A genetically-diverse mouse model reveals a complex gene-environment regulation of cognitive resilience and susceptibility to Alzheimer's disease

AUTHORS:

Amy R. Dunn[1], Harpreet Kaur[1], Miko Dai[1,2], Kevin Charland[1,3,4], Andrew R. Ouellette[1,5], Niran Hadad[1,6], Patricia H. Doyle[1,7], Glen H.G. Acosta[8], Elizabeth Litkowski[4], Timothy J. Hohman[9], Kristen M.S. O'Connell[1,3,5]*, Catherine C. Kaczorowski[1,3,4,5]*

*Co-senior authors

Affiliations:

1. The Jackson Laboratory, Mammalian Genetics, Bar Harbor, Maine, USA 04609
2. Washington University, Department of Neuroscience, St Louis, Missouri, USA 63130
3. Tufts University School of Medicine, Boston, Massachusetts, USA 02111
4. University of Michigan Medical School, Department of Neurology, Ann Arbor, Michigan, USA 48109
5. University of Maine Graduate School of Biomedical Science and Engineering, Orono, Maine, USA 04469
6. Transcriptional Genomics Institute (TGen), Phoenix, Arizona, USA 85004
7. University of Kentucky College of Medicine, Department of Neuroscience, Lexington, Kentucky, USA 40536
8. nChroma Bio, Preclinical Pharmacology, Boston, Massachusetts, USA 02215
9. Vanderbilt University School of Medicine, Department of Neurology, Nashville, Tennessee, USA 37232

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Highlights:

- Comprehensive phenotyping of genetically diverse mice (AD-BXDs) revealed cognitively resilient strains to extreme genetic and dietary risk factors
- Locus on chromosome 10 harbors resilience factor/s that buffer the impact of familial AD mutations, female sex, and high-fat high-sugar diet
- Human cognitive resilience genes are conserved in AD-BXDs
- About 10% of female AD-BXD mouse strains showed an improved AD-related cognitive outcome on a HFHS diet.

Abstract

Alzheimer's disease (AD) has a complex etiology arising from largely unknown interactions between genetic and environmental factors. Even in populations with highly penetrant, disease-causing familial Alzheimer's disease mutations, there is wide variation in disease onset and progression, suggesting that clinical symptoms are modified by genetics and environment. Identification of such modifiers is critical, as mechanisms that promote resilience to deleterious AD mutations, unhealthy diet, or aging represent promising therapeutic targets for AD and other causes of cognitive decline; global resilience factors that protect against multiple "hits" are among the highest priority for discovery. Both genetic and environmental protective factors in AD have been identified; however, interacting gene-environment (GxE) factors are incredibly difficult to study in human populations given complex genomes, poor self-reporting, data from underrepresented groups, and incompletely documented exposomes. Here, we used a population of mouse strains that model the polygenic nature of human AD to characterize individuals that display cognitive resilience to high-risk genetic and dietary perturbations to define and quantitate roles for genetics, sex, age, diet, and complex interactions that are nearly impossible to elucidate from humans or inbred AD mice. We found that some strains showed improved AD-related outcomes when fed a high-fat high-sugar (HFHS) diet, suggesting the need for personalized recommendations for dietary interventions in AD. We found a locus on Chr 10 that was associated with cognitive resilience to AD in females, and this association was strengthened by HFHS diet, directly pointing to an interaction between specific genetic and environmental factors in AD risk and resilience. In conclusion, this study is the first of its kind to explore characteristics of resilience and GxE interactions in a genetically diverse mouse model. We present a subset of strains that exemplify global cognitive resilience to be leveraged for deep mechanistic studies aimed toward development of resilience-based and personalized therapeutic interventions.

1.1 Introduction

Alzheimer's disease (AD) is a common and irreversible neurodegenerative disease. It is also highly heterogeneous, with individuals displaying a wide range of symptom profiles, severity, progression, and pathology. Individual genetic variation explains some of this phenotypic variability [1]. Even among patients who harbor the same causative genetic mutations the disease onset, progression, and pathology are surprisingly variable [2-5], suggesting that there are additional genetic modifiers that influence the penetrance of these dominant mutations. Dozens of genetic variants have been identified that increase the risk of sporadic AD [6]. A handful of genetic variants have been shown to be protective against familial or sporadic AD [7-13] in addition to protective molecular, cellular, and environmental factors [14-16]. These protective, or resilience, factors have been increasingly recognized as representing particularly powerful therapeutic approaches to AD—rather than inhibiting "risk" pathways, we may be able to engage and leverage "protective" pathways to prevent disease progression in many individuals [11, 17-20]. Moreover, mechanisms of resilience and susceptibility may vary across individuals, with many molecular strategies leading to the same observed phenotype of resilience, or resilience in one or more cognitive domains vs others.

Despite all the known genetic contributors to disease susceptibility/resilience, we are still unable to account for a majority of predicted genetic risk for AD [1]. One hypothesis for this so-called

missing heritability is that some genetic contributors interact with environmental factors, and these gene-environment (GxE) interactions may contribute significantly to AD risk and protection. These genetic contributors may have small effect sizes in some environmental contexts, or larger effect sizes when sensitized by other environmental factors, and/or known environmental contributors may only be meaningful depending on individuals' genetic context. Diet, for example, is a commonly considered environmental factor that may modify risk of AD [21], with variations of diets high in fiber, low in sugar and saturated fat (e.g., a Mediterranean or MIND diet) currently being tested as a lifestyle intervention to prevent progression to (or of) dementia in older adults. However, the reported effects of diet on cognitive function and AD risk are variable in both human and model organism studies [22]. The potential genetic factors contributing to the variable effects of diet/environment are difficult to disentangle in human studies given the incredible complexity of the human exposome and incomplete record and control over diet over the human lifespan. Mouse models of AD, then, are a viable option for studying effects of environment/diet on AD and cognition given their highly controlled environments; however, mouse models of AD historically lack genetic diversity (or at least tractable genetic diversity), so true genome-wide GxE studies have been impossible.

Here, we use the AD-BXD model of AD, which integrates well-characterized causative AD mutations of the 5XFAD transgenic model of AD with tractable and replicable genetic diversity of the BXD genetic reference panel [23-31]. This population provides an unprecedented opportunity to (1) identify resilient populations and determine the genetic factors leading to resilience, and (2) experimentally probe genetic modifiers of effects of environmental risk and protective factors of AD and aging.

2. Methods

2.1. AD-BXD generation

AD-BXD mice were generated as described previously [23] by crossing female B6-congenic mice hemizygous for the 5XFAD transgene (MMRRC Strain #034848-JAX; RRID:MMRRC_034848-JAX) [25] with males from 38 BXD strains and parental B6 and D2 strains for 41 AD-BXD strains total [24] (Fig. 1A). We included both DBA/2J (JAX Strain #000671; RRID:IMSR_JAX:000671) and DBA/2J-Gpnmb^{+}/SjJ (JAX Strain #007048; RRID:IMSR_JAX:007048) which has a corrected (functional) allele of *Gpnmb* as parental "D2" strains in this study, and B6D2F1 and B6xD2Gpnmb+ are abbreviated as "D2" and "D2G", respectively. We included an average of 6 biological replicates per BXD strain, sex, 5XFAD status (nontransgenic or carrier), diet (chow or HFHS diet), and terminal age timepoint (3 mo, 6 mo, or 14 mo), for a total of 7,312 mice who entered the study and 6,164 mice who completed the study. Mice were group-housed in pens of 2-5 sex-matched littermates of mixed genotypes. To capture phenotypic and molecular features of "preclinical", "early disease", and "advanced disease" stages, we assigned mice to 3 mo, 6 mo, and 14 mo terminal timepoints, respectively.

