

1 **Early memory deficits and extensive brain network disorganization in the *App<sup>NL-F</sup>/MAPT* double**  
2 **knock-in mouse model of familial Alzheimer's disease**

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33 **Abstract:**

34 A critical challenge in current research on AD is to clarify the relationship between early neuropathology  
35 and network dysfunction associated to the emergence of subtle memory alterations which announce  
36 disease onset. In the present work, the new generation *App<sup>NL-F</sup>/MAPT* double knock in (dKI) model was  
37 used to evaluate early stages of AD. The initial step of tau pathology was restricted to the perirhinal-  
38 entorhinal region, sparing the hippocampus. This discrete neuropathological sign was associated with  
39 deficits in the object-place associative memory, one of the earliest recognition memories affected in  
40 individuals at risk for developing AD. Analyses of task-dependent c-Fos activation was carried out in 22  
41 brain regions across the medial prefrontal cortex, claustrum, retrosplenial cortex, and medial temporal  
42 lobe. Initial hyperactivity was detected in the entorhinal cortex and the claustrum of dKI mice. The  
43 retention phase was associated to reduced network efficiency especially across cingulate cortical regions,  
44 which may be caused by a disruption of information flow through the retrosplenial cortex. Moreover, the  
45 relationship between network global efficiency and memory performance in the WT could predict  
46 memory loss in the dKI, further linking reduced network efficiency to memory dysfunction. Our results  
47 suggest that early perirhinal-entorhinal pathology is associated with local hyperactivity which spreads  
48 towards connected regions such as the claustrum, the medial prefrontal cortex and ultimately the key  
49 retrosplenial hub which is needed to relay information flow from frontal to temporal lobes. The similarity  
50 between our findings and those reported in the earliest stages of AD suggests that the *App<sup>NL-F</sup>/MAPT* dKI  
51 model has a high potential for generating key information on the initial stage of the disease.

52 **Abbreviations**

AD – Alzheimer’s disease

CLA – claustrum

DH – dorsal hippocampus

dKI - *App<sup>NL-F</sup>/MAPT* double knock-in mouse

FC – functional connectivity

mPFC – medial prefrontal cortex

MTC – medial temporal cortex

MTL – medial temporal lobe

OP – object-place association

PS – Pattern Separation

RSC – retrosplenial cortex

## 53 **Introduction**

54 Current diagnosis and treatment for Alzheimer's disease (AD) occurs too late, when physiological  
55 symptoms such as overt neurodegeneration have already reached an irreversible state (Selkoe, 2012).  
56 Hallmark pathologies of AD include insoluble amyloid plaques, initially formed in the medial prefrontal  
57 and posterior medial cortices indicative of the default mode network (DMN), and neurofibrillary tangles,  
58 initially formed in the medial temporal lobe (MTL). Combining functional neuroimaging with amyloid or  
59 tau PET reveals that spatial patterns of deposition induce functional network perturbations (Myers et al.,  
60 2014; Jones et al., 2017), and accompany cognitive decline (Sepulcre et al., 2017; Pereira et al., 2019).  
61 However, soluble amyloid peptide (A $\beta$ ) and hyperphosphorylated tau precursors are generally more toxic  
62 and accumulate in the brain decades prior to the onset of typical symptoms (Chen et al., 2017; Hill et al.,  
63 2020). This highlights the importance of focusing on the earliest stages to understand the origin of this  
64 devastating disease and to develop more proactive detection methods and therapies.

65 Amnesic mild cognitive impairment (aMCI) indicates a higher risk of progressing to AD and has been  
66 extensively studied over the last decade as a potential prodromal stage of AD (Petersen et al., 2009).  
67 Resting state functional connectivity (FC) has been evaluated in aMCI patients through the temporal  
68 correlation of signal fluctuations in fMRI, EEG, or MEG. In order to achieve a more rigorous  
69 understanding of FC disruption, graph theory has been used to evaluate the topology of functional  
70 networks. Healthy cognition likely requires efficient small world topology, combining local segregation  
71 between anatomically and functionally similar regions, and good integration between distant regions with  
72 the help of strongly connected hubs (Filippi et al., 2013, Bassett and Bullmore., 2006). In regards to  
73 aMCI, many studies have reported functional network perturbations within and between the DMN and the  
74 MTL, with reduced network integration along with reduced strength of cortical hubs (Drzezga et al.,  
75 2011; Wang et al., 2013a; Bai et al., 2013, Lin et al., 2019). However, these results are not entirely  
76 conclusive, as others have reported opposing or null results (Gardini et al., 2015; Grajski and Bressler,  
77 2019; Liang et al., 2020, Liu et al., 2012). This inconsistency may be indicative of the heterogeneity of  
78 aMCI staging, where the sense of perturbation (hyperconnectivity vs hypoconnectivity) is shown to  
79 change depending on the severity of the pathological state (Pusil et al., 2019; Jones et al., 2016). This  
80 highlights the importance of identifying earlier biomarkers to more precisely detect preclinical stages of  
81 AD, even preceding aMCI. Recent progress has led to the development of sensitive recognition memory  
82 paradigms, such as pattern separation and associative memory tasks, which can detect slight cognitive  
83 defects in subjective cognitive impairment and cognitively healthy elderly (Naveh-Benjamin, 2000, Sinha  
84 et al., 2018; Reagh et al., 2018). These tasks depend on specific interactions across DMN and MTL  
85 structures sensitive to early AD (Reagh and Yassa., 2014; Miller et al., 2014; Hales and Brewer, 2011;

86 Caviezel et al., 2020; Ritchey et al., 2020), and have been implemented to detect the earliest  
87 “asymptomatic” stages of preclinical AD (Rentz et al., 2011; Maass et al., 2019). By evaluating functional  
88 networks directly responsible for the earliest subtle memory deficits, we may achieve a clearer  
89 understanding of the neural networks first affected in the initial stages of AD.

90 Initial stages of AD are inevitably linked to discrete physiological and neuroanatomical perturbations,  
91 which should be more easily investigated in mouse models of AD (Searce-Levie et al, 2020). Using  
92 fMRI on young AD transgenic mice, increased soluble A $\beta$  (Shah et al, 2016; Shah et al., 2018), and  
93 regionally specific increases in neuroinflammation and phospho-tau (Degiorgis et al., 2020) have been  
94 linked to disruptions in resting state functional networks. As fMRI/EEG/MEG methods are currently  
95 difficult to implement in freely moving mice, ex-vivo imaging based on quantification of activity  
96 regulated expression of immediate early genes (IEG; c-fos, Egr1 and arc) may be used to evaluate  
97 memory related neural activation (Kinnavane et al, 2015). Using this technique, early-stage  
98 pathophysiology has been linked to individual changes in memory driven regional activity (Hamm et al.,  
99 2017), but not to functional networks. However, outside of AD, across-subjects correlations of activity  
100 regulated IEG levels have been used to assess functional networks directly related to memory (Tanimizu  
101 et al., 2017; Wheeler et al., 2013; Vetere et al., 2017). This approach can prove useful for evaluating  
102 memory driven FC in mouse models of AD.

103 Recently, knock-in mouse models have been created to express AD-related genes under endogenous  
104 mouse promoters (Saito et al., 2014). This removes gene overexpression and resulting potential artificial  
105 phenotypes, allowing for a more careful assessment of the initial stages of AD (Sasaguri et al., 2017). In  
106 the current study, we detected object-place (OP) associative memory deficits as the earliest sign of  
107 cognitive decline in the humanized *App*<sup>NL-F</sup>/*MAPT* double knock-in (dKI) mouse model. This model  
108 specifically expresses all six isoforms of tau and overexpresses A $\beta$ <sub>42</sub> which leads to marked amyloid  
109 deposition and typical pathological hyperphosphorylation of tau at an advanced age of 24 months (Saito  
110 et al., 2019). Given our specific interest in the early stages of AD, this model was chosen rather than the  
111 *App*<sup>NL-G-F</sup>/*xMAPT* dKIs because of the slower development of its neuropathology. This option seemed  
112 fortunate as the first memory deficits detected in the OP task coincided with the emergence of the earliest  
113 stages of abnormal tau phosphorylation within the entorhinal-perirhinal region and not in the  
114 hippocampus, indicative of an early pathological state. Task-dependent activity was then measured in  
115 regions implicated with this form of associative memory, including primarily the DMN/MTL, and FC  
116 was assessed using graph theory techniques. Our results show that initial memory decline in AD could be  
117 linked to specific topological changes in memory dependent functional networks.

## 118 **Results**

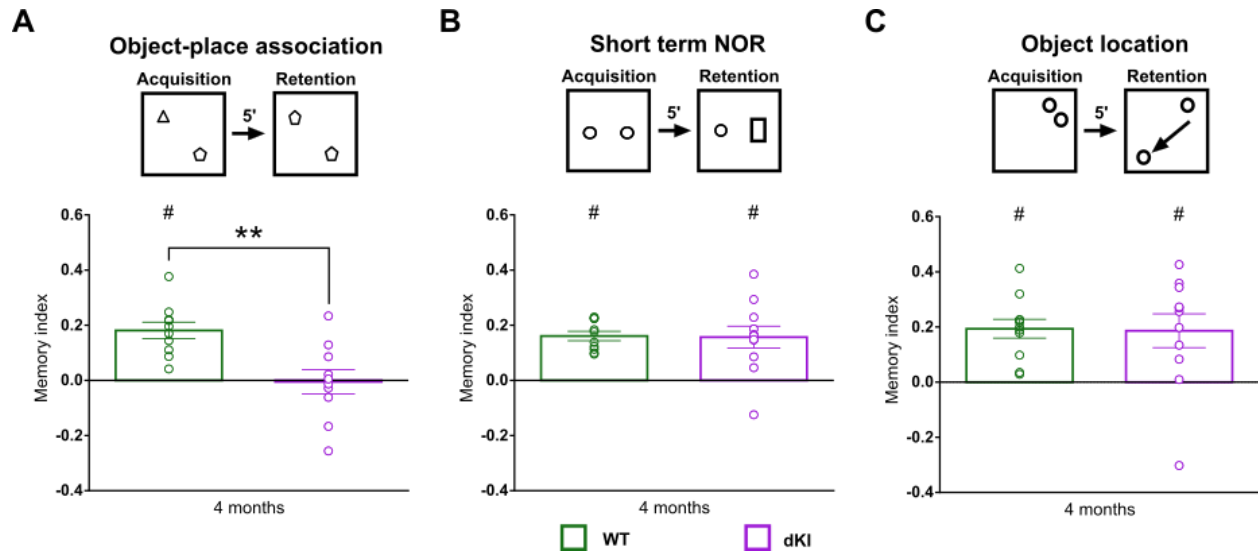
### 119 **Behavioural phenotyping reveals early alterations in object-place associative memory**

120 Pattern separation (PS) and OP associative memory performances are commonly affected in prodromal  
121 stages of AD and cognitively normal elderly (Yeung et al., 2018, Hampstead et al., 2018, Reagh et al.,  
122 2018, Reagh and Yassa, 2014), and in mouse models at pre-plaque deposition stages (Zhu et al., 2017;  
123 Hamm et al., 2017). In order to detect earliest sensitive recognition memory deficits, a preliminary study  
124 was made to evaluate OP and PS performance, as well as long term object recognition performance at 2,  
125 4, and 6 months of age (Figure 1—figure supplement 1). For OP and PS tasks, a short inter-trial-interval  
126 (ITI) of 5 minutes was chosen to ensure that deficits did not arise from a confounding perturbation of  
127 long-term memory consolidation. Given the high number of ages being tested and the difficult nature of  
128 the tasks, no results were significant in this preliminary experiment with multiple comparisons  
129 corrections. All groups succeeded in the long-term object recognition task and the PS task remained  
130 inconclusive because dKI's performances were highly variable (Figure 1—figure supplement 1A and 1B,  
131 respectively). On the other hand, a robust potential deficit could be seen at 4 months in the OP task, and  
132 persisted at 6 months of age (Figure 1—figure supplement 1C).

133 With a second independent cohort of mice, we confirmed that dKI mice were unable to detect a new OP  
134 association at 4 months of age (Figure 1A) (two sample t-test, WT vs. dKI:  $t(20) = 2.85$ ,  $P = 0.0098$ ; one  
135 sample t-test, against chance: dKI  $t(10)=0.3095$ ,  $p = 0.763$ ). On the other hand, 4-month-old mice did not  
136 show any deficits in short term novel object recognition (Figure 1B) (two sample t-test, WT vs. dKI:  $t(19)$   
137  $= .0934$ ,  $P = 0.9265$ ; one sample t-test, against chance: all  $ts>3.94$ ,  $ps<0.003$ ) and object location tasks  
138 (Figure 1C) (two sample t-test, WT vs. dKI:  $t(20) = 1.052$ ,  $P = 0.9173$ ; one sample t-test, against chance:  
139 all  $ts>3.03$ ,  $ps<0.013$ ). These results confirmed that the OP deficit was due to specific perturbations in  
140 associative memory rather than any separate impairments in the object recognition or spatial recognition.

