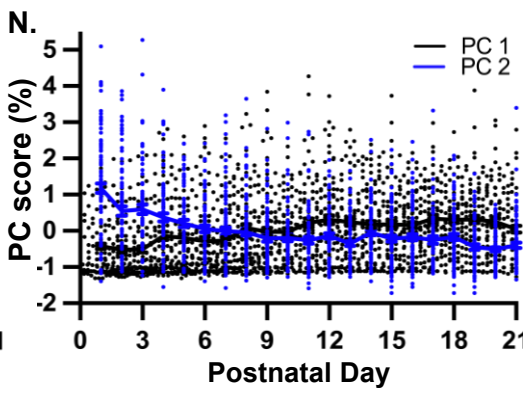
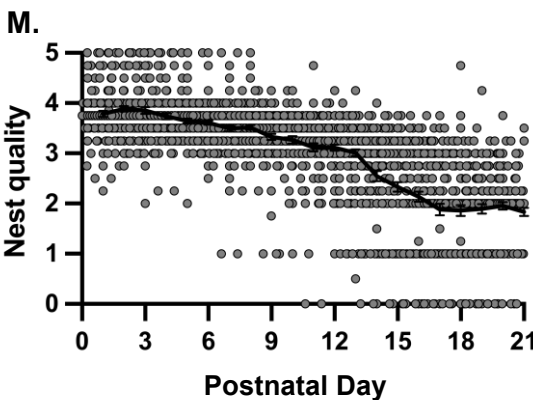
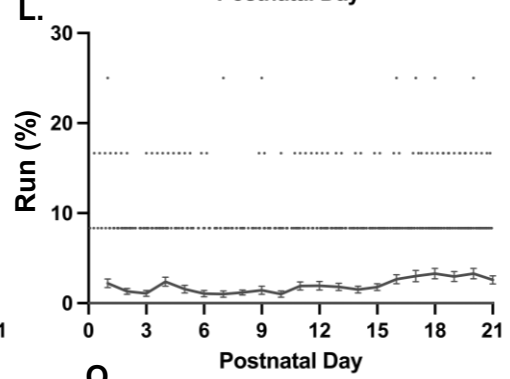
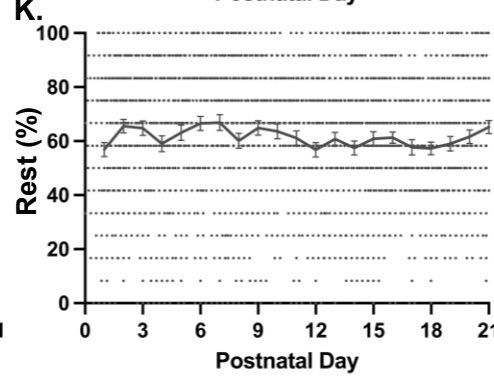
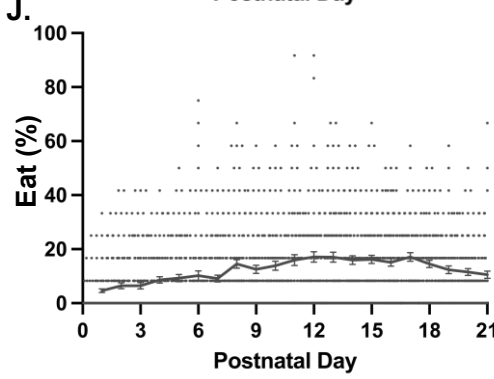
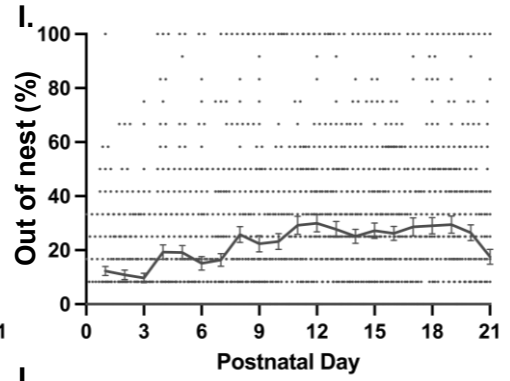
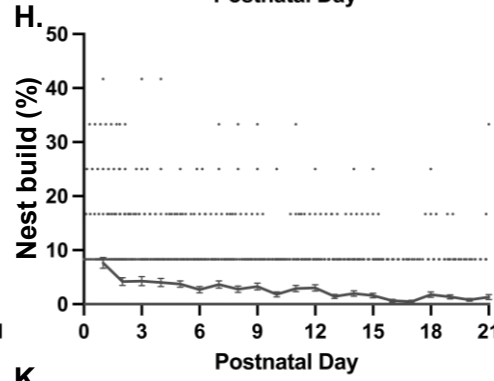
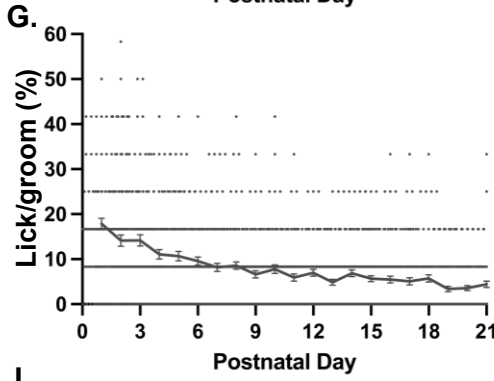
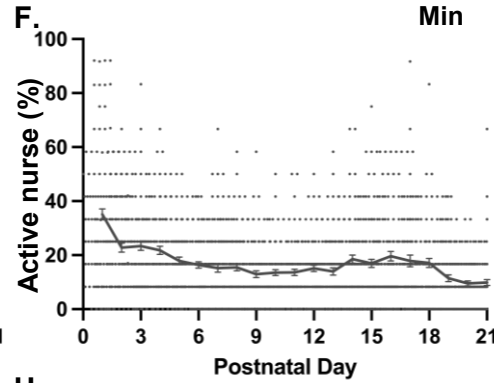
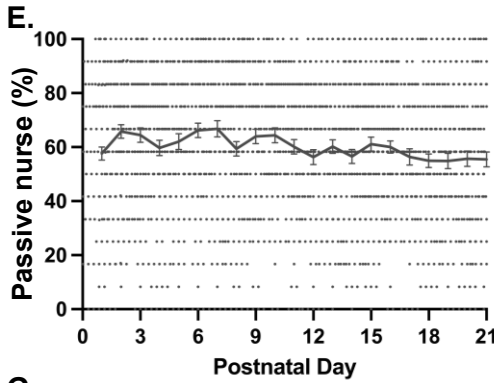
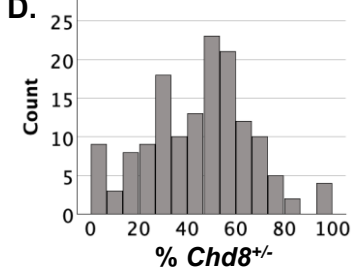
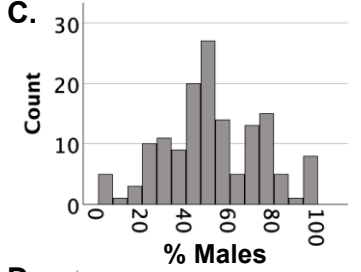
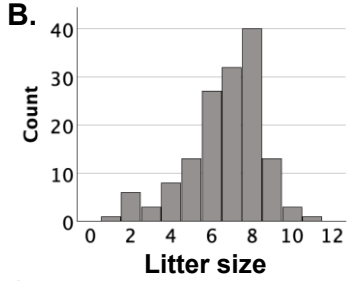