2.1.2. Diets

Mice were fed ad libitum either normal control diet ("chow"; LabDiet 5K0G, 16% fat, 61% carbohydrates (0.69% sucrose) [32], 22% protein by kcal, or a high-fat/high-sugar (HFHS) diet; Research Diets D12451, 45% fat, 35% carbohydrate (17.5% sucrose), and 20% protein by

kcal). Mice in the HFHS diet group were randomly assigned by pen to this diet condition and placed on HFHS diet at 2.5 ms of age following baseline cognitive testing at 2 mo of age.

2.2. Phenotyping pipeline

Mouse body weights were measured every two months, starting at 2 mo of age. Mice received longitudinal y-maze, every 2 or 4 mo throughout their lives starting at 2 mo of age, for a total of 12,639 individual y-maze tests. Body weights were taken at the same timepoints. A subset of mice (n=1-2 per strain/sex/genotype/diet group) received comprehensive metabolic phenotyping longitudinally at 4, 6, and 14mos of age, consisting of indirect calorimetry (IDC, 5 days), intraperitoneal glucose tolerance testing (IPGTT), and nuclear magnetic resonance (NMR) body composition analysis; of these metabolic phenotypes, NMR outcomes are included in the present report. At their terminal timepoints (3mo, 6mo, 14mos), mice additionally underwent sensorimotor testing (grip strength, inclined screen, and narrow beam tests) and contextual fear conditioning (CFC), which measured short-term memory (contextual fear acquisition, CFA, as measured by percent freezing in the post-shock 4 period) and long-term memory recall (contextual fear memory, CFM; see Fig. 1A). Before each behavioral testing timepoint, mice were habituated to transport and the testing room for one hour each day for three days. Each apparatus was thoroughly cleaned with 70% ethanol before and after tests to minimize detectable olfactory cues produced by previously tested mice. Details of each phenotype are as follows.

Y-maze: To measure spatial working memory, we conducted y-maze tests as described previously [33, 34]. Mice were placed in one arm of a three-arm plexiglass maze and allowed to explore for seven minutes. Visual cues were placed outside of each arm. Movement through the maze was tracked with AnyMaze software (Stoelting Co, Wood Dale, IL, USA). Entries into each of the three arms before returning to a previously-entered arm were recorded as “spontaneous alternations.” Percent correct spontaneous alternations was calculated as [spontaneous alternations/(total arm entries – 2)]*100. Mice who entered fewer than three arms during the testing period were excluded from analysis.

Sensorimotor: Sensorimotor function was assessed using narrow beam (balance and motor coordination), inclined screen (geotaxis), and grip strength (muscle strength) as described previously [29]. During the narrow beam test, mice were placed on a raised beam (1.2cm width) with a dark box and an open platform at either end. Latency to reach the end of the beam was recorded; faster times indicate better motor function. Animals who fell off the beam or who did not complete the task received the maximum score of 300 s. During the inclined screen assay, mice were placed head-down on a grid raised at a 45-degree angle. Latency to rotate to head-up orientation was recorded. Animals who fell off the grid or who did not complete the task received the maximum score of 300 s. During the grip strength test, mice were placed with their forearms gripped to a grid and pulled backward. Force in newtons required to pull the mouse off the grid was recorded as forelimb grip strength. A sensorimotor composite score was calculated by averaging the z-score-transformed score on each component (narrow beam, geotaxis, grip strength).

Nuclear magnetic resonance (NMR) analysis of body composition: To measure lean mass, fat mass, and water mass, animals underwent NMR body composition analysis. Awake animals were enclosed in a Plexiglas tube and placed into an Echo MRI (Houston TX, USA), where body composition was measured according to manufacturer’s protocols.

Contextual fear conditioning (CFC): To measure contextual fear acquisition and memory recall, we conducted CFC. Mice were placed in fear conditioning chambers (Actimetrics, Wilmette, IL, USA) and allowed to explore for a baseline period of 150s. They received four mild foot shocks (0.9mA, 1s duration) with an intershock period of 140s +/- 10s [33, 35-37]. Each “post-shock” period was defined as the 40s following the shock. Movement and percent time spent freezing were recorded using FreezeFrame software (Actimetrics). Percent time spent freezing in the fourth postshock period (PS4) was recorded as an index of short-term contextual memory (memory acquisition).

Twenty-four hours later, mice were returned to the chambers with the same context and allowed to explore for 10 min without receiving foot shocks. Percent time spent freezing during the 10 min exploration was recorded as long-term memory recall (contextual fear memory, CFM). Animals who exhibited >40% freezing during the baseline acclimation period prior to training were excluded from analysis. CFC was administered once per animal immediately before euthanasia and tissue harvest.

2.3. Survival analysis

While this study did not include a formal longevity paradigm, we did assess successful survival to the latest timepoint (14 mo). We categorized early deaths as “health-related” or “censored” study removals. “Health-related” deaths included spontaneous death due to age or illness, euthanasia for veterinary health reasons, and death due to aggressive behavior (aggressor or target). “Censored” deaths were not due to the health/behavioral status of the mouse and included euthanasia required as the result of environmental conditions, e.g., inability to group-house due to death of cage-mates. We then calculated rate of survival (excluding censored deaths) to 14 mo within each strain/genotype/sex/diet group as a measure of survivability and attrition.

2.4. Enzyme-linked immunosorbent assay (ELISA)

Following the completion of the phenotyping paradigm, mice were euthanized using isoflurane and brains were removed and hypothalamus, hippocampus, midbrain, cerebellum, and isocortex (frontal and rear) were sub-dissected for subsequent biochemical analysis. A total of 422 samples were collected from 41 strains n=395 5XFAD and n=27 non-transgenic mice at three different ages (3 mo, n= 137 (66M/71F), 6 mo, n=144 (67M/77F), 14 mo, n=141 (65M/76F) of both diets were used for quantification of beta-amyloid 1-42 (Aβ42). ELISAs were performed as described previously [23, 25]. Snap-frozen cortical tissues (post frontal lobe removal; ~25mg) were homogenized in PBS containing 1% TritonX-100 + protease inhibitor cocktail (Thermo Scientific, 78429) + DX reagent (Qiagen, 1011008) with a 5mm stainless steel bead (Qiagen, 69989) using a TissueLyser II system (QIAGEN) at $30s^{-1}$ for 3 minutes. Homogenates were then sonicated for 2x10 seconds and protein concentration was measure using NanoDrop 2000 UV-Vis Spectrophotometer (ThermoScientific, USA). Sample were diluted to 10µg/µl and mixed with 5M Guanidine HCl (GuHCl) for the final amyloid beta extraction. The GuHCl extraction was performed overnight at 4℃ on orbital shaker. Samples were then diluted appropriately and run in duplicate along with standards using a commercially available ELISA kit (Cat# 298-62401, FUJIFILM Wako Chemicals, VA, U.S.A). The absorbance was read at 450 nm

using a microplate reader (SpectraMax® i3x) from Molecular Devices LLC., CA, USA. The levels of Aβ42 are reported as picomoles per liter (pmol/L).

2.5. QTL mapping

QTL mapping was performed as described previously [34] using the R package R/qtl2 [38] using a Haley-Knott regression method and adjusting for kinship using the leave-one-chromosome-out (LOCO) method. Additive covariates were determined by identifying significant main effects of factors (Sex, Diet, Genotype, Age) on each given trait. Significance levels were determined using the scanone() function in R/qtl2 using 1,000 permutations.

2.6. Statistics

Statistics and data visualization were performed in R. Significant effects of diet/sex/age/AD status on cognitive and noncognitive outcomes were identified using linear regression/ANOVA. Multiple comparisons were corrected for with a Bonferroni adjustment and post-hoc comparisons from ANOVA were achieved with a Tukey's HSD test where appropriate and as noted.

3. Results

We generated a population of 41 AD-BXD strains and their nontransgenic Ntg-BXD littermates. Mice fed normal control diet (chow) were evaluated for baseline cognitive testing at 2 mo of age and then either kept on chow or switched to a HFHS. Mice received longitudinal y-maze, metabolic testing, and body weight measurement. They also received cross-sectional sensorimotor testing which measured grip strength, geotaxis on an inclined screen, and balance on a narrow beam. Prior to sacrifice at either 3, 6 or 14 mo, they received terminal contextual fear conditioning (Fig. 1A), which measured short term memory (contextual fear acquisition, CFA, as measured by percent freezing in the post shock 4 period) and memory recall (contextual fear memory, CFM).

