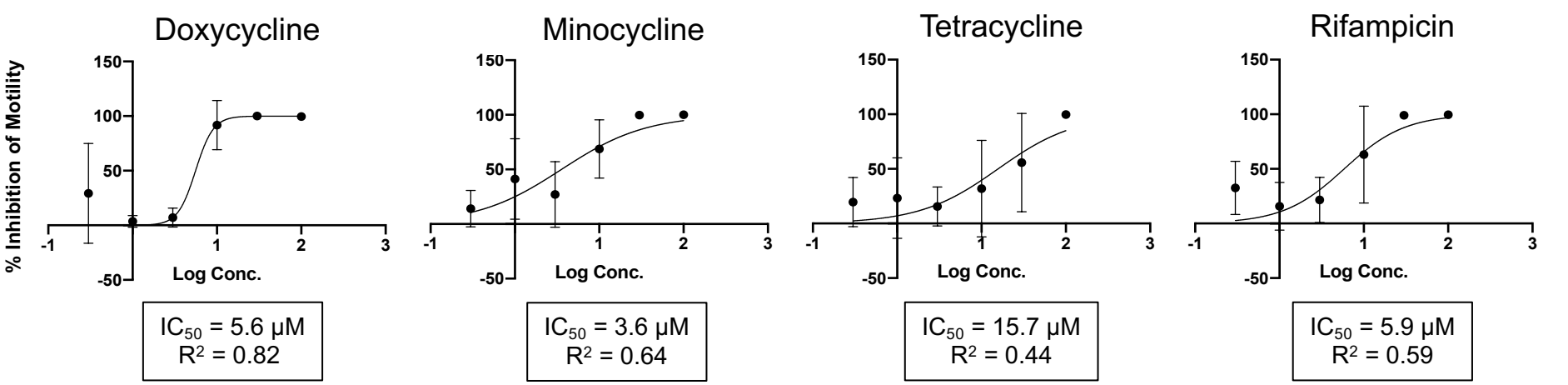


Figure S1: IC₅₀s of adult female and male worm motility on Day 6 of *in vitro* assays

FEMALES



MALES

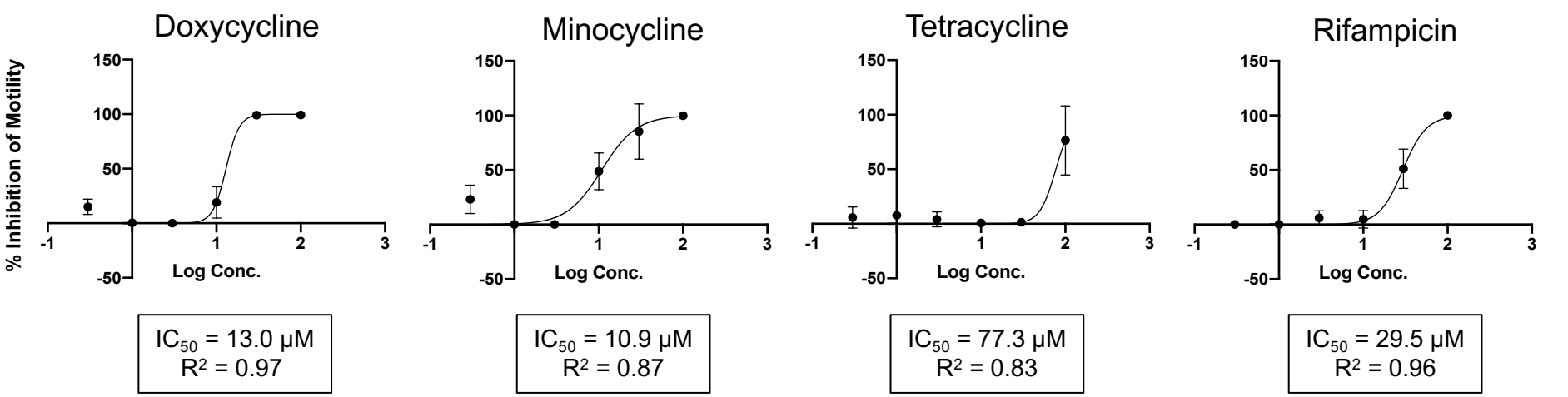


Fig. S1: Worm motility showed the expected relationship between antibiotic concentration and motility; i.e. higher antibiotic concentrations were associated with lower worm motility and lower antibiotic concentrations were associated with higher worm motility. Error bars show standard deviation of the mean.

