

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/Pupae Inspection Data.csv` for the pupae inspection. This script was most recently run on Wed Feb 26 12:33:18 2014.

C179-GAL4 Driver

This analysis is just for the C179 Driver.

Analysis of Pupae

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 pupae. The p-value for that comparason is **0.471** 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 **0.446**. This indicated that there was no significant effect on the number of pupae.

Gene	UAS	mean	se	sd	rel.error	n
Control	36304	117.56	15.02	45.05	38.32	9
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	129.43	19.61	55.48	42.86	8
Tsc1	35144					5

Table 1: Summary of the Number of Pupae by Driver and UAS for C179-GAL4

Analysis of Dead Pupae

A summary of the percent of dead pupae 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 pupae.

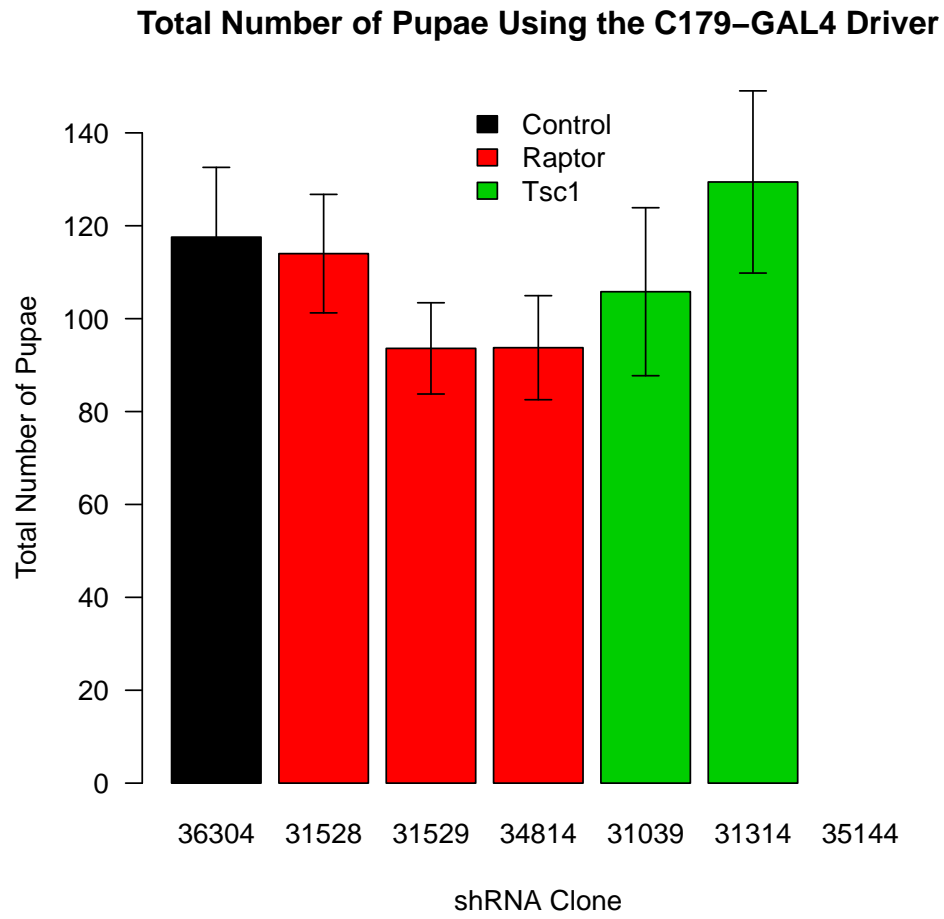


Figure 1: Total number of pupae counted. Presented as mean \pm standard error.