To assess main effects of experimental variables (age, diet, 5XFAD genotype, background strain (i.e., BXD strain), and sex) on metabolic and cognitive outcomes, we performed multivariate ANOVA on phenotype measures (Fig. 1B). As expected, age and sex accounted for most of the explained variance in body weight (51% and 21%, respectively), followed by background strain (9%) and 5XFAD genotype (3%). Much of the variance in cognitive traits was unexplained and could not be accounted for by the main effects of these experimental variables, as evidenced by residual variances of 23% or higher. Y-maze varied somewhat by background strain and age (20% and 15% respectively); contextual fear conditioning traits varied by background strain (42% and 67% for CFA and CFM, respectively), and CFA varied by 5XFAD genotype (15% of the variance). Notably, diet alone did not have a consistent effect on variance in cognitive traits; however, adding an interactive term (Age*Sex*Genotype*Strain*Diet) accounted for an additional 14-55% of the previously-unexplained variance in these traits (visualized in Fig. 1B as green segments), suggesting that these variables interact to influence metabolic and cognitive phenotypes in a strain-specific manner. We also calculated the heritability of these traits and found, consistent with our ANOVA results, that metabolic and cognitive traits were generally modestly heritable ([G_{var}/(G_{var}+E_{var})], where G_{var} = variance across strains and E_{var} = average within-strain variance); average overall h^2 range = 0.07-0.57; Table

1), again indicating that genetics contributes to a proportion of these traits, but that additional factors contribute to disease-related outcomes.

	Phenotype	Chow		HFHS	
		Female	Male	Female	Male
Ntg-BXD					
6mo	Body weight	0.36	0.39	0.44	0.46
	Y-maze	0.23	0.07	0.09	0.09
	CFA	0.21	0.20	0.24	0.20
	CFM	0.34	0.35	0.38	0.31
14mo	Body weight	0.44	0.57	0.53	0.54
	Y-maze	0.18	0.20	0.21	0.20
	CFA	0.22	0.24	0.27	0.26
	CFM	0.30	0.35	0.34	0.35
AD-BXD					
6mo	Body weight	0.45	0.38	0.41	0.49
	Y-maze	0.13	0.13	0.13	0.14
	CFA	0.21	0.29	0.23	0.20
	CFM	0.29	0.33	0.29	0.35
14mo	Body weight	0.43	0.56	0.46	0.52
	Y-maze	0.16	0.27	0.17	0.26
	CFA	0.20	0.23	0.27	0.32
	CFM	0.27	0.32	0.39	0.46
Overall					
	Body weight	0.46			
	Y-maze	0.17			
	CFA	0.24			
	CFM	0.34			

Table 1. Heritability estimates for body weight and cognitive traits at 6mo and 14mo in Ntg- and AD-BXDs on chow and HFHS, and overall (average of all groups)

GxE effects result in wide variation in cognitive and noncognitive outcomes in 14mo old mice

To assess the impact of GxE interactions on the progression and severity of AD-related phenotypes, we focused on outcomes in 14mo old Ntg and AD-BXD mice fed either chow or HFHS diets. Consistent with previous BXD and AD-BXD reports, there was a broad population-wide variation in disease-related outcomes such as body weight, cognitive performance, survival, and amyloid pathology in both diet groups (Fig. 1C)[23, 24, 29, 39].

Consistent with the widespread use of the HFHS model of diet-induced obesity (DIO), we expected that mice fed a HFHS diet would weigh more relative to chow-fed controls; in general, we did observe higher body weights in mice fed HFHS by 14 mo of age (effect of HFHS diet was +7.15g in 14 mo females and +7.28g in males; $p < 2E-16$ in both sexes). However, there was notable variation in the degree of weight gain, with some strains exhibiting little or even no diet-induced weight gain even after 8 months of HFHS feeding (see gray lines in Fig. 1C connecting chow- and HFHS-fed outcomes within each strain); these included Ntg male strain B6xBXD49, and 5XFAD male strain B6xBXD78; labeled in Fig. 1C). At a population level, HFHS

diet did not result in significant overall differences in cognition or a global increase in mortality. An exception to this was that Ntg males fed a HFHS diet performed better at 14 mo on long-term memory recall (CFM). Males fed a HFHS diet also performed overall better on y-maze when considering all age testing points (p=0.008; see Supplemental Fig. 1). We observed an overall increase in cortical Aβ1-42 at 14 mo in males fed a HFHS diet compared to males fed a normal control chow, but not in females. In all phenotypes observed, the degree and direction of response to HFHS diet varied by strain (Fig. 1C).

Along these lines, we observed significant diversity in cognitive trajectory over time in mice in both diet groups. In general, we observed AD-related cognitive decline in female mice across all cognitive domains; HFHS did not exacerbate cognitive decline in any domain.
Specifically, in females, we observed a significant effect of genotype, where 5XFAD females showed worse working memory performance (effect of 5XFAD transgene was -1.63 percentage points, p=0.006). Female mice also showed decline in working memory starting at 6mos of age (2-6 mo, p=0.003; 2-10 mo, p = 4.26E-05; 2-14 mo, p = 6.58E-05), and a decline in short- and long-term memory (CFA: 3-14mos, p<1.0E-07; CFM: 3-14mos, p=0.0001). Male mice carrying the 5XFAD transgene exhibited decline in working memory only by 10 mo of age (2-10mo, p=0.003; 2-14mo, p<1.0E-07), and HFHS-fed males showed better working memory performance overall (p=0.008). Finally, only female AD mice showed significant decline in short-term and long-term memory recall as measured by contextual fear conditioning (Supplemental Fig. 1). This observation that AD-related cognitive impairment occurs earlier and is more pronounced in females reflects the phenomenon in humans and animal models that females are more susceptible to AD [40-44].

A subset of strains show robust, global resilience against age- and AD-related cognitive decline independent of diet.

Resilience to AD is challenging to study in human populations, because of limited information regarding cognitive abilities throughout life, challenges accessing brain tissue from presymptomatic ages, and a lack of information regarding diet and other environmental exposures. Thus, characterizing resilience in a genetically diverse mouse population such as the AD-BXDs provides an unprecedented opportunity for discovering various molecular ,genetic, andbehavioral strategies that may all present as cognitive resilience.

To capture global cognitive resilience in AD, we incorporated all cognitive domains assayed (Y-maze/working memory, contextual fear acquisition/short-term memory, and contextual fear memory/long-term memory) and calculated a global resilience score. We took advantage of the reproducibility of the AD-BXD strains to adapt a common measure of human resilience—residual variance from the ‘expected’ cognitive function—to mice. This approach is used across human studies, where expected performance may be modeled given known characteristics (genetic risk, age, education, amyloid load, etc.) of the participants [45]. In our AD-BXD population, rather than modeling expected performance, we instead can consider the non-aged adult (i.e., 6mo) performance of Ntg, chow-fed animals as the “expected” performance based on that strain’s genetic background. Then, how much each strain deviates from this expected performance with age, transgene status, or environmental ‘hits’ (e.g., HFHS diet) is the residual variance that may be used to quantify relative resilience/susceptibility of that strain to these factors. This is visualized in Supplemental Fig. 2, where the line of slope = 1 is a theoretical expected performance if strains performed identically at 14mos to their Ntg/6mo/chow-fed baseline (i.e., if they experienced no age-, diet-, or AD-related cognitive decline). We repeated

this for each cognitive domain (working, short-term and long-term memory), and with a composite cognitive score incorporating all three assays (Fig. 2A). The composite cognitive score was calculated by scaling each test with a z-transformation, and summing the z-scores within each strain/sex/genotype/diet group. We measured distance from "expected" performance for each strain/sex/diet/genotype/age group for each cognitive domain. Finally, these values were scaled with a z-transformation and summed to obtain a "composite resilience score". In Fig. 2B, residual performance for each Ntg and AD-BXD strain at 14mos is quantified and plotted in order from least-to-most resilient female, chow-fed AD strain. Composite resilience scores around 0 or higher indicate that a strain performed similarly to their 6 mo/Ntg/chow-fed counterparts and represents cognitive resilience. We used this measure as a quantitative, continuous trait for further analyses of resilience and susceptibility.

