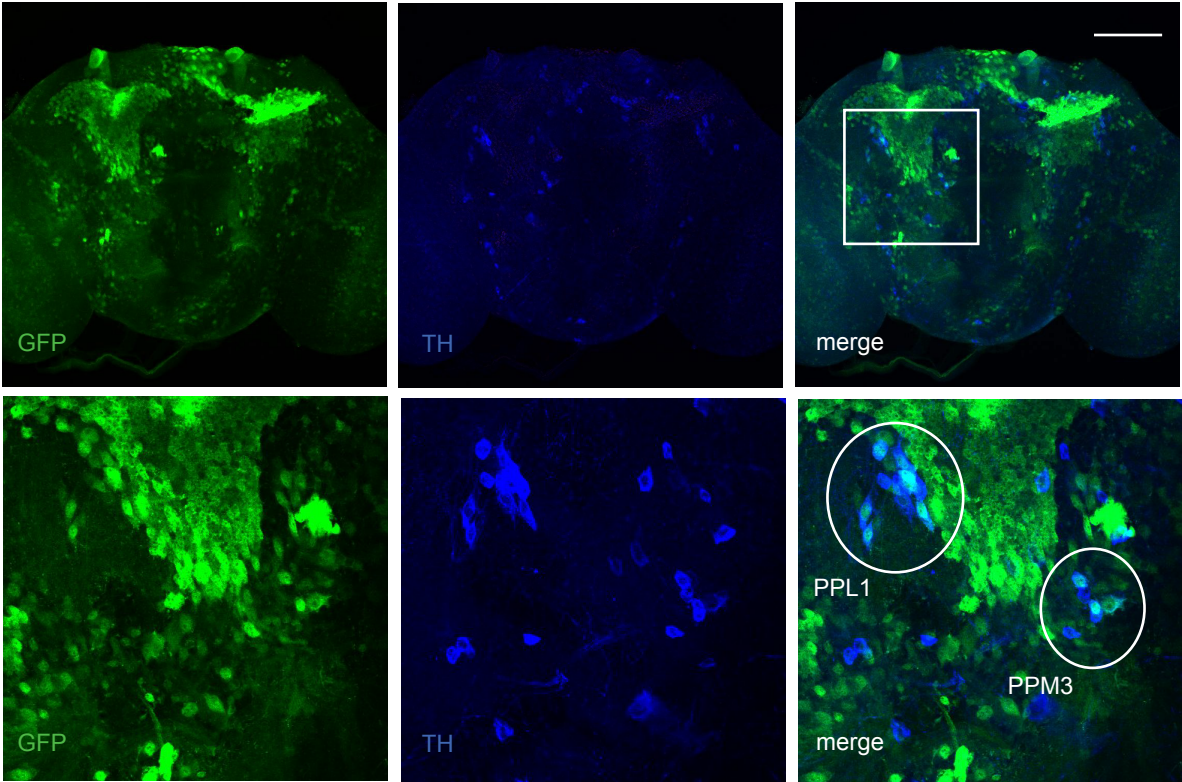