Figure S2: Female IC₅₀s *wsp* and *gst* copy numbers

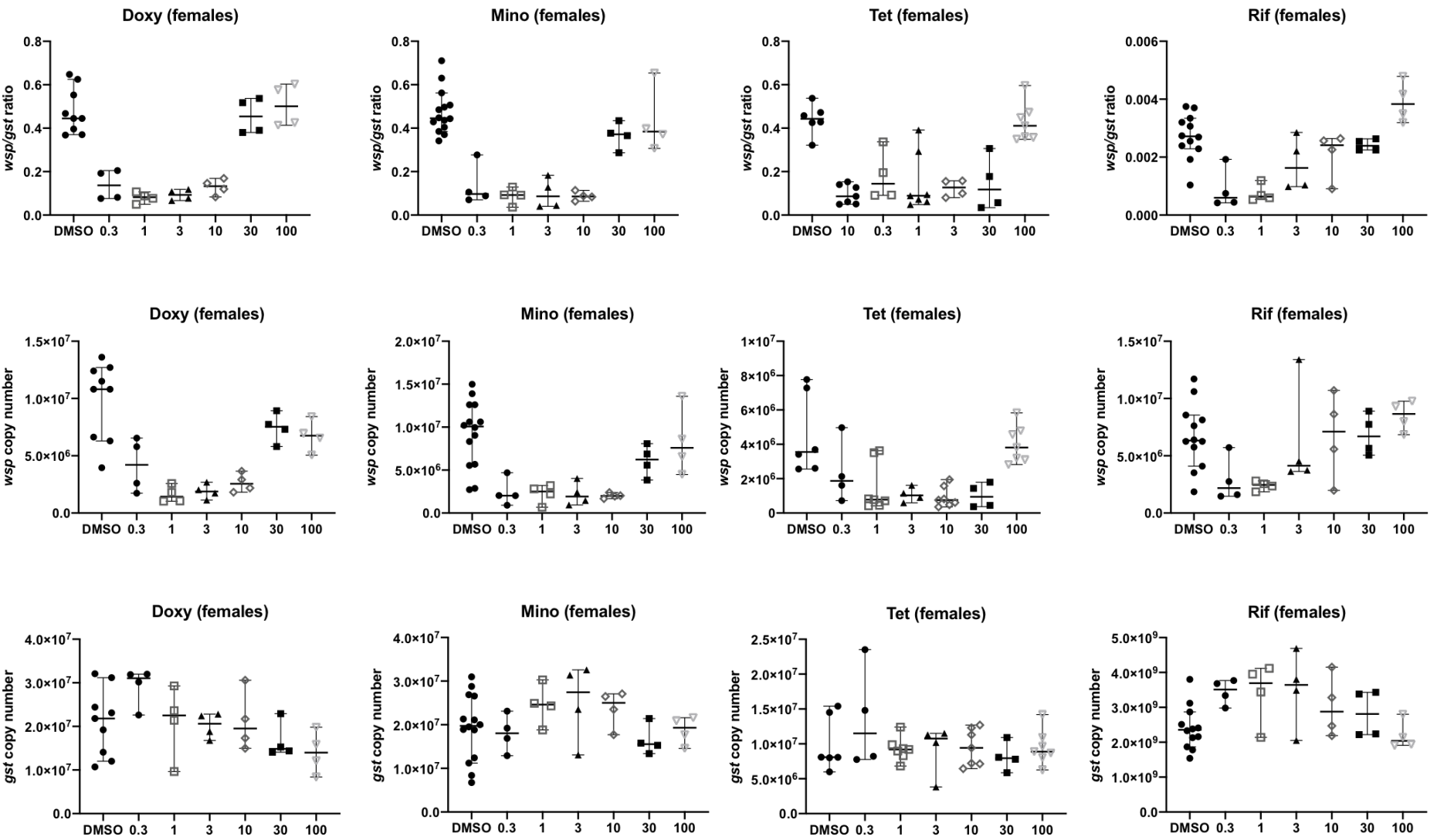


Figure S3: Male IC₅₀s *wsp* and *gst* copy numbers

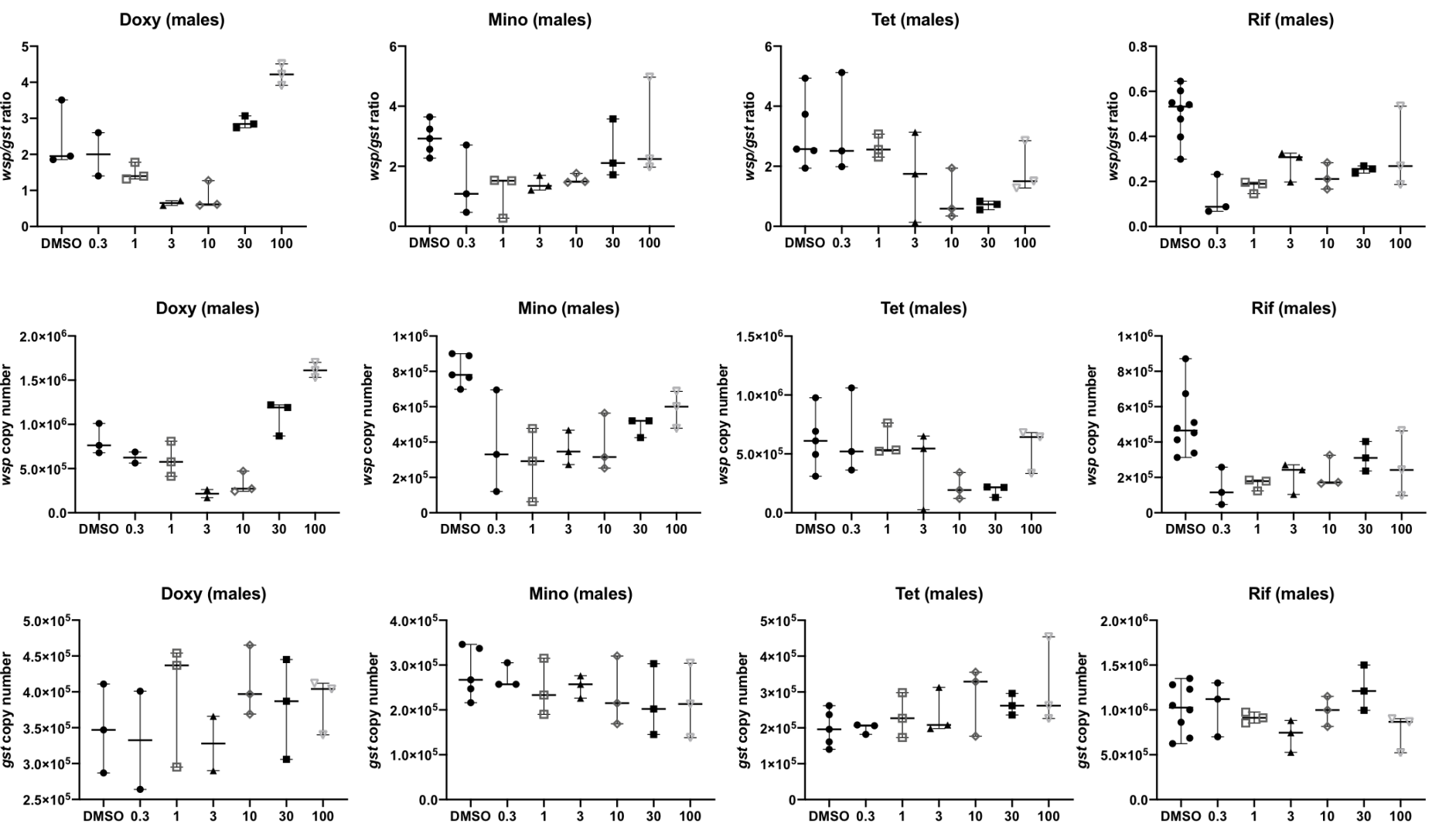