Notably, some strains consistently scored higher on cognitive resilience regardless of sex, AD mutation status, or diet. These robustly, globally resilient strains included mice from BXD strains 99, 124, and 14. This observed cognitive resilience does not appear to be related to survival bias: cognitive resilience is not mediated by a strain's 14 mo survival rate in a multiple linear regression model with genotype, sex, diet, and survival rate as predictors (df=1, F=2.2; p = 0.14 for survival rate). The degree of resilience in these strains hint at robust, cross-domain and environment-independent resilience factors.

Several strains show wide variation in resilience depending on sex, diet, or AD mutation status. For example, female B6-5XFAD mice are highly susceptible to cognitive decline (composite resilience score ≈ -3.5) when fed a chow diet, and this susceptibility is attenuated when fed a HFHS diet (composite resilience score ≈ -1). Males of the same strain, B6-5XFAD, are resilient to cognitive decline regardless of diet (composite resilience score ≈ +2.5). Such cognitive resilience to the 5XFAD transgene in males on a B6 background is consistent with our previous findings [33] and highlights the important sex differences in AD-related phenotypes.

Next, we calculated the difference in composite resilience scores between Ntg and AD-BXDs to assess the relative contribution of a general *resilience/susceptibility to cognitive aging* in observed resilience and susceptibility to AD-sensitized cognitive decline (Fig. 2D). Normal aging accounted for a significant amount of the variance in AD cognitive decline, as evidenced by an overall adjusted R^2 of 0.3 ($p < 2.25E-09$) and individual within-group correlations between 0.39-0.45 (Fig. 2C). Thus, much of an individual's resilience to AD may be mediated by a more general resilience to aging. However, there were exemplary strains that showed AD-specific vulnerability or resilience. In females, both chow- and HFHS-fed mice from strain 152 were relatively *resilient* to age-related cognitive decline, while showing significant *susceptibility* to AD-related cognitive decline. In chow-fed females, strain 66 showed especially resilient phenotypes in both AD and Ntg mice, and in males and HFHS-fed females, several strains showed greater resilience in AD compared to Ntg mice.

Resilience to cognitive decline in AD females is polygenetic, and this genetic association may be modified by diet

Next, using this global resilience score as a quantitative trait, we performed quantitative trait locus (QTL) mapping to determine if cognitive resilience in advanced AD is strongly modified by one or more loci. Likely reflecting the polygenetic nature of resilience, we did not find a single locus that was significantly associated with global cognitive resilience; however, mapping

resilience in 14 mo old female mice with diet as an additive covariate revealed a suggestive QTL on Chr 10 (Fig. 2E). The *B* genotype at this locus was associated with stronger cognitive resilience (Fig. 2F) in HFHS-fed females. This locus was not associated with resilience in males (Fig. 2G), supporting data from us and others that the genetic architecture of resilience varies by sex[8].

The suggestive locus on Chr 10 (defined as the interval around the peak remaining within 1.5LOD of the peak) is a 10.07MB region, Chr10:117.30-127.37MB with the peak at 121.64MB, The locus contains 176 genes, including 72 named genes and 105 gene models and unannotated genes.

Human resilience genes predict resilience in female, but not male, AD-BXD mice

Following this, we probed the translatable value of our model by assessing contribution of genes known to be associated with human resilience to global resilience in AD-BXDs. We identified variants across the BXD genomes in loci containing known human AD resilience genes, with human resilience defined by residual cognitive variance quantification analogous to our present quantification [17]; human resilience genes without variants between B6 and D2 genomes were excluded from this analysis. First, we calculated a “polygenic resilience score” (PResS) [33, 46] using homologs of loci previously associated with resilience in humans, including *App, Apoe, Picalm, Tmem106b, Casp7, Abca1, Abca7, Sorl1, Rab10, Rest, Plcg2,Treml2, Dlgap2, Klotho, Bdnf, and Ms4a6c*. The PResS was correlated with resilience in AD-BXD females regardless of diet, but not in males (Fig. 3A), supporting previous findings that genetic mechanisms of resilience vary by sex [8]. In a model with sex and diet covariates, the PResS still significantly predicts resilience, with an adjusted R^2 of 0.19 (p=8.87e-07). Importantly, this indicates that the genetic factors governing resilience in humans are relevant in AD-BXDs, underscoring their value as a tool to understand the mechanisms of resilience to AD and cognitive aging. Three observations suggest that the genetic drivers of resilience may vary by environmental context: first, the PResS was more strongly associated with resilience in chow-fed females vs. HFHS-fed females. Second, three individual loci (*Rab10*, *Abca1*, *Tmem106b)* were significantly associated with cognitive resilience at a nominal level in chow-fed females, but not in HFHS-fed females, one locus (*Rest*) was associated with resilience in HFHS-fed but not chow-fed females. *Dlgap2* was significantly negatively associated with resilience in females regardless of diet (Fig. 3B). Finally, we performed a mediation analysis to determine if the influence of genetic drivers of resilience are mediated through metabolic factors. Interestingly, in HFHS-fed females only, the relationship between genetic resilience and cognitive resilience was partially mediated by glucose tolerance. Specifically, there was a significant association between the area under the curve on a glucose tolerance test (GTT AUC) and the PResS (p = 0.017, where higher genetic resilience is associated with better glucose tolerance), and correcting for this relationship reduces significance of the association between the PResS and cognitive resilience (p = 0.016 vs p = 0.0038 uncorrected). This mediating relationship between the PResS, glucose tolerance, and cognitive resilience was not present in chow-fed females or in males.

To specifically test whether human sex-dependent genetic modifiers of AD risk impact cognitive outcomes in AD-BXDs, we assessed variants proximal to genes reported to have differential effects on AD risk in males and females [8]. Of this list, one gene (*Kat8*) showed a female-

specific association with our global cognitive resilience score. In humans, *Kat8* variants are associated specifically with male resilience to AD.

HFHS diet can drive resilience or susceptibility to AD-related cognitive decline, depending on strain

Finally, using CFM as our heritable and translatable individual cognitive measure, we identified subsets of strains that showed distinct responses to HFHS diet. Specifically, we compared CFM performance across AD-BXDs strains to identify significant effects of diet by strain in long-term recall trajectory (Fig. 4A). Intriguingly, only one strain showed a significant exacerbation of cognitive decline when fed a chronic HFHS diet: male B6xBXD66 (Fig. 4B). However, several strains, HFHS diet mitigated cognitive decline and pushed strains toward AD resilience, particularly in females: B6, B6xBXD125, B6xBXD14, and B6xBXD73 in females, and B6xBXD152 and B6xBXD154 in males. We saw no significant correlation between several noncognitive measures (sensorimotor function, body composition, fasting blood glucose or glucose tolerance, or respiratory quotient) and CFM in chow-fed nor HFHS-fed mice; however, chow-fed male AD-BXDs did show a nominally significant association between body weight and CFM, where higher body weight was associated with poorer cognitive function at 14 mo of age (Supplemental figure 3).

4. Discussion

The AD-BXDs are a uniquely valuable tool that has allowed us to assess resilience and susceptibility to AD. Moreover, they have provided the first opportunity to evaluate gene-environment interactions in AD in a genome-wide manner, revealing that resilience and GxE contributors to resilience are polygenic traits.

