Supplementary File S1

Genetic Signatures of Drug Response Variability in *Drosophila melanogaster*

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Supplementary Figures

Supplementary Tables

Supplementary Data

Raw and RMA normalized gene expression data for the wild type mass population and the F1 offspring is available at the Gene Expression Omnibus (GEO) with the accession number: GSE121643.

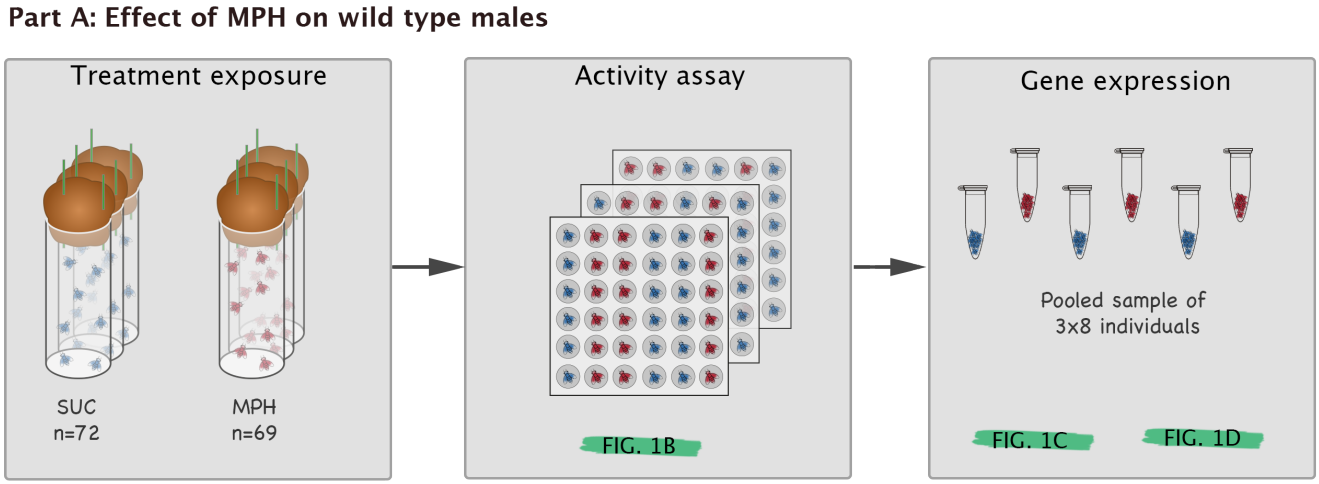

Figure S1A: Schematic overview of experimental setup for investigating effects of methylphenidate (MPH) on locomotor activity and on gene expression. Wild type males were exposed to either a solution containing sucrose (SUC, blue flies) or MPH (red flies) using a capillary feeding assay. The flies were exposed to the treatments for approximately 24h in groups of seven individuals. Hereafter, the flies were moved to the activity plates and individual movement tracks were obtained. After the behavioral assays the individuals were frozen down, and three replicates of eight individuals per treatment were randomly sampled and whole-genome gene expression data were obtained. Text collared in green refers to figures in the manuscript presenting results from the experiments.

Part B: Prediction of DGRP phenotypes, and identify candidate genes for MPH response

