

A genetic screen for *Drosophila* social isolation mutants and analysis of *sex pistol*

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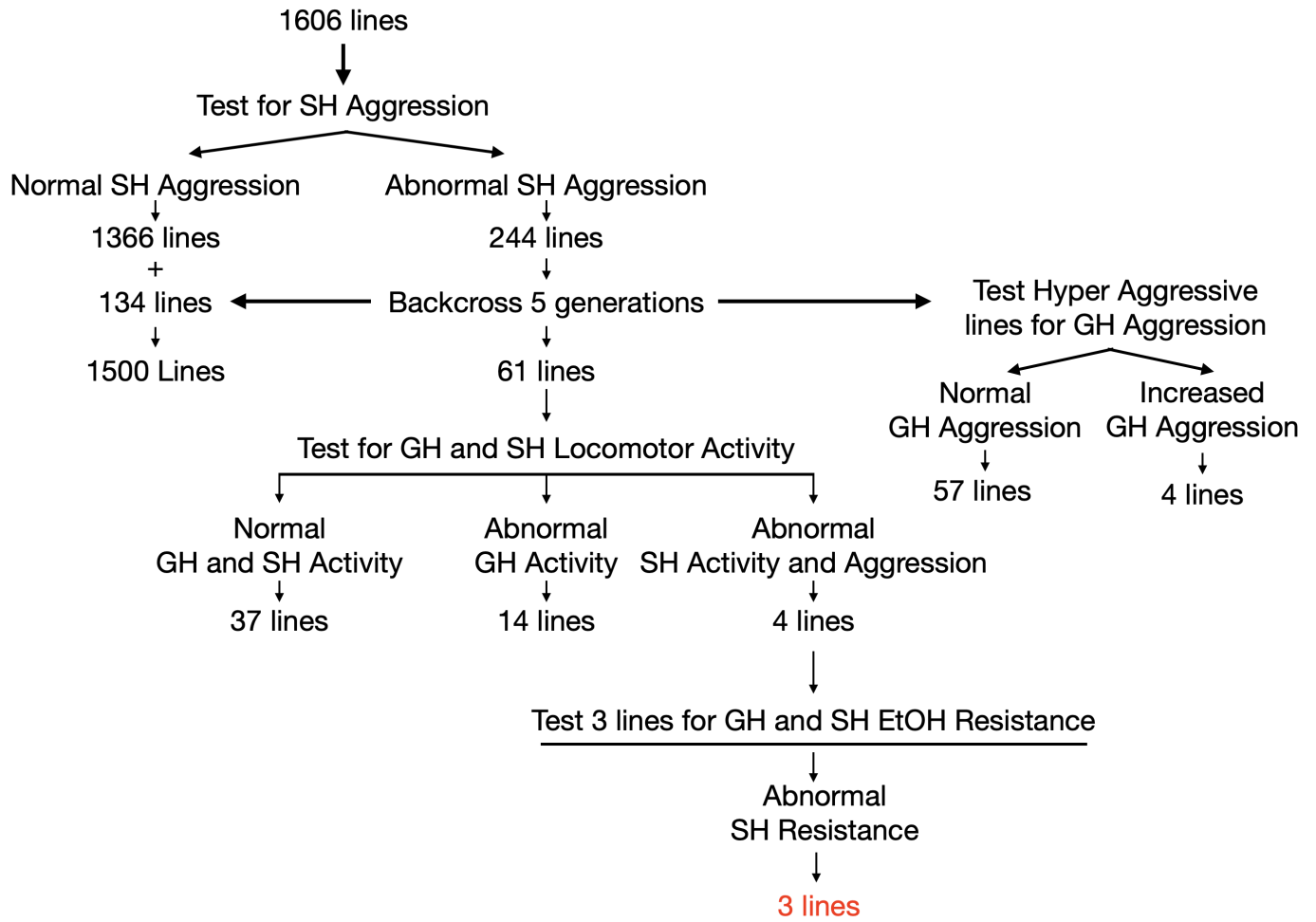


Figure S1) The strategy to identify isolation mutants from a population of 1606 *P-GAL4* insertions

