**Figure S1. q-q plots for GWAS.** q-q plots generated by the DGRP2 webtools are shown for the basal activity analysis (A-H) and the induced activity analysis (I-P).

**A. and I.** Phenotype averaged across both sexes, mixed model p-values.

**B. and J**. Phenotype averaged across both sexes, regression p-values.

**C. and K.** Phenotypic difference between sexes, mixed model p-values.

**D. and L.** Phenotypic difference between sexes, regression p-values.

**E. and M.** Female data, mixed model p-values.

**F. and N.** Female data, regression p-values.

**G. and O.** Male data, mixed model p-values.

**H. and P.** Male data, regression p-values.

**Figure S2. Histograms illustrating the variability in activity levels among the lines of the DGRP population.** All lines binned based on their mean activity levels (average number of beam crossings recorded by the activity monitor in a 5-minute interval; bin size: 10). The bins are indicated on the X-axis (average number of beam crosses recorded by the activity monitor in a 5 minute interval), and the number of lines in each bin is shown on the Y-axis.

**A.** Histogram for basal activity in females.

**B.** Histogram for induced activity in females.

**C.** Histogram for basal activity in males.

**D.** Histogram for induced activity in males.

**Figure S3. Correlation between activity levels and lifespan.** To investigate the relationship between animal activity and lifespan, animal activity levels (X-axis, average number of beam crossings recorded by the activity monitor in a 5-minute interval) are plotted against life span (Y-axis, days) for each DGRP strain.

**A.** Durham et al data - basal activity.

**B.** Durham et al data - induced activity.

**C.** Ivanov et al data – basal activity.

**D.** Ivanov et al data – induced activity.

**Figure S4. Genetic variants associated with basal and induced activity levels in a GWAS using combined data from both sexes.** Chromosomal location of genetic variants (X-axis) are plotted against the negative log of the p-value testing for the likelihood of the variant being associated with the measured phenotype (Y-axis). The blue line in each plot marks the p=10-5 significance level, while the red line marks p=10-7.

**A.** Manhattan plot for basal activity.

**B.** Manhattan plot for induced activity.

**Figure S5. Small areas of linkage disequilibrium (LD) exist for the genetic variants associated with basal and exercise-induced activity.**

Heat maps illustrating the extent of LD among the genetic variants identified as significantly contributing to the basal (**A**) and exercise-induced (**B**) activity levels. The level of LD is indicated by the color of each square with red indicating the highest level of LD and blue indicating the absence of LD. The genetic variants are plotted based on their chromosomal location (X-and Y-axis), with the diagonal showing areas of with the strongest LD due to close physical proximity.

**Figure S6. Knockdown of the polycomb group genes *Jarid2* and *Su(z)2* in muscle has limited impact on increased activity levels.** *Jarid2* and *Su(z)2* levels are decreased by expressing a UAS-controlled short hairpin construct targeting the gene of interest with a muscle Gal4 driver (*Mef2*). In all strip charts, mean activity levels are plotted on the Y-axis, with data from males shown in blue and data from females shown in red. The black dot marks the mean with the bars showing one standard deviation. Only for the induced activity data from the female *Su(z)2* knockdown is significantly altered compared to the parent strains (**D**).

**A.**  Strip chart showing the basal activity levels of animals with lower levels of *Jarid2* in muscle (*Jarid2* KD) as well as their parents (UAS siRNA line; muscle Gal4 line).

**B.** Strip chart showing the induced activity levels of animals with lower levels of *Jarid2* in muscle (*Jarid2* KD) as well as their parents (UAS siRNA line; muscle Gal4 line).

**C.** Strip chart showing the basal activity levels of animals with lower levels of *Su(z)2* in muscle (*Su(z)2* KD) as well as their parents (UAS siRNA line; muscle Gal4 line).

**D.** Box plot showing the induced activity levels of animals with lower levels of *Su(z)2* in muscle (*Su(z)2* KD) as well as their parents (UAS siRNA line; muscle Gal4 line).

**Figure S7. Neuronal knockdown of candidate genes has varied effects depending on sex and assay time.** Candidate genes identified in the GWASs were investigated further by targeted knockdown in neuronal tissues using an *elav* Gal4 driver and UAS-siRNA constructs. The experiments were carried out in two sets, I and II. In all strip charts, mean activity levels are plotted on the Y-axis, with data from males shown in blue and data from females shown in red. The black dot marks the overall mean. Activity levels from offspring of a cross between the Gal4 driver and UAS construct (KD) was compared using nonparametric Wilcoxon tests.

**A. Basal activity data I.** As no effect of “sex” was detected (Kruskal-Wallis rank sum test), data from both sexes was analyzed together. We detected a significant impact of *shot* knockdown on activity levels; however, this impact was only significant for the activity measures from the “AM” experiment (Wilcoxon test, p=0.002 and p=0.025 for the comparisons between the knockdown and driver/UAS construct).

**B.** **Induced activity data I.** In this data set, analyses were carried out separately by time of day (AM versus PM), as a significant effect of time was detected (Kruskal-Wallis rank sum test). Sex significantly impacted *rut* and *shot* knockdown, so these experiments were analyzed separately for each sex. We detected a significant impact of knockdown on activity levels for *shot* (AM, females; p=0.018 and p=0.042), and *Nrx-1* (AM, p=9.145e-05 and p=1.083e-05). The activity of *hts* knockdown animals is also different from both parental lines, but it is intermediate between the two parents (AM, p=0.015 and p=0.035; PM, p=0.001 and p=1.083e-05).

**C. Basal activity II.** In this data set, *MTA-like* knockdown significantly impacts activity in males (Wilcoxon test, p=0.002 and p=0.033). *Cirl* knockdown significantly impacts activity levels in a time and sex dependent manner (female, AM, Wilcoxon test, p=0.004 and p=0.008). *cpo* knockdown only impacts basal activity in males (0.0006 and 0.002) while *sh* knockdown impacts basal activity in males and females (Wilcoxon test; 0.0005 and 0.005; 0.004 and 0.011).

**D. Induced activity II.** No significant impacts were detected in this analysis.

**Supplemental Table S1. Strains used for candidate gene analysis.**  This table contains the genotypes and Bloomington stock numbers of the strains analyzed.

**Supplemental Table S2. List of DGRP lines included in this study.**

Table is separated by treatment and sex for each line. Y = Yes, N = No.

**Supplemental Table S3. Phenotypic measures used in the GWAS analysis.** Summary data as submitted to the DGRP GWAS webtool as well as raw data are provided.

**Supplemental Table S4. Candidate genetic variants identified as significant by the GWAS.** This table includes results from the basal activity GWAS and the exercise-induced activity GWAS.

**Supplemental Table S5. Quantitative genetic analysis of phenotypes.** This table presents various quantitative genetics measures such as variance partitioning, heritability, and genetic correlations.

**Supplemental Table S6. Detailed GO term enrichment analysis results for genetic variants associated with basal activity.** This table includes the complete GO term enrichment analysis results for the “Cellular component” and “Biological Process” categories for genetic variants associated with basal activity.