Supplemental Materials and Methods

Construction of the CoinFLP systems

CoinFLP constructs were constructed using traditional cloning techniques and inserted in attP40 on chromosome II using PhiC31 integration (BestGene).

To construct the plasmid used for *CoinFLP-Gal4*, we first PCR amplified the Gal4 coding sequence from pAct-FRT-CD2-FRT-Gal4 (Pignoni and Zipursky, 1997). Multiple restriction sites were added to the 5' end (Fse1-Not1-Age1-EcoRI-BglII) and Kpn1 to the 3' end with forward and reverse primers, respectively (sequence below). We ligated the resulting Gal4 fragment into pAct-FRT-stop-FRT-SV40polyA attB (Brittle et al., 2010), which had been purified after digesting with Fse1 and Kpn1. The resulting plasmid was named pAct-FRT-stop-Gal4 attB, which contains only one FRT site downstream of the *Actin5c* promoter. We ordered a plasmid containing a synthesized region containing two FRT3 sites that flank a wild-type FRT site (Integrated DNA Technologies (IDT)), called pNot1-FRT3-FRT-FRT3-EcoRI. This plasmid was cut with Not1/EcoRI to release FRT3-FRT-FRT3, which was ligated into pAct-FRT-stop-Gal4 attB. The resulting plasmid was named pAct-FRT-stop-FRT3-FRT-FRT3-Gal4 attB (Fig. S5) and was used to generate transgenic flies. This plasmid has been deposited with Addgene (52889).

To construct the plasmid used for CoinFLP-LexGAD/Gal4, we first ordered a plasmid from (IDT) containing the following elements from 5' to 3': FRT and FRT3 sites, a transcriptional stop sequence, a FRT site, a multiple cloning site containing EcoRI-Age1-Not1, and a FRT3 site. This plasmid was named pXho1-FRT-FRT3-stop-FRT-EcoRI-Age1-Not1-FRT3-BglII. We PCR amplified LexGAD coding and Hsp70 polyA sequence from pBPnlsLexA::GADflUw (Pfeiffer et al., 2010), adding EcoRI to 5' end and Not1 to 3' end with primers (sequence below). This PCR fragment was ligated into pXho1-FRT-FRT3-stop-FRT-EcoRI-Age1-Not1-FRT3-BglII with EcoRI/Not1. The resulting plasmid was named pXho1-FRT-FRT3-stop-FRT-LexGAD-FRT3-BglII. This plasmid was cut with Xho1/BglII and the released fragment was ligated into pAct-

FRT-stop-Gal4 attB cut with PspXI/BglII (the digested backbone lacks the FRT-stop sequence). The resulting plasmid is named pAct-FRT-FRT3-stop-FRT-LexGAD-FRT3-Gal4 attB (Fig. S6) and was used to generate transgenic flies. This plasmid has been deposited with Addgene (52890).

Oligonucleotides used (lower case = sequence added 5' to primer)
Fse1-Not1-Age1-EcoRI-BglII-Gal4-F
cgcgggccggccagcggccgcaccggtgaattcagatctTAAGCAAATAAACAAGCGCAG
Kpn1-Gal4-R
gcgcggtaccTTACTCTTTTTTTGGGTTTGGTG
EcoRI-LexGAD-F
cgggctgcaggaattccaaaATGCCACCCAAGAAGA
Not1-LexGAD-R
gacgcggcggccgcGATCTAAACGAGTTTTTAAGCAAACTC

Selecting RNAi lines toward genes used for the CoinFLP screen

To compile of list of genes in the genome that are refractory to screening by traditional mosaic analysis (mitotic recombination between *FRT* sites in *trans* on homologous chromosomes), we subtracted a list of genes distal to the commonly used *FRT* sites from the entire genome.

