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A second X chromosome contributes to resilience in a mouse model of Alzheimer's disease

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SUPPLEMENTARY MATERIALS
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Abstract

A major sex difference in Alzheimer's disease (AD) is that men with the disease die earlier than do women. In aging and preclinical AD, men also show more cognitive deficits. Here, we show that the X chromosome affects AD-related vulnerability in mice expressing the human amyloid precursor protein (hAPP), a model of AD. XY-hAPP mice genetically modified to develop testicles or ovaries showed worse mortality and deficits than did XX-hAPP mice with either gonad, indicating a sex chromosome effect. To dissect whether the absence of a second X chromosome or the presence of a Y chromosome conferred a disadvantage on male mice, we varied sex chromosome dosage. With or without a Y chromosome, hAPP mice with one X chromosome showed worse mortality and deficits than did those with two X chromosomes. Thus, adding a second X chromosome conferred resilience to XY males and XO females. In addition, the Y chromosome, its sex-determining region Y gene (*Sry*), or testicular development modified mortality in hAPP mice with one X chromosome such that XY males with testicles survived longer than did XY or XO females with ovaries. Furthermore, a second X chromosome conferred resilience potentially through the candidate gene *Kdm6a*, which does not undergo X-linked inactivation. In humans, genetic variation in *KDM6A* was linked to higher brain expression and associated with less cognitive decline in aging and preclinical AD, suggesting its relevance to human brain health. Our study suggests a potential role for sex chromosomes in modulating disease vulnerability related to AD.

INTRODUCTION

The expansion of translational neuroscience to investigate sex differences and their mechanistic underpinnings is of major consequence to human health (1). Understanding what makes one sex more vulnerable (or resilient) to aging and disease unravels new pathways to target with treatments that could benefit both sexes.

Alzheimer's disease (AD) is the most common neurodegenerative condition and a global health threat. In the absence of effective medical treatments, more than 50 million men and women worldwide will suffer from this devastating condition by 2050 (2). The burdens of the disease combined with failed clinical trials (3) warrant a deeper understanding of the heterogeneous nature of AD, with the goal of developing better therapies.

Being male or female, defined here as harboring a different sex chromosome complement (XY versus XX), is an understudied biologic variable that contributes heterogeneity to AD. Sex differences in AD reveal differing vulnerabilities in men and women (4, 5). Many more women have AD, largely due to their longevity (6) as they live to advanced ages, when AD risk and incidence is highest. In contrast, men with the disease die earlier in populations worldwide, indicating a male disadvantage with early-onset (7-9) and late-onset (10, 11) subtypes of AD. Furthermore, in aging and preclinical AD before the age of 85 years, men show worse cognition (12), more cognitive decline (13-15), and increased measures of neurodegeneration (16), despite similar deposition of amyloid and tau (15, 17), the pathological hallmarks of AD. This could underlie higher prevalence (18) and earlier onset of mild cognitive impairment (MCI) in men compared to women in some populations (19, 20). Here, we assess sex-biased mortality in AD by meta-analysis, investigate whether sex chromosomes affect vulnerability in a mouse model of AD, and test whether an X chromosome gene influences cognition in this mouse model.

RESULTS

Male sex and increased mortality in AD and the hAPP mouse model

We conducted a meta-analysis of data collected on mortality in human populations worldwide. Only longitudinal studies that defined the time variable as age of disease onset or duration of disease after onset were included; cross-sectional studies were excluded. Our meta-analysis showed that male sex increased risk for death in AD by 62% compared to female sex [male hazard ratio (HR) 1.63, CI 1.45 to 1.84, $P < 0.0001$; Fig. 1]. We then examined mortality in transgenic mice that expressed mutated forms of the human amyloid precursor protein (hAPP) (line J20) (21) and exhibited premature death, cognitive impairments, and pathological markers of the disease. Male hAPP mice died significantly earlier than did female hAPP mice on two genetic backgrounds, C57BL6/J ($P < 0.001$; Fig. 2A) and a mixed F1 generation of C57BL6/J crossed with FVB/N ($P < 0.05$; fig. S1).

Men and women undergo depletion of circulating gonadal hormones with aging (22-24), but mice do not (fig. S2) (25, 26). Because AD is a disease of aging, we simulated human reproductive aging in male and female nontransgenic and hAPP mice by gonadectomy to deplete circulating hormones (Fig. 2B) and assessed survival in gonadectomized male

and female hAPP mice. Male hAPP mice still died significantly faster than did female mice ($P < 0.05$; Fig. 2C). We explored whether hAPP mice showed a sex difference in cognitive functions independent of gonadal hormones. To reduce confounders, equalize hormones between sexes, and model reproductive aging of humans, we gonadectomized all nontransgenic and hAPP mice.

Male sex increases cognitive and molecular deficits in hAPP mice

We tested spatial learning and memory of gonadectomized mice in the Morris water maze and found that hAPP mice were impaired ($P < 0.05$; Fig. 2D). However, male hAPP mice traveled significantly longer distances to find the hidden platform than did females, indicating poorer learning capacity ($P < 0.05$; Fig. 2D). In a probe trial, male hAPP mice lacked memory retention, in contrast to all other groups (Fig. 2E). All mice located the target platform equally well when visible (Fig. 2D), and male and female mice within each group swam at equal speeds, although hAPP mice overall swam marginally slower ($P < 0.001$; fig. S3A).

In passive avoidance testing, which measures hippocampus- and amygdala-dependent fear memory, male hAPP mice, but not females, quickly reentered the dark chamber where they received a shock during training ($P < 0.05$; Fig. 2F). Male hAPP mice, but not females, lost the fear memory ($P < 0.05$; Fig. 2, G and H). Male vulnerability to deficits was significant with gonadectomy at young, middle, or old life stage ($P < 0.05$ to $P < 0.001$; fig. S4), across a range of cognitive and behavioral tasks ($P < 0.05$ to $P < 0.001$; fig. S4), and in an independent transgenic line of hAPP mice, hAPP-J9, which showed milder deficits ($P < 0.05$; fig. S5) (21, 27, 28).

Male hAPP mice showed significantly decreased expression of the neuronal activity–related protein calbindin ($P < 0.05$; Fig. 2I) in the hippocampus. Male and female hAPP mice did not differ in soluble β-amyloid (Aβ) (Fig. 2J) or protein expression of hAPP, total tau, and phospho-tau in the hippocampus (figs. S6, A to D, and S7) when cognitive and behavioral deficits had emerged (3 to 4 months). They also did not differ in amyloid plaque deposition (Fig. 2, K and L, and fig. S8) during middle age (14.5 to 15 months); however, females tended to show more plaques at a very old age (24 to 27 months) (fig. S9) as previously observed (29), despite decreased behavioral deficits compared to males.

We examined hAPP mRNA expression in the presence and absence of gonads and found that hAPP mRNA expression was equivalent across the experimental groups (fig. S10). Therefore, any unintentional gonadal hormone influences at the promoter of hAPP-J20 mice were not observed, a critical measure when directly comparing sexes in transgenic disease models.

Sex chromosomes mediate increased male vulnerability in hAPP mice

To dissect the etiology of male disadvantage related to AD after gonadectomy, we examined Four Core Genotype (FCG) (30, 31) mice. In normal mice and humans, the *Sry* gene on the Y chromosome encodes a protein that initiates development of testes followed by perinatal masculinization of the body and brain (32). In the FCG mouse model, *Sry* is transposed onto an autosome from the Y chromosome. This genetic manipulation enables generation

of XX and XY mice, each with either female ovarian (F, −Sry) or male testicular (M, +Sry) development: XX(F) ovaries, XX(M) testes, XY(F) ovaries, and XY(M) testes. A sex difference that varies by gonads is gonadal sex–mediated; one that varies by chromosome complement is sex chromosome–mediated (Fig. 3A).

We crossed FCG mice with hAPP mice to produce eight genotypes that included the four sex genotypes with or without hAPP (Fig. 3B). After sexual differentiation and reproductive maturity, we gonadectomized mice and assessed survival, cognition, and biochemical markers (Fig. 3C). XY-hAPP mice sexually differentiated as either male (M, testicular phenotype, +Sry) or female (F, ovarian phenotype, −Sry) died faster than did XX-hAPP mice of either gonadal phenotype (Fig. 3, D to F). In addition to the main effect of sex chromosomes, sex chromosomes interacted with gonadal phenotype in XY-hAPP mice. That is, XY-hAPP males (+Sry) survived longer than XY-hAPP females (−Sry) ($P < 0.05$; Fig. 3G), an effect not observed in XX-hAPP mice.

To determine whether sex chromosomes mediate male vulnerability to Aβ-related cognitive deficits, we tested mice in the Morris water maze. In finding the hidden platform, male or female XY-hAPP mice showed significantly worse learning than did male or female XX-hAPP mice ($P < 0.01$; Fig. 3, H and I, and fig. S11A). In contrast, all nontransgenic mice without hAPP learned similarly (Fig. 3, H and I, and fig. S11A). In a probe trial, XY-hAPP mice lacked memory retention (Fig. 3, J and K, and fig. S11B), whereas all XX (nontransgenic and hAPP) mice remembered, regardless of being male or female ($P < 0.05$; Fig. 3J and fig. S11B). All mice swam at equal speeds and located a visible target platform equally (fig. S3, B and C). In passive avoidance testing, male or female XY-hAPP mice showed significantly worse fear memory than did male or female XX-hAPP and nontransgenic mice ($P < 0.001$ and $P < 0.01$, respectively; fig. S12). As in non-FCG hAPP mice, male or female XX and XY mice did not differ in the amount of soluble Aβ in the hippocampus (fig. S6E) at the age of cognitive and behavioral testing.

