A versatile genetic tool for post-translational control of gene expression with a small molecule in *Drosophila melanogaster*

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Abstract

Several techniques have been developed in *Drosophila* to control gene expression temporally. While some of these techniques are incompatible with existing GAL4 lines, others suffer from side effects on physiology or behavior. Here, we describe a method of post-translational temporal control of gene expression which is compatible with the current library of transgenic reagents. We adopted a strategy to regulate protein degradation by fusing a protein of interest to a destabilizing domain (DD) derived from the *Escherichia coli* dihydrofolate reductase (ecDHFR). Trimethoprim (TMP), a stabilizing small molecule, binds to DD and blocks degradation of the chimeric protein. With a GFP-DD reporter, we show that this system is effective across different tissues and developmental stages in the fly. Notably, feeding flies with TMP can increase the expression level of GFP-DD up to 34 times in a dosage-dependent and reversible manner without altering the lifespan or behavior of the animal. To broaden the utility of our method, we engineered GAL80-DD flies that can be crossed to the available GAL4 lines to control the temporal pattern of gene expression with TMP. We also developed an inducible recombinase, FLP-DD, for high-efficiency sparse labeling and intersectional lineage analysis. Finally, we demonstrated the utility of the DD system in manipulating neuronal activity of sensory neurons. In summary, we have developed a system to control *in vivo* gene expression levels with negligible background, large dynamic range, and in a reversible manner, all by feeding a small molecule to *Drosophila melanogaster*.

Keywords: ecDHFR, destabilizing domain, *Drosophila*, degron, GAL80, flippase, sparse labeling

Introduction

Tools for precise spatial and temporal control of gene expression are essential to understand how neuronal circuits develop and function. For example, genetic knockdown of a target gene during a specific time and in a defined neuronal population permits the separation of its role in development from its contribution to circuit function in the adult stage. In the vinegar fly, *Drosophila melanogaster*, bipartite expression systems (GAL4/UAS, LexA/LexAop, QF/QUAS) provide a powerful means to control gene expression in a spatially selective manner(1–3). Several modifications of these expression systems have been made to permit temporal control over the exogenous transcription factors (GAL4, LexA or QF)(3–6). Much of the effort has been focused on using temperature or chemicals as a means to control the gene expression systems. Temperature-dependent expression systems have been previously engineered by the direct fusion of a heat-inducible promoter with a gene of interest(7), or by using a temperature sensitive allele of GAL80, $GAL80^{ts}$. In the GAL4/UAS system, $GAL80^{ts}$ efficiently suppresses GAL4-induced gene expression at low temperature (18 °C) but not at high temperature (29 °C)(5). Chemical-dependent tools include tetracycline-inducible systems(8, 9), steroid hormone-inducible GAL4/LexA hormone receptor chimeras(4, 10, 11) and the quinic acid-inducible QS/QF/QUAS system(3). For example, an RU486-inducible GAL4 was made by fusing the GAL4 DNA-binding domain to the human progesterone receptor and the p65 transcriptional activation domain(10). However, there are limitations associated with the existing tools for temporal control of gene expression. First, temperature can significantly alter a fly's physiology and behavior(12, 13), introducing confounding factors that cannot be directly addressed by additional control experiments. Moreover, these tools are unlikely to be suitable for the study of thermosensory circuits and related behaviors. Second, the utility of the current chemical-dependent tools is limited by the need to generate new transgenic stocks, such as new promoter-GAL4 lines. Additionally, the RU486-inducible Geneswitch system has been reported

to have developmental lethality associated with pan-neuronal induction of elav-Geneswitch at low dosage of RU486(14).

We propose an alternative chemically inducible system, in which gene expression is controlled at the post-translational stage, making it compatible with the existing library of GAL4 stocks. We adopted the destabilizing domain (DD) derived from dihydrofolate reductase (ecDHFR) of *E. coli* to control protein stability in a ligand-inducible manner(15, 16), a strategy that has been used to control gene expression in mice and worms previously(15–17). On fusing the destabilizing domain to a protein of interest, the chimeric protein is degraded by the proteasome, but its degradation is blocked by trimethoprim (TMP), a cell-permeable ligand (Fig. 1A)(15). Thus, the protein of interest can be temporally controlled by TMP. In this study, we optimized this technology and characterized its efficiency and dynamics *in vivo* in the fly brain. As a proof of its utility, we fused DD to GAL80 and chemically controlled GAL4-derived expression in a TMP-dependent manner. Additionally, by fusing DD to the FLP recombinase, we devised a strategy to control the recombination frequency within a neuronal population by controlling the concentration of TMP in fly food. We further used the destabilized FLP recombinase to refine the expression pattern arising from the intersection of two transgenic lines by temporally limiting the availability of TMP. Finally, we tested the effect of TMP on survival and behavior, and demonstrated that the DD system can be used to chemically manipulate neuronal activity of behaviorally relevant sensory neurons.

Results

Destabilized GFP. We first tested whether the ecDHFR-derived destabilizing domain (DD) can be used to control GFP expression levels. DD was genetically fused to the C-terminus of GFP and cloned into a 10XUAS construct (Fig. 1A, S1) to make *UAS-GFP-DD* transgenic flies. We expect that GFP expression should be conditioned on both the presence of the transcriptional

activator GAL4 and the availability of the stabilizing ligand TMP. The expression of GAL4 in select neuronal populations provides spatial specificity. Feeding these flies with TMP at a specific time could achieve temporal control of GFP expression.

Using the pan-neuronal *nsyb-GAL4* to drive GFP-DD expression, we observed robust GFP expression throughout the brain of adult flies fed with TMP (Fig. 1B). In the absence of TMP, GFP expression was low throughout the brain; this is consistent with the previous studies in mice and nematodes showing that DD is an effective tag to mark the fused protein for degradation(16, 17) (Fig. 1B). We next carried out experiments to determine the kinetics and dynamic range of this chemical induction system using *Orco-GAL4* to drive GFP-DD expression in olfactory sensory neurons. Feeding flies with food containing varying concentrations (0-5 mM) of TMP for 48 hours resulted in a dose-dependent change in GFP expression in the adult antennal lobe (Fig. 1C, D). The maximum GFP expression, induced by 1 mM TMP, was 34 times higher than that of control flies fed with the solvent-containing food (Fig 1D). Results from an experiment in which flies were fed for varying duration (0-60 hrs) with food containing 1 mM TMP show that GFP levels increase initially but reach saturation within 36 hours (Fig. 1E, F). To test if TMP-dependent GFP expression is reversible, we fed flies with food containing 1 mM TMP for 48 hours and then switched them to regular food (Fig. 1G). We observed that 73% of the initial GFP was degraded within 24 hours (Fig. 1H). In sum, using GFP as a test molecule, we show that genetic fusion of the ecDHFR-derived destabilizing domain confers instability to a protein of interest in *Drosophila*. Feeding flies with TMP can control protein levels in a reversible and dose-dependent manner with a large dynamic range.

