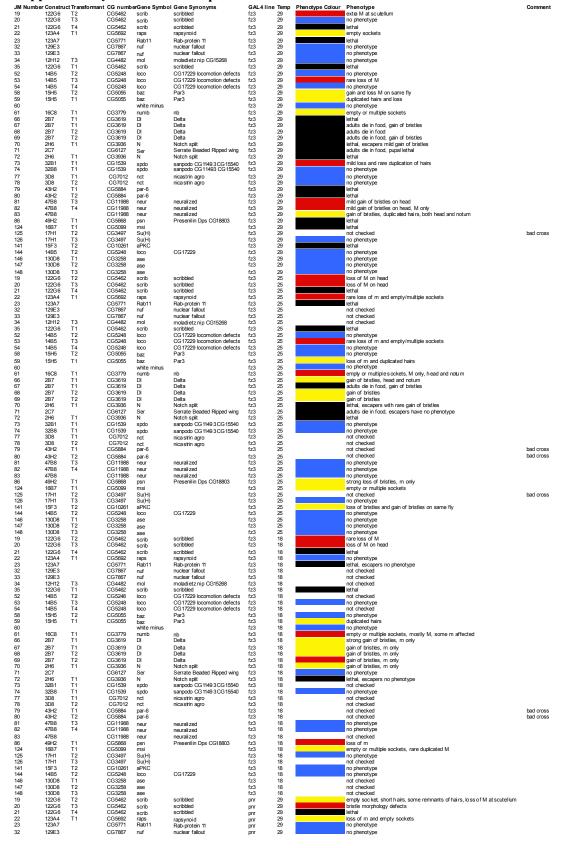
# SUPPLEMENTARY INFORMATION

#### **Supplementary Table 1. Bristle prescreen results**



#### Supplementary Table 1

			ant CC numb	orCono Sumbo	J Cana Sumanuma	CAL 4 lin	Tomo	Phenotype Colour	Phonotimo	Comment
33	129E3		CG7867	nuf	ol Gene Synonyms nuclear fallout	pnr	29	Phenotype Colour	not checked	bad cross
34 35	12H12 122G6	T3 T1	CG4482 CG5462	mol scrib	moladi etz ni p CG15268 scribbled	pnr	29 29		no phenotype lethal	
52 53	14B5	T2	CG5248	loco	CG 17229 locomotion defects	pnr	29 29		no phenotype loss of m and empty sockets	
54	14B5 14B5	T3 T4	CG5248 CG5248	loco loco	CG17229 locomotion defects CG17229 locomotion defects	pnr	29		no phenotype	
58 59	15H5 15H5	T2 T1	CG5055 CG5055	baz baz	Par3 Par3	pnr	29 29		duplicated hairs and loss duplicated hairs and loss	
60				white minus		pnr	29		no phenotype	
61 66	16C8 2B7	T1 T1	CG3779 CG3619	numb DI	nb Delta	pnr	29 29		strong empty or multiple sockets strong gain of bristles	
67	2B7	T1	CG3619	DI	Delta	pnr	29		strong gain of bristles	
68 69	2B7 2B7	T2 T2	CG3619 CG3619	DI DI	Delta Delta	pnr	29 29		strong gain of bristles strong gain of bristles	
70 71	2H6 2C7	T1	CG3936 CG6127	N Ser	Notch split Serrate Beaded Ripped wing	pnr	29 29		notum death malformation, loss of bristles, duplicated hairs no phenotype	
72	2H6	T1	CG3936	N	Notch split	pnr	29		notum death malformation, loss of bristles	
73 74	32B1 32B8	T1 T1	CG1539 CG1539	spdo spdo	sanpodo CG11493CG15540 sanpodo CG11493CG15540	pnr	29 29		stronger loss of bristles, rare duplication of hairs no phenotype	
77	3D8	T1	CG7012	nct	nica strin agro	pnr	29		no phenotype	
78 79	3D8 43H2	T2 T1	CG7012 CG5884	nct par-6	nica strin agro	pnr	29 29		no phenotype lethal	
80	43H2	T2	CG5884	par-6		pnr	29		lethal	
81 82	47B8 47B8	T3 T4	CG11988 CG11988	neur neur	neuralized neuralized	pnr	29 29		gain of bristles, duplicated hairs mild gain of bristles on head and notum	
83 86	47B8 49H2	T1	CG11988 CG5868	neur psn	neuralized Presenil in Dps CG18803	pnr	29 29		gain of bristles, duplicated hairs strong loss of bristles	
124	16B7	T1	CG5099	msi	т тезенит Бра ССТООО	pnr	29		strong duplication of hairs and empty/multiple sockets	
125 126	17H1 17H1	T2 T3	CG3497 CG3497	Su(H) Su(H)		pnr	29 29		no phenotype no phenotype	
141	15F3 14B5	T2	CG10261	aPKC	CG17229	pnr	29 29		lethal	
144 146	130D8	T2 T1	CG5248 CG3258	loco ase	CG 17229	pnr pnr	29		rare empty sockets no phenotype rare multiple sockets, loss of bristles	
147 148	130D8 130D8	T2 T3	CG3258 CG3258	ase ase		pnr	29		rare multiple sockets, loss of bristles no phenotype	
19	122G6	T2	CG5462	scrib	scribbled	pnr	29 25		loss of notum m and empty so ckets	
20 21	122G6 122G6	T3 T4	CG5462 CG5462	scrib scrib	scribbled scribbled	pnr pnr	25		rare loss of notum m and empty sockets lethal	
22	123A4	T1	CG5692	raps	rapsynoid	pnr	25 25		loss of m and empty/multiple sockets	
23 32	123A7 129E3		CG5771 CG7867	Rab11 nuf	Rab-protein 11 nuclear fallout	pnr	25 25		lethal? not checked	
33	129E3		CG7867	nuf	nuclear fallout	pnr	25		not checked	
34 35	12H12 122G6	T3 T1	CG4482 CG5462	mol scrib	moladi etz ni p CG15268 scribbled	pnr	25 25		not checked lethal	
52 53	14B5 14B5	T2 T3	CG5248 CG5248	loco loco	CG 17229 locomotion defects CG 17229 locomotion defects	pnr	25 25		no phenotype rare loss of m and empty/multiple sockets	
54	14B5	T4	CG5248	loco	CG 17229 locomotion defects	pnr	25		no phenotype	
58 59	15H5 15H5	T2 T1	CG5055 CG5055	baz baz	Par3 Par3	pnr pnr	25 25		loss of m and rare empty/multiple sockets, rare duplicated hairs loss of m and duplicated hairs	
60				white minus		pnr	25		no phenotype	
61 66	16C8 2B7	T1 T1	CG3779 CG3619	numb DI	nb Delta	pnr	25 25		strong empty or multiple sockets strong gain of bristles	
67	2B7	T1	CG3619	DI	Delta	pnr	25		gain of bristles, m only	
68 69	2B7 2B7	T2 T2	CG3619 CG3619	DI DI	Delta Delta	pnr	25 25		gain of bristles gain of bristles	
70 71	2H6 2C7	T1	CG3936 CG6127	N Ser	Notch split Serrate Beaded Ripped wing	pnr	25 25		gain of bristles, some notum death malformation	
72	2H6	T1	CG3936	N	Notch split	pnr	25		no phenotype notum death malformation, loss of bristles	
73 74	32B1 32B8	T1 T1	CG1539 CG1539	spdo spdo	sanpodo CG11493CG15540 sanpodo CG11493CG15540	pnr	25 25		no phenotype no phenotype	
77	3D8	T1	CG7012	nct	nica strin agro	pnr	25		not checked	
78 79	3D8 43H2	T2 T1	CG7012 CG5884	nct par-6	nica strin agro	pnr	25 25		not checked not checked	bad cross
80	43H2	T2	CG5884	par-6		pnr	25		lethal	
81 82	47B8 47B8	T3 T4	CG11988 CG11988	neur neur	neuralized neuralized	pnr pnr	25 25		no phenotype no phenotype	
83 86	47B8 49H2	T1	CG11988 CG5868	neur	neuralized	pnr	25 25		no phenotype	
124	16B7	T1	CG5099	psn msi	Presenil in Dps CG18803	pnr	25		strong loss of bristles empty or multiple sockets, duplicated hairs	
125 126	17H1 17H1	T2 T3	CG3497 CG3497	Su(H) Su(H)		pnr	25 25		no phenotype	
141	15F3	T2	CG10261	aPKC		pnr	25 25		no phenotype notum death malformation	
144 146	14B5 130D8	T2 T1	CG5248 CG3258	loco ase	CG17229	pnr	25 25		no phenotype no phenotype	
147	130D8	T2	CG3258	ase		pnr	25		no phenotype	
148 19	130D8 122G6	T3 T2	CG3258 CG5462	ase scrib	scribbled	pnr	25 18		no phenotype rare loss of bristles	
20 21	122G6 122G6	T3 T4	CG5462 CG5462	scrib scrib	scribbled scribbled	pnr	18 18		no phenotype lethal	
22	123A4	T1	CG5692	raps	rapsynoid	pnr	18		no phenotype	
23 32	123A7 129E3		CG5771 CG7867	Rab11 nuf	Rab-protein 11 nuclear fallout	pnr pnr	18 18		lethal not checked	
33	129E3		CG7867	nuf	nuclear fallout	pnr	18		not checked	
34 35	12H12 122G6	T3 T1	CG4482 CG5462	mol scrib	moladi etz ni p CG15268 scribbled	pnr	18 18		not checked lethal, escapers with strong no turn malformation	
52	14B5	T2	CG5248	loco	CG 17229 locomotion defects	pnr	18		not checked	
53 54	14B5 14B5	T3 T4	CG5248 CG5248	loco loco	CG 17229 locomotion defects CG 17229 locomotion defects	pnr	18 18		no phenotype not checked	
58 59	15H5 15H5	T2 T1	CG5055 CG5055	baz baz	Par3 Par3	pnr	18 18		no phenotype duplicated hairs	
60				white minus		pnr	18		no phenotype	
61 66	16C8 2B7	T1 T1	CG3779 CG3619	numb DI	nb Delta	pnr	18 18		strong empty or multiple sockets strong gain of bristles, m only	
67 68	2B7	T1	CG3619 CG3619	DI	Delta Delta	pnr	18		gain of bristles, m only	
69	2B7 2B7	T2 T2	CG3619 CG3619	DI DI	Delta	pnr	18 18		gain of bristles, m only not checked	bad cross
70	2H6	T1	CG3936	N	Notch split	pnr	18		gain of bristles, monly, rare notum death malformation	
71 72	2C7 2H6	T1	CG6127 CG3936	Ser N	Serrate Beaded Ripped wing Notch split	pnr	18 18		no phenotype lethal, escapers loss of bristles and notum death malformation	
73	32B1	T1	CG1539	spdo	sanpodo CG 1149 3 CG 15540	pnr	18		not checked	
74 77	32B8 3D8	T1 T1	CG1539 CG7012	spdo nct	sanpodo CG11493 CG15540 nica strin agro	pnr	18 18		no phenotype not checked	
78 79	3D8 43H2	T2 T1	CG7012 CG5884	nct par-6	nica strin agro	pnr	18 18		not checked not checked	had cross
80	43H2	T2	CG5884	par-6		pnr	18		not checked	bad cross
81 82	47B8 47B8	T3 T4	CG11988 CG11988	neur	neurali zed neurali zed	pnr	18 18		no phenotype no phenotype	
83	47B8		CG11988	neur	neurali zed	pnr	18		not checked	
86 124	49H2 16B7	T1 T1	CG5868 CG5099	psn msi	Presenil in Dps CG18803	pnr	18 18		stronger loss of bristles, rare duplication of hairs empty or multiple sockets	
125	17H1	T2	CG3497	Su(H)		pnr	18		no phenotype not checked	
126 141	17H1 15F3	T3 T2	CG3497 CG10261	Su(H) aPKC		pnr	18 18		rare empty or multiple sockets, loss of bristles	
144 146	14B5 130D8	T2 T1	CG5248 CG3258	loco	CG17229	pnr	18 18		no phenotype not checked	
147	130D8	T2	CG3258	ase ase		pnr	18		not checked	
148 19	130D8 122G6	T3 T2	CG3258 CG5462	ase scrib	scribbled	pnr sca	18 29		not checked no phenotype	
20	122G6	T3	CG5462	scrib	scribbled	sca	29		no phenotype	
21 22	122G6 123A4	T4 T1	CG5462 CG5692	scrib raps	scribbled rapsynoid	sca sca	29 29		lethal rare duplicated hair	
23	123A7		CG5771	Rab11	Rab-protein 11	sca	29		lethal	
32 33	129E3 129E3		CG7867 CG7867	nuf nuf	nuclear fallout nuclear fallout	sca sca	29 29		no phenotype not checked	bad cross
34	12H12	T3	CG4482	mol	moladi etz ni p CG15268	sca	29		no phenotype	
35 52	122G6 14B5	T1 T2	CG5462 CG5248	scrib loco	scribbled CG 17229 locomotion defects	sca sca	29 29		lethal no phenotype	
53 54	14B5 14B5	T3 T4	CG5248 CG5248	loco loco	CG 17229 locomotion defects CG 17229 locomotion defects	sca sca	29 29		rare loss of M no phenotype	
54 58	14B5 15H5	T2	CG5248 CG5055	baz	Par3	sca sca	29		gain and loss M on same fly	