A second X chromosome confers resilience to AD-related vulnerability in XY (male) and XO (female) hAPP mice

To further dissect causes of the sex chromosomal effects, we determined whether the presence of a Y or the lack of a second X chromosome conferred male disadvantage in hAPP mice. We investigated the XY* model (33, 34) of sex chromosomal biology in mice with and without hAPP. The Y* chromosome in XY* males contains an altered pseudoautosomal region that recombines abnormally with the X chromosome during meiosis. Progeny of XY* males crossed with XX females include four sex genotypes roughly equivalent to the following: XX and XO mice with ovaries and XY and XXY mice with testes. A sexual dimorphism that varies by the presence or absence of a Y is Y chromosome–mediated; one that varies by the presence of one versus two X's is X chromosome–mediated (Fig. 4A).

We crossed XY* males with hAPP females to produce eight genotypes of mice exhibiting varying dosages of X and Y chromosomes, with or without hAPP (Fig. 4B). We gonadectomized mice and then assessed survival, cognition, and biochemistry (Fig. 4C). Mice with one X chromosome (XY-hAPP and XO-hAPP) died significantly faster than

did those with two X chromosomes (XX-hAPP and XXY-hAPP) ($P < 0.01$; Fig. 4, D to F). Therefore, the addition of an X chromosome to XY-hAPP mice prevented male vulnerability, extending survival to that observed in XX-hAPP females. In addition to the main effect of X dose ($P < 0.01$; Fig. 4E), but not of Y (Fig. 4F), the Y interacted with the X; that is, XY-hAPP mice survived longer than XO-hAPP mice ($P < 0.01$; Fig. 4G).

We then tested whether the addition of an X to XY-hAPP mice reduced male vulnerability to cognitive deficits in the passive avoidance task (Fig. 4, H to J). Both male and female hAPP mice with one X chromosome (XY-hAPP and XO-hAPP) showed significant forgetting of fear memory ($P < 0.05$; Fig. 4, H to J), whereas those with two X chromosomes (XX-hAPP and XXY-hAPP) did not forget (Fig. 4, H to J). In contrast, all mice without hAPP had comparable and robust fear memory. As in FCG-hAPP mice, XY*-hAPP mice with 1X or 2X chromosomes did not differ in the amount of soluble Aβ in the hippocampus (fig. S6F). Thus, although hAPP mice with 1X or 2X chromosomes had comparable amounts of Aβ, hAPP mice with 2X chromosomes were less impaired.

A second X chromosome elevates *Kdm6a* expression independent of gonadal phenotype or the Y chromosome

We sought to understand how a second X chromosome could confer resilience, because XY and XX mice express only one active X due to X-chromosome inactivation in females. Whereas X-chromosome inactivation silences one X chromosome in mammalian XX cells, a small subset of X-linked genes escape X-chromosome inactivation and show transcription from both alleles, leading to higher expression in females (35-38). Of those, we focused on the gene lysine-specific demethylase 6a (*Kdm6a*; also known as *Utx*) encoding an H3K27 demethylase that consistently escapes X-chromosome inactivation in both mice and humans (39, 40). Loss-of-function mutations in *KDM6A* cause cognitive deficits in humans (41-46), and *Kdm6a* plays a post-developmental role in mouse synaptic plasticity and cognition (47).

We therefore examined *Kdm6a* expression in mouse brains. We first confirmed that *Kdm6a* escaped X-chromosome inactivation in the XX mouse brain through RNA fluorescence in situ hybridization (RNA FISH) (48) in mouse primary cortical neurons. Isolated XX neuronal nuclei with *Xist* RNA coating the inactive X chromosome, indicating X-chromosome inactivation, showed *Kdm6a* labeling at two sites, marking its transcription from both the active and inactive X chromosomes (Fig. 5A). In contrast, XY neurons showed only one site for transcription (Fig. 5A). Immunolabeling of Kdm6a protein in the adult hippocampus of XX and XY mice with a well-characterized antibody (49) showed a largely neuronal cytoplasmic staining pattern that was diffuse in both XX and XY mouse brains (Fig. 5B).

We assessed whether two X chromosomes increased expression of Kdm6a protein and mRNA in mouse hippocampus. Kdm6a protein expression was significantly higher in XX mice than in XY mice as measured by two antibodies ($P < 0.05$; Fig. 5, C and D). To determine whether the second X chromosome primarily governed higher expression, we assessed *Kdm6a* mRNA in FCG and XY* mice. As anticipated (50, 51), hippocampal *Kdm6a* was significantly elevated in XX mice with testes and ovaries ($P < 0.001$; Fig. 5E).

The presence of neither hAPP nor the Y chromosome altered this primary X-chromosome effect (Fig. 5F).

KDM6A expression is elevated in the brains of women, and *KDM6A* genetic variation in humans associates with cognitive resilience

We explored whether *KDM6A* mRNA expression was altered by sex in the brains of individuals with and without AD. We queried gene expression from a public dataset (GSE 15222; tables S1 and S2) accounting for age, postmortem interval, and sex. *KDM6A* expression was significantly higher in pathologically confirmed AD cases relative to controls in the temporal cortex, an area affected in early AD ($P = 3.64 \times 10^{-4}$; Fig. 6A). This increase was independently confirmed in two other public datasets of human postmortem gene expression in the temporal cortex, parahippocampal gyrus, and superior temporal gyrus (tables S1 and S3). In contrast, regions typically affected later or spared in AD such as the cerebellum showed no changes (tables S1 to S3). We then assessed *KDM6A* expression in brains of individuals identified as male or female in the GSE 15222 dataset. *KDM6A* expression was higher in females with ($P = 4.83 \times 10^{-4}$; Fig. 6B) and without AD ($P = 9.79 \times 10^{-4}$; Fig. 6B).

We then queried whether *KDM6A* expression, by proxy of a genetic variation, was associated with cognitive change over time. Using the Genotype-Tissue Expression project (GTEx) online portal of gene expression across tissues of nearly 1000 individuals (52), we searched for common variants associated with altered expression of *KDM6A*. The minor allele of one genetic variant, rs12845057, was associated with increased expression of *KDM6A* in the brain ($P = 7.0 \times 10^{-6}$). Frequency of the minor allele (A) is about 14% globally and 7% in Europeans (53). To test associations between the *KDM6A* variant and cognitive change, we queried the Alzheimer's Disease Neuroimaging Initiative (ADNI) dataset derived from a multisite study of individuals with both whole-genome sequencing and serial neuropsychological examinations ($n = 778$) that is enriched for individuals with MCI, a transition phase to AD. The minor allele was distributed equally among categories of cognitively normal ($n = 268$), MCI ($n = 465$), and AD ($n = 45$) individuals, indicating that it did not associate with disease risk (cohort demographics; table S4). Next, we used linear mixed-effects regression models to test for an association between the minor allele A of the *KDM6A* variant and cognitive change, accounting for baseline age, sex, education, and *APOE*ε4 dose. Increasing dose of the minor allele of the *KDM6A* variant was significantly associated with less cognitive decline over time using the Mini-Mental State Examination (MMSE) (β = 0.141, SE 0.035, $P = 0.00005$; Fig. 6C). This finding was consistent in another cognitive measure using the Alzheimer's Disease Assessment Scale (ADAS-cog) in overall function using the clinical dementia rating sum of boxes score (CDR), when assessing women only in all measures (fig. S13), and when assessing cognition in cognitively normal and in MCI individuals as subgroups (table S5).

Kdm6a knockdown in XX mouse neurons worsens, whereas *Kdm6a* overexpression in XY neurons attenuates Aβ toxicity in vitro

We next turned to experiments with primary wild-type mouse neurons exposed to recombinant Aβ1-42. The Aβ preparation was enriched for oligomers during the

experimental time frame, based on our previous characterization (54). XY mouse neurons were more vulnerable to Aβ-induced toxicity, in a dose-dependent manner, compared to XX neurons, using both the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay ($P < 0.001$; Fig. 7A) and the lactate dehydrogenase (LDH) assay ($P < 0.01$; fig. S14). In parallel with in vivo findings, neurons derived from XY* mice with one X chromosome (XY and XO) were significantly more vulnerable to Aβ toxicity than those with two X chromosomes (XX and XXY) ($P < 0.001$; Fig. 7, B and C). The protective effect of two X chromosomes was decreased by the Y chromosome ($P < 0.05$; Fig. 7B), indicating an X:Y interaction.

Given that *Kdm6a* escapes X-chromosome inactivation in XX mouse neurons and is increased in XX compared to XY mouse brains, we tested directly whether *Kdm6a* modulates neuronal susceptibility to Aβ toxicity in vitro. In XX mouse neurons, we decreased *Kdm6a* expression ($P < 0.01$; Fig. 7, D and E) to that found in XY neurons via lentivirus-mediated knockdown. Knockdown of *Kdm6a* in XX mouse neurons significantly worsened dose-dependent Aβ toxicity ($P < 0.01$; Fig. 7F) to a range observed in XY neurons. In XY mouse neurons, we increased *Kdm6a* expression to that found in XX neurons or higher ($P < 0.01$; Fig. 7, D and G) via lentivirus-mediated overexpression. Overexpression of *Kdm6a* in XY mouse neurons significantly attenuated dose-dependent Aβ toxicity ($P < 0.001$; Fig. 7H) to a range observed in XX neurons.