We then investigated whether the DD system is effective across different tissues and developmental stages. First, we observed TMP-dependent GFP expression in the larval brain (Fig. S2A). Second, similar results were obtained when GFP-DD was expressed selectively in olfactory sensory neurons, projection neurons, ovaries or the foreleg (Fig. S2B-E). Overall, we

show that the destabilization domain can be used in conjunction with the GAL4/UAS expression system. As TMP-dependent protein stabilization acts through post-translation modification of protein levels, it should also be compatible with other expression systems (LexA/LexAop, QF/QUAS). As a proof-of-principle, we generated a *13XLexAop-GFP-DD* transgenic fly line and observed similar TMP-dependent GFP expression in olfactory sensory neurons using the *Orco-LexA* driver line (Fig. S2F).

Destabilized GAL80. We next investigated whether expression of GAL80 could be controlled by TMP. Binding of GAL80 to GAL4 prevents GAL4-mediated transcriptional activation in the GAL4/UAS expression system(18). We engineered a chemically inducible GAL80 by fusing DD to the C-terminus of GAL80. GAL80-DD was cloned downstream of a pan-neuronal promoter, *n-synaptobrevin* (*nsyb*). Addition of GAL80-DD to the GAL4/UAS expression system could allow TMP to control gene expression. Indeed, we found that *nsyb-GAL80-DD* was able to suppress GAL4-dependent GFP expression in olfactory sensory neurons (Fig. 2A, B). This suppression of GFP expression in flies carrying the *Orco-GAL4*, *UAS-GFP* and *nsyb-GAL80-DD* transgenes was TMP-dependent (Fig. 2B). This feature can be used to control gene expression to perturb neuronal function in a stage-dependent manner. For example, RNAi expression could be targeted to specific neurons during the adult stage by removing TMP from the food, which causes the degradation of GAL80. As a proof-of-concept experiment, we fed flies with TMP throughout development and up to 3 days post eclosion (Fig. 2C, D). When flies were moved from TMP-containing food to regular food, GFP expression started to increase after 24 hours, and peaked at 72 hours post-TMP removal (Fig. $2C_1$, D_1). In contrast, flies fed with TMP continuously, from embryo to adult, showed low GFP expression throughout the course of the experiment (Fig. $2C_2$, D_2). Furthermore, flies that were raised on regular fly food throughout developmental and adult stages showed high GFP expression (Fig. $2C_3$, D_3). Together, these results suggest that fusion of the ecDHFR-derived destabilizing domain to GAL80 permits TMP

to control the GAL80 level, providing a chemically inducible system to control gene expression in a temporal manner.

Destabilized flippase. Flippase-mediated removal of a stop cassette has been widely used for lineage analysis and sparse neuronal labeling(18–20). Lineage analysis requires a transient high-level of flippase (FLP) at specific developmental stages. On the other hand, sparse neuronal labeling requires low-level FLP expression in post-mitotic neurons for the stochastic removal of a stop cassette. Owing to the large dynamic range of the DD system, we reasoned it could be used to control FLP expression at different levels by varying TMP concentrations in fly food, thereby accommodating both sparse labeling and lineage mapping. The heat-shock promoter has been used previously to drive different levels of FLP expression by varying the duration of the heat-shock pulses. However, heat-shock driven FLP activity cannot be limited to a subset of cells due to the ubiquitous expression of the heat shock promoter. This limitation restricts the utility of *hs-FLP* for lineage analysis in an intersectional manner.

We fused DD to the C-terminus of FLP and incorporated the coding sequence into a 10XUAS construct (*10XUAS-FLP-DD*). We tested the destabilized flippase in olfactory projection neurons using *GH146-GAL4* to drive *UAS-FLP-DD* and a GFP stop-cassette reporter, *UAS(FRT.STOP)CD8GFP*. In these flies, stabilization of FLP by TMP should permit FLP-mediated excision of the stop cassette, resulting in GFP expression. We observed that GFP expression in olfactory projection neurons was correlated with TMP dosage (Fig 3A, B). By varying the concentration of TMP (0.01 – 1 mM) in fly food, we could control the number of labeled projection neurons (Fig 3A). Furthermore, there were similar numbers of labeled neurons in both brain hemispheres for a given sample (Fig. 3B). For flies fed with standard fly food without TMP, 42% of the brain hemispheres had only one GFP-positive cell (Fig. 3C). This feature of FLP-DD can be used to generate single-cell clones at a reasonable probability for connectomics applications. As a proof-of-concept, we analyzed GFP-labeled neurons in the

brains of 36 flies fed with solvent only. Out of 72 brain hemispheres, 30 had only a single GFP-positive projection neuron (see Fig. 3D for examples). In summary, dose-dependent expression of FLP-DD can be effectively used to control the density of genetically manipulated cells within a population.

Restricting the activity of FLP-DD in a spatial and temporal manner should further refine expression patterns which arise from intersection of two expression systems (For eg. GAL4/UAS and QF/QUAS). To illustrate this principle, we focused on the intersection between *GH146-QF* and *NP21-GAL4*. It has been previously reported that the expression patterns for *NP21-GAL4* and *GH146-GAL4* overlap only in the DA1 lateral projection neurons (lPNs) in the adult brain(3), which we validated (Fig. $4A_1$,A_2). However, when UAS-FLP expression is driven by *NP21-GAL4*, the adult intersection pattern includes additional olfactory projection neurons, ellipsoid body neurons and neurons with cell bodies close to the lateral horn (Fig. $4B_1$,B_2). Similarly, when *QUAS-FLP* is driven by *GH146-QF*, the adult intersection pattern includes additional visual projection neurons (Fig $4C_1$,C_2). This discrepancy between the overlap and the intersection patterns arises because of the broader expression patterns for *GH146-QF* and *NP21-GAL4* before the adult stage. Thus, the stop cassette is prematurely excised during development in neurons outside of the overlapping adult pattern. We reasoned that the adult expression pattern can be recapitulated by limiting FLP-DD expression to the adult stage using TMP. Indeed, when *UAS-FLP-DD* is expressed by *the NP21-GAL4* line and 1 mM TMP is fed to flies only during the adult stage, GFP expression is limited only to DA1 lPNs in the whole brain (Fig $4D_1$,D_2). In comparison, flies fed with solvent alone did not have GFP expression in any neurons in the brain (Fig $4E_1$,E_2). On the other hand, flies fed with 1 mM TMP throughout development have GFP expression in additional olfactory projection neurons (Fig $4F_1$,F_2). We noted that the expression pattern in *UAS-FLP-DD* flies fed with 1mM TMP throughout development was a subset of that observed with *UAS-FLP* flies (Fig. 4B, F). It is possible that

TMP levels decline in the fly brain during metamorphosis after the larvae stop feeding. In fact, similar results have been observed in the context of RU486-induced FLP activity(21). To mitigate this potential decline of TMP, we fed flies with 10 mM TMP throughout the larval stage and obtained a large portion of the *UAS-FLP* expression pattern (Fig. 4B, S3). In sum, we show that TMP can be used to limit FLP-DD activity temporally in a way such that the intersection pattern is identical to the overlap in the adult expression patterns. In a previous study, an RU486-inducible FLP recombinase was constructed by fusing it with the human progesterone receptor (Flp-Switch)(21). Although this construct can be chemically induced similar to FLP-DD, further experiments will be required to compare the efficacy and dose-dependency of the two recombinases.