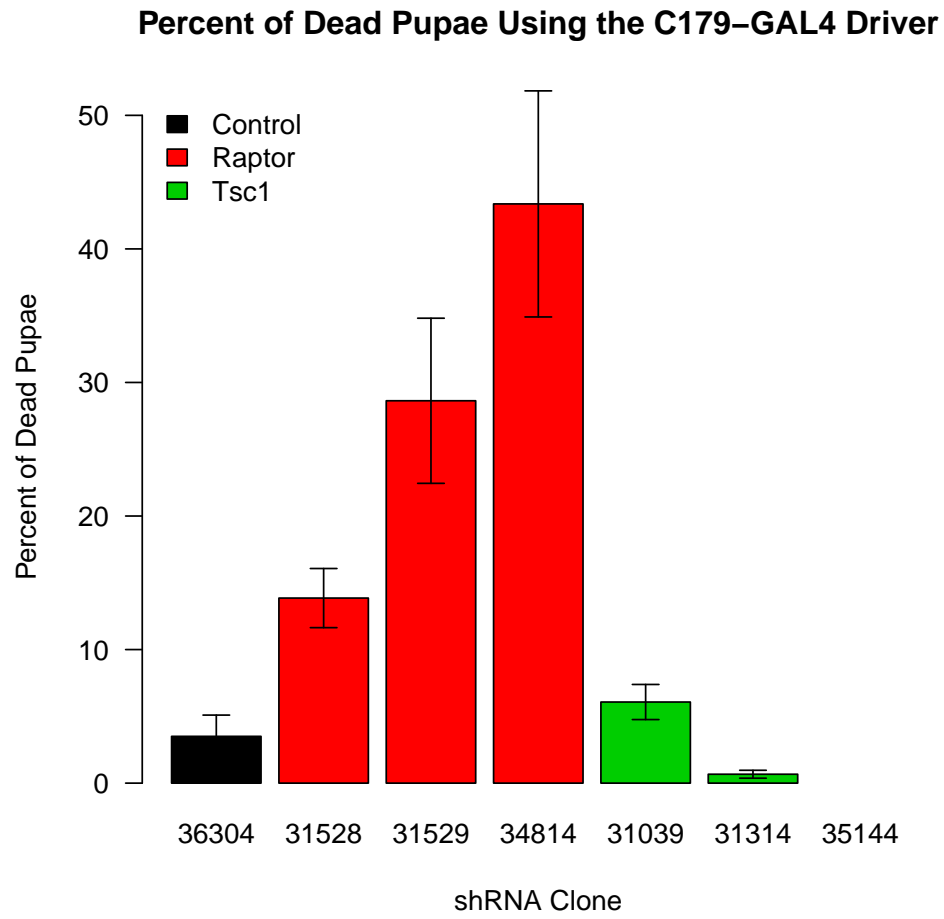


Figure 2: Proportion of pupae with dead flies. Presented as mean \pm standard error.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Driver	1	370.32	370.32	0.25	0.6169
UAS	5	6779.49	1355.90	0.93	0.4711
Residuals	49	71595.91	1461.14		

Table 2: ANOVA for the Number of Pupae by Driver and UAS for C179-GAL4

The p-value for that comparason is **1e-09** 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 **0.000603**. 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 **1e-06**. 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	3.50	1.59	4.78	136.52	9
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	0.67	0.30	0.84	125.51	8
Tsc1	35144					5

Table 3: Summary of Percent of Dead Pupae by Driver and UAS for C179-GAL4

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Driver	1	1852.63	1852.63	15.75	0.0002
UAS	5	10356.94	2071.39	17.61	0.0000
Residuals	49	5762.95	117.61		

Table 4: ANOVA for Percent of Dead Pupae by Driver and UAS for 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 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 **0** 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 **0.063**.

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 **2.15e-05** 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.

