File S1: Mapping of synthetic lethal mutation *27.89*

27.89 is located between the visible recessive markers dumpy and black

Recombination mapping was done by isolating recombinants between the *27.89 sub*^{131} chromosome and a chromosome that contained eight 2nd chromosome recessive visible phenotype markers: *aristaless* (*al*), *dumpy* (*dp*), *black* (*b*), *purple* (*pr*), *cinnabar* (*cn*), *curved* (*c*), *plexus* (*px*), *and speck* (*sp*) (Figure S 3). Flies that have had a crossover between the two chromosomes were identified by crossing to another chromosome with all of the markers. Crossovers were then tested to see if the *27.89* mutation remained on the recombinant chromosome by crossing to the *sub*^{1} allele and checking for synthetic lethality. Using the knowledge of which crossovers retained *27.89* one could deduce whether the mutation is to the left or right of each marker.

For the mapping of *27.89*. 59 recombinants were isolated. Nearly all of the recombinants that crossed over to the left of *dp* (the *al* recombinants) contained *27.89*. Most critically, the recombinants that crossed over in between *dp* and *b* (both the *al dp* recombinants as well as the double crossover *b pr cn* recombinants) showed a mixture of having or lacking *27.89*. These data suggest that 27.89 is likely located in between *dp* and *b.*

Mapping 27.89 to a 303 kilobase region using Single Nucleotide Polymorphisms

To map *27.89* at higher resolution, we used single nucleotide polymorphisms (SNPs) between *dp* and *b* (CHEN *et al.* 2008; CHEN *et al.* 2009). We isolated recombinants between the *27.89* chromosome and a chromosome of a different background so that there would be a large number of SNPs between the chromosomes. The other chromosome was marked with a Minos element (*Mi[GFP]*) inserted just to the left of *subito* (*sub*). Each individual recombinant was

tested for synthetic lethality and the location of the crossover relative to the SNP was determined by PCR followed by a restriction enzyme digest or sequencing of the amplified DNA (Figure S 4). For this SNP mapping scheme, a total of 594 recombinants that were al^+ dp^+ and GFP^- were collected from *al dp 27.89/Mi[GFP]* females. These were selected to isolate recombinants between *dumpy* and the Minos element while ensuring sub^{131} remained on the chromosome.

The SNP marker 939 was used to map the recombinants because is located just to the left of *black* and it was used to discard recombinants that occurred between *black* and *Mi[GFP]*. Similarly, the SNP 865 was used between it was located between *dumpy* and 939. The finding that 65 out of 66 recombinants that crossed over to the right of the SNP 939 were not synthetically lethal (i.e. they did not contain *27.89*), while all 45 of the recombinants that crossed over to the left of SNP 865 were synthetic lethal (i.e. they all contained *27.89*), is consistent with the previous mapping that *27.89* is between *dp* and *b* (Figure S 4A). More importantly, of the 28 recombinants between 865 and 939, 11 were synthetic lethal when crossed sub^1 and 17 were not. This mixture of recombinant types indicates that *27.89* is located between SNPs 865 and 939.

The 28 recombinants between 865 and 939 were tested with additional SNPs in the region 872, 889, and 894. 15 of the 28 recombinants crossed over between 894 and 939, all of which did not have *27.89*, implying that *27.89* is located to the left or very close to the right of 894 (Figure S 4B). 4 of the 28 recombinants crossed over in between 865 and 872, and all of these crossovers contained the *27.89* mutation suggesting that *27.89* is most likely located to the right or close to the left of 872 (Figure S 4E). The 9 remaining recombinants crossed over between 872 and 894, 7 of which retained *27.89* and 2 of which did not. The SNP 889 further divided these 9, into 6 crossovers between 872 and 889, all of which had *27.89*, and 3 crossovers between 889 and 894, of which one contained *27.89* and 2 did not (Figure S 4C and D). These

data indicate that *27.89* is located between 889 and 894, and likely closer to 889. This is a region of approximately 300 kb.

27.89 exhibits homozygous lethality, yet complements all deficiencies within the region between SNPs 872 and 894

The original *27.89* chromosome contained two mutations, *27.89* and sub^{131}. The sub^{131} allele was removed by isolating recombinants of the *27.89* sub^{131} chromosome as discussed above. By picking cn^{+} c^{-} recombinants (*curved* (*c*) is located a short distance to the left of *sub*) a stock was generated that carried only *27.89*. The recombinant *27.89* cn^{+} c^{-} chromosome was homozygous lethal. This could mean that *27.89* is a homozygous lethal mutation. Another possibility, however, was that there was another EMS induced lethal mutation elsewhere on the chromosome. To check if *27.89* is homozygous lethal or there is another EMS induced lethal on the chromosome, recombinants *al dp 27.89* sub^{131}, *27.89 b pr cn* sub^{131} and *27.89 c* were crossed to each, resulting in much of the original mutagenized chromosome remaining heterozygous. Even after removing much of the mutagenized chromosome we still failed to observe *27.89* homozygotes. Thus, these results support the conclusion *27.89* is homozygous lethal,.

We also attempted to map *27.89* using chromosomal deletions. Using the SNP mapping data, we crossed *27.89* to all deficiencies spanning the distance between SNPs 872 and 894 (Figure S 5). None of these deficiencies failed to complement *27.89* for lethality. To determine if the problem lies with the deficiencies, we acquired known homozygous lethal mutations in genes that the deficiencies are supposed to delete. Complementation tests were done between these mutations and their corresponding deficiencies, and it was determined that all of the deficiencies in the region that had complemented *27.89* failed to complement other known lethal mutations. Therefore, it is possible that *27.89* both fails to generate homozygotes yet is viable

when heterozygous to a deficiency. There are currently two explanations for this result, either *27.89* is a recessive hypermorph, that is viable over a deficiency, or the region between *dp* and *b* where *27.89* itself is located, contains a second site lethal mutation.

References

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