

Supplementary Figure 1. Full schematic depicting the G-TRACE analysis system. A) Individual Gal4expressing lines are crossed to the G-TRACE analysis test stock, which has the genotype UAS-RedStinger UAS-FLP Ubi-p63FRT>STOPFRT>nEGFP. B) Cells expressing Gal4 activate the expression of RFP and FLP recombinase, which in turn mediates the excision of the FRT-flanked STOP cassette. Subsequently, nEGFP is expressed from the Ubi-p63E promoter and is heritably maintained in all daughter cells, even cells in which Gal4 expression is subsequently downregulated ("POST-ACTIVE"). The circles at the bottom of the figure represent cells that appear red or yellow (left), when Gal4 activates RFP expression followed by EGFP expression, or green (right), when Gal4 expression has been downregulated such that cell only express the lineage-tracing marker EGFP.



Supplementary Figure 2. The G-TRACE analysis system recapitulates real-time expression patterns and reveals prior expression via lineage tracing. Comparison of lacZ expression patterns with G-TRACE expression patterns for three well-characterized genes in the late third instar wing imaginal disc. *hh-lacZ* expression (A, β gal) and *hh-gal4* expression (B, RFP) are restricted to the posterior (P) wing compartment. Lineage-traced EGFP expression (C) is also restricted to the P compartment indicating that *hh-gal4* expression is limited to P compartment cells throughout wing development; Scale bar: 50 microns. *ptc-lacZ* (D, β gal) and *ptc-gal4* (E, RFP) are expressed similarly in a stripe of cells in the anterior (A) compartment along the A/P boundary. By contrast, lineage tracing (F, EGFP) reveals that the entire A compartment either expressed *ptc-gal4* at one point or that all A compartment cells are derived from precursors, such as those at the A/P boundary, that expressed *ptc-gal4*; Scale bar: 50 microns. *dpp-lacZ* (G, β gal) and *dpp-gal4* (H, RFP) are, like *ptc* (compare to D and E), expressed in a stripe of A compartment cells at the A/P boundary. Despite the similarity in expression of real-time RFP patterns, the lineage-traced pattern for *dpp-gal4* (I, EGFP) is markedly different from that of *ptc-gal4* (compare to F), indicating that these Gal4 lines are differentially regulated during the course of wing disc development; Scale bar: 50 microns.



Supplementary Figure 3. A survey of G-TRACE patterns in the late larval brain. Low-magnification images (whole third-instar larval brain, first column) of several known and unknown Gal4-expressing lines showing real-time (RFP) and lineage-traced (EGFP) patterns throughout the late third instar brain (blue, DNA). Close-up images of single brain hemispheres detailing G-TRACE real-time (RFP, current, second column) and lineage-traced (EGFP, lineage, third column) expression patterns (MERGE, fourth column). A-D) OK107-gal4 expression is restricted to the mushroom body at this stage (B) but exhibits extensive lineage tracing in the optic lobe (C, D). E-H) Gal4-ET NP0114 expression is also highly restricted to the mushroom body (F) but traces only subset of cell clusters in the optic lobe (G, H). I-L) Like OK107-gal4 and NP0114, real-time expression of Gal4-ET NP0189 is highly restricted to the mushroom body (J), but traces significantly more cell clusters (K, L) than NP0114. M-P) gcm-gal4 exhibits real-time RFP expression in both a subset of mushroom body as well as the optic lobe (N), however most of the lineage-tracing is restricted to the optic lobe (O, P). Another observed pattern was one in which both the real-time and lineage-traced expression patterns were found to be restricted primarily to the optic lobes. Gal4-expressing lines that exhibit such a pattern include ptc-gal4 (Q-T), dome-gal4 (U-X), and upd-gal4 (Y-B'). While variable in extent, most Gal4-expressing lines examined were found to trace brain surface alia. Scale bars in Column 1 are 100 microns. Scale bars in Column 4 are 50 microns.



