Analysis of Longevity from Muscle TSC1/Raptor Flies

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Experimental Design

These data are stored in the **Data** subfolder. This script was most recently run on Thu Mar 20 10:25:48 2014. There has been a total of **3487** deaths, with **3435** of natural causes and **3385** of identifiable genotypes excluding accidental deaths. The oldest fly recorded so far was **661097** days old at time of death.

Gene Level Analysis

Number of Flies Examined

The total number of deaths for each cross and genotype ,removing deaths that were accidental or not due to natural causes, the data is shown in Table 1 and 2.

The distribution of deaths in the samples are shown at a gender level in Figure 1 and looking at the controls in Figure 2. To examine the controls, and whether there are effects of the GAL4 or the shRNA we examined the median age of the controls from each cross. These data are shown in Figure 3. Still need to do some statistics to test if this is relevant.

Driver	Gene	shRNA/GAL4	+/+	GAL4/+	GAL4/Tm6B	shRNA/Tm3,sb	Tm3,sb/Tm6B	NA
24B-Gal4	Raptor	0	0	0	20	8	10	0
24B-Gal4	Tsc1	1442	0	0	1018	1278	572	56
36304	Control	0	294	0	0	0	0	0
C179-Gal4	Control	0	0	225	0	0	0	0
C179-Gal4	Raptor	922	0	0	1134	0	0	394
C179-Gal4	Tsc1	0	0	0	0	0	0	576
Hand-Gal4	Raptor	1420	0	0	1228	1504	692	198
Hand-Gal4	Tsc1	1238	0	0	898	990	238	26

Table 1: Total Natural Deaths for Each Gene and Genotype

Survival Analysis

All of these are relative to the reference Genotype which is the knockdown (GAL4/shRNA). The key packages used in this analysis were R [1], lubridate [2], plyr [3] and survival [4, 5].
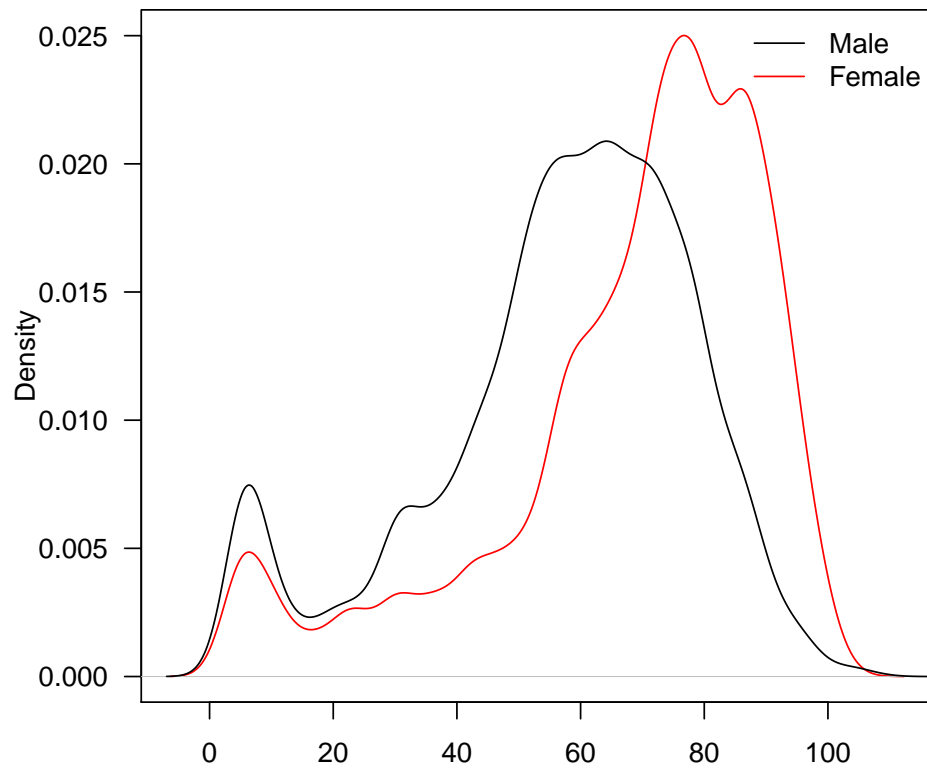
Natural Death Distribution, all Control Flies
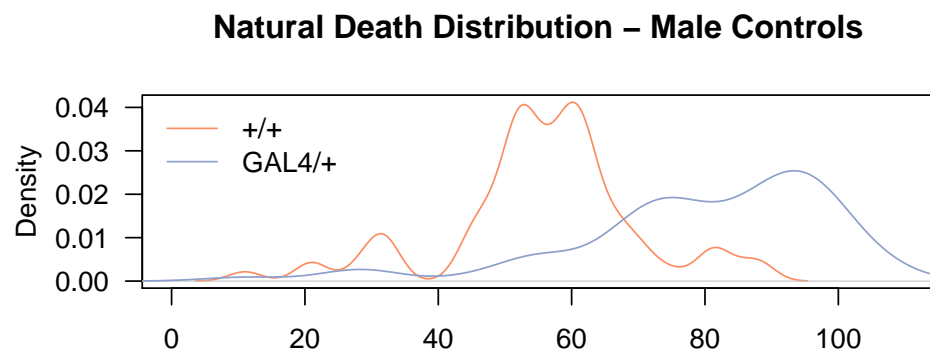
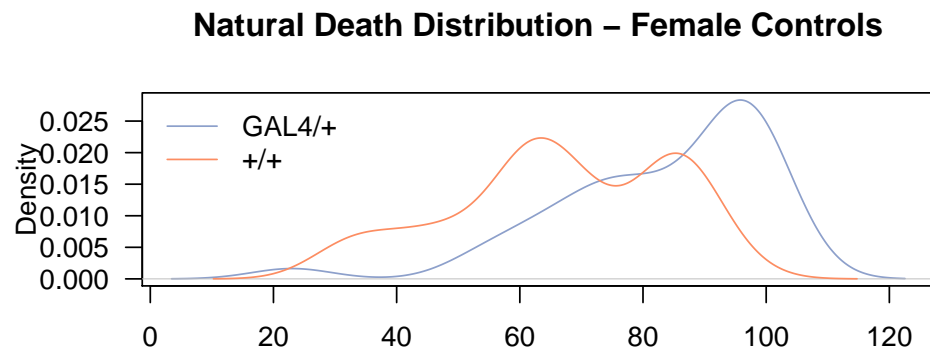

