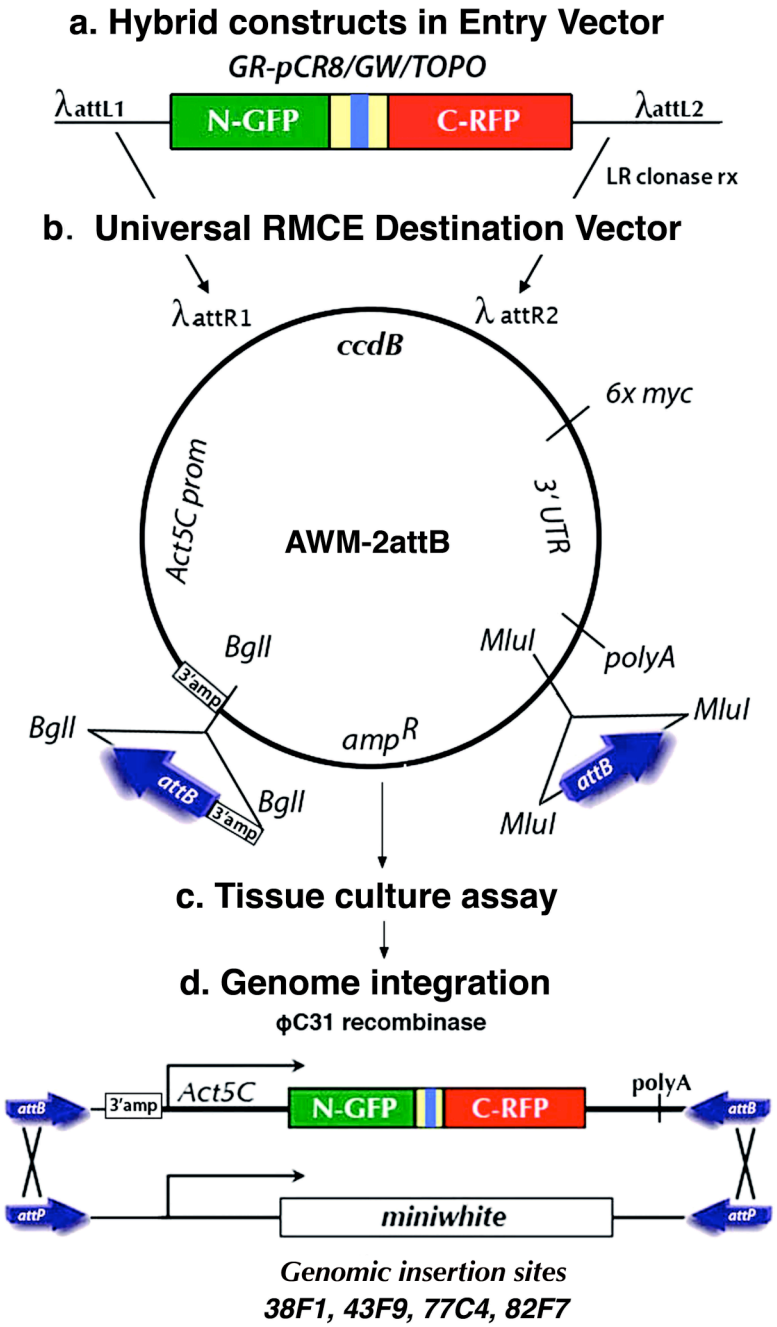
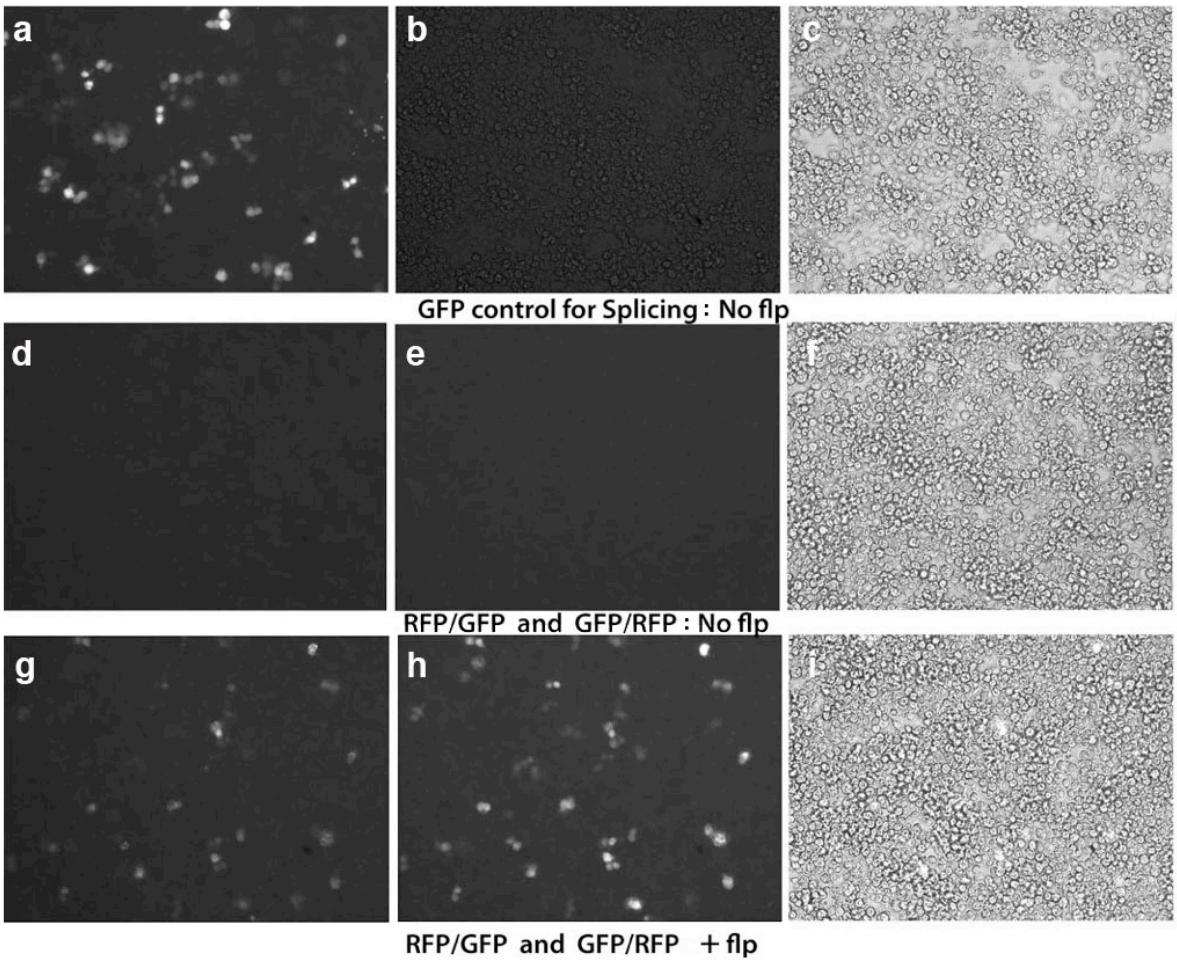
Supplementary Figure 1
TSG experimental strategy.