Supplementary Figure 4. G-TRACE analysis of late larval lymph glands. A) Schematic depicting lymph gland structure in late embryos and late larvae; only one half is depicted. The lymph gland primordium flanks dorsal vessel cardioblast and is anterior to pericardial cells that will develop into nephrocytes. The posterior signaling center (PSC) is already specified by this stage. In late 31L, the lymph gland has grown and the medullary and cortical zones have formed. The medullary zone consists of blood progenitor cells where as the cortical zone contains differentiating hemocytes. Real-time and lineage-traced expression of Cg-gal4 (B-D) is restricted to the cortical zone and absent from the medullary zone (MZ). Many RFP-negative, EGFP-positive cells can be observed in the cortical zone in C. Real-time expression of dome-gal4 (E-G) is restricted to the medullary zone, but lineage-traces both the medullary and cortical zones. Real-time and lineage-traced expression of Antp-gal4 (H-J) is restricted to the PSC. A few RFP-negative, EGFP-positive cells can be observed (J, arrows). Real-time expression of Dot-gal4 (K-M) is restricted to the PSC (K, arrow), but lineage-traces cells throughout the lymph gland. Scale bars are 50 microns.



Supplementary Figure 5. Temporal analysis of G-TRACE patterns with several Gal4 drivers. G-TRACE on gcmgal4 (A-D; inset: single optical section), NP0224 (E-H), and Dot-gal4 (I-L) lines at early and late developmental stages, as indicated. LG, lymph gland. PSC, posterior signaling center. 31L, third instar larvae; emb, embryo. Scale: (C-D, 25 μm; K-L, 10 μm; Others, 50 μm).



Supplementary Figure 6. The efficiency of Gal4-based lineage marking. G-TRACE analysis using Ubiquitin-gal4 (Ubi-gal4) essentially marks all cells, although some cells exhibit differences in level of expression. In the wing hinge regions (A-D) virtually all cells express EGFP however a few cells near the lateral edges have low or undetectable levels of RFP expression. Similarly, nearly all cells express EGFP in the wing pouch (E-H) although cells of the dorsal/ventral boundary exhibit low-level expression, suggesting that the Ubi-p63E promoter is less active in these cell types. This interpretation is consistent with the pattern of RFP expression (E), which is also low in the dorsal/ventral boundary. Based upon numerous examples like these, it appears that FLP-out and EGFP expression can occur in all cells, with the major limiting factor being the strength of the Gal4 line being used. For example, the hand gene is expressed throughout the cardiogenic mesoderm from which the lymph gland is derived. Furthermore, the hand-gal4 reporter line has been shown to recapitulate this pattern and be active in all such cells including the developing lymph gland. Using hand-gal4 with G-TRACE, however, often generates sectors of untraced cells in the lymph gland (I, arrowhead). Furthermore, these negative sectors are reproducibly located in the periphery of the primary lobes, indicating that precursors of these cells likely have lower levels of Gal4 activity relative to precursors of cells position medially. Indeed, examination of late embryo and first instar larvae (not shown) demonstrated that, although expressed throughout the lymph gland, cells located laterally, away from the midline, exhibited markedly lower level RFP expression (J, arrows). Dotted lines outline lymph gland cells and dorsal vessel cardioblasts (DV) visible in a single optical section. 31L, third instar larvae; emb, embryo.

Supplementary	Table 1. G-TRACE	expres	sion patt	erns in t	he late l	arval bra	ain				
		Expression			Combined	Dura in	Ontic Lobo		C II-		
Stock ID	ND Idoptifior	None	DED	ECED	DED	ECER		ECER		ECED	Comment
103526	NP0112		NEF	LOFF	NIP	LOFF	NEF	LGFP	NFF	LOFF	CB REP in MB?: minor EGEP in CB
103528	NP0114	-									CB RFP strong in MB
103535	NP0131										possible minor CB RFP
103539	NP0138										low sample number
103544	NP0158										minor RFP expression in CB
103546	NP0168										possible minor OL RFP
103548	NP0173										
103549	NP0176										
103555	NP0189										
103558	NP0219										
103561	NP0224										highly specific EGFP in laminar placode
103566	NP0239										low OL RFP, low CB EGFP
103568	NP0244										low expession levels
103573	NP0269										CB RFP/EGFP in MB? and elsewhere
103579	NP0293										
103586	NP0324										
103592	NP0346	-									CB RFP strong in MB
103697	NP0652										low RFP in CB/OL, low EGFP in CB
103701	NP0662										
103704	NP0675										CB RFP strong in MB
103707	NP0680										
103715	NP0699										possible OL EGFP
103/18	NP0708										RFP/EGFP in MB
103720	NP0723										
103730	NP0729										
103780	NP0829										RFP strong in CB, weaker in OL along furrow
103783	NP0837										
103795	NP0864										
103799	NP0873										
103803	NP0883										
103811	NP0902										
103830	NP0911 NP0949										CB REP strong in MB
105050	111 0545										
	Other Gal4 lines										
BL 8699	Hemese										
BL 1767	how[24B]										
BL 8860	Bx[MS1096]										
BL 2736	5108										
BL 6902 BL 3750	D0t		*								
BL 7011	Collagen										
BL 8641	daughterless		*								
	gcm										
BL 6773	Grunge								-		
	hand								-		
DI 054	hedgehog										
BL 854	OK107										
PG14 PG33	dome										
PG50											
	Peroxidasin										
BL 2017	patched[559.1]										
	serpent-Hemo										
	Ubx										
	upd[E132]	<u> </u>									
	LEGEND				Lines no	t scored.	103557	103797 1	03802	03810 F	1 3L 2721, Antp-gal4, BL 7031, BL 1553, BL 3039
	No expression										
	RFP expression										
	EGFP expression										
	sporadic	*									

