Using the Q system in *Drosophila melanogaster*

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In *Drosophila*, the GAL4/UAS/GAL80 repressible binary expression system is widely used to manipulate or mark tissues of interest. However, complex biological systems often require distinct transgenic manipulations of different cell populations. For this purpose, we recently developed the Q system, a second repressible binary expression system. We describe here the basic steps for performing a variety of Q system experiments *in vivo*. These include how to generate and use Q system reagents to express effector transgenes in tissues of interest, how to use the Q system in conjunction with the GAL4 system to generate intersectional expression patterns that precisely limit which tissues will be experimentally manipulated and how to use the Q system to perform mosaic analysis. The protocol described here can be adapted to a wide range of experimental designs.

INTRODUCTION

Binary expression is a powerful strategy for regulating the expression of an effector transgene for the purpose of investigating the development or function of cells and tissues in multicellular organisms. In such a strategy, one transgene contains a specific promoter driving an exogenous transcription factor, whereas the other transgene uses a promoter that is specifically activated only by the introduced transcription factor. An additional layer of control is afforded if the transcription factor itself can be specifically inhibited by an exogenous element. The yeast GAL4 system is such a repressible binary expression system, and it has revolutionized experimental manipulations in flies[1,2]. The GAL4 transcription factor binds to an upstream activation sequence (*UAS*) to induce expression of a reporter transgene (*UAS-geneX*) (where *geneX* is any gene of interest). Only when GAL4 and *UAS-geneX* are in the same animal is *geneX* expressed in the GAL4 expression pattern. Thousands of GAL4 lines have been characterized for tissue and developmental expression patterns in *Drosophila*, and can be used in combination with thousands of effector lines. Effector lines range from cell markers (e.g., membrane-tagged GFP) to signaling molecules (e.g., activated Ras) to inhibitory molecules (e.g., neurotoxins or RNAi constructs). Furthermore, GAL4 activity can be inhibited by GAL80, a natural suppressor of GAL4[3]. Thus, when GAL80 is co-expressed with GAL4, *UAS-geneX* reporters are silent. This allows for further effector refinement, including the Mosaic Analysis with a Repressible Cell Marker (MARCM) technique[3]. The combination of the three GAL4 components (GAL4, *UAS-geneX* and GAL80) allows for a rich diversity of experimental investigations.

Nonetheless, the GAL4 system has its limitations. *UAS-geneX* effectors can only be expressed in the single population of cells defined by GAL4. In complex cellular organisms, it is often desirable to express an effector in a fraction of a cellular population, and then examine the effects on the other population of cells. Likewise, one might want to differentially label and manipulate two different types of tissues—neurons labeled with GFP and glia labeled with red fluorescent protein (RFP). Such techniques would be invaluable for determining non-cellular autonomous effects (such as ligand/receptor interactions).

We have recently characterized the Q system for these and other purposes[4]; the protocol described here is based on this previous work. The Q system uses genes from the *qa* gene cluster of the filamentous fungus *Neurospora crassa*. This gene cluster, consisting of seven genes, is required for the catabolism of quinic acid (quinate) under conditions of limited glucose levels[5–9]. This gene cluster contains two regulatory genes: *qa-1f* (encoding a protein of 816 aa) and *qa-1s* (encoding a protein of 918 aa). qa-1f (shortened as QF hereafter) is a transcription factor, and qa-1s (shortened as QS hereafter) is a repressor of QF. The other five genes in the *qa* gene cluster encode enzymes or cofactors required for the catabolism of quinic acid. The promoters for the seven *qa* genes in *Neurospora* contain binding sites for QF, and expression of the *qa* genes can be induced by the QF transcription factor. The binding site for QF is the sequence '5′-GGRTAARYRYTTATCC-3′' (R is A/G, Y is C/T). Under normal growth conditions in which glucose is high, QS binds to and inhibits QF, and prevents expression of the *qa* gene cluster. However, when glucose is limiting and quinic acid is present, quinic acid binds to and inhibits QS. This releases QF from QS suppression, allowing QF to induce expression from the *qa* gene cluster. This results in the expression of the factors required for the catabolism of quinic acid as an energy source. In effect, the catabolite (quinic acid) controls expression of the genes required for its catabolism.

Development of the Q system in *Drosophila*

The Q system introduced into *Drosophila* consists of three components: the QF transcription factor, a *QUAS-geneX* effector and the QS suppressor (**Figs. 1** and **2**). The *QUAS* element contains five QF-binding sites and allows for robust QF-dependent expression of the effector. As such, the Q system contains the same three basic components (QF, *QUAS* and QS) as the analogous GAL4 system (GAL4, *UAS* and GAL80). In addition, the molecule quinic acid can inhibit the QS suppressor in flies fed a diet containing quinic acid (**Figs. 1** and **2**). This allows temporal control of the Q system on treatment with this non-toxic molecule (**Fig. 1**).

Applications of the method

The Q system contains the same basic components as the GAL4 system, and so can be used for the same applications as the GAL4 system: binary expression in a subset of tissues, and refinement of that expression by using the QS inhibitor and MARCM analysis[1,3,10] (**Figs. 2** and **3**). In addition, temporal control of QF activity can

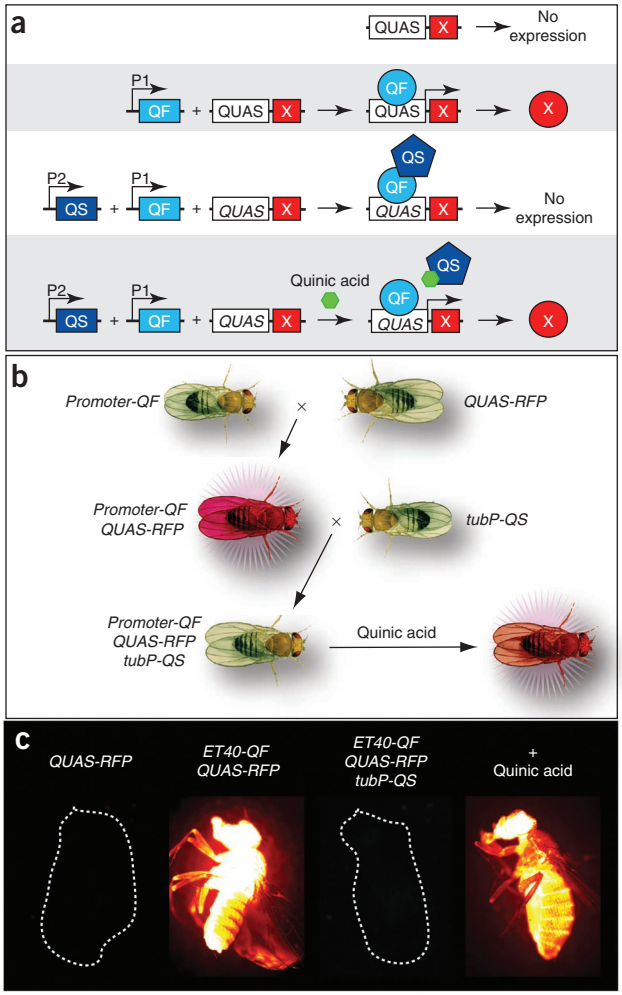

Figure 1 | Schematic and example of Q system components in *Drosophila*. (**a**) Schematic representing the function of the Q system components. P1 and P2 indicate promoter 1 and promoter 2. (**b**) Diagram illustrating a crossing scheme for Q system transgenic flies. tubP indicates the tubulin promoter. (**c**) Transgenic *Drosophila* examples of the genotypes shown in **b**. Transgenic flies not expressing the RFP reporter are outlined by a dashed white circle. The quinic acid–treated flies developed on quinic acid–containing fly food (see Step 6C). Images and schematics are reprinted with permission from reference 4.

be achieved by using quinic acid and QS expression (**Figs. 1c** and **4**). However, a key experimental advantage is obtained when the Q repressible binary system is used in conjunction with the GAL4-repressible binary system. **Figure 2** shows some of the possible applications achievable. Highlighted is the ability to define intersectional expression patterns, whereby finer precision of tissue manipulations can be achieved (**Figs. 5–8**). In addition, the Q system can be used for MARCM analysis (**Fig. 9**), which has a variety of *in vivo* applications[3,10–13]. As the Q system and GAL4 system function independently *in vivo*[4], Q-MARCM and GAL4-MARCM can be coupled to the same mitotic event. As such, an unlabeled progenitor cell would give rise by mitosis to one cell that is positively labeled by the Q system (as it lacks the QS repressor) and a sister cell that is positively labeled by the GAL4 system (as it lacks the GAL80 repressor). This is called 'coupled MARCM', as the segregation of the QS and GAL80 suppressors is coupled to the same mitotic event (**Fig. 10**). This allows for the differential marking and manipulation of all progeny from a single mitotic event. If segregation of the QS and GAL80 suppressor were not coupled to the same mitotic event, then the cell progeny could independently be labeled or unlabeled by the GAL4 or Q system. This is called 'independent double MARCM' (**Fig. 2**).

Comparisons with other methods

Binary expression systems. The bacterial *LexA/LexAop* binary expression system has also been used to express effectors independently of GAL4 (ref. 14). LexA contains a DNA-binding domain specific for the *LexA operator* (*LexAop*), yet it does not contain a transcriptional activation domain. In *Drosophila*, LexA is either fused to the viral acidic activation domain VP16 or the GAL4 activation domain (GAD). The LexA-VP16 protein is insensitive to GAL80, whereas the LexA-GAD protein can be inhibited by GAL80. The LexA/LexAop system does not contain an endogenous suppressor, and hence cannot be used to generate some intersectional expression patterns or for GAL4-independent MARCM analysis. The *LexAop-geneX* reporter also has a higher basal level of expression compared with *UAS-geneX* or *QUAS-geneX* reporters[4]. Nonetheless, recent progress has been made to optimize the LexA/LexAop binary expression system for use *in vivo*[15,16].