141 We then evaluated early AD pathophysiological changes in 4-month-old mice using western blotting  
142 (Figure 1—figure supplement 2A). Antibodies targeting the amyloid precursor protein, APP-cleaved  
143 carboxy terminal fragments, phosphorylated tau (Thr181) and total tau proteins were used. Wild-type  
144 mice expressed mainly APP  $\alpha$ -CTF fragments whereas dKI expressed mainly APP  $\beta$ -CTF fragments.  
145 Moreover, an increase in  $\beta$ -CTF/ $\alpha$ -CTF ratio was seen in both the hippocampus (Figure 1—figure  
146 supplement 2B) and the medial temporal cortex (MTC; including entorhinal, perirhinal, postrhinal  
147 cortices) (Figure 1—figure supplement 2C), indicating upregulated amyloidogenic APP processing. In  
148 addition, an increase of the tau phosphorylation degree on Thr181 was only seen in the MTC of dKI mice  
149 compared to wild-type mice while both models expressed similar levels of total tau proteins. This is

150 consistent with general consensus of the MTC as the initial area of tau staging (Braak and Braak, 1995).  
151 These results highlight that OP deficits occur in conjunction with considerably light pathophysiological  
152 changes, akin to an early preclinical AD stage.



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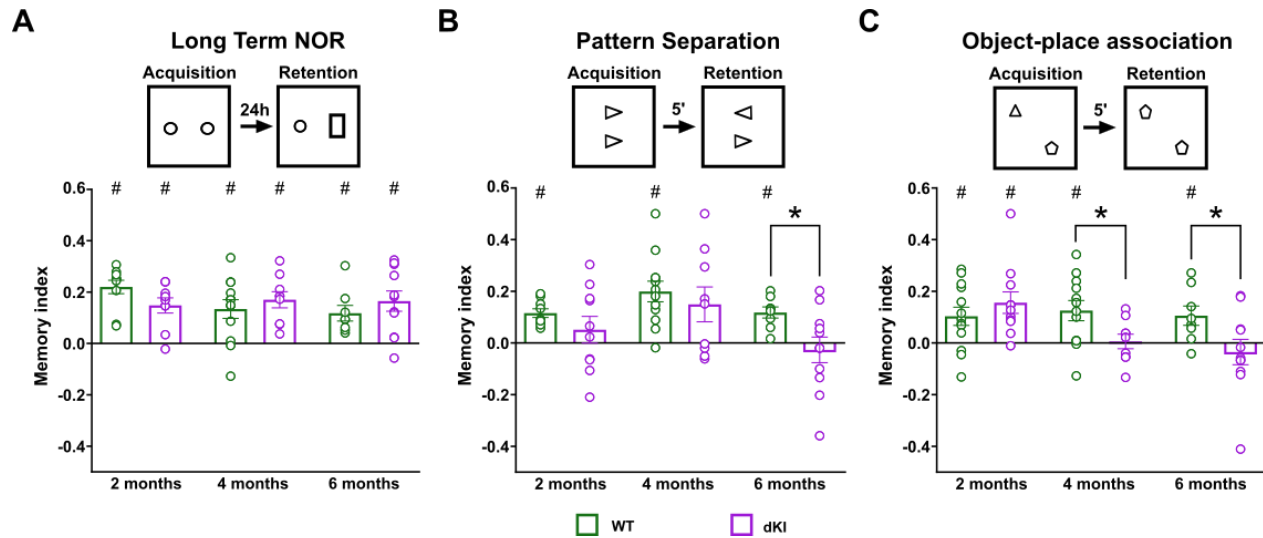
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**Figure 1.** Behavioral characterization of young dKI mice (WT n = 11, dKI n = 11). (A) At 4 months of age, dKI mice reproduced the potential deficit seen in the object-place association task during preliminary phenotyping (two sample t-test, WT vs. dKI:  $t(20) = 2.85$ ,  $P = 0.0098$ ; one sample t-test, against chance: dKI  $t(10) = 0.3095$ ,  $p = 0.763$ ). At the same age, dKI mice were normal in (B) object recognition (two sample t-test, WT vs. dKI:  $t(19) = .0934$ ,  $P = 0.9265$ ; one sample t-test, against chance: all  $t_s > 3.94$ ,  $p_s < 0.003$ ) (one WT mouse was removed due to being dropped before testing) and (C) spatial recognition (two sample t-test, WT vs. dKI:  $t(20) = 1.052$ ,  $P = 0.9173$ ; one sample t-test, against chance: all  $t_s > 3.03$ ,  $p_s < 0.013$ ), indicating that the loss in associative memory was not due to any singular loss in the short term object recognition or object location memory domains. Bar graphs represent the mean density ( $\pm$  SEM) \* difference between genotypes \*\* $p < .01$  (two sample t-test); #, difference from chance, # $p < .05$  (one sample t-test).



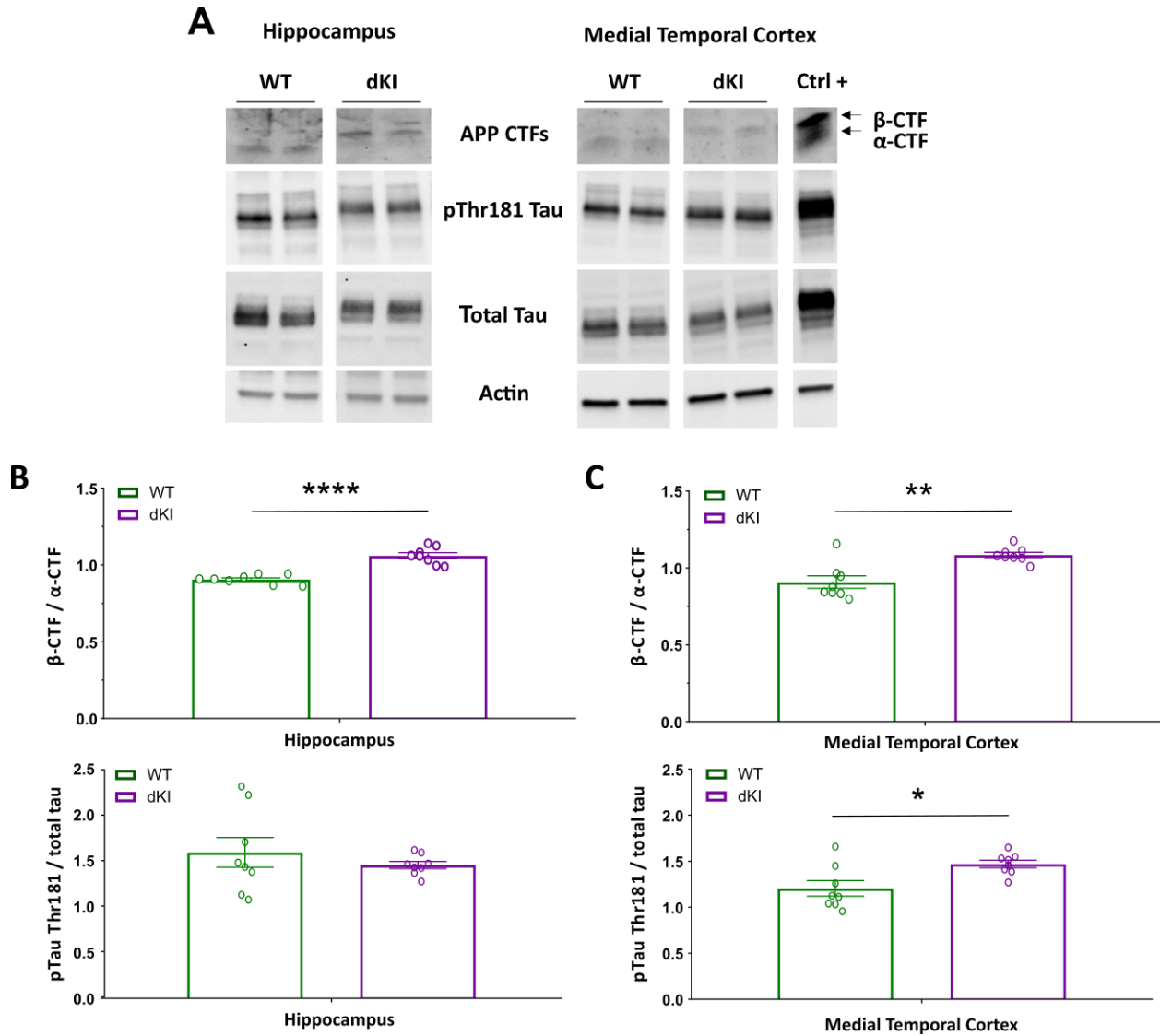
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168 **Figure 1 - figure supplement 1.** Preliminary phenotyping of young dKI mice with sensitive recognition memory paradigms.  
 169 Mice were tested at 2 months (WT n = 10, dKI n = 10), 4 months (WT n = 12, dKI n = 9) and at 6 months (WT n = 8, dKI n =  
 170 11). An additional cohort of (WT n = 3, dKI n = 1) was tested at 2 months in object-place association as the initial group did not  
 171 reach significance above 0. (A) In the long term object recognition task, dKI mice were completely unaffected. (B) In the pattern  
 172 separation task the dKI mice were highly variable making it so that they were not significant against chance at any age (one  
 173 sample t-test, against chance: all dKI  $t_s < .996$ ,  $p_s > 0.055$ ). However, between group significance was only obtained at 6 months of  
 174 age (WT vs. dKI:  $t(17) = 2.364$ ,  $P = 0.030$ ). This variability may represent a variety of factors including perhaps affected DG  
 175 neurogenesis (Scopa et al., 2020). (C) The earliest significant deficit in dKI mice appeared at 4 months in the object-place  
 176 association task, a deficit which was conserved at 6 months (two sample t-test, WT\_4mo vs. dKI\_4mo:  $t(20) = 2.325$ ,  $P = 0.0313$ ;  
 177 one sample t-test, against chance: dKI  $t(8) = 0.2086$ ,  $P = 0.8399$ ; two sample t-test, WT\_6mo vs. dKI\_6mo:  $t(17) = 2.132$ ,  $P =$   
 178  $0.0479$ ; one sample t-test, against chance: dKI  $t(10) = 0.7196$ ,  $P = 0.4883$ ). Although the variability seen during pattern  
 179 separation may be interesting, we chose to continue our study with the more robust object-place deficit. Bar graphs represent the  
 180 mean density ( $\pm$  SEM). difference between genotypes  $*p < .05$  (two sample t-test); difference from chance,  $\#p < .05$  (one sample t-  
 181 test). No multiple comparisons corrections were performed for these preliminary comparisons.

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186 **Figure 1 - figure supplement 2.** Early stage of Alzheimer-like disease in 4 month old male dKI mice. (A) Representative blots  
187 of APP-cleaved fragments, phosphorylated tau proteins on the threonine 181 site and of total tau proteins in the hippocampus and  
188 medial temporal cortex from WT and dKI mice. Analysis of the ratio of the  $\beta$ -CTF on  $\alpha$ -CTF fragments and the degree of tau  
189 phosphorylation on Thr181 in the (B) hippocampus and in the (C) medial temporal cortex. Results showed that dKI mice  
190 expressed mainly the  $\beta$ -CTF whereas the WT mice expressed mainly the  $\alpha$ -CTF and the  $\beta$ -CTF/ $\alpha$ -CTF ratio increased in both  
191 brain regions. Only the MTC displayed an increase of phosphorylated tau proteins. Bar graphs represent the mean density ( $\pm$   
192 SEM). \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  and \*\*\*\*  $p < 0.0001$  (two sample t-test).

193



## 194 **Assessing OP dependent changes in brain activity**

195 While encoding processes are known to be perturbed in AD patients (Granholm and Butters, 1988), recent  
196 studies in animal models also find dysfunctional retrieval of engrams as a mechanism for memory loss in  
197 early stages of AD-like pathology (Roy et al., 2016; Etter et al., 2019). We thus aimed to evaluate  
198 encoding versus recall dependent changes in brain activity in relation to the OP deficit, through  
199 independent evaluation of the two test-phases. Thus c-Fos activation was quantified in brains of mice that  
200 had underwent either the acquisition phase (*encoding*) (Figure 2A) or the retention phase (*recall*) (Figure  
201 2B) of the OP task. In order to account for the c-Fos protein expression curve, the ITI was extended to 3  
202 hours, the shortest delay that could permit definite isolation between test-phases. As with a 5-min ITI,  
203 dKI mice that underwent the retention phase after a 3-h ITI were likewise unable to detect the novel OP  
204 association (two sample t-test, WT vs. dKI:  $t(26) = 3.14$ ,  $P = 0.004$ ; one sample t-test, against chance: WT  
205  $t(13) = 3.93$ ,  $P = 0.002$ ; dKI  $t(13) = 0.139$ ,  $P = 0.891$ ). (Figure 2C). We then chose to evaluate 22 regions  
206 of interest (ROIs) encompassing subregions of the medial prefrontal cortex (mPFC), claustrum (CLA),  
207 retrosplenial cortex (RSC), dorsal hippocampus (DH) and medial temporal cortex (MTC) (Figure 2D-J).  
208 Neuronal hyperactivity is common feature in early AD and in young pre-plaque mice (Zott et al., 2019).  
209 We therefore first assessed test-phase and genotype dependent changes in regional activity.