Supplementary	elementary Table 2. G-TRACE expression patterns in the late larval eve-antenna imaginal disc								lisc											
		Express	sion																	
		None	Ubiquit	ous	Eve Spec	ific	Ant. Spe	cific	PMF		MF		AMF		PPM		Ant.	Ant.	Comment	
Stock ID	NP Identifier		RFP	EGFP	RFP	EGFP	RFP	EGFP	RFP	EGFP	RFP	EGFP	RFP	EGFP	RFP	EGFP	RFP	EGFP		
103526	NP0112			*															increased clone density in ventral antenna	
103535	NP0131																		minor RFP at edges of eye/ant peripodial/disc?	
103539	NP0138																		Ant. EGFP in A-1 and A-2	
103544	NP0158																		Ant. RFP/EGFP specific to A-1, A-2; minor, variable EGFP in eye	
103546	NP0168		*																	
103548	NP0173																			
103549	NP0176																			
103553	NP0188																			
103554	NP0189			*																
103557	NP0211																		appears RFP downregulated PMF	
103558	NP0219		*																	
103561	NP0224																		variable, RFP in small clusters	
103566	NP0239		*																RFP in small cell clusters throughout	
103568	NP0244																		RFP/EGFP in clusters at A1 ring of antenna	
103573	NP0269								*	*							*	*	increased clone density in ventral antenna; variable expression	
103579	NP0293															*			possible EGFP in anterior antenna	
103585	NP0319																			
103586	NP0324																			
103592	NP0346														*				few sporadic EGFP clones	
103697	NP0652																			
103701	NP0662																		near ventral(?) opitc stalk	
103704	NP0675																			
103707	NP0680			*																
103715	NP0699																			
103718	NP0708																			
103726	NP0723																		ventral EGFP in antenna, RFP unclear	
103729	NP0727																			
103730	NP0729																		specific expression in A1; eye glia express EGFP	
103780	NP0829			*															variable, RFP in small clusters, primarily in antenna	
103783	NP0837			*															variable, RFP in small clusters	
103795	NP0864			*															variable, RFP in small clusters, peripodial?	
103797	NP0870																		RFP expression high in antenna and eye glia	
103799	NP0873																		variable, RFP in small clusters, primarily in antenna	
103802	NP0882																		variable, RFP in small clusters	
103803	NP0883														*	*				
103810	NP0898																		RFP high in dorsal antenna; variable RFP in small clusters	
103811	NP0902																		RFP/EGFP expressed in three clusters in the A2 region	
103815	NP0911																			
103830	NP0949		*																	
	Other Gal4 lines																			
BL 2721	5015																		REP expression PME?	
BL 8699	Hemese																			
BL 1767	how[24B]																			
BL 7031	G115																			
BL 8860	Bx[MS1096]		*																	
BL 2736	5108																			
BL 6902	Dot		_																RFP/EGFP in the ventral side of the antenna disc	
BL 3750	C355																			
BL 7011	Collagen																		RFP/EGFP cluster in A3	
BL 8641	daughterless		*																	
DL 0//3	Grunge		——																Marianita, of alarma anatomian to the formation	
00140	nand mat (2)														-				Majority of clones posterior to the furrow	
PG142	cut (?)	-	l						I										KER HIGKS AKAT IN ONTENNO	
PG14	uome																			
PG53	SVI (?)			-																
PG50	N (?)																			
BL 3039	pannier[MD237]	_																	Restricted to anterior-most antenna and Bolwig's nerve	
01.0017	Peroxidasin								-										KFP/EGFP IN NEMOCYTES	
BL 2017	patched[559.1]	_							-										Verticle stripe at A/P boundary marked in antenna	
	serpent-Hemo																		REP/EGEP In circulating nemocytes	
	UDX		—																KEP is expressed in dorsal margin of eye; anterior-most region in antenna	
	upu(E132)			 															KEP is expressed near the optic stark; EGEP is expressed in ventral region of e	eye/antenna
	LECEND		-				Lines ask		1	ter meld	01 1552		DI 054							
	No expression			+			Lines not	scorea: 10	13328, An	r.p-gai4,	DL 1553,	ycm-gal4	, BL 854							
	NO expression			1																
	KEP expression			+			-							<u> </u>						
	corr expression			+											-					
	Apt	Antonce		<u> </u>					<u> </u>											
	DME	noctoria	r to the -	nombor-	notic furrer		-													
	ME	morph	n co une n	norpriode	near inito	r	-		-	-										
	AME	anterior	to the m	ornhoace	atic furrow				I					<u> </u>				-		
	DDM	poring di	al month	cape of the second	cacianow															
	PPP1	veripodi	ai membi														1	1		

