Figures and figure supplements

Large-scale animal model study uncovers altered brain pH and lactate levels as a transdiagnostic endophenotype of neuropsychiatric disorders involving cognitive impairment

Hideo Hagihara *et al.*

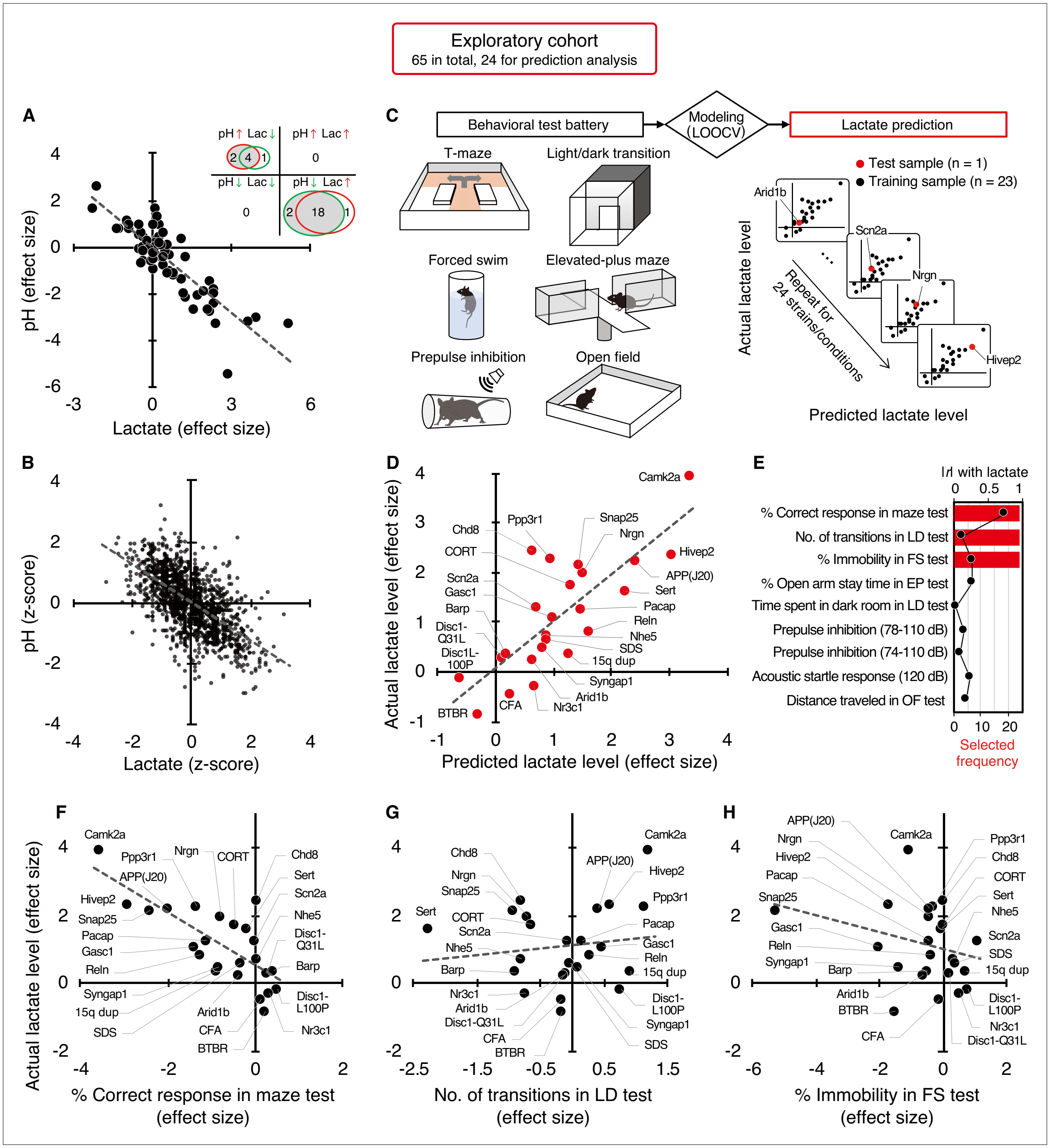

Figure 1. Increased brain lactate levels correlated with decreased pH are associated with poor working memory. (**A**) Venn diagrams show the number of strains/conditions of animal models with significant changes (*P*<0.05 compared with the corresponding controls) in brain pH and lactate levels in an exploratory cohort. Scatter plot shows the effect size-based correlations between pH and lactate levels of 65 strains/conditions of animals in the cohort. (**B**) Scatter plot showing the z-score-based correlations between pH and lactate levels of 1,239 animals in the cohort. A z-score was calculated for each animal within the strain/condition and used in this study. (**C**) Schematic diagram of the prediction analysis pipeline. Statistical learning models with leave-one-out cross-validation (LOOCV) were built using a series of behavioral data to predict brain lactate levels in 24 strains/conditions of mice in an exploratory cohort. (**D**) The scatter plot shows significant correlations between predicted and actual lactate levels. (**E**) Feature preference

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for constructing the model to predict brain lactate levels. Bar graphs indicate the selected frequency of behavioral indices in the LOOCV. Line graph indicates absolute correlation coefficient between brain lactate levels and each behavioral measure of the 24 strains/conditions of mice. *r*, Pearson's correlation coefficient. (F–H) Scatter plot showing correlations between actual brain lactate levels and measures of working memory (correct responses in maze test) (**F**), the number of transitions in the light/dark transition test (**G**), and the percentage of immobility in the forced swim test (**H**).