## 210 **Acquisition induces higher regional activity than retention**

211 All regions except the DH (phase effect: DH  $F(1, 192) = 1.85$ ,  $P = 0.175$ ; subregion x phase effect:  $F(3,$   
212  $192) = 5.32$ ,  $p = 0.002$ ; post-hoc Tukey: Acq vs Ret: CA1  $P = .052$ , CA3  $P = .383$ , DG\_ip  $P = .597$ ,  
213 DG\_sp  $P = .513$ ) showed higher levels of activity in the acquisition phase, although in the LEC this  
214 increase in activity was noticeably less drastic (phase effect: LEC  $F(1, 96) = 5.22$ ,  $P = 0.025$ ; all other Fs  
215  $> 25.6$ ,  $P_s < 0.001$ ). This can be expected in the initial phase as the mice are subjected to stronger changes  
216 with two new objects in previously unoccupied locations in respect to the retention phase, where the only  
217 change is a new OP association. During acquisition, the mice are actively encoding several new features,  
218 new objects and their unique location in the open-field. Moreover, this helps confirm the absence of  
219 residual c-Fos in the retention groups. If residual c-Fos lingered from encoding until perfusion after the  
220 retention phase, one would expect similar or increased c-Fos density during the retention phase, not a  
221 decrease.

## 222 **Hyperactivity in the claustrum and entorhinal cortex of dKI mice**

223 The CLA and LEC showed increased activation in dKI mice regardless of the test phase (genotype effect:  
224 CLA  $F(1, 96) = 8.29$ ,  $P = 0.005$ ; LEC  $F(1, 96) = 10.36$ ,  $P = 0.001$ ). The MEC revealed increased  
225 activation in dKI mice specifically during the acquisition phase (MEC: genotype effect:  $F(1, 96) = 25.64$ ,

226  $P < 0.001$ ; genotype x phase effect:  $F(1, 96) = 7.46$ ,  $P = 0.007$ ; post-hoc Tukey: WT vs dKI during  
 227 acquisition  $P < .001$ , WT vs dKI during retention  $P = 0.252$ ).

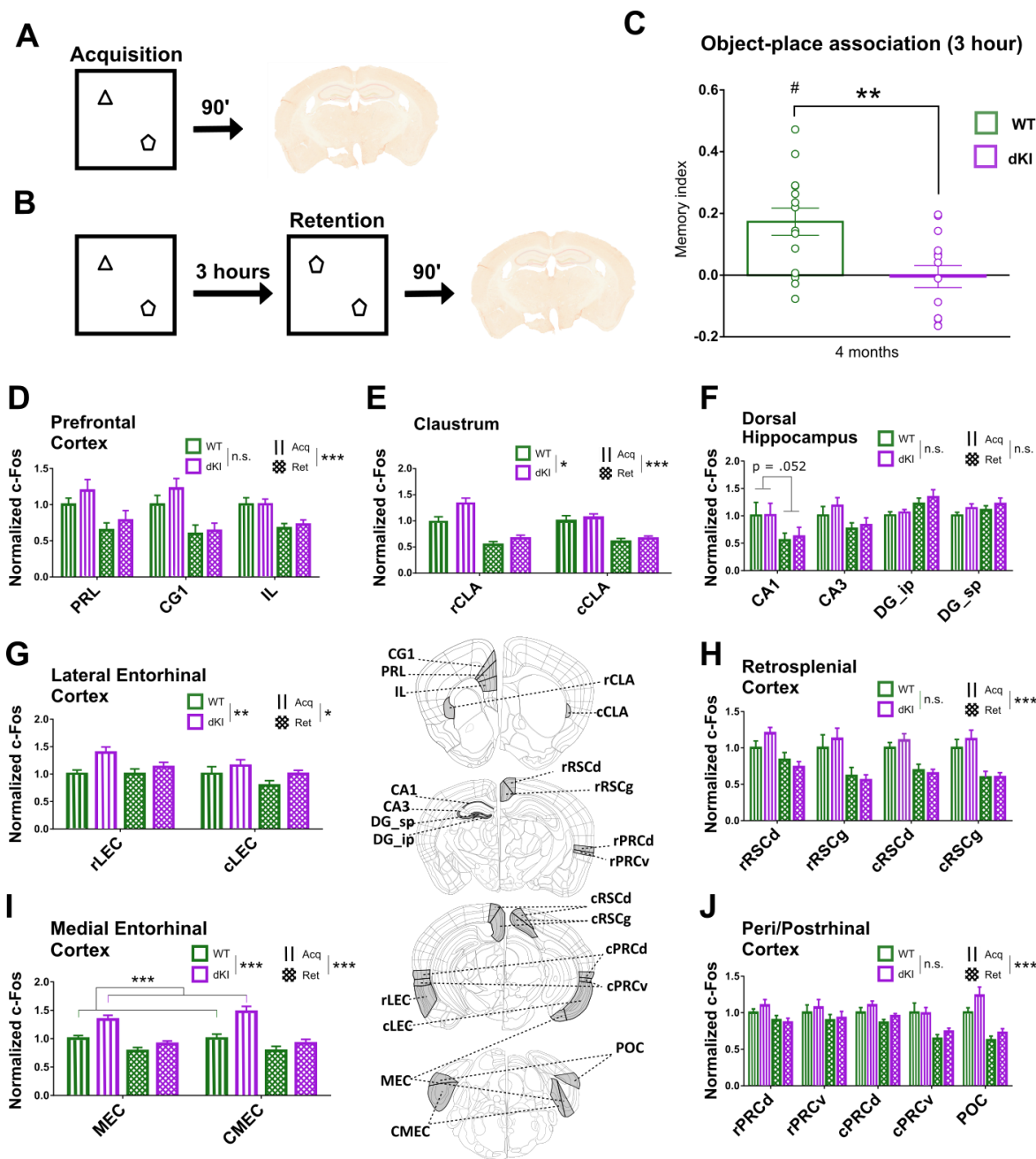
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Region	Acronym
Prelimbic cortex	PRL
Cingulate cortex	CG1
Infralimbic cortex	IL
Rostral claustrum	rCLA
Caudal claustrum	cCLA
Corpus ammoniss 1	CA1
Corpus ammoniss 3	CA3
Dentate gyrus suprapyramidal blade	DG_sp
Dentate gyrus infrapyramidal blade	DG_ip
Rostral retrosplenial cortex dysgranular (RSD)	rRSCd
Rostral retrosplenial cortex granular (RSG)	rRSCg
Caudal retrosplenial cortex dysgranular (RSD)	cRSCd
Caudal retrosplenial cortex granular (RSG)	cRSCg
Rostral perirhinal cortex dorsal (Ect)	rPRCd
Rostral perirhinal cortex ventral (PRh)	rPRCv
Caudal perirhinal cortex dorsal (Ect)	cPRCd
Caudal perirhinal cortex ventral (PRh)	cPRCv
Rostral lateral entorhinal cortex (DLEnt,DIEnt,VIent)	rLEC
Caudal lateral entorhinal cortex (DLEnt,DIEnt,VIent)	cLEC
Medial entorhinal cortex (MEnt)	MEC
Caudal medial entorhinal cortex (CEnt)	CMEC
Postrhinal cortex (Ect,PRh)	POC

229

230 **Table 1. List of evaluated regions.** Regions were chosen a priori based on their relevance to early AD pathology and to  
 231 associative memory processing. In parentheses are region identifications according to the third edition Franklin and Paxinos  
 232 Mouse Brain Atlas.

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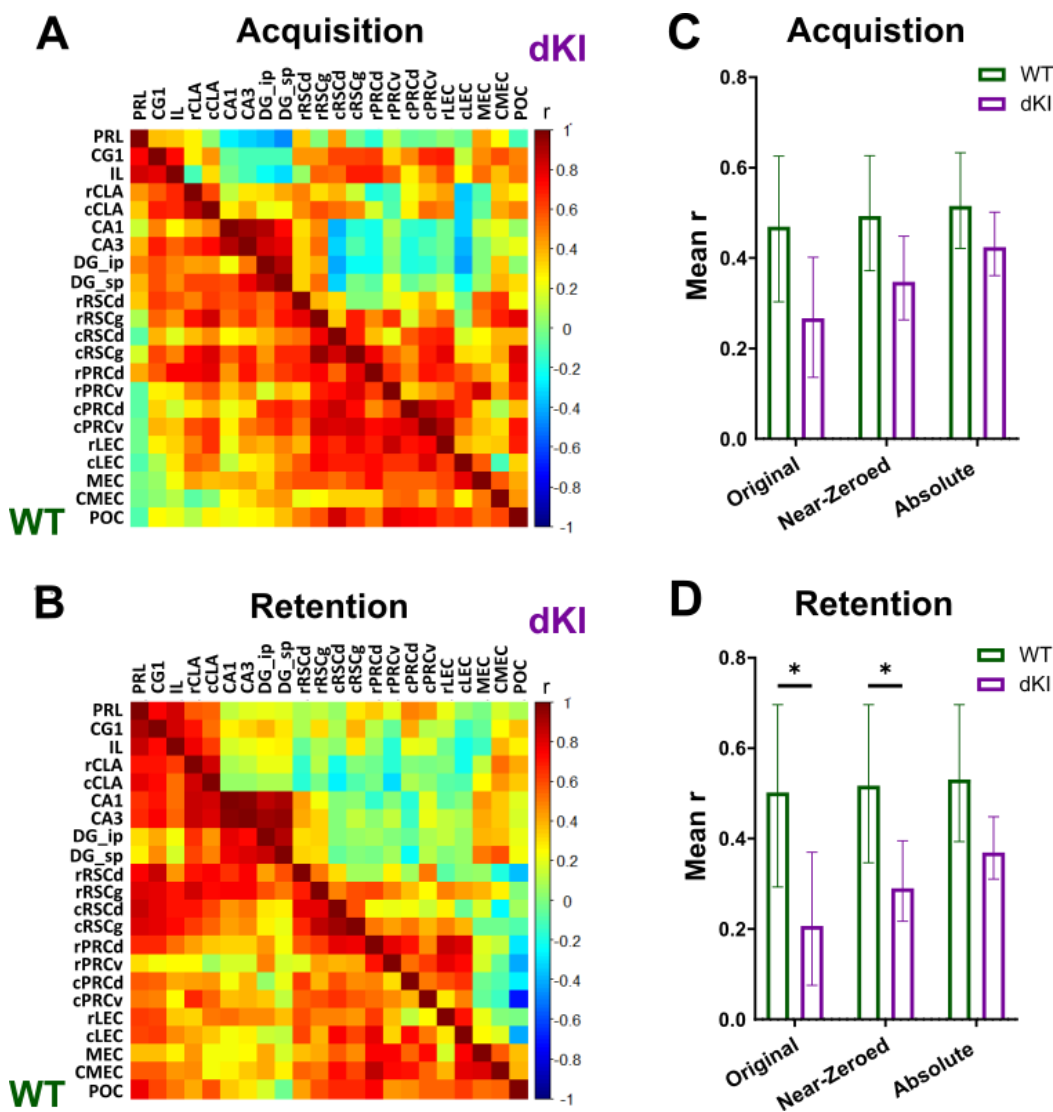
235 **Figure 2.** Regional activity during both phases of OP. Separate cohorts of WT and dKI mice were tested in either the (A)  
 236 acquisition phase only or (B) the entire OP3h task, i.e., acquisition and retention phases. Ninety minutes following one test  
 237 phase, brains were perfused and expression of the activity-regulated-gene, c-Fos, was evaluated immunohistochemically. (C) The  
 238 dKI mice that underwent the whole OP3h task reproduced a deficit in object- place associative memory. difference between  
 239 genotypes, \*\* $p < .01$  (two sample t-test); difference from chance, # $p < .05$  (one sample t-test). (D-J) Graphs that illustrate c-Fos  
 240 counts normalized to the WT-Acquisition group. For each region group a 3-factor ANOVA was performed for test-phase  
 241 (Acquisition-Lines, Retention-Grid), genotype (WT-green, dKI-purple) and subregion effects. Significance for test-phase  
 242 and genotype effects are depicted above each region graph; \* $p < .05$ , \*\* $p < .01$ , \*\*\* $p < .001$ . (D-E,G-J) There was an increase in activity  
 243 during the acquisition phase across all regions, except the (F) dorsal hippocampus. The (E) claustrum and (G) lateral entorhinal  
 244 cortex presented increases in activity in dKI mice regardless of the test-phase. The (I) medial entorhinal cortex however, was  
 245 hyperactive specifically during the acquisition phase \*\*\* $p < .001$  (Tukey post hoc). Bar graphs represent the mean density ( $\pm$   
 246 SEM). Subregion prefixes – r, rostral; c, caudal; suffixes – RSCd, dysgranular; RSCg, granular; PRCd, dorsal; PRCv, ventral; ip,  
 247 infrapyramidal; sp, suprapyramidal. Error bars indicate the SEM. Schematics indicating the general locations of brain regions  
 248 were adapted from Allen Mouse Brain Atlas derived vector images (Lein et al., 2007).

## 249 **Computing functional couplings**

250 Brain regions with correlated activity can be said to exhibit functional connectivity (FC), as co-  
251 modulations of activity can be a marker of inter-regional information sharing. In humans or head-  
252 restrained/anesthetized rodents recorded with EEG, MEG (for electrical signals) or fMRI (for metabolic  
253 rate), FC is computed from the covariance across time. In methods using regional expression of c-Fos, FC  
254 can be modeled by the covariance of regional activity across subjects to study memory driven networks in  
255 mice (Wheeler et al., 2013; Tanimizu et al., 2017). The validity of this approach has been confirmed using  
256 chemo-genetic techniques (Vetere et al., 2017).