We further investigated whether TMP has adverse effects on survival or behavior. Feeding adult flies with a defined medium containing TMP at 1 mM, a saturating dosage for GFP-DD induction, did not have any detectable effect on their survival (Fig. 5A). Furthermore, feeding adult flies with 1 mM TMP for 48 hours did not alter their locomotion speed (Fig. 5B) or their ability to locate an odor source (Fig. 5C). Finally, we tested if the DD system can be used to manipulate neuronal activity underlying behavior. We focused on the innate olfactory aversion to CO_2 in a T-maze assay. Olfactory aversion to CO_2 can be abolished by silencing Gr21a expressing sensory neurons(22). We chemically controlled the expression of tetanus toxin, a potent inhibitor of synaptic transmission(23), in Gr21a neurons using *nsyb-GAL80-DD* (Fig. 5D). *Gr21a-GAL4* derived tetanus toxin expression was blocked in the presence of GAL80-DD when flies were fed TMP, but not when they were fed the solvent (Fig. 5D). Accordingly, aversion to CO_2 was abolished only when flies expressing GAL80-DD were fed with the solvent, and not when they were fed with TMP (Fig. 5E). Overall, these results demonstrate that TMP is a relatively inert molecule at its working concentrations. Further, TMP can be used in conjunction with GAL80-DD to reversibly manipulate neuronal activity.

Discussion

Here we report a chemically inducible system to control gene expression in a dose-dependent and reversible manner. The DD system broadens the functionality of the *Drosophila* genetic toolkit as it provides an independent axis of control which can be used in combination with existing reagents. The DD system provides several advantages over existing chemical-dependent tools. The dose-dependency of TMP-induced DD stabilization can be exploited for titration of *in vivo* gene expression levels. In contrast to existing chemical tools, GAL80-DD can be combined with existing GAL4 lines to knockdown targeted genes by RNAi or perform neuronal silencing screens in a temporally refined manner. When TMP is withdrawn, the degradation kinetics of the DD fusion protein is most likely faster than that of the native protein. Thus, it is possible that the DD system offers fast temporal control in experiments which require reversible gene expression. Finally, it is worth noting that the cost of TMP is almost 150 times less than RU486 or quinic acid at their respective working concentrations, making the DD system conducive to large scale behavioral screens.

The applicability of the DD system for a given cell type is limited by two factors: 1) the cell type should have an active proteasome; 2) orally-fed TMP should be able to reach the cell. In the absence of TMP, the level of proteasome activity in a given cell type may influence the residual level of a DD chimeric protein. For instance, low proteasome activity may result in high residual levels of the chimeric protein. It may be possible to reduce the residual expression by co-expressing components of the protein degradation machinery, similar to how co-expression of Dicer enhances RNAi efficiency(24). As TMP is a cell-permeable ligand which can cross the blood-brain barrier, it should be accessible to all tissues during the adult and larval stages. However, the effectiveness of TMP during the pupal stage may depend on the expression level of DD protein and the concentration of TMP during the pupal stage. Due to the nature of chemical delivery, the utility of the DD system is also limited to applications which can tolerate

gene expression changes on the time scale of hours. However, it may be possible to achieve faster induction by using photocaged forms of trimethoprim(25). Finally, as trimethoprim is an antibiotic, experiments using the DD system should incorporate appropriate controls to rule out the effect of microbiome manipulation on the phenotype of interest.

In addition to chemically inducible forms of GAL80 and FLP, the DD technology can be used in flies for several other applications. DD can be knocked-in and fused to endogenous proteins to control their expression by limiting TMP feeding. This can be done using custom-designed genome editing strategies or by integration into the large number of available MiMIC sites within coding introns(26). DD can also be fused to a variety of effector genes for the purpose of inducible neuronal silencing or genome editing(27). Due to its inducible nature, GFP-DD can be coupled with knock-in GAL4 lines to compare gene expression in individual cells across time points spanning only a few hours, such as circadian fluctuation of gene expression. GFP-DD may also be useful as a sensor for proteasome activity.

In conclusion, we have developed a set of new tools for chemical control of gene expression in *Drosophila* which has broad-ranging applications and several advantages over existing tools of a similar nature. We characterized its efficiency and temporal limitations, and demonstrated its utility by engineering tools for chemical control of gene expression, recombination and neuronal activity.

Materials and Methods

Fly husbandry

Flies were raised on standard fly food (unless otherwise noted) at 25°C in a 12:12 light-dark cycle. All adult flies were aged for 3-7 days. The following transgenes were used in this study -

nsyb-GAL4(28) (BDSC_51941), Orco-GAL4(29) (BDSC_23292), UAS-GFP, GH146-GAL4(30)(BDSC_30026), UAS-(FRT.STOP)mCD8-GFP(3) (BDSC_30032) and UAS-(FRT.STOP)GFP.myr (BDSC_55810), UAS-6XmCherry-HA(31) (BDSC_52267), QUAS-6xGFP(BDSC_52264)(31), 20XUAS-FLPD5(32)(BDSC_55805), GH146-QF(3) (BDSC_30014), QUAS(FRT.STOP)GFP(3) (BDSC_30134), NP21-GAL4(33) (BDSC_30027), Actin5C-GAL4 (BDSC_4414), Orco-LexAVP16(34), Gr21-GAL4(35), UAS-TNT(23), 10XUAS-GFP-DD (this study), 10XUAS-FLP-DD (this study), nsyb-GAL80-DD (this study), 13XLexAop-GFP-DD (this study). See supplementary information for list of fly genotypes for every experiment and cloning details for generation of new stocks.

TMP feeding

Trimethoprim (Teknova Inc., CA) was maintained as a 100 mM stock solution in DMSO. To prepare food containing TMP for adult flies, standard fly food was heated to a liquid state. After cooling, TMP (or pure DMSO) was added to the food and vortexed to achieve a homogenous mixture of the required concentration. Food was poured into standard fly vials and allowed to solidify. Adult flies were transferred to new vials with TMP-containing food every 3 days. 1% DMSO was found to severely affect survival of larvae. Therefore, to feed flies with TMP from the embryo stage, pure TMP in powder form was mixed in fly food to attain the required concentration. Detailed information on the feeding regimen for every experiment can be found in supplementary information.

Histology

Tissues were dissected in cold PBS and fixed in 4% (w/v) paraformaldehyde for 3 minutes on ice in a microwave. Next, tissues were fixed in 4% (w/v) paraformaldehyde containing 0.25% Triton-X-100 for 3 minutes on ice in a microwave. Fixed tissues were placed in blocking solution (2% Triton X-100, 0.02% sodium azide and 10% normal goat serum in PBS) and

degassed in a vacuum chamber for 6 x 15 mins to expel tracheal air. For the purpose of quantification in Figures 1 and 2, samples were not immunostained. All samples for a given experiment were prepared and imaged in parallel to allow for comparison among them. Rabbit anti-GFP (Invitrogen A-11122, 1:200), mouse anti-bruchpilot nc82 (DSHB AB_2314866, 1:50), mouse anti-HA (Biolegend 901501, 1:500) and rabbit anti-TeTx antibody (POL 016, Statens Serum Institut, 1:1000) were used as primary antibodies in this study. Alexa Fluor 488 anti-rabbit immunoglobulin G (Invitrogen A-31628; 1:100) and Alexa Fluor 647 anti-mouse immunoglobulin G (Invitrogen A-21235, 1:100) were used as secondary antibodies. Brains were incubated in primary antibodies in dilution buffer (1% normal goat serum, 0.02% sodium azide and 0.25% Triton X-100 in PBS) for 48 hours at 4°C, rinsed for 3 x 15 mins in washing buffer (1% Triton X-100, 3% NaCl in PBS), incubated in secondary antibodies in dilution buffer for 24 hours at 4°C, and rinsed again for 3 x 15 mins in washing buffer. Samples were mounted in Focusclear (Cedarlane Labs) between glass coverslips separated by spacer rings.