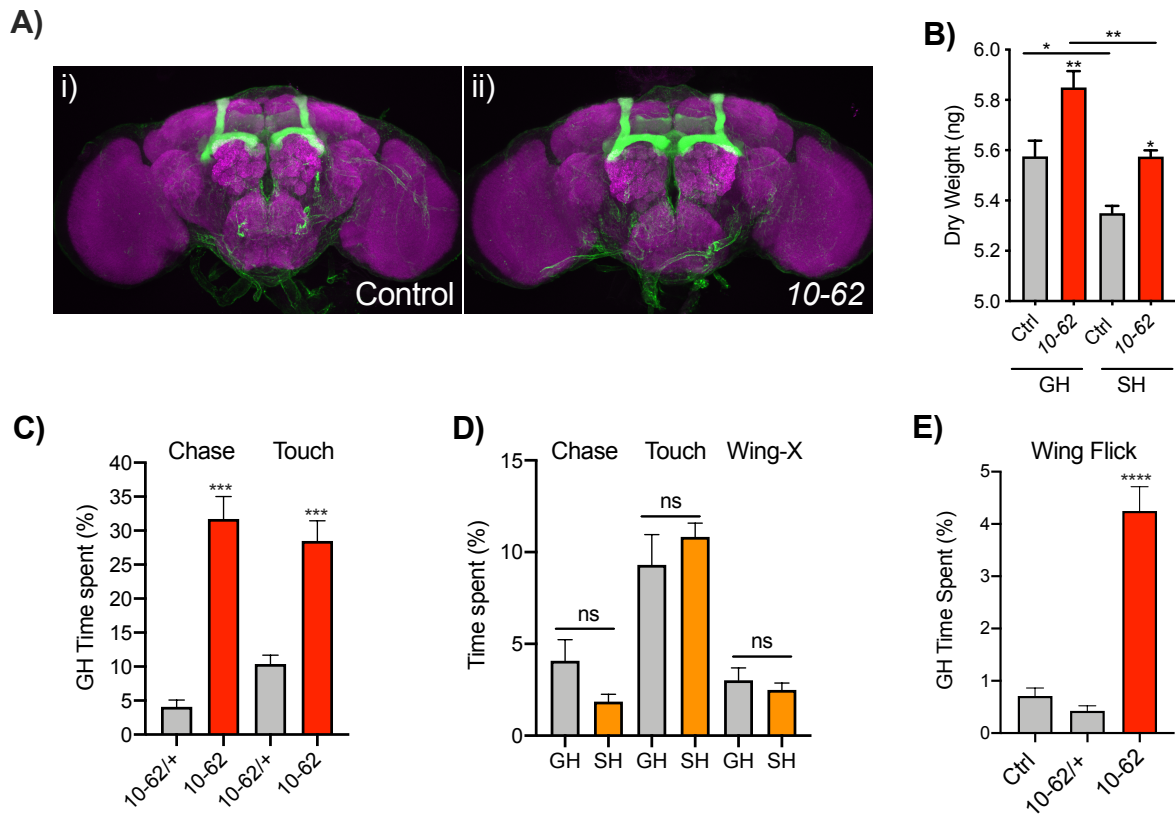


Figure S2) 10-62 characterization (A) Using Fas2 Antibody (green) to stain the mushroom bodies I detected no major morphological disruptions in the brain of 10-62. **(B)** 10-62 males weigh significantly more than control males when GH (** $p = 0.0087$) and SH (* $p = 0.0306$). Compared to the GH condition, both control (* $p = 0.0306$) and 10-62 male flies (** $p = 0.0087$) weighed less when SH ($n = 4$, One-Way ANOVA with Tukey's MCT). **(C)** GH 10-62 males also show increased chasing and touching when groups of 20 GH males were placed in the fly bowl ($p < 0.0002$; $n = 8$, Mann-Whitney Test). **(D)** Isolation did not increase chase, touch or single wing extension of screen control flies in the fly bowl ($p > 0.05$; $n = 6$ Unpaired t test). **(E)** GH 10-62 males have increased wing-flicks in the fly bowl (**** $p = 0.0001$; $n = 10$, One-way ANOVA with Tukey's MCT).