Figure 1: Histogram of Age Ranges for All Genotypes

Natural Death Distribution – Female Controls

Natural Death Distribution – Male Controls

Figure 2: Histogram of Age Ranges for Control Genotypes
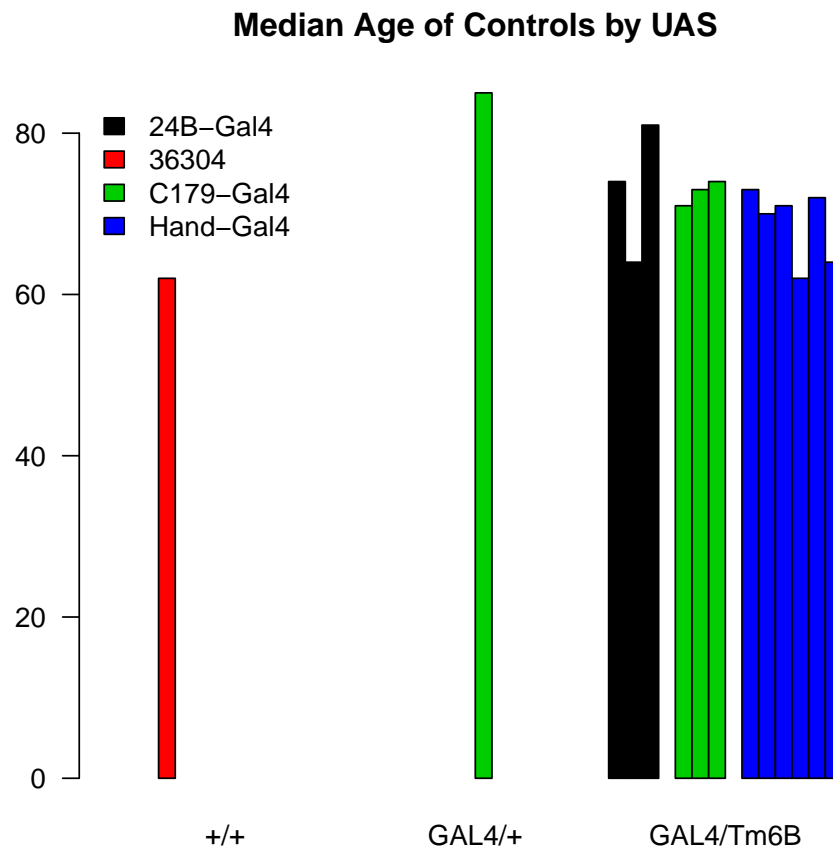
Figure 3: Median Ages for Control Genotypes

Driver	Gene	Gender	shRNA/GAL4	+/+	GAL4/+	GAL4/Tm6B	shRNA/Tm3,sb	Tm3,sb/Tm6B
24B-Gal4	Raptor	female	0	0	0	14	6	6
24B-Gal4	Raptor	male	0	0	0	6	2	4
24B-Gal4	Tsc1	female	712	0	0	536	678	322
24B-Gal4	Tsc1	male	730	0	0	482	600	250
36304	Control	female	0	142	0	0	0	0
36304	Control	male	0	152	0	0	0	0
C179-Gal4	Control	female	0	0	75	0	0	0
C179-Gal4	Control	male	0	0	150	0	0	0
C179-Gal4	Raptor	female	604	0	0	614	0	0
C179-Gal4	Raptor	male	318	0	0	520	0	0
C179-Gal4	Tsc1	female	0	0	0	0	0	0
C179-Gal4	Tsc1	male	0	0	0	0	0	0
Hand-Gal4	Raptor	female	710	0	0	680	888	424
Hand-Gal4	Raptor	male	710	0	0	548	616	268
Hand-Gal4	Tsc1	female	662	0	0	532	510	122
Hand-Gal4	Tsc1	male	576	0	0	366	480	116

Table 2: Total Natural Deaths by Gender for Each Gene and Genotype

24B-Gal4 Driver Analysis

This analysis is only for the three *Tsc1* shRNA alleles, since there were so few births in the *Raptor* knockdown alleles. The summary statistics from this analysis are shown in Table 3.

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null device
          1
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	n	logtest.p	waldtest.p	sctest.p
Tsc1	4304	0.00000	0.00000	0.00000

Table 3: Gene Level Tests for 24B-Gal4 Drivers

	n	logtest.p	waldtest.p	sctest.p
Tsc1 male	2056	0.000000000000000	0.00000	0.00000
Tsc1 female	2248	0.000000000000000	0.00000	0.00000

Table 4: Gene and Gender Level Tests for 24B-Gal4 Drivers

We performed a variety of survival tests for the was a 24B-Gal4 driven *Tsc1* knockdowns (see Tables 3 and 4). These tests removed the Tm3/Tm6B flies, which in all cases died prematurely. The logrank test was highly significant (p-=0) for the combined comparasons and both males (p=0) and females (p=0).

For post-hoc testing, we performed cox proportional hazard tests, shown in Tables 5, 6 and 7. All comparasons were highly significant all less than 0.00044. The instantaneous hazard ratios ranged from 0.47 to 2.16, in terms of that much less likely to die than the knockdown flies. These results are presented graphically in Figures 4 and 5.
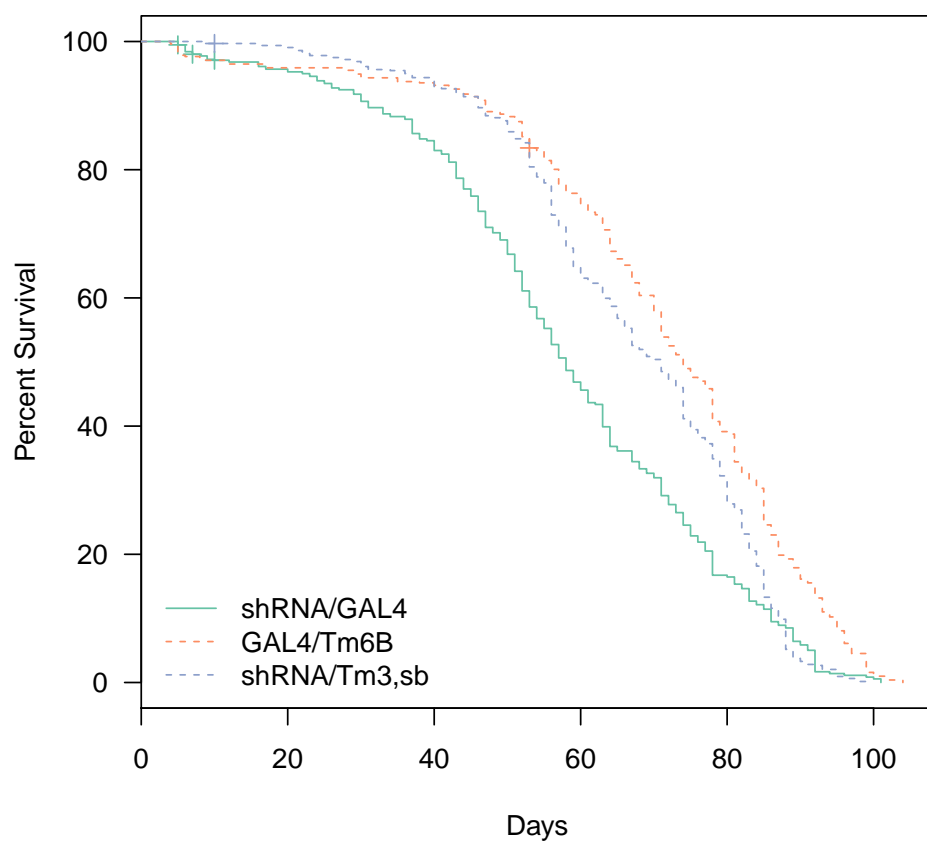
Figure 4: Survival Curve for 24B Driven Tsc1 Knockdown
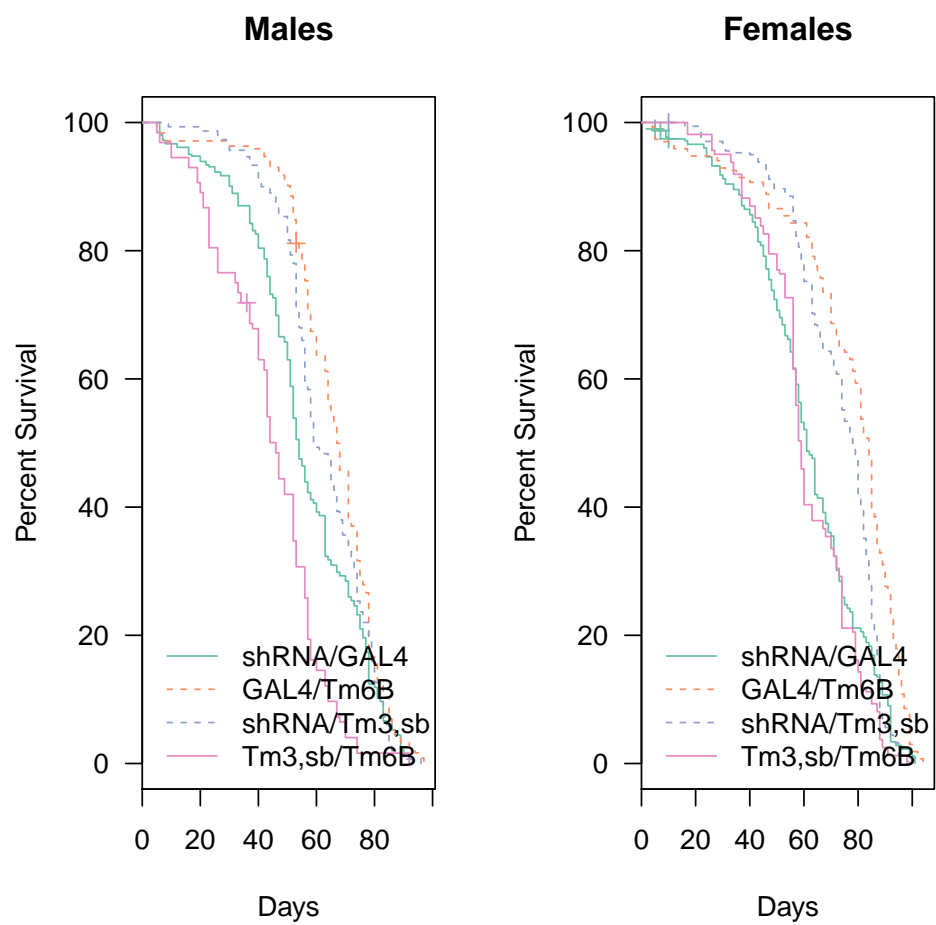
Figure 5: Survival Curves for 24B Driven Tsc1 Knockdown