Susceptibility and resilience to cognitive decline are heterogeneous traits not predicted by individual demographic factors

In AD-BXDs, the degree of cognitive decline by 14 mo of age was highly variable across strain, sex, and diet. Somewhat surprisingly, we did not observe a robust or global effect of HFHS diet on survival, AD pathology, or cognitive trajectory. In fact, depending on strain, HFHS diet was either neutral, protective, or damaging in terms of cognitive resilience in AD. This variability was striking and it provided an unprecedented opportunity to study both the strain differences in response to HFHS diet on AD-related cognitive phenotypes, and the differences in predictors of cognitive trajectory in chow- vs HFHS-fed animals.

Our study does provide additional support to a significant body of evidence that females are more susceptible to AD than males. Consistent with human disease, we observed the greatest susceptibility to AD-related cognitive decline in female AD-BXD mice. In fact, in all chow-fed female strains, AD mice exhibited greater cognitive decline compared to their Ntg counterparts. Male sex and HFHS diet resulted in improved cognitive outcomes in several strains, when compared to female or chow-fed genetic counterparts.

We hypothesized that some degree of resilience to AD was due to underlying resilience to general aging processes. In fact, we found a significant association between resilience to “normal” cognitive decline in middle aged Ntg animals and resilience to AD-related cognitive outcomes. These data suggest that there are likely common mechanisms between successful

normal cognitive aging and cognitive resilience to AD. However, many strains did exhibit selective vulnerability to AD. For example, in chow-fed females, strain B6xBXD152 is highly resilient to cognitive decline by age 14mo in Ntg animals, but highly *susceptible* to cognitive decline when harboring the 5XFAD transgene. Thus, some individuals likely harbor genetic susceptibility factors that overcome other factors that may promote successful cognitive aging.

To add to this complexity, some strains show dramatically different cognitive trajectories based on sex. B6xBXD152 females are particularly susceptible to AD-related cognitive decline; however, B6xBXD152 males are highly resilient to AD-related cognitive decline. Such strains may be particularly valuable in studying sex-dependent susceptibility and resilience in AD. Using the entire AD-BXD population, we next sought to identify potential genetic factors that could promote resilience in a sex-, diet-, or disease-specific manner.

Genetic predictors of resilience do not generalize to males in AD-BXDs

Several genes have been identified in previous studies as promoting cognitive resilience in humans [8, 17]. Using a polygenetic resilience score (PResS) generated using variants across these genes in the AD-BXDs, we found that these human resilience factors are also associated with resilience in females, but not strongly in males. The PResS was significantly associated with cognitive resilience in chow-fed AD females, regardless of metabolic outcomes. However, a portion of the protective effect of these resilience drivers was mediated via their association with glucose tolerance in HFHS-fed AD females. In HFHS-fed AD females, the PResS was associated with better glucose tolerance (lower AUC on a IP-GTT), suggesting that these genetic drivers of resilience may also improve metabolic outcomes on HFHS, which, in turn, lends protection against cognitive decline in AD. Thus, maintenance of metabolic health may be important in maintaining cognitive health, particularly in HFHS-fed individuals. We also examined known sex-specific modifiers of AD, and we found that one of these modifiers (*Kat8*) was uniquely associated with cognitive resilience in female AD-BXD mice but not in males. Finally, when we performed QTL mapping to identify novel mediators of cognitive resilience in AD-BXDs, our peak locus on Chr10 was associated with cognitive resilience in females, but not in males. Previous literature has suggested that the genetic architecture of resilience is sex-dependent, and our data support these findings. Our observation that the association between variants in these genes and resilience may also be modestly modified by diet, also hints that while genetic factors may be robust across environmental factors, the degree of protection may be influenced by environmental context.

Our suggestive resilience locus on Chr10 contains several promising candidate genes that could promote resilience in AD. One gene within this locus, *Slc16a7, w*hich encodes the protein neuronal monocarboxylate transporter MCT2, was previously identified by our group as mediating CFM in genetically diverse mice under various dietary interventions (e.g., caloric restriction)[47]. This strengthens our confidence that this locus may be one locus of many that is important in regulating dietary effects on cognitive function in aging and AD. Additionally, the several annotated genes contain missense mutations and thus may also represent candidates that are plausibly involved in the complex phenotype of cognitive resilience: *Cdk4*, kinase that is involved in response and metabolism of lipids and has been associated with obesity and type 2 diabetes; and *BC048403*, which encodes a subunit of KICSOTR, a component of the mTORC signaling pathway targeted by known resilience-promoting therapies such as rapamycin. Further validation of these candidates is warranted to evaluate the robustness of these candidates, as they may be potentially leveraged to promote resilience in people at risk for AD.

GxE in AD resilience: HFHS may be detrimental or protective, depending on genetic context

Previous studies have investigated how single AD-related genes may interact with certain environmental risk or protective factors that impact AD risk [48-62]. For example, educational attainment and lifetime cognitive activity are most strongly protective in *APOE4* carriers [58, 63], and *APOE4* carriers are particularly susceptible to AD risk associated with obesity, low physical activity, heavy metal exposure, and sleep disturbances[49, 53-55] [see [52] for a review of known GxE interactions in AD]. Thus, we expected some variability in response to HFHS in the AD-BXDs, and we sought to characterize these gene-by-diet interactions in a population-wide manner.

We identified several strains whose susceptibility and resilience varied by diet, underscoring the individual variability in response to environmental (and likely treatment) interventions. This is important to consider for future pharmacotherapeutics, but also for commonly recommended lifestyle interventions, such as the WW-FINGER studies [64-68] that show efficacy in preventing cognitive decline. For example, we observed several strains that would likely be characterized as good candidates for a low-fat, low-sugar dietary intervention, such as B6xBXD12 AD females, that were susceptible to cognitive decline on a HFHS diet but not on a low-fat diet. Conversely, our data suggest that some individuals may not benefit—and indeed may experience exacerbated cognitive decline--from low-fat, low-sugar diets: strain B6xBXD125 females, as an example, showed significant improvement of AD cognitive outcomes when fed a HFHS diet vs a low-fat, low-sugar diet.

One goal of these analyses was to identify models of the most robust examples of cognitive resilience in AD. Strains that showed resilient cognitive outcomes despite aging, high-risk AD mutations, and diet, may be considered “super resilient” strains: these strains were resilient to cognitive decline in aging and across cognitive domains, even with AD mutations and a HFHS diet. B6xBXD124, 99, and 14 were some such strains and thus may be good candidates for further studying robust mechanisms of resilience against genetic and environmental “hits” in AD.

It is worth noting that we and others have previously characterized B6 as a resilient strain and the B6 genome as harboring resilience factors [23, 69]. Our current study differs from these previous studies by scale (~900 mice in our previous study versus >6,500 in the current study) and power to detect sex differences. In fact, we did observe resilience in male B6 mice; only female B6 mice were susceptible to AD. Furthermore, female B6-5XFAD mice had improved cognitive outcomes when fed a HFHS diet. This sex difference highlights not only the need to study both sexes in AD and in AD resilience in sufficient numbers, but it also indicates that genetic and environmental contributors to resilience are different between males and females. Moreover, these data underscore the limited translational value of using only B6 males in preclinical interventions testing—a strategy which, heretofore, has been largely unsuccessful in effective drug development.

Translatability & Conclusion

This study represents a significant step toward understanding resilience to AD and in understanding individual differences in susceptibility to genetic and environmental risk factors. We have identified several strains that will be ideal candidates to help elucidate the nature of resilience to AD, whether that is global cognitive resilience, sex differences in resilience, or resilience to environmental sensitizers of AD. Moreover, we have aligned AD-BXD strains to

human molecular subtypes, again demonstrating the superior translatability of AD-BXDs to the human disease and providing a resource for deeper mechanistic studies using strains that are exemplars of resilience and susceptibility to AD in any given domain.