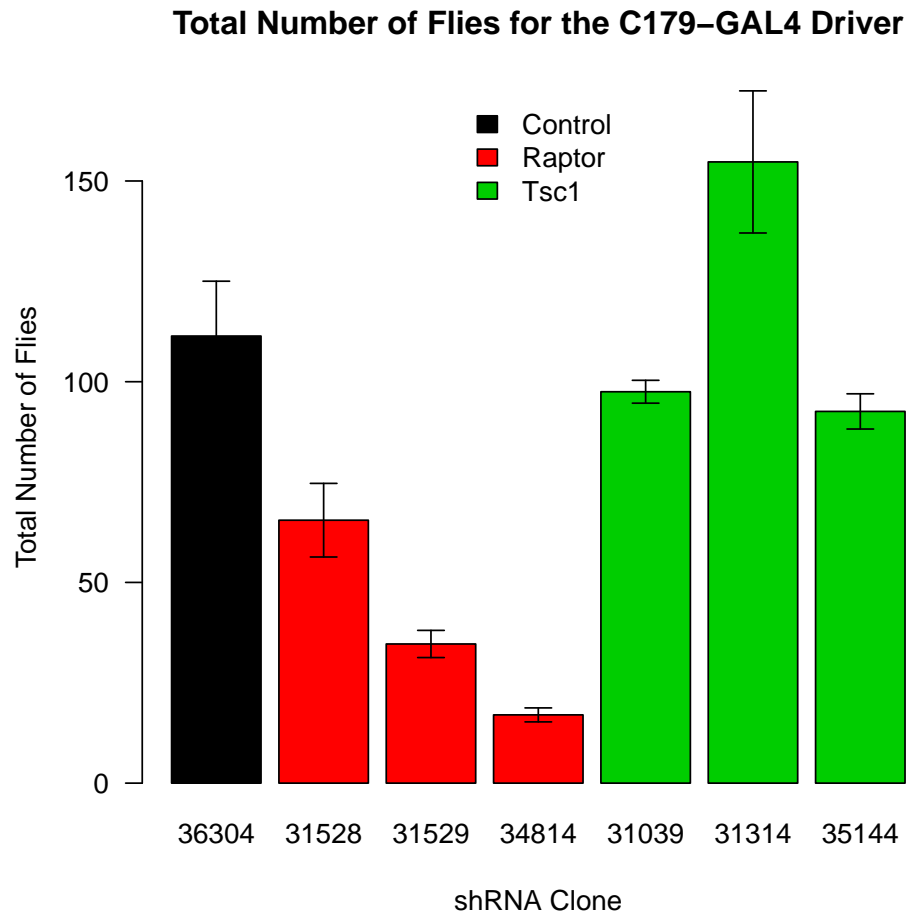


Figure 3: Total number of flies counted. Presented as mean \pm standard error.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
UAS	5	10356.94	2071.39	12.21	0.0000
Residuals	33	5597.26	169.61		

Table 5: ANOVA for Percent of Dead Pupae by UAS for C179-GAL4

	Effect Size (%)	p-value
31039 - 36304	2.6	0.9970976
31314 - 36304	-2.8	0.9927349
31528 - 36304	10.4	0.5157506
31529 - 36304	25.1	0.0071407
34814 - 36304	39.9	0.0000018

Table 6: Dunnett’s Test for the Percent of Dead Pupae 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 **2.8748e-06** 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 **0.189**.

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 **1.5e-05** 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 **0.02**.

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 **1.9151e-05** for the UAS for males and a p-value of **6e-05** 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.

