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Online Methods

Generation of Hybrid Constructs

We constructed the different *GR* and *RG* hybrid cassettes from EGFP[8] and monomeric RFP[9] sequences purchased from Invitrogen. We generated chimaeric sequences (**Supplementary Table 1)** by PCR amplification with appropriate overlapping primers (**Supplementary Table 2**) of three DNA segments : a 5' sequence encoding the N-terminus of one fluoresent protein (EGFP or mRFP1); an identical αTub84B intron designed by Harrison and Perrimon[10] containing the yeast FRT site; and a 3' sequence encoding the C-terminus of the complementary fluorescent protein (mRFP or EGFP, respectively). We interrupted the coding sequences *in silico* by systematic insertion of the FRT-intron sequence at different positions until a theoretical splicing efficiency of greater than 93% was attained for the computer-generated splice junctions (http://www.fruitfly.org/seq_tools/splice.html).

We verified the construct sequences by standard sequencing reactions. We inserted the chimaeric *GR* and *RG* constructs, and a positive control construct, *GFP-intronFRT-GFP*, or *GG*, into the Gateway entry vector supplied in the pCR8/GW/TOPO TA Cloning Kit. We determined insert orientation by restriction enzyme digestion or sequence analysis of junction fragments. We transferred candidate hybrid and control sequences to a recipient Gateway Destination Vector AWM (Invitrogen) modified as below. See **Supplementary Table 2** for primer details.

Generation of the universal RMCE Destination Vector AWM-2attB

Because the TSG strategy requires the recombination sites to be allelic, we cloned *GR* and *RG* into a vector that would permit us to use the ϕC31 integrase for targeted genome transformation[11] coupled with Recombination-Mediated-Cassette-Exchange (RMCE)[12]. In RMCE, the ϕC31 integrase catalyzes the exchange of DNA flanked by inversely-oriented ϕC31 attB sites with that of genomic sequences flanked by inversely-oriented ϕC31 attP sites. We chose the Gateway Destination Vector, AWM, containing the Actin5C promoter and modified this *in vitro* cloning vector for use in *in vivo Drosophila* transformation by placing attB sites on either side of the selectable cloning cassette to create AWM-2attB, a universal RMCE Destination Vector.

We inserted inverted attB sites into the Gateway AWM vector in a 3-step process (see **Supplementary Fig. 1b**): First step, we added an MluI site to each extremity of the attB sequence during PCR amplification of the pCA4 vector with attB-specific primers (Microbix; see **Supplementary Table 2** for primer details). We digested with MluI the resulting PCR fragment and the AWM vector (unique MluI site at 5529) and joined the sequences by ligation. We determined the orientation of inserted attB sites by PCR analysis and restriction enzyme digestion with respect to an external unique PmeI site (position 5520 in AWM). Second step, we added BglI sites to primers at both ends: one, directly; and the second, as part of a 203-nucleotide sequence from the 3' end of the *ampicillin resistance* (*amp*^{R}) gene, since BglI digestion interrupted the *amp*^{R} gene at this point (position 6277). We digested this sequence and the vector from step 1 with BglI and ligated them. We selected for correct orientation of the second attB site by restored ampicillin resistance of the plasmid, and we subsequently verified this by PCR analysis and restriction enzyme digestion relative to an external unique DrdI site (position 7181 in AWM). Thus, the 203 nt-displaced 3' *amp*^{R} sequence is present twice in this vector, separated by the attB stretch. Third step: LR clonase reaction. The final integration vector AWM-2attB retains cloning capacity, accepting ORFs placed in Gateway entry clones, through standard single-site recombination at lambda attR/attL sites, replacing the Gateway cassette with the desired ORF (http://www.invitrogen.com/content/sfs/manuals/pcr8gwtopo_man.pdf).

Tissue culture assay

We tested the ability of the *GR* and *RG* inserts in AWM-2attB to recombine after their transfection into *Drosophila* S2R+ cells in culture. Cells were cultured in Schneider's insect medium (Invitrogen), 10% Fetal Bovine Serum (SAFC), and Penicillin-Streptomycin (Gibco). We set up a series of transfections using AWM-2attB plasmids containing the *GR* and *RG* reciprocal hybrid cassettes as well as the control plasmid *GG*. In addition, an *Actin5C-GAL4* driver plasmid and a *UAS-FLP* target plasmid were also co-transfected to constitutively produce active FLP. Transfection of plasmids was performed using Effectene reagents (Qiagen) as described at www.flyrnai.org. Results are shown in **Supplementary Figure 2**. In this assay we generated doubly-marked (GFP + RPF) *Drosophila* cells that glowed yellow demonstrating FLP-dependent mitotic exchange in S2R+ cells and providing preliminary evidence that *GR* and *RG* would function as predicted in TSG. Signals were apparent after 24 hours and increased in intensity over three-five days when the cells were imaged.
It should be noted that the hybrid constructs exist as plasmids in this assay with no possibility of extensive alignment as in homologous fly chromosomes; however, even if exchange is quite inefficient, since the fundamental change is at the DNA level, the fluorescent protein pool is amplified and regenerated in the cell by constitutive *Actin5C*-driven expression of the restored functional RNA.