Intersectional expression patterns. Limiting GAL4 expression patterns can also be achieved by expressing GAL80 in the tissue of interest[3,17]. However, GAL80 expression patterns are difficult to determine, and GAL80 levels need to be higher than GAL4 for effective suppression. This can make it difficult to precisely define the resulting GAL4 expression pattern. A better approach, as detailed in **Figure 2** and Step 7A of the PROCEDURE, is to use a binary expression system to drive GAL80 expression. Similarly, the LexA/LexAop system could be used to refine GAL4 expression patterns. In this case, *LexA-VP16* would be used to drive *LexAop-GAL80*. However, given the lack of an independent repressor of LexA, the reciprocal experiment (using GAL4 to limit *LexAop-geneX* reporter expression) is not possible. This approach is possible using the Q system (Step 7B, **Fig. 5b**).

Limiting expression patterns to overlapping subsets is also achieved by using the 'split GAL4' method, in which GAL4 is split into two parts—one part containing the DNA-binding domain and the other part containing the activation domain[18]. The two GAL4 components can be reconstituted *in vivo* by the addition of leucine zippers to the split GAL4 proteins. This technique can achieve precise intersectional expression patterns[18]. However, split GAL4 cannot use existing characterized GAL4 lines for intersectional expression, the reconstituted GAL4 is not as robust as the original GAL4, and split GAL4 transgenes are not useful for many other purposes (in contrast to a new QF reagent that can be used for binary expression or MARCM experiments).

Mosaic labeling methods. Coupled MARCM allows the labeling of all progeny from a single mitotic event. It can also be used for independent gain- and loss-of-function genetic manipulations of both progeny. A number of other techniques also allow for the marking of both sister progeny.

'Dual expression control MARCM' uses *LexA-GAD* (the LexA DNA-binding domain fused to the GAL4 activation domain) in conjunction with GAL4-based MARCM to visualize progeny from a cell division[14]. This technique allows labeling of different

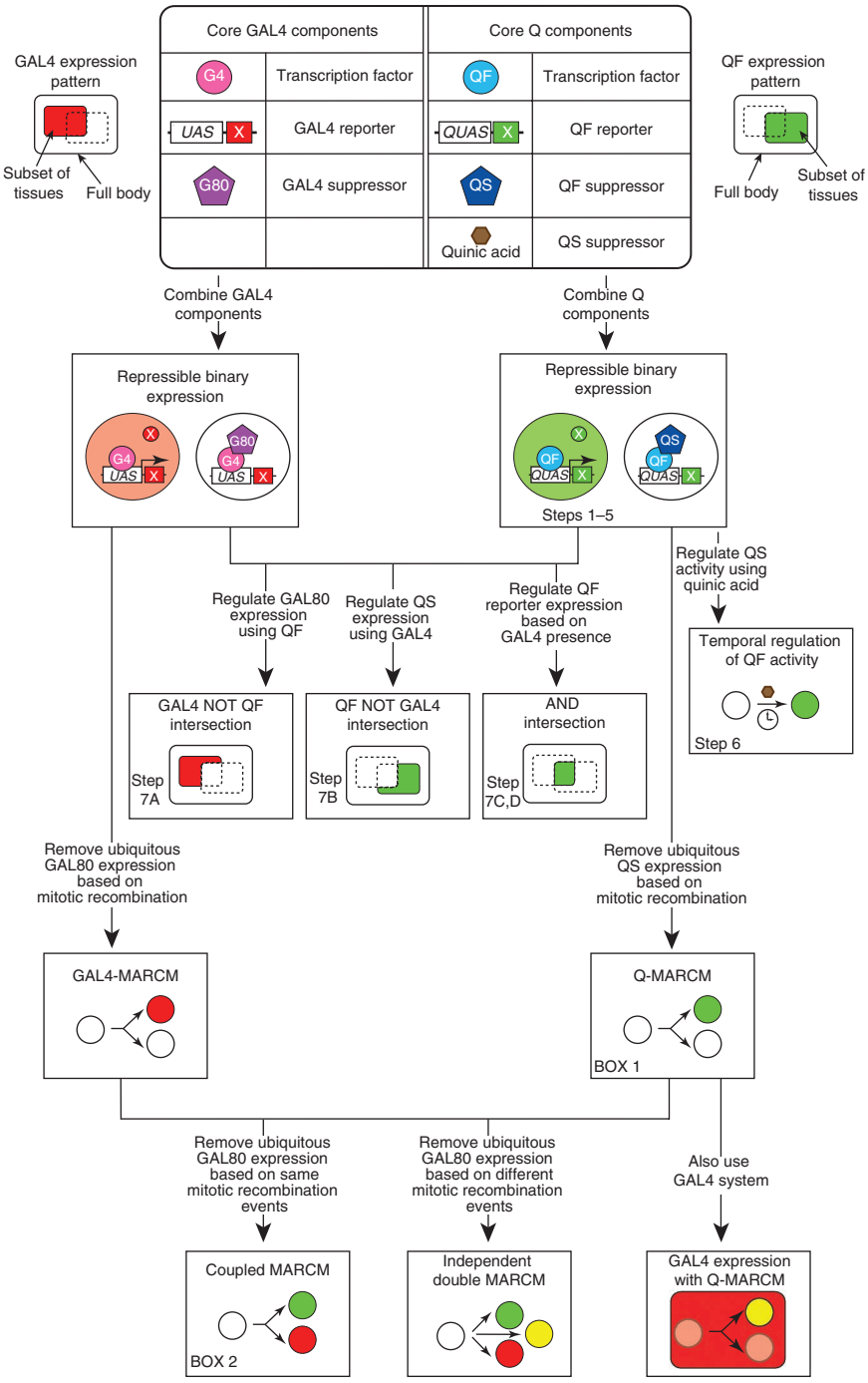

Figure 2 | Flowchart of example GAL4 and Q system applications. The main box illustrates the basic GAL4 and Q system components: the transcription factors GAL4 and QF, the GAL4 and QF reporters *UAS-geneX* and *QUAS-geneX* and the GAL4 and QF suppressors GAL80 and QS. In addition, the Q system includes a small drug inhibitor of QS (quinic acid). The manipulation and combination of these core components (arrows) allow for a number of *in vivo* applications. The PROCEDURE step describing the application is listed.

populations of cells (one labeled by the LexA driver and the other by the GAL4 driver) that arise from a common progenitor. However, as both *LexA-GAD* and GAL4 are suppressed by GAL80, this prevents labeling and manipulation of all progeny from a cell division. This technique has been used successfully for lineage analysis of certain neuronal populations[14,19].

'Twin-spot MARCM' uses UAS-inverse repeat (*UAS-IR*) transgenes as the source of repressors against two different fluorescent proteins. Similar in design to coupled MARCM, which uses the differential loss of *tubP-GAL80* and *tubP-QS* (each driven by the α-tubulin promoter), twin-spot MARCM follows the coupled loss of the *UAS-IR* repressors[20]. This creates two sibling cells, each losing one of the *RNAi repressor* genes. Twin-spot MARCM is simpler in design than coupled MARCM (as it uses fewer transgenes). However, both progeny are labeled by the same GAL4 driver, which could miss labeling of a cell progeny that lies outside this expression pattern. In addition, as the system is based on GAL4 only, cell progeny cannot be independently manipulated. Nonetheless, this technique is a powerful method for resolving the lineage pattern of a GAL4 expression pattern[20,21].

'Twin-spot generator' (TSG) does not use a binary expression system, but instead places two split chimeric fluorescent proteins on the same chromosome arm in *trans*[22]. On FLPase recombination enzyme/FLPase recognition target (FLP/FRT)-mediated recombination, the two fluorescent proteins are reconstituted and can be segregated to daughter cells. This is similar in design to the mouse mosaic analysis system with double markers (MADM) system for mosaic analysis[23]. The advantage of the TSG method over other methods that use a repressible binary system is the ability to examine clones shortly after clonal induction, as there is no perdurance of a repressor molecule. However, a major limitation is low marker expression as a result of the lack of binary system-based amplification. In addition, both markers are driven by a ubiquitous promoter, which severely limits the utility for tracking complex lineages. As TSG does not use a repressible binary system, cell progeny cannot be easily manipulated by effector transgene expression.

Limitations of the Q system. As the Q system has only been recently introduced, a number of Q reagents, such as *QUAS-geneX* effectors or *promoter-QF* lines, remain to be generated. However, as more studies use the Q system, the availability of useful reagents will grow. Alternatively, cases in which the GAL4 system is not sufficient, the LexA/LexAop system could be used if LexA system reagents have already been generated and validated for a tissue of interest, and experimental designs do not require an endogenous LexA suppressor.

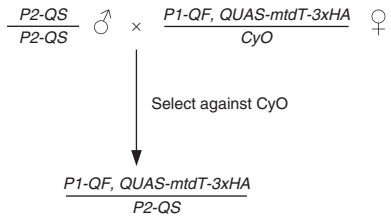

Figure 3 | Crossing scheme for tissue-specific QS suppression of QF. To simplify analysis of QS suppression on a QF-induced expression pattern, the *QUAS-geneX* reporter (*QUAS-mtdT-3xHA*) is recombined with *P1-QF*. Crossing this stable expression line with a *P2-QS* fly and selecting against the CyO balancer will result in progeny that have a subset of tissues no longer expressing the *QUAS-geneX* reporter. This can be directly compared with the original expression pattern.

Experimental design

Generation of QF transgenic flies. The first step for many Q system studies is the generation of Q system reagents for the manipulation of target tissues. The most straightforward approach is to clone a previously characterized enhancer/promoter region of interest into a QF DNA construct. A number of suitable QF DNA constructs are shown in **Supplementary Table 1**. There are two basic choices for cloning QF constructs: *pattB-QF-hsp70* and *pattB-QF-SV40* (pattB refers to a transformation vector that contains an *attB* Phi-C31 recognition sequence and a white$^+$ genetic marker). These constructs differ in their 3′ transcriptional terminators. SV40 terminators lead to increased mRNA stability and higher protein expression. We have found that, in most cases, this increased protein level is not necessary or desirable when generating QF constructs because of the potential toxicity of high QF expression in as-yet-unidentified tissues. We therefore recommend that the *pattB-QF-hsp70* construct be used for routine enhancer and promoter cloning.