Colour Key

medium to strong phenotypes rare, weak phenotypes no phenotype lethal (even if escapers are present)

#### Supplementary Table 1. Phenotype Colour Phenotype no phenotype no phenotype empty or multiple sockets week gain of bristles, lethal lethal GAL4 line Temposaca 29 care 29 t CG numb CG5055 Comment nb Deta Deta Deta Deta Notch split Serrate Beaded Ripped wing Notch split sanpodo CG 1149 3 CG 15540 sanpodo CG 1149 3 CG 15540 nica stri ngro nica stri ngro CG3779 CG3619 CG3619 CG3619 CG3619 CG36127 CG3936 CG1539 CG7012 CG7012 CG7012 CG5884 CG5884 CG11988 CG11988 CG11988 CG11988 CG11988 T1 T1 T1 T2 T2 T1 T1 T1 T1 T2 T1 T2 T3 T4 lethal leithal no phenotype no phenotype no phenotype leithal neurali zed neurali zed neurali zed Presenil in Dps CG18803 T1 T1 T2 CG3497 CG10261 CG5248 CG3258 CG3258 CG3258 CG3562 CG5462 CG5462 CG5662 CG57867 CG7867 CG7867 CG5482 CG5248 CG5248 CG5248 CG5248 CG5248 CG5055 CG5055 T3 T2 T1 T2 T3 T2 T3 T4 T1 no phenotype no phenotype no phenotype no phenotype rare duplicated hair, loss of M no phenotype no phenotype no phenotype no phenotype lethal no phenotype lethal not checked not checked not checked scribbled scribbled scribbled rapsynoid Rab-protein 11 nuclear fallout nuclear fallout moladietz nip CG15268 scribbled T3 T1 T2 T3 T4 T2 T1 not checked strong loss of bristles, M only no phenotype no phenotype no phenotype no phenotype adults die in food, rare duplicated hairs no phenotype empty or multiple sockets, M only, head and notum lether, phrate adults with gain of histes adults die in food, escapers have no phenotype no phenotype moladietz nip CG15268 scribbled CG17229 locomotion defects CG17229 locomotion defects CG17229 locomotion defects Par3 Par3 16C8 2B7 2B7 2B7 2B7 2H6 2C7 2H6 32B1 32B8 3D8 43H2 47B8 47B8 47B8 49H2 16B7 17H1 17H1 17H1 17F1 15F3 14B5 CG3779 CG3619 CG3619 CG3619 CG3619 CG3936 CG6127 CG3936 CG1539 CG1539 CG7012 CG7012 CG5884 CG5288 CG11988 CG11988 CG5099 CG3497 CG3497 CG3497 CG5248 T1 T1 T1 T2 T2 T1 Notch split Serrate Beaded Ripped wing Notch split sanpodo CG11493CG15540 sanpodo CG11493CG15540 nicastrin agro nicastrin agro lethal, escapers mild gain of bristles no phenotype lethal no phenotype not checked not checked loss of bistles, head and notum, M only T1 T1 T1 T1 T2 T1 T2 T3 T4 bad cross neurali zed Presenil in Dps CG18803 T1 T2 T3 T2 T2 T1 T2 T3 T2 T3 T4 T1 no phenotype lethal no phenotype lethal, escapers with strong loss of bristles, M only not checked not checked not checked not checked not phenotype not checked 130D8 130D8 130D8 122G6 122G6 123A4 123A7 129E3 129E3 12H12 122G6 14B5 14B5 14B5 14B5 15H5 CG3258 CG3258 CG3268 CG5462 CG5462 CG5462 CG57867 CG7867 CG7867 CG7862 CG5482 CG5248 CG5248 CG5248 CG5248 CG5065 scribbled scribbled scribbled rapsynoid Rab-protein 11 nuclear fallout nuclear fallout motion etz nip CG15288 scribbled CG17229 locomotion defects (CG17229 locomotion defects (CG17229) comotion defects T3 T1 T2 T3 T4 T2 T1 no phenotype not checked nb Delta Delta Delta Delta Notch split Serrate Beaded Ripped wing Notch split sanpodo CG 1149 3 CG 15540 16C8 2B7 2B7 2B7 2B7 2H6 2C7 2H6 32B1 32B8 3D8 3D8 T1 T1 T1 T2 T2 T1 CG3619 CG3619 CG3619 CG3619 CG3936 CG6127 CG3936 CG1539 CG1539 CG7012 CG7012 no phenotype no phenotype no phenotype no phenotype no phenotype no phenotype lethal T1 T1 T1 T2 T1 T2 T3 T4 ethal not checked not checked in ophenotype not checked not checked fare loss of M not checked no phenotype not checked no phenotype not checked no phenotype grave loss of M, rare duplication of hairs no phenotype sanpodo CG 1149 3 CG 15540 nica strin agro nica strin agro 43H2 43H2 47B8 47B8 47B8 49H2 16B7 17H1 17H1 15F3 14B5 130D8 130D8 CG5884 CG5884 CG11988 CG11988 CG11988 CG5868 CG5099 CG3497 CG3497 CG10261 CG5248 CG3258 CG3258 bad cross T1 T1 T2 T3 T2 T2 T1 T2 T3 rare loss of N no phenotype not checked no phenotype no phenotype not checked not checked not checked CG17229

To identify a suitable GAL4 driver line for the genome-wide screen we tested *fzIII*-GAL4, *sca*-GAL4 and *pnr*-GAL4 at 18, 25 and 29 °C. Table shows the gene targeted by RNAi, temperature, driver and resulting phenotype. (pdf; 116 kb)