Creation of transgenic lines

Target strains: The TSG protocol also called for *Drosophila* lines carrying genomic targets for RMCE. Here, we applied P-element transformation technology to *w* (white-eyed) flies in order to integrate target cassettes consisting of two inversely-oriented attP sites flanking the *miniwhite* gene[12], which codes for red eye color in the fly. We identified transformed flies by their colored eyes, and verified the presence of the attP sites by reverse PCR analyses. We screened lines derived from these flies for proximity of the target cassettes to the centromere. We chose this criterion because induction of MR close to centromeres maximizes the number of genes lying distal to the site of recombination and, therefore, the number of genes available for potential use in genetic mosaic analysis. One strain carrying a pUASTP2 target cassette at cytological position 82F7 was described previously[12]. We generated target cassette insertions at positions 38F2, 43F9, and 77C4 using *P[attP.w+.attP]*[14] via standard P-element-mediated germline transformation[15,16]. Together these four lines render about 80% of all autosomal genes potentially available for mosaic genetic analysis by TSG. Note that the pUASTP2 target cassette is juxtaposed to UAS and TATA sequences, while the *P[attP.w+.attP]* target cassettes have these sequences removed. All four strains carrying target cassettes are healthy as homozygous stocks. In order to facilitate further injections into these strains, we have introduced an X-chromosome carrying the ϕC31 integrase gene under the control of the *nanos* promotor into all four target strains[17].

TSG fly lines containing hybrid constructs: We used RMCE[12] to replace the target cassettes in the line carrying the insert at 82F7 with the *GR* and *RG* sequences, by co-injecting embryos with mRNA encoding the ϕC31 integrase and an AWM-2attB plasmid carrying either *GR* or *RG*. Successful insertions were tracked by loss of the red eye marker (*w+)* carried by the outgoing target cassettes, and we isolated putative TSG-competent flies through a screen for white-eyed flies in the F1 generation, after crosses to *y w* flies with the appropriate balancers. We balanced candidate TSG chromosomes over autosomal balancer chromosomes *CyO* and *TM3* and then made them homozygous. We confirmed the presence and orientation of the hybrid GR and *RG* constructs in each candidate strain by reverse PCR and sequence analysis. All white-eyed flies that were tested indeed carried an integrated hybrid construct (18/18 white-eyed F1 classes). We made the chromosome carrying the hybrid construct homozygous in subsequent standard genetic manipulations, and an X-chromosome was crossed in carrying

the *hs-FLP* gene[18]. One to three independent TSG lines were isolated for each *GR* and *RG* hybrid construct insertion.
See **Supplementary Table 3** for list of strains available.

Imaging
We dissected tissues in PBS and fixed in 4% paraformaldehyde in PBS for 20 minutes at room temperature. We washed and permeablized them in PBST (0.1% Triton X-100) for 10 minutes (3X). For **Fig. 2a-c**, with no antibody staining, we mounted larvae in Vectashield (Vector Laboratories, H-1000) ; for **Fig. 2d** with antibody staining, we incubated L2 larvae in a cocktail of primary antibodies diluted in 0.3% PBST overnight at room temperature. Primary antibodies: sheep anti-GFP (1/1000, Biogenesis), rabbit anti-DsRed (1/1000, Clontech), and rat anti-DECad (1:25, DSHB). Brains were washed (5 min, PBS, 3x) and incubated in secondary antibodies diluted in 0.3% PBST for 3 hr. Secondary antibodies (Molecular Probes): donkey anti-sheep Alexa488 (1/1000), donkey anti-rabbit Alexa555 (1/1000), goat anti-rat Alexa647 (1/200). After washing overnight, brains were mounted in Vectashield. For **Fig. 3a**, L3 larval imaginal discs were stained for two hours at RT with mouse anti-GFP (1:500, Invitrogen) and rabbit anti-DsRed (1:500, Clontech), followed by 4ºC overnight staining with secondary antibodies, goat anti-mouse Alexa488 and goat anti-rabbit Alexa568 (1:200 each, Molecular Probes) followed by DAPI (1 μg/ml in PBS) for five minutes. For **Fig. 2e-i and Fig. 3b** sheep anti-GFP (1/1000, Biogenesis), rabbit anti-DsRed (1/500, Clontech), and mouse anti-histone (Chemicon, 1/1000) were followed with secondaries, donkey anti-sheep Alexa488, goat anti-rabbit Alexa568, and goat anti-mouse Alexa647 (1/200 each, Molecular Probes). After washing, discs For **Fig. 2e-i and Fig. 3** were mounted in fluoromount-G (Southern Biotech). For Fig. **2a,b** and **d**, images were collected on a LeicaTCS SP2 AOBS confocal microscope system and processed with Leica confocal software imported into Adobe Photoshop7.0; for **Fig. 2c**, Nikon C1 confocal, Metamorph for imaging; for **Fig. 2e-i**, and **3**, BIO-RAD Radiance2000 confocal, Photoshop7.0 for imaging.

References

14. Bateman, J. R., & Wu, C. T. *Genetics* **180**, 1763-1766 (2008).
15. Rubin, G. M., & Spradling, A. C. *Science* **218**, 348-353 (1982).
16. Spradling, A. C., & Rubin, G. M. *Science* **218**, 341-347 (1982).
17. Bischof, J., Maeda, R. K., Hediger, M., Karch, F., & Basler, K. *Proc. Natl. Acad. Sci. USA* **104**, 3312-3317 (2007).
18. Chou, T. B., & Perrimon, N. *Genetics* **144**, 1673-1679 (1996).

AOP
This technique, adapted from Mosaic Analysis with Double Markers in mice, relies on mitotic recombination to reconstitute sequences encoding EGFP or mRFP. After cell division each daughter cell contains one fluorescent marker, causing a green and a red twin spot that can be traced through development.