There are three basic strategies for generating QF transgenics using previously characterized expression patterns. The first involves the cloning of gene promoters**.** In many cases, an enhancer and promoter region will be the genomic region immediately upstream of the ATG start site of a gene up to the preceding gene[24]. A PCR reaction that introduces flanking BamHI and EcoRI restriction sites can be used to amplify this genomic region for placement into the *pattB-QF-hsp70* construct.

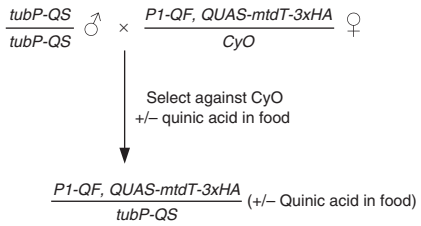

Figure 4 | Crossing scheme for ubiquitous QS-mediated suppression of QF coupled with quinic acid treatment. Ubiquitous QS expression is achieved by using a tubulin promoter to drive QS (*tubP-QS*). Crossing *tubP-QS* with a *P1-QF, QUAS-geneX* recombinant and selecting against the *CyO* balancer will result in a progeny that no longer expresses the *QUAS-geneX* effector in any tissues. This QS-mediated suppression can be inhibited by feeding developing flies quinic acid or by feeding adult flies quinic acid. If treated with quinic acid, the *QUAS-geneX* reporter induced by *P1-QF* will be expressed. Differing levels of QS suppression can be achieved by altering the concentration of quinic acid fed to the flies.

The second strategy to generate QF expression patterns of interest is to clone the genomic region associated with enhancer trap insertions. The expression pattern of an enhancer trap could be mimicked by cloning a large genomic region immediately preceding the insertion site of an enhancer trap[4,11,25]. In this case, a promoter would also need to be included, such as either the P-element promoter or the *Drosophila* synthetic core promoter (DSCP)[26], with the *QF-hsp70* cassette following the cloned genomic region.

When the above two approaches fail to recapitulate the expression pattern of interest, a third strategy is to clone a larger genomic region associated with the gene or enhancer trap insertion. Bacterial artificial chromosome (BAC) recombineering could be used to insert a *promoter-QF-hsp70* cassette into a larger genomic region (20 or 80 kb) to increase the likelihood of recapitulating a complex regulatory locus[27]. These BAC resources are compatible with PhiC31 integration for the generation of transgenic animals. The BAC constructs contain an *attB* site, and by using PhiC31 integrase, they allow

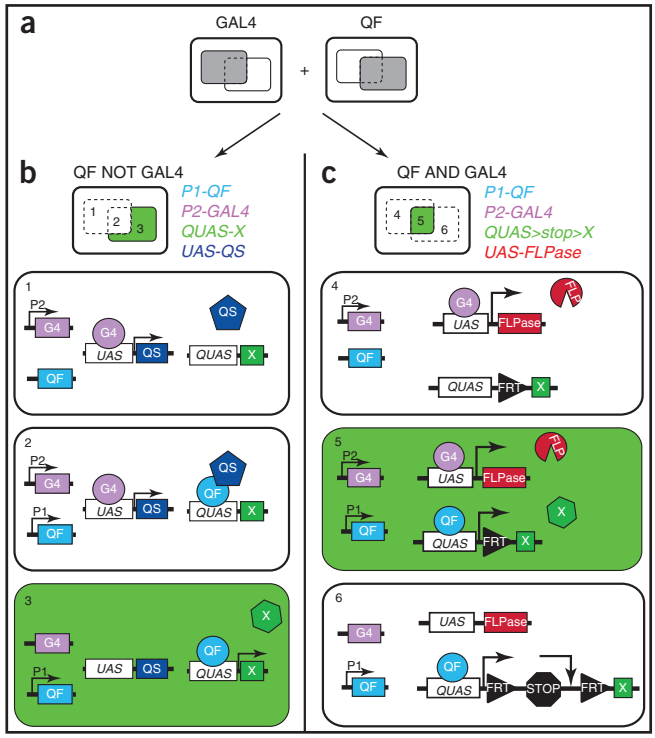

Figure 5 | Using the Q system with the GAL4 system for generating intersectional expression patterns. (**a**) The gray squares represent the extent of the GAL4 or QF expression pattern. (**b**) In the 'QF NOT GAL4' example, a GAL4 line (*P2-GAL4*) is used to drive expression of the QS suppressor (*UAS-QS*) to restrict *QUAS-geneX* expression. This results in a final expression pattern reflecting where QF is expressed but not where GAL4 is also expressed. Region 1 does not express the *QUAS-geneX* as *P1-QF* is not expressed in this region. Region 2 expresses both *P2-GAL4* and *P1-QF* but does not express the *QUAS-geneX* because of the expression of *QS*. Only region 3 expresses the Q*UAS-geneX*. See Step 7B. (**c**) In the 'QF AND GAL4' example, *QUAS-geneX* expression is limited to regions where both QF and GAL4 are expressed. The *QUAS-geneX* contains an '*FRT-transcription stop-FRT*' cassette (*>stop>*) between the *QUAS* promoter and the reporter gene. This cassette can be excised by the activity of the FLPase recombinase. Region 4 does not express the *QUAS>stop>geneX* as QF is not expressed in this region. Region 5 expresses the *QUAS>stop>geneX* as *P2-GAL4* induces *UAS-FLPase* expression, which removes the transcription stop cassette, allowing for *P2-QF*-induced expression. Region 6 does not express the *QUAS>stop>geneX* as *P2-GAL4* is not expressed in this region. '>' indicates *FRT*. See Step 7C.

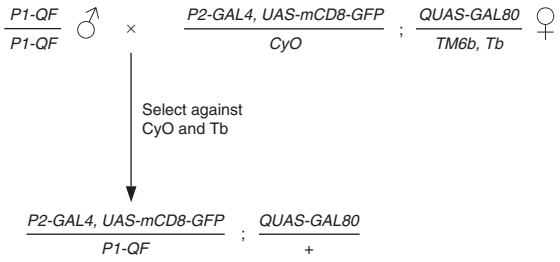

Figure 6 | Crossing scheme for GAL4 NOT QF intersectional experiments. For this NOT intersectional strategy to work, four components (*P1-QF, P2-GAL4, UAS-geneX* and *QUAS-GAL80*) need to be combined into a fly. In this example, a GAL4 NOT intersectional-ready female fly is represented. This fly contains a *P2-GAL4* line recombined with a *UAS-mCD8-GFP* marker, as well as the *QUAS-GAL80* transgene on the third chromosome. Crossing this stock to any QF line and selecting against the balancers will result in progeny that have reduced GAL4 expression based on the QF expression pattern. This simplifies the experimental setup for testing the intersectional results for many different QF lines.

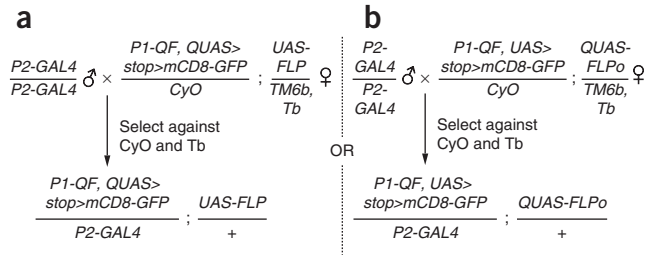

Figure 8 | Crossing scheme for QF AND GAL4 intersectional experiments. There are two strategies to perform an AND intersectional cross. (**a**,**b**) Both strategies require four components to be combined: *P1-QF* and *P2-GAL4* along with (**a**) *UAS-FLP, QUAS>stop>mCD8-GFP* or (**b**) *QUAS-FLPo, UAS>stop>mCD8-GFP*. In these examples, QF AND intersectional-ready flies are shown for each strategy. These AND intersectional-ready female flies contain all the necessary components except for the *P2-GAL4*. Crossing these stocks with any GAL4 line and selecting against the balancers will result in progeny that only have expression where both QF and GAL4 are expressed. These crossing schemes simplify the experimental design required to quickly test many different GAL4 lines for their intersection with a characterized QF line. Although both strategies limit expression to only regions where GAL4 and QF are expressed, they are not equivalent. In **a**, the resulting expression pattern is determined by the developmental expression pattern of the GAL4 line, and the final expression pattern of the QF line. Conversely, in **b**, the resulting expression pattern is determined by the developmental expression pattern of the QF line and the final expression pattern of the GAL4 line.

genomic insertion of large constructs into *attP* sites that have been placed at random locations in the *Drosophila* genome[28,29].

In some cases, generating *promoter-QF* transgenic lines might be difficult, especially for constructs that would result in widespread expression of QF. This could be due to QF being more toxic than GAL4. To reduce *QF* expression (and potential QF toxicity), the QF cDNA has been codon non-optimized for *Drosophila* expression. This allows for the generation of *promoter-QF* constructs that were previously difficult to generate, such as a pan-neuronal *synaptobrevin-QF* (C.J.P., unpublished data). Efforts are also underway to modify the *QF* gene to reduce its toxicity when broadly expressed (C.J.P., unpublished data).

In addition, success rates for generating QF transgenic lines can be improved when using P-element or piggyBac-based vectors instead of attB vectors (C.J.P., unpublished data). Alternative QF coding variants and QF cloning vectors that use piggyBac or P-elements are available from the authors on request.

Generation of QF enhancer trap lines. Enhancer trap lines can often give rise to an expression pattern that is difficult to reproduce by cloning. In addition, a small-scale enhancer trap screen can quickly generate many new expression patterns in parallel. A number of suitable QF enhancer trap DNA constructs are shown in **Supplementary Table 1**. These constructs can be injected with P-element transposase to generate new QF enhancer trap lines.