Genes distal to *FRT*s were defined by the known start/stop position of each chromosome arm and the insertion position of each *FRT* (Potter and Luo, 2010). For example, *FRT19A* is inserted at position X:19,804,903 on chromosome X. Genes distal to *FRT19A* are therefore in the range X:1..19,804,903. This was repeated for the four other commonly used *FRT* insertions *FRT40A* (2L:21,794,705), *FRT42D* (2R: 2,760,212), *FRT80B* (3L:23,095,809), and *FRT82B* (3R:278,974). We used QueryBuilder (flybase.org) to subtract this list from a list of all the genes in the genome (genome release 5.44 at the time of this analysis), resulting in a list of 863 genes, 302 of which are predicted protein coding, located in centromeric regions, the 4th chromosome, and the Y chromosome.

TRiP RNAi fly lines were selected based on our list of genes, resulting in 234 lines, targeting 175 different genes. Table S2 lists the TRiP RNAi stocks used.

Supplemental References

Brittle, A. L., Repiso, A., Casal, J., Lawrence, P. A. and Strutt, D. (2010). Four-jointed modulates growth and planar polarity by reducing the affinity of dachsous for fat. *Curr Biol* **20**, 803-810.

Pfeiffer, B. D., Ngo, T. T., Hibbard, K. L., Murphy, C., Jenett, A., Truman, J. W. and Rubin, G. M. (2010). Refinement of tools for targeted gene expression in *Drosophila*. *Genetics* **186**, 735-755.

Potter, C. J. and Luo, L. (2010). Splinkerette PCR for mapping transposable elements in *Drosophila*. *PLoS One* **5**, e10168.