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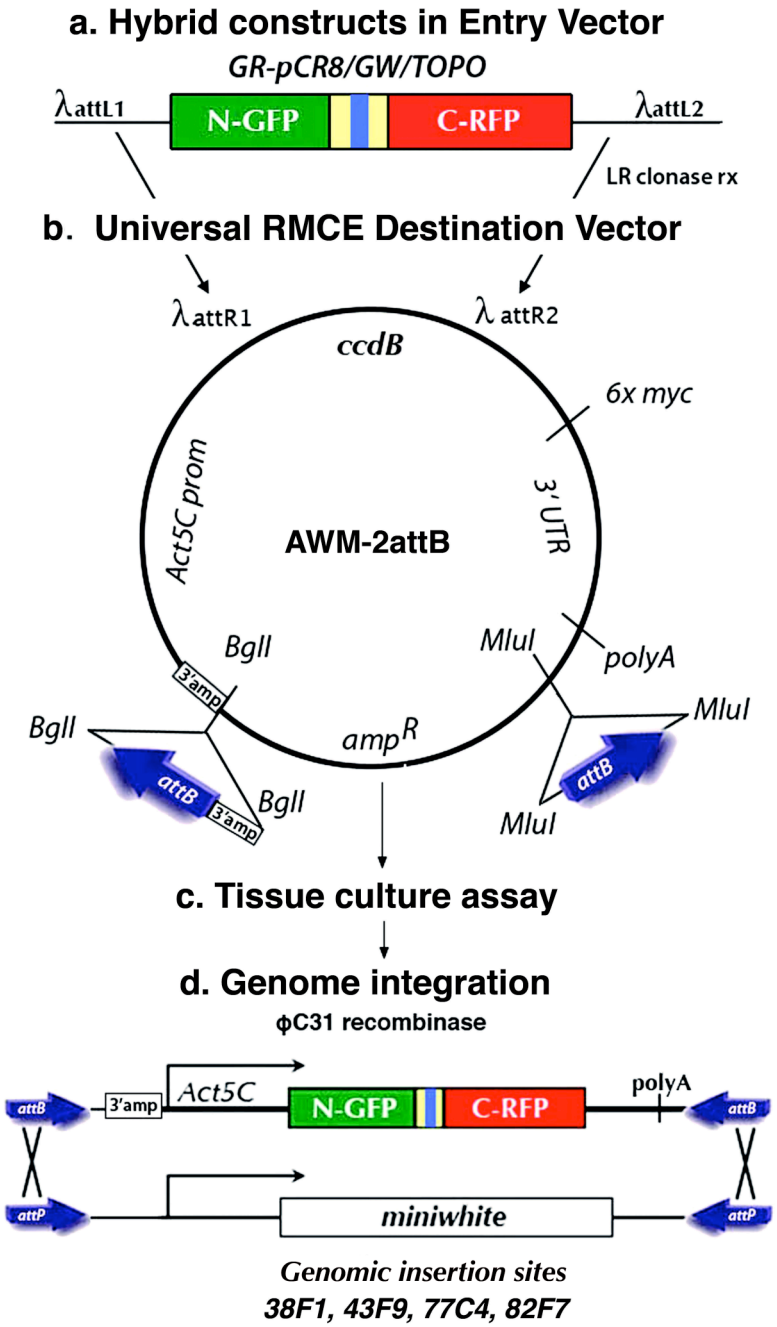
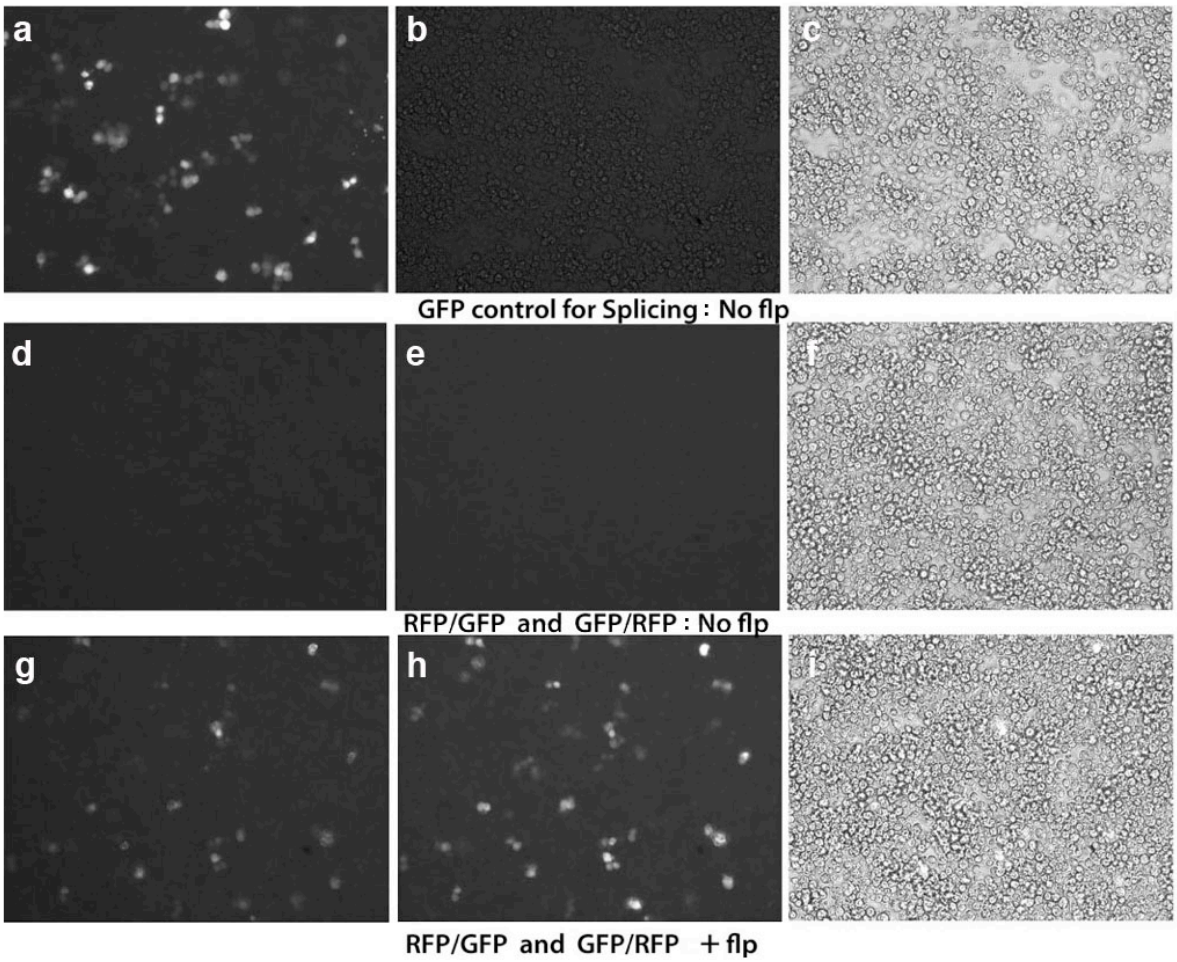
Supplementary Figure 1
TSG experimental strategy.