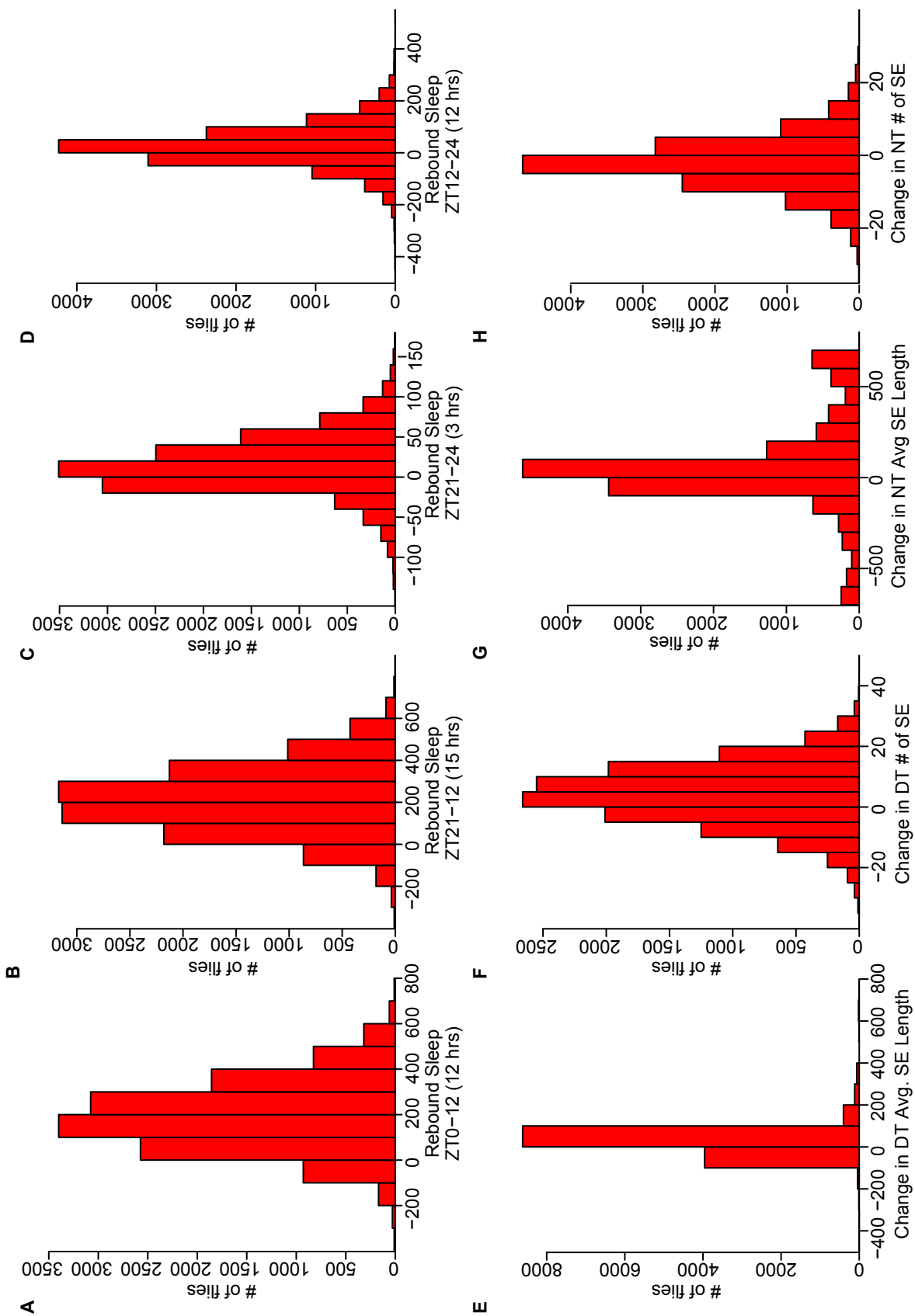