Supplementary	Table 3. G-TRACE	E expression patterns in the late larval wing imaginal disc												
		Express	sion											
		None	Ubiquit	ous	Compar	tment	Pouch		Hinge/I	Notum	PPM		Comment	
Stock ID	NP Identifier		RFP	EGFP	RFP	EGFP	RFP	EGFP	RFP	EGFP	RFP	EGFP		
103526	NP0112			*									minor REP in pouch, possible proneural clusters	
103528	NP0114												minor, variable	
103535	NP0131													
103539	NP0138												minor, variable	
103544	NP0158							*				*		
103546	NP0168		*										expressed in trachea	
103548	NP0173												expresse in trached	
102540	NP0176									<u> </u>				
103545	ND0199									<u> </u>			variable Bauch REP expression in small clusters	
103555	NP0100												variable Fouch Ri F expression in small clusters	
103554	NP0169												himor RFP III pouch, possible proheural clusters	
103557	NP0211									<u> </u>			niger KFP in pouch?, unclear	
103556	NP0219													
103501	NP0224									<u> </u>				
103566	NP0239									<u> </u>			FOED is the short	
103568	NP0244		*	*						<u> </u>			EGFP in tracheal	
103573	NP0269		· · ·											
103579	NP0293												RFP/EGFP in trachea	
103585	NP0319													
103586	NP0324													
103592	NP0346												possible trachea EGFP, few PPM clones	
103697	NP0652			_									minor RFP in pouch, possible proneural clusters	
103701	NP0662												RFP/EGFP in trachea	
103704	NP0675		L											
103707	NP0680												minor RFP in pouch, possible proneural clusters	
103715	NP0699												RFP expression primarily in pouch along A/P boundary	
103718	NP0708													
103726	NP0723												expressd in trachea	
103729	NP0727												minor, variable RFP	
103730	NP0729												RFP specific to hinge regions	
103780	NP0829												increased EGFP clone density at anterior compartment edge?	
103783	NP0837												minor, variable RFP	
103795	NP0864												minor, variable RFP	
103797	NP0870													
103799	NP0873												minor, variable REP throughout, strong REP cluster in notum	
103802	NP0882												minor, variable REP throughout	
103803	NP0883											*	minor variable REP throughout	
103811	NP0902													
103815	NP0011		*											
103830	NP0040													
105050	1110545		-											
	Other Gal4 lines													
BI 2721	5015													
DL 2721	Homoco													
BL 1767	how[24P]													
BL 7021	C115													
BL 7031	G115 Bu[MC1006]													
DL 0000	DX[M31090]													
BL 2736	5108									<u> </u>				
BL 6902	-255									<u> </u>				
BL 3750	C355		<u> </u>											
DL /U11	conagen					-		1		<u> </u>				
DL 8041	uaugnteriess		*					1		<u> </u>				
BL 1553	upp			I						<u> </u>			KFP expressed at A/P boundary. EGFP expressed in anterior compartment.	
BL 6//3	Grunge			I						<u> </u>				
	nand													
	hedgehog			<u> </u>									Posterior compartment	
PG142													RFP/EGFP restricted to notum; expressed in trachea.	
PG14	dome		L										Low sample	
PG33													RFP/EGFP in trachea	
PG50														
BL 3039	pannier[MD237]		L	L				L						
	Peroxidasin			L									RFP/EGFP in hemocytes	
BL 2017	patched[559.1]		L	L									RFP expressed in A/P boundary. EGFP expressed in anterior compartment.	
	serpent-Hemo			L										
	serpent													
	Ubx												RFP expressed in trachea	
	upd[E132]													
	LEGEND					Lines no	t scored:	103810,	Antp-gal4	, gcm-ga	14, BL 854	4		
	No expression													
	RFP expression													
	EGFP expression													
	sporadic	*	[
	PPM	peripodia	al membr	ane										