Kdm6a attenuates male vulnerability to cognitive impairments in XY-hAPP mice

We next determined whether increasing expression of Kdm6a attenuated male vulnerability to cognitive deficits in XY-hAPP mice. We gonadectomized XY nontransgenic and hAPP mice, injected lentivirus with (Kdm6A-OE) or without (control) the *Kdm6a* transgene bilaterally into the dentate gyrus, a region that affects spatial learning and memory, and analyzed mice behaviorally 1 month later (Fig. 8A). Lentiviral-mediated overexpression of *Kdm6a* in XY males increased *Kdm6a* mRNA expression in the dentate gyrus ($P < 0.05$; Fig. 8B) to that expected in XX females. In finding the hidden platform of the Morris water maze, XY-hAPP-Kdm6a-OE mice showed significantly better performance than XY-hAPP control mice measured by latency ($P < 0.001$; Fig. 8C) and learning ($P < 0.05$; Fig. 8D), quantified by comparing the last day of training to the first. Similarly, XY-hAPP-Kdm6a-OE mice showed significantly better learning than did XY-hAPP control mice measured by distance ($P < 0.001$; Fig. 8, E and F), although distance curves did not statistically differ. In a probe trial, XY-hAPP-Kdm6A-OE mice showed robust spatial memory retention, compared to XY-hAPP control mice ($P < 0.01$; Fig. 8, G and H) performing similarly to unimpaired nontransgenic mice. With the visible platform, hAPP mice swam marginally faster with longer distance than did nontransgenic mice; however, overexpression of *Kdm6a* did not alter either measure in either genotype (fig. S3, D and E). Further, increasing *Kdm6a* expression in XY mice did not alter hAPP-induced hyperactivity in the open field task and increased time spent in open arms in the elevated plus maze in hAPP mice (fig. S15).

DISCUSSION

Our data suggest a role for sex chromosomes in mice in countering deficits and toxicity related to AD in both sexes. A second X chromosome decreased mortality and brain dysfunction in gonadectomized male and female hAPP mice, without altering soluble Aβ or co-pathogenic proteins. A second X chromosome conferred resilience, in part, through the candidate gene *Kdm6a*, a histone demethylase gene that escapes X-chromosome inactivation, causing higher expression in cells with two X's compared to one X. Genetic variation of *KDM6A* linked to its increased brain expression was associated with slower cognitive decline in an aging population of individuals, including those with MCI.

Dissection of sex differences and their mechanistic underpinnings with powerful genetic tools provides opportunities to understand disease and unravel new sex-based pathways (55). Male sex is a major, underappreciated risk factor for rapid progression to death in AD (7-11), as confirmed by our meta-analysis (Fig. 1), and in other neurodegenerative conditions (56-59). These findings do not contradict the fact that more women have AD due to their longevity (6) and their increased risk or incidence after age 85 (4, 5, 12, 60), which together contribute to a higher lifetime risk of AD in women compared to men (61). When men get AD, they die faster (7-11). The male brain may be biologically older and more vulnerable, an idea supported by epigenetic (62) and metabolic studies (63) of humans.

In aging and preclinical AD, male sex may increase the likelihood of abnormalities favoring transition to clinical dementia. Men show worse memory function (12) and cognitive decline than do women (13-15), implying less compensation for similar subclinical brain pathology measured by positron emission tomography imaging of amyloid (12, 15). In studies of AD biomarkers (64), men show increased neurodegeneration (16, 17), a precursor for dementia. These findings could underlie earlier onset and increased incidence or prevalence of MCI observed in men from many (19, 20, 65-67), although not all (68-70), populations.

Recent studies of aging and AD [reviewed in (4)] indicate similar amyloid amounts in the brain (12, 15, 17, 71, 72) and cerebrospinal fluid (CSF) (71) of men and women, similar overall tau burden (73), but increased CSF and regional tau in women with high amyloid (71, 73). Likewise, AD pathology is similar between the sexes, up until older ages (72), when both pathology and risk of AD increases in women. Each sex may respond differently to comparable amounts of pathogenic proteins, a possibility observed in mice (74), which may explain why with similar tau loads, men show less neuro-structural preservation (75) and more cognitive impairment (76).

Congruent with human observations, soluble Aβ and amyloid deposition were similar between the sexes in our mice until very old age and did not explain male vulnerability at the neuronal or cognitive level. Other AD mouse models show very high amounts of Aβ with increased mortality in female mice (77-80) and are thus incongruent with our mouse findings. Given that no single model of AD fully recapitulates human AD, a disease with a wide clinical spectrum, we conducted cellular viability, cognitive, behavioral, synaptic, and mortality studies that collectively showed worse outcomes in primary neurons and gonadectomized male mice, a sex bias that persisted in our hAPP mice regardless of age

at hormone depletion, mouse strain, or genetic background. Our mouse studies focused on hAPP/Aβ-dependent abnormalities, representing a specific component of AD, a complex disease comprising multiple pathogenic proteins and risk factors.

We used gonadectomy to equate gonadal hormones between the sexes and simulate human reproductive aging, an approach distinctly different from previous studies of sex in AD-related models [reviewed in (81)]. Gonadectomy enables direct comparison of the sexes without confounding due to activational (short-acting) effects of ovarian and testicular hormones. This is of value, because ovarian hormones modulate Aβ, network dysfunction, and cognitive deficits in female hAPP mice (82, 83). Our experiments did not test the activational effects of hormonal treatments [reviewed in (82, 83)].

Sex chromosomes largely governed sex differences in vulnerability to mortality, cognitive dysfunction, molecular impairments, and cellular dysfunction in the FCG-hAPP mouse model. The XY genotype in hAPP mice that developed with ovaries or testes worsened measures, compared to the XX genotype that developed with ovaries or testes. Similarly, we recently found that sex chromosomes influenced mortality in mice during normal aging (84), suggesting action on fundamental pathways converging in aging and disease. The lack of a second X chromosome, rather than the presence of a Y chromosome, caused male disadvantage in animal and cellular models of AD in the XY* model. The presence of only one X chromosome (in XO females and XY males) consistently worsened hAPP/Aβ-related mortality, cognitive deficits, and cellular viability in both males and females, compared to two X chromosomes (in XX females and XXY males). The Y chromosome, the Y chromosome gene *Sry,* testes, or some combination of these decreased mortality in hAPP mice with one but not two X chromosomes. XY-hAPP males (+Sry) survived longer than did XY-hAPP females (−Sry) or XO-hAPP females (−Sry), indicating a potential protective role of the Sry protein or of testicular development itself in the XY, but not XX, genotype. Given that the X and Y chromosomes share homologous genes in pseudoautosomal regions, select Y genes may partially compensate for the lack of a second X chromosome.

Many factors influencing neural function reside on the X chromosome (85). Two X chromosomes could confer neural advantage through increased X dose arising from baseline escape of the inactive X chromosome. Whereas XY and XX organisms express one active X due to X-chromosome inactivation in females, select factors like the *Kdm6a* gene escape inactivation. Kdm6a is a histone demethylase that robustly and consistently escapes X-chromosome inactivation in female mice and humans (39, 40) and is enriched in the brain (51, 86, 87). The second X chromosome increased *Kdm6a* expression, independent of gonads or the Y chromosome, in our mice. This is important, because the Y paralog of *Kdm6a*, UTY (88), has high homology to *Kdm6a* (89) but a nearly inactive histone demethylation domain (90, 91). The presence of UTY in XY neurons and mice did not modify *Kdm6a*-mediated attenuation of AD-related toxicity in vitro or in vivo.

KDM6A expression in human brain was higher in females compared to males and in those with AD compared to controls. Because *KDM6A* loss-of-function mutations cause intellectual disability in humans (42-46) and *Kdm6a* elevation caused neural and cognitive

resilience in our mouse studies, it is interesting to speculate that increased *KDM6A* in AD could be a protective, compensatory response.

A common genetic variant in an intergenic region near *KDM6A*, rs12845057, was associated with greater expression in human brain. The minor allele frequency varies across populations, and about 13% of females and 6.5% of males carry it globally (53). In the current study of the ADNI cohort, increasing the minor allele dose was associated with cognitive resilience in individuals undergoing longitudinal testing over a decade, a finding consistent across clinical measures and when we assessed females only. Our analysis in males, who carry half the frequency, was likely limited by statistical power. In our subgroup analyses by clinical diagnosis, individuals with MCI showed the most resilience associated with the *KDM6A* minor allele, suggesting that increased KDM6A could modify clinical trajectory during the transitional period from MCI to AD. Whereas the ADNI cohort includes longitudinal data and multisite investigations, its limitations include a study of predominantly non-Hispanic, Caucasian populations within the United States. How broadly our findings extend to other populations remains to be determined.

In the current study, modestly increasing *Kdm6a* expression in XY mouse primary neurons and hippocampus of XY-hAPP mice attenuated hAPP/Aβ neurotoxicity and cognitive impairment. These findings suggest that minor elevation in *Kdm6a* transcription was sufficient to functionally increase neural resilience and partially reverse deficits in the XY-hAPP mice. Whether this requires histone demethylase activity is currently unknown. Kdm6a may act differently across cell types and biological systems. Whereas Kdm6a deletion in hippocampus impairs synaptic plasticity and cognition in mice (47), its deletion in immune $CD4^+$ T cells ameliorates the neuroimmune response in a mouse model of autoimmune encephalomyelitis (92). Thus, downstream actions of Kdm6a may be cell type specific.

Our study has several caveats and limitations. Our experiments do not exclude other potential contributions of X- or Y-based biological functions. A second X chromosome could contribute resilience through other baseline X escapee genes, epigenetic diversity derived from parent-of-X origin, or reactivation of the silent X chromosome. Furthermore, we did not study how the Y chromosome, its *Sry* gene, or testicular development contributed to a decreased mortality in hAPP mice with one X chromosome. Last, there are limitations to modeling AD in mice, including in each mouse model we used. Thus, we investigated several models and approaches, including mouse primary neurons, hAPP mice, human brain tissue expression data, and human cognitive data, and included several AD-related measures to increase the potential relevance of our findings. Collectively, these results imply that a second X chromosome, or genes that an X chromosome harbors, could contribute to counteracting AD vulnerability in both sexes.