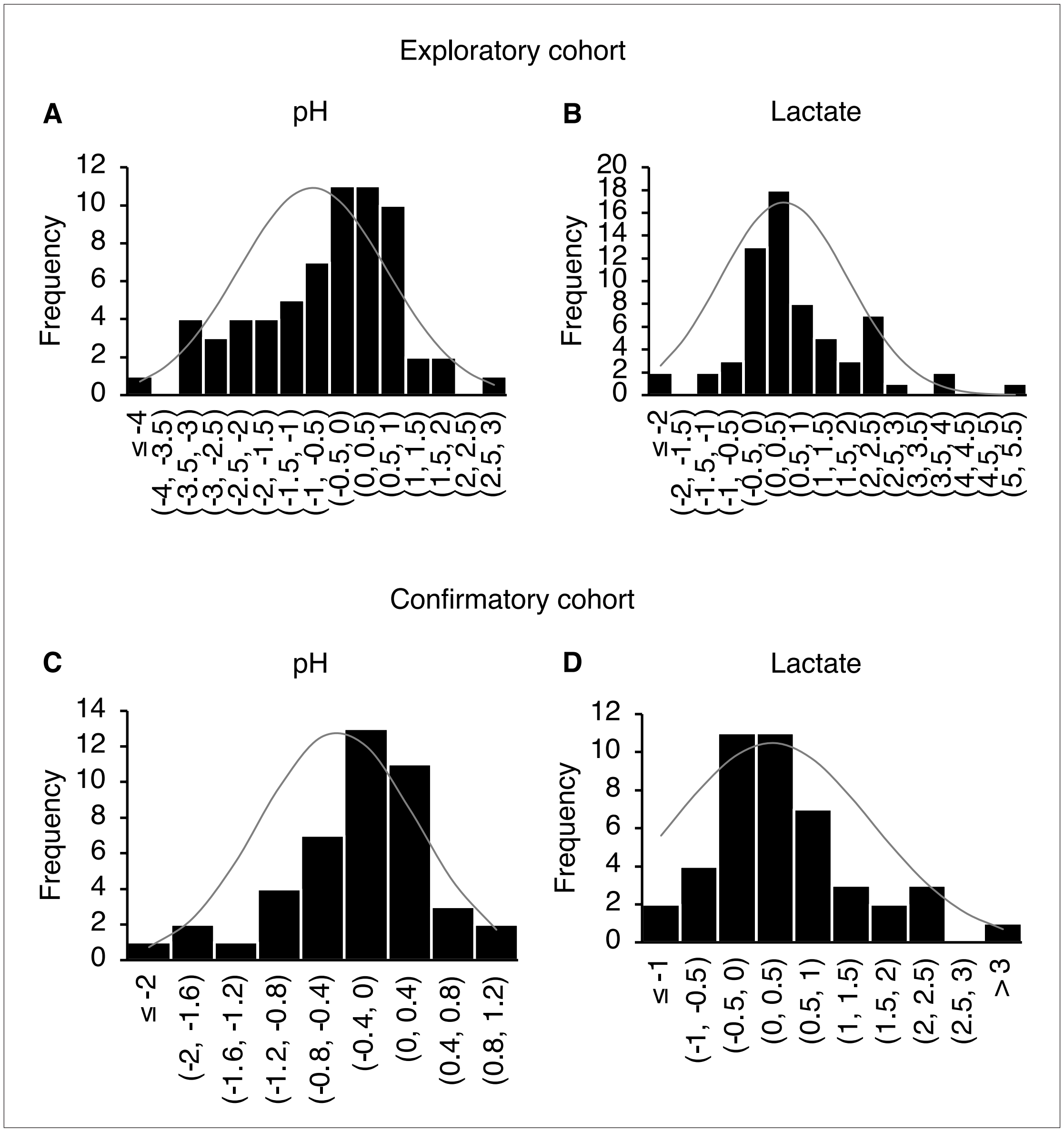

Figure 1—figure supplement 1. Normal distribution of effect size values for pH and lactate in the exploratory and confirmatory cohorts. (**A**) D=0.12, *P*=0.32. (**B**) D=0.15, *P*=0.088. (**C**) D=0.14, *P*=0.33. (**D**) D=0.18, *P*=0.10.

