Figures and figure supplements

R7 photoreceptor axon targeting depends on the relative levels of *lost and found* expression in R7 and its synaptic partners

Jessica Douthit *et al*

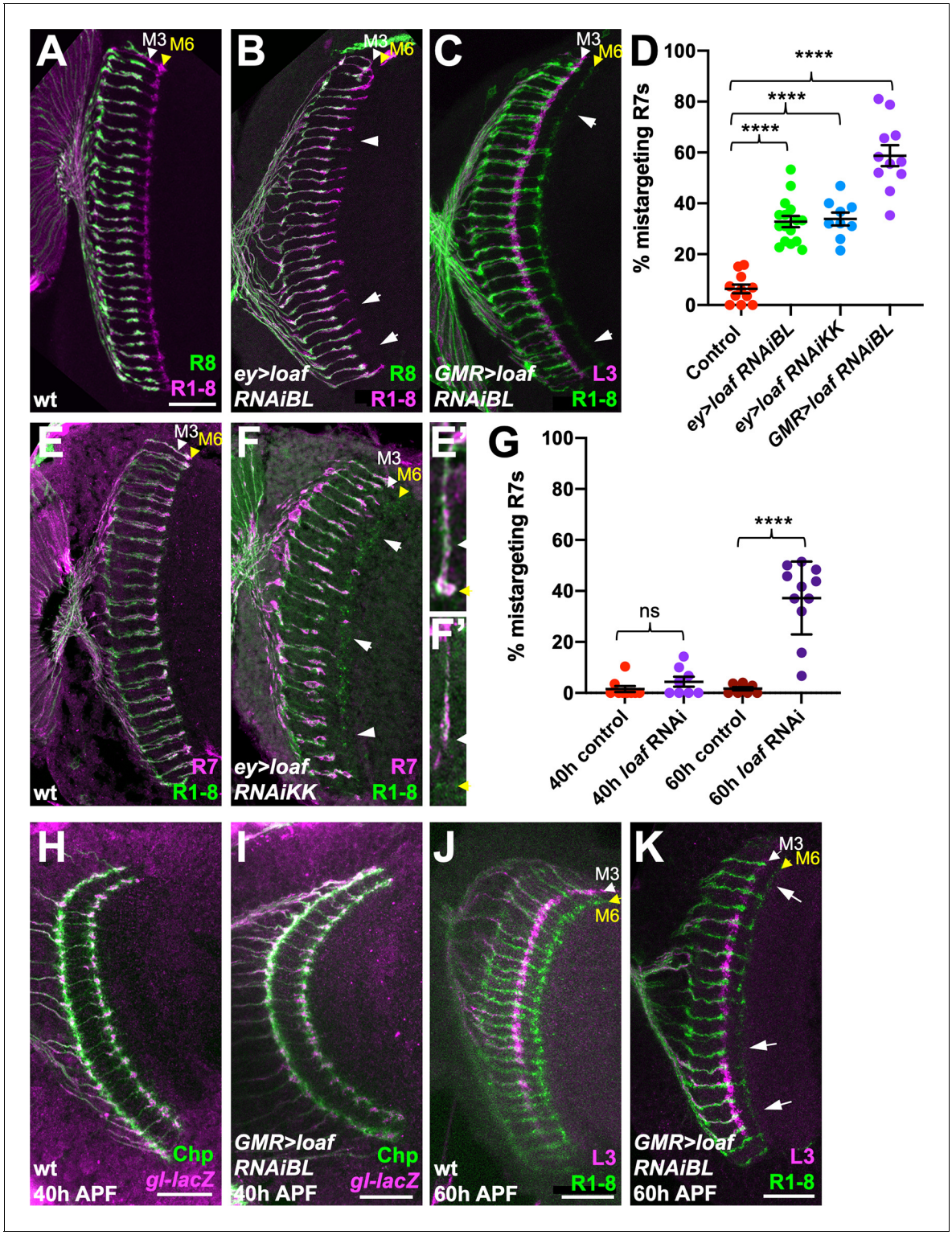

Figure 1. *loaf* RNAi in photoreceptors causes R7 mistargeting. (**A–C, E, F**) cryostat sections of adult heads stained for Chaoptin (Chp) to label all photoreceptor axons (magenta in A, B, green in C, E, F), *Rh5-GFP* and *Rh6-GFP* to label R8 (green in A, B), *22E09-LexA* driving *LexAop-myr-tdTomato* to label lamina neuron L3, which projects to the M3 layer (magenta in C) or *panR7-lacZ* to label R7 (magenta in E, F). (**A, E**) wild type; (**B**) *ey3.5-FLP, Act>CD2>GAL4; UAS-dcr2; UAS-loaf RNAiBL* (*P{TRiP.JF03040}attP2*); (**C**) *GMR-GAL4, UAS-dcr2; UAS-loaf RNAiBL*; (**F**) *ey3.5-FLP, Act>CD2>GAL4; UAS-dcr2; UAS-loaf RNAiKK* (*P{KK112220}VIE-260B*). Arrows show examples of R7 mistargeting. White arrowheads indicate the M3 layer and yellow arrowheads the M6 layer. (**E', F'**) show enlargements of single R7 axons from (**E, F**). (**D**) Quantification of the percentage of R7 axons that failed to reach

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the M6 layer in the same genotypes. n = 11 (control, *GMR > RNAiBL*), 16 (*ey>RNAiBL*), or 9 (*ey>RNAiKK*). ****p<0.0001 by unpaired t-test. Error bars show mean ± standard error of the mean (SEM) in this and all other graphs. (**H–K**) Pupal brains stained for Chp (green) and *glass* (*gl*)-*lacZ*, which labels all photoreceptor axons (magenta in H, **I**) or *22E09-LexA* driving *LexAop-myr-tdTomato* to label L3 neuronal processes in the M3 layer (magenta in J, K). (**H, I**) Forty hr after puparium formation (APF); (**J, K**) 60 hr APF. (**H, J**) wild type (*GMR-GAL4, UAS-dcr2/+*); (**I, K**) *GMR-GAL4, UAS-dcr2; UAS-loaf RNAiBL*. Loss of *loaf* does not prevent the initial targeting of R7 axons to their temporary layer at 40 hr APF, but many axons fail to project beyond that layer at 60 hr APF. (**G**) Quantification of the percentage of R7 axons that did not reach the appropriate layer for these genotypes and stages. n = 9 (40 h control), 8 (40 h RNAi, 60 hr control), or 11 (60 h RNAi). ****, p<0.0001 by unpaired t-test with Welch's correction; ns, not significant. Scale bars, 20 μm.

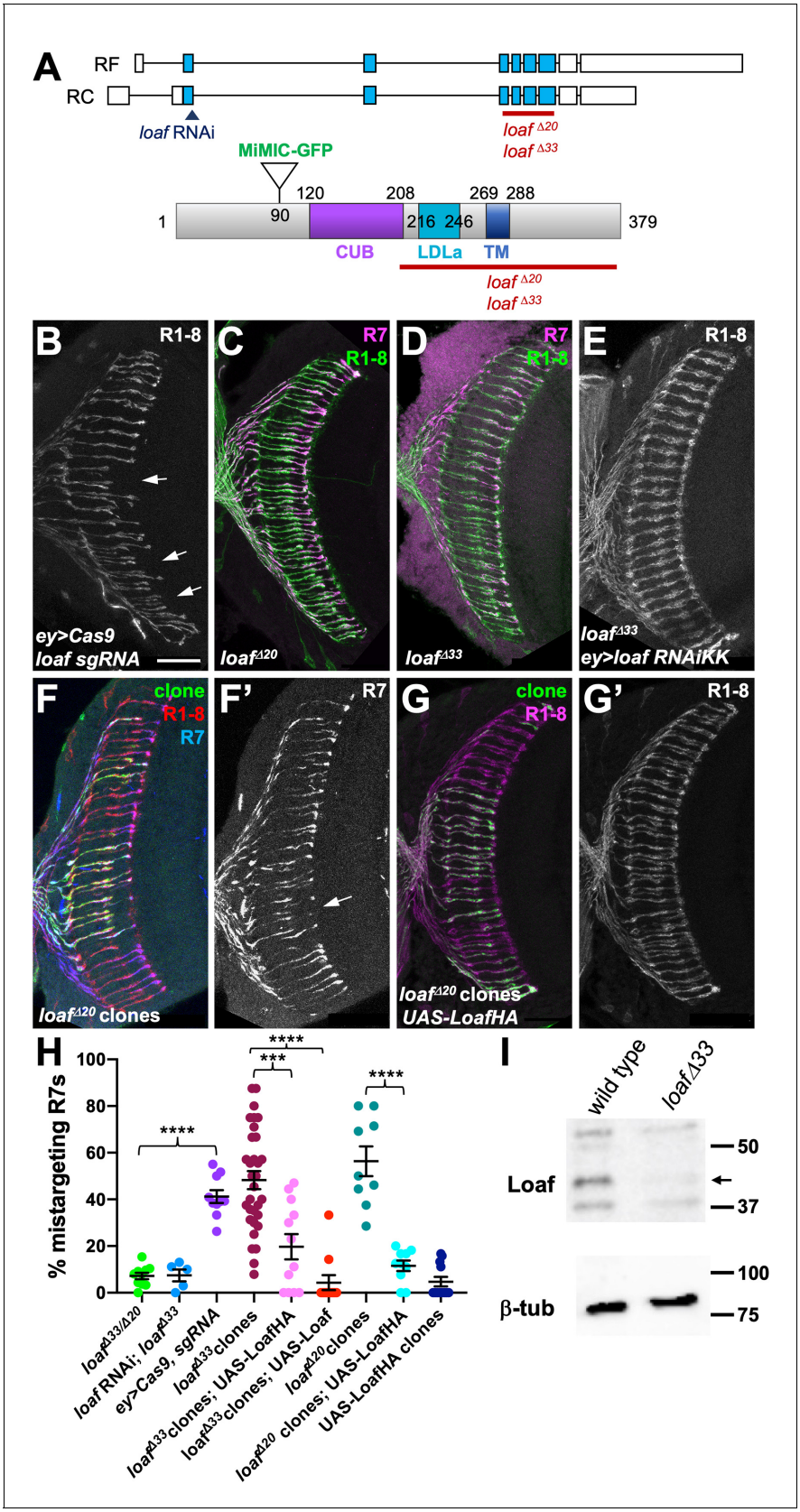

Figure 2. R7 is only affected by eye-specific loss of *loaf*. (**A**) Diagrams of the *loaf* gene and protein. Coding exons, which are identical for the two isoforms, are shown as blue boxes and non-coding exons as white boxes. The region targeted by both RNAi lines, the MiMIC GFP insertion and the