Figure S4: Female doxycycline time course *wsp* and *gst* copy numbers

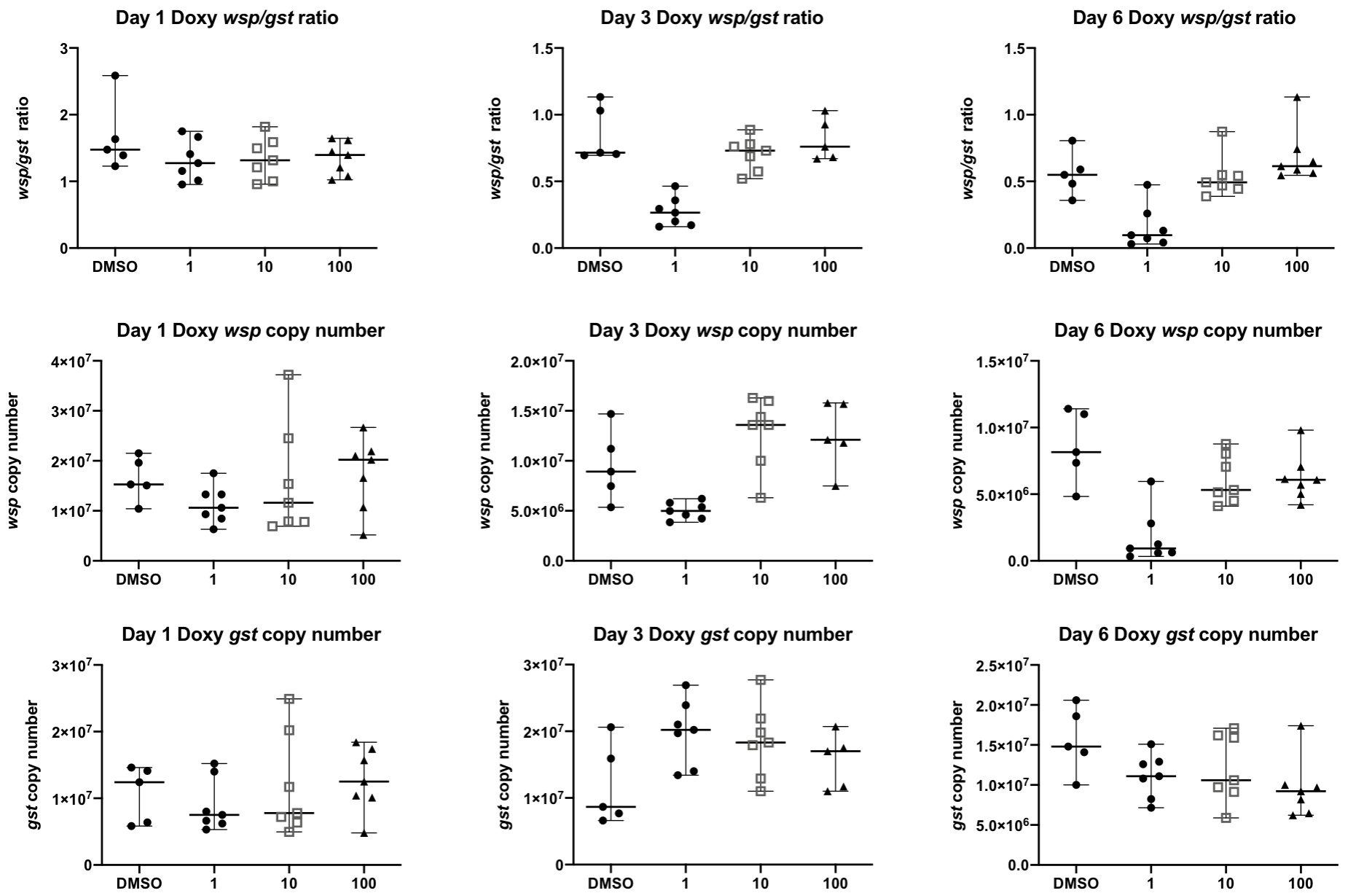
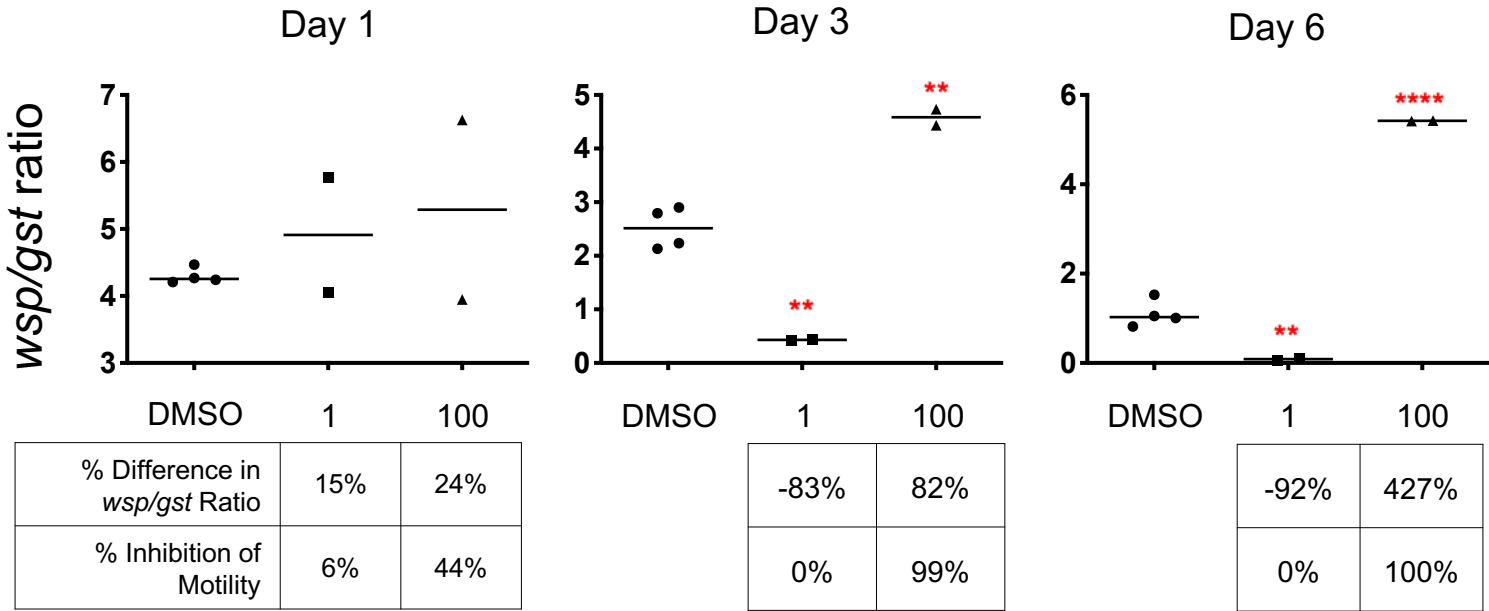