MATERIALS AND METHODS

Study design

The objectives of our study were to probe the association of sex-based mortality risk in AD using meta-analysis; investigate whether sex chromosomes modify vulnerability related

to AD in mice using molecular, cellular, neurogenetic, and behavioral approaches; and test in mice whether an X chromosome factor decreased male vulnerability related to AD. We used experimental models of AD (mice and their primary neurons) and human databases of both brain tissue expression and of clinical cognitive performance. All animal studies were approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco and conducted in compliance with the National Institutes of Health guidelines. For animal experiments, all studies were conducted in a blinded manner and included male and female mice across the lifespan in multiple cohorts at the ages and background strains indicated. Mouse studies used littermate controls along with randomization of mice, and experimentalists were blinded to the genotypes of the mice. In mouse studies, exclusion criteria (greater than 2 SDs above or below the mean) were defined a priori to ensure unbiased exclusion of outliers. We used transgenic mouse models of sex biology crossed with hAPP mice and also used mouse primary neurons exposed to varying doses of Aβ. We assessed several outcome measures including mortality, cognition, cell death, pathology, RNA and protein measures, and biochemistry. Cell culture treatments were carried out with vehicle or synthetic Aβ1-42 peptide previously characterized by atomic force microscopy, and relative neurotoxicity was assessed with MTT and LDH assays.

Our findings showing a statistical effect of the second X chromosome in contributing resilience across measures in mice and mouse primary neurons led us to study *Kdm6a*, an X-linked gene that escapes inactivation in mice and humans. We established that *Kdm6a* escapes X-chromosome inactivation in mouse primary neurons using RNA FISH. We then queried *KDM6A* expression in humans using established databases of brain tissues including the Mayo Clinic Brain Bank and Mount Sinai School of Medicine Brain Bank (RNA sequencing), Gene Expression Omnibus (RNA microarray), and GTEx. We examined clinical and cognitive trajectories using the ADNI database to assess the relevance of our findings to the human condition. Last, we tested whether elevating the expression of *Kdm6a* causally contributed resilience to AD-related deficits in mouse primary neurons and hAPP mice using lentiviral gene delivery methods.

Statistical analyses

Statistical analyses were carried out with GraphPad Prism (version 5.0) for *t* tests and log-rank tests for survival analyses. For FCG-hAPP mouse and XY*-hAPP mouse survival statistical analysis, Cox proportional hazards models were applied to determine main effects, and a multivariate Cox model was used to test interactions of main variables on survival. R (nmle package) was used for analyses of variance (ANOVAs), post hoc tests, and meta-analysis. Differences between two means were assessed by two-tailed *t* tests for all experiments unless indicated otherwise in a replication cohort. Differences among multiple means were assessed by two-way ANOVA. A mixed-model ANOVA was used for analyses of Morris water maze data and included effects of repeated measures. Only significant *P* values were stated for two-way ANOVA results. Unless indicated otherwise, multiple comparisons of post hoc *t* tests were corrected for with the Bonferroni-Holm (stepwise Bonferroni) procedure to control for a family-wise error rate of $\alpha = 0.05$. Linear mixed-effects models were fit in R (93) using the standard lme4 (94) package. In mouse studies, exclusion criteria (greater than 2 SDs above or below the mean) were defined a

priori to ensure unbiased exclusion of outliers. Error bars represent ±SEM. Null hypotheses were rejected at or below a *P* value of 0.05. All analyses for *KDM6A* human studies were performed using R version 3.5.2 unless otherwise stated. We used linear mixed-effects modeling with random intercepts to test whether the genetic variant identified via GTEx as a modifier of *KDM6A* expression in brain also affected cognitive and clinical changes in the ADNI cohort. We covaried for baseline age, sex, education, and *APOE*ε4 dose.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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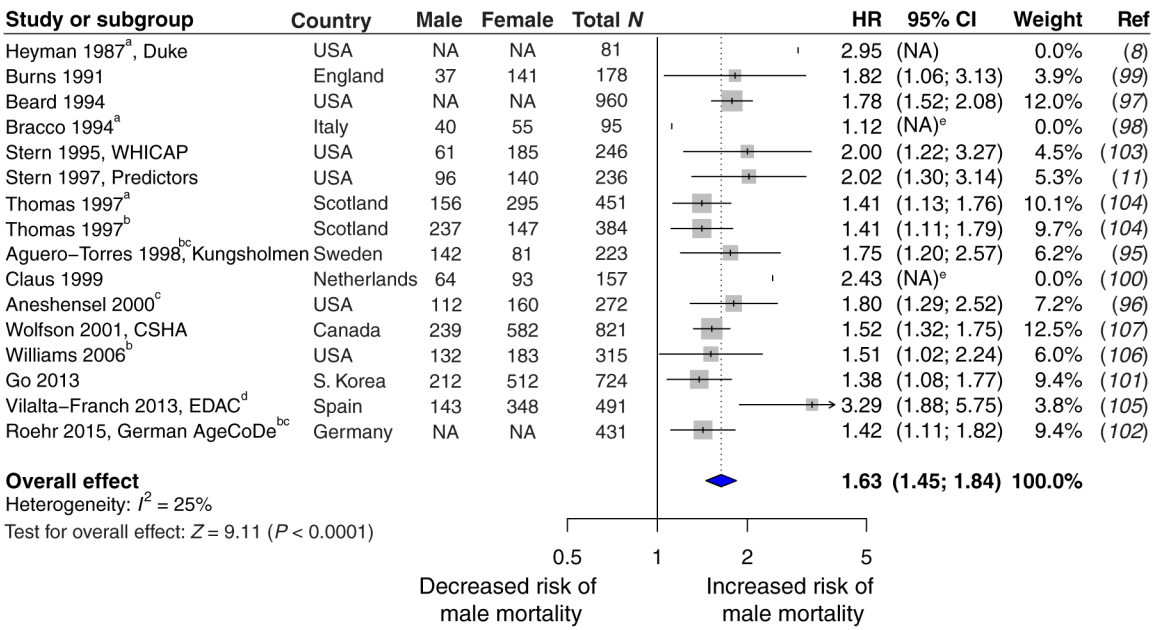

NA, not available.
[a]Early-onset AD
[b]Studies include dementia, vascular dementia, and/or AD with Lewy body dementia
[c]Additional stastical model(s) yielded similar results
[d]Cohort also studied in another publication, not included here
[e]HR calculated from mortality data provided in paper

Fig. 1. A meta-analysis of hazard ratios for male and female mortality in AD populations worldwide.

Hazard ratios (HRs) and 95% CIs are shown in a forest plot for studies (8, 11, 95-107) reporting male risk, compared to female risk, for death in longitudinal (and not cross-sectional) analysis of individuals with AD. Overall HR with 95% CI shown in bold indicates increased risk of male mortality (male, HR 1.63, CI 1.45 to 1.84; $P < 0.0001$). WHICAP, Washington Heights-Inwood Columbia Aging Project; CSHA, Canadian Study of Health and Aging; EDAC, Evolution of Dementia of the Alzheimer-type and Caregiver burden; AgeCoDe, Aging, Cognition, and Dementia in Primary Care Patients.