	Coef	SE	Hazard.Ratio	p
GenotypeGAL4/Tm6B	-0.629	0.042	1.876	0.000000000000000
GenotypeshRNA/Tm3,sb	-0.279	0.039	1.322	0.000000000000545
GenotypeTm3,sb/Tm6B	0.325	0.050	0.723	0.000000000062608

Table 5: Gene Level Cox Proportional Hazard Tests for 24B-Gal4 Drivers and Tsc1 Knockdown

	Coef	SE	Hazard.Ratio	p
GenotypeGAL4/Tm6B	-0.402	0.059	1.495	0.000000000012339
GenotypeshRNA/Tm3,sb	-0.195	0.055	1.215	0.000439707086347
GenotypeTm3,sb/Tm6B	0.756	0.075	0.470	0.000000000000000

Table 6: Gene Level Cox Proportional Hazard Tests for Males with 24B-Gal4 Drivers and Tsc1 Knockdown

C179 Driver Analysis

This analysis is only for the three *Raptor* shRNA alleles, since we did not examine the effects of Tsc1 knockdown with this driver. The summary statistics from this analysis are shown in Table 8.

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        1
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	Coef	SE	Hazard.Ratio	p
GenotypeGAL4/Tm6B	-0.772	0.058	2.163	0.00000000000000000
GenotypeshRNA/Tm3,sb	-0.276	0.054	1.318	0.00000036813820803
GenotypeTm3,sb/Tm6B	0.266	0.068	0.766	0.00008557375959850

Table 7: Gene Level Cox Proportional Hazard Tests for Females with 24B-Gal4 Drivers and Tsc1 Knockdown

	n	logtest.p	waldtest.p	sctest.p
Raptor	2277	0.00000	0.00000	0.00000

Table 8: Gene Level Tests for C179-Gal4 Drivers

We performed a variety of survival tests for the was a C179-Gal4 driven *Raptor* knockdowns (see Tables 8 and 9). These tests removed the Tm3/Tm6B flies, which in all cases died prematurely. The logrank test was not significant (p=0) for the combined comparason and females (p=0). There was a significant difference with the males (p=0).