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extent of the *loaf*$^{\Delta 20}$ and *loaf*$^{\Delta 33}$ deletions are indicated. These two deletions were independently generated and have minor sequence differences around the cut site. TM, transmembrane domain. (**B–G**) cryostat sections of adult heads stained for Chp (B, E, G', green in C, D, red in F, magenta in G), *panR7-lacZ* (F', magenta in C, D, blue in F), and GFP (green in F, G). (**B**) *ey3.5-FLP, Act>CD2>GAL4; loaf sgRNAs; UAS-Cas9P2;* (**C**) *loaf*$^{\Delta 20}$ homozygote; (**D**) *loaf*$^{\Delta 33}$ homozygote; (**E**) *ey3.5-FLP, Act>CD2>GAL4; UAS-dcr2/UAS-loaf RNAiKK; loaf*$^{\Delta 33}$*;* (**F**) *loaf*$^{\Delta 20}$ clones positively labeled with *lGMR-GAL4, UAS-GFP*; (**G**) *loaf*$^{\Delta 20}$ clones expressing *UAS-LoafHA* with *lGMR-GAL4*, positively labeled with GFP. Scale bar, 20 μm. (**H**) quantification of the percentage of R7 axons that failed to reach the M6 layer in the indicated genotypes. n = 10 (*loaf*$^{\Delta 33}$*/loaf*$^{\Delta 20}$*; ey>Cas9, sgRNA; loaf*$^{\Delta 20}$ *clones, UAS-LoafHA*), 5 (*loafRNAi; loaf*$^{\Delta 33}$), 32 (*loaf*$^{\Delta 33}$ clones), 12 (*loaf*$^{\Delta 33}$ clones, *UAS-LoafHA*; wild type clones, *UAS-LoafHA*), 11 (*loaf*$^{\Delta 33}$ clones, *UAS-Loaf*), or 9 (*loaf*$^{\Delta 20}$ clones). Error bars show mean ± SEM. ***, p<0.0005; ****, p<0.0001 by unpaired t-test, with Welch's correction when variances are significantly different. *loaf* homozygotes show little R7 mistargeting, but are resistant to the effect of *loaf* RNAi. R7 mistargeting is observed when *loaf* sgRNAs and Cas9 are expressed in the eye, and in clones homozygous for *loaf* alleles. This clonal phenotype is rescued by expressing *UAS-LoafHA* or *UAS-Loaf* in the mutant cells. (**I**) Western blot of extracts from wild type and *loaf*$^{\Delta 33}$ larval brains using an antibody to the cytoplasmic domain of Loaf and β-tubulin antibody as a loading control. Loaf protein (arrow) is absent in *loaf*$^{\Delta 33}$ mutants.

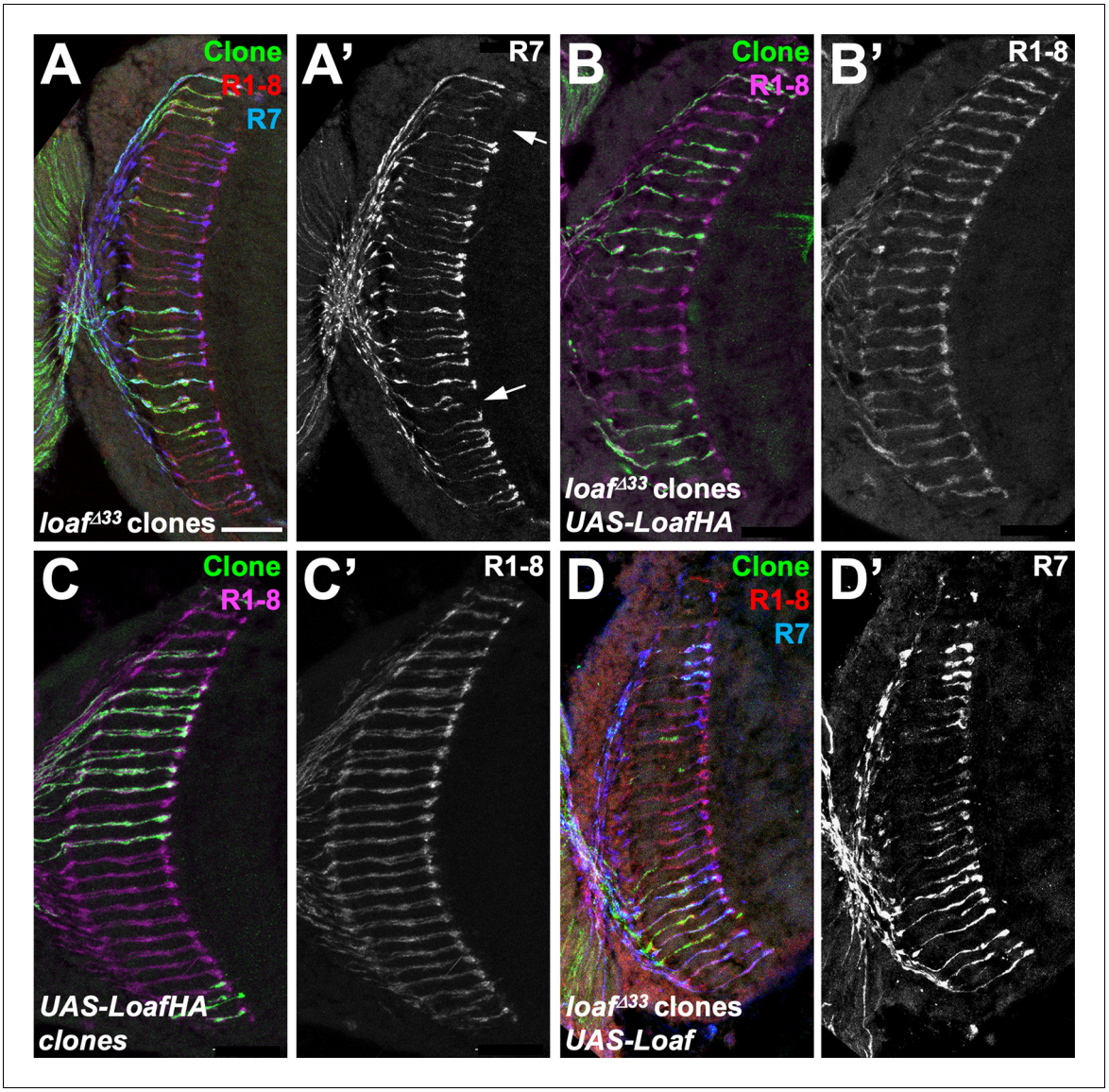

Figure 2—figure supplement 1. R7 mistargeting in *loaf* mutant clones is rescued by tagged or untagged Loaf. Cryostat sections of adult heads with clones positively labeled with GFP, stained for Chp (B', C', red in A, D, magenta in B, C), GFP (green), and *panR7-lacZ* (A', D', blue in A, D). (**A**) $loaf^{\Delta 33}$ clones; (**B**) $loaf^{\Delta 33}$ clones expressing *UAS-LoafHA* with *lGMR-GAL4*; (**C**) clones expressing *UAS-LoafHA* with *lGMR-GAL4*; (**D**) $loaf^{\Delta 33}$ clones expressing *UAS-Loaf* with *lGMR-GAL4*. Scale bar, 20 μm.