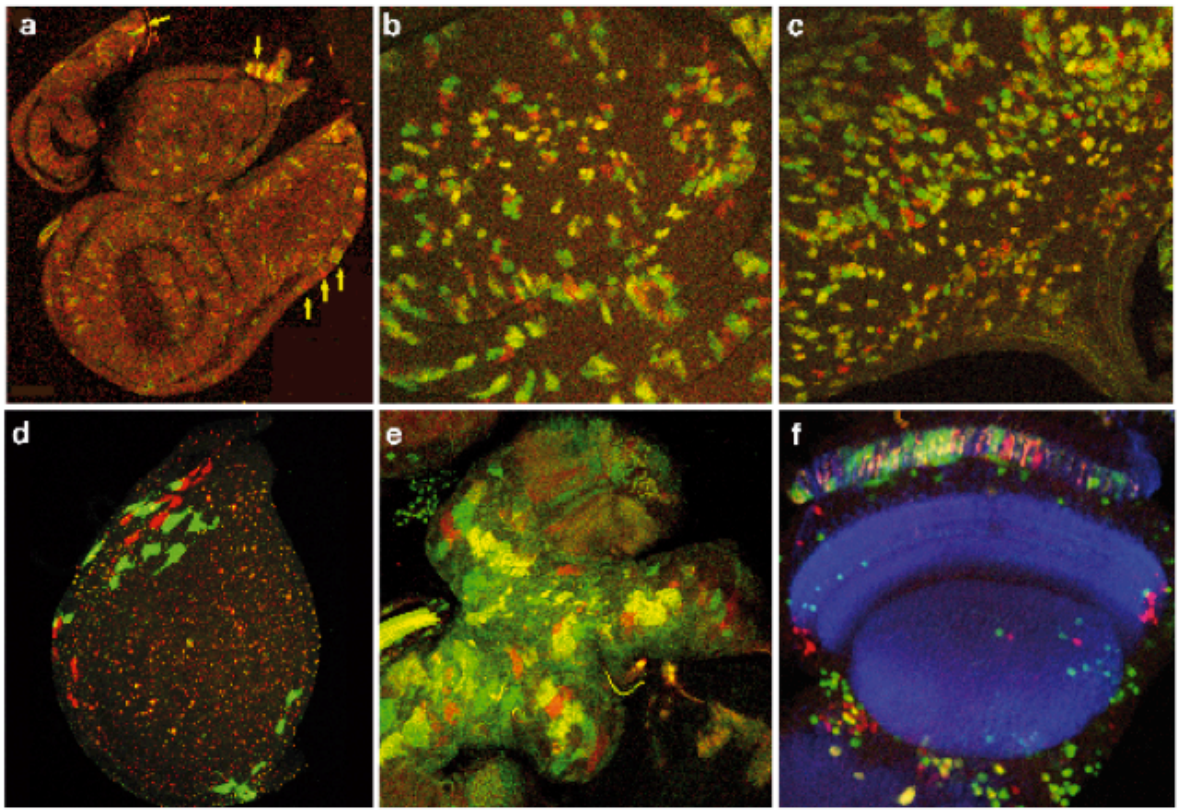
(a-d) Protocol. (a) *GR* construct in entry vector pCR8-GW-TOPO. (b) AWM-2attB accepts open reading frames (ORFs) from entry vector through LR clonase reaction. (c) Functional hybrid cassettes are identified by tissue culture assay. (d) Irreversible attB-attP recombination integrates expression cassettes at cytogenetic positions indicated.

Supplementary Figure 2
Identification of positive hybrid constructs by tissue culture assay.