For post-hoc testing, we performed cox proportional hazard tests, shown in Tables 10, 11 and 12. The instantaneous hazard ratios ranged from 0.74 to 2.95, in terms of that much less likely to die than the knockdown flies. These results are presented graphically in Figures 6 and 7.
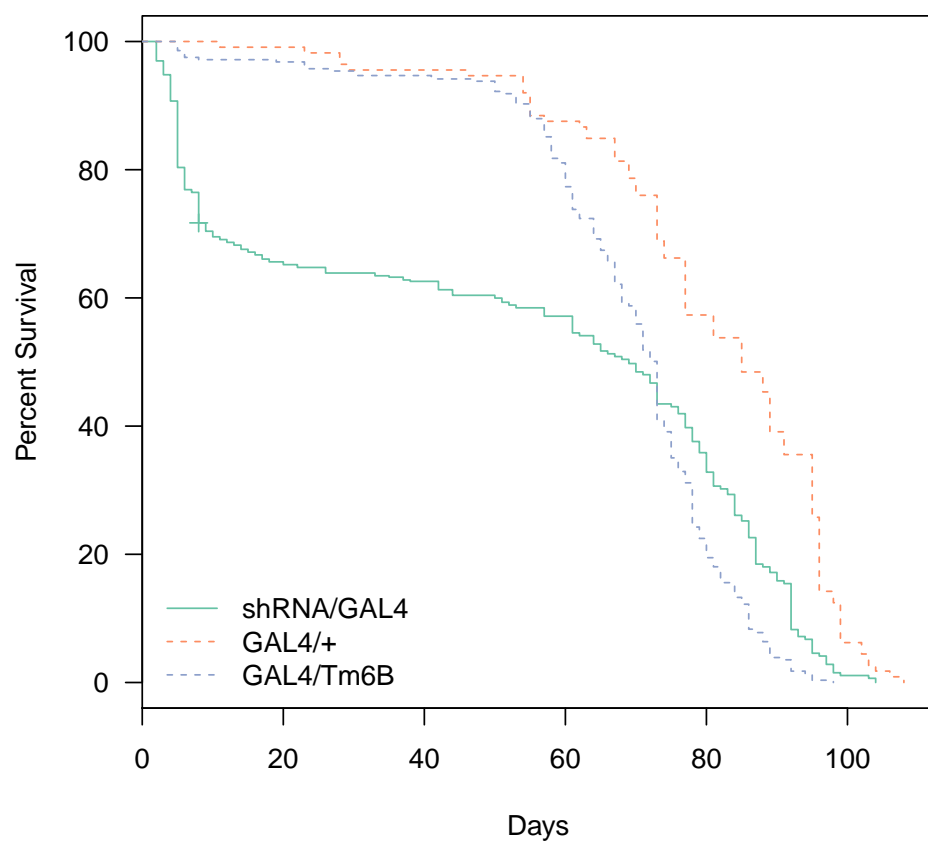
Figure 6: Survival Curve for C179 Driven Raptor Knockdown
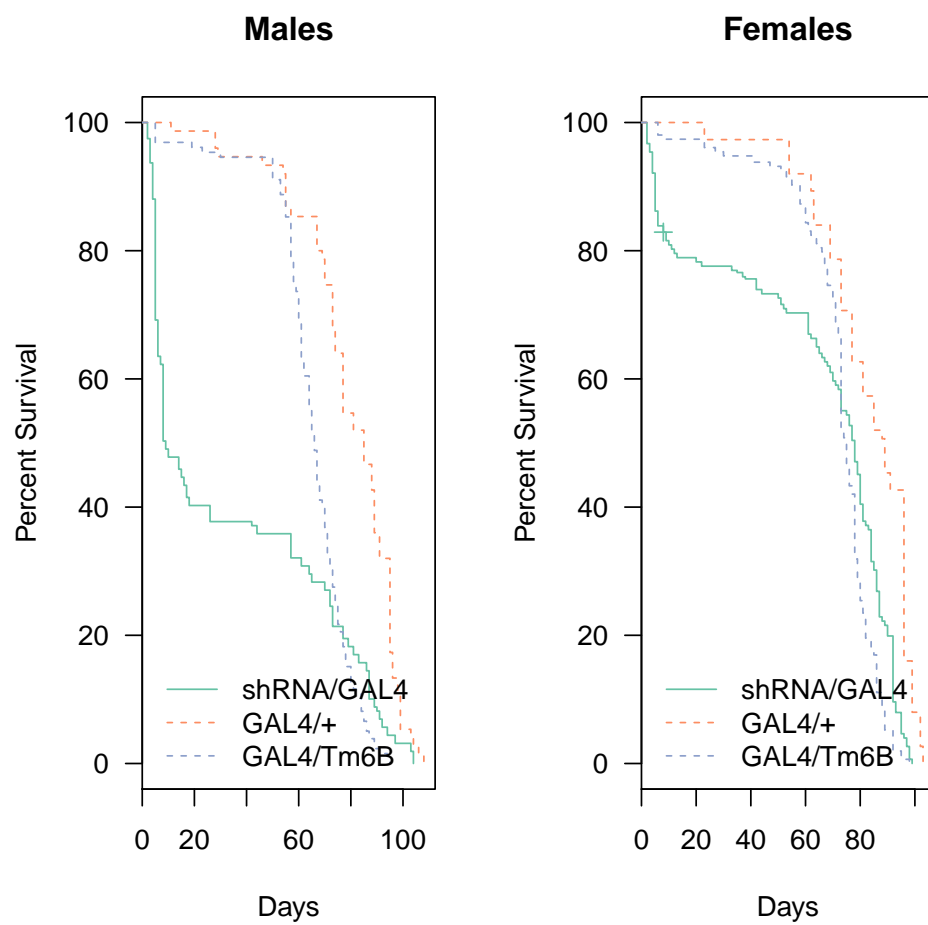
Figure 7: Survival Curves for C179 Driven Raptor Knockdown

	n	logtest.p	waldtest.p	sctest.p
Raptor male	984	0.00000	0.00000	0.00000
Raptor female	1293	0.00000	0.00000	0.00000

Table 9: Gene and Gender Level Tests for C179-Gal4 Drivers

	Coef	SE	Hazard.Ratio	p
GenotypeGAL4/+	-0.777	0.076	2.174	0.000
GenotypeGAL4/Tm6B	0.219	0.046	0.803	0.000

Table 10: Gene Level Cox Proportional Hazard Tests for C179-Gal4 Drivers and Raptor Knockdown

Hand-Gal4 Analysis

This analysis is for both the three *Tsc1* and the three *Raptor* shRNA alleles. The summary statistics from this analysis are shown in Table 13.

	Coef	SE	Hazard.Ratio	p
GenotypeGAL4/+	-1.082	0.101	2.950	0.0000000
GenotypeGAL4/Tm6B	-0.156	0.075	1.169	0.0381241

Table 11: Gene Level Cox Proportional Hazard Tests for Males with C179-Gal4 Drivers and Raptor Knockdown

	Coef	SE	Hazard.Ratio	p
GenotypeGAL4/+	-0.920	0.133	2.509	0.000
GenotypeGAL4/Tm6B	0.307	0.059	0.736	0.000

Table 12: Gene Level Cox Proportional Hazard Tests for Females with C179-Gal4 Drivers and Raptor Knockdown

Tsc1 Knockdown with Hand-Gal4

We performed a variety of survival tests for the was a Hand-Gal4 driven *Tsc1* knockdowns (see Tables 13 and 14). These tests removed the Tm3/Tm6B flies, which in all cases died prematurely. The logrank test was highly significant (p=0) for the combined comparasons and both males (p=0) and females (p=0).