Supplementary	ary Table 4. G-TRACE expression patterns in the late larval lymph gland																
		Express	ion														
		None	Ubiquit	ous	Primary		Seconda	ary	Tertiary		DV		PC		SS		Comment
Stock ID	NP Identifier		RFP	EGFP	RFP	EGFP	RFP	EGFP	RFP	EGFP	RFP	EGFP	RFP	EGFP	RFP	EGFP	
103526	NP0112																minor, variable
103528	NP0114																
103535	NP0131			*													minor variable
103535	NP0158																Innor, variable
103546	NP0158	-	-														
103548	NP0173																
103549	NP0176																
103553	NP0188																variable in lymph gland lobes
103554	NP0189																RFP in seven-up subset
103557	NP0211																
103558	NP0219																minor expression in primary lobe/cortical zone?
103561	NP0224																
103568	NP0244																expression in stringy stuff and ring gland
103573	NP0269																
103586	NP0324																
103592	NP0346																possibly in circulating hemocytes
103697	NP0652																
103701	NP0662																
103704	NP0675			*													
103/0/	NP0680																ible CCi
103726	NP0099																possible 55 expression
103729	NP0727			*													
103730	NP0729																
103780	NP0829																
103783	NP0837																minor, variable
103795	NP0864																minor, variable
103797	NP0870																RFP mostly in primary, minor in tertiary; mature cells?
103799	NP0873																
103802	NP0882																minor, variable
103803	NPU883																RER pattern unclears inconsistent
103811	NP0902																Kir pattern unclear, inconsistent
103815	NP0911																
103830	NP0949																EGFP also in ring gland
	Other Gal4 lines																
BL 2721	5015																
BL 8699	Hemese														-		RFP/EGFP expression may be in cortical zone
BL 1767	how[24B]																Expressed III PSC
BL 7031	G115																
BL 8860	Bx[MS1096]	-															
BL 2736	5108																
BL 6902	Dot																RFP expressed in the PSC
BL 3750	c355		*														
BL 7011	Collagen																RFP/EGFP in cortical zone
BL 8641	daughterless		*														RFP low in hemocytes relative to DV, etc.
DL 0773	Grunge																
	hedgehog					<u> </u>											RFP is in pericardial cells?
PG142																	RFP/EGFP restricted to mature hemocytes
PG14	dome																RFP expressed in medullary zone
PG33																	
PG50															L		
BL 3039	pannier[MD237]	<u> </u>													l		RFP/EGFP restricted to mature hemocytes
DI 2017	Peroxidasin																RFP/EGFP restricted to mature hemocytes
DL 2017	parcned[559.1]																
	serpent-nemo																Possible EGEP in primary lobe expression
	Ubx																RFP/EGFP possibly in pericardial cells
	upd[E132]																· · · · · · · · · · · · · · · · · · ·
	LEGEND	J	L		I										I		
	No expression					Lines not	t scored:	103579,	103585, 1	L03718, E	<u>L 1553, c</u>	cm-gal4,	BL 854				
	KFP expression																
	sporadic	*			-												
	DV	dorsal ve	essel														
	PC	pericardi	al cells														
	SS	stringy s	tuff, a lor	a, thin ce	ellular stru	icture ass	ociated w	ith secon	darv and	tertiary l	nhes						

Supplementary Table 5	Primers sequences for amplification of the Ubi p63E promoter
Forward	5' GAG AAT TCT GTC CGT ATC CTT TAG GC 3'
Reverse	5' GAG GTA CCT TGG ATT ATT CTG CGG GA 3'

Supplementary Data

G-TRACE expression patterns in four tissues











103802			
103803			
103810			
103811	A Contraction of the second seco		
103815		Contraction of the second	Constant of the second
103830			
5015			

Antp





collagen









daughterless







Constitution 1



Dpp

Gcm



Grunge









hand









hedgehog









Hemese









OK107



















































pannier

