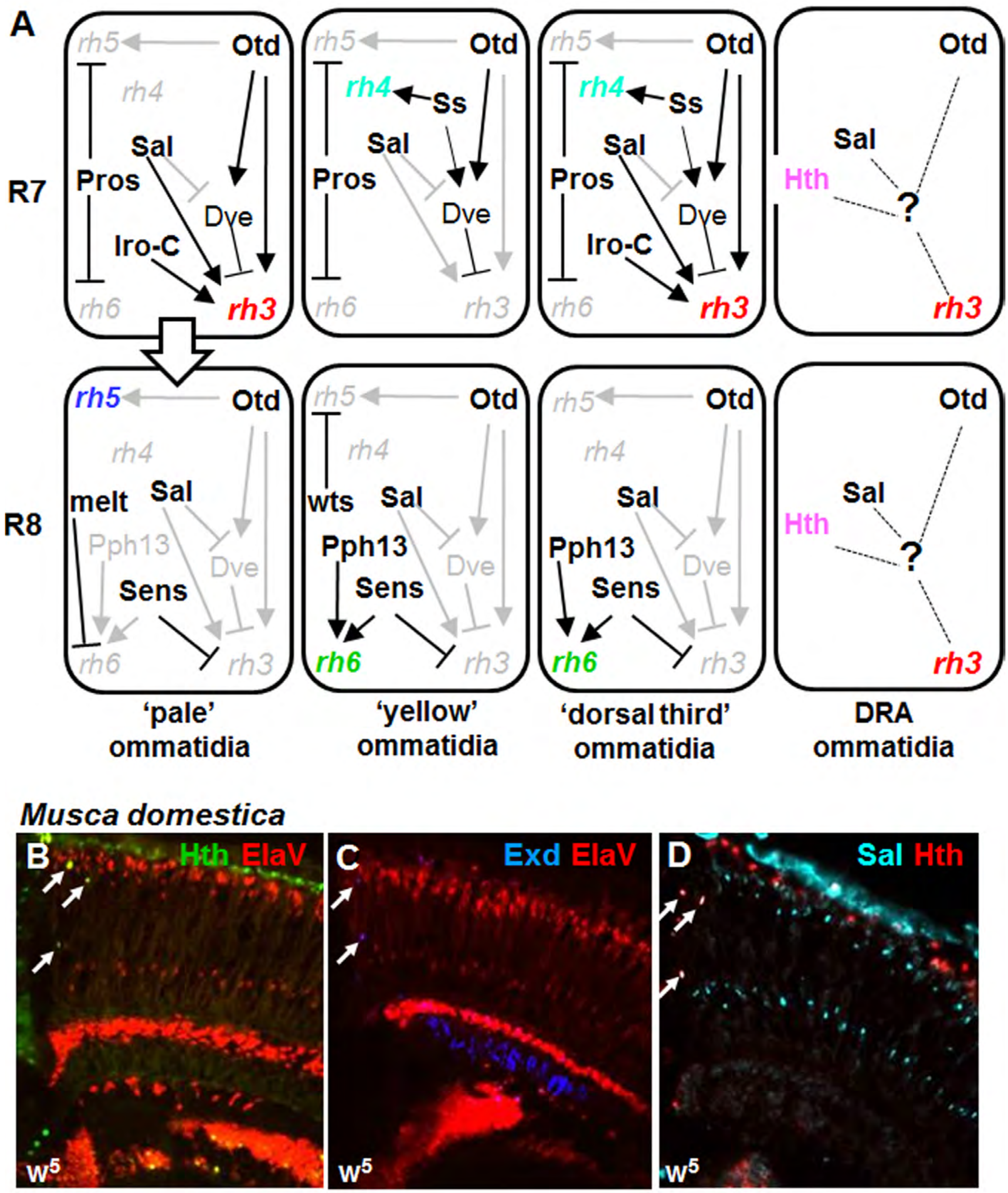
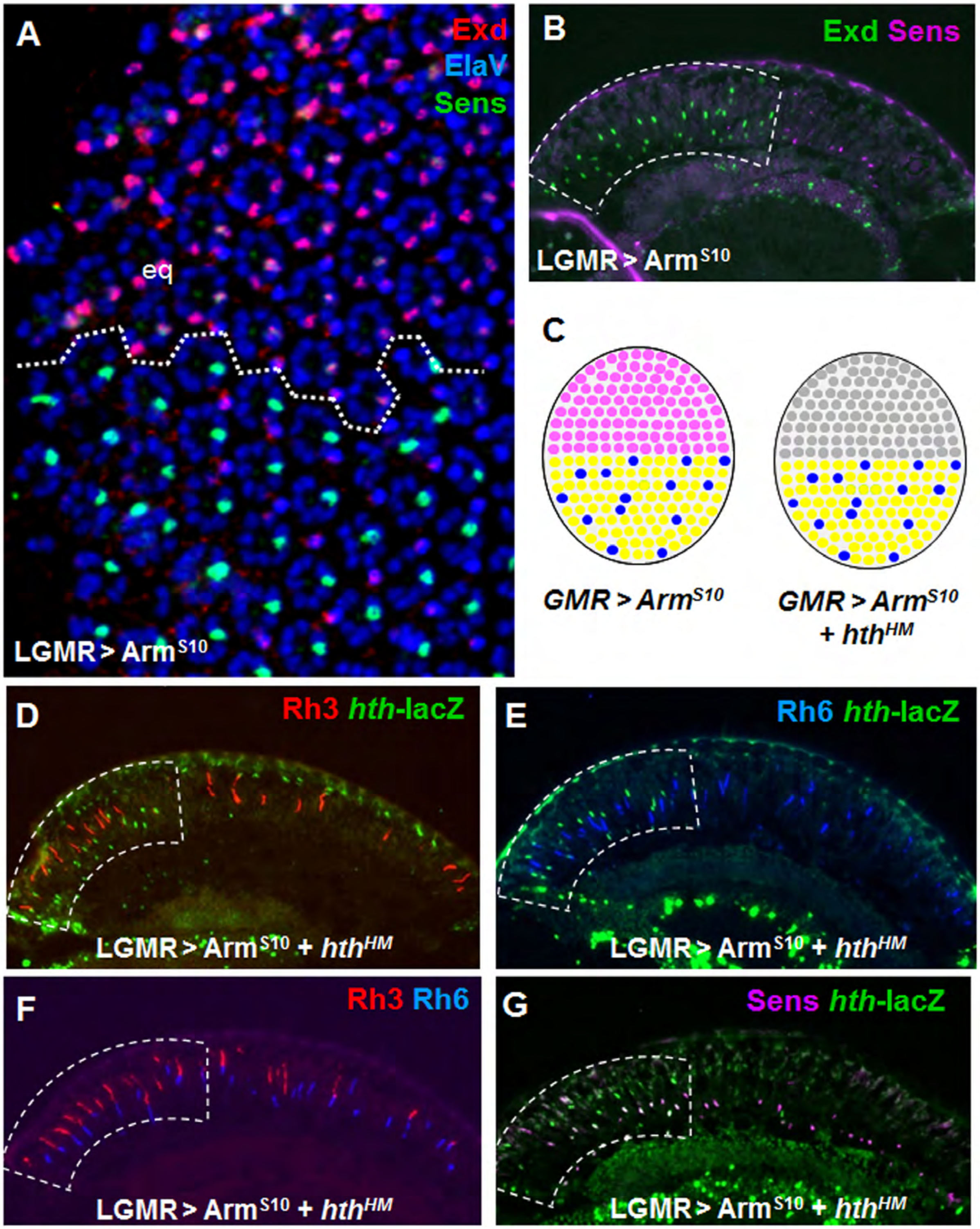
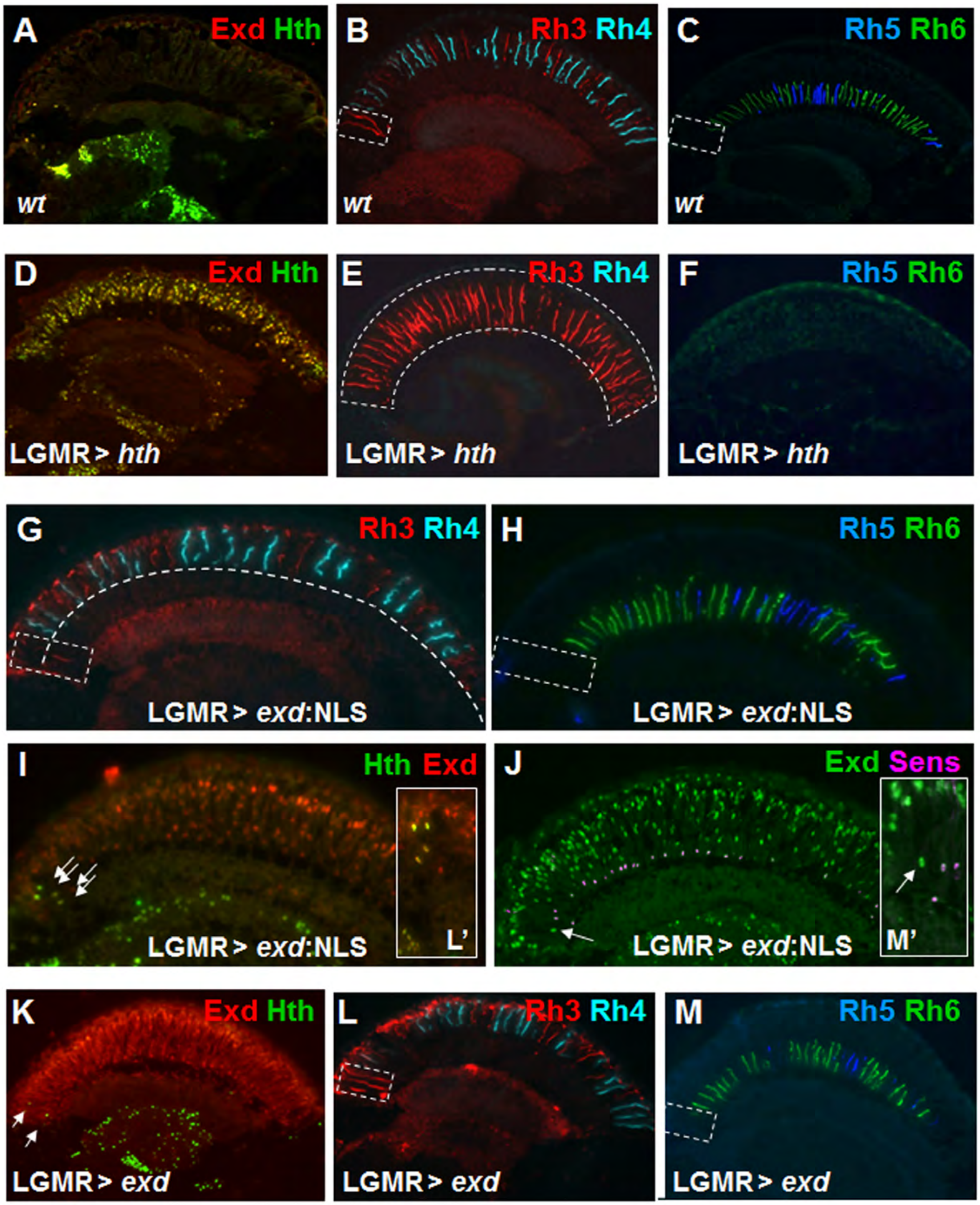


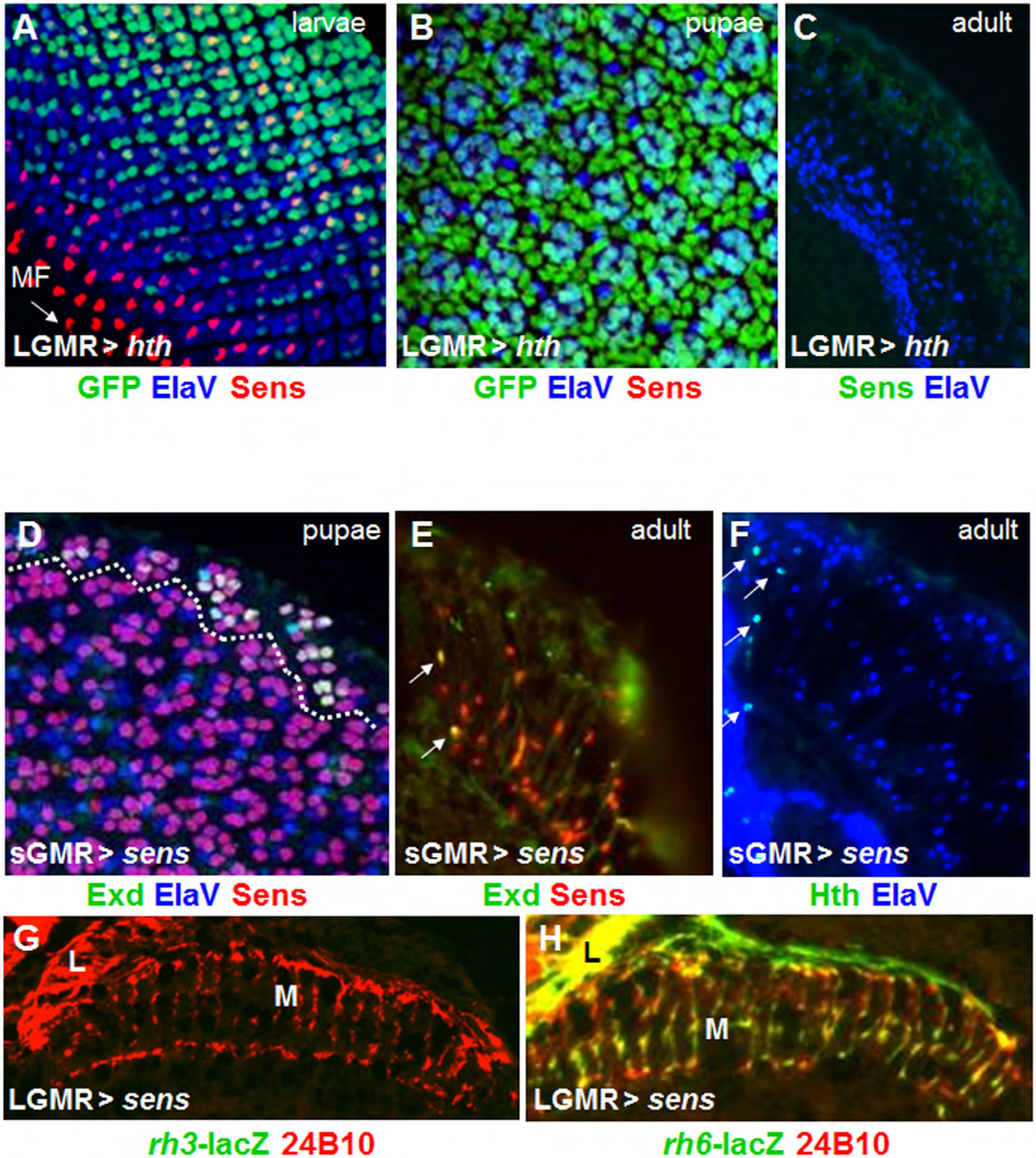
Supplemental Fig. S1. Introduction Model and Hth/Exd co-localization in *Musca*. (A) Model summarizing the transcriptional network specifying different ommatidial subtypes in *Drosophila* (from left to right): pale (p), yellow (y), 'dorsal third' (DT), and 'Dorsal Rim Area' (DRA) (after Johnston et al., 2011). Expression of Hth, Sal, and Otd in DRA ommatidia was known, yet their regulatory relationship remained to be examined. (B-D) Expression of Hth/Exd in DRA inner photoreceptors is conserved between different fly species. (B) Expression of Hth (green) and ElaV (red) in the DRA of *Musca domestica* w^5 (−/−) mutants. (C) Expression of *Musca domestica* Exd (blue) in the DRA R7 and R8, double-stained with Anti-ElaV (red). (D) Inner photoreceptors R7 and R8 in *Musca domestica* were labeled with Anti-Salm (cyan) and co-stained with Anti-Exd (red) in the DRA.