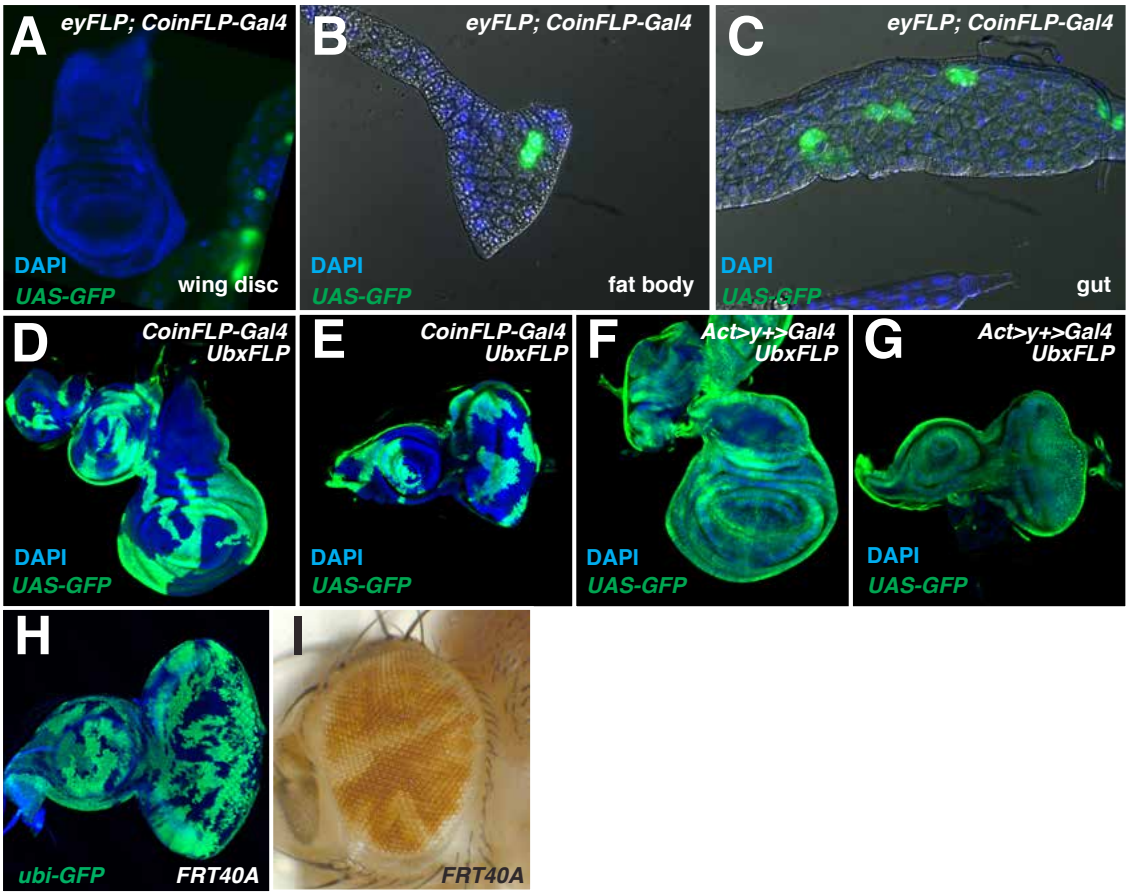

Figure S1: Additional characterization of the CoinFLP-Gal4 system. (A) A wing imaginal disc that does not express *UAS-GFP* when using *CoinFLP-Gal4* with *ey-FLP*. Sporadic expression of *UAS-GFP* in third-instar larvae using *CoinFLP-Gal4* in cells of the (B) fat body and (C) gut. (D-G) *Ubx-FLP*-driven recombination. (D) Haltere, leg, wing discs and (E) eye discs exhibit mosaic expression of *UAS-GFP* using *CoinFLP-Gal4*. (F) Haltere, leg, wing discs and (G) eye discs exhibit uniform expression of *UAS-GFP* using *Act>y+>Gal4*. (H-I) Traditional mitotic recombination between FRT40A sites using *ey-FLP*. (H) Mosaic eye-antennal disc, (I) mosaic adult eye. Genotypes: (A-C) *ey-FLP/+*; *CoinFLP-Gal4 UAS-GFP/+* (D-E) *Ubx-FLP/+*; *CoinFLP-Gal4 UAS-GFP/+* (F-G) *Ubx-FLP/+; Act>y+>Gal4 UAS-GFP/+* (H-I) *ey-FLP/+*; *FRT40A ubi-GFP/FRT40A*