A. Number of litters observed per postnatal day (P) and strain

Strain	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20	P21
B6-B6	13	10	7	8	7	6	10	15	11	7	7	9	10	12	15	12	6	7	6	8	12
B6-CC1	12	9	6	13	9	9	10	9	6	4	10	13	10	10	11	8	8	11	12	11	10
B6-CC10	7	6	6	6	4	6	7	8	7	6	7	6	8	7	9	7	5	6	7	9	5
B6-CC12	14	13	9	10	9	8	8	11	8	8	9	6	4	8	12	9	9	8	7	6	10
B6-CC13	5	3	4	3	3	7	5	5	4	4	3	3	7	6	5	5	2	7	4	6	7
B6-CC16	5	3	2	3	5	4	3	4	4	1	4	4	3	3	4	4	1	3	3	4	2
B6-CC2	3	4	4	6	6	4	4	3	5	4	5	4	5	5	2	4	5	6	4	5	4
B6-CC22	5	6	5	6	5	3	3	3	6	6	5	4	2	3	4	5	4	4	2	2	2
B6-CC24	6	5	3	2	5	5	6	7	6	4	3	5	5	7	7	3	6	3	5	6	7
B6-CC25	5	6	7	5	6	4	3	4	4	5	2	3	3	4	3	5	2	4	2	5	4
B6-CC28	5	2	4	4	5	5	5	4	4	3	5	5	5	4	5	5	3	6	4	5	4
B6-CC32	7	10	8	4	3	7	7	6	6	5	6	5	6	3	6	7	3	5	5	6	3
B6-CC44	4	3	5	5	6	6	5	5	2	6	4	6	6	5	6	2	4	5	5	5	5
B6-CC57	6	4	1	5	4	5	5	4	6	3	5	4	5	5	6	5	4	3	5	6	3
B6-CC7	7	6	8	5	4	5	4	5	2	3	3	3	5	5	3	4	1	5	3	5	5
B6-CC75	5	6	5	6	4	3	6	6	6	5	5	6	3	6	5	6	6	6	5	2	7