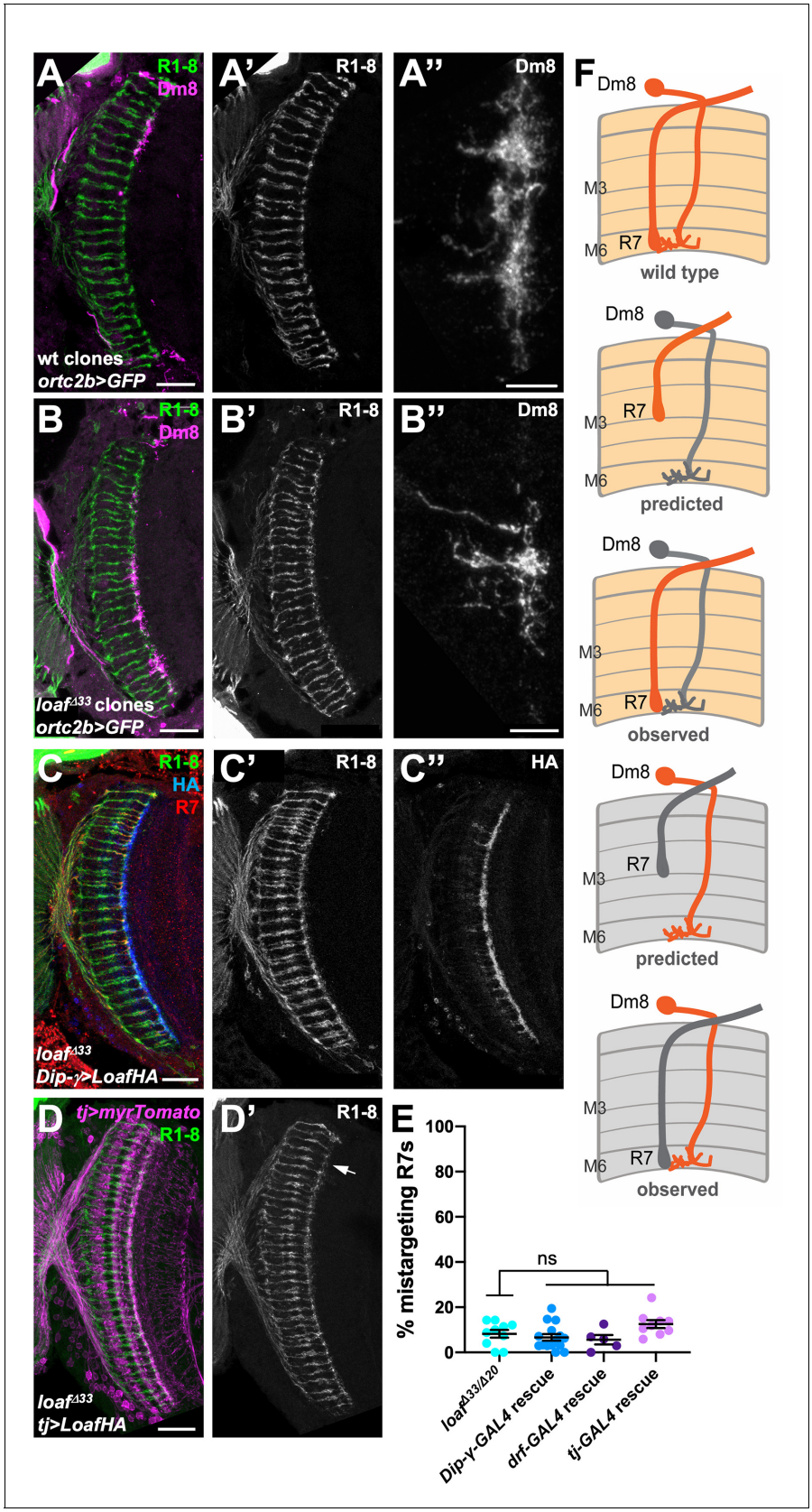

Figure 3. Changing the level of Loaf in Dm8 does not affect R7 targeting. (**A–D**) cryostat sections of adult heads stained for Chp (A'-D', green in A-D), GFP (A'', B'', magenta in A, B), *panR7-lacZ* (red in C), HA (C'', blue in C), or myrTomato (magenta in D). (**A**) wild type clones in which Dm8 is labeled

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with *ortc2b-GAL4, UAS-CD8GFP*; (**B**) $loaf^{\Delta 33}$ clones in which Dm8 is labeled with *ortc2b-GAL4, UAS-CD8GFP*. A'' and B'' show enlargements of labeled Dm8 dendrites. *loaf* mutant Dm8 dendrites and the R7 axons that target them have the normal position and morphology. (**C**) *UAS-LoafHA; DIP-γ-GAL4,* $loaf^{\Delta 33}/loaf^{\Delta 33}$; (**D**) *tj-GAL4/UAS-LoafHA;* $loaf^{\Delta 33}/loaf^{\Delta 33}$. The arrow in (D') indicates minor R7 mistargeting that was not statistically significant. Scale bars, 20 μm (A–D), 5 μm (A'', B''). (**E**) quantification of the percentage of R7 axons that failed to reach the M6 layer in the indicated genotypes. n = 10 ($loaf^{\Delta 33}/loaf^{\Delta 20}$; *DIP-γ -GAL4* rescue; *tj-GAL4* rescue), or 5 (*drf-GAL4* rescue). Error bars show mean ± SEM. ns, not significant by unpaired t-test. Expressing Loaf in Dm8 neurons in a *loaf* mutant does not cause R7 mistargeting. (**F**) diagrams explaining the predicted results if Loaf expression in R7 has to match its expression in Dm8. R7 and Dm8 both express Loaf (orange), which is also present in other cells in the brain. Removing *loaf* from Dm8 (gray) or expressing Loaf in Dm8 in a *loaf* mutant (gray in R7 and brain) would cause a mismatch and is predicted to result in R7 mistargeting. However, (A–E) show that there is no mistargeting in these situations (observed), indicating that Loaf does not act in Dm8 to regulate R7 targeting.

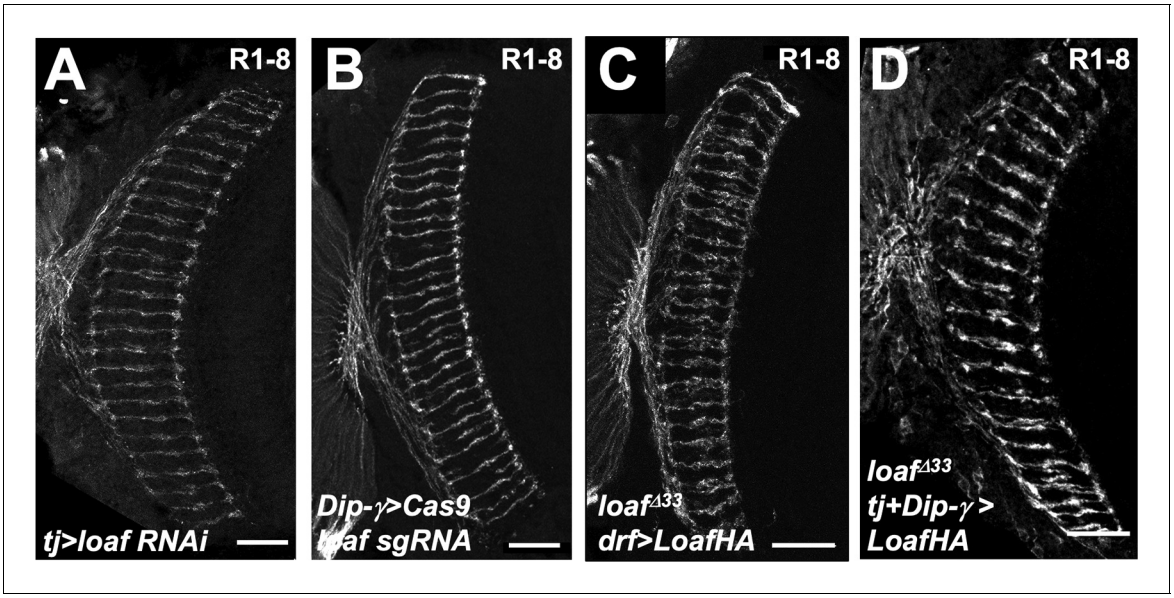

Figure 3—figure supplement 1. Changing Loaf levels in Dm8 has no effect. Cryostat sections of adult heads stained for Chp. (**A**) *tj-GAL4; loaf RNAiBL*; (**B**) *loaf sgRNAs; DIP-γ-GAL4/UAS-Cas9P2*; (**C**) *UAS-LoafHA; drf-GAL4, loaf$^{\Delta 33}$/loaf$^{\Delta 33}$*; (**D**) *tj-GAL4/UAS-LoafHA; DIP-γ-GAL4, loaf$^{\Delta 33}$/loaf$^{\Delta 33}$*. Scale bar, 20 μm.

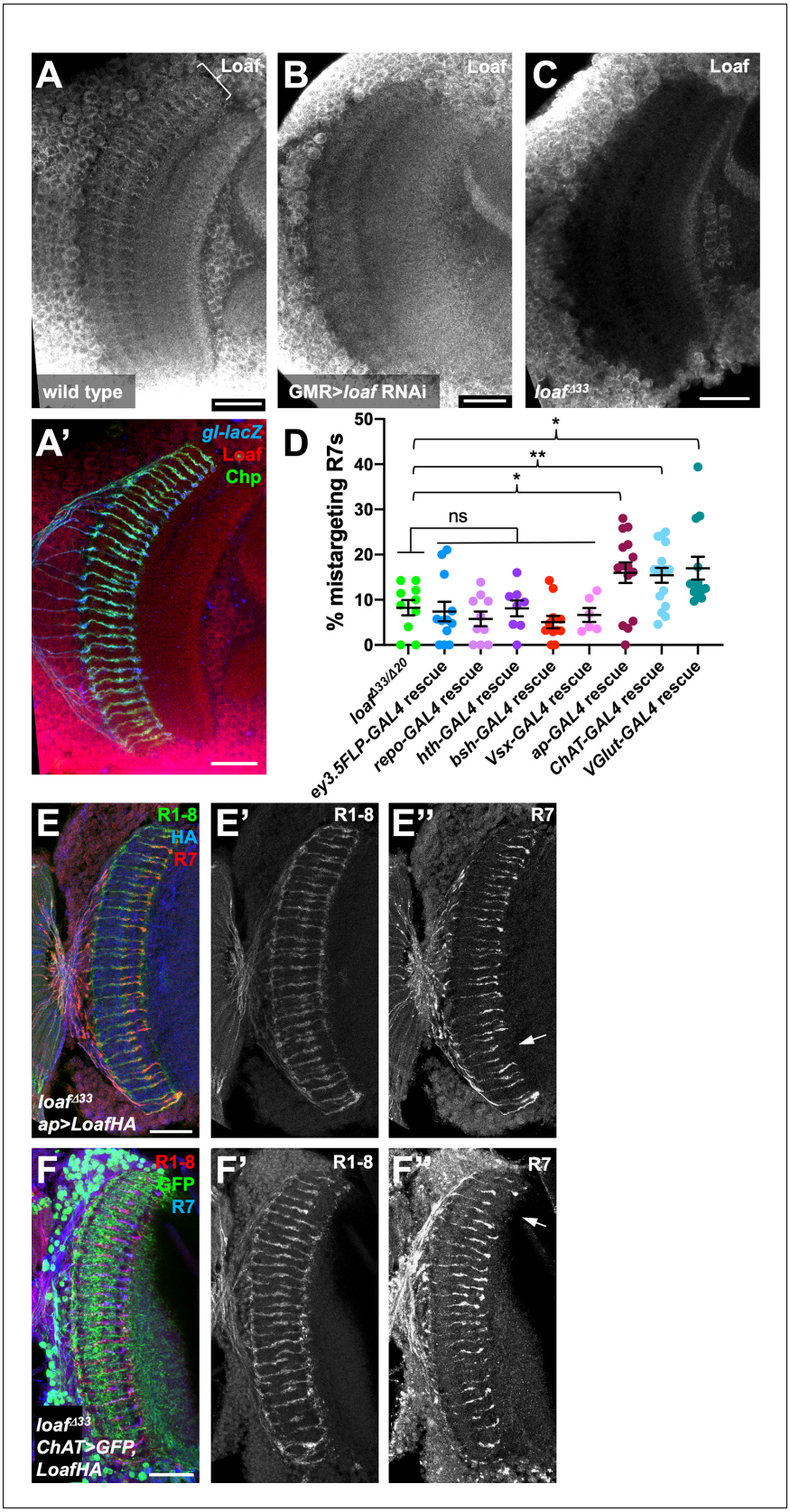

Figure 4. Loaf levels in cholinergic and glutamatergic neurons influence R7 targeting. (**A–C**) Pupal brains at 60 hr APF stained for Loaf (A-C, red in A'), Chp (green in A') and *gl-lacZ* (blue in A'). (**A**) wild type; (**B**) *GMR-GAL4, UAS-dcr2; UAS-loaf RNAiBL*; (**C**) *loaf*$^{\Delta 33}$. Loaf antibody staining in the medulla