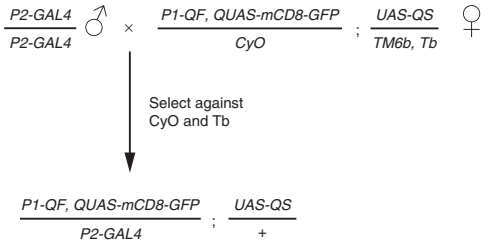

Figure 7 | Crossing scheme for QF NOT GAL4 intersectional experiments. For the NOT intersectional strategy to work, four components (*P1-QF, P2-GAL4, QUAS-geneX* and *UAS-QS*) need to be combined into a fly. In this example, a QF NOT intersectional-ready female fly is represented. This fly contains a *P1-QF* line recombined with a *QUAS-mCD8-GFP* marker as well as the *UAS-QS* transgene on the third chromosome. Crossing this stock with any GAL4 line and selecting against the balancers will result in progeny that have reduced QF expression based on the GAL4 expression pattern. This simplifies the experimental setup for testing the intersectional results for many different GAL4 lines.

Note that the available QF enhancer traps are P-element-based and use an SV40 terminator. Alternative QF enhancer traps that use hsp70 terminators or piggyBac vectors are available from the authors on request.

In addition, existing QF enhancer trap lines (**Supplementary Table 2**) can be mobilized by crossing with a stable P-element transposase (e.g., Δ2–3, Bloomington Stock no. 1798) to generate additional QF lines with new expression patterns. A small screen of ~25 lines has already identified QF enhancer trap lines that label trachea (*ET14-QF*), glia (*ET31-QF*), imaginal discs (*ET40-QF*) and many tissues including neuronal and epithelial (*ET49-QF*) tissues.

QF enhancer traps (and occasionally *promoter-QF* transgenes) can show tracheal expression, especially if the trapped enhancers are weak. This is likely to be due to a cryptic weak tracheal enhancer in the QF coding sequence. Constructs that use QF coding variants (and no longer contain the cryptic tracheal enhancer) show decreased or no tracheal expression in enhancer traps (C.J.P., unpublished data). In addition, *tracheal-promoter-QS* transgenic lines can be used to inhibit tracheal QF-induced reporter expression (C.J.P., unpublished data). These reagents are available from the authors on request.

Generation of *QUAS-geneX* effector lines. Another important Q system reagent is the QF-inducible reporter, *QUAS-geneX*. A number of *QUAS-geneX* transgenic flies are available (**Supplementary Table 2**). To simplify the generation of additional *QUAS-geneX* transgenic flies, the *pQUAST* vector (**Supplementary Table 1**) contains the same multicloning site as the *pUAST* vector (EcoRI-BglII-NotI-SacII-XhoI-KpnI-XbaI), which allows for easy exchange of inserts between *pUAST* and *pQUAST* vectors. If the *pUAST-geneX* plasmid is not available, genomic DNA from flies containing the *UAS-geneX* transgene can be used as the source of the *geneX* insert[4].

By using P-element–based transgenesis[30], many independent insertions of the same *QUAS-geneX* construct will be generated. It is often useful to keep a single transgenic line on each of the three

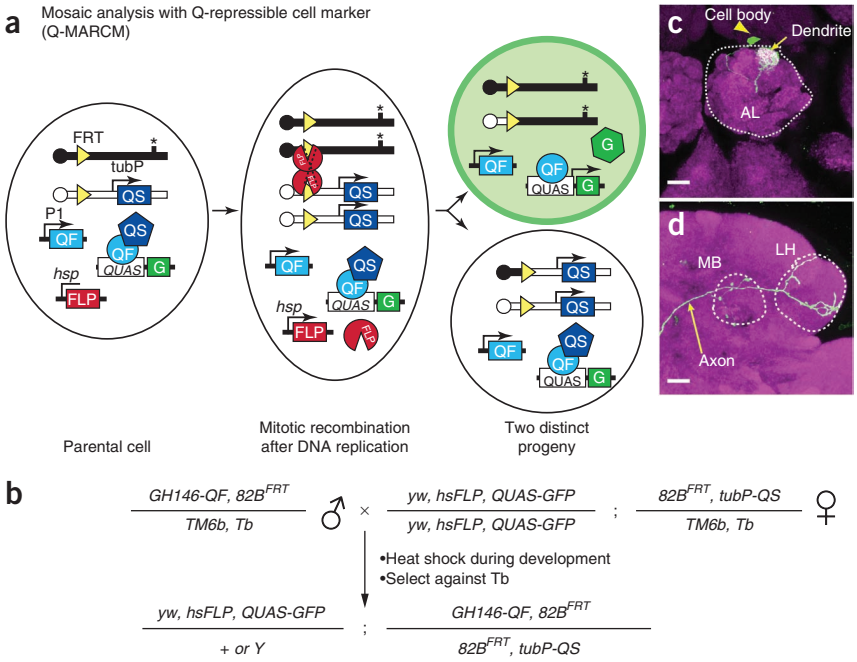

Figure 9 | Schematic and example of Q-based mosaic analysis with a repressible cell marker (Q-MARCM). (**a**) In a MARCM experiment, ubiquitous expression of the QS suppressor (driven by the tubulin promoter) is removed by a mitotic recombination event mediated by the FLP/FRT system, which allows for QF to activate *QUAS-geneX* reporters in a subset of cells. The parental cell contains sister chromosomes (black bars and white bars) containing the same *FRT* insertion (yellow triangles) distal to the centromere (circles). Distal to one of the *FRT* sites is the *tubP-QS* transgene. The other sister chromosome could contain a mutation of interest (*). *FLPase* expression is under control of a *heat-shock promoter* (*hsp*). A heat-shock pulse induces FLPase expression (red pacman) at or before mitosis. FLP/FRT-mediated mitotic recombination at the G2 phase of the cell cycle (dotted black cross) followed by the chromosome segregation, as shown here, causes the top cell progeny to lose both copies of *tubP-QS*, restore QF activity and become capable of expressing the *QUAS-GFP* marker (G). It also becomes homozygous for a mutation (*). *hsFLP*, *QF* and *QUAS-geneX* transgenes can be located on any other chromosome arm. Schematic modified from reference 4. (**b**–**d**) Example crossing strategy for the Q-MARCM experiment (**b**) shown in **c** and **d**. (**c**,**d**) Example of a single DL1 olfactory projection neuron labeled by Q-MARCM. The antennal lobe (AL), mushroom body calyx (MB) and lateral horn (LH) are outlined. Reprinted with permission from reference 4. Scale bars, 20 μm.

major chromosomes (X, 2nd, 3rd). Each transgenic line should be tested for inducibility and for lack of position effect. Even though most *QUAS-geneX* insertions are silent without a QF inducer, occasionally a *QUAS-geneX* line might be expressed because of induction of the minimal *hsp70* promoter by local strong enhancer elements. Such lines should be discarded.

Generation of QS effector lines. QS expression can be used to limit QF reporter expression patterns. Similar to the approaches for cloning QF transgenic animals, a promoter region known to express in defined tissues can be cloned into a *QS-SV40* transformation vector (**Supplementary Table 1**). For example, the EcoRI/KpnI-flanked tubulin promoter in *ptubP-QS-SV40* could be replaced with the promoter of choice. Alternatively, the *QS* coding region from *pBS-KS-QS* (that has restriction sites KpnI-ApaI-HindIII-EcoRI-QS-XbaI-NotI-EagI) could be cloned into an existing promoter-containing vector of choice.

For Q-MARCM experiments (**Box 1**), ubiquitous QS expression is required. Lines expressing ubiquitous QS (driven by the tubulin promoter) have been recombined with FRT sites

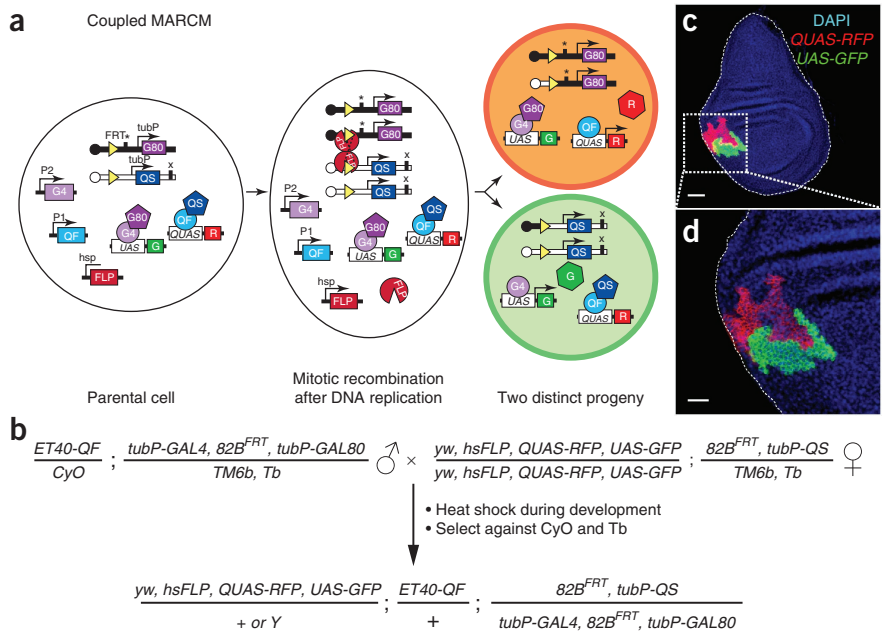

Figure 10 | Schematic and example of coupled MARCM. (**a**) In a coupled MARCM experiment, ubiquitous expression of the QS and GAL80 suppressors (driven by the tubulin promoter) are simultaneously segregated to different progeny by an experimentally induced mitotic recombination event. This results in two distinct progeny—one that has an active QF (due to loss of the QS suppressor) and the other that has an active GAL4 (due to loss of the GAL80 suppressor). See **Figure 9** for additional details. '*' and 'x' designate two independent mutations that can be rendered homozygous in sister progeny. *hsFLP*, *QF*, *GAL4*, *UAS-geneX* and *QUAS-geneX* transgenes can be located on any other chromosome arm. Schematic modified from reference 4. (**b**) Example crossing strategy for the coupled MARCM clone is shown in **c** and **d**. *ET40-QF* is a QF enhancer trap on the second chromosome that expresses QF in imaginal discs. (**c**,**d**) Example of a coupled MARCM clone in a third instar larval wing imaginal disc. Cell nuclei are labeled with 4,6-diamidino-2-phenylindole (DAPI). Larvae were heat shocked for 30 min at 48 h after egg laying. Scale bars, 20 μm.