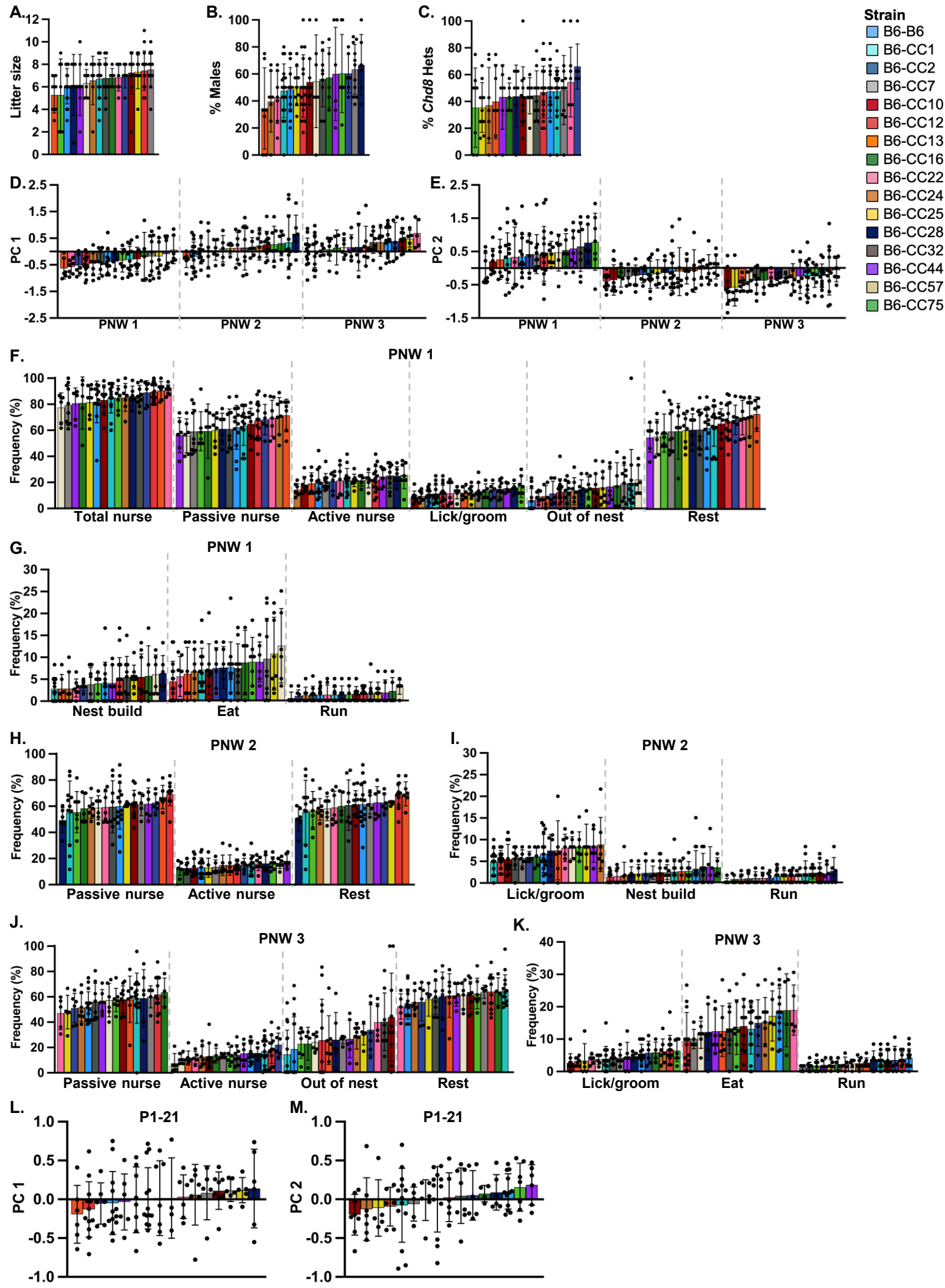


O.