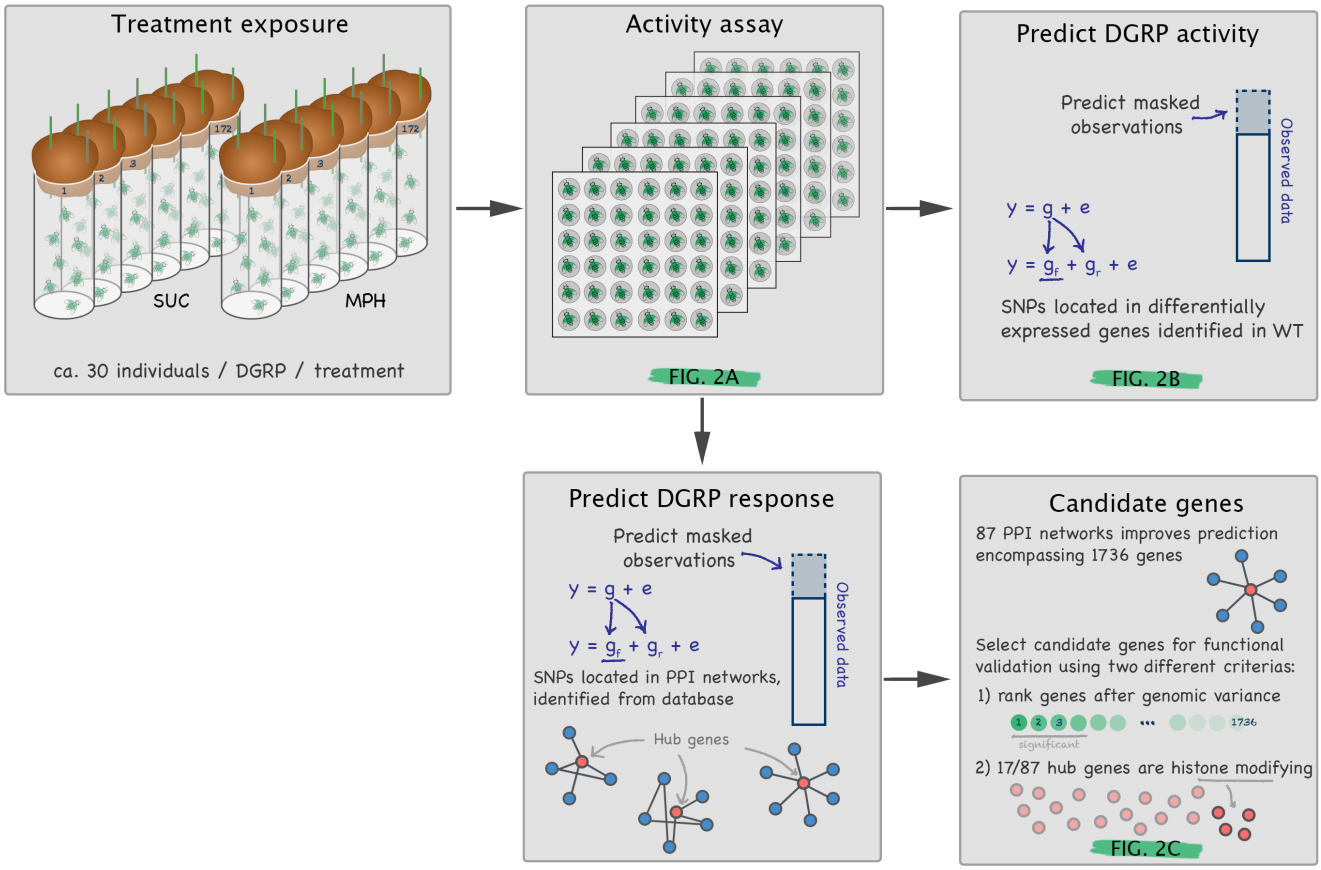

Figure S1B: Overview of the procedure for the DGRP experiments and identification of candidate genes. Approximately 30 individuals per DGRP line per treatment were exposed to either sucrose (SUC) or methylphenidate (MPH) using a capillary feeding assay in groups of seven individuals. After approximately 24h the flies were transferred to the activity plates and individual movement tracks were obtained. This procedure was done in blocks over the course of 46 days. Using the genomic feature prediction approach we predicted the DGRP locomotor activity phenotypes by creating feature sets using the gene expression data from the wild type males (Figure S1A). We then quantified the behavioral response to MPH as the within-DGRP line difference in locomotor activity between sucrose- and MPH treated individuals. Using the genomic feature prediction approach we predicted the behavioral response to MPH using feature sets defined using protein-protein interaction (PPI) networks from the STRING database. Among the PPI networks that significantly improved the accuracy of prediction (compared to the null model) we selected 36 genes for functional validation. The selection of candidate genes were based on two different criteria: 1) we ranked all the genes with the predictive PPI networks based on their overall contribution to the genomic variance, and the top 20 genes that contributed significantly were selected. 2) Among the predictive PPI networks, 20% (17/87) of the hub genes turned out to be involved in histone modifying processes (see Table S10 in File S1). Text collared in green refers to figures in the manuscript presenting results from the experiments.

