**Supplementary File 1.** Genotypes of flies in each experiment.

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| **Figure** | | **Genotype** |
| ***Figure 1-figure supplement 1*** | | |
|  | WT: *C155-GAL4/+ (or Y);; UAS-PlexB (WT)/+*  ∆Sema: *C155-GAL4/+ (or Y);; UAS-PlexB (∆Sema)/+*  GAPmut: *C155-GAL4/+ (or Y);; UAS-PlexB (GAPmut)/+*  ∆Rac1: *C155-GAL4/+ (or Y);; UAS-PlexB (∆Rac1)/+*  Rac1mut: *C155-GAL4/+ (or Y);; UAS-PlexB (Rac1mut)/+*  ∆Rho1: *C155-GAL4/+ (or Y);; UAS-PlexB (∆Rho1)/+*  Uncleav: *C155-GAL4/+ (or Y);; UAS-PlexB (Uncleav)/+*  CleavSec: *C155-GAL4/+ (or Y);; UAS-PlexB (CleavSec)/+*  CleavTMCyto: *C155-GAL4/+ (or Y);; UAS-PlexB (CleavTMCyto)/+* | |
| ***Figure 2*** | | |
| B | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  *plexB–/–: Pebbled-GAL4, UAS-mtdTomato/+ (or Y);;; plexB–/plexB–*  WT: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+; plexB–/plexB–* | |
| C | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  *plexB–/–: Pebbled-GAL4, UAS-mtdTomato/+ (or Y);;; plexB–/plexB–*  WT: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+; plexB–/plexB–*  ∆Sema: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (∆Sema)/+; plexB–/plexB–* | |
| D | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  *plexB–/–: Pebbled-GAL4, UAS-mtdTomato/+ (or Y);;; plexB–/plexB–*  WT: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+; plexB–/plexB–*  GAPmut: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (GAPmut)/+; plexB–/plexB–* | |
| E | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  *plexB–/–: Pebbled-GAL4, UAS-mtdTomato/+ (or Y);;; plexB–/plexB–*  WT: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+; plexB–/plexB–*  ∆Rac1: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (∆Rac1)/+; plexB–/plexB–* | |
| F | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  *plexB–/–: Pebbled-GAL4, UAS-mtdTomato/+ (or Y);;; plexB–/plexB–*  WT: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+; plexB–/plexB–*  Rac1mut: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (Rac1mut)/+; plexB–/plexB–* | |
| G | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  *plexB–/–: Pebbled-GAL4, UAS-mtdTomato/+ (or Y);;; plexB–/plexB–*  WT: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+; plexB–/plexB–*  ∆Rho1: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (∆Rho1)/+; plexB–/plexB–* | |
| H | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  *plexB–/–: Pebbled-GAL4, UAS-mtdTomato/+ (or Y);;; plexB–/plexB–*  WT: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+; plexB–/plexB–*  Uncleav: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (Uncleav)/+; plexB–/plexB–* | |
| I | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  *plexB–/–: Pebbled-GAL4, UAS-mtdTomato/+ (or Y);;; plexB–/plexB–*  WT: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+; plexB–/plexB–*  CleavSec: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (CleavSec)/+; plexB–/plexB–* | |
| J | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  *plexB–/–: Pebbled-GAL4, UAS-mtdTomato/+ (or Y);;; plexB–/plexB–*  WT: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+; plexB–/plexB–*  CleavTMCyto: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (CleavTMCyto)/+; plexB–/plexB–* | |
| K | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  *plexB–/–: Pebbled-GAL4, UAS-mtdTomato/+ (or Y);;; plexB–/plexB–*  WT: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+; plexB–/plexB–*  CleavSec+ CleavTMCyto:*Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (CleavSec)/ UAS-PlexB (CleavTMCyto); plexB–/plexB–* | |
| ***Figure 3*** | | |
| A | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  ORN>PlexB Overexpression: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+* | |
| B | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  WT: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+* | |
| C | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  WT: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+*  ∆Sema: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (∆Sema)/+* | |
| D | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  WT: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+*  GAPmut: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (GAPmut)/+* | |
| E | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  WT: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+*  ∆Rac1: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (∆Rac1)/+* | |
| F | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  WT: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+*  Rac1mut: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (Rac1mut)/+* | |
| G | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  WT: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+*  ∆Rho1: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (∆Rho1)/+* | |
| H | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  WT: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+*  Uncleav: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (Uncleav)/+* | |
| I | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  WT: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+*  CleavSec: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (CleavSec)/+* | |
| J | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  WT: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+*  CleavTMCyto: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (CleavTMCyto)/+* | |
| K | Control: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y)*  WT: *Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (WT)/+*  CleavSec+ CleavTMCyto:*Pebbled-GAL4, UAS-mtdTomato/+ (or Y);; UAS-PlexB (CleavSec)/ UAS-PlexB (CleavTMCyto)* | |
| ***Figure 4*** | | |
| A | Control: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+*  ORN>PlexB Overexpression: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+; UAS-PlexB (WT)/+* | |
| B | Control: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+*  WT: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+; UAS-PlexB (WT)/+* | |
| C | Control: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+*  WT: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+; UAS-PlexB (WT)/+*  ∆Sema: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+; UAS-PlexB (∆Sema)/+* | |
| D | Control: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+*  WT: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+; UAS-PlexB (WT)/+*  GAPmut: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+; UAS-PlexB (GAPmut)/+* | |
| E | Control: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+*  WT: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+; UAS-PlexB (WT)/+*  ∆Rac1: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+; UAS-PlexB (∆Rac1)/+* | |
| F | Control: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+*  WT: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+; UAS-PlexB (WT)/+*  Rac1mut: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+; UAS-PlexB (Rac1mut)/+* | |
| G | Control: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+*  WT: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+; UAS-PlexB (WT)/+*  ∆Rho1: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+; UAS-PlexB (∆Rho1)/+* | |
| H | Control: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+*  WT: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+; UAS-PlexB (WT)/+*  Uncleav: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+; UAS-PlexB (Uncleav)/+* | |
| I | Control: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+*  WT: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+; UAS-PlexB (WT)/+*  CleavSec: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+; UAS-PlexB (CleavSec)/+* | |
| J | Control: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+*  WT: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+; UAS-PlexB (WT)/+*  CleavTMCyto: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+; UAS-PlexB (CleavTMCyto)/+* | |
| K | Control: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+*  WT: *Pebbled-GAL4/+ (or Y); Or92a-rCD2/+; UAS-PlexB (WT)/+*  CleavSec+ CleavTMCyto:*Pebbled-GAL4/+ (or Y); Or92a-rCD2/+; UAS-PlexB (CleavSec)/ UAS-PlexB (CleavTMCyto)* | |