

Figure S1. Verification of *Pdf* FISH library, related to Figure 2. Examples of *Pdf* mRNA detection by fluorescent *in situ* hybridization (green) in brains from *yw ; Pdf{WT}; Pdf⁰¹* genomic rescue flies (top panels) and *Pdf⁰¹* null mutants (bottom panel). As expected, absence of signal is observed in *Pdf⁰¹* brains.

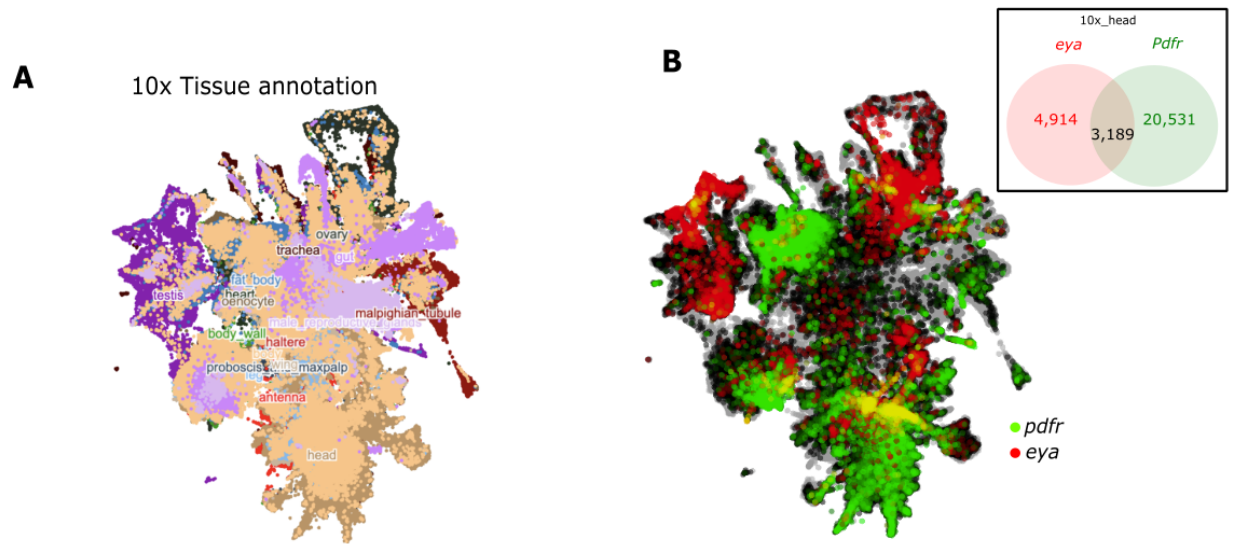


Figure S2. PDFR expression in *Drosophila* brains, related to Figure 4. (A) All cells from the 10x relaxed dataset colored and labeled by tissue as seen by UMAP^{S1}. (B) Same dataset now colored by *Pdfr* (green) and *eya* (red) expression. (B, Inset) Venn diagram depicting the number of cells from the head tissue that express *eya* (red), *Pdfr* (green) or both (overlap).