# Supplementary Table 3. GO Term over-representation among genes with similar phenotypes

GO Term	Number	P value
Growth and Proliferation Phenotypes ( <i>Poverprolif</i> >0)		
imaginal disc development	GO:0007444	2.34E-11
regulation of organ growth	GO:0046620	8.95E-08
cell proliferation	GO:0008283	4.33E-05
regulation of growth	GO:0040008	1.67E-04
Bristle Polarity Defects ( $P_{Polarity} \ge 4$ )	GO 0001 <b>53</b> 6	4.625.10
establishment of planar polarity	GO:0001736	4.62E-19
establishment of imaginal disc-derived wing hair	GO:0001737	2.87E-11
orientation		
Bristle Morphology Defects ( $P_{BMorph} > 0$ )		
mitochondrion	GO:0005739	8.75E-79
ribosome	GO:0005840	2.08E-39
primary metabolic process	GO:0044238	1.21E-13
cytoskeleton	GO:0005856	9.04E-05
translation	GO:0006412	9.16E-04
Asymmetric Cell Division	GG 000 <b>72</b> 00	0.005.10
nervous system development	GO:0007399	8.22E-12
asymmetric cell division	GO:0008356	5.23E-06
regulation of cell cycle	GO:0051726	1.28E-05
Notch signaling pathway	GO:0007219	2.96E-05
lateral inhibition	GO:0046331	7.80E-04
sensory organ precursor cell fate determination	GO:0016360	1.37E-03
spindle localization	GO:0051653	7.66E-03
Lateral Inhibition		
neurogenesis	GO:0022008	7.67E-10
Notch signaling pathway	GO:0007219	4.62E-05
cell fate commitment	GO:0045165	7.03E-05
lateral inhibition	GO:0046331	1.10E-03
imaginal disc-derived wing vein specification	GO:0007474	6.64E-03

Selected GO Terms and corresponding P-values for statistical over-representation compared to random gene sets of comparable sizes are shown for groups of genes that cause growth and proliferation, polarity, bristle morphology, asymmetric cell division and lateral inhibition phenotypes in the genome-wide screen. (doc; 79 kb)

#### Supplementary Table 4. False negative result data

Transformant ID	CG Number 5.7	Expected phenotype? (line)	Expected phenotype? (gene)
11257 15620	CG1007 CG10542	yes notum malformation death	yes notum malformation death
35507	CG11798	no	no
35508	CG11798	no	
10662	CG11988	yes	yes
44046 27044	CG12366 CG12676	yes lethal	yes yes
3087	CG12676	yes	,
938	CG12676	yes	
937 12647	CG12676 CG13281	yes lethal	lethal
12648	CG13281	lethal	Tetrial
13759	CG1520	yes	yes
13757	CG1520	yes	lothal
41134 44527	CG1725 CG17579	lethal yes	lethal yes
44528	CG17579	ves	,
43075	CG17697	yes (polarity)	yes
43077	CG17697	yes (polarity)	VOS
10855 43083	CG1856 CG18803	yes yes	yes yes
43082	CG18803	yes	,
28341	CG1903	lethal	lethal
19124 7769	CG2204 CG2534	no	no no
7005	CG2621	no no	no
16820	CG2855	yes	yes
16821	CG2855	yes	1/00
6858 6856	CG31020 CG31020	yes no	yes
14480	CG31020	no	no
14481	CG31152	no	
11690	CG32120	lethal	no lethal
9925 27187	CG3497 CG3619	no yes	yes
3720	CG3619	yes	,
37288	CG3619	yes	
37287	CG3619	yes	yes
7758	CG3779 CG3796	yes no	no
7756	CG3796	no	
7759	CG3796	no	20
5302 21469	CG3874 CG3874	no no	no
21468	CG3874	no	
47542	CG3874	no	
47543	CG3874	no	no
7796 7795	CG3929 CG3929	no no	110
1112	CG3936	yes	yes
27229	CG3936	yes	
27228 15565	CG3936 CG4260	yes lethal	lethal
15566	CG4260	lethal	100.101
21928	CG4713	lethal	lethal
8892 8893	CG4722 CG4722	no	yes
2914	CG5055	yes yes	yes
2915	CG5055	yes	
11784	CG5099	yes	yes
44895 41839	CG5099 CG5363	yes notum malformation death	notum malformation death
41838	CG5363	notum malformation death	notani manorination acati
24466	CG5460	yes	yes
33294	CG5820	lethal	yes
900 899	CG5820 CG5820	lethal lethal	
36301	CG5820	yes	
33295	CG5820	no	
27525	CG5841	yes	yes
27526 34340	CG5841 CG6235	yes yes	yes
34339	CG6235	yes	
35132	CG6897	yes	yes
35134 7376	CG6897 CG8075	yes	yes
48690	CG8075	yes no	no
6315	CG8384	yes	yes
6316	CG8384	yes	

Table indicates whether phenotypes in the genome-wide screen agree with published phenotypes for set of 42 genes previously described as members of the Notch signaling pathway or the asymmetric cell fate specification machinery. (pdf; 32 kb)

# Supplementary Table 5. Second UAS-hairpin transgenic line results

CG number 5.7 CG10210 CG10210	NIG-FLY	RNAi line ID 10210R-1 10210R-2	overlap 1st construct Same pheno. multi 1st lines?  0% no 0% no	Result (line) similar same	Result (gene) same
CG10261 CG10261	custom	1B1 T1 1B1 T2	0% 1 line 0% 1 line	lethal (p) lethal (p)	same (p)
CG10201 CG10618 CG10618	NIG-FLY	10618R-1 10618R-2	53% 1 line 53% 1 line	no pheno.	no pheno.
CG10776 CG10776	NIG-FLY	10776R-1 10776R-2	0% no 0% no	no pheno. no pheno.	no pheno.
CG10954 CG10954	NIG-FLY	10954R-1 10954R-4	40% 1 line 40% 1 line	same same	same
CG11101	NIG-FLY	11101R-2 11101R-3	0% no 0% no	similar (p)	similar (p)
CG11101 CG11940	NIG-FLY	11940R-2	0% no	similar (p)	similar
CG11940 CG11988	custom	11940R-3 1A3-5 T1	0% no 0% 1 line	similar same (p)	same (p)
CG12352	NIG-FLY	12352R-1	57% yes	same	same
CG12352		12352R-3	57% yes 0% 1 line	same (p)	same (n)
CG12366 CG12366	custom	1G3 1 T1 1G3 1 T2	0% 1 line	same (p) same (p)	same (p)
CG12484	NIG-FLY	12484R-1	0% yes	no pheno.	no pheno.
CG12484		12484R-2	0% yes	no pheno.	no nhono
CG12818 CG12818		12818R-1 12818R-3	59% no 59% no	no pheno. no pheno.	no pheno.
CG12880		12880R-1	0% no	no pheno.	no pheno.
CG13208		13208R-2	0% yes	same	same
CG13208 CG13472		13208R-3 13472R-1	0% yes 0% no	no pheno.	no pheno.
CG13472		13472R-3	0% no	no pheno.	no prieno.
CG13983	custom	1D4 b) T1	44% no	no pheno.	no pheno.
CG14721 CG14721		14721R-2 14721R-2	5% 1 line 5% 1 line	no pheno. similar	similar
CG14721 CG15543		15443R-1	63% 1 line	no pheno.	no pheno.
CG15543		15443R-2	63% 1 line	no pheno.	, , , , , , , , , , , , , , , , , , ,
CG15769		15769R-2	0% no	no pheno.	no pheno.
CG15769 CG16908		15769R-4 16908R-4	0% no 0% yes	no pheno. similar	similar
CG16993	NIG-FLY	16993R-1	0% no	same (p)	same (p)
CG16993 CG17559		16993R-2 17559R-2	0% no 36% no	same (p) no pheno.	no pheno.
CG17559	NIG-FLY	17559R-3	36% no	no pheno.	
CG17579 CG17657		17579R-1 17657R-1	0% yes 0% 1 line	similar (p)	similar (p)
CG17657		17657R-1 17657R-3	0% 1 line	same (p) same (p)	same (p)
CG17928		17928R-1	0% 1 line	no pheno.	no pheno.
CG17928		17928R-2 1804R-1	0% 1 line 0% no	no pheno.	different
CG1804 CG1804		1804R-1	0% no	different different	different
CG18214	NIG-FLY	18214R-1	0% no	different	different
CG18214		18214R-2	0% no	different	(-)
CG1903 CG2179		1903R-2 2179R-2	0% 1 line 0% 1 line	same (p) similar	same (p) similar
CG2183		2183R-1	59% no	no pheno.	no pheno.
CG31904		31904R-2	0% 1 line	same	same
CG31904 CG32707		31904R-3 4350R-2	0% 1 line 0% yes	same same	same
CG32707		4350R-1	0% yes	same	Jame
CG32732		12542R-3	0% 1 line	similar	similar
CG3298 CG3298		3298R-1 3298R-2	0% yes 0% yes	same same	same
CG3290 CG33193		13831R-1	0% yes	no pheno.	no pheno.
CG33193		13831R-2	0% no	no pheno.	·
CG33196		15637R-1 15637R-2	0% yes 0% yes	no pheno. similar	same
CG33196 CG33196		31724R-2	0% yes	same	
CG33196	NIG-FLY	31725R-1	0% yes	same	
CG33196		31725R-3	0% yes	same	
CG33196 CG33196		31727R-1 31727R-3	0% yes 0% yes	same same	
CG33205		32049R-1	0% yes	same	same
CG33205	NIG-FLY	32049R-2	0% yes	same	

