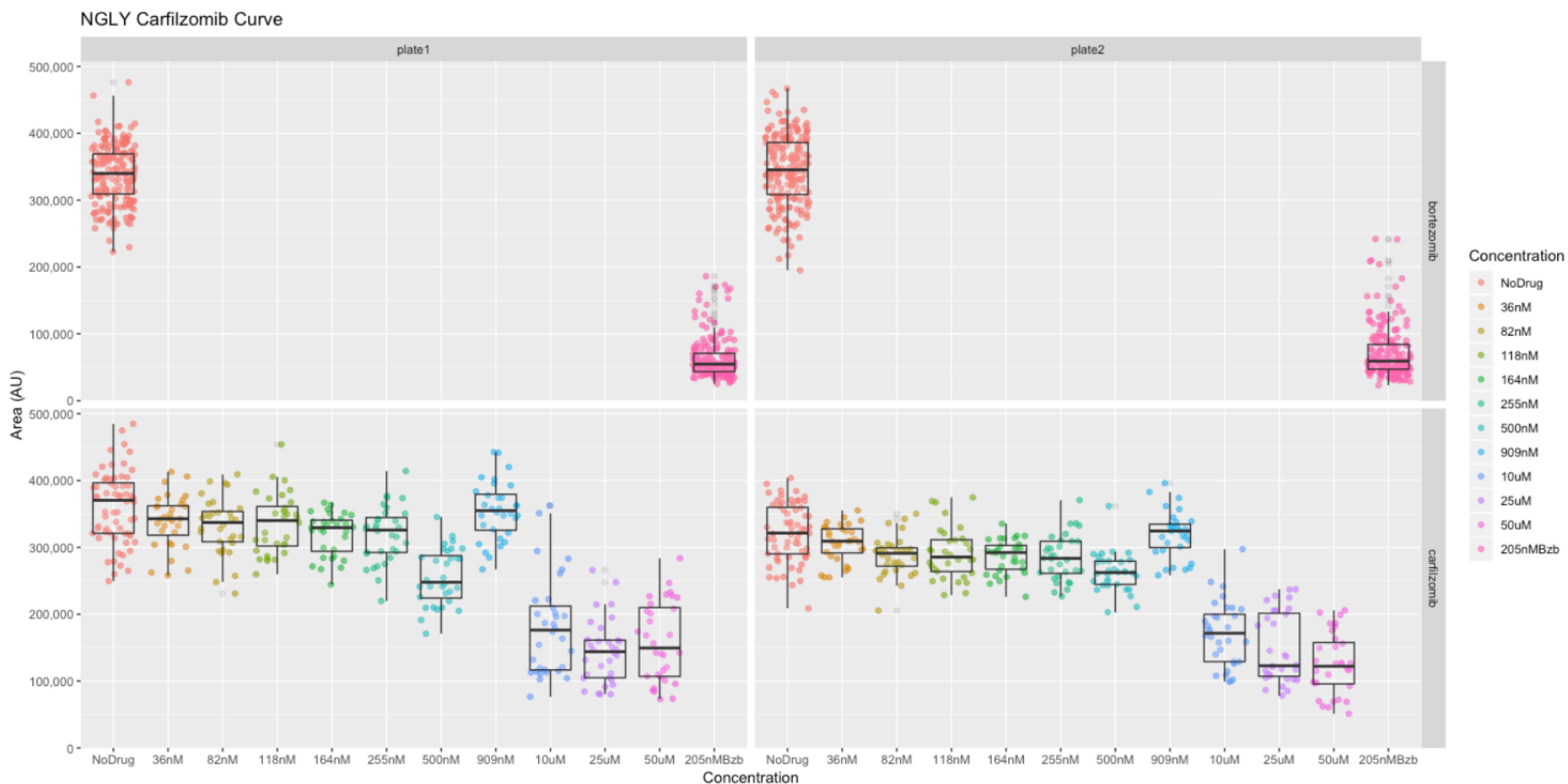
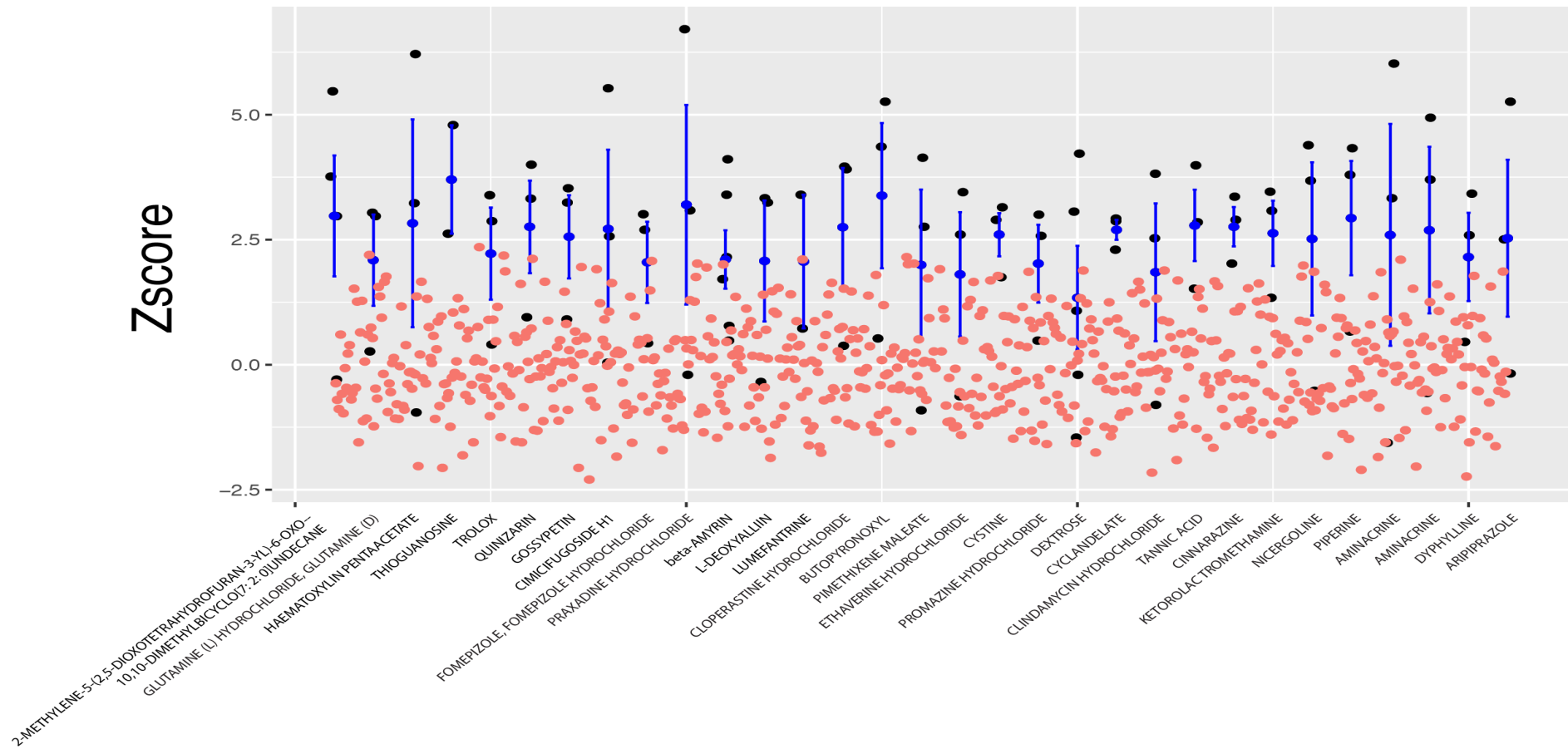


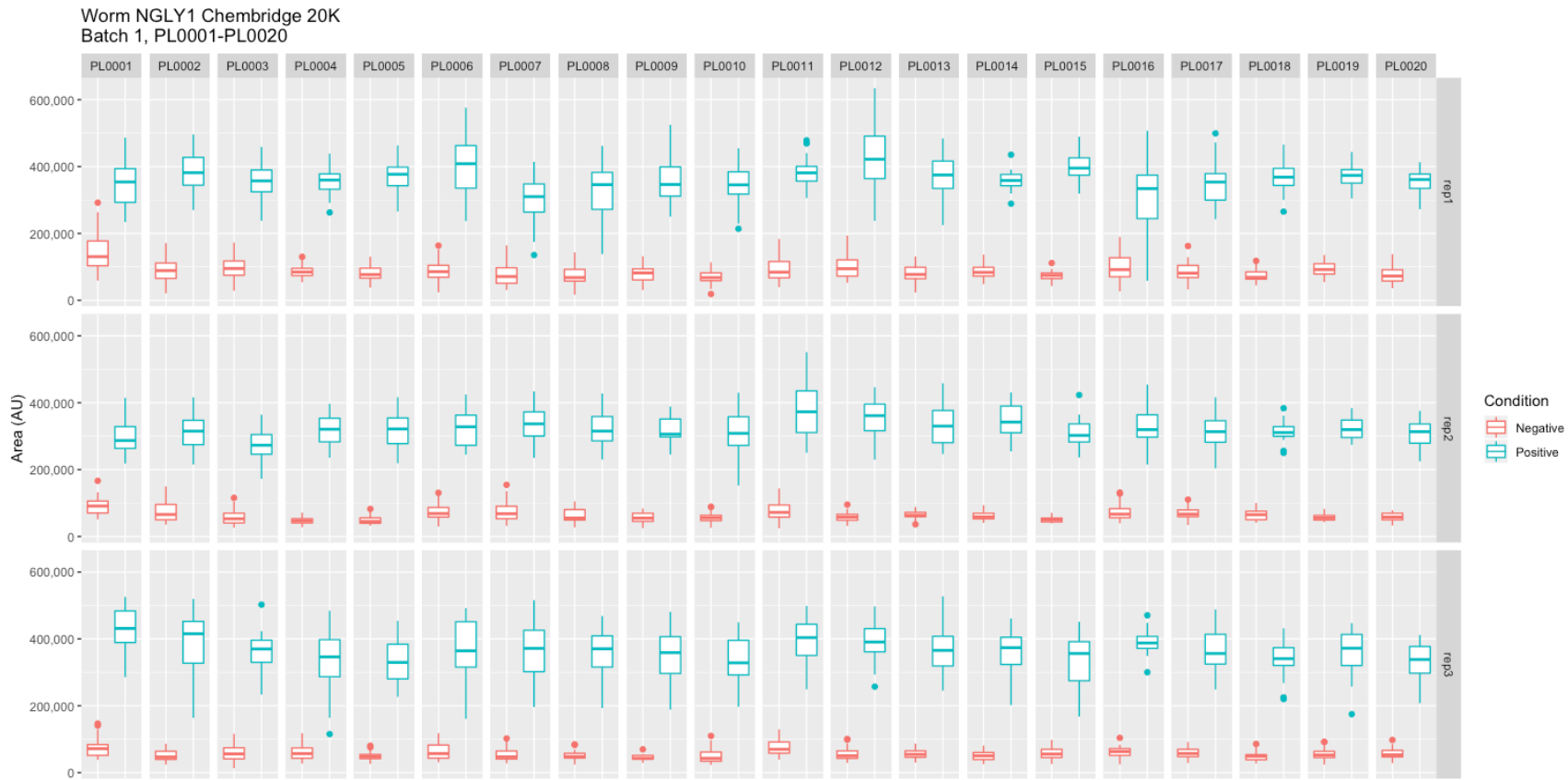
**Supplemental Material.** Summary of worm and fly cross-validation and secondary assays for worm and fly hit compounds from the Microsource Spectrum library (drug repurposing) and a Chembridge diversity library (drug discovery).



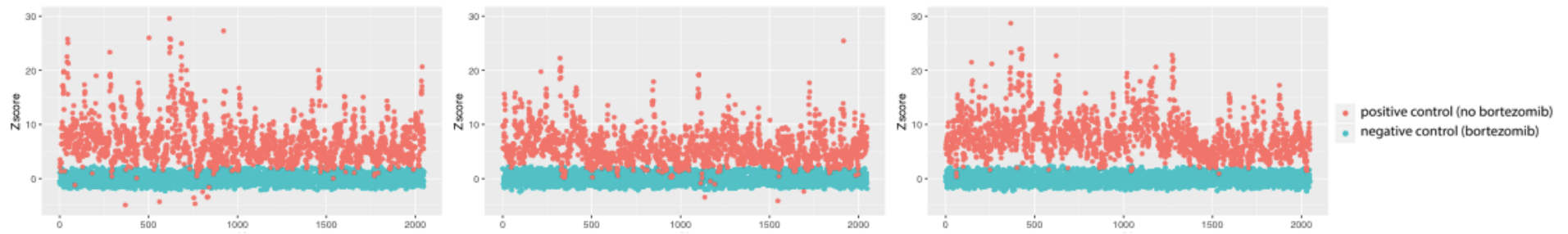
**Figure S1.** Carfilzomib dose-response experiment in *png-1* homozygous worms. Results from two technical replicates are shown. The y-axis is well area occupied by worms. As a comparison, the top panels show *png-1* homozygous worms treated with 205nM bortezomib. The bottom panels show *png-1* homozygous worms treated with ascending doses of carfilzomib.



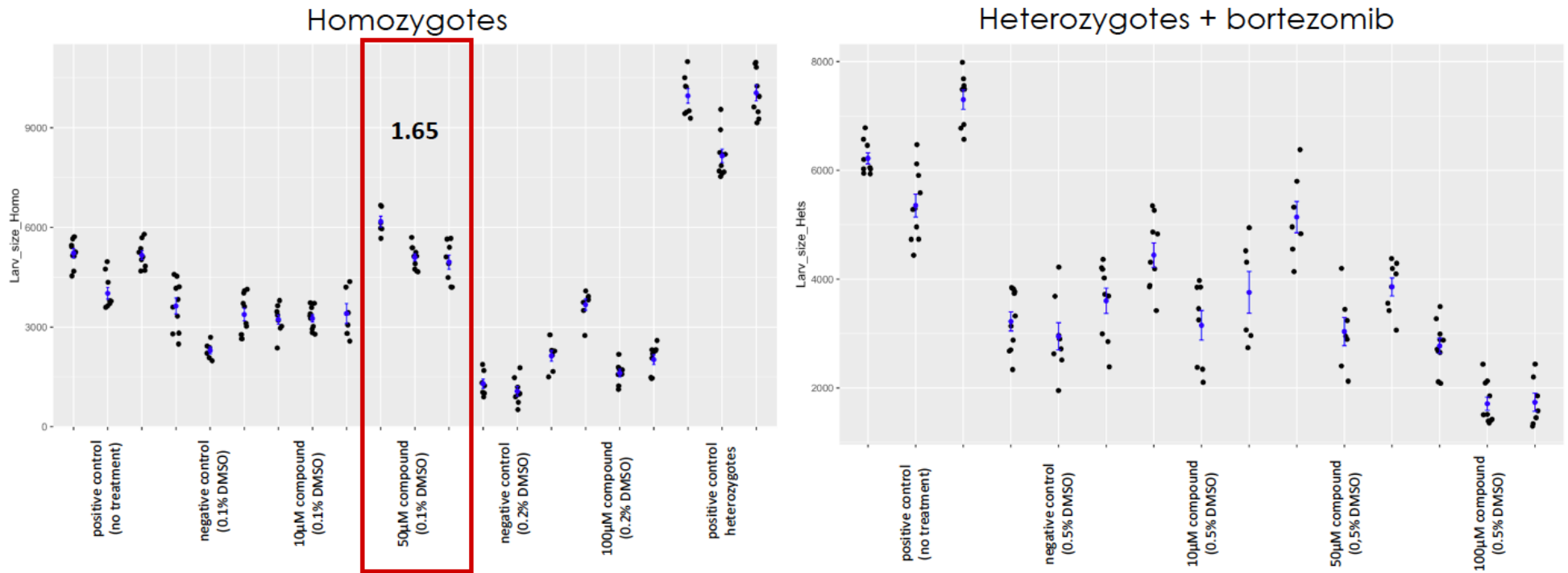
**Figure S2.** Z-score plot of 31 Microsource Spectrum repurposing hits from the *Pngl*<sup>+/-</sup> fly primary screen. Red circles denote negative controls, black circles denote hit compounds wells, and blue circles correspond to the average of replicates of hit compounds.



**Figure S3.** Box plots showing size separation of positive control versus negative control wells in the 20,240-compound lead discovery screen of *png-1* homozygous worms. Area (Arbitrary Units) refers to the area of each well that is occupied by worms.



**Figure S4.** Box plots showing size separation of positive control versus negative control wells in the 20,240-compound lead discovery screen of *Pngl*<sup>+/-</sup> fly larvae. Z scores were calculated as described in Methods and Materials.



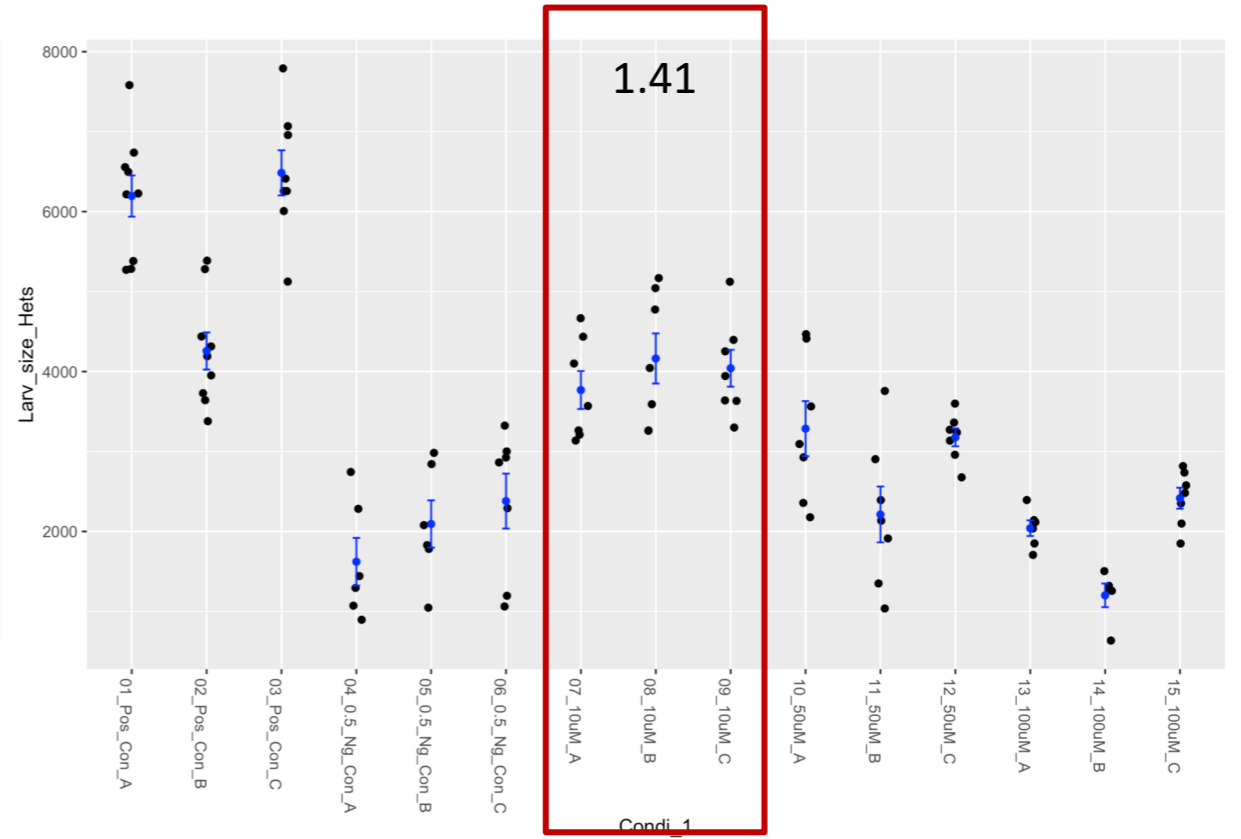
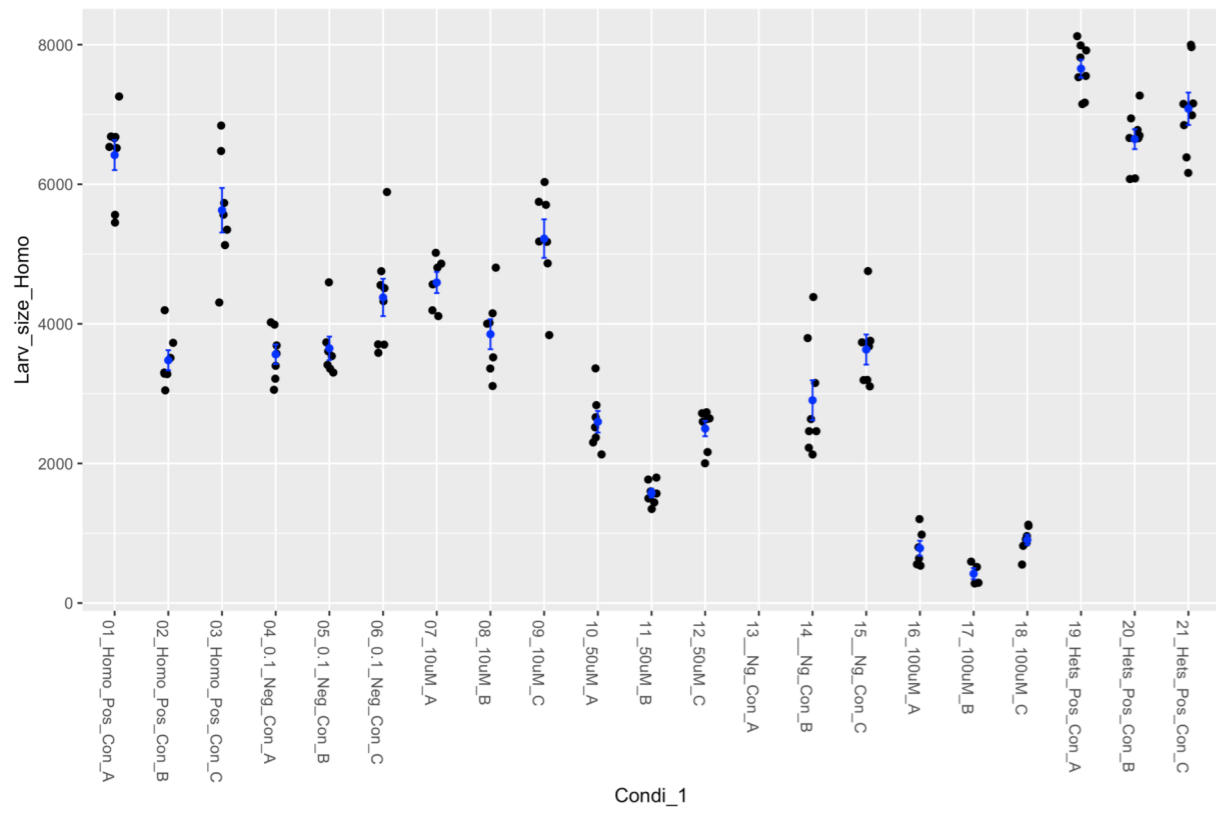
**Figure S5.** Fly *Pngl*<sup>-/-</sup> homozygote and 9µM bortezomib-treated *Pngl*<sup>+/-</sup> heterozygote larval size plots. Black dots represent individual larval sizes with means indicated by blue dots and standard deviations indicated by blue bars. Positive controls are untreated homozygotes. Negative controls are DMSO-treated homozygotes. 50µM sulforaphane fully rescues DMSO-treated homozygotes (red box). 1.65 refers to the fold change in larval size difference between sulforaphane-treated homozygotes and the negative control.

# Fly Microsource Spectrum Retests

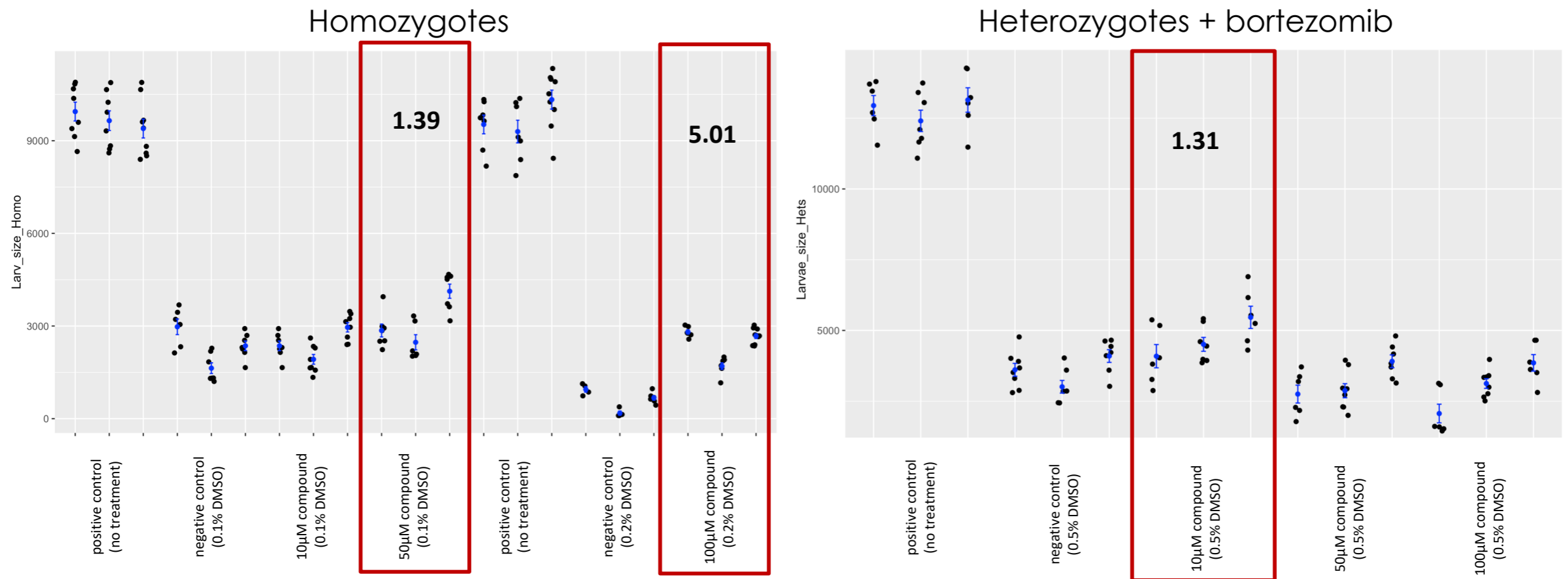
All compounds that scored as hits in the fly *Pngl*<sup>+/-</sup> Microsource Spectrum (drug repurposing) screen were retested in two fly secondary assays : rescue of *Pngl*<sup>-/-</sup> homozygotes and rescue of bortezomib-treated *Pngl*<sup>+/-</sup> heterozygotes. Red boxes indicate compounds that successfully retested. The number indicates the size ratio of compound-treated fly larvae to DMSO-treated control homozygous fly larvae. For example, 2 means that a compound increased the size of mutant larvae by twofold.

(Please note that some homozygote experiments included untreated heterozygote controls)

# Fly Microsource retest –2-METHYLENE-5-(2,5-DIOXOTETRAHYDROFURAN-3-YL)-6-OXO--10,10-DIMETHYL

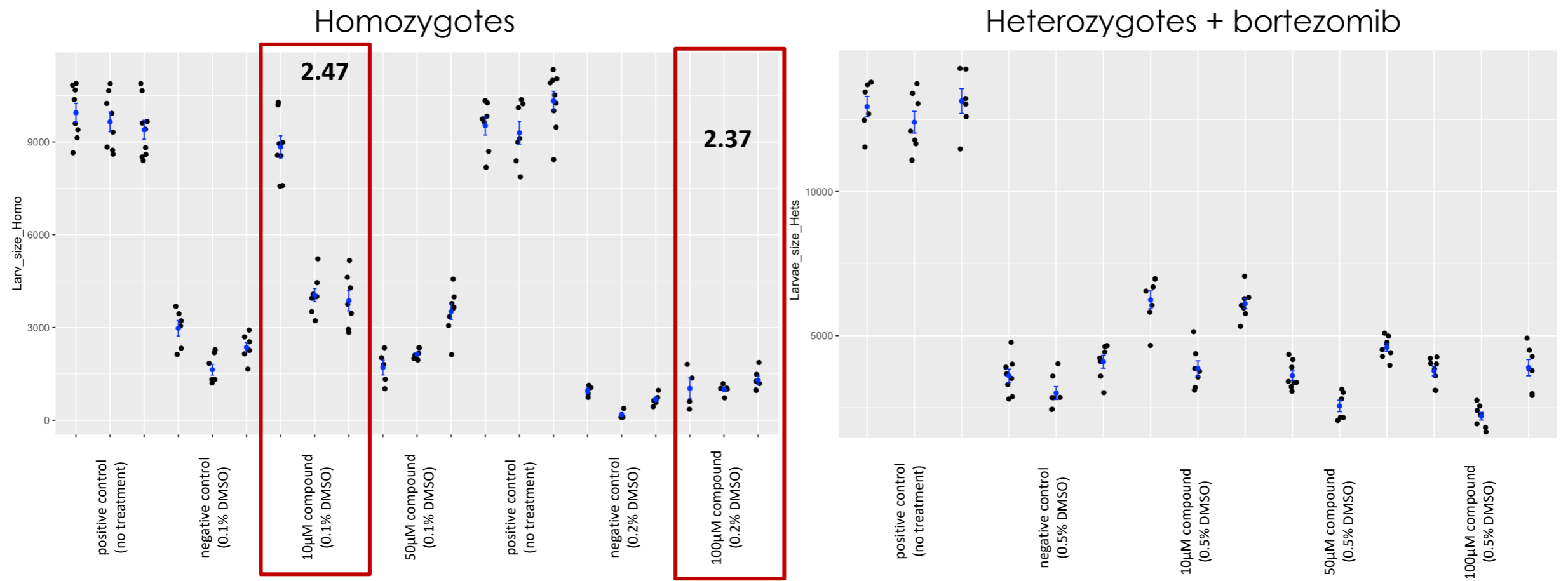


# Fly Microsource Retest – AMINACRINE



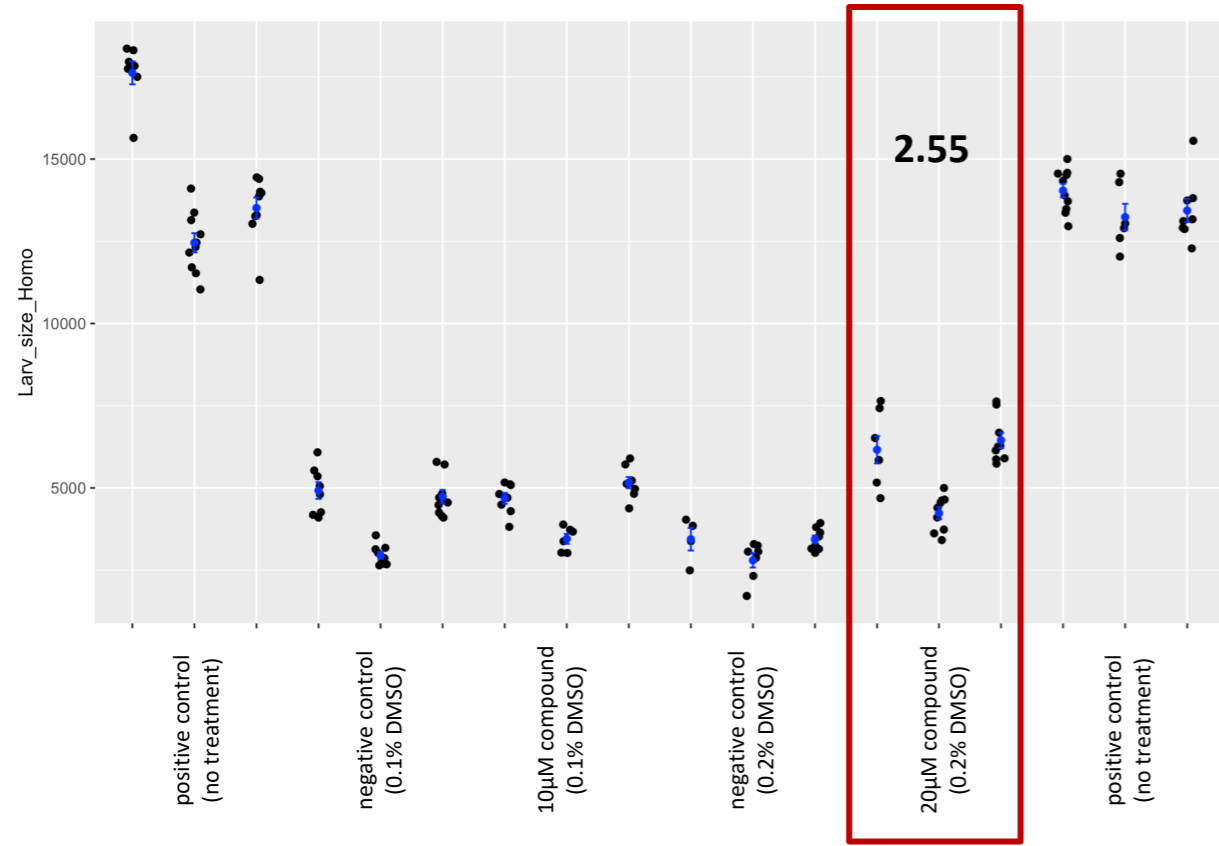


# Fly Microsource Retest – ARIPIIPRAZOLE

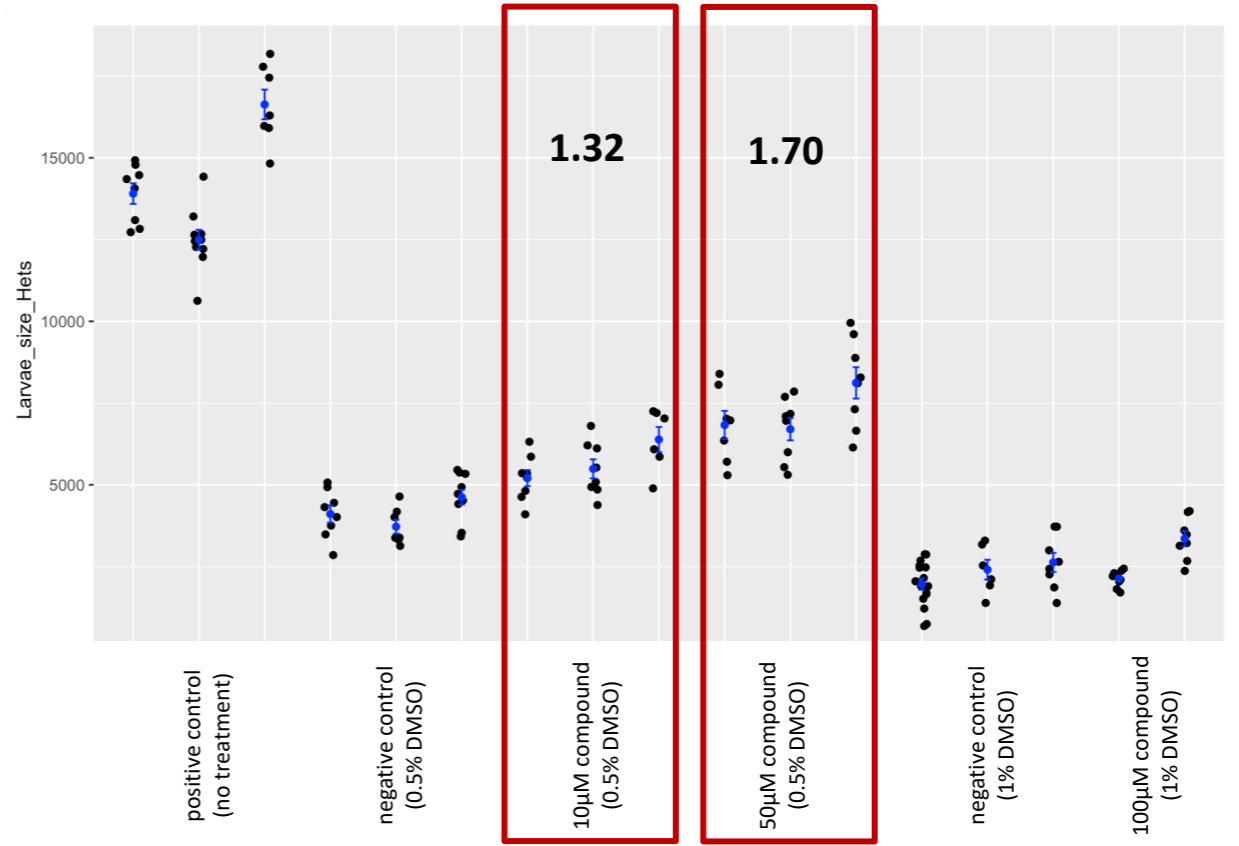


# Fly Microsource Retest – beta-AMYRIN

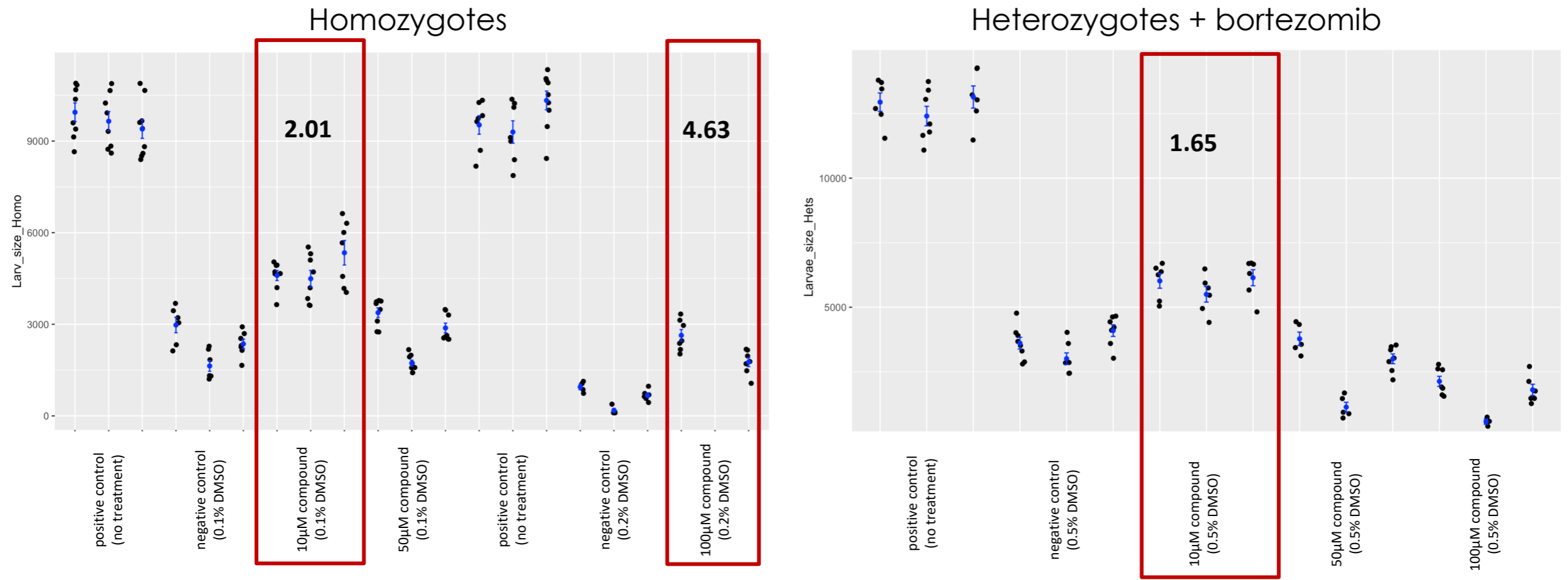
## Homozygotes



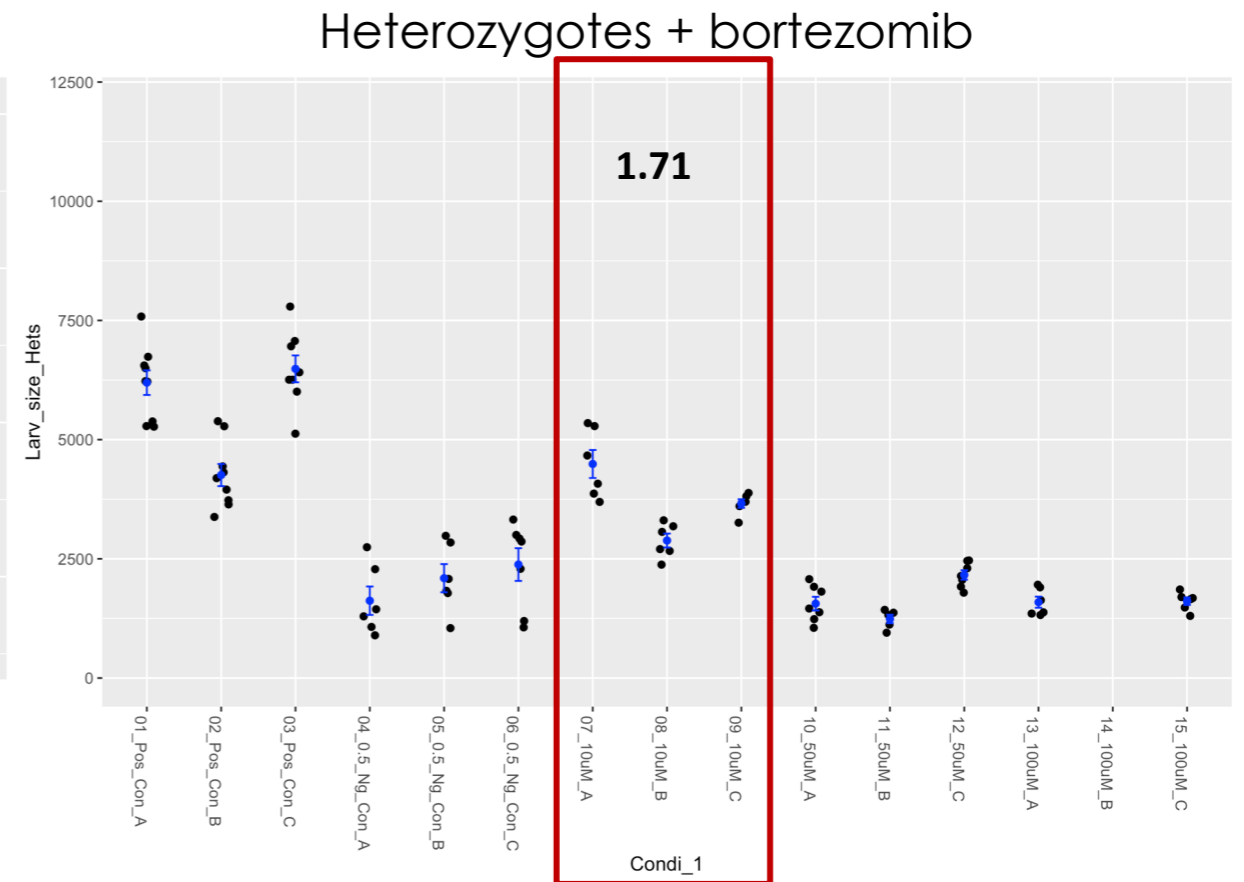
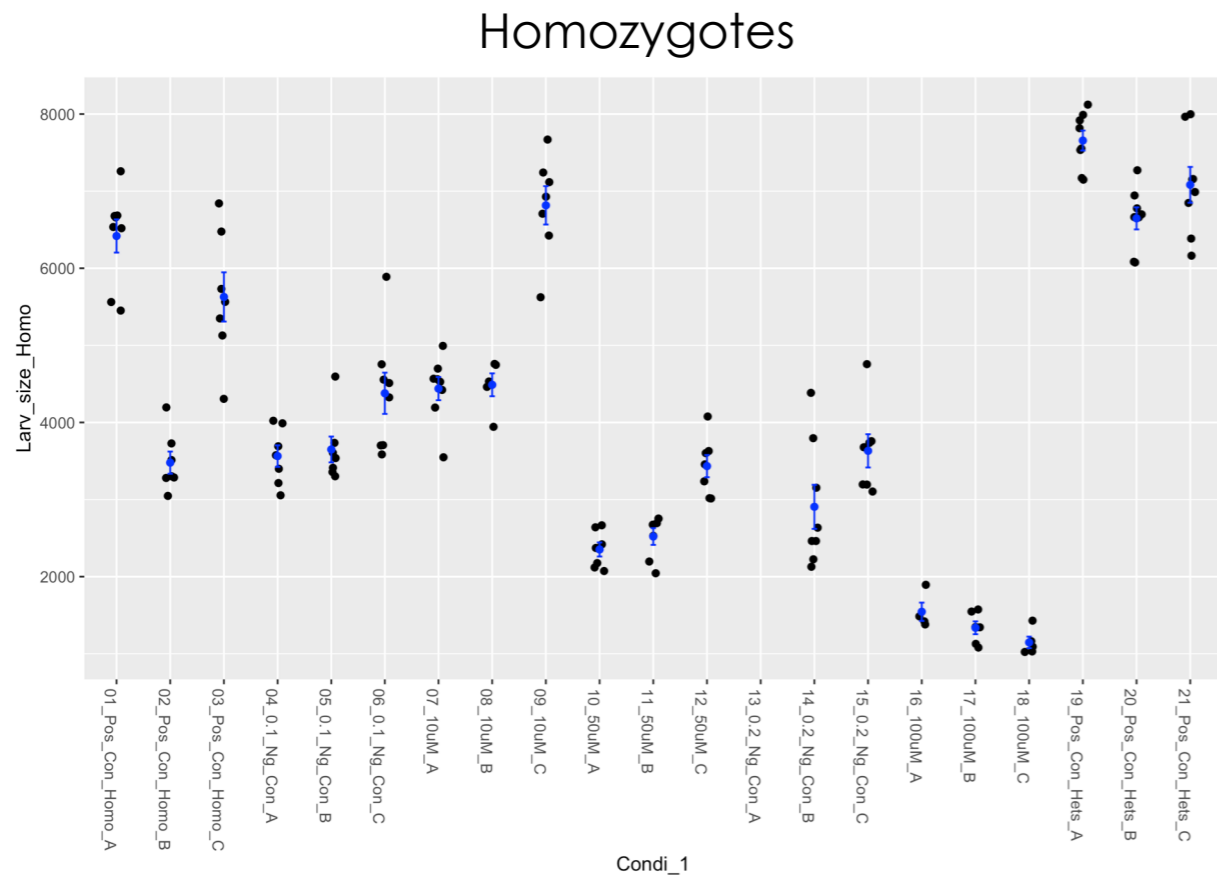
## Heterozygotes + bortezomib