Samples were imaged with a 10X/0.3 or 20X/0.75 objective using a Zeiss LSM 510 confocal microscope to collect Z-stacks at 2-µm intervals. During the course of an experiment, the laser power and gain were held constant to allow for comparison among images from different experimental conditions. To quantify GFP expression, maximum intensity Z-projections were prepared using ImageJ (NIH). Average fluorescent intensity in the background was subtracted from the sample fluorescent intensity and the result was used as a proxy for GFP expression.

T-maze assay

Flies were raised in standard fly food or food containing 1mM TMP from embryo to adult stages up to the time of the experiment. Behavioral tests were performed as described previously(36). About 30 flies were transferred from food vials into a 15 mL centrifuge tube (Fisher scientific, 14959B) using a funnel. The tube containing the flies was connected to the T-maze apparatus and the flies were transferred into a horizontal elevator in the dark. Flies were held in the

elevator for one minute before being pushed forward to choose between the test and the control arm. A fluorescent lamp was switched on at this point to phototactically draw flies out of the elevator. Flies were given one minute to choose between either arm, following which the elevator was retracted to separate the flies in the test arm from those in the control arm. The tubes serving as the test and the control arms were detached and flies in them were counted manually.

Flies were forced to choose between the control arm containing air and the test arm containing 0.28% CO_2 . 400 μL of 10% CO_2 was injected into the test arm using a syringe (Becton Dickinson, 10 mL). The position of the test arm and the control arm was alternated for each trial. The avoidance index was calculated as (no. of flies in the control arm - no. of flies in the test arm) /(no. of flies in the test arm + no. of flies in the control arm).

Survival assay

Adult flies were raised on a defined medium (1M sucrose, 1% agar) with 1 mM TMP or 1% DMSO from eclosion to death. Each experimental vial contained 5 male and 10 female flies. Flies were transferred to new vials every two days. Number of living flies was recorded every day.

Odor localization and locomotion assay

Odor localization ability and walking speed were measured using a setup described previously(37). Single flies were introduced in custom built chambers (60 mm diameter, 6 mm height) and tracked at 2 Hz under 660nm LED illumination using a custom software written in Labview (V.8.5, National Instruments). Wild type flies were fed with regular fly food containing 1 mM TMP or 1% DMSO for 48 hours before the experiment. The average walking speed of each fly during the first 50 seconds of each trial was determined using a custom macro with Igor Pro (V.6, Wavemetrics, Inc.). To perform the odor localization experiment, flies were transferred to

starvation vials containing water with 1 mM TMP or 1% DMSO in kimwipes (Kimberly-Clark) 24 hours prior to the experiment. 1% apple cider vinegar in low melting agarose was used as the odor source. Latency to localization is defined as the amount of time elapsed before a fly spends more than 5 seconds less than 5 mm from the odor source.

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Author contributions

S.S. and J.W.W. designed the research and wrote the paper. S.S. generated the reagents and performed the experiments detailed in this paper.

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Figure legends

Figure 1

GFP-DD expression and degradation kinetics. (A) Schematic showing the destabilizing domain (DD) system. ecDHFR = *E. coli.* Dihydrofolate reductase, POI = protein of interest, TMP = Trimethoprim. (B) TMP-dependent GFP expression in the adult brain. Flies were fed 1 mM TMP-containing food from embryo stage up to dissection. (C, D) Dose-dependent change in GFP-DD expression in the axonal terminals of olfactory sensory neurons. *Orco-Gal4, UAS-GFP-DD* flies were fed with TMP (0 – 5 mM) for 48 hours before dissection (n=5-6, $p<0.001$, $F=41.37$, one-way ANOVA with Tukey's post-hoc test). (E, F) GFP-DD expression is dependent on duration of TMP feeding. All flies were fed with fly food containing 1 mM TMP (n=5-6, $p<0.001$, $F= 87.34$, one-way ANOVA with Tukey's post-hoc test). (G, H) GFP degradation kinetics. Flies were fed with 1mM TMP for 48 hours and then switched to standard fly food. GFP-DD expression in the antennal lobe was observed at 12-hour intervals following the switch. (n=8-10, $p<0.001$, $F=71.43$, one-way ANOVA with Tukey's post-hoc test). Error bars indicate SEM. Significant differences between conditions ($p<0.05$) are denoted by different letters. Scale bar = 100 μm (B), 50 μm (C, E, G).

Figure 2

Chemically inducible control of GAL4-dependent expression using destabilized GAL80 (nsynaptobrevin-GAL80-DD). (A, B) GAL4-driven GFP expression in olfactory sensory neurons can be suppressed by destabilized GAL80 (*nsyb-GAL80-DD*) in a TMP-dependent manner (n=5, Unpaired t-test, two-tailed, t=-2.590 for A, t=-13.25 for B). (C, D) GAL80-DD can be used to temporally control GFP expression. (C_1, D_1) *Orco-Gal4, UAS-GFP, nsyb-GAL80-DD* flies were fed with food containing 1 mM TMP up to 3 days post-eclosion, following which flies were switched to standard fly food and dissected for quantification. GFP expression was

compared to flies fed with 1 mM TMP throughout (C_2, D_2) or solvent throughout (C_3, D_3) (n=4-5). 0 hour time point in C_1 and C_2 represent the same sample. Error bars indicate SEM. Scale bar = 50 μm (A,B), 25 μm (C).

Figure 3

Chemical control of recombination frequency using destabilized flippase (*10XUAS-FLP-DD*). (A) GFP expression in a sub-population of olfactory projection neurons following excision of the STOP cassette by FLP-DD (B) Number of GFP-positive projection neurons can be controlled by varying TMP dosage. The number of GFP-labeled cells within a sample is similar across both brain hemispheres. (C) Probability of labeling indicated number of projection neurons within a brain hemisphere for flies fed with solvent. 42% of hemispheres had a single GFP-labeled cell. (D) Sample single cell projection neurons labeled using FLP-DD. (Scale bar = 25 μm).

Figure 4

Refining intersection patterns by temporally limiting FLP-DD expression. (A) Z-stack projections showing expression patterns of *GH146-QF* (green) and *NP21-GAL4* (red). Both transgenic lines overlap in a single population of DA1 lPNs (arrow in A_2). Between one to three overlapping neurons can be observed across all samples. Antenna was ablated from the brain sample shown in A_2 to visualize projection neurons in the absence of sensory neuron axon terminals in the antennal lobe. Intersection using constitutively expressed flippase generates expanded patterns with additional expression in other olfactory (B) or visual (C) projection neurons. (D) Limiting FLP-DD expression by feeding 1 mM TMP only during adult stage results in GFP expression only in DA1-lPNs. (E) No GFP expression is observed in the absence of TMP. (F) GFP expression in additional olfactory projection neurons can be observed using FLP-DD if TMP is fed throughout development. (Scale bar = 25 μm).