Supplementary	y Table 5.				
CG number 5.7			overlap 1st construct Same pheno. multi 1st lines?		Result (gene)
CG33484	NIG-FLY		0% no	similar	similar
CG33484	NIG-FLY		0% no 0% no	similar	aama
CG33554 CG33554	NIG-FLY	2905R-3 2905R-7	0% no	no pheno. same	same
CG33956		15507R-2	0% yes	same	same
CG33956	NIG-FLY	15507R-4	0% yes	same	
CG34409	custom	1E8 3 T1	0% no	no pheno.	no pheno.
CG34409	custom	1E8 5 T1	0% no	no pheno.	
CG34409	custom	1E8 5 T2	0% no	no pheno.	
CG3619 CG3619	custom custom	1A10 T1 1A10 T2	0% yes 0% yes	same (p) same (p)	same (p)
CG3690	NIG-FLY		0% yes	no pheno.	no pheno.
CG3722	NIG-FLY		0% yes	same (p)	same (p)
CG3722	NIG-FLY	3722R-2	0% yes	same (p)	/
CG3779	NIG-FLY		66% 1 line	same (p)	same (p)
CG3779	NIG-FLY		66% 1 line	same (p)	
CG3891 CG3891	NIG-FLY NIG-FLY		67% yes	same	same
CG3936	NIG-FLY		67% yes 0% yes	same same (p)	same (p)
CG3936	NIG-FLY		0% yes	same (p)	Same (p)
CG4311	NIG-FLY		49% yes	same	same
CG4311	NIG-FLY	4311R-3	49% yes	same	
CG4633	NIG-FLY		0% yes	different	different
CG4633	NIG-FLY		0% yes	different	(-)
CG5055 CG5055	NIG-FLY NIG-FLY		0% yes 0% yes	same (p) same (p)	same (p)
CG5070	custom	1G11 T1	28% no	different	different
CG5070	custom	1G11 T2	28% no	different	amorone
CG5165	NIG-FLY		0% no	no pheno.	no pheno.
CG5441	custom	1C12 a) T1	0% yes	same	same
CG5460	custom	1D12 T1	0% 1 line	similar (p)	similar (p)
CG5460	custom	1D12 T2	0% 1 line	no pheno. (p)	no nhono
CG5608 CG5608	NIG-FLY NIG-FLY		19% 1 line 19% 1 line	no pheno. no pheno.	no pheno.
CG5640		5640R-3	0% yes	same	same
CG5640	NIG-FLY		0% yes	similar	
CG5680	NIG-FLY	5680R-1	62% yes	same (p)	same (p)
CG5680	NIG-FLY		62% yes	same (p)	
CG5857	custom	2B1 T2	0% yes	no pheno.	no pheno.
CG6098 CG6098	NIG-FLY NIG-FLY		0% yes 0% yes	same	same
CG6202	custom	2G1 T1	25% no	same same	same
CG6202	custom	2G1 T2	25% no	same	June
CG6255	NIG-FLY		0% no	no pheno.	no pheno.
CG6627	NIG-FLY		0% no	no pheno.	no pheno.
CG6883	NIG-FLY		0% yes	same	same
CG6883	NIG-FLY		0% yes	same	
CG7454 CG7454	NIG-FLY	7454R-1 7454R-3	50% no 50% no	no pheno.	no pheno.
CG7614	NIG-FLY		0% 1 line	no pneno. similar	similar
CG7614		7614R-3	0% 1 line	similar	Ontinia
CG7999		7999R-1	0% 1 line	same	same
CG7999		7999R-2	0% 1 line	same	
CG8142		8142R-2	70% 1 line	same	same
CG8142		8142R-3	70% 1 line	same	no nhono
CG8657 CG8657	NIG-FLY NIG-FLY		0% 1 line 0% 1 line	no pheno. no pheno.	no pheno.
CG8865	NIG-FLY		0% 1 line	different	different
CG8865		8865R-2	0% 1 line	different	
CG8890		8890R-2	0% 1 line	same (p)	same (p)
CG9249	NIG-FLY		0% yes?	similar	similar
CG9249		9249R-3	0% yes?	similar	
CG9596	NIG-FLY		0% no	same	same
CG9596 CG9901		9596R-2 9901R-2	0% no 0% yes	same	came
CG9901 CG9901		9901R-2 9901R-1	0% yes	similar same	same
300001	7110 I LI	200111	070 300	Curio	

Note: (p) denotes positive control genes in the Notch pathway or asymmetric cell division

Table indicates whether phenotypes with an independently generated transgenic RNAi line agree with phenotypes obtained in the genome-wide screen for a set of 73 genes. The sequence overlap with the original library transgenic line is shown. (pdf; 54 kb)

#### Supplementary Table 6. False positive result data

Transformant ID	CG Number 5.7	Gene Symbol	Expected phenotype?
9240	CG10385	msl-1	yes
9239	CG10385	msl-1	yes
13562 13563	CG10612 CG10612	Or83a Or83a	yes
4225	CG10888	Rh3	yes yes
31390	CG10986	g	yes
31391	CG10986	g	yes
39766	CG10986	g	yes
41369	CG10986	g	yes
41368 19691	CG10986 CG11680	g	yes
9354	CG13206	mle Or47b	yes yes
26805	CG14360	Or88a	yes
11321	CG1555	cn	yes
11322	CG1555	cn	yes
13730	CG15779	Tre	yes
12347 2560	CG15779 CG16724	Tre tra	yes
2561	CG16724	tra	yes yes
7210	CG16740	Rh2	yes
7723	CG16910	key	yes
28225	CG1980	dj	yes
28224	CG1980	dj	yes
13673 25591	CG2075 CG30170	aly bgcn	yes yes
25590	CG30170	bgcn	yes
25015	CG3029	or	yes
25014	CG3029	or	Expected? (notum malformation and overproliferation)
3875	CG31783	ninaD	yes
29356	CG3241	msl-2	yes
14535 26299	CG32498 CG32498	dnc dnc	Expected? (notum malformation death)
24616	CG32498	dnc	yes yes
24615	CG32498	dnc	yes
16717	CG32498	dnc	yes
14534	CG32498	dnc	Expected? (notum malformation death)
15444	CG32498	dnc	yes
18140 45688	CG32498	dnc	Expected? (notum malformation death)
45689	CG3331 CG3331	e e	yes yes
26211	CG3504	inaD	yes
34286	CG3525	eas	yes
34287	CG3525	eas	yes
21490	CG3620	norpA	yes
10374 40876	CG4215 CG4314	spel1 st	yes yes
40875	CG4314	st	yes
12045	CG4319	rpr	yes
27359	CG5125	ninaC	yes
27360	CG5125	ninaC	yes
1836	CG5192	Rh6	yes
23201 23199	CG5216 CG5216	Sir2 Sir2	yes yes
19834	CG5529	B-H1	yes
9253	CG5576	imd	yes
1748	CG5638	Rh7	yes
8933	CG6203	Fmr1	yes
13380 13381	CG7303 CG7303	Gr68a	yes
35240	CG7399	Gr68a Hn	yes yes
28041	CG7486	Dredd	yes
4365	CG8285	boss	yes
4366	CG8285	boss	yes
35876	CG8318	Nf1	Expected? (loss of bristles and notum malformation)
35877	CG8318	Nf1	yes
4867 4869	CG8536 CG8536	beta4GalNAcTA beta4GalNAcTA	yes yes
2998	CG8631	msl-3	yes
9827	CG9151	acj6	yes
29108	CG9347	ninaB	yes
3149	CG9771	Dip2	yes
3148	CG9771	Dip2	yes

Table indicates whether phenotypes in the genome-wide screen agree with published adult mutant phenotypes (or lack thereof) for a set of 43 non-essential genes. (pdf; 37 kb)