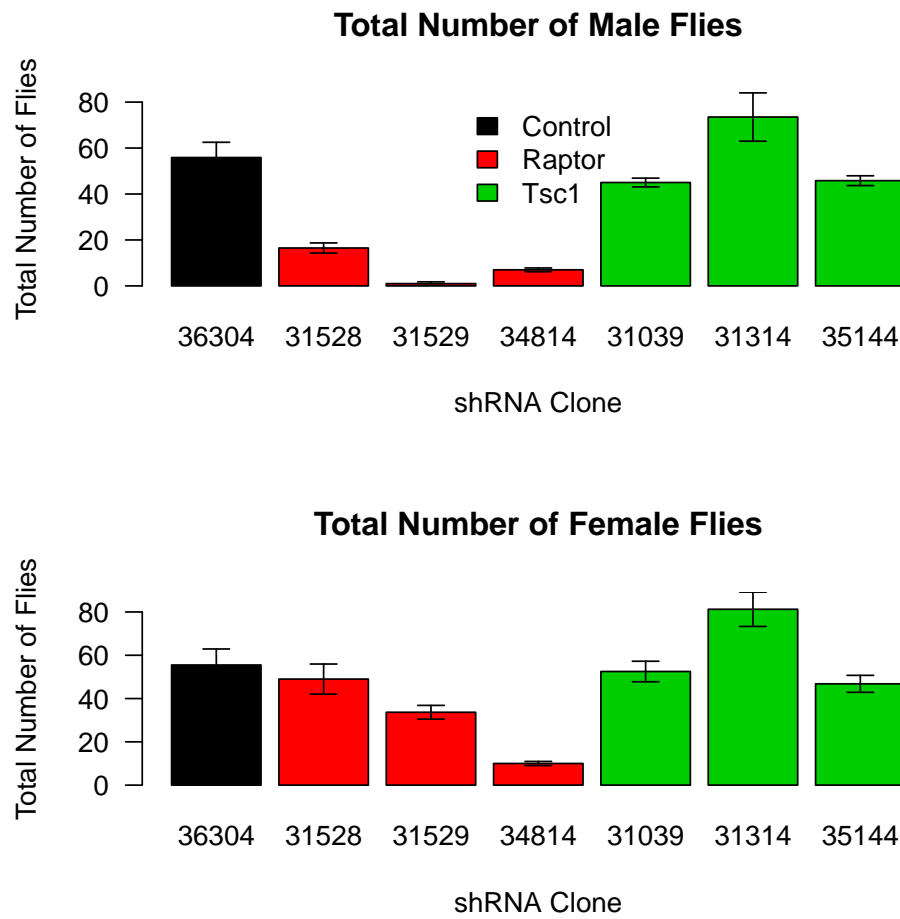


Figure 4: Total number of flies counted, separated by gender. Presented as mean \pm standard error.

Gene	UAS	mean	se	sd	rel.error	n
Control	36304	111.38	13.66	40.99	36.80	9
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	154.75	17.70	50.06	32.35	8
Tsc1	35144	92.60	4.39	9.81	10.60	5

Table 7: Summary of the Number of Flies by Driver and UAS for C179-GAL4

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Driver	1	5865.15	5865.15	4.97	0.0328
UAS	6	67665.63	11277.61	9.55	0.0000
Residuals	33	38968.24	1180.86		

Table 8: ANOVA for the Number of Flies by Driver and UAS for C179-GAL4

Mef-GAL4 Driver

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

	Effect Size (%)	p-value
31039 - 36304	-13.9	0.9942059
31314 - 36304	43.4	0.0991472
31528 - 36304	-45.9	0.4348492
31529 - 36304	-76.7	0.0178841
34814 - 36304	-94.4	0.0009590
35144 - 36304	-18.8	0.8906163

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	55.88	6.63	19.90	35.62	9
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	73.50	10.52	29.75	40.47	8
Tsc1	35144	45.80	2.13	4.76	10.40	5

Table 10: Summary of the Number of Male Flies by Driver and UAS for C179-GAL4

Analysis of Pupae

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 pupae. The p-value for that comparason is **0.006** 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 **0.128**. This indicated that there was no significant effect on the number of pupae.