# Fly Microsource Retest – BUTOPYRONOXYL

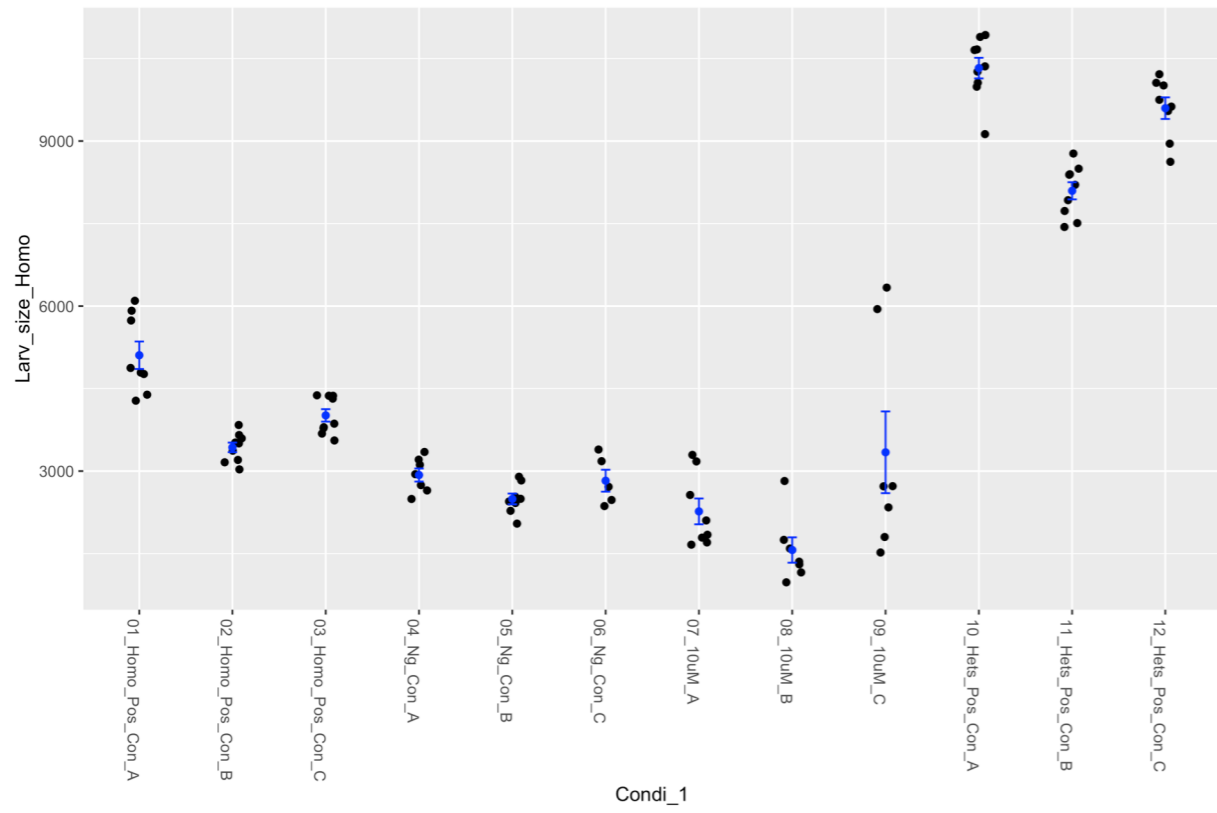


# Fly Microsource Retest – CIMICIFUGOSIDE

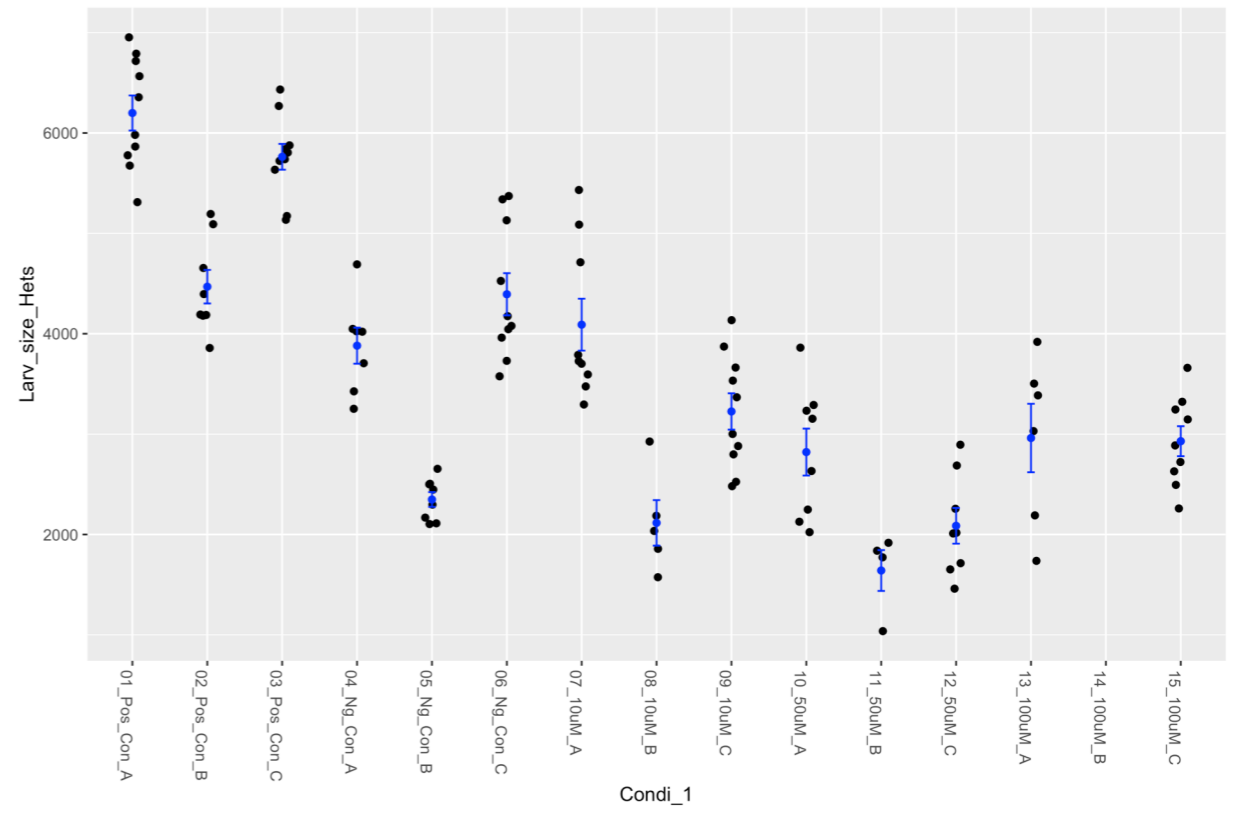


# Fly Microsource Retest – CINNARAZINE

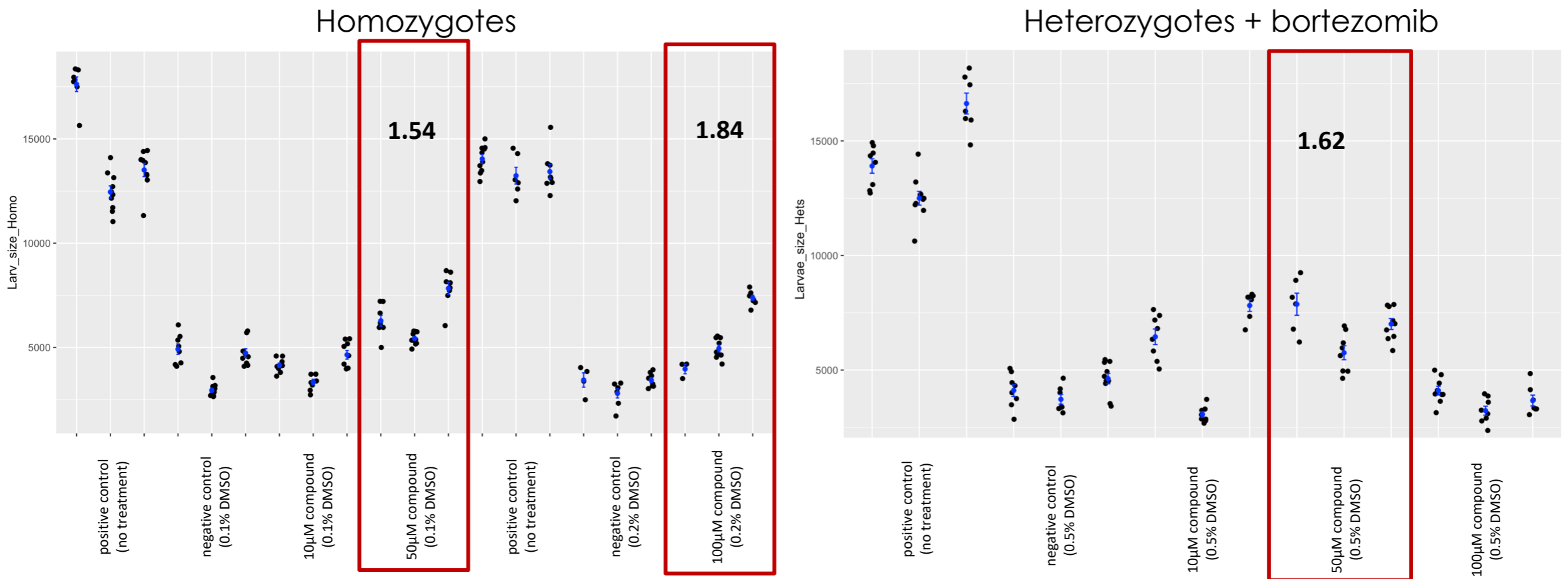
## Homozygotes



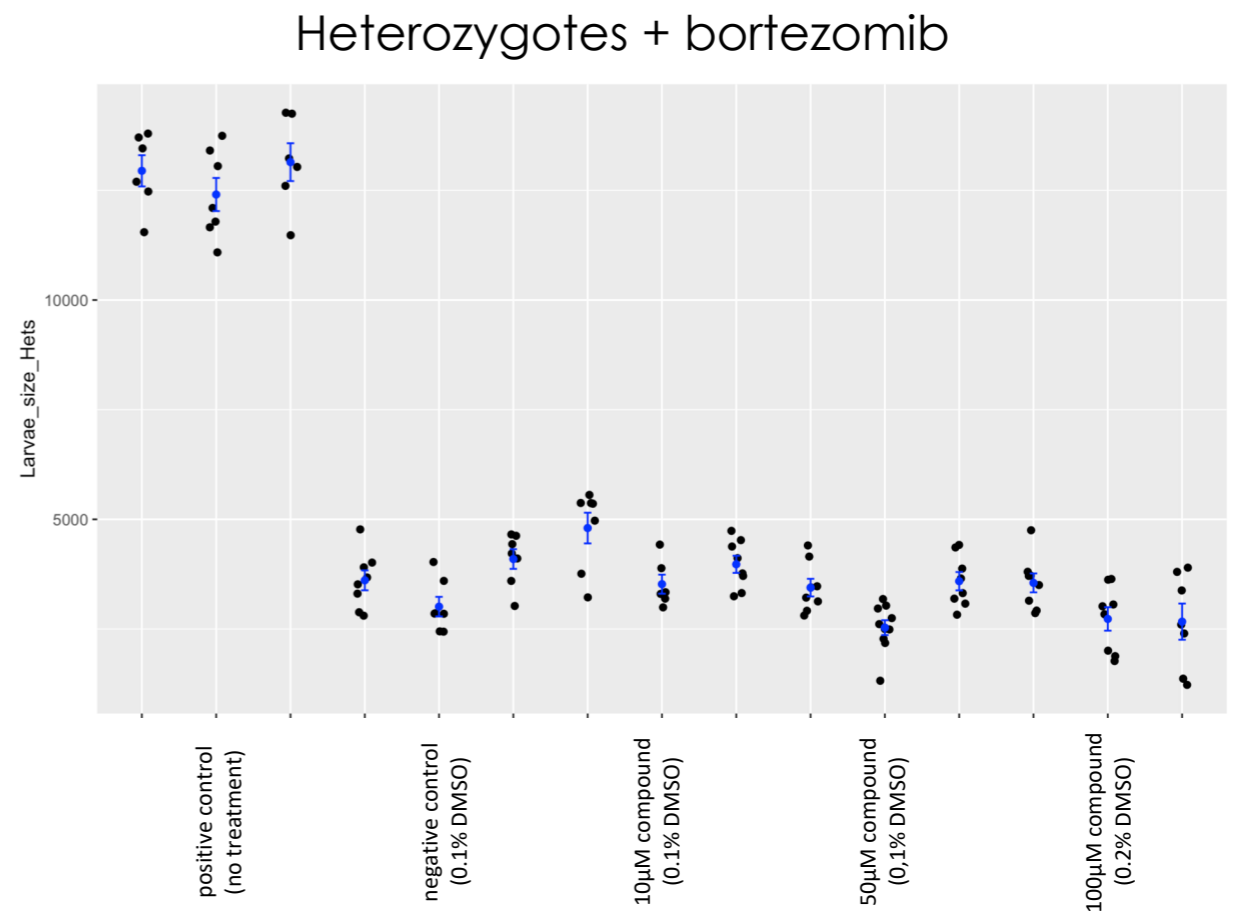
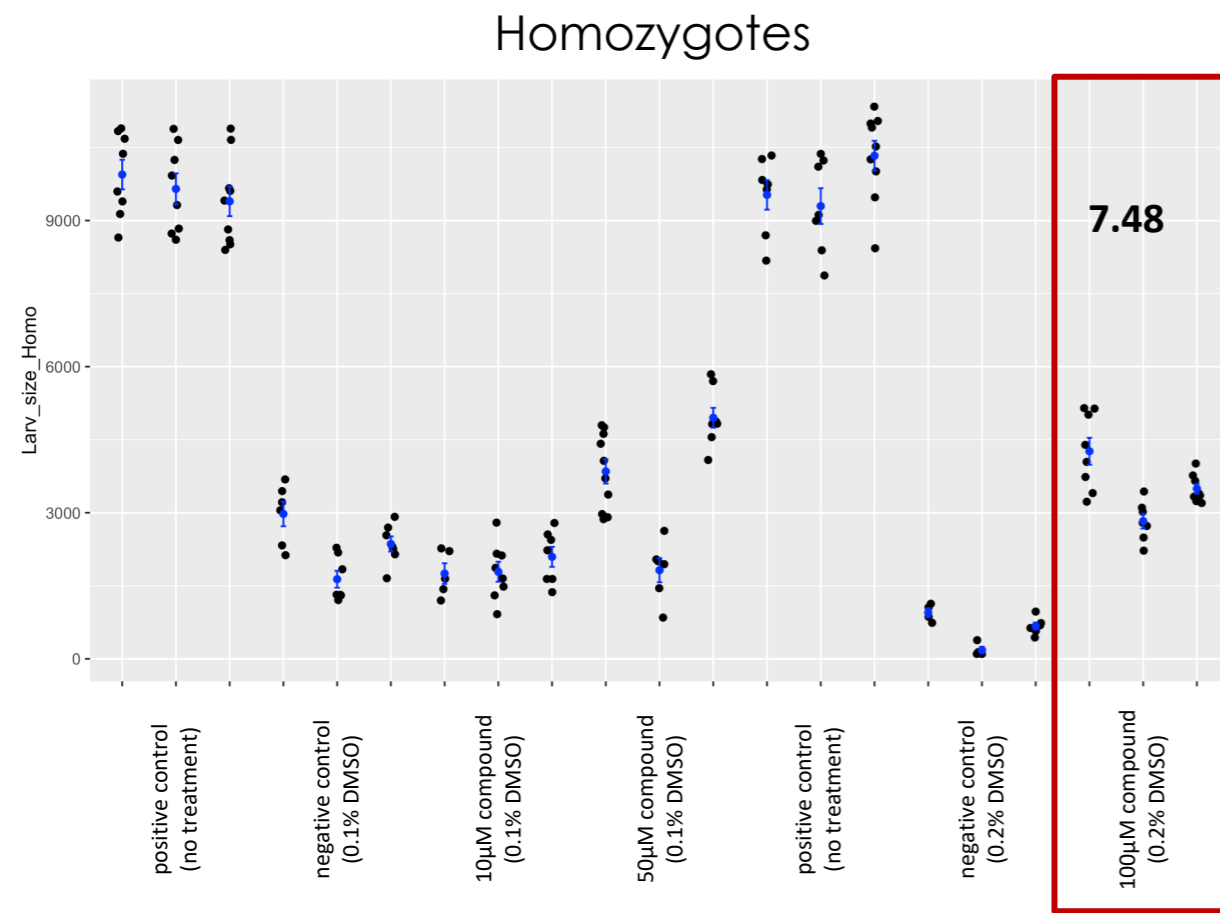
## Heterozygotes + bortezomib



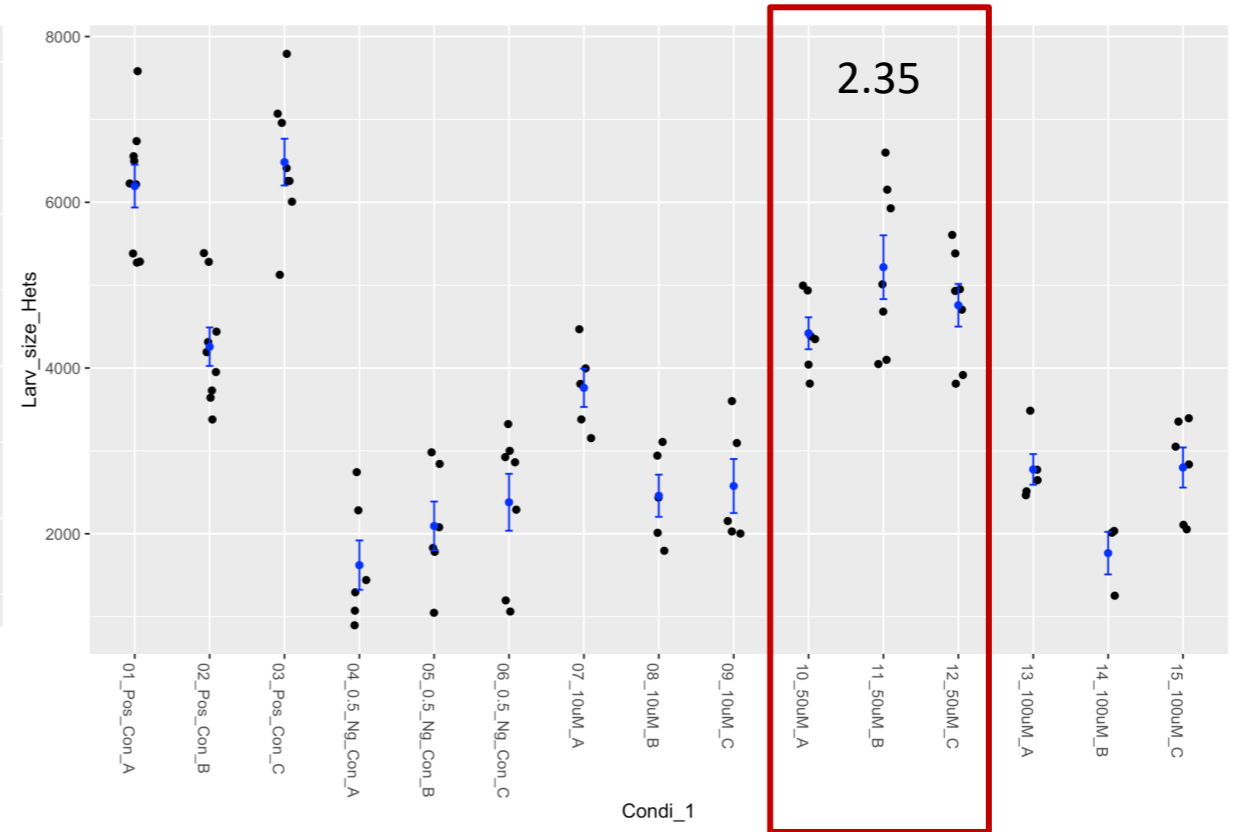
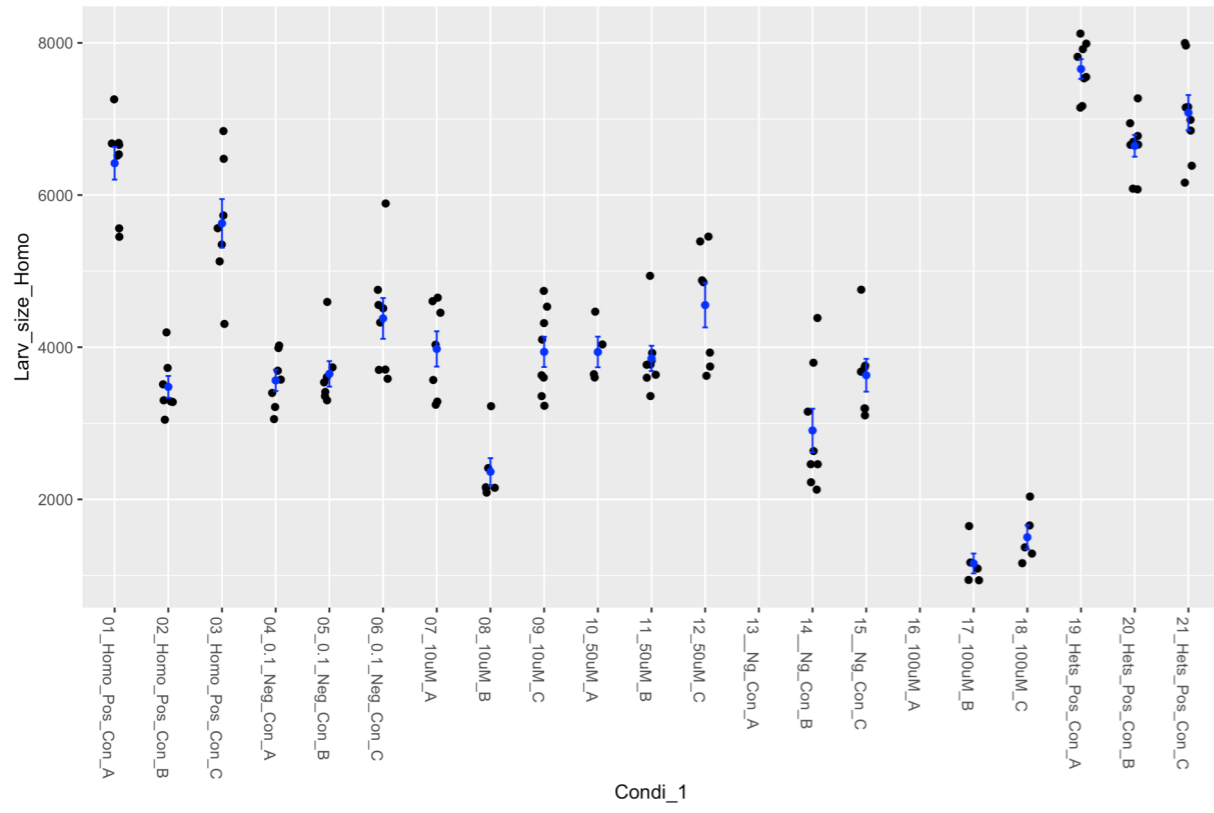
# Fly Microsource Retest – CLINDAMYCIN HYDROCHLORIDE



# Fly Microsource Retest – CLOPERASTINE HYDROCHLORIDE

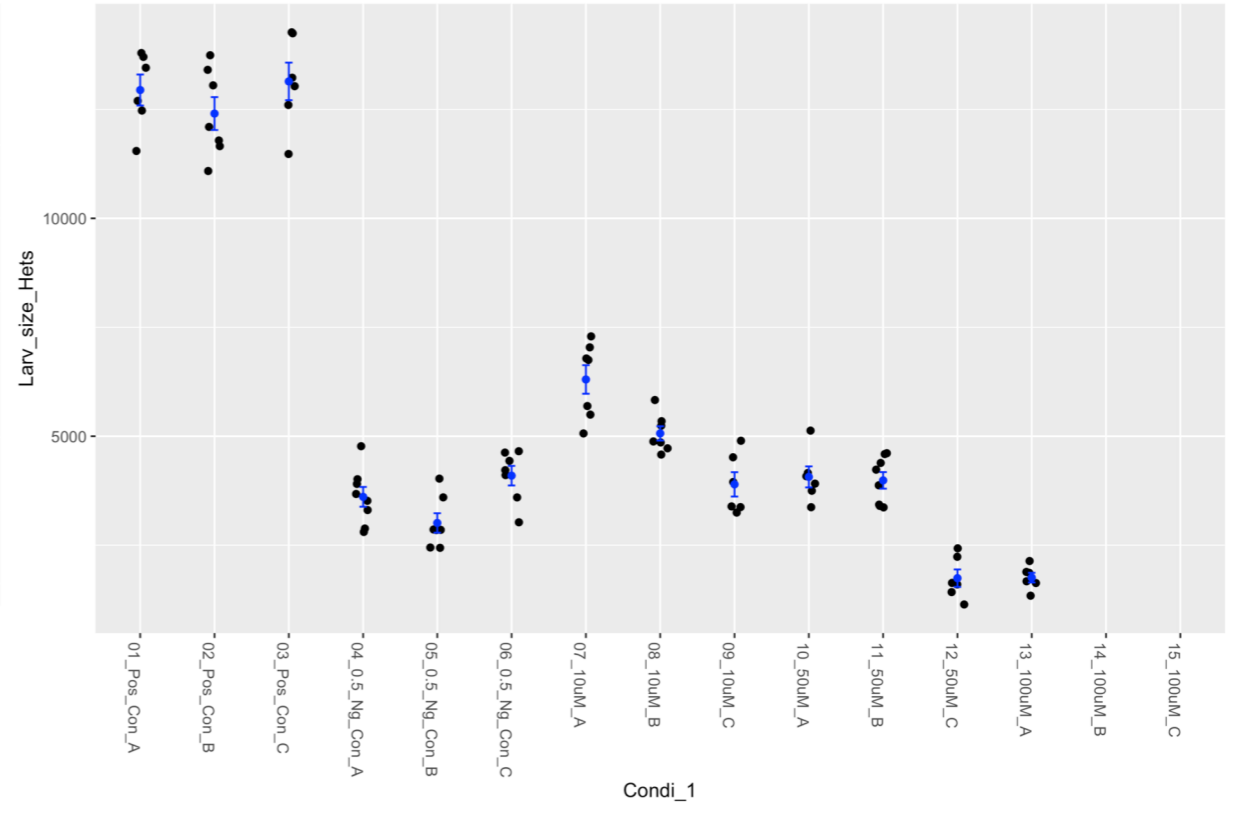
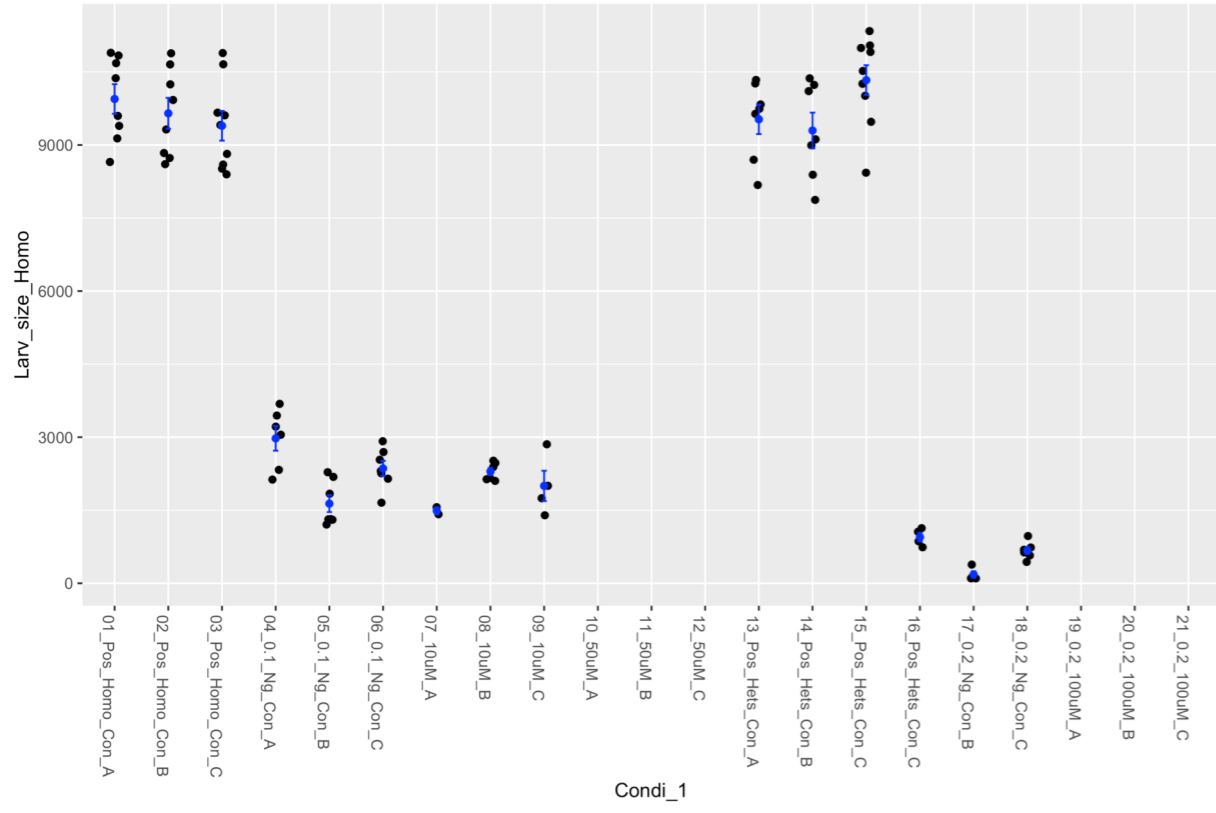


# Fly Microsource retest – CYCLANDELATE

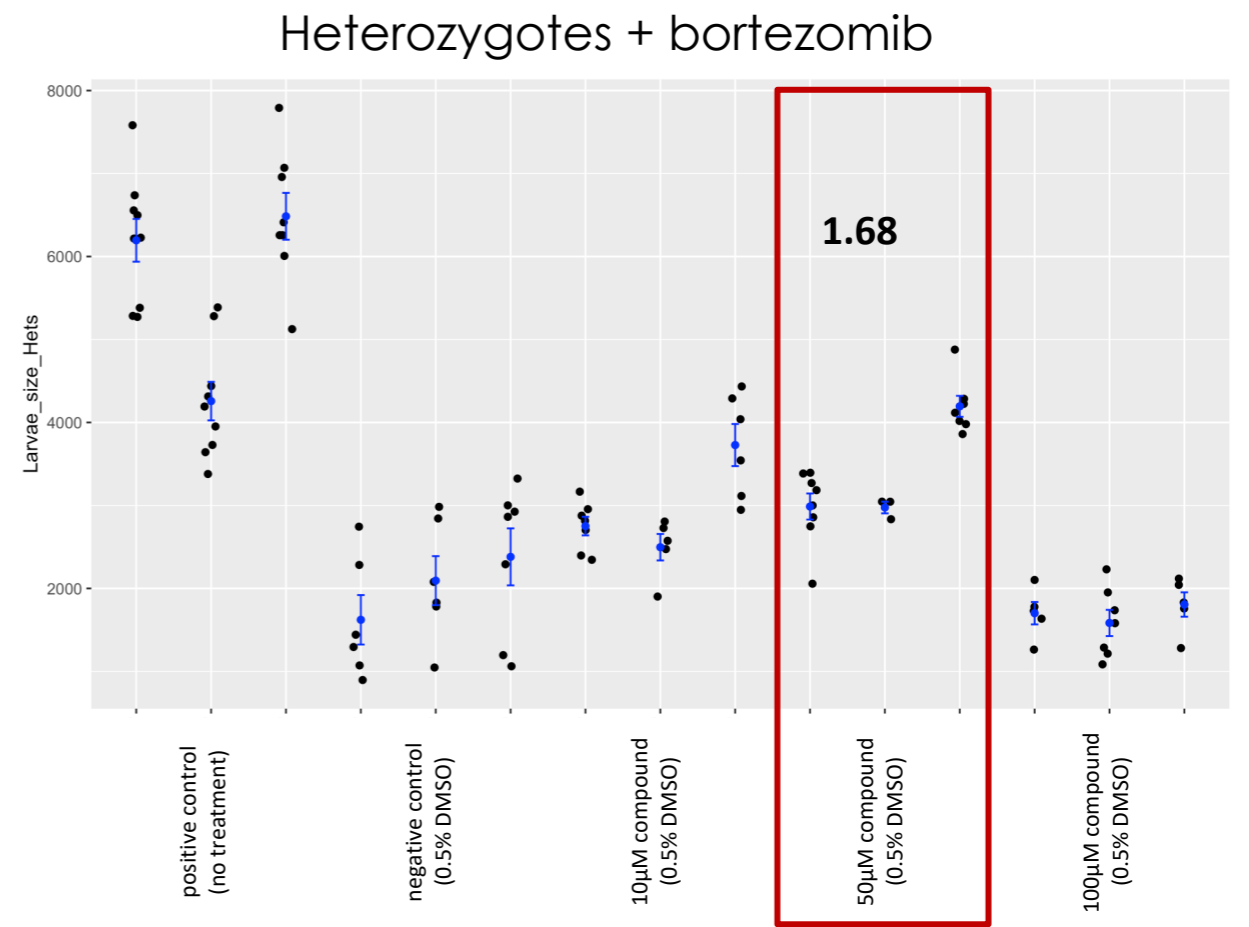
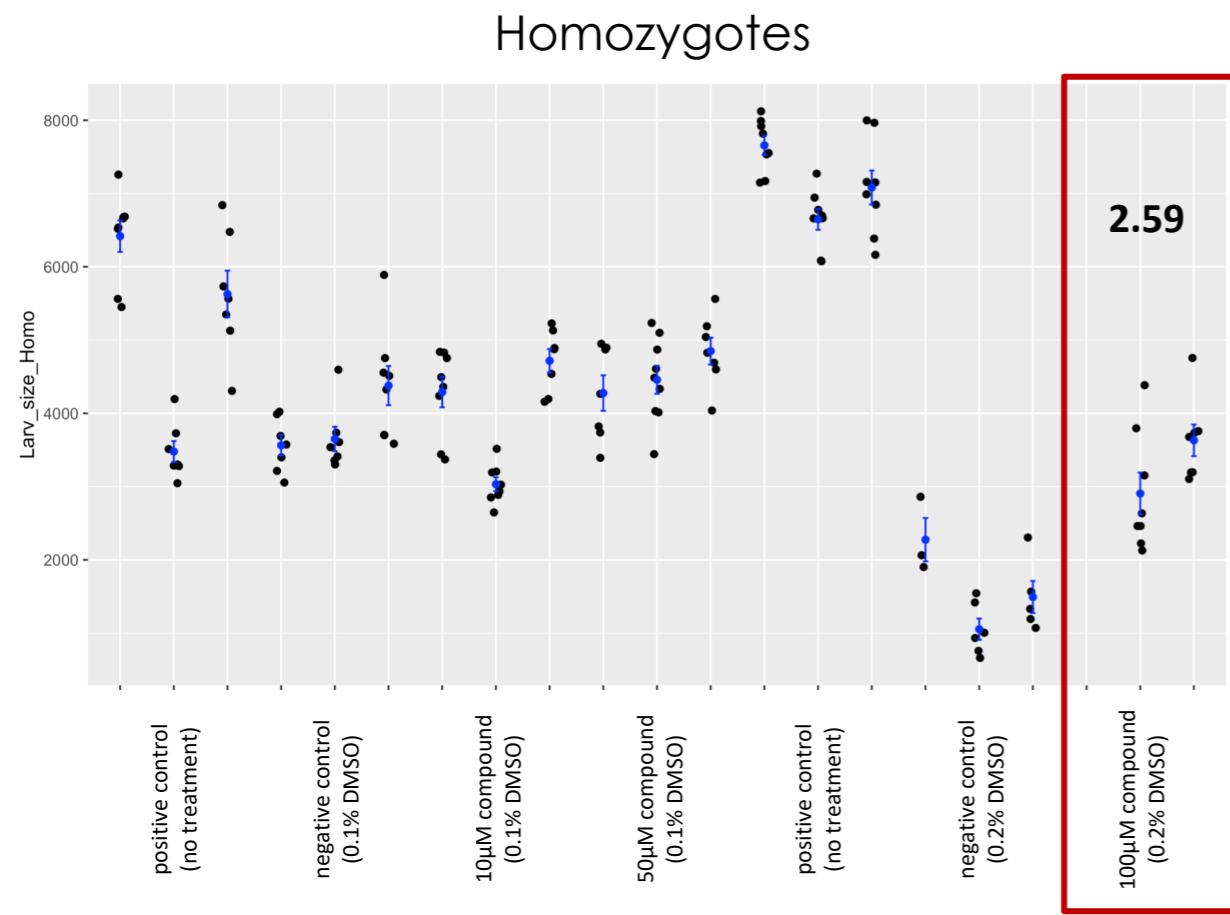




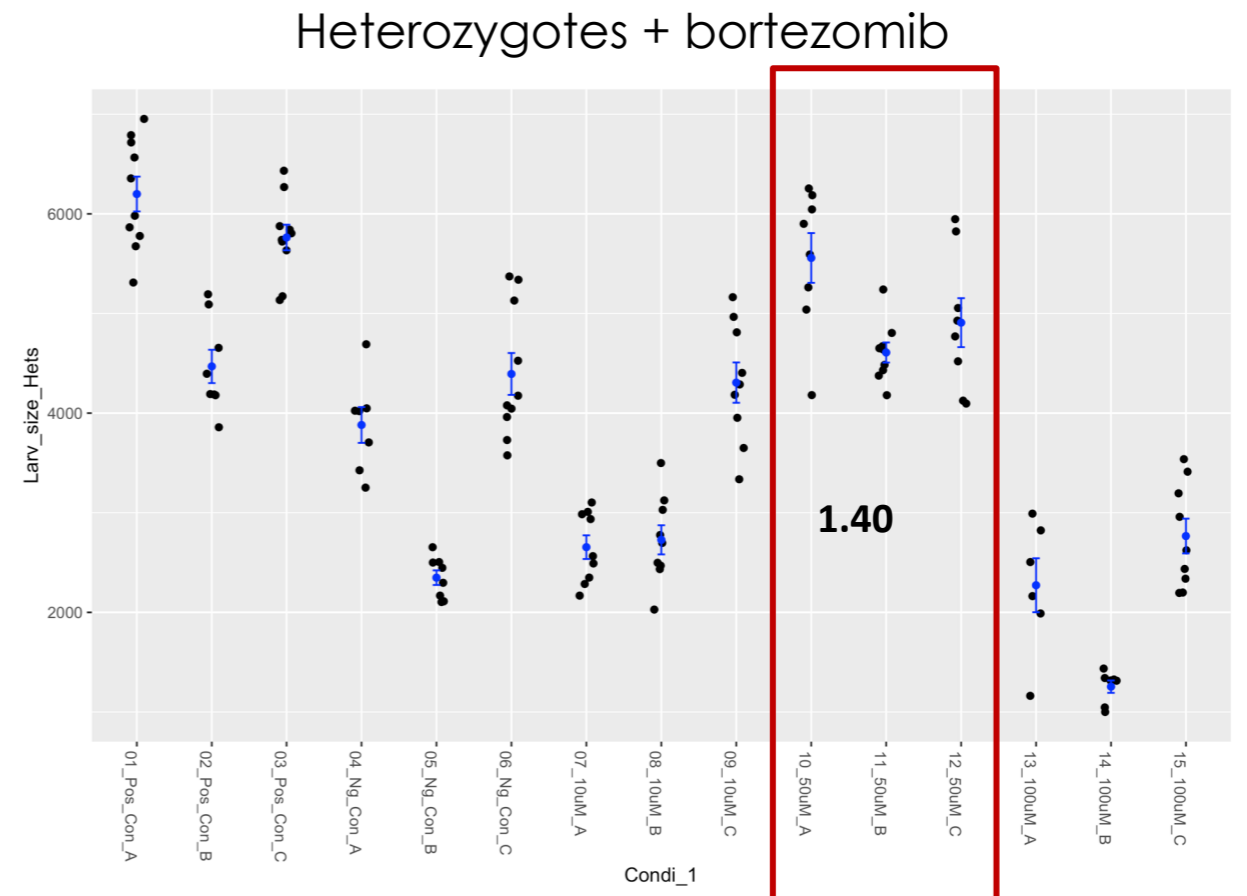
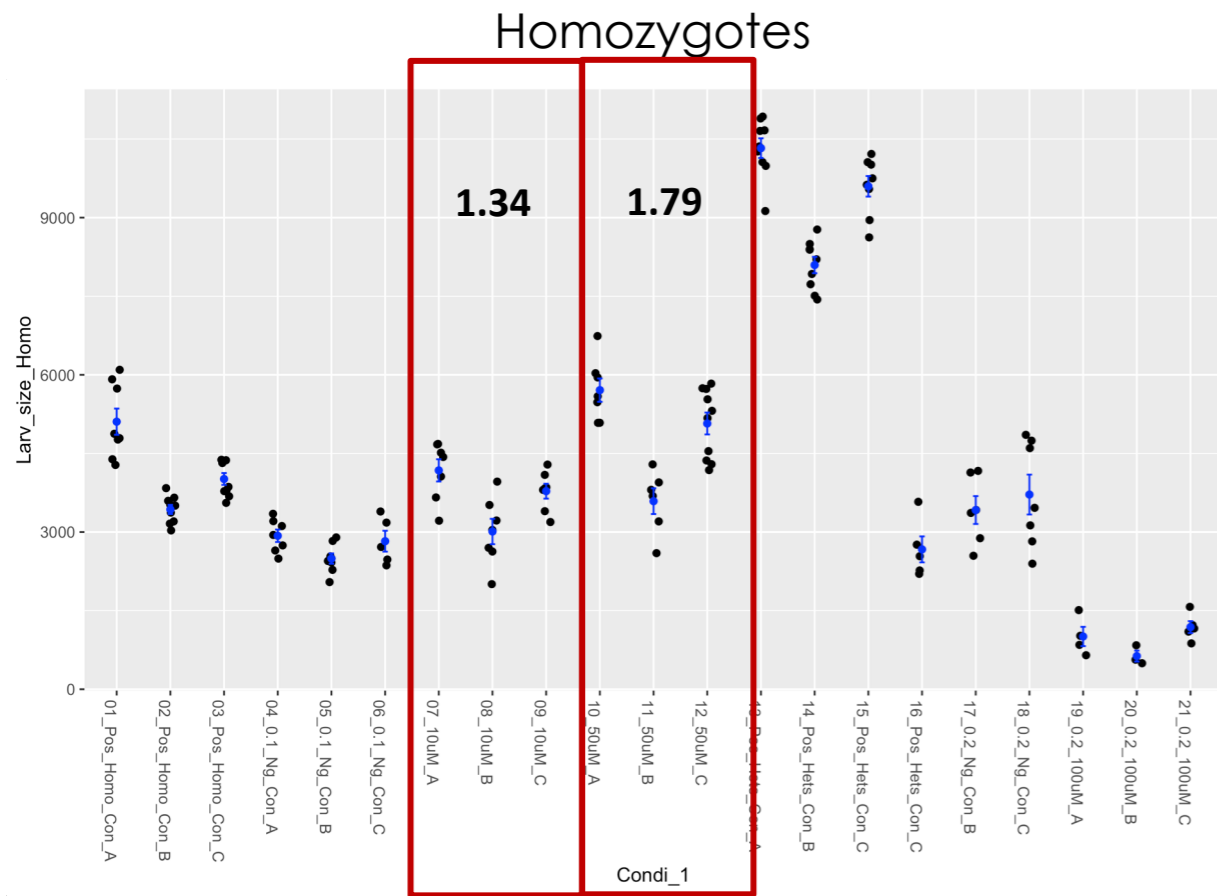
# Fly Microsource retest – CYSTINE



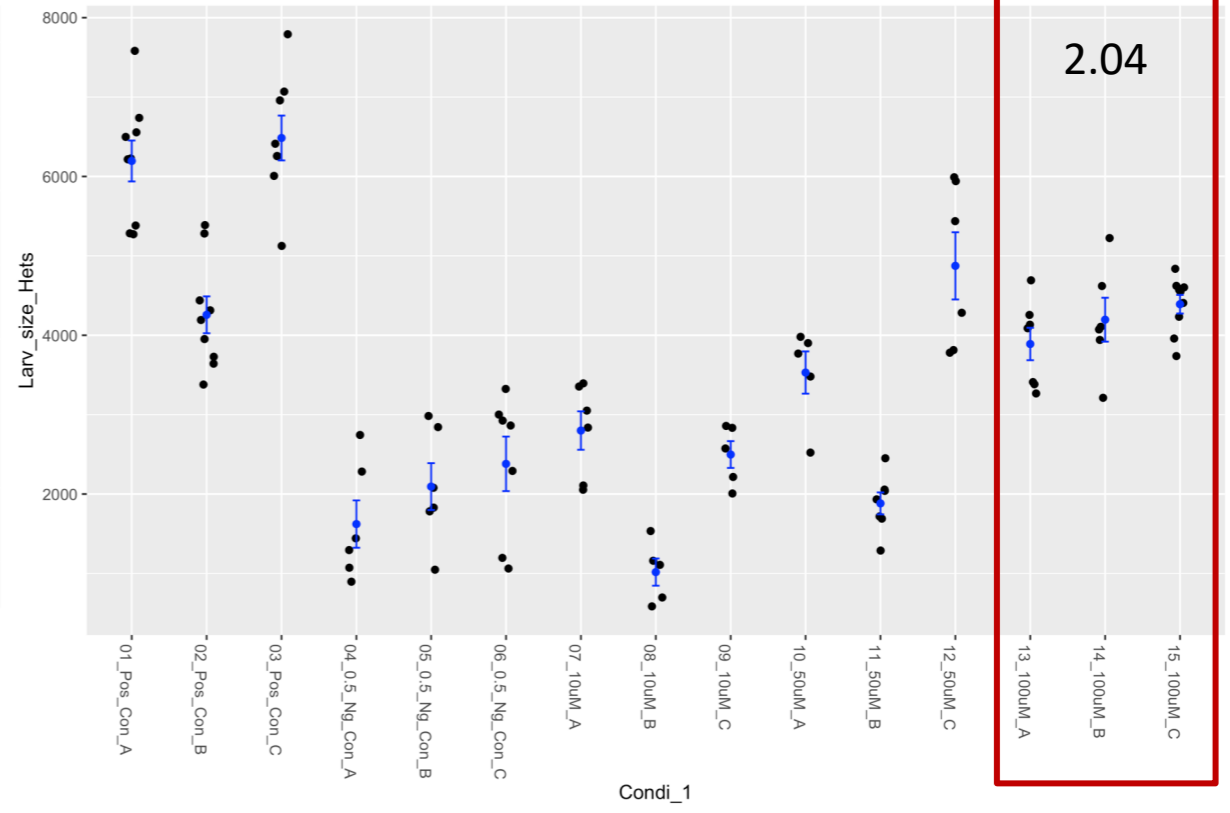
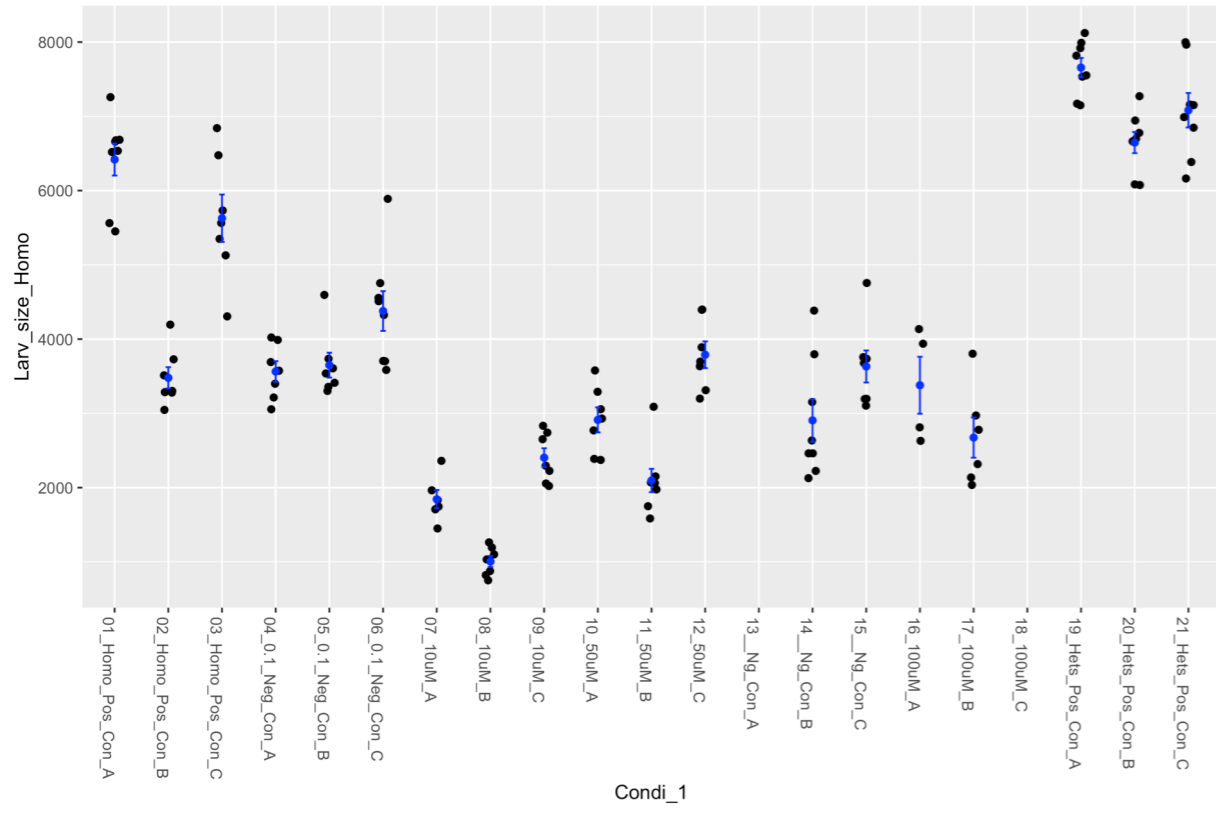
# Fly Microsource Retest – DYPHYLLINE