For post-hoc testing, we performed cox proportional hazard tests, shown in Tables 15, 16 and 17. All comparasons were highly significant all less than 0. The instantaneous hazard ratios ranged from 0.16 to 0.78, in terms of that much less likely to die than the knockdown flies. These results are presented graphically in Figures 8 and 9.
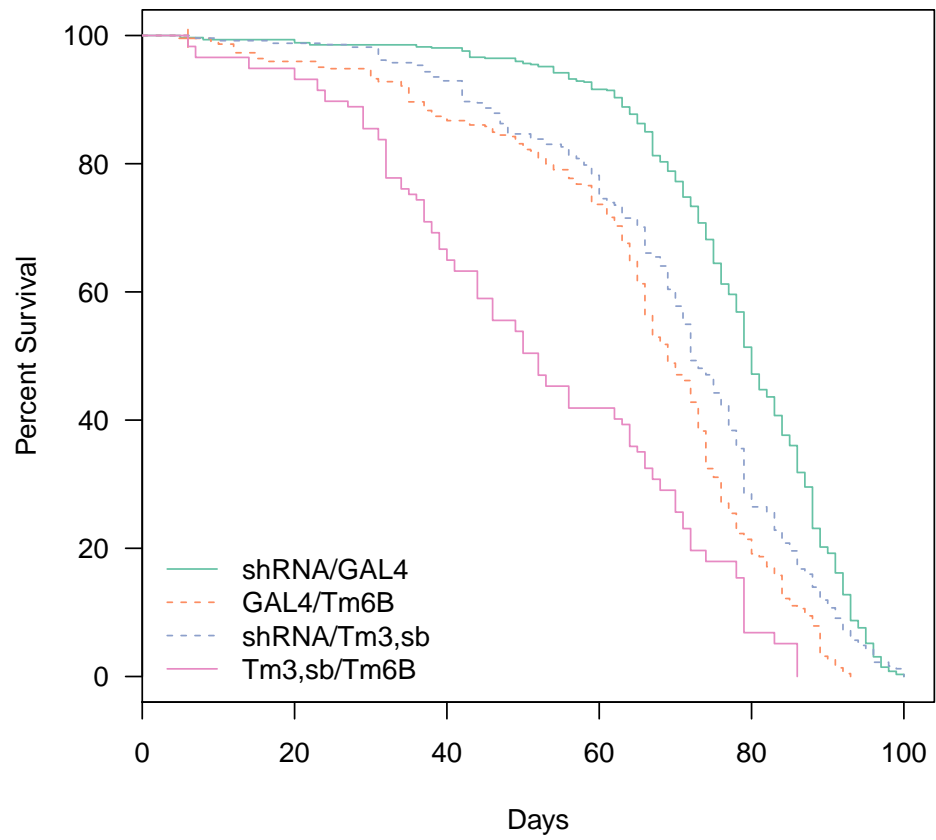
Figure 8: Survival Curve for Hand Driven Tsc1 Knockdown
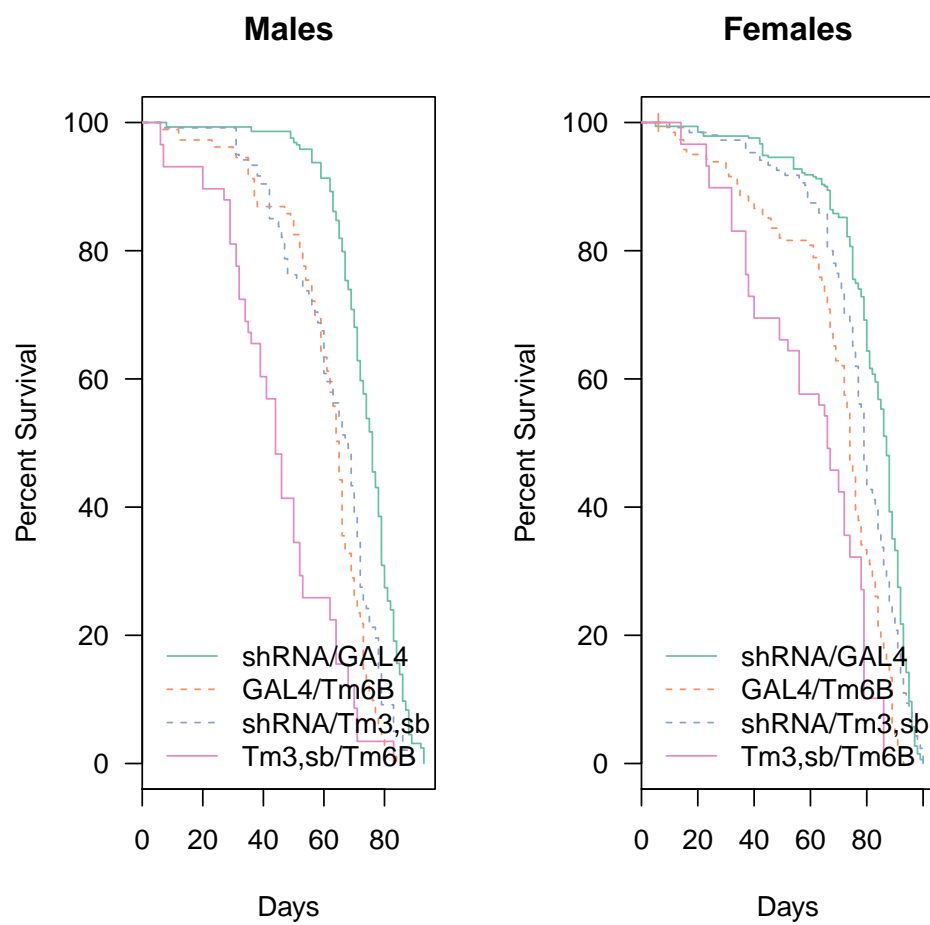
Figure 9: Survival Curves for Hand Driven Tsc1 Knockdown
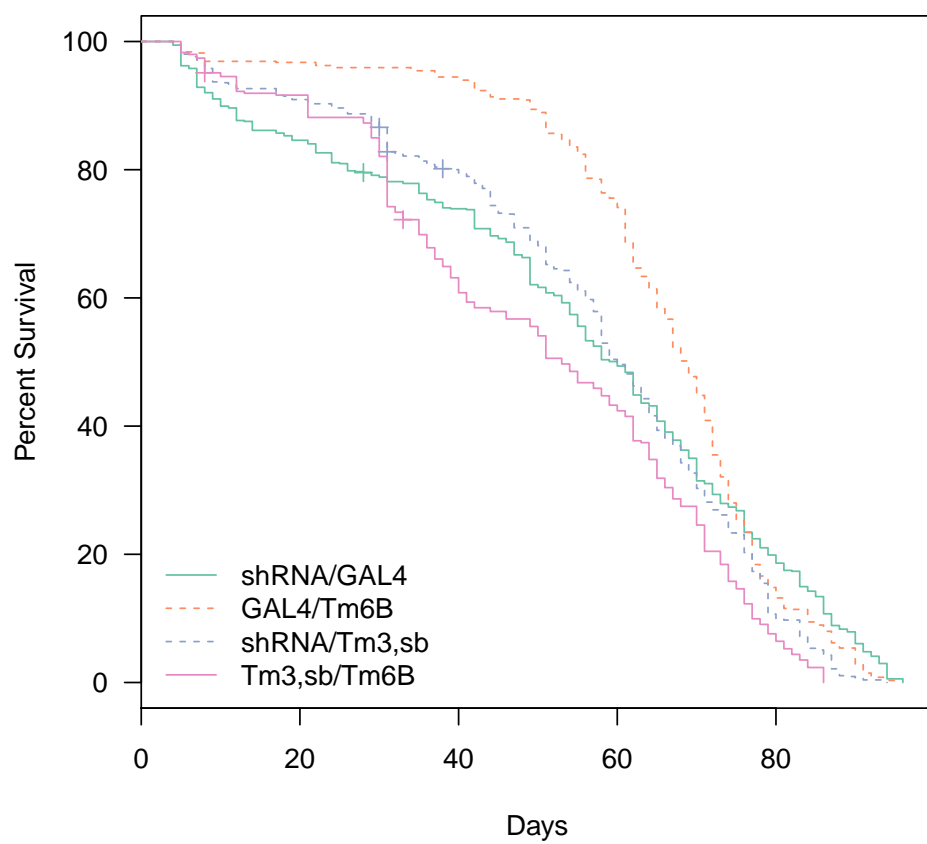
Figure 10: Survival Curve for Hand Driven Raptor Knockdown
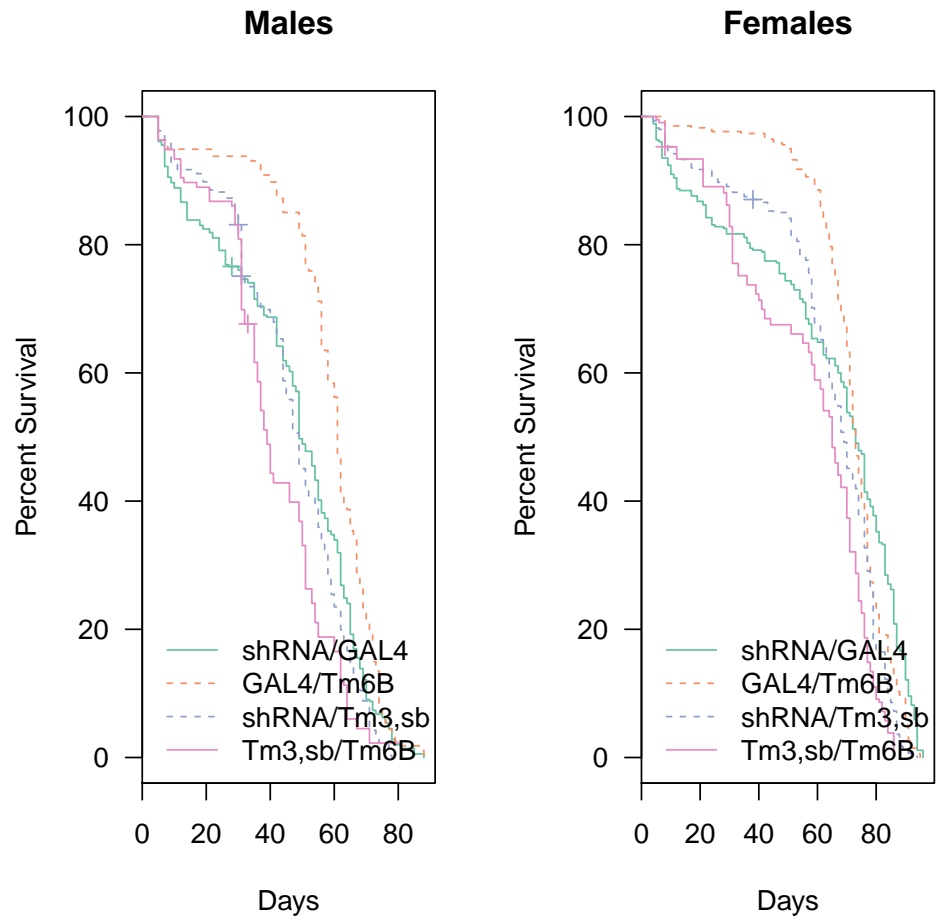
Figure 11: Survival Curves for Hand Driven Raptor Knockdown