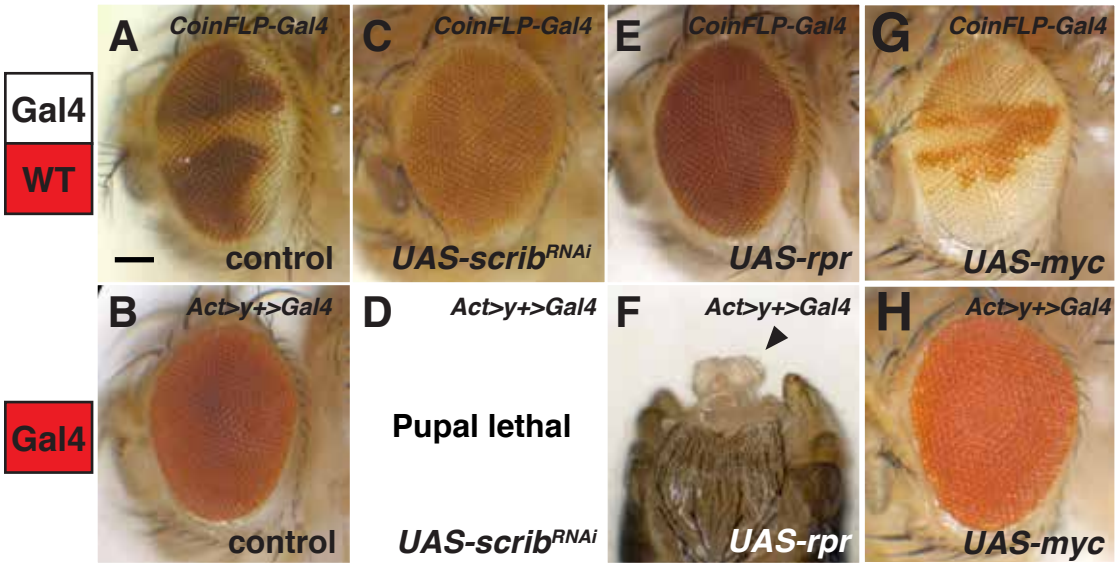

Figure S2: Mosaic eye phenotypes elicited with *CoinFLP-Gal4* and *ey-FLP*. (A-B) Control adult eyes (C-D) *UAS-scrib*^{RNAi}, (E-F) *UAS-rpr*, (G-H) *UAS-Myc*. (A, C, E, G) Mosaic eyes using *CoinFLP-Gal4* and *ey-FLP*. *UAS-white*^{RNAi} marks Gal4 expressing patches as white. (B, D, F, H) Eyes with *Act>y+>Gal4* and *ey-FLP*, uniformly expressing Gal4. Note: This genotype does not contain *UAS-white*^{RNAi} and Gal4 expressing cells are red. (F) Arrow head indicates remaining proboscis after head ablation. Scale bar: (A-H) 100µm. Genotypes: (A, C, E, G) *ey-FLP UAS-dcr2/+; CoinFLP-Gal4 /+; UAS-white*^{RNAi}*/UAS-X* (B, D, F, H)) *ey-FLP UAS-dcr2/+; Act>y+>Gal4 UAS-GFP/+; UAS-X/+*