Figure S1



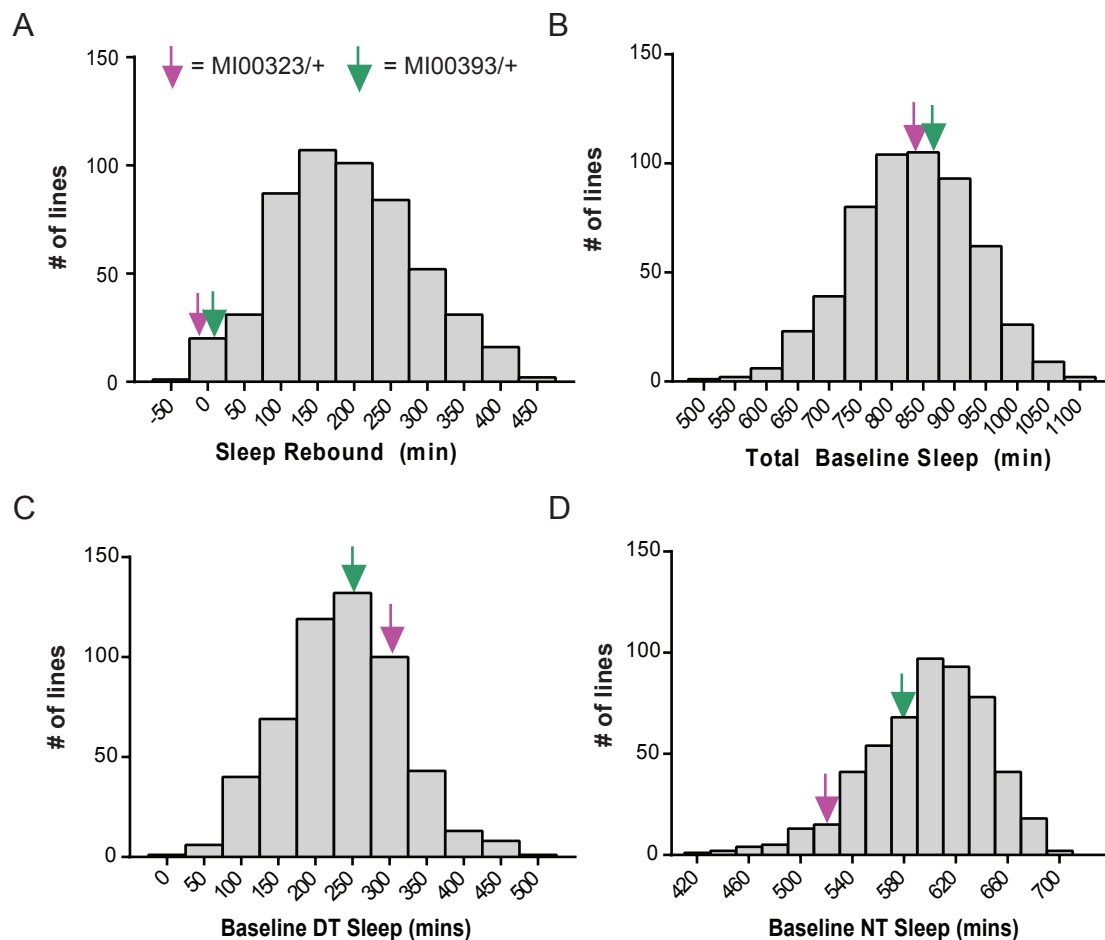
**Figure S1.** Coexpression of 104906-Gal4 with dopaminergic cells  
104906-Gal4>UAS-nGFP brains exhibit widespread GFP expression. Costaining with an anti-TH antibody reveals coexpression of GFP and TH in the PPL1 and PPM3 clusters. Scale bar = 100  $\mu$ m.

Figure S2



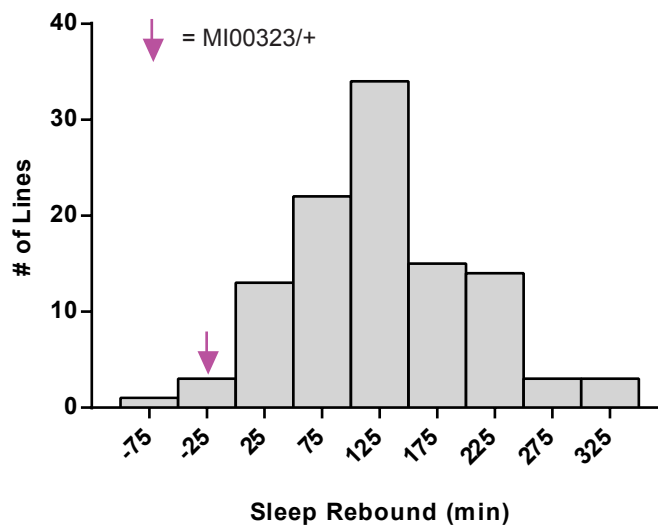
**Figure S2.** Changes in sleep parameters after thermogenetic sleep deprivation. **(A-D)** Histograms showing change in sleep amount for individual flies in the primary screen. An increase in sleep amount (rebound) relative to baseline is observed from Zeitgeber time (ZT)0-12 **(A)** and over the 15-h period from ZT21-12 in almost all flies **(B)**. Net rebound sleep during the 3 h immediately following sleep deprivation (ZT21-24) **(C)** and the first full night following sleep deprivation (ZT12-24) **(D)** can also be observed, but are less consistent, with many flies showing the opposite trend. **(E-H)** Histograms showing change in sleep bout architecture for individual flies in primary screen. During the day, flies tend to have longer **(E)** and a greater number **(F)** of sleep bouts following sleep deprivation. During the following night, sleep becomes more consolidated, with longer bouts **(G)** and fewer of them **(H)**. As with some of the sleep amount parameters discussed here, these trends are observed for the data set as a whole, but are not consistent across individual flies, with many flies exhibiting the opposite relationship.