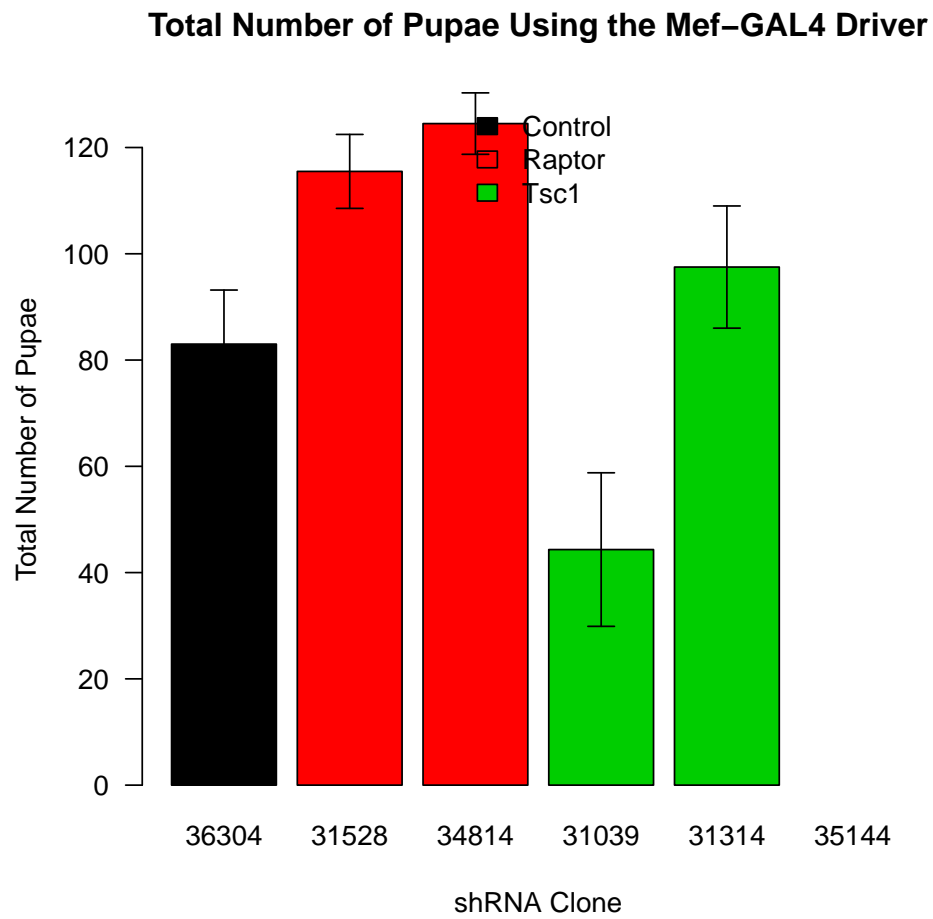


Figure 5: Total number of pupae counted. Presented as mean \pm standard error.

Gene	UAS	mean	se	sd	rel.error	n
Control	36304	55.50	7.41	22.23	40.05	9
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	81.25	7.93	22.44	27.61	8
Tsc1	35144	46.80	3.92	8.76	18.71	5

Table 11: Summary of the Number of Female Flies by Driver and UAS for C179-GAL4

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Driver	1	796.10	796.10	2.30	0.1389
UAS	6	20640.70	3440.12	9.94	0.0000
Residuals	33	11424.18	346.19		

Table 12: ANOVA for the Number of Male Flies by Driver and UAS for C179-GAL4

Analysis of Dead Pupae

A summary of the percent of dead pupae 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 pupae. 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 **0.001**. 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.