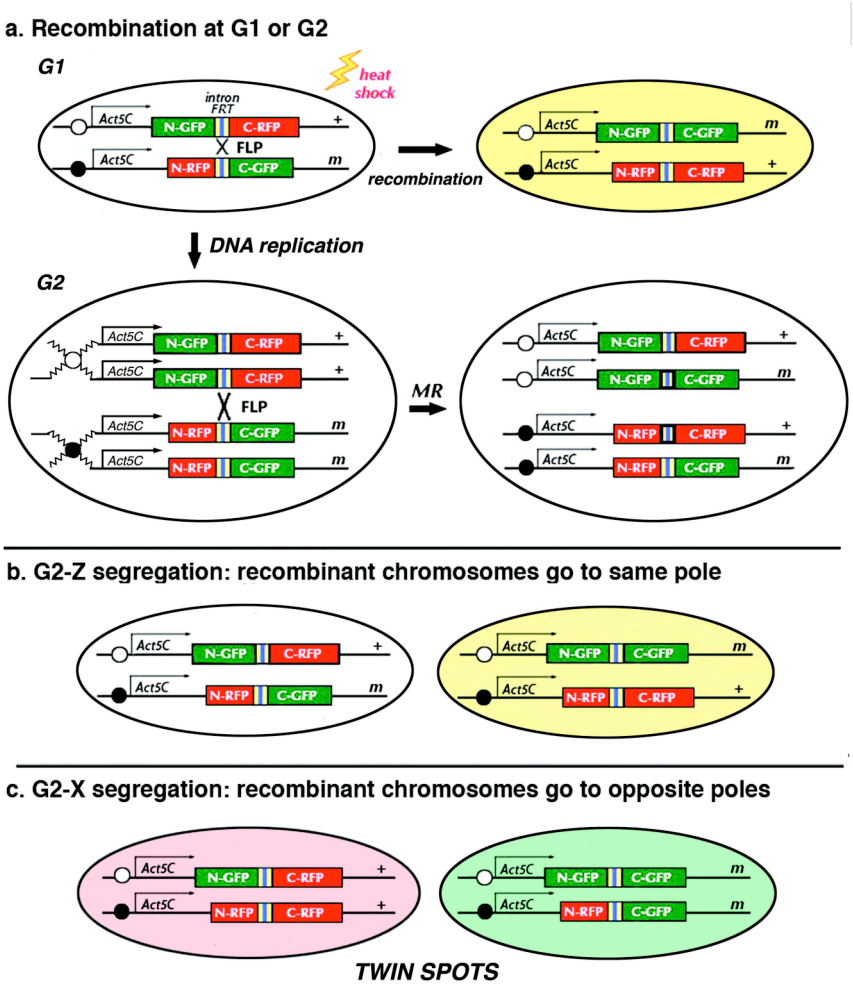
a-c. Transfection of control plasmid *GG*-AWM-2attB (non-hybrid, but interrupted, sequence *N-GFP[>]C-GFP* in AWM-2attB). Generation of GFP signal confirms that transcription, splicing and translation are functional. d-f. Cotransfection of *GR*-AWM-2attB and *RG*-AWM-2attB. Lack of any detectable signal demonstrates that the system is FLP-dependent. g-i. Cotransfection of *GR*-AWM-2attB and *RG*-AWM-2attB, plus plasmids containing *Act5C-GAL4* and *UAS-FLP*. Generation of both GFP and RFP signals in the same cell shows that hybrid cassettes in plasmid form function in cells in FLP-mediated exchange reactions, and that cassette exchange is fully reciprocal.

Supplementary Figure 3
Additional examples of TSG.



Examples of TSG showing red and green twin spots, and yellow clones after MR at 82F7 in the imaginal discs and brains of TSG flies providing evidence that clones can be induced everywhere. a and e: initial *GR* and *RG* cassettes. b-d and f: final *GR* and *RG* cassettes. Hs and dissection conditions: a-e: unstaged larvae, 30-45 min hs, dissected at wandering third instar larval stage; b-c: Mid-third instar larvae: 30 min hs, dissected 24 h later; d: hs, 72 h AED, dissected 120hAED; f: L3-96h: 15 min hs. Dissection 72 h after eclosion. a-c and e: No antibody staining. a-d. Imaginal discs. a. Haltere, top; leg, middle; wing, bottom. Arrows point to examples of clones in the peripodial epithelia. b. Wing. c. Eye. d. Projection of late third instar prothoracic leg disc, stained with anti-DsRed and anti-GFP. Twin spots were induced in peripodial epithelium, 72 h AED. e. Third instar larval brain. f. Adult optic lobe, lamina (upper distal), medulla (middle) and lobula (lower-proximal) stained with anti-DsRed, -GFP and -DNCad.

Supplementary Figure 4
TSG strategy in genetic mosaic analysis.



The FLP protein, supplied from a transgene driven by the heat shock (*hs*) promotor induces MR at the FRT site at desired times. (a) Top: G1 recombination between homologous chromosomes generates genotypically-identical *m*/+ yellow daughters. (Only one daughter is shown). Bottom, left: duplicated chromosomes at G2. Bottom right: chromatids after MR. (b) In G2-Z segregation, recombinant chromosomes go to the same pole to generate an *m*/+ colorless daughter and an *m*/+ yellow daughter. (c) In G2-X segregation, recombinant chromosomes go to opposite poles to generate twin spots: one +/+ red daughter and one *m/m* green daughter. Stocks currently available for mosaic analyses studies are listed in **Supplementary Table 3.** It should be noted that the *GR* and *RG* expression cassette transgenes are not marked.

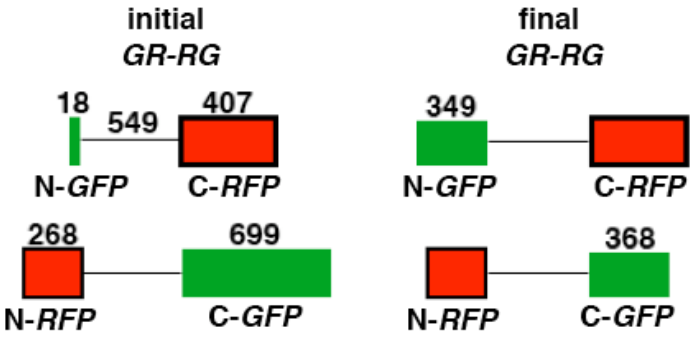
Supplementary Table 1
Nucleotide sequences of hybrid partner DNA.