# Supplementary Table 7. Wing assay results

	ID Lateral Inhibition		Wing Margin Notching	Broad Delta-like Veins	Positive in Su(H) reporter assay	Bent Up (Spoon-shaped) wing	Other wing phenotype or no phenotype	Known Notch Pathway Gene
3774 15425	Yes Yes	CG10021 CG10036	no no	no no	not tested not tested	no no		
11257	Yes	CG1007	no	yes, weak	no	no	ectopic bristles	yes
35469 16039	Yes Yes	CG10108 CG10210, CG10375	no no		not tested not tested	yes no		
16040	Yes	CG10377	no	no	no	no	wrinkled, small	
29715 3326	Yes Yes	CG10570 CG10657	no no	no no		no yes		
42245	Yes	CG10776	no	no	no	yes	terminal L4 and L5 are absent	
35489 35490	Yes Yes	CG10800 CG10800	yes yes			no no	wrinkled, small, tufts of trichomes wrinkled, small, tufts of trichomes	
45013	Yes	CG10954	no	no	not tested	yes		
27090 40631	Yes Yes	CG10975 CG10975	no no		no no	no yes	darker margin	
37812	Yes	CG11154	no	no	not tested	no		
10942 3046	Yes Yes	CG1119 CG11282	no no			yes no		
17541 39686	Yes	CG11286	yes	yes	yes	no		
2495	Yes Yes	CG11286 CG11592	yes no	yes no		no no		
38541 48786	Yes	CG11597 CG11637	no			no		
16258	Yes Yes	CG11659, CG6300	no no	no no		no yes	wrinkled	
16371 3335	Yes Yes	CG11940 CG11956	no no			no no	wrinkled	
3336	No	CG11956	no	no	not tested	no		
10662 31121	Yes Yes	CG11988 CG12065	no no			no no		yes
17043	Yes	CG1216	no	no	not tested	no		
43112 43113	Yes Yes	CG12265 CG12265	no yes			no no	wrinkled, small, tufts of trichomes	
23091	Yes	CG12333	no	no	not tested	no		
44046 25576	Yes Yes	CG12366 CG12484	yes no		yes not tested	no yes	wrinkled, small	yes
45761	Yes	CG12537	no	no	not tested	no		
938 31898	Yes Yes	CG12676 CG12818	no no			no no		yes
46248	No	CG12818	no	no	not tested	no		
15081 23873	Yes Yes	CG13208, CG7741 CG13349	no no			yes or lethal no		
32194	Yes	CG13472	no	no	not tested	yes		
43848 17334	Yes Yes	CG13586 CG13623	no no			yes yes		
17690 24481	Yes	CG13708 CG13827	no	no	not tested	no		
38003	Yes Yes	CG13983	no no		not tested	no no		
17340 45414	Yes Yes	CG14011 CG14011	no no			no yes	thin	
17349	Yes	CG14039	yes	yes	no	no	umi	
17471 32278	Yes Yes	CG14299 CG14544	no no			no no		
43588	Yes	CG14549	no	no	not tested	yes		
44064 44065	Yes Yes	CG14624 CG14624	no no	yes no	no not tested	yes no		
37757	Yes	CG14999	no	no	not tested	yes		
28552 14645	Yes Yes	CG15292 CG15307	no no	no no	not tested not tested	no no	wrinkled sprouting vein (L3)	
43402	Yes	CG15330	no	no	not tested	no		
11245 27045	Yes Yes	CG15367 CG15427	no no			no yes	wrinkled, supernumary trichomes	
20205	Yes	CG15429	no	no	not tested	no		
40877 40024	Yes	CG15443 CG15753	no			no von		
14404	Yes	CG15769	no no	no no	not tested not tested	yes yes		
33846 32767	No Yes	CG16713 CG16908	no no	no no	not tested not tested	no		
28758	Yes	CG17060	no	no	not tested	yes no		
14194 27056	Yes Yes	CG17081 CG17559	no no	no no	not tested no	no yes		
3861	Yes	CG17928	no	no	no	no	wrinkled margin	
969 27165	Yes No	CG1804 CG1804	no no	no no	no no	no no	wrinkled margin	
40137	Yes	CG18214	no	no	not tested	no		
12821 12822	No Yes	CG18332 CG18332	no no	yes no	no no	no no	wrinkled, thin, necrotic, ectopic bristles	
46043	Yes	CG18582, CG4013	no	yes, weak	no	no	flattened border	
46044 43082	Yes Yes	CG18582, CG4013 CG18803	no no	no yes	not tested no	no yes		ves
20799	Yes	CG2091	no	no	no	yes	flattened margin (weak)	,
20826 20829	Yes Yes	CG2161 CG2179	no no	no no	no no	no yes	furrows	
13762	Yes	CG2183	no	no	not tested	no	- Hand - Han - Shain, and - C	
20849 33509	Yes Yes	CG2222 CG2257	no no	no no	no not tested	yes no	altered pattern of hairs; parts of wing are delaminating wings bent down	
33510 7035	Yes Yes	CG2257 CG2938	no no	no	no not tested	no no	wings bent down, wrinkled, necrotic	
21763	Yes	CG2950	no	no no	not tested	no		
43880 44747	Yes Yes	CG30023, CG4013 CG30182	no no	no no	no not tested	yes yes or lethal		
35799	Yes	CG30321	no	no	not tested	no		
29193 33739	Yes Yes	CG30470 CG31007	no no	no yes, weak	no no	yes no	wrinkled, small	
38854	Yes	CG31012	no	no	not tested	no		
40207 27194	Yes Yes	CG31122 CG31140	no no	no no	not tested not tested	no yes		
14727	Yes	CG31238	no	no	no	yes		
9075 43430	Yes Yes	CG31292, CG3303 CG31389	no no	no no	not tested not tested	yes no		
30545	Yes	CG31612	no	no	not tested	no		
9425 8234	Yes Yes	CG31781 CG32060	no no	no no	not tested not tested	yes or lethal no		
34408	Yes	CG32130	no	yes, weak	no	yes		
37441 34446	Yes Yes	CG32136 CG32203	yes no	yes no	no not tested	no no	wrinkled, small, ectopic bristles	
14339	Yes	CG32206	no	no	not tested	no		
41875 41876	Yes Yes	CG32315 CG32315	no no	no no	not tested not tested	yes yes		
45459 24740	Yes	CG32315 CG32346	no	no	not tested not tested	yes	wrinkled	
	Yes Yes	CG32463	no no	no no	not tested	yes yes		
7255		CG32498	no	no	not tested	yes		
7255 18140	Yes		no	no no	not tested not tested	yes yes	thin	
7255 18140 27035 45071	Yes Yes	CG32676 CG32717	no		no	yes	furrows	
7255 18140 27035 45071 19428	Yes Yes Yes	CG32717 CG32717, CG4013	yes	yes, weak				
7255 18140 27035 45071 19428 25620 43370	Yes Yes Yes Yes Yes	CG32717 CG32717, CG4013 CG32732 CG32813	yes no no	no no	not tested no	yes yes	furrows	
7255 18140 27035 45071 19428 25620 43370 43751	Yes Yes Yes Yes Yes Yes Yes Yes	CG32717 CG32717, CG4013 CG32732 CG32813 CG3298	yes no no no	no no no	not tested no no	yes yes no		
7255 18140 27035 45071 19428 25620 43370 43751 29542 24801	Yes	CG32717 CG32717, CG4013 CG32732 CG32813 CG3298 CG33106, CG4013 CG33193	yes no no no no no no	no no no no	not tested no no not tested not tested	yes yes no yes yes yes	furrows necrotic	
7255 18140 27035 45071 19428 25620 43370 43751 29542 24801 25823	Yes	CG32717 CG32717, CG4013 CG32732 CG32813 CG3298 CG33106, CG4013 CG33193 CG33196	yes no no no no no no	no no no no no	not tested no no not tested not tested not tested not tested	yes yes no yes yes no	furrows	
7255 18140 27035 45071 19428 25620 43370 43751 29542 24801 25823 32275 21435	Yes	CG32717 CG32717, CG4013 CG32732 CG32813 CG3298 CG33106, CG4013 CG33193 CG33193 CG33203 CG33203	yes no	no n	not tested no no no no not tested	yes no yes yes no yes yes	furrows necrotic only 1 male adult, wrinkled, blistered, small	
7255 18140 27035 45071 19428 25620 43370 43751 29542 24801 25823 32275	Yes	CG32717 CG32717, CG4013 CG32732 CG32813 CG3298 CG33106, CG4013 CG33193 CG33196 CG33203	yes no	no no no no no no	not tested no no not tested not tested not tested not tested not tested	yes no yes yes no yes yes yes no yes	furrows necrotic	