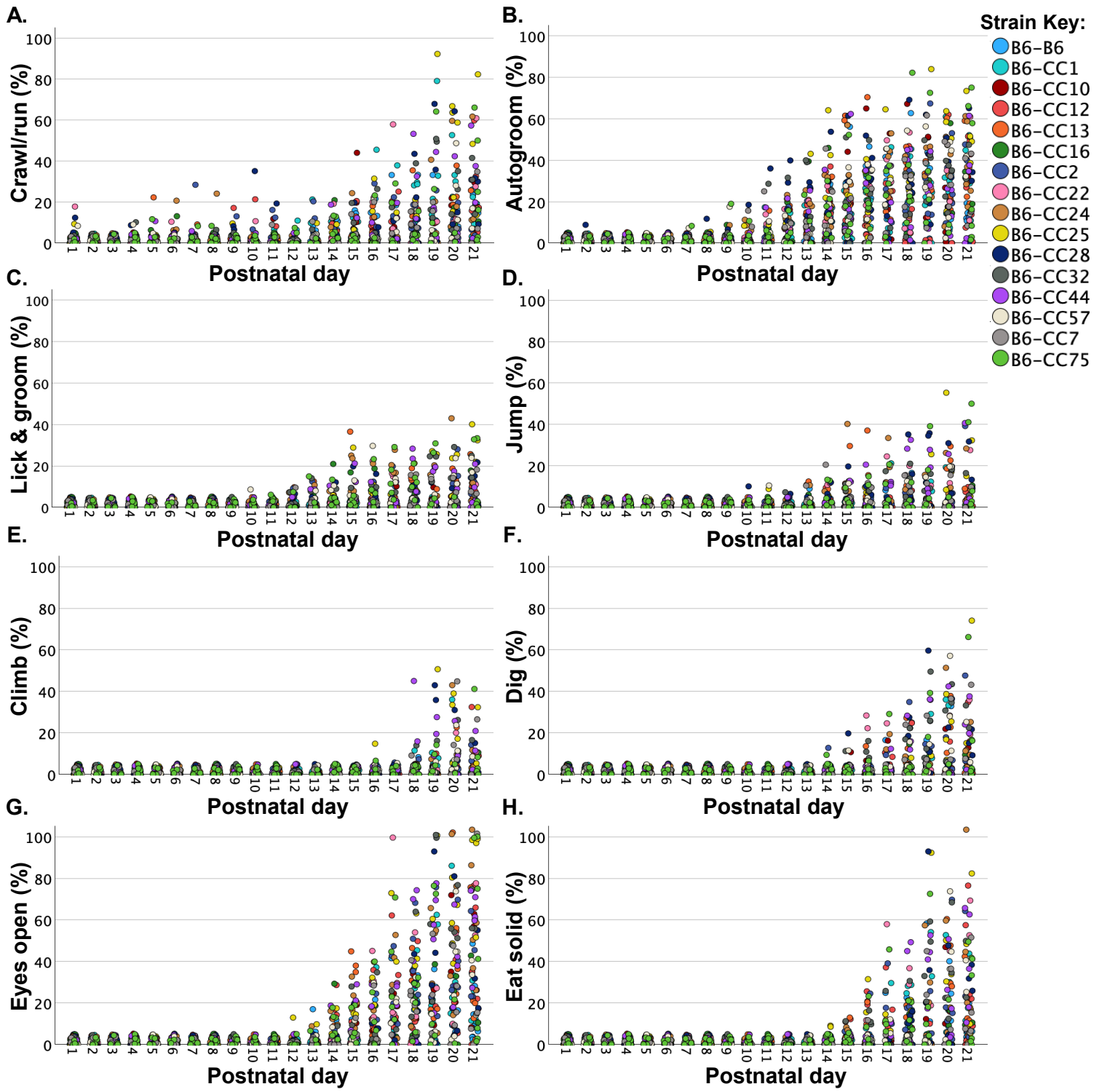
Correlation Coefficients	Litter size	% Males	% <i>Chd8</i> ^{+/-}
% Males	-0.11		
% <i>Chd8</i> ^{+/-}	0.20	0.02	
Nest quality	-0.52	0.22	-0.07
Passive nurse	-0.32	-0.08	-0.11
Active nurse	-0.05	-0.05	0.09
Lick & groom	-0.20	-0.02	-0.07
Nest build	-0.09	0.09	-0.04
Out of nest	0.48	-0.05	0.12
Eat	0.48	0.02	0.09
Rest	-0.32	-0.02	-0.11
Run	0.22	0.04	0.07
PC 1	0.41	0.00	0.16
PC 2	0.18	0.02	0.01

- Max + Max

Supplementary Figure 1: (A) The number of litters observed for each offspring strain are listed in the heatmap across each postnatal day (P). (B-D) Histograms of the litter size (B) and percentage of male (C) and *Chd8*^{+/−} (D) littermates per litter in the combined strain population. (E-L) Percent behavioral frequencies for B6-*Chd8*^{+/−} dams across P1-21 in the combined strain population. (M-N) Nest quality scores (M) and PC scores (N) over P1-21 in the combined strain population. Lines show means $\pm$ SEM. Dots show individual litters for each day. (O) The heatmap details Spearman's correlation between litter composition variables including litter size, the percentage of males per litter (% Males), and the percentage of *Chd8*^{+/−} pups per litter with nest quality scores, dam behaviors, and dam principal component (PC) scores across P1-21. Bold text indicates statistically significant correlations. Sample size (n= independent litters) SFig. 1B-D: n = 148. SFig. 1E-F: PND1–21: n = 109, 96, 84, 91, 85, 87, 91, 99, 87, 74, 83, 86, 87, 93, 103, 91, 69, 89, 79, 91, 90. SFig. 1M (PND1–21): n = 101, 91, 76, 82, 77, 77, 88, 89, 80, 68, 77, 79, 83, 90, 96, 86, 66, 86, 74, 87, 89. SFig. 1N (PND1–21): n = 108, 95, 83, 91, 85, 87, 91, 99, 87, 74, 83, 86, 87, 93, 103, 91, 69, 89, 79, 91, 90. SFig. 1O: Litter size, % Males, % *Chd8*^{+/−} = 148, Nest quality = 115; Passive nurse, Active nurse, Lick & groom, Nest build, Out of nest, Eat, Rest, Run, PC 1, and PC2 = 117.