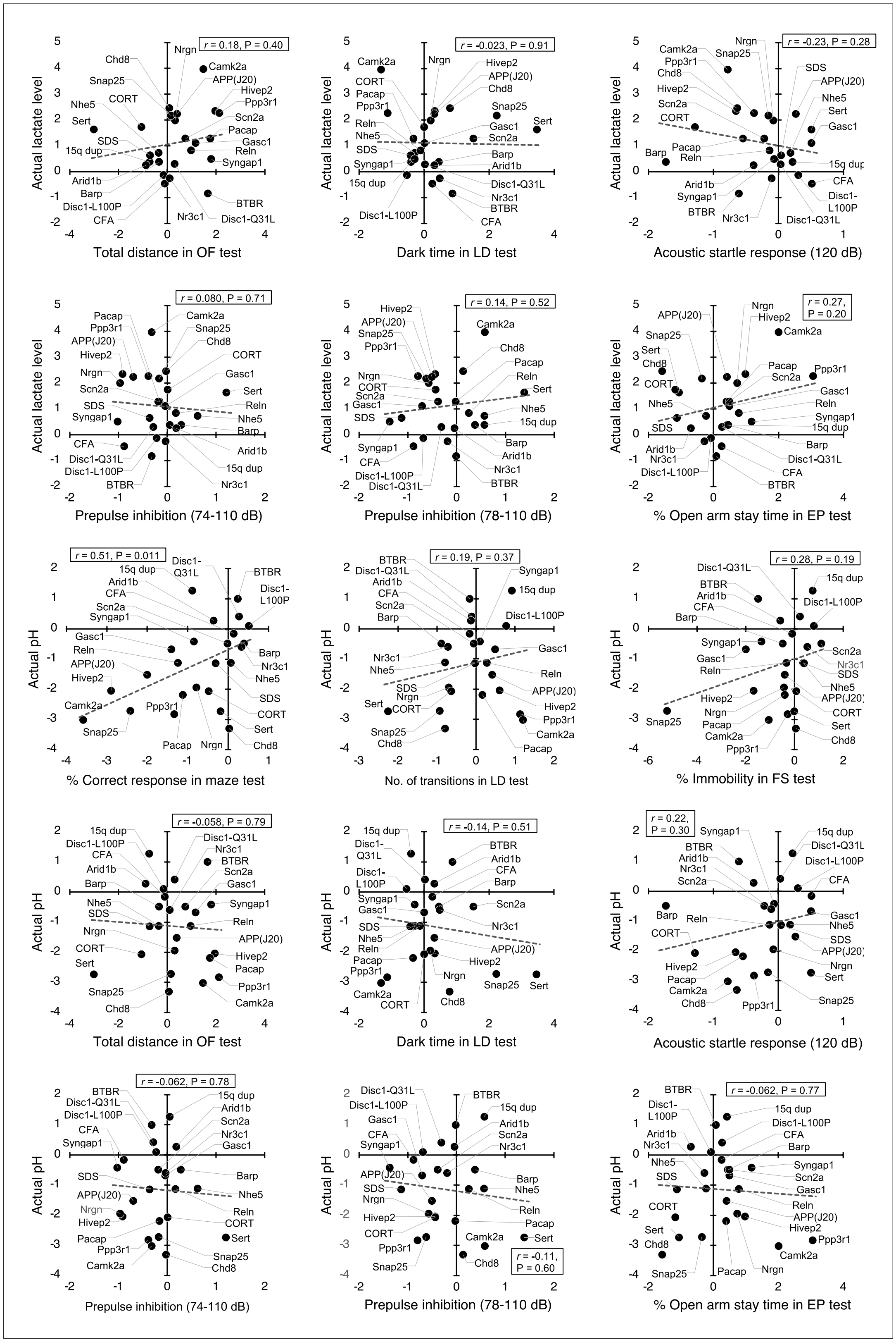

Figure 1—figure supplement 2. Correlations of brain lactate levels and pH with behavioral measures in an exploratory cohort. Scatter plots showing effect size-based correlations between actual lactate levels and pH, and behavioral measures. Data from 24 strains/conditions of mice used in the prediction analysis are shown. EP, elevated-plus maze; FS, forced swim test; LD, light/dark transition test; OF, open field test; *r*, Pearson's correlation coefficient.