Figure 5

Effect of TMP on survival and behavior (A) Survival of *Orco-GAL4/UAS-GFP-DD* flies fed with 1 mM TMP or solvent from eclosion to death. 14-15 flies per vial, four vials per condition. Error bars indicate SEM. (B) Walking speed of wild-type (CS) flies fed with 1 mM TMP or solvent for 48 hours prior to assay (n=148-161, Unpaired t-test, two-tailed). Bar indicates median. Whiskers indicate 90% percentile. (C) Percentage of flies reaching the food odor within 10 min. Food odor: 1% apple cider vinegar. (D) Tetanus toxin expression in the V antennal lobe glomerulus of flies fed with 1 mM TMP or solvent. (E) CO_2 avoidance index of flies fed with 1 mM TMP or solvent. One arm of the T-maze contained 0.28% (v/v) CO_2 and the other arm had air. GAL80-DD can rescue CO_2 aversion by suppressing TNT expression in the presence of TMP. n=11 per condition, two-way ANOVA indicated a significant interaction between feeding condition and genotype, $F=23.66$, $p<0.001$. Significant differences between conditions ($p<0.05$) are denoted by different letters (Tukey's post-hoc test). All flies were between 4-7 days old.

Figure S1

(A) Schematic of the *10XUAS-GFP-DD* construct. (B) DNA and protein sequence of the destabilizing domain.

Figure S2

TMP-dependent GFP expression in the larval brain (A), ovaries (B), olfactory projection neurons (C), foreleg (D) and olfactory sensory neurons (E). (F) TMP-dependent GFP expression in olfactory sensory neurons using the LexA/LexAop system. Scale bar = 50 μm (C, E, F), 100 μm (A, B), 150 μm (D).

Figure S3

Intersection pattern between *GH146-QF* and *NP21-GAL4* using FLP-DD in flies fed with 10 mM TMP throughout development.