	n	logtest.p	waldtest.p	sctest.p
Control				
Raptor	4838	0.0000000000000000	0.00000	0.00000
Tsc1	3350	0.0000000000000000	0.00000	0.00000

Table 13: Gene Level Tests for Hand-Gal4 Drivers

	n	logtest.p	waldtest.p	sctest.p
Tsc1 male	1538	0.0000000000000000	0.00000	0.00000
Tsc1 female	1812	0.0000000000000000	0.00000	0.00000
Raptor male	2142	0.0000000000000000	0.00000	0.00000
Raptor female	2696	0.0000000000000000	0.00000	0.00000

Table 14: Gene and Gender Level Tests for Hand-Gal4 Drivers

Raptor Knockdown with Hand-Gal4

We performed further survival tests for the was a Hand-Gal4 driven *Raptor* knockdowns (see Tables 13 and 14). These tests removed the Tm3/Tm6B flies, which in all cases died prematurely. The logrank test was highly significant (p=0) for the combined comparasons and both males (p=0) and females (p=0).

For post-hoc testing, we performed cox proportional hazard tests, shown in Tables 18, 19 and 20. All comparasons were highly significant all less than 8e-04. The instantaneous hazard ratios ranged from 0.444 to 1.51, in terms of that much less likely to die than the knockdown flies. These results are presented graphically in Figures 10 and 11.

	Coef	SE	Hazard.Ratio	p
GenotypeGAL4/Tm6B	0.766	0.045	0.465	0.000
GenotypeshRNA/Tm3,sb	0.346	0.043	0.708	0.000
GenotypeTm3,sb/Tm6B	1.390	0.073	0.249	0.000

Table 15: Gene Level Cox Proportional Hazard Tests for Hand-Gal4 Drivers and Tsc1 Knockdown

	Coef	SE	Hazard.Ratio	p
GenotypeGAL4/Tm6B	1.062	0.071	0.346	0.00000000000000000
GenotypeshRNA/Tm3,sb	0.680	0.063	0.507	0.00000000000000000
GenotypeTm3,sb/Tm6B	1.826	0.105	0.161	0.00000000000000000

Table 16: Gene Level Cox Proportional Hazard Tests for Males with Hand-Gal4 Drivers and Tsc1 Knockdown

Strain Level Analysis
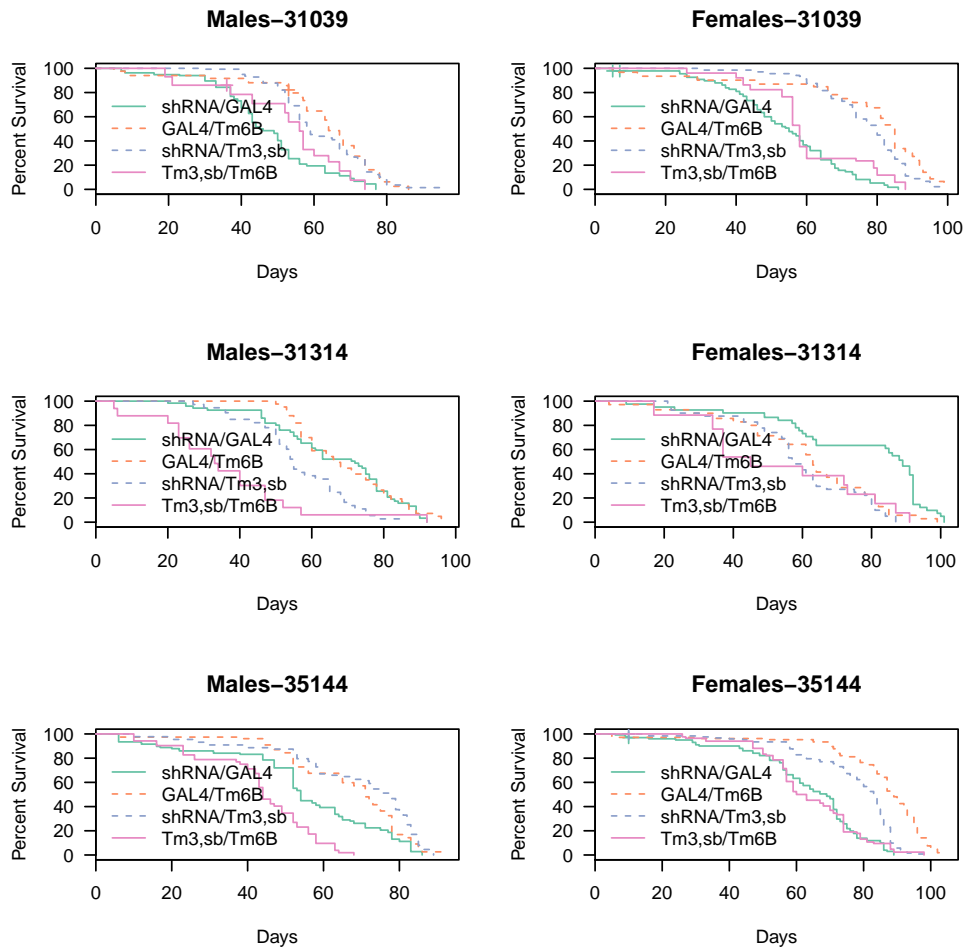
Strain Level Graphs for C179-Gal4 Driven Knockdowns