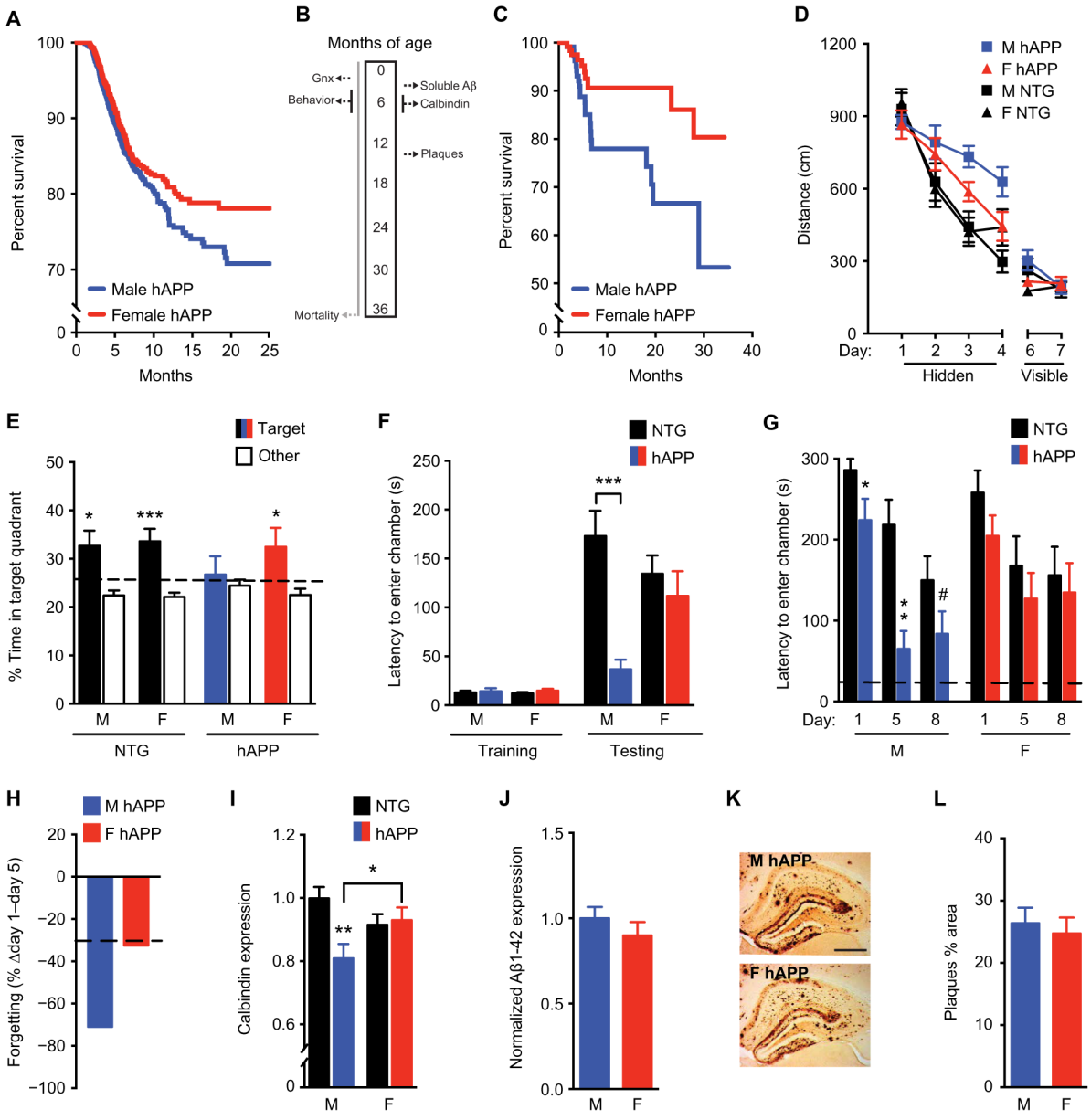

Fig. 2. Male sex increases mortality, cognitive deficits, and synaptic protein abnormalities in hAPP mice.

(**A**) Shown are Kaplan-Meier survival curves of male hAPP mice ($n = 1572$, blue) compared with female hAPP mice ($n = 1589$, red); all mice had intact gonads (log-rank test, $P < 0.001$). (**B**) All mice except those in (A) underwent gonadectomy (Gnx) at about 2.5 months of age; this was followed by behavioral testing conducted from 4 to 7 months of age and survival analysis conducted until 3 years of age. (**C**) Shown are Kaplan-Meier survival curves of male ($n = 116$) compared to female ($n = 123$) hAPP mice after gonadectomy (log-rank test, $P < 0.05$). (**D**) Shown are spatial learning curves of mice (age 4 to 7 months; $n = 10$ to 15 per group) tested in the Morris water maze during hidden platform training and when the platform was visible. Data points are daily average of total distance traveled to reach the platform over four trials. Mixed-model ANOVA for hidden training: female

hAPP versus male hAPP mice, $P < 0.05$. (**E**) A probe trial was conducted after hidden platform learning and removal of the escape platform. Percentage of time mice spent in the target quadrant of the maze, indicating memory for platform location, versus the average time spent in the other three quadrants is shown; *$P < 0.05$; ***$P < 0.001$. The dashed line represents chance performance (25%). (**F**) Shown is passive avoidance, fear memory of mice (age 3 to 3.5 months; $n = 7$ to 10 per group) reflected by latency to enter the dark chamber during training and testing 1 day after an electric shock to the foot. Two-way ANOVA: hAPP effect, $P < 0.01$; hAPP by sex interaction, $P < 0.05$. (**G**) Forgetting of passive avoidance memory in a separate cohort of mice (age 5 to 6 months; $n = 10$ to 12 per group), reflected by latency to enter a dark chamber 1, 5, and 8 days after a foot shock, was measured. The dashed line represents latency to enter the dark chamber during training, which did not differ among groups. (**H**) Percentage loss of fear memory from days 1 to 5 is shown. The dashed line represents the average for nontransgenic (NTG) animals. (**I**) Shown is quantitation of calbindin immunoreactivity in mouse dentate gyrus (age 5 to 7 months; $n = 11$ to 14 mice per group). Two-way ANOVA: hAPP effect, $P < 0.05$; hAPP by sex interaction, $P < 0.05$. Means are relative to NTG male control mice, arbitrarily defined as 1. (**J**) Soluble Aβ1-42 amounts in the mouse hippocampus determined by enzyme-linked immunosorbent assay (ELISA) are shown (age 3 months; $n = 8$ to 11 mice per group). (**K**) Representative immunostaining of hippocampal Aβ deposits in coronal brain sections from a male (top, M) and female (bottom, F) hAPP mouse (age 14.5 to 15 months). Scale bar, 200 μm; magnification, ×4. (**L**) Quantitation of percentage area covered by Aβ deposits in hAPP mice (age 14.5 to 15 months; $n = 11$ per group). Behavioral studies in male and female NTG and hAPP mice were performed across seven independent cohorts including in fig. S4. #$P = 0.06$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ [Bonferroni-Holm for (F), (G), and (I)]. Data are presented as means ± SEM.

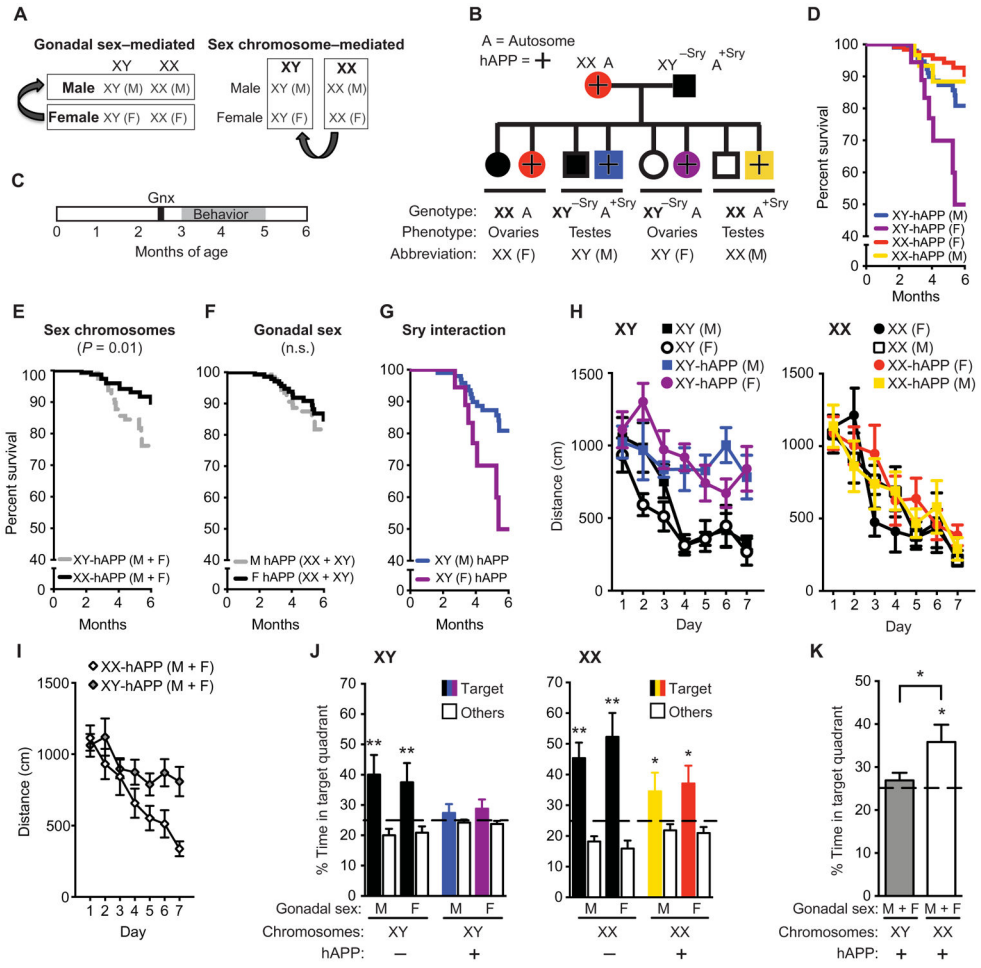

Fig. 3. Sex chromosomes mediate increased male vulnerability to mortality and cognitive impairments in hAPP mice.

(**A**) Strategy to identify the cause of sexual dimorphism using the FCG mouse model. (**B**) Diagram of the cross between hAPP and FCG transgenic mice is presented. FCG mice harbor a transposition of the *Sry* gene from the Y chromosome onto an autosome (A, autosome). Progeny include XX and XY mice, each with either ovarian (F) or testicular (M) development and with or without hAPP expression (hAPP, +). (**C**) Experimental strategy: All mice underwent gonadectomy at about 2.5 months of age, followed by behavioral testing and survival studies at 3 to 6 months of age. (**D** to **G**) In the Kaplan-Meier survival curves, (D) all groups of hAPP mice showed (E) a main effect of sex chromosomes on mortality (XY, HR 2.49, CI 1.21 to 5.14, $P < 0.01$) and (F) no main effect of gonadal sex on mortality ($P = 0.45$). (G) An interaction between sex chromosomes and gonadal sex indicated lower mortality in XY (male, M) compared to XY (female, F) mice (XY-M, HR 0.18, CI 0.03 to 0.92, $P < 0.05$). Analyses were by Cox proportional hazards for all groups: (XY-M: $n = 101$; XX-F: $n = 122$; XY-F: $n = 18$; XX-M: $n = 31$). (**H** and **I**) Spatial learning curves from the eight genotypes of mice tested altogether in the Morris water maze (age 3 to 5 months; $n = 5$ to 6 per group) show that (H) XY-hAPP mice (M or F) traveled longer distances to find the target platform, enabling escape from the water maze, than did XX-hAPP mice (M or F). This is highlighted in (I), where all XY-hAPP (M + F) mice were compared with

all XX-hAPP (M + F) mice. XX or XY mice without hAPP (M or F) learned similarly well. Data points are daily averages of total distance traveled to reach the platform over four trials. Mixed-model ANOVA: XX-hAPP versus XY-hAPP, $P < 0.01$. (**J** and **K**) A probe trial, during which the escape platform in the target quadrant was removed, tested for memory of the platform location in the eight genotypes of mice. Percentage of time spent in the target quadrant, indicating memory of the platform location, versus the average time spent in the other three quadrants showed that (J) XY-hAPP (M or F) mice did not favor the target quadrant, whereas XX-hAPP (M or F) mice did. The greater impairment of learning and memory in XY-hAPP mice is highlighted in (K) where all XY-hAPP (M + F) mice are compared with all XX-hAPP (M + F) mice. The dashed line represents chance performance. These findings were replicated in an independent cohort (fig. S11). *$P < 0.05$; **$P < 0.01$ versus chance performance of 25% (one-sample *t* tests) or as indicated by bracket (*t* test). Data are presented as means ± SEM. n.s., not significant.