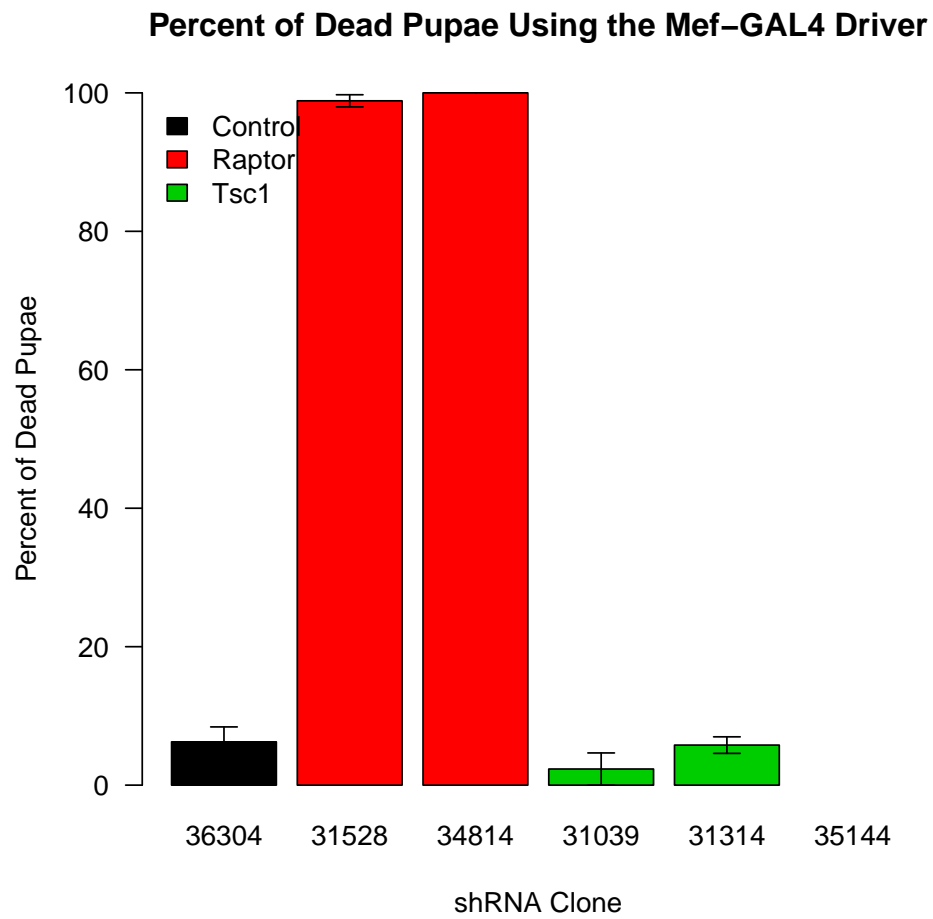


Figure 6: Proportion of pupae with dead flies. Presented as mean \pm standard error.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Driver	1	2339.56	2339.56	7.77	0.0087
UAS	6	15158.53	2526.42	8.39	0.0000
Residuals	33	9933.47	301.01		

Table 13: ANOVA for the Number of Female Flies by Driver and UAS for C179-GAL4

	Male Effect Size (%)	Male p-value	Female Effect Size (%)	Female p-value
31039 - 36304	-10.9	0.9658084	-3.0	0.9999517
31314 - 36304	17.6	0.3303420	25.7	0.0385952
31528 - 36304	-39.4	0.0774980	-6.5	0.9961487
31529 - 36304	-54.9	0.0015129	-21.8	0.3386861
34814 - 36304	-48.9	0.0017773	-45.5	0.0016291
35144 - 36304	-10.1	0.8994630	-8.7	0.9231479

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 **0.365** 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 **0.067**.

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 **0.177** 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 **0.332** 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 **0.054**.

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 **0.346** 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 **0.099**.

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 **0.082** for the UAS for males and a p-value of **0.256** 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].