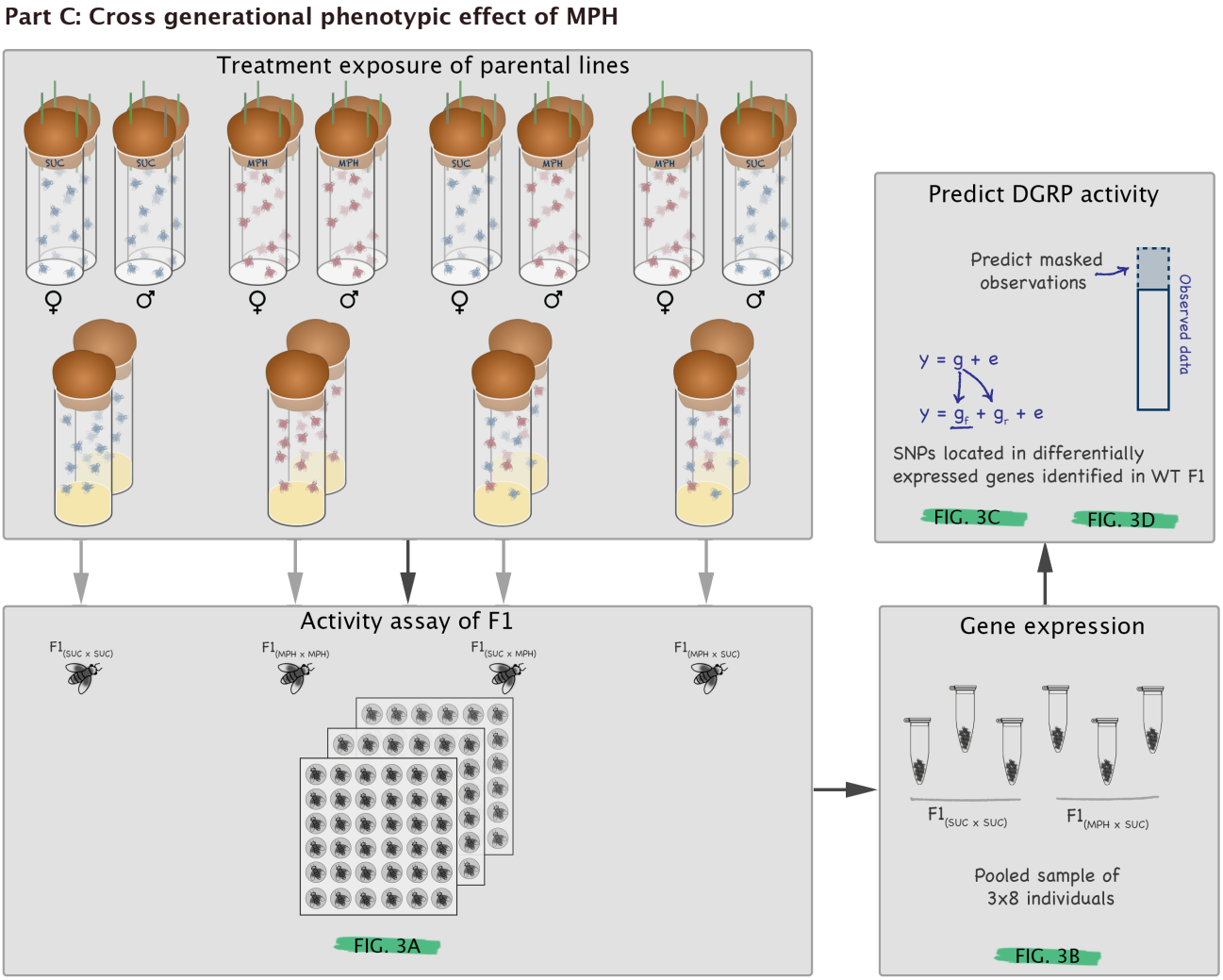

Figure S1C: One of the findings from the DGRP experiments (Figure S1B) was that a large proportion of hub genes of the predictive PPI networks were known to be involved in histone modifying processes. Because histone modifying processes are known to cause cross-generation effects, we investigated in a wild type population if MPH caused behavioral effects across generations. We set up different crosses exposing virgin males and females in different combinations to either sucrose (SUC, blue flies) or methylphenidate (MPH, red flies); thus, females and males to SUC, females and males to MPH, females to SUC and males to MPH, and females to MPH and males to SUC. After approximately 24h on capillary feeding assay the flies were transferred to vials containing food and allowed to reproduce. The F1 male offspring were transferred to the activity plates and individual movement tracks were obtained. F1 males from the cross between females and males exposed to SUC, and F1 males from the cross between females exposed to MPH and males to SUC (here the greatest phenotypic difference was observed, Figure 3A), were randomly sampled to obtain three replicates of eight individuals per cross type and whole-genome gene expression data were obtained. Using the genomic feature prediction approach we predicted the DGRP locomotor activity phenotypes by creating feature sets using the gene expression data from F1 wild type males. Text collared in green refers to figures in the manuscript presenting results from the experiments.

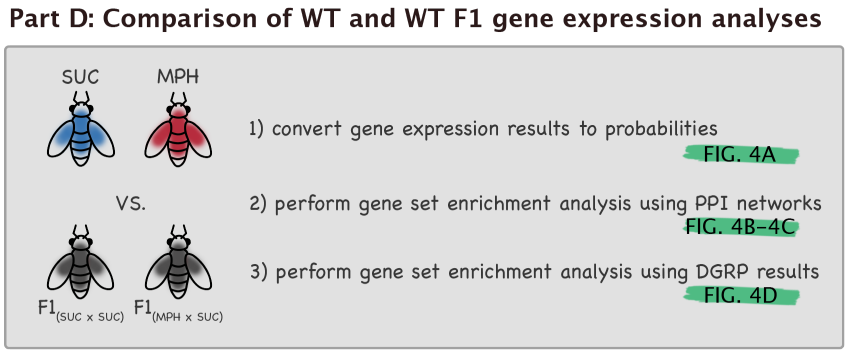

Figure S1D: The gene expression data of the wild type males (directly exposed flies, blue and red flies) and wild type F1 males (unexposed individuals, gray flies) were compared. Text collared in green refers to figures in the manuscript presenting results from the experiments.