# Fly Microsource Retest – EPALRESTAT

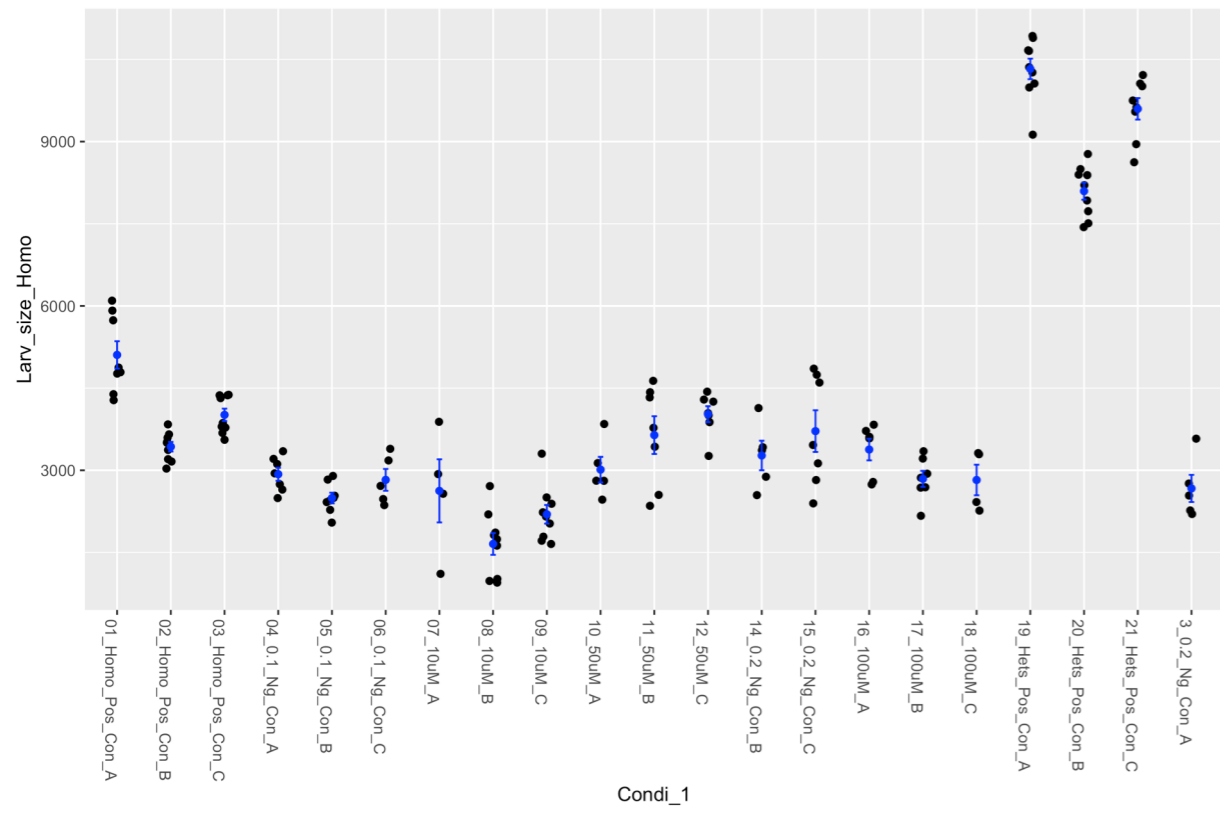


# Fly Microsource retest – ETHAVERINE HYDROCHLORIDE

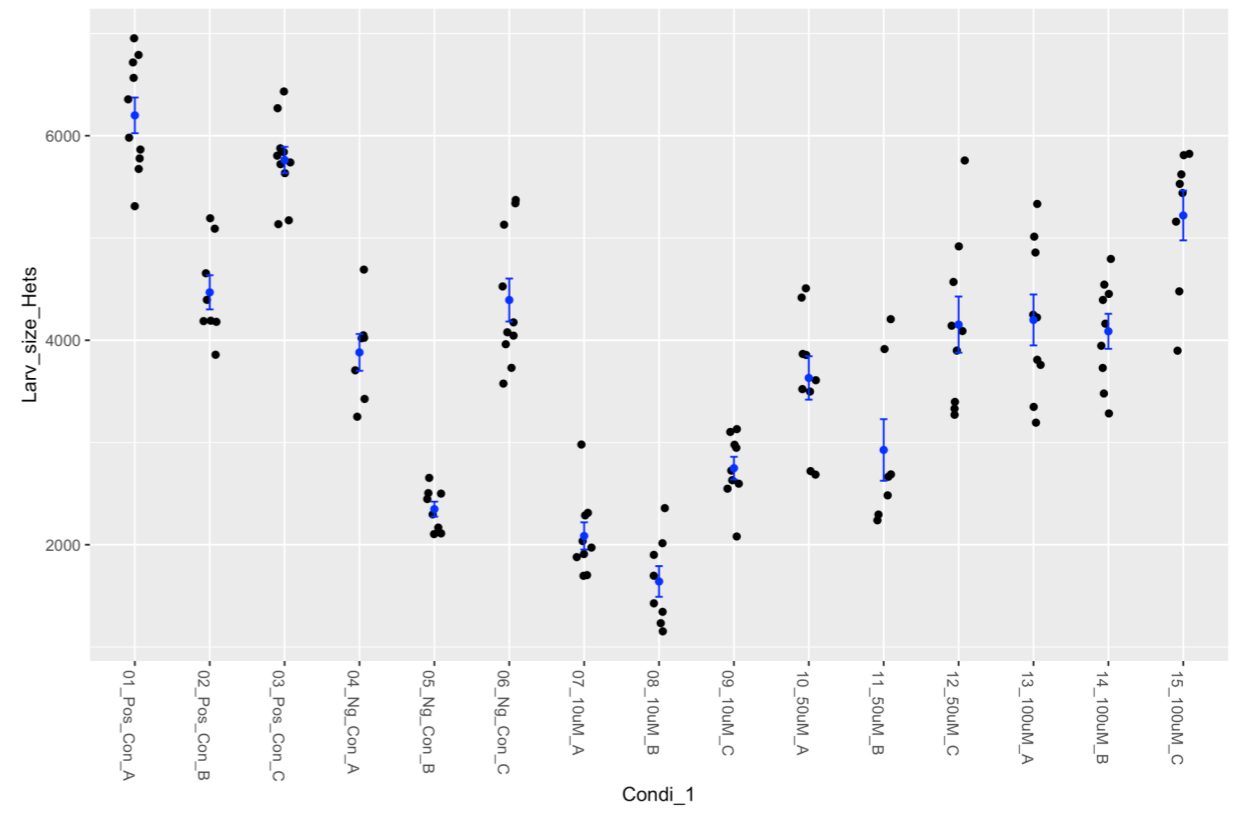


# Fly Microsource Retest – FOMEPIZOLE HYDROCHLORIDE

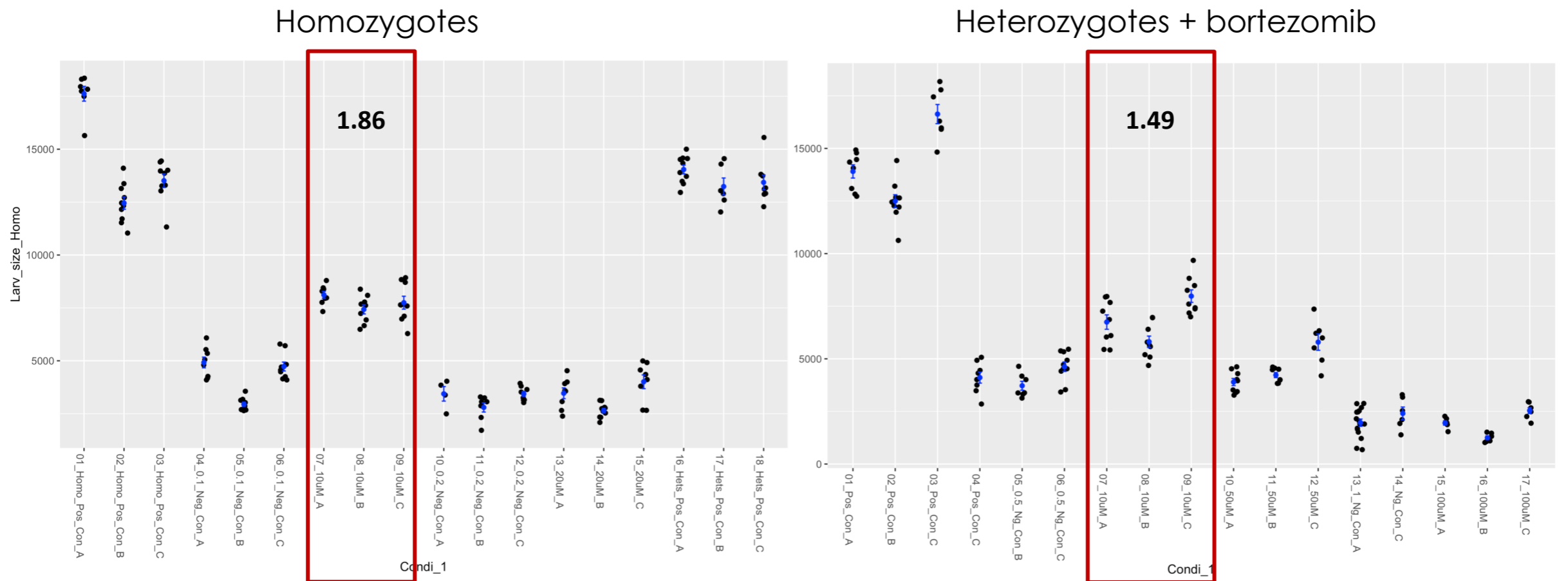
Homozygotes



Heterozygotes + bortezomib

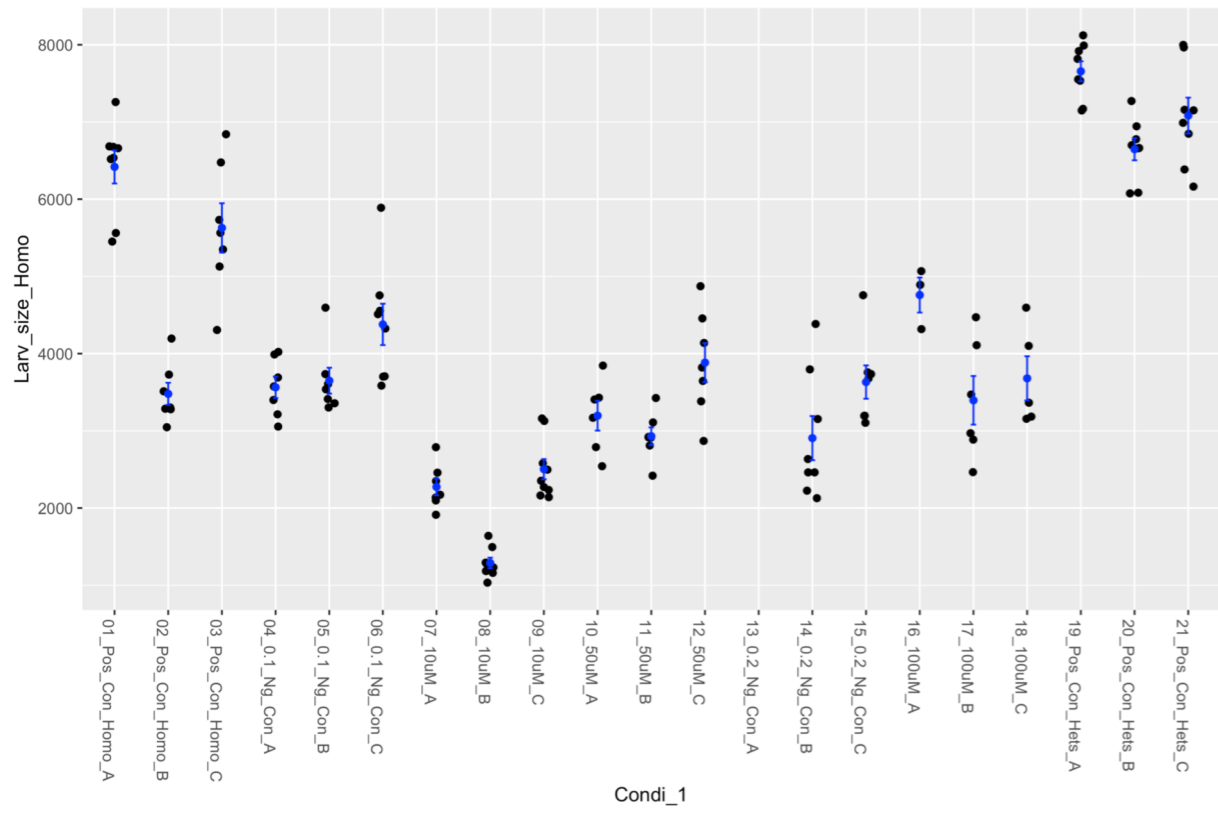


# Fly Microsource Retest – GLUTAMINE (L) HYDROCHLORIDE

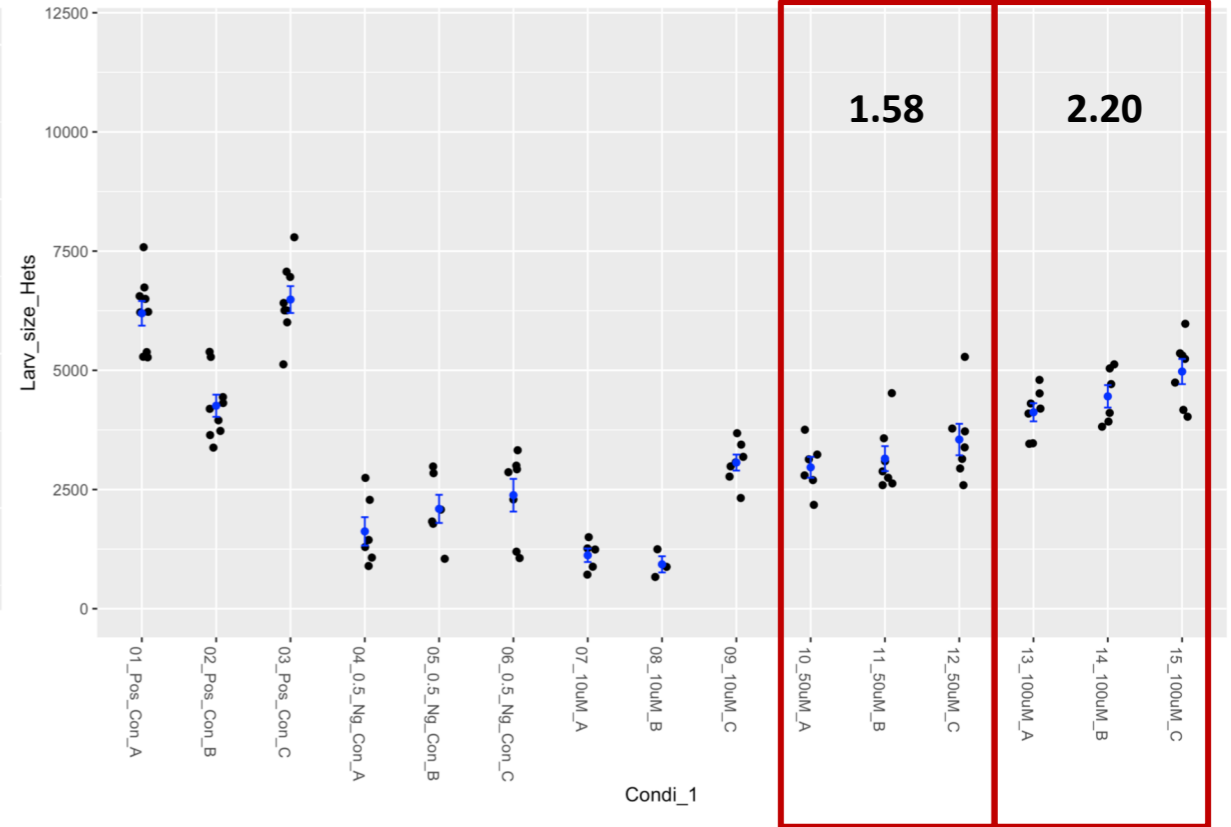


# Fly Microsource Retest – GOSSYPETIN

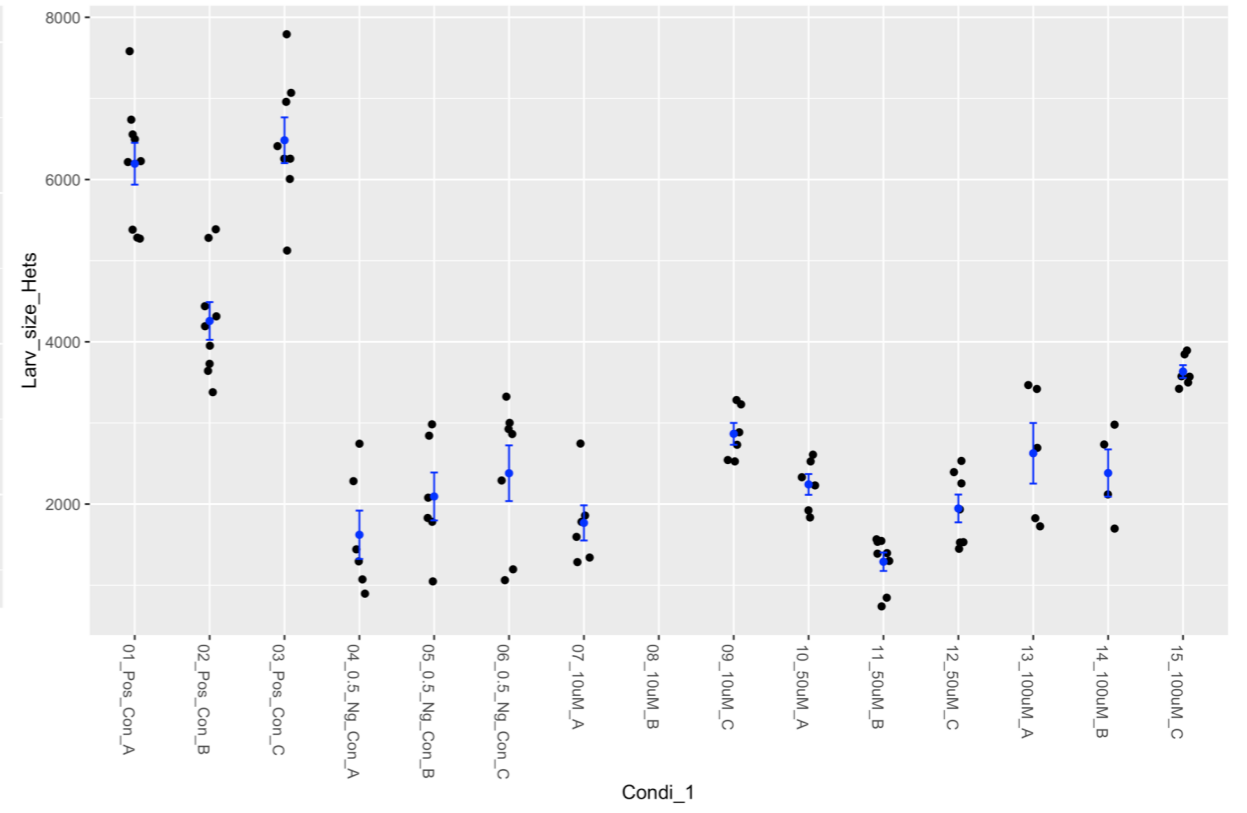
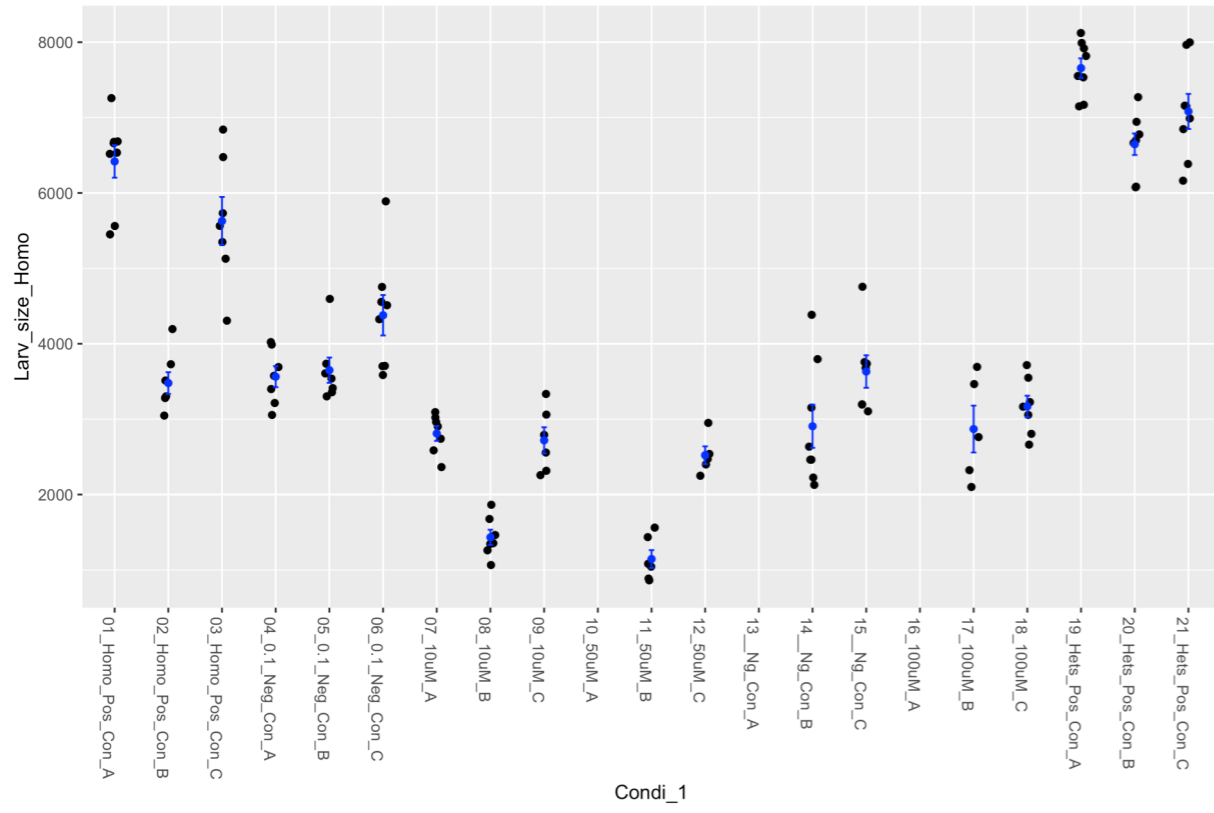
## Homozygotes



## Heterozygotes + bortezomib



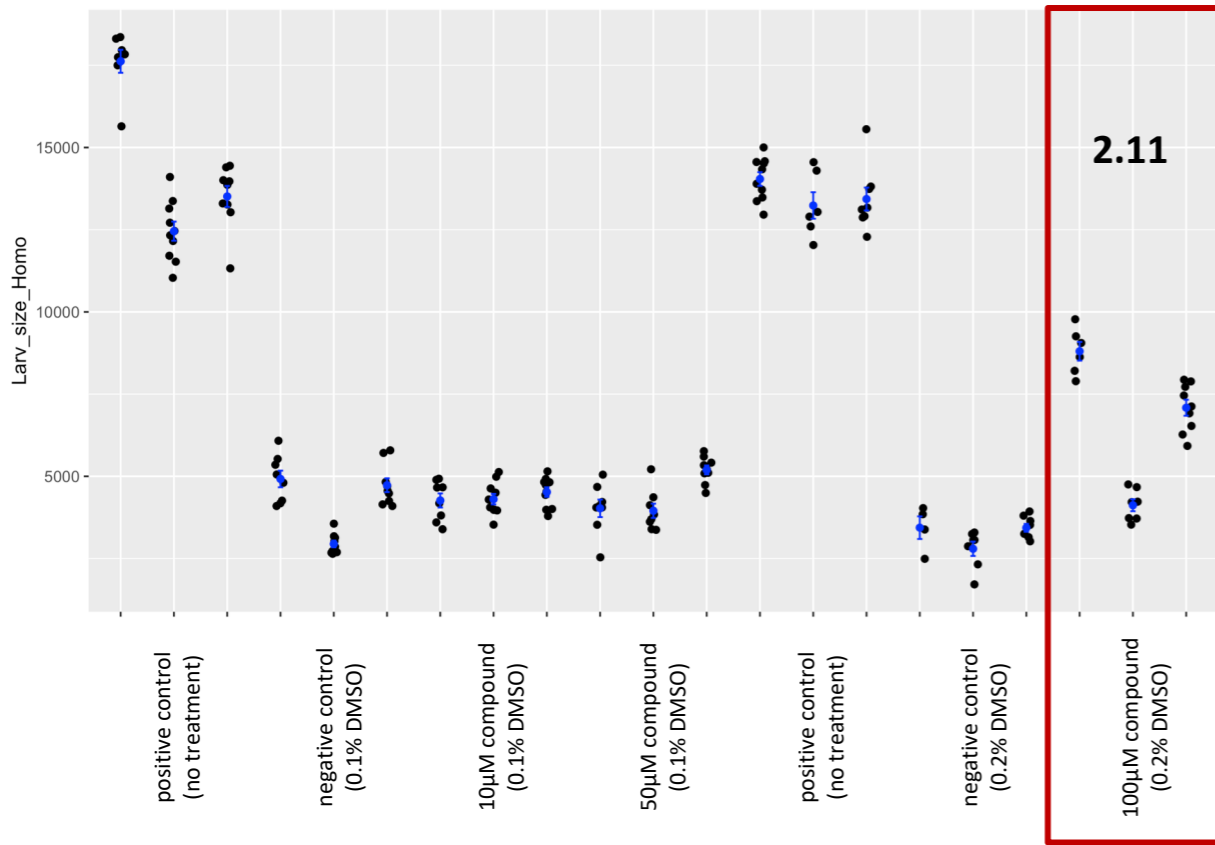
# Fly Microsource retest – HAEMATOXYLIN



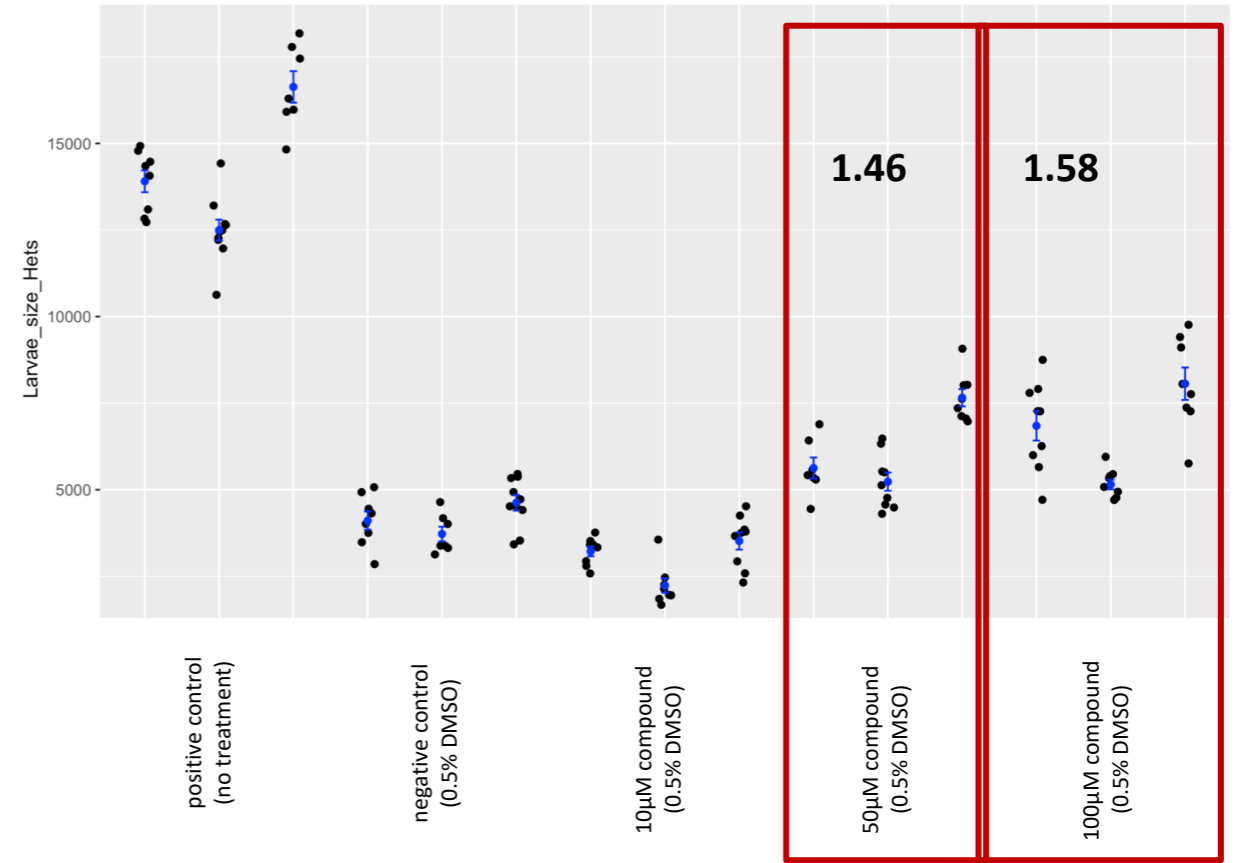


# Fly Microsource Retest – KETOROLAC TROMETHAMINE

## Homozygotes

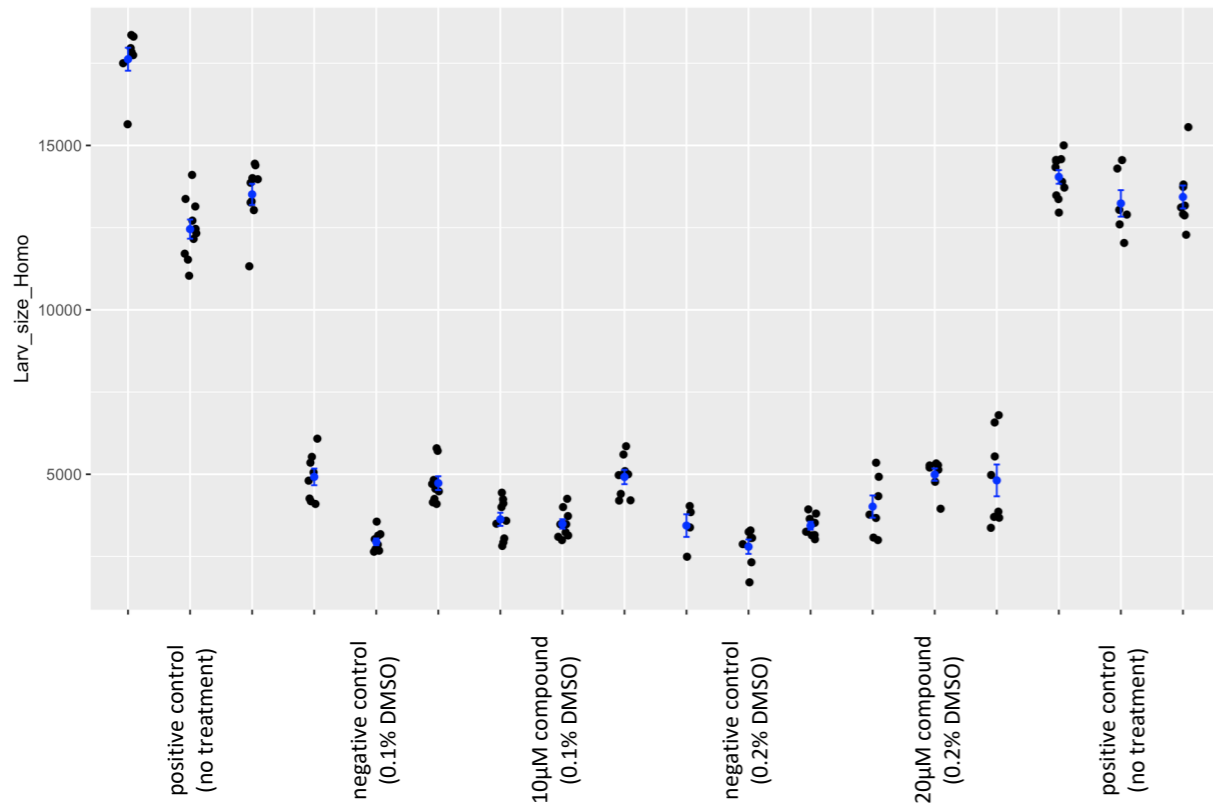


## Heterozygotes + bortezomib

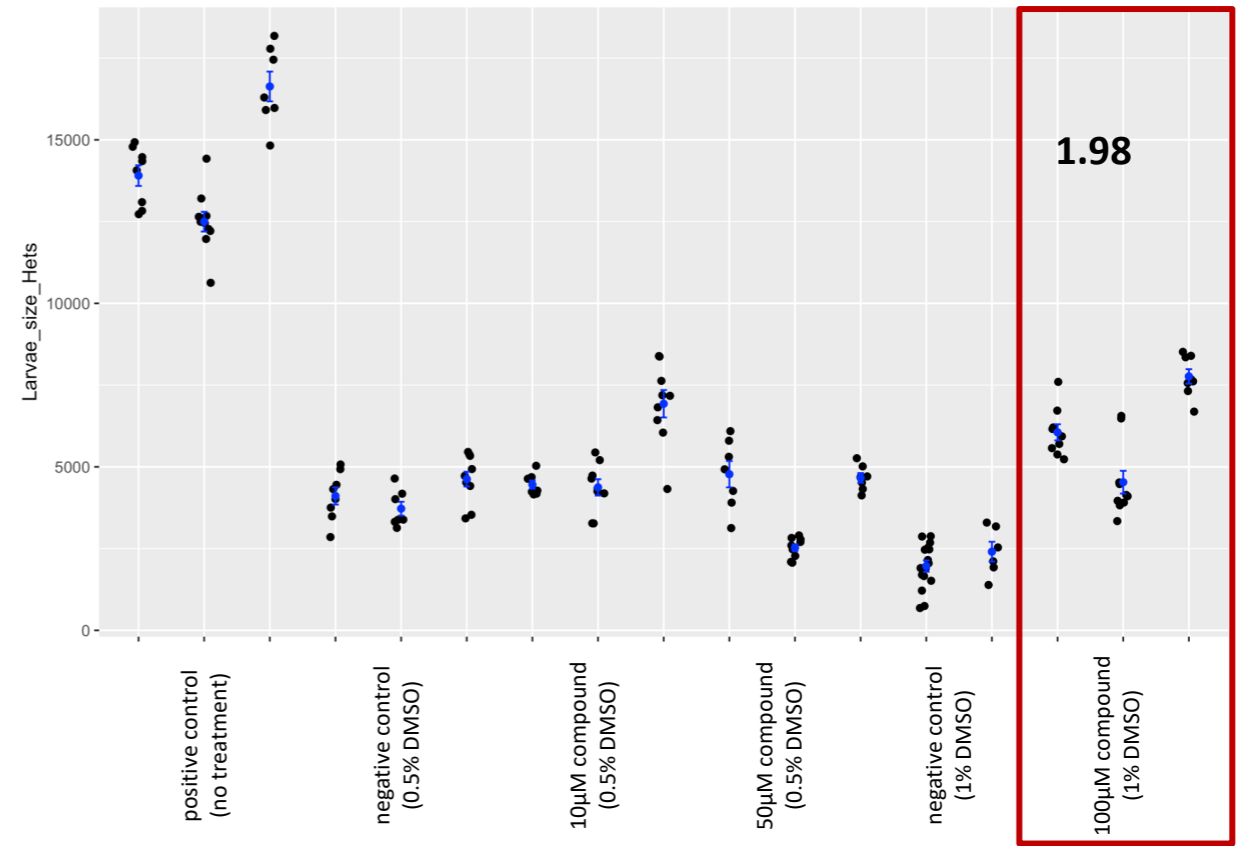


# Fly Microsource Retest – LUMEFANTRINE

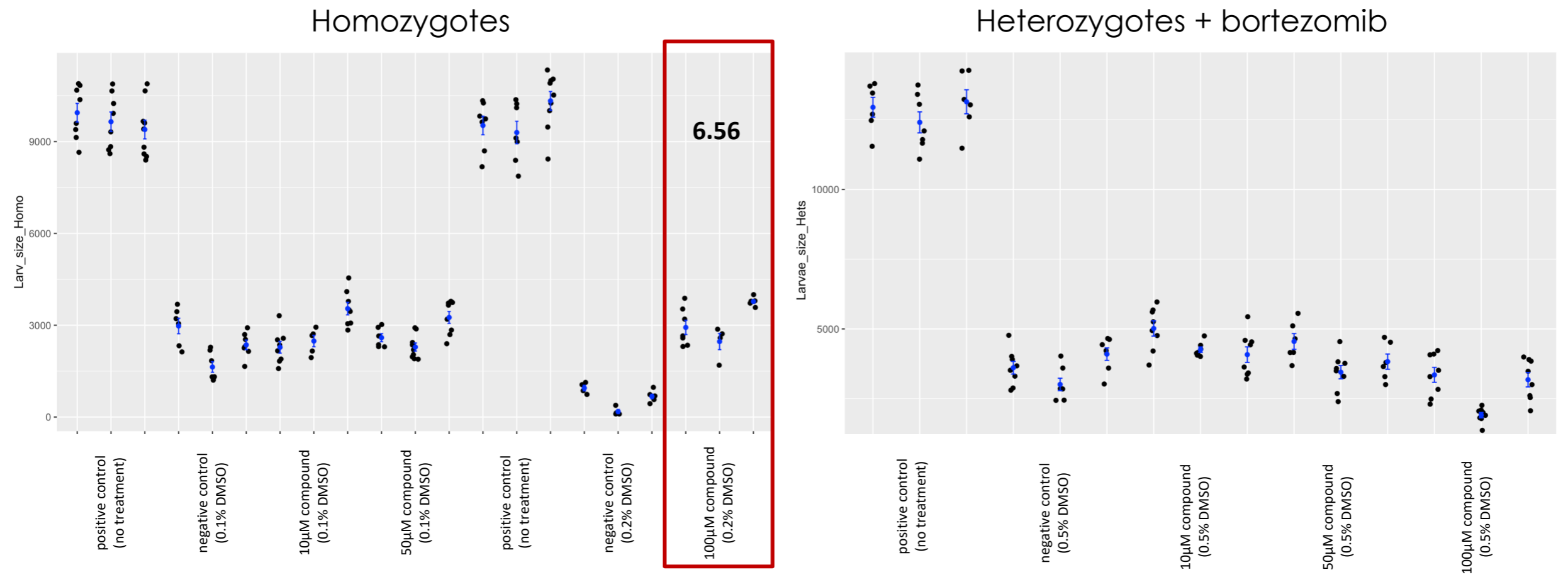
## Homozygotes



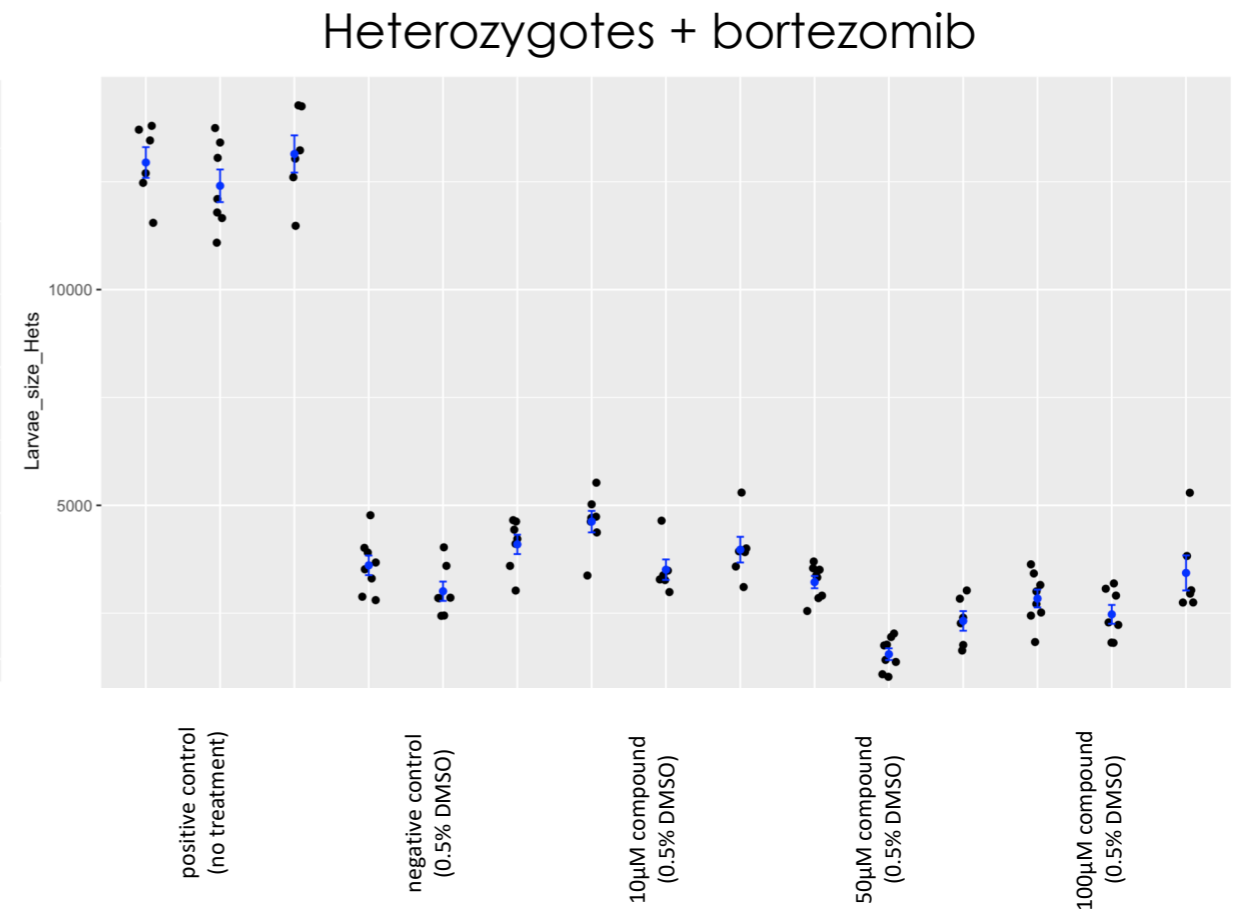
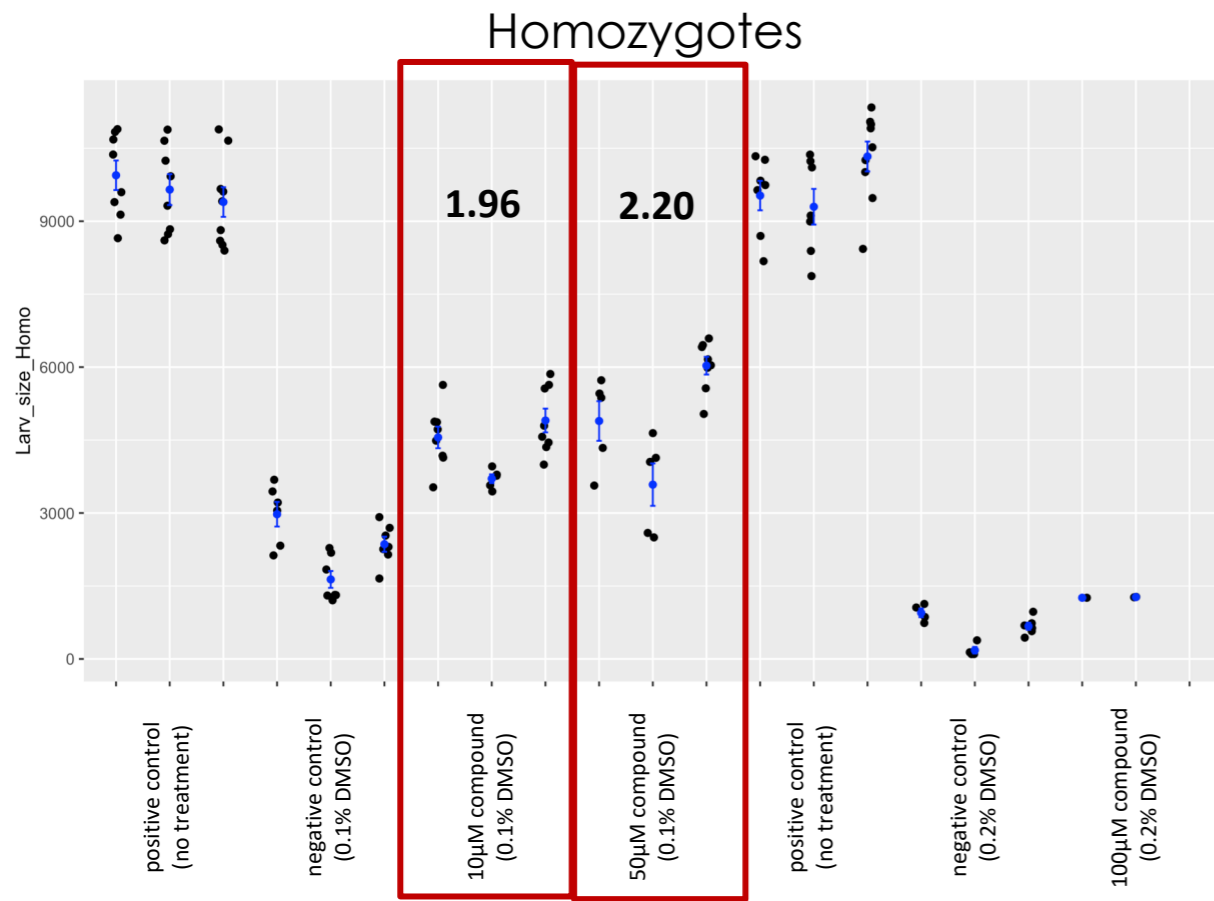
## Heterozygotes + bortezomib



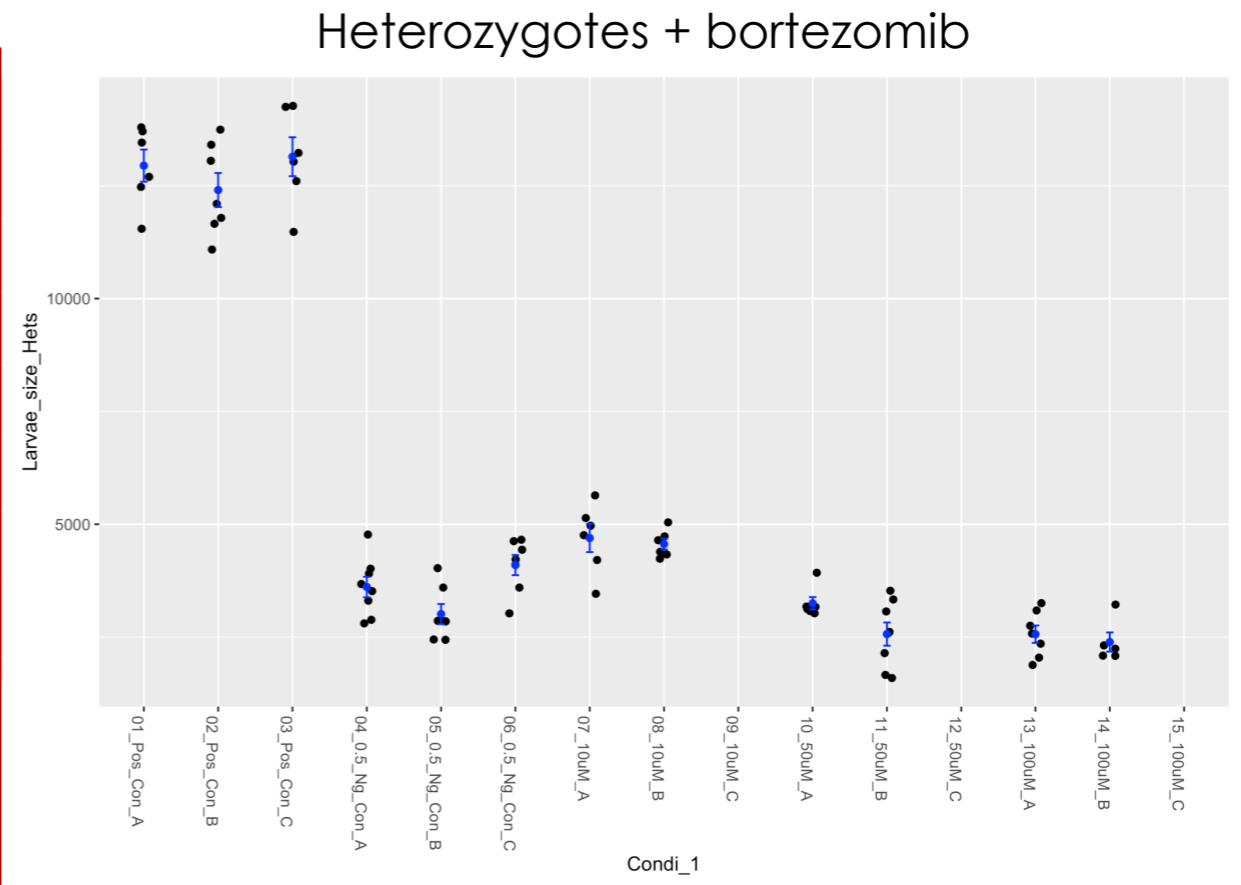
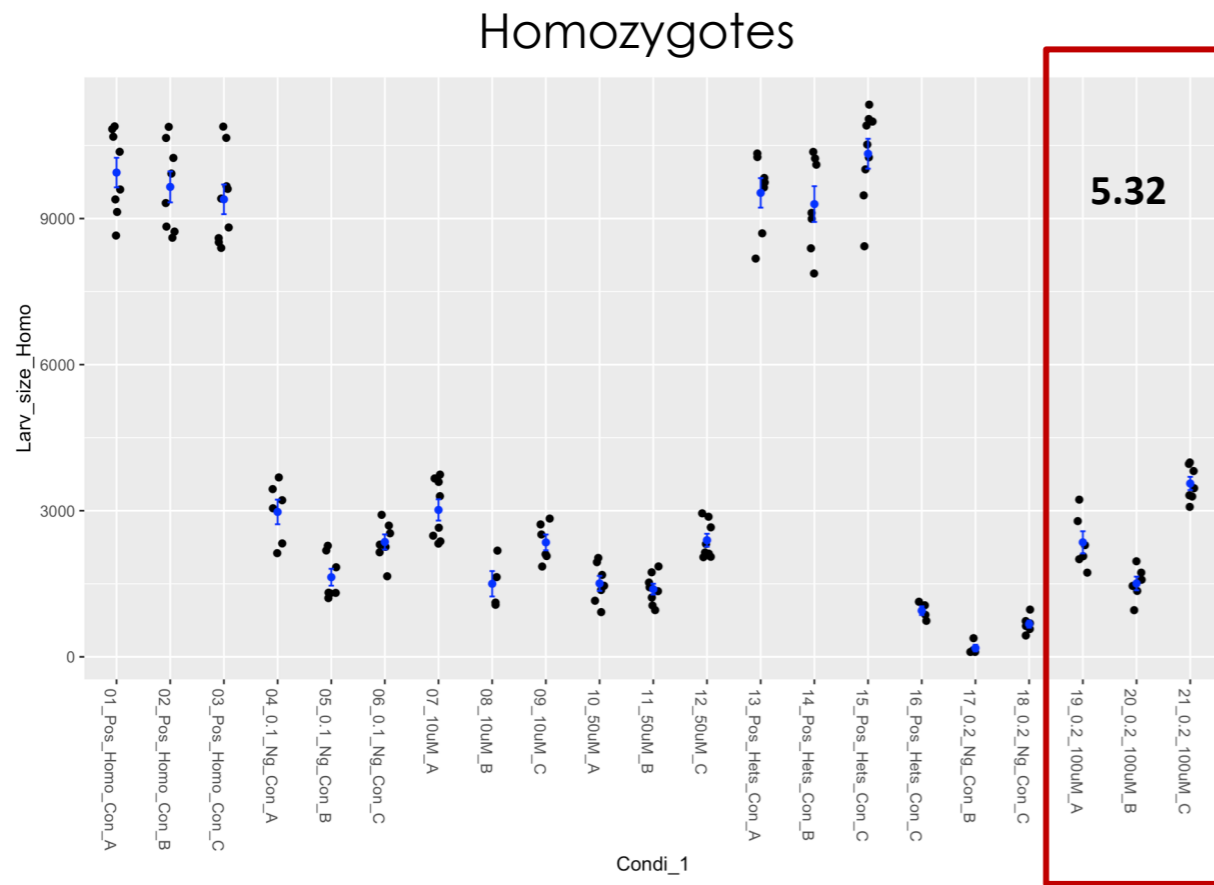
# Fly Microsource Retest – NICERGO LINE



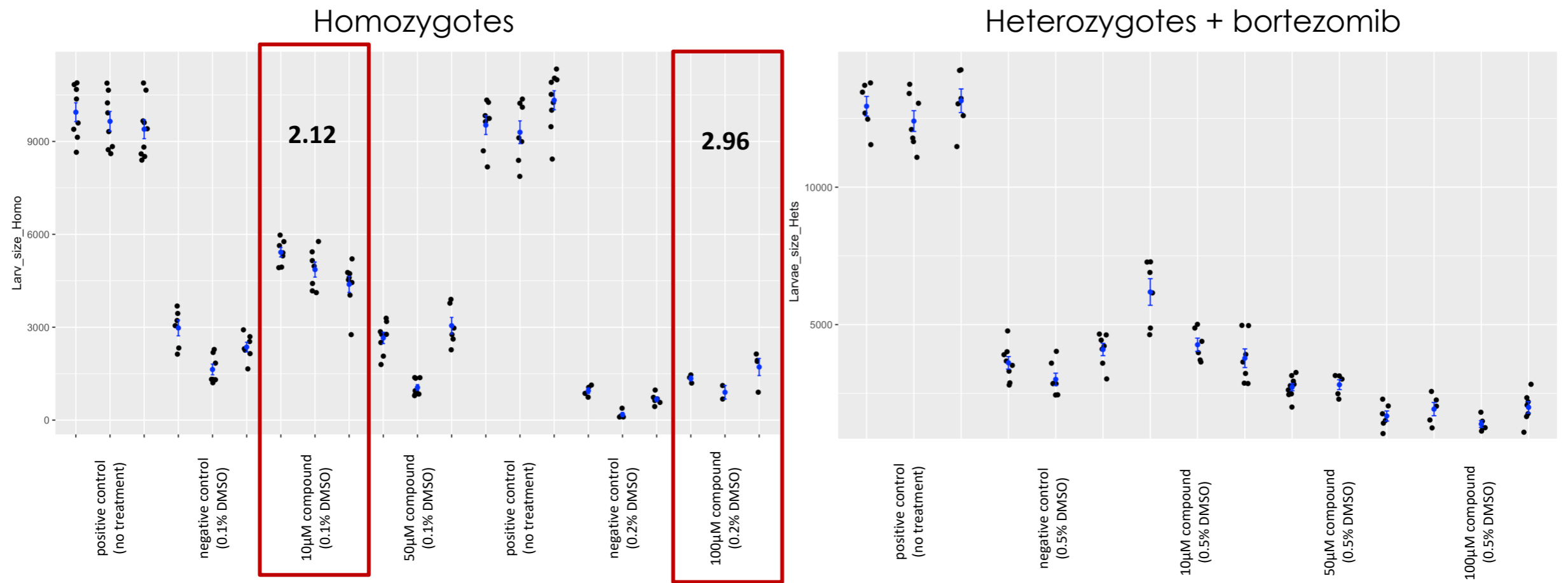
# Fly Microsource Retest – PIMETHIXENE MALEATE



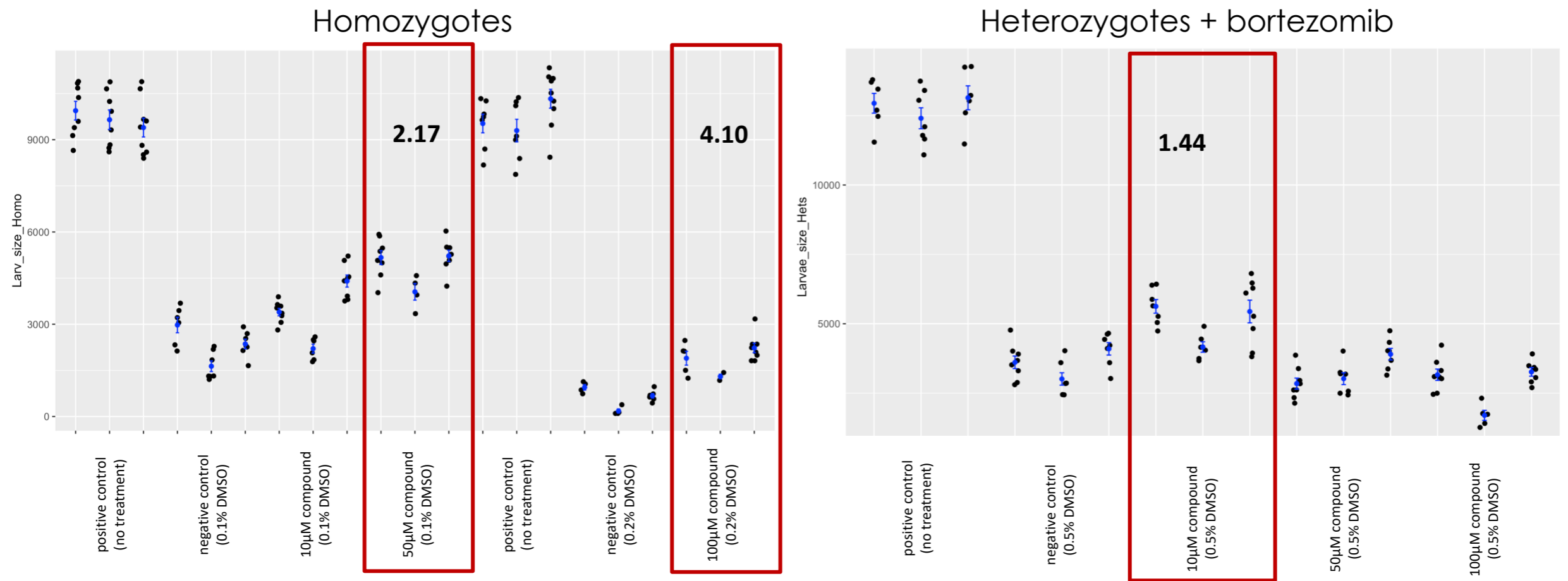
# Fly Microsource Retest – PIPERINE



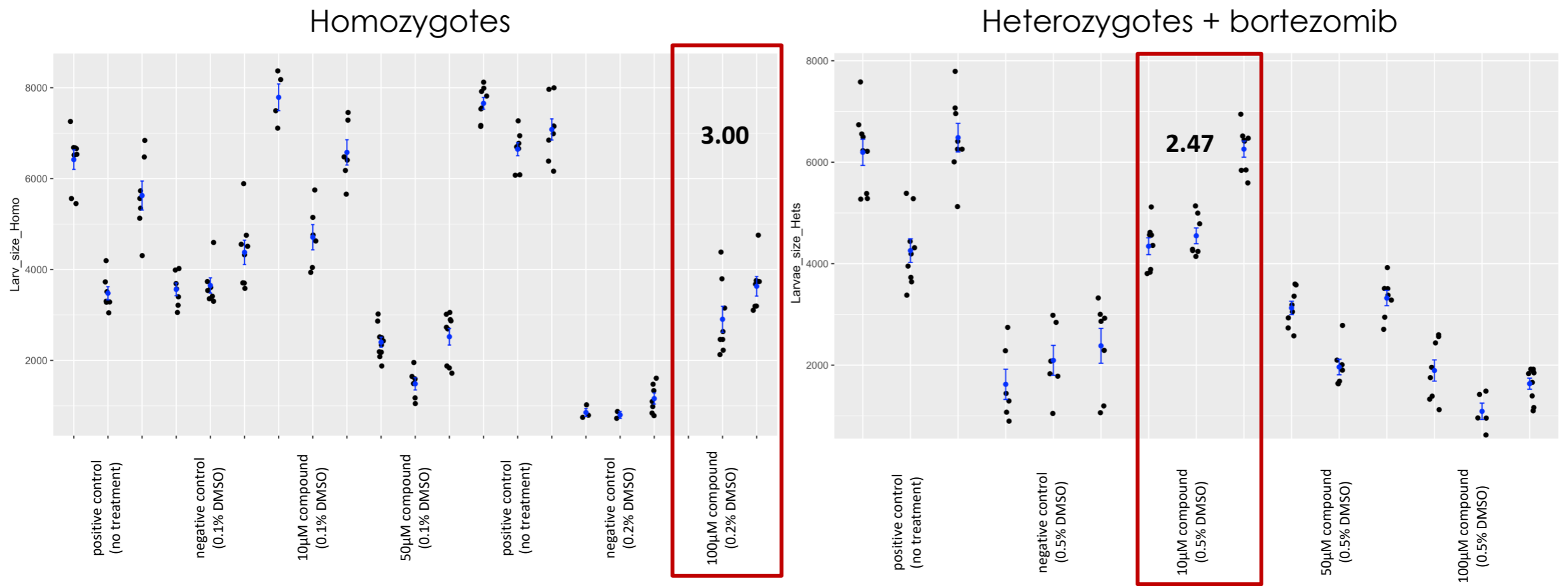
# Fly Microsource Retest – PRAXADINE HYDROCHLORIDE