257 From the c-Fos signal, we assessed FC by computing the inter-regional Spearman correlation coefficients  
258 ( $r$ ) for each Genotype-Phase group. Correlation matrices were used to visualize all possible correlations  
259 within each group (Figure 3A-B). We first assessed global FC strength by taking the mean  $r$  value of each  
260 matrix. Most FC couplings were positive, however we found a few weakly anti-correlated pairs of  
261 regions. In evaluating FC, there were 3 ways to consider these negative correlations; as disruptive, as  
262 very-weak or as contributing equally to positive correlations. All three cases were evaluated by taking the  
263 mean  $r$  with retained, near-zeroed, and absolute valued negative correlations respectively. In the near-  
264 zeroed case, negative correlations were reduced to a value of .006, the smallest positive correlation  
265 observed.

266 During acquisition, we found no significant change in global FC strength between WT and dKI groups,  
267 for any of the three ways to treat negative correlations in FC (Figure 3C). However, during retention,  
268 there was a decrease in global FC strength in dKI mice with respect to WT. This decrease could be proved  
269 significant when considering negative correlations as disruptive or as very weak, but not when their  
270 absolute value was taken (Figure 3D). This indicates that two phenomena coexist: first, a reduction of  
271 positive inter-regional correlations, corresponding to decreased “cooperation” between some regions;  
272 second, an increase in absolute strength of inter-regional negative correlations, corresponding to increased  
273 “conflict” between some regions.



274  
 275 **Figure 3.** Functional connectivity is depicted with matrices showing across subjects inter-regional Spearman correlations for c-  
 276 Fos expression during (A) retention and (B) acquisition. Colors reflect correlation strength (scale, right). We assessed global FC  
 277 strength by taking the mean r value of each matrix with retained, near-zeroed, and absolute valued negative correlations. In the  
 278 near-zeroed case, negative correlations were reduced to a value of .006, the smallest positive correlation observed. (C) We found  
 279 no change in global FC strength for any of the three cases during acquisition. (D) During retention however, there was a  
 280 significant decrease in global FC strength with the original and near-zeroed correlations, but not when they are absolute valued.  
 281 Bar graphs represent the mean bootstrap value, and the error bars represent the bootstrapped 95% confidence interval. \* -the 95%  
 282 CI for the difference  $\geq 0$

283

## 284 **Generating functional networks**

285 Each functional connectivity matrix can be considered as the adjacency matrix of a weighted undirected  
286 network (Rubinov and Sporns, 2010), and, as such, its organization can be assessed through the analysis  
287 of functional networks, using techniques arising from graph theory. From each matrix a functional  
288 network was generated as a fully connected weighted graph. In these graphs, the regions are represented  
289 as nodes and the functional connections between regions are represented by edges. The strength of a  
290 connection between two regions, as represented by the edge weight, is determined by their inter-regional  
291 correlation strength. As negative correlations are weak and pose difficulties of interpretation when dealing  
292 with many graph theory techniques, we decided for all following analyses to replace them with near-  
293 zeroed values when constructing our functional networks, in line with other studies of c-Fos derived  
294 functional networks in-vivo (Vetere et al., 2017). It has been shown that community organization of  
295 structural networks can change between healthy individuals and different stages of MCI (Pereira et al.,  
296 2016). We decided to evaluate community organization of each functional network to see if any  
297 consistent or phase specific changes could be detected between genotypes. This could also aid us in  
298 obtaining a qualitative understanding of organization of information flow in each network. Network  
299 communities were detected through a modularity maximization procedure (see Materials and Methods).  
300 This unsupervised algorithm separates the nodes into distinct groups (the communities), such that nodes  
301 within a community are more strongly interacting between them than with nodes in other communities.  
302 The separation in communities will partly stochastically vary for different runs of the algorithm and  
303 different bootstrap instances of the FC matrix. To extract a robust consensus set of communities we then  
304 computed allegiance matrices (Bassett et al., 2015), which depict the percentage of times that any given  
305 pair of regions is attributed to the same community among bootstraps (Figure 4).

## 306 **WT networks differentially engage the DH and mPFC**

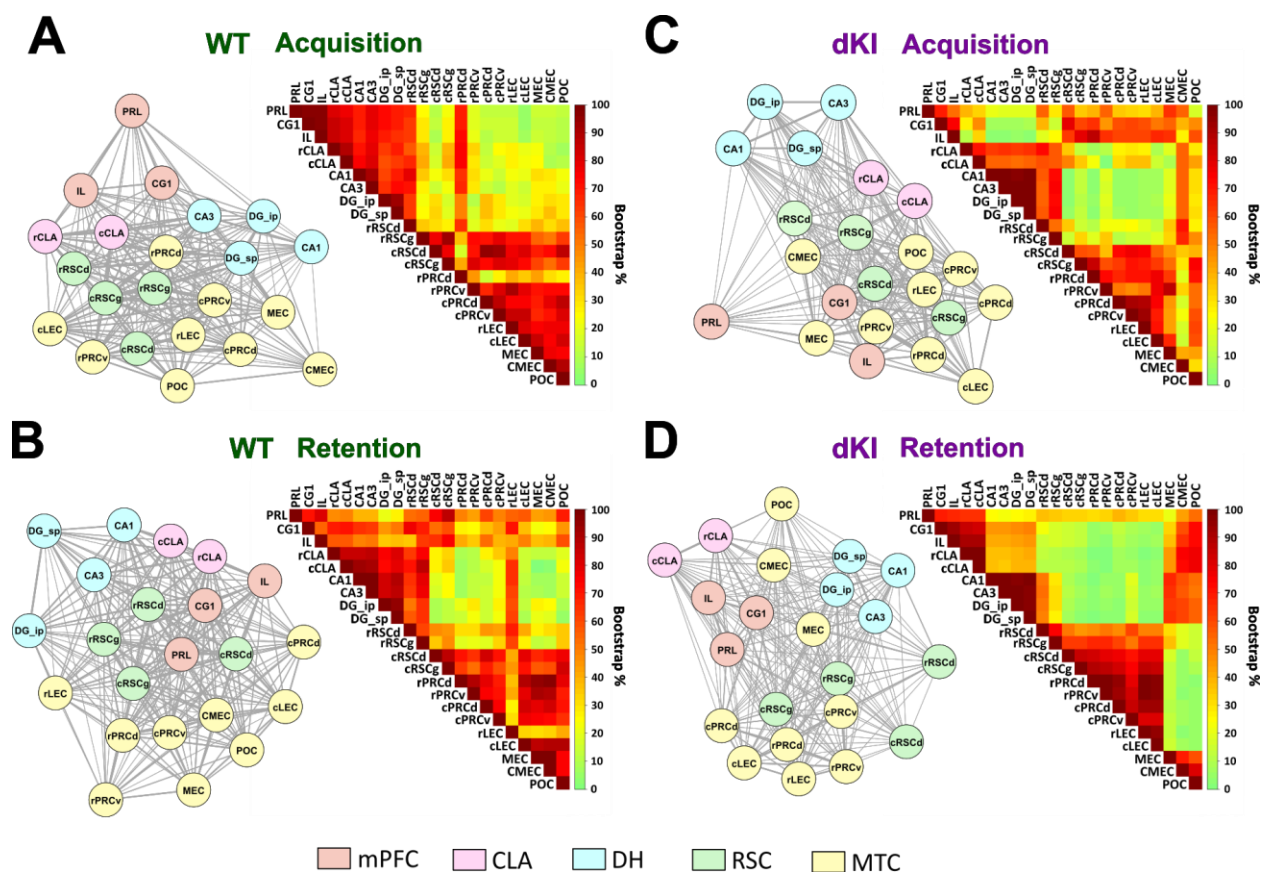
307 A stable community is found in both task phases encompassing most of the MTC and the cRSC (Figure  
308 4A,B; red area at the bottom right of each WT allegiance matrix). The main difference between the WT-  
309 Acquisition and WT-Retention groups was the differential involvement of the DH or mPFC with this  
310 MTC community. During acquisition, the DH shared a community primarily with the mPFC (Figure 4A;  
311 red area at top left the allegiance matrix). During retention, the mPFC shared allegiance across the  
312 network (Figure 4B; red area along the mPFC rows across the top of the allegiance matrix). This more  
313 global incorporation of the mPFC appeared to be driven largely through the RSC (Figure 4B; darker red  
314 square area located at the middle of the top three mPFC rows).

315 **dKI networks reveal consistent departures from WT community structure**

316 During acquisition, the DH community largely disengaged with the mPFC as compared to the WT-  
317 acquisition group (Figure 4C; green square at the top left of the allegiance matrix). Moreover, the DH  
318 appeared to be more strongly aligned to the CMEC, MEC, and POC as compared to the rest of the MTC  
319 (Figure 4C; the more red/orange area at the right of the DH rows).

320 During retention, both the mPFC and the DH disengaged with the MTC. This led to distinct communities  
321 containing mPFC/CLA, DH and the MTC (Figure 4D; three separate red squares of the allegiance  
322 matrix). Similarly to the acquisition phase, the MEC/CMEC/POC regions were heavily recruited by the  
323 mPFC and DH communities (Figure 4D; red area at top right), at the cost of disengagement with the MTC  
324 community (Figure 4D; green area at bottom right of the allegiance matrix). The consistent recruitment of  
325 the MEC/CMEC/POC in both dKI groups elucidates a possible role for these regions as compensatory  
326 hubs in this mouse model.

327



328

329 **Figure 4.** Functional network structure and community organization. Functional networks were computed as fully connected  
 330 weighted graphs, with regions as nodes (circles) and inter-regional correlation strengths as edge weights (line thickness). For  
 331 visual examination of networks, nodes were placed so that they lie closer to nodes with which they are strongly connected, and  
 332 were color coded according to a priori region subfields. Communities were detected through modularity maximization, which  
 333 finds communities of regions that have stronger connections with each other relative to the rest of the network. Communities  
 334 were detected across bootstrapped networks, and allegiance matrices were utilized to depict community stability as the  
 335 percentage of bootstraps that contain any given pair of regions in the same community. (A,B) In both WT groups there is a stable  
 336 MTC/cRSC community (red area at the bottom right of each WT allegiance matrix). (A) In the WT-Acq there is a stable  
 337 DH/mPFC community (red area at the top left of the allegiance matrix). (B) In the WT-Ret the mPFC shares community  
 338 allegiance with all of the network (red area along the mPFC rows across the top of the allegiance matrix) as mediated through the  
 339 RSC (darker red square in the middle of the top three mPFC rows). (C,D) Across both dKI groups, the MEC/CMEC/POC regions  
 340 appear to display a consistently modified community allegiance in respect to the WT, associating more with the DH/mPFC and  
 341 sometimes less with the rest of the MTC (red area at top right, some green areas at bottom right of each dKI allegiance matrix).

342



### 343 **Information flow in functional networks**

344 Efficient integration of information flow across functional networks is shown to aid in cognitive function  
345 (Wang et al., 2013a; Martinez et al., 2018). Interpreting network links as “pipes”, how well can a network  
346 allow information to flow will depend not only on how wide individual pipes are, but also on how pipes  
347 are disposed and aligned to form pipelines between the nodes that must communicate, without too many  
348 steps and bottlenecks. The general capacity for a network to sustain efficient flows is quantified in graph  
349 theory by metrics such as *global efficiency* (see Materials and Methods; Latora and Marchiori, 2001). It is  
350 important to note that even if a network has reduced global FC strength, its global efficiency may rest un-  
351 affected if there are well placed “hub” regions to facilitate indirect communication. To evaluate  
352 information flow at the regional level, *nodal strength* and *nodal efficiency* metrics are used. Nodal  
353 strength measures the degree to which each specific region can exchange information directly with all  
354 other regions of the network. A region with low strength may still be able to communicate with its  
355 network indirectly, again likely through hub regions. This indirect communication can be measured using  
356 nodal efficiency. Organization of information flow in a network can be examined through strength and  
357 efficiency distributions. Regions with higher strength and efficiency can be considered to contribute more  
358 to their network. High direct connectivity of a region, such as high strength, is also shown to describe  
359 potential hub regions (Vetere et al., 2017) which, as we have described, are needed to facilitate indirect  
360 information flow and maintain global efficiency. We compared information flow between WT and dKI  
361 networks within each test-phase. Organization of information flow was first evaluated through a  
362 qualitative examination of node strength and nodal efficiency distributions. To best visualize  
363 distributions, the strength/efficiency values were squared and normalized to the highest value within each  
364 network, and were displayed as necklace diagrams (acquisition - Figure 5A,B) (retention – Figure 5F,G).  
365 We then directly compared global efficiency and nodal strength/efficiency between genotypes in  
366 subsequent analyses (acquisition - Figure 5C,D,E) (retention - Figure 5H,I,J).