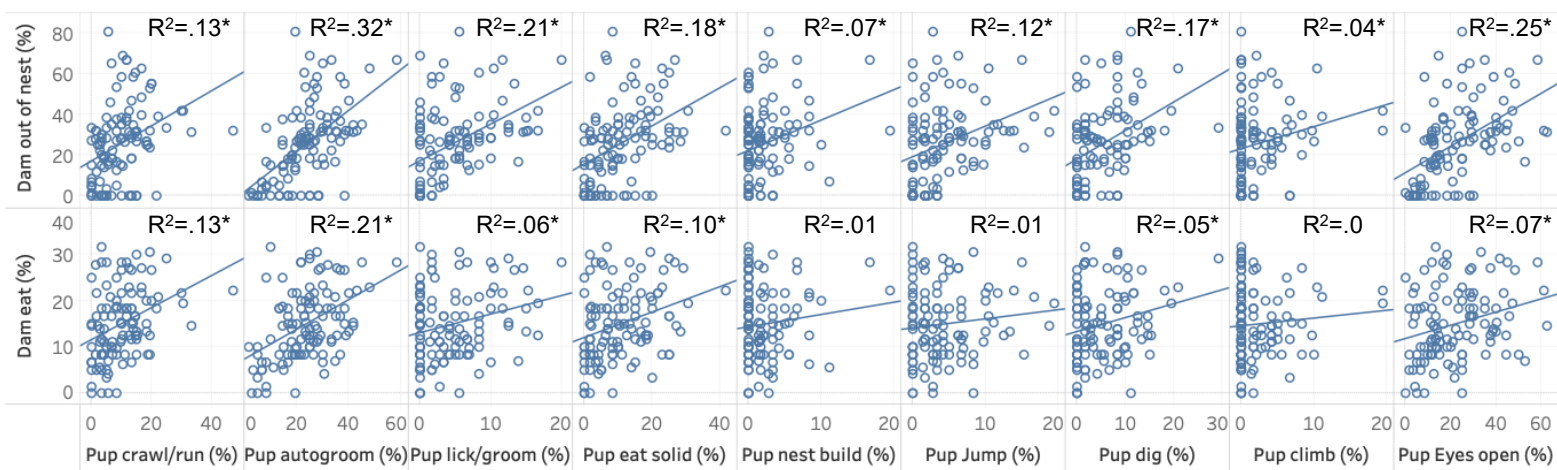
Supplementary Figure 2: (A-C) There were no significant differences in the mean litter size (A), percentage of male littermates (B), nor the percentage of *Chd8*^{+/-} littermates (C) across offspring strains born to B6-*Chd8*^{+/-} dams. (D-E) There were no significant differences in dam PC 1 and PC 2 scores between offspring strains during postnatal week (PNW) 1, 2, and 3. (F) There were also no significant differences between offspring strains in B6-*Chd8*^{+/-} dams' behaviors during PNW 1. (G-H) The means of B6-*Chd8*^{+/-} dams' behaviors that did not show significant differences across offspring strains are plotted. (I) Dam PC 1 and PC 2 scores across P1-21 did not differ significantly between strains. Bar graphs and standard error bars show +/- SEM. Dots show individual litters. Sample size (n= independent litters): SFig. 2A-M (strain order = B6-B6, B6-CC1, B6-CC10, B6-CC12, B6-CC13, B6-CC16, B6-CC2, B6-CC22, B6-CC24, B6-CC25, B6-CC28, B6-CC32, B6-CC44, B6-CC57, B6-CC7, B6-CC75): SFig. 2A-C: n = 15, 18, 12, 16, 7, 5, 6, 7, 7, 9, 6, 9, 6, 6, 11, 7. SFig. 2D-K: PNW1: n = 12, 13, 8, 15, 6, 5, 6, 7, 7, 8, 6, 8, 7, 6, 10, 7. PNW2: n = 15, 11, 11, 12, 6, 5, 6, 7, 7, 5, 6, 8, 7, 6, 5, 7. PNW3: n = 14, 14, 10, 12, 7, 4, 6, 5, 7, 5, 6, 8, 6, 6, 5, 7. SFig. 2L-M: n = 12, 13, 8, 12, 6, 4, 6, 6, 7, 5, 6, 8, 6, 6, 5, 7.