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neuropil is absent in the *loaf* mutant (**C**). Enriched staining in R7 axons (bracket in A) is lost when *loaf* is knocked down in photoreceptors (**B**). The antibody appears to cross-react with a protein present in medulla cell bodies, as this staining is still present in *loaf* mutant brains (**C**). (**D**) quantification of the percentage of R7 axons that failed to reach the M6 layer in the indicated genotypes. n = 10 (*loaf*$^{\Delta 33}$/*loaf*$^{\Delta 20}$*; repo-GAL4* rescue), 12 (*ey3.5-FLP, Act>CD2>GAL4* rescue), 8 (*hth-GAL4* rescue), 11 (*bsh-GAL4* rescue), 6 (*Vsx-GAL4* rescue), 15 (*ap-GAL4* rescue), 16 (*ChAT-GAL4* rescue), or 13 (*vGlut-GAL4* rescue). Error bars show mean ± SEM. *, p<0.05; **, p<0.01; ns, not significant by unpaired t-test. Expressing Loaf in cholinergic or glutamatergic neurons or in the precursors of cholinergic neurons with *ap-GAL4* in a *loaf* mutant causes R7 mistargeting. (**E, F**) cryostat sections of adult heads stained for Chp (E', F', green in E, red in F), *panR7-lacZ* (E'', F'', red in E, blue in F), HA (blue in E), or GFP (green in F). (**E**) *ap-GAL4/UAS-LoafHA; loaf*$^{\Delta 33}$; (**F**) *ChAT-GAL4, UAS-CD8GFP/UAS-LoafHA; loaf*$^{\Delta 33}$. Scale bars, 20 μm.

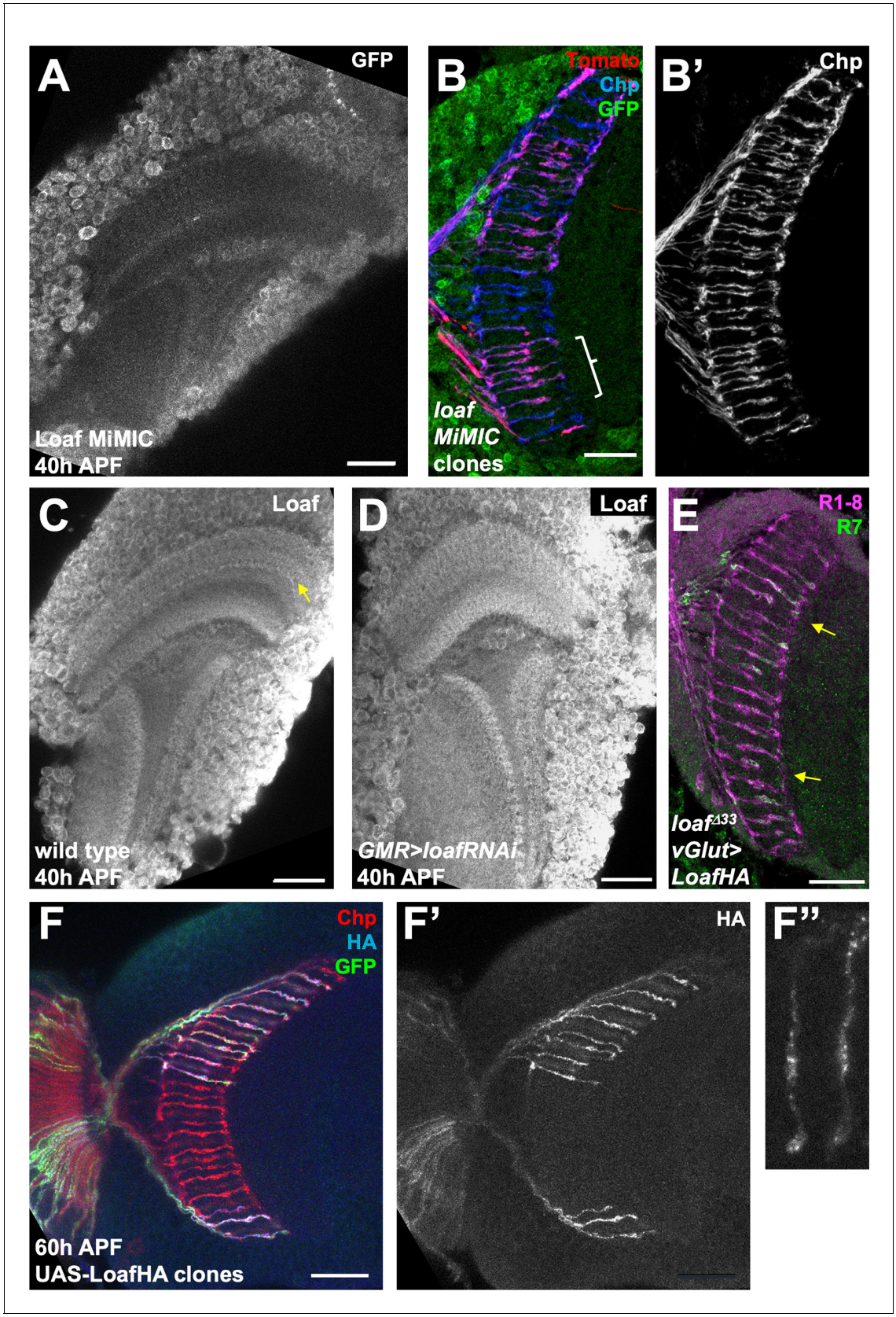

Figure 4—figure supplement 1. Loaf is expressed in many cells and enriched in R7 terminals. (**A**) *Mi{PT-GFSTF.1}CG6024*$^{MI00316\text{-}GFSTF.1}$ brain at 40 hr APF stained for GFP. GFP-tagged Loaf is mostly confined to cell bodies and only weakly present in the neuropil. (**B**) Cryostat section of an adult head in

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which *Mi{PT-GFSTF.1}CG6024*$^{MI00316\text{-}GFSTF.1}$ homozygous clones are marked with *UAS-myr-tdTomato* (red) and their axons are stained for Chp (B', blue in B). GFP-Loaf is stained in green. R7 mistargeting is observed in some clones homozygous for the insertion (bracket), indicating that it disrupts the function of the protein. (**C, D**) pupal brains at 40 hr APF stained for Loaf. (**C**) wild type; (**D**) *GMR-GAL4, UAS-dcr2; UAS-loaf RNAiBL*. Loaf is enriched in R7 terminals at this stage (yellow arrow in C) and this staining is absent when *loaf* is knocked down in photoreceptors. (**E**) Cryostat section of a *vGlut-GAL4/UAS-LoafHA; loaf*$^{\Delta 33}$ adult head stained for Chp (magenta) and *panR7-lacZ* (green). Arrows indicate mistargeted R7 axons. (**F**) 60 hr APF brain in which LoafHA is misexpressed in clones of photoreceptors with *lGMR-GAL4*, marked with GFP (green) and stained for Chp (red) and HA (F', F'', blue in F). (**F''**) shows an enlargement of two R7 axons. LoafHA is transported to R7 axons and terminals. Scale bars, 20 μm.