Figure S5: Male time course assay results

MALES
Doxycycline



Rifampicin

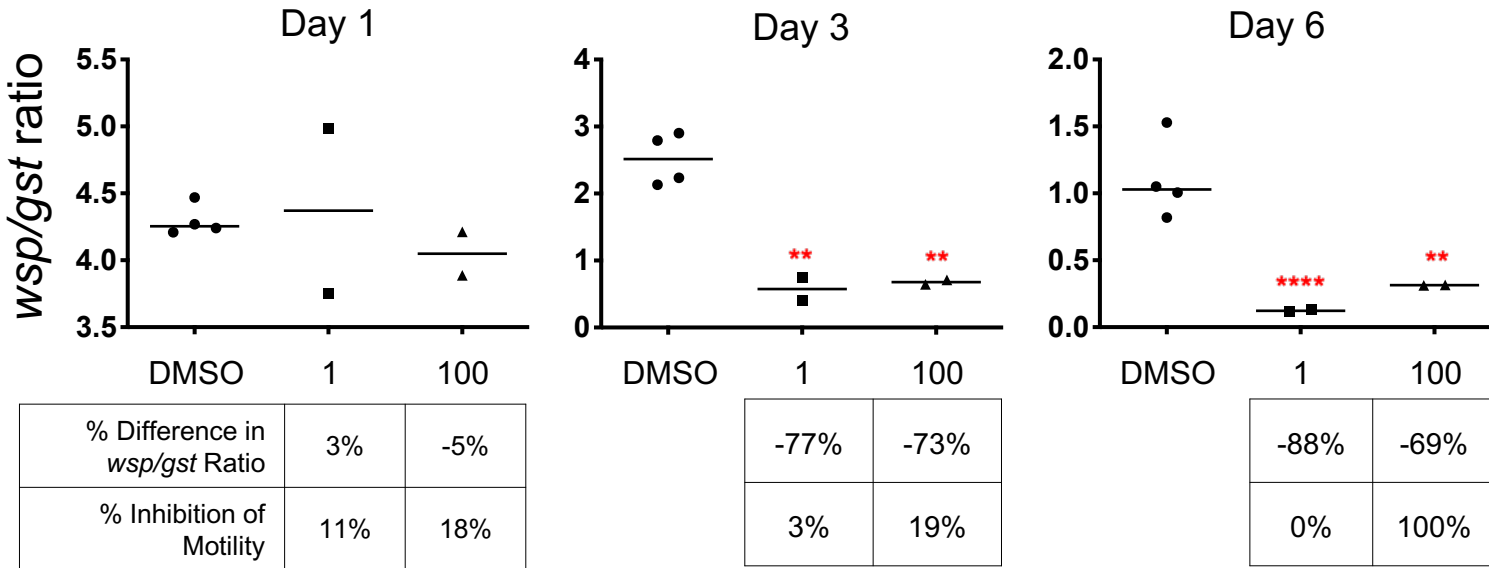


Fig. S5: Male time course assay results

A time course experiment was conducted to determine the effects of doxycycline, tetracycline and rifampicin on *B. pahangi* adult males and *Wolbachia* titers at low (1 μ M) and high (100 μ M) concentrations at three time points (Day 1, 3 and 6).