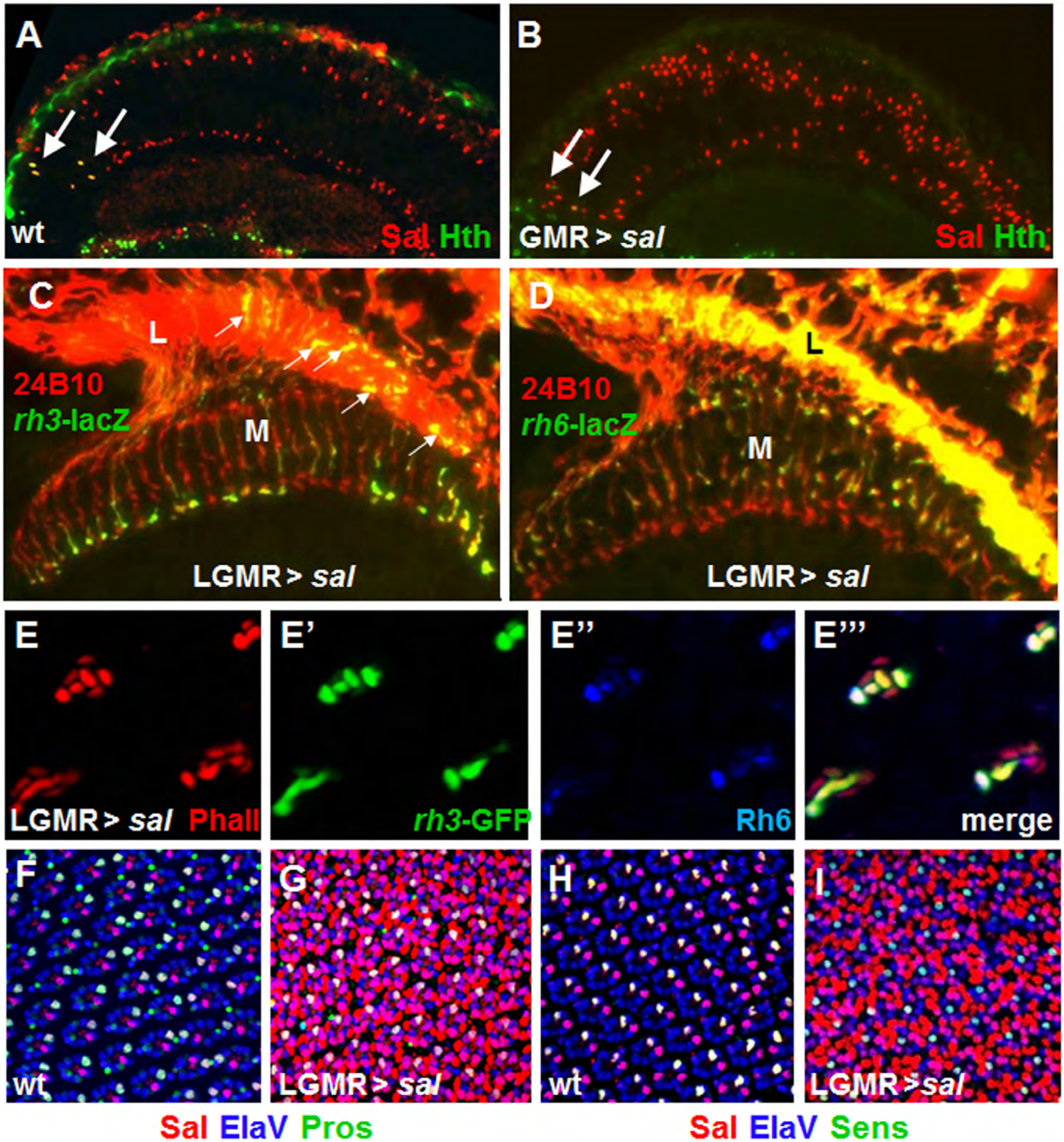
Supplemental Fig. S2. Exclusion between Exd and Sens in expanded DRA′s. (A) Overexpression of the constitutively active wingless pathway effector Armadillo (Arm^{S10}) in all photoreceptors led to the expansion of DRA ommatidia throughout the entire dorsal half of the eye, stopping at the equator (dashed line). Expression of Exd (red) and Sens (green) was mutually exclusive in these supernumerary DRA ommatidia. (B) Exclusion between Exd (green) and Sens (pink) in LGMR>Arm^{S10} flies persisted until adulthood. (C) Schematic summarizing the altered retinal mosaic of LGMR>Arm^{S10} flies (left), as well as the one expected from flies expressing an additional copy of dominant negative Hth (UAS-*hth*^{HM}). In these flies, the entire dorsal eye should be transformed into odd-coupled Rh3/Rh6 ommatidia (shown as grey circles). (D,E) Adult markers expressed in the expanded DRA of GMR>Arm^{S10}+*hth*^{HM} flies: R7 cells co-expressed Hth (green) and Rh3 (red) (D), whereas R8 cells expressed Hth (green) and Rh6 (blue). (F) As a result, all dorsal ommatidia were ‘odd-coupled’, expressing Rh3 (red) and Rh6 (blue) in the same ommatidia. (G) Co-expression of Hth (green) and Sens (pink) in all dorsal ommatidia, in GMR>Arm^{S10} + *hth*^{HM} flies.