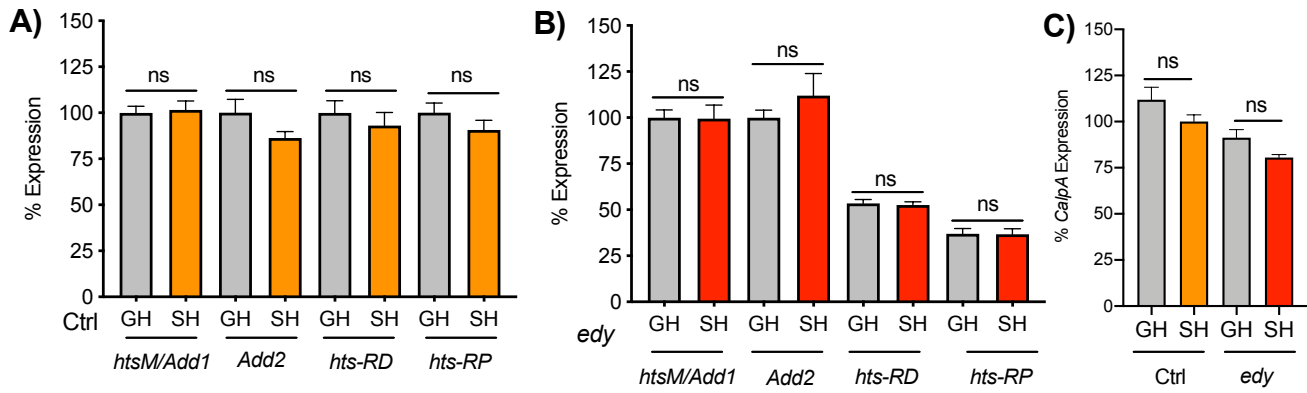


Figure S3) Isolation does not affect *hts* or *CalpA* transcription. Four major *hts* transcripts are unaffected by isolation in both **(A)** control and **(B)** *sxp* flies ($p > 0.05$, $n = 3 - 6$, Unpaired t-test). **(C)** *CalpA* is unaffected by isolation in both control and *sxp* flies ($p > 0.05$, $n = 3$, Unpaired t-test).

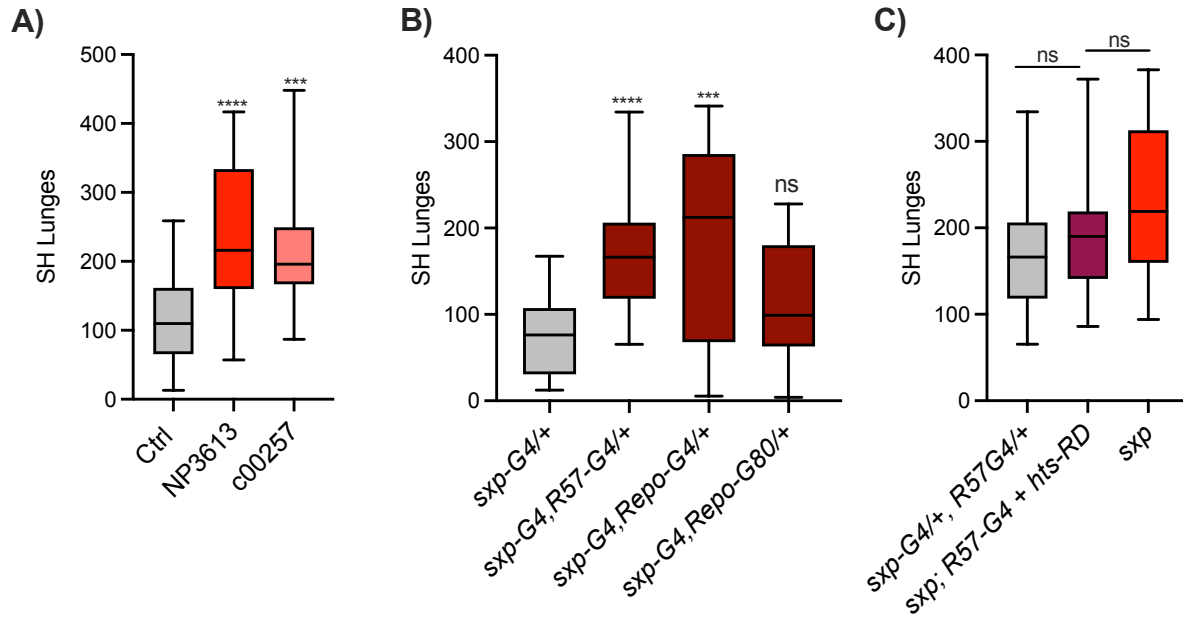


Figure S4) Analysis of *hts* aggression. (A) *hts* P-*GAL4*, NP3613 that inserts close to *sxp-GAL4*, was hyperaggressive (**** $p < 0.0001$, $n = 22-24$) as was *hts* P-Bac C00257, that inserts $\sim 15\text{kb}$ 5' of *CalpA* (***) $p = 0.0001$, $n = 24$, Kruskal-Wallis Test, with Dunn's MCT). **(B)** The combination of *sxp-G4/+* with *R57C10-GAL4/+* (pan-neuronal), *Repo-GAL4/+* (pan-glia) or *dILP3-GAL4/+* (insulin neurons), but not *Repo-GAL80/+*, increased SH aggression (**** $p < 0.0001$ $n = 20-25$, Kruskal-Wallis Test with Dunn's MCT). **(C)** Rescue by pan-neuronal expression of *hts-RD* was inconclusive ($p > 0.05$, $n = 24-25$), Kruskal-Wallis Test with Dunn's MCT).