Wolbachia titer and motility of male worms treated with doxycycline and rifampicin followed a similar pattern as female worms. Low concentration doxycycline reduced *Wolbachia* titers but did not affect worm motility. High concentration doxycycline killed worms, but *Wolbachia* titers increased 4X by Day 6. Rifampicin reduced *Wolbachia* titers on Day 3 and Day 6 at both low and high concentrations, but worms were only killed with 100 μ M rifampicin on Day 6. *Wolbachia* titers were measured by *wsp/gst* ratio; medians are shown. X-axis labels show antibiotic concentration in μ M. The percentage difference in *wsp/gst* ratio as compared to DMSO controls is shown below each antibiotic concentration. Negative percentages signify a decrease in *Wolbachia* titers and positive percentages indicate that titers were higher than controls. The percent inhibition of motility is shown below each antibiotic concentration as well; 0% inhibition indicates that worms were as motile as controls and 100% inhibition indicates that the worms were fully immotile. Red asterisks indicate statistical significance of the difference between *wsp/gst* ratios in treated worms and DMSO controls. ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05. Statistical significance of inhibition of motility can be found in Supplemental Table S2.

Figure S6: Male doxycycline time course *wsp* and *gst* copy numbers

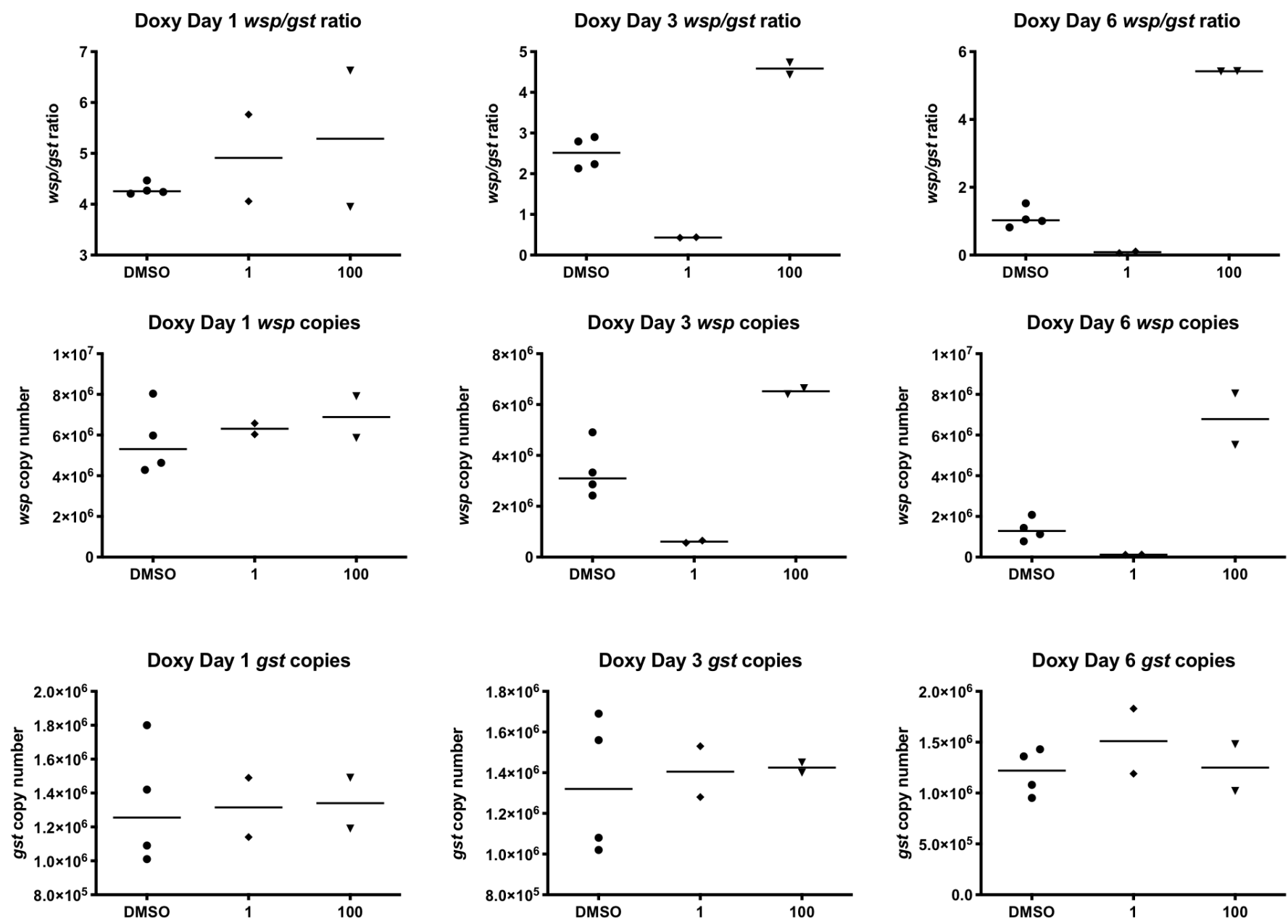


Figure S7: Female tetracycline time course *wsp* and *gst* copy numbers

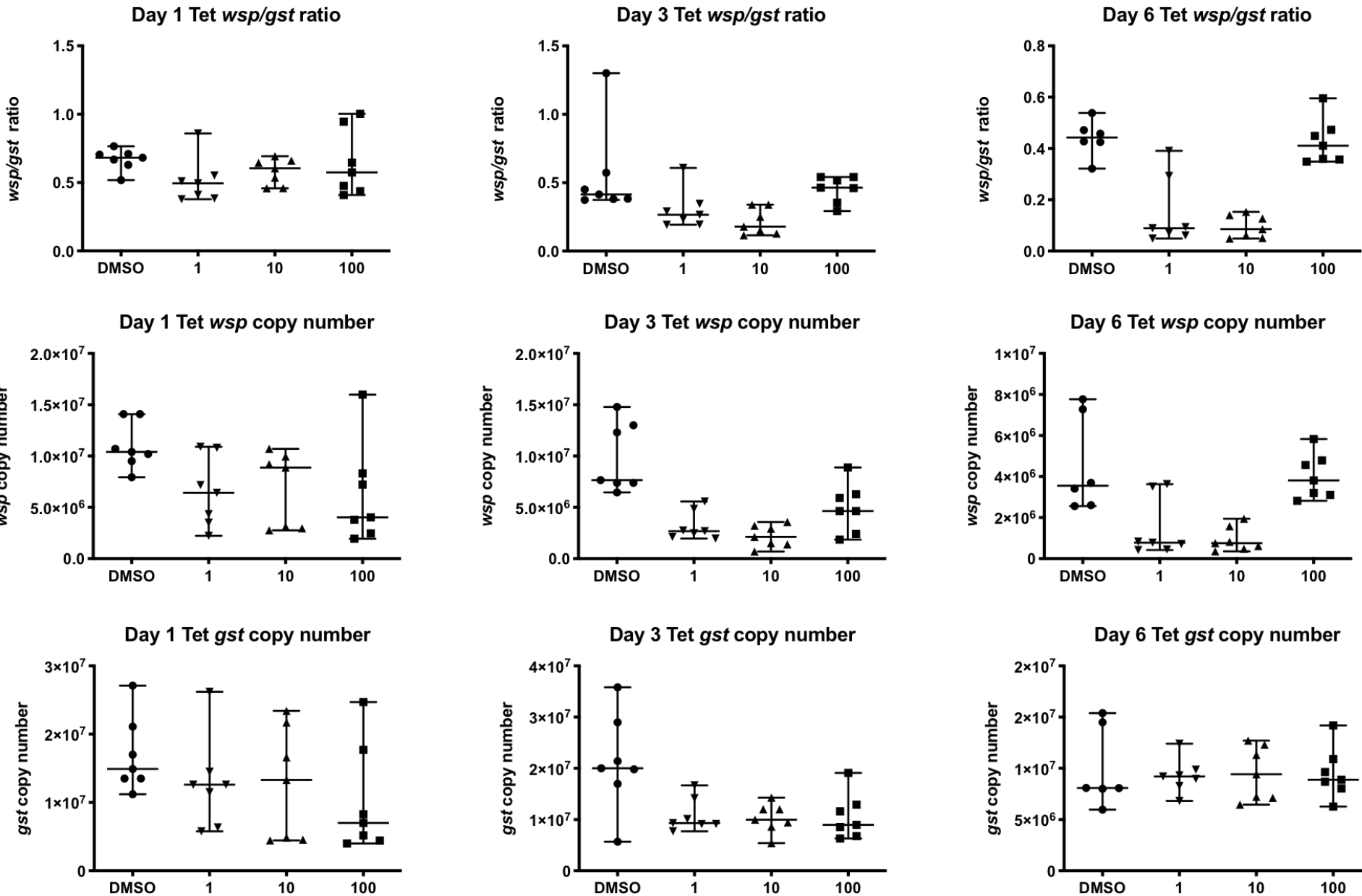


Figure S8: Female rifampicin time course *wsp* and *gst* copy numbers

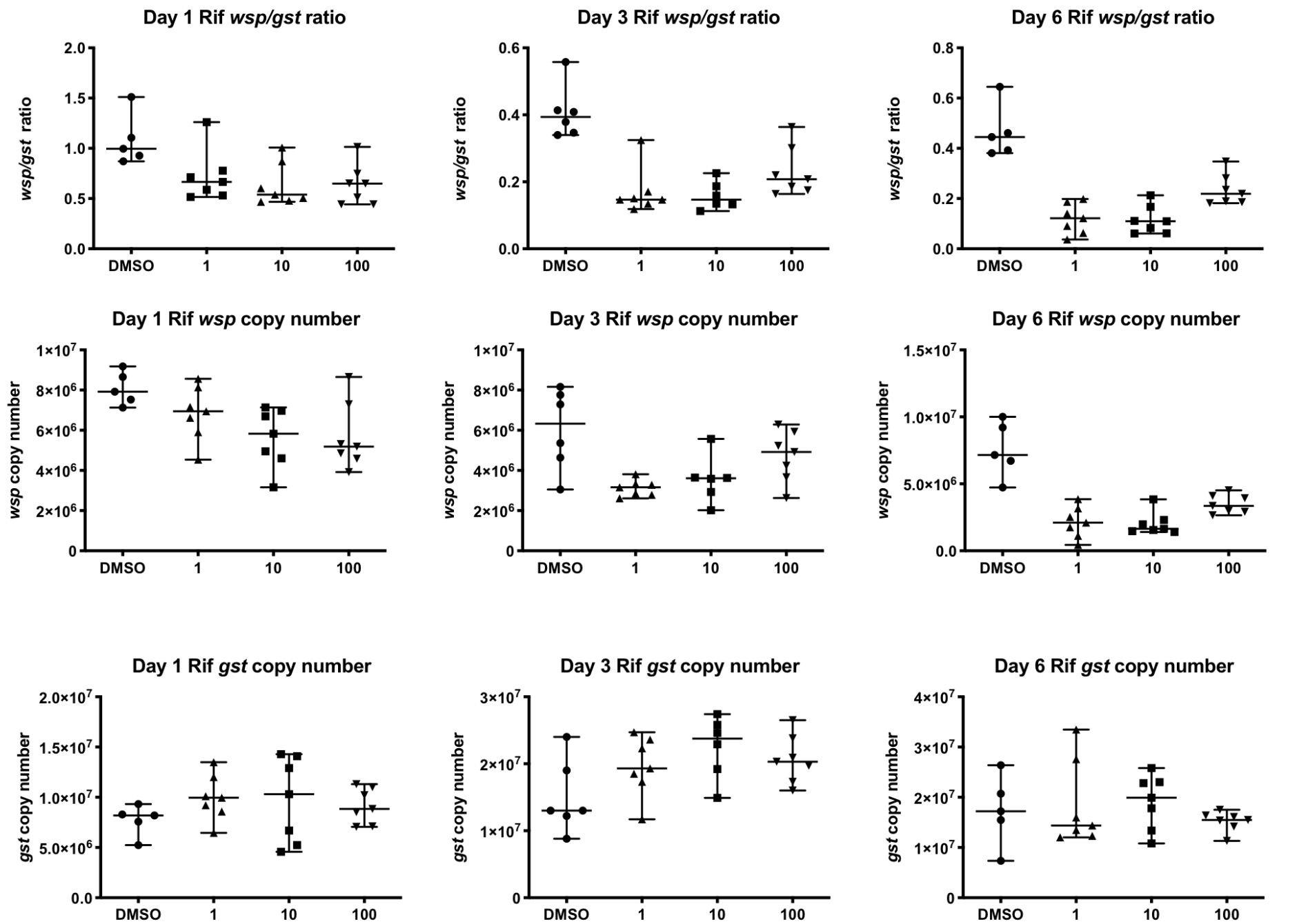
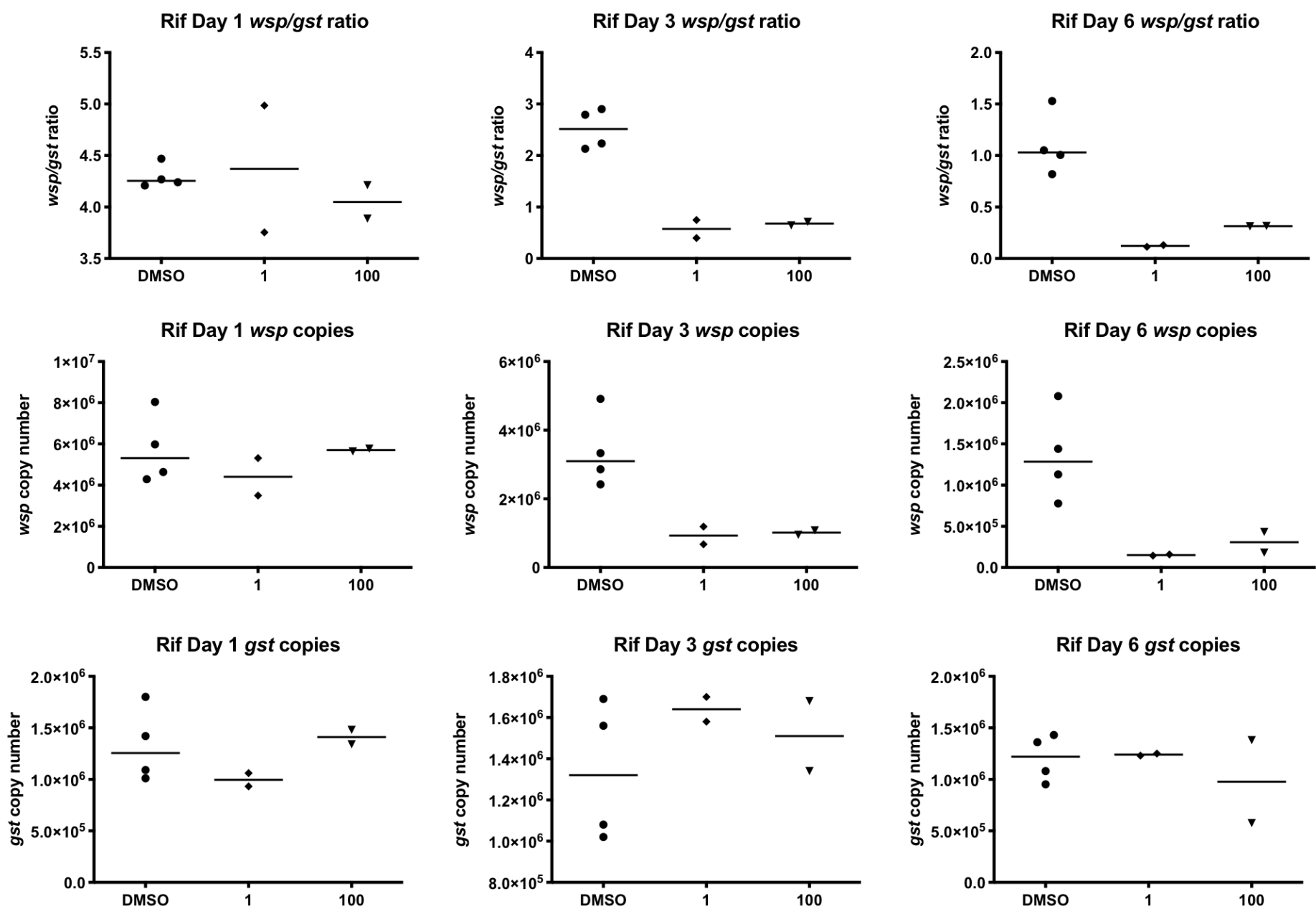