# Fly Microsource Retest – PROMAZINE HYDROCHLORIDE

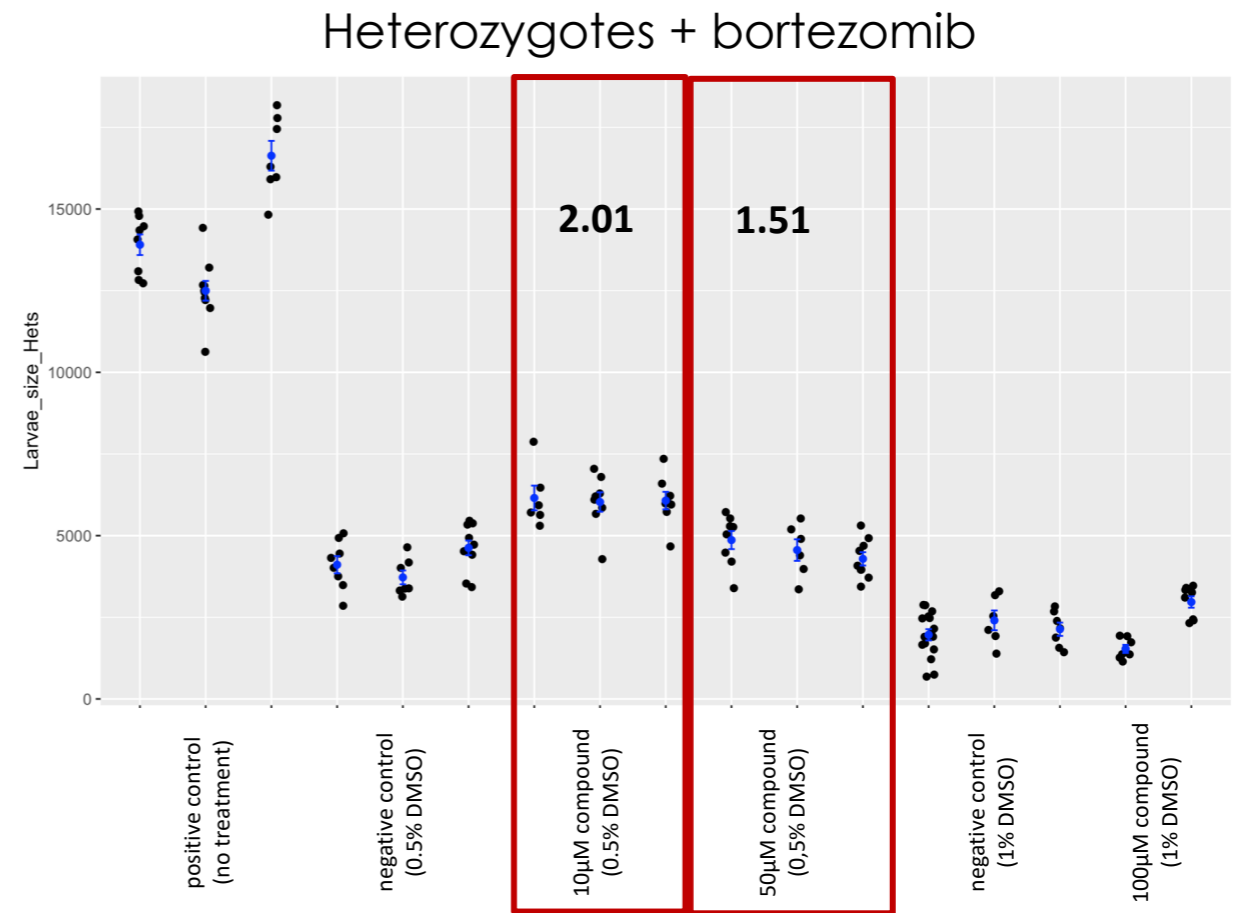
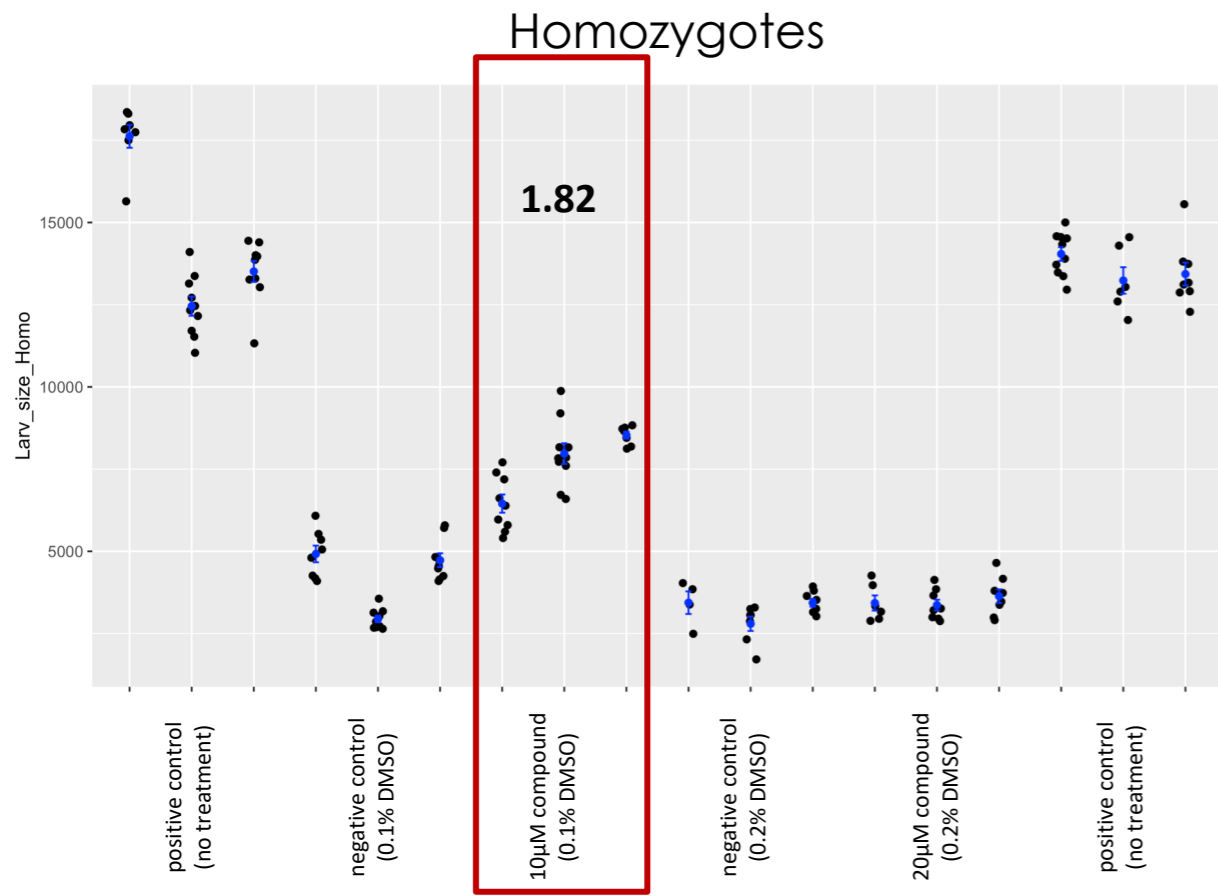


# Fly Microsource Retest – PYRIMETHAMINE

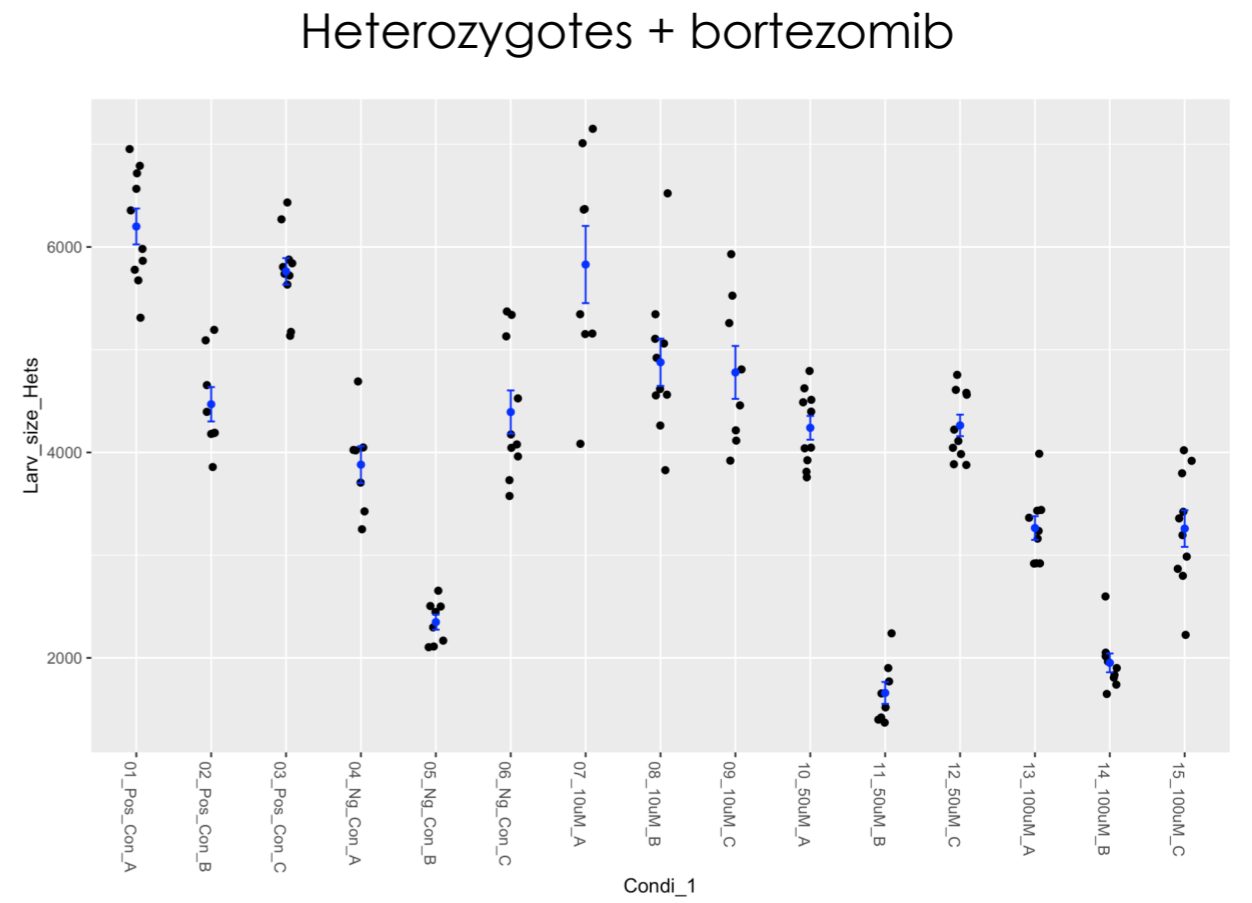
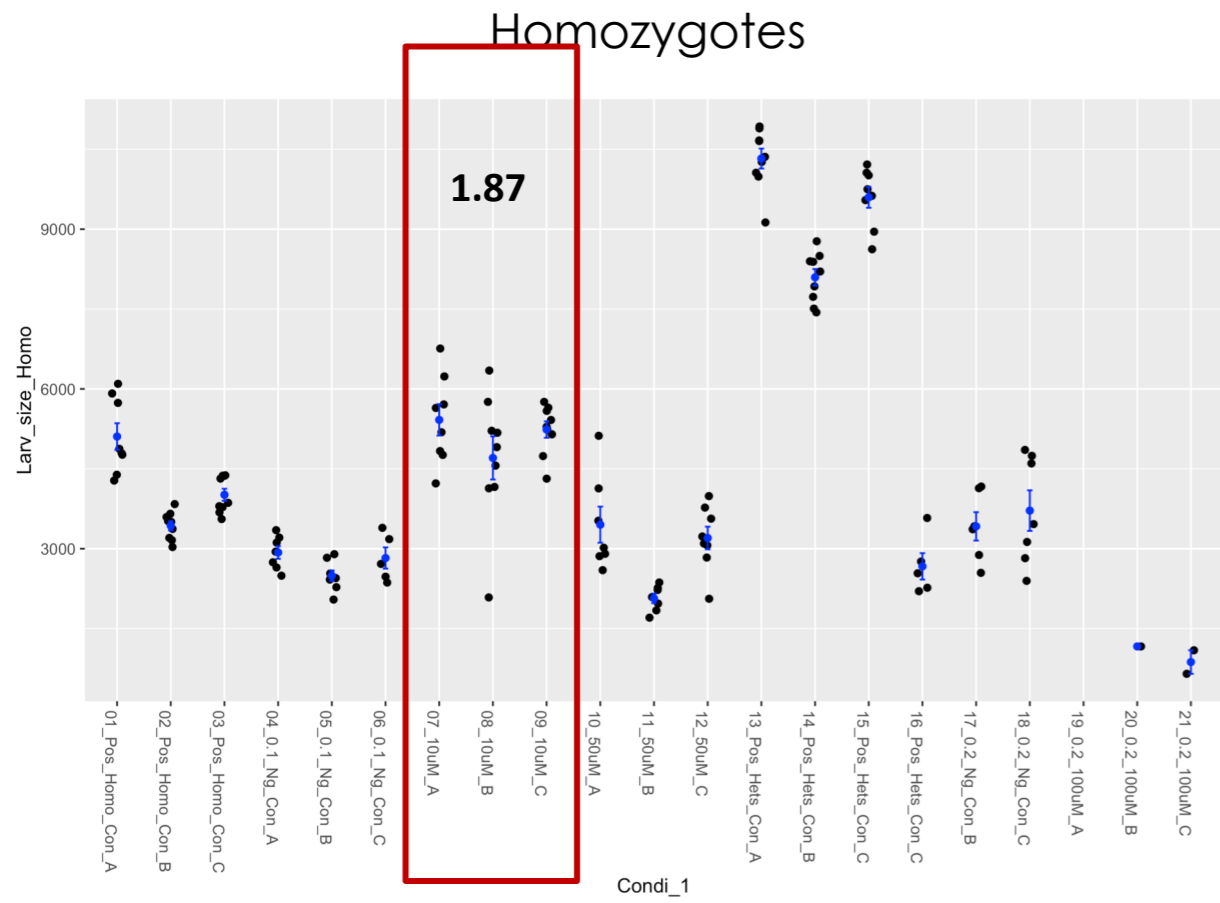




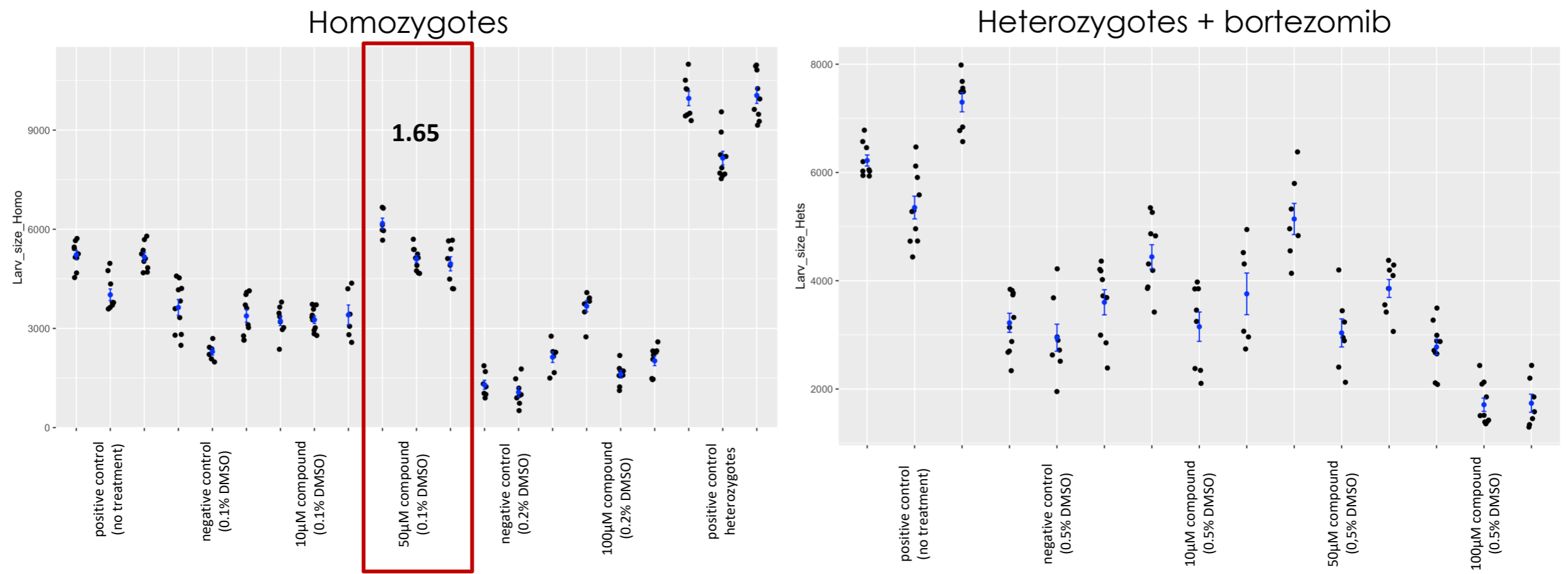
# Fly Microsource Retest – QUINIZARIN



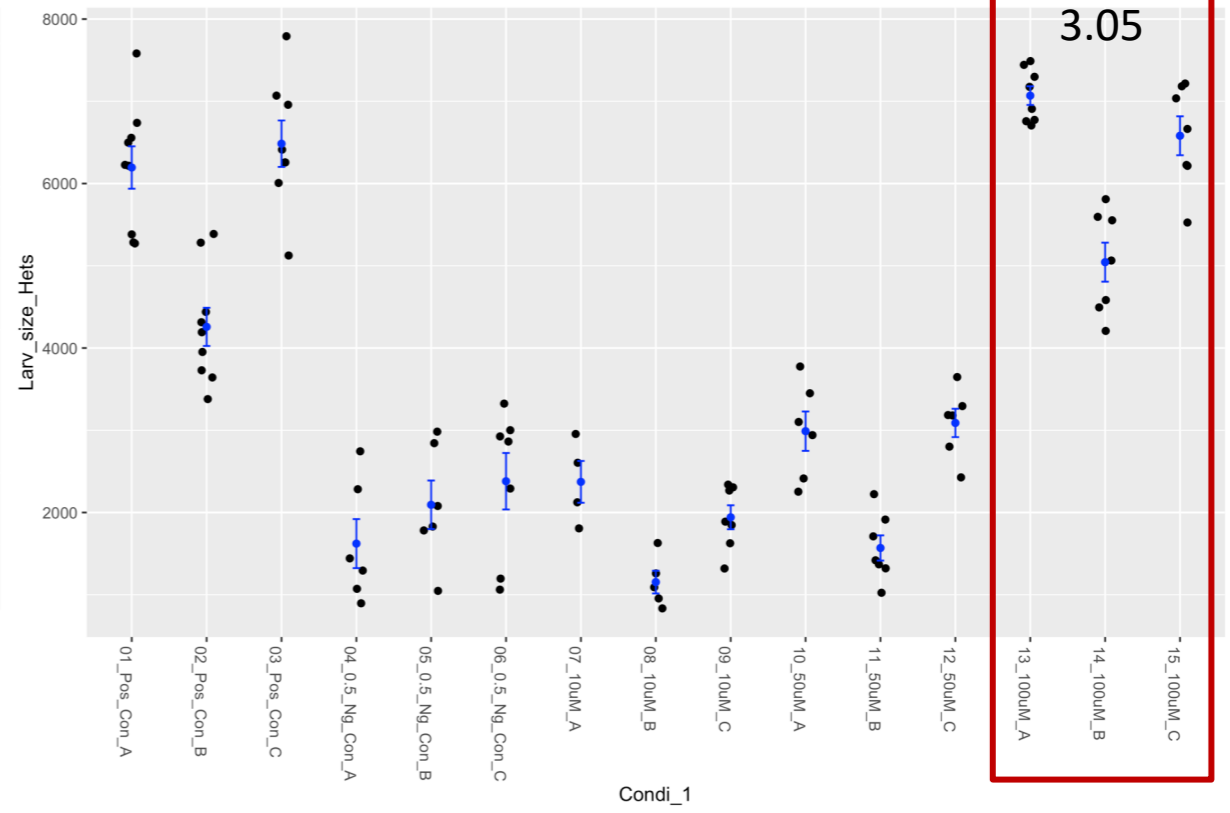
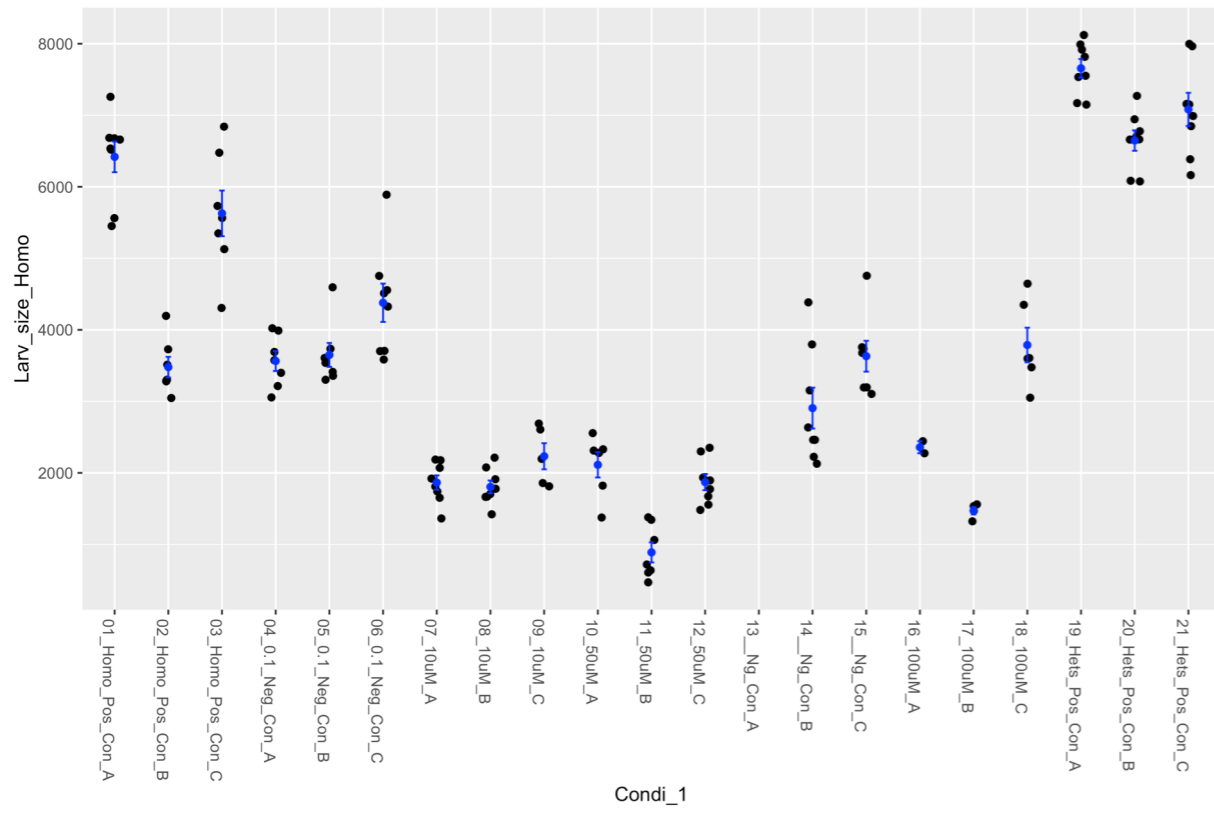
# Fly Microsource Retest – RHETSININE



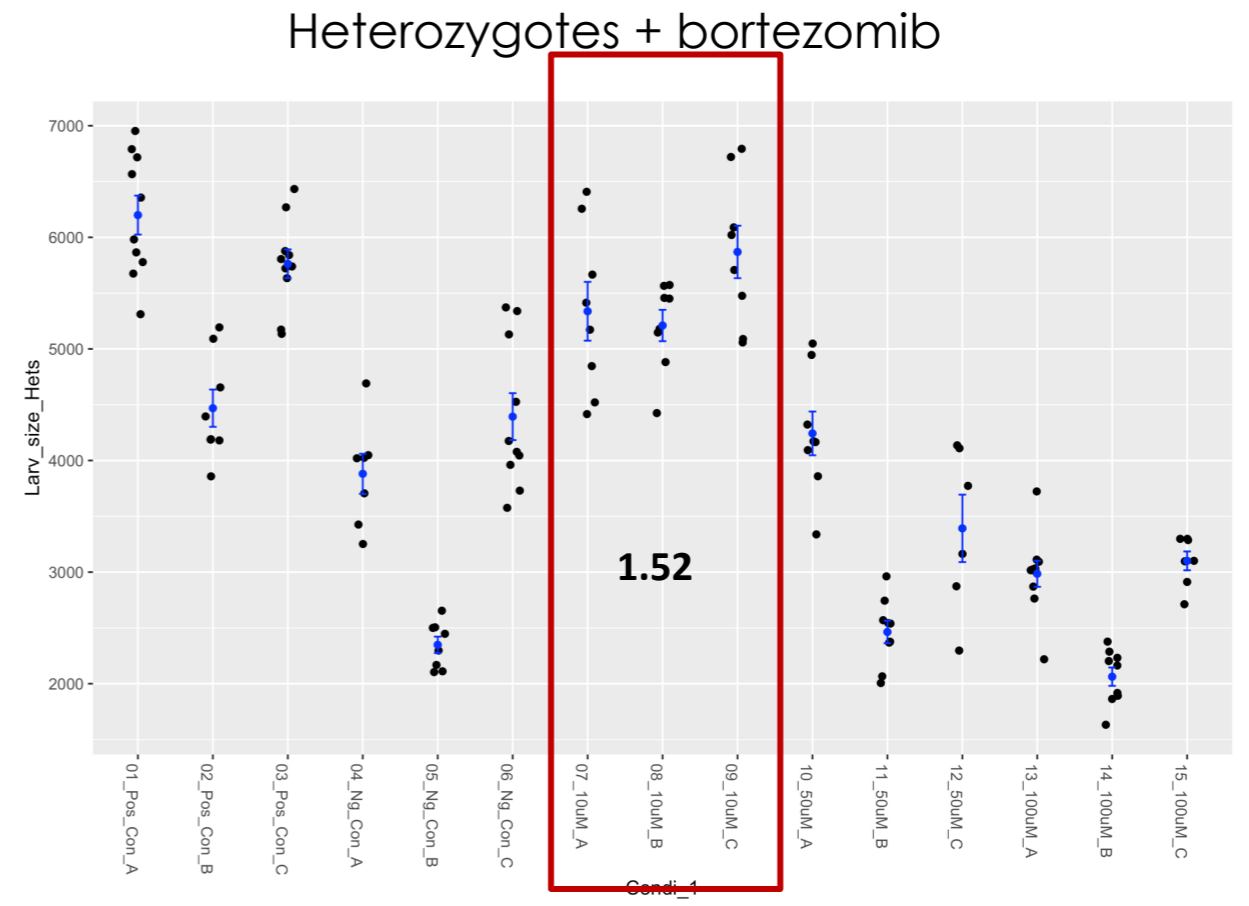
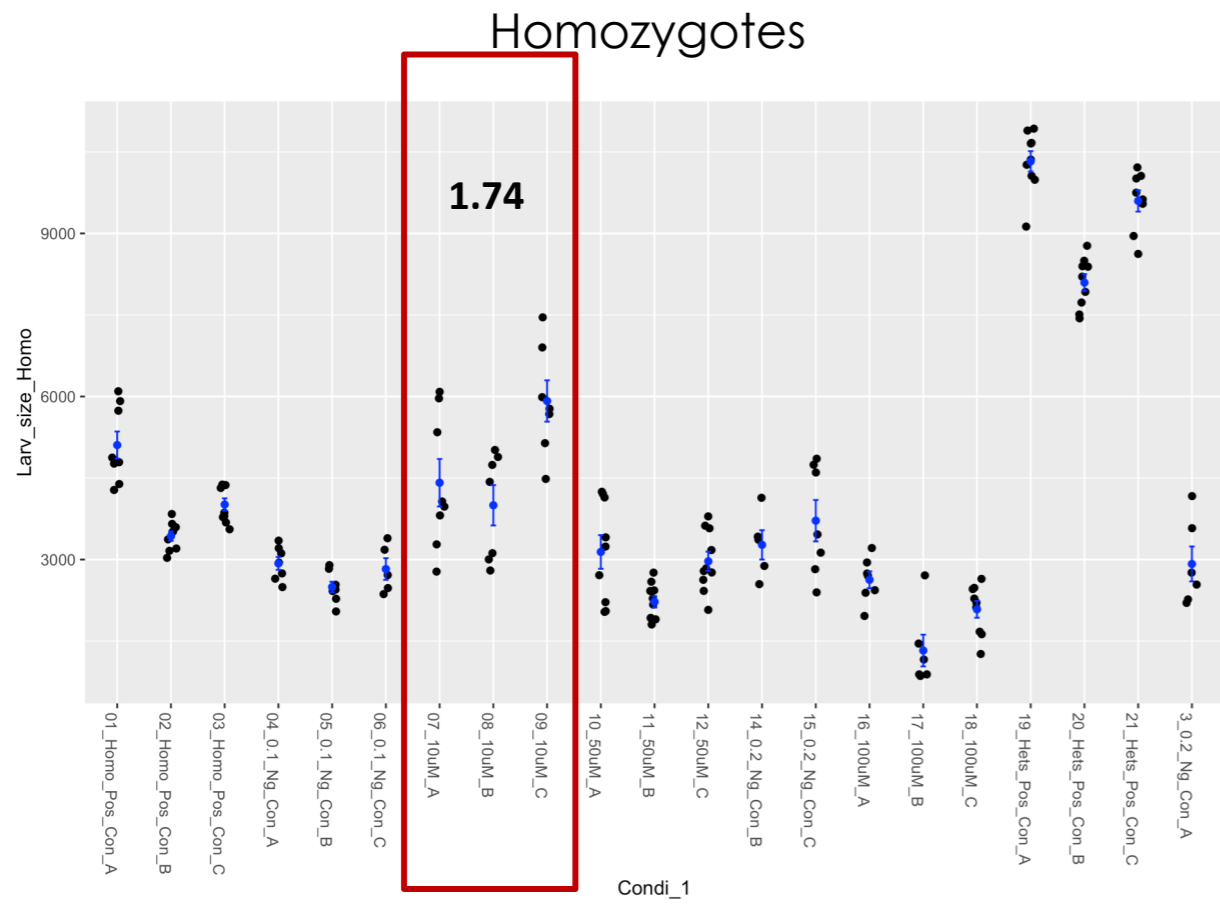
# Fly GSF tool compound test – SULFORAPHANE



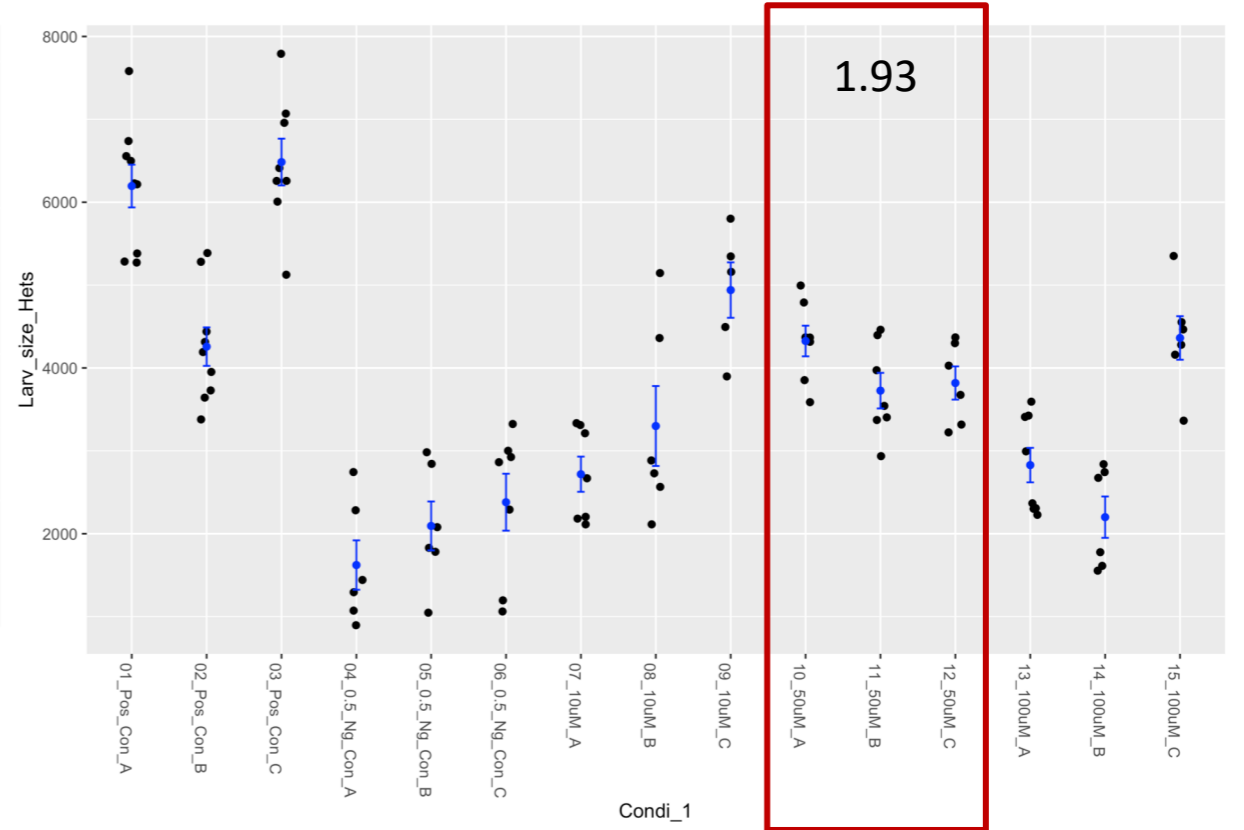
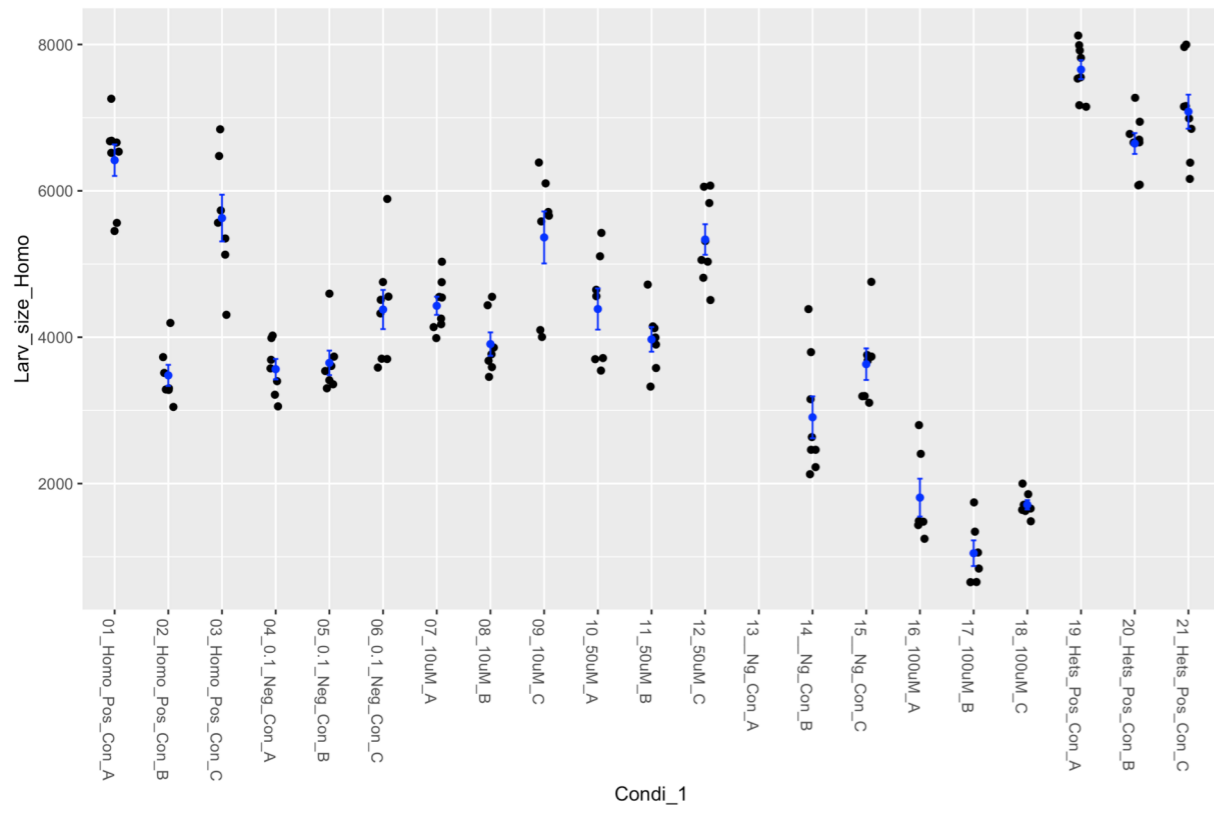
# Fly Microsource retest – TANNIC ACID



# Fly Microsource Retest – THIOGUANOSINE



# Fly Microsource retest – TROLOX

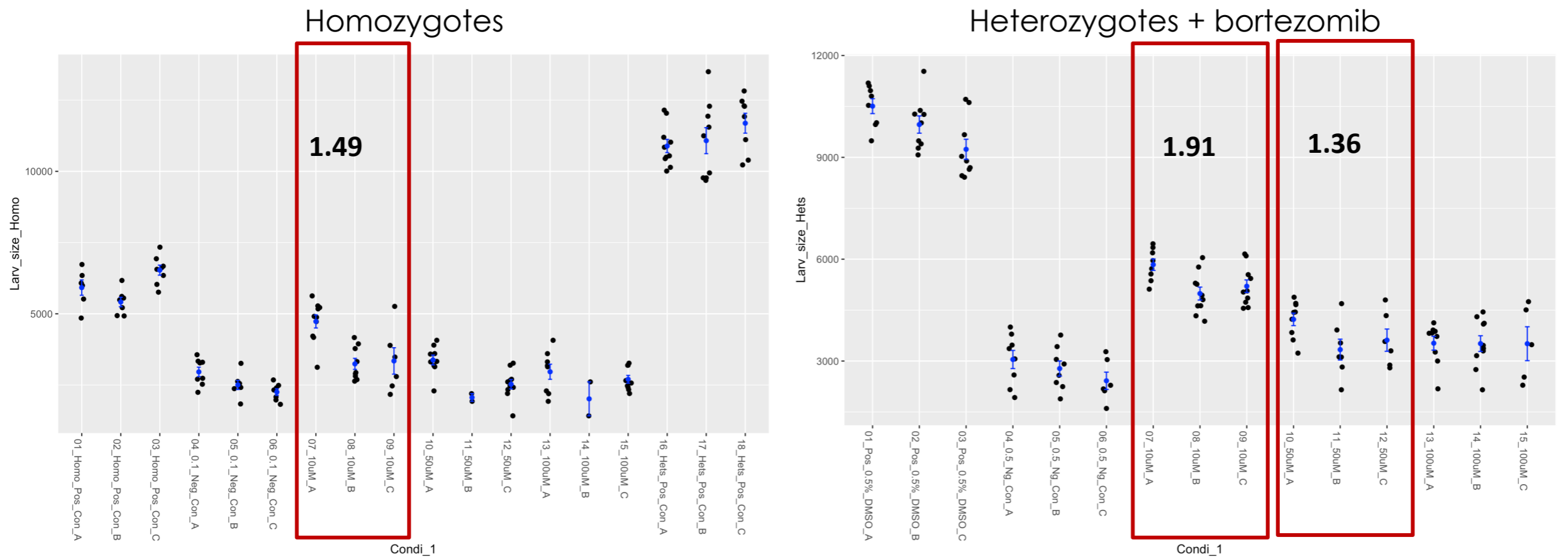


# Fly Worm MS cross validation

All compounds that scored as hits in the worm *png-1* Microsource Spectrum (drug repurposing) screen were cross-validated in two fly secondary assays: rescue of *Pngl*<sup>-/-</sup> homozygotes and rescue of bortezomib-treated *Pngl*<sup>+/-</sup> heterozygotes. Red boxes indicate compounds that successfully retested. The number indicates the size ratio of compound-treated fly larvae to untreated control homozygous fly larvae.

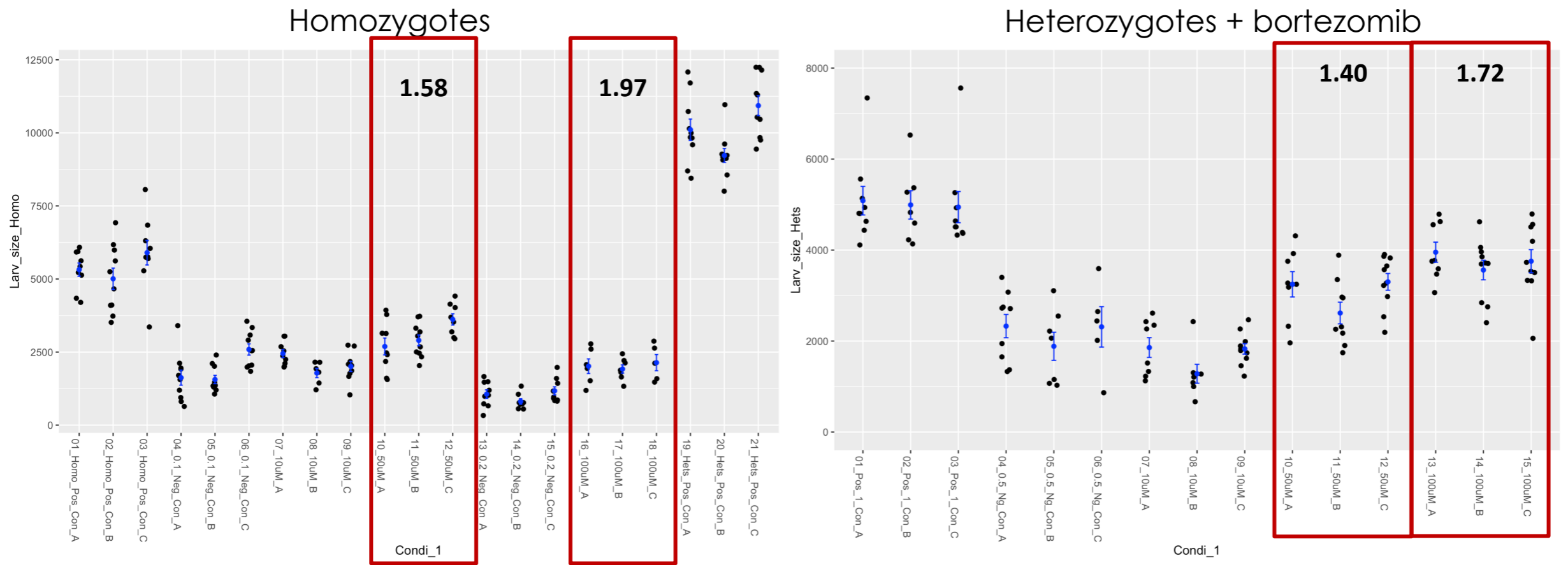
(Please note that all homozygote experiments in this section included an untreated heterozygote control)