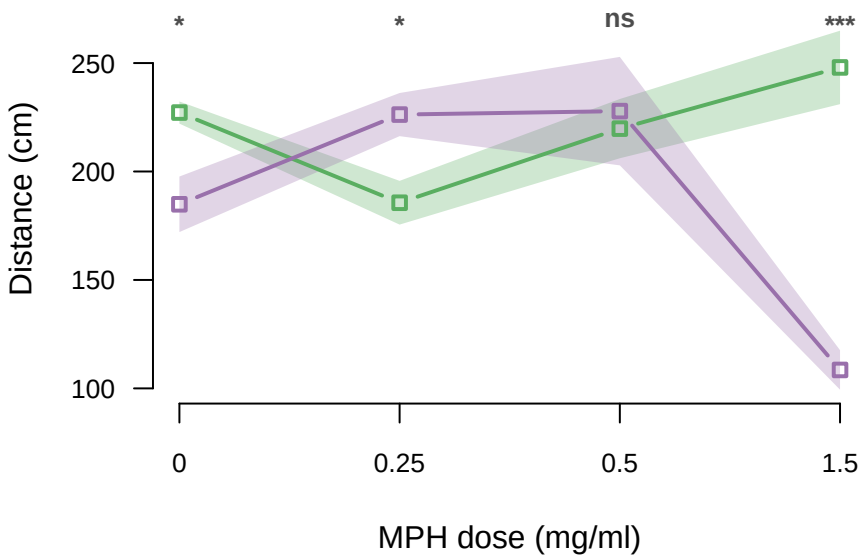

Figure S2: Dose response curve for two isogenic lines (purple: $\text{DGRP}_{\text{RAL361}}$, green: $\text{DGRP}_{\text{RAL381}}$). Between 15-18 individual flies from each isogenic line were tested at four levels of MPH (0 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1.5 mg/ml) using the same experimental setup as described for the DGRP experiments. The shaded area around the two curves shows the standard error of the mean. The symbols above the two curves indicate level of significance ($*$: $p < 0.05$, $***$: $p < 0.001$, ns: not significant) comparing activity bewteen the two isogenic lines at each dose of MPH using a t-test.

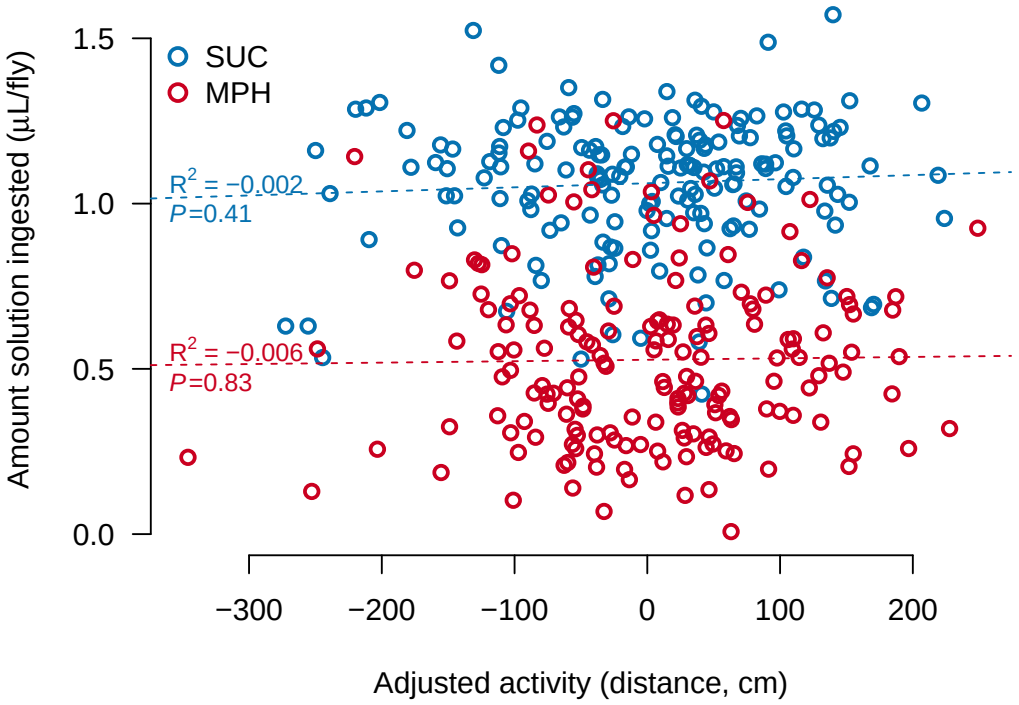

Figure S3: Average amount of solution (sucrose (SUC) or methylphenidate (MPH)) ingested per DGRP line as function of distance moved. Dashed lines are the regression of amount of solution ingested on adjusted activity for the two treatments separately. The variance explained by the linear fit (R^2), and the P values from testing if the slopes are different from zero are shown in the plot.