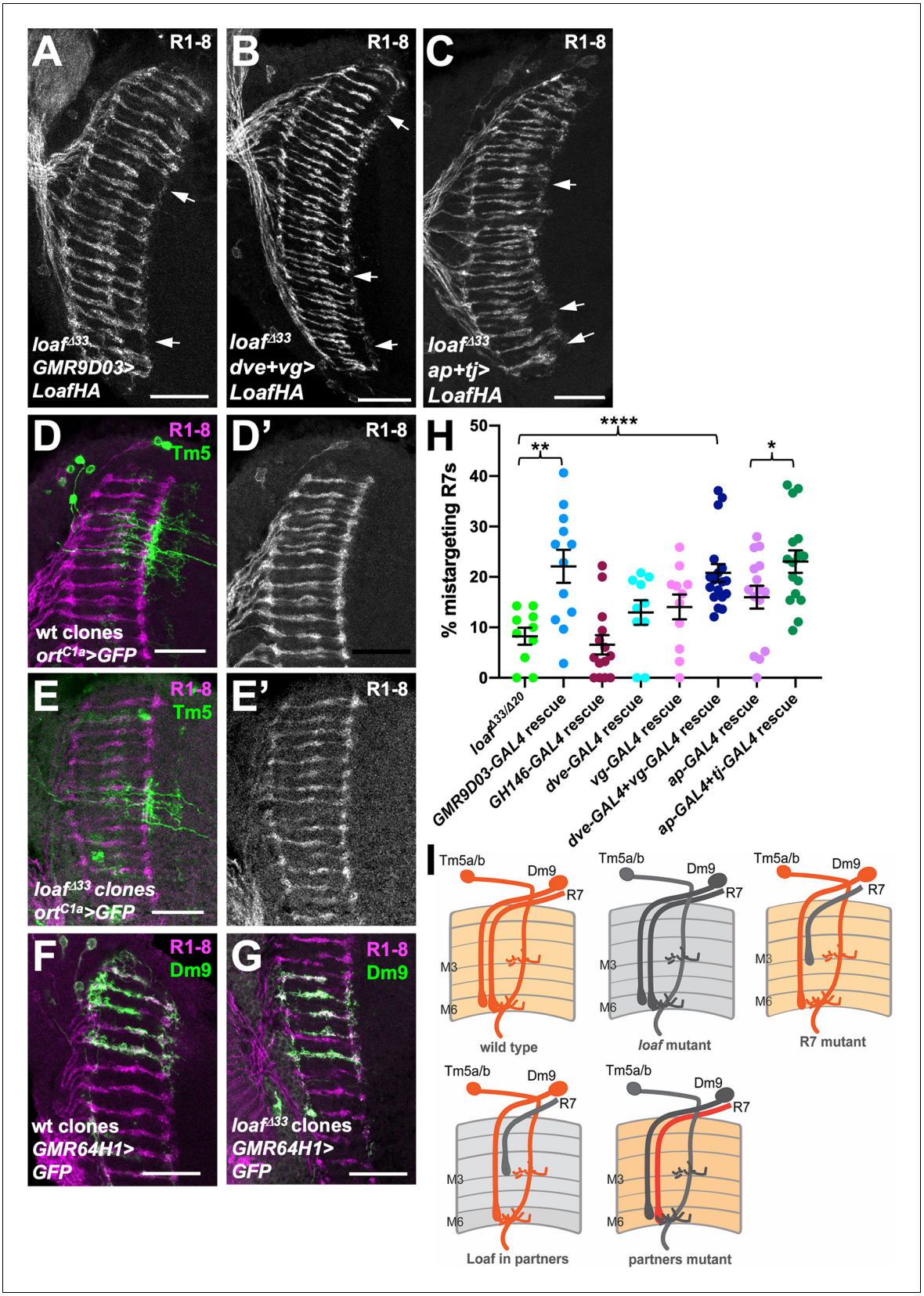

Figure 5. Expressing Loaf in synaptic partners of R7 in a *loaf* mutant causes mistargeting. (**A–C**) cryostat sections of adult heads stained for Chp. (**A**) *UAS-LoafHA; GMR9D03-GAL4, loaf$^{\Delta 33}$/loaf$^{\Delta 33}$*; (**B**) *dve-GAL4, vg-GAL4/UAS-LoafHA; loaf$^{\Delta 33}$*; (**C**) *ap-GAL4, tj-GAL4/UAS-LoafHA; loaf$^{\Delta 33}$*. Expressing Loaf

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in populations of neurons that form synapses with R7 in a *loaf* mutant background causes R7 mistargeting. (**D–G**) cryostat sections of adult heads in which clones generated with *hs-FLP* are labeled in green with UAS-CD8-GFP and R1-8 are stained with anti-Chp (D', E', magenta in D-G). (**D**) wild type and (**E**) $loaf^{\Delta 33}$ mutant clones in which Tm5a/b/c and Tm20 are labeled with ort^{C1a}*-GAL4*. The genotypes are (**D**) *hsFLP, UAS-GFP; ortc1a-GAL4/CyO; FRT80/tub-GAL80, FRT80;* (**E**) *hsFLP, UAS-GFP; ortc1a-GAL4/CyO;* $loaf^{\Delta 33}$*, FRT80/tub-GAL80, FRT80.* (**F**) wild type and (**G**) $loaf^{\Delta 33}$ mutant clones in which Dm9 cells are labeled with *GMR64H1-GAL4*. The genotypes are (**F**) *hsFLP, UAS-GFP; GMR64H1-GAL4, FRT80/tub-GAL80, FRT 80;* (**G**) *hsFLP, UAS-GFP; GMR64H1-GAL4,* $loaf^{\Delta 33}$*, FRT80/tub-GAL80, FRT 80.* The morphologies of wild type and *loaf* mutant Tm5 and Dm9 cells appear similar. (**H**) Quantification of the percentage of R7 axons that failed to reach the M6 layer in the indicated genotypes. n = 10 ($loaf^{\Delta 33}/loaf^{\Delta 20}$*; dve-GAL4* rescue), 12 (*GMR9D03-GAL4* rescue), 14 (*GH146-GAL4* rescue), 11 (*vg-GAL4* rescue), 18 (*dve-GAL4 + vg* GAL4 rescue), 15 (*ap-GAL4* rescue), or 16 (*ap-GAL4 +tj* GAL4 rescue). Error bars show mean ± SEM. *p<0.05; **p<0.01; ****p<0.0001 by unpaired t-test. (**I**) model showing that the presence of Loaf in Dm9 or Tm5a/b when *loaf* is absent in R7 causes R7 mistargeting. Scale bars, 20 μm.

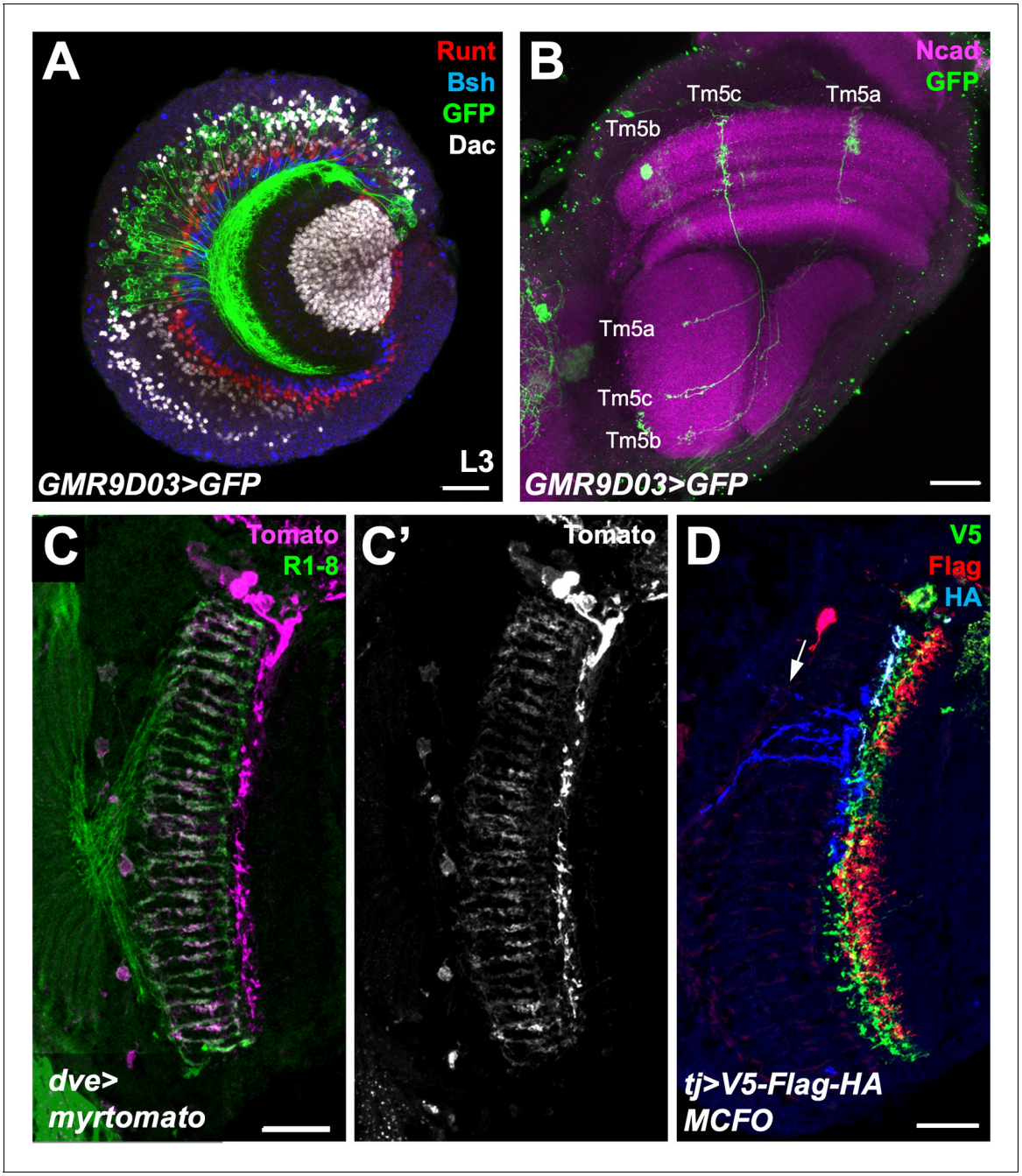

Figure 5—figure supplement 1. GAL4 drivers for Tm5a/b and Dm9. (**A**) A third instar larval brain expressing *UAS-CD8-GFP* with *GMR9D03-GAL4*, stained for GFP (green), Dac (white), Runt (red) and Bsh (blue). *GMR9D03-GAL4*-expressing cell bodies do not express Bsh, Runt or Dac, consistent with Tm5a/b identity, and are restricted to the dorsal half of the medulla cortex. (**B**) Adult brain expressing *UAS>stop> CD4-tdGFP* with *GMR9D03-GAL4* after a 7 min heat shock at late L3 to induce the expression of *hs-FLP2PEST*, stained for GFP (green) and Ncad (magenta). Cells with the morphology and projection pattern of Tm5a/b/c are labeled with GFP, showing that the *GMR9D03* enhancer is expressed in these cell types. (**C**) Cryostat section of adult head expressing *UAS-myr-tdTomato* with dve^{NP3428}*-GAL4*, stained for Tomato (C', magenta in C) and Chp (green). dve^{NP3428}*-GAL4* labels cells with the morphology and projection pattern of Dm9. (**D**) *tj-GAL4* expressing cells labeled with V5 (green), FLAG (red), and HA (blue) using the MultiColor FlpOut (MCFO) system. The cell labeled in blue (arrow) resembles a Dm11 cell. Scale bars, 20 μm.