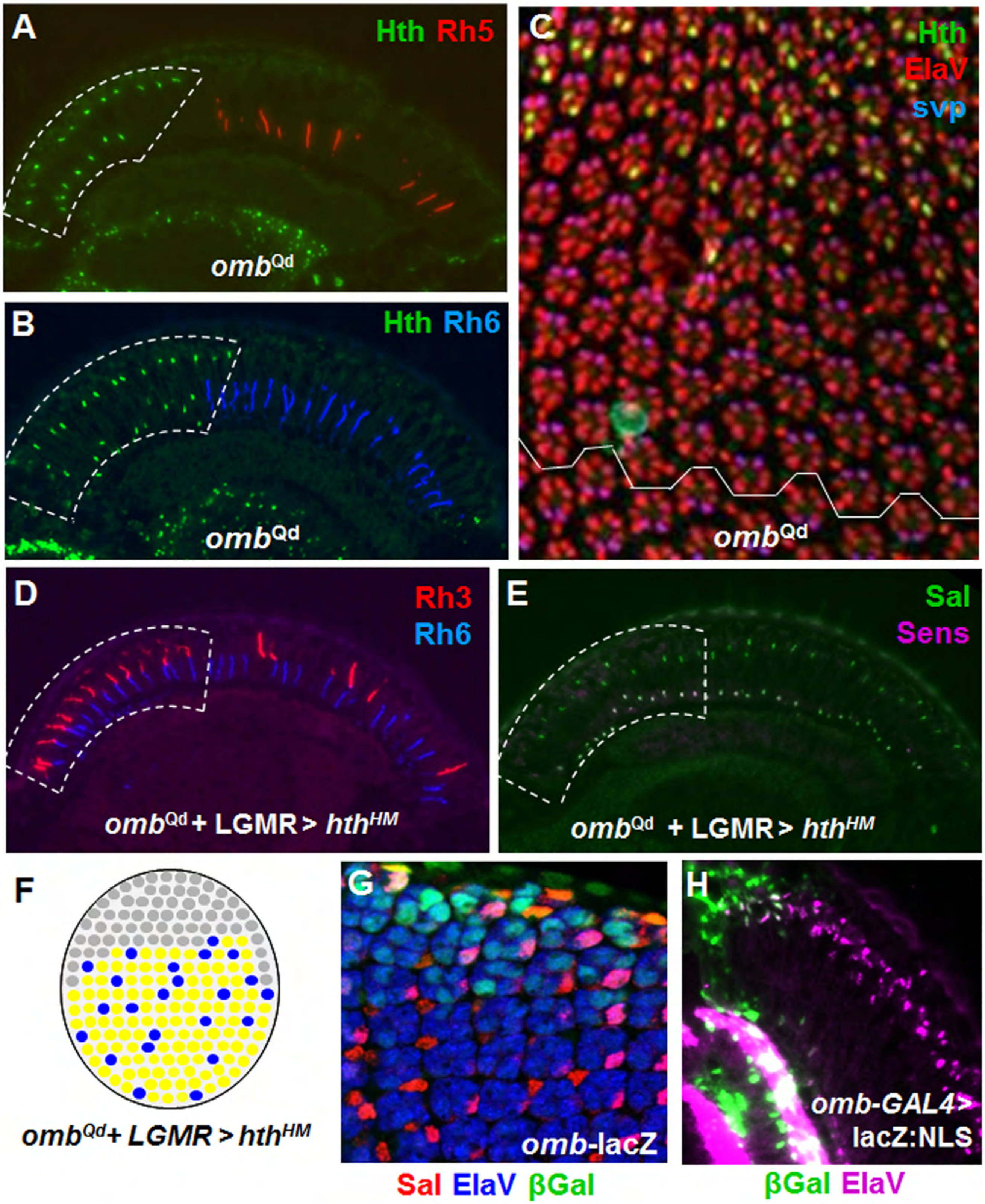
Supplemental Fig. S3. Exd and Hth gain-of-function experiments. (A-C). Adult wild type controls. (A) Co-expression of Hth (green) and Exd (red) in DRA R7 and R8 of the adult retina (white arrows). B. Wild-type expression of R7 opsins Rh3 (red) and Rh4 (cyan). Note expression of Rh3 in DRA R8 cells (dashed box). (C) Wild-type expression of R8 opsins Rh5 (blue) and Rh6 (green); both were excluded from the DRA (dashed box). (D-F) Homothorax gain-of-function. D. Ectopic overexpression of Hth led to nuclear localization of Exd (red) in all photoreceptors. (E) Expression of Rh3 (red) was expanded into all inner photoreceptors R7 and R8; expression of Rh4 (cyan) was lost. (F) Expression of both R8 opsins is lost in GMR>*hth* flies. G-J. Forced nuclear localization of Exd is not sufficient to induce the DRA fate. (G) R7 rhodopsin expression was normal, when UAS-Exd:NLS was over-expressed in all photoreceptors using LGMR-GAL4. Like in wild type flies, Rh3 (red) was expressed in the R8 cell layer only in DRA ommatidia (white dashed box). (H) R8 opsin expression was normal in GMR>*exd*:NLS flies, Rh5 (blue) and Rh6 (green) being excluded from the DRA (dashed white box). (I) Homothorax expression (green) was not altered by ectopic Exd:NLS (white arrows, inset). (J) Ectopic Exd:NLS (green) did not repress Sens (pink). However, R8 cells in the endogenous DRA still repressed Sens (white arrows, inset). (K-M) Extradenticle gain-of-function. (K) Ectopic expression of Exd (red) had no effect on Hth expression (green), which remained specifically expressed in DRA R7 and R8 (white arrows). (L) Expression of R7 opsins Rh3 (red) and Rh4 (cyan) is normal in GMR>*exd* flies. (M) Expression of R8 opsins Rh5 (blue) and Rh6 (green) is normal in GMR>*exd* flies.