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FIGURE LEGENDS

Figure 1. Study design and experimental factors contributing to weight/cognitive outcomes. (A) Mice underwent baseline cognitive phenotyping at 2 mo of age and were placed on high-fat/high-sugar (HFHS) diet at 2.5 mo of age (or continued on normal chow diet). They received cross-sectional and longitudinal phenotyping through their terminal timepoints of 3, 6, or 14 mo of age. (B) Percent variance explained in various traits by all main variables was quantified by the proportion of sums of squares contributed by a given factor. A substantial portion (14-55%) of the variance was accounted for by an interaction of these factors. Twenty-three to 91% of the variance in these selected traits was unexplained by these factors. (C) Individual strains had substantial variability in direction and degree of body weight and cognitive response to HFHS diet at 14 mo, as well as cortical amyloid levels at 14 mo and survival to 14 mo. HFHS diet led to higher body weights in all sex/genotype groups overall. Nontransgenic males fed a HFHS diet had better long-term memory at 14 mo, and 5XFAD males had higher amyloid at 14 mo. Selected strains that were extreme examples of high/low values on these traits are labeled.

Figure 2. Global resilience varies by AD status, sex, and diet, and is likely under polygenetic control. (A) Global resilience scores were calculated by generating a “residual variance” score for each strain/sex/diet group by determining the distance from expected performance of 14mo old animals relative to their baseline/chow-fed/Ntg controls for each cognitive domain. These scores were then normalized and summed to generate a composite resilience score representative of cognitive resilience across cognitive domains. (B) Composite resilience scores for both Ntg and AD-BXD strains are plotted in order of increasing resilience for each AD-BXD strain in their respective sex/diet groups. Connecting lines between genotypes indicate the distance between Ntg and AD resilience. (C) AD composite resilience scores plotted against Ntg composite resilience scores. Pearson’s R and p-value are displayed. There is a significant positive correlation between Ntg and AD composite residuals, suggesting common mechanisms conferring resilience to AD and normal aging. (D) The difference between

AD and Ntg composite resilience scores are plotted, where lower values represent specifically lower AD-related resilience. (E) Using QTL mapping with diet as an additive covariate, we identified a suggestive locus on Chr10 associated with composite resilience in female AD-BXD mice. (F) The B allele at this locus was associated with higher resilience. (G) This locus was specifically associated with resilience in HFHS-fed vs chow-fed females, and was not associated with resilience in males.

Figure 3. Human AD resilience genes predict resilience in AD-BXDs. (A) We calculated a polygenetic resilience score (PResS) based on human resilience genes. This PResS was significantly predictive of global resilience in female AD-BXD strains, but not in males. (B) Generating an odds ratio for individual reslience loci revealed that many of these were more strongly associated with resilience in chow-fed females compared to HFHS diet-fed females, particularly *Rab10*, *Abca1*, and *Tmem106b*, for which the *D* allele was significantly associated with higher resilience in chow-fed, but not HFHS-fed female AD-BXDs. However, *Rest* genotype was significantly associated with resilience in HFHS-fed females, and *Dlgap2* was associated with resilience regardless of diet, where the D allele was associated with lower resilience. (C) Out of genes that have been reported to have sex-specific associations with AD resilience, we found one gene that was significantly associated with resilience in females but not males, *Kat8*.

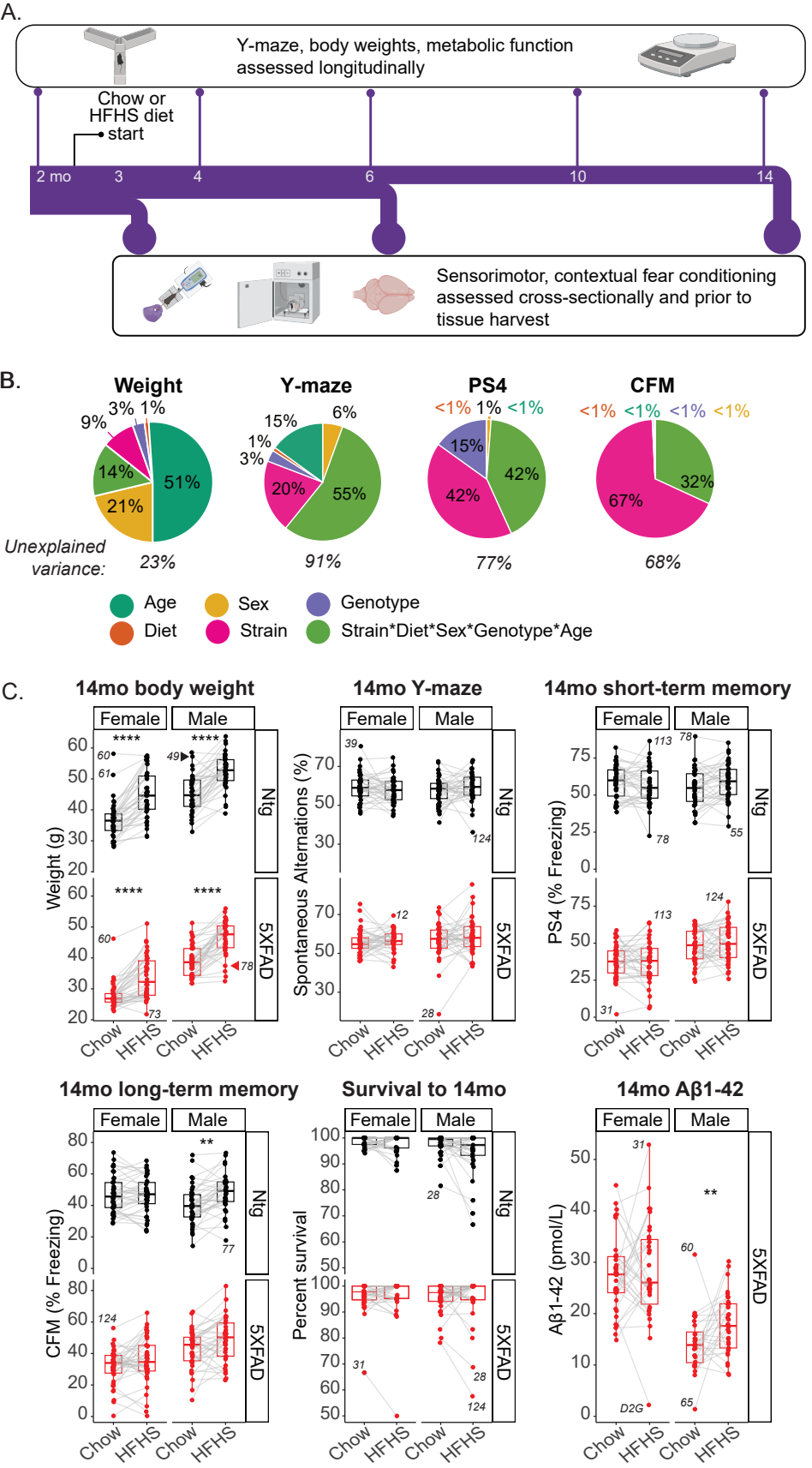
Figure 4. Several strains showed improved cognitive outcomes when fed a HFHS. (A) A handful of AD strains show significant difference between chow- and HFHS-fed CFM performance (4 female AD-BXD, B6-5XFAD, B6xBXD125, B6xBXD14, B6xBXD73; and 2 male, B6xBXD152 and B6xBXD154). One male AD strain showed significantly worsened cognitive outcomes when fed a HFHS diet compared to chow diet: B6xBXD66. (B) We observed several examples of HFHS-fed AD animals performing better compared to Ntg animals at 14mos on CFM. Males and female AD mice from strain B6xBXD66 showed significantly greater cognitive decline on HFHS diet.