Hybrid Partner	Sequence Name	Sequence	Sequence length[c]	Comments
GR initial	5'GFP[b]	ATGGTGAGCA AGGGCGAG	18	Final

construct[a]			(1-18) [d]	reconstructed GFP product gave punctate signal
	intron	gtgagtactt taaaaaaaaa tctagtgaaa taatgctgaa aagaaatttg tgtgggcaaa attcaatggg caaaaacgcg atgcggcttt ttctcaaaat ggcggccggc ctgcgttttt tcctcaaaag tgatgacgtc atgcctgttt ttttttttg ttcgcaatga ggaatggctc ttaaaaTCTA GGATCCCGGA AGTTCCTATT CTCTAGAAAG TATAGGAACT TCGAATtcta gataaaaaaa atattcatta tttctatgct gctggaacgc ttcattaatc ttaaaaattc taaattcggt taccatgata cttcgacgca taactgtaga ttttggatag aattaaagag aaaatggcga gagagtaaaa ttccggcgtc ggcaaagtag agcaaaaaaa tcagtatacc atttagctac ctctctcact cgcacgcagt gccggctcaa gttgggcgcg gctctgcaat tatcgatttt cttggggtgt gtaactaatc atccgttttc ccttcctcct catccacag	539	Same for all constructs (FRT sequence in upper case letters)
	3'RFP	GCTTCAAGTG GGAGCGCGTG ATGAACTTCG AGGACGGCGG CGTGGTGACC GTGACCCAGG ACTCCTCCCT GCAGGACGGC GAGTTCATCT ACAAGGTGAA GCTGCGCGGC ACCAACTTCC CCTCCGACGG CCCCGTAATG CAGAAGAAGA CCATGGGCTG GGAGGCCTCC ACCGAGCGGA TGTACCCCGA GGACGGCGCC CTGAAGGGCG AGATCAAGAT GAGGCTGAAG CTGAAGGACG GCGGCCACTA CGACGCCGAG GTCAAGACCA CCTACATGGC CAAGAAGCCC GTGCAGCTGC CCGGCGCCTA CAAGACCGAC ATCAAGCTGG ACATCACCTC CCACAACGAG GACTACACCA TCGTGGAACA GTACGAGCGC GCCGAGGGCC GCCACTCCAC CGGCGCG	407 (269-675)	Same for all constructs
RG	5'RFP	ATGGCCTCCT CCGAGGACGT CATCAAGGAG TTCATGCGCT TCAAGGTGCG CATGGAGGGC TCCGTGAACG GCCACGAGTT CGAGATCGAG GGCGAGGGCG AGGGCCGCCC CTACGAGGGC ACCCAGACCG CCAAGCTGAA GGTGACCAAG GGCGGCCCCC TGCCCTTCGC CTGGGACATC CTGTCCCCTC AGTTCCAGTA CGGCTCCAAG GCCTACGTGA AGCACCCCGC CGACATCCCC GACTACTTGA AGCTGTCCTT CCCCGAGG	268 (1-268)	Same for all constructs
	3' GFP	GAGCTGTTCA CCGGGGTGGT GCCCATCCTG GTCGAGCTGG ACGGCGACGT AAACGGCCAC AAGTTCAGCG TGTCCGGCGA GGGCGAGGGC GATGCCACCT ACGGCAAGCT GACCCTGAAG TTCATCTGCA CCACCGGCAA GCTGCCCGTG CCCTGGCCCA CCCTCGTGAC CACCCTGACC TACGGCGTGC AGTGCTTCAG CCGCTACCCC GACCACATGA AGCAGCACGA CTTCTTCAAG TCCGCCATGC CCGAAGGCTA CGTCCAGGAG CGCACCATCT TCTTCAAGGA CGACGGCAAC TACAAGACCC GCGCCGAGGT GAAGTTCGAG GGCGACACCC TGGTGAACCG CATCGAGCTG AAGGGCATCG ACTTCAAGGA GGACGGCAAC ATCCTGGGGC ACAAGCTGGA GTACAACTAC AACAGCCACA ACGTCTATAT CATGGCCGAC AAGCAGAAGA ACGGCATCAA GGTGAACTTC AAGATCCGCC ACAACATCGA GGACGGCAGC GTGCAGCTCG CCGACCACTA CCAGCAGAAC ACCCCCATCG GCGACGGCCC CGTGCTGCTG CCCGACAACC ACTACCTGAG CACCCAGTCC GCCCTGAGCA AAGACCCCAA CGAGAAGCGC GATCACATGG TCCTGCTGGA GTTCGTGACC GCCGCCGGGA TCACTCTCGG CATGGACGAG	699 (19-717)	