Figure 1

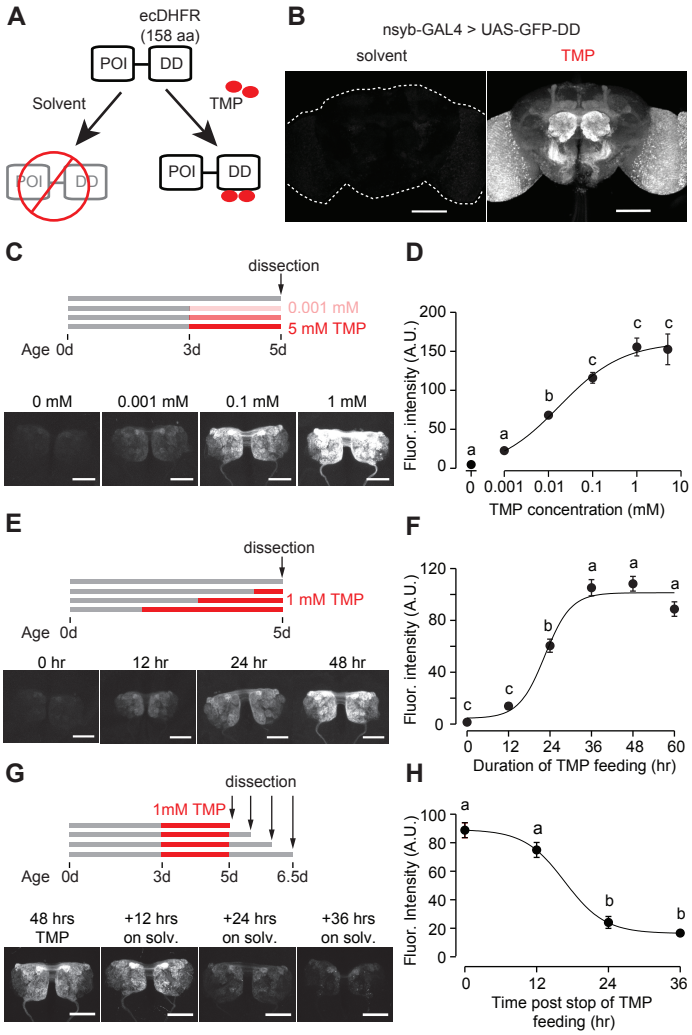


Figure 2

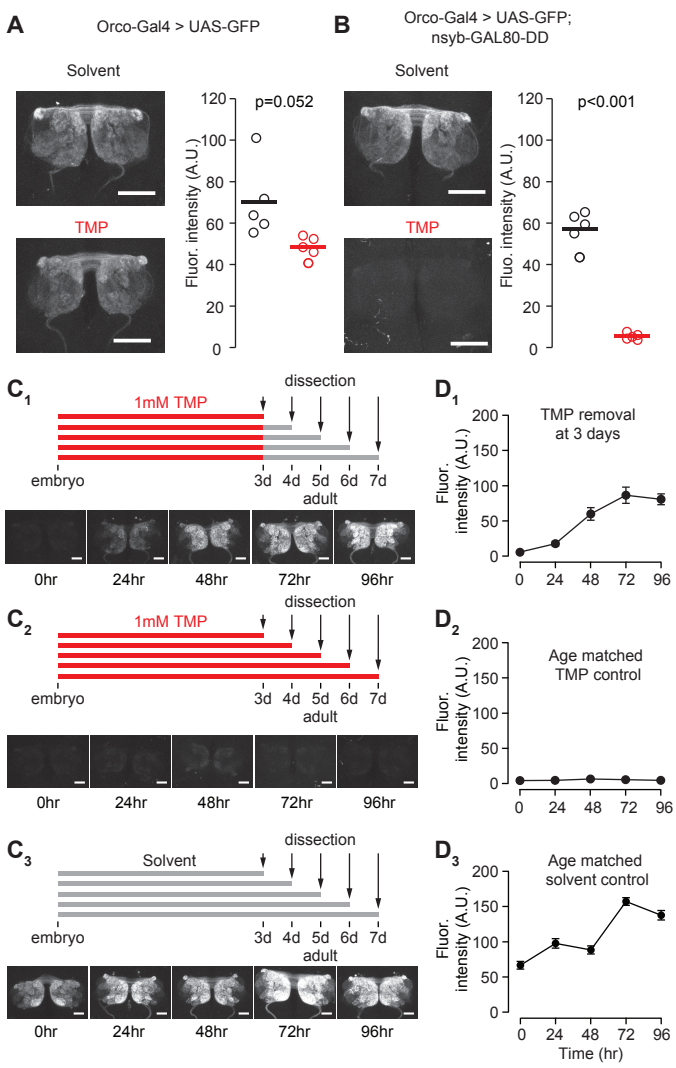


Figure 3

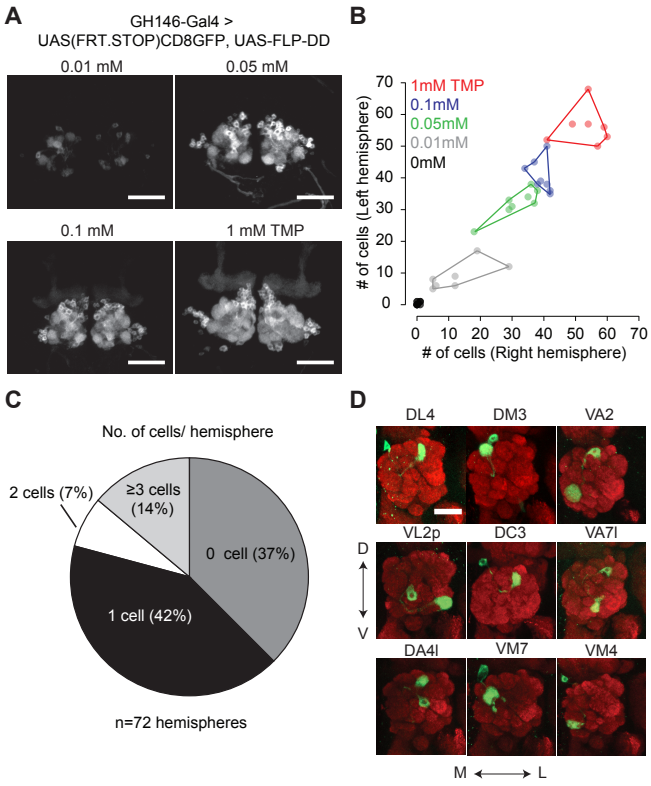


Figure 4

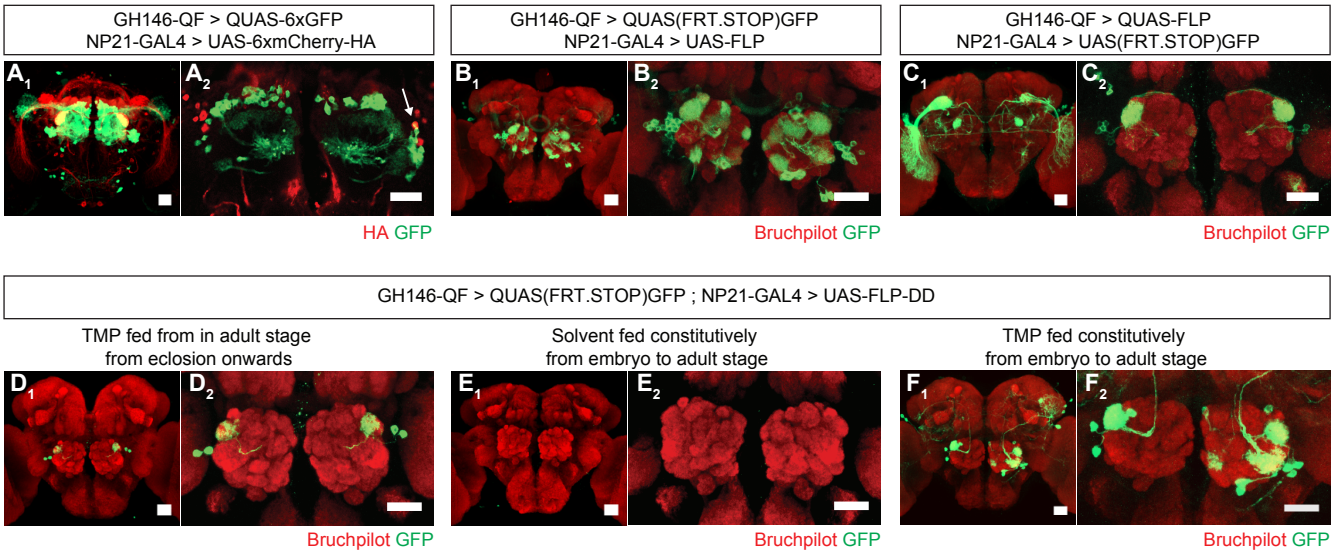


Figure 5

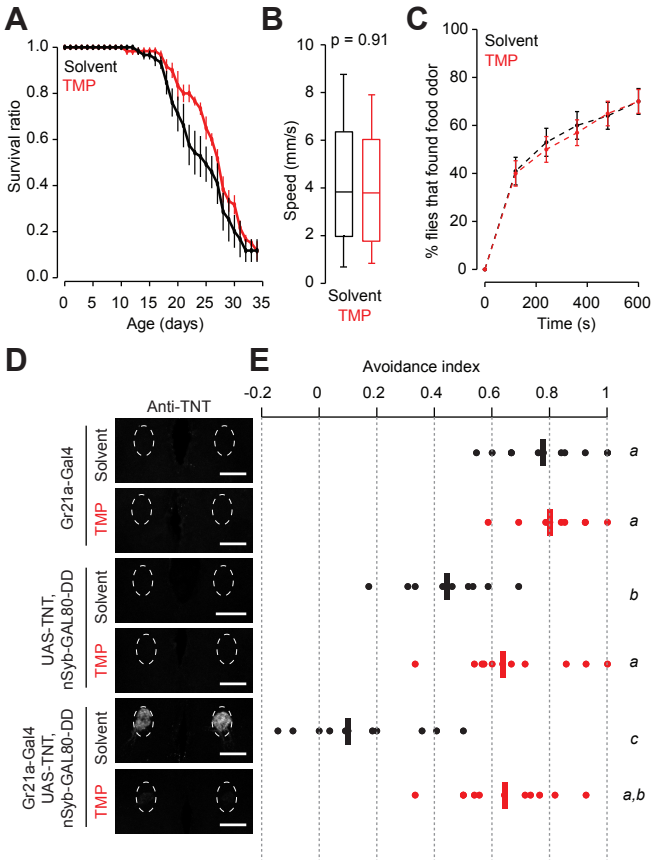


Figure S1

A

POI = Protein of Interest
DD = Destabilizing domain

B

```
          10        20        30        40        50        60        70        80        90       100       110       120
           *         *         *         *         *         *         *         *         *         *         *         *
1 NNNNNNNNNNNNNNNNNNCTCGAGATCTCCCTGATTGCCGCCCTGGCCGTGGACCACGTGATTGGCATGGAAAATGCCATGCCCTGGAACCTGCCCGCCGATCTGGCCTGGTTCAAGCGC 120
  X  X  X  X  X  X  L  E  I  S  L  I  A  A  L  A  V  D  H  V  I  G  M  E  N  A  M  P  W  N  L  P  A  D  L  A  W  F  K  R
                        >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
                        DD
                   >>>>>>
                   Linker
>>>>>>>>>>>>>>>>>>>
C-term of POI

          130       140       150       160       170       180       190       200       210       220       230       240
           *         *         *         *         *         *         *         *         *         *         *         *
121 AACACCTTGGACAAGCCCGTGATCATGGGCCGCCACACTTGGGAAAGCATCGGTCGTCCCTTGCCCGGCCGCAAGGATATCATTCTGAGCAGCCAGCCGTCCACCGACGATCGCGTCACT 240
  N  T  L  D  K  P  V  I  M  G  R  H  T  W  E  S  I  G  R  P  L  P  G  R  K  D  I  I  L  S  S  Q  P  S  T  D  D  R  V  T
>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
DD

          250       260       270       280       290       300       310       320       330       340       350       360
           *         *         *         *         *         *         *         *         *         *         *         *
241 TGGGTGAAGAGCGTCGACGAGGCCATCGCTGCCTGCGGAGATGTCCCGGAGATCATGGTGATCGGAGGAGGACGTGTCTACGAGCAGTTCCTGCCCAAGGCCCAGAAGCTCTACCTGACC 360
  W  V  K  S  V  D  E  A  I  A  A  C  G  D  V  P  E  I  M  V  I  G  G  G  R  V  Y  E  Q  F  L  P  K  A  Q  K  L  Y  L  T
>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
DD

          370       380       390       400       410       420       430       440       450       460       470       480
           *         *         *         *         *         *         *         *         *         *         *         *
361 CATATCGATGCCGAGGTGGAGGGAGATACCCACTTCCCCGACTACGAGCCCGACGGTTGGGAGTCGGTGTTCTCGGAGTTCCACGACGCCGACGCCCAGAACTCGCACTCGTACTGCTTC 480
  H  I  D  A  E  V  E  G  D  T  H  F  P  D  Y  E  P  D  G  W  E  S  V  F  S  E  F  H  D  A  D  A  Q  N  S  H  S  Y  C  F
>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
DD

          490       500
           *         *
481 GAGATTCTGGAGCGCCGTTAA  501
  E  I  L  E  R  R  *
>>>>>>>>>>>>>>>>>>>>
DD
```