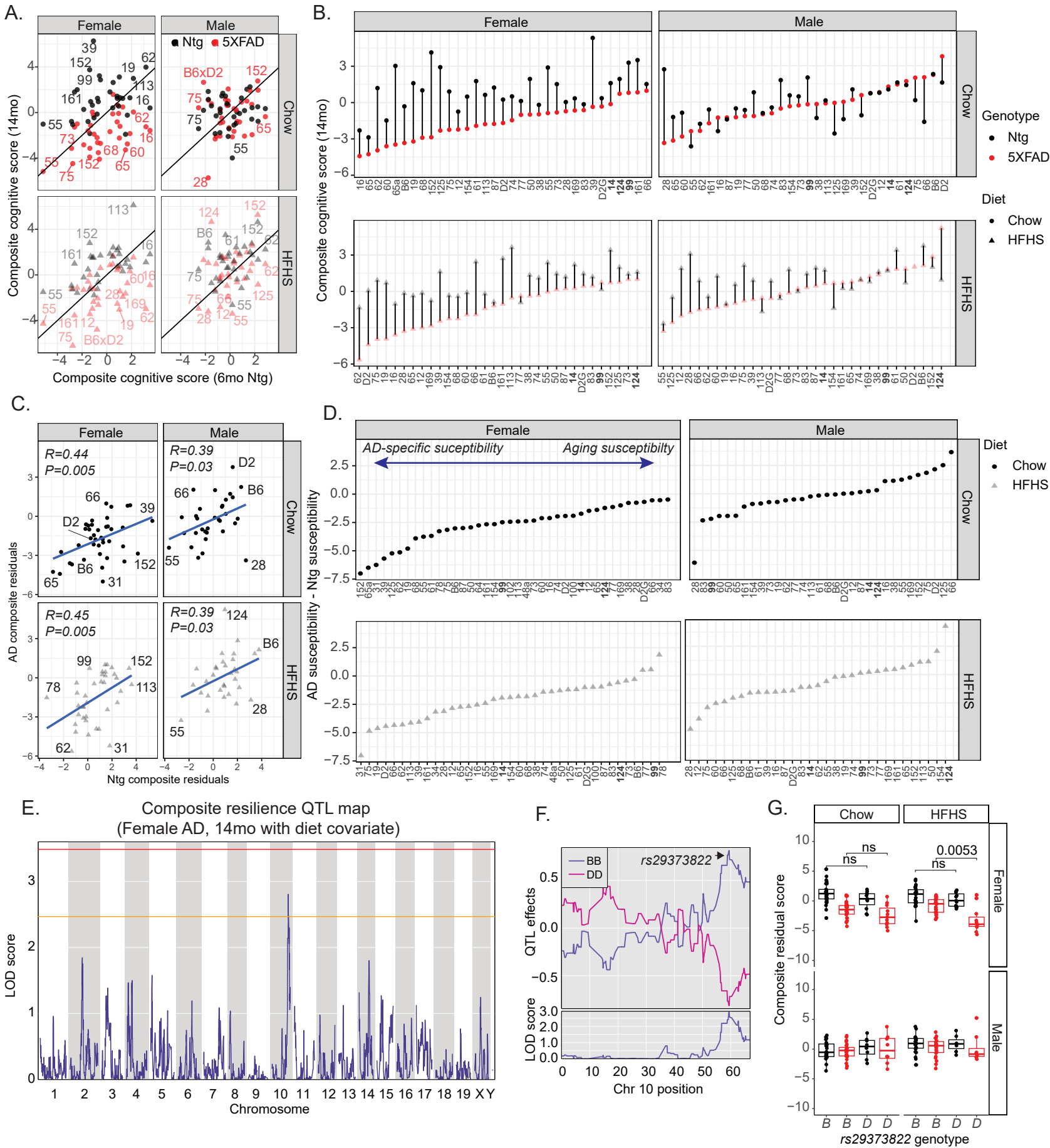
Supplemental figure 1. Trajectories of weight and cognitive function in Ntg and AD-BXDs by sex, genotype, and diet. Body weight varied significantly by age, genotype, diet, and interactions between these factors in both males and females by ANOVA. Differing letters denote statistically significant differences between diet/age/genotype groups. By ANOVA, Ymaze varied by age (females), and age, diet with no interaction between these factors. Asterisks denote statistically significant differences in performance across age groups. In females, short-term memory (PS4) varied by age, genotype, and an interaction between these factors. Differences between age/genotype groups are denoted by differing letters. In males, short-term memory varied by genotype; asterisks denote significant genotype differences in short-term memory at 6 and 14mos. Finally, CFM varied by age and genotype (females) or age and diet (males). Significant differences between age/genotype or age/diet groups are denoted by differing letters.

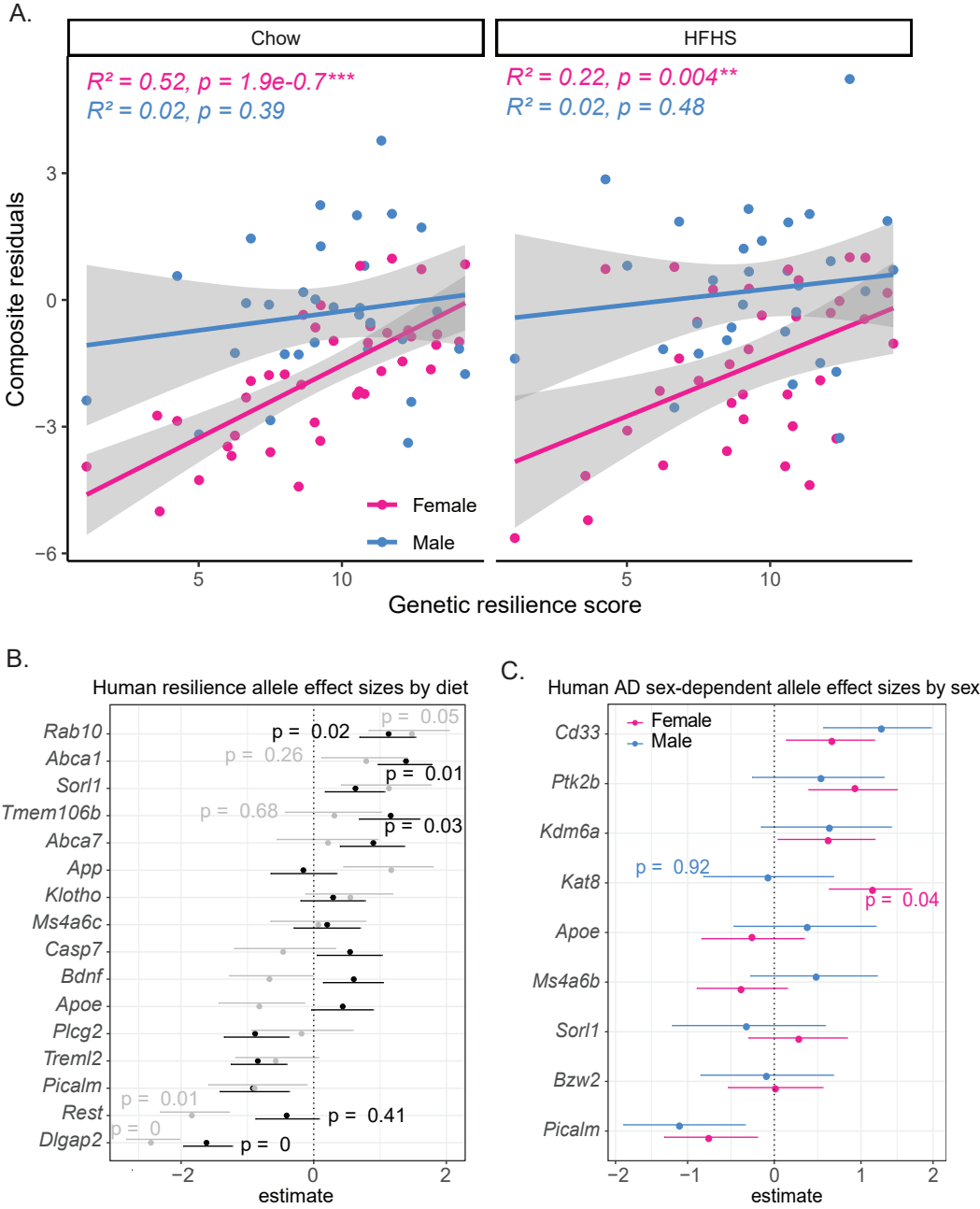
Supplemental figure 2. Calculating residual performance compared to 6mo/Ntg/chow-fed controls across each cognitive assay. These plots detail the individual cognitive assay data incorporated into the composite resilience score described in Fig. 2.