Figure 12: Survival Curves for 24B Driven Tsc1 Knockdown

	Coef	SE	Hazard.Ratio	p
GenotypeGAL4/Tm6B	0.922	0.062	0.398	0.00000000000000000
GenotypeshRNA/Tm3,sb	0.249	0.059	0.779	0.00002702178604086
GenotypeTm3,sb/Tm6B	1.480	0.104	0.228	0.00000000000000000

Table 17: Gene Level Cox Proportional Hazard Tests for Females with Hand-Gal4 Drivers and Tsc1 Knockdown

	Coef	SE	Hazard.Ratio	p
GenotypeGAL4/Tm6B	-0.081	0.039	1.084	0.0404290946
GenotypeshRNA/Tm3,sb	0.206	0.038	0.814	0.0000000497
GenotypeTm3,sb/Tm6B	0.469	0.048	0.626	0.0000000000

Table 18: Gene Level Cox Proportional Hazard Tests for Hand-Gal4 Drivers and Raptor Knockdown

Strain Level Graphs for C179-Gal4 Driven Knockdowns
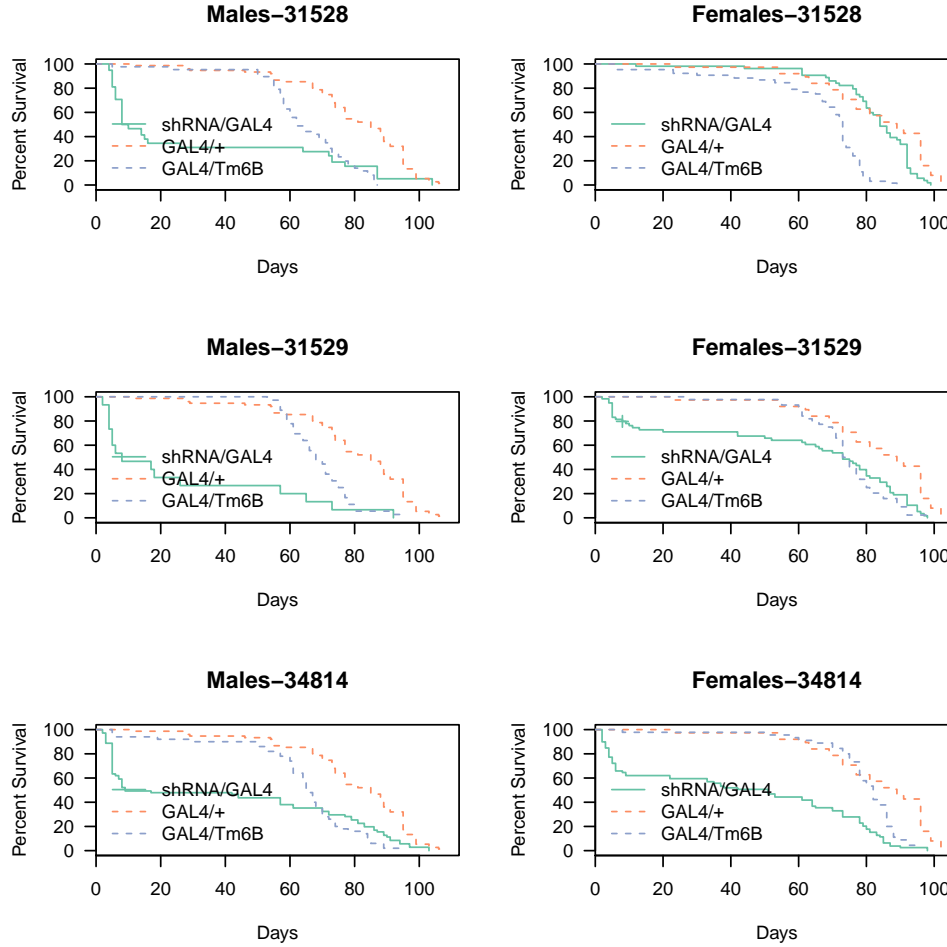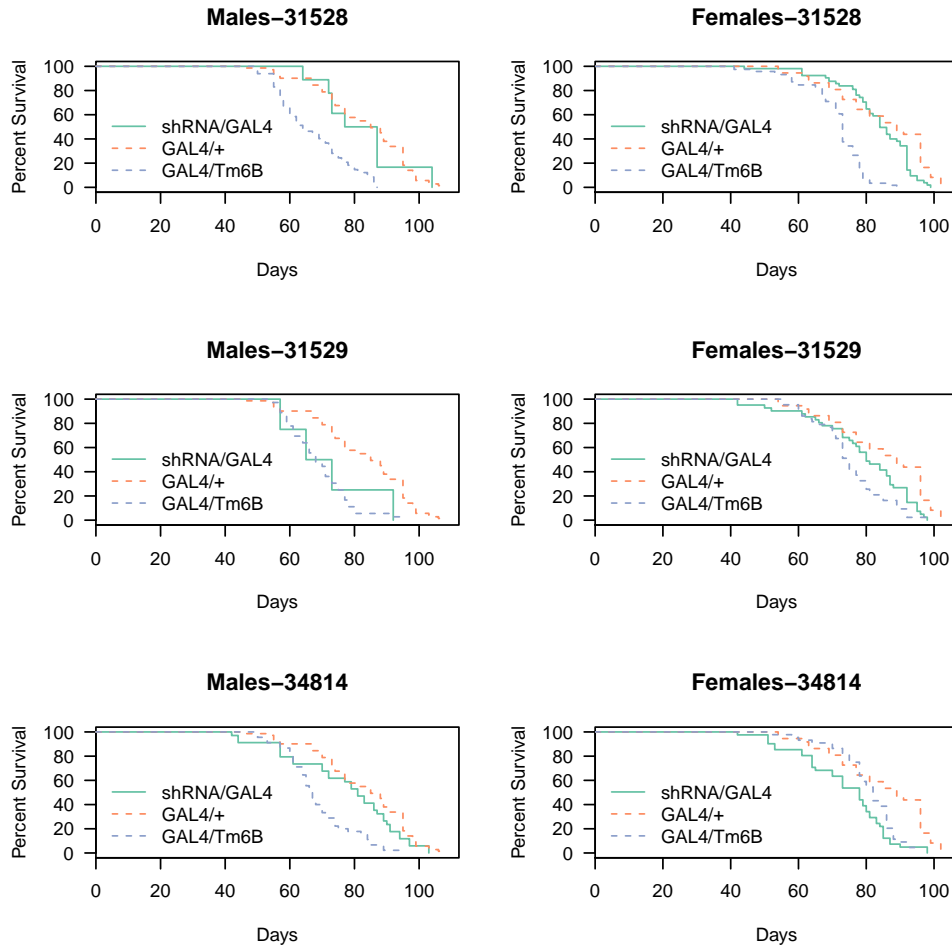
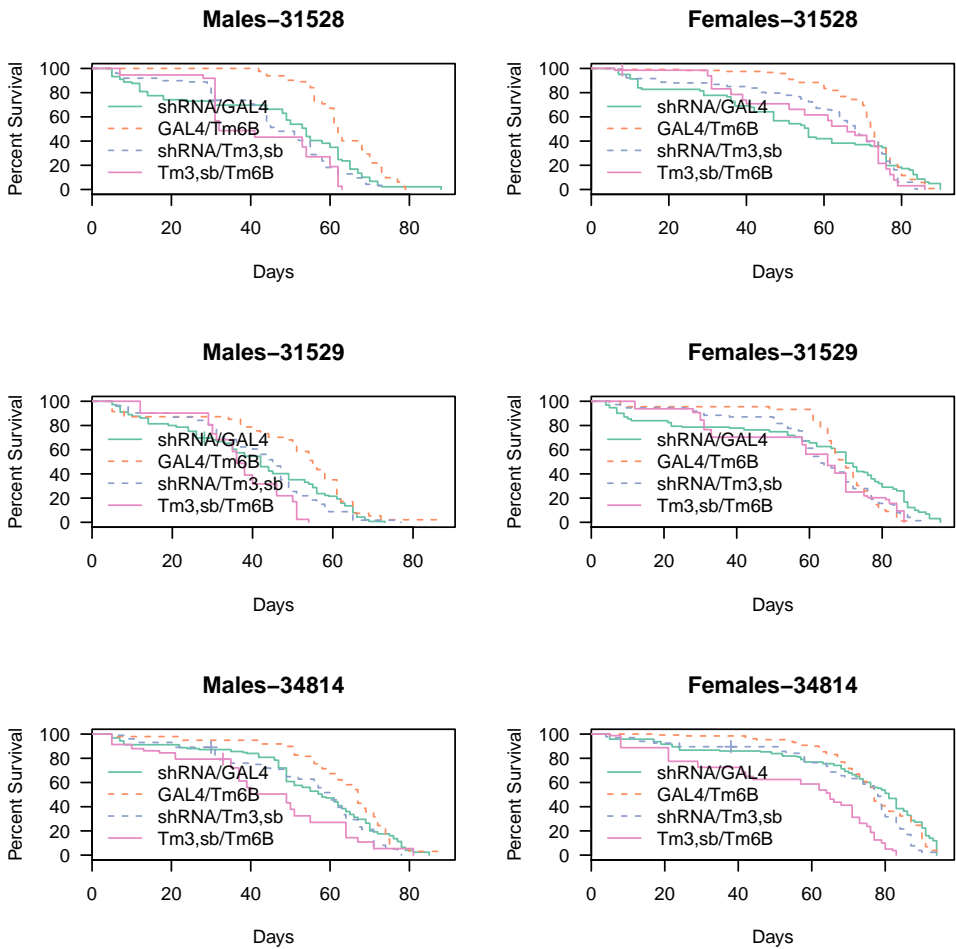|	Coef	SE	Hazard.Ratio	p
GenotypeGAL4/Tm6B	-0.410	0.057	1.508	0.0000000000
GenotypeshRNA/Tm3,sb	0.201	0.056	0.818	0.0002905583
GenotypeTm3,sb/Tm6B	0.474	0.072	0.622	0.0000000001