BOX 1 | Q-MARCM EXPERIMENTS ● TIMING VARIABLE, DEPENDING ON GENERATION OF FLY STOCKS (1–5 GENERATIONS, ~ 2-10 WEEKS)

MARCM experiments can serve a variety of purposes, including generating mosaic tissues that are mutant for a gene of interest or for identifying the anatomy of a single neuron. Any QF driver line can be used for Q-MARCM experiments (**Fig. 9**). The protocol below for performing MARCM experiments is adapted from *Nature Protocols*[10].

Generate Q-MARCM-ready flies

1. Use standard genetic techniques to introduce the following genetic components into a single fly: (i) *FLP recombinase* under the control of a heat-shock promoter, (ii) a *QUAS-geneX* reporter to visualize the Q-MARCM clone, such as *QUAS-mCD8-GFP*, and (iii) an FRT site and *tubP-QS* recombined onto the chromosome arm of interest (**Fig. 9b**). *tubP-QS* insertions recombined with FRT sites are available for each major chromosome arm (**Supplementary Table 2**).

▲ CRITICAL STEP This balanced stock is a valuable reagent and should be maintained for future experiments.

Generate a *promoter-QF* line that is Q-MARCM ready

2. Use standard genetic techniques to combine a QF line (e.g., *GH146-QF*) with an FRT chromosome that uses the same FRT site as the Q-MARCM–ready flies generated in the previous step. For example, to be compatible with an $82B^{FRT}$, *tubP-QS* stock, an $82B^{FRT}$ line with *GH146-QF* could be used. The *GH146-QF* insertion can occur on any chromosome arm.

▲ CRITICAL STEP This balanced stock is a valuable reagent and should be maintained for future experiments.

▲ CRITICAL STEP The *promoter-QF* insertion can be located distal to the desired FRT (e.g., $82B^{FRT}$, *promoter-QF*). However, as this chromosome arm will become homozygous after the mitotic recombination event, it might affect the tissue of interest in cases in which the transgene insertion disrupts proper gene functions. It is recommended instead to position the *promoter-QF* insertion on any other chromosome arm. If possible, recombine the *promoter-QF* onto the chromosome arm opposite to the used *FRT* (e.g., *promoter-QF,* $82B^{FRT}$), which can simplify future MARCM experiments.

Perform Q-MARCM cross and generate MARCM clones

3. Cross five to ten *promoter-QF* MARCM-ready males with 10–20 *Q-MARCM*-ready virgins in a freshly yeasted vial. Depending on the birth date of the tissues of interest, heat shock the progeny in a 37 °C water bath for 30 min to 2 h (see ref. 10 for additional details). For example, to generate olfactory projection neuron clones, a 1.5-h heat-shock procedure can be performed from embryonic to third instar stages. For imaginal wing disc MARCM clones, a 30 min heat-shock procedure is performed at 48 h after egg laying.

▲ CRITICAL STEP The developmental time point and extent of the heat shock needs to be experimentally determined for each target tissue. The Q-MARCM-ready flies often contain an *hsFLP* insertion on the X chromosome (e.g., **Fig. 9b**). Using females of these flies for the Q-MARCM cross will ensure that both male and female progeny will contain Q-MARCM clones.

Analyze and examine Q-MARCM clones

4. Analyze Q-MARCM clones using an appropriate technique[10]; live or fixed tissues can be used.

? TROUBLESHOOTING

for every chromosome arm as well as inserted into the CyO and TM6B balancers (**Supplementary Table 2**). In addition, by using a *UAS-QS* transgenic animal (**Supplementary Table 2**), GAL4 patterns can be used to direct QS expression with the purpose of limiting QF expression patterns (see Step 7B; **Fig. 5b**).

Potential applications of the Q system. The Q system can be used for a variety of *in vivo* applications. In many cases, the experiment in question will determine which *QUAS-geneX* effector is used. **Table 1** presents a sampling of possible studies, the *geneX* effectors for *QUAS-geneX* constructs that might be used, and the method of detection or analysis.

TABLE 1 | Example applications of the Q system.

Application	*geneX* for *QUAS-geneX*	Detection/analysis method	References
Labeling tissues	*mCD8-GFP* *mtdT-3xHA* *CD2-HRP*	Live imaging Immunohistochemistry Electron microscopy	3,4,31,32
Marking different cellular compartments	*EYFP-Mito* (mitochondria) *EYFP-Golgi* (golgi) DenMark (dendrites) *synaptotagmin-HA* (presynaptic termini) *nuclearLacZ* (nucleus) *GFP-α-tubu*lin (microtubules)	Live imaging Immunohistochemistry	12,33–35

(continued)

TABLE 1 | Example applications of the Q system (continued).

Application	*geneX* for *QUAS-geneX*	Detection/analysis method	References
Ectopically expressing a gene of interest	*Tsc1/Tsc2* (cell growth/proliferation) *Akt* (cell growth) *TβH* (enzyme for synthesis of octopamine)	Live imaging Electron microscopy Immunohistochemistry Behavior	36–38
Cell ablation	*reaper* *hid* *grim*	Immunohistochemistry	39–41
Report cell activity	*GCAMP3* (neural activity) *tGPH* (PIP3 signaling)	Two-photon microscopy Immunohistochemistry	42,43
Gene knockdown	Interfering DNA against *geneX* (RNAi) microRNA against *geneX*	Behavior Live imaging Immunohistochemistry	44,45
Neuronal activation	*Channel Rhodopsin* (blue light activation) *TRPA1* (high temperature activation) *TRPM8* (low temperature activation)	Behavior Calcium imaging	46–49
Neuronal inactivation	*shibire^{ts1}* (inhibits vesicle recycling) *Kir2.1* (hyperpolarizes neuron) *tetanus toxin* (cleaves synaptobrevin)	Behavior	50–53
Mosaic analysis	Reporter (to label clones and/or mutant tissue)	Immunohistochemistry	3,10,54

MATERIALS

REAGENTS

- Q system cloning vectors (many Q system cloning vectors (**Supplementary Table 1**) are available from Addgene (http://www.addgene.org/pgvec1?identifier=Luo.p9EJQGBAq0qGJ7t4LCsvD2Yax9w&cmd=findpub))
- *Drosophila* fly stocks (many Q system fly stocks are available from the Bloomington Stock Center (**Supplementary Table 2**; http://flystocks.bio.indiana.edu/Browse/misc-browse/Qintro.htm))
- Quinic acid (Sigma-Aldrich, cat. no. 138622)
- Active dry yeast (Red Star Active Dry Yeast, http://Flystuff.com, cat. no. 62–103)
- Propionic acid (http://Flystuff.com, cat. no. 20–271)

EQUIPMENT

- Standard fly-culturing equipment
- Wide Polystyrene Vials (cat. no. 32–110, http://Flystuff.com)
- Fly vial plugs (Wide plugs, cat. no. 49–101, http://Flystuff.com)
- Dissecting microscope (Stemi 2000, Zeiss)
- Fluorescent dissecting microscope (Stereo Discovery V8 Pentafluar, Zeiss)
- RFP filter cube for V8 Pentafluar (KSC 295–834D DS RED, Zeiss)
- GFP filter cube for V8 Penatfluar (KSC 295–814D GFP CUBE, Zeiss)
- Water bath set at 37 °C for heat-shock (if using heat-shock promoter for FLP expression during MARCM experiments)
- Humidified 25 °C incubator to maintain fly crosses (Environmental Chamber 3940, Forma Scientific)
- Imaging microscope and software (Zeiss LSM 510 confocal microscope, Zeiss)
- Sharp forceps for brain dissections (Ted Pella, cat. no. 503, Dumont Biology Grade Tweezers Style 3)
- Three-well glass dissection dishes (Fisher Scientific, cat. no. 21–379)

REAGENT SETUP

Quinic acid solution Dissolve quinic acid in water to achieve the desired concentration; saturated concentration is ~300 mg ml^{-1} (roughly equivalent to 1.56 M). The solution may need to be incubated at 37 °C for ~15 min to help dissolve the quinic acid. The solution can be stored as 3.5-ml aliquots (makes approximately ten quinic acid vials) at −20 °C for months, but repeated freeze/thaw cycles should be avoided.

Propionic acid (0.5% (wt/vol)) In 1-liter bottle, mix 5 g of propionic acid with 999 ml of water. This is a stable solution that can be stored for months at room temperature (22–25 °C).

Yeast paste In a small container, mix approximately equal volumes of active dry yeast with 0.5% (wt/vol) propionic acid. Mix with metal spatula until yeast paste has dissolved. Mix in additional dried yeast as needed to achieve creamy peanut butter consistency. Yeast paste should be stored at 4 °C when not in use. The yeast paste in the container should be replaced when it begins to smell sour, usually in ~2-3 weeks.

EQUIPMENT SETUP

Quinic acid–containing vials Poke approximately ten holes into the medium of standard fly vials with wooden sticks. Apply ~300 µl of quinic acid solution to the medium, making sure all holes are covered. Cover the vials with cotton plugs and allow them to dry on the benchtop overnight. Vials should be used fresh (within 3–4 d if stored at 22–25 °C), but they can be stored at 4 °C for ~2 weeks.