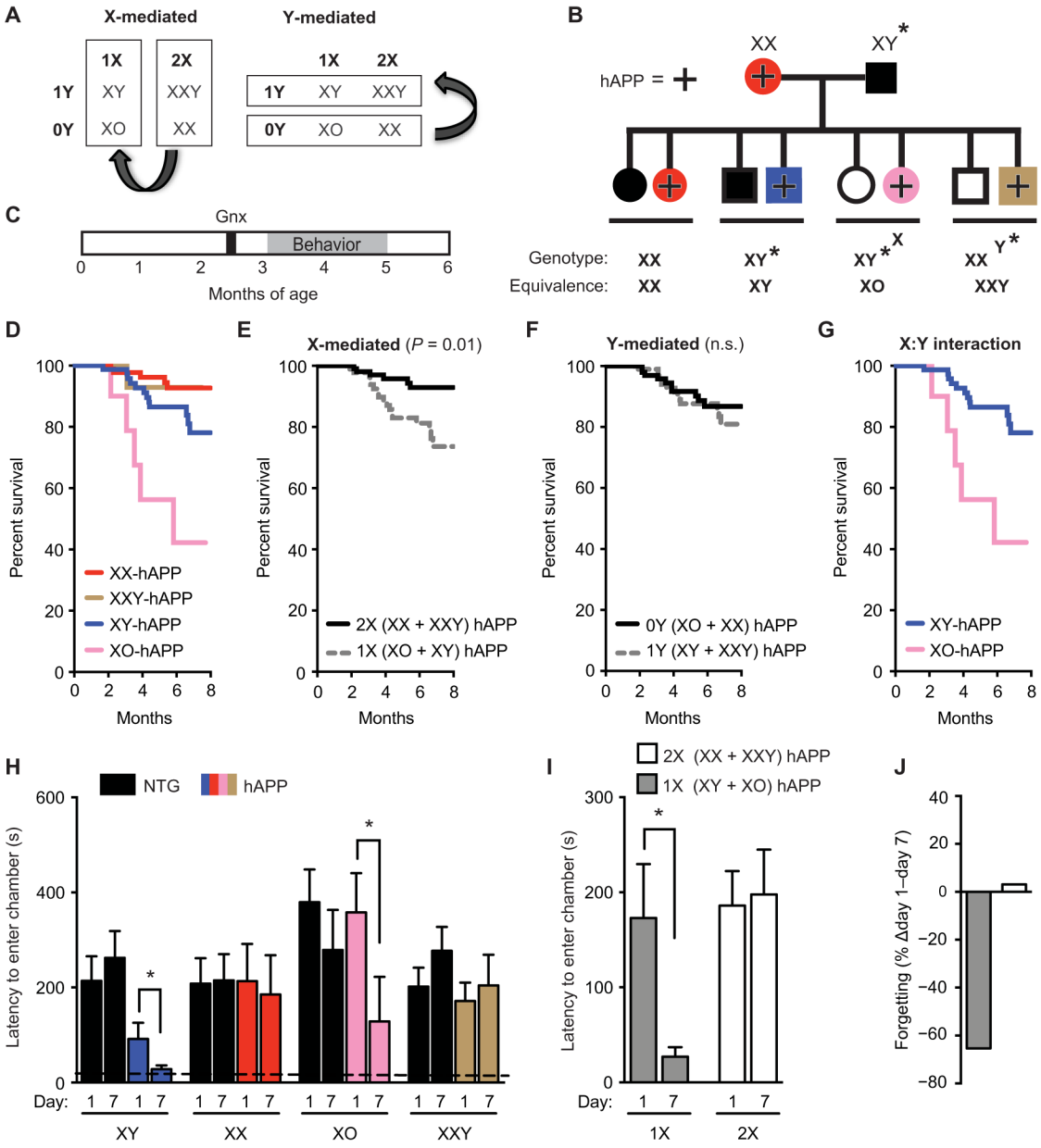

Fig. 4. A second X chromosome confers resilience against AD-related cognitive impairments in XY (male) and XO (female) hAPP mice.

(**A**) Strategy to identify whether the sex chromosome effect depends on the X or Y chromosome. (**B**) Diagram of mouse cross used in this experiment. hAPP females (XX, hAPP) were crossed with XY* males that harbored an altered pseudoautosomal region on the Y chromosome, allowing abnormal crossover with the X chromosome during meiosis (33, 34). The cross resulted in offspring of eight genotypes, each of the sex chromosome genotypes, with or without hAPP. The equivalent number of X and Y chromosomes for each genotype is shown. (**C**) Experimental strategy: All mice underwent gonadectomy at 2.5 months of age followed by behavioral testing and survival studies between 3 and 6 months of age. (**D** to **G**) In the Kaplan-Meier survival curves in (D), all hAPP mice show (E) a main

effect of X chromosome dose on mortality (2X, HR 0.2, $P < 0.01$, CI 0.12 to 0.75) and (F) no main effect of a Y chromosome on mortality ($P = 0.53$). (G) An interaction between X and Y chromosomes showed lower mortality in the presence of Y (or male gonadal type) when X dose = 1 (XY versus XO, HR 0.23, $P < 0.01$, CI 0.08 to 0.64). Analyses were by Cox proportional hazards for all groups (XY: $n = 79$, XX: $n = 88$; XO: $n = 10$; XXY: $n = 15$ mice). (**H** to **J**) Shown is testing of mice in the passive avoidance task, measured by latency to enter the dark chamber 1 and 7 days after a foot shock (age 3 to 5 months; $n = 4$ to 16 per group). (H) Abnormal loss of fear memory in hAPP mice of XY and XO genotypes is shown. Two-way repeated measures ANOVA: X dose effect, $P < 0.05$. The dashed line represents latency to enter the dark chamber during training, which did not differ among the groups. (I) Greater loss of fear memory in hAPP mice with 1X compared to 2X chromosomes is presented. (J) Percent loss of fear memory in hAPP mice with 1X compared to 2X chromosomes is shown. $*P < 0.05$ as indicated by bracket (Bonferroni-Holm). Data are presented as means ± SEM.

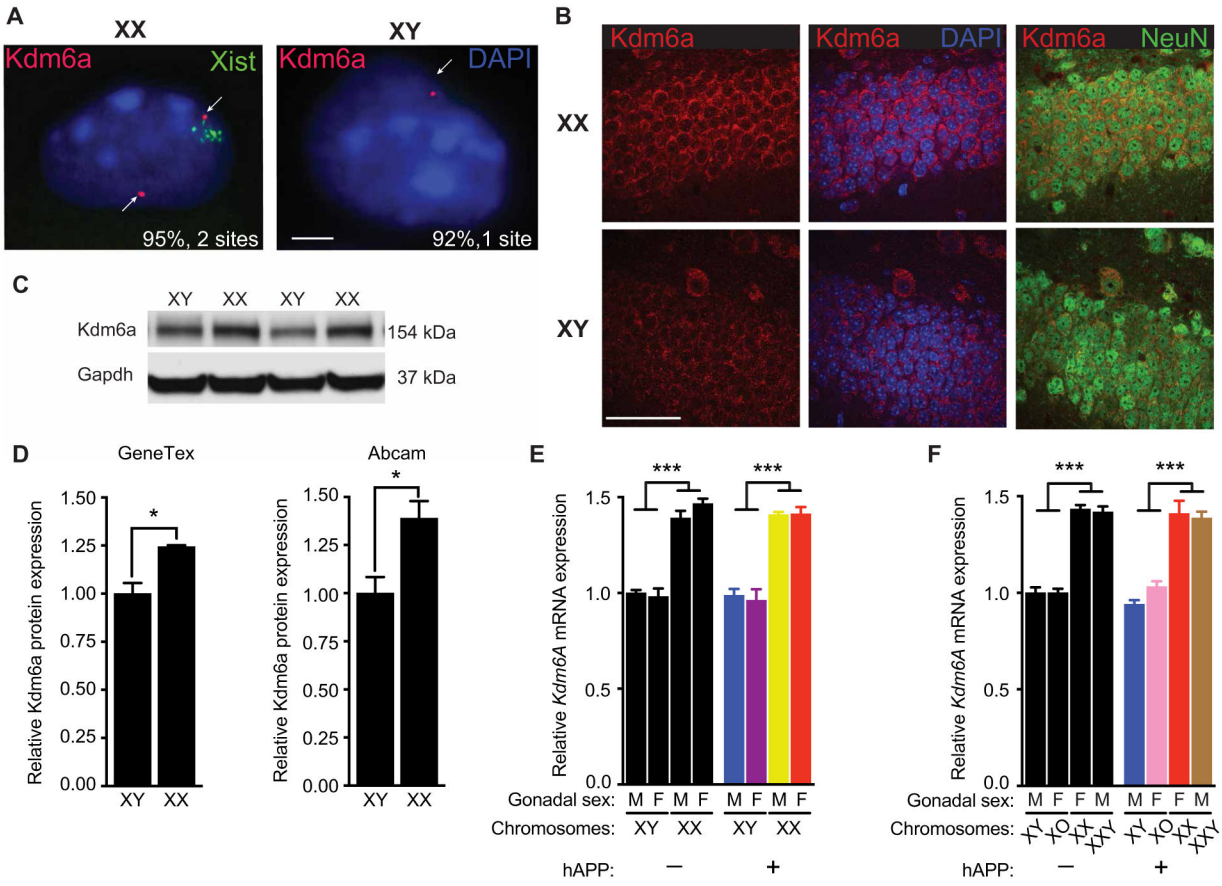

Fig. 5. A second X chromosome elevates *Kdm6a* expression independent of gonads or the Y chromosome in mice.

