

# Analysis of Birth Ratios for Muscle TSC1/Raptor Flies – Second Crosses

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## Experimental Design

This was for the second batch of *C179-Gal4* + *Raptor/Tsc1* knockdown crosses.

These data are stored in `/Volumes/bridges_lab/Hatfield/Drosophila/Births`, with the raw data saved in `../Data/Birth Data.csv` for the births and `../Data/Larvae Inspection Data.csv` for the larvae inspection. This script was most recently run on Mon Feb 24 09:06:43 2014.

## C179-GAL4 Driver

This analysis is just for the C179 Driver.

## Analysis of Larvae

We inspected the pupae from vials, 21 days after the cross was set up and counted how many pupae were present, and how many had dead flies in them. These data are summarized in Table 1 and in Figure 1. To analyse these data, first we did an ANOVA testing for whether the UAS has an effect on the percentage of dead larvae. The p-value for that comparason is **0.325** for the UAS. The results of this ANOVA are shown in Table 2. To test if the driver alone had an effect with did a Student's t-test comparing the *+/+* and *C179/+* progeny. The p-value for the Driver having an effect is **0.759**. This indicated that there was no significant effect on the number of larvae.

Gene	UAS	mean	se	sd	rel.error	n
Control	36304	113.00	19.07	50.45	44.64	7
Raptor	31528	114.00	12.76	28.52	25.02	5
Raptor	31529	93.60	9.82	21.96	23.46	5
Raptor	34814	93.75	11.20	33.60	35.84	9
Tsc1	31039	105.80	18.08	40.43	38.21	5
Tsc1	31314	142.60	25.05	61.37	43.04	6
Tsc1	35144					3

Table 1: Summary of the Number of Larvae by Driver and UAS

## Analysis of Dead Larvae

A summary of the percent of dead larvae for each cross is shown in Table 3 and in Figure 2. To analyse these data, first we did an ANOVA testing for whether the UAS has an effect on the percentage of dead larvae.

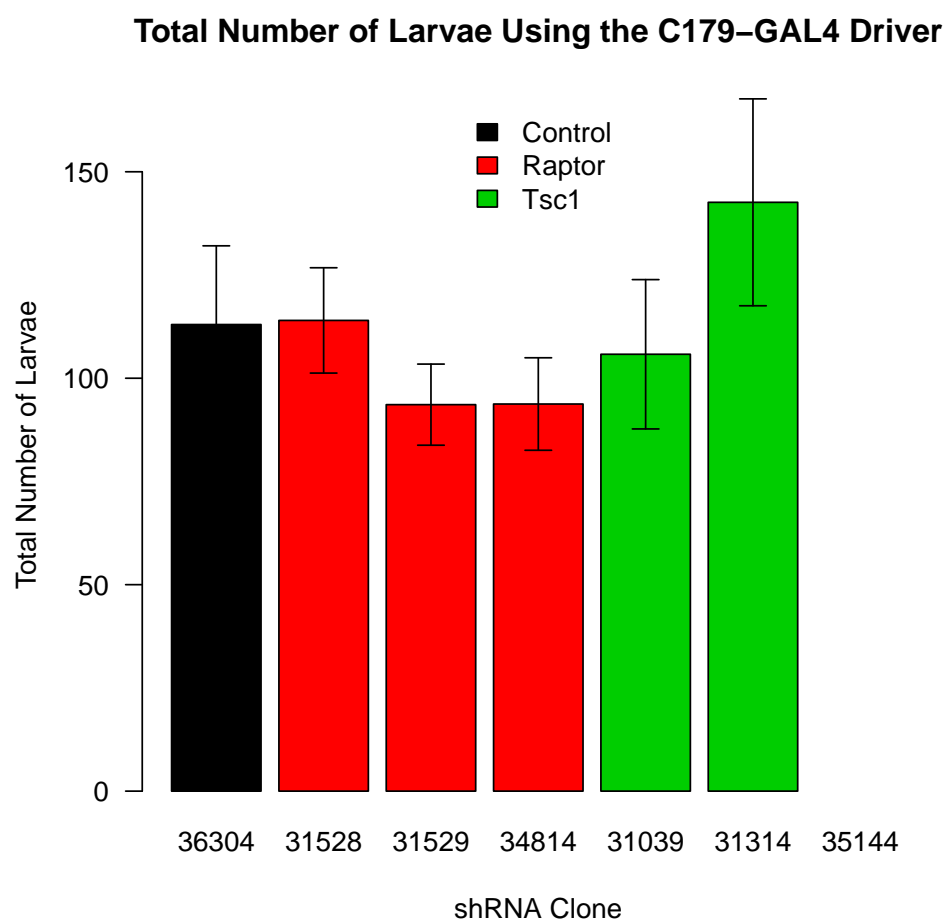


Figure 1: Total number of larvae counted. Presented as mean  $\pm$  standard error.