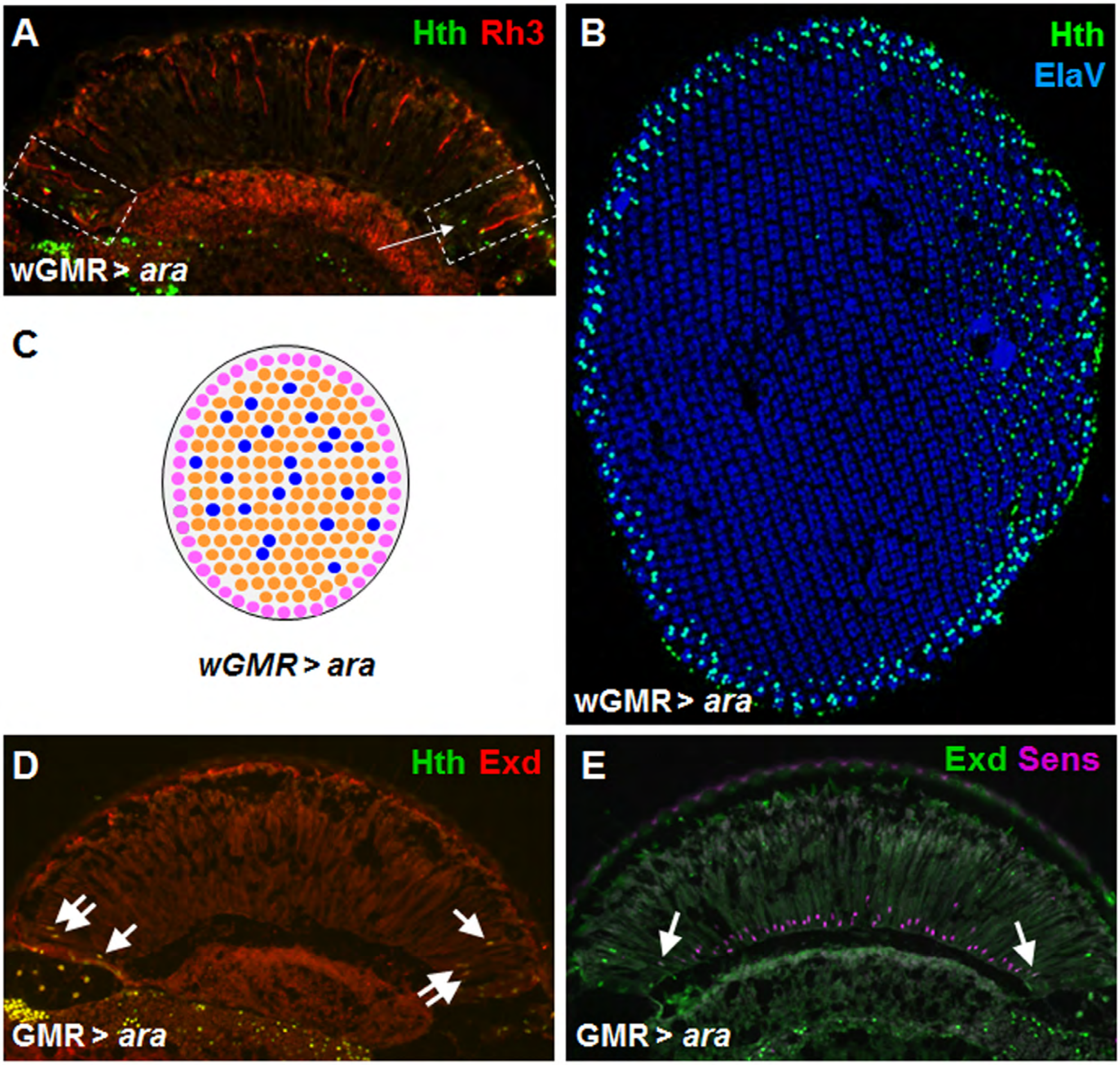
Supplemental Fig. S4. Gain-of-function of Homothorax and Senseless. (A-C) Repression of Sens expression by overexpressed Hth. (A) Early Sens expression (red) in 3rd instar larval eye discs was not affected by overexpression of Hth (green). MF=morphogenetic furrow. (B) During pupation, Sens expression (red) was lost, in GMR>*hth* flies. (C) Sens expression (green) was undetectable in the adult retina of GMR>*hth* flies. (D,E) Overexpression of Sens (red) did not repress Exd (green) in pupae (D), or in the adult retina (E). (F) Overexpression of Sens did not affect Hth expression (green). (G) Ectopic expression of Sens led to a loss of *rh3* expression in the adult retina. Labeled were projections of flies carrying *rh3*-lacZ transgenes (green). No βGal protein was detected in R7 terminals in the Medulla (M). (H) Ectopic expression of Sens led to strong activation of *rh6* expression: projections of flies carrying *rh6*-lacZ transgenes (green) showed strong signals in R8 terminals in the Medulla (M), as well as in the Lamina (L).



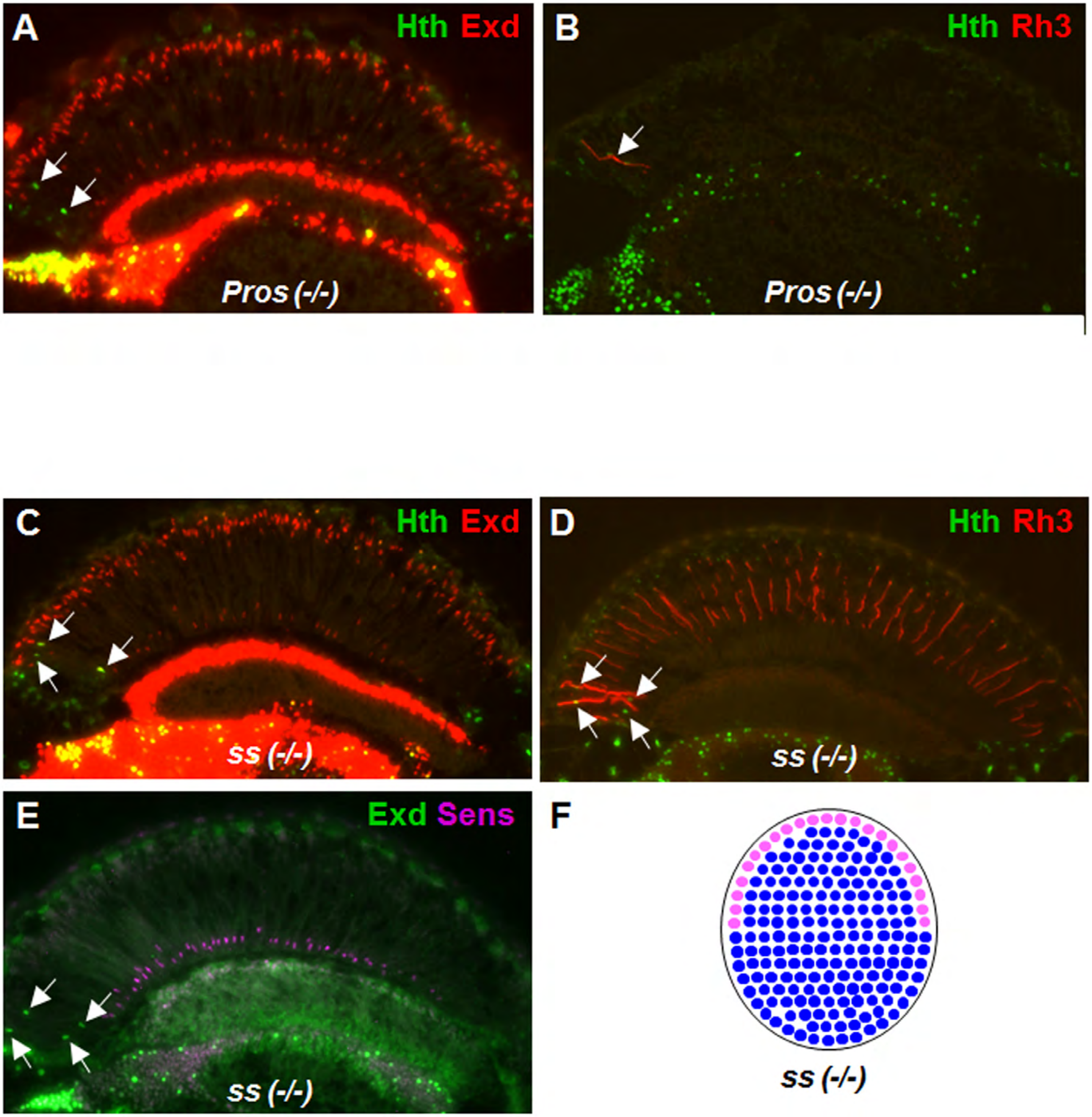
Supplemental Fig. S5. Spalt gain-of-function phenotypes. (A,B) Overexpression of 'Spalt major' (Salm) does not induce Hth in the adult retina. Hth expression (green) in the adult DRA (A) is not altered by overexpression of Salm (red), using LGMR-GAL4 (B). (C-E) Ectopic overexpression of Salm leads to an activation of Rh3 and Rh6. (C) Ectopic expression of Salm (LGMR>*Salm*) led to activation of *rh3-lacZ* expression: photoreceptor projections of flies carrying *rh3*-lacZ transgenes (green) showed strong signals