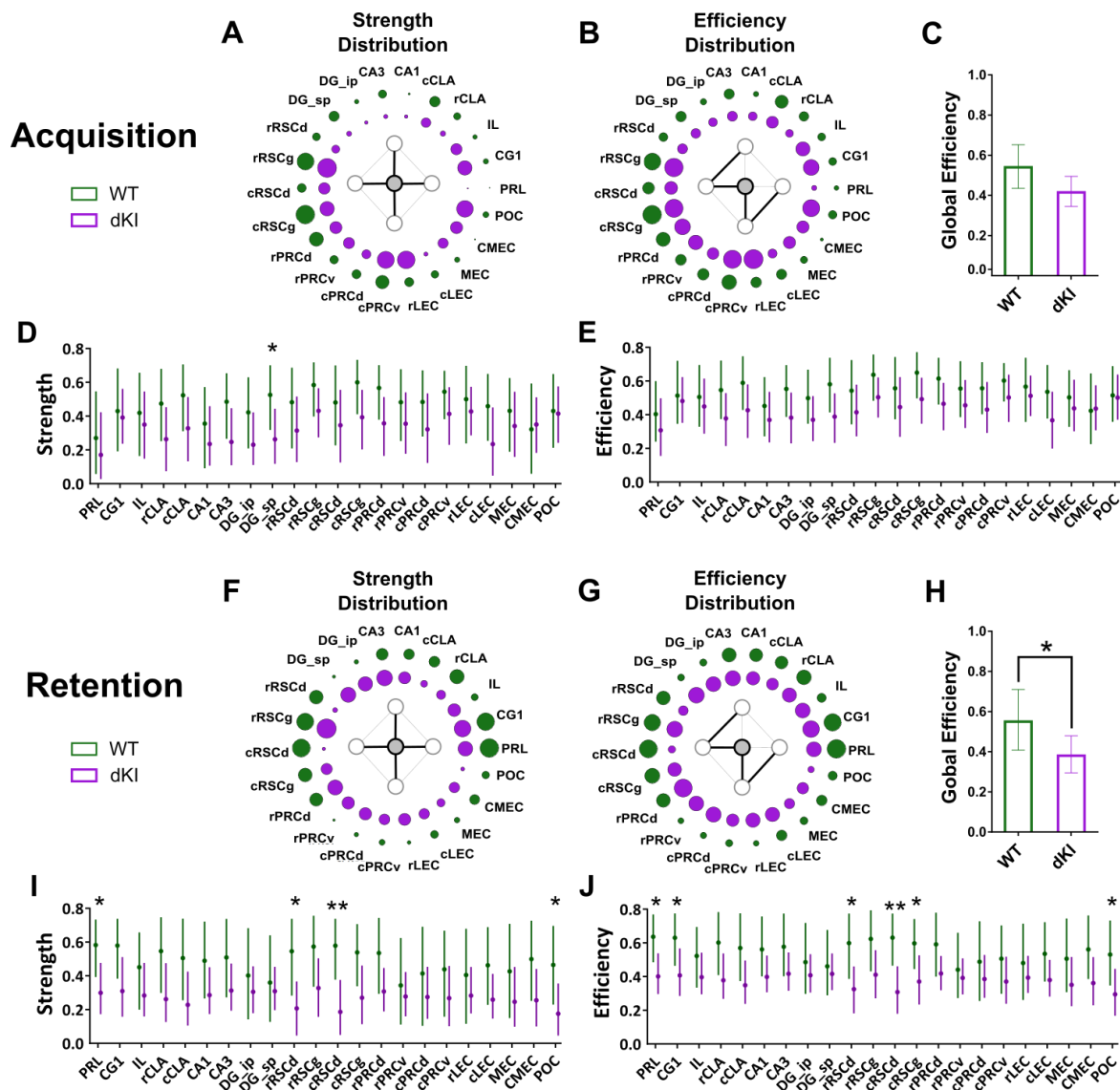
### 367 **Acquisition– the DG\_sp maintains efficiency despite a drop in strength**

368 During acquisition, strength and efficiency distributions were largely conserved between genotypes with  
369 strong emphasis on RSC and MTC regions. Although, in the dKI there appeared to be additional  
370 involvement of mPFC, MEC and POC (Figure 5A,B). Nevertheless, global efficiency was not severely  
371 affected in the dKI network (Figure 5C). The unique significant drop in nodal strength was seen in the  
372 DG\_sp (Figure 5D), but as there was no drop in nodal efficiency (Figure 5E), it could still effectively  
373 communicate with the rest of the network indirectly. This maintenance of indirect communication, despite  
374 drops in direct communication, reflects the potential importance of the rRSC or MEC/CMEC/POC  
375 compensatory hubs.

376 **Retention – severe losses in network efficiency, especially across the cingulate cortex**

377 During retention, heavy emphasis was placed on mPFC and RSC regions in the WT network. In the dKI  
378 network this emphasis was largely lost and the distributions take on a more homogenous structure, though  
379 the rRSCg and mPFC subregions still appeared to be among the most involved (Figure 5F,G). Global  
380 efficiency was significantly reduced in the dKI network (Figure 5H), with sharp drops in strength seen in  
381 the PRL and the POC, but most severely in both subregions of the RSCd (Figure 5I). Loss in nodal  
382 efficiency was even more prevalent, with additional reductions in the CG1 and the cRSCg (Figure 5J).  
383 These results suggest that retention dependent functional integration across the cingulate cortex is  
384 severely disrupted in the dKI, and may be linked to reduced hub strength of the RSCd.

385



386

387 **Figure 5.** Information flow in functional networks. Network organization of information flow was assessed through examination  
 388 of node strength and nodal efficiency distributions in both test-phases (acquisition, retention). These distributions were visualized  
 389 using necklace diagrams, where circle size reflects the within network normalized (A,G) strength<sup>2</sup> and (B,H) efficiency<sup>2</sup>. Global  
 390 efficiency was then compared directly between genotypes to assess (C,F) network integration. (D,I) Nodal strength and (E,J)  
 391 nodal efficiency were compared between genotypes to assess region dependent changes in direct and indirect information flow,  
 392 respectively. (A,B) During acquisition, strength and efficiency distributions were roughly conserved between genotypes, with  
 393 strong emphasis on the RSC and MTC regions, although in the dKI, mPFC, MEC and POC subregions were much more  
 394 involved. (C) Global efficiency was not severely affected in dKI mice. (D) A drop in region strength was seen in the DGsp, (E)  
 395 but its efficiency was unaffected. (G,H) During retention, heavy emphasis was placed on mPFC and RSC regions in the WT  
 396 network. In the dKI, this emphasis is largely lost. (F) Global efficiency of the dKI network was significantly reduced as  
 397 compared to the WT network. (I) Reductions in region strength were seen in the POC, PRL, and most severely in both subregions  
 398 of the RSCd. (J) Reductions in efficiency was retained among these regions, with additional losses in the CG1 and the cRSCg.  
 399 Bar graphs and dot graphs represent the mean bootstrap value, and the error bars represent the bootstrapped 95% confidence  
 400 interval. \* -the 95% CI for the difference  $\geq 0$  ; \*\* -the 99% CI for the difference  $\geq 0$

401

## 402 **Relating network efficiency and memory**

403 Finally, we addressed the question of whether the reduction of OP memory performance in dKI mice  
404 could be accounted for by the reduction in network efficiency observed during retention. Our aim was  
405 thus to determine if a relationship could be found between global efficiency and memory index in the WT  
406 and be used to predict dKI memory loss. This was assessed through correlation testing across sub-  
407 sampled WT populations, where each sub-sample corresponded to the removal of one mouse. From each  
408 sub-sample the average memory index and resulting global efficiency were calculated, and correlation  
409 significance was tested with Pearson and Spearman correlation coefficients.

### 410 **Global efficiency positively correlates with an exploration adjusted memory index, and predicts** 411 **memory deficiency in the dKI**

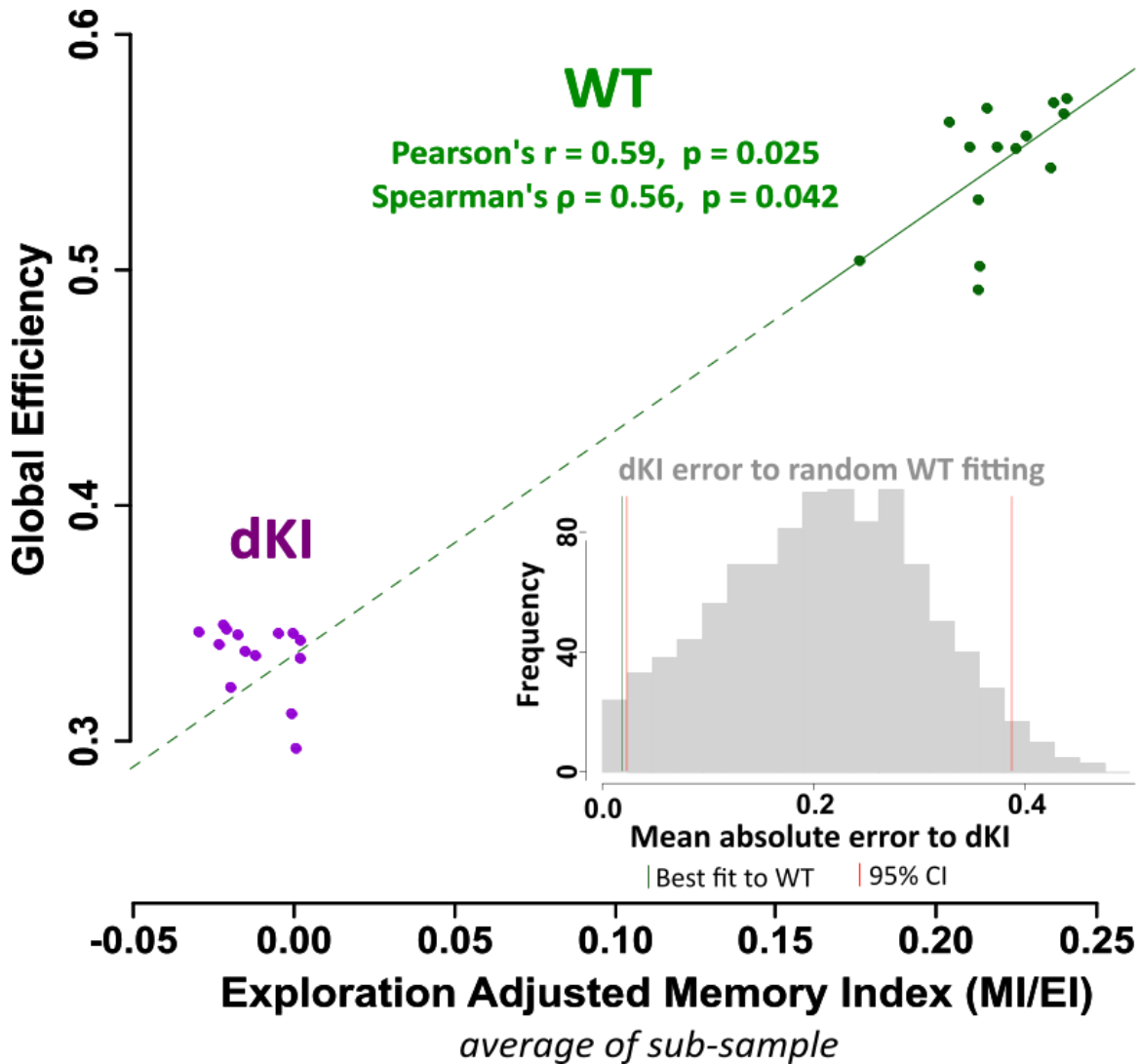
412 No significant correlation was found between global efficiency and memory index across WT sub-  
413 samples (Figure 6 - figure supplement 1A). However, we noticed that of the mice with high memory  
414 index, those with greater exploration times (total time exploring both objects) appeared to have a weaker  
415 contribution to global efficiency. This hinted a potential dampening effect of general object exploration  
416 on memory driven FC. Note that increases in exploration time themselves may be interpreted as a further  
417 sign of memory function alteration, beyond reductions in the previously defined memory index MI. We  
418 thus defined an exploration index EI, quantifying the exploration time of the mouse normalized to the  
419 group and used it to compute as well an exploration adjusted memory index as the memory index divided  
420 by the exploration index (MI/EI). Decrease of such an exploration adjusted memory index may reflect  
421 both MI decrease and EI increase, thus summarizing both probed facets of memory-related behavior  
422 alteration. As in the case of MI alone, we did not find a significant correlation between global efficiency  
423 and EI index (Figure 6 - figure supplement 1B). A significant positive correlation was found however  
424 between global efficiency and the adjusted memory index MI/EI across the WT sub-sampled networks  
425 (Figure 6).

426 Moreover, we could show that extrapolating the best fit line for the WT ensemble to drops in global  
427 efficiency as large as the ones observed in the dKI ensemble also predicted the observed dKI decrease of  
428 the MI/EI behavioral performance index. This is illustrated in Figure 6 where the dashed continuation of  
429 the green line (the extrapolation of the best fit line to the WT ensemble) intersects the cloud of purple  
430 points (the ensemble of dKI sub-samples). The prediction of dKI sub-samples MI/EI index based on  
431 extrapolating the law fitted on WT sub-samples yielded errors way smaller than what expected at chance  
432 level, as we could verify via comparison with a null distribution of prediction errors constructed from  
433 1000 randomly fit models to the WT ensemble. This is shown in the inset histogram of Figure 6, where

434 the green vertical line (prediction error of best fit to WT) lies to the left of the leftmost red vertical line  
435 (lower end of the null distribution 95% CI).

436 This suggests that subjects with a high memory index but low total exploration time are thus more likely  
437 to have higher global efficiency. Contrary to memory index, there is no detectable significant difference  
438 in total object exploration time between the WT and dKI groups (Figure 6 - figure supplement 1C).  
439 Memory index is thus a more sensitive metric of subtle and complex behavioral changes, which are  
440 tracked by a decrease of global efficiency from WT to dKI. These results support reduced network  
441 integration as a potential factor for impairment of OP memory recall in the dKI.