**Figure S3**



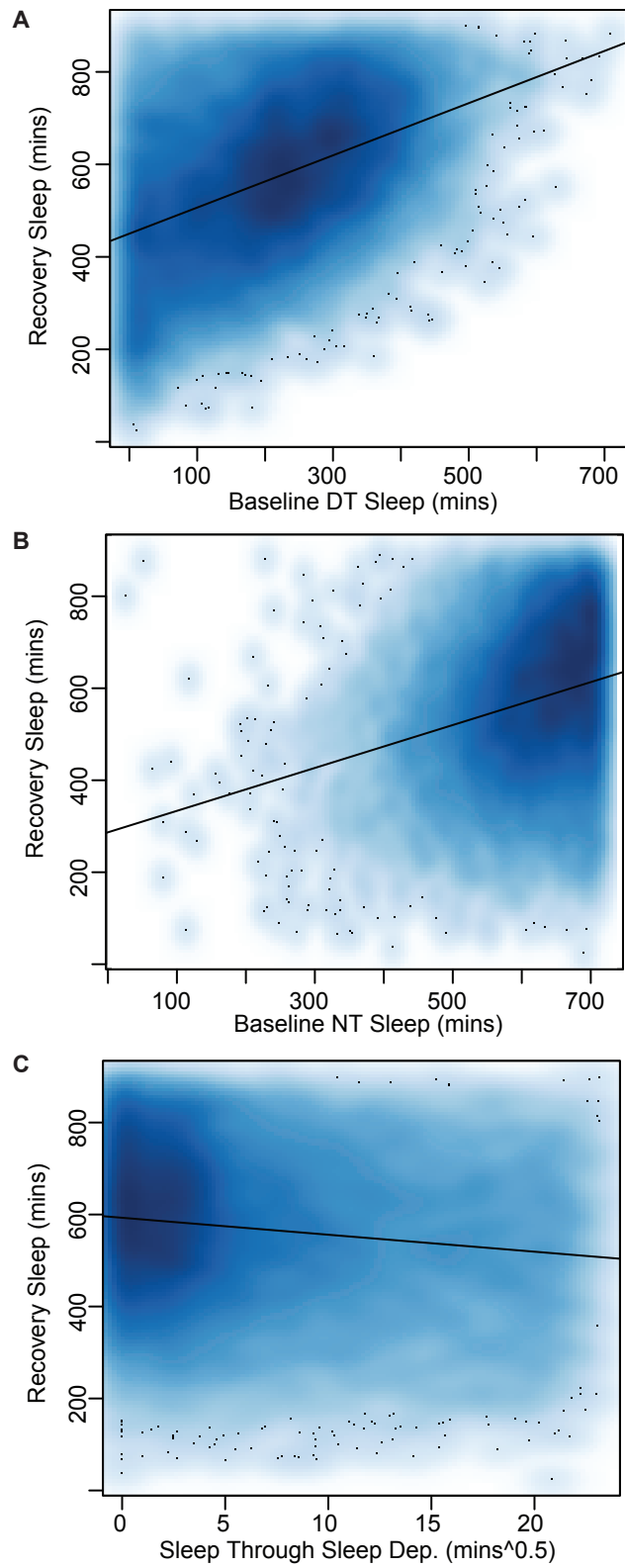
**Figure S3.** Baseline sleep is not reduced in hits with reduced rebound compared to other MiMIC lines tested in the screen. **(A-D)** Histograms depicting values for **(A)** sleep recovered (Zeitgeber time 21-12) **(B)** total baseline sleep amount, **(C)** baseline daytime (DT) and **(D)** baseline nighttime (NT) sleep for all MiMIC lines run as heterozygotes in the screen. Arrows depict baseline sleep for MI00323/+ and MI00393/+ in the first four independent experiments for each of these lines, run in parallel with the primary screen. Overall baseline sleep values for MI00323/+ and MI00393/+ are within the normal range compared to other lines tested in the screen. We do observe a shift in the timing of sleep for MI00323/+ toward greater DT sleep, which we discuss in more detail later. Although in this data set we observe a reduction in baseline NT sleep for MI00323/+, we do not see significant differences in baseline NT sleep between this line and a control line discussed in Figure 4.