in R7 terminals in the Medulla (M), as well as in the Lamina (L) (white arrows). (D) Ectopic expression of Salm led to strong expansion of *rh6*-lacZ transgenes (green) into R8 terminals in the Medulla (M), as well as in the Lamina (L). (E) Confocal images of whole mounted adult retinas from LGMR>Salm flies: expression of *rh3*-GFP (green, E′) and Anti-Rh6 (blue, E'') were detected in several photoreceptors per ommatidium, leading to co-expression between them. (F,G) Overexpression of Spalt does not induce R7 markers. Pros expression (green) in pupal R7 cells (F) was not altered by overexpression of Sal (red) (G). (H,I) Overexpression of Spalt does not induce R8 markers. Sens expression (green) in pupal R8 cells (H) is not altered by overexpression of Sal (red) (I).



Supplemental Fig. S6. More *omb*^{Qd} characterization and *omb* expression in the eye. (A,B) Exclusion of R8 opsins Rh5 (red, A) and Rh6 (blue, B) from the expanded DRA in *omb*^{Qd} mutants, labeled with Hth (green). (C) The expanded DRA in *omb*^{Qd} mutants stops several rows before the equator: whole-mounted pupal retina labeled with Hth (green) and *svp*-lacZ (blue), visualizing the equator of the eye (white line). (D,E) Overexpression dominant-negative Hth^{HM} transformed all expanded DRA ommatidia of *omb*^{Qd} mutants into 'odd-couples': only Rh3 (red) / Rh6 (Blue) coupled ommatidia are found in the dorsal retina (dashed box) (D). Dorsal ommatidia co-express Exd (green) and Sens (pink) (E). (F) Schematic summarizing the ommatidial mosaic of *omb*^{Qd}+GMR>*hth*^{HM} flies. (G,H) Optomotorblind (*omb*) expression at the eye margins of the developing retina. (G) Weak expression of *omb*-lacZ (green) in ommatidia at the dorsal margin of a 3rd instar larval disc. (H) Weak expression of *omb*-GAL4 (green) in photoreceptor neurons at the dorsal margin of the adult retina.