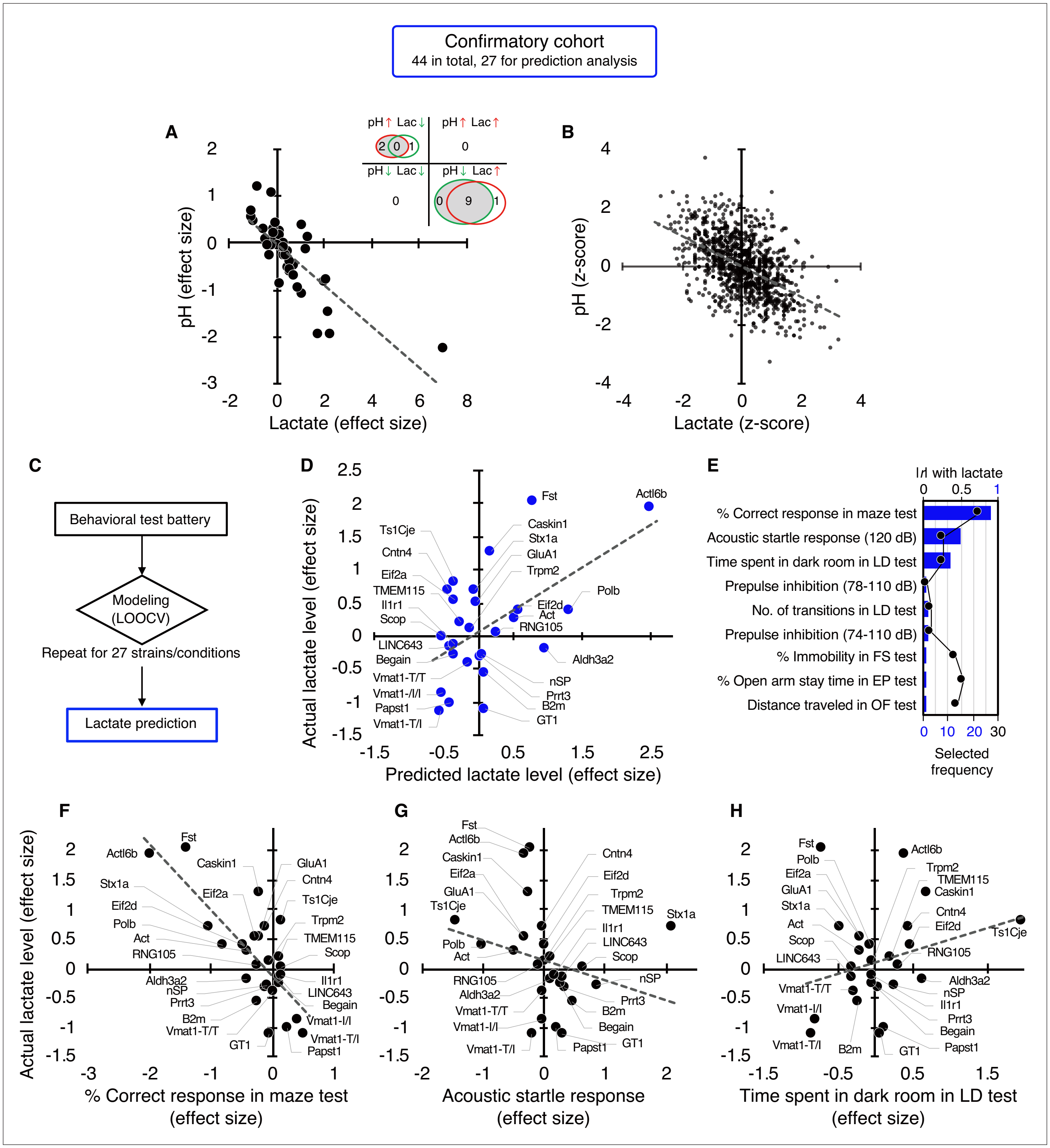

Figure 2. Studies in an independent confirmatory cohort validate the negative correlation of brain lactate levels with pH and the association of increased lactate with poor working memory. (**A**) Venn diagrams show the number of strains/conditions of animal models with significant changes (P<0.05 compared with the corresponding controls) in brain pH and lactate levels in a confirmatory cohort. Scatter plot shows the effect size-based correlations between pH and lactate levels of 44 strains/conditions of animals in the cohort. (**B**) Scatter plot showing the z-score-based correlations between pH and lactate levels of 1,055 animals in the cohort. (**C**) Statistical learning models with leave-one-out cross-validation (LOOCV) were built using a series of behavioral data to predict brain lactate levels in 27 strains/conditions of mice in the confirmatory cohort. (**D**) The scatter plot shows significant correlations between predicted and actual lactate levels. (**E**) Feature preference for constructing the model to predict brain lactate levels. Bar

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graphs indicate the selected frequency of behavioral indices in the LOOCV. Line graph indicates absolute correlation coefficient between brain lactate levels and each behavioral index of the 27 strains of mice. *r*, Pearson's correlation coefficient. (F–H) Scatter plots showing correlations between actual brain lactate levels and working memory measures (correct responses in the maze test) (**F**), the acoustic startle response at 120 dB (**G**), and the time spent in dark room in the light/dark transition test (**H**). Figure supplements.

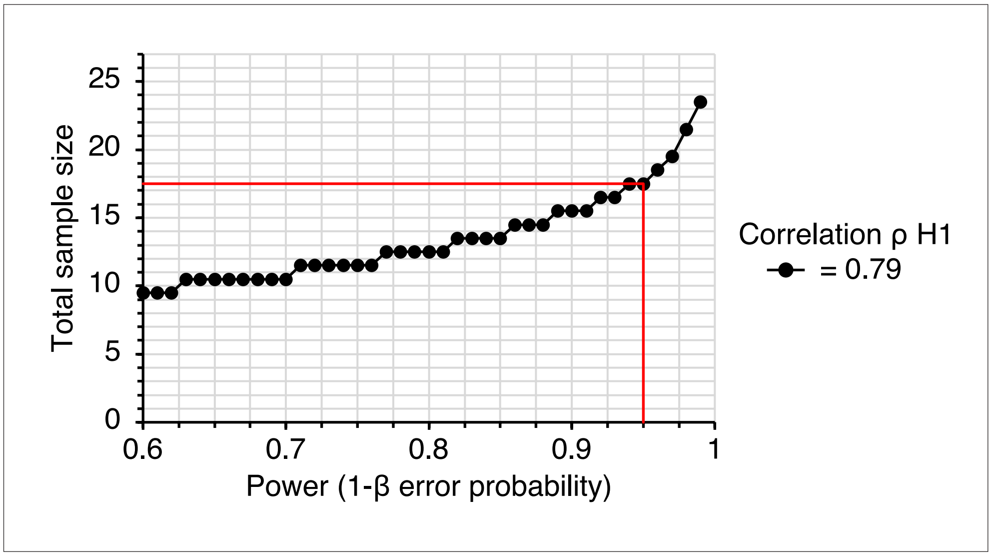

Figure 2—figure supplement 1. A *priori* power analysis to estimate the optimum sample size for the confirmatory experiment. Input parameters: tails = two, correlation | ρ | H1=0.79, α error probability = 0.01, power (1–β error probability)=0.95, correlation | ρ | H0=0. Output parameters: total sample size = 18, actual power = 0.95. The red line indicates 1–β=0.95.