#### Supplementary Table 7.

335		n CG number 5.7		Broad Delta-like Veins	Positive in Su(H) reporter assay		Other wing phenotype or no phenotype	Known Notch Pathway
176	Yes Yes	CG33324 CG33688	no no	yes no	no not tested	no no	sprouting vein (L4)	
552	Yes	CG33696	no	no	not tested	yes	oproduing voin (E4)	
97	Yes	CG34315, CG408		no	not tested	yes		
70	Yes	CG34319	no	no	not tested	yes		
7 '5	Yes	CG34341 CG34343, CG401	no 2 no	yes, weak	no not tested	no waa		
5	Yes Yes	CG34345, CG401	3 no yes	no yes	yes	yes no		
3	Yes	CG3443	no	no	not tested	yes		
	Yes	CG3619	yes	yes	no	no		yes
7	Yes	CG3619	yes	yes	no	no	thin	yes
7	Yes	CG3619	yes	yes	not tested	no	wrinkled, blistered	yes
3	Yes	CG3619	yes	yes	no	no	wrinkled, blistered	yes
9	Yes	CG3690	no	no	not tested	no		
)	Yes Yes	CG3845 CG3891	no	no	not tested no	yes	wrinkled	
1	Yes	CG3927	no no	no no	not tested	yes no		
	Yes	CG3936	yes	yes	yes	no	wrinkled	yes
3	Yes	CG3936	yes	yes	yes	no	wrinkled, small	yes
9	Yes	CG3936	yes	yes	yes	no	wrinkled	yes
9	Yes	CG4013	no	no	not tested	yes		
S I	Yes	CG4013, CG8254		no	no	yes	,	
	Yes	CG4039	no	no	no	no	furrows	
	Yes Yes	CG4125 CG4158	no no	no yes, weak	not tested no	no no		
3	Yes	CG4178	no	no	not tested	no		
	Yes	CG4311	no	no	not tested	no		
	Yes	CG4412	no	no	not tested	no	wrinkled, small	
	Yes	CG4531	no	yes	no	no		
	Yes	CG4722	no	no	no	yes	wrinkled	yes
	Yes	CG4733	no	no	not tested	yes		
	Yes	CG4733	no	no	not tested	yes		
	Yes	CG4760 CG4952	no	no no	not tested not tested	yes		
	Yes Yes	CG4952 CG5165	no no	no no	not tested not tested	yes no		
	Yes	CG5313	no	no	not tested	yes		
	Yes	CG5362	no	no	not tested	yes	wrinkled	
	Yes	CG5488	no	no	not tested	no		
9	Yes	CG5608	yes	yes	yes	no		
; ;	Yes	CG5674	no	no	not tested	yes		
	Yes	CG5841	yes	yes	yes	no	blistered, ectopic bristles	yes
	Yes	CG5940	no	no	no	no	wrinkled, small, tufts of trichomes	
	Yes Yes	CG5953 CG6098	no no	no no	no not tested	yes no		
	Yes	CG6098	no	no	not tested	no		
	Yes	CG6124	no	no	not tested	no		
	Yes	CG6146	no	no	not tested	yes		
	Yes	CG6255	no	no	not tested	no		
	Yes	CG6308	no	no	not tested	no		
,	Yes	CG6315	no	no	not tested	yes		
3	Yes	CG6315	no	no	not tested	yes		
5	Yes	CG6444	no	no	not tested	yes		
8 6	Yes	CG6453 CG6509	no no	no no	not tested	no	thin	
9	Yes Yes	CG6627	no no	no no	no not tested	no no	Unin	
23	Yes	CG6838	no	no	not tested	no no		
14	Yes	CG6883	no	no	not tested	yes		
32	Yes	CG6889	no	no	not tested	no		
}	Yes	CG6975	no	no	not tested	yes	wrinkled	
8	Yes	CG7015	no	no	not tested	yes		
i3	Yes	CG7417	no	no	not tested	no		
5	Yes	CG7417	no	no	not tested	no		
99	Yes	UG7454	no	no	not tested	no		
41 29	Yes Yes	CG7631 CG7655	no	no	no	no no	wrinkled, small, ectopic bristles, vein material in E cell	
9	Yes		no	no	not tested		actonic brietlae marninal cancon brietlae partially deformed	
12	Yes	CG7668 CG7714	no no	yes no	no not tested	yes no	ectopic bristles, marginal sensory bristles partially deformed	
2	Yes	CG7883	no	no	not tested	yes	small, wing margin is less brown and narrower	
i3	Yes	CG7935	no	no	not tested	yes	,	
75	Yes	CG8021	yes	yes	yes	no		
8	Yes	CG8094	no	no	not tested	yes		
8	Yes	CG8136	yes	yes	yes	yes	thin	
1 3	Yes	CG8142	no	no	not tested	yes	wrinkled margin	
	Yes	CG8239 CG8355	no no	No weak	not tested	yes		
0 3	Yes Yes	CG8355 CG8355	no no	yes, weak yes, weak	no no	no no		
	Yes	CG8384	no	no	not tested	no		yes
	Yes	CG8384	no	yes, weak	no	ves		yes
2	Yes	CG8386	no	no	not tested	yes or lethal		
3	Yes	CG8472	no	no	no	no	wrinkled, small	
В	Yes	CG8489	no	no	not tested	no		
2	Yes	CG8556	no	no	not tested	no		
2	Yes Yes	CG8725 CG8743	no no	no no	not tested	yes ves or lethal	larger	
,	Yes	CG8743 CG8743	no no	no no	not tested no	yes or lethal yes	larger	
,	Yes	CG8765	no	no	not tested	no		
5	Yes	CG8946	no	no	not tested	no		
	Yes	CG8994	no	no	not tested	no		
;	Yes	CG9032	no	no	not tested	no		
5	Yes	CG9032	no	no	not tested	yes	wrinkled	
7	Yes	CG9194	no	no	not tested	no		
3	Yes	CG9249	no	no	not tested	no	wrinkled, parts of wing are delaminating	
	No	CG9249	no	no	not tested	yes		
	Yes Yes	CG9270	no	no	not tested	no	inlined bilintered excell	
2		CG9415 CG9473	no no	no	not tested not tested	no	wrinkled, blistered, small wrinkled	
			110	no		no		
	Yes				not tested			
	Yes Yes	CG9518	no	no	not tested	no no	additional vein parallel to L3	
	Yes		no no	no no	not tested not tested not tested	no	additional vein parallel to L3	
	Yes Yes Yes	CG9518 CG9596	no	no	not tested		additional vein parallel to L3	

Results of the *MS1096*-GAL4 wing secondary screen describing phenotypes in wing margin notching, wing vein broadening and other phenotypes for genes in the lateral inhibition category. Results of the Su(H)-*lacZ* reporter assay for genes whose knockdown caused wing margin notching or broad wing vein phenotypes in the *MS1096*-GAL4 secondary screen are also included. (pdf; 71 kb)