# Fly WormMS cross validation – 3,4-DIDESMETHYL-5-DESHYDROXY-3'-ETHOXYSCLEROIN



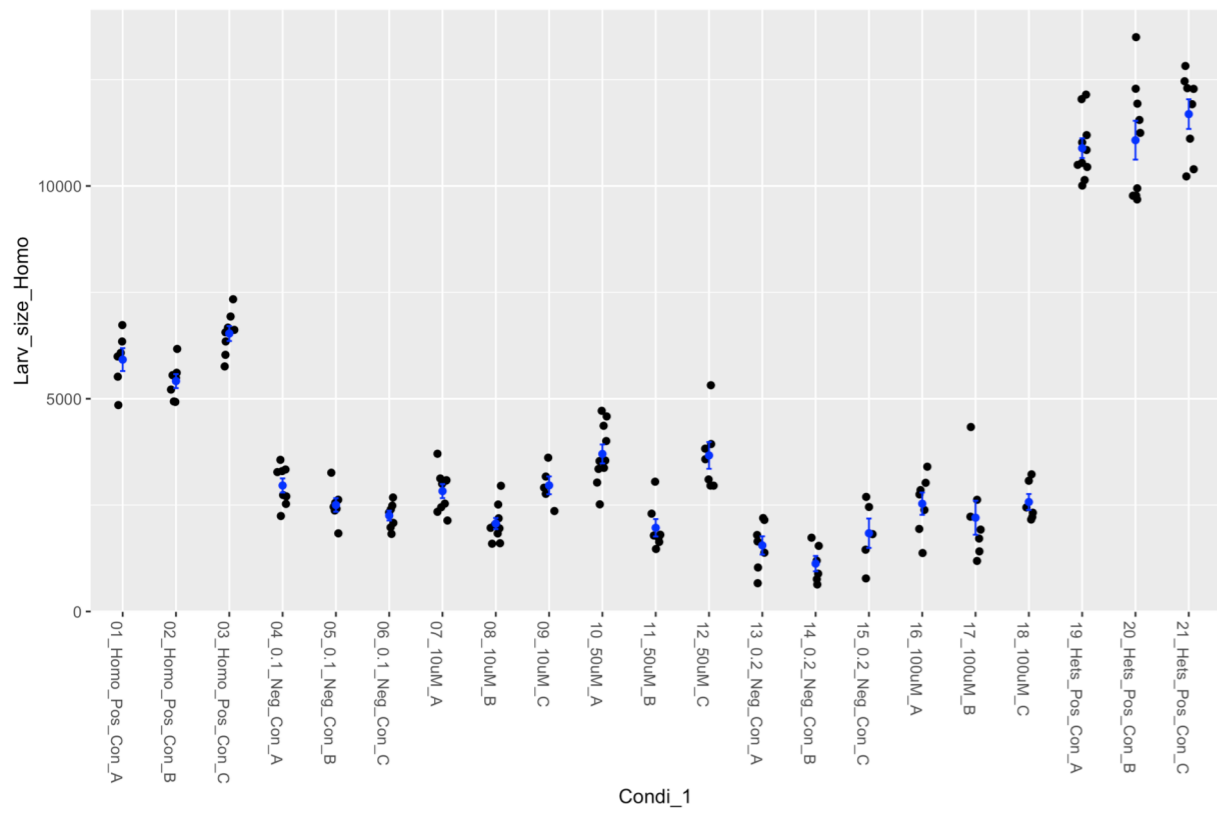


# Fly WormMS cross validation – BENSERAZIDE

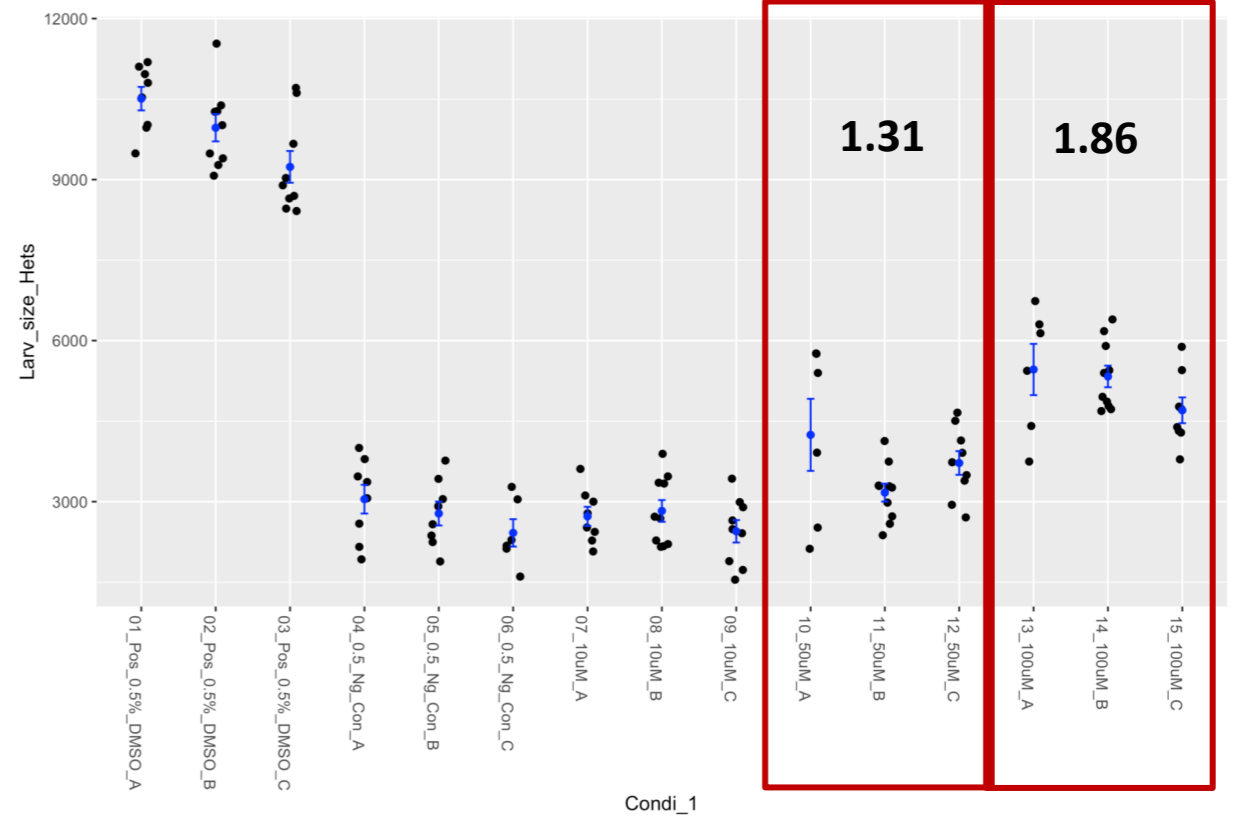


# Fly WormMS cross validation – ELLAGIC ACID

Homozygotes

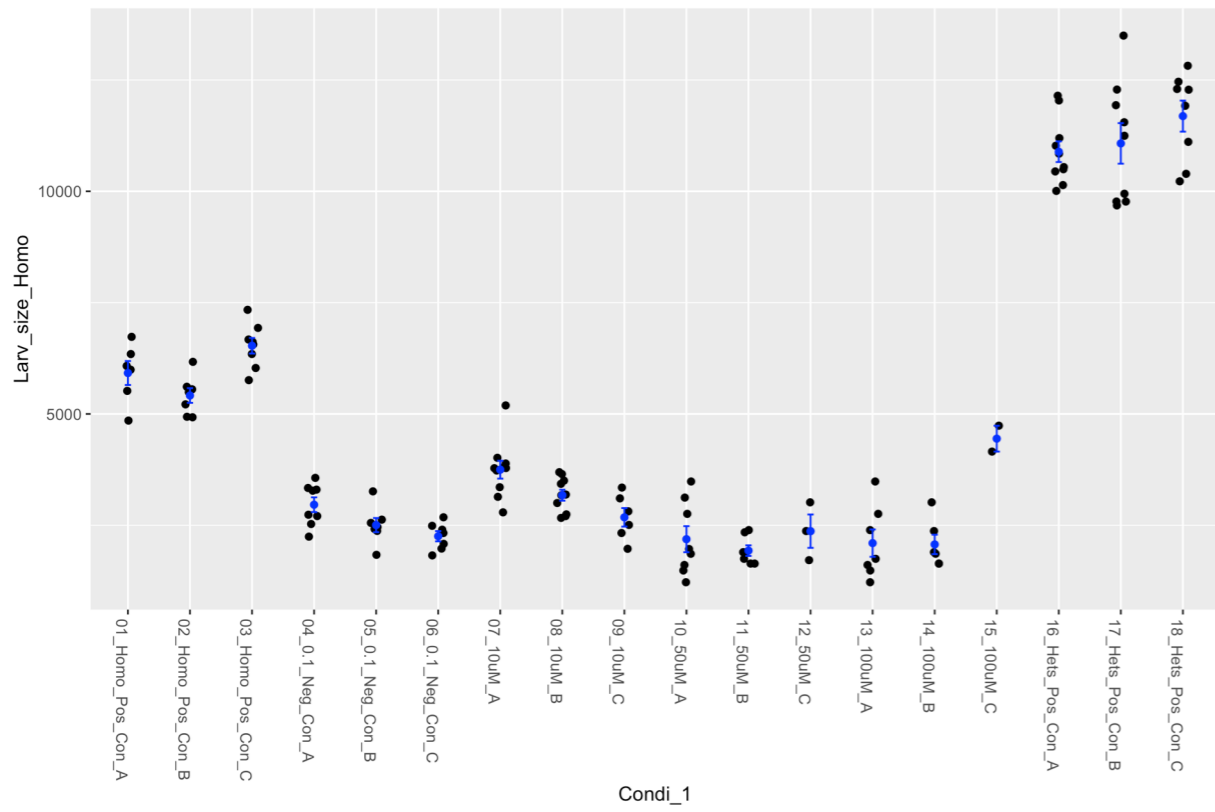


Heterozygotes + bortezomib

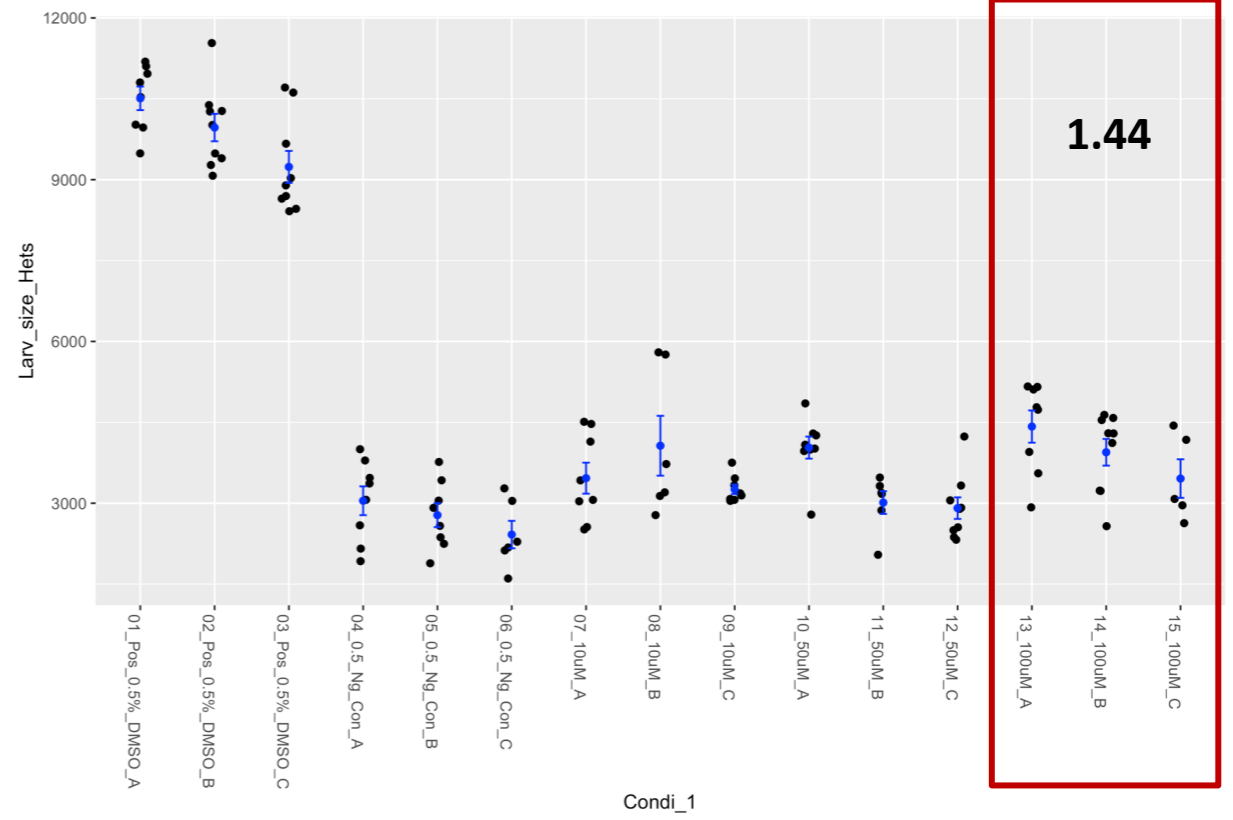


# Fly WormMS cross validation – EPICATECHIN MONOGALLATE

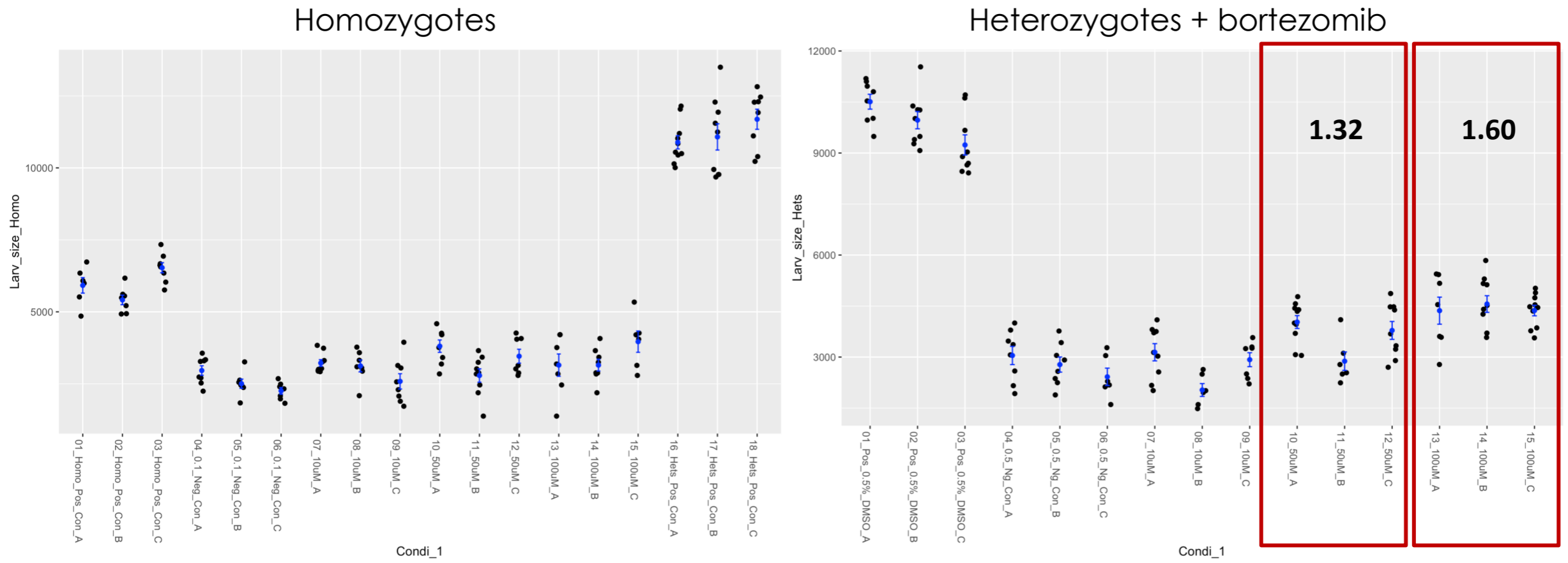
Homozygotes



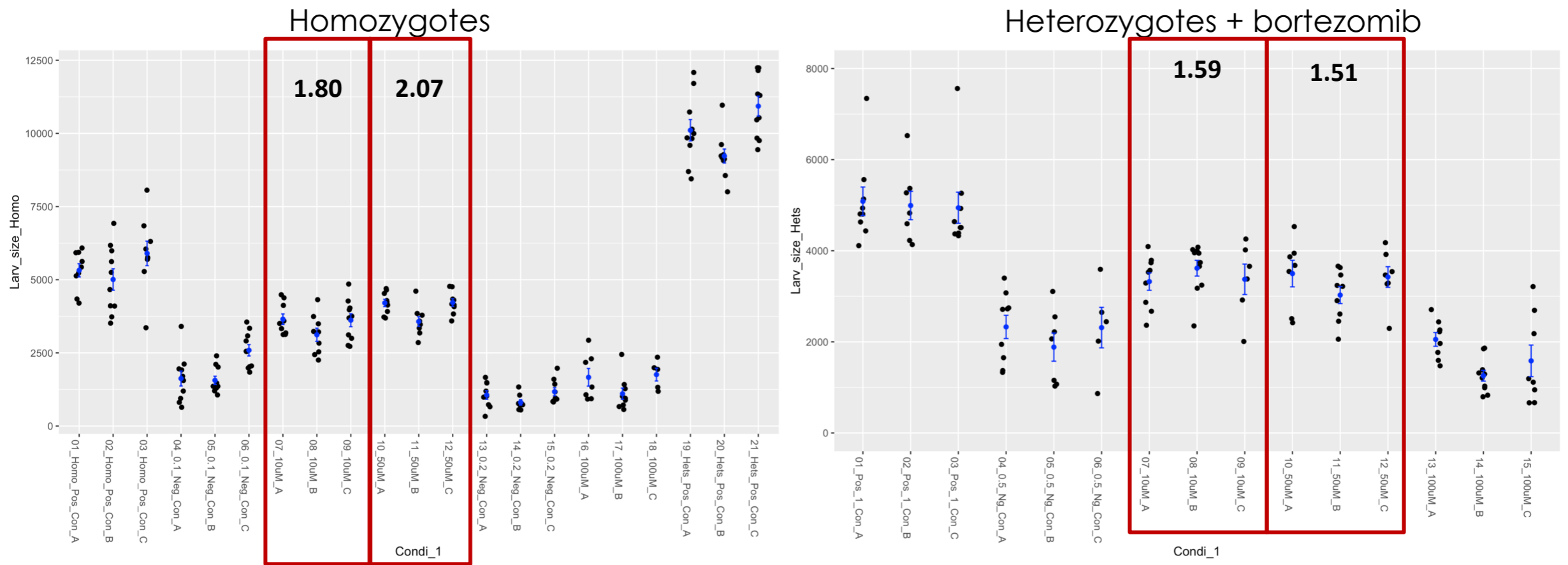
Heterozygotes + bortezomib



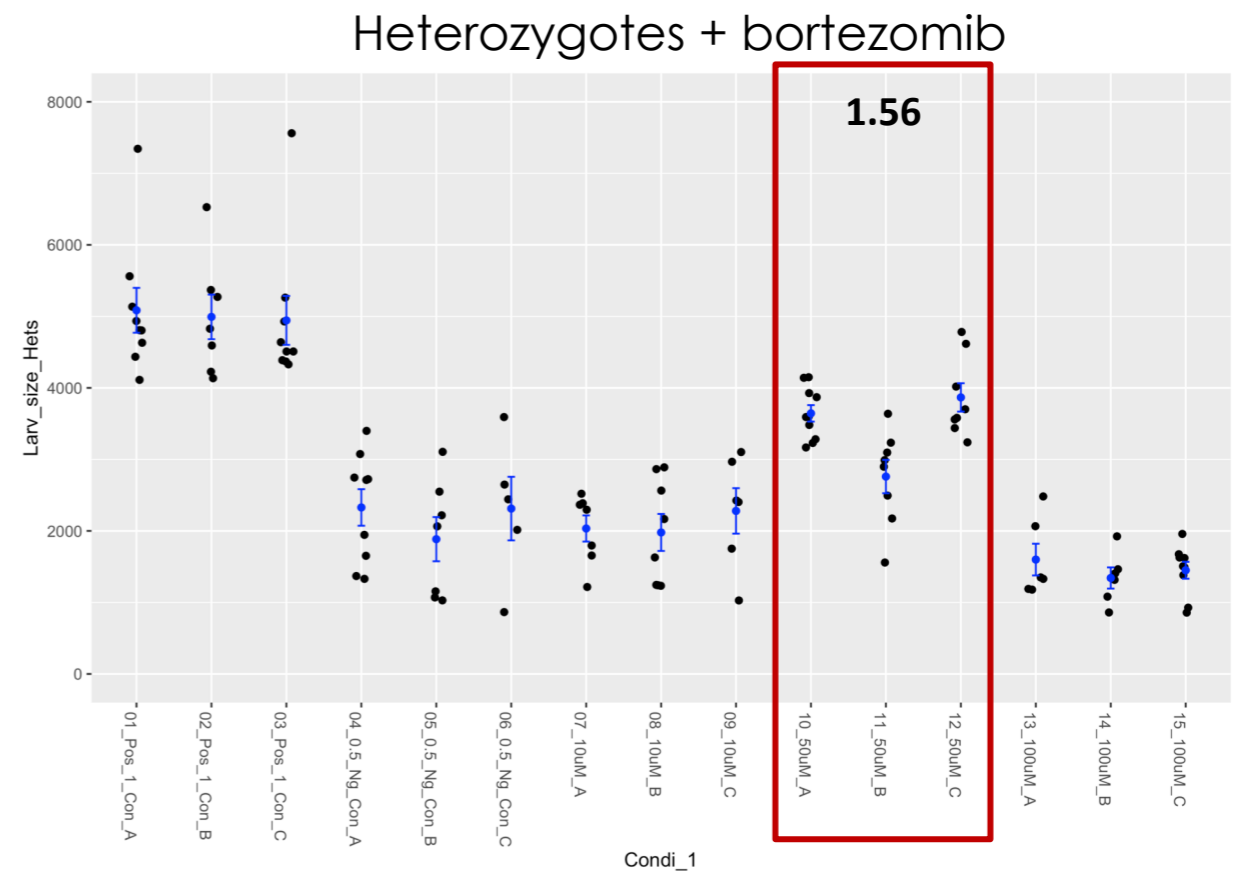
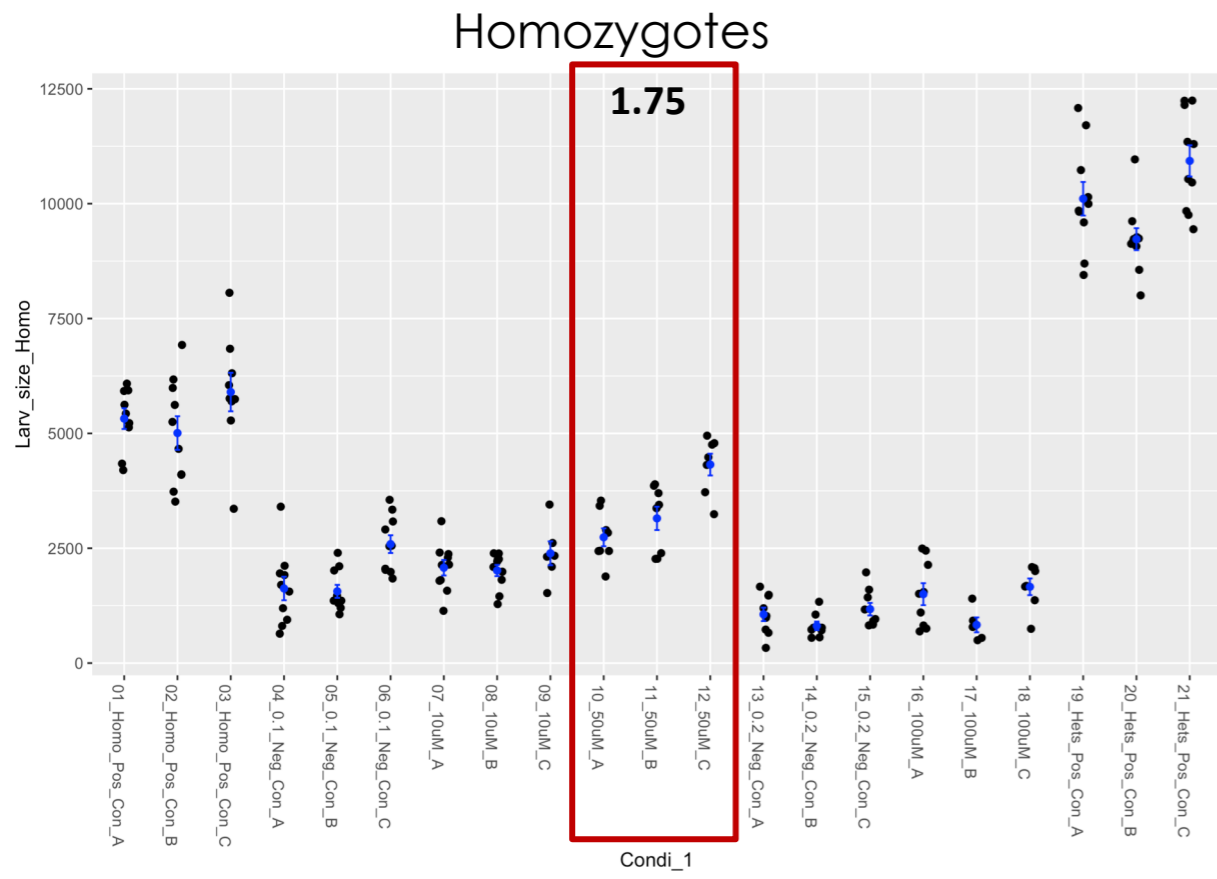
# Fly WormMS cross validation – KOPARIN



# Fly WormMS cross validation – PHENYLBUATAZONE

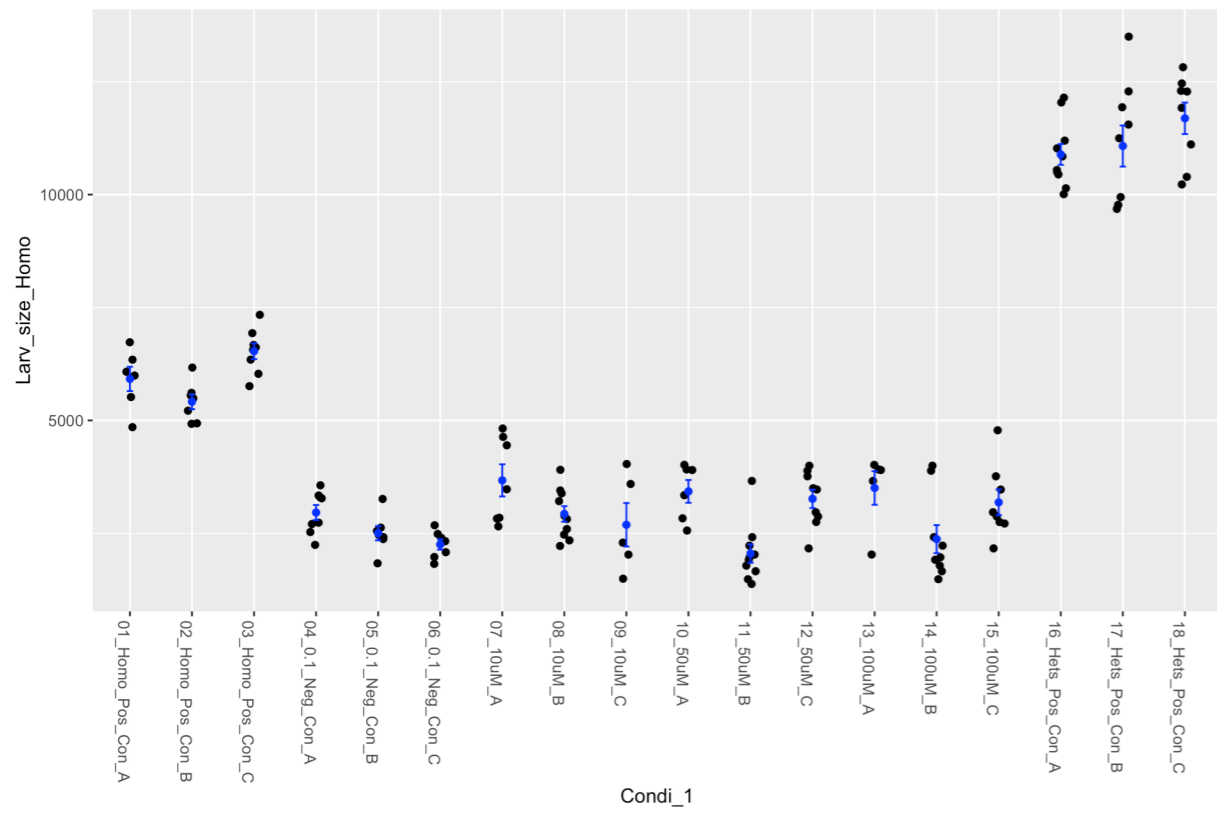


# Fly WormMS cross validation – POMIFERIN

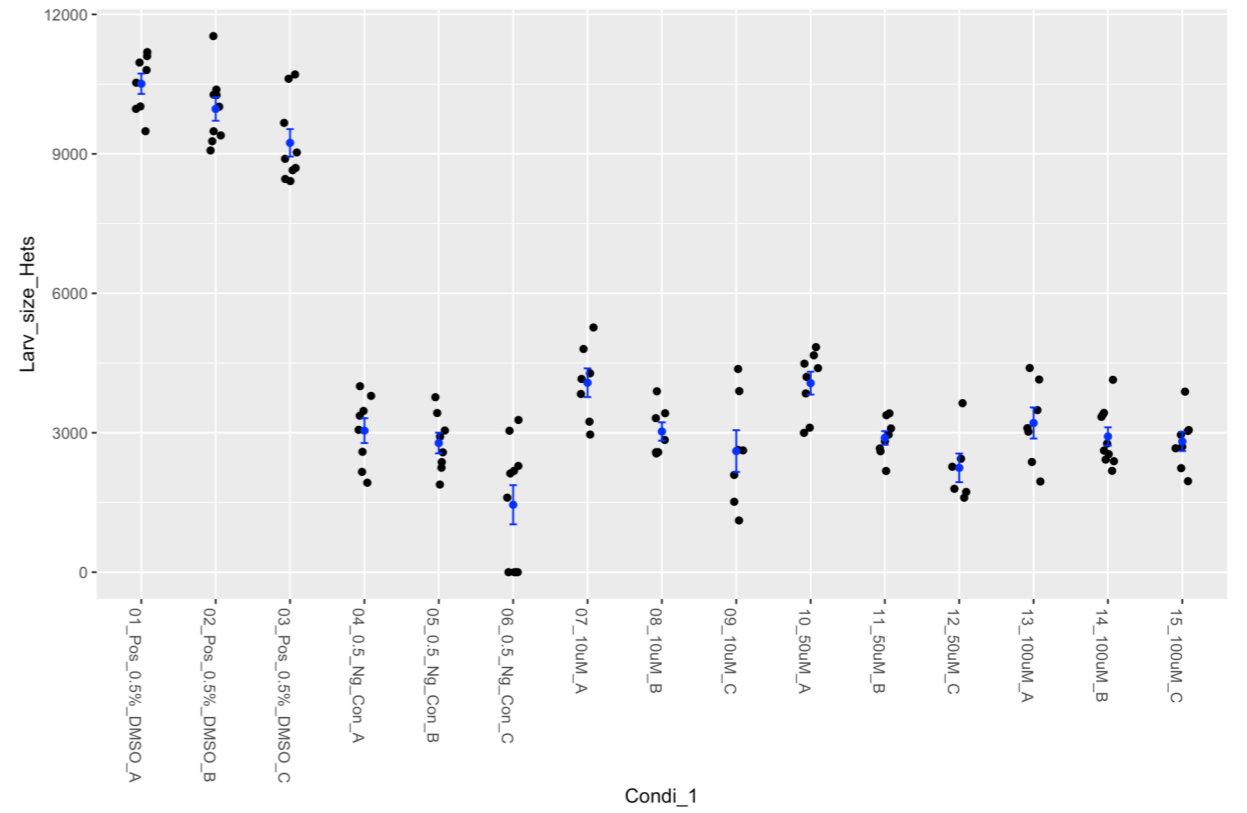


# Fly WormMS cross validation – PURPUROGALLIN-4-CARBOXYLIC ACID

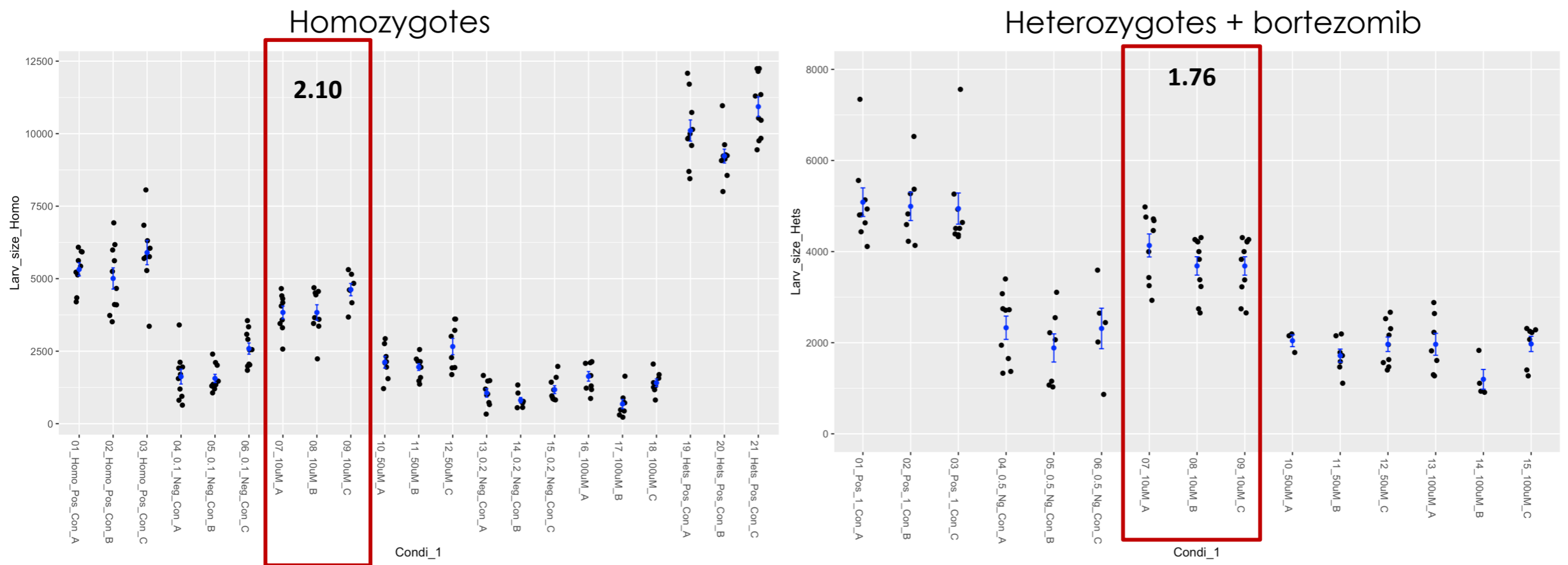
Homozygotes



Heterozygotes + bortezomib

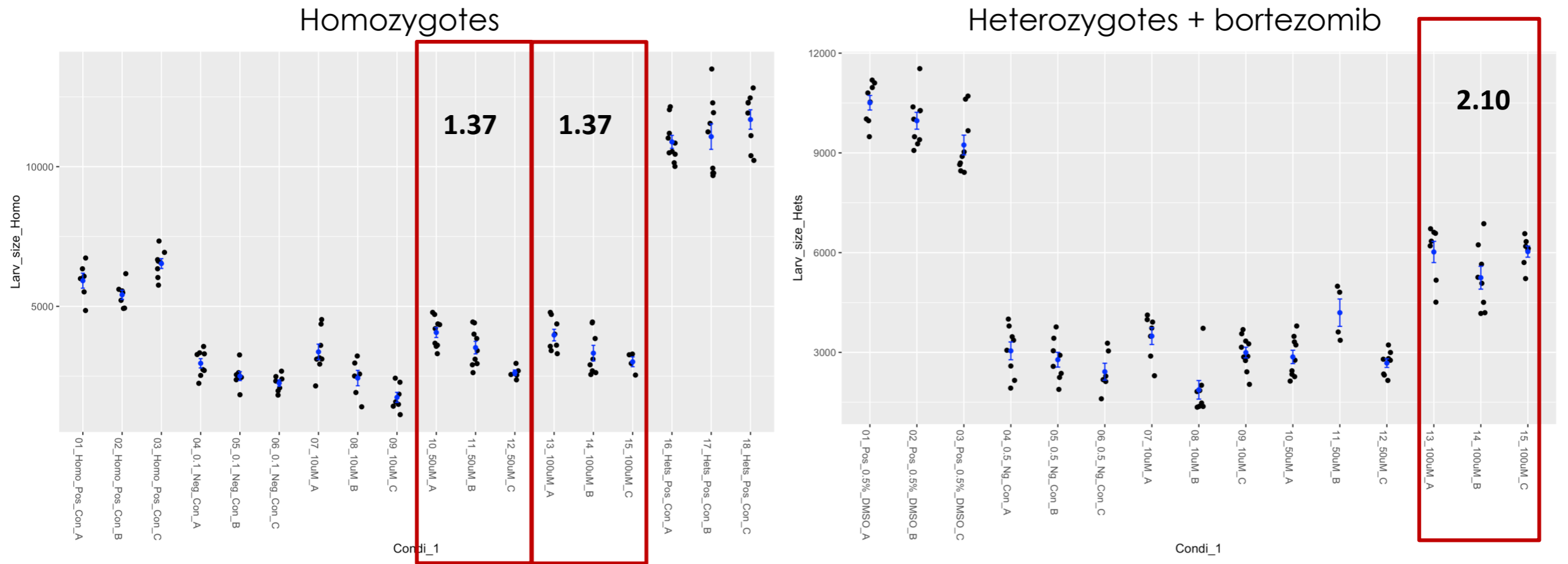


# Fly WormMS cross validation – QUERCETIN



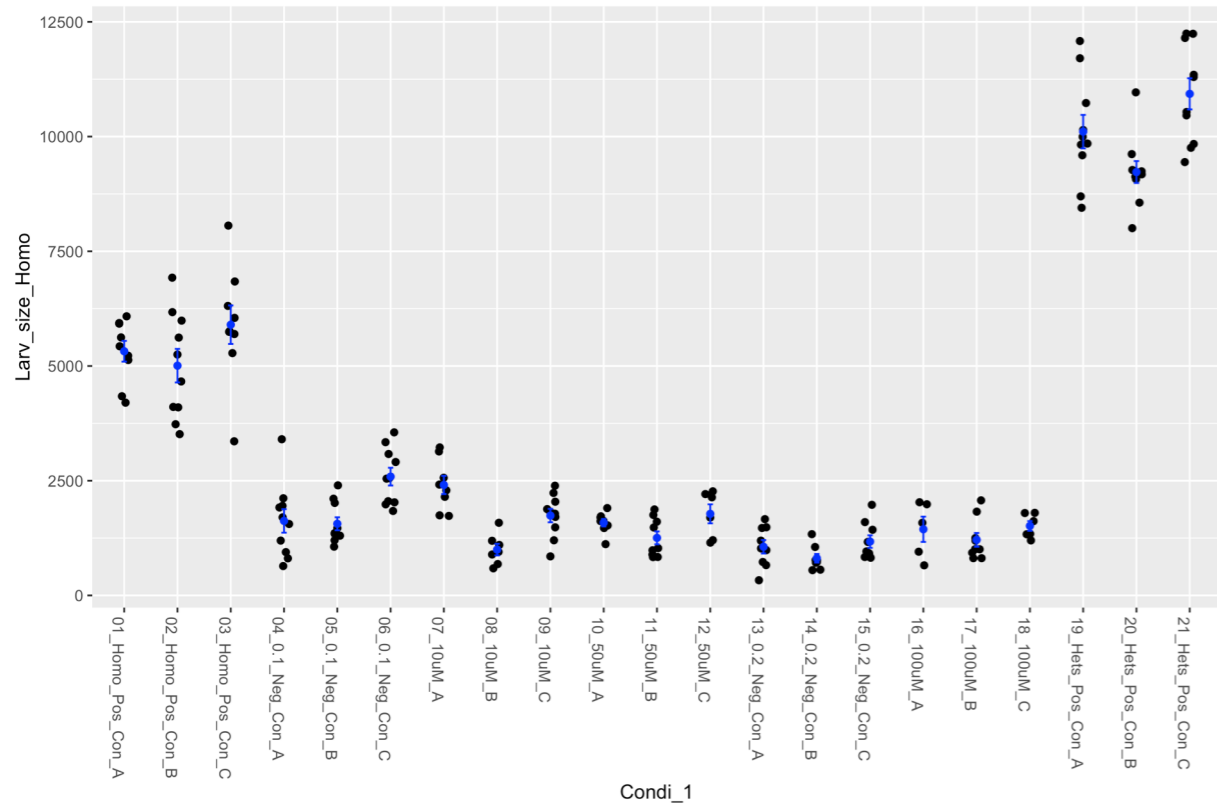


# Fly WormMS cross validation – THEAFLAVIN MONOGALLATE

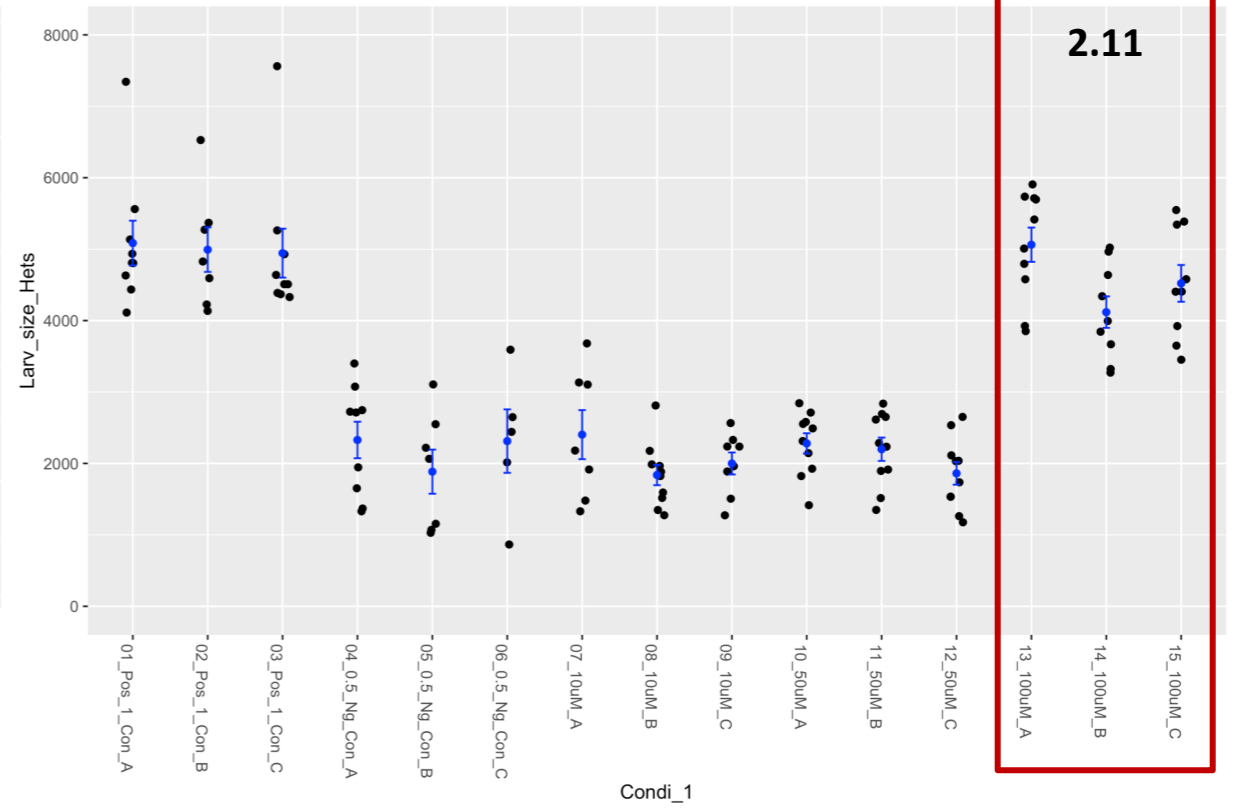


# Fly WormMS cross validation – TRIAMCINOLONE

## Homozygotes



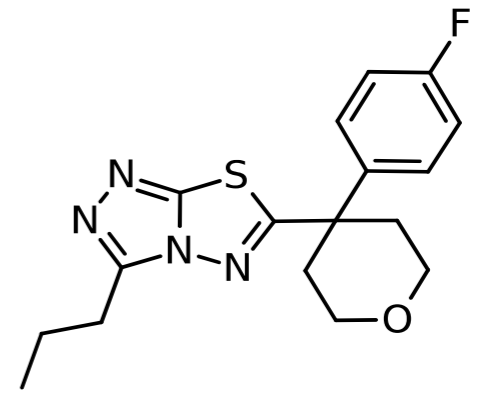
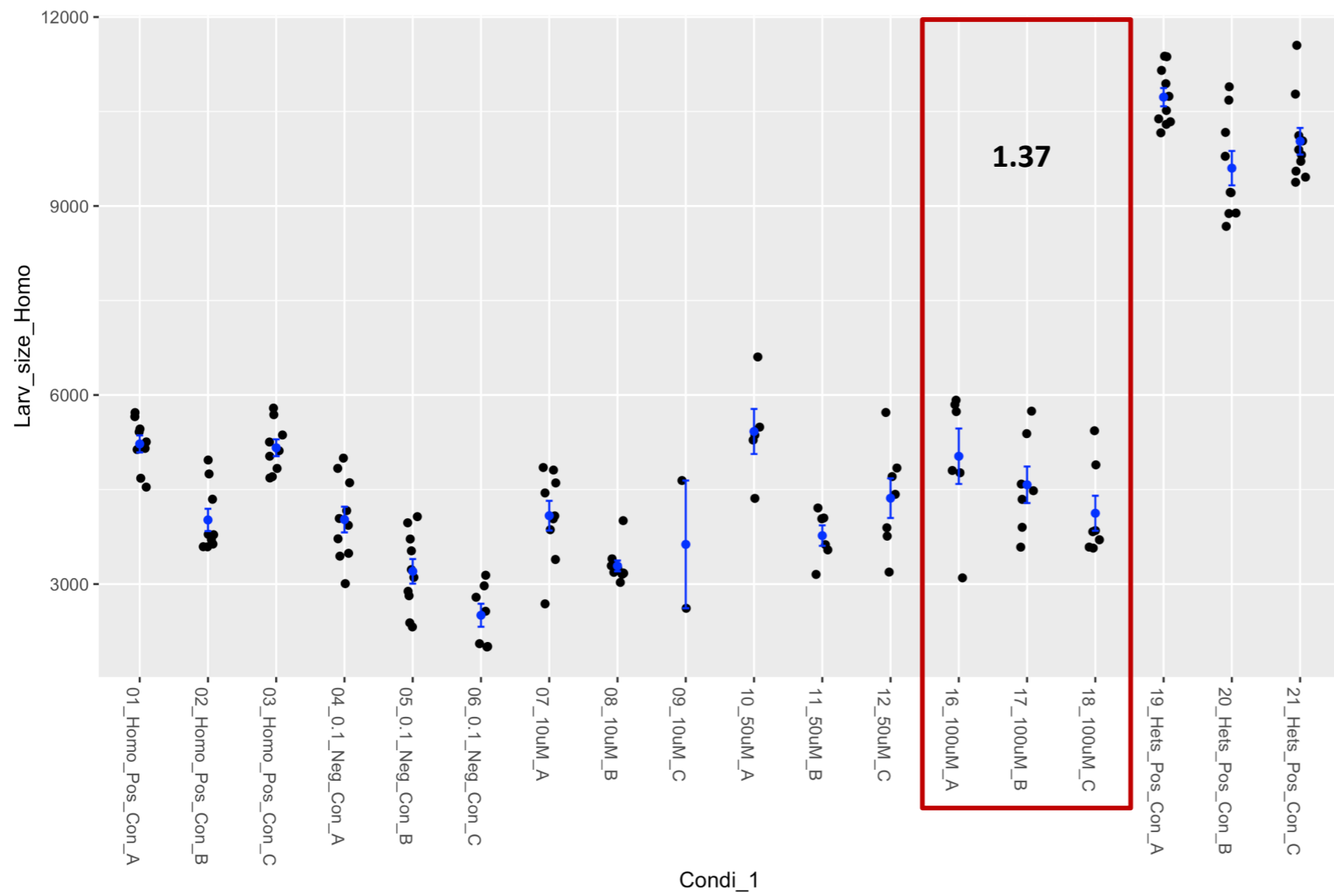
## Heterozygotes + bortezomib



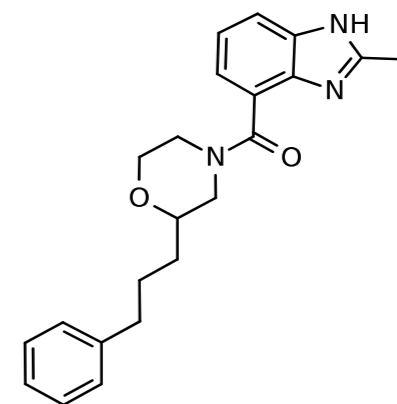
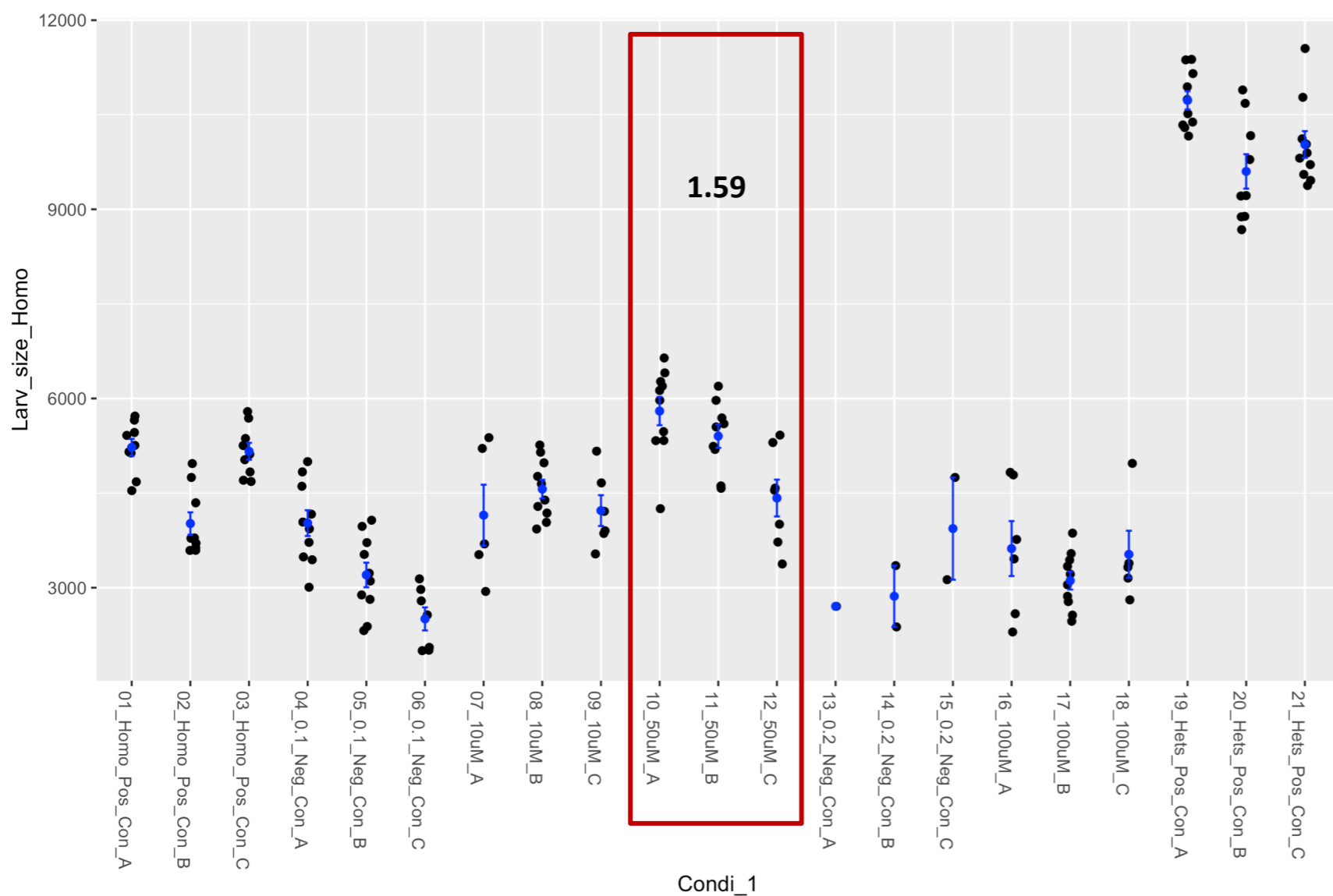
# Fly Chembridge Retests

All compounds that scored as hits in the fly *Pngl*<sup>+/-</sup> Chembridge (drug discovery) screen were retested in two fly secondary assays : rescue of *Pngl*<sup>-/-</sup> homozygotes and rescue of bortezomib-treated *Pngl*<sup>+/-</sup> heterozygotes. Red boxes indicate compounds that successfully retested. The number indicates the size ratio of compound-treated fly larvae to DMSO-treated control homozygous fly larvae. For example, 2 means that a compound increased the size of mutant larvae by twofold.