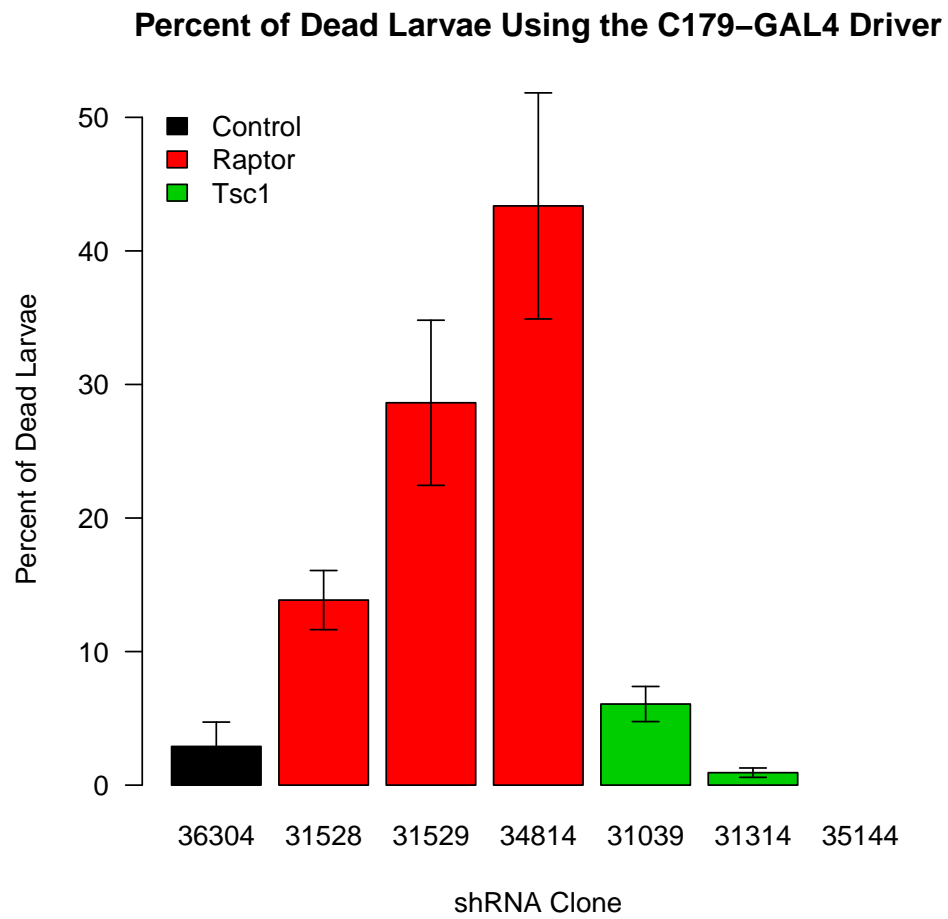


Figure 2: Proportion of larvae with dead flies. Presented as mean  $\pm$  standard error.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Driver	1	82.07	82.07	0.05	0.8159
UAS	5	8978.27	1795.65	1.20	0.3249
Residuals	44	65838.64	1496.33		

Table 2: ANOVA for the Number of Larvae by Driver and UAS

The p-value for that comparason is **1.7e-08** for the UAS. The results of this ANOVA are shown in Table 4. To test if the driver alone had an effect with did a Student’s t-test comparing the  $+/+$  and  $C179/+$  progeny. The p-value for the Driver having an effect is **0.000348**. We therefore excluded the  $+/+$  progeny from the analysis and compared with the  $C179-Gal4/+$  progeny as a control.

The ANOVA for this comparason yielded a p-value for the UAS of **1.2e-05**. The results of this ANOVA are in Table 5. Since this ANOVA was significant, we did a post-hoc test to look at each strain relative to the control. To test for differences compared to the  $C179-GAL4/+$  control strain, we did a Dunnett’s test. The results of this test are shown in Table 6.

Gene	UAS	mean	se	sd	rel.error	n
Control	36304	2.90	1.82	4.83	166.65	7
Raptor	31528	13.86	2.21	4.95	35.73	5
Raptor	31529	28.63	6.18	13.82	48.28	5
Raptor	34814	43.37	8.46	25.39	58.55	9
Tsc1	31039	6.07	1.31	2.94	48.41	5
Tsc1	31314	0.93	0.35	0.86	92.11	6
Tsc1	35144					3

Table 3: Summary of Percent of Dead Larvae by Driver and UAS

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Driver	1	2261.89	2261.89	17.49	0.0001
UAS	5	9556.95	1911.39	14.78	0.0000
Residuals	44	5689.01	129.30		

Table 4: ANOVA for Percent of Dead Larvae by Driver and UAS

## Analysis of Total Number of Flies

We inspected the total number of flies eclosed up to 21 days after the cross was set up. These data are summarized in Table 7 and in Figure 3. To analyse these data, first we did an ANOVA testing for whether the UAS has an effect on the number of eclosed flies. The p-value for that comparason is **1e-04** for the UAS. The results of this ANOVA are shown in Table 8. To test if the driver alone had an effect with did a Student’s t-test comparing the  $+/+$  and  $C179/+$  progeny. The p-value for the Driver having an effect is **0.24**.

Due to the significant ANOVA we performed a post-hoc Dunnett’s test using the  $C179/+$  progeny as the controls. An ANOVA analysis excluding the  $+/+$  flies had a p-value of **0.0001651** for the UAS. The results of that test are in Table 9. This test indicates that each of the three Raptor shRNA knockdowns cause a reduction in the number of flies.