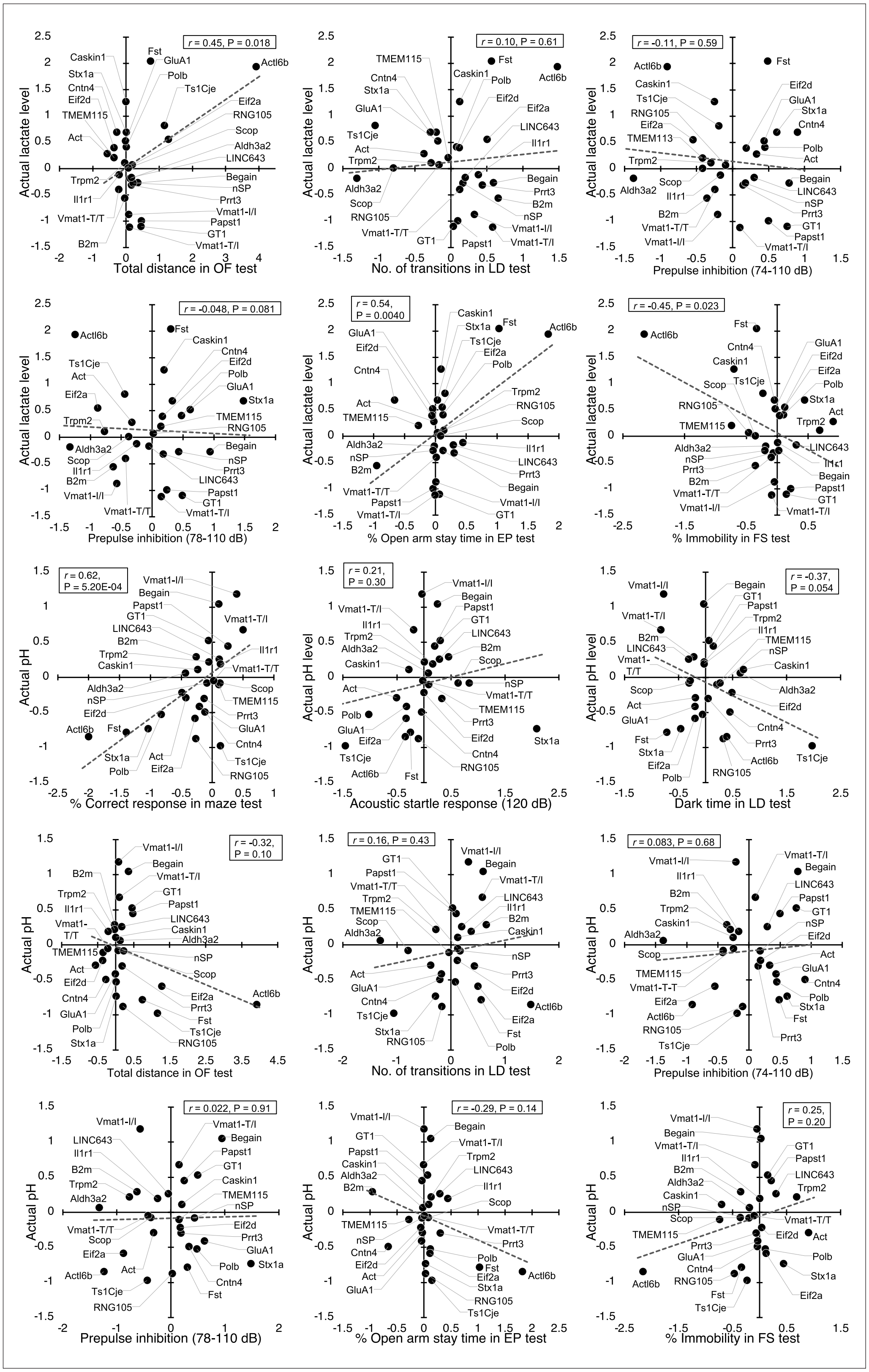

Figure 2—figure supplement 2. Correlations of brain lactate levels and pH with behavioral measures in a confirmatory cohort. Scatter plots showing effect size-based correlations between actual lactate levels and pH, and behavioral measures. Data from 27 strains/conditions of mice used in the prediction analysis are shown. EP, elevated-plus maze; FS, forced swim test; LD, light/dark transition test; OF, open field test; *r*, Pearson's correlation coefficient.