PROCEDURE

Performing repressible binary expression experiments ● TIMING ~15 d

1| In a yeasted vial, cross three to five *promoter1-QF* transgenic flies with three to five transgenic flies containing the appropriate *QUAS-geneX* reporter (**Figs. 1b** and **2**; **Table 1** and **Supplementary Table 2**).

2| Depending on the goal of the experiment and the identity of *geneX*, determine the effect of binary expression on F_1 progeny at an appropriate developmental stage using an appropriate method (see **Table 1**). Alternatively, if *promoter1-QF* and *QUAS-geneX* are on the same chromosome, you may wish to proceed directly to Step 3 to generate a stable binary expression stock for subsequent analyses.
? TROUBLESHOOTING

Generating a stable binary expression stock ● TIMING 2–3 generations ~25–30 d

3| It is often convenient to recombine the *promoter1-QF* and *QUAS-geneX* reporter onto the same chromosome for future expression experiments. This requires that the *promoter1-QF* and *QUAS-geneX* are both located on the same chromosome. Common *QUAS-geneX* reporters are available with insertions on each of the three major chromosomes (**Supplementary Table 2**). Choose five to ten virgin F_1 females of genotype *promoter1-QF/QUAS-geneX* from the progeny in Step 1 and cross with a balancer stock.
▲ CRITICAL STEP To get a successful recombinant, it is essential to use F_1 heterozygote females as meiotic recombination occurs only in females and not in males.

4| Select a single male progeny that contains both copies of the selectable marker (usually two copies of the mini-white+ gene) and set up individual crosses with virgin females from an appropriate balancer stock. Carry out appropriate sib-crosses with the progeny to generate a balanced *promoter1-QF/QUAS-geneX* stock derived from each original male.
▲ CRITICAL STEP Single males are used for establishing balanced recombinant stocks as recombination does not occur in males. The use of single male crosses ensures that the generated stock will be genetically homogeneous.
▲ CRITICAL STEP If the expression pattern of the *promoter1-QF/QUAS-geneX* reporter can be visualized in live animals, this expression activity can be used to select for recombinant animals (instead of scoring for both copies of the selectable marker).

5| If desired, use the balanced stocks to analyze the effects of binary expression. Alternatively, proceed to Step 6 to repress or temporally control binary expression or to Step 7 to carry out intersectional experiments in conjunction with the GAL4 system.

Repression and temporal control of QF-induced binary expression ● TIMING 1 generation ~10–15 d

6| QF-induced *QUAS-geneX* expression can be effectively silenced by the presence of QS. To refine a QF expression pattern, for example, to remove a subset of QF-labeled tissues, follow option A. To completely abolish QF expression, for example, when performing quinic acid treatment experiments, follow option B. QS suppression of QF-induced reporters can be relieved by quinic acid treatment, resulting in temporal suppression of QF (**Figs. 1**, **2** and **4**). To relieve QS suppression of QF during larval development, follow option C. To relieve QS suppression of QF only in adult animals, follow option D. Ubiquitous expression of QS that is linked to a mitotic recombination event can also be used for MARCM (Q-MARCM; **Box 1**). Coupling both GAL4-based MARCM and Q-based MARCM to the same mitotic event can be used for coupled MARCM (**Box 2**).

(A) Expressing QS in a subset of tissues

(i) Generate (or select an existing) *promoter2-QS* line that results in the desired expression pattern of QS. Cross *promoter2-QS* flies with *promoter1-QF/QUAS-geneX* flies (generated in Step 4; **Fig. 3**) and maintain in standard fly food vials.

(ii) Depending on the aim of the experiment, either use an appropriate method to analyze the effects of QS in F_1 progeny with the genotype *promoter1-QF/QUAS-geneX promoter2-QS* or raise F_1 to adulthood and proceed to Step 6D to relieve QS-mediated suppression of QF using quinic acid. In the former case, in which QS is expressed, the *QUAS-geneX* reporter will no longer be expressed even if *QF* is present. As a control, reporter expression without QS presence should also be examined, that is, in parental flies of genotype *promoter1-QF/QUAS-geneX*.
▲ CRITICAL STEP *Promoter2-QS* transgenic lines should express QS in the same pattern as *promoter2-QF* transgenic animals that use the same promoter. This should be verified by crossing the *promoter2-QS* transgenic fly with a *promoter2-QF,QUAS-geneX* recombinant fly to confirm that the entire *promoter2-QF*-reported expression pattern is silenced. Different insertions of the *promoter2-QS* might need to be tested to find a line that effectively suppresses *promoter2-QF*.
? TROUBLESHOOTING

(B) Expressing QS in all tissues

(i) Ubiquitous expression of QS can be achieved by using the tubulin promoter to drive QS (*tubP-QS*). Select an appropriate *tubP-QS* stock (**Supplementary Table 2**) and cross with stable *promoter1-QF/QUAS-geneX* lines (from Step 4); maintain on standard fly food.

BOX 2 | COUPLED MARCM EXPERIMENTS ● TIMING VARIABLE, DEPENDING ON GENERATION OF FLY STOCKS (1–6 GENERATIONS, ~2- TO 12 WEEKS)

To label or manipulate all progeny of a mitotic division, coupled MARCM experiments can be used (**Fig. 10**). This involves combining both Q-MARCM and GAL4-MARCM techniques.

Generate coupled MARCM-ready flies containing *tubP-QS*

1. Use standard genetic techniques to introduce the following genetic components into a single fly: (i) *FLP recombinase* under the control of a heat-shock promoter, (ii) a *QUAS-geneX* reporter to visualize the Q-MARCM clone, such as *QUAS-mtdT-3xHA*, (iii) a *UAS-geneX* reporter to visualize GAL4 MARCM clones, such as *UAS-mCD8-GFP* and (iv) an FRT site and *tubP-QS* recombined onto the chromosome arm of interest (**Fig. 10b**). *tubP-QS* insertions recombined with FRT sites are available for each major chromosome arm (**Supplementary Table 2**).

▲ CRITICAL STEP This balanced stock is a valuable reagent and should be maintained for future experiments. This fly line could also be used for Q-MARCM experiments.

Generate coupled MARCM-ready flies containing *tubP-GAL80*

2. Use standard genetic techniques to introduce the following genetic components into a single fly: (i) *tubP-GAL80* recombined distally to an FRT chromosome that uses the same FRT site as the coupled MARCM-ready flies generated in the previous step, (ii) *promoter2-GAL4* and (iii) *promoter1-QF* (**Fig. 10b**).

▲ CRITICAL STEP This balanced stock is a valuable reagent and should be maintained for future experiments.

▲ CRITICAL STEP The *promoter2-GAL4* and *promoter1-QF* insertions can technically be located on any chromosome arm to generate coupled MARCM clones. However, as mentioned for Q-MARCM in **Box 1**, it is best to avoid recombining these reagents distal to the FRT site being used, in case these lines, when homozygous, disrupt endogenous gene functions. The crossing scheme diagrammed in **Figure 10b** allows different *promoter-GAL4* or *promoter-QF* lines to be used with the same coupled MARCM-ready flies. However, *promoter2-GAL4* and/or *promoter1-QF* could also be combined to other components in the previous step. The positioning of such components depends on the simplicity of generating a compatible coupled MARCM stock.

Perform coupled MARCM cross and generate coupled MARCM clones

3. In a freshly yeasted vial, cross five to ten coupled MARCM-ready males containing *tubP-GAL80* with 10–20 *Q-MARCM*-ready virgins containing *tubP-QS*. Depending on the birth date of the tissues of interest, heat shock the progeny in a 37 °C water bath for 30 min to 2 h.

▲ CRITICAL STEP The developmental time point and extent of the heat shock needs to be experimentally determined for each target tissue. The coupled MARCM-ready flies with the *tubP-QS* often contain an *hsFLP* insertion on the X chromosome (e.g., see **Fig. 10b**). Using females of these flies for the Q-MARCM cross will ensure that both male and female progeny will contain Q-MARCM clones.

Analyze and examine coupled MARCM clones

4. Analyze coupled MARCM clones using an appropriate technique[4,10]; live or fixed tissues can be used.

▲ CRITICAL STEP It is highly recommended to use a *promoter1-QF/QUAS-geneX* recombinant for ubiquitous QS experiments. As the outcome of *tubP-QS* experiments is lack of expression, it is vital to know, with 100% certainty, that both *promoter1-QF* and *QUAS-geneX* components are present. The lack of either of these components will appear identical to *tubP-QS* suppression.

(ii) Depending on the aim of the experiment, either examine the F_1 progeny for suppression of QF using an appropriate method (**Table 1**) or raise F_1 to adulthood and proceed to Step 6D to relieve QS-mediated suppression of QF using quinic acid. In the former case, the effects of ubiquitous QS expression can be confirmed by the lack of signal from the *QUAS-geneX* reporter. As a control for effectiveness of *tubP-QS*, reporter expression of parental flies of genotype *promoter1-QF/QUAS-geneX* can be examined.

? TROUBLESHOOTING

(C) Quinic acid treatment of developing flies

(i) Prepare fresh quinic acid–containing food vials (see REAGENT SETUP).

■ PAUSE POINT Quinic acid fly food can be stored for up to 2 weeks if kept at 4 °C.

(ii) Cross approximately ten *tubP-QS* animals with approximately ten *promoter1-QF/QUAS-geneX* animals (from Step 4) and let them lay eggs in quinic acid-containing food vials for 6–12 h. Transfer adults to fresh quinic acid food vials at approximately every 12 h to prevent overcrowding of progeny. The developing larval progeny will ingest sufficient quinic acid for suppression of QS and re-expression of the *QUAS-geneX* effector (**Fig. 1c**).

▲ CRITICAL STEP Alternatively, to target a specific developmental period, crosses could be set up on standard fly food and larvae at the required developmental stages transferred to grape plates or food containing quinic acid.

▲ CRITICAL STEP Quinic acid suppression of QS occurs within ~2 h of animals being placed on quinic acid-containing plates[4]. However, different tissues might respond differently to quinic acid feeding, owing to variations in proliferation rates or the extent of exposure to quinic acid. To reduce the level of quinic acid suppression, lower concentrations of quinic acid solution can be used when generating quinic acid food vials.