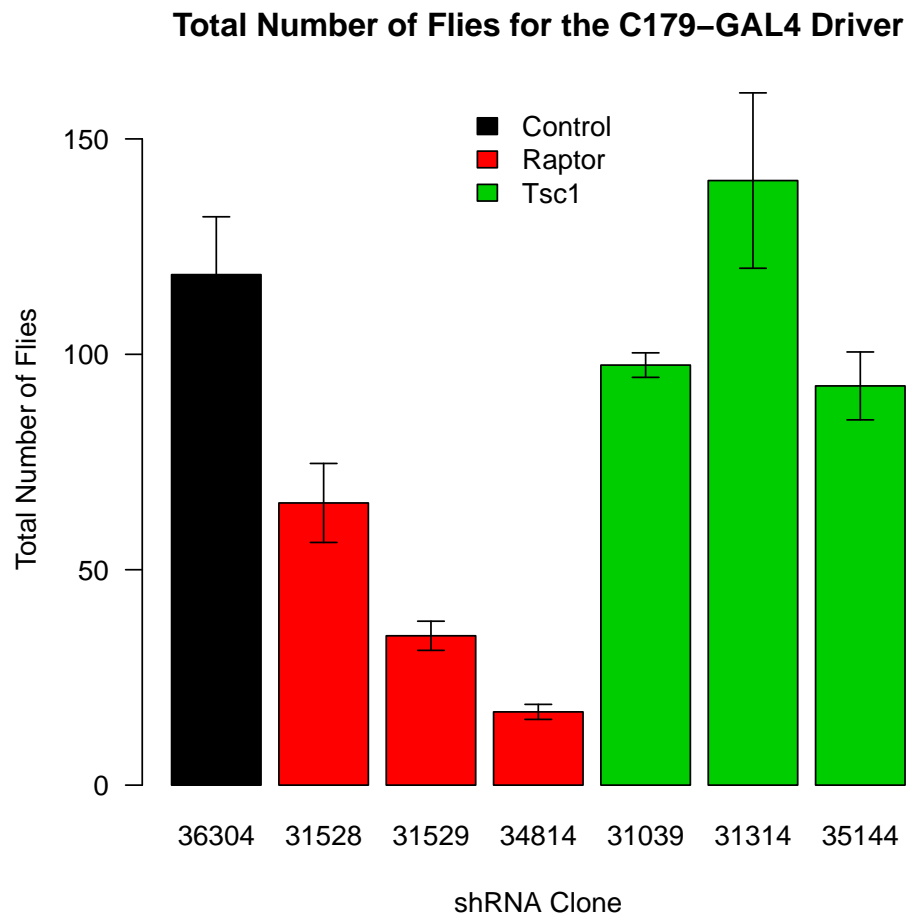


Figure 3: Total number of flies counted. Presented as mean  $\pm$  standard error.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
UAS	5	9556.95	1911.39	9.98	0.0000
Residuals	29	5552.98	191.48		

Table 5: ANOVA for Percent of Dead Larvae by UAS

	Effect Size (%)	p-value
31039 - 36304	3.2	0.9947626
31314 - 36304	-2.0	0.9994602
31528 - 36304	11.0	0.5534662
31529 - 36304	25.7	0.0156059
34814 - 36304	40.5	0.0000264

Table 6: Dunnett’s Test for the Percent of Dead Larvae Comparing to Control (C179-Gal4/+)

## Analysis of Number of Flies by Gender

For the flies which had been born, we could separate these data based on gender. The separated data is summarized in Tables 10 and 11. These data are graphed in Figure 4.

For Males, first we did an ANOVA testing for whether the UAS has an effect on the number of eclosed flies. The p-value for that comparason is **5.51675e-05** for the UAS. The results of this ANOVA are shown in Table 12. To test if the driver alone had an effect with did a Student’s t-test comparing the +/+ and *C179/+* progeny. The p-value for the Driver having an effect is **0.496**.

For Females, first we did an ANOVA testing for whether the UAS has an effect on the number of eclosed flies. The p-value for that comparason is **0.000109** for the UAS. The results of this ANOVA are shown in Table 13. To test if the driver alone had an effect with did a Student’s t-test comparing the +/+ and *C179/+* progeny. The p-value for the Driver having an effect is **0.1**.

Due to the significant ANOVA we performed a post-hoc Dunnett’s test using the *C179/+* progeny as the controls. An ANOVA analysis excluding the +/+ flies had a p-value of **0.000246494** for the UAS for males and a p-value of **0.00023** for the UAS for the females. The results of that test are in Tables 14. This test indicates that each of the three Raptor shRNA knockdowns cause a reduction in the number of male flies, but only 34814 had significantly less females.