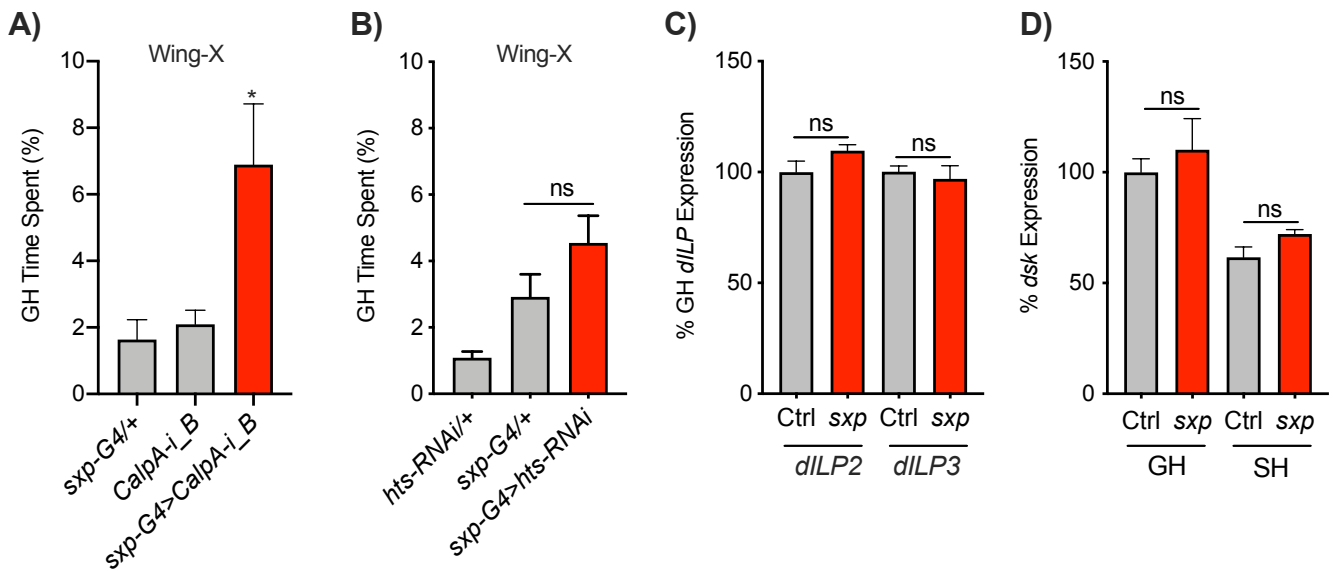


Figure S5) Knockdown of *hts* in *sxp-GAL4* neurons did not increase male-male courtship **(A)** Driving an independent insertion of *UAS-CalpA-RNAi* with *sxp-G4* increased GH single-wing extensions (* $p < 0.037$, $n = 5-6$, One-way ANOVA with Tukey's MCT). **(B)** Driving a *UAS-hts-RNAi* with *sxp-G4* did not increase single-wing extensions ($p > 0.05$ $n = 8$, One-way ANOVA with Tukey's MCT). **(C)** *dILP2* and *dILP3* expression levels are unaffected in GH *sxp* ($p > 0.05$, $n = 6$, Unpaired t-test). **(D)** *Dsk* expression is unaffected in GH and SH *sxp* ($p = 0.632$, 0.608 , $n = 4$, Two-way ANOVA with Sidak's MCT).

Supplementary Tables and Figure Legends

Table S1) Tables of the 46 hyper-aggressive mutants and 15 hypo-aggressive mutants identified from the screen, the P-element insertion site and candidate gene.

Table S2) Tables showing GH and SH daytime activity of SH aggressive mutants.

Table S3) Table showing 4 aggression and activity mutants.

Table S4) Tables showing GH and SH ethanol resistance of 3 aggressive and activity mutants.

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