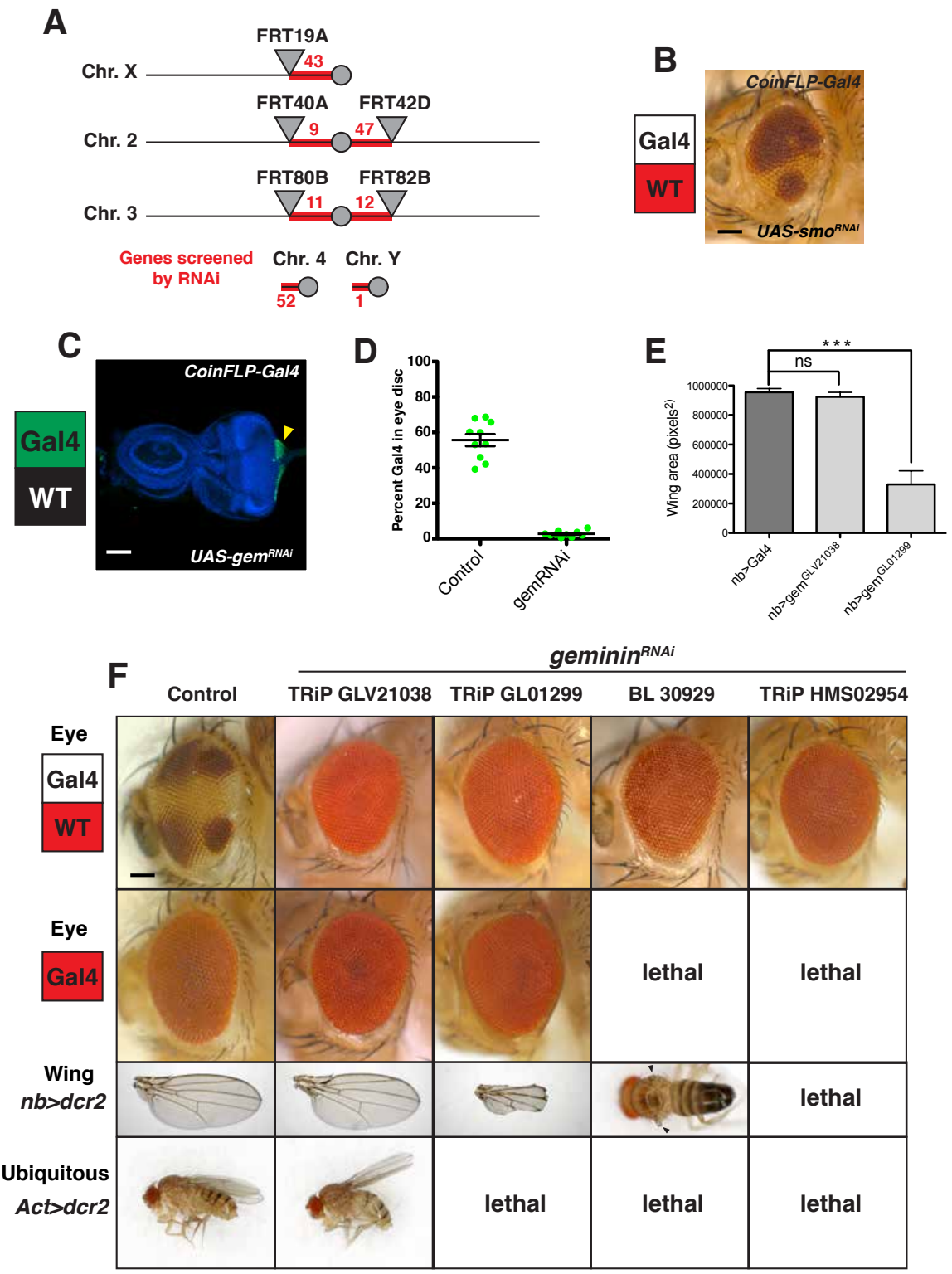

Figure S3: **Additional characterization from *CoinFLP-Gal4* screen.** (A) Locations of genes screened. (B) *UAS-smo*^{RNAi} mosaic adult eye using *CoinFLP-Gal4* and *ey-FLP*. Mutant cells are marked as white with *UAS-white*^{RNAi}. (C) *UAS-gem*^{RNAi} mosaic eye-antennal disc using *CoinFLP-Gal4* and *ey-FLP*. Mutant cells are marked with *UAS-GFP* (green). (D) Quantification of *UAS-gem*^{RNAi} mutant clone area in the eye-antennal

disc. Mean=3%, N=10 discs, s.d.= 1.6%, p<.001. (E) Quantification adult wing size from expression of *UAS-gem^{RNAi}* in the wing imaginal disc. N=30 wings, *** = p<.001 (F) Adult phenotypes from expression of different *UAS-gem^{RNAi}* transgenes. The lines are shown from left to right in order of increasing severity of phenotypes: TRiP GLV21038 < TRiP GL01299 < BL 30929 < TRiP HMS02954. Scale bar: (B-C, F) 100µm. Genotypes: (B) *ey-FLP UAS-dcr2/+; CoinFLP-Gal4 /+; UAS-white^{RNAi}/UAS-smo^{RNAi}* (C) *ey-FLP UAS-dcr2/+; CoinFLP-Gal4 UAS-GFP/+; +/UAS-gem^{RNAi}* (F) 1st row: *ey-FLP UAS-dcr2/+; CoinFLP-Gal4 /+; UAS-white^{RNAi}/UAS-X* 2nd row: *ey-FLP UAS-dcr2/+; Act>y+>Gal4 UAS-GFP/+; UAS-X/+* 3rd row: *UAS-dcr2/+; nb-Gal4/+; UAS-X/+* 4th row: *UAS-dcr2/+; act-Gal4/+; UAS-X/+*