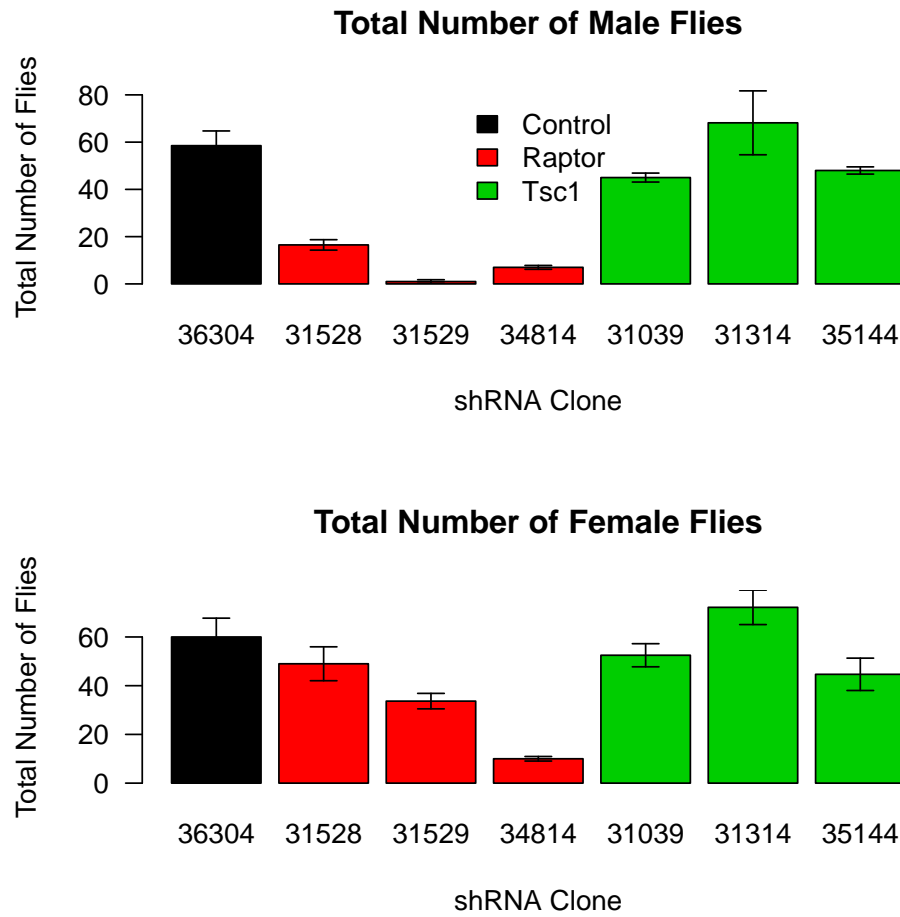


Figure 4: Total number of flies counted, separated by gender. Presented as mean  $\pm$  standard error.

Gene	UAS	mean	se	sd	rel.error	n
Control	36304	118.50	13.43	35.52	29.98	7
Raptor	31528	65.50	9.17	20.51	31.31	5
Raptor	31529	34.67	3.39	7.57	21.84	5
Raptor	34814	17.00	1.74	5.23	30.75	9
Tsc1	31039	97.50	2.85	6.36	6.53	5
Tsc1	31314	140.33	20.35	49.86	35.53	6
Tsc1	35144	92.67	7.88	13.65	14.73	3

Table 7: Summary of the Number of Flies by Driver and UAS

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Driver	1	2314.78	2314.78	2.15	0.1543
UAS	6	51905.18	8650.86	8.05	0.0001
Residuals	26	27954.04	1075.16		

Table 8: ANOVA for the Number of Flies by Driver and UAS

## Mef-GAL4 Driver

This analysis is just for the *Mef1c-GAL4* Driver.



	Effect Size (%)	p-value
31039 - 36304	-21.0	0.9417726
31314 - 36304	21.8	0.7581839
31528 - 36304	-53.0	0.2543785
31529 - 36304	-83.8	0.0085874
34814 - 36304	-101.5	0.0006569
35144 - 36304	-25.8	0.7828801

Table 9: Dunnett’s Test for the Number of Flies Comparing to Control (C179-Gal4/+)

Gene	UAS	mean	se	sd	rel.error	n
Control	36304	58.50	6.23	16.48	28.17	7
Raptor	31528	16.50	2.21	4.95	30.00	5
Raptor	31529	1.00	0.77	1.73	173.21	5
Raptor	34814	7.00	0.82	2.45	34.99	9
Tsc1	31039	45.00	1.90	4.24	9.43	5
Tsc1	31314	68.17	13.52	33.13	48.60	6
Tsc1	35144	48.00	1.53	2.65	5.51	3

Table 10: Summary of the Number of Male Flies by Driver and UAS

## Analysis of Larvae

We inspected the pupae from vials, 21 days after the cross was set up and counted how many pupae were present, and how many had dead flies in them. These data are summarized in Table 15 and in Figure 5. To analyse these data, first we did an ANOVA testing for whether the UAS has an effect on the percentage of dead larvae. The p-value for that comparason is **0.135** for the UAS. The results of this ANOVA are shown in Table 16. To test if the driver alone had an effect with did a Student’s t-test comparing the +/+ and *Mef*/+ progeny. The p-value for the Driver having an effect is **0.219**. This indicated that there was no significant effect on the number of larvae.

