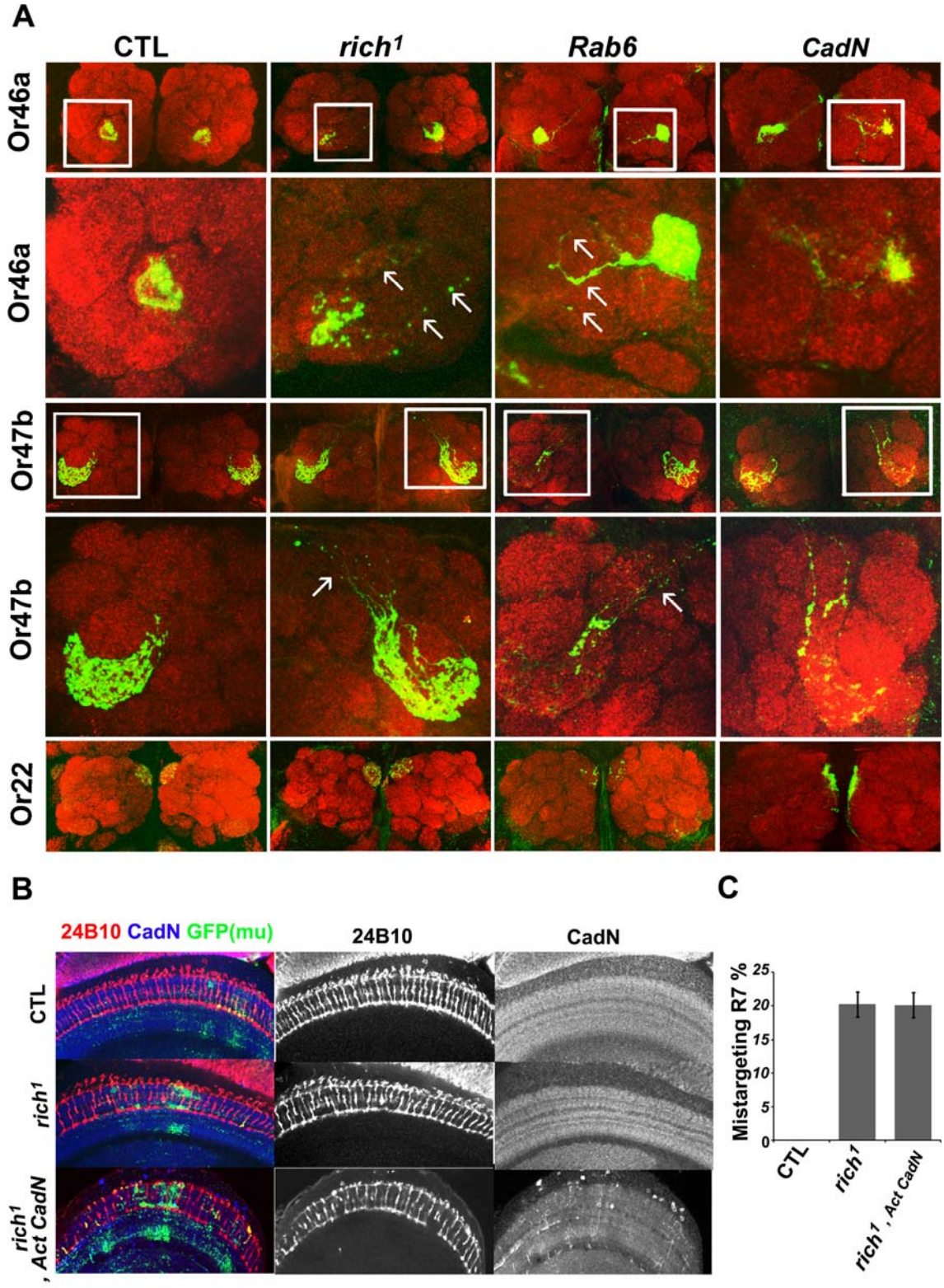

A
CTL
rich1
Rab6
CadN (PR)
CadN 24B10
CadN
B
Fold change
1.2
1.0
0.8
0.6
0.4
0.2
CTL
CadN
rich1

Figure S8


A
CTL
rich1
Rab6
CadN
Or46a
Or46a
Or47b
Or47b
Or22
B
24B10 CadN GFP(mu)
24B10
CadN
CTL
rich1
rich1 , Act CadN
C
Mistargeting R7 %
25
20
15
10
5
0
CTL
rich1
rich1 , Act CadN