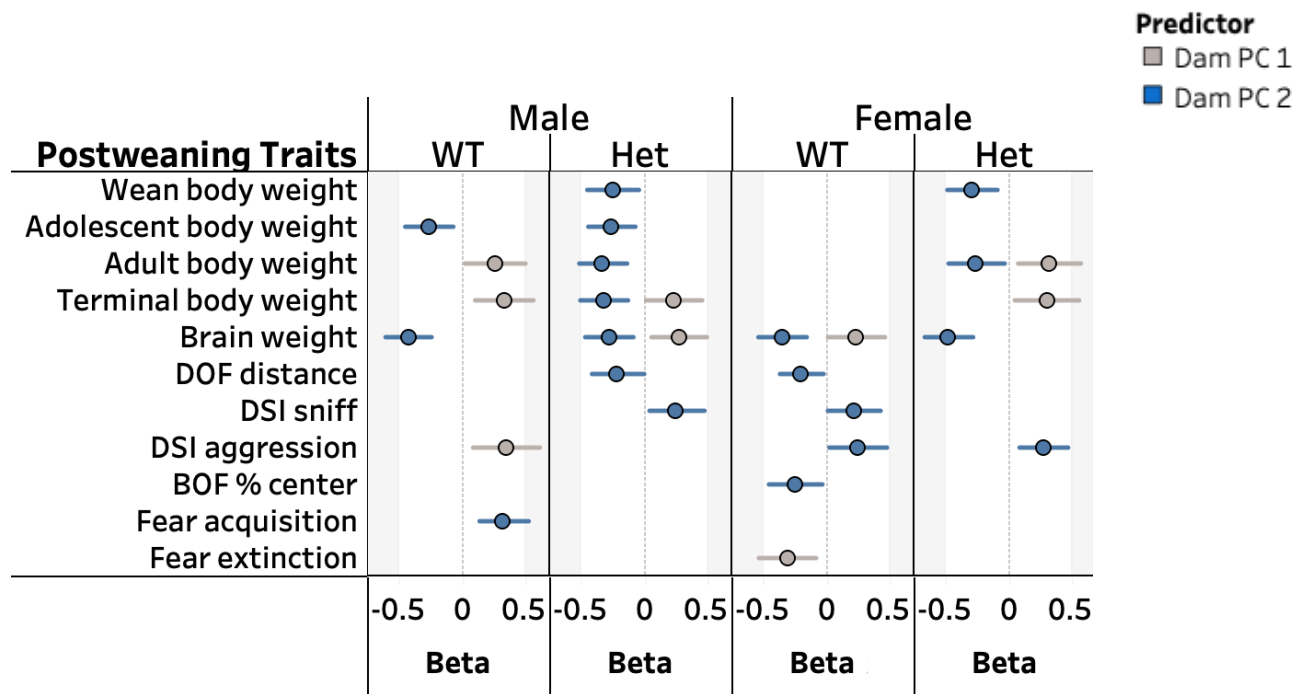
Supplementary Figure 3: (A-I) The frequency of behaviors observed across offspring strain groups from postnatal day 1-21 including the percent frequency of pup crawling/running (A), autogrooming (B), licking and grooming (C), jumping (D) climbing (E), digging (F), eyes open (G), and eating solid food (H). Different colors indicate different strain groups, as detailed in the key. Each circle reflects one litter. Same size (n=independent litters) SFig. 3A-H: B6-B6: P1-P21 = 12, 10, 7, 7, 6, 6, 9, 13, 11, 7, 7, 7, 9, 11, 14, 11, 6, 7, 6, 7, 11. B6-CC1: P1-P21 = 9, 7, 6, 8, 6, 6, 7, 7, 4, 3, 8, 9, 7, 7, 9, 7, 6, 9, 9, 7, 7. B6-CC10: P1-P21 = 3, 3, 2, 2, 1, 1, 2, 3, 4, 3, 3, 3, 4, 4, 4, 4, 3, 3, 3, 4, 4. B6-CC12: P1-P21 = 9, 7, 5, 5, 4, 3, 5, 6, 5, 6, 7, 3, 3, 8, 9, 7, 6, 7, 4, 4, 8. B6-CC13: P1-P21 = 5, 3, 4, 3, 3, 7, 5, 5, 4, 4, 3, 3, 7, 6, 5, 5, 2, 7, 4, 6, 7. B6-CC16: P1-P21 = 5, 3, 2, 3, 5, 4, 3, 4, 4, 1, 4, 4, 3, 3, 4, 4, 1, 3, 3, 4, 2. B6-CC2: P1-P21 = 3, 4, 4, 6, 6, 4, 4, 3, 5, 4, 5, 4, 5, 5, 2, 4, 5, 6, 4, 5, 4. B6-CC22: P1-P21 = 5, 6, 5, 6, 5, 3, 3, 3, 6, 6, 5, 4, 2, 3, 4, 5, 4, 4, 2, 2, 2. B6-CC24: P1-P21 = 6, 5, 3, 2, 5, 5, 6, 7, 6, 4, 3, 5, 5, 7, 7, 3, 6, 3, 5, 6, 7. B6-CC25: P1-P21 = 5, 6, 7, 5, 6, 4, 3, 4, 4, 5, 2, 3, 3, 4, 3, 5, 2, 4, 2, 5, 4. B6-CC28: P1-P21 = 5, 2, 4, 4, 5, 5, 5, 4, 4, 3, 5, 5, 5, 4, 5, 5, 3, 6, 4, 5, 4. B6-CC32: P1-P21 = 7, 10, 8, 4, 3, 7, 7, 6, 6, 5, 6, 5, 6, 3, 6, 7, 3, 5, 5, 6, 3. B6-CC44: P1-P21 = 4, 3, 5, 5, 6, 6, 5, 5, 2, 6, 4, 6, 6, 5, 6, 2, 4, 5, 5, 5, 5. B6-CC57: P1-P21 = 6, 4, 1, 5, 4, 5, 5, 4, 6, 3, 5, 4, 5, 5, 6, 5, 4, 3, 5, 6, 3. B6-CC7: P1-P21 = 7, 6, 8, 5, 4, 5, 4, 5, 2, 3, 3, 3, 5, 5, 3, 4, 1, 5, 3, 5, 5. B6-CC75: P1-P21 = 5, 6, 5, 6, 4, 3, 6, 6, 6, 5, 5, 6, 3, 6, 5, 6, 6, 6, 5, 2, 7.