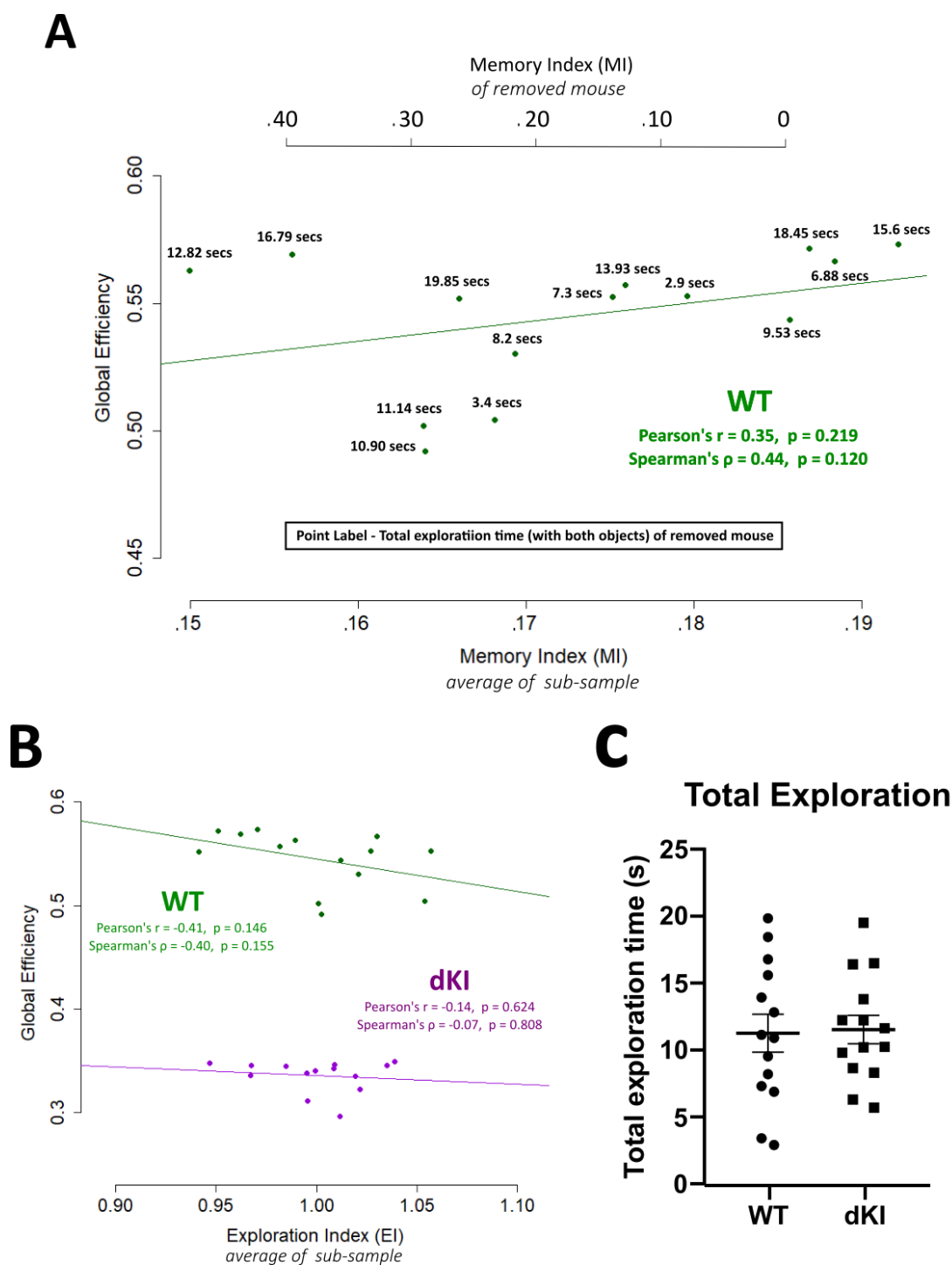
442



443

444 **Figure 6.** Association between network efficiency and exploration adjusted memory index “MI/EI”. Sub-samples were  
445 generated from each retention group by resampling  $n-1$  mice without replacement. From each sub-sample, the average MI/EI and  
446 the network global efficiency were computed, where each sub-sample is depicted as point on the plot. The relationship between  
447 global efficiency and MI/EI was evaluated across only the WT sub-samples (solid green line), and a significant positive linear  
448 relationship was found. Interestingly, when this linear law across WT sub-samples was extrapolated down (dashed green line) to  
449 the reduced global efficiency of the dKI sub-samples, it could predict their reduction in MI/EI (the intersection of the dashed  
450 green line to the cloud of purple points). The prediction of dKI sub-samples MI/EI index based on extrapolating the law fitted on  
451 WT sub-samples yielded errors way smaller than what expected at chance level, as we could verify via comparison with a null  
452 distribution of prediction errors constructed from 1000 randomly fit models. See inset histogram: the green vertical line (dKI  
453 error to best fit of WT) lies to the left of the leftmost red vertical line (lower end of the null distribution 95% CI).

454



455

456 **Figure 6-figure supplement 1** Associations of memory index “MI” and exploration index “EI” to network efficiency. (A) There  
 457 was no relationship found between global efficiency and memory index. However, we noticed that of the mice with high memory  
 458 index, those with greater exploration times (total time exploring both objects) appeared to have a weaker contribution to global  
 459 efficiency. To take into account an exploration disruption effect on memory related FC, an exploration adjusted memory index  
 460 was computed as the memory index divided by the exploration index (MI/EI), where the exploration index (EI) is the exploration  
 461 time of the mouse normalized to the group. (B) We verified that there was no direct relationship found between global efficiency  
 462 and EI. (C) There was also no difference in total exploration between WT and dKI mice.

## 463 Discussion

464 The current necessity to learn more about initial steps of AD pathology prompted us to investigate brain  
465 network alterations associated to the encoding and retrieval phases of the first recognition memory  
466 paradigm affected in the late-onset *App<sup>NL F</sup>/MAPT* dKI mouse model of the disease (Saito et al, 2019).  
467 Here we show that the neuropathology expressed by 4-month-old dKI mice at the time of their earliest  
468 recognition deficit is reminiscent of an early preclinical stage. Quantification of c-Fos activation  
469 highlighted an abnormally increased activity in the entorhinal cortex and the claustrum, two entities  
470 known to be vulnerable to AD pathology. Finally, extensive analyses of network connectivity point to a  
471 disorganization of internal communication between and within the MTL and interconnected regions  
472 where the RSC seems to play a pivotal “hub” role which could be disrupted in dKI mice.

### 473 Object-place recognition deficit as an early marker of emerging AD neuropathology

474 In the battery of five recognition memory tasks, the object-place associative memory task was the first to  
475 detect a deficit in dKI mice at 4 months of age. This recognition deficit occurred 2 months before spatial  
476 pattern separation deficits and with intact long term object recognition memory up to 6 months of age.  
477 The object-place task seems to be highly sensitive to early amyloid pathology because it was also  
478 impaired at pre-plaque stages in more aggressive models of AD (Bonardi et al, 2016; Hamm et al., 2017).  
479 In TgCRND8 mice it was specifically associated with increased levels of  $\beta$ -CTF in the hippocampus  
480 (Bonardi et al, 2016; Hamm et al., 2017). A $\beta$  levels of *App<sup>NL F</sup>/MAPT* dKI were not evaluated in this  
481 study, but the seminal work characterizing the parental single KI *App<sup>NL F</sup>* mice already showed increased  
482 cortical levels of the neurotoxic species A $\beta_{42}$  at the age of 2 months (Saito et al, 2014). This early  
483 accumulation of A $\beta$  in 2-month *App<sup>NL F</sup>* mice was associated with significant cell loss and hyperexcitation  
484 in the LEC, whereas the same alterations appeared several months later in the CA1 (Petrache et al., 2019).  
485 Here, we show for the first time evidence for early onset of AD pathology in 4-month-old dKI mice with  
486 specific increases in levels of  $\beta$ -CTF and phosphorylated tau labeled with AT270 antibodies in the MTC,  
487 but not in the dorsal hippocampus. These results suggest that early abnormal levels of tau phosphorylation  
488 are still restricted to the MTC at the age of 4 months. This is of particular importance because the PRC-  
489 EC pathway has long been seen as a primary site of neurodegenerative event and dysfunction that  
490 characterizes the earliest preclinical Braak stage I of AD tau deposition (Kahn et al, 2013; Braak and  
491 Braak, 1995; Hyman et al, 1984). Interestingly, significant worsening of neuropsychological markers of  
492 PRC and EC functionality were detected more than 10 years before AD diagnosis (Hirni et al, 2016).  
493 More recently, Yeung et al., 2018, showed that object-place recognition performance was predicted by  
494 anterolateral EC volume in the elderly. This result is in agreement with studies attributing a key role of



495 the equivalent rodent LEC in the network supporting association of an object with a place or a scene  
496 (Deshmukh et al, 2012; Wilson et al, 2013a,b; Chao et al 2016). It must be noted that deficits in the  
497 pattern separation task in 6-month-old dKIs suggest subsequent development of functional alterations  
498 within the DG-CA3 region which is in agreement with studies in aMCI patients and transgenic mouse  
499 models of AD (Yassa et al, 2010; Zhu et al, 2017). All these findings strongly suggest that 4-month-old  
500 dKI mice recapitulate early impairment in recognition memory and specific vulnerability of the PRH-EC  
501 region to both amyloid and tau pathologies as found in preclinical stages of AD (Kahn et al, 2014).

#### 502 **Distributed increase in c-Fos activation possibly reflecting spreading of pathological hyperactivity**

503 We detected high level of c-Fos expression in the CLA and the LEC of dKI mice regardless of the test-  
504 phase suggesting that both structures would be hyperactivated during memory encoding and recall. On the  
505 other hand, the hyperactivation of the MEC restricted to the acquisition phase could be have been  
506 triggered by active encoding of the new environment. Well documented in mouse models of AD, early  
507 hyperactivity within the LEC appears as a consequence of local increase in amyloid pathology and it is  
508 considered as a major factor driving propagation of both A $\beta$  and tau pathology to its main outputs  
509 especially the hippocampus (Xu et al., 2015; Nuriel et al., 2017; Rodriquez et al., 2020). The CLA has  
510 been less extensively studied, but a few studies show evidence of A $\beta$  accumulation and  
511 neurodegeneration in this region (Qin et al, 2013; Ogomori et al., 1989; Gustafson et al., 1998).  
512 Interestingly, an early study in Alzheimer's patients found neuronal loss only within a CLA subregion  
513 strongly connected with the EC (Morys et al., 1996). Thus, EC may also propagate hyperactivity,  
514 exporting amyloid and tau pathology to the CLA as well as other limbic cortices in preclinical stages of  
515 AD (Bonthius et al., 2005). Note that an alternative hypothesis has been proposed with a central role of  
516 the CLA in the spreading of AD pathology in the brain (Avila and Perry, 2021). Being largely  
517 interconnected with most cortical areas (Wang et al, 2017), hyperactivity of CLA may initiate or  
518 aggravate spontaneous the cortical network hypersynchrony and task-induced hyperactivation found in  
519 elderly at risk and in early MCI (Mueller and Weiner, 2017; Corriveau-Lecavalier et al., 2020).

520 Early-stage hyperactivity has also been proposed to reflect compensatory mechanisms which would first  
521 help maintaining and then worsen cognitive function through the spreading of neuropathological  
522 processes and a deleterious impact on cognitive-related network organization and functioning (Corriveau-  
523 Lecavalier et al., 2020). There is some indication for a reorganization of network information flow in the  
524 dKI mice. We saw through the community organization of dKI functional networks during both phases,  
525 that the MEC and POC are more integrated with mPFC and DH communities (Figure 4C,D). This change  
526 in community allegiance may be a consequence of an initial hyperactive state within the EC, much like

527 artificial stimulation of brain structures can enforce specific functional networks (Warren et al., 2019). In  
528 certain cases, this shift towards stronger outbound allegiance may help facilitate alternative  
529 communication between the MTC and interconnected regions, as seen with the maintenance of indirect  
530 information flow of the DG\_sp during acquisition (Figure 5D,E). The apparition of compensatory hubs  
531 may be one way through which hyperactivity could initially help memory processing during the  
532 preclinical AD.

### 533 **mPFC and RSC are disrupted during object-place associative memory retention**

534 The mPFC and RSC were the most heavily utilized regions in WT mice during retention phase, and direct  
535 and indirect information flow through these regions was significantly reduced in dKI mice (Figure 5F).  
536 The specificity of this dysfunction to the retention phase is not surprising as the mPFC and RSC are  
537 shown to be more involved in the retrieval and/or editing of memory traces rather than encoding *per se*  
538 (Mitchell et al., 2018). Lesioning the RSC disrupts object-place associative memory in rodents (Parron  
539 and Save., 2004), and communication between the mPFC-MTC is also shown to be essential (Chao et al.,  
540 2016; Hernandez et al., 2017). The RSC is proposed to play a pivotal “hub” role in facilitating this  
541 communication as it exhibits strong structural connections with both the mPFC and the MTC (Sugar et  
542 al., 2011, Vann et al., 2009). In humans, the posterior cingulate cortex (PCC) and RSC, close equivalents  
543 to the rodent RSC (Lu et al., 2012, Stafford et al., 2014, Vogt and Paxinos, 2014), are shown to support  
544 structural connectivity between the mPFC and MTL, essential to associative memory networks and the  
545 DMN (Greicius et al., 2009, Miller et al., 2014). The hubness of the RSC during retrieval is clearly  
546 supported by the community organization of our functional networks, where it is well positioned to  
547 facilitate communication between the mPFC and MTL communities (Figure 4B). The severe loss in  
548 strength seen in the dysgranular RSC may therefore indicate an object-place recall-dependent roadblock  
549 for mPFC-MTL communication.