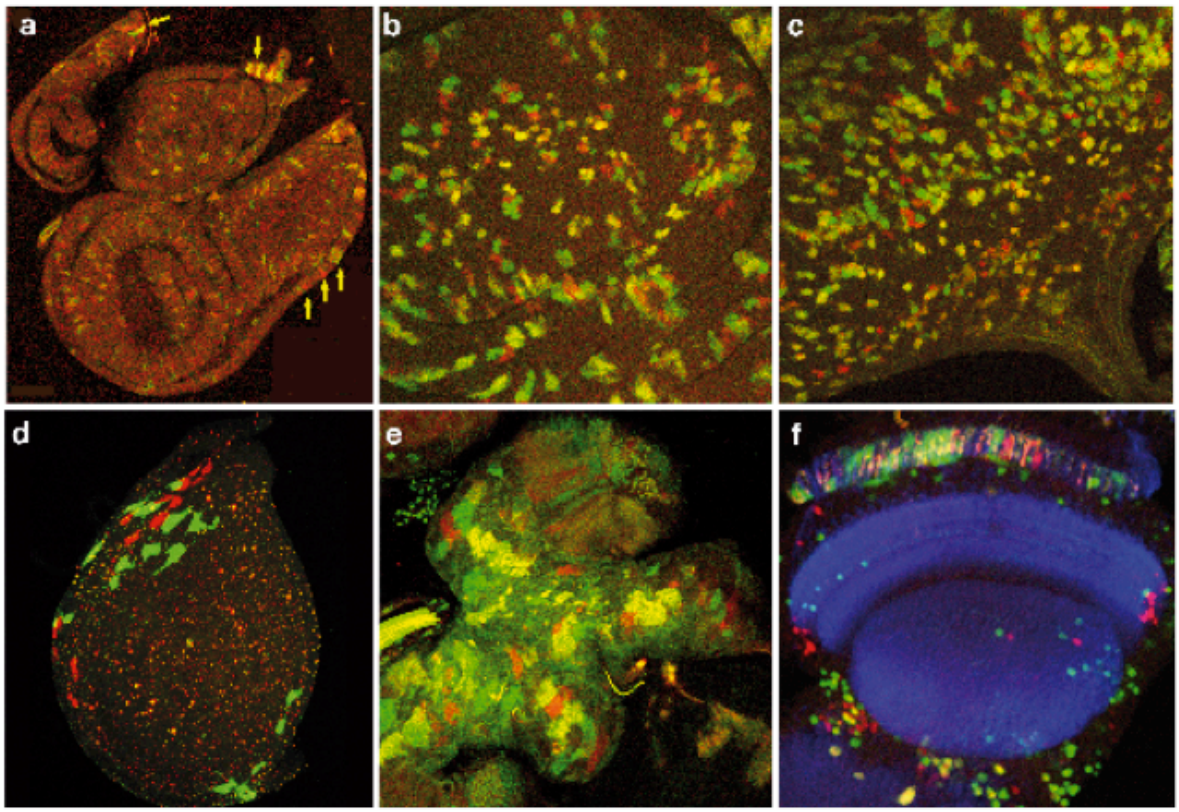
(a-d) Protocol. (a) *GR* construct in entry vector pCR8-GW-TOPO. (b) AWM-2attB accepts open reading frames (ORFs) from entry vector through LR clonase reaction. (c) Functional hybrid cassettes are identified by tissue culture assay. (d) Irreversible attB-attP recombination integrates expression cassettes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