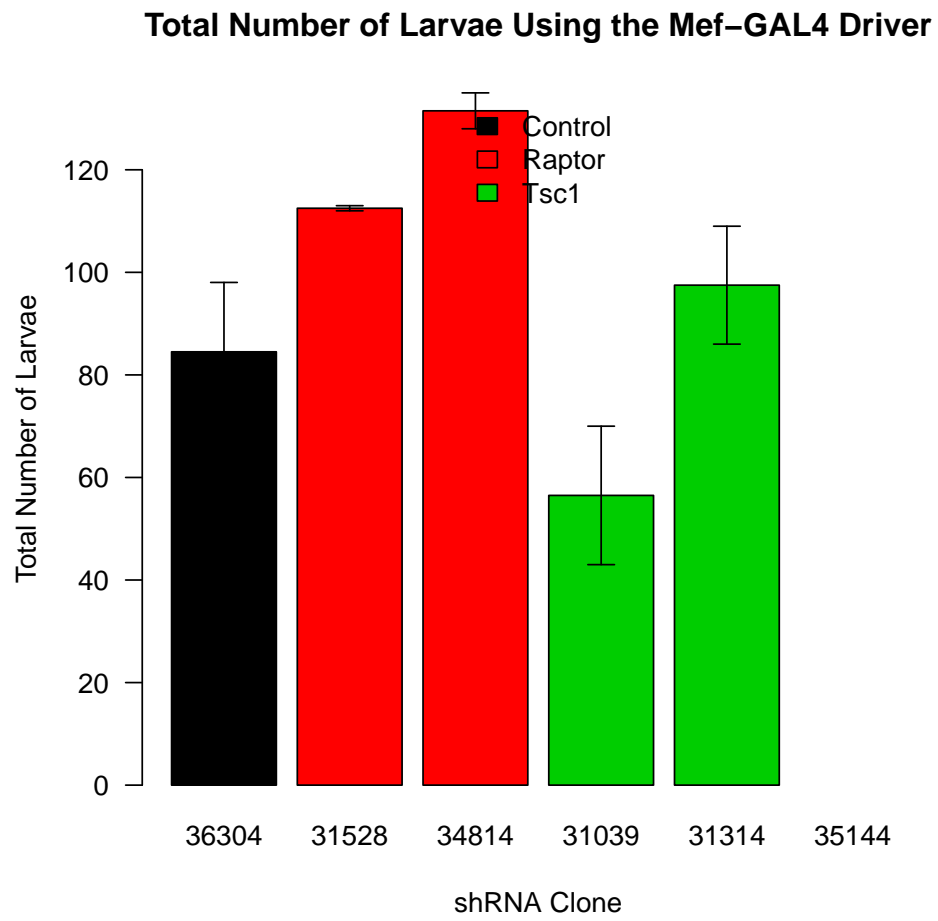


Figure 5: Total number of larvae counted. Presented as mean  $\pm$  standard error.

Gene	UAS	mean	se	sd	rel.error	n
Control	36304	60.00	7.73	20.45	34.08	7
Raptor	31528	49.00	6.96	15.56	31.75	5
Raptor	31529	33.67	3.17	7.09	21.07	5
Raptor	34814	10.00	0.94	2.83	28.28	9
Tsc1	31039	52.50	4.74	10.61	20.20	5
Tsc1	31314	72.17	7.08	17.34	24.02	6
Tsc1	35144	44.67	6.64	11.50	25.75	3

Table 11: Summary of the Number of Female Flies by Driver and UAS

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Driver	1	225.20	225.20	0.64	0.4312
UAS	6	17066.71	2844.45	8.07	0.0001
Residuals	26	9158.71	352.26		

Table 12: ANOVA for the Number of Male Flies by Driver and UAS

## Analysis of Dead Larvae

A summary of the percent of dead larvae for each cross is shown in Table 17 and in Figure 2. To analyse these data, first we did an ANOVA testing for whether the UAS has an effect on the percentage of dead larvae. The p-value for that comparason is **0** for the UAS. The results of this ANOVA are shown in Table 18. To test if the driver alone had an effect with did a Student’s t-test comparing the  $+/+$  and  $Mef/+$  progeny. The p-value for the Driver having an effect is **0.034**. We therefore excluded the  $+/+$  progeny from the analysis and compared with the  $Mef-Gal4/+$  progeny as a control.

The ANOVA for this comparason yielded a p-value for the UAS of **0**. The results of this ANOVA are in Table 19. Since this ANOVA was significant, we did a post-hoc test to look at each strain relative to the control. To test for differences compared to the  $Mef-GAL4/+$  control strain, we did a Dunnett’s test. The results of this test are shown in Table 20.