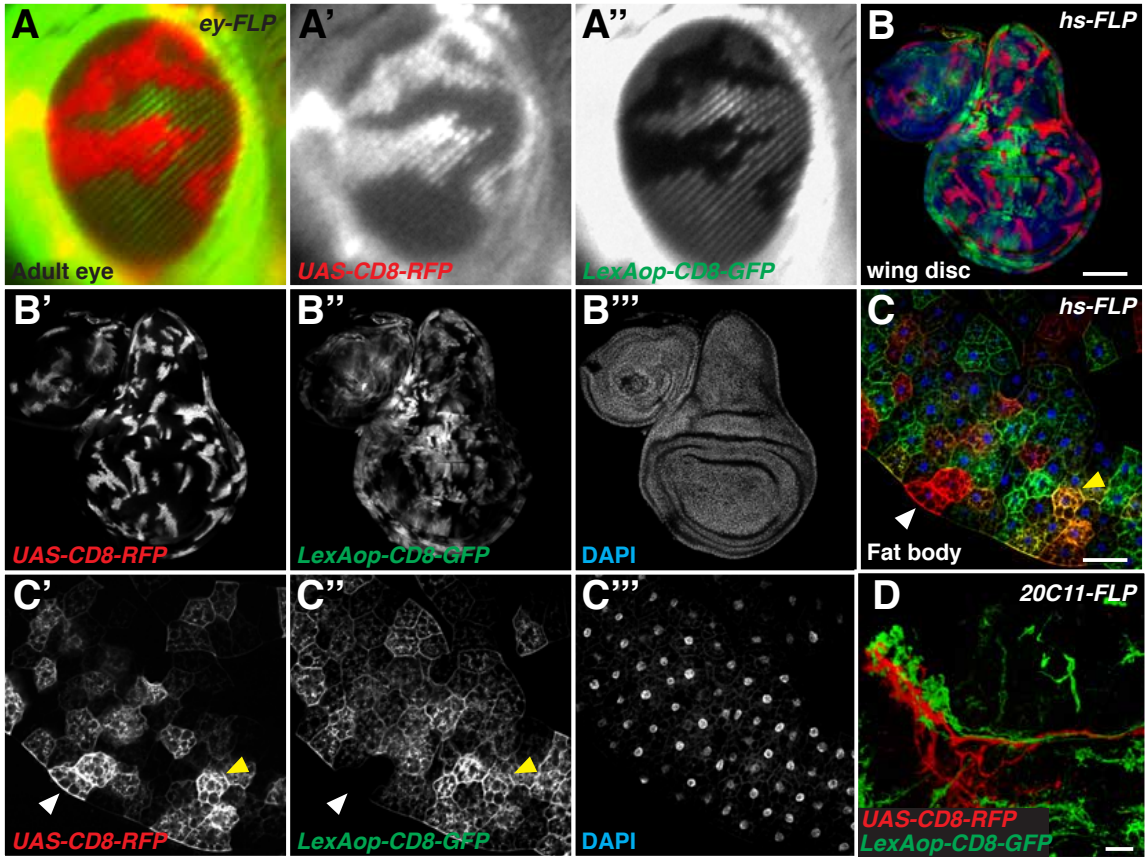

Figure S4: **Additional characterization of CoinFLP-LexGAD/Gal4 system.** (A-D) Tissues with *CoinFLP-LexGAD/Gal4* and different FLP transgenes. LexGAD expressing cells are marked with *LexAop-CD8-GFP* (green) and Gal4 cells with *UAS-CD8-RFP* (red). (A-A'') Adult eyes composed of only Gal4 or LexGAD clones using *ey-FLP*. Anterior is to the left. (B-B''') Confocal section showing Gal4- and LexGAD-expressing clones generated in the wing disc and (C-C''') fat body. White arrowhead indicates a cell expressing predominantly Gal4 while the yellow arrowhead indicates a cell that expresses both Gal4 and LexGAD. (D)Two neighboring neurons, each expressing either Gal4 or LexGAD induced by *20C11-FLP*. Scalebar: (B-C''') 100µm, (D) 10µm. Genotypes: (A) *ey-FLP/UAS-CD8-RFP, lexAop-CD8-GFP; CoinFLP-LexGAD|Gal4/+* (B) *sens-FLP/UAS-CD8:RFP, LexAop-CD8:GFP; CoinFLP-LexGAD|Gal4/+* (C-D''') *hs-FLP/UAS-CD8:RFP, LexAop-CD8:GFP; CoinFLP-LexGAD|Gal4/+* (E) *20C11-FLP (Chr.?)/UAS-CD8:RFP, LexAop-CD8:GFP; CoinFLP-LexGAD|Gal4/+*

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        Actin P.                    PspXI
CTAGTGGATCAGCTTGCATGCCTGCAGGTCCCCTCGAGGGGACTCTAGCTAGAGGATCC

                    FRT
CGGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGAATTGACTAAAGCCAAATAGAA