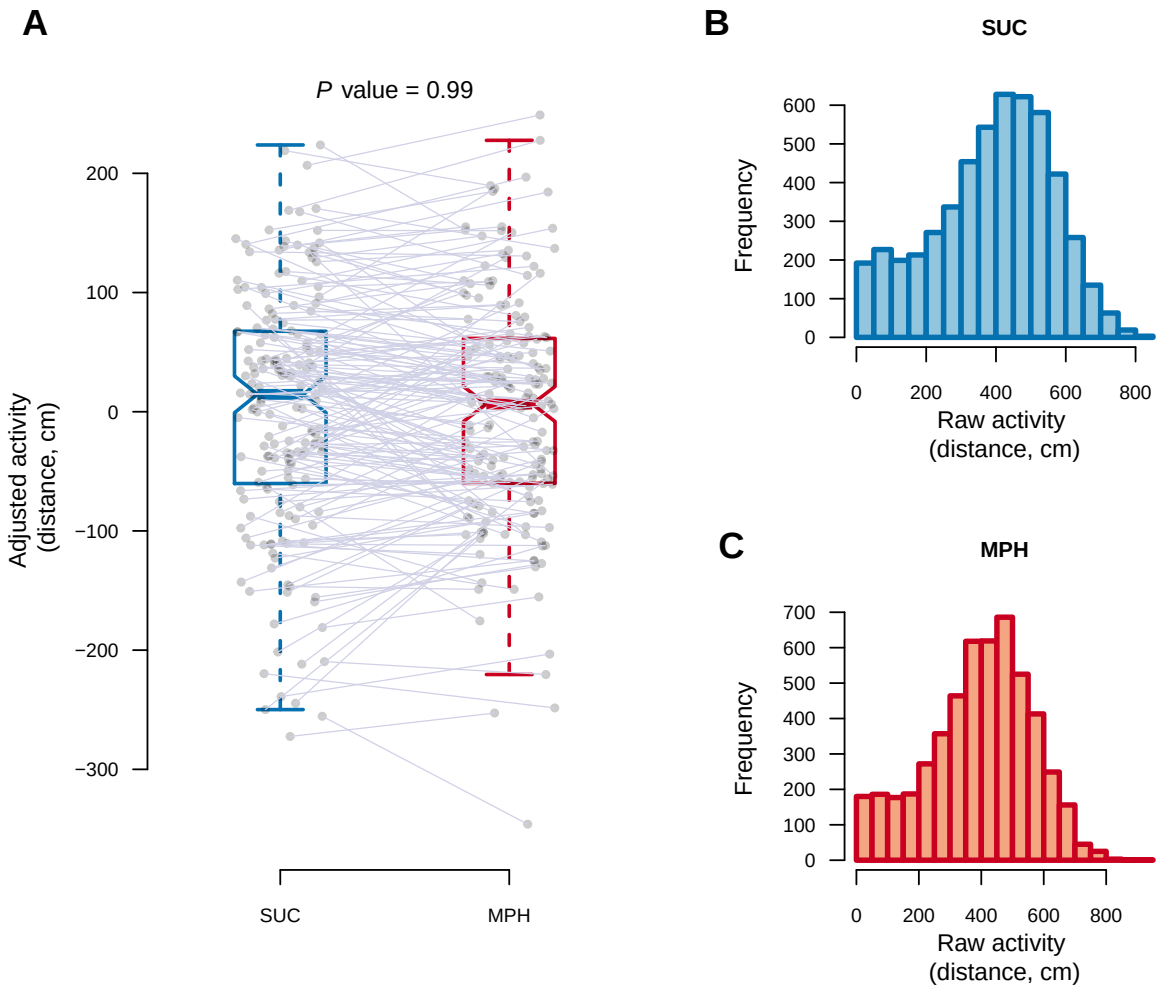

Figure S4: DGRP activity (adjusted values) after treatment with sucrose (SUC) or methylphenidate (MPH). (**A**) Box plots showing the distribution of mean locomotor activity for 172 DGRP lines with prior exposure to SUC or MPH. The scatter points indicate the DGRP line mean (approximately 30 males per DGRP line/treatment), and lines connecting two points represents the same DGRP line. The *P* value comparing the overall effect of MPH treatment is shown. (**B**) Histogram of raw activity data of SUC treated DGRP lines. (**C**) Histogram of raw activity data of MPH treated DGRP lines.

