

GENETICS

Supporting Information

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Note

Frequent Unanticipated Alleles of *lethal giant larvae* in *Drosophila* Second Chromosome Stocks

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FILE S1**Supplemental Experimental Procedures***Bloomington deficiency screening*

We stained collections of Bloomington embryos with rabbit anti-Miranda (gift of Fumio Matsuzaki) and identified stocks with Mendelian segregation of delocalized Miranda in neuroblasts. We subsequently tested the entire current 2nd chromosome collection (DK2) for failure to complement *lgl[4]*.

Bruinfly FRT-P lethal screening

We induced germline clones in *hs-FLP/+; P lethal FRT40A/ovo[D], FRT40A* females by heat-shocking for 1 hr at 38°C during the first instar. We mated these to sibling males and prepared cuticles from 16-24 hr embryos, which were scored for the presence of epithelial patterning defects. We subsequently tested 4-10 individual flies from 24 randomly selected Bruinfly stocks for failure to complement *lgl[4]*.

Analysis of insensitive

We analyzed thorax clones in *Ubx-FLP/+; insv [23B]* (or *insv[23L]*), *FRT40A/ubi-GFP, FRT40A* animals. We dissected 24 hr APF pupae and stained these with rabbit anti-GFP (Molecular Probes), rat anti-Elav Developmental Studies Hybridoma Bank, DSHB), mouse anti-Prospero (DSHB), rat anti-Suppressor of Hairless (gift of Francois Schweisguth), rabbit anti-Numb (gift of Yuh Nung Jan), rabbit anti-Lgl (gift of Scott Goode), followed by Alexa-coupled secondary antibodies (Molecular Probes). Following the determination that *insv* chromosomes harbored *lgl*, we performed rescue tests in *Ubx-FLP/+; tub-Gal80, FRT40A/lgl, insv [23B], FRT40A; neur-Gal4, UAS-lgl-GFP* (or *UAS-Histone2B-RFP*) animals.

TABLE S1**Complementation tests of the DK2 Bloomington Deficiency Kit with *lgI[4]***

| stock name | | Df name | single fly cross to <i>lgI[4]</i> | complements <i>lgI[4]</i> ? |
|------------|------|-----------------|-----------------------------------|-----------------------------|
| DK2-1 | 3638 | Df(2L)net-PMF | 1 | yes |
| | | | 2 | yes |
| DK2-2 | 6283 | Df(2L)BSC4 | 1 | yes |
| | | | 2 | yes |
| DK2-3 | 8672 | Df(2L)BSC106 | 1 | yes |
| | | | 2 | yes |
| DK2-4 | 6608 | Df(2L)BSC16 | 1 | yes |
| | | | 2 | yes |
| DK2-5 | 3084 | Df(2L)ast2 | 1 | yes |
| | | | 2 | yes |
| DK2-7 | 7144 | Df(2L)BSC37 | 1 | yes |
| | | | 2 | yes |
| DK2-8 | 6648 | Df(2L)dpp[d14] | 1 | yes |
| | | | 2 | yes |
| DK2-9 | 90 | Df(2L)C144 | 1 | yes |
| | | | 2 | yes |
| DK2-10 | 1567 | Df(2L)JS17 | 1 | yes |
| | | | 2 | yes |
| DK2-11 | 6875 | Df(2L)BSC28 | 1 | yes |
| | | | 2 | yes |
| DK2-12 | 6965 | Df(2L)BSC31 | 1 | yes |
| | | | 2 | yes |
| DK2-13 | 6507 | Df(2L)drm-P2 | 1 | yes |
| | | | 2 | yes |
| DK2-14 | 5330 | Df(2L)ed1 | 1 | yes |
| | | | 2 | yes |
| DK2-15 | 693 | Df(2L)sc19-8 | 1 | yes |
| | | | 2 | yes |
| DK2-16 | 9270 | Df(2L)ED250 | 1 | yes |
| | | | 2 | yes |
| DK2-17 | 8835 | Df(2L)BSC110 | 1 | yes |
| | | | 2 | yes |
| DK2-18 | 8674 | Df(2L)BSC109 | 1 | yes |
| | | | 2 | yes |
| DK2-19 | 7497 | Df(2L)Exel6011 | 1 | yes |
| | | | 2 | yes |
| DK2-20 | 781 | Df(2L)cl-h3 | 1 | yes |
| | | | 2 | yes |
| DK2-21 | 490 | Df(2L)E110 | 1 | yes |
| | | | 2 | yes |
| DK2-22 | 6299 | Df(2L)BSC5 | 1 | yes |
| | | | 2 | yes |
| DK2-23 | 6338 | Df(2L)BSC6 | 1 | yes |
| | | | 2 | yes |
| DK2-24 | 6374 | Df(2L)BSC7 | 1 | yes |
| | | | 2 | yes |
| DK2-25 | 2414 | Df(2L)spd[j2] | 1 | yes |
| | | | 2 | yes |
| DK2-26 | 5420 | Df(2L)Dwee1-W05 | 1 | yes |
| | | | 2 | yes |
| DK2-27 | 4956 | Df(2L)XE-3801 | 1 | yes |
| | | | 2 | yes |
| DK2-28 | 7147 | Df(2L)BSC41 | 1 | yes |