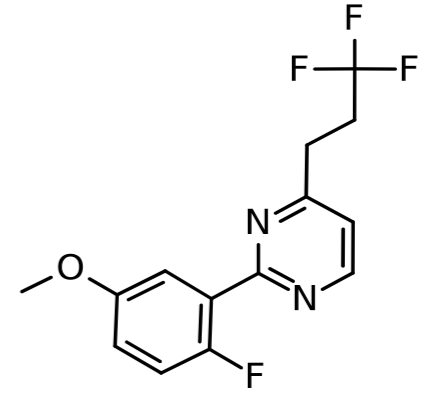
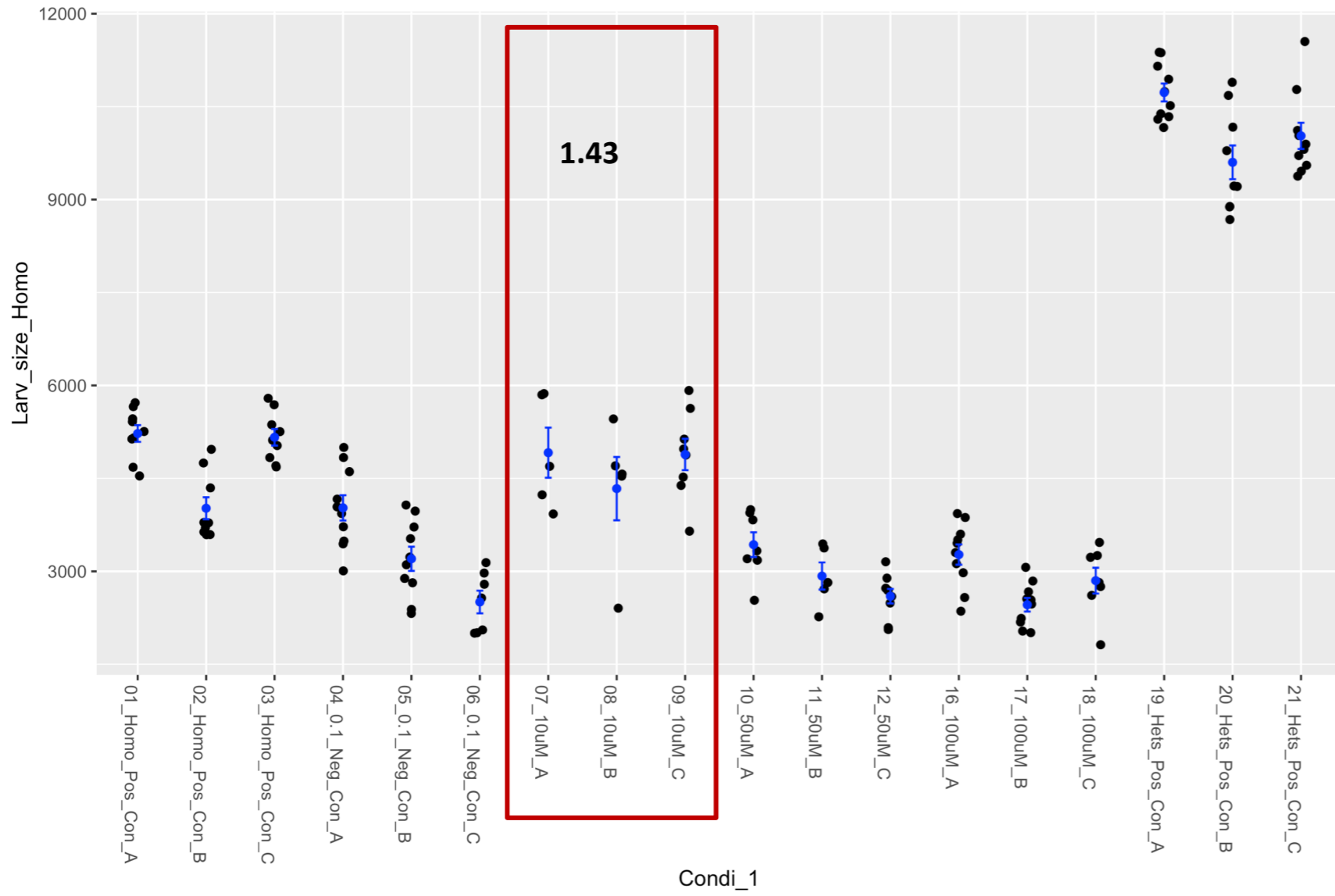
# Fly Chembridge retest – Supplier ID: 10343708



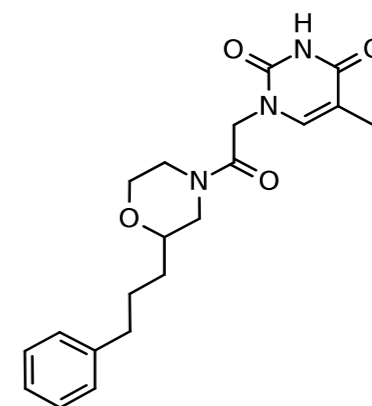
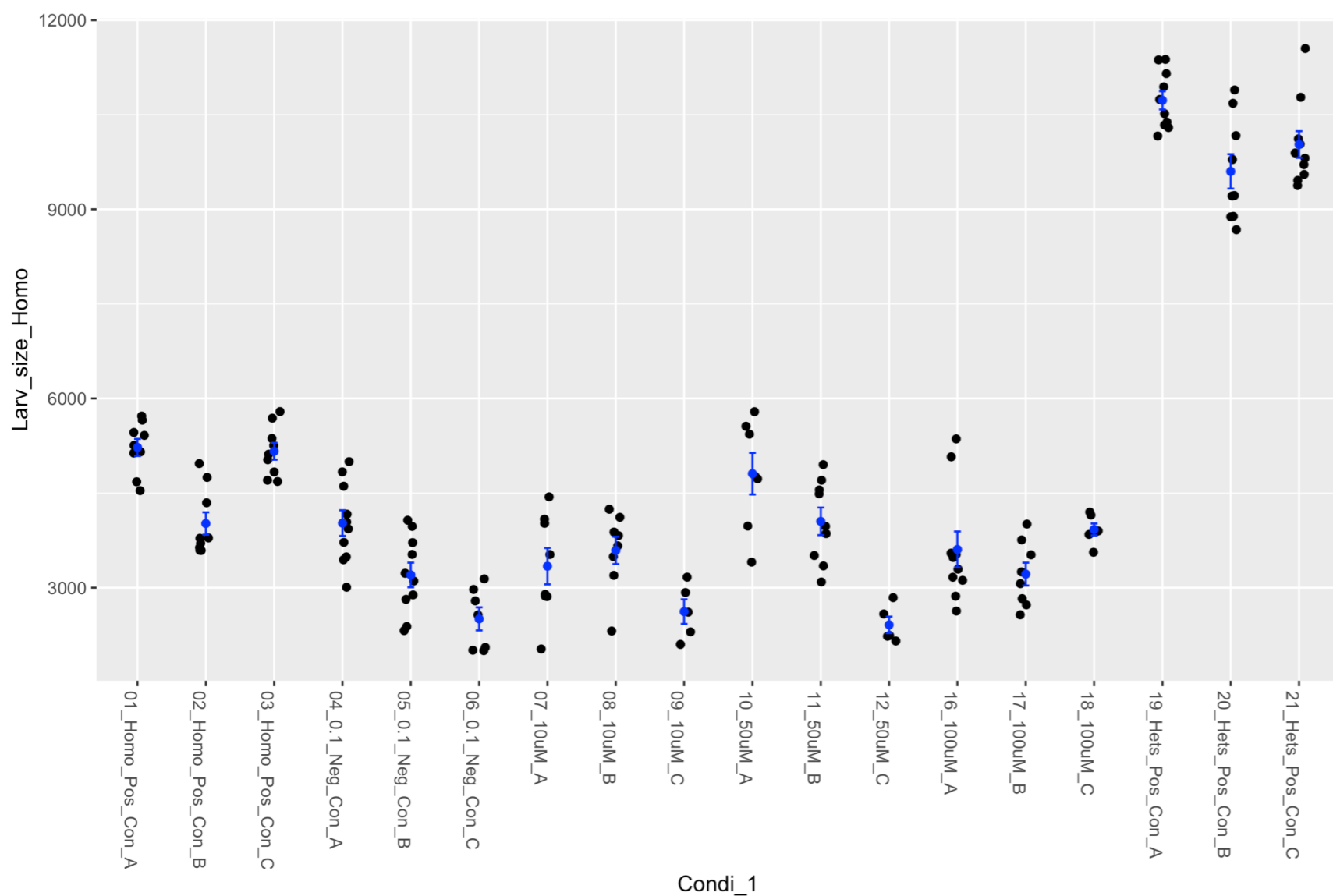
# Fly Chembridge retest – Supplier ID: 11646952



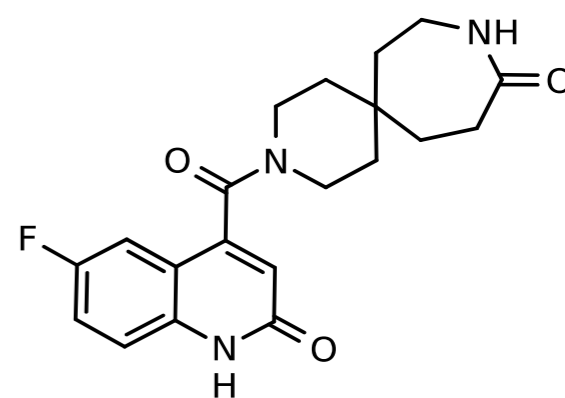
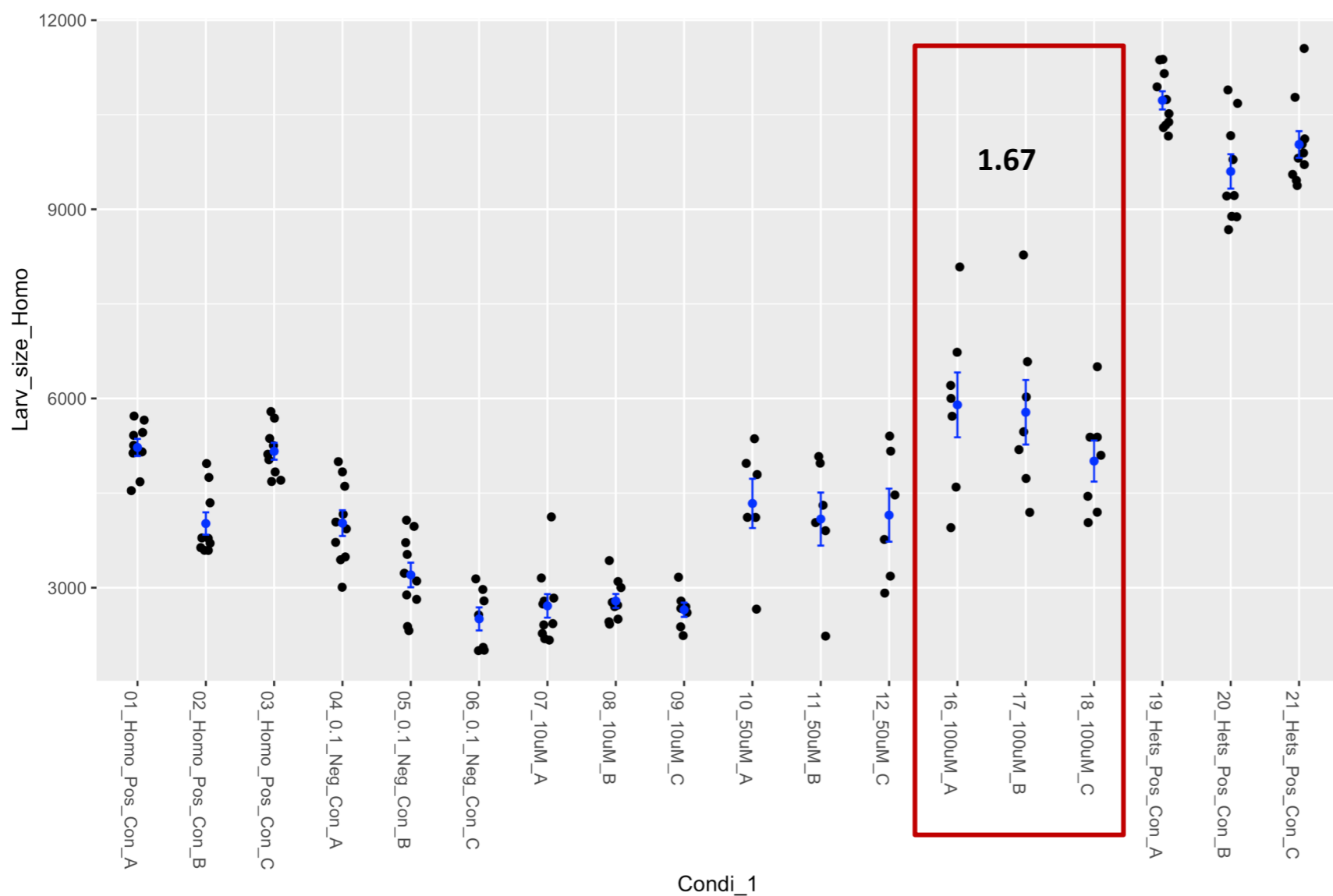
# Fly Chembridge retest – Supplier ID: 22934709



# Fly Chembridge retest – Supplier ID: 26889292

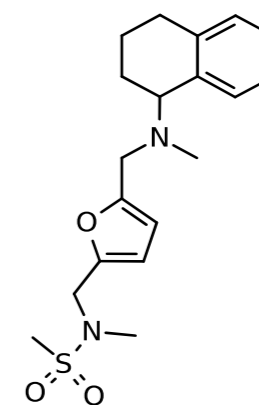
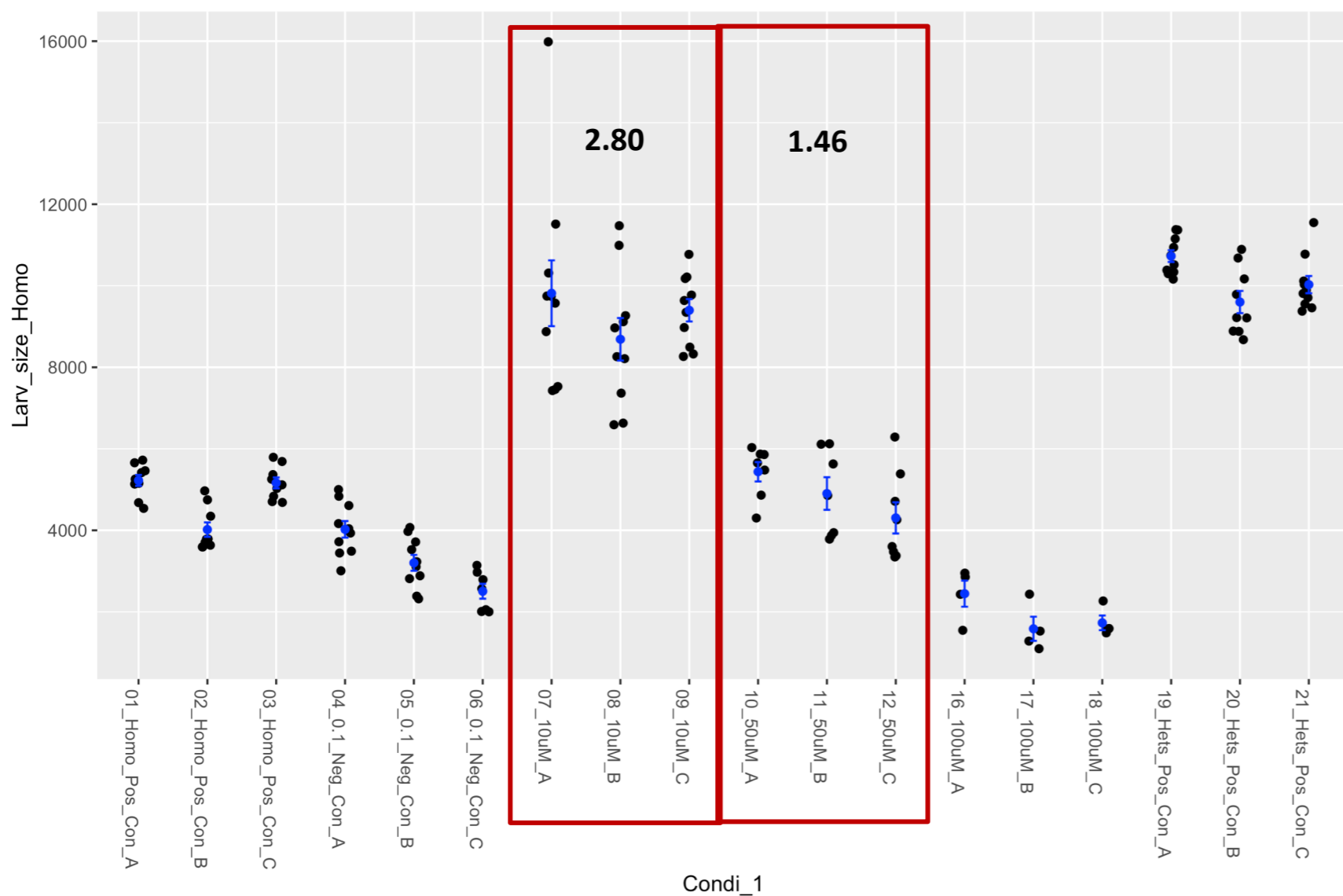


# Fly Chembridge retest – Supplier ID: 35527962

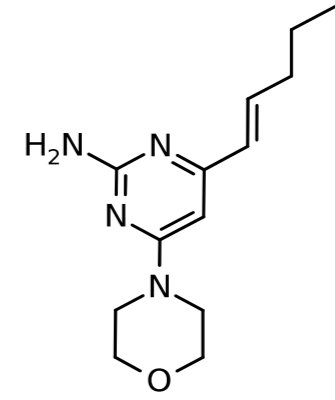
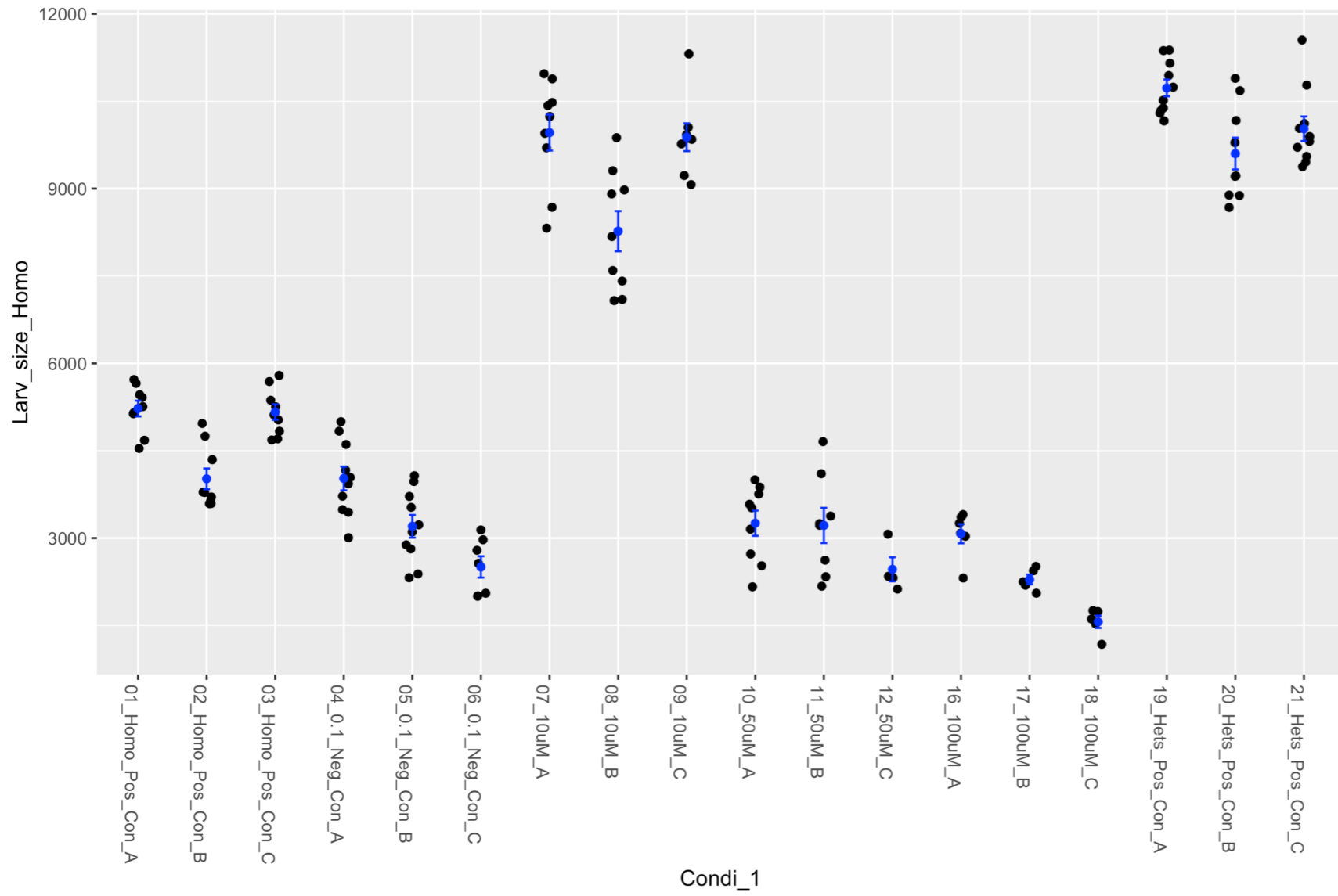




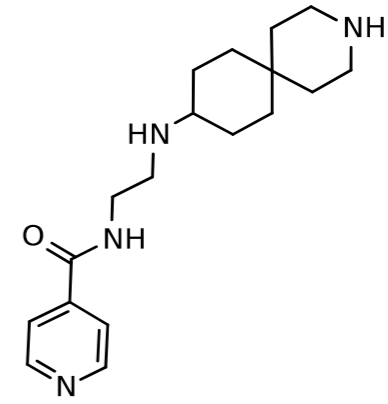
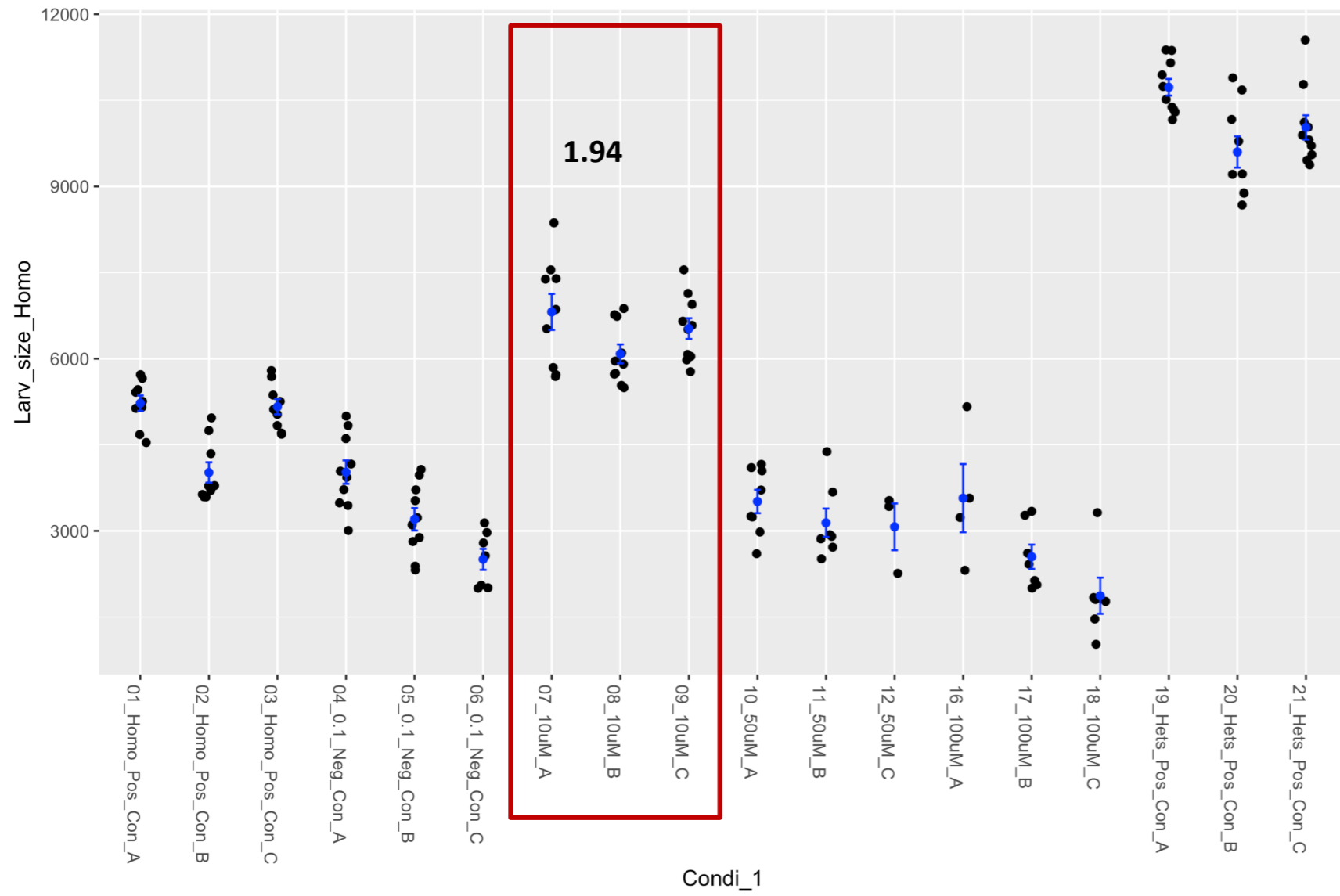
# Fly Chembridge retest – Supplier ID: 40966617



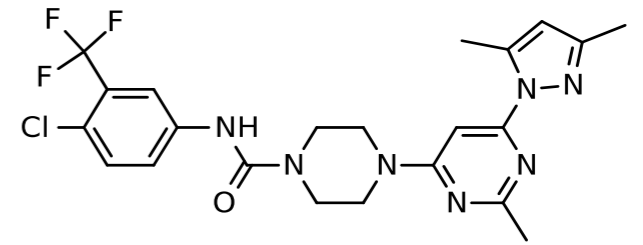
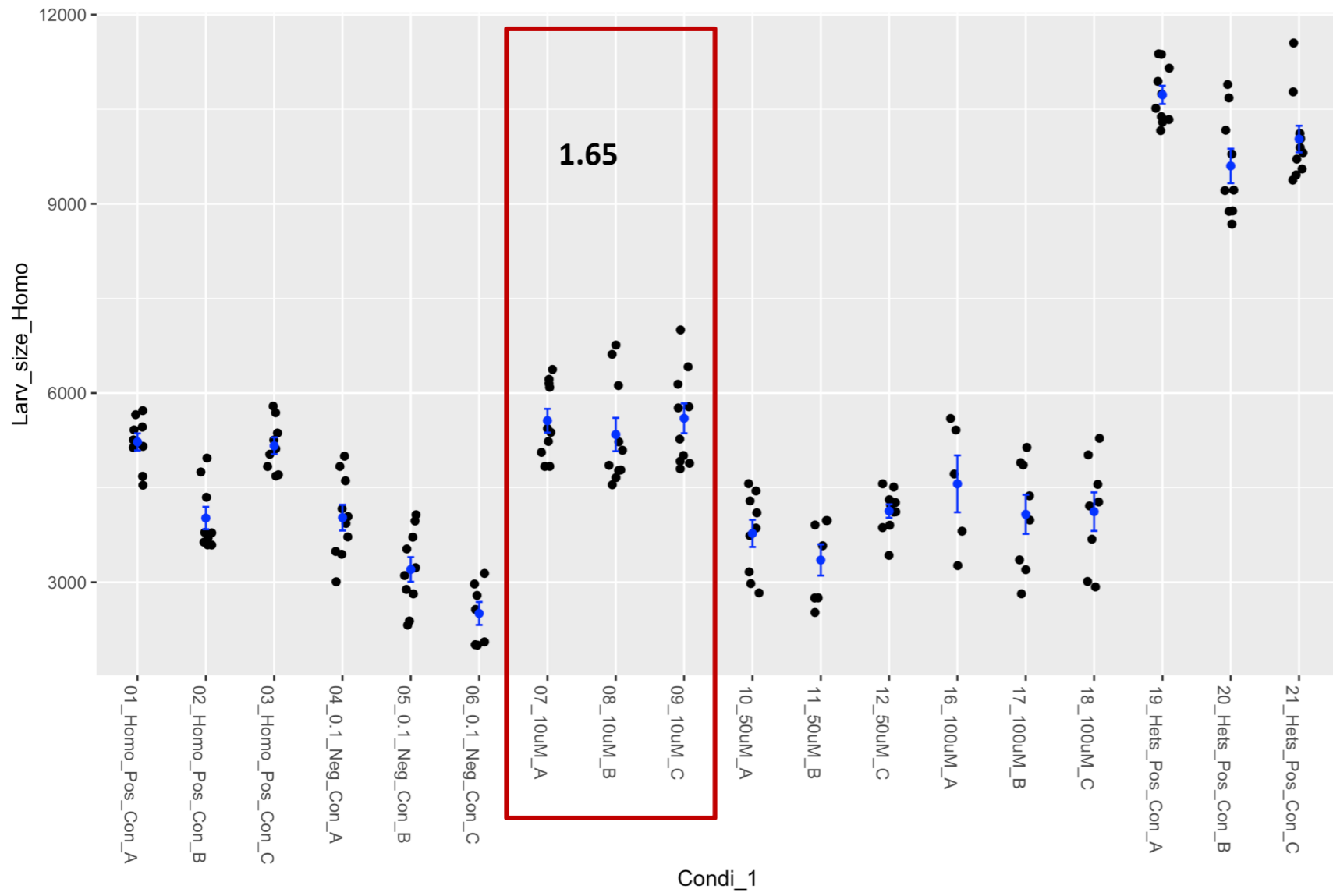
# Fly Chembridge retest – Supplier ID: 58095314



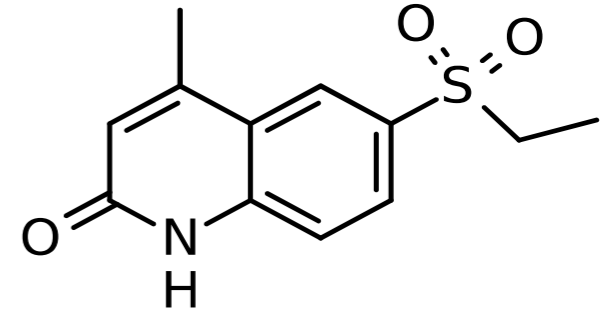
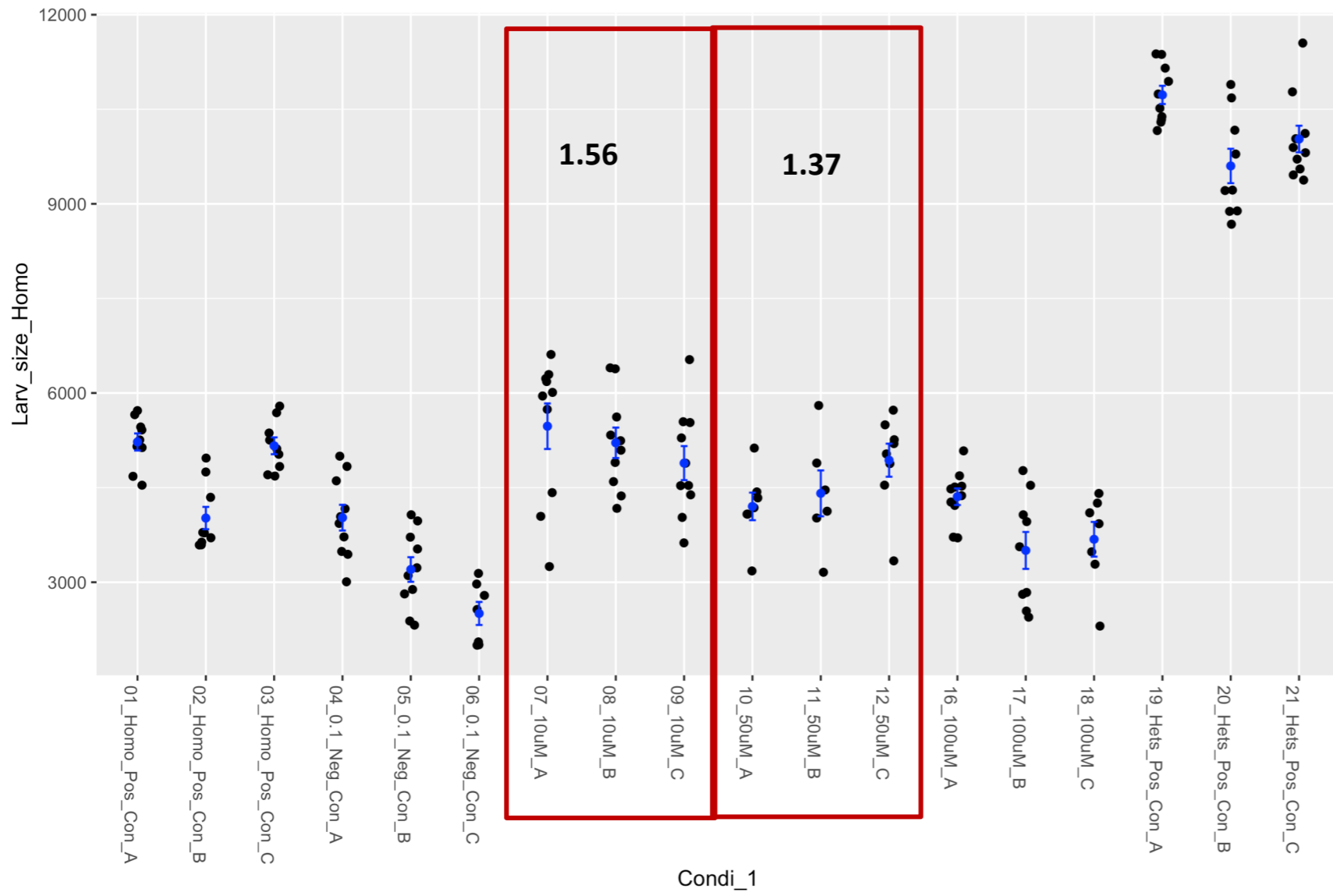
# Fly Chembridge retest – Supplier ID: 89890646



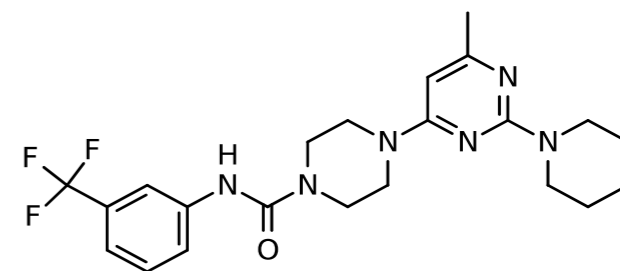
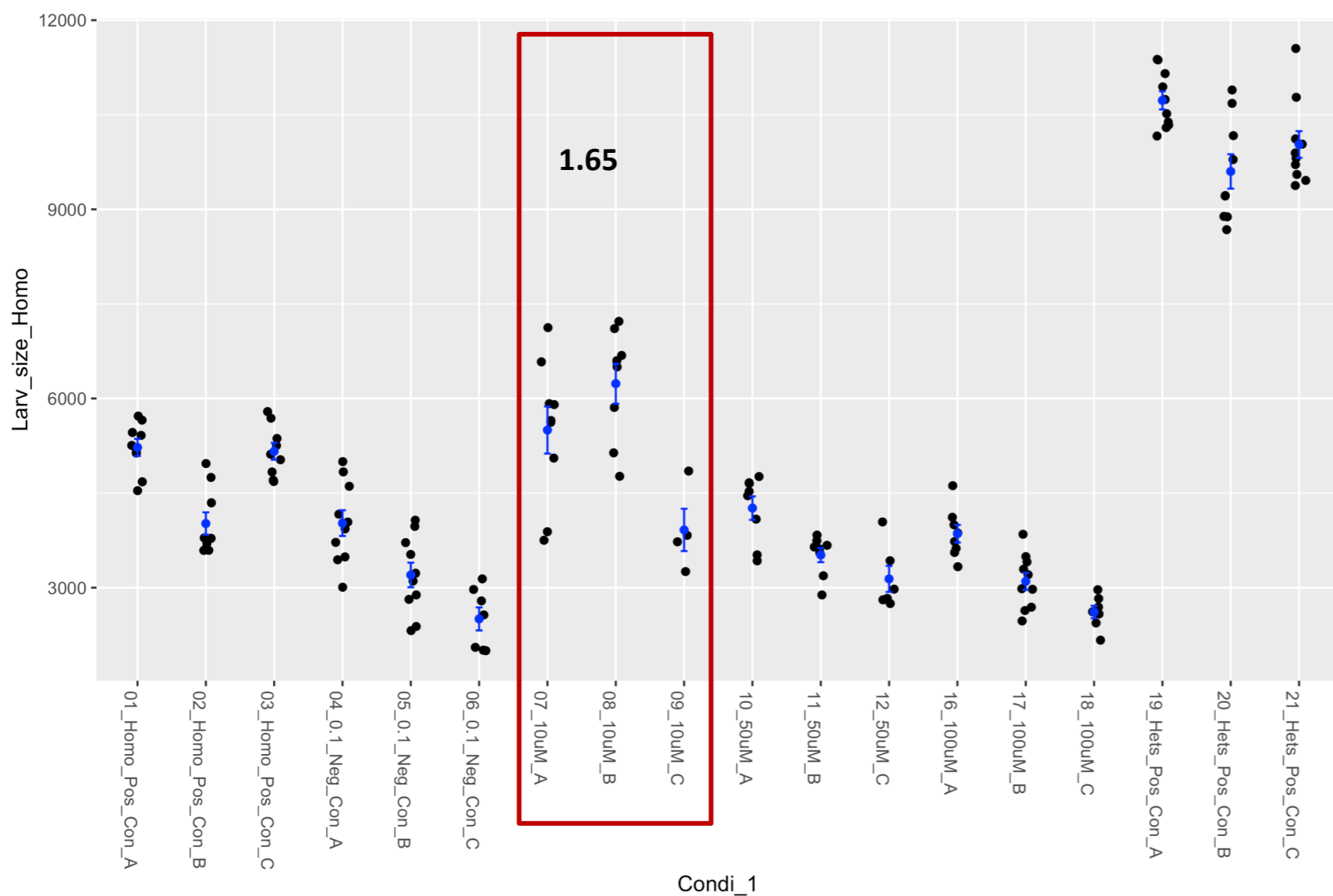
# Fly Chembridge retest – Supplier ID: 9206326



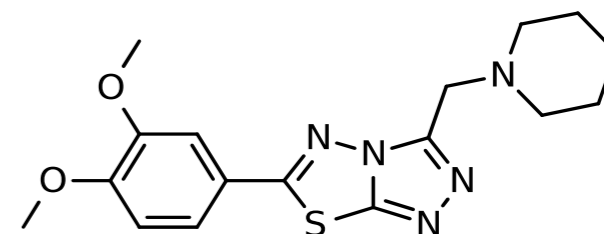
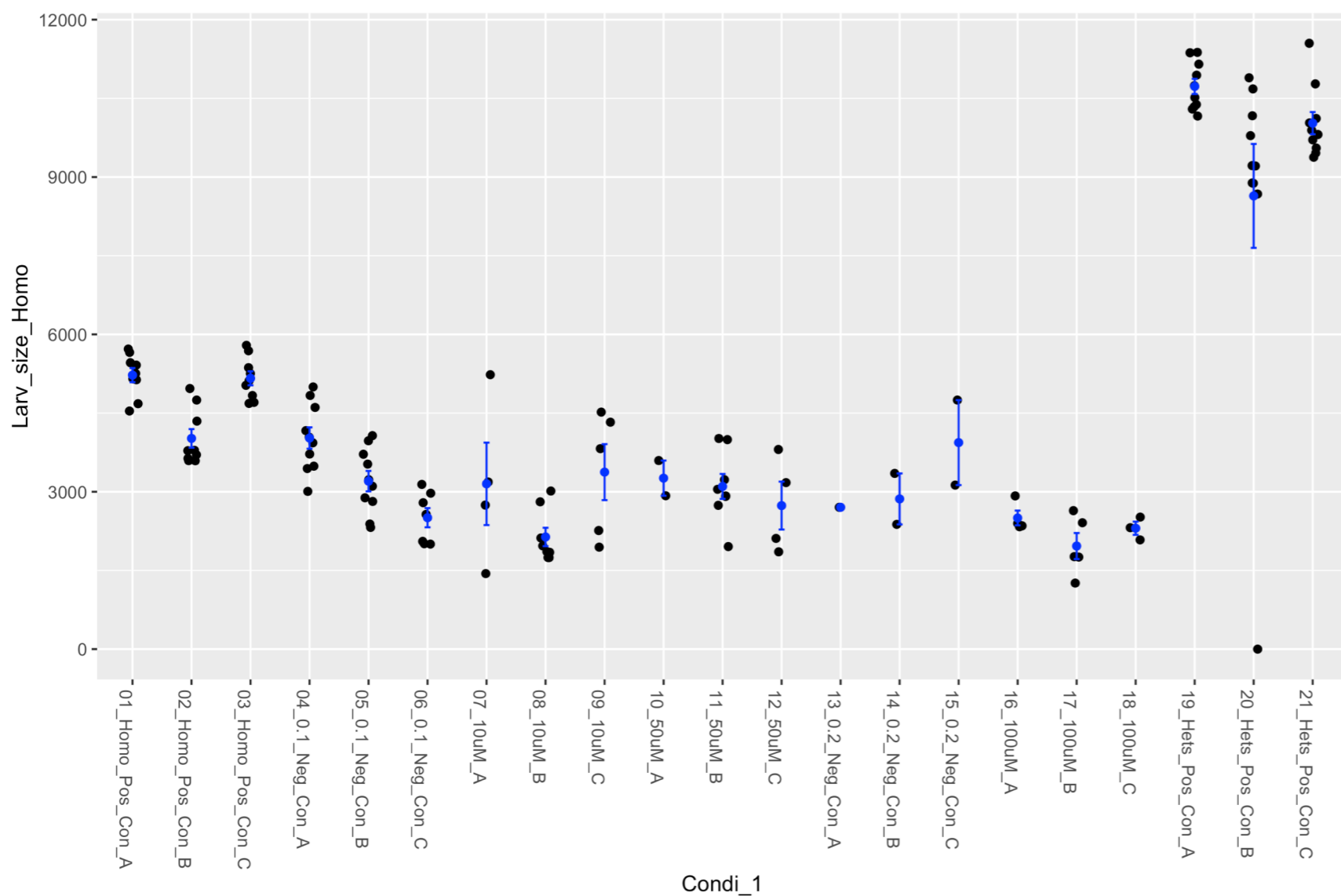
# Fly Chembridge retest – Supplier ID: 9233974



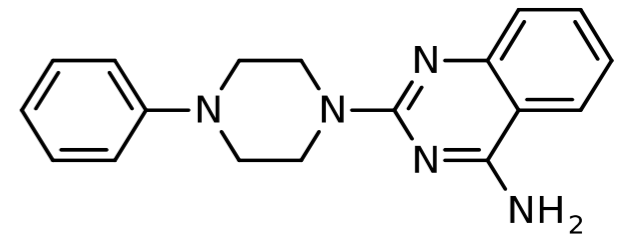
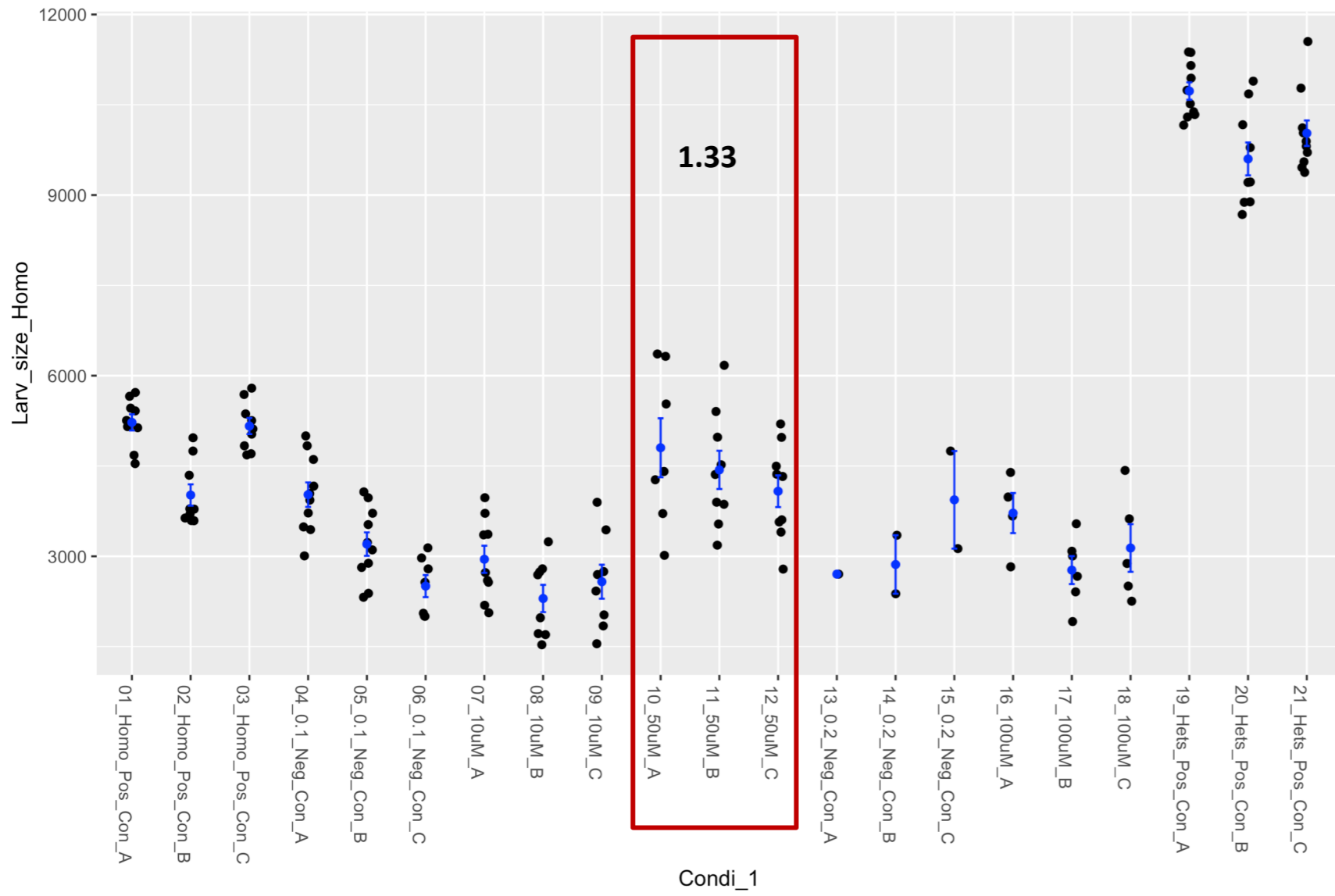
# Fly Chembridge retest – Supplier ID: 9251119



# Fly Chembridge retest – Supplier ID: 9251633

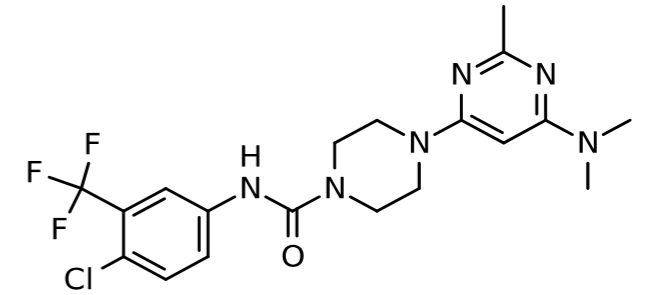
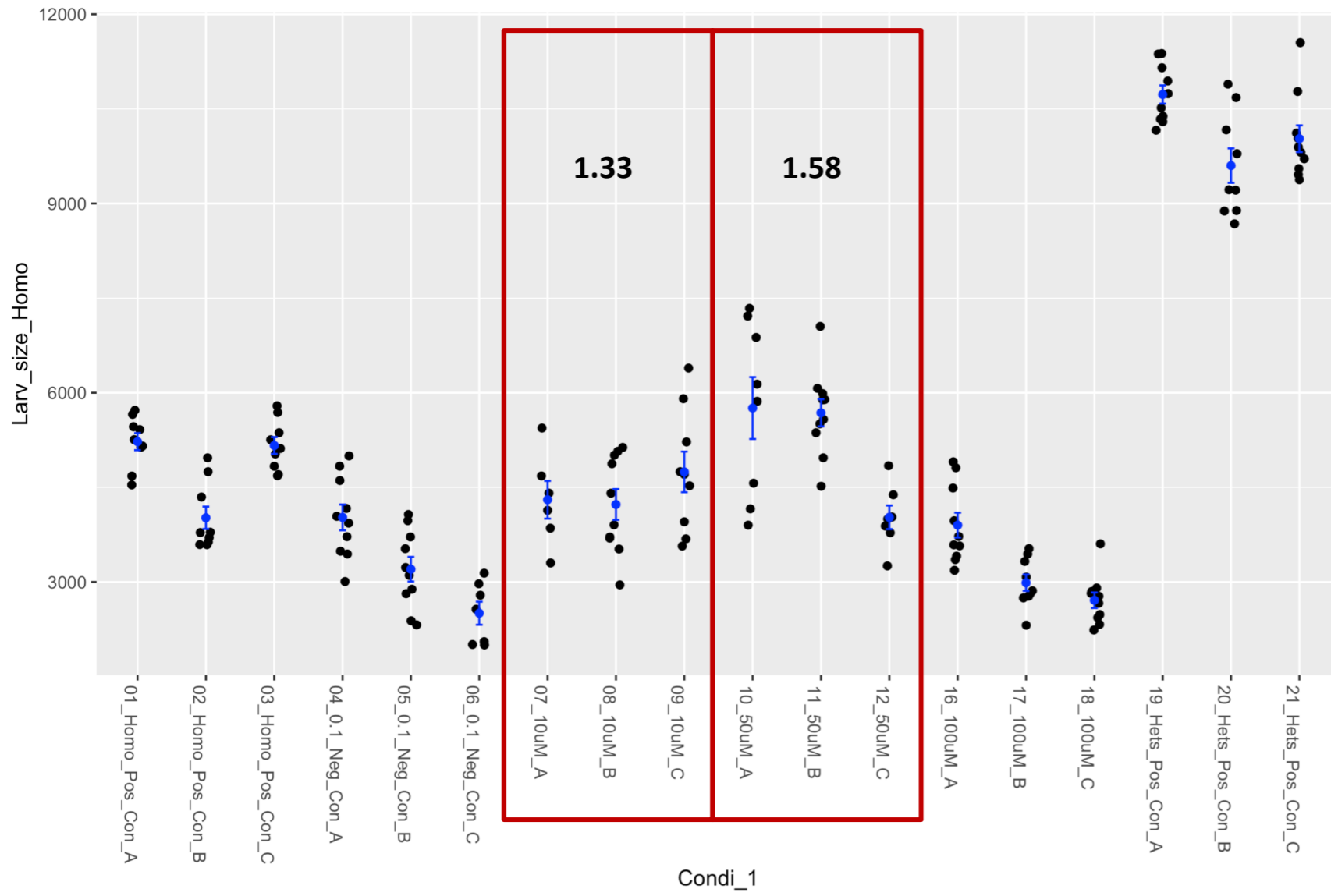


# Fly Chembridge retest – Supplier ID: 9262220

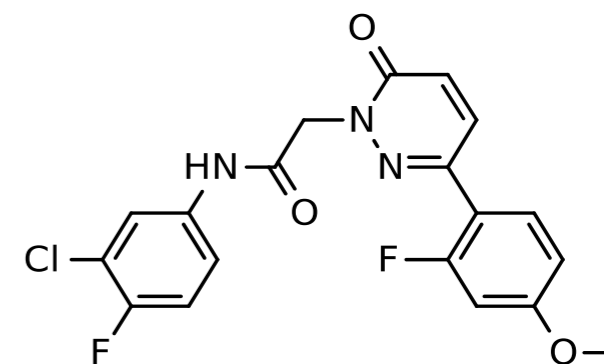
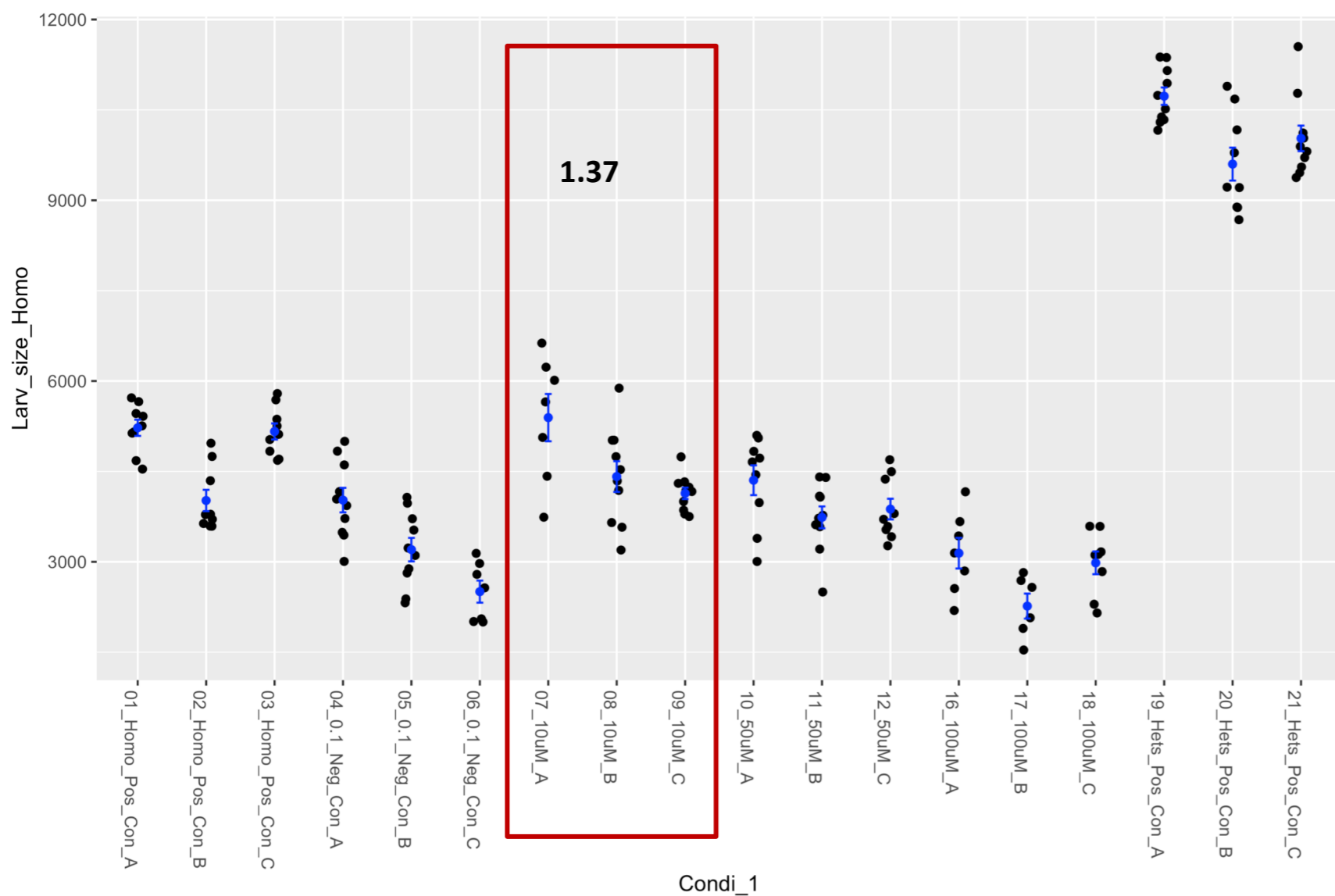