Percentage of litters excluded from time to reach developmental milestone analysis				
Strain	Eye opening	Eat solid food	Digging	Jumping
B6-B6	0	14%	57%	57%
B6-CC1	0	18%	36%	45%
B6-CC10	0	0	17%	100%
B6-CC12	0	11%	55%	44%
B6-CC13	0	29%	14%	14%
B6-CC16	0	0	0	25%
B6-CC2	0	0	17%	17%
B6-CC22	0	0	17%	0
B6-CC24	0	0	17%	14%
B6-CC25	0	0	0	0
B6-CC28	17%	17%	17%	17%
B6-CC32	0	0	0	0
B6-CC44	0	0	17%	0
B6-CC57	0	0	17%	17%
B6-CC7	0	20%	0	0
B6-CC75	0	17%	57%	17%

Supplementary Figure 4: The percentage of litters that were excluded from analyses for strain differences in the age to reach developmental milestones. Litters were excluded because they were not observed until postnatal day 21 and they failed to display the behavior by the last postnatal day observed.



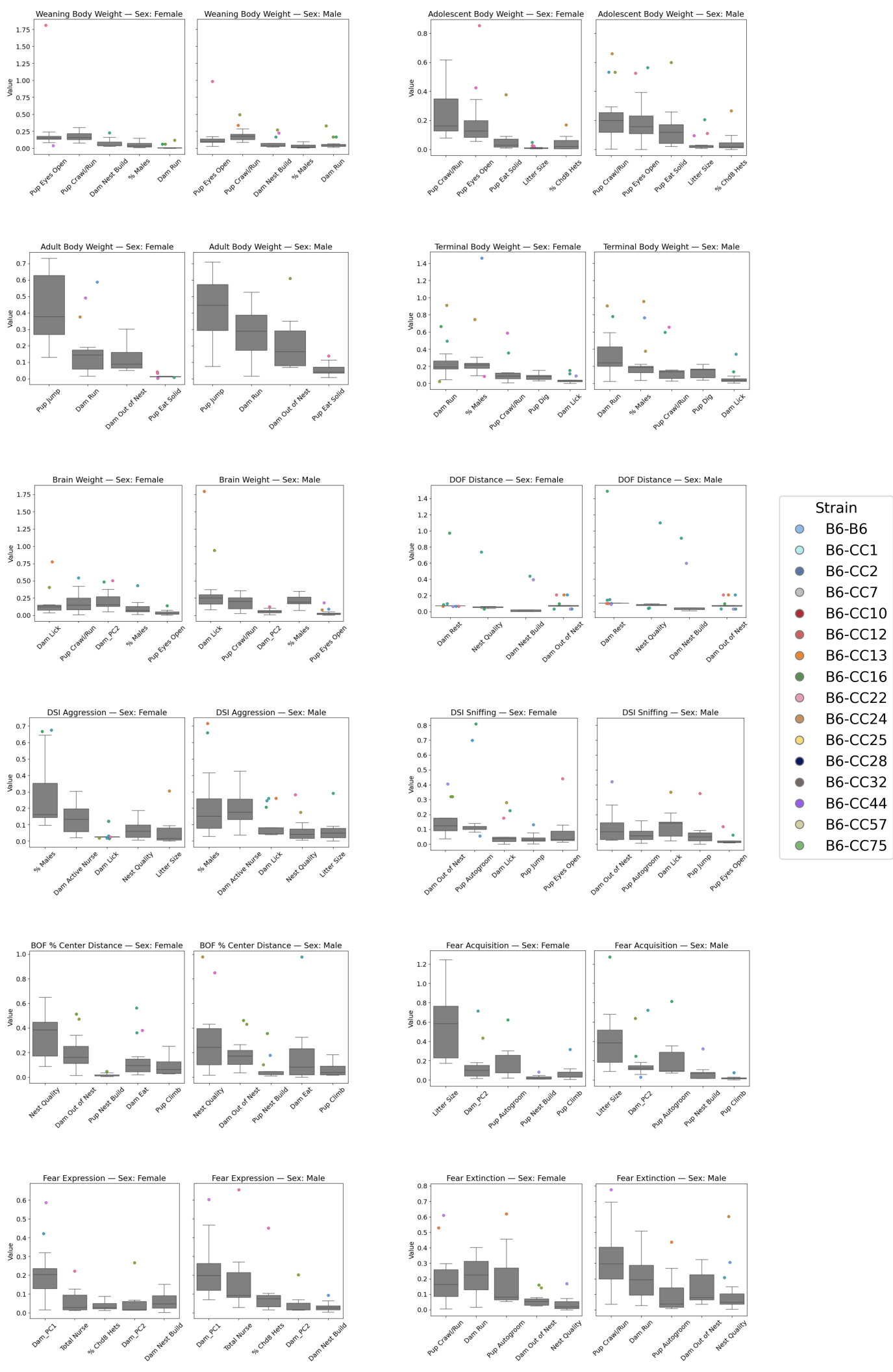
Supplementary Figure 5: Scatter plots show the correlation between dam out of nest (top row) and eat (bottom row) percent frequency and pup behaviors during postnatal week (PNW) 3. Each point represents one dam-litter pair. R^2 values represent the proportion of variance in dam behavior explained by the corresponding pup measure. N=612 total observations of 132 litters across postnatal days 15-21.