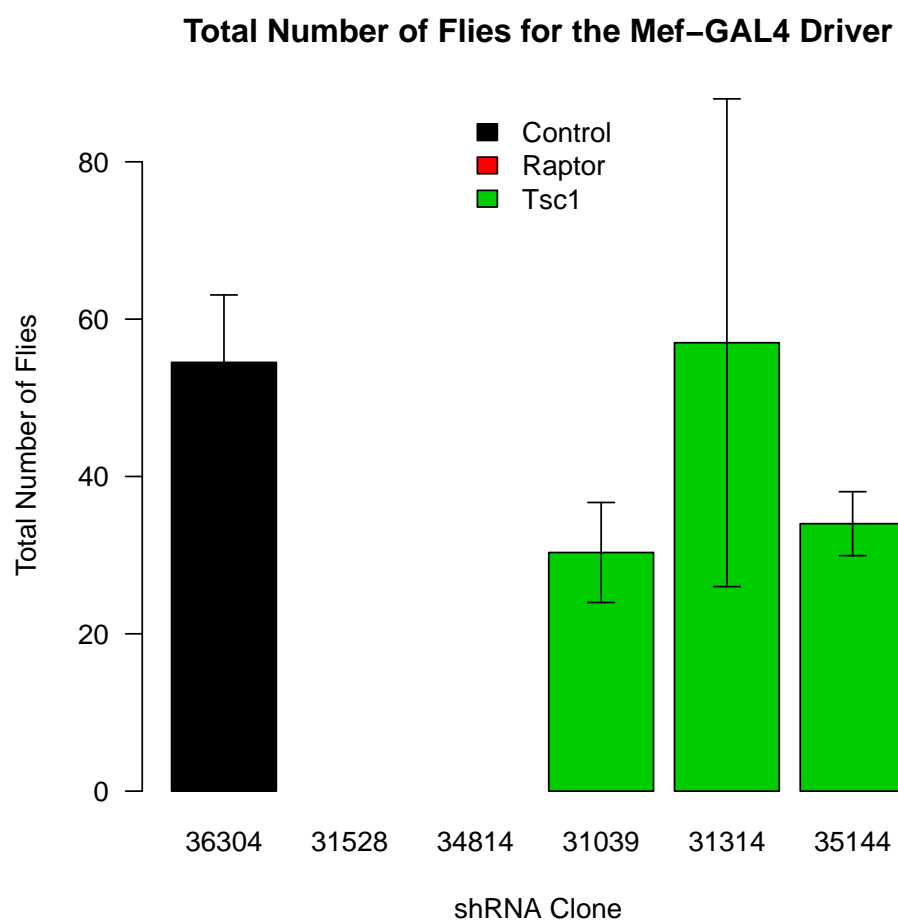


Figure 7: Total number of flies counted. Presented as mean \pm standard error.

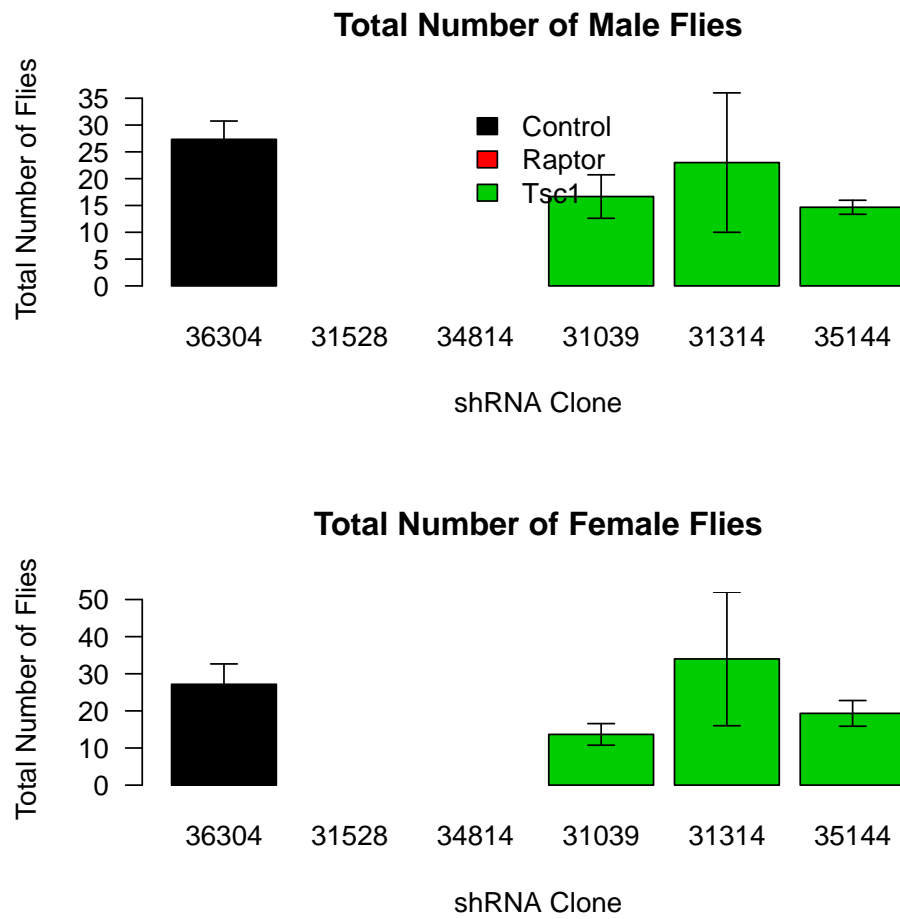


Figure 8: Total number of flies counted, separated by gender. Presented as mean \pm standard error.