Figure S9: Male rifampicin time course *wsp* and *gst* copy numbers



SUPPLEMENTARY TABLE S1

FEMALES

Conc (μM)	Doxycycline			Minocycline			Tetracycline			Rifampicin		
	% diff in <i>wsp/gst</i>	Significance	Motility IC ₅₀	% diff in <i>wsp/gst</i>	Significance	Motility IC ₅₀	% diff in <i>wsp/gst</i>	Significance	Motility IC ₅₀	% diff in <i>wsp/gst</i>	Significance	Motility IC ₅₀
100	13%	ns	5.6 μM	-14%	ns	3.6 μM	-7%	ns	15.7 μM	41%	ns	5.9 μM
30	2%	ns		-17%	ns		-74%	*		-12%	ns	
10	-70%	ns		-81%	**		-81%	**		-11%	ns	
3	-79%	*		-81%	**		-71%	ns		-40%	ns	
1	-81%	**		-79%	**		-80%	*		-76%	*	
0.3	-69%	ns		-78%	**		-67%	ns		-78%	*	

MALES

Conc (μM)	Doxycycline			Minocycline			Tetracycline			Rifampicin		
	% diff in <i>wsp/gst</i>	Significance	Motility IC ₅₀	% diff in <i>wsp/gst</i>	Significance	Motility IC ₅₀	% diff in <i>wsp/gst</i>	Significance	Motility IC ₅₀	% diff in <i>wsp/gst</i>	Significance	Motility IC ₅₀
100	116%	ns	13.0 μM	-23%	ns	10.9 μM	-42%	ns	77.3 μM	-50%	ns	29.5 μM
30	46%	ns		-28%	ns		-72%	ns		-52%	ns	
10	-69%	ns		-49%	ns		-77%	ns		-60%	ns	
3	-67%	ns		-54%	ns		-32%	ns		-42%	ns	
1	-28%	ns		-48%	ns		-1%	ns		-64%	**	
0.3	3%	ns		-63%	ns		-2%	ns		-83%	**	

Table S1: Statistical significance of changes in *wsp/gst* ratios in IC₅₀ assays

Table S1 shows the percent difference in *wsp/gst* ratios between antibiotic treated worms and DMSO control worms, along with their statistical significance. Negative percentage differences indicate a decrease in *Wolbachia* titers; positive percentages indicate higher *Wolbachia* titers than controls. ns = not significant (P > 0.05), * P < 0.05; ** P < 0.01. Red lines demarcate the concentrations between which the IC₅₀ falls.

SUPPLEMENTARY TABLE S2 FEMALES

	Doxycycline [1 µM]			Doxycycline [10 µM]			Doxycycline [100 µM]		
	Day 1	Day 3	Day 6	Day 1	Day 3	Day 6	Day 1	Day 3	Day 6
% difference in <i>wsp/gst</i> ratios	-14%	-63%	-82%	-11%	2%	-10%	-5%	6%	12%
Significance	ns	p < 0.0001	p < 0.001	ns	ns	ns	ns	ns	ns
% difference in motility	0%	0%	-6%	0%	-96%	-99%	-90%	-99%	-99%
Significance	ns	ns	ns	ns	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001

	Tetracycline [1 µM]			Tetracycline [10 µM]			Tetracycline [100 µM]		
	Day 1	Day 3	Day 6	Day 1	Day 3	Day 6	Day 1	Day 3	Day 6
% difference in <i>wsp/gst</i> ratios	-28%	-36%	-80%	-11%	-57%	-81%	-16%	12%	-7%
Significance	ns	p < 0.05	p < 0.05	ns	p < 0.001	p < 0.01	ns	ns	ns
% difference in motility	0%	0%	-2%	0%	0%	-12%	-8%	-57%	-100%
Significance	ns	ns	ns	ns	ns	ns	ns	p < 0.0001	p < 0.0001

	Rifampicin [1 µM]			Rifampicin [10 µM]			Rifampicin [100 µM]		
	Day 1	Day 3	Day 6	Day 1	Day 3	Day 6	Day 1	Day 3	Day 6
% difference in <i>wsp/gst</i> ratios	-33%	-63%	-73%	-46%	-63%	-75%	-35%	-47%	-51%
Significance	ns	p < 0.0001	p < 0.0001	p < 0.01	p < 0.0001	p < 0.0001	p < 0.01	p < 0.01	p < 0.001
% difference in motility	0%	0%	0%	0%	0%	0%	0%	-31%	-100%
Significance	ns	ns	ns	ns	ns	ns	ns	p < 0.0001	p < 0.0001

MALES

	Doxycycline [1 µM]			Doxycycline [100 µM]		
	Day 1	Day 3	Day 6	Day 1	Day 3	Day 6
% difference in <i>wsp/gst</i> ratios	15%	-83%	-92%	24%	82%	427%
Significance	ns	p < 0.01	p < 0.01	ns	p < 0.01	p < 0.0001
% difference in motility	-6%	0%	0%	-44%	-99%	-100%
Significance	ns	ns	ns	p < 0.0001	p < 0.0001	p < 0.0001

	Rifampicin [1 µM]			Rifampicin [100 µM]		
	Day 1	Day 3	Day 6	Day 1	Day 3	Day 6
% difference in <i>wsp/gst</i> ratios	3%	-77%	-88%	-5%	-73%	-69%
Significance	ns	p < 0.01	p = 0.0001	ns	p < 0.01	p < 0.01
% difference in motility	-11%	-3%	0%	-18%	-19%	-100%
Significance	ns	ns	ns	p < 0.01	p < 0.05	p < 0.0001

Table S2: Statistical significance of changes in *wsp/gst* ratios in time course assays

Table S2 shows the percent difference in *wsp/gst* ratios between antibiotic treated worms and DMSO control worms and the percent decrease in motility, along with the statistical significance. Statistically significant differences are colored green.