Supplementary Figure 6: Results from regression models examining the associations between PC 1 (gray circles) and PC 2 (blue circles) scores and offspring postweaning traits (y-axis) after controlling for litter size and the percentage of male and *Chd8*^{+/-} littermates per litter. Only significant standardized beta coefficients (x-axis) are shown. The horizontal lines denote 95% confidence intervals. Dam PC scores differentially predicted offspring postweaning outcomes in WT and *Chd8*^{+/-} males and females. Gray shading represents large effect sizes ($\beta \geq |0.50|$). Sample size: n = 109 (female Het), 113 (female WT), 111 (male WT), and 107 (male Het).

Litter variable		Chd8 Het effect	Sex	Age	r	R ²
↑ Dam out of nest	↓	Decrease in body weight	Males	Weaning	0.77	0.62
↑ Dam rest	↓	Decrease in body weight	Females	Weaning	0.8	0.3
↑ Nest quality	↓	Decrease in body weight	Females	Adolescence, adult	0.75, 0.59	0.418, .243
↑ Dam run	↑	Decrease in body weight	Females	Adolescence, adult, terminal	-0.55, -0.56, -0.53	0.287, .338, .335
↑ Dam lick/groom	↓	Increased brain weight	Females	Adult	0.55	0.318
↑ Total nurse	↓	Increased DSI aggression	Males	Adult	0.55	0.26
↑ % Males	↑	Increased DSI aggression	Females	Adult	0.74	0.441
↑ Dam nest build	↑	Increased DSI aggression	Males & females	Adult	0.54, 0.53	.207, .286
↑ pup nest build	↑	Decrease in locomotion	Males	Adult	-0.56	0.264
↑ % Chd8+/-	↓	Decrease in locomotion	Females	Adult	0.62	0.463
↑ Pup crawl/run	↓	Increased BOF anxiety	Males	Adult	0.65	0.31
↑ Total nurse	↓	Decrease in body weight	Females	Weaning	0.67	0.175
↑ Passive nurse	↓	Decrease in body weight	Females	Weaning	0.72	0.246
↑ Pup crawl/run	↓	Increase in DSI sniffing	Males	Adult	-0.59	0.247
↑ Litter size	↑↓	Bidirectional effect on fear acquisition	Females	Adult	0.74	0.532
↑ Total nurse	↓	Increased fear expression	Females	Adult	0.55	0.26
↑ Pup jump	↑↓	Bidirectional effect on fear acquisition	Males	Adult	-0.52	0.339
↑ Nest quality	↑	Increased BOF anxiety	Females	Adult	-0.6	0.195
↑ Pup drink	↑↓	Bidirectional effect on fear acquisition	Males	Adult	-0.54	0.184
↑ Pup drink	↓	Increased fear expression	Males	Adult	-0.52	0.175
↑ % Chd8+/-	↑↓	Bidirectional effect on fear extinction	Females	Adult	-0.5	0.171
↑ Total nurse	↑↓	Bidirectional effect on fear extinction	Females	Adult	-0.54	0.076
↑ Dam eat	↑	Decrease in body weight	Males	Adolescence	-0.53	0.127

Supplementary Figure 7: Results of univariate linear regressions testing litter variable predictors across postnatal days 1-21 on Cohen's D strain effect sizes on postweaning traits due to *Chd8* heterozygosity. The arrows indicate the direction of the litter observation variable and the corresponding direction of the effect on postweaning traits. Green arrows indicate a decrease in the *Chd8*^{+/-} effect while red arrows indicate an increase. Spearman's correlation coefficients (r) are listed, as well as the percent of variance accounted for in the regression models (R²). Only significant effects are included in the table. Sample size: n = 109 (female Het), 113 (female WT), 111 (male WT), and 107 (male Het).



Supplementary Figure 8: The distribution of SHAP values for each model is displayed through box plots. Outlier SHAP values ($1.5 \times$ Interquartile Range) are shown as dots, colored by the corresponding strain. See related Figure 5.