Figure S4



**Figure S4.** Histogram of rebound sleep for all heterozygous MiMIC lines that displayed high daytime baseline sleep (>300 min). In the primary screen MI00323/+ did not have daytime sleep above 300 min but would have still been an outlier for recovery sleep when compared with other MiMIC insertions with high daytime baseline sleep.

Figure S5



**Figure S5.** Relationships of recovery sleep with baseline sleep and sleep through thermogenetic sleep deprivation. **(A-C)** Smoothed scatter plots depict relationships between sleep during the recovery period and **(A)** baseline daytime (DT) sleep **(B)** baseline nighttime (NT) sleep and **(C)** square root transformation (see Table 3) of sleep through the thermogenetic stimulation (sleep through sleep deprivation) in primary screen.

**Table S1**

	# Genotypes	# Flies	Sleep Lost	Sleep Rebound (15hrs)	Baseline Sleep (Night)	Baseline Sleep (Day)	Sleep Through Sleep Dep.	Recovery Sleep (15hrs)
KG het	308	2340	441.8 ± 74.1	227.8 ± 144.6	607.1 ± 68.8	248.7 ± 107.4	24 ± 56.1	617.6 ± 147.9
KG III	263	1923	285.2 ± 155.8	222.7 ± 162.6	638.4 ± 73.3	199.8 ± 124.9	192.2 ± 160.9	583.2 ± 166.5
KG X	141	1019	336.3 ± 138	207.8 ± 149.4	527.3 ± 114.1	209.4 ± 119	59.5 ± 109.4	548.7 ± 156.4
MI het	566	4343	413.5 ± 100.9	195.8 ± 152.9	596.1 ± 80.1	229.7 ± 117	40.8 ± 79.5	567.3 ± 148.8
MI III	430	3177	356.8 ± 138	206.1 ± 161.8	631.9 ± 84.6	162.7 ± 132.4	116.7 ± 129	527.2 ± 180
MI X	58	443	362 ± 118.7	270.3 ± 172.7	536.9 ± 103.2	229.1 ± 121.2	51.3 ± 91.4	621.7 ± 152

Summary statistics (mean ± standard deviation, all values in minutes) for all collections of insertions screened. "Het" designates heterozygous insertion mutants and III and X indicate homozygous insertions on the 3rd and X chromosomes.