| | | | | |
|--------|------|----------------------|---|-----|
| | | | 2 | yes |
| DK2-29 | 9502 | Df(2L)BSC142 | 1 | yes |
| | | | 2 | yes |
| DK2-30 | 140 | Df(2L)Trf-C6R31 | 1 | yes |
| | | | 2 | yes |
| DK2-31 | 179 | Df(2L)TE29Aa-11 | 1 | yes |
| | | | 2 | yes |
| DK2-32 | 8836 | Df(2L)BSC111 | 1 | yes |
| | | | 2 | yes |
| DK2-33 | 9298 | Df(2L)ED611 | 1 | yes |
| | | | 2 | yes |
| DK2-34 | 2892 | Df(2L)N22-14 | 1 | no |
| | | | 2 | no |
| DK2-35 | 6478 | Df(2L)BSC17 | 1 | yes |
| | | | 2 | yes |
| DK2-36 | 1045 | Df(2L)Mdh | 1 | yes |
| | | | 2 | yes |
| DK2-37 | 8469 | Df(2L)BSC50 | 1 | yes |
| | | | 2 | yes |
| DK2-38 | 3366 | Df(2L)J2 | 1 | yes |
| | | | 2 | yes |
| DK2-39 | 9503 | Df(2L)BSC143 | 1 | no |
| | | | 2 | no |
| DK2-40 | 7142 | Df(2L)BSC32 | 1 | yes |
| | | | 2 | yes |
| DK2-41 | 9505 | Df(2L)BSC145 | 1 | yes |
| | | | 2 | yes |
| DK2-42 | 7143 | Df(2L)BSC36 | 1 | yes |
| | | | 2 | yes |
| DK2-43 | 5869 | Df(2L)FCK-20 | 1 | yes |
| | | | 2 | yes |
| DK2-44 | 3079 | Df(2L)Prl | 1 | no |
| | | | 2 | no |
| DK2-45 | 6999 | Df(2L)BSC30 | 1 | no |
| | | | 2 | no |
| DK2-47 | 3138 | Df(2L)b87e25 | 1 | no |
| | | | 2 | no |
| DK2-48 | 9506 | Df(2L)BSC147 | 1 | yes |
| | | | 2 | yes |
| DK2-49 | 3588 | Df(2L)TE35BC-24 | 1 | yes |
| | | | 2 | yes |
| DK2-50 | 1491 | Df(2L)r10 | 1 | yes |
| | | | 2 | yes |
| DK2-51 | 2583 | Df(2L)cact-255rv64 | 1 | no |
| | | | 2 | no |
| DK2-52 | 420 | Df(2L)TW137 | 1 | yes |
| | | | 2 | yes |
| DK2-53 | 567 | Df(2L)pr-A16 | 1 | yes |
| | | | 2 | yes |
| DK2-54 | 167 | Df(2L)TW161 | 1 | yes |
| | | | 2 | yes |
| DK2-55 | 7531 | Df(2L)Exel6049 | 1 | yes |
| | | | 2 | yes |
| DK2-56 | 9510 | Df(2L)BSC151 | 1 | yes |
| | | | 2 | yes |
| DK2-57 | 4959 | Df(2L)C' | 1 | yes |
| | | | 2 | yes |
| DK2-58 | 749 | In(2R)bw[VDe2L]Cy[R] | 1 | yes |