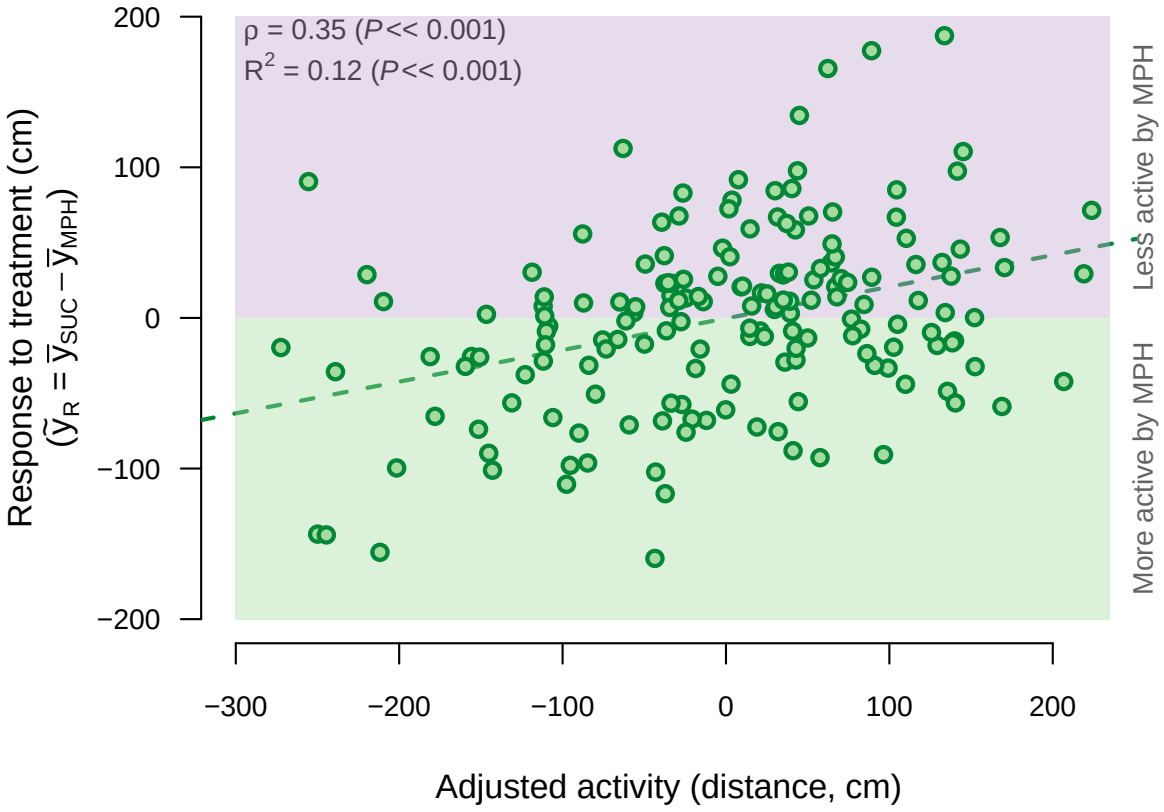

Figure S5: Response to treatment ($\tilde{y}_R$) as function of adjusted activity data of DGRP lines exposed to the sucrose treatment. The dashed line is the regression line (variance explained (R^2), and P value of the slope estimate is shown), and Pearson's correlation coefficient (ρ, with associated P value) is shown in the plot. The purple area ($\tilde{y}_R > 0$) is where DGRP lines became less active by MPH, whereas the green area ($\tilde{y}_R < 0$) is where DGRP lines became more active by MPH.

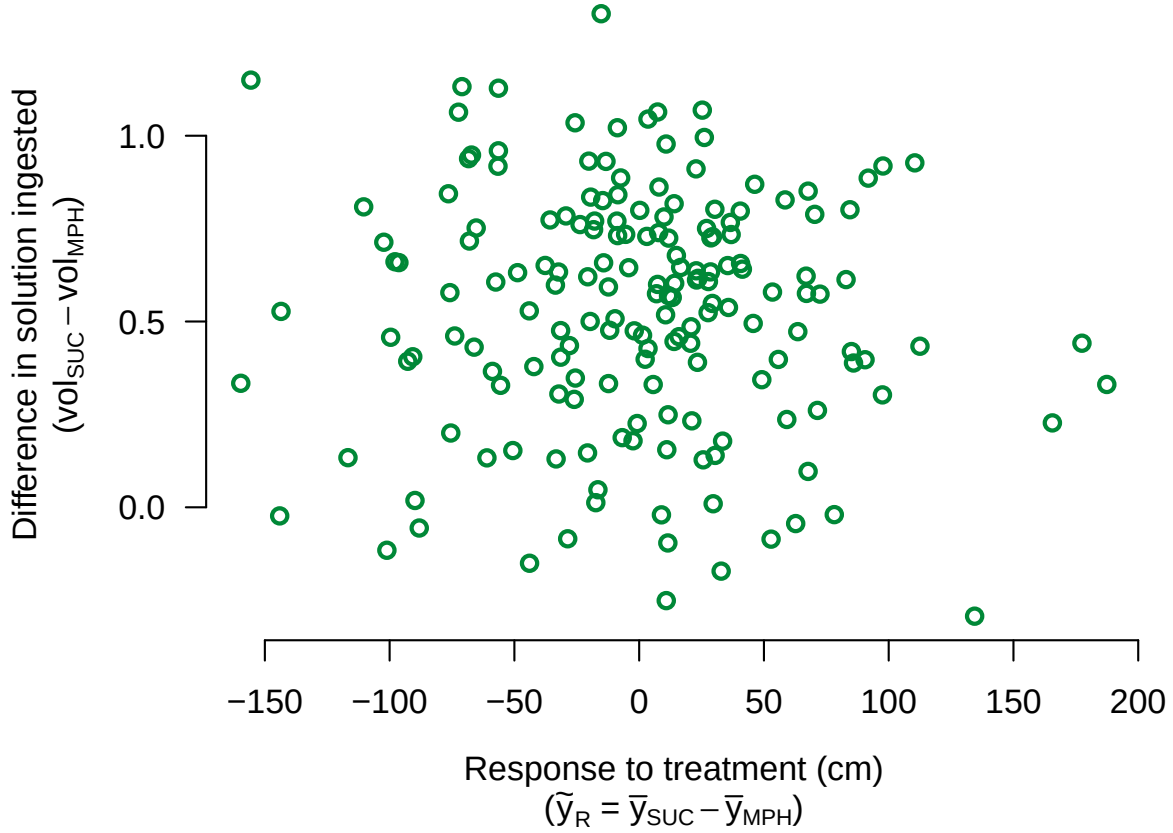

Figure S6: Difference in how much DGRP lines ingested of SUC and MPH treatment as a function of response to treatment ($\tilde{y}_R$).

Table S1: Stock information on transgenic *D. melanogaster* lines used for phenotypic assessment of putative candidate genes for response to treatment with methylphenidate. VDRC: Vienna *Drosophila* Stock Center (http://stockcenter.vdrc.at/) BDSC: Bloomington *Drosophila* Stock Center (https://bdsc.indiana.edu/). Viability: Plus sign indicates if the offspring carrying the *UAS*-GAL4 construct was viable and used for phenotypic assessment.