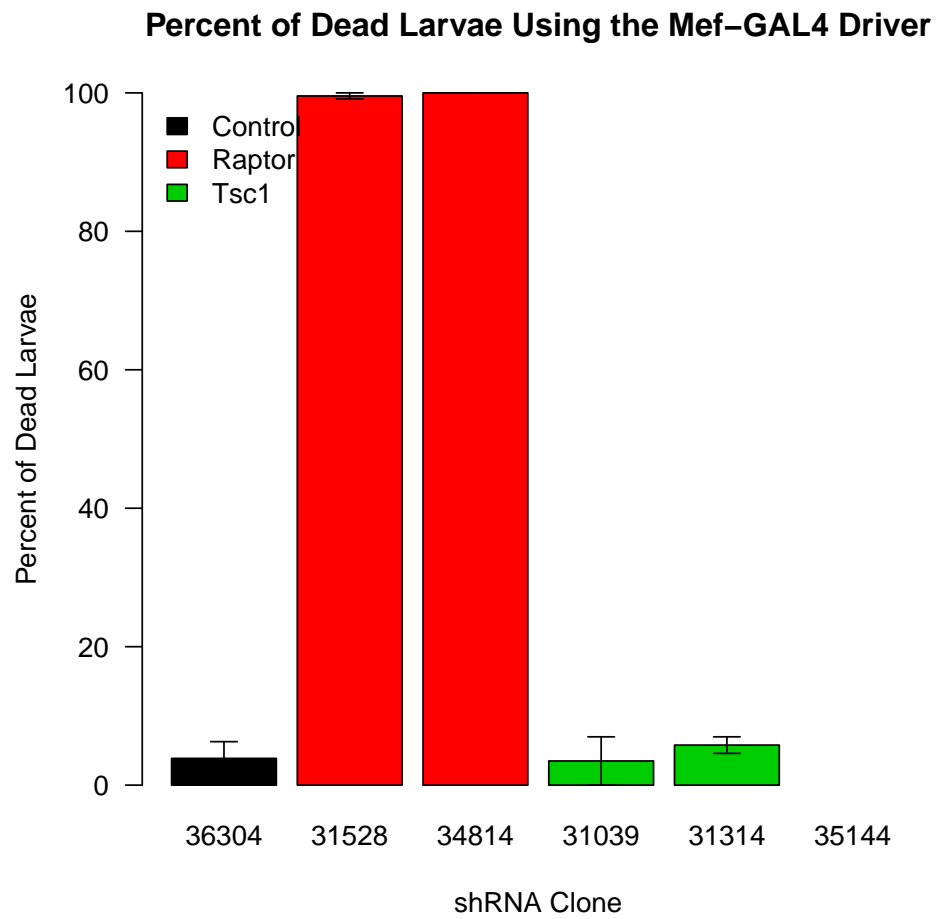


Figure 6: Proportion of larvae with dead flies. Presented as mean  $\pm$  standard error.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Driver	1	1095.96	1095.96	4.49	0.0437
UAS	6	10815.99	1802.66	7.39	0.0001
Residuals	26	6340.67	243.87		

Table 13: ANOVA for the Number of Female Flies by Driver and UAS

	Male Effect Size (%)	Male p-value	Female Effect Size (%)	Female p-value
31039 - 36304	-13.5	0.9179171	-7.5	0.9832569
31314 - 36304	9.7	0.9136034	12.2	0.6104077
31528 - 36304	-42.0	0.0712432	-11.0	0.9077411
31529 - 36304	-57.5	0.0023740	-26.3	0.1121668
34814 - 36304	-51.5	0.0028252	-50.0	0.0003960
35144 - 36304	-10.5	0.9476610	-15.3	0.5830047

Table 14: Dunnett’s Test for the Number of Males and Females Comparing to Control (C179-Gal4/+)

## Analysis of Total Number of Flies

We inspected the total number of flies eclosed up to 21 days after the cross was set up. These data are summarized in Table 21 and in Figure 7. To analyse these data, first we did an ANOVA testing for whether the UAS has an effect on the number of eclosed flies. The p-value for that comparason is **0.669** for the UAS. The results of this ANOVA are shown in Table 8. To test if the driver alone had an effect with did a Student’s t-test comparing the +/+ and *Mef*/+ progeny. The p-value for the Driver having an effect is **0.103**.

Due to the significant ANOVA we performed a post-hoc Dunnett’s test using the *Mef*/+ progeny as the controls. An ANOVA analysis excluding the +/+ flies had a p-value of **0.528** for the UAS. The results of that test are in Table 23. This test indicates that each of the three Raptor shRNA knockdowns cause a reduction in the number of flies.

## Analysis of Number of Flies by Gender

For the flies which had been born, we could separate these data based on gender. The separated data is summarized in Tables 10 and 11. These data are graphed in Figure 4.

For Males, first we did an ANOVA testing for whether the UAS has an effect on the number of eclosed flies. The p-value for that comparason is **0.685** for the UAS. The results of this ANOVA are shown in Table 26. To test if the driver alone had an effect with did a Student’s t-test comparing the +/+ and *Mef*/+ progeny. The p-value for the Driver having an effect is **0.069**.

For Females, first we did an ANOVA testing for whether the UAS has an effect on the number of eclosed flies. The p-value for that comparason is **0.62** for the UAS. The results of this ANOVA are shown in Table 13. To test if the driver alone had an effect with did a Student’s t-test comparing the +/+ and *Mef*/+ progeny. The p-value for the Driver having an effect is **0.176**.

Due to the significant ANOVA we performed a post-hoc Dunnett’s test using the *Mef*/+ progeny as the controls. An ANOVA analysis excluding the +/+ flies had a p-value of **0.416** for the UAS for males and a p-value of **0.576** for the UAS for the females. The results of that test are in Tables 14. This test indicates that each of the three Raptor shRNA knockdowns cause a reduction in the number of male flies, but only 34814 had significantly less females.

The key packages used in this analysis were R [1], plyr [2], reshape2 [3] and multcomp [4].