Gene	UAS	mean	se	sd	rel.error	n
Control	36304	83.00	10.18	24.92	30.03	6
Raptor	31528	115.50	6.96	13.92	12.05	4
Raptor	34814	124.50	5.78	11.56	9.29	4
Tsc1	31039	44.33	14.45	25.03	56.45	3
Tsc1	31314	97.50	11.50	16.26	16.68	2
Tsc1	35144					6

Table 15: Summary of the Number of Pupae by Driver and UAS for Mef-GAL4

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Driver	1	929.17	929.17	1.22	0.2784
UAS	4	13720.83	3430.21	4.50	0.0058
Residuals	30	22873.64	762.45		

Table 16: ANOVA for the Number of Pupae by Driver and UAS for Mef-GAL4

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	6.25	2.16	5.29	84.62	6
Raptor	31528	98.85	0.88	1.75	1.78	4
Raptor	34814	100.00	0.00	0.00	0.00	4
Tsc1	31039	2.33	2.33	4.03	173.21	3
Tsc1	31314	5.78	1.19	1.69	29.22	2
Tsc1	35144					6

Table 17: Summary of Percent of Dead Pupae by Driver and UAS for Mef-GAL4

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Driver	1	15284.09	15284.09	1309.64	0.0000
UAS	4	41248.47	10312.12	883.61	0.0000
Residuals	30	350.11	11.67		

Table 18: ANOVA for Percent of Dead Pupae by Driver and UAS for Mef-GAL4

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
UAS	4	41248.47	10312.12	782.82	0.0000
Residuals	14	184.42	13.17		

Table 19: ANOVA for Percent of Dead Pupae by UAS for Mef-GAL4

	Effect Size (%)	p-value
31039 - 36304	-3.9	0.4185101
31314 - 36304	-0.5	0.9996276
31528 - 36304	92.6	0.0000000
34814 - 36304	93.7	0.0000000

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

Gene	UAS	mean	se	sd	rel.error	n
Control	36304	54.50	8.58	21.01	38.55	6
Raptor	31528					4
Raptor	34814					4
Tsc1	31039	30.33	6.36	11.02	36.31	3
Tsc1	31314	57.00	31.00	43.84	76.91	2
Tsc1	35144	34.00	4.07	9.96	29.29	6

Table 21: Summary of the Number of Flies by Driver and UAS for Mef-GAL4

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Driver	1	3495.59	3495.59	5.44	0.0297
UAS	3	2151.36	717.12	1.12	0.3648
Residuals	21	13488.17	642.29		

Table 22: ANOVA for the Number of Flies by Driver and UAS for Mef-GAL4

	Effect Size (%)	p-value
31039 - 36304	-24.2	0.2450584
31314 - 36304	2.5	0.9975220
35144 - 36304	-20.5	0.2200041

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	27.33	3.41	8.36	30.58	6
Raptor	31528					4
Raptor	34814					4
Tsc1	31039	16.67	4.06	7.02	42.14	3
Tsc1	31314	23.00	13.00	18.38	79.93	2
Tsc1	35144	14.67	1.31	3.20	21.85	6

Table 24: Summary of the Number of Male Flies by Driver and UAS for Mef-GAL4

Gene	UAS	mean	se	sd	rel.error	n
Control	36304	27.17	5.49	13.44	49.46	6
Raptor	31528					4
Raptor	34814					4
Tsc1	31039	13.67	2.91	5.03	36.83	3
Tsc1	31314	34.00	18.00	25.46	74.87	2
Tsc1	35144	19.33	3.46	8.48	43.85	6

Table 25: Summary of the Number of Female Flies by Driver and UAS for Mef-GAL4

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Driver	1	1024.73	1024.73	6.86	0.0160
UAS	3	540.90	180.30	1.21	0.3317
Residuals	21	3137.33	149.40		

Table 26: ANOVA for the Number of Male Flies by Driver and UAS for Mef-GAL4

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Driver	1	735.07	735.07	3.74	0.0667
UAS	3	687.64	229.21	1.17	0.3461
Residuals	21	4126.83	196.52		

Table 27: ANOVA for the Number of Female Flies by Driver and UAS for Mef-GAL4

	Male Effect Size (%)	Male p-value	Female Effect Size (%)	Female p-value
31039 - 36304	-10.7	0.2046979	-13.5	0.3366209
31314 - 36304	-4.3	0.8642396	6.8	0.8540058
35144 - 36304	-12.7	0.0457884	-7.8	0.5956404

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