AATTATTCAGTTCCTGGCTTAAGTTTTTAAAAGTGATATTATTTATTTGGTTGTAACCA

                         Hsp70 3'UTR ("Stop")
ACCAAAAGAATGTAAATAACTAATACATAATTATGTTAGTTTTAAGTTAGCAACAAATT

GATTTTAGCTATATTAGCTACTTGGTTAATAAATAGAATATATTTATTTAAAGATAATT

GCGTTTTTATTGTCAGGGAGTGAGTTTGCTTAAAAACTCGTTTAGATCCACTAGTTCTA

        FseI      Not1
GTTGCGGCCGGCCAGCGGCCGCTGGGTTCAGGCATAGAGGAGTTGACGACTACTTCCAA

GGCAGTACGTGACGTAACGGCCCTTAAAGTGATCAGTCCTTATTGCTTTGTCTGCCGGA

               FRT3
AGTTCCTATTCTTCAAATAGTATAGGAACTTCGTGTGAGGGAGCGTATTTGGGAAGTTC

              FRT
CTATTCTCTAGAAAGTATAGGAACTTCCCCGCTTACCACCATACATCCAAATCACTACG

                            FRT3
GCTCGCTTGAAGTTCCTATTCTTCAAATAGTATAGGAACTTCGATGGTGTAGTGTTGCG

   EcoRI  BglII
GTGGAATTCAGATCTTAAGCAAATAAACAAGCGCAGCTGAACAAGCTAAACAATCTGCA

                               Gal4(2646bp) Kpn1     SV40 3'UTR
GCCCAAGCTTGAAGCAAGCCTCCTGAAAGATG...TAAGGTACCTAGATCT...TCT
```

Figure S5: DNA sequence of the CoinFLP-Gal4 transgene. Sequence displayed corresponds to the 3' end of the Actin promoter through the SV40 3'UTR in the pAct-FRT-stop-FRT3-FRT-FRT3-Gal4 attB plasmid. Bold underlined text indicates restriction enzyme sites that are unique in the plasmid.

```
        Actin P.                    Xho1            FRT
CTAGTGGATCAGCTTGCATGCCTGCAGGTCCCCTCGAGGAAGTTCCTATTCTCTAGAAA

                                                  FRT3
GTATAGGAACTTCCCCGCGTACCACCAGACAGCGAAGTTCCTATTCTTCAAATAGTATA

GGAACTTCGAATTGACTAAAGCCAAATAGAAAATTATTCAGTTCCTGGCTTAAGTTTTT

AAAAGTGATATTATTTATTTGGTTGTAACCAACCAAAAGAATGTAAATAACTAATACAT

                        Hsp70 3'UTR ("Stop")
AATTATGTTAGTTTTAAGTTAGCAACAAATTGATTTTAGCTATATTAGCTACTTGGTTA

ATAAATAGAATATATTTATTTAAAGATAATTGCGTTTTTATTGTCAGGGAGTGAGTTTG

CTTAAAAACTCGTTTAGATCCACTAGTTCTAGTTGCGGCCGGCCGCAACTAGAGGATCC

                    FRT                     EcoRI   LexGAD(2853bp)
CGGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGAATTCCAAAATG...TAGGTTT

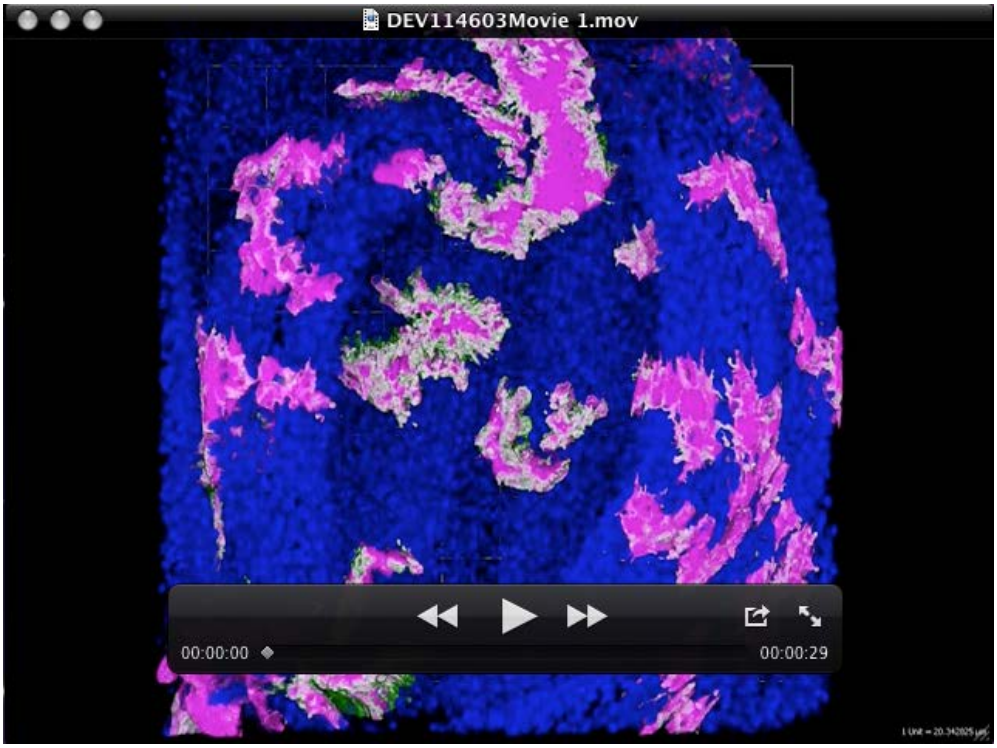
Pmel        Hsp70 3'UTR(246bp) Not1          FRT3
AAACAAGCTTATCGATACCGTC...ATCGCGGCCGCGAAGTTCCTATTCTTCAAATAGT