**Table S2**

Group	Variable	$\beta$ coefficient	Standard Error	P-value
MI Het	Intercept (mins)	567.3	2.01	<0.001
MI Het	Daytime Sleep	0.40	0.018	<0.001
MI Het	Nighttime Sleep	0.49	0.026	<0.001
MI Het	Sleep Through Sleep Dep.* (mins <sup>0.5</sup> )	-4.07	0.434	<0.001
MI (III)	Intercept (mins)	527.2	2.67	<0.001
MI (III)	Daytime Sleep	0.66	0.021	<0.001
MI (III)	Nighttime Sleep	0.40	0.032	<0.001
MI (III)	Sleep Through Sleep Dep.* (mins <sup>0.5</sup> )	-5.67	0.449	<0.001
MI (X)	Intercept (mins)	621.7	6.65	<0.001
MI (X)	Daytime Sleep	0.19	0.058	<0.001
MI (X)	Nighttime Sleep	0.47	0.068	0.0011
MI (X)	Sleep Through Sleep Dep.* (mins <sup>0.5</sup> )	-3.99	1.247	0.0015
KG het	Intercept (mins)	617.6	2.70	<0.001
KG het	Daytime Sleep	0.47	0.026	<0.001
KG het	Nighttime Sleep	0.52	0.041	<0.001
KG het	Sleep Through Sleep Dep.* (mins <sup>0.5</sup> )	-4.98	0.707	<0.001
KG (III)	Intercept (mins)	583.2	3.1	<0.001
KG (III)	Daytime Sleep	0.53	0.025	<0.001
KG (III)	Nighttime Sleep	0.59	0.045	<0.001
KG (III)	Sleep Through Sleep Dep.* (mins <sup>0.5</sup> )	-9.92	0.469	<0.001
KG (X)	Intercept (mins)	548.66	4.23	<0.001
KG (X)	Daytime Sleep	0.47	0.039	<0.001
KG (X)	Nighttime Sleep	0.33	0.041	<0.001
KG (X)	Sleep Through Sleep Dep.* (mins <sup>0.5</sup> )	-2.67	0.756	<0.001

Regression of recovery sleep against daytime and nighttime baseline sleep and sleep through sleep deprivation for each collection of insertions screened. \* = square root transformation. Baseline daytime (DT) sleep, baseline nighttime (NT) sleep, and sleep through the thermogenetic stimulation (sleep through sleep dep.) were regressed on recovery sleep in a multiple linear regression model.  $\beta$  coefficients and standard error for coefficients are reported in the table.

**Table S3**

Group	Model	Variable	RMSE (mins)	R <sup>2</sup>	adjusted R <sup>2</sup>	ΔRMSE (mins)	ΔR <sup>2</sup>	Δadjusted R <sup>2</sup>
MI Het	Reduced Model	Intercept	148.8	-	-	-	-	-
MI Het	Reduced Model	Experimental Run	139.6	0.124	0.120	9.2	0.124	0.120
MI Het	Reduced Model	Genotype	122.1	0.414	0.327	17.5	0.290	0.207
MI Het	Full Model	Intercept	148.8	-	-	-	-	-
MI Het	Full Model	Daytime Sleep	139.0	0.128	0.127	9.8	0.128	0.127
MI Het	Full Model	Nighttime Sleep	134.0	0.190	0.190	5.0	0.062	0.062
MI Het	Full Model	Sleep Through Sleep Dep*	132.7	0.206	0.205	1.3	0.016	0.016
MI Het	Full Model	Experimental Run	126.5	0.281	0.277	6.2	0.075	0.072
MI Het	Full Model	Genotype	112.7	0.502	0.427	13.8	0.220	0.149
MI (III)	Reduced Model	Intercept	180.0	-	-	-	-	-
MI (III)	Reduced Model	Experimental Run	174.5	0.066	0.060	5.5	0.066	0.060
MI (III)	Reduced Model	Genotype	144.3	0.444	0.358	30.3	0.378	0.298
MI (III)	Full Model	Intercept	180.0	-	-	-	-	-
MI (III)	Full Model	Daytime Sleep	157.1	0.238	0.238	22.9	0.238	0.238
MI (III)	Full Model	Nighttime Sleep	154.3	0.265	0.265	2.8	0.027	0.027
MI (III)	Full Model	Sleep Through Sleep Dep*	150.6	0.300	0.300	3.7	0.035	0.035
MI (III)	Full Model	Experimental Run	147.9	0.330	0.325	2.7	0.029	0.025
MI (III)	Full Model	Genotype	128.7	0.559	0.489	19.2	0.229	0.164
MI (X)	Reduced Model	Intercept	152.0	-	-	-	-	-
MI (X)	Reduced Model	Genotype	136.9	0.29	0.19	15.1	0.293	0.189
MI (X)	Full Model	Intercept	152.0	-	-	-	-	-
MI (X)	Full Model	Daytime Sleep	148.3	0.050	0.048	3.7	0.050	0.048
MI (X)	Full Model	Nighttime Sleep	141.4	0.138	0.134	6.8	0.087	0.085
MI (X)	Full Model	Sleep Through Sleep Dep*	140.0	0.157	0.152	1.5	0.020	0.018
MI (X)	Full Model	Genotype	123.8	0.427	0.336	16.2	0.269	0.185