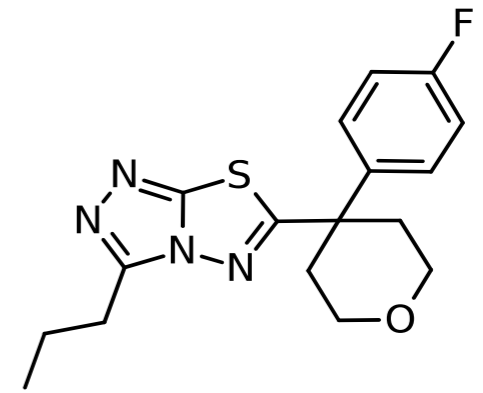
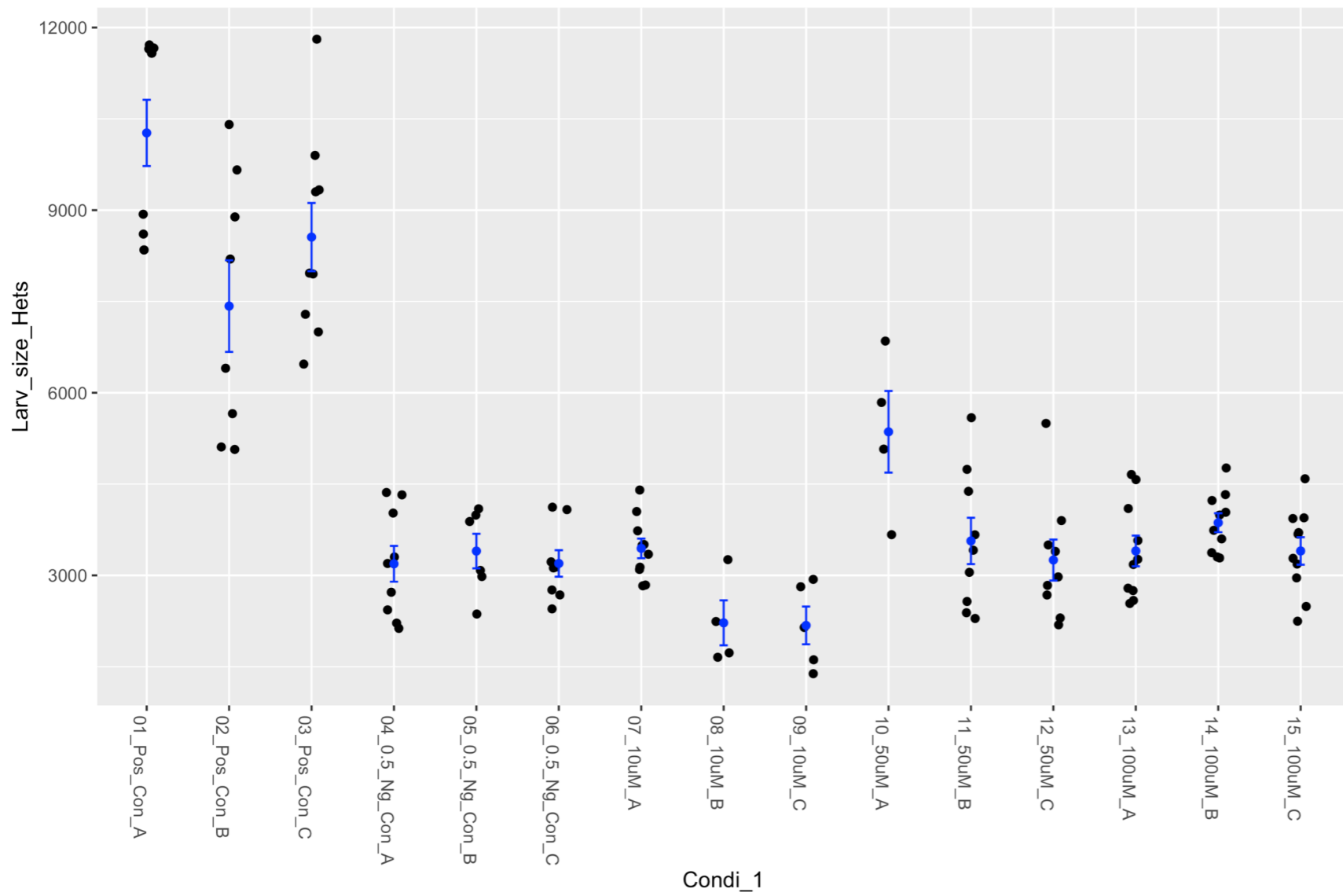
# Fly Chembridge retest – Supplier ID: 9262955



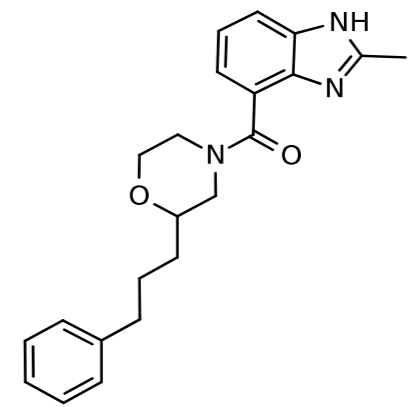
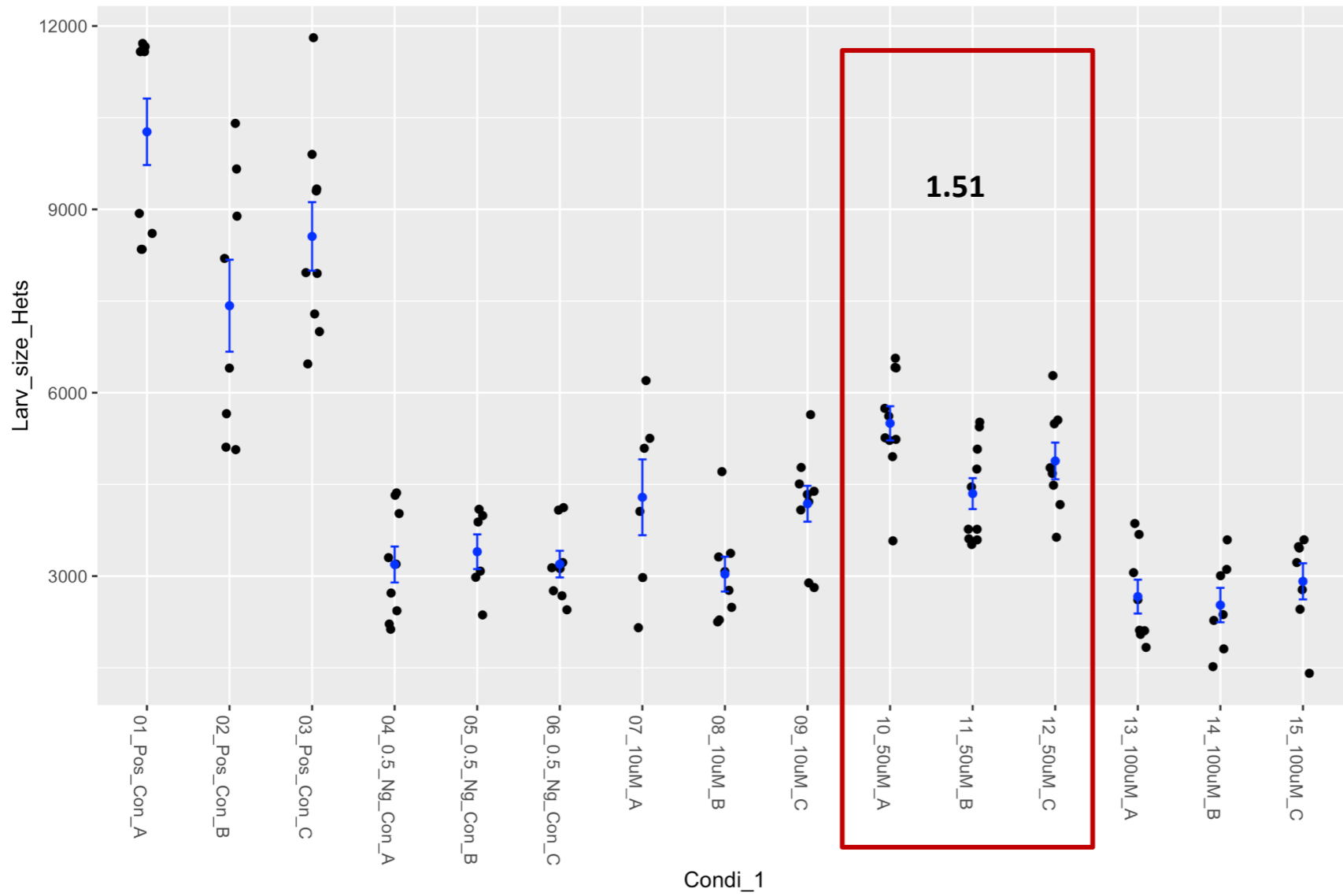
# Fly Chembridge retest – Supplier ID: 9290684



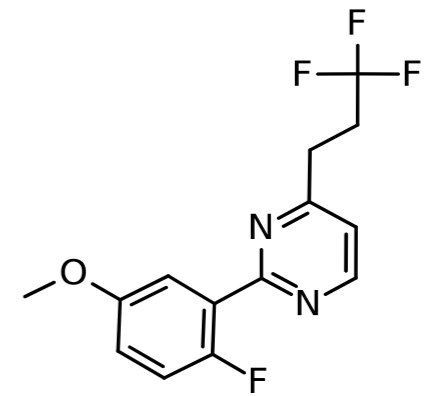
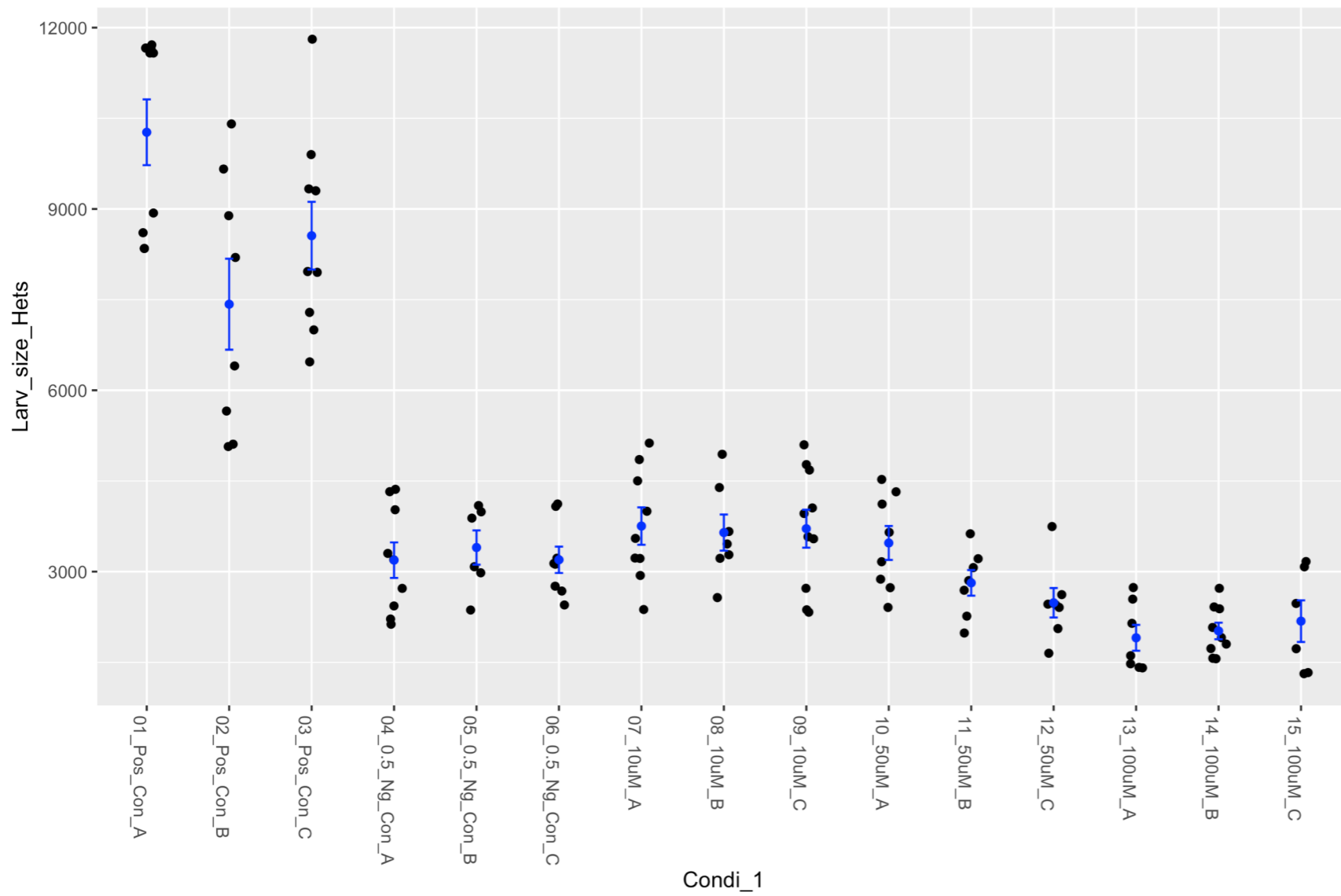
# Fly Chembridge retest – Supplier ID: 10343708



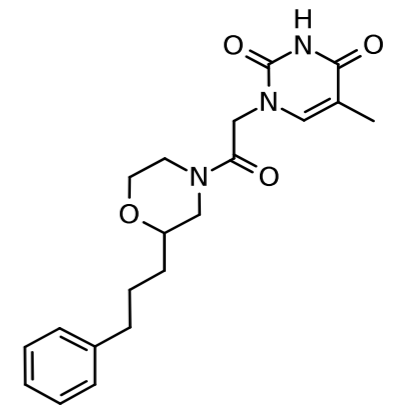
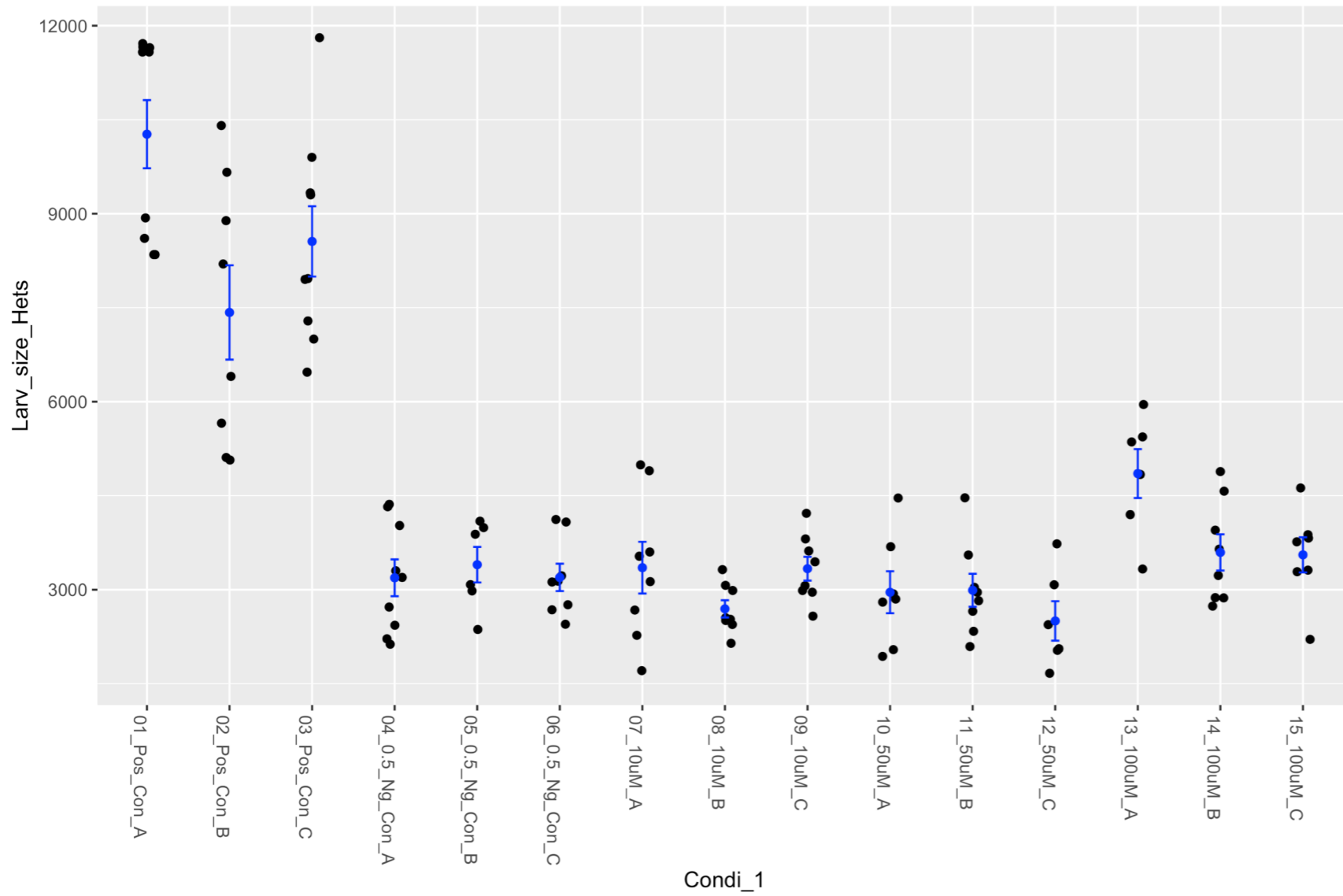
# Fly Chembridge retest – Supplier ID: 11646952



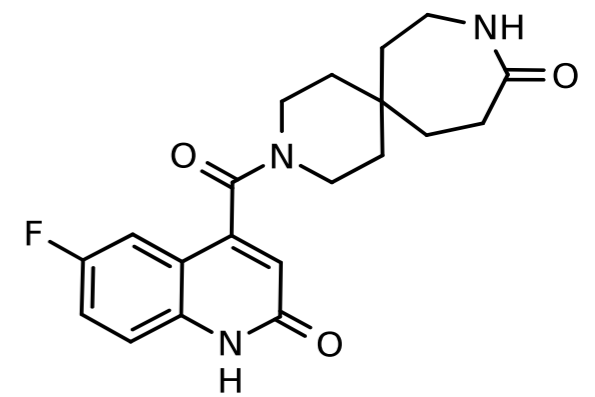
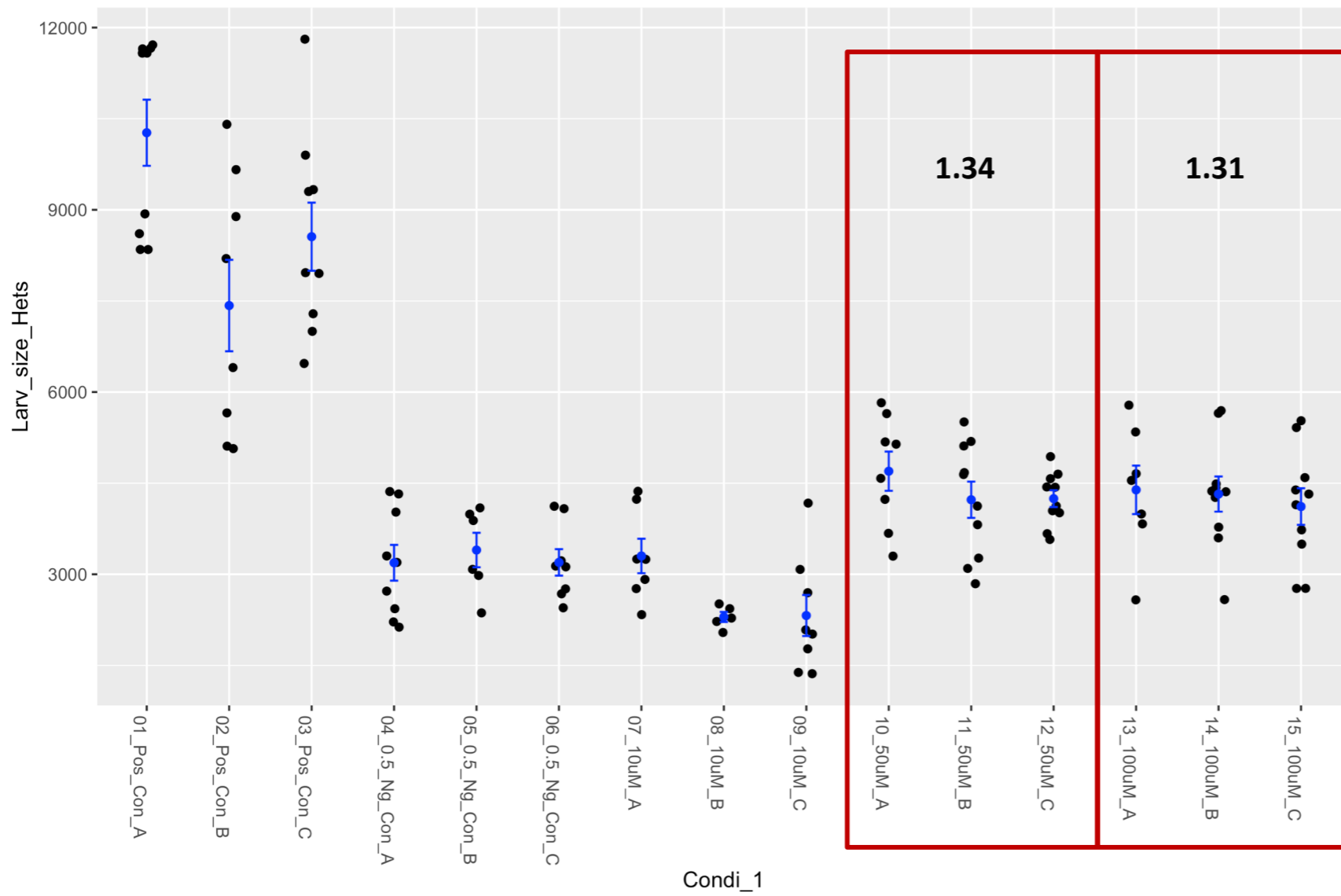
# Fly Chembridge retest – Supplier ID: 22934709



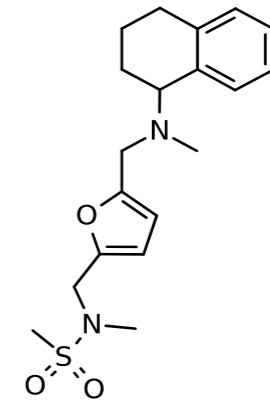
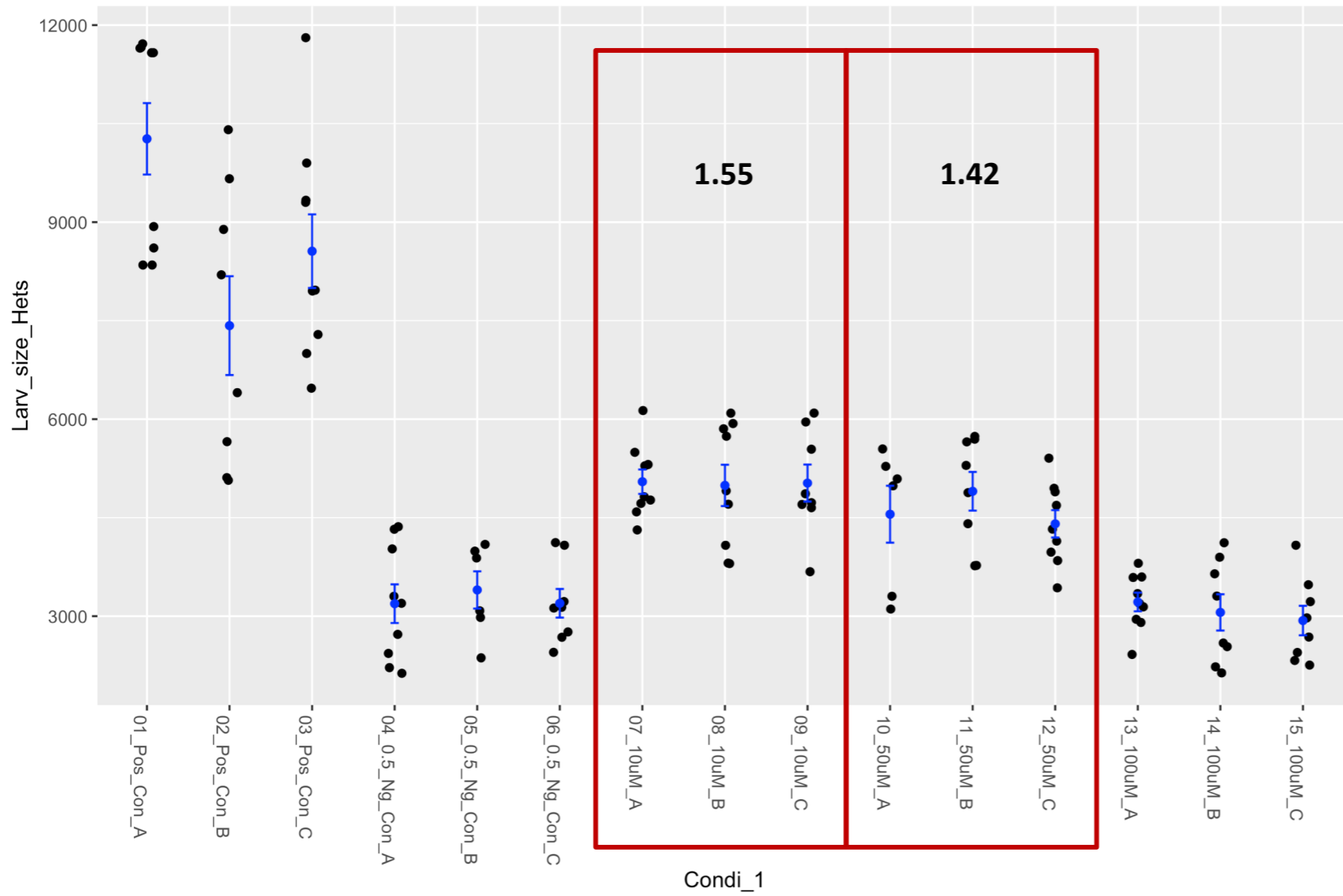
# Fly Chembridge retest – Supplier ID: 26889292



# Fly Chembridge retest – Supplier ID: 35527962

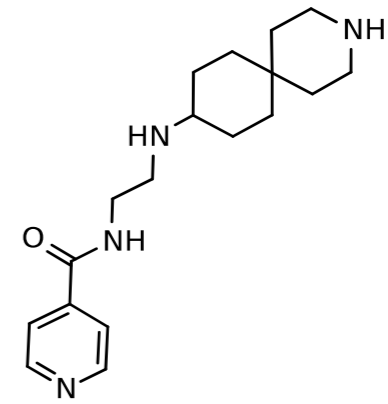
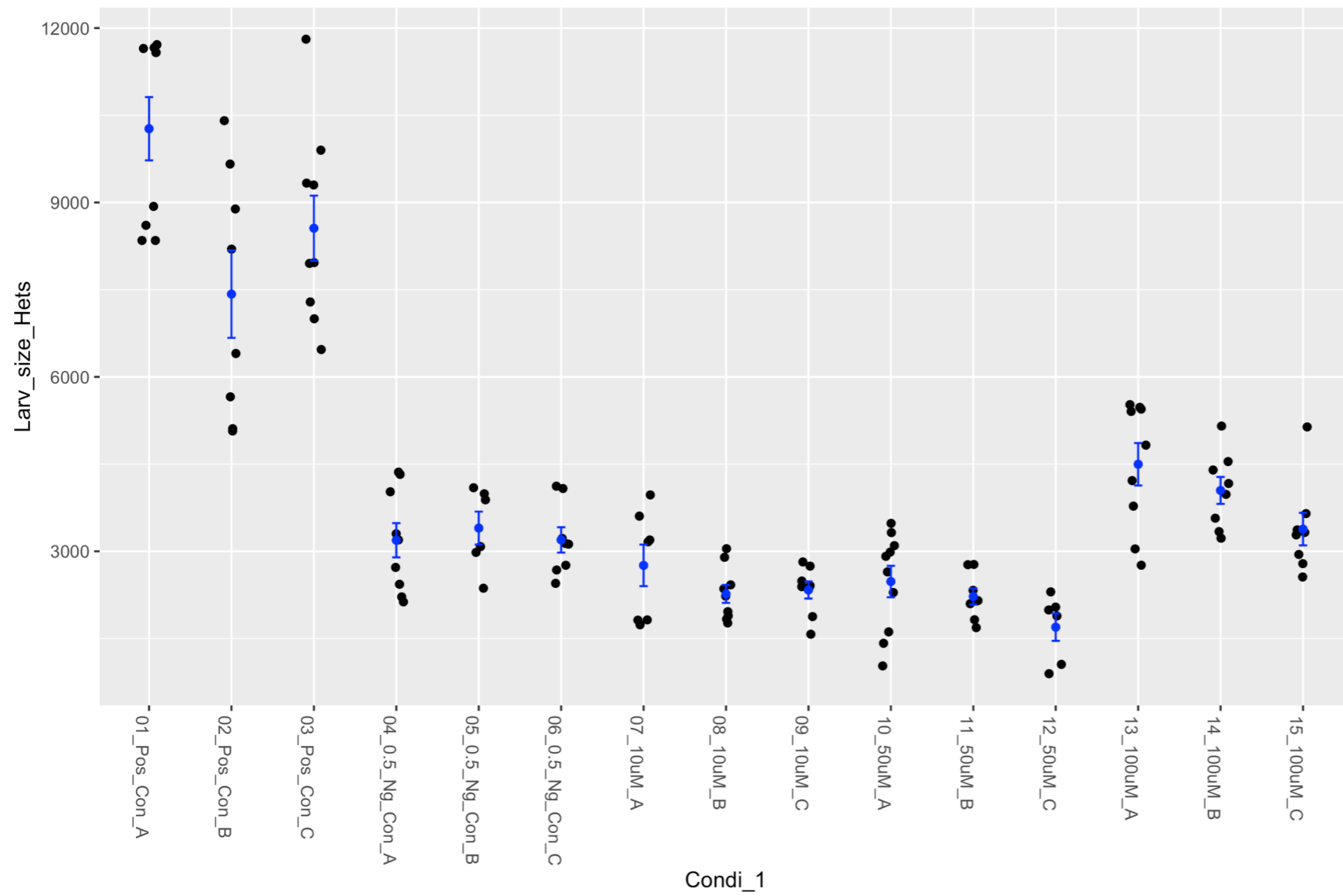


# Fly Chembridge retest – Supplier ID: 40966617

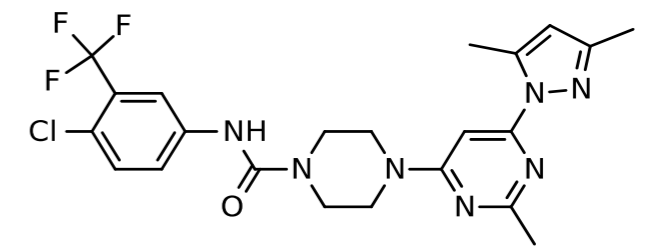
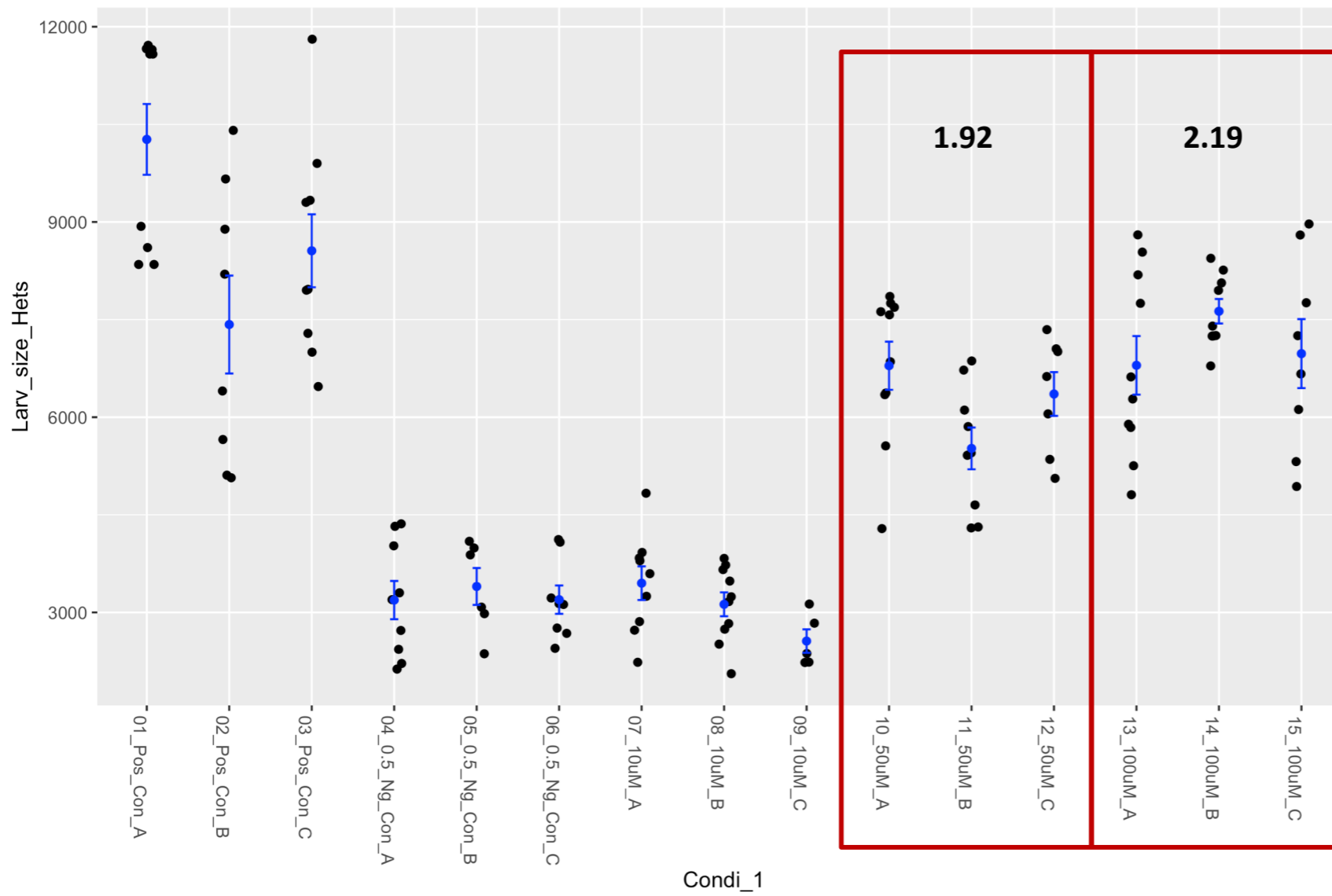




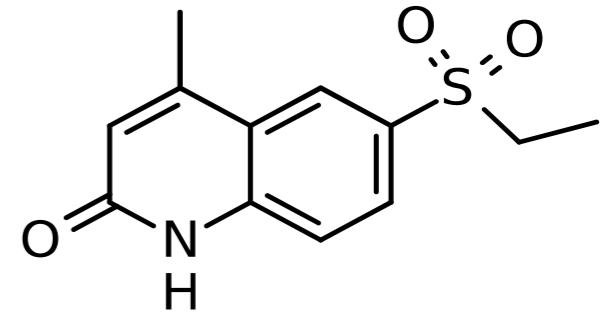
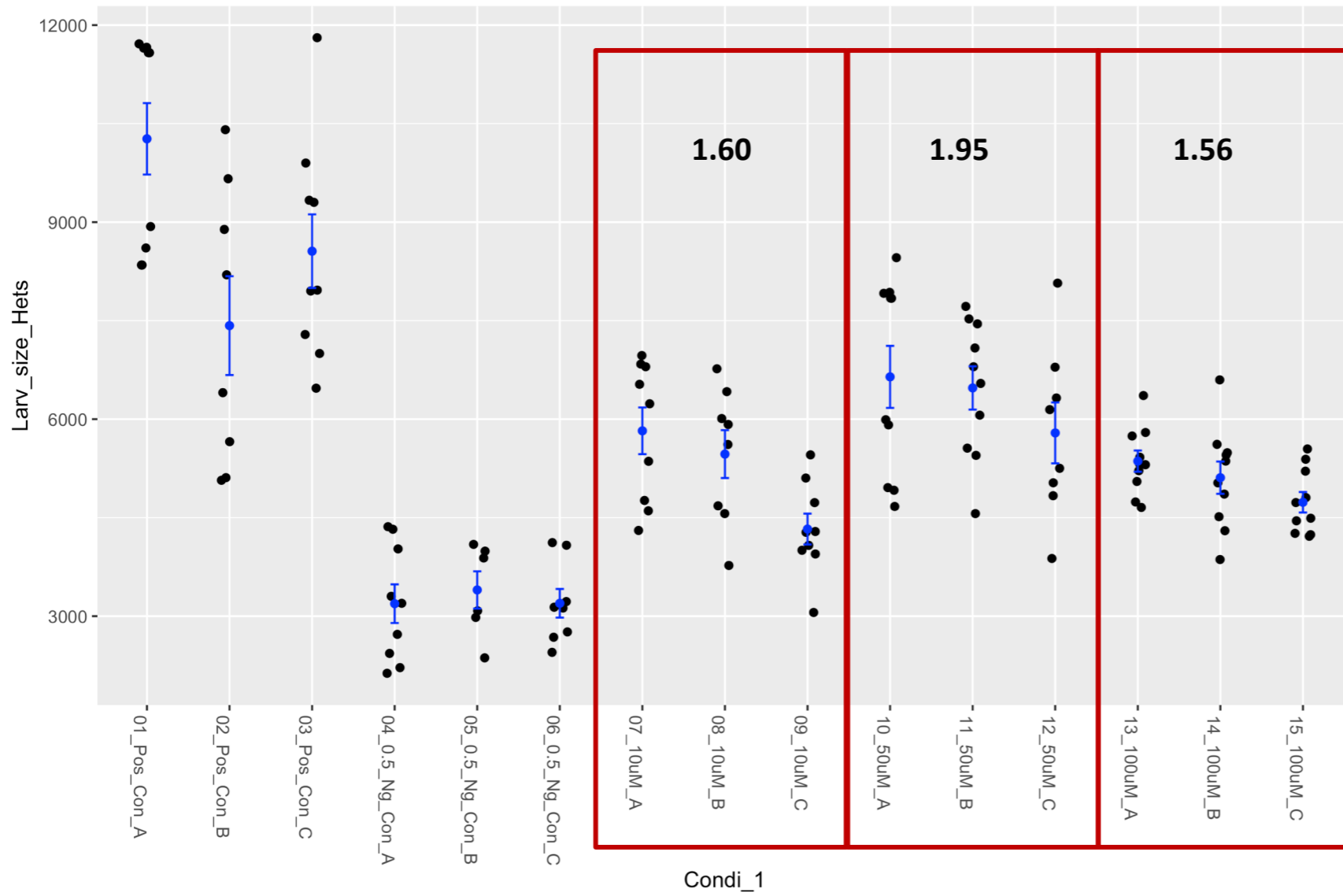
# Fly Chembridge retest – Supplier ID: 89890646



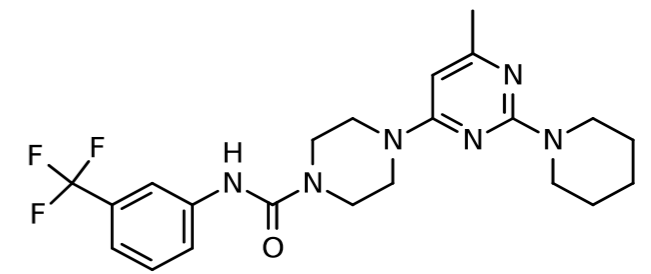
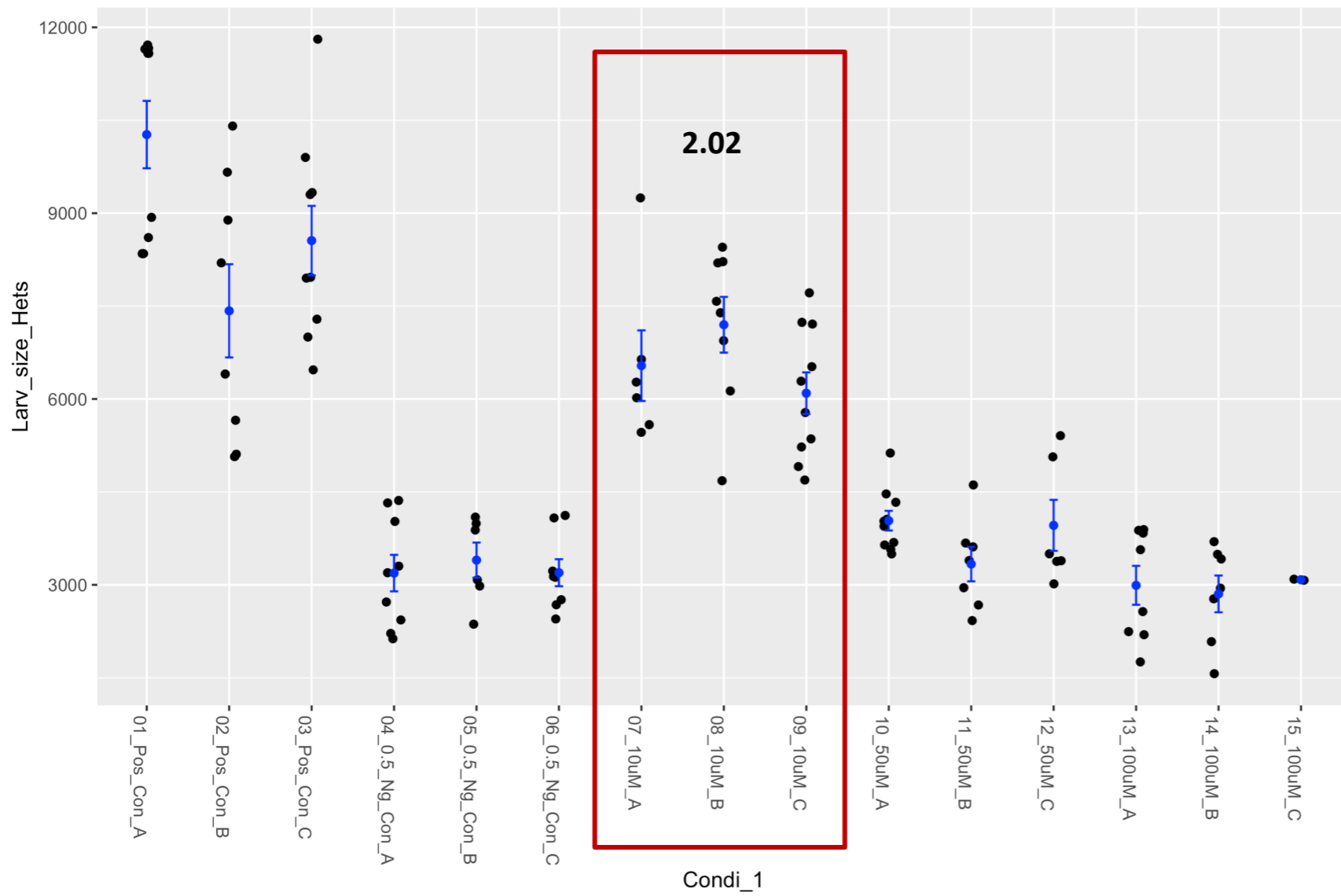
# Fly Chembridge retest – Supplier ID: 9206326



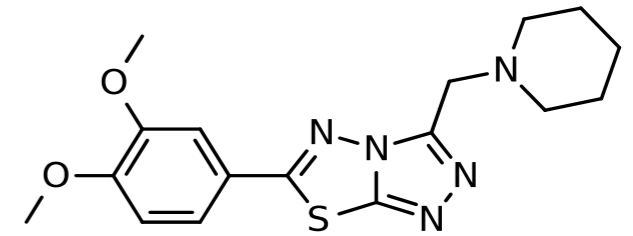
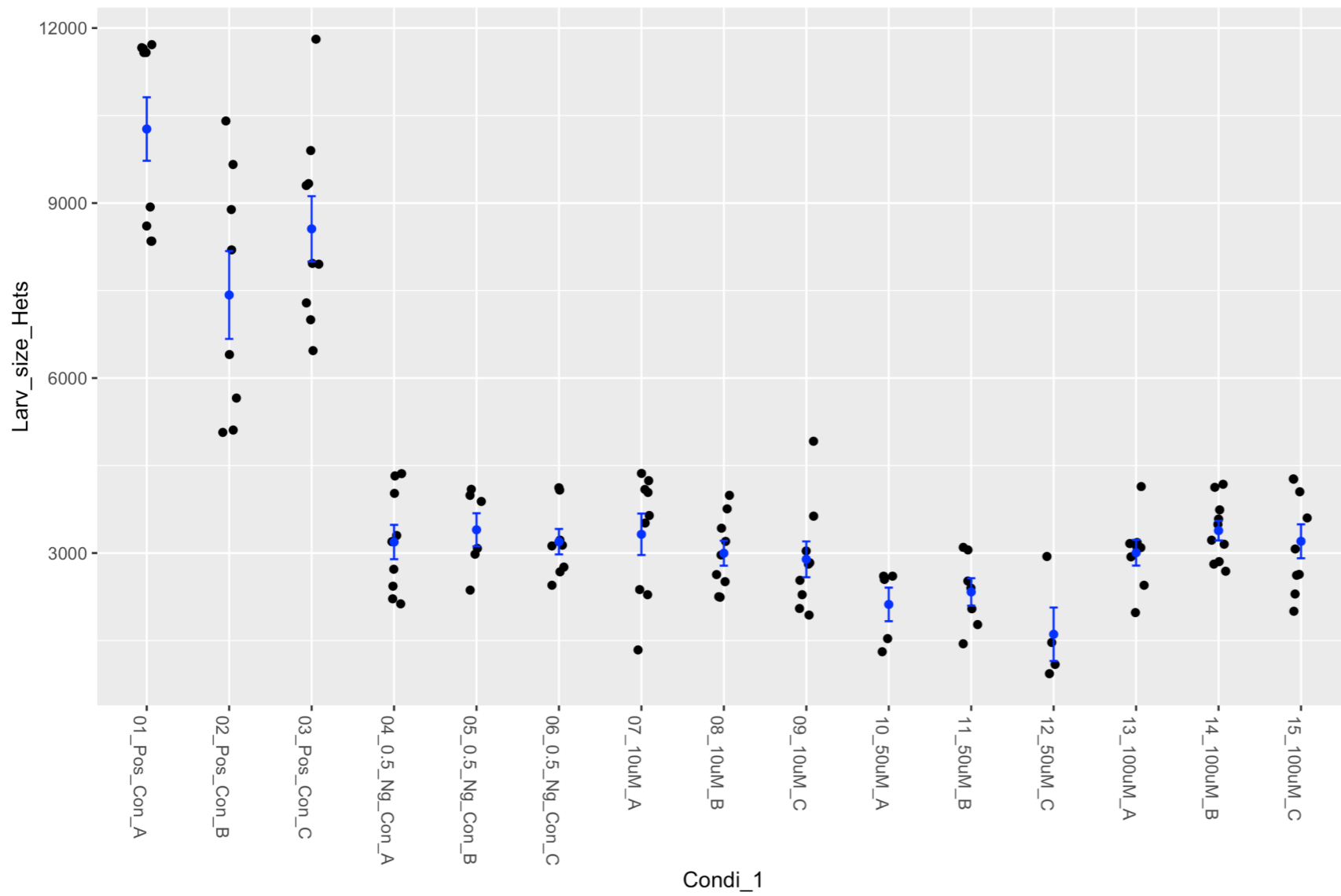
# Fly Chembridge retest – Supplier ID: 9233974



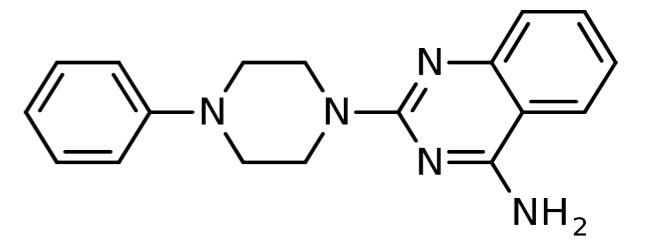
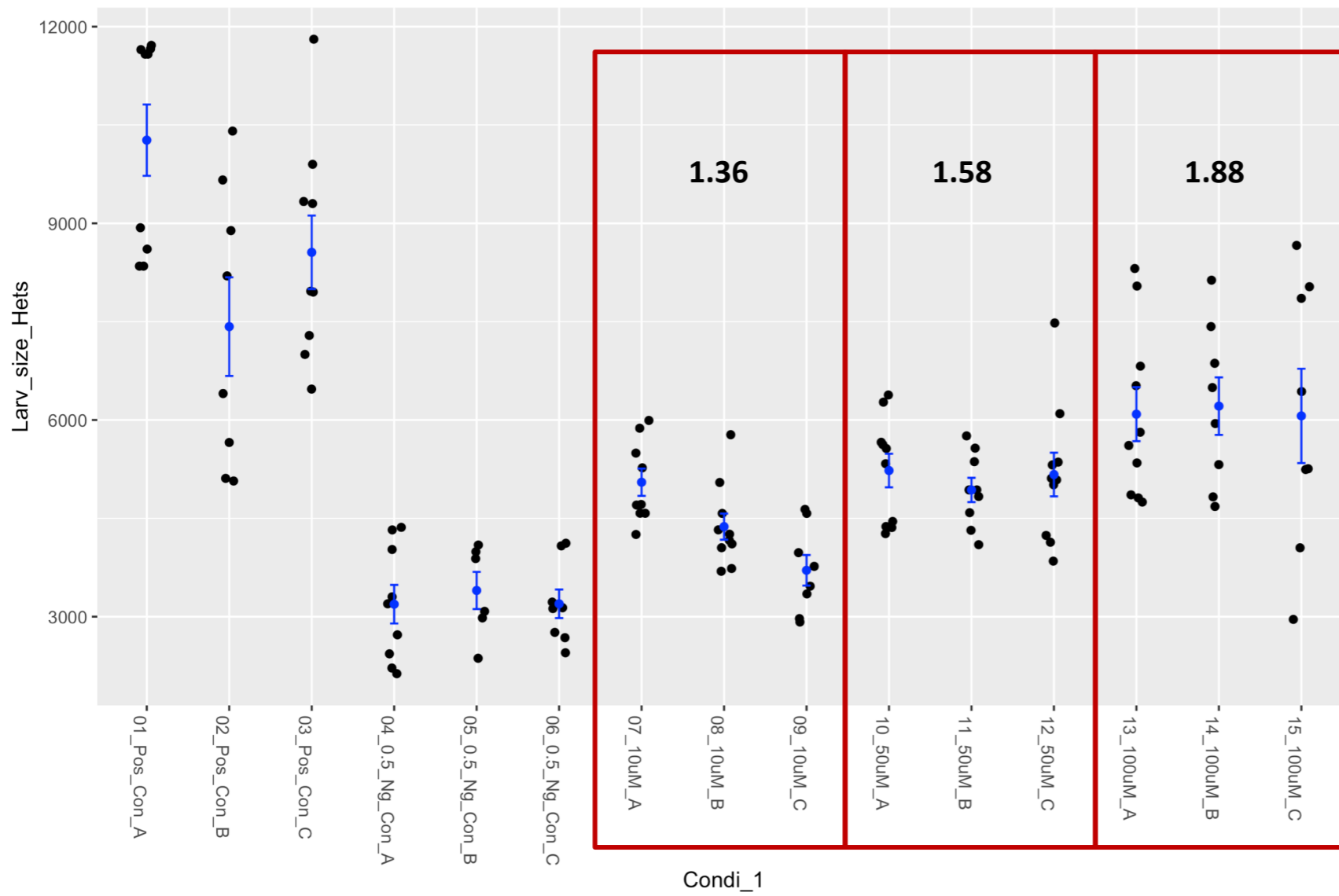
# Fly Chembridge retest – Supplier ID: 9251119