Figure S2

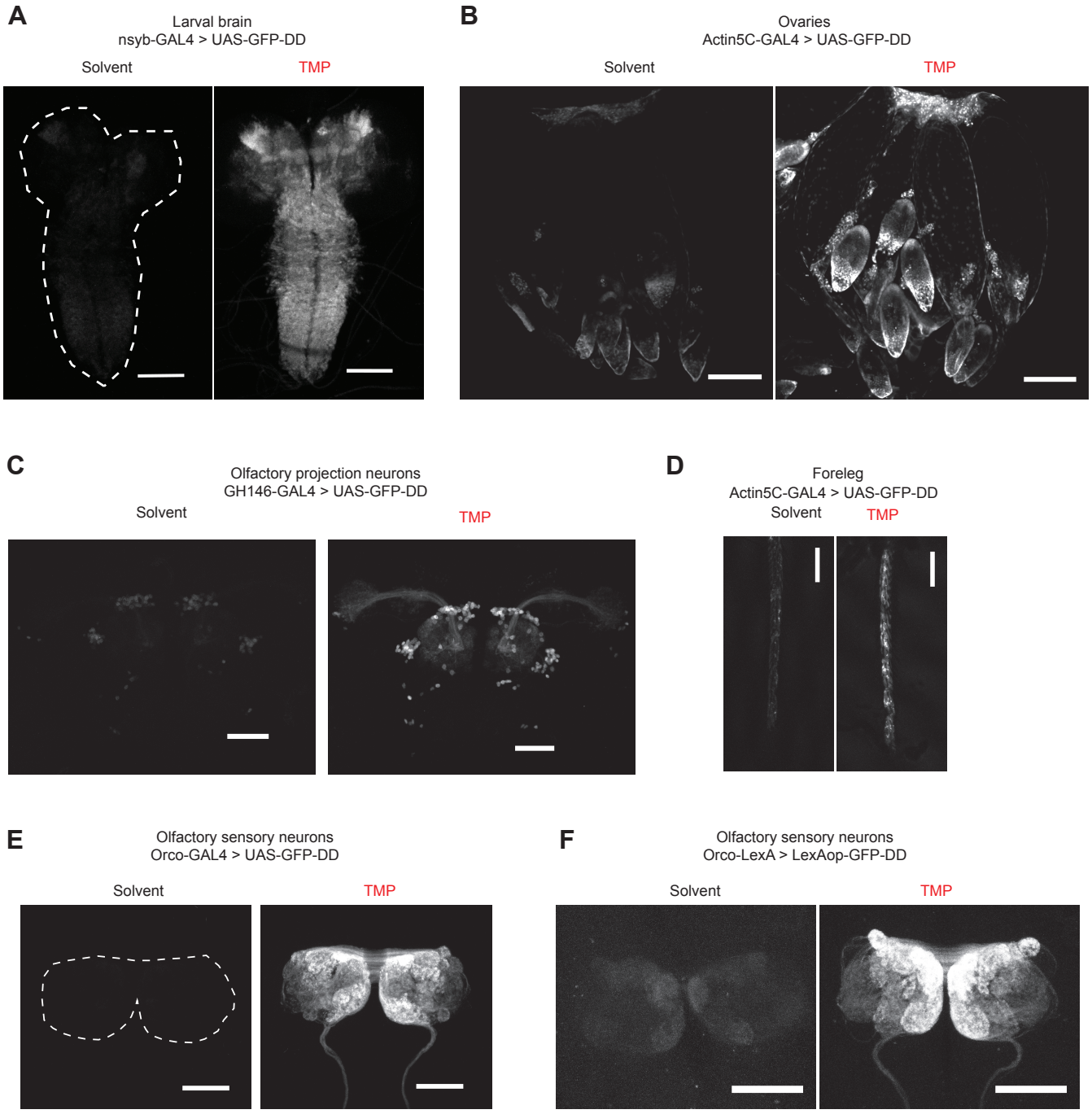
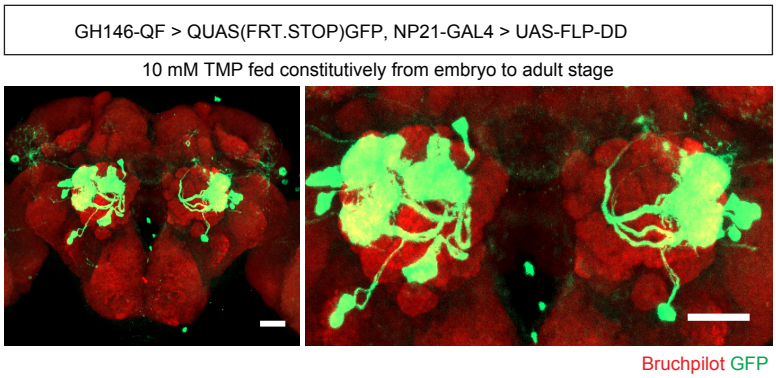


Figure S3



Supplementary methods

Transgenic fly generation

Drosophila codon optimized destabilized domain (DD) was synthesized with 5' *XhoI* and 3' *XbaI* overhangs by Genewiz, Inc. (La Jolla, CA). Plasmids were generated using standard protocols for PCR, restriction digestion and ligation.

Destabilized GFP

To generate the 10XUAS-GFP-DD fly, DD was ligated to the c-terminus of GFP in the pJFRC81 vector (1). GFP was subcloned from the pJFRC81 plasmid using primer P1 and P2. DD was ligated to the c-terminus of GFP using the *XhoI* cut site. GFP-DD was ligated into the pJFC81 vector between the *PshAI* and *XbaI* cut sites. To generate the 13XLexAop2-GFP-DD fly, GFP-DD was cut from the 10XUAS-GFP-DD and ligated to the pJFRC95 plasmid(1) between the *NotI* and *XbaI* sites. Both GFP-DD constructs were transformed using phiC31 integrase mediated recombination into the attP2 landing site(2) by Genetic Services Inc. (Cambridge, MA).