| | | | | |
|--------|------|-----------------|---|-----|
| DK2-59 | 739 | Df(2R)M41A4 | 2 | yes |
| | | | 1 | yes |
| DK2-60 | 1007 | Df(2R)nap9 | 2 | yes |
| | | | 1 | yes |
| | | | 2 | yes |
| DK2-61 | 1888 | Df(2R)ST1 | 1 | no |
| | | | 2 | no |
| DK2-62 | 3368 | Df(2R)cn9 | 1 | no |
| | | | 2 | no |
| DK2-63 | 198 | Df(2R)H3C1 | 1 | yes |
| | | | 2 | yes |
| DK2-65 | 3591 | Df(2R)Np5 | 1 | yes |
| | | | 2 | yes |
| DK2-66 | 4966 | Df(2R)w45-30n | 1 | yes |
| | | | 2 | yes |
| DK2-67 | 6917 | Df(2R)BSC29 | 1 | yes |
| | | | 2 | yes |
| DK2-69 | 1743 | Df(2R)B5 | 1 | yes |
| | | | 2 | yes |
| DK2-70 | 1702 | Df(2R)X1 | 1 | yes |
| | | | 2 | yes |
| DK2-71 | 190 | Df(2R)en-A | 1 | no |
| | | | 2 | no |
| DK2-72 | 1145 | Df(2R)en30 | 1 | yes |
| | | | 2 | yes |
| DK2-73 | 7145 | Df(2R)BSC39 | 1 | yes |
| | | | 2 | yes |
| DK2-74 | 4960 | Df(2R)CB21 | 1 | no |
| | | | 2 | no |
| DK2-75 | 7146 | Df(2R)BSC40 | 1 | yes |
| | | | 2 | yes |
| DK2-76 | 5879 | Df(2R)BSC3 | 1 | yes |
| | | | 2 | yes |
| DK2-77 | 754 | Df(2R)vg-C | 1 | yes |
| | | | 2 | yes |
| DK2-80 | 7875 | Df(2R)Exel7130 | 1 | yes |
| | | | 2 | yes |
| DK2-81 | 9496 | Df(2R)BSC134 | 1 | no |
| | | | 2 | no |
| DK2-82 | 7876 | Df(2R)Exel7131 | 1 | yes |
| | | | 2 | yes |
| DK2-85 | 3520 | Df(2R)Jp8 | 1 | yes |
| | | | 2 | yes |
| DK2-86 | 7445 | Df(2R)BSC49 | 1 | yes |
| | | | 2 | yes |
| DK2-87 | 7414 | Df(2R)BSC44 | 1 | yes |
| | | | 2 | yes |
| DK2-88 | 9596 | Df(2R)BSC161 | 1 | yes |
| | | | 2 | yes |
| DK2-90 | 5574 | Df(2R)k10408 | 1 | yes |
| | | | 2 | yes |
| DK2-91 | 7441 | Df(2R)BSC45 | 1 | yes |
| | | | 2 | yes |
| DK2-92 | 6779 | Df(2R)14H10Y-53 | 1 | yes |
| | | | 2 | yes |
| DK2-93 | 6780 | Df(2R)14H10W-35 | 1 | yes |
| | | | 2 | yes |
| DK2-94 | 1547 | Df(2R)PC4 | 1 | yes |