550 One question lies as to why the dysgranular RSC was more severely disrupted than the granular part. The  
551 dysgranular cortex is comparatively more connected to visual and sensory processing areas, making it  
552 ideal for processing and identifying local and distal cues for allocentric orientation. Lesioning the  
553 dysgranular RSC alone is enough to have rats shift from allocentric to egocentric strategies in a spatial  
554 memory task (Vann and Aggleton., 2005). The dysgranular RSC contains landmark dependent head  
555 direction cells, which may help orient the mice in the open field with respect to the objects (Jacob et al.,  
556 2016), and decreased strength of the dysgranular RSC may contribute to their dysfunction.

557 Our findings also corroborate those of human studies showing that the mPFC and the PCC/RSC are often  
558 disrupted in evaluations of functional and structural connectivity in aMCI (Catheline et al., 2010; Wang et

559 al., 2013c). The especially severe disruption of the RSC reflects clinical observations of the human PCC  
560 as one of the most, if not the most, consistently disrupted regions in evaluations of resting state FC of  
561 aMCI (Badhwar et al., 2017; Eyler et al., 2019). The mPFC and the PCC/RSC are also the first regions to  
562 display amyloid deposition (Palmqvist et al., 2017). The PCC/RSC shows reduced connectivity in pre-  
563 plaque APOE  $\epsilon$ 4 carriers at risk for developing AD (Wang et al., 2013b; Jones et al., 2016), and very  
564 early increases in PCC amyloid deposition correlate with face-name associative memory deficits in  
565 subjective cognitive decline (Sanabria et al., 2017). Initial pathologies of amyloid in the mPFC/PCC/RSC  
566 and tau in the MTC may thus have a combined negative effect on associative memory through reducing  
567 memory-recall dependent FC.

### 568 **Decreased global efficiency during retention predicts memory deficits in dKI mice**

569 In WT mice, we detected a positive relationship between global efficiency of the retention network and  
570 memory performance when taking into account a disruptive effect of total object exploration. The  
571 exploration disruption on memory driven FC can perhaps be explained by increased focus and a more  
572 conscious exploration of objects for mice with a low exploration index, in contrast to a more haphazard  
573 exploration of objects for mice with high exploration index. This may also indicate a disruptive effect of  
574 simultaneous sensory or motor network activation. Regardless, dKI mice presented no difference in object  
575 exploration as compared to the WT mice, making it an unlikely behavioral link to reduced global  
576 efficiency in these mice.

577 Global efficiency of c-Fos derived fear memory networks has already been shown as a reliable measure  
578 for predicting memory performance in mice (Vetere et al., 2017). Such findings are consistent with  
579 clinical observations linking increased global efficiency to better cognitive function (Li et al., 2009;  
580 Stanley et al., 2015). The relationship across the WT group can be extrapolated down to predict memory  
581 performance in the dKI. This provides further evidence that their drop in memory performance is directly  
582 linked to deficient information transfer of the memory retention network. There is increasing evidence  
583 that memory loss in AD pathology is related to dysfunctional recall, as it can be rescued by aiding  
584 retrieval processes (i.e., activating the “silent” engram; Roy et al., 2016, Perusini et al., 2017). Perhaps  
585 early associative memory deficits of dKI mice could be rescued by activating “silent” inefficient  
586 functional networks. In humans, noninvasive brain stimulation has been extensively studied as a potential  
587 therapeutic tool for AD on regions such as the dorsolateral mPFC with mixed results (Weiler et al., 2020).  
588 However, electromagnetic stimulation of the parietal cortex was shown to support associative memory by  
589 increasing memory-retrieval dependent FC strength of an associated parietal-RSC-hippocampal network  
590 (Wang et al., 2014; Warren et al., 2019). Taken together with our study, these results suggest that the

591 PCC/RSC, or more laterally accessible and functionally similar regions such as the parietal cortex, may  
592 be more effective targets for improving associative memory in AD.

### 593 **Contrast with resting state fMRI in other mouse models of AD**

594 Contrary to our results, studies that evaluate resting state networks in pre-plaque or early-NFT mouse  
595 models of AD predominantly find cortical and hippocampal-cortical hyperconnectivity, while  
596 hypoconnectivity appears at later stages (Asaad and Lee, 2018). This suggests that perturbations in resting  
597 state FC may predict memory FC perturbations, but do not directly mirror them. Moreover, the mouse  
598 models used to evaluate pre-aggregate resting state FC presented either amyloid pathology (Shah et al.,  
599 2016; Shah et al., 2018) or tau pathology (Degiorgis et al., 2020) but not both concurrently. It has been  
600 shown with combined resting state fMRI and PET imaging in humans that increased A $\beta$  alone is  
601 associated with hyperconnectivity of the DMN while combined A $\beta$  and Tau pathologies reveal  
602 hypoconnectivity (Schultz et al., 2017). Whether these contrasting results reflect differences between  
603 “resting state vs memory driven FC” or differences in “amyloid/tau pathological staging” will be more  
604 thoroughly understood once resting state fMRI is directly measured in young dKI mice.

605 In conclusion, our results suggest that the present dKI model was caught at the very beginning of its  
606 neuropathology as it was restricted to the MTC region, leaving the dorsal hippocampus quite preserved.  
607 The local MTC pathology of these mice was associated with EC and CLA hyperactivity which would  
608 most likely spread towards densely interconnected regions such as the mPFC, the DH and the RSC.  
609 Retrieval dependent communication between cingulate areas and the MTL was disrupted, and can be  
610 potentially linked to reduced dysgranular RSC hub strength. The similarity between our findings in the  
611 dKI model and those reported in the earliest stages of the disease suggests that the *App*<sup>NL-F</sup> version of the  
612 dKI model has a high potential for generating new discoveries on the earliest stage of AD.

613

614

## 615 **Methods**

### 616 **Animals**

617 The *App*<sup>NL-F</sup>/*MAPT* double knock-in (dKI) mice were produced through crossing homozygotes of two  
618 single knock-in (KI) mouse lines for the humanized *App*<sup>NL-F</sup> and *MAPT* genes, and then through the  
619 crossing of the resulting doubly heterozygote mice to obtain dKI and non-knock-in WT line founders.  
620 After three generation of homozygous breeding, the dKI mouse line was backcrossed with C57BL/6J  
621 mice (Janvier Laboratories, Le Genest Saint Isle, France) in order to limit genetic drift between dKI mice  
622 and their WT controls. The *App*<sup>NL-F</sup> gene contains a humanized A $\beta$  fragment with Beyreuther/Iberian and  
623 Swedish FAD mutations, and the human *MAPT* gene expresses all 6 isoforms of tau found in humans.  
624 Both single KI mouse lines were produced by T Saido and T Saito (RIKEN Brain Science Institute,  
625 JAPAN) and sent to us by the RIKEN BioResource Center. Mice were group-housed with food and water  
626 ad libitum, nesting material, and additional food pellets on bedding to promote natural behavioral  
627 patterns. The animal room was under controlled temperature (23 °C  $\pm$  1°C) and a 12/12-hour light/dark  
628 cycle (lights on at 8.00 AM). Procedures were in compliance with rules of the European Community  
629 Council Directive 2010-63 and French Department of Agriculture Directive 2013-118 and approved by  
630 the local review board (CREMEAS: APAFIS#9848). Animal facilities were approved for animal  
631 experimentation (H 67-482-13).

### 632 **Behavioral Testing**

633 Behavioral testing took place during the light phase. Mice were single-housed for 1 week before testing.  
634 Spontaneous object exploration tests were carried out in an open field (100cmx100cmx50cm, Ugo Basile,  
635 Italy) with dark grey acrylic walls and a grey metal floor. The open field was evenly illuminated by three  
636 indirect halogen lights (open field center, 15 lux), and a radio gave background noise from 1.5m away  
637 (open field center, 45  $\pm$  5 dB). Nine different sets of objects were used: two for the habituation phases,  
638 two for the long-term novel object recognition task, two for the OP task, two for the short-term novel  
639 object recognition task, and one for the object location task. These objects differed in size (10 to 20 cm),  
640 material (metal, glass, or plastic), shape, and color. Each object was available in duplicate or triplicate.  
641 Ethanol (30%) was used to clean the objects and the open field between each trial. Object exploration  
642 time was recorded and defined as the nose pointing toward the object within 2 cm. Gnawing and climbing  
643 of objects were not counted as exploration time.

### 644 **Habituation**

645 Before testing, all mice received two days of habituation. On the first day, they were given a habituation  
646 trial of 10 min with two identical objects placed in the open field. On the second day they were given two  
647 10 min trials with a different set of two identical objects, with the trials separated by an inter trial interval  
648 (ITI) of 5' that the mice spent in their home cage. For the OP task with a 3-hour ITI, the second day  
649 habituation procedure was applied with an ITI of 3 hours.

#### 650 **Preliminary testing cohorts**

651 Mice were tested at 2 months (WT n = 10, dKI n = 10), 4 months (WT n = 12, dKI n = 9) and at 6 months  
652 (WT n = 8, dKI n = 11). An additional cohort of (WT n = 3, dKI n = 1) was tested at 2 months in object-  
653 place association as the initial WT group did not reach significance above 0. For pattern separation one  
654 two month old WT mouse was removed due to the wrong objects accidentally being placed during the  
655 retention phase.

#### 656 **Short term, pattern separation and long-term novel object recognition**

657 Mice were tested at 4 months (WT n = 11, dKI n = 11) in the short term OR task. One WT mouse was  
658 removed from the analysis due to being dropped before the task. Mice explored two identical objects  
659 during a 10-min acquisition trial, and were returned to their home cage for an ITI of 5 min (short term,  
660 pattern separation) or 24 hours (long term). Thereafter, mice were given a 10-min retention trial, where  
661 one of the familiar objects was replaced by an unfamiliar new one. For the pattern separation task, objects  
662 were made of legos, the novel object had the same composition of colored lego blocks but a different  
663 pattern. Exploration of the objects was recorded during the 6 minutes (4 minutes for pattern separation)  
664 following the initial exploration of object for each mouse. The memory index was calculated as:

$$\text{Memory index} = \frac{\text{Time at replaced object} - \text{Time at unchanged object}}{\text{Time at both objects}}$$

#### 665 **Short term object-place association**

666 To validate the potential deficit seen during preliminary phenotyping, an additional cohort (WT n = 11,  
667 dKI n = 11) was tested in the object-place association task. Mice explored two different objects during a  
668 10-min acquisition trial, and were returned to their home cage for an ITI of 5 minutes. During the 10-min  
669 retention trial, one of the objects was replaced by a copy of the other. The mice had to detect the  
670 mismatch between one object and its actual location in the open field. Exploration of the objects was  
671 recorded during the 4 minutes following initiation of object exploration episode for each mouse. The  
672 memory index was calculated as with the long-term object recognition task.

#### 673 **Short term novel object location**

674 Mice were tested at 4 months (WT n = 11, dKI n =11). Mice explored two identical objects during a 10-  
675 min acquisition trial, and were returned to their home cage for an ITI of 5 minutes. During a 10-min  
676 retention trial, one of the objects was moved 55 cm from its original position for the object location task.  
677 Exploration of the objects was evaluated during the 6 minutes following the initiation of object  
678 exploration for each mouse. During both phases, all objects were placed equidistant from the walls. The  
679 memory index was calculated as:

$$\text{Memory index} = \frac{\text{Time at moved object} - \text{Time at unmoved object}}{\text{Time at both objects}}$$

## 680 **Western Blotting**

681 Four-month-old male mice were sacrificed by cervical dislocation (n=8 per group), and their brains were  
682 carefully dissected on ice. Hippocampi and medial temporal cortex were quickly removed, frozen in  
683 liquid nitrogen and then stored at  $-80^{\circ}\text{C}$  until their use. Tissues were homogenized in 10 volumes of ice-  
684 cold radioimmunoprecipitation assay buffer containing protease inhibitor cocktail (Sigma-Aldrich, St.  
685 Louis, Missouri), phosphatase inhibitor cocktail (PhosStop, Roche Life Science, Penzberg, Germany),  
686 and 1 mM phenylmethylsulfonyl fluoride (Sigma-Aldrich). After centrifugation at 20,000g for 20 min at  
687  $4^{\circ}\text{C}$ , supernatants were aliquoted for immunoblot analysis. Brains extracts of Tg2576 and Thy-tau22 mice  
688 were homogeneized and separated in parallel to our samples as a positive control for APP proteins and  
689 cleaved fragments and for tau proteins, respectively. Protein concentration was measured using the Bio-  
690 Rad Protein Assay (Bio-Rad, Hercules, California). Thirty or 20 microgramms were respectively loaded  
691 on 4-20% precast gel (Mini-Protean TGX precast gels, Bio-Rad) for APP and tau proteins. After  
692 electrophoresis and transfer to nitrocellulose membranes using the Trans-Blot Turbo System (Bio-Rad),  
693 membranes were incubated with 5% skimmed milk for 1 hour at room temperature and then with primary  
694 antibodies diluted in 2% bovine serum albumin (Sigma-Aldrich) in tris-buffered saline 0.05% Tween 20  
695 (Sigma-Aldrich) overnight at  $4^{\circ}\text{C}$ . After washes, membranes were incubated with anti-mouse or anti-  
696 rabbit immunoglobulins conjugated to horseradish peroxidase (Jackson ImmunoResearch, West Grove,  
697 Pennsylvania) for development with enhanced ECL chemiluminescence detection kit (Thermo Fisher  
698 Scientific, Waltham, Massachusetts). After detection, all membranes were re-probed with anti-actin  
699 antibody for normalization of total protein. The primary antibodies used were the rabbit polyclonal anti-  
700 APP, C terminus (Sigma-Aldrich), the rabbit polyclonal anti-tau (B19, generously gifted by JP Brion,  
701 ULB, Belgium), the mouse monoclonal anti-phospho-Tau Thr181 (AT270, ThermoScientific), the mouse  
702 monoclonal anti-actin (Sigma-Aldrich) and the rabbit polyclonal anti-actin (Sigma-Aldrich). The  
703 secondary antibodies used were the peroxidase-conjugated AffiniPure goat anti-mouse and goat anti-  
704 rabbit (Jackson ImmunoResearch). The quantification of the band intensity acquired with the ChemiDoc

705 Imaging system (Bio-Rad) was performed by densitometry analysis using the ImageJ program. For each  
706 mouse, the phosphorylation degree was calculated as the ratio of total phosphorylated Thr181 tau proteins  
707 on total tau proteins. The ratio of APP-cleaved fragments was calculated as the total of APP  $\beta$ -CTF on  $\alpha$ -  
708 CTF fragments.