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



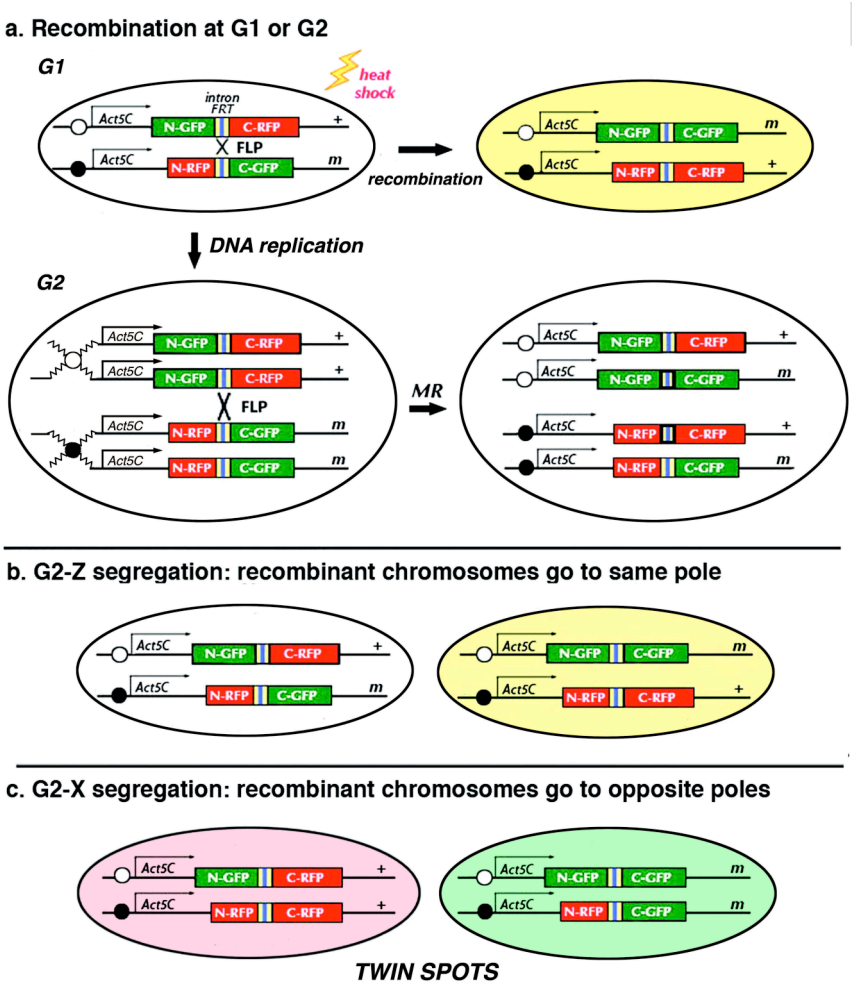
a-c. Transfection of control plasmid *GG*-AWM-2attB (non-hybrid, but interrupted, sequence *N-GFP[>]C-GFP* in AWM-2attB). Generation of GFP signal confirms that transcription, splicing and translation are functional. d-f. Cotransfection of *GR*-AWM-2attB and *RG*-AWM-2attB. Lack of any detectable signal 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.