		CTGTACAAA		
GR	5'GFP	ATGGTGAGCA AGGGCGAGGA GCTGTTCACC GGGGTGGTGC CCATCCTGGT CGAGCTGGAC GGCGACGTAA ACGGCCACAA GTTCAGCGTG TCCGGCGAGG GCGAGGGCGA TGCCACCTAC GGCAAGCTGA CCCTGAAGTT CATCTGCACC ACCGGCAAGC TGCCCGTGCC CTGGCCCACC CTCGTGACCA CCCTGACCTA CGGCGTGCAG TGCTTCAGCC GCTACCCCGA CCACATGAAG CAGCACGACT TCTTCAAGTC CGCCATGCCC GAAGGCTACG TCCAGGAGCG CACCATCTTC TTCAAGGACG ACGGCAACTA CAAGACCCGC GCCGAGGTGA AGTTCGAGG	349 (1-349)	Final reconstructed GFP product gives homogeneous signal
RG	3'GFP	GCGACACCCT GGTGAACCGC ATCGAGCTGA AGGGCATCGA CTTCAAGGAG GACGGCAACA TCCTGGGGCA CAAGCTGGAG TACAACTACA ACAGCCACAA CGTCTATATC ATGGCCGACA AGCAGAAGAA CGGCATCAAG GTGAACTTCA AGATCCGCCA CAACATCGAG GACGGCAGCG TGCAGCTCGC CGACCACTAC CAGCAGAACA CCCCCATCGG CGACGGCCCC GTGCTGCTGC CCGACAACCA CTACCTGAGC ACCCAGTCCG CCCTGAGCAA AGACCCCAAC GAGAAGCGCG ATCACATGGT CCTGCTGGAG TTCGTGACCG CCGCCGGGAT CACTCTCGGC ATGGACGAGC TGTACAAA	368 (350-717)	"

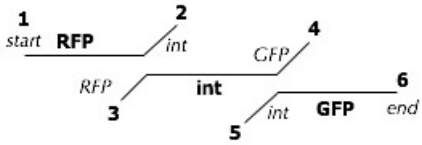
[a]We significantly improved GFP signal quality by splitting the EGFP coding sequence at position 349 and generating a new reciprocal pair of hybrid sequences, the final *GR* and *RG* constructs, which were again inserted at 82F7 to create new TSG fly lines. Heat shock treatment of the progeny from these mated *GR* and *RG* lines produced green fluorescent signals that were significantly more homogeneous in both green and yellow clones. [b]CACC was added at the beginning of all 5' sequences to favor translation. [c]For 5' sequences, numbering begins at the ATG. [d]numbers in parentheses show nucleotide number in the uninterrupted coding sequences for GFP and RFP. Diagram : heavily outlined RFP component sequences are invariant. Numbers refer to length in nucleotides.



Supplementary Table 2
PCR primers for construction of hybrid cassettes and insertion of attB sites.

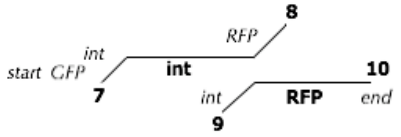
Primers used in constructing initial *RG*

1	sensRFP1_16	CACCATGGCCTCCTCCGAGG
2	asint491RFP269	ttat ttcactagat ttttttttaa agtactcacC CTCGGGGAAG GACAGCT
3	sensRFP268int33	AGCTGTCCTT CCCCGAGGgt gagtacttta aaaaaaaatc tagtgaaata a
4	asint491GFP19	CACCCCGGTG AACAGCTCCT gtggatgagg aggaagg
5	sensint491GFP19	ccttcctcct catccacagG AGCTGTTCAC CGGGGTG
6	as GFP1457	TTTGTACAGCT CGTCCATGC



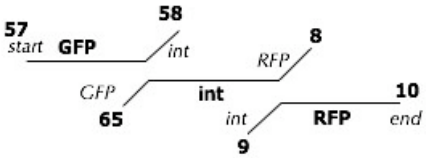
Primers used in constructing initial *GR*

7	sensGFP18int33	CACCATGGTGAGCA AGGGCGAGgt gagtacttta aaaaaaaatc tagtgaaata a
8	asint491RFP239	AC GCGCTCCCAC TTGAAGCctg tggatgagga ggaagg
9	sensint491GFP19	ccttcctcct catccacagG CTTCAAGTGG GAGCGCGT
10	as RFP916	CGCGCCGGTG GAGT



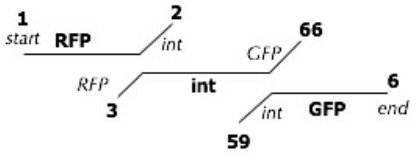
New primers used in constructing final *GR*

57	57sens newGFP1_16	CACCATGGTGAGCA AGGGCG
58	58asnewGFP350_1	ttatttcactagatttttttttaaagtactcacCCTCGAACTTCACCTCGG
65	65sensnewGFP1_350	CCGAGGTGAAGTTCGAGGgtgagtactttaaaaaaaaatctagtgaaataa



New primers used in constructing final *RG*

59	59sens newGFP871	ccttcctcc tcatccacag GCGACACCCTGGTGAACC
66	66as newGFP871	GGTTCACCAGGGTGTCGCctgtggatgaggaggaagg



Primers used in inserting 2 attB sequences and restoring *ampR* gene

31	Sens 4enz MluMfe1-45	acgcgtctcg agcaattgaa gcttATGTAG GTCACGGTCT CGAAG
32	as4enzMluMfe1-39	acgcgtgggc cccaattgcc taggATGCCC GCCGTGACC
35	sens soe amp 1to11	GCCCTTCCGG CTGGC
36	as soe amp 220 to176	CTTCGAGA CCGTGACCTA CATGTTACCA ATGCTTAATCAGTGAGG
37	Sens soeamp 176to220	CCTCA CTGATTAAGC ATTGGTAACA TGTAGGTCAC GGTCTCGAAG
39	as soeamp2enz 1to38	GCCGGAAGGG Ccctaggggg cccATGCCCG CCGTGACC

Supplementary Table 3

TSG fly stocks available.