Target gene	Stock center	Stock No.	Genotype	Viability
Arp2	VDRC	101999	P{KK108910}VIE-260B	
ash2	VDRC	100718	P{KK108086}VIE-260B	
betaTub60D	VDRC	102052	P{KK110523}VIE-260B	
Caf1-55	VDRC	105838	P{KK102930}VIE-260B	
Cfp1	VDRC	110690	P{KK101246}VIE-260B	+
CG18418	VDRC	102109	P{KK110768}VIE-260B	+
CG7886	VDRC	109607	P{KK100967}VIE-260B	+
CG9314	VDRC	101690	P{KK105455}VIE-260B	
Cpr64Ab	VDRC	105517	P{KK113267}VIE-260B	+
dom	VDRC	7789	w[1118];P{GD1420}v7789	
Dpy-30L1	VDRC	27625	w[1118]; P{GD11928}v27625	+
escl	VDRC	103747	P{KK102406}VIE-260B	+
fest	VDRC	106300	P{KK106182}VIE-260B	
HDAC11	VDRC	108098	P{KK101558}VIE-260B	
Jarid2	VDRC	109290	P{KK101518}VIE-260B	+
l(2)09851	VDRC	110333	P{KK100751}VIE-260B	
MED31	VDRC	101488	P{KK108845}VIE-260B	
Mnn1	VDRC	110376	P{KK101050}VIE-260B	+
mRpS28	VDRC	107181	P{KK109300}VIE-260B	+
Myo61F	VDRC	110682	P{KK101033}VIE-260B	+
Nurf-38	VDRC	103776	P{KK102637}VIE-260B	
PpD6	VDRC	104211	P{KK104384}VIE-260B	
Rbbp5	VDRC	106139	P{KK102877}VIE-260B	
Smurf	VDRC	107349	P{KK103197}VIE-260B	+
Sod1	VDRC	108307	P{KK102426}VIE-260B	+
ss	VDRC	108732	P{KK107561}VIE-260B	
Su(dx)	VDRC	103814	P{KK102798}VIE-260B	
Su(var)3-3	VDRC	106147	P{KK102965}VIE-260B	+
trh	VDRC	101509	P{KK108906}VIE-260B	
trx	VDRC	108122	P{KK100756}VIE-260B	
Vhl	VDRC	108920	P{KK111257}VIE-260B	
Wdr82	VDRC	25246	w[1118]; P{GD9340}v25246	
wds	VDRC	105371	P{KK100120}VIE-260B	
XNP	VDRC	101568	P{KK103859}VIE-260B	+
Yeti	VDRC	102960	P{KK113635}VIE-260B	
YL-1	VDRC	107951	P{KK100166}VIE-260B	
(RNAi host KK)	BDSC	60100	y,w[1118];P{attP,y[+],w[3‘]}	
(RNAi host GD)	BDSC	60000	w1118	
(*tubulin*-GAL4 driver)	BDSC	5138	y[1] w[*]; P{w[+mC]=tubP-GAL4}LL7/TM3, Sb[1], Ser[1]	

Table S2: Association between chromosomal inversions and *Wolbachia* infection status with locomotor activity for DGRP flies treated with sucrose (SUC) or methylphenidate (MPH) treatment. Statistical significance is denoted with asterisk (∗).

	SUC			**MPH**		
Effect	χ^2	**d.f.**	P **value**	χ^2	**d.f.**	P **value**
2L_t*	7.43	2	0.024	11.13	2	0.0038
2R_NS	0.88	2	0.64	0.22	2	0.9
2R_Y1	0.53	1	0.46	0.41	1	0.52
2R_Y2	0.55	1	0.46	0.0017	1	0.97
2R_Y3	0.88	1	0.35	1.52	1	0.22
2R_Y4	0.88	1	0.35	1.52	1	0.22
3L_P	3.52	2	0.17	0.16	2	0.92
3L_M	0.012	1	0.91	0.26	1	0.61
3R_P*	11.43	2	0.003	9.24	2	0.009
3R_K	1.27	2	0.53	0.49	2	0.78
3R_Mo	0.29	2	0.86	1.6	2	0.45
3R_C	0.83	1	0.36	1.7	1	0.19
Wolbachia	0.16	1	0.69	0.027	1	0.87

Table S3: Using maximum likelihood methods we assessed the effect of experimental factors, such as feed intake, placement, day and plate on DGRP locomotor activity. Statistical significance is denoted with asterisk (∗).