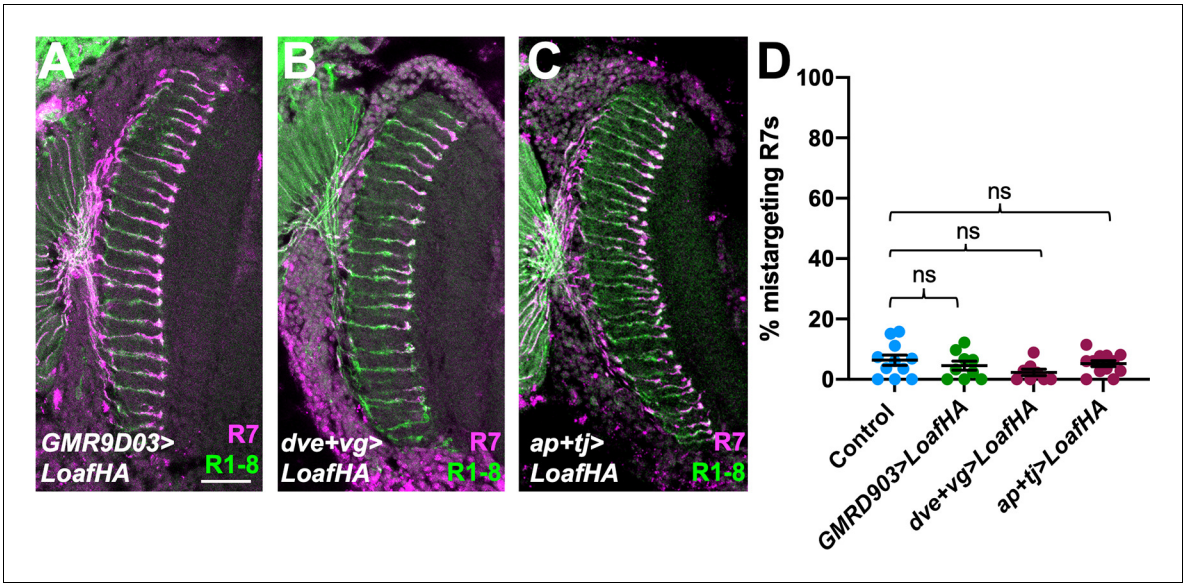

Figure 5—figure supplement 2. Overexpression of Loaf in the synaptic partners of R7 does not cause mistargeting. (**A–C**) Cryostat sections of adult heads stained for Chp (green) and *panR7-lacZ* (magenta). (**A**) *GMR9D03-GAL4; UAS-LoafHA*; (**B**) *dve-GAL4, vg-GAL4; UAS-LoafHA*; (**C**) *ap-GAL4, tj-GAL4; UAS-LoafHA*. Overexpression of Loaf in these cell types does not cause significant mistargeting. Scale bar, 20 μm. (**D**) Quantification of the percentage of R7 axons that failed to reach the M6 layer in these genotypes. n = 11 (control), 9 (*GMR9D03 > LoafHA*), 3 (*dve +vg* > LoafHA), or 12 (*ap +tj* > LoafHA). ns, not significant by unpaired t-test.

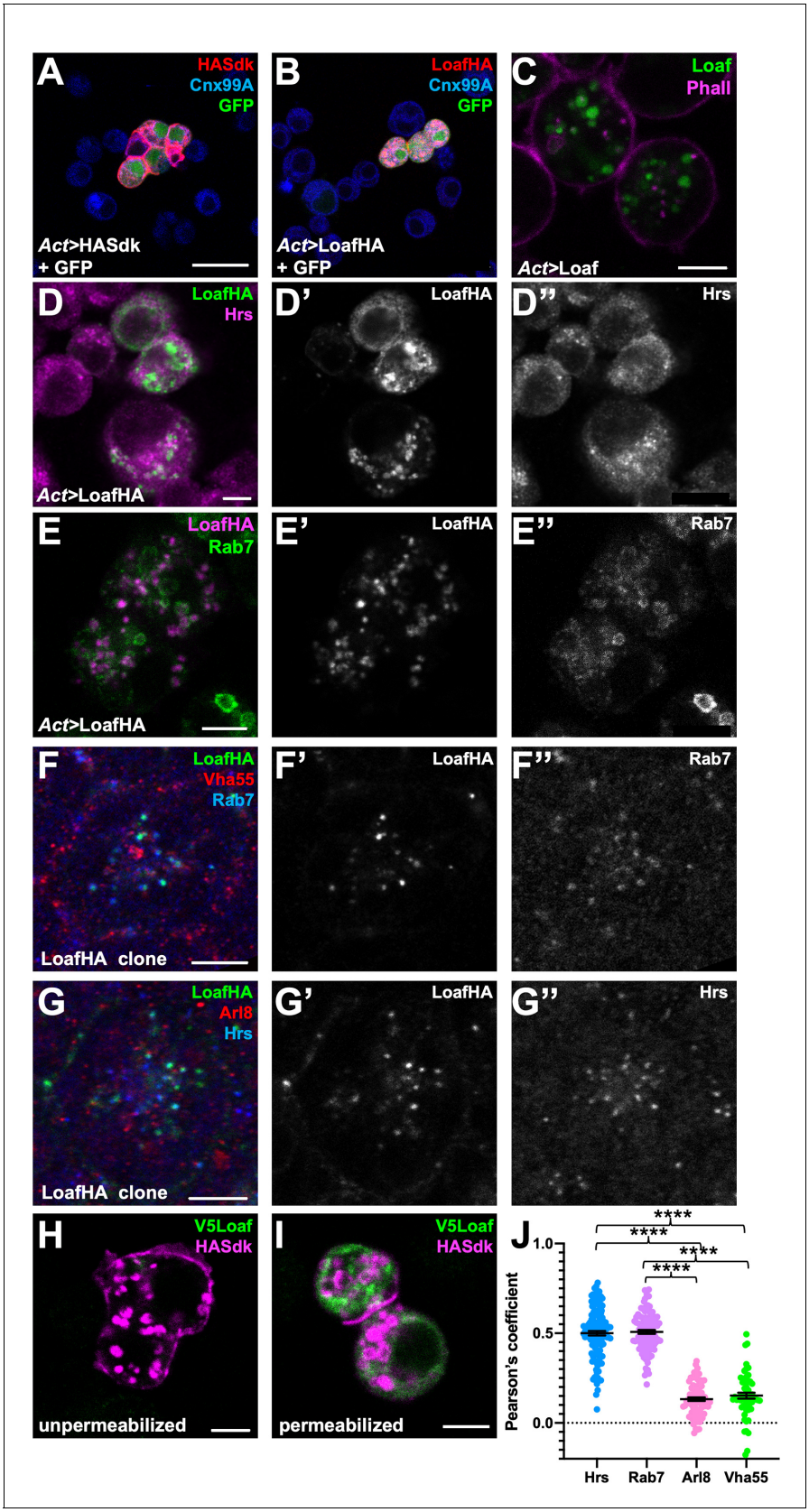

Figure 6. Loaf localizes to endosomes. (**A, B**) S2 cells transfected with *Act-GAL4*, *UAS-GFP*, and *UAS-HASdk* (**A**) or *UAS-LoafHA* (**B**) and allowed to aggregate, stained for GFP (green), HA (red) and the ER marker Calnexin 99A (Cnx99A, blue). Sdk localizes to cell contacts and induces aggregation,

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but Loaf does not. (**C**) S2 cells transfected with *Act-GAL4* and *UAS-Loaf*, stained with anti-Loaf (green) and Phalloidin (magenta). (**D, E**) S2 cells transfected with *Act-GAL4* and *UAS-LoafHA*, stained for HA (D', E', green in D, magenta in E), Hrs (D'', magenta in D), or Rab7 (E'', green in E). Loaf localizes to intracellular vesicles that show some colocalization with Hrs and Rab7. (**F, G**) Ommatidia from 42 hr APF pupal retinas in clones expressing UAS-LoafHA, stained for HA (F', G', green in F, G) Rab7 (F'', blue in F), Vha55 (red in F), Hrs (G'', blue in G), and Arl8 (red in G). Loaf colocalizes with the endosomal markers Rab7 and Hrs, but not the lysosomal markers Vha55 and Arl8, in photoreceptors in vivo. (**H, I**) S2 cells transfected with *Act-GAL4*, *UAS-HASdk*, and *UAS-V5Loaf* and incubated with antibodies to HA (magenta) and V5 (green) at room temperature prior to fixation (**H**) or after fixation and permeabilization (**I**). Sdk is detected on the cell surface and in internalized vesicles without fixation, but Loaf is not. (**J**) Quantification of the colocalization of LoafHA with Hrs, Rab7, Arl8, and Vha55 in 42 hr APF retinas by Pearson's correlation. n = 131 ommatidia from 19 retinas (Hrs), 100 ommatidia from 19 retinas (Rab7), 85 ommatidia from 16 retinas (Arl8) or 59 ommatidia from 11 retinas (Vha55). Error bars show mean ± SEM. ****p<0.0001. Scale bars, 20 μm (**A**) or 5 μm (**C–I**).