Destabilized GAL80

To generate the nsyb-GAL80-DD fly, DD was ligated to the c-terminus of GAL80. GAL80 was subcloned with 5' *EcoRI* and 3' *XhoI* overhangs from pAC-GAL80 plasmid (Addgene #24346) using primers P3 and P4. DD was subcloned from the 10XUAS-GFP-DD plasmid with 5' *XhoI* and 3' *AatII* overhangs using primers P5 and P6. GAL80-DD was triple ligated between *EcoRI* and *AatII* sites in the cut nsyb-GAL4-hsp70 plasmid (Addgene #46107)(3). The resulting construct was transformed using phiC31 integrase mediated recombination into the VK00005 landing site(2) by Genetic Services Inc. (Cambridge, MA).

Destabilized FLP

The 10XUAS-FLP-DD plasmid was generated by ligating DD to the c-terminus of FLPD5. FLPD5 was subcloned with 5' *NotI* and 3' *XhoI* overhangs from pCaSpeR-DEST5 (DGRC #1031) using primers P7 and P8. FLP was ligated between the *NotI* and *XhoI* sites in the cut 10XUAS-GFP-DD plasmid. The construct was transformed using phiC31 integrase-mediated recombination into the attP2 landing site by Bestgene Inc. (Chino Hills, CA).

Primers

Primer No.	Sequence
P1	GGAGTAGTCCCGATATTGGTTG
P2	TTCATCTCGAGCTTGTAGAGCTCATCCATGCCGT
P3	ATCATCGACAGCCGAATTCCAACATGGACTACAACAAGAGATCTTCG
P4	GCGGCAATCAGGGAGATCTCGAGTAAACTATAATGCGAGATATT
P5	CTGGTTTCCAAACTGATCGGTC
P6	CGACGGTATCGATAGACGTCTATTAACGGCGCTCCAGAATCTCGAA
P7	TACTTCAGGCGGCCGCGGCTGGAGGGTACCAACTTAAAAAAAAAAATCAAAATG CCACAATTTGATATATTATGT
P8	ATCAGGGAGATCTCGAGTATGCGTCTATTTATGTAGGATG

Table of genotypes and feeding condition

Figure	Genotype	Feeding condition
1B	*w⁻ ; + ; nsyb-GAL4/10XUAS-GFP-DD*	Flies fed with standard fly food or food containing 1mM TMP from embryo stage to adult up to dissection
1C, D	*w⁻ ; + ; Orco-GAL4/10XUAS-GFP-DD*	Flies fed with standard fly food containing 0 – 5 mM TMP for 48 hours
1E, F	*w⁻ ; + ; Orco-GAL4/10XUAS-GFP-DD*	Flies fed with standard fly food containing 1 mM TMP for 0 - 60 hours
1G, H	*w⁻ ; + ; Orco-GAL4/10XUAS-GFP-DD*	Flies fed with standard fly food containing 1 mM TMP for 48 hours, and then moved to standard fly food without TMP for 0-36 hours
2A	*w⁻ ; UAS-GFP/+ ; Orco-GAL4/+*	Flies fed with standard fly food or food containing 1 mM TMP from embryo stage to adult up to dissection
2B	*w⁻ ; UAS-GFP/+ ; Orco-GAL4/nsyb-GAL80-DD*	Flies fed with standard fly food or food containing 1 mM TMP from embryo stage to adult up to dissection
2C, D	*w⁻ ; UAS-GFP/+ ; Orco-GAL4/nsyb-GAL80-DD*	C1, D1: Flies fed with food containing 1mM TMP from embryo stage to 3 days post eclosion. Flies were then transferred to standard food without TMP up to dissection. C2, D2: Flies fed with standard food from embryo stage to adult up to dissection. C3, D3: Flies fed with food containing 1mM TMP from embryo to adult up to dissection.
3A, B	*w⁻ ; GH146-GAL4/+ ; UAS(FRT.STOP)mCD8GFP/ 10XUAS-FLP-DD*	Flies fed with standard fly food containing 0 - 1mM TMP from embryo stage up to dissection.
3C,D	*w⁻ ; GH146-GAL4/+ ; UAS(FRT.STOP)mCD8GFP/ 10XUAS-FLP-DD*	Flies fed with standard fly food without TMP from embryo stage up to dissection.
4A	*w⁻; GH146-QF/UAS-6xmcherry-HA; NP21-GAL4/QUAS-6xGFP*	Flies fed with standard fly food from embryo stage up to dissection
4B	*w⁻; GH146-QF/ QUAS(FRT.STOP)mCD8GFP ; NP21-GAL4/ UAS-FLP*	Flies fed with standard fly food from embryo stage up to dissection
4C	*w⁻; GH146-QF/ UAS(FRT.STOP)GFP.myr; NP21-GAL4/ QUAS-FLP*	Flies fed with standard fly food from embryo stage up to dissection
		D: Flies fed with standard fly food without TMP from embryo stage up to eclosion. After eclosion, flies were switched to

4D-F	*w⁻; GH146-QF/ QUAS(FRT.STOP)mCD8GFP ; NP21-GAL4/ UAS-FLP-DD*	food containing 1mM TMP for 4-5 days before dissection. E: Flies fed with standard fly food from embryo stage up to dissection F: Flies fed with fly food containing 1mM TMP from embryo stage up to dissection.
5A	*w⁻ ; + ; Orco-GAL4/10XUAS-GFP-DD*	Flies fed with 1mM TMP or 1% DMSO in 1M sucrose, 1% agar from eclosion to death
5B	Wild-type (Canton-S)	Flies fed with 1mM TMP or 1% DMSO in standard fly food for 48 hours before behavioral assay.
5B,C	Wild-type (Canton-S)	Flies fed with 1mM TMP or 1% DMSO in standard fly food for 24 hours, following which flies were transferred to starvation vials containing water with 1mM TMP or 1% DMSO for 24 hours before behavioral assay.
5D, E	*w⁻/+ ; Gr21a-GAL4/+ ; +* *w⁻/+ ; UAS-TNT/+ ; nsyb-GAL80-DD/+* *w⁻/+; Gr21a-GAL4, UAS-TNT/+; nsyb-GAL80-DD/+*	Flies fed with standard fly food or food containing 1mM TMP from embryo stage to adult up to behavioral assay
S2A	*w⁻ ; + ; nsyb-GAL4/10XUAS-GFP-DD*	Flies fed with standard fly food or food containing 1mM TMP from embryo to larval stage up to dissection.
S2B	*w⁻; Actin5C-GAL4/+ ; 10XUAS-GFP-DD/+*	Flies fed with 1mM TMP or 1% DMSO in standard fly food for 48 hours before dissection.
S2C	*w⁻ ; GH146-GAL4/+ ; 10XUAS-GFP-DD/+*	Flies fed with 1mM TMP or 1% DMSO in standard fly food for 48 hours before dissection.
S2D	*w⁻ ; Actin5C-GAL4/+ ; 10XUAS-GFP-DD/+*	Flies fed with 1mM TMP or 1% DMSO in standard fly food for 48 hours before dissection.
S2E	*w⁻; + ; Orco-GAL4/10XUAS-GFP-DD*	Flies fed with standard fly food or food containing 1mM TMP from embryo to adult stage.
S2F	*w⁻; + ; Orco-LexA/13xLexAop-GFP-DD/+*	Flies fed with 1mM TMP or 1% DMSO in standard fly food for 48 hours before dissection.
S3	*w⁻; GH146-QF/ QUAS(FRT.STOP)mCD8GFP ; NP21-GAL4/ UAS-FLP-DD*	Flies fed with fly food containing 10mM TMP from embryo stage up to dissection.

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