(iii) Analyze expression at the appropriate developmental stage using an appropriate technique (**Table 1**).
? TROUBLESHOOTING

(D) Quinic acid treatment of adult flies

(i) Place adults of genotype *tubP-QS+promoter1-QF/QUAS-geneX* (Step 6B(ii)) in a fresh food vial containing quinic acid solution (**Fig. 4**).
▲ CRITICAL STEP Although quinic acid–mediated relief of ubiquitous QS expression is detailed here, tissue-specific *promoter2-QS* expression can also be relieved by quinic acid treatments, as described above by using flies generated as described in Step 6A.
? TROUBLESHOOTING

(ii) Analyze adult flies for suppression of QS (as monitored by QF-induced *QUAS-geneX* expression) using an appropriate method (**Table 1**). Weak suppression of QS is seen within 6 h of being transferred to quinic acid–containing vials, but is most notable within 24 h (ref. 4).

(iii) For continued suppression, transfer flies to fresh quinic acid–containing food vials every 24–48 h. Quinic acid is nontoxic to flies and can be supplemented in their diet with no adverse effects.

Performing intersectional expression experiments ● TIMING Variable

7| There are 12 intersectional expression patterns possible by using GAL4 and QF systems together (examples are shown in **Figs. 2**, **5** and **11**). Each of these 12 intersectional expression patterns represent an effector expression profile that is a subset of the GAL4 and QF expression patterns used in the experiment. See reference 4 for a full list of expression patterns possible, including required genotypes. Below are details for three of the intersectionals that illustrate the basic principles for performing these genetic experiments. Choose option A to use QF expression patterns to limit the extent of GAL4 expression patterns. Choose option B to use GAL4 expression patterns to limit the extent of QF expression patterns. Choose options C or D to limit expression of an effector to only tissues that express both GAL4 and QF transgenes.

▲ CRITICAL STEP Even though the strategies in options C and D reflect the overlapping intersection between QF and GAL4, they are not equivalent. Whichever line is driving FLPase expression will capture the entire developmental profile of that expression pattern, which could be much broader than the expression pattern at the target stage (e.g., the adult stage). The final effector expression level is reflected by whichever transcription factor is driving the final effector transgene (e.g., QF driving *QUAS>geneX*).

(A) GAL4 NOT QF intersectional experiments

(i) Recombine *promoter2-GAL4* and the *UAS-geneX* onto the same chromosome and generate a balanced stock (as described in Steps 3 and 4 for *promoter1-QF* and *QUAS-geneX*).
▲ CRITICAL STEP This balanced stock is a valuable reagent and should be kept for future experiments.

(ii) To this *promoter2-GAL4/UAS-geneX* stock, cross in a *QUAS-GAL80* transgene and generate a balanced stock (**Fig. 6**). *QUAS-GAL80* transgenes are available on each chromosome (**Supplementary Table 2**).
▲ CRITICAL STEP This balanced stock is a valuable reagent and should be kept for future experiments.

(iii) Cross a *promoter1-QF* to the *promoter2-GAL4/UAS-geneX*; *QUAS-GAL80* stock (**Fig. 6**). Select progeny that contain all four genetic components required (*promoter1-QF*, *promoter2-GAL4*, *UAS-geneX* and *QUAS-GAL80*; **Fig. 6** and **Supplementary Table 2**). As a control, also choose animals that do not contain the *QUAS-GAL80* transgene (e.g., select for animals containing the balancer chromosome marked by the *Tubby* mutation in **Fig. 6**) for analysis.
▲ CRITICAL STEP These genetic components may be located on any chromosome just as long as the progeny contains all four components. The scheme above is designed to simplify the testing of many different *promoter-QF* lines on altering GAL4 expression patterns.

(iv) Analyze *UAS-geneX* expression using an appropriate technique (**Table 1**).
▲ CRITICAL STEP *UAS-geneX* effector expression will be refined based on the expression pattern of the *promoter1-QF*. For example, if *promoter1-QF* overlaps a portion of the *promoter2-GAL4* expression pattern, then the overlapping tissues would no longer express the *UAS-geneX* effector.

(B) QF NOT GAL4 intersectional experiments

(i) Recombine *promoter1-QF* and the *QUAS-geneX* onto the same chromosome and generate a balanced stock (see Steps 3 and 4).

(ii) To the *promoter1-QF/QUAS-geneX* stock, cross in a *UAS-QS* transgene (**Supplementary Table 2**) and generate a balanced stock (**Fig. 7**).
▲ CRITICAL STEP This balanced stock is a valuable reagent and should be maintained for future experiments.

(iii) Cross a *promoter2-GAL4* with the *promoter1-QF/QUAS-geneX*; *UAS-QS* stock (**Fig. 7**). Select progeny that contain all four genetic components required (*promoter1-QF*, *promoter2-GAL4*, *QUAS-geneX* and *UAS-QS*; **Figs. 5b** and **7**; **Supplementary Table 2**). As a control, also choose animals that do not contain the *UAS-QS* transgene (e.g., select for animals containing the *Tubby* balancer chromosome in **Fig. 7**) for imaging.

▲ CRITICAL STEP These genetic components may be located on any chromosome, just as long as the progeny contains all four components. The scheme shown in **Figure 7** is designed to simplify the testing of many different *promoter-GAL4* lines for their effects on QF expression patterns.

(iv) Analyze *QUAS-geneX* expression using an appropriate technique (**Table 1**).

▲ CRITICAL STEP *QUAS-geneX* effector expression will be refined based on the expression pattern of the *promoter2-GAL4*. For example, if *promoter2-GAL4* is *tubulin-GAL4*, then there would be no expression of the *QUAS-geneX* effector. If *promoter2-GAL4* overlaps a portion of the *promoter1-QF* expression pattern, then only the overlapping tissues would no longer express the *QUAS-geneX* effector (**Fig. 5b**).

(C) QF AND GAL4 intersectional experiment (developmental profile of *promoter-GAL4*)

(i) Recombine *promoter1-QF* with a QUAS 'FLP-out' reporter, such as *QUAS>stop>mCD8-GFP* (**Supplementary Table 2**) and generate a balanced stock.

▲ CRITICAL STEP This balanced stock is a valuable reagent and should be kept for future experiments.

(ii) To the *promoter1-QF*, *QUAS>stop>mCD8-GFP* stock, cross in a *UAS-FLP* transgene and generate a balanced stock (**Fig. 8a**).

▲ CRITICAL STEP This balanced stock is a valuable reagent and should be kept for future experiments.

(iii) Cross *promoter2-GAL4* animals with the *promoter1-QF*, *QUAS>stop>mCD8-GFP*; *UAS-FLP* stock. Select progeny that contain all four genetic components required for QUAS reporter expression (**Fig. 8a**). In this case, GAL4 will drive FLPase expression, which will excise the transcription stop from the *QUAS>stop>mCD8-GFP* effector. QF is then able to induce expression from the resulting *QUAS>mCD8-GFP* transgene (**Fig. 5c**). As a control, also select animals that do not contain the *UAS-FLP* transgene (e.g., select for animals containing the dominant *Tubby* mutation, which marks the balancer chromosome in **Fig. 8a**) for imaging.

▲ CRITICAL STEP These four genetic components may be located on any chromosome, just as long as the progeny contains all four components. The scheme shown in **Figure 8a** is designed to simplify the testing of many different *promoter-GAL4* lines to determine their overlapping expression pattern with a *promoter1-QF* line. Unbalanced lines can be used for these experiments, as only when all four components are together will there be any reporter expression. However, using unbalanced lines will reduce the efficiency of the cross and increase the number of animals that need to be processed to ensure a positive result.

(iv) Analyze *QUAS>mCD8-GFP* expression by immunohistochemistry or on live animals by fluorescent microscopy (**Table 1**).

? TROUBLESHOOTING

(D) QF AND GAL4 intersectional experiment (developmental profile of *promoter-QF*)

(i) Recombine *promoter1-QF* with a UAS 'FLP-out' reporter, such as *UAS>stop>mCD8-GFP* (**Supplementary Table 2**) and generate a balanced stock.

▲ CRITICAL STEP This balanced stock is a valuable reagent and should be kept for future experiments.

(ii) To the *promoter1-QF*, *UAS>stop>mCD8-GFP* stock, cross in a *QUAS-FLPo* transgene (**Supplementary Table 2**) and generate a balanced stock (**Fig. 8b**).

▲ CRITICAL STEP This balanced stock is a valuable reagent and should be kept for future experiments.

(iii) Cross *promoter2-GAL4* animals with the *promoter1-QF*, *UAS>stop>mCD8-GFP*; *QUAS-FLPo* stock. Select progeny that contain all four genetic components required for UAS reporter expression (**Fig. 8b**). In this case, QF will drive FLPase expression, which will excise the transcription stop from the *UAS>stop>mCD8-GFP* effector. GAL4 is then able to induce expression from the resulting *UAS>mCD8-GFP* transgene. As a control, also choose animals that do not contain the *QUAS-FLPo* transgene (e.g., select for the *Tubby* animals in **Fig. 8b**) for imaging.

▲ CRITICAL STEP These four genetic components may be located on any chromosome just as long as the progeny contains all four components. The scheme shown in **Figure 8b** is designed to simplify the testing of many different *promoter-GAL4* lines to determine their overlapping expression pattern with a *promoter1-QF* line. Unbalanced lines can be used for these experiments, as only when all four components are together will there be any reporter expression. However, using unbalanced lines will reduce the efficiency of the cross and increase the number of animals that need to be processed to ensure a positive result.

(iv) Analyze *UAS>mCD8-GFP* expression by immunohistochemistry or by using a fluorescent dissecting scope (**Table 1**).

? TROUBLESHOOTING

? TROUBLESHOOTING

Troubleshooting advice can be found in **Table 2**.

TABLE 2 | Troubleshooting table.