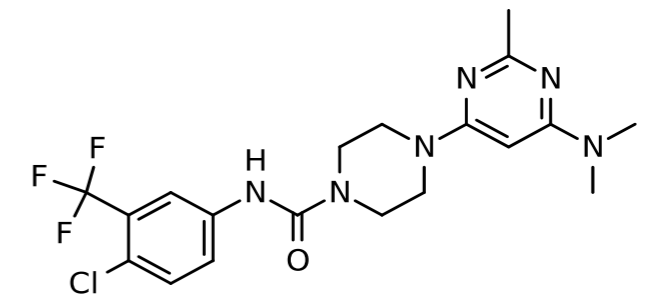
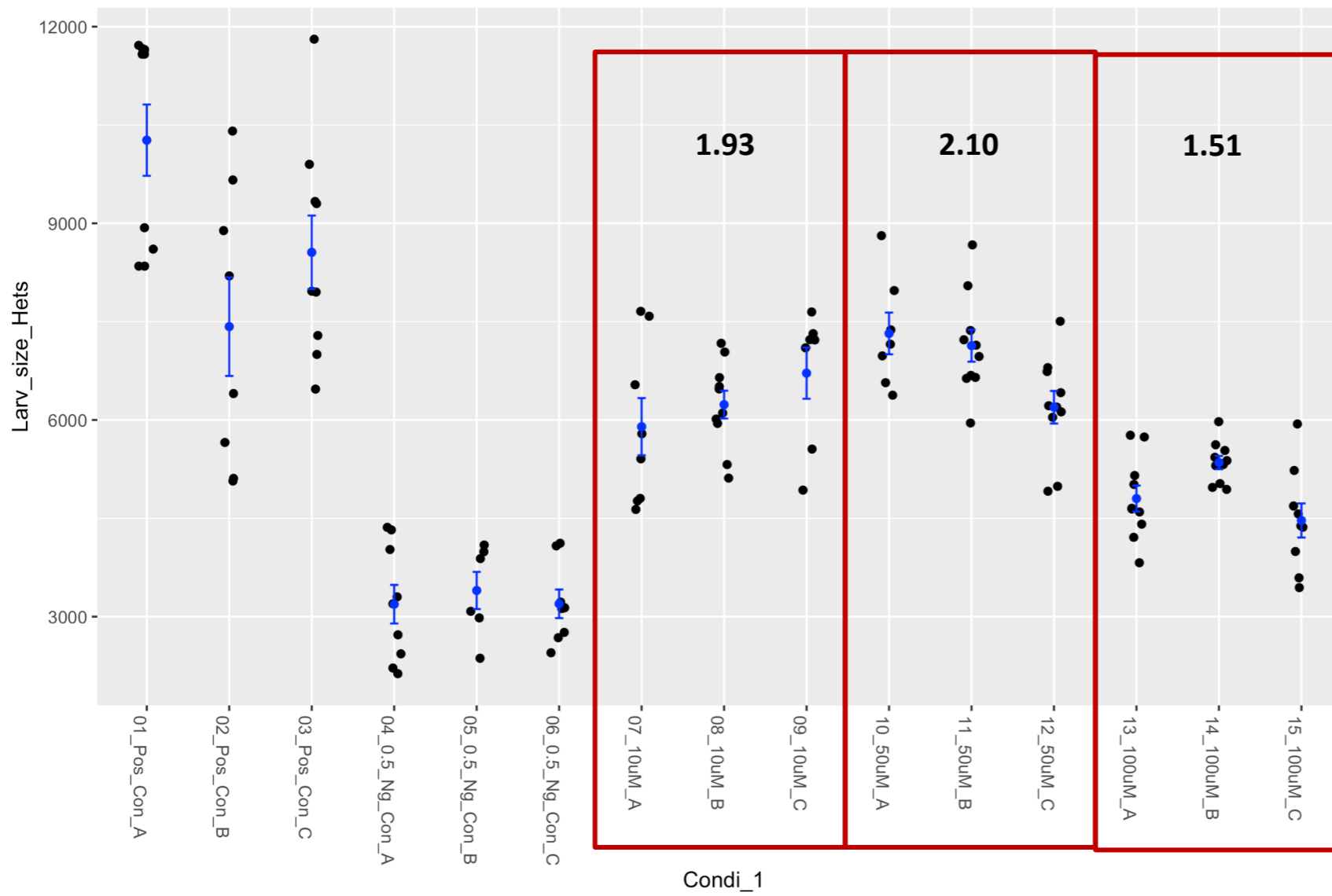
# Fly Chembridge retest – Supplier ID: 9251633



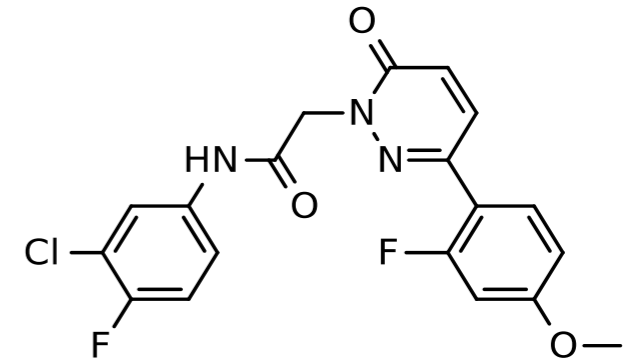
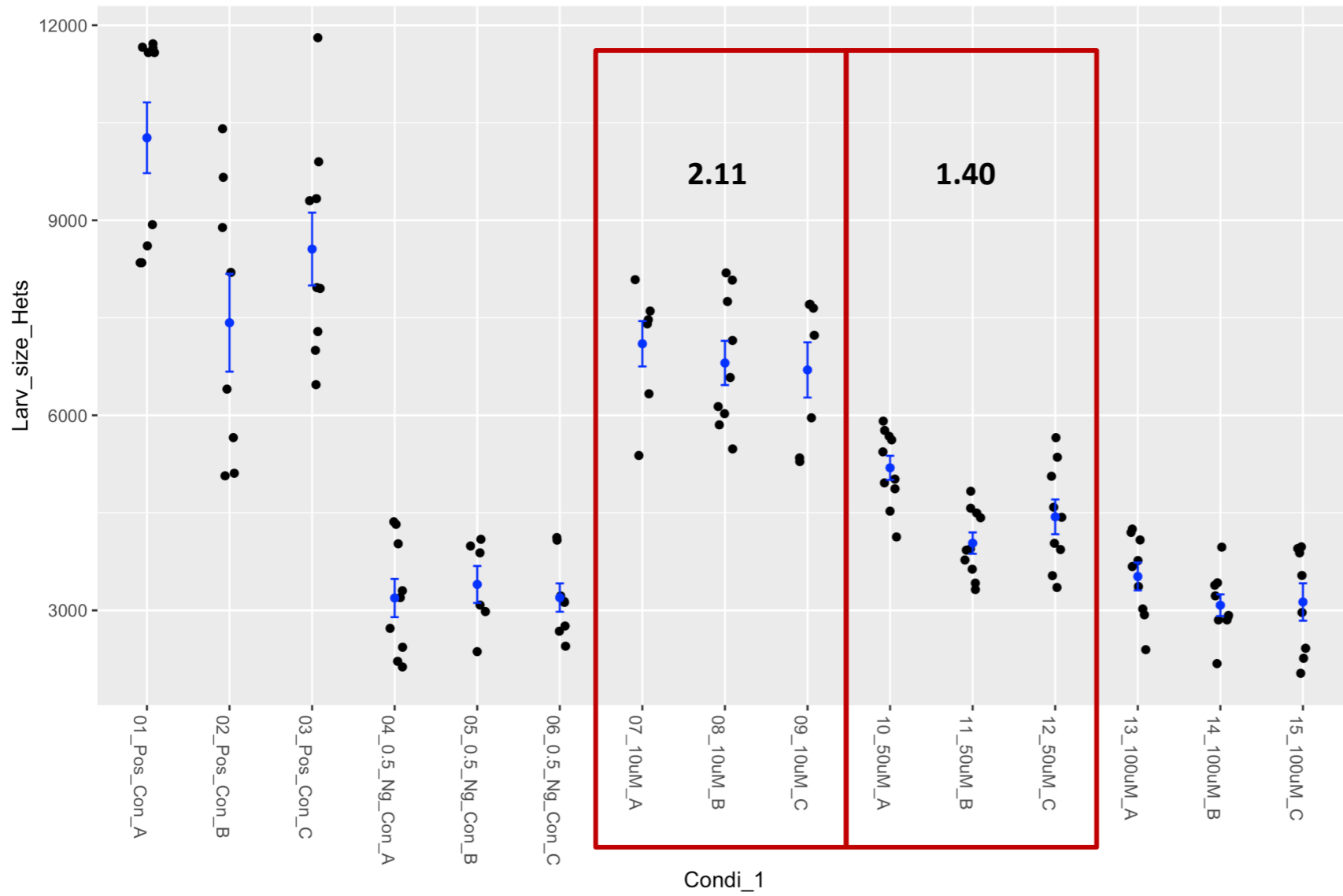
# Fly Chembridge retest – Supplier ID: 9262220



# Fly Chembridge retest – Supplier ID: 9262955



# Fly Chembridge retest – Supplier ID: 9290684

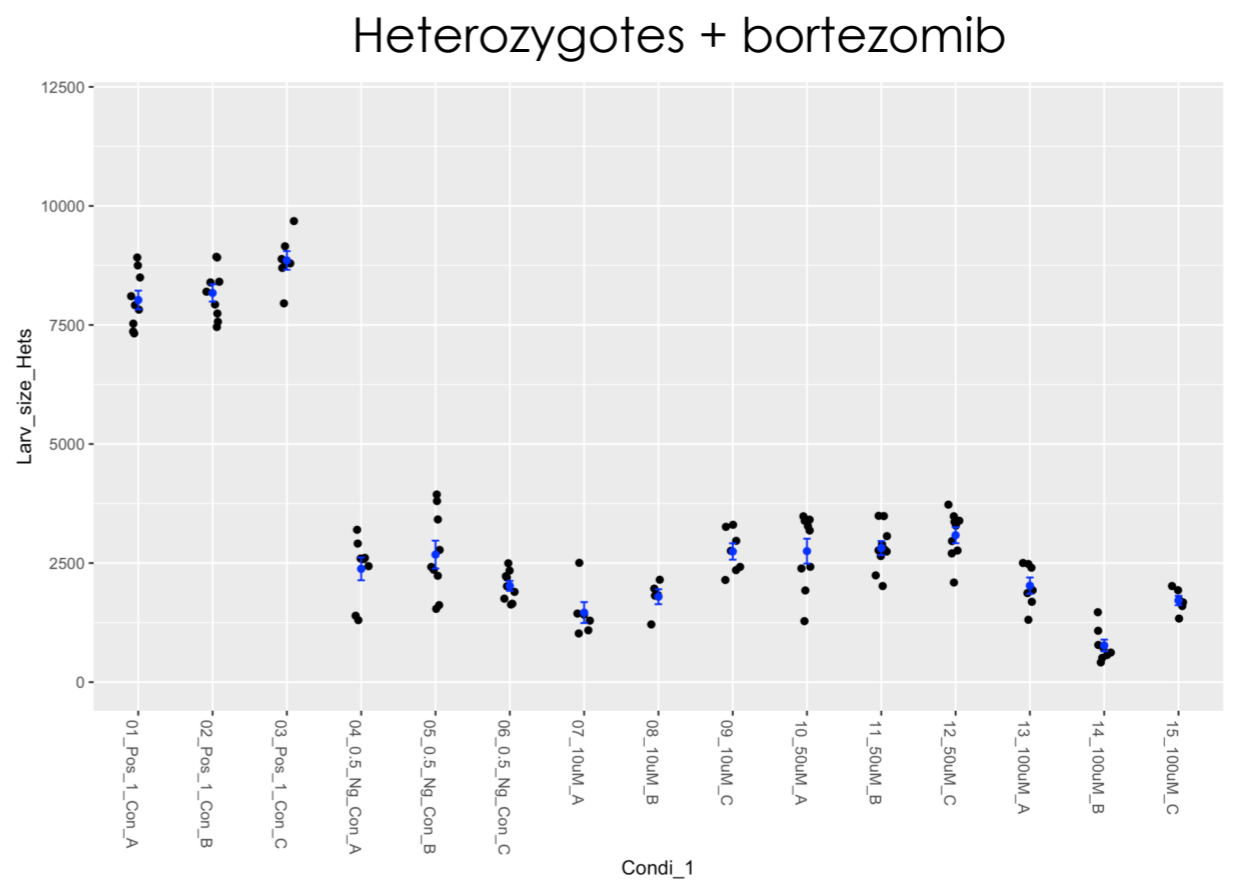
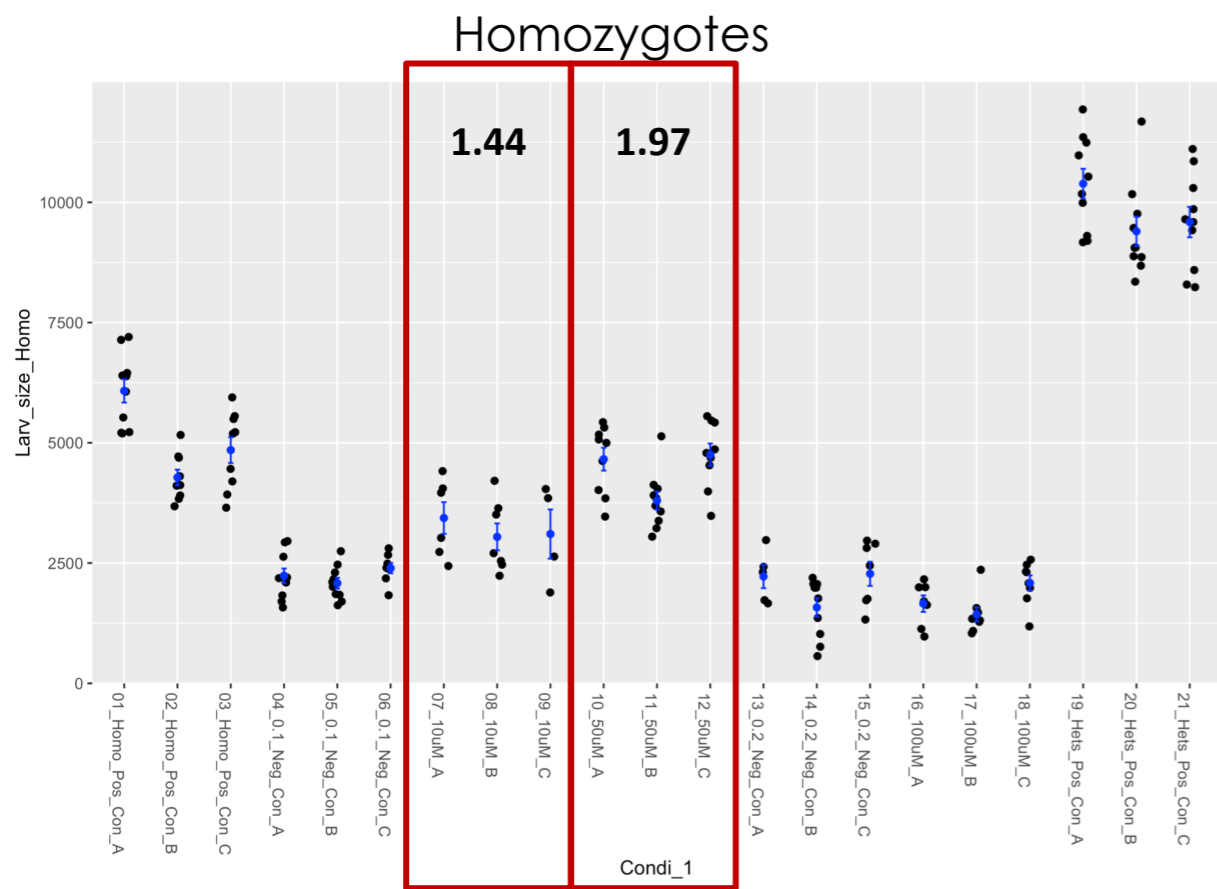




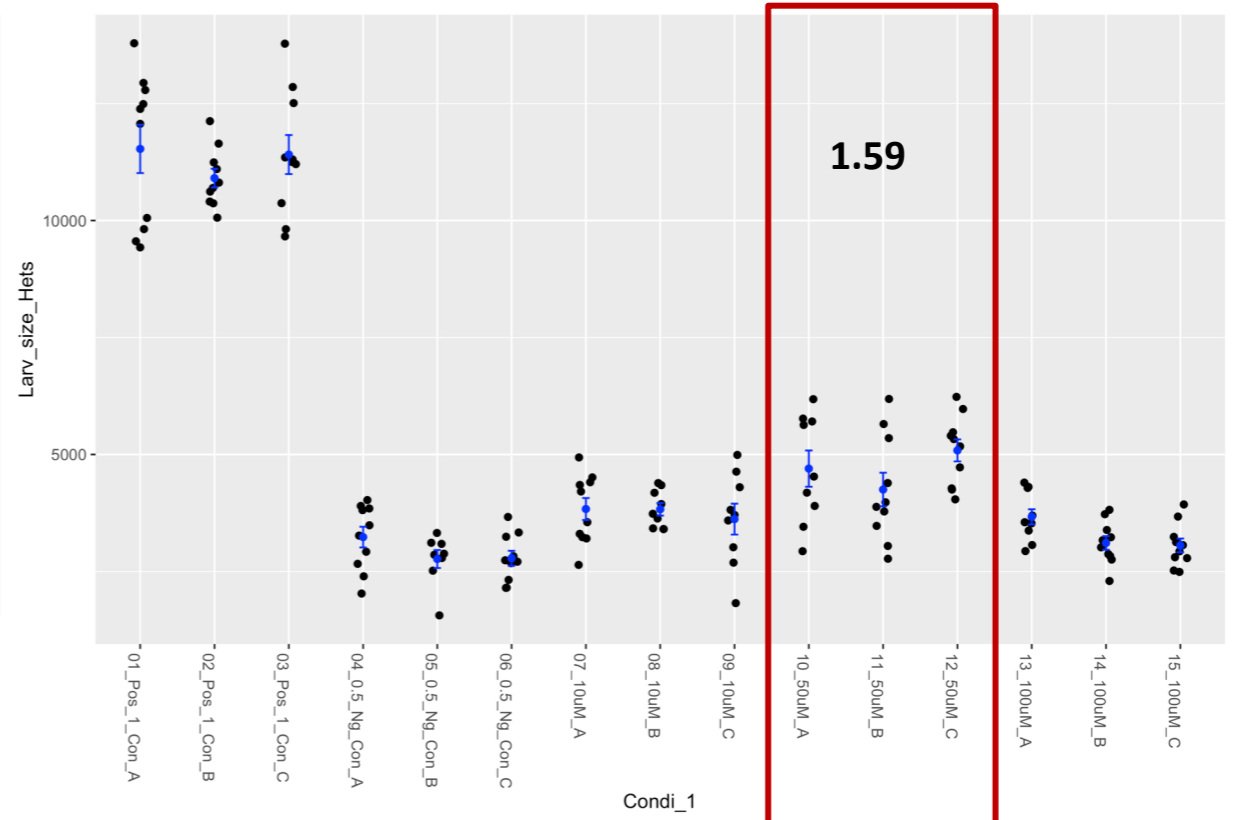
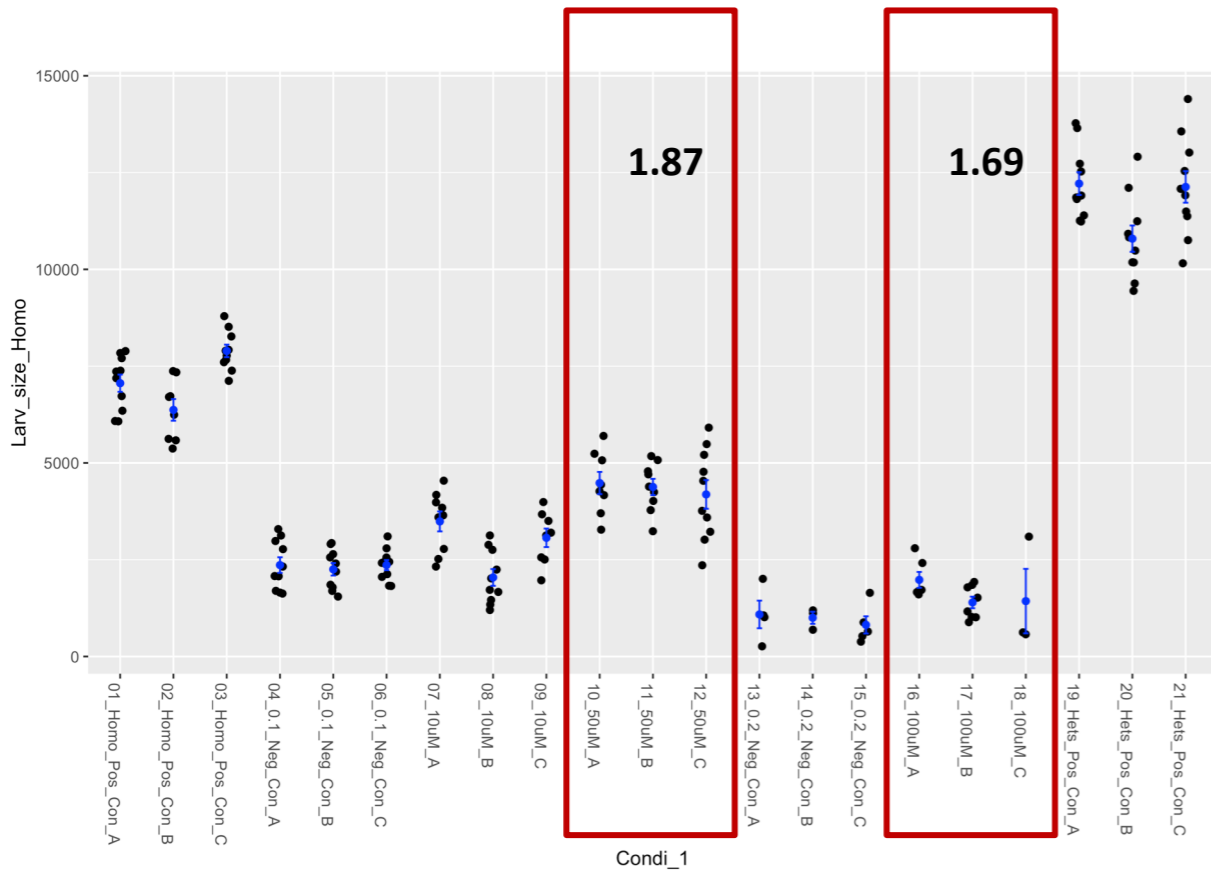
# Fly retest of Chembridge Worm hits

All compounds that scored as hits in the worm *png-1* screen were cross-validated in two fly assay paradigms: rescue of *Pngl*<sup>-/-</sup> homozygotes and rescue of bortezomib-treated *Pngl*<sup>+/-</sup> heterozygotes. Red boxes indicate compounds that successfully retested. The number indicates the size ratio of compound-treated fly larvae to untreated control homozygous fly larvae.

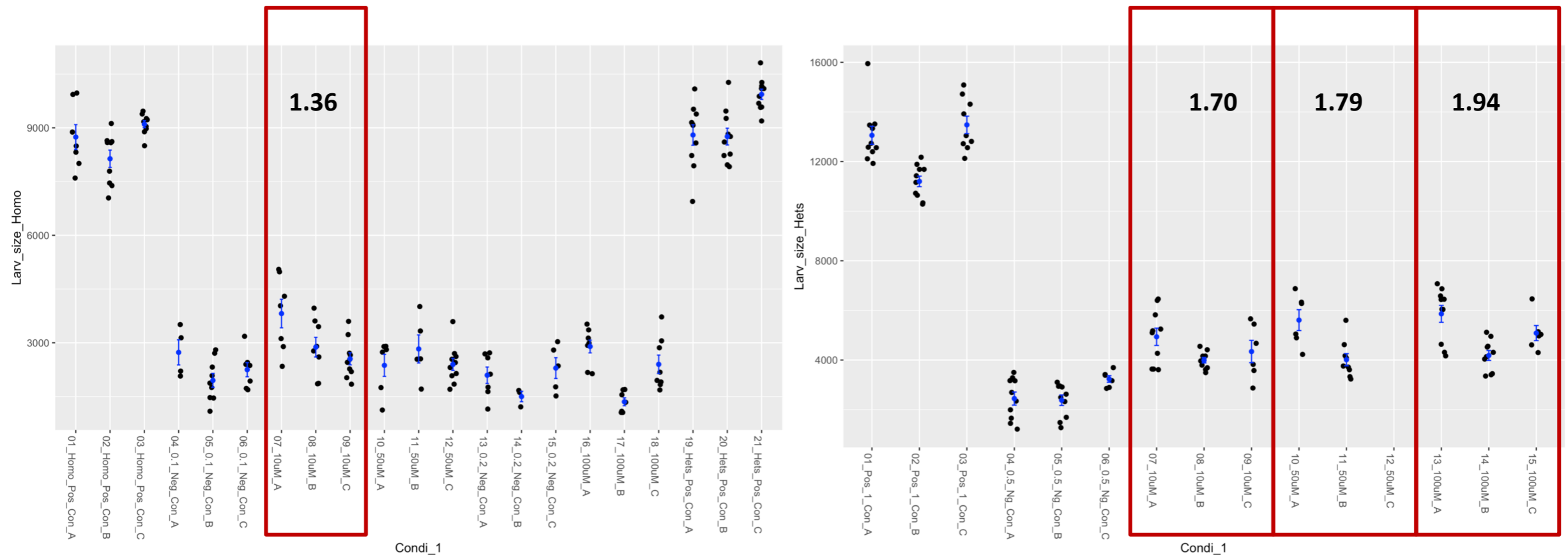
# Fly Chembridge retest – Supplier ID: 4141722



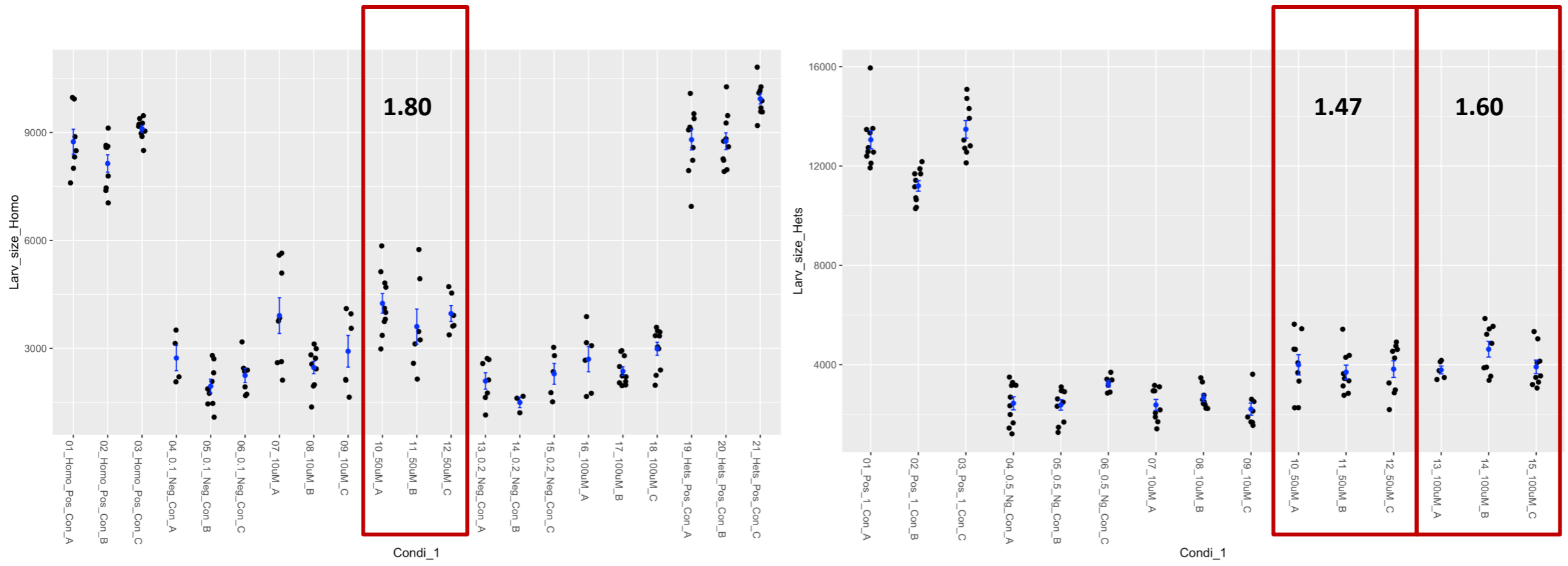
# Fly Chembridge retest – Supplier ID: 9223573



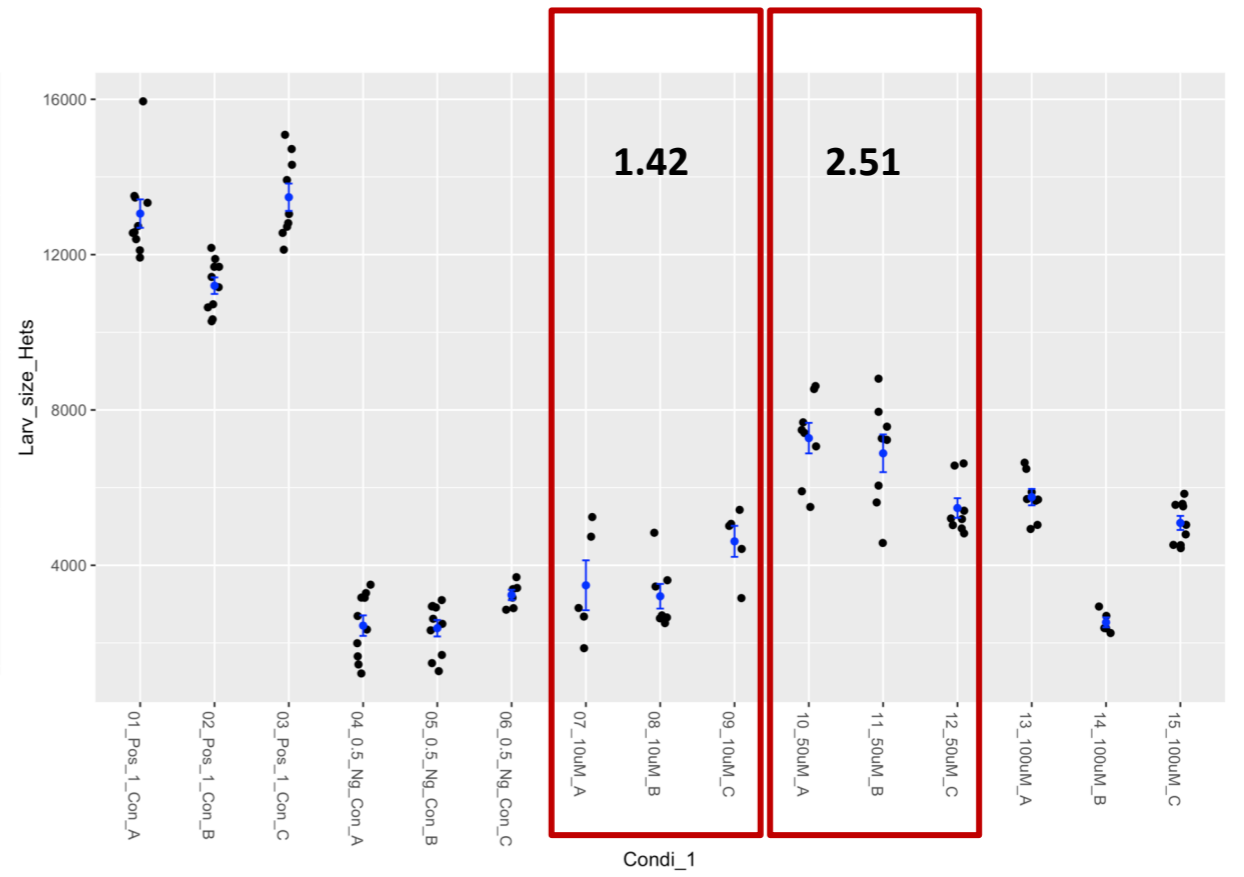
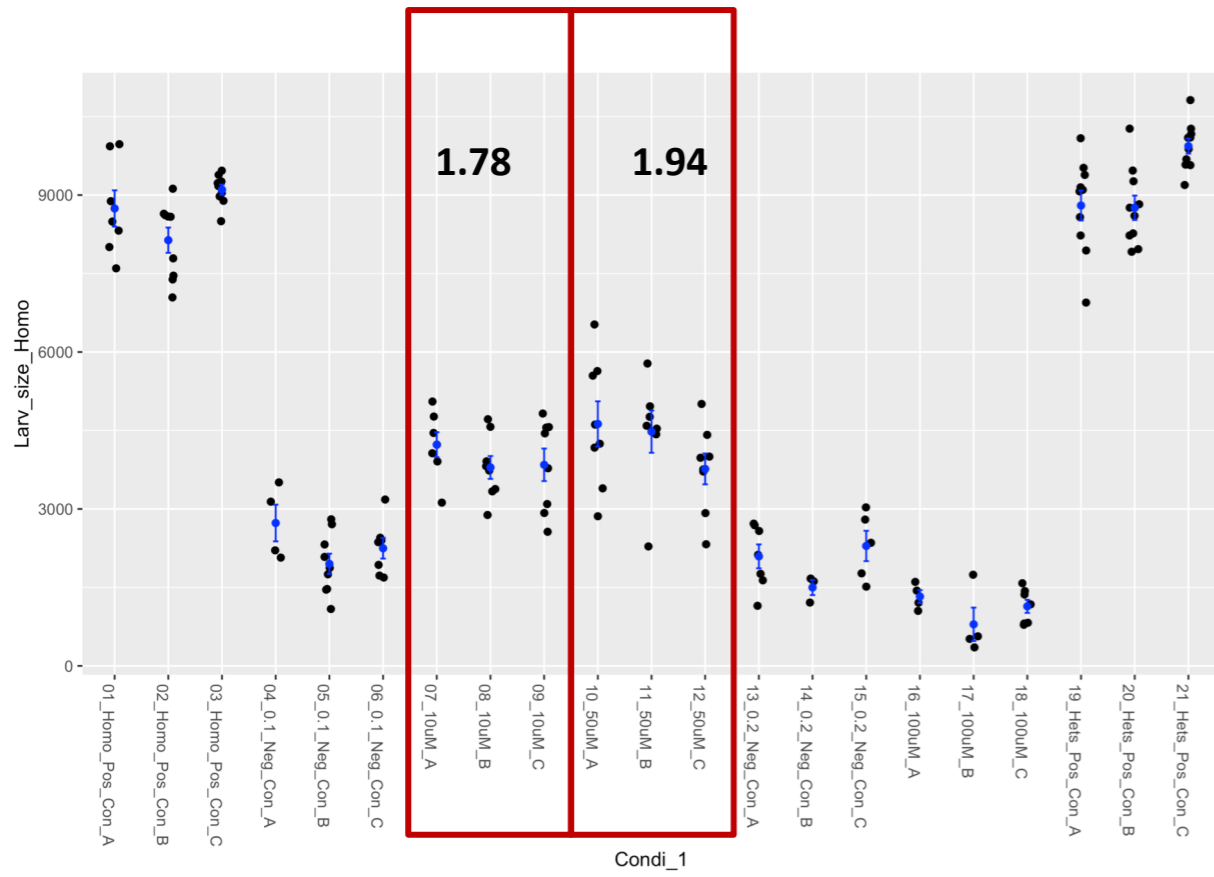
# Fly Chembridge retest – Supplier ID: 9268787



# Fly Chembridge retest – Supplier ID: 9283120



# Fly Chembridge retest – Supplier ID: 9313992

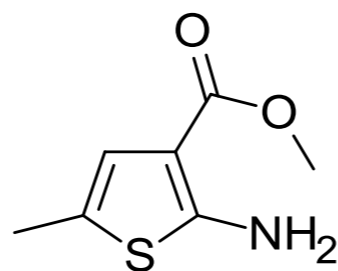


## Bortezomib-only

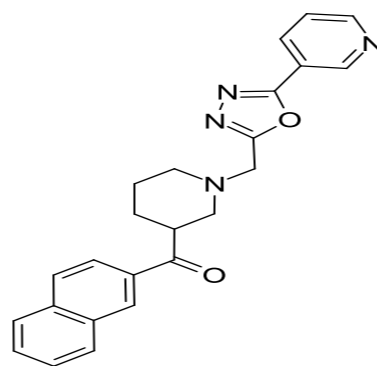
Supplier ID

Structure

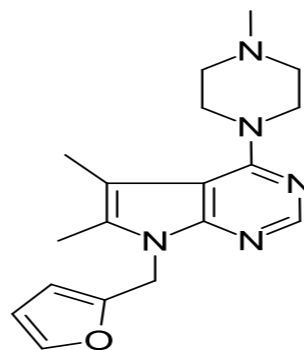
4141722



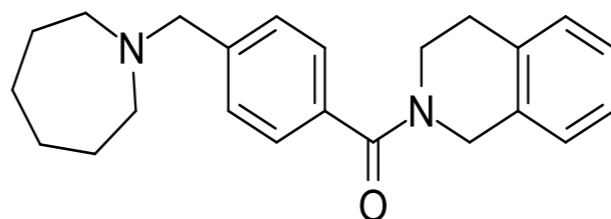
83371714



9193234



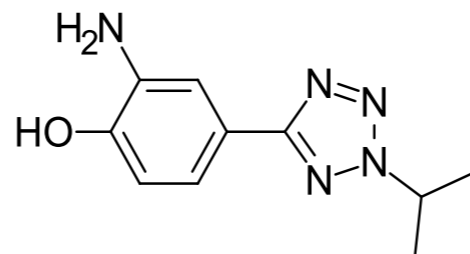
9223573



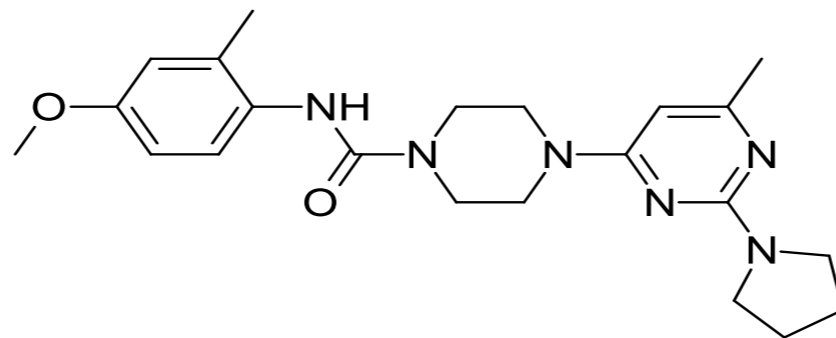
Chembridge hits from the worm *png-1* screen that only retested in the bortezomib-treated worm secondary assay, i.e., it failed to rescue carfilzomib-treated worms.

## Bortezomib-only

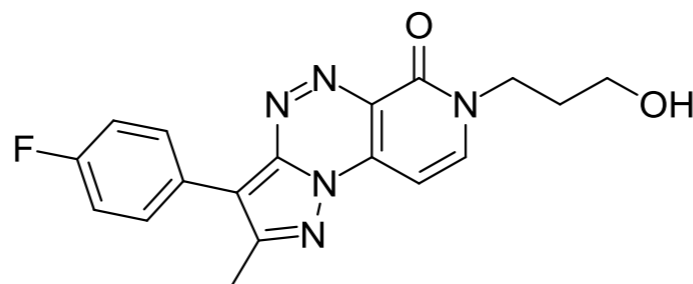
9243480



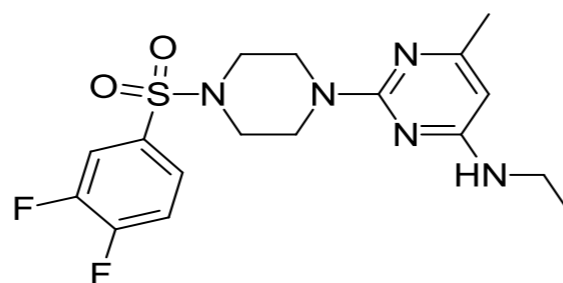
9253438



9258723



9266225

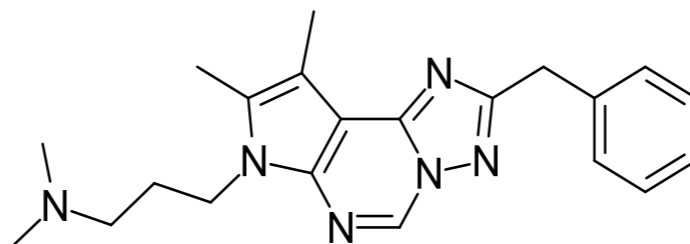


Chembridge hits from the worm *png-1* screen that only retested in the bortezomib-treated worm secondary assay, i.e., it failed to rescue carfilzomib-treated worms.

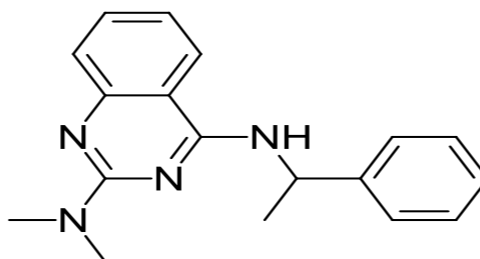


## Bortezomib-only

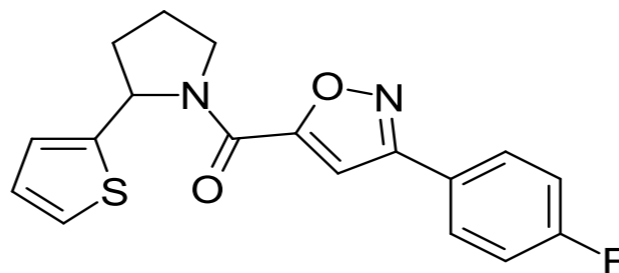
9266617



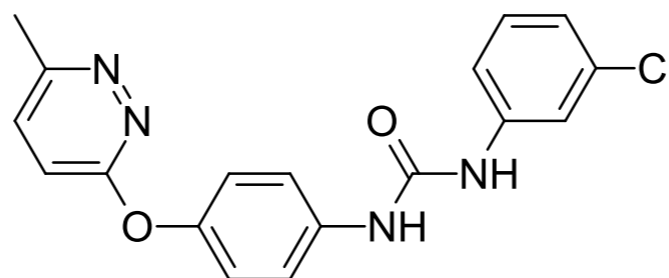
9268787



9283120



9313992



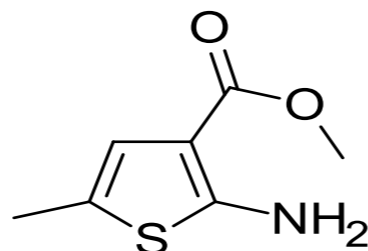
Chembridge hits from the worm *png-1* screen that only retested in the bortezomib-treated worm secondary assay, i.e., it failed to rescue carfilzomib-treated worms.

## Carfilzomib-only

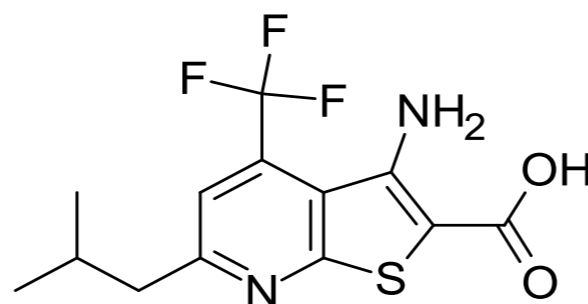
Supplier ID

Structure

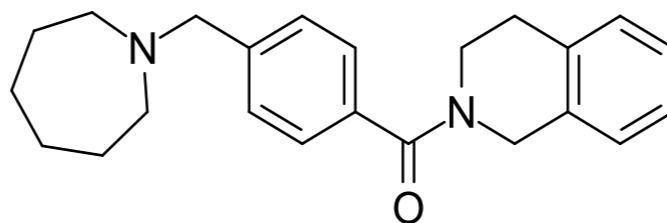
4141722



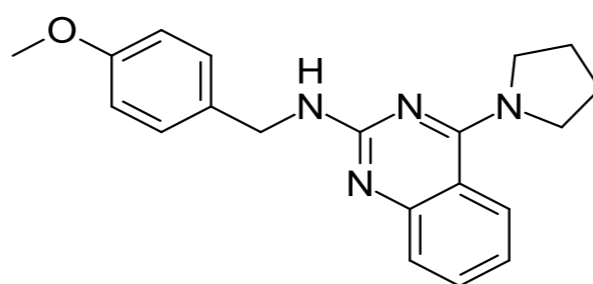
9223301



9223573



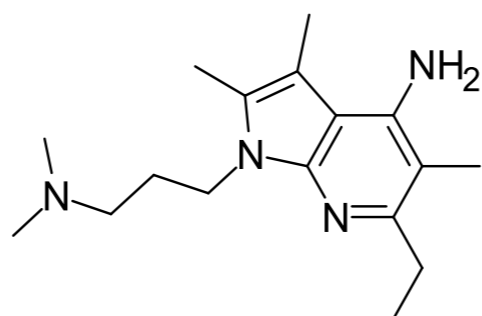
9236078



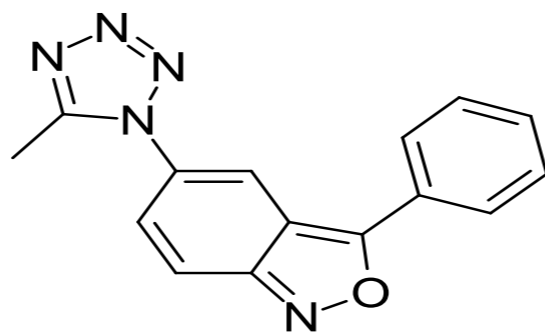
Chembridge hits from the worm *png-1* screen that only retested in the carfilzomib-treated worm secondary assay, i.e., failed to rescue in bortezomib-treated worms. These compounds are likely weak or borderline hits in bortezomib.

## Carfilzomib-only

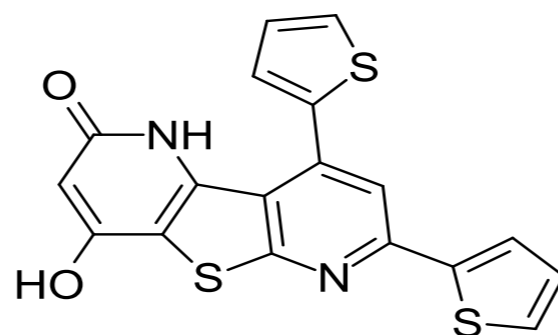
9256157



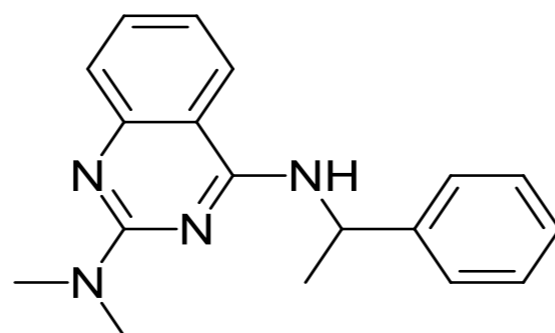
9257006



9260754



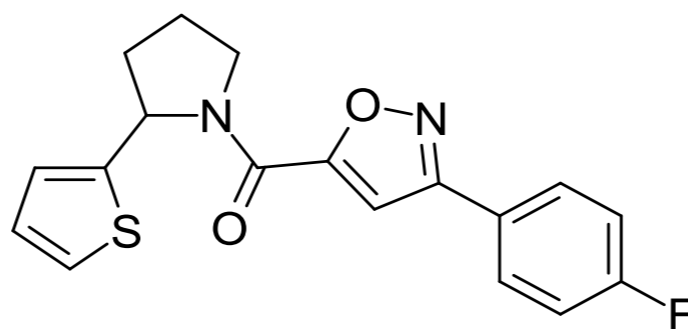
9268787



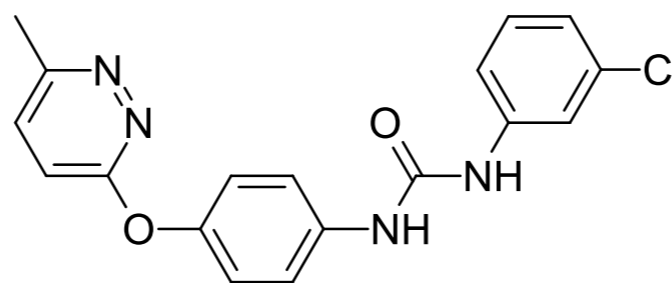
Chembridge hits from the worm *png-1* screen that only retested in the carfilzomib-treated worm secondary assay, i.e., failed to rescue in bortezomib-treated worms. These compounds are likely weak or borderline hits in bortezomib.

## Carfilzomib-only

9283120

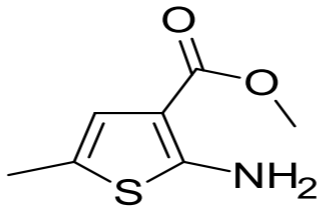
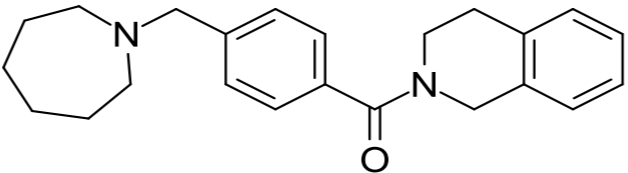
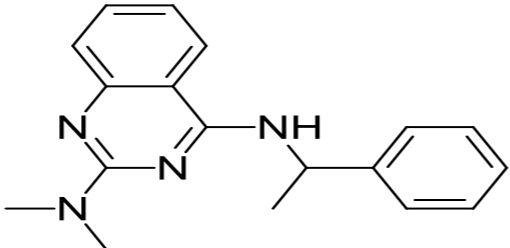
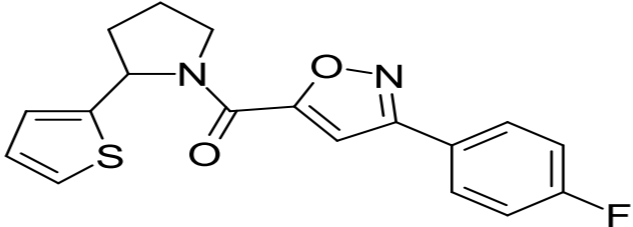
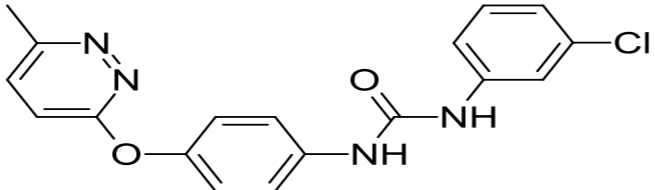


9313992



Chembridge hits from the worm *png-1* screen that only retested in the carfilzomib-treated worm secondary assay, i.e., failed to rescue in bortezomib-treated worms. These compounds are likely weak or borderline hits in bortezomib.

## Bortezomib and carfilzomib

Supplier ID	Structure
4141722	 <chem>Cc1cc(N)c(C(=O)OC)s1</chem>
9223573	 <chem>C1CCN(C1)Cc2ccc(cc2)C(=O)N3CCc4ccccc43</chem>
9268787	 <chem>CN(C)C1=NC=C(NC(=O)C)N1c2ccccc2</chem>
9283120	 <chem>CC(=O)Nc1cc2oc(C1=CC=C(C=C1)F)n2</chem>
9313992	 <chem>CC(=O)Nc1ccc(NC(=O)N)cc1</chem>

Chembridge hits from the worm *png-1* screen that retested in both bortezomib- and carfilzomib-treated worm secondary assays.