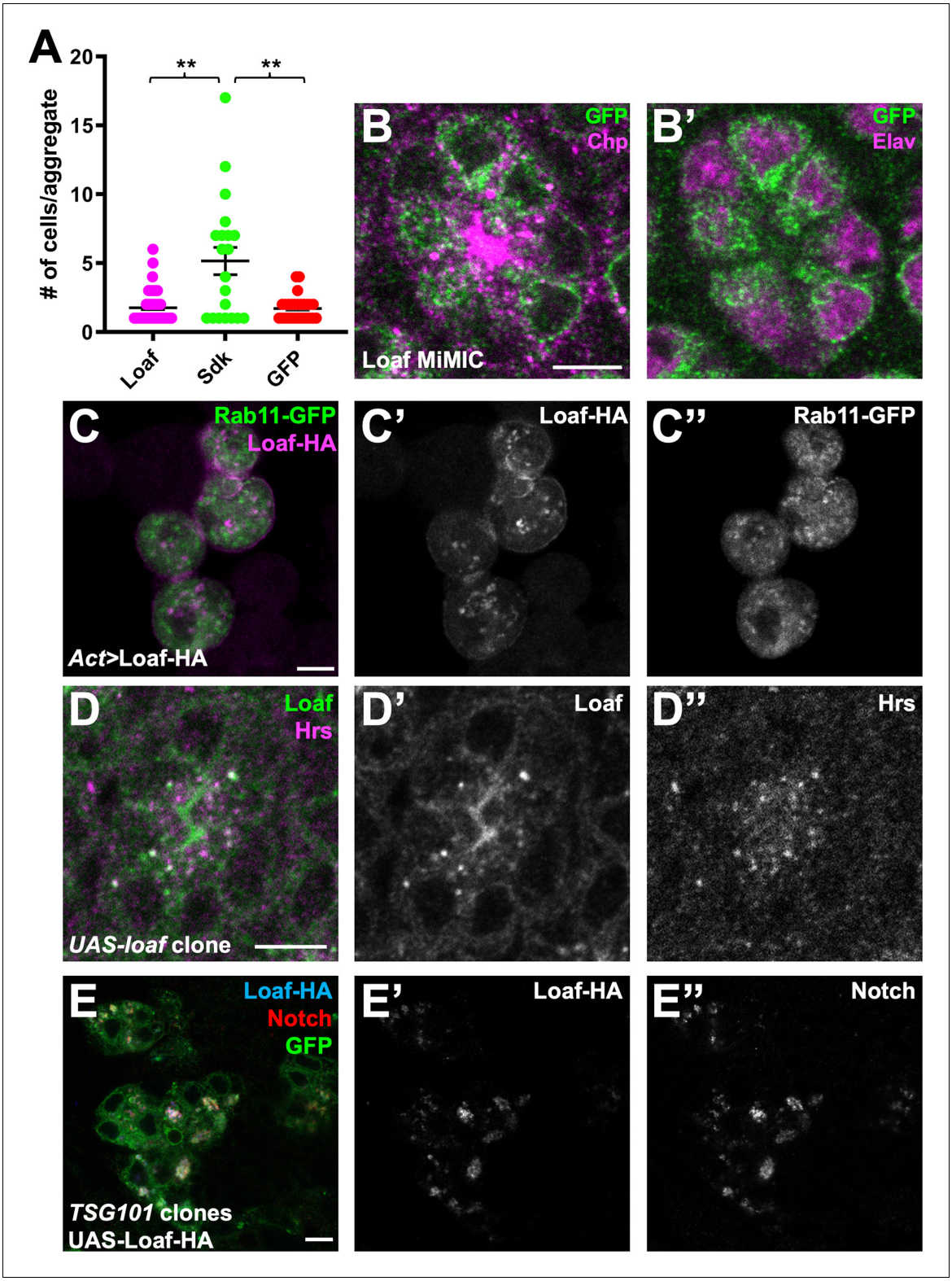

Figure 6—figure supplement 1. Loaf localizes to endosomes and does not mediate cell aggregation. (**A**) Quantification of the number of cells per aggregate for S2 cells transfected with *Act-GAL4* and *UAS-LoafHA*, *UAS-HASdk*, or *UAS-GFP*. n = 63 (Loaf), 20 (Sdk), or 27 (GFP). **, p<0.01 by unpaired t-test with Welch's correction. (**B**) Ommatidium from a Loaf protein trap *Mi{PT-GFSTF.1}CG6024*$^{MI00316\text{-}GFSTF.1}$ retina, stained for GFP (green), Chp (magenta in B), and Elav (magenta in B'). Endogenously tagged LoafGFP is present just inside the plasma membrane marked by Chp and in the cytoplasm close to the rhabdomere. (**C**) S2 cells transfected with *Act-GAL4*, *UAS-LoafHA*, and *UAS-Rab11-GFP*, stained for HA (C', magenta in C) and

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GFP (C'', green in C). Loaf does not colocalize with Rab11. (**D**) Ommatidium from a clone expressing *UAS-Loaf* in a 42 hr APF retina, stained for Loaf (D', green in D) and Hrs (D'', magenta in D). Untagged Loaf colocalizes with Hrs in vivo. (**E**) 42 hr APF retina with a *TSG101* clone in which *UAS-LoafHA* was expressed with *lGMR-GAl4*, positively labeled with GFP and stained for GFP (green), HA (E', blue in E), and Notch (E'', red in E). Loss of *TSG101* causes accumulation of enlarged endosomes in which Loaf colocalizes with internalized Notch. Scale bars, 5 μm.

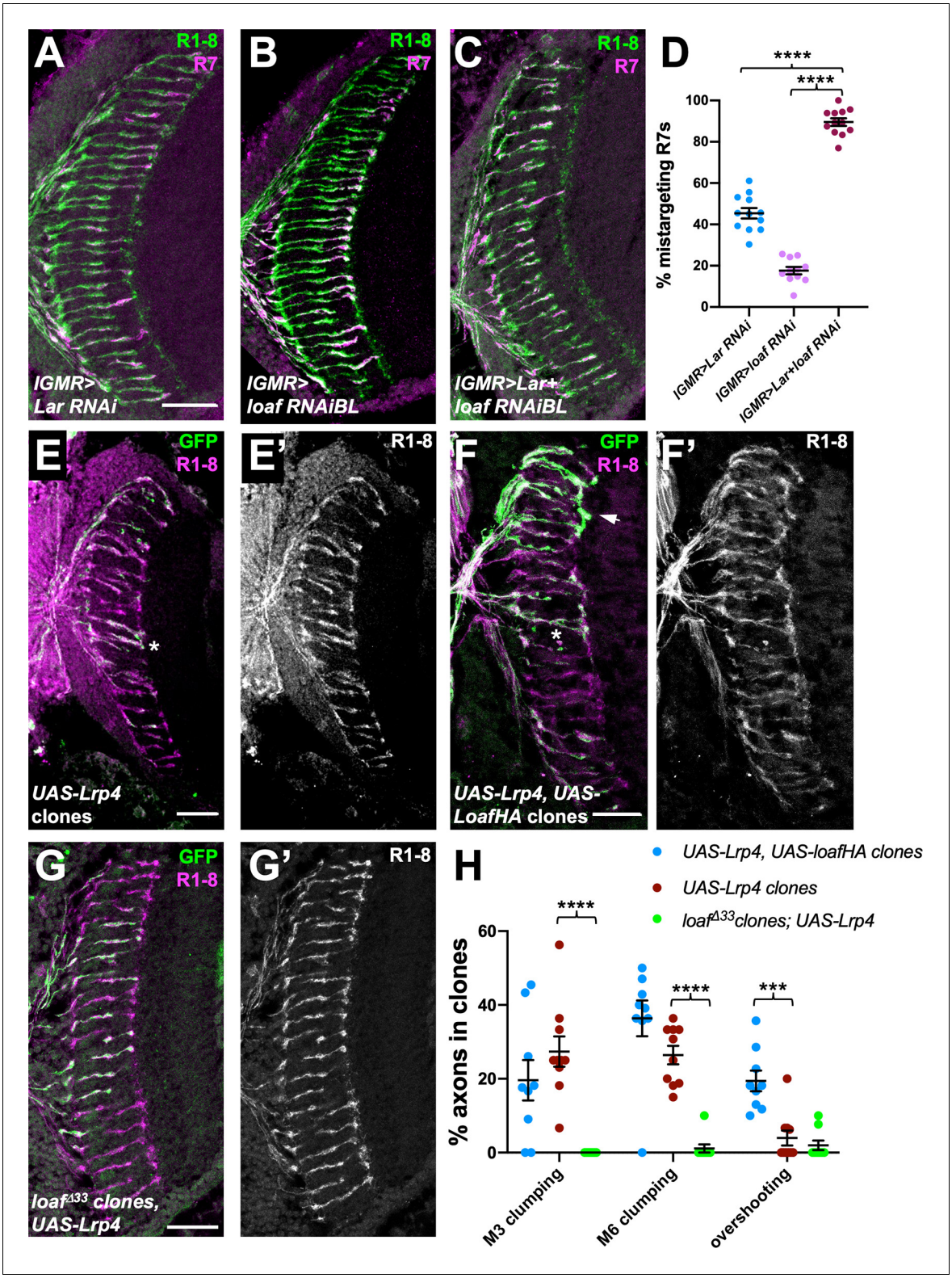

Figure 7. *loaf* genetically interacts with *Lar* and *Lrp4*. (**A–C**) Cryostat sections of adult heads stained for Chp (green) and *panR7-lacZ* (magenta). (**A**) *IGMR-GAL4, UAS-dcr2/UAS-Lar RNAi*; (**B**) *IGMR-GAL4, UAS-dcr2; UAS-loaf RNAiBL*; (**C**) *IGMR-GAL4, UAS-dcr2/UAS-Lar RNAi; UAS-loaf RNAiBL*. With this driver, *Lar* RNAi induces moderate and *loaf* RNAi mild R7 mistargeting, but the combination has a severe phenotype. (**D**) Quantification of the percentage of R7 axons that failed to reach the M6 layer in the indicated genotypes. n = 12 (*Lar RNAi*, *Lar RNAi +loaf RNAi*) or 11 (*loaf RNAi*). ****, p<0.0001 by unpaired t-test. (**E–G**) Cryostat sections of adult heads with clones positively labeled with GFP, stained for Chp (E', F', G', magenta in E-G)

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and GFP (green). (**E**) Clones expressing *UAS-Lrp4* with *lGMR-GAL4*. (**F**) Clones expressing *UAS-Lrp4* and *UAS-LoafHA* with *lGMR-GAL4*. (**C**) $loaf^{\Delta 33}$ clones in which *UAS-Lrp4* is expressed with *lGMR-GAL4*. (**H**) Quantification of the percentage of labeled R7s of each genotype that show R7 axons clumping together in the M3 (asterisk in F) or M6 (asterisk in E) layers or overshooting the M6 layer (arrow in F). n = 9 heads (*UAS-Lrp4, UASLoafHA; loaf*$^{\Delta 33}$ clones, *UAS-Lrp4*) or 10 (*UAS-Lrp4*). Error bars show mean ± SEM. ***p<0.001; ****p<0.0001 by multiple t-tests with two-stage linear step-up procedure. Scale bars, 20 μm.