Designation	Docking site	Stock no.	Genotype[c]
GrR_38[a]_10[b]_Flp	38F1	10	*Df(1) y ac, w^{1118} Flp^{12} ; Act5C-N-GFP[>]C-RFP*[c]

RGr_38_20-4_Flp	”	20-4	*Df(1) y ac, w^{1118} Flp^{12} ; Act5C-N-RFP[>]C-GFP*
CD8GrR_77_43_Flp	77C4	43	*Df(1) y ac, w^{1118} Flp^{22} ; Act5C-N-CD8 [d]GFP[>]* C-*RFP*
CD8GrR_77_13_Flp	”	13	*Df(1) y ac, w^{1118} Flp^{22} ; Act5C-N-CD8 [d]GFP[>]* C-*RFP*
CD8GrR_77_24_Flp	”	13	*Df(1) y ac, w^{1118} Flp^{22} ; Act5C-N-CD8 [d]GFP[>]* C-*RFP*
CD8RGr_77_6_Flp	”	13	*Df(1) y ac, w^{1118} Flp^{22} ; Act5C-N-CD8 [d]RFP[>]* C-*GFP*
GrR_82_18-2_Flp	82F	18-2	*Df(1) y ac, w^{1118} Flp^{22} ; Act5C-N-GFP [>]* C-*RFP*
GrR_82_25-2_Flp	”	25-2	*Df(1) y ac, w^{1118} Flp^{22} ; Act5C-N-GFP[>]* C-*RFP*
RGr_82_20-4_Flp	”	20-4	*Df(1) y ac, w^{1118} Flp^{22} ; Act5C-N-RFP[>]C-GFP*
RGr_82_25-3_Flp	”	25-3	*Df(1) y ac, w^{1118} Flp^{22} ; Act5C-N-RFP[>]C-GFP*
Target attP line-38F1	38F1		*y w P{y+. nos-int.NLS}*[e]; *P[attP.w+.attP]*
Target attP line - 43F9	43F9		*y w P{y+. nos-int.NLS}*; *P[attP.w+.attP]*
Target attP line -77C	77C4		*y w P{y+. nos-int.NLS}*; *P[attP.w+.attP]*
Target attP line - 82F7	82F7		*y w P{y+. nos-int.NLS}*; *P[attP.w+.attP]*

[a]corresponds to cytogenetic position of docking site [b] Stock no. [c] *[>]* represents *FRT*-containing intron[6]. All stocks were checked in a preliminary round of experiments to verify that green clones were produced. (Red clones were not always visible without antibody staining.) Thereafter, GR stocks 82_18-2_Flp and 82_25-2_Flp, and RG stocks 82_20-4 and 82_25-3_Flp were routinely used. [d]These constructs carry the CD8 complement sequence at the 5' ends. [e]To facilitate the injection process, we have introduced an X-chromosome carrying the ϕC31 integrase under the control of the *nanos* promotor[1].

Supplementary Table 4

Ratios of red/green twins to yellow clones in imaginal discs and brains.

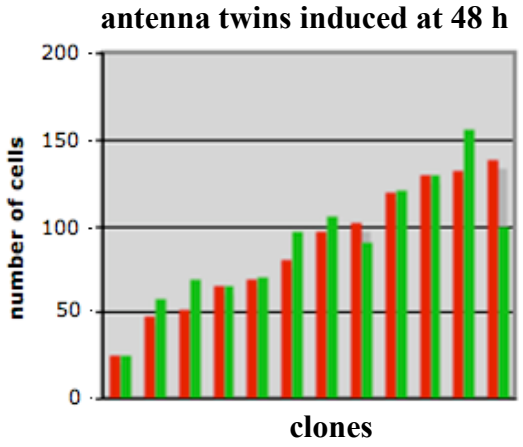
Tissue	# samples	# clones	time of clone induction	time of dissection	hs conditions (37^{o})	% green/red twins	% yellow clones
Eye-antennal discs	23	232	48 h AED	120	10, 15 or 20 min	51	49
Leg discs	57	80	”	”	”	54	46
Larval brain	62	335	2º instar	3-6 h later	40	33	67

If we assume that the frequency of G2-Z segregation (if it occurs at all[2,3]) is constant in different cell types, then the differences in the relative frequencies of green/red twin spots (from G2-X segregation) and yellow clones (from either G0 and G1 recombination or G2-Z segregation) in different tissues most likely reflect differences in the fraction of cells in G1 and G2.

Supplementary Table 5

Clone cell counts and doubling times.

Antenna induced at 48 h harvest 120 h			Eye induced at 48 h harvest 120 h	
	number of red cells	number of green cells	number of red cells	number of green cells
	26	26	29	39
	48	57	33	29
	51	69	42	26
	65	65	45	40
	69	70	58	58
	80	97	58	83
	97	106	60	47
	102	90	73	100
	120	121	92	111
	129	129	120	122
	132	156	129	170
	138	100	180	138
average	**88**	**90**	**77**	**80**
Cell doubling time		**9.6 hours**		**9.9 hours**



Antenna induced at 72 h harvest 120 h			Eye induced at 72 h harvest 120 h	
	number of red cells	number of green cells	number of red cells	number of green cells
	4	3	2	2
	5	11	2	2
	8	9	2	3
	9	14	2	3
	10	10	3	2
	10	8	3	2
	15	9	3	4
			4	2
			4	3
			4	6
			6	3
			6	3
			6	7
			8	4
			8	4
			8	8
			8	10
			9	13
			10	12

			10	6
			12	12
			12	12
			13	11
			14	10
			14	15
			18	14
			18	14
			18	18
			20	16
average	**9**	**9**	**9**	**8**
Cell doubling time		**11.5 hours**		**11.9 hours**