(**A**) Representative fluorescence in situ hybridization images for *Kdm6a* and *Xist* (RNA FISH) expression in XX (top) and XY (bottom) primary mouse neuronal nuclei. *Kdm6a* is shown in red, *Xist* is shown in green, and 4’,6-diamidino-2-phenylindole (DAPI) nuclear stain is shown in blue. Nascent *Kdm6a* transcripts appear as red fluorescent puncta at the site of transcription (indicated by white arrows). *Xist* RNA remains associated with the inactive X chromosome and is detected only in XX cells. Inset numbers indicate the percentage of nuclei with two sites of nascent *Kdm6a* accumulation in XX cells and one site in XY cells (*n* = 100 cells). Scale bar, 2 μm. (**B**) Representative confocal images of Kdm6a staining (left), Kdm6a with DAPI staining (middle), and Kdm6a with Neuronal nuclei (NeuN) staining (right) in the hippocampal dentate gyrus region of a gonadectomized nontransgenic (NTG) female XX mouse (top row) and a gonadectomized NTG male XY mouse (bottom row). Kdm6a is shown in red, DAPI nuclear stain is shown in blue, and NeuN is shown in green. Scale bar, 50 μm; magnification, ×100. (**C** and **D**) Western blot representative image (C) and subsequent quantification (D) of Kdm6a protein expression in the hippocampus of gonadectomized NTG XX female and XY male mice. Bands represent individual mouse samples. (C) Representative images show samples bound by the GeneTex antibody, and (D) quantification is given for both GeneTex and Abcam rabbit anti-Kdm6a antibodies; Kdm6a was normalized using glyceraldehyde phosphate dehydrogenase (GAPDH) as a loading control. Means are relative to NTG XY male control mice, arbitrarily defined as 1 (age 3.4

to 3.6 months; $n = 3$ mice per group). Gonadectomized NTG XX female mice show higher Kdm6a protein expression. Two-tailed t test, $^{*}P < 0.05$. (**E** and **F**) Hippocampal *Kdm6a* mRNA expression in (E) FCG mice (age 3.5 to 5.5 months; $n = 6$ to 26 mice per group) and (F) XY* mice (age 5.5 to 7.5 months; $n = 4$ to 17 mice per group) with and without hAPP, shown relative to XY male mice without hAPP. Two-way ANOVA: sex chromosome effect, $^{***}P < 0.001$ and X dose effect, $^{***}P < 0.001$. Data are presented as means ± SEM in (D) to (F). $^{*}P < 0.05$; $^{***}P < 0.001$ (Bonferroni-Holm).

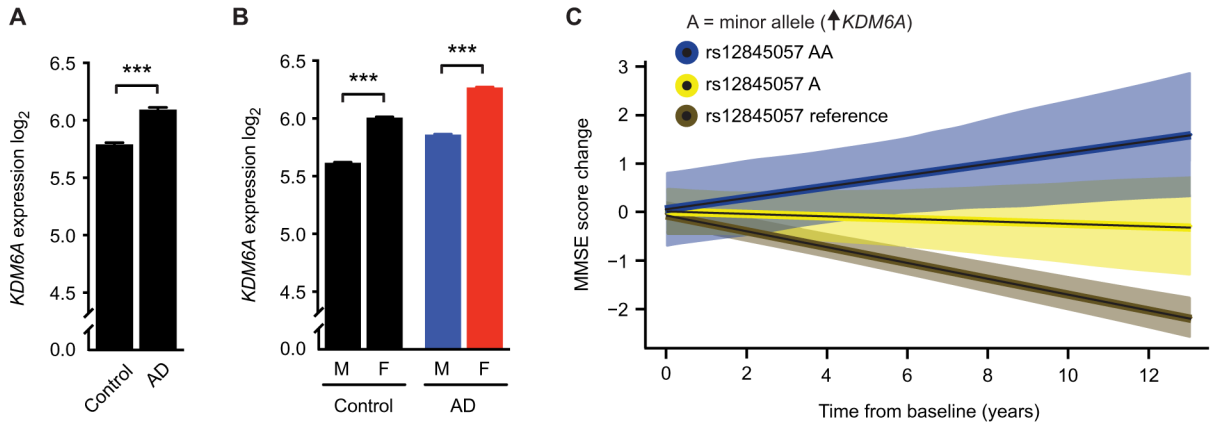

Fig. 6. *KDM6A* genetic variation associates with cognitive resilience in humans.

(**A**) Shown is human *KDM6A* RNA expression via RNA sequencing and microarray in the temporal and parahippocampal cortex of individuals without (control, $n = 135$) and with AD ($n = 86$) (***$P = 3.64 \times 10^{-4}$). (**B**) Shown is human *KDM6A* RNA expression via RNA sequencing and microarray in individuals identified as male (M) or female (F) without (M, $n = 75$; F, $n = 60$; ***$P = 9.79 \times 10^{-4}$) and with AD (M, $n = 37$; F, $n = 49$; ***$P = 4.83 \times 10^{-4}$). Expression data were analyzed by linear models accounting for effects of postmortem interval and age at death. (**C**) Shown is cognitive change with 95% CIs in 778 individuals of the ADNI cohort (cognitively normal, 268; MCI, 465; AD, 45), who carried two alleles (AA, blue, $n = 8$ all female), one allele (A, yellow, $n = 78$), or no allele (noncarriers, reference, brown, $n = 692$) for the rs12845057 variant of the *KDM6A* gene associated with increased *KDM6A* RNA expression in brain (table S4). Cognition was measured by the MMSE score. Increasing dose of the minor allele was associated with slower rates of cognitive decline over time ($\beta = 0.141$, SE 0.035, $P = 0.00005$). Cognitive data were analyzed by linear models accounting for effects of baseline age, sex, education, and *APOE*ε4 dose. Data are presented as means ± SEM in (A) and (B). ***$P < 0.001$ (Bonferroni-Holm).

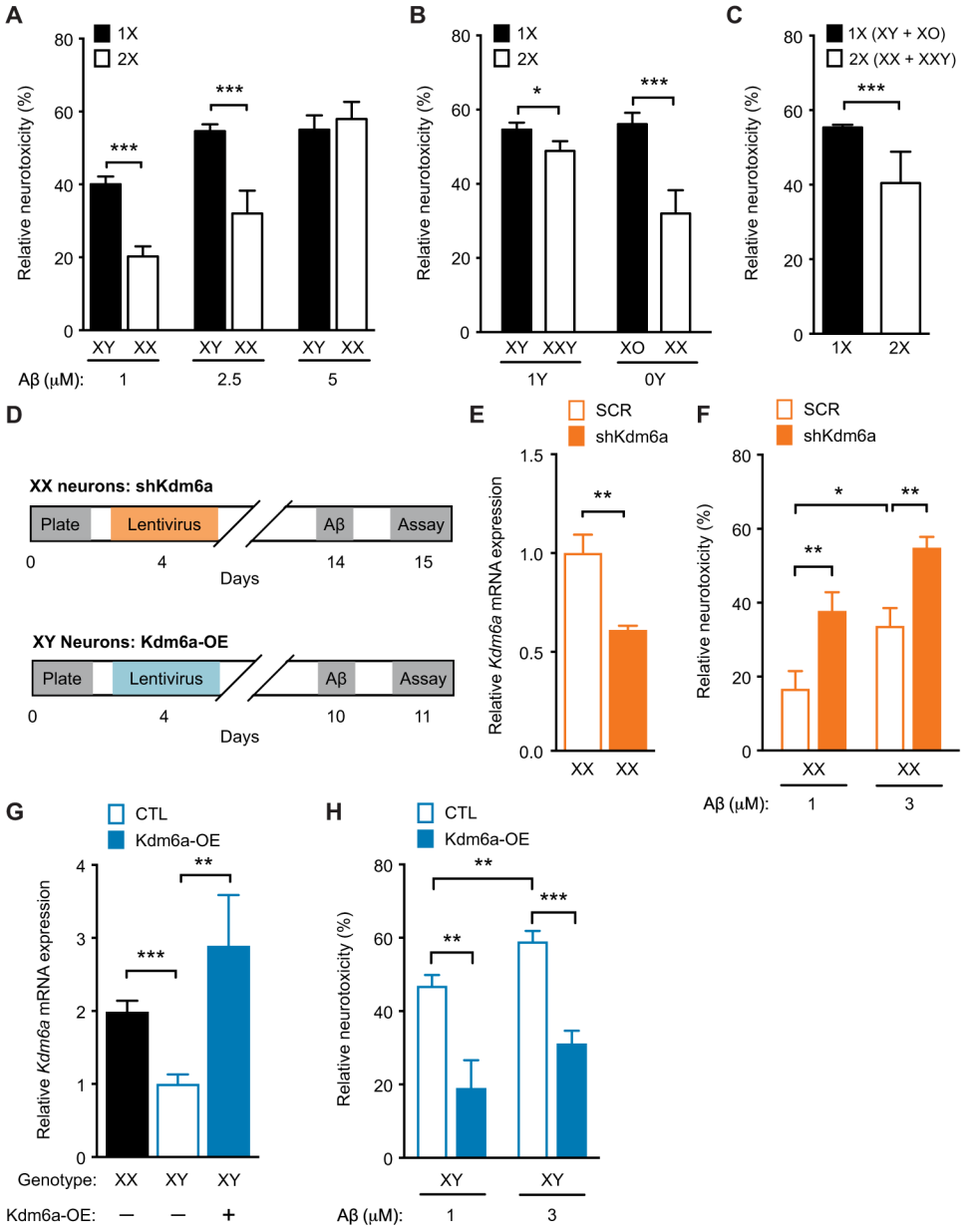

Fig. 7. *Kdm6a* knockdown in XX mouse neurons worsens, whereas *Kdm6a* overexpression in XY neurons attenuates Aβ toxicity in vitro.