## Supplementary Table 8. Live imaging analysis for SOP specification results

35469         CG10108         9.0         too many SOP cells           29715         CG10570         10.0         no SOP cells           3326         CG10657         7.0         normal number, size SOP cells           42245         CG10776         9.0         no SOP cells           35489         CG10800         10.0         no SOP cells           35490         CG10800         9.0         few SOP cells, big SOP cells           37812         CG11154         9.0         small SOP cells           10942         CG1119         8.0         normal number, size SOP cells           3046         CG11282         8.0         normal number, size SOP cells           2495         CG11592         9.0         lethal           38541         CG11597         10.0         no SOP cells           43112         CG12265         10.0         few SOP cells, big SOP cells           43113         CG12265         10.0         few SOP cells, big SOP cells           31741         CG12333         9.0         normal number, size SOP cells           44046         CG13623         7.0         normal number, size SOP cells           17690         CG13708         10.0         no SOP cells           <	no n
3326         CG10657         7.0         normal number, size SOP cells           42245         CG10776         9.0         no SOP cells           35489         CG10800         10.0         no SOP cells           35490         CG10800         9.0         few SOP cells, big SOP cells           37812         CG11154         9.0         small SOP cells           10942         CG1119         8.0         normal number, size SOP cells           3046         CG11282         8.0         normal number, size SOP cells           2495         CG11592         9.0         lethal           38541         CG11597         10.0         no SOP cells           43112         CG12265         10.0         few SOP cells, big SOP cells           43113         CG12265         10.0         few SOP cells, big SOP cells           23091         CG12333         9.0         no SOP cells           31741         CG12352         7.0         normal number, size SOP cells           44046         CG13663         9.0         too many SOP cells           17690         CG13708         10.0         no SOP cells           17471         CG14299         10.0         no SOP cells           32278	no n
42245         CG10776         9.0         no SOP cells           35489         CG10800         10.0         no SOP cells           35490         CG10800         9.0         few SOP cells, big SOP cells           37812         CG11154         9.0         small SOP cells           10942         CG1119         8.0         normal number, size SOP cells           3046         CG11282         8.0         normal number, size SOP cells           2495         CG11592         9.0         lethal           38541         CG11597         10.0         no SOP cells           43112         CG12265         10.0         few SOP cells, big SOP cells           43113         CG12265         10.0         few SOP cells, big SOP cells           23091         CG12333         9.0         no SOP cells           31741         CG12352         7.0         normal number, size SOP cells           44046         CG12366         9.0         too many SOP cells           17690         CG13708         10.0         no SOP cells           17471         CG14299         10.0         no SOP cells           32278         CG14544         9.0         normal number, size SOP cells           32279	no n
35489         CG10800         10.0         no SOP cells           35490         CG10800         9.0         few SOP cells, big SOP cells           37812         CG11154         9.0         small SOP cells           10942         CG1119         8.0         normal number, size SOP cells           3046         CG11282         8.0         normal number, size SOP cells           2495         CG11592         9.0         lethal           38541         CG11597         10.0         no SOP cells           43112         CG12265         10.0         few SOP cells, big SOP cells           43113         CG12265         10.0         few SOP cells, big SOP cells           23091         CG12333         9.0         no SOP cells           31741         CG12352         7.0         normal number, size SOP cells           44046         CG12366         9.0         too many SOP cells           17690         CG13708         10.0         no SOP cells           17690         CG13708         10.0         no SOP cells           17471         CG14299         10.0         no SOP cells           32278         CG14544         9.0         normal number, size SOP cells           32279	no n
35490         CG10800         9.0         few SOP cells, big SOP cells           37812         CG11154         9.0         small SOP cells           10942         CG1119         8.0         normal number, size SOP cells           3046         CG11282         8.0         normal number, size SOP cells           2495         CG11592         9.0         lethal           38541         CG11597         10.0         no SOP cells           43112         CG12265         10.0         few SOP cells, big SOP cells           43113         CG12265         10.0         few SOP cells, big SOP cells           23091         CG12333         9.0         no SOP cells           31741         CG12352         7.0         normal number, size SOP cells           44046         CG12366         9.0         too many SOP cells           17690         CG13708         10.0         no SOP cells           17690         CG13708         10.0         no SOP cells           17471         CG14299         10.0         no SOP cells           32278         CG14544         9.0         normal number, size SOP cells           32279         CG14549         8.0         normal number, size SOP cells	no n
37812         CG11154         9.0         small SOP cells           10942         CG1119         8.0         normal number, size SOP cells           3046         CG11282         8.0         normal number, size SOP cells           2495         CG11592         9.0         lethal           38541         CG11597         10.0         no SOP cells           43112         CG12265         10.0         few SOP cells, big SOP cells           43113         CG12265         10.0         few SOP cells, big SOP cells           23091         CG12333         9.0         no SOP cells           31741         CG12352         7.0         normal number, size SOP cells           44046         CG12366         9.0         too many SOP cells           17334         CG13623         7.0         normal number, size SOP cells           17690         CG13708         10.0         no SOP cells           24481         CG13827         10.0         no SOP cells           17471         CG14299         10.0         no SOP cells           32278         CG14544         9.0         normal number, size SOP cells           32279         CG14544         8.0         normal number, size SOP cells	no n
10942         CG1119         8.0         normal number, size SOP cells           3046         CG11282         8.0         normal number, size SOP cells           2495         CG11592         9.0         lethal           38541         CG11597         10.0         no SOP cells           43112         CG12265         10.0         few SOP cells, big SOP cells           43113         CG12265         10.0         few SOP cells, big SOP cells           23091         CG12333         9.0         no SOP cells           31741         CG12352         7.0         normal number, size SOP cells           44046         CG12366         9.0         too many SOP cells           17334         CG13623         7.0         normal number, size SOP cells           17690         CG13708         10.0         no SOP cells           24481         CG13827         10.0         no SOP cells           17471         CG14299         10.0         no SOP cells           32278         CG14544         9.0         normal number, size SOP cells           32279         CG14544         8.0         normal number, size SOP cells           37757         CG14999         8.0         normal number, size SOP cells </td <td>no no n</td>	no n
3046         CG11282         8.0         normal number, size SOP cells           2495         CG11592         9.0         lethal           38541         CG11597         10.0         no SOP cells           43112         CG12265         10.0         few SOP cells, big SOP cells           43113         CG12265         10.0         few SOP cells, big SOP cells           23091         CG12333         9.0         no SOP cells           31741         CG12352         7.0         normal number, size SOP cells           44046         CG12366         9.0         too many SOP cells           17334         CG13623         7.0         normal number, size SOP cells           17690         CG13708         10.0         no SOP cells           24481         CG13827         10.0         no SOP cells           17471         CG14299         10.0         no SOP cells           32278         CG14544         9.0         normal number, size SOP cells           32279         CG14544         8.0         normal number, size SOP cells           37757         CG14999         8.0         normal number, size SOP cells	no yes, Notch no
2495         CG11592         9.0         lethal           38541         CG11597         10.0         no SOP cells           43112         CG12265         10.0         few SOP cells, big SOP cells           43113         CG12265         10.0         few SOP cells, big SOP cells           23091         CG12333         9.0         no SOP cells           31741         CG12352         7.0         normal number, size SOP cells           44046         CG12366         9.0         too many SOP cells           17334         CG13623         7.0         normal number, size SOP cells           17690         CG13708         10.0         no SOP cells           24481         CG13827         10.0         no SOP cells           17471         CG14299         10.0         no SOP cells           32278         CG14544         9.0         normal number, size SOP cells           32279         CG14544         8.0         normal number, size SOP cells           37757         CG14999         8.0         normal number, size SOP cells	no no no no no no no no yes, Notch no
38541         CG11597         10.0         no SOP cells           43112         CG12265         10.0         few SOP cells, big SOP cells           43113         CG12265         10.0         few SOP cells, big SOP cells           23091         CG12333         9.0         no SOP cells           31741         CG12352         7.0         normal number, size SOP cells           44046         CG12366         9.0         too many SOP cells           17334         CG13623         7.0         normal number, size SOP cells           17690         CG13708         10.0         no SOP cells           24481         CG13827         10.0         no SOP cells           17471         CG14299         10.0         no SOP cells           32278         CG14544         9.0         normal number, size SOP cells           32279         CG14544         8.0         normal number, size SOP cells           3588         CG14549         8.0         normal number, size SOP cells           37757         CG14999         8.0         normal number, size SOP cells	no no no no no yes, Notch no
43112       CG12265       10.0       few SOP cells, big SOP cells         43113       CG12265       10.0       few SOP cells, big SOP cells         23091       CG12333       9.0       no SOP cells         31741       CG12352       7.0       normal number, size SOP cells         44046       CG12366       9.0       too many SOP cells         17334       CG13623       7.0       normal number, size SOP cells         17690       CG13708       10.0       no SOP cells         24481       CG13827       10.0       no SOP cells         17471       CG14299       10.0       no SOP cells         32278       CG14544       9.0       normal number, size SOP cells         32279       CG14544       8.0       normal number, size SOP cells         43588       CG14549       8.0       normal number, size SOP cells         37757       CG14999       8.0       normal number, size SOP cells	no no no no yes, Notch no
43113         CG12265         10.0         few SOP cells, big SOP cells           23091         CG12333         9.0         no SOP cells           31741         CG12352         7.0         normal number, size SOP cells           44046         CG12366         9.0         too many SOP cells           17334         CG13623         7.0         normal number, size SOP cells           17690         CG13708         10.0         no SOP cells           24481         CG13827         10.0         no SOP cells           17471         CG14299         10.0         no SOP cells           32278         CG14544         9.0         normal number, size SOP cells           32279         CG14544         8.0         normal number, size SOP cells           43588         CG14549         8.0         normal number, size SOP cells           37757         CG14999         8.0         normal number, size SOP cells	no no no yes, Notch no
43113         CG12265         10.0         few SOP cells, big SOP cells           23091         CG12333         9.0         no SOP cells           31741         CG12352         7.0         normal number, size SOP cells           44046         CG12366         9.0         too many SOP cells           17334         CG13623         7.0         normal number, size SOP cells           17690         CG13708         10.0         no SOP cells           24481         CG13827         10.0         no SOP cells           17471         CG14299         10.0         no SOP cells           32278         CG14544         9.0         normal number, size SOP cells           32279         CG14544         8.0         normal number, size SOP cells           43588         CG14549         8.0         normal number, size SOP cells           37757         CG14999         8.0         normal number, size SOP cells	no no no yes, Notch no
23091         CG12333         9.0         no SOP cells           31741         CG12352         7.0         normal number, size SOP cells           44046         CG12366         9.0         too many SOP cells           17334         CG13623         7.0         normal number, size SOP cells           17690         CG13708         10.0         no SOP cells           24481         CG13827         10.0         no SOP cells           17471         CG14299         10.0         no SOP cells           32278         CG14544         9.0         normal number, size SOP cells           32279         CG14544         8.0         normal number, size SOP cells           43588         CG14549         8.0         normal number, size SOP cells           37757         CG14999         8.0         normal number, size SOP cells	no yes, Notch no
31741         CG12352         7.0         normal number, size SOP cells           44046         CG12366         9.0         too many SOP cells           17334         CG13623         7.0         normal number, size SOP cells           17690         CG13708         10.0         no SOP cells           24481         CG13827         10.0         no SOP cells           17471         CG14299         10.0         no SOP cells           32278         CG14544         9.0         normal number, size SOP cells           32279         CG14544         8.0         normal number, size SOP cells           43588         CG14549         8.0         normal number, size SOP cells           37757         CG14999         8.0         normal number, size SOP cells	no yes, Notch no
44046         CG12366         9.0         too many SOP cells           17334         CG13623         7.0         normal number, size SOP cells           17690         CG13708         10.0         no SOP cells           24481         CG13827         10.0         no SOP cells           17471         CG14299         10.0         no SOP cells           32278         CG14544         9.0         normal number, size SOP cells           32279         CG14544         8.0         normal number, size SOP cells           43588         CG14549         8.0         normal number, size SOP cells           37757         CG14999         8.0         normal number, size SOP cells	yes, Notch no
17334         CG13623         7.0         normal number, size SOP cells           17690         CG13708         10.0         no SOP cells           24481         CG13827         10.0         no SOP cells           17471         CG14299         10.0         no SOP cells           32278         CG14544         9.0         normal number, size SOP cells           32279         CG14544         8.0         normal number, size SOP cells           43588         CG14549         8.0         normal number, size SOP cells           37757         CG14999         8.0         normal number, size SOP cells	no
17690         CG13708         10.0         no SOP cells           24481         CG13827         10.0         no SOP cells           17471         CG14299         10.0         no SOP cells           32278         CG14544         9.0         normal number, size SOP cells           32279         CG14544         8.0         normal number, size SOP cells           43588         CG14549         8.0         normal number, size SOP cells           37757         CG14999         8.0         normal number, size SOP cells	no no no no no no
24481         CG13827         10.0         no SOP cells           17471         CG14299         10.0         no SOP cells           32278         CG14544         9.0         normal number, size SOP cells           32279         CG14544         8.0         normal number, size SOP cells           43588         CG14549         8.0         normal number, size SOP cells           37757         CG14999         8.0         normal number, size SOP cells	no no no no no
17471         CG14299         10.0         no SOP cells           32278         CG14544         9.0         normal number, size SOP cells           32279         CG14544         8.0         normal number, size SOP cells           43588         CG14549         8.0         normal number, size SOP cells           37757         CG14999         8.0         normal number, size SOP cells	no no no no no
32278         CG14544         9.0         normal number, size SOP cells           32279         CG14544         8.0         normal number, size SOP cells           43588         CG14549         8.0         normal number, size SOP cells           37757         CG14999         8.0         normal number, size SOP cells	no no no no
32279         CG14544         8.0         normal number, size SOP cells           43588         CG14549         8.0         normal number, size SOP cells           37757         CG14999         8.0         normal number, size SOP cells	no no no
43588 CG14549 8.0 normal number, size SOP cells 37757 CG14999 8.0 normal number, size SOP cells	no no
37757 CG14999 8.0 normal number, size SOP cells	no
28552 CG15292 8.0 normal number, size SOP cells	no
14645 CG15307 10.0 no SOP cells	no
11245 CG15367 9.0 normal number, size SOP cells	no
20205 CG15429 10.0 no SOP cells	no
40877 CG15443 9.0 no SOP cells	no
14404 CG15769 7.0 few SOP cells	no
32767 CG16908 10.0 normal number, size SOP cells	no
14194 CG17081 10.0 no SOP cells	no
40137 CG18214 9.0 few SOP cells	no
43082 CG18803 9.0 too many SOP cells	yes, Notch
13762 CG2183 8.0 normal number, size SOP cells	no
20849 CG2222 9.0 normal number, size SOP cells	no
7035 CG2938 9.0 no SOP cells	no
44747 CG30182 9.0 normal number, size SOP cells	no
35799 CG30321 10.0 no SOP cells	no
30545 CG31612 9.0 no SOP cells	no
9425 CG31781 8.0 normal number, size SOP cells	no
34446 CG32203 9.0 no SOP cells	no
14339 CG32206 9.0 no SOP cells	no
41875 CG32315 8.0 normal number, size SOP cells	no
41876 CG32315 8.0 normal number, size SOP cells	no
45459 CG32315 8.0 normal number, size SOP cells	no
7255 CG32463 10.0 no SOP cells	no
43370 CG32813 10.0 no SOP cells	no
43751 CG3298 9.0 lethal	no
48476 CG33688 10.0 no SOP cells	no
33197 CG34315, CG4088 7.0 normal number, size SOP cells	no
18682 CG34345 8.0 too many SOP cells	
20105 CG34345 9.0 too many SOP cells	no no
	yes, Notch, ACD
,	
37287 CG3619 7.0 too many SOP cells	yes, Notch, ACD
37288 CG3619 8.0 too many SOP cells	yes, Notch, ACD
28359 CG3690 10.0 no SOP cells	no
29674 CG3927 8.0 normal number, size SOP cells	no
1112 CG3936 9.0 too many SOP cells	yes, Notch, ACD
27228 CG3936 9.0 too many SOP cells	yes, Notch, ACD