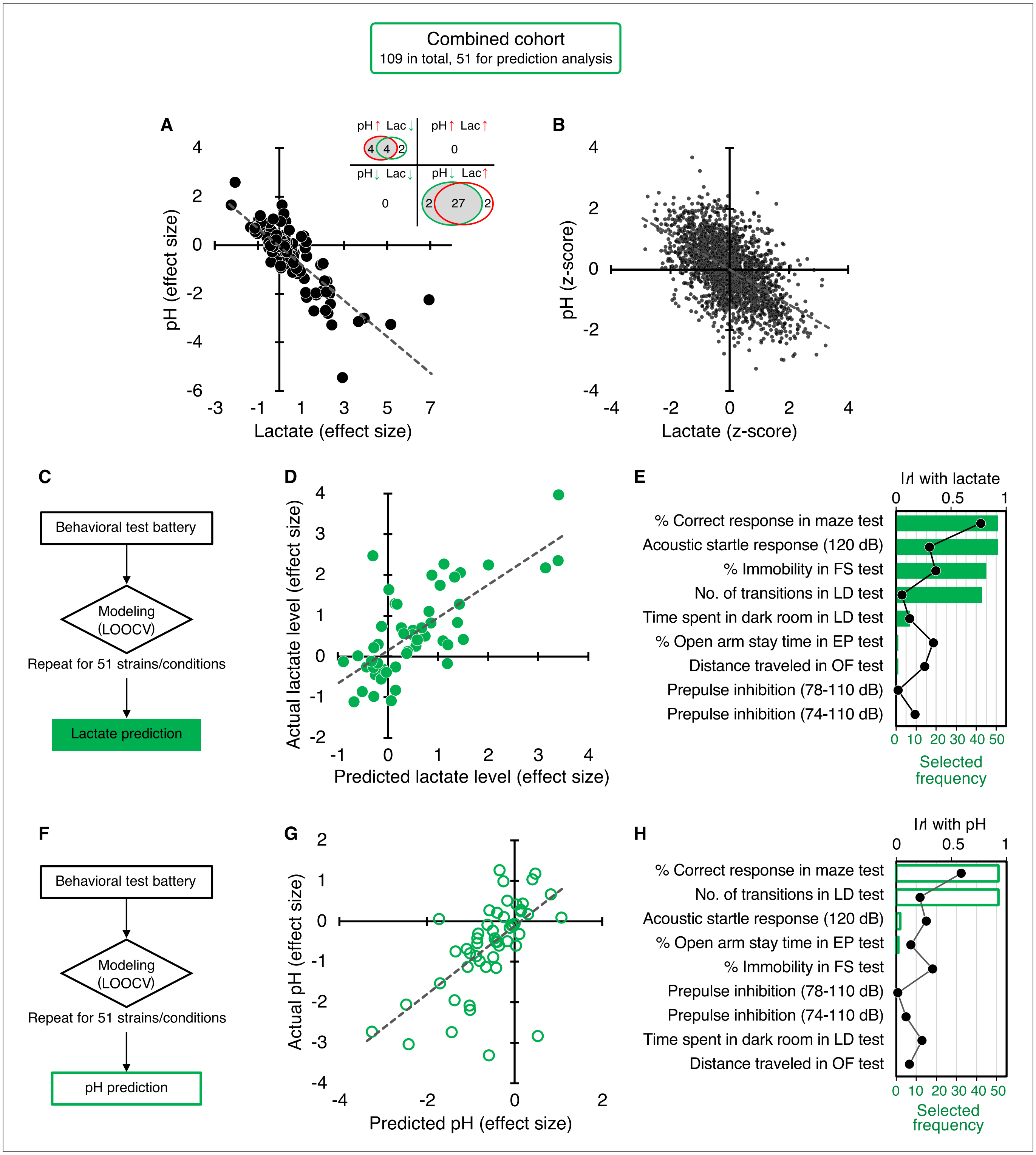

Figure 2—figure supplement 3. Correlation of increased brain lactate levels and decreased pH and their associations with poor working memory: studies in a combined cohort. (**A**) Venn diagrams show the number of strains/conditions of animal models with significant changes (*P*<0.05 compared to the corresponding controls) in brain pH and lactate levels in a combined cohort. Scatter plot shows the effect size-based correlations between pH and lactate levels of 109 strains/conditions of animals combined. (**B**) Scatter plot showing z-score-based correlations between pH and lactate levels of 2,294 animals combined. A z-score was calculated for each animal within strain/condition. (**C–H**) Prediction of brain lactate levels (**C–E**) and pH (**F–H**) from behavioral outcomes in 51 strains/conditions of animals. The scatter plot shows correlations between predicted and actual lactate levels (**D**) and pH values (**G**). Feature preference for constructing the model to predict brain lactate levels (**E**) and pH (**H**). Bar graphs indicate the selected frequency of

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behavioral indices in the LOOCV. Line graph shows the absolute correlation coefficient between brain lactate levels and pH, and each behavioral index of 51 mouse strains. *r*, Pearson's correlation coefficient.