Variance explained by variables in a hierarchical multiple linear regression model for each collection of insertions screened. \* = square root transformation. For both the reduced model and the full model, variables were added hierarchically in the order listed based on expected biological relationships. The total Root Mean Square Error (RMSE), R<sup>2</sup>, and adjusted R<sup>2</sup> for the models after the addition of each variable, and the change in these values compared to the model without the variable are reported. All reported variables significantly improved the model with p<0.001 (variance ratio test).

**Table S3 (con't)**

Group	Model	Variable	RMSE (mins)	R <sup>2</sup>	adjusted R <sup>2</sup>	ΔRMSE (mins)	ΔR <sup>2</sup>	Δadjusted R <sup>2</sup>
KG Het	Reduced Model	Intercept	147.9	-	-	-	-	-
KG Het	Reduced Model	Experimental Run	142.6	0.075	0.071	5.4	0.075	0.071
KG Het	Reduced Model	Genotype	116.4	0.462	0.381	26.2	0.387	0.310
KG Het	Full Model	Intercept	147.9	-	-	-	-	-
KG Het	Full Model	Daytime Sleep	135.7	0.159	0.158	12.2	0.159	0.158
KG Het	Full Model	Nighttime Sleep	131.8	0.206	0.206	3.9	0.047	0.047
KG Het	Full Model	Sleep Through Sleep Dep.*	130.5	0.223	0.222	1.3	0.016	0.016
KG Het	Full Model	Experimental Run	125.3	0.287	0.283	5.2	0.064	0.061
KG Het	Full Model	Genotype	106.1	0.553	0.485	19.1	0.266	0.202
KG (III)	Reduced Model	Intercept	166.5	-	-	-	-	-
KG (III)	Reduced Model	Experimental Run	163.8	0.037	0.031	2.6	0.037	0.031
KG (III)	Reduced Model	Genotype	134.3	0.438	0.349	29.6	0.401	0.318
KG (III)	Full Model	Intercept	166.5	-	-	-	-	-
KG (III)	Full Model	Daytime Sleep	152.0	0.166	0.166	14.4	0.166	0.166
KG (III)	Full Model	Nighttime Sleep	150.1	0.188	0.187	1.9	0.022	0.021
KG (III)	Full Model	Sleep Through Sleep Dep.*	135.2	0.341	0.340	14.9	0.154	0.153
KG (III)	Full Model	Experimental Run	132.0	0.376	0.371	3.2	0.034	0.031
KG (III)	Full Model	Genotype	116.0	0.581	0.514	16.0	0.206	0.143
KG (X)	Reduced Model	Intercept	156.4	-	-	-	-	-
KG (X)	Reduced Model	Experimental Run	151.9	0.062	0.056	4.5	0.062	0.056
KG (X)	Reduced Model	Genotype	138.0	0.328	0.221	13.9	0.266	0.165
KG (X)	Full Model	Intercept	156.4	-	-	-	-	-
KG (X)	Full Model	Daytime Sleep	140.1	0.199	0.198	16.3	0.199	0.198
KG (X)	Full Model	Nighttime Sleep	135.9	0.246	0.245	4.2	0.048	0.047
KG (X)	Full Model	Sleep Through Sleep Dep.*	135.1	0.256	0.253	0.8	0.009	0.008
KG (X)	Full Model	Experimental Run	132.9	0.284	0.277	2.2	0.028	0.024
KG (X)	Full Model	Genotype	123.8	0.461	0.373	9.1	0.177	0.096