	SUC			**MPH**		
Effect	χ^2	**d.f.**	P **value**	χ^2	**d.f.**	P **value**
Feed intake	0.18	1	0.67	0.20	1	0.65
Placement*	18.0	1	2.2×10^{-5}	48.51	1	3.3×10^{-12}
Day*	373.3	45	2.2×10^{-16}	360.63	44	2.2×10^{-16}
Plate*	118.97	11	2.2×10^{-16}	106.73	12	2.2×10^{-16}

Table S4: Estimated quantitative genetic parameters for DGRP males exposed to the sucrose (SUC) or methylphenidate (MPH), for an interaction model between treatments, and for the response to treatment (the within-line mean difference in adjusted phenotypic values). The parameters includes REML variance components (σ^2_p: phenotypic, σ^2_g: genetic, $\sigma^2_{g:t}$: interaction between genetic and treatment effect, σ^2_e: residual) and broad- (H^2) and narrow-sense heritability (h^2) using raw phenotypes (y) and adjusted phenotypes ($\tilde{y}$) for models assuming independence among DGRP lines (**I**) or using the realized relationship estimated using genome-wide SNP data (**G**). The mean predictive ability (PA) with standard errors (SE) for predictive DGRP activity after control or MPH treatment, and predicting the response to treatment

	SUC			**MPH**			**SUC:MPH**		**Response**
	$\mathbf{I}_y$	$\mathbf{I}_{\tilde{y}}$	$\mathbf{G}_{\tilde{y}}$	$\mathbf{I}_y$	$\mathbf{I}_{\tilde{y}}$	$\mathbf{G}_{\tilde{y}}$	$\mathbf{I}_y$	$\mathbf{I}_{\tilde{y}}$	$\mathbf{G}_{\tilde{y}}$
σ^2_p	26884.38	25339.47	20750.31	25940.52	24336.04	20079.76	26548.08	24831.39	2406.119
σ^2_g	11273.99	9891.214	5298.233	10527.17	9082.402	4824.449	9893.707	8224.294	1147.696
$\sigma^2_{g:t}$							1166.163	1255.372	
σ^2_e	15610.39	15448.255	15452.081	15413.35	15253.638	15255.313	15578.213	15351.726	1258.422
H^2	0.42	0.39		0.41	0.37		0.42	0.38	
h^2			0.26			0.24			0.48
PA			0.14 (0.03)			0.12 (0.03)			0.30 (0.04)

Table S10: Subset of hub genes from the list of significant predictive gene networks that has evidence of being related to histone processes, such as histone methylation and acetylation, and chromatin folding. The list is based upon the information from flybase.org accessed January 28, 2019. Rows highlighted in bold are the genes investigated in the current study with *UAS*-GAL4 knockdown. The list of experimental evidence for each gene is by no means exhaustive, but solely focuses on a few selected known functions related to histones and chromatin. For the full list of known experimental evidence visit flybase.org and look up each gene.

FlyBase protein ID	FlyBase gene name	FlyBase gene symbol	Experimental evidence
FBpp0084040	absent, small, or homeotic discs 2	*ash2*	Histone H3-K4 methylation[2, 6].
FBpp0082511	Chromatin assembly factor 1, p55 subunit	*Caf1-55*	Histone deacetylase activity and chromatin assembly [17, 20].
FBpp0071262	CXXC finger protein 1	*Cfp1* (or *CXX1*)	Histone H3-K4 methylation [11].
FBpp0071529	domino	*dom*	Histone acetylation and histone exchange [9].
FBpp0079701	Dpy-30-like 1	*Dpy-30L1*	Histone H3-K4 methylation [11].
FBpp0079870	escl	*escl*	Histone H3-K27 methylation [13].
FBpp0076196	Jumonji, AT rich interactive domain 2	*Jarid2*	Regulation of histone H3-K27 methylation [8].
FBpp0110277	Menin 1	*Mnn1*	Histone H3-K4 methylation [11].
FBpp0078059	Polycomb	*Pc*	Chromatin binding [12] and histone H3-K4 [3].
FBpp0077900	Retinoblastoma binding protein 5	*Rbbp5*	Histone H3-K4 methylation [6, 11].
FBpp0074594	Suppressor of variegation 3-3	*Su(var)3-3*	Histone H3-K4 demethylation [16, 18].
FBpp0082406	trithorax	*trx*	Histone H3-K4 methylation [3, 15].
FBpp0079266	WD repeat domain 82	*Wdr82*	Histone H3-K4 methylation [6, 11].
FBpp0300790	will die slowly	*wds*	Histone H3-K4 methylation [6, 11].
FBpp0084212	XNP	*XNP*	Chromating silencing [5].
FBpp0288749	Yeti	*Yeti*	Regulation of H3-K4 and H3-K9 methylation [10]
FBpp0079735	YL-1	*YL-1*	Histone acetylation and exchange [9].

Table S13: Genes that previously have been found to respond to different types of drugs, which we also identified.

Drug exposure	**Gene symbols**	**References**
Methamphetamine	*CG10562, CG11893, CG12766, CG13658, CG13659, CG31288, CG33514, CG5724, CG6908, CG9360, Cyp12a5, Cyp12d1-d, Cyp12d1-p, Cyp4e2, Cyp6a2, Cyp9b2, GstE6, Mef2, Ugt36Ba, Ugt36Bc, Ugt86Da, Ugt86Dd*	[19]
Insecticides	*Cyp12d1-d, Cyp12d1-p, Cyp6a2, Cyp6a8*	[4, 7, 21]
Nicotine	*phu*	[14]
Caffeine	*Cyp6a2, Cyp6a8*	[1]

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[6] Hallson G., *et al.* (2012) dSet1 is the main H3K4 di- and tri-methyltransferase throughout *Drosophila* development. *Genetics* 190: 91–100.

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