Supplemental Fig. S7. The role of the *Iroquois* complex in inducing ventral margins. (A) Induction of Hth (green) and Rh3 (red) expression at the ventral rim of adult flies overexpressing Iro-C factor Araucan (*ara*) in all photoreceptors. Rh3 is missing from some marginal R8 cells (arrow). (B) One or two rows of DRA ommatidia could be induced all around the retina, by over- expressing an *Iro-C* complex member (in this case: UAS-*ara*). Pupal retina labeled with Hth (green) and ElaV (blue). (C) Schematic summarizing the ommatidial mosaic of GMR>*ara* flies. (D) Co-expression of Hth (green) and Exd (red) in R7 and R8 cells at the dorsal and ventral margins of the adult eye (white arrows), in flies overexpressing Araucan (Ara) in all photoreceptors (GMR>*ara*). (E) Exd (green) did not always exclude Sens (pink) expression at dorsal and ventral margins in GMR>*ara* flies (white arrows).



Supplemental Fig. S8. Factors specifying R7 cell fates play no role in the DRA. (A) Expression of Hth (green) in the DRA was normal in whole mutant Prospero (*pros*) (−/−) eyes. (B) DRA ommatidia displayed normal expression of Rh3 (red) and Hth (green) in the absence of Pros. (C) Expression of Hth (green) was normal *spineless* (−/−) mutant eyes. (D) DRA ommatidia expressed Rh3 (red) and Hth (green) in the absence of Ss. (E) In *ss* (−/−) mutant eyes, Exd (green) excluded Sens expression (pink) in DRA R8 cells (white arrows). (F) Schematic summarizing the *ss* (−/−) retinal mosaic. Despite the drastic re-arrangement of the p/y mosaic, DRA ommatidia were specified normally.