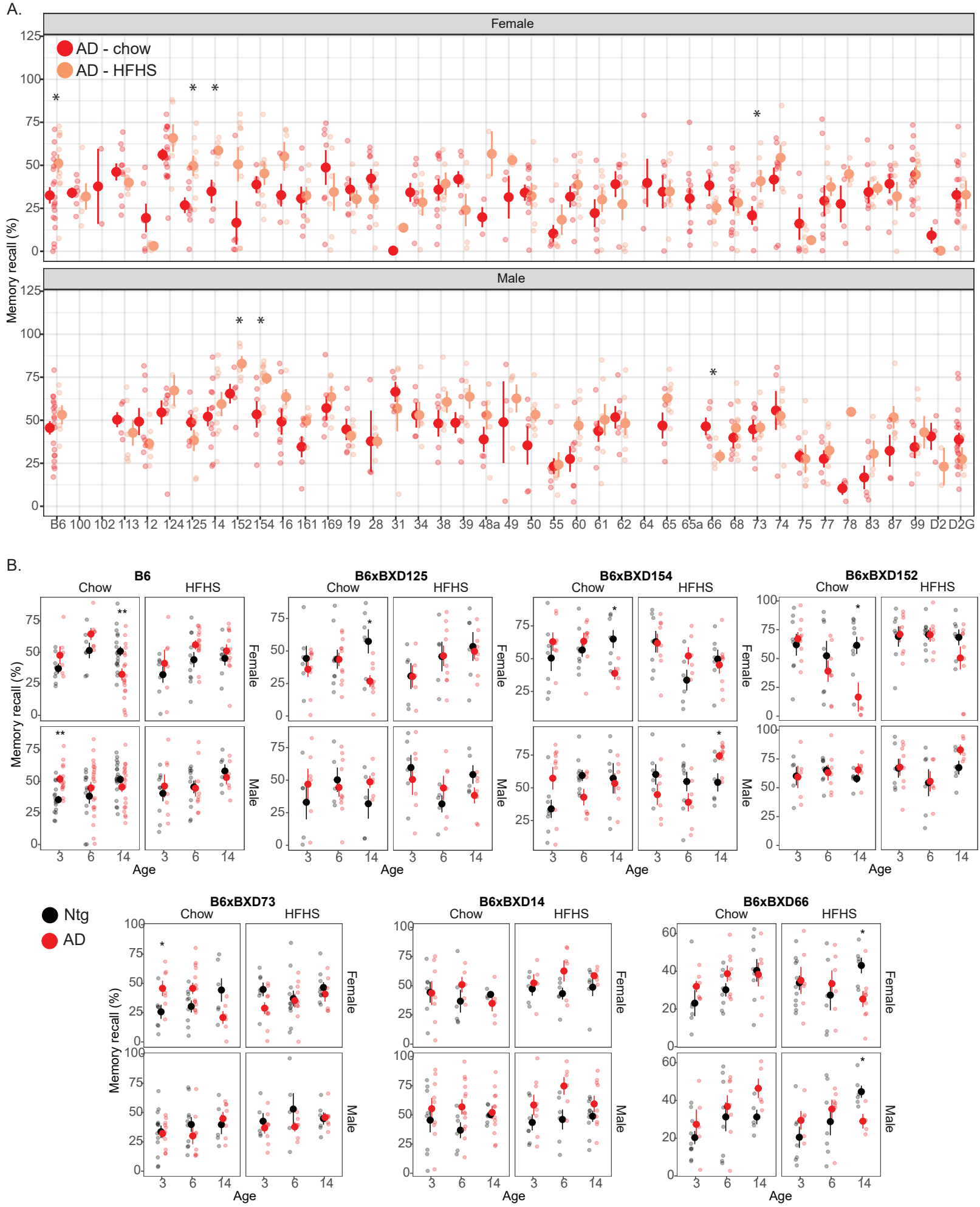
Supplemental figure 3. Little correlation between noncognitive traits and cognitive performance in 14mo AD mice. Sensorimotor function, glucose tolerance, body composition, and metabolic rate as represented by respiratory quotient (RQ) show no correlation with long-term memory (CFM) in 14 AD male or female mice fed either a chow or HFHS diet. This was

also true for body weight in every group except male AD mice fed a chow diet, where higher body weight was significantly negatively correlated with CFM (nominal p-value = 0.037).


A.
Y-maze, body weights, metabolic function assessed longitudinally
Chow or HFHS diet start
2 mo
3
4
6
10
14
Sensorimotor, contextual fear conditioning assessed cross-sectionally and prior to tissue harvest
B.
Weight
Y-maze
PS4
CFM
Unexplained variance:
23%
91%
77%
68%
Age
Sex
Genotype
Diet
Strain
Strain*Diet*Sex*Genotype*Age
C.
14mo body weight
14mo Y-maze
14mo short-term memory
14mo long-term memory
Survival to 14mo
14mo Aβ1-42
Female
Male
Ntg
5XFAD
Weight (g)
Spontaneous Alternations (%)
PS4 (% Freezing)
CFM (% Freezing)
Percent survival
Aβ1-42 (pmol/L)
Chow
HFHS
D2G


A.
Female
Male
Ntg
5XFAD
Chow
HFHS
Composite cognitive score (14mo)
Composite cognitive score (6mo Ntg)
B.
Female
Male
Chow
HFHS
Composite cognitive score (14mo)
Genotype
Ntg
5XFAD
Diet
Chow
HFHS
C.
Female
Male
R=0.44
P=0.005
R=0.39
P=0.03
R=0.45
P=0.005
R=0.39
P=0.03
Chow
HFHS
AD composite residuals
Ntg composite residuals
D.
Female
Male
AD-specific suceptibility
Aging susceptibilty
Chow
HFHS
AD susceptibility - Ntg susceptibility
Diet
Chow
HFHS
E.
Composite resilience QTL map
(Female AD, 14mo with diet covariate)
LOD score
Chromosome
F.
BB
DD
rs29373822
QTL effects
LOD score
Chr 10 position
G.
Chow
HFHS
Female
Male
ns
ns
ns
0.0053
Composite residual score
B B D D B B D D
rs29373822 genotype


A.
Chow
HFHS
R² = 0.52, p = 1.9e-0.7***
R² = 0.02, p = 0.39
R² = 0.22, p = 0.004**
R² = 0.02, p = 0.48
Composite residuals
3
0
−3
−6
5
10
Female
Male
Genetic resilience score
B.
Human resilience allele effect sizes by diet
Rab10
Abca1
Sorl1
Tmem106b
Abca7
App
Klotho
Ms4a6c
Casp7
Bdnf
Apoe
Plcg2
Treml2
Picalm
Rest
Dlgap2
p = 0.05
p = 0.02
p = 0.26
p = 0.01
p = 0.68
p = 0.03
p = 0.01
p = 0.41
p = 0
p = 0
−2
0
2
estimate
C.
Human AD sex-dependent allele effect sizes by sex
Female
Male
Cd33
Ptk2b
Kdm6a
Kat8
Apoe
Ms4a6b
Sorl1
Bzw2
Picalm
p = 0.92
p = 0.04
−2
−1
0
1
2
estimate


A.
Female
Male
AD - chow
AD - HFHS
Memory recall (%)
B6 100 102 113 12 124 125 14 152 154 16 161 169 19 28 31 34 38 39 48a 49 50 55 60 61 62 64 65 65a 66 68 73 74 75 77 78 83 87 99 D2 D2G
B.
B6
B6xBXD125
B6xBXD154
B6xBXD152
B6xBXD73
B6xBXD14
B6xBXD66
Chow
HFHS
Female
Male
Memory recall (%)
Age
Ntg
AD