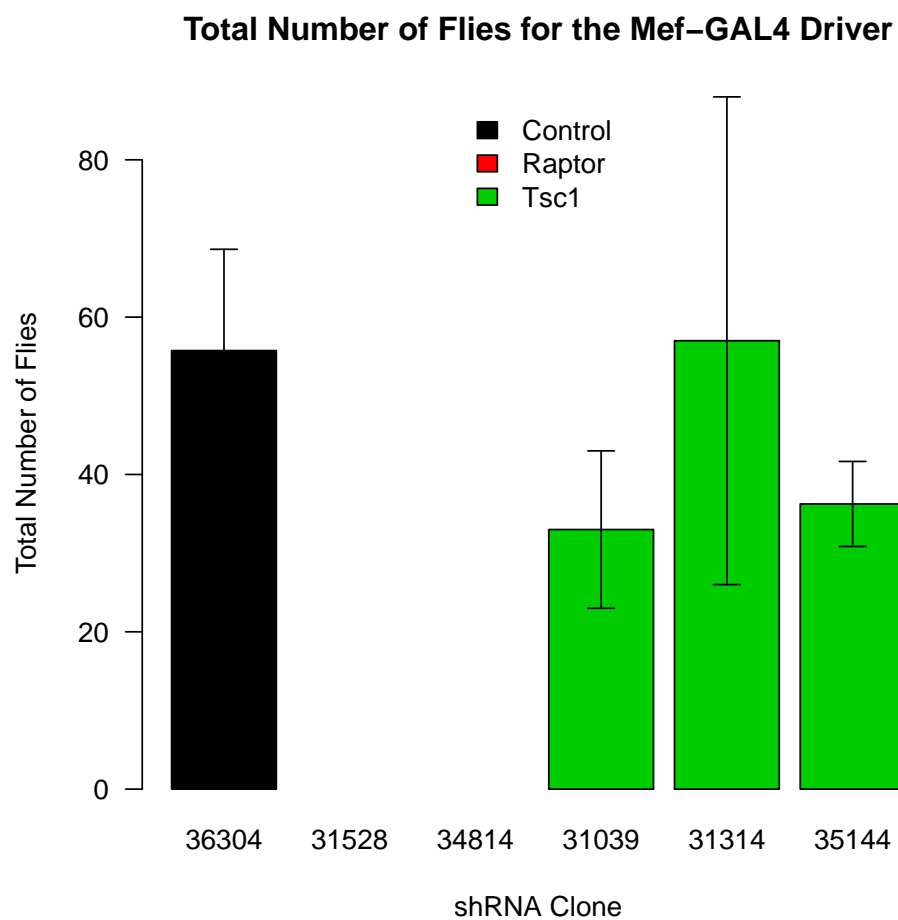


Figure 7: Total number of flies counted. Presented as mean  $\pm$  standard error.

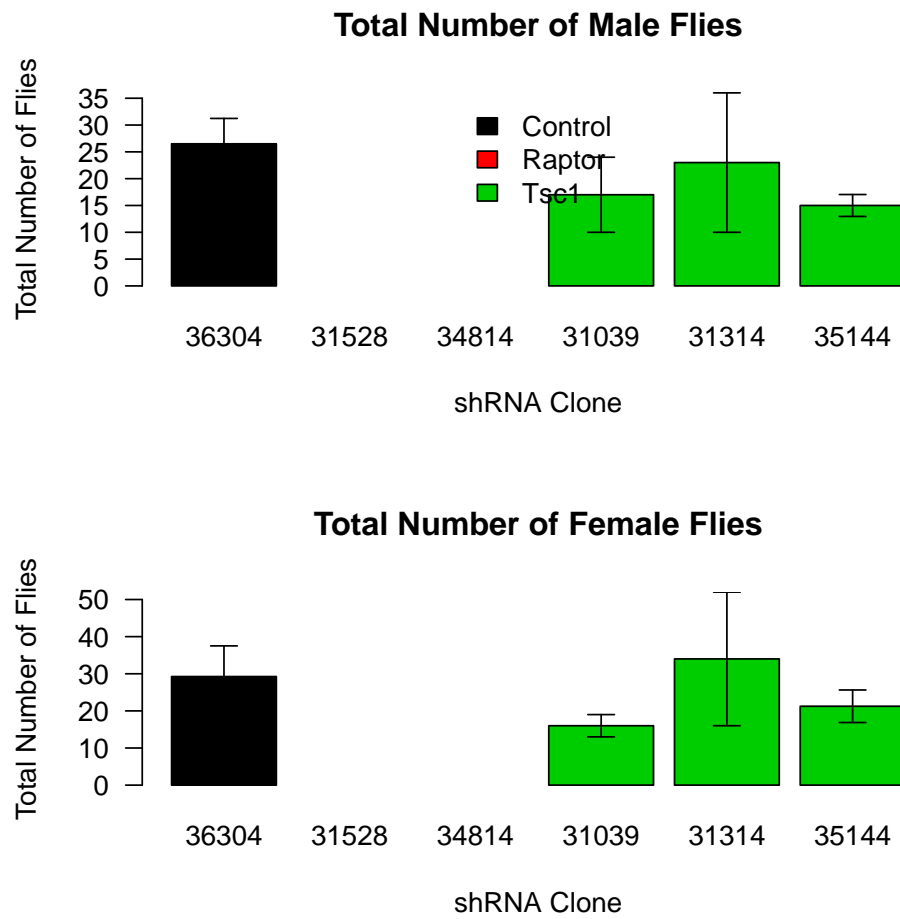


Figure 8: Total number of flies counted, separated by gender. Presented as mean  $\pm$  standard error.