### 709 **Object-place association with a 3-hour ITI for ex-vivo imaging**

710 The same protocol as with the short-term object-place task was followed, except with an ITI of 3-hours.  
711 The “Acquisition” set of WT (N=12) and dKI (N=12) mice were only tested in the acquisition phase. The  
712 “Retention” set of WT (N=14) and dKI (N=14) mice were tested for the entire task, acquisition and  
713 retention phases included. At the end of testing mice were left in their home cage in a quiet dim room (9  
714 lux,  $35 \pm 5$  dB) next to the testing room for 90 minutes, after which they were taken for brain perfusion  
715 and removal.

### 716 **Perfusion and tissue preparation.**

717 Mice were killed with an overdose of sodium pentobarbital (105 mg/kg intraperitoneally) and  
718 transcardially perfused in 0.1% heparin phosphate-buffered saline (PBS) and 4% paraformaldehyde [PFA;  
719 in phosphate buffer (PB) pH7.4; 4°C]. Brains were removed, postfixed in a 4% PFA solution for 24h, and  
720 cryoprotected in a saccharose solution (20% in PB, 0.1 M; pH 7.4; 4°C) for 48 hours before being frozen  
721 with isopentane ( $-40^{\circ}\text{C}$ ) and subsequently stored at  $-80^{\circ}\text{C}$ . Forty  $\mu\text{m}$  coronal cryostat sections were cut  
722 from the anterior to the posterior of the brain. For the medial prefrontal cortex (mPFC), claustrum (CLA),  
723 dorsal hippocampus (DH), medial entorhinal cortex (MEC), and postrhinal cortex (POC) every 4<sup>th</sup> section  
724 created a set (spacing of 160  $\mu\text{m}$ ), and for the retrosplenial cortex (RSC), lateral entorhinal cortex (LEC),  
725 and perirhinal cortex (PRC) every 6<sup>th</sup> section (spacing of 240  $\mu\text{m}$ ).

### 726 **Immunohistochemistry**

727 Brain sections were given three 10 min washes in 0.1 M phosphate-buffered saline (PBS), followed by a  
728 30 min incubation in 1%  $\text{H}_2\text{O}_2$ . They were washed for 5 min with ultra-pure water and again three times  
729 for 10 min in 0.1 M PBS. This was followed by a 45 min blocking incubation in a 5% natal goat serum  
730 (NGS) diluted in a “diluent” solution consisting of 0.1 M PBS, and 0.5% triton. The sections were  
731 incubated at room temperature for 2 days in a 1/15000 dilution of rabbit-anti-cFos (Synaptic Systems,  
732 Göttingen, Germany) primary antibody in diluent containing 2% NGS. After 2 days the sections were first  
733 given two 10 min washes in 0.1 M PBS, and then incubated for 2 hours at room temperature in a 1/500  
734 dilution of biotinylated mouse-anti-rabbit (Vector Laboratories, Burlingame, California) secondary  
735 antibody in diluent containing 2% NGS. The sections were given two 10 min washes in PBS, followed by



736 a 45 min incubation in the avidin/biotin (Vector Laboratories) solution. This was followed by three 10  
737 min washes in 0.1 M PBS and a 10 min wash in phosphate buffer. The sections were finally revealed with  
738 a 10 min incubation in 3,3-diaminobenzidine (Vector Laboratories). Images of whole brain sections were  
739 taken at 20x magnification using a Hamamatsu NanoZoomer S60 digital slide scanner (Hamamatsu  
740 Photonics K.K., Hamamatsu City, Japan) for offline quantification of c-Fos expression.

#### 741 **c-Fos Imaging**

742 Neuronal activation is associated with increases in intracellular calcium levels, which in turn leads to the  
743 rapid up-regulation of immediate early genes such as c-fos. The quantification of c-Fos protein levels can  
744 thus be used to derive a measure of neuronal activity (Tischmeyer and Grimm, 1999). c-Fos expression  
745 was analyzed in 22 regions of interest (ROIs) (see Table S1), including sub-regions of the PFC, CLA,  
746 DH, RSC, PRC, POC and EC. Image processing was done using ImageJ (National Institute of Health,  
747 Bethesda, MD). ROIs were anatomically defined according to the atlas of Franklin and Paxinos (2008).  
748 For c-Fos quantification, the images were transformed into 8-bit grayscale. A grayscale threshold was set  
749 at a consistent level for each region by an experimenter blind to group condition. Only c-Fos positive  
750 nuclei with a grayscale intensity below the threshold and an area between 25–300  $\mu\text{m}^2$  were counted. At  
751 least three brain sections were processed per ROI. Mean c-Fos density was calculated for each ROI as the  
752 quantity of c-Fos marked nuclei per  $\text{mm}^2$ , normalized to the WT-Acquisition group. ROIs were grouped  
753 into region subfields whenever anatomically and functionally justified. These groupings reduce the  
754 number of comparisons and, thereby, restrict Type 1 errors. Three-way ANOVAs compared test-phases  
755 (acquisition or retention), genotypes (WT or dKI) and subregions for each subfield. When an interaction  
756 was significant, the simple effects were examined.

#### 757 **Functional Connectivity**

758 From the c-Fos signals, functional connectivity (FC) was assessed by computing the between subject  
759 inter-regional spearman correlations for each Genotype-Phase group. Spearman correlations, rather than  
760 Pearson correlations, were used to account for potential outlier effects and the relatively small sample  
761 sizes. Correlation matrices were used to visualize all possible pairwise inter-regional correlations within  
762 each group. To assess global FC strength the mean  $r$  was calculated with retained, near-zeroed, and  
763 absolute valued negative correlations respectively. In the near-zeroed case, negative correlations were  
764 reduced to a value of .006, the smallest positive correlation observed.

#### 765 **Generating functional networks as fully connected weighted graphs**

766 From each correlation matrix, a functional network was generated as a fully connected weighted graph.  
767 The edges weights of the graph reflect inter-regional spearman correlation strengths and the nodes reflect  
768 regions. Negative correlations can be interesting, but complicate considerably graph analyses as various  
769 algorithm variants exist to handle them (e.g. in community detection) and there is no obvious criterion to  
770 choose one variant over others. For the sake of clarity, we thus treated negative correlations in most  
771 analyses (unless explicitly mentioned) as near-zero positive value of correlation (minimum edge weight  
772 of 0.006, see above). In efficiency analyses, this corresponds to interpreting negative correlations as open  
773 but difficult paths for information transfer. All graph construction and graph analysis were done through  
774 the igraph (Csardi G and Nepusz T, 2006) package on R (R Core Team, 2017).

### 775 **Bootstrapping confidence intervals**

776 For all network metrics, confidence intervals were computed through bootstrapping. This involves  
777 resampling subjects with replacement 1000 times, each time regenerating a functional network, then  
778 recalculating the estimate of interest. The 95% quartile of the bootstrap distribution was taken as the 95%  
779 confidence interval. Confidence intervals for the difference were used to test for differences between  
780 genotype groups (Wright et al., 2011). Groups were considered different to a  $P < .05$  if the 95 %  
781 confidence interval for the difference  $\geq 0$ , and to a  $P < .01$  if the 99 % confidence interval for the difference  
782  $\geq 0$ .

### 783 **Community Analysis**

784 Networks with high modularity,  $Q$ , have strong connections between the nodes within communities and  
785 relatively weaker connections between nodes of different communities.

$$Q = \frac{1}{2m} \sum_{i \neq j \in N} \left[ w_{ij} - \frac{s_i s_j}{2m} \right] \delta(c_i, c_j)$$

786 where  $N$  denotes the set of all nodes in the network,  $m$  denotes the total number of edges in the network,  
787  $w(i, j)$  denotes the edge weight between a node  $i$  and another node  $j$ ,  $s$  denotes the sum of a node's edge  
788 weights,  $c$  denotes the community to which a node belongs, and  $\delta(c_i, c_j)$  indicates if the compared nodes  
789 are in the same community (  $\delta(c_i, c_j)$  is 1 if  $c_i = c_j$ , and 0 otherwise ). Communities were detected in  
790 each bootstrap through finding the maximum modularity across all possible community partitions. This  
791 modularity maximization computation was done through the *cluster\_optimal* function of the igraph  
792 package. This transforms modularity maximization into an integer programming problem, and calls the  
793 GNU Linear Programming Kit (GLPK) to solve that. See Brandes et al, 2008 for more details. This  
794 computationally expensive detection method of evaluating all possible community partitions for

795 modularity maximization (in contrast to less expensive methods that infer modularity, as through greedy  
796 optimization, i.e. the Louvain method), could be permitted due to the relatively small number of nodes in  
797 our network. Allegiance matrices were used to assess community stability across bootstraps, by depicting  
798 the percentage of bootstraps ( $n = 1000$ ) that contain any given pair of regions within the same  
799 community.

## 800 **Information flow**

801 Nodal strength,  $\mathbf{s}$ , is traditionally calculated as the sum of a node's edge weights. In a fully connected  
802 network this is directly proportional to the average of a node's edge weights. The average,  $\mathbf{s}_{average}$ , was  
803 used in our case for better comparison with nodal efficiency.

$$s_{average}(i) = \frac{1}{n-1} \sum_{j \in N} w_{ij}$$

804 where  $N$  denotes the set of all nodes in the network,  $n$  denotes the total number of nodes in the network,  
805 and  $w_{ij}$  denotes the edge weight between a node  $i$  and another node  $j$ .

806 For efficiency metrics, edge lengths were first computed as inverted edge weights. Nodal efficiency,  
807  $E_{nodal}$ , was calculated as the average inverse shortest path length between a region and all other regions  
808 of the network.

$$E_{nodal}(i) = \frac{1}{n-1} \sum_{j \in N} \frac{1}{d_{ij}}$$

809 where  $d_{ij}$  denotes the length of the shortest path (lowest sum of edge lengths) between a node  $i$  and  
810 another node  $j$ . Global efficiency,  $E_{global}$ , was calculated as the average inverse shortest path length of  
811 the network.

$$E_{global} = \frac{1}{n(n-1)} \sum_{i \neq j \in N} \frac{1}{d_{ij}}$$

812

## 813 **Assessing the impact of global efficiency on memory deficits**

814 Subsamples were generated from each retention group by resampling  $n-1$  mice without replacement. This  
815 procedure generates a set of subsamples of size  $n-1$ , where the number of subsamples is the number of  
816 mice,  $n$ . From each subsample, the average memory index and the resulting network global efficiency

817 were computed. Correlation significance between memory index and global efficiency was computed  
818 through both Pearson and Spearman correlation coefficients. To compute the exploration adjusted  
819 memory index, first the exploration index was defined for each mouse as total time exploring both objects  
820 normalized to the group average:

$$\text{Exploration Index (EI)} = \frac{\text{Time exploring both objects of mouse}}{\text{Average time exploring both objects across the group}}$$

821 The exploration adjusted memory index was then defined as the memory index divided by the exploration  
822 index.

$$\text{Exploration adjusted memory index (MI/EI)} = \frac{\text{Memory Index (MI)}}{\text{Exploration Index (EI)}}$$

823 To assess the significance of the intersection of the WT linear fit with the dKI subsample population,  
824 1000 randomly fit linear models to the WT were generated by permuting the y labels of the WT  
825 subsample population and recalculating the best fit line each time. The mean absolute error to the dKI  
826 subsamples was calculated for each random model and their distribution is presented as a histogram. The  
827 95% confidence interval was considered as the 95% quartile of the distribution, and compared to the dKI  
828 mean absolute error to the original WT fit.

829

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