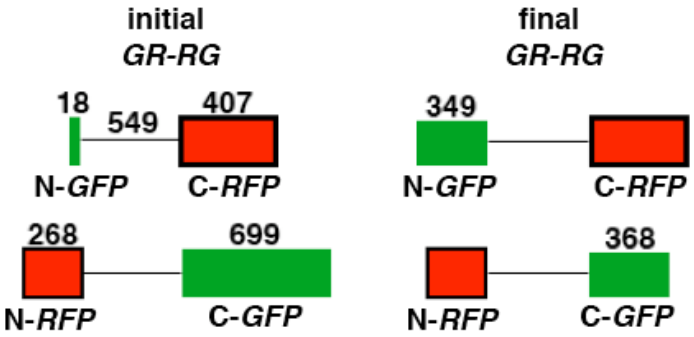
Supplementary Table 1
Nucleotide sequences of hybrid partner DNA.

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)	"

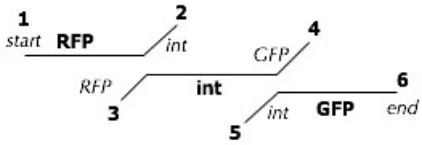
[a]We significantly improved GFP signal quality by splitting the EGFP coding sequence at position 349 and generating a new reciprocal pair of hybrid sequences, the final *GR* and *RG* constructs, which were again inserted at 82F7 to create new TSG fly lines. Heat shock treatment of the progeny from these 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.

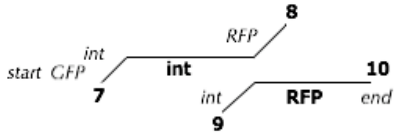
Primers used in constructing initial *RG*

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



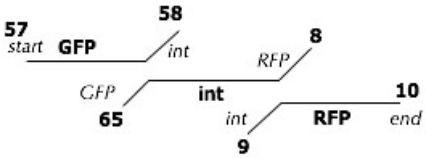
Primers used in constructing initial *GR*

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



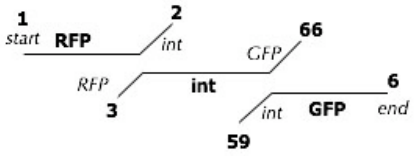
New primers used in constructing final *GR*

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.

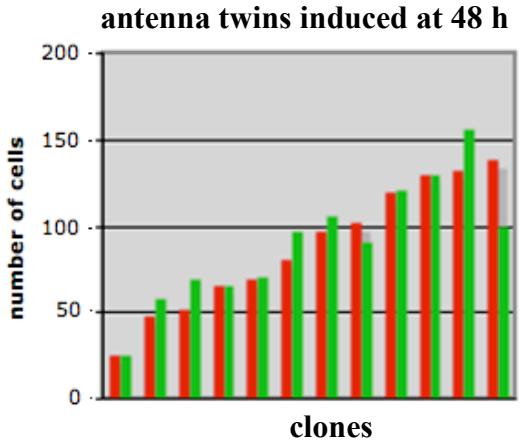
Tissue	# samples	# clones	time of clone induction	time of dissection	hs conditions (37^{o})	% green/red twins	% yellow clones
Eye-antennal discs	23	232	48 h AED	120	10, 15 or 20 min	51	49
Leg discs	57	80	”	”	”	54	46
Larval brain	62	335	2º instar	3-6 h later	40	33	67

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**