Gene	UAS	mean	se	sd	rel.error	n
Control	36304	84.50	13.53	27.06	32.03	4
Raptor	31528	112.50	0.50	0.71	0.63	2
Raptor	34814	131.50	3.50	4.95	3.76	2
Tsc1	31039	56.50	13.50	19.09	33.79	2
Tsc1	31314	97.50	11.50	16.26	16.68	2
Tsc1	35144					4

Table 15: Summary of the Number of Larvae by Driver and UAS

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Driver	1	977.17	977.17	1.15	0.2956
UAS	4	6692.00	1673.00	1.97	0.1352
Residuals	22	18728.94	851.32		

Table 16: ANOVA for the Number of Larvae by Driver and UAS

## References

- [1] R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, 2013.
- [2] Hadley Wickham. The split-apply-combine strategy for data analysis. *Journal of Statistical Software*, 40(1):1–29, 2011.
- [3] Hadley Wickham. Reshaping data with the reshape package. *Journal of Statistical Software*, 21(12):1–20, 2007.
- [4] Torsten Hothorn, Frank Bretz, and Peter Westfall. Simultaneous inference in general parametric models. *Biometrical Journal*, 50(3):346–363, 2008.

## Session Information

- R version 3.0.2 (2013-09-25), x86\_64-apple-darwin10.8.0
- Locale: en\_US.UTF-8/en\_US.UTF-8/en\_US.UTF-8/C/en\_US.UTF-8/en\_US.UTF-8
- Base packages: base, datasets, graphics, grDevices, methods, splines, stats, utils
- Other packages: bibtex 0.3-6, multcomp 1.3-2, mvtnorm 0.9-9997, plyr 1.8, reshape2 1.2.2, survival 2.37-7, TH.data 1.0-3, xtable 1.7-1
- Loaded via a namespace (and not attached): grid 3.0.2, lattice 0.20-24, sandwich 2.3-0, stringr 0.6.2, tools 3.0.2, zoo 1.7-10



Gene	UAS	mean	se	sd	rel.error	n
Control	36304	3.86	2.42	4.83	125.06	4
Raptor	31528	99.56	0.44	0.63	0.63	2
Raptor	34814	100.00	0.00	0.00	0.00	2
Tsc1	31039	3.49	3.49	4.93	141.42	2
Tsc1	31314	5.78	1.19	1.69	29.22	2
Tsc1	35144					4

Table 17: Summary of Percent of Dead Larvae by Driver and UAS

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Driver	1	7414.33	7414.33	698.17	0.0000
UAS	4	24342.45	6085.61	573.05	0.0000
Residuals	22	233.63	10.62		

Table 18: ANOVA for Percent of Dead Larvae by Driver and UAS

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
UAS	4	24342.45	6085.61	436.47	0.0000
Residuals	7	97.60	13.94		

Table 19: ANOVA for Percent of Dead Larvae by UAS

	Effect Size (%)	p-value
31039 - 36304	-0.4	0.9998801
31314 - 36304	1.9	0.9446428
31528 - 36304	95.7	0.0000000
34814 - 36304	96.1	0.0000000

Table 20: Dunnett's Test for the Percent of Dead Larvae Comparing to Control (Mef-Gal4/+)

Gene	UAS	mean	se	sd	rel.error	n
Control	36304	55.75	12.87	25.75	46.18	4
Raptor	31528					2
Raptor	34814					2
Tsc1	31039	33.00	10.00	14.14	42.85	2
Tsc1	31314	57.00	31.00	43.84	76.91	2
Tsc1	35144	36.25	5.41	10.81	29.83	4

Table 21: Summary of the Number of Flies by Driver and UAS

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Driver	1	2871.41	2871.41	3.41	0.0848
UAS	3	1339.17	446.39	0.53	0.6689
Residuals	15	12646.38	843.09		

Table 22: ANOVA for the Number of Flies by Driver and UAS

	Effect Size (%)	p-value
31039 - 36304	-22.7	0.5950296
31314 - 36304	1.2	0.9998454
35144 - 36304	-19.5	0.5613855

Table 23: Dunnett's Test for the Number of Flies Comparing to Control (Mef-Gal4/+)

Gene	UAS	mean	se	sd	rel.error	n
Control	36304	26.50	4.73	9.47	35.73	4
Raptor	31528					2
Raptor	34814					2
Tsc1	31039	17.00	7.00	9.90	58.23	2
Tsc1	31314	23.00	13.00	18.38	79.93	2
Tsc1	35144	15.00	2.04	4.08	27.22	4

Table 24: Summary of the Number of Male Flies by Driver and UAS

Gene	UAS	mean	se	sd	rel.error	n
Control	36304	29.25	8.26	16.52	56.48	4
Raptor	31528					2
Raptor	34814					2
Tsc1	31039	16.00	3.00	4.24	26.52	2
Tsc1	31314	34.00	18.00	25.46	74.87	2
Tsc1	35144	21.25	4.39	8.77	41.27	4

Table 25: Summary of the Number of Female Flies by Driver and UAS

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Driver	1	957.68	957.68	4.81	0.0445
UAS	3	302.00	100.67	0.51	0.6845
Residuals	15	2988.87	199.26		

Table 26: ANOVA for the Number of Male Flies by Driver and UAS

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Driver	1	512.53	512.53	2.07	0.1711
UAS	3	452.17	150.72	0.61	0.6201
Residuals	15	3719.50	247.97		

Table 27: ANOVA for the Number of Female Flies by Driver and UAS

	Male Effect Size (%)	Male p-value	Female Effect Size (%)	Female p-value
31039 - 36304	-9.5	0.5847329	-13.2	0.6365505
31314 - 36304	-3.5	0.9576999	4.8	0.9683683
35144 - 36304	-11.5	0.3022776	-8.0	0.8014972

Table 28: Dunnett's Test for the Number of Males and Females Comparing to Control (Mef-Gal4/+)