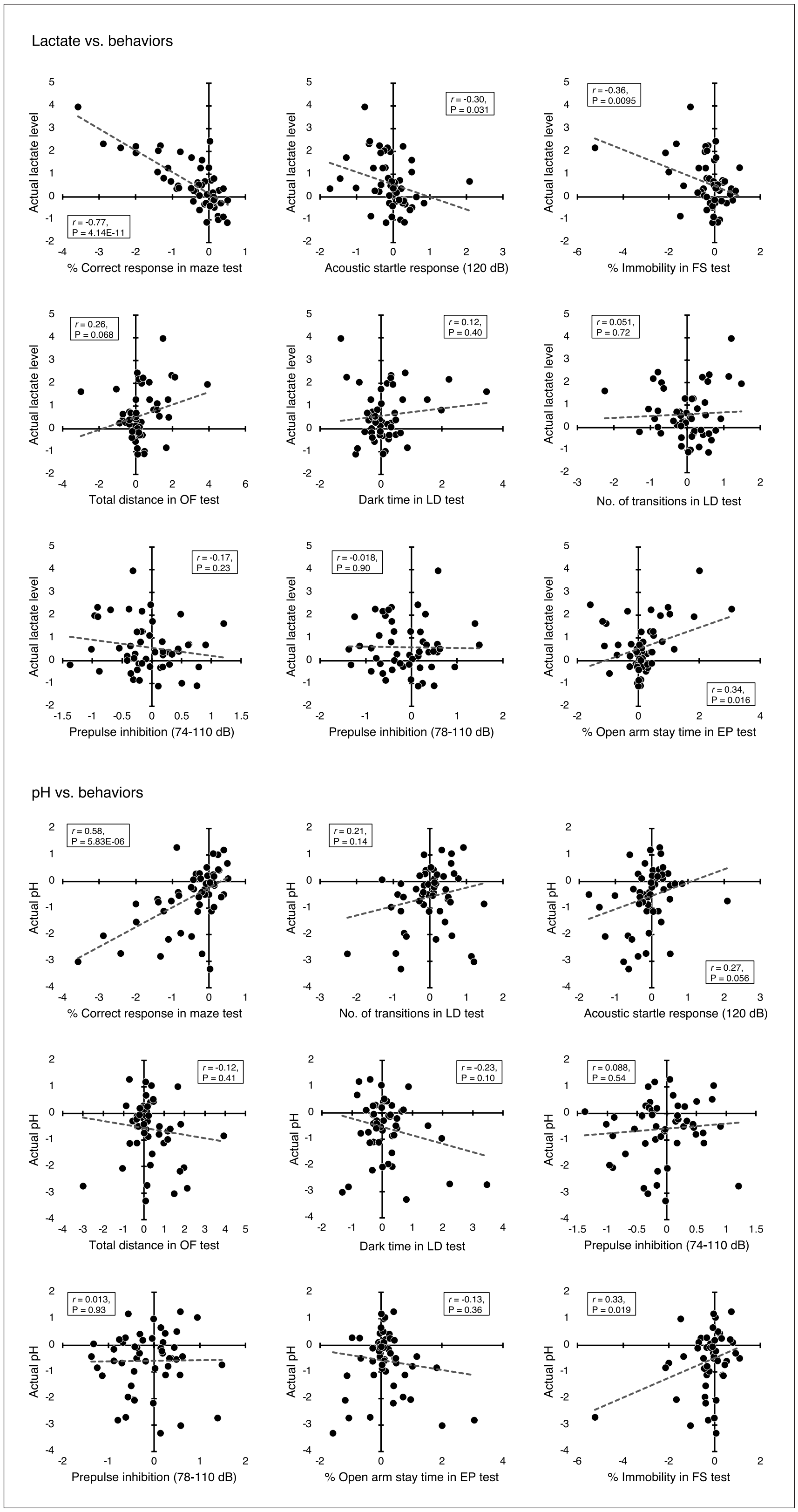

Figure 2—figure supplement 4. Correlations of brain lactate levels and pH with behavioral measures in a combined cohort. Scatter plots showing effect size-based correlations between actual lactate levels and pH, and behavioral measures. Data from 51 strains/conditions of mice used in the prediction analysis are shown. EP, elevated-plus maze; FS, forced swim test; LD, light/dark transition test; OF, open field test; *r*, Pearson's correlation coefficient.

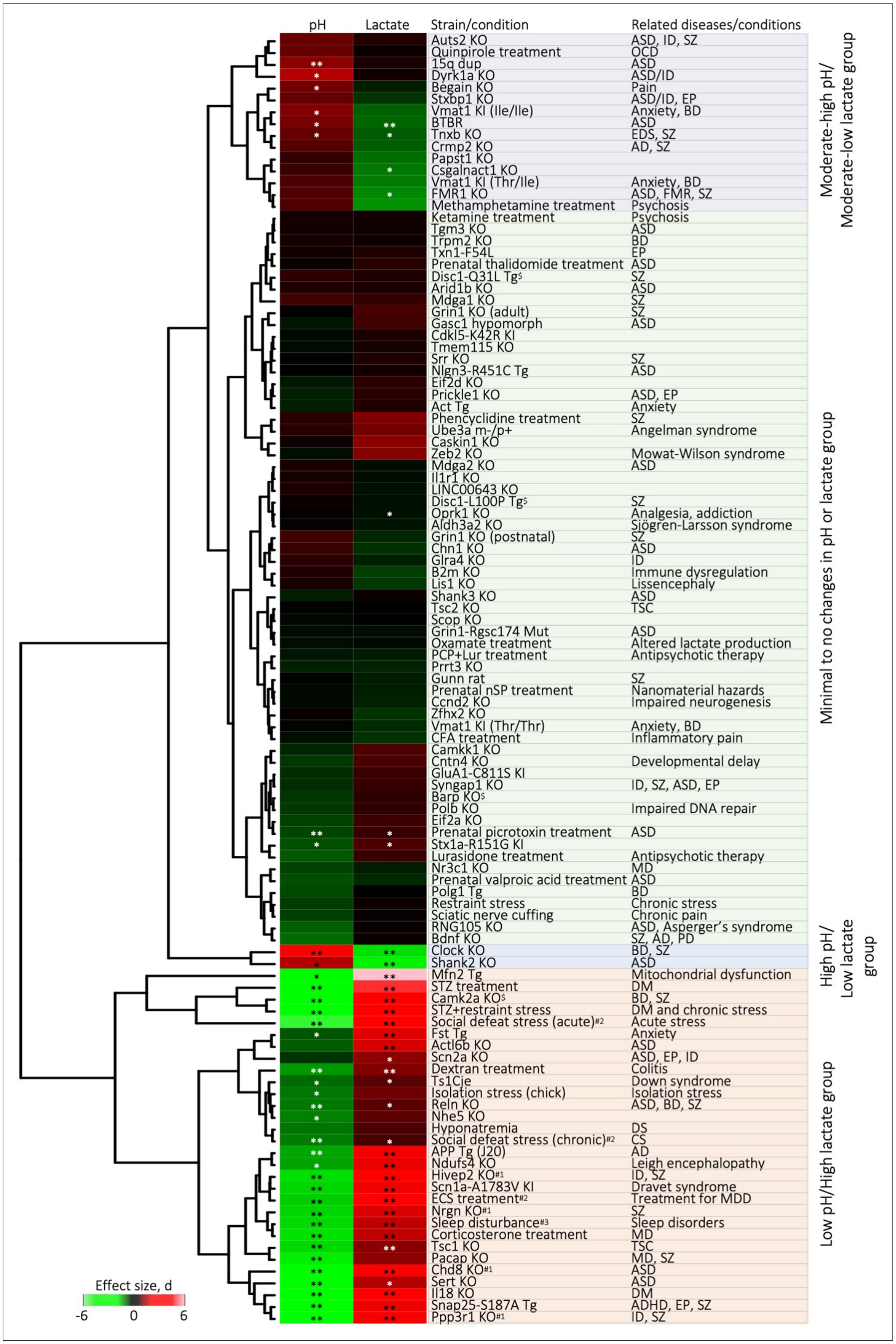

Figure 2—figure supplement 5. Hierarchical clustering of 109 strains/conditions of animals with respect to brain pH and lactate levels. The effect size was calculated for each strain/condition and was used in this analysis. #1pH and lactate data have been previously reported (***Hagihara et al., 2018***). #2Lactate data have been reported previously (***Hagihara et al., 2021a***). #3Lactate data have been submitted elsewhere. Asterisks indicate significant