Table 19: Gene Level Cox Proportional Hazard Tests for Males with Hand-Gal4 Drivers and Raptor Knockdown

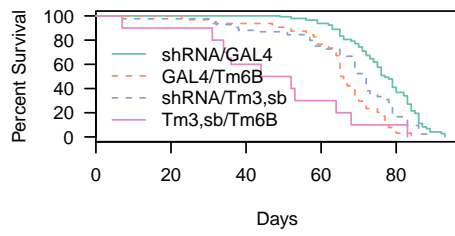|	Coef	SE	Hazard.Ratio	p
GenotypeGAL4/Tm6B	0.184	0.055	0.832	0.0007603801
GenotypeshRNA/Tm3,sb	0.468	0.052	0.626	0.0000000000
GenotypeTm3,sb/Tm6B	0.811	0.065	0.444	0.0000000000

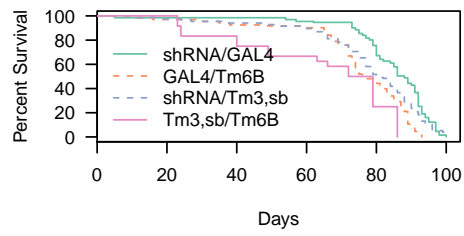Table 20: Gene Level Cox Proportional Hazard Tests for Females with Hand-Gal4 Drivers and Raptor Knockdown

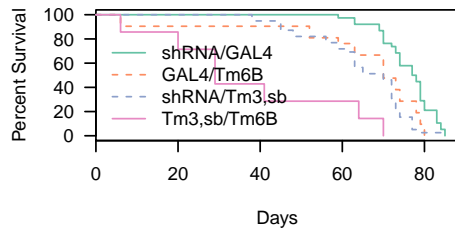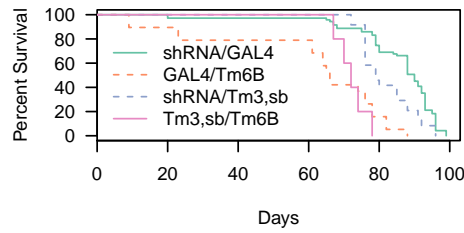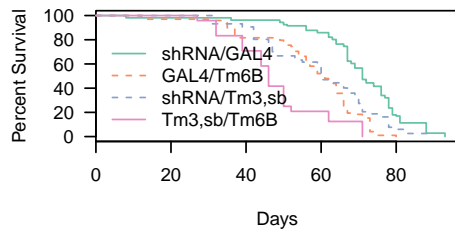# Strain Level Graphs for Hand-Gal4 Driven Knockdowns

Males−31528

Females−31528

Males−31529

Females−31529

Males−34814

Females−34814

Males−31039

Percent Survival vs Days

- shRNA/GAL4
- GAL4/Tm6B
- shRNA/Tm3,sb
- Tm3,sb/Tm6B

Females−31039

Percent Survival vs Days

- shRNA/GAL4
- GAL4/Tm6B
- shRNA/Tm3,sb
- Tm3,sb/Tm6B

Males−31314

Percent Survival vs Days

- shRNA/GAL4
- GAL4/Tm6B
- shRNA/Tm3,sb
- Tm3,sb/Tm6B

Females−31314

Percent Survival vs Days

- shRNA/GAL4
- GAL4/Tm6B
- shRNA/Tm3,sb
- Tm3,sb/Tm6B

Males−35144

Percent Survival vs Days

- shRNA/GAL4
- GAL4/Tm6B
- shRNA/Tm3,sb
- Tm3,sb/Tm6B

Females−35144

Percent Survival vs Days

- shRNA/GAL4
- GAL4/Tm6B
- shRNA/Tm3,sb
- Tm3,sb/Tm6B

References

[1] R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, 2013.

[2] Garrett Grolemund and Hadley Wickham. Dates and times made easy with lubridate. *Journal of Statistical Software*, 40(3):1–25, 2011.

[3] Hadley Wickham. The split-apply-combine strategy for data analysis. *Journal of Statistical Software*, 40(1):1–29, 2011.

[4] Terry M Therneau. *A Package for Survival Analysis in S*, 2013. R package version 2.37-4.

[5] Terry M. Therneau and Patricia M. Grambsch. *Modeling Survival Data: Extending the Cox Model*. Springer, New York, 2000.

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 0.3-5, bitops 1.0-5, lubridate 1.3.0, plyr 1.8, RColorBrewer 1.0-5, RCurl 1.95-4.1, reshape2 1.2.2, survival 2.37-4, xtable 1.7-1

- Loaded via a namespace (and not attached): digest 0.6.3, memoise 0.1, stringr 0.6.2, tools 3.0.2