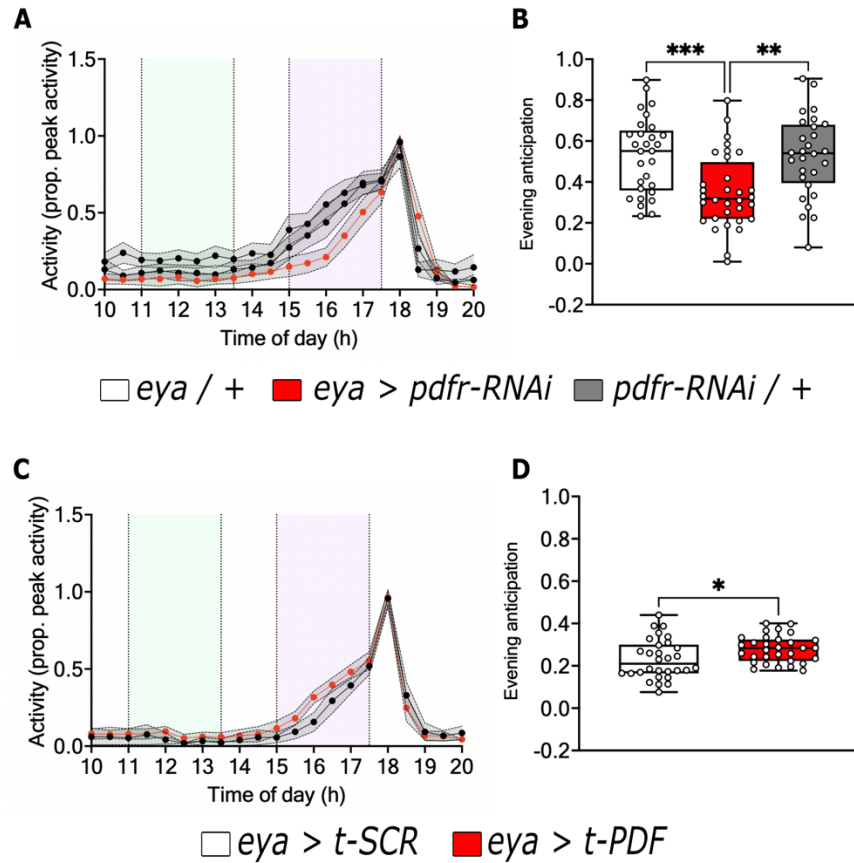


Figure S3. PDF modulates evening anticipation through *eya*⁺ cells, related to Figure 4. (A-B) Evening anticipation in locomotion activity of flies where the PDF receptor was knocked down in *eya*⁺ cells (*eya* > *Pdfr-RNAi* red line and box) compared to controls (*eya* / + and *Pdfr-RNAi* / +, black and gray lines, respectively) in 12:12 LD cycles at 25°C. (C-D) Evening anticipation of flies where a membrane tethered version of PDF was expressed in *eya*⁺ cells (*eya* > *t-PDF* red line and box) compared to the expression of a scrambled version of the peptide (*eya* > *t-SCR* black line and box). Green and purple boxes in A and C denote the windows used for the calculation of the evening anticipation. Data in B were analyzed with one-way ANOVA followed by Dunnett's multiple comparison test, unpaired t-test in D. Number of flies used were *eya* / + n = 29, *eya* > *Pdfr-RNAi* n = 32, *Pdfr-RNAi* / + n = 28, *eya* > *t-SCR* n = 31, *eya* > *t-PDF* n = 31, *eya* / + n = 30.

Sequence (5' to 3')	Sequence Name
agcaggagacttgcgaaatga	PDF_1
caggaactgagcacggaaca	PDF_2
attagtccgaggagctggaa	PDF_3
gacaaggtagctgttagcgag	PDF_4
gcaaatggccagaagcacia	PDF_5
tgtactccttgcgacacatag	PDF_6
ttgttgaaccagtcgaggag	PDF_7
cggatagcgacagagagtgg	PDF_8
ccaaggagtctcgaggatc	PDF_9
agttgattagctccgagttg	PDF_10
ttcttgggcagactcaacia	PDF_11
taatccttcagcattttccg	PDF_12
taaattcttgtccaggttcc	PDF_13
cttcgacgcacaaaaaatc	PDF_14
ttatctgaggattttcatt	PDF_15
tattagatgactacaccggc	PDF_16
aagttatacggctttttct	PDF_17
ggctacttgatatttttct	PDF_18
gtttatttcagcagtaagcc	PDF_19
tgtaccagattcaagtcga	PDF_20

Table S1. Library for *Pdf* detection by FISH originally from Long et al.^{S2}, related to Figure 2.

Supplemental References

- S1. Li, H., Janssens, J., de Waegeneer, M., Kolluru, S.S., Davie, K., Gardeux, V., Saelens, W., David, F.P.A., Brbić, M., Spanier, K., et al. (2022). Fly Cell Atlas: A single-nucleus transcriptomic atlas of the adult fruit fly. *Science* 375. [10.1126/science.abk2432](https://doi.org/10.1126/science.abk2432).
- S2. Long, X., Colonell, J., Wong, A.M., Singer, R.H., and Lionnet, T. (2017). Quantitative mRNA imaging throughout the entire *Drosophila* brain. *Nat. Methods* 14, 703–706. [10.1038/nmeth.4309](https://doi.org/10.1038/nmeth.4309).