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effects of genotype/condition. *$p < 0.05$, **$p < 0.01$; unpaired t-test, or one-way or two-way ANOVA followed by post hoc Tukey's multiple comparison test. Detailed statistical analysis is shown in ***Supplementary file 3***. AD, Alzheimer's disease; ADHD, attention-deficit/hyperactivity disorder; ASD, autism spectrum disorders; BD, bipolar disorder; CS, chronic stress; DM, diabetes mellitus; EDS, Ehlers-Danlos syndrome; DS, depression symptom; EP, epilepsy; FMR, Fragile X mental retardation; ID, intellectual disability, KI, knock-in; KO, knock out; MD, major depressive disorder; OCD, obsessive-compulsive disorder; PD, Parkinson's disease; SZ, schizophrenia; Tg, transgenic; TSC, tuberous sclerosis complex.

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	Dependent variable:			
	pH		Lactate	
Variable	$\lvert t \rvert$	P-value	$\lvert t \rvert$	P-value
Intercept	1630	< 0.0001	138.9	< 0.0001
Age	0.2815	0.7784	0.6189	0.5361
Sex	0.4591	0.6463	0.8773	0.3806
Storage duration	2.464	0.0139	0.2065	0.8364

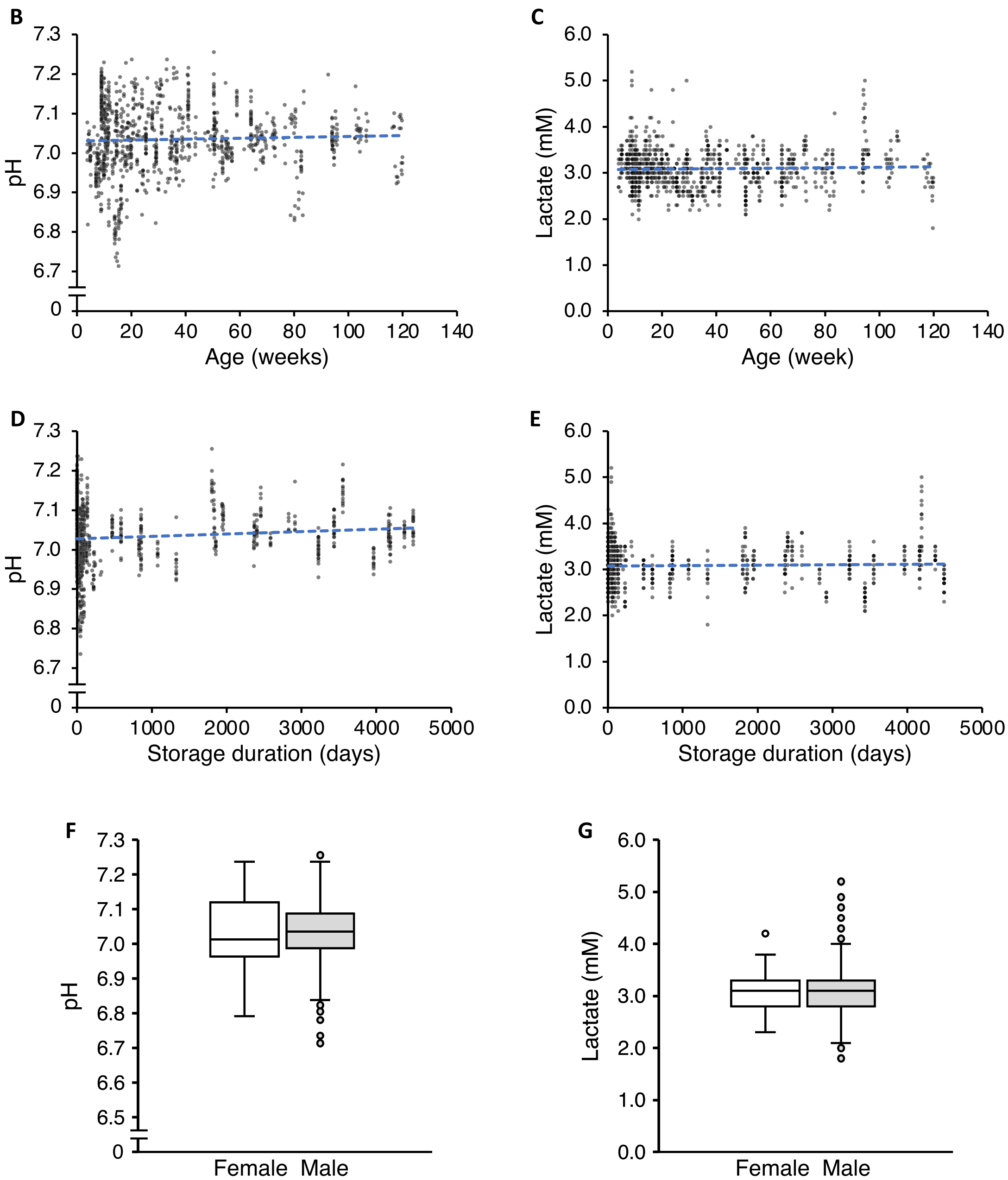

Figure 2—figure supplement 6. Effects of age, sex, and storage duration on brain pH and lactate levels. (**A**) Multivariate linear regression analysis. (**B, C**) Scatter plots showing correlations between age at sampling and raw pH (**B**), and lactate values (**C**) in wild-type/control animals. (**D, E**) Scatter plots showing correlations between storage duration and pH (**D**), and lactate values (**E**) in the wild-type/control animals. (**F, G**) Box plots of pH (**F**) and lactate values (**G**) in wild-type/control animals of each sex.