Step	Problem	Possible reason	Solution
2	No reporter expression with *promoter-QF* line	QF line is not expressed	Try *promoter-QF* insertion at different genomic loci
		QF kills expressing cells	Verify whether cells are dying by co-labeling cells with antibody marker or GAL4/UAS marker. Try a weaker *promoter-QF* line
		Reporter expression is low	Use two copies of reporter or *promoter-QF* line. Use different reporters
6A	QS expression cannot inhibit QF	QS expression is too low	Use extra copies of QS transgenic lines
6B		QS is not expressed in same cells as QF	Use different QS transgene
6C	Quinic acid not inhibiting QS	Quinic acid solution is too old	Make fresh quinic acid solution
6D		Quinic acid solution is not concentrated enough	Make a saturated 300 mg ml^{-1} quinic acid solution
		QS expression is too high	Try different QS transgenic line (e.g., *tubP-QS #9B*, Bloomington Stock no. 30022)
7C	Intersection of QF AND GAL4 shows no expression	FRT-STOP-FRT reporter is weak	Use extra copies of the FRT-STOP-FRT reporter
7D		QF and GAL4 are not expressed in the same cells	Try a different QF or GAL4 line
		Expression of QF or GAL4 is weak at examined stage	There are two approaches for the "AND" intersection. They differ by which transcription factor is the final readout and which is the developmental readout. The final readout might be weak at the examined stage. Try the alternative method
		All four required genetic components are not in the same fly	Check crossing strategy to ensure that selected progenies contain all four components
7C	Intersection of QF AND GAL4 shows stochasticity in labeled tissues	Low FLPase expression	Use extra copy of FLPase or reporter
			Use codon-optimized FLPase for higher expression
7D		Low expression of GAL4 enhancer trap	Perform intersection using different components (e.g., different *UAS-FLPase* line or use *UAS-FLPase*, *QUAS>stop>reporter* instead of *QUAS-FLPase*, *UAS>stop>reporter*)
Box 1	Few or no Q MARCM clones	MARCM stocks have broken down	Check that all components (e.g., *hsFLP*, FRT sites) are still present
		Heat shock done during the wrong developmental period or for too short a time	Try heat shocking at earlier developmental time points. Try heat shocking for longer time periods (e.g., 1.5–2 h at 37 °C)

● TIMING

Step 1, ~10 d (1 Fly generation)
Step 2, ~5 d For immunohistochemistry and imaging
Step 3, ~10 d (1 Fly generation)

Step 4, 2 Fly generations (~20 d)
Step 5, Variable depending on experimental design; ~5 d if staining and imaging are required
Step 6A, 1 Generation for cross; ~5 d if staining and imaging are required
Step 6B, 1 Generation for cross; ~5 d if staining and imaging are required
Step 6C, 1 Generation for cross; variable depending on extent of quinic acid feeding during development
Step 6D, 1 Generation for cross; adult feeding of quinic acid can continue as long as necessary for the experiment
Step 7A, ~4 Fly generations to generate required stocks; 1 fly generation to perform intersectional experiment; ~5 d for staining and imaging if required
Step 7B, Variable depending on necessity to generate appropriate fly stocks: 1–5 fly generations, and ~5 d for immunohistochemistry and imaging if required
Step 7C, Variable: 1–5 fly generations and ~5 d for imaging
Step 7D, Variable: 1–5 fly generations and ~5 d for imaging
Box 1, Variable: 1–5 fly generations and ~5 d for imaging
Box 2, Variable: 1–6 fly generations and ~5 d for imaging

ANTICIPATED RESULTS

When a *promoter-QF* and *QUAS-geneX* are combined in the same fly, there will be induced expression of *geneX*. However, when the *QUAS-geneX* is alone, there will be no effector expression. **Figure 1c** shows adult flies that contain the *QUAS-mtdT-3xHA* reporter alone or when combined with a QF enhancer trap line. When the QS suppressor is also introduced, this will block QF activity and keep *QUAS-geneX* reporters silent. **Figure 1c** also shows adult flies whose broad QF-induced expression of *QUAS-mtdT-3xHA* has been silenced by ubiquitous expression of QS. QS-mediated suppression can itself be inhibited by treating flies with quinic acid. Quinic acid can be fed to developing animals by supplementing their food with quinic acid, and larvae will ingest enough quinic acid for efficient QS suppression in many tissues. **Figure 1c** shows an adult fly that was previously suppressed by ubiquitous QS but was relieved from such QS suppression by developing on fly food containing quinic acid. Similar quinic acid-mediated re-expression of QF-induced genes can also be carried out in adult animals.

By combining the GAL4 and Q systems together, more refined expression patterns can be achieved (**Figs. 5** and **11**). These are called intersectional expression experiments, as the final expression pattern depends on the intersection between the QF and GAL4 expression domains. Such intersectional expression experiments could be used to target expression of an effector to a carefully defined target tissue, bypassing confounding effects due to more widespread expression. The outcome of the intersectional experiment depends on the additional genetic components that are used with the *promoter1-QF* and *promoter2-GAL4* lines. By using a *UAS-QS* transgene, GAL4 expression can be used to effectively limit a QF expression pattern. An example of this QF NOT GAL4 intersection is shown in **Figure 11d**. Similarly, by using a *QUAS-GAL80* transgene, QF expression can be used to effectively limit a GAL4 expression pattern. An example of this GAL4 NOT QF intersection is shown in **Figure 11e**. A powerful expressional refinement approach is to limit effector expression only to tissues that express both QF and GAL4. An example of this GAL4 AND QF intersection is shown in **Figure 11f**. This approach can effectively limit effector expression to a very small subset of cells. As the expression pattern of *promoter1-QF* and *promoter2-GAL4* can be easily determined, targeting expression to a desired population of cells only requires picking and choosing the right intersectional combination of GAL4 and QF lines.

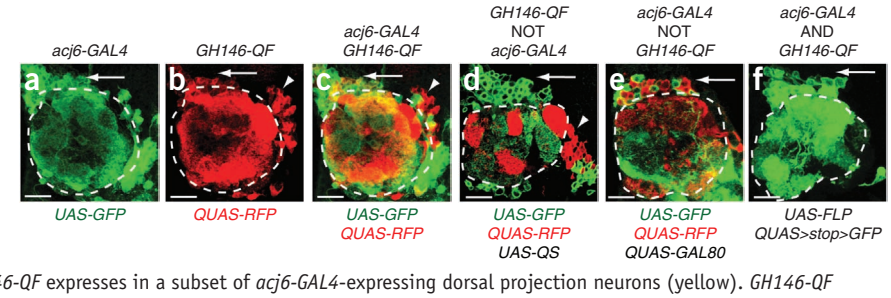

Figure 11 | Example intersectional expression experiments between GAL4 and QF olfactory projection neuron lines. (**a**) Shown is the antennal lobe innervation of *acj6-GAL4* projection neurons labeled by *UAS-mCD8-GFP*. The antennal lobe is circled. The arrow in all panels points to the dorsal population of projection neuron cell bodies. (**b**) Shown is the antennal lobe innervation of *GH146-QF* labeled by *QUAS-mtdT-3xHA*. The arrowhead in all panels points to a GH146$^+$ lateral population of projection neuron cell bodies. (**c**) *GH146-QF* expresses in a subset of *acj6-GAL4*-expressing dorsal projection neurons (yellow). *GH146-QF* and *acj6-GAL4* do not express in the same population of lateral projection neurons. (**d**) Example of the *GH146-QF* NOT *acj6-GAL4* intersectional expression pattern. *QUAS-mtdT-3xHA* is no longer expressed in any of the dorsal projection neurons (arrow) due to *acj6-GAL4* expression (green) driving *UAS-QS*. The lateral *GH146-QF* projection neurons remain labeled (arrowhead) as they do not express *acj6-GAL4*. (**e**) Example of the *acj6-GAL4* NOT *GH146-QF* intersectional expression pattern. *UAS-mCD8-GFP* is no longer expressed in a subset of dorsal projection neurons because of *GH146-QF* expression (red) driving *QUAS-GAL80*. (**f**) Example of the *acj6-GAL4* AND *GH146-QF* intersectional expression pattern. The *QUAS>GFP* reporter is only expressed in a subset of dorsal projection neurons that coexpress both *acj6-GAL4* and *GH146-QF* (arrow). The lateral projection neurons are not labeled. GFP, mouse CD8 membrane protein fused to GFP (mCD8-GFP); RFP, membrane targeted tandem Tomato C-terminally tagged with 3 hemagglutinin motifs (mtdT-3xHA); Scale bars, 20 μm. Panels **d** and **f** are reprinted with permission from reference 4.

Ubiquitous QS expression can effectively silence QF-induced reporter expression. By using mitotic recombination to differentially segregate a *tubP-QS* transgene, one population of cells will no longer have the *tubP-QS* transgene and hence will be released from QS suppression. These cells that are positively labeled (e.g., marked by a *QUAS-CD8-GFP* reporter) can also be made homozygous mutants for a gene of interest. This technique is called Q-MARCM and is a powerful approach to genetically manipulate and label a small number of cells, or even a single cell. An example of a Q-MARCM clone that labels a single olfactory projection neuron is shown in **Figure 9c**,**d**.

The MARCM technique was originally developed for the GAL4 system[3]. In this case, ubiquitous expression of the GAL4 suppressor, GAL80, is differentially segregated to cell progeny based on a mitotic recombination event. As the GAL4 system and the Q system function independently, these two mosaic labeling techniques can be combined together in coupled MARCM (**Fig. 10**). An example of a coupled MARCM clone in the wing imaginal disc is shown in **Figure 10c**,**d**. A QF-marked clone could be homozygous mutant for a gene of interest and/or express an effector gene. Similarly, the GAL4-marked clone could be homozygous for a different gene of interest and/or express a different effector gene. Such experiments could prove useful in addressing cell-cell communication or cell nonautonomous effects.

Note: Supplementary information is available via the HTML version of this article.

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