(**A** to **C**) Vulnerability of mouse primary neurons was tested by the MTT assay. For each genotype, cell toxicity was calculated as a percentage of the corresponding vehicle-treated group, 24 hours after treatment with increasing doses of Aβ. (A) Mouse primary cortical XY neurons showed greater vulnerability than did XX neurons after exposure to vehicle or increasing doses of Aβ (n = 8 to 40 wells per experimental group from 8 to 10 pups per genotype, from four independent litters). Two-way ANOVA: sex chromosome effect, $P < 0.01$; Aβ dose effect, $P < 0.001$; interaction, $P < 0.05$. (B) Toxicity of Aβ in neurons of varying X and Y chromosome dosage derived from littermate pups of XY* males crossed with nontransgenic (NTG) females, with genotypes roughly equivalent to XO, XX, XY,

and XX, exposed to vehicle or Aβ (2.5 μM) (n = 15 to 45 wells per experimental group from 7 to 10 pups per genotype, from four independent litters). Two-way ANOVA: X effect, $P < 0.0001$; Y effect, not significant; X by Y interaction, $P < 0.05$. (C) Main effect of X chromosome dose shows increased Aβ toxicity in neurons with 1X (XO and XY combined) compared to those with 2X chromosomes (XX and XXY combined). (**D**) Experimental strategy of lentivirus-mediated knockdown of *Kdm6a* in XX mouse primary cortical neurons (top) and *Kdm6a* overexpression in XY mouse primary cortical neurons (bottom). (**E**) Shown is *Kdm6a* mRNA expression in neurons transfected with lentivirus expressing scrambled (SCR) or short hairpin (sh) *Kdm6a* for knockdown expressed relative to XX SCR (n = 5 to 6 wells per experimental group from eight XX pups, from two litters). Two-tailed t test, $^{**}P < 0.01$. (**F**) Shown is Aβ toxicity in XX neurons treated with SCR or sh*Kdm6a* and exposed to vehicle or Aβ (1 and 3 μM); knockdown of *Kdm6a* worsened Aβ toxicity (n = 24 to 25 wells per experimental group from 14 XX pups, from three independent litters). Two-way ANOVA: *Kdm6a* effect, $P < 0.001$; Aβ effect, $P < 0.001$; *Kdm6a* by Aβ interaction, $P = 0.99$. (**G**) *Kdm6a* mRNA expression in neurons transfected with lentivirus expressing control (CTL) or overexpressing *Kdm6a* (*Kdm6a*-OE), shown relative to control XY neurons (n = 3 to 8 wells per experimental group from 12 XY pups, from two independent litters). One-way ANOVA, $P < 0.001$. (**H**) Shown is Aβ toxicity in XY neurons transfected with lentivirus expressing control or overexpressing *Kdm6a* (*Kdm6a* OE) and exposed to vehicle or Aβ (1 and 3 μM); overexpression of *Kdm6a* attenuated Aβ toxicity (n = 12 to 13 wells per experimental group from 26 XY pups, from three independent litters). Two-way ANOVA: *Kdm6a* effect, $P < 0.001$; Aβ effect, $P = 0.01$; *Kdm6a* by Aβ interaction, $P = 0.99$. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (Bonferroni-Holm). Data are presented as means ± SEM.

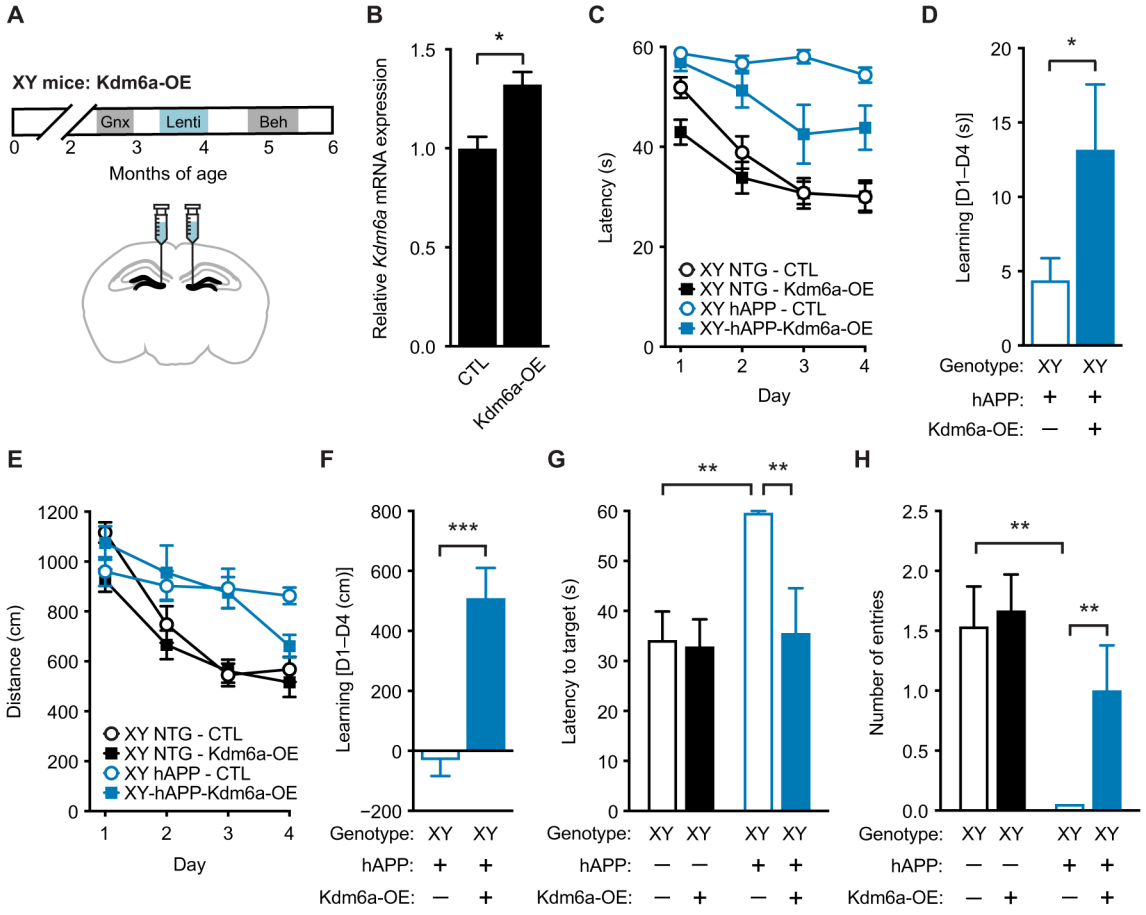

Fig. 8. *Kdm6a* overexpression in hippocampus attenuates male vulnerability to cognitive impairments in XY-hAPP mice.

(**A**) Experimental strategy: XY mice were gonadectomized and injected with lentivirus expressing control or overexpressing *Kdm6a* (*Kdm6a* OE) into the dentate gyrus of the hippocampus; animals were then tested on behavioral tasks. (**B**) Shown is *Kdm6a* mRNA expression measured in dentate gyrus of mice injected with lentivirus expressing control or overexpressing *Kdm6a* (*Kdm6a* OE) ($n = 3$ mice per experimental group), relative to XY control; *t* test, $^*P < 0.05$. (**C** to **F**) Spatial learning task results for the four experimental groups of XY mice tested in the Morris water maze (age 5 to 5.5 months; $n = 7$ to 15 per group). XY-hAPP-*Kdm6a*-OE mice exhibited (C) decreased latency to find the target escape platform (mixed-model ANOVA: XY-hAPP-CTL versus XY-hAPP-*Kdm6a*-OE, $P < 0.001$) and (D) a better learning index of latency during hidden platform training, measured by the difference in performance of each mouse at day 4 from average group performance on day 1 (D1 to D4). (E) XY-hAPP-*Kdm6a*-OE mice did not travel a statistically decreased distance to find the target platform but (F) showed better learning in the distance traveled during hidden platform training. (**G** and **H**) Probe trial results 24 hours after completion of hidden platform learning, indicating spatial memory of the escape platform location, showed that XY-hAPP-*Kdm6a*-OE mice had attenuated spatial deficits including decreased (G) latency to target platform and (H) increased number of entries into the target zone, compared to

XY-hAPP-CTL mice. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ [Bonferroni-Holm for (G) and (H)]. Data are presented as means ± SEM.