#### Supplementary Table 8.

Transformant ID	CG Number 5.7	Loss of Bristles	Phenotype in Live Imaging Assay	Positive Control Gene?
27229	CG3936	10.0	too many SOP cells	yes, Notch, ACD
13661	CG4039	9.0	normal number, size SOP cells	no
35583	CG4178	10.0	no SOP cells	no
35385	CG4412	9.0	no SOP cells	no
3238	CG4531	7.0	too many SOP cells	no
21536	CG4760	8.0	few SOP cells	no
34953	CG5165	9.0	no SOP cells	no
37761	CG5313	8.0	normal number, size SOP cells	no
32421	CG5940	10.0	few SOP cells, big SOP cells	yes, ACD
27566	CG6098	9.0	normal number, size SOP cells	no
27567	CG6098	9.0	normal number, size SOP cells	no
30274	CG6308	9.0	no SOP cells	no
42799	CG6627	9.0	no SOP cells	no
35123	CG6838	10.0	no SOP cells	no
14099	CG7454	9.0	no SOP cells	no
12429	CG7655	9.0	no SOP cells	no
14392	CG7714	10.0	no SOP cells	no
40322	CG7883	9.0	lethal	no
38963	CG7935	7.0	small SOP cells	no
35338	CG8094	7.0	normal number, size SOP cells	no
10881	CG8142	7.0	normal number, size SOP cells	no
24253	CG8239	7.0	normal number, size SOP cells	no
28243	CG8472	10.0	no SOP cells	no
50350	CG8556	9.0	lethal	no
28247	CG8765	9.0	no SOP cells	no
23335	CG9032	9.0	normal number, size SOP cells	no
23685	CG9032	9.0	few SOP cells	no
42211	CG9249	8.0	normal number, size SOP cells	no
29962	CG9270	10.0	no SOP cells	no
27026	CG9596	9.0	normal number, size SOP cells	no
24193	CG9731	9.0	too many SOP cells	no

Live imaging secondary screen results indicating phenotypes observed on SOP number and size for knockdown of genes with  $P_{Loss} \ge 7$  in the genome-wide screen. (pdf; 45 kb)

## **Supplementary Table 9. Lineage analysis results**

TransformantID	CG Number 5.7	Phenotype in Lineage Staining
3326	CG10657	notum too malformed to get good staining
10942	CG1119	less than 4 cells per organ
3046	CG11282	cell fate transformation, 2 neuron 2 sheath or 3 neuron 1 sheath
31741	CG12352	notum too malformed, potential rare cell fate transformation
32278	CG14544	no phenotype - could be differentiation problem
32279	CG14544	no phenotype - could be differentiation problem
43588	CG14549	less than 4 cells per organ
37757	CG14999	no phenotype - could be differentiation problem
28552	CG15292	unhealthy cells- weak cut staining, otherwise no phenotype
11245	CG15367	less than 4 cells per organ
32767	CG16908	cell fate transformation, 2 neuron 2 sheath or 3 neuron 1 sheath
13762	CG2183	cell fate transformation, 2 neuron 2 sheath
20849	CG2222	less than 4 cells per organ
45459	CG32315	very few unhealthy cells, otherwise no phenotype
41875	CG32315	unhealthy cells
41876	CG32315	unhealthy cells
33197	CG34315, CG4088	less than 4 cells per organ
29674	CG3927	cell fate transformation, 2 neuron 2 sheath or 3 neuron 1 sheath
37761	CG5313	less than 4 cells per organ
27566	CG6098	less than 4 cells per organ
27567	CG6098	less than 4 cells per organ
35338	CG8094	cell fate transformation, 2 neuron 2 sheath or 3 neuron 1 sheath
10881	CG8142	no phenotype - could be differentiation problem
24253	CG8239	no phenotype - could be differentiation problem
42211	CG9249	cell fate transformation, 2 neuron 2 sheath or 3 neuron 1 sheath
27026	CG9596	no phenotype - could be differentiation problem

Results of antibody staining lineage analysis are indicated for genes with no apparent phenotype in the live imaging secondary screen. (pdf; 34 kb)

## Supplementary Table 10. Primer Sequences for Custom Second RNAi lines

RNAi line	CG number	Primer 1 Sequence	Primer 2 Sequence
ID	5.7		
1B1 T1	CG10261	GCGTCTAGAATGCAGAAAATGCCCTCGCAAAT	CGCGAATTCAATCCCGGCGCTTGAGGAAC
1B1 T2	CG10261	GCGTCTAGAATGCAGAAAATGCCCTCGCAAAT	CGCGAATTCAATCCCGGCGCTTGAGGAAC
1A3- 5 T1	CG11988	GCGTCTAGACGACCAGCAGCAGCACCGATT	CGCGAATTCTGGTGTAGGTGCGGATGACG
1G3 1 T1	CG12366	GCGTCTAGAGTGAGAGCCTGTGAGCACGTCAAG	CGCGAATTCGATAAAGTGGTTCGACTGTCCGAGAATG
1G3 1 T2	CG12366	GCGTCTAGAGTGAGAGCCTGTGAGCACGTCAAG	CGCGAATTCGATAAAGTGGTTCGACTGTCCGAGAATG
1D4 b) T1	CG13983	GCGTCTAGACGCCACTCTGGTTGCCTTTTG	CGCGAATTCTGCATGTTGTTGGGGTTGATGA
1E8 3 T1	CG34409	${\tt GCGTCTAGAGATTTGGGATGGTGCGGATAGTGA}$	CGCGAATTCCCAGGGGCGGACCTTGATCT
1E8 5 T1	CG34409	${\tt GCGTCTAGAGATTTGGGATGGTGCGGATAGTGA}$	CGCGAATTCCCAGGGGCGGACCTTGATCT
1E8 5 T2	CG34409	${\tt GCGTCTAGAGATTTGGGATGGTGCGGATAGTGA}$	CGCGAATTCCCAGGGGCGGACCTTGATCT
1A10	CG3619	GCGTCTAGAGCGGACGAGTGTCTCATGTACGG	CGCGAATTCGACCACGGATCGCTGCTGTTG
T1 1A10 T2	CG3619	GCGTCTAGAGCGGACGAGTGTCTCATGTACGG	CGCGAATTCGACCACGGATCGCTGCTGTTG
1G11	CG5070	GCGTCTAGACCCTAGCGAAGCCGGGAGAAG	CGCGAATTCGCCCACAAAGCCGCTGGAATA
T1 1G11	CG5070	GCGTCTAGACCCTAGCGAAGCCGGGAGAAG	CGCGAATTCGCCCACAAAGCCGCTGGAATA
T2 1C12	CG5441	GCGTCTAGAGCCAGAGCGACGGACTCCAA	CGCGAATTCGCTTCGGGAACACAATGCCTCA
a) T1 1D12	CG5460	GCGTCTAGAACAACGGCAGCAGCAGCAATAAC	CGCGAATTCGTGGGCTTGGACGCAGTAGCTC
T1 1D12	CG5460	GCGTCTAGAACAACGGCAGCAGCAGCAATAAC	CGCGAATTCGTGGGCTTGGACGCAGTAGCTC
T2 2B1	CG5857	GCGTCTAGAACCCGAAGGTGCCAAGACGAC	CGCGAATTCCAGTTCCTCTCCAGGGGGTAGCA
T2 2G1	CG6202	GCGTCTAGAGGAGGATGTGGCGGAACAGGT	CGCGAATTCGCAGTAGGGCTCCGATGAGTGC
T1 2G1 T2	CG6202	GCGTCTAGAGGAGGATGTGGCGGAACAGGT	CGCGAATTCGCAGTAGGGCTCCGATGAGTGC