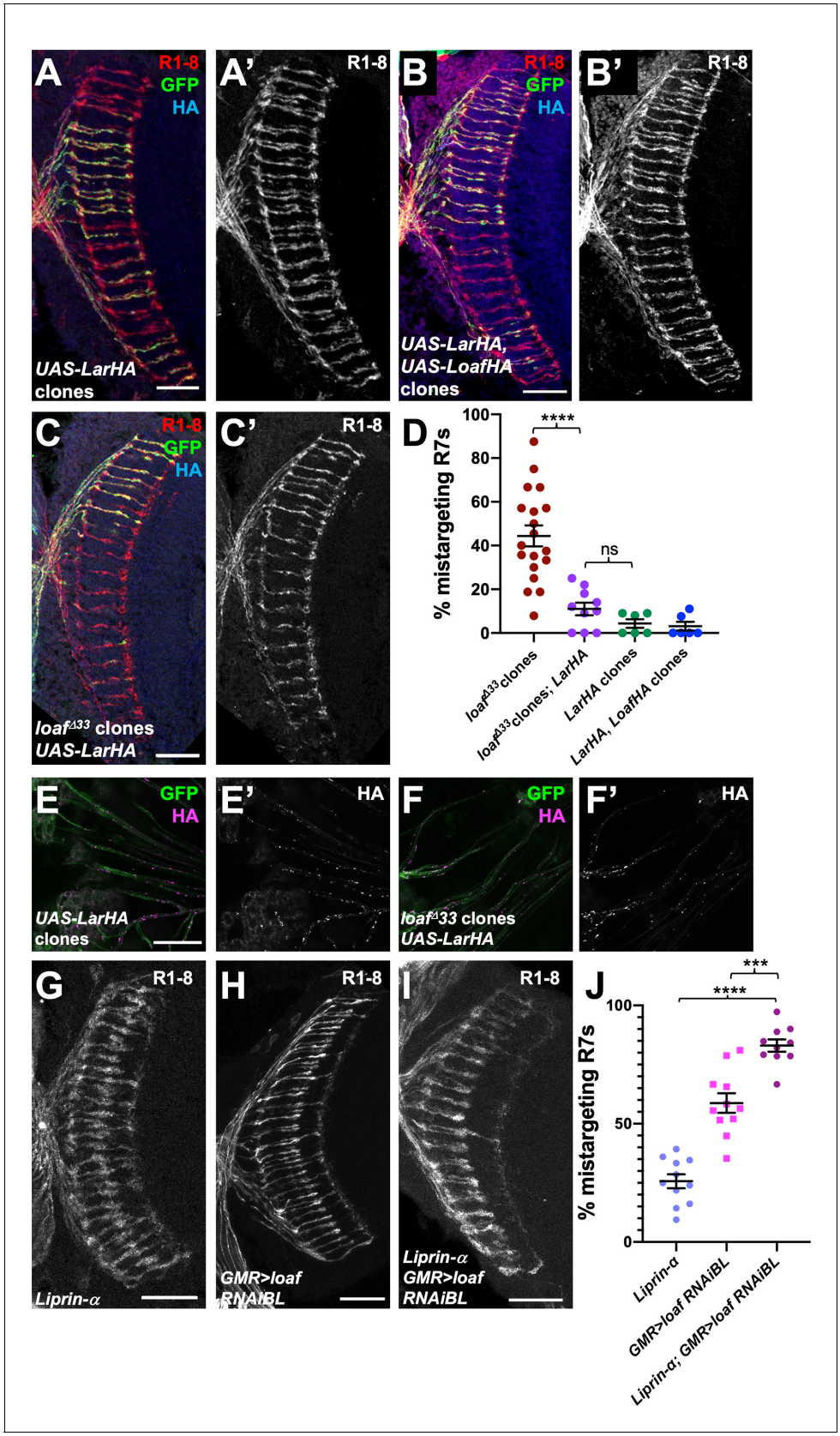

Figure 7—figure supplement 1. Lar overexpression can compensate for loss of Loaf. (**A–C**) Cryostat sections of adult heads with clones positively labeled with GFP, stained for Chp (A', B', C', red in A-C), GFP (green) and HA (blue). (**A**) Clones expressing *UAS-LarHA* with *lGMR-GAL4*. (**B**) Clones

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expressing *UAS-LarHA* and *UAS-LoafHA* with *lGMR-GAL4*. (**C**) $loaf^{\Delta 33}$ clones in which *UAS-LarHA* is expressed with *lGMR-GAL4*. (**D**) Quantification of the percentage of R7 axons that failed to reach the M6 layer in the indicated genotypes. Expressing *Lar* rescues mistargeting in *loaf* mutant clones. n = 19 ($loaf^{\Delta 33}$ clones), 10 ($loaf^{\Delta 33}$ clones; *LarHA*), or 6 (*LarHA* clones; *LarHA*, *LoafHA* clones). Error bars show mean ± SEM. ****p<0.0001; ns, not significant by unpaired t-test with Welch's correction. (**E, F**) photoreceptor axons from clones in 42 hr APF retinas positively labeled with GFP, stained for GFP (green) and HA (E', F', magenta in E, F). (**E**) Wild-type clones expressing *UAS-LarHA* with *lGMR-GAL4*; (**F**) $loaf^{\Delta 33}$ clones in which *UAS-LarHA* is expressed with *lGMR-GAL4*. Lar is transported into axons in wild type or *loaf* mutant photoreceptors. (**G–I**) Cryostat sections of adult heads stained for Chp. (**G**) *Liprin-α*^{oos}; (**H**) *GMR-GAL4/UAS-loafRNAiBL*; (**I**) *Liprin-α*^{oos}*; GMR-GAL4/UAS-loafRNAiBL*. (**J**) Quantification of the percentage of R7 axons that failed to reach the M6 layer in the indicated genotypes. n = 11 (*Liprin-α*, *GMR > loafRNAiBL*) or 10 (*Liprin-α; GMR > loafRNAiBL*). ****p<0.0001; ***p=0.0001. Scale bars, 20 μm.

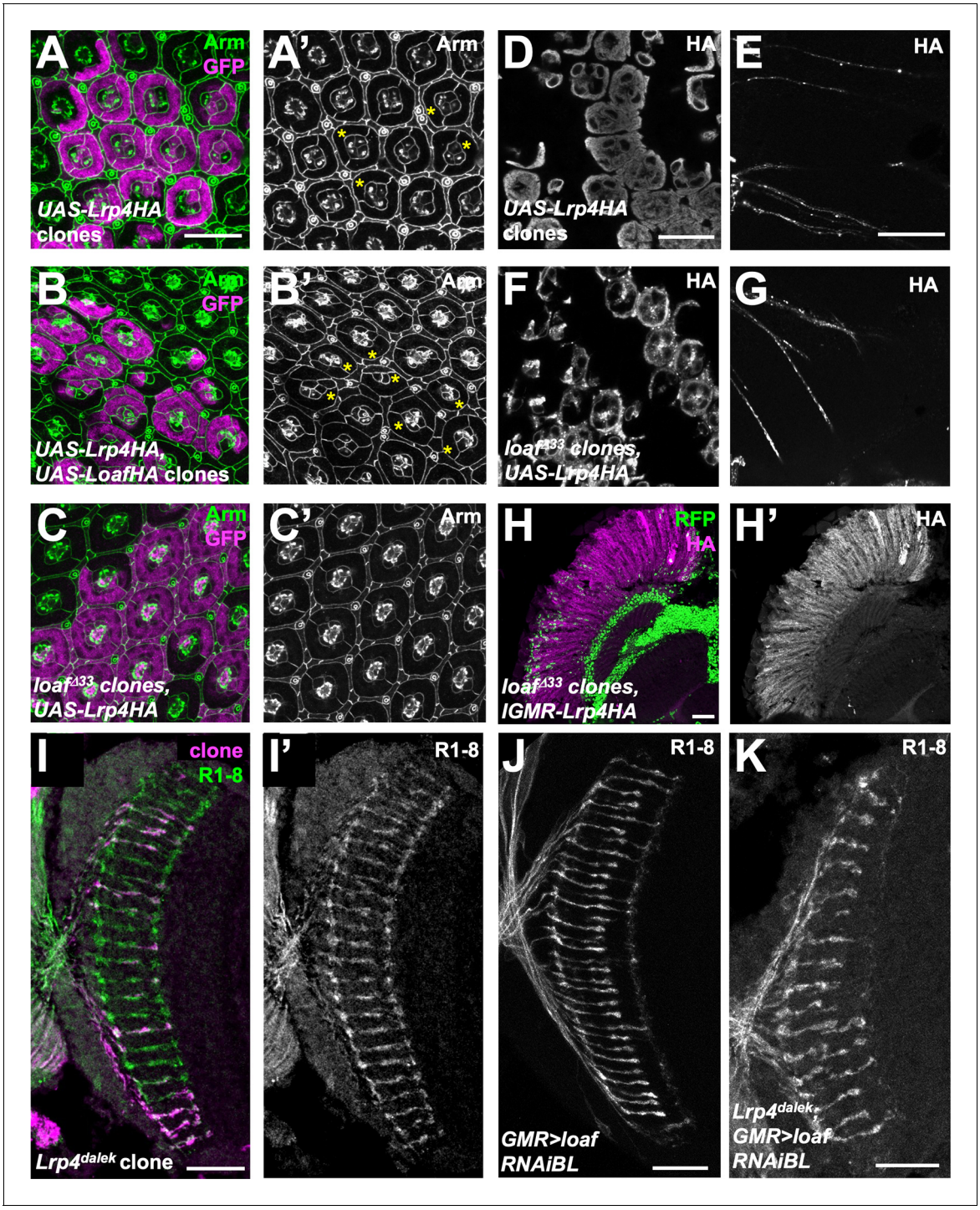

Figure 7—figure supplement 2. Lrp4 is not the only effector of Loaf. (**A–C**) 42 hr APF retinas in which clones are positively labeled with GFP, stained for GFP (magenta) and Arm (A'-C', green in A-C). (**A**) Clones expressing *UAS-Lrp4* with *lGMR-GAL4*; (**B**) clones expressing *UAS-Lrp4* and *UAS-LoafHA* with *lGMR-GAL4*; (**C**) $loaf^{\Delta 33}$ clones in which *UAS-Lrp4HA* is expressed with *lGMR-GAL4*. The ordered lattice structure of cone and pigment cells at the apical surface of the retina shows abnormalities (asterisks) when Lrp4 is overexpressed. These are more severe with Loaf coexpression and rescued in *loaf* mutant clones. (**D–G**) 42 hr APF pupal retinas in which *UAS-Lrp4HA* is expressed with *lGMR-GAL4* in wild-type clones (**D, E**) or $loaf^{\Delta 33}$ mutant clones (**F, G**), stained for HA. (**D, F**) show photoreceptor cell bodies and (**E, G**) show their axons. Lrp4 is still transported into axons in *loaf* mutant clones, but has a different appearance in cell bodies. (**H**) Cryostat section of an adult head in which *UAS-Lrp4HA* is expressed in all cells with *lGMR-GAL4*, stained for HA (H', magenta in H). $loaf^{\Delta 33}$ clones are marked by the absence of nuclear RFP (green). The level of Lrp4 protein is unaffected by loss of *loaf*. (**I–K**) Cryostat sections of adult heads stained for Chp (I', J, K, green in I) and GFP to positively label clones (magenta in I). (**I**) $Lrp4^{dalek}$ clones do not show R7

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mistargeting. (**J, K**) *GMR-GAL4* driving *UAS-loaf RNAiBL* causes R7 mistargeting in an otherwise wild-type background (**J**) and in an *Lrp4*^{dalek} background (**K**). Scale bars, 20 μm.