            BglII
ATAGGAACTTCAGATCTTAAGCAAATAAACAAGCGCAGCTGAACAAGCTAAACAATCTG

                                Gal4(2646bp) Kpn1   SV40 3'UTR
CAGCCCAAGCTTGAAGCAAGCCTCCTGAAAGATG...TAAGGTACCTAGATCT...TCT

BamHI
GGATCC
```

Figure S6: DNA sequence of the CoinFLP-LexGAD/Gal4 transgene. Sequence displayed corresponds to the 3' end of the Actin promoter through the SV40 3'UTR in the pAct-FRT-FRT3-stop-FRT-LexGAD-FRT3-Gal4 attB plasmid. Bold underlined text indicates restriction enzyme sites that are unique in the plasmid.



Movie 1: 3D reconstruction and flyc through of cell contacts in the wing disc. Wing imaginal disc with clone boundaries marked by GRASP. Clones expressing spGFP1t 10 are labeled magenta, GRASP fluorescence is green, and nuclei are labeled with DAPI (blue). spGFP11 clones are not labeled and nearly surround spGFP1-10 clones. A confocal stack of the pouch of a 3rd instar wing imaginal disc was processed with Volocity software to produce a 3D reconstruction and fly-through to better visualize the extent of cell contact visualized by GRASP. Genotype: *hs-FLP/+; CoinFLP-LexGAD|Gal4, LexAop-CD2-RFP/+; UAS-CD4-spGFP1-10, LexAop-CD4-spGFP11/+*

Table S1.xls: **Comparison of different *eyFLP* transgenes in the context of the CoinFLP system.** Expression of Gal4 is induced by *eyFLP* transgenes when paired with either *Act>y+>Gal4* or *CoinFLP-Gal4* transgenes. A *UAS-GFP* was included to visualize Gal4 expression. Several tissues in the 3rd instar larvae were inspected for GFP expression with the different *ey-FLP* transgenes. *ey-FLP* in stock #BL5580 has the most restricted expression in the eye imaginal disc.

Click here to Download Table S1

Table S2.xls: *CoinFLP-Gal4* screen phenotypes elicited by UAS-RNAi. List of adult phenotypes induced by RNAi of corresponding genes. Adult mosaic eye phenotypes were identified in a primary screen using *CoinFLP-Gal4*. Adult wing phenotypes were scored in parallel using *nubbin-Gal4*, which expresses Gal4 in the larval wing imaginal disc. For cases in which RNAi expression in the mosaic eye lead to the elimination of mutant tissue, these RNAi lines were scored in a secondary assay that uses uniform Gal4 expression in the eye.

Click here to Download Table S2

Table S3.xls: Useful *CoinFLP-Gal4* and *CoinFLP-LexGAD/Gal4* stocks. List of Drosophila stocks for conducting experiments using the *CoinFLP-Gal4* and *CoinFLP-LexGAD/Gal4* transgenes.

Click here to Download Table S3