| | | | | |
|---------|------|----------------|---|-----|
| | | | 2 | yes |
| DK2-95 | 757 | Df(2R)P34 | 1 | no |
| | | | 2 | no |
| DK2-96 | 6866 | Df(2R)BSC26 | 1 | no |
| | | | 2 | no |
| DK2-97 | 6647 | Df(2R)BSC22 | 1 | yes |
| | | | 2 | yes |
| DK2-98 | 3467 | Df(2R)AA21 | 1 | yes |
| | | | 2 | yes |
| DK2-99 | 7896 | Df(2R)Exel7162 | 1 | yes |
| | | | 2 | yes |
| DK2-100 | 6609 | Df(2R)BSC19 | 1 | yes |
| | | | 2 | yes |
| DK2-101 | 5246 | Df(2R)Egfr5 | 1 | yes |
| | | | 2 | yes |
| DK2-103 | 3909 | Df(2R)59AD | 1 | yes |
| | | | 2 | yes |
| DK2-104 | 7273 | Df(2R)vir130 | 1 | yes |
| | | | 2 | yes |
| DK2-106 | 9691 | Df(2R)BSC155 | 1 | yes |
| | | | 2 | yes |
| DK2-107 | 2604 | Df(2R)Px2 | 1 | yes |
| | | | 2 | yes |
| DK2-108 | 9069 | Df(2R)ED4065 | 1 | yes |
| | | | 2 | yes |
| DK2-110 | 4961 | Df(2R)Kr10 | 1 | yes |
| | | | 2 | yes |

Two male flies were individually tested from each stock. For stocks that failed to complement *lgl*, an additional single fly cross was performed as confirmation.

TABLE S2**Complementation tests of 24 randomly selected FRT40A Bruinfly lines with *lgl*[4]**

| Kyoto ID | Bruinfly ID | insertion site | annotated gene disrupted | single fly crosses that fail to complement <i>lgl</i> [4] |
|----------|-------------|-----------------------------|--------------------------------|---|
| 111624 | 14733 | 023E01_ | CG3347 | 0 of 8 |
| 111512 | 13053 | 35D04 | gliotactin | 10 of 10 |
| 111516 | 13097 | 026B05 | Kruppel homolog 1 | 10 of 10 |
| 111066 | 20404 | 035F01 | Cropped | 0 of 4 |
| 111067 | 10363 | 029E04-029E06 | raw | 0 of 4 |
| 111079 | 10386 | 033A01-033A02 | crooked legs | 0 of 4 |
| 111083 | 10391 | 038B03-038B05 | nebbish | 0 of 4 |
| 111097 | 10435 | 031D01 | no mitochondrial derivative | 0 of 4 |
| 111106 | 10451 | 024E01_ | turtle | 5 of 10 |
| 111108 | 10453 | 030A02-030A06 | taiman | 0 of 4 |
| 111111 | 10457 | 023B06 | overgrown hematopoietic organs | 0 of 4 |
| 111122 | 10473 | 025C01 | viking | 0 of 10 |
| 111275 | 10959 | 022C01 | CG31672 | 0 of 4 |
| 111278 | 10965 | 023F03 | Pdsw | 0 of 4 |
| 111334 | 11115 | 036A11 | cytochrome C proximal | 0 of 4 |
| 111356 | 11166 | 021C02 | ebi | 0 of 4 |
| 111366 | 11212 | 024C05 | lethal (2) k16918 | 0 of 4 |
| 111369 | 11218 | 034A04 | Target of rapamycin | 0 of 4 |
| 111429 | 12169 | 021E02_ | dribble | 0 of 4 |
| 111431 | 12173 | 035B08 | moladietz | 0 of 4 |
| 111462 | 12309 | 033A01-033A02 | unknown | 0 of 4 |
| 111463 | 12310 | 023D01-023D02,029A03-029A05 | unknown | 0 of 4 |
| 111558 | 13692 | 032F02 | CG6509 | 7 of 10 |
| 111564 | 13853 | 038A01 | Lar leukocyte antigen related | 8 of 8 |

Initially, 4-6 male flies were individually tested from each stock. For stocks that exhibited some failure to complement *lgl*, additional single fly crosses were performed to assay a total of 10 chromosomes from each stock. The gliotactin, Kr-H1, and Lar insertions were 100% *lgl*, whereas the turtle and CG6509 insertions were 50-70% *lgl*.